약학박사학위논문

Part I : 천연 **iridoid lactone**의 최초 전합성

Part II: **Ginsenoside Rk1** 구조 기반의 **anti-apoptotic agent**의 개발

Part I : **First total synthesis of natural iridoid lactone**

Part II: **Cholesterol derived novel anti-apoptotic agent on the structural basis of ginsenoside Rk1**

2011년 8월

서울대학교 대학원

제약학과 약품제조화학전공

이 수 진

Table of contents

Part I - First total synthesis of natural iridoid lactone

국문 초록 iii

List of Tables v

List of Figures vi

List of Schemes vii

Abbriviations viii

I. Introduction 1

1. Iridoid 2
   1) Previous synthetic approaches for iridoid lactones 5
2. Pd(0)-catalyzed cyclization 9
   1) Previous studies about Pd(0)-catalyzed cyclization 10
   2) Synthetic applications of bicyclic lactones 12
   3) Transformation of bicyclic lactone 14

II. Result and discussions 16

1. Pd(0)-catalyzed cyclization with substituted allyl carbonate 16
2. Total synthesis of natural iridoid lactone 20

III. Conclusion 33

IV. Experimental 34

V. References 55

VI. Appendix 60

VII. Abstract 98

Part I:천연 iridoid lactone의 최초 전합성

Part I: First total synthesis of natural iridoid lactone

국문 초록

Cyclopentane system은 의약품 및 생리활성을 갖는 천연물에 광범위하게 존재하는 구조로서, 다양한 입체 선택적인 cyclopentane system의 합성법이 여러 연구자들에 의해 개발, 발전되어 왔다. Iridoid 화합물은 *cis*-fused cyclopentane system을 갖는 천연물의 통칭으로, 주로 약용 식물로 이용되던 천연 자원으로부터 다양한 구조의 iridoid가 보고되었다. 그 중 최근 분리, 보고된 (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one의 특징적인 구조와 생리활성에 대한 기대로, 전합성을 시도하였다.

본 연구실에서 개발한 intramolecular Pd(0)-catalyzed allylic alkylation을 응용하여 iridoid 화합물의 합성을 계획하였다. C4에 alkyl기를 도입하기 위해 trisubstituted allyl carbonate가 도입된 γ-lactone을 합성 전구체로 계획하였다. 이를 합성하기 위해 합성 효율성이 높은 silicon-tether를 이용한 분자내 ring closing metathesis를 수행하였고, Pd(0)-catalyzed cyclization을 수행해 좋은 수율과 입체 선택적으로 bicyclic lactone을 합성하였다. 또한 이를 추가로 변환하여 iridoid의 핵심 골격인 *cis*-fused cyclopentane system을 효율적으로 합성하였다. C7-Hydroxymethyl을 입체선택적으로 도입하고자 cyclopentenol에 silicon-tether를 이용한 intramolecular radical cyclization, Tamao-Fleming oxidation을 수행하여 효과적으로 합성을 완료하였다.

본 연구를 통해 본 연구실에서 개발한 substituted vinyl기가 도입된 bicyclic lactone을 중간체로 이용하여 천연물의 합성에 적용할 수 있음을 확인하였고, 향후에 다양한 생리활성을 갖는 iridoid, iridoalkaloid등의 천연물 합성에 응용할 수 있을 것이라 기대한다.

주요어: (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one,

iridoid lactone, cyclopentane, Pd(0)-catalyzed cyclization, bicyclic lactone, silicon-tethered radical cyclization, Tamao-Fleming oxidation.

학 번: 2006-30449

List of Tables

Table 1. Cross metathesis with γ-lactone 18

Table 2. Pd(0)-catalyzed cyclization of 66 24

Table 3. Oxidation to cyclopentenone 26

List of Figures

Figure 1. Structures of cyclopentanoids 1
Figure 2. Structures of iridoids 2
Figure 3. Proposed biosynthetic process for iridoids 4
Figure 4. Palldium catalyzed allylic alkylation 10
Figure 5. Synthetic approach for branched allyl carbonate 16
Figure 6. Plausible transition state of Pd(0)-catalyzed cyclization 20
Figure 7. Target iridoid lactone 21
Figure 8. Bicyclopentenone coordinated with (*S*)-CBS oxazaborolidine 28
Figure 9. 3D-structure of bicyclic enone 28
Figure 10. 1, 6 hydrogen transfer 32

List of Schemes

Scheme 1. The first synthesis of nepetalactone 5
Scheme 2. Synthesis of *ent*-(-)-nepetalactone 6
Scheme 3. Synthesis of nepetalactone via oxidative radical cyclization 6
Scheme 4. Synthesis of (-)-7-deoxyloganin via NHC catalyzed rearrangement of α,β-unsaturated enolester 7
Scheme 5. Synthesis of (+)-geniposide via phosphine-catalyzed [3+2] cycloaddition 8
Scheme 6. Pd(0)-catalyzed allylic alkylation of acyclic allylic precursors 10
Scheme 7. Pd(0)-catalyzed cyclization with γ-lactone..... 11
Scheme 8. Synthetic applications with bicyclic lactone-formal synthesis of carbaprostacyclin 12
Scheme 9. Total synthesis of (+)-brefeldin A..... 13
Scheme 10. Total synthesis of bacillariolideⅢ 14
Scheme 11. Transformations of bicyclic lactone 15
Scheme 12. Syntheses of branched allyl carbonate γ-lactones..... 17
Scheme 13. Pd(0)-catalyzed cyclization with branched γ-lactone allylic carbonate... 19
Scheme 14. Retrosynthetic approach 21
Scheme 15. Synthesis of bicyclic pentanone 22
Scheme 16. Revised retrosynthetic approach 23
Scheme 17. Synthesis of γ-lactone 64d..... 23
Scheme 18. Attempt with bicyclic system 25
Scheme 19. Basic work up with cyclopentanone 27
Scheme 20. Revised retrosynthetic approach 29
Scheme 21. Synthesis of radical cyclization precursor 82 30
Scheme 22. Completion of synthesis of iridoid lactone 1..... 31

Abbriviations

br: broad singlet

BMCMDS: (bromomethyl) dimethylchlorosilane

calcd: calculated

CBS: Corey-Bakshi-Shibata

d: doublet

DBU: 1,8- diazabicyclo[5.4.0]undec-7-ene

DMAP: 4-dimethylaminopyridine

DMF: *N,N*-dimethylformamide

DMP: Dess-Martin periodinane

DMSO: dimethylsulfoxide

dr; diastereomeric ratio

EtOAc: ethylacetate

FAB: fast atom bombardment

GPP: geranylpyrophosphate

HRMS: high resolution mass spectroscopy

HWE: Horner-Wadsworth-Emmons reaction

IR: infrared

LiTMP: lithium tetramethylpiperidide

LRMS: low resolution mass spectroscopy

m: multiplet

Me: methyl

MeOH: methyl alcohol

NHC: *N*-heterocyclic carbene

NMO: 4-methylmorpholine *N*-oxide

NMR: nuclear magnetic resonance

OBO: 2,6,7-trioxabicyclo[2.2.2]octane

PDC: pyridinium dichromate

Ph: phenyl

PPTS: pyridinium *p*-toluene sulfonate

q: quartet

RCM: ring-closing metathesis

s: singlet

SAR: structure-activity relationship

t: triplet

TBAF: tetra-*n*-butylammonium fluoride

TBS: *t*-butyldimethylsilyl

TFAA: trifluoroacetic anhydride

THF: tetrahydrofuran

TLC: thin layer chromatography

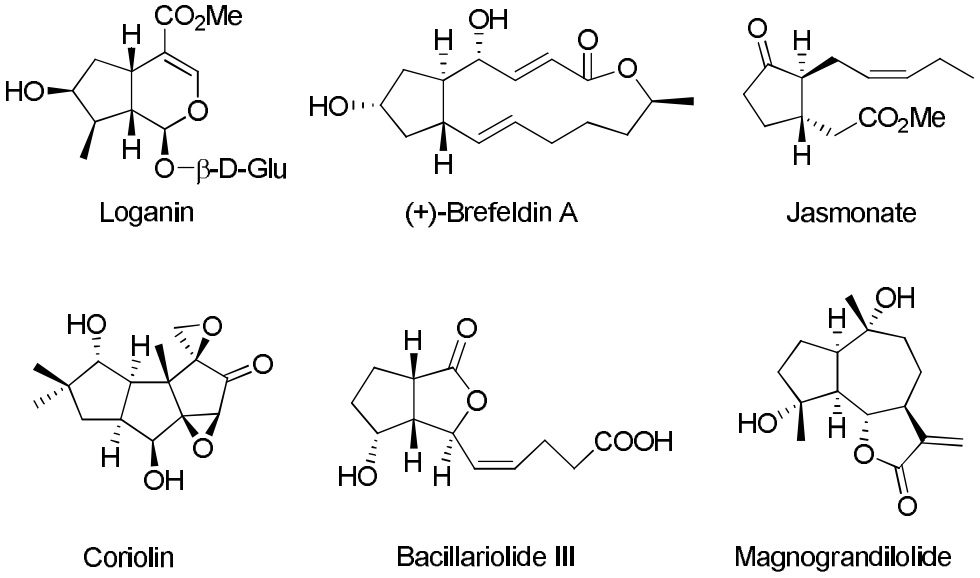
TPAP: tetra-*n*-propylammonium perruthenate

TsOH: *p*-toluene sulfonic acid

I. Introduction

Cyclopentane system is an abundant structure in nature and bioactive chemicals, such as prostaglandins, iridoids, sesquiterpenoids and guaianolides. (Figure 1) And it has been reported that the cyclopentanoids represented various activities, such as, antibacterial, antiviral, antiproliferative, antipyretic and anti-hypertensive effects.[1] Thus, considerable effort has been devoted in the past decades to the development of new methods for the construction of five-membered carbocycles, since they play a fundamental role in synthetic organic chemistry, as both targets and intermediates.

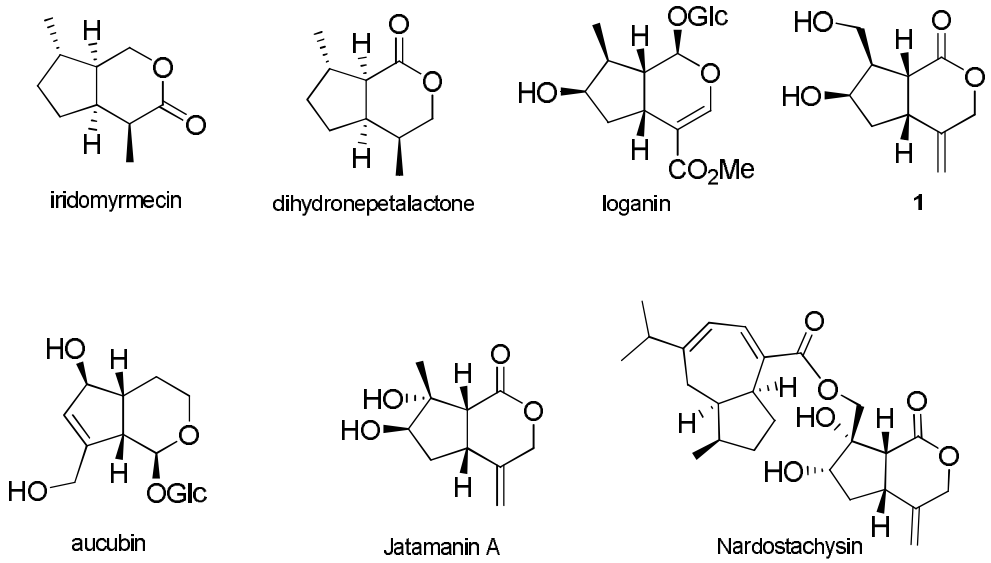
Figure 1. Structures of cyclopentanoids



The construction of five-membered carbocycles has attracted extensive attention among synthetic chemists. Considerable diverse methodologies emerged and were summarized in a series of reviews, which can be divided in two general categories: procedures based on the sequential functionalization of cyclopentanoid precursors, such as cyclopentene[2] and cyclopentadiene,[3] and

ring-formation routes from acyclic molecules, some being applied as key steps in the restructuring of readily available chiral materials, such as carbohydrates,[4] quinic acid,[5] and cyclohexadiene diols, into carbocycles. [6] The search for new, more efficient, and versatile antitumor, anticancer, antibacterial, antimicrobial, etc. agents is among the priorities of the worldwide science. In particular, a serious part of the efforts of the leading scientific and industrial chemical organizations nowadays is directed toward the synthesis of natural and biologically active products, and the improvement of the routes for the preparation of cyclopentanoid moieties is still growing at a steady pace.

Figure 2. Structures of iridoids



1. Iridoid

Iridoids are large class of naturally occurring compounds with over 300 members in the family

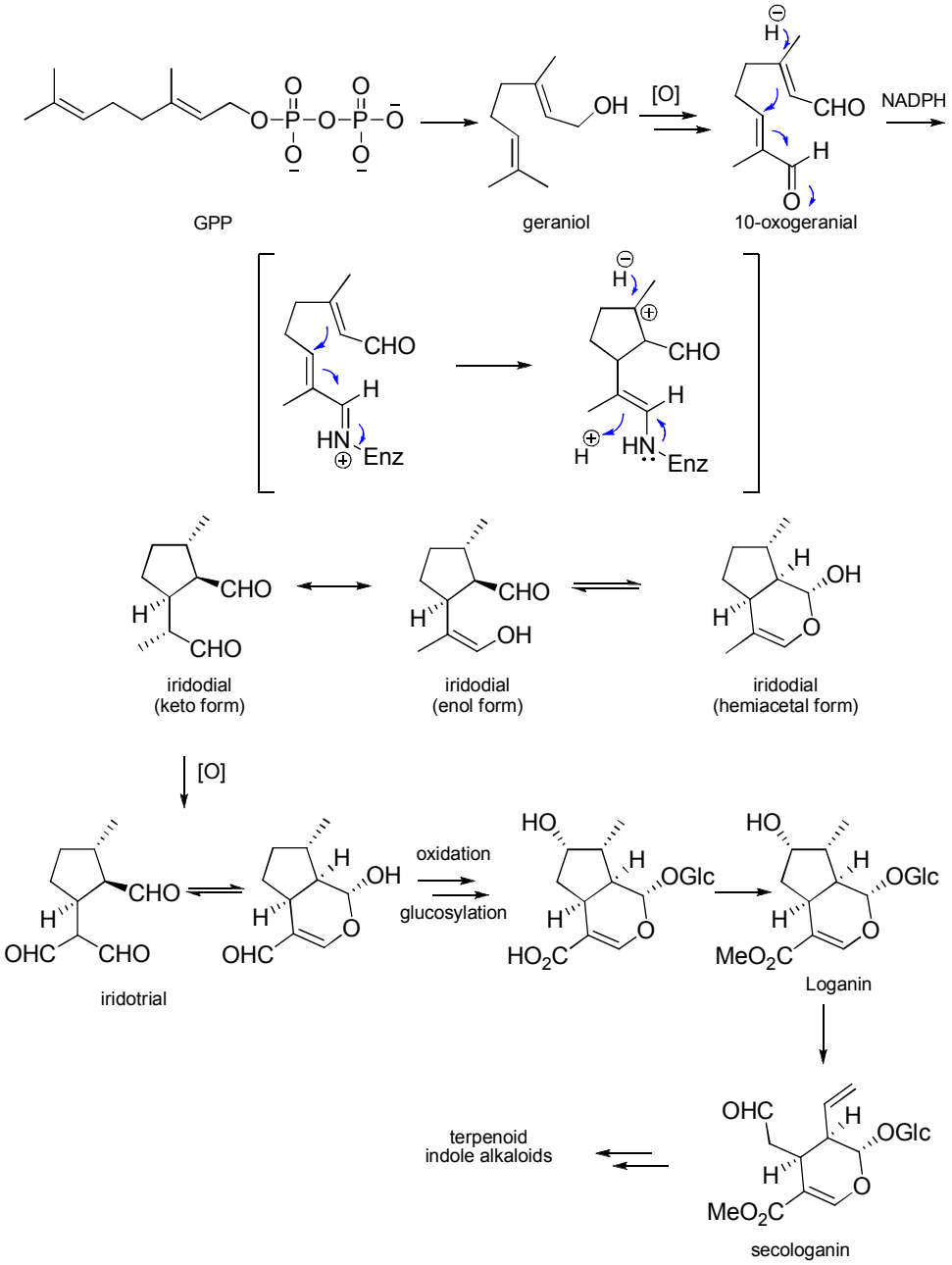
and still expanding. The first isolated iridoid was isolated from the secretion of ants belonging to the genus *Iridomyrmex,* the name of 'iridoid' was originated from that fact.[7] The iridoids have been found in a number of folk medicinal plants used as bitter tonics, sedatives, hypotensives, antipyretics, cough medicines, remidies for wounds and skin disorders. This fact encouraged to investigate the bioactivities of these phytochemicals. Structurally, iridoids represent a highly oxygenated monoterpenoids skeleton characterized by *cis*-fused cyclopenta[*c*]pyran. The representative structures of iridoids are presented at Figure 2.

The iridoid lactones can be further subdivided into two groups, type I and type II, depending on the regiochemical orientation of the lactone with respect to the functionalized cyclopentane ring. In type I iridoids typified by iridomyrmecin the lactone carbonyl is at C3, whereas in type II lactones such as dihydronepetalactone the carbonyl is at C1.

Proposed biosynthetic pathway is shown at Figure 3. Geraniol was produced by geranylpyrophosphate (GPP) by enzyme reaction. Geraniol underwent hydroxylation and oxidation reactions to 10-oxogeranial. The skeleton of iridoid arises from iridodial, which is formulated by cyclization as reductive Michael addition with hydride on the dialdehyde. Or imine formation by enzyme assisted carbocationic cyclization to cyclopentane.[8] The imine mediated pathway is more acceptable for the natural synthetic mode.

Iridodial was found to serve in the chemical defense system of many insect species,[9] and iridodial is the key precursor of numerous iridoids, possessing methyl group at C4, such as nepetalactone.[10] Further oxidized iridotrial has converted to loganin via oxidation, glucosylation and methylation. Loganin is a key intermediate in the pathway to a range of complex terpenoid indole alkaloids and tetrahydroisoquinoline alkaloids. And secologanin, which has been produced by oxidative cleavage from loganin, is a key intermediate for secoiridoids.

Figure 3. Proposed biosynthetic process for iridoids

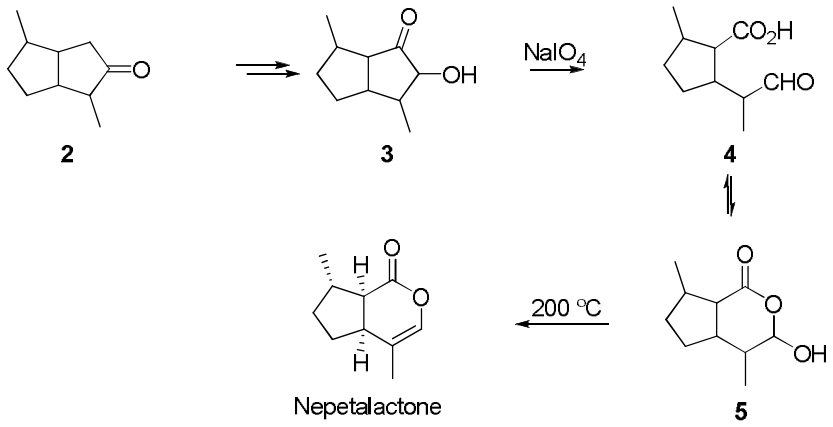


The various biological activities of iridoids have continuously attracted interests from both biologists and chemists. The unique *cis*-fused cyclopenta[*c*]pyran ring system has presented a variety of challenges for chemical synthesis and in the analysis of biological activities. Numerous syntheses of iridoids have been described in the literature.

1) Previous synthetic approaches for iridoid lactones

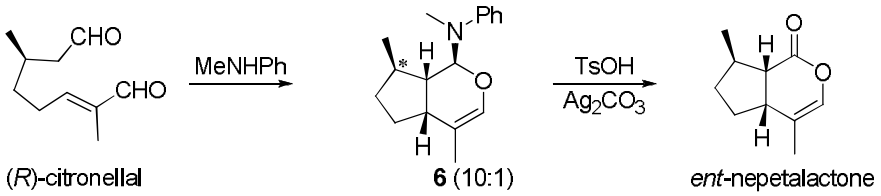
The representative synthetic approaches for type II iridoids are subscribed as below.
The key features of these syntheses involved the formation of cyclopentane and stereoselective introduction of pyran ring system to cylopentane.

Scheme 1. The first synthesis of nepetalactone



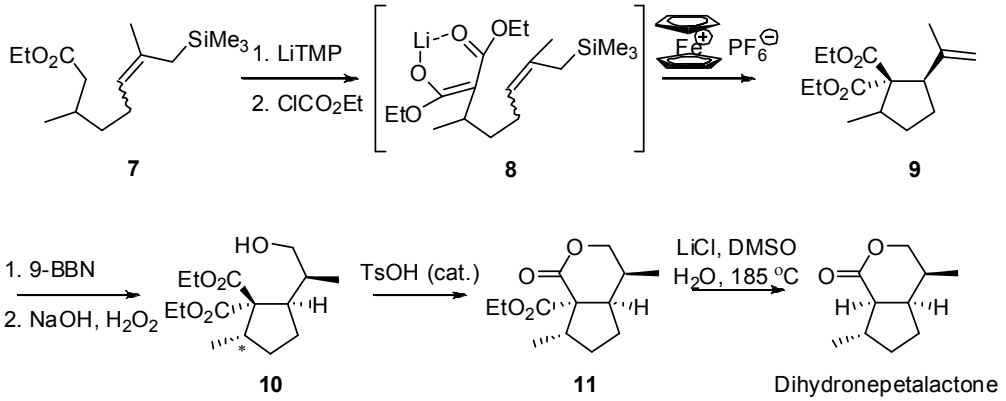
The first synthesized type II iridoid was nepetalactone.[11] Sakan et al. achieved α-ketol **3** as a key intermediate from bicyclooctanone [3.3.0] **2** and oxidative cleavage afforded to aldehyde acid **4**. The synthesis has accomplished by pyrolytic dehydration from lactol **5**. This piece of work was not a stereoselective synthesis. There are so many synthetic achieves since this synthesis has achieved. Here represent asymmetric syntheses by sigmatropic rearrangement.

Scheme 2. Synthesis of *ent*-(-)-nepetalactone



Schreiber et al. synthesized *ent*-nepetalactone via intramolecular enal-enamine cycloaddition [2+4].[12] (*R*)-citronellal treated with methyl phenylamine afforded enamine, and then furnished intramolecualr cycloaddition of enamine and enal provided a separable 10:1 mixture of the desired dihydropyran product **6** and its methyl epimer in 84% yield. Equilibration furnished a better 25:1 ratio of diastereomers, although in lower yields (60-65%). Then the dihydropyran **6** was hydrolyzed to unsaturated lactol, which was oxidized with silver carbonate (Fetizon oxidation) to afford *ent*-nepetalactone. This intramolecular cycloaddition strategy has inspired many other synthetic chemists.[13]

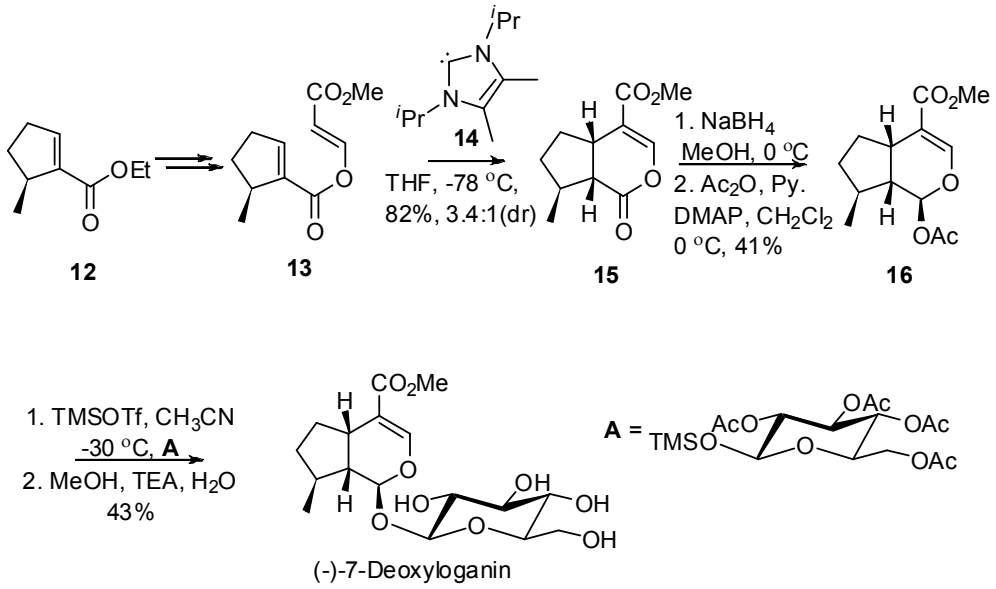
Scheme 3. Synthesis of nepetalactone via oxidative radical cyclization



Recently, numerous research papers have been published on the synthetic approaches to iridoids.

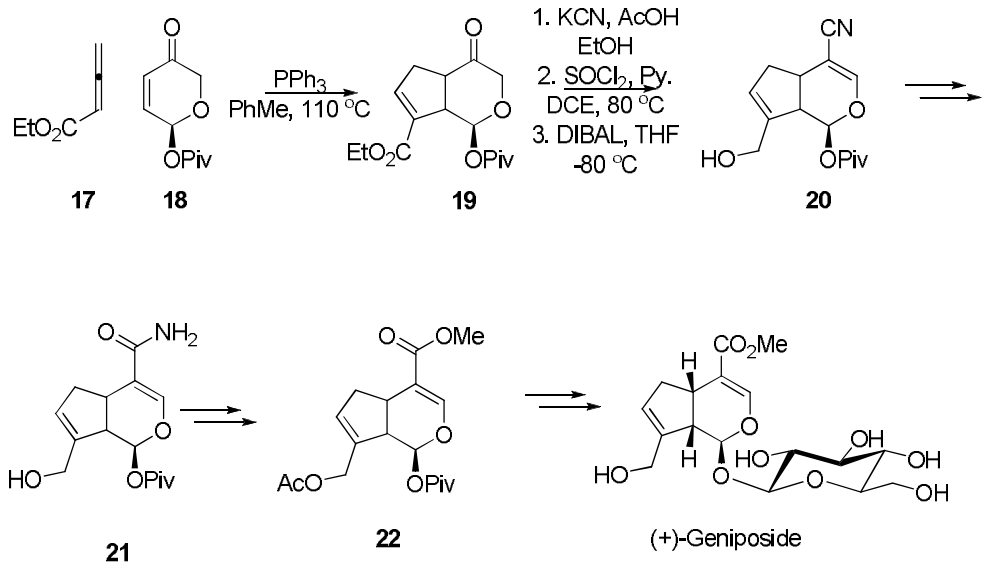
And the stereoselective synthesis of iridoid lactone has been carried out very successfully. As shown in Scheme 3, cyclopentane system embedded isoprene and ester **9** was synthesized via oxidative radical cyclization. [14] ω-Silylcitronellate **7** was subjected to the tandem alkoxycarbonylation/ oxidative cyclization reaction by deprotonation with lithium tetramethylpiperidide (LiTMP), ethylchloroformate and ferrocenium hexafluorohphosphate. The product cyclopentane **9** was isolated as a 2:1 *trans:cis*-diastereomeric mixture. The inseparable diastereomeric mixture was carried through to hydroboration/ oxidation to provide the separable alcohol **10**. The alcohol **10** was submitted to lactonization induced by catalytic amount of TsOH in CH_2Cl_2 to provide the lactone **11**. Then, Krapcho dealkoxycarbonylation afforded dihydronepetalactone.

Scheme 4. Synthesis of (-)-7-deoxyloganin via NHC catalyzed rearrangement of α,β-unsaturated enolester



Another synthetic tactic was *N*-heterocyclic carbene (NHC) rearrangemet with α,β-unsaturated enolester, which provided bicycle lactone system[15] (Scheme 4). NHCs have become universal ligands in organometallic and inorganic coordination chemistry.[16] Because of their specific coordination chemistry, NHCs both stabilize and activate metal centers in quite different key catalytic steps of organic syntheses, such as C-H activation, C-C, C-O, C-N bond formation. According to Candish and Lupton, NHC (**14**) catalyzed rearrangement with the α,β-unsaturated enolester **13** afforded bicyclic lactone **15** successfully, though the diastereomeric ratio was slightly low (3.4:1). Concommitant carbonyl reduction, glycosylation and global deprotection provided (-)-7-deoxyloganin.

Scheme 5. Synthesis of (+)-geniposide via phosphine-catalyzed [3+2] cycloaddition



17 **18** **19** **20** **21** **22**

The more recent approach for iridoid synthesis was contributed by Jones and Krische.[17] Phosphine-catalyzed [3+2] cycloaddition with ethyl-2,3-butadienoate **17** and α,β-unsatutated lactol **18** (Achmatowicz enone[18]) afforded bicyclic lactol **19** as shown in Scheme 5. This bicyclic lactol

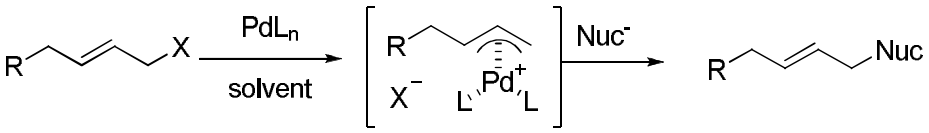
19 was converted to the cyanohydrins, which upon elimination furnished the α,β-unsatutated nitrile **20**. Nitrile converted to methyl ester **22** via primary amide **21**. Glycosidation of lactol employing the trichloroacetimidate as the glycosyl donor delivered the β-glucoside and then global deprotection provided (+)-geniposide.

Throughout these pieces of works, most synthetic efforts had been focused on the deoxygenated iridoids, since it is difficult to achieve to synthesize highly oxidized cyclopentane system enantioselectively. With this notion in mind, we took on the challenge in the iridoid lactone synthesis, which relies on our endeavor of Pd(0)-catalyzed cyclization method.

2. Pd(0)-catalyzed cyclization

Development of asymmetric metal-catalyzed reactions has played a significant role in allowing synthetic access to biologically important molecules. Since Pd(0) catalyzed alkylation has developed by Jiro Tsuji and co-workers, development of asymmetric metal-catalyzed reaction has been manipulated by a lot of chemist groups.[19] The most widely utilized reactions involve dihydroxylation, epoxidation or hydrogenation, and these reactions generate only one type of bond, either a C-O bond C-H bond. In contrast, asymmetric allylic alkylation reaction forms diverse type of bonds, such as C-C bond, C-N bond, C-S bond and C-O bond. Above all, palladium catalyzed cyclization has been utilized as one of the most powerful synthetic tools for carbocycle construction due to its highly versatile selectivities. The schematic introduction of Pd(0)-catalyzed allylic alkylation has shown in Figure 4.

Figure 4. Palldium catalyzed allylic alkylation

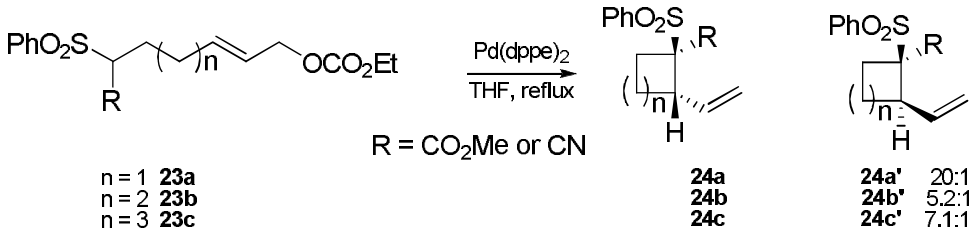


Formation of π-allylpalladium complex takes place by the oxidative addition of palladium to allylic compounds, such as allyl carbonates, allylic ester or halides. The π-allylpalladium complex is a resonance form of σ-allylpalladium complex and a coordinated π-bond. The π-allylpalladium complex formation involves inversion of stereochemistry, and the attack of the soft carbon mucleophile on the π-allylpalladium complex is also inversion.[20] Thus, the stereochemistry of product is retention.

1) Previous studies about Pd(0)-catalyzed cyclization

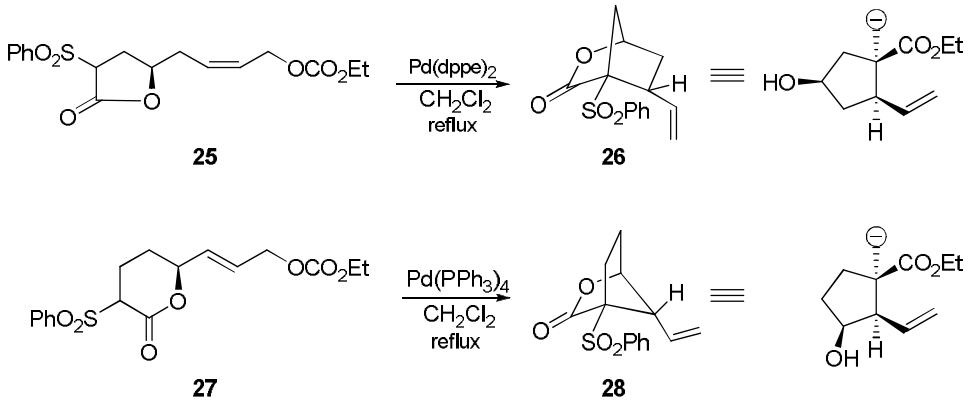
In our laboratory, it has developed Pd(0)-catalyzed intramolecular allylic alkylation. At first, Pd(0)-catalyzed cyclization reaction was investigated with the acyclic allyl precursors **23a-23c** (Scheme 6). Possessed two anion stabilizing groups, benzenesulfonate and ester, or nitrile, allyl carbonates afforded the 1,1,2-stereoselective triisubstitutued cyclobutane **24a**, cyclopentane **24b** and cyclohexane **24c** with good chemical yields.[21]

Scheme 6. Pd(0)-catalyzed allylic alkylation of acyclic allylic precursors



However, it is difficult to control of the stereogenic center at nucleophilic carbon possessing two anion stabilizing groups, because the stereocontrol at this center as well as the ultimate loss of the stereochemistry upon removal of the anion stabilizing auxiliary. Accordingly, 1,2-diastereocontrol of the new two stereogenic centers in Pd(0)-catalyzed cyclization of allylic precursor as well as retention of the established stereochemistries reamin as formidable tasks. To enhance the ability of application to natural products synthesis, the chiral hydroxyl group has introduced to alkyl chain, thus the concomitant γ-, or δ-lactone has provided. With γ-, or δ-lactone **25** or **27**, Pd(0)-catalyzed cyclization reaction was successfully performed to afford the bicyclic lactone **26** or **28** with high diastereoselectivity (Scheme 7). Particularly, desulfonylation of the bicyclic lactone **26** or **28** afforded trisubstituted cyclopentane with retention of stereochemistry. The functional groups at the trisubstituted cyclopentane systems are all *cis* relationship, which are laborious to synthesis. The synthetic utility of the optically active *cis*-trisubstituted cyclopentane obtained by this sequence has been demonstrated by some applications to the stereoselective synthesis of natural products.

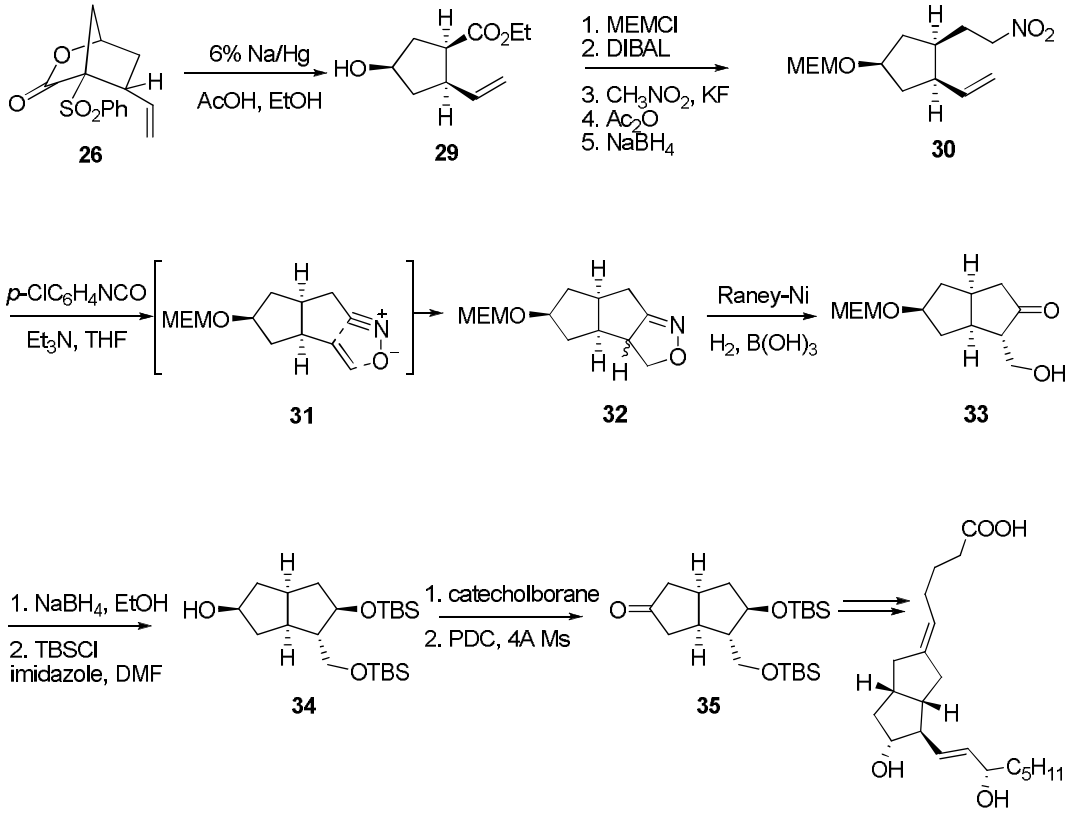
Scheme 7. Pd(0)-catalyzed cyclization with γ-lactone



2) Synthetic applications of bicyclic lactones

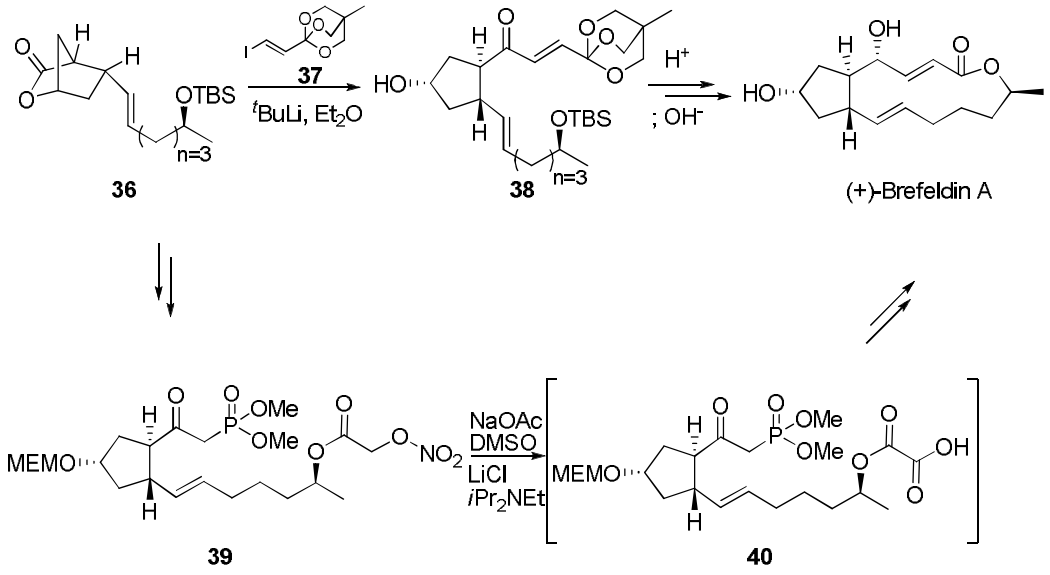
With these bicyclic lactones, the synthetic applications for natural products and bioactive compounds have been reported in our laboratory. The bicyclic lactone **26** was selectively converted to *cis*-trisubstituted cyclopentane system **29**. The *cis*-trisubstituted cyclopentane **29** was protected with methoxyethyl chloride then the intramolecular nitrile oxide-olefin cycloaddition afforded to the hydroxy ketone **33** via **32**. Stereoselective reduction of the ketone **33** afforded the cyclopentanol **34** and then straightforwardly transformed to known intermediate **35** of carbaprostacyclin[22] (Scheme 8).

Scheme 8. Synthetic applications with bicyclic lactone-formal synthesis of carbaprostacyclin



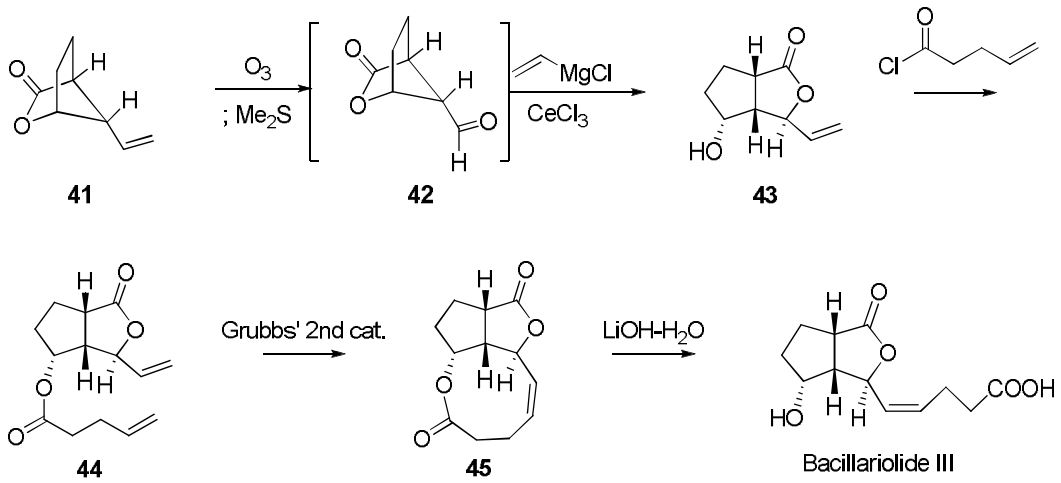
In our laboratory, the total synthesis of (+)-brefeldin A, employing the stereoselective Pd(0)-catalyzed cyclization has been reported. (Scheme 9) (+)-Brefeldin A has been one of the most attractive targets for the synthetic chemists due to its wide range of biological activities and characteristic structure. To introduce the three carbon synthon to lactone carbonyl at **36**, the (*E*)-1-(2-iodovinyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**37**, OBO ortho ester) was adapted as an equivalent of the carboxyl group. Especially, the OBO ortho ester **37** was an excellent carboxyl equivalent, since it is resistant to attacks by strong nucleophiles and redily hydrolyzed under mild conditions.[23] On the other hand, as a part of ongoing studies directed toward the synthesis of the diversified brefeldin A analogs, more practical and versatile synthetic strategy was investigated. The salient feature of the synthesis includes the intramolecular HWE olefination of **40**.[24] Through this tactic, brefeldin A-amide analog synthesis was also accomplished.[25]

Scheme 9. Total synthesis of (+)-brefeldin A



From the bicyclic lactone **28**, the total synthesis of bacillariolide Ⅲ, which was isolated from the marine diatom *Pseudonitzschia multiseries*, was accomplished.[26] The key feature of this synthesis involves the stereoselective vinylcerium addition to bicyclic lactone aldehyde **42**, which induces its facile transformation into the bicyclic lactone skeleton **43** and efficient introduction of the (Z)-pentenoic acid side chain by RCM of **44** (Scheme 10).

Scheme 10. Total synthesis of bacillariolideⅢ

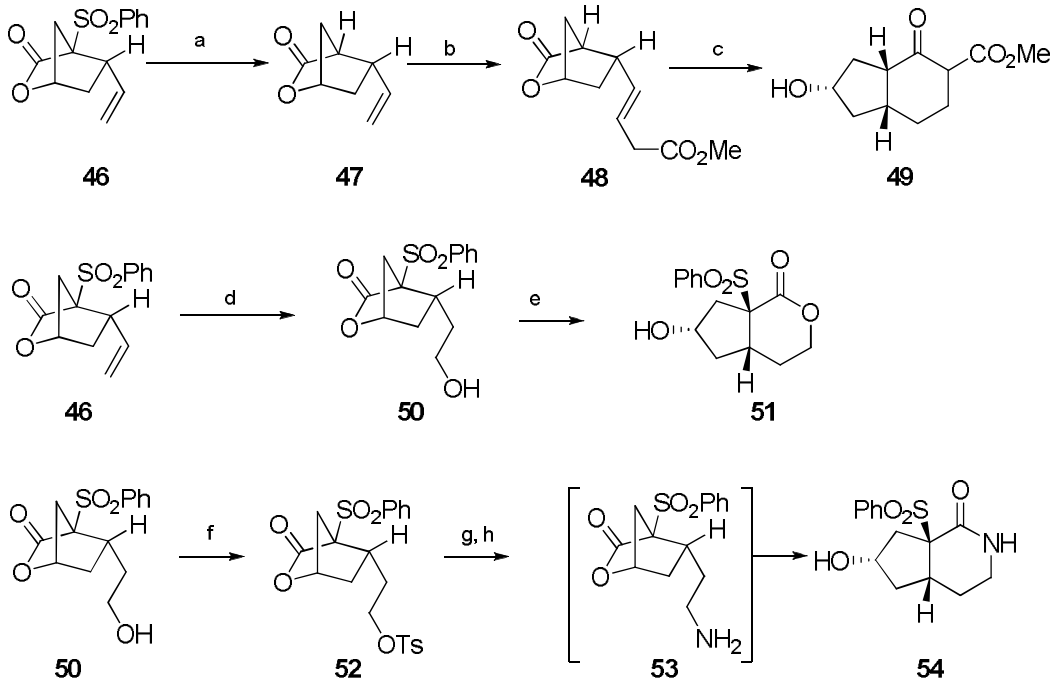


3) Transformation of bicyclic lactone

Also, the bicyclic lactone was easily transformed to other bicyclic systems (Scheme 9). At first, desulfonylated bicyclic lactone **46** underwent cross metathesis with acrolate, hydrogenation of **48** and then base promoted isomerization afforded 5-6 membered ring *cis*-fused carbocycle **49**. Secondly, hydroboration of vinyl afforded to the primary alcohol **50**, transesterification was accomplished to bicyclic lactone **51** by DBU. And the primary alcohol **50** was substituted to azide

via tosylate **52**, and successive Staudinger reaction gave the bicyclic lactam **54**. [27]

Scheme 11. Transformations of bicyclic lactone



Reagents: (a) Na/Hg, $B(OH)_3$, MeOH, 55%; (b) Grubbs' 2nd catalyst, methyl acrolate, CH_2Cl_2, 92%; (c) H_2, Pd/C, then KO^tBu, benzene, 70% for 2 steps; (d) catechol borane, $Rh(PPh_3)_3Cl$, THF, then H_2O_2, pH 7.0 buffer, 67% (e) DBU, CH_3CN, 72%; (f) TsCl, Et_3N, DMAP, CH_2Cl_2, 97%; (g) NaN_3, DMF, 88%; (h) PPh_3, wet THF, 68%.

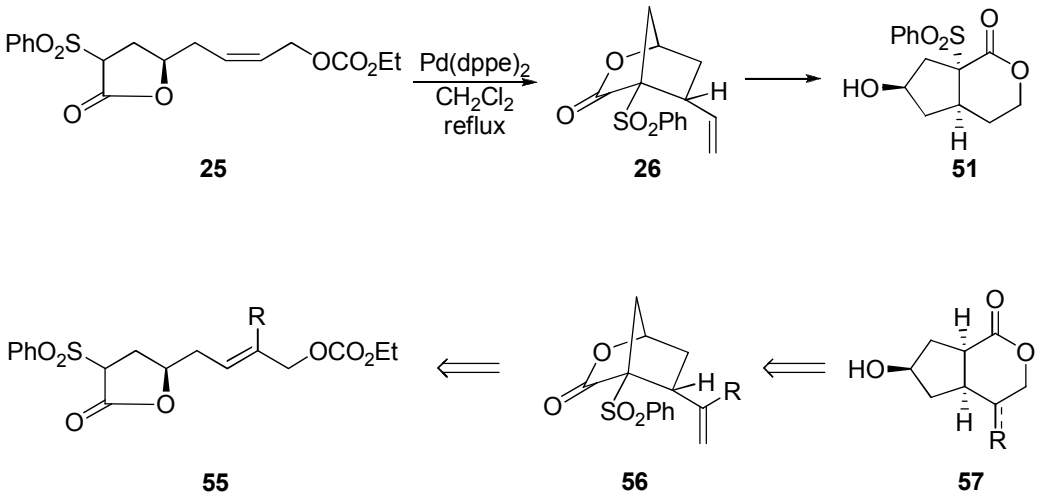
Throughout these transformations, it is well-known that the bicyclic lactone is an efficient synthetic intermediate, the synthetic approaches for natural products is on-going.

II. Result and discussions

1. Pd(0)-catalyzed cyclization with substituted allyl carbonate

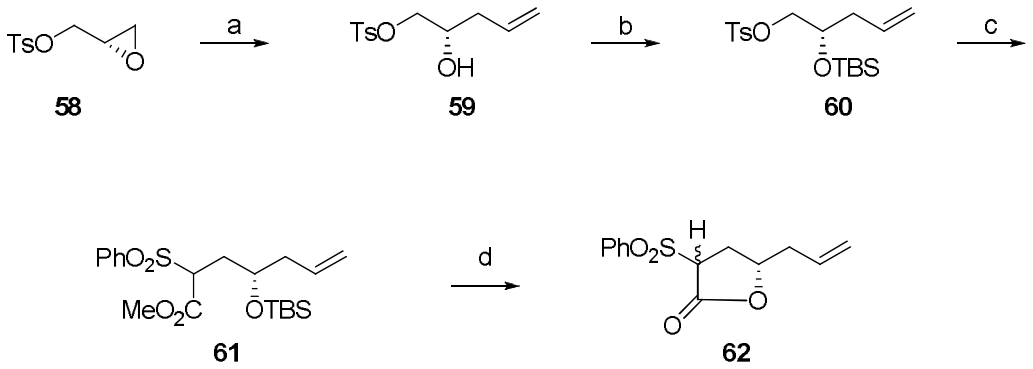
Previously, it has been reported that highly stereoselective construction of unique bicyclic lactones via intramoelcular Pd(0)-catalyzed cyclization has been accomplished in our lab. The [2.1.2] or [2.2.1] bicyclic lactones could be utilized as excellent equivalents of contra thermodynamic *cis*-trisubstituted cyclopentanes which have been importantly been utilized for total synthesis of bioactive natural products as described before. These applications implied high utility of the bicyclic lactone architectures. However, iridoids or other bioactive natural products, which have bicyclic structure embedded natural products, possess alkyl substituent at C4 (Figure 6). And to address these substituents after the bicyclic ring formation is a laborious task. Hence, the development of more transformative bicyclic lactone was needed. That has driven us to focus on the investigation of the more transformative bicyclic lactone. Thus we envisaged the branched allyl carbonate for Pd(0)-catalyzed cyclization.

Figure 5. Synthetic approach for branched allyl carbonate



As shown in Figure 5, substituted vinyl bicyclic lactone **56** could be transformation to bicyclic lactone **57**, which has substituent at C4. The branched allyl carbonate γ-lactone **55** would be obtained via cross metathesis with γ-lactone and diverse allyl carbonates. The synthesis of γ-lactone is shown below (Scheme 12).

Scheme 12. Synthesis of γ-lactone

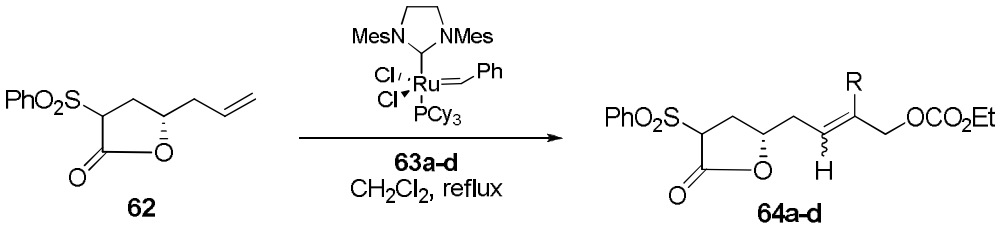


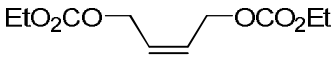
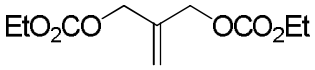
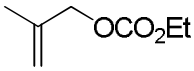
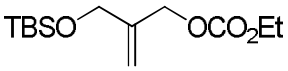
Reagents: (a) vinylmagnesium bromide, Li_2CuCl_4, THF; (b) imidazole, TBSCl, DMF, 0 ºC, 88% for 2 steps; (c) methyl benzenesulfonyl acetate, NaH, DMF, 80 ºC, 77%; (d) TBAF, THF, 78%.

Vinyl addition to tosylate glycidol **58** and TBS protection afforded **60**, alkylation with methyl benzenesulfonate and TBS deprotection gave γ-lactone **62** (Scheme 12). At the alkylation step, diastereomeric mixture was obtained and the concomitant γ-lactone **62** was also inseparable diastereomeric mixture. γ-Lactone **62** underwent cross metathesis with allyl carbonates afforded precursors of Pd(0)-catalyzed cyclization (Table 1).

The cross metathesis product **63a** and **64b** were obtained *E* isomers dominantly, but **63d** was obtained *E/Z* mixtures, which were inseparable. Thus, with these branched allyl carbonate γ-lactone, we proceeded Pd(0)-catalyzed cyclization at the same conditions.

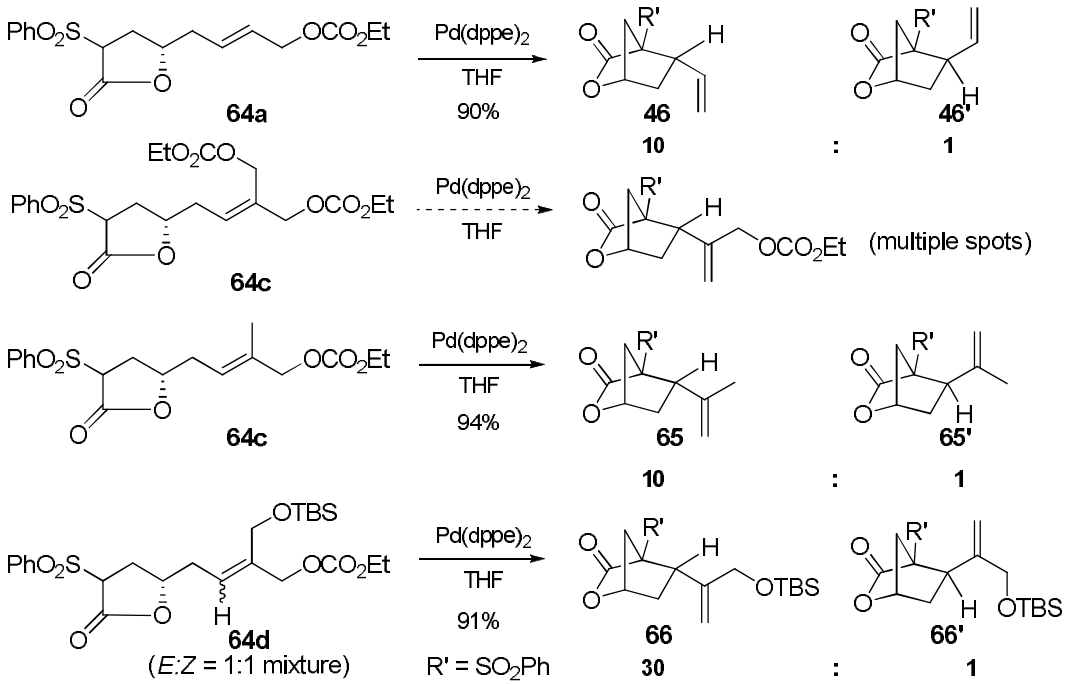
Table 1. Cross metathesis with γ-lactone



Entry	Cross metathesis partner	Yield	product
1	 **63a**	48	**64a**
2	 **63b**	49	**64b**
3	 **63c**	33	**64c**
4	 **63d**	43	**64d**

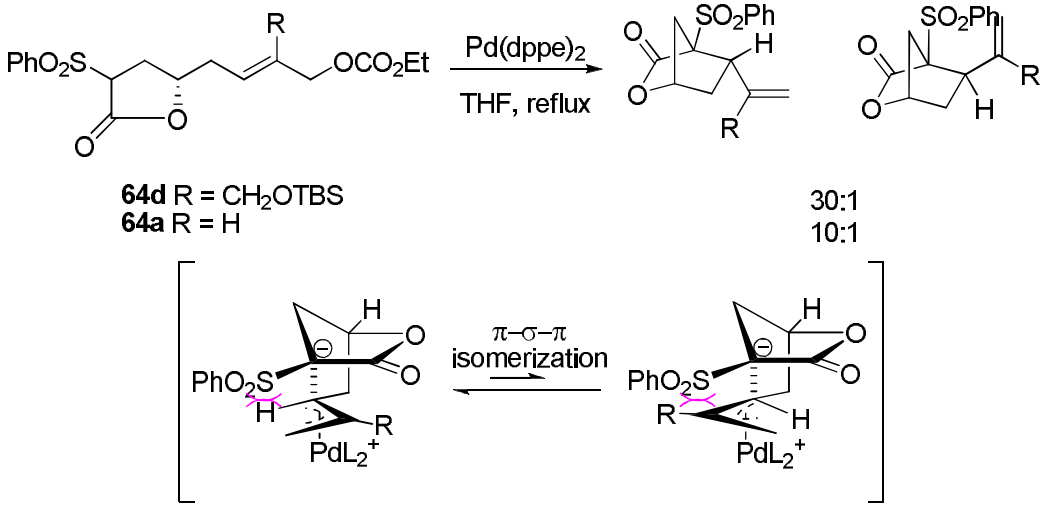
Similar to linear allyl carbonate, metallyl carbonate and hydroxymethyl allyl carbonate were represented good precursors for Pd(0)-catalyzed cyclization. These branched allyl carbonates underwent Pd(0)-catalyzed cyclization with excellent chemical yields and diastereoselectivities from 10:1 to 30:1 (Scheme 13). Exceptionally, diallylcarbonate **64b** did not afford desired bicyclic lactone. It seemed that initially diallylcarbonate **64b** underwent the Pd(0)-catalyzed cyclization, but further unexpected reactions occurred with the allyl carbonate at bicyclic lactone, provided multiple unidentified products.

Scheme 13. Pd(0)-catalyzed cyclization with branched allyl carbonate



The diastereomeric ratio was enhanced with branched allyl carbonate, which would explain the steric repulsion between benzensulfonate and methylene or hydrogen as shown in Figure 6. The excellent stereoselectivity is likely due to the presence of the silyloxymethyl substituent, given the lower stereoselectivity (10:1) for the disubstituted alkene **64a** under the same conditions as shown in Figure 6. In particular, the *E/Z* isomers of **64d** were executed the Pd(0)-catalyzed cyclization with high diastereoselectivity, which proved the Pd(0)-catalyzed C-C bond cleavage/formation sequence would reinforce high diasteroselectivity of the cyclization process for [2.1.2] bicyclic lactone followed by π-σ-π isomerization. This type of facial preference is well supported by our previous report.[28] And the stereochemistry of benzenesulfonyl group was negligible, because of α-deprotonation with basic condition.

Figure 6. Plausible transition state of Pd(0)-catalyzed cyclization

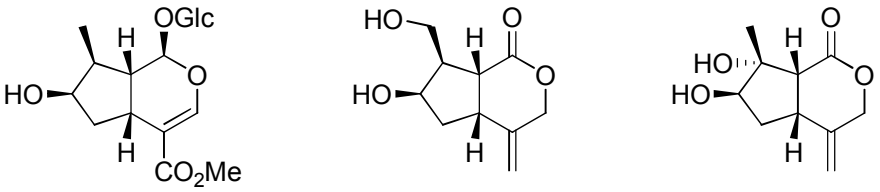


2. Total synthesis of natural iridoid lactone

In the cyclopentanoids, we have been interested in a recently reported iridoid lactone, which is structurally related to loganin, and consists of a C7-substituted C6-hydroxycyclopenta[*c*]lactone, as illustrated in Figure 8. The unique structures and biological activities of iridoid lactone was concerned the synthetic interests. Iridoid lactone **1** was isolated from the roots and rhizomes of *Nardostachys chinensis* Batalin (Valerianaceae).[29] This plant has been used as a Chinese folk medicine and its roots and rhizomes exhibit a variety of biological activities, such as sedative, antimalarial, antinociceptive, cytotoxic, and enhancement of nerve growth factor activities.[30] However, the paucity of its key component **1** has hampered the systematic research of the iridoid, including structure-activity relationship (SAR) analysis and further pharmacological studies. Thus, we have worked on the development of an efficient synthetic route to the new iridoid lactone **1** (Figure 8). The iridoid lactone **1** is structurally very interests. It has contiguous, various functional groups, such as hydroxyl, hydroxymethyl, *exo*-methylene, and lactone. Moreover, the 4-

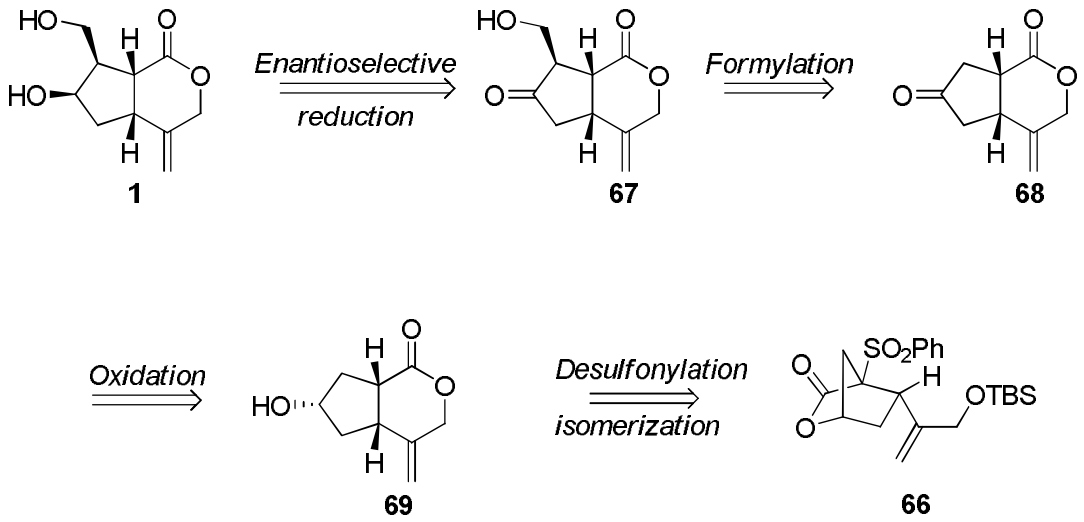
stereogenic centers are contiguous. This challengeable structure as well as the curiosity for biological activity led us to synthesis.

Figure 7. Target iridoid lactone



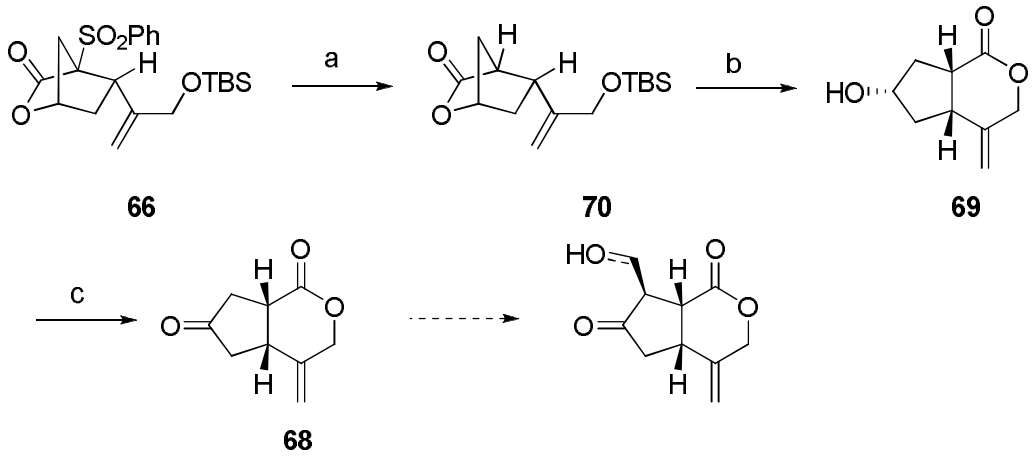
To establish the bicyclic lactone structure, we planned to synthesize the bicyclic lactone via Pd(0)-catalyzed cyclization. Our retrosynthetic approach for iridoid lactone **1** is illustrated in Scheme 14. We hypothesized that the bicyclic lactone **66** might serve as a synthetic precursor of iridoid lactone through three potentially straightforward transformations: (a) oxidation of cyclopentanol **69** to cyclopentanone **68**, (b) regioselective α-formylation, and (c) enantioselective reduction of C6 carbonyl group.

Scheme 14. Retrosynthetic approach



With the bicyclic lactone **66** in hand, we attempted to isomerize bicyclic system for the iridoid skeleton. Desulfonylated bicylcic lactone was isomerized to concomitant bicyclic lactone **69** and oxidized to cyclopentanone **68**. To address the regioselective hydroxymethylation, various reaction conditions were considered. A direct α-hydroxymethylation with HCHO[31] or the introduction of a hydroxymethyl equivalent via α-methylenation with Eschenmoser's salt[32] as well as formylation with $HCO_2CH_2CF_3$[33] to the cyclopentanone did not provide satisfactory results.

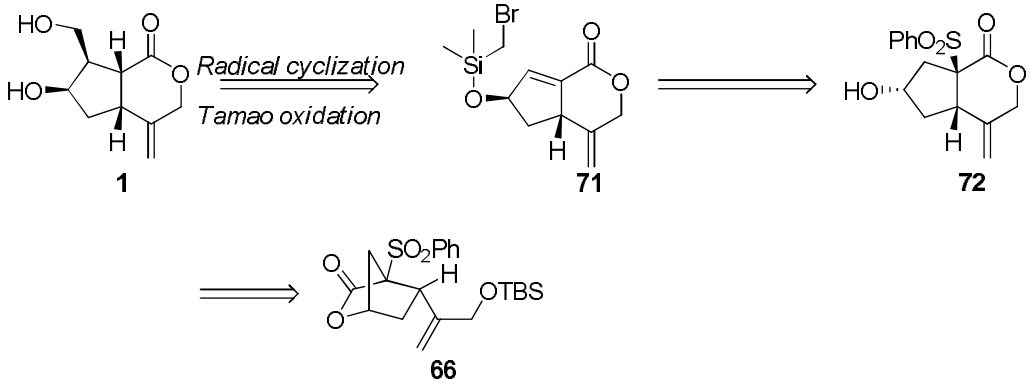
Scheme 15. Synthesis of bicyclic pentanone



Reagents: (a) Na/Hg, $B(OH)_3$, MeOH, 56%; (b) TBAF, THF, 73%; (c) TPAP, NMO, CH_2Cl_2, 67%.

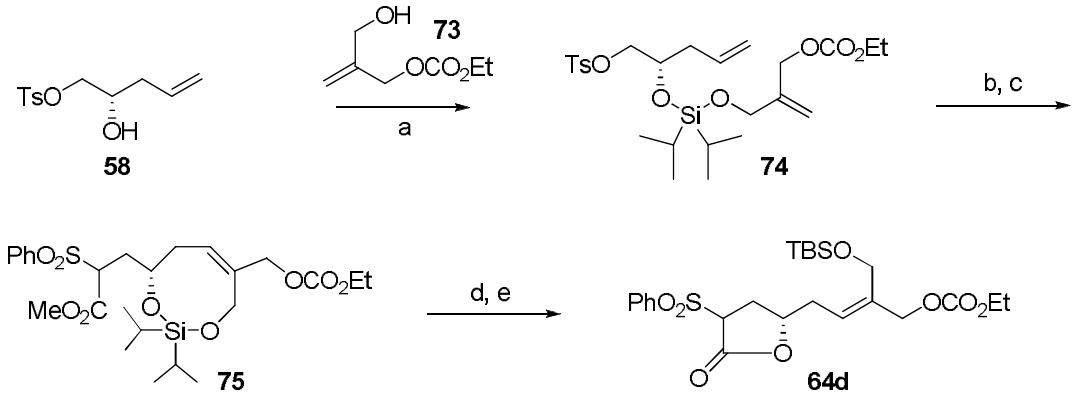
Throughout the results, it turned out that the regioselective direct α-formylation or hydroxymethylation was not successful for our system. It was suspected that the hydrogens at ring junction were labile at basic condition. As a matter of fact, the allylic hydrogen at C5 of **68** was easily deprotonated with general bases. So we envisaged cyclopentenone to establish the regio-, and chemoselectivity for hydroxymethylation.

Scheme 16. Revised retrosynthetic approach



The process involves the efficient regio- and stereoselective elaboration of the C7 hydroxymethyl substituent through the silicon-tethered intramolecular radical cyclization[34] of cyclopentene system **71**, followed by Tamao-Fleming oxidation.[35] Cyclopentanol **72** is generated by functional group transformation from bicyclic lactone **66**, which can be efficiently constructed by the palladium-catalyzed cyclization of allylic carbonate.

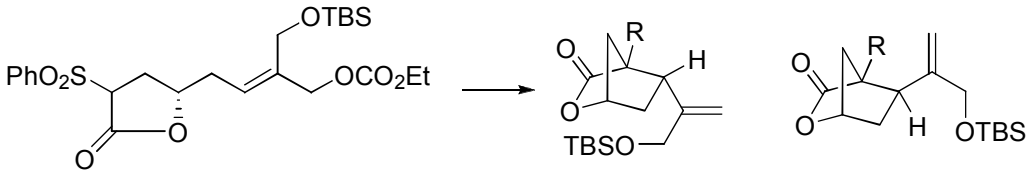
Scheme 17. Synthesis of γ-lactone 64d



Reagents: (a) $^{i}Pr_2SiCl_2$, imidazole, CH_2Cl_2, 0 ºC, 78%; (b) methyl benzenesulfonyl acetate, NaH, DMF, 80 ºC, (c) Grubbs' 2nd catalyst, CH_2Cl_2, reflux, 67% for 2 steps; (d) TBAF, THF; (e) TBSCl, imidazole, CH_2Cl_2, 76% for 2 steps.

The synthesis commenced with the preparation of the requisite γ-lactone **64d** for Pd(0)-catalyzed allylic alkylation, as illustrated in Scheme 17. To address the tri-substituted alkene unit, we initially attempted olefin cross metathesis of the vinyl γ-lactone with the hydroxymethyl substituted allyl carbonate to produce the Pd(0)-catalyzed cyclization precursor. However, intermolecular cross metathesis led to γ-lactone **64d** with only 60% yield. Thus, we turned our attention to silicon-tethered ring-closing metathesis (RCM),[36] which is considered superior in terms of entropic benefit and concomitant alcohol protection. In addition, (*Z*)-form alkene was obtained solely. That is because (*Z*)-conformation is favorable at 8-membered cyclic compound.

Table 2. Pd(0)-catalyzed cyclization of 66



64d **66** **66'**

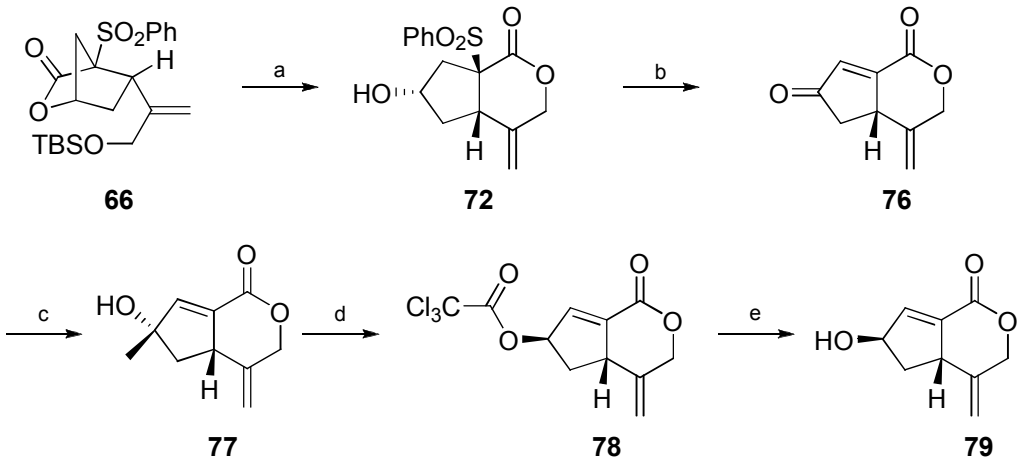
Entry	Catalyst	solvent	Yield[a]	Ratio[b] (**66**:**66'**)
1	$Pd(dppe)_2$	THF	91	30:1
2	$Pd(dppe)_2$	CH_2Cl_2	90	3.5:1
3	$Pd(PPh_3)_4$	THF	91	1:1
4	$Pd(PPh_3)_4$	CH_2Cl_2	90	2.5:1
5	$Pd(PPh_3)_4$	DMSO	78	1:1
6	$Pd(dppp)_2$[c]	THF	78	10:1
7	$Pd(dppb)_2$[c]	THF	89	25:1

All reactions were conducted in reflux condition. [b]Isolated yields. [c]Determined by 300-MHz ^{1}H-NMR spectra of the crude diastereomeric mixtures. [d] Pre-mixed $Pd(OAc)_2$ and dppp/dppb in THF was added to a solution of **64d**.

Alkoxysilane **74** was prepared from the known hydroxy tosylate **58** with a 78% yield. Alkylation of tosylate **74** with the anion of methyl phenylsulfonyl acetate and subsequent RCM provided the cyclic *bis*-alkoxysilane **75**. Removal of the tethered silicon of *bis*-alkoxysilane **75** with TBAF and the spontaneous relactonization of the resulting hydroxy ester provided the γ-lactone intermediate, which was protected with TBSCl to afford allylic carbonate **64d**.

We next conducted a survey of stereoselective Pd(0)-catalyzed cyclization of **64d** under a variety of reaction conditions as summarized in Table 2. Stereoselective cyclization of **64d** in the presence of $Pd(dppe)_2$ in THF afforded the desired product of **66** with a 91% yield along with a minor product **66'** (entry 1). Comparison with the result at Scheme 13, at this time, the precursor **64d** was only *Z*-isomer and the cyclization result was same as the result with *E/Z* mixtures. That might be the evidence of π-σ-π isomerization. Cyclization of **64d** in the presence of $Pd(PPh_3)_4$ resulted in low stereoselectivity regardless of the solvent (entry 3-5). Cyclization in the presence of $Pd(OAc)_2$ with dppp or dppb (entry 6, 7) also showed good stereoselectivities, although the yield was slightly lower.

Scheme 18. Attempt with bicyclic system

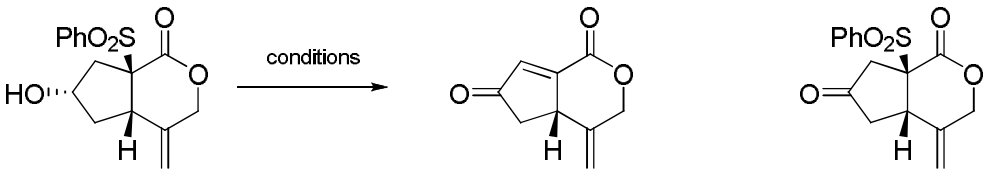


Reagents: (a) TBAF, THF, rt, 2 h, 80%; (b) DMP, rt, 16 h, CH_2Cl_2, 88%; (c) CBS, $BH_3 \cdot SMe_2$, CH_2Cl_2, 45-80%; (d) Cl_3CCOOH, DIAD, PPh_3, THF; (e) SiO_2, THF, $MeOH/H_2O$, 20% for 2 steps.

The oxidation and concomitant β-elimination of sulfonate afforded to cyclopentenone **76**.

The desulfonylation for making a variety of double bonds has been used in natural products synthesis as a potential procedure. The reductive elimination of sulfonyl group has been carried out by using aluminium amalgam, sodium amalgam, and zinc-acetic acid,[37] and many examples of base-induced desulfonylation to α,β-unsaturated carbonyl compounds have been reported.[38] Several attempts for cyclopentenone via oxidation and concomitant β-elimination of sulfonate have described as below (Table 3).

Table 3. Oxidation to cyclopentenone



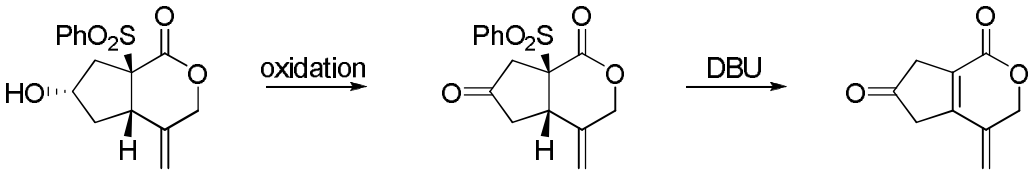
72 **76** **80**

condition	Yield **76**	**72**	**80**
DMP, $NaHCO_3$, CH_2Cl_2	56%		30%
$(COCl)_2$, Et_3N, DMSO	-	80%	-
TFAA, Et_3N, DMSO			67%
TPAP, NMO, CH_2Cl_2	-	100%	-
PCC, silica gel, CH_2Cl_2	-	-	85%
DMP, CH_2Cl_2	88%	-	-

With the basic conditions, such as Swern oxidation with oxally chloride or Ley oxidation, the β-elimination as well as the oxidation of alcohol was not executed, the cyclopentanol **72** was recovered. Interestingly, in PCC oxidation condition or Swern oxidation with TFAA, the oxidation of cyclopentanol **72** to cyclopentanone **80** was accomplished successfully, however β-elinmination of sulfonate did not occur. Moreover, after oxidation of cyclopentanone **80** followed by basic work

up with DBU, product **81** was obtained by the β-elinmination of sulfonate with allylic proton at C5 (Scheme 19).

Scheme 19. Basic work up with cyclopentanone

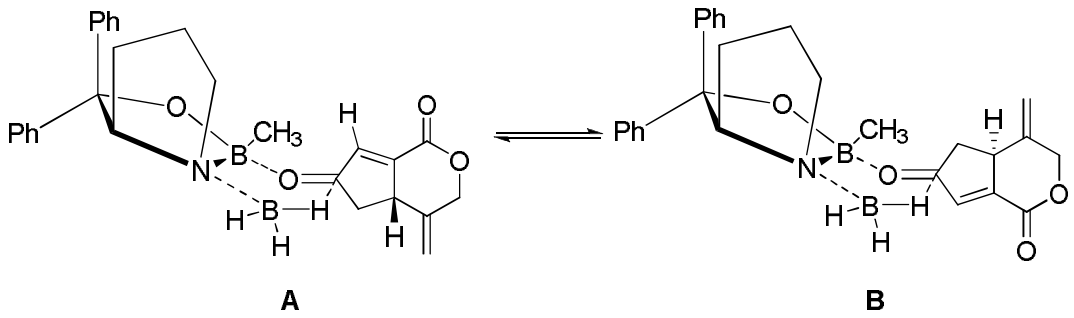


72 **80** **81**

Gratifyingly, the oxidation of cyclopentanol with *Dess-Martin* periodinane yielded cyclopentenone **76** in acidic condition. When $NaHCO_3$ was co-treated for neutral condition, the β-eliminated product **76** was obtained only 56% and cyclopentanone **80** was obtained about 30%. However, when *Dess-Martin* periodinane was only treated the desired product **76** was obtained upto 88% as sole product. There are a few examples for β-elinmination of sulfonate in acidic condition.[39]

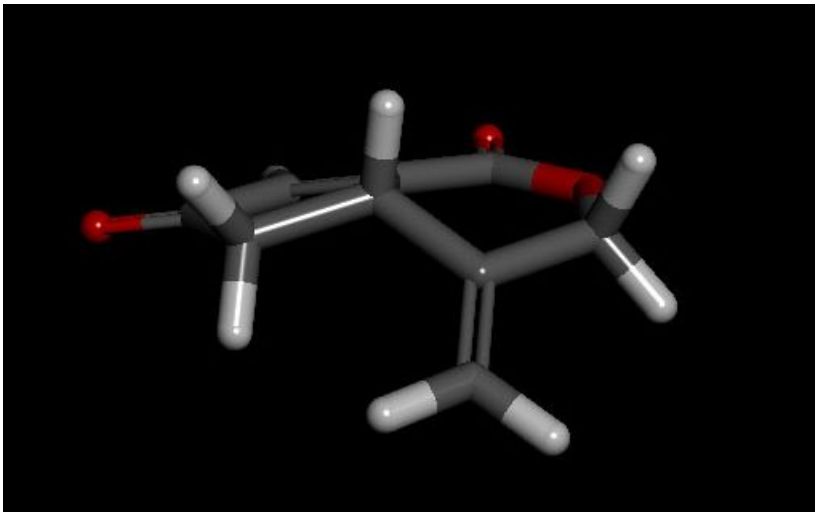
With the cyclopentenone **76** in hand, the enantioselective reduction of cyclopentenone to cyclopentenol **79** was attempted (Scheme 18). As shown at Figure 8, we expected the coordination of cyclopentenone **76** with (*S*)-Me-CBS oxazaborolidine would be preferred **B** because of the steric interaction with methyl group.[40]

Figure 8. Bicyclopentenone coordinated with (*S*)-CBS oxazaborolidine



However, the enantioselective reduction of cyclopentenone to cyclopentenol was not successful with the bicyclic system. It seemed that the 3D structure of bicyclic lactone might interfere with the approach of hydride from the concave face (Figure 9). Considering the result that reduction of cyclopentenone **76** with (*R*)-CBS afforded the same cyclopentenol **77** as sole product in 74% yield, we assured the the face selectivity had relevance to the structure of bicyclic system.

Figure 9. 3D-structure of bicyclic enone

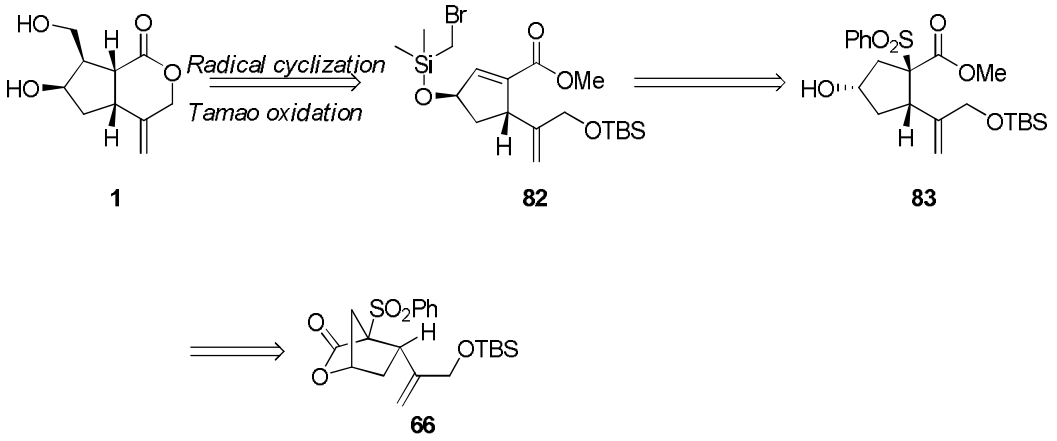


With the cyclopentenol **77**, Mitsunobu reaction with Cl_3CCO_2H and acidic hydrolysis[41] afforded

the desired cyclopentenol **79**, although the chemical yield of 2-step was too low (Scheme 18).

So instead of the bicyclic system, we envisaged the monocyclic system. Instead of relactonization of bicyclic lactone **66** to bicyclic lactone **72**, transesterification with sodium methoxide might provide cyclopentanol **83** (Scheme 20).

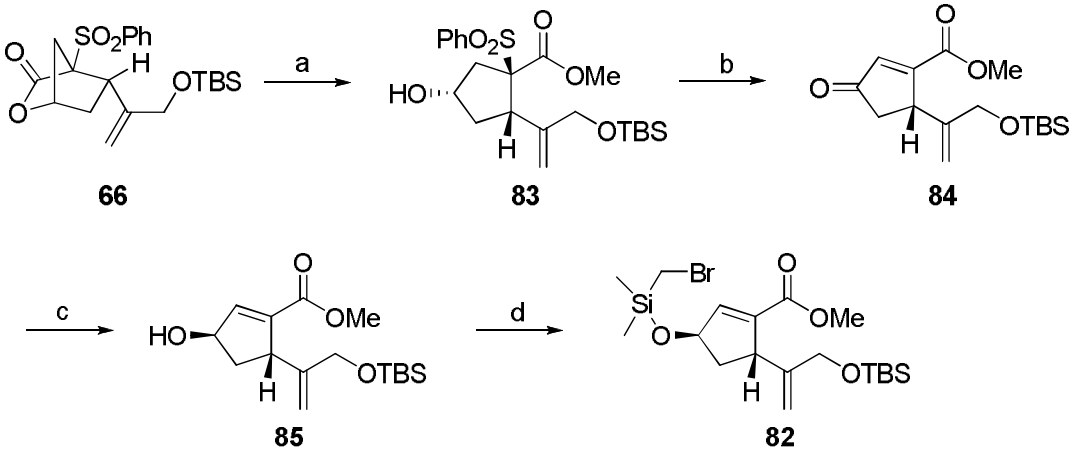
Scheme 20. Revised retrosynthetic approach



1 **82** **83** **66**

With the requisite building block **66** in hand, we focused our attention to the facile introduction of the hydroxymethyl substituent at C7. Convenient methanolysis of bicyclic lactone **66** afforded cyclopentanol **83**, which was subjected to Dess-Martin oxidation to produce enone **84** after concomitant β-elimination of sulfonate (scheme 21). Stereoselective reduction of cyclopentenone **84** with (*S*)-Me-CBS afforded cyclopentenol **85** with the correct stereochemistry.

Scheme 21. Synthesis of radical cyclization precursor 82

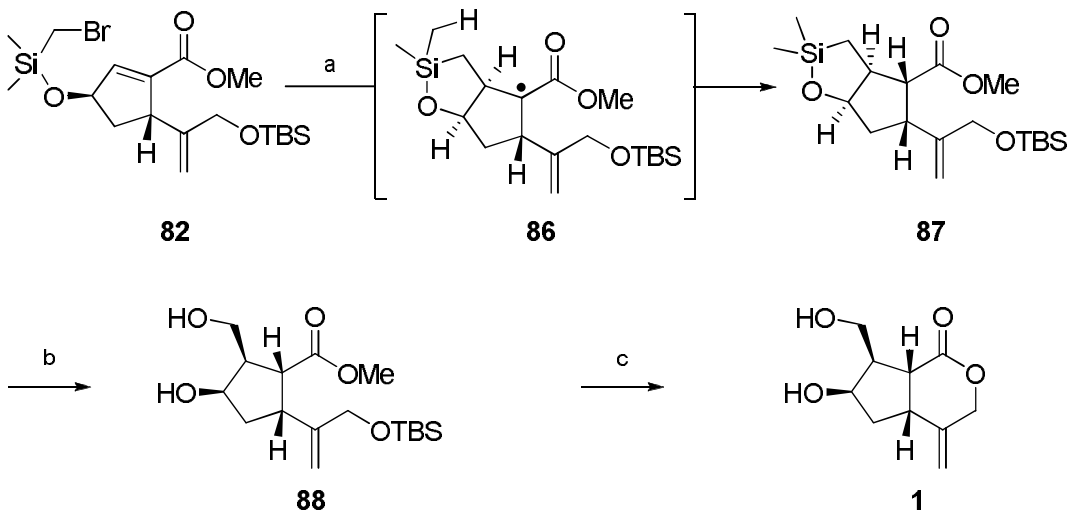


Reagents: (a) NaOMe, MeOH, 93%; (b) *Dess-Martine* periodinane, CH_2Cl_2, 91%; (c) (*S*)-CBS, $BH_3 \cdot SMe_2$, CH_2Cl_2, 68%; (d) (Bromomethyl) dimethylchlorosilane, DMAP, Et_3N, benzene, 0 ºC, 85%.

Silylation of cyclopentenol **85** with (bromomethyl) dimethylchlorosilane yielded the bromosilyl ether **82**, which was directly subjected to the radical cyclization (Scheme 22). Intramolecular radical cyclization of the labile silyl ether intermediate with Et_3B[42] and Bu_3SnH afforded the desired *cis*-fused oxasilole **87** in good yield. Organoboranes are widely recognized as excellent sources of free-radicals and can participate in free-radical chain reactions.[43] In particular, over classical initiators, Et_3B offers the great advantage of being efficient even at low temperature (-78 ºC), thus it proved to be particularly important for the development of stereoselective radical reactions and for radical reactions involving thermally unstable adducts or products.[44] The mechanistic aspect has been recognized that the nucleohemolytic substitution reaction.[45] With Et_3B, very reactive radical initiator as referred above, the initiation step was allowed at 0 ºC to ambient temperature, the *cis*-fused oxasole ring was produced, which stereogenic center was derived by the cyclopentenol's stereochemistry. And the newly generated C8 stereogenic center

could be established by an intramolecular 1, 6-hydrogen atom transfer from the proximal silylmethyl group to C8.

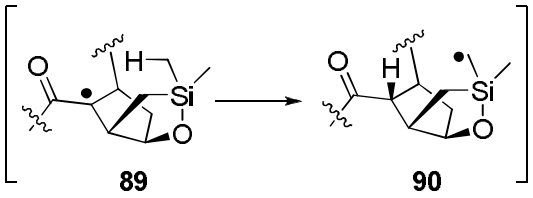
Scheme 22. Completion of synthesis of iridoid lactone 1



Reagents; (a) Et_3B, Bu_3SnH, benzene, 0 ºC; (b) $KHCO_3$, H_2O_2, KF, THF/MeOH, 60% for 2 steps; (c) $TsOH \cdot H_2O$, CH_2Cl_2, 66%.

According to the Little et al, the fused ring system allows the intramolecular 1,6 hydrogen transfer to the radical center.[46] In the radical quenching portion of the 5-exo, trig cyclization step via an intramolecular hydrogen atom transfer from the proximal silylmethyl group of silacycle **89** to the radical center located C8 at **90**. Once transferred, the silylmethly radical **90** is presumably quenched intermolecularly by tributyl tin hydride to afford an oxasilole (Figure 10).

Figure 10. 1, 6 hydrogen transfer



Subsequent Tamao-Fleming oxidation of oxasilole **87** gave diol **88**. Finally lactone iridoid **1** was obtained in good yield by TBS deprotection with TsOH,[47] followed by a concomintant lactonization. The structure of **1** was confirmed by comparison of its spectral data with those of the authentic sample (optical rotation, ^{1}H-NMR, ^{13}C-NMR, IR, HRMS).[48]

III. Conclusion

We has developed the useful method for synthesizing trisubstituted cyclopentane systems via Pd(0)-catalyzed cyclization. In particular, branched allyl carbonated γ-lactone yielded diastereoselective bicyclic lactone with good chemical yield. With the bicyclic lactone, we accomplished the first total synthesis of (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydro-cyclopenta[*c*]pyran-1(3*H*)-one in 13 steps. The key features of the synthesis involve the stereoselective and efficient preparation of the cyclopentanol intermediate *via* Pd(0)-catalyzed cyclization and then the silicon-tethered radical cyclization followed by Tamao-Fleming oxidation for the stereoselective introduction of the C7-hydroxymethyl substituent. This versatile synthetic approach is expected to be widely utilized for the synthesis of related iridoids and iridoalkaloids.

IV. Experimental

General procedure

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran and Et_2O were distilled from sodium benzophenone ketyl. Dichloromethane, triethylamine, acetonitrile and pyridine were freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried at 100 ºC. Air and moisture sensitive reactions were performed under argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Optical rotations were measured with JASCO DIP-1000 or JASCO P-1020 digital polarimeter at ambient temperature using 100 mm cell of 2 mL capacity. Infrared spectra were recorded on a Perkin-Elmer 1710 FT-IR spectrometer. Mass spectra were obtained with VG Trio-2 GC-MS instrument. High resolution mass spectra were obtained with JEOL JMS-AX 505WA instrument. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 (300 MHz), Varian Gemini 2000 (300 MHz), Brucker Analytik AVANCE digital 400 (400MHz), or AVANCE digital 500 (500MHz) spectrometer as solutions in deuteriochloroform ($CDCl_3$). Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane and are referenced to the deuterated solvent ($CHCl_3$). ^1H-NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad singlet; m, multiplet and/or multiple resonance), number of protons, and coupling constant in hertz (Hz).

(*S*)-2-Hydroxypent-4-enyl 4-methylbenzenesulfonate (59)

To a solution of lithium tetrachlorocuprate (0.1 M solution in THF, 6.6 ml, 0.66 mmol), vinylmagnesium bromoide (1.0 M solution in THF, 17 ml, 17 mmol) was added at -40 ℃. After stirred for 1 h, a solution of tosylated glycidol (2.96 g, 13 mmol) in THF (35 ml) was added dropwise by dropping funnel *ca.* 1 h at -40 ℃ and stirred for 1 h. The reaction mixture was quenched with aqueous NH_4Cl and warmed to ambient temperature and then, extracted with ether. The aqueous layers were extracted with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 3.19 g (96%) of hydroxy tosylate **59** as a colorless oil: $[\alpha]_D^{25}$ 3.24 (*c* 0.1, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.76 (dd, 2H, *J* = 6.6 Hz), 7.32 (d, 2H, *J* = 8.07 Hz), 5.77-5.64 (m, 1H), 5.05 (m, 2H), 4.0 (m, 1H), 3.92-3.83 (m, 2H), 2.41 (s, 3H), 2.26-2.13 (m, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 145.0, 132.8, 132.5, 129.9, 127.9, 118.7, 73.0, 68.5, 60.4, 37.3, 21.0; IR (neat) ν_{max} 3541, 2921, 1598, 1357, 1178, 1097, 978, 922, 818, 668 cm^{-1}; LRMS (FAB) *m/z* 257 ($M + H^+$); HRMS (FAB) Calcd for $C_{12}H_{17}O_4S$ ($M + H^+$): 257.0769, found 257.0848.

(*S*)-2-(*tert*-Butyldimethylsilyloxy) pent-4-enyl 4-methylbenzenesulfonate (60)

To a solution of hydroxy tosylate **59** (9.0 g, 33.3 mmol) and imidazole (3.4 g, 50.0 mmol) in DMF (30 ml) was added tertiarybutyl dimethylsilyl chloride (7.5g, 50.0 mmol) at 0 ℃. After stirred for 5 h, the reaction mixture was quenched with water and warmed to ambient temperature and then, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 11.0 g (90%) of silyl ether **60** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, 2H, *J* = 8.43 Hz), 7.32 (d, 2H, *J* = 7.86 Hz), 5.76-5.62 (m, 1H), 5.02-4.96 (m, 2H), 3.90-3.78(m, 3H), 2.42 (s, 3H),

2.22-2.02 (m, 2H), 0.81 (s, 9H), -0.04 (d, 6H, J = 1.2 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 144.8, 140.9, 133.2, 129.8, 128.0, 120.8, 72.6, 69.5, 38.7, 25.7, 21.6, 18.0, -4.7, -4.8; IR (neat) ν_{max} 2931, 2857, 1463, 1364, 1255, 1181, 983, 835, 665 cm^{-1}; LRMS (FAB) *m/z* 371 (M + H$^+$); HRMS (FAB) Calcd for $C_{18}H_{31}O_4S$ Si(M + H$^+$): 371.1712, found 371.1705.

(4*S*)-Methyl 4-(*tert*-butyldimethylsilyloxy)-2-(phenylsulfonyl) hept-6-enoate (61)

To a suspension of NaH (4.8 g, 120 mmol) in DMF (10 ml) was added benzenesulfonyl acetate (23.7 g, 120 mmol) in DMF (30 ml) at 0 ºC. After stirred for 3 h, to the reaction mixture was added **60** (11 g, 30 mmol) in DMF (40 ml) at 0 ºC, and then heated to 80 ºC for 7 days. The reaction mixture was quenched with water, and cooled to ambient temperature and then, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 20) to afford 9.5 g (77%) of silyl ether **61** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.83-7.51 (m, 5H), 5.04-4.95 (m, 2H), 4.19-4.05 (m, 1H), 3.62 (s, 3H), 0.83 (s, 9H), 0.01 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.2, 134.1, 133.1, 129.2, 129.0, 118.1, 77.1, 69.6, 68.7, 67.5, 52.7, 41.8, 33.3, 25.7, 17.9; IR (neat) ν_{max} 2953, 2857, 1743, 1444, 1325, 1149, 836, 778 cm^{-1}; LRMS (FAB) *m/z* 413 (M + H$^+$); HRMS (FAB) Calcd for $C_{20}H_{32}O_5S$ Si (M + H$^+$): 413.1818, found 413.1813.

(5*S*)-5-Allyl-3-(phenylsulfonyl) dihydrofuran-2(3*H*)-one (62)

To a solution of **61** (5.7 g, 13.8 mmol) in THF (70 ml) was added TBAF (1.0 M in THF, 27.5 ml, 27.5 mmol). After stirred for 16 h, the reaction mixture was quenched with saturated NH_4Cl, and then, extracted with EtOAc. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford

3.85 g (78%) of lactone **62** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.94-7.86 (m, 2H), 7.69-7.63 (m, 1H), 7.54 (t, 2H, *J* = 7.59 Hz), 5.65 (m, 1H), 5.13 (d, 2H, *J* = 8.43 Hz), 4.79-4.40 (m, 1H), 4.01 (t, 0.4H, *J* = 9.69 Hz), 4.00 (dd, 0.6H, *J* = 10.1 Hz, 3.1 Hz), 3.00 (dq, 0.6H, *J* = 14.7 Hz, 3.3 Hz), 2.75-2.25 (m, 3.4H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167, 136, 134, 131, 130, 129, 120, 77, 64, 39, 28; IR (neat) ν_{max} 2938, 1769, 1448, 1321, 1189, 934, 687 cm^{-1}; LRMS (FAB) *m/z* 267 ($M + H^+$); HRMS (FAB) Calcd for $C_{13}H_{15}O_4S$ ($M + H^+$): 267.0691, found 267.0688.

(*Z*)-But-2-ene-1,4-diyl diethyl dicarbonate (63a)

To a solution of 1,4-butene diol (10.0 g, 119.5 mmol) in CH_2Cl_2 (100 ml) were added pyridine (21 ml, 262.9 mmol) and ethyl chloroformate (28 ml, 298 mmol) at 0 ºC. After stirred for 4 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 3) to afford 24 g (76%) of dicarbonate **63a** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.23 (s, 2H), 4.55 (s, 4H), 4.10 (q, 4H, *J* = 7.14 Hz), 1.19 (t, 6H, *J* = 7.14 Hz); LRMS (FAB) *m/z* 255 ($M + Na^+$).

Diethyl 2-methylenepropane-1,3-diyl dicarbonate (63b)

To a solution of 2-methylene-1,3-propan diol (1.0 g, 11.3 mmol) in CH_2Cl_2 (20 ml) were added pyridine (2 ml, 25 mmol) and ethyl chloroformate (2.7 ml, 28.3 mmol) at 0 ºC. After stirred for 1 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford 2.7 g (100%) of dicarbonate **63b** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.23 (s, 2H), 4.55 (s, 4H), 4.10 (q, 4H, *J* = 7.14 Hz), 1.19 (t, 6H, *J* =

7.14 Hz); IR (neat) ν_{max} 2922, 1743, 1475, 1150, 926; cm^{-1}; LRMS (FAB) *m/z* 255 ($M + Na^+$).

Ethyl 2-Methylallyl carbonate (63c)

To a solution of methallyl alcohol (1.0 g, 13.9 mmol) in CH_2Cl_2 (25 ml) were added pyridine (1.44 ml, 18 mmol) and ethyl chloroformate (1.6 ml, 16.6 mmol) at 0 ºC. After stirred for 1 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford 1.37 g (68%) of dicarbonate **63c** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.21 (s, 1H), 5.20 (s, 1H), 4.60 (s, 2H), 4.10 (m, 2H), 1.37 (t, 3H, *J* = 7.14 Hz), 0.99 (s, 3H), 0.89 (s, 9H), 0.02 (s, 6H); LRMS (FAB) *m/z* 145 ($M + H^+$).

2-((*tert*-Butyldimethylsilyloxy) methyl)allyl ethyl carbonate (63d)

To a suspension of NaH (438 mg, 11 mmol) in THF (5 ml) was added diol (965 mg, 11 mmol) and stirred for 1 h. The TBSCl was then added, and vigourous stirring was continued for 1 h. the mixture was poured into ether, washed with 10% aqueous K_2CO_3 and brine, dried ($MgSO_4$) and concentrated *in vacuo.* To the crude silyl ether in CH_2Cl_2 (15 ml) were added pyridine (1.1 ml, 14.3 mmol) and ethyl chloroformate (1.3 ml, 13.2 mmol) at 0 ºC. After stirred for 1 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford 2.4 g (80% for 2 steps) of dicarbonate **63d** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.21 (s, 1H), 5.20 (s, 1H), 4.60 (s, 2H), 4.10 (m, 4H), 1.37 (t, 3H, *J* = 7.14 Hz), 0.86 (s, 9H), 0.02 (s, 6H); IR (neat) ν_{max} 2853, 1687, 1443, 1149, 836 cm^{-1}; LRMS (FAB) *m/z* 275 ($M + H^+$).

Ethyl -4-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl) but-2-enyl carbonate (64a)

To a mixture of lactone **62** (100 mg, 0.38 mmol) and diethyl carbonate **63a** (262 mg, 1.13 mmol) in CH_2Cl_2 (6 ml) was added Grubbs' 2nd catalyst (32 mg, 0.038 mmol). The reaction mixture was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 67.5 mg (48%) of **64a** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.93-7.51 (m, 5H), 5.69-5.66 (m, 2H), 4.81-4.72 (m, 0.7H), 4.51 (d, 2H, *J* = 4.0 Hz), 4.19-4.0 (m, 3.3H), 3.02-3.0 (m, 0.7 Hz), 2.77-2.66 (m, 0.3H), 2.58-2.23 (m, 3H), 1.27-1.16 (m, 3H); IR (neat) ν_{max} 2955, 1745, 1449, 1261, 1193, 1009, 722 cm^{-1}; LRMS (FAB) *m/z* 369 (M + H$^+$).

Diethyl 2-(2-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl)ethylidene)propane-1,3-diyl dicarbonate (64b)

To a mixture of lactone **62** (100 mg, 0.38 mmol) and dicatbonate **63b** (262 mg, 1.13 mmol) in CH_2Cl_2 (5 ml) was added Grubbs' 2nd catalyst (32 mg, 0.038 mmol). The reaction mixture was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 88 mg (49%) of **64b** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.9-7.5 (m, 5H), 5.44 (d, 1H, *J* = 7.4 Hz), 4.8-4.7 (m, 1H), 4.49-4.45 (m, 2H), 3.08-3.02 (m, 1H), 2.58-2.28 (m, 4H), 1.58 (s, 3H), 1.23 (m, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 155.0, 136.7, 134.6, 129.5, 129.2, 78.8, 72.3, 64.8, 33.2, 29.0, 14.2, 14.1; LRMS (FAB) *m/z* 383 (M + H$^+$).

Ethyl -2-methyl-4-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl)but-2-enyl carbonate (64c)

To a mixture of lactone **62** (395 mg, 1.48 mmol) and methyally catbonate **63c** (214 mg, 1.48 mmol) in CH_2Cl_2 (10 ml) was added Grubbs' 2nd catalyst (62.8 mg, 0.074 mmol). The reaction mixture

was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 184 mg (33%) of **64c** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.9-7.5 (m, 5H), 5.44 (d, 1H, *J* = 7.4 Hz), 4.8-4.7 (m, 1H), 4.49-4.45 (m, 2H), 3.08-3.02 (m, 1H), 2.58-2.28 (m, 4H), 1.58 (s, 3H), 1.23 (m, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 155.0, 136.7, 134.6, 129.5, 129.2, 78.8, 72.3, 64.8, 33.2, 29.0, 14.2, 14.1; IR (neat) ν_{max} 2922, 1743, 1475, 1319, 1150, 1008, 728 cm^{-1}; LRMS (FAB) *m/z* 383 ($M + H^+$); HRMS (FAB) Calcd for $C_{18}H_{23}O_7S$ ($M + H^+$): 383.1165, found 383.1162.

2-((*tert*-Butyldimethylsilyloxy) methyl)-4-((2*S*)-5-oxo-4-(phenylsulfonyl)tetrahydrofuran-2-yl)but-2-enyl ethyl carbonate (64d)

To a mixture of lactone **62** (194 mg, 0.73 mmol) and silyloxyallyl catbonate **63d** (200 mg, 0.73 mmol) in CH_2Cl_2 (10 ml) was added Grubbs' 2nd catalyst (31 mg, 0.036 mmol). The reaction mixture was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 230 mg (43%) of **64d** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.0-7.5 (m, 5H), 5.5 (t, 1H, *J* = 7.4 Hz), 4.8-4.4 (m, 3H), 4.29-4.0 (m, 4H), 3.0-2.3 (m, 5H), 1.25 (m, 3H), 0.85 (s, 9H), 0.01 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.4, 155, 137.5, 134.7, 129.6, 129.3, 127.9, 123.2, 78.9, 68.9, 64.7, 62.5, 58.6, 33, 29, 25.7, 18.3, 14.2.

(*Z*)-2-((*tert*-Butyldimethylsilyloxy)methyl)-4-((2*S*)-5-oxo-4-(phenylsulfonyl)tetrahydrofuran-2-yl)but-2-enyl ethyl carbonate (64d)

To a solution of allyl alcohol **S75** (2.4 g, 6.0 mmol) in CH_2Cl_2 (40 mL) at 0 ºC were added TBSCl (904 mg, 6.0 mmol), imidazole (443 mg, 6.5 mmol). After stirring for 3 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2 and aqueous layer was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was

purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 3.07 g (99%) of silyl ether **64d** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.92 (dd, 2H, *J* = 7.4 Hz, 20 Hz), 7.68 (t, 1H, *J* = 7.4 Hz), 7.56 (t, 2H, *J* = 7.8 Hz), 5.52 (t, 1H, *J* = 7.4 Hz), 4.78-4.46 (m, 1H), 4.60 (d, 1H, 8.8Hz), 4.22-4.05 (m, 5H), 3.02-2.74 (m, 1H), 2.55 (m, 2H), 2.68-2.30 (m, 1H), 1.28-1.20 (m, 3H), 0.85 (m, 9H), 0.05 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.4, 167.1, 154.9, 138.3, 137.8, 136.8, 136.7, 134.7, 134.6, 129.6, 129.4, 123.2, 122.7, 78.6, 68.9, 64.8, 64.0, 58.8, 58.7, 33.2, 32.8, 28.7, 28.2, 25.8, 18.2, 17.4, 17.2, 14.3, 12.8; IR (neat) ν_{max} 2926, 2856, 1744, 1516, 1458, 1321, 1258, 1151, 1083, 840, 781 cm^{-1}; LRMS (FAB) *m/z* 513 (M + H$^+$); HRMS (FAB) Calcd for $C_{24}H_{37}O_8SSi$ (M + H$^+$): 513.1978, found 513.1971.

4-(Phenylsulfonyl)5-(pro-1-en-2yl)-2-oxa-bicyclo[2.2.1]heptan-3-one (65)

To a refluxing solution of methally carbonate **64c** (12.5 mg, 0.033 mmol) in CH_2Cl_2 (2 ml) was added $Pd(dppe)_2$ (5.9 mg, 0.0065 mmol). AfAfter stirring for 15 h the reaction mixture was cooled to ambient temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 9 mg (94%) of bicyclic lactone **65** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.08-7.52 (m, 5H), 4.97 (s, 1H), 4.85 (dt, 2H, *J* = 13.7 Hz, 2.4 Hz, 1.3 Hz), 3.65 (q, 1H, *J* = 6.0 Hz), 2.5-2.30 (m, 2H), 2.26 (s, 1H), 1.97-1.92 (m, 1H), 1.64 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 168.4, 141.4, 134.5, 130.5, 128.7, 116.6, 77.0, 74.6, 45.8, 45.6, 37.0, 19.7; IR (neat) ν_{max} 2922, 1784, 1452, 1321, 1158, 1115, 723 cm^{-1}; LRMS (FAB) *m/z* 315 (M + Na$^+$); HRMS (FAB) Calcd for $C_{15}H_{16}O_4SNa$ (M + Na$^+$): 315.0667, found 315.0668.

5-(3-(*tert*-Butyldimethylsilyloxy)prop-1-en-2-yl)-4-(phenylsulfonyl)-2-oxabicyclo[2.2.1]heptan-3-one (66)

To a refluxing solution of silyl ether **64d** (250 mg, 0.49 mmol) in THF (2 mL) was added

$Pd(dppe)_2$ (44 mg, 50 μmol). After stirring for 1 h the reaction mixture was cooled to ambient temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 170 mg (89%) of bicyclic lactone **66** as a colorless oil: $[\alpha]_D^{25}$ -51.4 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02(m, 2H), 7.67-7.50 (m, 3H), 5.24(s, 1H), 5.04(s, 1H), 4.81(s, 1H), 4.21(s, 2H), 3.69 (dd, 1H, *J* = 5.5 Hz, 11.2 Hz), 2.53 (m, 1H), 2.30 (m, 2H), 2.09-2.00 (m, 1H), 0.90 (s, 9H), 0.04 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.9, 145.5, 136.8, 134.5, 130.2, 128.9, 113.8, 77.2, 75.4, 65.9, 45.9, 39.6, 39.0, 25.8, 18.2, -5.3; IR (neat) ν_{max} 2927, 1779, 1448, 1318, 1258, 1151, 1083, 840, 755, 726, 689 cm^{-1}; LRMS (FAB) *m/z* 423 (M + H$^+$); HRMS (FAB) Calcd for $C_{21}H_{31}O_5SSi$ (M + H$^+$): 423.1661, found 423.1658.

Minor 66': $[\alpha]_D^{25}$ -7.54 (c 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.03(d, 2H, *J* = 8.2 Hz), 7.62 (t, 1H, *J* = 7.4 Hz), 7.49 (t, 2H, *J* = 7.8 Hz), 5.41 (s, 1H), 5.10 (s, 1H), 4.90 (s, 1H), 4.50 (d, 1H, *J* = 9.4 Hz), 4.29 (d, 1H, *J* = 13.9 Hz), 3.08 (m, 1H), 2.65 (d, 1H, *J* = 10.7 Hz), 2.46 (d, 1H, *J* = 10.7 Hz), 2.36 (d, 1H, 6.6 Hz), 0.89 (s, 9H), 0.08 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 169.6, 144.8, 137.2, 134.3, 130.4, 128.5, 116.6, 77.7, 75.1, 65.9, 41.3, 40.6, 38.8, 25.8, 18.2, -5.3.

4-Methylenehexahydrocyclopenta[c]pyran-1,6-dione (68)

To a solution of cyclopentanol **70** (11 mg, 0.036 mmol) in CH_2Cl_2 were added TPAP (2 mg, 0.005 mmol) and NMO (8 mg, 0.07 mmol). The reaction mixture was stirred for 4 h and filtered with celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 :1) to afford 4 mg (67%) of cyclopentanone **68** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.33 (s, 1H), 5.12 (br, 1H), 4.92 (d, 1H, *J* = 12.8 Hz), 4.67 (d, 1H, *J* = 12.8 Hz), 3.04 (d, 1H, *J* = 20.88 Hz), 2.65 (m, 1H), 2.43 (m, 1H), 2.22 (m, 1H), 1.84 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 213.5, 172.2, 140.4, 115.6, 71.7, 42.9, 39.8, 38.9, 37.9; IR (neat) ν_{max} 2924, 2855, 1741, 1096, 804 cm^{-1}; LRMS (FAB) *m/z* 167 (M+H$^+$); HRMS (FAB) Calcd for $C_9H_{11}O_3$ (M + H$^+$): 167.0708, found 167.0706.

6-Hydroxy-4-methylenehexahydrocyclopenta[c]pyran-1(3*H*)-one (69)

To a solution of bicyclic lactone **70** (140 mg, 0.33 mmol) in THF (9 mL) was added TBAF (1.0 M solution in THF, 0.4 mL, 0.4 mmol) was added. The reaction mixture was stirred for 2 h and quenched with aqueous NH_4Cl. This mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 91 mg (90%) of cyclopentanol **69** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.08 (br, 1H), 5.00 (br, 1H), 4.74 (d, 1H, *J* = 12.3 Hz), 4.59 (d, 1H, *J* = 12 Hz), 4.27 (m, 1H), 2.60 (br, 1H), 2.28-2.04 (m, 3H), 1.61-1.52 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 174.6, 141.3, 113.7, 72.0, 71.0, 42.2, 40.1, 39.8, 38.7, 20.9, 14.0; IR (neat) ν_{max} 3414, 2931, 1722, 1380, 1039, 922 cm^{-1}; LRMS (FAB) m/z 169 (M + H$^+$).

5-(3-(*tert*-Butyldimethylsilyloxy)prop-1-en-2-yl)-2-oxabicyclo[2.2.1]heptan-3-one (70)

To a mixture of bicyclic lactone **66** (142 mg, 0.51 mmol) and boric acid (316 mg, 5.1 mmol) in methanol (5 ml) was added 6% Na/Hg (1.56 g, 4.08 mmol) and stirred for 48 h. The reaction mixture was quenched with saturated NH_4Cl, and then extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 3) to afford 38.6 mg (56%) of bicyclic lactone **70** as a white solid: ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.47 (s, 1H), 5.14 (s, 1H), 4.94 (s, 1H), 4.18 (q, 2H, *J* = 13.7 Hz), 3.07 (m, 1H), 3.01 (s, 1H), 2.22 (d, 1H, *J* = 10.5 Hz), 2.14 (m, 1H), 1.89 (m, 2H), 0.92 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C-NMR (CD_3OD, 125 MHz) δ 179.1, 149.3, 111.0, 82.9, 67.7, 42.8, 40.7, 33.8, 27.2, 19.9, -4.3, -4.4; IR (neat) ν_{max} 2955, 2928, 1789, 1255, 1084, 837 cm^{-1}; LRMS (FAB) *m/z* 283 (M+H$^+$); HRMS (FAB) Calcd for $C_{15}H_{26}O_3Si$ (M + H$^+$): 282.1651, found 282.1688.

6-((Bromomethyl)dimethylsilyloxy)-4-methylene-4,4a,5,6-tetrahydrocyclopenta[c]pyran-

1(3*H*)-one (71)

To a solution of cyclopetenol **79** (1 mg, 0.006 mmol) in CH_2Cl_2 (0.5 ml) were added BMDMCS (0.1 M in benzene, 0. 9 ml, 0.09 mmol), Et_3N (1.4 μl, 0.09 mmol) and DMAP (1.2 mg, 0.01 mmol) at 0 ºC. After stirred for 1 h at the ambient temperature, the reaction mixture was concentrated *in vacuo* to give as colorless oil. The crude silylether **71** was used in the next step without further purification: ^{1}H-NMR (C_6D_6, 300 MHz) δ 6.73 (br, 1H), 5.15 (s, 1H), 5.12 (m, 1H), 4.99 (s, 1H), 4.82 (d, 1H, *J* = 13.4 Hz), 4.70 (d, 1H, *J* = 13 Hz), 3.46 (br, 1H), 2.74 (m, 1H), 2.47 (s, 2H), 1.90 (m, 1H), 0.27 (s, 6H); LRMS (FAB) *m/z* 317 ($M + H^+$).

6-Hydroxy-4-methylene-7a-(phenylsulfonyl)hexahydrocyclopenta[c]pyran-1(3*H*)-one (72)

To a solution of bicyclic lactone **66** (168 mg, 0.4 mmol) in THF (10 mL), TBAF (1.0 M solution in THF, 0.48 mL, 0.48 mmol) was added. The reaction mixture was stirred for 2 h and quenched with aqueous NH_4Cl. This mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 98 mg (80%) of bicyclic lactone **72** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.80 (m, 2H), 7.66 (m,1H), 7.54 (m, 2H), 5.19 (m, 3H), 4.72 (d, 1H, *J* = 12 Hz), 4.39 (m, 1H), 4.05 (m, 1H), 2.3 (m, 3H), 2.0 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 167.1, 139.2, 135.2, 134.5, 130.4, 128.7, 114.5, 77.1, 72.4, 71.3, 44.9, 40.9, 39.9; IR (neat) ν_{max} 3508, 1725, 1377, 1301, 1238, 1135, 1076, 919, 719 cm^{-1}; LRMS (FAB) m/z 309 ($M + H^+$); HRMS (FAB) Calcd for $C_{15}H_{17}O_5S$ ($M + H^+$): 309.0797, found 309.0789.

Ethyl 2-(Hhydroxymethyl)allyl carbonate (73)

To a solution of 2-methylene-1,3-propanediol (2.17 g, 24.6 mmol) in anhydrous CH_2Cl_2 (25 ml) was added pyridine (3.98 ml, 49.3 mmol) at -10 ºC and stirred for 10 minutes. To the mixture was added a solution of ethylchloroformate (2.46 ml, 25.9 mmol) in anhydrous CH_2Cl_2 (25 ml) by

dropping fonnel for 40 minutes. After stirring for 2 h, the reaction mixture was quenched with H_2O, extracted with EtOAc and then concentrated *in vacuo*. The residue was purified by flash chromatography by silica gel (EtOAc : *n*- Hexane = 1 : 2) to afford 2.8 g of allyl carbonate **73** (71%) as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.18 (s, 1H), 5.13 (s, 1H), 4.59 (s, 2H), 4.12 (q, 2H, *J* = 7.2 Hz), 4.07 (s, 2H), 2.68 (s, 1H), 1.23 (t, 3H, *J* = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 155.0, 142.7, 114.3, 67.7, 64.0, 63.1, 14.0; IR (neat) ν_{max} 3428, 2986, 1747, 1260, 1011 cm^{-1}; LRMS (FAB) *m/z* 161 ($M + H^+$); HRMS (FAB) Calcd for $C_7H_{13}O_4$ ($M + H^+$): 161.0814, found 161.0810.

(*S*)-2-Allyl-4,4-diisopropyl-7-methylene-10-oxo-3,5,9,11-tetraoxa-4-silatridecyl 4-methylbenzenesulfonate (74)

Dichlorodiisopropylsilane (2.36 ml, 13.1 mmol) was added to imidazole (2.44 g, 35.8 mmol) in CH_2Cl_2 (15 ml) at 0 ºC. The solution was stirred for *ca*. 5 minutes, then the hydroxy tosylate **59** (3.22 g, 11.9 mmol) in CH_2Cl_2 (10 ml) was added dropwise by dropping funnel *ca.*1 h at 0 ºC. After the mixture was stirred for 10 minutes at 0 ºC, a solution of the allylic alcohol **73** (1.91 g, 11.9 mmol) in CH_2Cl_2 (10 mL) was added at 0 ºC. The reaction mixture was warmed to the room temperature and stirred for 16 h. The reaction mixture filtrated with hexane, then concentrated *in vacuo* to afford the crude oil, which was purified by flash chromatography on silica gel (EtOAc : *n*-hexane = 1 : 7) to afford 5.2 g (82%) of *bis*-alkoxysilane **74** as a colorless oil: $[\alpha]_D^{25}$ 1.65 (*c* 0.5, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.75 (d, 2H, *J* = 8.2 Hz), 7.30 (d, 2H, *J* = 8.1 Hz), 5.76-5.62 (m, 1H), 5.22(s, 1H), 5.15 (s, 1H), 5.02-4.97 (m, 2H), 4.59 (s, 2H), 4.24 (s, 2H), 4.17 (q, 2H, *J* = 7.1 Hz), 4.05 (m, 1H), 3.89 (m, 2H), 2.41 (s, 3H), 2.25 (m, 2H), 1.27 (t, 3H, *J* = 7.1 Hz), 0.96 (m, 14H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 154.9, 144.7, 142.2, 132.8, 132.7, 129.7, 127.9, 118.3, 113.6, 113.3, 72.1, 69.2, 67.6, 64.0, 63.3, 38.4, 21.5, 17.2, 17.1, 14.2, 12.1, 12.0; IR (neat) ν_{max} 2945, 2867, 1748, 1259, 1178, 1097, 989, 815 cm^{-1}; LRMS (FAB) *m/z* 529 ($M + H^+$).

(12*S*)-Methyl 12-allyl-10,10-diisopropyl-7-methylene-4-oxo-14-(phenylsulfonyl)-3,5,9,11-tetraoxa-10-silapentadecan-15-oate (S74)

To a suspension of NaH (1.98 g, 49.5 mmol) in DMF (10 ml) was slowly added solution of benzene sulfonate (10.6 g, 49.5 mmol) in DMF (20 ml) at 0 ºC. After the mixture was stirred for 1 h at 0 ºC, *bis*-alkoxysilane **74** (8 g, 15.1 mmol) in DMF (20 ml) was added. The reaction mixture was stirred at 80 ºC for 24 h and quenched with aqueous NH_4Cl solution. This mixture was extracted by ether. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1: 5) to afford 4.696 g (54 %) of the alkylated product **S74** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.80 (m, 2H), 7.63 (m, 1H), 7.50 (m, 2H), 5.71-5.60 (m, 1H), 5.21 (d, 1H, *J* = 16.5 Hz), 5.13 (d, 1H, *J* = 8.7 Hz), 5.02-4.95 (m, 2H), 4.58 (d, 1H, *J* = 12.3 Hz), 4.24-4.20 (m, 3H), 4.17-4.11 (m, 2H), 4.03-3.83 (m, 1H), 3.58 (s, 3H), 2.35-2.04 (m, 4H), 1.25 (t, 3H, *J* = 7.1 Hz), 1.20 (t, 2H, *J* = 7.1 Hz), 1.00-0.89 (m, 14H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 170.9, 166.5, 166.2, 154.9, 142.1, 137.5, 134.0, 133.1, 132.9, 129.5, 129.2, 129.1, 129.0, 128.9, 128.8, 118.2, 118.1, 113.4, 113.3, 69.1, 67.6, 67.5, 67.3, 63.9, 63.3, 63.2, 60.2, 52.8, 52.6, 41.7, 32.9, 32.7, 20.9, 17.2, 17.1, 17.0, 16.9, 14.1, 12.2, 12.0, 11.8; IR (neat) ν_{max} 2946, 2867, 1746, 1448, 1259, 884 cm^{-1}; LRMS (FAB) *m/z* 571.

Methyl 3-((*S*,*Z*)-7-((ethoxycarbonyloxy)methyl)-2,2-diisopropyl-5,8-dihydro-4*H*-1,3,2-dioxasilocin-4-yl)-2-(phenylsulfonyl)propanoate (75)

To a refluxing solution of benzensulfonate **S74** (4.6 g, 8.06 mmol) in CH_2Cl_2 (600 mL) were added Grubbs' 2nd catalyst (171 mg, 0.20 mmol). After stirring of the mixture for 0.5 h, the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1: 5) to afford 4.26 g (97%) of cyclic dioxasilane **75** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.80 (m, 2H), 7.62 (m, 1H), 7.51 (m, 2H), 5.72 (t, 1H, *J* = 8.6 Hz),

4.57 (d, 1H, *J* = 12.7 Hz), 4.50 (d, 1H, *J* = 12.5 Hz), 4.29-4.21 (m, 1.3H), 4.14-4.09 (m, 3H), 3.75-3.70 (m, 0.7H), 3.58 (s, 2H), 3.51 (s, 1H), 2.53-2.48 (m, 0.3H), 2.46-2.41 (m, 0.7H), 2.25-2.04 (m, 3H), 1.23 (m, 3H), 0.94-0.79 (m, 14H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 170.8, 166.4, 166.0, 154.7, 138.5, 138.4, 137.2, 137.0, 134.1,129.1, 129.0, 128.9, 127.4, 71.9, 70.0, 69.8, 69.7, 68.5, 67.7, 63.9, 60.1, 59.6, 52.6, 35.1, 34.7, 34.4, 20.8, 17.2, 17.0, 16.9, 14.0, 12.6, 12.5, 12.0, 11.8; IR (neat) ν_{max} 2945, 2866, 1745, 1463, 1256, 1084 cm^{-1}; LRMS (FAB) *m/z* 543 (M + H$^+$); HRMS (FAB) Calcd for $C_{25}H_{39}O_9SSi$ (M + H$^+$): 543.2084, found 543.2074.

Ethyl (*E*)-2-(hydroxymethyl)-4-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl)but-2-enyl carbonate (S75)

To a solution of cyclic dioxasilane **75** (2.76 g, 5.08 mmol) in THF (50 mL), TBAF (1.0 M solution in THF, 5.6 mL, 5.6 mmol) was added. The reaction mixture was stirred for 2 h and quenched with aqueous NH_4Cl. This mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 1.48 g (73%) of allyl alcohol **S75** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.9-7.55 (m, 5H), 5.75-5.60 (m, 1H), 5.22 (d, 1H, *J* = 12.6 Hz), 4.85-4.48 (m, 4H), 4.19-4.05 (m, 3H), 3.10-2.29 (m, 4H), 1.26 (m, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 155.1, 138.1, 137.8, 136.5, 134.7, 129.5, 129.3, 129.2, 125.6, 125.2, 78.6, 77.1, 69.5, 64.8, 64.2, 63.9, 58.1, 32.8, 28.8, 14.2; IR (neat) ν_{max} 3531, 2983, 1744, 1448, 1261, 1149, 1084, 1009, 937, 873 cm^{-1}; LRMS (FAB) *m/z* 399 (M + H$^+$); HRMS (FAB) Calcd for $C_{18}H23O_8S$ (M + H$^+$): 399.1114, found 399.1102.

(*S*)-4-Methylene-3,4,4a,5-tetrahydrocyclopenta[c]pyran-1,6-dione (76)

To a solution of cyclopentanol **72** (11 mg, 0.036 mmol) in CH_2Cl_2 (1 ml) was added *Dess-Martin* periodinane (23 mg, 0.054 mmol) at the ambient temperature and the reaction mixture was stirred

for 16 h. After quenching with H_2O, the mixture was extracted with EtOAc and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 3.3 mg (56%) of cyclopentenone **76** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.73 (d, 1H, *J* = 2.9 Hz), 5.30 (s, 1H), 5.13 (s, 1H), 5.02 (d, 1H, *J* = 13.2 Hz), 4.84 (d, 1H, *J* = 7.8 Hz), 3.89 (m, 1H), 2.84 (dd, 1H, *J* = 6.2 Hz, 18.5 Hz), 2.65 (dd, 1H, *J* = 3.6 Hz, 18.5 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 209.0, 152.0, 138.2, 136.8, 127.6, 112.6, 70.5, 42.8, 42.1, 39.4; IR (neat) ν_{max} 2929, 1725, 1612, 1462, 1250, 1110, 838 cm^{-1}; LRMS (FAB) m/z 165 (M + H$^+$).

6-Hydroxy-4-methylene-4,4a,5,6-tetrahydrocyclopenta[c]pyran-1(3*H*)-one (77)

To a flamed flask were added (*S*)-Me-CBS-oxazaborolidine (1.0 M solution in toluene, 0.37 ml, 0.36 mmol) and borane-SMe_2 complex (2.0 M in THF, 0.09 ml, 0.18 mmol) at the ambient temperature. After stirring for 10 minutes, cyclopentenone **76** (6 mg, 0.04 mmol) in CH_2Cl_2 (2 ml) was added at 0 ℃ and stirred for 10 minutes. After quenching with *i*PrOH, the mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 2 : 1) to afford 5 mg (80%) of cyclopentenol **77** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.79 (s, 1H), 5.16 (s, 1H), 5.08 (q, 1H, *J* = 7.3 Hz), 5.0 (s, 1H), 4.84 (d, 1H, *J* = 13 Hz), 4,72 (d, 1H, *J* = 13 Hz), 3.72 (br, 1H), 2.82 (td, 1H, *J* = 13 Hz, 6.8 Hz), 1.84 (m, 1H), 1.78 (d, 1H, *J* = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 162, 145, 140, 134, 111, 76, 73, 44, 40; IR cm^{-1} 3399, 2925, 1722, 1633, 1245, 1050, 916; LRMS (FAB) m/z 167 (M + H$^+$); HRMS (FAB) Calcd for $C_9H_{11}O_3$ (M + H$^+$): 167.0708, found 167.0702.

4-Methylene-1-oxo-1,3,4,4a,5,6-hexahydrocyclopenta[c]pyran-6-yl 2,2,2-trichloroacetate (78)

To a solution of cyclopentenol **77** (3.4 mg, 0.02 mmol) in THF (0.6 ml) were added trichloroacetic acid (13 mg, 0.082 mmol) and triphenyl phosphine (21 mg, 0.082 mmol). And then, DIAD (0.016

ml, 0.082 mmol) was added dropwise. After stirred for 4 h, the reaction mixture was quenched with $NaHCO_3$, the mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 6 mg (96%) of trichloroacetate **78** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.88 (t, 1H, *J* = 2.7 Hz), 5.80 (d, 1H, *J* = 5.7 Hz), 5.27 (s, 1H), 5.06 (s, 1H), 4.91 (d, 1H, *J* = 13 Hz), 4.71 (d, 1H, *J* =13 Hz), 3.93 (br, 1H), 2.53 (dd, 1H, *J* =14.6 Hz, 6 Hz), 2.39 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 161.5, 152.3, 139.8, 138.1, 112.0, 82.5, 65.4, 64.1, 45.0, 36.1; LRMS (FAB) m/z 312 ($M + H^+$).

6-Hydroxy-4-methylene-4,4a,5,6-tetrahydrocyclopenta[c]pyran-1(3*H*)-one (79)

To a solution of trichloroacetate **78** (6 mg, 0.019 mmol) in THF/MeOH (1:1, 1ml) was added silica gel (20 mg). The reaction mixture was heated to 40 ℃. The reaction mixture was stirred for 8 h and filtered with celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane =) to afford 1 mg (31%) of cyclopentenol **79** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.84 (t, 1H, *J* = 2.9 HZ), 5.15 (s, 1H), 5.01 (s, 1H), 4.88 (m, 2H), 4.71 (d, 1H, *J* = 13Hz), 3.87 (br, 1H), 2.30 (dd, 1H, *J* = 6.5 Hz, 20 Hz), 2.21 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 162.9, 145.3, 140.8, 134.8, 111.4, 76.1, 73.1, 44.9, 40.3; IR cm^{-1} 3399, 2925, 1722, 1633, 1245, 1050, 916; LRMS (FAB) m/z 167 ($M + H^+$); HRMS (FAB) Calcd for $C_9H_{11}O_3$ ($M + H^+$): 167.0708, found 167.0702.

4-Methylene-7a-(phenylsulfonyl)hexahydrocyclopenta[c]pyran-1,6-dione (80)

-Swern oxidation condition

DMSO (0.02 ml, 0.28 mmol) was dissolved in CH_2Cl_2 (1 ml) at -78 ℃ and treated with TFAA (0.02 ml, 0.14 mmol). The solution was allowed to stir 10 min at -78 ℃ and then, treated dropwise with cyclopentanol **79** (10 mg, 0.03 mmol) in CH_2Cl_2 (1 ml). The solution was

allowed to stir for 1 h at -78 ºC and then, treated with Et_3N (0.05 ml, 0.36 mmol). The solution was allowed to warm to 0 ºC and stirred for 1h. The reaction mixture was quenched with water, aqueous phase wasextracted with CH_2Cl_2 and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 2 : 1) to afford 8 mg (80%) of cyclopentanone **80** as a colorless oil.

-PCC oxidation condition

To a solution of cyclopentanol **79** (3 mg, 0.01 mmol) in CH_2Cl_2 (0.6 ml) were added PCC (3 mg, 0.015 mmol) and silica gel (6 mg). After stirred for 4 h, the reaction mixture filtered with celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 2 :1) to afford 2.4 mg (85%) of cyclopentanone **80** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.82-7.50 (m, 5H), 5.22-5.15 (m, 3H), 4.73 (d, 1H, J = 12.6 Hz), 4.42 (m, 1H), 4.07 (m, 1H), 2.40 (m, 3H), 2.01 (br, 1H); IR cm^{-1} 2924, 1717, 1261, 745; LRMS (FAB) m/z 307 (M + H$^+$).

4-Methylene-3,4-dihydrocyclopenta[c]pyran-1,6(5*H*,7*H*)-dione (81)

To a solution of cyclopentanone **80** (4 mg, 0.013 mmol) in THF (0.8 ml) was added DBU (3 μl, 0.022 mmol). The reaction mixture was refluxed for 16 h, and then cooled. The reaction mixture was quenched with aqueous NH_4Cl, concentrated *in vacuo*. The residue was diluted with EtOAc, the organic layer were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 1 mg (47%) of ccyclopentenone **81** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.41 (s, 1H), 5.26 (s, 1H), 5.08 (s, 2H), 3.30 (m, 4H); IR cm^{-1} 2925, 1633, 1245, 1050; LRMS (FAB) m/z 165 (M+H).

(1*R*,2*S*,4*S*)-Methyl 2-(3-(tert-butyldimethylsilyloxy)prop-1-en-2-yl)-4-hydroxy-1-(phenylsulfonyl) cyclopentanecarboxylate (83)

To a solution of bicyclic lactone **66** (78 mg, 0.19 mmol) in methanol (10 ml) was added sodium

methoxide (12 mg, 0.20 mmol) at the ambient temperature. After stirred for 1 h, the reaction mixture was quenched with aqueous NH_4Cl, concentrated *in vacuo*. The residue was diluted with EtOAc, the organic layer were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 78 mg (93%) of cyclopentanol **83** as a colorless oil: $[\alpha]_D^{25}$ -104.5 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.89 (d, 2H, *J* = 7.6 Hz), 7.61 (t, 1H, *J* = 7.4 Hz), 7.50 (t, 2H, *J* = 7.6 Hz), 5.23 (s, 1H), 4.98 (s, 1H), 4.48 (m, 1H), 4.03 (d, 1H, *J* = 14.6 Hz), 3.85 (d, 1H, *J* = 14.6 Hz), 3.52 (s, 3H), 3.30 (t, 1H, *J*= 8.7 Hz), 2.80 (dd, 1H, *J* = 6.8 Hz, 14.8 Hz), 2.58 (dd, 1H, *J* = 7.8 Hz, 14.8 Hz), 2.37 (m, 1H), 1.92-1.84 (m, 1H), 0.85 (m, 9H), -0.01 (m, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 167.6, 148.0, 136.8, 134.1, 130.6, 128.7, 111.7, 83.3, 70.9, 65.6, 52.5, 43.0, 41.8, 41.5, 25.8, 18.1, -5.4; IR (neat) ν_{max} 3458, 2953, 2929, 2856, 1738, 1447, 1308, 1254, 1140, 1079, 837 cm^{-1}; LRMS (FAB) *m/z* 455 (M + H$^+$); HRMS (FAB) Calcd for $C_{22}H_{35}O_6SSi$ (M + H$^+$): 455.1924, found 455.1932.

(*S*)-Methyl 5-(3-(tert-butyldimethylsilyloxy)prop-1-en-2-yl)-3-oxocyclopent-1-enecarboxylate (84)

To a solution of cyclopentanol **83** (48 mg, 0.11 mmol) in CH_2Cl_2 (5 ml) was added Dess-Martin periodinane (49 mg, 0.12 mmol) at the ambient temperature and the reaction mixture was stirred for 16 h. After quenching with H_2O, the mixture was extracted with CH_2Cl_2 and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 30 mg (91%) of cyclopentenone **84** as a colorless oil: $[\alpha]_D^{25}$ 751.4 (*c* 0.33, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.73 (d, 1H, *J* = 1.8 Hz), 5.06 (s, 1H), 4.73 (s, 1H), 4.14 (s, 2H), 3.80 (s, 3H), 3.77 (m, 1H), 2.76 (dd, 1H, *J* = 7.2 Hz, 19.1 Hz), 2.38 (dd, 1H, *J*= 1.9 Hz, 19.1 Hz), 0.88 (s, 9H), 0.04 (m, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 208.2, 165.1, 164.4, 148.9, 137.8, 110.4, 65.8, 52.4, 44.3, 42.4, 25.8, 18.3, -5.4; IR (neat) ν_{max} 2954, 2929, 2857, 1725, 1612, 1462, 1250, 1110, 838 cm^{-1}; LRMS (FAB) *m/z* 311 (M + H$^+$); HRMS (FAB) Calcd for

$C_{16}H_{27}O_4Si$ ($M + H^+$): 311.1679, found 311.1670.

(3*R*,5*S*)-Methyl 5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)-3-hydroxycyclopent-1-enecarboxylate (85)

To a flamed flask were added (*S*)-Me-CBS-oxazaborolidine (1.0 M solution in toluene, 3 μl, 3 μmol) and borane-SMe_2 complex (2.0 M in THF, 8 μl, 0.016 mmol) at the ambient temperature. After stirring for 10 minutes, cyclopentenone **84** (10 mg, 0.03 mmol) in THF (1 ml) was added at -78 ºC and stirred for 10 minutes. After quenching with *i*PrOH, the mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 6 mg (60%) of cyclopentenol **85** as a colorless oil: $[\alpha]_D^{25}$ 362.3 (*c* 0.33, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.77 (s, 1H), 5.06 (m, 1H), 4.98 (s, 1H), 4.67 (s, 1H), 3.69 (s, 3H), 3.68 (m, 1H), 2.02 (m, 1H), 1.88 (m, 1H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 164.9, 150.0, 144.5, 140.1, 107.8, 76.5, 65.6, 51.6, 46.0, 42.5, 25.8, 18.3, -5.4; IR (neat) ν_{max} 3435, 2953, 2930, 2857, 1725, 1436, 1256, 1110, 721 cm^{-1}; LRMS (ESI) *m/z* 313 ($M + H^+$); HRMS (FAB) Calcd for $C_{16}H_{29}O_4Si$ ($M + H^+$): 313.1835, found 313.1826.

(3*R*,5*S*)-Methyl 3-((bromomethyl)dimethylsilyloxy)-5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)cyclopent-1-enecarboxylate (82)

To a solution of cyclopetenol **85** (5 mg, 0.016 mmol) in bezene (1 ml) were added BMDMCS (0.1 M in benzene, 0.3 ml, 0.03 mmol), Et_3N (6 μl, 0.04 mmol) and DMAP (1.2 mg, 0.01 mmol) at 0 ºC. After stirred for 2 h at the ambient temperature, the reaction mixture was concentrated *in vacuo* to give as colorless oil. The crude silylether **82** was used in the next step without further purification: $[\alpha]_D^{20}$ 52.4 (*c* 0.50, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.70 (t, 1H), 5.11 (m, 1H), 4.98 (s, 1H), 4.66 (s, 1H), 4.10 (m, 2H), 3.68 (s, 3H), 3.61 (m, 1H), 2.45 (s, 2H), 2.23 (m, 1H),

2.07 (m, 1H), 0.89 (m, 9H), 0.26 (s, 6H), 0.05 (s, 6H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 164.8, 151.1, 145.7, 139.7, 107.5, 76.2, 65.9, 57.8, 42.6, 38.2, 26.0, 7.7, -1.0, -5.3; IR (neat) ν_{max} 2954, 2930, 2857, 1726, 1436, 1361, 1255, 1113, 837 cm^{-1}; LRMS (FAB) *m/z* 463 (M + H$^+$); HRMS (FAB) Calcd for $C_{19}H_{36}BrO_4Si_2$ (M + H$^+$): 463.1318, found 463.1321.

Methyl 5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)-2,2-dimethylhexahydro-2*H*-cyclopenta[d][1,2] oxasilole-4-carboxylate (87)

To a solution of silylether **82** (7 mg, 0.016 mmol) in benzene were added Et_3B (1.0 M in hexane, 0.016ml, 3 μmol) and Bu_3SnH (5 μl, 0.019 mmol) at 0 ℃. After stirred for 2h at ambient temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 3.8 mg (62%) of oxasilole **87** as a colorless oil: $[\alpha]_D^{20}$ 3.2 (*c* 0.50, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.12 (s, 1H), 4.80 (s, 1H), 4.27 (s, 1H), 4.09 (d, 1H, *J* = 14.3 Hz), 4.02 (d, 1H, *J* = 14.3 Hz), 3.53 (s. 3H), 3.14 (m, 1H), 2.77 (dd, 1H, *J* = 7.75 Hz, 10.1 Hz), 2.54 (m, 1H), 2.44 (s, 1H), 2.07-2.02 (m, 1H), 1.88 (dd, 1H, *J* = 6.65 Hz, 13.2 Hz), 0.90 (m, 9H), 0.78 (m, 1H), 0.69 (m, 1H), 0.09 (s, 6H), 0.04 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 163.7, 139.5, 108.7, 73.8, 65.9, 55.8, 51.6, 44.2, 41.8, 39.2, 25.9, 18.3, 1.7, -5.4; IR (neat) ν_{max} 2957, 2925, 2871, 2855, 1736, 1462, 1253, 842 cm^{-1}; LRMS (FAB) *m/z* 385 (M + H$^+$); HRMS (FAB) Calcd for $C_{19}H_{37}O_4Si_2$ (M + H$^+$): 385.2230, found 385.2216.

Methyl 5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)-3-hydroxy-2-(hydroxymethyl) cyclopentane carboxylate (88)

To a solution of oxasilole **87** (3.5 mg, 9 μmol) in THF/ MeOH (1:1, 1 ml) were added potassium bicarbonate (1.8 mg, 0.018 mmol), potassium fluoride (2.1 mg, 0.036 mmol) and aqueous hydrogen peroxide (30% aqueous solution, 0.02ml, 0.18 mmol) at ambient temperature. After

stirred for 16 h, to the reaction mixture was added well-grounded $Na_2S_2O_3$ (70 mg, 1.2 *eq* of H_2O_2), and then diluted with Et_2O, then filtrated with celite pad. The filtrate was concentrated *in vacuo*, the residue was purified by flash column chromatography on silica gel (EtOAc : Hexane = 2 : 1) to afforded 1.6 mg (50%) of diol **88** as a colorless oil: $[\alpha]_D^{20}$ –5.60 (*c* 0.50, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.13 (s, 1H), 4.79 (s, 1H), 4.60 (m, 1H), 4.10 (m, 2H), 3.94 (dd, 1H, *J* = 4.0 Hz, 11.0 Hz), 3.72 (m, 1H), 3.54 (s, 3H), 3.16 (m, 2H), 2.69 (m, 1H), 2.15 (m, 1H), 1.81 (m, 1H), 0.89 (m, 9H), 0.05 (s, 6H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.6, 148.0, 119.6, 108.8, 74.7, 65.9, 62.3, 51.4, 48.3, 41.9, 40.2, 25.8, 18.3, -5.4; IR (neat) ν_{max} 3468, 2928, 2856, 1735, 1682, 1460, 838 cm^{-1}; LRMS (FAB) *m/z* 345 (M + H$^+$); HRMS (FAB) Calcd for $C_{17}H_{33}O_5Si$ (M + H$^+$): 345.2097, found 345.2092.

6-Hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta [*c*]pyran-1 (3*H*)-one (1)

To a solution of hydroxymethyl cyclopentanol **88** (6 mg, 0.017 mmol) in CH_2Cl_2 (1.5 ml) was added TsOH-H_2O (5mg, 0.026 mmol) at the ambient temperature and stirred overnight. The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-Hexane = 2 : 1 →CH_2Cl_2 : MeOH = 10 : 1) to afford of iridoid **1** 2.4 mg (66%) as a coloress needle: mp 110-112 ℃; $[\alpha]_D^{25}$ -13.0 (*c* 0.01, MeOH); ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.18 (s, 1H), 5.05 (s, 1H), 4.73 (m, 2H), 4.37 (t, 1H, *J* = 3.5 Hz), 3.86 (dd, 1H, *J* = 4.8 Hz, 10.8 Hz), 3.79 (dd, 1H, *J* = 8 Hz, 10.7 Hz), 3.45 (m, 1H), 3.01 (dd, 1H, *J* = 9.6 Hz, 11.2 Hz), 2.40 (m, 1H), 2.14 (m, 1H), 1.65 (m, 1H); ^{13}C-NMR (CD_3OD, 75 MHz) δ 178.1, 144.9, 114.5, 74.2, 73.2, 62.6, 52.8, 45.6, 42.1, 41.6; IR (neat) ν_{max} 3361, 2925, 2853, 1728, 1659, 1467, 1378, 1241, 1171, 1037, 917 cm^{-1}; LRMS (FAB) *m/z* 199 (M + H$^+$); HRMS (FAB) Calcd for $C_{10}H_{15}O_4$ (M + H$^+$): 199.0970, found 199.0977.

V. References

1 (a) El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod*, **1980**, *43*, 649-707. (b) Boros, C. A.; Stermitz, F. R. *J. Nat. Prod*. **1991**, *54*, 1173-1246. (c) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2007**, *55*, 159-222. (d) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2007**, *55*, 689-728. (e) Tundis, R.; Loizzo, M. R.; Menichini, F.; Statti, G. A.; Menichini, F. *Mini-Rev. Med. Chem.* **2008**, *8*, 399-420. (f) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2009**, *57*, 765-796. (g) Paquette, L. A. *Top. Curr. Chem*. **1984**, *119*, 1-160.

2 (a) Kobayashi, Y.; *Curr. Org. Chem.* **2003**, *7,* 133-147. (b) Leemans, E.; D'hooghe, M.; De Kimpe, N. *Chem. Rev*. **2011**, *111*, 3268-3333.

3 (a) *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984. (b) Gothelf, K. V.; Jørgensen, K. A. *Chem. Rev.* **1998**, *98*, 863-909. (c) Li, C.-L.; Liu, R.-S. *Chem. Rev.* **2000**, *100*, 3127-3161. (d) Klunder, A. J. H.; Zhu, J.; Zwanenburg, B. *Chem. Rev.* **1999**, *99*, 1163-1190. (e) Gibson, S. E.; Lewis, S. E.; Mainolfi, N. *J. Organomet. Chem.* **2004**, *689*, 3873-3890. (f) Aubert, C.; Buisine, O.; Malacria, M. *Chem. Rev.* **2002**, *102*, 813-834. (g) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875-1916.

4 (a) Hudlicky, T.; Price, J. D. *Chem. Rev.* **1989**, *89*, 1467.-1486. (b) Borthwick, A. D.; Biggadike, K. *Tetrahedron* **1992**, *48*, 571-623. (c) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319-4347.

5 (a) Barco, A.; Benetti, S.; De Risi, C.; Marchetti, P.; Pollini, G. P.; Zanirato, V. *Tetrahedron: Asymmetry* **1997**, *8*, 3515-3545. (b) Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229-9272. (c) Dalko, P. I.; Sinay, P. *Angew. Chem., Int. Ed.* **1999**, *38*, 773-777.

6 (a) Arjona, O.; Go´mez, A. M.; Lo´pez, J. C.; Plumet, J. *Chem. Rev.* **2007**, *107*, 1919-2036.

7 (a) El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod*, **1980**, *43*, 649-707. (b) Boros, C. A.; Stermitz, F. R. *J. Nat. Prod*. **1991**, *54*, 1173-1246. (c) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2007**, *55*, 159-222.

8 (a) Dewick, P. M. (2009) *Medicinal natural products: A biosynthetic approach. 3rd ed.* England:

John Wiley & Sons Ltd. 206-223. (b) Tietz, L.-T. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 828-841. (c) Banthorpe, D. V.; Charlwood, B. V.; Francis, M. J. O. *Chem. Rev.*, **1972**, *72*, 115-155.

9 (a) Scheffrahn, R. H.; Gaston, L. K.; Sims, J. J.; Rust, M. K. Environ. *Entomol*. **1984**, *13*, 1502-1506. (b) Tomalski, M. D.; Blum, M. S.; Jones, T.H.; Fales, H. M.; Howard, D. F.; Passera, L. *J. Chem. Ecol*. **1987**, *13*, 253-263. (c) Huth, A.; Dettner, K. *J. Chem. Ecol*. **1990**, *16*, 2691-2711. (d) Lorenz, M.; Boland, W.; Dettner, K. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 912-914.

10 Inouye, H. *Planta Med.* **1978**, *33*, 193-216.

11 Sakan, T.; Fujino, A.; Murai, F.; Suzui, A. *Bull. Chem. Soc. Jpn.* **1960**, *33*, 1737.

12 Schreiber, S. L.; Meyers, H. V.; Wiberg, K. B. *J. Am. Chem. Soc.* **1986**, *108*, 8274.

13 For recently references; (a) Mukherjee, S.; Yang, J. W.; Hoffmann, S. *Chem. Rev.* **2007**, *107*, 5471-5569. (b) Radtke L, Willot, M; Sun, H. Y.; *Angew. Chem., Int. Ed.* **2011**, *50*, 3998-4002. (c) Beckett, J. S.; Beckett J. D.; Hofferberth, J. E.; *Org. Lett.* **2010**, *12*, 1408-1411.

14 Carroll, G. L.; Allan, A. K.; Schwaebe, M. K.; Little, R. D. *Org. Lett*. **2000**, *2*, 2531-2534.

15 Candish, L.; Lupton, D. W. *Org. Lett.*, **2010**, *12*, 4836-4839.

16 Herrmann, W. A. *Angew. Chem. Int. Ed.* **2002**, *41*, 1290-1309.

17 Jones, R. A.; Krische, M. *Org. Lett*. **2009**, *11*, 1849-1851.

18 Achmatowicz, O.; Szechner, B.; Maurin, J. K.; *Tetrahedron* **1997**, *53*, 6035-6044.

19 Trost, B. M.; Van Vranken, D. L. *Chem. Rev.* **1996**, *96*, 395-422.

20 Tsuji, J. (2004) *Palladium reagents and catalysts: new perspectives for the 21st century.* England: John Wiley & Sons Ltd. 1-18.

21 Suh, Y.-G.; Lee, Y.-C.; Ko, J.-A.; Kim, S.-A.; Kim, J.-Y.; Jung, J.-K.; Seo, S.-Y.; Lee, K.-H. *Synthetic Commun.* **2000**, *30*, 3053-3060.

22 Suh, Y.-G.; Jung, J.-K.; Kim, S.-A.; Shin, D.-Y.; Min, K.-H. *Tetrahedron Lett.* **1997**, *38*, 3911-3914.

23 (a) Suh, Y.-G.; Jung, J.-K.; Suh, B.-C.; Lee, Y.-C.; Kim, S.-A. *Tetrahedron Lett*. **1998**, *39*, 5377-5380. (b) Suh, Y.-G.; *Tetrahedron Lett.* **2001**, *42*, 1691-1694. (c) Suh, Y.-G.; Jung, J.-K.; Seo, S.-Y.; Min, K.-H.; Shin, D.-Y.; Lee, Y.-S.; Kim, S.-H.; Park, H.-J. *J. Org. Chem*. **2002**, *67*, 4127-4137.

24 Seo, S.-Y.; Jung, J.-K.; Paek, S.-M.; Lee, Y.-S.; Kim, S.-H.; Suh, Y.-G. *Tetrahedron Lett*. **2006**, *47*, 6527-6530.

25 Paek, S.-M.; Seo, S.-Y.; Min, K.-H.; Shin, D.-M.; Chung, Y.-K.; Suh, Y.-G. *Heterocycles*, **2007**, *71*, 1059-1066.

26 Seo, S.-Y.; Jung, J.-K.; Paek, S.-M.; Lee, Y.-S.; Kim, S.-H.; Lee, K.-O.; Suh, Y.-G. *Org. Lett*. **2004**, *6*, 429-432.

27 Lee, S. (2006) *Studies on the iridoid syntheses employing stereoselective construction of the tri-substituted cyclopentanes*, Unpublished thesis (M.A.), Seoul National University.

28 Han, Y.-T.; Paek, S.-M.; Lee, S.; Jung, J.-W.; Jung, J.-K.; Seo, S.-Y.; Lee, J.; Suh, Y.-G. *Tetrahedron Lett*. **2010**, *51*, 2697-2699.

29 Zhang, Y.; Lu, Y.; Zhang, L.; Zheng,Q.-T.; Xu,L-Z.; Yang, S.-L. *J. Nat. Prod*. **2005**, *68*, 1131-1134.

30 Tanitsu, M.; Takaya, Y.; Akasaka, M.; Niwa, M.; Oshima, Y. *Phytochemistry* **2002**, *59*, 845-849, and references cited therein.

31 Kokubo, M.; Ogawa, C.; Kobayashi, S. *Angew. Chem. Int. Ed.* **2008**, *47*, 6909-6911.

32 Schreiber, J.; Maag, H.; Hashimoto, N.; Eschenmoser, A. *Angew. Chem., Int. Ed*. **1971**, *10*, 330-331.

33 Zayia, G. H. *Org. Lett.* **1999**, *1*, 989-992.

34 (a) Nishiyama, H.; Kitajima, T.; Matsumoto, M.; Itoh, K. *J. Org. Chem.* **1984**, *49*, 2298-2300. (b) Stork, G.; Kahn, M. *J. Am. Chem. Soc*. **1985**, *107*, 500-501. (c) Jenkins, P. R.; Wood, A. J.

Tetrahedron Lett. **1997**, *38*, 1853-1856. (d) Kessabi, F. M.; Winkler, T.; Luft, J. A. R.; Houk, K. N. *Org. Lett*. **2008**, *10*, 2255-2258.

35 Tamao, K.; Ishida, N.; Kumada, M. *J. Org. Chem*. **1983**, *48*, 2120-2122.

36 Evans, P. A.; Cui, J.; Gharpure, S. J.; Polosukhin, A.; Zhang, H.-R. *J. Am. Chem. Soc*. **2003**, *125*, 14702-14703.

37 J. Strating (1961) *Organic Sulfur Compounds.* Pergamon Press, New York: N. Y.: Chapter 15.

38 (a) Conrad, P. C.; Fuchs, P. L. *J. Am. Chem. Soc*. **1978**, *100*, 346-348. (b) Cheng, W.-C.; Kurth, M. K. *J. Org. Chem.* **2002**, *67*, 4387-4391. (b) Nantz, M. H.; Radisson, X.; Fuchs, P. L. *Synthetic Commun.* **1987**, *17*, 55-69.

39 Yoshida, T.; Saito, S. *Chem. Lett*, **1982**, 165-168.

40 (a) Lanver, A.; Schmalz, H.-G. *Eur. J. Org. Chem.* **2005**, 1444-1458. (b) Schemalz, H.-G.; Jope, H. *Tetrahedron* **1998**, *54*, 3457-3464.

41 (a) Lipshutz, B. H.; Miller, T. A. *Tetrahedron Lett.* **1990**, *37*, 5253-5256. (b) Hughes, D. L.; Reamer, R. A. *J. Org. Chem.* **1996**, *61*, 2967-2971. (c) Saiah, M.; Bessodes, M.; Antonakis, K. *Tetrahedron Lett.* **1992**, *30*, 4317-4320. (d) Cook, A. F.; Maichuk, D. T. *J. Org. Chem.* **1970**, *35*, 1940-1943. (e) Wagner, J.; Anders, H.; Rohrbach, S.; Wagner, D.; Oberer, L.; France, J. *J. Org. Che.* **2005**, *70*, 9588-9590.

42 Matsumoto, K.; Miura, K.;Oshima, K.; Utimoto, K. *Tetrahedron Lett*. **1992**, *33*, 7031-7034, and references therein.

43 (a) Kabalka, G. W.; Brown, H. C.; Suzuki, A.; Honma, S.; Arase, A.; Itoh, M. *J. Am. Chem. Soc*. **1970**, *92*, 710-712. (b) Davies, A. G.; Griller, D.; Roberts, B. P.; Tudor, R. *J. Chem. Soc. D* **1970**, 640 (c) Davies, A. G.; Maki, T.; Roberts, B. P. *J. Chem. Soc. Perkin Trans. 2* **1972**, 744-747. (d) Krusic, P. J.; Kochi, J. K. *J. Am. Chem. Soc.* **1969**, *91*, 3942-3944. (e) Ollivier, C.; Renaud, P. *Chem. Rev.* **2001**, *101*, 3415-3434.

44 (a) Nozaki, K.; Oshima, K.; Utimoto, K. *J. Am. Chem. Soc.* **1987**, *109*, 2547-2549. (b) Guindon, Y.; Lavalle´e, J.-F.; Boisvert, L.; Chabot, C.; Delorme, D.; Yoakim, C.; Hall, D.; Lemieux, R.; Simoneau, B. *Tetrahedron Lett.* **1991**, *32*, 27-30. (c) Satoh, S.; Sodeoka, M.; Sasai, H.; Shibasaki, M. *J. Org. Chem.* **1991**, *56*, 2278-2280.

45 Carra, C.; Scaiano, J. C. *Eur. J. Org. Chem.* **2008**, *26*, 4454-4459.

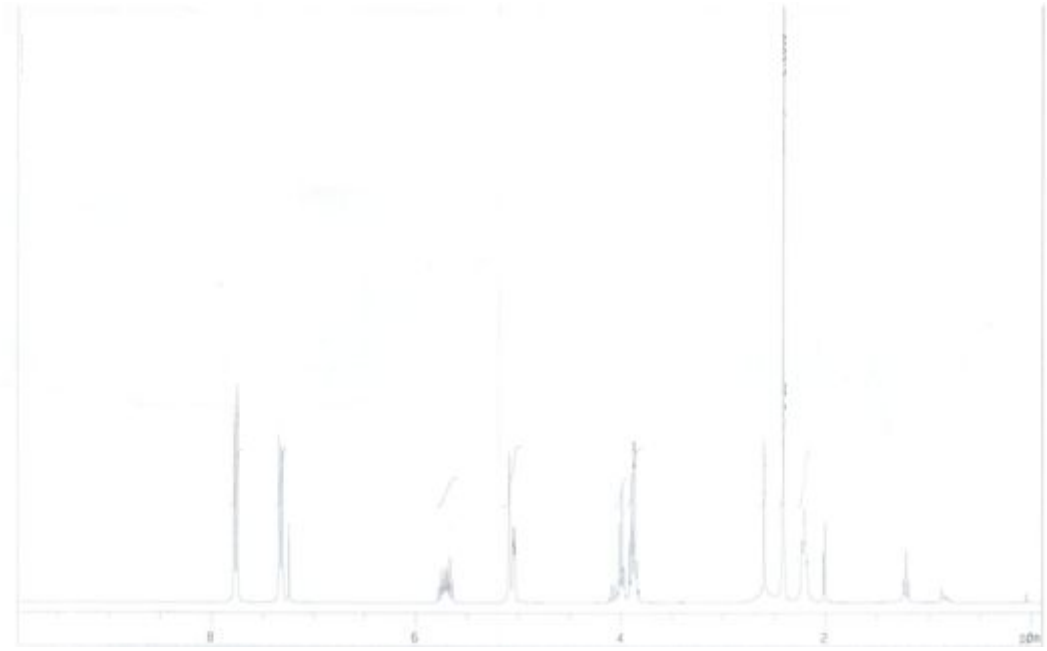
46 (a) Carroll, G. L.; Allan, A. K.; Schwaebe, M. K.; Little, R. D. *Org. Lett*. **2000**, *2*, 2531-2534. (b) Devin, P.; Fensterbank, L.; Malacria, M. *J. Org. Chem*. **1998**, *63*, 6764-6765.

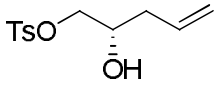
47 (a) Thomas, E. J.; Williams, A. C. *J. Chem. Soc., Chem. Commun*. **1987**, *13*, 992-994. (b) Jahn, U.; Hartmann, P.; Kaasalainen, E. *Org. Lett*. **2004**, *6*, 257-260.

48 We have reported the successful result previously: Lee, S.; Paek, S.-M.; Yun, H.; Kim, N.-J.; Suh, Y.-G. *Org. Lett.* **2011**, *13*, 3344-3347.

