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A Thesis for the Degree of Doctor of Philosophy in Pharmacology

Studies on the Novel Bioactive Terpenoids and Saponins from a Tropical Marine Sponge, *Lipastrotethya* sp.

August 2015

by
Jung-Ho Lee

Natural Products Science Major, College of Pharmacy
Doctoral Course in the Graduate School
Seoul National University

Abstract

Studies on the Novel Bioactive Terpenoids and Saponins from a Tropical Marine Sponge, *Lipastrotethya* sp.

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College of Pharmacy
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Seoul National University

Researches on marine natural products have been grown rapidly from the late 1960s upward with developments of methods of isolation, analysis, and structure determination. After marine natural products are proven as potent sources therapeutic agents that may be directly used as drugs or served as lead structures for drug development due to their high novelty of bioactivities and chemical structures, there were enormous efforts to investigate new drugs and achievements

Since the 1960s, more than 20,000 compounds were discovered

from marine organisms and over 1000 compounds are reported annually after 2008. Majority of novel compounds, approximately 75%, were isolated from marine invertebrates belonging to the phyla Porifera and Coelenterate. Due to its wide distribution, the diversity in species, convenience of collection, and a wide range of chemical class for metabolites, sponges takes important place in marine natural products and are very highly regarded as subject of study.

This study is searching novel bioactive marine natural products from a torpical marine sponge, *Lipastrotethya* sp. which was collected in Micronesia. The research aimed to improve the availability of marine natural products in the industry and essentioal technologies needed to develop lead compounds for natural product-based drug discovery. By the chemical methods, spectroscopic analysis, and bioactivity tests, the sponge, *Lipastrotethya* sp., was selected and pure 25 compounds were isolated from the extract by various chromatographic methods. Finally, 14 novel compounds and 11 known compounds are determined using combined spectroscopic and chemical analyses. There were 7 compounds of pouoside-class, 5 compounds of corresponsive sapogenins, and 13 compounds of sarasinoside-class. All isolates have been examined under various bioactivity: cytotoxicity test (K562 human erythroleukemia cell,

A549 lung carcinoma cell), antimicrobial activity test (gram-positive bacteria, gram-negative bacteria, and pathogenic fungi), enzyme-inhibitory activity test (Na^+/K^+ ATPase, Isocitrate lyase, Sortase A). Several compounds displayed significant cytotoxic, and/or enzyme-inhibitory activities.

1. Triterpene Galactosides of the Pouoside Class and Corresponding Aglycones from the Sponge *Lipastrotethya* sp.

Nine new triterpene galactosides and aglycones, along with three known compounds from the rare pouoside class, were isolated from the tropical sponge *Lipastrotethya* sp. collected from Micronesia. The structures of these new compounds were determined by combined spectroscopic methods and designated as pouosides F-I (**4**, **8**, **10**, and **12**) and pouogenins A-E (**5**–**7**, **9**, and **11**). The absolute configurations of the asymmetric centers and the cyclohexenone moiety, which had been previously undetermined, were assigned by NOESY analyses and Mosher's methods. Several of these compounds exhibited weak cytotoxicity against the K562 cell-line.

2. Nortriterpene Glycosides of the Sarasinoside Class from the Sponge

Lipastrotethya sp.

Five new nortriterpene glycosides, along with eight known compounds of the sarasinoside class, were isolated from the tropical sponge *Lipastrotethya* sp. collected from Chuuk, Micronesia. The structures of these new compounds were determined by combined spectroscopic and chemical methods and designated as sarasinosides N–R (**21**–**25**). The aglycone portions of **22**–**25** were found to be unprecedented among nortriterpeneoids on the basis of extensive NMR analyses. Several of these compounds exhibited cytotoxicity against A549 and K562 cell lines as well as weak inhibitory activity against Na^+/K^+-ATPase.

Key words: sponge, marine natural products, structure determination, bioactivity, terpenoid, saponin

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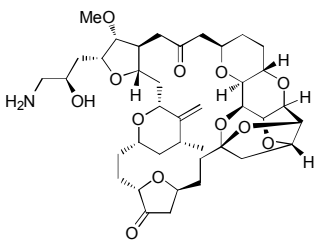
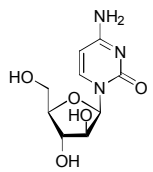
I. Introduction

Researches on marine natural products as sources of bioactive metabolites that may be directly used as drugs or served as lead structures for drug development started intensively in the late 1960s.[1] The discovery of prostaglandin A_2 and analogs in the gorgonian *Plexaura homomalla* is usually considered as the turning point for marine natural products chemistry. These compounds had been discovered as important mediators involved in inflammatory diseases, fever and pain and showed possibilities for drug discovery from marine organisms.[1,2] There is another example. In 1951, the unusual nucleosides spongouridin and spongothymidin from the sponge *Cryptotethya crypta* were reported. These compounds served as lead structures for the development of nowadays commercially important anti-viral drugs such as Ara-A (vidarabine^{TM}) and the anticancer drug for leukemia, Ara-C (cytarabine^{TM}). And it also motivate to investigate marine natural compounds.[1,3] Within commercialization of NMR (nuclear magnetic resonance) spectroscopes and mass spectrometers, methods of isolation, analysis, and structure determination are developed, and marine natural products chemistry had been grown rapidly.

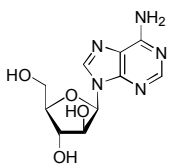
After marine natural products are proven as potent sources of novel therapeutic agents due to their high novelty of bioactivities and



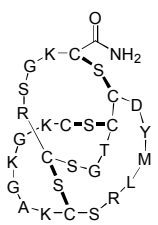
Eribulin (HalavenTM) : antitumor
(Sponge *Lissodendoryx* sp. Halichondrin B derivative)



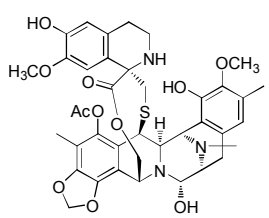
Ara-C (Cytarabine™) : antitumor
(Sponge *Cryptotethya crypta)*



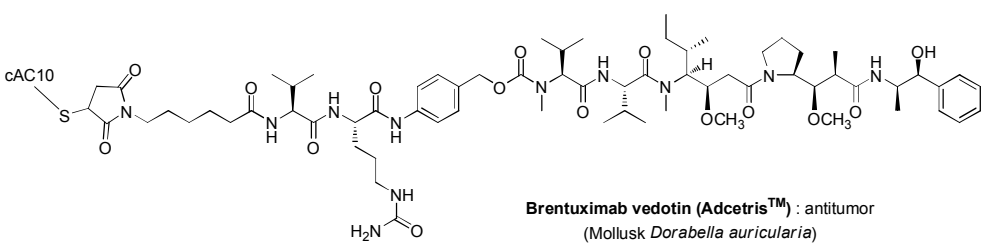
Ara-A (Vidarabine™) : antiviral
(Sponge *Cryptotethya crypta*)



Zinconotide (Prialt™) : analgesic
(Cone sanail *Conus magus*)



Ecteinascidin 743 (Yondelis™) : antitumor
(Ascidian *Ecteinascidia turbinata*)



Brentuximab vedotin (Adcetris™) : antitumor
(Mollusk *Dorabella auricularia*)

Figure 1. Chemical structures of the approved drugs deriving from or inspired by a marine natural product.

chemical structures, there were enormous efforts to investigate new drugs and achievements. Examples approved by the FDA (US Food and Drug Administration) are shown here (Figure 1); Prialt™ (zinconotide, analgesic-cone snail), Cytarabine™ (Ara-C, antitumor-sponge), Vidarabine™ (Ara-A, antiviral-sponge), Halaven™ (eribulin mesylate, antitumor-sponge), Yondelis™ (ecteinascidin 743, antitumor-tunicate), and Adcetris™ (brentuximab vedotin, antitumor-mollusk). Also numorous drug candidates are in clinical or preclinical trials in now.[4,5]

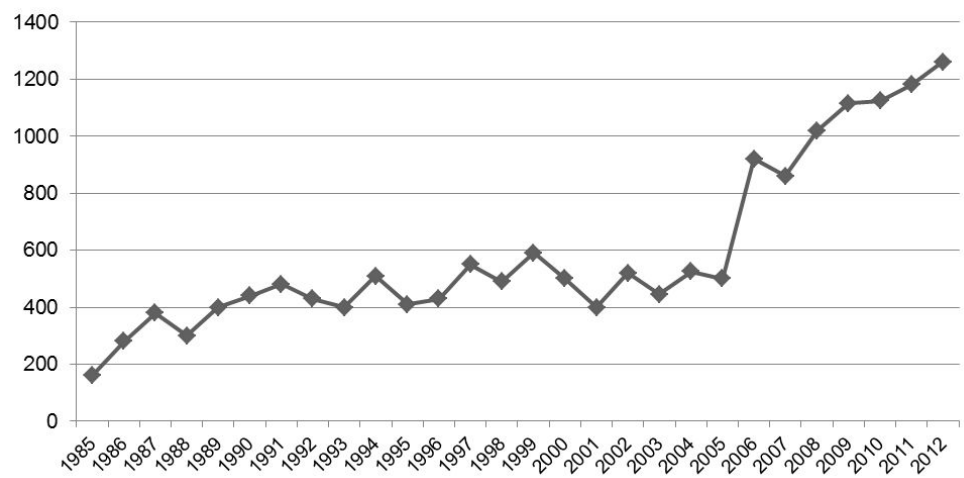


Figure 2. Variation in number of new marine natural products for 1985-2012.[7]

Since the 1960s, more than 20,000 compounds were discovered from marine organisms.[6] Between 1985 and 2012, 15,000 compounds were reported and among these more than 4000 have bioactibities.[7]

Futhermore, over 1000 compounds are discovered from marine organisms annually after 2008 (Figure 2). Majority of novel compounds, approximately 75%, were isolated from marine invertebrates belonging to the phyla Porifera and Coelenterate (Figure 3). Similarly, the major bioactive compounds were from Porifera and Coelenterate, accounting for 56.9% of the total bioactive compounds.[6,7]

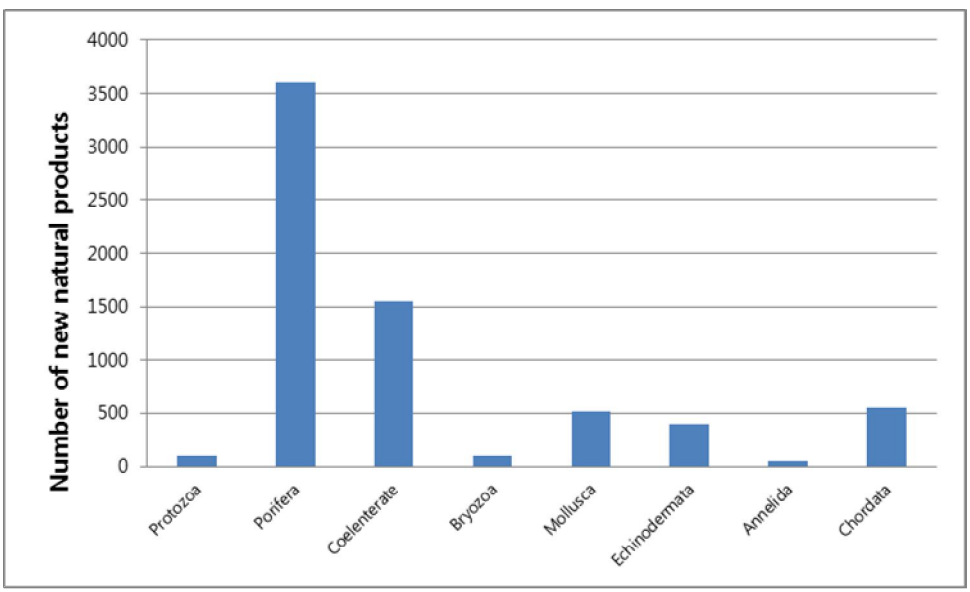
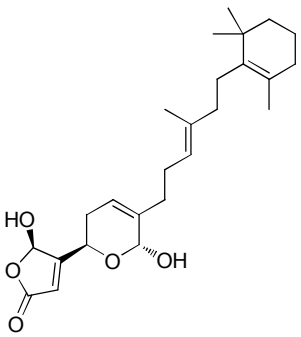


Figure 3. The number of novel compounds isolated from marine invertebrate between 1985 and 2008.[6]

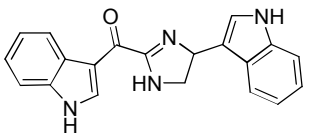
Sponges (phylum Porifera) are one of the most diverse marine organisms, over 8000 species and inhabit a wide variety of marine and fresh water ecosystems and are found throughout tropical, temperate, and

polar regions, ranging from tidal zone to depths exceeding 8,800 m.[8] Due to the diversity of species and convenience to collect samples, it have been dealt with the research object of marine natural products frequently. Furthermore, in the early stage sponge-derived compounds had shown high proportion of the appearance frequency of bioactive compounds and it made sponges to be researched intensively. Among the reported novel compounds from the marine organisms, more than 30% are derived from Porifera, and it is the highest frequency as a phylum during the entire marine organisms. Following the reports, the ratio showing bioactivities from novel compounds is 30.5%. This proportion is just average (28.5%) in the marine organisms including microorganisms and algae.[6,7]

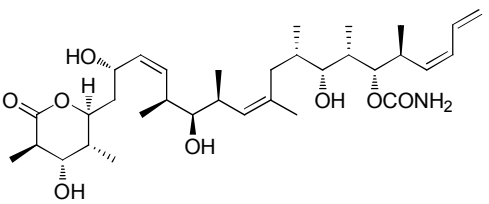
Sponge-derived compounds span a very wide range of chemical classes; terpenoides, steroids, aminoacids, linear and cyclic peptides, polycyclic aromatics, polyketides, nucleosides, and mixed biogenetic products.[9] That means almost all structure classes are come out from sponges. Probabley, we can understand how they can do it when we see their ecology. Sponges are sessile benthic orgamisms and pump water to acquire nutrients from the surrounding seawater. Their primary nutrition sources are planktons and sometimes metabolites from planktons are concentrated in sponges.[10] Also, it is reported sponges contain many



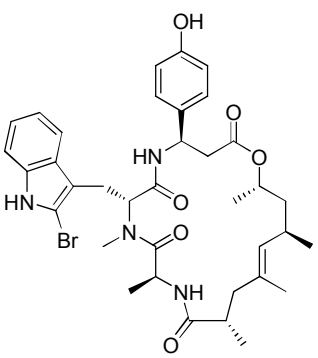
Manoalide : anti-inflammatory, analgesic
(*Luffariella variabilis*)



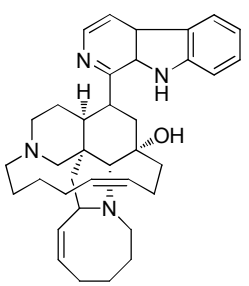
Topsentin A : antitumor
(*Topsentia genitrix*)



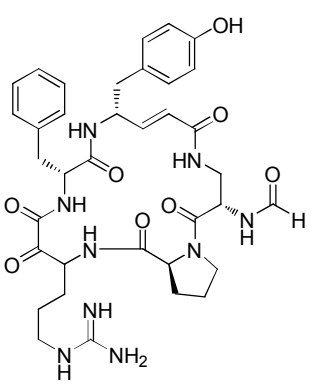
Discodermolide : immunosuppressive
(*Discodermia dissoluta*)



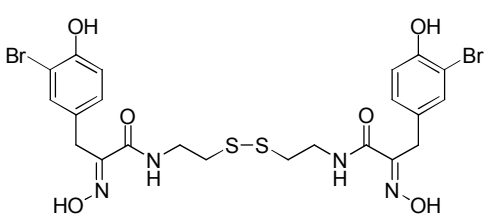
Jaspamide : antifungal
(*Jaspis* sp.)



Manzamine A : antimalaria, tuberculosis, HIV
(*Acanthostrongylophora* sp.)



Cyclotheonamide A :thrombin inhibitor
(*Theonella* sp.)



Psammaplin A : antitumor, HDAc inhibitory
(*Psammaplysilla* sp.)

Figure 4. Examples of potential drug leads derived from marine sponges

symbionts such as epiphytes, microalgae, eubacteria, cyanobacteria, actinomycetes, and fungi.[11] Therefore, it makes us infer there are metabolites of symbionts apart from sponge-origin substances, and some evidances were reported.[10-13]

Compounds derived from sponges reported various bioactivities. Some of these are already commercially available or are being developed for use in antitumor, antifungal, antivirual, enzyme-inhibitor, anti-inflamatory, analergic, and immunosuppresor etc. Except commercialized drugs which are mentioned above, there are considerable drug candidates in clinical or preclinical tests including manoalide (anti-inflammatory, analgesic; *Luffariella* sp.), topsentin A (antitumor; *Topsentia* sp.), jaspamide (antifungal; *Jaspis* sp.), discodermolide (immunosuppressive; *Discodermia dissolute*), cyclotheonamide A (thrombin inhibitor; *Theonella* sp.), manzamine A (antimalarial, tuberculosis, HIV; *Acanthostrongylophora* sp.), and psammaplin A (antitumor, HDAc inhibitory; *Psammaplysilla* sp.) (Figure 4).

This study is searching for novel bioactive natural products from a torpical marine sponge, *Lipastrotethya* sp. which was collected in Micronesia. The research aimed to improve the availability of marine natural products in the industry and essentioal technologies needed to

develop lead compounds for natural product-based drug discovery. By the chemical methods, spectroscopic analysis, and bioactivity tests, the sponge, *Lipastrotethya* sp., was selected and pure 25 compounds were isolated from the extract by various chromatographic methods. Finally, 14 novel compounds and 11 known compounds are determined using combined spectroscopic and chemical analyses. These include 7 compounds of pouoside-class, 5 compounds of corresponsive sapogenins, and 13 compounds of sarasinoside-class. All isolates have been examined under various bioactivity: cytotoxicity test (K562 human erythroleukemia cell, A549 lung carcinoma cell), antimicrobial activity test (gram-positive bacteria, gram-negative bacteria, and pathogenic fungi), enzyme-inhibitory activity test (Na^+/K^+ ATPase, isocitrate lyase, sortase A). Several compounds displayed significant cytotoxic, and/or enzyme-inhibitory activities.

II. Stuedies on the Metabolites from a Marine sponge

1. Triterpene Galactosides of the Pouoside Class and Corresponding Aglycones from the Sponge *Lipastrotethya* sp.

Nine new triterpene galactosides and aglycones, along with three known compounds from the rare pouoside class, were isolated from the tropical sponge *Lipastrotethya* sp. collected from Micronesia. The structures of these new compounds were determined by combined spectroscopic methods and designated as pouosides F-I (**4**, **8**, **10**, and **12**) and pouogenins A-E (**5**–**7**, **9**, and **11**). The absolute configurations of the asymmetric centers and the cyclohexenone moiety, which had been previously undetermined, were assigned by NOESY analyses and Mosher's methods. Several of these compounds exhibited weak cytotoxicity against the K562 cell-line.

1-1. Introduction

Marine sponges produce a wide variety of terpenes and related metabolites possessing diverse carbon skeletons and bioactivities.[9] However, triterpenes and triterpene saponins are relatively rare and represent only a minor fraction of the known sponge-derived terpenoids. Of these compounds, pouosides are a class of cytotoxic triterpene

galactosides that have been isolated from the tropical sponge *Asteropus* sp. collected from the Truk Lagoon.[14] The symmetric carbon skeletons of these compounds are reminiscent of carotenoids and are unique among sponge-derived terpenoids. Despite the numerous works on marine sponges, compounds of this structural class have not been investigated since the first report on pouosides A-E by Schmitz[14] several decades ago.

During the course of our search for bioactive metabolites from tropical marine organisms, we encountered the sponge *Lipastrotethya* sp. (order Halichondrida, family Dictyonellidae), the organic extract of which exhibited weak cytotoxicity (LC_{50} 146 μg/mL) against the K562 cell-line. Bioassay-guided separation of the extract yielded sarasinosides, widely-recognized nortriterpene pentaglycosides, as the major constituents.[15-21] However, moderately polar vacuum flash chromatographic fractions yielded several compounds of a different structural class. Here we report the structures of nine new triterpenes of the pouoside class along with three other known compounds of the same structural class (**1**–**3**).[14] The new compounds were designated as the new triterpene galactosides pouosides F-I (**4**, **8**, **10**, and **12**) and their aglycones, pouogenins A-E (**5**–**7**, **9**, and **11**), respectively. In addition, the absolute configurations of the asymmetric carbon centers and the cyclohexenone moiety were

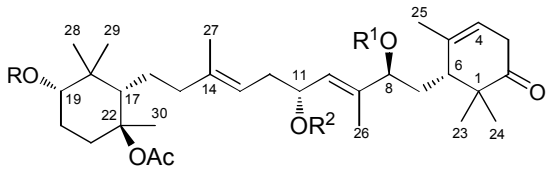
determined for the first time using a series of Mosher's esterification methods and NOESY analyses. Several of these compounds showed moderate cytotoxicity against K562.

1-2. Results and discussion

The sponge samples were lyophilized, macerated, and repeatedly extracted with CH_2Cl_2 and MeOH. The combined extracts were separated by solvent-partitioning followed by ODS vacuum flash chromatography, and then repeated ODS-HPLC. The major constituents were identified as sarasinosides A_1 and A_3 by combined spectroscopic methods and a comparison of ^1H and ^{13}C NMR data with authentic samples.[17,19]

In addition to the sarasinosides, 12 other compounds were isolated by the same HPLC separation methods. The structures of compounds **1**–**3** were identified as pouosides A, D, and B, respectively, on the basis of spectroscopic analyses and a comparison of NMR data with the literature.[14]

The molecular formula of pouoside F (**4**) was $C_{38}H_{62}O_{11}$ as determined by HRFABMS analysis. The ^{13}C and ^1H NMR data of this compound were similar to those of **1** and implied a mono-acetylated



	R	R^1	R^2
1	A	Ac	Ac
2	B	Ac	Ac
3	A	Ac	H
4	A	H	H
5	H	H	H
6	H	Ac	Ac
7	H	Ac	H

	R	R^1
8	A	OAc
9	H	OAc
10	A	=O
11	H	=O

	R
12	A

Figure 5. Chemical structures of isolated compounds (**1**-**12**).

triterpene glycoside. The triterpene nature of this compound was revealed by the presence of eight upfield methyl signals in the NMR data (Tables 1). Also, the ^{13}C NMR spectrum contained signals corresponding to a ketone (δ_C 217.4), an acetoxy group (δ_C 172.2 and 23.0), and three double bonds (six signals at δ_C 141.0–119.0), which were precisely matched with their corresponding signals in the ^{1}H NMR spectrum and absorption bands at 1730 and 1710 cm^{-1} in the IR spectrum. Similarly, the presence of a sugar unit was revealed by characteristic signals at of δ_C 107.3 and 77–62 and δ_H 4.3–3.4. Overall, these NMR features clearly indicated that **4** was an analog of pouoside A (**1**).

Given this information, the structure of **4** was determined by a combination of 2-D NMR experiments. First, the presence of a sugar moiety was secured by a combination of COSY and *g*HSQC data for the signals in the aforementioned NMR regions. Similarities in the chemical shifts and splitting patterns of these signals with those of **1** suggested the presence of a D-galactose group that was confirmed by hydrolysis of **4** followed by GC analysis with authentic sugars. The pyranose nature of this galactose group was supported by long-range correlations at H-1'/C-5' and H-5'/C-1' in the *g*HMBC data. In addition, the β-configuration at the anomeric center was also assigned by its carbon chemical shift (δ_C

107.3) and large $^{1}H/^{1}H$ coupling constant ($J_{1',2'}$ = 7.5 Hz). Taken together, these data indicate the presence of a β-D-galactopyranose unit on compound **4**.

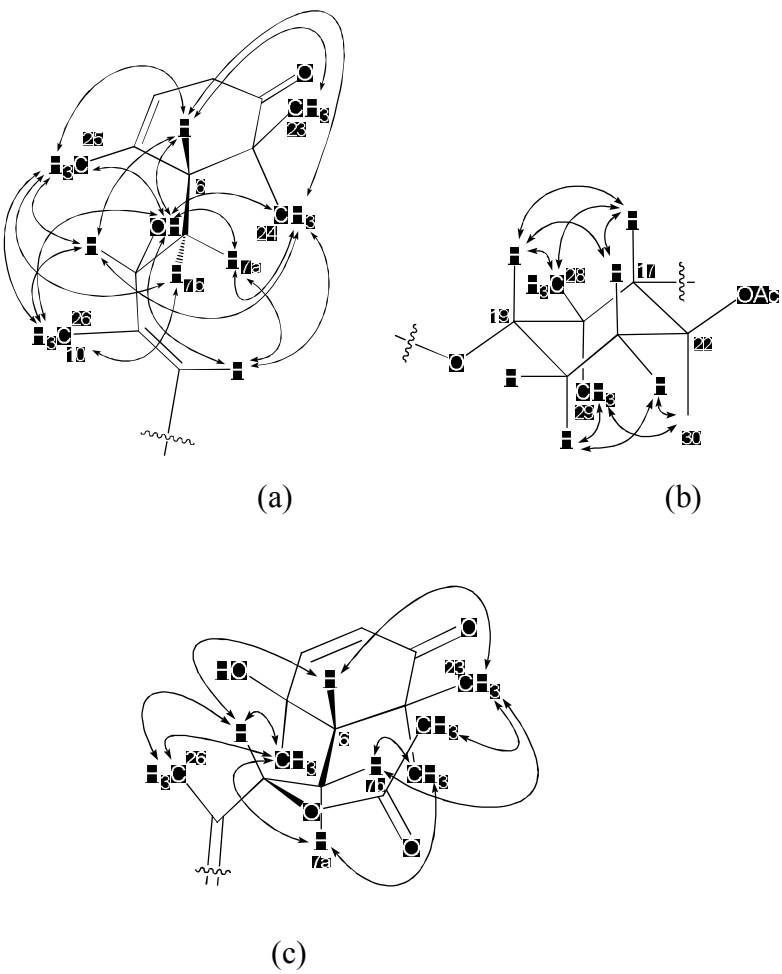


Figure 6. Selected NOESY correlations for compound **4** (a, b) and **8** (c)

The structure of the aglycone was also determined by combined NMR analyses. In particular, several long-range correlations between upfield methyl protons and neighboring ring carbons in the *g*HMBC data revealed the presence of 4,6,6-trimethyl-β,γ-cyclohexenone (C-1–C-6) and 1,1,4-trimethylcyclohexane (C-17–C-22) moieties. The downfield shift of C-19 at δ_C 89.0, the HMBC correlation H-19/C-1', and the NOESY cross peak at H-19/H-1' indicated a galactose moiety at this location via a glycosidic linkage.

The remaining part of the molecule, including two trisubstituted double bonds, two oxymethine groups, and two vinyl methyl groups made up a linear terpenoidal chain (C-7–C-16) based on COSY (H-7~H-8, H-10~H-13, H-15~H-16) and *g*HMBC (H-8/C-7, C-9, C-10, C-26; H-11/C-9, C-10, C-12, C-13; H-26/C-8, C-9, C-10; H-27/C-13, C-14, C-15) data. Connections of this chain with the cyclic moieties were also established with these data: COSY (H-6–H-8, H-15–H-17) and *g*HMBC (H-6/C-7, C-8; H-8/C-6; H-15/C-17). Of the oxygenated locations at C-8, C-11, and C-22, the remaining acetoxy group was placed at C-22 by the proton chemical shifts of H-8 (δ_H 3.95) and H-11 (δ_H 4.32). Thus, pouoside F (**4**) was identified as a triterpene galactoside.

Pouoside F possessed asymmetric double bonds at C-9 and C-13.

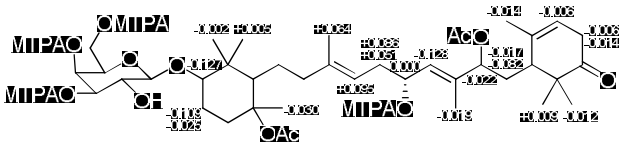
The geometries at these bonds were both *E* based on the upfield shifts of vinyl methyl carbons (δ_C 12.9 and 16.6 for C-26 and C-27, respectively) and cross-peaks at H-8/H-10, H-11/H-26, H-12/H-27, and H-13/H-15 in the NOESY data. This compound also possessed asymmetric carbon centers at C-6, C-8, C-11, C-17, C-19, and C-22 whose relative configurations had previously only been partially determined. Among these, the relative configurations of those in the 6-membered rings and vicinity were assigned by proton coupling constants and NOESY analyses. Firstly the anti-orientations were assigned for both H-6/H-7b (δ_H 1.14) and H-7a (δ_H 1.68)/H-8 by the large vicinal coupling constants ($J_{6,7b}$ = 9.5 Hz, $J_{7a,8}$ = 10.0 Hz). Given this information, the NOESY data showed cross-peaks at H-4/H-23, H-6/H23, H-24, H-25, and H-23/H-25, placing H-6 at the pseudoequatorial position in the cyclohexenone ring. Additional NOE cross-peaks were found at H-10/H-7a, H-24, and H-25/H-26, supporting the 6*R** and 8*S** configurations. More crucial evidences were found by NOESY data obtained in pyridine-d_5 (Supporting Information). The 8-OH proton at δ 6.35 showed cross-peaks at 8-OH/H-6, H-7a, H-8, H-10, H-24, H-25, and H-26. Among these, the cross-peak at 8-OH/H-6 was crucial for the removal of the alternative 6*S** and 8*S** configurations (Figure 6). Similarly the NOESY data of the cyclohexane moiety showed

conspicuous cross-peaks at H-17/H-19, H-17/H-21β (δ_H 1.77), H-17/H-28, H-19/H-21β, H-19/H-28, H-20α (δ_H 1.57)/H-21α (δ_H 2.48), H-20α/H-29, H-21α/H-30, and H-29/H-30. These 1,3-diaxial-type cross-peaks readily established the 17*S**, 19*S**, 22*S** configurations (Figure 6). The absolute configurations at these centers and the remaining C-11 were determined by a series of Mosher esterification reactions and are discussed below along with those of the other compounds.

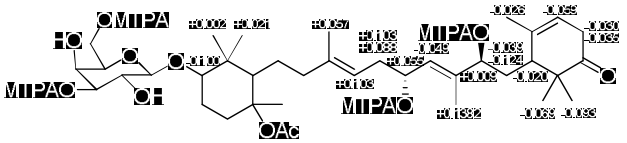
The molecular formula of pouogenin A (**5**) was $C_{32}H_{52}O_6$ based on HRFABMS data. The ^1H and ^{13}C NMR spectra of this compound were similar to those of **4** except, most noticeably, for the disappearance of signals corresponding to the galactose unit. This interpretation was confirmed by a combination of 2-D NMR experiments and further supported by the shift of the H-19 oxymethine proton (**4**: δ_C 89.0, δ_H 3.30; **5**: δ_C 78.3, δ_H 3.27). Thus, **5** was structurally defined as the aglycone of **4**.

A structurally related compound, pouogenin B (**6**), was isolated as colorless oil with the molecular formula $C_{36}H_{56}O_8$ as determined by HRFABMS. As for **5**, the removal of a sugar unit was apparent from the NMR data, implying that **6** is a triterpene triacetate. A combination of 2-D NMR data showed that this compound was the aglycone of **1**. This conclusion is consistent with noticeable shifts of the H-19 oxymethine

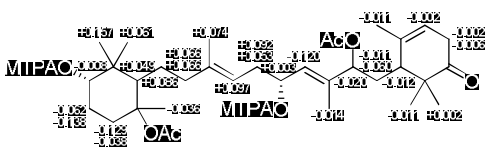
proton (**1**: δ_C 89.0, δ_H 3.30; **6**: δ_C 78.3, δ_H 3.27) as well as downfield shifts of the H-8 and H-11 oxymethine protons (**5**: δ3.95 and 4.32; **6**: δ4.97 and 5.41 for H-8 and H-11, respectively) from that of **5**.



(a)



(b)



(c)

Figure 7. Results of MTPA esterification for compound **3**, **4**, and **7** (a-c)

The NMR spectroscopic features of another aglycone, pouogenin C (**7**) ($C_{34}H_{54}O_7$), were similar to those of the other aglycones (Tables 4). 2-D NMR analyses revealed that this compound possessed the same carbon framework as **5** and **6** with structural differences occurring only in the oxygenated functionalities at C-8, C-11, or C-22. Proton chemical

shifts and HMBC correlation analyses placed a hydroxy group at C-11 (δ_H 4.30) and acetoxy groups at the C-8 and C-22, respectively. Thus, **7** was structurally defined as the aglycone of pouoside B (**3**), which is consistent with the chemical shifts of the oxymethine group at C-19 (**3**: δ_C 89.0, δ_H 3.30; **7**: δ_C 78.4, δ_H 3.27).

The molecular formula of pouoside G (**8**) was deduced as $C_{40}H_{64}O_{13}$ by HRFABMS. A detailed examination of its ^{13}C and ^{1}H NMR spectra revealed that this compound is another triterpene galactoside. However, significant differences were found in the carbon and proton signals of the cyclohexenone moiety (C-1–C-6). The carbon signals corresponding to the C-4 trisubstituted double bond were replaced with those of a disubstituted double bond [δ_C 158.0 (CH) and 125.3 (CH)]. A corresponding difference was also found in the ^{1}H NMR data in which new signals appeared at δ_H 6.73 (1H, d, J = 10.0 Hz) and 5.80 (1H, d, J = 10.0 Hz). In addition, the C-3 methylene carbon of **1**-**7** disappeared and the C-5 quaternary carbon atom (δ_C 72.0) appeared. These changes were accommodated by a migration of the C-4 double bond to C-3 and concomitant attachment of a hydroxy group at C-5. These changes were confirmed by 2-D NMR data including key long-range correlations at H-3/C-1, C-5, H-4/C-2, C-6, and H-25/C-4, C-5, C-6 in the *g*HMBC data.

The relative configurations at C-5 and C-6 were established as 5*S** and 6*S** based on cross-peaks at H-6/H-8, H-6/H-23 (δ 1.21), H-7a (δ 1.95)/H-24, H-7a/H-25, H-7b (δ 1.78)/H-23, H-7b/H-24 (δ 1.02), H-8/H-25, H-8/H-26, H-23/8-OAc, H-24/H-25, and H-25/H-26 in the NOESY data (Figure 6). Thus the structure of pouoside G (**8**) was that of a 3-ene-5-hydroxy derivative of pouoside B (**2**).

The aglycone of compound **8**, pouogenin D (**9**), was also isolated as a colorless oil with a molecular formula of $C_{32}H_{52}O_7$. With the exception of signals derived from the galactose moiety, the carbon and proton NMR data were very similar to those of **8**. All of the proton-proton and proton-carbon correlations observed in the 2-D NMR experiments were also virtually identical to those of **8**, and the removal of the galactose unit from C-19 was further confirmed by characteristic shifts of the methine (**8**: δ_C 88.4, δ_H 3.29, **9**: δ_C 78.4, δ_H 3.27).

The molecular formula of pouoside H (**10**) was established as $C_{38}H_{60}O_{12}$ by HRFABMS. The NMR spectra of this compound were reminiscent of those of other pouosides, and particularly with those of **8**, with a galactose unit and two 6-membered rings as the most apparent structural features. However, the ^{13}C NMR data indicated oxidation of a hydroxymethine to a ketone (δ 203.8) that was consistent with the shift [**8**:

δ_H 5.45 (1H, dd, *J* = 9.0, 5.0 Hz); **10**: δ_H 6.57 (1H, br d, *J* = 8.0 Hz)] of a proton signal in the ^{1}H NMR data. The ketone was placed at C-8 based on long-range correlations at H-6/C-8, H-7/C-8, H-10/C-8, C-26, and H-26/C-8, C-9, C-10 in the *g*HMBC data. Thus, the structure of pouoside H (**10**) was determined to be that of an 8-oxo analog of pouoside G (**8**).

The aglycone of pouoside H (**10**), pouogenin E (**11**), was isolated as a colorless oil with a molecular formula of $C_{32}H_{50}O_7$ based on HR-negative-FABMS analysis. As found for the other triterpene galactosides and corresponding aglycones, the NMR data of this compound were highly reminiscent of those obtained from pouoside H (**10**). Based on the results of combined NMR analyses, all of the carbons and protons are in the same locations as in **10** (Tables 7).

The molecular formula of another triterpene galactoside, pouoside I (**12**), was determined as $C_{40}H_{62}O_{11}$ by mass analysis. The spectroscopic data of this compound were very similar to those of the other triterpene galactosides. However, the ^{13}C NMR spectrum revealed an exo-double bond [δ 149.4 (C), 108.6 (CH_2)] that coincided with the exo-methylene protons [δ 4.84 (1H, br s), 4.55 (1H, br s)] in the ^{1}H NMR data. This group was securely established at C-22 (30) based on long-range correlations H-16/C-22, H-17/C-22, C-30, H-20/C-22, H-21/C-22, C-30, and H-30/ C-21,

C-22 in the *g*HMBC data. The remainder of the structure, including the galactose moiety, was identical to that of **1** according to combined 2-D NMR analyses. Thus, the structure of pouoside I (**12**) was that of a 22-deacetoxy derivative of pouoside A (**1**).

A comparison of ^{13}C and ^{1}H NMR data revealed nearly identical chemical shifts for both carbons and protons among the core triterpenes of all the compounds **1**–**12** (Tables 1–9). Thus, the results of NOESY experiments indicated the same 5*S**, 6*R** (6*S** for 8–11 but the same orientation with others), 8*S**, 17*S**, 19*S**, and 22*S** relative configurations at the same locations for these compounds. Based on a comparison of chemical shifts, the relative configuration at C-11, which was not determinable by NMR experiments, is also thought to be the same among these compounds.

Absolute configurations were assigned using Mosher's method on selected compounds. First, MTPA esterification of **3** with both (-)- and (+)-MTPA chloride unambiguously established the 11*R* configuration (Figure 7a). The same reactions with **4** resulted in the formation of bis-MTPA esters at the aglycone. Despite the proximity of the C-8 and C-11 asymmetric centers, the results clearly established 8*S* and 11*R* configurations based on differences in proton chemical shifts at H-10 and

H-26. These two protons are located between two ester centers, and their chemical shifts were significantly different than those of the **3**-MTPA esters. The chemical shifts of protons at the remaining locations were not significantly shifted (Figure 7b). Finally, a 19*S* configuration was established at the galactoside position by MTPA esterification of pouogenin C (**7)** and an 11*R* configuration was again confirmed by this reaction (Figure 7c). Thus, the absolute configurations of the asymmetric centers were unambiguously determined as 6*R*, 8*S*, 11*R*, 17*S*, 19*S*, and 22*S* (except **12**) for **1–7** and **12**, and 5*S*, 6*S*, 8*S* (except **10** and **11**), 11*R*, 17*S*, 19*S*, and 22*S* for **8**–**11**.

A previous paper on pouosides A-E reported moderate cytotoxicity of pouoside A towards a P388 cell-line (ED_{50} 1.5 μg/mL).[14] The compounds reported herein, exhibited weak to no activity against a K562 human erythroleukemia cell-line (IC_{50} 17.0, 12.5, >100, >100, 14.8, 38.9, 14.6, >100, 27.5, >100, 18.9, and >100 μM for **1**–**12**, respectively, n=3; IC_{50} 13.6 μM for doxorubicin). With the exception of **1** and **2**, the aglycones were far more cytotoxic than the triterpene galactosides. Compounds **5**, **7**, and **11**, for example, showed inhibition properties comparable to those of doxorubicin against this cell line. None of these compounds showed significant antimicrobial activity against selected

Gram-positive and Gram-negative bacteria and pathogenic fungi.[22]

1-3. Experimental section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter using a 1-cm cell. UV spectra were recorded on a Hitachi U-3010 spectrophotometer and IR spectra were recorded on a JASCO 300E FT-IR spectrometer. NMR spectra were recorded in MeOH-d_4 containing Me_4Si as an internal standard on Bruker Avance 500 and 600 spectrometers. Proton and carbon NMR spectra were measured at 600 and 150 MHz (**4**, **6**, **8**, **9**, **11** and **12**), and 500 and 125 MHz (**5**, **7** and **10**), respectively. Mass spectrometric data were obtained at the Korea Basic Science Institute (Daegu, Korea) and were acquired using a JEOL JMS 700 mass spectrometer with *meta*-nitrobenzyl alcohol (NBA) as a matrix for the FABMS. HPLC was performed on a SpectraSystem p2000 equipped with a SpectraSystem RI-150 refractive index detector. Gas chromatography was performed on a Younglin YL6100 plus chromatograph equipped with an FID. All solvents were spectroscopic grade or distilled from glass prior to use.

Animal Material. Specimens of *Lipastrotethya* sp. (voucher collection number 102CH-232) were collected by hand using SCUBA

equipment off the shore of Weno Island, Chuuk state, Federated States of Micronesia, at a depth of 15 m on February 18, 2010. The sponge was globular in shape and beige in alcohol, measured 130 × 170 mm and was 45 mm thick. This structure was attached to the sea bottom by a few to several stalks up to 5 cm long and 1 cm in diameter. The surface was warty but smooth and the texture was elastic in alcohol. The consistency was cartilaginous. The skeleton was a network of oxea and strongyle (400–1000 × 3–20 μm). The specimens were similar to those of *Lipastrotethya rhaphoxea* de Laubenfels, 1954, in general morphology except that *L. rhaphoxea* had larger spicules, reaching 5000 x 15 μm, with smaller spicules in the dermis measuring only 50 × 1 μm. A voucher specimen (registry No. Spo. 57) was deposited at the Natural History Museum, Hannam University, Korea under the curatorship of C.J.S.

Extraction and Isolation. Freshly collected specimens were immediately frozen and stored at –25 °C until use. Lyophilized specimens were macerated and repeatedly extracted with MeOH (2 L × 3) and CH_2Cl_2 (2 L × 3). The combined extracts (112.7 g) were successively partitioned between H_2O (99.3 g) and *n*-BuOH (24.8 g); the latter fraction was repartitioned between H_2O-MeOH (15:85) (13.72 g) and *n*-hexane (7.72 g). An aliquot of the former layer (5.70 g) was separated by C_{18}

reversed-phase vacuum flash chromatography using a sequential mixture of MeOH and H_2O as eluents (six fractions in gradient, H_2O-MeOH, from 50:50 to 0:100), acetone, and finally EtOAc.

Based on the results of ^1H NMR and cytotoxicity analyses, the fractions eluted with H_2O-MeOH (30:70) (0.33 g), H_2O-MeOH (20:80) (0.74 g), and H_2O-MeOH (10:90) (1.10 g) were chosen for separation. The fraction eluted with H_2O-MeOH (30:70) was separated by semi-preparative reversed-phase HPLC (YMC-ODS column, 10 mm × 250 mm; H_2O-MeOH, 35:65), yielding two peaks rich with secondary metabolites. Further purification of the first peak by reversed-phase HPLC (YMC-Pack CN column, 10 mm × 250 mm; H_2O-MeOH, 65:35) provided compound **10** as a colorless oil while purification of the second peak by reversed-phase HPLC (H_2O-MeOH, 60:40) yielded compound **11**.

The H_2O-MeOH (20:80) fraction was separated by reversed-phase HPLC (YMC-ODS column; H_2O-MeOH, 30:70) to yield, in order of elution, compounds **4**, **8**, **3**, **5**, and **9**. These metabolites were then purified by reversed-phase HPLC (YMC-Pack CN column; H_2O-MeOH, 65:35 for **4** and **8**, and H_2O-MeOH, 60:40 for **3, 5** and **9**, respectively).

The H_2O-MeOH (10:90) fraction was separated and purified by reversed-phase HPLC (YMC-ODS column; H_2O-MeOH, 20:80) yielding, in order of elution, compounds **7**, **1**, **2**, **6**, and **12**. These metabolites were then purified by HPLC (YMC-Pack CN column; H_2O-MeOH, 50:50 for **1** and **7**, H_2O-MeCN, 62:38 for **2**, and H_2O-MeOH, 65:35 for **6** and **12**, respectively). The purified metabolites were isolated in the following amounts: 48.0, 3.7, 72.4, 24.6, 17.0, 3.8, 20.2, 16.2, 17.8, 3.8, 4.6, and 3.5 mg of **1-12**, respectively.

Pouoside A (1): Colorless oil; $[\alpha]_D^{25}$ –26.2 (*c* 0.50, MeOH)

Pouoside D (2): Colorless oil; $[\alpha]_D^{25}$ –25.8 (*c* 0.48, MeOH)

Pouoside B (3): Colorless oil; $[\alpha]_D^{25}$ –40.8 (*c* 0.65, MeOH)

Pouoside F (4): Colorless oil; $[\alpha]_D^{25}$ –29.0 (*c* 0.35, MeOH); IR (ZnSe) ν_{max} 3400 (br), 1730, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 717.4194 $[M + Na]^+$ (calcd for $C_{38}H_{62}O_{11}Na$, 717.4190).

Pouogenin A (5): Colorless oil; $[\alpha]_D^{25}$ –49.5 (*c* 0.40, MeOH); IR (ZnSe) ν_{max} 3420 (br), 1730, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 555.3663 $[M + Na]^+$ (calcd for $C_{32}H_{52}O_6Na$, 555.3662).

Pouogenin B (6): Colorless oil; $[\alpha]_D^{25}$ –24.0 (*c* 0.40, MeOH); IR (ZnSe) ν_{max} 3400 (br), 1733, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 639.3876 $[M + Na]^+$ (calcd for $C_{36}H_{56}O_8Na$, 639.3873).

Pouogenin C (7): Colorless oil; $[\alpha]_D^{25}$ –26.3 (*c* 0.60, MeOH); IR (ZnSe) ν_{max} 3450 (br), 1730, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 597.3771 $[M + Na]^+$ (calcd for $C_{34}H_{54}O_7Na$, 597.3767).

Pouoside G (8): Colorless oil; $[\alpha]_D^{25}$ –13.4 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 224 (2.42), 273 (1.45) nm; IR (ZnSe) ν_{max} 3400 (br), 1729, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 775.4247 $[M + Na]^+$ (calcd for $C_{40}H_{64}O_{13}Na$, 775.4245).

Pouogenin D (9): Colorless oil; $[\alpha]_D^{25}$ –25.9 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 224 (2.41), 275 (1.47) nm; IR (ZnSe) ν_{max} 3430, 1730, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 613.3719 $[M + Na]^+$ (calcd for $C_{34}H_{54}O_8Na$, 613.3716).

Pouoside H (10): Colorless oil; $[\alpha]_D^{25}$ –11.5 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 224 (2.43), 275 (1.47) nm; IR (ZnSe) ν_{max} 3400 (br), 1728, 1711 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 731.3987 $[M + Na]^+$ (calcd for $C_{38}H_{60}O_{12}Na$, 731.3982).

Pouogenin E (11): Colorless oil; $[\alpha]_D^{25}$ –5.0 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 224 (2.43), 2.73 (1.46) nm; IR (ZnSe) ν_{max} 3420, 1729, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 545.3484 $[M - H]^-$ (calcd for $C_{32}H_{49}O_7$, 545.3478).

Pouoside I (12): Colorless oil; $[\alpha]_D^{25}$ –11.5 (*c* 0.35, MeOH); IR (ZnSe) ν_{max} 3430 (br), 1732, 1710 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 741.4195 $[M + Na]^+$ (calcd for $C_{40}H_{62}O_{11}Na$, 741.4190).

Esterification with (–)-(*R*)-α-Methyoxy-α-(trifluoromethyl) phenylacetyl (MTPA) Chloride. Esterifications of compounds **3**, **4**, and **7** were carried out as follows. A total of 20 μL (–)-(*R*)-MTPA chloride was added to a solution containing 1.1–2.0 mg of the alcoholic compound in 100 μL dry pyridine. The mixture was allowed to stand under N_2 at room temperature for 2 h. After the consumption of starting material was confirmed by thin-layer chromatography (TLC), 0.5 mL H_2O-MeOH (10:90) was added. The solvents were removed under vacuum and the residue was separated by reversed-phase HPLC (YMC-ODS column, 10 mm × 250 mm; H_2O-MeOH, 10:90) to give 1.0–2.1 mg (*S*)-MTPA esters (**3a**, **4a**, and **7a**). The corresponding (*R*)-MTPA esters (**3b**, **4b**, and **7b**) were also obtained from the same esterification reactions with (+)-(*S*)-

MTPA chloride. Selected ^{1}H NMR (CD_3OD, 500 MHz) of **3a**: δ_H 5.675 (1 H, ddd, *J* = 9.0, 7.0, and 7.0 Hz, H-11), 5.279 (1 H, d, *J* = 9.0 Hz, H-10), 5.150 (1 H, dd, *J* = 7.0 and 7.0 Hz, H-13), 4.935 (1 H, dd, *J* = 9.5 and 3.5 Hz, H-8), 2.488 (1 H, ddd, *J* = 14.0, 7.0, and 7.0 Hz, H-12), 2.337 (1 H, ddd, *J* = 14.0, 7.0, and 7.0 Hz, H-12), 2.112 (1 H, m, H-6), 1.881 (1 H, m, H-7), 1.728 (3 H, s, H-26), 1.635 (3 H, s, H-27), and 1.366 (1 H, m, H-7); LR-ESIMS *m/z* 1624.7 $[M + Na]^+$ (calcd for $C_{80}H_{92}F_{12}O_{20}Na$, 1624.6). Selected ^{1}H NMR (CD_3OD, 500 MHz) of **3b**: δ_H 5.675 (1 H, ddd, *J* = 9.0, 6.5 and 6.5 Hz, H-11), 5.407 (1 H, d, *J* = 9.0 Hz, H-10), 5.0554 (1 H, dd, *J* = 7.0 and 7.0 Hz, H-13), 4.958 (1 H, dd, *J* = 9.5 and 3.4 Hz, H-8), 2.403 (1 H, ddd, *J* =14.0, 7.0, and 7.0 Hz, H-12), 2.285 (1 H, ddd, *J* = 14.0, 7.0, and 7.0 Hz, H-12), 2.140 (1 H, dd, *J* = 6.5, 5.0 Hz, H-6), 1.899 (1 H, m, H-7), 1.747 (3 H, s, H-26), 1.571 (3 H, s, H-27), and 1.447 (1 H, m, H-7); LR-ESIMS *m/z* 1624.7 $[M + Na]^+$ (calcd for $C_{80}H_{92}F_{12}O_{20}Na$, 1624.6). Selected ^{1}H NMR (CD_3OD, 500 MHz) of **4a**: δ_H 5.719 (1 H, ddd, *J* = 9.0, 6.0, and 6.0 Hz, H-11), 5.432 (1 H, d, *J* = 9.0 Hz, H-10), 5.422 (1 H, br s, H-4), 5.270 (1 H, dd, *J* = 9.0 and 4.0 Hz, H-8), 5.176 (1 H, dd, *J* = 7.6 and 7.6 Hz, H-13), 2.906 (1 H, m, H-3), 2.681 (1 H, m, H-3), 2.494 (1 H, ddd, *J* = 14.0, 7.0, and 7.0 Hz, H-12), 2.371 (1 H, ddd, *J* = 14.0, 7.0, and 7.0 Hz, H-12), 2.278 (1 H, m, H-6), 1.871 (1 H, m, H-7), 1.748 (3 H, s, H-25),

1.748 (3 H, s, H-26), 1.630 (3 H, s, H-27), 1.338 (1 H, m, H-7), 1.067 (3 H, s, H-23), and 0.925 (3 H, s, H-24); LR-ESIMS m/z 1582.4 $[M + Na]^+$ (calcd for $C_{78}H_{90}F_{12}O_{19}Na$, 1582.6). Selected ^1H NMR (CD_3OD) of **4b**: δ_H 5.664 (1 H, ddd, J = 9.0, 6.0, and 6.0), 5.480 (1 H, br s, H-4), 5.480 (1 H, d, J = 9.0 Hz, H-10), 5.261 (1 H, dd, J = 8.5 and 4.5 Hz, H-8), 5.073 (1 H, dd, J = 7.0 and 7.0 Hz, H-13), 2.936 (1 H, m, H-3), 2.716 (1 H, m, H-3), 2.391 (1 H, ddd, J = 14.0, 7.0, and 7.0 Hz, H-12), 2.298 (1 H, m, H-6), 2.282 (1 H, m, H-12), 1.910 (1 H, m. H-7), 1.774 (3 H, s, H-25), 1.610 (3 H, s, H-26), 1.573 (3 H, s, H-27), 1.462 (1 H, m, H-7), 1.136 (3 H, s, H-23), and 1.018 (3 H, s, H-24); LR-ESIMS *m/z* 1582.4 $[M + Na]^+$ (calcd for $C_{78}H_{90}F_{12}O_{19}Na$, 1582.6). Selected ^1H NMR (CD_3OD, 500 MHz) of **7a**: δ_H 4.775 (1 H, m, H-19), 2.442 (1 H, m, H-21), 1.895 (1 H, m, H-20), 1.843 (1 H, m, H-21), 1.743 (1 H, m, H-17), 1.582 (1 H, m, H-20), 1.472 (2 H, m, H-16), 1.458 (3 H, s, H-30), 0.983 (3 H, s, H-28), and 0.860 (3 H, s, H-29); LR-ESIMS m/z 1030.4 $[M + Na]^+$ (calcd for $C_{54}H_{68}F_6O_{11}Na$, 1030.5). Selected ^1H NMR (CD_3OD, 500 MHz) of **7b**: δ_H 4.784 (1 H, m, H-19), 2.571 (1 H, br s, H-21), 1.957 (1 H, m, H-20), 1.881 (1 H, m, H-21), 1.720 (1 H, m, H-20), 1.694 (1 H, m, H-17), 1.493 (3 H, s, H-30), 1.387 (2 H, m, H-16), 0.826 (3 H, s, H-28), and 0.799 (3 H, s, H-29); LR-ESIMS m/z 1030.4 $[M + Na]^+$ (calcd for $C_{54}H_{68}F_6O_{11}Na$, 1030.5).

Acid Hydrolysis and GC Analysis of Compound 4. A solution of **4** (4.0 mg) in 3 *N* HCl (0.5 mL) was stirred at 80 °C for 5 h. After being cooled to room temperature, the solution was concentrated by evaporation under a stream of N_2. The residue was re-dissolved in a solution of L-cysteine methyl ester hydrochloride (2.3 mg) in pyridine (0.5 mL) and the solution was stirred at 60 °C for 1.5 h. After being cooled and concentrated, the mixture was treated with hexamethyldisilazane (HMDS) in TMSCl (50 μL, *v*/*v* = 2:1) in pyridine (0.5 mL) and the solution was stirred at 60 °C for 30 min. After drying the solution with a stream of N_2, the residue was separated with H_2O and CH_2Cl_2 (1 mL, *v*/*v* = 1:1). The CH_2Cl_2 layer was analyzed by GC using an HP5 column (0.32 mm × 30 m). The injector and detector were both held at 200 °C. A temperature gradient was applied to the GC oven; the initial temperature was maintained at 60 °C for 3 min, then ramped to 200 °C at 4 °C/min, and maintained at 200 °C for 3 min. A peak corresponding to a hydrolysate sugar derivative was detected at 31.89 min. Retention times for authentic samples after being treated simultaneously with HMDS/TMSCl were 31.87 (D-galactose), and 30.58 min (L-galactose), respectively. Co-injection of the hydrolysate with the authentic silylated D-galactose gave single peaks at 31.90 min.

Biological Assays. Cytotoxicity assays were performed in accordance with the protocols in references 23 and 24.

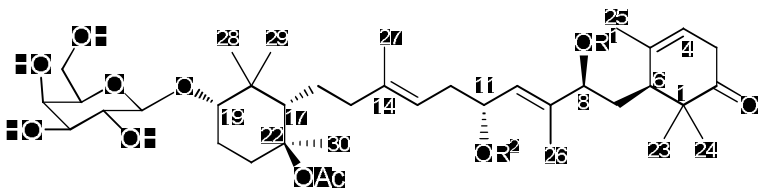


Table 1. ^{13}C and ^{1}H NMR assignment for compound **4** in MeOD-d_4

Position	δ_C	δ_H
1	48.6, C	
2	217.4, C	
3	38.7, CH_2	2.89, ddq (22.5, 4.5, 1.0)
		2.71, ddq (22.5, 3.0, 1.0)
4	119.0, CH	5.50, m
5	141.0, C	
6	50.0, CH	2.27, dd (9.5, 4.5)
7	38.8, CH_2	1.68, ddd (13.5, 10.0, 4.5)
		1.14, ddd (13.5, 9.5, 3.0)
8	75.6, CH	3.95, dd (10.0, 3.0)
9	141.0, C	
10	129.1, CH	5.37, br d (9.0)
11	69.2, CH	4.32, ddd (9.0, 7.0, 6.5)
12	37.3, CH_2	2.29, ddd (14.0, 7.0, 6.5)
		2.14, ddd (14.0, 7.0, 7.0)
13	121.0, CH	5.17, br t (7.0)
14	139.0, C	
15	44.0, CH_2	2.14, m
		2.01, ddd (12.5, 12.5, 5.0)
16	26.4, CH_2	1.55, m
		1.46, m
17	54.5, CH	1.60, m

18	42.1, C	
19	89.0, CH	3.30, dd (11.5, 4.0)
20	28.3, CH_2	2.06, dddd (13.5, 4.0, 4.0, 4.0)
		1.57, dddd (13.5, 11.5, 11.5, 4.0)
21	36.3, CH_2	2.48, ddd (13.5, 4.0, 4.0)
		1.77, ddd (13.5, 11.5, 4.0)
22	88.2, C	
23	26.0, CH_3	1.19, s
24	21.6, CH_3	1.06, s
25	25.0, CH_3	1.91, d (1.0)
26	13.0, CH_3	1.59, d (1.0)
27	16.7, CH_3	1.64, br s
28	28.6, CH_3	1.10, s
29	16.7, CH_3	0.88, s
30	20.5, CH_3	1.47, s
1’	107.3, CH	4.28, d (7.5)
2’	73.1, CH	3.50, dd (7.5, 9.5)
3’	75.2, CH	3.43, dd (9.5, 3.5)
4’	70.3, CH	3.81, dd (3.5, 0.5)
5’	76.5, CH	3.48, dt (0.5, 6.0)
6’	62.5, CH_2	3.71, d (6.0)
22-OAc	172.2, C	
	23.0, CH_3	1.93, s

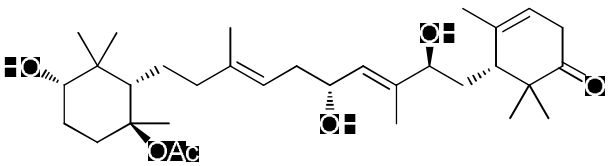


Table 2. ^{13}C and ^{1}H NMR assignment for compound **5** in MeOD-d_4

Position	δ_C	δ_H
1	48.7, C	
2	217.3, C	
3	39.0, CH_2	2.89, ddq (22.5, 4.5, 1.0)
		2.72, ddq (22.5, 3.0, 1.0)
4	118.9, CH	5.49, m
5	141.0, C	
6	50.0, CH	2.27, dd (9.5, 4.5)
7	38.8, CH_2	1.68, ddd (13.5, 10.0, 4.5)
		1.15, ddd (13.5, 9.5, 3.0)
8	75.6, CH	3.95, dd (10.0, 3.0)
9	141.0, C	
10	129.1, CH	5.37, br d (9.0)
11	69.1, CH	4.32, ddd (9.0, 7.0, 6.5)
12	37.3, CH_2	2.29, ddd (14.0, 7.0, 6.5)
		2.15, ddd (14.0, 7.0, 7.0)
13	121.0, CH	5.18, br t (7.0)
14	139.0, C	
15	44.0, CH_2	2.15, m
		2.01, ddd (12.5, 12.5, 5.0)
16	26.5, CH_2	1.55, m
		1.46, m
17	54.3, CH	1.57, m

18	41.7, C	
19	78.3, CH	3.27, dd (11.5, 4.0)
20	28.7, CH_2	1.68, dddd (13.5, 4.0, 4.0, 4.0)
		1.53, dddd (13.5, 11.5, 11.5, 4.0)
21	36.4, CH_2	2.51, ddd (13.5, 4.0, 4.0)
		1.77, ddd (13.5, 11.5, 4.0)
22	88.3, C	
23	25.9, CH_3	1.19, s
24	21.6, CH_3	1.06, s
25	24.9, CH_3	1.91, d (1.0)
26	12.9, CH_3	1.60, d (1.0)
27	16.6, CH_3	1.64, br s
28	29.0, CH_3	1.02, s
29	16.0, CH_3	0.80, s
30	20.4, CH_3	1.47, s
22-OAc	172.1, C	
	23.0, CH_3	1.93, s

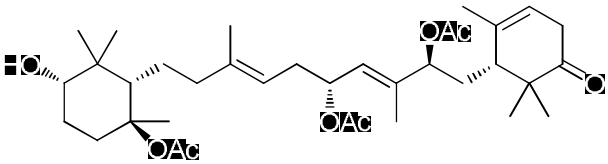


Table 3. ^{13}C and ^{1}H NMR assignment for compound **6** in MeOD-d_4

Position	δ_C	δ_H
1	48.6, C	
2	216.6, C	
3	38.7, CH_2	2.93, ddq (22.5, 4.5, 1.0)
		2.72, ddq (22.5, 3.0, 1.0)
4	119.6, CH	5.47, m
5	139.2, C	
6	51.0, CH	2.12, dd (6.5, 5.0)
7	35.6, CH_2	1.88, ddd (14.5, 9.0, 5.0)
		1.42, ddd (14.5, 6.5, 4.5)
8	78.4, CH	4.97, dd (9.0, 4.5)
9	139.2, C	
10	127.6, CH	5.32, br d (9.0)
11	72.0, CH	5.41, ddd (9.0, 7.0, 6.5)
12	34.1, CH_2	2.39, ddd (14.0, 7.0, 6.5)
		2.24, ddd (14.0, 7.0, 7.0)
13	119.5, CH	5.08, br t (7.0)
14	140.6, C	
15	43.9, CH_2	2.14, ddd (12.5, 12.5, 5.0)
		2.00, m
16	26.6, CH_2	1.51, m
		1.44, m
17	54.3, CH	1.56, m

18	41.8, C	
19	78.3, CH	3.27, dd (11.5, 4.0)
20	29.0, CH_2	1.69, dddd (13.5, 4.0, 4.0, 4.0)
		1.54, dddd (13.5, 11.5, 11.5, 4.0)
21	36.4, CH_2	2.52, ddd (13.5, 4.0, 4.0)
		1.76, ddd (13.5, 11.5, 4.0)
22	88.3, C	
23	26.5, CH_3	1.21, s
24	21.7, CH_3	1.07, s
25	23.9, CH_3	1.79, d (1.0)
26	13.0, CH_3	1.66, d (1.0)
27	16.6, CH_3	1.65, br s
28	28.8, CH_3	1.02, s
29	16.1, CH_3	0.81, s
30	20.4, CH_3	1.47, s
8-OAc	171.7, C	
	21.1, CH_3	2.01, s
11-OAc	172.1, C	
	21.2, CH_3	1.98, s
22-OAc	172.1, C	
	23.0, CH_3	1.93, s

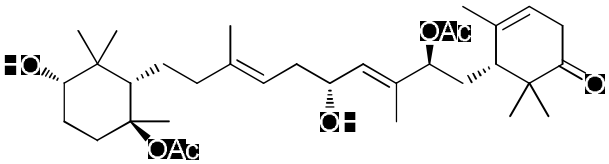


Table 4. ^{13}C and ^{1}H NMR assignment for compound **7** in MeOD-d_4

Position	δ_C	δ_H
1	48.6, C	
2	216.7, C	
3	38.9, CH_2	2.93, ddq (22.5, 4.5, 1.0)
		2.72, ddq (22.5, 3.0, 1.0)
4	119.6, CH	5.48, m
5	139.5, C	
6	51.1, CH	2.16, dd (8.0, 4.5)
7	36.0, CH_2	1.92, ddd (14.5, 10.0, 4.5)
		1.42, ddd (14.5, 8.0, 3.0)
8	78.4, CH	4.96, dd (10.0, 3.0)
9	139.5, C	
10	131.6, CH	5.33, br d (9.0)
11	68.9, CH	4.30, ddd (9.0, 7.0, 6.5)
12	37.2, CH_2	2.29, ddd (14.0, 7.0, 6.5)
		2.13, ddd (14.0, 7.0, 7.0)
13	120.7, CH	5.12, br t (7.0)
14	139.3, C	
15	44.0, CH_2	2.15, m
		1.98, ddd (12.5, 12.5, 5.0)
16	26.6, CH_2	1.56, m
		1.46, m
17	54.4, CH	1.57, m

18	41.8, C	
19	78.4, CH	3.27, dd (11.5, 4.0)
20	29.0, CH_2	1.69, dddd (13.5, 4.0, 4.0, 4.0)
		1.53, dddd (13.5, 11.5, 11.5, 4.0)
21	36.4, CH_2	2.52, ddd (13.5, 4.0, 4.0)
		1.77, ddd (13.5, 11.5, 4.0)
22	88.4, C	
23	26.4, CH_3	1.21, s
24	21.6, CH_3	1.09, s
25	24.1, CH_3	1.81, d (1.0)
26	13.1, CH_3	1.61, d (1.0)
27	16.7, CH_3	1.63, br s
28	28.8, CH_3	1.02, s
29	16.1, CH_3	0.81, s
30	21.2, CH_3	1.47, s
8-OAc	171.9, C	
	21.2, CH_3	1.99, s
22-OAc	172.1, C	
	23.0, CH_3	1.93, s

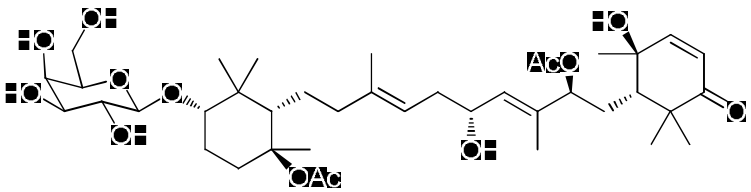


Table 5. ^{13}C and ^{1}H NMR assignment for compound **8** in MeOD-d_4

Position	δ_C	δ_H
1	46.7, C	
2	206.2, C	
3	125.3, CH	5.80, d (10.0)
4	158.0, CH	6.73, d (10.0)
5	72.0, C	
6	50.0, CH	2.06, dd (7.0, 4.0)
7	31.0, CH_2	1.95, ddd (15.0, 7.0, 5.0)
		1.78, ddd (15.0, 9.0, 4.0)
8	80.3, CH	5.45, dd (9.0, 5.0)
9	137.1, C	
10	131.5, CH	5.39, br d (9.0)
11	68.9, CH	4.34, ddd (9.0, 7.0, 6.0)
12	37.2, CH_2	2.31, ddd (14.0, 7.0, 6.0)
		2.16, ddd (14.0, 7.0, 7.0)
13	120.7, CH	5.15, br t (7.0)
14	139.2, C	
15	44.1, CH_2	2.14, m
		2.00, m
16	26.4, CH_2	1.54, m
		1.47, m
17	54.4, CH	1.61, m
18	42.1, C	
19	88.9, CH	3.29, dd (11.5, 4.0)

20	28.3, CH_2	2.04, dddd (13.5, 4.0, 4.0, 4.0)
		1.59, dddd (13.5, 11.5, 11.5, 4.0)
21	36.4, CH_2	2.47, ddd (13.5, 4.0, 4.0)
		1.78, ddd (13.5, 11.5, 4.0)
22	88.2, C	
23	25.3, CH_3	1.21, s
24	22.1, CH_3	1.02, s
25	23.3, CH_3	1.35, s
26	13.1, CH_3	1.71, d (1.0)
27	16.7, CH_3	1.65, br s
28	28.6, CH_3	1.10, s
29	16.7, CH_3	0.89, s
30	20.4, CH_3	1.47, s
1’	107.3, CH	4.27, d (7.5)
2’	73.1, CH	3.49, dd (7.5, 9.5)
3’	75.2, CH	3.42, dd (9.5, 3.5)
4’	70.3, CH	3.81, dd (3.5, 0.5)
5’	76.5, CH	3.48, dt (0.5, 6.0)
6’	62.5, CH_2	3.70, d (6.0)
8-OAc	172.1, C	
	21.3, CH_3	2.02, s
22-OAc	172.1, C	
	23.0, CH_3	1.93, s

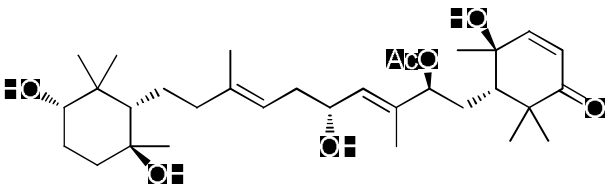


Table 6. ^{13}C and ^{1}H NMR assignment for compound **9** in MeOD-d_4

Position	δ_C	δ_H
1	46.7, C	
2	206.2, C	
3	125.3, CH	5.80, d (10.0)
4	158.0, CH	6.73, d (10.0)
5	72.0, C	
6	50.0, CH	2.06, dd (7.0, 4.0)
7	31.0, CH_2	1.94, ddd (15.0, 7.0, 5.0)
		1.78, ddd (15.0, 9.0, 4.0)
8	80.3, CH	5.45, dd (9.0, 5.0)
9	137.1, C	
10	131.5, CH	5.39, br d (9.0)
11	68.9, CH	4.34, ddd (9.0, 7.0, 6.0)
12	37.2, CH_2	2.30, ddd (14.0, 7.0, 6.0)
		2.15, ddd (14.0, 7.0, 7.0)
13	120.7, CH	5.16, br t (7.0)
14	139.2, C	
15	44.1, CH_2	2.14, m
		1.99, m
16	26.6, CH_2	1.56 m
		1.46 m
17	54.4, CH	1.57 m

18	41.8, C	
19	78.4, CH	3.27 dd (11.5, 4.0)
20	29.0, CH_2	1.68, dddd (13.5, 4.0, 4.0, 4.0)
		1.53, dddd (13.5, 11.5, 11.5, 4.0)
21	36.4, CH_2	2.51, ddd (13.5, 4.0, 4.0)
		1.76, ddd (13.5, 11.5, 4.0)
22	88.3, C	
23	25.2, CH_3	1.21, s
24	22.1, CH_3	1.02, s
25	23.3, CH_3	1.35, s
26	13.0, CH_3	1.71, d (1.0)
27	16.7, CH_3	1.65, br s
28	28.8, CH_3	1.02, s
29	16.1, CH_3	0.81, s
30	20.4, CH_3	1.47, s
8-OAc	172.1, C	
	21.3, CH_3	2.02, s
22-OAc	172.1, C	
	23.0, CH_3	1.93, s

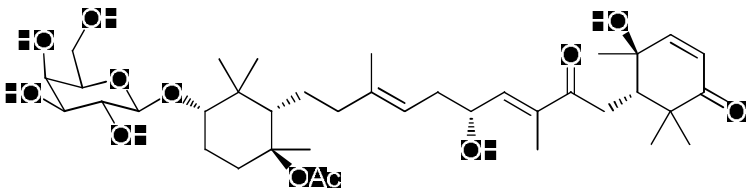


Table 7. ^{13}C and ^{1}H NMR assignment for compound **10** in MeOD-d_4

Position	δ_C	δ_H
1	46.7, C	
2	205.8, C	
3	125.5, CH	5.81, d (10.0)
4	157.7, CH	6.79, d (10.0)
5	71.6, C	
6	51.1, CH	2.86, dd (8.0, 3.5)
7	35.1, CH_2	3.00, dd (17.5, 8.0)
		2.85, dd (17.5, 3.5)
8	203.8, C	
9	137.7, C	
10	144.0, CH	6.57, br d (8.0)
11	69.6, CH	4.54, ddd (8.0, 7.5, 6.5)
12	36.5, CH_2	2.41, ddd (14.0, 7.5, 6.5)
		2.29, ddd (14.0, 7.5, 7.5)
13	120.0, CH	5.19, br t (7.5)
14	139.9, C	
15	43.8, CH_2	2.14, ddd (12.5, 12.5, 5.0)
		2.04, ddd (12.5, 12.5, 4.5)
16	26.3, CH_2	1.56, dddd (12.5, 12.5, 12.5, 5.0)
		1.44, m
17	54.4, CH	1.59, m
18	42.0, C	
19	88.9, CH	3.28, dd (11.5, 4.0)

20	28.2, CH_2	2.05, dddd (13.5, 4.0, 4.0, 4.0)
		1.58, dddd (13.5, 11.5, 11.5, 4.0)
21	36.2, CH_2	2.48, ddd (13.5, 4.0, 4.0)
		1.76, ddd (13.5, 11.5, 4.0)
22	88.2, C	
23	25.1, CH_3	1.01, s
24	21.8, CH_3	1.06, s
25	23.2, CH_3	1.34, s
26	12.8, CH_3	1.80, d (1.0)
27	16.6, CH_3	1.66, br s
28	28.5, CH_3	1.09, s
29	16.6, CH_3	0.88, s
30	20.5, CH_3	1.47, s
1’	107.2, CH	4.28, d (7.5)
2’	73.1, CH	3.50, dd (7.5, 9.5)
3’	75.2, CH	3.43, dd (9.5, 3.5)
4’	70.3, CH	3.82, dd (3.5, 0.5)
5’	76.4, CH	3.48, dt (0.5, 6.0)
6’	62.5, CH_2	3.71, d (6.0)
22-OAc	172.1, C	
	22.9, CH_3	1.92, s

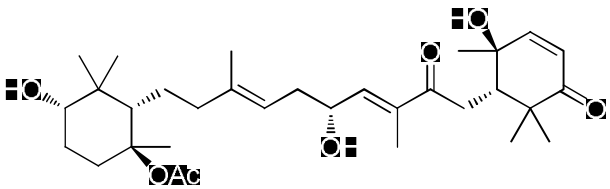


Table 8. ^{13}C and ^{1}H NMR assignment for compound **11** in MeOD-d_4

Position	δ_C	δ_H
1	46.8, C	
2	205.8, C	
3	125.6, CH	5.82, d (10.0)
4	157.7, CH	6.77, d (10.0)
5	71.7, C	
6	51.1, CH	2.86, dd (8.0, 3.5)
7	35.1, CH_2	3.02, dd (17.5, 8.0)
		2.84, dd (17.5, 3.5)
8	203.8, C	
9	137.8, C	
10	144.0, CH	6.57, br d (8.0)
11	69.6, CH	4.54, ddd (8.0, 7.5, 6.5)
12	36.5, CH_2	2.41, ddd (14.0, 7.5, 6.5)
		2.28, ddd (14.0, 7.5, 7.5)
13	120.1, CH	5.19, br t (7.5)
14	140.0, C	
15	44.0, CH_2	2.15, ddd (12.5, 12.5, 5.0)
		2.02, ddd (12.5, 12.5, 4.5)
16	26.5, CH_2	1.53 m
		1.44 dddd (12.5, 12.5, 5.0, 3.0)
17	54.3, CH	1.55 m
18	41.8, C	
19	78.4, CH	3.26 dd (11.5, 4.0)

20	29.0, CH_2	1.69, dddd (13.5, 4.0, 4.0, 4.0)
		1.52, dddd (13.5, 11.5, 11.5, 4.0)
21	36.4, CH_2	2.52, ddd (13.5, 4.0, 4.0)
		1.76, ddd (13.5, 11.5, 4.0)
22	88.3, C	
23	25.1, CH_3	1.01, s
24	21.8, CH_3	1.06, s
25	23.1, CH_3	1.34, s
26	12.9, CH_3	1.79, d (1.0)
27	16.7, CH_3	1.66, br s
28	28.8, CH_3	1.01, s
29	16.0, CH_3	0.80, s
30	20.3, CH_3	1.46, s
22-OAc	172.1, C	
	23.0, CH_3	1.91, s

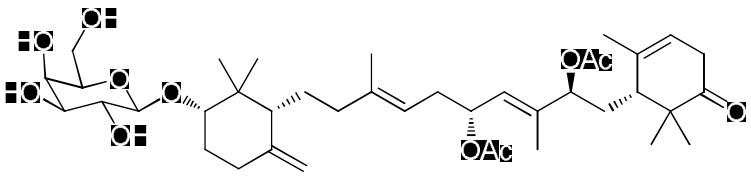


Table 9. ^{13}C and ^{1}H NMR assignment for compound **12** in MeOD-d_4

Position	δ_C	δ_H
1	48.9, C	
2	216.7, C	
3	38.2, CH_2	2.89, ddq (22.5, 4.5, 1.0)
		2.68, ddq (22.5, 3.0, 1.0)
4	119.5, CH	5.46, m
5	139.3, C	
6	51.0, CH	2.11, dd (6.5, 5.0)
7	35.6, CH_2	1.87, ddd (14.5, 9.0, 5.0)
		1.42, ddd (14.5, 6.5, 4.5)
8	78.4, CH	4.97, dd (9.0, 4.5)
9	139.1, C	
10	127.6, CH	5.31, br d (9.0)
11	72.2, CH	5.39, ddd (9.0, 7.0, 6.5)
12	34.0, CH_2	2.38, ddd (14.0, 7.0, 7.0)
		2.29, ddd (14.0, 7.5, 7.5)
13	119.5, CH	5.03, br t (7.0)
14	140.2, C	
15	39.7, CH_2	2.04, m
		1.77, m
16	24.8, CH_2	1.66, m
		1.55, m
17	52.7, CH	1.67, m
18	42.0, C	
19	88.4, CH	3.35, dd (9.5, 4.5)

20	32.4, CH_2	2.02, dddd (12.5, 4.5, 4.5, 4.5)
		1.64, m
21	34.0, CH_2	2.31, ddd (12.5, 4.5, 4.5)
		1.95, ddd (12.5, 11.5, 4.5)
22	149.4, C	
23	26.5, CH_3	1.20, s
24	21.7, CH_3	1.07, s
25	23.9, CH_3	1.79, d (1.0)
26	13.0, CH_3	1.66, d (1.0)
27	16.5, CH_3	1.61, br s
28	26.7, CH_3	1.07, s
29	17.5, CH_3	0.79, s
30	108.6, CH_2	4.84, br s
		4.55, br s
1’	107.3, CH	4.27, d (7.5)
2’	73.2, CH	3.51, dd (7.5, 9.5)
3’	75.3, CH	3.43, dd (9.5, 3.5)
4’	70.3, CH	3.82, dd (3.5, 0.5)
5’	76.4, CH	3.47, dt (0.5, 6.0)
6’	62.4, CH_2	3.72, dd (6.0, 11.0)
		3.69, dd (6.0, 11.0)
8-OAc	171.8, C	
	21.1, CH_3	2.01, s
11-OAc	172.2, C	
	21.2, CH_3	1.99, s

2. Nortriterpene Glycosides of the Sarasinoside Class from the Sponge *Lipastrotethya* sp.

Five new nortriterpene glycosides, along with eight known compounds of the sarasinoside class, were isolated from the tropical sponge *Lipastrotethya* sp. collected from Chuuk, Micronesia. The structures of these new compounds were determined by combined spectroscopic and chemical methods and designated as sarasinosides N-R (**21-25**). The aglycone portions of **22-25** were found to be unprecedented among nortriterpeneoids on the basis of extensive NMR analyses. Several of these compounds exhibited moderate cytotoxicity against A549 and K562 cell-lines as well as weak inhibitory activity against Na^+/K^+-ATPase.

2-1. Introduction

Sponges are widely recognized as prolific sources of marine natural products with diverse biogenetic origins and bioactivities.[9] However, saponins possessing triterpene or steroidal aglycones are relatively rare and occupy only a minor fraction of the known sponge-derived metabolites. Most notable examples of sponge-derived saponins

include erylosides, pouosides, and sarasinosides.[14-21,25-28] Of these compounds, sarasinosides are norlanostane nortriterpene glycosides isolated from tropical sponges of the genera Asteropus and Melophlus.[15-21,27] These compounds are reported to possess antimicrobial,[19,20] cytotoxic,[16,17] and piscicidal[17] activities.

Recently, we reported the isolation and structural determination of cytotoxic triterpene galactosides, pouosides, and corresponding aglycone metabolites from the sponge Lipastrotethya sp. (order Halichondrida, family Dictyonellidae), collected from Chuuk, Micronesia.[28] As noted in that study, however, the crude extract of this sponge contained sarasinoside A_1 (**13**) as the major metabolite. Subsequent chromatographic analyses of the polar fractions of the crude extract yielded several saponins of the same structural class. Herein, we report the structures of sarasinosides N–R (**21**–**25**), new constituents of the sarasinoside class. Sarasinoside N is structurally analogous to sarasinoside A_1, possessing a triglycoside moiety, while sarasinosides O–R possess nortriterpene aglycones with distinct oxidation patterns. In addition, a new semi-synthetic derivative (**14**) was isolated as a natural product. Several of these compounds exhibited moderate cytotoxicity against K562 and A549 cell lines as well as weak inhibition against Na+/K+-ATPase. Structure-

activity characterizations of these compounds provided important information about the role of the aglycone and glycoside portions on cytotoxicity.

2-2. Results and discussion

The sponge samples were lyophilized, macerated, and repeatedly extracted with CH_2Cl_2 and MeOH. The combined extracts were separated by solvent-partitioning followed by ODS vacuum flash chromatography and then repeated ODS-HPLC. The major constituent was identified as sarasinoside A_1 (**13**), in addition to the minor component sarasinosides A_2 (**15**), A_3 (**16**), H_1 (**17**), H_2 (**18**), J (**19**), and M (**20**), by combined spectroscopic methods and ^1H and ^{13}C NMR data to authentic samples and compare to literature citations.[16,17,19,20] Also isolated was a sarasinoside derivative (**14**), which was originally reported as a glycolysis product from sarasinoside A_1.[17]

Sarasinoside N (**21**) was isolated as a white amorphous solid, which was analyzed for $C_{50}H_{80}N_2O_{16}$ by HRFABMS analysis supported by ^{13}C NMR data. The spectral data of this compound were reminiscent of those from **13**, suggesting the nortriterpene saponin nature. Detailed examination of the ^{13}C and ^1H NMR data revealed that these compounds

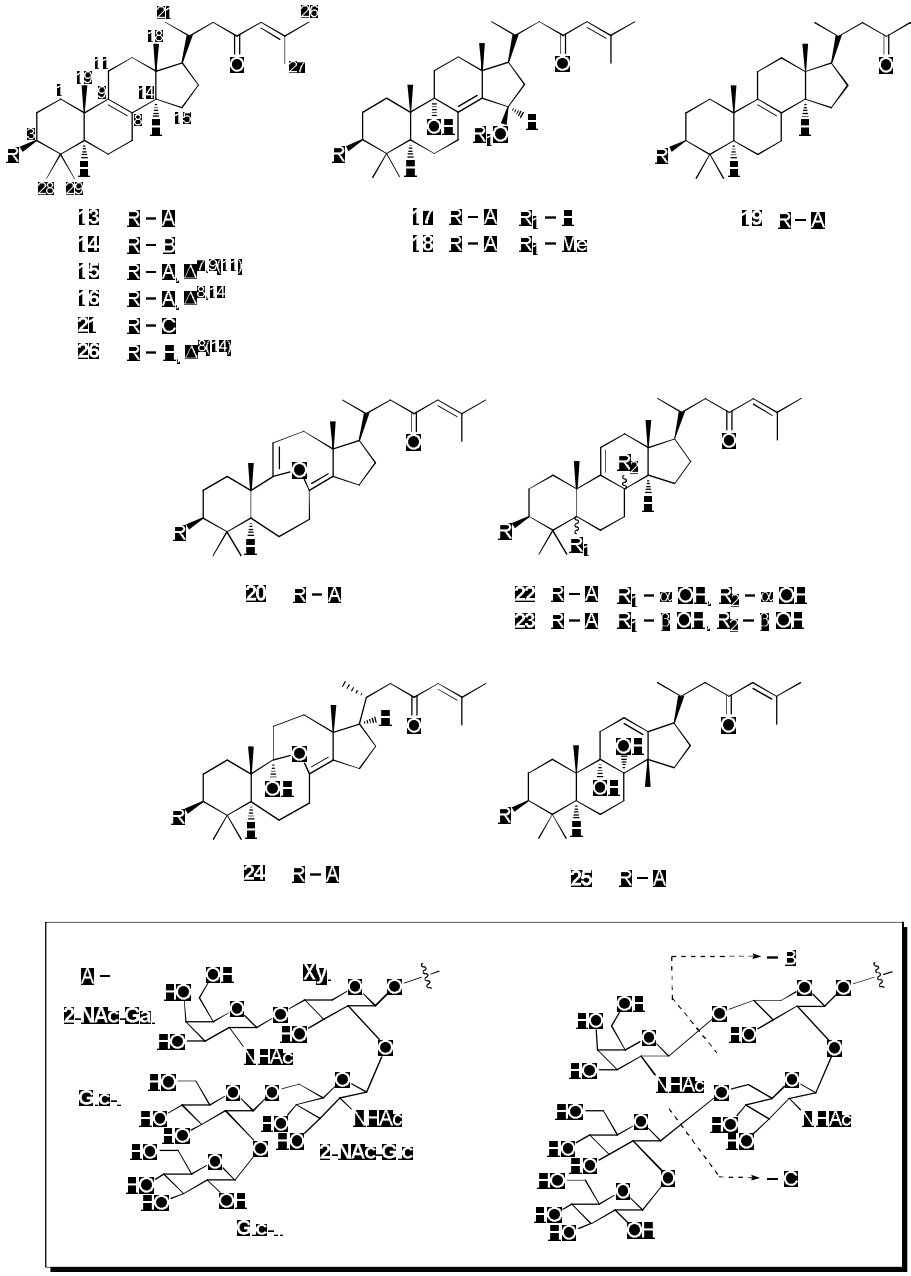


Figure 8. Chemical structures of isolated compounds (**13-26**).

have the same aglycone moiety; thus, structural differences occurred only in their glycoside portion (Table 11). The number of carbon signals in the ^{13}C NMR spectra of **21** suggested the presence of a triglycoside moiety that was comparable to the pentaglycoside of **13**. This interpretation was further supported by the number of characteristic anomeric signals in the NMR data (δ_H 4.88-4.37, δ_C 106.0-102.5) (Table 11). These sugar units were identified by an acid-catalyzed hydrolysis of **21** followed by GC analysis that clearly showed the presence of a xylose, a 2-*N*-acetylglucosamine, and a 2-*N*-acetylgalactosamine (Experimental Section). The cyclization pattern of these sugar residues was determined by 2D NMR experiments. After assigning the individual NMR signals for these sugar units by extensive COSY, TOCSY, and *g*HSQC analysis (Table 11), *g*HMBC experiments showed long-range correlations at H-1'/C-5', H-5'/C-1', H-5''/C-1'', H-1'''/C-5''', and H-5'''/C-1''', thus revealing the pyranose nature of all of these sugars (Figure 9).