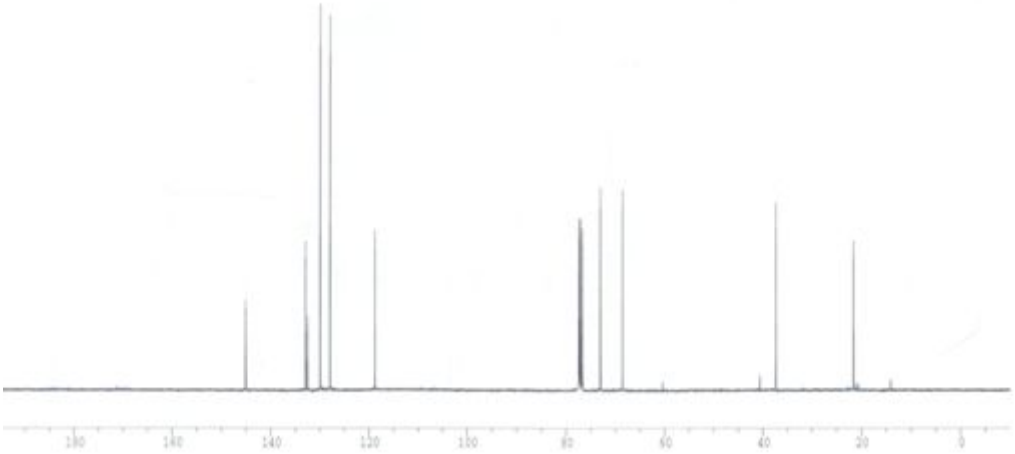
VI. Appendix

▼ **59, ^{1}H-NMR, 300 MHz**

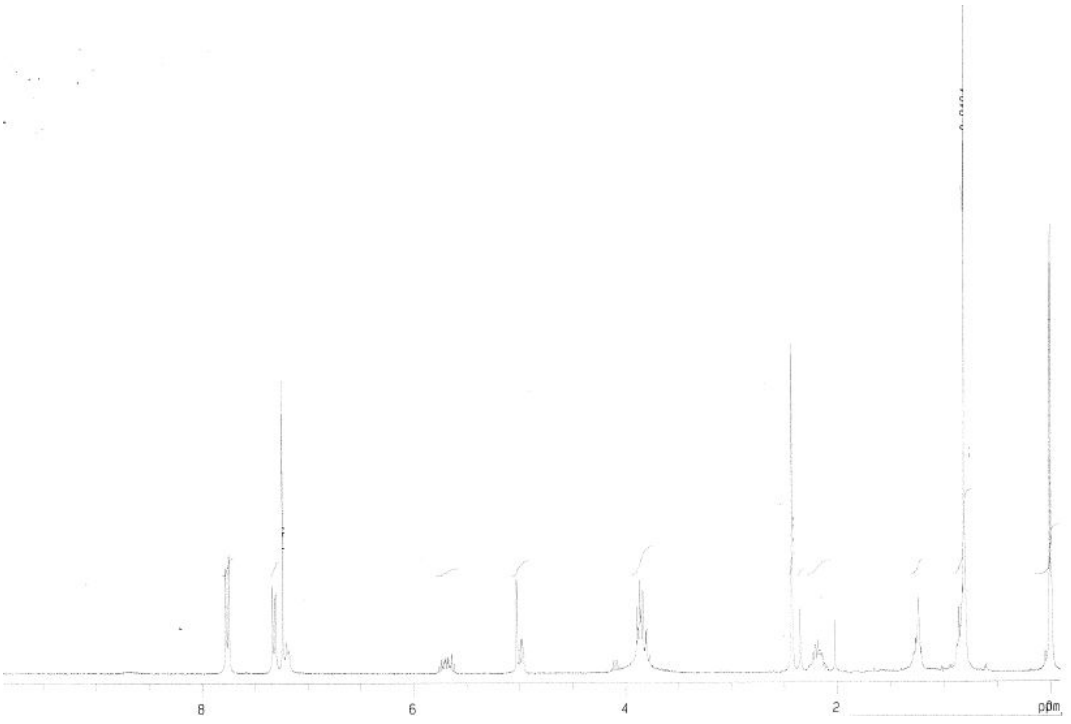




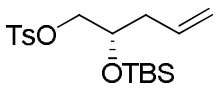
▼ **59, ^{13}C-NMR, 75 MHz**

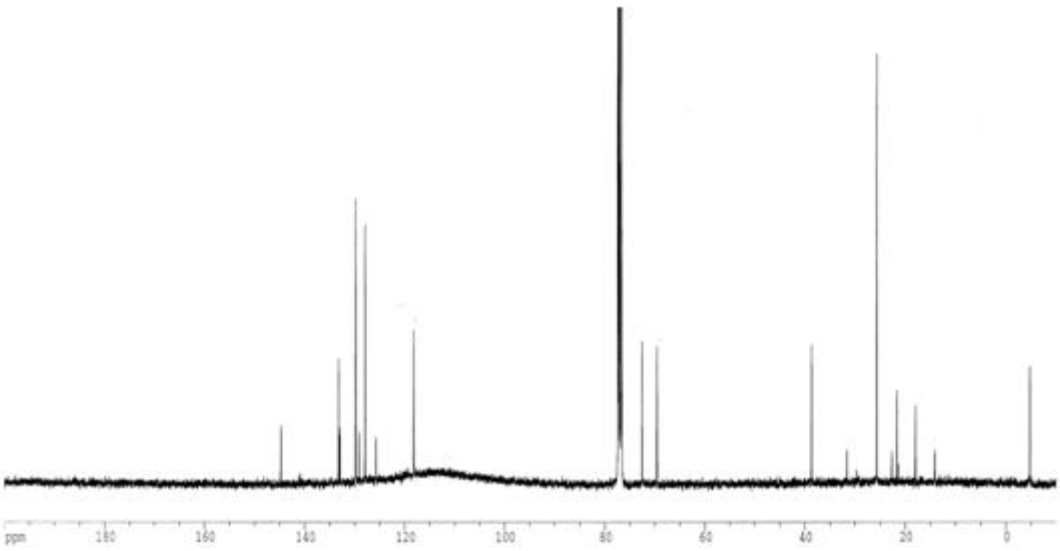


▼ **60, ^{1}H-NMR, 300 MHz**

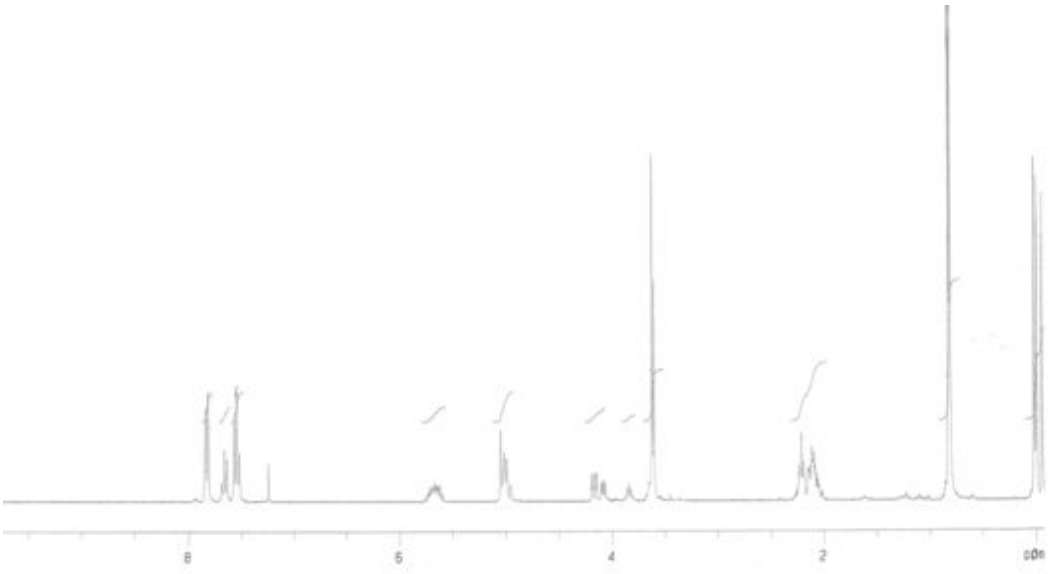


▼ **60, ^{13}C-NMR, 75 MHz**

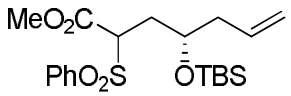


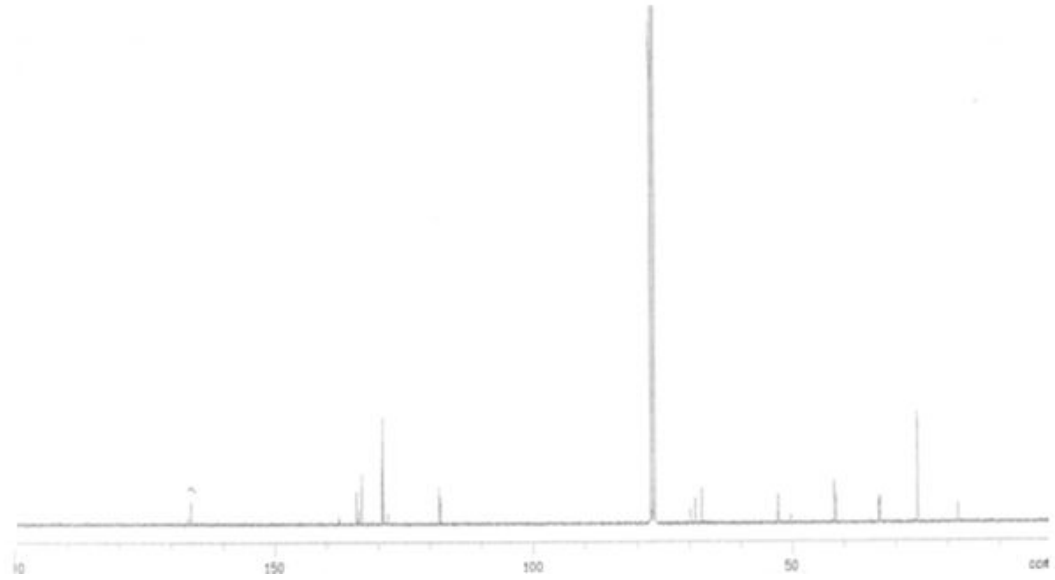


▼ **61, ^{1}H-NMR, 300 MHz**

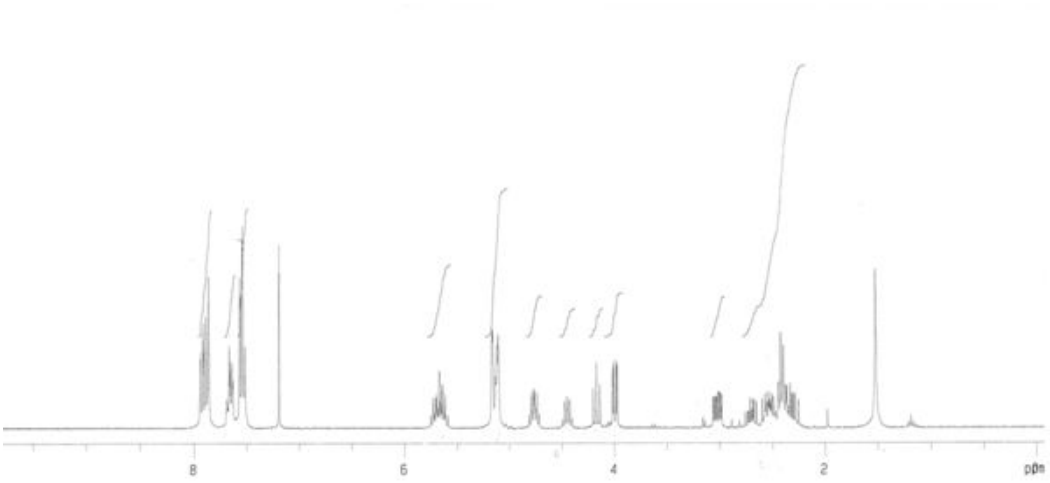


▼ **61, ^{13}C-NMR, 75 MHz**

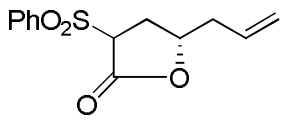


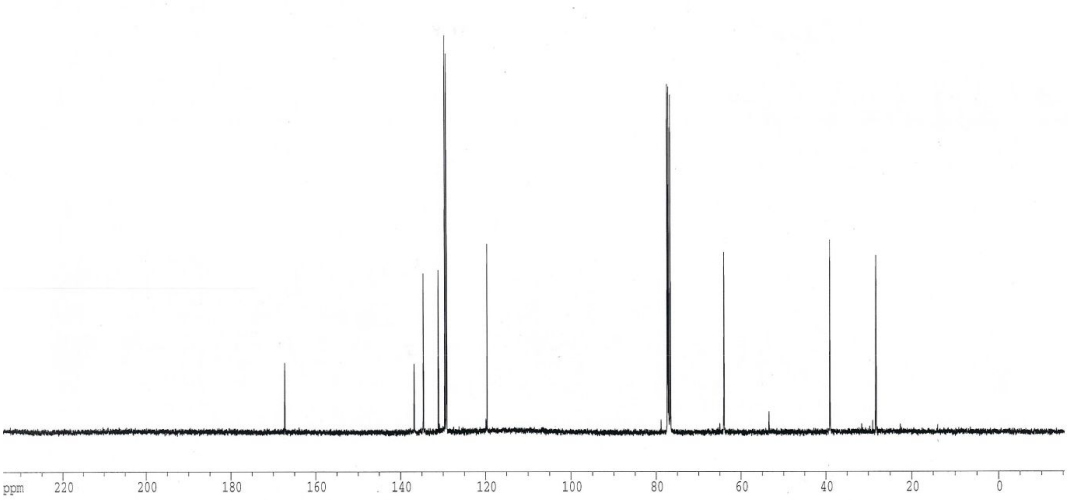


▼ **62, ^{1}H-NMR, 300 MHz**

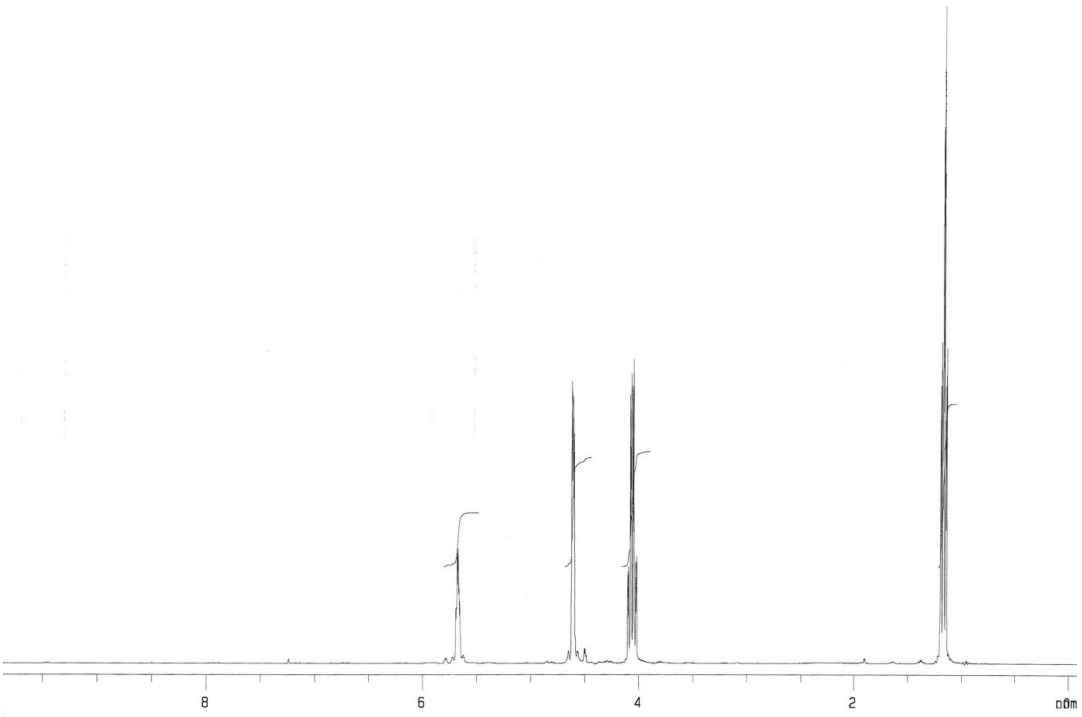


▼ **62, ^{13}C-NMR, 75 MHz**

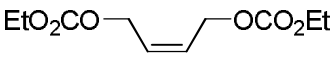


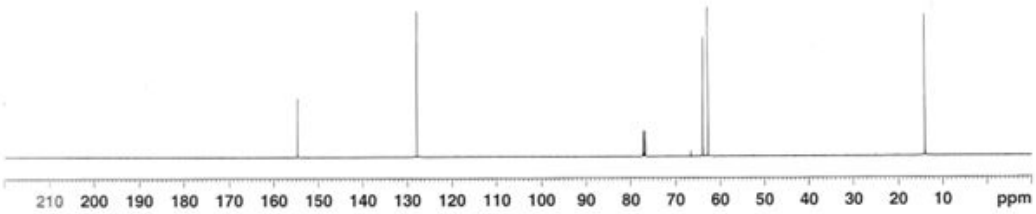


▼ **63a, ^{1}H-NMR, 300 MHz**

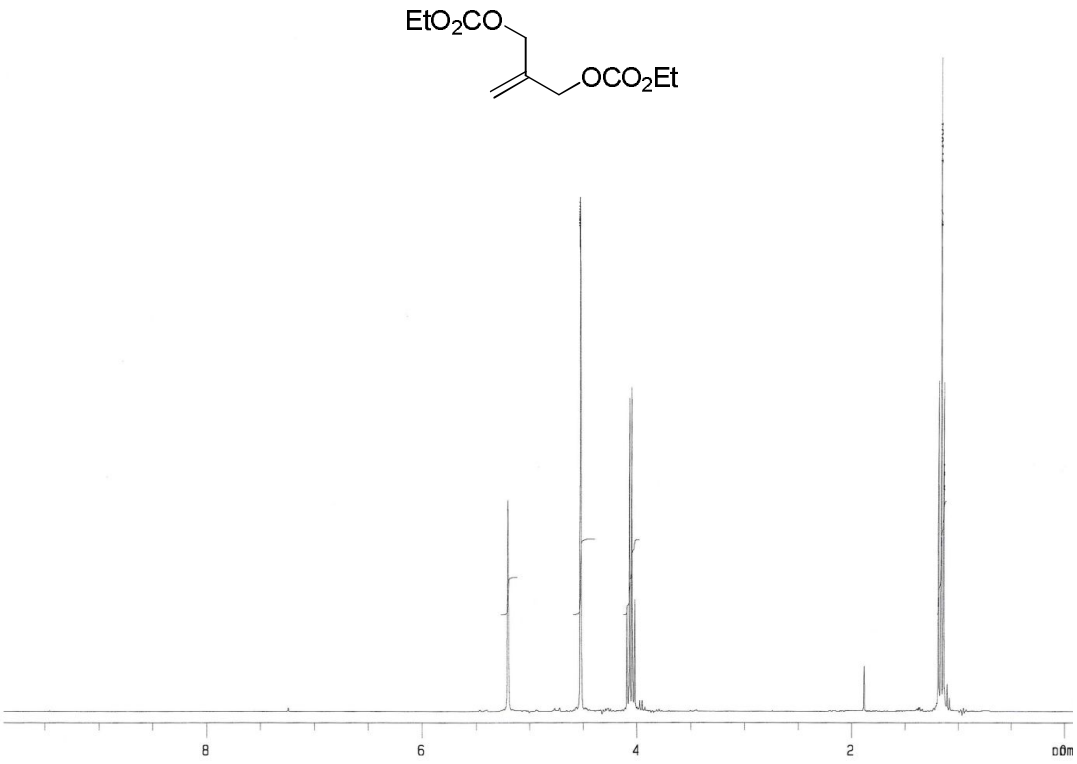


▼ **63a, ^{13}C-NMR, 125 MHz**

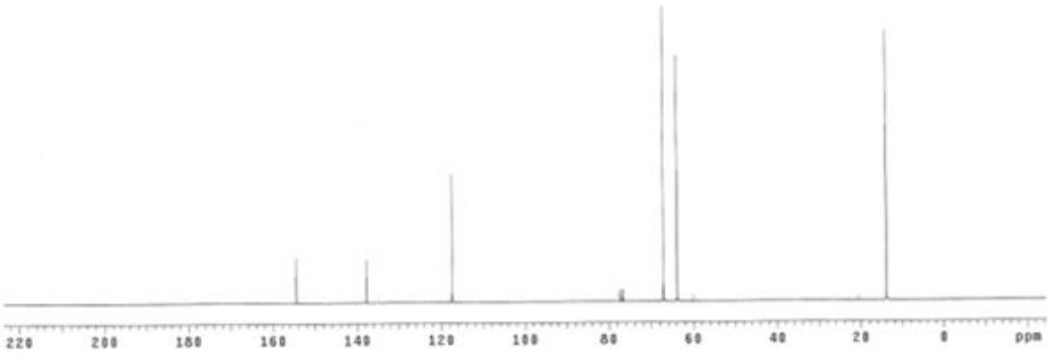




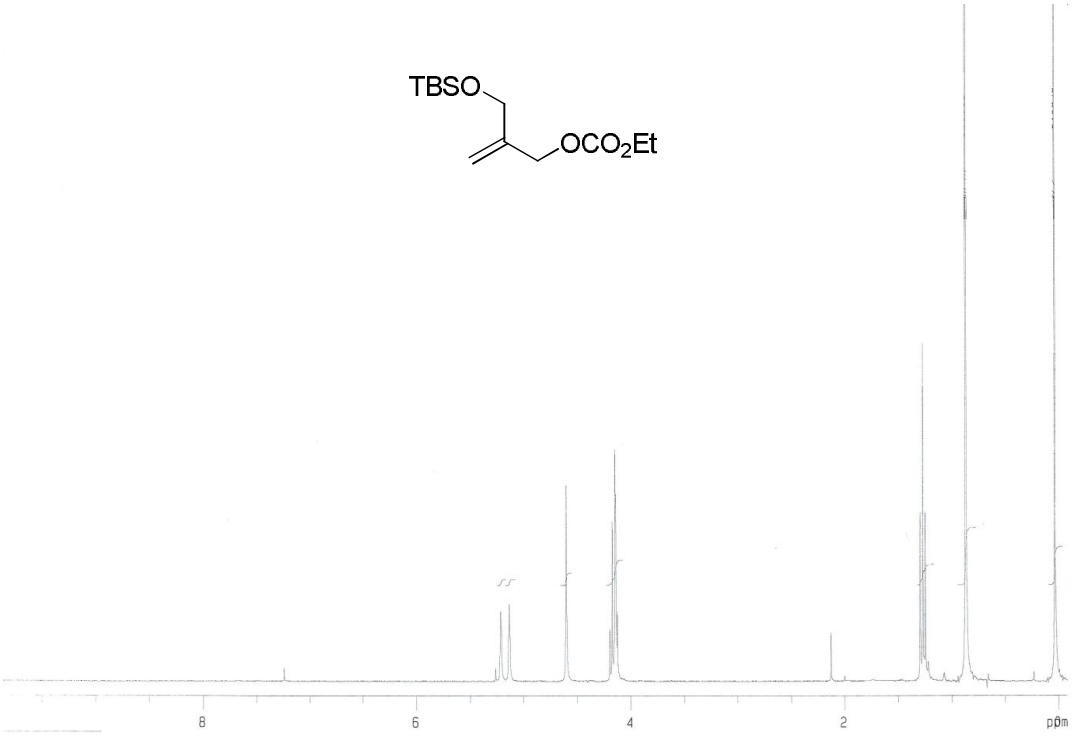
▼ **63b, ^{1}H-NMR, 300 MHz**



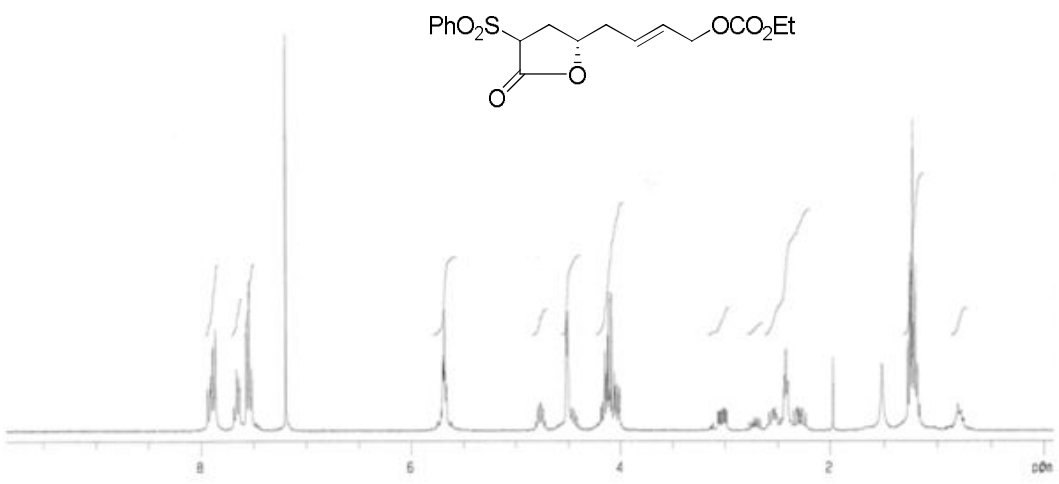
▼ **63b, ^{13}C-NMR, 75 MHz**



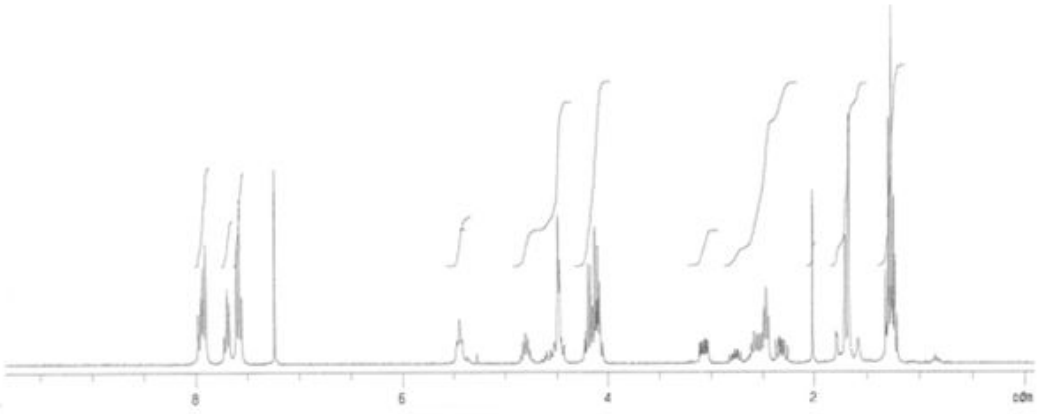
▼ **63d, ^{1}H-NMR, 300 MHz**



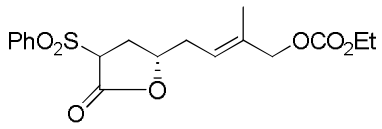
▼ **64a, ^{1}H-NMR, 300 MHz**

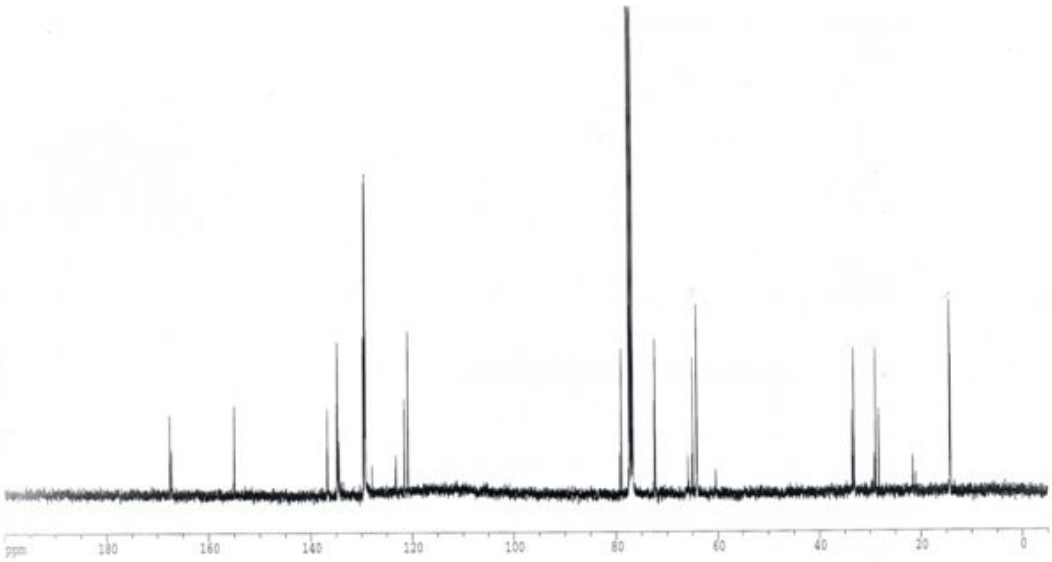


▼ **64c, ^{1}H-NMR, 300 MHz**

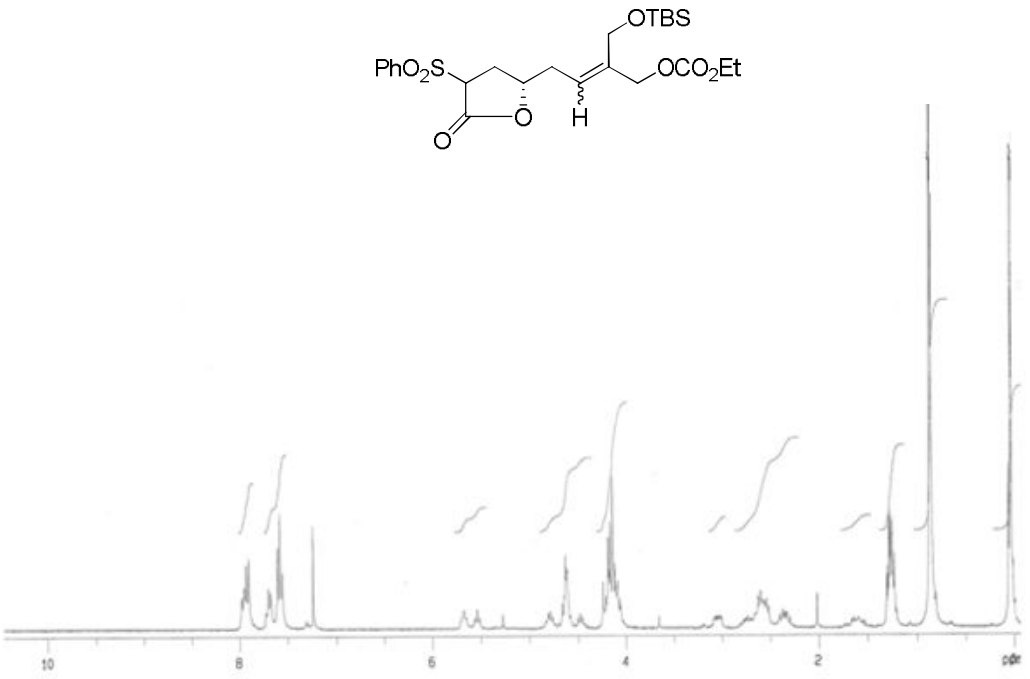


▼ **64c, ^{13}C-NMR, 75 MHz**

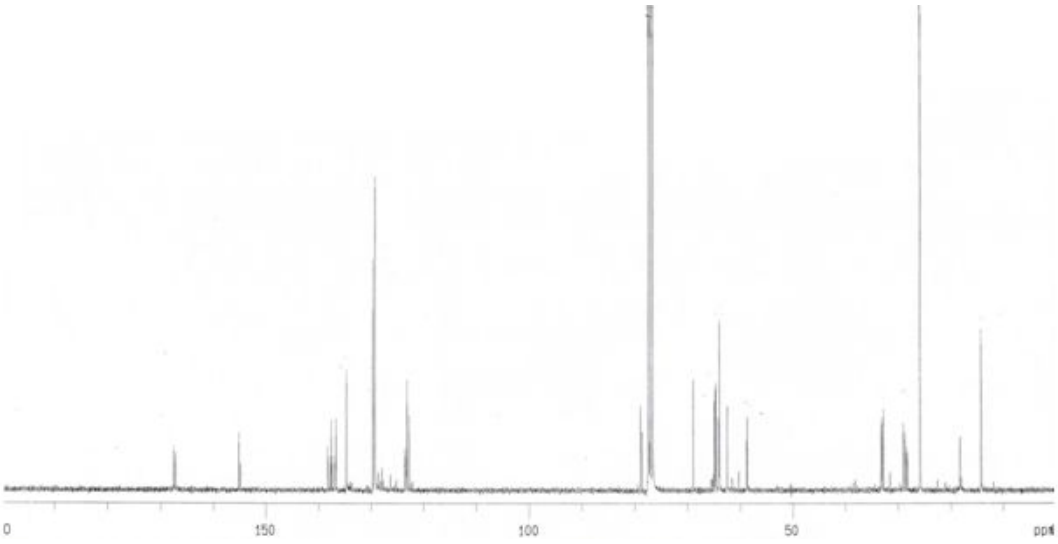




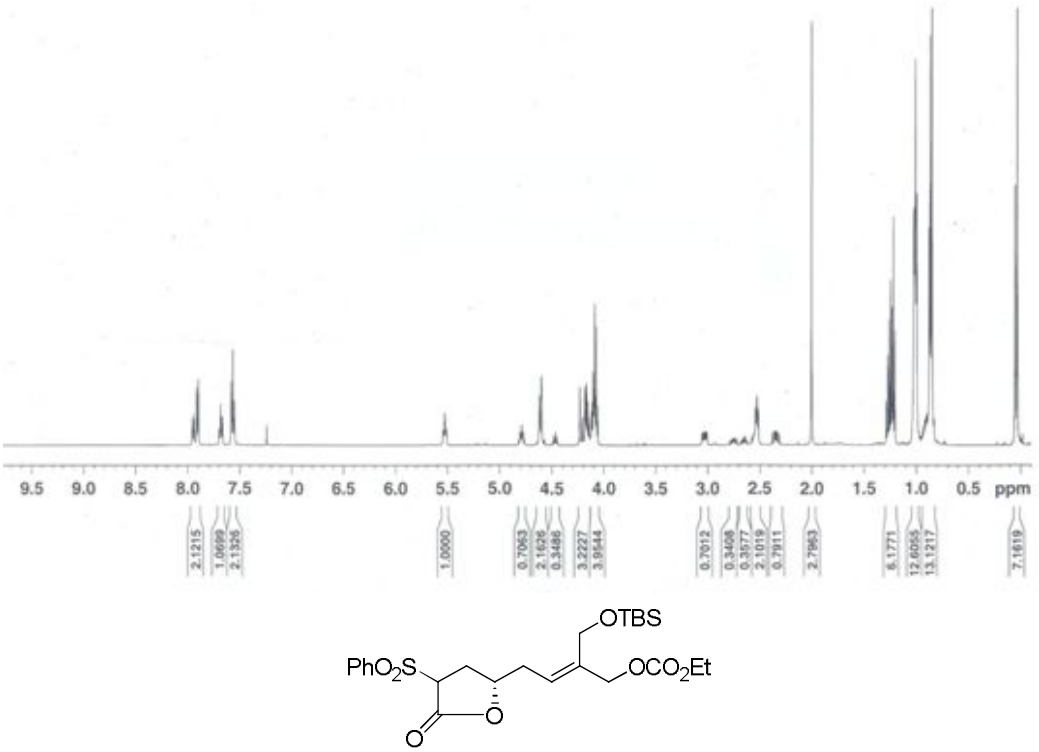
▼ **64d, *E/Z* mix ^{1}H-NMR, 300 MHz**



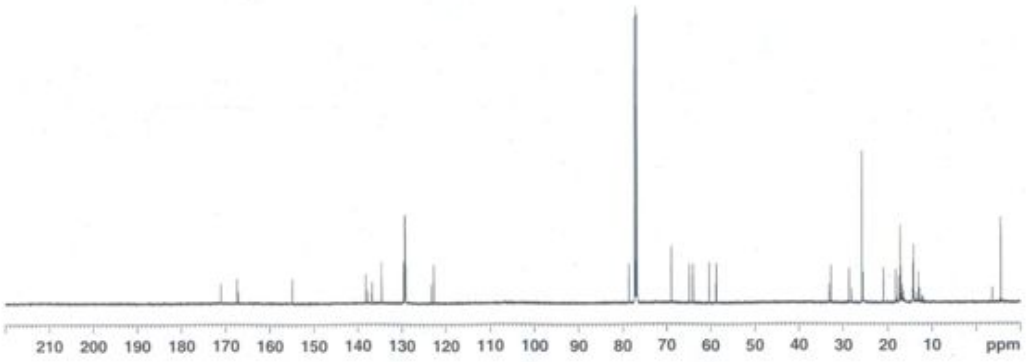
▼ **64d, ^{13}C-NMR, 75 MHz**



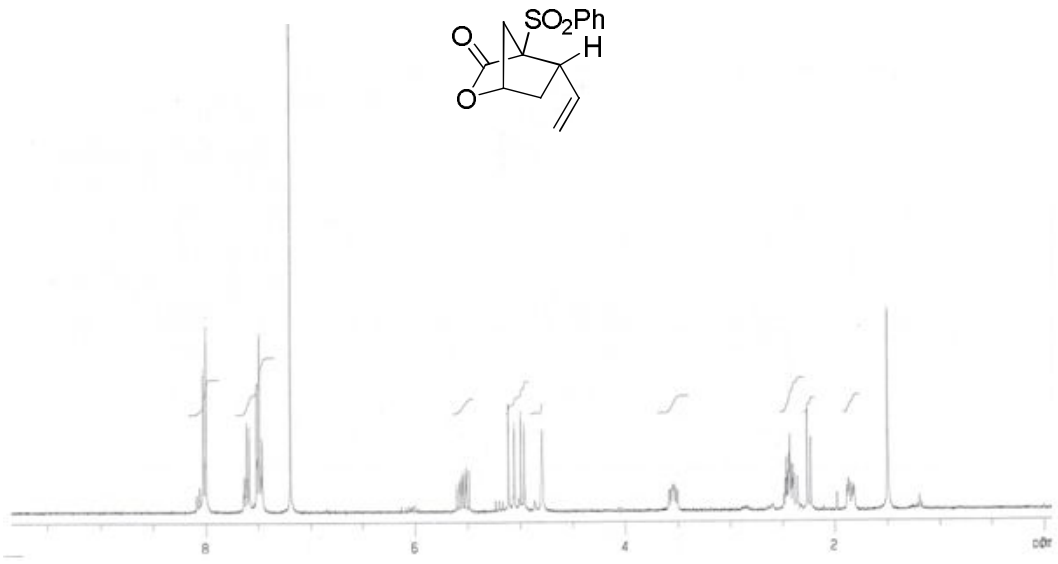
▼ ***Z*-64d, ^{1}H-NMR, 500 MHz**



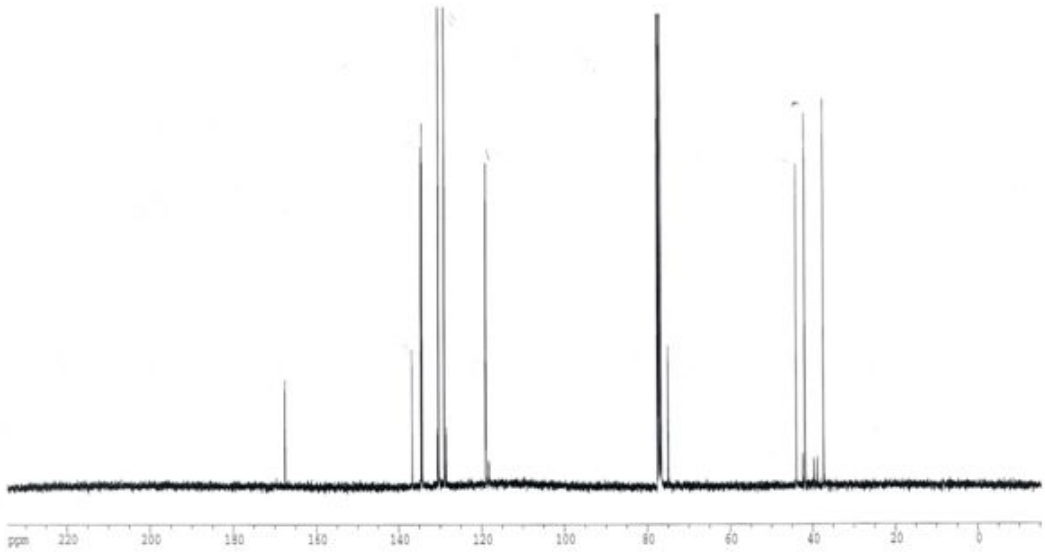
▼ ***Z*-64d, ^{13}C-NMR, 125 MHz**



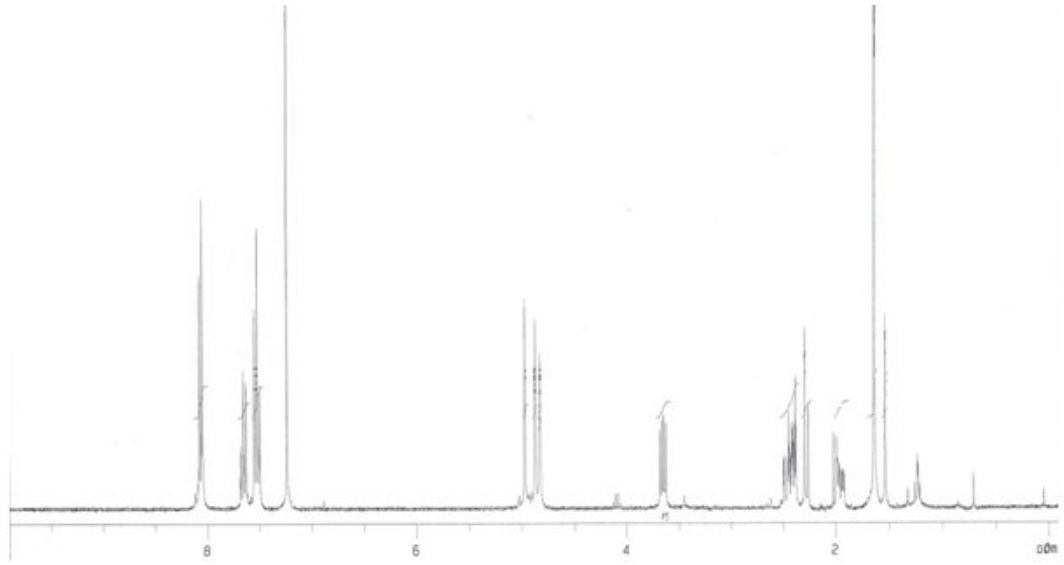
▼ **46, ^{1}H-NMR, 300 MHz**



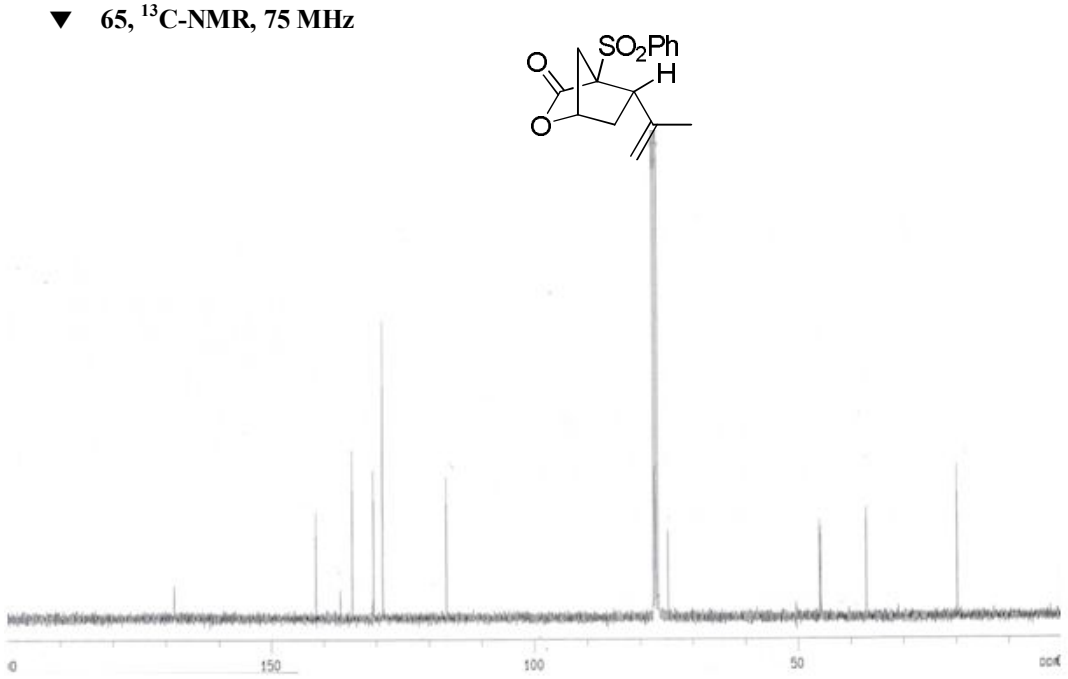
▼ **46 ^{13}C-NMR, 75 MHz**



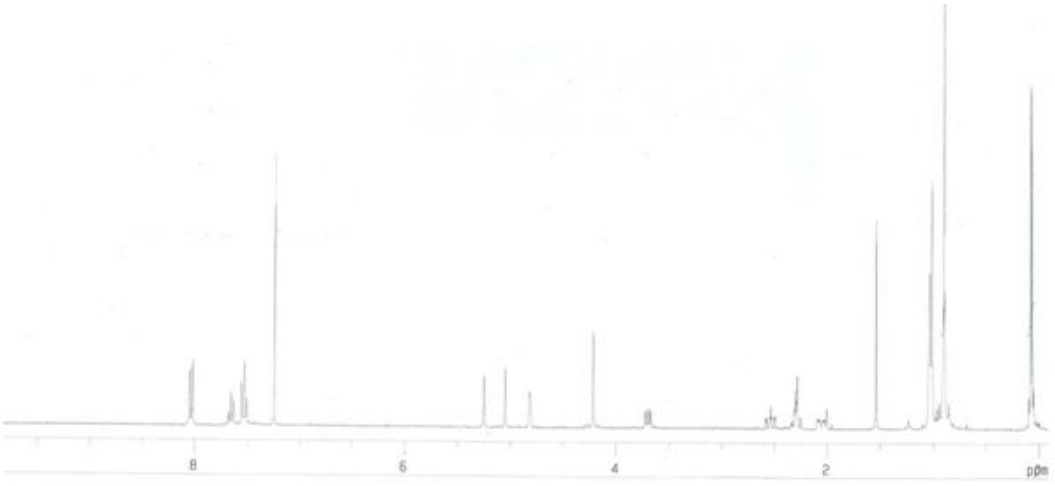
▼ **65, ^{1}H-NMR, 300 MHz**

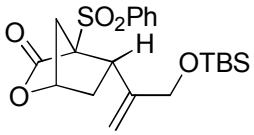


▼ **65, ^{13}C-NMR, 75 MHz**

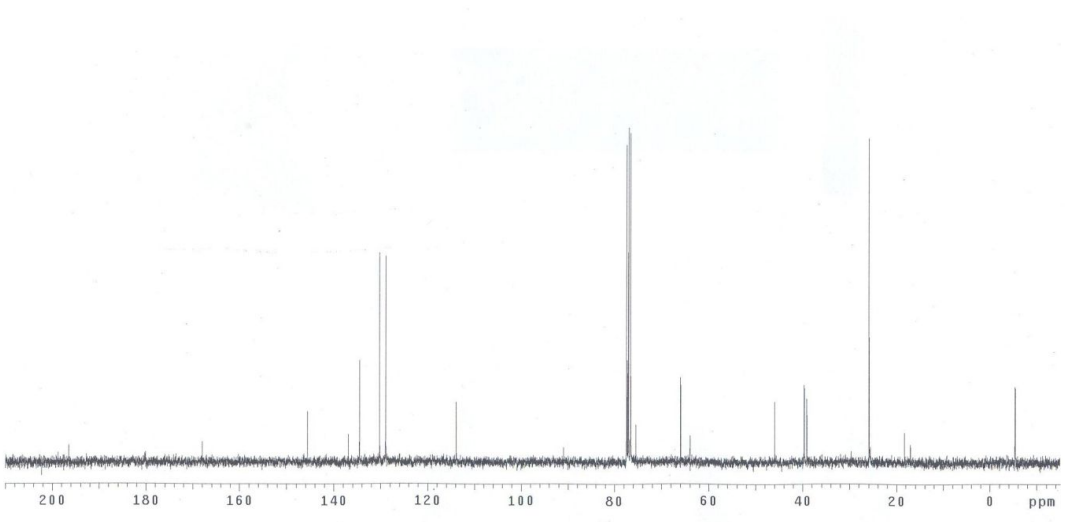


▼ **66, ^{1}H-NMR, 300 MHz**

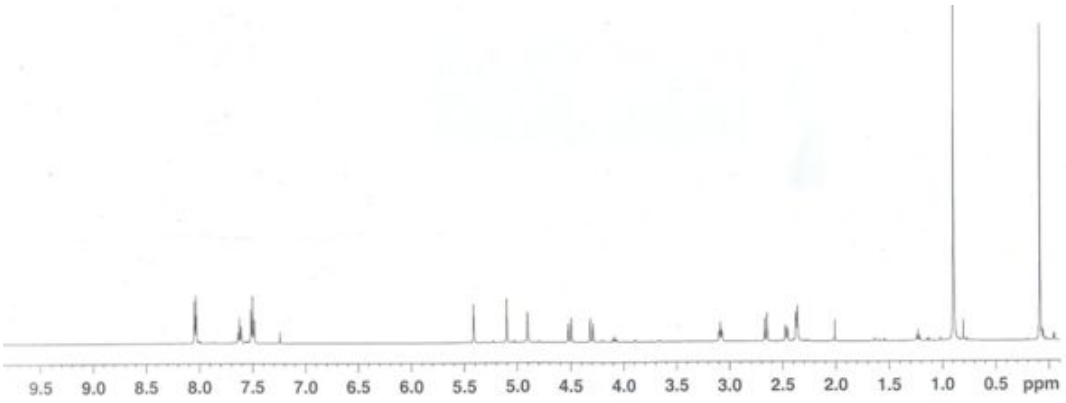




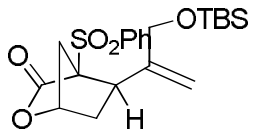
▼ **66 ^{13}C-NMR, 75 MHz**

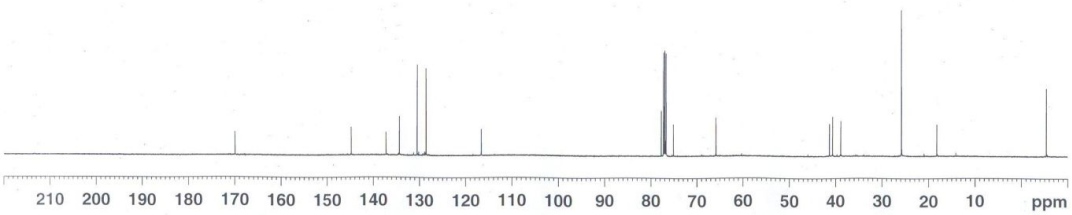


▼ **66', ^{1}H-NMR, 500 MHz**

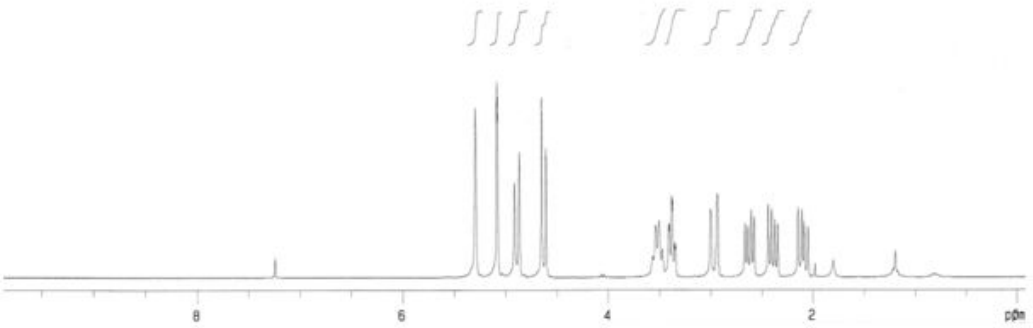


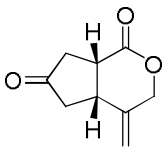
▼ **66', ^{13}C-NMR, 125 MHz**



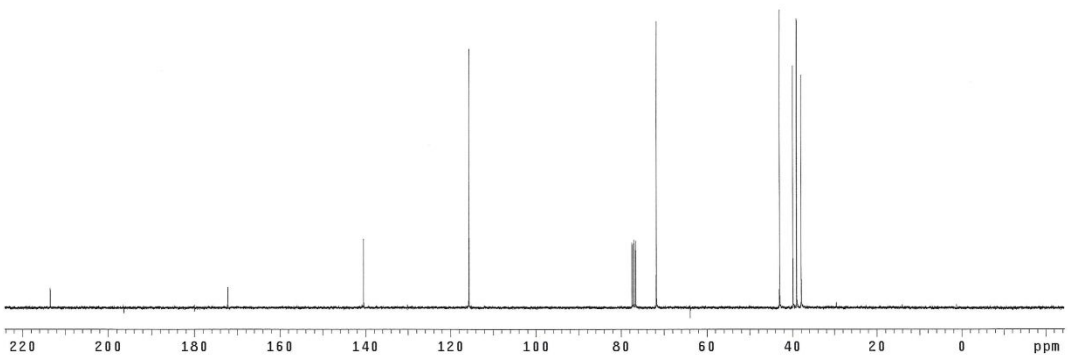


▼ **68, ^{1}H-NMR, 300 MHz**

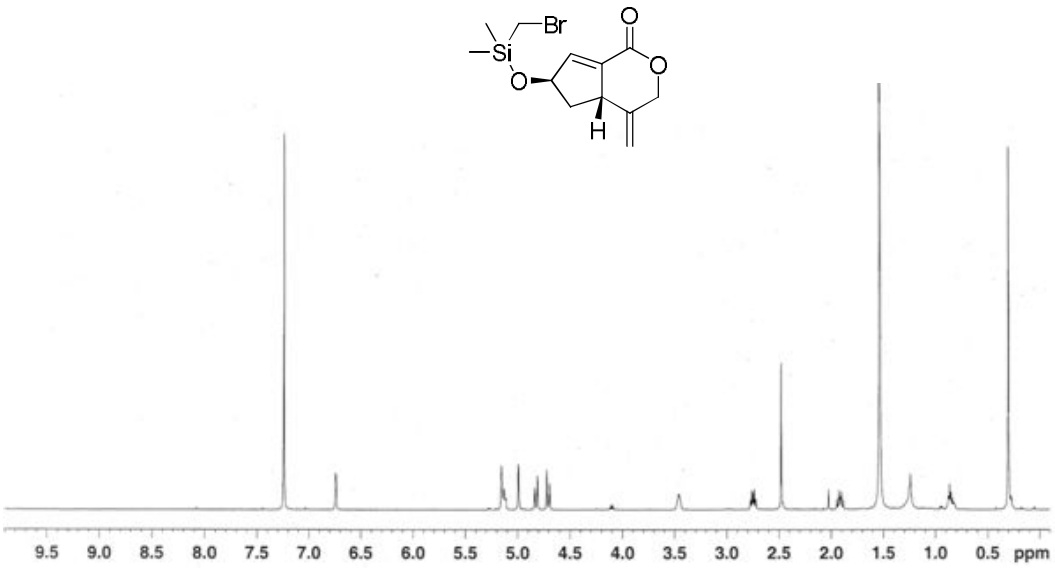




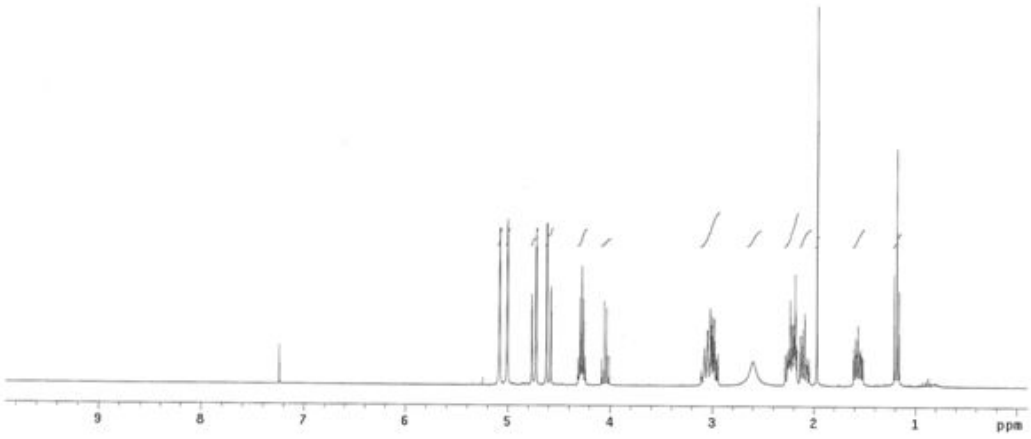
68, ^{13}C-NMR, 75 MHz

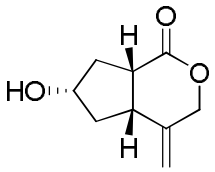


▼ **71, ^{1}H-NMR, 500 MHz**

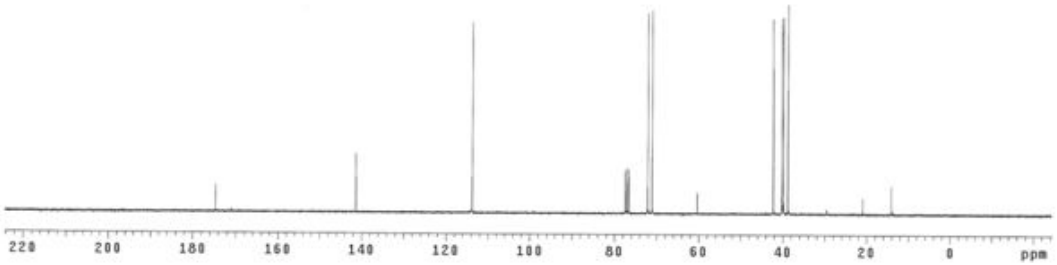


▼ **69, ^{1}H-NMR, 300 MHz**

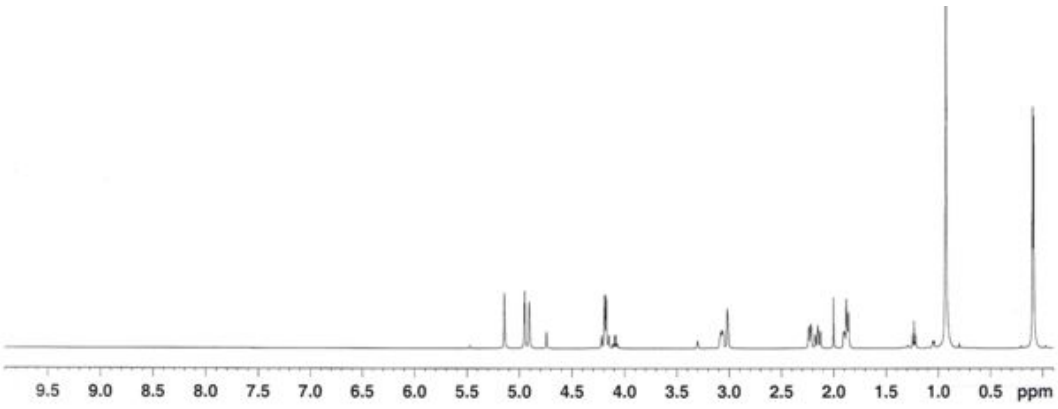


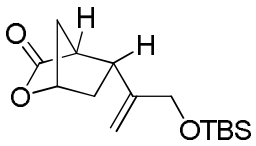


▼ **69, ^{13}C-NMR, 75 MHz**

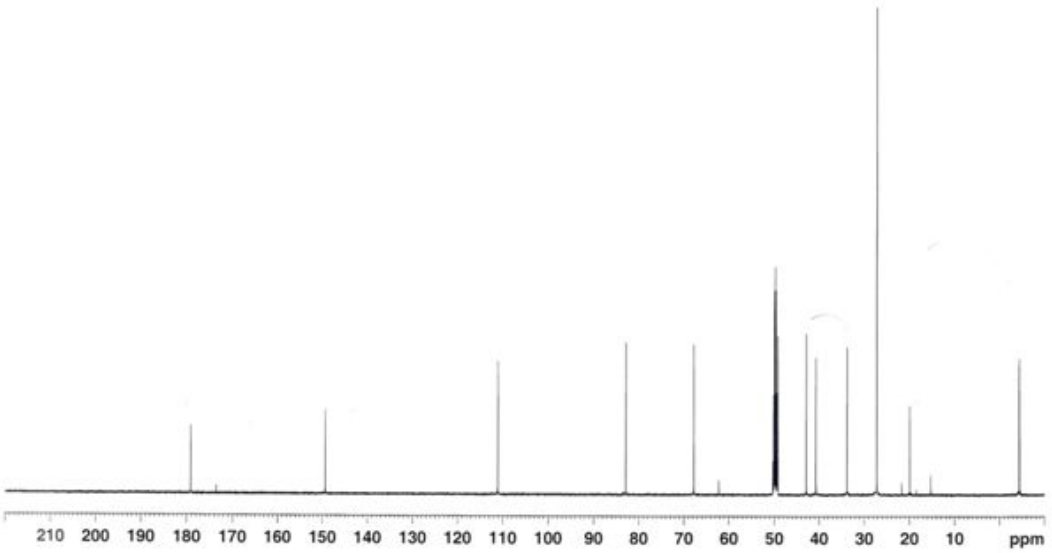


▼ **70, ^{1}H-NMR, 500 MHz**

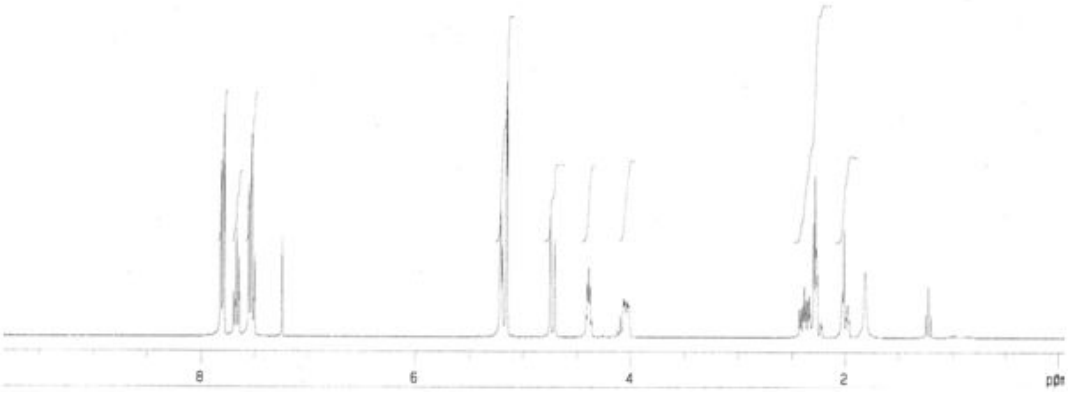


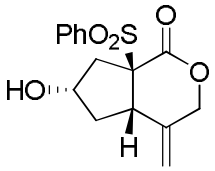


▼ **70, ^{13}C-NMR, 125 MHz**

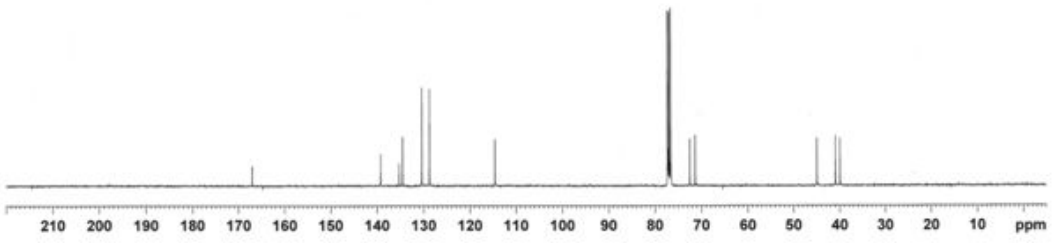


▼ **72, ^{1}H-NMR, 300 MHz**

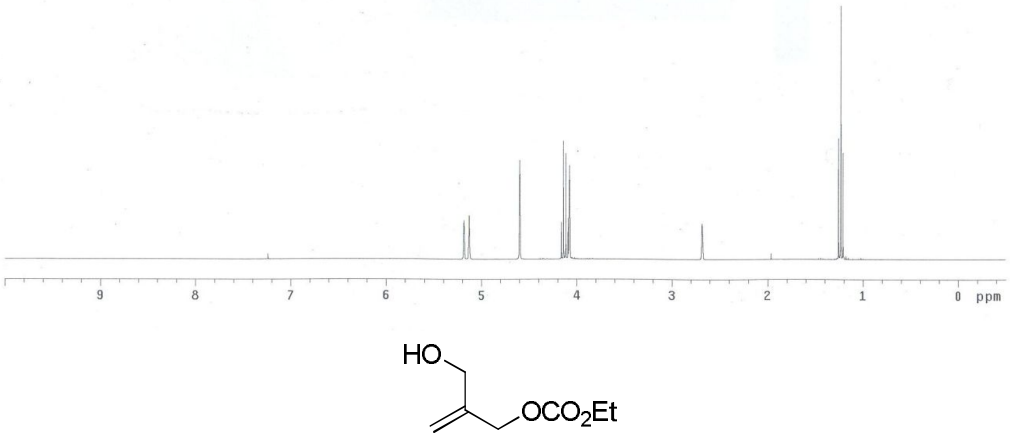




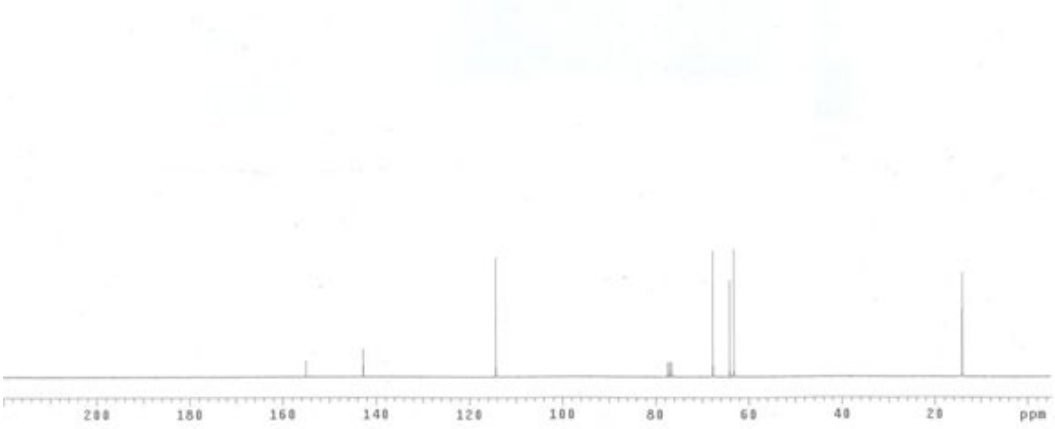
▼ **72, ^{13}C-NMR, 125 MHz**



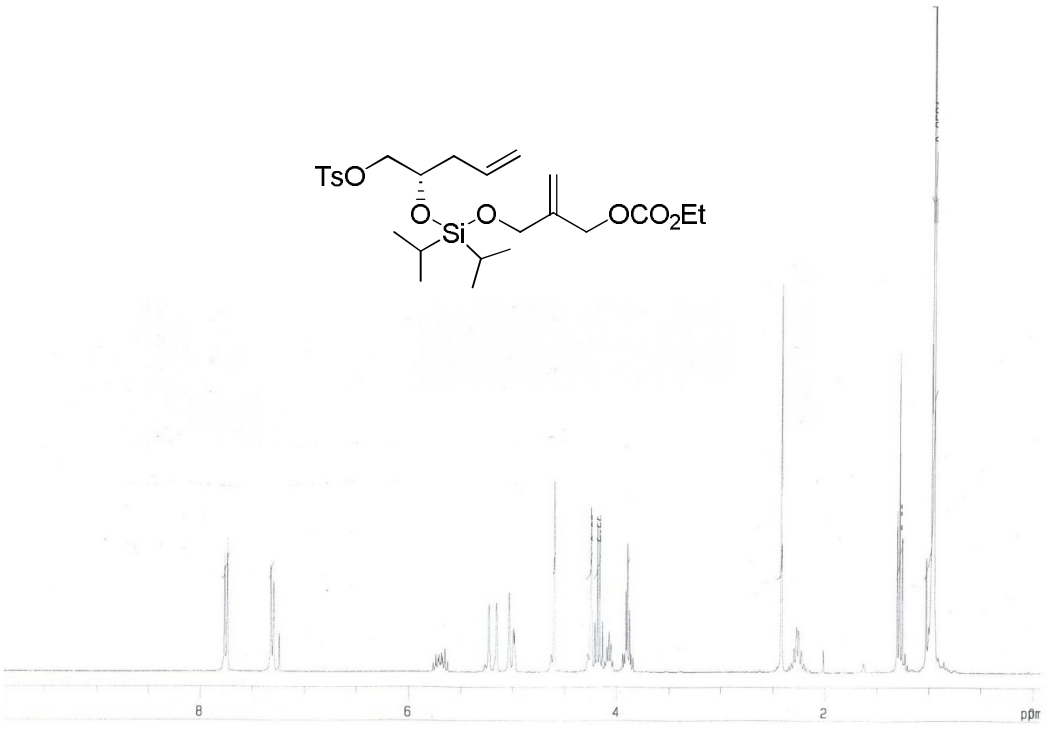
▼ **73, ^{1}H-NMR, 300 MHz**



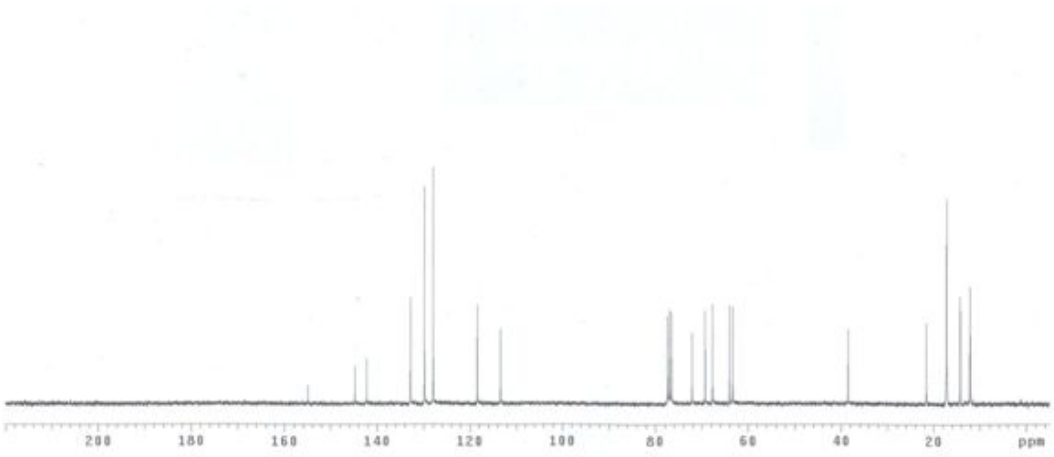
▼ **73, ^{13}C-NMR, 75 MHz**



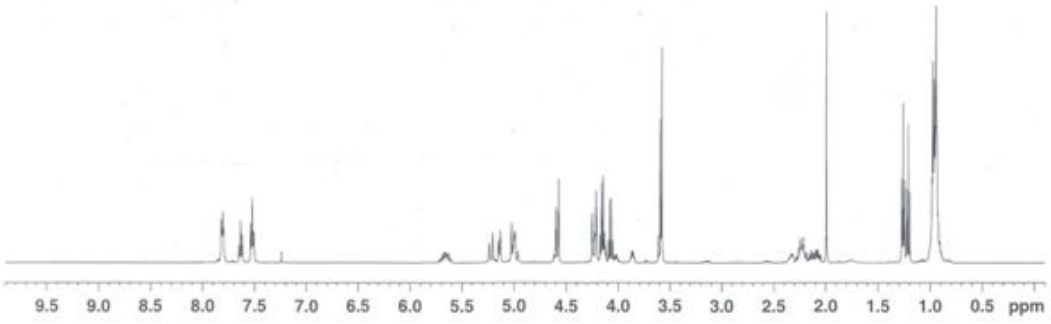
▼ **74, ^{1}H-NMR, 300 MHz**



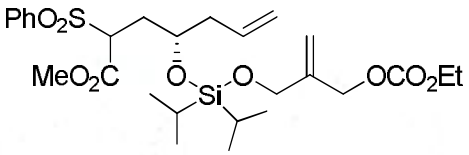
▼ **74, ^{13}C-NMR, 75 MHz**

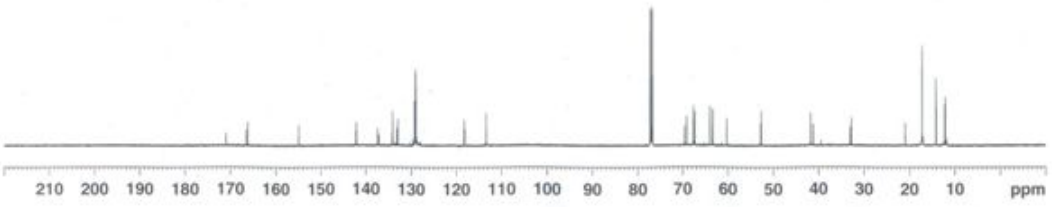


▼ **S74, ^{1}H-NMR, 500 MHz**

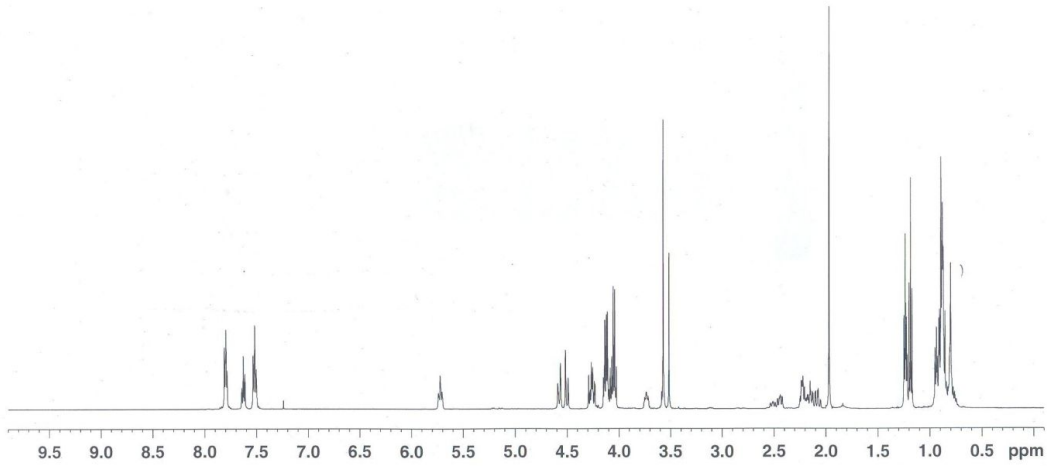


▼ **S74, ^{13}C-NMR, 125 MHz**

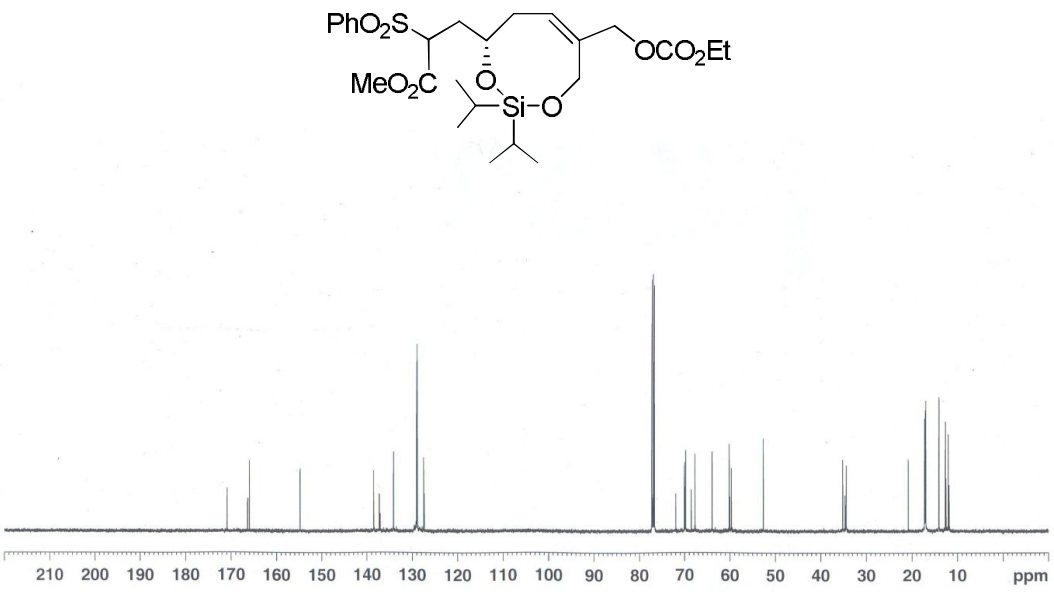




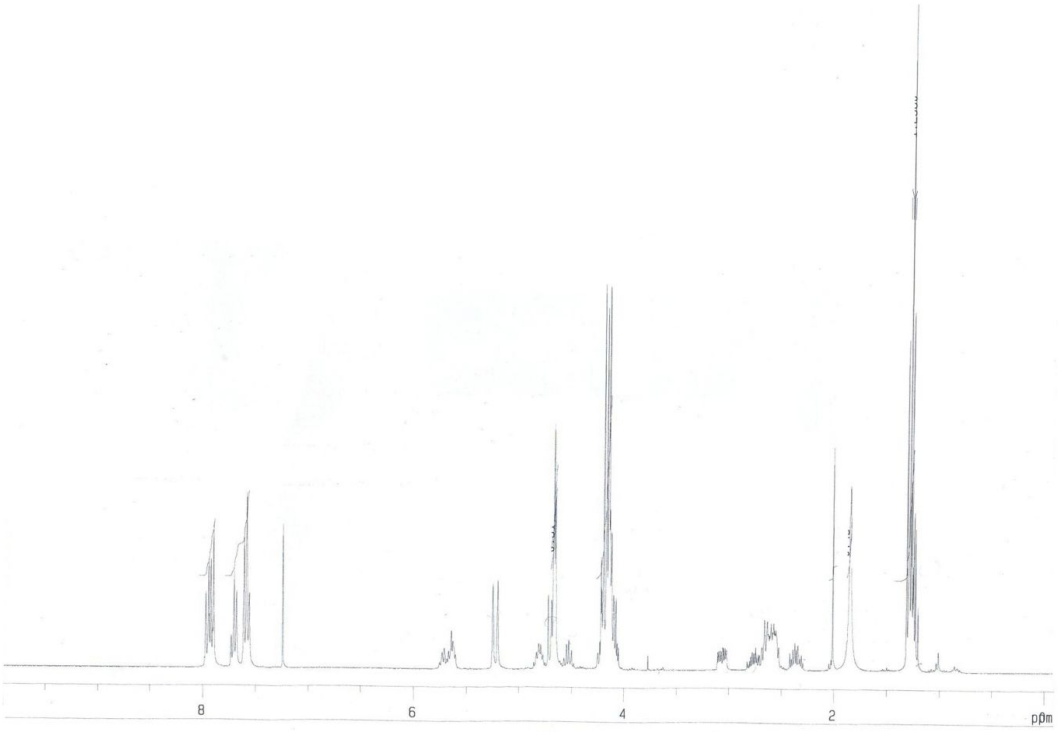
▼ **75, ^{1}H-NMR, 500 MHz**

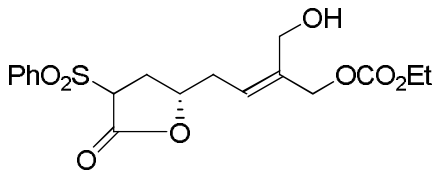


▼ **75, ^{13}C-NMR, 125 MHz**

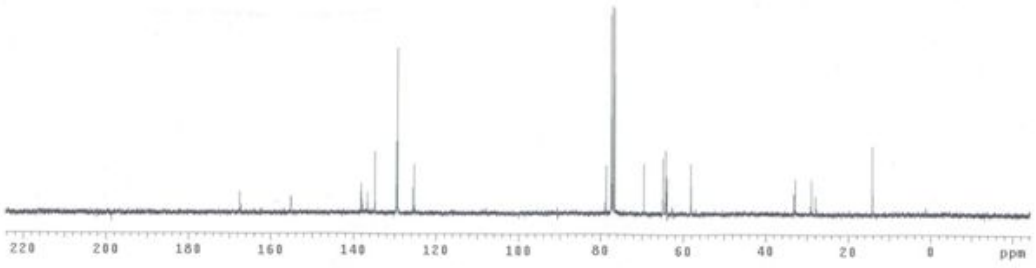


▼ **S75, ^{1}H-NMR, 300 MHz**

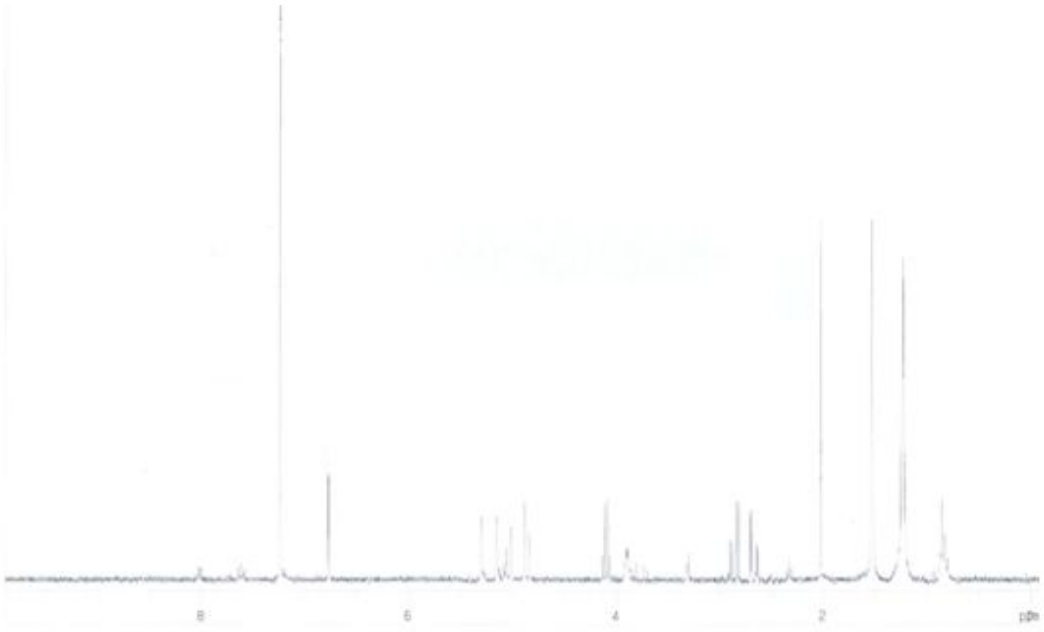




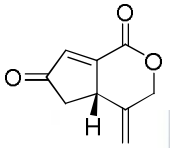
▼ **S75, ^{13}C-NMR, 75 MHz**

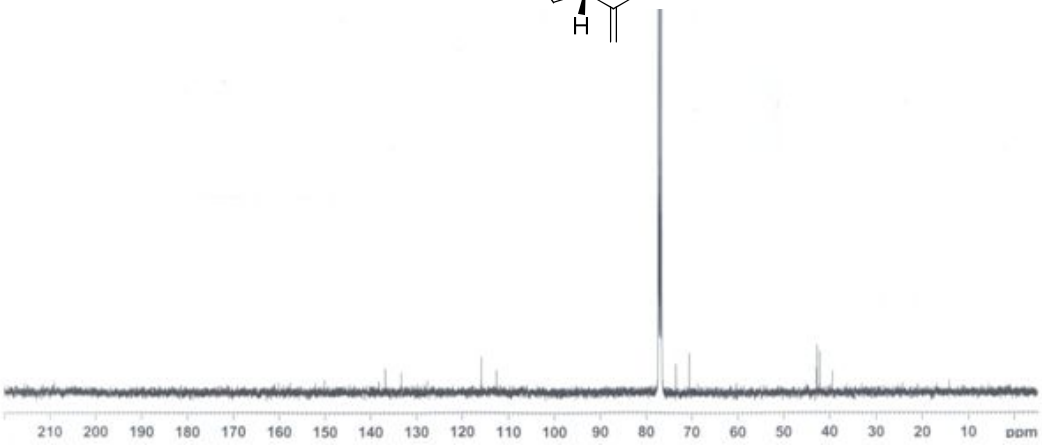


▼ **76, ^{1}H-NMR, 300 MHz**

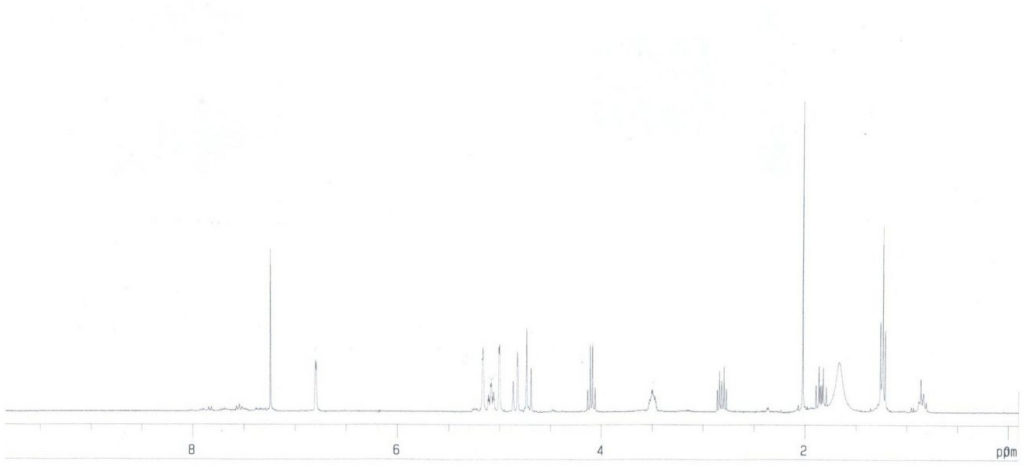


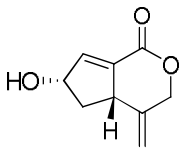
▼ **76, ^{13}C-NMR, 75 MHz**



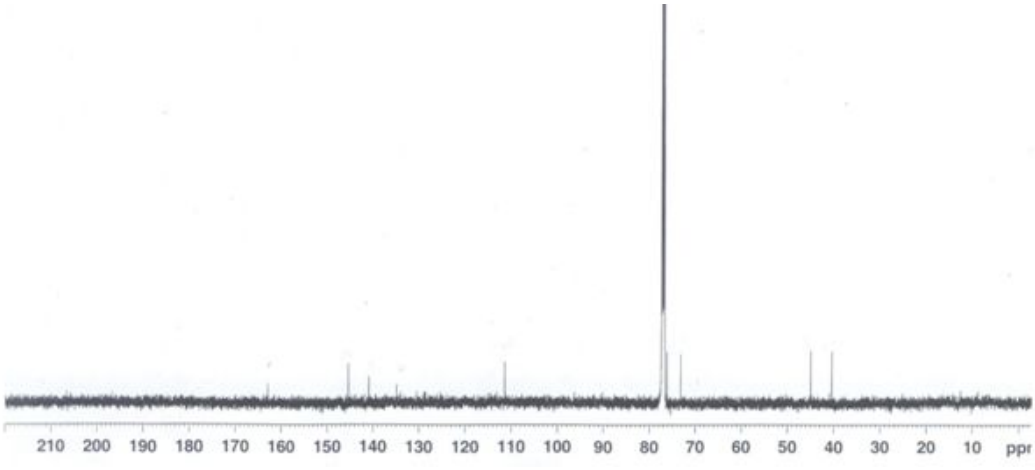


▼ **77, ^{1}H-NMR, 500 MHz**

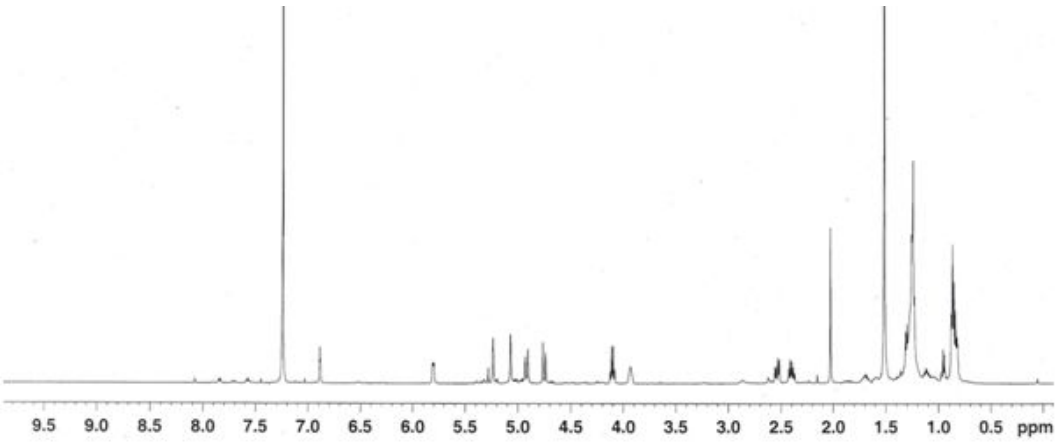


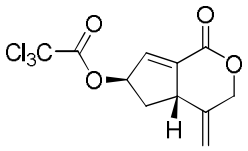


▼ **77, ^{13}C-NMR, 100 MHz**

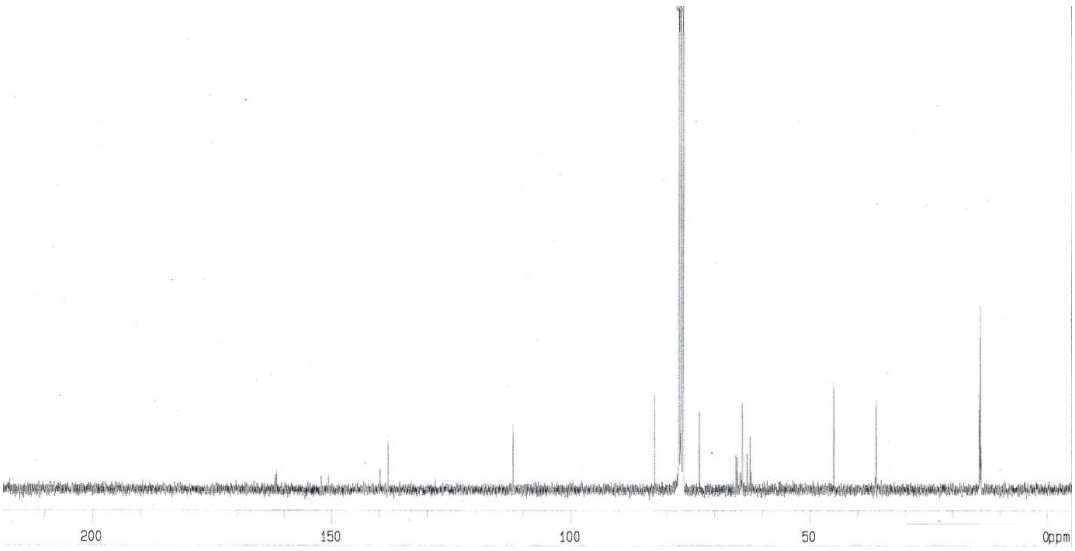


▼ **78, ^{1}H-NMR, 500 MHz**

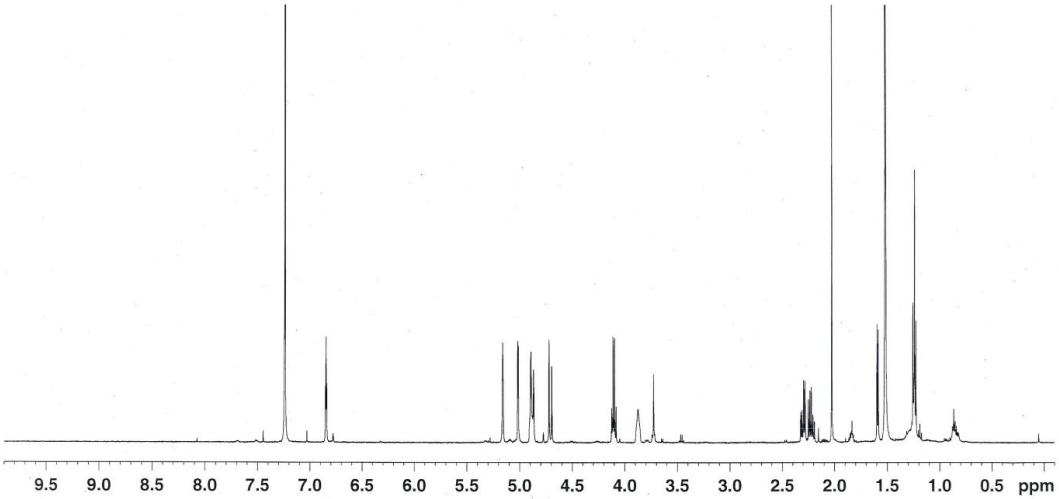




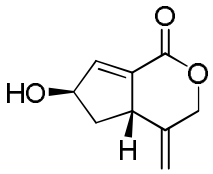
▼ **78, ^{13}C-NMR, 100 MHz**

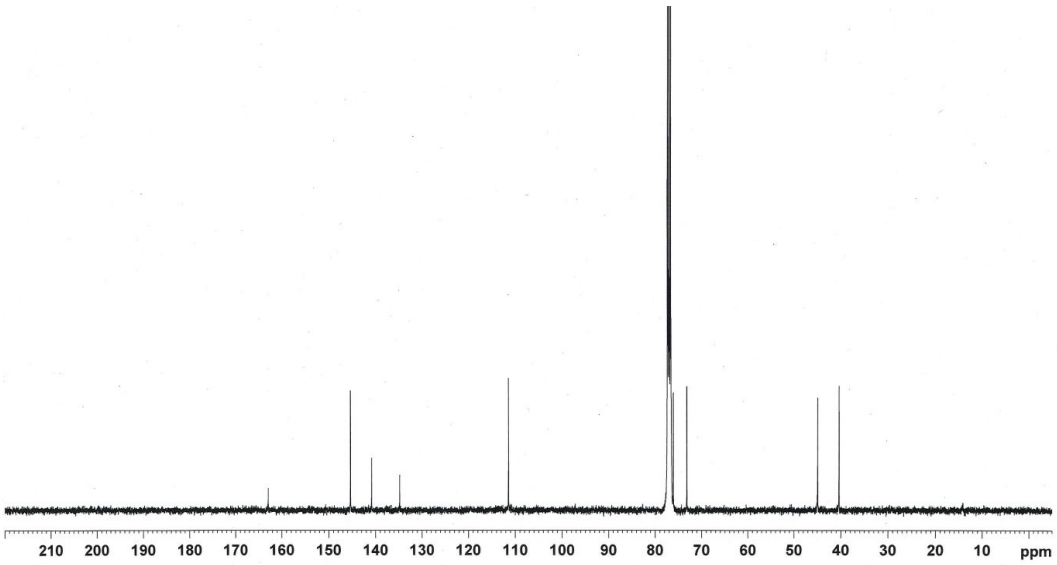


▼ **79, ^{1}H-NMR, 500 MHz**

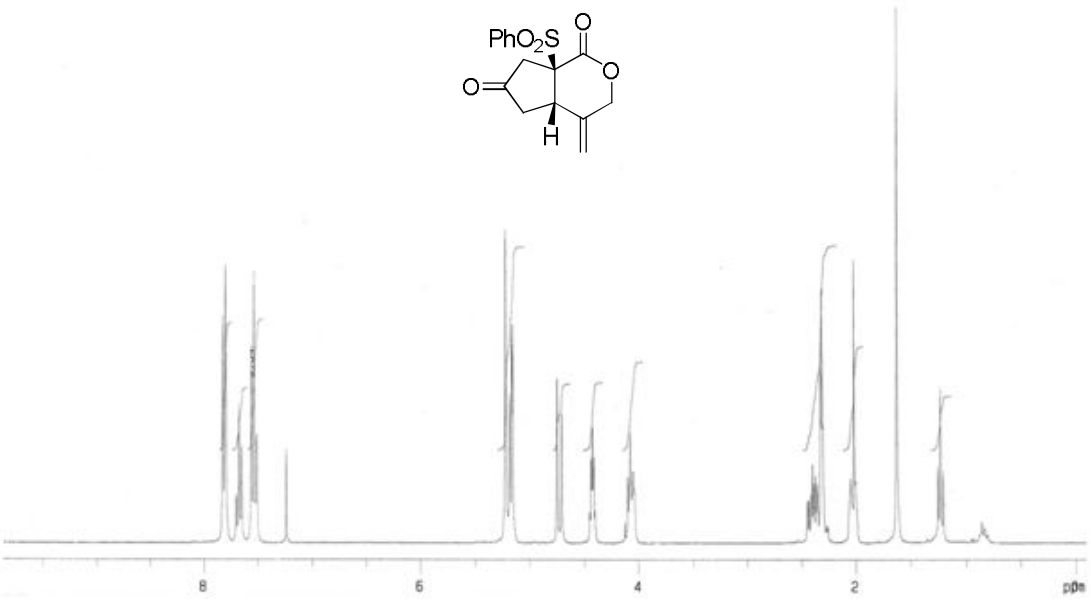


▼ **79, ^{13}C-NMR, 100 MHz**

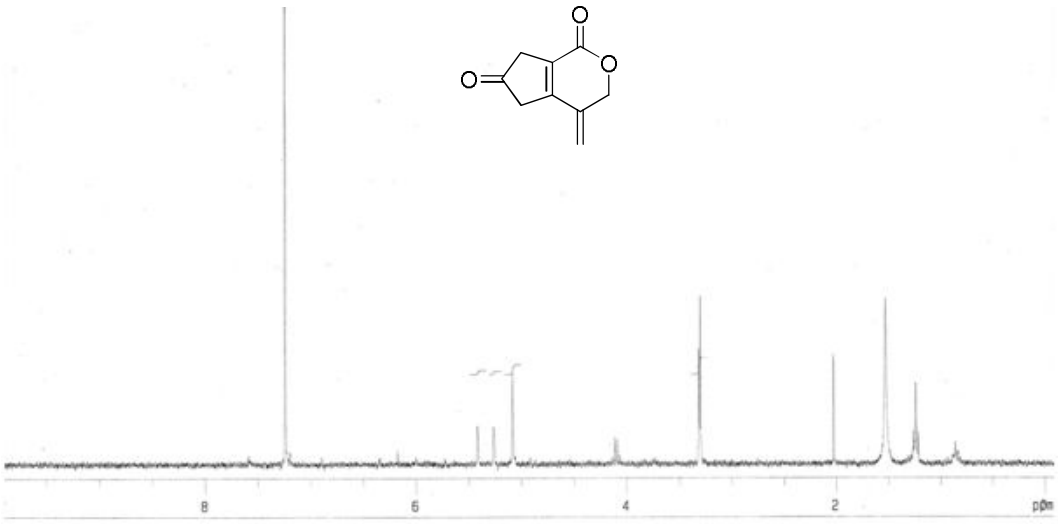




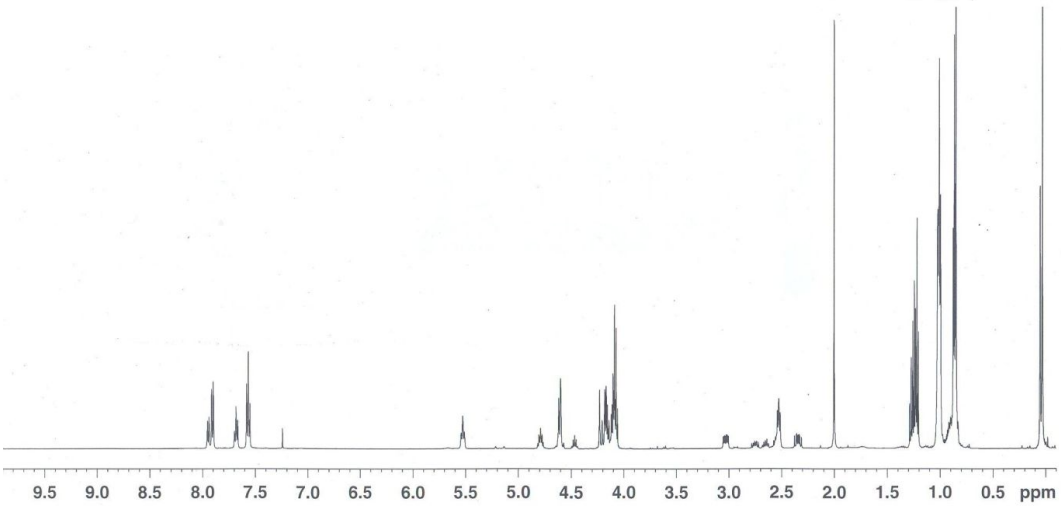
▼ **80, ^{1}H-NMR, 300 MHz**



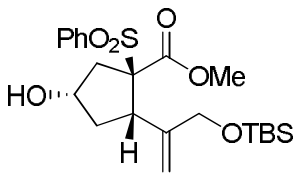
▼ **81, ^{1}H-NMR, 300 MHz**

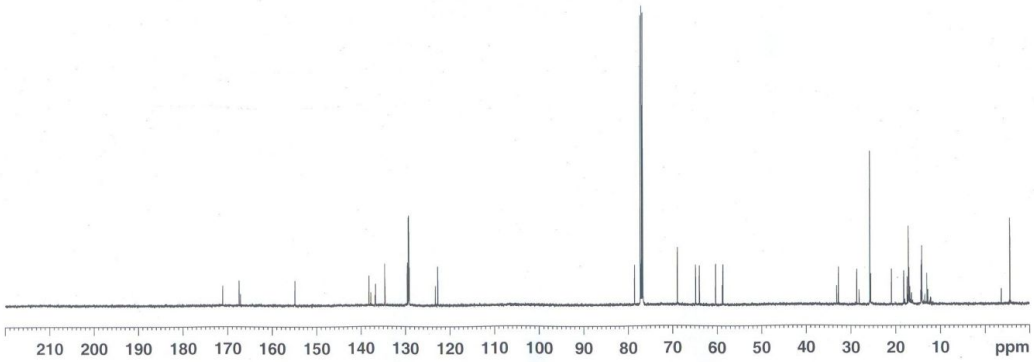


▼ **83, ^{1}H-NMR, 400 MHz**

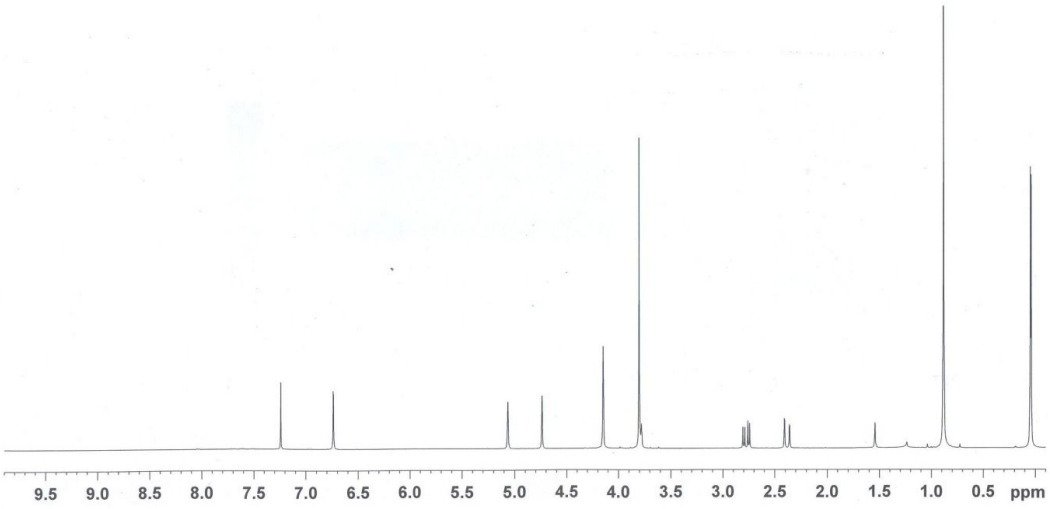


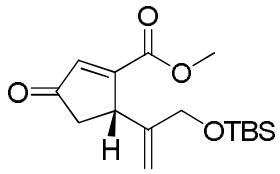
▼ **83, ^{13}C-NMR, 100 MHz**



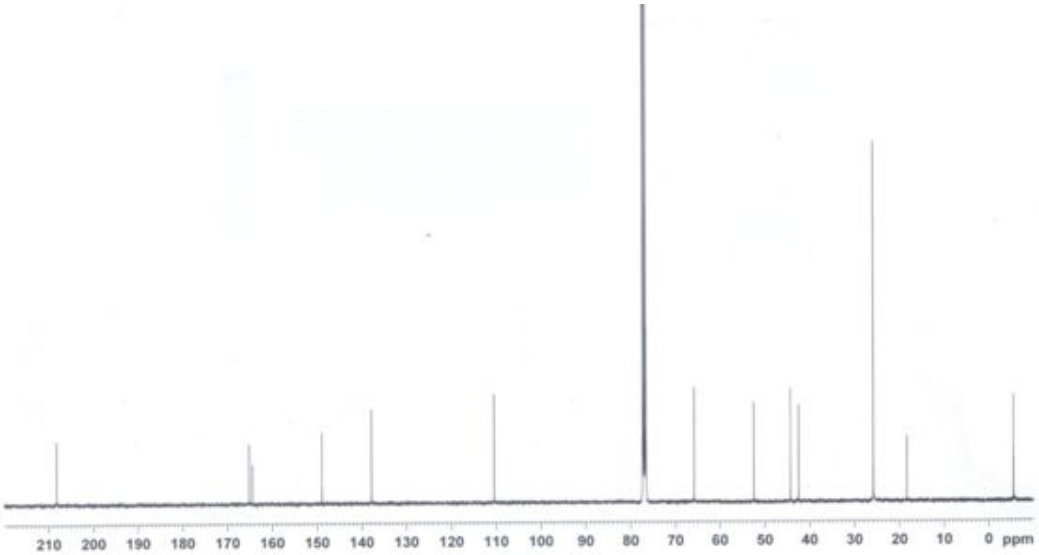


▼ **84, ^{1}H-NMR, 400 MHz**

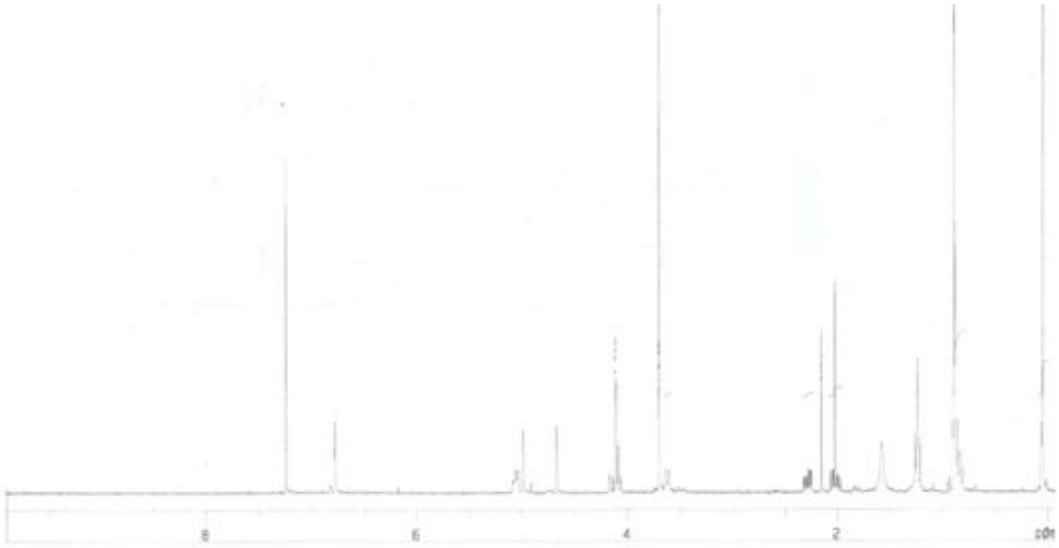




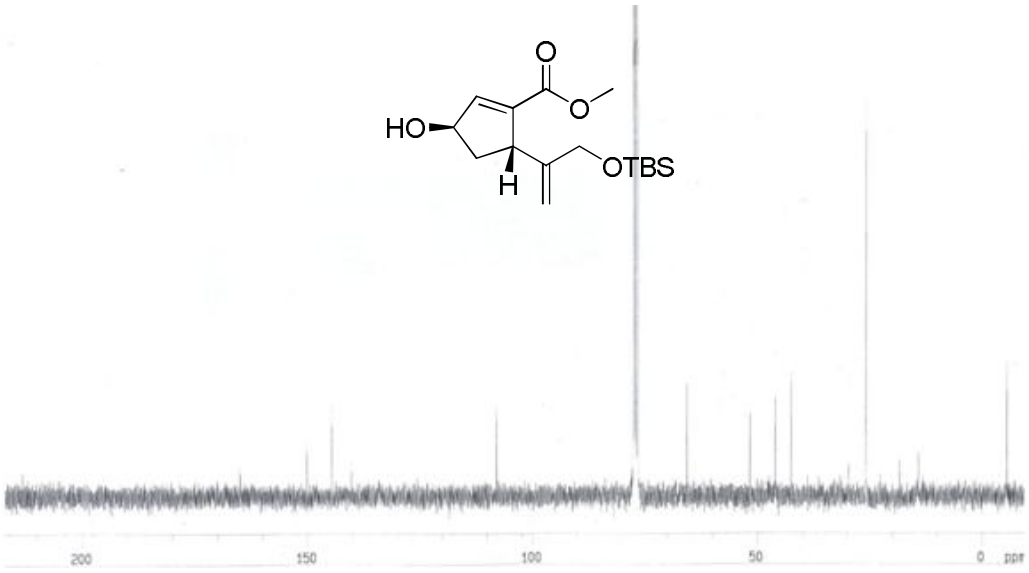
▼ **84, ^{13}C-NMR, 125 MHz**



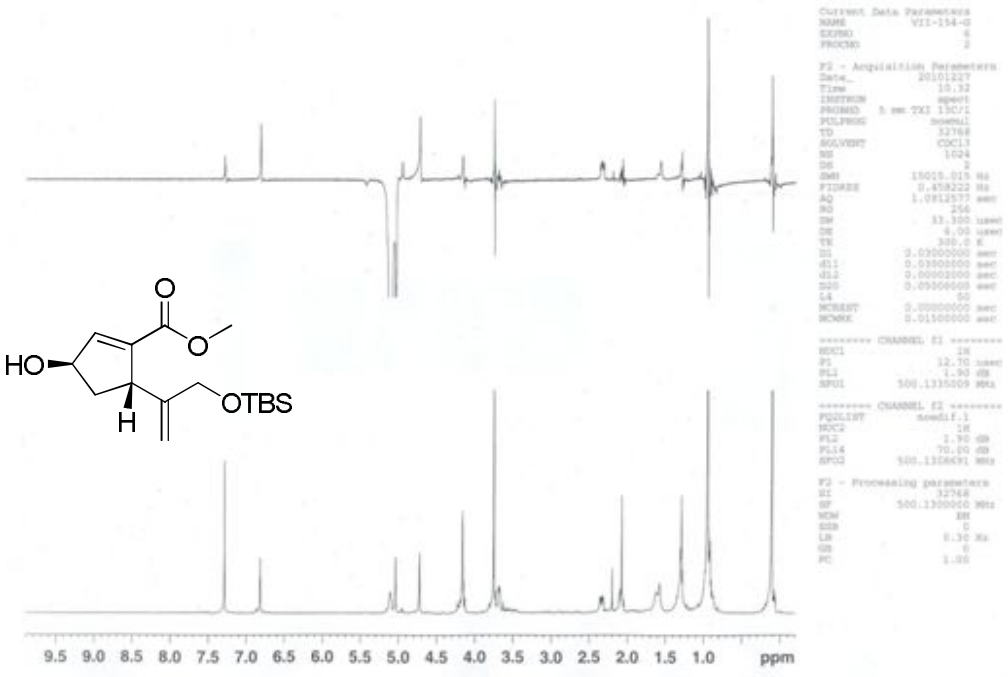
▼ **85, ^{1}H-NMR, 300 MHz**

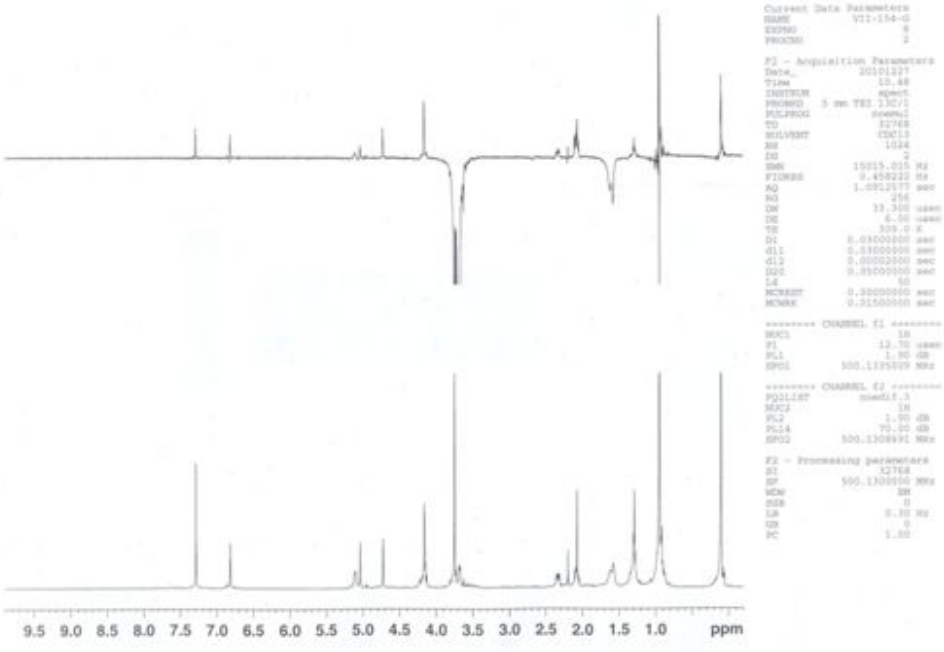


▼ **85, ^{13}C-NMR, 75 MHz**

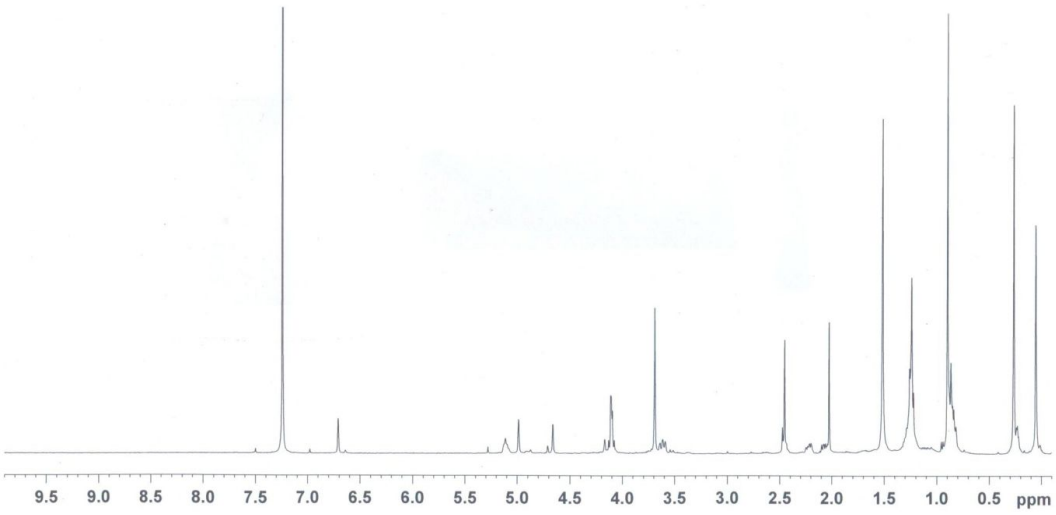


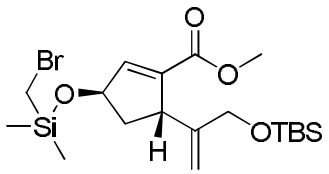
▼ **NOE Study of 85**



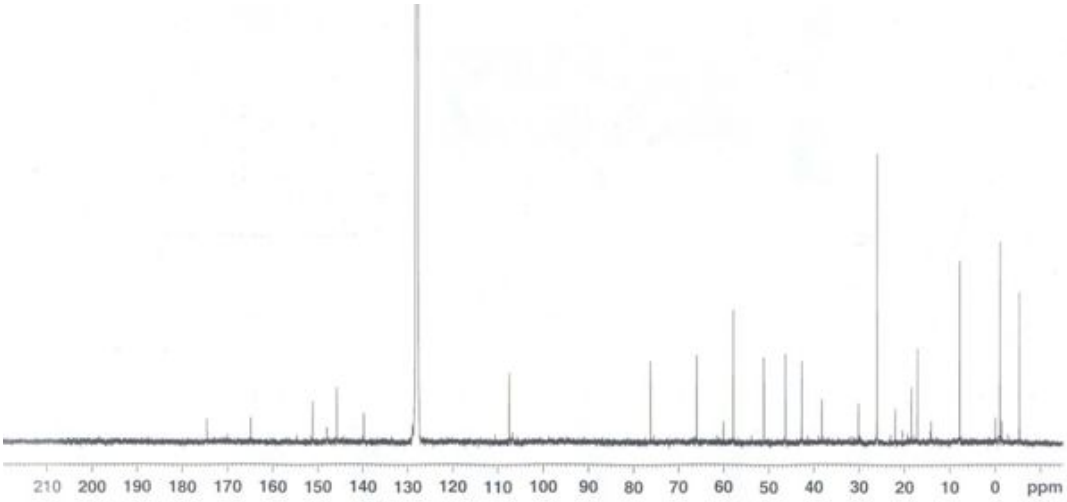


▼ **82, ^{1}H-NMR, 300 MHz**

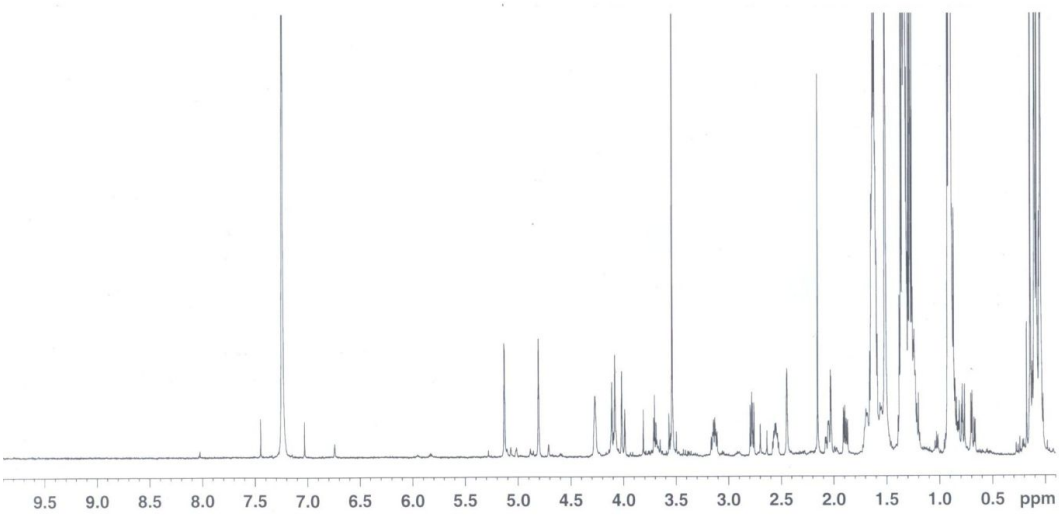




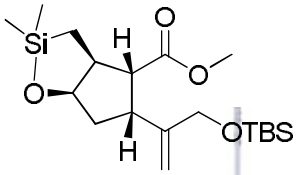
▼ **82, ^{13}C-NMR, 125 MHz**

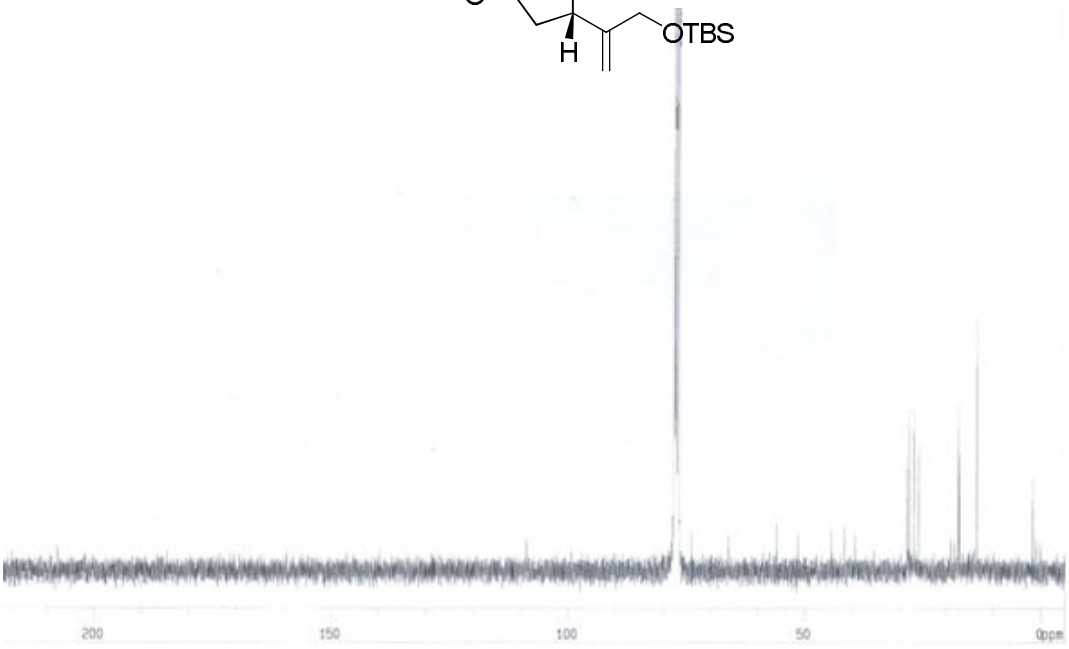


▼ **87, ^{1}H-NMR, 300 MHz**

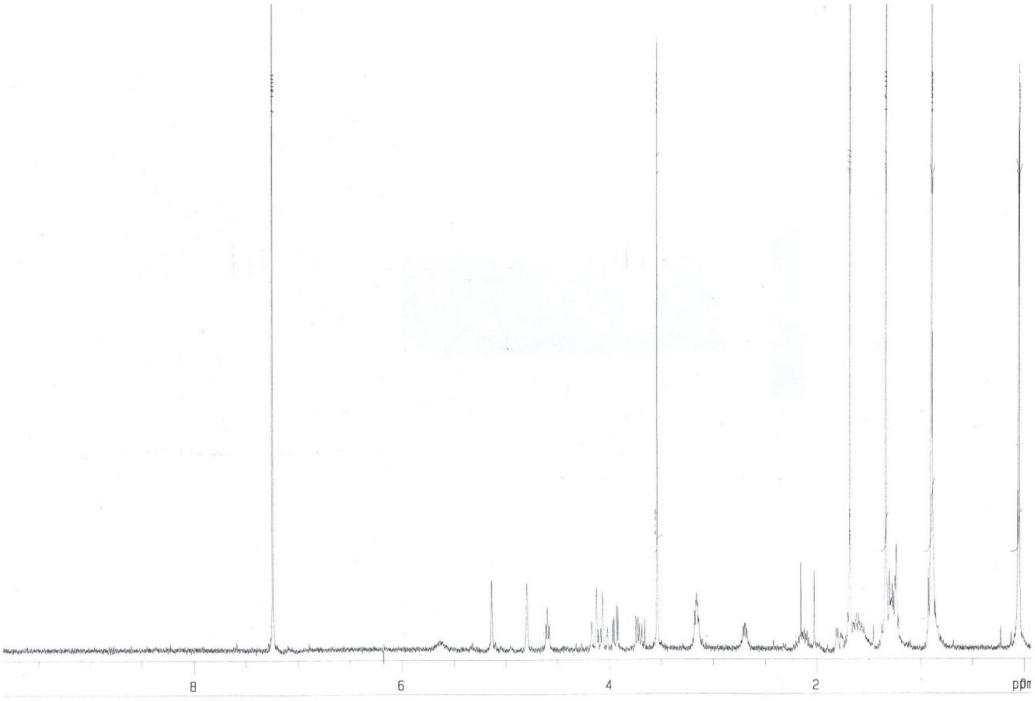


▼ **87, ^{13}C-NMR, 75 MHz**

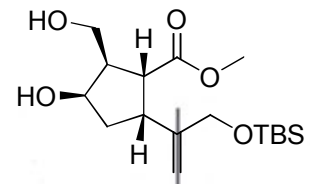


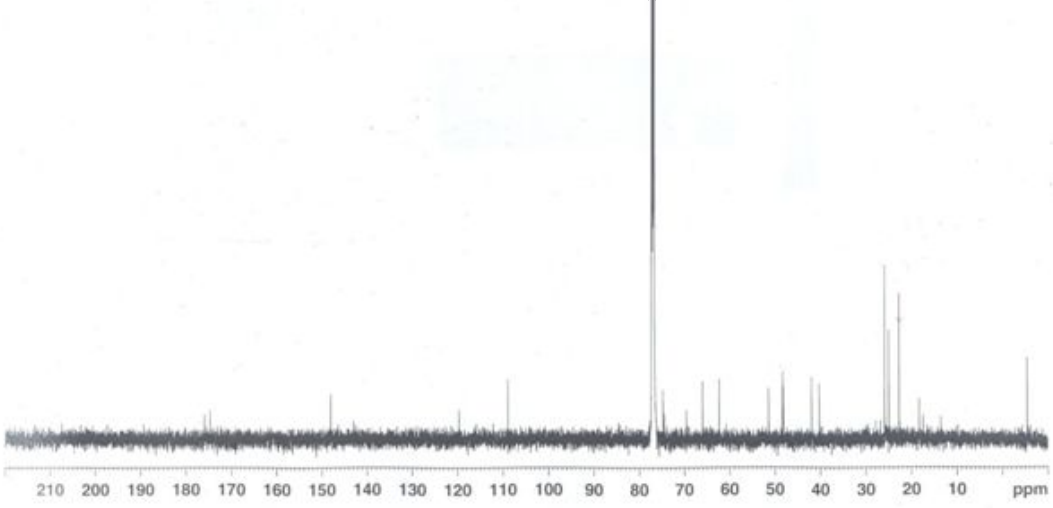


▼ **88, ^{1}H-NMR, 300 MHz**

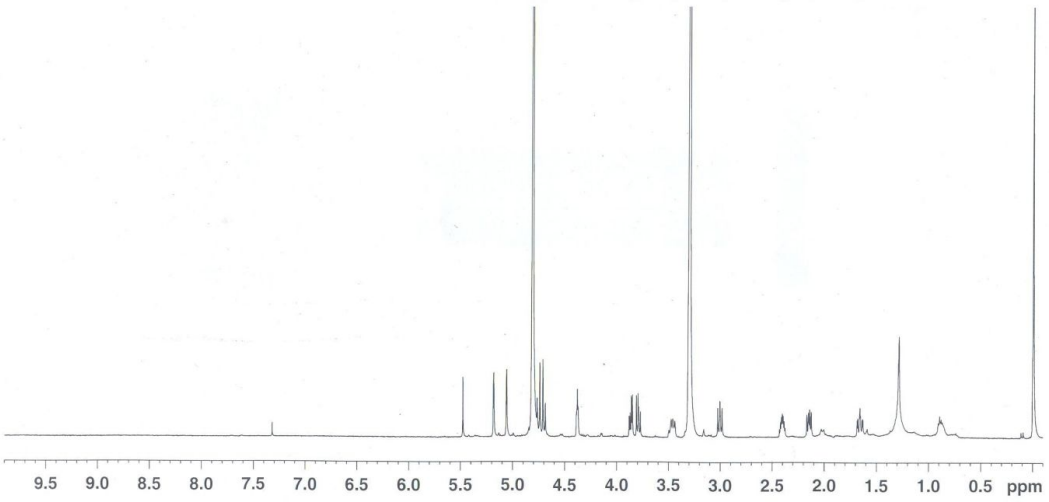


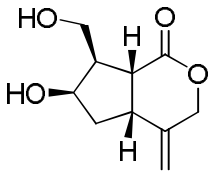
▼ **88, ^{13}C-NMR, 125 MHz**



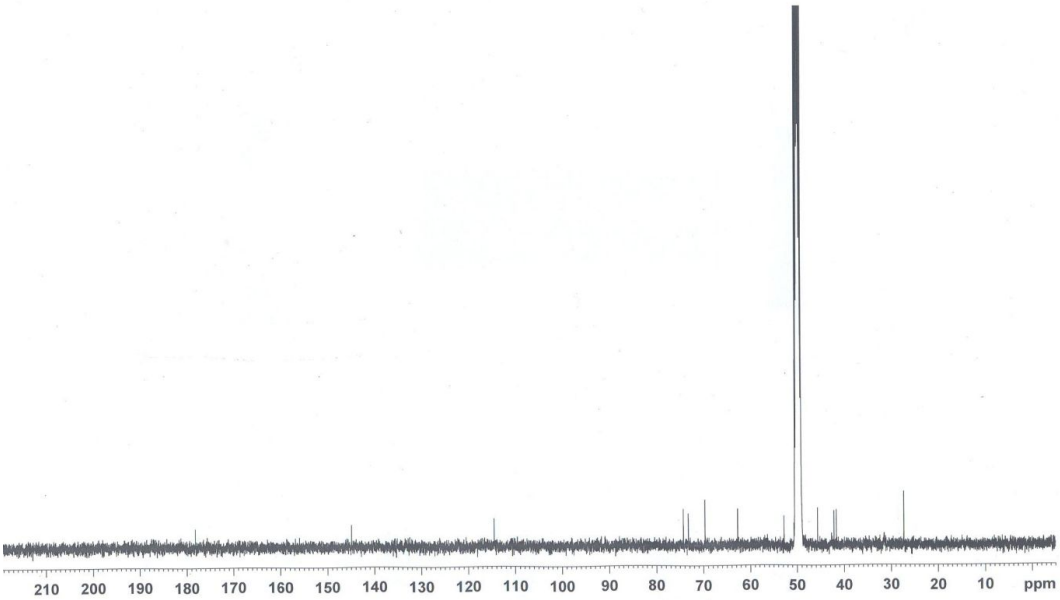


▼ **1, ^{1}H-NMR, 500 MHz**





▼ **1, ^{13}C-NMR, 125 MHz**



VII. Abstract

First total synthesis of natural iridoid lactone

Sujin Lee
College of pharmacy
Department of manufacturing pharmacy
The Graduate School
Seoul National University

Iridoids are a large family of natural monoterpenoids products, and structurally characterized by a *cis*-fused cyclopenta[*c*]pyran ring system. The unique *cis*-fused cyclopenta[*c*]pyran ring system has presented a variety of challenges for chemical synthesis and in the analysis of biological activities. In particular, we have been interested in a recently reported iridoid lactone, which is structurally related to loganin and consists of a C7-substituted C6-hydroxycyclopenta[*c*]lactone. Iridoid lactone, (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1 (3*H*)-one, was isolated from the roots and rhizomes of *Nardostachys chinensis* Batalin (Valerianaceae). This plant has been used as a Chinese folk medicine and its roots and rhizomes exhibit a variety of biological activities, such as sedative, antimalarial, antinociceptive, cytotoxic, and enhancement of nerve growth factor activities. Thus, the unique structural feature as well as the unknown biological activity of iridoid lactone led us to synthesize the recently reported iridoid lactone.

In this connection, we accomplished the first total synthesis of (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1 (3*H*)-one (**1**) in 13 steps. The key features of the synthesis involve the stereoselective and efficient preparation of the bicyclic lactone *via* Pd(0)-catalyzed cyclization, which is easily transformated to cyclopentanol intermediate and then the silicon-tethered radical cyclization followed by Tamao-Fleming oxidation for the

stereoselective introduction of the C7-hydroxymethyl substituent. This versatile synthetic approach is expected to be widely utilized for the synthesis of iridoids and iridoalkaloids.

Key words: (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one, iridoid lactone, iridoid, Pd(0)-catalyzed cyclization, bicyclic lactoen, silicon-tethered radical cyclization, Tamao-Fleming oxidation.

Student number: 2006-30449

Part II: Ginsenoside Rk1 구조 기반의 anti-apoptotic agent의 개발

Part II: Cholesterol derived novel anti-apoptosis agents on the structural basis of ginsenoside Rk1

Table of Contents

Part II: Cholesterol derived novel anti-apoptosis agents on the structural basis of ginsenoside Rk1

국문 초록 iii
List of Figures iv
List of Schemes v
Abbriviations vi
I. INTRODUCTION 1
1. Endothelial cells 1
2. Apoptosis 2
3. Ginsenoside 4
II. RESULT AND DISCUSSION 8
1. Preliminary study 8
2. Phytosterol screening 10
3. Synthesis of pseudosugar derivatives 16
4. Biological activity against serum deprived apoptosis 19
1) MTT assay on HUVECs 19
2) DAPI/ TUNEL assay 23
III. Conclusion 26
IV. Experimental 27
V. References 42
VI. Appendix 47
VII. Abstract 64

국문 초록

혈관 내벽은 혈관내피세포의 단일막으로 이루어져 있다. 혈관 내피세포의 항상성 유지가 혈관의 생명활동을 유지하는 중요한 요인이다. 혈관내피세포의 세포사멸에 의한 혈관의 구조변화와 기능저하가 동맥경화, 동종이식편에 의한 맥관장애, 울혈성 심부전 등의 여러 질병과 연관된다는 연구결과가 보고되고 있다. 따라서 혈관내피세포의 세포사멸 억제제가 혈관질환의 새로운 치료제로서 연구 가치가 있으리라 생각된다.

최근, 인삼으로부터 분리 보고된 ginsenoside Rg3, Rk1이 세포사멸 억제효과가 있는 것으로 발표되었다. 하지만 이들 ginsenoside들은 다당체를 함유하고 있어 산성 조건에서 화학적으로 불안정하고, 자연에서 얻기에는 양적인 면에서 한계가 있기 때문에 이들 구조를 기반으로 하여 화학적으로 비교적 안정하면서 세포사멸 억제효과를 갖는 유도체들의 합성을 계획하였다. Ginsenoside의 뼈대인 스테로이드 골격을 유지하면서 ginsenoside의 활성을 유지할 수 있는 여러 phytosterol을 검색하였고, C3 hydroxyl기에 활성에 중요한 역할을 하는 glucoside를 대체할 bioisoster를 도입하고자 하였다. 이를 통해 상업적으로 손쉽게 구입 가능한 콜레스테롤을 기반으로 하여 다양한 유도체를 합성하였고, 이들의 세포사멸억제 효과를 확인하였다.

주요어; 혈관내피세포, 세포사멸, 동맥경화, 동종이식편에 의한 맥관장애, 울혈성 심부전 ginsenoside Rg3, Rk1, phytosterol, 콜레스테롤, 세포사멸 억제효과

학 번: 2006-30449

List of Figures

Figure 1. Schematic structure of blood vessel (a) and tight junction (b) 1
Figure 2. Schematic representation of apoptosis and necrosis 3
Figure 3. Small molecules inhibiting apoptosis of endothelial cells.....4
Figure 4. Structures of ginsenosides.....6
Figure 5. (20*S*)Rg3 protects endothelial cells from serum-deprived apoptosis 8
Figure 6. MTT assay result for processed Ginseng extraction 9
Figure 7. Synthetic approach for ginsenoside derivatives 10
Figure 8. Structures of phytosterols.....11
Figure 9. MTT assay on HUVECs 14
Figure 10. Morphology of HUVEC.....15
Figure 11. Effect of compounds on apoptosis of HUVECs: 5 μg/ml treatment 19
Figure 12. Effect of compounds on apoptosis of HUVECs: 10 μg/ml treatment.....20
Figure 13. Morphological changes of HUVEC 21
Figure 14. Inhibition of compounds 7, and 9 on apoptosis of HRECs.....22
Figure 15. Morphological changes of HUVEC 22
Figure 16. Changes in nuclear morphology accessed by DAPI staining.....24
Figure 17. TUNEL assays at various concentrations of 7 and 9 24

List of Schemes

Scheme 1. Synthesis of stigmasterol derivatives **11**
Scheme 2. Synthesis of β-sitosterol derivatives **12**
Scheme 3. Synthesis of sitostanol derivatives **13**
Scheme 4. Synthesis of cholesterol derivatives **13**
Scheme 5. Synthesis of cholestanol derivatives **14**
Scheme 6. Synthesis of dihydropyran derivatives **16**
Scheme 7. Synthesis of acyclic analogs **17**
Scheme 8. Synthesis of bulky analogs **18**

Abbriviations

BrdUTP: 5-bromo-2'-deoxyuridine 5'-triphosphate

bs: broad singlet

t-Bu: *tert*-butyl

calcd: calculated

CHF: congestive heart failure

CNS: central nervous system

d: doublet

DAPI: 4'-6-diamino-2-phenylindole

DHP: 3,4-dihydro-*2H*-pyran

DMF: *N,N*-dimethylformamide

EC: endothelial cell

FAB: fast atom bombardment

HREC: human retina endothelial cell

HRMS: high resolution mass spectroscopy

HUVEC: human umbilical vein endothelial cell

IR: infrared

LRMS: low resolution mass spectroscopy

m: multiplet

Me: methyl

MeOH: methyl alcohol

NMR: nuclear magnetic resonance

P. ginseng: Panax ginseng

Ph: phenyl

PPD: protopanaxadiols

PPT: protopanaxatriols

PPTS: pyridinium *p*-toluene sulfonate

PTSA: *p*-toluene sulfonic acid

q: quartet

s: singlet

SAR: structure activity relationship

t: triplet

TdT: terminal deoxynucleotidyl transferase

THF: tetrahydrofuran

TLC: thin layer chromatography

TUNEL: Terminal Transferase dUTP Nick End Labeling

F-dUTP: fluorescent-tagged deoxyuridine triphosphate nucleotides

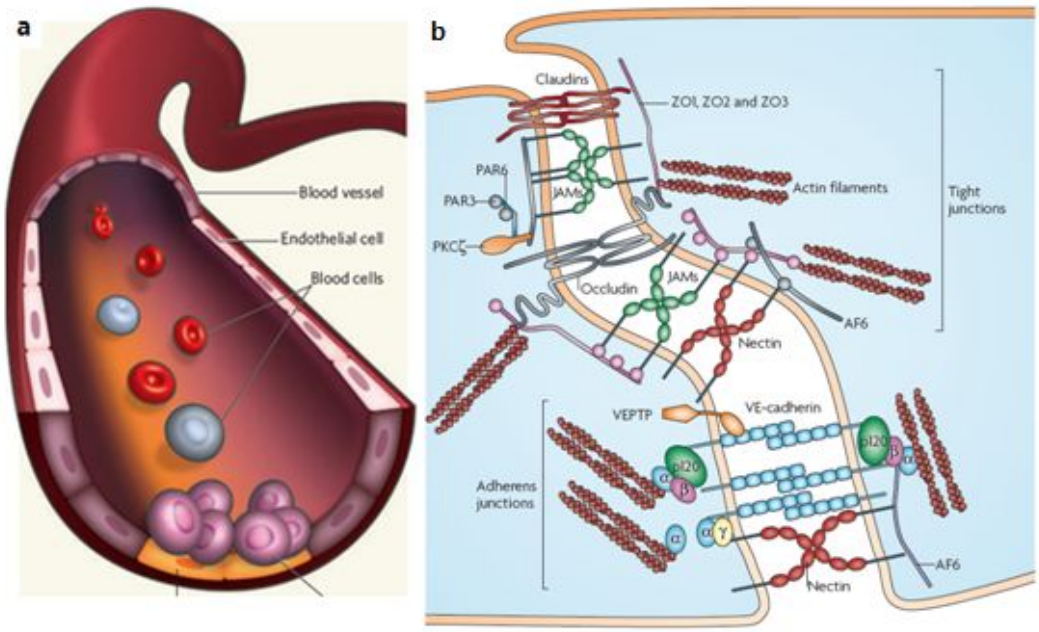
VEC: vascular endothelial cell

I. INTRODUCTION

1. Endothelial cells

The blood vessel has a monolayer of endothelial cell (EC) lining (Figure 1a) [1] located at the interface between the vascular and perivascular compartments. In this location, the ECs have two different roles. First, they establish the communication between circulating blood and underlying tissues. The ECs behave as a permeable filter, allowing the selective exchange of solutes and water between the luminal and abluminal sides of the barrier. Permeability follows two distinct pathways. The transcellular route crosses apical and basal cell membranes and is mostly mediated by channels, carriers, pumps, and vesicles. In contrast, the paracellular route passes through intercellular and lateral spaces between contacting cells and is mediated by the tight junction (Figure 1b). [2] Tight junctions are composed of integral membrane proteins (e.g. occludin, claudins, and junctional adhesion molecules [JAMs]) and intracellular proteins (e.g. ZO-1 and cingulin). [3]

Figure 1. Schematic structure of blood vessel (a) and tight junction (b)



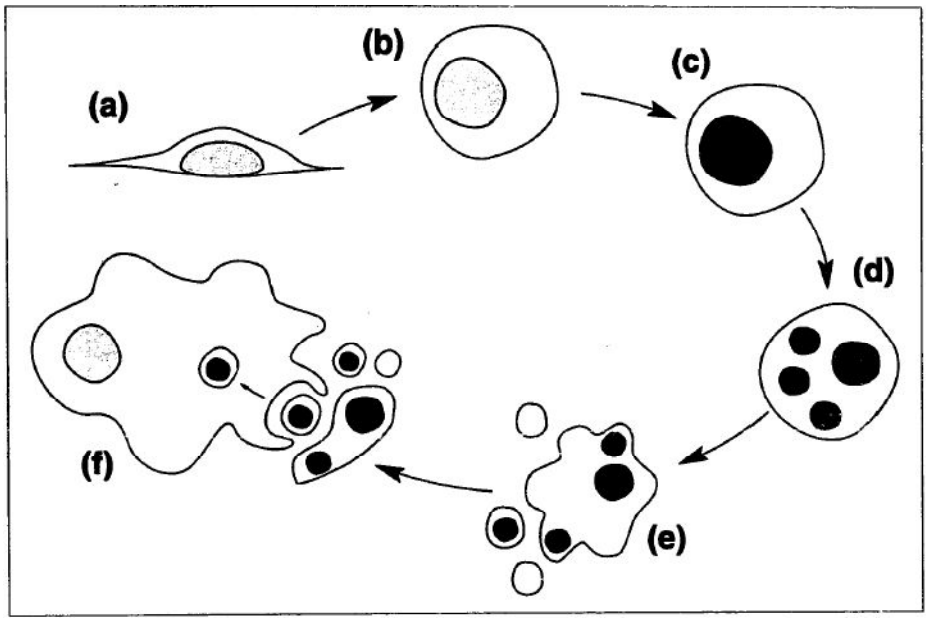
Second, ECs act as a barrier between the vascular and extravascular compartments, thus maintaining tissue homeostasis and preventing thrombosis. The location of ECs ensures their simultaneous and constant exposure to a wide variety of stimuli, many of which have the potential to induce or prevent apoptosis of the cells. It has been suggested that disturbed endothelial function could account for a large portion of all cardiovascular diseases,[4] such as atherosclerosis, allograft vasculopathy, hypertension, congestive heart failure (CHF), and other diseases (e.g. systemic sclerosis, diabetes, and thrombotic thrombocytopenic purpura). [5]

2. Apoptosis

There are two major types of cell death: necrosis and apoptosis. Necrosis occurs following diverse traumatisms including hyperthermia, ischemia, and physical injury or appears as a result of the exposure to cytotoxic cytokines or substances. Apoptosis, on the other hand, is a kind of programmed cell death. It has recently gained considerable interest because it has been appreciated as a regulatory mechanism in organ growth, wound repair, tumor genesis, and immune response.

Apoptosis is a normal occurrence in which an orchestrated sequence of events leads to the cell death. As represented schematically in Figure 2,[6] death by apoptosis is a neat, orderly process characterized by the overall shrinkage in volume of the cell and its nucleus, the loss of adhesion to neighboring cells, the formation of blebs at the cell surface, the dissection of the chromatin into small fragments, and the rapid engulfment by phagocytosis. Necrotic cells lyse and release their intracellular components, including DNA and RNA, into the extracellular space. Waste-management nucleases digest these extracellular nucleic acids, and inflammatory reactions occur. Apoptosis, however, is characterized by distinct morphological changes. Membrane fusion early in apoptosis causes fluid loss and therefore cell shrinkage. Chromatin condenses and is deposited on the inner surface of the nuclear membrane. Macromolecular synthesis decreases and usually cellular DNA is cleaved at internucleosomal sites, resulting in fragmentation of DNA.

Figure 2. Schematic representation of apoptosis and necrosis



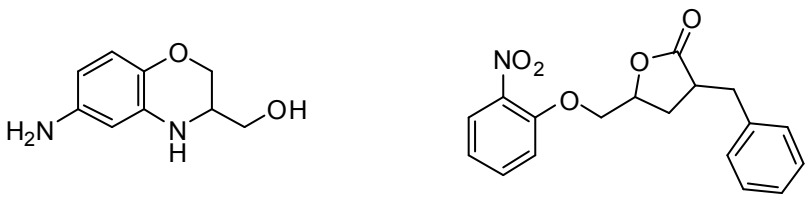
Upon receipt of a signal to undergo apoptosis, an adherent cell (a) rounds up (b) and rapidly condenses its DNA (c), thought to be a consequence of DNA fragmentation. This is rapidly followed by separation of the nucleus into discrete masses of condensed chromatin (d) and, finally, fragmentation of the cell into several membrane-bound vesicles (apoptotic bodies) (e), which are rapidly recognized and phagocytosed by macrophages or neighbouring cells (f).

This DNA is eventually released from cells in membrane-bound vesicles, and the vesicles are eliminated by phagocytic cells. Since intracellular contents are not released to interstitial fluid, inflammation does not occur. [7]

It is an active process of cell death that occurs in many important physiological conditions, such as embryonic development and tissue remodeling. Apoptosis is involved during angiogenesis in de novo tissue formation and wound healing. Moreover, many diseases are associated with either too much or too little apoptosis, including cancer, sepsis, autoimmunity, and neurodegenerative diseases. [8] In particular, the endothelium is a critical site for the control of apoptosis during processes such as inflammation, vascular remodeling, and angiogenesis. Although the suppression of EC apoptosis is required for the maintenance of blood vessel integrity and for angiogenesis, the

activation of apoptosis has been suggested to contribute to pathological tissue destruction during vascular injury, inflammation, atherosclerosis, and allograft arteriopathy. [9] Therefore, the inhibition of vascular endothelial cell (VEC) apoptosis is considered as a new target for diverse cardiovascular diseases. EC perturbing agents such as inflammatory cytokines, lipopolysaccharides, reactive oxygen species, and oxidized low-density lipoprotein (LDL) are known to induce EC apoptosis. [10]

Figure 3. Small molecules inhibiting apoptosis of endothelial cells



ABO 3BDO

Considering these preliminary reports, the inhibition of VEC apoptosis has been considered as a novel approach for the treatment of various physiological disorders such as cardiovascular disease. A few reports have addressed the development of anti-apoptotic agents from natural products [11] to small molecules.[12] Miao et al. discovered lead compounds for anti-apoptotic activity on human umbilical vein endothelial cells (HUVECs) through the library screening (Figure 3). These molecules inhibit against oxidized LDL-induced apoptosis and increase intergrin β4, NF-kB, and P53 in HUVECs.

3. Ginsenoside

Ginseng (*Panax ginseng* C. A. Meyer, Araliaceae) is one of the most popular herbal medicines in the Orient. [13] It has been used for over 2000 years in the belief that it is a panacea and promotes longevity. Recently, there has been a renewed interest in investigating ginseng pharmacology using

biochemical and molecular biological techniques. The pharmacological effects of ginseng have been demonstrated in the central nervous system (CNS) [14] and in cardiovascular, endocrine,[15] and immune systems.[16] In addition, ginseng and its constituents have been ascribed anti-neoplastic, anti-stress, and antioxidant activities. [17]

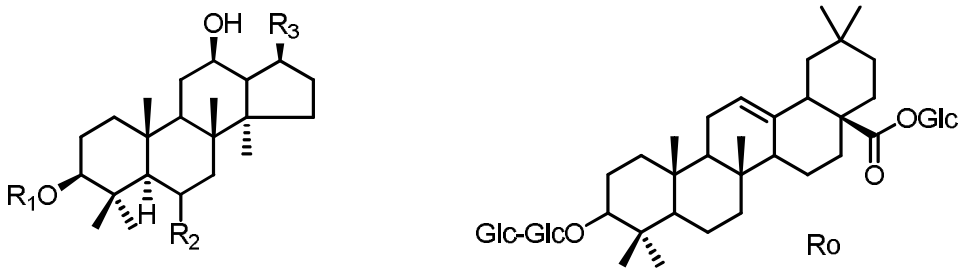
The major components found in most ginseng spices are ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids. Among those components, the most pharmacological active components are ginsenosides.[18] Ginsenosides are a family of steroids named steroidal saponins; they have also been named ginsenoside saponins, triterpenoid saponins, or dammarane derivatives under previous classifications. Ginsenosides possess a triterpene structure as a backbone and have a structural diversity.

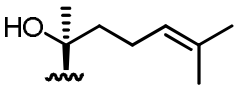
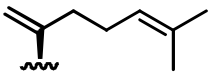
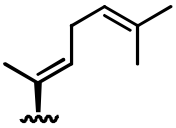
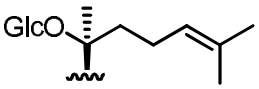
Structurally, ginsenosides possess the four successive trans-fused ring steroidal skeletons with a modified side chain at C20, and β-OH at C3, which is usually attached to sugar residue such as glucose, rhamnose, xylose, and arabinose. Based on the number of hydroxyl groups on their dammarane type triterpene, ginsenosides are generally divided into three groups: protopanaxadiols (PPDs), protopanaxatriols (PPTs), and oleanane type. The representative structures of ginsenosides are shown in Figure 4. Rb1, Rb2, Rc, and Rd are examples of PPDs, which have two hydroxyl groups at C3 and C20. Re, Rf, Rg1, and Rg2 are examples of PPTs, which have three hydroxyl groups at C3, C6, and C20. The oleanane type ginsenoside is only one ginsenoside, Ro (Figure 4). [19]

Ginseng is widely used in traditional Chinese medicine and Western herbal preparations, numerous researches to evaluate the biological activities of ginsenosides have been undertaken. Although the extracts from fresh or processed *P. ginseng* have been extensively tested for their pharmacological effects, the precise biological functions and underlying action mechanisms of pure molecules from ginseng extract are still largely unknown. Some literature reports suggest that ginseng extract has both wound-healing and anti-tumor effects through a reciprocal role in the

vascular system.[20]

Figure 4. Structures of ginsenosides



ginsenoside	R_1	R_2	R_3
Rg3 (PPD)	Glc-Glc-	H_2	
Rk1 (PPD)	Glc-Glc-	H_2	
Rg5 (PPD)	Glc-Glc-	H_2	
Rg1 (PPT)	H	OGlc	

Ginsenosides Rg1 and Rb1 have been demonstrated to have opposing activities in the vasculature. Rg1 promotes functional neovascularization in vivo and proliferation, chemoinvasion, and tubulogenesis of ECs in vitro, whereas Rb1 suppresses the earliest step in angiogenesis by inhibiting the chemoinvasion of ECs.[21] These results suggest that ginsenosides are structurally related but functionally different, depending on the side chain moieties attached to the triterpene backbone. In particular, (3β,12β)-12,20-Dihydroxydammar-24-en-3-yl 2-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranoside (ginsenoside Rg3) exerts many pharmacological activities such as anti-tumor, anti-inflammatory, neuroprotection against the cerebral ischemia-induced injury, and a

vasodilation effect.[22] Interestingly, ginsenoside Rg3 has been recently revealed to have anti-apoptotic activity via the inhibition of a mitochondrial caspase pathway.[23] Moreover, 3β,12β-dihydroxydammar-20(21),24-diene-3-*O*-β-*D*-glucopyrano-syl(12)-β-*D*-gluco-pyranoside (ginsenoside Rk1), isolated from heat-processed ginseng (Sun Ginseng),[24] has more effective anti-apoptotic activity on ECs than Rg3. Considering these reports, we focused on the development of anti-apoptotic agents on HUVECs based on the structure of both ginsenosides. However, ginsenosides are generally labile under acidic conditions, and their composition is variable depending on the process of extraction. Thus, it is difficult to determine the precise biological functions and underlying mechanisms.[25]

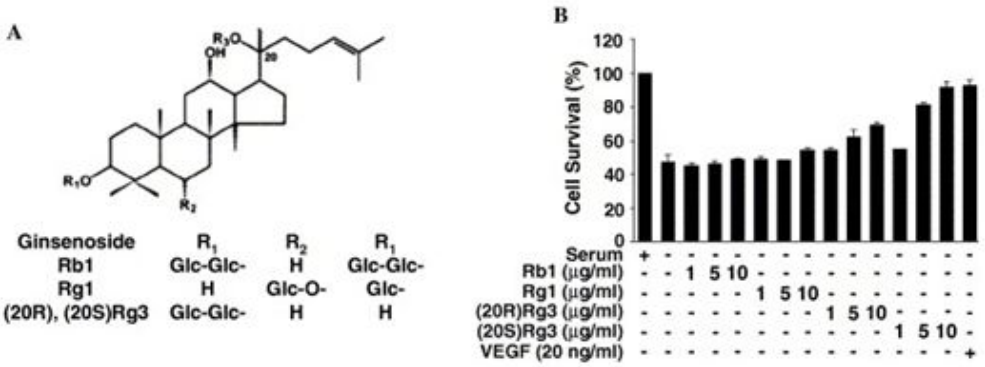
To date, most research has focused on the activities of natural ginsenosides, and few studies have reported on the structure activity relationship with ginsenoside modification or derivatives synthesis.[26] On the basis of these preliminary studies, we planned to investigate a preliminary structure-activity relationship (SAR) study of ginsenosides Rg3 and Rk1 on the anti-apoptotic effect. At first, we designed and synthesized diverse phytosterol analogs for the elucidation of the SAR.

II. RESULT AND DISCUSSION

1. Preliminary study

As recently reported,[23] some ginsenosides were treated on apoptosis-induced human ECs by serum deprivation[10] (Figure 5). Interestingly, the data showed that both Rg1 and Rb1 have no significant effect on EC survival up to the concentration 10 μg/ml, whereas Rg3 has a significant activity at the same concentration. Moreover, ginsenoside Rg3 epimers also exhibited differential effects. (20*S*)Rg3 promoted EC survival to an extent comparable to 20 ng/ml VEGF, but (20*R*)Rg3 showed only a minimal effect. These results indicate that the precise structural characteristics of ginsenosides such as side chains, glucosyl elimination, and epimerization of C20 are required for their vascular effects.

Figure 5. (20*S*)Rg3 protects endothelial cells from serum-deprived apoptosis

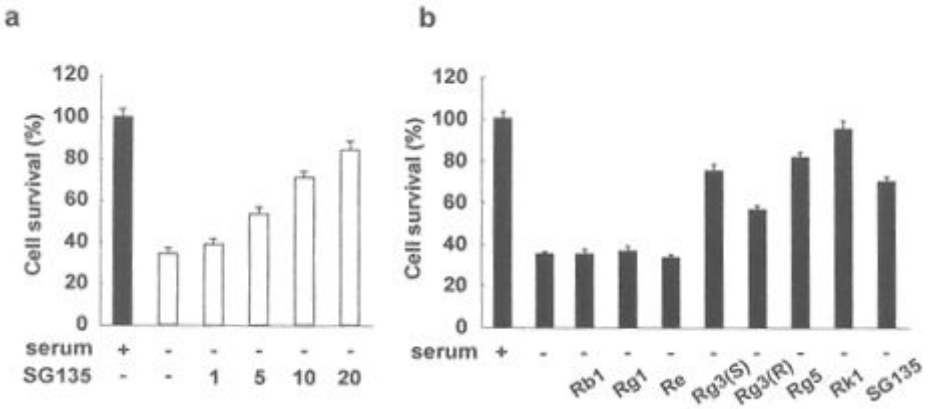


(A) Chemical structure of ginsenosides Rg1, Rb1, (20*R*)Rg3, and (20*S*)Rg3. (B) HUVECs (1 × 105 cells/well) were plated onto six-well plates in 1 ml of M199 containing 20% fetal bovine serum. Next day, the cells were switched to serum-free M199 with various concentrations of Rg1, Rb1, 20(*R*)Rg3 or 20(*S*)Rg3. After 36 h, cell viability was determined by trypan blue exclusion. Viability of control cells was set at 100% and viability relative to the control is presented.

The triterpene backbone structure of ginsenoside Rg3 is similar to the cholestane structure, which is a general backbone structure of phytosterols. The hydroxyl group at C6 seemed not to influence

the apoptotic activity (shown as Rg1). The glucosyl group attached at C3 OH is crucial for the activity, and the stereochemistry of C20 slightly affects to the activity from comparison of Rb1 to Rg3. On the other hand, ginsenosides extracted from heat-processed ginseng (SG 135) and crude extraction were examined, and ginsenoside Rk1 showed the most effective activity (Figure 6).

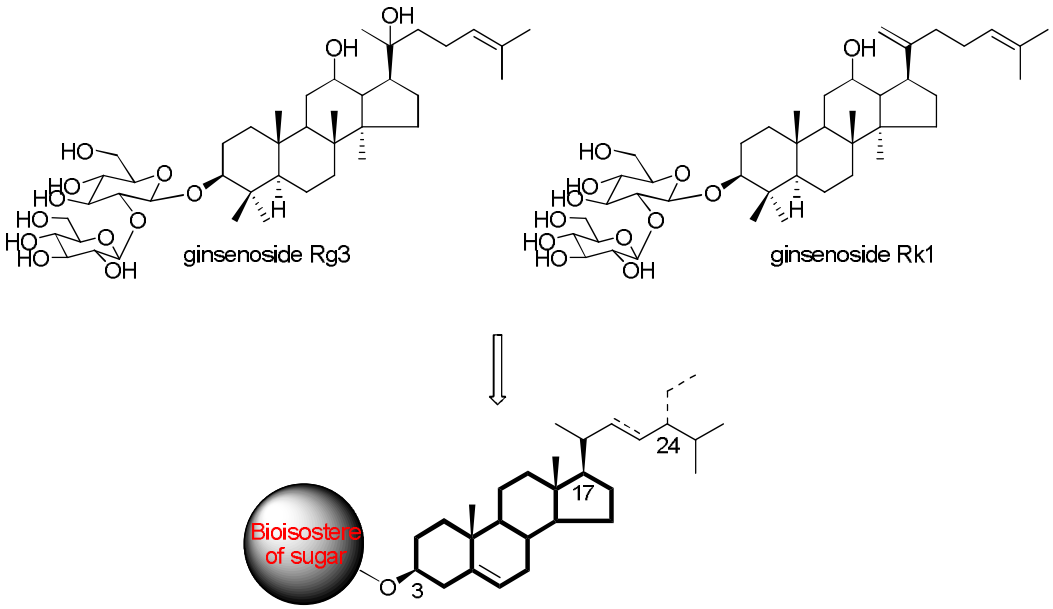
Figure 6. MTT assay result for processed Ginseng extraction



(a) The viability on HUVECs with the serum-free conditions with various concentrations of SG135. (b) HUVECs cell viability with various ginsenosides.

Taking these results together, the glucosyl group at C3 is considered to be crucial. Thus, we planned to determine three major considerations: 1) the effect of sugar moiety at the C3 hydroxyl group, 2) the steric influence of the alkyl chain at C24, and 3) the influence of steroidal skeleton structures. The research began with the selection of proper phytosterols, which contain simple steroidal backbone structures (Figure 7). Thus, our studies commenced with the synthesis of pseudosugar derivatives of several phytosterols, which are structurally simple and commercially available steroids.

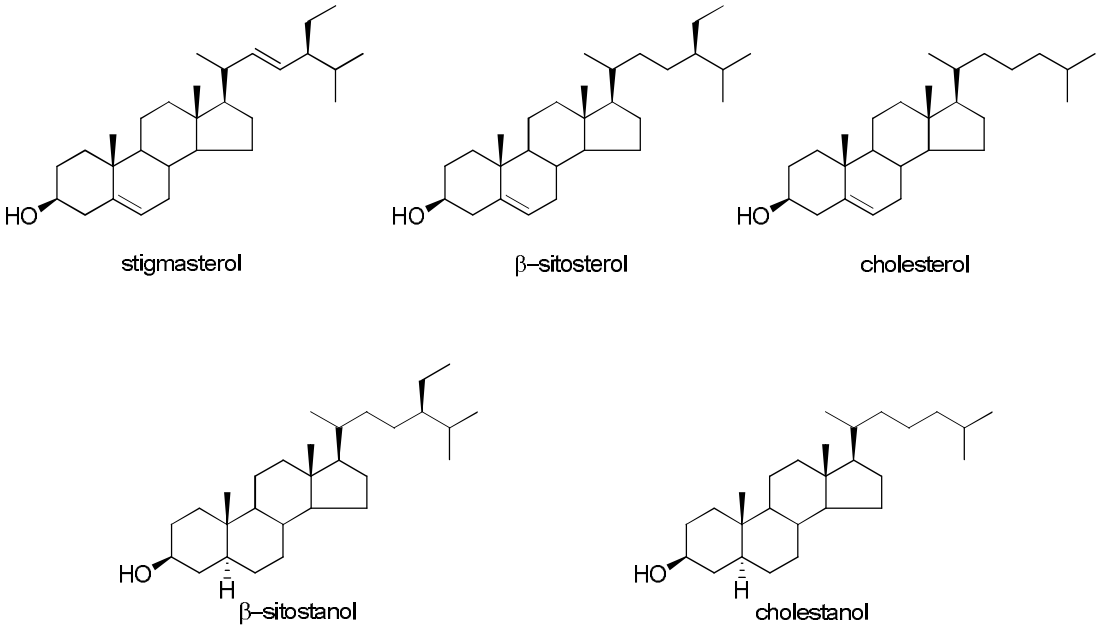
Figure 7. Synthetic approach for ginsenoside derivatives



2. Phytosterol screening

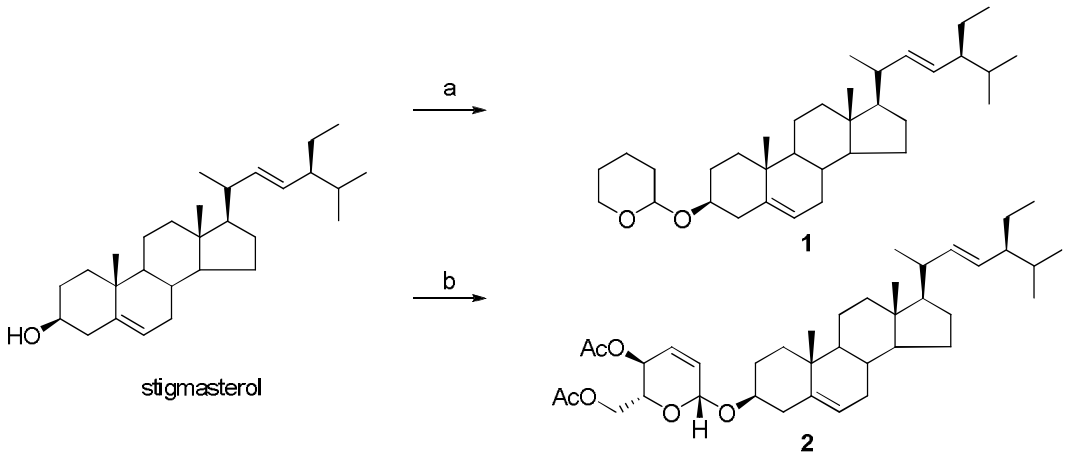
Phytosterols, also referred to as plant sterols, are common plant and vegetable constituents and are therefore normal constituents of the human diet. They are structurally related to cholesterol but differ from it in the structure of the side chain. They consist of a steroidal skeleton with a hydroxyl group attached to the C3 and an aliphatic side chain attached to the C17 (Figure 8). Commercially, phytosterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil, or corn oil, or from so-called tall oil, which is a by-product of the manufacture of wood pulp. [27] These sterols can be hydrogenated to obtain phytostanols. The most common phytosterols and phytostanols are sitosterol (3β-stigmast-5-en-3ol), sitostanol (3β,5α-stigmastan-3-ol), and stigmasterol (3β-stigmasta-5,22-dien-3-ol). Each commercial source has its own typical composition.

Figure 8. Structures of phytosterols



To determine the influence of the side chain at C20, we planned to synthesize simple pseudosugar derivatives such as tetrahydropyrans (THPs) and dihydropyran moieties with those phytosterols. The synthetic schemes are shown below (Scheme 1-3).

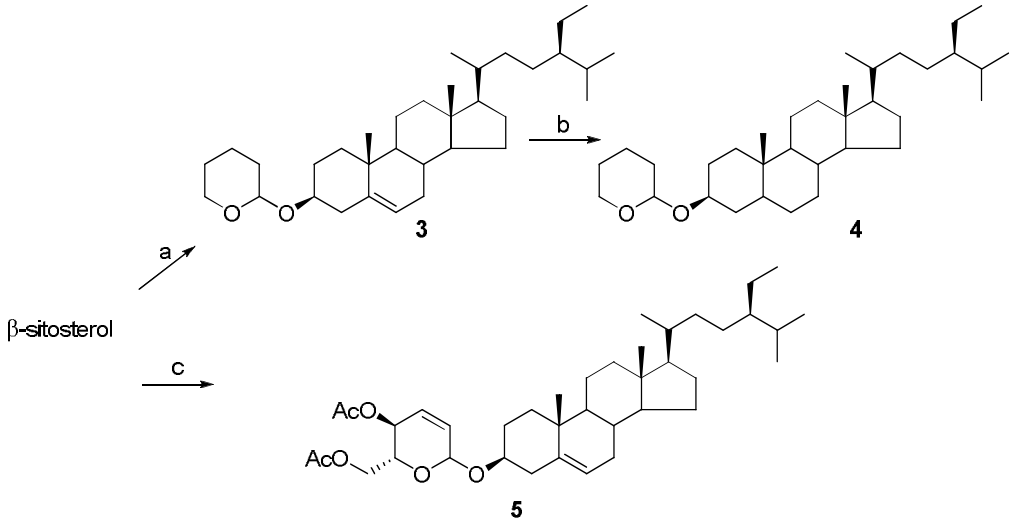
Scheme 1. Synthesis of stigmasterol derivatives



Reagents; (a) DHP, PPTS, CH_2Cl_2, 68%; (b) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 79%.

At first, the addition of 3,4-dihydro-*2H*-pyran and pyridinium *p*-toluene sulfonate into stigmasterol in CH_2Cl_2 afforded to compound **1**. The dihydropyran analog **2** was synthesized by the addition of 3,4,6-*Tri*-*O*-acetyl-*D*-glucal into the stigmasterol solution in the presence of boron trifluoride (Scheme 1)

Scheme 2. Synthesis of β-sitosterol derivatives

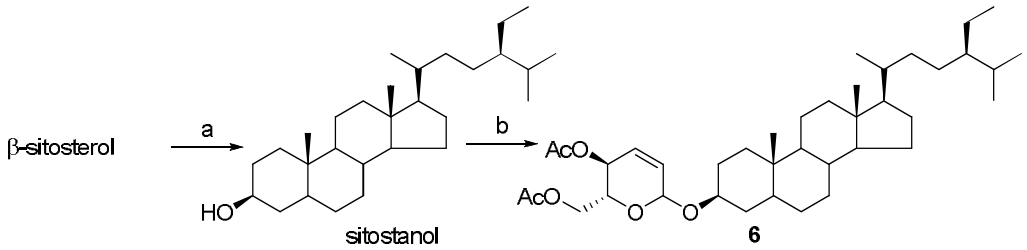


Reagents; (a) DHP, PPTS, CH_2Cl_2, 88%; (b) H_2, Pd/C, EtOAc, 82%; (c) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 82%.

Next, THP and dihydropyran moieties were introduced to β-sitosterol[28] (Scheme 2). THP derivative **3** was synthesized under acidic conditions with dihydropyran, and hydrogenation with palladium activated on carbon afforded sitostanol THP analog **4**. Dihydropyran analog **5** was synthesized with triacetyl glucal.

Sitostanol was synthesized by hydrogenation of β-sitosterol with Pd/C. The dihydropyran analog **6** was synthesized by the addition of 3,4,6-*Tri*-*O*-acetyl-*D*-glucal into the β-sitosterol solution in the presence of boron trifluoride (Scheme 3).

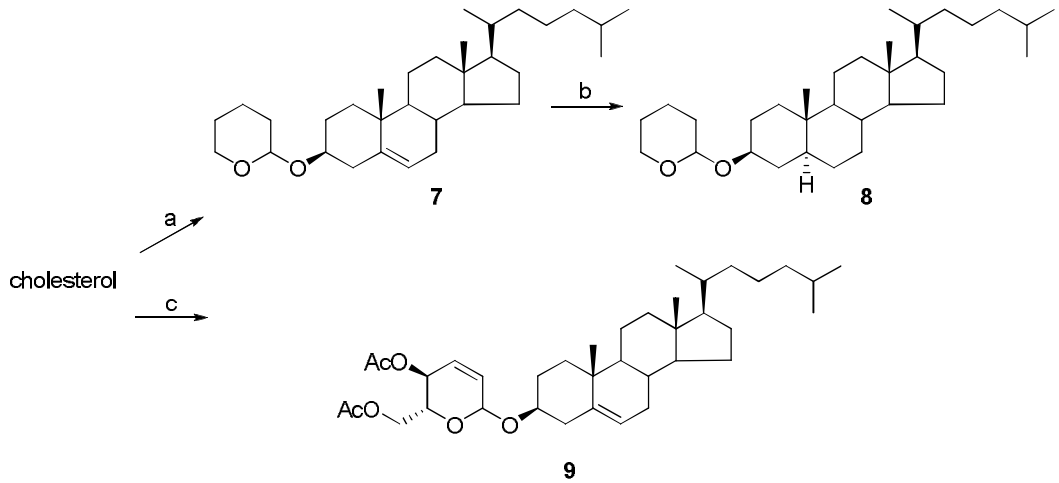
Scheme 3. Synthesis of sitostanol derivatives



Reagents; (a) H_2, Pd/C, EtOAc, 92%; (b)3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 78%.

At Scheme 4, the cholesterol derivatives **7** and **9** and cholestanol derivative **8** were produced under the same reaction conditions described above.

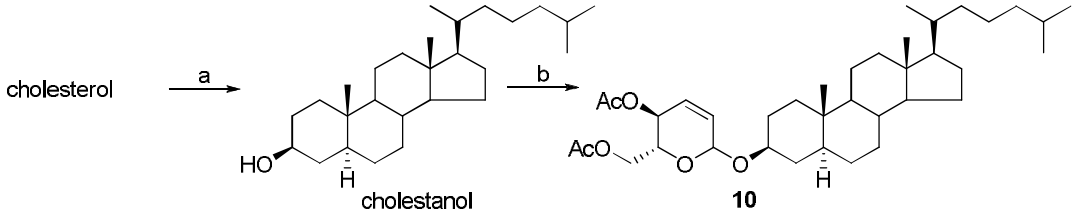
Scheme 4. Synthesis of cholesterol derivatives



Reagents; (a) DHP, PPTS, CH_2Cl_2, 68%; (b) H_2, Pd/C, EtOAc, 98%; (c) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 79%.

At Scheme 5, the synthesis of the cholestanol derivative is described. Hydrogenation of cholesterol with Pd/C afforded cholestanol. In particular, the stereochemistry at C5 of cholestanol was determined by comparison with ^{13}C-NMR data from the literature. [29] With cholestanol, dihydropyran analog **10** was synthesized.

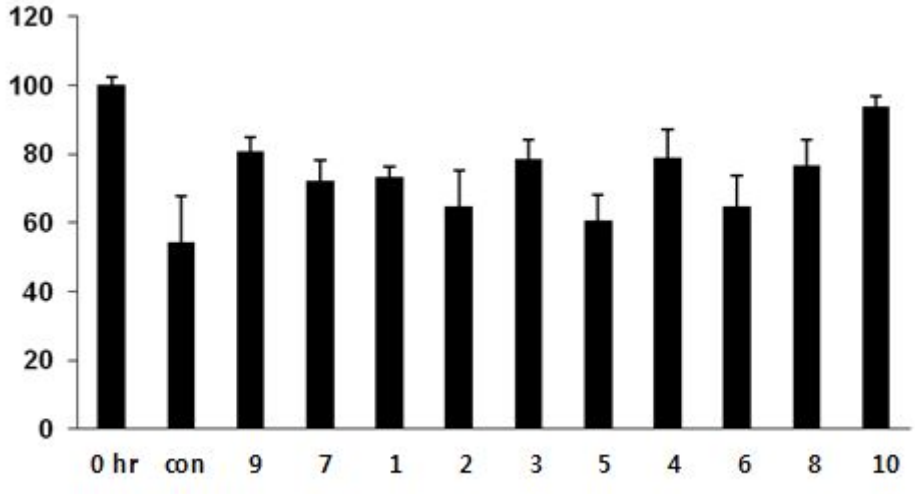
Scheme 5. Synthesis of cholestanol derivatives



Reagents; (a) H_2, Pd/C, EtOAc, 98%; (c) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 82%.

With the 10 pseudosugar analogs of phytosterols in hand, the MTT assay was executed for the anti-apoptotic activities on HUVECs. The results are shown in Figure 9.

Figure 9. MTT assay on HUVECs



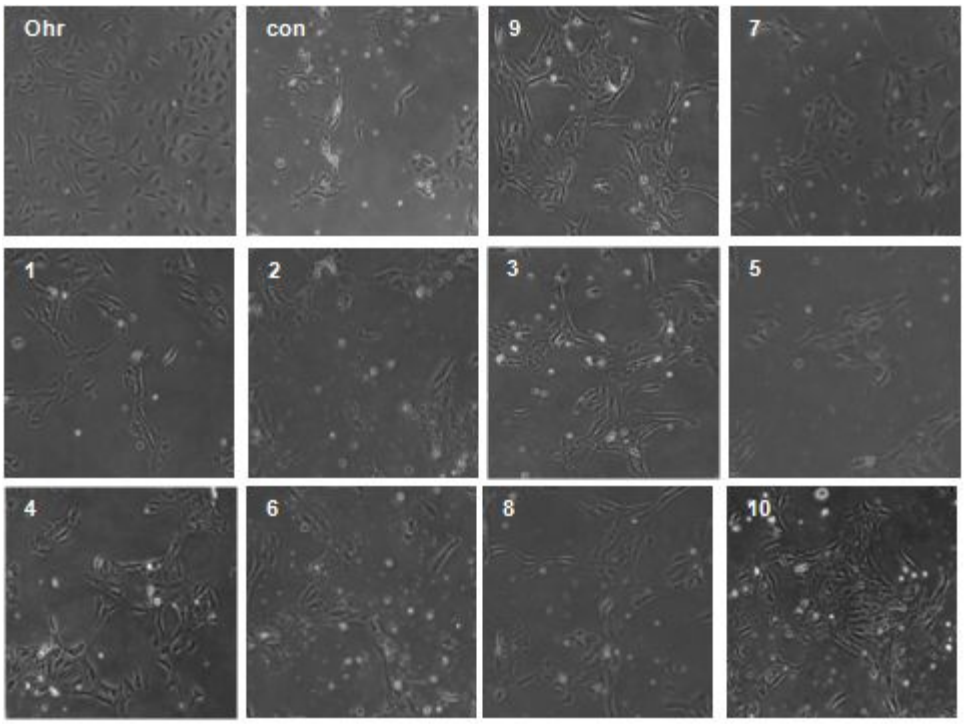
Anti-apoptotic activities of the synthetic analogs on HUVECs at 10 μg/ml treatment. 0 hr; the viability of the cells cultured in the medium without any derivatives. Con; the viability of the cells cultures in the medium containing DMSO used as a vehicle control.

In order to evaluate the effects of the compounds in HUVEC apoptosis, the given model of HUVEC apoptosis induced by deprivation of serum and growth factors[30] was used. The results of the MTT assay showed that cholesterol and cholestanol derivatives (**9**, **3**, **4**, and **10**) have anti-

apoptotic activities on ECs (Figure 9). It is suggested that the side chain at C24 affects the anti-apoptotic activity compared with stimgasterol, β-sitosterol to cholesterol. The branched side chains embedded analogs, such as stimgasterol or β-sitosterol analogs, are slightly less active.

Next, we observed the morphological changes associated with the physiological and pathological processes in HUVECs. When the cells detach from the culture dish bottom and become round, they will enter into apoptosis processes. However, if HUVECs elongate and bend into rings, they will form microvessels. [31] In our experiments, the elongated HUVECs were found with compounds **9**, **3**, **4**, and **10**. The data suggested that cholesterol or cholestanol possessing compounds might be capable of inhibiting the apoptosis

Figure 10. Morphology of HUVEC

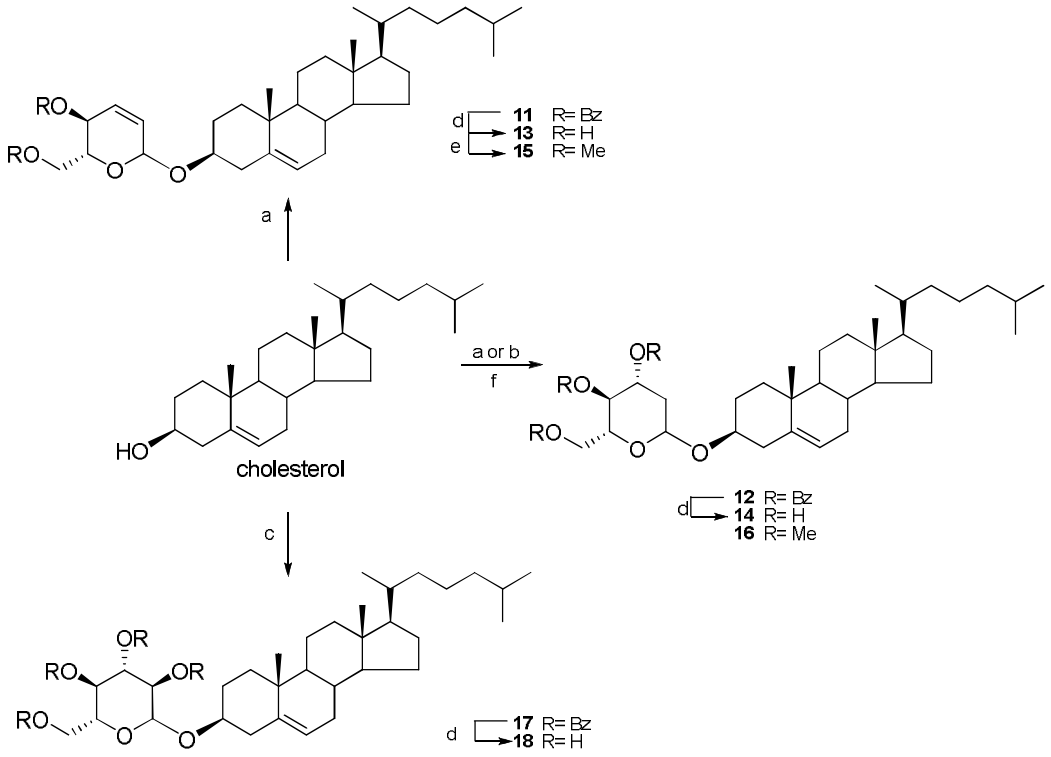


The morphological changes of HUVECs on analogs treated at 10 μg/ml for 48 hr. 0 hr, the morphologies of cells cultured in the medium without any derivatives. Con, the morphologies of cells cultures in the medium containing DMSO used as a vehicle control.

3. Synthesis of pseudosugar derivatives

Considering the previous results with ginsenosides, we had assurance about the relationship between activity and sugar moiety; thus, the studies proceeded with the pseudosugar derivatives synthesis. From the results of the phytosterol assay, we chose cholesterol as the steroidal backbone. As bioisosteres of the glucosyl residue, cyclic ether (di-or THP), acyclic ether (alkyl ether), and aryl groups were introduced to C3 OH of cholesterol. The THP derivative was synthesized already. Thus, the synthesis commenced with an introduction of dihydropyran to C3 alcohol of cholesterol as the substituents of glucosyl moiety as shown in Scheme 6.

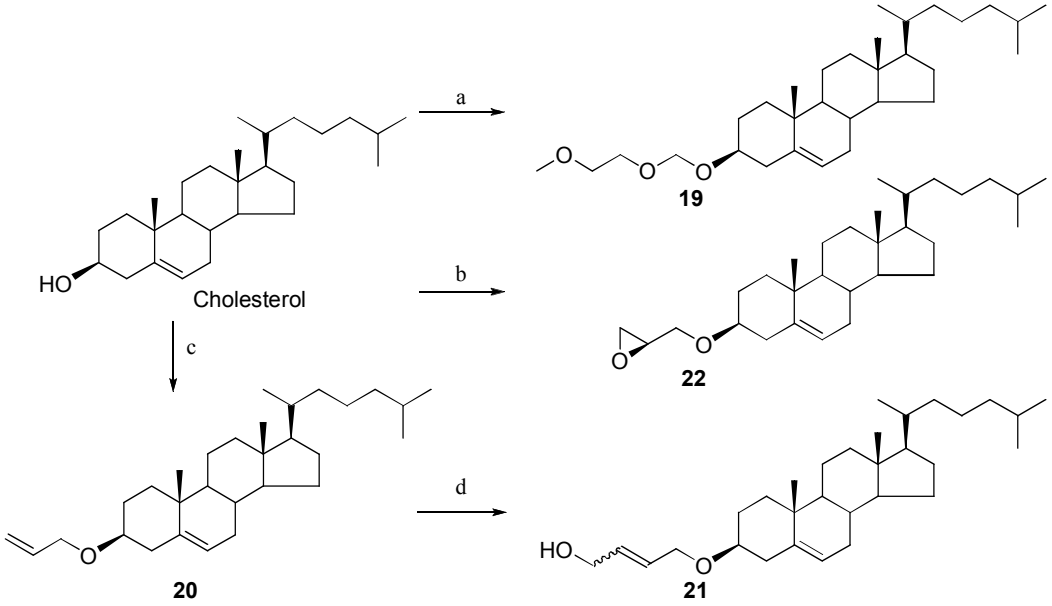
Scheme 6. Synthesis of dihydropyran derivatives



Reagents; (a) 3,4,6-*tri*-*O*-benzoyl-*D*-glucal, TsOH, CH_2Cl_2, 43%; (b) 3,4,6-*tri*-*O*-methoxy-*D*-glucal, CAN, CH_3CN, 26%; (c) 2,3,4,6-tetrabenzoyl-*D*-gluconosyl bromide, Ag_2CO_3, CH_2Cl_2, 45%; (d) NaOMe, MeOH/CH_2Cl_2, 60%; (e) MeI, NaH, THF, 0 ^oC, 32% (f) 3,4,6-*tri*-*O*-methyl-*D*-glucal, CAN, CH_3CN, 2% for **16**.

Treatment of *tri-O*-benzoyl glucal to cholesterol in acidic media was afforded to **11** and **12** mixtures, which could be separated by column chromatography. Removal of benzoyl group of **11** and **12** by using NaOMe afforded to **13** and **14**, which could be transformed to **15** and **16** by the addition of NaH and iodomethane, respectively.

Scheme 7. Synthesis of acyclic analogs



Reagents; (a) $^{i}Pr_2NEt$, CH_2Cl_2, MEMCl, 48%; (b) NaH, THF/DMF (1:1), 2*S*-(+)-glycidol tosylate, 45%; (c) NaH, THF/DMF (1:1), allyl bromide, reflux, 36%; (d) Grubbs' 2nd catalyst, 1,4-butene diol, CH_2Cl_2, reflux, 30%.

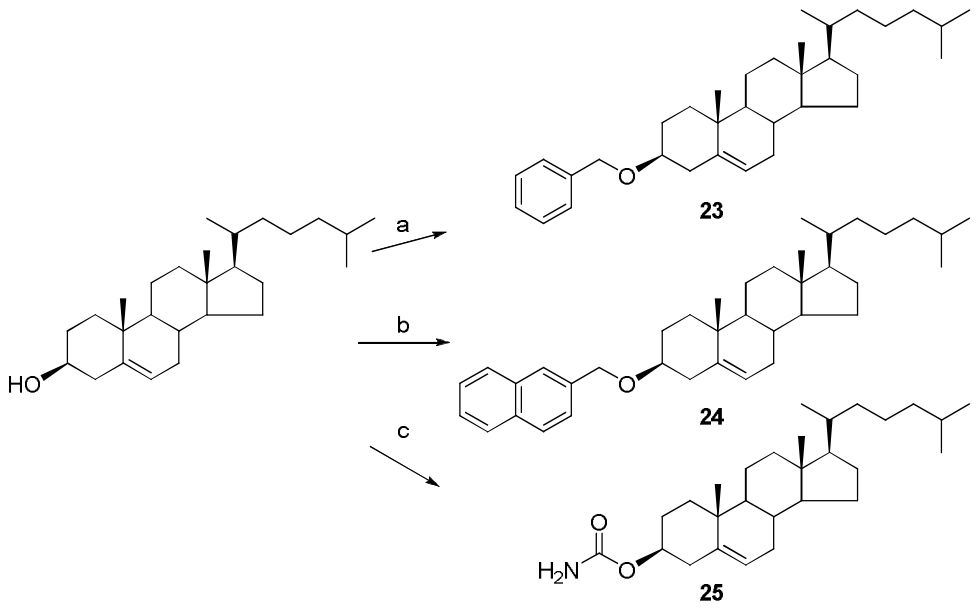
Analog **18** could be prepared by coupling cholesterol with 2,3,4,6-tetrabenzoyl-*D*-glucopyranosyl bromide in the presence of silver triflate, followed by debenzoylation as described above.

Next, we synthesized a series of cholesterol analogs linked with the acyclic or alkyl ether moieties (Scheme 7). Analog **19** was synthesized by reaction with MEMCl in the presence of Hunig's base. Allylation of cholesterol by treatment with sodium hydride and allyl bromide in refluxing

THF/DMF provided allyl ether **20**. Cross metathesis of **20** with 1,4-butene diol by employing Grubbs' 2nd generation catalyst afforded **21**. Reaction of cholesterol with sodium hydride and then with (2*S*)-glycidyl tosylate afforded **22**.

To confirm the spatial influence of glucosyl residue, aryl moieties as a carbohydrate equivalent were also introduced to the cholesterol scaffold. Analogs **23** and **24** were prepared by *O*-alkylation with benzyl bromide and bromomethyl naphthalene under reaction conditions of sodium hydride and TBAI in refluxing THF. Treatment of cholesterol with trichloroacetyl isocyanate gave carbamate **25** (Scheme 8).

Scheme 8. Synthesis of bulky analogs



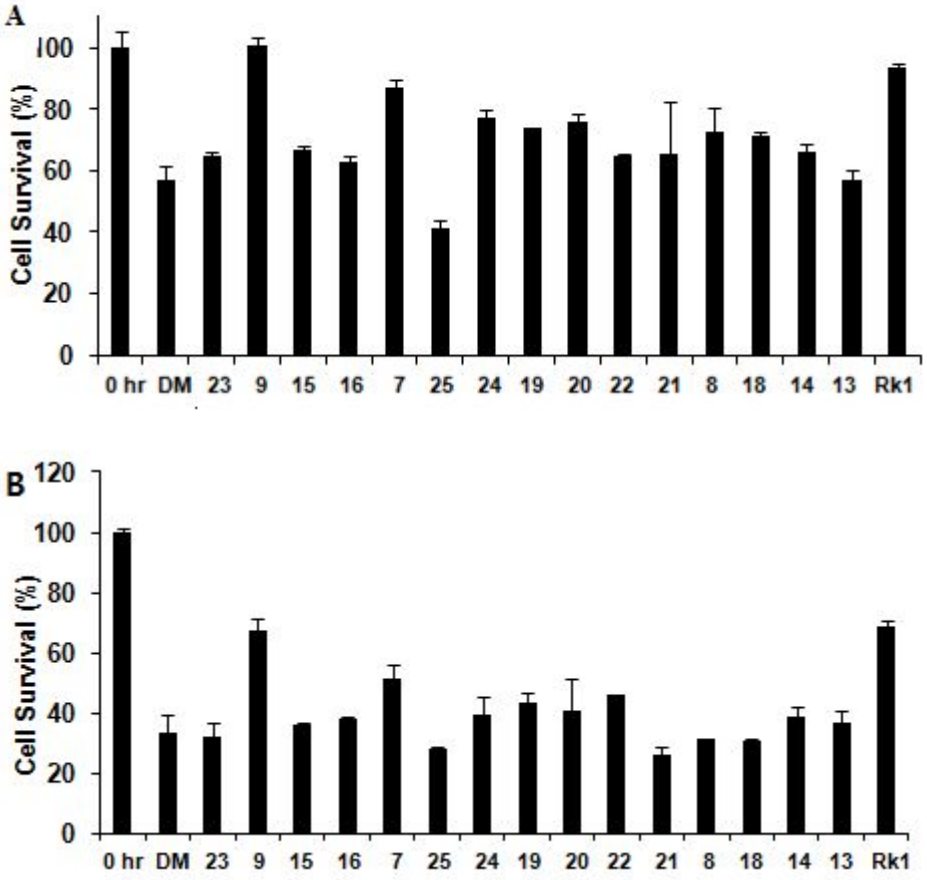
Reagents; (a) NaH, TBAI, benzyl bromide, THF, reflux, 30%; (b) NaH, TBAI, (2-bromomethyl) naphthalene, THF, reflux, 30%; (c) $Cl_3CCONCO$, $CHCl_3$, 31%.

4. Biological activity against serum deprived apoptosis

1) MTT assay on HUVECs

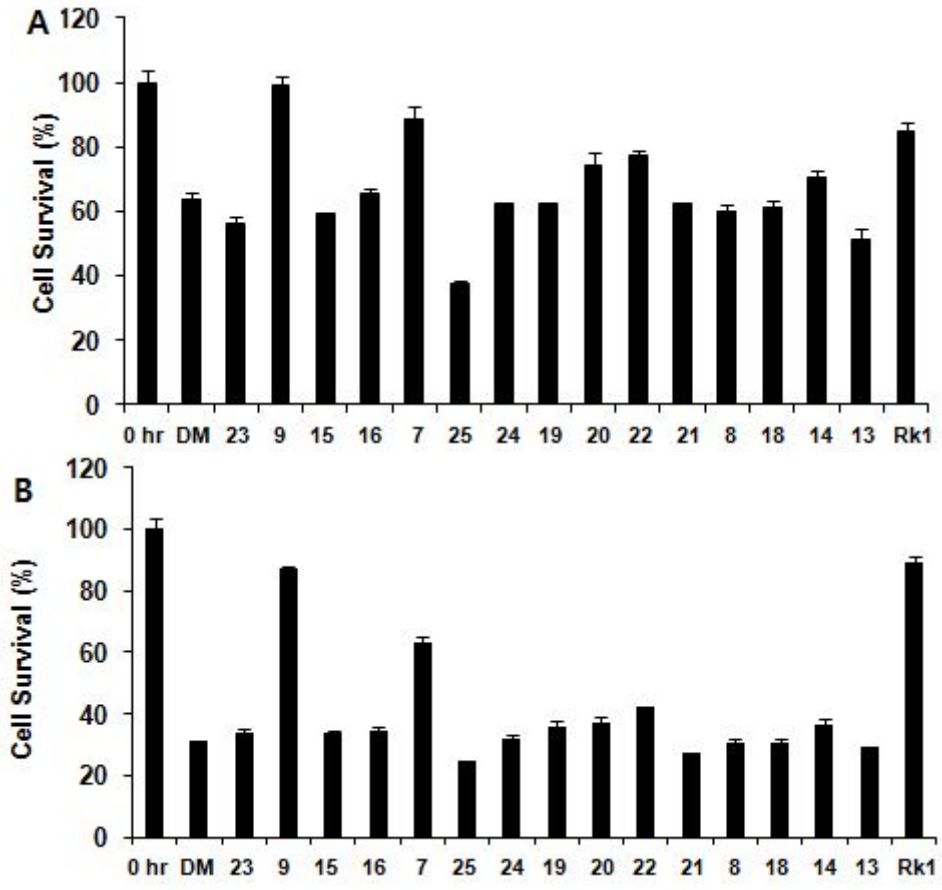
The inhibitory effects of the cholesterol analogs were investigated on the growth factor deprivation-induced HUVEC cell death using the MTT colorimetric method at concentrations of 5 μg/ml and 10 μg/ml for 24 h and 48 h, respectively. The results of the MTT assay are summarized in Figures 11 and 12.

Figure 11. Effect of compounds on apoptosis of HUVECs: 5 μg/ml treatment



(A) 5 μg/ml for 24 h treatment, (B) 5 μg/ml, 48 h treatment. 0 hr; the viability of the cells cultured in the medium without any derivatives. DM; the viability of the cells cultured in the medium containing DMSO used as a vehicle control. **Rk1**; the viability treated with ginsenoside Rk1.

Figure 12. Effect of compounds on apoptosis of HUVECs: 10 μg/ml treatment



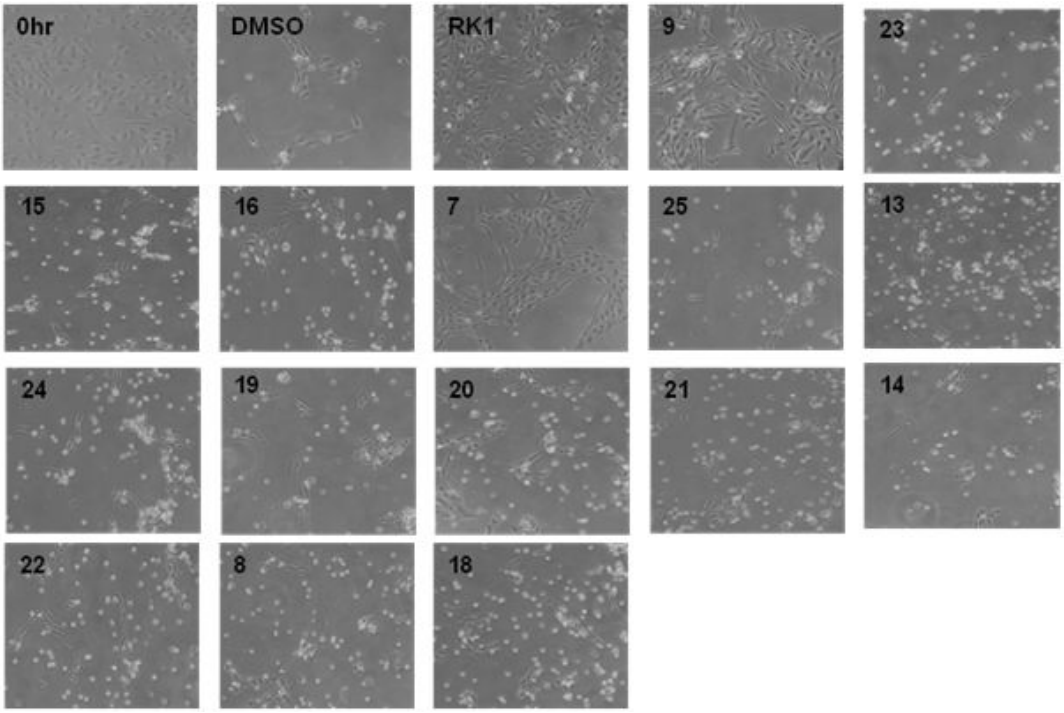
(A) 10 μg/ml for 24 h treatment, (B) 10 μg/ml for 48 h treatment. 0 h; the viability of the cells cultured in the medium without any derivatives. DM; the viability of the cells cultured in the medium containing DMSO used as a vehicle control. **Rk1**; the viability treated with ginsenoside Rk1.

It is noticeable that the acetyl dihydropyran derivative **11** was more potent than ginsenoside Rk1, which has known as one of the potent anti-apoptotic agents. In contrast, analogs **13** and **15** possessing methoxy or hydroxyl dihydropyran exhibited less potent activity compared to Rk1. This result revealed that the acyl group of the carbohydrate equivalents is important to manifest the inhibitory effect of the cholesterol analogs. The THP analog **7** also exhibited comparable activity to that of Rk1 after 24-h treatment. However, it was slightly less potent than Rk1 after 48 h

treatment. Analogs **14**, **16**, and **18** possessing the substituted pyran moiety did not show any significant inhibitory activity. Analogs **19**, **20**, **21**, and **22** possessing the acyclic carbohydrate equivalents exhibited moderate inhibitory activities at the concentration of 5 μg/ml. It is also noteworthy that the cholesteryl THP analog **7** was more potent than the dihydrocholesteryl THP analog **8**.

Next, we observed the morphological changes associated with the physiological and pathological processes in HUVECs, as described ahead (Figure 13). Treated with compounds **7** and **9, t**he elongated HUVECs were observed. The data suggested that particularly dihydro-, tetrahydrocholesterol derivatives might inhibit the apoptosis of ECs.