The connectivity of sugar residues was also determined by *g*HMBC experiments in which the characteristic 3-bond correlations were found for all of the glycosidic linkages among these sugars and aglycone. This HMBC-based linkage was further supported by the ROESY experiments in which cross-peaks were found at H-3/H-1', H-2'/H-1'',

and H-4'/H-1'''. Overall, the NMR spectra showed that **21** had the same branched sugar assembly as **13**. Thus, the structure of sarasinoside N (**21**) was unambiguously determined to be a triglycosidic derivative of sarasinoside A_1 (**13**).

The molecular formula of sarasinoside O (**22**) was deduced as $C_{62}H_{100}N_2O_{28}$ by combined HRMS and ^{13}C NMR analyses. The NMR data of this compound were very similar to those of sarasinoside A_1 (**13**). Detailed examination of the NMR data showed that the pentaglycoside portion of **13** was intact in **22**, which was confirmed by acidic hydrolysis followed by GC analysis of the sugar hydrolysates (Experimental Section). However, the ^{13}C NMR spectra of this compound showed the replacement of the C-8 double bond (δ_C 137.4 C, 128.8 C) of **13** with a trisubstituted bond (δ_C 156.2 C, 112.6 CH), which corresponded to a newly apparent signal (δ_H 5.03, 1 H) in the ^1H NMR spectra (Tables 12). The ^{13}C NMR data also showed the appearance of two oxy-quaternary carbons at δ 93.8 and 87.3.

These changes at the aglycone portion were determined by a combination of 2D NMR analyses. After adequately assigning all of the proton-bearing carbons and their protons by COSY and *g*HSQC data, the connectivity of the partial structures was defined by *g*HMBC experiments

(Figure 9). That is, the placement of a hydroxy group at C-5 was determined by long-range couplings of a quaternary carbon at δ 93.8 with singlet methyl protons at δ 1.26 (H-19), 1.05 (H-28), and 1.18 (H-29). The trisubstituted double bond was placed at C-9(11) based on correlations of the olefinic proton and carbon with neighboring ones, specifically H-19/C-9 and H-11/C-13. Finally, the remaining hydroxy group was placed at C-8 by the key HMBC correlation, H-11/C-8. In addition, the pyranose nature of sugar units and their assembly pattern were defined to be the same as in **13** by these 2D NMR experiments (Table 12).

The configurations at the asymmetric centers were assigned by ROESY analysis (Figure 10). The *trans* A/B ring juncture with the 5α-OH was secured by the NOE cross-peaks at H-2β/H-19, H-2β/H-28, H-6α/H-29, H-6β/H-19, H-6β/H-28, and H-19/H-28. Similarly, the *trans* B/C ring juncture with the 8α-OH was assigned by those at H-7α/H-15β, H-7β/H-18, and H-7β/H-19. Thus, the structure of sarasinoside O (**22**) was determined to be a $\Delta^{9(11)}$-5α,8α-dihydroxy derivative of sarasinoside A_1 (**13**).

The molecular formula of sarasinoside P (**23**) was established to be $C_{62}H_{100}N_2O_{28}$, the same as **22**, by HRFABMS analysis. The spectroscopic data of this compound were also very similar to those of **22**,

thereby suggesting the nortriterpene pentaglycoside nature of this compound. However, careful examination of the ^{13}C and ^{1}H NMR data revealed that signals at the ring portion of the aglycones shifted noticeably, while those at the side chain and sugar moieties were intact (Tables 13). The most significant differences occurred for the signals of carbons and protons at the A/B and B/C ring junctures and vicinity, implying the structural differences at these junctures. This interpretation was supported by the combined 2D NMR analyses, which defined an identical planar structure for **22** and **23**, despite the significant NMR chemical shifts between these compounds (Figure 9).

The configurational differences were determined by ROESY experiments. The NOE cross-peaks at H-1α/H-6α, H-3/H-6α, H-6α/H-29, H-6β/H-29, and H-19/H-28 suggested a 5β-OH, thus a *cis* A/B ring juncture. Similarly, the 8β-OH and the accompanying *cis* B/C ring juncture were also assigned by the H-7α/H-14 and H-18/H-19 cross-peaks. This interpretation was further supported by the lack of an H-7β/H-18 cross-peak that was crucial for the 8α-OH assignment in **22**. Thus, the structure of sarasinoside P (**23**) was determined to be the 5,8-bisepimer of sarasinoside O (**22**). To the best of our knowledge, this is the first reported example of sarasinosides with *cis* A/B ring junctures.

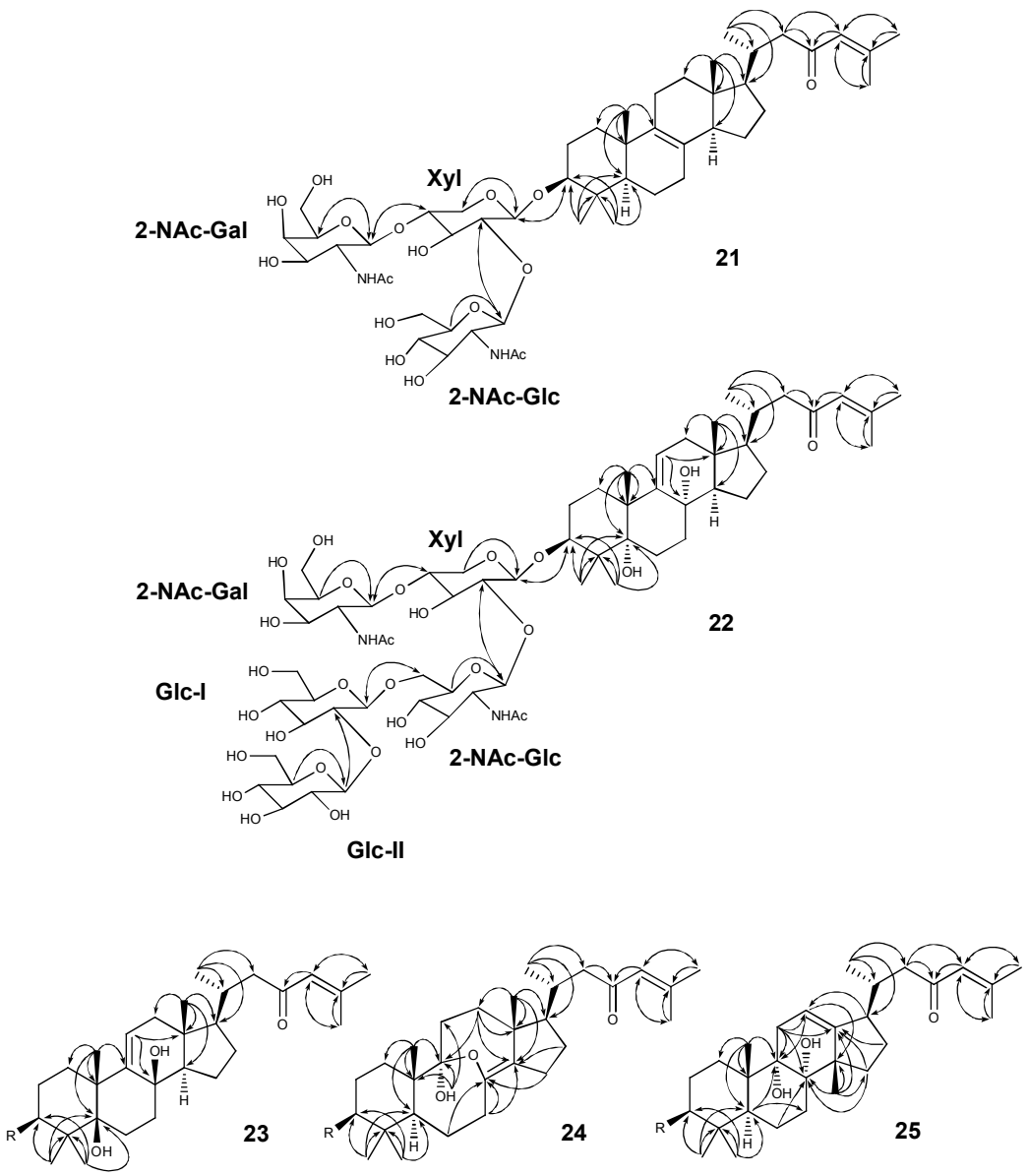


Figure 9. *g*HMBC correlations for compounds **21–25**

Another minor constituent, sarasinoside Q (**24**), was isolated as a white amorphous solid. The molecular formula of **24** was found to be $C_{62}H_{100}N_2O_{28}$ by HRFABMS and ^{13}C NMR analyses. The NMR data of this compound showed the typical characteristics of sarasinosides, namely twenty-nine carbons including seven methyls, a terminal unsaturated ketone, and pentaglycoside. The most conspicuous features of the ^{13}C NMR data of this compound were signals that corresponded to a tetrasubstituted double bond at δ 142.3 and 129.4 and an oxy-quaternary carbon at δ 107.6 (Table 14). The placements of these groups were determined by the *g*HMBC experiments. Long-range correlations of the olefinic carbons with the neighboring H-6, H-7, H-12, H-15, H-16, and H-18 placed this double bond at C-8 (14). Similarly, the oxy-quaternary carbon was placed at C-9 by its long-range correlations with H-12 and H-19. This interpretation and the identification of the functionality to be a hemiacetal group was further supported by the long-range correlations of the hydroxyl group (δ_H 7.05 in pyridine-d_5, Supporting Information) with C-9, C-10, and C-11 (Figure 9).

The configurations of **24** were assigned by ROESY experiments. Several cross-peaks among the protons in the A and B rings, coupled with chemical shifts of the NMR signals for the A ring, suggested a *trans*

juncture. The newly apparent C-9 hydroxyl group was determined to be α-oriented to the B/C juncture by its NOE cross-peaks with H-1α, H-5, H-11α, and H-12α. Thus, the structure of sarasinoside Q (**24**) was determined to be the 9,11-hydrated derivative of the congener sarasinoside M (**20**).

Finally, the molecular formula of sarasinoside R (**25**) was determined to be $C_{62}H_{100}N_2O_{28}$, identical to several congeners such as **17**, **22**, and **23**, by combined HRFABMS and ^{13}C NMR analyses. Although the NMR data of this compound were highly reminiscent of other pentaglycosidic sarasinosides, the ^{1}H and ^{13}C NMR signals for the ring portion of the aglycone showed significant shifts (Tables 15). Based on the matching of protons and their bearing carbons by the COSY and *g*HSQC experiments, the planar structure of the ring portion was determined by *g*HMBC experiments. Long-range couplings with the H-19 methyl and H-5 methine protons placed a hydroxyl group at C-9 (δ_C 72.2). Similarly, couplings with a methyl proton (δ_H 1.02) placed four carbons (δ_C 148.4, 70.0, 49.2, and 33.7) in the vicinity. However, the lack of long-range coupling of this methyl proton with C-17 (δ_C 50.3), as was observed in other compounds, suggested the migration of this methyl group from C-18. This problem was solved by placement of the oxy-quaternary carbon (δ_C

70.0) at C-8 by its long-range correlation with H-6, thereby assigning the methyl group to be attached at C-14. The remaining trisubstituted double bond was also placed at C-12 by several correlations of the olefinic carbons and protons with neighboring ones. Thus, the planar structure of the aglycone portion of **25** was determined to be a C-18 methyl-rearranged nortriterpene, which is unprecedented among the sarasinosides.

The configurations at the C-8, C-9, and C-14 asymmetric centers at the ring junctures were determined by ROESY experiments. First, the *trans* A/B ring juncture was defined by a series of cross-peaks: H-1α/H-3, H-1α/H-5/, H-3/H-5, H-6β/H-19, H-6β/H-28, and H-19/H-28. Then, additional cross-peaks at H-6β/H-18, H-11β/H-18, H-11β/H-19, H-15α/H-17, H-15β/H-18, H-16β/H-18, and H-18/H-19 unambiguously defined the *cis* B/C ring juncture with both 8-OH and 9-OH in the α-orientation. In addition, the C-18 methyl group was placed in β-orientation to the C/D juncture, which is consistent with the 1,2-migration of this methyl group from the original norlanostane skeleton (Figure 10). Thus, sarasinoside R (**25**) was determined to be a nortriterpene pentaglycoside possessing a rearranged norlanostane aglycone.

Previous studies of the bioactivities of sarasinosides reported antimicrobial, cytotoxic, and piscicidal activities for these compounds.

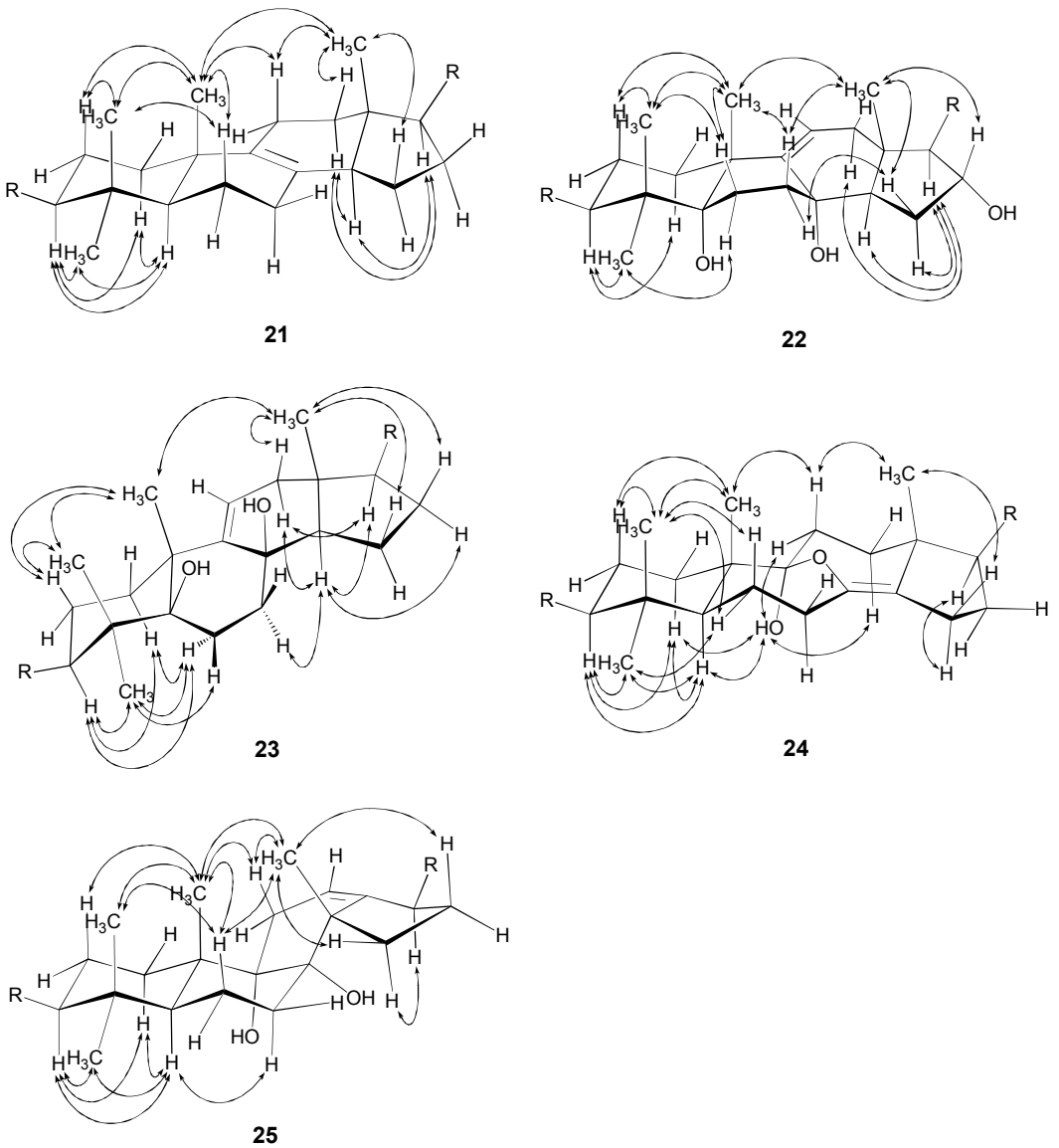


Figure 10. Selected NOE correlations for the aglycone portion of compounds **21–25**.

Table 10. Results of Cytotoxicity tests

Compound	K562 LC_{50} (μM)	A549 LC_{50} (μM)
13	7.4	7.6
14	18.7	9.1
15	8.5	7.6
16	24.8	10.1
17	37.4	14.2
18	42.7	>100
19	20.8	10.3
20	12.1	7.7
21	20.2	10.7
22	61.5	54.7
23	11.6	17.9
24	17.9	14.0
25	>100	14.7
26	69.4	41.6
doxorubicin	4.7	1.7

However, the antimicrobial activity is controversial, possibly due to the selection of different microbial strains. In our study, all of these compounds were inactive (MIC > 100 μg/mL) against selected strains of Gram-positive (*Bacillus subtilis* ATCC 6633, *Micrococcus luteus* IFO 12708, and *Staphylococcus aureus* ATCC 6538p) and Gram-negative (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 3851, and *Salmonella typhimurium* ATCC 14028) bacteria and pathogenic fungi (*Aspergillus fumigatus* HIC 6094, *Candida albicans* ATCC 10231, *Trichophyton mentagrophyte*s IFO 40996, and *Trichophyton rubrum* IFO 9185). Neither of these compounds were noticeably active (IC_{50} > 50 μg/mL) against microbial-derived sortase A and isocitrate lyase, key enzymes of microbial metabolism. Therefore, the antimicrobial activity of sarasinosides is either strain specific or should be re-investigated more thoroughly.

Several of these compounds showed moderate cytotoxicity against K562 leukemia and A549 lung carcinoma strains (Table 10). Although the degree of inhibition differed among the strains, a few significant structure-activity relationships were found for the sarasinosides. Comparison of the activity among **13**, **14**, **21**, and **26**, a glycolysis product from **13**, showed that the cytotoxicity significantly depends on the presence of glycoside

portion. However, the size or units of the sugar moieties only weakly influenced the activity. As shown from **13** and **19**, the loss of the terminal double bond at C-24 weakly decreased the cytotoxicity. This result suggested that the presence of hydroxyl groups at the aglycones negatively contributed to the cytotoxicity (**13**, **17**, **18**, **22**, **23**, and **25**; **20** and **24**). It is also noteworthy that configurations at the ring juncture significantly attributed to the bioactivity (**22** and **23**). In addition, compounds **13**, **14**, and **21** weakly inhibited (IC_{50} 60.0, 59.4, and 54.1 μg/mL for **13**, **14**, and **21**, respectively) Na^+/K^+-ATPase, while the remaining ones were inactive (IC_{50} > 100 μg/mL).[22]

2-3. Experimental section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter using a 1-cm cell. UV spectra were recorded on a Hitachi U-3010 spectrophotometer and IR spectra were recorded on a JASCO 300E FT-IR spectrometer. NMR spectra were recorded in MeOH-d_4 containing Me_4Si as an internal standard on Bruker Avance 500 and 600 spectrometers. Proton and carbon NMR were measured at 600 and 150 MHz (**13**–**18**, **20**, **21**, **25**, and **26**) and 500 and 125 MHz (**19**, and **22**–**24**), respectively. Mass spectrometric data were

obtained at the Korea Basic Science Institute (Daegu, Korea) and were acquired using a JEOL JMS 700 mass spectrometer with *meta*-nitrobenzyl alcohol (NBA) as a matrix for the FABMS. HPLC was performed on a SpectraSystem p2000 equipped with a SpectraSystem RI-150 refractive index detector. All solvents were spectroscopic grade or distilled from glass prior to use.

Animal Material. Specimens of *Lipastrotethya* sp. (voucher collection number 102CH-232) were collected by hand using SCUBA equipment off the shore of Weno Island, Chuuk state, Federated States of Micronesia, at a depth of 15 m on February 18, 2010. The morphological features of the specimens were reported previously. A voucher specimen (registry No. Spo. 57) was deposited at the Natural History Museum, Hannam University, Korea under the curatorship of C.J.S.

Extraction and Isolation. Freshly collected specimens were immediately frozen and stored at –25 °C until use. Lyophilized specimens were macerated and repeatedly extracted with MeOH (2 L × 3) and CH_2Cl_2 (2 L × 3). The combined extracts (112.7 g) were successively partitioned between H_2O (99.3 g) and *n*-BuOH (24.8 g); the latter fraction was repartitioned between H_2O-MeOH (15:85) (13.72 g) and *n*-hexane

(7.72 g). An aliquot of the former layer (9.22 g) was separated by C_{18} reversed-phase vacuum flash chromatography using sequential mixtures of MeOH and H_2O as eluents (six fractions in gradient, H_2O-MeOH, from 50:50 to 0:100), acetone, and finally EtOAc.

Based on the results of ^1H NMR and cytotoxicity analyses, the fractions eluted with H_2O-MeOH (30:70) (0.33 g), H_2O-MeOH (20:80) (0.74 g), and H_2O-MeOH (10:90) (1.10 g) were chosen for separation. The fraction eluted with H_2O-MeOH (30:70) was separated by semi-preparative reversed-phase HPLC (YMC-ODS column, 10 mm × 250 mm; H_2O-MeOH, 35:65), yielding two peaks rich with secondary metabolites. Further purification of the first peak by reversed-phase HPLC (YMC-Pack CN column, 10 mm × 250 mm; H_2O-MeOH, 65:35) isolated compound **18** as an amorphous solid, while purification of the second peak yielded compound **17**.

The H_2O-MeOH (20:80) fraction was separated by reversed-phase HPLC (YMC-ODS column; H_2O-MeOH, 30:70) to yield, in order of elution, compounds **24**, **19**, **25**, and **23**. These metabolites were then purified by reversed-phase HPLC (YMC-Pack CN column; H_2O-MeOH, 60:40).

The H_2O-MeOH (10:90) fraction was separated and purified by reversed-phase HPLC (YMC-ODS column; H_2O-MeOH, 20:80) yielding, in order of elution, compounds **20**, **22**, **16**, **15**, **13**, **14**, and **21**. These metabolites were then purified by HPLC (YMC-Pack CN column; H_2O-MeCN, 65:35 for **20**, **22**, **16**, and **15** and YMC-Pack ODS column; H_2O-MeOH, 25:75 for **13**, **14**, and **21**, respectively). The purified metabolites were isolated in the following amounts: 721.8, 9.2, 18.2, 16.4, 53.6, 18.2, 15.0, 39.6, 4.9, 4.5, 3.6, 18.2, and 6.5 mg of **13**–**25**, respectively.

Sarasinoside N (**21**): white amorphous solid; $[\alpha]_D^{25}$ –10.0 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 236 (2.01) nm; IR (ZnSe) ν_{max} 3400 (br), 2940, 1650 (br), 1565, 1380 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1, 2, and 3, respectively; HRFABMS *m*/*z* 987.5409 $[M + Na]^+$ (calcd for $C_{50}H_{80}N_2O_{16}Na$, 987.5406).

Sarasinoside O (**22**): White amorphous solid; $[\alpha]_D^{25}$ –2.6 (*c* 0.75, MeOH); UV (MeOH) λ_{max} (log ε) 238 (2.05) nm; IR (ZnSe) ν_{max} 3400 (br), 2930, 1650 (br), 1570, 1450, 1375 cm^{-1}; ^1H and ^{13}C NMR data of the aglycone, see Tables 1, 2, and 3, respectively; HRFABMS *m*/*z* 1325.6221 $[M – H_2O + Na]^+$ (calcd for $C_{62}H_{98}N_2O_{27}Na$, 1325.6255).

Sarasinoside P (**23**): White amorphous solid; $[\alpha]_D^{25}$ –6.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 238 (1.75) nm; IR (ZnSe) ν_{max} 3400 (br), 2930, 1650 (br), 1580, 1450, 1375 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ^1H NMR data of the sugar portion (CD_3OD) Xyl δ_H 4.26 (1H, d, *J* = 7.8 Hz, H-1'), 3.88 (H-2'), 3.85 (H-5'), 3.65 (H-4'), 3.49 (1H, dd, *J* = 9.1, 9.1 Hz, H-3'), 3.14 (1H, dd, *J* = 11.3, 10.2 Hz, H-5'); 2-NAc-Glc δ_H 4.81 (1H, d, *J* = 8.6 Hz, H-1''), 4.10 (1H, br d, *J* = 12.6 Hz, H-6''), 3.74 (1H, dd, *J* = 12.6, 7.6 Hz, H-6''), 3.66 (H-2'', H-5''), 3.37 (H-3''), 3.15 (1H, dd, *J* = 9.8, 8.7 Hz, H-4''), 1.97 (3H, s, NHAc); Glc-I δ_H 5.03 (1H, d, *J* = 7.9 Hz, H-1'''), 3.82 (1H, dd, *J* = 11.8, 2.5 Hz, H-6'''), 3.70 (H-6'''), 3.67 (H-3'''), 3.43 (1H, dd, *J* = 9.8, 5.2, 2.5 Hz, H-5'''), 3.37 (H-2'''), 3.31 (H-4'''); Glc-II δ_H 4.61 (1H, d, *J* = 7.4 Hz, H-1''''), 3.87 (H-6''''), 3.72 (1H, dd, *J* = 12.5, 6.0 Hz, H-6''''), 3.40 (H-2'''', H-3''''), 3.32 (H-4''''), 3.27 (1H, ddd, *J* = 9.5, 5.5, 2.3 Hz, H-5''''); 2-NAc-Gal δ_H 4.48 (1H, d, *J* = 8.4 Hz, H-1'''''), 3.88 (H-6'''''), 3.85 (H-2'''''), 3.78 (H-4'''''), 3.71 (H-6'''''), 3.62 (H-3''''', H-5'''''), 1.98 (3H, s, NHAc); ^{13}C NMR data of the sugar portion (CD_3OD) Xyl δ_C 106.4 (C-1'), 79.9 (C-4'), 78.0 (C-2'), 77.5 (C-3'), 64.3 (C-5'); 2-NAc-Glc δ_C 174.3 (NHAc), 102.1 (C-1''), 78.0 (C-5''), 77.0 (C-3''), 72.4 (C-4''), 70.1 (C-6''), 57.9 (C-2''), 23.0 (NHAc); Glc-I δ_C 102.6 (C-1'''), 83.6 (C-2'''), 77.9 (C-3'''), 77.0

(C-5’’’), 71.6 (C-4’’’), 62.8 (C-6’’’); Glc-II δ_C 105.8 (C-1’’’’), 78.3 (C-5’’’’), 77.7 (C-3’’’’), 75.8 (C-2’’’’), 71.5 (C-4’’’’), 62.6 (C-6’’’’); 2-NAc-Gal δ_C 173.9 (NHAc), 102.7 (C-1’’’’’), 77.2 (C-5’’’’’), 72.8 (C-3’’’’’), 69.9 (C-4’’’’’), 63.1 (C-6’’’’’), 54.6 (C-2’’’’’), 23.0 (NHAc); HRFABMS *m*/*z* 1341.6031 $[M - H_2O + K]^+$ (calcd for $C_{62}H_{98}N_2O_{27}K$, 1341.5994).

Sarasinoside Q (**24**): White amorphous solid; $[\alpha]_D^{25}$ –29.1 (*c* 0.80, MeOH); UV (MeOH) λ_{max} (log ε) 240 (1.96) nm; IR (ZnSe) ν_{max} 3400 (br), 2930, 1645 (br), 1565, 1375 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ^1H NMR data of the sugar portion (CD_3OD) Xyl δ_H 4.29 (1H, d, *J* = 7.8 Hz, H-1’), 3.90 (1H, dd, *J* = 9.1, 7.8 Hz, H-2’), 3.86 (1H, dd, *J* = 11.4, 5.5 Hz, H-5’), 3.67 (H-4’), 3.50 (1H, dd, *J* = 9.1, 9.1 Hz, H-3’), 3.14 (1H, dd, *J* = 11.4, 10.0 Hz, H-5’); 2-NAc-Glc δ_H 4.82 (H-1’’), 4.08 (1H, br d, *J* = 12.5 Hz, H-6’’), 3.77 (1H, dd, *J* = 12.5, 7.4 Hz, H-6’’), 3.67 (H-2’’, H-5’’), 3.40 (H-3’’), 3.14 (1H, dd, *J* = 8.9, 8.9 Hz, H-4’’), 1.99 (3H, s, NHAc); Glc-I δ_H 5.08 (1H, d, *J* = 7.9 Hz, H-1’’’), 3.81 (1H, dd, *J* = 11.8, 2.5 Hz, H-6’’’), 3.69 (H-3’’’, H-6’’’), 3.45 (1H, ddd, *J* = 9.4, 5.4, 2.5 Hz, H-5’’’), 3.37 (1H, dd, *J* = 9.2, 7.9 Hz, H-2’’’), 3.31 (H-4’’’); Glc-II δ_H 4.61 (1H, d, *J* = 7.5 Hz, H-1’’’’), 3.88 (H-6’’’’), 3.72 (1H, dd, *J* = 12.2, 6.0 Hz, H-6’’’’), 3.40 (H-2’’’’, H-3’’’’), 3.32 (H-4’’’’), 3.27 (1H, ddd, *J* = 9.6, 6.0, 2.4 Hz, H-5’’’’); 2-NAc-Gal δ_H 4.48 (1H, d, *J* = 8.4 Hz, H-1’’’’’),

3.89 (H-6’’’’’), 3.87 (H-2’’’’’), 3.79 (H-4’’’’’), 3.71 (H-6’’’’’), 3.63 (H-3’’’’’, H-5’’’’’), 1.98 (3H, s, NHAc); ^{13}C NMR data of the sugar portion (CD_3OD) Xyl δ_C 106.6 (C-1’), 79.8 (C-4’), 78.0 (C-2’), 77.8 (C-3’), 64.2 (C-5’); 2-NAc-Glc δ_C 173.9 (NHAc), 102.0 (C-1’’), 78.2 (C-5’’), 77.0 (C-3’’), 72.4 (C-4’’), 70.1 (C-6’’), 57.9 (C-2’’), 23.0 (NHAc); Glc-I δ_C 102.6 (C-1’’’), 83.4 (C-2’’’), 78.0 (C-3’’’), 77.2 (C-5’’’), 71.6 (C-4’’’), 62.6 (C-6’’’); Glc-II δ_C 105.7 (C-1’’’’), 78.2 (C-3’’’’, C-5’’’’), 75.9 (C-2’’’’), 71.5 (C-4’’’’), 62.7 (C-6’’’’); 2-NAc-Gal δ_C 174.2 (NHAc), 102.6 (C-1’’’’’), 77.2 (C-5’’’’’), 72.9 (C-3’’’’’), 69.9 (C-4’’’’’), 63.1 (C-6’’’’’), 54.5 (C-2’’’’’), 23.0 (NHAc); HRFABMS *m/z* 1343.6366 $[M + Na]^+$ (calcd for $C_{62}H_{100}N_2O_{28}Na$, 1343.6360).

Sarasinoside R (**25**): White amorphous solid; $[\alpha]_D^{25}$ −12.4 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 238 (1.60) nm; IR (ZnSe) ν_{max} 3400 (br), 2930, 1645 (br), 1570, 1450, 1380 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ^{1}H NMR data of the sugar portion (CD_3OD) Xyl δ_H 4.28 (1H, d, *J* = 7.8 Hz, H-1’), 3.90 (1H, dd, *J* = 9.2, 7.8 Hz, H-2’), 3.86 (H-5’), 3.68 (H-4’), 3.51 (1H, dd, *J* = 9.2, 9.2 Hz, H-3’), 3.16 (1H, dd, *J* = 10.7, 10.7 Hz, H-5’); 2-NAc-Glc δ_H 4.82 (H-1’’), 4.09 (1H, br d, *J* = 12.6 Hz, H-6’’), 3.76 (1H, dd, *J* = 12.6, 7.6 Hz, H-6’’), 3.67 (H-2’’, H-5’’), 3.41 (H-3’’), 3.14 (1H, dd, *J* = 8.9, 8.9 Hz, H-4’’), 1.98 (3H, s, NHAc); Glc-I

δ_H 5.07 (1H, d, *J* = 7.9 Hz, H-1’’’), 3.82 (1H, dd, *J* = 11.8, 2.5 Hz, H-6’’’), 3.70 (H-6’’’), 3.69 (H-3’’’), 3.45 (1H, ddd, *J* = 10.1, 5.2, 2.5 Hz, H-5’’’), 3.38 (1H, dd, *J* = 9.2, 7.9 Hz, H-2’’’), 3.32 (H-4’’’); Glc-II δ_H 4.61 (1H, d, *J* = 7.3 Hz, H-1’’’’), 3.88 (1H, br d, *J* = 12.4 Hz, H-6’’’’), 3.72 (1H, dd, *J* = 12.4, 6.0 Hz, H-6’’’’), 3.40 (H-2’’’’, H-3’’’’), 3.32 (H-4’’’’), 3.27 (1H, ddd, *J* = 9.5, 6.0, 2.0 Hz, H-5’’’’); 2-NAc-Gal δ_H 4.49 (1H, d, *J* = 7.8 Hz, H-1’’’’’), 3.88 (H-6’’’’’), 3.85 (H-2’’’’’), 3.79 (H-4’’’’’), 3.71 (H-6’’’’’), 3.63 (H-3’’’’’, H-5’’’’’), 1.98 (3H, s, NHAc); ^{13}C NMR data of the sugar portion (CD_3OD) Xyl δ_C 106.8 (C-1’), 79.7 (C-4’), 78.0 (C-2’), 77.4 (C-3’), 64.3 (C-5’); 2-NAc-Glc δ_C 174.3 (NHAc), 102.0 (C-1’’), 77.9 (C-5’’), 77.0 (C-3’’), 72.3 (C-4’’), 70.1 (C-6’’), 57.9 (C-2’’), 23.1 (NHAc); Glc-I δ_C 102.5 (C-1’’’), 83.3 (C-2’’’), 77.9 (C-3’’’), 77.0 (C-5’’’), 71.7 (C-4’’’), 62.7 (C-6’’’); Glc-II δ_C 105.8 (C-1’’’’), 78.3 (C-5’’’’), 77.7 (C-3’’’’), 75.9 (C-2’’’’), 71.5 (C-4’’’’), 62.7 (C-6’’’’); 2-NAc-Gal δ_C 173.9 (NHAc), 102.6 (C-1’’’’’), 77.3 (C-5’’’’’), 72.9 (C-3’’’’’), 69.9 (C-4’’’’’), 63.2 (C-6’’’’’), 54.6 (C-2’’’’’), 23.1 (NHAc); HRFABMS *m*/*z* 1325.6223 [M – H_2O + Na]$^+$ (calcd for $C_{62}H_{98}N_2O_{27}Na$, 1325.6255).

Acidic Hydrolysis of Sarasinoside A_1 (13).[16,17] A mixture of **13** (25.1 mg) and 3 N HCl was stirred at 65 °C for 1 h. After cooling, the solution was concentrated by blowing with N_2 and then extracted with

MeOH. The MeOH extract was purified by reversed-phase HPLC (YMC-Pack ODS column, 10 mm × 250 mm; H_2O-MeOH, 10:90) to furnish a sapogenol **26** (3.6 mg); LR-FABMS *m*/*z* 427.50 $[M + H]^+$ (calcd for $C_{29}H_{47}O_2$, 427.36).

Acid Hydrolysis and GC Analysis of Compounds 9 and 10.[19,28] A solution of **9** (1.4 mg) in 3 N HCl (0.5 mL) was stirred at 80 °C for 5 h. After being cooled to room temperature, the solution was concentrated by evaporation under a stream of N_2. The residue was treated with hexamethyldisilazane (HMDS) in TMSCl (50 μL, *v*/*v* = 2:1) in pyridine (0.5 mL), and the solution was stirred at 60 °C for 30 min. After drying the solution with a stream of N_2, the residue was separated with H_2O and CH_2Cl_2 (1 mL, *v*/*v* = 1:1). The CH_2Cl_2 layer was analyzed by GC using an HP5 column (0.32 mm × 30 m). The injector and detector were both held at 200 °C. A temperature gradient was applied to the GC oven; the initial temperature was maintained at 60 °C for 3 min, then ramped to 200 °C at 4 °C/min, and maintained at 200 °C for 3 min. Peaks of hydrolysate were detected at 27.02, 31.51, and 36.73 min. Retention times for authentic samples after being treated simultaneously with HMDS/TMSCl were 26.56 (L-xylose), 27.01 (D-xylose), 31.51 (2-*N*-acetyl-D-galactosamine), 32.53 (D-glucose), 32.62 (L-glucose), and 36.72 min (2-*N*-acetyl-D-

glucosamine). Co-injection of the hydrolysate with the authentic silylated D-xylose, 2-*N*-acetyl-D-galactosamine, and 2-*N*-acetyl-D-glucosamine gave single peaks at 27.02, 31.49, and 36.73 min, respectively. A solution of **22** (3.1 mg) was prepared in a similar manner and peaks of hydrolysate were detected at 27.01, 31.51, 32.52, and 36.71 min. Co-injection of the hydrolysate with the authentic silylated D-xylose, 2-*N*-acetyl-D-glucosamine, D-glucose, and 2-*N*-acetyl-D-galactosamine gave single peaks at 27.01, 31.51, 32.52, and 36.73 min, respectively.

Biological Assays. Cytotoxicity assays were performed in accordance with the protocols in references (23) and (24).

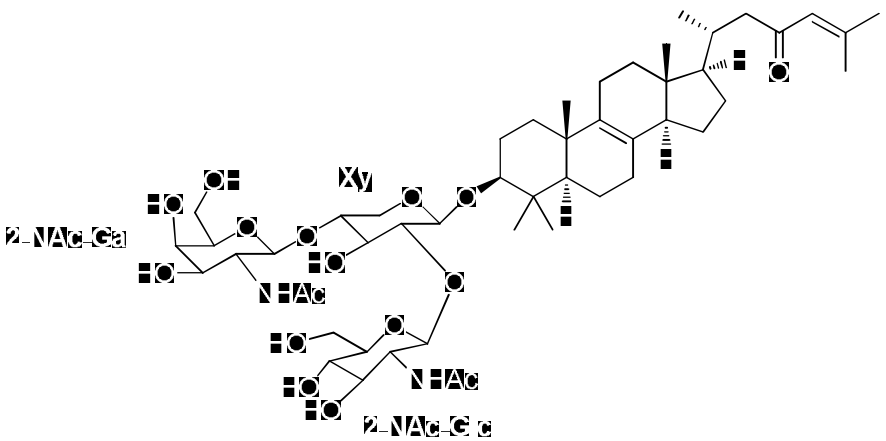


Table 11. ^{13}C and ^{1}H NMR assignment for compound **21** in MeOD-d_4

Position	δ_C	δ_H
1	37.1, CH_2	1.23, ddd (13.0, 13.0, 3.5)
		1.70, m
2	27.8, CH_2	1.87, dddd (13.0, 3.5, 3.5, 3.5)
		1.73, dddd (13.0, 13.0, 12.0, 3.5)
3	91.8, CH	3.16, dd (12.0, 3.5)
4	40.7, C	
5	52.0, CH	1.11, dd (12.5, 1.4)
6	19.4, CH_2	1.71, m
		1.51, dddd (12.5, 12.5, 12.5, 6.7)
7	29.5, CH_2	1.87, m
		1.98, m
8	129.0, C	
9	137.2, C	
10	37.9, C	
11	23.0, CH_2	1.97, m
		2.07, m
12	38.2, CH_2	1.39, ddd (11.9, 10.3, 2.3)
		1.97, m

	13	43.4, C	
	14	53.2, CH	2.06, m
	15	24.8, CH_2	1.62, m
			1.35, m
	16	30.0, CH_2	1.86, m
			1.31, m
	17	56.3, CH	1.19, m
	18	11.7, CH_3	0.67, s
	19	20.3, CH_3	1.01, s
	20	35.1, CH	1.97, m
	21	20.1, CH_3	0.91, d (6.4)
	22	52.4, CH_2	2.52, dd (14.9, 2.9)
			2.09, m
	23	204.1, C	
	24	125.3, CH	6.16, br s
	25	157.1, C	
	26	20.9, CH_3	2.11, d (1.0)
	27	27.7, CH_3	1.90, d (1.0)
	28	16.6, CH_3	0.89, s
	29	28.3, CH_3	1.08, s
Xyl	1’	106.0, CH	4.37, d (7.6)
	2’	79.0, CH	3.61, m
	3’	77.5, CH	3.52, dd (8.8, 8.8)
	4’	79.3, CH	3.59, ddd (10.7, 8.8, 5.5)
	5’	64.3, CH2	3.88, dd (12.0, 5.5)
			3.18, dd (12.0, 10.7)
2-NAc-	1’’	102.5, CH	4.88, d (8.4)
Glc	2’’	58.1, CH	3.65, dd (10.3, 8.4)
	3’’	76.7, CH	3.41, dd (10.3, 8.8)
	4’’	72.9, CH	3.11, dd (8.8, 9.8)

	5’’	78.6, CH	3.22, ddd (9.8, 7.6, 2.2)
	6’’	63.8, CH_2	3.84, dd (12.0, 2.2)
			3.56, dd (12.0, 7.6)
	Ac	23.2, CH_3	1.98, s
		174.1, C	
2-NAc-	1’’’	102.5, CH	4.44, d (8.7)
	2’’’	54.5, CH	3.89, dd (10.6, 8.7)
	3’’’	73.0, CH	3.60, m
	4’’’	69.8, CH	3.81, m
	5’’’	77.4, CH	3.52, ddd (7.4, 4.3, 0.8)
	6’’’	62.9, CH	3.80, dd (11.6, 7.4)
			3.70, dd (11.6, 4.3)
	Ac	23.2, CH_3	1.97, s
		174.3, C	

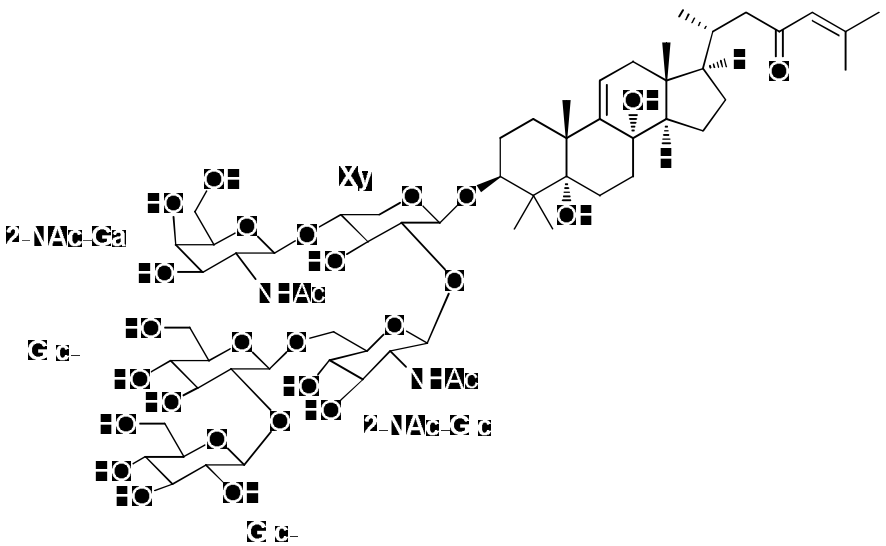


Table 12. ^{13}C and ^{1}H NMR assignment for compound **22** in MeOD-d_4

Position	δ_C	δ_H
1	39.2, CH_2	1.74, ddd (12.5, 12.5, 3.5)
		1.46, ddd (12.5, 3.5, 3.5)
2	28.5, CH_2	1.69, dddd (12.5, 3.5, 3.5, 3.5)
		1.74, dddd (12.5, 12.5, 12.5, 3.5)
3	88.2, CH	3.43, m
4	41.8, C	
5	93.8, C	
6	27.8, CH_2	1.90, m
		1.88, ddd (10.5, 10.5, 4.0)
7	29.0, CH_2	1.70, m
		1.49, ddd (10.5, 10.5, 3.5)
8	87.3, C	
9	156.2, C	
10	45.9, C	
11	112.6, CH	5.03, dd (3.9, 3.0)
12	43.0, CH_2	2.22, dd (17.0, 3.9)

			1.95, m
	13	43.9, C	
	14	52.4, CH	1.98, m
	15	21.6, CH_2	1.67, m
			1.61, m
	16	29.5, CH_2	1.92, m
			1.34, m
	17	58.1, CH	1.32, m
	18	15.4, CH_3	0.75, s
	19	23.4, CH_3	1.26, s
	20	34.3, CH	1.97, m
	21	19.7, CH_3	0.91, d (6.5)
	22	52.3, CH_2	2.52, dd (15.0, 3.0)
			2.12, dd (15.0, 9.8)
	23	203.9, C	
	24	125.3, CH	6.17, br s
	25	157.0, C	
	26	20.8, CH_3	2.11, d (1.0)
	27	27.6, CH_3	1.90, d (1.0)
	28	19.0, CH_3	1.05, s
	29	21.8, CH_3	1.18, s
Xyl	1’	106.0, CH	4.37, d (7.6)
	2’	79.0, CH	3.61, m
	3’	77.5, CH	3.52, dd (8.8, 8.8)
	4’	79.3, CH	3.59, ddd (10.7, 8.8, 5.5)
	5’	64.3, CH2	3.88, dd (12.0, 5.5)
			3.18, dd (12.0, 10.7)
2-NAc-	1’’	102.5, CH	4.88, d (8.4)
Glc	2’’	58.1, CH	3.65, dd (10.3, 8.4)
	3’’	76.7, CH	3.41, dd (10.3, 8.8)

	4''	72.9, CH	3.11, dd (8.8, 9.8)
	5''	78.6, CH	3.22, ddd (9.8, 7.6, 2.2)
	6''	63.8, CH_2	3.84, dd (12.0, 2.2)
			3.56, dd (12.0, 7.6)
	Ac	23.2, CH_3	1.98, s
		174.1, C	
Glc-I	1'''	102.7, CH	5.07, d (7.9)
	2'''	83.5, CH	3.38, dd (7.9, 9.1)
	3'''	78.0, CH	3.69, m
	4'''	71.7, CH	3.31, m
	5'''	77.2, CH	3.45, ddd (9.8, 5.7, 2.5)
	6'''	62.7, CH_2	3.83, dd (11.5, 2.5)
			3.70, m
Glc-II	1''''	105.8, CH	4.61, d (7.4)
	2''''	75.9, CH	3.40, m
	3''''	77.0, CH	3.40, m
	4''''	71.5, CH	3.31, m
	5''''	78.3, CH	3.27, ddd (9.5, 6.0, 2.0)
	6''''	62.7, CH_2	3.87, br d (11.7)
			3.72, dd (11.7, 6.0)
2-NAc-	1'''''	102.5, CH	4.49, d (8.4)
Gal	2'''''	54.6, CH	3.85, m
	3'''''	72.8, CH	3.63, m
	4'''''	69.9, CH	3.79, m
	5'''''	77.4, CH	3.63, m
	6'''''	63.1, CH	3.87, m
			3.71, m
	Ac	23.0, CH_3	1.99, s
		173.9, C	

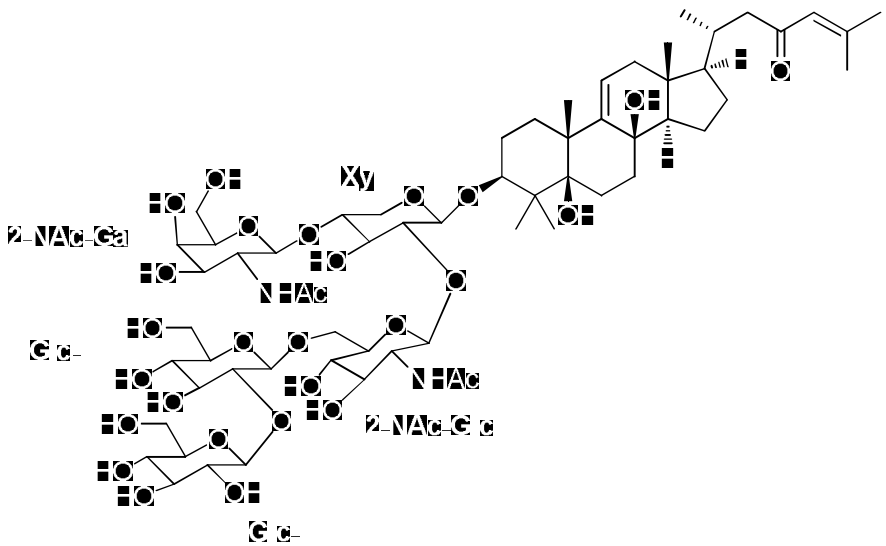


Table 13. ^{13}C and ^{1}H NMR assignment for compound **23** in MeOD-d_4

Position	δ_C	δ_H
1	32.3, CH_2	1.57, ddd (12.5, 12.5, 4.0)
		1.84, m
2	28.4, CH_2	1.92, m
		1.84, m
3	87.7, CH	3.33, m
4	45.0, C	
5	84.2, C	
6	24.6, CH_2	1.80, m
		2.28, m
7	33.0, CH_2	1.40, ddd (12.5, 12.5, 1.9)
		2.21, ddd (12.5, 12.5, 7.0)
8	76.6, C	
9	150.6, C	
10	43.7, C	
11	118.7, CH	5.46, dd (7.4, 1.6)
12	42.0, CH_2	2.29, dd (16.9, 7.4)

			1.81, m
	13	42.9, C	
	14	57.8, CH	1.62, m
	15	19.8, CH_2	1.51, m
			1.61, m
	16	29.3, CH_2	1.88, m
			1.30, m
	17	56.4, CH	1.17, m
	18	12.7, CH_3	0.77, s
	19	30.3, CH_3	1.52, s
	20	34.4, CH	1.93, m
	21	19.9, CH_3	0.89, d (6.5)
	22	52.1, CH_2	2.52, dd (15.0, 3.1)
			2.08, dd (15.0, 9.9)
	23	203.9, C	
	24	125.3, CH	6.15, br s
	25	157.0, C	
	26	20.8, CH_3	2.10, d (1.0)
	27	27.6, CH_3	1.90, d (1.0)
	28	19.1, CH_3	1.15, s
	29	25.9, CH_3	1.18, s
Xyl	1’	106.4 CH	4.26, d (7.8)
	2’	78.0, CH	3.88, m
	3’	77.5, CH	3.49, dd (9.1, 9.1)
	4’	79.9, CH	3.65, m
	5’	64.3, CH2	3.85, m
			3.14, dd (11.3, 10.2)
2-NAc-	1’’	102.1, CH	4.81, d (8.6)
Glc	2’’	57.9, CH	3.66, m
	3’’	77.0, CH	3.37, m

	4’’	72.4, CH	3.15, dd (9.8, 8.7)
	5’’	78.0, CH	3.66, m
	6’’	70,1, CH_2	4.10, br d (12.6)
			3.74, dd (12.6, 7.6)
	Ac	23.0, CH_3	1.97, s
		174.3, C	
Glc-I	1’’’	102.6, CH	5.03, d (7.9)
	2’’’	83.6, CH	3.37, dd (7.9, 9.1)
	3’’’	77.9, CH	3.67, m
	4’’’	71.6, CH	3.31, m
	5’’’	77.0, CH	3.43, ddd (9.8, 5.2, 2.5)
	6’’’	62.8, CH_2	3.82, dd (11.8, 2.5)
			3.70, m
Glc-II	1’’’’	105.8, CH	4.61, d (7.4)
	2’’’’	75.8, CH	3.40, m
	3’’’’	77.7, CH	3.40, m
	4’’’’	71.5, CH	3.32, m
	5’’’’	78.3, CH	3.27, ddd (9.5, 5.5, 2.3)
	6’’’’	62.6, CH_2	3.87, m
			3.72, dd (12.5, 6.0)
2-NAc-	1’’’’’	102.7, CH	4.48, d (8.4)
Gal	2’’’’’	54.6, CH	3.85, m
	3’’’’’	72.8, CH	3.62, m
	4’’’’’	69.9, CH	3.78, m
	5’’’’’	77.5, CH	3.62, m
	6’’’’’	63.1, CH	3.88, m
			3.71, m
	Ac	23.0, CH_3	1.98, s
		173.9, C	

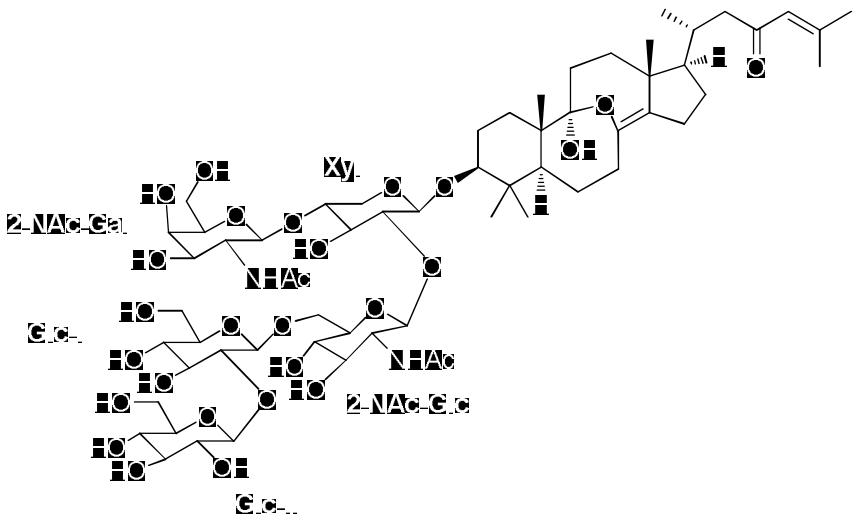


Table 14. ^{13}C and ^{1}H NMR assignment for compound **24** in MeOD-d_4

Position	δ_C	δ_H
1	36.4, CH_2	2.19, ddd (13.5, 13.5, 3.0)
		1.23, ddd (13.5, 3.0, 3.0)
2	26.7, CH_2	1.70, m
		1.60, dddd (13.5, 13.5, 11.5, 3.0)
3	91.8, CH	3.11, dd (11.5, 3.5)
4	42.3, C	
5	46.3, CH	1.95, br d (12.5)
6	26.7, CH_2	1.50, dddd (12.5, 12.5, 12.5, 4.7)
		1.70, m
7	32.0, CH_2	1.87, m
		2.34, m
8	142.3, C	
9	107.6, C	
10	48.3, C	
11	31.3, CH_2	1.59, m
		2.44, br dd (13.5, 13.5)
12	34.0, CH_2	2.36, m

			1.67, m
	13	49.7, C	
	14	129.4, C	1.62, m
	15	24.5, CH_2	2.00, m
			2.07, m
	16	28.9, CH_2	1.86, m
			1.40, m
	17	54.9, CH	1.39, m
	18	17.8, CH_3	0.97, s
	19	25.3, CH_3	1.29, s
	20	33.3, CH	2.02, m
	21	20.85, CH_3	0.94, d (6.4)
	22	51.8, CH_2	2.54, dd (15.0, 2.7)
			2.12, dd (15.0, 10.1)
	23	203.9, C	
	24	125.3, CH	6.15, br s
	25	157.0, C	
	26	20.89, CH_3	2.11, d (1.0)
	27	27.7, CH_3	1.90, d (1.0)
	28	17.1, CH_3	0.97, s
	29	29.3, CH_3	1.15, s
Xyl	1’	106.6, CH	4.29, d (7.8)
	2’	78.0, CH	3.90, dd (9.1, 7.8)
	3’	77.8, CH	3.50, dd (9.1, 9.1)
	4’	79.8, CH	3.67, m
	5’	64.2, CH_2	3.86, dd (11.4, 5.5)
			3.14, dd (11.4, 10.0)
2-NAc-	1’’	102.0, CH	4.82, m
Glc	2’’	57.9, CH	3.67, m
	3’’	77.0, CH	3.40, m

	4’’	72.4, CH	3.14, dd (8.9, 8.9)
	5’’	78.2, CH	3.67, m
	6’’	70.1, CH_2	4.08, br d (12.5)
			3.77, dd (12.5, 7.4)
	Ac	23.0, CH_3	1.99, s
		173.9, C	
Glc-I	1’’’	102.6, CH	5.08, d (7.9)
	2’’’	83.4, CH	3.37, dd (9.2, 7.9)
	3’’’	78.0, CH	3.69, m
	4’’’	71.6, CH	3.31, m
	5’’’	77.2, CH	3.45, ddd (9.4, 5.4, 2.5)
	6’’’	62.6, CH_2	3.81, dd (11.8, 2.5)
			3.69, m
Glc-II	1’’’’	105.7, CH	4.61, d (7.5)
	2’’’’	75.9, CH	3.40, m
	3’’’’	78.2, CH	3.40, m
	4’’’’	71.5, CH	3.32, m
	5’’’’	78.2, CH	3.27, ddd (9.6, 6.0, 2.4)
	6’’’’	62.7, CH_2	3.88, m
			3.72, dd (12.2, 6.0)
2-NAc-	1’’’’’	102.6, CH	4.48, d (8.4)
Gal	2’’’’’	54.5, CH	3.87, m
	3’’’’’	72.9, CH	3.63, m
	4’’’’’	69.9, CH	3.79, m
	5’’’’’	77.2, CH	3.63, m
	6’’’’’	63.1, CH	3.89, m
			3.71, m
	Ac	23.0, CH_3	1.98, s
		174.2, C	

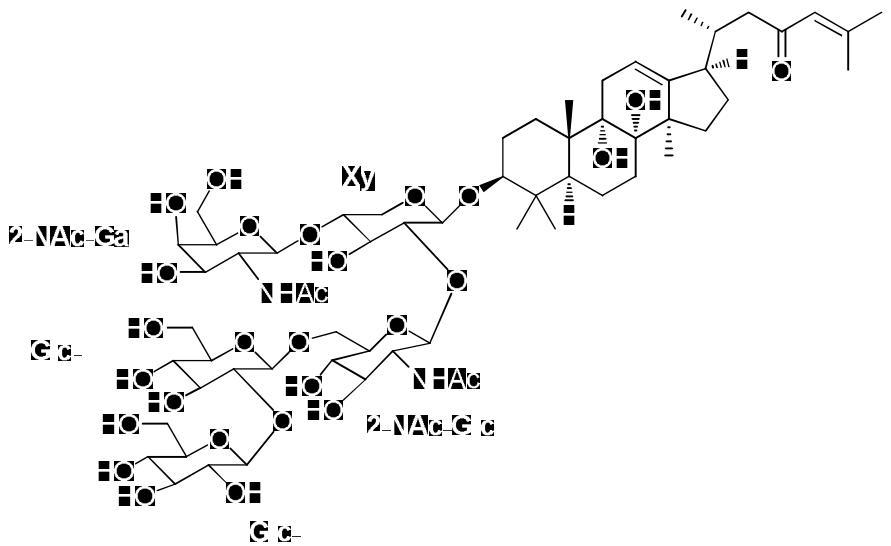


Table 15. ^{13}C and ^{1}H NMR assignment for compound **25** in MeOD-d_4

Position	δ_C	δ_H
1	34.5, CH_2	1.32, m
		1.73, m
2	27.8, CH_2	1.89, m
		1.72, m
3	91.5, CH	3.03, dd (11.8, 4.2)
4	40.3, C	
5	44.8, CH	1.48, dd (12.5, 1.8)
6	24.8, CH_2	2.17, m
		1.69, m
7	18.2, CH_2	1.49, m
		1.34, m
8	70.0, C	
9	72.2, C	
10	37.4, C	
11	25.2, CH_2	2.37, dd (17.0, 7.1)
		2.48, dd (17.0, 1.2)

	12	115.1, CH	5.18, br d (7.1)
	13	148.2, C	
	14	49.2, C	
	15	33.7, CH_2	1.54, m
			1.54, m
	16	25.5, CH_2	1.69, m
			1.43, ddd (11.0, 11.0, 3.5)
	17	50.3, CH	2.49, m
	18	22.1, CH_3	1.02, s
	19	18.2, CH_3	1.13, s
	20	33.2, CH	2.24, m
	21	18.8, CH_3	0.90, d (6.6)
	22	48.4, CH_2	2.44, dd (14.5, 2.3)
			2.16, dd (14.5, 10.7)
	23	203.9, C	
	24	125.1, CH	6.14, br s
	25	157.1, C	
	26	20.9, CH_3	2.10, d (1.0)
	27	27.7, CH_3	1.89, d (1.0)
	28	17.2, CH_3	0.95, s
	29	29.0, CH_3	1.07, s
Xyl	1’	106.8, CH	4.28, d (7.8)
	2’	78.0, CH	3.90, d (9.2, 7.8)
	3’	77.4, CH	3.51, dd (9.2, 9.2)
	4’	79.7, CH	3.68, m
	5’	64.3, CH2	3.86, m
			3.16, dd (10.7, 10.7)
2-NAc-	1’’	102.0, CH	4.82, m
Glc	2’’	57.9, CH	3.65, m
	3’’	77.0, CH	3.41, m

	4’’	72.3, CH	3.14, dd (8.9, 8.9)
	5’’	77.9, CH	3.67, m
	6’’	70.1, CH_2	4.09, br d (12.6)
			3.76, dd (12.6, 7.6)
	Ac	23.1, CH_3	1.98, s
		174.3, C	
Glc-I	1’’’	102.5, CH	5.07, d (7.9)
	2’’’	83.3, CH	3.38, dd (7.9, 9.2)
	3’’’	77.9, CH	3.69, m
	4’’’	71.7, CH	3.32, m
	5’’’	77.0, CH	3.45, ddd (10.1, 5.2, 2.5)
	6’’’	62.7, CH_2	3.82, dd (11.8, 2.5)
			3.70, m
Glc-II	1’’’’	105.8, CH	4.61, d (7.3)
	2’’’’	75.9, CH	3.40, m
	3’’’’	77.7, CH	3.40, m
	4’’’’	71.5, CH	3.32, m
	5’’’’	78.3, CH	3.27, ddd (9.5, 6.0, 2.0)
	6’’’’	62.7, CH_2	3.88, br d (12.4)
			3.72, dd (12.4, 6.0)
2-NAc-	1’’’’’	102.6, CH	4.49, d (7.8)
Gal	2’’’’’	54.6, CH	3.85, m
	3’’’’’	72.9, CH	3.63, m
	4’’’’’	69.9, CH	3.79, m
	5’’’’’	77.3, CH	3.63, m
	6’’’’’	63.2, CH	3.88, m
			3.71, m
	Ac	23.1, CH_3	1.98, s
		173.9, C	

III. Conclusion

During a period of the Doctor of Philosophy studies, effort have been focused on the study of marine sponge derived natural products research. That goes to the isolation and structure determination of novel substances from a selected organism based upon chemical analysis and bioassay.

From a selected sponge, *Lipatrotethya* sp. 25 compounds have been isolated and 14 novel compounds among these have been structurally elucidated by combined chemical and spectral methods. The structures of these 25 compounds are 7 pouoside-class, 5 compounds of corresponding sapogenins, and 13 compounds of sarasinoside-class.

Wide range of bioassay related to anticancer, antimicrobial, and enzyme-inhibitory activities have been performed for the isolates. Some of them showed potent bioactivities.

In conclusion, this study has successfully demonstrated the biomedical potential of a marine organism. Compounds and an organism obtained during research, as well as techniques developed throughout the research, will be valuable assets for related works in the future.

Summary

The purpose of this work is the development of new lead compounds, information, and essential techniques for drug-related advanced research based upon chemical investigation of a sponge.

To achieve this goal, a sponge were systematically collected from various location of Micronesia. One specimen has been selected for chemical investigation on the basis of combined results of bioactivity tests, TLC and proton NMR analysis of the crude organic extracts. Solvent extraction of freeze-dried sample was performed. The extrat obtained was fractionated by liquid-liquid partitioning followed by various chromatographic separation techniques including thin layer chromatography, vacuum liquid chromatography, and preparative high-performance liquid chromatography. Structure elucidation is achieved using various spectroscopic techniques, especially 2-D NMR and mass spectrometry analysis.

From the selected sponge, 25 compounds have been isolated and 14 new compounds among these have been structurally elucidated by combined chemical and spectral methods. The structures of these 25

compounds are 7 pouoside-class, 5 compounds of corresponding sapogenins, and 13 compounds of sarasinoside-class.

Wide range of bioassay related to anticancer, antimicrobial, and enzyme-inhibitory activities have been performed for the isolates. Some of them showed potent bioactivities. Especially, sortase A (SrtA) and isocitrate lyase (ICL) were selected as target enzymes since they are not found in mammals and play crucial roles in the virulence or survival of various human-pathogenic bacteria and fungi. Na^+/K^+-ATPase plays crucial role in cellular function, the development of a new type less toxic natural regulators of this pump is an attractive prospect. Several compounds displayed significant cytotoxic, antifungal, and/or enzyme-inhitory activities.

1. Triterpene Galactosides of the Pouoside Class and Corresponding Aglycones from the Sponge *Lipastrotethya* sp.

Nine new triterpene galactosides and aglycones, along with three known compounds from the rare pouoside class, were isolated from the tropical sponge *Lipastrotethya* sp. collected from Micronesia. The structures of these new compounds were determined by combined

spectroscopic methods and designated as pouosides F-I (**4**, **8**, **10**, and **12**) and pouogenins A-E (**5**–**7**, **9**, and **11**). The absolute configurations of the asymmetric centers and the cyclohexenone moiety, which had been previously undetermined, were assigned by NOESY analyses and Mosher's methods. Several of these compounds exhibited weak cytotoxicity against the K562 cell-line.

2. Nortriterpene Glycosides of the Sarasinoside Class from the Sponge *Lipastrotethya* sp.

Five new nortriterpene glycosides, along with eight known compounds of the sarasinoside class, were isolated from the tropical sponge *Lipastrotethya* sp. collected from Chuuk, Micronesia. The structures of these new compounds were determined by combined spectroscopic and chemical methods and designated as sarasinosides N–R (**9–13**). The aglycone portions of **10**–**13** were found to be unprecedented among nortriterpeneoids on the basis of extensive NMR analyses. Several of these compounds exhibited moderate cytotoxicity against A549 and K562 cell lines as well as weak inhibitory activity against Na^+/K^+-ATPase.

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APPENDIX A:
NMR Spectroscopic Data of Isolated Compounds

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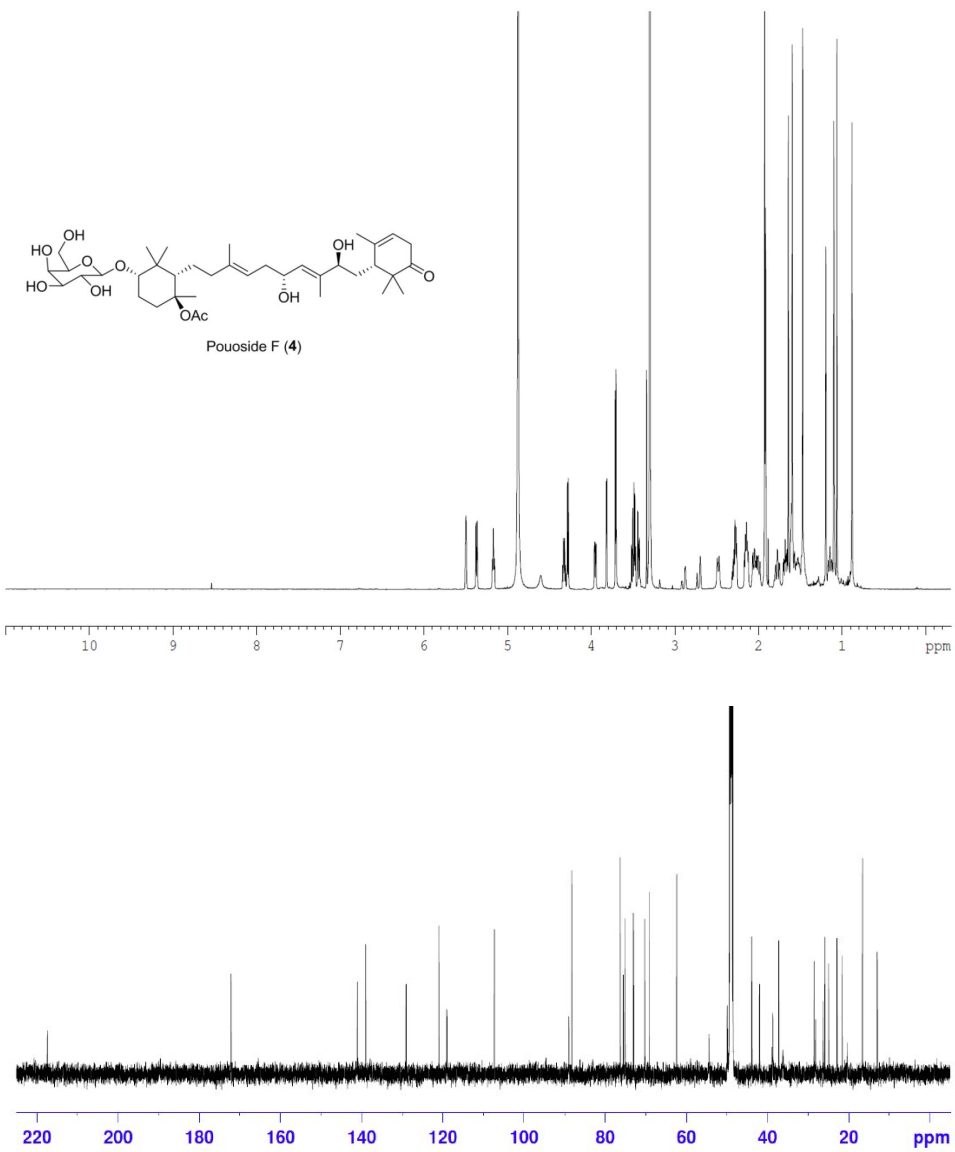


Figure A1. ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra of Compound **4**

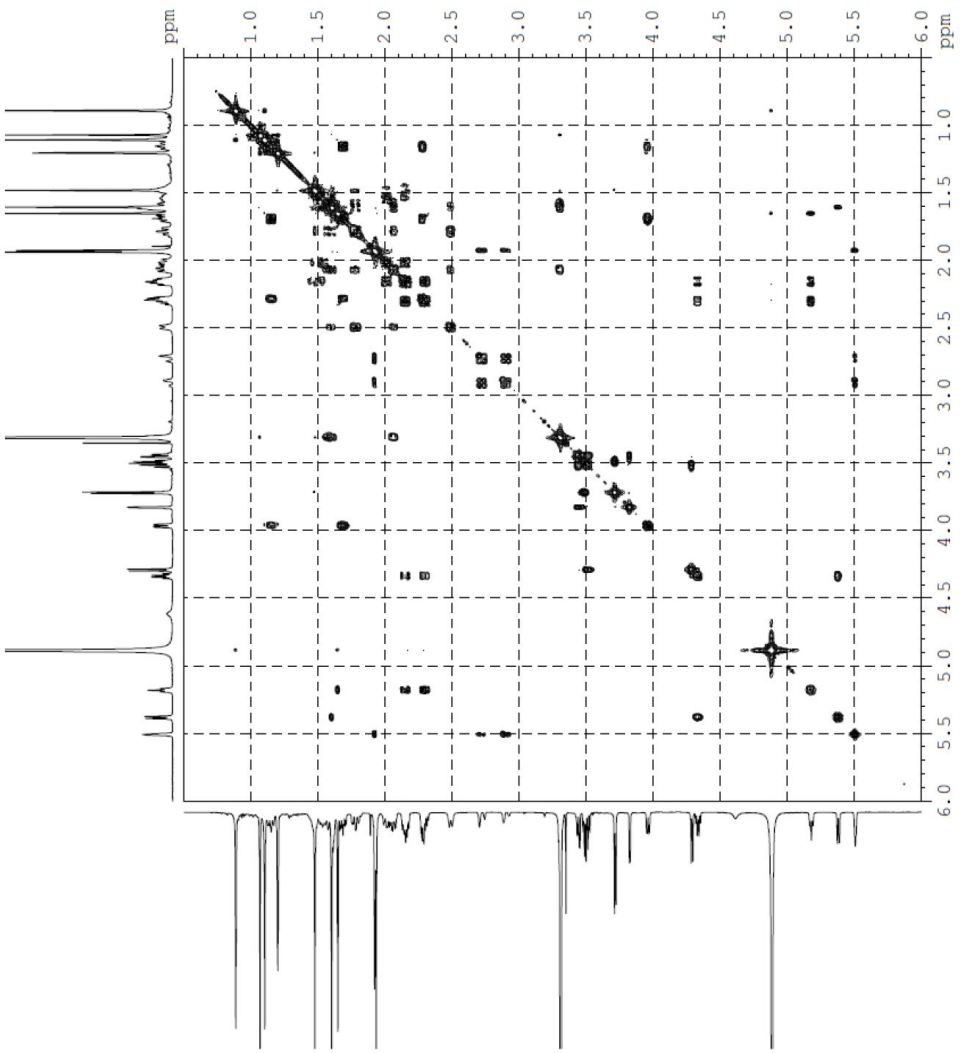
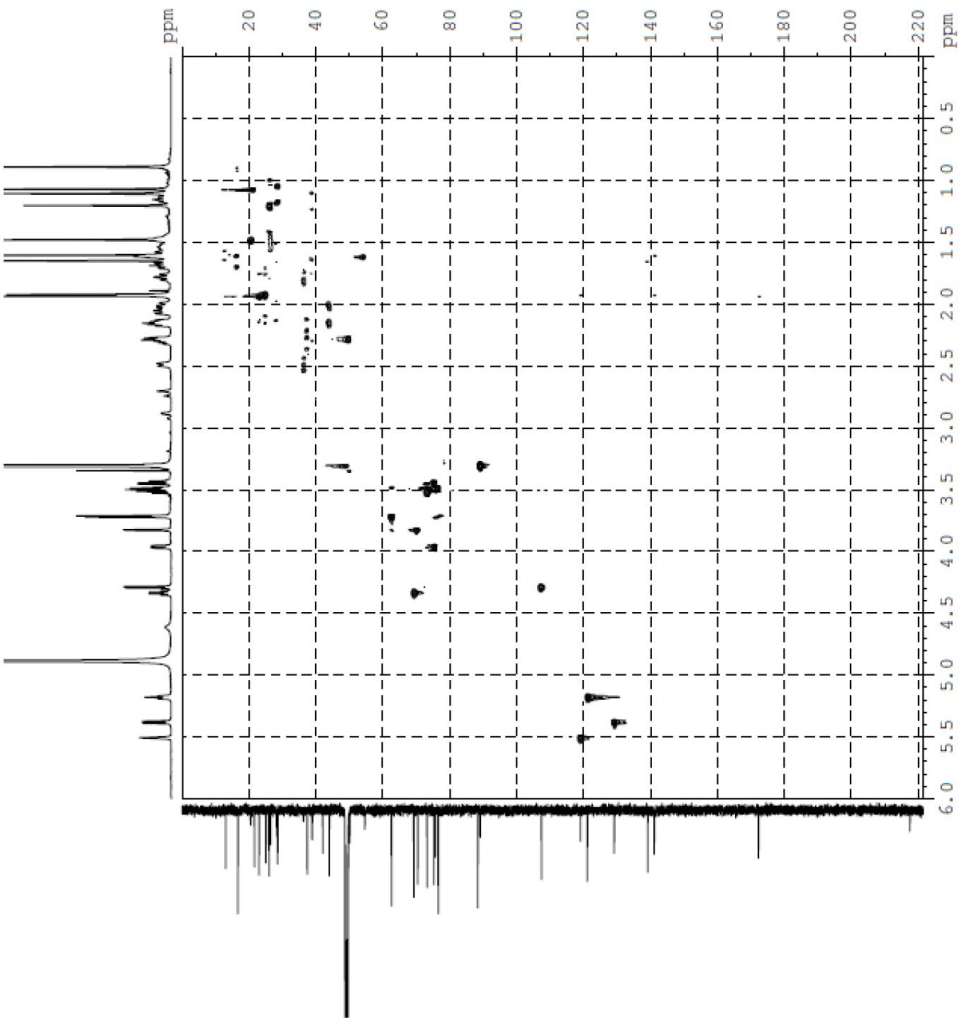


Figure A2. The COSY (600 MHz, MeOH-d_4) spectrum of Compound **4**



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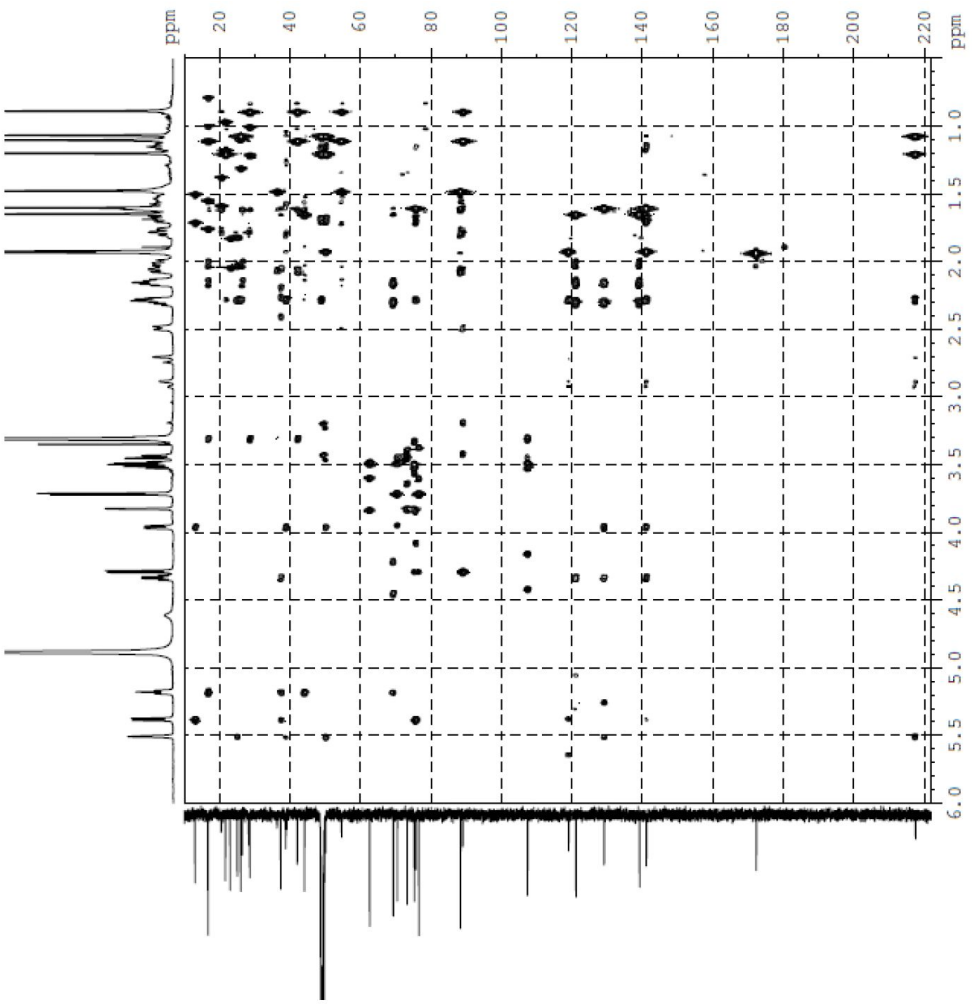