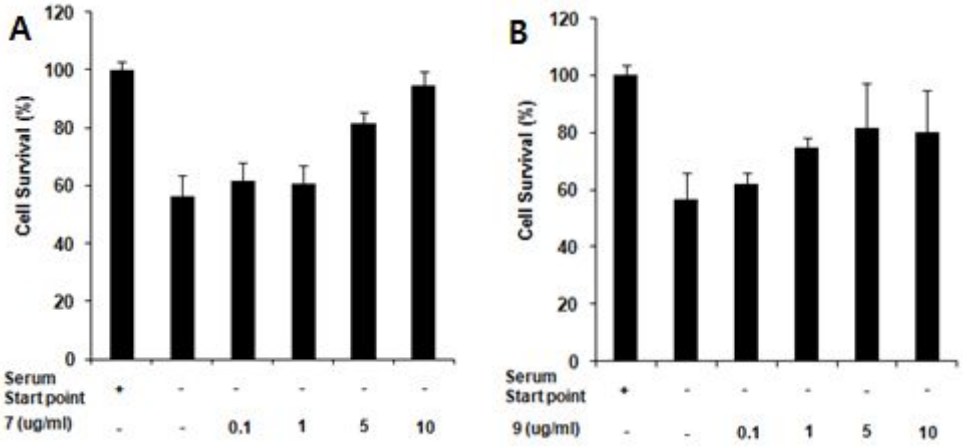
Figure 13. Morphological changes of HUVEC



The morphological changes of HUVECs on analogs treated at 10 μg/ml for 48 hr. 0 hr; the morphologies of cells cultured in the medium without any derivatives. Con; the morphologies of cells cultures in the medium containing DMSO used as a vehicle control.

For further investigation of the cell-protective effect of analogs **7** and **9**, their anti-apoptotic effect on cell viability in human retina endothelial cells (HRECs) was examined. In addition, their dose-dependent activities were confirmed by the cell survival counting assay, which was performed at 0.1 μg/ml to 10 μg/ml, respectively. The concentration-dependent cell viabilities by both analogs **7** and **9** are well revealed in Figure 14.

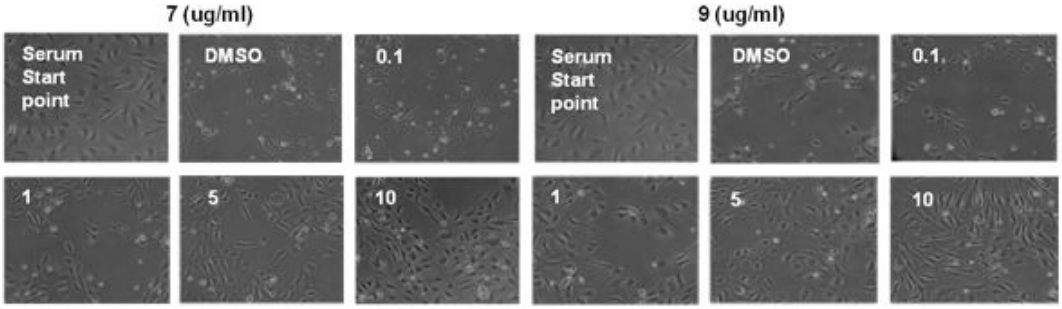
Figure 14. Inhibition of compounds 7, and 9 on apoptosis of HRECs



Inhibitory effects by analogs **7** and **9** on apoptosis of HRECs. (A) Treatment with analog **7** at sequential concentration from 0.1 μg/ml to 10 μg/ml for 48 h. (B) Treatment with analog **9** at sequential concentration from 0.1 μg/ml to 10 μg/ml for 48 h.

And the morphological changes have shown the correlation of the activities with the concentrations of the analogs **7** and **9** (Figure 15).

Figure 15. Morphological changes of HUVEC

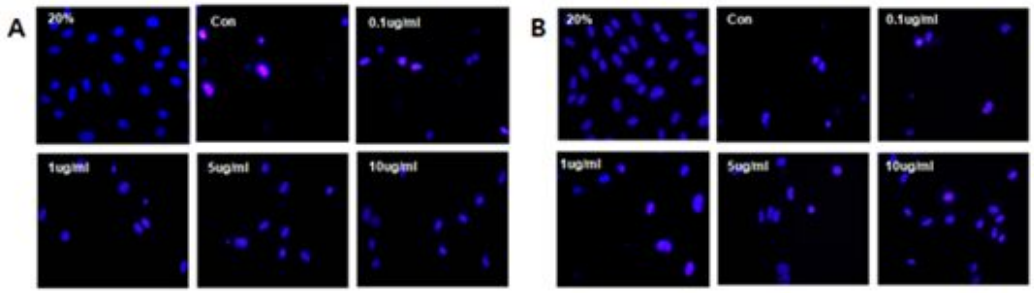


2) DAPI/ TUNEL assay

Apoptotic activity in the cytoplasmic extracts can be examined by the measurement of enzymatic caspase activity by western blots of proteins processed during apoptosis (caspases and their substrates) or by using isolated cell nuclei as indicators for apoptotic factors inducing nuclear morphological changes and/or DNA fragmentation. In 1993, a paper first described that a cell-free system can mimic characteristic features of apoptosis in intact cells. [32] Indeed, it has become clear that the apoptotic pathways acting in the cytoplasm function independently from the nucleus, and thus cell-free systems appear to be appropriate model systems that represent at least part of the apoptotic machinery and signaling mechanisms. Also, signaling pathways such as the caspase cascade have been studied under cell-free conditions, providing insight into activation patterns and inhibitor specificities. [33] On the other hand, there are cell-based assay systems. Apoptosis could be characterized biochemically by the activation of endonuclease activity that contributed to massive morphological changes in nuclei and ultimately to cell death. Endonuclease activity in individual nuclei was determined by a terminal deoxynucleotidyl transferase (TdT). 4',6'-Diamidino-2-phenylindole.2HCl (DAPI) forms a specific complex with DNA, and this fact has been used for the quantitative fluorometric estimation of DNA. [34] The fluorescent dye DAPI complexes with DNA to give a product with a fluorescence intensity about 20 times greater than that of the dye alone. Cells stained with DAPI and examined by fluorescence microscopy show nuclear DNA as well as mitochondrial and chloroplast DNA. This lack of interference from other cell components prompted us to develop a simple fluorometric assay for the DNA content of crude cellular homogenates using DAPI. [35] Among these assay systems, we tested the DAPI and terminal transferase dUTP nick end labeling (TUNEL) assay.

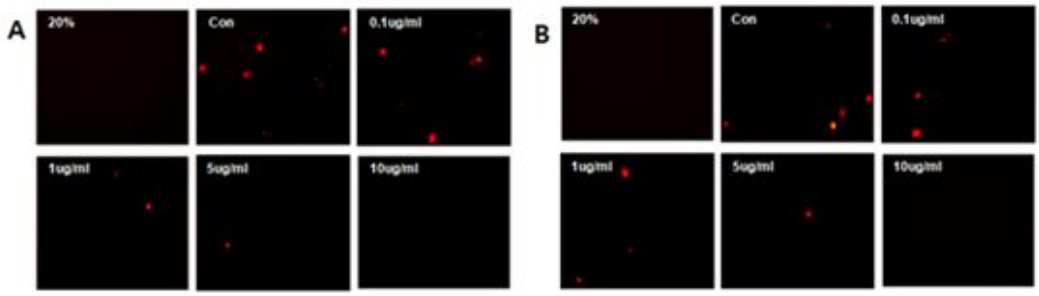
At first, morphological changes in the nuclear chromatin in cells undergoing apoptosis were detected by staining with the DNA-binding fluorochrome, DAPI. HUVECs were grown on glass coverslips and treated with analogs **7** and **9** in the presence or absence of serum for 48 h. Cells were washed twice with phosphate buffered saline (PBS) and fixed by incubation in 4% paraformaldehyde for 30 min. Following washing with PBS, cells were incubated in a DAPI solution (1 μg/ml) for 30 min in the dark. Cells were then washed with PBS and subjected to fluorescence microscopy (Figure 16).

Figure 16. Changes in nuclear morphology accessed by DAPI staining.



(A) Cells treated with **9** of various concentrations for 48 h; (B) Cells treated with **7** of various concentrations for 48 h.

Figure 17. TUNEL assays at various concentrations of 7 and 9



(A) Cells treated with **9** of various concentrations for 48 h; (B) Cells treated with **7** of various concentrations for 48 h.

Next, the TUNEL assay was executed. The TUNEL assay is a method used to detect DNA degradation in apoptotic cells because one of the hallmarks of late-stage apoptosis is the fragmentation of nuclear chromatin, which results in a multitude of 3'-hydroxyl termini of DNA ends. [36] This property can be used to identify apoptotic cells by labeling the DNA breaks with fluorescent-tagged deoxyuridine triphosphate nucleotides (F-dUTP). The addition of the deoxythymidine analog 5-bromo-2'-deoxyuridine 5'-triphosphate (BrdUTP) to the TdT reaction serves to label the break sites. Once incorporated into the DNA, BrdU can be detected by an anti-BrdU antibody using standard immunohistochemical techniques. Non-apoptotic cells do not incorporate much of the F-dUTP because of the absence of exposed 3'-hydroxyl DNA ends. This enzyme labeled the 3' OH ends of DNA for visualization with a secondary detection system using a colorimetric substrate for peroxidase. The TdT-mediated dUTP-biotin nick-end labeling (TUNEL) assay stained apoptotic nuclei red for the expression of endogenous endonucleases.

The TUNEL assay, as shown in Figure 17, specifically detecting DNA fragmentation, also revealed that analogs **7** and **9** suppressed the apoptotic cell death induced by serum deprivation in a dose-dependent manner. We have reported parts of these synthetic products and biological assay results. [37]

III. Conclusion

In conclusion, we have developed novel anti-apoptotic agents based on the ginsenoside Rk1 and Rg3, providing valuable information for the development of new anti-apoptotic agents. In particular, we replaced the ginsenosides, which are chemically unstable and difficult to be supplied sustainably, to the easily accessible phytosterol backbone. Furthermore, the analogs **7** and **9** possessing the cholesterol scaffold and the cyclic carbohydrate equivalents exhibited excellent cell survival activities, which are equipotent to that of ginsenoside Rk1. In addition, these analogs exhibited dose-dependent cell viability on the HREC line. The excellent human EC protective activities of our novel Rk1-based anti-apoptotic agents allow us to envisage their valuable application for the treatment of vascular disorders. Currently, systematic studies on their therapeutic applications, including in vivo testing of the potent sterol analogs as well as their mechanistic aspects, are in good progress. [38]

IV. Experimental

General Experimental

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane, triethylamine, acetonitrile and pyridine were freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried at 100 °C. Air and moisture sensitive reactions were performed under an argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Infrared spectra were recorded on a Perkin-Elmer 1710 FT-IR spectrometer. Mass spectra were obtained with VG Trio-2 GC-MS instrument. High-resolution mass spectra were obtained with JEOL JMS-AX 505WA instrument. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 (300 MHz), Varian Gemini 2000 (300 MHz), Brucker Analytik AVANCE digital 400 (400MHz), or AVANCE digital 500 (500MHz) spectrometer as solutions in deuteriochloroform ($CDCl_3$). Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane and are referenced to the deuterated solvent ($CHCl_3$). ^{1}H-NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad singlet; m, multiplet and/or multiple resonance), number of protons, and coupling constant in hertz (Hz).

2-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*S*,*E*)-5-Ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (1)

To a solution of stigmasterol (100 mg, 0.24 mmol) in CH_2Cl_2 (4 mL) was added dihydropyran (0.033 mL, 0.36 mmol) and PPTS (6 mg, 0.24 mmol). After stirring for 4 h, the reaction mixture was quenched with H_2O, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 82 mg (68%) of **1** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.32 (m, 1H), 5.12 (dd, 1H, *J* = 8.43 Hz, 15.0 Hz), 4.99 (dd, 1H, *J* = 8.6 Hz, 15.0 Hz), 4.69 (m, 1H), 3.88 (m, 1H), 3.47 (m, 2H), 2.34 (m, 2H), 1.97-1.06 (m, 29H), 0.98 (s, 6H), 0.80 (m, 9H), 0.67 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 140.9, 138.3, 129.1, 121.5, 96.7, 75.9, 62.8, 56.8, 51.2, 50.1, 42.1, 40.4, 39.6, 38.7, 37.4, 37.1, 36.7, 31.8, 31.2, 29.6, 28.8, 27.9, 25.4, 25.3, 24.3, 21.1, 21.0, 20.06, 21.01, 19.9, 19.3, 18.9, 12.2, 12.0; LRMS (FAB+) *m/z* 497(M+H).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*S*,*E*)-5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (2)

To a solution of 3,4,6-triacetyl glucal (198 mg, 0.7 mmol) in THF (4 mL) was added stigmasterol (100 mg, 0.24 mmol) and $BF_3{\cdot}OEt_2$ (0.09 ml, 0.7 mmol). After stirring for 18 h, the reaction mixture was quenched with saturated NH_4Cl and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 119 mg (79%) of **2** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.81 (m, 2H), 5.30 (m, 2H), 5.16 (br, 1H), 5.10 (d, 1H, *J* = 8.4 Hz), 4.98 (dd, 1H, *J* = 8.4 Hz, 15.0 Hz), 4.17 (m, 3H), 3.53 (m, 1H), 2.35

(m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 2.02-1.81 (m, 6H), 1.68 (m, 1H), 1.53 (m, 9H), 1.25-0.75 (m, 22H), 0.67 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 170.7, 170.2, 140.6, 138.2, 129.2, 128.8, 128.0, 121.7, 92.7, 76.5, 66.7, 65.3, 63.1, 56.7, 55.8, 51.1, 50.1, 42.1, 40.4, 40.3, 39.5, 37.0, 36.6, 31.8, 28.8, 28.1, 25.3, 24.2, 21.1, 21.0, 20.9, 20.9, 20.7, 19.2, 18.9, 12.2, 11.9; LR-MS (FAB+) *m/z* 647 (M+Na).

2-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (3)

To a solution of β-sitosterol (200 mg, 0.48 mmol) in CH_2Cl_2 (6 mL) was added dihydropyran (0.07 mL, 0.72 mmol) and PPTS (12 mg, 0.05 mmol). After stirring for 2 h, the reaction mixture was quenched with H_2O, and then diluted with CH_2Cl_2. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 164 mg (68%) of **3** as a white solid: FT-IR (neat) ν_{max} 2921, 1460, 1112 cm^{-1}; ^{1}H -NMR ($CDCl_3$, 300 MHz) δ 5.32 (br, 1H), 4.69 (br, 1H), 3.89 (m, 1H), 3.47 (m, 2H), 2.34 (m, 2H), 2.00-1.00 (m, 33H), 0.98 (s, 3H), 0.89 (d, 3H, *J* = 6.26 Hz), 0.83 (q, 9H, *J* = 7.14 Hz), 0.65 (s, 3H); ^{13}C -NMR ($CDCl_3$, 75 MHz) δ 140.9, 121.5, 96.8, 75.9, 62.7, 56.7, 56.0, 50.1, 45.7, 42.2, 40.2, 39.7, 38.7, 37.4, 47.1, 36.7, 36.0, 33.8, 31.8, 31.2, 29.6, 29.0, 28.2, 25.9, 25.4, 24.2, 23.0, 21.0, 20.0, 19.7, 19.3, 18.9, 18.7, 11.9; LRMS (FAB+) *m/z* 499 (M+H).

2-((3*S*,10*S*,13*R*,17*R*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (4)

To a solution of **3 (**68 mg**,** 0.14 mmol) in EtOAc (4 mL) was added 10% activated palladium carbon. The resulting mixture was hydrogenated for 24 h and then filtered through a pad of Celite. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 :

30) afforded 56 mg (82 %) of **4** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 4.68 (s, 1H), 3.89 (m, 1H), 3.50 (m, 2H), 1.94-0.77 (m, 53H), 0.61 (s, 3H); LRMS (FAB+) *m/z* 523 (M+Na).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*R*)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (5)

To a solution of 3,4,6-triacetyl glucal (984 mg, 3.62 mmol) in THF (6 mL) was added β-sitosterol (500 mg, 1.21 mmol) and $BF_3 \cdot OEt_2$ (0.45 ml, 3.62 mmol). After stirring for 2 h, the reaction mixture was quenched with saturated $NaHCO_3$ and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 7) afforded 620 mg (82%) of **5** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.81 (m, 2H), 5.32 (d, 1H, *J* = 4.77 Hz), 5.25 (d, 1H, *J* = 9.33 Hz), 5.14 (s, 1H), 4.16 (m, 3H), 3.51 (m, 1H), 2.34 (m, 2H), 2.06 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.00-1.81 (m, 5H), 1.74-1.00 (m, 22H), 0.97 (s, 3H), 0.88 (d, 3H, *J* = 6.24 Hz), 0.80 (q, 6H, *J* = 6.96 Hz), 0.64 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 170.4, 169.9, 140.5, 128.6, 128.2, 121.6, 92.5, 77.9, 66.6, 65.1, 62.9, 56.2, 55.8, 49.9, 45.5, 42.1, 40.2, 39.5, 36.9, 36.4, 35.9, 33.7, 31.7, 31.6, 28.9, 28.0, 25.8, 24.1, 22.8, 20.8, 20.7, 20.6, 19.6, 19.1, 18.8, 18.6, 18.5, 12.0, 11.8; LRMS (FAB+) *m/z* 649 (M+Na).

(3*S*,10*S*,13*R*,17*R*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-ol (Sitostanol)

To a solution of β-sitosterol (225 mg**,** 0.54 mmol) in EtOAc (8 mL) was added 10% activated palladium carbon. The resulting mixture was hydrogenated for 2 h and then filtered through a pad of Celite. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 209 mg (92 %) of **sitostanol** as a white solid: FT-IR (neat) ν_{max} 3350,

1464, 1029 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.55 (m, 1H), 1.93 (d, 1H, *J* = 12.09 Hz), 1.82-0.72 (m, 45 H), 0.58 (s, 3H); LRMS (FAB+) *m/z* 417 (M+H).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,10*S*,13*R*,17*R*)-17-((2*R*,5*R*)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (6)

To a solution of 3,4,6-triacetyl glucal (275 mg, 1.01 mmol) in THF (5 mL) was added sitostanol (140 mg, 0.337 mmol) and $BF_3 \cdot OEt_2$ (0.125 ml, 1.01 mmol). After stirring for 4 h, the reaction mixture was quenched with water and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 165 mg (78%) of **6** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.80 (m, 2H), 5.26 (d, 1H, *J* = 9.15 Hz), 5.14 (s, 1H), 4.16 (m, 3H), 3.59 (m, 1H), 2.06 (s, 3H), 2.04 (s, 3H), 1.93 (m, 1H), 1.82-0.77 (m, 46H), 0.61 (s, 3H); ^{13}C -NMR ($CDCl_3$, 75 MHz) δ 170.6, 170.2, 128.7, 128.6, 92.9, 77.8, 66.6, 65.2, 63.1, 56.3, 56.0, 54.2, 45.7, 45.0, 42.5, 39.9, 36.8, 36.2, 26.0, 35.4, 33.8, 32.0, 29.0, 28.8, 28.1, 28.0, 25.9, 24.1, 22.9, 21.1, 20.8, 20.7, 19.7, 18.9, 18.5, 12.1, 11.9, 11.8; LRMS (FAB+) *m/z* 651 (M+Na).

2-((3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (7)

To a solution of cholesterol (100 mg, 0.26 mmol) in CH_2Cl_2 (1.5 mL) was added dihydropyran (0.18 mL, 1.97 mmol) and PTSA (12 mg, 0.07 mmol). After stirring for 4 h, the reaction mixture was quenched with H_2O, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash

column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 82 mg (68%) of **7** as a white solid: FT-IR (neat) ν_{max} 2921, 1460, 1112 cm^{-1}; ^1H -NMR ($CDCl_3$, 300 MHz) δ 5.32 (br, 1H), 4.69 (br, 1H), 3.88 (m, 1H), 3.54-3.43 (m, 2H), 2.34-2.28 (m, 2H), 2.00-1.66 (m, 7H), 1.59-1.00 (m, 21H), 0.98 (s, H), 0.86 (ddd, 9H, *J* = 15.3 Hz, 6.4 Hz, 1.2 Hz), 0.65 (s, 3H); LRMS (FAB+) *m/z* 471 (M+H).

2-((3*S*,10*S*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (8)

To a solution of **7 (**40 mg**,** 0.01 mmol) in EtOAc (3 mL) was added 10% activated palladium carbon. The resulting mixture was hydrogenated for 1 h and then filtered through a pad of Celite. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 37 mg (78 %) of **8** as a white solid: FT-IR (neat) ν_{max} 2930, 1738, 1464, 1029 cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz) δ 4.69 (m, 1H), 3.89 (m, 1H), 3.60-3.41 (m, 2H), 1.92 (m, 1H), 1.82-1.39 (m, 13H), 1.35-0.94 (m, 15H), 0.85 (ddd, 9H, *J* = 10.5 Hz, 6.6 Hz, 1.3 Hz), 0.77 (s, 3H), 0.62 (s, 3H); LRMS (FAB+) *m/z* 473 (M+H).

((2R,3S)-3-Acetoxy-6-((3S,10R,13R,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (9)

To a solution of 3,4,6-triacetyl glucal (2.0 g, 7.5 mmol) in ether (20 mL) was added cholesterol (1.0 g, 2.5 mmol) and $BF_3 \cdot OEt_2$ (1.3 ml, 10 mmol). After stirring for 18 h, the reaction mixture was quenched with a saturated $NaHCO_3$ and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 660 mg (43%) of **9** as a white solid: ^1H-NMR ($CDCl_3$, 300 MHz) δ 5.83 (m, 2H), 5.33 (br, 1H), 5.27 (dd,

1H, *J* = 9.15 Hz, 1.47 Hz), 5.14 (br, 1H), 4.17 (m, 3H), 3.53 (m, 1H), 2.37 (m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 1.99-1.01 (m, 26H), 0.97 (s, 3H), 0.84 (dd, 9H, *J* = 6.6 Hz, 14.3 Hz), 0.65 (s, 3H); FT-IR (neat) ν_{max} 2938, 1464, 1377, 1261, 1199, 1024 cm^{-1}; LRMS (FAB+) *m/z* 621 (M+Na).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,5*S*,10*S*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)hexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (10)

To a solution of 3,4,6-triacetyl glucal (95 mg, 0.35 mmol) in THF (2 mL) was added cholestanol (45 mg, 0.116 mmol) and $BF_3 \cdot OEt_2$ (0.043 ml, 0.35 mmol). After stirring for 18 h, the reaction mixture was quenched with a saturated $NaHCO_3$ and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 57 mg (82%) of **10** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.82 (m, 2H), 5.26 (d, 1H, *J* = 9.15 Hz), 5.14, (s, 1H), 4.18 (m, 3H), 3.60 (m, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 1.93 (d, 1H, *J* = 12.09 Hz), 1.78-0.93 (m, 30H), 0.85 (dd, 9H, *J* = 6.5 Hz, 10.08 Hz), 0.77 (s, 3H), 0.61 (s, 3H); LRMS (FAB+) *m/z* 623 (M+Na).

((2*R*,3*S*)-3-(Benzoyloxy)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl benzoate (11)

To a solution of 3,4,6-tribenzoyl glucal (320 mg, 0.7 mmol) in CH_2Cl_2 (10 mL) was added cholesterol (108 mg, 0.28 mmol) and PTSA (11 mg, 0.06 mmol). After stirring for 18 h, the reaction mixture was quenched with water and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 15) afforded 33 mg

(23%) of **11** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02-8.00 (m, 4H), 7.57-7.32 (m, 6H), 6.04 (d, 1H, *J* = 10.2 Hz), 5.87 (dd, 1H, *J* = 10.2 Hz, 2.0 Hz), 5.61 (d, 1H, *J* = 8.9 Hz), 5.22 (br, 1H), 5.11 (br, 1H), 4.58-4.38 (m, 4H), 3.59 (m, 1H), 3.65 (dd, 1H, *J* = 11.0 Hz, 7.9 Hz), 2.37-2.33 (m, 2H), 2.02-0.83 (m, 38H), 0.65 (s, 3H); LRMS (FAB+) *m/z* 745 (M+Na).

(2*R*,3*S*,4*R*)-2-(Benzoyloxymethyl)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran-3,4-diyl dibenzoate (12)

To a solution of 3,4,6-tribenzoyl glucal (320 mg, 0.7 mmol) in CH_2Cl_2 (10 mL) was added cholesterol (108 mg, 0.28 mmol) and PTSA (11 mg, 0.06 mmol). After stirring for 18 h, the reaction mixture was quenched with water and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 15) afforded 60 mg (26%) of **12** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02-7.89 (m, 6H), 7.58-7.32 (m, 9H), 5.47 (m, 2H), 5.30 (br, 1H), 5.19 (br, 1H), 4.87 (m, 1H), 4.53-4.47 (m, 3H), 3.97 (m, 1H), 3.55 (m, 1H), 2.52-2.15 (m, 3H), 2.02-0.83 (m, 20H), 0.65 (s, 3H); LRMS (FAB+) *m/z* 867 (M+Na); HRMS (FAB+) calcd for $C_{54}H_{68}O_8Na$ (M+Na): 867.4812, found 867.4816.

(2*R*,3*S*)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-Dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15, 16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-2-(hydroxymethyl)-3,6-dihydro-2*H*-pyran-3-ol (13)

To a refluxing solution of sodium (2.5 mg, 0.1 mmol) in MeOH (2 mL) was added a solution of the **11** (33 mg, 0.46 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 1.2 h, cooled to ambient temperature, and then quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with ether and the organic phase was washed with brine, dried over $MgSO_4$ and

concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes : MeOH = 1 : 1 : 0.05) afforded 14 mg (60%) of **13** as a white solid: FT-IR (neat) ν_{max} 3463, 2933, 1461, 1049 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.95 (d, 1H, *J* = 10.2 Hz), 5.75 (dt, 1H, *J* = 10.2 Hz, 2.2 Hz), 5.35 (br, 1H), 5.13 (br, 1H), 4.21 (br, 1H), 3.87 (br, 2H), 3.79-3.73 (m, 1H), 3.57-3.48 (m, 1H), 2.36 (br, 2H), 2.02-1.80 (m, 8H), 0.99 (s, 6H), 0.88 (ddd, 12H, *J* = 18.7 Hz, 6.6 Hz, 1.7 Hz), 0.67 (s, 3H); LRMS (FAB+) *m/z* 537 (M+Na).

(2*R*,3*S*,4*R*)-6-((3*S*,10*R*,13*R*,17*R*)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-1*H*-cyclopenta[α] phenanthren-3-yloxy)-tetrahydro-2-(hydroxymethyl)-2*H*-pyran-3,4-diol (14)

To a refluxing solution of sodium (3 mg, 0.12 mmol) in MeOH (3 mL) was slowly added a solution of **12** (57 mg, 0.067 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred for 1.2 h, cooled to ambient temperature, and quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with ether and the organic phase was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes : MeOH = 2 : 1 : 0.05) afforded 30 mg (84%) of **14** as a white solid: FT-IR (neat) ν_{max} 3394, 2929, 1461, 1070 cm^{-1}; ^{1}H -NMR ($CDCl_3$, 300 MHz) δ 5.32 (br, 1H), 5.05 (d, 0.6H, *J* = 2.1 Hz), 4.67 (d, 0.4H, *J* = 9.3 Hz), 3.98 (br, 0.6H), 3.85-3.81 (m, 2H), 3.66 (m, 1H), 3.55-3.38 (m, 2H), 3.25 (br, 0.4H), 2.31-2.07 (m, 4H), 1.99-1.78 (m, 5H), 1.62-0.99 (m, 20H), 0.97 (s, 6H), 0.86 (ddd, 12H, *J* = 19.5 Hz, 5.2 Hz, 1.8 Hz), 0.64 (s, 3H); LRMS (FAB+) *m/z* 555 (M+Na).

(2*R*,3*S*)-3-Methoxy-2-(methoxymethyl)-6-((3*S*)-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16, 17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran (15)

To a suspension of NaH (33.8 mg, 0.85 mmol) in THF (1 mL) was added a solution of **13** (87 mg,

0.17 mmol) in THF (3 mL) at 0 ºC. After stirring for 1 h, iodomethane (0.11 mL, 1.69 mmol) was slowly added to the reaction mixture. After stirring for an additional 16 h, the reaction mixture was quenched with water, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 30 mg (32%) of **15** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.03 (d, 1H, *J* = 10.23 Hz), 5.73 (d, 1H, *J* = 11.19 Hz), 5.32 (br, 1H), 5.12 (d, 1H, *J* = 2.55 Hz), 3.87 (br, 2H), 3.59 (m, 3H), 3.40 (s, 3H), 3.37 (s, 3H), 2.33 (m, 2H), 2.01-1.71 (m, 5H), 1.55-1.09 (m, 17H), 0.96 (s, 3H), 0.95-0.82 (m, 12H), 0.64 (s, 3H); LRMS (FAB+) *m/z* 565(M+Na).

(2*R*,3*S*,4*R*)-3,4-Dimethoxy-2-(methoxymethyl)-6-((3*S*,17*R*)-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (16)

A glycal (288 mg, 1.53 mmol) and cholesterol (592 mg, 1.53 mmol) were combined with a catalytic amount (5 mol%) of CAN (41 mg) in anhydrous CH_3CN (6 ml) and stirred for 12 h. The reaction mixture was extracted with ether and washed with $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated in a vacuum. The residue was purified by column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 20 mg (2.3%) of **16** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.27 (s, 2H), 5.05 (s, 1H), 3.63 (m, 2H), 3.52 (s, 3H), 3.42 (s, 3H), 3.40 (m, 1H), 3.38 (s, 3H), 3.16 (m, 1H), 2.20 (m, 2H), 1.95-0.99 (m, 24H), 0.97 (s, 3H), 0.85 (dd, 12H, *J* = 6.4 Hz, 13.7 Hz), 0.64 (s, 3H); LRMS (FAB+) *m/z* 597 (M+Na).

(2*R*,3*R*,4*S*,5*R*)-2-(Benzoyloxymethyl)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yloxy)tetrahydro-2*H*-pyran-3,4,5-triyl tribenzoate (17)

To a solution of cholesterol (150 mg, 0.39 mmol) in CH_2Cl_2 (5 mL) was added 4A molecular sieve (500 mg), 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (244 mg, 0.37 mmol) and a solution of silver triflate (105 mg, 0.41 mmol) in dry toluene (1 mL) at -20 °C. After stirring for 2 h, the reaction mixture was filtered through a pad of silica gel. The filtrate was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 140 mg (45%) of **17** as a pale yellow oil: FT-IR (neat) ν_{max} 2929, 1501, 1226 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.99-7.79 (m, 8H), 7.54-7.7.26 (m, 12H), 5.87 (t, 1H, *J* = 9.7 Hz), 5.60 (t, 1H, *J* = 9.7 Hz), 5.47 (dd, 1H, *J* = 9.7 Hz, 7.9 Hz), 5.20 (d, 1H, *J* = 4.7 Hz), 4.91 (d, 1H, *J* = 7. 9 Hz), 4.60-4.46 (m, 2H), 4.12 (m, 1H), 3.50 (m, 1H), 2.15 (br, 2H), 2.01-1.65 (m, 6H), 1.56-0.71 (m, 21H), 0.63 (s, 3H) ; LRMS (FAB+) *m/z* 867 (M+Na).

(3*R*,4*S*,5*S*,6*R*)-2-((3*S*,10*R*,13*R*,17*R*)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-1*H*-cyclopenta[α] phenanthren-3-yloxy)-tetrahydro-6-(hydroxymethyl)-2*H*-pyran-3,4,5-triol (18)

To a refluxing solution of sodium (4 mg, 0.16 mmol) in MeOH (5 mL) was slowly added a solution of **17** (90 mg, 0.11 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 0.5 h, cooled to ambient temperature, and quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with ether and the organic phase was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (CH_2Cl_2 : MeOH = 7 : 1) afforded 22 mg (36%) of **18** as a white solid: FT-IR (neat) ν_{max} 3428, 2936, 1632, 1377, 1025 cm^{-1}; ^{1}H-NMR (Pyridine *d*-5, 300 MHz) δ 5.33 (br, 1H), 5.06 (d, 1H, *J* = 7.7 Hz), 4.57 (d, 1H, *J* = 9.5 Hz), 4.41 (dd, 1H, *J* = 11.7, 4.9 Hz), 4.29 (m, 2H), 4.09-3.91 (m, 3H), 2.72 (d, 1H, *J* = 13.0 Hz), 2.46 (m, 1H), 2.10-1.68 (m, 6H), 1.55-0.89 (m, 33H), 0.63 (s, 3H); LRMS (FAB+) *m/z* 571 (M+Na); HRMS (FAB+) calcd for $C_{33}H_{56}O_6Na$(M+Na): 571.3975, found

571.3974.

(3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-3-[(2-methoxyethoxy)methoxy]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (19)

To a solution of cholesterol (97 mg, 0.25 mmol) in CH_2Cl_2 (1 mL) was added diisopropylethylamine (0.09 mL, 0.5 mmol). After stirring for 1 h, methoxyethoxymethyl chloride (0.3 mL, 2.5 mmol) was added at 0 °C. After stirring for an additional 1 h, the reaction mixture was quenched with saturated aqueous NH_4Cl, and then diluted with EtOAc. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 57 mg (48%) of **19** as a white solid: FT-IR (neat) ν_{max} 2935, 1463, 1375, 1112 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.31 (d, 1H, *J* = 5.3 Hz), 4.75 (s, 2H), 3.70-3.67 (m, 2H), 3.55-3.52 (m, 2H), 3.42 (m, 1H), 3.37 (s, 3H), 2.31-2.26 (m, 2H), 2.00-1.77 (m, 5H), 1.59-1.01 (m, 20H), 0.97 (s, 3H), 0.85 (ddd, 9H, *J* = 14.7, 6.6, 1.3 Hz) 0.65 (s, 3H); LRMS (FAB+) *m/z* 497 (M+ Na).

(3*S*,10*R*,13*R*,17*R*)-3-(Allyloxy)-17-[(1*R*)-1,5-dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16, 17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (20)

To a suspension of NaH (41 mg, 1.03 mmol) in DMF/THF (1: 1, 1 mL) was added a solution of cholesterol (200 mg. 0.52 mmol) in a mixture of DMF/THF (2 mL). After stirring for 1 h, allyl bromide (0.22 mL, 2.59 mmol) was slowly added to the reaction mixture. After stirring for an additional 16 h, the reaction mixture was quenched with water, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 80 mg (36%) of **20** as a colorless oil: FT-IR (neat) ν_{max} 2929, 1462, 1375, 1094 cm^{-1};

^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.87 (m, 1H), 5.32 (d, 1H, *J* = 5.3 Hz), 5.28-5.11 (m, 2H), 4.00 (dt, 2H, *J* = 5.7 Hz, 1.3 Hz), 3.22-3.15 (m, 1H), 2.37-2.20 (m, 2H), 2.01-0.80 (m, H), 0.65 (s, 3H); LRMS (FAB+) *m/z* 426 (M+).

4-((3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-2-buten-1-ol (21)

To a solution of **20** (38 mg, 0.09 mmol) in CH_2Cl_2 (1 mL) was added the 2nd generation Grubbs' catalyst (4 mg, 0.01 mmol) and 1,4-butene diol (0.2 mL). After stirring for 1 h, the mixture was concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 12 mg (30 %) of **21** as a colorless oil: FT-IR (neat) ν_{max} 3394, 2932, 1735, 1464 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.91-5.75 (m, 2H), 5.32 (d, 1H, *J* = 5.1 Hz), 4.14 (d, 2H, *J* = 8.4 Hz), 4.00 (d, 2H, *J* = 5.1 Hz), 3.18 (m, 1H), 2.36-2.15 (m, 2H), 2.00-1.74 (m, 5H), 1.53-1.00 (m, 21H), 0.97 (s, 3H), 0.86 (ddd, 9H, *J* = 14.8 Hz, 6.6 Hz, 1.1 Hz), 0.65 (s, 3H) ; LRMS (FAB+) *m/z* 456 (M+).

(2*R*)-2-[((3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)methyl]oxirane (22)

To a suspension of NaH **(**60% dispersion on mineral oil, 42 mg, 1.1 mmol) in a mixture of DMF/THF (2 mL) was slowly added a solution of cholesterol (200 mg, 0.52 mmol) in a mixture of DMF/THF (1/1 mL). After stirring for 30 minute, a solution of 2S-(+)-glycidyl tosylate in DMF/THF (0.5/0.5 mL) was slowly added. The reaction mixture was vigorously stirred for 26 h at ambient temperature, quenched with saturated aqueous NH_4Cl, and then diluted with ether. The organic phase was washed with 10% aqueous NaOH and brine, dried over $MgSO_4$ and

concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 101 mg (45%) of **22** as a white solid: FT-IR (neat) ν_{max} 2938, 1465, 1107cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.32 (d, 1H, *J* = 5.3 Hz), 3.70 (dd, 1H, *J* = 11.3 Hz, 3.3 Hz), 3.43 (dd, 1H, *J* = 11.3 Hz, 5.7 Hz), 3.25-3.09 (m, 2H), 2.78 (t, 1H, *J* = 4.9 Hz), 2.59 (dd, 1H, *J* = 4.9 Hz, 2.7 Hz), 2.38-2.12 (m, 2H), 2.02- 1.80 (m, 5H), 1.53-1.00 (m, 21H), 0.97 (s, 3H), 0.85 (ddd, 9H, *J* = 15.3 Hz, 6.6 Hz, 1.3 Hz), 0.64 (s, 3H); LRMS (FAB+) *m/z* 441 (M+H).

(3*S*,17*R*)-3-(Benzyloxy)-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (23)

To a suspension of NaH (42 mg, 1.04 mmol) in THF (1 mL) was added a solution of cholesterol (100 mg, 0.26 mmol) in THF (3 mL) at 0 ºC. After stirring for 0.5 h, benzyl bromide (0.12 mL, 1.04 mmol) and tetrabutyl ammonium iodide (14 mg, 0.04 mmol) was added to the reaction mixture. The resulting mixture was refluxed for 16 h and then quenched with a saturated solution of NH_4Cl, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 30) afforded 45 mg (32%) of **23** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.85-6.74 (m, 5H), 4.87-4.83 (m, 3H), 4.04 (s, 2H), 2.75 (m, 1H), 1.93 (m, 1H), 1.75 (m, 1H), 1.55-1.32 (m, 5H), 1.07-0.37 (m, 34H), 0.20 (s, 3H); LRMS (FAB+) *m/z* 499 (M+Na).

(3*S*,17*R*)-17-((*R*)-6-Methylheptan-2-yl)-3-(naphthalen-2-ylmethoxy)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (24)

To a suspension of NaH (42 mg, 1.04 mmol) in THF (1 mL) was added a solution of cholesterol (100 mg, 0.26 mmol) in THF (3 mL) at 0 ºC. After stirring for 0.5 h, 2-bromomethyl naphthalene (69 mg, 0.31 mmol) and tetrabutyl ammonium iodide (29 mg, 0.08 mmol) was added to the

reaction mixture. After stirred for 4 h and then quenched with water, and then diluted with ether. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 15) afforded 13 mg (10%) of **24** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.79 (m, 4H), 7.45 (m, 3H), 5.33 (br, 1H), 4.70 (s, 2H), 3.31 (m, 1H), 2.46-2.26 (m, 2H), 2.00-1.74 (m, 5H), 1.61-1.04 (m, 24H), 1.00 (s, 3H), 0.85 (dd, 9H, *J* = 6.42 Hz, 13.9 Hz), 0.65 (s, 3H); LRMS (FAB+) *m/z* 549 (M+Na).

(3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yl carbamate (25)

To a solution of cholesterol (20 mg, 0.05 mmol) in chloroform (1 mL) was added tricholoroacetyl isocyanate (0.05 mL, 0.42 mmol). After stirring for 2 h, the reaction mixture was diluted with chloroform, followed by filtration through a pad of aluminium oxide. The filtrate was concentrated in vacuo to afforded 7 mg (31%) of **25** as a white solid: FT-IR (neat) ν_{max} 3458, 2932, 1704 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.35 (br, 1H), 4.49-4.43 (m, 3H), 2.33-2.22 (m, 2H), 1.97-1.76 (m, 6H), 1.55-0.98 (m, 22H), 0.94 (s, 3H), 0.82 (ddd, 9H, *J* = 15.3, 6.6, 1.2 Hz), 0.60 (s, 3H); LRMS (FAB+) *m/z* 452 (M+Na).

V. References

1 Yoshimoto, M.; Yoder, M. C. *Nature* **2009**, *457*, 801-803.

2 (a) Lemichez, E.; Lecuit, M.; Nassif, X.; Bourdoulous, S. *Nature Rev. Microbiol.***2010**, *8*, 93-104. (b) Matter, K.; Balda, M.S. *Nature Rev. Mol. Cell. Biol.* **2003***, 4*, 225-236.

3 (a) Bazzoni, G. *Thromb Haemost*, **2006**, *95*, 36-42. (b) Bazzoni, G.; Dejana, E. *Physiol. Rev.* **2004**, *84*, 869-901.

4 (a) Gerber, H.-P.; Dixit, V.; Ferrara, N. *J. Biol. Chem.* **1998**, *273*, 13313-13316. (b) Weis, S. M.; Cheresh, D.A. *Nature*, **2005**, *437*, 497-504. (c) Sanz, J.; Fayad, Z.A. *Nature*, **2008**, *451*, 953-957.

5 (a) Tricot, O.; Mallat, Z.; Heymes, C.; Belmin, J.; Leseche, G.; Tedgui, A. *Circulation* **2000**, *101*, 2450–2453; (b) Segura, A.; Serrano, G.; Gonzalez, M.; Abad, C.; Claveria, L.; Gomez, A.; Bernad, C.; Martinez, H.; Riese, H. *FASEB J*. **2002**, *16*, 833–841; (c) Chavakis, E.; Dimmeler, S. Arterioscler. *Thromb. Vasc. Biol*. **2002**, *22*, 887–893; (d) Hunt, B. J. *Lupus* **2000**, *9*, 189–193; (e) Choy, J.; Granville, D.; Hunt, D. J. *Mol. Cell. Cardiol*. **2001**, *33*, 1673–1690; (f) Rossig, L.; Haendeler, J.; Mallat, Z. *J. Am. Coll. Cardiol*. **2000**, *36*, 2081–2089; (g) Walsh, K.; Smith, R. C.; Kim, H. S. *Circ. Res*. **2000**, *87*, 184–188; (h) Libby, P.; Ridker, P. M.; Maseri, A. *Circulation* **2002**, *105*, 1135–1143. References related to retina; (i) Wong, P. *Biochem. Cell Biol*. **1994**, *72*, 489-498. (j) Levin, L.A.; Louhab, A. *Arch. Ophthalmol*. **1996**, *114*, 488-491. (k) Barber, A. J.; Lieth, E.; Khin, S. A.; Antonetti, D. A.; Buchanan, A. G.; Gardner, T. W. *J. Clin. Invest.* **1998**, *102*, 783-791.

6 (a) Poon, I.K.; Hulett, M.D.; Parish, C.P. *Cell Death Differ.* **2010**, *17,* 381–397. (b) Nartin, S. J.; Green, D. R.; Cotter, T. G. *Trends Biochem. Sci*. **1994**, *19*, 26-30.

7 (a) Samejima, K.; Earnshaw, W. C. *Nat. Rev.* **2005**, *6*, 677-688. (b) Elmore, S. *Toxicol. Pathol.* **2007**, *35*, 495-516.

8 (a) Kam, P.C.; Ferch, N.I. *Anaesthesia* **2000**, *55*, 1081–1093. (b) Chamond, R.R.; Anon, J.C.; Guerra, Pasadas, C.M.A. *Alergol Immunol Clin,* **1999**, *14*, 367-374. (c) Yuan, J.; Yankner, B.A. *Nature* **2000**, *407*, 802-809. (d) Martin, L.J. *Int. J. Mol. Med.* **2001**, *7*, 455-478. (e) Martin, J.B. *N.*

Engl. J. Med. **1999**, *340*, 1970-1980. (f) Krysko, D. V.; D'Herde, K.; Vandenabeele, P. *Apoptosis* **2006**, *11*, 1709-1726. (g) Tricot, O.; Mallat, Z.; Heymes, C.; Belmin, J.; Leseche, G.; Tedgui, A. *Circulation* **2000**, *101*, 2450-2453. (h) Segura, A.; Serrano, G.; Gonzalez, M.; Abad, C.; Claveria, L.; Gomez, A.; Bernad, C.; Martinez, H.; Riese, H. *FASEB J*. **2002**, *16*, 833-841.

9 (a) Stefanec, T. *Chest* **2000**, *117*, 841-854. (b) O'Connell, B.J.; Genest Jr. J. *Circulation*, **2001**, *104*, 1978-1983.

10 (a) Dimmeler, S.; Zeiher, A.M. *Circ. Res.* **2000**, *87*, 434-439. (b) Eissner, G.; Kohlhuber, F.; Grell, M.; Ueffing, M.; Scheurich, P. ; Hieke, A.; Multhoff, G.; Bornkamm, G.W.; Holler, E. *Blood* **1995**, *86*, 4184-4193. (c) Chavakis, E.; Dimmeler, S. *Arterioscler. Thromb. Vasc. Biol*. **2002**, *22*, 887-893. (d) Hunt, B. J. *Lupus* **2000**, *9*, 189-193. (e) Choy, J.; Granville, D.; Hunt, D. J. *Mol. Cell. Cardiol*. **2001**, *33*, 1673-1690. (f) Rossig, L.; Haendeler, J.; Mallat, Z. *J. Am. Coll. Cardiol*. **2000**, *36*, 2081-2089. (g) Walsh, K.; Smith, R. C.; Kim, H. S. Circ. Res. 2000, 87, 184–188; (h) Libby, P.; Ridker, P. M.; Maseri, A. *Circulation* **2002**, *105*, 1135-1143.

11 (a) Alvarez, R. J.; Gips, S. J.; Moldovan, N.; Wilhide, C. C.; Milliken, E. E.; Hoang, A. T.; Hruban, R. H.; Silverman, H. S.; Dang, C. V.; Goldschmidt-Clermont, P. J. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 372-381. (b) Chen, D.-F.; Zhang, H.-L.; Du, S.-H.; Li, H.; Zhou, J.-H.; Li, Y.-W.; Zeng, H.-P.; Hua, Z.-C.; *Steroids* **2010**, *75*, 1119-1126.

12 (a) Liu, X.; Zhao, J.; Xu, J.; Zhao, B.; Zhang, Y.; Zhang, S.; Miao, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2896-2900. (b) Tan, C.-B.; Gao, M.; Xu, W.R.; Yang, X.-Y.; Zhu, X.-M.; Du, G.-H. *Biol. Pharm. Bull.* **2009**, *32*, 1359-1363. (c) Rodrigues, C.; Steer, C.J. *Expert Opin. Investig. Drugs* **2001**, *10*, 1243-1253. (d) Stirling, D. P.; Koochesfahani, K. M.; Steeves, J. D.; Tetzlaff, W. *Neuroscientist* **2005**, *11*, 308-323.

13 (a) Lü, J.-M.; Yao, Q.; Chen, C. *Curr Vasc Pharmacol*. **2009**, *7*, 293-302. (b) Helms, S. *Altern. Med. Rev.* **2004**, *9*, 259-274. (c) Bames, A.S.; Powell-Griner, E.; McFann, K.; Nahin, R.L. *Adv.*

Data. **2004**, *343*, 1-19. (d) Borchers, A.T.; Keen, C.L.; Stern, J.S.; Gershwin, M.E. *Am. J. Clin. Nutr.* **2000**, *72*, 339-347.

14 (a) Ekshyyan, O.; Aw, T. Y. Curr. *Neurovasc. Res*. **2004**, *1*, 355–371; (b) Mattson, M. *Nat. Rev. Mol. Cell Biol.* **2000**, *2*, 120-129.

15 (a) Cho, J.; Park, W.; Lee, S.; Ahn, W.; Lee, Y. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 3510-3515. (b) Al-Dujaili, E.; Chalmers, R.; Sharp, M. *Endocrine Abstracts* **2007**, *13*, 285. (c) Shang, W.; Yang, Y.; Zhou, L.; Jiang, B.; Jin, H.; Chen, M. *J. Endocrinol.* **2008**, *198*, 561-569.

16 (a) Owen, R. *Drugs Today* **1981**, *17*, 343-351. (b) Rivera, E.; Hu, S.; Concha, C. *Vaccine* **2003**, *21*, 1149-1157. (c) Hu, S.; Concha, C.; Lin, F.; Persson, W. K. *Immunol. Immunopathol.* **2003**, *91*, 29-37.

17 (a) Yue, P.; Wong, D.; Wu, P.; Leung, P.; Mak, N.; Yeung, H.; Liu, L.; Cai, Z.; Jiang, Z.-H.; Fan, T.; Wong, R. *Biochem. Pharmacol*. **2006**, *72*, 437-445. (b) Cheng, L.-Q.; Na, J.; Bang, M.-H.; Kim, M.-K.; Yang, D.-C. *Phytochemistry* **2008**, *69*, 218-224. (c) Tian, J.; Fu, F.; Geng, M.; Jiang, Y.; Yang, J.; Jiang, W.; Wang, C.; Liu, K. *Neurosci. Lett*. **2005**, *374*, 92-97.

18 (a) Hasegawa, H. *J Pharmacol Sci*. **2004**, *95*, 153-157. (b) Liu, C. X.; Xiao, P. G. *J. Ethnopharmacol*. **1992**, *36*, 27-38.

19 Yun, T.K. *Lancet Oncol.* **2001**, *2*, 49-55.

20 (a) Morisaki, N.; Watanabe, S.; Tezuka, M.; Zenibayashi, M.; Shiina, R.; Koyama, N.; Kanzaki, T.; Saito, Y.; *Br. J. Pharmacol.* **1995**, *115*, 188-1193. (b) Sato, K.; Mochizuki, M.; Saiki, I.; Yoo, Y.C.; Samukawa, K.; Azuma, I. *Biol. Pharm. Bull.* **1994**, *17*, 635-639.

21 (a) Sengupta, S.; Toh, S.A.; Sellers, L.A.; Skepper, J.N.; Koolwijk, P.; Leung, H.W.; Yeung, H.W.; Wong, R.N.; Sasisekharan, R.; Fan, T.P. *Circulation* **2004**, *110*, 1219-1225. (b) Liu, X.; Kim, C.N.; Yang, J.; Jemmerson, R.; Wang, X. *Cell* **1996**, *86*, 147-157. (c) Leung, K. W.; Pon, Y. L.; Wong, R. N. S.; Wong, A. S. T. *J. Biol. Chem.* **2006**, *281*, 36280-36288.

22 a) Yue, P.; Wong, D.; Wu, P.; Leung, P.; Mak, N.; Yeung, H.; Liu, L.; Cai, Z.; Jiang, Z.-H.; Fan, T.; Wong, R. *Biochem. Pharmacol*. **2006**, *72*, 437-445. (b) Cheng, L.-Q.; Na, J.; Bang, M.-H.; Kim, M.-K.; Yang, D.-C. *Phytochemistry* **2008**, *69*, 218-224. (c) Tian, J.; Fu, F.; Geng, M.; Jiang, Y.; Yang, J.; Jiang, W.; Wang, C.; Liu, K. *Neurosci. Lett.* **2005**, *374*, 92-97.

23 Min, J.-K.; Kim, J.-H.; Cho, Y.-L.; Maeng, Y.-S.; Lee, S.-J.; Pyun, B.-J.; Kim, Y.-M.; Park, J. H.; Kwon, Y.-G. *Biochem. Bioph. Res. Co.* **2006**, *349*, 987-994.

24 Park, I. H.; Kim, N. Y.; Han, S. B.; Kim, J. M.; Kwon, S. W.; Kim, H. J.; Park, M. K.; Park, J. H. *Arch. Pharm. Res.* **2002**, *25*, 428-432.

25 (a) Shibata, S.; Ando, T.; Tanaka, O. *Chem. Pharm. Bull*. **1966**, *14*, 1157-1161; (b) Han, B. H.; Park, M. H.; Han, Y. N.; Woo, L. K.; Sankawa, U.; Yahara, S.; Tanaka,O. *Planta Med*. **1982**, *44*, 146-149; (c) Yuan, C.-S.; Wu, J. *Am. J. Clin. Nutr*. **2002**, *75*, 600-601.

26 (a) Kim, J.-H.; Hong, Y.-H.; Lee, J.-H.; Kim, D.-H.; Nam, G.; Jeong, S. M.; Lee, B.-H.; Lee, S.-M.; Nah, S.-Y. *Mol. Cells* **2005**, *19*, 137-142. (b) Lei, J.; Li, X.; Gong, X.-J.; Zheng, Y.-N. *Molecules* **2007**, *12*, 2140-2150. (c) Danieli, B.; Falcone, L.;Monti, D.; Riva, S.; Gebhardt, S.; Schubert-Zsilavecz, M. *J. Org. Chem.* **2001**, 66, 262-269.

27 *Chemical and Technical Assessment* **2008**, 1-13.

28 β-Sitosterol contained campersterol and sitostanol; the purity of β-sitosterol is up to 70%. The reagent was purchased from Sigma-Aldrich Co.

29 Jursic, B. S.; Upadhyay, S. K.; Creech, C. C.; Neumann, D. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7372-7375.

30 Hogg, N.; Browning, J.; Howard T.; Winterford C.; Fitzpatrick D.; Gobé G. *Endothelium* **1999**, *7*, 35-49.

31 Oishi, K.; Kobayashi, A.; Fujii, K.; Kanehira, D.; Ito, Y.; Uchida, M. K. *J. Pharm. Sci.* **2004**, *96*, 208.

32 (Lazebnik et al., *J. Cell Biol*. **1993**, *123*, 7-22

33 (a) Mesner, P. W.; Bible, K. C.; Martins, L. M.; Kottke, T. J.; Srinivasula, S. M.; Svingen, P. A.; Chilcote, T. J.; Basi, G. S.; Tung, J. S.; Krajewski, S.; Reed, J. C.; Alnemri, E. S.; Earnshaw, W. C.; Kaufmann, S. H. *J. Biol. Chem.* **1999**, *274*, 22635-22645. (b) Faleiro, L.; Kobayashi, R.; Fearnhead, H.; Lazebnik, Y. *EMBO* **1997**, *16*, 2271-2281. (c) Takahashi, A.; Hirata, H.; Yonehara, S.; Imai, Y.; Lee, K.-K.; Moyer, R. W.; Turner, P. C.; Mesner, P. W.; Okazaki, T.; Sawai, H.; Kishi, S.; Yamamoto, K.; Okuma, M.; Sasada, M. *Oncogene* **1997**, *14*, 2741-2752

34 Kapuscinski, J.; Skoczylas, B. *Anal. Biochem.* **1977**, *83*, 252-257.

35 Brunk, C.F.; Jones, K. C.; James, T. W. *Anal. Biochem.* **1979**, *92*, 497-500.

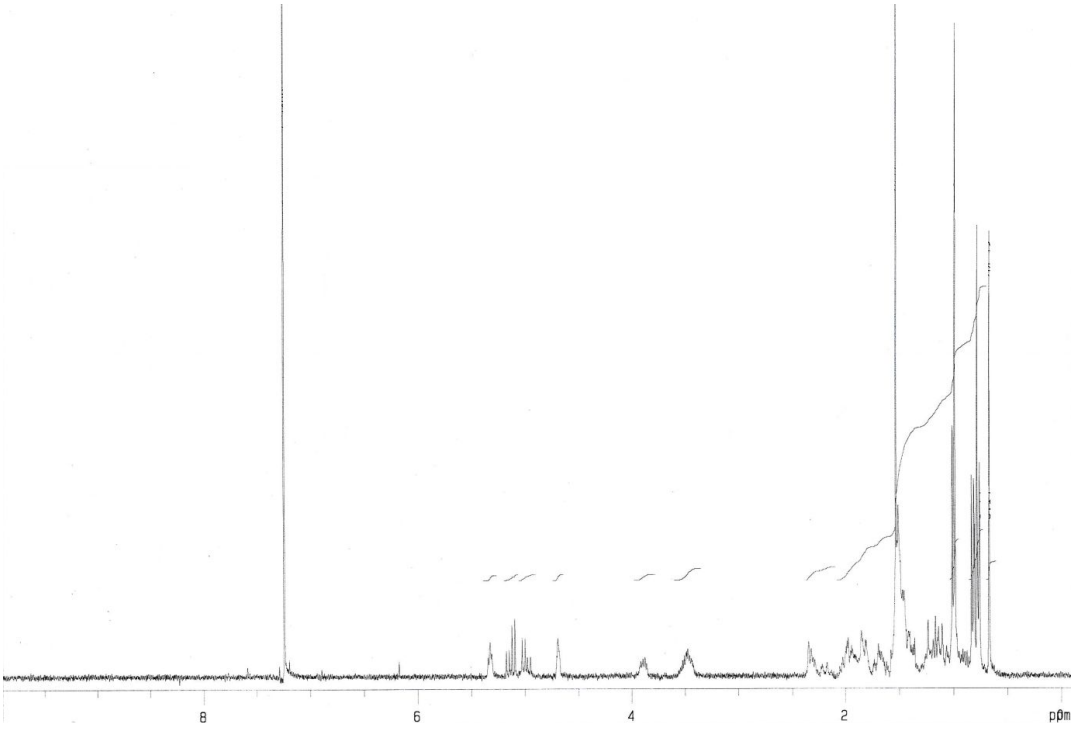
36 Carrillo-Vico, A.; Lardone, P. J.; Naji, L.; Ferna´ndez-Santos, J. M.; Martı´n-Lacave, I.; Guerrero, J. M.; Calvo, J. R. *J. Pineal Res*. **2005**, *39*, 400-408.

37 Parts of these synthetic products and biological assay results have been reported by us. Lee, S.; Maharjan, S.; Kim, K.; Kim, N.-J.; Choi, H.-J.; Kwon, Y. G.; Suh, Y.-G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7102-7105.

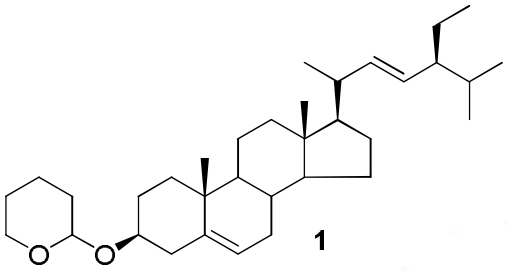
38 Maharjan, S.; Lee, S.; Agrawal, V.; Choi, H.-J.; Maeng, Y.-S.; Kim, K.; Kim, N.-J.; Suh, Y.-G.; Kwon, Y.-G. *Eur. J. Pharmacol.* **2011**, *657*, 35-40.

VI. Appendix

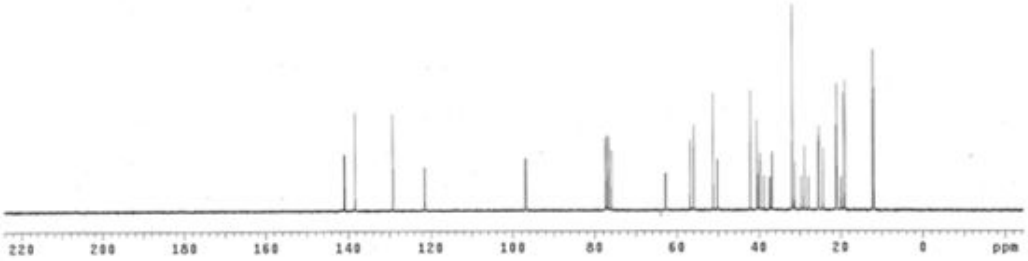
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



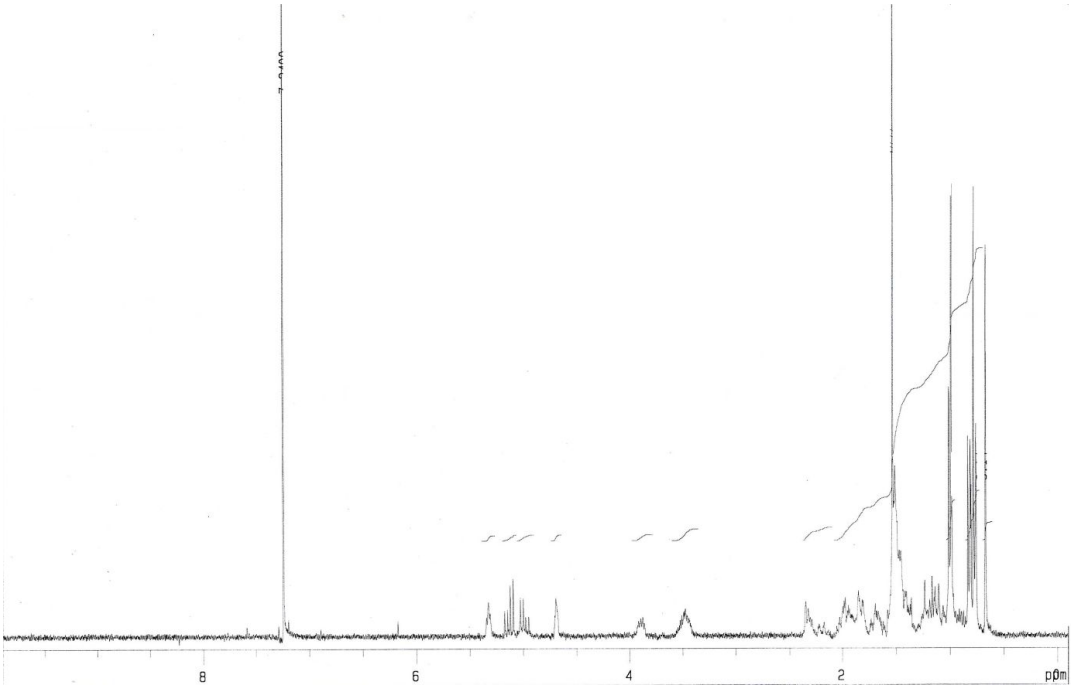
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



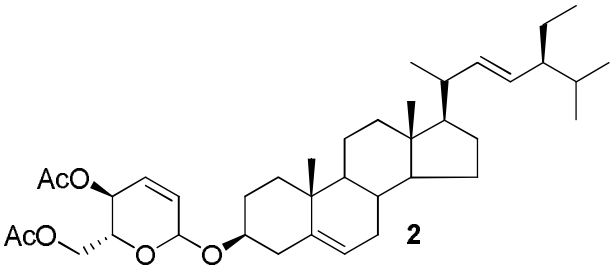
1



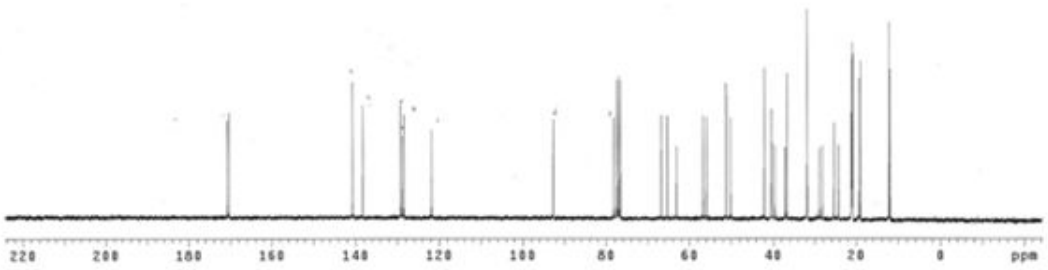
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



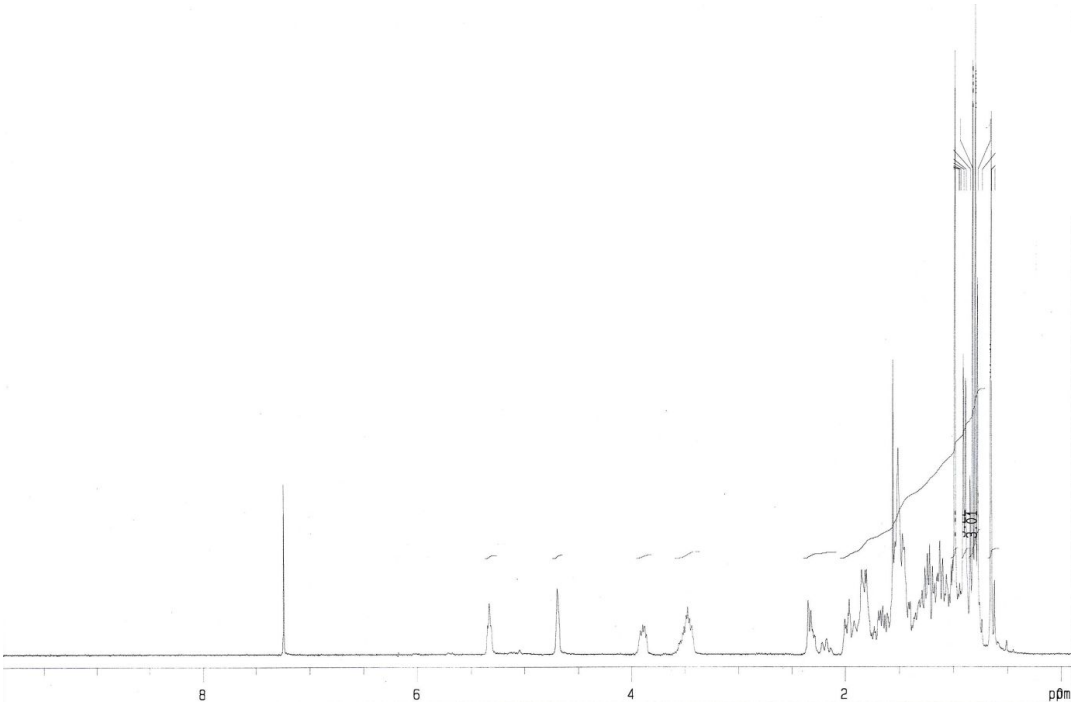
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



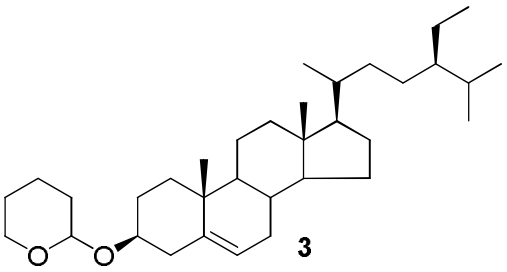
2



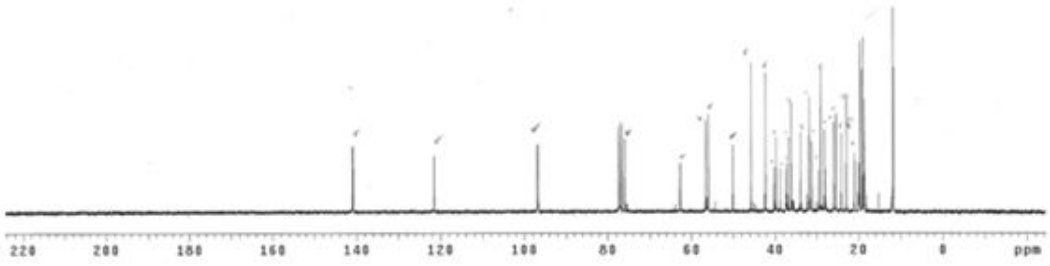
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



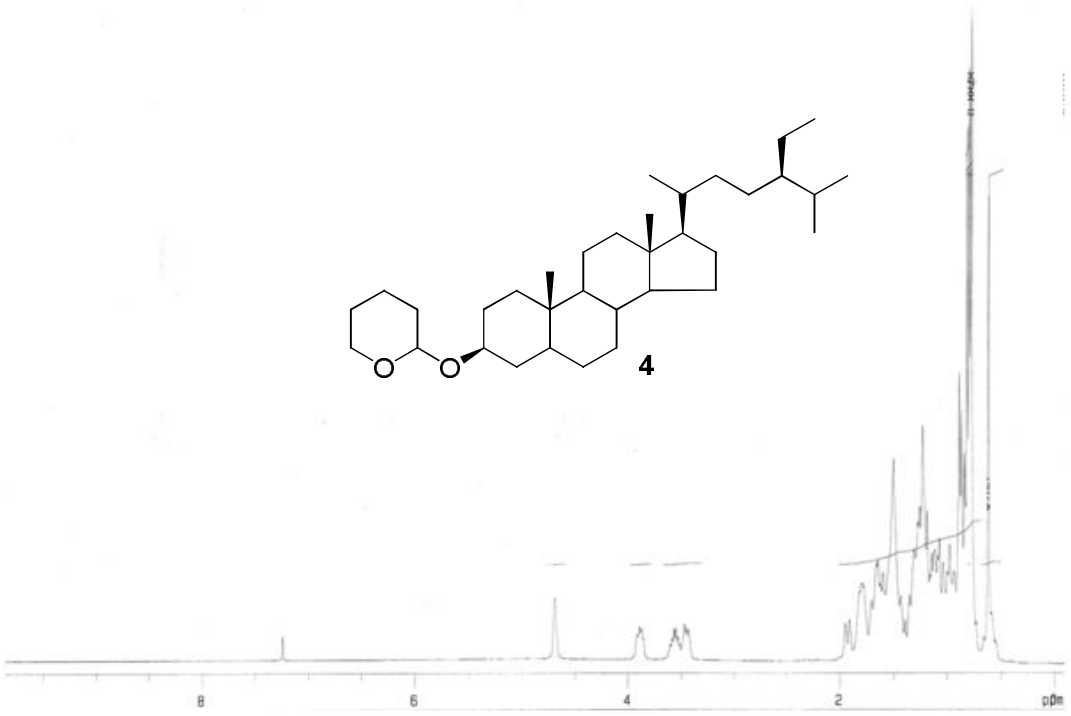
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



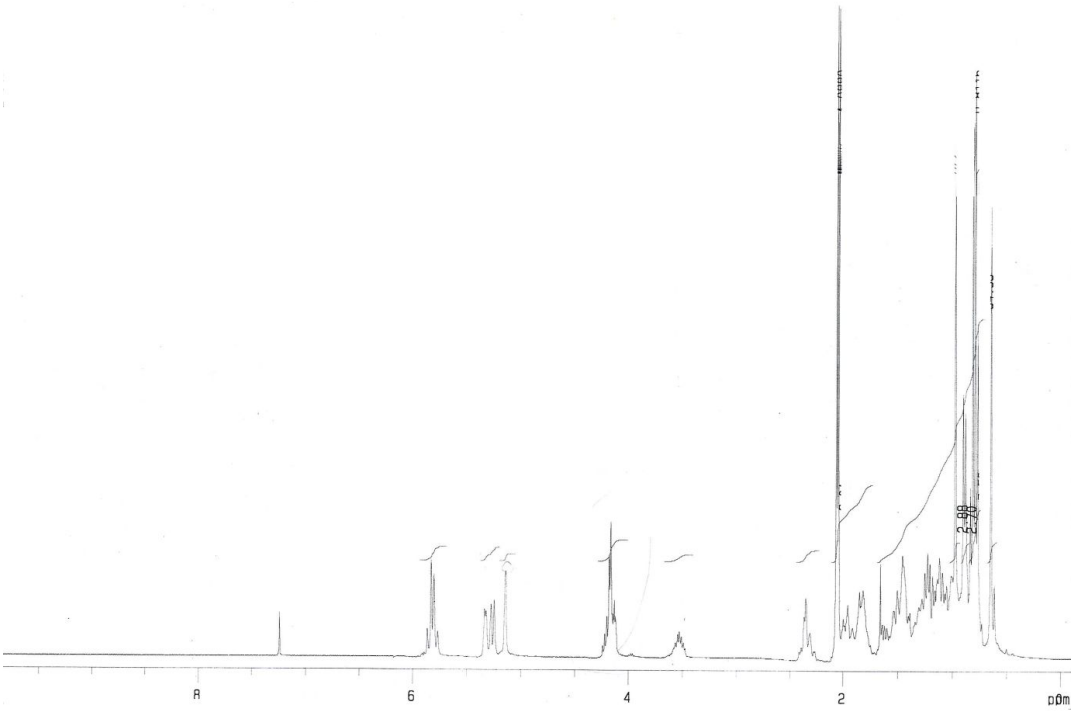
3



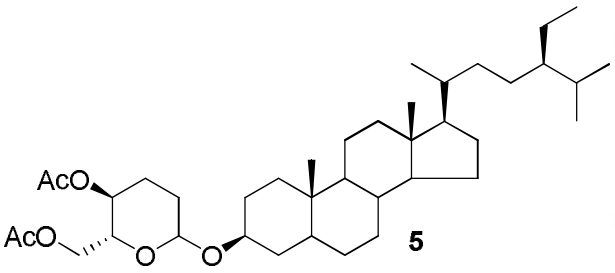
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



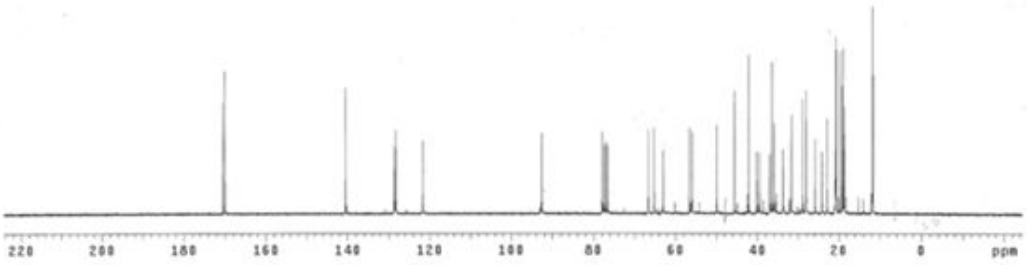
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



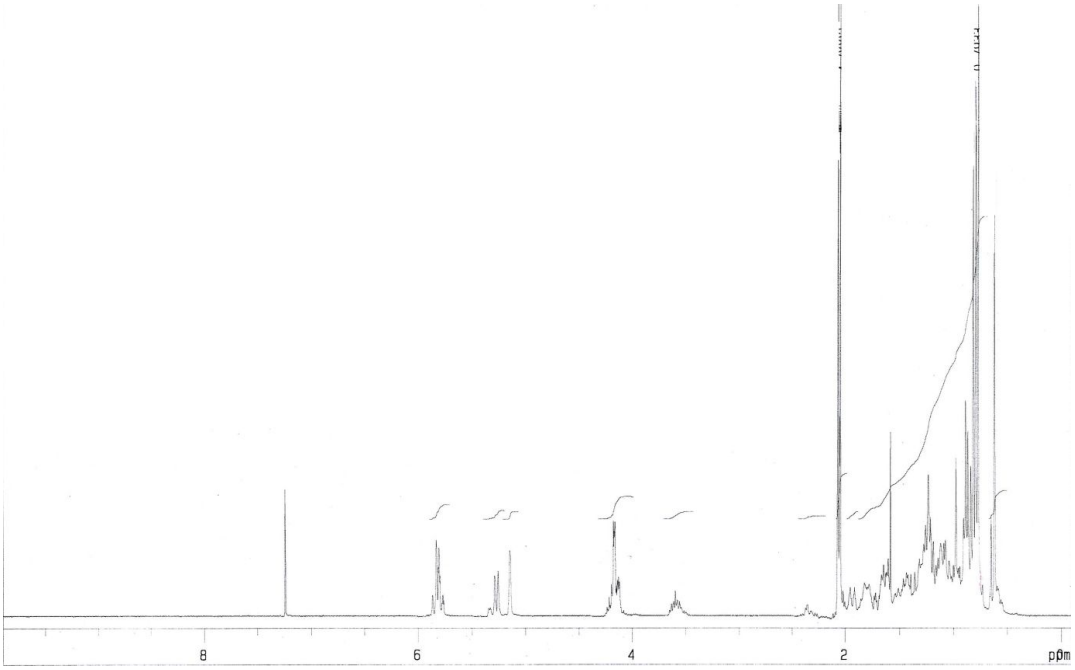
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



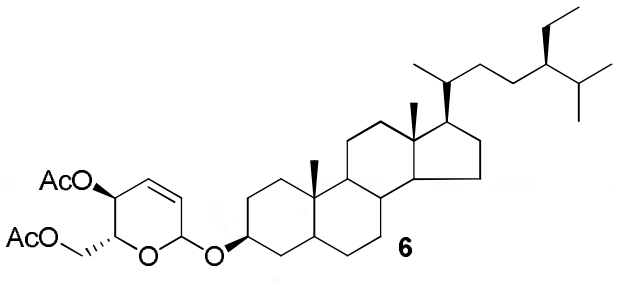
5



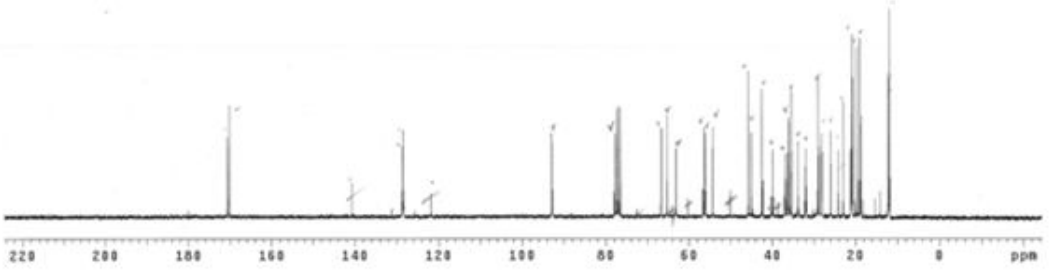
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



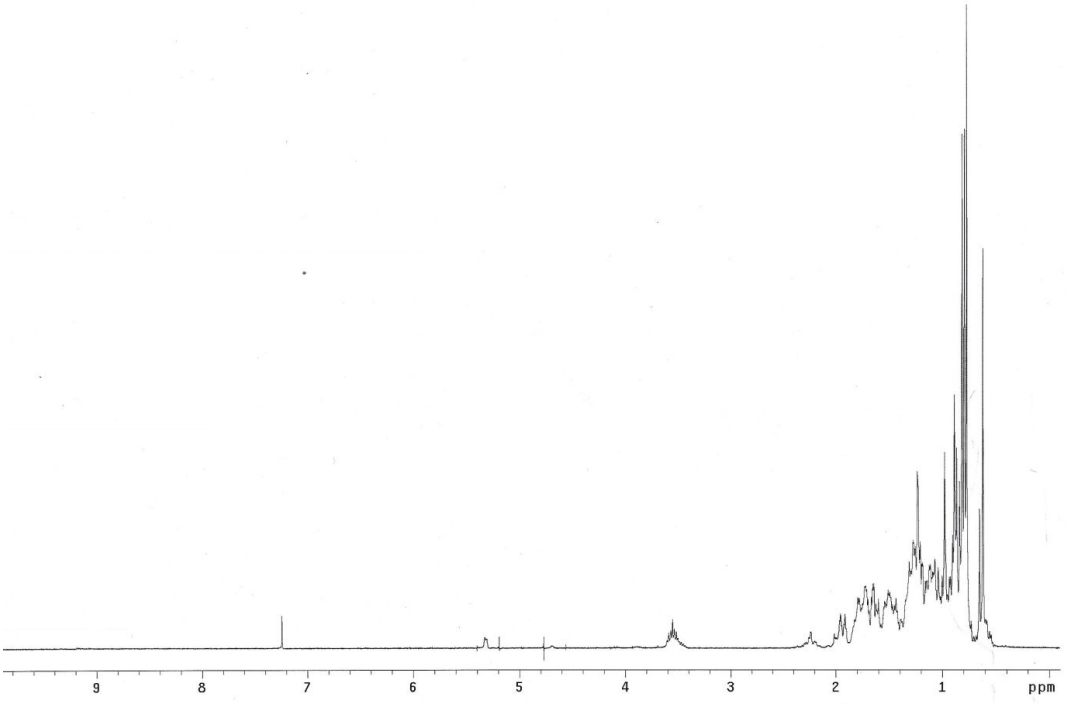
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



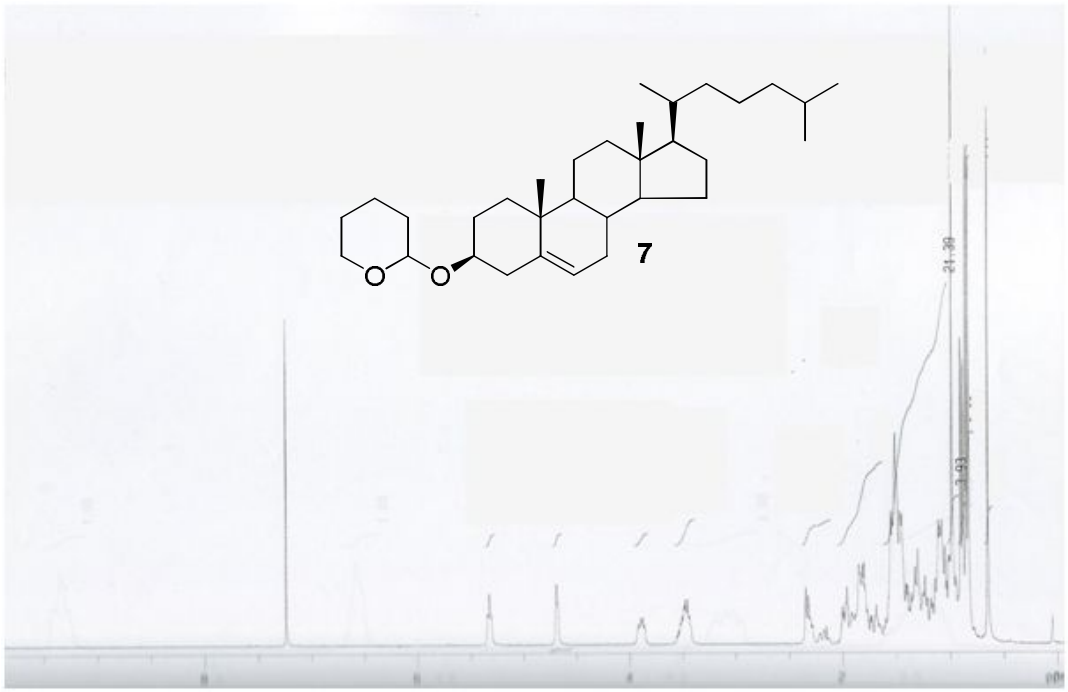
6



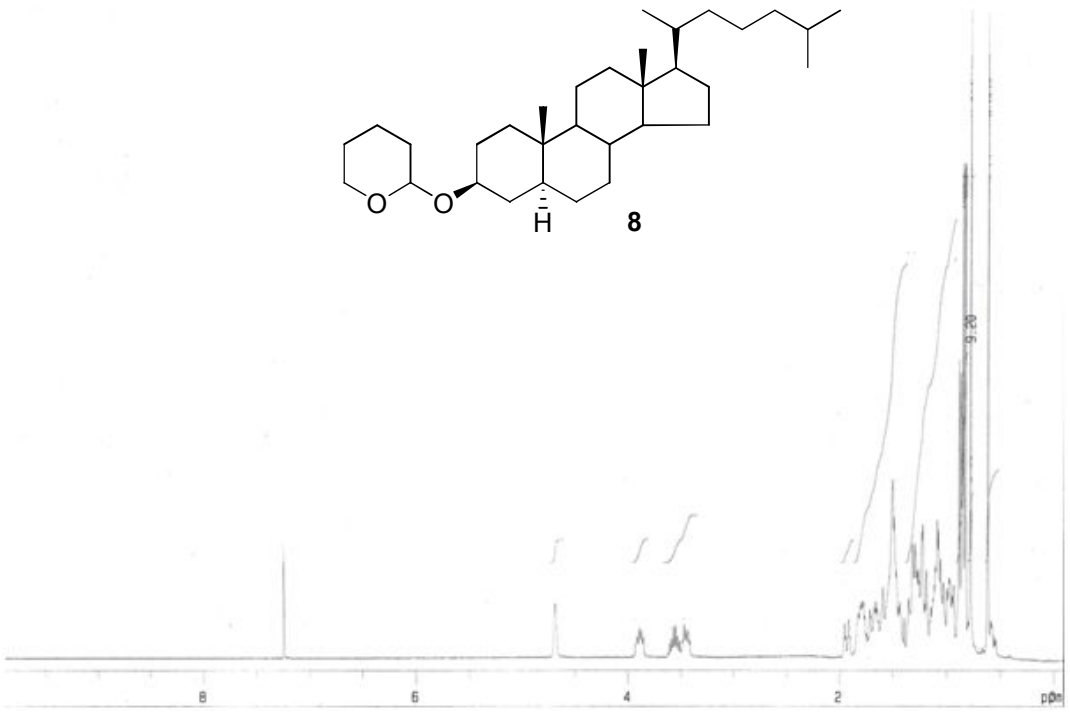
▼ **Sitostanol, ^{1}H-NMR (300 MHz, $CDCl_3$)**



▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**

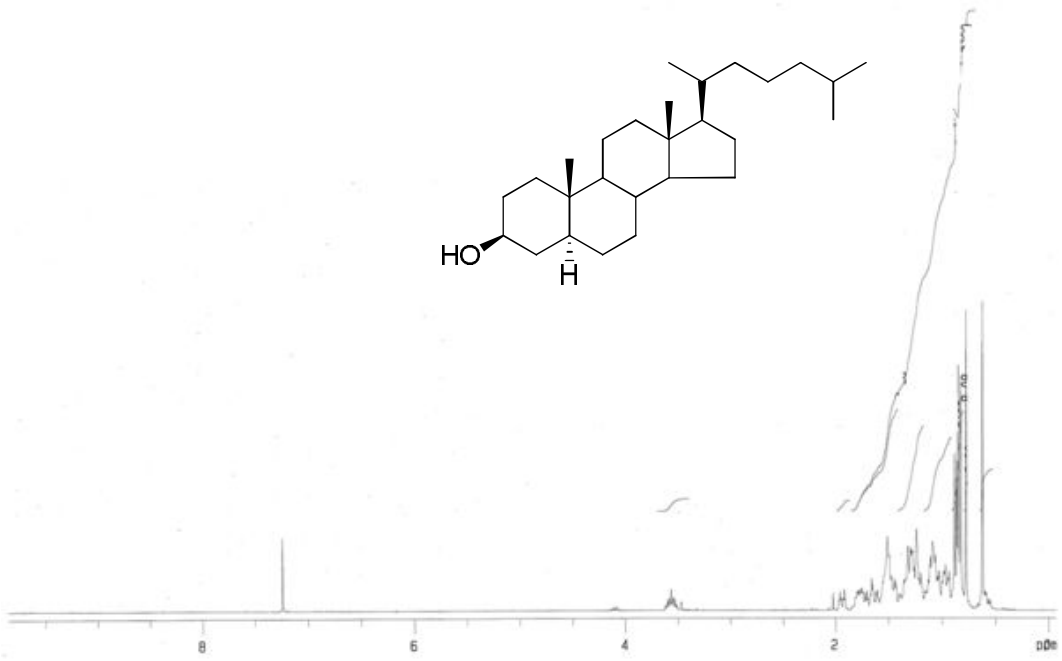


8

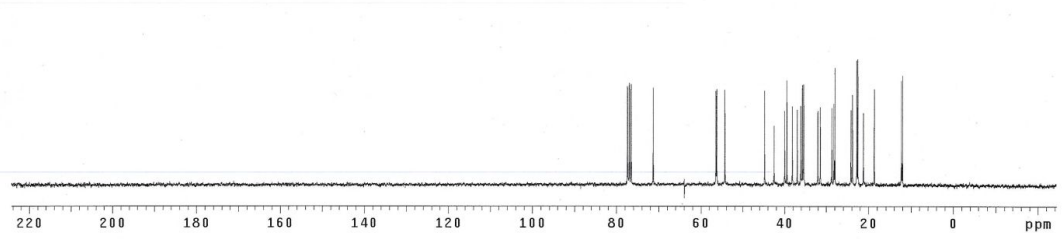
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**

9

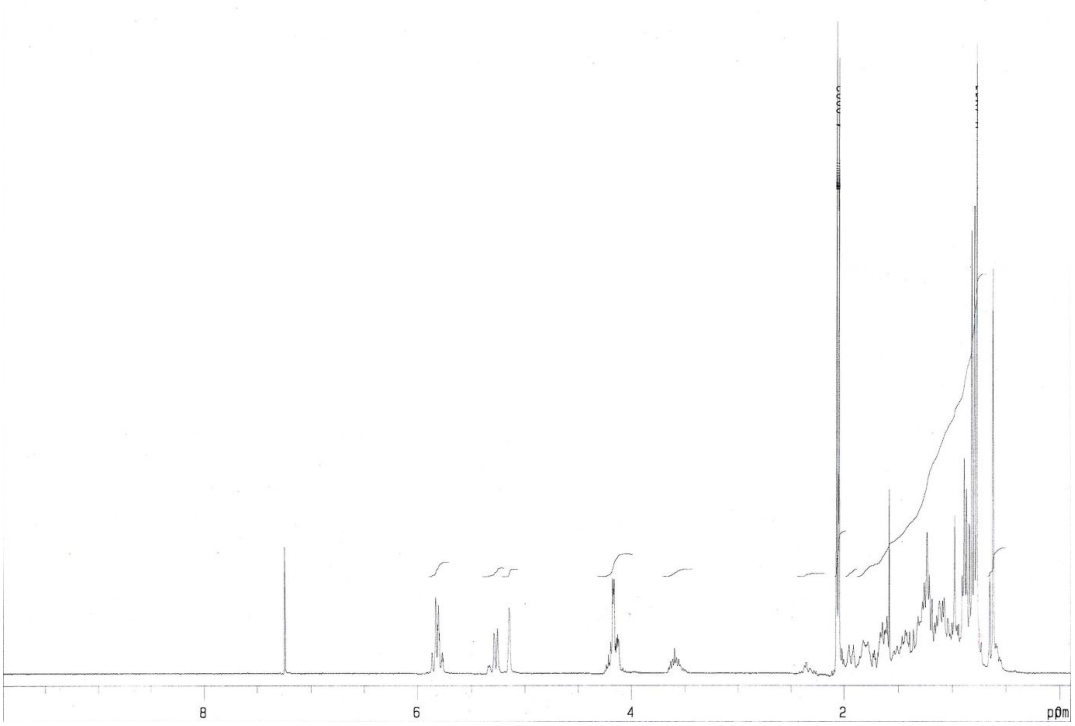
▼ **Cholestanol, ^{1}H-NMR (300 MHz, $CDCl_3$)**



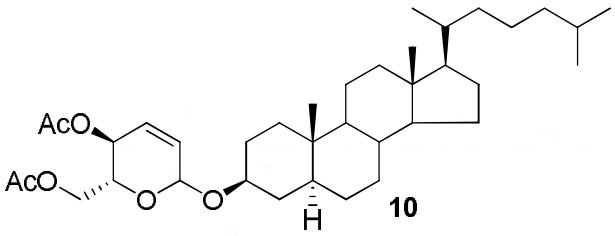
▼ **Cholestanol, ^{13}C-NMR (75 MHz, $CDCl_3$)**



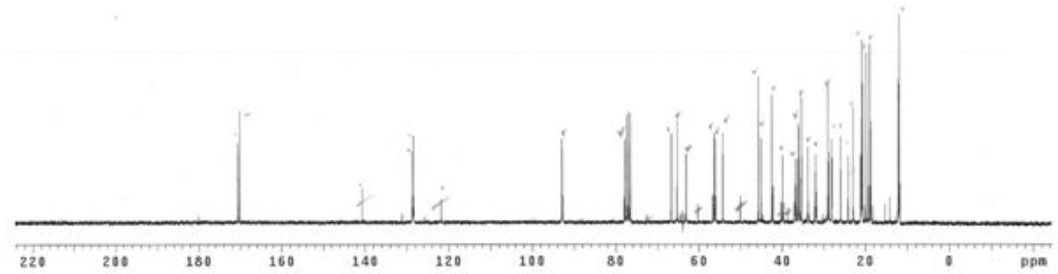
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



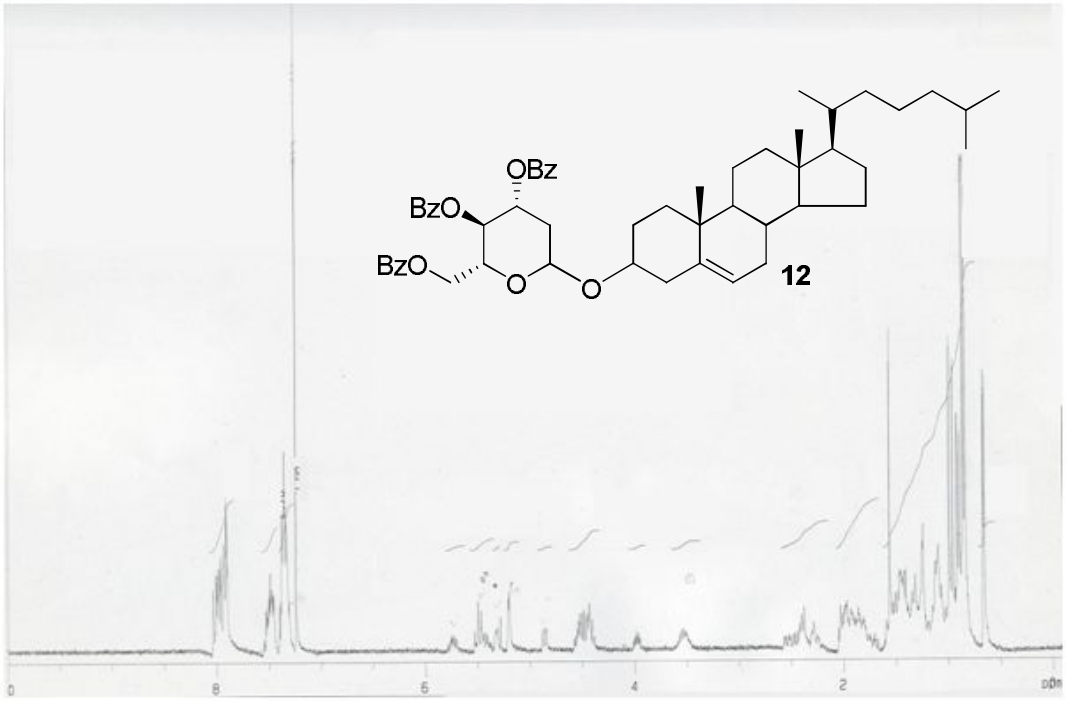
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



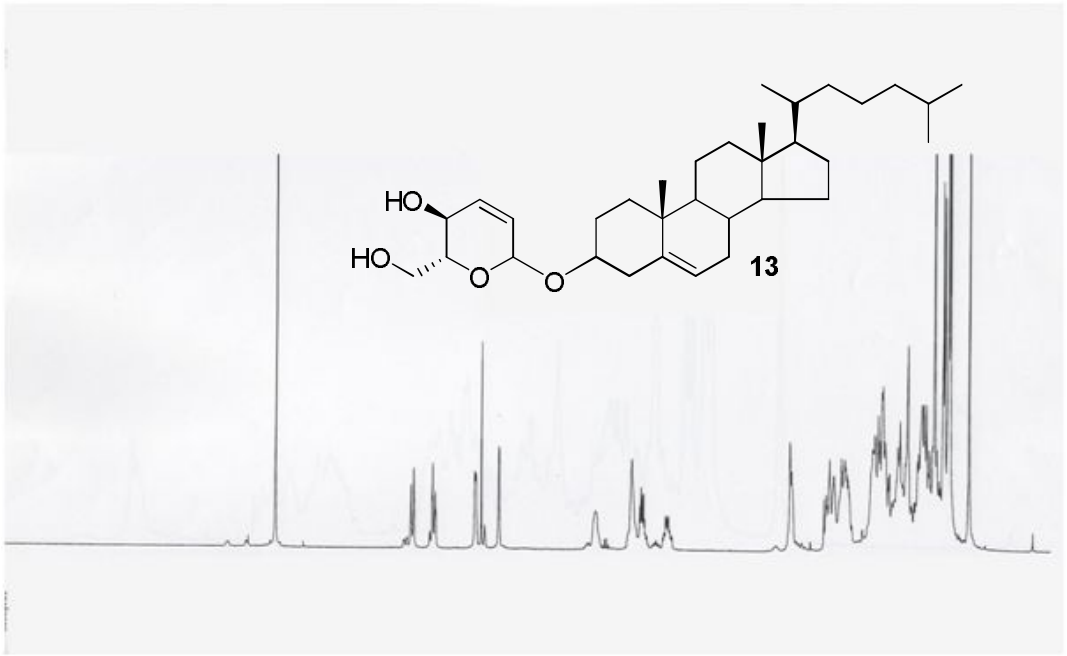
10



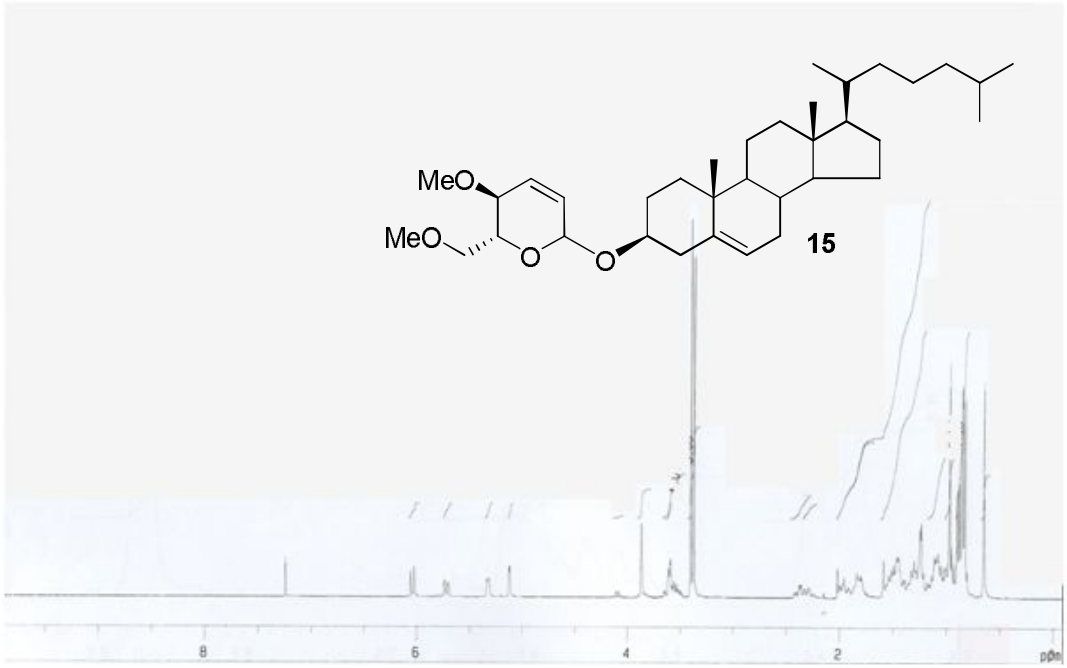
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



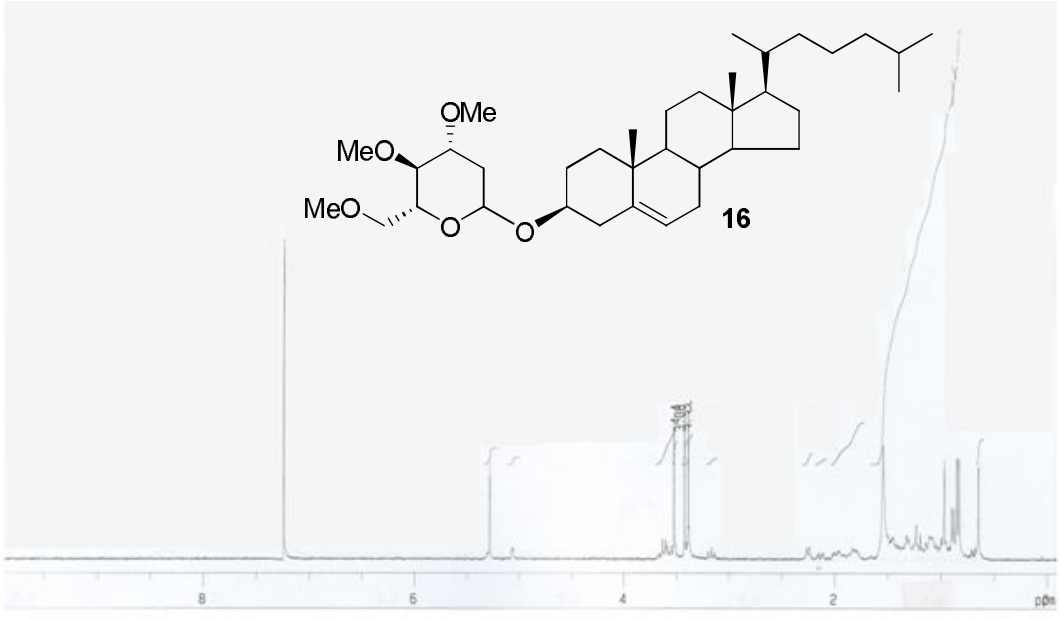
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



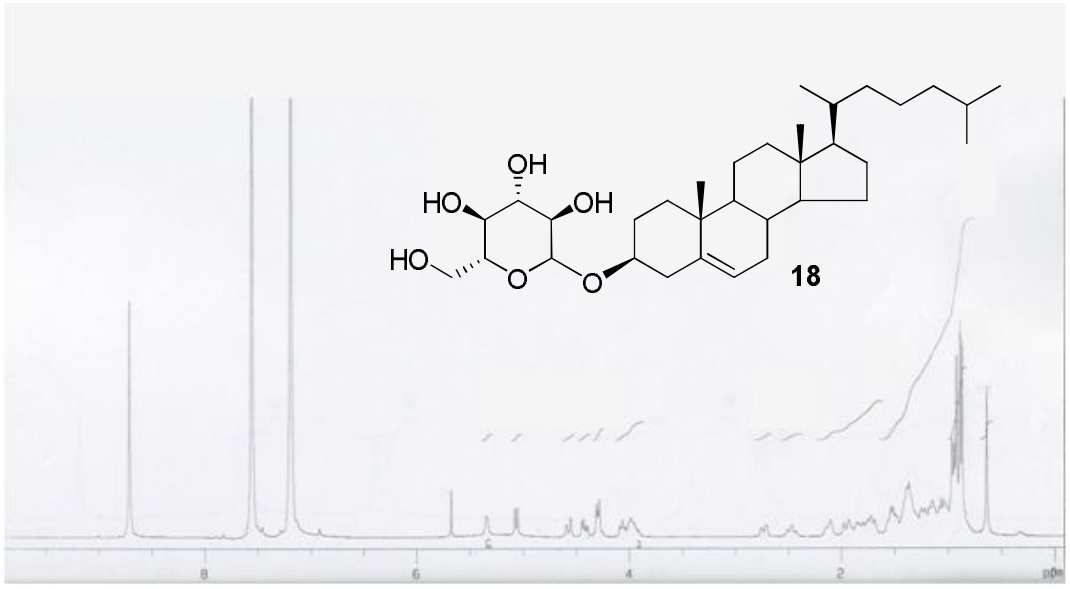
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



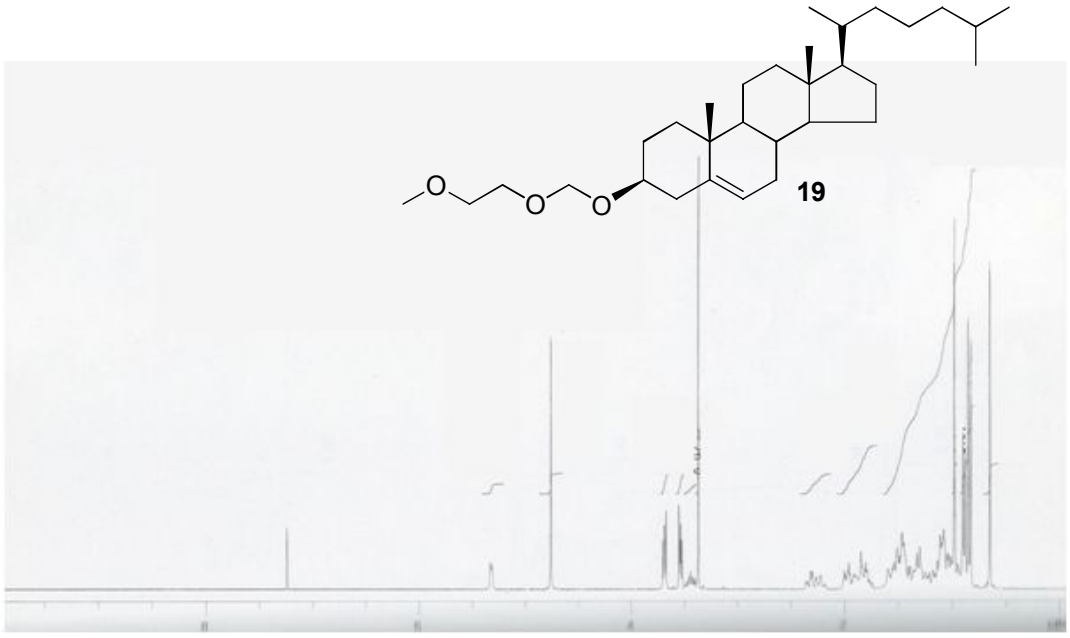
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



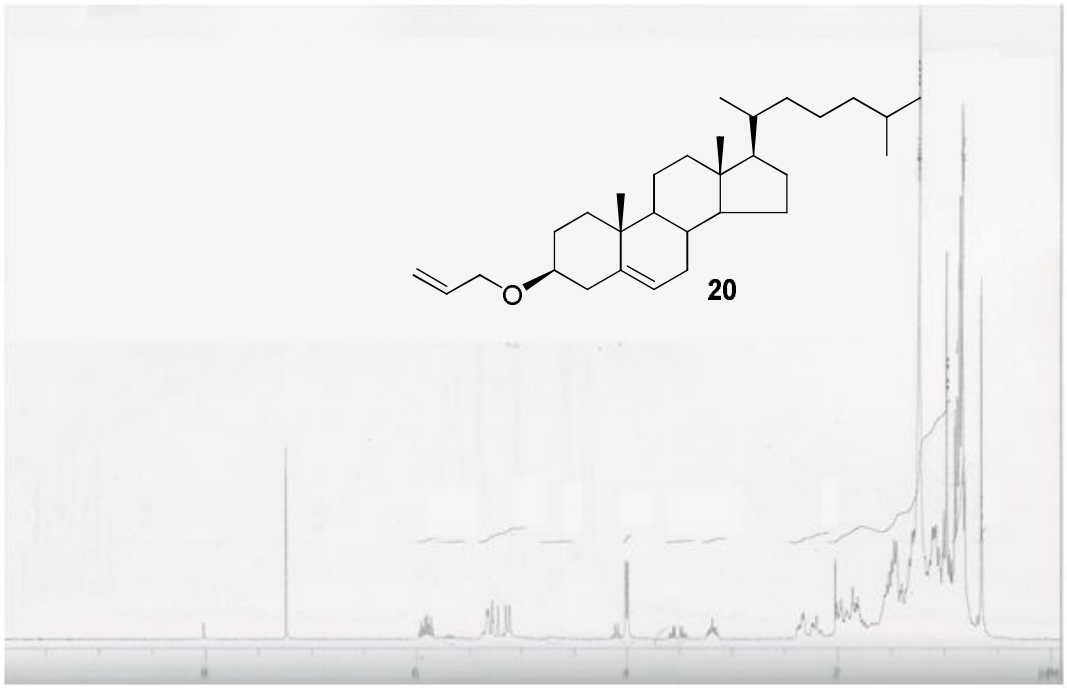
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



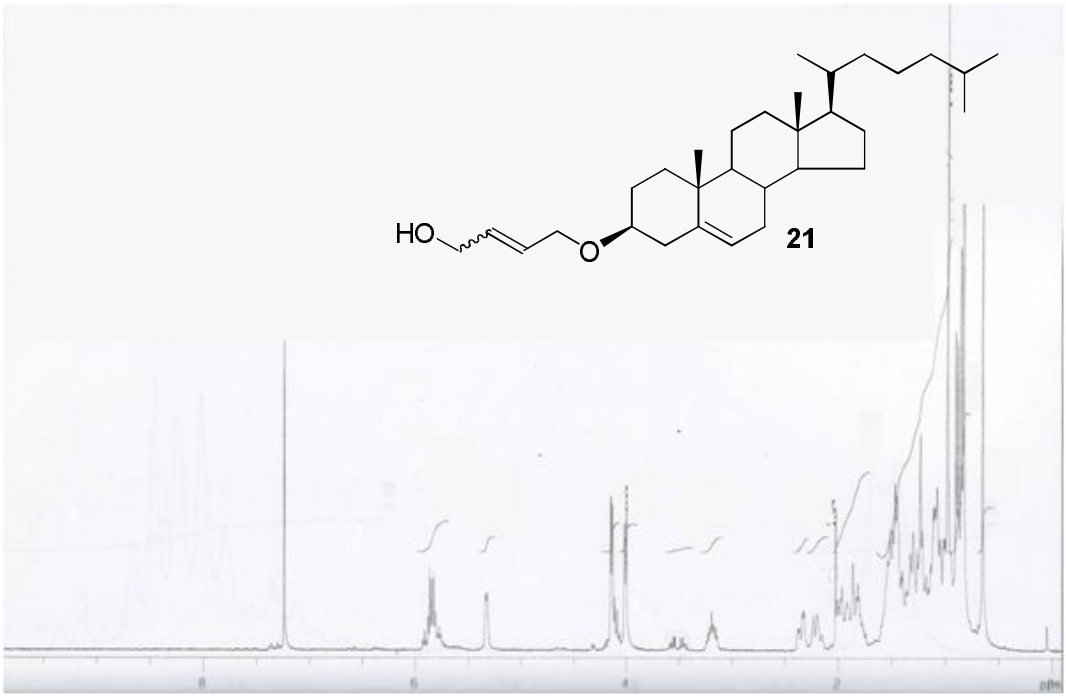
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



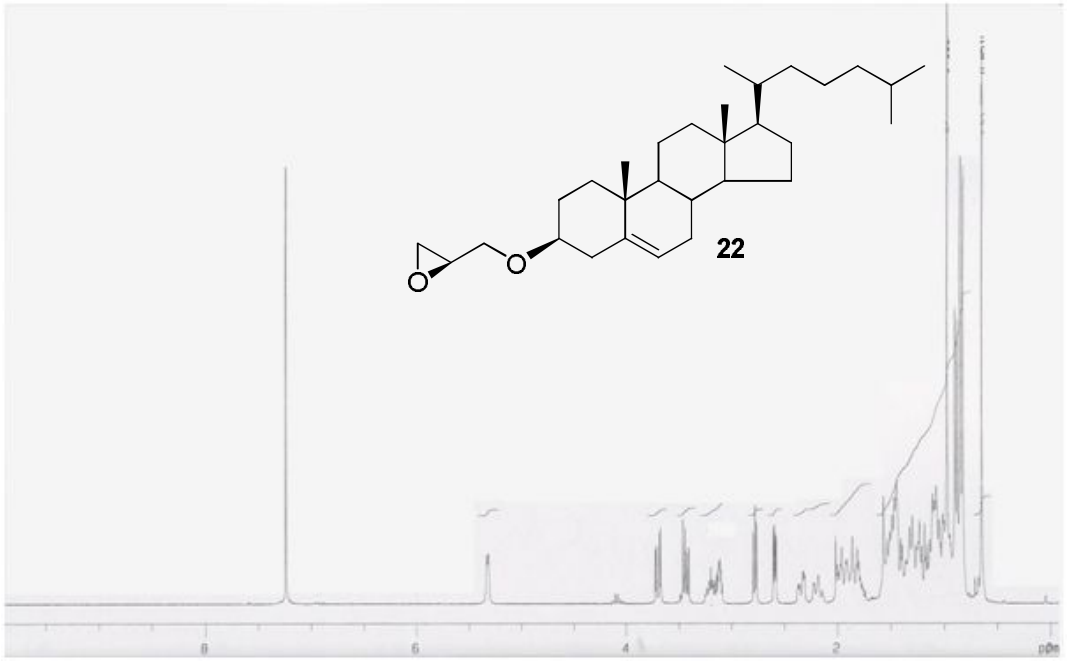
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



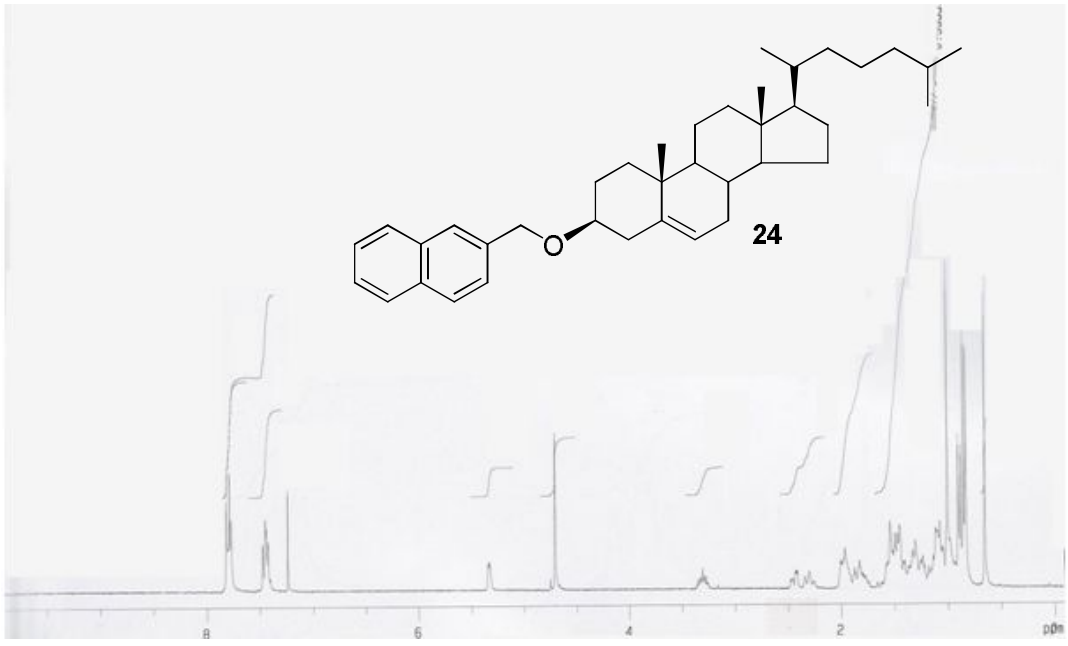
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



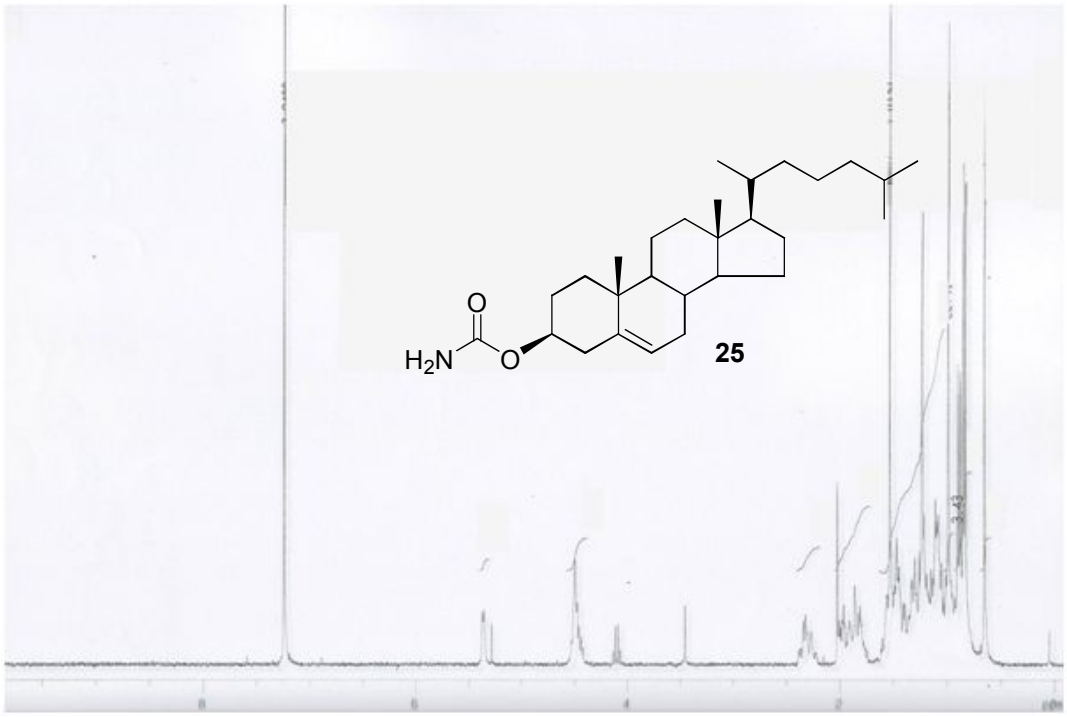
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



VII. Abstract

Cholesterol derived novel anti-apoptotic agent on the structural basis of ginsenoside Rk1

Sujin Lee
College of pharmacy
Department of manufacturing pharmacy
The Graduate School
Seoul National University

The endothelial cell monolayer constructs the lining of the blood vessel. In this particular position, different stimuli activate a cascade of proteases (caspases) that execute a program of cellular self-destruction by cleaving cellular structures at specific sites in a strictly controlled manner. This leads to the disassembly of the cell into small apoptotic bodies without spillage of cellular contents or significant inflammatory responses.

Recently, some ginsenosides isolated from *P. ginseng* have represented the anti-apoptotic activities on HUVECs. However, ginsenosides are generally labile under acidic conditions. And the compositions of ginsenosides in ginseng are variable depending on the growth environment, growth period, and process of extraction. In this connection, we planned to synthesize the anti-apoptotic agents from the simple and easily accessible phytosterol derivatives.

We discovered that the glucosyl moiety at C3 hydroxyl group is essential for anti-apoptotic activity through the comparison of some ginsenosides. We first searched the proper phytosterol for the replacement of the ginsenoside triterpene backbone. In particular, cholesterol, which has the linear side chain at C24, was the best choice. Then, the diverse bioisoster of glucosyl moiety at C3 hydroxyl group was substituted. Cyclic ether, acyclic ether, and spatial interactive bulky substituents were adapted to the C3 hydroxyl group.

With those analogs in hand, we tested the MTT assay on HUVECs. Throughout, the analogs **7** and **9** possessing the cholesterol scaffold and the cyclic carbohydrate equivalents exhibited

excellent cell survival activities, which are equipotent to that of ginsenoside Rk1. In addition, these analogs exhibited dose-dependent cell viability on the HREC line. The excellent human EC protective activities of our novel Rk1-based anti-apoptotic agents allow us to envisage their valuable application for the treatment of vascular disorders. Currently, systematic studies on their therapeutic applications, including in vivo testing of the potent sterol analogs as well as their mechanistic aspects, are in good progress.

Key words: human endothelial cell, apoptosis, cholesterol derivatives, ginsenoside Rg3, Rk1, phytosterol.

Student number: 2006-30449

creative commons
COMMONS DEED

저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

약학박사학위논문

Part I : 천연 **iridoid lactone**의 최초 전합성

Part II: **Ginsenoside Rk1** 구조 기반의 **anti-apoptotic agent**의 개발

Part I : **First total synthesis of natural iridoid lactone**

Part II: **Cholesterol derived novel anti-apoptotic agent on the structural basis of ginsenoside Rk1**

2011년 8월

서울대학교 대학원

제약학과 약품제조화학전공

이 수 진

Table of contents

Part I - First total synthesis of natural iridoid lactone

국문 초록 iii

List of Tables v

List of Figures vi

List of Schemes vii

Abbriviations viii

I. Introduction 1

1. Iridoid 2
   1) Previous synthetic approaches for iridoid lactones 5
2. Pd(0)-catalyzed cyclization 9
   1) Previous studies about Pd(0)-catalyzed cyclization 10
   2) Synthetic applications of bicyclic lactones 12
   3) Transformation of bicyclic lactone 14

II. Result and discussions 16

1. Pd(0)-catalyzed cyclization with substituted allyl carbonate 16
2. Total synthesis of natural iridoid lactone 20

III. Conclusion 33

IV. Experimental 34

V. References 55

VI. Appendix 60

VII. Abstract 98

Part I:천연 iridoid lactone의 최초 전합성

Part I: First total synthesis of natural iridoid lactone

국문 초록

Cyclopentane system은 의약품 및 생리활성을 갖는 천연물에 광범위하게 존재하는 구조로서, 다양한 입체 선택적인 cyclopentane system의 합성법이 여러 연구자들에 의해 개발, 발전되어 왔다. Iridoid 화합물은 *cis*-fused cyclopentane system을 갖는 천연물의 통칭으로, 주로 약용 식물로 이용되던 천연 자원으로부터 다양한 구조의 iridoid가 보고되었다. 그 중 최근 분리, 보고된 (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one의 특징적인 구조와 생리활성에 대한 기대로, 전합성을 시도하였다.

본 연구실에서 개발한 intramolecular Pd(0)-catalyzed allylic alkylation을 응용하여 iridoid 화합물의 합성을 계획하였다. C4에 alkyl기를 도입하기 위해 trisubstituted allyl carbonate가 도입된 γ-lactone을 합성 전구체로 계획하였다. 이를 합성하기 위해 합성 효율성이 높은 silicon-tether를 이용한 분자내 ring closing metathesis를 수행하였고, Pd(0)-catalyzed cyclization을 수행해 좋은 수율과 입체 선택적으로 bicyclic lactone을 합성하였다. 또한 이를 추가로 변환하여 iridoid의 핵심 골격인 *cis*-fused cyclopentane system을 효율적으로 합성하였다. C7-Hydroxymethyl을 입체선택적으로 도입하고자 cyclopentenol에 silicon-tether를 이용한 intramolecular radical cyclization, Tamao-Fleming oxidation을 수행하여 효과적으로 합성을 완료하였다.

본 연구를 통해 본 연구실에서 개발한 substituted vinyl기가 도입된 bicyclic lactone을 중간체로 이용하여 천연물의 합성에 적용할 수 있음을 확인하였고, 향후에 다양한 생리활성을 갖는 iridoid, iridoalkaloid등의 천연물 합성에 응용할 수 있을 것이라 기대한다.

주요어: (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one,

iridoid lactone, cyclopentane, Pd(0)-catalyzed cyclization, bicyclic lactone, silicon-tethered radical cyclization, Tamao-Fleming oxidation.

학 번: 2006-30449

List of Tables

Table 1. Cross metathesis with γ-lactone 18

Table 2. Pd(0)-catalyzed cyclization of 66 24

Table 3. Oxidation to cyclopentenone 26

List of Figures

Figure 1. Structures of cyclopentanoids 1
Figure 2. Structures of iridoids 2
Figure 3. Proposed biosynthetic process for iridoids 4
Figure 4. Palldium catalyzed allylic alkylation 10
Figure 5. Synthetic approach for branched allyl carbonate 16
Figure 6. Plausible transition state of Pd(0)-catalyzed cyclization 20
Figure 7. Target iridoid lactone 21
Figure 8. Bicyclopentenone coordinated with (*S*)-CBS oxazaborolidine 28
Figure 9. 3D-structure of bicyclic enone 28
Figure 10. 1, 6 hydrogen transfer 32

List of Schemes

Scheme 1. The first synthesis of nepetalactone 5
Scheme 2. Synthesis of *ent*-(-)-nepetalactone 6
Scheme 3. Synthesis of nepetalactone via oxidative radical cyclization 6
Scheme 4. Synthesis of (-)-7-deoxyloganin via NHC catalyzed rearrangement of α,β-unsaturated enolester 7
Scheme 5. Synthesis of (+)-geniposide via phosphine-catalyzed [3+2] cycloaddition 8
Scheme 6. Pd(0)-catalyzed allylic alkylation of acyclic allylic precursors 10
Scheme 7. Pd(0)-catalyzed cyclization with γ-lactone..... 11
Scheme 8. Synthetic applications with bicyclic lactone-formal synthesis of carbaprostacyclin 12
Scheme 9. Total synthesis of (+)-brefeldin A..... 13
Scheme 10. Total synthesis of bacillariolideⅢ 14
Scheme 11. Transformations of bicyclic lactone 15
Scheme 12. Syntheses of branched allyl carbonate γ-lactones..... 17
Scheme 13. Pd(0)-catalyzed cyclization with branched γ-lactone allylic carbonate... 19
Scheme 14. Retrosynthetic approach 21
Scheme 15. Synthesis of bicyclic pentanone 22
Scheme 16. Revised retrosynthetic approach 23
Scheme 17. Synthesis of γ-lactone 64d..... 23
Scheme 18. Attempt with bicyclic system 25
Scheme 19. Basic work up with cyclopentanone 27
Scheme 20. Revised retrosynthetic approach 29
Scheme 21. Synthesis of radical cyclization precursor 82 30
Scheme 22. Completion of synthesis of iridoid lactone 1..... 31

Abbriviations

br: broad singlet

BMCMDS: (bromomethyl) dimethylchlorosilane

calcd: calculated

CBS: Corey-Bakshi-Shibata

d: doublet

DBU: 1,8- diazabicyclo[5.4.0]undec-7-ene

DMAP: 4-dimethylaminopyridine

DMF: *N,N*-dimethylformamide

DMP: Dess-Martin periodinane

DMSO: dimethylsulfoxide

dr; diastereomeric ratio

EtOAc: ethylacetate

FAB: fast atom bombardment

GPP: geranylpyrophosphate

HRMS: high resolution mass spectroscopy

HWE: Horner-Wadsworth-Emmons reaction

IR: infrared

LiTMP: lithium tetramethylpiperidide

LRMS: low resolution mass spectroscopy

m: multiplet

Me: methyl

MeOH: methyl alcohol

NHC: *N*-heterocyclic carbene

NMO: 4-methylmorpholine *N*-oxide

NMR: nuclear magnetic resonance

OBO: 2,6,7-trioxabicyclo[2.2.2]octane

PDC: pyridinium dichromate

Ph: phenyl

PPTS: pyridinium *p*-toluene sulfonate

q: quartet

RCM: ring-closing metathesis

s: singlet

SAR: structure-activity relationship

t: triplet

TBAF: tetra-*n*-butylammonium fluoride

TBS: *t*-butyldimethylsilyl

TFAA: trifluoroacetic anhydride

THF: tetrahydrofuran

TLC: thin layer chromatography

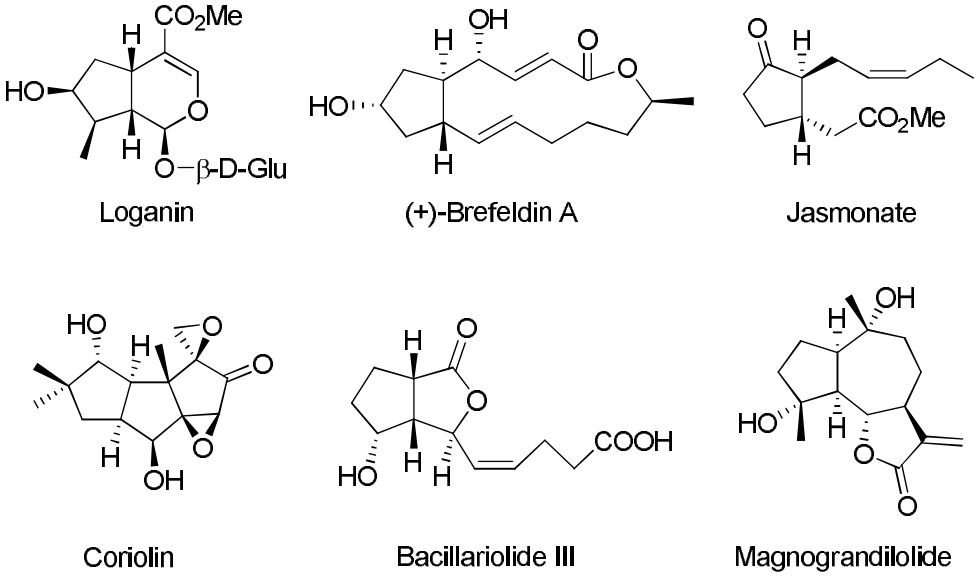
TPAP: tetra-*n*-propylammonium perruthenate

TsOH: *p*-toluene sulfonic acid

I. Introduction

Cyclopentane system is an abundant structure in nature and bioactive chemicals, such as prostaglandins, iridoids, sesquiterpenoids and guaianolides. (Figure 1) And it has been reported that the cyclopentanoids represented various activities, such as, antibacterial, antiviral, antiproliferative, antipyretic and anti-hypertensive effects.[1] Thus, considerable effort has been devoted in the past decades to the development of new methods for the construction of five-membered carbocycles, since they play a fundamental role in synthetic organic chemistry, as both targets and intermediates.

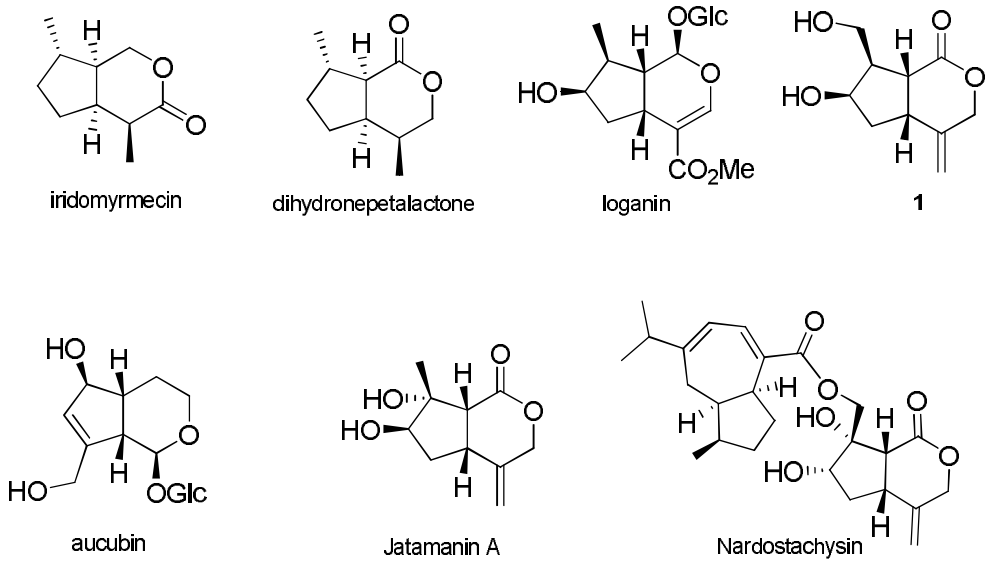
Figure 1. Structures of cyclopentanoids



The construction of five-membered carbocycles has attracted extensive attention among synthetic chemists. Considerable diverse methodologies emerged and were summarized in a series of reviews, which can be divided in two general categories: procedures based on the sequential functionalization of cyclopentanoid precursors, such as cyclopentene[2] and cyclopentadiene,[3] and

ring-formation routes from acyclic molecules, some being applied as key steps in the restructuring of readily available chiral materials, such as carbohydrates,[4] quinic acid,[5] and cyclohexadiene diols, into carbocycles. [6] The search for new, more efficient, and versatile antitumor, anticancer, antibacterial, antimicrobial, etc. agents is among the priorities of the worldwide science. In particular, a serious part of the efforts of the leading scientific and industrial chemical organizations nowadays is directed toward the synthesis of natural and biologically active products, and the improvement of the routes for the preparation of cyclopentanoid moieties is still growing at a steady pace.

Figure 2. Structures of iridoids



1. Iridoid

Iridoids are large class of naturally occurring compounds with over 300 members in the family

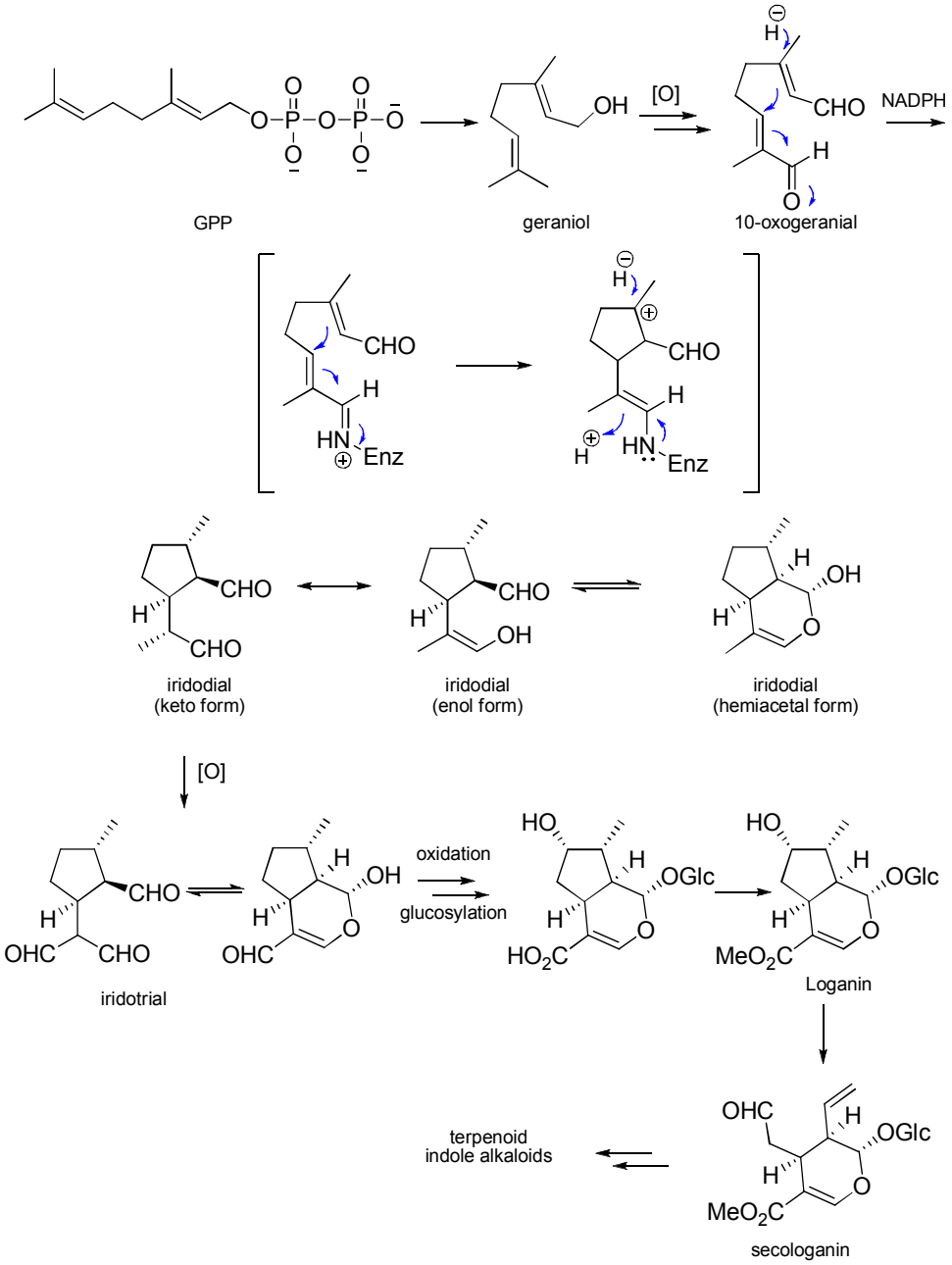
and still expanding. The first isolated iridoid was isolated from the secretion of ants belonging to the genus *Iridomyrmex,* the name of 'iridoid' was originated from that fact.[7] The iridoids have been found in a number of folk medicinal plants used as bitter tonics, sedatives, hypotensives, antipyretics, cough medicines, remidies for wounds and skin disorders. This fact encouraged to investigate the bioactivities of these phytochemicals. Structurally, iridoids represent a highly oxygenated monoterpenoids skeleton characterized by *cis*-fused cyclopenta[*c*]pyran. The representative structures of iridoids are presented at Figure 2.

The iridoid lactones can be further subdivided into two groups, type I and type II, depending on the regiochemical orientation of the lactone with respect to the functionalized cyclopentane ring. In type I iridoids typified by iridomyrmecin the lactone carbonyl is at C3, whereas in type II lactones such as dihydronepetalactone the carbonyl is at C1.

Proposed biosynthetic pathway is shown at Figure 3. Geraniol was produced by geranylpyrophosphate (GPP) by enzyme reaction. Geraniol underwent hydroxylation and oxidation reactions to 10-oxogeranial. The skeleton of iridoid arises from iridodial, which is formulated by cyclization as reductive Michael addition with hydride on the dialdehyde. Or imine formation by enzyme assisted carbocationic cyclization to cyclopentane.[8] The imine mediated pathway is more acceptable for the natural synthetic mode.

Iridodial was found to serve in the chemical defense system of many insect species,[9] and iridodial is the key precursor of numerous iridoids, possessing methyl group at C4, such as nepetalactone.[10] Further oxidized iridotrial has converted to loganin via oxidation, glucosylation and methylation. Loganin is a key intermediate in the pathway to a range of complex terpenoid indole alkaloids and tetrahydroisoquinoline alkaloids. And secologanin, which has been produced by oxidative cleavage from loganin, is a key intermediate for secoiridoids.

Figure 3. Proposed biosynthetic process for iridoids

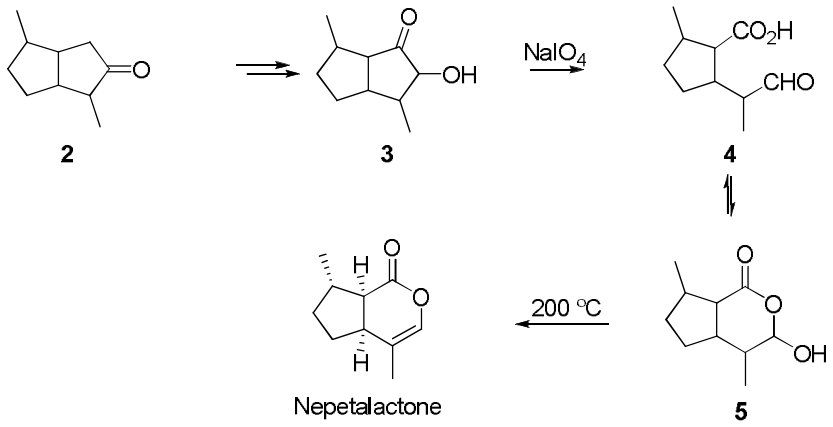


The various biological activities of iridoids have continuously attracted interests from both biologists and chemists. The unique *cis*-fused cyclopenta[*c*]pyran ring system has presented a variety of challenges for chemical synthesis and in the analysis of biological activities. Numerous syntheses of iridoids have been described in the literature.

1) Previous synthetic approaches for iridoid lactones

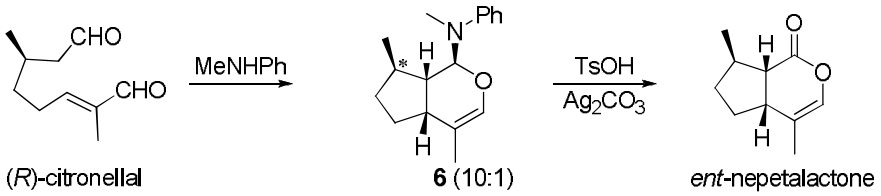
The representative synthetic approaches for type II iridoids are subscribed as below.
The key features of these syntheses involved the formation of cyclopentane and stereoselective introduction of pyran ring system to cylopentane.

Scheme 1. The first synthesis of nepetalactone



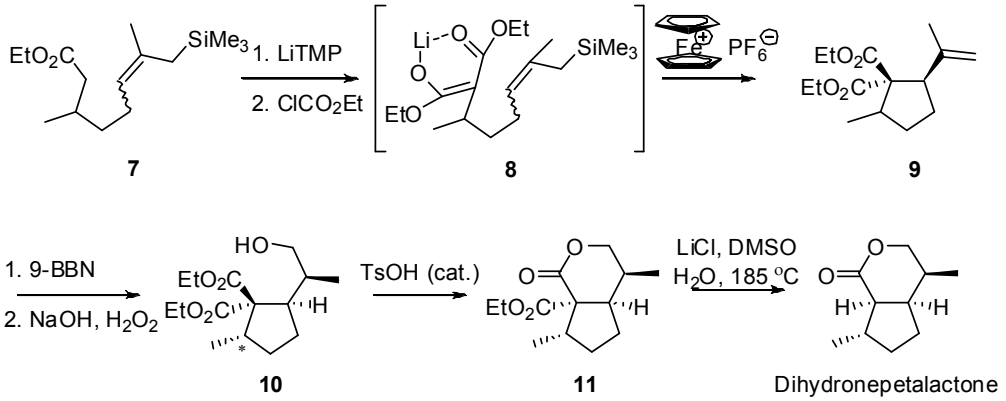
The first synthesized type II iridoid was nepetalactone.[11] Sakan et al. achieved α-ketol **3** as a key intermediate from bicyclooctanone [3.3.0] **2** and oxidative cleavage afforded to aldehyde acid **4**. The synthesis has accomplished by pyrolytic dehydration from lactol **5**. This piece of work was not a stereoselective synthesis. There are so many synthetic achieves since this synthesis has achieved. Here represent asymmetric syntheses by sigmatropic rearrangement.

Scheme 2. Synthesis of *ent*-(-)-nepetalactone



Schreiber et al. synthesized *ent*-nepetalactone via intramolecular enal-enamine cycloaddition [2+4].[12] (*R*)-citronellal treated with methyl phenylamine afforded enamine, and then furnished intramolecualr cycloaddition of enamine and enal provided a separable 10:1 mixture of the desired dihydropyran product **6** and its methyl epimer in 84% yield. Equilibration furnished a better 25:1 ratio of diastereomers, although in lower yields (60-65%). Then the dihydropyran **6** was hydrolyzed to unsaturated lactol, which was oxidized with silver carbonate (Fetizon oxidation) to afford *ent*-nepetalactone. This intramolecular cycloaddition strategy has inspired many other synthetic chemists.[13]

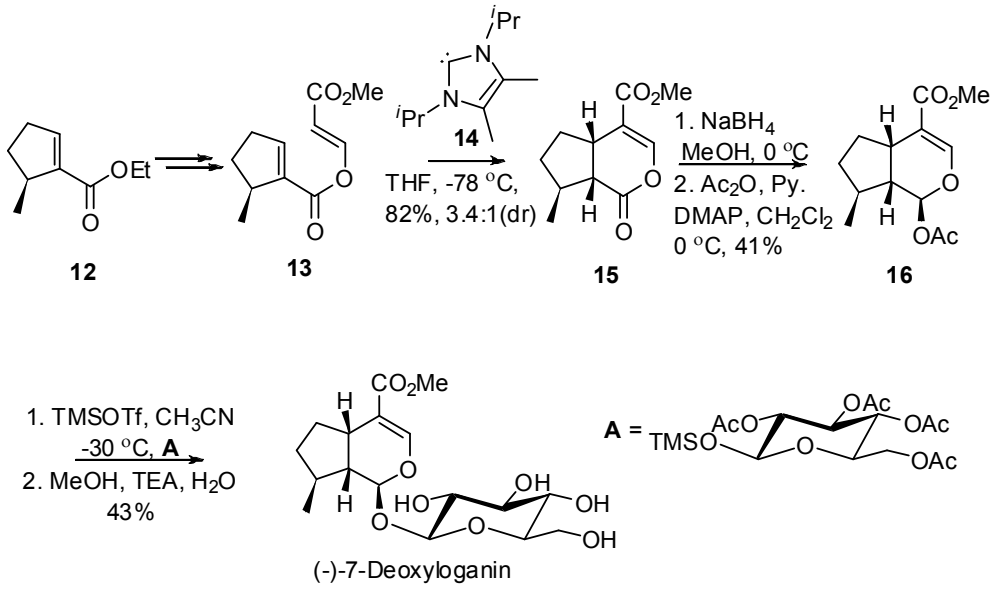
Scheme 3. Synthesis of nepetalactone via oxidative radical cyclization



Recently, numerous research papers have been published on the synthetic approaches to iridoids.

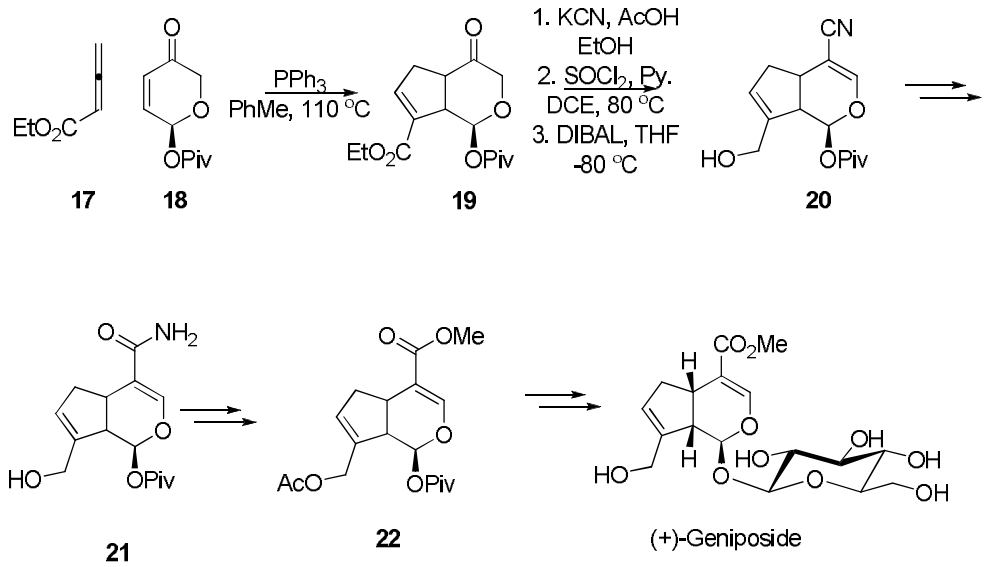
And the stereoselective synthesis of iridoid lactone has been carried out very successfully. As shown in Scheme 3, cyclopentane system embedded isoprene and ester **9** was synthesized via oxidative radical cyclization. [14] ω-Silylcitronellate **7** was subjected to the tandem alkoxycarbonylation/ oxidative cyclization reaction by deprotonation with lithium tetramethylpiperidide (LiTMP), ethylchloroformate and ferrocenium hexafluorohphosphate. The product cyclopentane **9** was isolated as a 2:1 *trans:cis*-diastereomeric mixture. The inseparable diastereomeric mixture was carried through to hydroboration/ oxidation to provide the separable alcohol **10**. The alcohol **10** was submitted to lactonization induced by catalytic amount of TsOH in CH_2Cl_2 to provide the lactone **11**. Then, Krapcho dealkoxycarbonylation afforded dihydronepetalactone.

Scheme 4. Synthesis of (-)-7-deoxyloganin via NHC catalyzed rearrangement of α,β-unsaturated enolester



Another synthetic tactic was *N*-heterocyclic carbene (NHC) rearrangemet with α,β-unsaturated enolester, which provided bicycle lactone system[15] (Scheme 4). NHCs have become universal ligands in organometallic and inorganic coordination chemistry.[16] Because of their specific coordination chemistry, NHCs both stabilize and activate metal centers in quite different key catalytic steps of organic syntheses, such as C-H activation, C-C, C-O, C-N bond formation. According to Candish and Lupton, NHC (**14**) catalyzed rearrangement with the α,β-unsaturated enolester **13** afforded bicyclic lactone **15** successfully, though the diastereomeric ratio was slightly low (3.4:1). Concommitant carbonyl reduction, glycosylation and global deprotection provided (-)-7-deoxyloganin.

Scheme 5. Synthesis of (+)-geniposide via phosphine-catalyzed [3+2] cycloaddition



17 **18** **19** **20** **21** **22**

The more recent approach for iridoid synthesis was contributed by Jones and Krische.[17] Phosphine-catalyzed [3+2] cycloaddition with ethyl-2,3-butadienoate **17** and α,β-unsatutated lactol **18** (Achmatowicz enone[18]) afforded bicyclic lactol **19** as shown in Scheme 5. This bicyclic lactol

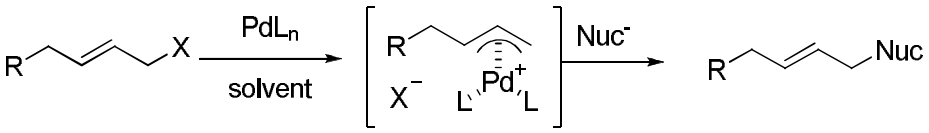
19 was converted to the cyanohydrins, which upon elimination furnished the α,β-unsatutated nitrile **20**. Nitrile converted to methyl ester **22** via primary amide **21**. Glycosidation of lactol employing the trichloroacetimidate as the glycosyl donor delivered the β-glucoside and then global deprotection provided (+)-geniposide.

Throughout these pieces of works, most synthetic efforts had been focused on the deoxygenated iridoids, since it is difficult to achieve to synthesize highly oxidized cyclopentane system enantioselectively. With this notion in mind, we took on the challenge in the iridoid lactone synthesis, which relies on our endeavor of Pd(0)-catalyzed cyclization method.

2. Pd(0)-catalyzed cyclization

Development of asymmetric metal-catalyzed reactions has played a significant role in allowing synthetic access to biologically important molecules. Since Pd(0) catalyzed alkylation has developed by Jiro Tsuji and co-workers, development of asymmetric metal-catalyzed reaction has been manipulated by a lot of chemist groups.[19] The most widely utilized reactions involve dihydroxylation, epoxidation or hydrogenation, and these reactions generate only one type of bond, either a C-O bond C-H bond. In contrast, asymmetric allylic alkylation reaction forms diverse type of bonds, such as C-C bond, C-N bond, C-S bond and C-O bond. Above all, palladium catalyzed cyclization has been utilized as one of the most powerful synthetic tools for carbocycle construction due to its highly versatile selectivities. The schematic introduction of Pd(0)-catalyzed allylic alkylation has shown in Figure 4.

Figure 4. Palldium catalyzed allylic alkylation

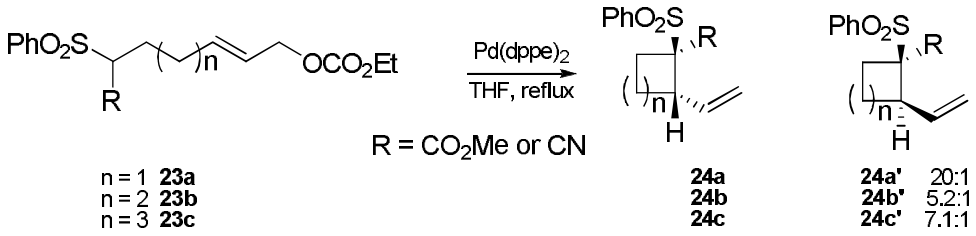


Formation of π-allylpalladium complex takes place by the oxidative addition of palladium to allylic compounds, such as allyl carbonates, allylic ester or halides. The π-allylpalladium complex is a resonance form of σ-allylpalladium complex and a coordinated π-bond. The π-allylpalladium complex formation involves inversion of stereochemistry, and the attack of the soft carbon mucleophile on the π-allylpalladium complex is also inversion.[20] Thus, the stereochemistry of product is retention.

1) Previous studies about Pd(0)-catalyzed cyclization

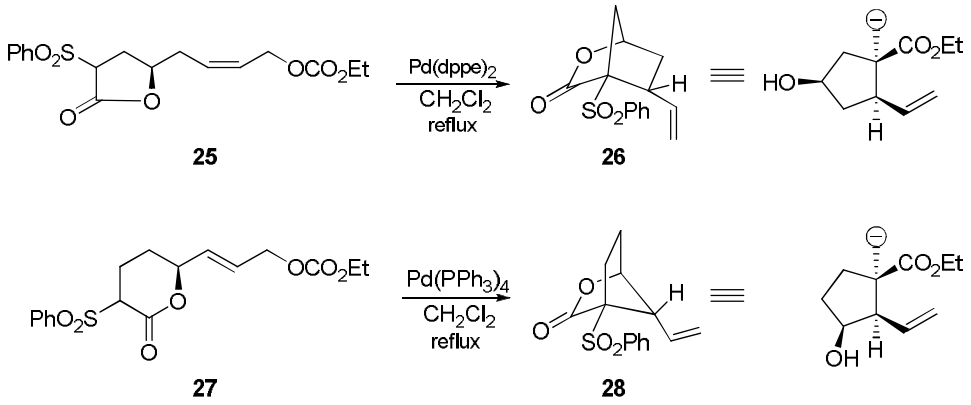
In our laboratory, it has developed Pd(0)-catalyzed intramolecular allylic alkylation. At first, Pd(0)-catalyzed cyclization reaction was investigated with the acyclic allyl precursors **23a-23c** (Scheme 6). Possessed two anion stabilizing groups, benzenesulfonate and ester, or nitrile, allyl carbonates afforded the 1,1,2-stereoselective triisubstitutued cyclobutane **24a**, cyclopentane **24b** and cyclohexane **24c** with good chemical yields.[21]

Scheme 6. Pd(0)-catalyzed allylic alkylation of acyclic allylic precursors



However, it is difficult to control of the stereogenic center at nucleophilic carbon possessing two anion stabilizing groups, because the stereocontrol at this center as well as the ultimate loss of the stereochemistry upon removal of the anion stabilizing auxiliary. Accordingly, 1,2-diastereocontrol of the new two stereogenic centers in Pd(0)-catalyzed cyclization of allylic precursor as well as retention of the established stereochemistries reamin as formidable tasks. To enhance the ability of application to natural products synthesis, the chiral hydroxyl group has introduced to alkyl chain, thus the concomitant γ-, or δ-lactone has provided. With γ-, or δ-lactone **25** or **27**, Pd(0)-catalyzed cyclization reaction was successfully performed to afford the bicyclic lactone **26** or **28** with high diastereoselectivity (Scheme 7). Particularly, desulfonylation of the bicyclic lactone **26** or **28** afforded trisubstituted cyclopentane with retention of stereochemistry. The functional groups at the trisubstituted cyclopentane systems are all *cis* relationship, which are laborious to synthesis. The synthetic utility of the optically active *cis*-trisubstituted cyclopentane obtained by this sequence has been demonstrated by some applications to the stereoselective synthesis of natural products.

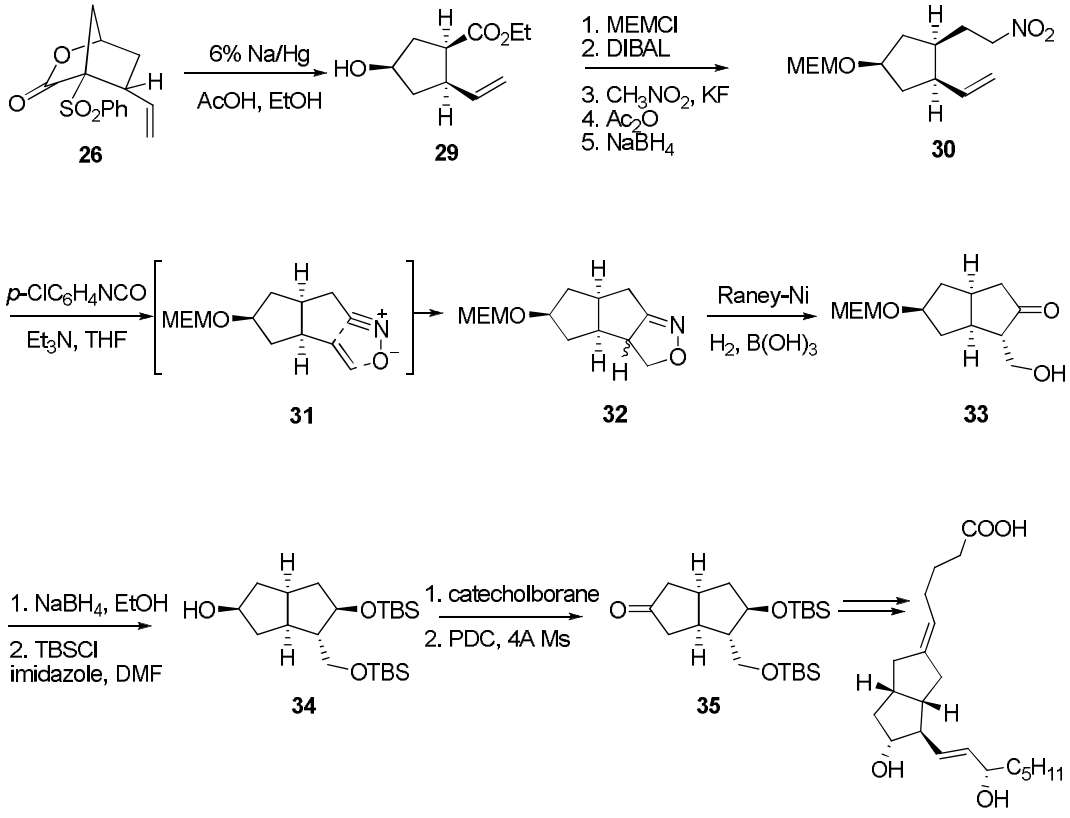
Scheme 7. Pd(0)-catalyzed cyclization with γ-lactone



2) Synthetic applications of bicyclic lactones

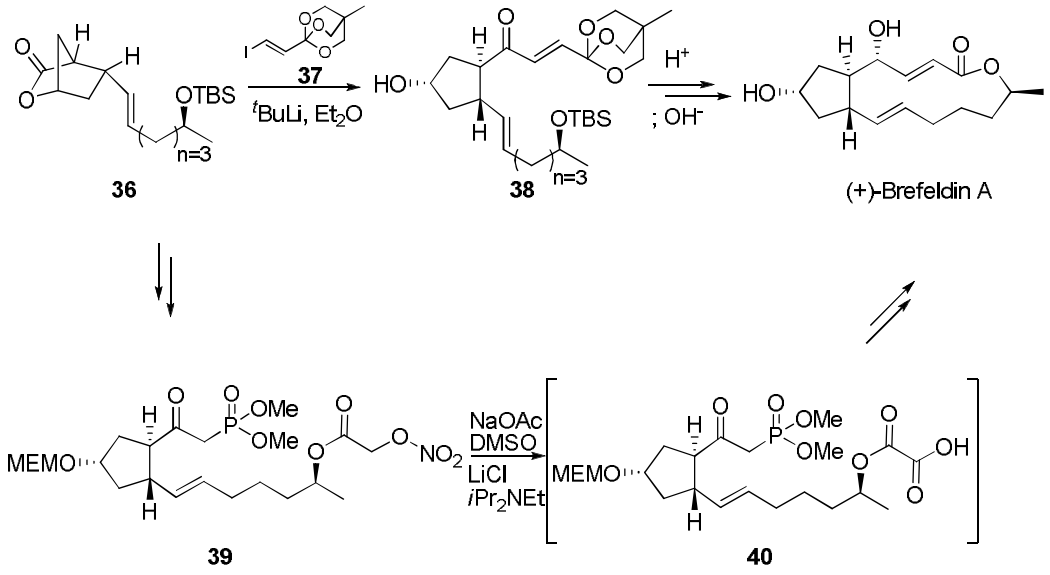
With these bicyclic lactones, the synthetic applications for natural products and bioactive compounds have been reported in our laboratory. The bicyclic lactone **26** was selectively converted to *cis*-trisubstituted cyclopentane system **29**. The *cis*-trisubstituted cyclopentane **29** was protected with methoxyethyl chloride then the intramolecular nitrile oxide-olefin cycloaddition afforded to the hydroxy ketone **33** via **32**. Stereoselective reduction of the ketone **33** afforded the cyclopentanol **34** and then straightforwardly transformed to known intermediate **35** of carbaprostacyclin[22] (Scheme 8).

Scheme 8. Synthetic applications with bicyclic lactone-formal synthesis of carbaprostacyclin



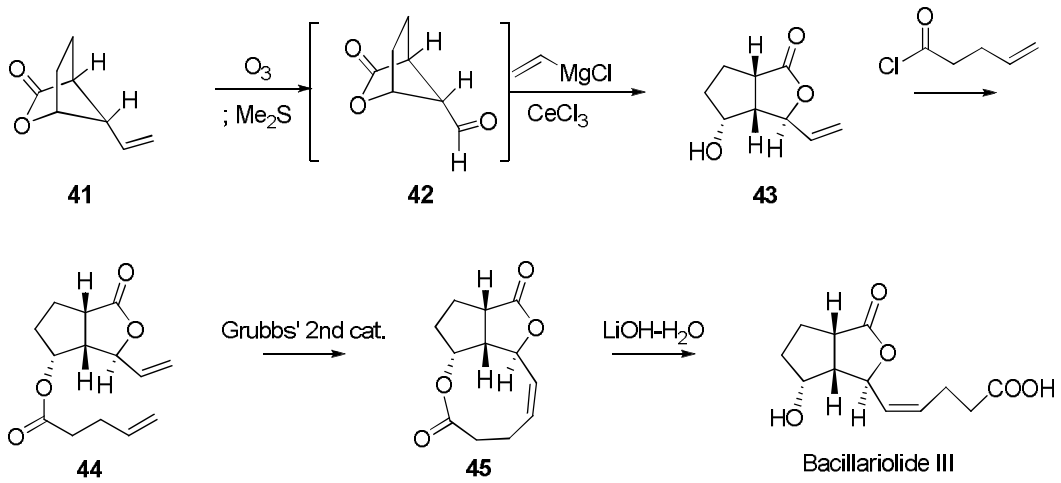
In our laboratory, the total synthesis of (+)-brefeldin A, employing the stereoselective Pd(0)-catalyzed cyclization has been reported. (Scheme 9) (+)-Brefeldin A has been one of the most attractive targets for the synthetic chemists due to its wide range of biological activities and characteristic structure. To introduce the three carbon synthon to lactone carbonyl at **36**, the (*E*)-1-(2-iodovinyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**37**, OBO ortho ester) was adapted as an equivalent of the carboxyl group. Especially, the OBO ortho ester **37** was an excellent carboxyl equivalent, since it is resistant to attacks by strong nucleophiles and redily hydrolyzed under mild conditions.[23] On the other hand, as a part of ongoing studies directed toward the synthesis of the diversified brefeldin A analogs, more practical and versatile synthetic strategy was investigated. The salient feature of the synthesis includes the intramolecular HWE olefination of **40**.[24] Through this tactic, brefeldin A-amide analog synthesis was also accomplished.[25]

Scheme 9. Total synthesis of (+)-brefeldin A



From the bicyclic lactone **28**, the total synthesis of bacillariolide Ⅲ, which was isolated from the marine diatom *Pseudonitzschia multiseries*, was accomplished.[26] The key feature of this synthesis involves the stereoselective vinylcerium addition to bicyclic lactone aldehyde **42**, which induces its facile transformation into the bicyclic lactone skeleton **43** and efficient introduction of the (Z)-pentenoic acid side chain by RCM of **44** (Scheme 10).

Scheme 10. Total synthesis of bacillariolideⅢ

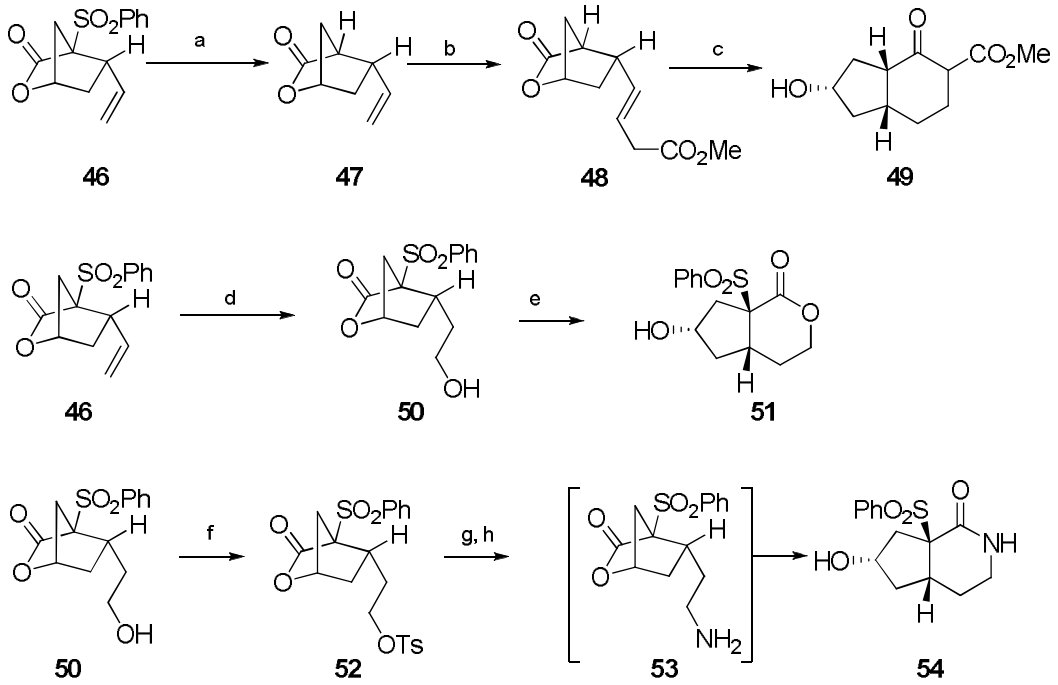


3) Transformation of bicyclic lactone

Also, the bicyclic lactone was easily transformed to other bicyclic systems (Scheme 9). At first, desulfonylated bicyclic lactone **46** underwent cross metathesis with acrolate, hydrogenation of **48** and then base promoted isomerization afforded 5-6 membered ring *cis*-fused carbocycle **49**. Secondly, hydroboration of vinyl afforded to the primary alcohol **50**, transesterification was accomplished to bicyclic lactone **51** by DBU. And the primary alcohol **50** was substituted to azide

via tosylate **52**, and successive Staudinger reaction gave the bicyclic lactam **54**. [27]

Scheme 11. Transformations of bicyclic lactone



Reagents: (a) Na/Hg, $B(OH)_3$, MeOH, 55%; (b) Grubbs' 2nd catalyst, methyl acrolate, CH_2Cl_2, 92%; (c) H_2, Pd/C, then KO^tBu, benzene, 70% for 2 steps; (d) catechol borane, $Rh(PPh_3)_3Cl$, THF, then H_2O_2, pH 7.0 buffer, 67% (e) DBU, CH_3CN, 72%; (f) TsCl, Et_3N, DMAP, CH_2Cl_2, 97%; (g) NaN_3, DMF, 88%; (h) PPh_3, wet THF, 68%.

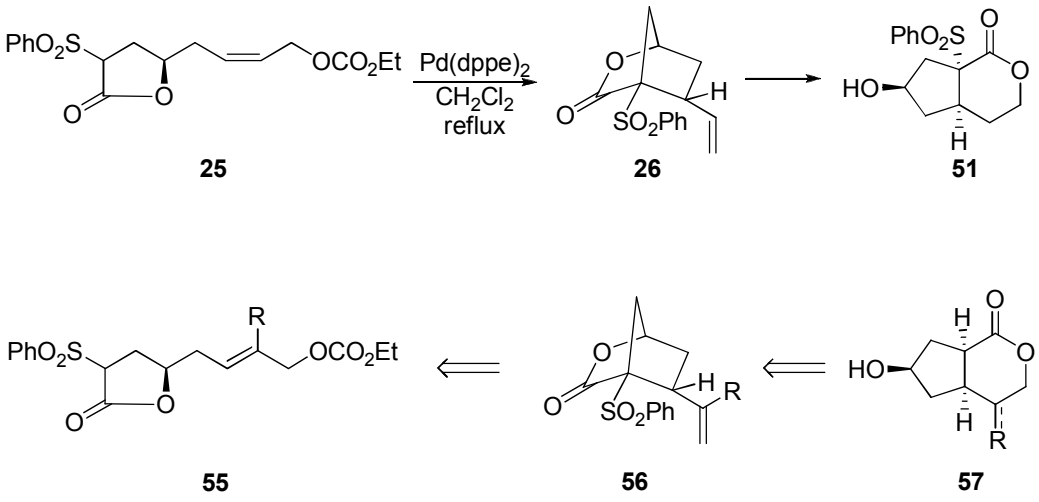
Throughout these transformations, it is well-known that the bicyclic lactone is an efficient synthetic intermediate, the synthetic approaches for natural products is on-going.

II. Result and discussions

1. Pd(0)-catalyzed cyclization with substituted allyl carbonate

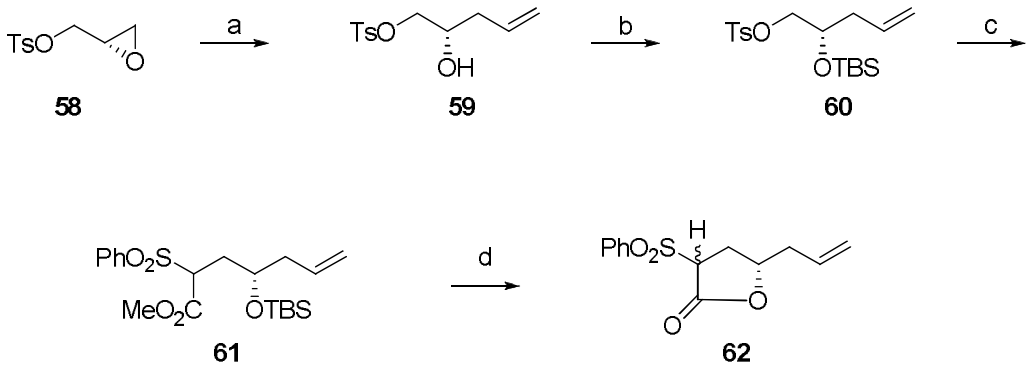
Previously, it has been reported that highly stereoselective construction of unique bicyclic lactones via intramoelcular Pd(0)-catalyzed cyclization has been accomplished in our lab. The [2.1.2] or [2.2.1] bicyclic lactones could be utilized as excellent equivalents of contra thermodynamic *cis*-trisubstituted cyclopentanes which have been importantly been utilized for total synthesis of bioactive natural products as described before. These applications implied high utility of the bicyclic lactone architectures. However, iridoids or other bioactive natural products, which have bicyclic structure embedded natural products, possess alkyl substituent at C4 (Figure 6). And to address these substituents after the bicyclic ring formation is a laborious task. Hence, the development of more transformative bicyclic lactone was needed. That has driven us to focus on the investigation of the more transformative bicyclic lactone. Thus we envisaged the branched allyl carbonate for Pd(0)-catalyzed cyclization.

Figure 5. Synthetic approach for branched allyl carbonate



As shown in Figure 5, substituted vinyl bicyclic lactone **56** could be transformation to bicyclic lactone **57**, which has substituent at C4. The branched allyl carbonate γ-lactone **55** would be obtained via cross metathesis with γ-lactone and diverse allyl carbonates. The synthesis of γ-lactone is shown below (Scheme 12).

Scheme 12. Synthesis of γ-lactone

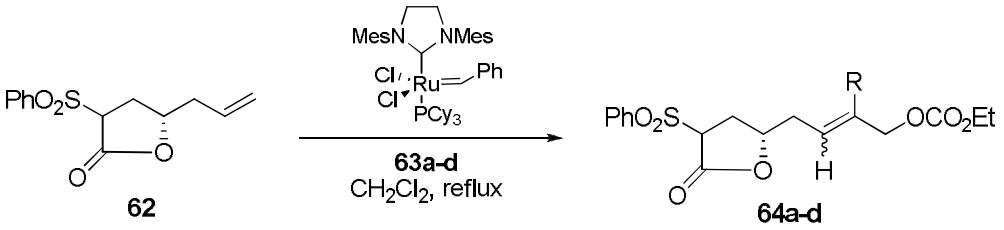


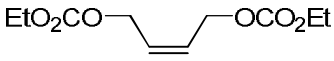
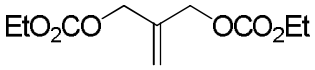
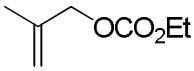
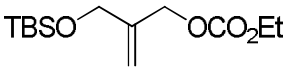
Reagents: (a) vinylmagnesium bromide, Li_2CuCl_4, THF; (b) imidazole, TBSCl, DMF, 0 ºC, 88% for 2 steps; (c) methyl benzenesulfonyl acetate, NaH, DMF, 80 ºC, 77%; (d) TBAF, THF, 78%.

Vinyl addition to tosylate glycidol **58** and TBS protection afforded **60**, alkylation with methyl benzenesulfonate and TBS deprotection gave γ-lactone **62** (Scheme 12). At the alkylation step, diastereomeric mixture was obtained and the concomitant γ-lactone **62** was also inseparable diastereomeric mixture. γ-Lactone **62** underwent cross metathesis with allyl carbonates afforded precursors of Pd(0)-catalyzed cyclization (Table 1).

The cross metathesis product **63a** and **64b** were obtained *E* isomers dominantly, but **63d** was obtained *E/Z* mixtures, which were inseparable. Thus, with these branched allyl carbonate γ-lactone, we proceeded Pd(0)-catalyzed cyclization at the same conditions.

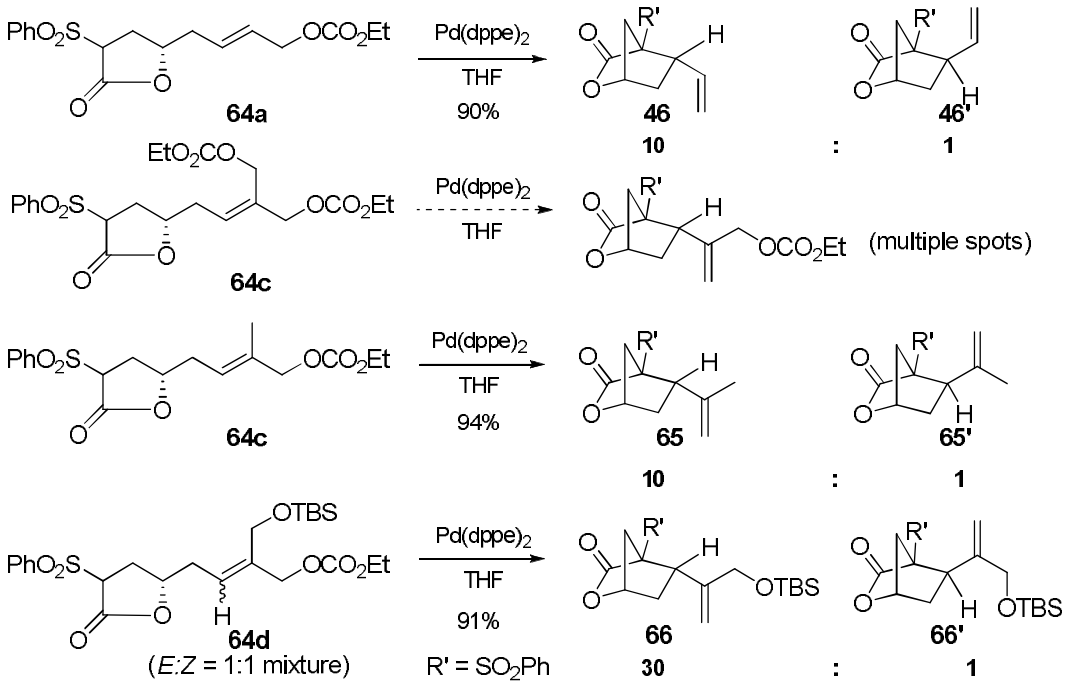
Table 1. Cross metathesis with γ-lactone



Entry	Cross metathesis partner	Yield	product
1	 **63a**	48	**64a**
2	 **63b**	49	**64b**
3	 **63c**	33	**64c**
4	 **63d**	43	**64d**

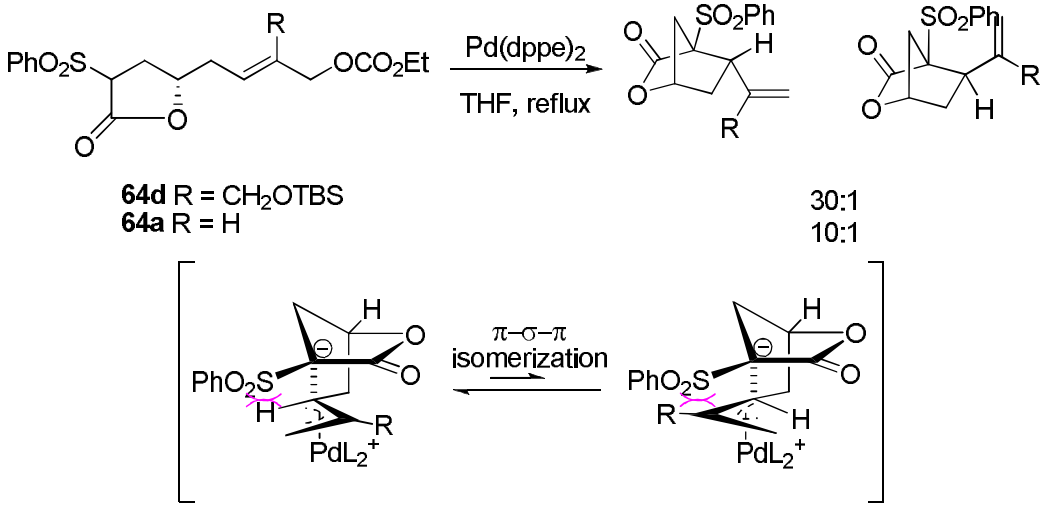
Similar to linear allyl carbonate, metallyl carbonate and hydroxymethyl allyl carbonate were represented good precursors for Pd(0)-catalyzed cyclization. These branched allyl carbonates underwent Pd(0)-catalyzed cyclization with excellent chemical yields and diastereoselectivities from 10:1 to 30:1 (Scheme 13). Exceptionally, diallylcarbonate **64b** did not afford desired bicyclic lactone. It seemed that initially diallylcarbonate **64b** underwent the Pd(0)-catalyzed cyclization, but further unexpected reactions occurred with the allyl carbonate at bicyclic lactone, provided multiple unidentified products.

Scheme 13. Pd(0)-catalyzed cyclization with branched allyl carbonate



The diastereomeric ratio was enhanced with branched allyl carbonate, which would explain the steric repulsion between benzensulfonate and methylene or hydrogen as shown in Figure 6. The excellent stereoselectivity is likely due to the presence of the silyloxymethyl substituent, given the lower stereoselectivity (10:1) for the disubstituted alkene **64a** under the same conditions as shown in Figure 6. In particular, the *E/Z* isomers of **64d** were executed the Pd(0)-catalyzed cyclization with high diastereoselectivity, which proved the Pd(0)-catalyzed C-C bond cleavage/formation sequence would reinforce high diasteroselectivity of the cyclization process for [2.1.2] bicyclic lactone followed by π-σ-π isomerization. This type of facial preference is well supported by our previous report.[28] And the stereochemistry of benzenesulfonyl group was negligible, because of α-deprotonation with basic condition.