Figure A4. The *g*HMBC (600 MHz, MeOH-d_4) spectrum of Compound **4**

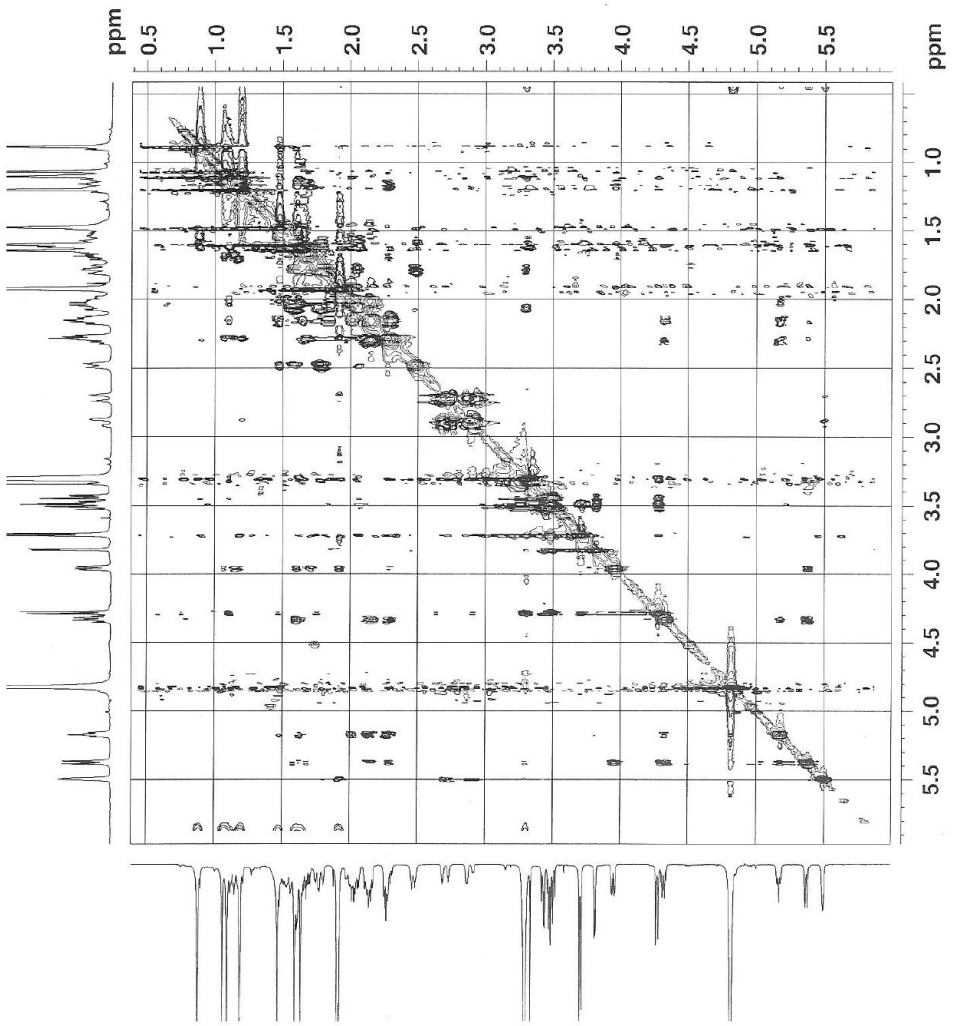

Figure A5. The NOESY (600 MHz, MeOH-d_4) spectrum of Compound **4**

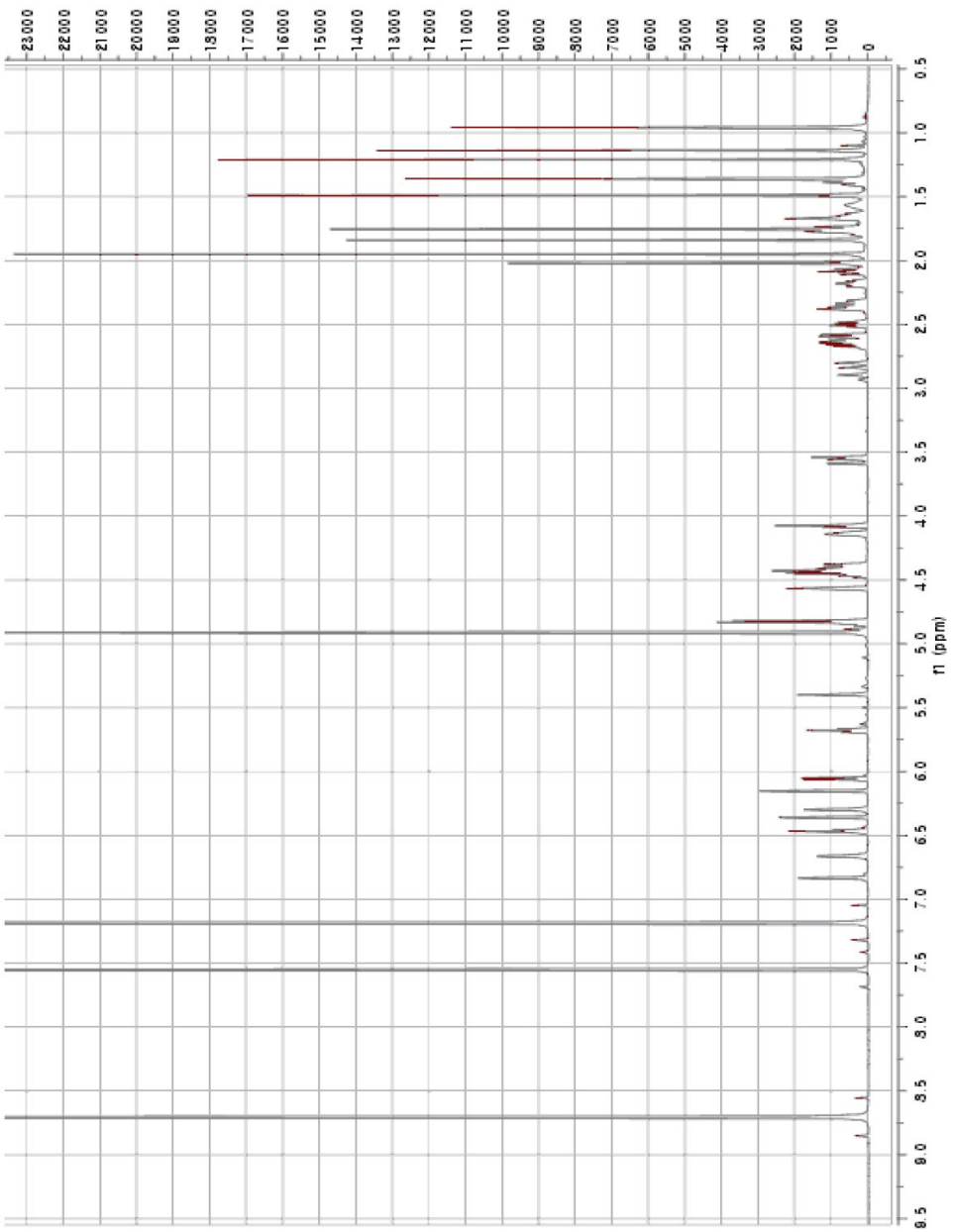

Figure A6. The ^{1}H-NMR (600 MHz, Pyridine-d_5) spectrum of Compound **4**

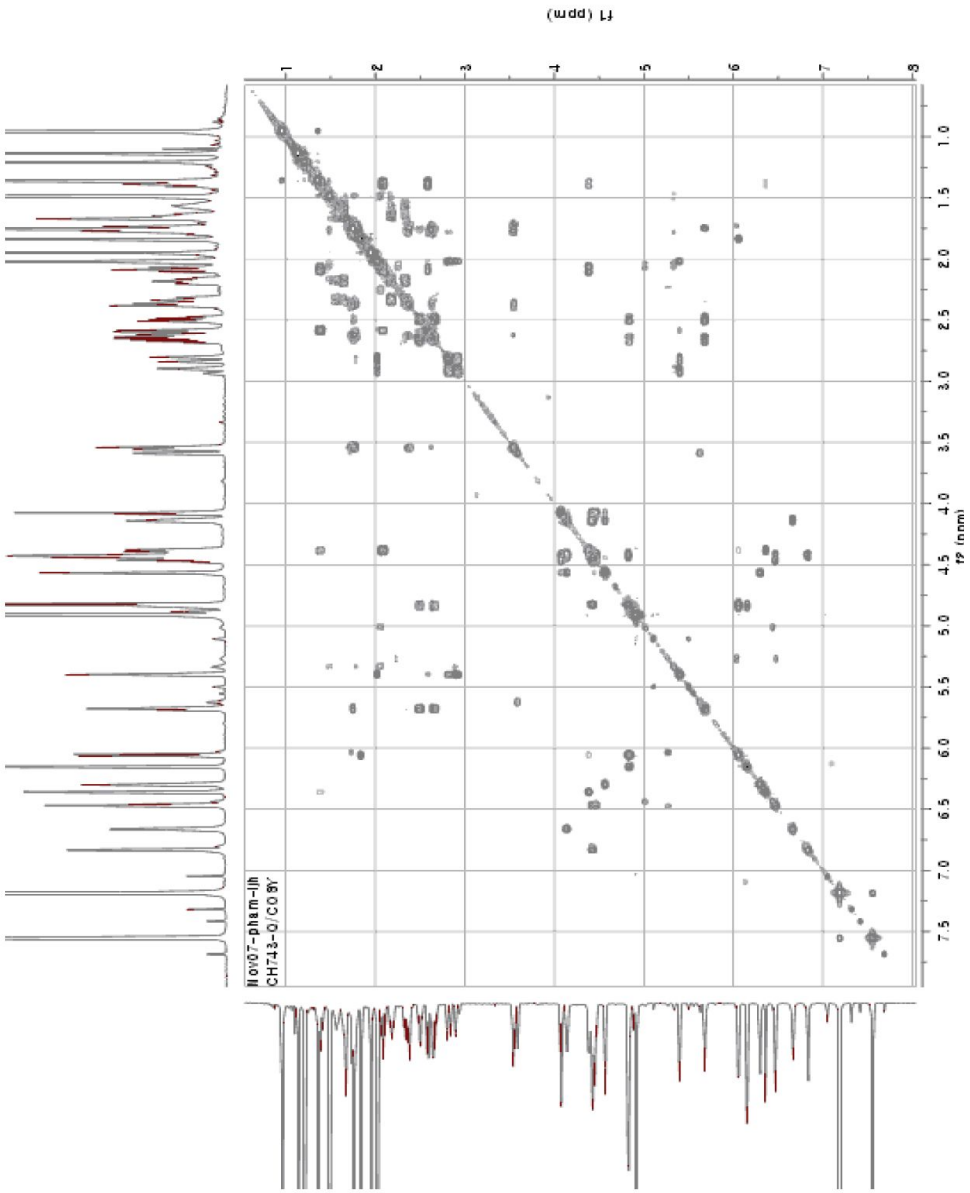

Figure A7. The COSY (600 MHz, Pyridine-d_5) spectrum of Compound **4**

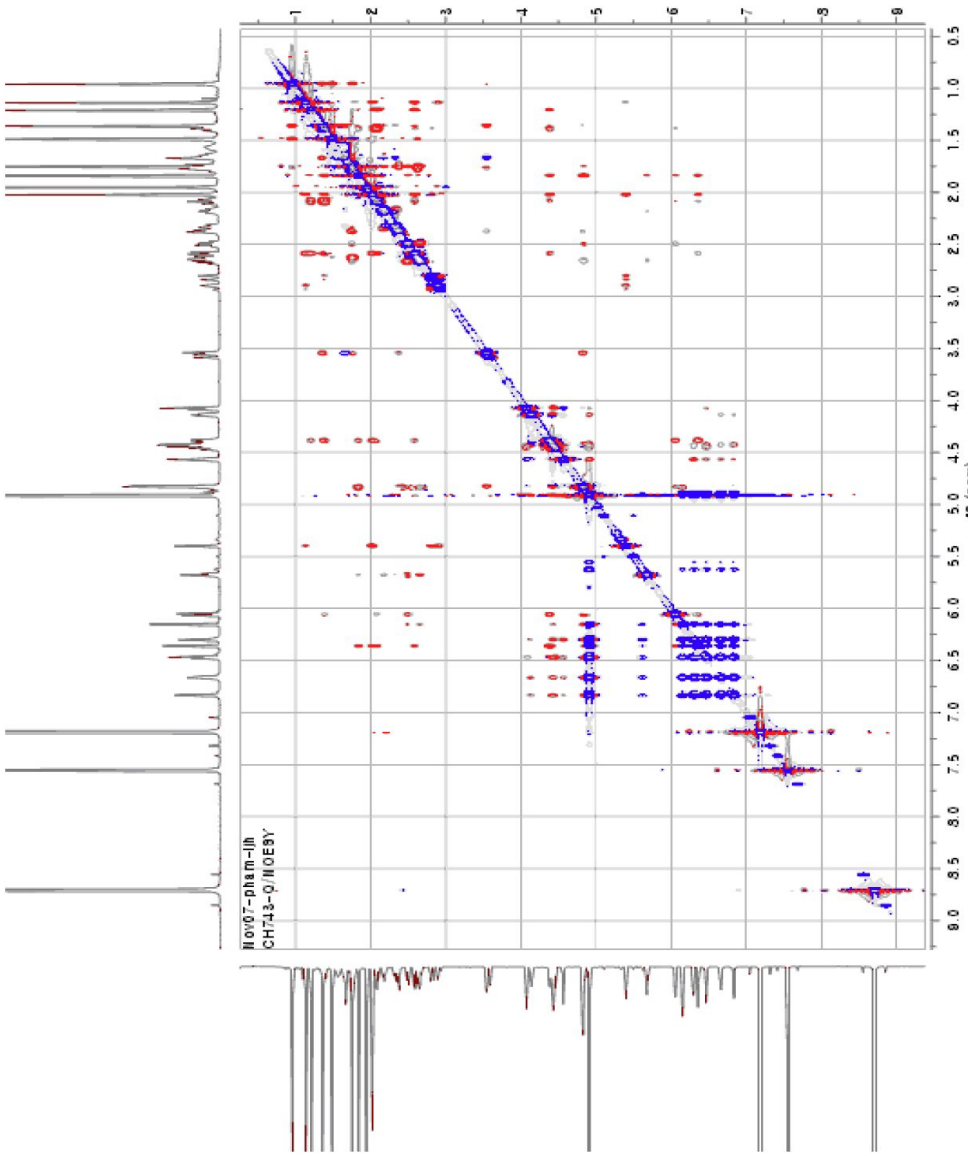

Figure A8. The NOESY (600 MHz, Pyridine-d_5) spectrum of Compound

4

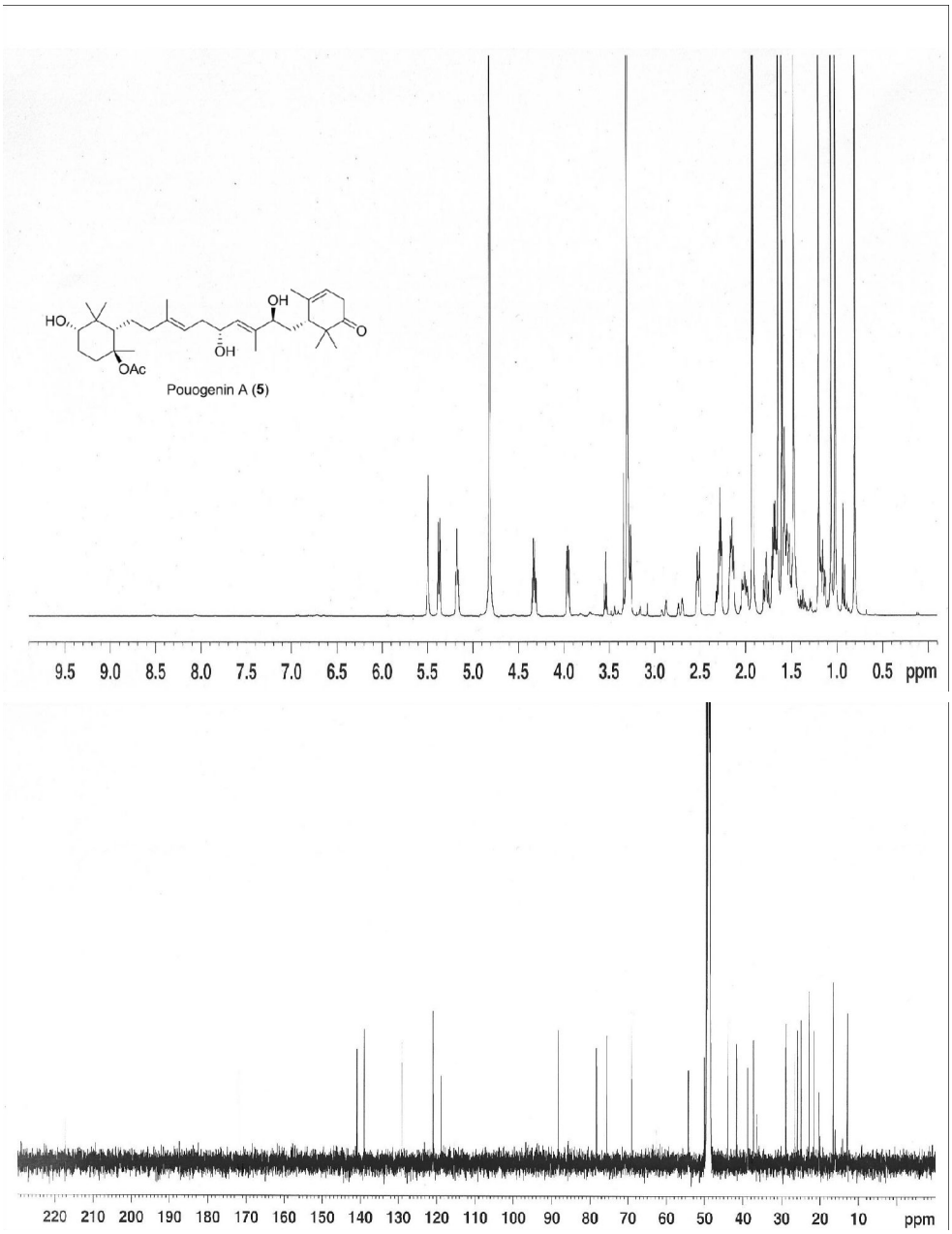

Figure A9. The ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra of Compound **5**

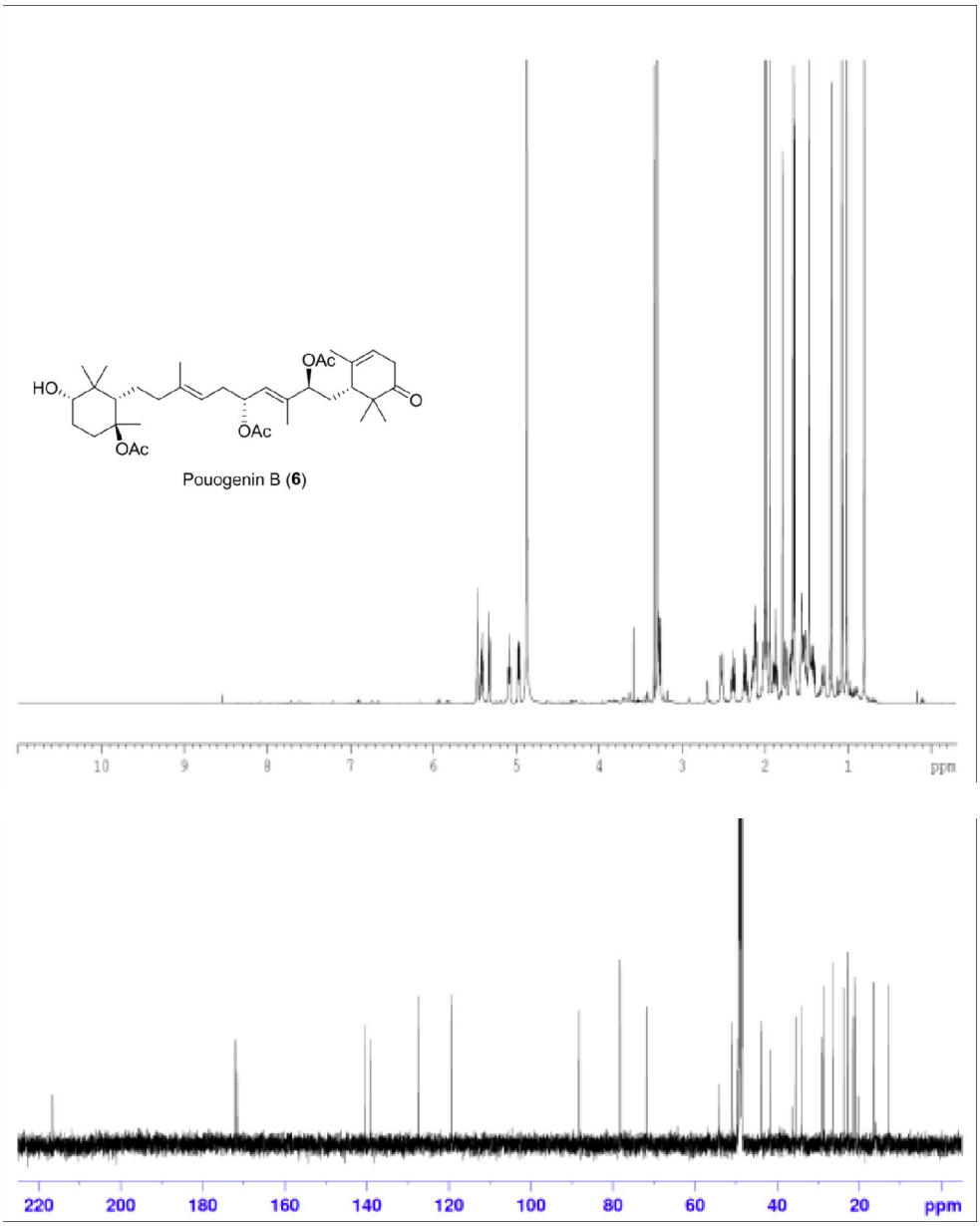

Figure A10. The ^{1}H NMR (600MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra of Compound **6**

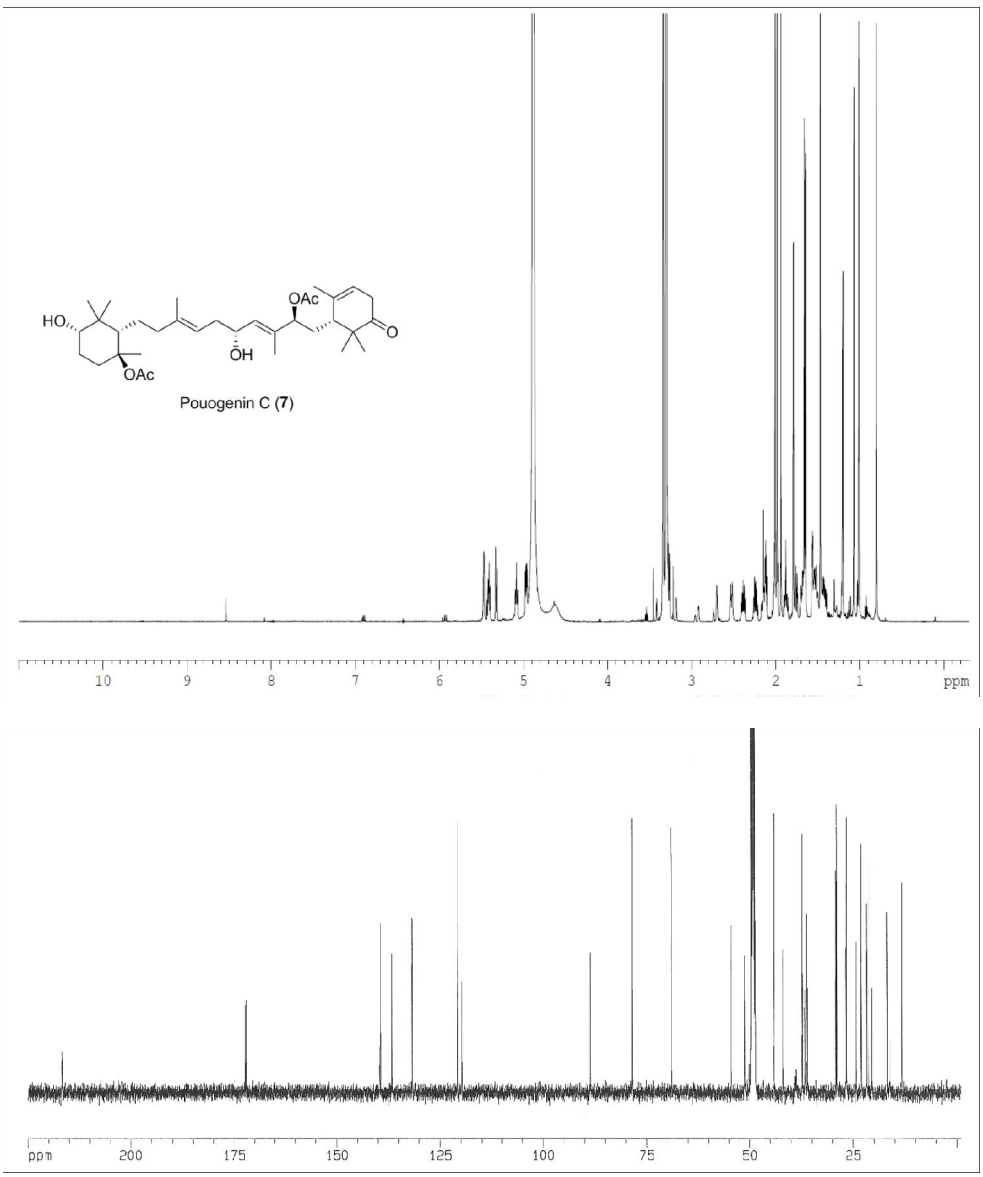

Figure A11. The ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra of Compound **7**

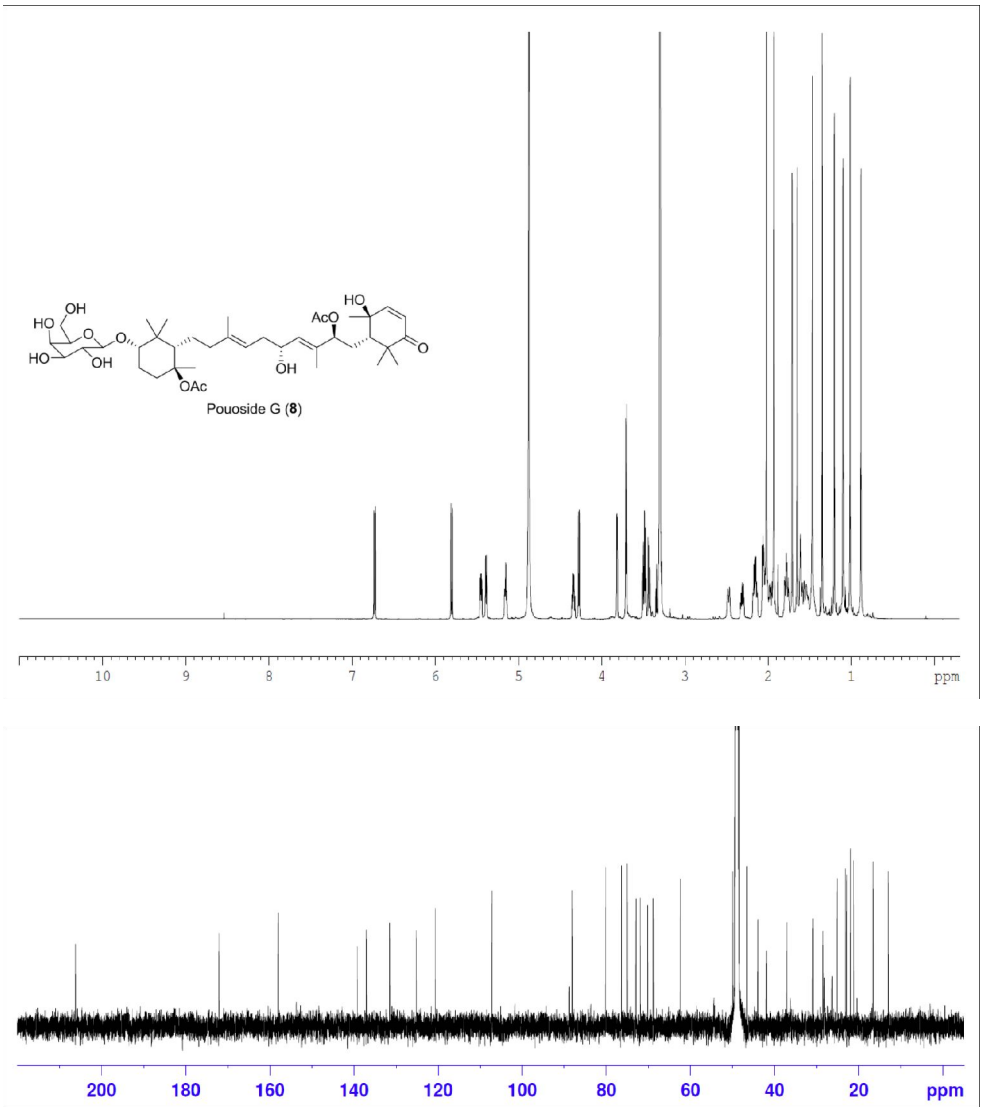

Figure A12. The ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra of Compound **8**

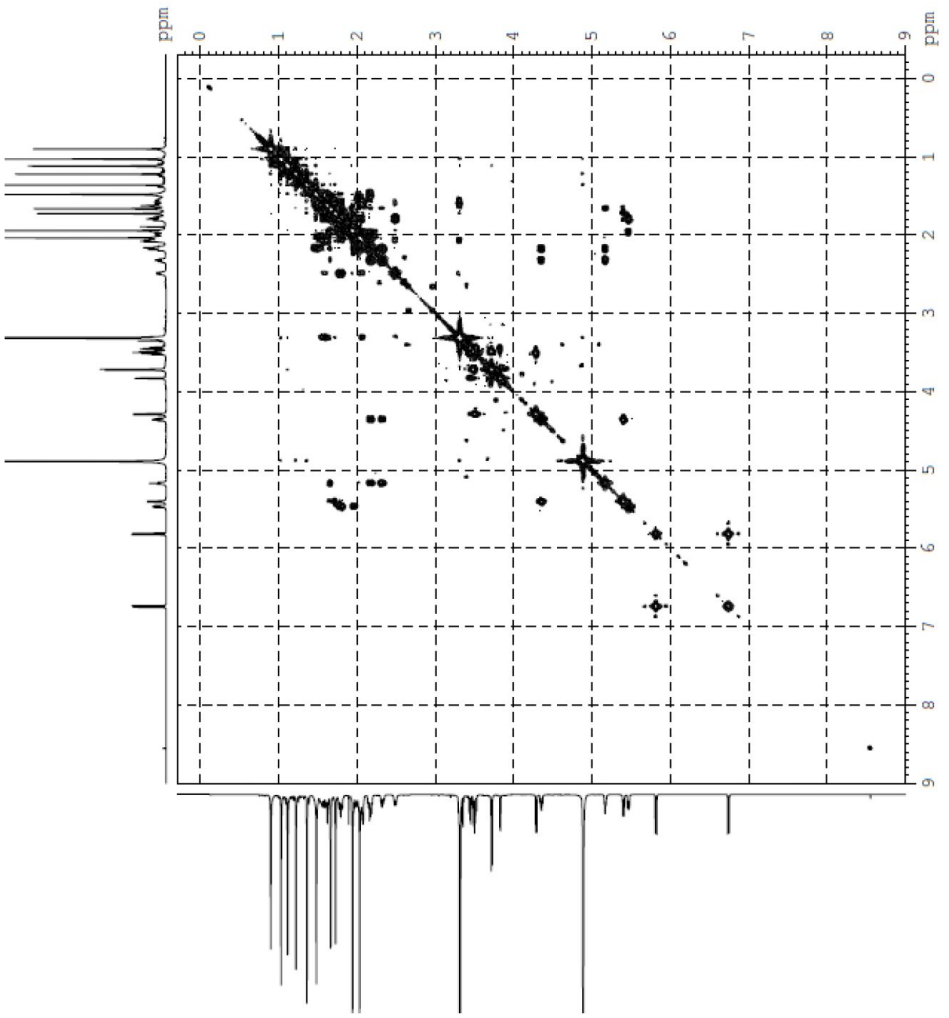

Figure A13. The COSY (600 MHz, MeOH-d_4) spectrum of Compound **8**

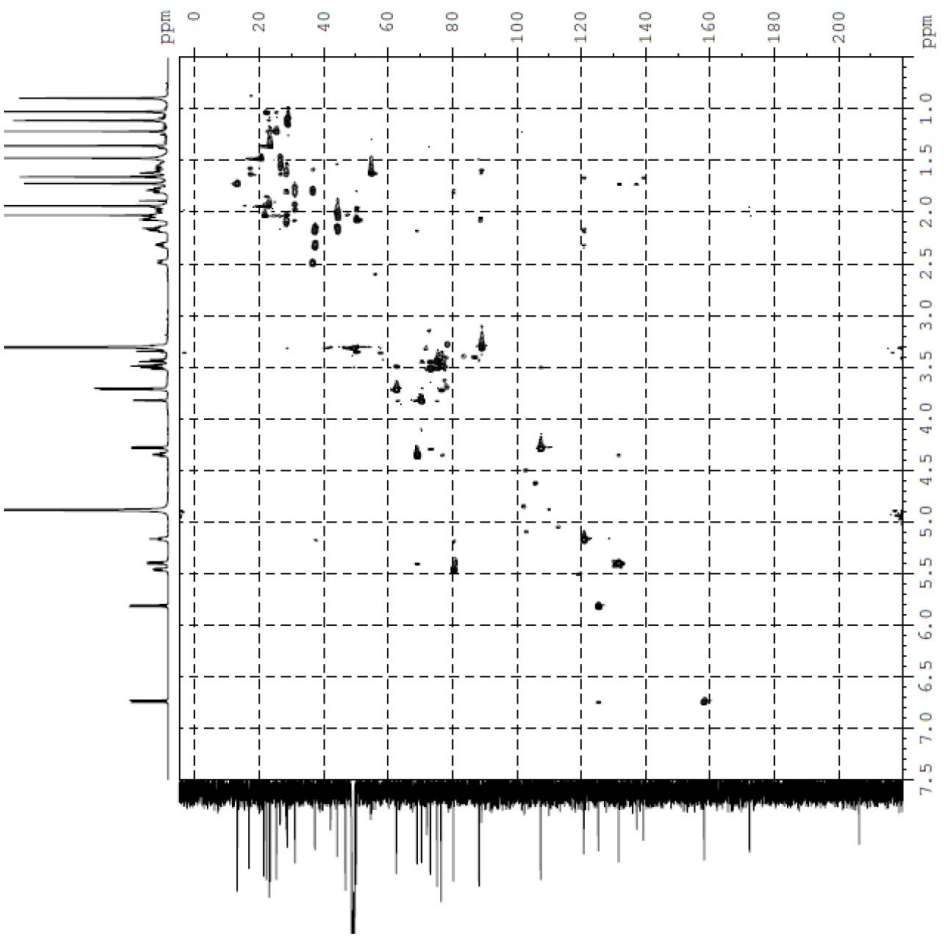

Figure A14. The *g*HSQC (600 MHz, MeOH-d_4) spectrum of Compound **8**

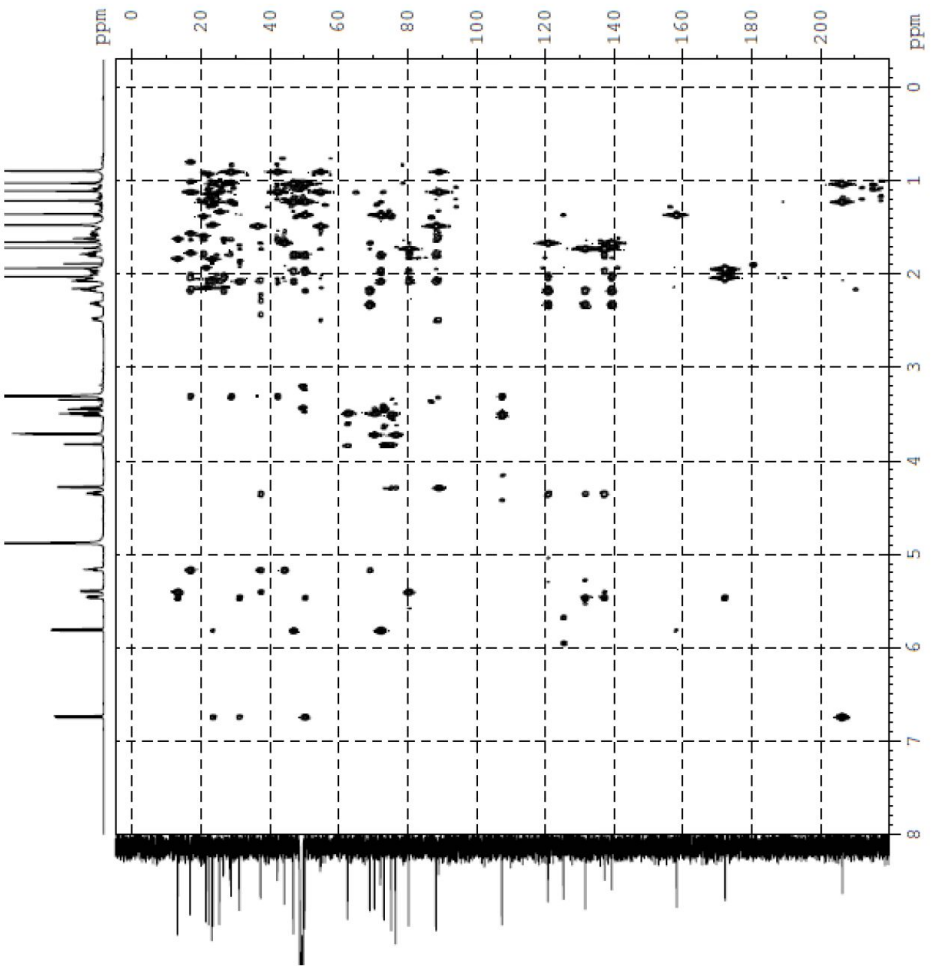

Figure A15. The *g*HMBC (600 MHz, MeOH-d_4) spectrum of Compound

8

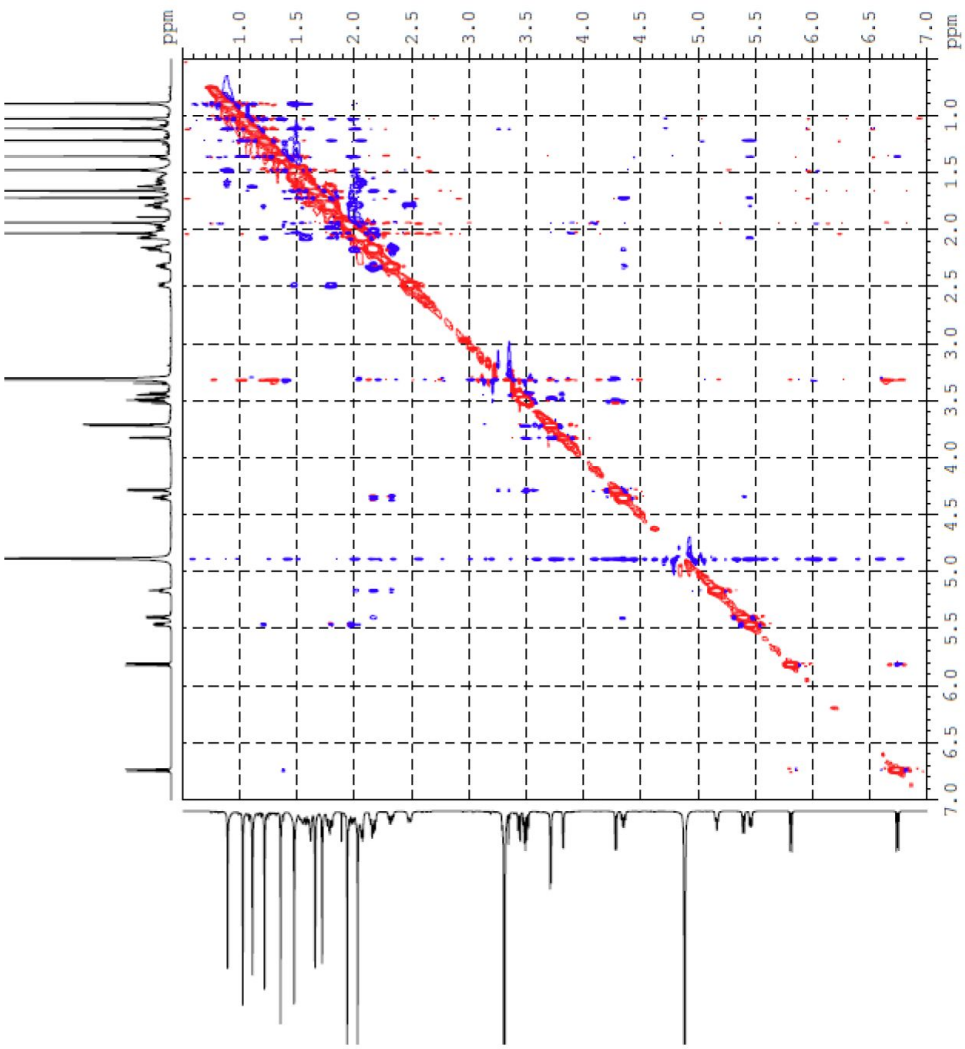

Figure A16. The NOESY (600 MHz, MeOH-d_4) spectrum of Compound **8**

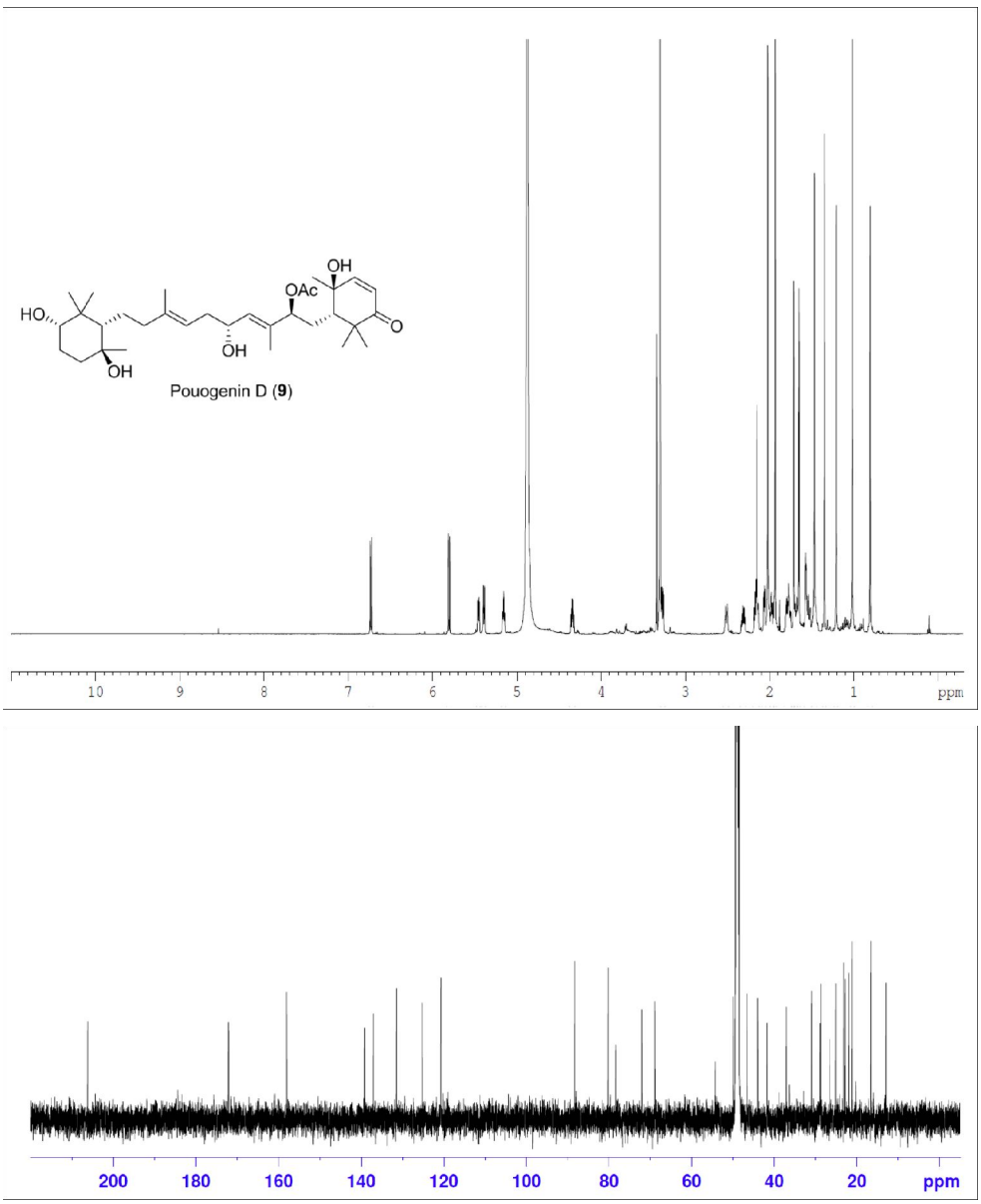

Figure A17. The ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra of Compound **9**

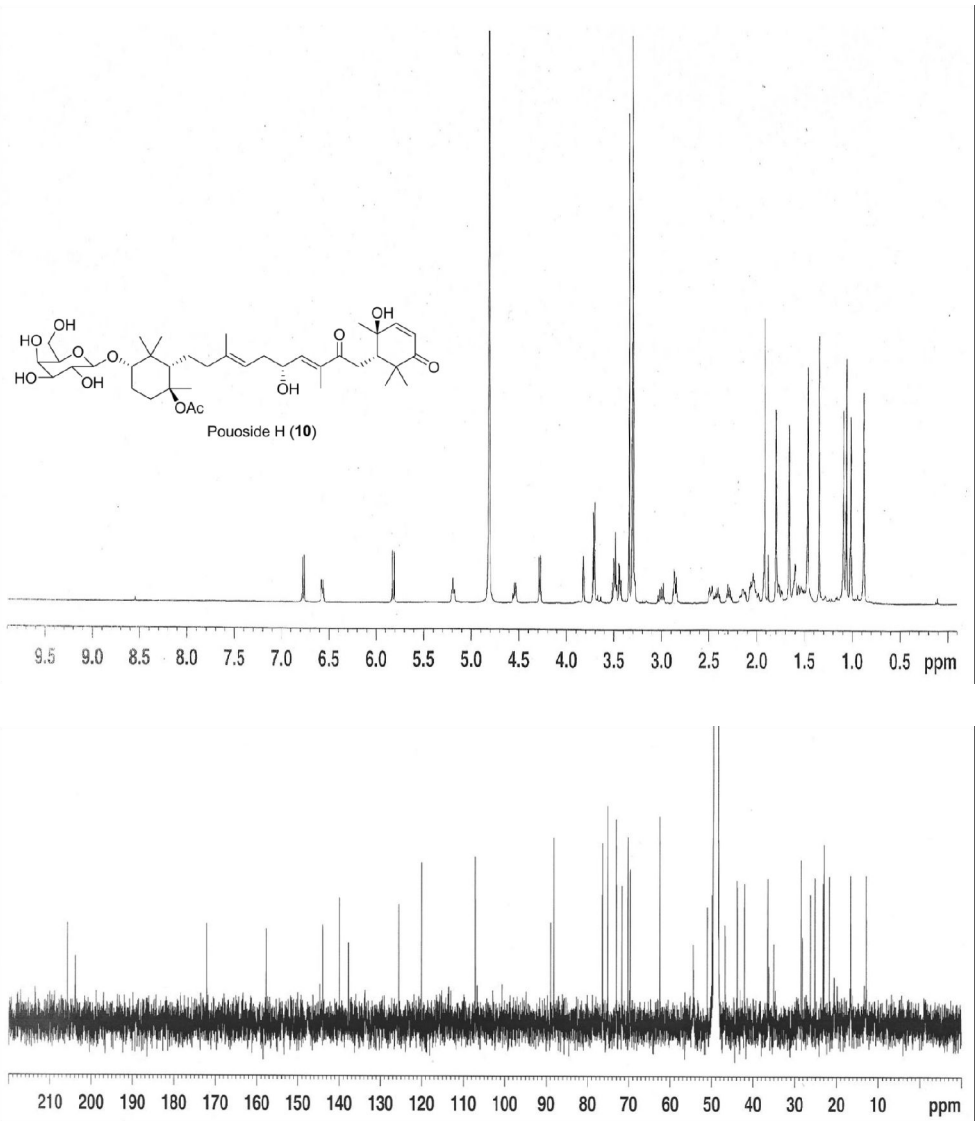

Figure A18. The ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra of Compound **10**

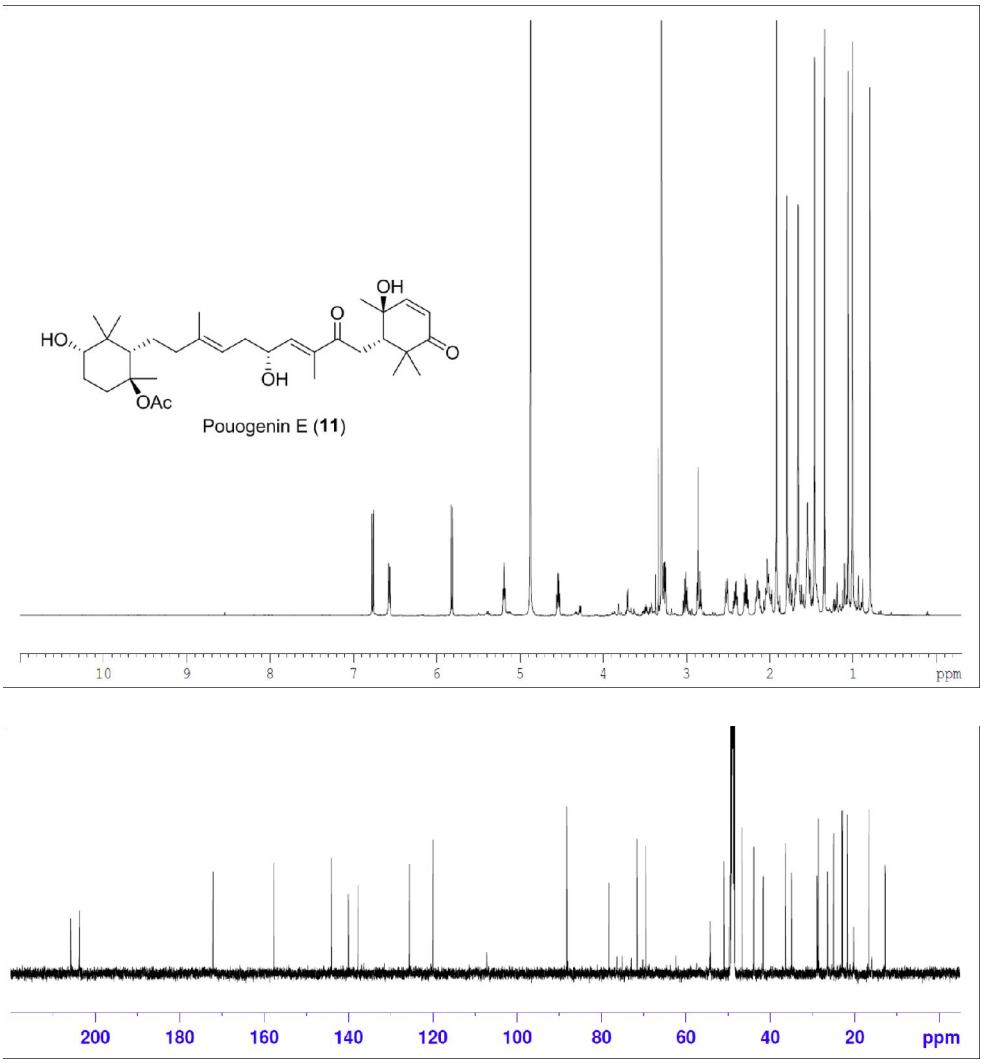

Figure A19. The ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra of Compound **11**