Figure 6. Plausible transition state of Pd(0)-catalyzed cyclization

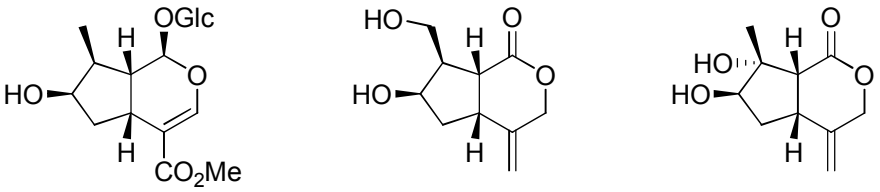


2. Total synthesis of natural iridoid lactone

In the cyclopentanoids, we have been interested in a recently reported iridoid lactone, which is structurally related to loganin, and consists of a C7-substituted C6-hydroxycyclopenta[*c*]lactone, as illustrated in Figure 8. The unique structures and biological activities of iridoid lactone was concerned the synthetic interests. Iridoid lactone **1** was isolated from the roots and rhizomes of *Nardostachys chinensis* Batalin (Valerianaceae).[29] This plant has been used as a Chinese folk medicine and its roots and rhizomes exhibit a variety of biological activities, such as sedative, antimalarial, antinociceptive, cytotoxic, and enhancement of nerve growth factor activities.[30] However, the paucity of its key component **1** has hampered the systematic research of the iridoid, including structure-activity relationship (SAR) analysis and further pharmacological studies. Thus, we have worked on the development of an efficient synthetic route to the new iridoid lactone **1** (Figure 8). The iridoid lactone **1** is structurally very interests. It has contiguous, various functional groups, such as hydroxyl, hydroxymethyl, *exo*-methylene, and lactone. Moreover, the 4-

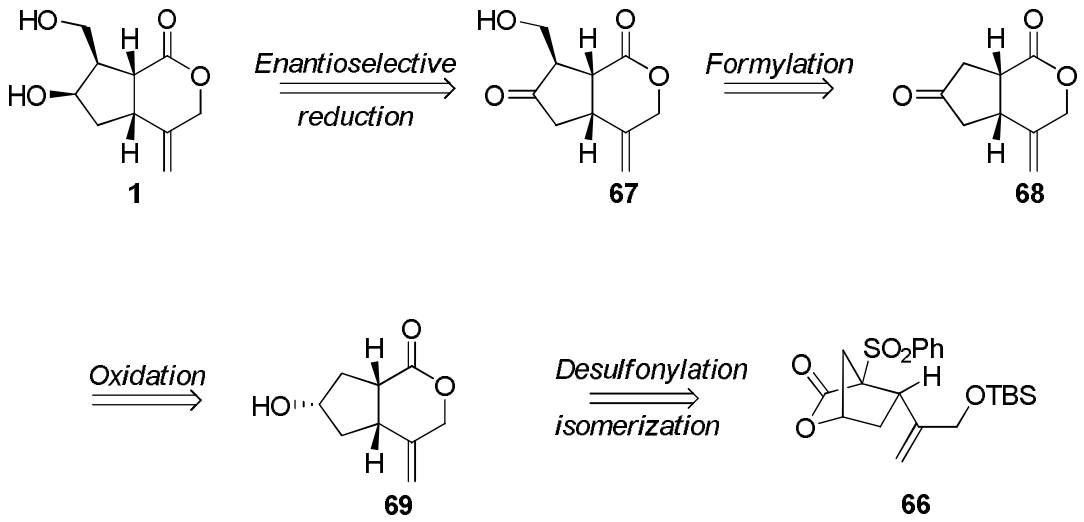
stereogenic centers are contiguous. This challengeable structure as well as the curiosity for biological activity led us to synthesis.

Figure 7. Target iridoid lactone



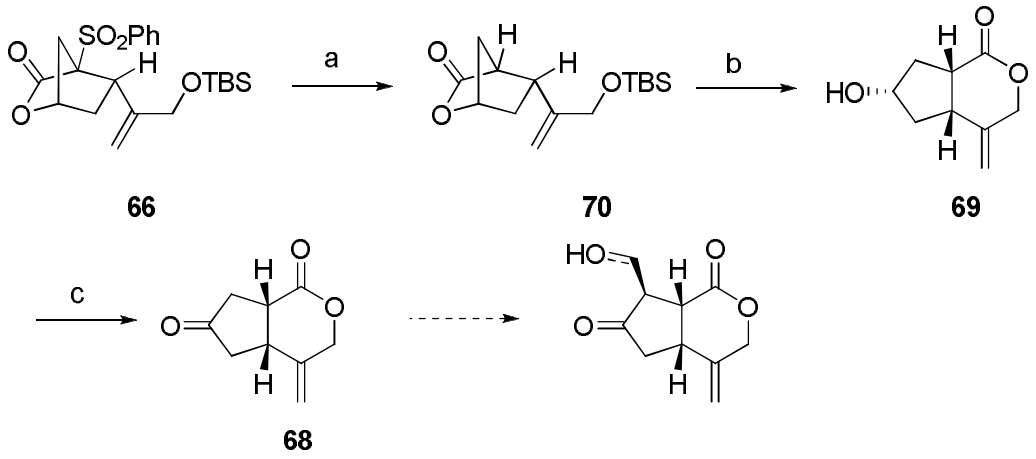
To establish the bicyclic lactone structure, we planned to synthesize the bicyclic lactone via Pd(0)-catalyzed cyclization. Our retrosynthetic approach for iridoid lactone **1** is illustrated in Scheme 14. We hypothesized that the bicyclic lactone **66** might serve as a synthetic precursor of iridoid lactone through three potentially straightforward transformations: (a) oxidation of cyclopentanol **69** to cyclopentanone **68**, (b) regioselective α-formylation, and (c) enantioselective reduction of C6 carbonyl group.

Scheme 14. Retrosynthetic approach



With the bicyclic lactone **66** in hand, we attempted to isomerize bicyclic system for the iridoid skeleton. Desulfonylated bicylcic lactone was isomerized to concomitant bicyclic lactone **69** and oxidized to cyclopentanone **68**. To address the regioselective hydroxymethylation, various reaction conditions were considered. A direct α-hydroxymethylation with HCHO[31] or the introduction of a hydroxymethyl equivalent via α-methylenation with Eschenmoser's salt[32] as well as formylation with $HCO_2CH_2CF_3$[33] to the cyclopentanone did not provide satisfactory results.

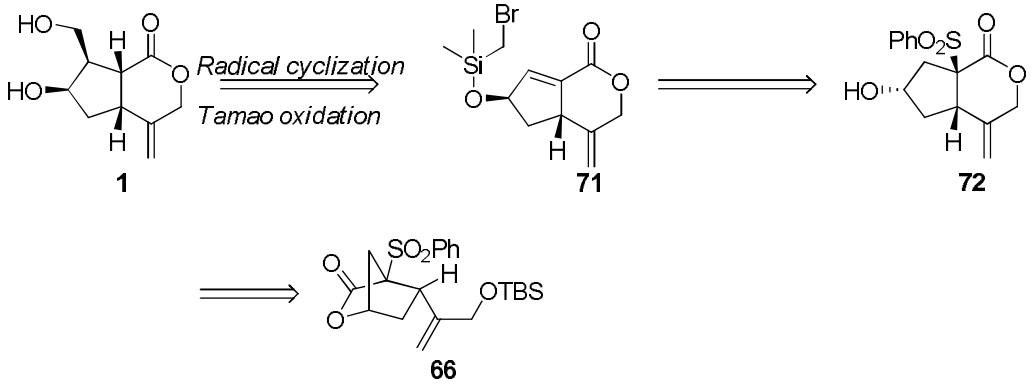
Scheme 15. Synthesis of bicyclic pentanone



Reagents: (a) Na/Hg, $B(OH)_3$, MeOH, 56%; (b) TBAF, THF, 73%; (c) TPAP, NMO, CH_2Cl_2, 67%.

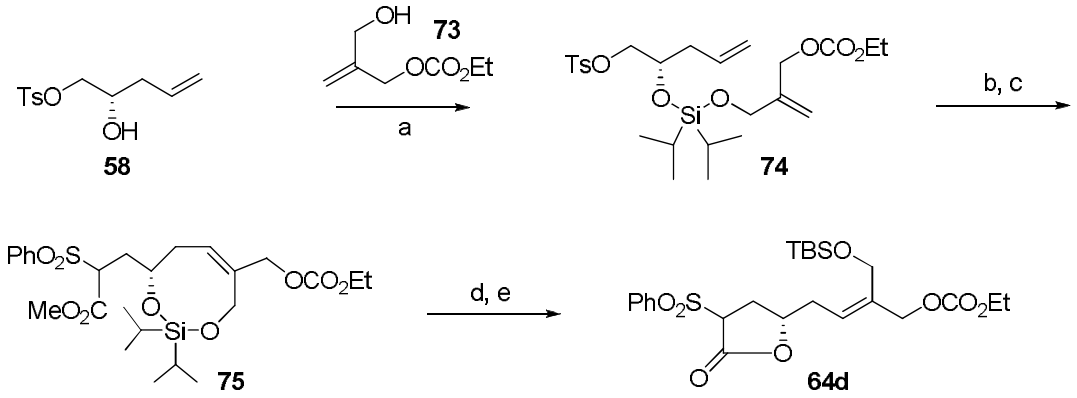
Throughout the results, it turned out that the regioselective direct α-formylation or hydroxymethylation was not successful for our system. It was suspected that the hydrogens at ring junction were labile at basic condition. As a matter of fact, the allylic hydrogen at C5 of **68** was easily deprotonated with general bases. So we envisaged cyclopentenone to establish the regio-, and chemoselectivity for hydroxymethylation.

Scheme 16. Revised retrosynthetic approach



The process involves the efficient regio- and stereoselective elaboration of the C7 hydroxymethyl substituent through the silicon-tethered intramolecular radical cyclization[34] of cyclopentene system **71**, followed by Tamao-Fleming oxidation.[35] Cyclopentanol **72** is generated by functional group transformation from bicyclic lactone **66**, which can be efficiently constructed by the palladium-catalyzed cyclization of allylic carbonate.

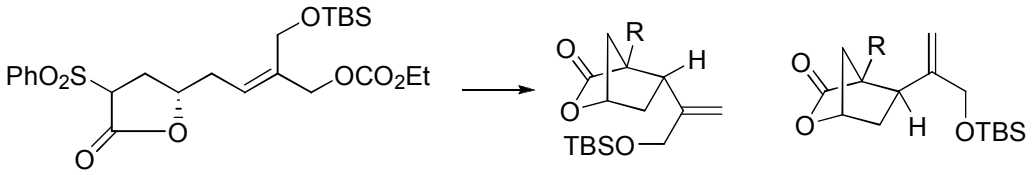
Scheme 17. Synthesis of γ-lactone 64d



Reagents: (a) $^{i}Pr_2SiCl_2$, imidazole, CH_2Cl_2, 0 ºC, 78%; (b) methyl benzenesulfonyl acetate, NaH, DMF, 80 ºC, (c) Grubbs' 2nd catalyst, CH_2Cl_2, reflux, 67% for 2 steps; (d) TBAF, THF; (e) TBSCl, imidazole, CH_2Cl_2, 76% for 2 steps.

The synthesis commenced with the preparation of the requisite γ-lactone **64d** for Pd(0)-catalyzed allylic alkylation, as illustrated in Scheme 17. To address the tri-substituted alkene unit, we initially attempted olefin cross metathesis of the vinyl γ-lactone with the hydroxymethyl substituted allyl carbonate to produce the Pd(0)-catalyzed cyclization precursor. However, intermolecular cross metathesis led to γ-lactone **64d** with only 60% yield. Thus, we turned our attention to silicon-tethered ring-closing metathesis (RCM),[36] which is considered superior in terms of entropic benefit and concomitant alcohol protection. In addition, (*Z*)-form alkene was obtained solely. That is because (*Z*)-conformation is favorable at 8-membered cyclic compound.

Table 2. Pd(0)-catalyzed cyclization of 66



64d **66** **66'**

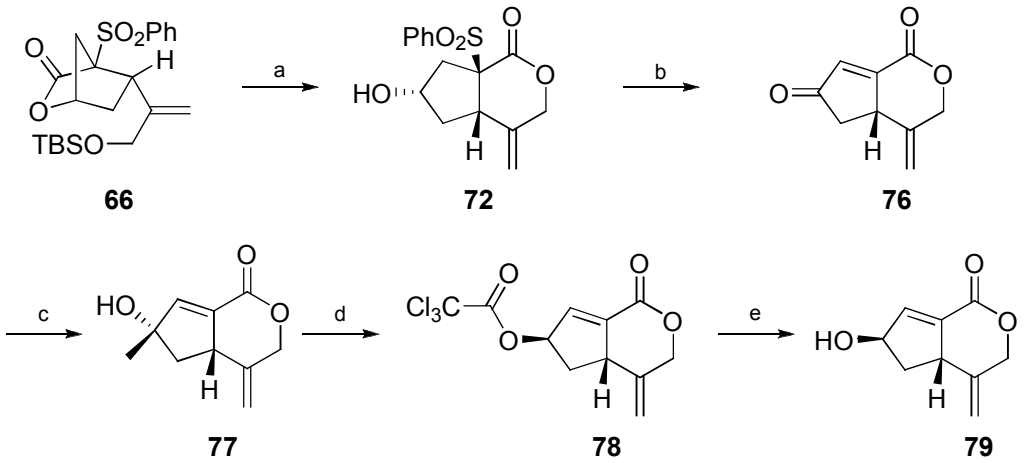
Entry	Catalyst	solvent	Yield[a]	Ratio[b] (**66**:**66'**)
1	$Pd(dppe)_2$	THF	91	30:1
2	$Pd(dppe)_2$	CH_2Cl_2	90	3.5:1
3	$Pd(PPh_3)_4$	THF	91	1:1
4	$Pd(PPh_3)_4$	CH_2Cl_2	90	2.5:1
5	$Pd(PPh_3)_4$	DMSO	78	1:1
6	$Pd(dppp)_2$[c]	THF	78	10:1
7	$Pd(dppb)_2$[c]	THF	89	25:1

All reactions were conducted in reflux condition. [b]Isolated yields. [c]Determined by 300-MHz ^{1}H-NMR spectra of the crude diastereomeric mixtures. [d] Pre-mixed $Pd(OAc)_2$ and dppp/dppb in THF was added to a solution of **64d**.

Alkoxysilane **74** was prepared from the known hydroxy tosylate **58** with a 78% yield. Alkylation of tosylate **74** with the anion of methyl phenylsulfonyl acetate and subsequent RCM provided the cyclic *bis*-alkoxysilane **75**. Removal of the tethered silicon of *bis*-alkoxysilane **75** with TBAF and the spontaneous relactonization of the resulting hydroxy ester provided the γ-lactone intermediate, which was protected with TBSCl to afford allylic carbonate **64d**.

We next conducted a survey of stereoselective Pd(0)-catalyzed cyclization of **64d** under a variety of reaction conditions as summarized in Table 2. Stereoselective cyclization of **64d** in the presence of $Pd(dppe)_2$ in THF afforded the desired product of **66** with a 91% yield along with a minor product **66'** (entry 1). Comparison with the result at Scheme 13, at this time, the precursor **64d** was only *Z*-isomer and the cyclization result was same as the result with *E/Z* mixtures. That might be the evidence of π-σ-π isomerization. Cyclization of **64d** in the presence of $Pd(PPh_3)_4$ resulted in low stereoselectivity regardless of the solvent (entry 3-5). Cyclization in the presence of $Pd(OAc)_2$ with dppp or dppb (entry 6, 7) also showed good stereoselectivities, although the yield was slightly lower.

Scheme 18. Attempt with bicyclic system

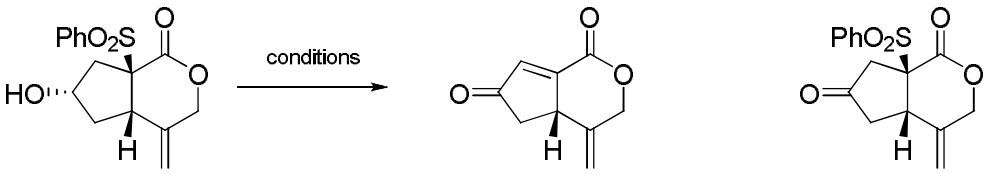


Reagents: (a) TBAF, THF, rt, 2 h, 80%; (b) DMP, rt, 16 h, CH_2Cl_2, 88%; (c) CBS, $BH_3 \cdot SMe_2$, CH_2Cl_2, 45-80%; (d) Cl_3CCOOH, DIAD, PPh_3, THF; (e) SiO_2, THF, $MeOH/H_2O$, 20% for 2 steps.

The oxidation and concomitant β-elimination of sulfonate afforded to cyclopentenone **76**.

The desulfonylation for making a variety of double bonds has been used in natural products synthesis as a potential procedure. The reductive elimination of sulfonyl group has been carried out by using aluminium amalgam, sodium amalgam, and zinc-acetic acid,[37] and many examples of base-induced desulfonylation to α,β-unsaturated carbonyl compounds have been reported.[38] Several attempts for cyclopentenone via oxidation and concomitant β-elimination of sulfonate have described as below (Table 3).

Table 3. Oxidation to cyclopentenone



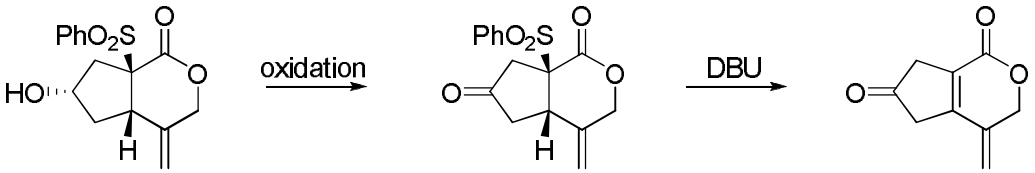
72 **76** **80**

condition	Yield **76**	**72**	**80**
DMP, $NaHCO_3$, CH_2Cl_2	56%		30%
$(COCl)_2$, Et_3N, DMSO	-	80%	-
TFAA, Et_3N, DMSO			67%
TPAP, NMO, CH_2Cl_2	-	100%	-
PCC, silica gel, CH_2Cl_2	-	-	85%
DMP, CH_2Cl_2	88%	-	-

With the basic conditions, such as Swern oxidation with oxally chloride or Ley oxidation, the β-elimination as well as the oxidation of alcohol was not executed, the cyclopentanol **72** was recovered. Interestingly, in PCC oxidation condition or Swern oxidation with TFAA, the oxidation of cyclopentanol **72** to cyclopentanone **80** was accomplished successfully, however β-elinmination of sulfonate did not occur. Moreover, after oxidation of cyclopentanone **80** followed by basic work

up with DBU, product **81** was obtained by the β-elinmination of sulfonate with allylic proton at C5 (Scheme 19).

Scheme 19. Basic work up with cyclopentanone

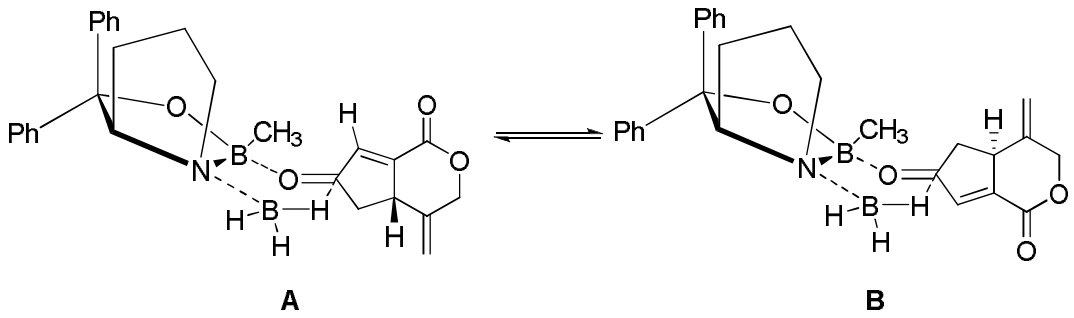


72 **80** **81**

Gratifyingly, the oxidation of cyclopentanol with *Dess-Martin* periodinane yielded cyclopentenone **76** in acidic condition. When $NaHCO_3$ was co-treated for neutral condition, the β-eliminated product **76** was obtained only 56% and cyclopentanone **80** was obtained about 30%. However, when *Dess-Martin* periodinane was only treated the desired product **76** was obtained upto 88% as sole product. There are a few examples for β-elinmination of sulfonate in acidic condition.[39]

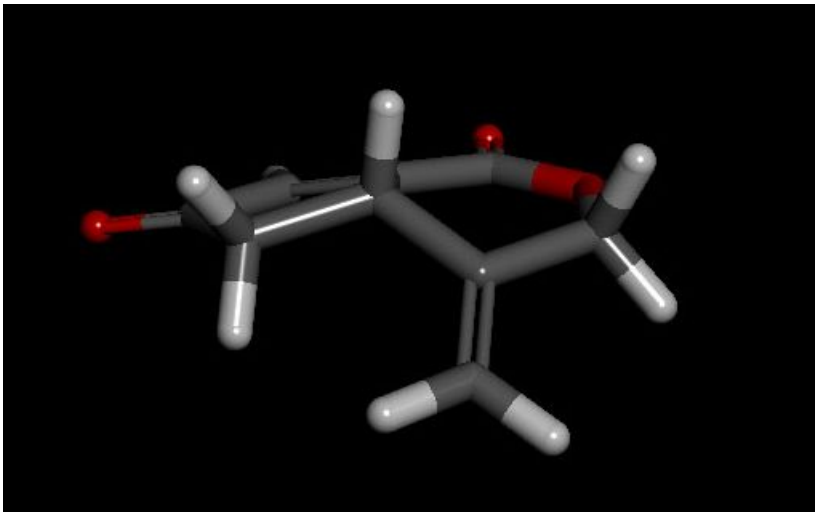
With the cyclopentenone **76** in hand, the enantioselective reduction of cyclopentenone to cyclopentenol **79** was attempted (Scheme 18). As shown at Figure 8, we expected the coordination of cyclopentenone **76** with (*S*)-Me-CBS oxazaborolidine would be preferred **B** because of the steric interaction with methyl group.[40]

Figure 8. Bicyclopentenone coordinated with (*S*)-CBS oxazaborolidine



However, the enantioselective reduction of cyclopentenone to cyclopentenol was not successful with the bicyclic system. It seemed that the 3D structure of bicyclic lactone might interfere with the approach of hydride from the concave face (Figure 9). Considering the result that reduction of cyclopentenone **76** with (*R*)-CBS afforded the same cyclopentenol **77** as sole product in 74% yield, we assured the the face selectivity had relevance to the structure of bicyclic system.

Figure 9. 3D-structure of bicyclic enone

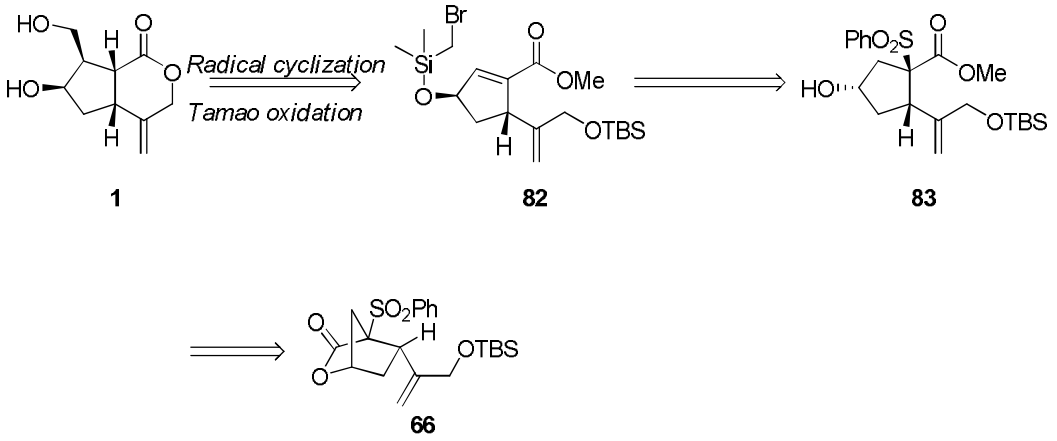


With the cyclopentenol **77**, Mitsunobu reaction with Cl_3CCO_2H and acidic hydrolysis[41] afforded

the desired cyclopentenol **79**, although the chemical yield of 2-step was too low (Scheme 18).

So instead of the bicyclic system, we envisaged the monocyclic system. Instead of relactonization of bicyclic lactone **66** to bicyclic lactone **72**, transesterification with sodium methoxide might provide cyclopentanol **83** (Scheme 20).

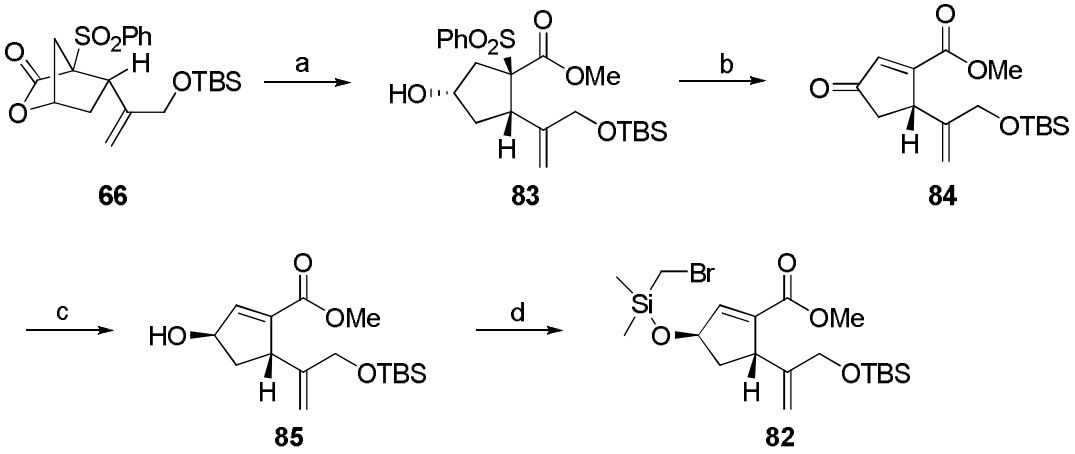
Scheme 20. Revised retrosynthetic approach



1 **82** **83** **66**

With the requisite building block **66** in hand, we focused our attention to the facile introduction of the hydroxymethyl substituent at C7. Convenient methanolysis of bicyclic lactone **66** afforded cyclopentanol **83**, which was subjected to Dess-Martin oxidation to produce enone **84** after concomitant β-elimination of sulfonate (scheme 21). Stereoselective reduction of cyclopentenone **84** with (*S*)-Me-CBS afforded cyclopentenol **85** with the correct stereochemistry.

Scheme 21. Synthesis of radical cyclization precursor 82

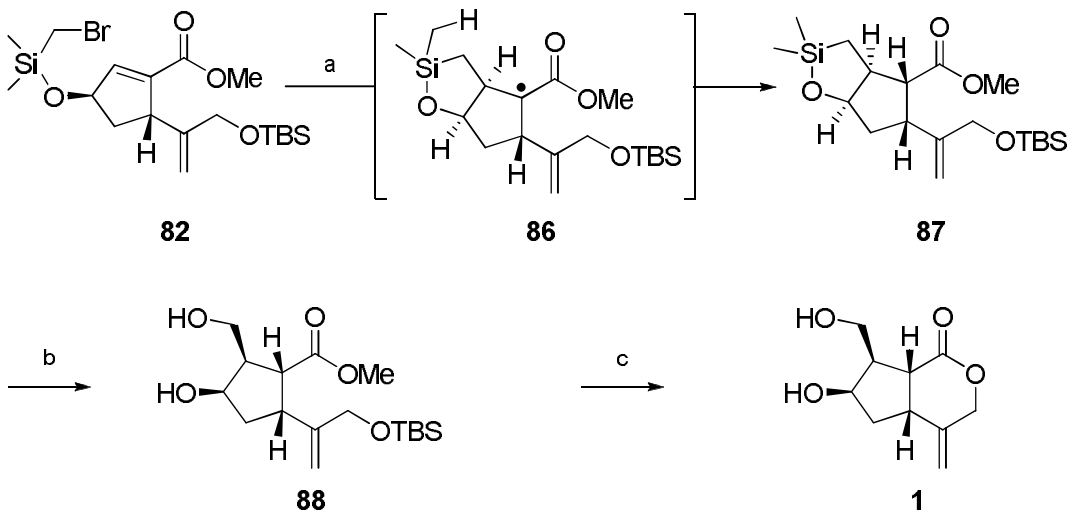


Reagents: (a) NaOMe, MeOH, 93%; (b) *Dess-Martine* periodinane, CH_2Cl_2, 91%; (c) (*S*)-CBS, $BH_3 \cdot SMe_2$, CH_2Cl_2, 68%; (d) (Bromomethyl) dimethylchlorosilane, DMAP, Et_3N, benzene, 0 ºC, 85%.

Silylation of cyclopentenol **85** with (bromomethyl) dimethylchlorosilane yielded the bromosilyl ether **82**, which was directly subjected to the radical cyclization (Scheme 22). Intramolecular radical cyclization of the labile silyl ether intermediate with Et_3B[42] and Bu_3SnH afforded the desired *cis*-fused oxasilole **87** in good yield. Organoboranes are widely recognized as excellent sources of free-radicals and can participate in free-radical chain reactions.[43] In particular, over classical initiators, Et_3B offers the great advantage of being efficient even at low temperature (-78 ºC), thus it proved to be particularly important for the development of stereoselective radical reactions and for radical reactions involving thermally unstable adducts or products.[44] The mechanistic aspect has been recognized that the nucleohemolytic substitution reaction.[45] With Et_3B, very reactive radical initiator as referred above, the initiation step was allowed at 0 ºC to ambient temperature, the *cis*-fused oxasole ring was produced, which stereogenic center was derived by the cyclopentenol's stereochemistry. And the newly generated C8 stereogenic center

could be established by an intramolecular 1, 6-hydrogen atom transfer from the proximal silylmethyl group to C8.

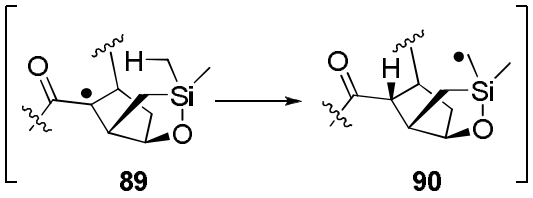
Scheme 22. Completion of synthesis of iridoid lactone 1



Reagents; (a) Et_3B, Bu_3SnH, benzene, 0 ºC; (b) $KHCO_3$, H_2O_2, KF, THF/MeOH, 60% for 2 steps; (c) $TsOH \cdot H_2O$, CH_2Cl_2, 66%.

According to the Little et al, the fused ring system allows the intramolecular 1,6 hydrogen transfer to the radical center.[46] In the radical quenching portion of the 5-exo, trig cyclization step via an intramolecular hydrogen atom transfer from the proximal silylmethyl group of silacycle **89** to the radical center located C8 at **90**. Once transferred, the silylmethly radical **90** is presumably quenched intermolecularly by tributyl tin hydride to afford an oxasilole (Figure 10).

Figure 10. 1, 6 hydrogen transfer



Subsequent Tamao-Fleming oxidation of oxasilole **87** gave diol **88**. Finally lactone iridoid **1** was obtained in good yield by TBS deprotection with TsOH,[47] followed by a concomintant lactonization. The structure of **1** was confirmed by comparison of its spectral data with those of the authentic sample (optical rotation, ^{1}H-NMR, ^{13}C-NMR, IR, HRMS).[48]

III. Conclusion

We has developed the useful method for synthesizing trisubstituted cyclopentane systems via Pd(0)-catalyzed cyclization. In particular, branched allyl carbonated γ-lactone yielded diastereoselective bicyclic lactone with good chemical yield. With the bicyclic lactone, we accomplished the first total synthesis of (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydro-cyclopenta[*c*]pyran-1(3*H*)-one in 13 steps. The key features of the synthesis involve the stereoselective and efficient preparation of the cyclopentanol intermediate *via* Pd(0)-catalyzed cyclization and then the silicon-tethered radical cyclization followed by Tamao-Fleming oxidation for the stereoselective introduction of the C7-hydroxymethyl substituent. This versatile synthetic approach is expected to be widely utilized for the synthesis of related iridoids and iridoalkaloids.

IV. Experimental

General procedure

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran and Et_2O were distilled from sodium benzophenone ketyl. Dichloromethane, triethylamine, acetonitrile and pyridine were freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried at 100 ºC. Air and moisture sensitive reactions were performed under argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Optical rotations were measured with JASCO DIP-1000 or JASCO P-1020 digital polarimeter at ambient temperature using 100 mm cell of 2 mL capacity. Infrared spectra were recorded on a Perkin-Elmer 1710 FT-IR spectrometer. Mass spectra were obtained with VG Trio-2 GC-MS instrument. High resolution mass spectra were obtained with JEOL JMS-AX 505WA instrument. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 (300 MHz), Varian Gemini 2000 (300 MHz), Brucker Analytik AVANCE digital 400 (400MHz), or AVANCE digital 500 (500MHz) spectrometer as solutions in deuteriochloroform ($CDCl_3$). Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane and are referenced to the deuterated solvent ($CHCl_3$). ^1H-NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad singlet; m, multiplet and/or multiple resonance), number of protons, and coupling constant in hertz (Hz).

(*S*)-2-Hydroxypent-4-enyl 4-methylbenzenesulfonate (59)

To a solution of lithium tetrachlorocuprate (0.1 M solution in THF, 6.6 ml, 0.66 mmol), vinylmagnesium bromoide (1.0 M solution in THF, 17 ml, 17 mmol) was added at -40 ℃. After stirred for 1 h, a solution of tosylated glycidol (2.96 g, 13 mmol) in THF (35 ml) was added dropwise by dropping funnel *ca.* 1 h at -40 ℃ and stirred for 1 h. The reaction mixture was quenched with aqueous NH_4Cl and warmed to ambient temperature and then, extracted with ether. The aqueous layers were extracted with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 3.19 g (96%) of hydroxy tosylate **59** as a colorless oil: $[\alpha]_D^{25}$ 3.24 (*c* 0.1, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.76 (dd, 2H, *J* = 6.6 Hz), 7.32 (d, 2H, *J* = 8.07 Hz), 5.77-5.64 (m, 1H), 5.05 (m, 2H), 4.0 (m, 1H), 3.92-3.83 (m, 2H), 2.41 (s, 3H), 2.26-2.13 (m, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 145.0, 132.8, 132.5, 129.9, 127.9, 118.7, 73.0, 68.5, 60.4, 37.3, 21.0; IR (neat) ν_{max} 3541, 2921, 1598, 1357, 1178, 1097, 978, 922, 818, 668 cm^{-1}; LRMS (FAB) *m/z* 257 ($M + H^+$); HRMS (FAB) Calcd for $C_{12}H_{17}O_4S$ ($M + H^+$): 257.0769, found 257.0848.

(*S*)-2-(*tert*-Butyldimethylsilyloxy) pent-4-enyl 4-methylbenzenesulfonate (60)

To a solution of hydroxy tosylate **59** (9.0 g, 33.3 mmol) and imidazole (3.4 g, 50.0 mmol) in DMF (30 ml) was added tertiarybutyl dimethylsilyl chloride (7.5g, 50.0 mmol) at 0 ℃. After stirred for 5 h, the reaction mixture was quenched with water and warmed to ambient temperature and then, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 11.0 g (90%) of silyl ether **60** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, 2H, *J* = 8.43 Hz), 7.32 (d, 2H, *J* = 7.86 Hz), 5.76-5.62 (m, 1H), 5.02-4.96 (m, 2H), 3.90-3.78(m, 3H), 2.42 (s, 3H),

2.22-2.02 (m, 2H), 0.81 (s, 9H), -0.04 (d, 6H, J = 1.2 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 144.8, 140.9, 133.2, 129.8, 128.0, 120.8, 72.6, 69.5, 38.7, 25.7, 21.6, 18.0, -4.7, -4.8; IR (neat) ν_{max} 2931, 2857, 1463, 1364, 1255, 1181, 983, 835, 665 cm^{-1}; LRMS (FAB) *m/z* 371 (M + H$^+$); HRMS (FAB) Calcd for $C_{18}H_{31}O_4S$ Si(M + H$^+$): 371.1712, found 371.1705.

(4*S*)-Methyl 4-(*tert*-butyldimethylsilyloxy)-2-(phenylsulfonyl) hept-6-enoate (61)

To a suspension of NaH (4.8 g, 120 mmol) in DMF (10 ml) was added benzenesulfonyl acetate (23.7 g, 120 mmol) in DMF (30 ml) at 0 ºC. After stirred for 3 h, to the reaction mixture was added **60** (11 g, 30 mmol) in DMF (40 ml) at 0 ºC, and then heated to 80 ºC for 7 days. The reaction mixture was quenched with water, and cooled to ambient temperature and then, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 20) to afford 9.5 g (77%) of silyl ether **61** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.83-7.51 (m, 5H), 5.04-4.95 (m, 2H), 4.19-4.05 (m, 1H), 3.62 (s, 3H), 0.83 (s, 9H), 0.01 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.2, 134.1, 133.1, 129.2, 129.0, 118.1, 77.1, 69.6, 68.7, 67.5, 52.7, 41.8, 33.3, 25.7, 17.9; IR (neat) ν_{max} 2953, 2857, 1743, 1444, 1325, 1149, 836, 778 cm^{-1}; LRMS (FAB) *m/z* 413 (M + H$^+$); HRMS (FAB) Calcd for $C_{20}H_{32}O_5S$ Si (M + H$^+$): 413.1818, found 413.1813.

(5*S*)-5-Allyl-3-(phenylsulfonyl) dihydrofuran-2(3*H*)-one (62)

To a solution of **61** (5.7 g, 13.8 mmol) in THF (70 ml) was added TBAF (1.0 M in THF, 27.5 ml, 27.5 mmol). After stirred for 16 h, the reaction mixture was quenched with saturated NH_4Cl, and then, extracted with EtOAc. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford

3.85 g (78%) of lactone **62** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.94-7.86 (m, 2H), 7.69-7.63 (m, 1H), 7.54 (t, 2H, *J* = 7.59 Hz), 5.65 (m, 1H), 5.13 (d, 2H, *J* = 8.43 Hz), 4.79-4.40 (m, 1H), 4.01 (t, 0.4H, *J* = 9.69 Hz), 4.00 (dd, 0.6H, *J* = 10.1 Hz, 3.1 Hz), 3.00 (dq, 0.6H, *J* = 14.7 Hz, 3.3 Hz), 2.75-2.25 (m, 3.4H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167, 136, 134, 131, 130, 129, 120, 77, 64, 39, 28; IR (neat) ν_{max} 2938, 1769, 1448, 1321, 1189, 934, 687 cm^{-1}; LRMS (FAB) *m/z* 267 ($M + H^+$); HRMS (FAB) Calcd for $C_{13}H_{15}O_4S$ ($M + H^+$): 267.0691, found 267.0688.

(*Z*)-But-2-ene-1,4-diyl diethyl dicarbonate (63a)

To a solution of 1,4-butene diol (10.0 g, 119.5 mmol) in CH_2Cl_2 (100 ml) were added pyridine (21 ml, 262.9 mmol) and ethyl chloroformate (28 ml, 298 mmol) at 0 ºC. After stirred for 4 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 3) to afford 24 g (76%) of dicarbonate **63a** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.23 (s, 2H), 4.55 (s, 4H), 4.10 (q, 4H, *J* = 7.14 Hz), 1.19 (t, 6H, *J* = 7.14 Hz); LRMS (FAB) *m/z* 255 ($M + Na^+$).

Diethyl 2-methylenepropane-1,3-diyl dicarbonate (63b)

To a solution of 2-methylene-1,3-propan diol (1.0 g, 11.3 mmol) in CH_2Cl_2 (20 ml) were added pyridine (2 ml, 25 mmol) and ethyl chloroformate (2.7 ml, 28.3 mmol) at 0 ºC. After stirred for 1 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford 2.7 g (100%) of dicarbonate **63b** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.23 (s, 2H), 4.55 (s, 4H), 4.10 (q, 4H, *J* = 7.14 Hz), 1.19 (t, 6H, *J* =

7.14 Hz); IR (neat) ν_{max} 2922, 1743, 1475, 1150, 926; cm^{-1}; LRMS (FAB) *m/z* 255 ($M + Na^+$).

Ethyl 2-Methylallyl carbonate (63c)

To a solution of methallyl alcohol (1.0 g, 13.9 mmol) in CH_2Cl_2 (25 ml) were added pyridine (1.44 ml, 18 mmol) and ethyl chloroformate (1.6 ml, 16.6 mmol) at 0 ºC. After stirred for 1 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford 1.37 g (68%) of dicarbonate **63c** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.21 (s, 1H), 5.20 (s, 1H), 4.60 (s, 2H), 4.10 (m, 2H), 1.37 (t, 3H, *J* = 7.14 Hz), 0.99 (s, 3H), 0.89 (s, 9H), 0.02 (s, 6H); LRMS (FAB) *m/z* 145 ($M + H^+$).

2-((*tert*-Butyldimethylsilyloxy) methyl)allyl ethyl carbonate (63d)

To a suspension of NaH (438 mg, 11 mmol) in THF (5 ml) was added diol (965 mg, 11 mmol) and stirred for 1 h. The TBSCl was then added, and vigourous stirring was continued for 1 h. the mixture was poured into ether, washed with 10% aqueous K_2CO_3 and brine, dried ($MgSO_4$) and concentrated *in vacuo.* To the crude silyl ether in CH_2Cl_2 (15 ml) were added pyridine (1.1 ml, 14.3 mmol) and ethyl chloroformate (1.3 ml, 13.2 mmol) at 0 ºC. After stirred for 1 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford 2.4 g (80% for 2 steps) of dicarbonate **63d** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.21 (s, 1H), 5.20 (s, 1H), 4.60 (s, 2H), 4.10 (m, 4H), 1.37 (t, 3H, *J* = 7.14 Hz), 0.86 (s, 9H), 0.02 (s, 6H); IR (neat) ν_{max} 2853, 1687, 1443, 1149, 836 cm^{-1}; LRMS (FAB) *m/z* 275 ($M + H^+$).

Ethyl -4-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl) but-2-enyl carbonate (64a)

To a mixture of lactone **62** (100 mg, 0.38 mmol) and diethyl carbonate **63a** (262 mg, 1.13 mmol) in CH_2Cl_2 (6 ml) was added Grubbs' 2nd catalyst (32 mg, 0.038 mmol). The reaction mixture was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 67.5 mg (48%) of **64a** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.93-7.51 (m, 5H), 5.69-5.66 (m, 2H), 4.81-4.72 (m, 0.7H), 4.51 (d, 2H, *J* = 4.0 Hz), 4.19-4.0 (m, 3.3H), 3.02-3.0 (m, 0.7 Hz), 2.77-2.66 (m, 0.3H), 2.58-2.23 (m, 3H), 1.27-1.16 (m, 3H); IR (neat) ν_{max} 2955, 1745, 1449, 1261, 1193, 1009, 722 cm^{-1}; LRMS (FAB) *m/z* 369 (M + H$^+$).

Diethyl 2-(2-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl)ethylidene)propane-1,3-diyl dicarbonate (64b)

To a mixture of lactone **62** (100 mg, 0.38 mmol) and dicatbonate **63b** (262 mg, 1.13 mmol) in CH_2Cl_2 (5 ml) was added Grubbs' 2nd catalyst (32 mg, 0.038 mmol). The reaction mixture was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 88 mg (49%) of **64b** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.9-7.5 (m, 5H), 5.44 (d, 1H, *J* = 7.4 Hz), 4.8-4.7 (m, 1H), 4.49-4.45 (m, 2H), 3.08-3.02 (m, 1H), 2.58-2.28 (m, 4H), 1.58 (s, 3H), 1.23 (m, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 155.0, 136.7, 134.6, 129.5, 129.2, 78.8, 72.3, 64.8, 33.2, 29.0, 14.2, 14.1; LRMS (FAB) *m/z* 383 (M + H$^+$).

Ethyl -2-methyl-4-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl)but-2-enyl carbonate (64c)

To a mixture of lactone **62** (395 mg, 1.48 mmol) and methyally catbonate **63c** (214 mg, 1.48 mmol) in CH_2Cl_2 (10 ml) was added Grubbs' 2nd catalyst (62.8 mg, 0.074 mmol). The reaction mixture

was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 184 mg (33%) of **64c** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.9-7.5 (m, 5H), 5.44 (d, 1H, *J* = 7.4 Hz), 4.8-4.7 (m, 1H), 4.49-4.45 (m, 2H), 3.08-3.02 (m, 1H), 2.58-2.28 (m, 4H), 1.58 (s, 3H), 1.23 (m, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 155.0, 136.7, 134.6, 129.5, 129.2, 78.8, 72.3, 64.8, 33.2, 29.0, 14.2, 14.1; IR (neat) ν_{max} 2922, 1743, 1475, 1319, 1150, 1008, 728 cm^{-1}; LRMS (FAB) *m/z* 383 ($M + H^+$); HRMS (FAB) Calcd for $C_{18}H_{23}O_7S$ ($M + H^+$): 383.1165, found 383.1162.

2-((*tert*-Butyldimethylsilyloxy) methyl)-4-((2*S*)-5-oxo-4-(phenylsulfonyl)tetrahydrofuran-2-yl)but-2-enyl ethyl carbonate (64d)

To a mixture of lactone **62** (194 mg, 0.73 mmol) and silyloxyallyl catbonate **63d** (200 mg, 0.73 mmol) in CH_2Cl_2 (10 ml) was added Grubbs' 2nd catalyst (31 mg, 0.036 mmol). The reaction mixture was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 230 mg (43%) of **64d** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.0-7.5 (m, 5H), 5.5 (t, 1H, *J* = 7.4 Hz), 4.8-4.4 (m, 3H), 4.29-4.0 (m, 4H), 3.0-2.3 (m, 5H), 1.25 (m, 3H), 0.85 (s, 9H), 0.01 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.4, 155, 137.5, 134.7, 129.6, 129.3, 127.9, 123.2, 78.9, 68.9, 64.7, 62.5, 58.6, 33, 29, 25.7, 18.3, 14.2.

(*Z*)-2-((*tert*-Butyldimethylsilyloxy)methyl)-4-((2*S*)-5-oxo-4-(phenylsulfonyl)tetrahydrofuran-2-yl)but-2-enyl ethyl carbonate (64d)

To a solution of allyl alcohol **S75** (2.4 g, 6.0 mmol) in CH_2Cl_2 (40 mL) at 0 ºC were added TBSCl (904 mg, 6.0 mmol), imidazole (443 mg, 6.5 mmol). After stirring for 3 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2 and aqueous layer was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was

purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 3.07 g (99%) of silyl ether **64d** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.92 (dd, 2H, *J* = 7.4 Hz, 20 Hz), 7.68 (t, 1H, *J* = 7.4 Hz), 7.56 (t, 2H, *J* = 7.8 Hz), 5.52 (t, 1H, *J* = 7.4 Hz), 4.78-4.46 (m, 1H), 4.60 (d, 1H, 8.8Hz), 4.22-4.05 (m, 5H), 3.02-2.74 (m, 1H), 2.55 (m, 2H), 2.68-2.30 (m, 1H), 1.28-1.20 (m, 3H), 0.85 (m, 9H), 0.05 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.4, 167.1, 154.9, 138.3, 137.8, 136.8, 136.7, 134.7, 134.6, 129.6, 129.4, 123.2, 122.7, 78.6, 68.9, 64.8, 64.0, 58.8, 58.7, 33.2, 32.8, 28.7, 28.2, 25.8, 18.2, 17.4, 17.2, 14.3, 12.8; IR (neat) ν_{max} 2926, 2856, 1744, 1516, 1458, 1321, 1258, 1151, 1083, 840, 781 cm^{-1}; LRMS (FAB) *m/z* 513 (M + H$^+$); HRMS (FAB) Calcd for $C_{24}H_{37}O_8SSi$ (M + H$^+$): 513.1978, found 513.1971.

4-(Phenylsulfonyl)5-(pro-1-en-2yl)-2-oxa-bicyclo[2.2.1]heptan-3-one (65)

To a refluxing solution of methally carbonate **64c** (12.5 mg, 0.033 mmol) in CH_2Cl_2 (2 ml) was added $Pd(dppe)_2$ (5.9 mg, 0.0065 mmol). AfAfter stirring for 15 h the reaction mixture was cooled to ambient temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 9 mg (94%) of bicyclic lactone **65** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.08-7.52 (m, 5H), 4.97 (s, 1H), 4.85 (dt, 2H, *J* = 13.7 Hz, 2.4 Hz, 1.3 Hz), 3.65 (q, 1H, *J* = 6.0 Hz), 2.5-2.30 (m, 2H), 2.26 (s, 1H), 1.97-1.92 (m, 1H), 1.64 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 168.4, 141.4, 134.5, 130.5, 128.7, 116.6, 77.0, 74.6, 45.8, 45.6, 37.0, 19.7; IR (neat) ν_{max} 2922, 1784, 1452, 1321, 1158, 1115, 723 cm^{-1}; LRMS (FAB) *m/z* 315 (M + Na$^+$); HRMS (FAB) Calcd for $C_{15}H_{16}O_4SNa$ (M + Na$^+$): 315.0667, found 315.0668.

5-(3-(*tert*-Butyldimethylsilyloxy)prop-1-en-2-yl)-4-(phenylsulfonyl)-2-oxabicyclo[2.2.1]heptan-3-one (66)

To a refluxing solution of silyl ether **64d** (250 mg, 0.49 mmol) in THF (2 mL) was added

$Pd(dppe)_2$ (44 mg, 50 μmol). After stirring for 1 h the reaction mixture was cooled to ambient temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 170 mg (89%) of bicyclic lactone **66** as a colorless oil: $[\alpha]_D^{25}$ -51.4 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02(m, 2H), 7.67-7.50 (m, 3H), 5.24(s, 1H), 5.04(s, 1H), 4.81(s, 1H), 4.21(s, 2H), 3.69 (dd, 1H, *J* = 5.5 Hz, 11.2 Hz), 2.53 (m, 1H), 2.30 (m, 2H), 2.09-2.00 (m, 1H), 0.90 (s, 9H), 0.04 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.9, 145.5, 136.8, 134.5, 130.2, 128.9, 113.8, 77.2, 75.4, 65.9, 45.9, 39.6, 39.0, 25.8, 18.2, -5.3; IR (neat) ν_{max} 2927, 1779, 1448, 1318, 1258, 1151, 1083, 840, 755, 726, 689 cm^{-1}; LRMS (FAB) *m/z* 423 (M + H$^+$); HRMS (FAB) Calcd for $C_{21}H_{31}O_5SSi$ (M + H$^+$): 423.1661, found 423.1658.

Minor 66': $[\alpha]_D^{25}$ -7.54 (c 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.03(d, 2H, *J* = 8.2 Hz), 7.62 (t, 1H, *J* = 7.4 Hz), 7.49 (t, 2H, *J* = 7.8 Hz), 5.41 (s, 1H), 5.10 (s, 1H), 4.90 (s, 1H), 4.50 (d, 1H, *J* = 9.4 Hz), 4.29 (d, 1H, *J* = 13.9 Hz), 3.08 (m, 1H), 2.65 (d, 1H, *J* = 10.7 Hz), 2.46 (d, 1H, *J* = 10.7 Hz), 2.36 (d, 1H, 6.6 Hz), 0.89 (s, 9H), 0.08 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 169.6, 144.8, 137.2, 134.3, 130.4, 128.5, 116.6, 77.7, 75.1, 65.9, 41.3, 40.6, 38.8, 25.8, 18.2, -5.3.

4-Methylenehexahydrocyclopenta[c]pyran-1,6-dione (68)

To a solution of cyclopentanol **70** (11 mg, 0.036 mmol) in CH_2Cl_2 were added TPAP (2 mg, 0.005 mmol) and NMO (8 mg, 0.07 mmol). The reaction mixture was stirred for 4 h and filtered with celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 :1) to afford 4 mg (67%) of cyclopentanone **68** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.33 (s, 1H), 5.12 (br, 1H), 4.92 (d, 1H, *J* = 12.8 Hz), 4.67 (d, 1H, *J* = 12.8 Hz), 3.04 (d, 1H, *J* = 20.88 Hz), 2.65 (m, 1H), 2.43 (m, 1H), 2.22 (m, 1H), 1.84 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 213.5, 172.2, 140.4, 115.6, 71.7, 42.9, 39.8, 38.9, 37.9; IR (neat) ν_{max} 2924, 2855, 1741, 1096, 804 cm^{-1}; LRMS (FAB) *m/z* 167 (M+H$^+$); HRMS (FAB) Calcd for $C_9H_{11}O_3$ (M + H$^+$): 167.0708, found 167.0706.

6-Hydroxy-4-methylenehexahydrocyclopenta[c]pyran-1(3*H*)-one (69)

To a solution of bicyclic lactone **70** (140 mg, 0.33 mmol) in THF (9 mL) was added TBAF (1.0 M solution in THF, 0.4 mL, 0.4 mmol) was added. The reaction mixture was stirred for 2 h and quenched with aqueous NH_4Cl. This mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 91 mg (90%) of cyclopentanol **69** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.08 (br, 1H), 5.00 (br, 1H), 4.74 (d, 1H, *J* = 12.3 Hz), 4.59 (d, 1H, *J* = 12 Hz), 4.27 (m, 1H), 2.60 (br, 1H), 2.28-2.04 (m, 3H), 1.61-1.52 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 174.6, 141.3, 113.7, 72.0, 71.0, 42.2, 40.1, 39.8, 38.7, 20.9, 14.0; IR (neat) ν_{max} 3414, 2931, 1722, 1380, 1039, 922 cm^{-1}; LRMS (FAB) m/z 169 (M + H$^+$).

5-(3-(*tert*-Butyldimethylsilyloxy)prop-1-en-2-yl)-2-oxabicyclo[2.2.1]heptan-3-one (70)

To a mixture of bicyclic lactone **66** (142 mg, 0.51 mmol) and boric acid (316 mg, 5.1 mmol) in methanol (5 ml) was added 6% Na/Hg (1.56 g, 4.08 mmol) and stirred for 48 h. The reaction mixture was quenched with saturated NH_4Cl, and then extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 3) to afford 38.6 mg (56%) of bicyclic lactone **70** as a white solid: ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.47 (s, 1H), 5.14 (s, 1H), 4.94 (s, 1H), 4.18 (q, 2H, *J* = 13.7 Hz), 3.07 (m, 1H), 3.01 (s, 1H), 2.22 (d, 1H, *J* = 10.5 Hz), 2.14 (m, 1H), 1.89 (m, 2H), 0.92 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C-NMR (CD_3OD, 125 MHz) δ 179.1, 149.3, 111.0, 82.9, 67.7, 42.8, 40.7, 33.8, 27.2, 19.9, -4.3, -4.4; IR (neat) ν_{max} 2955, 2928, 1789, 1255, 1084, 837 cm^{-1}; LRMS (FAB) *m/z* 283 (M+H$^+$); HRMS (FAB) Calcd for $C_{15}H_{26}O_3Si$ (M + H$^+$): 282.1651, found 282.1688.

6-((Bromomethyl)dimethylsilyloxy)-4-methylene-4,4a,5,6-tetrahydrocyclopenta[c]pyran-

1(3*H*)-one (71)

To a solution of cyclopetenol **79** (1 mg, 0.006 mmol) in CH_2Cl_2 (0.5 ml) were added BMDMCS (0.1 M in benzene, 0. 9 ml, 0.09 mmol), Et_3N (1.4 μl, 0.09 mmol) and DMAP (1.2 mg, 0.01 mmol) at 0 ºC. After stirred for 1 h at the ambient temperature, the reaction mixture was concentrated *in vacuo* to give as colorless oil. The crude silylether **71** was used in the next step without further purification: ^{1}H-NMR (C_6D_6, 300 MHz) δ 6.73 (br, 1H), 5.15 (s, 1H), 5.12 (m, 1H), 4.99 (s, 1H), 4.82 (d, 1H, *J* = 13.4 Hz), 4.70 (d, 1H, *J* = 13 Hz), 3.46 (br, 1H), 2.74 (m, 1H), 2.47 (s, 2H), 1.90 (m, 1H), 0.27 (s, 6H); LRMS (FAB) *m/z* 317 ($M + H^+$).

6-Hydroxy-4-methylene-7a-(phenylsulfonyl)hexahydrocyclopenta[c]pyran-1(3*H*)-one (72)

To a solution of bicyclic lactone **66** (168 mg, 0.4 mmol) in THF (10 mL), TBAF (1.0 M solution in THF, 0.48 mL, 0.48 mmol) was added. The reaction mixture was stirred for 2 h and quenched with aqueous NH_4Cl. This mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 98 mg (80%) of bicyclic lactone **72** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.80 (m, 2H), 7.66 (m,1H), 7.54 (m, 2H), 5.19 (m, 3H), 4.72 (d, 1H, *J* = 12 Hz), 4.39 (m, 1H), 4.05 (m, 1H), 2.3 (m, 3H), 2.0 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 167.1, 139.2, 135.2, 134.5, 130.4, 128.7, 114.5, 77.1, 72.4, 71.3, 44.9, 40.9, 39.9; IR (neat) ν_{max} 3508, 1725, 1377, 1301, 1238, 1135, 1076, 919, 719 cm^{-1}; LRMS (FAB) m/z 309 ($M + H^+$); HRMS (FAB) Calcd for $C_{15}H_{17}O_5S$ ($M + H^+$): 309.0797, found 309.0789.

Ethyl 2-(Hhydroxymethyl)allyl carbonate (73)

To a solution of 2-methylene-1,3-propanediol (2.17 g, 24.6 mmol) in anhydrous CH_2Cl_2 (25 ml) was added pyridine (3.98 ml, 49.3 mmol) at -10 ºC and stirred for 10 minutes. To the mixture was added a solution of ethylchloroformate (2.46 ml, 25.9 mmol) in anhydrous CH_2Cl_2 (25 ml) by

dropping fonnel for 40 minutes. After stirring for 2 h, the reaction mixture was quenched with H_2O, extracted with EtOAc and then concentrated *in vacuo*. The residue was purified by flash chromatography by silica gel (EtOAc : *n*- Hexane = 1 : 2) to afford 2.8 g of allyl carbonate **73** (71%) as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.18 (s, 1H), 5.13 (s, 1H), 4.59 (s, 2H), 4.12 (q, 2H, *J* = 7.2 Hz), 4.07 (s, 2H), 2.68 (s, 1H), 1.23 (t, 3H, *J* = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 155.0, 142.7, 114.3, 67.7, 64.0, 63.1, 14.0; IR (neat) ν_{max} 3428, 2986, 1747, 1260, 1011 cm^{-1}; LRMS (FAB) *m/z* 161 ($M + H^+$); HRMS (FAB) Calcd for $C_7H_{13}O_4$ ($M + H^+$): 161.0814, found 161.0810.

(*S*)-2-Allyl-4,4-diisopropyl-7-methylene-10-oxo-3,5,9,11-tetraoxa-4-silatridecyl 4-methylbenzenesulfonate (74)

Dichlorodiisopropylsilane (2.36 ml, 13.1 mmol) was added to imidazole (2.44 g, 35.8 mmol) in CH_2Cl_2 (15 ml) at 0 ºC. The solution was stirred for *ca*. 5 minutes, then the hydroxy tosylate **59** (3.22 g, 11.9 mmol) in CH_2Cl_2 (10 ml) was added dropwise by dropping funnel *ca.*1 h at 0 ºC. After the mixture was stirred for 10 minutes at 0 ºC, a solution of the allylic alcohol **73** (1.91 g, 11.9 mmol) in CH_2Cl_2 (10 mL) was added at 0 ºC. The reaction mixture was warmed to the room temperature and stirred for 16 h. The reaction mixture filtrated with hexane, then concentrated *in vacuo* to afford the crude oil, which was purified by flash chromatography on silica gel (EtOAc : *n*-hexane = 1 : 7) to afford 5.2 g (82%) of *bis*-alkoxysilane **74** as a colorless oil: $[\alpha]_D^{25}$ 1.65 (*c* 0.5, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.75 (d, 2H, *J* = 8.2 Hz), 7.30 (d, 2H, *J* = 8.1 Hz), 5.76-5.62 (m, 1H), 5.22(s, 1H), 5.15 (s, 1H), 5.02-4.97 (m, 2H), 4.59 (s, 2H), 4.24 (s, 2H), 4.17 (q, 2H, *J* = 7.1 Hz), 4.05 (m, 1H), 3.89 (m, 2H), 2.41 (s, 3H), 2.25 (m, 2H), 1.27 (t, 3H, *J* = 7.1 Hz), 0.96 (m, 14H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 154.9, 144.7, 142.2, 132.8, 132.7, 129.7, 127.9, 118.3, 113.6, 113.3, 72.1, 69.2, 67.6, 64.0, 63.3, 38.4, 21.5, 17.2, 17.1, 14.2, 12.1, 12.0; IR (neat) ν_{max} 2945, 2867, 1748, 1259, 1178, 1097, 989, 815 cm^{-1}; LRMS (FAB) *m/z* 529 ($M + H^+$).

(12*S*)-Methyl 12-allyl-10,10-diisopropyl-7-methylene-4-oxo-14-(phenylsulfonyl)-3,5,9,11-tetraoxa-10-silapentadecan-15-oate (S74)

To a suspension of NaH (1.98 g, 49.5 mmol) in DMF (10 ml) was slowly added solution of benzene sulfonate (10.6 g, 49.5 mmol) in DMF (20 ml) at 0 ºC. After the mixture was stirred for 1 h at 0 ºC, *bis*-alkoxysilane **74** (8 g, 15.1 mmol) in DMF (20 ml) was added. The reaction mixture was stirred at 80 ºC for 24 h and quenched with aqueous NH_4Cl solution. This mixture was extracted by ether. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1: 5) to afford 4.696 g (54 %) of the alkylated product **S74** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.80 (m, 2H), 7.63 (m, 1H), 7.50 (m, 2H), 5.71-5.60 (m, 1H), 5.21 (d, 1H, *J* = 16.5 Hz), 5.13 (d, 1H, *J* = 8.7 Hz), 5.02-4.95 (m, 2H), 4.58 (d, 1H, *J* = 12.3 Hz), 4.24-4.20 (m, 3H), 4.17-4.11 (m, 2H), 4.03-3.83 (m, 1H), 3.58 (s, 3H), 2.35-2.04 (m, 4H), 1.25 (t, 3H, *J* = 7.1 Hz), 1.20 (t, 2H, *J* = 7.1 Hz), 1.00-0.89 (m, 14H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 170.9, 166.5, 166.2, 154.9, 142.1, 137.5, 134.0, 133.1, 132.9, 129.5, 129.2, 129.1, 129.0, 128.9, 128.8, 118.2, 118.1, 113.4, 113.3, 69.1, 67.6, 67.5, 67.3, 63.9, 63.3, 63.2, 60.2, 52.8, 52.6, 41.7, 32.9, 32.7, 20.9, 17.2, 17.1, 17.0, 16.9, 14.1, 12.2, 12.0, 11.8; IR (neat) ν_{max} 2946, 2867, 1746, 1448, 1259, 884 cm^{-1}; LRMS (FAB) *m/z* 571.

Methyl 3-((*S*,*Z*)-7-((ethoxycarbonyloxy)methyl)-2,2-diisopropyl-5,8-dihydro-4*H*-1,3,2-dioxasilocin-4-yl)-2-(phenylsulfonyl)propanoate (75)

To a refluxing solution of benzensulfonate **S74** (4.6 g, 8.06 mmol) in CH_2Cl_2 (600 mL) were added Grubbs' 2nd catalyst (171 mg, 0.20 mmol). After stirring of the mixture for 0.5 h, the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1: 5) to afford 4.26 g (97%) of cyclic dioxasilane **75** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.80 (m, 2H), 7.62 (m, 1H), 7.51 (m, 2H), 5.72 (t, 1H, *J* = 8.6 Hz),

4.57 (d, 1H, *J* = 12.7 Hz), 4.50 (d, 1H, *J* = 12.5 Hz), 4.29-4.21 (m, 1.3H), 4.14-4.09 (m, 3H), 3.75-3.70 (m, 0.7H), 3.58 (s, 2H), 3.51 (s, 1H), 2.53-2.48 (m, 0.3H), 2.46-2.41 (m, 0.7H), 2.25-2.04 (m, 3H), 1.23 (m, 3H), 0.94-0.79 (m, 14H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 170.8, 166.4, 166.0, 154.7, 138.5, 138.4, 137.2, 137.0, 134.1,129.1, 129.0, 128.9, 127.4, 71.9, 70.0, 69.8, 69.7, 68.5, 67.7, 63.9, 60.1, 59.6, 52.6, 35.1, 34.7, 34.4, 20.8, 17.2, 17.0, 16.9, 14.0, 12.6, 12.5, 12.0, 11.8; IR (neat) ν_{max} 2945, 2866, 1745, 1463, 1256, 1084 cm^{-1}; LRMS (FAB) *m/z* 543 (M + H$^+$); HRMS (FAB) Calcd for $C_{25}H_{39}O_9SSi$ (M + H$^+$): 543.2084, found 543.2074.

Ethyl (*E*)-2-(hydroxymethyl)-4-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl)but-2-enyl carbonate (S75)

To a solution of cyclic dioxasilane **75** (2.76 g, 5.08 mmol) in THF (50 mL), TBAF (1.0 M solution in THF, 5.6 mL, 5.6 mmol) was added. The reaction mixture was stirred for 2 h and quenched with aqueous NH_4Cl. This mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 1.48 g (73%) of allyl alcohol **S75** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.9-7.55 (m, 5H), 5.75-5.60 (m, 1H), 5.22 (d, 1H, *J* = 12.6 Hz), 4.85-4.48 (m, 4H), 4.19-4.05 (m, 3H), 3.10-2.29 (m, 4H), 1.26 (m, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 155.1, 138.1, 137.8, 136.5, 134.7, 129.5, 129.3, 129.2, 125.6, 125.2, 78.6, 77.1, 69.5, 64.8, 64.2, 63.9, 58.1, 32.8, 28.8, 14.2; IR (neat) ν_{max} 3531, 2983, 1744, 1448, 1261, 1149, 1084, 1009, 937, 873 cm^{-1}; LRMS (FAB) *m/z* 399 (M + H$^+$); HRMS (FAB) Calcd for $C_{18}H23O_8S$ (M + H$^+$): 399.1114, found 399.1102.

(*S*)-4-Methylene-3,4,4a,5-tetrahydrocyclopenta[c]pyran-1,6-dione (76)

To a solution of cyclopentanol **72** (11 mg, 0.036 mmol) in CH_2Cl_2 (1 ml) was added *Dess-Martin* periodinane (23 mg, 0.054 mmol) at the ambient temperature and the reaction mixture was stirred

for 16 h. After quenching with H_2O, the mixture was extracted with EtOAc and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 3.3 mg (56%) of cyclopentenone **76** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.73 (d, 1H, *J* = 2.9 Hz), 5.30 (s, 1H), 5.13 (s, 1H), 5.02 (d, 1H, *J* = 13.2 Hz), 4.84 (d, 1H, *J* = 7.8 Hz), 3.89 (m, 1H), 2.84 (dd, 1H, *J* = 6.2 Hz, 18.5 Hz), 2.65 (dd, 1H, *J* = 3.6 Hz, 18.5 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 209.0, 152.0, 138.2, 136.8, 127.6, 112.6, 70.5, 42.8, 42.1, 39.4; IR (neat) ν_{max} 2929, 1725, 1612, 1462, 1250, 1110, 838 cm^{-1}; LRMS (FAB) m/z 165 (M + H$^+$).

6-Hydroxy-4-methylene-4,4a,5,6-tetrahydrocyclopenta[c]pyran-1(3*H*)-one (77)

To a flamed flask were added (*S*)-Me-CBS-oxazaborolidine (1.0 M solution in toluene, 0.37 ml, 0.36 mmol) and borane-SMe_2 complex (2.0 M in THF, 0.09 ml, 0.18 mmol) at the ambient temperature. After stirring for 10 minutes, cyclopentenone **76** (6 mg, 0.04 mmol) in CH_2Cl_2 (2 ml) was added at 0 ℃ and stirred for 10 minutes. After quenching with *i*PrOH, the mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 2 : 1) to afford 5 mg (80%) of cyclopentenol **77** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.79 (s, 1H), 5.16 (s, 1H), 5.08 (q, 1H, *J* = 7.3 Hz), 5.0 (s, 1H), 4.84 (d, 1H, *J* = 13 Hz), 4,72 (d, 1H, *J* = 13 Hz), 3.72 (br, 1H), 2.82 (td, 1H, *J* = 13 Hz, 6.8 Hz), 1.84 (m, 1H), 1.78 (d, 1H, *J* = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 162, 145, 140, 134, 111, 76, 73, 44, 40; IR cm^{-1} 3399, 2925, 1722, 1633, 1245, 1050, 916; LRMS (FAB) m/z 167 (M + H$^+$); HRMS (FAB) Calcd for $C_9H_{11}O_3$ (M + H$^+$): 167.0708, found 167.0702.

4-Methylene-1-oxo-1,3,4,4a,5,6-hexahydrocyclopenta[c]pyran-6-yl 2,2,2-trichloroacetate (78)

To a solution of cyclopentenol **77** (3.4 mg, 0.02 mmol) in THF (0.6 ml) were added trichloroacetic acid (13 mg, 0.082 mmol) and triphenyl phosphine (21 mg, 0.082 mmol). And then, DIAD (0.016

ml, 0.082 mmol) was added dropwise. After stirred for 4 h, the reaction mixture was quenched with $NaHCO_3$, the mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 6 mg (96%) of trichloroacetate **78** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.88 (t, 1H, *J* = 2.7 Hz), 5.80 (d, 1H, *J* = 5.7 Hz), 5.27 (s, 1H), 5.06 (s, 1H), 4.91 (d, 1H, *J* = 13 Hz), 4.71 (d, 1H, *J* =13 Hz), 3.93 (br, 1H), 2.53 (dd, 1H, *J* =14.6 Hz, 6 Hz), 2.39 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 161.5, 152.3, 139.8, 138.1, 112.0, 82.5, 65.4, 64.1, 45.0, 36.1; LRMS (FAB) m/z 312 ($M + H^+$).

6-Hydroxy-4-methylene-4,4a,5,6-tetrahydrocyclopenta[c]pyran-1(3*H*)-one (79)

To a solution of trichloroacetate **78** (6 mg, 0.019 mmol) in THF/MeOH (1:1, 1ml) was added silica gel (20 mg). The reaction mixture was heated to 40 ℃. The reaction mixture was stirred for 8 h and filtered with celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane =) to afford 1 mg (31%) of cyclopentenol **79** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.84 (t, 1H, *J* = 2.9 HZ), 5.15 (s, 1H), 5.01 (s, 1H), 4.88 (m, 2H), 4.71 (d, 1H, *J* = 13Hz), 3.87 (br, 1H), 2.30 (dd, 1H, *J* = 6.5 Hz, 20 Hz), 2.21 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 162.9, 145.3, 140.8, 134.8, 111.4, 76.1, 73.1, 44.9, 40.3; IR cm^{-1} 3399, 2925, 1722, 1633, 1245, 1050, 916; LRMS (FAB) m/z 167 ($M + H^+$); HRMS (FAB) Calcd for $C_9H_{11}O_3$ ($M + H^+$): 167.0708, found 167.0702.

4-Methylene-7a-(phenylsulfonyl)hexahydrocyclopenta[c]pyran-1,6-dione (80)

-Swern oxidation condition

DMSO (0.02 ml, 0.28 mmol) was dissolved in CH_2Cl_2 (1 ml) at -78 ℃ and treated with TFAA (0.02 ml, 0.14 mmol). The solution was allowed to stir 10 min at -78 ℃ and then, treated dropwise with cyclopentanol **79** (10 mg, 0.03 mmol) in CH_2Cl_2 (1 ml). The solution was

allowed to stir for 1 h at -78 ºC and then, treated with Et_3N (0.05 ml, 0.36 mmol). The solution was allowed to warm to 0 ºC and stirred for 1h. The reaction mixture was quenched with water, aqueous phase wasextracted with CH_2Cl_2 and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 2 : 1) to afford 8 mg (80%) of cyclopentanone **80** as a colorless oil.

-PCC oxidation condition

To a solution of cyclopentanol **79** (3 mg, 0.01 mmol) in CH_2Cl_2 (0.6 ml) were added PCC (3 mg, 0.015 mmol) and silica gel (6 mg). After stirred for 4 h, the reaction mixture filtered with celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 2 :1) to afford 2.4 mg (85%) of cyclopentanone **80** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.82-7.50 (m, 5H), 5.22-5.15 (m, 3H), 4.73 (d, 1H, J = 12.6 Hz), 4.42 (m, 1H), 4.07 (m, 1H), 2.40 (m, 3H), 2.01 (br, 1H); IR cm^{-1} 2924, 1717, 1261, 745; LRMS (FAB) m/z 307 (M + H$^+$).

4-Methylene-3,4-dihydrocyclopenta[c]pyran-1,6(5*H*,7*H*)-dione (81)

To a solution of cyclopentanone **80** (4 mg, 0.013 mmol) in THF (0.8 ml) was added DBU (3 μl, 0.022 mmol). The reaction mixture was refluxed for 16 h, and then cooled. The reaction mixture was quenched with aqueous NH_4Cl, concentrated *in vacuo*. The residue was diluted with EtOAc, the organic layer were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 1 mg (47%) of ccyclopentenone **81** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.41 (s, 1H), 5.26 (s, 1H), 5.08 (s, 2H), 3.30 (m, 4H); IR cm^{-1} 2925, 1633, 1245, 1050; LRMS (FAB) m/z 165 (M+H).

(1*R*,2*S*,4*S*)-Methyl 2-(3-(tert-butyldimethylsilyloxy)prop-1-en-2-yl)-4-hydroxy-1-(phenylsulfonyl) cyclopentanecarboxylate (83)

To a solution of bicyclic lactone **66** (78 mg, 0.19 mmol) in methanol (10 ml) was added sodium

methoxide (12 mg, 0.20 mmol) at the ambient temperature. After stirred for 1 h, the reaction mixture was quenched with aqueous NH_4Cl, concentrated *in vacuo*. The residue was diluted with EtOAc, the organic layer were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 78 mg (93%) of cyclopentanol **83** as a colorless oil: $[\alpha]_D^{25}$ -104.5 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.89 (d, 2H, *J* = 7.6 Hz), 7.61 (t, 1H, *J* = 7.4 Hz), 7.50 (t, 2H, *J* = 7.6 Hz), 5.23 (s, 1H), 4.98 (s, 1H), 4.48 (m, 1H), 4.03 (d, 1H, *J* = 14.6 Hz), 3.85 (d, 1H, *J* = 14.6 Hz), 3.52 (s, 3H), 3.30 (t, 1H, *J*= 8.7 Hz), 2.80 (dd, 1H, *J* = 6.8 Hz, 14.8 Hz), 2.58 (dd, 1H, *J* = 7.8 Hz, 14.8 Hz), 2.37 (m, 1H), 1.92-1.84 (m, 1H), 0.85 (m, 9H), -0.01 (m, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 167.6, 148.0, 136.8, 134.1, 130.6, 128.7, 111.7, 83.3, 70.9, 65.6, 52.5, 43.0, 41.8, 41.5, 25.8, 18.1, -5.4; IR (neat) ν_{max} 3458, 2953, 2929, 2856, 1738, 1447, 1308, 1254, 1140, 1079, 837 cm^{-1}; LRMS (FAB) *m/z* 455 (M + H$^+$); HRMS (FAB) Calcd for $C_{22}H_{35}O_6SSi$ (M + H$^+$): 455.1924, found 455.1932.

(*S*)-Methyl 5-(3-(tert-butyldimethylsilyloxy)prop-1-en-2-yl)-3-oxocyclopent-1-enecarboxylate (84)

To a solution of cyclopentanol **83** (48 mg, 0.11 mmol) in CH_2Cl_2 (5 ml) was added Dess-Martin periodinane (49 mg, 0.12 mmol) at the ambient temperature and the reaction mixture was stirred for 16 h. After quenching with H_2O, the mixture was extracted with CH_2Cl_2 and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 30 mg (91%) of cyclopentenone **84** as a colorless oil: $[\alpha]_D^{25}$ 751.4 (*c* 0.33, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.73 (d, 1H, *J* = 1.8 Hz), 5.06 (s, 1H), 4.73 (s, 1H), 4.14 (s, 2H), 3.80 (s, 3H), 3.77 (m, 1H), 2.76 (dd, 1H, *J* = 7.2 Hz, 19.1 Hz), 2.38 (dd, 1H, *J*= 1.9 Hz, 19.1 Hz), 0.88 (s, 9H), 0.04 (m, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 208.2, 165.1, 164.4, 148.9, 137.8, 110.4, 65.8, 52.4, 44.3, 42.4, 25.8, 18.3, -5.4; IR (neat) ν_{max} 2954, 2929, 2857, 1725, 1612, 1462, 1250, 1110, 838 cm^{-1}; LRMS (FAB) *m/z* 311 (M + H$^+$); HRMS (FAB) Calcd for

$C_{16}H_{27}O_4Si$ ($M + H^+$): 311.1679, found 311.1670.

(3*R*,5*S*)-Methyl 5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)-3-hydroxycyclopent-1-enecarboxylate (85)

To a flamed flask were added (*S*)-Me-CBS-oxazaborolidine (1.0 M solution in toluene, 3 μl, 3 μmol) and borane-SMe_2 complex (2.0 M in THF, 8 μl, 0.016 mmol) at the ambient temperature. After stirring for 10 minutes, cyclopentenone **84** (10 mg, 0.03 mmol) in THF (1 ml) was added at -78 ºC and stirred for 10 minutes. After quenching with *i*PrOH, the mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 6 mg (60%) of cyclopentenol **85** as a colorless oil: $[\alpha]_D^{25}$ 362.3 (*c* 0.33, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.77 (s, 1H), 5.06 (m, 1H), 4.98 (s, 1H), 4.67 (s, 1H), 3.69 (s, 3H), 3.68 (m, 1H), 2.02 (m, 1H), 1.88 (m, 1H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 164.9, 150.0, 144.5, 140.1, 107.8, 76.5, 65.6, 51.6, 46.0, 42.5, 25.8, 18.3, -5.4; IR (neat) ν_{max} 3435, 2953, 2930, 2857, 1725, 1436, 1256, 1110, 721 cm^{-1}; LRMS (ESI) *m/z* 313 ($M + H^+$); HRMS (FAB) Calcd for $C_{16}H_{29}O_4Si$ ($M + H^+$): 313.1835, found 313.1826.

(3*R*,5*S*)-Methyl 3-((bromomethyl)dimethylsilyloxy)-5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)cyclopent-1-enecarboxylate (82)

To a solution of cyclopetenol **85** (5 mg, 0.016 mmol) in bezene (1 ml) were added BMDMCS (0.1 M in benzene, 0.3 ml, 0.03 mmol), Et_3N (6 μl, 0.04 mmol) and DMAP (1.2 mg, 0.01 mmol) at 0 ºC. After stirred for 2 h at the ambient temperature, the reaction mixture was concentrated *in vacuo* to give as colorless oil. The crude silylether **82** was used in the next step without further purification: $[\alpha]_D^{20}$ 52.4 (*c* 0.50, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.70 (t, 1H), 5.11 (m, 1H), 4.98 (s, 1H), 4.66 (s, 1H), 4.10 (m, 2H), 3.68 (s, 3H), 3.61 (m, 1H), 2.45 (s, 2H), 2.23 (m, 1H),

2.07 (m, 1H), 0.89 (m, 9H), 0.26 (s, 6H), 0.05 (s, 6H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 164.8, 151.1, 145.7, 139.7, 107.5, 76.2, 65.9, 57.8, 42.6, 38.2, 26.0, 7.7, -1.0, -5.3; IR (neat) ν_{max} 2954, 2930, 2857, 1726, 1436, 1361, 1255, 1113, 837 cm^{-1}; LRMS (FAB) *m/z* 463 (M + H$^+$); HRMS (FAB) Calcd for $C_{19}H_{36}BrO_4Si_2$ (M + H$^+$): 463.1318, found 463.1321.

Methyl 5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)-2,2-dimethylhexahydro-2*H*-cyclopenta[d][1,2] oxasilole-4-carboxylate (87)

To a solution of silylether **82** (7 mg, 0.016 mmol) in benzene were added Et_3B (1.0 M in hexane, 0.016ml, 3 μmol) and Bu_3SnH (5 μl, 0.019 mmol) at 0 ℃. After stirred for 2h at ambient temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 3.8 mg (62%) of oxasilole **87** as a colorless oil: $[\alpha]_D^{20}$ 3.2 (*c* 0.50, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.12 (s, 1H), 4.80 (s, 1H), 4.27 (s, 1H), 4.09 (d, 1H, *J* = 14.3 Hz), 4.02 (d, 1H, *J* = 14.3 Hz), 3.53 (s. 3H), 3.14 (m, 1H), 2.77 (dd, 1H, *J* = 7.75 Hz, 10.1 Hz), 2.54 (m, 1H), 2.44 (s, 1H), 2.07-2.02 (m, 1H), 1.88 (dd, 1H, *J* = 6.65 Hz, 13.2 Hz), 0.90 (m, 9H), 0.78 (m, 1H), 0.69 (m, 1H), 0.09 (s, 6H), 0.04 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 163.7, 139.5, 108.7, 73.8, 65.9, 55.8, 51.6, 44.2, 41.8, 39.2, 25.9, 18.3, 1.7, -5.4; IR (neat) ν_{max} 2957, 2925, 2871, 2855, 1736, 1462, 1253, 842 cm^{-1}; LRMS (FAB) *m/z* 385 (M + H$^+$); HRMS (FAB) Calcd for $C_{19}H_{37}O_4Si_2$ (M + H$^+$): 385.2230, found 385.2216.

Methyl 5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)-3-hydroxy-2-(hydroxymethyl) cyclopentane carboxylate (88)

To a solution of oxasilole **87** (3.5 mg, 9 μmol) in THF/ MeOH (1:1, 1 ml) were added potassium bicarbonate (1.8 mg, 0.018 mmol), potassium fluoride (2.1 mg, 0.036 mmol) and aqueous hydrogen peroxide (30% aqueous solution, 0.02ml, 0.18 mmol) at ambient temperature. After

stirred for 16 h, to the reaction mixture was added well-grounded $Na_2S_2O_3$ (70 mg, 1.2 *eq* of H_2O_2), and then diluted with Et_2O, then filtrated with celite pad. The filtrate was concentrated *in vacuo*, the residue was purified by flash column chromatography on silica gel (EtOAc : Hexane = 2 : 1) to afforded 1.6 mg (50%) of diol **88** as a colorless oil: $[\alpha]_D^{20}$ –5.60 (*c* 0.50, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.13 (s, 1H), 4.79 (s, 1H), 4.60 (m, 1H), 4.10 (m, 2H), 3.94 (dd, 1H, *J* = 4.0 Hz, 11.0 Hz), 3.72 (m, 1H), 3.54 (s, 3H), 3.16 (m, 2H), 2.69 (m, 1H), 2.15 (m, 1H), 1.81 (m, 1H), 0.89 (m, 9H), 0.05 (s, 6H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.6, 148.0, 119.6, 108.8, 74.7, 65.9, 62.3, 51.4, 48.3, 41.9, 40.2, 25.8, 18.3, -5.4; IR (neat) ν_{max} 3468, 2928, 2856, 1735, 1682, 1460, 838 cm^{-1}; LRMS (FAB) *m/z* 345 (M + H$^+$); HRMS (FAB) Calcd for $C_{17}H_{33}O_5Si$ (M + H$^+$): 345.2097, found 345.2092.

6-Hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta [*c*]pyran-1 (3*H*)-one (1)

To a solution of hydroxymethyl cyclopentanol **88** (6 mg, 0.017 mmol) in CH_2Cl_2 (1.5 ml) was added TsOH-H_2O (5mg, 0.026 mmol) at the ambient temperature and stirred overnight. The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-Hexane = 2 : 1 →CH_2Cl_2 : MeOH = 10 : 1) to afford of iridoid **1** 2.4 mg (66%) as a coloress needle: mp 110-112 ℃; $[\alpha]_D^{25}$ -13.0 (*c* 0.01, MeOH); ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.18 (s, 1H), 5.05 (s, 1H), 4.73 (m, 2H), 4.37 (t, 1H, *J* = 3.5 Hz), 3.86 (dd, 1H, *J* = 4.8 Hz, 10.8 Hz), 3.79 (dd, 1H, *J* = 8 Hz, 10.7 Hz), 3.45 (m, 1H), 3.01 (dd, 1H, *J* = 9.6 Hz, 11.2 Hz), 2.40 (m, 1H), 2.14 (m, 1H), 1.65 (m, 1H); ^{13}C-NMR (CD_3OD, 75 MHz) δ 178.1, 144.9, 114.5, 74.2, 73.2, 62.6, 52.8, 45.6, 42.1, 41.6; IR (neat) ν_{max} 3361, 2925, 2853, 1728, 1659, 1467, 1378, 1241, 1171, 1037, 917 cm^{-1}; LRMS (FAB) *m/z* 199 (M + H$^+$); HRMS (FAB) Calcd for $C_{10}H_{15}O_4$ (M + H$^+$): 199.0970, found 199.0977.

V. References

1 (a) El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod*, **1980**, *43*, 649-707. (b) Boros, C. A.; Stermitz, F. R. *J. Nat. Prod*. **1991**, *54*, 1173-1246. (c) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2007**, *55*, 159-222. (d) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2007**, *55*, 689-728. (e) Tundis, R.; Loizzo, M. R.; Menichini, F.; Statti, G. A.; Menichini, F. *Mini-Rev. Med. Chem.* **2008**, *8*, 399-420. (f) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2009**, *57*, 765-796. (g) Paquette, L. A. *Top. Curr. Chem*. **1984**, *119*, 1-160.

2 (a) Kobayashi, Y.; *Curr. Org. Chem.* **2003**, *7,* 133-147. (b) Leemans, E.; D'hooghe, M.; De Kimpe, N. *Chem. Rev*. **2011**, *111*, 3268-3333.

3 (a) *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984. (b) Gothelf, K. V.; Jørgensen, K. A. *Chem. Rev.* **1998**, *98*, 863-909. (c) Li, C.-L.; Liu, R.-S. *Chem. Rev.* **2000**, *100*, 3127-3161. (d) Klunder, A. J. H.; Zhu, J.; Zwanenburg, B. *Chem. Rev.* **1999**, *99*, 1163-1190. (e) Gibson, S. E.; Lewis, S. E.; Mainolfi, N. *J. Organomet. Chem.* **2004**, *689*, 3873-3890. (f) Aubert, C.; Buisine, O.; Malacria, M. *Chem. Rev.* **2002**, *102*, 813-834. (g) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875-1916.

4 (a) Hudlicky, T.; Price, J. D. *Chem. Rev.* **1989**, *89*, 1467.-1486. (b) Borthwick, A. D.; Biggadike, K. *Tetrahedron* **1992**, *48*, 571-623. (c) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319-4347.

5 (a) Barco, A.; Benetti, S.; De Risi, C.; Marchetti, P.; Pollini, G. P.; Zanirato, V. *Tetrahedron: Asymmetry* **1997**, *8*, 3515-3545. (b) Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229-9272. (c) Dalko, P. I.; Sinay, P. *Angew. Chem., Int. Ed.* **1999**, *38*, 773-777.

6 (a) Arjona, O.; Go´mez, A. M.; Lo´pez, J. C.; Plumet, J. *Chem. Rev.* **2007**, *107*, 1919-2036.

7 (a) El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod*, **1980**, *43*, 649-707. (b) Boros, C. A.; Stermitz, F. R. *J. Nat. Prod*. **1991**, *54*, 1173-1246. (c) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2007**, *55*, 159-222.

8 (a) Dewick, P. M. (2009) *Medicinal natural products: A biosynthetic approach. 3rd ed.* England:

John Wiley & Sons Ltd. 206-223. (b) Tietz, L.-T. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 828-841. (c) Banthorpe, D. V.; Charlwood, B. V.; Francis, M. J. O. *Chem. Rev.*, **1972**, *72*, 115-155.

9 (a) Scheffrahn, R. H.; Gaston, L. K.; Sims, J. J.; Rust, M. K. Environ. *Entomol*. **1984**, *13*, 1502-1506. (b) Tomalski, M. D.; Blum, M. S.; Jones, T.H.; Fales, H. M.; Howard, D. F.; Passera, L. *J. Chem. Ecol*. **1987**, *13*, 253-263. (c) Huth, A.; Dettner, K. *J. Chem. Ecol*. **1990**, *16*, 2691-2711. (d) Lorenz, M.; Boland, W.; Dettner, K. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 912-914.

10 Inouye, H. *Planta Med.* **1978**, *33*, 193-216.

11 Sakan, T.; Fujino, A.; Murai, F.; Suzui, A. *Bull. Chem. Soc. Jpn.* **1960**, *33*, 1737.

12 Schreiber, S. L.; Meyers, H. V.; Wiberg, K. B. *J. Am. Chem. Soc.* **1986**, *108*, 8274.

13 For recently references; (a) Mukherjee, S.; Yang, J. W.; Hoffmann, S. *Chem. Rev.* **2007**, *107*, 5471-5569. (b) Radtke L, Willot, M; Sun, H. Y.; *Angew. Chem., Int. Ed.* **2011**, *50*, 3998-4002. (c) Beckett, J. S.; Beckett J. D.; Hofferberth, J. E.; *Org. Lett.* **2010**, *12*, 1408-1411.

14 Carroll, G. L.; Allan, A. K.; Schwaebe, M. K.; Little, R. D. *Org. Lett*. **2000**, *2*, 2531-2534.

15 Candish, L.; Lupton, D. W. *Org. Lett.*, **2010**, *12*, 4836-4839.

16 Herrmann, W. A. *Angew. Chem. Int. Ed.* **2002**, *41*, 1290-1309.

17 Jones, R. A.; Krische, M. *Org. Lett*. **2009**, *11*, 1849-1851.

18 Achmatowicz, O.; Szechner, B.; Maurin, J. K.; *Tetrahedron* **1997**, *53*, 6035-6044.

19 Trost, B. M.; Van Vranken, D. L. *Chem. Rev.* **1996**, *96*, 395-422.

20 Tsuji, J. (2004) *Palladium reagents and catalysts: new perspectives for the 21st century.* England: John Wiley & Sons Ltd. 1-18.

21 Suh, Y.-G.; Lee, Y.-C.; Ko, J.-A.; Kim, S.-A.; Kim, J.-Y.; Jung, J.-K.; Seo, S.-Y.; Lee, K.-H. *Synthetic Commun.* **2000**, *30*, 3053-3060.

22 Suh, Y.-G.; Jung, J.-K.; Kim, S.-A.; Shin, D.-Y.; Min, K.-H. *Tetrahedron Lett.* **1997**, *38*, 3911-3914.

23 (a) Suh, Y.-G.; Jung, J.-K.; Suh, B.-C.; Lee, Y.-C.; Kim, S.-A. *Tetrahedron Lett*. **1998**, *39*, 5377-5380. (b) Suh, Y.-G.; *Tetrahedron Lett.* **2001**, *42*, 1691-1694. (c) Suh, Y.-G.; Jung, J.-K.; Seo, S.-Y.; Min, K.-H.; Shin, D.-Y.; Lee, Y.-S.; Kim, S.-H.; Park, H.-J. *J. Org. Chem*. **2002**, *67*, 4127-4137.

24 Seo, S.-Y.; Jung, J.-K.; Paek, S.-M.; Lee, Y.-S.; Kim, S.-H.; Suh, Y.-G. *Tetrahedron Lett*. **2006**, *47*, 6527-6530.

25 Paek, S.-M.; Seo, S.-Y.; Min, K.-H.; Shin, D.-M.; Chung, Y.-K.; Suh, Y.-G. *Heterocycles*, **2007**, *71*, 1059-1066.

26 Seo, S.-Y.; Jung, J.-K.; Paek, S.-M.; Lee, Y.-S.; Kim, S.-H.; Lee, K.-O.; Suh, Y.-G. *Org. Lett*. **2004**, *6*, 429-432.

27 Lee, S. (2006) *Studies on the iridoid syntheses employing stereoselective construction of the tri-substituted cyclopentanes*, Unpublished thesis (M.A.), Seoul National University.

28 Han, Y.-T.; Paek, S.-M.; Lee, S.; Jung, J.-W.; Jung, J.-K.; Seo, S.-Y.; Lee, J.; Suh, Y.-G. *Tetrahedron Lett*. **2010**, *51*, 2697-2699.

29 Zhang, Y.; Lu, Y.; Zhang, L.; Zheng,Q.-T.; Xu,L-Z.; Yang, S.-L. *J. Nat. Prod*. **2005**, *68*, 1131-1134.

30 Tanitsu, M.; Takaya, Y.; Akasaka, M.; Niwa, M.; Oshima, Y. *Phytochemistry* **2002**, *59*, 845-849, and references cited therein.

31 Kokubo, M.; Ogawa, C.; Kobayashi, S. *Angew. Chem. Int. Ed.* **2008**, *47*, 6909-6911.

32 Schreiber, J.; Maag, H.; Hashimoto, N.; Eschenmoser, A. *Angew. Chem., Int. Ed*. **1971**, *10*, 330-331.

33 Zayia, G. H. *Org. Lett.* **1999**, *1*, 989-992.

34 (a) Nishiyama, H.; Kitajima, T.; Matsumoto, M.; Itoh, K. *J. Org. Chem.* **1984**, *49*, 2298-2300. (b) Stork, G.; Kahn, M. *J. Am. Chem. Soc*. **1985**, *107*, 500-501. (c) Jenkins, P. R.; Wood, A. J.

Tetrahedron Lett. **1997**, *38*, 1853-1856. (d) Kessabi, F. M.; Winkler, T.; Luft, J. A. R.; Houk, K. N. *Org. Lett*. **2008**, *10*, 2255-2258.

35 Tamao, K.; Ishida, N.; Kumada, M. *J. Org. Chem*. **1983**, *48*, 2120-2122.

36 Evans, P. A.; Cui, J.; Gharpure, S. J.; Polosukhin, A.; Zhang, H.-R. *J. Am. Chem. Soc*. **2003**, *125*, 14702-14703.

37 J. Strating (1961) *Organic Sulfur Compounds.* Pergamon Press, New York: N. Y.: Chapter 15.

38 (a) Conrad, P. C.; Fuchs, P. L. *J. Am. Chem. Soc*. **1978**, *100*, 346-348. (b) Cheng, W.-C.; Kurth, M. K. *J. Org. Chem.* **2002**, *67*, 4387-4391. (b) Nantz, M. H.; Radisson, X.; Fuchs, P. L. *Synthetic Commun.* **1987**, *17*, 55-69.

39 Yoshida, T.; Saito, S. *Chem. Lett*, **1982**, 165-168.

40 (a) Lanver, A.; Schmalz, H.-G. *Eur. J. Org. Chem.* **2005**, 1444-1458. (b) Schemalz, H.-G.; Jope, H. *Tetrahedron* **1998**, *54*, 3457-3464.

41 (a) Lipshutz, B. H.; Miller, T. A. *Tetrahedron Lett.* **1990**, *37*, 5253-5256. (b) Hughes, D. L.; Reamer, R. A. *J. Org. Chem.* **1996**, *61*, 2967-2971. (c) Saiah, M.; Bessodes, M.; Antonakis, K. *Tetrahedron Lett.* **1992**, *30*, 4317-4320. (d) Cook, A. F.; Maichuk, D. T. *J. Org. Chem.* **1970**, *35*, 1940-1943. (e) Wagner, J.; Anders, H.; Rohrbach, S.; Wagner, D.; Oberer, L.; France, J. *J. Org. Che.* **2005**, *70*, 9588-9590.

42 Matsumoto, K.; Miura, K.;Oshima, K.; Utimoto, K. *Tetrahedron Lett*. **1992**, *33*, 7031-7034, and references therein.

43 (a) Kabalka, G. W.; Brown, H. C.; Suzuki, A.; Honma, S.; Arase, A.; Itoh, M. *J. Am. Chem. Soc*. **1970**, *92*, 710-712. (b) Davies, A. G.; Griller, D.; Roberts, B. P.; Tudor, R. *J. Chem. Soc. D* **1970**, 640 (c) Davies, A. G.; Maki, T.; Roberts, B. P. *J. Chem. Soc. Perkin Trans. 2* **1972**, 744-747. (d) Krusic, P. J.; Kochi, J. K. *J. Am. Chem. Soc.* **1969**, *91*, 3942-3944. (e) Ollivier, C.; Renaud, P. *Chem. Rev.* **2001**, *101*, 3415-3434.

44 (a) Nozaki, K.; Oshima, K.; Utimoto, K. *J. Am. Chem. Soc.* **1987**, *109*, 2547-2549. (b) Guindon, Y.; Lavalle´e, J.-F.; Boisvert, L.; Chabot, C.; Delorme, D.; Yoakim, C.; Hall, D.; Lemieux, R.; Simoneau, B. *Tetrahedron Lett.* **1991**, *32*, 27-30. (c) Satoh, S.; Sodeoka, M.; Sasai, H.; Shibasaki, M. *J. Org. Chem.* **1991**, *56*, 2278-2280.

45 Carra, C.; Scaiano, J. C. *Eur. J. Org. Chem.* **2008**, *26*, 4454-4459.

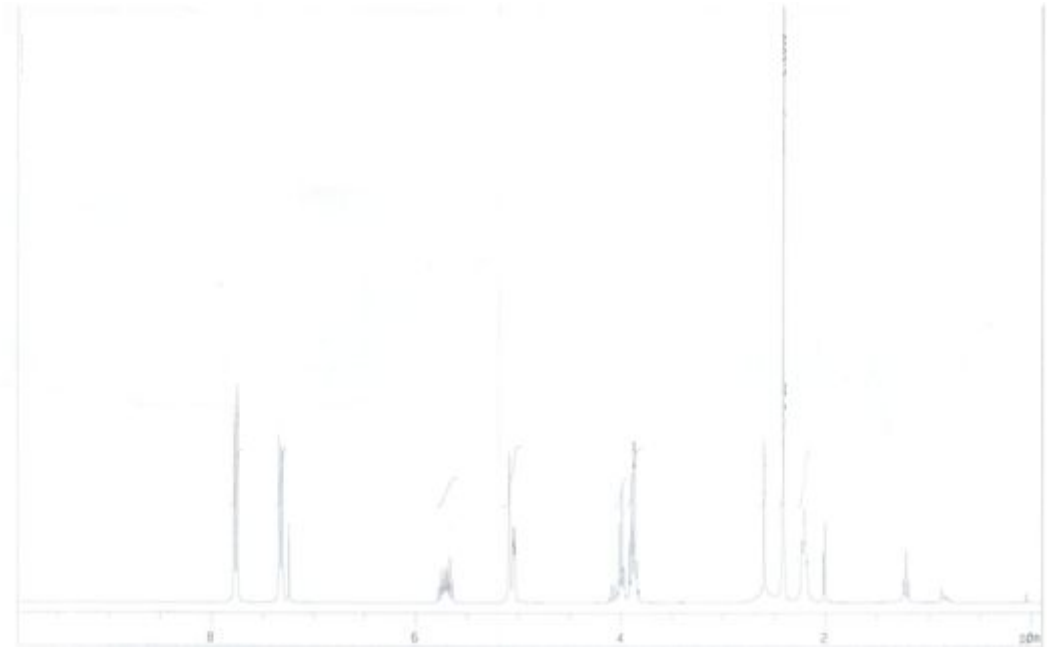
46 (a) Carroll, G. L.; Allan, A. K.; Schwaebe, M. K.; Little, R. D. *Org. Lett*. **2000**, *2*, 2531-2534. (b) Devin, P.; Fensterbank, L.; Malacria, M. *J. Org. Chem*. **1998**, *63*, 6764-6765.

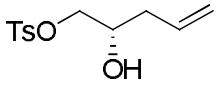
47 (a) Thomas, E. J.; Williams, A. C. *J. Chem. Soc., Chem. Commun*. **1987**, *13*, 992-994. (b) Jahn, U.; Hartmann, P.; Kaasalainen, E. *Org. Lett*. **2004**, *6*, 257-260.

48 We have reported the successful result previously: Lee, S.; Paek, S.-M.; Yun, H.; Kim, N.-J.; Suh, Y.-G. *Org. Lett.* **2011**, *13*, 3344-3347.

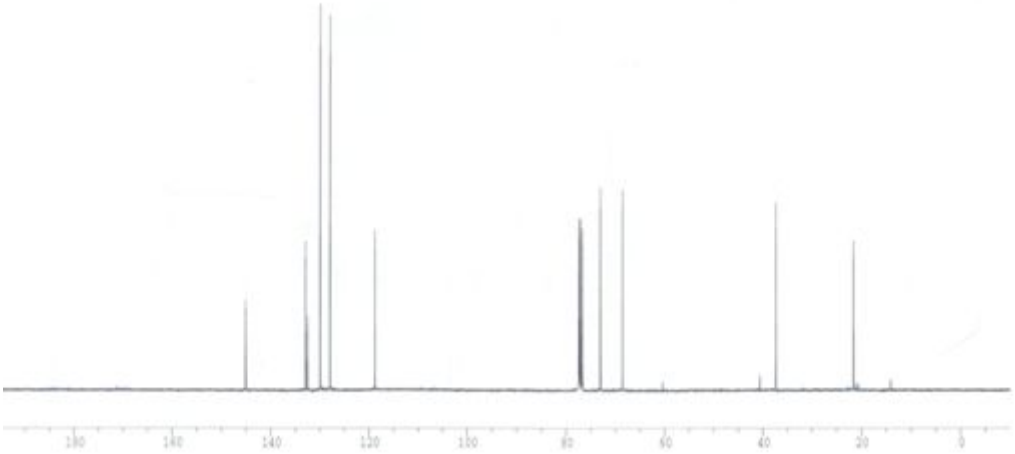
VI. Appendix

▼ **59, ^{1}H-NMR, 300 MHz**

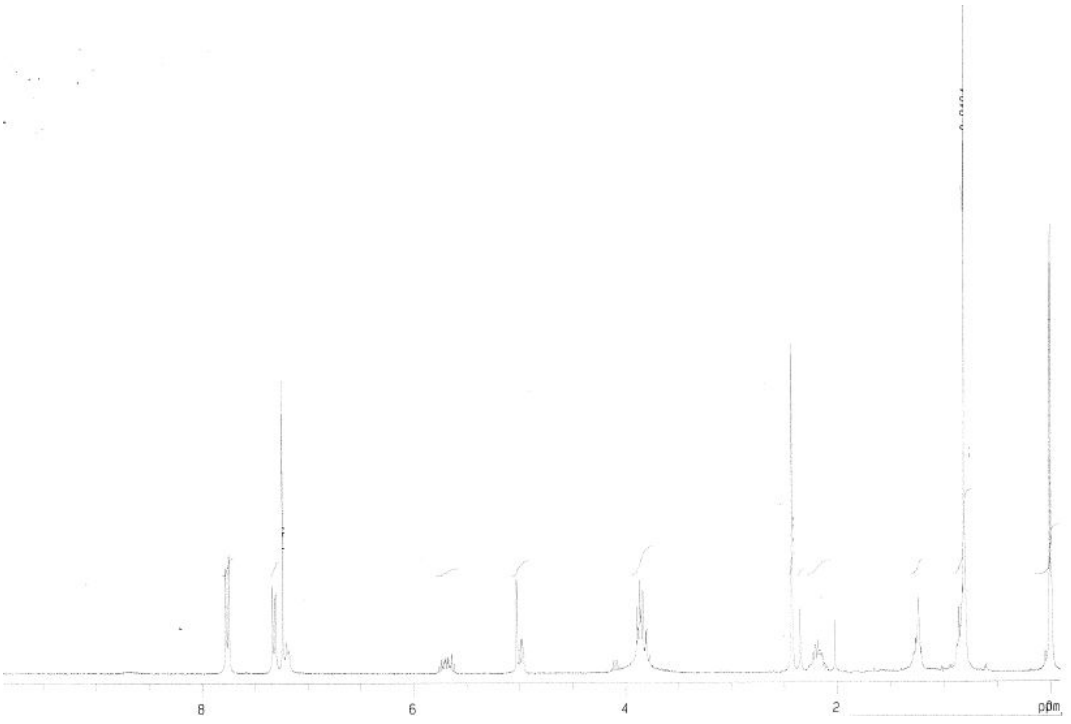




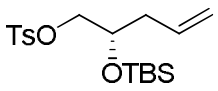
▼ **59, ^{13}C-NMR, 75 MHz**

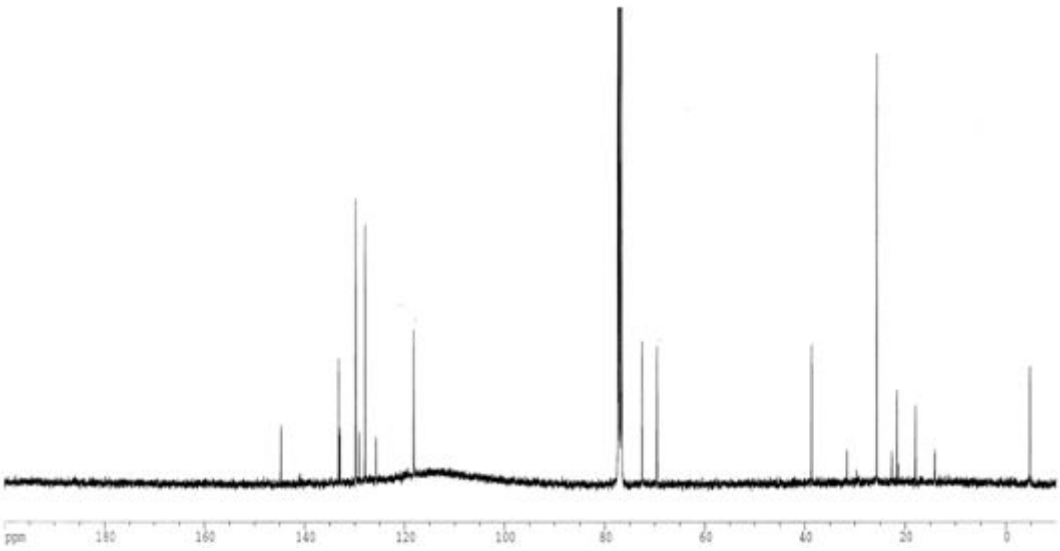


▼ **60, ^{1}H-NMR, 300 MHz**

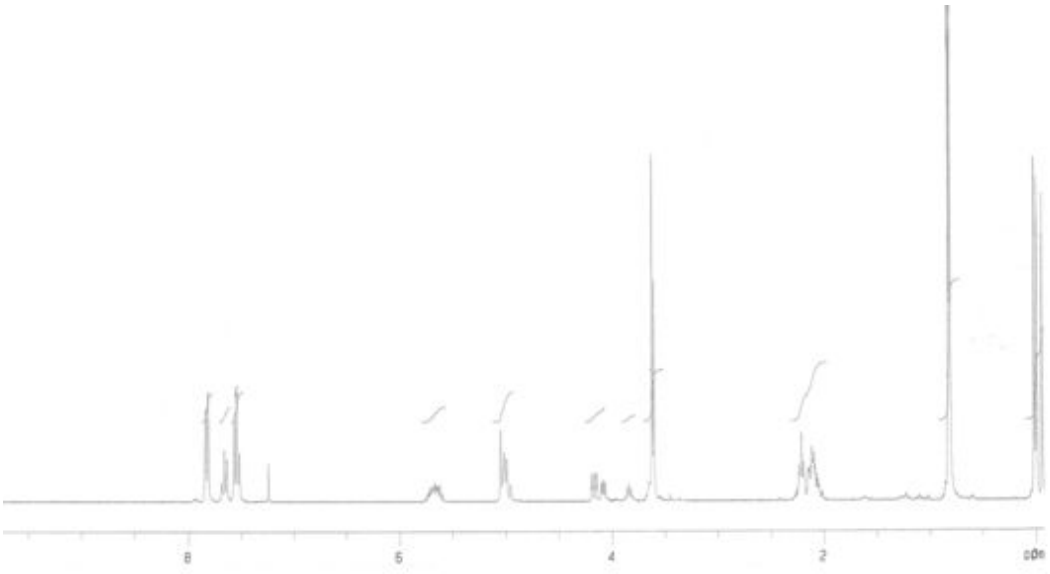


▼ **60, ^{13}C-NMR, 75 MHz**

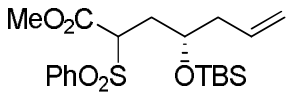


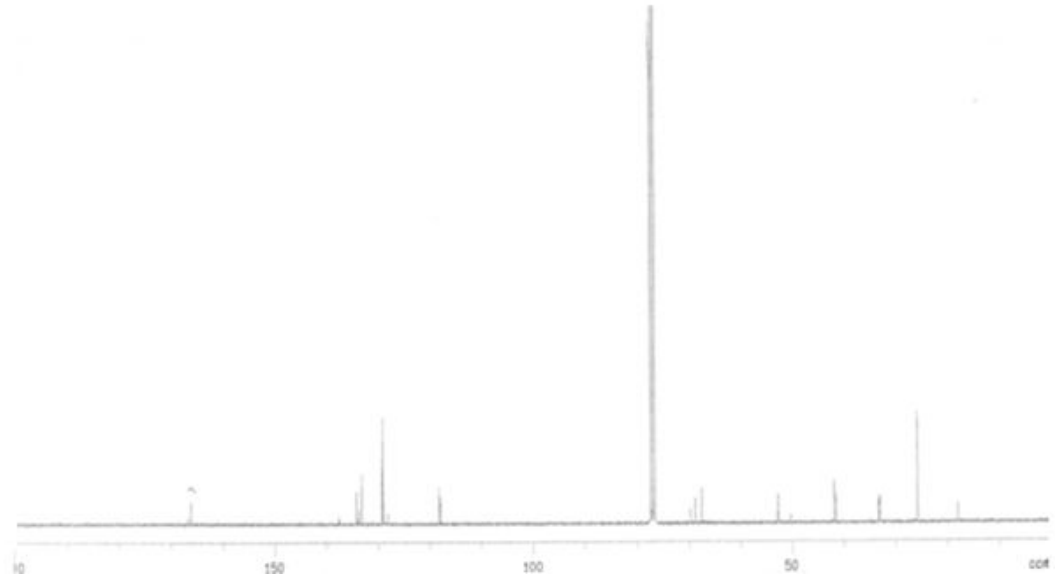


▼ **61, ^{1}H-NMR, 300 MHz**

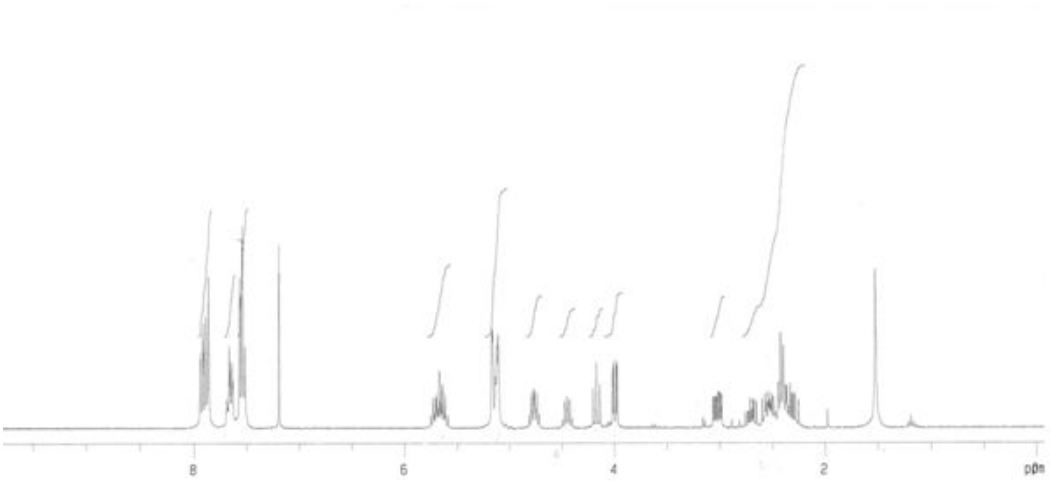


▼ **61, ^{13}C-NMR, 75 MHz**

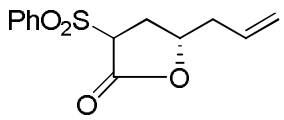


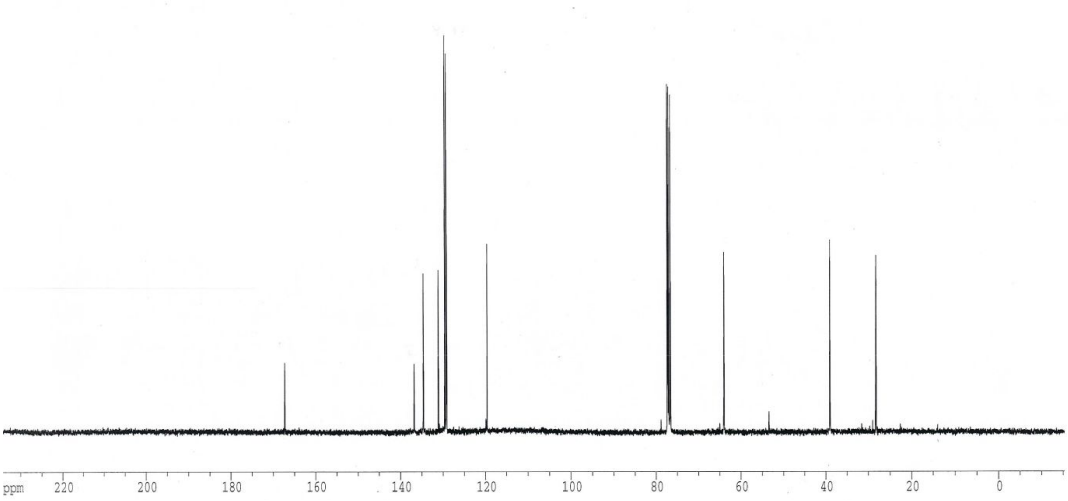


▼ **62, ^{1}H-NMR, 300 MHz**

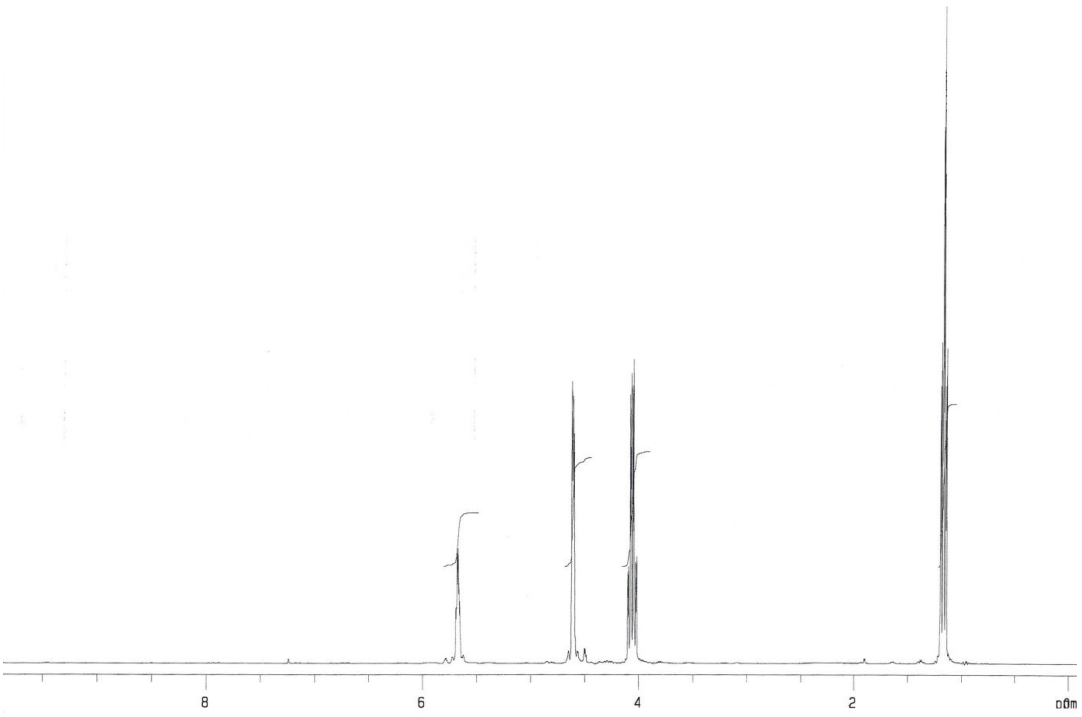


▼ **62, ^{13}C-NMR, 75 MHz**

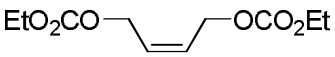


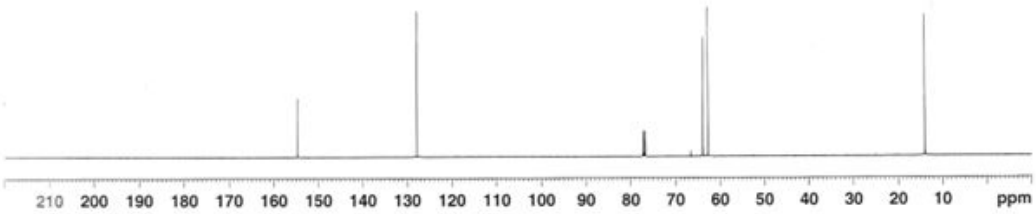


▼ **63a, ^{1}H-NMR, 300 MHz**

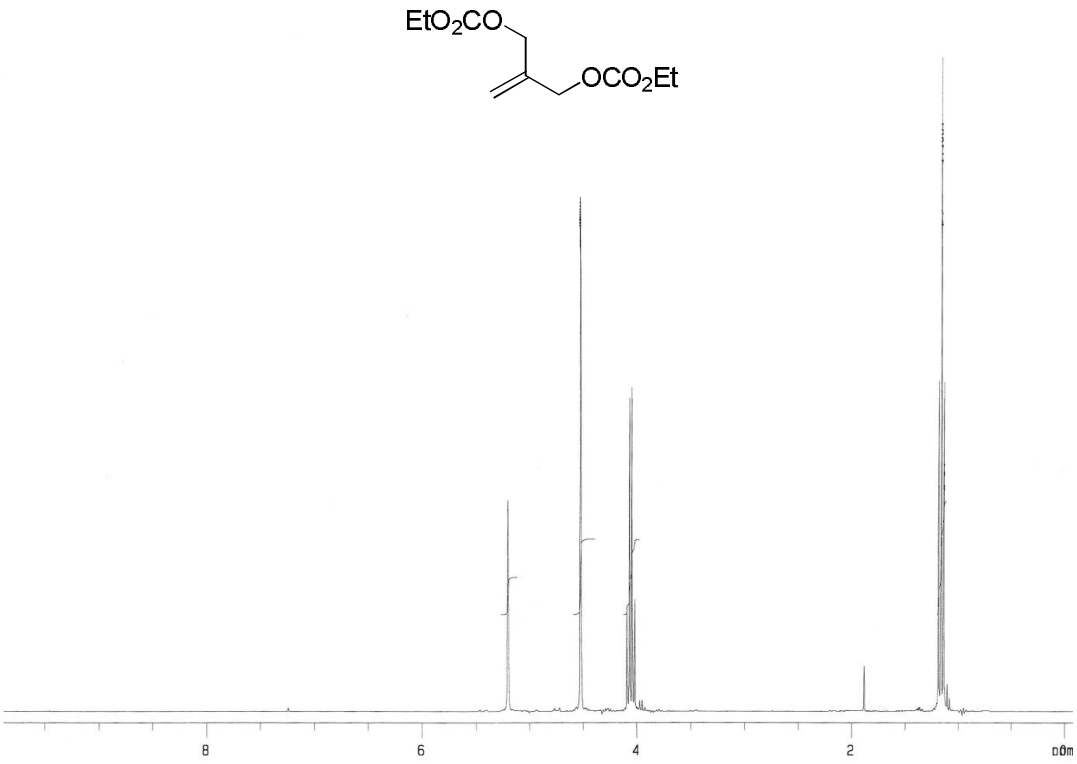


▼ **63a, ^{13}C-NMR, 125 MHz**

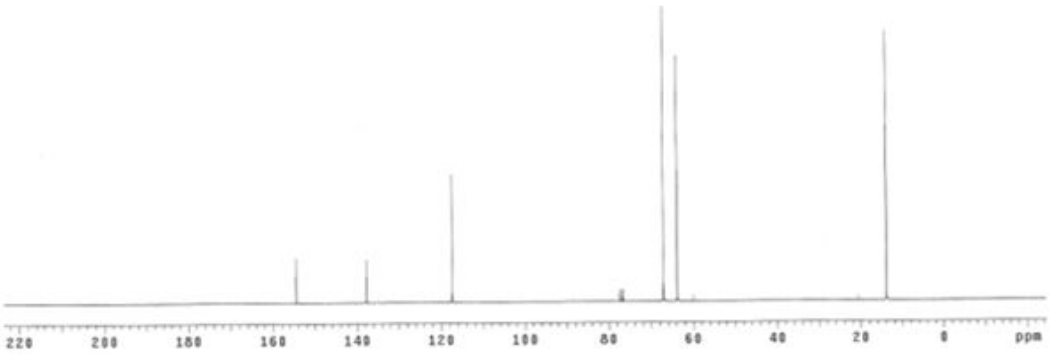




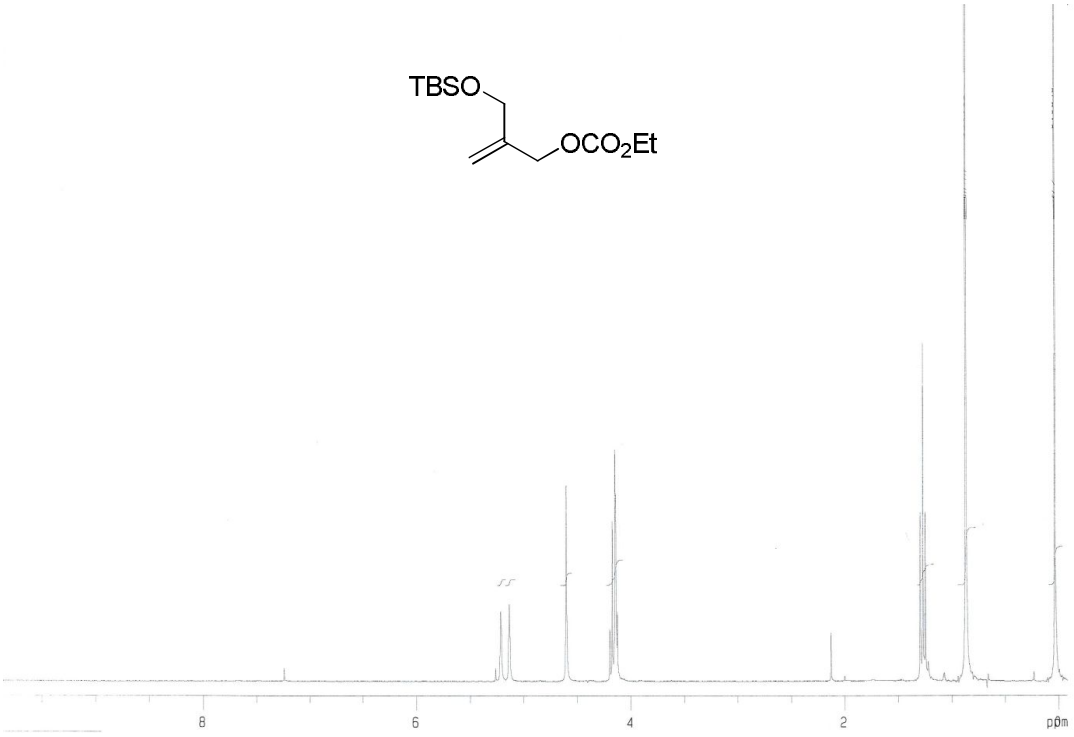
▼ **63b, ^{1}H-NMR, 300 MHz**



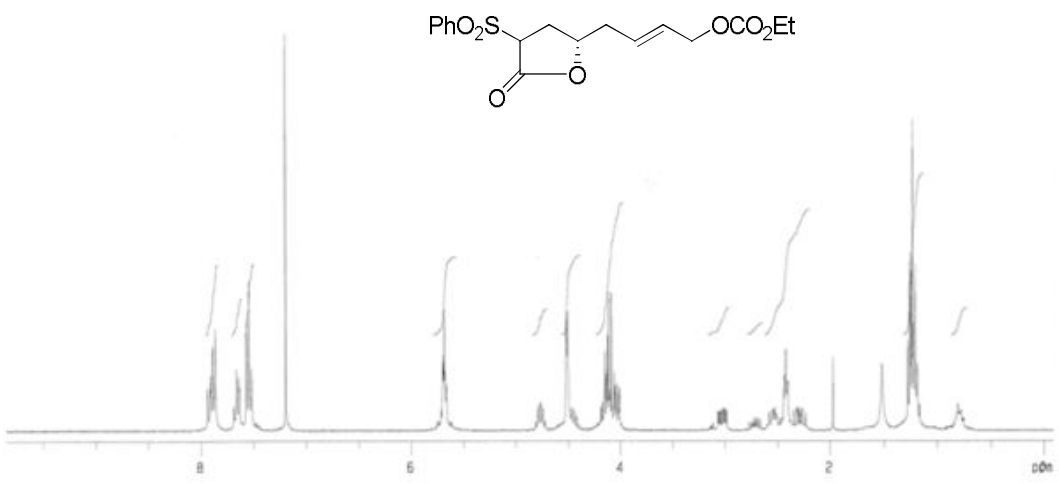
▼ **63b, ^{13}C-NMR, 75 MHz**



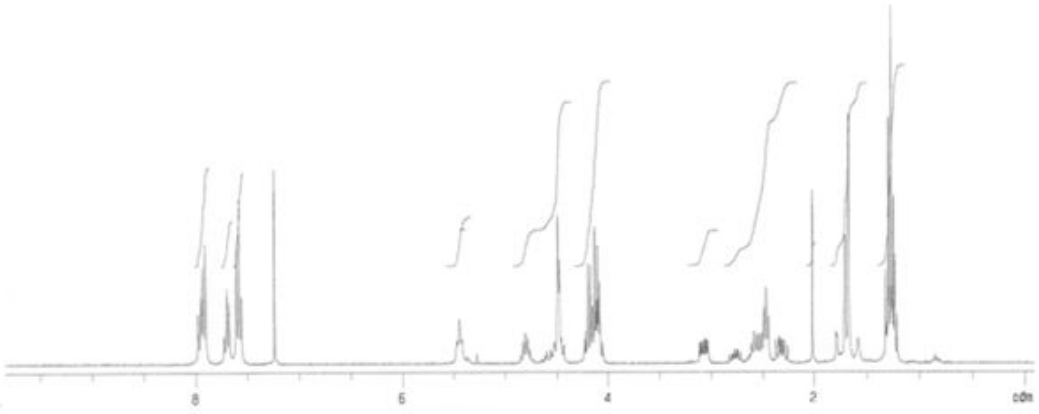
▼ **63d, ^{1}H-NMR, 300 MHz**



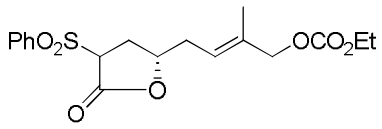
▼ **64a, ^{1}H-NMR, 300 MHz**

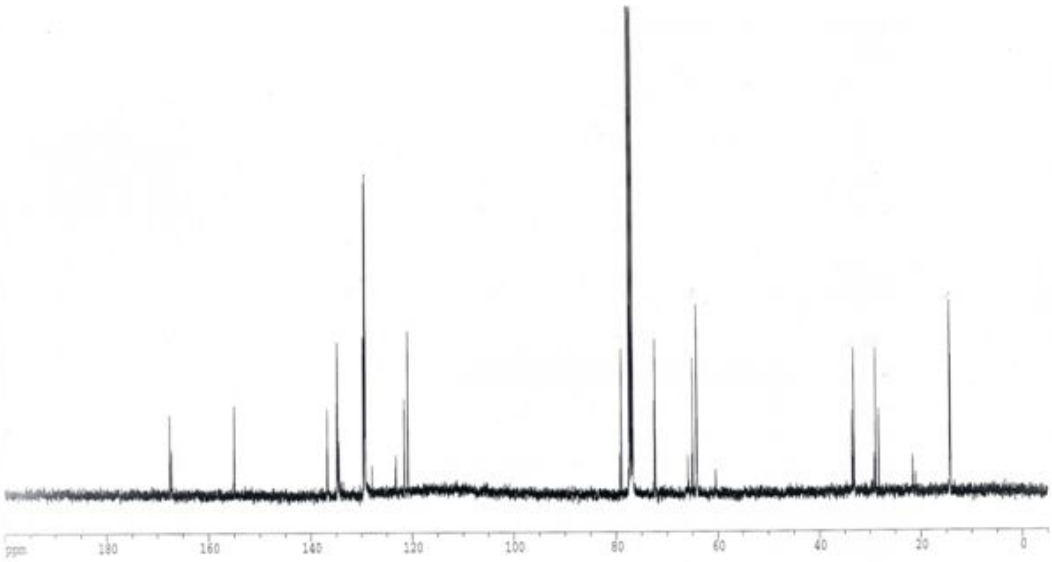


▼ **64c, ^{1}H-NMR, 300 MHz**

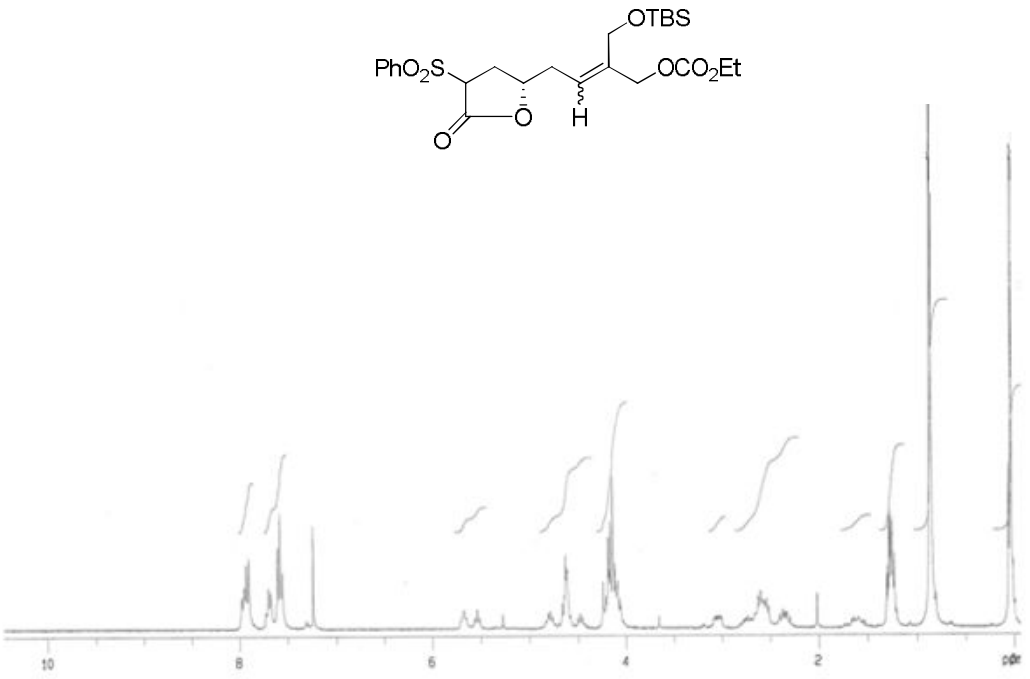


▼ **64c, ^{13}C-NMR, 75 MHz**

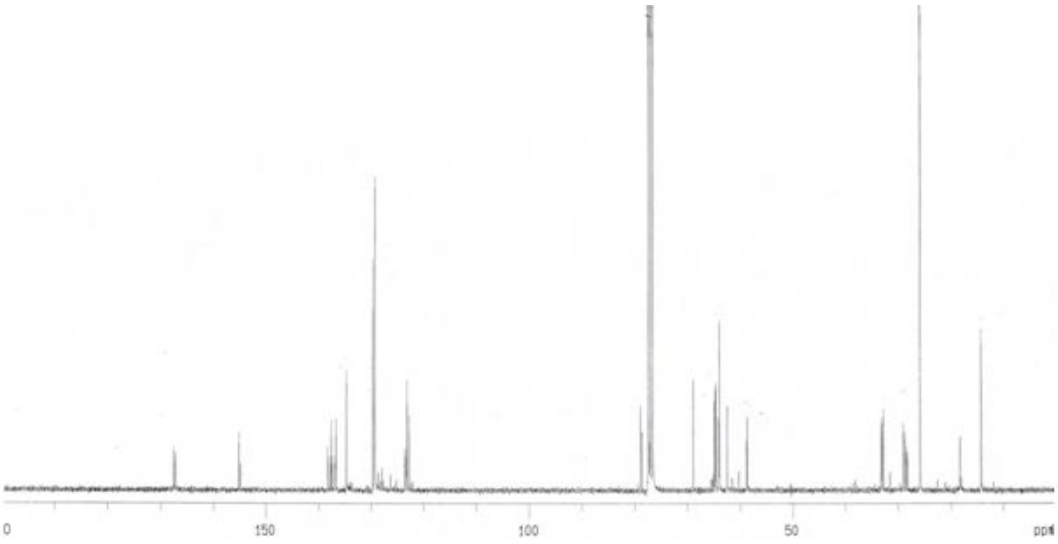




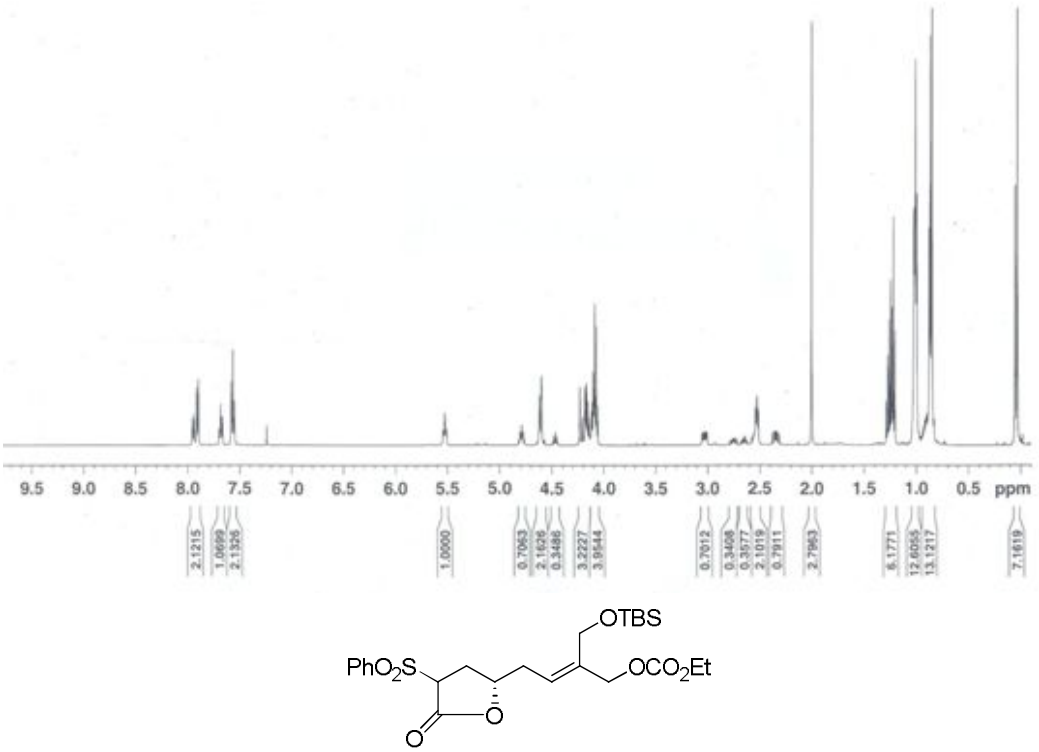
▼ **64d, *E/Z* mix ^{1}H-NMR, 300 MHz**



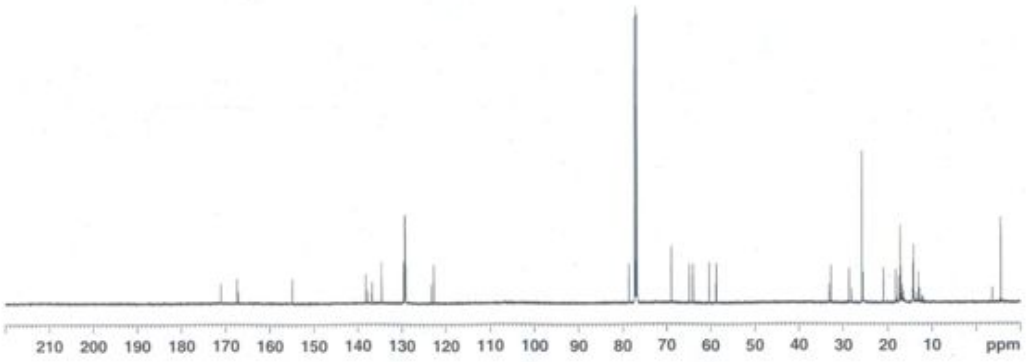
▼ **64d, ^{13}C-NMR, 75 MHz**



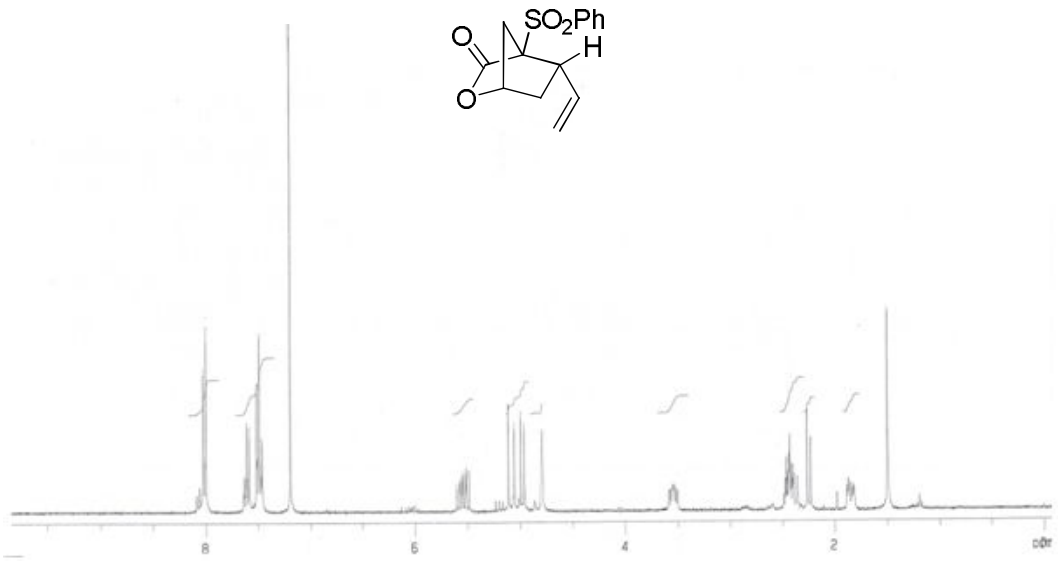
▼ ***Z*-64d, ^{1}H-NMR, 500 MHz**



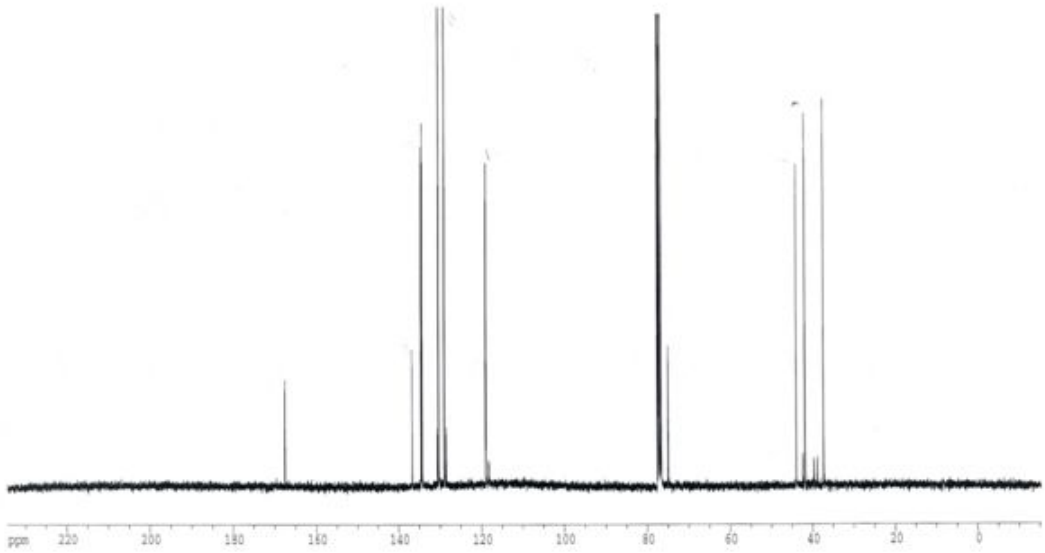
▼ ***Z*-64d, ^{13}C-NMR, 125 MHz**



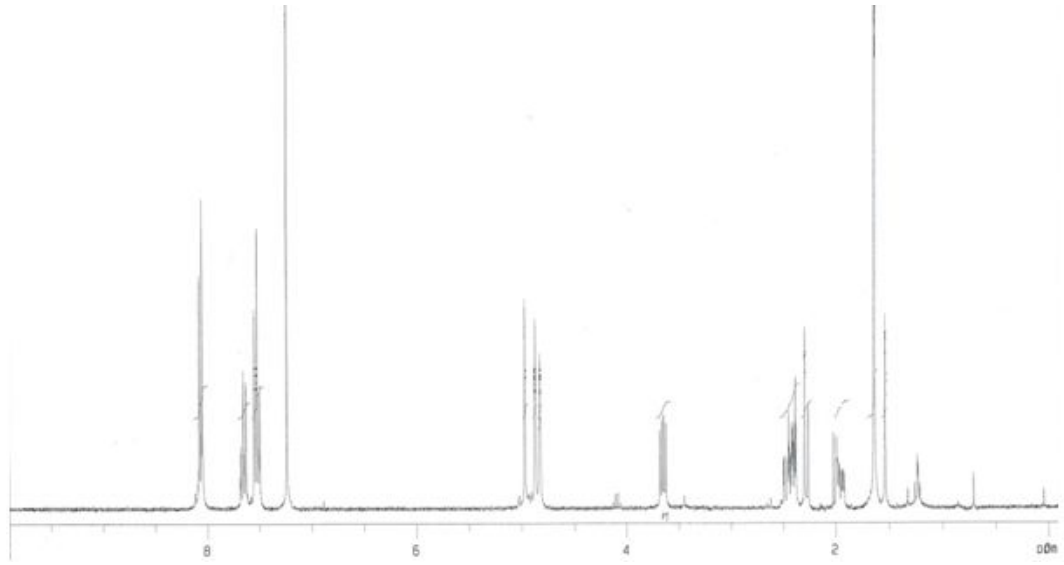
▼ **46, ^{1}H-NMR, 300 MHz**



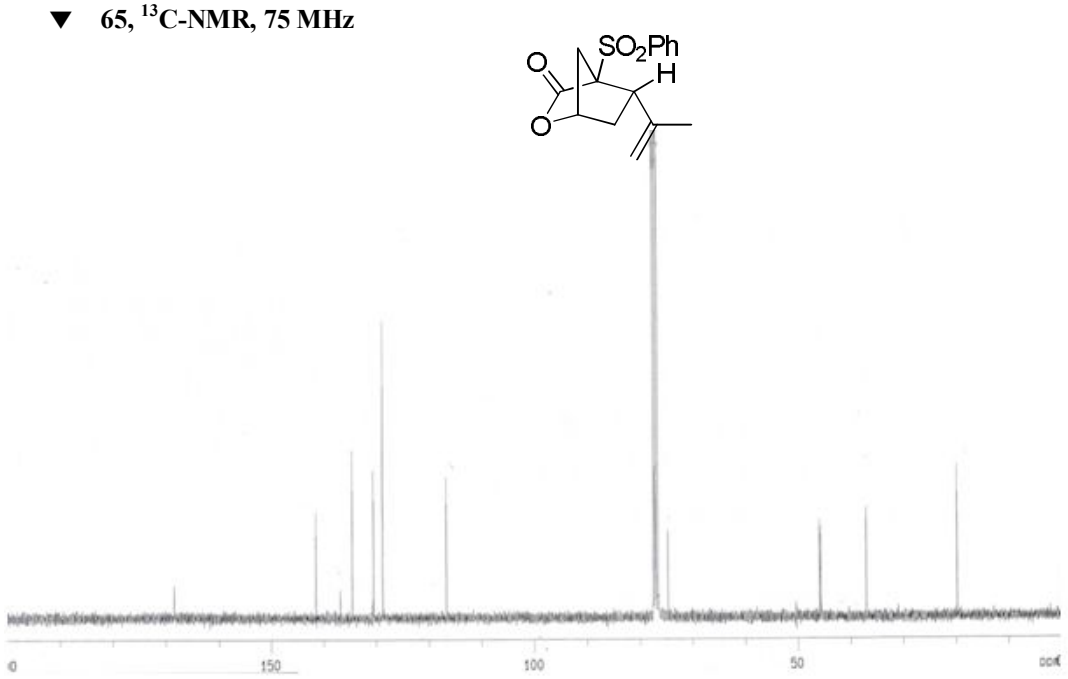
▼ **46 ^{13}C-NMR, 75 MHz**



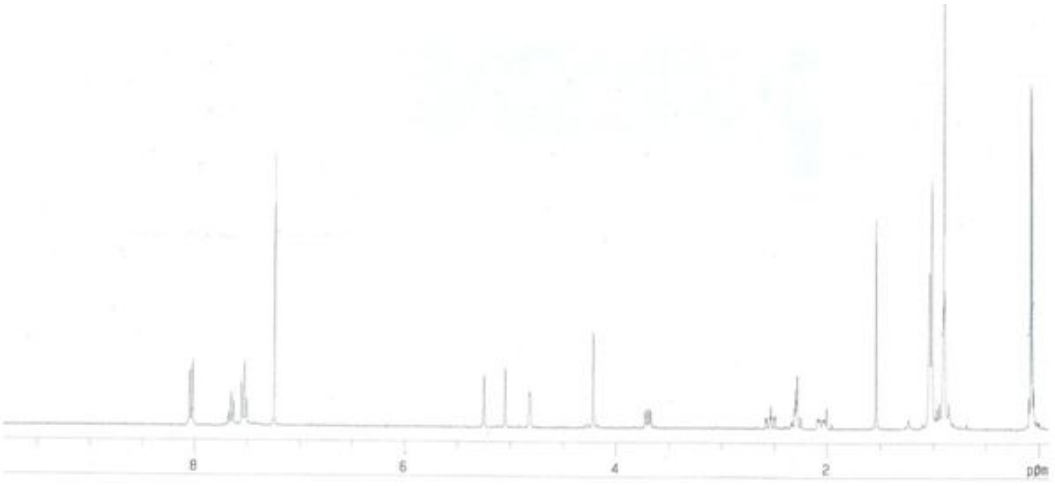
▼ **65, ^{1}H-NMR, 300 MHz**

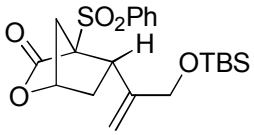


▼ **65, ^{13}C-NMR, 75 MHz**

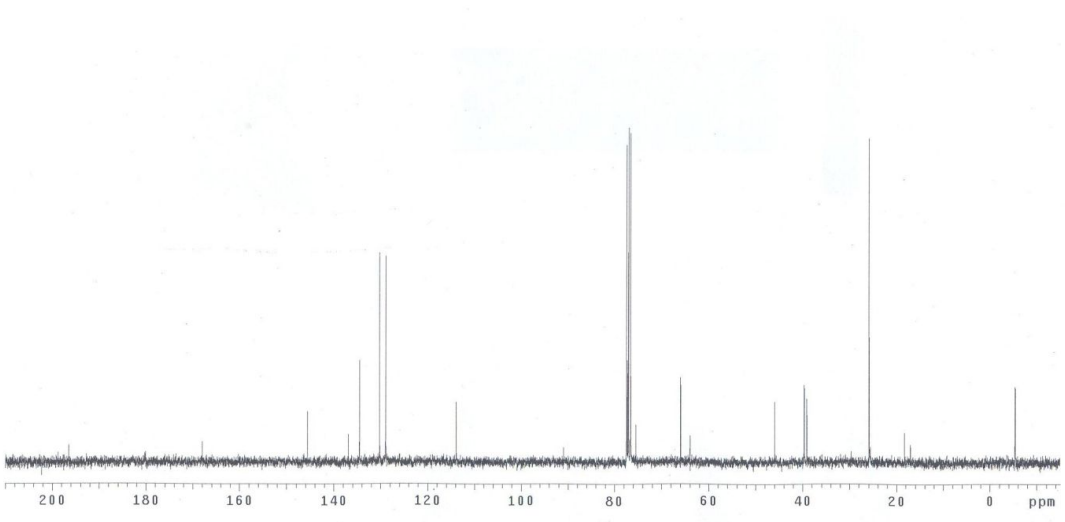


▼ **66, ^{1}H-NMR, 300 MHz**

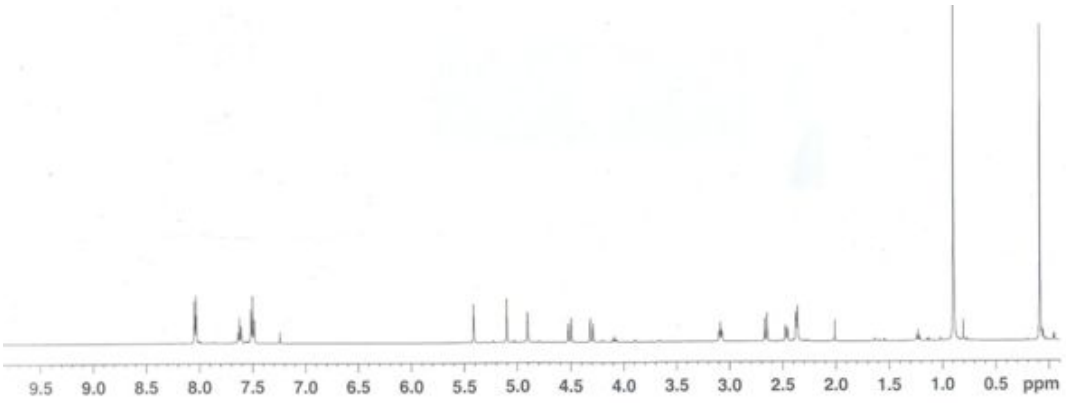




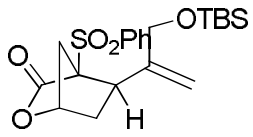
▼ **66 ^{13}C-NMR, 75 MHz**

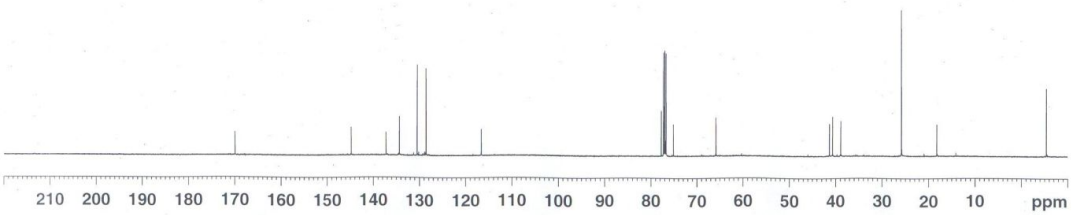


▼ **66', ^{1}H-NMR, 500 MHz**

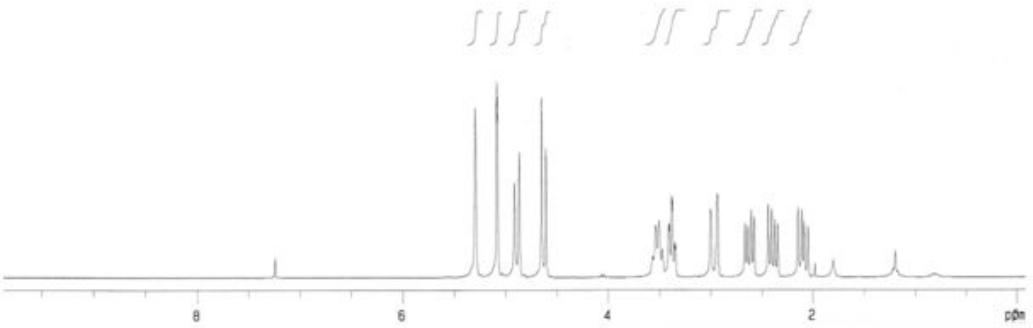


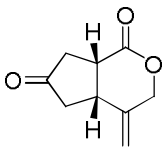
▼ **66', ^{13}C-NMR, 125 MHz**



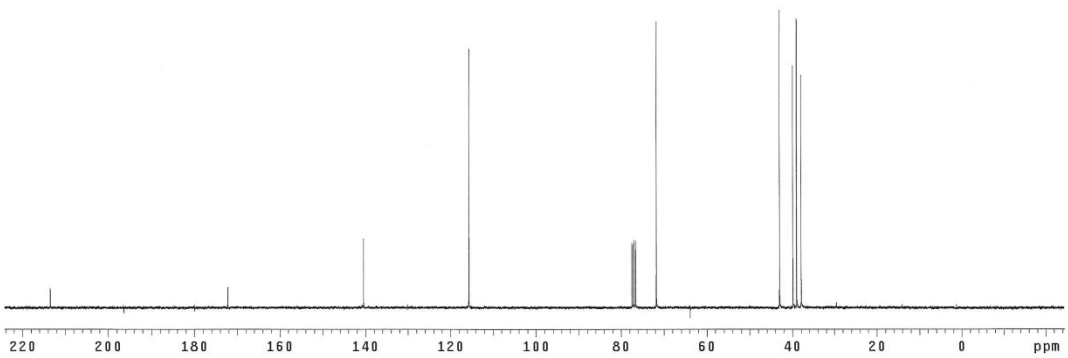


▼ **68, ^{1}H-NMR, 300 MHz**

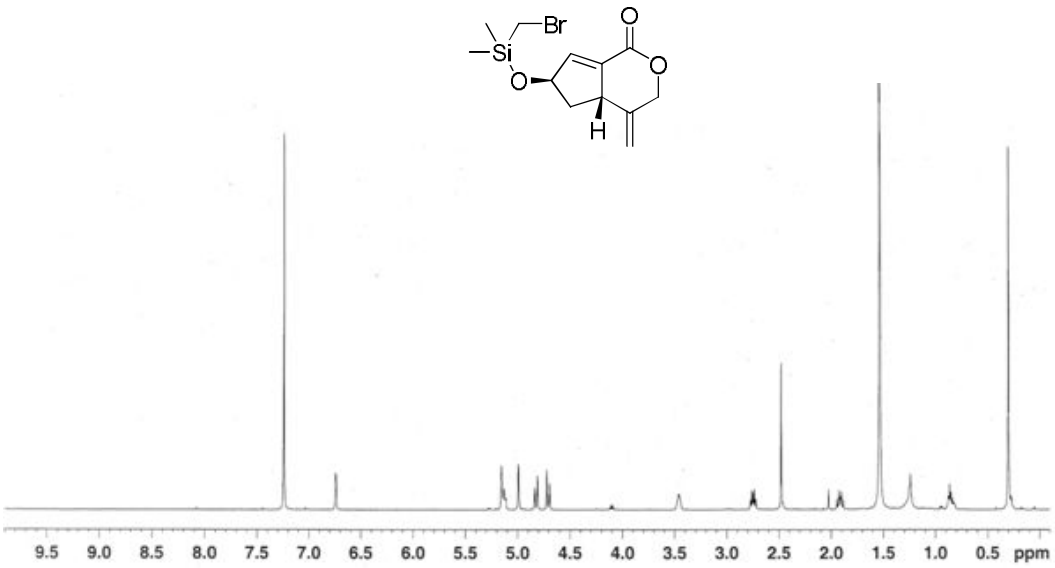




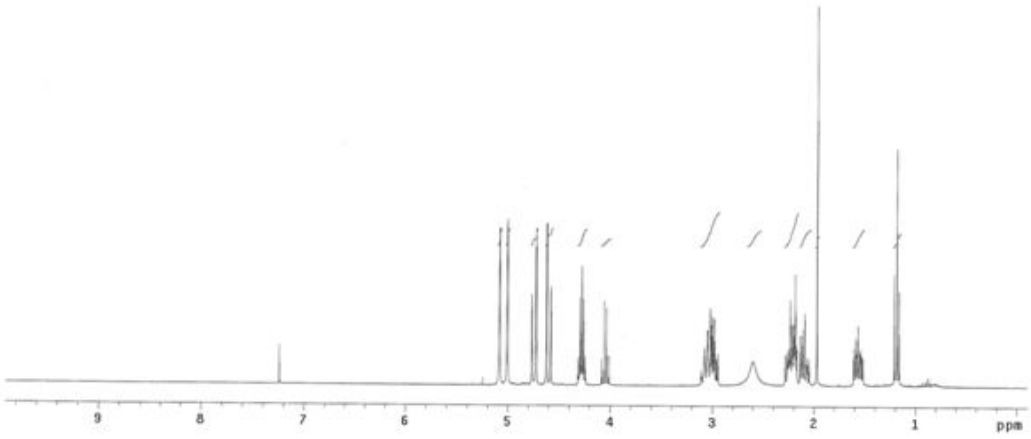
68, ^{13}C-NMR, 75 MHz

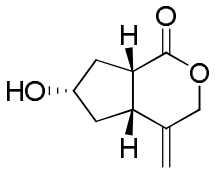


▼ **71, ^{1}H-NMR, 500 MHz**

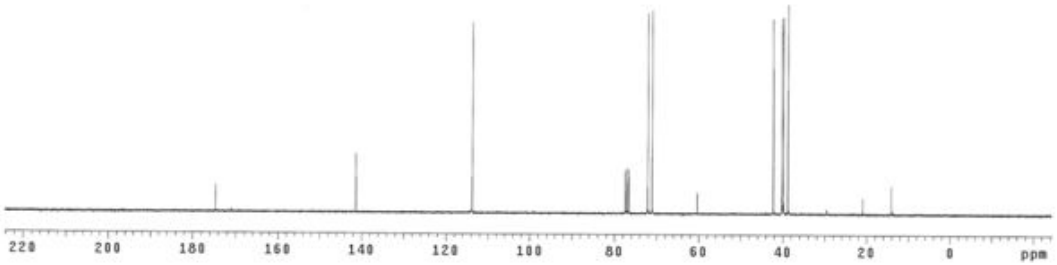


▼ **69, ^{1}H-NMR, 300 MHz**

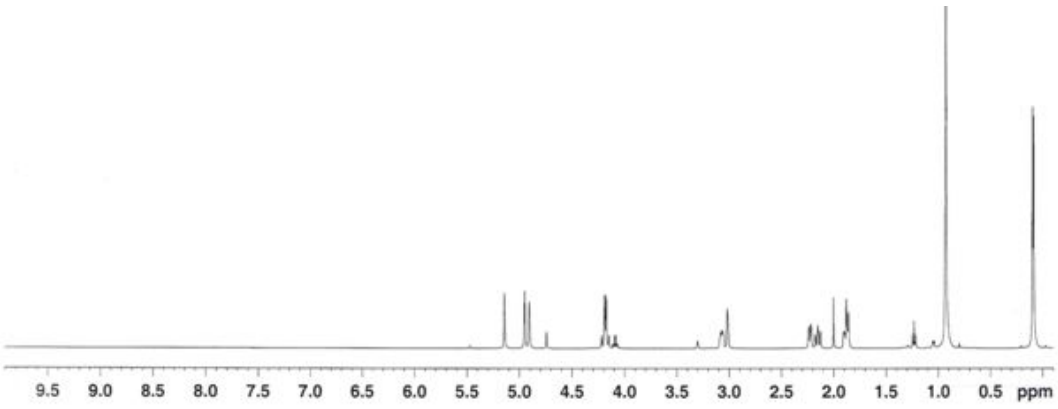


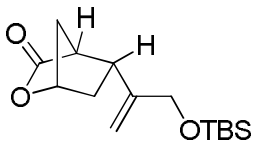


▼ **69, ^{13}C-NMR, 75 MHz**

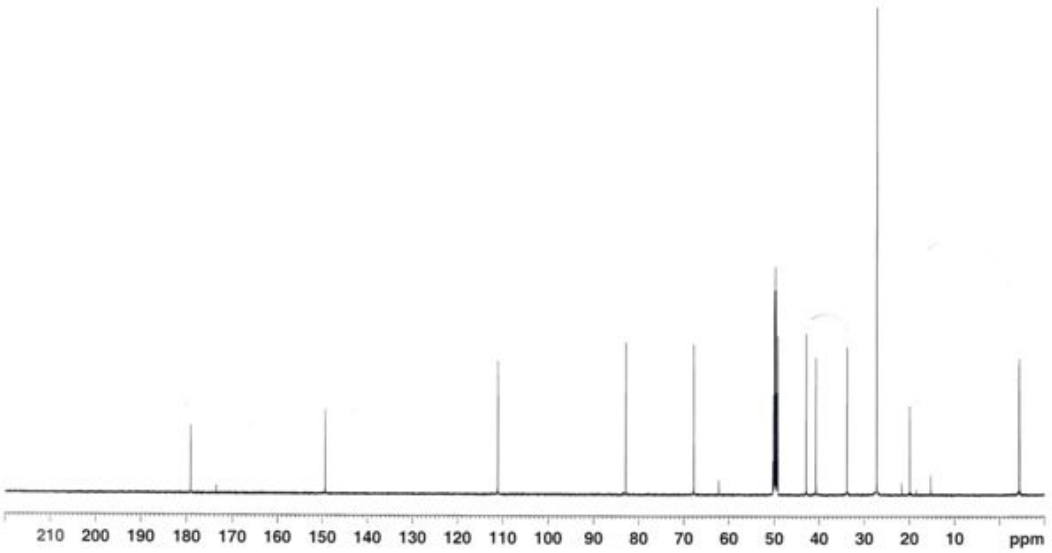


▼ **70, ^{1}H-NMR, 500 MHz**

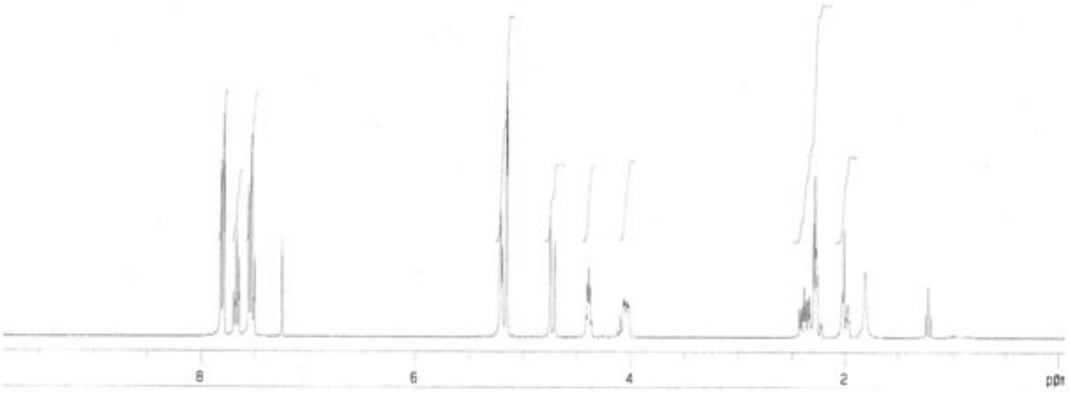


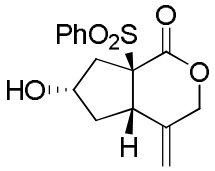


▼ **70, ^{13}C-NMR, 125 MHz**

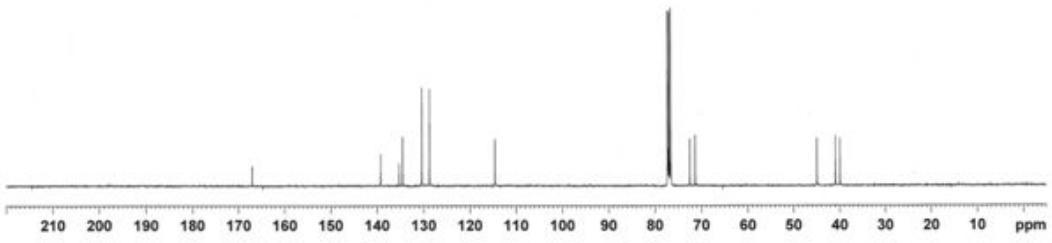


▼ **72, ^{1}H-NMR, 300 MHz**

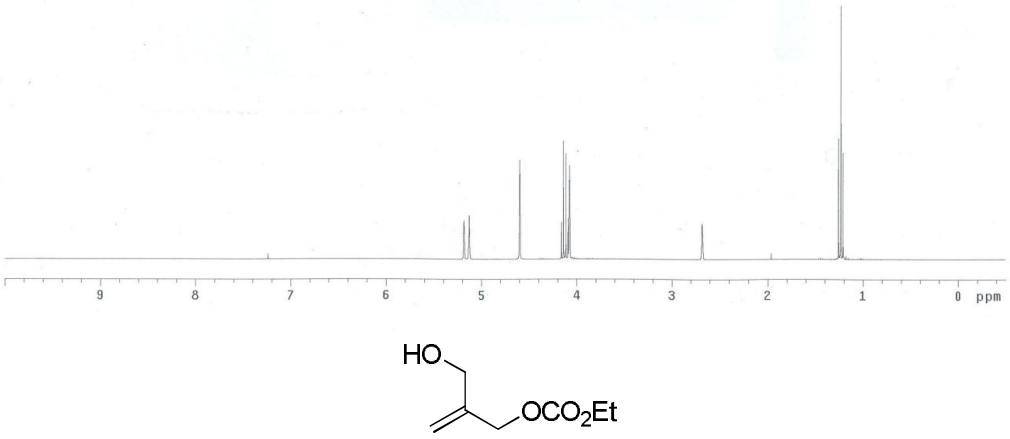




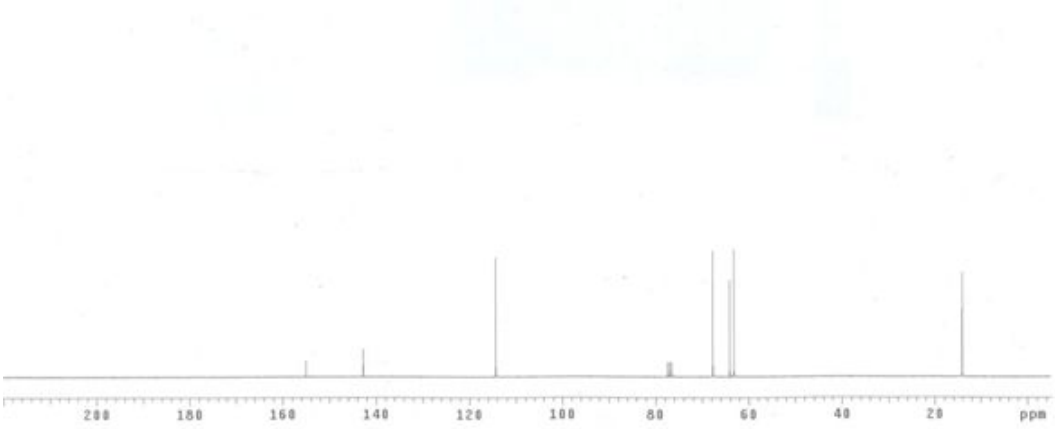
▼ **72, ^{13}C-NMR, 125 MHz**



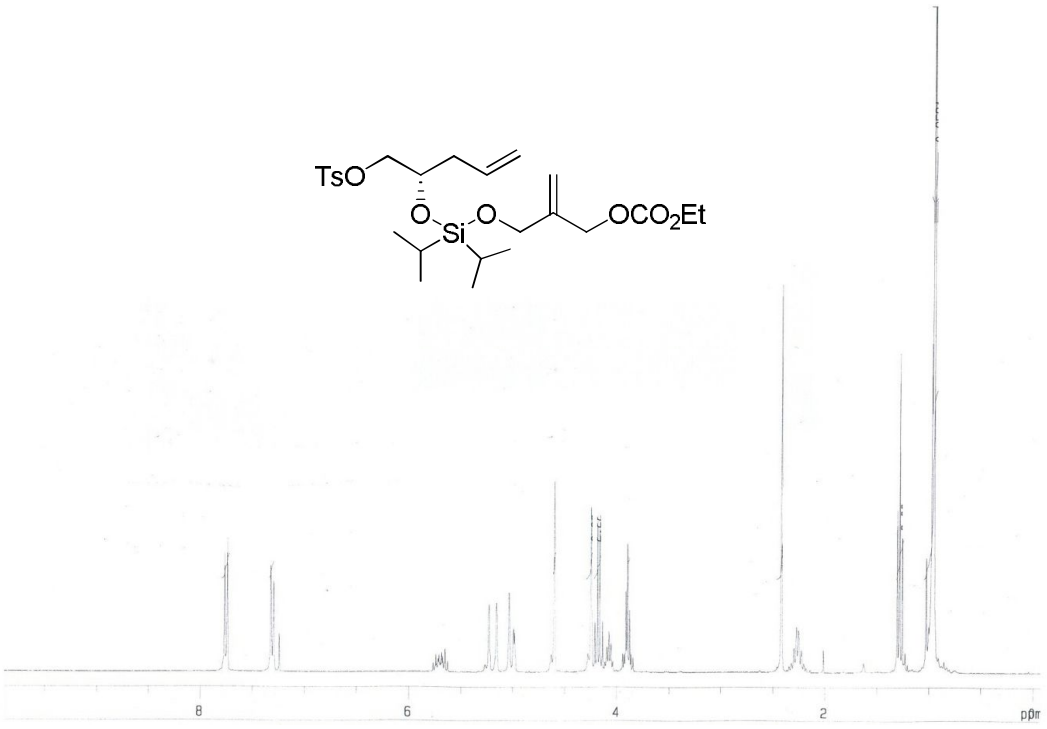
▼ **73, ^{1}H-NMR, 300 MHz**



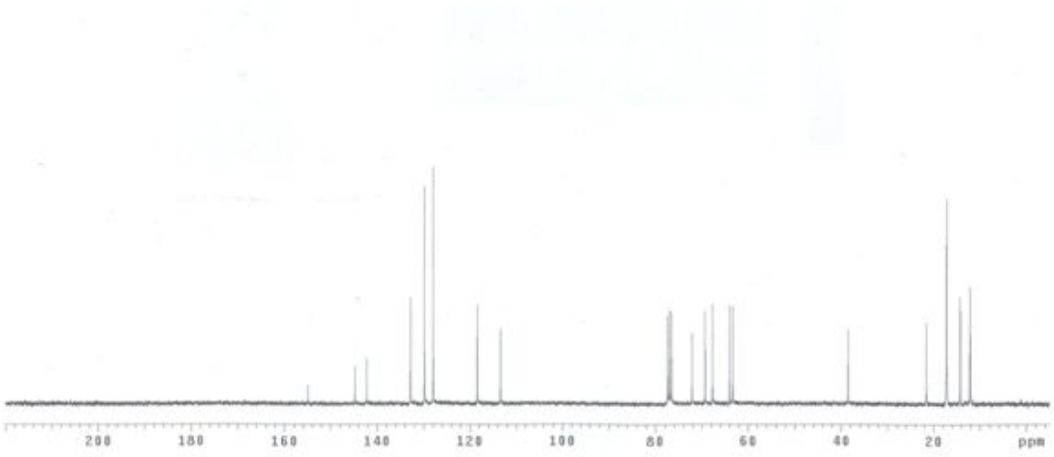
▼ **73, ^{13}C-NMR, 75 MHz**



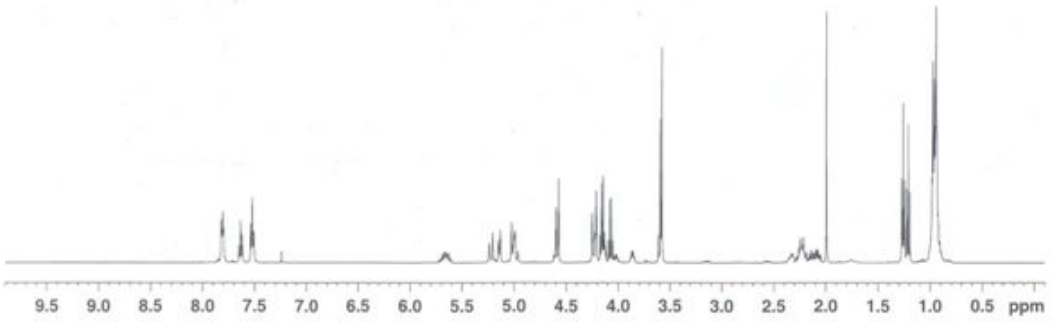
▼ **74, ^{1}H-NMR, 300 MHz**



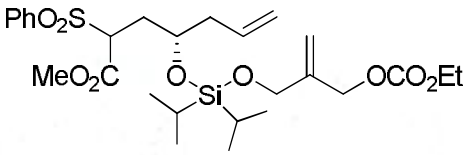
▼ **74, ^{13}C-NMR, 75 MHz**

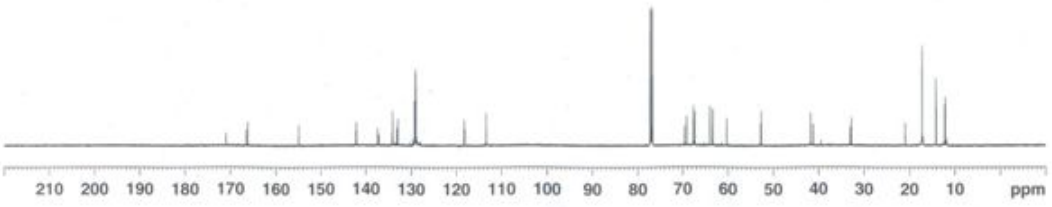


▼ **S74, ^{1}H-NMR, 500 MHz**

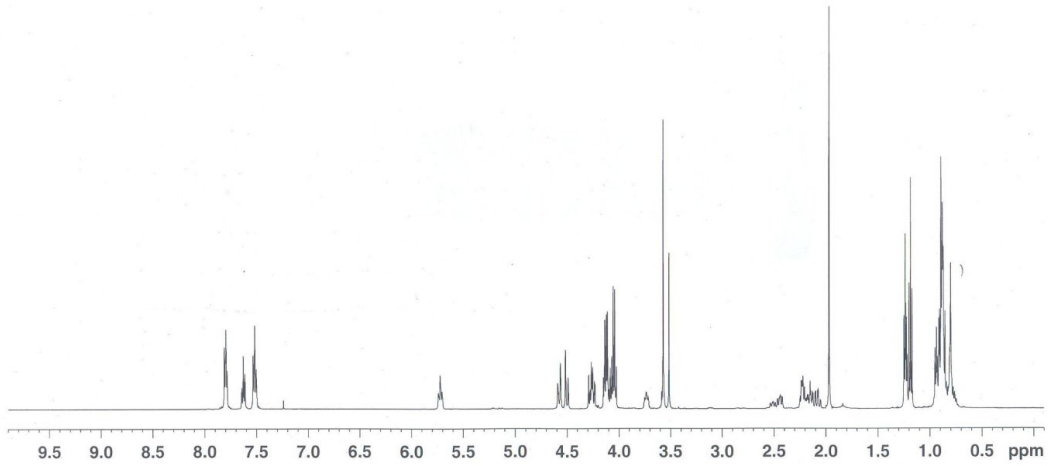


▼ **S74, ^{13}C-NMR, 125 MHz**

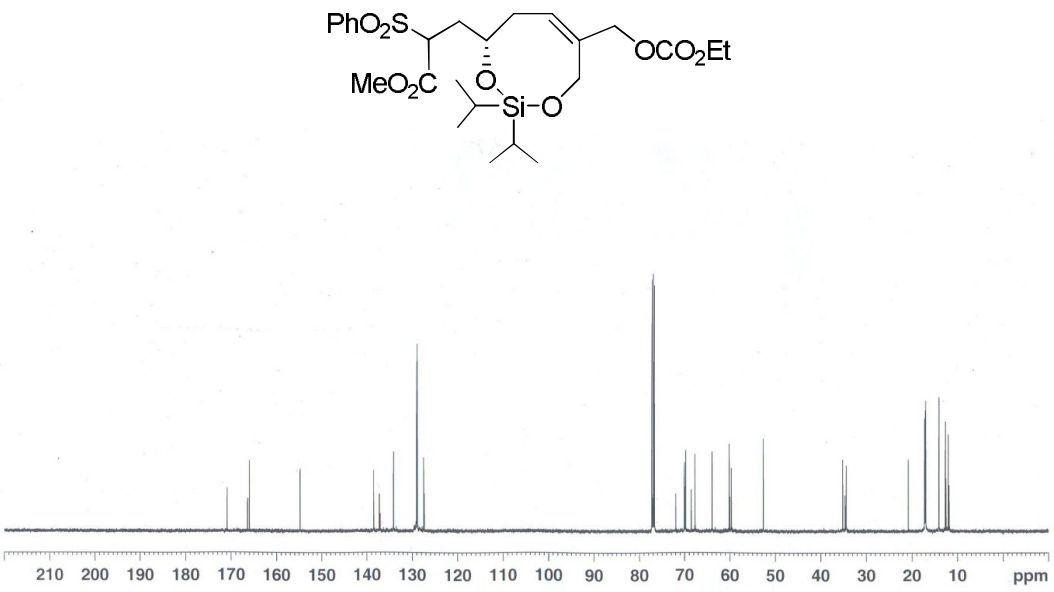




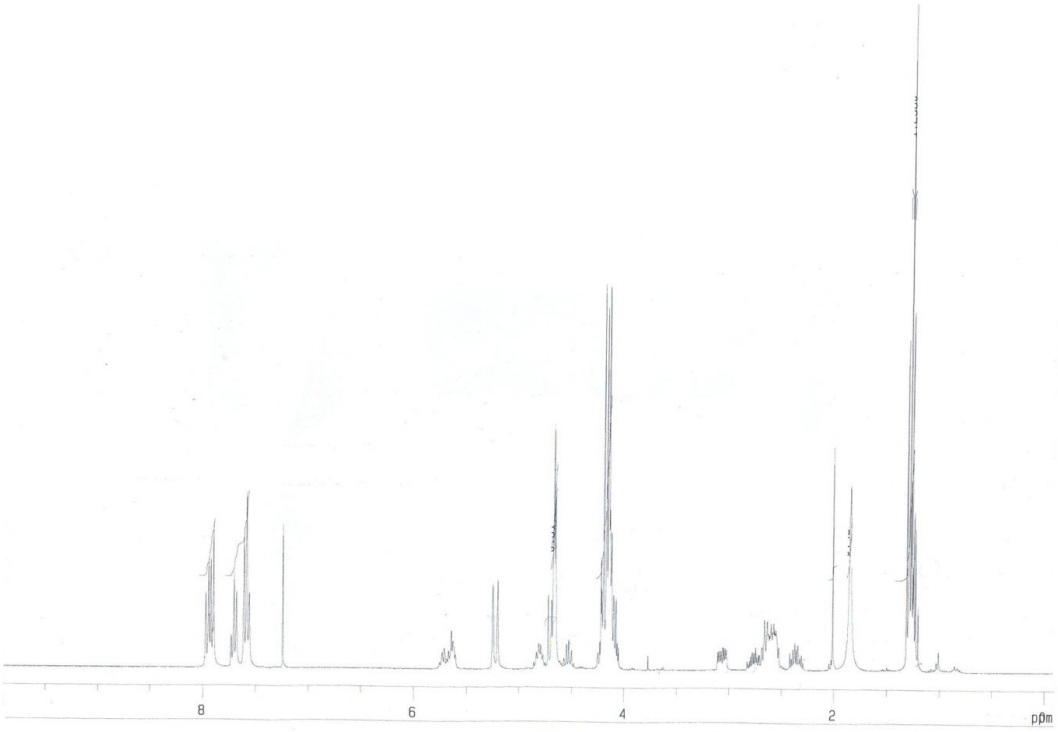
▼ **75, ^{1}H-NMR, 500 MHz**

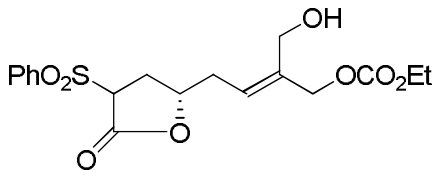


▼ **75, ^{13}C-NMR, 125 MHz**

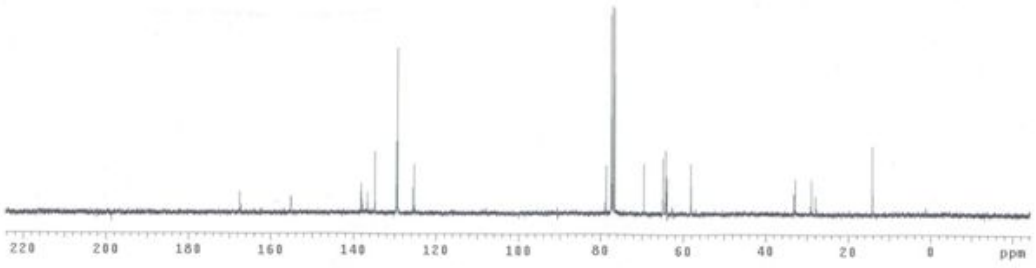


▼ **S75, ^{1}H-NMR, 300 MHz**

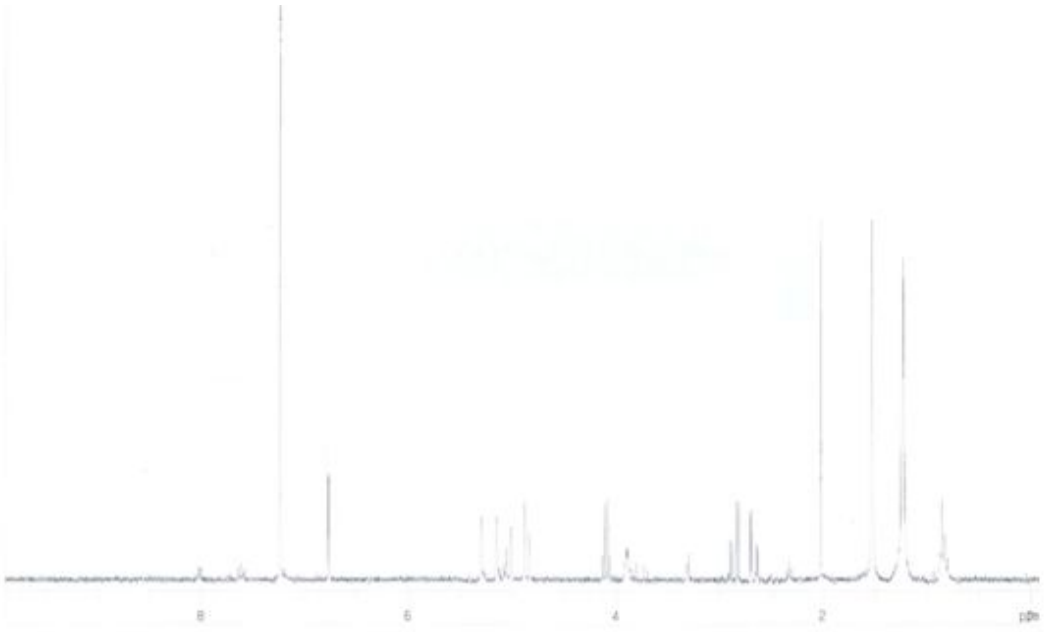




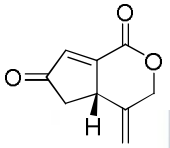
▼ **S75, ^{13}C-NMR, 75 MHz**

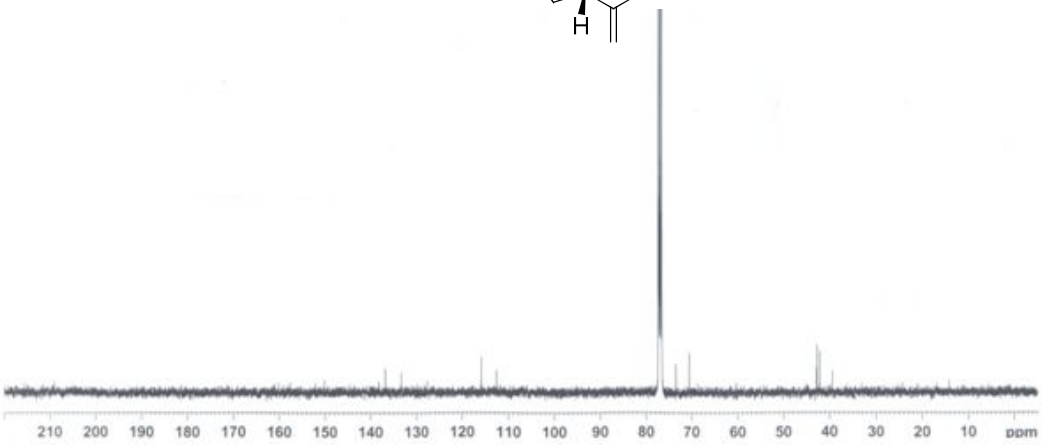


▼ **76, ^{1}H-NMR, 300 MHz**

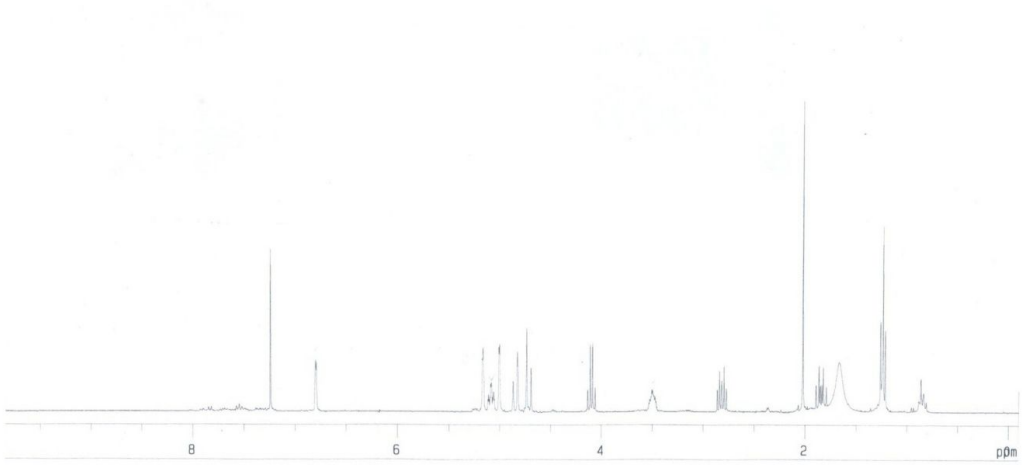


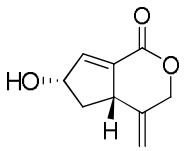
▼ **76, ^{13}C-NMR, 75 MHz**



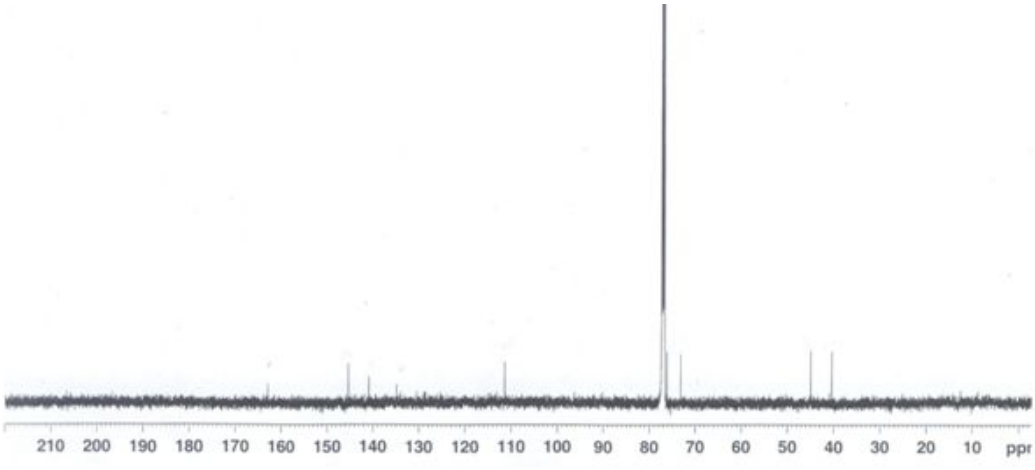


▼ **77, ^{1}H-NMR, 500 MHz**

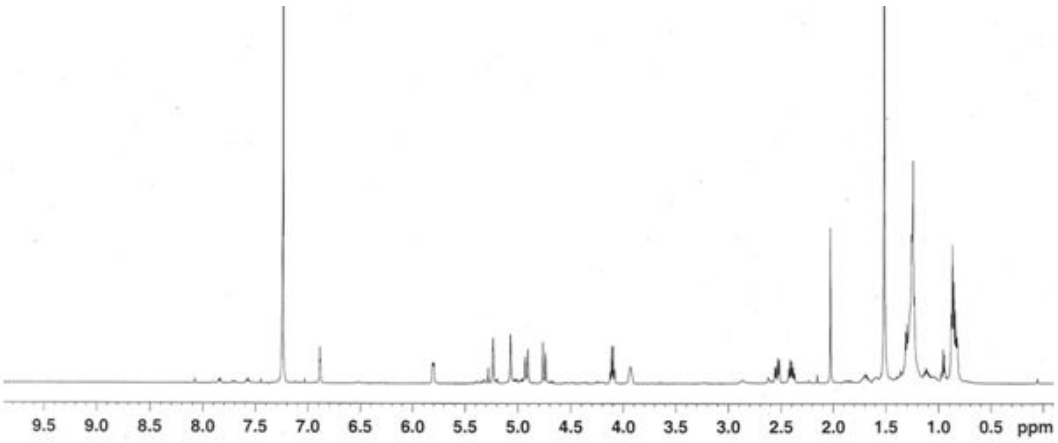


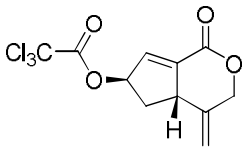


▼ **77, ^{13}C-NMR, 100 MHz**

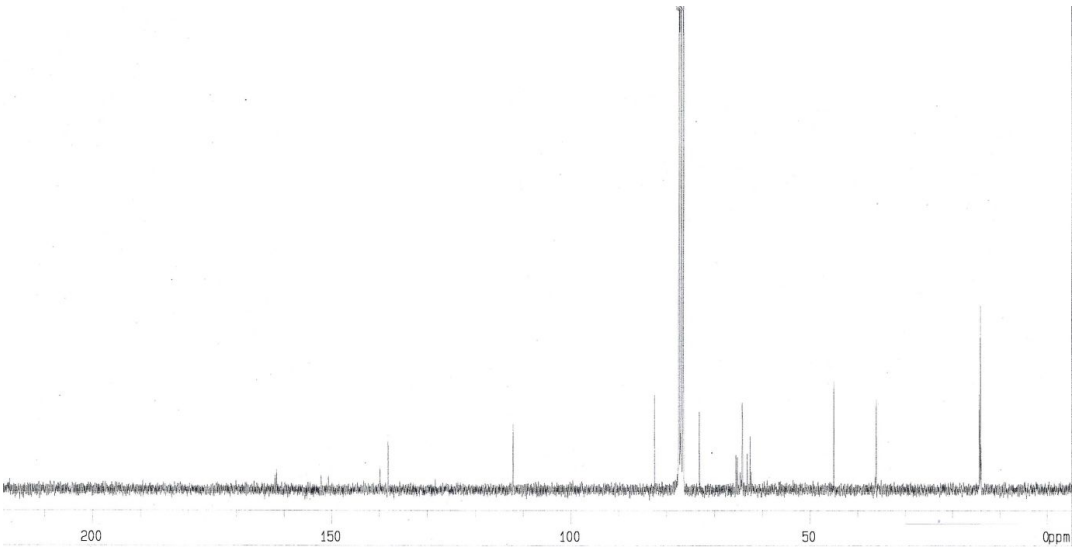


▼ **78, ^{1}H-NMR, 500 MHz**

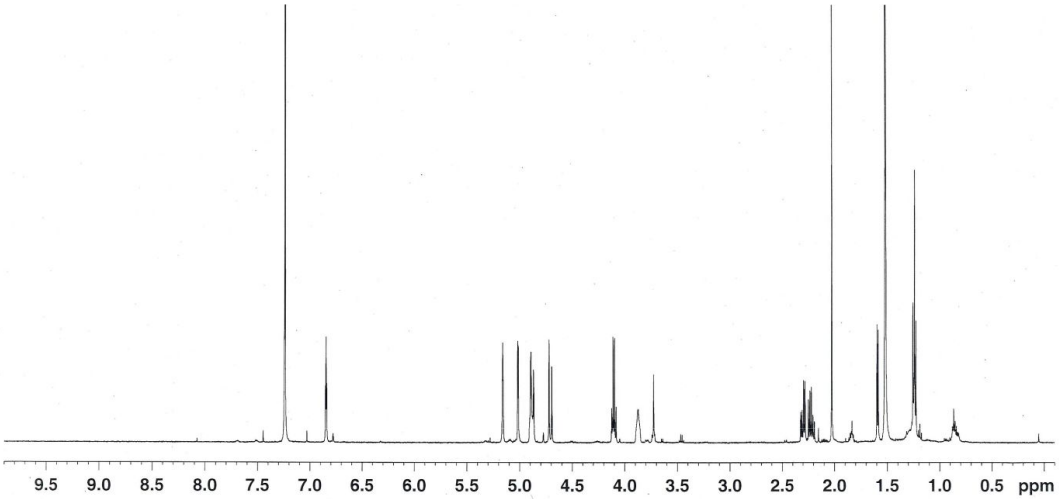




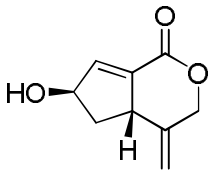
▼ **78, ^{13}C-NMR, 100 MHz**

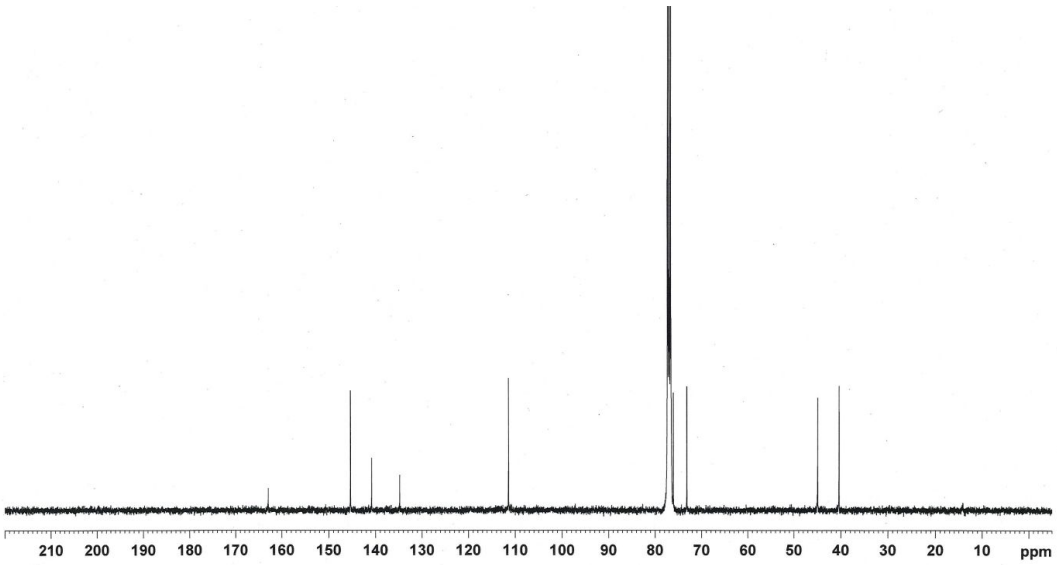


▼ **79, ^{1}H-NMR, 500 MHz**

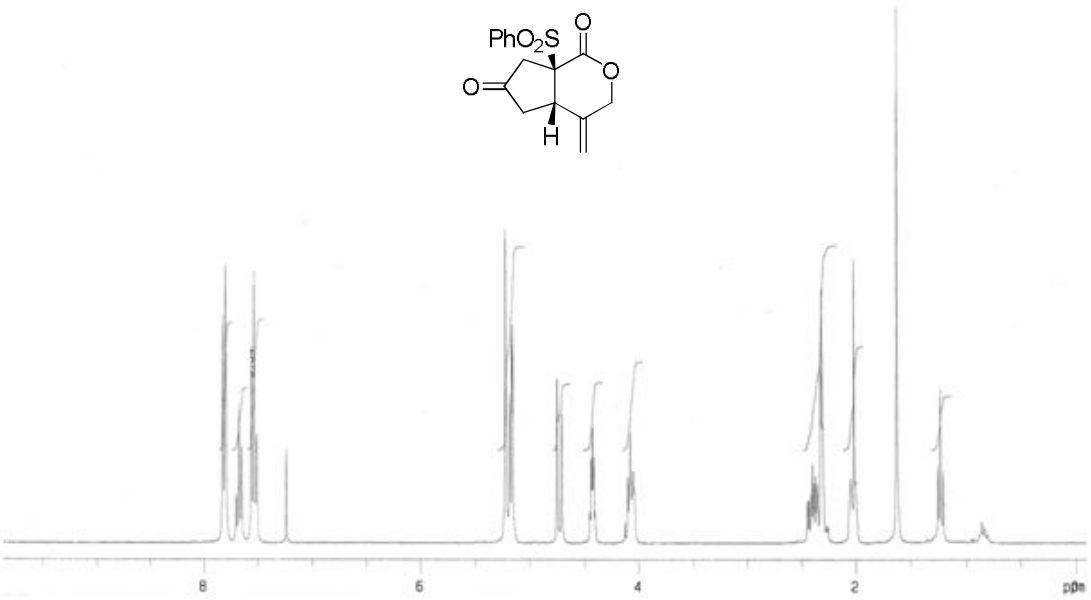


▼ **79, ^{13}C-NMR, 100 MHz**

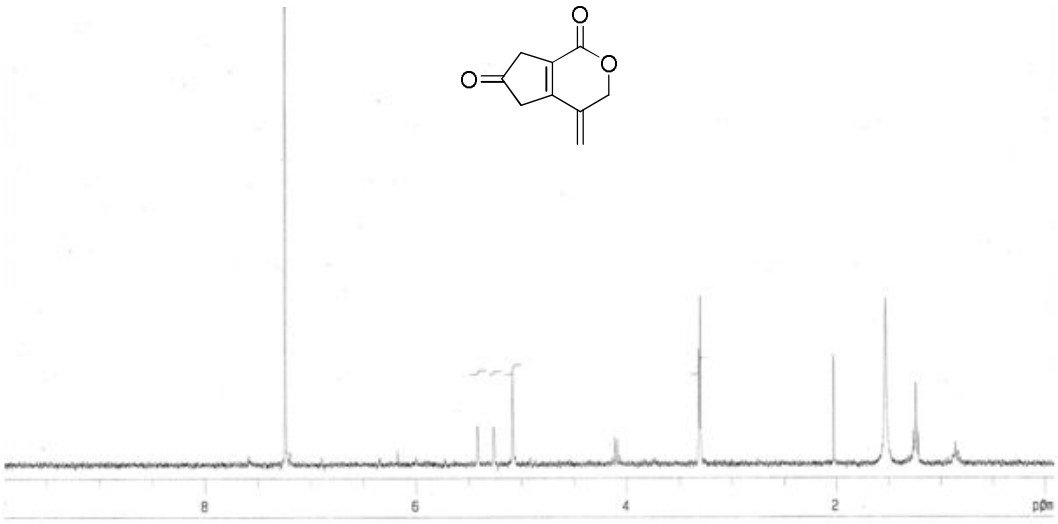




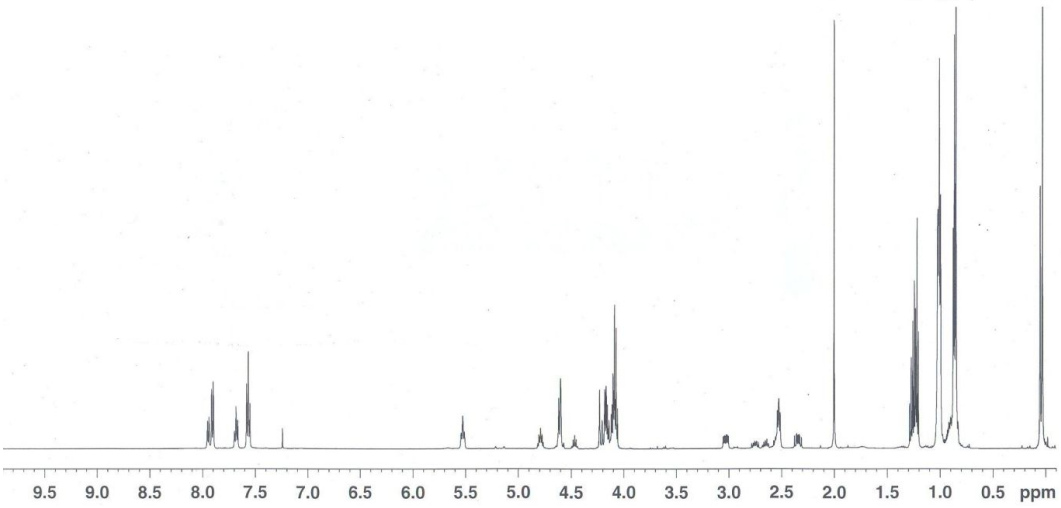
▼ **80, ^{1}H-NMR, 300 MHz**



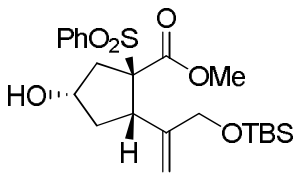
▼ **81, ^{1}H-NMR, 300 MHz**

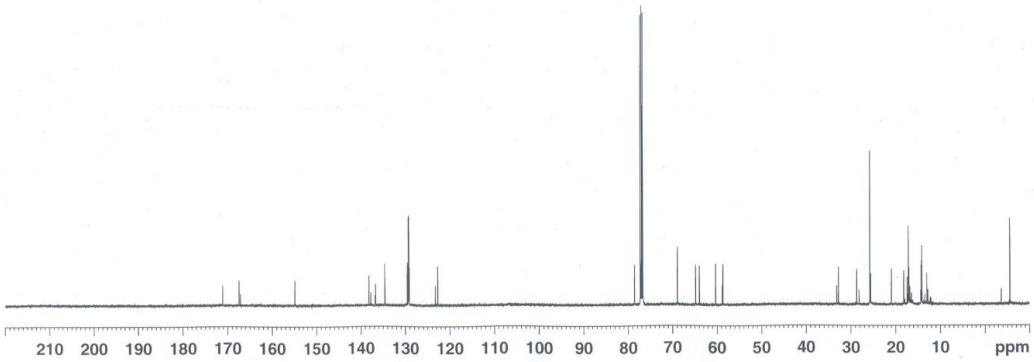


▼ **83, ^{1}H-NMR, 400 MHz**

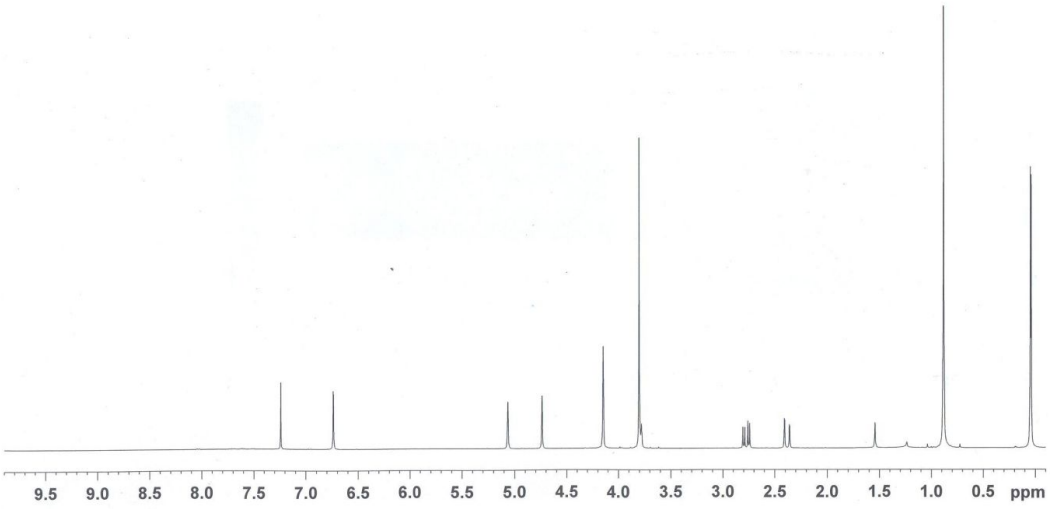


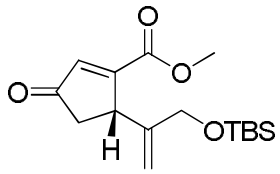
▼ **83, ^{13}C-NMR, 100 MHz**



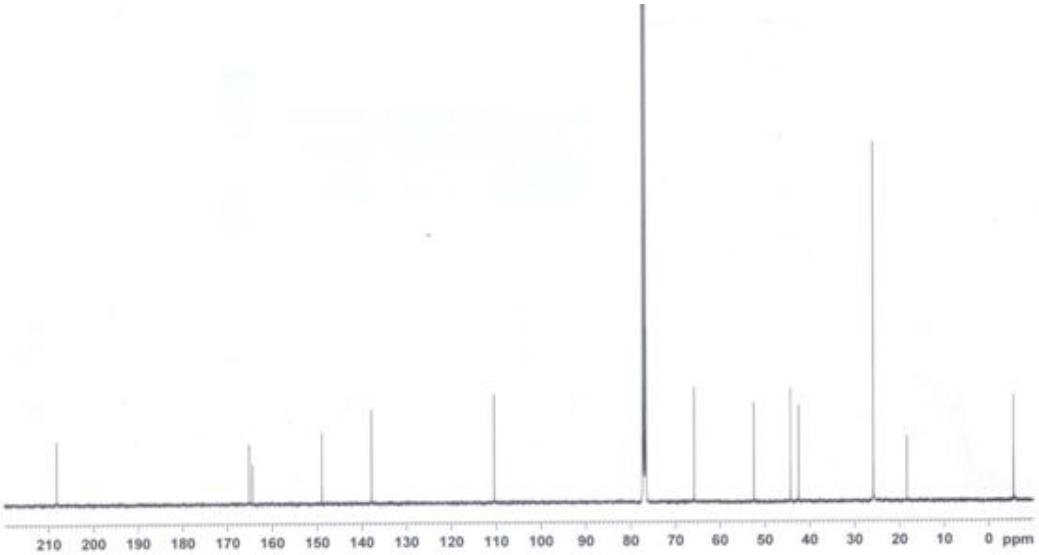


▼ **84, ^{1}H-NMR, 400 MHz**

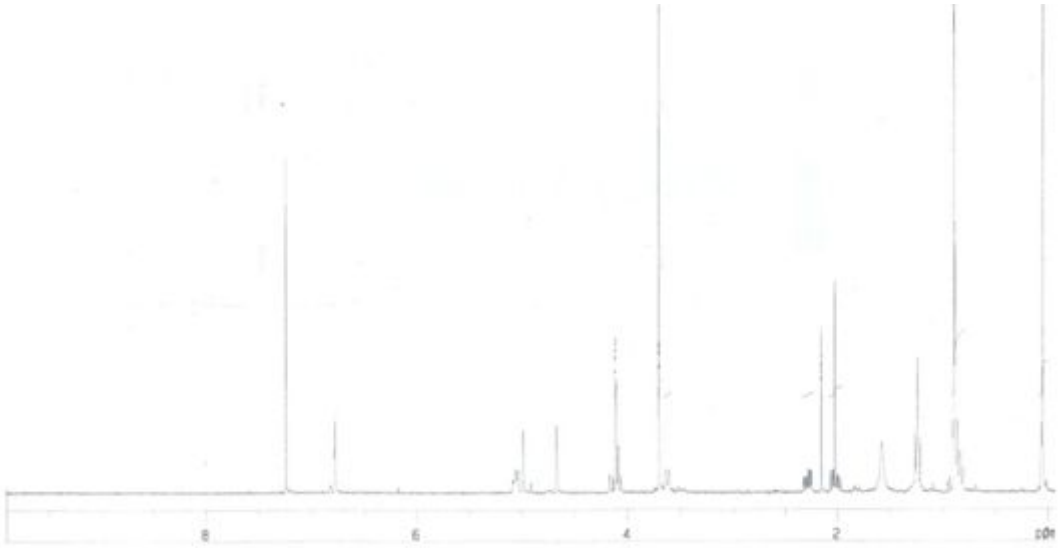




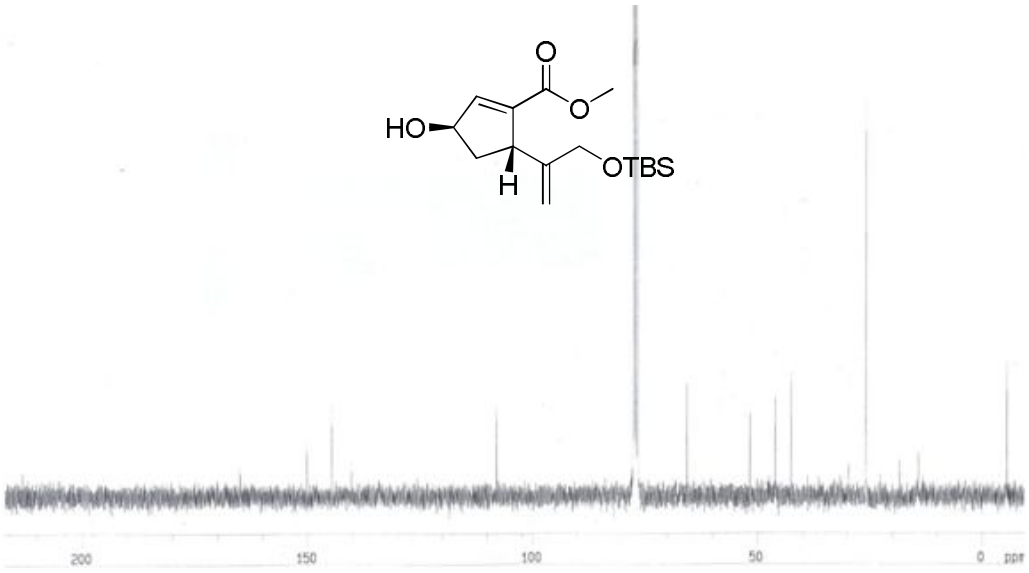
▼ **84, ^{13}C-NMR, 125 MHz**



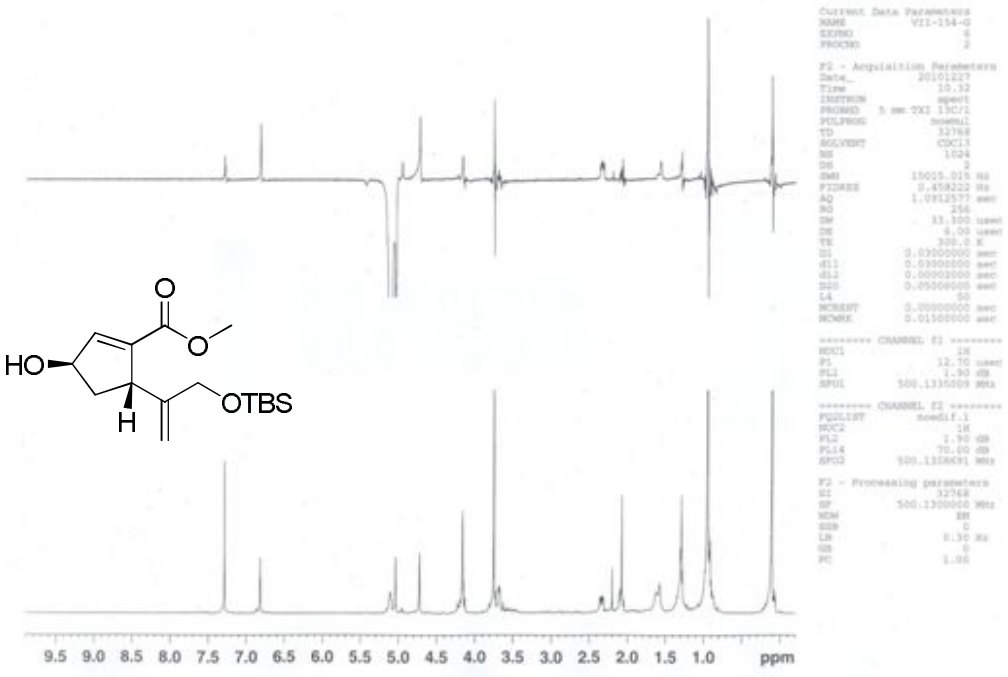
▼ **85, ^{1}H-NMR, 300 MHz**

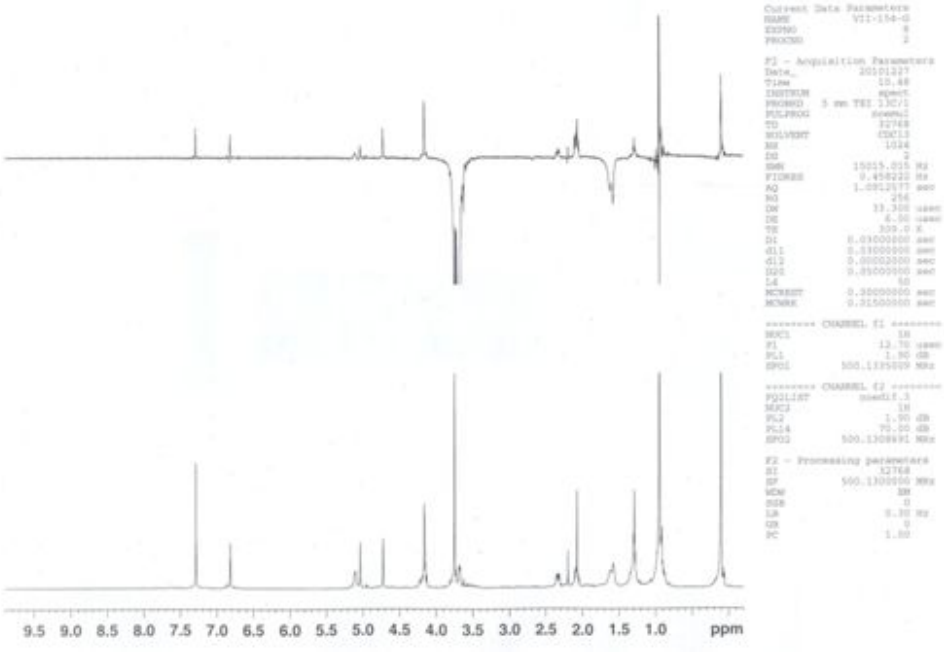


▼ **85, ^{13}C-NMR, 75 MHz**

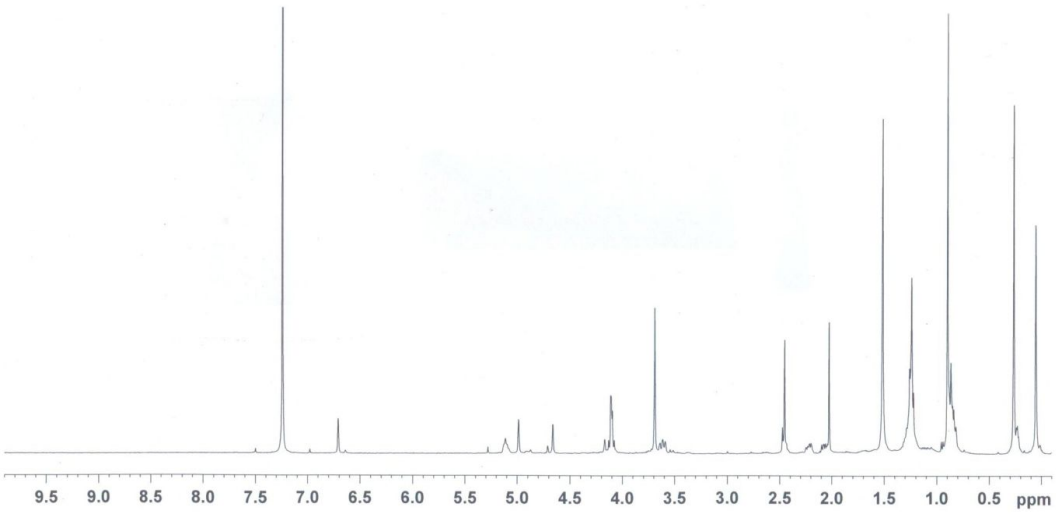


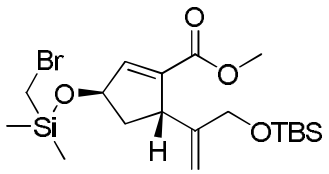
▼ **NOE Study of 85**



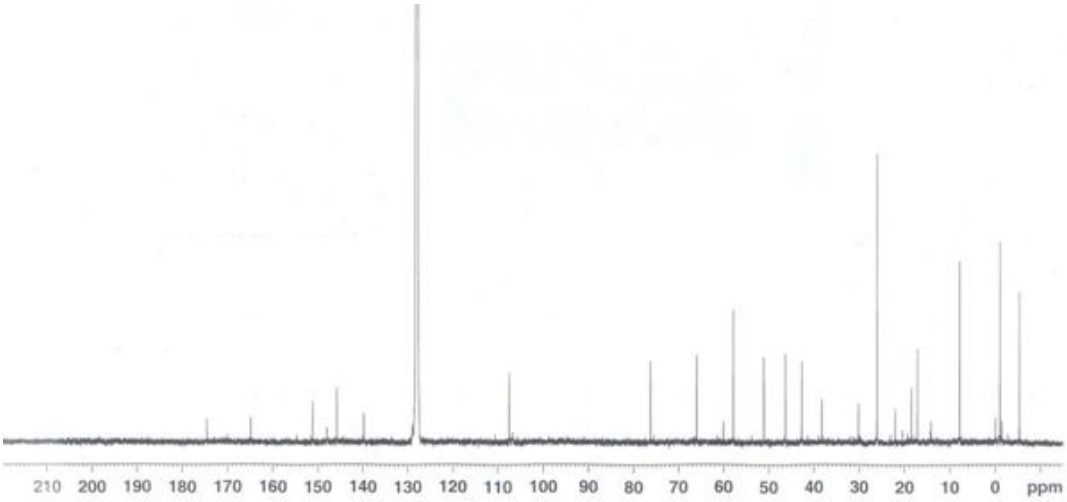


▼ **82, ^{1}H-NMR, 300 MHz**

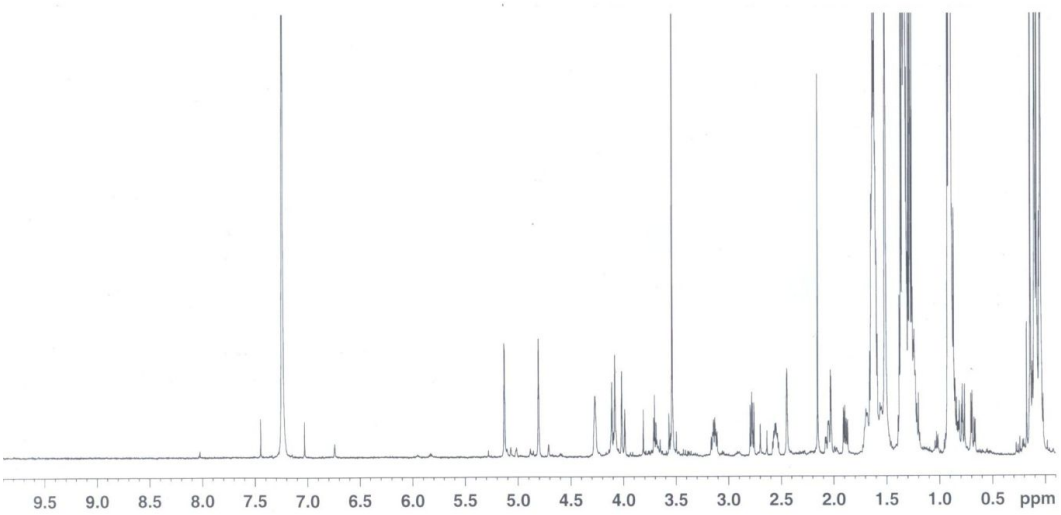




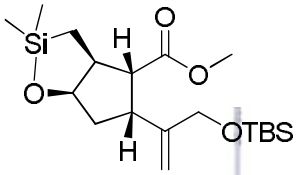
▼ **82, ^{13}C-NMR, 125 MHz**

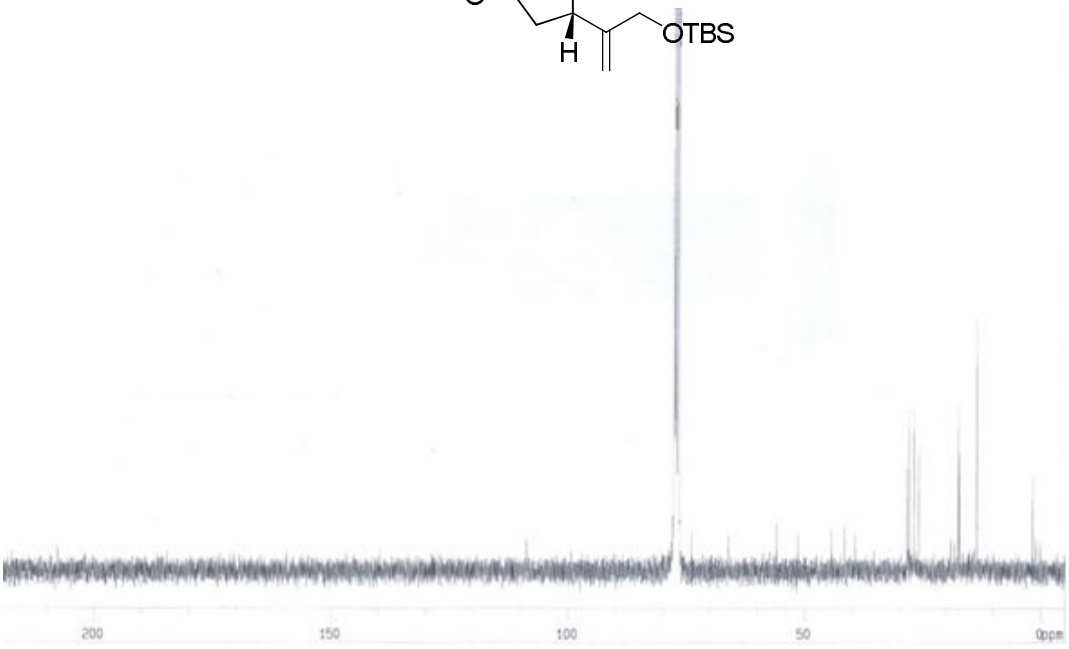


▼ **87, ^{1}H-NMR, 300 MHz**

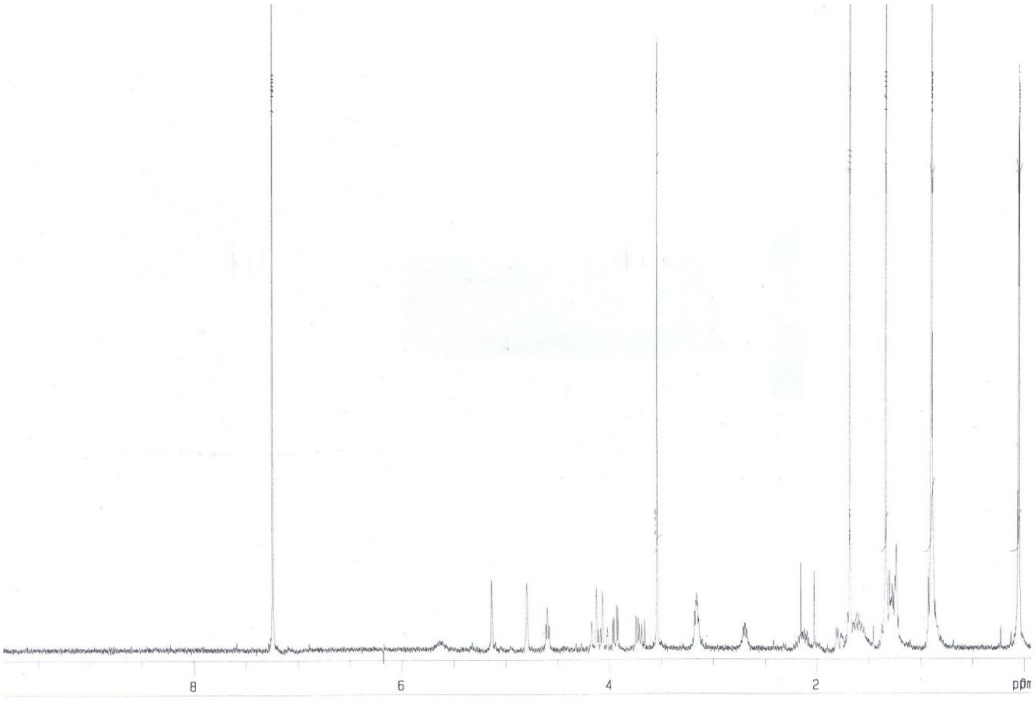


▼ **87, ^{13}C-NMR, 75 MHz**

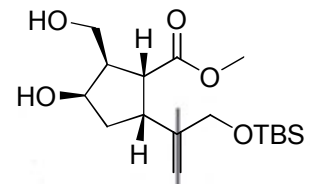


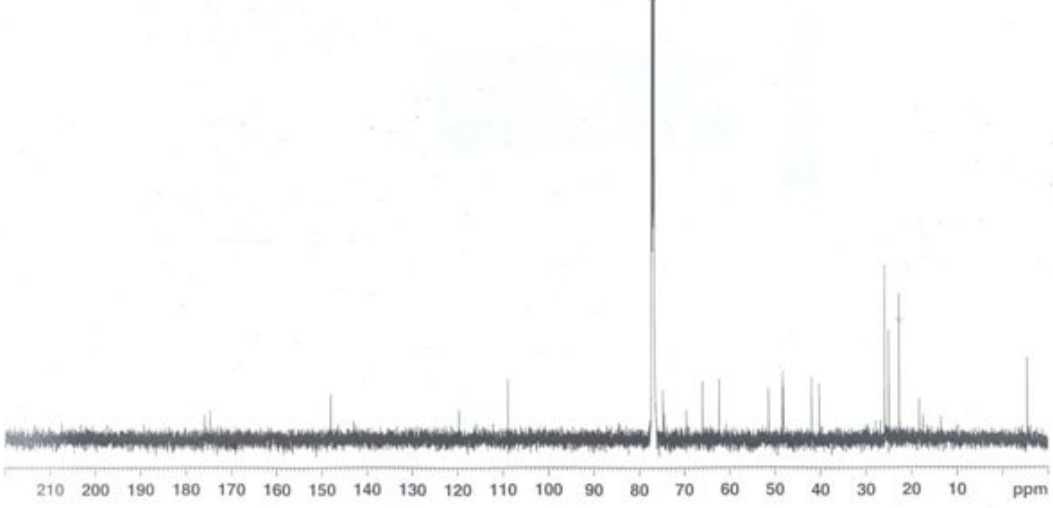


▼ **88, ^{1}H-NMR, 300 MHz**

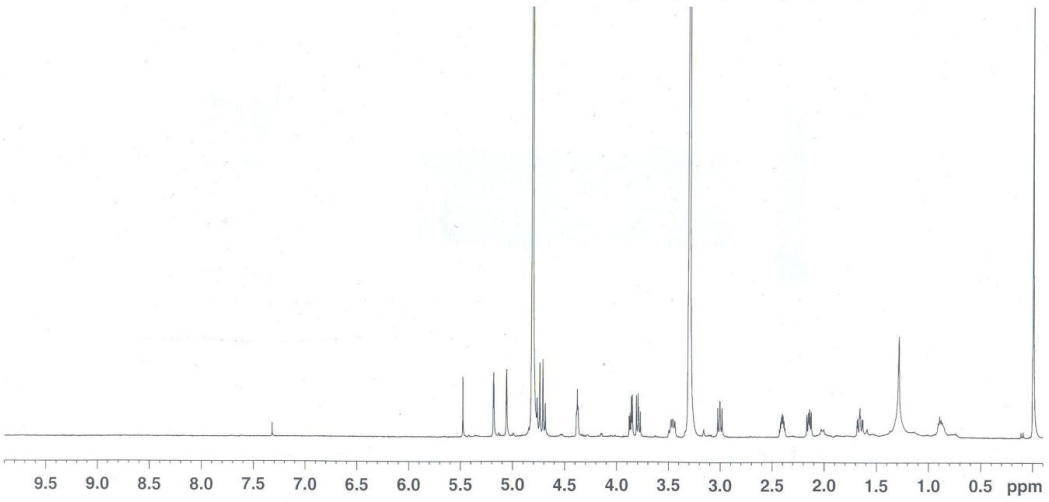


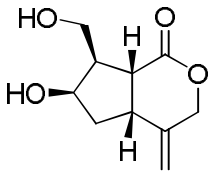
▼ **88, ^{13}C-NMR, 125 MHz**



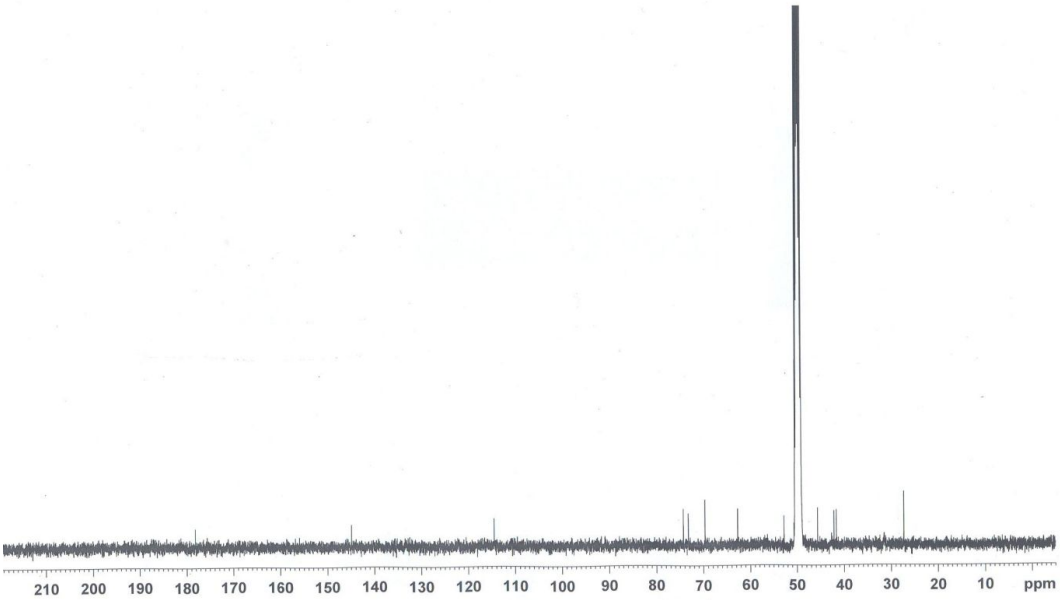


▼ **1, ^{1}H-NMR, 500 MHz**





▼ **1, ^{13}C-NMR, 125 MHz**



VII. Abstract

First total synthesis of natural iridoid lactone

Sujin Lee
College of pharmacy
Department of manufacturing pharmacy
The Graduate School
Seoul National University

Iridoids are a large family of natural monoterpenoids products, and structurally characterized by a *cis*-fused cyclopenta[*c*]pyran ring system. The unique *cis*-fused cyclopenta[*c*]pyran ring system has presented a variety of challenges for chemical synthesis and in the analysis of biological activities. In particular, we have been interested in a recently reported iridoid lactone, which is structurally related to loganin and consists of a C7-substituted C6-hydroxycyclopenta[*c*]lactone. Iridoid lactone, (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1 (3*H*)-one, was isolated from the roots and rhizomes of *Nardostachys chinensis* Batalin (Valerianaceae). This plant has been used as a Chinese folk medicine and its roots and rhizomes exhibit a variety of biological activities, such as sedative, antimalarial, antinociceptive, cytotoxic, and enhancement of nerve growth factor activities. Thus, the unique structural feature as well as the unknown biological activity of iridoid lactone led us to synthesize the recently reported iridoid lactone.

In this connection, we accomplished the first total synthesis of (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1 (3*H*)-one (**1**) in 13 steps. The key features of the synthesis involve the stereoselective and efficient preparation of the bicyclic lactone *via* Pd(0)-catalyzed cyclization, which is easily transformated to cyclopentanol intermediate and then the silicon-tethered radical cyclization followed by Tamao-Fleming oxidation for the

stereoselective introduction of the C7-hydroxymethyl substituent. This versatile synthetic approach is expected to be widely utilized for the synthesis of iridoids and iridoalkaloids.

Key words: (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one, iridoid lactone, iridoid, Pd(0)-catalyzed cyclization, bicyclic lactoen, silicon-tethered radical cyclization, Tamao-Fleming oxidation.

Student number: 2006-30449

Part II: Ginsenoside Rk1 구조 기반의 anti-apoptotic agent의 개발

Part II: Cholesterol derived novel anti-apoptosis agents on the structural basis of ginsenoside Rk1

Table of Contents

Part II: Cholesterol derived novel anti-apoptosis agents on the structural basis of ginsenoside Rk1

국문 초록 iii
List of Figures iv
List of Schemes v
Abbriviations vi
I. INTRODUCTION 1
1. Endothelial cells 1
2. Apoptosis 2
3. Ginsenoside 4
II. RESULT AND DISCUSSION 8
1. Preliminary study 8
2. Phytosterol screening 10
3. Synthesis of pseudosugar derivatives 16
4. Biological activity against serum deprived apoptosis 19
1) MTT assay on HUVECs 19
2) DAPI/ TUNEL assay 23
III. Conclusion 26
IV. Experimental 27
V. References 42
VI. Appendix 47
VII. Abstract 64

국문 초록

혈관 내벽은 혈관내피세포의 단일막으로 이루어져 있다. 혈관 내피세포의 항상성 유지가 혈관의 생명활동을 유지하는 중요한 요인이다. 혈관내피세포의 세포사멸에 의한 혈관의 구조변화와 기능저하가 동맥경화, 동종이식편에 의한 맥관장애, 울혈성 심부전 등의 여러 질병과 연관된다는 연구결과가 보고되고 있다. 따라서 혈관내피세포의 세포사멸 억제제가 혈관질환의 새로운 치료제로서 연구 가치가 있으리라 생각된다.

최근, 인삼으로부터 분리 보고된 ginsenoside Rg3, Rk1이 세포사멸 억제효과가 있는 것으로 발표되었다. 하지만 이들 ginsenoside들은 다당체를 함유하고 있어 산성 조건에서 화학적으로 불안정하고, 자연에서 얻기에는 양적인 면에서 한계가 있기 때문에 이들 구조를 기반으로 하여 화학적으로 비교적 안정하면서 세포사멸 억제효과를 갖는 유도체들의 합성을 계획하였다. Ginsenoside의 뼈대인 스테로이드 골격을 유지하면서 ginsenoside의 활성을 유지할 수 있는 여러 phytosterol을 검색하였고, C3 hydroxyl기에 활성에 중요한 역할을 하는 glucoside를 대체할 bioisoster를 도입하고자 하였다. 이를 통해 상업적으로 손쉽게 구입 가능한 콜레스테롤을 기반으로 하여 다양한 유도체를 합성하였고, 이들의 세포사멸억제 효과를 확인하였다.

주요어; 혈관내피세포, 세포사멸, 동맥경화, 동종이식편에 의한 맥관장애, 울혈성 심부전 ginsenoside Rg3, Rk1, phytosterol, 콜레스테롤, 세포사멸 억제효과

학 번: 2006-30449

List of Figures

Figure 1. Schematic structure of blood vessel (a) and tight junction (b) 1
Figure 2. Schematic representation of apoptosis and necrosis 3
Figure 3. Small molecules inhibiting apoptosis of endothelial cells.....4
Figure 4. Structures of ginsenosides.....6
Figure 5. (20*S*)Rg3 protects endothelial cells from serum-deprived apoptosis 8
Figure 6. MTT assay result for processed Ginseng extraction 9
Figure 7. Synthetic approach for ginsenoside derivatives 10
Figure 8. Structures of phytosterols.....11
Figure 9. MTT assay on HUVECs 14
Figure 10. Morphology of HUVEC.....15
Figure 11. Effect of compounds on apoptosis of HUVECs: 5 μg/ml treatment 19
Figure 12. Effect of compounds on apoptosis of HUVECs: 10 μg/ml treatment.....20
Figure 13. Morphological changes of HUVEC 21
Figure 14. Inhibition of compounds 7, and 9 on apoptosis of HRECs.....22
Figure 15. Morphological changes of HUVEC 22
Figure 16. Changes in nuclear morphology accessed by DAPI staining.....24
Figure 17. TUNEL assays at various concentrations of 7 and 9 24

List of Schemes

Scheme 1. Synthesis of stigmasterol derivatives **11**
Scheme 2. Synthesis of β-sitosterol derivatives **12**
Scheme 3. Synthesis of sitostanol derivatives **13**
Scheme 4. Synthesis of cholesterol derivatives **13**
Scheme 5. Synthesis of cholestanol derivatives **14**
Scheme 6. Synthesis of dihydropyran derivatives **16**
Scheme 7. Synthesis of acyclic analogs **17**
Scheme 8. Synthesis of bulky analogs **18**

Abbriviations

BrdUTP: 5-bromo-2'-deoxyuridine 5'-triphosphate

bs: broad singlet

t-Bu: *tert*-butyl

calcd: calculated

CHF: congestive heart failure

CNS: central nervous system

d: doublet

DAPI: 4'-6-diamino-2-phenylindole

DHP: 3,4-dihydro-*2H*-pyran

DMF: *N,N*-dimethylformamide

EC: endothelial cell

FAB: fast atom bombardment

HREC: human retina endothelial cell

HRMS: high resolution mass spectroscopy

HUVEC: human umbilical vein endothelial cell

IR: infrared

LRMS: low resolution mass spectroscopy

m: multiplet

Me: methyl

MeOH: methyl alcohol

NMR: nuclear magnetic resonance

P. ginseng: Panax ginseng

Ph: phenyl

PPD: protopanaxadiols

PPT: protopanaxatriols

PPTS: pyridinium *p*-toluene sulfonate

PTSA: *p*-toluene sulfonic acid

q: quartet

s: singlet

SAR: structure activity relationship

t: triplet

TdT: terminal deoxynucleotidyl transferase

THF: tetrahydrofuran

TLC: thin layer chromatography

TUNEL: Terminal Transferase dUTP Nick End Labeling

F-dUTP: fluorescent-tagged deoxyuridine triphosphate nucleotides

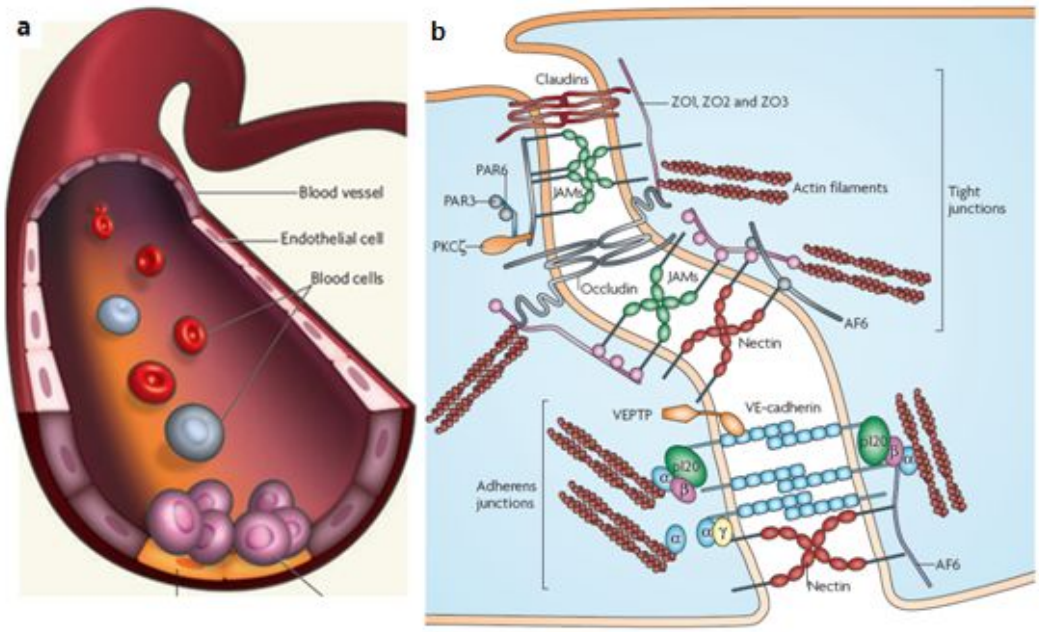
VEC: vascular endothelial cell

I. INTRODUCTION

1. Endothelial cells

The blood vessel has a monolayer of endothelial cell (EC) lining (Figure 1a) [1] located at the interface between the vascular and perivascular compartments. In this location, the ECs have two different roles. First, they establish the communication between circulating blood and underlying tissues. The ECs behave as a permeable filter, allowing the selective exchange of solutes and water between the luminal and abluminal sides of the barrier. Permeability follows two distinct pathways. The transcellular route crosses apical and basal cell membranes and is mostly mediated by channels, carriers, pumps, and vesicles. In contrast, the paracellular route passes through intercellular and lateral spaces between contacting cells and is mediated by the tight junction (Figure 1b). [2] Tight junctions are composed of integral membrane proteins (e.g. occludin, claudins, and junctional adhesion molecules [JAMs]) and intracellular proteins (e.g. ZO-1 and cingulin). [3]

Figure 1. Schematic structure of blood vessel (a) and tight junction (b)



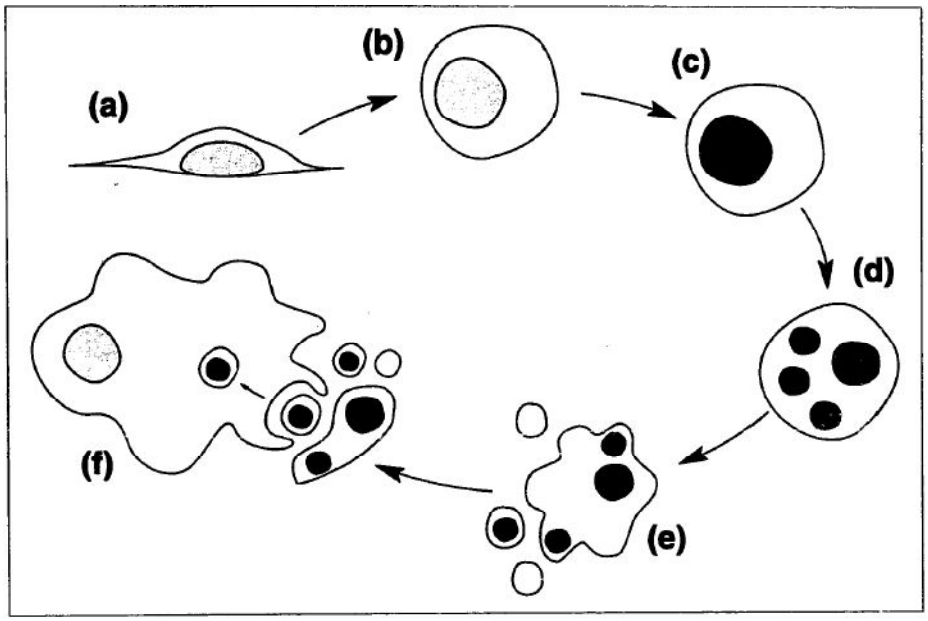
Second, ECs act as a barrier between the vascular and extravascular compartments, thus maintaining tissue homeostasis and preventing thrombosis. The location of ECs ensures their simultaneous and constant exposure to a wide variety of stimuli, many of which have the potential to induce or prevent apoptosis of the cells. It has been suggested that disturbed endothelial function could account for a large portion of all cardiovascular diseases,[4] such as atherosclerosis, allograft vasculopathy, hypertension, congestive heart failure (CHF), and other diseases (e.g. systemic sclerosis, diabetes, and thrombotic thrombocytopenic purpura). [5]

2. Apoptosis

There are two major types of cell death: necrosis and apoptosis. Necrosis occurs following diverse traumatisms including hyperthermia, ischemia, and physical injury or appears as a result of the exposure to cytotoxic cytokines or substances. Apoptosis, on the other hand, is a kind of programmed cell death. It has recently gained considerable interest because it has been appreciated as a regulatory mechanism in organ growth, wound repair, tumor genesis, and immune response.

Apoptosis is a normal occurrence in which an orchestrated sequence of events leads to the cell death. As represented schematically in Figure 2,[6] death by apoptosis is a neat, orderly process characterized by the overall shrinkage in volume of the cell and its nucleus, the loss of adhesion to neighboring cells, the formation of blebs at the cell surface, the dissection of the chromatin into small fragments, and the rapid engulfment by phagocytosis. Necrotic cells lyse and release their intracellular components, including DNA and RNA, into the extracellular space. Waste-management nucleases digest these extracellular nucleic acids, and inflammatory reactions occur. Apoptosis, however, is characterized by distinct morphological changes. Membrane fusion early in apoptosis causes fluid loss and therefore cell shrinkage. Chromatin condenses and is deposited on the inner surface of the nuclear membrane. Macromolecular synthesis decreases and usually cellular DNA is cleaved at internucleosomal sites, resulting in fragmentation of DNA.

Figure 2. Schematic representation of apoptosis and necrosis



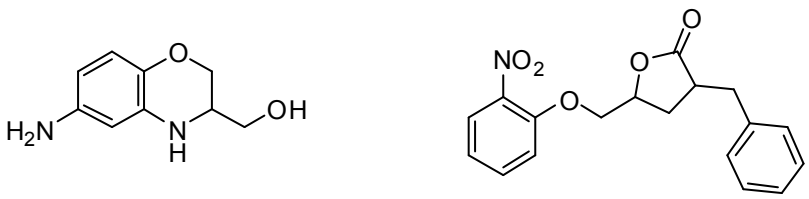
Upon receipt of a signal to undergo apoptosis, an adherent cell (a) rounds up (b) and rapidly condenses its DNA (c), thought to be a consequence of DNA fragmentation. This is rapidly followed by separation of the nucleus into discrete masses of condensed chromatin (d) and, finally, fragmentation of the cell into several membrane-bound vesicles (apoptotic bodies) (e), which are rapidly recognized and phagocytosed by macrophages or neighbouring cells (f).

This DNA is eventually released from cells in membrane-bound vesicles, and the vesicles are eliminated by phagocytic cells. Since intracellular contents are not released to interstitial fluid, inflammation does not occur. [7]

It is an active process of cell death that occurs in many important physiological conditions, such as embryonic development and tissue remodeling. Apoptosis is involved during angiogenesis in de novo tissue formation and wound healing. Moreover, many diseases are associated with either too much or too little apoptosis, including cancer, sepsis, autoimmunity, and neurodegenerative diseases. [8] In particular, the endothelium is a critical site for the control of apoptosis during processes such as inflammation, vascular remodeling, and angiogenesis. Although the suppression of EC apoptosis is required for the maintenance of blood vessel integrity and for angiogenesis, the

activation of apoptosis has been suggested to contribute to pathological tissue destruction during vascular injury, inflammation, atherosclerosis, and allograft arteriopathy. [9] Therefore, the inhibition of vascular endothelial cell (VEC) apoptosis is considered as a new target for diverse cardiovascular diseases. EC perturbing agents such as inflammatory cytokines, lipopolysaccharides, reactive oxygen species, and oxidized low-density lipoprotein (LDL) are known to induce EC apoptosis. [10]

Figure 3. Small molecules inhibiting apoptosis of endothelial cells



ABO 3BDO

Considering these preliminary reports, the inhibition of VEC apoptosis has been considered as a novel approach for the treatment of various physiological disorders such as cardiovascular disease. A few reports have addressed the development of anti-apoptotic agents from natural products [11] to small molecules.[12] Miao et al. discovered lead compounds for anti-apoptotic activity on human umbilical vein endothelial cells (HUVECs) through the library screening (Figure 3). These molecules inhibit against oxidized LDL-induced apoptosis and increase intergrin β4, NF-kB, and P53 in HUVECs.

3. Ginsenoside

Ginseng (*Panax ginseng* C. A. Meyer, Araliaceae) is one of the most popular herbal medicines in the Orient. [13] It has been used for over 2000 years in the belief that it is a panacea and promotes longevity. Recently, there has been a renewed interest in investigating ginseng pharmacology using

biochemical and molecular biological techniques. The pharmacological effects of ginseng have been demonstrated in the central nervous system (CNS) [14] and in cardiovascular, endocrine,[15] and immune systems.[16] In addition, ginseng and its constituents have been ascribed anti-neoplastic, anti-stress, and antioxidant activities. [17]

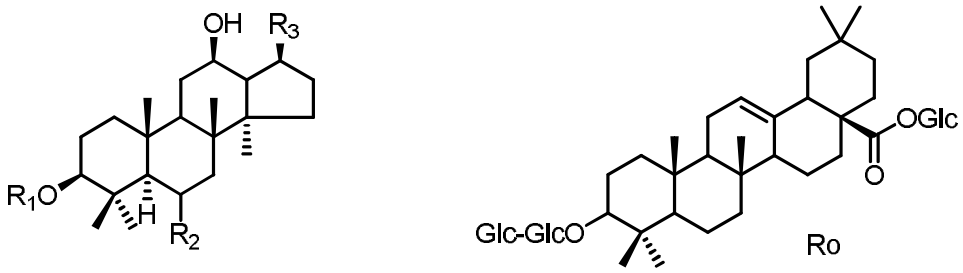
The major components found in most ginseng spices are ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids. Among those components, the most pharmacological active components are ginsenosides.[18] Ginsenosides are a family of steroids named steroidal saponins; they have also been named ginsenoside saponins, triterpenoid saponins, or dammarane derivatives under previous classifications. Ginsenosides possess a triterpene structure as a backbone and have a structural diversity.

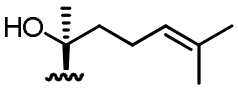
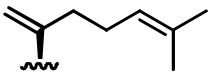
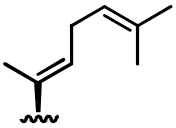
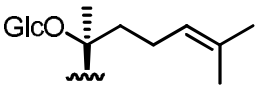
Structurally, ginsenosides possess the four successive trans-fused ring steroidal skeletons with a modified side chain at C20, and β-OH at C3, which is usually attached to sugar residue such as glucose, rhamnose, xylose, and arabinose. Based on the number of hydroxyl groups on their dammarane type triterpene, ginsenosides are generally divided into three groups: protopanaxadiols (PPDs), protopanaxatriols (PPTs), and oleanane type. The representative structures of ginsenosides are shown in Figure 4. Rb1, Rb2, Rc, and Rd are examples of PPDs, which have two hydroxyl groups at C3 and C20. Re, Rf, Rg1, and Rg2 are examples of PPTs, which have three hydroxyl groups at C3, C6, and C20. The oleanane type ginsenoside is only one ginsenoside, Ro (Figure 4). [19]

Ginseng is widely used in traditional Chinese medicine and Western herbal preparations, numerous researches to evaluate the biological activities of ginsenosides have been undertaken. Although the extracts from fresh or processed *P. ginseng* have been extensively tested for their pharmacological effects, the precise biological functions and underlying action mechanisms of pure molecules from ginseng extract are still largely unknown. Some literature reports suggest that ginseng extract has both wound-healing and anti-tumor effects through a reciprocal role in the

vascular system.[20]

Figure 4. Structures of ginsenosides



ginsenoside	R_1	R_2	R_3
Rg3 (PPD)	Glc-Glc-	H_2	
Rk1 (PPD)	Glc-Glc-	H_2	
Rg5 (PPD)	Glc-Glc-	H_2	
Rg1 (PPT)	H	OGlc	

Ginsenosides Rg1 and Rb1 have been demonstrated to have opposing activities in the vasculature. Rg1 promotes functional neovascularization in vivo and proliferation, chemoinvasion, and tubulogenesis of ECs in vitro, whereas Rb1 suppresses the earliest step in angiogenesis by inhibiting the chemoinvasion of ECs.[21] These results suggest that ginsenosides are structurally related but functionally different, depending on the side chain moieties attached to the triterpene backbone. In particular, (3β,12β)-12,20-Dihydroxydammar-24-en-3-yl 2-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranoside (ginsenoside Rg3) exerts many pharmacological activities such as anti-tumor, anti-inflammatory, neuroprotection against the cerebral ischemia-induced injury, and a

vasodilation effect.[22] Interestingly, ginsenoside Rg3 has been recently revealed to have anti-apoptotic activity via the inhibition of a mitochondrial caspase pathway.[23] Moreover, 3β,12β-dihydroxydammar-20(21),24-diene-3-*O*-β-*D*-glucopyrano-syl(12)-β-*D*-gluco-pyranoside (ginsenoside Rk1), isolated from heat-processed ginseng (Sun Ginseng),[24] has more effective anti-apoptotic activity on ECs than Rg3. Considering these reports, we focused on the development of anti-apoptotic agents on HUVECs based on the structure of both ginsenosides. However, ginsenosides are generally labile under acidic conditions, and their composition is variable depending on the process of extraction. Thus, it is difficult to determine the precise biological functions and underlying mechanisms.[25]

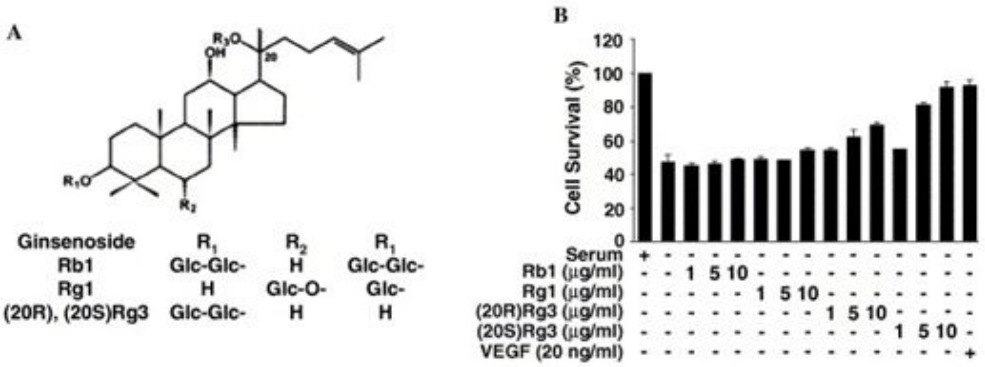
To date, most research has focused on the activities of natural ginsenosides, and few studies have reported on the structure activity relationship with ginsenoside modification or derivatives synthesis.[26] On the basis of these preliminary studies, we planned to investigate a preliminary structure-activity relationship (SAR) study of ginsenosides Rg3 and Rk1 on the anti-apoptotic effect. At first, we designed and synthesized diverse phytosterol analogs for the elucidation of the SAR.

II. RESULT AND DISCUSSION

1. Preliminary study

As recently reported,[23] some ginsenosides were treated on apoptosis-induced human ECs by serum deprivation[10] (Figure 5). Interestingly, the data showed that both Rg1 and Rb1 have no significant effect on EC survival up to the concentration 10 μg/ml, whereas Rg3 has a significant activity at the same concentration. Moreover, ginsenoside Rg3 epimers also exhibited differential effects. (20*S*)Rg3 promoted EC survival to an extent comparable to 20 ng/ml VEGF, but (20*R*)Rg3 showed only a minimal effect. These results indicate that the precise structural characteristics of ginsenosides such as side chains, glucosyl elimination, and epimerization of C20 are required for their vascular effects.

Figure 5. (20*S*)Rg3 protects endothelial cells from serum-deprived apoptosis

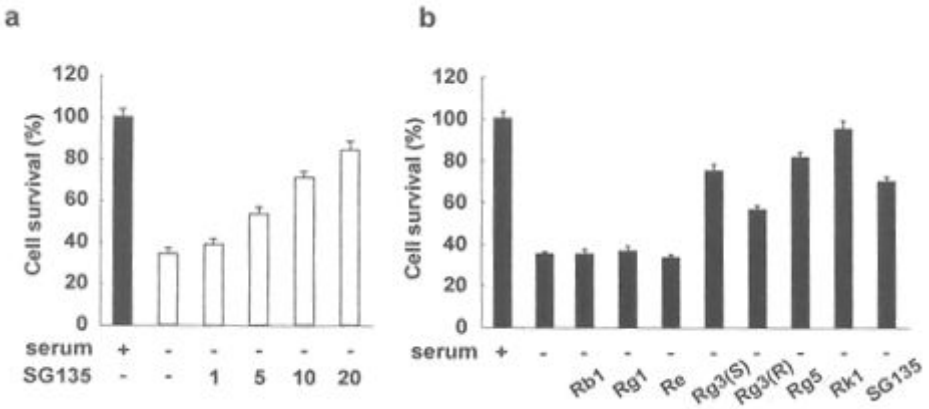


(A) Chemical structure of ginsenosides Rg1, Rb1, (20*R*)Rg3, and (20*S*)Rg3. (B) HUVECs (1 × 105 cells/well) were plated onto six-well plates in 1 ml of M199 containing 20% fetal bovine serum. Next day, the cells were switched to serum-free M199 with various concentrations of Rg1, Rb1, 20(*R*)Rg3 or 20(*S*)Rg3. After 36 h, cell viability was determined by trypan blue exclusion. Viability of control cells was set at 100% and viability relative to the control is presented.

The triterpene backbone structure of ginsenoside Rg3 is similar to the cholestane structure, which is a general backbone structure of phytosterols. The hydroxyl group at C6 seemed not to influence

the apoptotic activity (shown as Rg1). The glucosyl group attached at C3 OH is crucial for the activity, and the stereochemistry of C20 slightly affects to the activity from comparison of Rb1 to Rg3. On the other hand, ginsenosides extracted from heat-processed ginseng (SG 135) and crude extraction were examined, and ginsenoside Rk1 showed the most effective activity (Figure 6).

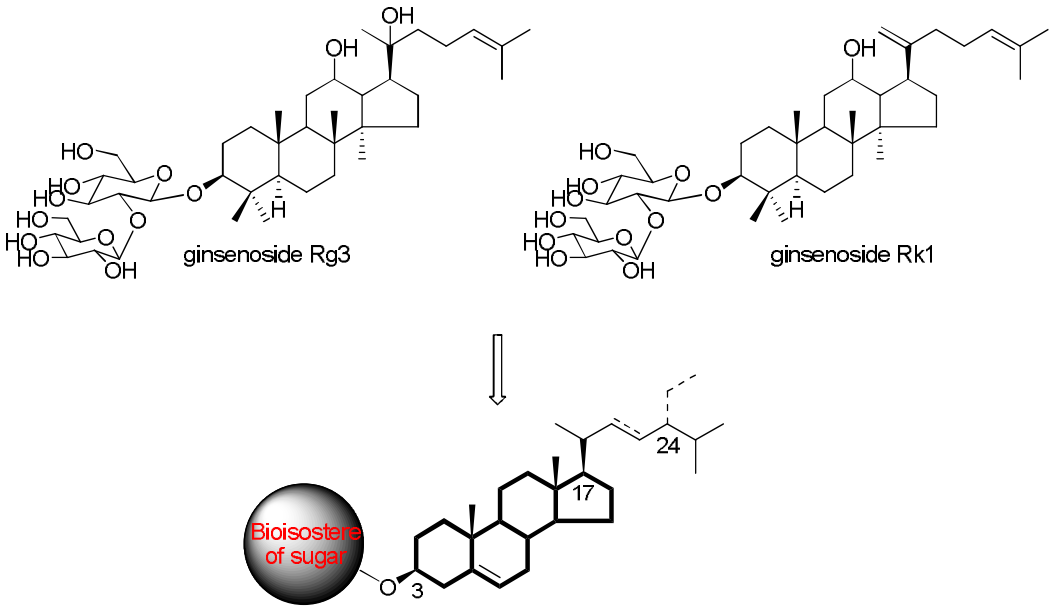
Figure 6. MTT assay result for processed Ginseng extraction



(a) The viability on HUVECs with the serum-free conditions with various concentrations of SG135. (b) HUVECs cell viability with various ginsenosides.

Taking these results together, the glucosyl group at C3 is considered to be crucial. Thus, we planned to determine three major considerations: 1) the effect of sugar moiety at the C3 hydroxyl group, 2) the steric influence of the alkyl chain at C24, and 3) the influence of steroidal skeleton structures. The research began with the selection of proper phytosterols, which contain simple steroidal backbone structures (Figure 7). Thus, our studies commenced with the synthesis of pseudosugar derivatives of several phytosterols, which are structurally simple and commercially available steroids.

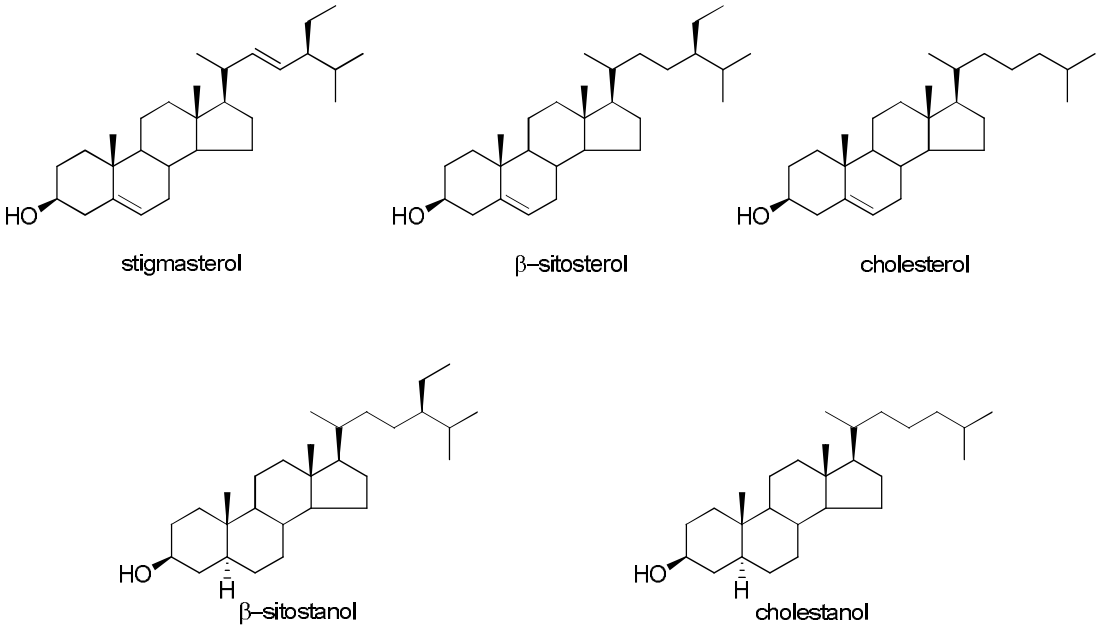
Figure 7. Synthetic approach for ginsenoside derivatives



2. Phytosterol screening

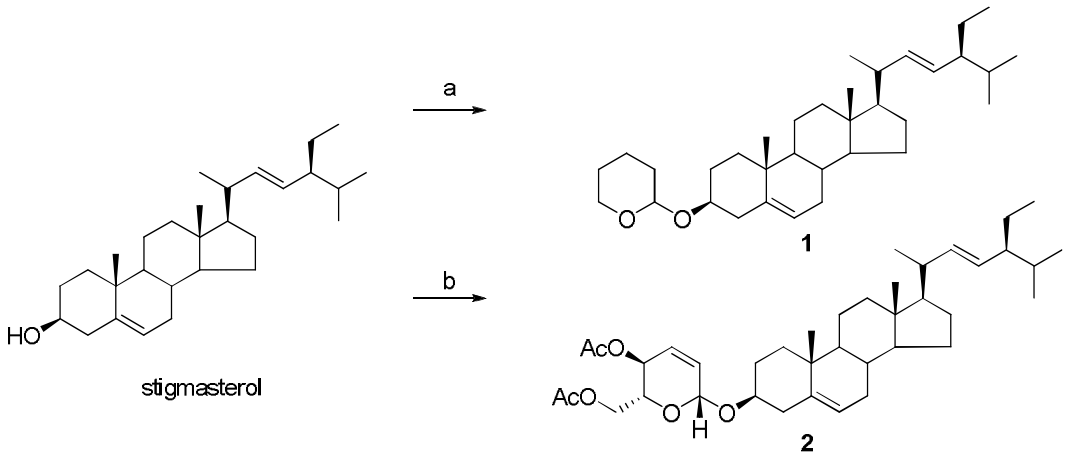
Phytosterols, also referred to as plant sterols, are common plant and vegetable constituents and are therefore normal constituents of the human diet. They are structurally related to cholesterol but differ from it in the structure of the side chain. They consist of a steroidal skeleton with a hydroxyl group attached to the C3 and an aliphatic side chain attached to the C17 (Figure 8). Commercially, phytosterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil, or corn oil, or from so-called tall oil, which is a by-product of the manufacture of wood pulp. [27] These sterols can be hydrogenated to obtain phytostanols. The most common phytosterols and phytostanols are sitosterol (3β-stigmast-5-en-3ol), sitostanol (3β,5α-stigmastan-3-ol), and stigmasterol (3β-stigmasta-5,22-dien-3-ol). Each commercial source has its own typical composition.

Figure 8. Structures of phytosterols



To determine the influence of the side chain at C20, we planned to synthesize simple pseudosugar derivatives such as tetrahydropyrans (THPs) and dihydropyran moieties with those phytosterols. The synthetic schemes are shown below (Scheme 1-3).

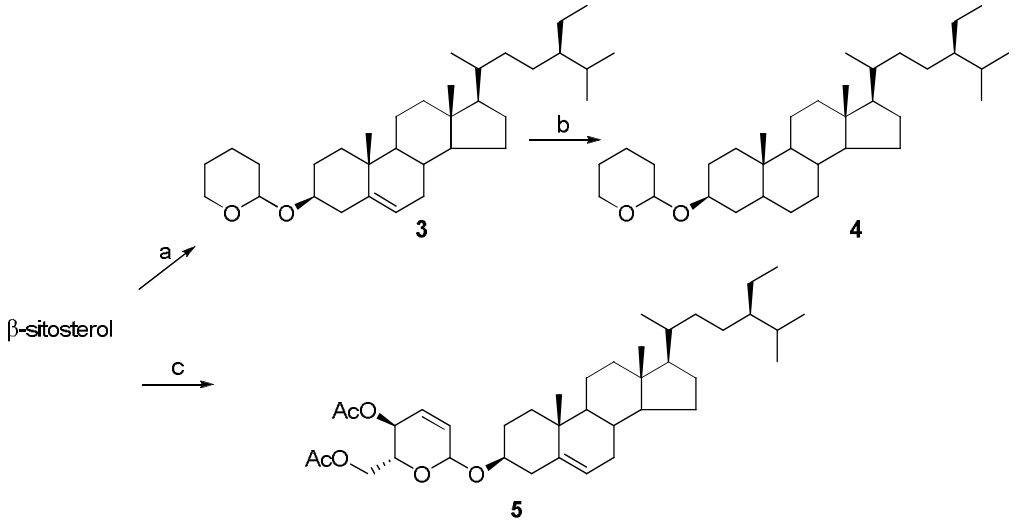
Scheme 1. Synthesis of stigmasterol derivatives



Reagents; (a) DHP, PPTS, CH_2Cl_2, 68%; (b) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 79%.

At first, the addition of 3,4-dihydro-*2H*-pyran and pyridinium *p*-toluene sulfonate into stigmasterol in CH_2Cl_2 afforded to compound **1**. The dihydropyran analog **2** was synthesized by the addition of 3,4,6-*Tri*-*O*-acetyl-*D*-glucal into the stigmasterol solution in the presence of boron trifluoride (Scheme 1)

Scheme 2. Synthesis of β-sitosterol derivatives

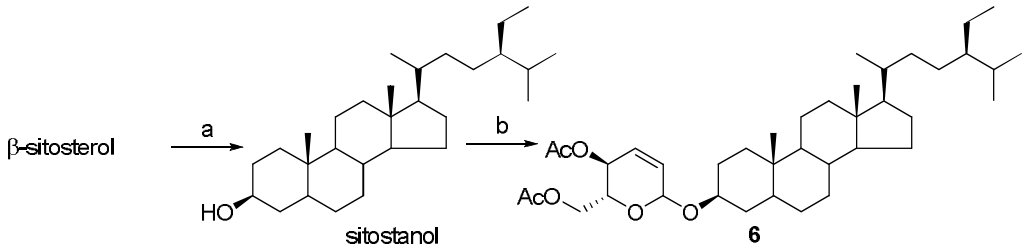


Reagents; (a) DHP, PPTS, CH_2Cl_2, 88%; (b) H_2, Pd/C, EtOAc, 82%; (c) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 82%.

Next, THP and dihydropyran moieties were introduced to β-sitosterol[28] (Scheme 2). THP derivative **3** was synthesized under acidic conditions with dihydropyran, and hydrogenation with palladium activated on carbon afforded sitostanol THP analog **4**. Dihydropyran analog **5** was synthesized with triacetyl glucal.

Sitostanol was synthesized by hydrogenation of β-sitosterol with Pd/C. The dihydropyran analog **6** was synthesized by the addition of 3,4,6-*Tri*-*O*-acetyl-*D*-glucal into the β-sitosterol solution in the presence of boron trifluoride (Scheme 3).

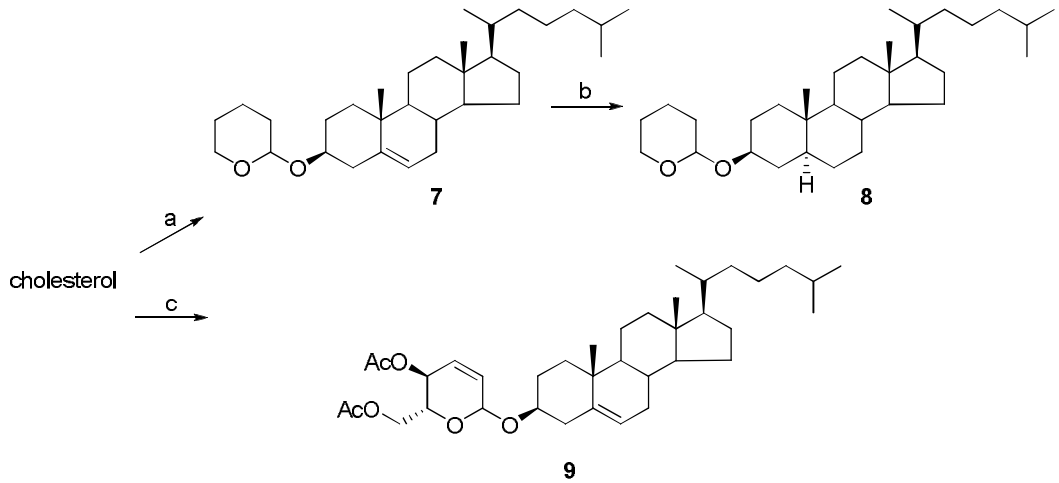
Scheme 3. Synthesis of sitostanol derivatives



Reagents; (a) H_2, Pd/C, EtOAc, 92%; (b)3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 78%.

At Scheme 4, the cholesterol derivatives **7** and **9** and cholestanol derivative **8** were produced under the same reaction conditions described above.

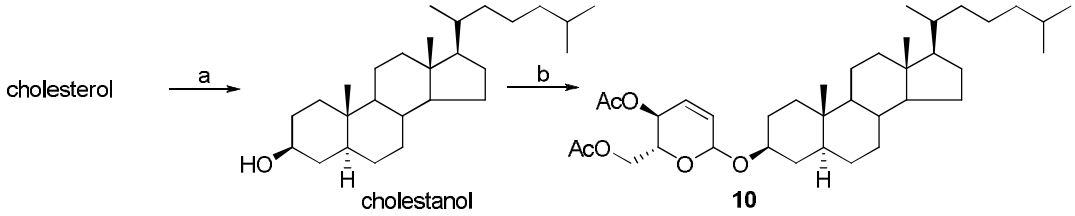
Scheme 4. Synthesis of cholesterol derivatives



Reagents; (a) DHP, PPTS, CH_2Cl_2, 68%; (b) H_2, Pd/C, EtOAc, 98%; (c) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 79%.

At Scheme 5, the synthesis of the cholestanol derivative is described. Hydrogenation of cholesterol with Pd/C afforded cholestanol. In particular, the stereochemistry at C5 of cholestanol was determined by comparison with ^{13}C-NMR data from the literature. [29] With cholestanol, dihydropyran analog **10** was synthesized.

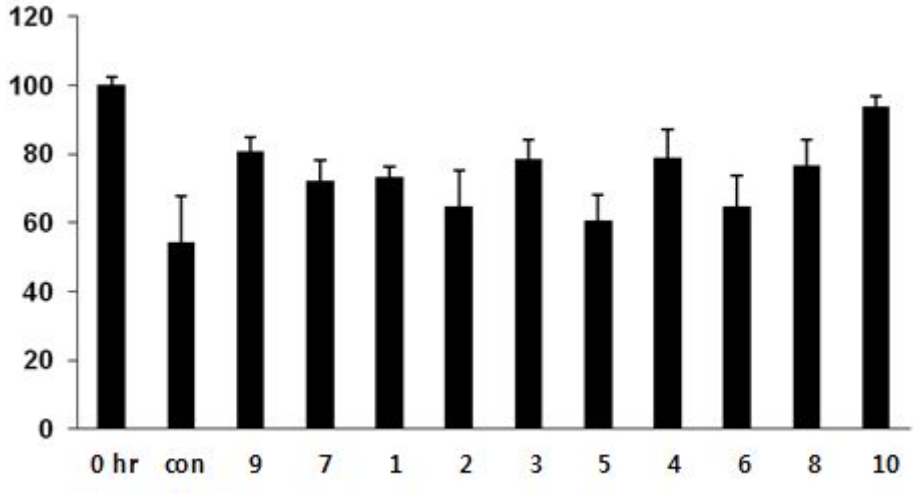
Scheme 5. Synthesis of cholestanol derivatives



Reagents; (a) H_2, Pd/C, EtOAc, 98%; (c) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 82%.

With the 10 pseudosugar analogs of phytosterols in hand, the MTT assay was executed for the anti-apoptotic activities on HUVECs. The results are shown in Figure 9.

Figure 9. MTT assay on HUVECs



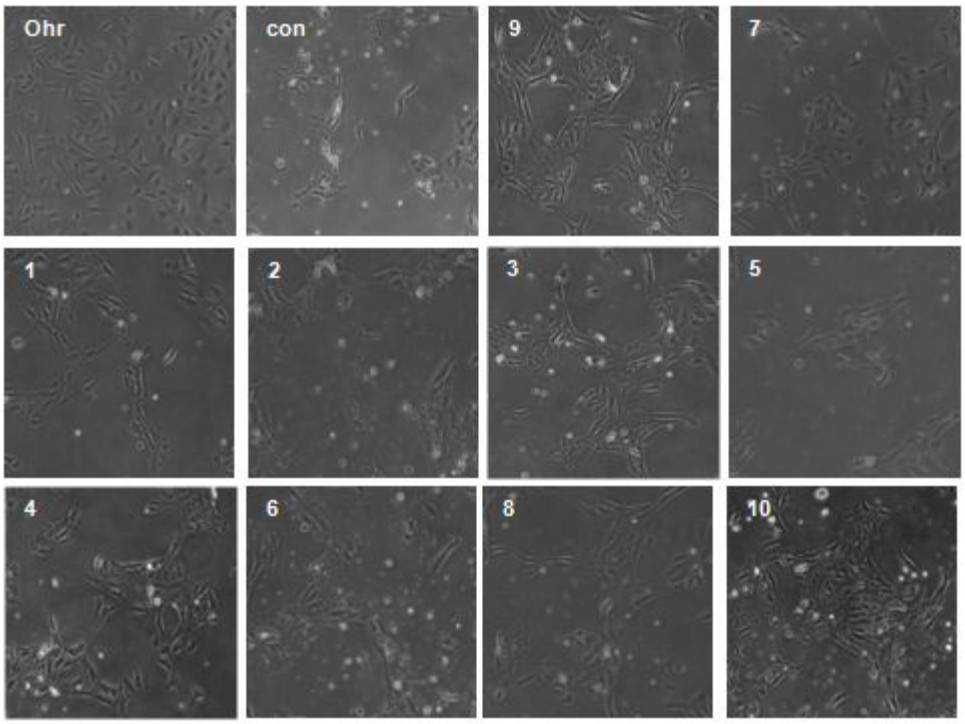
Anti-apoptotic activities of the synthetic analogs on HUVECs at 10 μg/ml treatment. 0 hr; the viability of the cells cultured in the medium without any derivatives. Con; the viability of the cells cultures in the medium containing DMSO used as a vehicle control.

In order to evaluate the effects of the compounds in HUVEC apoptosis, the given model of HUVEC apoptosis induced by deprivation of serum and growth factors[30] was used. The results of the MTT assay showed that cholesterol and cholestanol derivatives (**9**, **3**, **4**, and **10**) have anti-

apoptotic activities on ECs (Figure 9). It is suggested that the side chain at C24 affects the anti-apoptotic activity compared with stimgasterol, β-sitosterol to cholesterol. The branched side chains embedded analogs, such as stimgasterol or β-sitosterol analogs, are slightly less active.

Next, we observed the morphological changes associated with the physiological and pathological processes in HUVECs. When the cells detach from the culture dish bottom and become round, they will enter into apoptosis processes. However, if HUVECs elongate and bend into rings, they will form microvessels. [31] In our experiments, the elongated HUVECs were found with compounds **9**, **3**, **4**, and **10**. The data suggested that cholesterol or cholestanol possessing compounds might be capable of inhibiting the apoptosis

Figure 10. Morphology of HUVEC

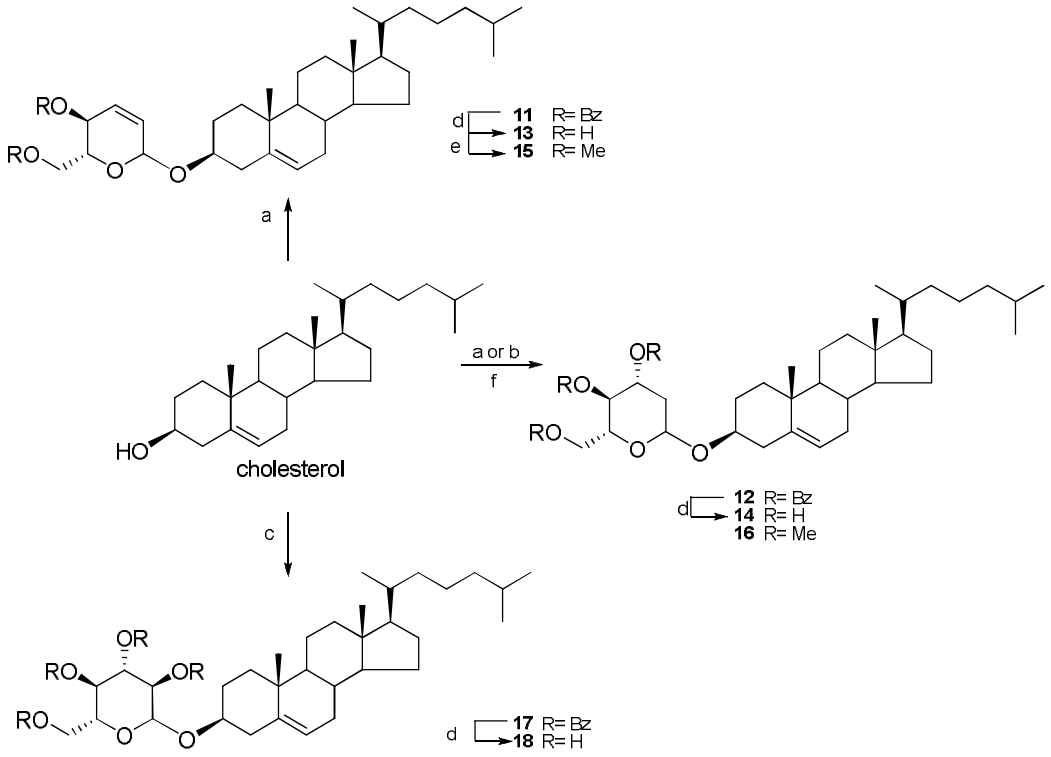


The morphological changes of HUVECs on analogs treated at 10 μg/ml for 48 hr. 0 hr, the morphologies of cells cultured in the medium without any derivatives. Con, the morphologies of cells cultures in the medium containing DMSO used as a vehicle control.

3. Synthesis of pseudosugar derivatives

Considering the previous results with ginsenosides, we had assurance about the relationship between activity and sugar moiety; thus, the studies proceeded with the pseudosugar derivatives synthesis. From the results of the phytosterol assay, we chose cholesterol as the steroidal backbone. As bioisosteres of the glucosyl residue, cyclic ether (di-or THP), acyclic ether (alkyl ether), and aryl groups were introduced to C3 OH of cholesterol. The THP derivative was synthesized already. Thus, the synthesis commenced with an introduction of dihydropyran to C3 alcohol of cholesterol as the substituents of glucosyl moiety as shown in Scheme 6.

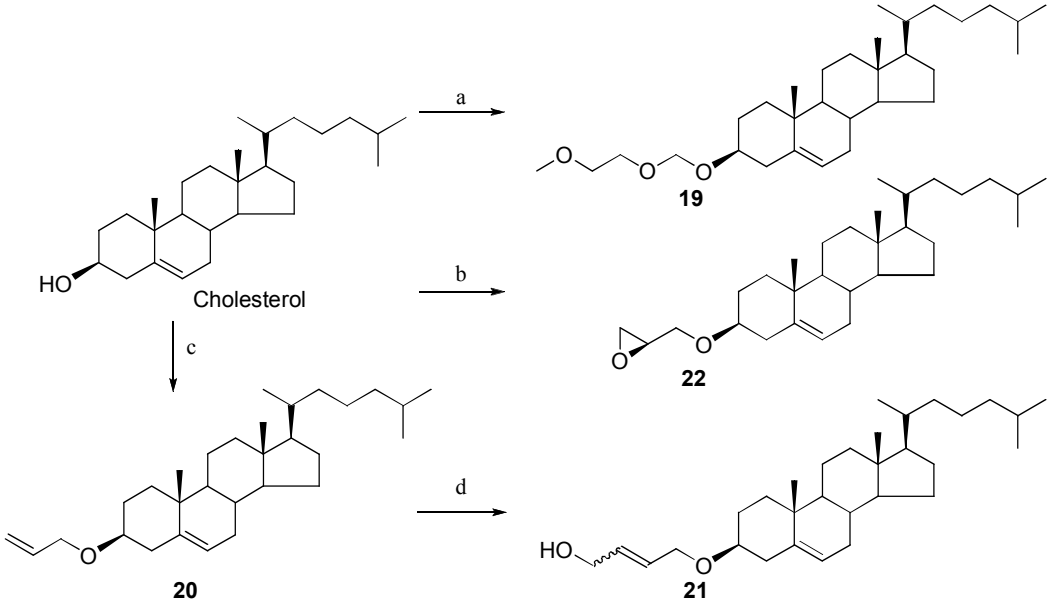
Scheme 6. Synthesis of dihydropyran derivatives



Reagents; (a) 3,4,6-*tri*-*O*-benzoyl-*D*-glucal, TsOH, CH_2Cl_2, 43%; (b) 3,4,6-*tri*-*O*-methoxy-*D*-glucal, CAN, CH_3CN, 26%; (c) 2,3,4,6-tetrabenzoyl-*D*-gluconosyl bromide, Ag_2CO_3, CH_2Cl_2, 45%; (d) NaOMe, MeOH/CH_2Cl_2, 60%; (e) MeI, NaH, THF, 0 ^oC, 32% (f) 3,4,6-*tri*-*O*-methyl-*D*-glucal, CAN, CH_3CN, 2% for **16**.

Treatment of *tri-O*-benzoyl glucal to cholesterol in acidic media was afforded to **11** and **12** mixtures, which could be separated by column chromatography. Removal of benzoyl group of **11** and **12** by using NaOMe afforded to **13** and **14**, which could be transformed to **15** and **16** by the addition of NaH and iodomethane, respectively.

Scheme 7. Synthesis of acyclic analogs



Reagents; (a) $^{i}Pr_2NEt$, CH_2Cl_2, MEMCl, 48%; (b) NaH, THF/DMF (1:1), 2*S*-(+)-glycidol tosylate, 45%; (c) NaH, THF/DMF (1:1), allyl bromide, reflux, 36%; (d) Grubbs' 2nd catalyst, 1,4-butene diol, CH_2Cl_2, reflux, 30%.

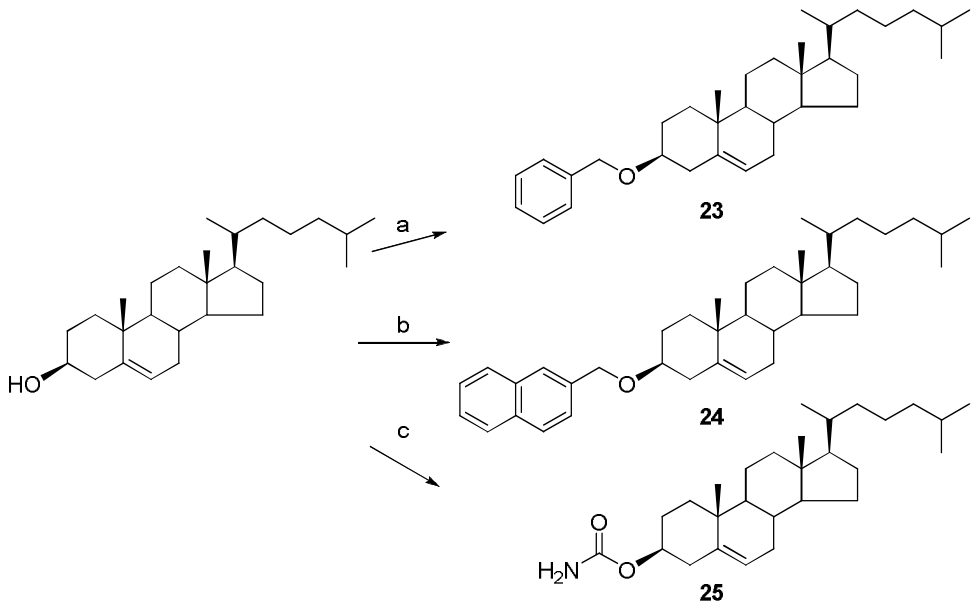
Analog **18** could be prepared by coupling cholesterol with 2,3,4,6-tetrabenzoyl-*D*-glucopyranosyl bromide in the presence of silver triflate, followed by debenzoylation as described above.

Next, we synthesized a series of cholesterol analogs linked with the acyclic or alkyl ether moieties (Scheme 7). Analog **19** was synthesized by reaction with MEMCl in the presence of Hunig's base. Allylation of cholesterol by treatment with sodium hydride and allyl bromide in refluxing

THF/DMF provided allyl ether **20**. Cross metathesis of **20** with 1,4-butene diol by employing Grubbs' 2nd generation catalyst afforded **21**. Reaction of cholesterol with sodium hydride and then with (2*S*)-glycidyl tosylate afforded **22**.

To confirm the spatial influence of glucosyl residue, aryl moieties as a carbohydrate equivalent were also introduced to the cholesterol scaffold. Analogs **23** and **24** were prepared by *O*-alkylation with benzyl bromide and bromomethyl naphthalene under reaction conditions of sodium hydride and TBAI in refluxing THF. Treatment of cholesterol with trichloroacetyl isocyanate gave carbamate **25** (Scheme 8).

Scheme 8. Synthesis of bulky analogs



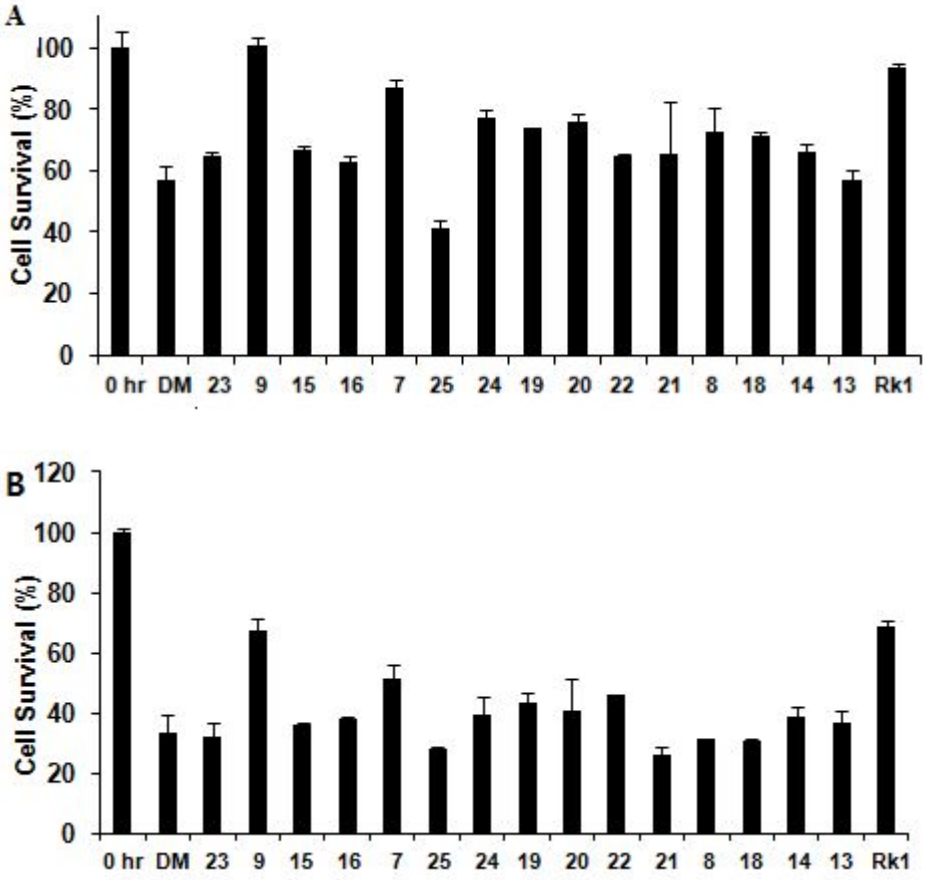
Reagents; (a) NaH, TBAI, benzyl bromide, THF, reflux, 30%; (b) NaH, TBAI, (2-bromomethyl) naphthalene, THF, reflux, 30%; (c) $Cl_3CCONCO$, $CHCl_3$, 31%.

4. Biological activity against serum deprived apoptosis

1) MTT assay on HUVECs

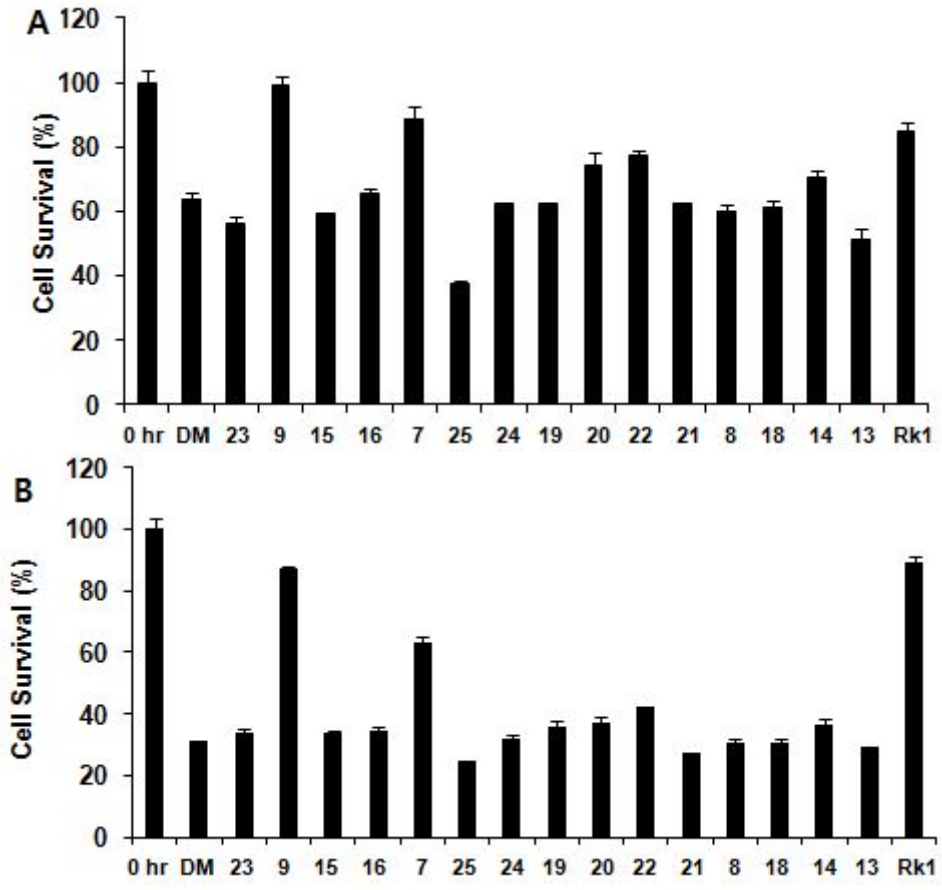
The inhibitory effects of the cholesterol analogs were investigated on the growth factor deprivation-induced HUVEC cell death using the MTT colorimetric method at concentrations of 5 μg/ml and 10 μg/ml for 24 h and 48 h, respectively. The results of the MTT assay are summarized in Figures 11 and 12.

Figure 11. Effect of compounds on apoptosis of HUVECs: 5 μg/ml treatment



(A) 5 μg/ml for 24 h treatment, (B) 5 μg/ml, 48 h treatment. 0 hr; the viability of the cells cultured in the medium without any derivatives. DM; the viability of the cells cultured in the medium containing DMSO used as a vehicle control. **Rk1**; the viability treated with ginsenoside Rk1.

Figure 12. Effect of compounds on apoptosis of HUVECs: 10 μg/ml treatment



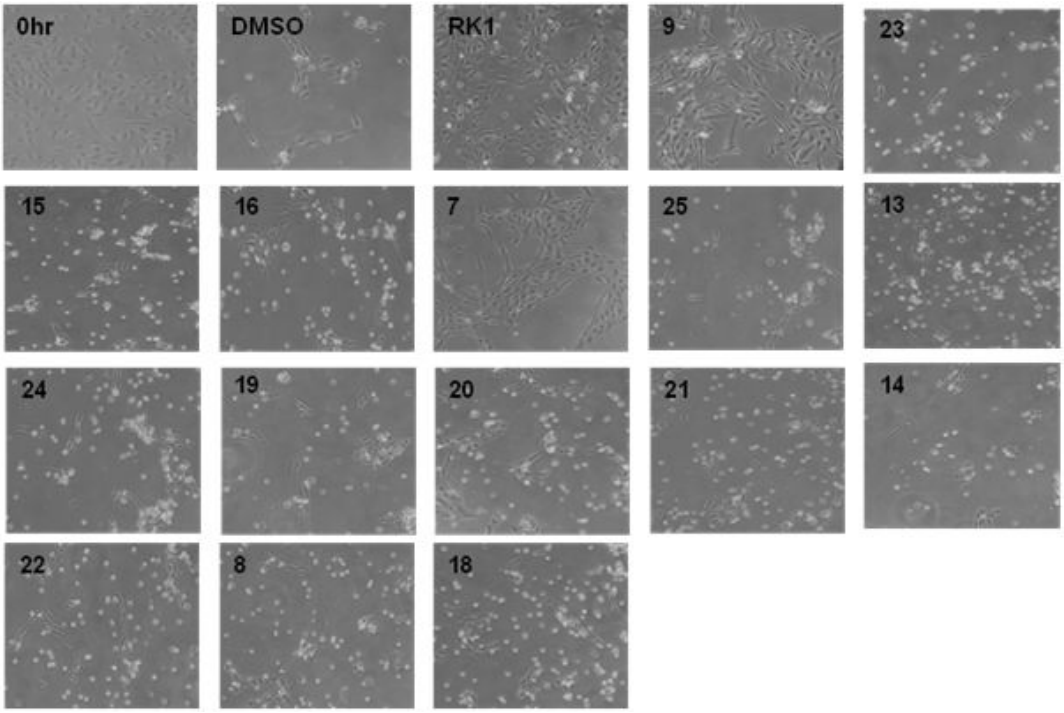
(A) 10 μg/ml for 24 h treatment, (B) 10 μg/ml for 48 h treatment. 0 h; the viability of the cells cultured in the medium without any derivatives. DM; the viability of the cells cultured in the medium containing DMSO used as a vehicle control. **Rk1**; the viability treated with ginsenoside Rk1.

It is noticeable that the acetyl dihydropyran derivative **11** was more potent than ginsenoside Rk1, which has known as one of the potent anti-apoptotic agents. In contrast, analogs **13** and **15** possessing methoxy or hydroxyl dihydropyran exhibited less potent activity compared to Rk1. This result revealed that the acyl group of the carbohydrate equivalents is important to manifest the inhibitory effect of the cholesterol analogs. The THP analog **7** also exhibited comparable activity to that of Rk1 after 24-h treatment. However, it was slightly less potent than Rk1 after 48 h

treatment. Analogs **14**, **16**, and **18** possessing the substituted pyran moiety did not show any significant inhibitory activity. Analogs **19**, **20**, **21**, and **22** possessing the acyclic carbohydrate equivalents exhibited moderate inhibitory activities at the concentration of 5 μg/ml. It is also noteworthy that the cholesteryl THP analog **7** was more potent than the dihydrocholesteryl THP analog **8**.

Next, we observed the morphological changes associated with the physiological and pathological processes in HUVECs, as described ahead (Figure 13). Treated with compounds **7** and **9, t**he elongated HUVECs were observed. The data suggested that particularly dihydro-, tetrahydrocholesterol derivatives might inhibit the apoptosis of ECs.

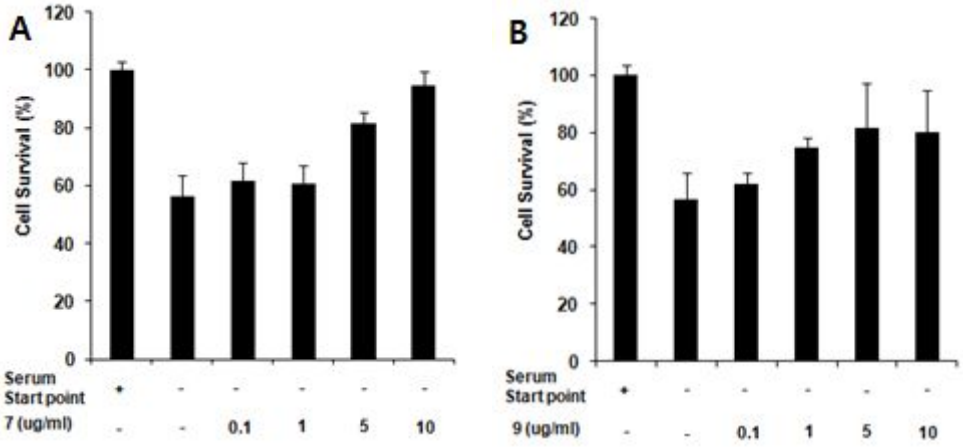
Figure 13. Morphological changes of HUVEC



The morphological changes of HUVECs on analogs treated at 10 μg/ml for 48 hr. 0 hr; the morphologies of cells cultured in the medium without any derivatives. Con; the morphologies of cells cultures in the medium containing DMSO used as a vehicle control.

For further investigation of the cell-protective effect of analogs **7** and **9**, their anti-apoptotic effect on cell viability in human retina endothelial cells (HRECs) was examined. In addition, their dose-dependent activities were confirmed by the cell survival counting assay, which was performed at 0.1 μg/ml to 10 μg/ml, respectively. The concentration-dependent cell viabilities by both analogs **7** and **9** are well revealed in Figure 14.

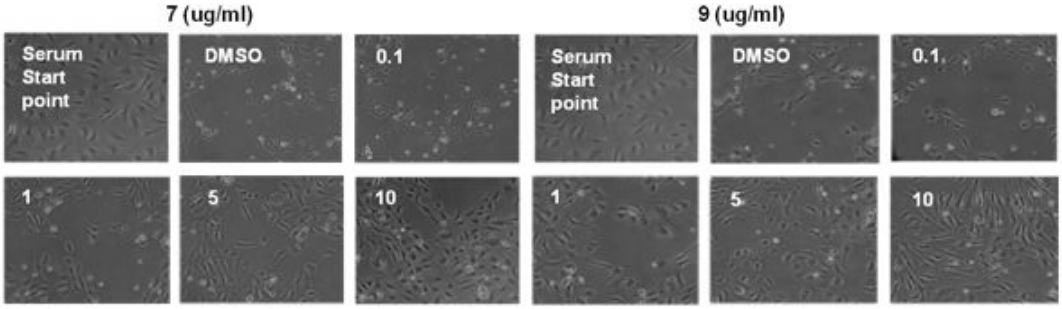
Figure 14. Inhibition of compounds 7, and 9 on apoptosis of HRECs



Inhibitory effects by analogs **7** and **9** on apoptosis of HRECs. (A) Treatment with analog **7** at sequential concentration from 0.1 μg/ml to 10 μg/ml for 48 h. (B) Treatment with analog **9** at sequential concentration from 0.1 μg/ml to 10 μg/ml for 48 h.

And the morphological changes have shown the correlation of the activities with the concentrations of the analogs **7** and **9** (Figure 15).

Figure 15. Morphological changes of HUVEC

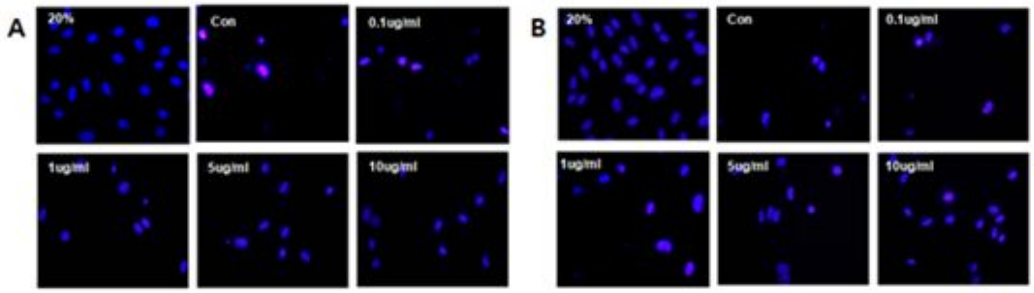


2) DAPI/ TUNEL assay

Apoptotic activity in the cytoplasmic extracts can be examined by the measurement of enzymatic caspase activity by western blots of proteins processed during apoptosis (caspases and their substrates) or by using isolated cell nuclei as indicators for apoptotic factors inducing nuclear morphological changes and/or DNA fragmentation. In 1993, a paper first described that a cell-free system can mimic characteristic features of apoptosis in intact cells. [32] Indeed, it has become clear that the apoptotic pathways acting in the cytoplasm function independently from the nucleus, and thus cell-free systems appear to be appropriate model systems that represent at least part of the apoptotic machinery and signaling mechanisms. Also, signaling pathways such as the caspase cascade have been studied under cell-free conditions, providing insight into activation patterns and inhibitor specificities. [33] On the other hand, there are cell-based assay systems. Apoptosis could be characterized biochemically by the activation of endonuclease activity that contributed to massive morphological changes in nuclei and ultimately to cell death. Endonuclease activity in individual nuclei was determined by a terminal deoxynucleotidyl transferase (TdT). 4',6'-Diamidino-2-phenylindole.2HCl (DAPI) forms a specific complex with DNA, and this fact has been used for the quantitative fluorometric estimation of DNA. [34] The fluorescent dye DAPI complexes with DNA to give a product with a fluorescence intensity about 20 times greater than that of the dye alone. Cells stained with DAPI and examined by fluorescence microscopy show nuclear DNA as well as mitochondrial and chloroplast DNA. This lack of interference from other cell components prompted us to develop a simple fluorometric assay for the DNA content of crude cellular homogenates using DAPI. [35] Among these assay systems, we tested the DAPI and terminal transferase dUTP nick end labeling (TUNEL) assay.

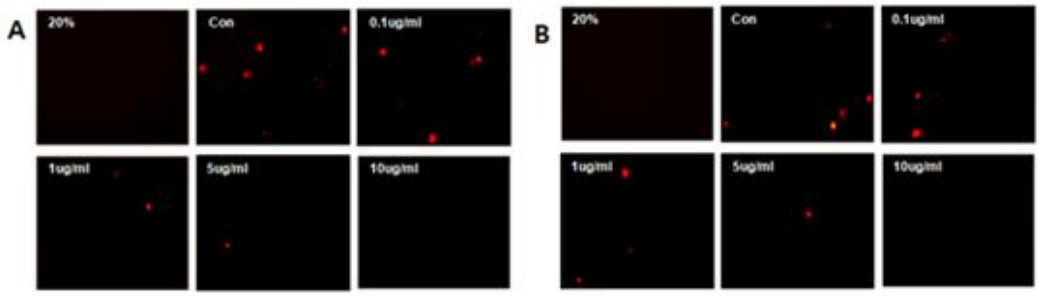
At first, morphological changes in the nuclear chromatin in cells undergoing apoptosis were detected by staining with the DNA-binding fluorochrome, DAPI. HUVECs were grown on glass coverslips and treated with analogs **7** and **9** in the presence or absence of serum for 48 h. Cells were washed twice with phosphate buffered saline (PBS) and fixed by incubation in 4% paraformaldehyde for 30 min. Following washing with PBS, cells were incubated in a DAPI solution (1 μg/ml) for 30 min in the dark. Cells were then washed with PBS and subjected to fluorescence microscopy (Figure 16).

Figure 16. Changes in nuclear morphology accessed by DAPI staining.



(A) Cells treated with **9** of various concentrations for 48 h; (B) Cells treated with **7** of various concentrations for 48 h.

Figure 17. TUNEL assays at various concentrations of 7 and 9



(A) Cells treated with **9** of various concentrations for 48 h; (B) Cells treated with **7** of various concentrations for 48 h.

Next, the TUNEL assay was executed. The TUNEL assay is a method used to detect DNA degradation in apoptotic cells because one of the hallmarks of late-stage apoptosis is the fragmentation of nuclear chromatin, which results in a multitude of 3'-hydroxyl termini of DNA ends. [36] This property can be used to identify apoptotic cells by labeling the DNA breaks with fluorescent-tagged deoxyuridine triphosphate nucleotides (F-dUTP). The addition of the deoxythymidine analog 5-bromo-2'-deoxyuridine 5'-triphosphate (BrdUTP) to the TdT reaction serves to label the break sites. Once incorporated into the DNA, BrdU can be detected by an anti-BrdU antibody using standard immunohistochemical techniques. Non-apoptotic cells do not incorporate much of the F-dUTP because of the absence of exposed 3'-hydroxyl DNA ends. This enzyme labeled the 3' OH ends of DNA for visualization with a secondary detection system using a colorimetric substrate for peroxidase. The TdT-mediated dUTP-biotin nick-end labeling (TUNEL) assay stained apoptotic nuclei red for the expression of endogenous endonucleases.

The TUNEL assay, as shown in Figure 17, specifically detecting DNA fragmentation, also revealed that analogs **7** and **9** suppressed the apoptotic cell death induced by serum deprivation in a dose-dependent manner. We have reported parts of these synthetic products and biological assay results. [37]

III. Conclusion

In conclusion, we have developed novel anti-apoptotic agents based on the ginsenoside Rk1 and Rg3, providing valuable information for the development of new anti-apoptotic agents. In particular, we replaced the ginsenosides, which are chemically unstable and difficult to be supplied sustainably, to the easily accessible phytosterol backbone. Furthermore, the analogs **7** and **9** possessing the cholesterol scaffold and the cyclic carbohydrate equivalents exhibited excellent cell survival activities, which are equipotent to that of ginsenoside Rk1. In addition, these analogs exhibited dose-dependent cell viability on the HREC line. The excellent human EC protective activities of our novel Rk1-based anti-apoptotic agents allow us to envisage their valuable application for the treatment of vascular disorders. Currently, systematic studies on their therapeutic applications, including in vivo testing of the potent sterol analogs as well as their mechanistic aspects, are in good progress. [38]

IV. Experimental

General Experimental

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane, triethylamine, acetonitrile and pyridine were freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried at 100 °C. Air and moisture sensitive reactions were performed under an argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Infrared spectra were recorded on a Perkin-Elmer 1710 FT-IR spectrometer. Mass spectra were obtained with VG Trio-2 GC-MS instrument. High-resolution mass spectra were obtained with JEOL JMS-AX 505WA instrument. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 (300 MHz), Varian Gemini 2000 (300 MHz), Brucker Analytik AVANCE digital 400 (400MHz), or AVANCE digital 500 (500MHz) spectrometer as solutions in deuteriochloroform ($CDCl_3$). Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane and are referenced to the deuterated solvent ($CHCl_3$). ^{1}H-NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad singlet; m, multiplet and/or multiple resonance), number of protons, and coupling constant in hertz (Hz).

2-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*S*,*E*)-5-Ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (1)

To a solution of stigmasterol (100 mg, 0.24 mmol) in CH_2Cl_2 (4 mL) was added dihydropyran (0.033 mL, 0.36 mmol) and PPTS (6 mg, 0.24 mmol). After stirring for 4 h, the reaction mixture was quenched with H_2O, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 82 mg (68%) of **1** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.32 (m, 1H), 5.12 (dd, 1H, *J* = 8.43 Hz, 15.0 Hz), 4.99 (dd, 1H, *J* = 8.6 Hz, 15.0 Hz), 4.69 (m, 1H), 3.88 (m, 1H), 3.47 (m, 2H), 2.34 (m, 2H), 1.97-1.06 (m, 29H), 0.98 (s, 6H), 0.80 (m, 9H), 0.67 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 140.9, 138.3, 129.1, 121.5, 96.7, 75.9, 62.8, 56.8, 51.2, 50.1, 42.1, 40.4, 39.6, 38.7, 37.4, 37.1, 36.7, 31.8, 31.2, 29.6, 28.8, 27.9, 25.4, 25.3, 24.3, 21.1, 21.0, 20.06, 21.01, 19.9, 19.3, 18.9, 12.2, 12.0; LRMS (FAB+) *m/z* 497(M+H).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*S*,*E*)-5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (2)

To a solution of 3,4,6-triacetyl glucal (198 mg, 0.7 mmol) in THF (4 mL) was added stigmasterol (100 mg, 0.24 mmol) and $BF_3{\cdot}OEt_2$ (0.09 ml, 0.7 mmol). After stirring for 18 h, the reaction mixture was quenched with saturated NH_4Cl and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 119 mg (79%) of **2** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.81 (m, 2H), 5.30 (m, 2H), 5.16 (br, 1H), 5.10 (d, 1H, *J* = 8.4 Hz), 4.98 (dd, 1H, *J* = 8.4 Hz, 15.0 Hz), 4.17 (m, 3H), 3.53 (m, 1H), 2.35

(m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 2.02-1.81 (m, 6H), 1.68 (m, 1H), 1.53 (m, 9H), 1.25-0.75 (m, 22H), 0.67 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 170.7, 170.2, 140.6, 138.2, 129.2, 128.8, 128.0, 121.7, 92.7, 76.5, 66.7, 65.3, 63.1, 56.7, 55.8, 51.1, 50.1, 42.1, 40.4, 40.3, 39.5, 37.0, 36.6, 31.8, 28.8, 28.1, 25.3, 24.2, 21.1, 21.0, 20.9, 20.9, 20.7, 19.2, 18.9, 12.2, 11.9; LR-MS (FAB+) *m/z* 647 (M+Na).

2-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (3)

To a solution of β-sitosterol (200 mg, 0.48 mmol) in CH_2Cl_2 (6 mL) was added dihydropyran (0.07 mL, 0.72 mmol) and PPTS (12 mg, 0.05 mmol). After stirring for 2 h, the reaction mixture was quenched with H_2O, and then diluted with CH_2Cl_2. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 164 mg (68%) of **3** as a white solid: FT-IR (neat) ν_{max} 2921, 1460, 1112 cm^{-1}; ^{1}H -NMR ($CDCl_3$, 300 MHz) δ 5.32 (br, 1H), 4.69 (br, 1H), 3.89 (m, 1H), 3.47 (m, 2H), 2.34 (m, 2H), 2.00-1.00 (m, 33H), 0.98 (s, 3H), 0.89 (d, 3H, *J* = 6.26 Hz), 0.83 (q, 9H, *J* = 7.14 Hz), 0.65 (s, 3H); ^{13}C -NMR ($CDCl_3$, 75 MHz) δ 140.9, 121.5, 96.8, 75.9, 62.7, 56.7, 56.0, 50.1, 45.7, 42.2, 40.2, 39.7, 38.7, 37.4, 47.1, 36.7, 36.0, 33.8, 31.8, 31.2, 29.6, 29.0, 28.2, 25.9, 25.4, 24.2, 23.0, 21.0, 20.0, 19.7, 19.3, 18.9, 18.7, 11.9; LRMS (FAB+) *m/z* 499 (M+H).

2-((3*S*,10*S*,13*R*,17*R*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (4)

To a solution of **3 (**68 mg**,** 0.14 mmol) in EtOAc (4 mL) was added 10% activated palladium carbon. The resulting mixture was hydrogenated for 24 h and then filtered through a pad of Celite. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 :

30) afforded 56 mg (82 %) of **4** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 4.68 (s, 1H), 3.89 (m, 1H), 3.50 (m, 2H), 1.94-0.77 (m, 53H), 0.61 (s, 3H); LRMS (FAB+) *m/z* 523 (M+Na).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*R*)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (5)

To a solution of 3,4,6-triacetyl glucal (984 mg, 3.62 mmol) in THF (6 mL) was added β-sitosterol (500 mg, 1.21 mmol) and $BF_3 \cdot OEt_2$ (0.45 ml, 3.62 mmol). After stirring for 2 h, the reaction mixture was quenched with saturated $NaHCO_3$ and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 7) afforded 620 mg (82%) of **5** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.81 (m, 2H), 5.32 (d, 1H, *J* = 4.77 Hz), 5.25 (d, 1H, *J* = 9.33 Hz), 5.14 (s, 1H), 4.16 (m, 3H), 3.51 (m, 1H), 2.34 (m, 2H), 2.06 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.00-1.81 (m, 5H), 1.74-1.00 (m, 22H), 0.97 (s, 3H), 0.88 (d, 3H, *J* = 6.24 Hz), 0.80 (q, 6H, *J* = 6.96 Hz), 0.64 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 170.4, 169.9, 140.5, 128.6, 128.2, 121.6, 92.5, 77.9, 66.6, 65.1, 62.9, 56.2, 55.8, 49.9, 45.5, 42.1, 40.2, 39.5, 36.9, 36.4, 35.9, 33.7, 31.7, 31.6, 28.9, 28.0, 25.8, 24.1, 22.8, 20.8, 20.7, 20.6, 19.6, 19.1, 18.8, 18.6, 18.5, 12.0, 11.8; LRMS (FAB+) *m/z* 649 (M+Na).

(3*S*,10*S*,13*R*,17*R*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-ol (Sitostanol)

To a solution of β-sitosterol (225 mg**,** 0.54 mmol) in EtOAc (8 mL) was added 10% activated palladium carbon. The resulting mixture was hydrogenated for 2 h and then filtered through a pad of Celite. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 209 mg (92 %) of **sitostanol** as a white solid: FT-IR (neat) ν_{max} 3350,

1464, 1029 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.55 (m, 1H), 1.93 (d, 1H, *J* = 12.09 Hz), 1.82-0.72 (m, 45 H), 0.58 (s, 3H); LRMS (FAB+) *m/z* 417 (M+H).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,10*S*,13*R*,17*R*)-17-((2*R*,5*R*)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (6)

To a solution of 3,4,6-triacetyl glucal (275 mg, 1.01 mmol) in THF (5 mL) was added sitostanol (140 mg, 0.337 mmol) and $BF_3 \cdot OEt_2$ (0.125 ml, 1.01 mmol). After stirring for 4 h, the reaction mixture was quenched with water and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 165 mg (78%) of **6** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.80 (m, 2H), 5.26 (d, 1H, *J* = 9.15 Hz), 5.14 (s, 1H), 4.16 (m, 3H), 3.59 (m, 1H), 2.06 (s, 3H), 2.04 (s, 3H), 1.93 (m, 1H), 1.82-0.77 (m, 46H), 0.61 (s, 3H); ^{13}C -NMR ($CDCl_3$, 75 MHz) δ 170.6, 170.2, 128.7, 128.6, 92.9, 77.8, 66.6, 65.2, 63.1, 56.3, 56.0, 54.2, 45.7, 45.0, 42.5, 39.9, 36.8, 36.2, 26.0, 35.4, 33.8, 32.0, 29.0, 28.8, 28.1, 28.0, 25.9, 24.1, 22.9, 21.1, 20.8, 20.7, 19.7, 18.9, 18.5, 12.1, 11.9, 11.8; LRMS (FAB+) *m/z* 651 (M+Na).

2-((3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (7)

To a solution of cholesterol (100 mg, 0.26 mmol) in CH_2Cl_2 (1.5 mL) was added dihydropyran (0.18 mL, 1.97 mmol) and PTSA (12 mg, 0.07 mmol). After stirring for 4 h, the reaction mixture was quenched with H_2O, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash

column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 82 mg (68%) of **7** as a white solid: FT-IR (neat) ν_{max} 2921, 1460, 1112 cm^{-1}; ^1H -NMR ($CDCl_3$, 300 MHz) δ 5.32 (br, 1H), 4.69 (br, 1H), 3.88 (m, 1H), 3.54-3.43 (m, 2H), 2.34-2.28 (m, 2H), 2.00-1.66 (m, 7H), 1.59-1.00 (m, 21H), 0.98 (s, H), 0.86 (ddd, 9H, *J* = 15.3 Hz, 6.4 Hz, 1.2 Hz), 0.65 (s, 3H); LRMS (FAB+) *m/z* 471 (M+H).

2-((3*S*,10*S*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (8)

To a solution of **7 (**40 mg**,** 0.01 mmol) in EtOAc (3 mL) was added 10% activated palladium carbon. The resulting mixture was hydrogenated for 1 h and then filtered through a pad of Celite. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 37 mg (78 %) of **8** as a white solid: FT-IR (neat) ν_{max} 2930, 1738, 1464, 1029 cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz) δ 4.69 (m, 1H), 3.89 (m, 1H), 3.60-3.41 (m, 2H), 1.92 (m, 1H), 1.82-1.39 (m, 13H), 1.35-0.94 (m, 15H), 0.85 (ddd, 9H, *J* = 10.5 Hz, 6.6 Hz, 1.3 Hz), 0.77 (s, 3H), 0.62 (s, 3H); LRMS (FAB+) *m/z* 473 (M+H).

((2R,3S)-3-Acetoxy-6-((3S,10R,13R,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (9)

To a solution of 3,4,6-triacetyl glucal (2.0 g, 7.5 mmol) in ether (20 mL) was added cholesterol (1.0 g, 2.5 mmol) and $BF_3 \cdot OEt_2$ (1.3 ml, 10 mmol). After stirring for 18 h, the reaction mixture was quenched with a saturated $NaHCO_3$ and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 660 mg (43%) of **9** as a white solid: ^1H-NMR ($CDCl_3$, 300 MHz) δ 5.83 (m, 2H), 5.33 (br, 1H), 5.27 (dd,

1H, *J* = 9.15 Hz, 1.47 Hz), 5.14 (br, 1H), 4.17 (m, 3H), 3.53 (m, 1H), 2.37 (m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 1.99-1.01 (m, 26H), 0.97 (s, 3H), 0.84 (dd, 9H, *J* = 6.6 Hz, 14.3 Hz), 0.65 (s, 3H); FT-IR (neat) ν_{max} 2938, 1464, 1377, 1261, 1199, 1024 cm^{-1}; LRMS (FAB+) *m/z* 621 (M+Na).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,5*S*,10*S*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)hexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (10)

To a solution of 3,4,6-triacetyl glucal (95 mg, 0.35 mmol) in THF (2 mL) was added cholestanol (45 mg, 0.116 mmol) and $BF_3 \cdot OEt_2$ (0.043 ml, 0.35 mmol). After stirring for 18 h, the reaction mixture was quenched with a saturated $NaHCO_3$ and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 57 mg (82%) of **10** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.82 (m, 2H), 5.26 (d, 1H, *J* = 9.15 Hz), 5.14, (s, 1H), 4.18 (m, 3H), 3.60 (m, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 1.93 (d, 1H, *J* = 12.09 Hz), 1.78-0.93 (m, 30H), 0.85 (dd, 9H, *J* = 6.5 Hz, 10.08 Hz), 0.77 (s, 3H), 0.61 (s, 3H); LRMS (FAB+) *m/z* 623 (M+Na).

((2*R*,3*S*)-3-(Benzoyloxy)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl benzoate (11)

To a solution of 3,4,6-tribenzoyl glucal (320 mg, 0.7 mmol) in CH_2Cl_2 (10 mL) was added cholesterol (108 mg, 0.28 mmol) and PTSA (11 mg, 0.06 mmol). After stirring for 18 h, the reaction mixture was quenched with water and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 15) afforded 33 mg

(23%) of **11** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02-8.00 (m, 4H), 7.57-7.32 (m, 6H), 6.04 (d, 1H, *J* = 10.2 Hz), 5.87 (dd, 1H, *J* = 10.2 Hz, 2.0 Hz), 5.61 (d, 1H, *J* = 8.9 Hz), 5.22 (br, 1H), 5.11 (br, 1H), 4.58-4.38 (m, 4H), 3.59 (m, 1H), 3.65 (dd, 1H, *J* = 11.0 Hz, 7.9 Hz), 2.37-2.33 (m, 2H), 2.02-0.83 (m, 38H), 0.65 (s, 3H); LRMS (FAB+) *m/z* 745 (M+Na).

(2*R*,3*S*,4*R*)-2-(Benzoyloxymethyl)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran-3,4-diyl dibenzoate (12)

To a solution of 3,4,6-tribenzoyl glucal (320 mg, 0.7 mmol) in CH_2Cl_2 (10 mL) was added cholesterol (108 mg, 0.28 mmol) and PTSA (11 mg, 0.06 mmol). After stirring for 18 h, the reaction mixture was quenched with water and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 15) afforded 60 mg (26%) of **12** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02-7.89 (m, 6H), 7.58-7.32 (m, 9H), 5.47 (m, 2H), 5.30 (br, 1H), 5.19 (br, 1H), 4.87 (m, 1H), 4.53-4.47 (m, 3H), 3.97 (m, 1H), 3.55 (m, 1H), 2.52-2.15 (m, 3H), 2.02-0.83 (m, 20H), 0.65 (s, 3H); LRMS (FAB+) *m/z* 867 (M+Na); HRMS (FAB+) calcd for $C_{54}H_{68}O_8Na$ (M+Na): 867.4812, found 867.4816.

(2*R*,3*S*)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-Dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15, 16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-2-(hydroxymethyl)-3,6-dihydro-2*H*-pyran-3-ol (13)

To a refluxing solution of sodium (2.5 mg, 0.1 mmol) in MeOH (2 mL) was added a solution of the **11** (33 mg, 0.46 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 1.2 h, cooled to ambient temperature, and then quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with ether and the organic phase was washed with brine, dried over $MgSO_4$ and

concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes : MeOH = 1 : 1 : 0.05) afforded 14 mg (60%) of **13** as a white solid: FT-IR (neat) ν_{max} 3463, 2933, 1461, 1049 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.95 (d, 1H, *J* = 10.2 Hz), 5.75 (dt, 1H, *J* = 10.2 Hz, 2.2 Hz), 5.35 (br, 1H), 5.13 (br, 1H), 4.21 (br, 1H), 3.87 (br, 2H), 3.79-3.73 (m, 1H), 3.57-3.48 (m, 1H), 2.36 (br, 2H), 2.02-1.80 (m, 8H), 0.99 (s, 6H), 0.88 (ddd, 12H, *J* = 18.7 Hz, 6.6 Hz, 1.7 Hz), 0.67 (s, 3H); LRMS (FAB+) *m/z* 537 (M+Na).

(2*R*,3*S*,4*R*)-6-((3*S*,10*R*,13*R*,17*R*)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-1*H*-cyclopenta[α] phenanthren-3-yloxy)-tetrahydro-2-(hydroxymethyl)-2*H*-pyran-3,4-diol (14)

To a refluxing solution of sodium (3 mg, 0.12 mmol) in MeOH (3 mL) was slowly added a solution of **12** (57 mg, 0.067 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred for 1.2 h, cooled to ambient temperature, and quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with ether and the organic phase was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes : MeOH = 2 : 1 : 0.05) afforded 30 mg (84%) of **14** as a white solid: FT-IR (neat) ν_{max} 3394, 2929, 1461, 1070 cm^{-1}; ^{1}H -NMR ($CDCl_3$, 300 MHz) δ 5.32 (br, 1H), 5.05 (d, 0.6H, *J* = 2.1 Hz), 4.67 (d, 0.4H, *J* = 9.3 Hz), 3.98 (br, 0.6H), 3.85-3.81 (m, 2H), 3.66 (m, 1H), 3.55-3.38 (m, 2H), 3.25 (br, 0.4H), 2.31-2.07 (m, 4H), 1.99-1.78 (m, 5H), 1.62-0.99 (m, 20H), 0.97 (s, 6H), 0.86 (ddd, 12H, *J* = 19.5 Hz, 5.2 Hz, 1.8 Hz), 0.64 (s, 3H); LRMS (FAB+) *m/z* 555 (M+Na).

(2*R*,3*S*)-3-Methoxy-2-(methoxymethyl)-6-((3*S*)-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16, 17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran (15)

To a suspension of NaH (33.8 mg, 0.85 mmol) in THF (1 mL) was added a solution of **13** (87 mg,

0.17 mmol) in THF (3 mL) at 0 ºC. After stirring for 1 h, iodomethane (0.11 mL, 1.69 mmol) was slowly added to the reaction mixture. After stirring for an additional 16 h, the reaction mixture was quenched with water, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 30 mg (32%) of **15** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.03 (d, 1H, *J* = 10.23 Hz), 5.73 (d, 1H, *J* = 11.19 Hz), 5.32 (br, 1H), 5.12 (d, 1H, *J* = 2.55 Hz), 3.87 (br, 2H), 3.59 (m, 3H), 3.40 (s, 3H), 3.37 (s, 3H), 2.33 (m, 2H), 2.01-1.71 (m, 5H), 1.55-1.09 (m, 17H), 0.96 (s, 3H), 0.95-0.82 (m, 12H), 0.64 (s, 3H); LRMS (FAB+) *m/z* 565(M+Na).

(2*R*,3*S*,4*R*)-3,4-Dimethoxy-2-(methoxymethyl)-6-((3*S*,17*R*)-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (16)

A glycal (288 mg, 1.53 mmol) and cholesterol (592 mg, 1.53 mmol) were combined with a catalytic amount (5 mol%) of CAN (41 mg) in anhydrous CH_3CN (6 ml) and stirred for 12 h. The reaction mixture was extracted with ether and washed with $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated in a vacuum. The residue was purified by column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 20 mg (2.3%) of **16** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.27 (s, 2H), 5.05 (s, 1H), 3.63 (m, 2H), 3.52 (s, 3H), 3.42 (s, 3H), 3.40 (m, 1H), 3.38 (s, 3H), 3.16 (m, 1H), 2.20 (m, 2H), 1.95-0.99 (m, 24H), 0.97 (s, 3H), 0.85 (dd, 12H, *J* = 6.4 Hz, 13.7 Hz), 0.64 (s, 3H); LRMS (FAB+) *m/z* 597 (M+Na).

(2*R*,3*R*,4*S*,5*R*)-2-(Benzoyloxymethyl)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yloxy)tetrahydro-2*H*-pyran-3,4,5-triyl tribenzoate (17)

To a solution of cholesterol (150 mg, 0.39 mmol) in CH_2Cl_2 (5 mL) was added 4A molecular sieve (500 mg), 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (244 mg, 0.37 mmol) and a solution of silver triflate (105 mg, 0.41 mmol) in dry toluene (1 mL) at -20 °C. After stirring for 2 h, the reaction mixture was filtered through a pad of silica gel. The filtrate was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 140 mg (45%) of **17** as a pale yellow oil: FT-IR (neat) ν_{max} 2929, 1501, 1226 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.99-7.79 (m, 8H), 7.54-7.7.26 (m, 12H), 5.87 (t, 1H, *J* = 9.7 Hz), 5.60 (t, 1H, *J* = 9.7 Hz), 5.47 (dd, 1H, *J* = 9.7 Hz, 7.9 Hz), 5.20 (d, 1H, *J* = 4.7 Hz), 4.91 (d, 1H, *J* = 7. 9 Hz), 4.60-4.46 (m, 2H), 4.12 (m, 1H), 3.50 (m, 1H), 2.15 (br, 2H), 2.01-1.65 (m, 6H), 1.56-0.71 (m, 21H), 0.63 (s, 3H) ; LRMS (FAB+) *m/z* 867 (M+Na).

(3*R*,4*S*,5*S*,6*R*)-2-((3*S*,10*R*,13*R*,17*R*)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-1*H*-cyclopenta[α] phenanthren-3-yloxy)-tetrahydro-6-(hydroxymethyl)-2*H*-pyran-3,4,5-triol (18)

To a refluxing solution of sodium (4 mg, 0.16 mmol) in MeOH (5 mL) was slowly added a solution of **17** (90 mg, 0.11 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 0.5 h, cooled to ambient temperature, and quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with ether and the organic phase was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (CH_2Cl_2 : MeOH = 7 : 1) afforded 22 mg (36%) of **18** as a white solid: FT-IR (neat) ν_{max} 3428, 2936, 1632, 1377, 1025 cm^{-1}; ^{1}H-NMR (Pyridine *d*-5, 300 MHz) δ 5.33 (br, 1H), 5.06 (d, 1H, *J* = 7.7 Hz), 4.57 (d, 1H, *J* = 9.5 Hz), 4.41 (dd, 1H, *J* = 11.7, 4.9 Hz), 4.29 (m, 2H), 4.09-3.91 (m, 3H), 2.72 (d, 1H, *J* = 13.0 Hz), 2.46 (m, 1H), 2.10-1.68 (m, 6H), 1.55-0.89 (m, 33H), 0.63 (s, 3H); LRMS (FAB+) *m/z* 571 (M+Na); HRMS (FAB+) calcd for $C_{33}H_{56}O_6Na$(M+Na): 571.3975, found

571.3974.

(3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-3-[(2-methoxyethoxy)methoxy]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (19)

To a solution of cholesterol (97 mg, 0.25 mmol) in CH_2Cl_2 (1 mL) was added diisopropylethylamine (0.09 mL, 0.5 mmol). After stirring for 1 h, methoxyethoxymethyl chloride (0.3 mL, 2.5 mmol) was added at 0 °C. After stirring for an additional 1 h, the reaction mixture was quenched with saturated aqueous NH_4Cl, and then diluted with EtOAc. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 57 mg (48%) of **19** as a white solid: FT-IR (neat) ν_{max} 2935, 1463, 1375, 1112 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.31 (d, 1H, *J* = 5.3 Hz), 4.75 (s, 2H), 3.70-3.67 (m, 2H), 3.55-3.52 (m, 2H), 3.42 (m, 1H), 3.37 (s, 3H), 2.31-2.26 (m, 2H), 2.00-1.77 (m, 5H), 1.59-1.01 (m, 20H), 0.97 (s, 3H), 0.85 (ddd, 9H, *J* = 14.7, 6.6, 1.3 Hz) 0.65 (s, 3H); LRMS (FAB+) *m/z* 497 (M+ Na).

(3*S*,10*R*,13*R*,17*R*)-3-(Allyloxy)-17-[(1*R*)-1,5-dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16, 17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (20)

To a suspension of NaH (41 mg, 1.03 mmol) in DMF/THF (1: 1, 1 mL) was added a solution of cholesterol (200 mg. 0.52 mmol) in a mixture of DMF/THF (2 mL). After stirring for 1 h, allyl bromide (0.22 mL, 2.59 mmol) was slowly added to the reaction mixture. After stirring for an additional 16 h, the reaction mixture was quenched with water, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 80 mg (36%) of **20** as a colorless oil: FT-IR (neat) ν_{max} 2929, 1462, 1375, 1094 cm^{-1};

^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.87 (m, 1H), 5.32 (d, 1H, *J* = 5.3 Hz), 5.28-5.11 (m, 2H), 4.00 (dt, 2H, *J* = 5.7 Hz, 1.3 Hz), 3.22-3.15 (m, 1H), 2.37-2.20 (m, 2H), 2.01-0.80 (m, H), 0.65 (s, 3H); LRMS (FAB+) *m/z* 426 (M+).

4-((3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-2-buten-1-ol (21)

To a solution of **20** (38 mg, 0.09 mmol) in CH_2Cl_2 (1 mL) was added the 2nd generation Grubbs' catalyst (4 mg, 0.01 mmol) and 1,4-butene diol (0.2 mL). After stirring for 1 h, the mixture was concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 12 mg (30 %) of **21** as a colorless oil: FT-IR (neat) ν_{max} 3394, 2932, 1735, 1464 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.91-5.75 (m, 2H), 5.32 (d, 1H, *J* = 5.1 Hz), 4.14 (d, 2H, *J* = 8.4 Hz), 4.00 (d, 2H, *J* = 5.1 Hz), 3.18 (m, 1H), 2.36-2.15 (m, 2H), 2.00-1.74 (m, 5H), 1.53-1.00 (m, 21H), 0.97 (s, 3H), 0.86 (ddd, 9H, *J* = 14.8 Hz, 6.6 Hz, 1.1 Hz), 0.65 (s, 3H) ; LRMS (FAB+) *m/z* 456 (M+).

(2*R*)-2-[((3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)methyl]oxirane (22)

To a suspension of NaH **(**60% dispersion on mineral oil, 42 mg, 1.1 mmol) in a mixture of DMF/THF (2 mL) was slowly added a solution of cholesterol (200 mg, 0.52 mmol) in a mixture of DMF/THF (1/1 mL). After stirring for 30 minute, a solution of 2S-(+)-glycidyl tosylate in DMF/THF (0.5/0.5 mL) was slowly added. The reaction mixture was vigorously stirred for 26 h at ambient temperature, quenched with saturated aqueous NH_4Cl, and then diluted with ether. The organic phase was washed with 10% aqueous NaOH and brine, dried over $MgSO_4$ and

concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 101 mg (45%) of **22** as a white solid: FT-IR (neat) ν_{max} 2938, 1465, 1107cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.32 (d, 1H, *J* = 5.3 Hz), 3.70 (dd, 1H, *J* = 11.3 Hz, 3.3 Hz), 3.43 (dd, 1H, *J* = 11.3 Hz, 5.7 Hz), 3.25-3.09 (m, 2H), 2.78 (t, 1H, *J* = 4.9 Hz), 2.59 (dd, 1H, *J* = 4.9 Hz, 2.7 Hz), 2.38-2.12 (m, 2H), 2.02- 1.80 (m, 5H), 1.53-1.00 (m, 21H), 0.97 (s, 3H), 0.85 (ddd, 9H, *J* = 15.3 Hz, 6.6 Hz, 1.3 Hz), 0.64 (s, 3H); LRMS (FAB+) *m/z* 441 (M+H).

(3*S*,17*R*)-3-(Benzyloxy)-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (23)

To a suspension of NaH (42 mg, 1.04 mmol) in THF (1 mL) was added a solution of cholesterol (100 mg, 0.26 mmol) in THF (3 mL) at 0 ºC. After stirring for 0.5 h, benzyl bromide (0.12 mL, 1.04 mmol) and tetrabutyl ammonium iodide (14 mg, 0.04 mmol) was added to the reaction mixture. The resulting mixture was refluxed for 16 h and then quenched with a saturated solution of NH_4Cl, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 30) afforded 45 mg (32%) of **23** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.85-6.74 (m, 5H), 4.87-4.83 (m, 3H), 4.04 (s, 2H), 2.75 (m, 1H), 1.93 (m, 1H), 1.75 (m, 1H), 1.55-1.32 (m, 5H), 1.07-0.37 (m, 34H), 0.20 (s, 3H); LRMS (FAB+) *m/z* 499 (M+Na).

(3*S*,17*R*)-17-((*R*)-6-Methylheptan-2-yl)-3-(naphthalen-2-ylmethoxy)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (24)

To a suspension of NaH (42 mg, 1.04 mmol) in THF (1 mL) was added a solution of cholesterol (100 mg, 0.26 mmol) in THF (3 mL) at 0 ºC. After stirring for 0.5 h, 2-bromomethyl naphthalene (69 mg, 0.31 mmol) and tetrabutyl ammonium iodide (29 mg, 0.08 mmol) was added to the

reaction mixture. After stirred for 4 h and then quenched with water, and then diluted with ether. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 15) afforded 13 mg (10%) of **24** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.79 (m, 4H), 7.45 (m, 3H), 5.33 (br, 1H), 4.70 (s, 2H), 3.31 (m, 1H), 2.46-2.26 (m, 2H), 2.00-1.74 (m, 5H), 1.61-1.04 (m, 24H), 1.00 (s, 3H), 0.85 (dd, 9H, *J* = 6.42 Hz, 13.9 Hz), 0.65 (s, 3H); LRMS (FAB+) *m/z* 549 (M+Na).

(3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yl carbamate (25)

To a solution of cholesterol (20 mg, 0.05 mmol) in chloroform (1 mL) was added tricholoroacetyl isocyanate (0.05 mL, 0.42 mmol). After stirring for 2 h, the reaction mixture was diluted with chloroform, followed by filtration through a pad of aluminium oxide. The filtrate was concentrated in vacuo to afforded 7 mg (31%) of **25** as a white solid: FT-IR (neat) ν_{max} 3458, 2932, 1704 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.35 (br, 1H), 4.49-4.43 (m, 3H), 2.33-2.22 (m, 2H), 1.97-1.76 (m, 6H), 1.55-0.98 (m, 22H), 0.94 (s, 3H), 0.82 (ddd, 9H, *J* = 15.3, 6.6, 1.2 Hz), 0.60 (s, 3H); LRMS (FAB+) *m/z* 452 (M+Na).

V. References

1 Yoshimoto, M.; Yoder, M. C. *Nature* **2009**, *457*, 801-803.

2 (a) Lemichez, E.; Lecuit, M.; Nassif, X.; Bourdoulous, S. *Nature Rev. Microbiol.***2010**, *8*, 93-104. (b) Matter, K.; Balda, M.S. *Nature Rev. Mol. Cell. Biol.* **2003***, 4*, 225-236.

3 (a) Bazzoni, G. *Thromb Haemost*, **2006**, *95*, 36-42. (b) Bazzoni, G.; Dejana, E. *Physiol. Rev.* **2004**, *84*, 869-901.

4 (a) Gerber, H.-P.; Dixit, V.; Ferrara, N. *J. Biol. Chem.* **1998**, *273*, 13313-13316. (b) Weis, S. M.; Cheresh, D.A. *Nature*, **2005**, *437*, 497-504. (c) Sanz, J.; Fayad, Z.A. *Nature*, **2008**, *451*, 953-957.

5 (a) Tricot, O.; Mallat, Z.; Heymes, C.; Belmin, J.; Leseche, G.; Tedgui, A. *Circulation* **2000**, *101*, 2450–2453; (b) Segura, A.; Serrano, G.; Gonzalez, M.; Abad, C.; Claveria, L.; Gomez, A.; Bernad, C.; Martinez, H.; Riese, H. *FASEB J*. **2002**, *16*, 833–841; (c) Chavakis, E.; Dimmeler, S. Arterioscler. *Thromb. Vasc. Biol*. **2002**, *22*, 887–893; (d) Hunt, B. J. *Lupus* **2000**, *9*, 189–193; (e) Choy, J.; Granville, D.; Hunt, D. J. *Mol. Cell. Cardiol*. **2001**, *33*, 1673–1690; (f) Rossig, L.; Haendeler, J.; Mallat, Z. *J. Am. Coll. Cardiol*. **2000**, *36*, 2081–2089; (g) Walsh, K.; Smith, R. C.; Kim, H. S. *Circ. Res*. **2000**, *87*, 184–188; (h) Libby, P.; Ridker, P. M.; Maseri, A. *Circulation* **2002**, *105*, 1135–1143. References related to retina; (i) Wong, P. *Biochem. Cell Biol*. **1994**, *72*, 489-498. (j) Levin, L.A.; Louhab, A. *Arch. Ophthalmol*. **1996**, *114*, 488-491. (k) Barber, A. J.; Lieth, E.; Khin, S. A.; Antonetti, D. A.; Buchanan, A. G.; Gardner, T. W. *J. Clin. Invest.* **1998**, *102*, 783-791.

6 (a) Poon, I.K.; Hulett, M.D.; Parish, C.P. *Cell Death Differ.* **2010**, *17,* 381–397. (b) Nartin, S. J.; Green, D. R.; Cotter, T. G. *Trends Biochem. Sci*. **1994**, *19*, 26-30.

7 (a) Samejima, K.; Earnshaw, W. C. *Nat. Rev.* **2005**, *6*, 677-688. (b) Elmore, S. *Toxicol. Pathol.* **2007**, *35*, 495-516.

8 (a) Kam, P.C.; Ferch, N.I. *Anaesthesia* **2000**, *55*, 1081–1093. (b) Chamond, R.R.; Anon, J.C.; Guerra, Pasadas, C.M.A. *Alergol Immunol Clin,* **1999**, *14*, 367-374. (c) Yuan, J.; Yankner, B.A. *Nature* **2000**, *407*, 802-809. (d) Martin, L.J. *Int. J. Mol. Med.* **2001**, *7*, 455-478. (e) Martin, J.B. *N.*

Engl. J. Med. **1999**, *340*, 1970-1980. (f) Krysko, D. V.; D'Herde, K.; Vandenabeele, P. *Apoptosis* **2006**, *11*, 1709-1726. (g) Tricot, O.; Mallat, Z.; Heymes, C.; Belmin, J.; Leseche, G.; Tedgui, A. *Circulation* **2000**, *101*, 2450-2453. (h) Segura, A.; Serrano, G.; Gonzalez, M.; Abad, C.; Claveria, L.; Gomez, A.; Bernad, C.; Martinez, H.; Riese, H. *FASEB J*. **2002**, *16*, 833-841.

9 (a) Stefanec, T. *Chest* **2000**, *117*, 841-854. (b) O'Connell, B.J.; Genest Jr. J. *Circulation*, **2001**, *104*, 1978-1983.

10 (a) Dimmeler, S.; Zeiher, A.M. *Circ. Res.* **2000**, *87*, 434-439. (b) Eissner, G.; Kohlhuber, F.; Grell, M.; Ueffing, M.; Scheurich, P. ; Hieke, A.; Multhoff, G.; Bornkamm, G.W.; Holler, E. *Blood* **1995**, *86*, 4184-4193. (c) Chavakis, E.; Dimmeler, S. *Arterioscler. Thromb. Vasc. Biol*. **2002**, *22*, 887-893. (d) Hunt, B. J. *Lupus* **2000**, *9*, 189-193. (e) Choy, J.; Granville, D.; Hunt, D. J. *Mol. Cell. Cardiol*. **2001**, *33*, 1673-1690. (f) Rossig, L.; Haendeler, J.; Mallat, Z. *J. Am. Coll. Cardiol*. **2000**, *36*, 2081-2089. (g) Walsh, K.; Smith, R. C.; Kim, H. S. Circ. Res. 2000, 87, 184–188; (h) Libby, P.; Ridker, P. M.; Maseri, A. *Circulation* **2002**, *105*, 1135-1143.

11 (a) Alvarez, R. J.; Gips, S. J.; Moldovan, N.; Wilhide, C. C.; Milliken, E. E.; Hoang, A. T.; Hruban, R. H.; Silverman, H. S.; Dang, C. V.; Goldschmidt-Clermont, P. J. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 372-381. (b) Chen, D.-F.; Zhang, H.-L.; Du, S.-H.; Li, H.; Zhou, J.-H.; Li, Y.-W.; Zeng, H.-P.; Hua, Z.-C.; *Steroids* **2010**, *75*, 1119-1126.

12 (a) Liu, X.; Zhao, J.; Xu, J.; Zhao, B.; Zhang, Y.; Zhang, S.; Miao, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2896-2900. (b) Tan, C.-B.; Gao, M.; Xu, W.R.; Yang, X.-Y.; Zhu, X.-M.; Du, G.-H. *Biol. Pharm. Bull.* **2009**, *32*, 1359-1363. (c) Rodrigues, C.; Steer, C.J. *Expert Opin. Investig. Drugs* **2001**, *10*, 1243-1253. (d) Stirling, D. P.; Koochesfahani, K. M.; Steeves, J. D.; Tetzlaff, W. *Neuroscientist* **2005**, *11*, 308-323.

13 (a) Lü, J.-M.; Yao, Q.; Chen, C. *Curr Vasc Pharmacol*. **2009**, *7*, 293-302. (b) Helms, S. *Altern. Med. Rev.* **2004**, *9*, 259-274. (c) Bames, A.S.; Powell-Griner, E.; McFann, K.; Nahin, R.L. *Adv.*

Data. **2004**, *343*, 1-19. (d) Borchers, A.T.; Keen, C.L.; Stern, J.S.; Gershwin, M.E. *Am. J. Clin. Nutr.* **2000**, *72*, 339-347.

14 (a) Ekshyyan, O.; Aw, T. Y. Curr. *Neurovasc. Res*. **2004**, *1*, 355–371; (b) Mattson, M. *Nat. Rev. Mol. Cell Biol.* **2000**, *2*, 120-129.

15 (a) Cho, J.; Park, W.; Lee, S.; Ahn, W.; Lee, Y. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 3510-3515. (b) Al-Dujaili, E.; Chalmers, R.; Sharp, M. *Endocrine Abstracts* **2007**, *13*, 285. (c) Shang, W.; Yang, Y.; Zhou, L.; Jiang, B.; Jin, H.; Chen, M. *J. Endocrinol.* **2008**, *198*, 561-569.

16 (a) Owen, R. *Drugs Today* **1981**, *17*, 343-351. (b) Rivera, E.; Hu, S.; Concha, C. *Vaccine* **2003**, *21*, 1149-1157. (c) Hu, S.; Concha, C.; Lin, F.; Persson, W. K. *Immunol. Immunopathol.* **2003**, *91*, 29-37.

17 (a) Yue, P.; Wong, D.; Wu, P.; Leung, P.; Mak, N.; Yeung, H.; Liu, L.; Cai, Z.; Jiang, Z.-H.; Fan, T.; Wong, R. *Biochem. Pharmacol*. **2006**, *72*, 437-445. (b) Cheng, L.-Q.; Na, J.; Bang, M.-H.; Kim, M.-K.; Yang, D.-C. *Phytochemistry* **2008**, *69*, 218-224. (c) Tian, J.; Fu, F.; Geng, M.; Jiang, Y.; Yang, J.; Jiang, W.; Wang, C.; Liu, K. *Neurosci. Lett*. **2005**, *374*, 92-97.

18 (a) Hasegawa, H. *J Pharmacol Sci*. **2004**, *95*, 153-157. (b) Liu, C. X.; Xiao, P. G. *J. Ethnopharmacol*. **1992**, *36*, 27-38.

19 Yun, T.K. *Lancet Oncol.* **2001**, *2*, 49-55.

20 (a) Morisaki, N.; Watanabe, S.; Tezuka, M.; Zenibayashi, M.; Shiina, R.; Koyama, N.; Kanzaki, T.; Saito, Y.; *Br. J. Pharmacol.* **1995**, *115*, 188-1193. (b) Sato, K.; Mochizuki, M.; Saiki, I.; Yoo, Y.C.; Samukawa, K.; Azuma, I. *Biol. Pharm. Bull.* **1994**, *17*, 635-639.

21 (a) Sengupta, S.; Toh, S.A.; Sellers, L.A.; Skepper, J.N.; Koolwijk, P.; Leung, H.W.; Yeung, H.W.; Wong, R.N.; Sasisekharan, R.; Fan, T.P. *Circulation* **2004**, *110*, 1219-1225. (b) Liu, X.; Kim, C.N.; Yang, J.; Jemmerson, R.; Wang, X. *Cell* **1996**, *86*, 147-157. (c) Leung, K. W.; Pon, Y. L.; Wong, R. N. S.; Wong, A. S. T. *J. Biol. Chem.* **2006**, *281*, 36280-36288.

22 a) Yue, P.; Wong, D.; Wu, P.; Leung, P.; Mak, N.; Yeung, H.; Liu, L.; Cai, Z.; Jiang, Z.-H.; Fan, T.; Wong, R. *Biochem. Pharmacol*. **2006**, *72*, 437-445. (b) Cheng, L.-Q.; Na, J.; Bang, M.-H.; Kim, M.-K.; Yang, D.-C. *Phytochemistry* **2008**, *69*, 218-224. (c) Tian, J.; Fu, F.; Geng, M.; Jiang, Y.; Yang, J.; Jiang, W.; Wang, C.; Liu, K. *Neurosci. Lett.* **2005**, *374*, 92-97.

23 Min, J.-K.; Kim, J.-H.; Cho, Y.-L.; Maeng, Y.-S.; Lee, S.-J.; Pyun, B.-J.; Kim, Y.-M.; Park, J. H.; Kwon, Y.-G. *Biochem. Bioph. Res. Co.* **2006**, *349*, 987-994.

24 Park, I. H.; Kim, N. Y.; Han, S. B.; Kim, J. M.; Kwon, S. W.; Kim, H. J.; Park, M. K.; Park, J. H. *Arch. Pharm. Res.* **2002**, *25*, 428-432.

25 (a) Shibata, S.; Ando, T.; Tanaka, O. *Chem. Pharm. Bull*. **1966**, *14*, 1157-1161; (b) Han, B. H.; Park, M. H.; Han, Y. N.; Woo, L. K.; Sankawa, U.; Yahara, S.; Tanaka,O. *Planta Med*. **1982**, *44*, 146-149; (c) Yuan, C.-S.; Wu, J. *Am. J. Clin. Nutr*. **2002**, *75*, 600-601.

26 (a) Kim, J.-H.; Hong, Y.-H.; Lee, J.-H.; Kim, D.-H.; Nam, G.; Jeong, S. M.; Lee, B.-H.; Lee, S.-M.; Nah, S.-Y. *Mol. Cells* **2005**, *19*, 137-142. (b) Lei, J.; Li, X.; Gong, X.-J.; Zheng, Y.-N. *Molecules* **2007**, *12*, 2140-2150. (c) Danieli, B.; Falcone, L.;Monti, D.; Riva, S.; Gebhardt, S.; Schubert-Zsilavecz, M. *J. Org. Chem.* **2001**, 66, 262-269.

27 *Chemical and Technical Assessment* **2008**, 1-13.

28 β-Sitosterol contained campersterol and sitostanol; the purity of β-sitosterol is up to 70%. The reagent was purchased from Sigma-Aldrich Co.

29 Jursic, B. S.; Upadhyay, S. K.; Creech, C. C.; Neumann, D. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7372-7375.

30 Hogg, N.; Browning, J.; Howard T.; Winterford C.; Fitzpatrick D.; Gobé G. *Endothelium* **1999**, *7*, 35-49.

31 Oishi, K.; Kobayashi, A.; Fujii, K.; Kanehira, D.; Ito, Y.; Uchida, M. K. *J. Pharm. Sci.* **2004**, *96*, 208.

32 (Lazebnik et al., *J. Cell Biol*. **1993**, *123*, 7-22

33 (a) Mesner, P. W.; Bible, K. C.; Martins, L. M.; Kottke, T. J.; Srinivasula, S. M.; Svingen, P. A.; Chilcote, T. J.; Basi, G. S.; Tung, J. S.; Krajewski, S.; Reed, J. C.; Alnemri, E. S.; Earnshaw, W. C.; Kaufmann, S. H. *J. Biol. Chem.* **1999**, *274*, 22635-22645. (b) Faleiro, L.; Kobayashi, R.; Fearnhead, H.; Lazebnik, Y. *EMBO* **1997**, *16*, 2271-2281. (c) Takahashi, A.; Hirata, H.; Yonehara, S.; Imai, Y.; Lee, K.-K.; Moyer, R. W.; Turner, P. C.; Mesner, P. W.; Okazaki, T.; Sawai, H.; Kishi, S.; Yamamoto, K.; Okuma, M.; Sasada, M. *Oncogene* **1997**, *14*, 2741-2752

34 Kapuscinski, J.; Skoczylas, B. *Anal. Biochem.* **1977**, *83*, 252-257.

35 Brunk, C.F.; Jones, K. C.; James, T. W. *Anal. Biochem.* **1979**, *92*, 497-500.

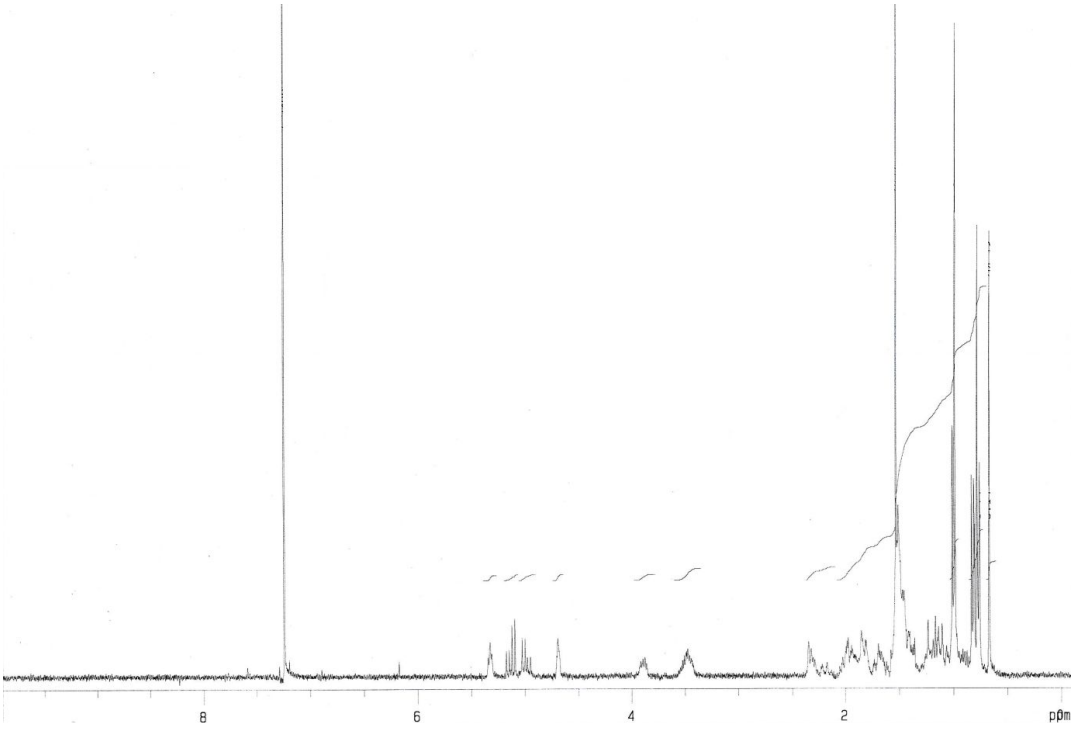
36 Carrillo-Vico, A.; Lardone, P. J.; Naji, L.; Ferna´ndez-Santos, J. M.; Martı´n-Lacave, I.; Guerrero, J. M.; Calvo, J. R. *J. Pineal Res*. **2005**, *39*, 400-408.

37 Parts of these synthetic products and biological assay results have been reported by us. Lee, S.; Maharjan, S.; Kim, K.; Kim, N.-J.; Choi, H.-J.; Kwon, Y. G.; Suh, Y.-G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7102-7105.

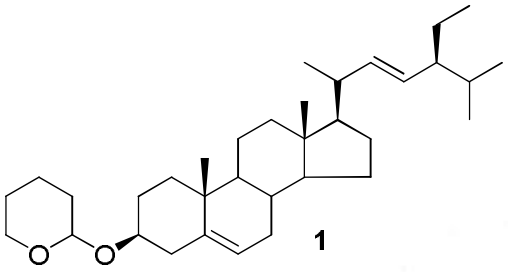
38 Maharjan, S.; Lee, S.; Agrawal, V.; Choi, H.-J.; Maeng, Y.-S.; Kim, K.; Kim, N.-J.; Suh, Y.-G.; Kwon, Y.-G. *Eur. J. Pharmacol.* **2011**, *657*, 35-40.