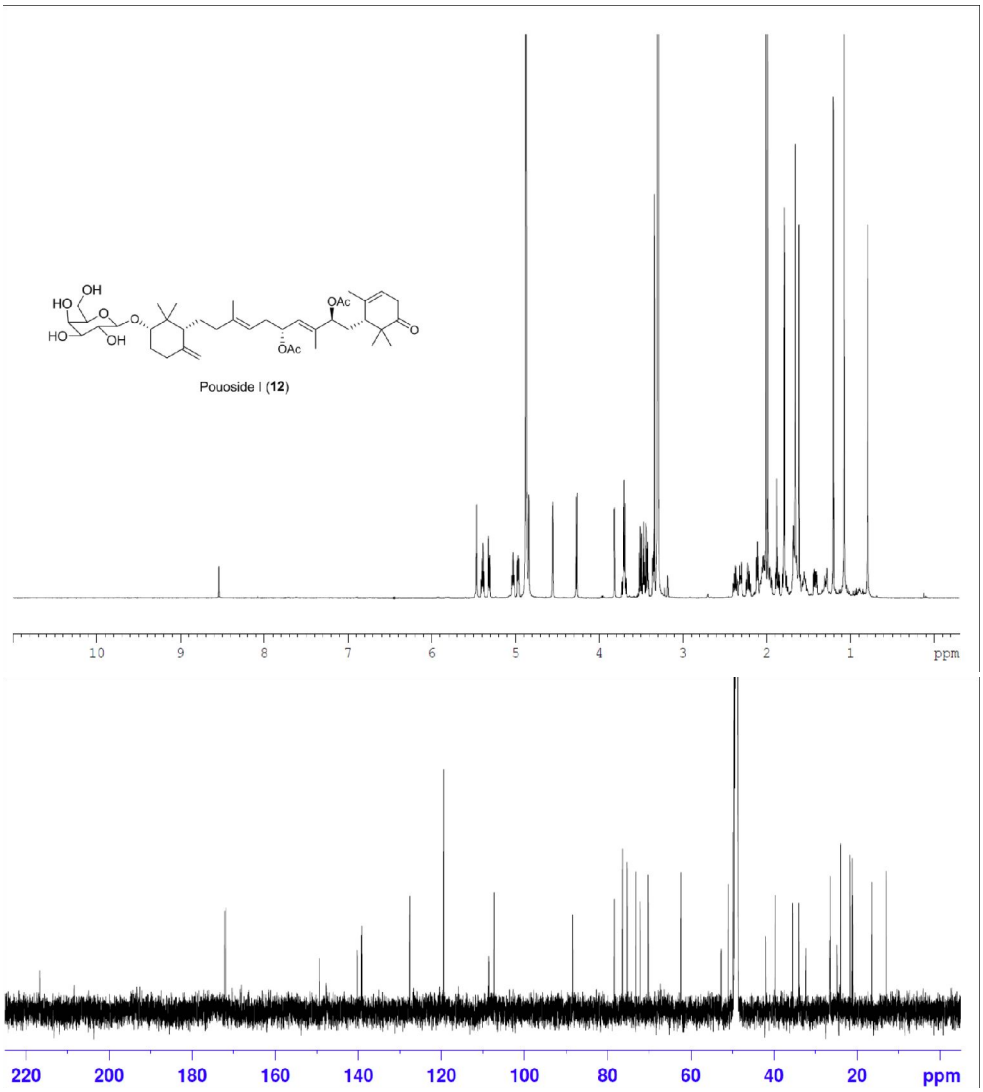

Figure A20. The ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra of Compound **12**

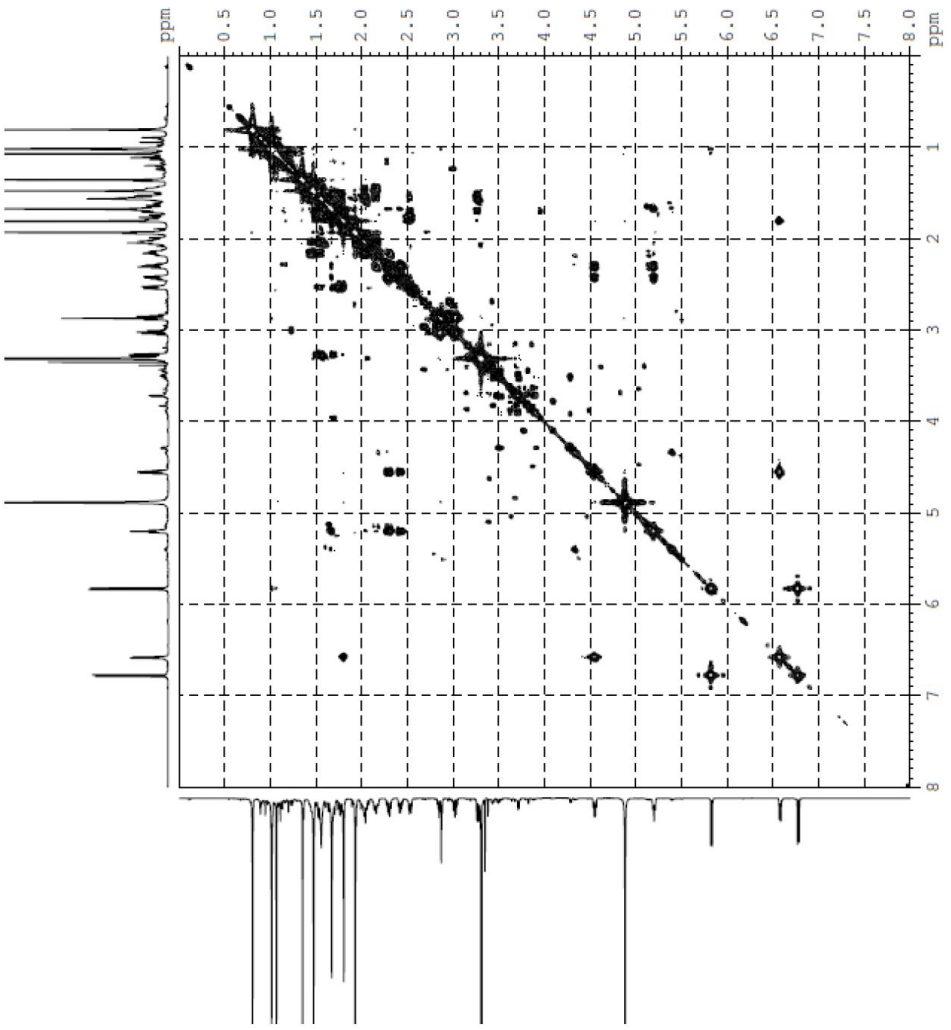

Figure A21. The COSY (600 MHz, MeOH-d_4) spectrum of Compound **12**

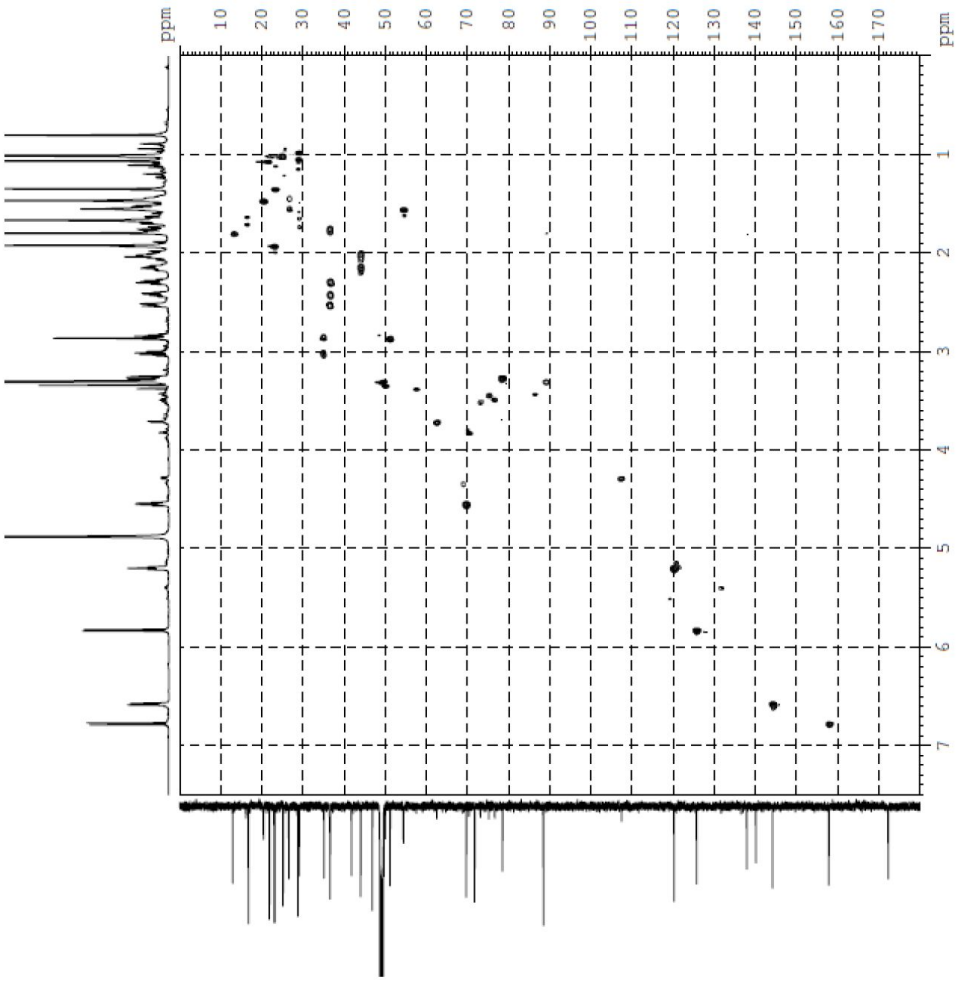

Figure A22. The *g*HSQC (600 MHz, MeOH-d_4) spectrum of Compound

12

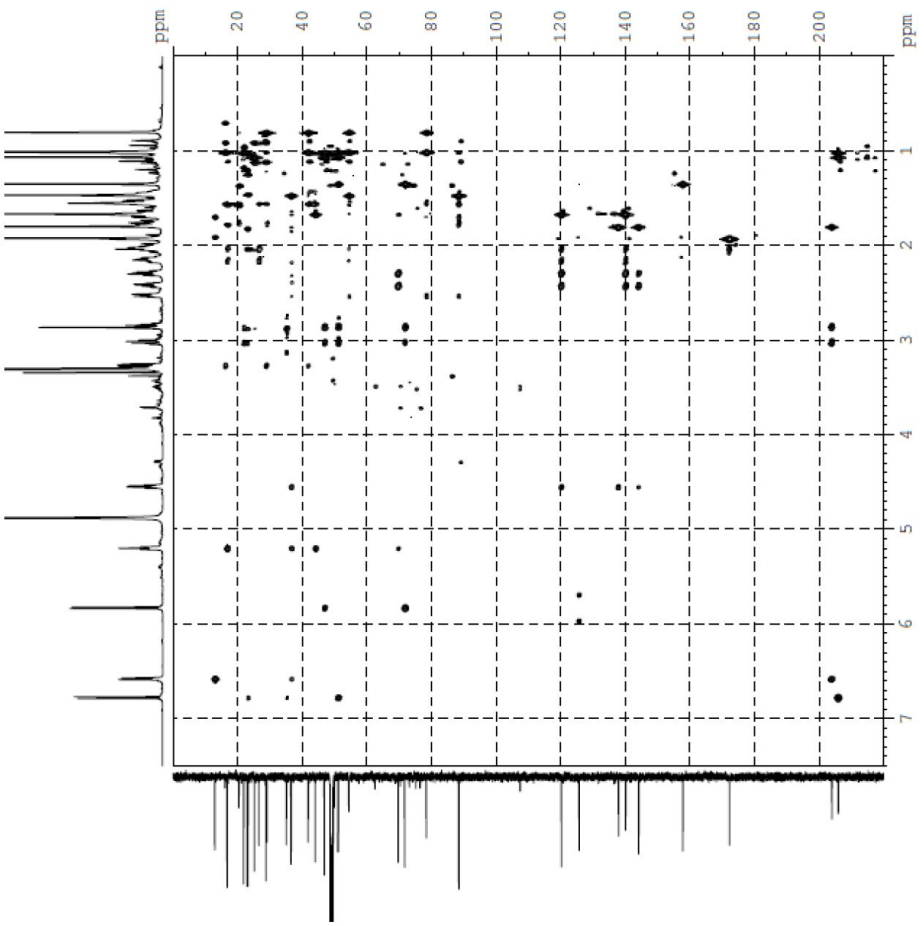

Figure A23. The *g*HMBC (600 MHz, MeOH-d_4) spectrum of Compound

12

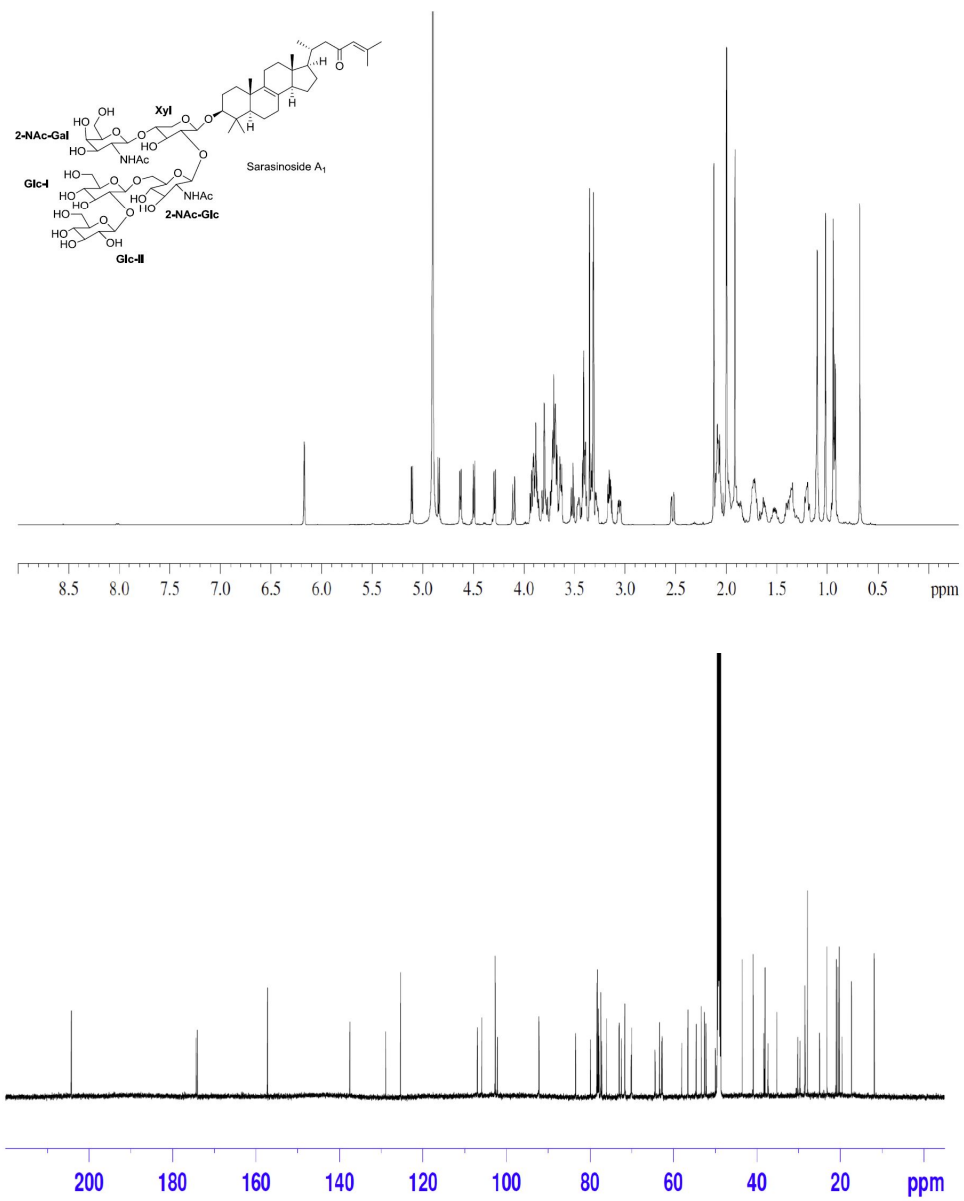

Figure A24. The ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra of Compound **13**

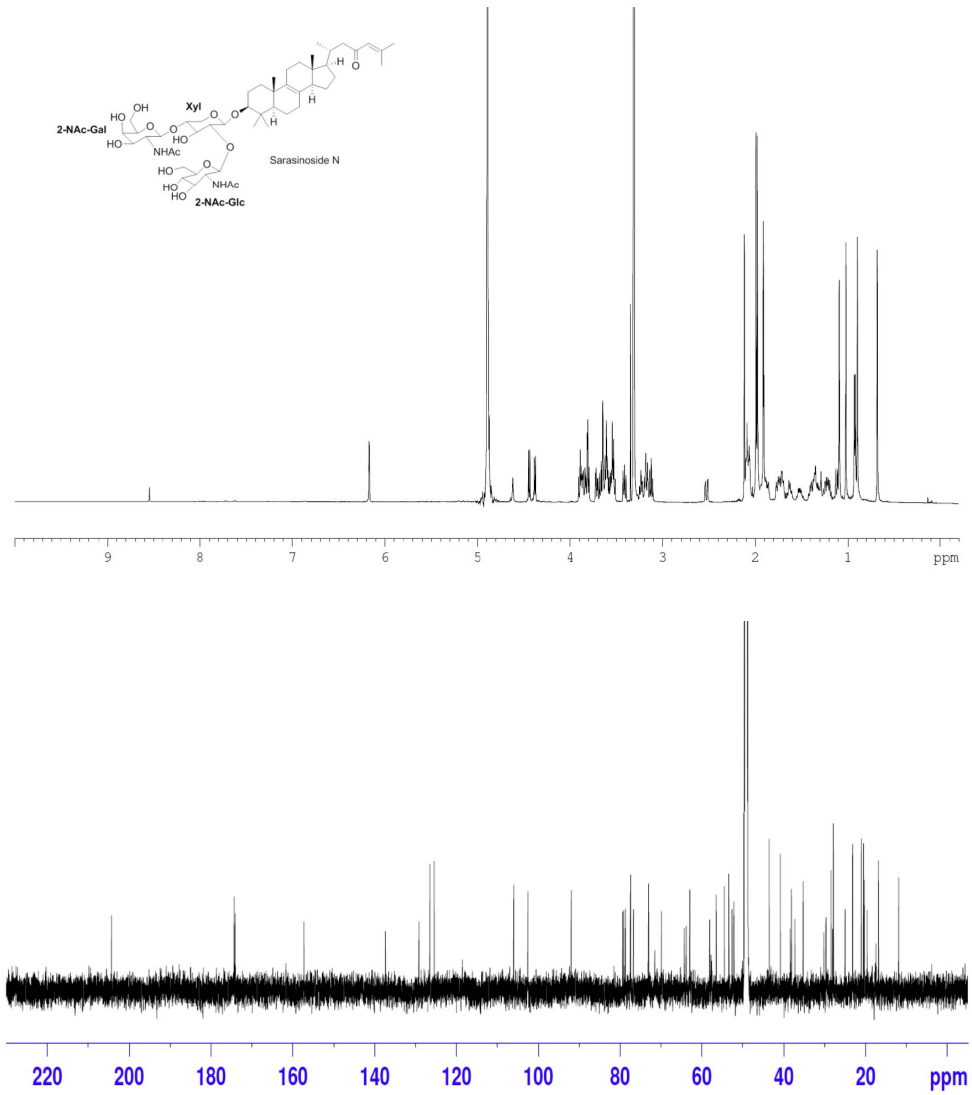

Figure A25. The ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra of Compound **21**

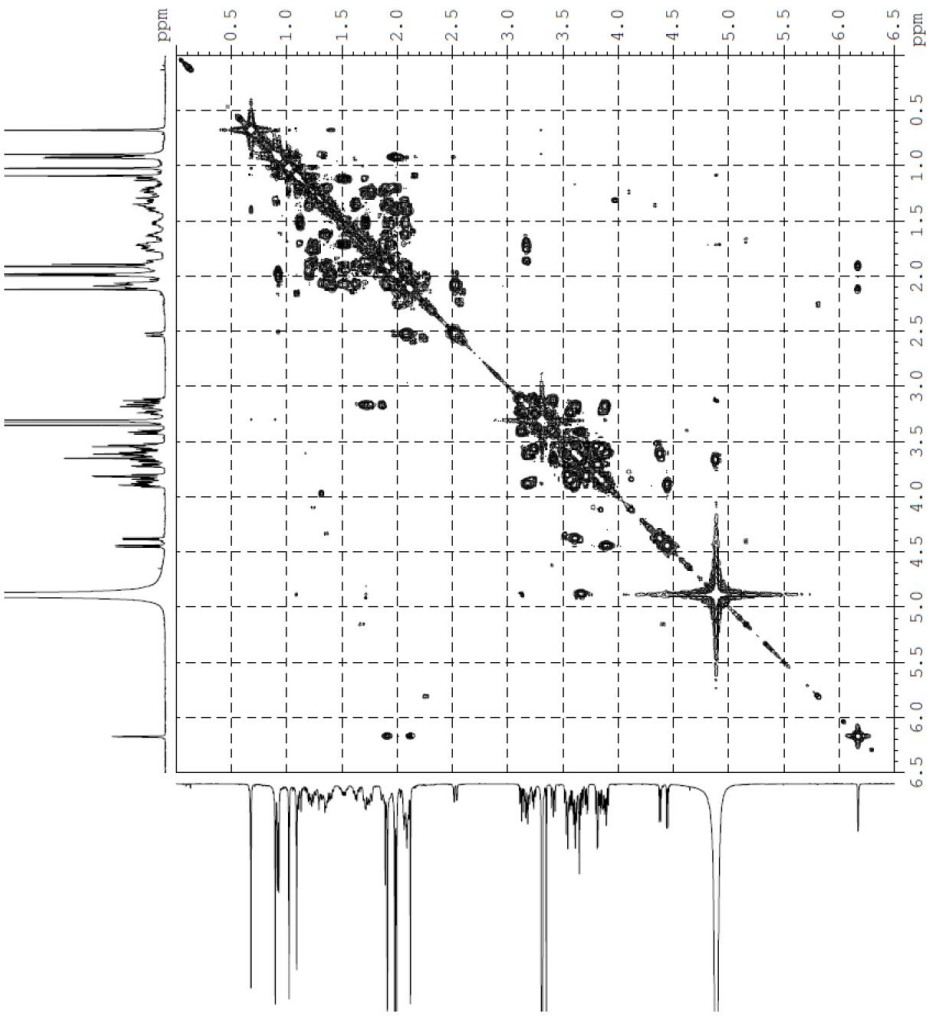

Figure A26. The COSY (600 MHz, MeOH-d_4) spectrum of Compound **21**

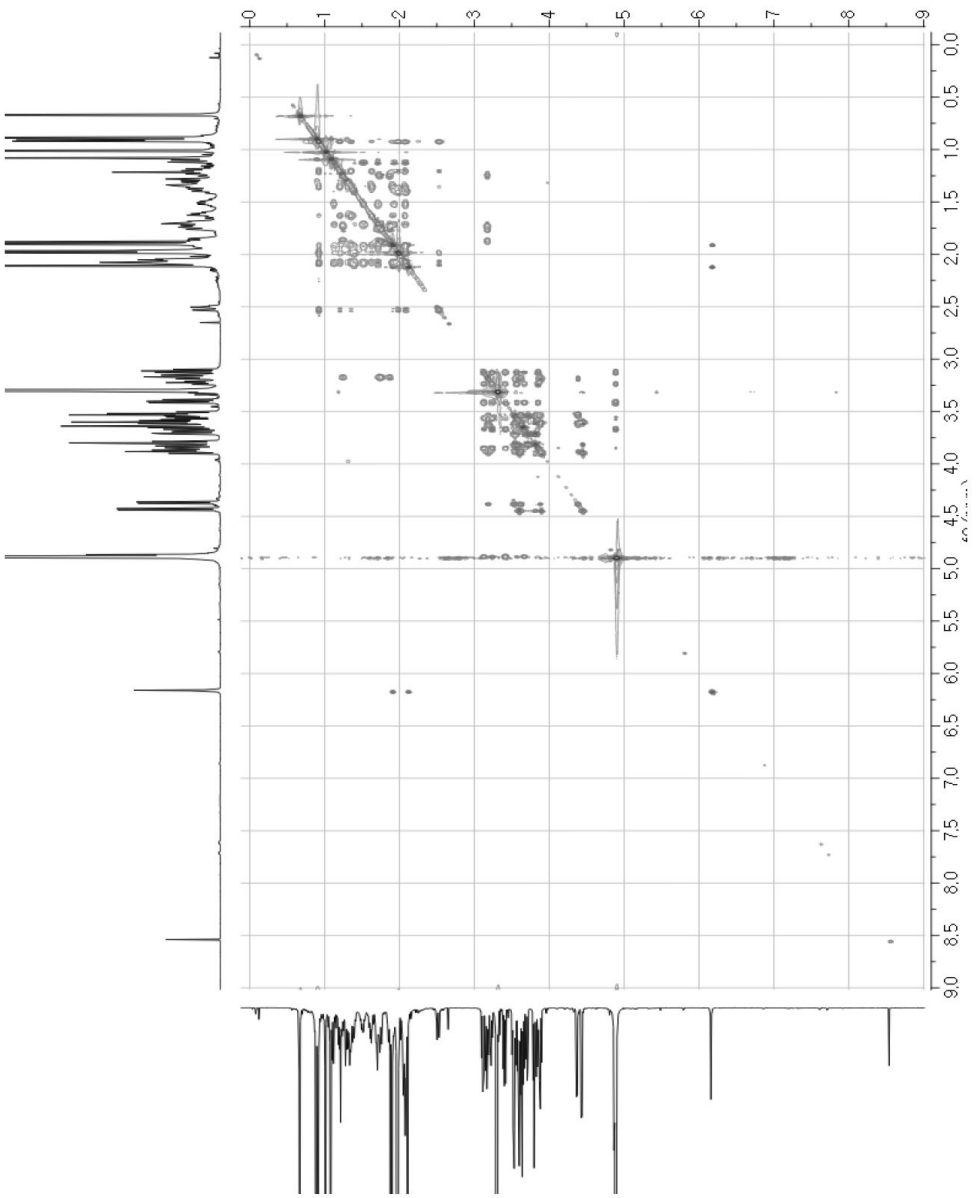

Figure A27. The TOCSY (600 MHz, MeOH-d_4) spectrum of Compound

21

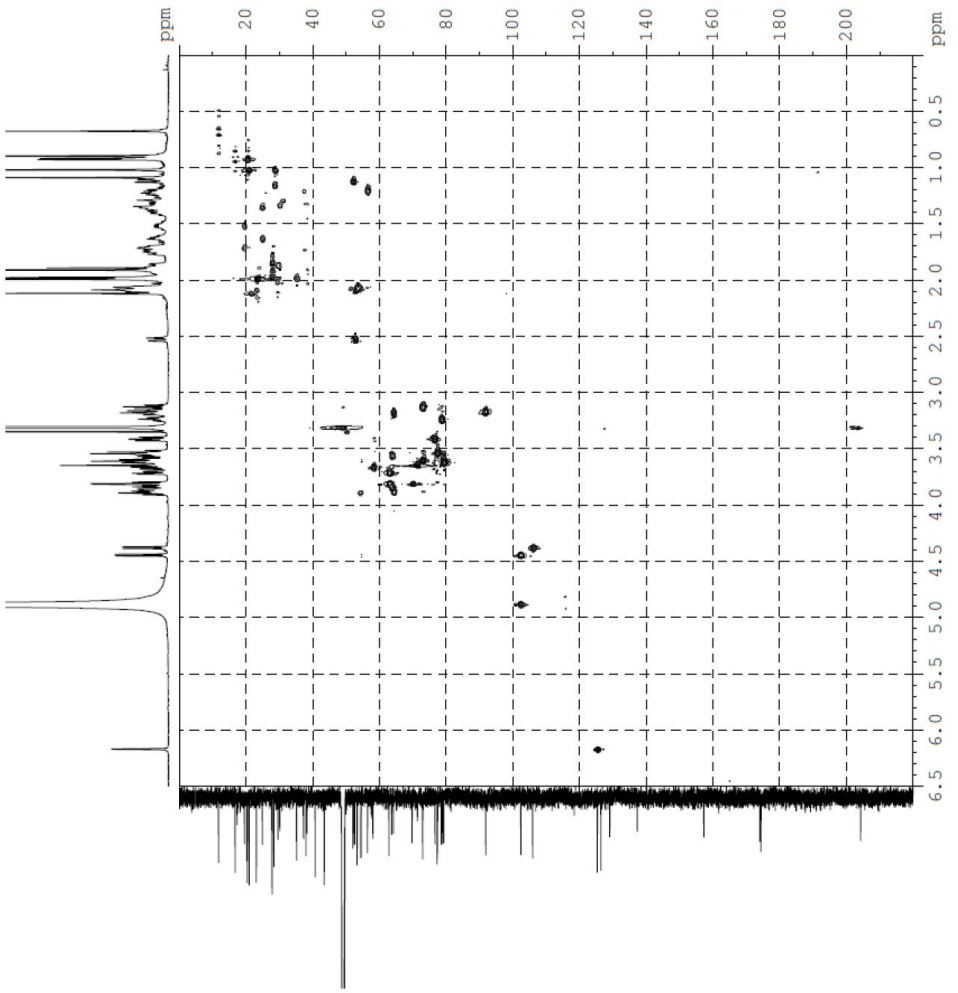

Figure A28. The *g*HSQC (600 MHz, MeOH-d_4) spectrum of Compound

21

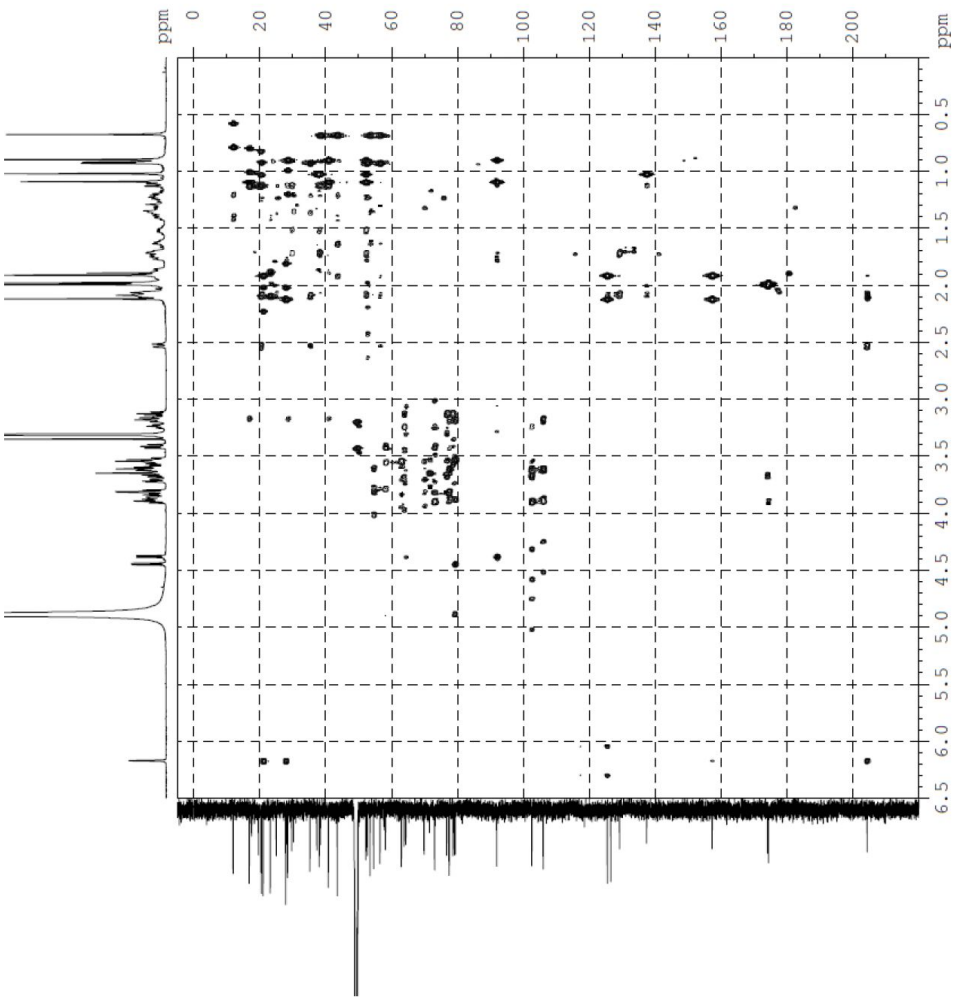

Figure A29. The *g*HMBC (600 MHz, MeOH-d_4) spectrum of Compound

21

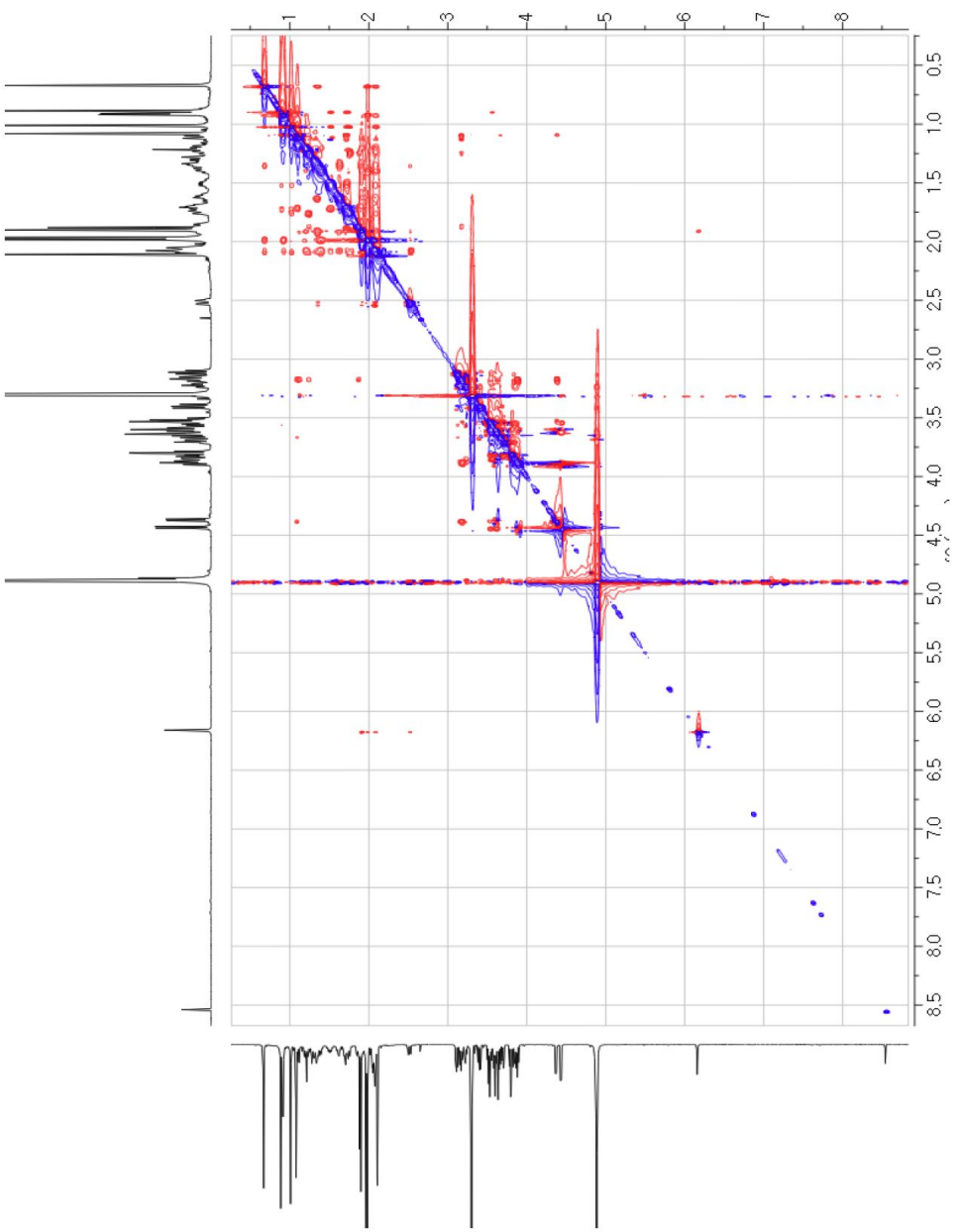

Figure A30. The ROESY (600 MHz, MeOH-d_4) spectrum of Compound

21

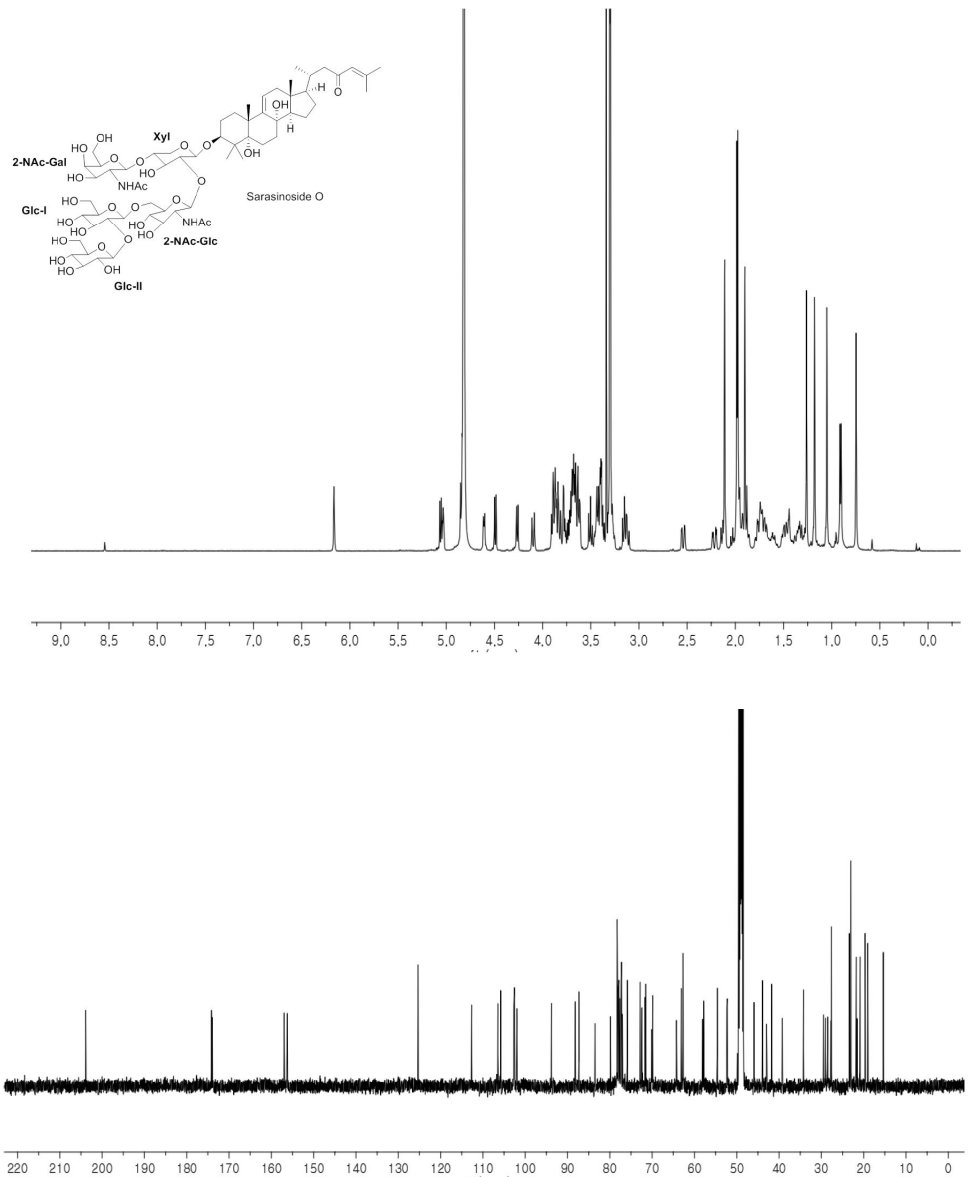

Figure A31. The ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra of Compound **22**