VI. Appendix

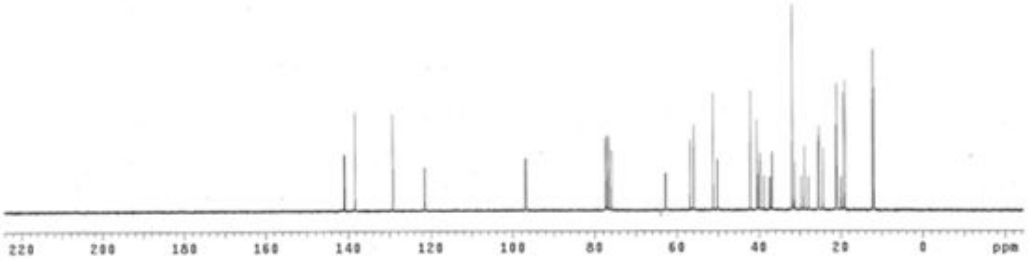
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



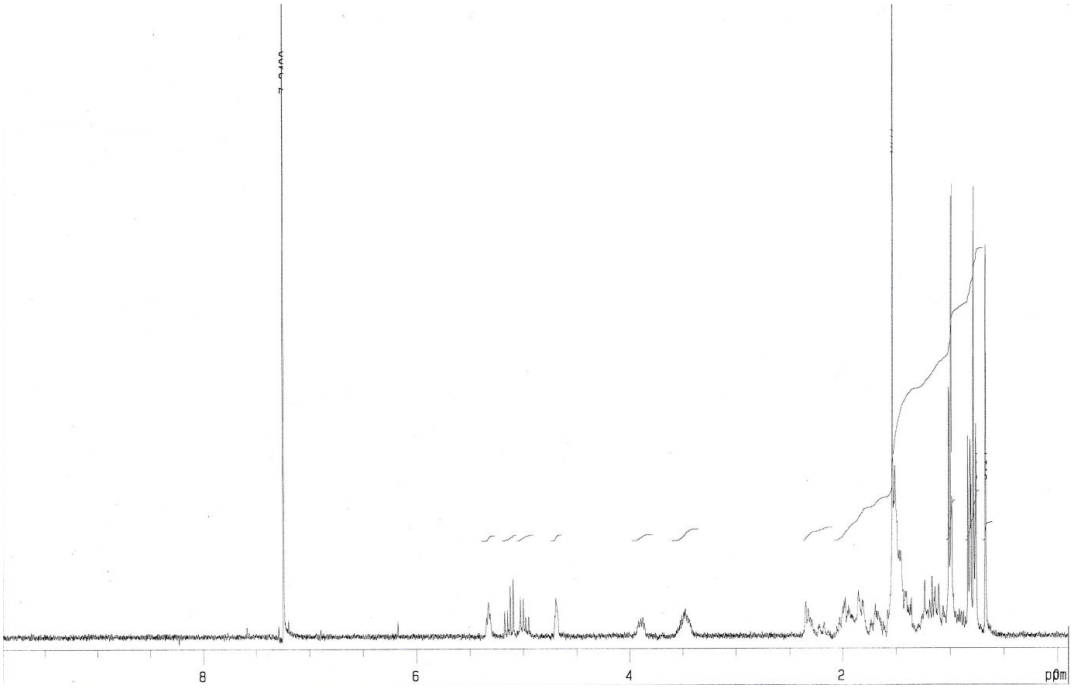
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



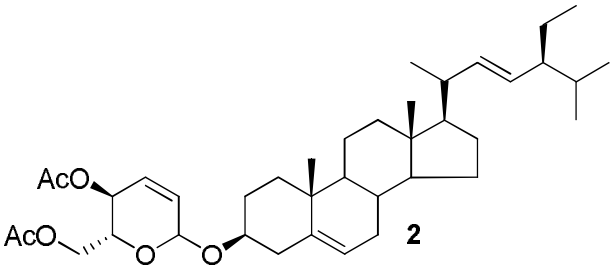
1



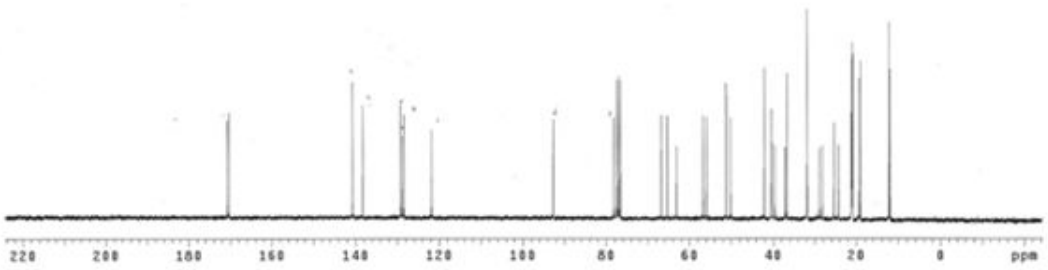
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



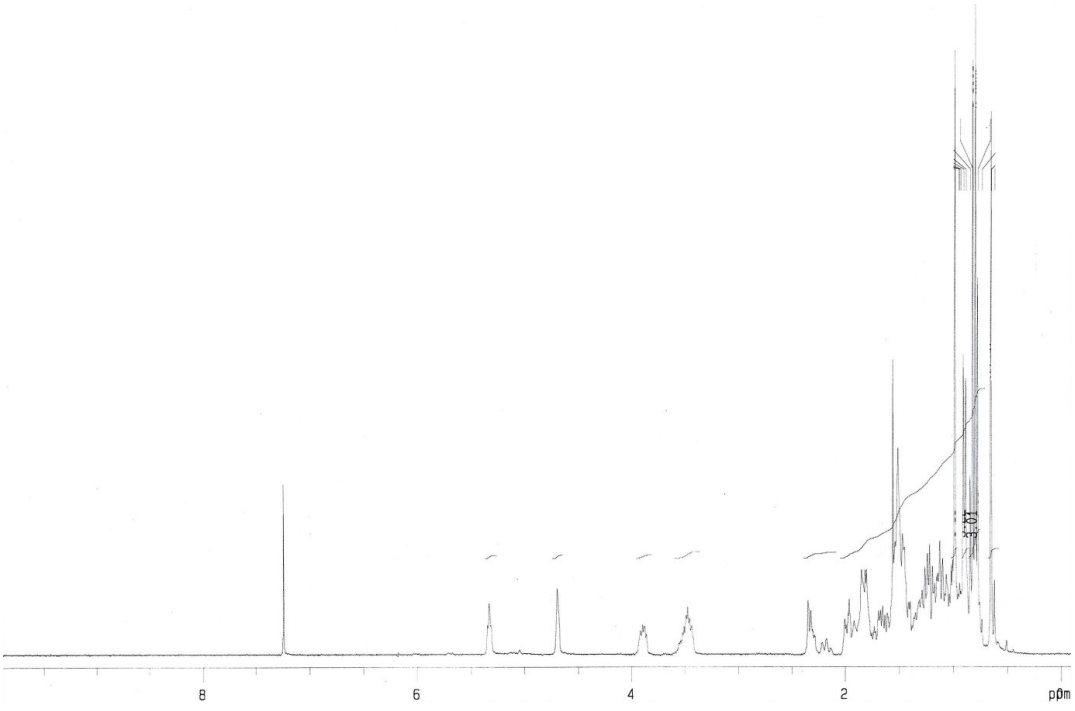
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



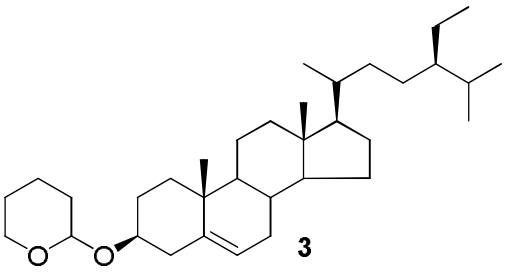
2



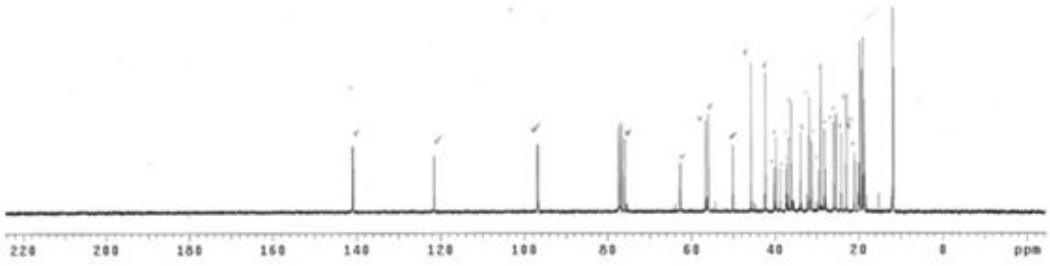
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



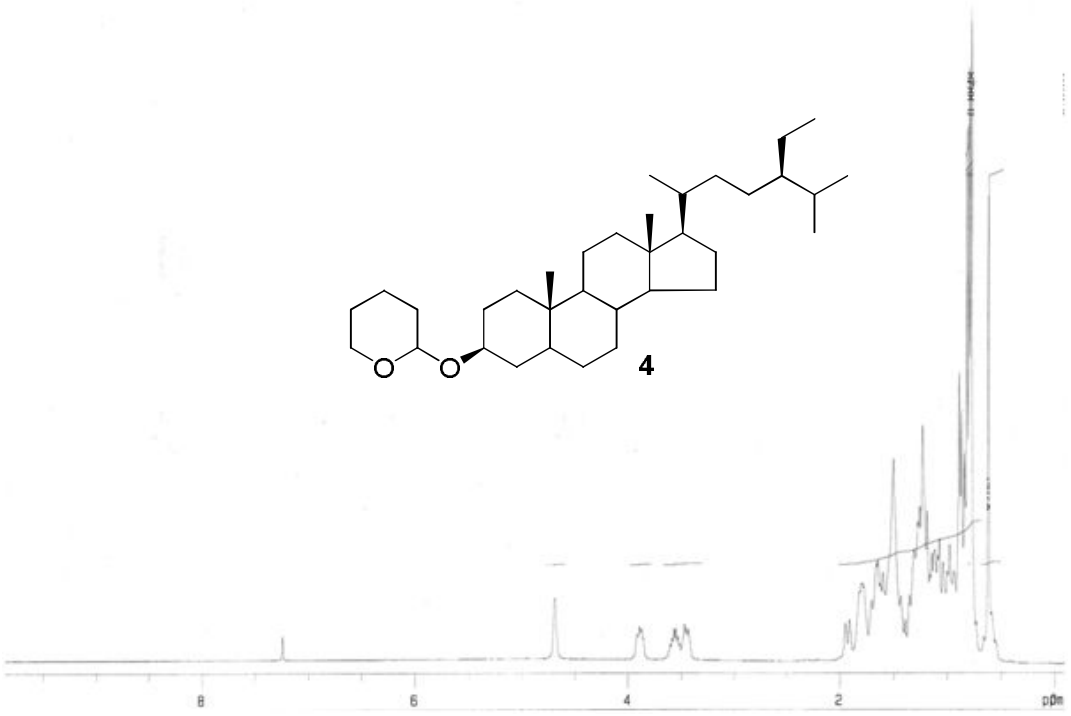
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



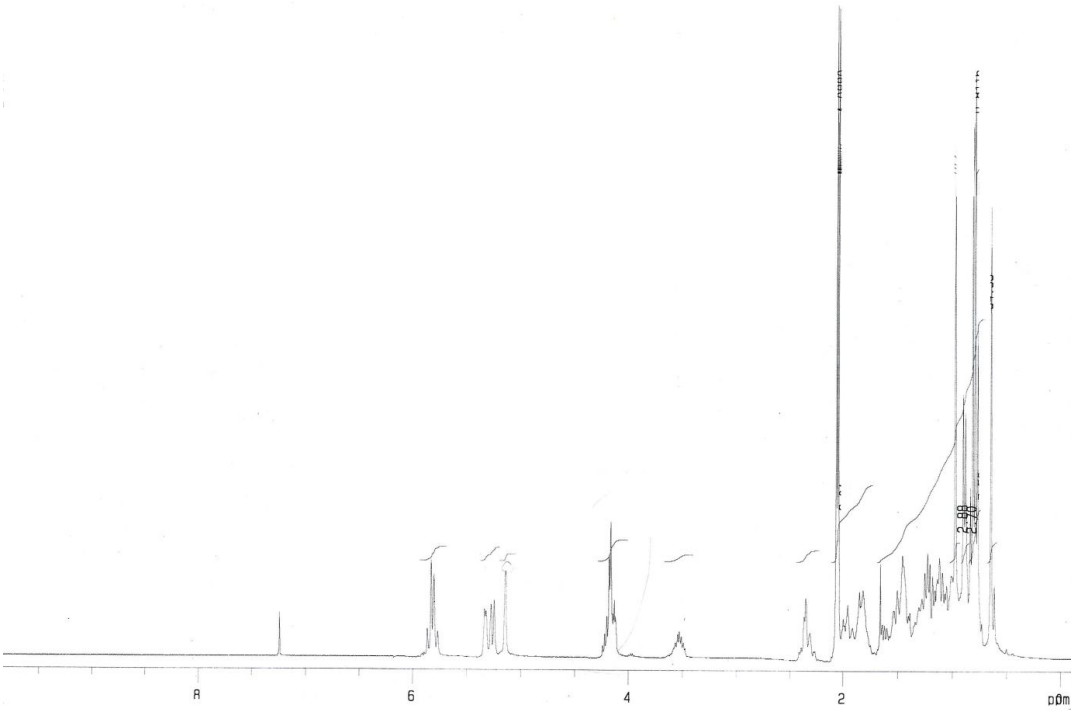
3



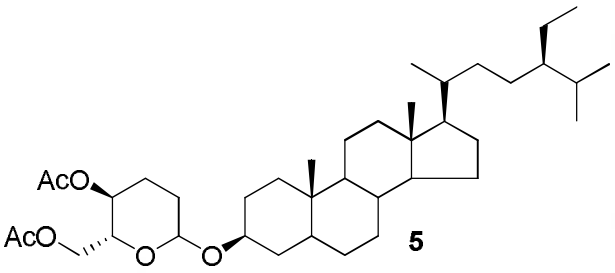
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



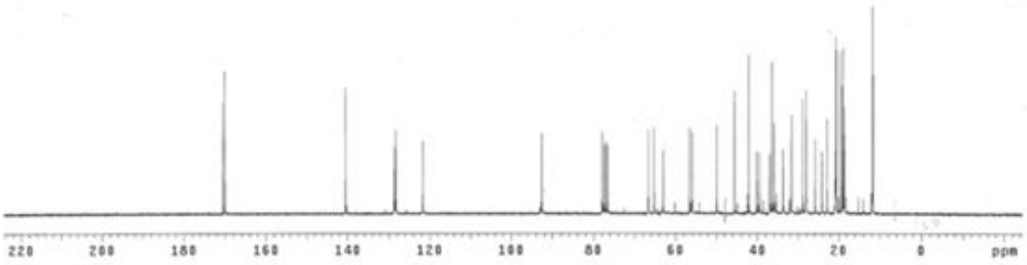
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



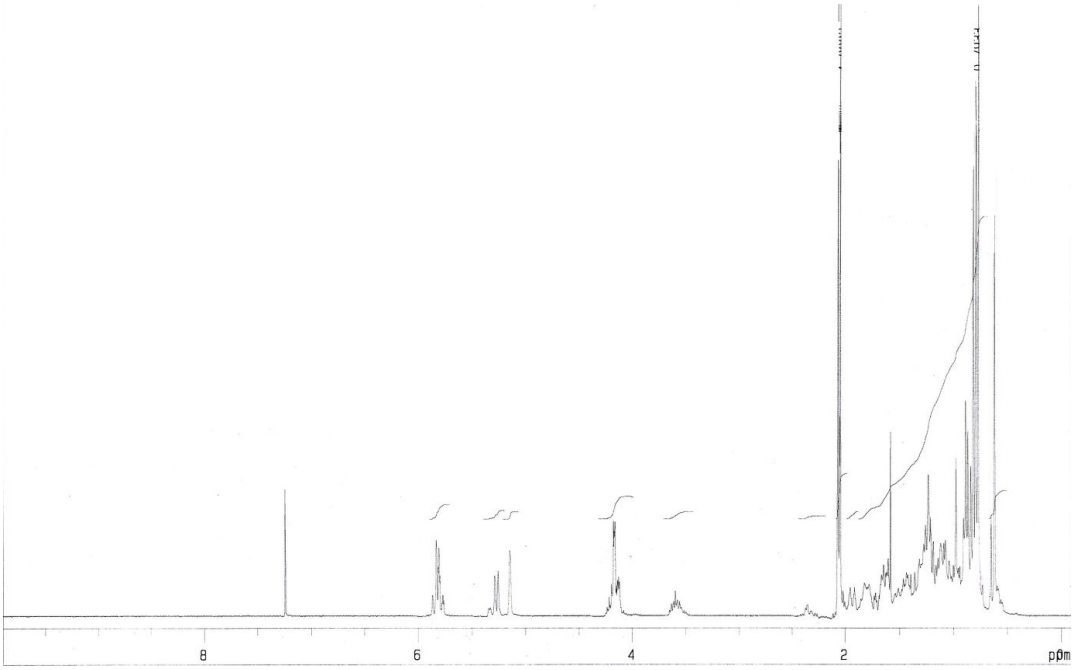
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



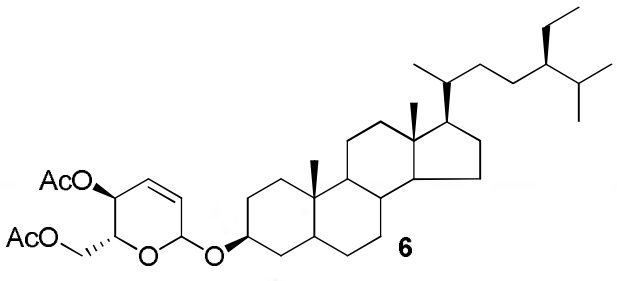
5



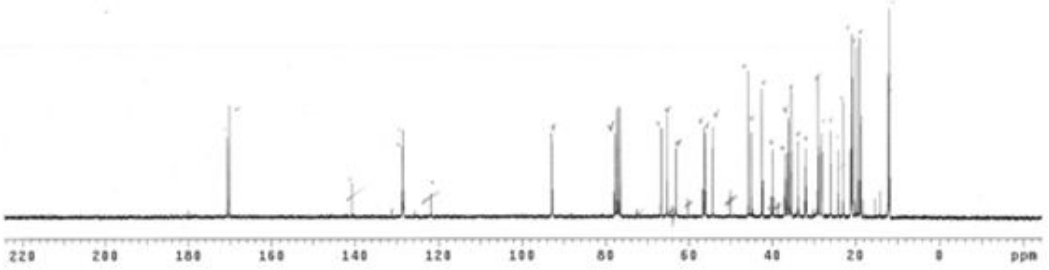
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



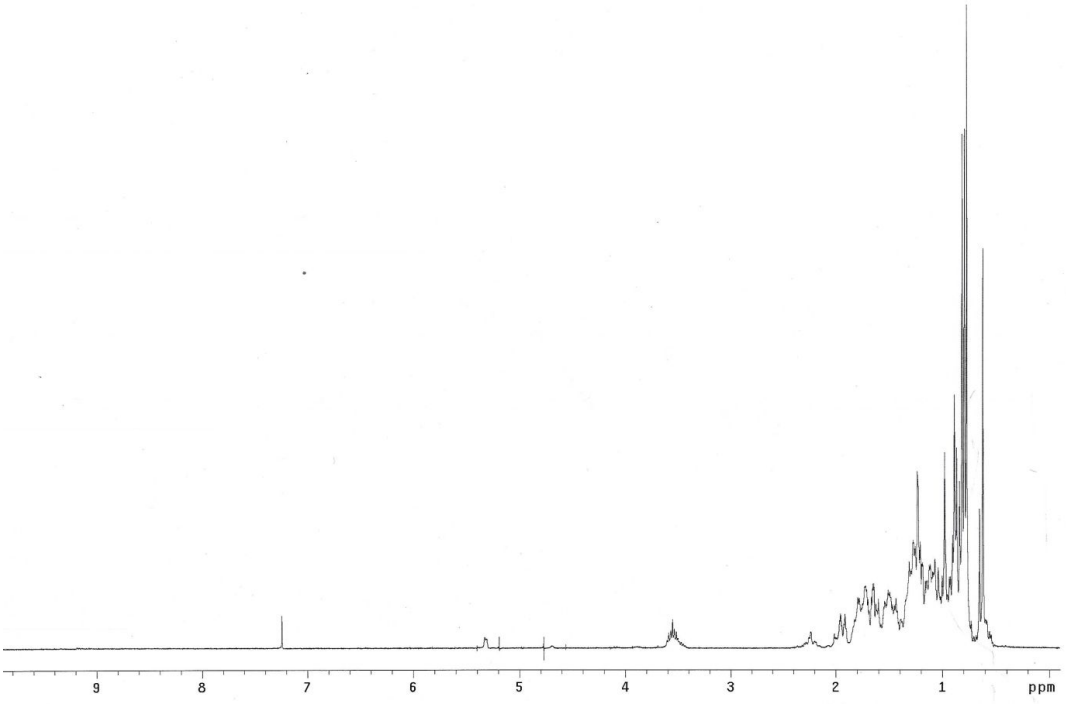
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



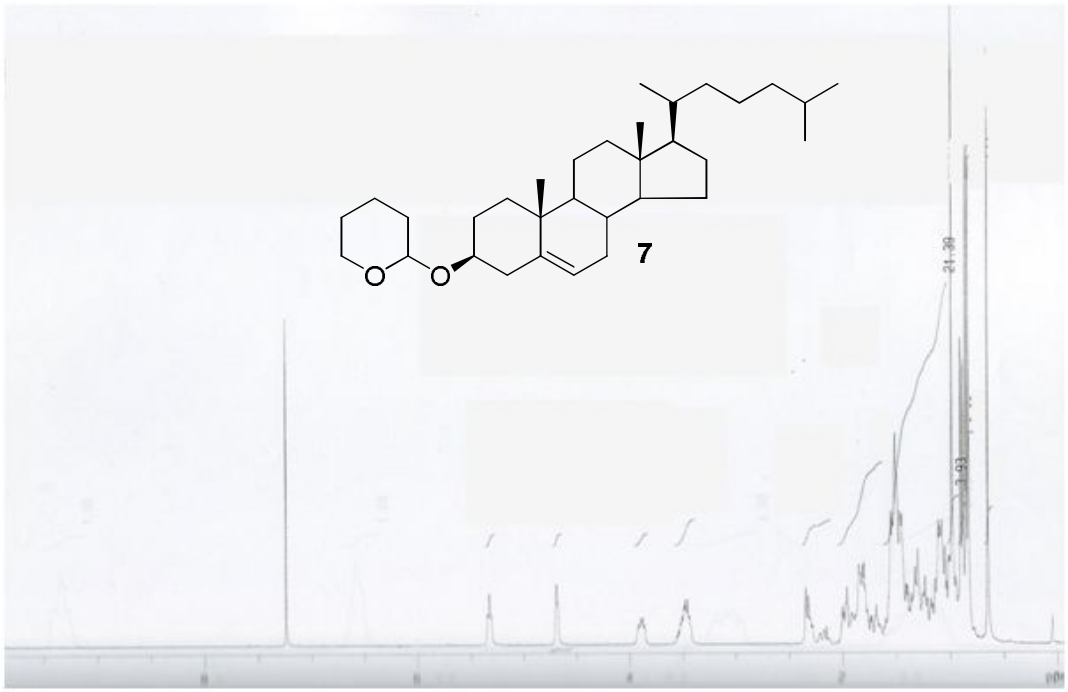
6



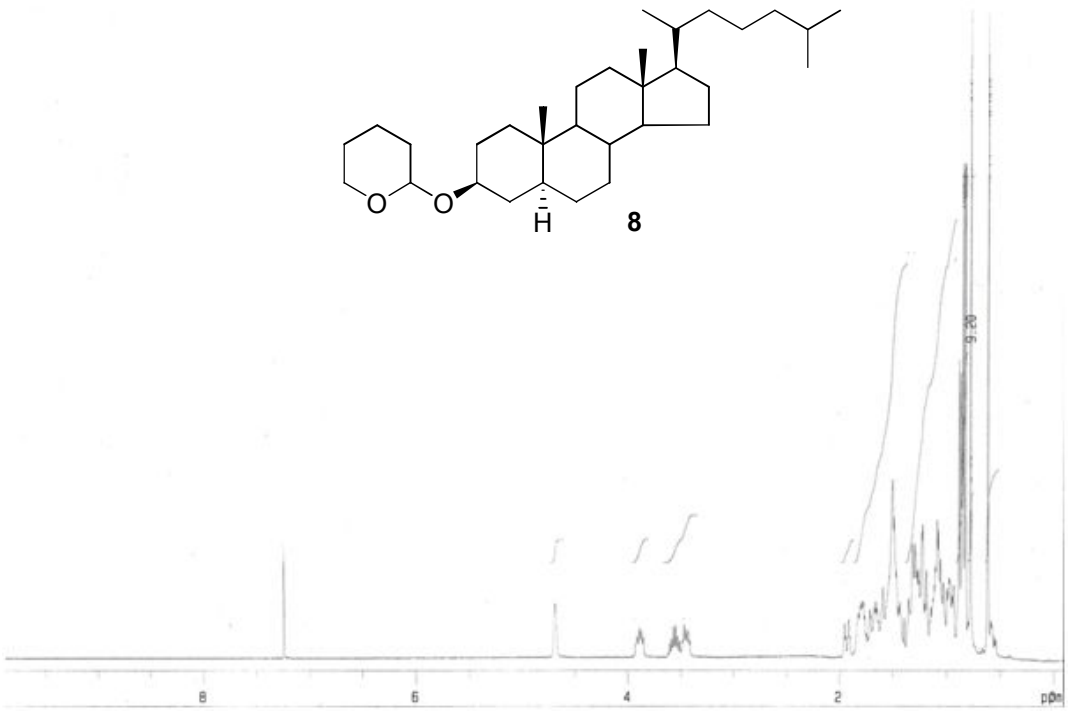
▼ **Sitostanol, ^{1}H-NMR (300 MHz, $CDCl_3$)**



▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**

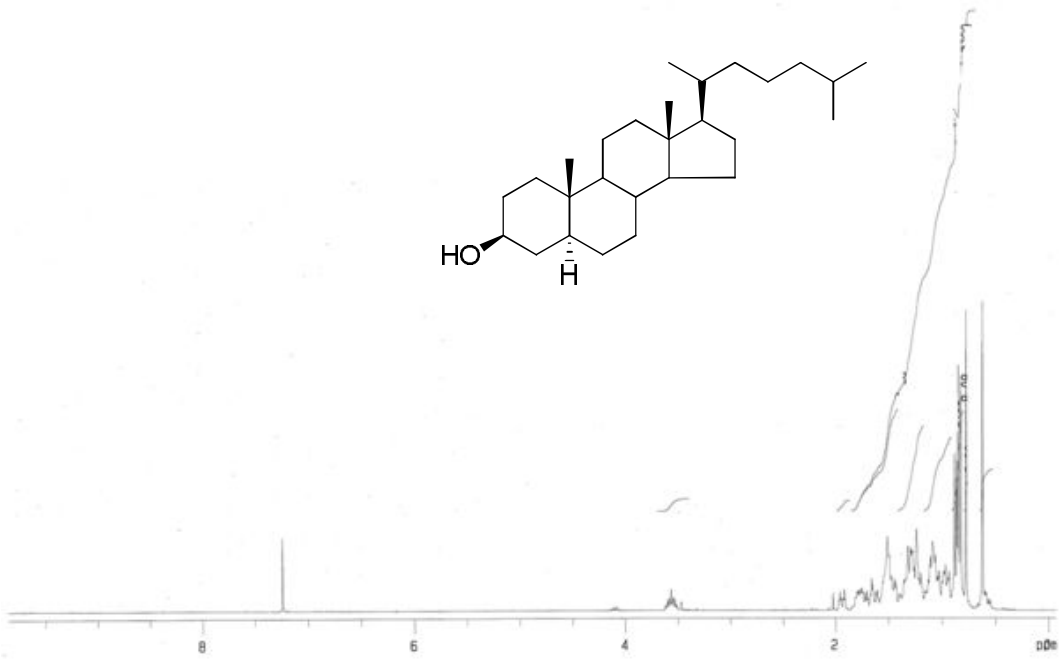


8

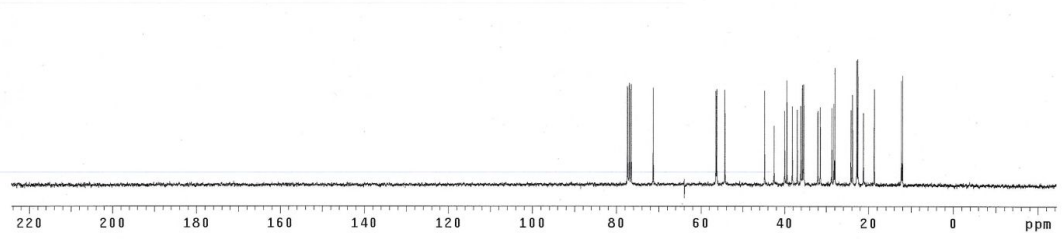
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**

9

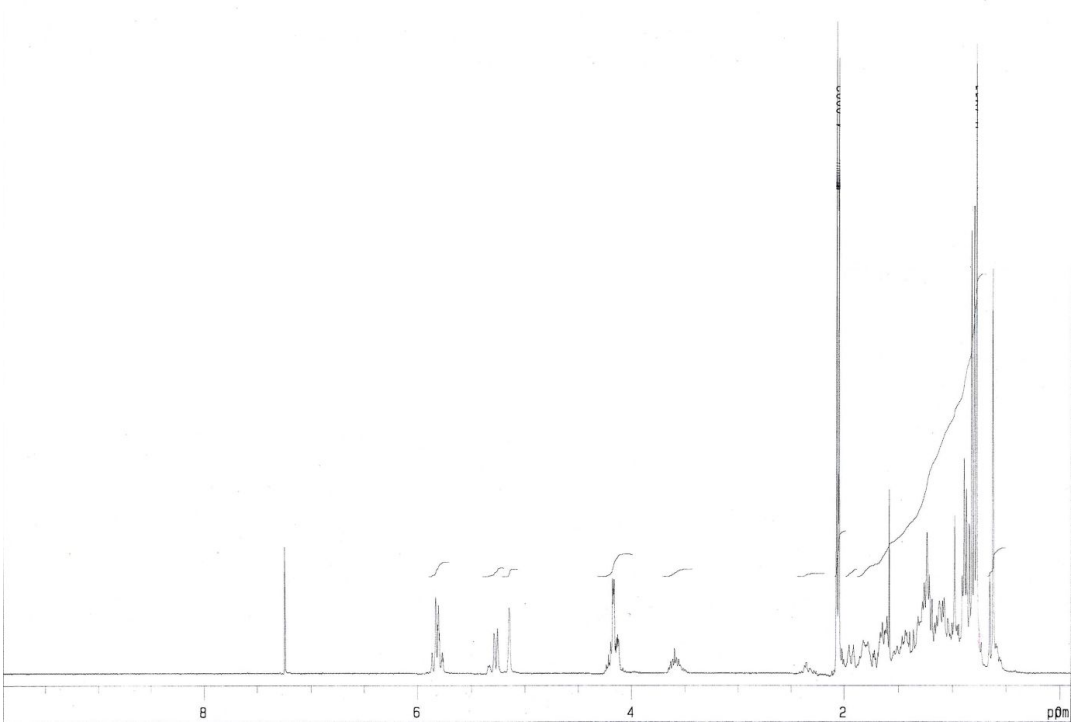
▼ **Cholestanol, ^{1}H-NMR (300 MHz, $CDCl_3$)**



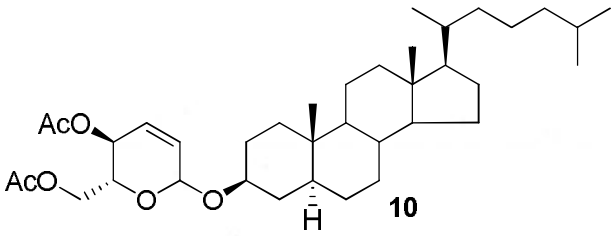
▼ **Cholestanol, ^{13}C-NMR (75 MHz, $CDCl_3$)**



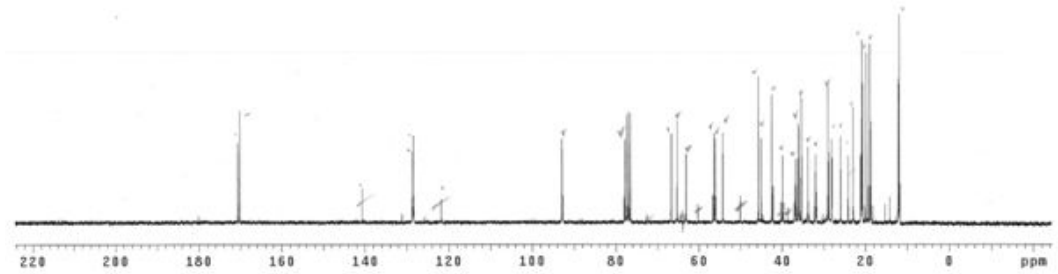
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



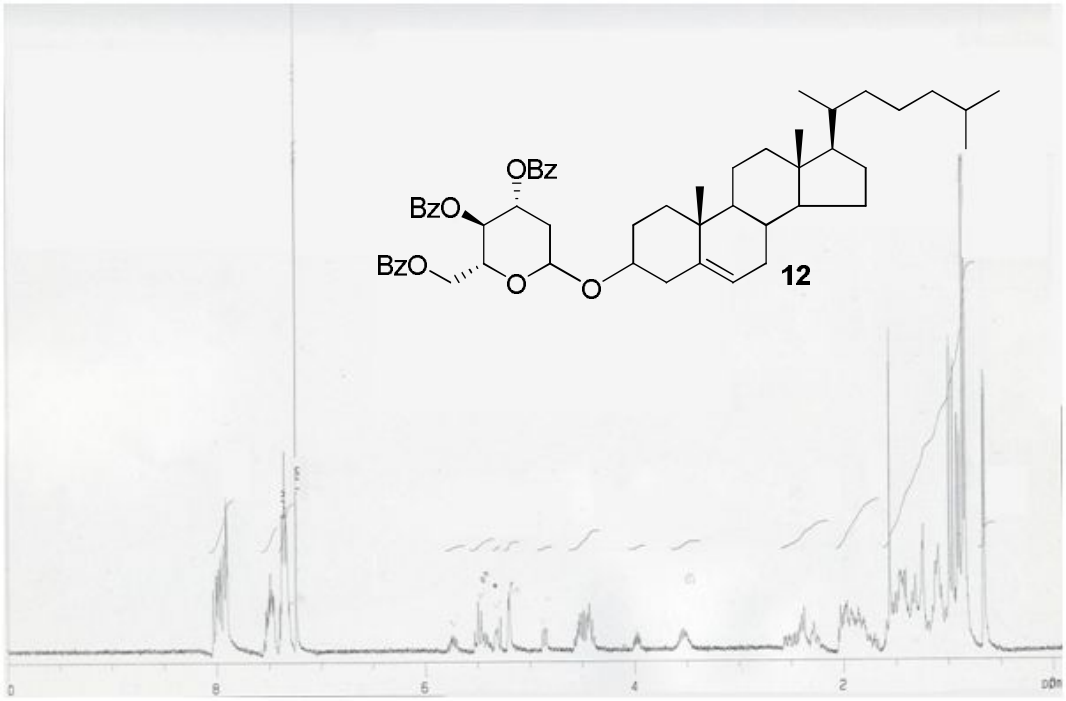
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



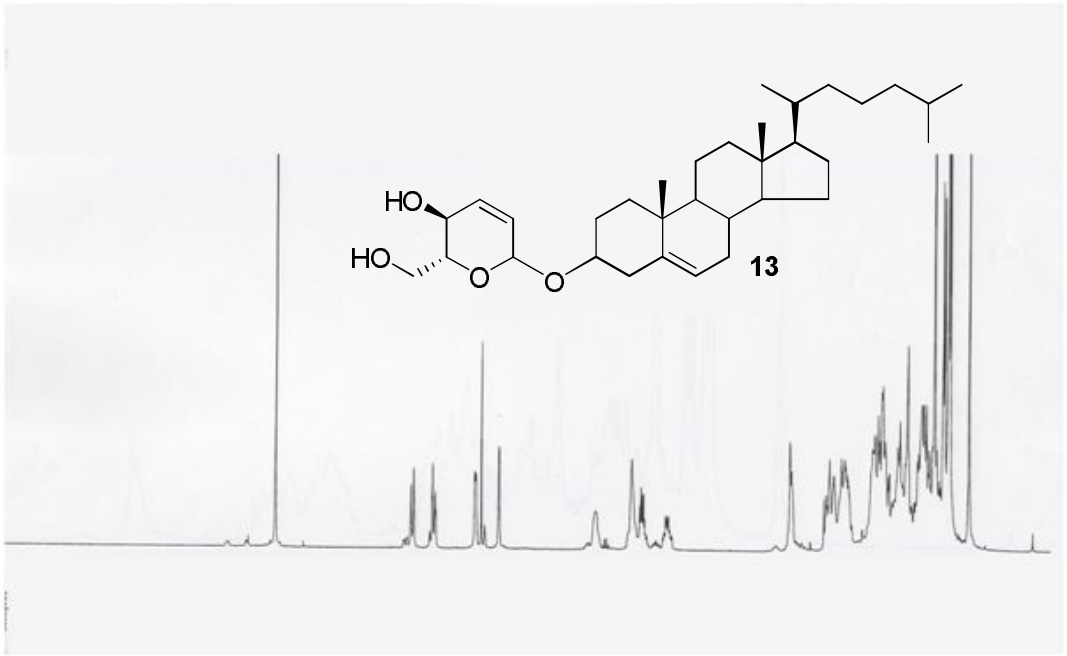
10



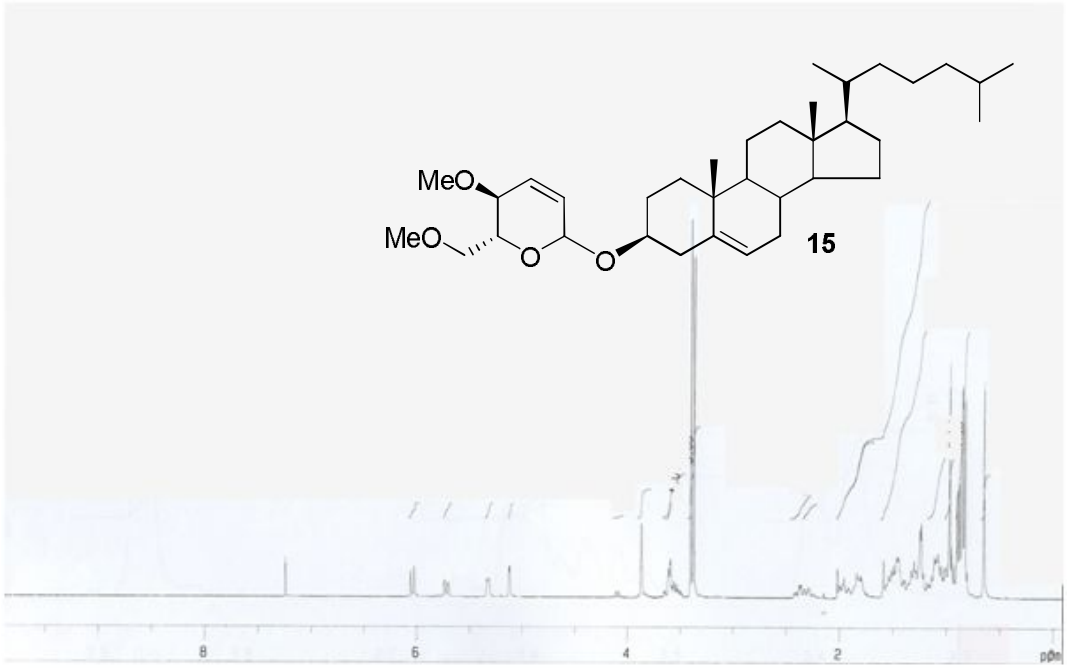
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



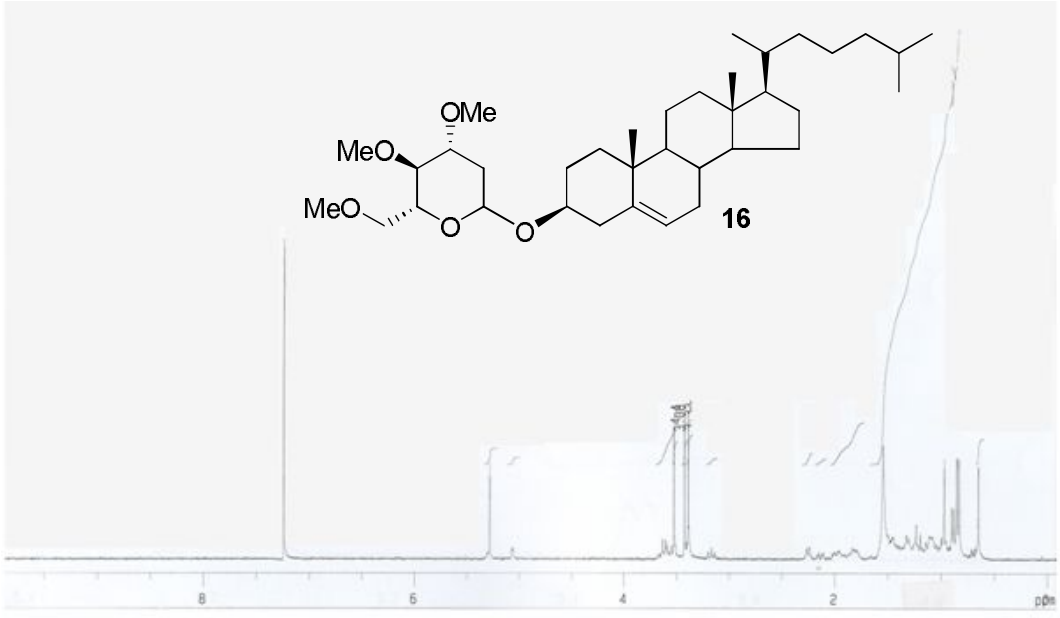
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



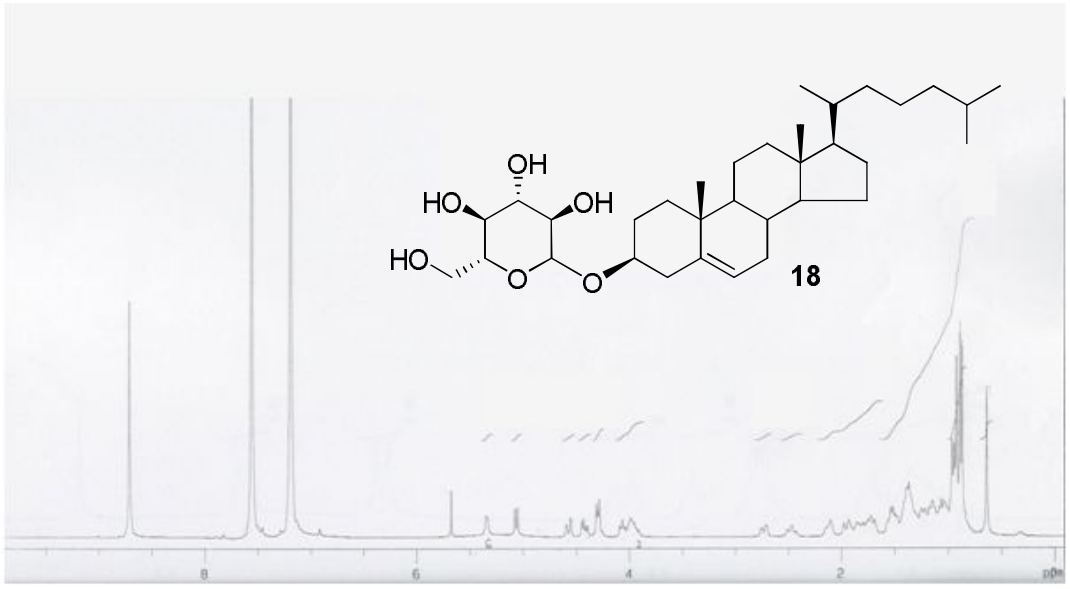
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



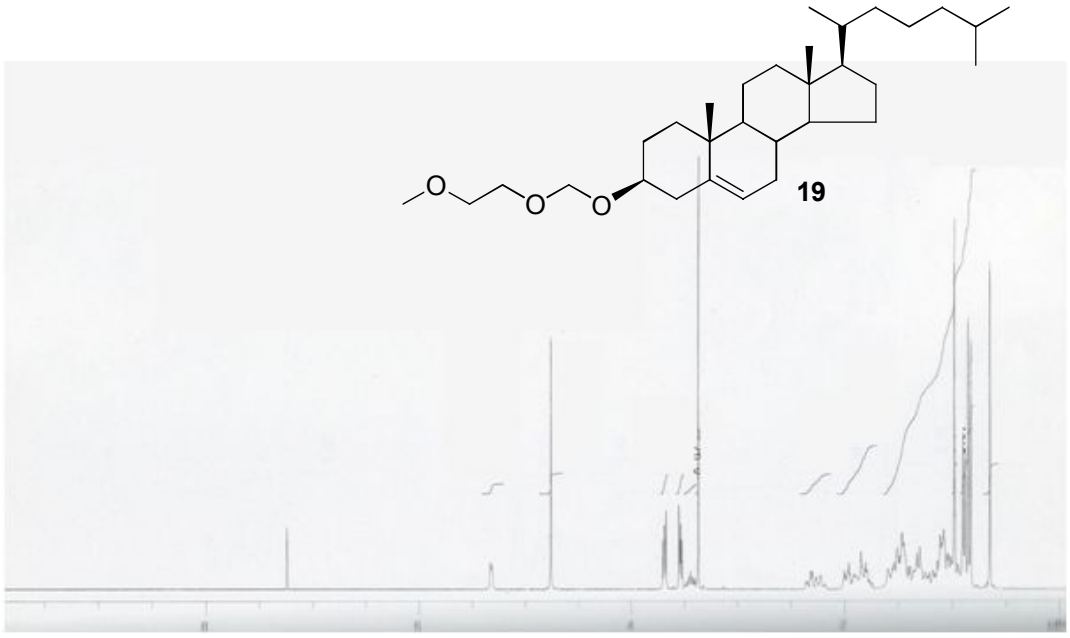
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



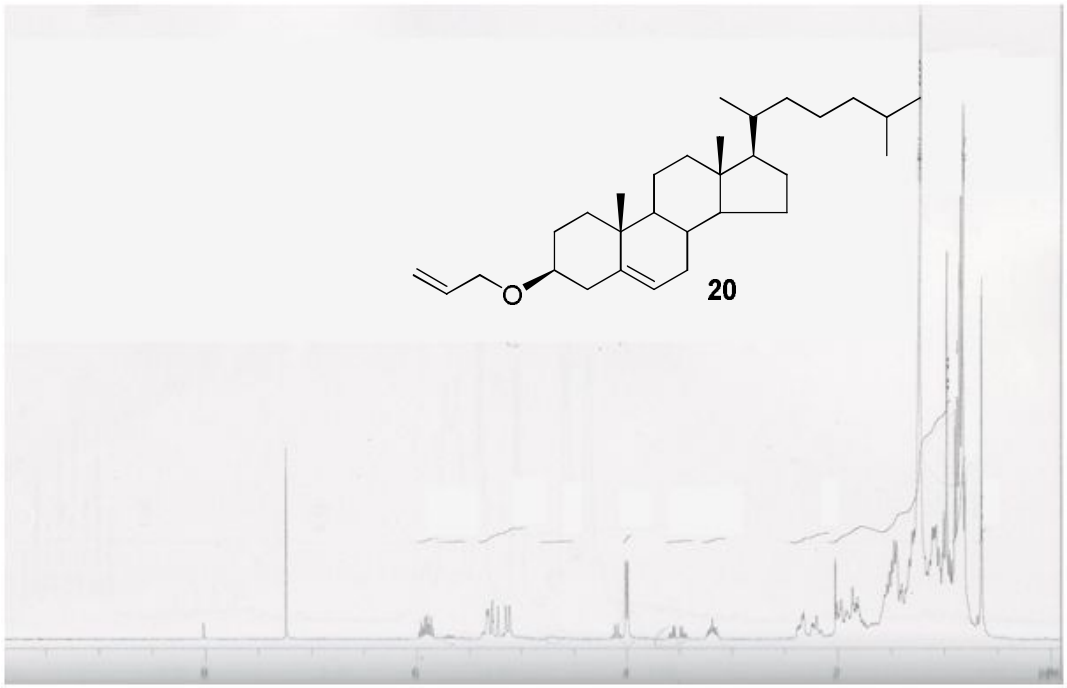
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



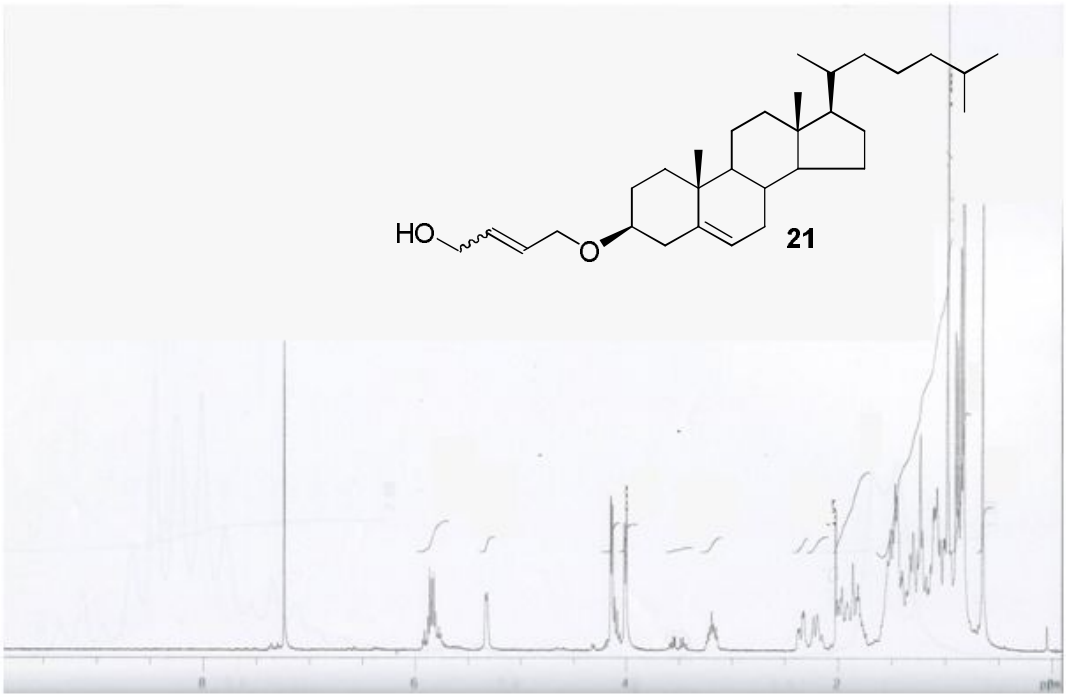
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



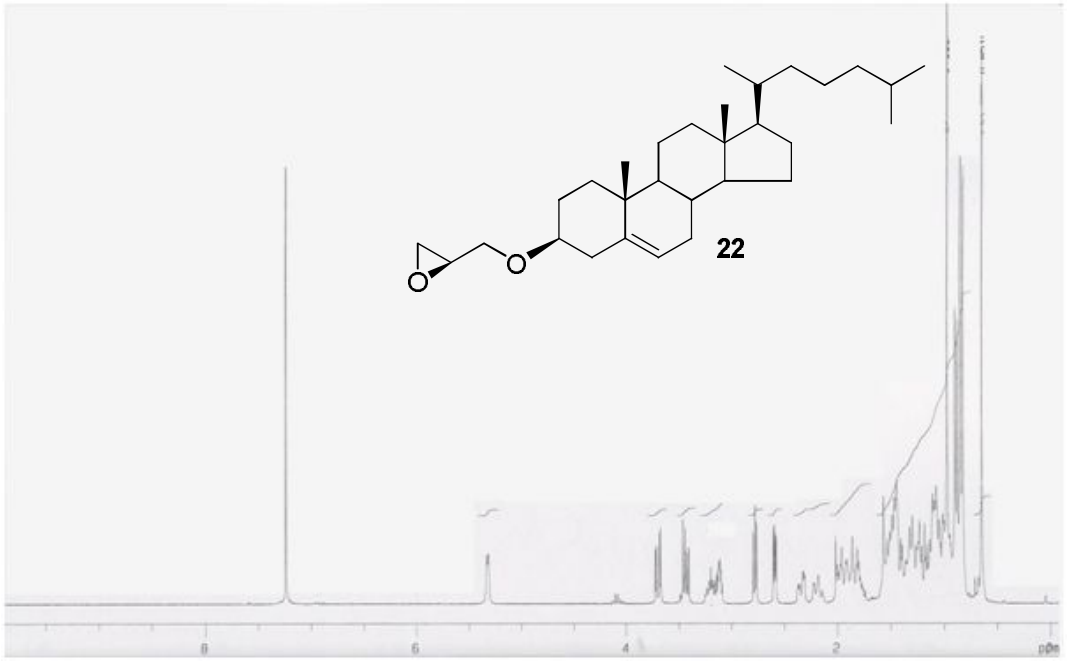
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



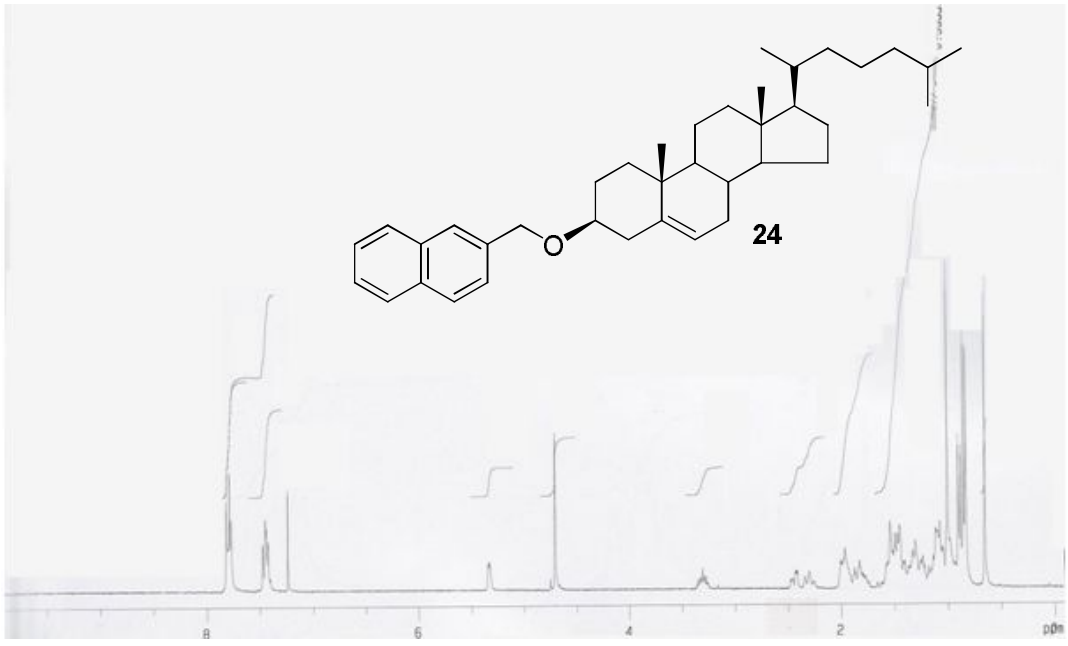
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



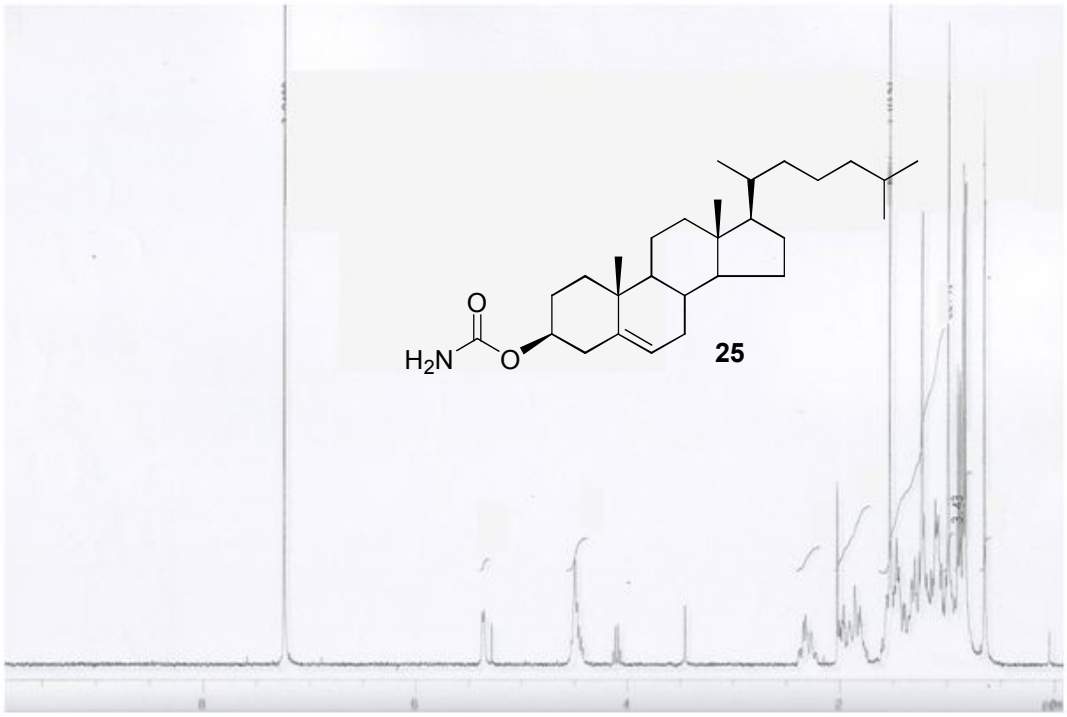
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



VII. Abstract

Cholesterol derived novel anti-apoptotic agent on the structural basis of ginsenoside Rk1

Sujin Lee
College of pharmacy
Department of manufacturing pharmacy
The Graduate School
Seoul National University

The endothelial cell monolayer constructs the lining of the blood vessel. In this particular position, different stimuli activate a cascade of proteases (caspases) that execute a program of cellular self-destruction by cleaving cellular structures at specific sites in a strictly controlled manner. This leads to the disassembly of the cell into small apoptotic bodies without spillage of cellular contents or significant inflammatory responses.

Recently, some ginsenosides isolated from *P. ginseng* have represented the anti-apoptotic activities on HUVECs. However, ginsenosides are generally labile under acidic conditions. And the compositions of ginsenosides in ginseng are variable depending on the growth environment, growth period, and process of extraction. In this connection, we planned to synthesize the anti-apoptotic agents from the simple and easily accessible phytosterol derivatives.

We discovered that the glucosyl moiety at C3 hydroxyl group is essential for anti-apoptotic activity through the comparison of some ginsenosides. We first searched the proper phytosterol for the replacement of the ginsenoside triterpene backbone. In particular, cholesterol, which has the linear side chain at C24, was the best choice. Then, the diverse bioisoster of glucosyl moiety at C3 hydroxyl group was substituted. Cyclic ether, acyclic ether, and spatial interactive bulky substituents were adapted to the C3 hydroxyl group.

With those analogs in hand, we tested the MTT assay on HUVECs. Throughout, the analogs **7** and **9** possessing the cholesterol scaffold and the cyclic carbohydrate equivalents exhibited

excellent cell survival activities, which are equipotent to that of ginsenoside Rk1. In addition, these analogs exhibited dose-dependent cell viability on the HREC line. The excellent human EC protective activities of our novel Rk1-based anti-apoptotic agents allow us to envisage their valuable application for the treatment of vascular disorders. Currently, systematic studies on their therapeutic applications, including in vivo testing of the potent sterol analogs as well as their mechanistic aspects, are in good progress.

Key words: human endothelial cell, apoptosis, cholesterol derivatives, ginsenoside Rg3, Rk1, phytosterol.

Student number: 2006-30449

creative commons
COMMONS DEED

저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

약학박사학위논문

Part I : 천연 **iridoid lactone**의 최초 전합성

Part II: **Ginsenoside Rk1** 구조 기반의 **anti-apoptotic agent**의 개발

Part I : **First total synthesis of natural iridoid lactone**

Part II: **Cholesterol derived novel anti-apoptotic agent on the structural basis of ginsenoside Rk1**

2011년 8월

서울대학교 대학원

제약학과 약품제조화학전공

이 수 진

Table of contents

Part I - First total synthesis of natural iridoid lactone

국문 초록 iii

List of Tables v

List of Figures vi

List of Schemes vii

Abbriviations viii

I. Introduction 1

1. Iridoid 2
   1) Previous synthetic approaches for iridoid lactones 5
2. Pd(0)-catalyzed cyclization 9
   1) Previous studies about Pd(0)-catalyzed cyclization 10
   2) Synthetic applications of bicyclic lactones 12
   3) Transformation of bicyclic lactone 14

II. Result and discussions 16

1. Pd(0)-catalyzed cyclization with substituted allyl carbonate 16
2. Total synthesis of natural iridoid lactone 20

III. Conclusion 33

IV. Experimental 34

V. References 55

VI. Appendix 60

VII. Abstract 98

Part I:천연 iridoid lactone의 최초 전합성

Part I: First total synthesis of natural iridoid lactone

국문 초록

Cyclopentane system은 의약품 및 생리활성을 갖는 천연물에 광범위하게 존재하는 구조로서, 다양한 입체 선택적인 cyclopentane system의 합성법이 여러 연구자들에 의해 개발, 발전되어 왔다. Iridoid 화합물은 *cis*-fused cyclopentane system을 갖는 천연물의 통칭으로, 주로 약용 식물로 이용되던 천연 자원으로부터 다양한 구조의 iridoid가 보고되었다. 그 중 최근 분리, 보고된 (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one의 특징적인 구조와 생리활성에 대한 기대로, 전합성을 시도하였다.

본 연구실에서 개발한 intramolecular Pd(0)-catalyzed allylic alkylation을 응용하여 iridoid 화합물의 합성을 계획하였다. C4에 alkyl기를 도입하기 위해 trisubstituted allyl carbonate가 도입된 γ-lactone을 합성 전구체로 계획하였다. 이를 합성하기 위해 합성 효율성이 높은 silicon-tether를 이용한 분자내 ring closing metathesis를 수행하였고, Pd(0)-catalyzed cyclization을 수행해 좋은 수율과 입체 선택적으로 bicyclic lactone을 합성하였다. 또한 이를 추가로 변환하여 iridoid의 핵심 골격인 *cis*-fused cyclopentane system을 효율적으로 합성하였다. C7-Hydroxymethyl을 입체선택적으로 도입하고자 cyclopentenol에 silicon-tether를 이용한 intramolecular radical cyclization, Tamao-Fleming oxidation을 수행하여 효과적으로 합성을 완료하였다.

본 연구를 통해 본 연구실에서 개발한 substituted vinyl기가 도입된 bicyclic lactone을 중간체로 이용하여 천연물의 합성에 적용할 수 있음을 확인하였고, 향후에 다양한 생리활성을 갖는 iridoid, iridoalkaloid등의 천연물 합성에 응용할 수 있을 것이라 기대한다.

주요어: (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one,

iridoid lactone, cyclopentane, Pd(0)-catalyzed cyclization, bicyclic lactone, silicon-tethered radical cyclization, Tamao-Fleming oxidation.

학 번: 2006-30449

List of Tables

Table 1. Cross metathesis with γ-lactone 18

Table 2. Pd(0)-catalyzed cyclization of 66 24

Table 3. Oxidation to cyclopentenone 26

List of Figures

Figure 1. Structures of cyclopentanoids 1
Figure 2. Structures of iridoids 2
Figure 3. Proposed biosynthetic process for iridoids 4
Figure 4. Palldium catalyzed allylic alkylation 10
Figure 5. Synthetic approach for branched allyl carbonate 16
Figure 6. Plausible transition state of Pd(0)-catalyzed cyclization 20
Figure 7. Target iridoid lactone 21
Figure 8. Bicyclopentenone coordinated with (*S*)-CBS oxazaborolidine 28
Figure 9. 3D-structure of bicyclic enone 28
Figure 10. 1, 6 hydrogen transfer 32

List of Schemes

Scheme 1. The first synthesis of nepetalactone 5
Scheme 2. Synthesis of *ent*-(-)-nepetalactone 6
Scheme 3. Synthesis of nepetalactone via oxidative radical cyclization 6
Scheme 4. Synthesis of (-)-7-deoxyloganin via NHC catalyzed rearrangement of α,β-unsaturated enolester 7
Scheme 5. Synthesis of (+)-geniposide via phosphine-catalyzed [3+2] cycloaddition 8
Scheme 6. Pd(0)-catalyzed allylic alkylation of acyclic allylic precursors 10
Scheme 7. Pd(0)-catalyzed cyclization with γ-lactone..... 11
Scheme 8. Synthetic applications with bicyclic lactone-formal synthesis of carbaprostacyclin 12
Scheme 9. Total synthesis of (+)-brefeldin A..... 13
Scheme 10. Total synthesis of bacillariolideⅢ 14
Scheme 11. Transformations of bicyclic lactone 15
Scheme 12. Syntheses of branched allyl carbonate γ-lactones..... 17
Scheme 13. Pd(0)-catalyzed cyclization with branched γ-lactone allylic carbonate... 19
Scheme 14. Retrosynthetic approach 21
Scheme 15. Synthesis of bicyclic pentanone 22
Scheme 16. Revised retrosynthetic approach 23
Scheme 17. Synthesis of γ-lactone 64d..... 23
Scheme 18. Attempt with bicyclic system 25
Scheme 19. Basic work up with cyclopentanone 27
Scheme 20. Revised retrosynthetic approach 29
Scheme 21. Synthesis of radical cyclization precursor 82 30
Scheme 22. Completion of synthesis of iridoid lactone 1..... 31

Abbriviations

br: broad singlet

BMCMDS: (bromomethyl) dimethylchlorosilane

calcd: calculated

CBS: Corey-Bakshi-Shibata

d: doublet

DBU: 1,8- diazabicyclo[5.4.0]undec-7-ene

DMAP: 4-dimethylaminopyridine

DMF: *N,N*-dimethylformamide

DMP: Dess-Martin periodinane

DMSO: dimethylsulfoxide

dr; diastereomeric ratio

EtOAc: ethylacetate

FAB: fast atom bombardment

GPP: geranylpyrophosphate

HRMS: high resolution mass spectroscopy

HWE: Horner-Wadsworth-Emmons reaction

IR: infrared

LiTMP: lithium tetramethylpiperidide

LRMS: low resolution mass spectroscopy

m: multiplet

Me: methyl

MeOH: methyl alcohol

NHC: *N*-heterocyclic carbene

NMO: 4-methylmorpholine *N*-oxide

NMR: nuclear magnetic resonance

OBO: 2,6,7-trioxabicyclo[2.2.2]octane

PDC: pyridinium dichromate

Ph: phenyl

PPTS: pyridinium *p*-toluene sulfonate

q: quartet

RCM: ring-closing metathesis

s: singlet

SAR: structure-activity relationship

t: triplet

TBAF: tetra-*n*-butylammonium fluoride

TBS: *t*-butyldimethylsilyl

TFAA: trifluoroacetic anhydride

THF: tetrahydrofuran

TLC: thin layer chromatography

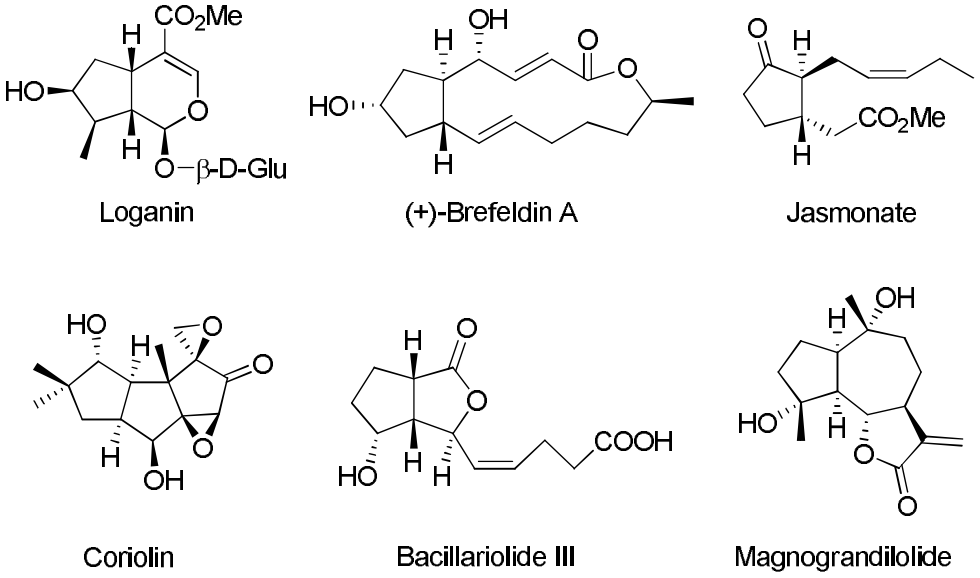
TPAP: tetra-*n*-propylammonium perruthenate

TsOH: *p*-toluene sulfonic acid

I. Introduction

Cyclopentane system is an abundant structure in nature and bioactive chemicals, such as prostaglandins, iridoids, sesquiterpenoids and guaianolides. (Figure 1) And it has been reported that the cyclopentanoids represented various activities, such as, antibacterial, antiviral, antiproliferative, antipyretic and anti-hypertensive effects.[1] Thus, considerable effort has been devoted in the past decades to the development of new methods for the construction of five-membered carbocycles, since they play a fundamental role in synthetic organic chemistry, as both targets and intermediates.

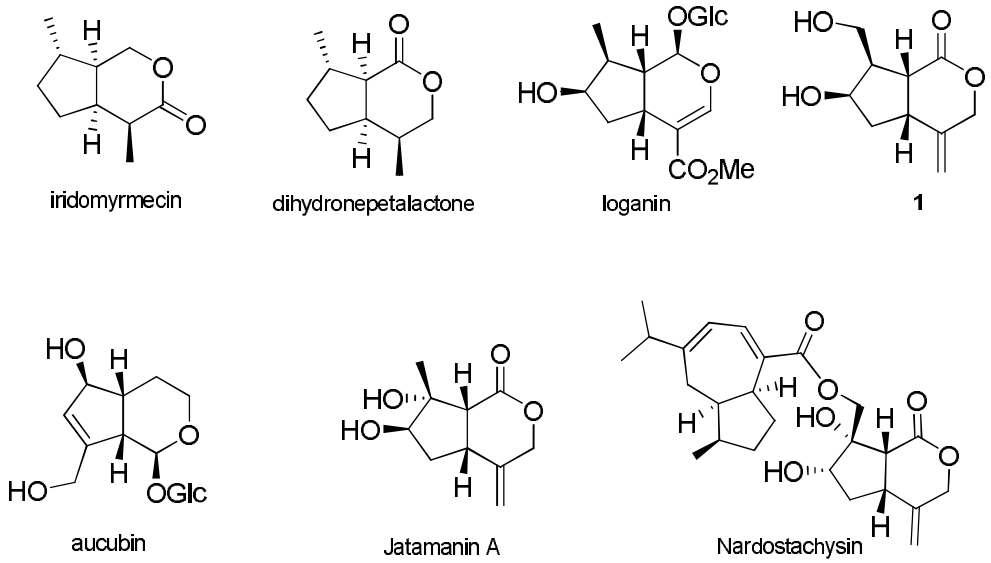
Figure 1. Structures of cyclopentanoids



The construction of five-membered carbocycles has attracted extensive attention among synthetic chemists. Considerable diverse methodologies emerged and were summarized in a series of reviews, which can be divided in two general categories: procedures based on the sequential functionalization of cyclopentanoid precursors, such as cyclopentene[2] and cyclopentadiene,[3] and

ring-formation routes from acyclic molecules, some being applied as key steps in the restructuring of readily available chiral materials, such as carbohydrates,[4] quinic acid,[5] and cyclohexadiene diols, into carbocycles. [6] The search for new, more efficient, and versatile antitumor, anticancer, antibacterial, antimicrobial, etc. agents is among the priorities of the worldwide science. In particular, a serious part of the efforts of the leading scientific and industrial chemical organizations nowadays is directed toward the synthesis of natural and biologically active products, and the improvement of the routes for the preparation of cyclopentanoid moieties is still growing at a steady pace.

Figure 2. Structures of iridoids



1. Iridoid

Iridoids are large class of naturally occurring compounds with over 300 members in the family

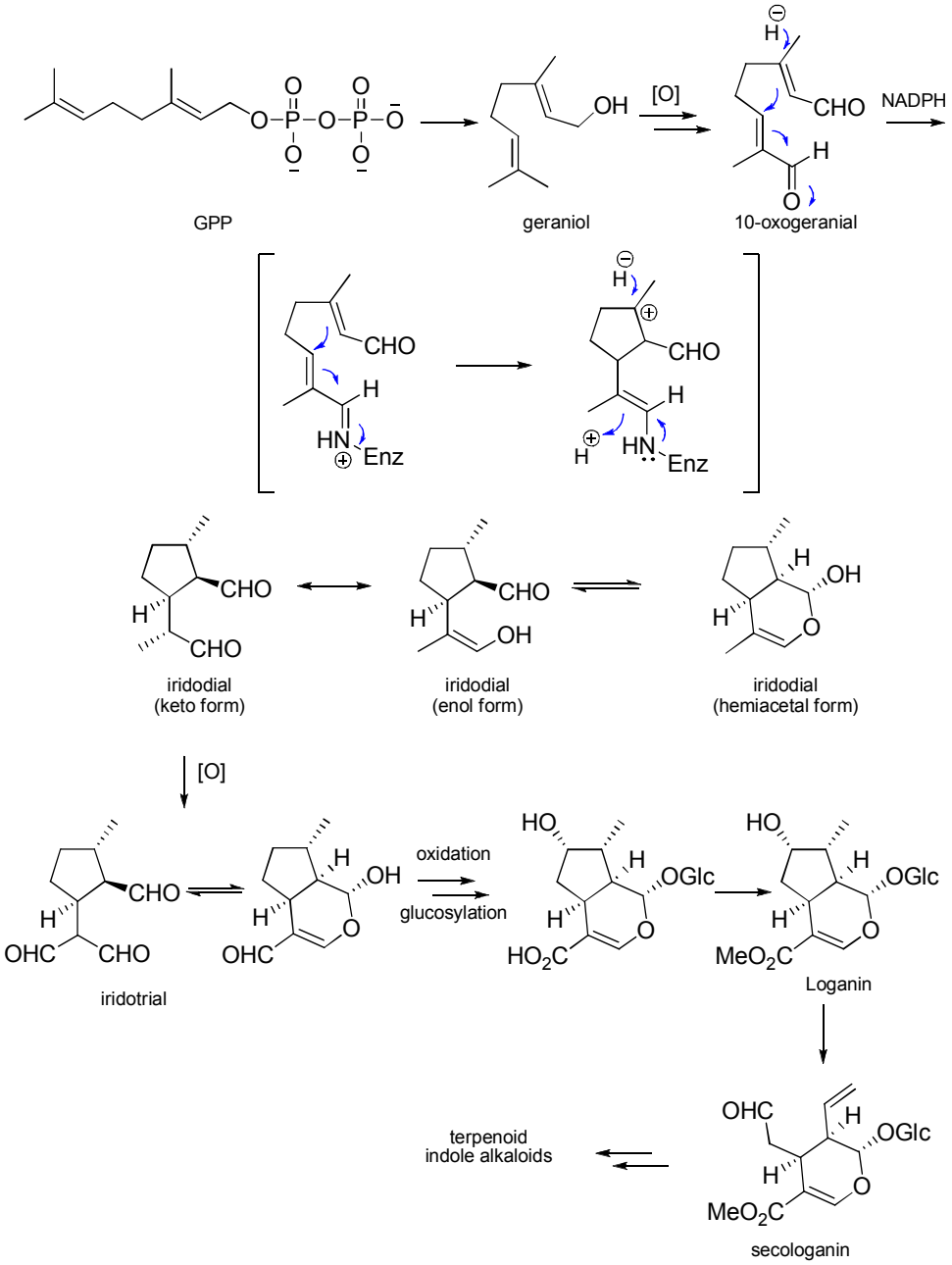
and still expanding. The first isolated iridoid was isolated from the secretion of ants belonging to the genus *Iridomyrmex,* the name of 'iridoid' was originated from that fact.[7] The iridoids have been found in a number of folk medicinal plants used as bitter tonics, sedatives, hypotensives, antipyretics, cough medicines, remidies for wounds and skin disorders. This fact encouraged to investigate the bioactivities of these phytochemicals. Structurally, iridoids represent a highly oxygenated monoterpenoids skeleton characterized by *cis*-fused cyclopenta[*c*]pyran. The representative structures of iridoids are presented at Figure 2.

The iridoid lactones can be further subdivided into two groups, type I and type II, depending on the regiochemical orientation of the lactone with respect to the functionalized cyclopentane ring. In type I iridoids typified by iridomyrmecin the lactone carbonyl is at C3, whereas in type II lactones such as dihydronepetalactone the carbonyl is at C1.

Proposed biosynthetic pathway is shown at Figure 3. Geraniol was produced by geranylpyrophosphate (GPP) by enzyme reaction. Geraniol underwent hydroxylation and oxidation reactions to 10-oxogeranial. The skeleton of iridoid arises from iridodial, which is formulated by cyclization as reductive Michael addition with hydride on the dialdehyde. Or imine formation by enzyme assisted carbocationic cyclization to cyclopentane.[8] The imine mediated pathway is more acceptable for the natural synthetic mode.

Iridodial was found to serve in the chemical defense system of many insect species,[9] and iridodial is the key precursor of numerous iridoids, possessing methyl group at C4, such as nepetalactone.[10] Further oxidized iridotrial has converted to loganin via oxidation, glucosylation and methylation. Loganin is a key intermediate in the pathway to a range of complex terpenoid indole alkaloids and tetrahydroisoquinoline alkaloids. And secologanin, which has been produced by oxidative cleavage from loganin, is a key intermediate for secoiridoids.

Figure 3. Proposed biosynthetic process for iridoids

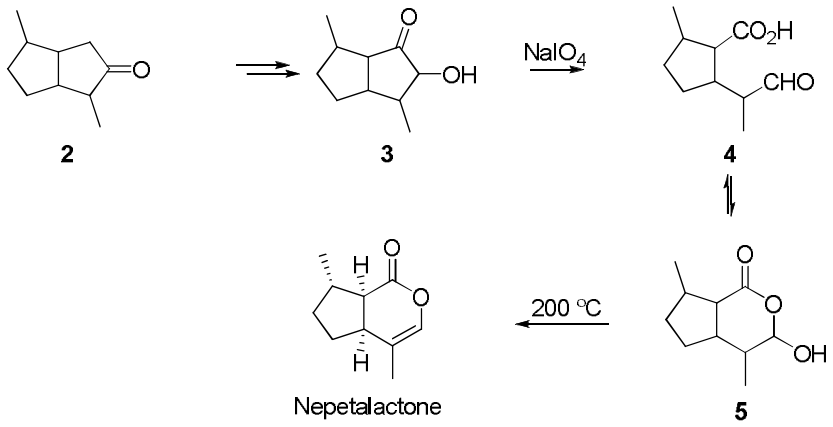


The various biological activities of iridoids have continuously attracted interests from both biologists and chemists. The unique *cis*-fused cyclopenta[*c*]pyran ring system has presented a variety of challenges for chemical synthesis and in the analysis of biological activities. Numerous syntheses of iridoids have been described in the literature.

1) Previous synthetic approaches for iridoid lactones

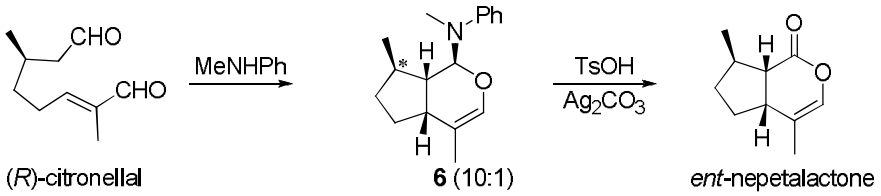
The representative synthetic approaches for type II iridoids are subscribed as below.
The key features of these syntheses involved the formation of cyclopentane and stereoselective introduction of pyran ring system to cylopentane.

Scheme 1. The first synthesis of nepetalactone



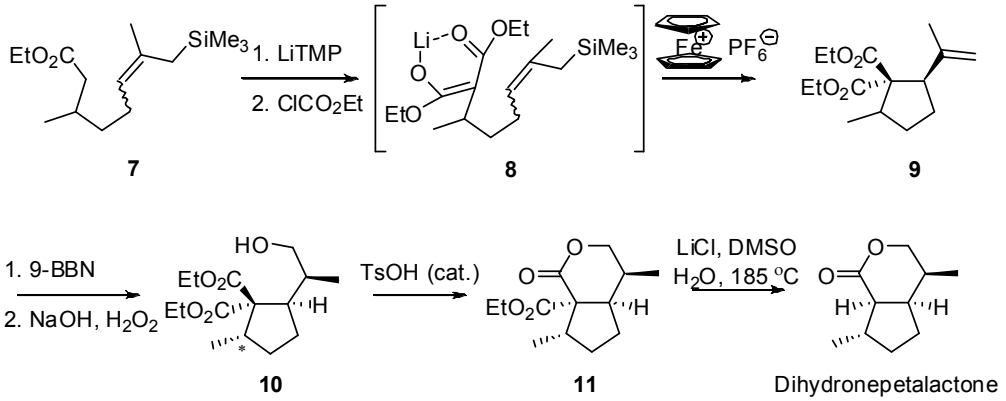
The first synthesized type II iridoid was nepetalactone.[11] Sakan et al. achieved α-ketol **3** as a key intermediate from bicyclooctanone [3.3.0] **2** and oxidative cleavage afforded to aldehyde acid **4**. The synthesis has accomplished by pyrolytic dehydration from lactol **5**. This piece of work was not a stereoselective synthesis. There are so many synthetic achieves since this synthesis has achieved. Here represent asymmetric syntheses by sigmatropic rearrangement.

Scheme 2. Synthesis of *ent*-(-)-nepetalactone



Schreiber et al. synthesized *ent*-nepetalactone via intramolecular enal-enamine cycloaddition [2+4].[12] (*R*)-citronellal treated with methyl phenylamine afforded enamine, and then furnished intramolecualr cycloaddition of enamine and enal provided a separable 10:1 mixture of the desired dihydropyran product **6** and its methyl epimer in 84% yield. Equilibration furnished a better 25:1 ratio of diastereomers, although in lower yields (60-65%). Then the dihydropyran **6** was hydrolyzed to unsaturated lactol, which was oxidized with silver carbonate (Fetizon oxidation) to afford *ent*-nepetalactone. This intramolecular cycloaddition strategy has inspired many other synthetic chemists.[13]

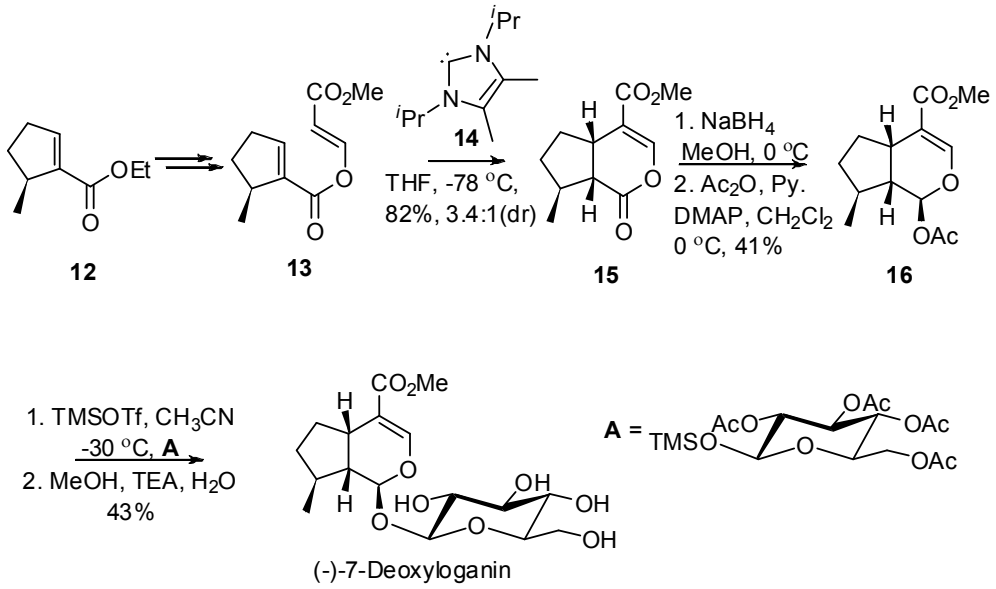
Scheme 3. Synthesis of nepetalactone via oxidative radical cyclization



Recently, numerous research papers have been published on the synthetic approaches to iridoids.

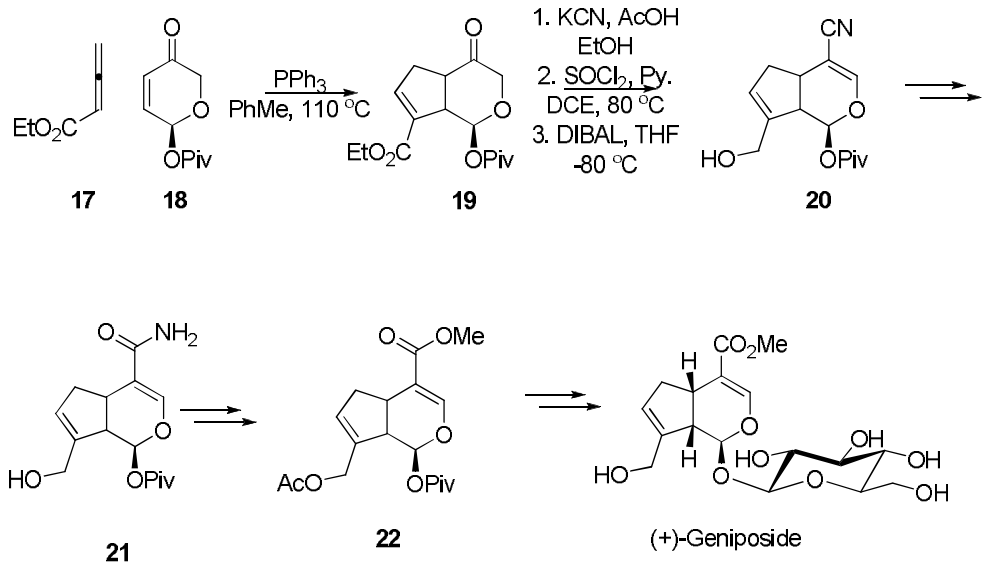
And the stereoselective synthesis of iridoid lactone has been carried out very successfully. As shown in Scheme 3, cyclopentane system embedded isoprene and ester **9** was synthesized via oxidative radical cyclization. [14] ω-Silylcitronellate **7** was subjected to the tandem alkoxycarbonylation/ oxidative cyclization reaction by deprotonation with lithium tetramethylpiperidide (LiTMP), ethylchloroformate and ferrocenium hexafluorohphosphate. The product cyclopentane **9** was isolated as a 2:1 *trans:cis*-diastereomeric mixture. The inseparable diastereomeric mixture was carried through to hydroboration/ oxidation to provide the separable alcohol **10**. The alcohol **10** was submitted to lactonization induced by catalytic amount of TsOH in CH_2Cl_2 to provide the lactone **11**. Then, Krapcho dealkoxycarbonylation afforded dihydronepetalactone.

Scheme 4. Synthesis of (-)-7-deoxyloganin via NHC catalyzed rearrangement of α,β-unsaturated enolester



Another synthetic tactic was *N*-heterocyclic carbene (NHC) rearrangemet with α,β-unsaturated enolester, which provided bicycle lactone system[15] (Scheme 4). NHCs have become universal ligands in organometallic and inorganic coordination chemistry.[16] Because of their specific coordination chemistry, NHCs both stabilize and activate metal centers in quite different key catalytic steps of organic syntheses, such as C-H activation, C-C, C-O, C-N bond formation. According to Candish and Lupton, NHC (**14**) catalyzed rearrangement with the α,β-unsaturated enolester **13** afforded bicyclic lactone **15** successfully, though the diastereomeric ratio was slightly low (3.4:1). Concommitant carbonyl reduction, glycosylation and global deprotection provided (-)-7-deoxyloganin.

Scheme 5. Synthesis of (+)-geniposide via phosphine-catalyzed [3+2] cycloaddition



17 **18** **19** **20** **21** **22**

The more recent approach for iridoid synthesis was contributed by Jones and Krische.[17] Phosphine-catalyzed [3+2] cycloaddition with ethyl-2,3-butadienoate **17** and α,β-unsatutated lactol **18** (Achmatowicz enone[18]) afforded bicyclic lactol **19** as shown in Scheme 5. This bicyclic lactol

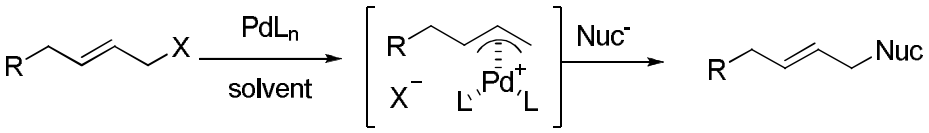
19 was converted to the cyanohydrins, which upon elimination furnished the α,β-unsatutated nitrile **20**. Nitrile converted to methyl ester **22** via primary amide **21**. Glycosidation of lactol employing the trichloroacetimidate as the glycosyl donor delivered the β-glucoside and then global deprotection provided (+)-geniposide.

Throughout these pieces of works, most synthetic efforts had been focused on the deoxygenated iridoids, since it is difficult to achieve to synthesize highly oxidized cyclopentane system enantioselectively. With this notion in mind, we took on the challenge in the iridoid lactone synthesis, which relies on our endeavor of Pd(0)-catalyzed cyclization method.

2. Pd(0)-catalyzed cyclization

Development of asymmetric metal-catalyzed reactions has played a significant role in allowing synthetic access to biologically important molecules. Since Pd(0) catalyzed alkylation has developed by Jiro Tsuji and co-workers, development of asymmetric metal-catalyzed reaction has been manipulated by a lot of chemist groups.[19] The most widely utilized reactions involve dihydroxylation, epoxidation or hydrogenation, and these reactions generate only one type of bond, either a C-O bond C-H bond. In contrast, asymmetric allylic alkylation reaction forms diverse type of bonds, such as C-C bond, C-N bond, C-S bond and C-O bond. Above all, palladium catalyzed cyclization has been utilized as one of the most powerful synthetic tools for carbocycle construction due to its highly versatile selectivities. The schematic introduction of Pd(0)-catalyzed allylic alkylation has shown in Figure 4.

Figure 4. Palldium catalyzed allylic alkylation

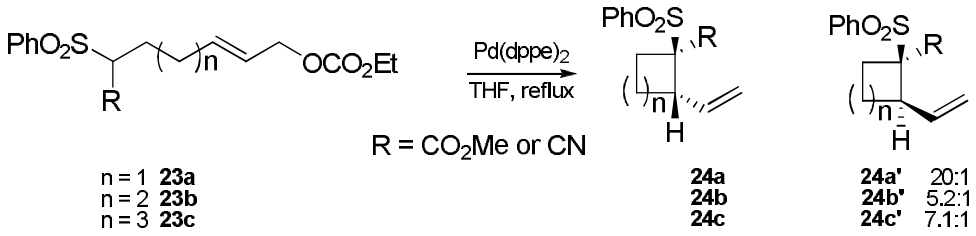


Formation of π-allylpalladium complex takes place by the oxidative addition of palladium to allylic compounds, such as allyl carbonates, allylic ester or halides. The π-allylpalladium complex is a resonance form of σ-allylpalladium complex and a coordinated π-bond. The π-allylpalladium complex formation involves inversion of stereochemistry, and the attack of the soft carbon mucleophile on the π-allylpalladium complex is also inversion.[20] Thus, the stereochemistry of product is retention.

1) Previous studies about Pd(0)-catalyzed cyclization

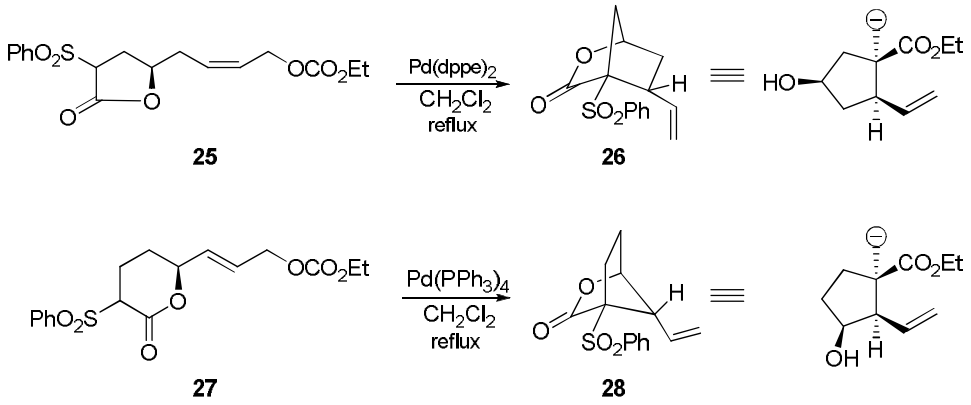
In our laboratory, it has developed Pd(0)-catalyzed intramolecular allylic alkylation. At first, Pd(0)-catalyzed cyclization reaction was investigated with the acyclic allyl precursors **23a-23c** (Scheme 6). Possessed two anion stabilizing groups, benzenesulfonate and ester, or nitrile, allyl carbonates afforded the 1,1,2-stereoselective triisubstitutued cyclobutane **24a**, cyclopentane **24b** and cyclohexane **24c** with good chemical yields.[21]

Scheme 6. Pd(0)-catalyzed allylic alkylation of acyclic allylic precursors



However, it is difficult to control of the stereogenic center at nucleophilic carbon possessing two anion stabilizing groups, because the stereocontrol at this center as well as the ultimate loss of the stereochemistry upon removal of the anion stabilizing auxiliary. Accordingly, 1,2-diastereocontrol of the new two stereogenic centers in Pd(0)-catalyzed cyclization of allylic precursor as well as retention of the established stereochemistries reamin as formidable tasks. To enhance the ability of application to natural products synthesis, the chiral hydroxyl group has introduced to alkyl chain, thus the concomitant γ-, or δ-lactone has provided. With γ-, or δ-lactone **25** or **27**, Pd(0)-catalyzed cyclization reaction was successfully performed to afford the bicyclic lactone **26** or **28** with high diastereoselectivity (Scheme 7). Particularly, desulfonylation of the bicyclic lactone **26** or **28** afforded trisubstituted cyclopentane with retention of stereochemistry. The functional groups at the trisubstituted cyclopentane systems are all *cis* relationship, which are laborious to synthesis. The synthetic utility of the optically active *cis*-trisubstituted cyclopentane obtained by this sequence has been demonstrated by some applications to the stereoselective synthesis of natural products.

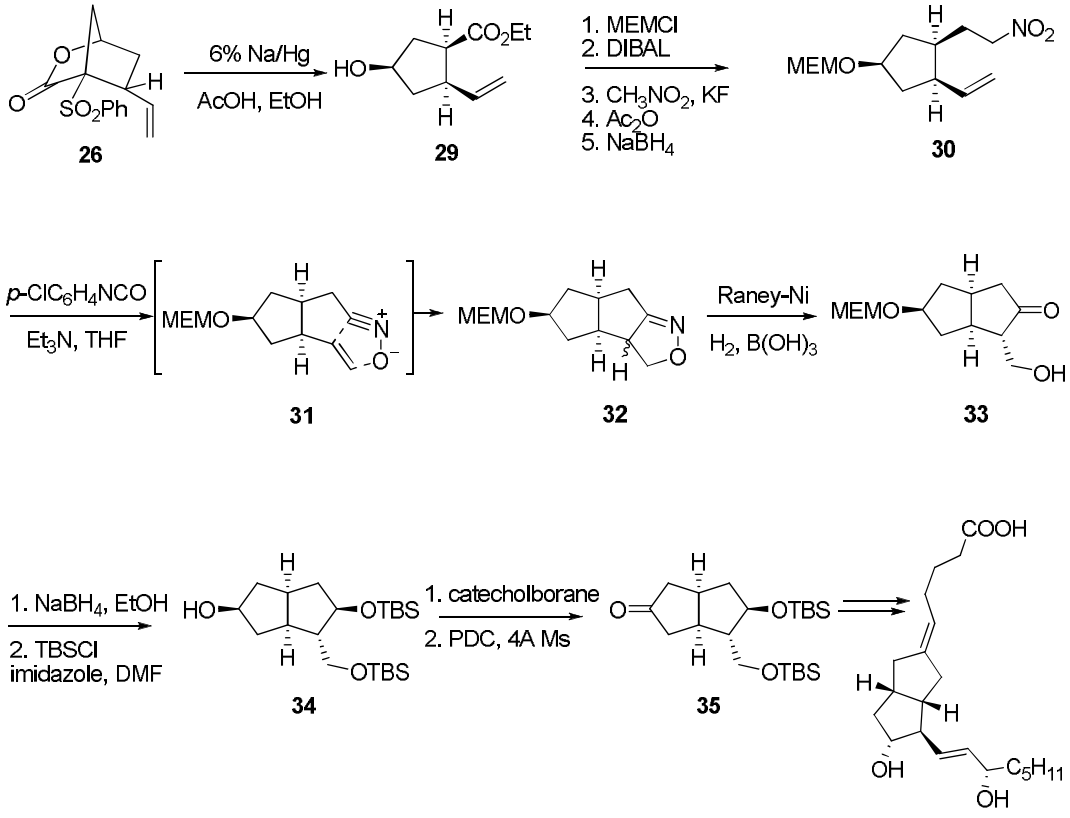
Scheme 7. Pd(0)-catalyzed cyclization with γ-lactone



2) Synthetic applications of bicyclic lactones

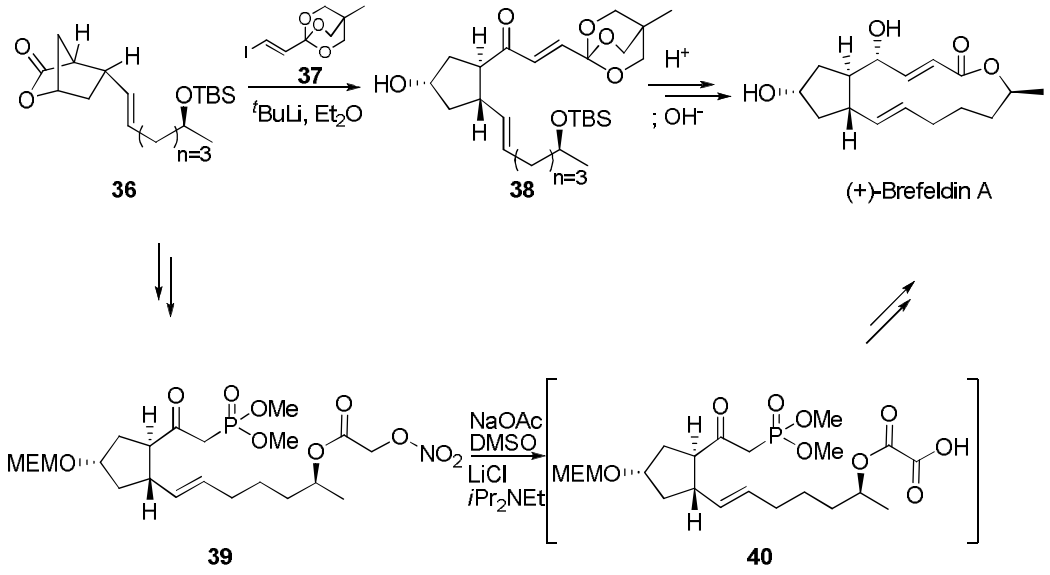
With these bicyclic lactones, the synthetic applications for natural products and bioactive compounds have been reported in our laboratory. The bicyclic lactone **26** was selectively converted to *cis*-trisubstituted cyclopentane system **29**. The *cis*-trisubstituted cyclopentane **29** was protected with methoxyethyl chloride then the intramolecular nitrile oxide-olefin cycloaddition afforded to the hydroxy ketone **33** via **32**. Stereoselective reduction of the ketone **33** afforded the cyclopentanol **34** and then straightforwardly transformed to known intermediate **35** of carbaprostacyclin[22] (Scheme 8).

Scheme 8. Synthetic applications with bicyclic lactone-formal synthesis of carbaprostacyclin



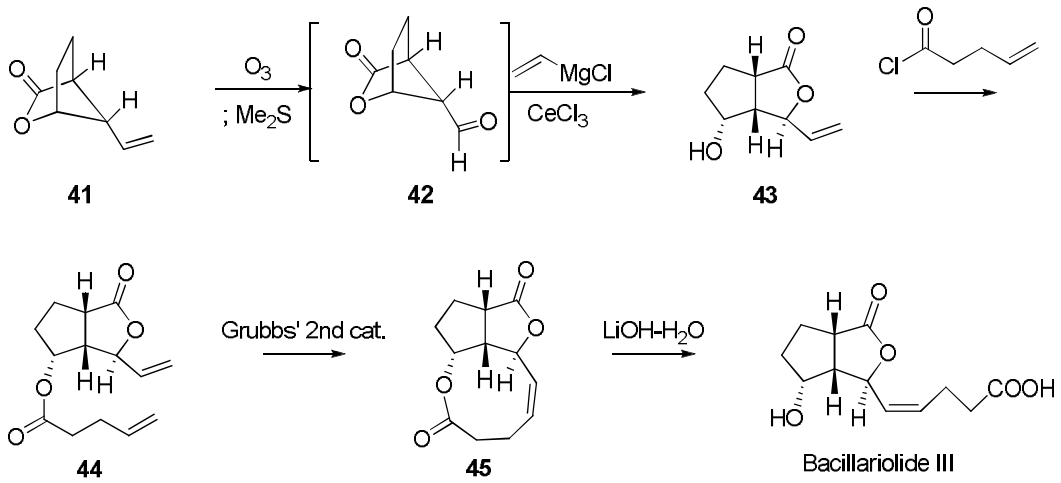
In our laboratory, the total synthesis of (+)-brefeldin A, employing the stereoselective Pd(0)-catalyzed cyclization has been reported. (Scheme 9) (+)-Brefeldin A has been one of the most attractive targets for the synthetic chemists due to its wide range of biological activities and characteristic structure. To introduce the three carbon synthon to lactone carbonyl at **36**, the (*E*)-1-(2-iodovinyl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**37**, OBO ortho ester) was adapted as an equivalent of the carboxyl group. Especially, the OBO ortho ester **37** was an excellent carboxyl equivalent, since it is resistant to attacks by strong nucleophiles and redily hydrolyzed under mild conditions.[23] On the other hand, as a part of ongoing studies directed toward the synthesis of the diversified brefeldin A analogs, more practical and versatile synthetic strategy was investigated. The salient feature of the synthesis includes the intramolecular HWE olefination of **40**.[24] Through this tactic, brefeldin A-amide analog synthesis was also accomplished.[25]

Scheme 9. Total synthesis of (+)-brefeldin A



From the bicyclic lactone **28**, the total synthesis of bacillariolide Ⅲ, which was isolated from the marine diatom *Pseudonitzschia multiseries*, was accomplished.[26] The key feature of this synthesis involves the stereoselective vinylcerium addition to bicyclic lactone aldehyde **42**, which induces its facile transformation into the bicyclic lactone skeleton **43** and efficient introduction of the (Z)-pentenoic acid side chain by RCM of **44** (Scheme 10).

Scheme 10. Total synthesis of bacillariolideⅢ

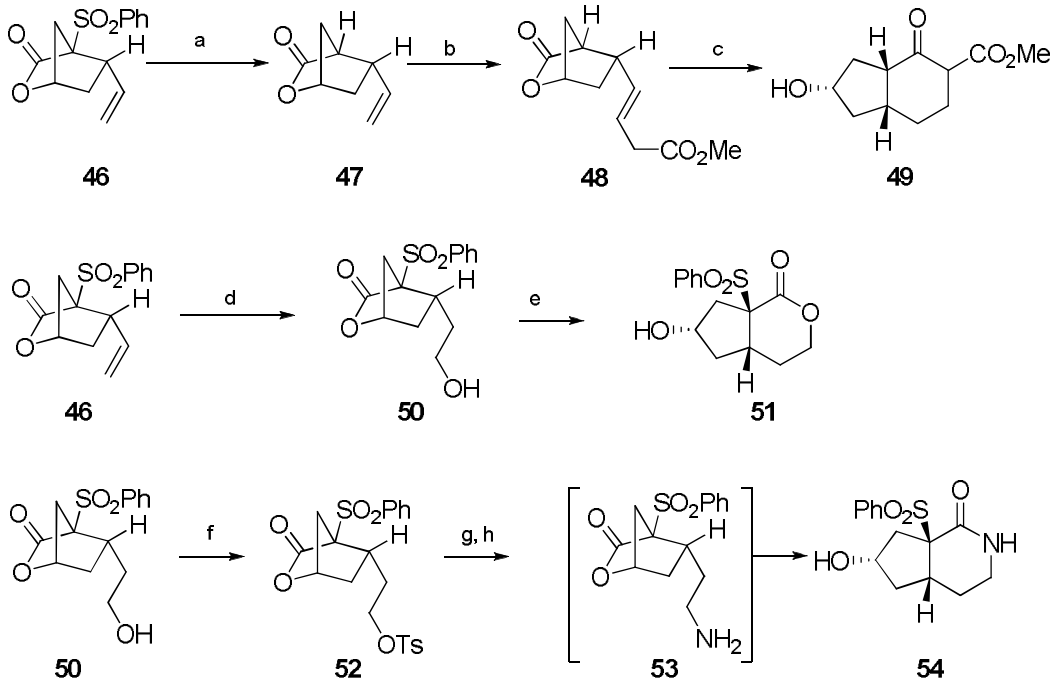


3) Transformation of bicyclic lactone

Also, the bicyclic lactone was easily transformed to other bicyclic systems (Scheme 9). At first, desulfonylated bicyclic lactone **46** underwent cross metathesis with acrolate, hydrogenation of **48** and then base promoted isomerization afforded 5-6 membered ring *cis*-fused carbocycle **49**. Secondly, hydroboration of vinyl afforded to the primary alcohol **50**, transesterification was accomplished to bicyclic lactone **51** by DBU. And the primary alcohol **50** was substituted to azide

via tosylate **52**, and successive Staudinger reaction gave the bicyclic lactam **54**. [27]

Scheme 11. Transformations of bicyclic lactone



Reagents: (a) Na/Hg, $B(OH)_3$, MeOH, 55%; (b) Grubbs' 2nd catalyst, methyl acrolate, CH_2Cl_2, 92%; (c) H_2, Pd/C, then KO^tBu, benzene, 70% for 2 steps; (d) catechol borane, $Rh(PPh_3)_3Cl$, THF, then H_2O_2, pH 7.0 buffer, 67% (e) DBU, CH_3CN, 72%; (f) TsCl, Et_3N, DMAP, CH_2Cl_2, 97%; (g) NaN_3, DMF, 88%; (h) PPh_3, wet THF, 68%.

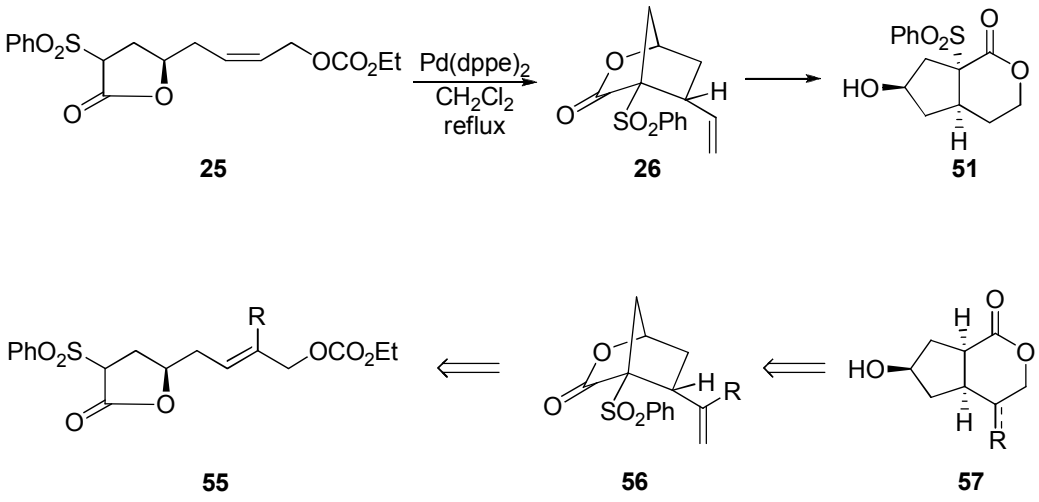
Throughout these transformations, it is well-known that the bicyclic lactone is an efficient synthetic intermediate, the synthetic approaches for natural products is on-going.

II. Result and discussions

1. Pd(0)-catalyzed cyclization with substituted allyl carbonate

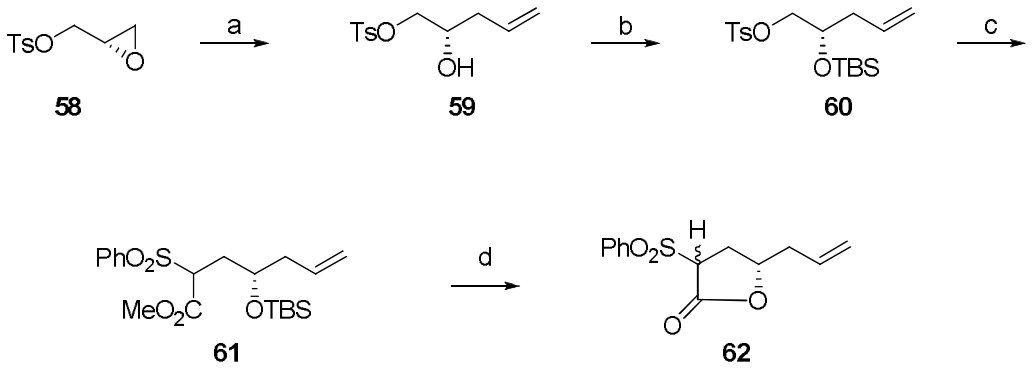
Previously, it has been reported that highly stereoselective construction of unique bicyclic lactones via intramoelcular Pd(0)-catalyzed cyclization has been accomplished in our lab. The [2.1.2] or [2.2.1] bicyclic lactones could be utilized as excellent equivalents of contra thermodynamic *cis*-trisubstituted cyclopentanes which have been importantly been utilized for total synthesis of bioactive natural products as described before. These applications implied high utility of the bicyclic lactone architectures. However, iridoids or other bioactive natural products, which have bicyclic structure embedded natural products, possess alkyl substituent at C4 (Figure 6). And to address these substituents after the bicyclic ring formation is a laborious task. Hence, the development of more transformative bicyclic lactone was needed. That has driven us to focus on the investigation of the more transformative bicyclic lactone. Thus we envisaged the branched allyl carbonate for Pd(0)-catalyzed cyclization.

Figure 5. Synthetic approach for branched allyl carbonate



As shown in Figure 5, substituted vinyl bicyclic lactone **56** could be transformation to bicyclic lactone **57**, which has substituent at C4. The branched allyl carbonate γ-lactone **55** would be obtained via cross metathesis with γ-lactone and diverse allyl carbonates. The synthesis of γ-lactone is shown below (Scheme 12).

Scheme 12. Synthesis of γ-lactone

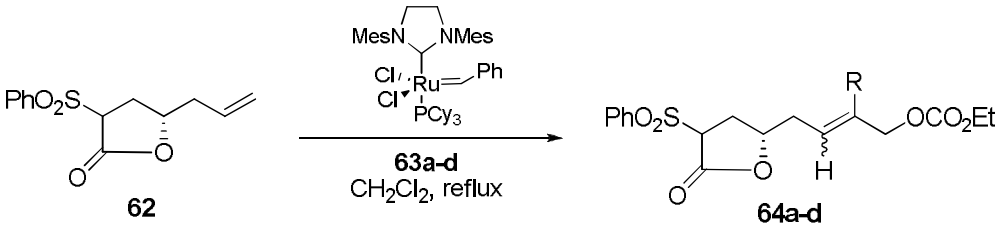


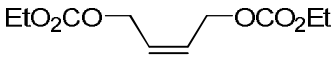
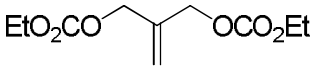
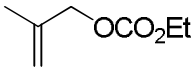
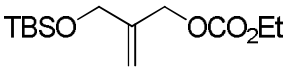
Reagents: (a) vinylmagnesium bromide, Li_2CuCl_4, THF; (b) imidazole, TBSCl, DMF, 0 ºC, 88% for 2 steps; (c) methyl benzenesulfonyl acetate, NaH, DMF, 80 ºC, 77%; (d) TBAF, THF, 78%.

Vinyl addition to tosylate glycidol **58** and TBS protection afforded **60**, alkylation with methyl benzenesulfonate and TBS deprotection gave γ-lactone **62** (Scheme 12). At the alkylation step, diastereomeric mixture was obtained and the concomitant γ-lactone **62** was also inseparable diastereomeric mixture. γ-Lactone **62** underwent cross metathesis with allyl carbonates afforded precursors of Pd(0)-catalyzed cyclization (Table 1).

The cross metathesis product **63a** and **64b** were obtained *E* isomers dominantly, but **63d** was obtained *E/Z* mixtures, which were inseparable. Thus, with these branched allyl carbonate γ-lactone, we proceeded Pd(0)-catalyzed cyclization at the same conditions.

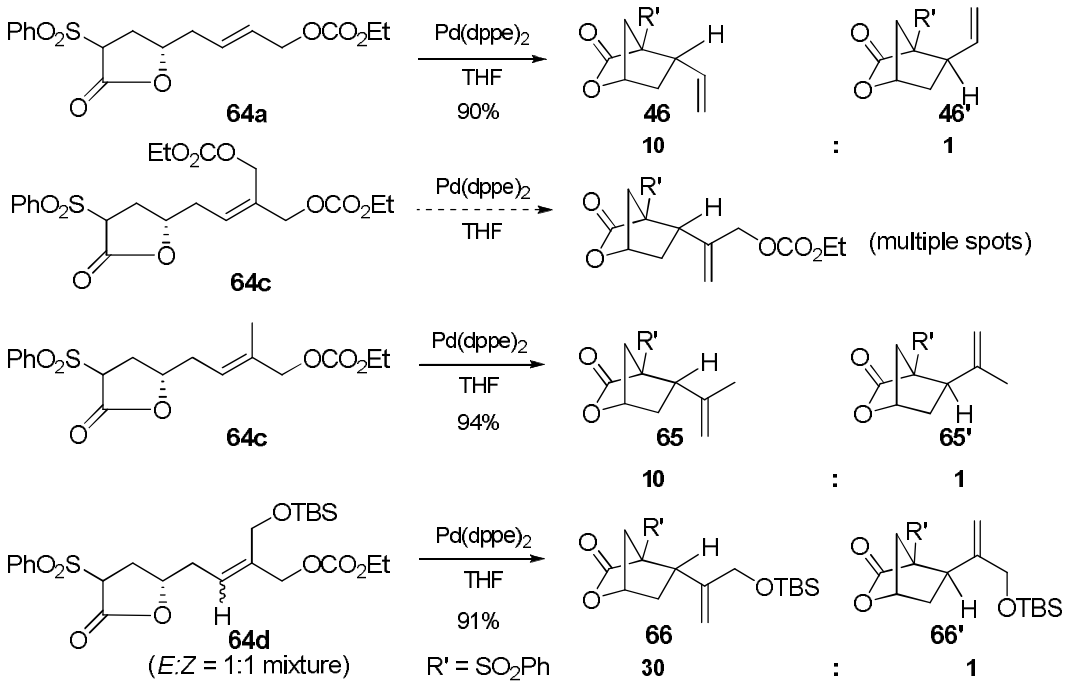
Table 1. Cross metathesis with γ-lactone



Entry	Cross metathesis partner	Yield	product
1	 **63a**	48	**64a**
2	 **63b**	49	**64b**
3	 **63c**	33	**64c**
4	 **63d**	43	**64d**

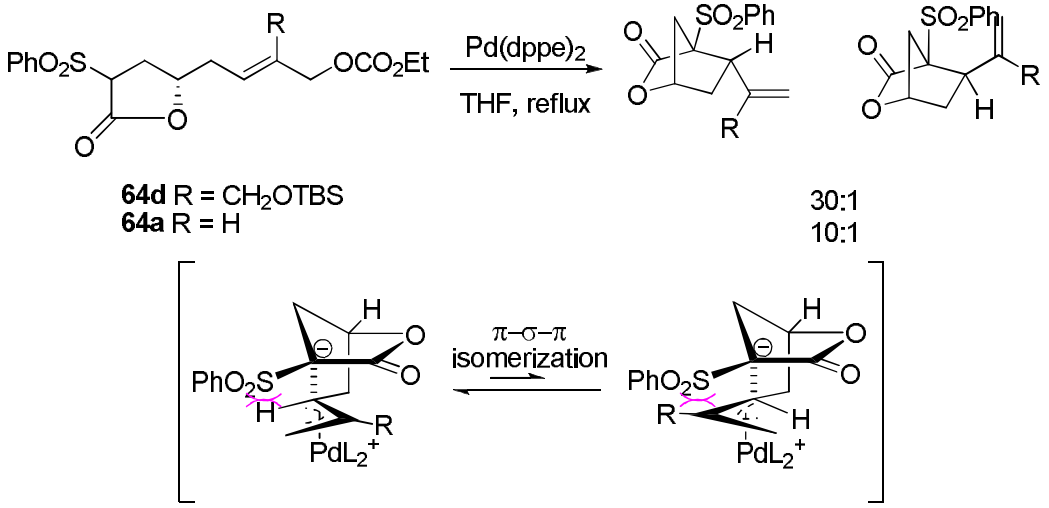
Similar to linear allyl carbonate, metallyl carbonate and hydroxymethyl allyl carbonate were represented good precursors for Pd(0)-catalyzed cyclization. These branched allyl carbonates underwent Pd(0)-catalyzed cyclization with excellent chemical yields and diastereoselectivities from 10:1 to 30:1 (Scheme 13). Exceptionally, diallylcarbonate **64b** did not afford desired bicyclic lactone. It seemed that initially diallylcarbonate **64b** underwent the Pd(0)-catalyzed cyclization, but further unexpected reactions occurred with the allyl carbonate at bicyclic lactone, provided multiple unidentified products.

Scheme 13. Pd(0)-catalyzed cyclization with branched allyl carbonate



The diastereomeric ratio was enhanced with branched allyl carbonate, which would explain the steric repulsion between benzensulfonate and methylene or hydrogen as shown in Figure 6. The excellent stereoselectivity is likely due to the presence of the silyloxymethyl substituent, given the lower stereoselectivity (10:1) for the disubstituted alkene **64a** under the same conditions as shown in Figure 6. In particular, the *E/Z* isomers of **64d** were executed the Pd(0)-catalyzed cyclization with high diastereoselectivity, which proved the Pd(0)-catalyzed C-C bond cleavage/formation sequence would reinforce high diasteroselectivity of the cyclization process for [2.1.2] bicyclic lactone followed by π-σ-π isomerization. This type of facial preference is well supported by our previous report.[28] And the stereochemistry of benzenesulfonyl group was negligible, because of α-deprotonation with basic condition.

Figure 6. Plausible transition state of Pd(0)-catalyzed cyclization

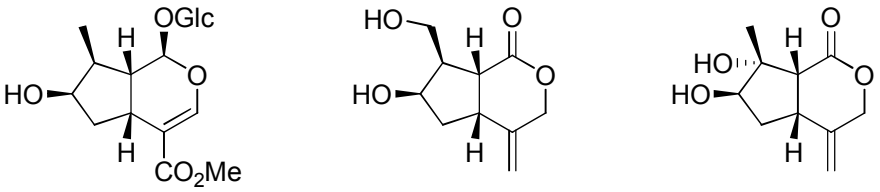


2. Total synthesis of natural iridoid lactone

In the cyclopentanoids, we have been interested in a recently reported iridoid lactone, which is structurally related to loganin, and consists of a C7-substituted C6-hydroxycyclopenta[*c*]lactone, as illustrated in Figure 8. The unique structures and biological activities of iridoid lactone was concerned the synthetic interests. Iridoid lactone **1** was isolated from the roots and rhizomes of *Nardostachys chinensis* Batalin (Valerianaceae).[29] This plant has been used as a Chinese folk medicine and its roots and rhizomes exhibit a variety of biological activities, such as sedative, antimalarial, antinociceptive, cytotoxic, and enhancement of nerve growth factor activities.[30] However, the paucity of its key component **1** has hampered the systematic research of the iridoid, including structure-activity relationship (SAR) analysis and further pharmacological studies. Thus, we have worked on the development of an efficient synthetic route to the new iridoid lactone **1** (Figure 8). The iridoid lactone **1** is structurally very interests. It has contiguous, various functional groups, such as hydroxyl, hydroxymethyl, *exo*-methylene, and lactone. Moreover, the 4-

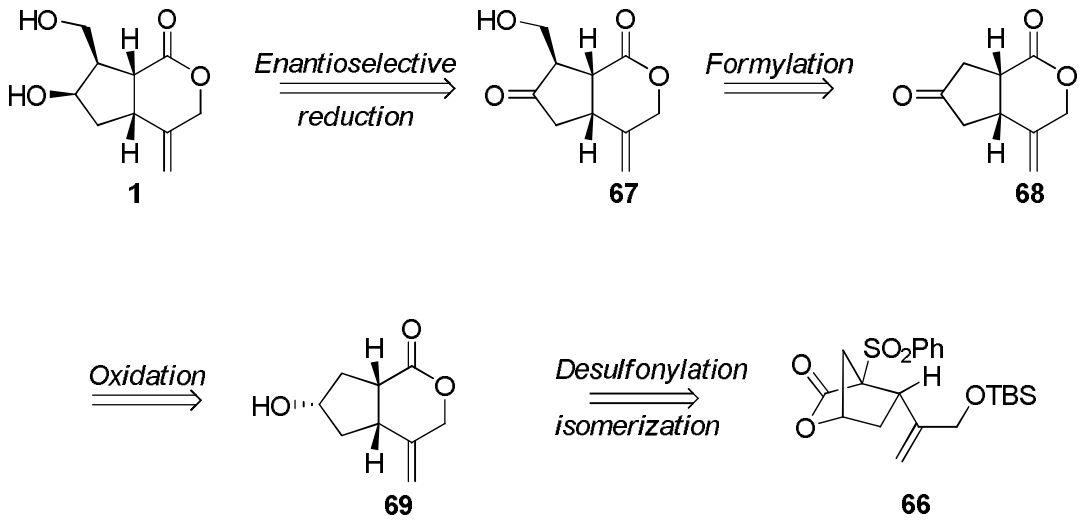
stereogenic centers are contiguous. This challengeable structure as well as the curiosity for biological activity led us to synthesis.

Figure 7. Target iridoid lactone



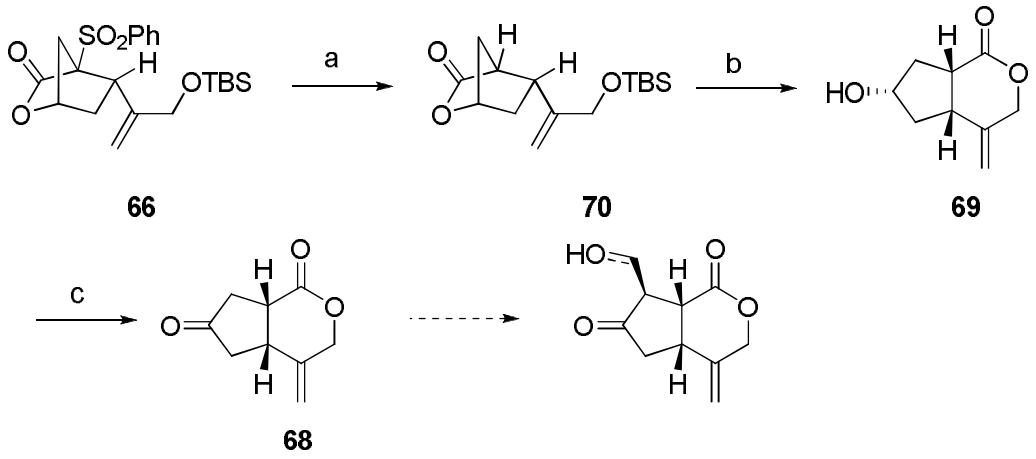
To establish the bicyclic lactone structure, we planned to synthesize the bicyclic lactone via Pd(0)-catalyzed cyclization. Our retrosynthetic approach for iridoid lactone **1** is illustrated in Scheme 14. We hypothesized that the bicyclic lactone **66** might serve as a synthetic precursor of iridoid lactone through three potentially straightforward transformations: (a) oxidation of cyclopentanol **69** to cyclopentanone **68**, (b) regioselective α-formylation, and (c) enantioselective reduction of C6 carbonyl group.

Scheme 14. Retrosynthetic approach



With the bicyclic lactone **66** in hand, we attempted to isomerize bicyclic system for the iridoid skeleton. Desulfonylated bicylcic lactone was isomerized to concomitant bicyclic lactone **69** and oxidized to cyclopentanone **68**. To address the regioselective hydroxymethylation, various reaction conditions were considered. A direct α-hydroxymethylation with HCHO[31] or the introduction of a hydroxymethyl equivalent via α-methylenation with Eschenmoser's salt[32] as well as formylation with $HCO_2CH_2CF_3$[33] to the cyclopentanone did not provide satisfactory results.

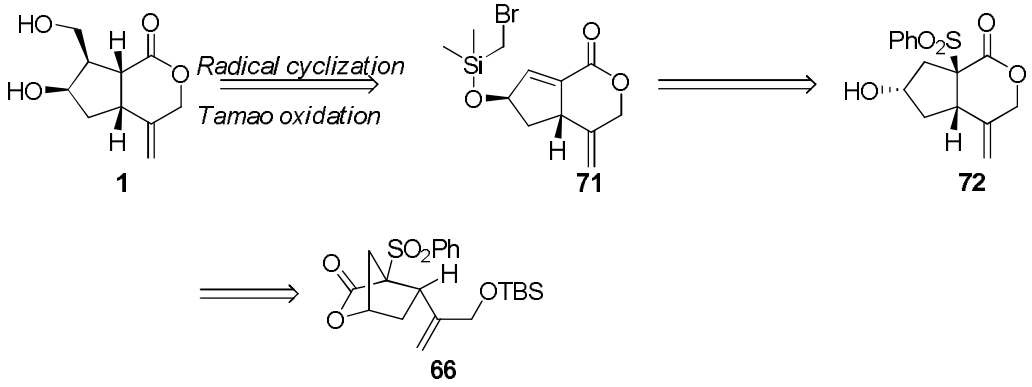
Scheme 15. Synthesis of bicyclic pentanone



Reagents: (a) Na/Hg, $B(OH)_3$, MeOH, 56%; (b) TBAF, THF, 73%; (c) TPAP, NMO, CH_2Cl_2, 67%.

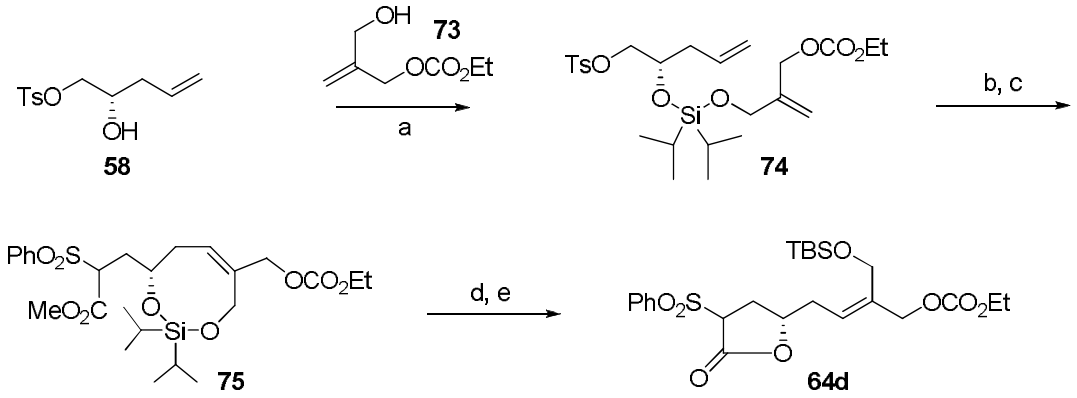
Throughout the results, it turned out that the regioselective direct α-formylation or hydroxymethylation was not successful for our system. It was suspected that the hydrogens at ring junction were labile at basic condition. As a matter of fact, the allylic hydrogen at C5 of **68** was easily deprotonated with general bases. So we envisaged cyclopentenone to establish the regio-, and chemoselectivity for hydroxymethylation.

Scheme 16. Revised retrosynthetic approach



The process involves the efficient regio- and stereoselective elaboration of the C7 hydroxymethyl substituent through the silicon-tethered intramolecular radical cyclization[34] of cyclopentene system **71**, followed by Tamao-Fleming oxidation.[35] Cyclopentanol **72** is generated by functional group transformation from bicyclic lactone **66**, which can be efficiently constructed by the palladium-catalyzed cyclization of allylic carbonate.

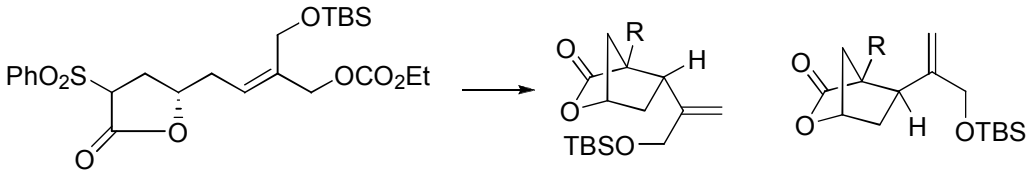
Scheme 17. Synthesis of γ-lactone 64d



Reagents: (a) $^{i}Pr_2SiCl_2$, imidazole, CH_2Cl_2, 0 ºC, 78%; (b) methyl benzenesulfonyl acetate, NaH, DMF, 80 ºC, (c) Grubbs' 2nd catalyst, CH_2Cl_2, reflux, 67% for 2 steps; (d) TBAF, THF; (e) TBSCl, imidazole, CH_2Cl_2, 76% for 2 steps.

The synthesis commenced with the preparation of the requisite γ-lactone **64d** for Pd(0)-catalyzed allylic alkylation, as illustrated in Scheme 17. To address the tri-substituted alkene unit, we initially attempted olefin cross metathesis of the vinyl γ-lactone with the hydroxymethyl substituted allyl carbonate to produce the Pd(0)-catalyzed cyclization precursor. However, intermolecular cross metathesis led to γ-lactone **64d** with only 60% yield. Thus, we turned our attention to silicon-tethered ring-closing metathesis (RCM),[36] which is considered superior in terms of entropic benefit and concomitant alcohol protection. In addition, (*Z*)-form alkene was obtained solely. That is because (*Z*)-conformation is favorable at 8-membered cyclic compound.

Table 2. Pd(0)-catalyzed cyclization of 66



64d **66** **66'**

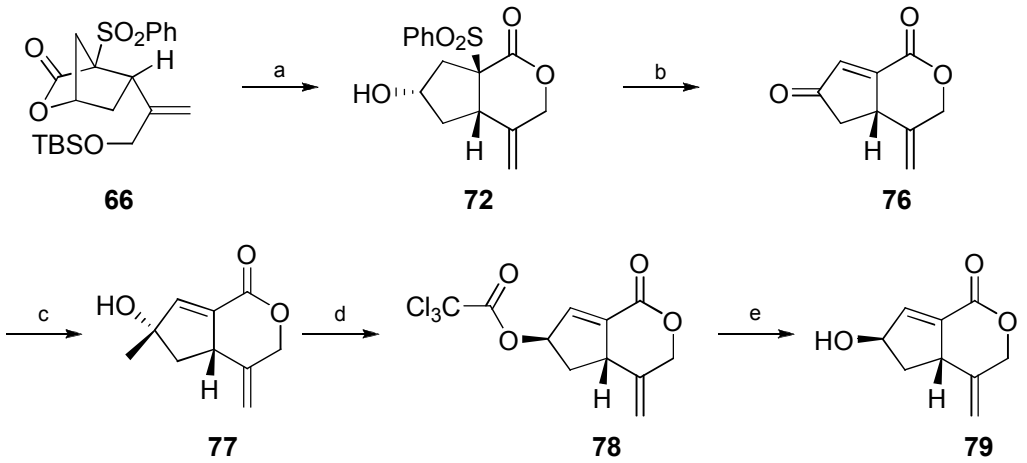
Entry	Catalyst	solvent	Yield[a]	Ratio[b] (**66**:**66'**)
1	$Pd(dppe)_2$	THF	91	30:1
2	$Pd(dppe)_2$	CH_2Cl_2	90	3.5:1
3	$Pd(PPh_3)_4$	THF	91	1:1
4	$Pd(PPh_3)_4$	CH_2Cl_2	90	2.5:1
5	$Pd(PPh_3)_4$	DMSO	78	1:1
6	$Pd(dppp)_2$[c]	THF	78	10:1
7	$Pd(dppb)_2$[c]	THF	89	25:1

All reactions were conducted in reflux condition. [b]Isolated yields. [c]Determined by 300-MHz ^{1}H-NMR spectra of the crude diastereomeric mixtures. [d] Pre-mixed $Pd(OAc)_2$ and dppp/dppb in THF was added to a solution of **64d**.

Alkoxysilane **74** was prepared from the known hydroxy tosylate **58** with a 78% yield. Alkylation of tosylate **74** with the anion of methyl phenylsulfonyl acetate and subsequent RCM provided the cyclic *bis*-alkoxysilane **75**. Removal of the tethered silicon of *bis*-alkoxysilane **75** with TBAF and the spontaneous relactonization of the resulting hydroxy ester provided the γ-lactone intermediate, which was protected with TBSCl to afford allylic carbonate **64d**.

We next conducted a survey of stereoselective Pd(0)-catalyzed cyclization of **64d** under a variety of reaction conditions as summarized in Table 2. Stereoselective cyclization of **64d** in the presence of $Pd(dppe)_2$ in THF afforded the desired product of **66** with a 91% yield along with a minor product **66'** (entry 1). Comparison with the result at Scheme 13, at this time, the precursor **64d** was only *Z*-isomer and the cyclization result was same as the result with *E/Z* mixtures. That might be the evidence of π-σ-π isomerization. Cyclization of **64d** in the presence of $Pd(PPh_3)_4$ resulted in low stereoselectivity regardless of the solvent (entry 3-5). Cyclization in the presence of $Pd(OAc)_2$ with dppp or dppb (entry 6, 7) also showed good stereoselectivities, although the yield was slightly lower.

Scheme 18. Attempt with bicyclic system

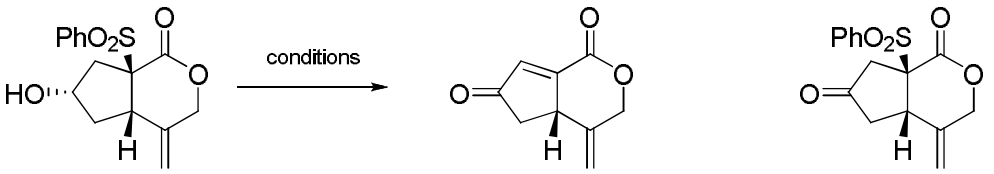


Reagents: (a) TBAF, THF, rt, 2 h, 80%; (b) DMP, rt, 16 h, CH_2Cl_2, 88%; (c) CBS, $BH_3 \cdot SMe_2$, CH_2Cl_2, 45-80%; (d) Cl_3CCOOH, DIAD, PPh_3, THF; (e) SiO_2, THF, $MeOH/H_2O$, 20% for 2 steps.

The oxidation and concomitant β-elimination of sulfonate afforded to cyclopentenone **76**.

The desulfonylation for making a variety of double bonds has been used in natural products synthesis as a potential procedure. The reductive elimination of sulfonyl group has been carried out by using aluminium amalgam, sodium amalgam, and zinc-acetic acid,[37] and many examples of base-induced desulfonylation to α,β-unsaturated carbonyl compounds have been reported.[38] Several attempts for cyclopentenone via oxidation and concomitant β-elimination of sulfonate have described as below (Table 3).

Table 3. Oxidation to cyclopentenone



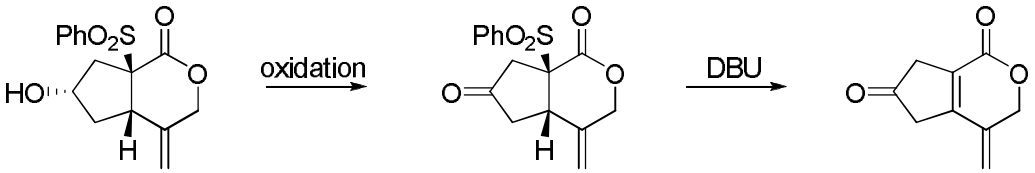
72 **76** **80**

condition	Yield **76**	**72**	**80**
DMP, $NaHCO_3$, CH_2Cl_2	56%		30%
$(COCl)_2$, Et_3N, DMSO	-	80%	-
TFAA, Et_3N, DMSO			67%
TPAP, NMO, CH_2Cl_2	-	100%	-
PCC, silica gel, CH_2Cl_2	-	-	85%
DMP, CH_2Cl_2	88%	-	-

With the basic conditions, such as Swern oxidation with oxally chloride or Ley oxidation, the β-elimination as well as the oxidation of alcohol was not executed, the cyclopentanol **72** was recovered. Interestingly, in PCC oxidation condition or Swern oxidation with TFAA, the oxidation of cyclopentanol **72** to cyclopentanone **80** was accomplished successfully, however β-elinmination of sulfonate did not occur. Moreover, after oxidation of cyclopentanone **80** followed by basic work

up with DBU, product **81** was obtained by the β-elinmination of sulfonate with allylic proton at C5 (Scheme 19).

Scheme 19. Basic work up with cyclopentanone

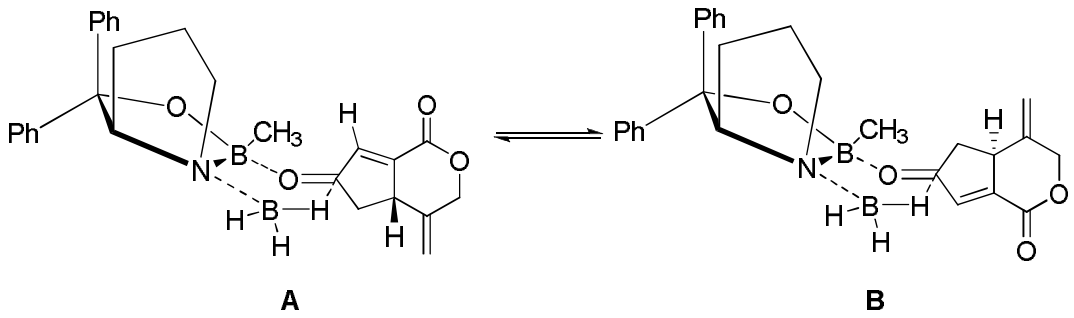


72 **80** **81**

Gratifyingly, the oxidation of cyclopentanol with *Dess-Martin* periodinane yielded cyclopentenone **76** in acidic condition. When $NaHCO_3$ was co-treated for neutral condition, the β-eliminated product **76** was obtained only 56% and cyclopentanone **80** was obtained about 30%. However, when *Dess-Martin* periodinane was only treated the desired product **76** was obtained upto 88% as sole product. There are a few examples for β-elinmination of sulfonate in acidic condition.[39]

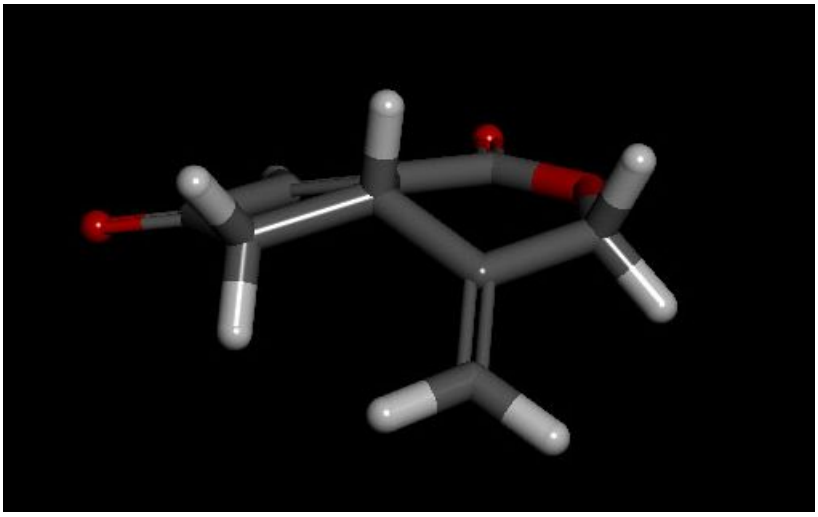
With the cyclopentenone **76** in hand, the enantioselective reduction of cyclopentenone to cyclopentenol **79** was attempted (Scheme 18). As shown at Figure 8, we expected the coordination of cyclopentenone **76** with (*S*)-Me-CBS oxazaborolidine would be preferred **B** because of the steric interaction with methyl group.[40]

Figure 8. Bicyclopentenone coordinated with (*S*)-CBS oxazaborolidine



However, the enantioselective reduction of cyclopentenone to cyclopentenol was not successful with the bicyclic system. It seemed that the 3D structure of bicyclic lactone might interfere with the approach of hydride from the concave face (Figure 9). Considering the result that reduction of cyclopentenone **76** with (*R*)-CBS afforded the same cyclopentenol **77** as sole product in 74% yield, we assured the the face selectivity had relevance to the structure of bicyclic system.

Figure 9. 3D-structure of bicyclic enone

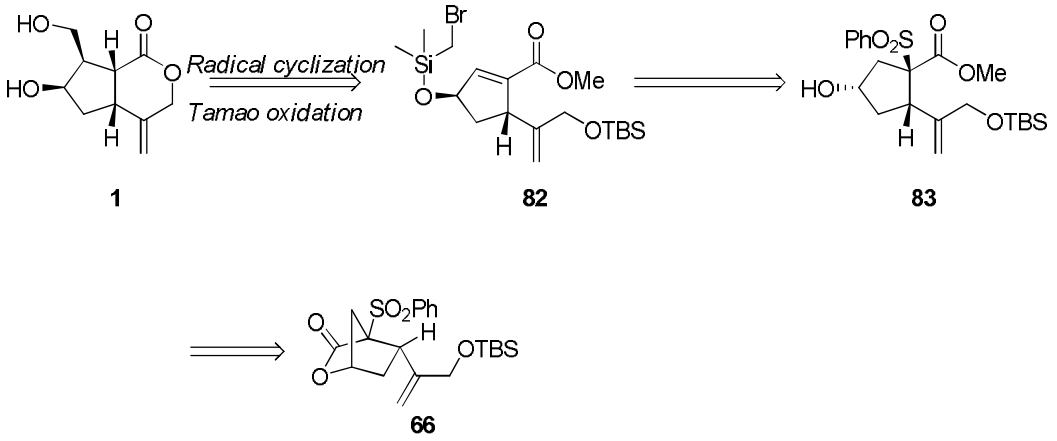


With the cyclopentenol **77**, Mitsunobu reaction with Cl_3CCO_2H and acidic hydrolysis[41] afforded

the desired cyclopentenol **79**, although the chemical yield of 2-step was too low (Scheme 18).

So instead of the bicyclic system, we envisaged the monocyclic system. Instead of relactonization of bicyclic lactone **66** to bicyclic lactone **72**, transesterification with sodium methoxide might provide cyclopentanol **83** (Scheme 20).

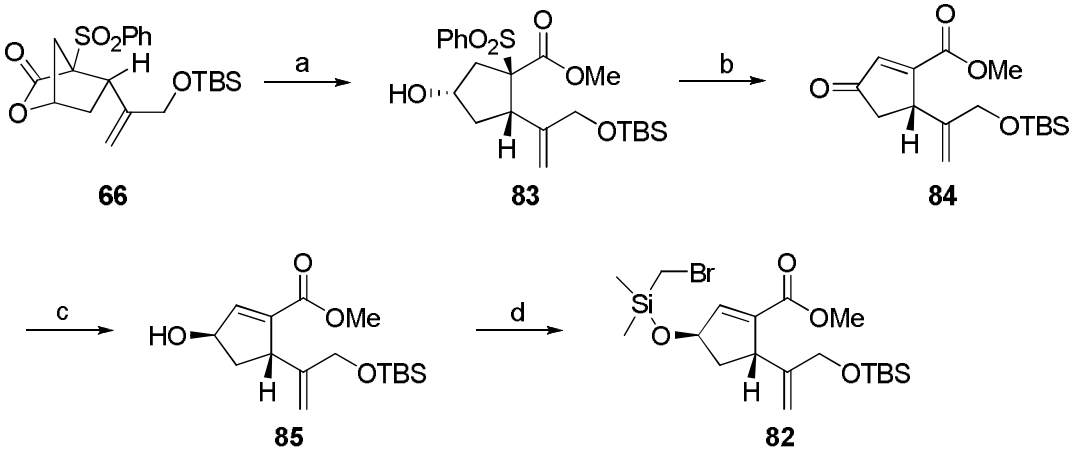
Scheme 20. Revised retrosynthetic approach



1 **82** **83** **66**

With the requisite building block **66** in hand, we focused our attention to the facile introduction of the hydroxymethyl substituent at C7. Convenient methanolysis of bicyclic lactone **66** afforded cyclopentanol **83**, which was subjected to Dess-Martin oxidation to produce enone **84** after concomitant β-elimination of sulfonate (scheme 21). Stereoselective reduction of cyclopentenone **84** with (*S*)-Me-CBS afforded cyclopentenol **85** with the correct stereochemistry.

Scheme 21. Synthesis of radical cyclization precursor 82

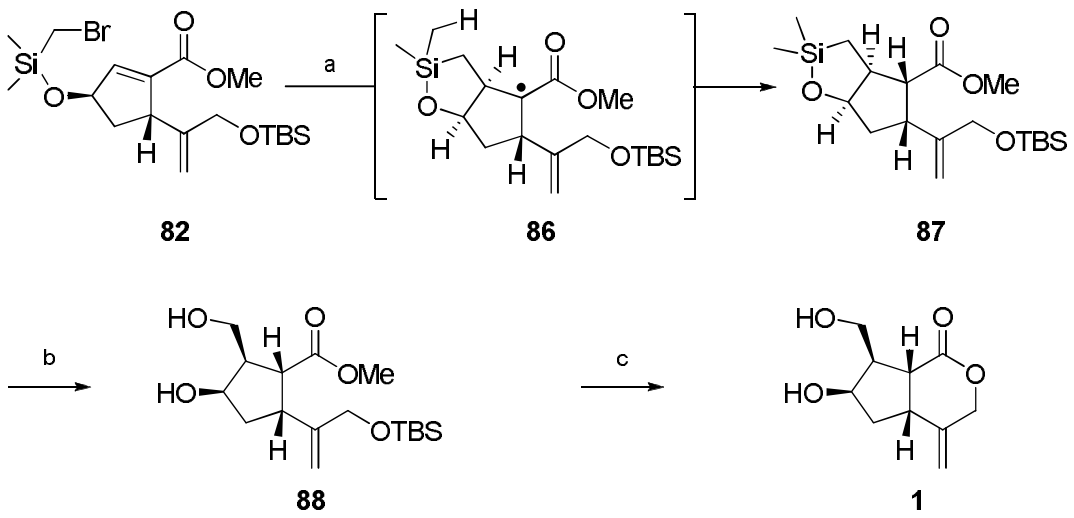


Reagents: (a) NaOMe, MeOH, 93%; (b) *Dess-Martine* periodinane, CH_2Cl_2, 91%; (c) (*S*)-CBS, $BH_3 \cdot SMe_2$, CH_2Cl_2, 68%; (d) (Bromomethyl) dimethylchlorosilane, DMAP, Et_3N, benzene, 0 ºC, 85%.

Silylation of cyclopentenol **85** with (bromomethyl) dimethylchlorosilane yielded the bromosilyl ether **82**, which was directly subjected to the radical cyclization (Scheme 22). Intramolecular radical cyclization of the labile silyl ether intermediate with Et_3B[42] and Bu_3SnH afforded the desired *cis*-fused oxasilole **87** in good yield. Organoboranes are widely recognized as excellent sources of free-radicals and can participate in free-radical chain reactions.[43] In particular, over classical initiators, Et_3B offers the great advantage of being efficient even at low temperature (-78 ºC), thus it proved to be particularly important for the development of stereoselective radical reactions and for radical reactions involving thermally unstable adducts or products.[44] The mechanistic aspect has been recognized that the nucleohemolytic substitution reaction.[45] With Et_3B, very reactive radical initiator as referred above, the initiation step was allowed at 0 ºC to ambient temperature, the *cis*-fused oxasole ring was produced, which stereogenic center was derived by the cyclopentenol's stereochemistry. And the newly generated C8 stereogenic center

could be established by an intramolecular 1, 6-hydrogen atom transfer from the proximal silylmethyl group to C8.

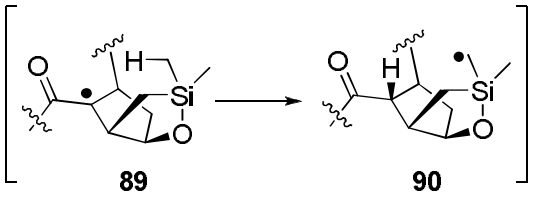
Scheme 22. Completion of synthesis of iridoid lactone 1



Reagents; (a) Et_3B, Bu_3SnH, benzene, 0 ºC; (b) $KHCO_3$, H_2O_2, KF, THF/MeOH, 60% for 2 steps; (c) $TsOH \cdot H_2O$, CH_2Cl_2, 66%.

According to the Little et al, the fused ring system allows the intramolecular 1,6 hydrogen transfer to the radical center.[46] In the radical quenching portion of the 5-exo, trig cyclization step via an intramolecular hydrogen atom transfer from the proximal silylmethyl group of silacycle **89** to the radical center located C8 at **90**. Once transferred, the silylmethly radical **90** is presumably quenched intermolecularly by tributyl tin hydride to afford an oxasilole (Figure 10).

Figure 10. 1, 6 hydrogen transfer



Subsequent Tamao-Fleming oxidation of oxasilole **87** gave diol **88**. Finally lactone iridoid **1** was obtained in good yield by TBS deprotection with TsOH,[47] followed by a concomintant lactonization. The structure of **1** was confirmed by comparison of its spectral data with those of the authentic sample (optical rotation, ^{1}H-NMR, ^{13}C-NMR, IR, HRMS).[48]

III. Conclusion

We has developed the useful method for synthesizing trisubstituted cyclopentane systems via Pd(0)-catalyzed cyclization. In particular, branched allyl carbonated γ-lactone yielded diastereoselective bicyclic lactone with good chemical yield. With the bicyclic lactone, we accomplished the first total synthesis of (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydro-cyclopenta[*c*]pyran-1(3*H*)-one in 13 steps. The key features of the synthesis involve the stereoselective and efficient preparation of the cyclopentanol intermediate *via* Pd(0)-catalyzed cyclization and then the silicon-tethered radical cyclization followed by Tamao-Fleming oxidation for the stereoselective introduction of the C7-hydroxymethyl substituent. This versatile synthetic approach is expected to be widely utilized for the synthesis of related iridoids and iridoalkaloids.

IV. Experimental

General procedure

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran and Et_2O were distilled from sodium benzophenone ketyl. Dichloromethane, triethylamine, acetonitrile and pyridine were freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried at 100 ºC. Air and moisture sensitive reactions were performed under argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Optical rotations were measured with JASCO DIP-1000 or JASCO P-1020 digital polarimeter at ambient temperature using 100 mm cell of 2 mL capacity. Infrared spectra were recorded on a Perkin-Elmer 1710 FT-IR spectrometer. Mass spectra were obtained with VG Trio-2 GC-MS instrument. High resolution mass spectra were obtained with JEOL JMS-AX 505WA instrument. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 (300 MHz), Varian Gemini 2000 (300 MHz), Brucker Analytik AVANCE digital 400 (400MHz), or AVANCE digital 500 (500MHz) spectrometer as solutions in deuteriochloroform ($CDCl_3$). Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane and are referenced to the deuterated solvent ($CHCl_3$). ^1H-NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad singlet; m, multiplet and/or multiple resonance), number of protons, and coupling constant in hertz (Hz).

(*S*)-2-Hydroxypent-4-enyl 4-methylbenzenesulfonate (59)

To a solution of lithium tetrachlorocuprate (0.1 M solution in THF, 6.6 ml, 0.66 mmol), vinylmagnesium bromoide (1.0 M solution in THF, 17 ml, 17 mmol) was added at -40 ℃. After stirred for 1 h, a solution of tosylated glycidol (2.96 g, 13 mmol) in THF (35 ml) was added dropwise by dropping funnel *ca.* 1 h at -40 ℃ and stirred for 1 h. The reaction mixture was quenched with aqueous NH_4Cl and warmed to ambient temperature and then, extracted with ether. The aqueous layers were extracted with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 3.19 g (96%) of hydroxy tosylate **59** as a colorless oil: $[\alpha]_D^{25}$ 3.24 (*c* 0.1, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.76 (dd, 2H, *J* = 6.6 Hz), 7.32 (d, 2H, *J* = 8.07 Hz), 5.77-5.64 (m, 1H), 5.05 (m, 2H), 4.0 (m, 1H), 3.92-3.83 (m, 2H), 2.41 (s, 3H), 2.26-2.13 (m, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 145.0, 132.8, 132.5, 129.9, 127.9, 118.7, 73.0, 68.5, 60.4, 37.3, 21.0; IR (neat) ν_{max} 3541, 2921, 1598, 1357, 1178, 1097, 978, 922, 818, 668 cm^{-1}; LRMS (FAB) *m/z* 257 ($M + H^+$); HRMS (FAB) Calcd for $C_{12}H_{17}O_4S$ ($M + H^+$): 257.0769, found 257.0848.

(*S*)-2-(*tert*-Butyldimethylsilyloxy) pent-4-enyl 4-methylbenzenesulfonate (60)

To a solution of hydroxy tosylate **59** (9.0 g, 33.3 mmol) and imidazole (3.4 g, 50.0 mmol) in DMF (30 ml) was added tertiarybutyl dimethylsilyl chloride (7.5g, 50.0 mmol) at 0 ℃. After stirred for 5 h, the reaction mixture was quenched with water and warmed to ambient temperature and then, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 11.0 g (90%) of silyl ether **60** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.76 (d, 2H, *J* = 8.43 Hz), 7.32 (d, 2H, *J* = 7.86 Hz), 5.76-5.62 (m, 1H), 5.02-4.96 (m, 2H), 3.90-3.78(m, 3H), 2.42 (s, 3H),

2.22-2.02 (m, 2H), 0.81 (s, 9H), -0.04 (d, 6H, J = 1.2 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 144.8, 140.9, 133.2, 129.8, 128.0, 120.8, 72.6, 69.5, 38.7, 25.7, 21.6, 18.0, -4.7, -4.8; IR (neat) ν_{max} 2931, 2857, 1463, 1364, 1255, 1181, 983, 835, 665 cm^{-1}; LRMS (FAB) *m/z* 371 (M + H$^+$); HRMS (FAB) Calcd for $C_{18}H_{31}O_4S$ Si(M + H$^+$): 371.1712, found 371.1705.

(4*S*)-Methyl 4-(*tert*-butyldimethylsilyloxy)-2-(phenylsulfonyl) hept-6-enoate (61)

To a suspension of NaH (4.8 g, 120 mmol) in DMF (10 ml) was added benzenesulfonyl acetate (23.7 g, 120 mmol) in DMF (30 ml) at 0 ºC. After stirred for 3 h, to the reaction mixture was added **60** (11 g, 30 mmol) in DMF (40 ml) at 0 ºC, and then heated to 80 ºC for 7 days. The reaction mixture was quenched with water, and cooled to ambient temperature and then, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 20) to afford 9.5 g (77%) of silyl ether **61** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.83-7.51 (m, 5H), 5.04-4.95 (m, 2H), 4.19-4.05 (m, 1H), 3.62 (s, 3H), 0.83 (s, 9H), 0.01 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.2, 134.1, 133.1, 129.2, 129.0, 118.1, 77.1, 69.6, 68.7, 67.5, 52.7, 41.8, 33.3, 25.7, 17.9; IR (neat) ν_{max} 2953, 2857, 1743, 1444, 1325, 1149, 836, 778 cm^{-1}; LRMS (FAB) *m/z* 413 (M + H$^+$); HRMS (FAB) Calcd for $C_{20}H_{32}O_5S$ Si (M + H$^+$): 413.1818, found 413.1813.

(5*S*)-5-Allyl-3-(phenylsulfonyl) dihydrofuran-2(3*H*)-one (62)

To a solution of **61** (5.7 g, 13.8 mmol) in THF (70 ml) was added TBAF (1.0 M in THF, 27.5 ml, 27.5 mmol). After stirred for 16 h, the reaction mixture was quenched with saturated NH_4Cl, and then, extracted with EtOAc. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford

3.85 g (78%) of lactone **62** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.94-7.86 (m, 2H), 7.69-7.63 (m, 1H), 7.54 (t, 2H, *J* = 7.59 Hz), 5.65 (m, 1H), 5.13 (d, 2H, *J* = 8.43 Hz), 4.79-4.40 (m, 1H), 4.01 (t, 0.4H, *J* = 9.69 Hz), 4.00 (dd, 0.6H, *J* = 10.1 Hz, 3.1 Hz), 3.00 (dq, 0.6H, *J* = 14.7 Hz, 3.3 Hz), 2.75-2.25 (m, 3.4H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167, 136, 134, 131, 130, 129, 120, 77, 64, 39, 28; IR (neat) ν_{max} 2938, 1769, 1448, 1321, 1189, 934, 687 cm^{-1}; LRMS (FAB) *m/z* 267 ($M + H^+$); HRMS (FAB) Calcd for $C_{13}H_{15}O_4S$ ($M + H^+$): 267.0691, found 267.0688.

(*Z*)-But-2-ene-1,4-diyl diethyl dicarbonate (63a)

To a solution of 1,4-butene diol (10.0 g, 119.5 mmol) in CH_2Cl_2 (100 ml) were added pyridine (21 ml, 262.9 mmol) and ethyl chloroformate (28 ml, 298 mmol) at 0 ºC. After stirred for 4 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 3) to afford 24 g (76%) of dicarbonate **63a** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.23 (s, 2H), 4.55 (s, 4H), 4.10 (q, 4H, *J* = 7.14 Hz), 1.19 (t, 6H, *J* = 7.14 Hz); LRMS (FAB) *m/z* 255 ($M + Na^+$).

Diethyl 2-methylenepropane-1,3-diyl dicarbonate (63b)

To a solution of 2-methylene-1,3-propan diol (1.0 g, 11.3 mmol) in CH_2Cl_2 (20 ml) were added pyridine (2 ml, 25 mmol) and ethyl chloroformate (2.7 ml, 28.3 mmol) at 0 ºC. After stirred for 1 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford 2.7 g (100%) of dicarbonate **63b** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.23 (s, 2H), 4.55 (s, 4H), 4.10 (q, 4H, *J* = 7.14 Hz), 1.19 (t, 6H, *J* =

7.14 Hz); IR (neat) ν_{max} 2922, 1743, 1475, 1150, 926; cm^{-1}; LRMS (FAB) *m/z* 255 ($M + Na^+$).

Ethyl 2-Methylallyl carbonate (63c)

To a solution of methallyl alcohol (1.0 g, 13.9 mmol) in CH_2Cl_2 (25 ml) were added pyridine (1.44 ml, 18 mmol) and ethyl chloroformate (1.6 ml, 16.6 mmol) at 0 ºC. After stirred for 1 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford 1.37 g (68%) of dicarbonate **63c** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.21 (s, 1H), 5.20 (s, 1H), 4.60 (s, 2H), 4.10 (m, 2H), 1.37 (t, 3H, *J* = 7.14 Hz), 0.99 (s, 3H), 0.89 (s, 9H), 0.02 (s, 6H); LRMS (FAB) *m/z* 145 ($M + H^+$).

2-((*tert*-Butyldimethylsilyloxy) methyl)allyl ethyl carbonate (63d)

To a suspension of NaH (438 mg, 11 mmol) in THF (5 ml) was added diol (965 mg, 11 mmol) and stirred for 1 h. The TBSCl was then added, and vigourous stirring was continued for 1 h. the mixture was poured into ether, washed with 10% aqueous K_2CO_3 and brine, dried ($MgSO_4$) and concentrated *in vacuo.* To the crude silyl ether in CH_2Cl_2 (15 ml) were added pyridine (1.1 ml, 14.3 mmol) and ethyl chloroformate (1.3 ml, 13.2 mmol) at 0 ºC. After stirred for 1 h, the reaction mixture was quenched with water, extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 5) to afford 2.4 g (80% for 2 steps) of dicarbonate **63d** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.21 (s, 1H), 5.20 (s, 1H), 4.60 (s, 2H), 4.10 (m, 4H), 1.37 (t, 3H, *J* = 7.14 Hz), 0.86 (s, 9H), 0.02 (s, 6H); IR (neat) ν_{max} 2853, 1687, 1443, 1149, 836 cm^{-1}; LRMS (FAB) *m/z* 275 ($M + H^+$).

Ethyl -4-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl) but-2-enyl carbonate (64a)

To a mixture of lactone **62** (100 mg, 0.38 mmol) and diethyl carbonate **63a** (262 mg, 1.13 mmol) in CH_2Cl_2 (6 ml) was added Grubbs' 2nd catalyst (32 mg, 0.038 mmol). The reaction mixture was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 67.5 mg (48%) of **64a** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.93-7.51 (m, 5H), 5.69-5.66 (m, 2H), 4.81-4.72 (m, 0.7H), 4.51 (d, 2H, *J* = 4.0 Hz), 4.19-4.0 (m, 3.3H), 3.02-3.0 (m, 0.7 Hz), 2.77-2.66 (m, 0.3H), 2.58-2.23 (m, 3H), 1.27-1.16 (m, 3H); IR (neat) ν_{max} 2955, 1745, 1449, 1261, 1193, 1009, 722 cm^{-1}; LRMS (FAB) *m/z* 369 (M + H$^+$).

Diethyl 2-(2-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl)ethylidene)propane-1,3-diyl dicarbonate (64b)

To a mixture of lactone **62** (100 mg, 0.38 mmol) and dicatbonate **63b** (262 mg, 1.13 mmol) in CH_2Cl_2 (5 ml) was added Grubbs' 2nd catalyst (32 mg, 0.038 mmol). The reaction mixture was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 88 mg (49%) of **64b** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.9-7.5 (m, 5H), 5.44 (d, 1H, *J* = 7.4 Hz), 4.8-4.7 (m, 1H), 4.49-4.45 (m, 2H), 3.08-3.02 (m, 1H), 2.58-2.28 (m, 4H), 1.58 (s, 3H), 1.23 (m, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 155.0, 136.7, 134.6, 129.5, 129.2, 78.8, 72.3, 64.8, 33.2, 29.0, 14.2, 14.1; LRMS (FAB) *m/z* 383 (M + H$^+$).

Ethyl -2-methyl-4-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl)but-2-enyl carbonate (64c)

To a mixture of lactone **62** (395 mg, 1.48 mmol) and methyally catbonate **63c** (214 mg, 1.48 mmol) in CH_2Cl_2 (10 ml) was added Grubbs' 2nd catalyst (62.8 mg, 0.074 mmol). The reaction mixture

was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 184 mg (33%) of **64c** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.9-7.5 (m, 5H), 5.44 (d, 1H, *J* = 7.4 Hz), 4.8-4.7 (m, 1H), 4.49-4.45 (m, 2H), 3.08-3.02 (m, 1H), 2.58-2.28 (m, 4H), 1.58 (s, 3H), 1.23 (m, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 155.0, 136.7, 134.6, 129.5, 129.2, 78.8, 72.3, 64.8, 33.2, 29.0, 14.2, 14.1; IR (neat) ν_{max} 2922, 1743, 1475, 1319, 1150, 1008, 728 cm^{-1}; LRMS (FAB) *m/z* 383 ($M + H^+$); HRMS (FAB) Calcd for $C_{18}H_{23}O_7S$ ($M + H^+$): 383.1165, found 383.1162.

2-((*tert*-Butyldimethylsilyloxy) methyl)-4-((2*S*)-5-oxo-4-(phenylsulfonyl)tetrahydrofuran-2-yl)but-2-enyl ethyl carbonate (64d)

To a mixture of lactone **62** (194 mg, 0.73 mmol) and silyloxyallyl catbonate **63d** (200 mg, 0.73 mmol) in CH_2Cl_2 (10 ml) was added Grubbs' 2nd catalyst (31 mg, 0.036 mmol). The reaction mixture was refluxed for 4 h, and then concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 230 mg (43%) of **64d** as a yellowish oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.0-7.5 (m, 5H), 5.5 (t, 1H, *J* = 7.4 Hz), 4.8-4.4 (m, 3H), 4.29-4.0 (m, 4H), 3.0-2.3 (m, 5H), 1.25 (m, 3H), 0.85 (s, 9H), 0.01 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.4, 155, 137.5, 134.7, 129.6, 129.3, 127.9, 123.2, 78.9, 68.9, 64.7, 62.5, 58.6, 33, 29, 25.7, 18.3, 14.2.

(*Z*)-2-((*tert*-Butyldimethylsilyloxy)methyl)-4-((2*S*)-5-oxo-4-(phenylsulfonyl)tetrahydrofuran-2-yl)but-2-enyl ethyl carbonate (64d)

To a solution of allyl alcohol **S75** (2.4 g, 6.0 mmol) in CH_2Cl_2 (40 mL) at 0 ºC were added TBSCl (904 mg, 6.0 mmol), imidazole (443 mg, 6.5 mmol). After stirring for 3 h, the reaction mixture was quenched with H_2O and extracted with CH_2Cl_2 and aqueous layer was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was

purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 3.07 g (99%) of silyl ether **64d** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.92 (dd, 2H, *J* = 7.4 Hz, 20 Hz), 7.68 (t, 1H, *J* = 7.4 Hz), 7.56 (t, 2H, *J* = 7.8 Hz), 5.52 (t, 1H, *J* = 7.4 Hz), 4.78-4.46 (m, 1H), 4.60 (d, 1H, 8.8Hz), 4.22-4.05 (m, 5H), 3.02-2.74 (m, 1H), 2.55 (m, 2H), 2.68-2.30 (m, 1H), 1.28-1.20 (m, 3H), 0.85 (m, 9H), 0.05 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.4, 167.1, 154.9, 138.3, 137.8, 136.8, 136.7, 134.7, 134.6, 129.6, 129.4, 123.2, 122.7, 78.6, 68.9, 64.8, 64.0, 58.8, 58.7, 33.2, 32.8, 28.7, 28.2, 25.8, 18.2, 17.4, 17.2, 14.3, 12.8; IR (neat) ν_{max} 2926, 2856, 1744, 1516, 1458, 1321, 1258, 1151, 1083, 840, 781 cm^{-1}; LRMS (FAB) *m/z* 513 (M + H$^+$); HRMS (FAB) Calcd for $C_{24}H_{37}O_8SSi$ (M + H$^+$): 513.1978, found 513.1971.

4-(Phenylsulfonyl)5-(pro-1-en-2yl)-2-oxa-bicyclo[2.2.1]heptan-3-one (65)

To a refluxing solution of methally carbonate **64c** (12.5 mg, 0.033 mmol) in CH_2Cl_2 (2 ml) was added $Pd(dppe)_2$ (5.9 mg, 0.0065 mmol). AfAfter stirring for 15 h the reaction mixture was cooled to ambient temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 9 mg (94%) of bicyclic lactone **65** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.08-7.52 (m, 5H), 4.97 (s, 1H), 4.85 (dt, 2H, *J* = 13.7 Hz, 2.4 Hz, 1.3 Hz), 3.65 (q, 1H, *J* = 6.0 Hz), 2.5-2.30 (m, 2H), 2.26 (s, 1H), 1.97-1.92 (m, 1H), 1.64 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 168.4, 141.4, 134.5, 130.5, 128.7, 116.6, 77.0, 74.6, 45.8, 45.6, 37.0, 19.7; IR (neat) ν_{max} 2922, 1784, 1452, 1321, 1158, 1115, 723 cm^{-1}; LRMS (FAB) *m/z* 315 (M + Na$^+$); HRMS (FAB) Calcd for $C_{15}H_{16}O_4SNa$ (M + Na$^+$): 315.0667, found 315.0668.

5-(3-(*tert*-Butyldimethylsilyloxy)prop-1-en-2-yl)-4-(phenylsulfonyl)-2-oxabicyclo[2.2.1]heptan-3-one (66)

To a refluxing solution of silyl ether **64d** (250 mg, 0.49 mmol) in THF (2 mL) was added

$Pd(dppe)_2$ (44 mg, 50 μmol). After stirring for 1 h the reaction mixture was cooled to ambient temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 170 mg (89%) of bicyclic lactone **66** as a colorless oil: $[\alpha]_D^{25}$ -51.4 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02(m, 2H), 7.67-7.50 (m, 3H), 5.24(s, 1H), 5.04(s, 1H), 4.81(s, 1H), 4.21(s, 2H), 3.69 (dd, 1H, *J* = 5.5 Hz, 11.2 Hz), 2.53 (m, 1H), 2.30 (m, 2H), 2.09-2.00 (m, 1H), 0.90 (s, 9H), 0.04 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.9, 145.5, 136.8, 134.5, 130.2, 128.9, 113.8, 77.2, 75.4, 65.9, 45.9, 39.6, 39.0, 25.8, 18.2, -5.3; IR (neat) ν_{max} 2927, 1779, 1448, 1318, 1258, 1151, 1083, 840, 755, 726, 689 cm^{-1}; LRMS (FAB) *m/z* 423 (M + H$^+$); HRMS (FAB) Calcd for $C_{21}H_{31}O_5SSi$ (M + H$^+$): 423.1661, found 423.1658.

Minor 66': $[\alpha]_D^{25}$ -7.54 (c 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.03(d, 2H, *J* = 8.2 Hz), 7.62 (t, 1H, *J* = 7.4 Hz), 7.49 (t, 2H, *J* = 7.8 Hz), 5.41 (s, 1H), 5.10 (s, 1H), 4.90 (s, 1H), 4.50 (d, 1H, *J* = 9.4 Hz), 4.29 (d, 1H, *J* = 13.9 Hz), 3.08 (m, 1H), 2.65 (d, 1H, *J* = 10.7 Hz), 2.46 (d, 1H, *J* = 10.7 Hz), 2.36 (d, 1H, 6.6 Hz), 0.89 (s, 9H), 0.08 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 169.6, 144.8, 137.2, 134.3, 130.4, 128.5, 116.6, 77.7, 75.1, 65.9, 41.3, 40.6, 38.8, 25.8, 18.2, -5.3.

4-Methylenehexahydrocyclopenta[c]pyran-1,6-dione (68)

To a solution of cyclopentanol **70** (11 mg, 0.036 mmol) in CH_2Cl_2 were added TPAP (2 mg, 0.005 mmol) and NMO (8 mg, 0.07 mmol). The reaction mixture was stirred for 4 h and filtered with celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 :1) to afford 4 mg (67%) of cyclopentanone **68** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.33 (s, 1H), 5.12 (br, 1H), 4.92 (d, 1H, *J* = 12.8 Hz), 4.67 (d, 1H, *J* = 12.8 Hz), 3.04 (d, 1H, *J* = 20.88 Hz), 2.65 (m, 1H), 2.43 (m, 1H), 2.22 (m, 1H), 1.84 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 213.5, 172.2, 140.4, 115.6, 71.7, 42.9, 39.8, 38.9, 37.9; IR (neat) ν_{max} 2924, 2855, 1741, 1096, 804 cm^{-1}; LRMS (FAB) *m/z* 167 (M+H$^+$); HRMS (FAB) Calcd for $C_9H_{11}O_3$ (M + H$^+$): 167.0708, found 167.0706.

6-Hydroxy-4-methylenehexahydrocyclopenta[c]pyran-1(3*H*)-one (69)

To a solution of bicyclic lactone **70** (140 mg, 0.33 mmol) in THF (9 mL) was added TBAF (1.0 M solution in THF, 0.4 mL, 0.4 mmol) was added. The reaction mixture was stirred for 2 h and quenched with aqueous NH_4Cl. This mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 91 mg (90%) of cyclopentanol **69** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.08 (br, 1H), 5.00 (br, 1H), 4.74 (d, 1H, *J* = 12.3 Hz), 4.59 (d, 1H, *J* = 12 Hz), 4.27 (m, 1H), 2.60 (br, 1H), 2.28-2.04 (m, 3H), 1.61-1.52 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 174.6, 141.3, 113.7, 72.0, 71.0, 42.2, 40.1, 39.8, 38.7, 20.9, 14.0; IR (neat) ν_{max} 3414, 2931, 1722, 1380, 1039, 922 cm^{-1}; LRMS (FAB) m/z 169 (M + H$^+$).

5-(3-(*tert*-Butyldimethylsilyloxy)prop-1-en-2-yl)-2-oxabicyclo[2.2.1]heptan-3-one (70)

To a mixture of bicyclic lactone **66** (142 mg, 0.51 mmol) and boric acid (316 mg, 5.1 mmol) in methanol (5 ml) was added 6% Na/Hg (1.56 g, 4.08 mmol) and stirred for 48 h. The reaction mixture was quenched with saturated NH_4Cl, and then extracted with ether. The aqueous layers were extracted with ether, and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 3) to afford 38.6 mg (56%) of bicyclic lactone **70** as a white solid: ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.47 (s, 1H), 5.14 (s, 1H), 4.94 (s, 1H), 4.18 (q, 2H, *J* = 13.7 Hz), 3.07 (m, 1H), 3.01 (s, 1H), 2.22 (d, 1H, *J* = 10.5 Hz), 2.14 (m, 1H), 1.89 (m, 2H), 0.92 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C-NMR (CD_3OD, 125 MHz) δ 179.1, 149.3, 111.0, 82.9, 67.7, 42.8, 40.7, 33.8, 27.2, 19.9, -4.3, -4.4; IR (neat) ν_{max} 2955, 2928, 1789, 1255, 1084, 837 cm^{-1}; LRMS (FAB) *m/z* 283 (M+H$^+$); HRMS (FAB) Calcd for $C_{15}H_{26}O_3Si$ (M + H$^+$): 282.1651, found 282.1688.

6-((Bromomethyl)dimethylsilyloxy)-4-methylene-4,4a,5,6-tetrahydrocyclopenta[c]pyran-

1(3*H*)-one (71)

To a solution of cyclopetenol **79** (1 mg, 0.006 mmol) in CH_2Cl_2 (0.5 ml) were added BMDMCS (0.1 M in benzene, 0. 9 ml, 0.09 mmol), Et_3N (1.4 μl, 0.09 mmol) and DMAP (1.2 mg, 0.01 mmol) at 0 ºC. After stirred for 1 h at the ambient temperature, the reaction mixture was concentrated *in vacuo* to give as colorless oil. The crude silylether **71** was used in the next step without further purification: ^{1}H-NMR (C_6D_6, 300 MHz) δ 6.73 (br, 1H), 5.15 (s, 1H), 5.12 (m, 1H), 4.99 (s, 1H), 4.82 (d, 1H, *J* = 13.4 Hz), 4.70 (d, 1H, *J* = 13 Hz), 3.46 (br, 1H), 2.74 (m, 1H), 2.47 (s, 2H), 1.90 (m, 1H), 0.27 (s, 6H); LRMS (FAB) *m/z* 317 ($M + H^+$).

6-Hydroxy-4-methylene-7a-(phenylsulfonyl)hexahydrocyclopenta[c]pyran-1(3*H*)-one (72)

To a solution of bicyclic lactone **66** (168 mg, 0.4 mmol) in THF (10 mL), TBAF (1.0 M solution in THF, 0.48 mL, 0.48 mmol) was added. The reaction mixture was stirred for 2 h and quenched with aqueous NH_4Cl. This mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 98 mg (80%) of bicyclic lactone **72** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.80 (m, 2H), 7.66 (m,1H), 7.54 (m, 2H), 5.19 (m, 3H), 4.72 (d, 1H, *J* = 12 Hz), 4.39 (m, 1H), 4.05 (m, 1H), 2.3 (m, 3H), 2.0 (m, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 167.1, 139.2, 135.2, 134.5, 130.4, 128.7, 114.5, 77.1, 72.4, 71.3, 44.9, 40.9, 39.9; IR (neat) ν_{max} 3508, 1725, 1377, 1301, 1238, 1135, 1076, 919, 719 cm^{-1}; LRMS (FAB) m/z 309 ($M + H^+$); HRMS (FAB) Calcd for $C_{15}H_{17}O_5S$ ($M + H^+$): 309.0797, found 309.0789.

Ethyl 2-(Hhydroxymethyl)allyl carbonate (73)

To a solution of 2-methylene-1,3-propanediol (2.17 g, 24.6 mmol) in anhydrous CH_2Cl_2 (25 ml) was added pyridine (3.98 ml, 49.3 mmol) at -10 ºC and stirred for 10 minutes. To the mixture was added a solution of ethylchloroformate (2.46 ml, 25.9 mmol) in anhydrous CH_2Cl_2 (25 ml) by

dropping fonnel for 40 minutes. After stirring for 2 h, the reaction mixture was quenched with H_2O, extracted with EtOAc and then concentrated *in vacuo*. The residue was purified by flash chromatography by silica gel (EtOAc : *n*- Hexane = 1 : 2) to afford 2.8 g of allyl carbonate **73** (71%) as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.18 (s, 1H), 5.13 (s, 1H), 4.59 (s, 2H), 4.12 (q, 2H, *J* = 7.2 Hz), 4.07 (s, 2H), 2.68 (s, 1H), 1.23 (t, 3H, *J* = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 155.0, 142.7, 114.3, 67.7, 64.0, 63.1, 14.0; IR (neat) ν_{max} 3428, 2986, 1747, 1260, 1011 cm^{-1}; LRMS (FAB) *m/z* 161 ($M + H^+$); HRMS (FAB) Calcd for $C_7H_{13}O_4$ ($M + H^+$): 161.0814, found 161.0810.

(*S*)-2-Allyl-4,4-diisopropyl-7-methylene-10-oxo-3,5,9,11-tetraoxa-4-silatridecyl 4-methylbenzenesulfonate (74)

Dichlorodiisopropylsilane (2.36 ml, 13.1 mmol) was added to imidazole (2.44 g, 35.8 mmol) in CH_2Cl_2 (15 ml) at 0 ºC. The solution was stirred for *ca*. 5 minutes, then the hydroxy tosylate **59** (3.22 g, 11.9 mmol) in CH_2Cl_2 (10 ml) was added dropwise by dropping funnel *ca.*1 h at 0 ºC. After the mixture was stirred for 10 minutes at 0 ºC, a solution of the allylic alcohol **73** (1.91 g, 11.9 mmol) in CH_2Cl_2 (10 mL) was added at 0 ºC. The reaction mixture was warmed to the room temperature and stirred for 16 h. The reaction mixture filtrated with hexane, then concentrated *in vacuo* to afford the crude oil, which was purified by flash chromatography on silica gel (EtOAc : *n*-hexane = 1 : 7) to afford 5.2 g (82%) of *bis*-alkoxysilane **74** as a colorless oil: $[\alpha]_D^{25}$ 1.65 (*c* 0.5, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.75 (d, 2H, *J* = 8.2 Hz), 7.30 (d, 2H, *J* = 8.1 Hz), 5.76-5.62 (m, 1H), 5.22(s, 1H), 5.15 (s, 1H), 5.02-4.97 (m, 2H), 4.59 (s, 2H), 4.24 (s, 2H), 4.17 (q, 2H, *J* = 7.1 Hz), 4.05 (m, 1H), 3.89 (m, 2H), 2.41 (s, 3H), 2.25 (m, 2H), 1.27 (t, 3H, *J* = 7.1 Hz), 0.96 (m, 14H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 154.9, 144.7, 142.2, 132.8, 132.7, 129.7, 127.9, 118.3, 113.6, 113.3, 72.1, 69.2, 67.6, 64.0, 63.3, 38.4, 21.5, 17.2, 17.1, 14.2, 12.1, 12.0; IR (neat) ν_{max} 2945, 2867, 1748, 1259, 1178, 1097, 989, 815 cm^{-1}; LRMS (FAB) *m/z* 529 ($M + H^+$).

(12*S*)-Methyl 12-allyl-10,10-diisopropyl-7-methylene-4-oxo-14-(phenylsulfonyl)-3,5,9,11-tetraoxa-10-silapentadecan-15-oate (S74)

To a suspension of NaH (1.98 g, 49.5 mmol) in DMF (10 ml) was slowly added solution of benzene sulfonate (10.6 g, 49.5 mmol) in DMF (20 ml) at 0 ºC. After the mixture was stirred for 1 h at 0 ºC, *bis*-alkoxysilane **74** (8 g, 15.1 mmol) in DMF (20 ml) was added. The reaction mixture was stirred at 80 ºC for 24 h and quenched with aqueous NH_4Cl solution. This mixture was extracted by ether. The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1: 5) to afford 4.696 g (54 %) of the alkylated product **S74** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.80 (m, 2H), 7.63 (m, 1H), 7.50 (m, 2H), 5.71-5.60 (m, 1H), 5.21 (d, 1H, *J* = 16.5 Hz), 5.13 (d, 1H, *J* = 8.7 Hz), 5.02-4.95 (m, 2H), 4.58 (d, 1H, *J* = 12.3 Hz), 4.24-4.20 (m, 3H), 4.17-4.11 (m, 2H), 4.03-3.83 (m, 1H), 3.58 (s, 3H), 2.35-2.04 (m, 4H), 1.25 (t, 3H, *J* = 7.1 Hz), 1.20 (t, 2H, *J* = 7.1 Hz), 1.00-0.89 (m, 14H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 170.9, 166.5, 166.2, 154.9, 142.1, 137.5, 134.0, 133.1, 132.9, 129.5, 129.2, 129.1, 129.0, 128.9, 128.8, 118.2, 118.1, 113.4, 113.3, 69.1, 67.6, 67.5, 67.3, 63.9, 63.3, 63.2, 60.2, 52.8, 52.6, 41.7, 32.9, 32.7, 20.9, 17.2, 17.1, 17.0, 16.9, 14.1, 12.2, 12.0, 11.8; IR (neat) ν_{max} 2946, 2867, 1746, 1448, 1259, 884 cm^{-1}; LRMS (FAB) *m/z* 571.

Methyl 3-((*S*,*Z*)-7-((ethoxycarbonyloxy)methyl)-2,2-diisopropyl-5,8-dihydro-4*H*-1,3,2-dioxasilocin-4-yl)-2-(phenylsulfonyl)propanoate (75)

To a refluxing solution of benzensulfonate **S74** (4.6 g, 8.06 mmol) in CH_2Cl_2 (600 mL) were added Grubbs' 2nd catalyst (171 mg, 0.20 mmol). After stirring of the mixture for 0.5 h, the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1: 5) to afford 4.26 g (97%) of cyclic dioxasilane **75** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.80 (m, 2H), 7.62 (m, 1H), 7.51 (m, 2H), 5.72 (t, 1H, *J* = 8.6 Hz),

4.57 (d, 1H, *J* = 12.7 Hz), 4.50 (d, 1H, *J* = 12.5 Hz), 4.29-4.21 (m, 1.3H), 4.14-4.09 (m, 3H), 3.75-3.70 (m, 0.7H), 3.58 (s, 2H), 3.51 (s, 1H), 2.53-2.48 (m, 0.3H), 2.46-2.41 (m, 0.7H), 2.25-2.04 (m, 3H), 1.23 (m, 3H), 0.94-0.79 (m, 14H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 170.8, 166.4, 166.0, 154.7, 138.5, 138.4, 137.2, 137.0, 134.1,129.1, 129.0, 128.9, 127.4, 71.9, 70.0, 69.8, 69.7, 68.5, 67.7, 63.9, 60.1, 59.6, 52.6, 35.1, 34.7, 34.4, 20.8, 17.2, 17.0, 16.9, 14.0, 12.6, 12.5, 12.0, 11.8; IR (neat) ν_{max} 2945, 2866, 1745, 1463, 1256, 1084 cm^{-1}; LRMS (FAB) *m/z* 543 (M + H$^+$); HRMS (FAB) Calcd for $C_{25}H_{39}O_9SSi$ (M + H$^+$): 543.2084, found 543.2074.

Ethyl (*E*)-2-(hydroxymethyl)-4-((2*S*)-5-oxo-4-(phenylsulfonyl) tetrahydrofuran-2-yl)but-2-enyl carbonate (S75)

To a solution of cyclic dioxasilane **75** (2.76 g, 5.08 mmol) in THF (50 mL), TBAF (1.0 M solution in THF, 5.6 mL, 5.6 mmol) was added. The reaction mixture was stirred for 2 h and quenched with aqueous NH_4Cl. This mixture was extracted with EtOAc and the organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 1.48 g (73%) of allyl alcohol **S75** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.9-7.55 (m, 5H), 5.75-5.60 (m, 1H), 5.22 (d, 1H, *J* = 12.6 Hz), 4.85-4.48 (m, 4H), 4.19-4.05 (m, 3H), 3.10-2.29 (m, 4H), 1.26 (m, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 167.5, 155.1, 138.1, 137.8, 136.5, 134.7, 129.5, 129.3, 129.2, 125.6, 125.2, 78.6, 77.1, 69.5, 64.8, 64.2, 63.9, 58.1, 32.8, 28.8, 14.2; IR (neat) ν_{max} 3531, 2983, 1744, 1448, 1261, 1149, 1084, 1009, 937, 873 cm^{-1}; LRMS (FAB) *m/z* 399 (M + H$^+$); HRMS (FAB) Calcd for $C_{18}H23O_8S$ (M + H$^+$): 399.1114, found 399.1102.

(*S*)-4-Methylene-3,4,4a,5-tetrahydrocyclopenta[c]pyran-1,6-dione (76)

To a solution of cyclopentanol **72** (11 mg, 0.036 mmol) in CH_2Cl_2 (1 ml) was added *Dess-Martin* periodinane (23 mg, 0.054 mmol) at the ambient temperature and the reaction mixture was stirred

for 16 h. After quenching with H_2O, the mixture was extracted with EtOAc and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 3.3 mg (56%) of cyclopentenone **76** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.73 (d, 1H, *J* = 2.9 Hz), 5.30 (s, 1H), 5.13 (s, 1H), 5.02 (d, 1H, *J* = 13.2 Hz), 4.84 (d, 1H, *J* = 7.8 Hz), 3.89 (m, 1H), 2.84 (dd, 1H, *J* = 6.2 Hz, 18.5 Hz), 2.65 (dd, 1H, *J* = 3.6 Hz, 18.5 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 209.0, 152.0, 138.2, 136.8, 127.6, 112.6, 70.5, 42.8, 42.1, 39.4; IR (neat) ν_{max} 2929, 1725, 1612, 1462, 1250, 1110, 838 cm^{-1}; LRMS (FAB) m/z 165 (M + H$^+$).

6-Hydroxy-4-methylene-4,4a,5,6-tetrahydrocyclopenta[c]pyran-1(3*H*)-one (77)

To a flamed flask were added (*S*)-Me-CBS-oxazaborolidine (1.0 M solution in toluene, 0.37 ml, 0.36 mmol) and borane-SMe_2 complex (2.0 M in THF, 0.09 ml, 0.18 mmol) at the ambient temperature. After stirring for 10 minutes, cyclopentenone **76** (6 mg, 0.04 mmol) in CH_2Cl_2 (2 ml) was added at 0 ℃ and stirred for 10 minutes. After quenching with *i*PrOH, the mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 2 : 1) to afford 5 mg (80%) of cyclopentenol **77** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.79 (s, 1H), 5.16 (s, 1H), 5.08 (q, 1H, *J* = 7.3 Hz), 5.0 (s, 1H), 4.84 (d, 1H, *J* = 13 Hz), 4,72 (d, 1H, *J* = 13 Hz), 3.72 (br, 1H), 2.82 (td, 1H, *J* = 13 Hz, 6.8 Hz), 1.84 (m, 1H), 1.78 (d, 1H, *J* = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 162, 145, 140, 134, 111, 76, 73, 44, 40; IR cm^{-1} 3399, 2925, 1722, 1633, 1245, 1050, 916; LRMS (FAB) m/z 167 (M + H$^+$); HRMS (FAB) Calcd for $C_9H_{11}O_3$ (M + H$^+$): 167.0708, found 167.0702.

4-Methylene-1-oxo-1,3,4,4a,5,6-hexahydrocyclopenta[c]pyran-6-yl 2,2,2-trichloroacetate (78)

To a solution of cyclopentenol **77** (3.4 mg, 0.02 mmol) in THF (0.6 ml) were added trichloroacetic acid (13 mg, 0.082 mmol) and triphenyl phosphine (21 mg, 0.082 mmol). And then, DIAD (0.016

ml, 0.082 mmol) was added dropwise. After stirred for 4 h, the reaction mixture was quenched with $NaHCO_3$, the mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 6 mg (96%) of trichloroacetate **78** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.88 (t, 1H, *J* = 2.7 Hz), 5.80 (d, 1H, *J* = 5.7 Hz), 5.27 (s, 1H), 5.06 (s, 1H), 4.91 (d, 1H, *J* = 13 Hz), 4.71 (d, 1H, *J* =13 Hz), 3.93 (br, 1H), 2.53 (dd, 1H, *J* =14.6 Hz, 6 Hz), 2.39 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 161.5, 152.3, 139.8, 138.1, 112.0, 82.5, 65.4, 64.1, 45.0, 36.1; LRMS (FAB) m/z 312 ($M + H^+$).

6-Hydroxy-4-methylene-4,4a,5,6-tetrahydrocyclopenta[c]pyran-1(3*H*)-one (79)

To a solution of trichloroacetate **78** (6 mg, 0.019 mmol) in THF/MeOH (1:1, 1ml) was added silica gel (20 mg). The reaction mixture was heated to 40 ℃. The reaction mixture was stirred for 8 h and filtered with celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane =) to afford 1 mg (31%) of cyclopentenol **79** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 6.84 (t, 1H, *J* = 2.9 HZ), 5.15 (s, 1H), 5.01 (s, 1H), 4.88 (m, 2H), 4.71 (d, 1H, *J* = 13Hz), 3.87 (br, 1H), 2.30 (dd, 1H, *J* = 6.5 Hz, 20 Hz), 2.21 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 162.9, 145.3, 140.8, 134.8, 111.4, 76.1, 73.1, 44.9, 40.3; IR cm^{-1} 3399, 2925, 1722, 1633, 1245, 1050, 916; LRMS (FAB) m/z 167 ($M + H^+$); HRMS (FAB) Calcd for $C_9H_{11}O_3$ ($M + H^+$): 167.0708, found 167.0702.

4-Methylene-7a-(phenylsulfonyl)hexahydrocyclopenta[c]pyran-1,6-dione (80)

-Swern oxidation condition

DMSO (0.02 ml, 0.28 mmol) was dissolved in CH_2Cl_2 (1 ml) at -78 ℃ and treated with TFAA (0.02 ml, 0.14 mmol). The solution was allowed to stir 10 min at -78 ℃ and then, treated dropwise with cyclopentanol **79** (10 mg, 0.03 mmol) in CH_2Cl_2 (1 ml). The solution was

allowed to stir for 1 h at -78 ºC and then, treated with Et_3N (0.05 ml, 0.36 mmol). The solution was allowed to warm to 0 ºC and stirred for 1h. The reaction mixture was quenched with water, aqueous phase wasextracted with CH_2Cl_2 and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 2 : 1) to afford 8 mg (80%) of cyclopentanone **80** as a colorless oil.

-PCC oxidation condition

To a solution of cyclopentanol **79** (3 mg, 0.01 mmol) in CH_2Cl_2 (0.6 ml) were added PCC (3 mg, 0.015 mmol) and silica gel (6 mg). After stirred for 4 h, the reaction mixture filtered with celite pad. The filtrate was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 2 :1) to afford 2.4 mg (85%) of cyclopentanone **80** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.82-7.50 (m, 5H), 5.22-5.15 (m, 3H), 4.73 (d, 1H, J = 12.6 Hz), 4.42 (m, 1H), 4.07 (m, 1H), 2.40 (m, 3H), 2.01 (br, 1H); IR cm^{-1} 2924, 1717, 1261, 745; LRMS (FAB) m/z 307 (M + H$^+$).

4-Methylene-3,4-dihydrocyclopenta[c]pyran-1,6(5*H*,7*H*)-dione (81)

To a solution of cyclopentanone **80** (4 mg, 0.013 mmol) in THF (0.8 ml) was added DBU (3 μl, 0.022 mmol). The reaction mixture was refluxed for 16 h, and then cooled. The reaction mixture was quenched with aqueous NH_4Cl, concentrated *in vacuo*. The residue was diluted with EtOAc, the organic layer were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 1 mg (47%) of ccyclopentenone **81** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.41 (s, 1H), 5.26 (s, 1H), 5.08 (s, 2H), 3.30 (m, 4H); IR cm^{-1} 2925, 1633, 1245, 1050; LRMS (FAB) m/z 165 (M+H).

(1*R*,2*S*,4*S*)-Methyl 2-(3-(tert-butyldimethylsilyloxy)prop-1-en-2-yl)-4-hydroxy-1-(phenylsulfonyl) cyclopentanecarboxylate (83)

To a solution of bicyclic lactone **66** (78 mg, 0.19 mmol) in methanol (10 ml) was added sodium

methoxide (12 mg, 0.20 mmol) at the ambient temperature. After stirred for 1 h, the reaction mixture was quenched with aqueous NH_4Cl, concentrated *in vacuo*. The residue was diluted with EtOAc, the organic layer were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 1) to afford 78 mg (93%) of cyclopentanol **83** as a colorless oil: $[\alpha]_D^{25}$ -104.5 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.89 (d, 2H, *J* = 7.6 Hz), 7.61 (t, 1H, *J* = 7.4 Hz), 7.50 (t, 2H, *J* = 7.6 Hz), 5.23 (s, 1H), 4.98 (s, 1H), 4.48 (m, 1H), 4.03 (d, 1H, *J* = 14.6 Hz), 3.85 (d, 1H, *J* = 14.6 Hz), 3.52 (s, 3H), 3.30 (t, 1H, *J*= 8.7 Hz), 2.80 (dd, 1H, *J* = 6.8 Hz, 14.8 Hz), 2.58 (dd, 1H, *J* = 7.8 Hz, 14.8 Hz), 2.37 (m, 1H), 1.92-1.84 (m, 1H), 0.85 (m, 9H), -0.01 (m, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 167.6, 148.0, 136.8, 134.1, 130.6, 128.7, 111.7, 83.3, 70.9, 65.6, 52.5, 43.0, 41.8, 41.5, 25.8, 18.1, -5.4; IR (neat) ν_{max} 3458, 2953, 2929, 2856, 1738, 1447, 1308, 1254, 1140, 1079, 837 cm^{-1}; LRMS (FAB) *m/z* 455 (M + H$^+$); HRMS (FAB) Calcd for $C_{22}H_{35}O_6SSi$ (M + H$^+$): 455.1924, found 455.1932.

(*S*)-Methyl 5-(3-(tert-butyldimethylsilyloxy)prop-1-en-2-yl)-3-oxocyclopent-1-enecarboxylate (84)

To a solution of cyclopentanol **83** (48 mg, 0.11 mmol) in CH_2Cl_2 (5 ml) was added Dess-Martin periodinane (49 mg, 0.12 mmol) at the ambient temperature and the reaction mixture was stirred for 16 h. After quenching with H_2O, the mixture was extracted with CH_2Cl_2 and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 30 mg (91%) of cyclopentenone **84** as a colorless oil: $[\alpha]_D^{25}$ 751.4 (*c* 0.33, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.73 (d, 1H, *J* = 1.8 Hz), 5.06 (s, 1H), 4.73 (s, 1H), 4.14 (s, 2H), 3.80 (s, 3H), 3.77 (m, 1H), 2.76 (dd, 1H, *J* = 7.2 Hz, 19.1 Hz), 2.38 (dd, 1H, *J*= 1.9 Hz, 19.1 Hz), 0.88 (s, 9H), 0.04 (m, 6H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 208.2, 165.1, 164.4, 148.9, 137.8, 110.4, 65.8, 52.4, 44.3, 42.4, 25.8, 18.3, -5.4; IR (neat) ν_{max} 2954, 2929, 2857, 1725, 1612, 1462, 1250, 1110, 838 cm^{-1}; LRMS (FAB) *m/z* 311 (M + H$^+$); HRMS (FAB) Calcd for

$C_{16}H_{27}O_4Si$ ($M + H^+$): 311.1679, found 311.1670.

(3*R*,5*S*)-Methyl 5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)-3-hydroxycyclopent-1-enecarboxylate (85)

To a flamed flask were added (*S*)-Me-CBS-oxazaborolidine (1.0 M solution in toluene, 3 μl, 3 μmol) and borane-SMe_2 complex (2.0 M in THF, 8 μl, 0.016 mmol) at the ambient temperature. After stirring for 10 minutes, cyclopentenone **84** (10 mg, 0.03 mmol) in THF (1 ml) was added at -78 ºC and stirred for 10 minutes. After quenching with *i*PrOH, the mixture was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 2) to afford 6 mg (60%) of cyclopentenol **85** as a colorless oil: $[\alpha]_D^{25}$ 362.3 (*c* 0.33, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.77 (s, 1H), 5.06 (m, 1H), 4.98 (s, 1H), 4.67 (s, 1H), 3.69 (s, 3H), 3.68 (m, 1H), 2.02 (m, 1H), 1.88 (m, 1H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 164.9, 150.0, 144.5, 140.1, 107.8, 76.5, 65.6, 51.6, 46.0, 42.5, 25.8, 18.3, -5.4; IR (neat) ν_{max} 3435, 2953, 2930, 2857, 1725, 1436, 1256, 1110, 721 cm^{-1}; LRMS (ESI) *m/z* 313 ($M + H^+$); HRMS (FAB) Calcd for $C_{16}H_{29}O_4Si$ ($M + H^+$): 313.1835, found 313.1826.

(3*R*,5*S*)-Methyl 3-((bromomethyl)dimethylsilyloxy)-5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)cyclopent-1-enecarboxylate (82)

To a solution of cyclopetenol **85** (5 mg, 0.016 mmol) in bezene (1 ml) were added BMDMCS (0.1 M in benzene, 0.3 ml, 0.03 mmol), Et_3N (6 μl, 0.04 mmol) and DMAP (1.2 mg, 0.01 mmol) at 0 ºC. After stirred for 2 h at the ambient temperature, the reaction mixture was concentrated *in vacuo* to give as colorless oil. The crude silylether **82** was used in the next step without further purification: $[\alpha]_D^{20}$ 52.4 (*c* 0.50, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.70 (t, 1H), 5.11 (m, 1H), 4.98 (s, 1H), 4.66 (s, 1H), 4.10 (m, 2H), 3.68 (s, 3H), 3.61 (m, 1H), 2.45 (s, 2H), 2.23 (m, 1H),

2.07 (m, 1H), 0.89 (m, 9H), 0.26 (s, 6H), 0.05 (s, 6H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 164.8, 151.1, 145.7, 139.7, 107.5, 76.2, 65.9, 57.8, 42.6, 38.2, 26.0, 7.7, -1.0, -5.3; IR (neat) ν_{max} 2954, 2930, 2857, 1726, 1436, 1361, 1255, 1113, 837 cm^{-1}; LRMS (FAB) *m/z* 463 (M + H$^+$); HRMS (FAB) Calcd for $C_{19}H_{36}BrO_4Si_2$ (M + H$^+$): 463.1318, found 463.1321.

Methyl 5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)-2,2-dimethylhexahydro-2*H*-cyclopenta[d][1,2] oxasilole-4-carboxylate (87)

To a solution of silylether **82** (7 mg, 0.016 mmol) in benzene were added Et_3B (1.0 M in hexane, 0.016ml, 3 μmol) and Bu_3SnH (5 μl, 0.019 mmol) at 0 ℃. After stirred for 2h at ambient temperature, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc : *n*-hexane = 1 : 10) to afford 3.8 mg (62%) of oxasilole **87** as a colorless oil: $[\alpha]_D^{20}$ 3.2 (*c* 0.50, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.12 (s, 1H), 4.80 (s, 1H), 4.27 (s, 1H), 4.09 (d, 1H, *J* = 14.3 Hz), 4.02 (d, 1H, *J* = 14.3 Hz), 3.53 (s. 3H), 3.14 (m, 1H), 2.77 (dd, 1H, *J* = 7.75 Hz, 10.1 Hz), 2.54 (m, 1H), 2.44 (s, 1H), 2.07-2.02 (m, 1H), 1.88 (dd, 1H, *J* = 6.65 Hz, 13.2 Hz), 0.90 (m, 9H), 0.78 (m, 1H), 0.69 (m, 1H), 0.09 (s, 6H), 0.04 (m, 6H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 163.7, 139.5, 108.7, 73.8, 65.9, 55.8, 51.6, 44.2, 41.8, 39.2, 25.9, 18.3, 1.7, -5.4; IR (neat) ν_{max} 2957, 2925, 2871, 2855, 1736, 1462, 1253, 842 cm^{-1}; LRMS (FAB) *m/z* 385 (M + H$^+$); HRMS (FAB) Calcd for $C_{19}H_{37}O_4Si_2$ (M + H$^+$): 385.2230, found 385.2216.

Methyl 5-(3-(*tert*-butyldimethylsilyloxy)prop-1-en-2-yl)-3-hydroxy-2-(hydroxymethyl) cyclopentane carboxylate (88)

To a solution of oxasilole **87** (3.5 mg, 9 μmol) in THF/ MeOH (1:1, 1 ml) were added potassium bicarbonate (1.8 mg, 0.018 mmol), potassium fluoride (2.1 mg, 0.036 mmol) and aqueous hydrogen peroxide (30% aqueous solution, 0.02ml, 0.18 mmol) at ambient temperature. After

stirred for 16 h, to the reaction mixture was added well-grounded $Na_2S_2O_3$ (70 mg, 1.2 *eq* of H_2O_2), and then diluted with Et_2O, then filtrated with celite pad. The filtrate was concentrated *in vacuo*, the residue was purified by flash column chromatography on silica gel (EtOAc : Hexane = 2 : 1) to afforded 1.6 mg (50%) of diol **88** as a colorless oil: $[\alpha]_D^{20}$ –5.60 (*c* 0.50, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.13 (s, 1H), 4.79 (s, 1H), 4.60 (m, 1H), 4.10 (m, 2H), 3.94 (dd, 1H, *J* = 4.0 Hz, 11.0 Hz), 3.72 (m, 1H), 3.54 (s, 3H), 3.16 (m, 2H), 2.69 (m, 1H), 2.15 (m, 1H), 1.81 (m, 1H), 0.89 (m, 9H), 0.05 (s, 6H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.6, 148.0, 119.6, 108.8, 74.7, 65.9, 62.3, 51.4, 48.3, 41.9, 40.2, 25.8, 18.3, -5.4; IR (neat) ν_{max} 3468, 2928, 2856, 1735, 1682, 1460, 838 cm^{-1}; LRMS (FAB) *m/z* 345 (M + H$^+$); HRMS (FAB) Calcd for $C_{17}H_{33}O_5Si$ (M + H$^+$): 345.2097, found 345.2092.

6-Hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta [*c*]pyran-1 (3*H*)-one (1)

To a solution of hydroxymethyl cyclopentanol **88** (6 mg, 0.017 mmol) in CH_2Cl_2 (1.5 ml) was added TsOH-H_2O (5mg, 0.026 mmol) at the ambient temperature and stirred overnight. The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography on silica gel (EtOAc : *n*-Hexane = 2 : 1 →CH_2Cl_2 : MeOH = 10 : 1) to afford of iridoid **1** 2.4 mg (66%) as a coloress needle: mp 110-112 ℃; $[\alpha]_D^{25}$ -13.0 (*c* 0.01, MeOH); ^{1}H-NMR (CD_3OD, 500 MHz) δ 5.18 (s, 1H), 5.05 (s, 1H), 4.73 (m, 2H), 4.37 (t, 1H, *J* = 3.5 Hz), 3.86 (dd, 1H, *J* = 4.8 Hz, 10.8 Hz), 3.79 (dd, 1H, *J* = 8 Hz, 10.7 Hz), 3.45 (m, 1H), 3.01 (dd, 1H, *J* = 9.6 Hz, 11.2 Hz), 2.40 (m, 1H), 2.14 (m, 1H), 1.65 (m, 1H); ^{13}C-NMR (CD_3OD, 75 MHz) δ 178.1, 144.9, 114.5, 74.2, 73.2, 62.6, 52.8, 45.6, 42.1, 41.6; IR (neat) ν_{max} 3361, 2925, 2853, 1728, 1659, 1467, 1378, 1241, 1171, 1037, 917 cm^{-1}; LRMS (FAB) *m/z* 199 (M + H$^+$); HRMS (FAB) Calcd for $C_{10}H_{15}O_4$ (M + H$^+$): 199.0970, found 199.0977.

V. References

1 (a) El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod*, **1980**, *43*, 649-707. (b) Boros, C. A.; Stermitz, F. R. *J. Nat. Prod*. **1991**, *54*, 1173-1246. (c) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2007**, *55*, 159-222. (d) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2007**, *55*, 689-728. (e) Tundis, R.; Loizzo, M. R.; Menichini, F.; Statti, G. A.; Menichini, F. *Mini-Rev. Med. Chem.* **2008**, *8*, 399-420. (f) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2009**, *57*, 765-796. (g) Paquette, L. A. *Top. Curr. Chem*. **1984**, *119*, 1-160.

2 (a) Kobayashi, Y.; *Curr. Org. Chem.* **2003**, *7,* 133-147. (b) Leemans, E.; D'hooghe, M.; De Kimpe, N. *Chem. Rev*. **2011**, *111*, 3268-3333.

3 (a) *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley: New York, 1984. (b) Gothelf, K. V.; Jørgensen, K. A. *Chem. Rev.* **1998**, *98*, 863-909. (c) Li, C.-L.; Liu, R.-S. *Chem. Rev.* **2000**, *100*, 3127-3161. (d) Klunder, A. J. H.; Zhu, J.; Zwanenburg, B. *Chem. Rev.* **1999**, *99*, 1163-1190. (e) Gibson, S. E.; Lewis, S. E.; Mainolfi, N. *J. Organomet. Chem.* **2004**, *689*, 3873-3890. (f) Aubert, C.; Buisine, O.; Malacria, M. *Chem. Rev.* **2002**, *102*, 813-834. (g) Agrofoglio, L. A.; Gillaizeau, I.; Saito, Y. *Chem. Rev.* **2003**, *103*, 1875-1916.

4 (a) Hudlicky, T.; Price, J. D. *Chem. Rev.* **1989**, *89*, 1467.-1486. (b) Borthwick, A. D.; Biggadike, K. *Tetrahedron* **1992**, *48*, 571-623. (c) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319-4347.

5 (a) Barco, A.; Benetti, S.; De Risi, C.; Marchetti, P.; Pollini, G. P.; Zanirato, V. *Tetrahedron: Asymmetry* **1997**, *8*, 3515-3545. (b) Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229-9272. (c) Dalko, P. I.; Sinay, P. *Angew. Chem., Int. Ed.* **1999**, *38*, 773-777.

6 (a) Arjona, O.; Go´mez, A. M.; Lo´pez, J. C.; Plumet, J. *Chem. Rev.* **2007**, *107*, 1919-2036.

7 (a) El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod*, **1980**, *43*, 649-707. (b) Boros, C. A.; Stermitz, F. R. *J. Nat. Prod*. **1991**, *54*, 1173-1246. (c) Dinda, B.; Debnath, S.; Harigaya, Y. *Chem. Pharm. Bull.* **2007**, *55*, 159-222.

8 (a) Dewick, P. M. (2009) *Medicinal natural products: A biosynthetic approach. 3rd ed.* England:

John Wiley & Sons Ltd. 206-223. (b) Tietz, L.-T. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 828-841. (c) Banthorpe, D. V.; Charlwood, B. V.; Francis, M. J. O. *Chem. Rev.*, **1972**, *72*, 115-155.

9 (a) Scheffrahn, R. H.; Gaston, L. K.; Sims, J. J.; Rust, M. K. Environ. *Entomol*. **1984**, *13*, 1502-1506. (b) Tomalski, M. D.; Blum, M. S.; Jones, T.H.; Fales, H. M.; Howard, D. F.; Passera, L. *J. Chem. Ecol*. **1987**, *13*, 253-263. (c) Huth, A.; Dettner, K. *J. Chem. Ecol*. **1990**, *16*, 2691-2711. (d) Lorenz, M.; Boland, W.; Dettner, K. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 912-914.

10 Inouye, H. *Planta Med.* **1978**, *33*, 193-216.

11 Sakan, T.; Fujino, A.; Murai, F.; Suzui, A. *Bull. Chem. Soc. Jpn.* **1960**, *33*, 1737.

12 Schreiber, S. L.; Meyers, H. V.; Wiberg, K. B. *J. Am. Chem. Soc.* **1986**, *108*, 8274.

13 For recently references; (a) Mukherjee, S.; Yang, J. W.; Hoffmann, S. *Chem. Rev.* **2007**, *107*, 5471-5569. (b) Radtke L, Willot, M; Sun, H. Y.; *Angew. Chem., Int. Ed.* **2011**, *50*, 3998-4002. (c) Beckett, J. S.; Beckett J. D.; Hofferberth, J. E.; *Org. Lett.* **2010**, *12*, 1408-1411.

14 Carroll, G. L.; Allan, A. K.; Schwaebe, M. K.; Little, R. D. *Org. Lett*. **2000**, *2*, 2531-2534.

15 Candish, L.; Lupton, D. W. *Org. Lett.*, **2010**, *12*, 4836-4839.

16 Herrmann, W. A. *Angew. Chem. Int. Ed.* **2002**, *41*, 1290-1309.

17 Jones, R. A.; Krische, M. *Org. Lett*. **2009**, *11*, 1849-1851.

18 Achmatowicz, O.; Szechner, B.; Maurin, J. K.; *Tetrahedron* **1997**, *53*, 6035-6044.

19 Trost, B. M.; Van Vranken, D. L. *Chem. Rev.* **1996**, *96*, 395-422.

20 Tsuji, J. (2004) *Palladium reagents and catalysts: new perspectives for the 21st century.* England: John Wiley & Sons Ltd. 1-18.

21 Suh, Y.-G.; Lee, Y.-C.; Ko, J.-A.; Kim, S.-A.; Kim, J.-Y.; Jung, J.-K.; Seo, S.-Y.; Lee, K.-H. *Synthetic Commun.* **2000**, *30*, 3053-3060.

22 Suh, Y.-G.; Jung, J.-K.; Kim, S.-A.; Shin, D.-Y.; Min, K.-H. *Tetrahedron Lett.* **1997**, *38*, 3911-3914.

23 (a) Suh, Y.-G.; Jung, J.-K.; Suh, B.-C.; Lee, Y.-C.; Kim, S.-A. *Tetrahedron Lett*. **1998**, *39*, 5377-5380. (b) Suh, Y.-G.; *Tetrahedron Lett.* **2001**, *42*, 1691-1694. (c) Suh, Y.-G.; Jung, J.-K.; Seo, S.-Y.; Min, K.-H.; Shin, D.-Y.; Lee, Y.-S.; Kim, S.-H.; Park, H.-J. *J. Org. Chem*. **2002**, *67*, 4127-4137.

24 Seo, S.-Y.; Jung, J.-K.; Paek, S.-M.; Lee, Y.-S.; Kim, S.-H.; Suh, Y.-G. *Tetrahedron Lett*. **2006**, *47*, 6527-6530.

25 Paek, S.-M.; Seo, S.-Y.; Min, K.-H.; Shin, D.-M.; Chung, Y.-K.; Suh, Y.-G. *Heterocycles*, **2007**, *71*, 1059-1066.

26 Seo, S.-Y.; Jung, J.-K.; Paek, S.-M.; Lee, Y.-S.; Kim, S.-H.; Lee, K.-O.; Suh, Y.-G. *Org. Lett*. **2004**, *6*, 429-432.

27 Lee, S. (2006) *Studies on the iridoid syntheses employing stereoselective construction of the tri-substituted cyclopentanes*, Unpublished thesis (M.A.), Seoul National University.

28 Han, Y.-T.; Paek, S.-M.; Lee, S.; Jung, J.-W.; Jung, J.-K.; Seo, S.-Y.; Lee, J.; Suh, Y.-G. *Tetrahedron Lett*. **2010**, *51*, 2697-2699.

29 Zhang, Y.; Lu, Y.; Zhang, L.; Zheng,Q.-T.; Xu,L-Z.; Yang, S.-L. *J. Nat. Prod*. **2005**, *68*, 1131-1134.

30 Tanitsu, M.; Takaya, Y.; Akasaka, M.; Niwa, M.; Oshima, Y. *Phytochemistry* **2002**, *59*, 845-849, and references cited therein.

31 Kokubo, M.; Ogawa, C.; Kobayashi, S. *Angew. Chem. Int. Ed.* **2008**, *47*, 6909-6911.

32 Schreiber, J.; Maag, H.; Hashimoto, N.; Eschenmoser, A. *Angew. Chem., Int. Ed*. **1971**, *10*, 330-331.

33 Zayia, G. H. *Org. Lett.* **1999**, *1*, 989-992.

34 (a) Nishiyama, H.; Kitajima, T.; Matsumoto, M.; Itoh, K. *J. Org. Chem.* **1984**, *49*, 2298-2300. (b) Stork, G.; Kahn, M. *J. Am. Chem. Soc*. **1985**, *107*, 500-501. (c) Jenkins, P. R.; Wood, A. J.

Tetrahedron Lett. **1997**, *38*, 1853-1856. (d) Kessabi, F. M.; Winkler, T.; Luft, J. A. R.; Houk, K. N. *Org. Lett*. **2008**, *10*, 2255-2258.

35 Tamao, K.; Ishida, N.; Kumada, M. *J. Org. Chem*. **1983**, *48*, 2120-2122.

36 Evans, P. A.; Cui, J.; Gharpure, S. J.; Polosukhin, A.; Zhang, H.-R. *J. Am. Chem. Soc*. **2003**, *125*, 14702-14703.

37 J. Strating (1961) *Organic Sulfur Compounds.* Pergamon Press, New York: N. Y.: Chapter 15.

38 (a) Conrad, P. C.; Fuchs, P. L. *J. Am. Chem. Soc*. **1978**, *100*, 346-348. (b) Cheng, W.-C.; Kurth, M. K. *J. Org. Chem.* **2002**, *67*, 4387-4391. (b) Nantz, M. H.; Radisson, X.; Fuchs, P. L. *Synthetic Commun.* **1987**, *17*, 55-69.

39 Yoshida, T.; Saito, S. *Chem. Lett*, **1982**, 165-168.

40 (a) Lanver, A.; Schmalz, H.-G. *Eur. J. Org. Chem.* **2005**, 1444-1458. (b) Schemalz, H.-G.; Jope, H. *Tetrahedron* **1998**, *54*, 3457-3464.

41 (a) Lipshutz, B. H.; Miller, T. A. *Tetrahedron Lett.* **1990**, *37*, 5253-5256. (b) Hughes, D. L.; Reamer, R. A. *J. Org. Chem.* **1996**, *61*, 2967-2971. (c) Saiah, M.; Bessodes, M.; Antonakis, K. *Tetrahedron Lett.* **1992**, *30*, 4317-4320. (d) Cook, A. F.; Maichuk, D. T. *J. Org. Chem.* **1970**, *35*, 1940-1943. (e) Wagner, J.; Anders, H.; Rohrbach, S.; Wagner, D.; Oberer, L.; France, J. *J. Org. Che.* **2005**, *70*, 9588-9590.

42 Matsumoto, K.; Miura, K.;Oshima, K.; Utimoto, K. *Tetrahedron Lett*. **1992**, *33*, 7031-7034, and references therein.

43 (a) Kabalka, G. W.; Brown, H. C.; Suzuki, A.; Honma, S.; Arase, A.; Itoh, M. *J. Am. Chem. Soc*. **1970**, *92*, 710-712. (b) Davies, A. G.; Griller, D.; Roberts, B. P.; Tudor, R. *J. Chem. Soc. D* **1970**, 640 (c) Davies, A. G.; Maki, T.; Roberts, B. P. *J. Chem. Soc. Perkin Trans. 2* **1972**, 744-747. (d) Krusic, P. J.; Kochi, J. K. *J. Am. Chem. Soc.* **1969**, *91*, 3942-3944. (e) Ollivier, C.; Renaud, P. *Chem. Rev.* **2001**, *101*, 3415-3434.

44 (a) Nozaki, K.; Oshima, K.; Utimoto, K. *J. Am. Chem. Soc.* **1987**, *109*, 2547-2549. (b) Guindon, Y.; Lavalle´e, J.-F.; Boisvert, L.; Chabot, C.; Delorme, D.; Yoakim, C.; Hall, D.; Lemieux, R.; Simoneau, B. *Tetrahedron Lett.* **1991**, *32*, 27-30. (c) Satoh, S.; Sodeoka, M.; Sasai, H.; Shibasaki, M. *J. Org. Chem.* **1991**, *56*, 2278-2280.

45 Carra, C.; Scaiano, J. C. *Eur. J. Org. Chem.* **2008**, *26*, 4454-4459.

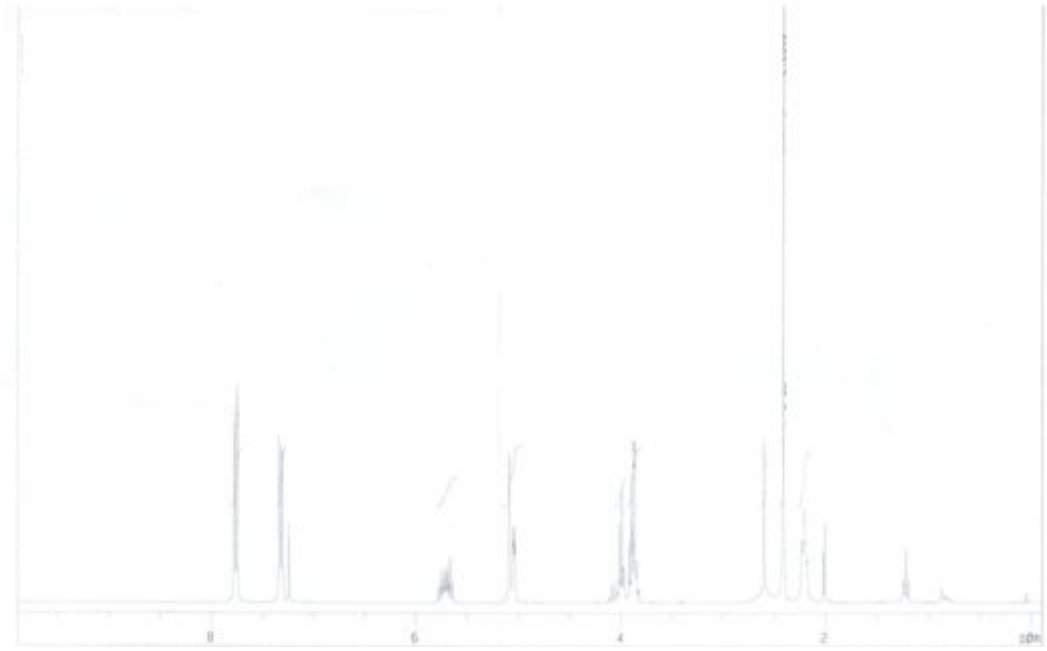
46 (a) Carroll, G. L.; Allan, A. K.; Schwaebe, M. K.; Little, R. D. *Org. Lett*. **2000**, *2*, 2531-2534. (b) Devin, P.; Fensterbank, L.; Malacria, M. *J. Org. Chem*. **1998**, *63*, 6764-6765.

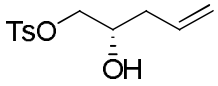
47 (a) Thomas, E. J.; Williams, A. C. *J. Chem. Soc., Chem. Commun*. **1987**, *13*, 992-994. (b) Jahn, U.; Hartmann, P.; Kaasalainen, E. *Org. Lett*. **2004**, *6*, 257-260.

48 We have reported the successful result previously: Lee, S.; Paek, S.-M.; Yun, H.; Kim, N.-J.; Suh, Y.-G. *Org. Lett.* **2011**, *13*, 3344-3347.

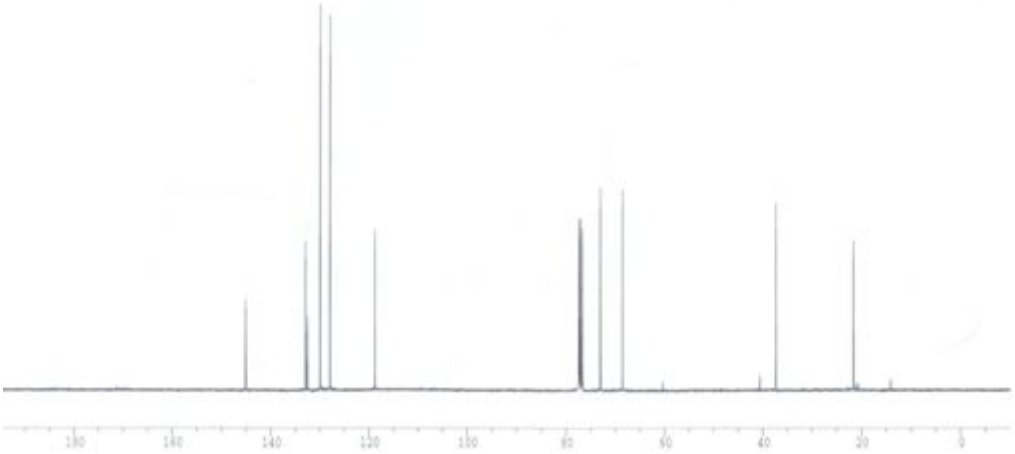
VI. Appendix

▼ **59, ^{1}H-NMR, 300 MHz**

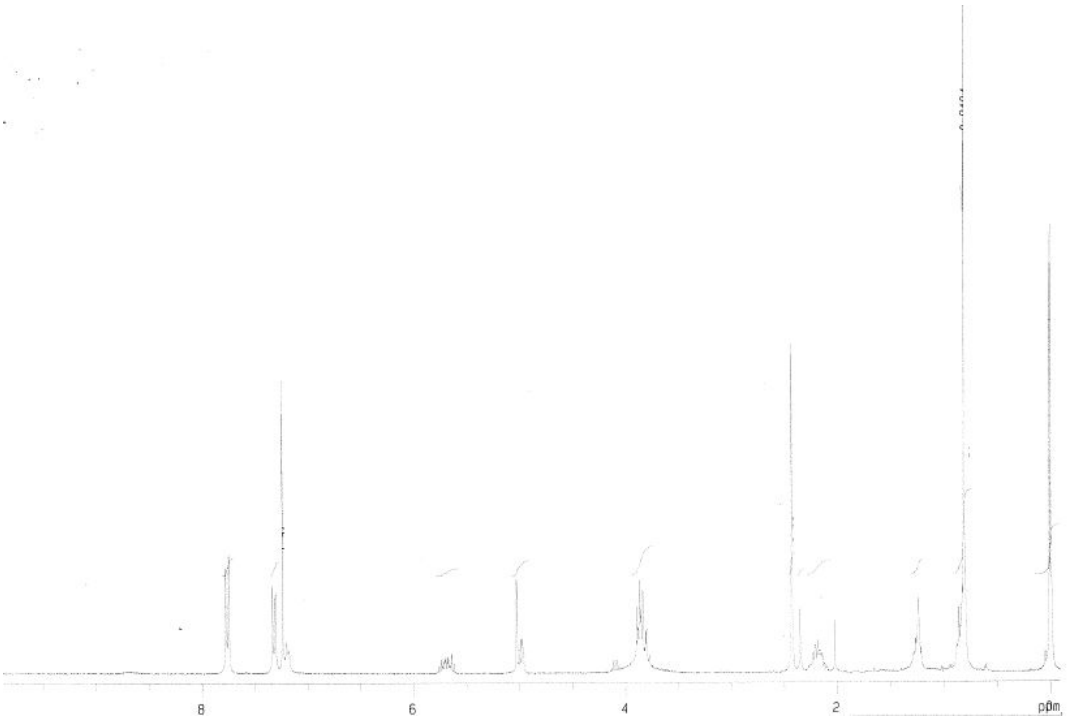




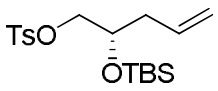
▼ **59, ^{13}C-NMR, 75 MHz**

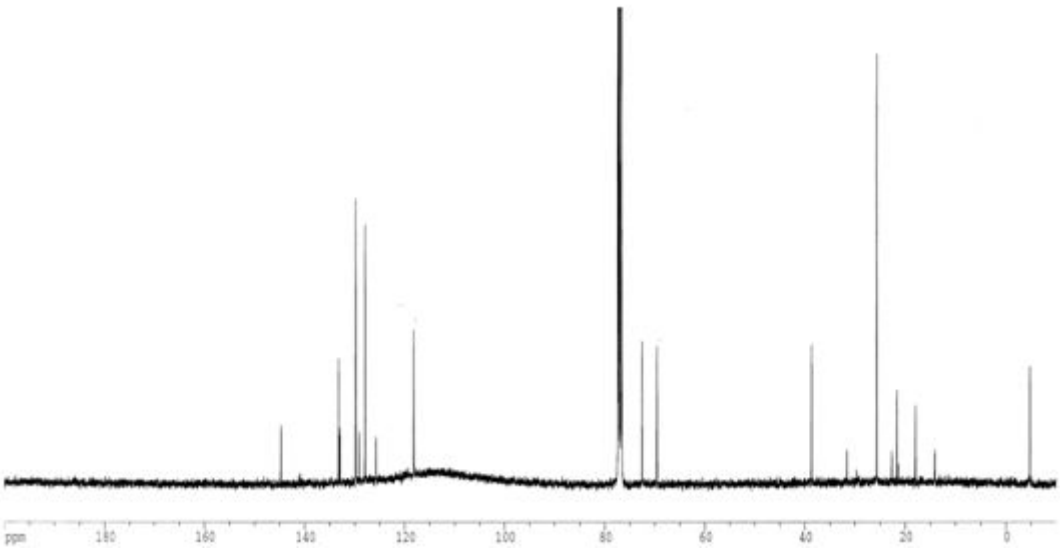


▼ **60, ^{1}H-NMR, 300 MHz**

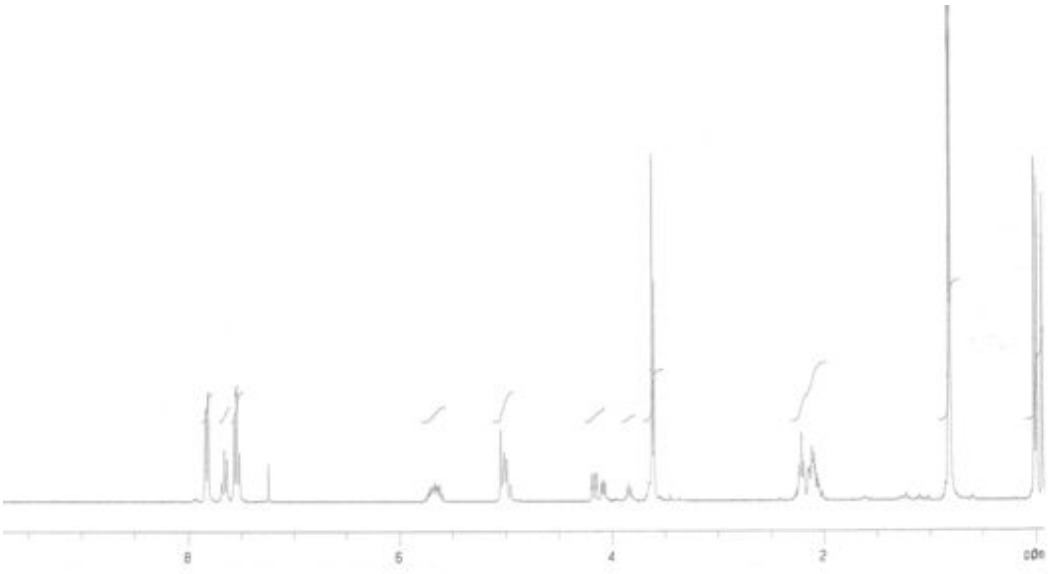


▼ **60, ^{13}C-NMR, 75 MHz**

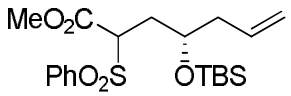


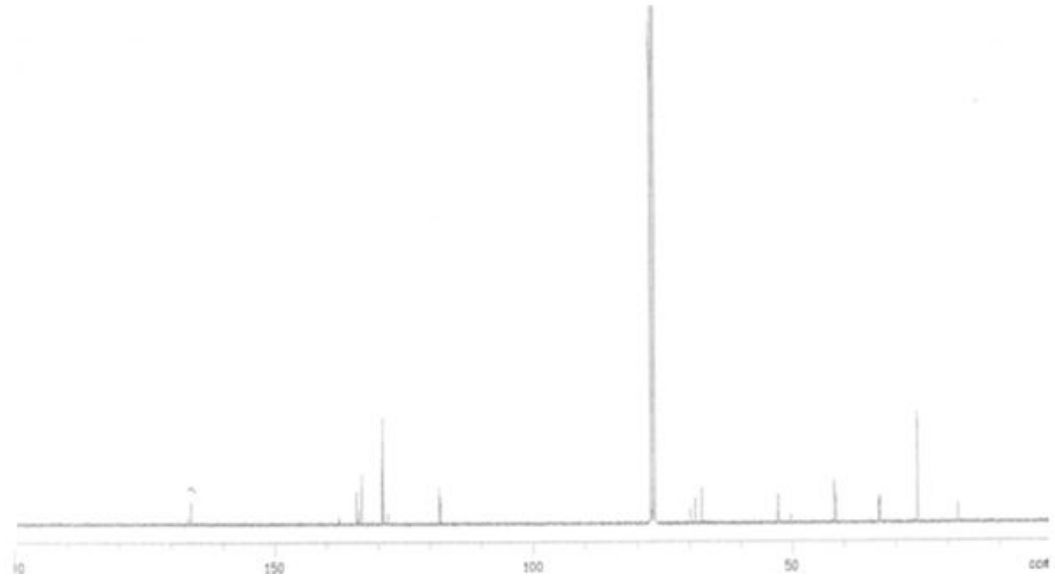


▼ **61, ^{1}H-NMR, 300 MHz**

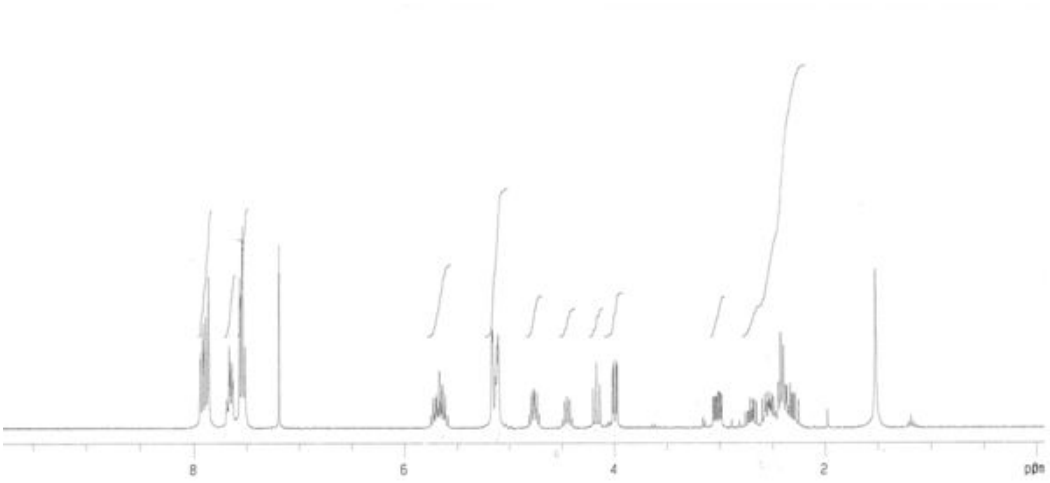


▼ **61, ^{13}C-NMR, 75 MHz**

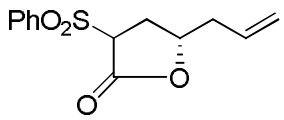


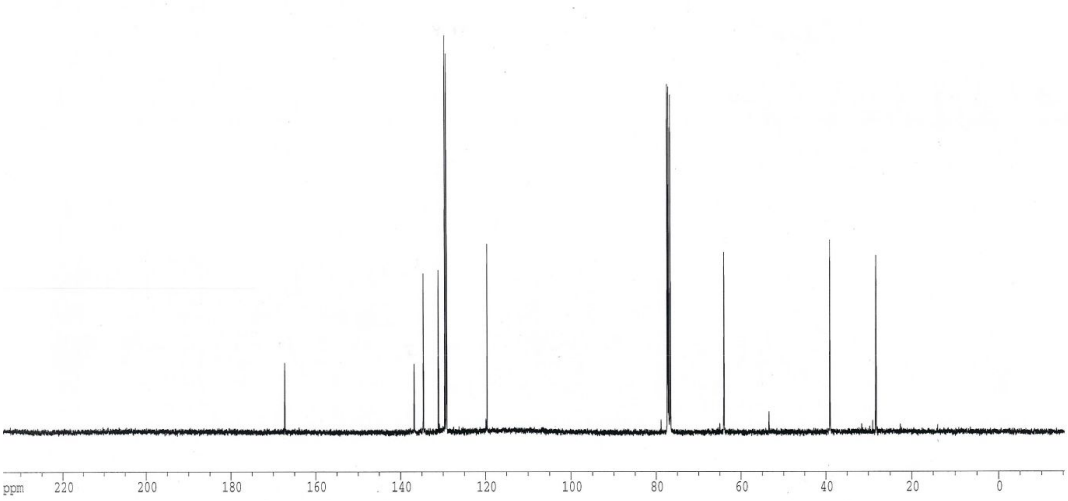


▼ **62, ^{1}H-NMR, 300 MHz**

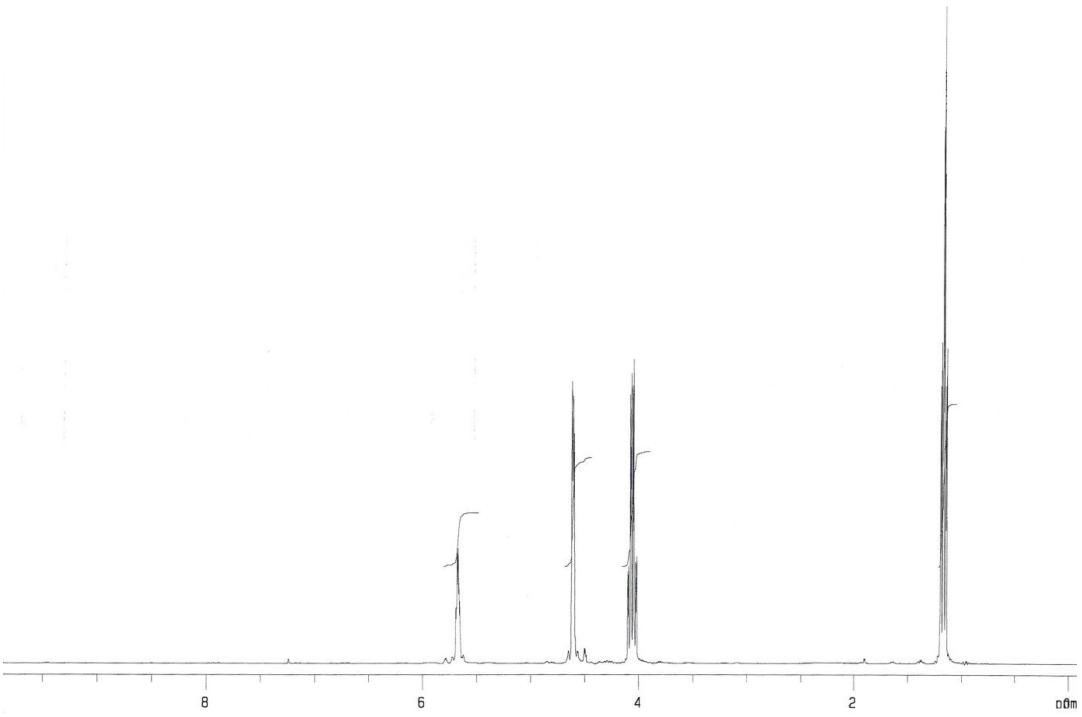


▼ **62, ^{13}C-NMR, 75 MHz**

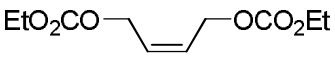


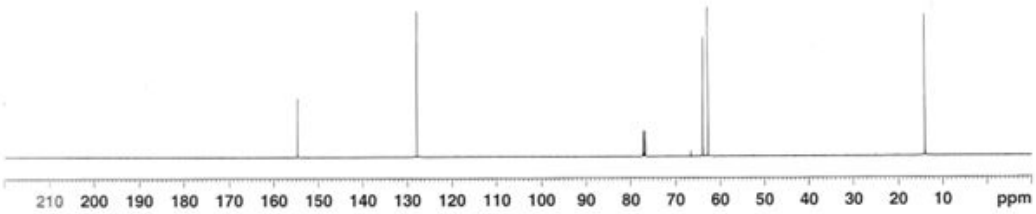


▼ **63a, ^{1}H-NMR, 300 MHz**

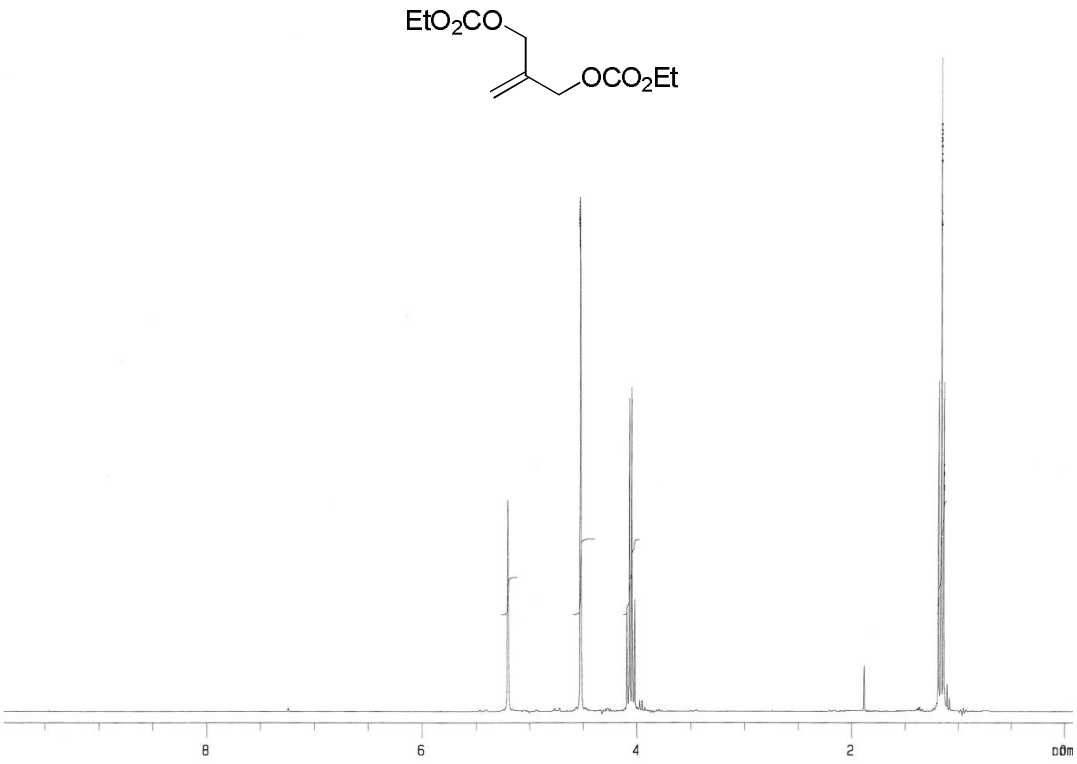


▼ **63a, ^{13}C-NMR, 125 MHz**

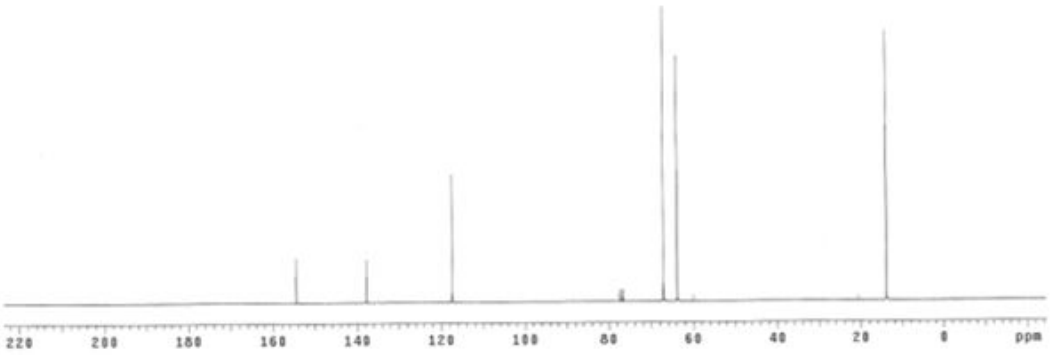




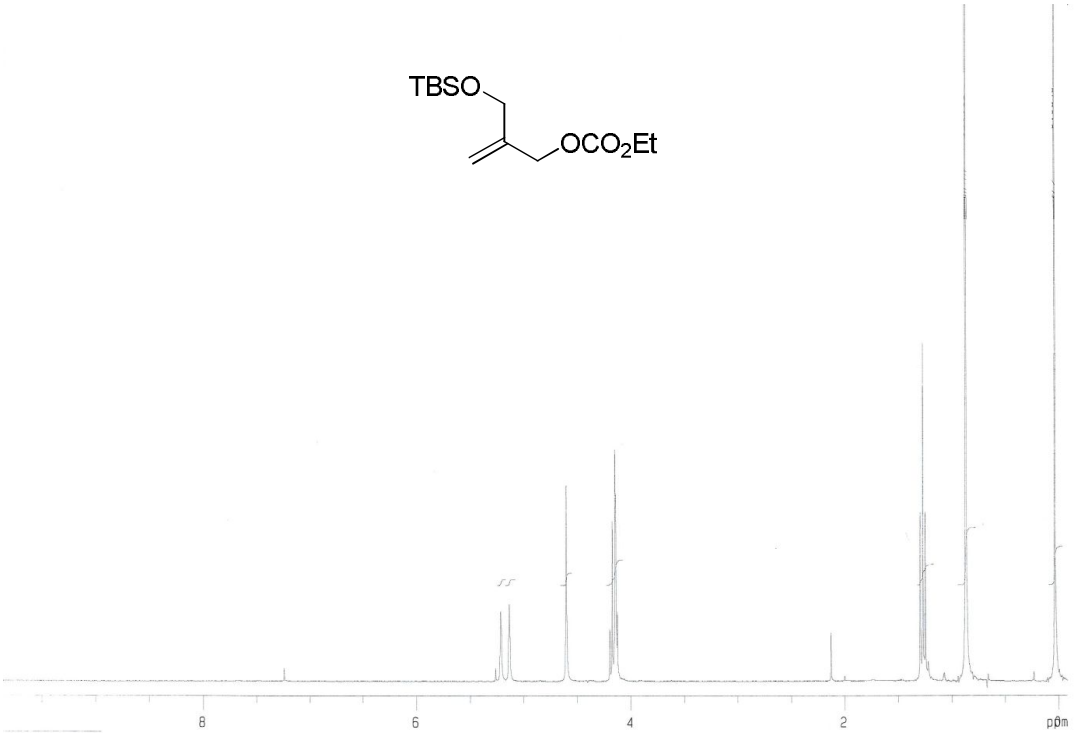
▼ **63b, ^{1}H-NMR, 300 MHz**



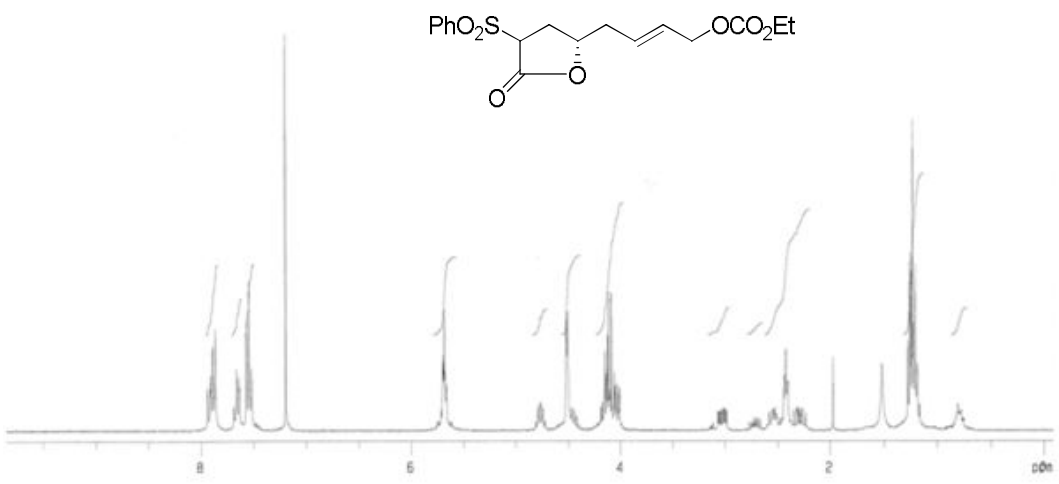
▼ **63b, ^{13}C-NMR, 75 MHz**



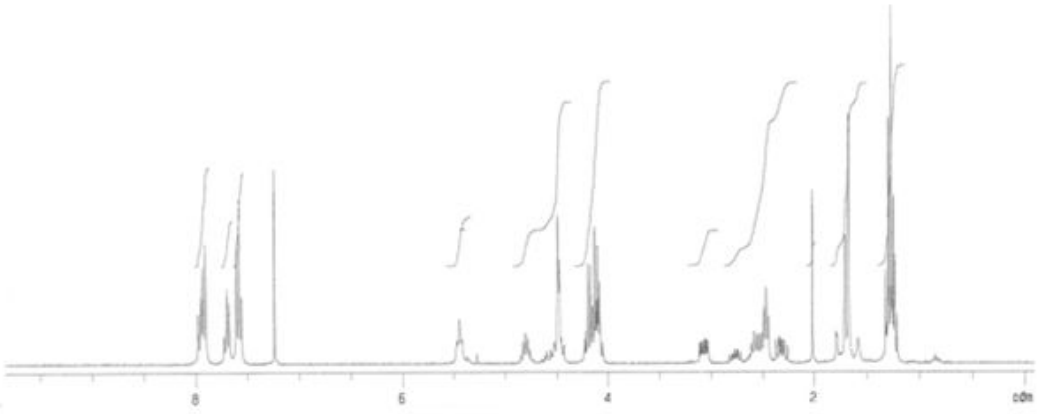
▼ **63d, ^{1}H-NMR, 300 MHz**



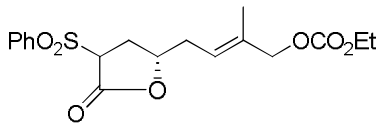
▼ **64a, ^{1}H-NMR, 300 MHz**

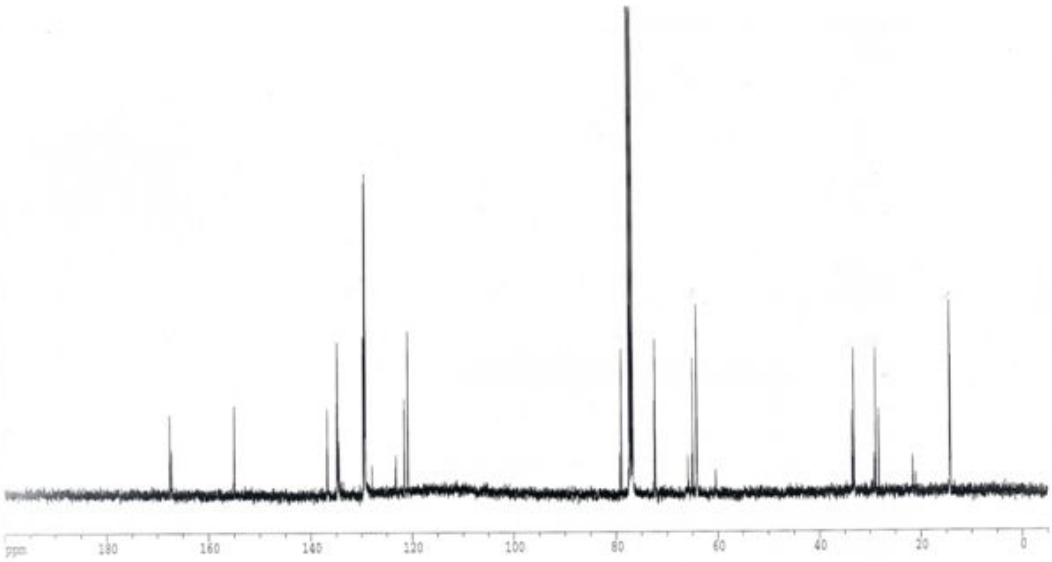


▼ **64c, ^{1}H-NMR, 300 MHz**

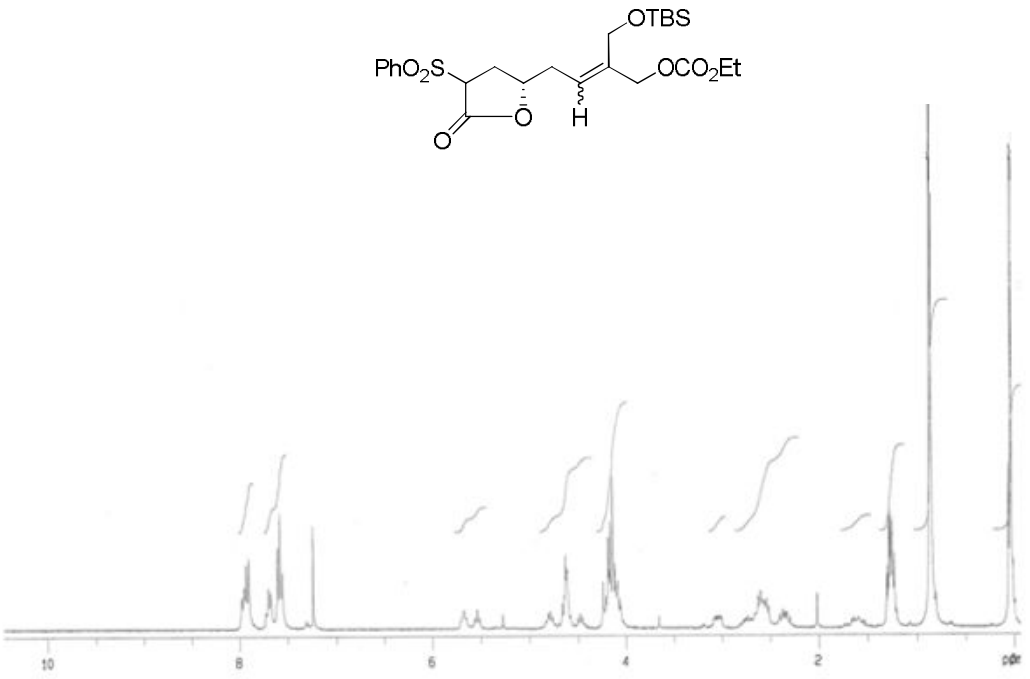


▼ **64c, ^{13}C-NMR, 75 MHz**

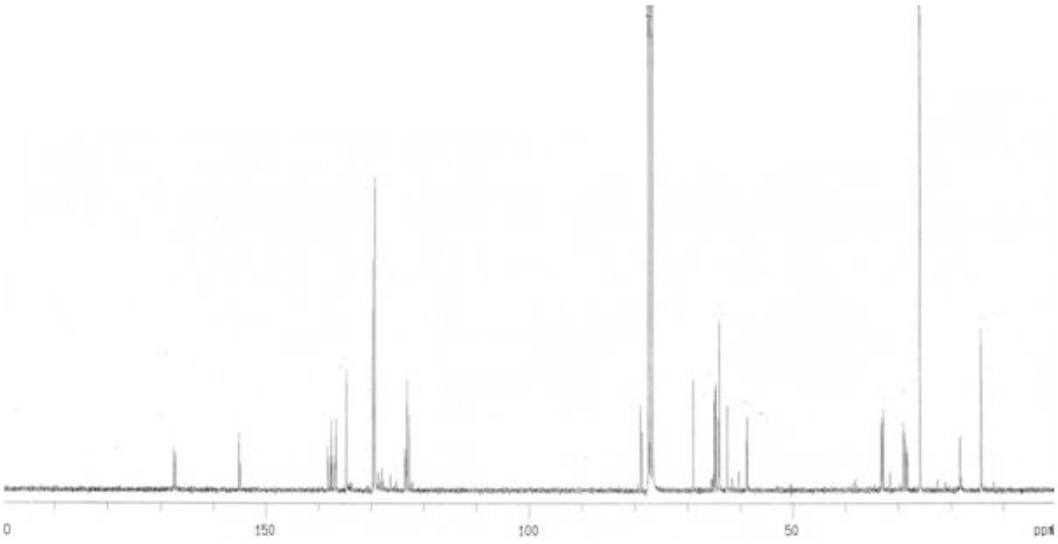




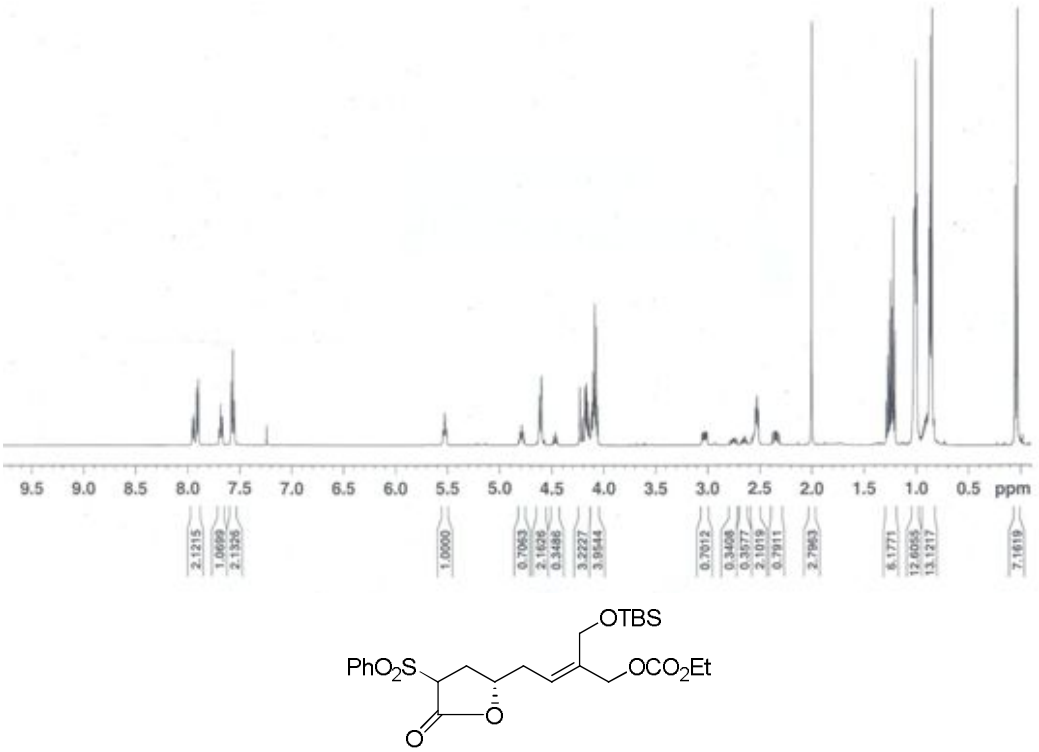
▼ **64d, *E/Z* mix ^{1}H-NMR, 300 MHz**



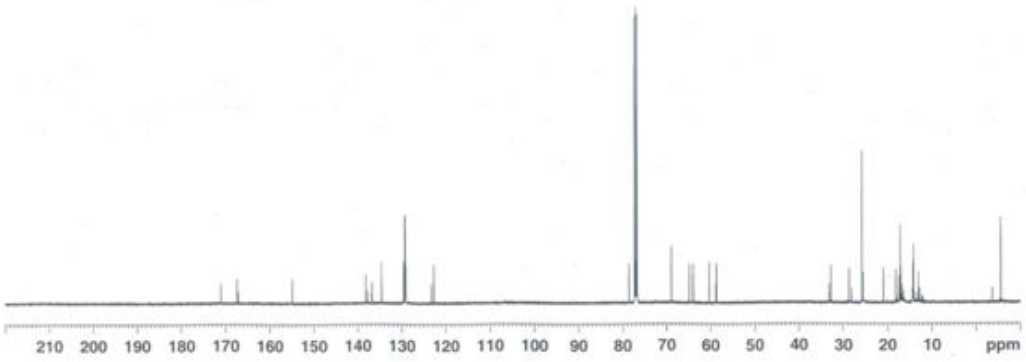
▼ **64d, ^{13}C-NMR, 75 MHz**



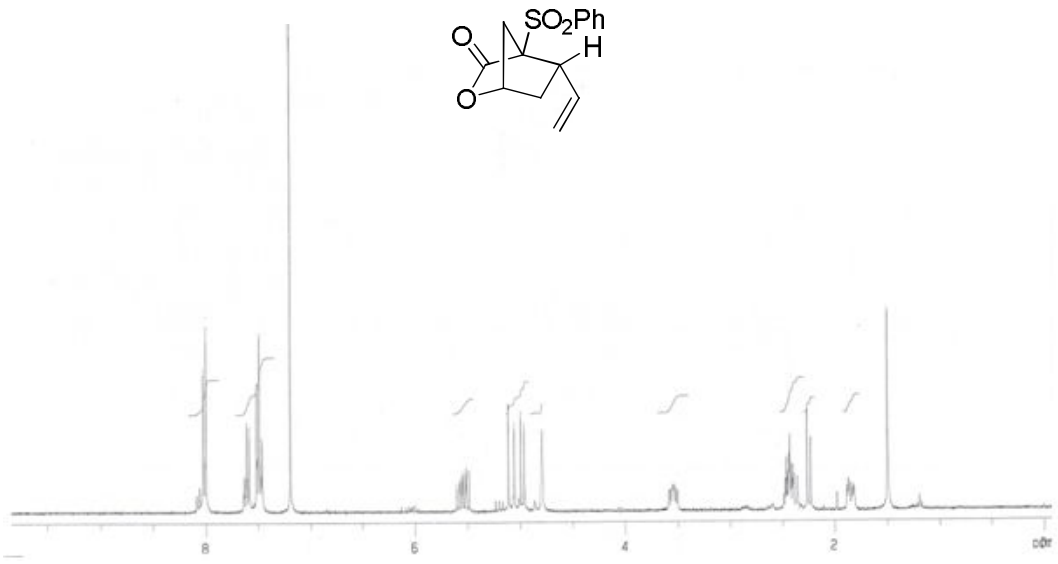
▼ ***Z*-64d, ^{1}H-NMR, 500 MHz**



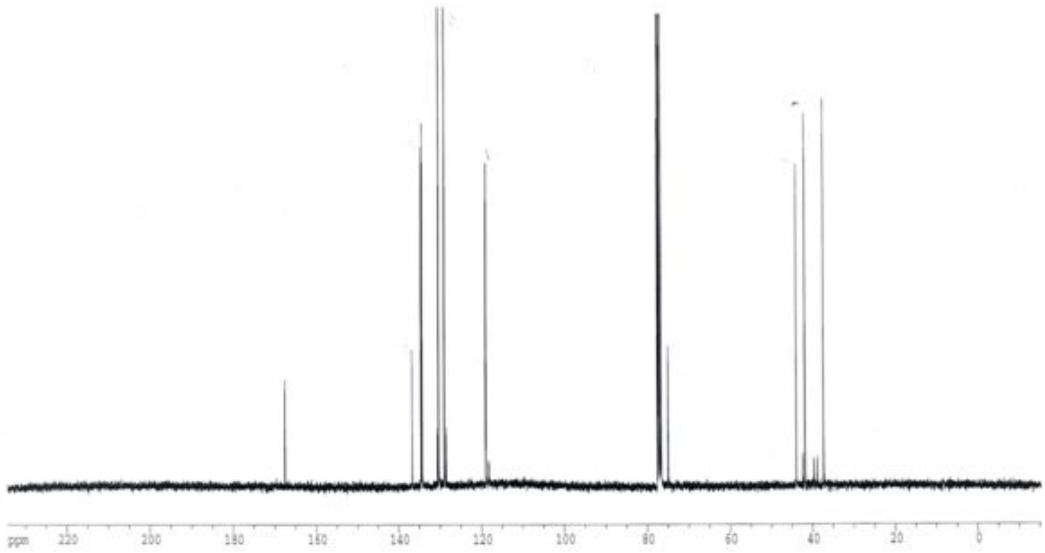
▼ ***Z*-64d, ^{13}C-NMR, 125 MHz**



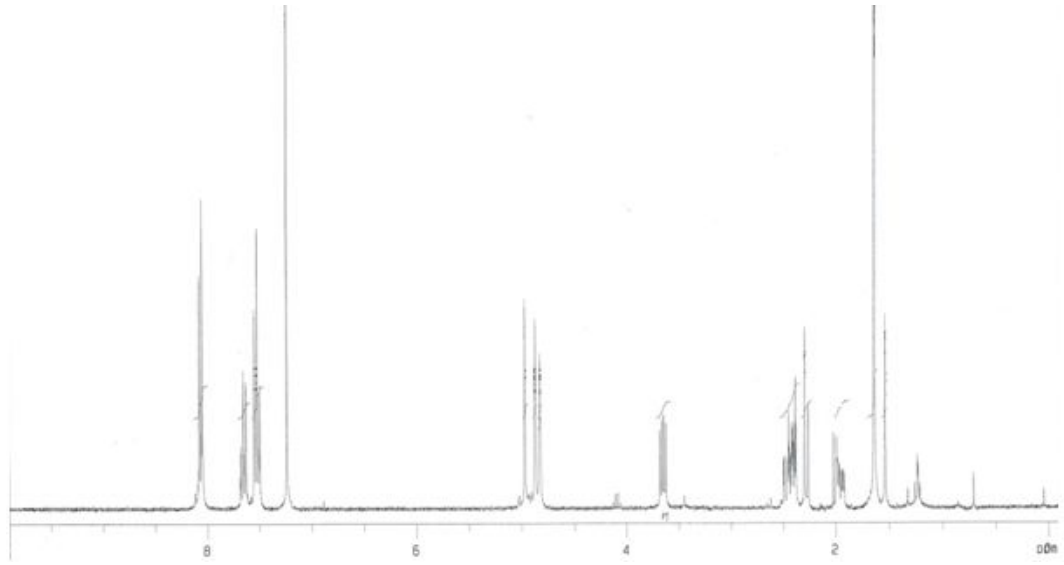
▼ **46, ^{1}H-NMR, 300 MHz**



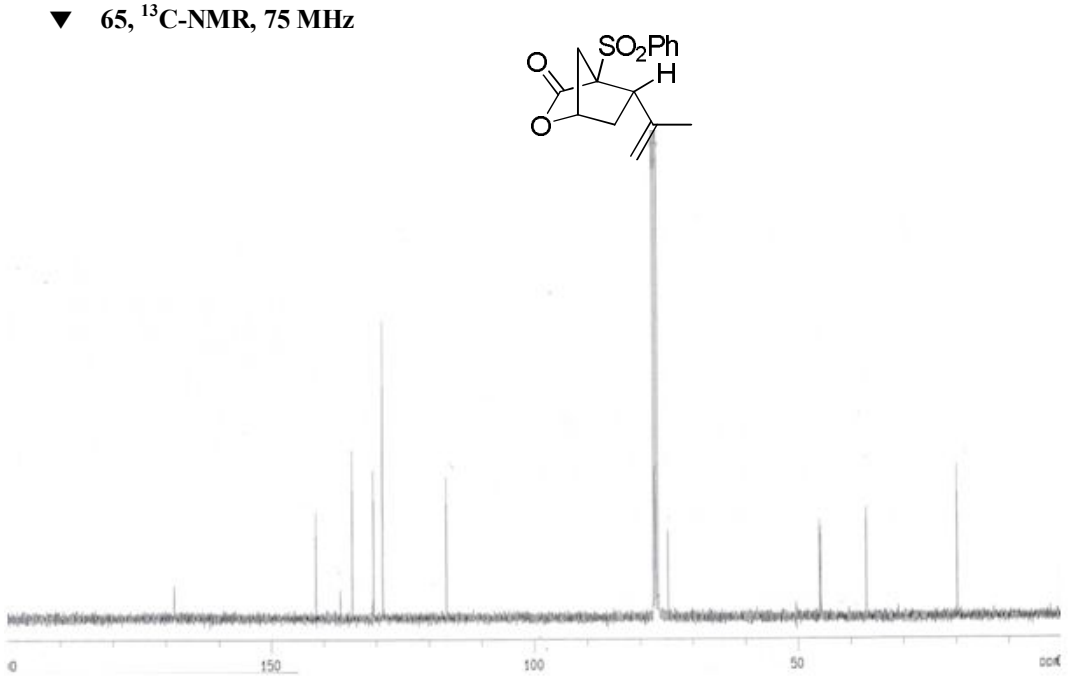
▼ **46 ^{13}C-NMR, 75 MHz**



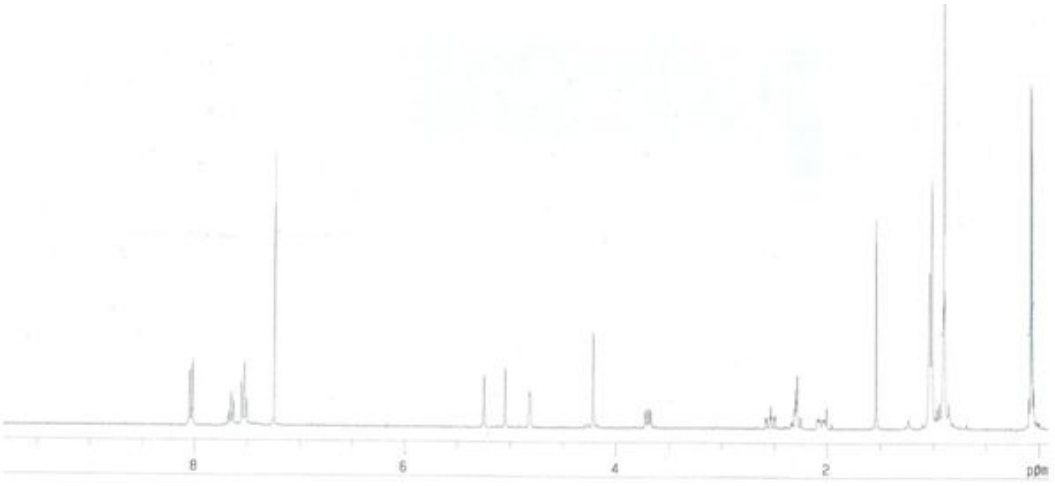
▼ **65, ^{1}H-NMR, 300 MHz**

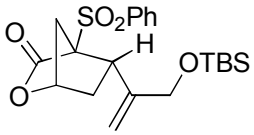


▼ **65, ^{13}C-NMR, 75 MHz**

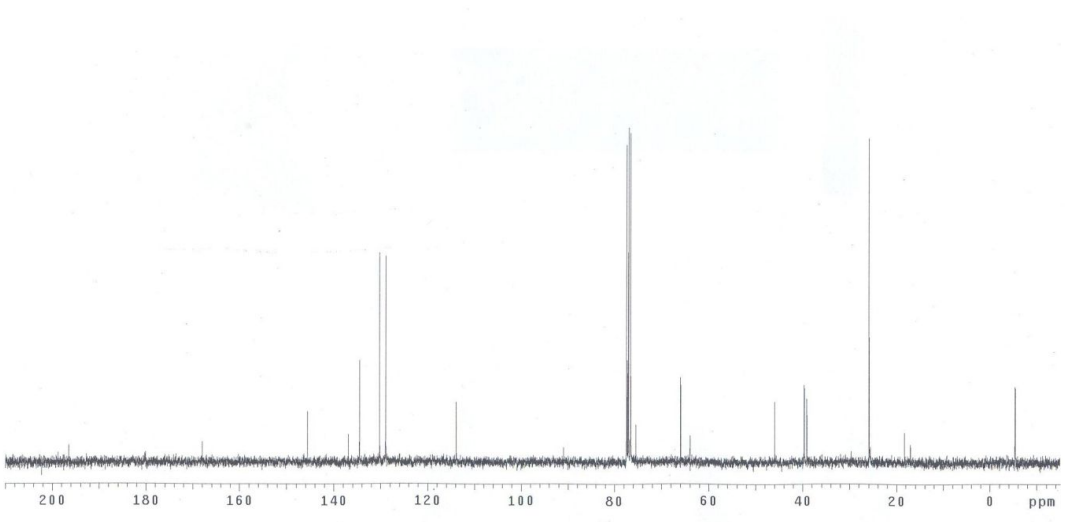


▼ **66, ^{1}H-NMR, 300 MHz**

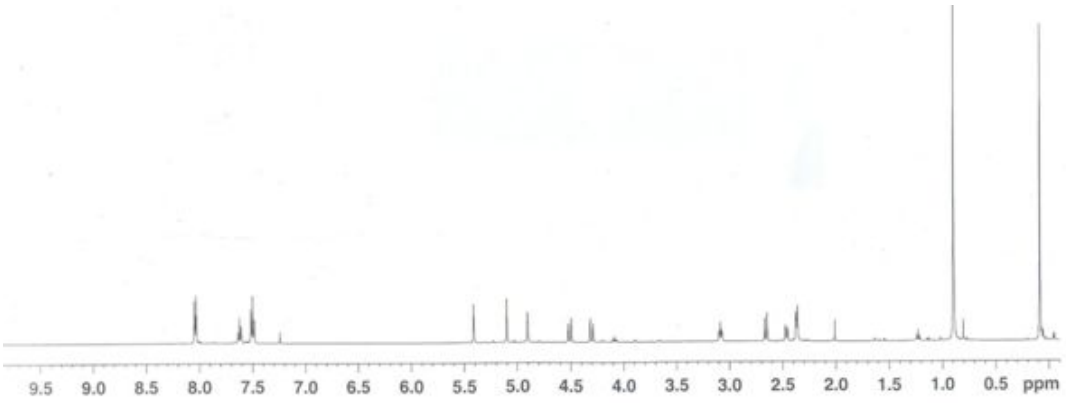




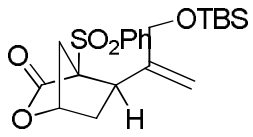
▼ **66 ^{13}C-NMR, 75 MHz**

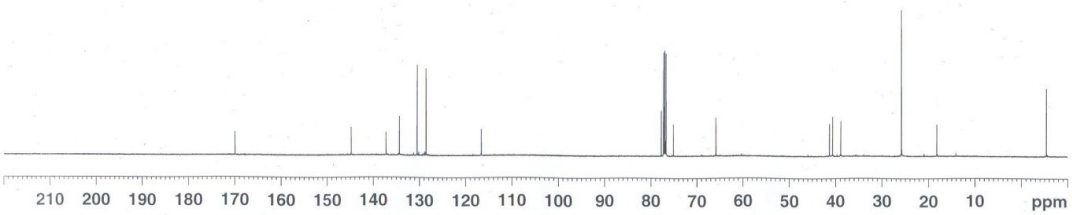


▼ **66', ^{1}H-NMR, 500 MHz**

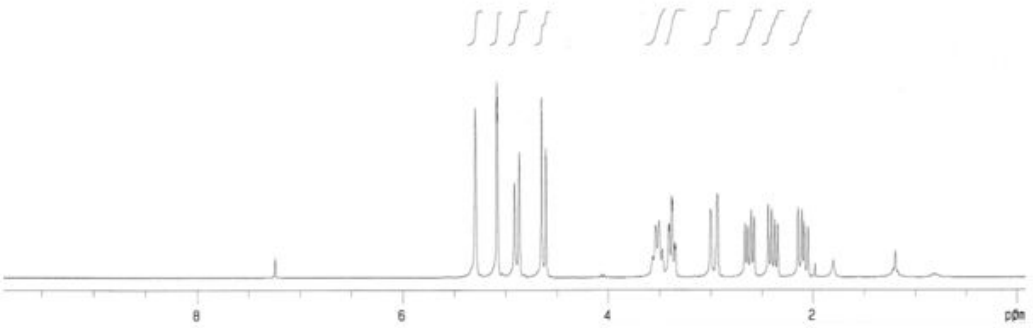


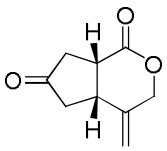
▼ **66', ^{13}C-NMR, 125 MHz**



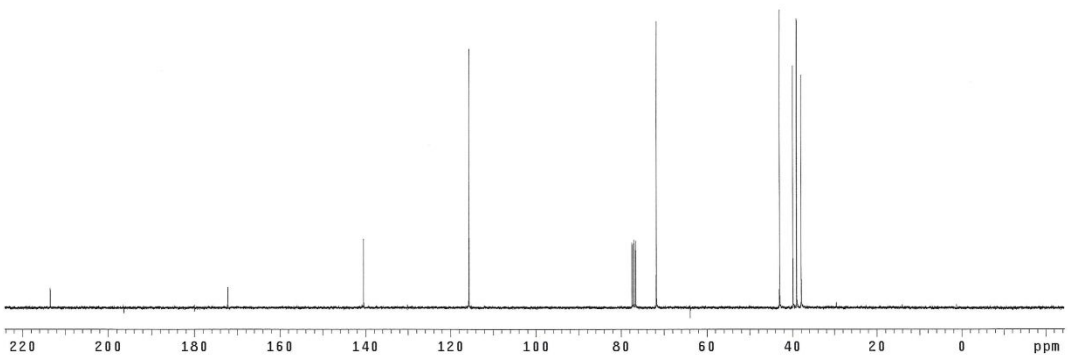


▼ **68, ^{1}H-NMR, 300 MHz**

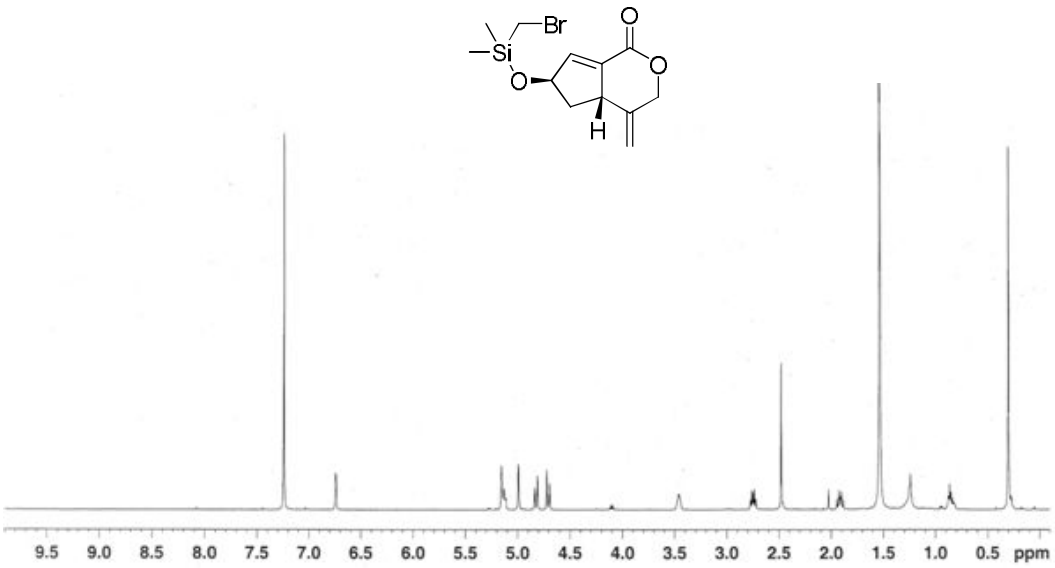




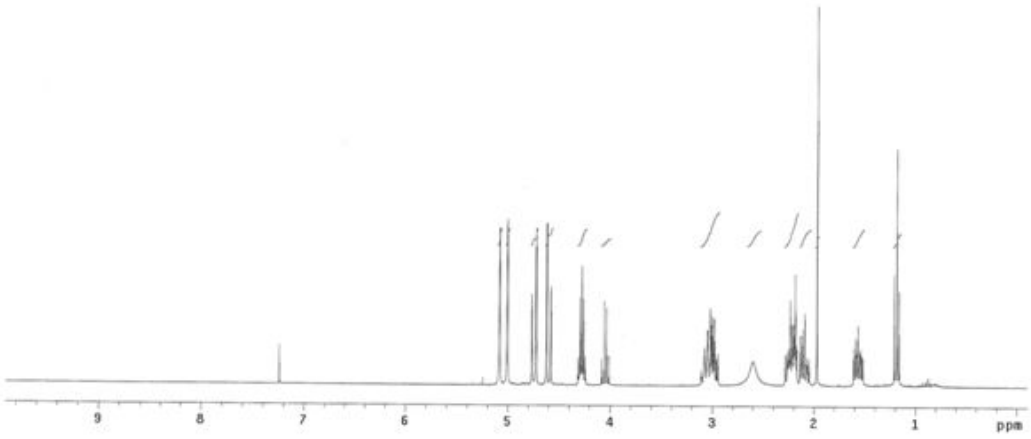
68, ^{13}C-NMR, 75 MHz

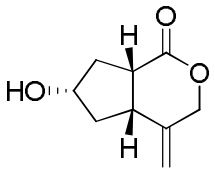


▼ **71, ^{1}H-NMR, 500 MHz**

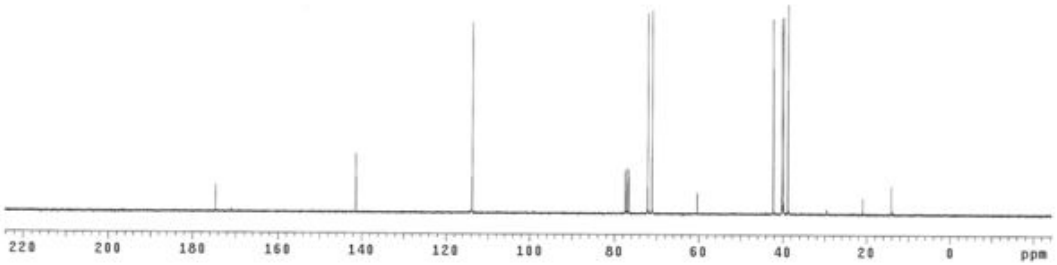


▼ **69, ^{1}H-NMR, 300 MHz**

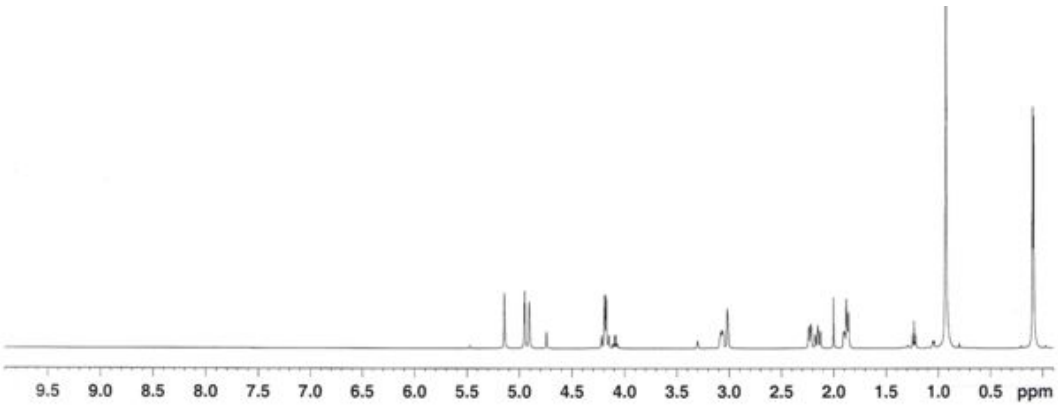


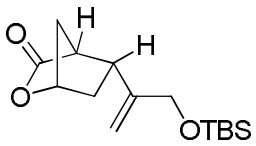


▼ **69, ^{13}C-NMR, 75 MHz**

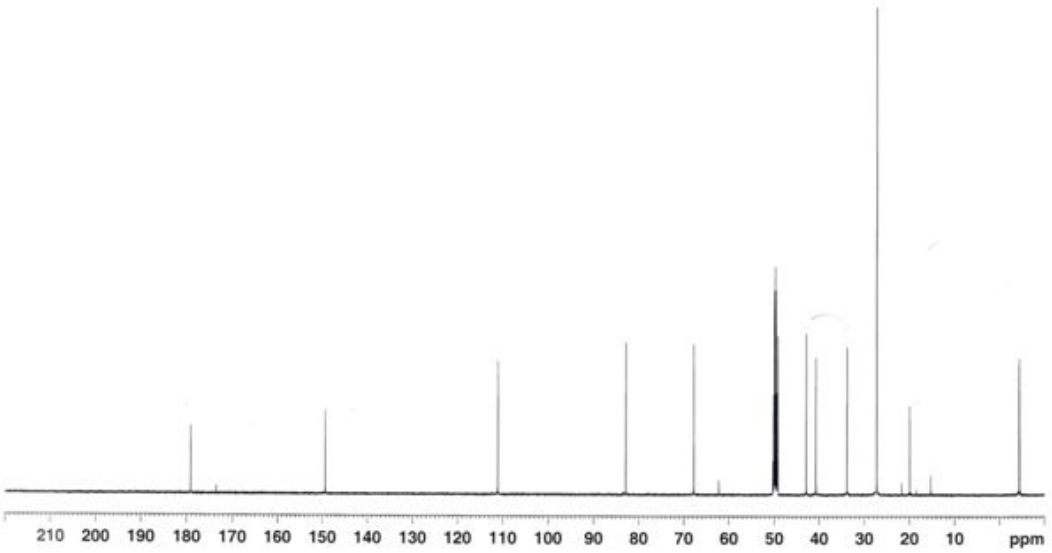


▼ **70, ^{1}H-NMR, 500 MHz**

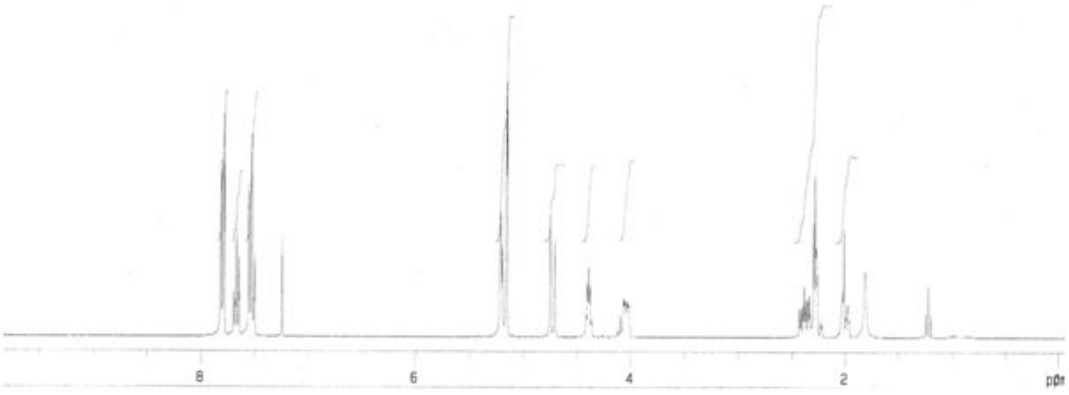


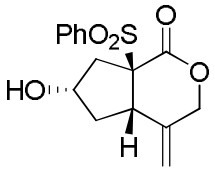


▼ **70, ^{13}C-NMR, 125 MHz**

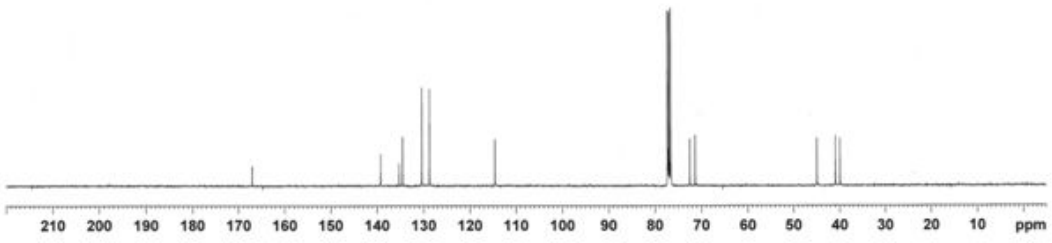


▼ **72, ^{1}H-NMR, 300 MHz**

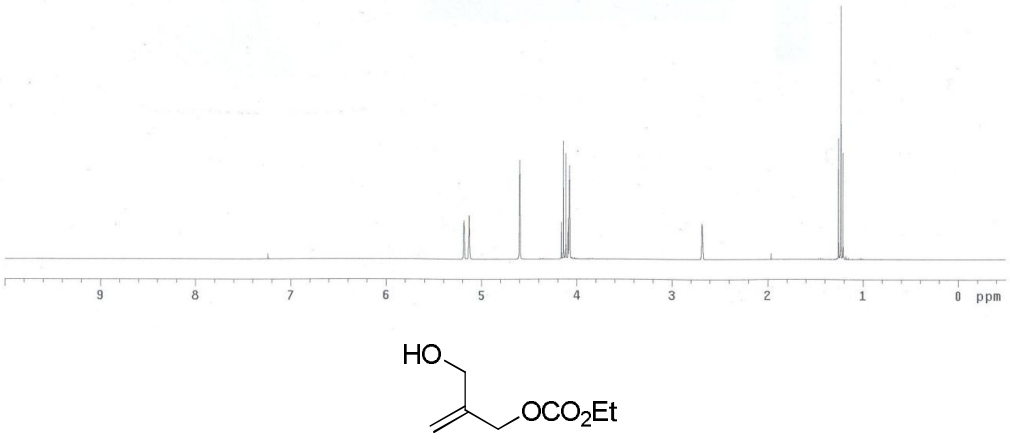




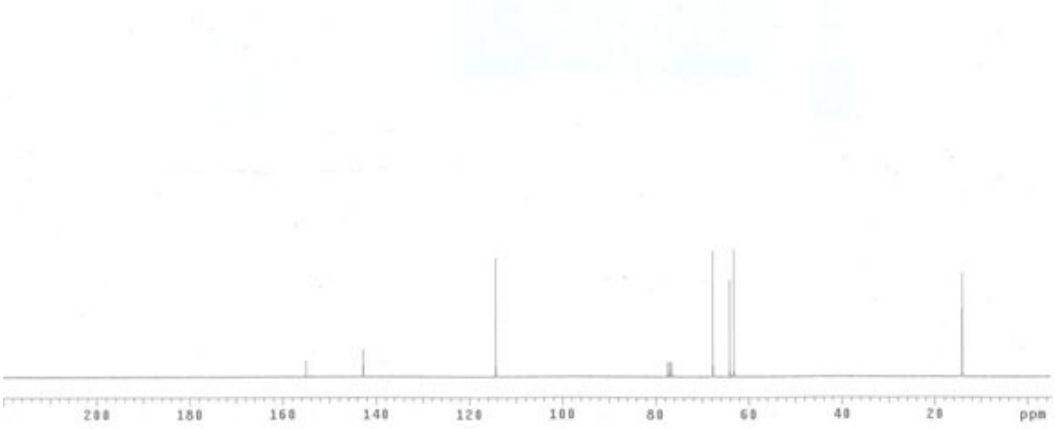
▼ **72, ^{13}C-NMR, 125 MHz**



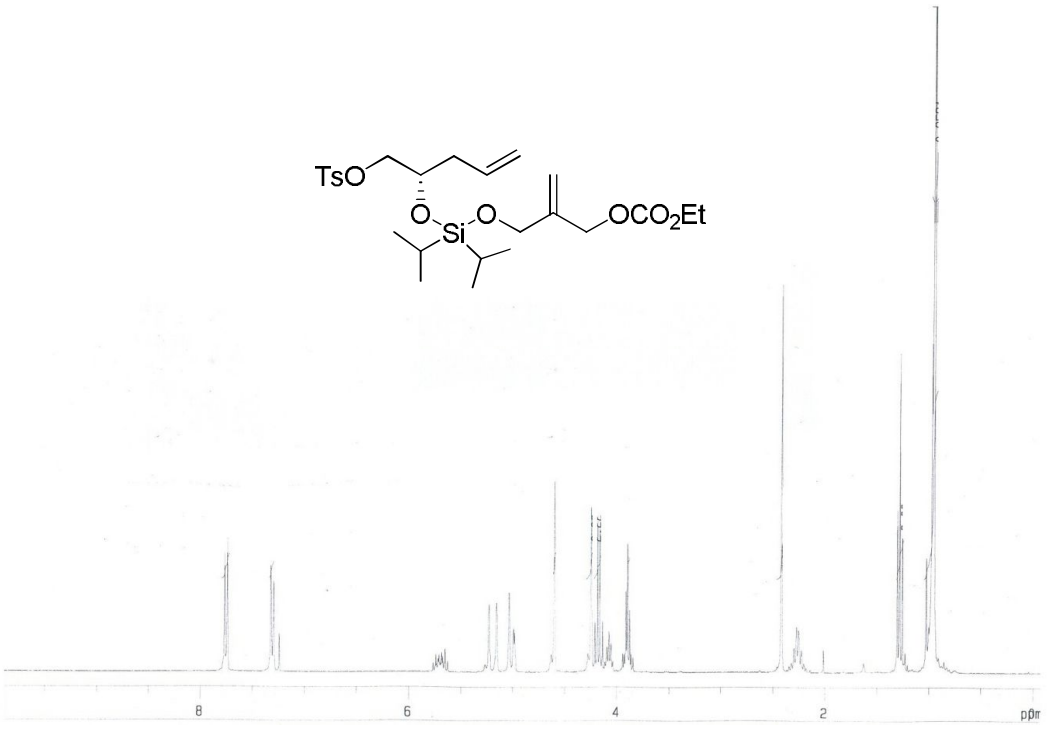
▼ **73, ^{1}H-NMR, 300 MHz**



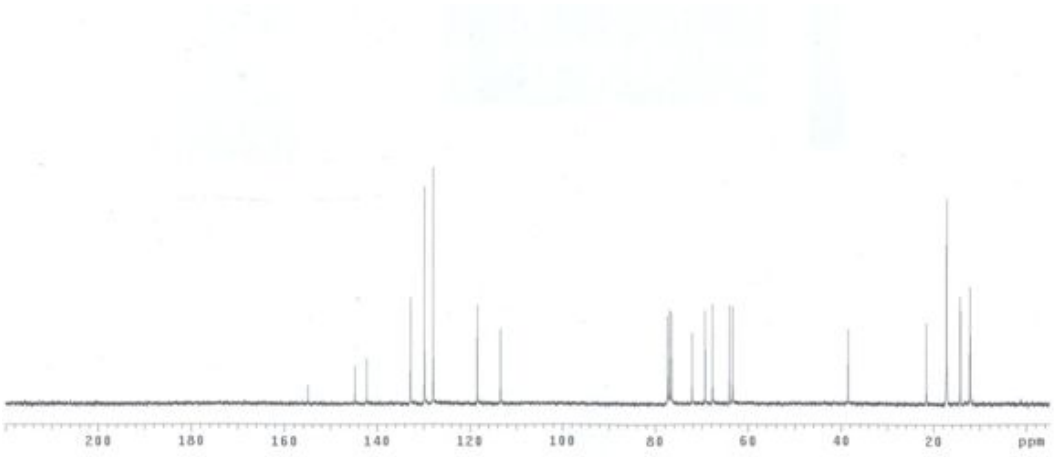
▼ **73, ^{13}C-NMR, 75 MHz**



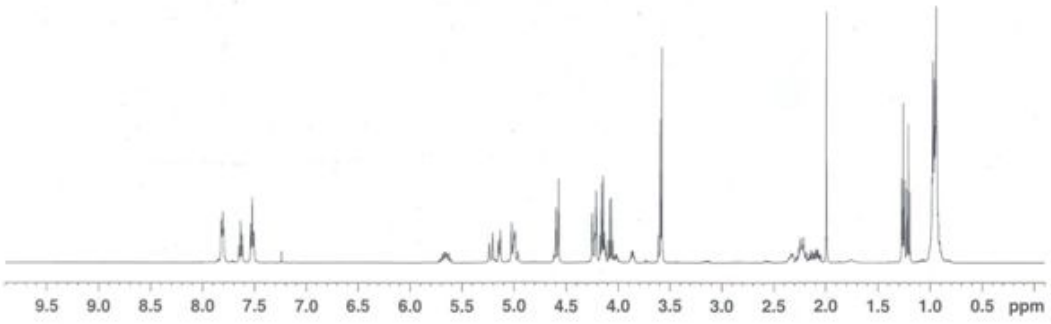
▼ **74, ^{1}H-NMR, 300 MHz**



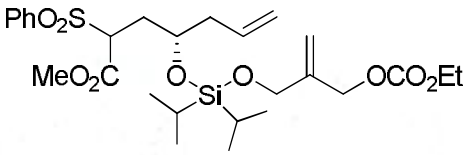
▼ **74, ^{13}C-NMR, 75 MHz**

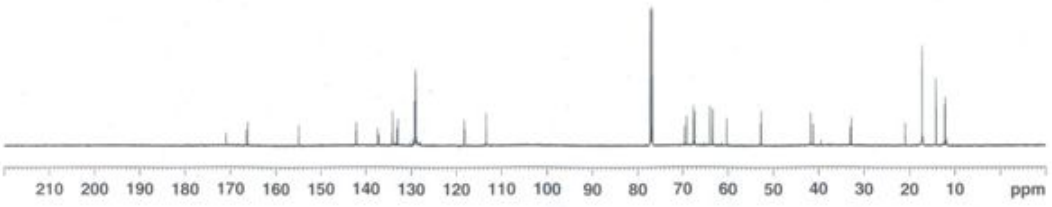


▼ **S74, ^{1}H-NMR, 500 MHz**

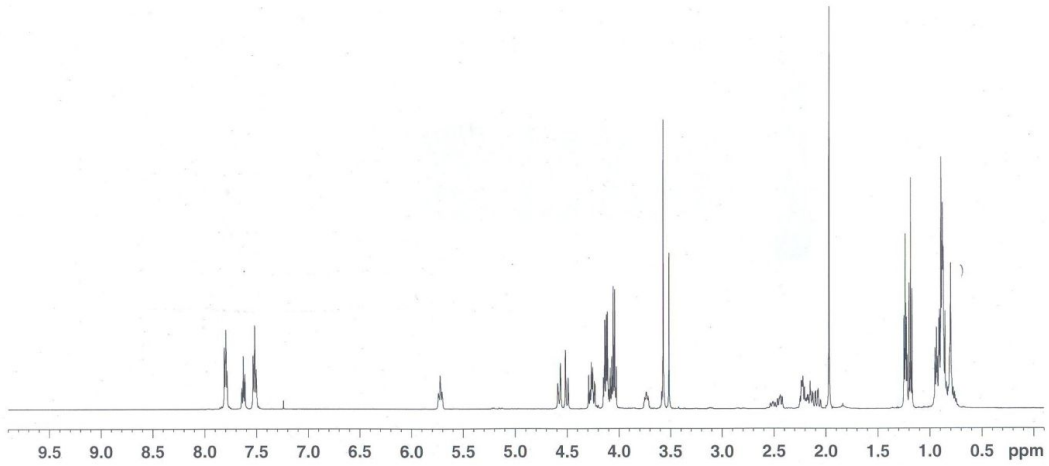


▼ **S74, ^{13}C-NMR, 125 MHz**

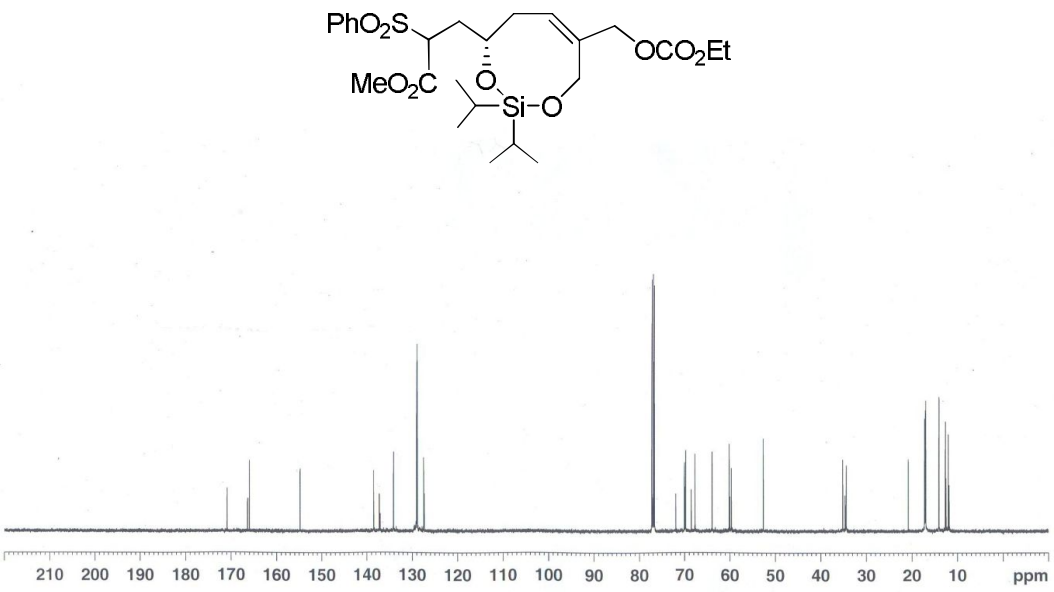




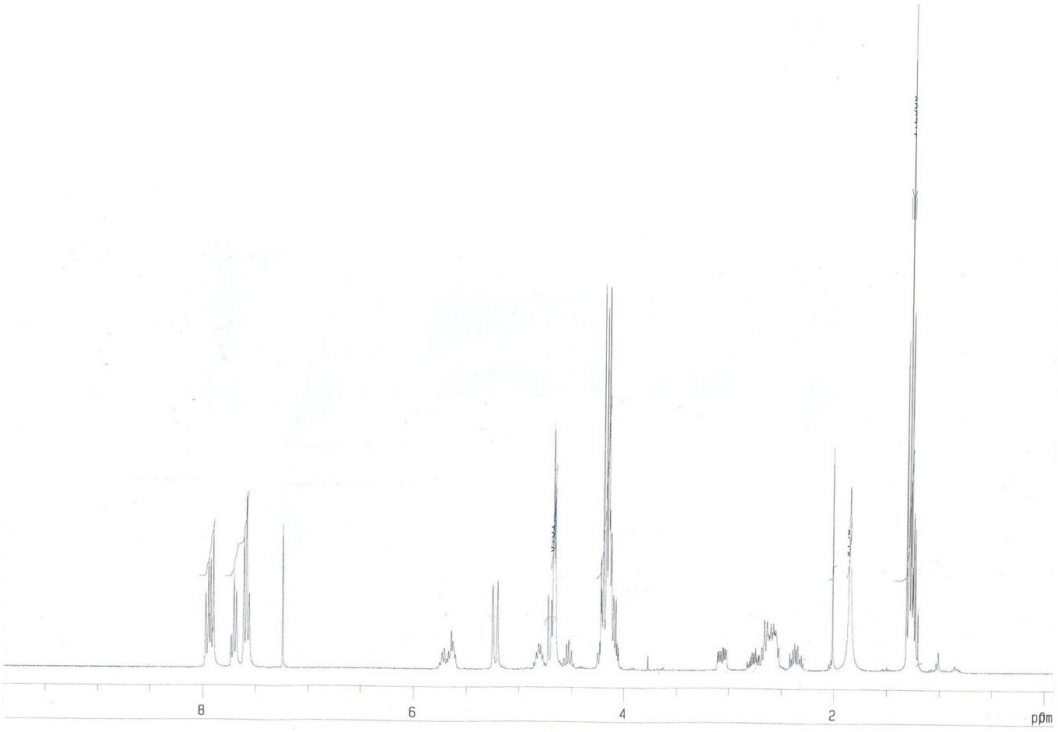
▼ **75, ^{1}H-NMR, 500 MHz**

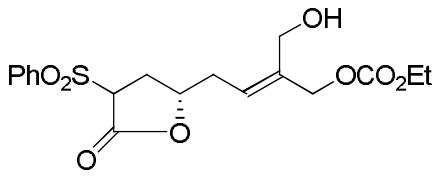


▼ **75, ^{13}C-NMR, 125 MHz**

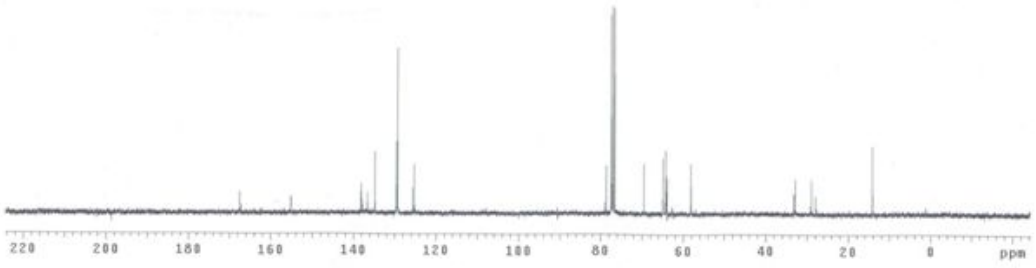


▼ **S75, ^{1}H-NMR, 300 MHz**

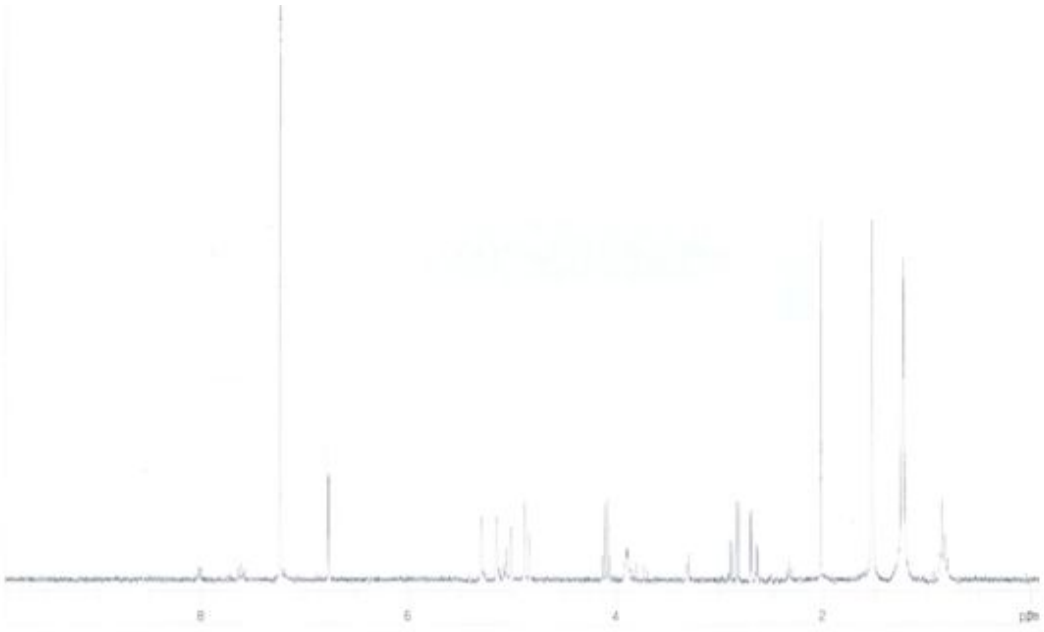




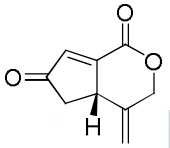
▼ **S75, ^{13}C-NMR, 75 MHz**

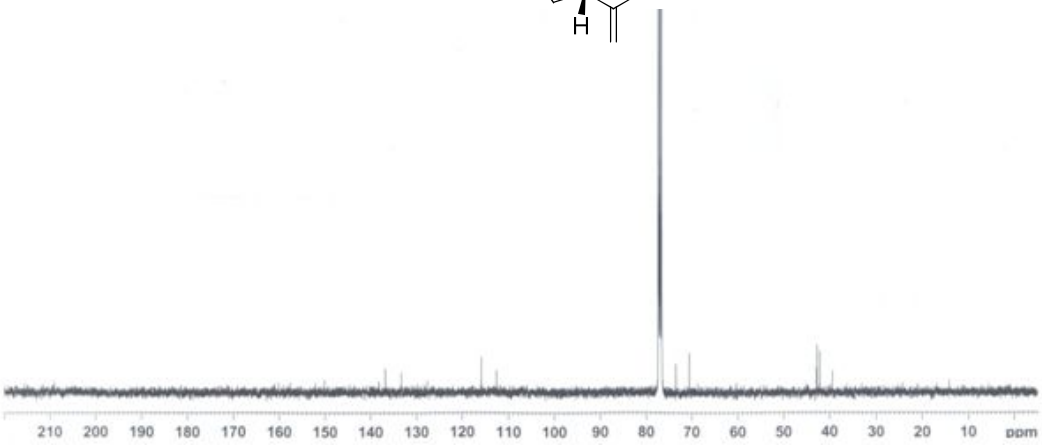


▼ **76, ^{1}H-NMR, 300 MHz**

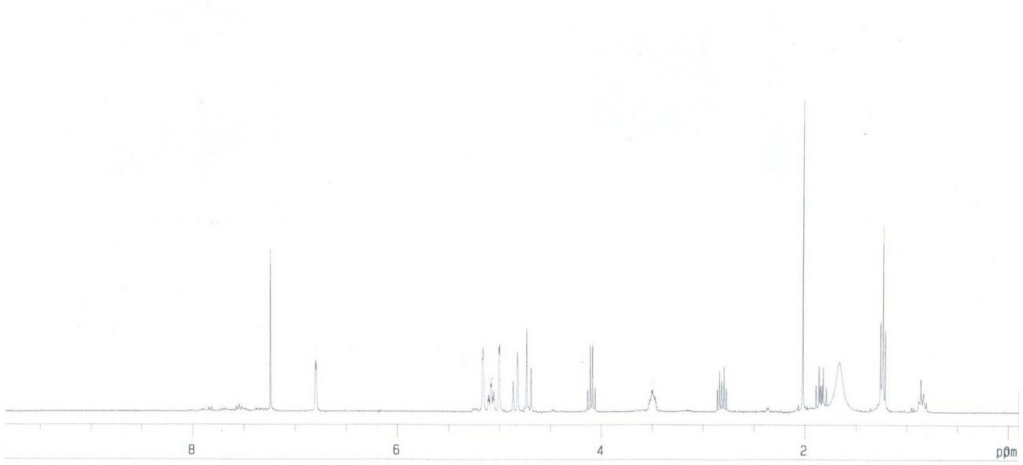


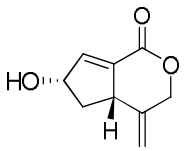
▼ **76, ^{13}C-NMR, 75 MHz**



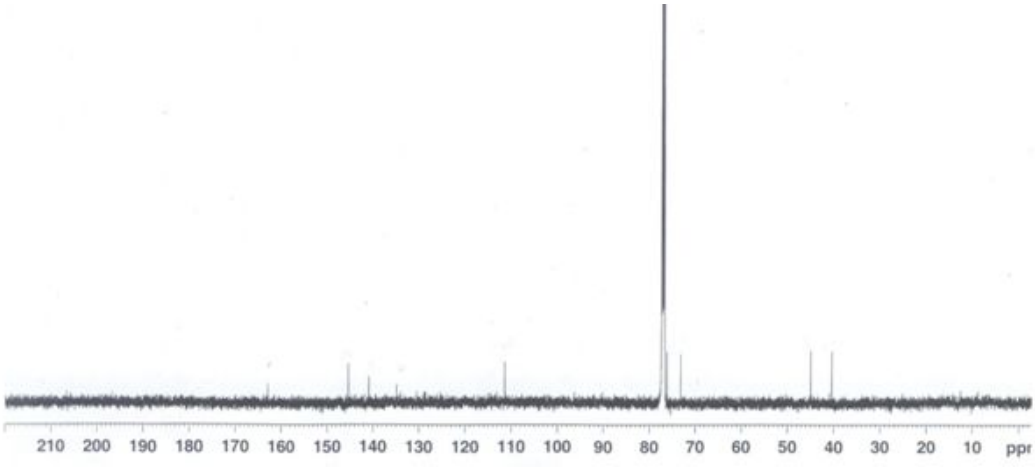


▼ **77, ^{1}H-NMR, 500 MHz**

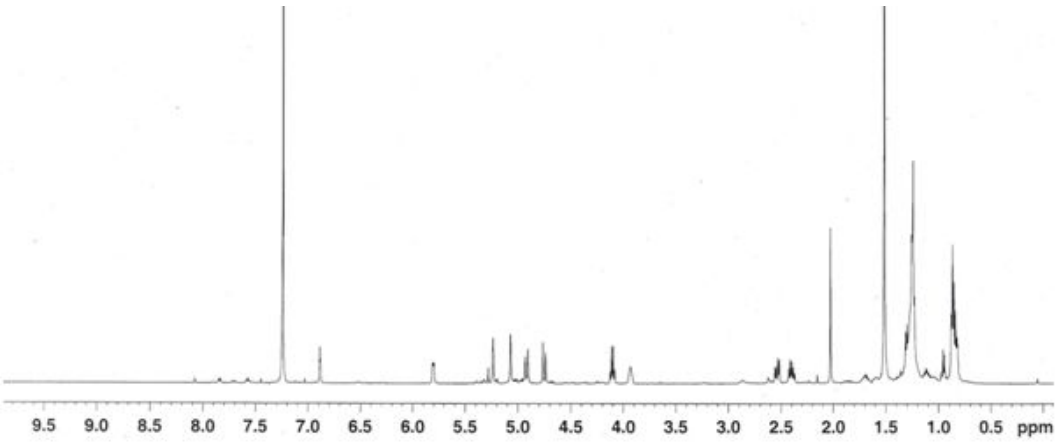


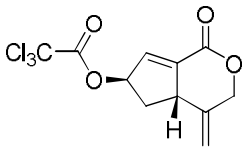


▼ **77, ^{13}C-NMR, 100 MHz**

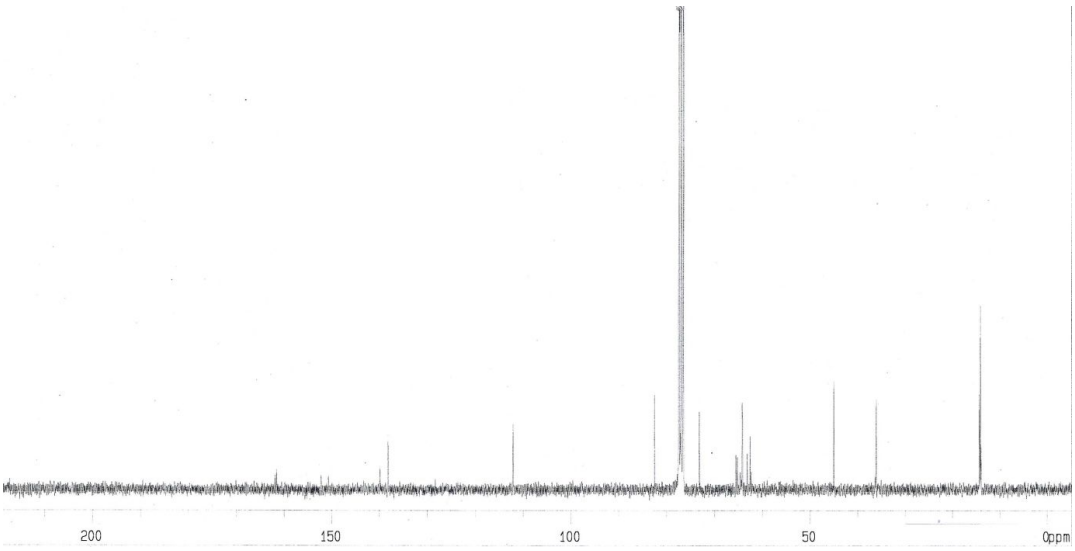


▼ **78, ^{1}H-NMR, 500 MHz**

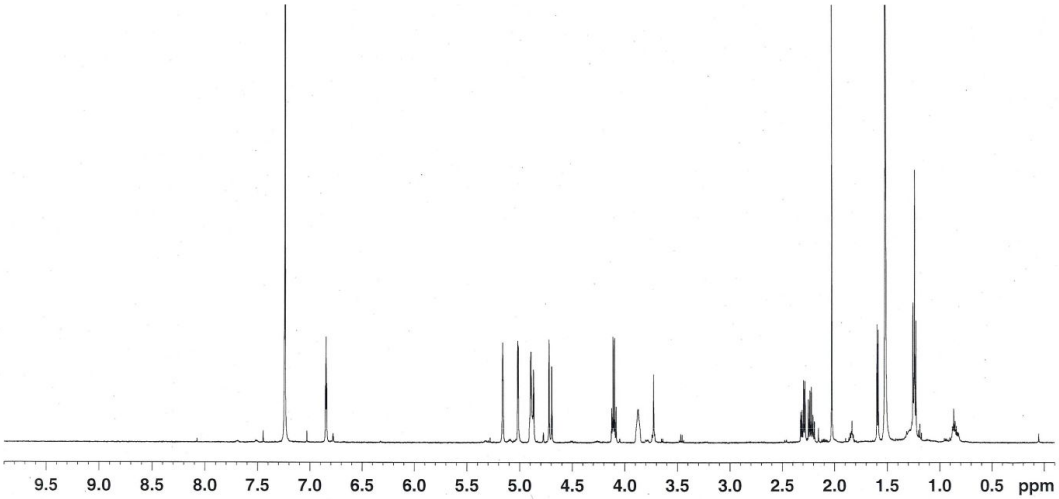




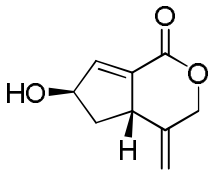
▼ **78, ^{13}C-NMR, 100 MHz**

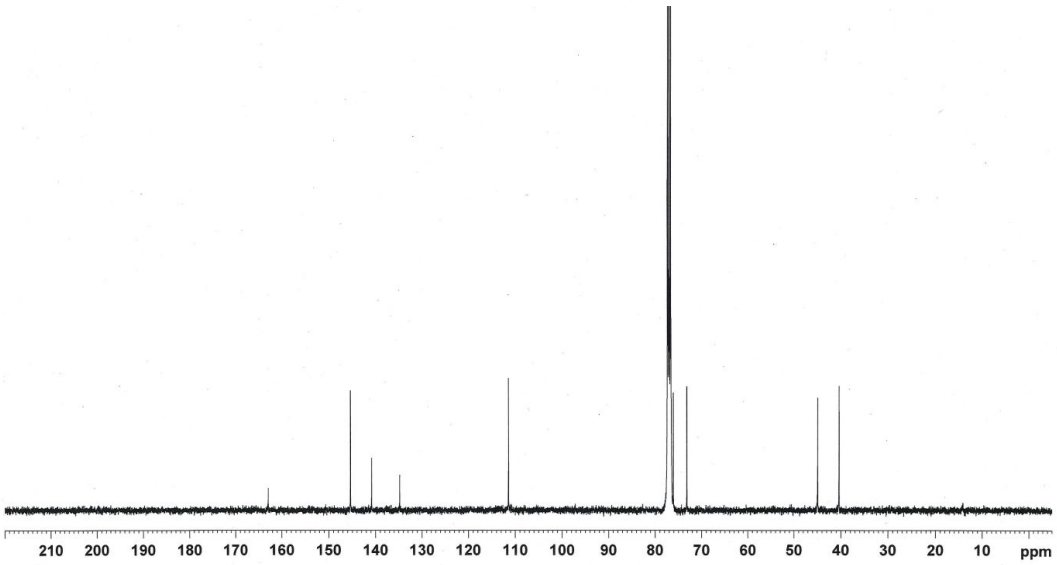


▼ **79, ^{1}H-NMR, 500 MHz**

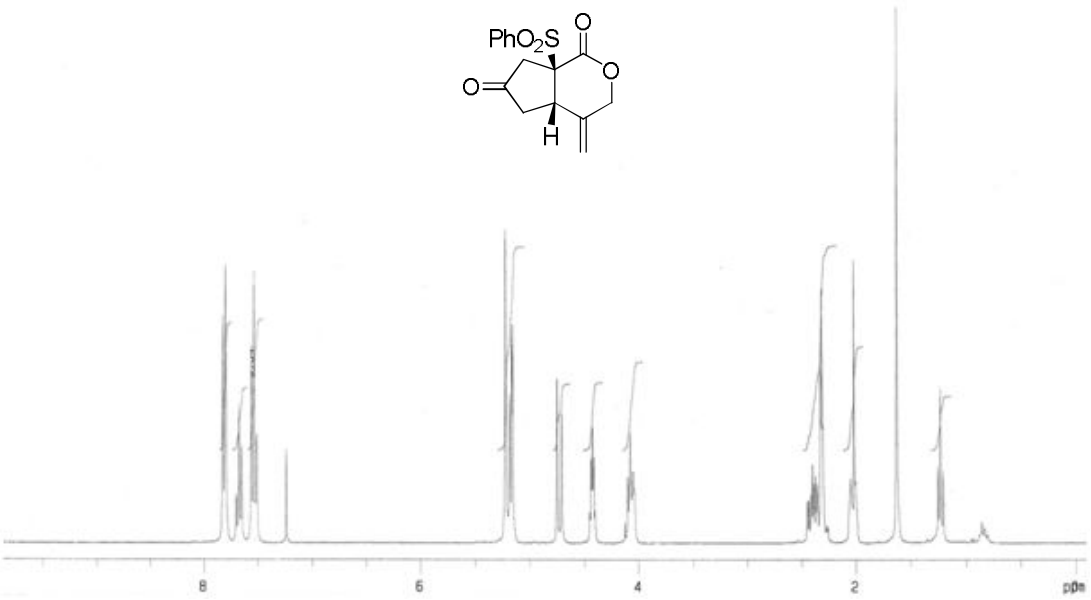


▼ **79, ^{13}C-NMR, 100 MHz**

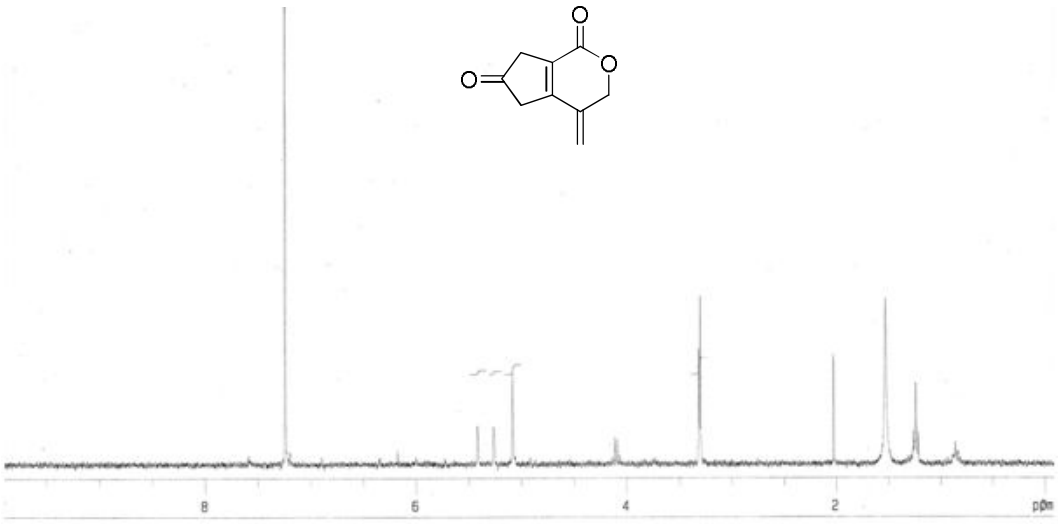




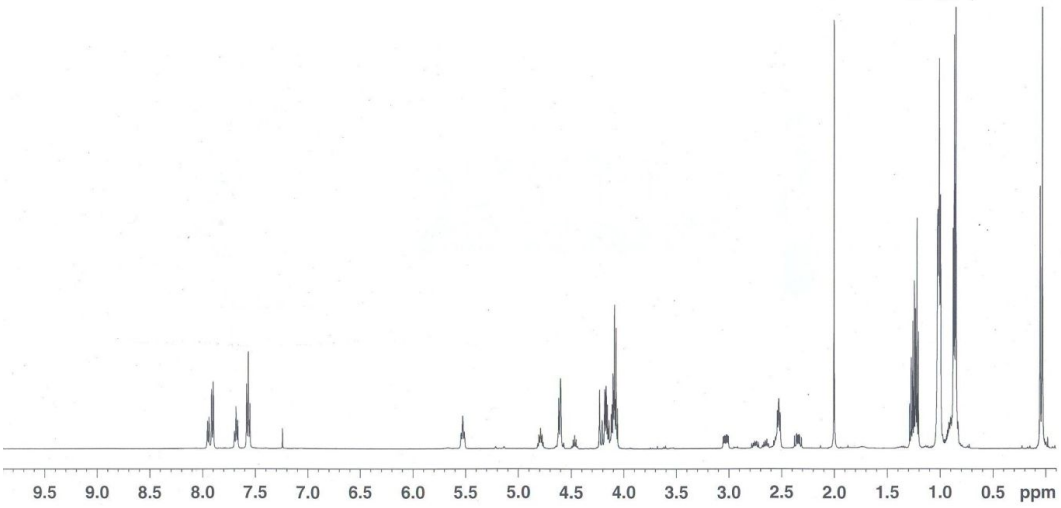
▼ **80, ^{1}H-NMR, 300 MHz**



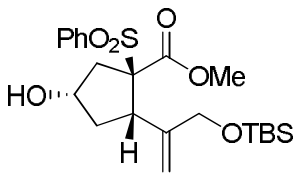
▼ **81, ^{1}H-NMR, 300 MHz**

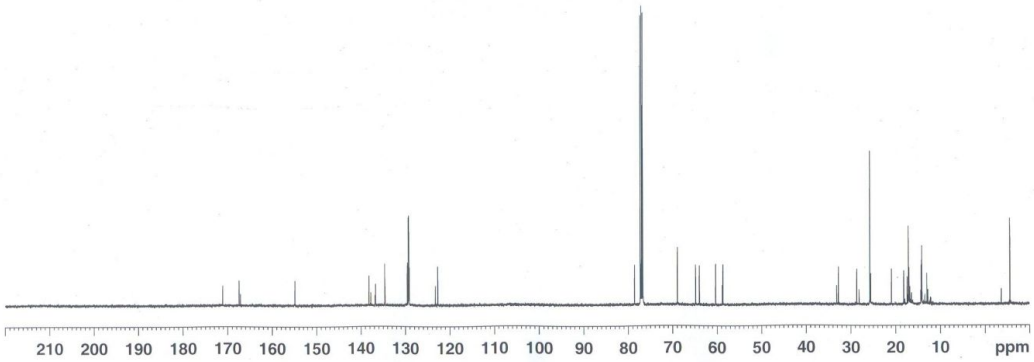


▼ **83, ^{1}H-NMR, 400 MHz**

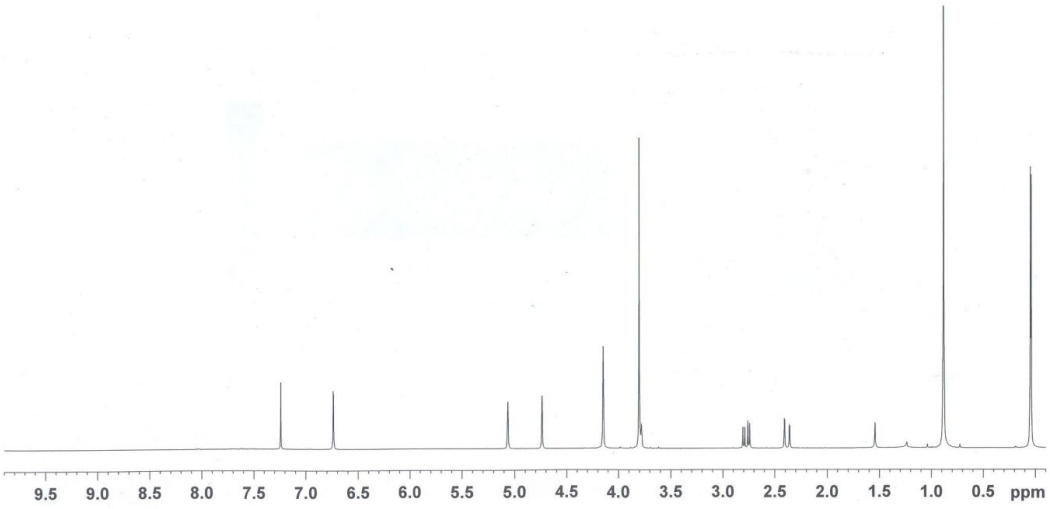


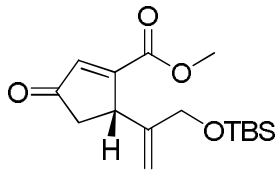
▼ **83, ^{13}C-NMR, 100 MHz**



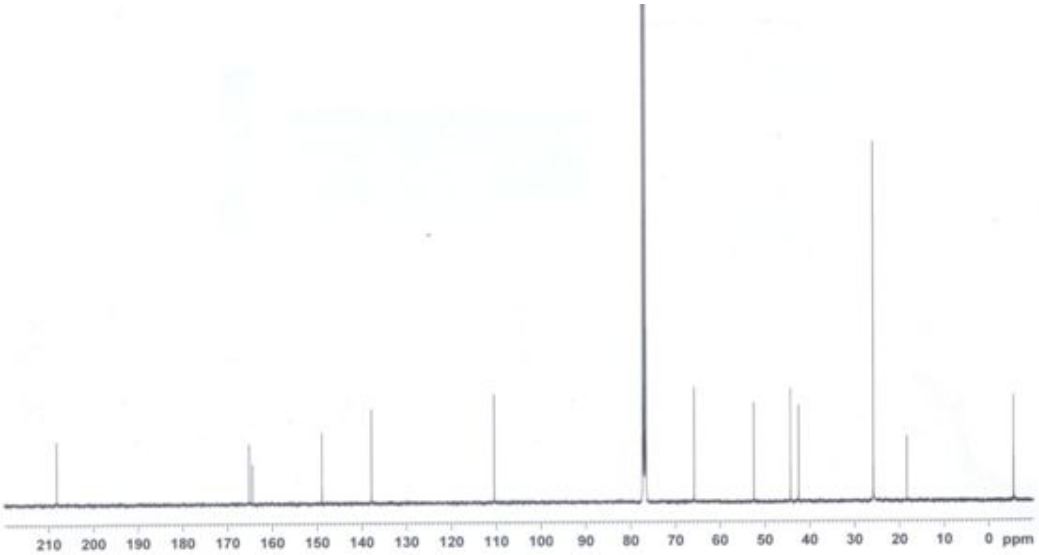


▼ **84, ^{1}H-NMR, 400 MHz**

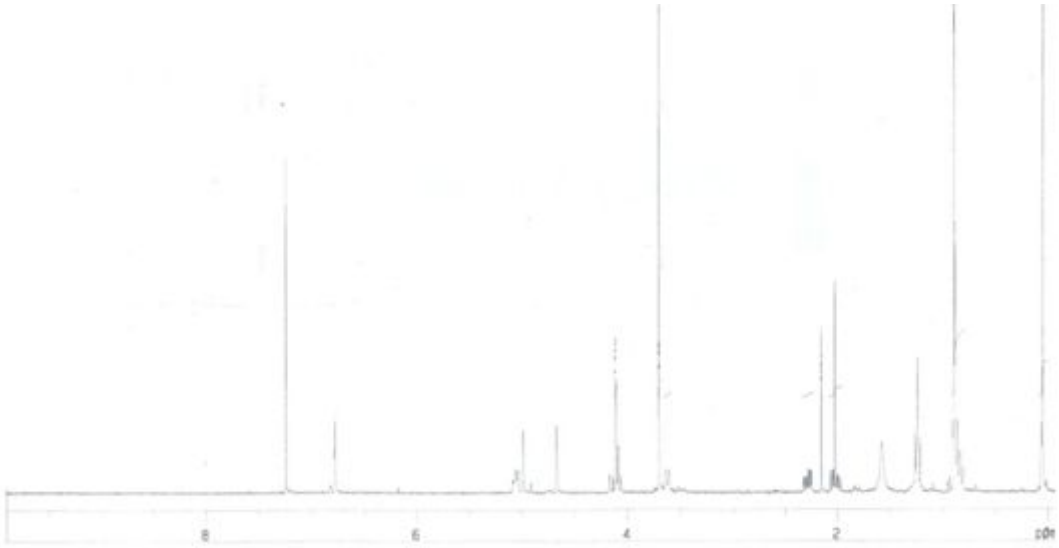




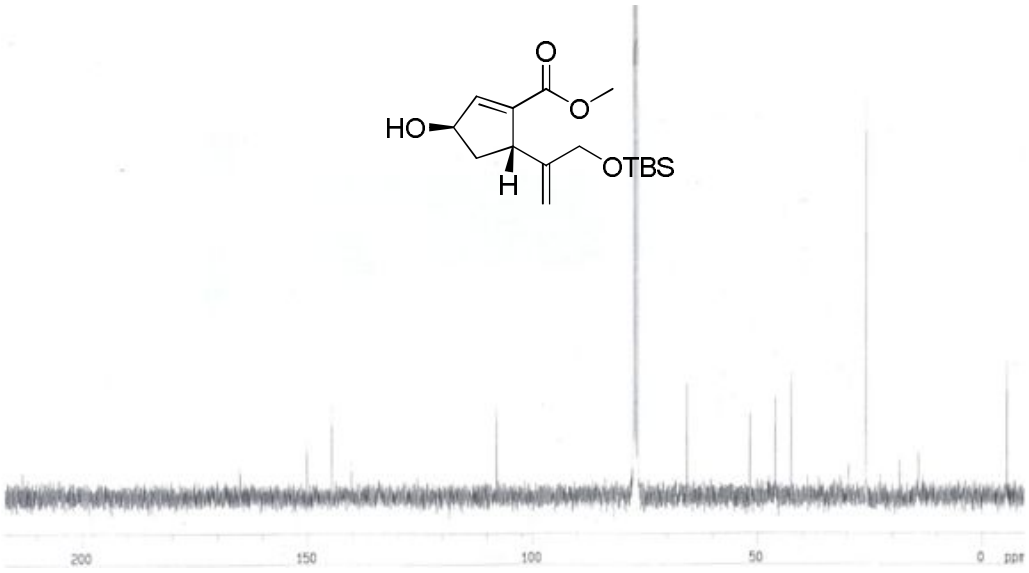
▼ **84, ^{13}C-NMR, 125 MHz**



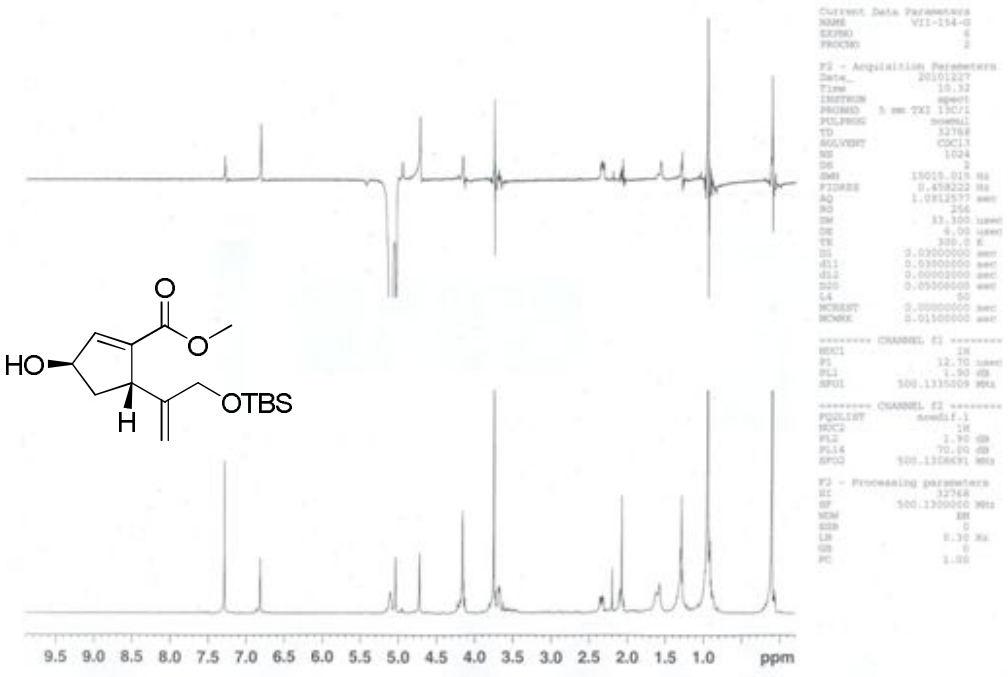
▼ **85, ^{1}H-NMR, 300 MHz**

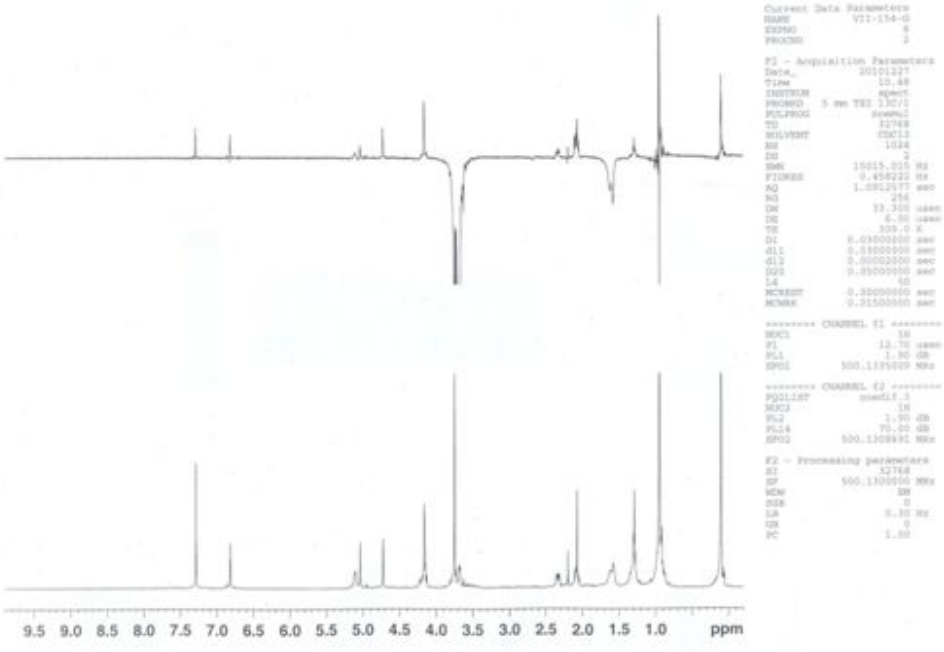


▼ **85, ^{13}C-NMR, 75 MHz**

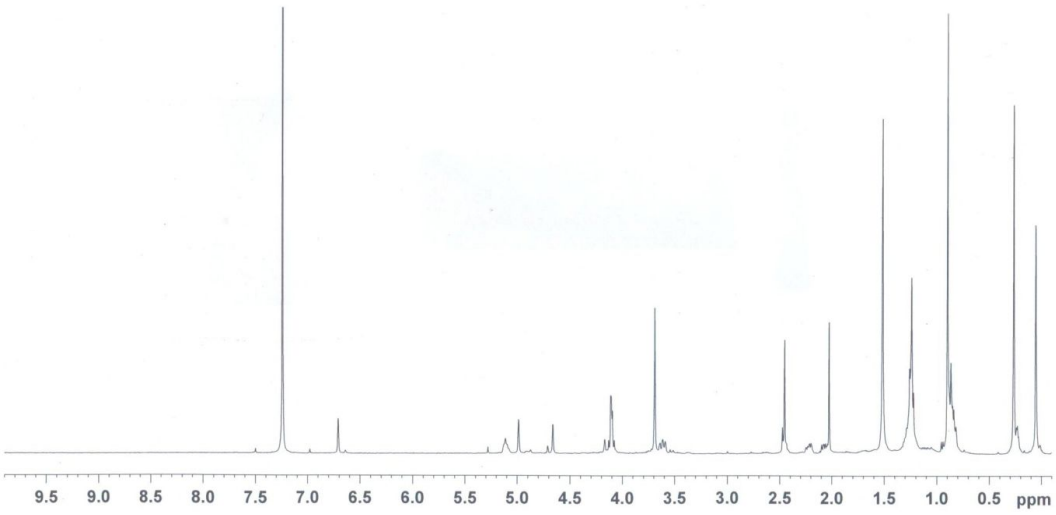


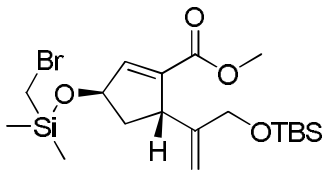
▼ **NOE Study of 85**



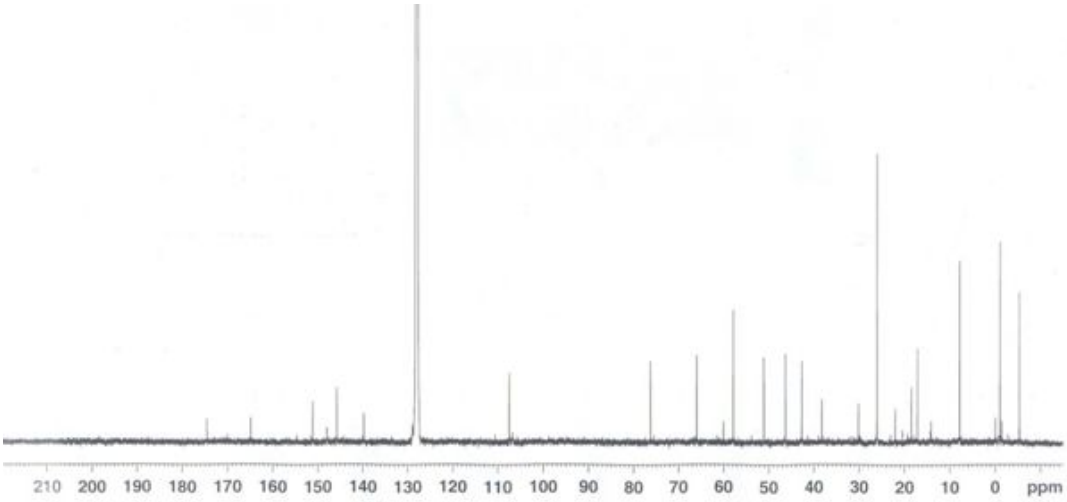


▼ **82, ^{1}H-NMR, 300 MHz**

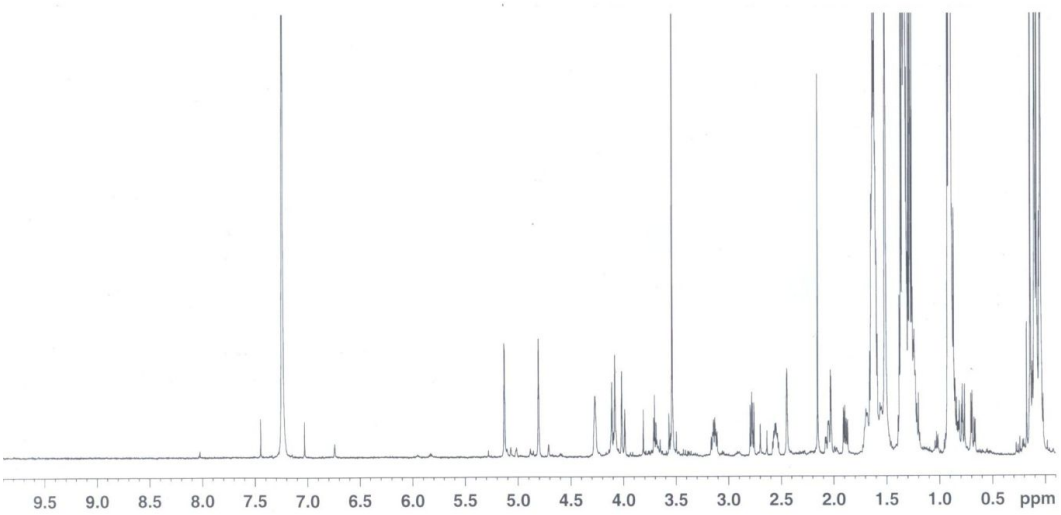




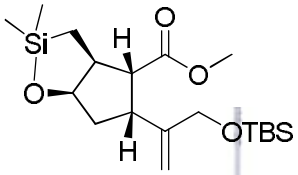
▼ **82, ^{13}C-NMR, 125 MHz**

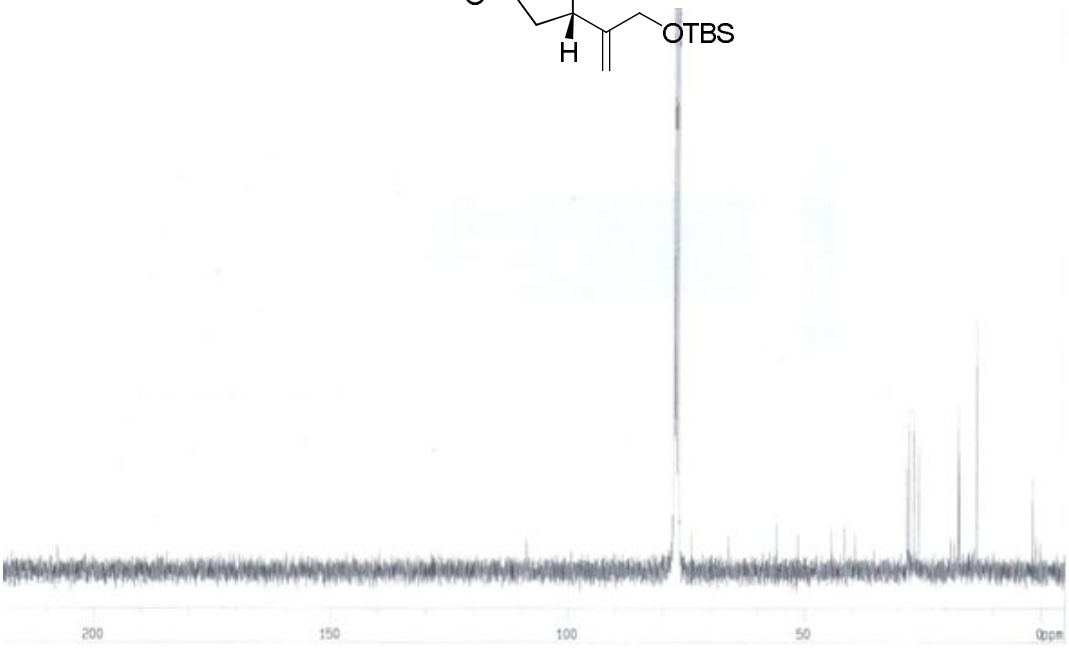


▼ **87, ^{1}H-NMR, 300 MHz**

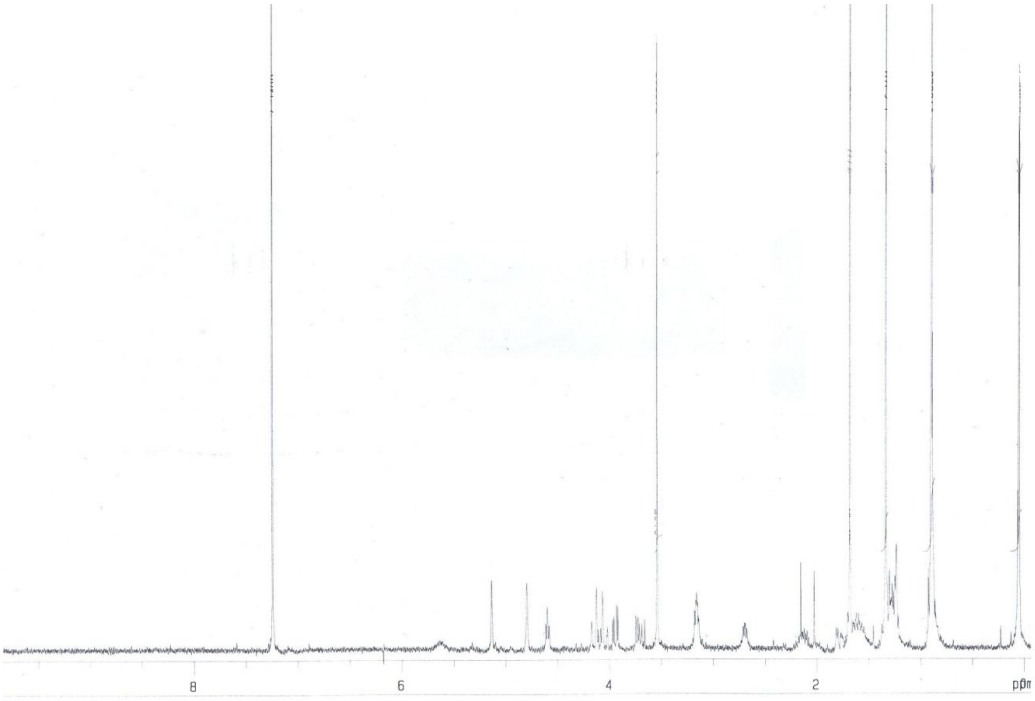


▼ **87, ^{13}C-NMR, 75 MHz**

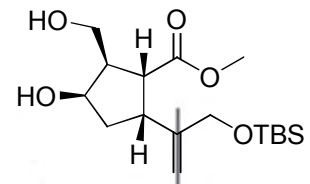


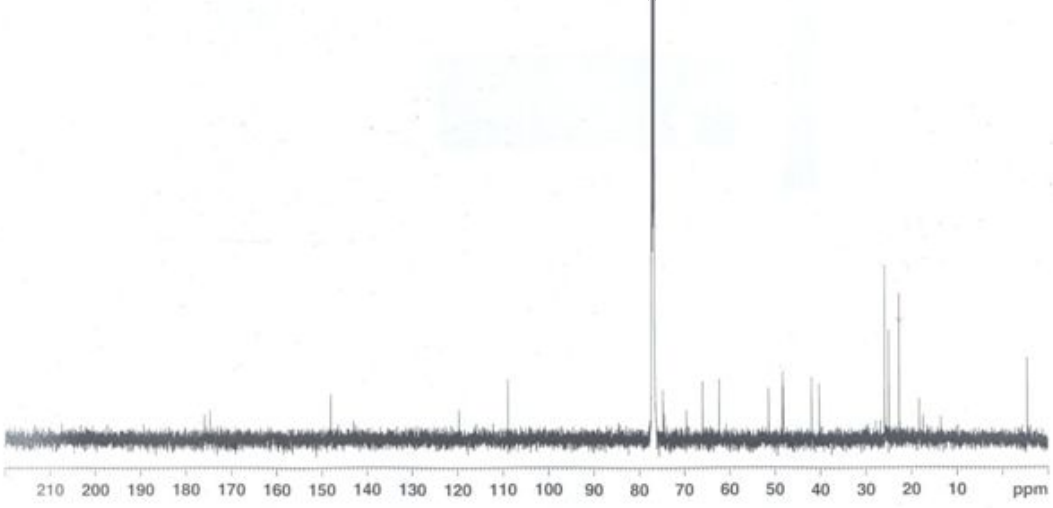


▼ **88, ^{1}H-NMR, 300 MHz**

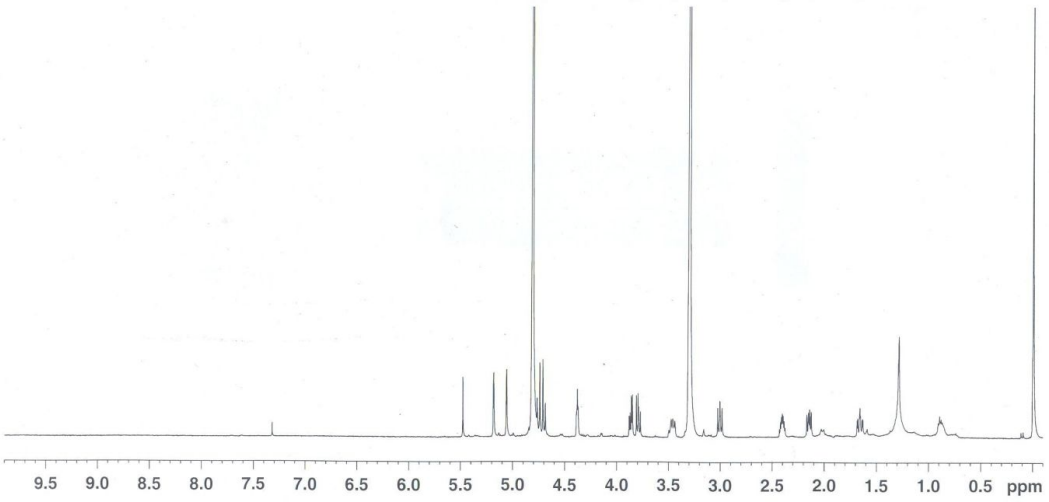


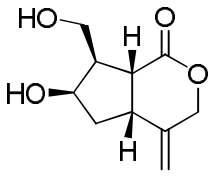
▼ **88, ^{13}C-NMR, 125 MHz**



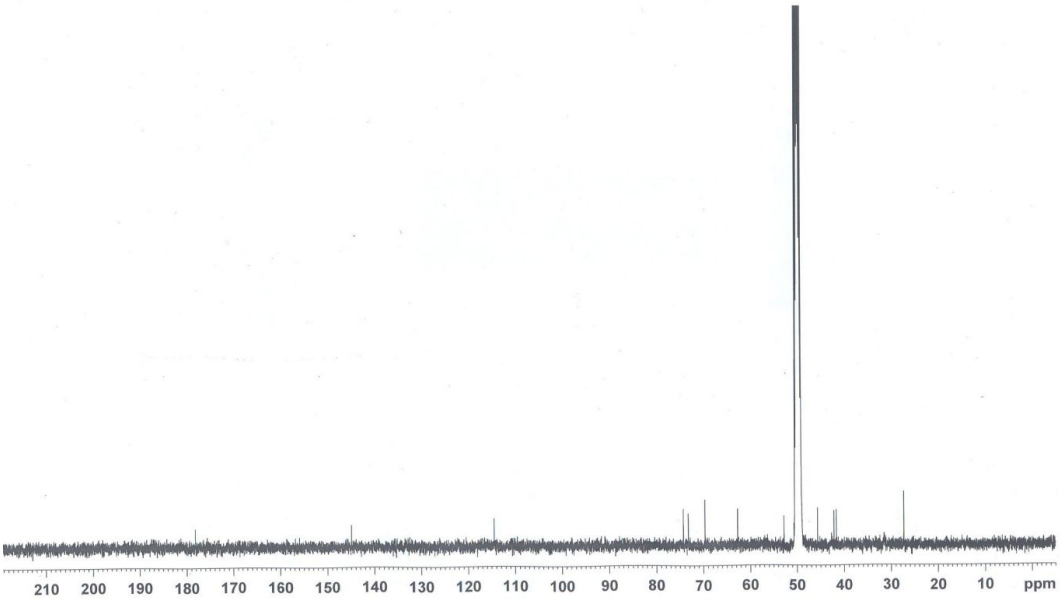


▼ **1, ^{1}H-NMR, 500 MHz**





▼ **1, ^{13}C-NMR, 125 MHz**



VII. Abstract

First total synthesis of natural iridoid lactone

Sujin Lee
College of pharmacy
Department of manufacturing pharmacy
The Graduate School
Seoul National University

Iridoids are a large family of natural monoterpenoids products, and structurally characterized by a *cis*-fused cyclopenta[*c*]pyran ring system. The unique *cis*-fused cyclopenta[*c*]pyran ring system has presented a variety of challenges for chemical synthesis and in the analysis of biological activities. In particular, we have been interested in a recently reported iridoid lactone, which is structurally related to loganin and consists of a C7-substituted C6-hydroxycyclopenta[*c*]lactone. Iridoid lactone, (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1 (3*H*)-one, was isolated from the roots and rhizomes of *Nardostachys chinensis* Batalin (Valerianaceae). This plant has been used as a Chinese folk medicine and its roots and rhizomes exhibit a variety of biological activities, such as sedative, antimalarial, antinociceptive, cytotoxic, and enhancement of nerve growth factor activities. Thus, the unique structural feature as well as the unknown biological activity of iridoid lactone led us to synthesize the recently reported iridoid lactone.

In this connection, we accomplished the first total synthesis of (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1 (3*H*)-one (**1**) in 13 steps. The key features of the synthesis involve the stereoselective and efficient preparation of the bicyclic lactone *via* Pd(0)-catalyzed cyclization, which is easily transformated to cyclopentanol intermediate and then the silicon-tethered radical cyclization followed by Tamao-Fleming oxidation for the

stereoselective introduction of the C7-hydroxymethyl substituent. This versatile synthetic approach is expected to be widely utilized for the synthesis of iridoids and iridoalkaloids.

Key words: (+)-6-hydroxy-7-(hydroxymethyl)-4-methylenehexahydrocyclopenta[*c*]pyran-1(3*H*)-one, iridoid lactone, iridoid, Pd(0)-catalyzed cyclization, bicyclic lactoen, silicon-tethered radical cyclization, Tamao-Fleming oxidation.

Student number: 2006-30449

Part II: Ginsenoside Rk1 구조 기반의 anti-apoptotic agent의 개발

Part II: Cholesterol derived novel anti-apoptosis agents on the structural basis of ginsenoside Rk1

Table of Contents

Part II: Cholesterol derived novel anti-apoptosis agents on the structural basis of ginsenoside Rk1

국문 초록 iii
List of Figures iv
List of Schemes v
Abbriviations vi
I. INTRODUCTION 1
1. Endothelial cells 1
2. Apoptosis 2
3. Ginsenoside 4
II. RESULT AND DISCUSSION 8
1. Preliminary study 8
2. Phytosterol screening 10
3. Synthesis of pseudosugar derivatives 16
4. Biological activity against serum deprived apoptosis 19
1) MTT assay on HUVECs 19
2) DAPI/ TUNEL assay 23
III. Conclusion 26
IV. Experimental 27
V. References 42
VI. Appendix 47
VII. Abstract 64

국문 초록

혈관 내벽은 혈관내피세포의 단일막으로 이루어져 있다. 혈관 내피세포의 항상성 유지가 혈관의 생명활동을 유지하는 중요한 요인이다. 혈관내피세포의 세포사멸에 의한 혈관의 구조변화와 기능저하가 동맥경화, 동종이식편에 의한 맥관장애, 울혈성 심부전 등의 여러 질병과 연관된다는 연구결과가 보고되고 있다. 따라서 혈관내피세포의 세포사멸 억제제가 혈관질환의 새로운 치료제로서 연구 가치가 있으리라 생각된다.

최근, 인삼으로부터 분리 보고된 ginsenoside Rg3, Rk1이 세포사멸 억제효과가 있는 것으로 발표되었다. 하지만 이들 ginsenoside들은 다당체를 함유하고 있어 산성 조건에서 화학적으로 불안정하고, 자연에서 얻기에는 양적인 면에서 한계가 있기 때문에 이들 구조를 기반으로 하여 화학적으로 비교적 안정하면서 세포사멸 억제효과를 갖는 유도체들의 합성을 계획하였다. Ginsenoside의 뼈대인 스테로이드 골격을 유지하면서 ginsenoside의 활성을 유지할 수 있는 여러 phytosterol을 검색하였고, C3 hydroxyl기에 활성에 중요한 역할을 하는 glucoside를 대체할 bioisoster를 도입하고자 하였다. 이를 통해 상업적으로 손쉽게 구입 가능한 콜레스테롤을 기반으로 하여 다양한 유도체를 합성하였고, 이들의 세포사멸억제 효과를 확인하였다.