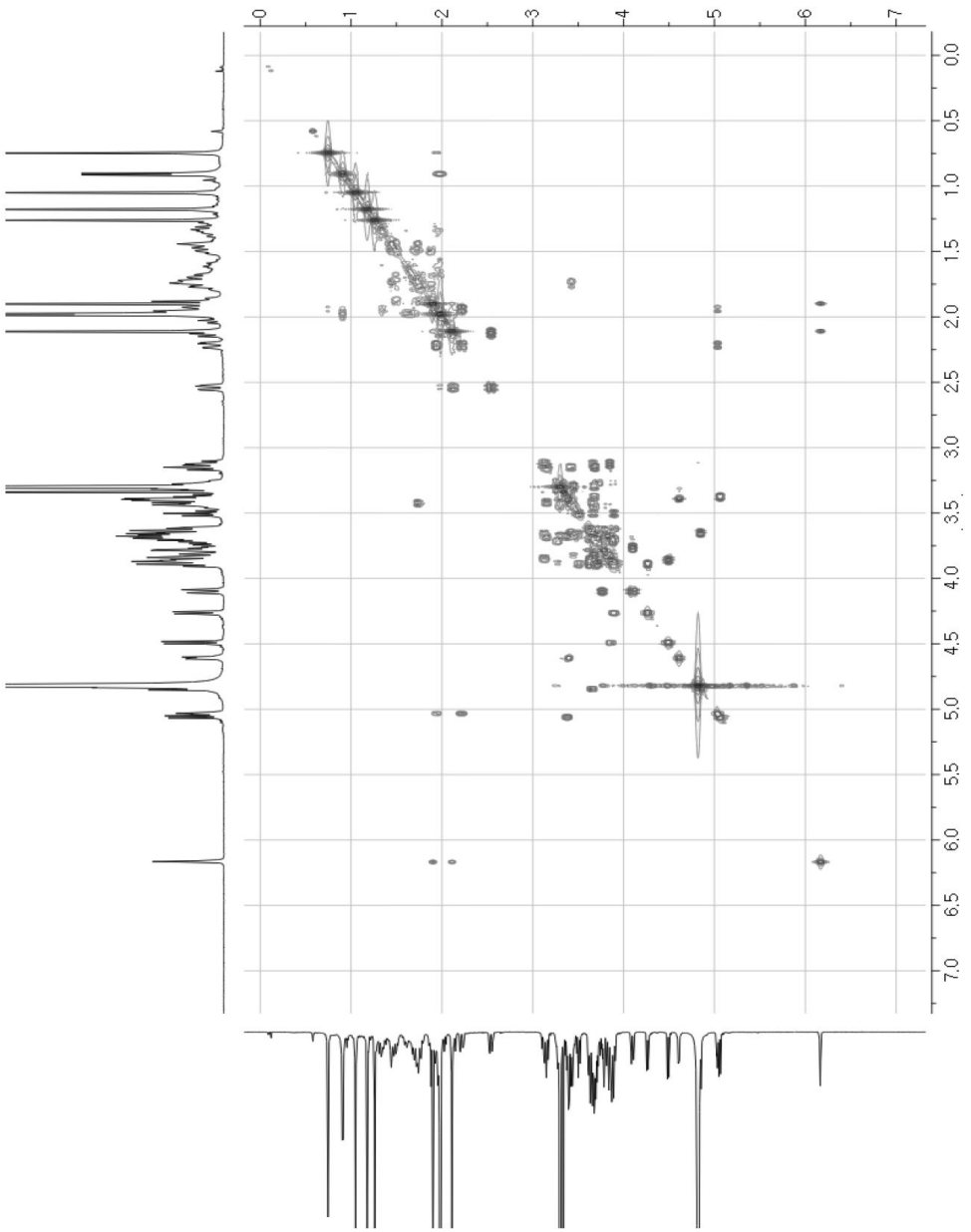

Figure A32. The COSY (500 MHz, MeOH-d_4) spectrum of Compound **22**

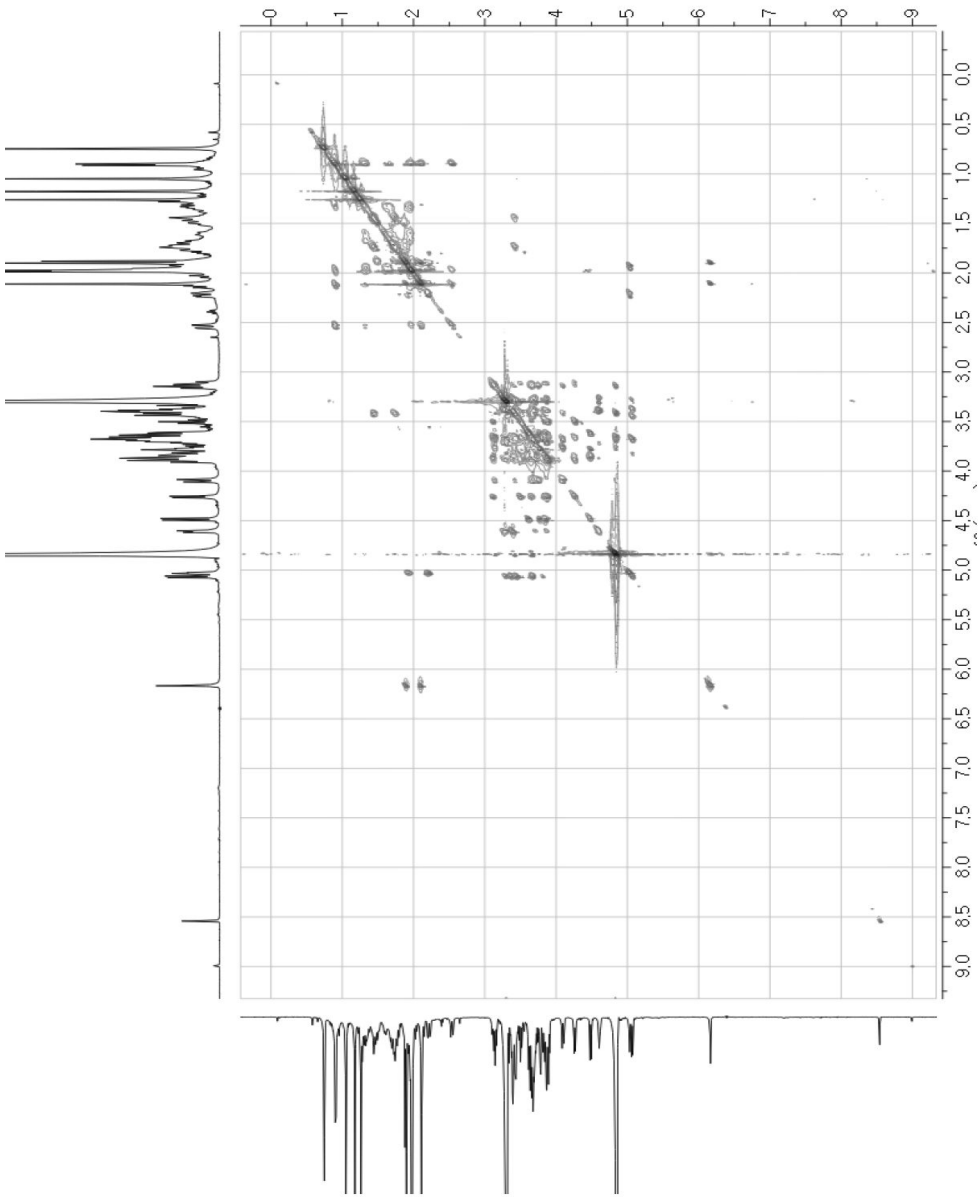

Figure A33. The TOCSY (500 MHz, MeOH-d_4) spectrum of Compound

22

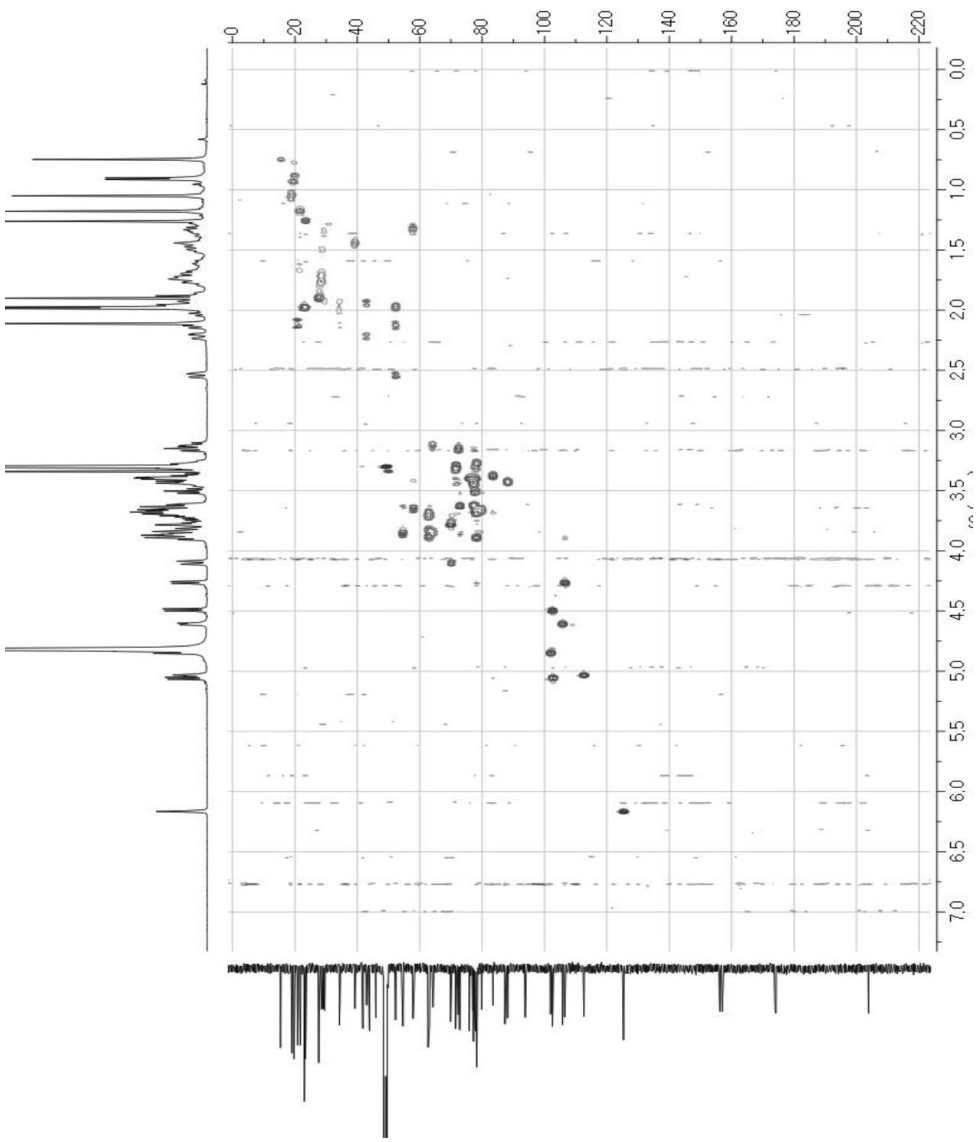

Figure A34. The *g*HSQC (500 MHz, MeOH-d_4) spectrum of Compound

22

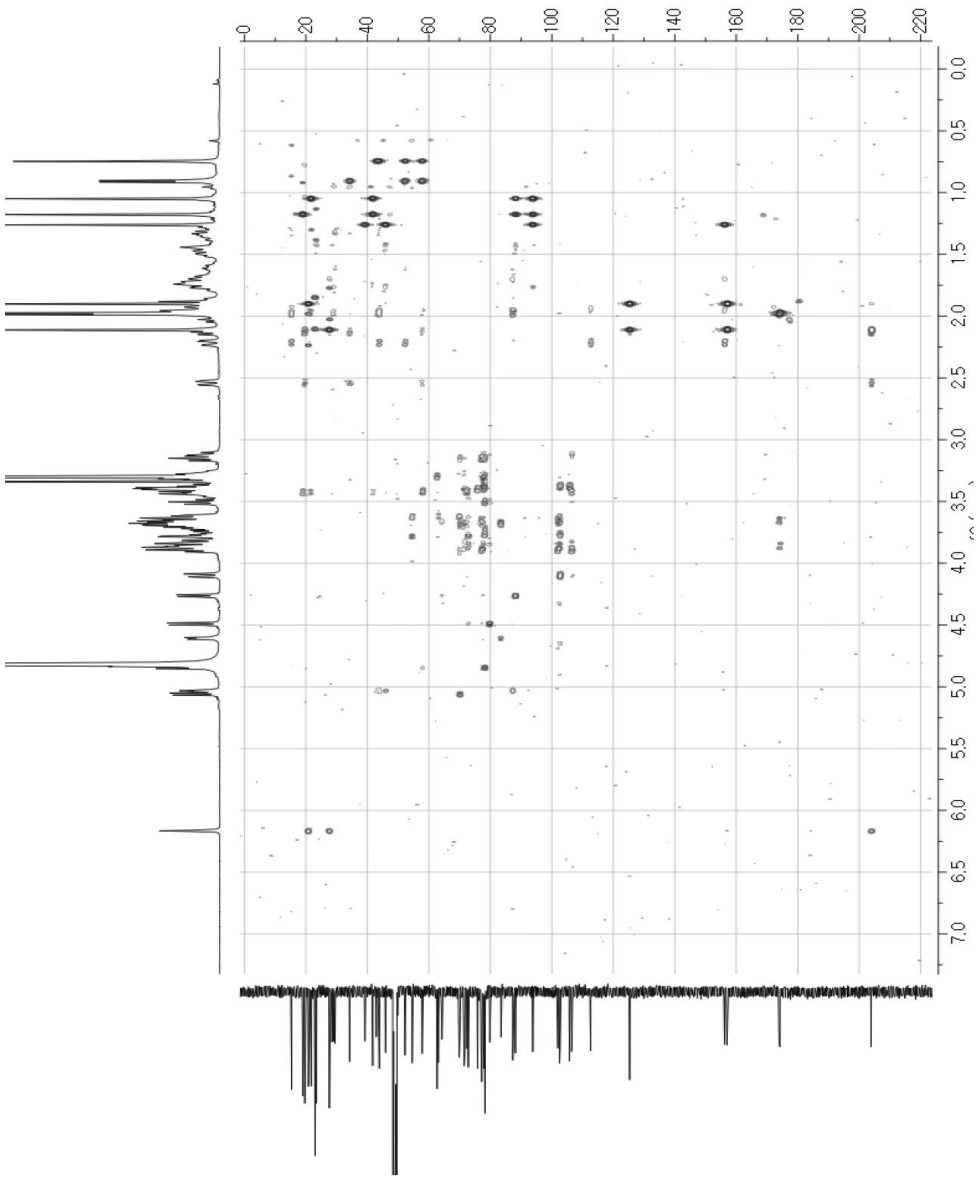

Figure A35. The *g*HMBC (500 MHz, MeOH-d_4) spectrum of Compound

22

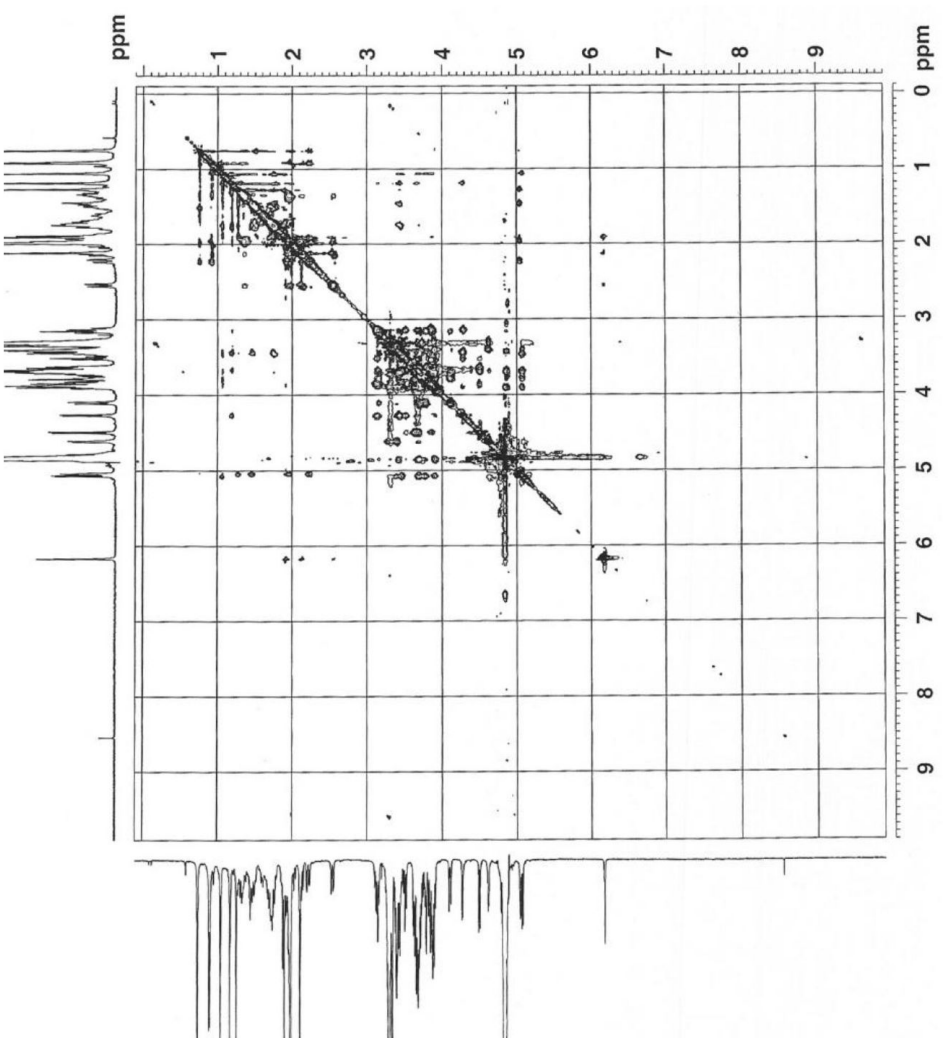

Figure A36. The ROESY (500 MHz, MeOH-d_4) spectrum of Compound

22

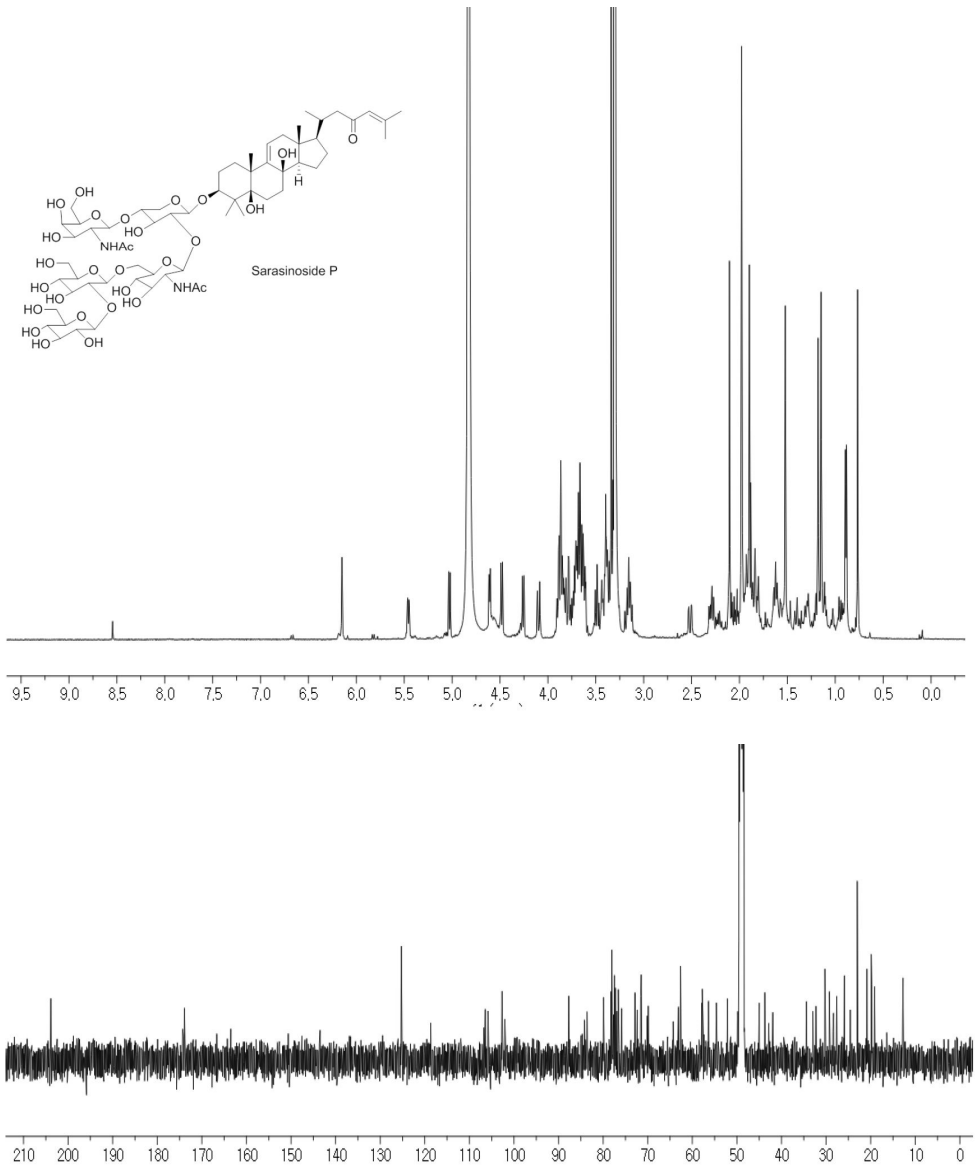

Figure A37. The ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra of Compound **23**

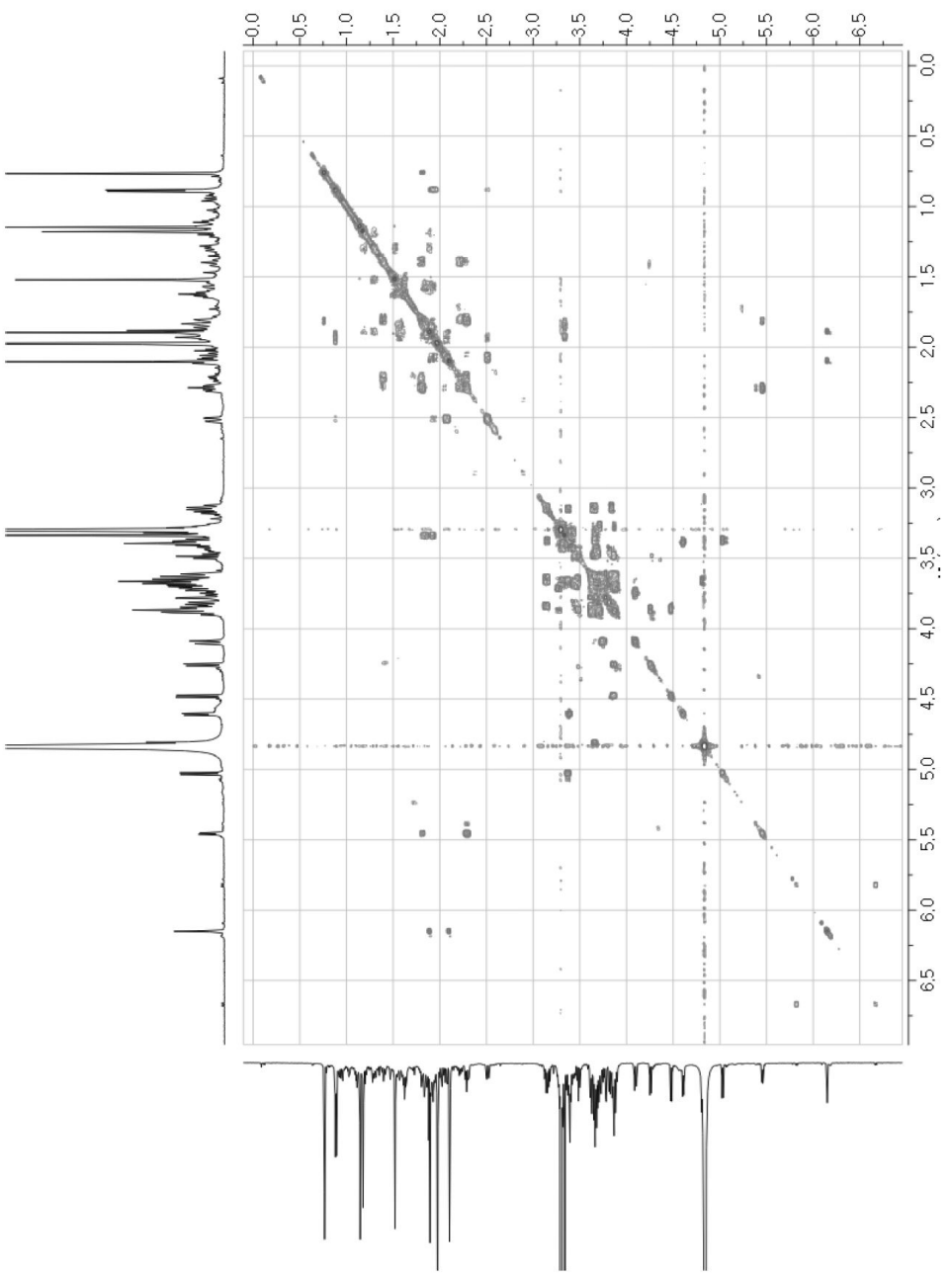

Figure A38. The COSY (500 MHz, MeOH-d_4) spectrum of Compound **23**

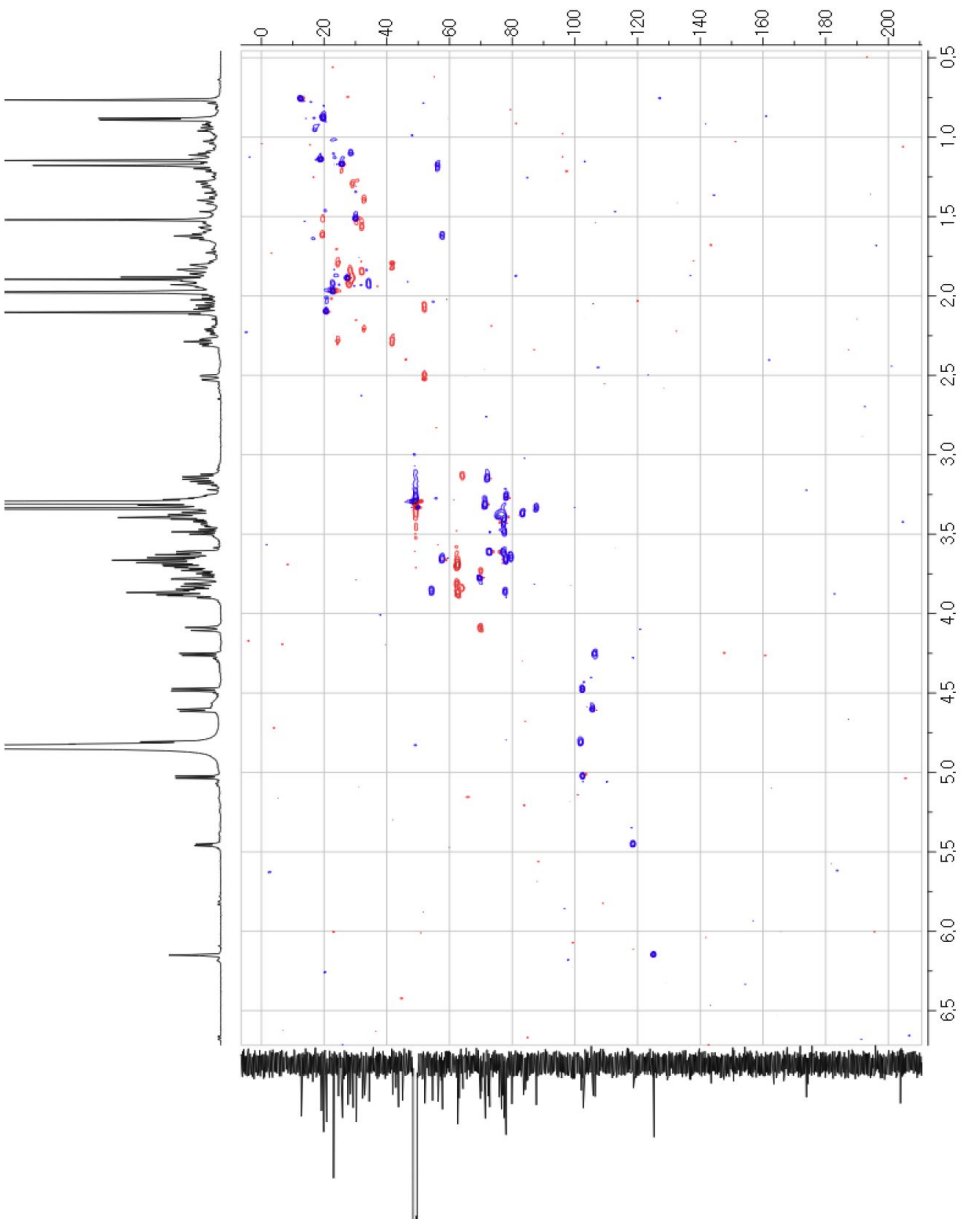

Figure A39. The *g*HSQC (500 MHz, MeOH-d_4) spectrum of Compound

23

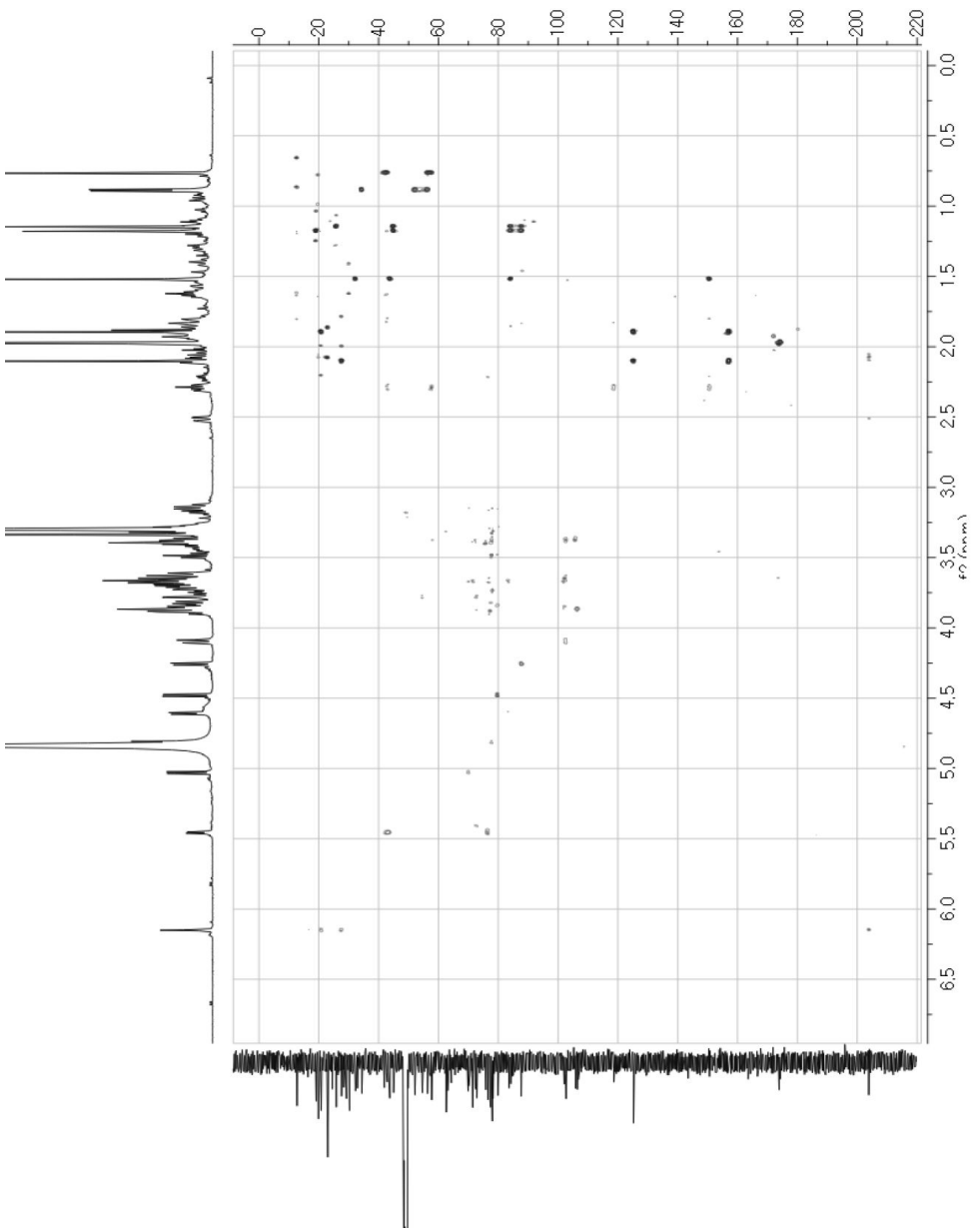

Figure A40. The *g*HMBC (500 MHz, MeOH-d_4) spectrum of Compound

23

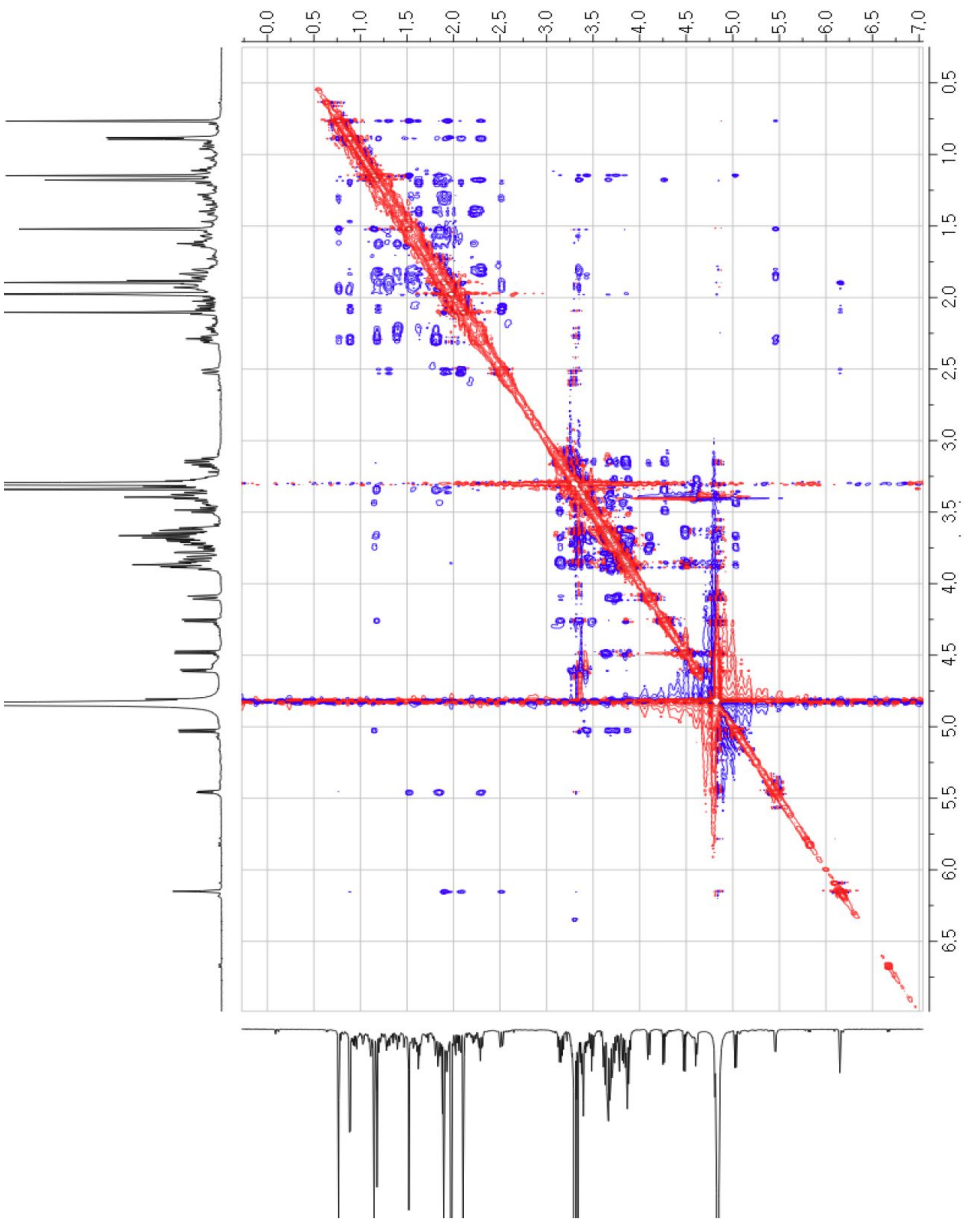

Figure A41. The ROESY (500 MHz, MeOH-d_4) spectrum of Compound

23

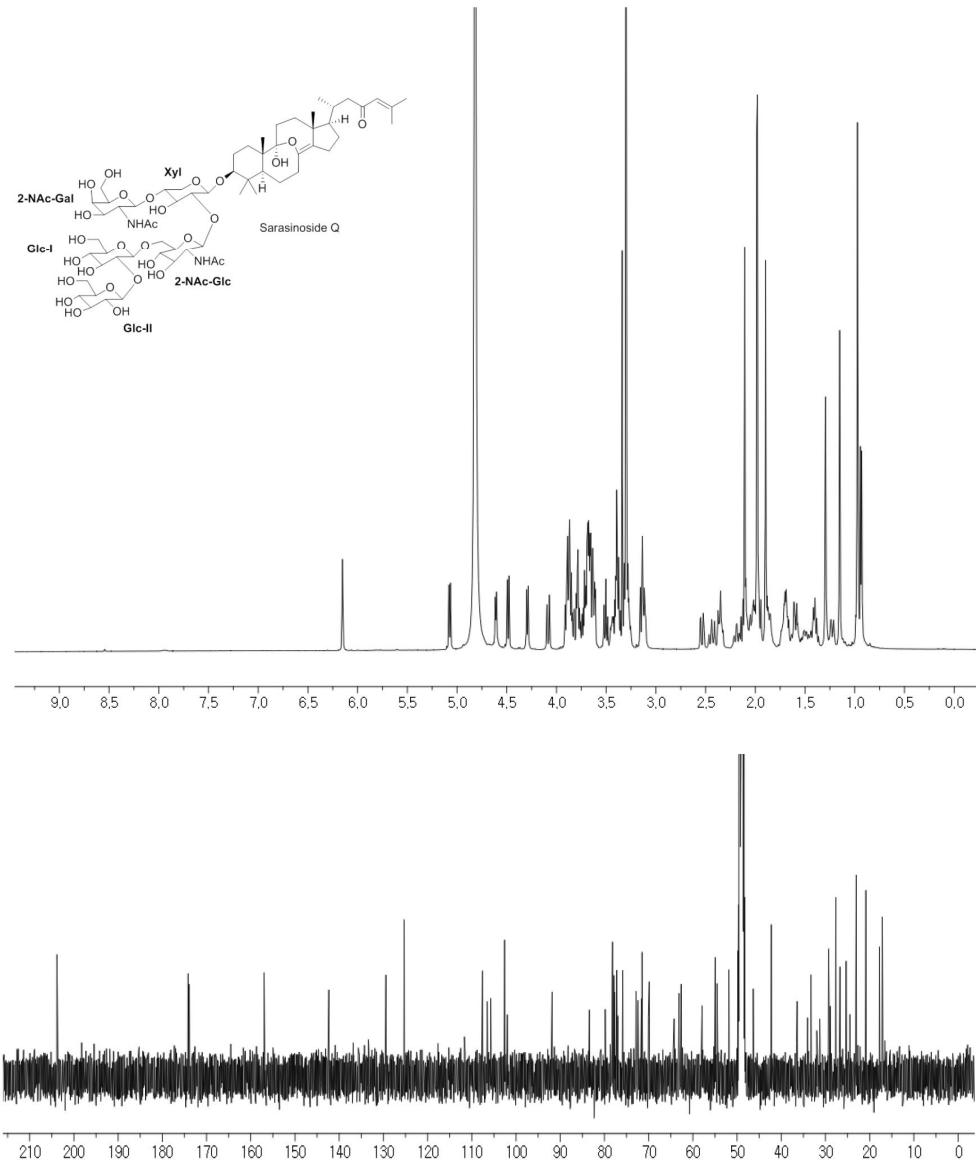

Figure A42. The ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra of Compound **24**