주요어; 혈관내피세포, 세포사멸, 동맥경화, 동종이식편에 의한 맥관장애, 울혈성 심부전 ginsenoside Rg3, Rk1, phytosterol, 콜레스테롤, 세포사멸 억제효과

학 번: 2006-30449

List of Figures

Figure 1. Schematic structure of blood vessel (a) and tight junction (b) 1
Figure 2. Schematic representation of apoptosis and necrosis 3
Figure 3. Small molecules inhibiting apoptosis of endothelial cells.....4
Figure 4. Structures of ginsenosides.....6
Figure 5. (20*S*)Rg3 protects endothelial cells from serum-deprived apoptosis 8
Figure 6. MTT assay result for processed Ginseng extraction 9
Figure 7. Synthetic approach for ginsenoside derivatives 10
Figure 8. Structures of phytosterols.....11
Figure 9. MTT assay on HUVECs 14
Figure 10. Morphology of HUVEC.....15
Figure 11. Effect of compounds on apoptosis of HUVECs: 5 μg/ml treatment 19
Figure 12. Effect of compounds on apoptosis of HUVECs: 10 μg/ml treatment.....20
Figure 13. Morphological changes of HUVEC 21
Figure 14. Inhibition of compounds 7, and 9 on apoptosis of HRECs.....22
Figure 15. Morphological changes of HUVEC 22
Figure 16. Changes in nuclear morphology accessed by DAPI staining.....24
Figure 17. TUNEL assays at various concentrations of 7 and 9 24

List of Schemes

Scheme 1. Synthesis of stigmasterol derivatives **11**
Scheme 2. Synthesis of β-sitosterol derivatives **12**
Scheme 3. Synthesis of sitostanol derivatives **13**
Scheme 4. Synthesis of cholesterol derivatives **13**
Scheme 5. Synthesis of cholestanol derivatives **14**
Scheme 6. Synthesis of dihydropyran derivatives **16**
Scheme 7. Synthesis of acyclic analogs **17**
Scheme 8. Synthesis of bulky analogs **18**

Abbriviations

BrdUTP: 5-bromo-2'-deoxyuridine 5'-triphosphate

bs: broad singlet

t-Bu: *tert*-butyl

calcd: calculated

CHF: congestive heart failure

CNS: central nervous system

d: doublet

DAPI: 4'-6-diamino-2-phenylindole

DHP: 3,4-dihydro-*2H*-pyran

DMF: *N,N*-dimethylformamide

EC: endothelial cell

FAB: fast atom bombardment

HREC: human retina endothelial cell

HRMS: high resolution mass spectroscopy

HUVEC: human umbilical vein endothelial cell

IR: infrared

LRMS: low resolution mass spectroscopy

m: multiplet

Me: methyl

MeOH: methyl alcohol

NMR: nuclear magnetic resonance

P. ginseng: Panax ginseng

Ph: phenyl

PPD: protopanaxadiols

PPT: protopanaxatriols

PPTS: pyridinium *p*-toluene sulfonate

PTSA: *p*-toluene sulfonic acid

q: quartet

s: singlet

SAR: structure activity relationship

t: triplet

TdT: terminal deoxynucleotidyl transferase

THF: tetrahydrofuran

TLC: thin layer chromatography

TUNEL: Terminal Transferase dUTP Nick End Labeling

F-dUTP: fluorescent-tagged deoxyuridine triphosphate nucleotides

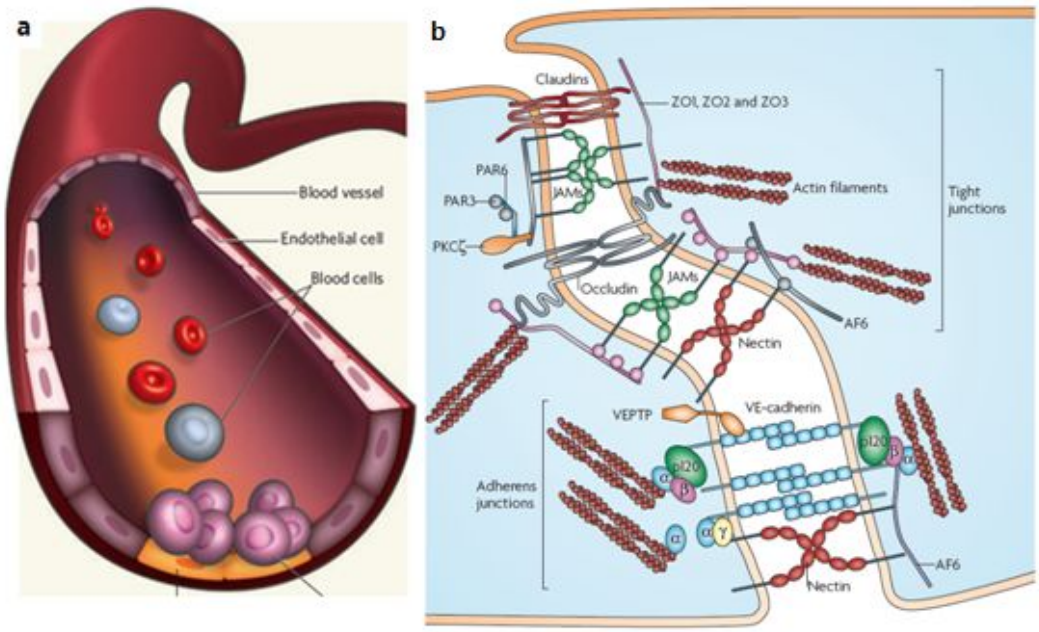
VEC: vascular endothelial cell

I. INTRODUCTION

1. Endothelial cells

The blood vessel has a monolayer of endothelial cell (EC) lining (Figure 1a) [1] located at the interface between the vascular and perivascular compartments. In this location, the ECs have two different roles. First, they establish the communication between circulating blood and underlying tissues. The ECs behave as a permeable filter, allowing the selective exchange of solutes and water between the luminal and abluminal sides of the barrier. Permeability follows two distinct pathways. The transcellular route crosses apical and basal cell membranes and is mostly mediated by channels, carriers, pumps, and vesicles. In contrast, the paracellular route passes through intercellular and lateral spaces between contacting cells and is mediated by the tight junction (Figure 1b). [2] Tight junctions are composed of integral membrane proteins (e.g. occludin, claudins, and junctional adhesion molecules [JAMs]) and intracellular proteins (e.g. ZO-1 and cingulin). [3]

Figure 1. Schematic structure of blood vessel (a) and tight junction (b)



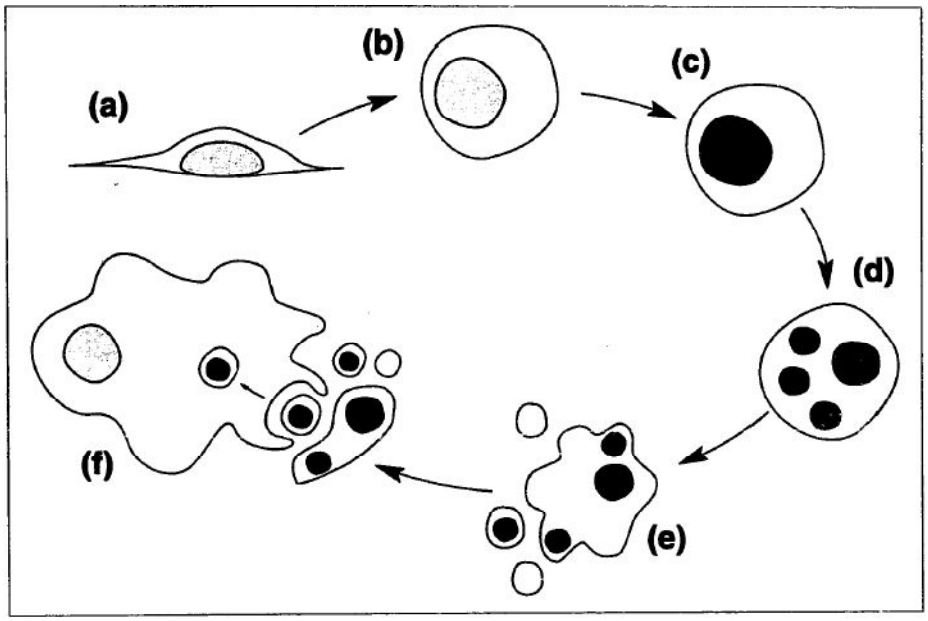
Second, ECs act as a barrier between the vascular and extravascular compartments, thus maintaining tissue homeostasis and preventing thrombosis. The location of ECs ensures their simultaneous and constant exposure to a wide variety of stimuli, many of which have the potential to induce or prevent apoptosis of the cells. It has been suggested that disturbed endothelial function could account for a large portion of all cardiovascular diseases,[4] such as atherosclerosis, allograft vasculopathy, hypertension, congestive heart failure (CHF), and other diseases (e.g. systemic sclerosis, diabetes, and thrombotic thrombocytopenic purpura). [5]

2. Apoptosis

There are two major types of cell death: necrosis and apoptosis. Necrosis occurs following diverse traumatisms including hyperthermia, ischemia, and physical injury or appears as a result of the exposure to cytotoxic cytokines or substances. Apoptosis, on the other hand, is a kind of programmed cell death. It has recently gained considerable interest because it has been appreciated as a regulatory mechanism in organ growth, wound repair, tumor genesis, and immune response.

Apoptosis is a normal occurrence in which an orchestrated sequence of events leads to the cell death. As represented schematically in Figure 2,[6] death by apoptosis is a neat, orderly process characterized by the overall shrinkage in volume of the cell and its nucleus, the loss of adhesion to neighboring cells, the formation of blebs at the cell surface, the dissection of the chromatin into small fragments, and the rapid engulfment by phagocytosis. Necrotic cells lyse and release their intracellular components, including DNA and RNA, into the extracellular space. Waste-management nucleases digest these extracellular nucleic acids, and inflammatory reactions occur. Apoptosis, however, is characterized by distinct morphological changes. Membrane fusion early in apoptosis causes fluid loss and therefore cell shrinkage. Chromatin condenses and is deposited on the inner surface of the nuclear membrane. Macromolecular synthesis decreases and usually cellular DNA is cleaved at internucleosomal sites, resulting in fragmentation of DNA.

Figure 2. Schematic representation of apoptosis and necrosis



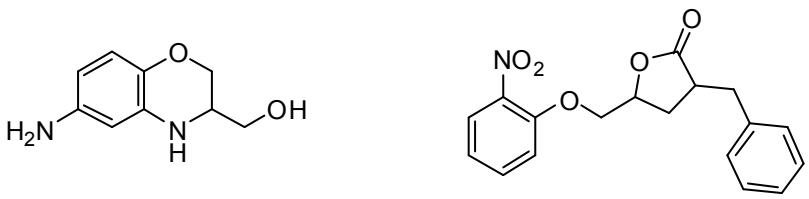
Upon receipt of a signal to undergo apoptosis, an adherent cell (a) rounds up (b) and rapidly condenses its DNA (c), thought to be a consequence of DNA fragmentation. This is rapidly followed by separation of the nucleus into discrete masses of condensed chromatin (d) and, finally, fragmentation of the cell into several membrane-bound vesicles (apoptotic bodies) (e), which are rapidly recognized and phagocytosed by macrophages or neighbouring cells (f).

This DNA is eventually released from cells in membrane-bound vesicles, and the vesicles are eliminated by phagocytic cells. Since intracellular contents are not released to interstitial fluid, inflammation does not occur. [7]

It is an active process of cell death that occurs in many important physiological conditions, such as embryonic development and tissue remodeling. Apoptosis is involved during angiogenesis in de novo tissue formation and wound healing. Moreover, many diseases are associated with either too much or too little apoptosis, including cancer, sepsis, autoimmunity, and neurodegenerative diseases. [8] In particular, the endothelium is a critical site for the control of apoptosis during processes such as inflammation, vascular remodeling, and angiogenesis. Although the suppression of EC apoptosis is required for the maintenance of blood vessel integrity and for angiogenesis, the

activation of apoptosis has been suggested to contribute to pathological tissue destruction during vascular injury, inflammation, atherosclerosis, and allograft arteriopathy. [9] Therefore, the inhibition of vascular endothelial cell (VEC) apoptosis is considered as a new target for diverse cardiovascular diseases. EC perturbing agents such as inflammatory cytokines, lipopolysaccharides, reactive oxygen species, and oxidized low-density lipoprotein (LDL) are known to induce EC apoptosis. [10]

Figure 3. Small molecules inhibiting apoptosis of endothelial cells



ABO 3BDO

Considering these preliminary reports, the inhibition of VEC apoptosis has been considered as a novel approach for the treatment of various physiological disorders such as cardiovascular disease. A few reports have addressed the development of anti-apoptotic agents from natural products [11] to small molecules.[12] Miao et al. discovered lead compounds for anti-apoptotic activity on human umbilical vein endothelial cells (HUVECs) through the library screening (Figure 3). These molecules inhibit against oxidized LDL-induced apoptosis and increase intergrin β4, NF-kB, and P53 in HUVECs.

3. Ginsenoside

Ginseng (*Panax ginseng* C. A. Meyer, Araliaceae) is one of the most popular herbal medicines in the Orient. [13] It has been used for over 2000 years in the belief that it is a panacea and promotes longevity. Recently, there has been a renewed interest in investigating ginseng pharmacology using

biochemical and molecular biological techniques. The pharmacological effects of ginseng have been demonstrated in the central nervous system (CNS) [14] and in cardiovascular, endocrine,[15] and immune systems.[16] In addition, ginseng and its constituents have been ascribed anti-neoplastic, anti-stress, and antioxidant activities. [17]

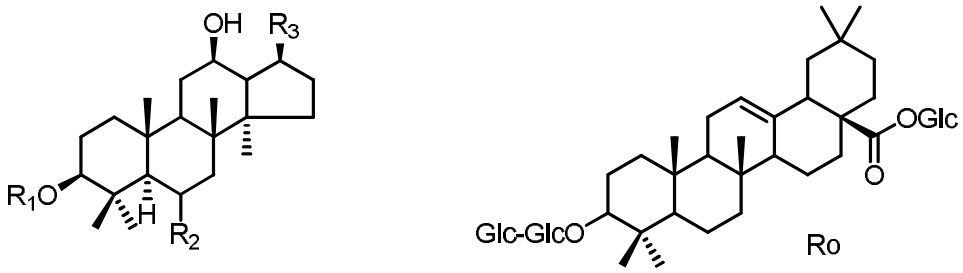
The major components found in most ginseng spices are ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids. Among those components, the most pharmacological active components are ginsenosides.[18] Ginsenosides are a family of steroids named steroidal saponins; they have also been named ginsenoside saponins, triterpenoid saponins, or dammarane derivatives under previous classifications. Ginsenosides possess a triterpene structure as a backbone and have a structural diversity.

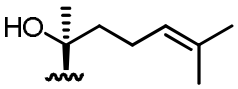
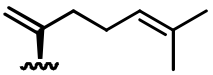
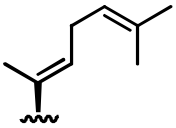
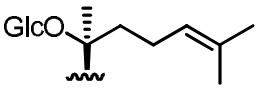
Structurally, ginsenosides possess the four successive trans-fused ring steroidal skeletons with a modified side chain at C20, and β-OH at C3, which is usually attached to sugar residue such as glucose, rhamnose, xylose, and arabinose. Based on the number of hydroxyl groups on their dammarane type triterpene, ginsenosides are generally divided into three groups: protopanaxadiols (PPDs), protopanaxatriols (PPTs), and oleanane type. The representative structures of ginsenosides are shown in Figure 4. Rb1, Rb2, Rc, and Rd are examples of PPDs, which have two hydroxyl groups at C3 and C20. Re, Rf, Rg1, and Rg2 are examples of PPTs, which have three hydroxyl groups at C3, C6, and C20. The oleanane type ginsenoside is only one ginsenoside, Ro (Figure 4). [19]

Ginseng is widely used in traditional Chinese medicine and Western herbal preparations, numerous researches to evaluate the biological activities of ginsenosides have been undertaken. Although the extracts from fresh or processed *P. ginseng* have been extensively tested for their pharmacological effects, the precise biological functions and underlying action mechanisms of pure molecules from ginseng extract are still largely unknown. Some literature reports suggest that ginseng extract has both wound-healing and anti-tumor effects through a reciprocal role in the

vascular system.[20]

Figure 4. Structures of ginsenosides



ginsenoside	R_1	R_2	R_3
Rg3 (PPD)	Glc-Glc-	H_2	
Rk1 (PPD)	Glc-Glc-	H_2	
Rg5 (PPD)	Glc-Glc-	H_2	
Rg1 (PPT)	H	OGlc	

Ginsenosides Rg1 and Rb1 have been demonstrated to have opposing activities in the vasculature. Rg1 promotes functional neovascularization in vivo and proliferation, chemoinvasion, and tubulogenesis of ECs in vitro, whereas Rb1 suppresses the earliest step in angiogenesis by inhibiting the chemoinvasion of ECs.[21] These results suggest that ginsenosides are structurally related but functionally different, depending on the side chain moieties attached to the triterpene backbone. In particular, (3β,12β)-12,20-Dihydroxydammar-24-en-3-yl 2-*O*-β-*D*-glucopyranosyl-β-*D*-glucopyranoside (ginsenoside Rg3) exerts many pharmacological activities such as anti-tumor, anti-inflammatory, neuroprotection against the cerebral ischemia-induced injury, and a

vasodilation effect.[22] Interestingly, ginsenoside Rg3 has been recently revealed to have anti-apoptotic activity via the inhibition of a mitochondrial caspase pathway.[23] Moreover, 3β,12β-dihydroxydammar-20(21),24-diene-3-*O*-β-*D*-glucopyrano-syl(12)-β-*D*-gluco-pyranoside (ginsenoside Rk1), isolated from heat-processed ginseng (Sun Ginseng),[24] has more effective anti-apoptotic activity on ECs than Rg3. Considering these reports, we focused on the development of anti-apoptotic agents on HUVECs based on the structure of both ginsenosides. However, ginsenosides are generally labile under acidic conditions, and their composition is variable depending on the process of extraction. Thus, it is difficult to determine the precise biological functions and underlying mechanisms.[25]

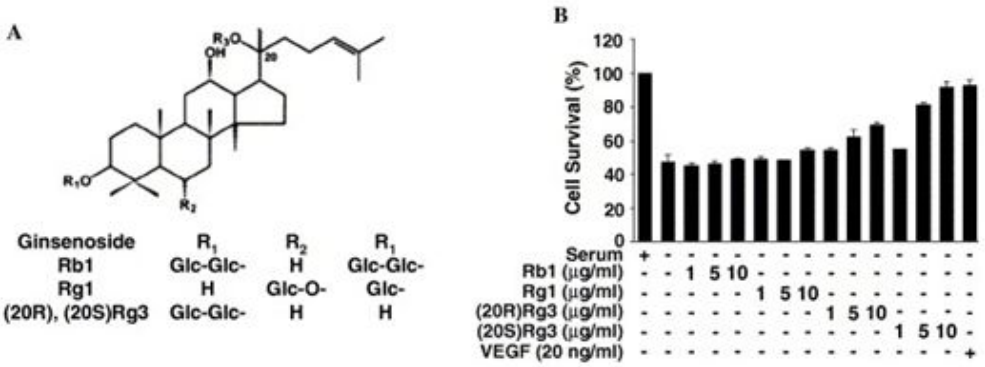
To date, most research has focused on the activities of natural ginsenosides, and few studies have reported on the structure activity relationship with ginsenoside modification or derivatives synthesis.[26] On the basis of these preliminary studies, we planned to investigate a preliminary structure-activity relationship (SAR) study of ginsenosides Rg3 and Rk1 on the anti-apoptotic effect. At first, we designed and synthesized diverse phytosterol analogs for the elucidation of the SAR.

II. RESULT AND DISCUSSION

1. Preliminary study

As recently reported,[23] some ginsenosides were treated on apoptosis-induced human ECs by serum deprivation[10] (Figure 5). Interestingly, the data showed that both Rg1 and Rb1 have no significant effect on EC survival up to the concentration 10 μg/ml, whereas Rg3 has a significant activity at the same concentration. Moreover, ginsenoside Rg3 epimers also exhibited differential effects. (20*S*)Rg3 promoted EC survival to an extent comparable to 20 ng/ml VEGF, but (20*R*)Rg3 showed only a minimal effect. These results indicate that the precise structural characteristics of ginsenosides such as side chains, glucosyl elimination, and epimerization of C20 are required for their vascular effects.

Figure 5. (20*S*)Rg3 protects endothelial cells from serum-deprived apoptosis

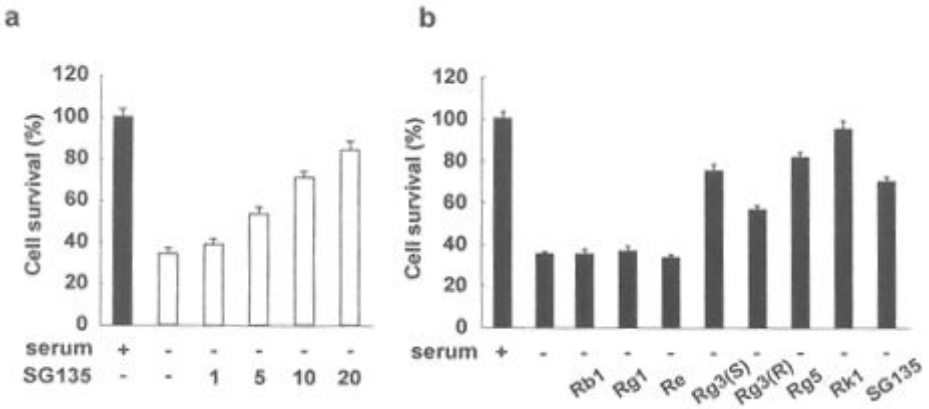


(A) Chemical structure of ginsenosides Rg1, Rb1, (20*R*)Rg3, and (20*S*)Rg3. (B) HUVECs (1 × 105 cells/well) were plated onto six-well plates in 1 ml of M199 containing 20% fetal bovine serum. Next day, the cells were switched to serum-free M199 with various concentrations of Rg1, Rb1, 20(*R*)Rg3 or 20(*S*)Rg3. After 36 h, cell viability was determined by trypan blue exclusion. Viability of control cells was set at 100% and viability relative to the control is presented.

The triterpene backbone structure of ginsenoside Rg3 is similar to the cholestane structure, which is a general backbone structure of phytosterols. The hydroxyl group at C6 seemed not to influence

the apoptotic activity (shown as Rg1). The glucosyl group attached at C3 OH is crucial for the activity, and the stereochemistry of C20 slightly affects to the activity from comparison of Rb1 to Rg3. On the other hand, ginsenosides extracted from heat-processed ginseng (SG 135) and crude extraction were examined, and ginsenoside Rk1 showed the most effective activity (Figure 6).

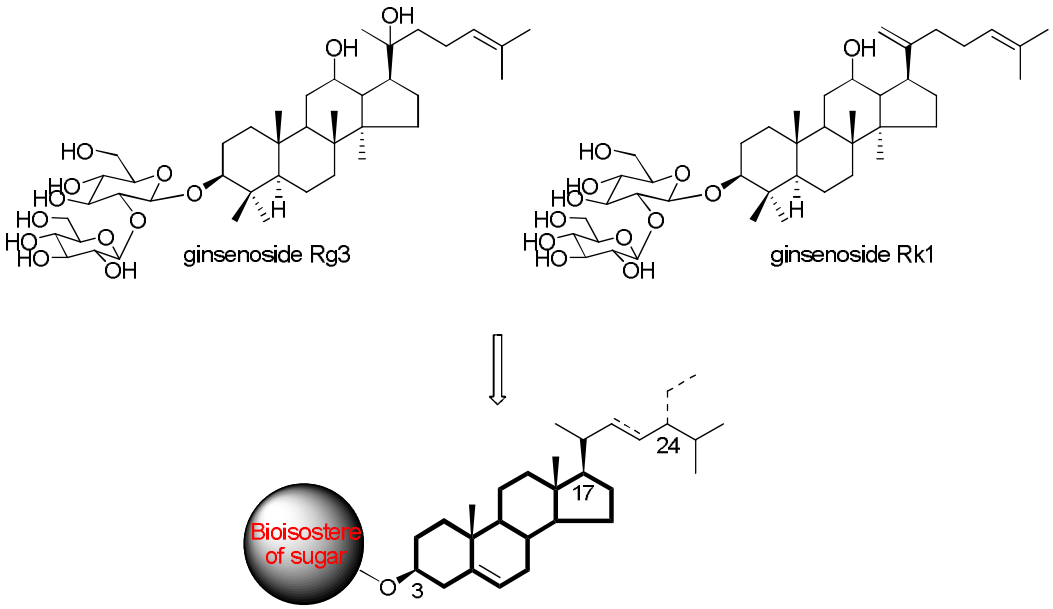
Figure 6. MTT assay result for processed Ginseng extraction



(a) The viability on HUVECs with the serum-free conditions with various concentrations of SG135. (b) HUVECs cell viability with various ginsenosides.

Taking these results together, the glucosyl group at C3 is considered to be crucial. Thus, we planned to determine three major considerations: 1) the effect of sugar moiety at the C3 hydroxyl group, 2) the steric influence of the alkyl chain at C24, and 3) the influence of steroidal skeleton structures. The research began with the selection of proper phytosterols, which contain simple steroidal backbone structures (Figure 7). Thus, our studies commenced with the synthesis of pseudosugar derivatives of several phytosterols, which are structurally simple and commercially available steroids.

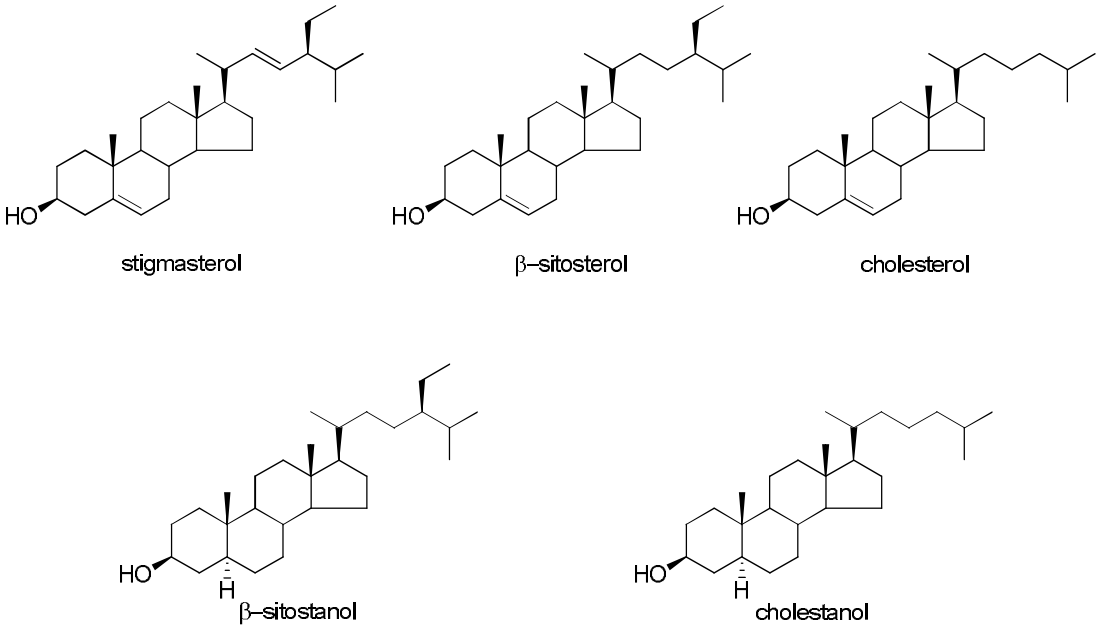
Figure 7. Synthetic approach for ginsenoside derivatives



2. Phytosterol screening

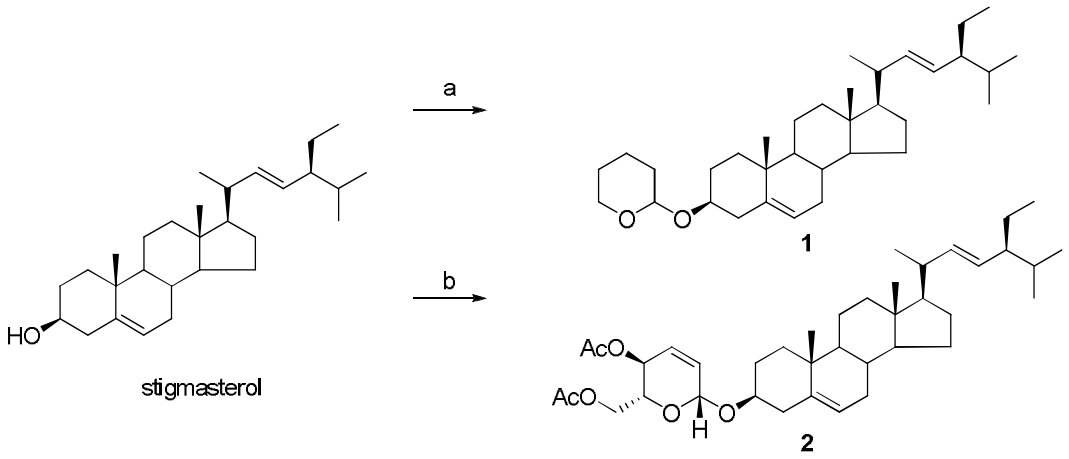
Phytosterols, also referred to as plant sterols, are common plant and vegetable constituents and are therefore normal constituents of the human diet. They are structurally related to cholesterol but differ from it in the structure of the side chain. They consist of a steroidal skeleton with a hydroxyl group attached to the C3 and an aliphatic side chain attached to the C17 (Figure 8). Commercially, phytosterols are isolated from vegetable oils, such as soybean oil, rapeseed (canola) oil, sunflower oil, or corn oil, or from so-called tall oil, which is a by-product of the manufacture of wood pulp. [27] These sterols can be hydrogenated to obtain phytostanols. The most common phytosterols and phytostanols are sitosterol (3β-stigmast-5-en-3ol), sitostanol (3β,5α-stigmastan-3-ol), and stigmasterol (3β-stigmasta-5,22-dien-3-ol). Each commercial source has its own typical composition.

Figure 8. Structures of phytosterols



To determine the influence of the side chain at C20, we planned to synthesize simple pseudosugar derivatives such as tetrahydropyrans (THPs) and dihydropyran moieties with those phytosterols. The synthetic schemes are shown below (Scheme 1-3).

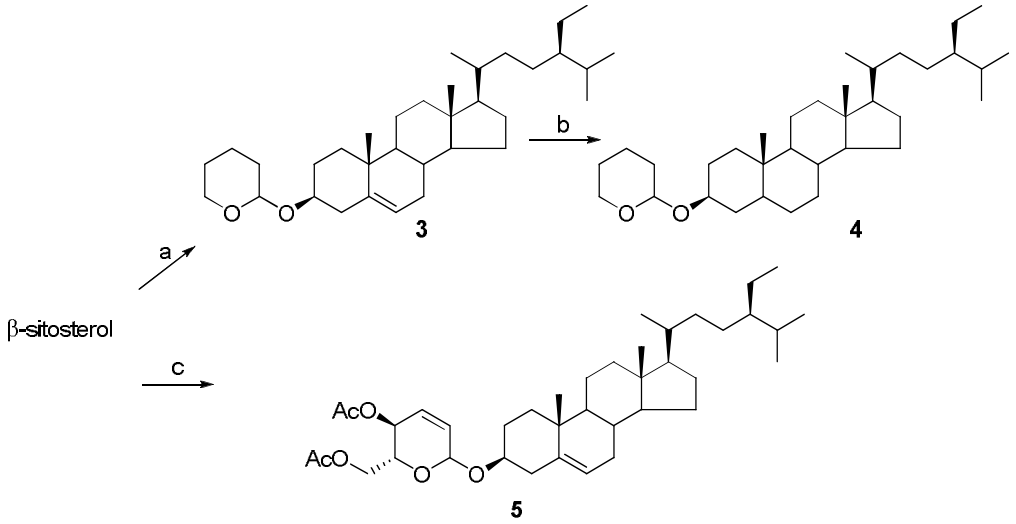
Scheme 1. Synthesis of stigmasterol derivatives



Reagents; (a) DHP, PPTS, CH_2Cl_2, 68%; (b) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 79%.

At first, the addition of 3,4-dihydro-*2H*-pyran and pyridinium *p*-toluene sulfonate into stigmasterol in CH_2Cl_2 afforded to compound **1**. The dihydropyran analog **2** was synthesized by the addition of 3,4,6-*Tri*-*O*-acetyl-*D*-glucal into the stigmasterol solution in the presence of boron trifluoride (Scheme 1)

Scheme 2. Synthesis of β-sitosterol derivatives

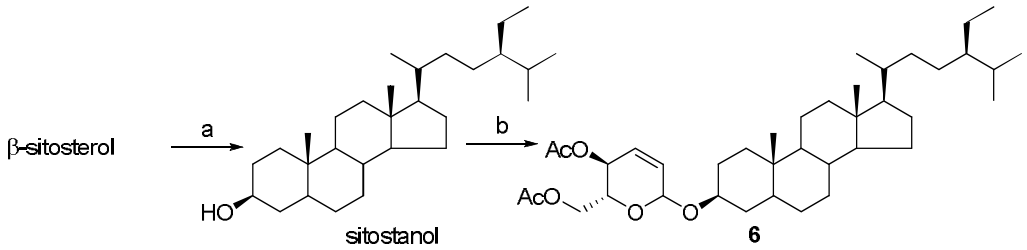


Reagents; (a) DHP, PPTS, CH_2Cl_2, 88%; (b) H_2, Pd/C, EtOAc, 82%; (c) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 82%.

Next, THP and dihydropyran moieties were introduced to β-sitosterol[28] (Scheme 2). THP derivative **3** was synthesized under acidic conditions with dihydropyran, and hydrogenation with palladium activated on carbon afforded sitostanol THP analog **4**. Dihydropyran analog **5** was synthesized with triacetyl glucal.

Sitostanol was synthesized by hydrogenation of β-sitosterol with Pd/C. The dihydropyran analog **6** was synthesized by the addition of 3,4,6-*Tri*-*O*-acetyl-*D*-glucal into the β-sitosterol solution in the presence of boron trifluoride (Scheme 3).

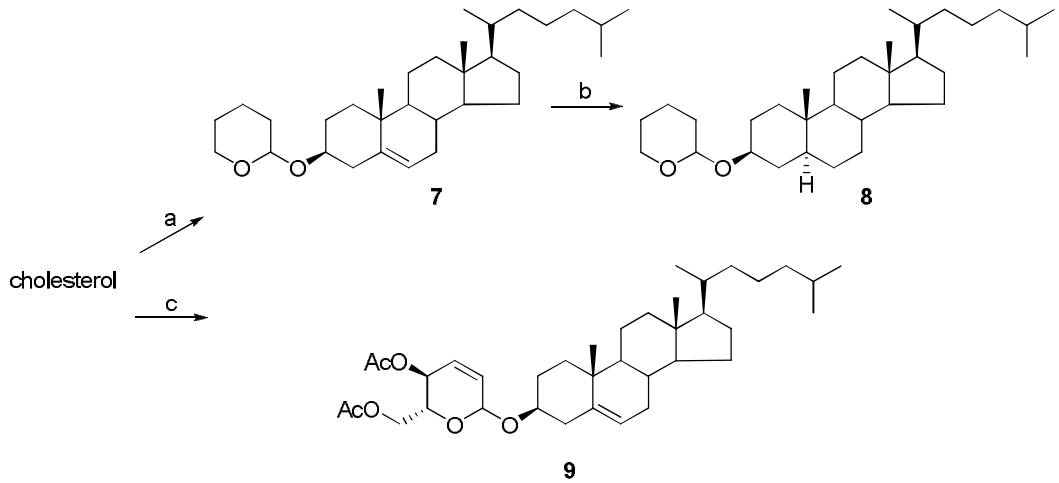
Scheme 3. Synthesis of sitostanol derivatives



Reagents; (a) H_2, Pd/C, EtOAc, 92%; (b)3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 78%.

At Scheme 4, the cholesterol derivatives **7** and **9** and cholestanol derivative **8** were produced under the same reaction conditions described above.

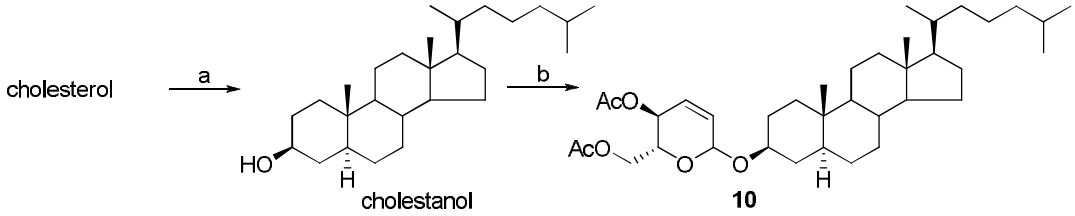
Scheme 4. Synthesis of cholesterol derivatives



Reagents; (a) DHP, PPTS, CH_2Cl_2, 68%; (b) H_2, Pd/C, EtOAc, 98%; (c) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 79%.

At Scheme 5, the synthesis of the cholestanol derivative is described. Hydrogenation of cholesterol with Pd/C afforded cholestanol. In particular, the stereochemistry at C5 of cholestanol was determined by comparison with ^{13}C-NMR data from the literature. [29] With cholestanol, dihydropyran analog **10** was synthesized.

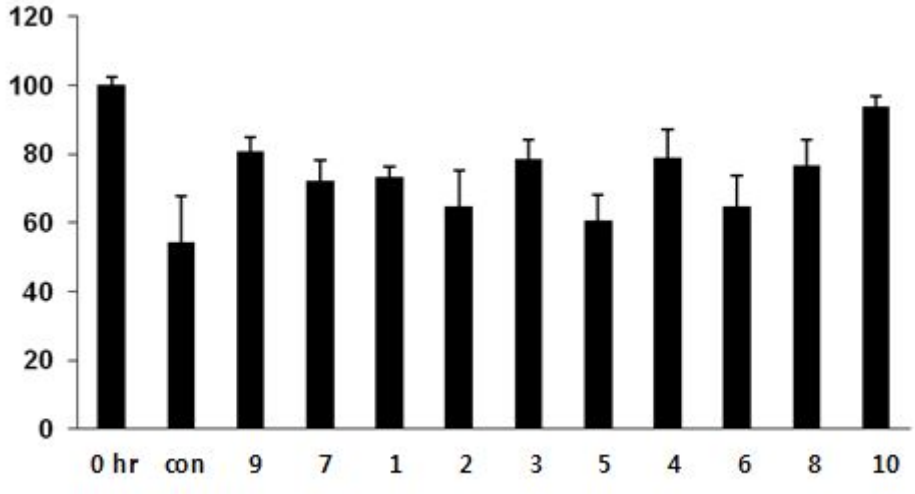
Scheme 5. Synthesis of cholestanol derivatives



Reagents; (a) H_2, Pd/C, EtOAc, 98%; (c) 3,4,6- *tri*-*O*-acetyl-*D*-glucal, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 82%.

With the 10 pseudosugar analogs of phytosterols in hand, the MTT assay was executed for the anti-apoptotic activities on HUVECs. The results are shown in Figure 9.

Figure 9. MTT assay on HUVECs



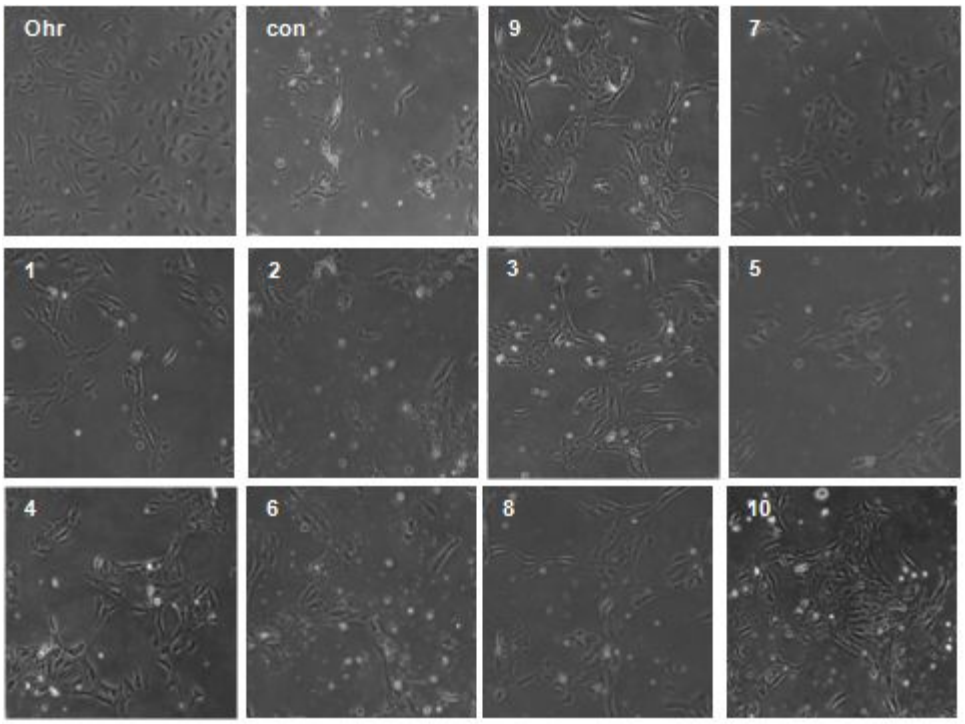
Anti-apoptotic activities of the synthetic analogs on HUVECs at 10 μg/ml treatment. 0 hr; the viability of the cells cultured in the medium without any derivatives. Con; the viability of the cells cultures in the medium containing DMSO used as a vehicle control.

In order to evaluate the effects of the compounds in HUVEC apoptosis, the given model of HUVEC apoptosis induced by deprivation of serum and growth factors[30] was used. The results of the MTT assay showed that cholesterol and cholestanol derivatives (**9**, **3**, **4**, and **10**) have anti-

apoptotic activities on ECs (Figure 9). It is suggested that the side chain at C24 affects the anti-apoptotic activity compared with stimgasterol, β-sitosterol to cholesterol. The branched side chains embedded analogs, such as stimgasterol or β-sitosterol analogs, are slightly less active.

Next, we observed the morphological changes associated with the physiological and pathological processes in HUVECs. When the cells detach from the culture dish bottom and become round, they will enter into apoptosis processes. However, if HUVECs elongate and bend into rings, they will form microvessels. [31] In our experiments, the elongated HUVECs were found with compounds **9**, **3**, **4**, and **10**. The data suggested that cholesterol or cholestanol possessing compounds might be capable of inhibiting the apoptosis

Figure 10. Morphology of HUVEC

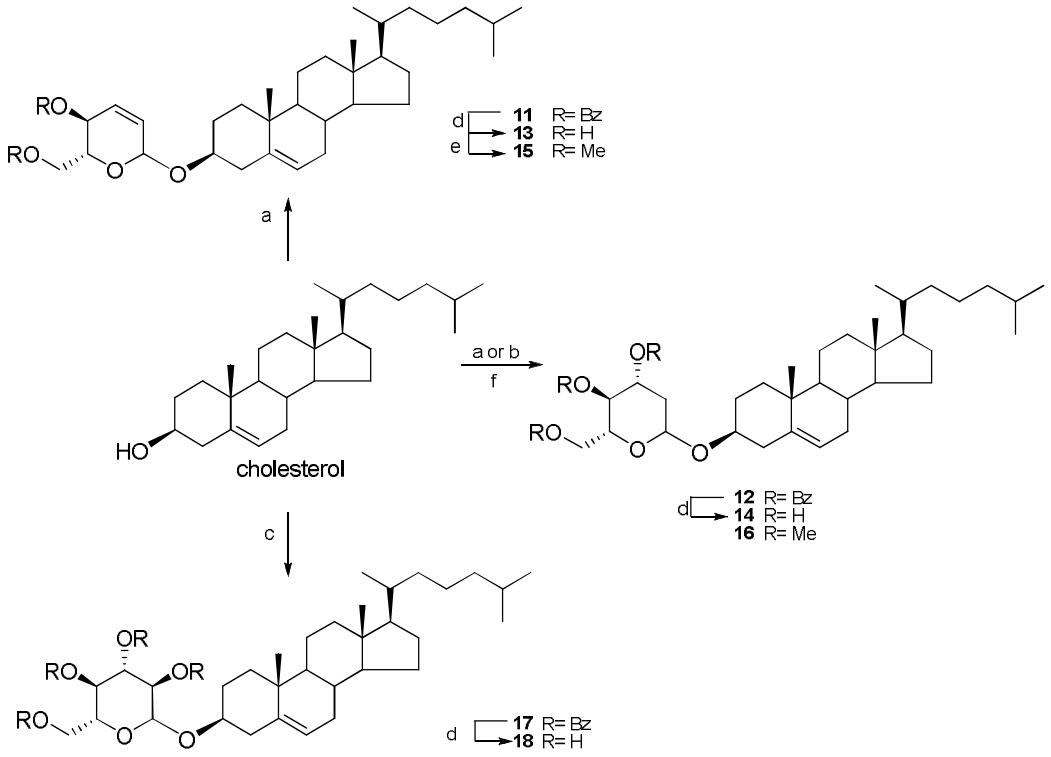


The morphological changes of HUVECs on analogs treated at 10 μg/ml for 48 hr. 0 hr, the morphologies of cells cultured in the medium without any derivatives. Con, the morphologies of cells cultures in the medium containing DMSO used as a vehicle control.

3. Synthesis of pseudosugar derivatives

Considering the previous results with ginsenosides, we had assurance about the relationship between activity and sugar moiety; thus, the studies proceeded with the pseudosugar derivatives synthesis. From the results of the phytosterol assay, we chose cholesterol as the steroidal backbone. As bioisosteres of the glucosyl residue, cyclic ether (di-or THP), acyclic ether (alkyl ether), and aryl groups were introduced to C3 OH of cholesterol. The THP derivative was synthesized already. Thus, the synthesis commenced with an introduction of dihydropyran to C3 alcohol of cholesterol as the substituents of glucosyl moiety as shown in Scheme 6.

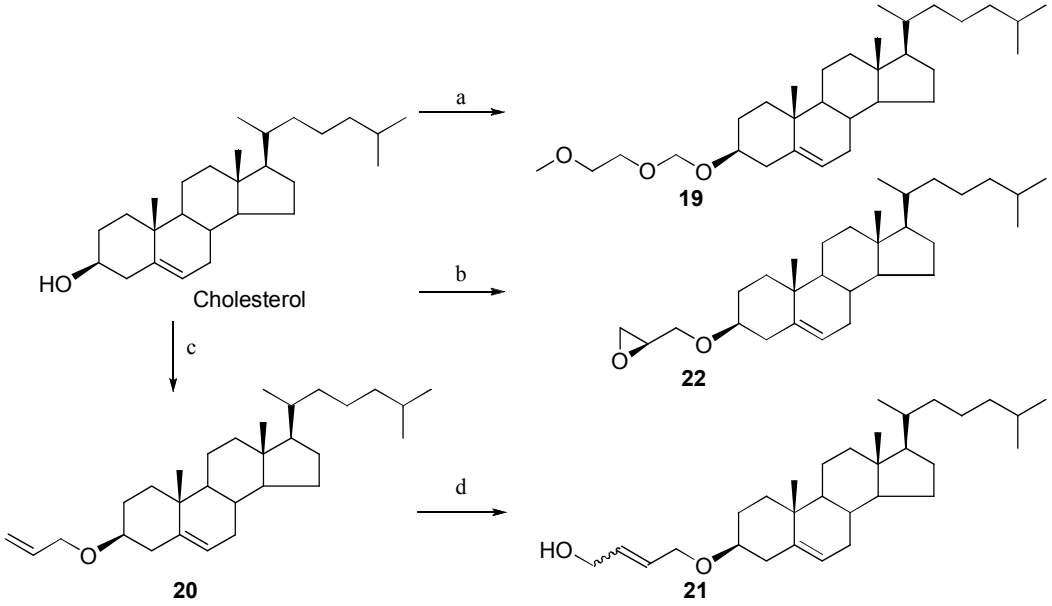
Scheme 6. Synthesis of dihydropyran derivatives



Reagents; (a) 3,4,6-*tri*-*O*-benzoyl-*D*-glucal, TsOH, CH_2Cl_2, 43%; (b) 3,4,6-*tri*-*O*-methoxy-*D*-glucal, CAN, CH_3CN, 26%; (c) 2,3,4,6-tetrabenzoyl-*D*-gluconosyl bromide, Ag_2CO_3, CH_2Cl_2, 45%; (d) NaOMe, MeOH/CH_2Cl_2, 60%; (e) MeI, NaH, THF, 0 ^oC, 32% (f) 3,4,6-*tri*-*O*-methyl-*D*-glucal, CAN, CH_3CN, 2% for **16**.

Treatment of *tri-O*-benzoyl glucal to cholesterol in acidic media was afforded to **11** and **12** mixtures, which could be separated by column chromatography. Removal of benzoyl group of **11** and **12** by using NaOMe afforded to **13** and **14**, which could be transformed to **15** and **16** by the addition of NaH and iodomethane, respectively.

Scheme 7. Synthesis of acyclic analogs



Reagents; (a) $^{i}Pr_2NEt$, CH_2Cl_2, MEMCl, 48%; (b) NaH, THF/DMF (1:1), 2*S*-(+)-glycidol tosylate, 45%; (c) NaH, THF/DMF (1:1), allyl bromide, reflux, 36%; (d) Grubbs' 2nd catalyst, 1,4-butene diol, CH_2Cl_2, reflux, 30%.

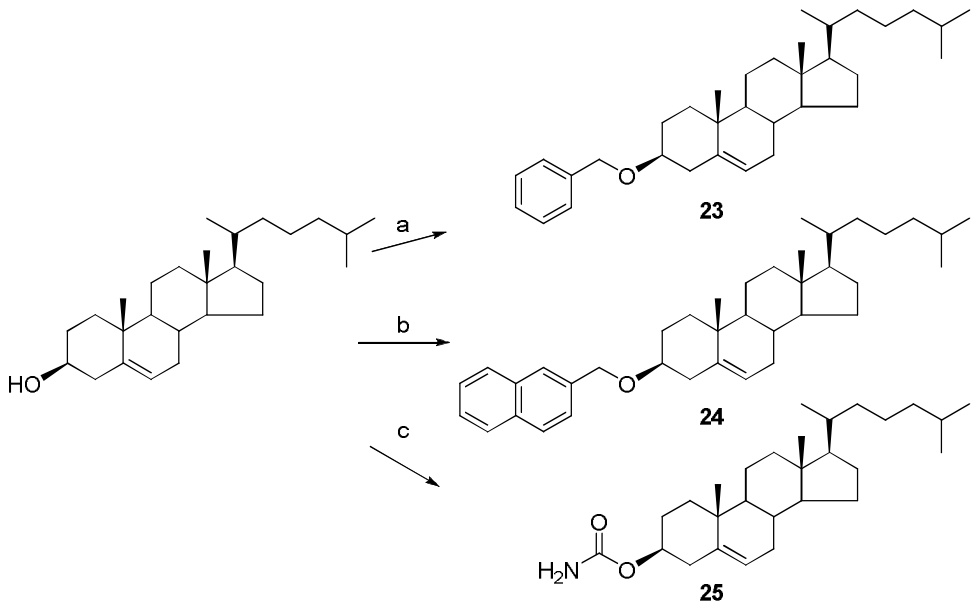
Analog **18** could be prepared by coupling cholesterol with 2,3,4,6-tetrabenzoyl-*D*-glucopyranosyl bromide in the presence of silver triflate, followed by debenzoylation as described above.

Next, we synthesized a series of cholesterol analogs linked with the acyclic or alkyl ether moieties (Scheme 7). Analog **19** was synthesized by reaction with MEMCl in the presence of Hunig's base. Allylation of cholesterol by treatment with sodium hydride and allyl bromide in refluxing

THF/DMF provided allyl ether **20**. Cross metathesis of **20** with 1,4-butene diol by employing Grubbs' 2nd generation catalyst afforded **21**. Reaction of cholesterol with sodium hydride and then with (2*S*)-glycidyl tosylate afforded **22**.

To confirm the spatial influence of glucosyl residue, aryl moieties as a carbohydrate equivalent were also introduced to the cholesterol scaffold. Analogs **23** and **24** were prepared by *O*-alkylation with benzyl bromide and bromomethyl naphthalene under reaction conditions of sodium hydride and TBAI in refluxing THF. Treatment of cholesterol with trichloroacetyl isocyanate gave carbamate **25** (Scheme 8).

Scheme 8. Synthesis of bulky analogs



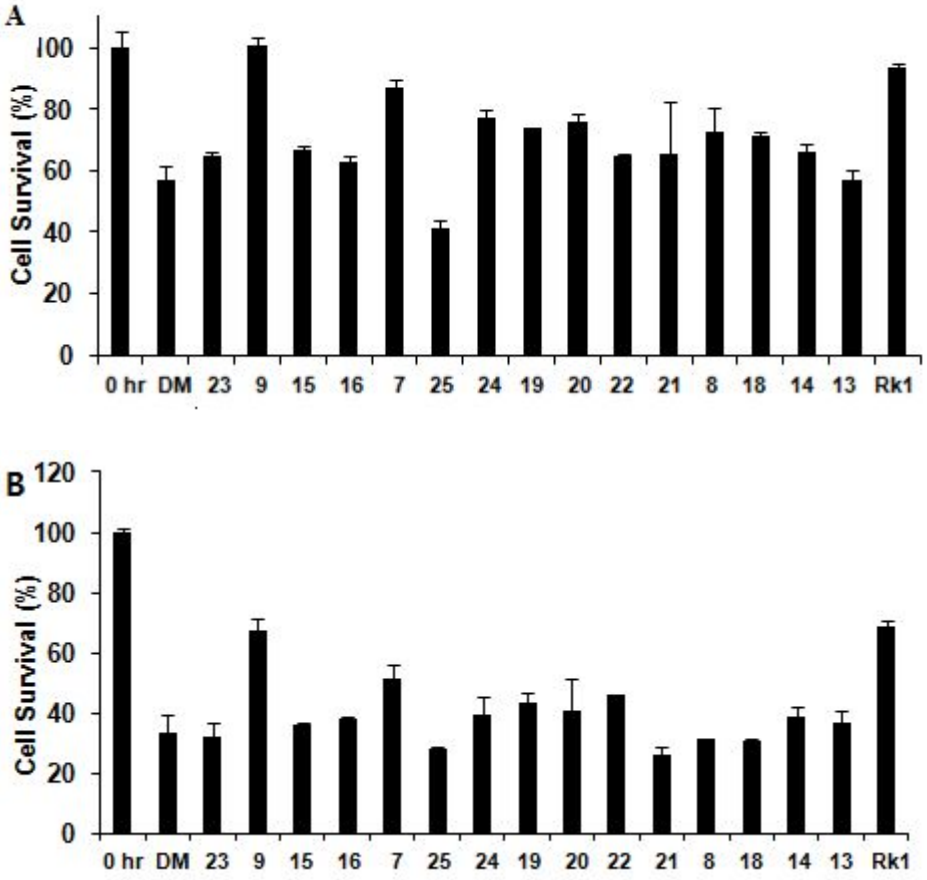
Reagents; (a) NaH, TBAI, benzyl bromide, THF, reflux, 30%; (b) NaH, TBAI, (2-bromomethyl) naphthalene, THF, reflux, 30%; (c) $Cl_3CCONCO$, $CHCl_3$, 31%.

4. Biological activity against serum deprived apoptosis

1) MTT assay on HUVECs

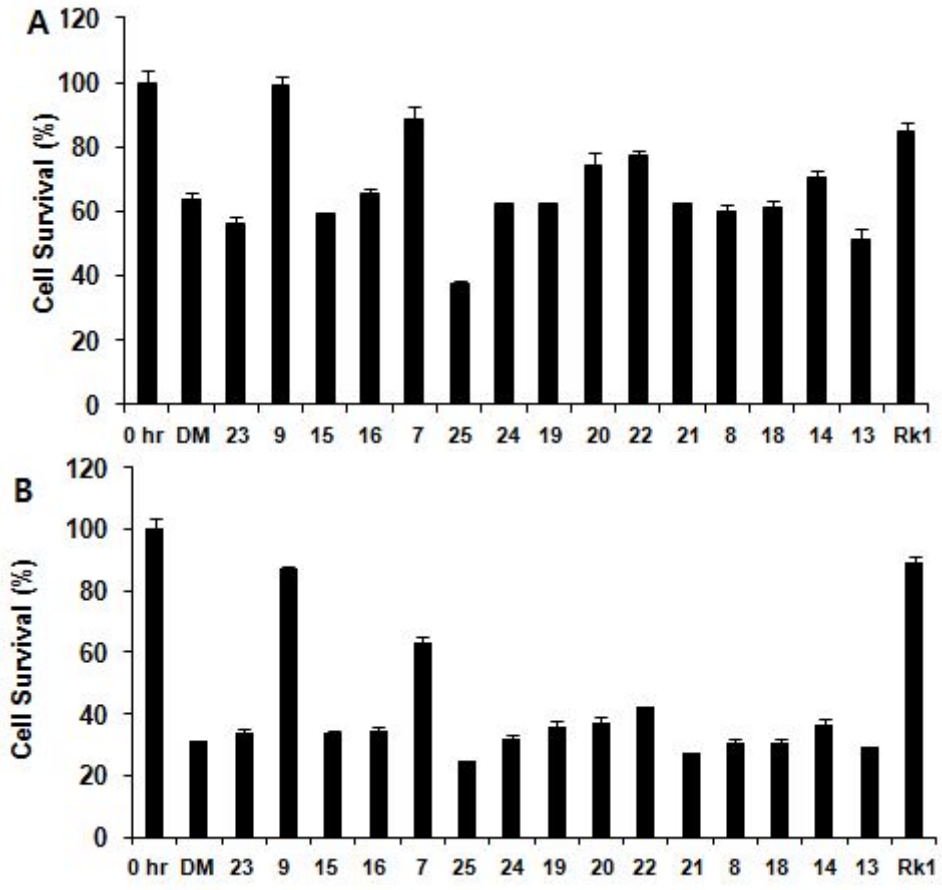
The inhibitory effects of the cholesterol analogs were investigated on the growth factor deprivation-induced HUVEC cell death using the MTT colorimetric method at concentrations of 5 μg/ml and 10 μg/ml for 24 h and 48 h, respectively. The results of the MTT assay are summarized in Figures 11 and 12.

Figure 11. Effect of compounds on apoptosis of HUVECs: 5 μg/ml treatment



(A) 5 μg/ml for 24 h treatment, (B) 5 μg/ml, 48 h treatment. 0 hr; the viability of the cells cultured in the medium without any derivatives. DM; the viability of the cells cultured in the medium containing DMSO used as a vehicle control. **Rk1**; the viability treated with ginsenoside Rk1.

Figure 12. Effect of compounds on apoptosis of HUVECs: 10 μg/ml treatment



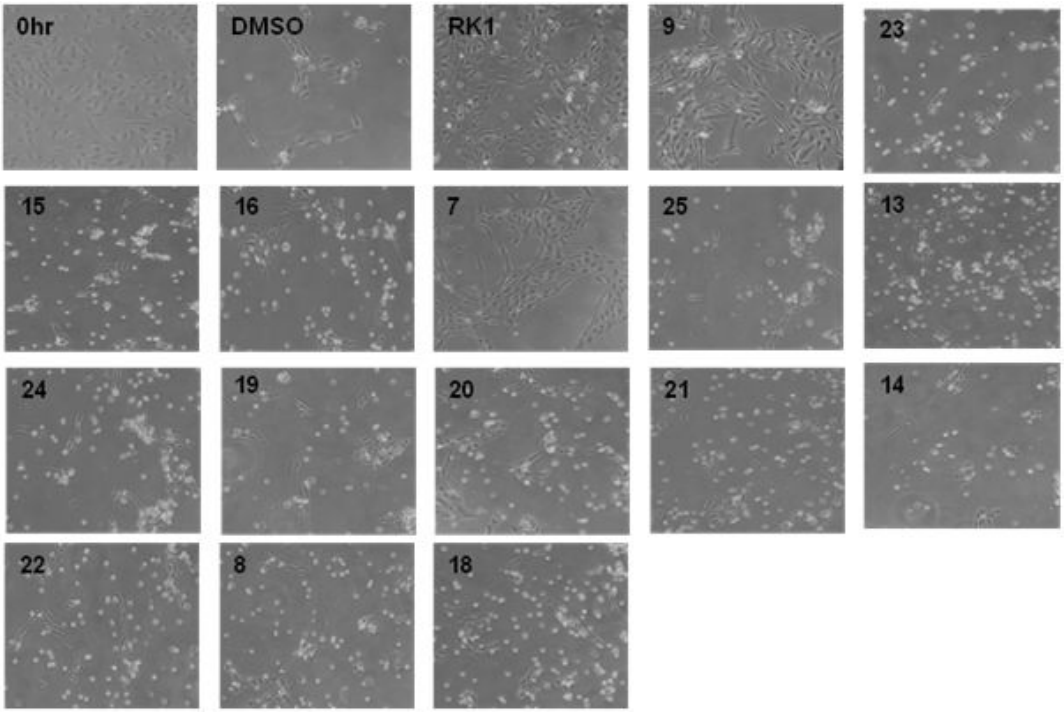
(A) 10 μg/ml for 24 h treatment, (B) 10 μg/ml for 48 h treatment. 0 h; the viability of the cells cultured in the medium without any derivatives. DM; the viability of the cells cultured in the medium containing DMSO used as a vehicle control. **Rk1**; the viability treated with ginsenoside Rk1.

It is noticeable that the acetyl dihydropyran derivative **11** was more potent than ginsenoside Rk1, which has known as one of the potent anti-apoptotic agents. In contrast, analogs **13** and **15** possessing methoxy or hydroxyl dihydropyran exhibited less potent activity compared to Rk1. This result revealed that the acyl group of the carbohydrate equivalents is important to manifest the inhibitory effect of the cholesterol analogs. The THP analog **7** also exhibited comparable activity to that of Rk1 after 24-h treatment. However, it was slightly less potent than Rk1 after 48 h

treatment. Analogs **14**, **16**, and **18** possessing the substituted pyran moiety did not show any significant inhibitory activity. Analogs **19**, **20**, **21**, and **22** possessing the acyclic carbohydrate equivalents exhibited moderate inhibitory activities at the concentration of 5 μg/ml. It is also noteworthy that the cholesteryl THP analog **7** was more potent than the dihydrocholesteryl THP analog **8**.

Next, we observed the morphological changes associated with the physiological and pathological processes in HUVECs, as described ahead (Figure 13). Treated with compounds **7** and **9, t**he elongated HUVECs were observed. The data suggested that particularly dihydro-, tetrahydrocholesterol derivatives might inhibit the apoptosis of ECs.

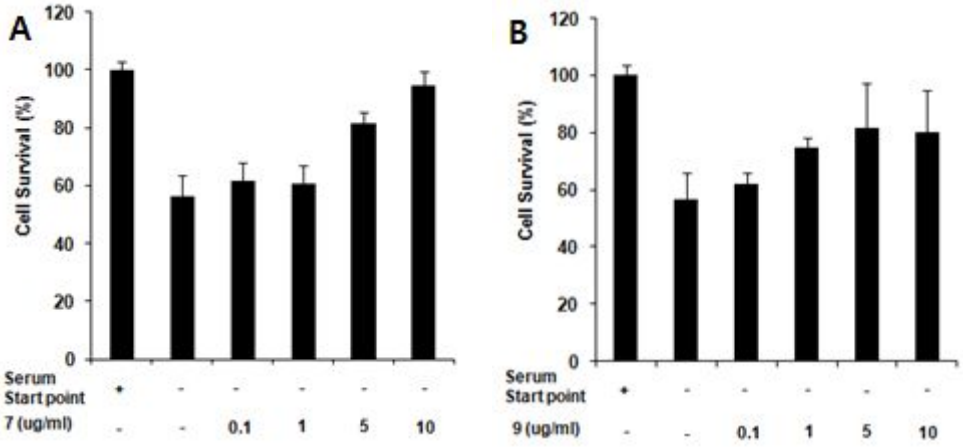
Figure 13. Morphological changes of HUVEC



The morphological changes of HUVECs on analogs treated at 10 μg/ml for 48 hr. 0 hr; the morphologies of cells cultured in the medium without any derivatives. Con; the morphologies of cells cultures in the medium containing DMSO used as a vehicle control.

For further investigation of the cell-protective effect of analogs **7** and **9**, their anti-apoptotic effect on cell viability in human retina endothelial cells (HRECs) was examined. In addition, their dose-dependent activities were confirmed by the cell survival counting assay, which was performed at 0.1 μg/ml to 10 μg/ml, respectively. The concentration-dependent cell viabilities by both analogs **7** and **9** are well revealed in Figure 14.

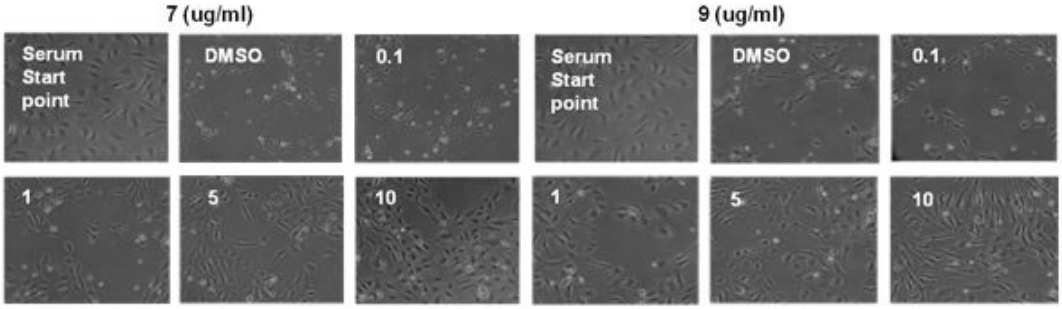
Figure 14. Inhibition of compounds 7, and 9 on apoptosis of HRECs



Inhibitory effects by analogs **7** and **9** on apoptosis of HRECs. (A) Treatment with analog **7** at sequential concentration from 0.1 μg/ml to 10 μg/ml for 48 h. (B) Treatment with analog **9** at sequential concentration from 0.1 μg/ml to 10 μg/ml for 48 h.

And the morphological changes have shown the correlation of the activities with the concentrations of the analogs **7** and **9** (Figure 15).

Figure 15. Morphological changes of HUVEC

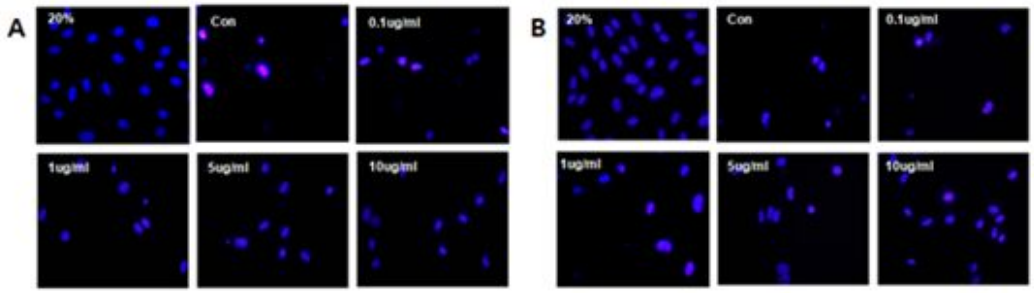


2) DAPI/ TUNEL assay

Apoptotic activity in the cytoplasmic extracts can be examined by the measurement of enzymatic caspase activity by western blots of proteins processed during apoptosis (caspases and their substrates) or by using isolated cell nuclei as indicators for apoptotic factors inducing nuclear morphological changes and/or DNA fragmentation. In 1993, a paper first described that a cell-free system can mimic characteristic features of apoptosis in intact cells. [32] Indeed, it has become clear that the apoptotic pathways acting in the cytoplasm function independently from the nucleus, and thus cell-free systems appear to be appropriate model systems that represent at least part of the apoptotic machinery and signaling mechanisms. Also, signaling pathways such as the caspase cascade have been studied under cell-free conditions, providing insight into activation patterns and inhibitor specificities. [33] On the other hand, there are cell-based assay systems. Apoptosis could be characterized biochemically by the activation of endonuclease activity that contributed to massive morphological changes in nuclei and ultimately to cell death. Endonuclease activity in individual nuclei was determined by a terminal deoxynucleotidyl transferase (TdT). 4',6'-Diamidino-2-phenylindole.2HCl (DAPI) forms a specific complex with DNA, and this fact has been used for the quantitative fluorometric estimation of DNA. [34] The fluorescent dye DAPI complexes with DNA to give a product with a fluorescence intensity about 20 times greater than that of the dye alone. Cells stained with DAPI and examined by fluorescence microscopy show nuclear DNA as well as mitochondrial and chloroplast DNA. This lack of interference from other cell components prompted us to develop a simple fluorometric assay for the DNA content of crude cellular homogenates using DAPI. [35] Among these assay systems, we tested the DAPI and terminal transferase dUTP nick end labeling (TUNEL) assay.

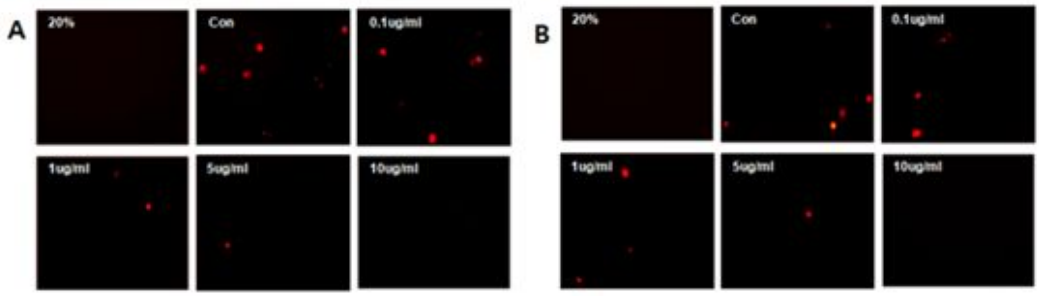
At first, morphological changes in the nuclear chromatin in cells undergoing apoptosis were detected by staining with the DNA-binding fluorochrome, DAPI. HUVECs were grown on glass coverslips and treated with analogs **7** and **9** in the presence or absence of serum for 48 h. Cells were washed twice with phosphate buffered saline (PBS) and fixed by incubation in 4% paraformaldehyde for 30 min. Following washing with PBS, cells were incubated in a DAPI solution (1 μg/ml) for 30 min in the dark. Cells were then washed with PBS and subjected to fluorescence microscopy (Figure 16).

Figure 16. Changes in nuclear morphology accessed by DAPI staining.



(A) Cells treated with **9** of various concentrations for 48 h; (B) Cells treated with **7** of various concentrations for 48 h.

Figure 17. TUNEL assays at various concentrations of 7 and 9



(A) Cells treated with **9** of various concentrations for 48 h; (B) Cells treated with **7** of various concentrations for 48 h.

Next, the TUNEL assay was executed. The TUNEL assay is a method used to detect DNA degradation in apoptotic cells because one of the hallmarks of late-stage apoptosis is the fragmentation of nuclear chromatin, which results in a multitude of 3'-hydroxyl termini of DNA ends. [36] This property can be used to identify apoptotic cells by labeling the DNA breaks with fluorescent-tagged deoxyuridine triphosphate nucleotides (F-dUTP). The addition of the deoxythymidine analog 5-bromo-2'-deoxyuridine 5'-triphosphate (BrdUTP) to the TdT reaction serves to label the break sites. Once incorporated into the DNA, BrdU can be detected by an anti-BrdU antibody using standard immunohistochemical techniques. Non-apoptotic cells do not incorporate much of the F-dUTP because of the absence of exposed 3'-hydroxyl DNA ends. This enzyme labeled the 3' OH ends of DNA for visualization with a secondary detection system using a colorimetric substrate for peroxidase. The TdT-mediated dUTP-biotin nick-end labeling (TUNEL) assay stained apoptotic nuclei red for the expression of endogenous endonucleases.

The TUNEL assay, as shown in Figure 17, specifically detecting DNA fragmentation, also revealed that analogs **7** and **9** suppressed the apoptotic cell death induced by serum deprivation in a dose-dependent manner. We have reported parts of these synthetic products and biological assay results. [37]

III. Conclusion

In conclusion, we have developed novel anti-apoptotic agents based on the ginsenoside Rk1 and Rg3, providing valuable information for the development of new anti-apoptotic agents. In particular, we replaced the ginsenosides, which are chemically unstable and difficult to be supplied sustainably, to the easily accessible phytosterol backbone. Furthermore, the analogs **7** and **9** possessing the cholesterol scaffold and the cyclic carbohydrate equivalents exhibited excellent cell survival activities, which are equipotent to that of ginsenoside Rk1. In addition, these analogs exhibited dose-dependent cell viability on the HREC line. The excellent human EC protective activities of our novel Rk1-based anti-apoptotic agents allow us to envisage their valuable application for the treatment of vascular disorders. Currently, systematic studies on their therapeutic applications, including in vivo testing of the potent sterol analogs as well as their mechanistic aspects, are in good progress. [38]

IV. Experimental

General Experimental

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Dichloromethane, triethylamine, acetonitrile and pyridine were freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Reaction flasks were dried at 100 °C. Air and moisture sensitive reactions were performed under an argon atmosphere. Flash column chromatography was performed using silica gel 60 (230-400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Infrared spectra were recorded on a Perkin-Elmer 1710 FT-IR spectrometer. Mass spectra were obtained with VG Trio-2 GC-MS instrument. High-resolution mass spectra were obtained with JEOL JMS-AX 505WA instrument. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 (300 MHz), Varian Gemini 2000 (300 MHz), Brucker Analytik AVANCE digital 400 (400MHz), or AVANCE digital 500 (500MHz) spectrometer as solutions in deuteriochloroform ($CDCl_3$). Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane and are referenced to the deuterated solvent ($CHCl_3$). ^{1}H-NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad singlet; m, multiplet and/or multiple resonance), number of protons, and coupling constant in hertz (Hz).

2-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*S*,*E*)-5-Ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (1)

To a solution of stigmasterol (100 mg, 0.24 mmol) in CH_2Cl_2 (4 mL) was added dihydropyran (0.033 mL, 0.36 mmol) and PPTS (6 mg, 0.24 mmol). After stirring for 4 h, the reaction mixture was quenched with H_2O, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 82 mg (68%) of **1** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.32 (m, 1H), 5.12 (dd, 1H, *J* = 8.43 Hz, 15.0 Hz), 4.99 (dd, 1H, *J* = 8.6 Hz, 15.0 Hz), 4.69 (m, 1H), 3.88 (m, 1H), 3.47 (m, 2H), 2.34 (m, 2H), 1.97-1.06 (m, 29H), 0.98 (s, 6H), 0.80 (m, 9H), 0.67 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 140.9, 138.3, 129.1, 121.5, 96.7, 75.9, 62.8, 56.8, 51.2, 50.1, 42.1, 40.4, 39.6, 38.7, 37.4, 37.1, 36.7, 31.8, 31.2, 29.6, 28.8, 27.9, 25.4, 25.3, 24.3, 21.1, 21.0, 20.06, 21.01, 19.9, 19.3, 18.9, 12.2, 12.0; LRMS (FAB+) *m/z* 497(M+H).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*S*,*E*)-5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (2)

To a solution of 3,4,6-triacetyl glucal (198 mg, 0.7 mmol) in THF (4 mL) was added stigmasterol (100 mg, 0.24 mmol) and $BF_3{\cdot}OEt_2$ (0.09 ml, 0.7 mmol). After stirring for 18 h, the reaction mixture was quenched with saturated NH_4Cl and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 119 mg (79%) of **2** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.81 (m, 2H), 5.30 (m, 2H), 5.16 (br, 1H), 5.10 (d, 1H, *J* = 8.4 Hz), 4.98 (dd, 1H, *J* = 8.4 Hz, 15.0 Hz), 4.17 (m, 3H), 3.53 (m, 1H), 2.35

(m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 2.02-1.81 (m, 6H), 1.68 (m, 1H), 1.53 (m, 9H), 1.25-0.75 (m, 22H), 0.67 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 170.7, 170.2, 140.6, 138.2, 129.2, 128.8, 128.0, 121.7, 92.7, 76.5, 66.7, 65.3, 63.1, 56.7, 55.8, 51.1, 50.1, 42.1, 40.4, 40.3, 39.5, 37.0, 36.6, 31.8, 28.8, 28.1, 25.3, 24.2, 21.1, 21.0, 20.9, 20.9, 20.7, 19.2, 18.9, 12.2, 11.9; LR-MS (FAB+) *m/z* 647 (M+Na).

2-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (3)

To a solution of β-sitosterol (200 mg, 0.48 mmol) in CH_2Cl_2 (6 mL) was added dihydropyran (0.07 mL, 0.72 mmol) and PPTS (12 mg, 0.05 mmol). After stirring for 2 h, the reaction mixture was quenched with H_2O, and then diluted with CH_2Cl_2. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 164 mg (68%) of **3** as a white solid: FT-IR (neat) ν_{max} 2921, 1460, 1112 cm^{-1}; ^{1}H -NMR ($CDCl_3$, 300 MHz) δ 5.32 (br, 1H), 4.69 (br, 1H), 3.89 (m, 1H), 3.47 (m, 2H), 2.34 (m, 2H), 2.00-1.00 (m, 33H), 0.98 (s, 3H), 0.89 (d, 3H, *J* = 6.26 Hz), 0.83 (q, 9H, *J* = 7.14 Hz), 0.65 (s, 3H); ^{13}C -NMR ($CDCl_3$, 75 MHz) δ 140.9, 121.5, 96.8, 75.9, 62.7, 56.7, 56.0, 50.1, 45.7, 42.2, 40.2, 39.7, 38.7, 37.4, 47.1, 36.7, 36.0, 33.8, 31.8, 31.2, 29.6, 29.0, 28.2, 25.9, 25.4, 24.2, 23.0, 21.0, 20.0, 19.7, 19.3, 18.9, 18.7, 11.9; LRMS (FAB+) *m/z* 499 (M+H).

2-((3*S*,10*S*,13*R*,17*R*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (4)

To a solution of **3 (**68 mg**,** 0.14 mmol) in EtOAc (4 mL) was added 10% activated palladium carbon. The resulting mixture was hydrogenated for 24 h and then filtered through a pad of Celite. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 :

30) afforded 56 mg (82 %) of **4** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 4.68 (s, 1H), 3.89 (m, 1H), 3.50 (m, 2H), 1.94-0.77 (m, 53H), 0.61 (s, 3H); LRMS (FAB+) *m/z* 523 (M+Na).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,10*R*,13*R*,17*R*)-17-((2*R*,5*R*)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (5)

To a solution of 3,4,6-triacetyl glucal (984 mg, 3.62 mmol) in THF (6 mL) was added β-sitosterol (500 mg, 1.21 mmol) and $BF_3 \cdot OEt_2$ (0.45 ml, 3.62 mmol). After stirring for 2 h, the reaction mixture was quenched with saturated $NaHCO_3$ and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 7) afforded 620 mg (82%) of **5** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.81 (m, 2H), 5.32 (d, 1H, *J* = 4.77 Hz), 5.25 (d, 1H, *J* = 9.33 Hz), 5.14 (s, 1H), 4.16 (m, 3H), 3.51 (m, 1H), 2.34 (m, 2H), 2.06 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.00-1.81 (m, 5H), 1.74-1.00 (m, 22H), 0.97 (s, 3H), 0.88 (d, 3H, *J* = 6.24 Hz), 0.80 (q, 6H, *J* = 6.96 Hz), 0.64 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 170.4, 169.9, 140.5, 128.6, 128.2, 121.6, 92.5, 77.9, 66.6, 65.1, 62.9, 56.2, 55.8, 49.9, 45.5, 42.1, 40.2, 39.5, 36.9, 36.4, 35.9, 33.7, 31.7, 31.6, 28.9, 28.0, 25.8, 24.1, 22.8, 20.8, 20.7, 20.6, 19.6, 19.1, 18.8, 18.6, 18.5, 12.0, 11.8; LRMS (FAB+) *m/z* 649 (M+Na).

(3*S*,10*S*,13*R*,17*R*)-17-((2*R*,5*R*)-5-Ethyl-6-methylheptan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-ol (Sitostanol)

To a solution of β-sitosterol (225 mg**,** 0.54 mmol) in EtOAc (8 mL) was added 10% activated palladium carbon. The resulting mixture was hydrogenated for 2 h and then filtered through a pad of Celite. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 209 mg (92 %) of **sitostanol** as a white solid: FT-IR (neat) ν_{max} 3350,

1464, 1029 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.55 (m, 1H), 1.93 (d, 1H, *J* = 12.09 Hz), 1.82-0.72 (m, 45 H), 0.58 (s, 3H); LRMS (FAB+) *m/z* 417 (M+H).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,10*S*,13*R*,17*R*)-17-((2*R*,5*R*)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (6)

To a solution of 3,4,6-triacetyl glucal (275 mg, 1.01 mmol) in THF (5 mL) was added sitostanol (140 mg, 0.337 mmol) and $BF_3 \cdot OEt_2$ (0.125 ml, 1.01 mmol). After stirring for 4 h, the reaction mixture was quenched with water and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 165 mg (78%) of **6** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.80 (m, 2H), 5.26 (d, 1H, *J* = 9.15 Hz), 5.14 (s, 1H), 4.16 (m, 3H), 3.59 (m, 1H), 2.06 (s, 3H), 2.04 (s, 3H), 1.93 (m, 1H), 1.82-0.77 (m, 46H), 0.61 (s, 3H); ^{13}C -NMR ($CDCl_3$, 75 MHz) δ 170.6, 170.2, 128.7, 128.6, 92.9, 77.8, 66.6, 65.2, 63.1, 56.3, 56.0, 54.2, 45.7, 45.0, 42.5, 39.9, 36.8, 36.2, 26.0, 35.4, 33.8, 32.0, 29.0, 28.8, 28.1, 28.0, 25.9, 24.1, 22.9, 21.1, 20.8, 20.7, 19.7, 18.9, 18.5, 12.1, 11.9, 11.8; LRMS (FAB+) *m/z* 651 (M+Na).

2-((3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (7)

To a solution of cholesterol (100 mg, 0.26 mmol) in CH_2Cl_2 (1.5 mL) was added dihydropyran (0.18 mL, 1.97 mmol) and PTSA (12 mg, 0.07 mmol). After stirring for 4 h, the reaction mixture was quenched with H_2O, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash

column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 82 mg (68%) of **7** as a white solid: FT-IR (neat) ν_{max} 2921, 1460, 1112 cm^{-1}; ^1H -NMR ($CDCl_3$, 300 MHz) δ 5.32 (br, 1H), 4.69 (br, 1H), 3.88 (m, 1H), 3.54-3.43 (m, 2H), 2.34-2.28 (m, 2H), 2.00-1.66 (m, 7H), 1.59-1.00 (m, 21H), 0.98 (s, H), 0.86 (ddd, 9H, *J* = 15.3 Hz, 6.4 Hz, 1.2 Hz), 0.65 (s, 3H); LRMS (FAB+) *m/z* 471 (M+H).

2-((3*S*,10*S*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (8)

To a solution of **7 (**40 mg**,** 0.01 mmol) in EtOAc (3 mL) was added 10% activated palladium carbon. The resulting mixture was hydrogenated for 1 h and then filtered through a pad of Celite. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 37 mg (78 %) of **8** as a white solid: FT-IR (neat) ν_{max} 2930, 1738, 1464, 1029 cm^{-1}; ^1H-NMR ($CDCl_3$, 300 MHz) δ 4.69 (m, 1H), 3.89 (m, 1H), 3.60-3.41 (m, 2H), 1.92 (m, 1H), 1.82-1.39 (m, 13H), 1.35-0.94 (m, 15H), 0.85 (ddd, 9H, *J* = 10.5 Hz, 6.6 Hz, 1.3 Hz), 0.77 (s, 3H), 0.62 (s, 3H); LRMS (FAB+) *m/z* 473 (M+H).

((2R,3S)-3-Acetoxy-6-((3S,10R,13R,17R)-10,13-dimethyl-17-((R)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (9)

To a solution of 3,4,6-triacetyl glucal (2.0 g, 7.5 mmol) in ether (20 mL) was added cholesterol (1.0 g, 2.5 mmol) and $BF_3 \cdot OEt_2$ (1.3 ml, 10 mmol). After stirring for 18 h, the reaction mixture was quenched with a saturated $NaHCO_3$ and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 660 mg (43%) of **9** as a white solid: ^1H-NMR ($CDCl_3$, 300 MHz) δ 5.83 (m, 2H), 5.33 (br, 1H), 5.27 (dd,

1H, *J* = 9.15 Hz, 1.47 Hz), 5.14 (br, 1H), 4.17 (m, 3H), 3.53 (m, 1H), 2.37 (m, 2H), 2.07 (s, 3H), 2.05 (s, 3H), 1.99-1.01 (m, 26H), 0.97 (s, 3H), 0.84 (dd, 9H, *J* = 6.6 Hz, 14.3 Hz), 0.65 (s, 3H); FT-IR (neat) ν_{max} 2938, 1464, 1377, 1261, 1199, 1024 cm^{-1}; LRMS (FAB+) *m/z* 621 (M+Na).

((2*R*,3*S*)-3-Acetoxy-6-((3*S*,5*S*,10*S*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)hexadecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl acetate (10)

To a solution of 3,4,6-triacetyl glucal (95 mg, 0.35 mmol) in THF (2 mL) was added cholestanol (45 mg, 0.116 mmol) and $BF_3 \cdot OEt_2$ (0.043 ml, 0.35 mmol). After stirring for 18 h, the reaction mixture was quenched with a saturated $NaHCO_3$ and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 57 mg (82%) of **10** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.82 (m, 2H), 5.26 (d, 1H, *J* = 9.15 Hz), 5.14, (s, 1H), 4.18 (m, 3H), 3.60 (m, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 1.93 (d, 1H, *J* = 12.09 Hz), 1.78-0.93 (m, 30H), 0.85 (dd, 9H, *J* = 6.5 Hz, 10.08 Hz), 0.77 (s, 3H), 0.61 (s, 3H); LRMS (FAB+) *m/z* 623 (M+Na).

((2*R*,3*S*)-3-(Benzoyloxy)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran-2-yl)methyl benzoate (11)

To a solution of 3,4,6-tribenzoyl glucal (320 mg, 0.7 mmol) in CH_2Cl_2 (10 mL) was added cholesterol (108 mg, 0.28 mmol) and PTSA (11 mg, 0.06 mmol). After stirring for 18 h, the reaction mixture was quenched with water and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 15) afforded 33 mg

(23%) of **11** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02-8.00 (m, 4H), 7.57-7.32 (m, 6H), 6.04 (d, 1H, *J* = 10.2 Hz), 5.87 (dd, 1H, *J* = 10.2 Hz, 2.0 Hz), 5.61 (d, 1H, *J* = 8.9 Hz), 5.22 (br, 1H), 5.11 (br, 1H), 4.58-4.38 (m, 4H), 3.59 (m, 1H), 3.65 (dd, 1H, *J* = 11.0 Hz, 7.9 Hz), 2.37-2.33 (m, 2H), 2.02-0.83 (m, 38H), 0.65 (s, 3H); LRMS (FAB+) *m/z* 745 (M+Na).

(2*R*,3*S*,4*R*)-2-(Benzoyloxymethyl)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)tetrahydro-2*H*-pyran-3,4-diyl dibenzoate (12)

To a solution of 3,4,6-tribenzoyl glucal (320 mg, 0.7 mmol) in CH_2Cl_2 (10 mL) was added cholesterol (108 mg, 0.28 mmol) and PTSA (11 mg, 0.06 mmol). After stirring for 18 h, the reaction mixture was quenched with water and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 15) afforded 60 mg (26%) of **12** as a pale yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.02-7.89 (m, 6H), 7.58-7.32 (m, 9H), 5.47 (m, 2H), 5.30 (br, 1H), 5.19 (br, 1H), 4.87 (m, 1H), 4.53-4.47 (m, 3H), 3.97 (m, 1H), 3.55 (m, 1H), 2.52-2.15 (m, 3H), 2.02-0.83 (m, 20H), 0.65 (s, 3H); LRMS (FAB+) *m/z* 867 (M+Na); HRMS (FAB+) calcd for $C_{54}H_{68}O_8Na$ (M+Na): 867.4812, found 867.4816.

(2*R*,3*S*)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-Dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15, 16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-2-(hydroxymethyl)-3,6-dihydro-2*H*-pyran-3-ol (13)

To a refluxing solution of sodium (2.5 mg, 0.1 mmol) in MeOH (2 mL) was added a solution of the **11** (33 mg, 0.46 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 1.2 h, cooled to ambient temperature, and then quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with ether and the organic phase was washed with brine, dried over $MgSO_4$ and

concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes : MeOH = 1 : 1 : 0.05) afforded 14 mg (60%) of **13** as a white solid: FT-IR (neat) ν_{max} 3463, 2933, 1461, 1049 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.95 (d, 1H, *J* = 10.2 Hz), 5.75 (dt, 1H, *J* = 10.2 Hz, 2.2 Hz), 5.35 (br, 1H), 5.13 (br, 1H), 4.21 (br, 1H), 3.87 (br, 2H), 3.79-3.73 (m, 1H), 3.57-3.48 (m, 1H), 2.36 (br, 2H), 2.02-1.80 (m, 8H), 0.99 (s, 6H), 0.88 (ddd, 12H, *J* = 18.7 Hz, 6.6 Hz, 1.7 Hz), 0.67 (s, 3H); LRMS (FAB+) *m/z* 537 (M+Na).

(2*R*,3*S*,4*R*)-6-((3*S*,10*R*,13*R*,17*R*)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-1*H*-cyclopenta[α] phenanthren-3-yloxy)-tetrahydro-2-(hydroxymethyl)-2*H*-pyran-3,4-diol (14)

To a refluxing solution of sodium (3 mg, 0.12 mmol) in MeOH (3 mL) was slowly added a solution of **12** (57 mg, 0.067 mmol) in CH_2Cl_2 (3 mL). The reaction mixture was stirred for 1.2 h, cooled to ambient temperature, and quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with ether and the organic phase was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes : MeOH = 2 : 1 : 0.05) afforded 30 mg (84%) of **14** as a white solid: FT-IR (neat) ν_{max} 3394, 2929, 1461, 1070 cm^{-1}; ^{1}H -NMR ($CDCl_3$, 300 MHz) δ 5.32 (br, 1H), 5.05 (d, 0.6H, *J* = 2.1 Hz), 4.67 (d, 0.4H, *J* = 9.3 Hz), 3.98 (br, 0.6H), 3.85-3.81 (m, 2H), 3.66 (m, 1H), 3.55-3.38 (m, 2H), 3.25 (br, 0.4H), 2.31-2.07 (m, 4H), 1.99-1.78 (m, 5H), 1.62-0.99 (m, 20H), 0.97 (s, 6H), 0.86 (ddd, 12H, *J* = 19.5 Hz, 5.2 Hz, 1.8 Hz), 0.64 (s, 3H); LRMS (FAB+) *m/z* 555 (M+Na).

(2*R*,3*S*)-3-Methoxy-2-(methoxymethyl)-6-((3*S*)-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16, 17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yloxy)-3,6-dihydro-2*H*-pyran (15)

To a suspension of NaH (33.8 mg, 0.85 mmol) in THF (1 mL) was added a solution of **13** (87 mg,

0.17 mmol) in THF (3 mL) at 0 ºC. After stirring for 1 h, iodomethane (0.11 mL, 1.69 mmol) was slowly added to the reaction mixture. After stirring for an additional 16 h, the reaction mixture was quenched with water, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 30 mg (32%) of **15** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.03 (d, 1H, *J* = 10.23 Hz), 5.73 (d, 1H, *J* = 11.19 Hz), 5.32 (br, 1H), 5.12 (d, 1H, *J* = 2.55 Hz), 3.87 (br, 2H), 3.59 (m, 3H), 3.40 (s, 3H), 3.37 (s, 3H), 2.33 (m, 2H), 2.01-1.71 (m, 5H), 1.55-1.09 (m, 17H), 0.96 (s, 3H), 0.95-0.82 (m, 12H), 0.64 (s, 3H); LRMS (FAB+) *m/z* 565(M+Na).

(2*R*,3*S*,4*R*)-3,4-Dimethoxy-2-(methoxymethyl)-6-((3*S*,17*R*)-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yloxy)tetrahydro-2*H*-pyran (16)

A glycal (288 mg, 1.53 mmol) and cholesterol (592 mg, 1.53 mmol) were combined with a catalytic amount (5 mol%) of CAN (41 mg) in anhydrous CH_3CN (6 ml) and stirred for 12 h. The reaction mixture was extracted with ether and washed with $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated in a vacuum. The residue was purified by column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 20 mg (2.3%) of **16** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.27 (s, 2H), 5.05 (s, 1H), 3.63 (m, 2H), 3.52 (s, 3H), 3.42 (s, 3H), 3.40 (m, 1H), 3.38 (s, 3H), 3.16 (m, 1H), 2.20 (m, 2H), 1.95-0.99 (m, 24H), 0.97 (s, 3H), 0.85 (dd, 12H, *J* = 6.4 Hz, 13.7 Hz), 0.64 (s, 3H); LRMS (FAB+) *m/z* 597 (M+Na).

(2*R*,3*R*,4*S*,5*R*)-2-(Benzoyloxymethyl)-6-((3*S*,10*R*,13*R*,17*R*)-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[a]phenanthren-3-yloxy)tetrahydro-2*H*-pyran-3,4,5-triyl tribenzoate (17)

To a solution of cholesterol (150 mg, 0.39 mmol) in CH_2Cl_2 (5 mL) was added 4A molecular sieve (500 mg), 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (244 mg, 0.37 mmol) and a solution of silver triflate (105 mg, 0.41 mmol) in dry toluene (1 mL) at -20 °C. After stirring for 2 h, the reaction mixture was filtered through a pad of silica gel. The filtrate was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 140 mg (45%) of **17** as a pale yellow oil: FT-IR (neat) ν_{max} 2929, 1501, 1226 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.99-7.79 (m, 8H), 7.54-7.7.26 (m, 12H), 5.87 (t, 1H, *J* = 9.7 Hz), 5.60 (t, 1H, *J* = 9.7 Hz), 5.47 (dd, 1H, *J* = 9.7 Hz, 7.9 Hz), 5.20 (d, 1H, *J* = 4.7 Hz), 4.91 (d, 1H, *J* = 7. 9 Hz), 4.60-4.46 (m, 2H), 4.12 (m, 1H), 3.50 (m, 1H), 2.15 (br, 2H), 2.01-1.65 (m, 6H), 1.56-0.71 (m, 21H), 0.63 (s, 3H) ; LRMS (FAB+) *m/z* 867 (M+Na).

(3*R*,4*S*,5*S*,6*R*)-2-((3*S*,10*R*,13*R*,17*R*)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-Tetradecahydro-10,13-dimethyl-17-((*R*)-6-methylheptan-2-yl)-1*H*-cyclopenta[α] phenanthren-3-yloxy)-tetrahydro-6-(hydroxymethyl)-2*H*-pyran-3,4,5-triol (18)

To a refluxing solution of sodium (4 mg, 0.16 mmol) in MeOH (5 mL) was slowly added a solution of **17** (90 mg, 0.11 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 0.5 h, cooled to ambient temperature, and quenched with saturated aqueous NH_4Cl. The reaction mixture was diluted with ether and the organic phase was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (CH_2Cl_2 : MeOH = 7 : 1) afforded 22 mg (36%) of **18** as a white solid: FT-IR (neat) ν_{max} 3428, 2936, 1632, 1377, 1025 cm^{-1}; ^{1}H-NMR (Pyridine *d*-5, 300 MHz) δ 5.33 (br, 1H), 5.06 (d, 1H, *J* = 7.7 Hz), 4.57 (d, 1H, *J* = 9.5 Hz), 4.41 (dd, 1H, *J* = 11.7, 4.9 Hz), 4.29 (m, 2H), 4.09-3.91 (m, 3H), 2.72 (d, 1H, *J* = 13.0 Hz), 2.46 (m, 1H), 2.10-1.68 (m, 6H), 1.55-0.89 (m, 33H), 0.63 (s, 3H); LRMS (FAB+) *m/z* 571 (M+Na); HRMS (FAB+) calcd for $C_{33}H_{56}O_6Na$(M+Na): 571.3975, found

571.3974.

(3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-3-[(2-methoxyethoxy)methoxy]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (19)

To a solution of cholesterol (97 mg, 0.25 mmol) in CH_2Cl_2 (1 mL) was added diisopropylethylamine (0.09 mL, 0.5 mmol). After stirring for 1 h, methoxyethoxymethyl chloride (0.3 mL, 2.5 mmol) was added at 0 °C. After stirring for an additional 1 h, the reaction mixture was quenched with saturated aqueous NH_4Cl, and then diluted with EtOAc. The organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 57 mg (48%) of **19** as a white solid: FT-IR (neat) ν_{max} 2935, 1463, 1375, 1112 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.31 (d, 1H, *J* = 5.3 Hz), 4.75 (s, 2H), 3.70-3.67 (m, 2H), 3.55-3.52 (m, 2H), 3.42 (m, 1H), 3.37 (s, 3H), 2.31-2.26 (m, 2H), 2.00-1.77 (m, 5H), 1.59-1.01 (m, 20H), 0.97 (s, 3H), 0.85 (ddd, 9H, *J* = 14.7, 6.6, 1.3 Hz) 0.65 (s, 3H); LRMS (FAB+) *m/z* 497 (M+ Na).

(3*S*,10*R*,13*R*,17*R*)-3-(Allyloxy)-17-[(1*R*)-1,5-dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16, 17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (20)

To a suspension of NaH (41 mg, 1.03 mmol) in DMF/THF (1: 1, 1 mL) was added a solution of cholesterol (200 mg. 0.52 mmol) in a mixture of DMF/THF (2 mL). After stirring for 1 h, allyl bromide (0.22 mL, 2.59 mmol) was slowly added to the reaction mixture. After stirring for an additional 16 h, the reaction mixture was quenched with water, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 20) afforded 80 mg (36%) of **20** as a colorless oil: FT-IR (neat) ν_{max} 2929, 1462, 1375, 1094 cm^{-1};

^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.87 (m, 1H), 5.32 (d, 1H, *J* = 5.3 Hz), 5.28-5.11 (m, 2H), 4.00 (dt, 2H, *J* = 5.7 Hz, 1.3 Hz), 3.22-3.15 (m, 1H), 2.37-2.20 (m, 2H), 2.01-0.80 (m, H), 0.65 (s, 3H); LRMS (FAB+) *m/z* 426 (M+).

4-((3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)-2-buten-1-ol (21)

To a solution of **20** (38 mg, 0.09 mmol) in CH_2Cl_2 (1 mL) was added the 2nd generation Grubbs' catalyst (4 mg, 0.01 mmol) and 1,4-butene diol (0.2 mL). After stirring for 1 h, the mixture was concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 5) afforded 12 mg (30 %) of **21** as a colorless oil: FT-IR (neat) ν_{max} 3394, 2932, 1735, 1464 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.91-5.75 (m, 2H), 5.32 (d, 1H, *J* = 5.1 Hz), 4.14 (d, 2H, *J* = 8.4 Hz), 4.00 (d, 2H, *J* = 5.1 Hz), 3.18 (m, 1H), 2.36-2.15 (m, 2H), 2.00-1.74 (m, 5H), 1.53-1.00 (m, 21H), 0.97 (s, 3H), 0.86 (ddd, 9H, *J* = 14.8 Hz, 6.6 Hz, 1.1 Hz), 0.65 (s, 3H) ; LRMS (FAB+) *m/z* 456 (M+).

(2*R*)-2-[((3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yloxy)methyl]oxirane (22)

To a suspension of NaH **(**60% dispersion on mineral oil, 42 mg, 1.1 mmol) in a mixture of DMF/THF (2 mL) was slowly added a solution of cholesterol (200 mg, 0.52 mmol) in a mixture of DMF/THF (1/1 mL). After stirring for 30 minute, a solution of 2S-(+)-glycidyl tosylate in DMF/THF (0.5/0.5 mL) was slowly added. The reaction mixture was vigorously stirred for 26 h at ambient temperature, quenched with saturated aqueous NH_4Cl, and then diluted with ether. The organic phase was washed with 10% aqueous NaOH and brine, dried over $MgSO_4$ and

concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 10) afforded 101 mg (45%) of **22** as a white solid: FT-IR (neat) ν_{max} 2938, 1465, 1107cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.32 (d, 1H, *J* = 5.3 Hz), 3.70 (dd, 1H, *J* = 11.3 Hz, 3.3 Hz), 3.43 (dd, 1H, *J* = 11.3 Hz, 5.7 Hz), 3.25-3.09 (m, 2H), 2.78 (t, 1H, *J* = 4.9 Hz), 2.59 (dd, 1H, *J* = 4.9 Hz, 2.7 Hz), 2.38-2.12 (m, 2H), 2.02- 1.80 (m, 5H), 1.53-1.00 (m, 21H), 0.97 (s, 3H), 0.85 (ddd, 9H, *J* = 15.3 Hz, 6.6 Hz, 1.3 Hz), 0.64 (s, 3H); LRMS (FAB+) *m/z* 441 (M+H).

(3*S*,17*R*)-3-(Benzyloxy)-17-((*R*)-6-methylheptan-2-yl)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (23)

To a suspension of NaH (42 mg, 1.04 mmol) in THF (1 mL) was added a solution of cholesterol (100 mg, 0.26 mmol) in THF (3 mL) at 0 ºC. After stirring for 0.5 h, benzyl bromide (0.12 mL, 1.04 mmol) and tetrabutyl ammonium iodide (14 mg, 0.04 mmol) was added to the reaction mixture. The resulting mixture was refluxed for 16 h and then quenched with a saturated solution of NH_4Cl, and then diluted with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 30) afforded 45 mg (32%) of **23** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.85-6.74 (m, 5H), 4.87-4.83 (m, 3H), 4.04 (s, 2H), 2.75 (m, 1H), 1.93 (m, 1H), 1.75 (m, 1H), 1.55-1.32 (m, 5H), 1.07-0.37 (m, 34H), 0.20 (s, 3H); LRMS (FAB+) *m/z* 499 (M+Na).

(3*S*,17*R*)-17-((*R*)-6-Methylheptan-2-yl)-3-(naphthalen-2-ylmethoxy)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthrene (24)

To a suspension of NaH (42 mg, 1.04 mmol) in THF (1 mL) was added a solution of cholesterol (100 mg, 0.26 mmol) in THF (3 mL) at 0 ºC. After stirring for 0.5 h, 2-bromomethyl naphthalene (69 mg, 0.31 mmol) and tetrabutyl ammonium iodide (29 mg, 0.08 mmol) was added to the

reaction mixture. After stirred for 4 h and then quenched with water, and then diluted with ether. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated in vacuo. Purification of the residue via flash column chromatography on silica gel (EtOAc : hexanes = 1 : 15) afforded 13 mg (10%) of **24** as a white solid: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.79 (m, 4H), 7.45 (m, 3H), 5.33 (br, 1H), 4.70 (s, 2H), 3.31 (m, 1H), 2.46-2.26 (m, 2H), 2.00-1.74 (m, 5H), 1.61-1.04 (m, 24H), 1.00 (s, 3H), 0.85 (dd, 9H, *J* = 6.42 Hz, 13.9 Hz), 0.65 (s, 3H); LRMS (FAB+) *m/z* 549 (M+Na).

(3*S*,10*R*,13*R*,17*R*)-17-[(1*R*)-1,5-Dimethylhexyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[α]phenanthren-3-yl carbamate (25)

To a solution of cholesterol (20 mg, 0.05 mmol) in chloroform (1 mL) was added tricholoroacetyl isocyanate (0.05 mL, 0.42 mmol). After stirring for 2 h, the reaction mixture was diluted with chloroform, followed by filtration through a pad of aluminium oxide. The filtrate was concentrated in vacuo to afforded 7 mg (31%) of **25** as a white solid: FT-IR (neat) ν_{max} 3458, 2932, 1704 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.35 (br, 1H), 4.49-4.43 (m, 3H), 2.33-2.22 (m, 2H), 1.97-1.76 (m, 6H), 1.55-0.98 (m, 22H), 0.94 (s, 3H), 0.82 (ddd, 9H, *J* = 15.3, 6.6, 1.2 Hz), 0.60 (s, 3H); LRMS (FAB+) *m/z* 452 (M+Na).

V. References

1 Yoshimoto, M.; Yoder, M. C. *Nature* **2009**, *457*, 801-803.

2 (a) Lemichez, E.; Lecuit, M.; Nassif, X.; Bourdoulous, S. *Nature Rev. Microbiol.***2010**, *8*, 93-104. (b) Matter, K.; Balda, M.S. *Nature Rev. Mol. Cell. Biol.* **2003***, 4*, 225-236.

3 (a) Bazzoni, G. *Thromb Haemost*, **2006**, *95*, 36-42. (b) Bazzoni, G.; Dejana, E. *Physiol. Rev.* **2004**, *84*, 869-901.

4 (a) Gerber, H.-P.; Dixit, V.; Ferrara, N. *J. Biol. Chem.* **1998**, *273*, 13313-13316. (b) Weis, S. M.; Cheresh, D.A. *Nature*, **2005**, *437*, 497-504. (c) Sanz, J.; Fayad, Z.A. *Nature*, **2008**, *451*, 953-957.

5 (a) Tricot, O.; Mallat, Z.; Heymes, C.; Belmin, J.; Leseche, G.; Tedgui, A. *Circulation* **2000**, *101*, 2450–2453; (b) Segura, A.; Serrano, G.; Gonzalez, M.; Abad, C.; Claveria, L.; Gomez, A.; Bernad, C.; Martinez, H.; Riese, H. *FASEB J*. **2002**, *16*, 833–841; (c) Chavakis, E.; Dimmeler, S. Arterioscler. *Thromb. Vasc. Biol*. **2002**, *22*, 887–893; (d) Hunt, B. J. *Lupus* **2000**, *9*, 189–193; (e) Choy, J.; Granville, D.; Hunt, D. J. *Mol. Cell. Cardiol*. **2001**, *33*, 1673–1690; (f) Rossig, L.; Haendeler, J.; Mallat, Z. *J. Am. Coll. Cardiol*. **2000**, *36*, 2081–2089; (g) Walsh, K.; Smith, R. C.; Kim, H. S. *Circ. Res*. **2000**, *87*, 184–188; (h) Libby, P.; Ridker, P. M.; Maseri, A. *Circulation* **2002**, *105*, 1135–1143. References related to retina; (i) Wong, P. *Biochem. Cell Biol*. **1994**, *72*, 489-498. (j) Levin, L.A.; Louhab, A. *Arch. Ophthalmol*. **1996**, *114*, 488-491. (k) Barber, A. J.; Lieth, E.; Khin, S. A.; Antonetti, D. A.; Buchanan, A. G.; Gardner, T. W. *J. Clin. Invest.* **1998**, *102*, 783-791.

6 (a) Poon, I.K.; Hulett, M.D.; Parish, C.P. *Cell Death Differ.* **2010**, *17,* 381–397. (b) Nartin, S. J.; Green, D. R.; Cotter, T. G. *Trends Biochem. Sci*. **1994**, *19*, 26-30.

7 (a) Samejima, K.; Earnshaw, W. C. *Nat. Rev.* **2005**, *6*, 677-688. (b) Elmore, S. *Toxicol. Pathol.* **2007**, *35*, 495-516.

8 (a) Kam, P.C.; Ferch, N.I. *Anaesthesia* **2000**, *55*, 1081–1093. (b) Chamond, R.R.; Anon, J.C.; Guerra, Pasadas, C.M.A. *Alergol Immunol Clin,* **1999**, *14*, 367-374. (c) Yuan, J.; Yankner, B.A. *Nature* **2000**, *407*, 802-809. (d) Martin, L.J. *Int. J. Mol. Med.* **2001**, *7*, 455-478. (e) Martin, J.B. *N.*

Engl. J. Med. **1999**, *340*, 1970-1980. (f) Krysko, D. V.; D'Herde, K.; Vandenabeele, P. *Apoptosis* **2006**, *11*, 1709-1726. (g) Tricot, O.; Mallat, Z.; Heymes, C.; Belmin, J.; Leseche, G.; Tedgui, A. *Circulation* **2000**, *101*, 2450-2453. (h) Segura, A.; Serrano, G.; Gonzalez, M.; Abad, C.; Claveria, L.; Gomez, A.; Bernad, C.; Martinez, H.; Riese, H. *FASEB J*. **2002**, *16*, 833-841.

9 (a) Stefanec, T. *Chest* **2000**, *117*, 841-854. (b) O'Connell, B.J.; Genest Jr. J. *Circulation*, **2001**, *104*, 1978-1983.

10 (a) Dimmeler, S.; Zeiher, A.M. *Circ. Res.* **2000**, *87*, 434-439. (b) Eissner, G.; Kohlhuber, F.; Grell, M.; Ueffing, M.; Scheurich, P. ; Hieke, A.; Multhoff, G.; Bornkamm, G.W.; Holler, E. *Blood* **1995**, *86*, 4184-4193. (c) Chavakis, E.; Dimmeler, S. *Arterioscler. Thromb. Vasc. Biol*. **2002**, *22*, 887-893. (d) Hunt, B. J. *Lupus* **2000**, *9*, 189-193. (e) Choy, J.; Granville, D.; Hunt, D. J. *Mol. Cell. Cardiol*. **2001**, *33*, 1673-1690. (f) Rossig, L.; Haendeler, J.; Mallat, Z. *J. Am. Coll. Cardiol*. **2000**, *36*, 2081-2089. (g) Walsh, K.; Smith, R. C.; Kim, H. S. Circ. Res. 2000, 87, 184–188; (h) Libby, P.; Ridker, P. M.; Maseri, A. *Circulation* **2002**, *105*, 1135-1143.

11 (a) Alvarez, R. J.; Gips, S. J.; Moldovan, N.; Wilhide, C. C.; Milliken, E. E.; Hoang, A. T.; Hruban, R. H.; Silverman, H. S.; Dang, C. V.; Goldschmidt-Clermont, P. J. *Biochem. Biophys. Res. Commun.* **1997**, *237*, 372-381. (b) Chen, D.-F.; Zhang, H.-L.; Du, S.-H.; Li, H.; Zhou, J.-H.; Li, Y.-W.; Zeng, H.-P.; Hua, Z.-C.; *Steroids* **2010**, *75*, 1119-1126.

12 (a) Liu, X.; Zhao, J.; Xu, J.; Zhao, B.; Zhang, Y.; Zhang, S.; Miao, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2896-2900. (b) Tan, C.-B.; Gao, M.; Xu, W.R.; Yang, X.-Y.; Zhu, X.-M.; Du, G.-H. *Biol. Pharm. Bull.* **2009**, *32*, 1359-1363. (c) Rodrigues, C.; Steer, C.J. *Expert Opin. Investig. Drugs* **2001**, *10*, 1243-1253. (d) Stirling, D. P.; Koochesfahani, K. M.; Steeves, J. D.; Tetzlaff, W. *Neuroscientist* **2005**, *11*, 308-323.

13 (a) Lü, J.-M.; Yao, Q.; Chen, C. *Curr Vasc Pharmacol*. **2009**, *7*, 293-302. (b) Helms, S. *Altern. Med. Rev.* **2004**, *9*, 259-274. (c) Bames, A.S.; Powell-Griner, E.; McFann, K.; Nahin, R.L. *Adv.*

Data. **2004**, *343*, 1-19. (d) Borchers, A.T.; Keen, C.L.; Stern, J.S.; Gershwin, M.E. *Am. J. Clin. Nutr.* **2000**, *72*, 339-347.

14 (a) Ekshyyan, O.; Aw, T. Y. Curr. *Neurovasc. Res*. **2004**, *1*, 355–371; (b) Mattson, M. *Nat. Rev. Mol. Cell Biol.* **2000**, *2*, 120-129.

15 (a) Cho, J.; Park, W.; Lee, S.; Ahn, W.; Lee, Y. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 3510-3515. (b) Al-Dujaili, E.; Chalmers, R.; Sharp, M. *Endocrine Abstracts* **2007**, *13*, 285. (c) Shang, W.; Yang, Y.; Zhou, L.; Jiang, B.; Jin, H.; Chen, M. *J. Endocrinol.* **2008**, *198*, 561-569.

16 (a) Owen, R. *Drugs Today* **1981**, *17*, 343-351. (b) Rivera, E.; Hu, S.; Concha, C. *Vaccine* **2003**, *21*, 1149-1157. (c) Hu, S.; Concha, C.; Lin, F.; Persson, W. K. *Immunol. Immunopathol.* **2003**, *91*, 29-37.

17 (a) Yue, P.; Wong, D.; Wu, P.; Leung, P.; Mak, N.; Yeung, H.; Liu, L.; Cai, Z.; Jiang, Z.-H.; Fan, T.; Wong, R. *Biochem. Pharmacol*. **2006**, *72*, 437-445. (b) Cheng, L.-Q.; Na, J.; Bang, M.-H.; Kim, M.-K.; Yang, D.-C. *Phytochemistry* **2008**, *69*, 218-224. (c) Tian, J.; Fu, F.; Geng, M.; Jiang, Y.; Yang, J.; Jiang, W.; Wang, C.; Liu, K. *Neurosci. Lett*. **2005**, *374*, 92-97.

18 (a) Hasegawa, H. *J Pharmacol Sci*. **2004**, *95*, 153-157. (b) Liu, C. X.; Xiao, P. G. *J. Ethnopharmacol*. **1992**, *36*, 27-38.

19 Yun, T.K. *Lancet Oncol.* **2001**, *2*, 49-55.

20 (a) Morisaki, N.; Watanabe, S.; Tezuka, M.; Zenibayashi, M.; Shiina, R.; Koyama, N.; Kanzaki, T.; Saito, Y.; *Br. J. Pharmacol.* **1995**, *115*, 188-1193. (b) Sato, K.; Mochizuki, M.; Saiki, I.; Yoo, Y.C.; Samukawa, K.; Azuma, I. *Biol. Pharm. Bull.* **1994**, *17*, 635-639.

21 (a) Sengupta, S.; Toh, S.A.; Sellers, L.A.; Skepper, J.N.; Koolwijk, P.; Leung, H.W.; Yeung, H.W.; Wong, R.N.; Sasisekharan, R.; Fan, T.P. *Circulation* **2004**, *110*, 1219-1225. (b) Liu, X.; Kim, C.N.; Yang, J.; Jemmerson, R.; Wang, X. *Cell* **1996**, *86*, 147-157. (c) Leung, K. W.; Pon, Y. L.; Wong, R. N. S.; Wong, A. S. T. *J. Biol. Chem.* **2006**, *281*, 36280-36288.

22 a) Yue, P.; Wong, D.; Wu, P.; Leung, P.; Mak, N.; Yeung, H.; Liu, L.; Cai, Z.; Jiang, Z.-H.; Fan, T.; Wong, R. *Biochem. Pharmacol*. **2006**, *72*, 437-445. (b) Cheng, L.-Q.; Na, J.; Bang, M.-H.; Kim, M.-K.; Yang, D.-C. *Phytochemistry* **2008**, *69*, 218-224. (c) Tian, J.; Fu, F.; Geng, M.; Jiang, Y.; Yang, J.; Jiang, W.; Wang, C.; Liu, K. *Neurosci. Lett.* **2005**, *374*, 92-97.

23 Min, J.-K.; Kim, J.-H.; Cho, Y.-L.; Maeng, Y.-S.; Lee, S.-J.; Pyun, B.-J.; Kim, Y.-M.; Park, J. H.; Kwon, Y.-G. *Biochem. Bioph. Res. Co.* **2006**, *349*, 987-994.

24 Park, I. H.; Kim, N. Y.; Han, S. B.; Kim, J. M.; Kwon, S. W.; Kim, H. J.; Park, M. K.; Park, J. H. *Arch. Pharm. Res.* **2002**, *25*, 428-432.

25 (a) Shibata, S.; Ando, T.; Tanaka, O. *Chem. Pharm. Bull*. **1966**, *14*, 1157-1161; (b) Han, B. H.; Park, M. H.; Han, Y. N.; Woo, L. K.; Sankawa, U.; Yahara, S.; Tanaka,O. *Planta Med*. **1982**, *44*, 146-149; (c) Yuan, C.-S.; Wu, J. *Am. J. Clin. Nutr*. **2002**, *75*, 600-601.

26 (a) Kim, J.-H.; Hong, Y.-H.; Lee, J.-H.; Kim, D.-H.; Nam, G.; Jeong, S. M.; Lee, B.-H.; Lee, S.-M.; Nah, S.-Y. *Mol. Cells* **2005**, *19*, 137-142. (b) Lei, J.; Li, X.; Gong, X.-J.; Zheng, Y.-N. *Molecules* **2007**, *12*, 2140-2150. (c) Danieli, B.; Falcone, L.;Monti, D.; Riva, S.; Gebhardt, S.; Schubert-Zsilavecz, M. *J. Org. Chem.* **2001**, 66, 262-269.

27 *Chemical and Technical Assessment* **2008**, 1-13.

28 β-Sitosterol contained campersterol and sitostanol; the purity of β-sitosterol is up to 70%. The reagent was purchased from Sigma-Aldrich Co.

29 Jursic, B. S.; Upadhyay, S. K.; Creech, C. C.; Neumann, D. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7372-7375.

30 Hogg, N.; Browning, J.; Howard T.; Winterford C.; Fitzpatrick D.; Gobé G. *Endothelium* **1999**, *7*, 35-49.

31 Oishi, K.; Kobayashi, A.; Fujii, K.; Kanehira, D.; Ito, Y.; Uchida, M. K. *J. Pharm. Sci.* **2004**, *96*, 208.

32 (Lazebnik et al., *J. Cell Biol*. **1993**, *123*, 7-22

33 (a) Mesner, P. W.; Bible, K. C.; Martins, L. M.; Kottke, T. J.; Srinivasula, S. M.; Svingen, P. A.; Chilcote, T. J.; Basi, G. S.; Tung, J. S.; Krajewski, S.; Reed, J. C.; Alnemri, E. S.; Earnshaw, W. C.; Kaufmann, S. H. *J. Biol. Chem.* **1999**, *274*, 22635-22645. (b) Faleiro, L.; Kobayashi, R.; Fearnhead, H.; Lazebnik, Y. *EMBO* **1997**, *16*, 2271-2281. (c) Takahashi, A.; Hirata, H.; Yonehara, S.; Imai, Y.; Lee, K.-K.; Moyer, R. W.; Turner, P. C.; Mesner, P. W.; Okazaki, T.; Sawai, H.; Kishi, S.; Yamamoto, K.; Okuma, M.; Sasada, M. *Oncogene* **1997**, *14*, 2741-2752

34 Kapuscinski, J.; Skoczylas, B. *Anal. Biochem.* **1977**, *83*, 252-257.

35 Brunk, C.F.; Jones, K. C.; James, T. W. *Anal. Biochem.* **1979**, *92*, 497-500.

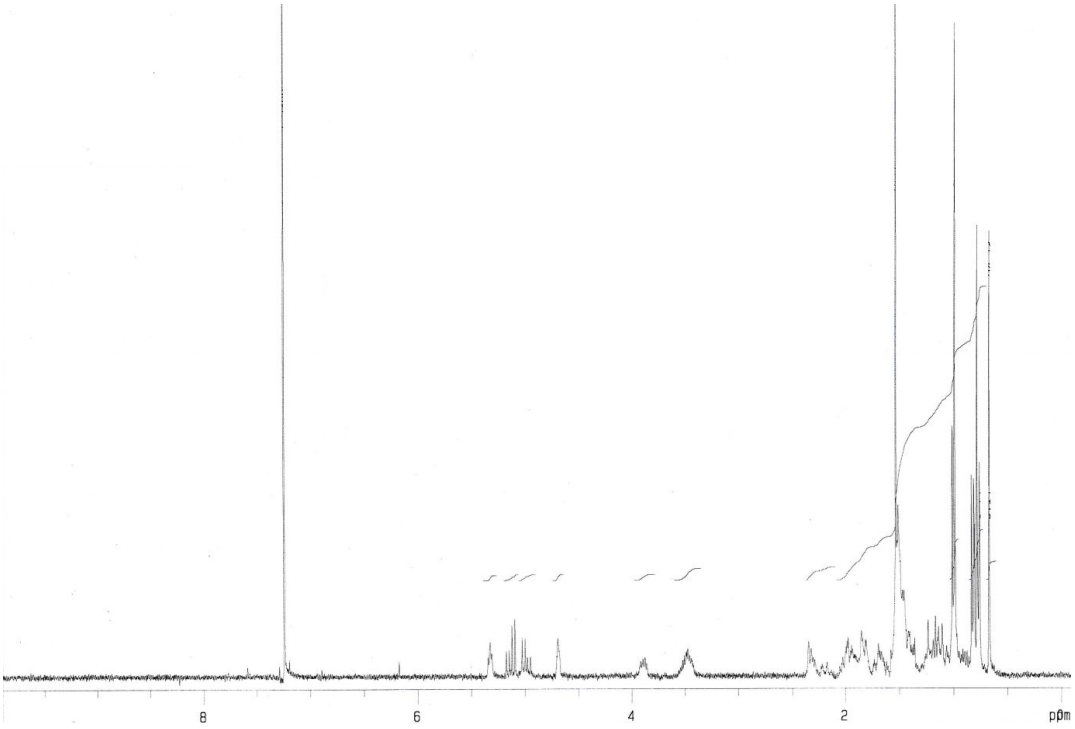
36 Carrillo-Vico, A.; Lardone, P. J.; Naji, L.; Ferna´ndez-Santos, J. M.; Martı´n-Lacave, I.; Guerrero, J. M.; Calvo, J. R. *J. Pineal Res*. **2005**, *39*, 400-408.

37 Parts of these synthetic products and biological assay results have been reported by us. Lee, S.; Maharjan, S.; Kim, K.; Kim, N.-J.; Choi, H.-J.; Kwon, Y. G.; Suh, Y.-G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7102-7105.

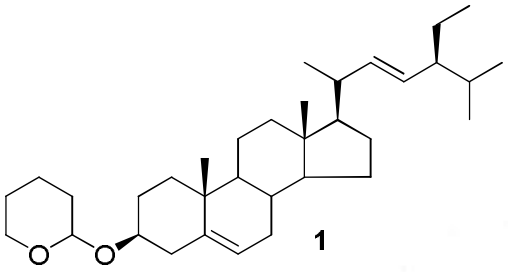
38 Maharjan, S.; Lee, S.; Agrawal, V.; Choi, H.-J.; Maeng, Y.-S.; Kim, K.; Kim, N.-J.; Suh, Y.-G.; Kwon, Y.-G. *Eur. J. Pharmacol.* **2011**, *657*, 35-40.

VI. Appendix

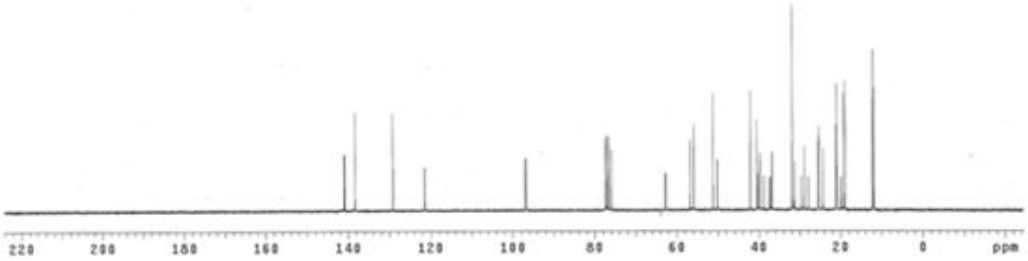
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



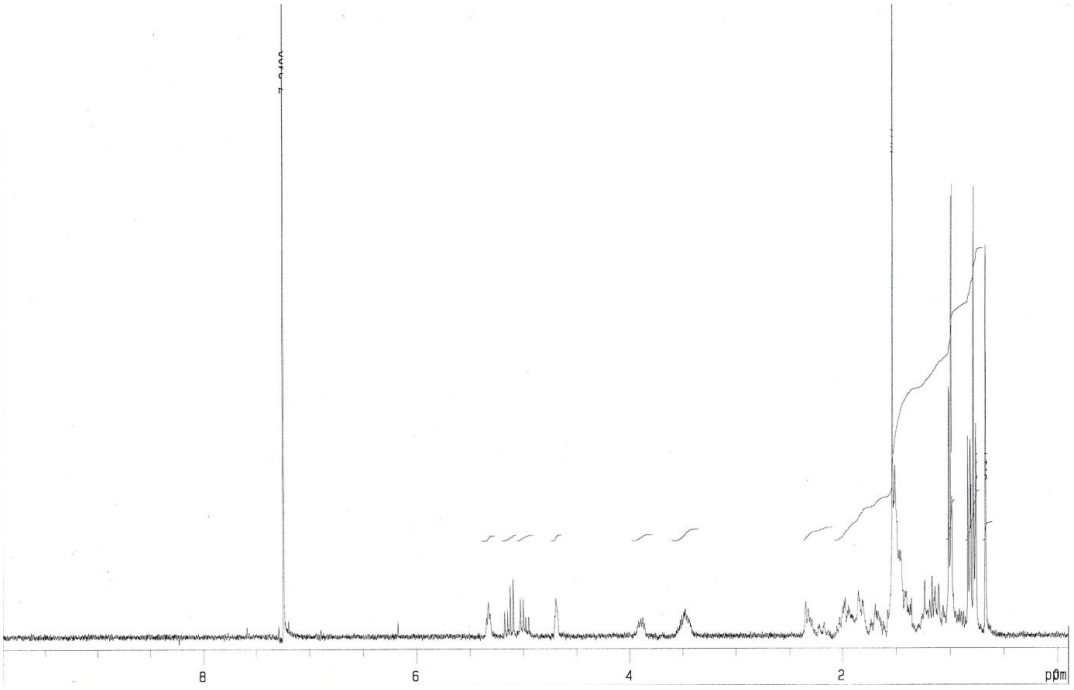
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



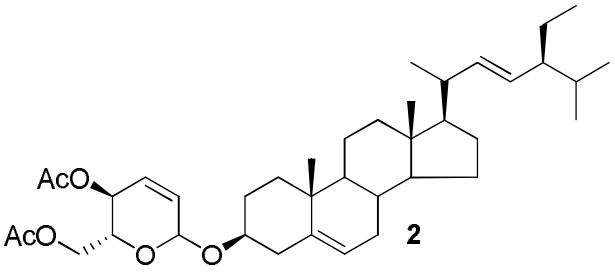
1



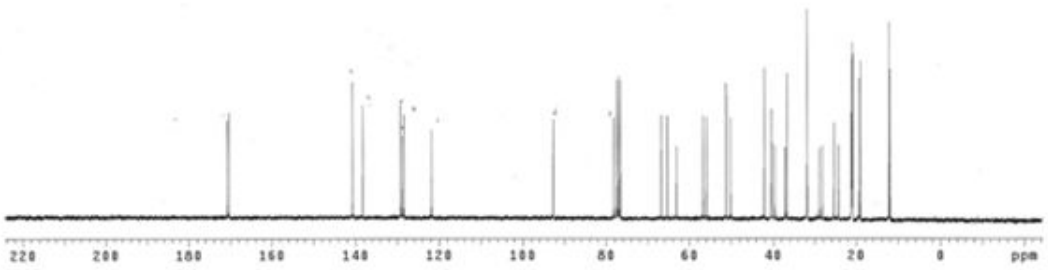
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



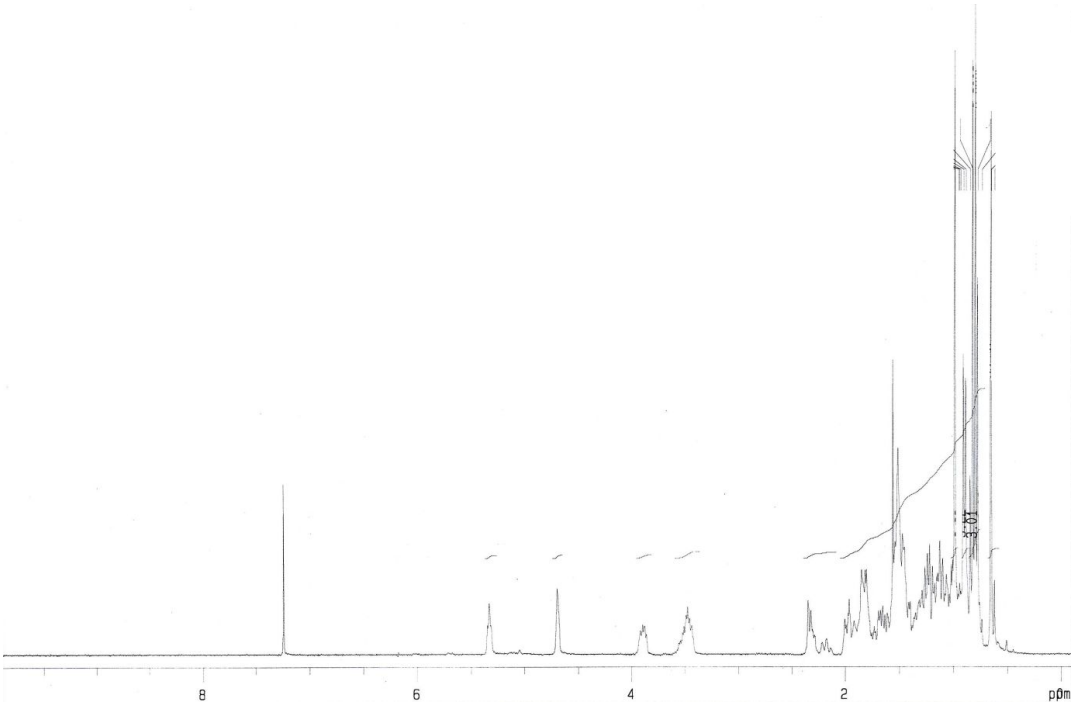
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



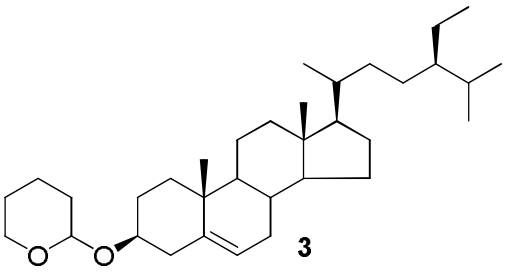
2



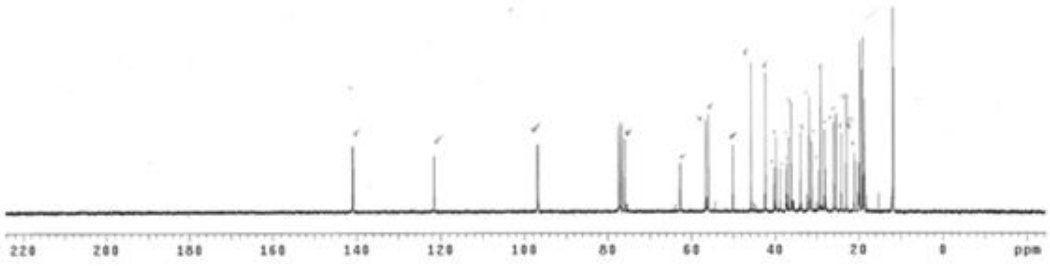
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



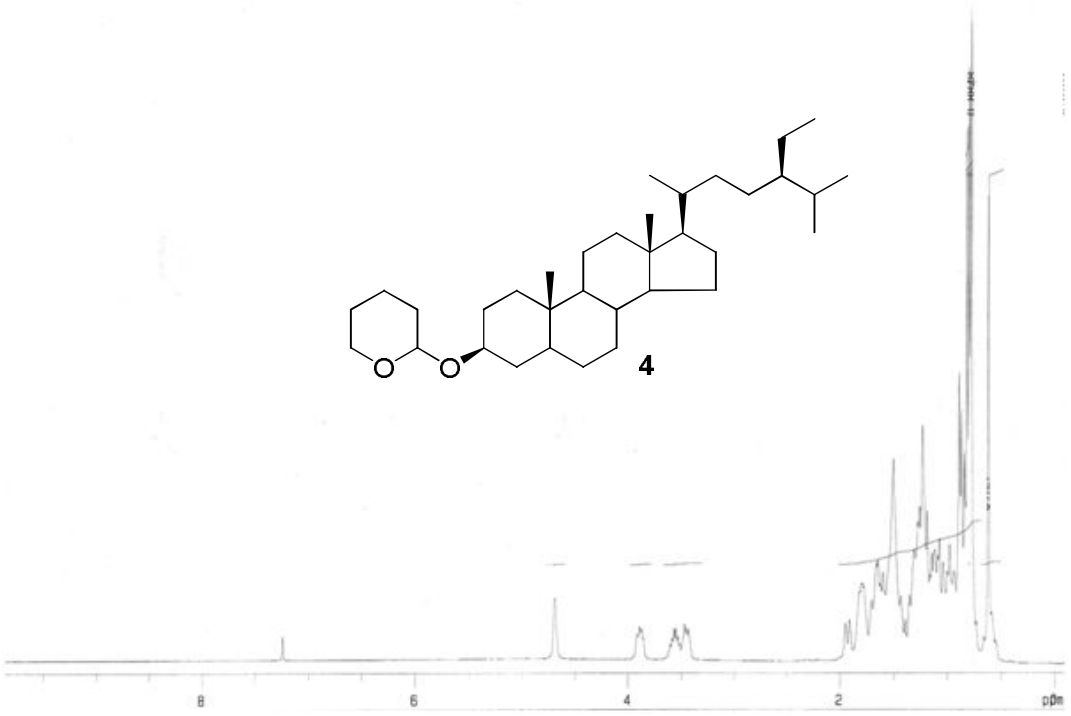
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



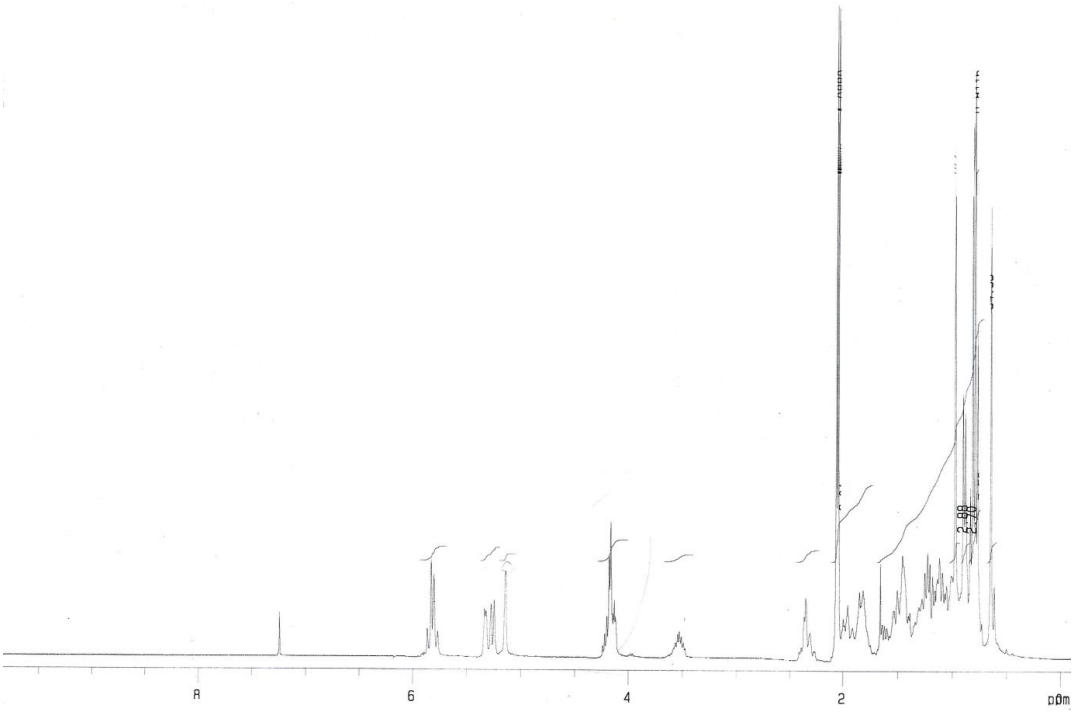
3



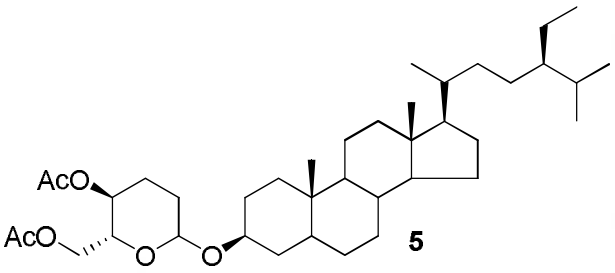
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



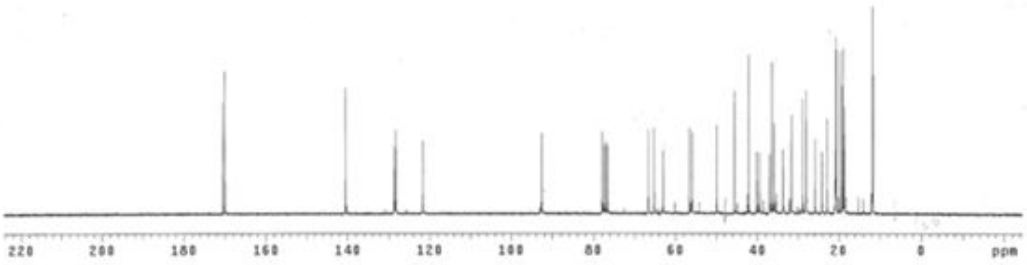
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



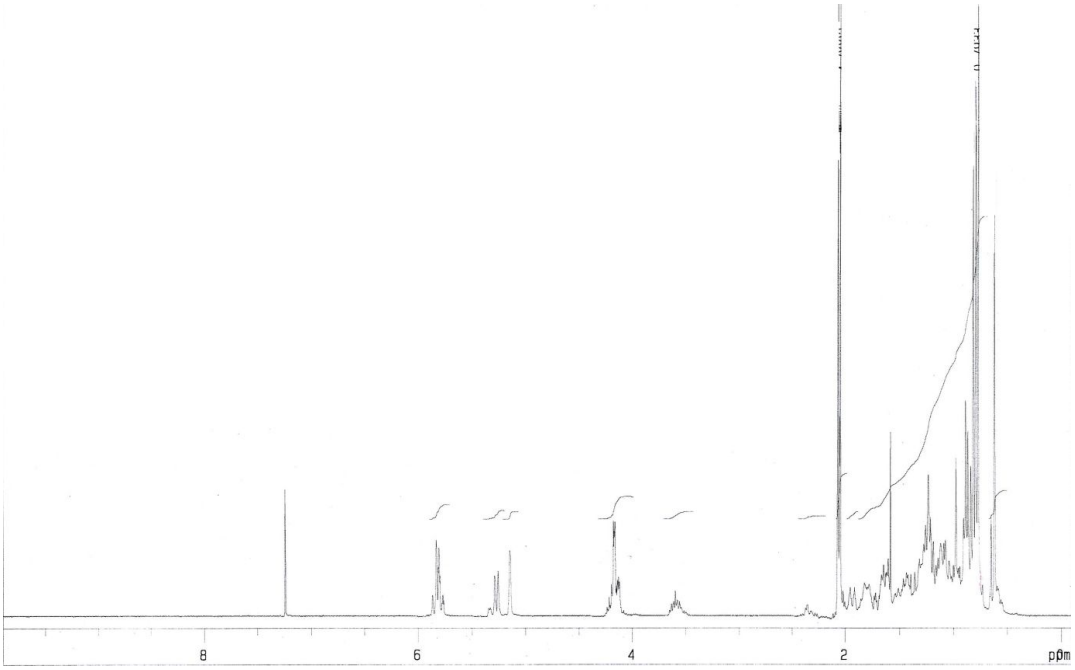
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



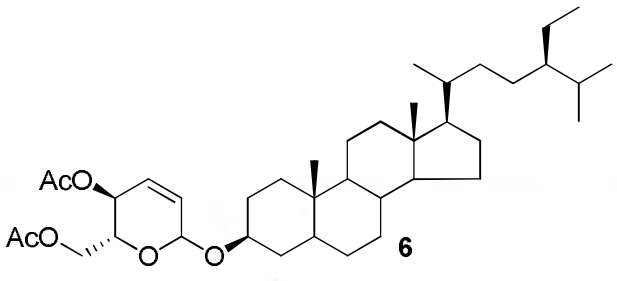
5



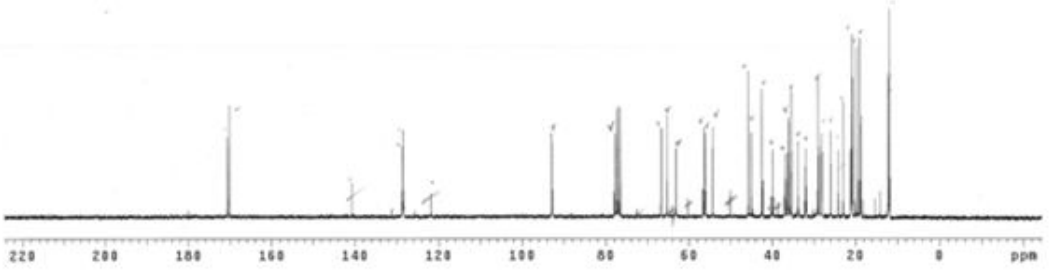
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



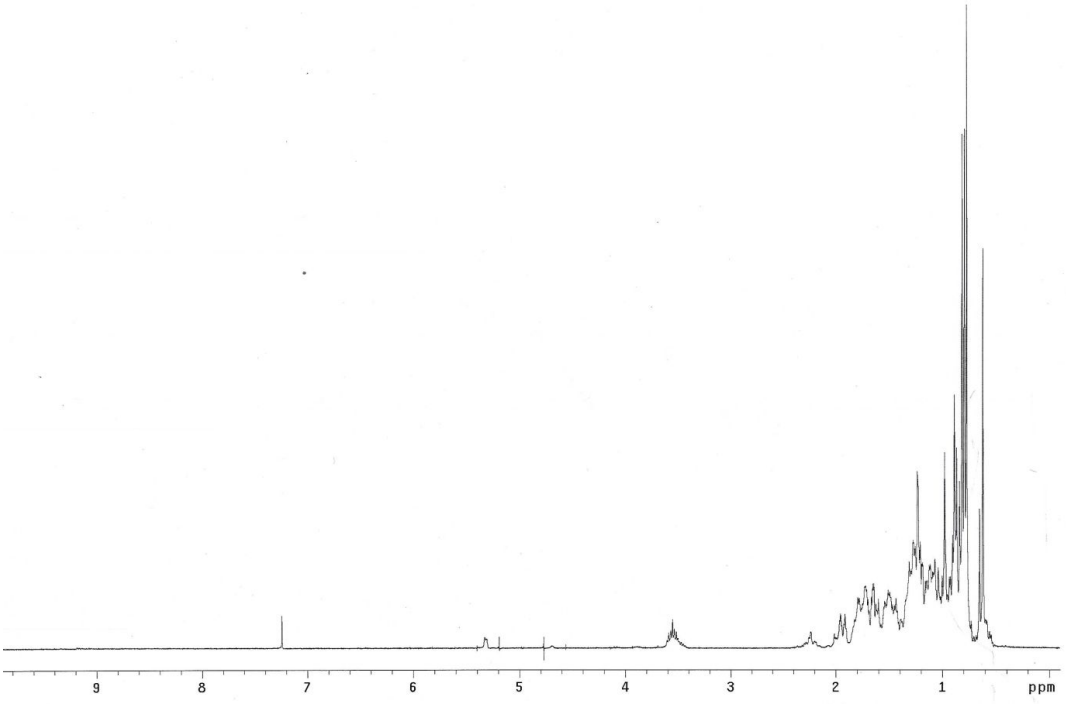
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



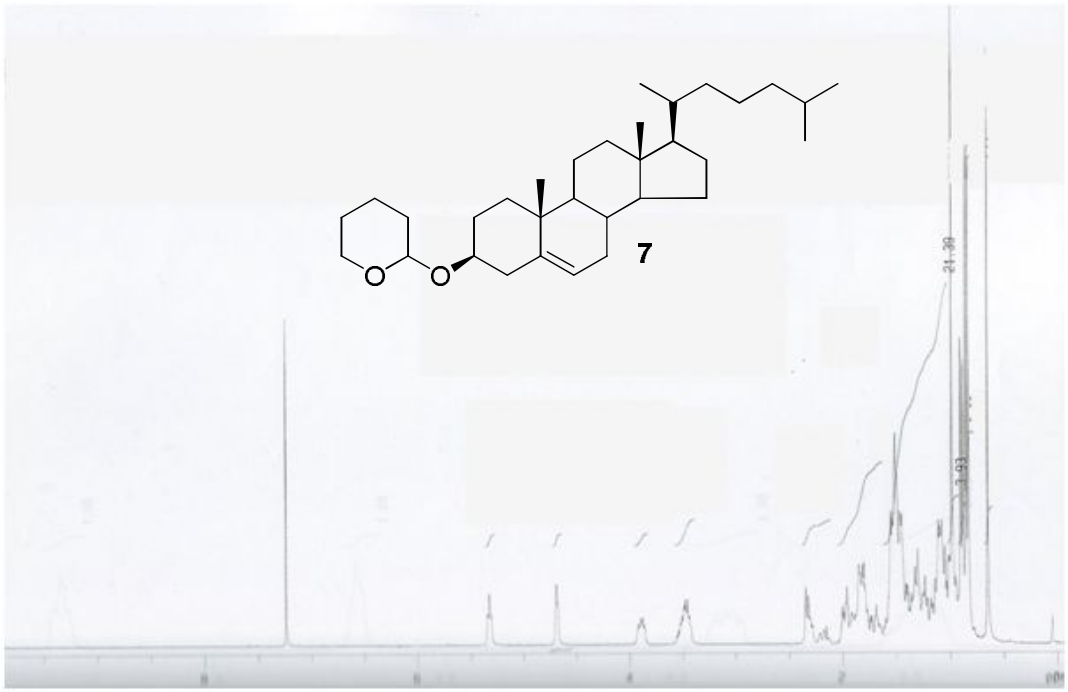
6



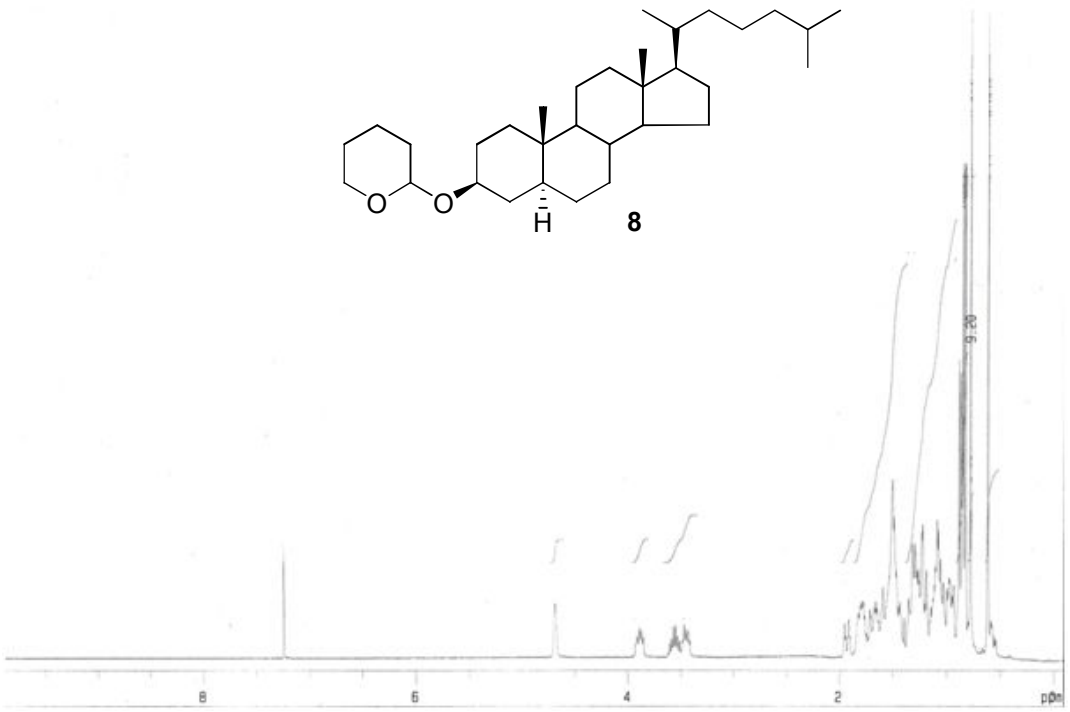
▼ **Sitostanol, ^{1}H-NMR (300 MHz, $CDCl_3$)**



▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**

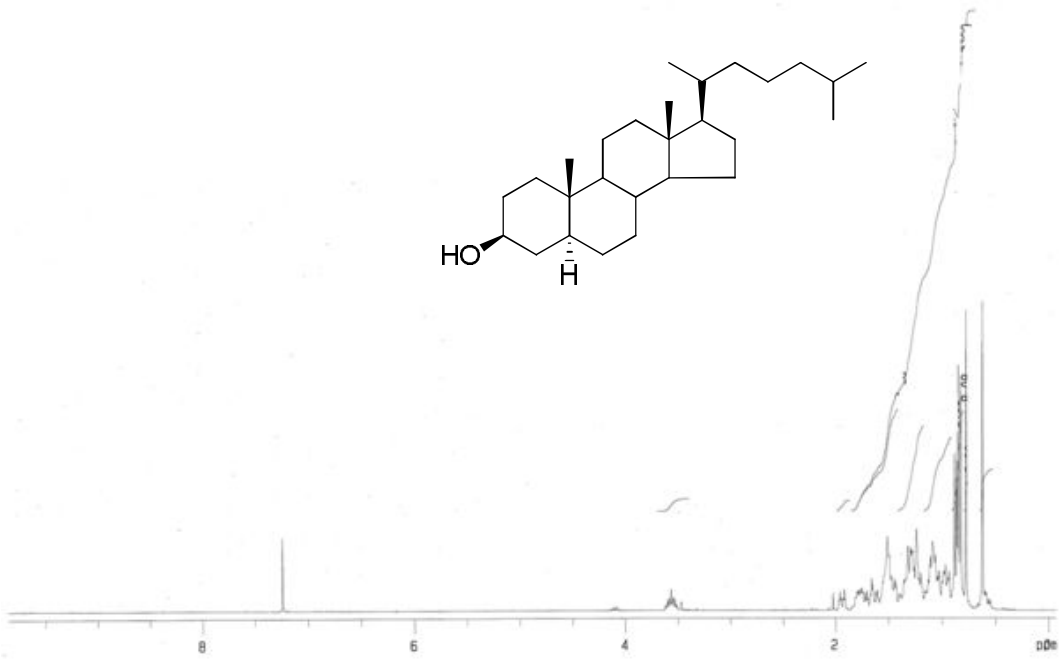


8

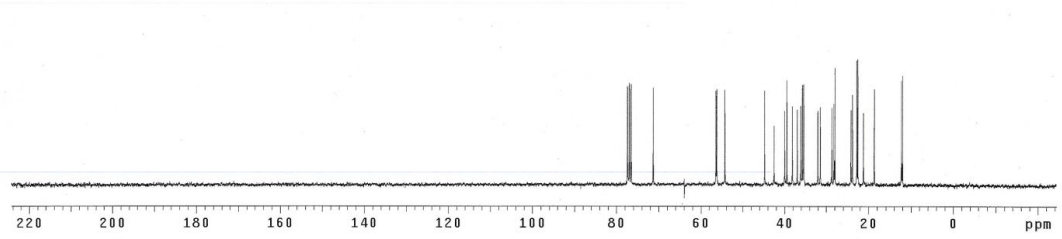
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**

9

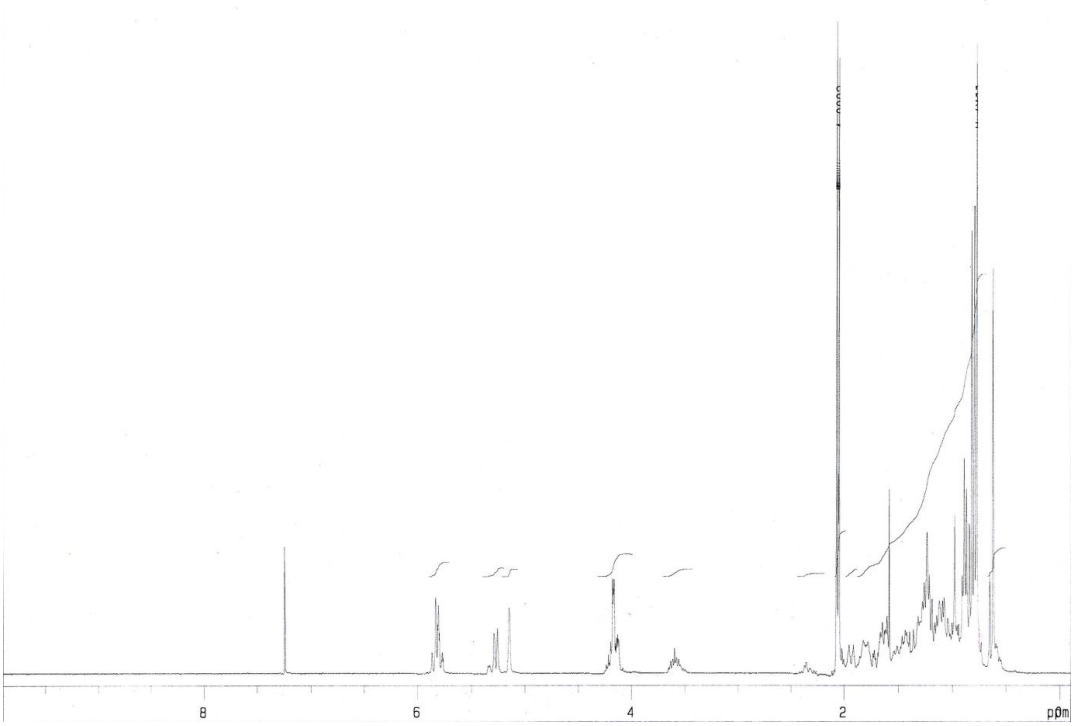
▼ **Cholestanol, ^{1}H-NMR (300 MHz, $CDCl_3$)**



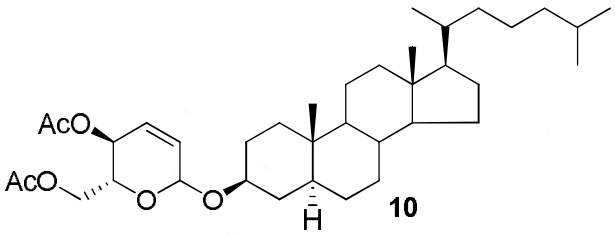
▼ **Cholestanol, ^{13}C-NMR (75 MHz, $CDCl_3$)**



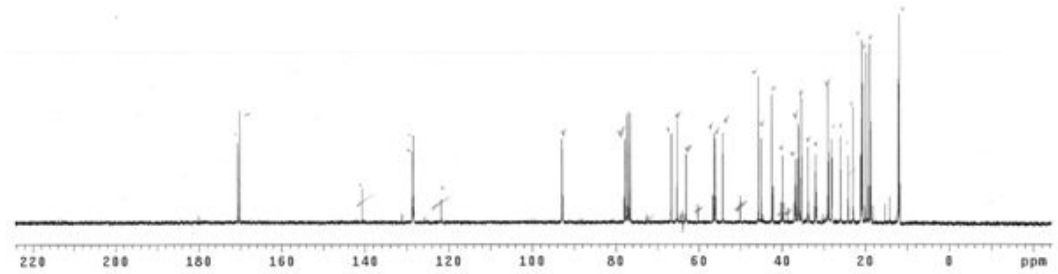
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



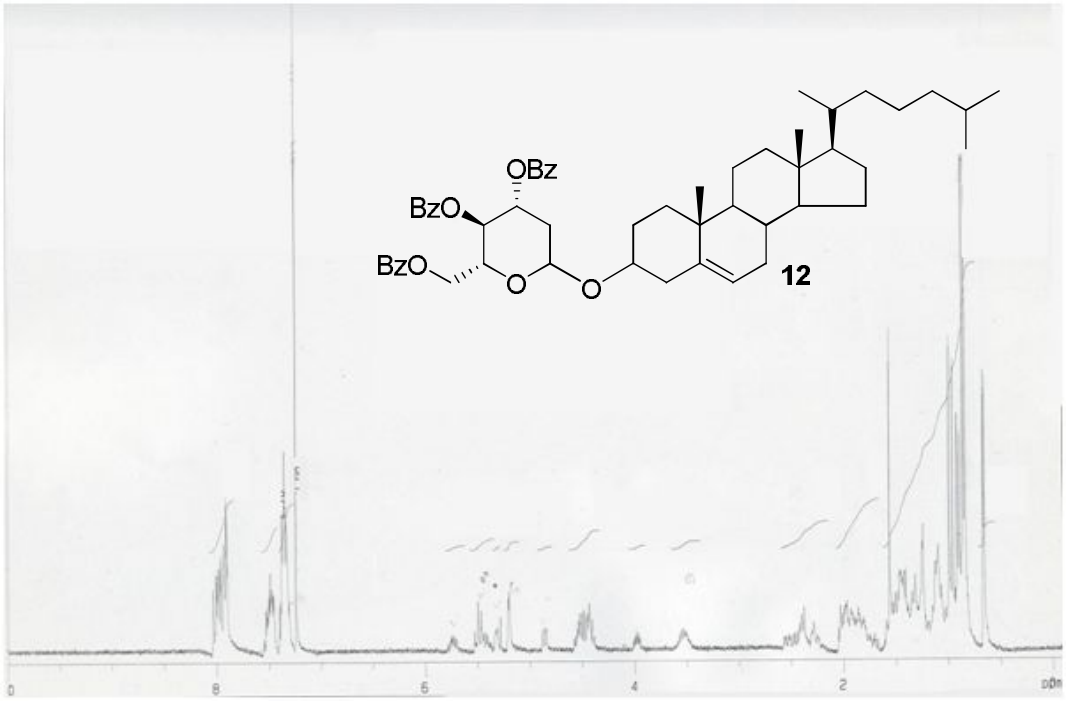
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



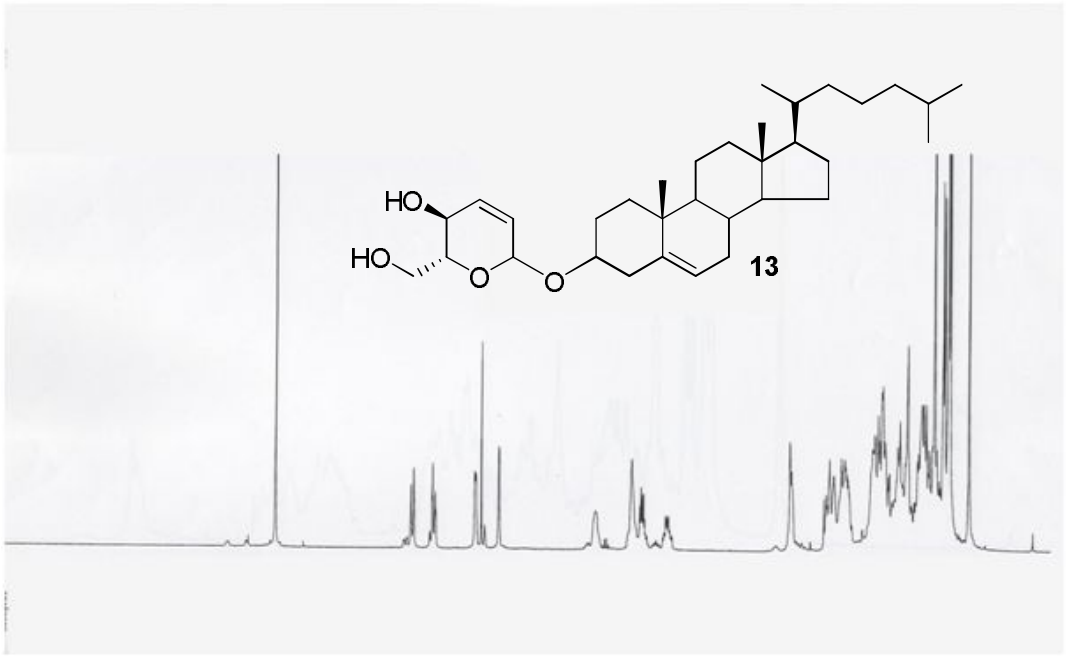
10



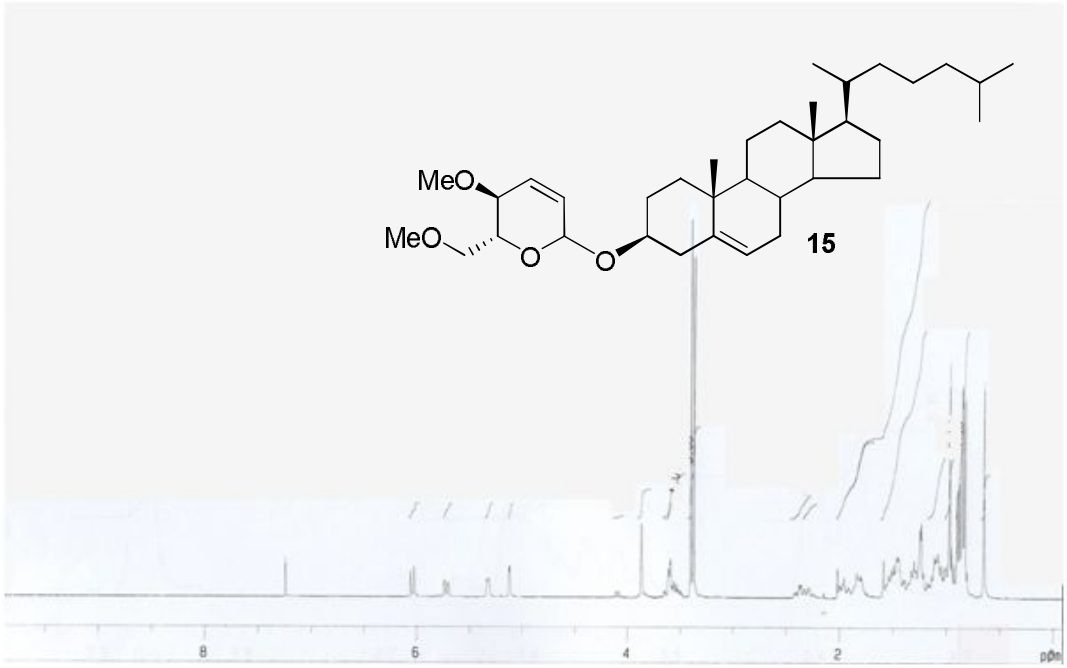
▼ ^{13}C-NMR (75 MHz, $CDCl_3$)



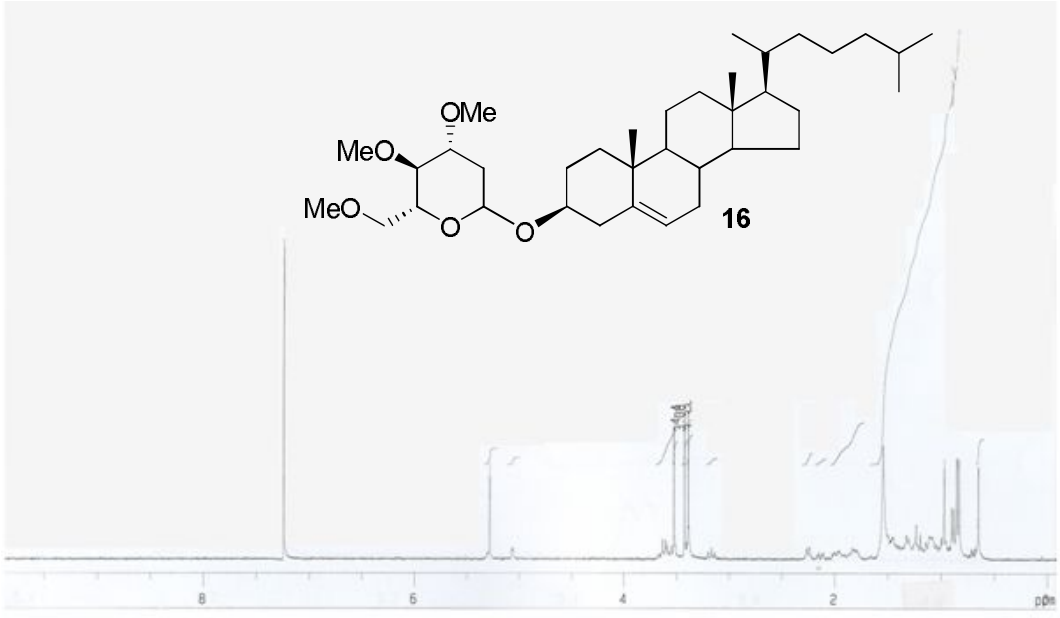
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



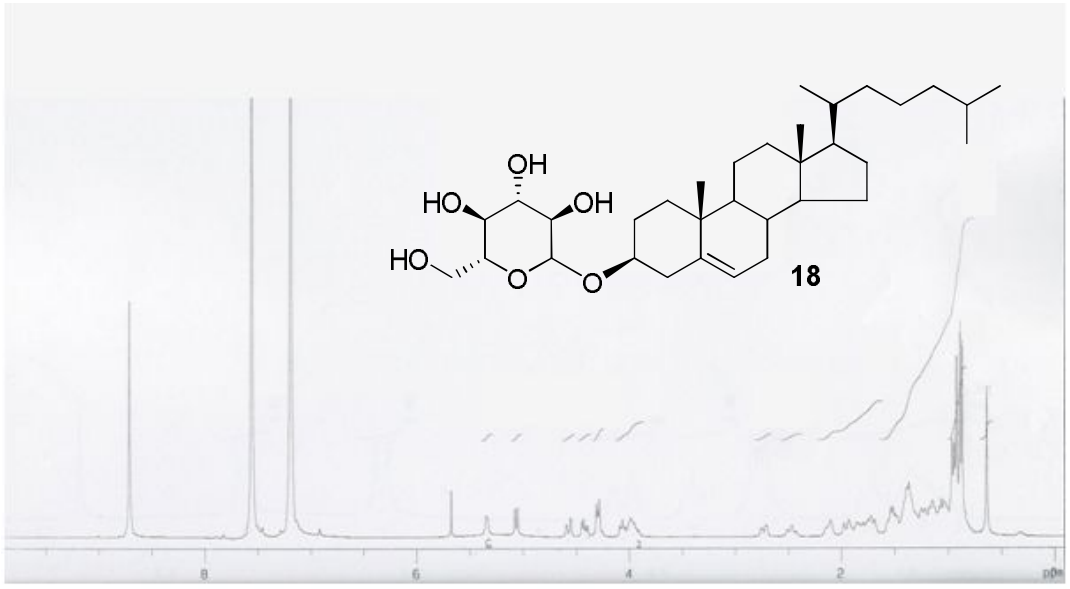
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



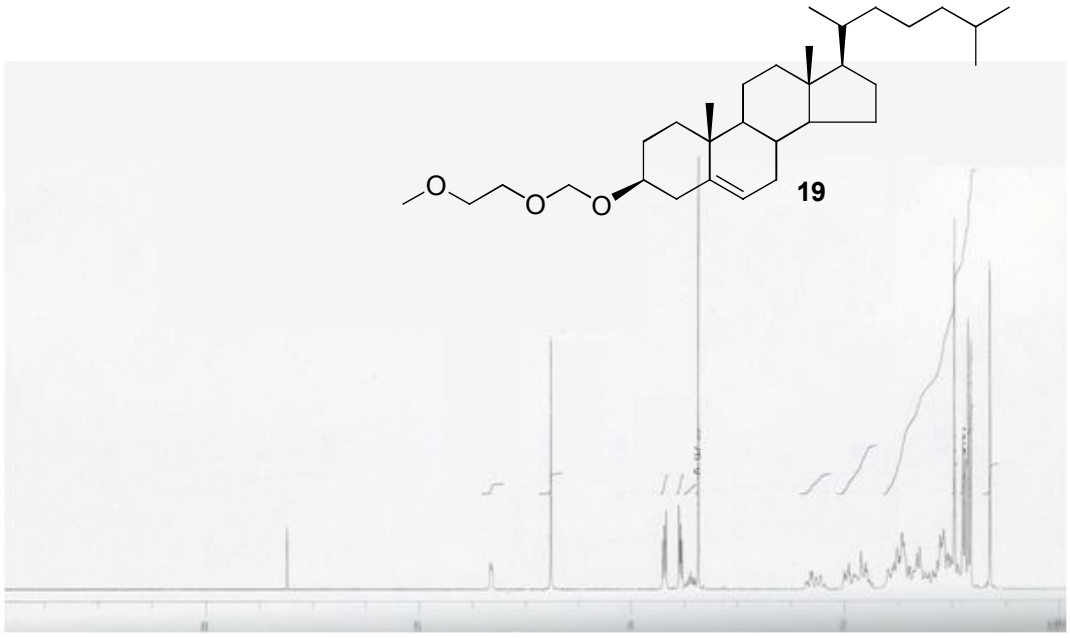
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



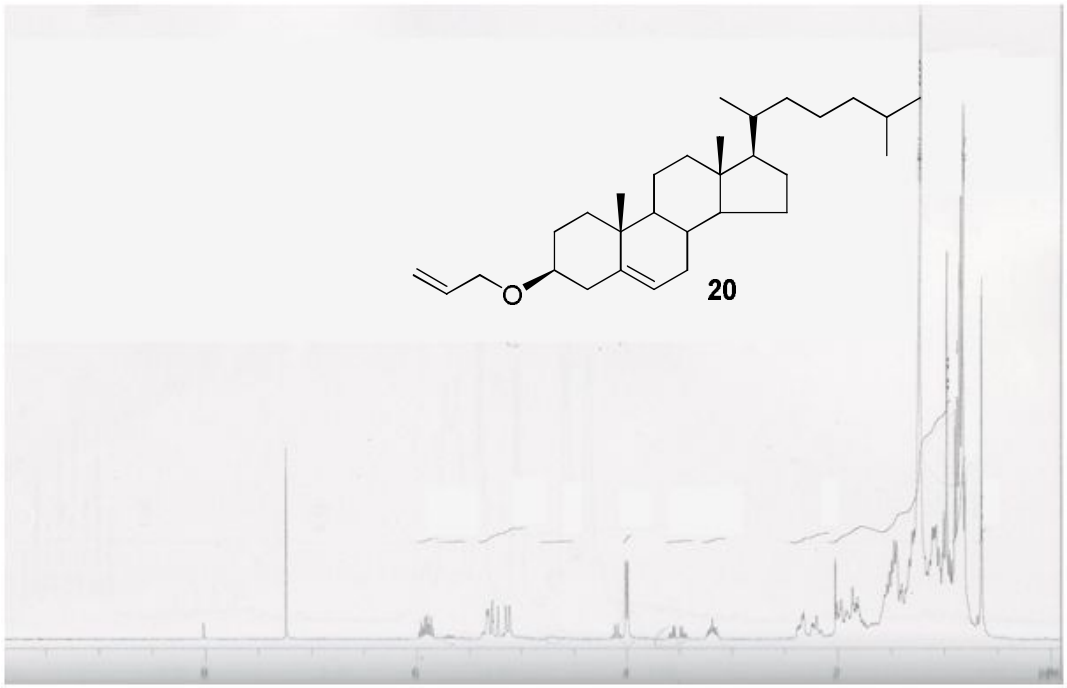
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



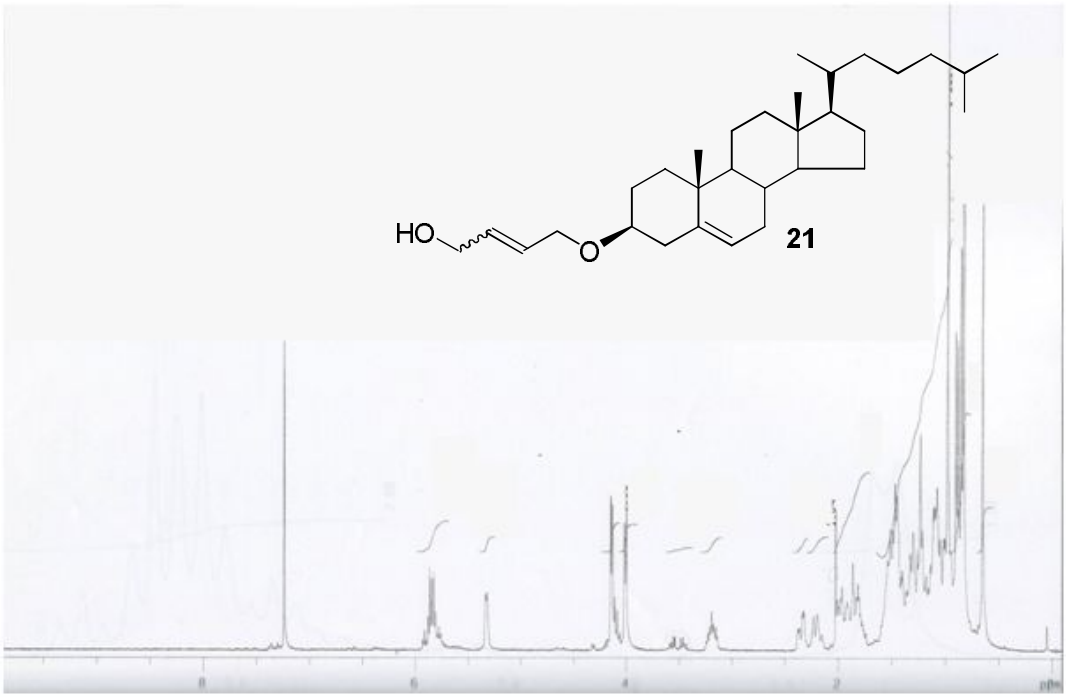
▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



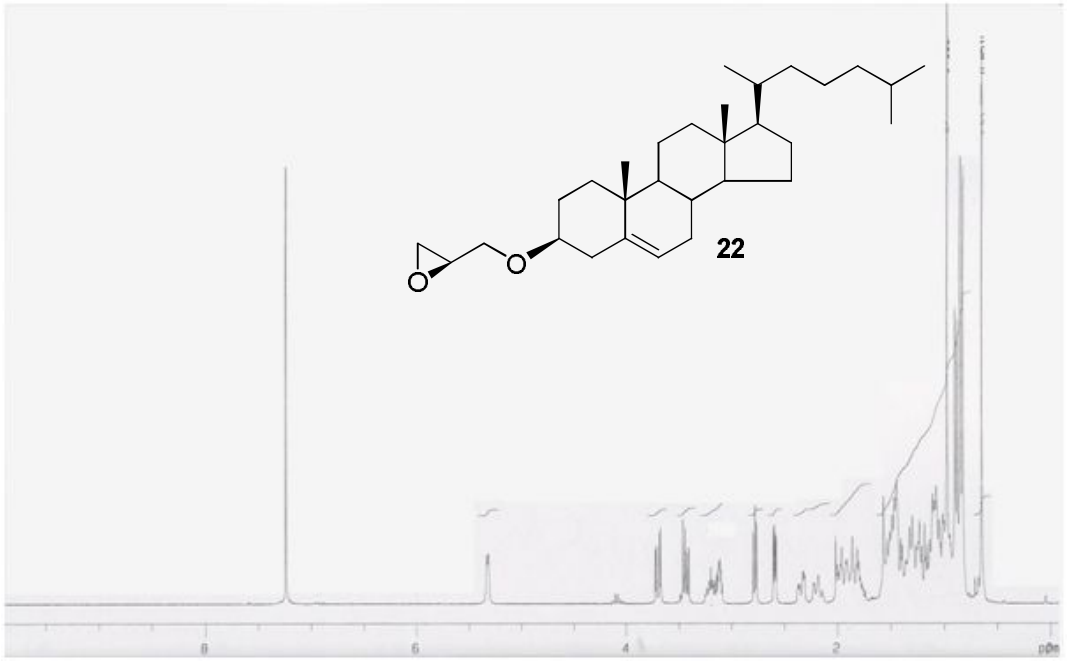
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



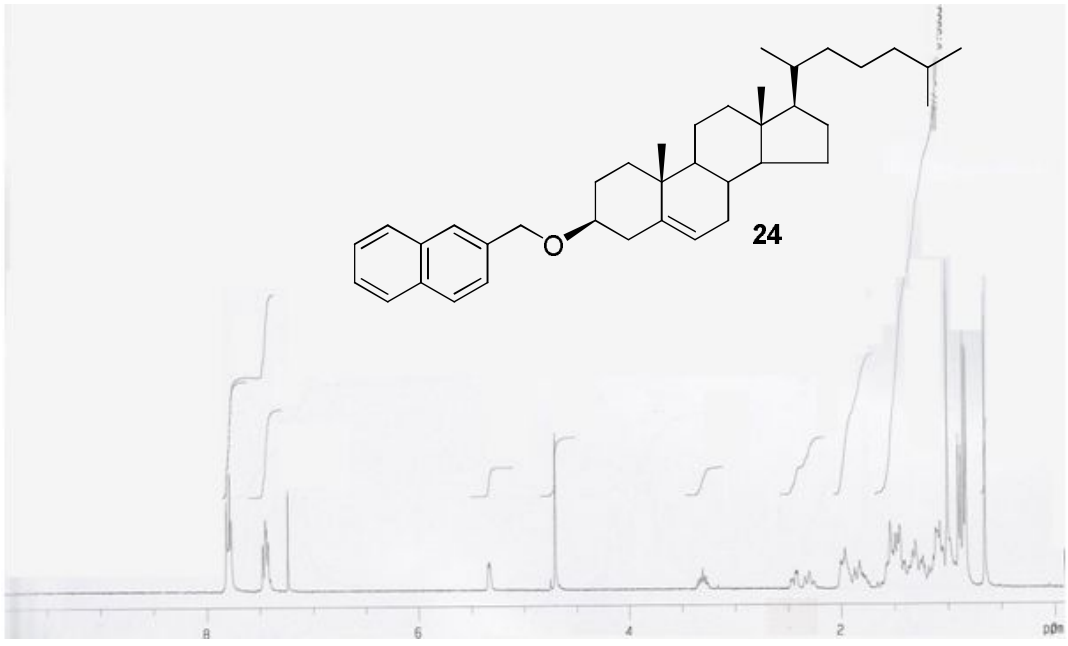
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



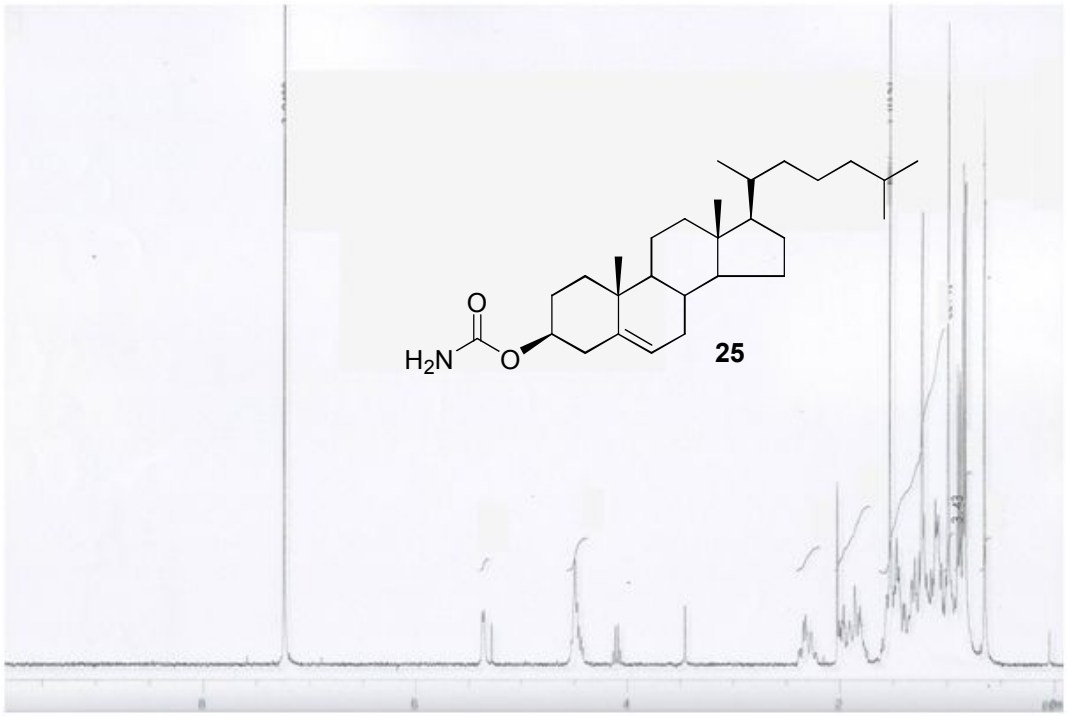
▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



▼ ^{1}H-NMR (300 MHz, $CDCl_3$)



▼ **^{1}H-NMR (300 MHz, $CDCl_3$)**



VII. Abstract

Cholesterol derived novel anti-apoptotic agent on the structural basis of ginsenoside Rk1

Sujin Lee
College of pharmacy
Department of manufacturing pharmacy
The Graduate School
Seoul National University

The endothelial cell monolayer constructs the lining of the blood vessel. In this particular position, different stimuli activate a cascade of proteases (caspases) that execute a program of cellular self-destruction by cleaving cellular structures at specific sites in a strictly controlled manner. This leads to the disassembly of the cell into small apoptotic bodies without spillage of cellular contents or significant inflammatory responses.

Recently, some ginsenosides isolated from *P. ginseng* have represented the anti-apoptotic activities on HUVECs. However, ginsenosides are generally labile under acidic conditions. And the compositions of ginsenosides in ginseng are variable depending on the growth environment, growth period, and process of extraction. In this connection, we planned to synthesize the anti-apoptotic agents from the simple and easily accessible phytosterol derivatives.

We discovered that the glucosyl moiety at C3 hydroxyl group is essential for anti-apoptotic activity through the comparison of some ginsenosides. We first searched the proper phytosterol for the replacement of the ginsenoside triterpene backbone. In particular, cholesterol, which has the linear side chain at C24, was the best choice. Then, the diverse bioisoster of glucosyl moiety at C3 hydroxyl group was substituted. Cyclic ether, acyclic ether, and spatial interactive bulky substituents were adapted to the C3 hydroxyl group.

With those analogs in hand, we tested the MTT assay on HUVECs. Throughout, the analogs **7** and **9** possessing the cholesterol scaffold and the cyclic carbohydrate equivalents exhibited

excellent cell survival activities, which are equipotent to that of ginsenoside Rk1. In addition, these analogs exhibited dose-dependent cell viability on the HREC line. The excellent human EC protective activities of our novel Rk1-based anti-apoptotic agents allow us to envisage their valuable application for the treatment of vascular disorders. Currently, systematic studies on their therapeutic applications, including in vivo testing of the potent sterol analogs as well as their mechanistic aspects, are in good progress.

Key words: human endothelial cell, apoptosis, cholesterol derivatives, ginsenoside Rg3, Rk1, phytosterol.

Student number: 2006-30449