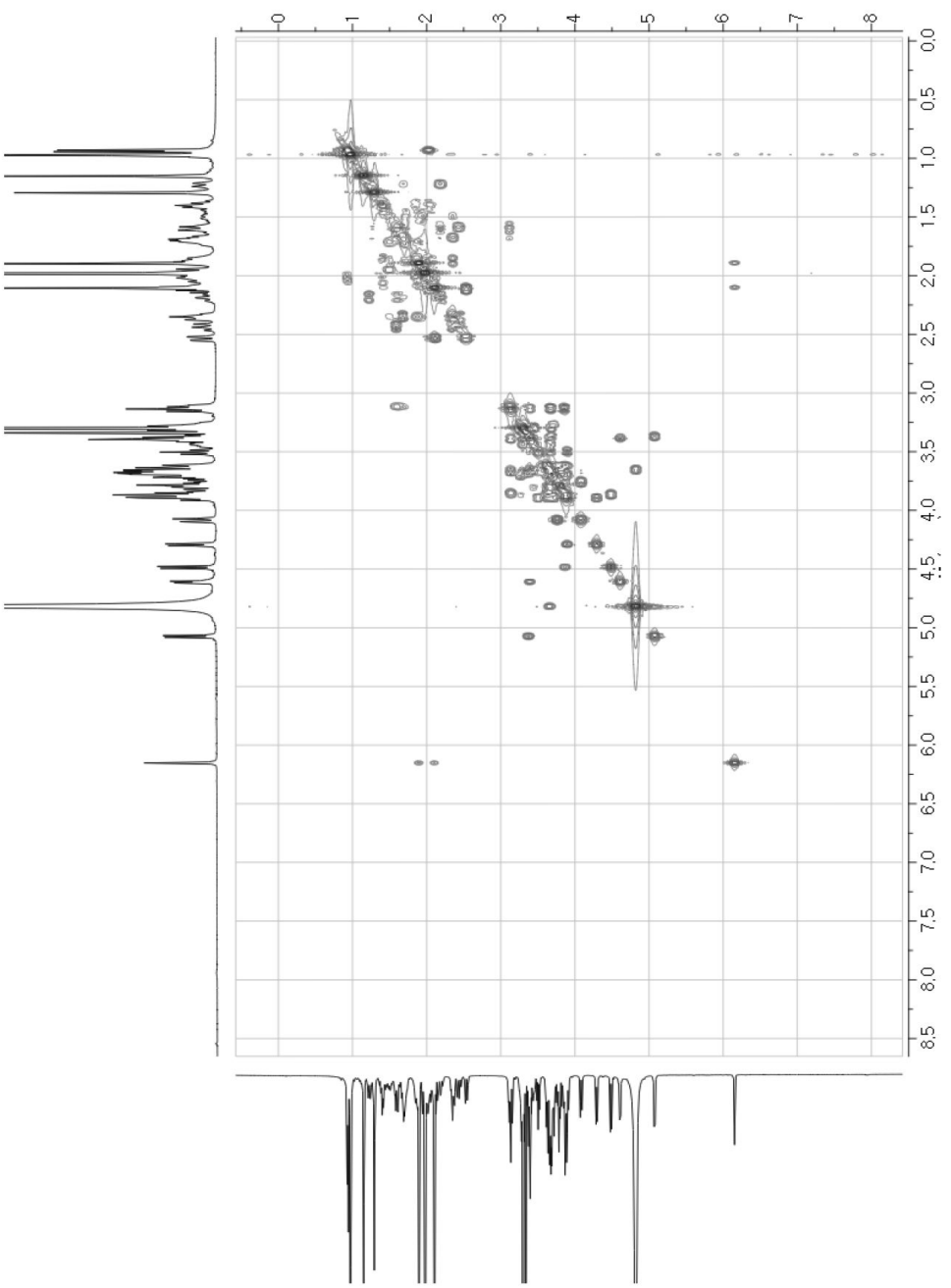

Figure A43. The COSY (500 MHz, MeOH-d_4) spectrum of Compound **24**

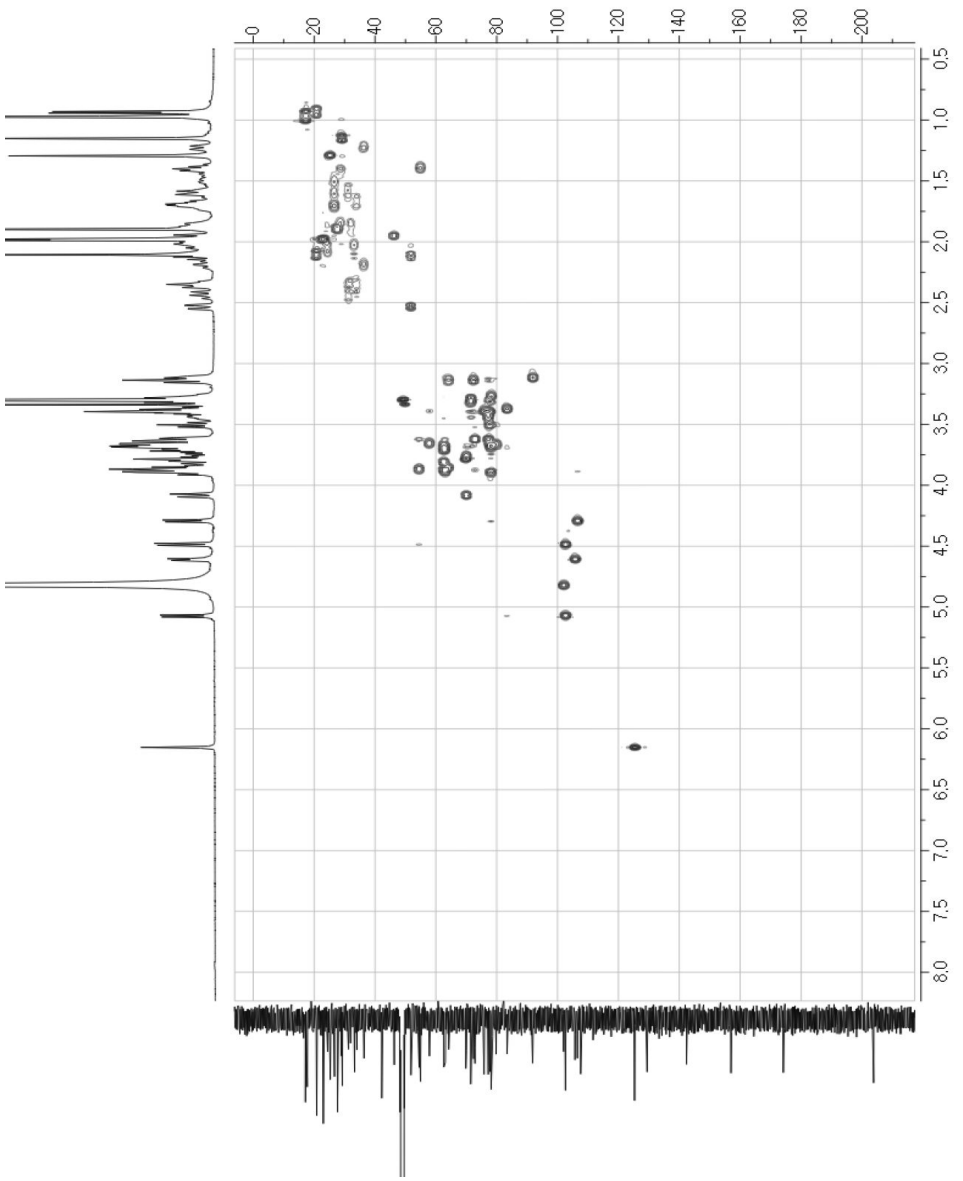

Figure A44. The *g*HSQC (500 MHz, MeOH-d_4) spectrum of Compound

24

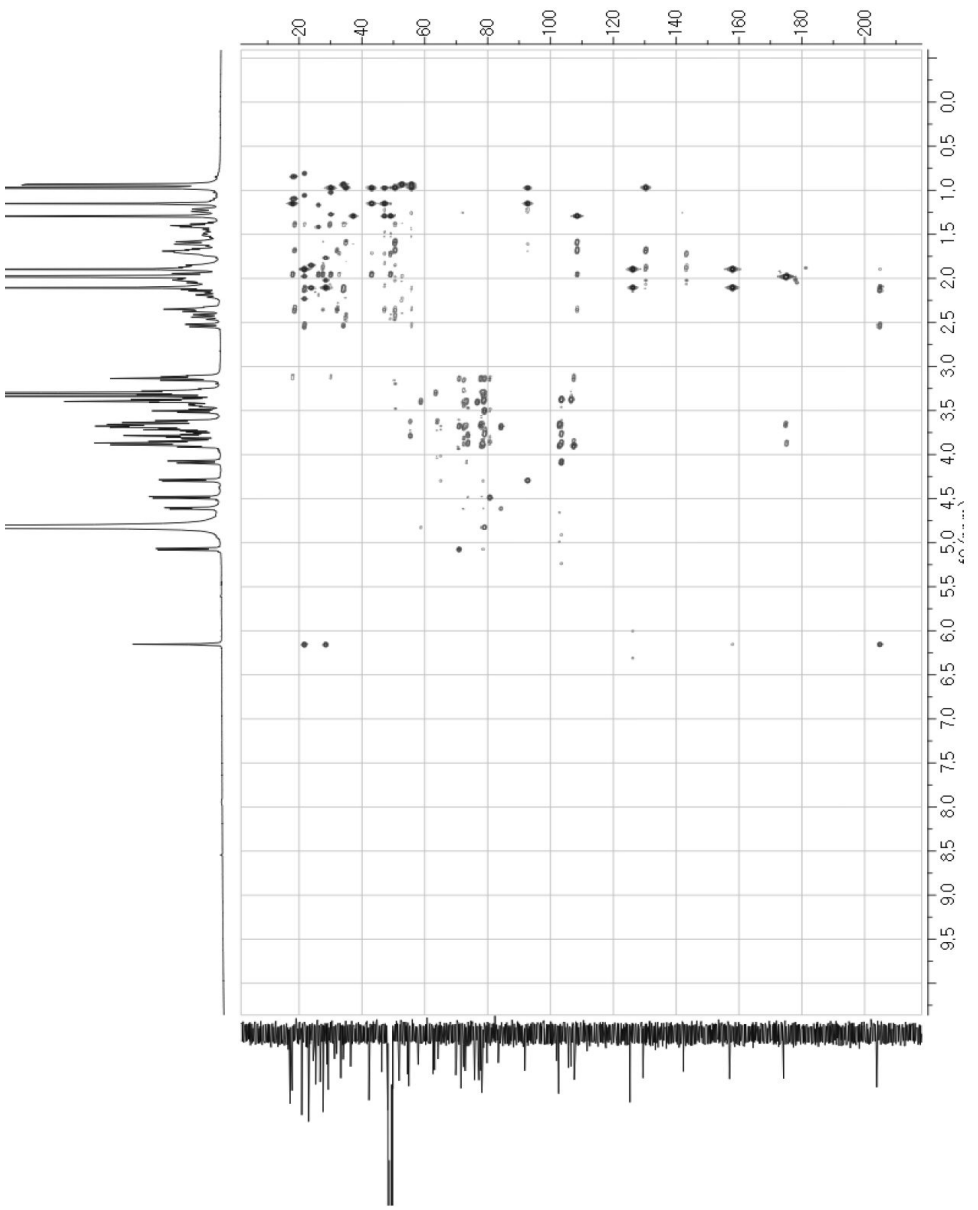

Figure A45. The *g*HMBC (500 MHz, MeOH-d_4) spectrum of Compound

24

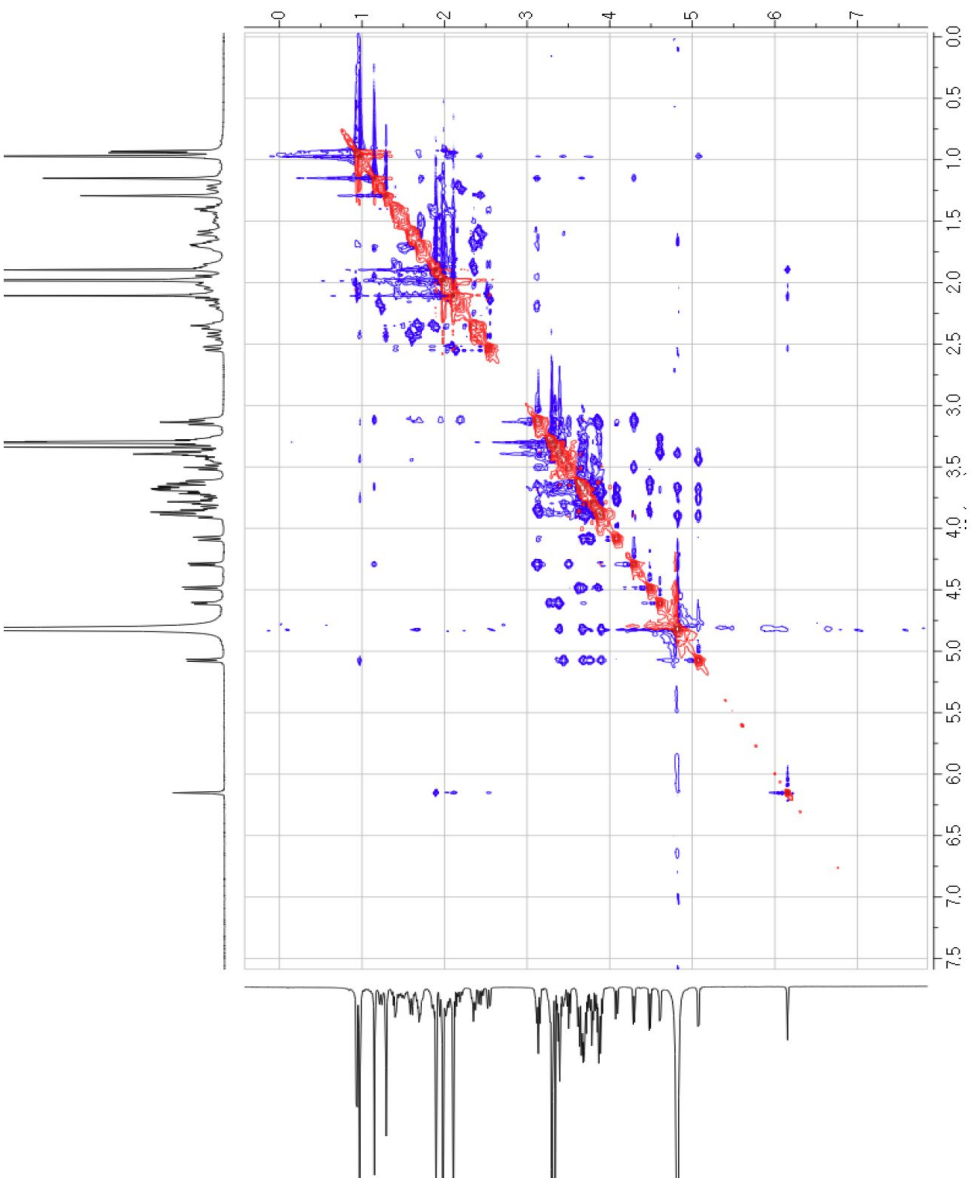

Figure A46. The ROESY (500 MHz, MeOH-d_4) spectrum of Compound

24

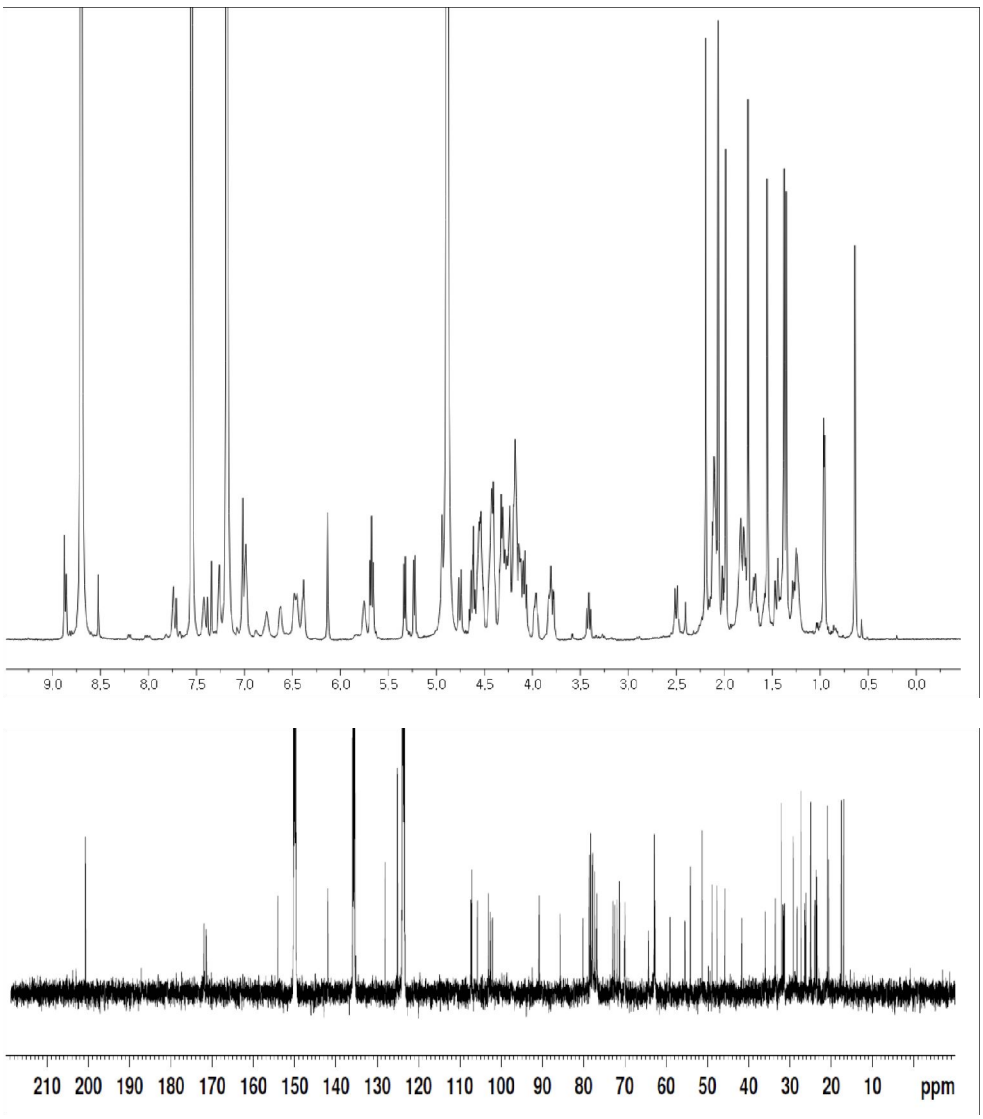

Figure A47. The ^{1}H NMR (500 MHz, Pyridine-d_5) and ^{13}C NMR (125 MHz, Pyridine-d_5) spectra of Compund **24**

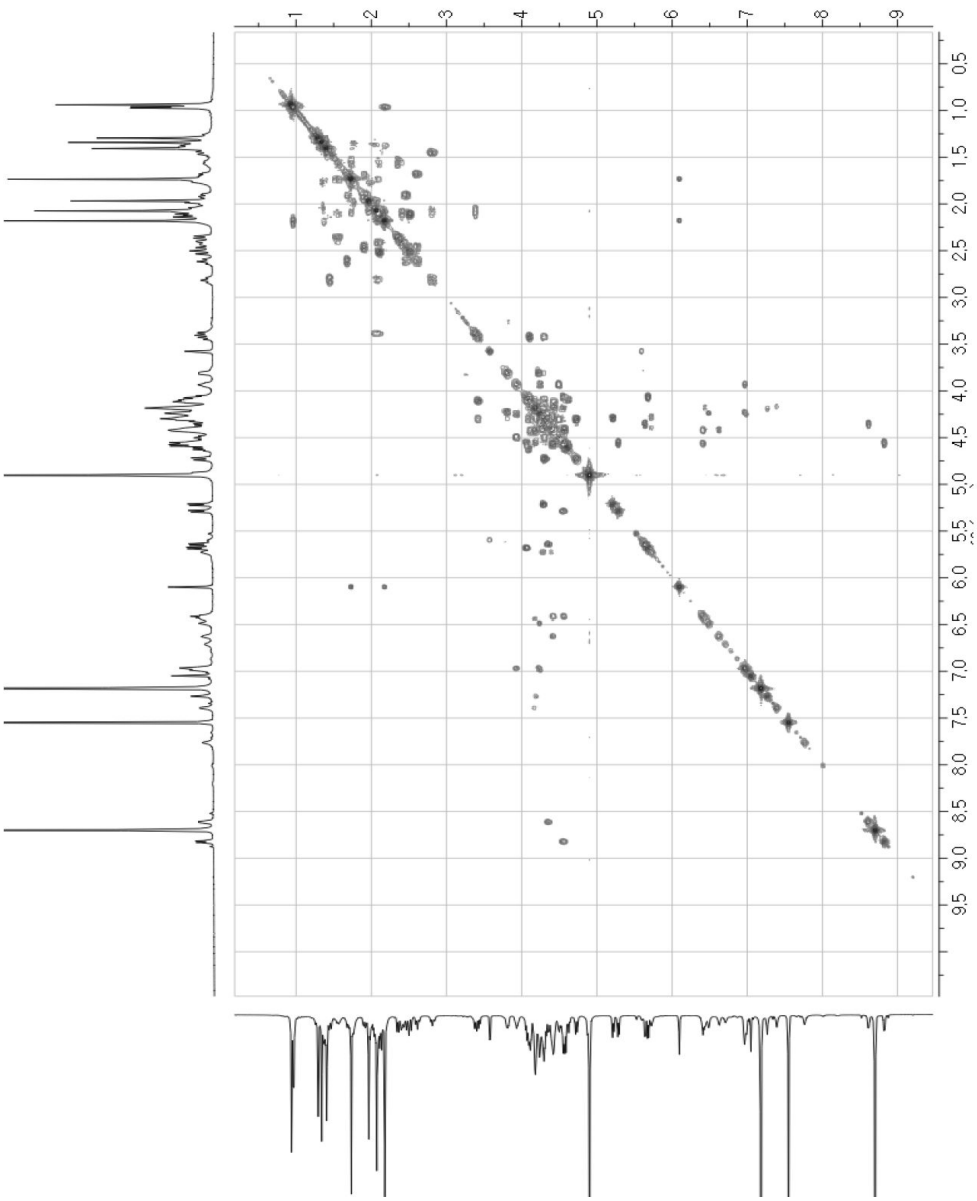

Figure A48. The COSY (500 MHz, Pyridine-d_5) spectrum of Compound

24

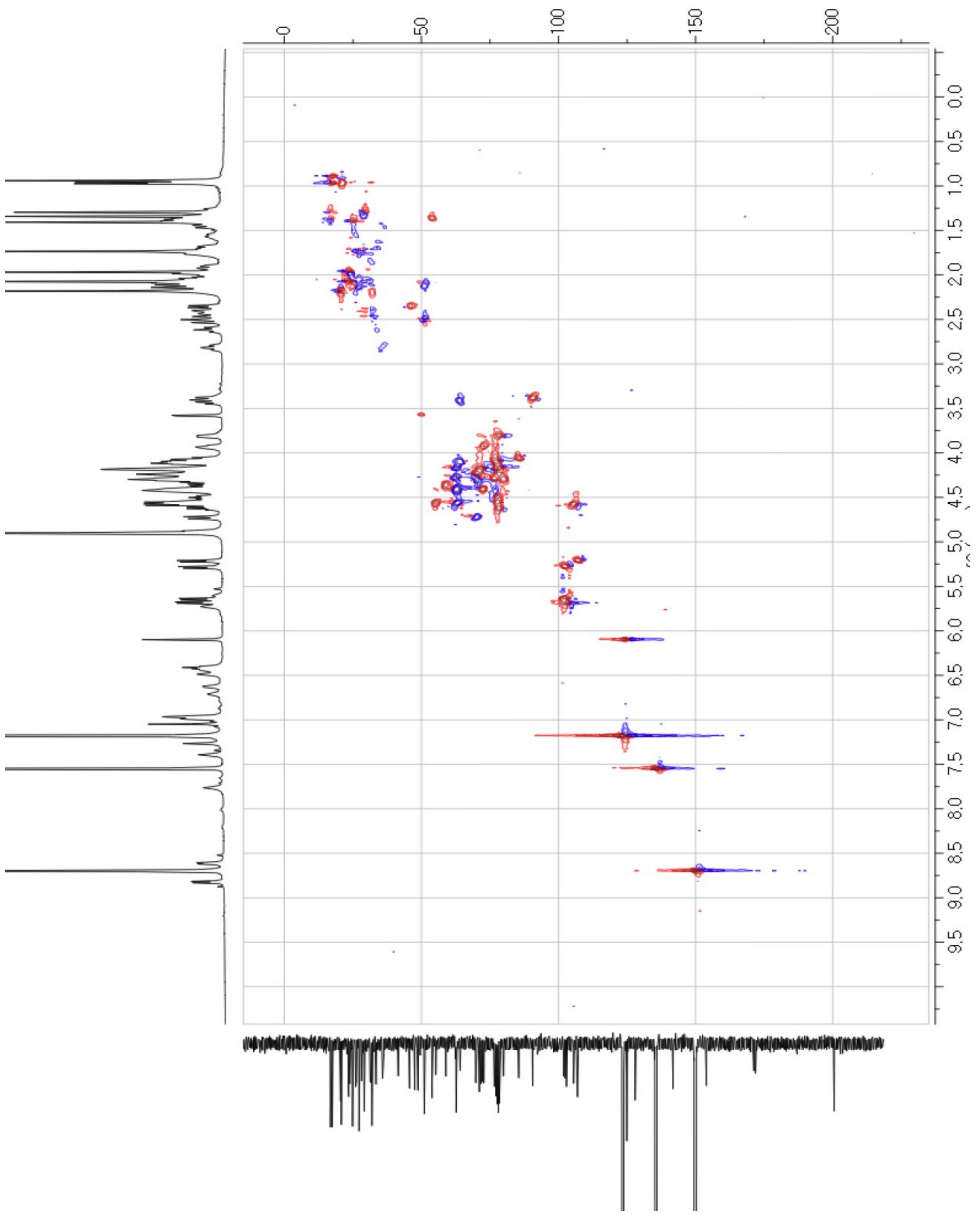

Figure A49. The *g*HSQC (500 MHz, MeOH-d_4) spectrum of Compound

24

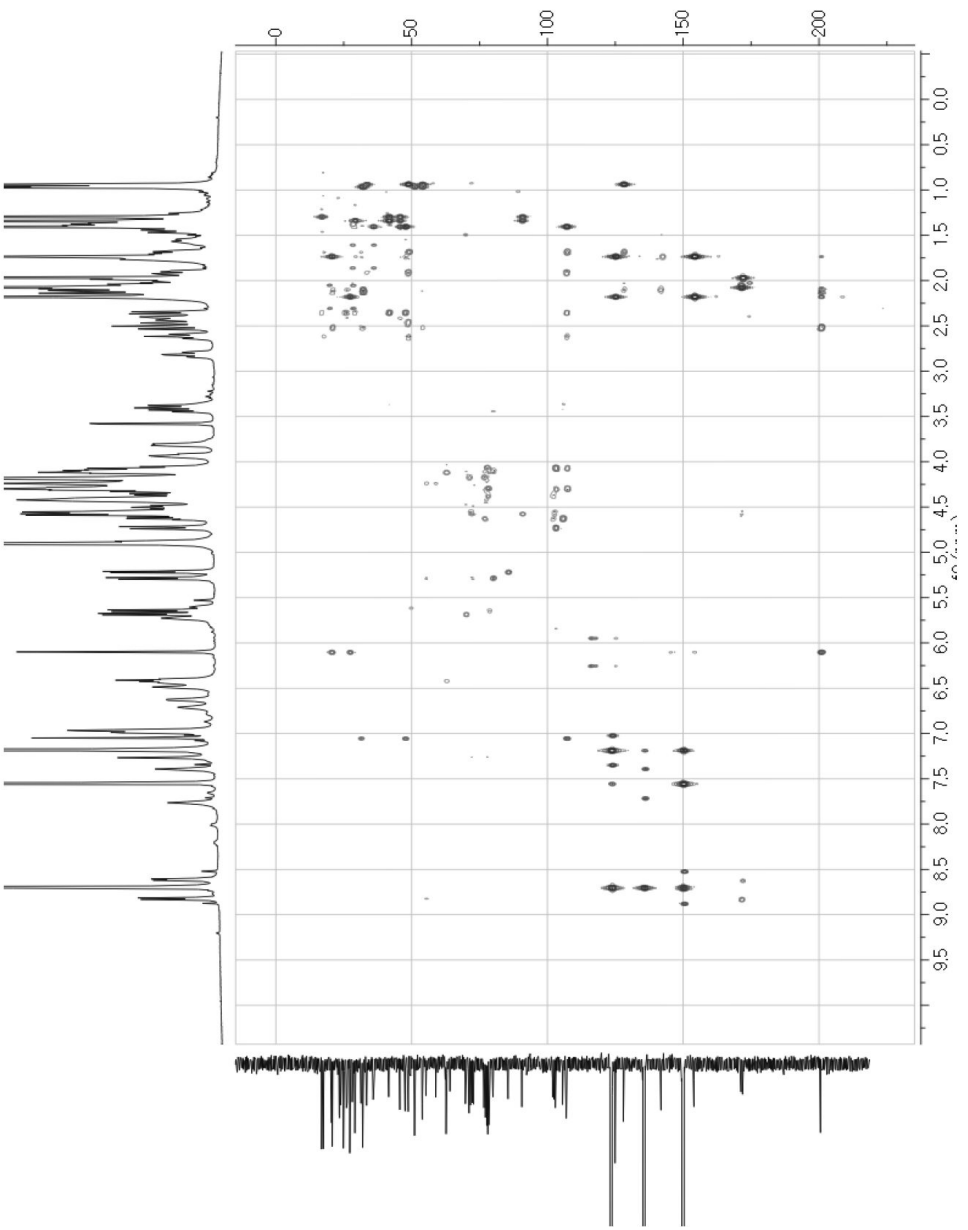

Figure A50. The *g*HMBC (500 MHz, Pyridine-d_5) spectrum of Compound

24

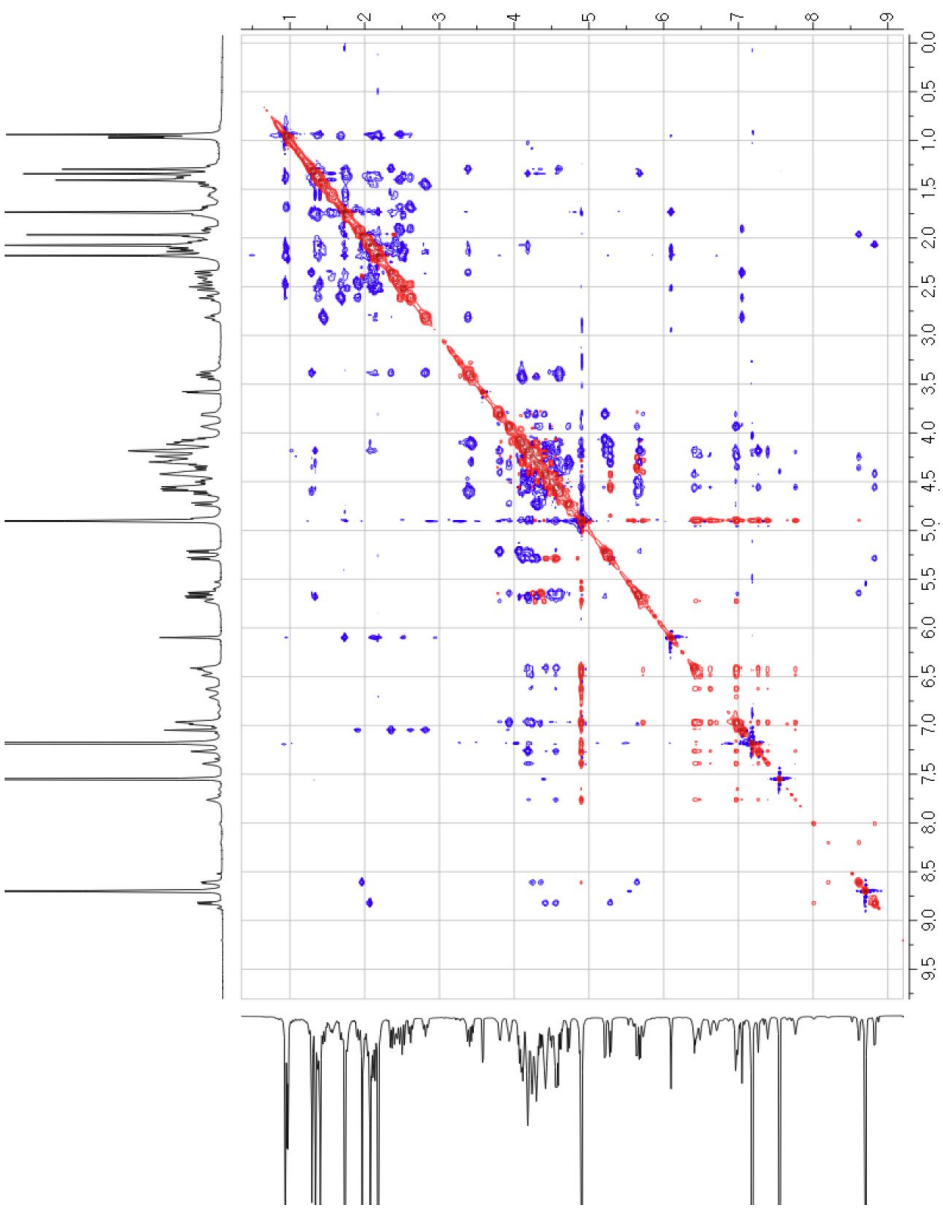

Figure A51. The ROESY (500 MHz, Pyridine-d_5) spectrum of Compound

24

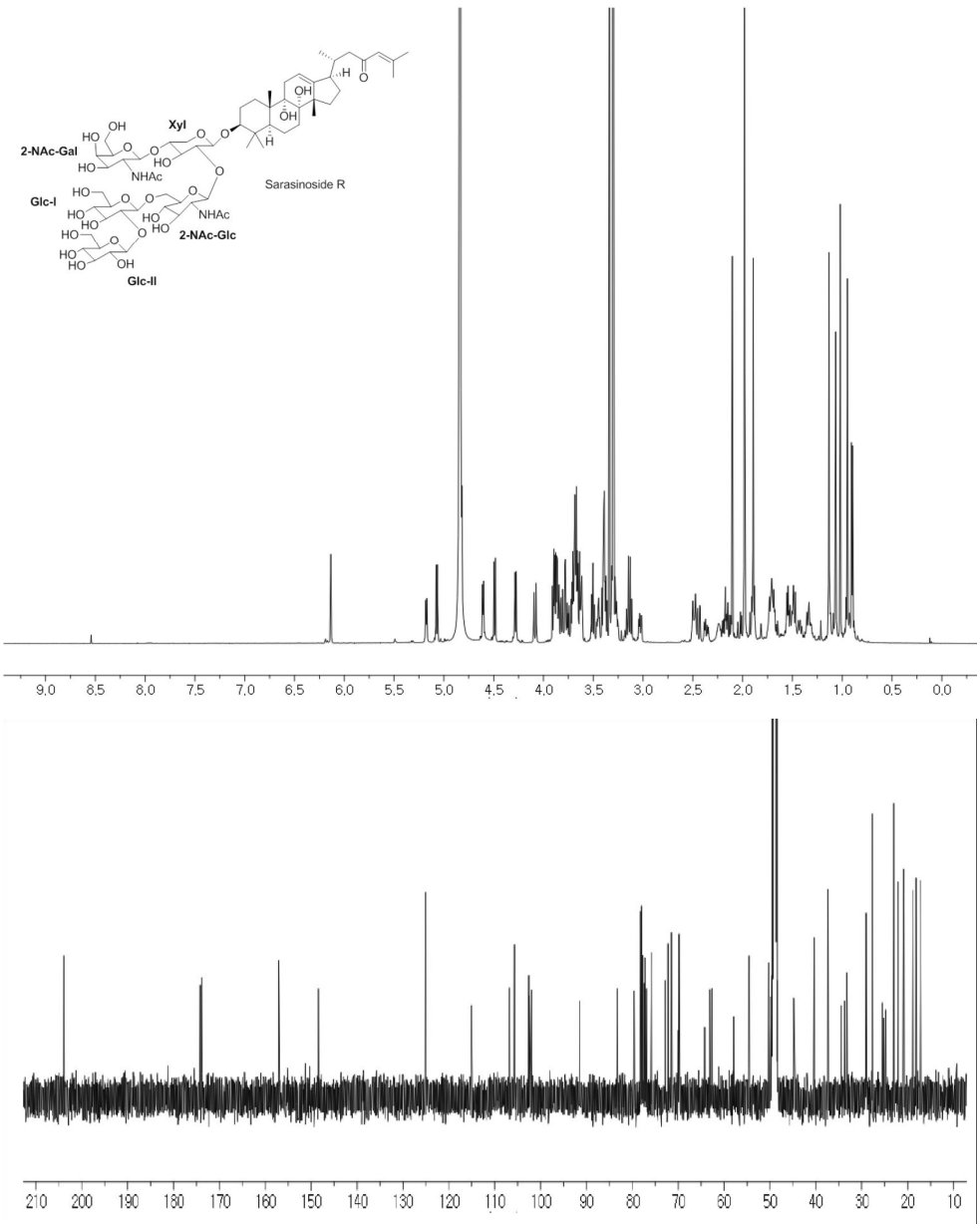

Figure A52. The ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra of Compound **25**

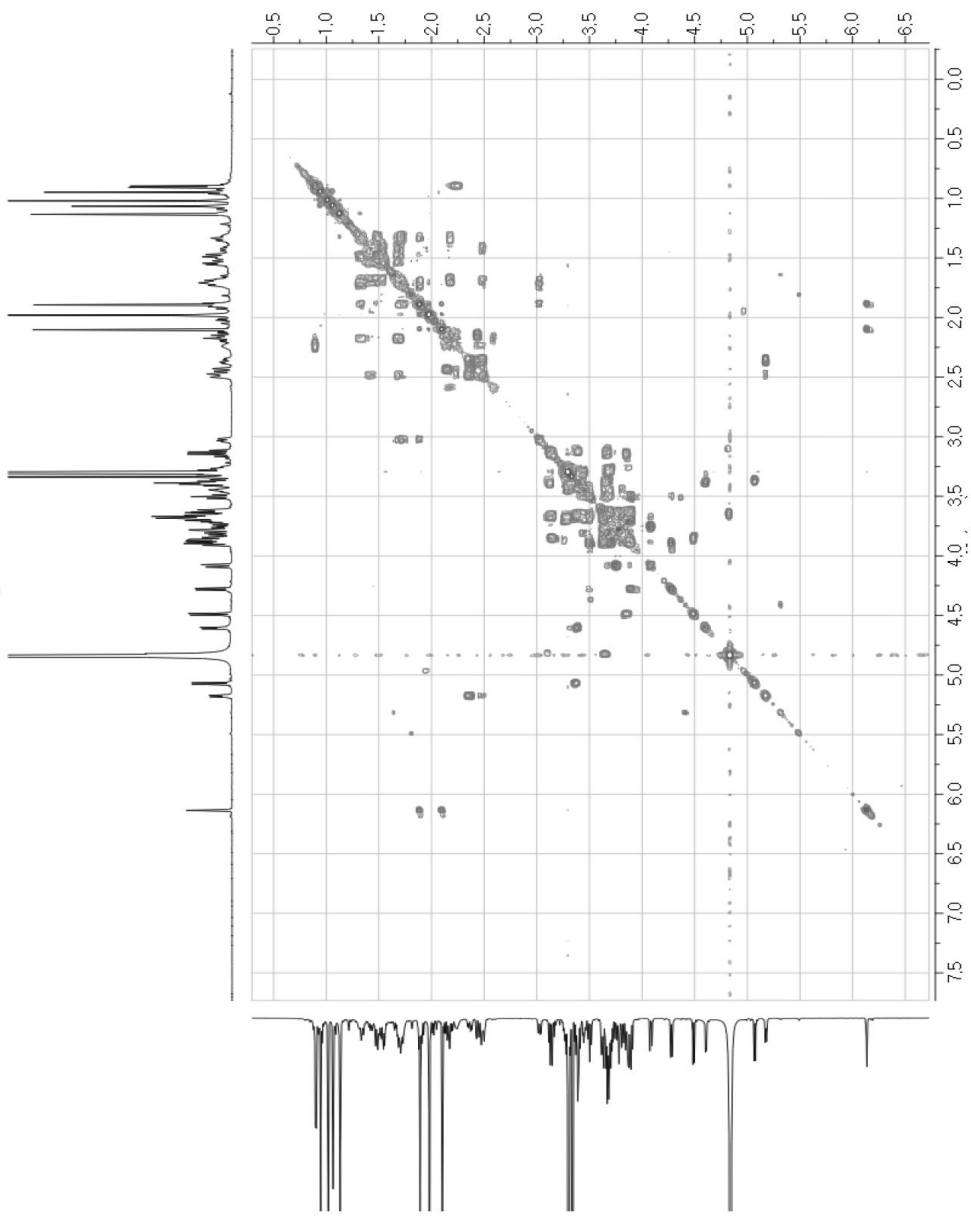

Figure A53. The COSY (600 MHz, MeOH-d_4) spectrum of Compound **25**

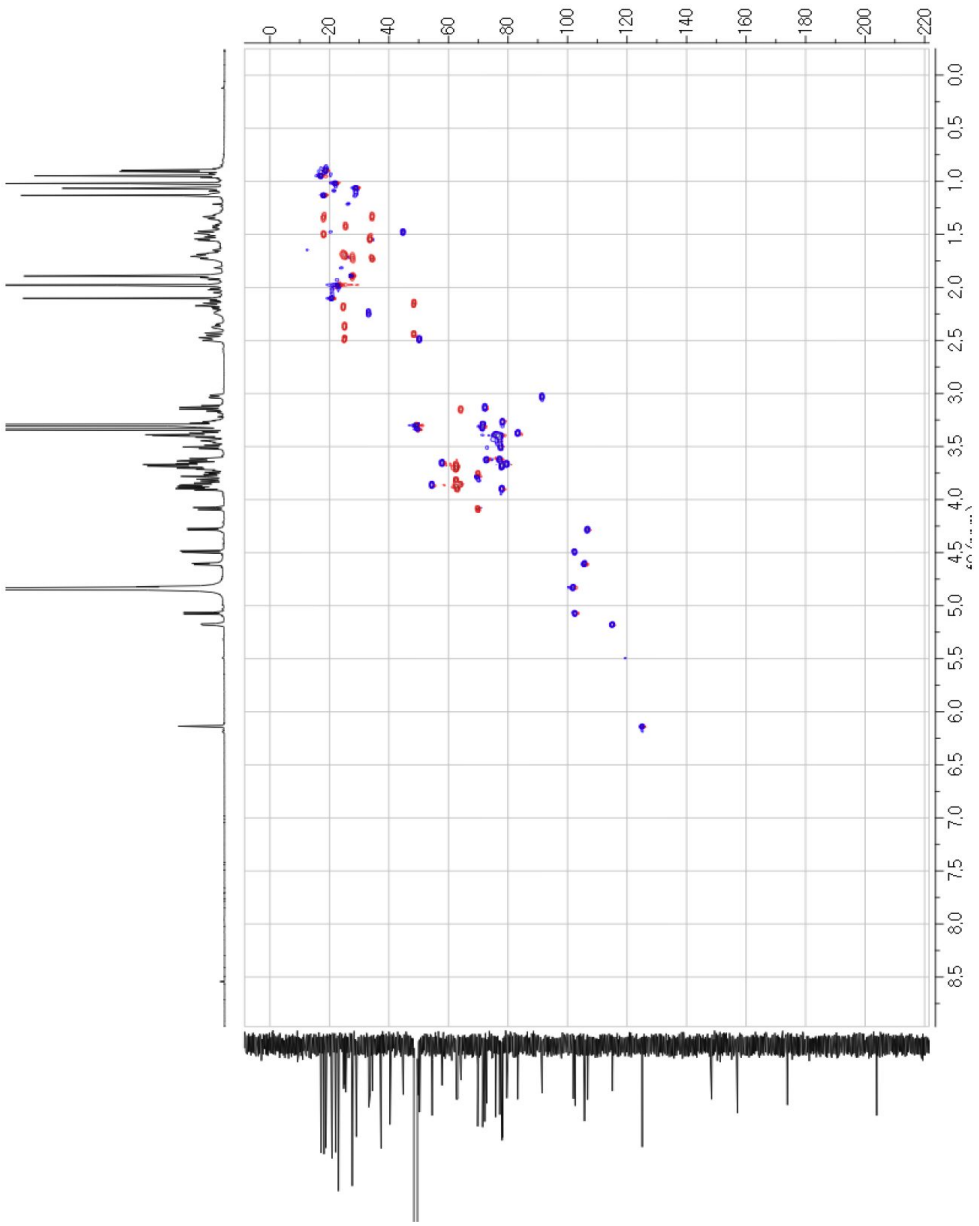

Figure A54. The *g*HSQC (600 MHz, MeOH-d_4) spectrum of Compound

25

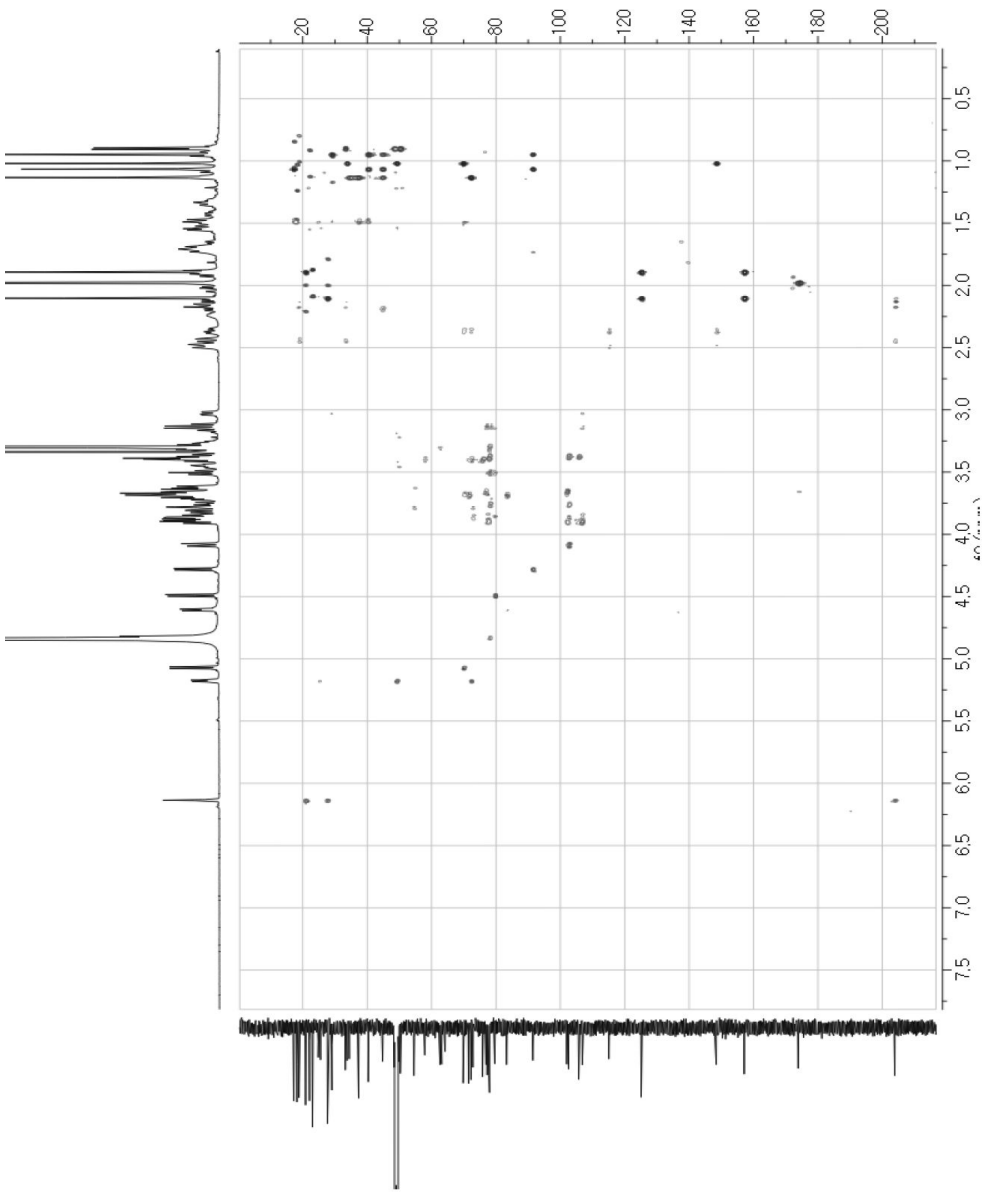

Figure A55. The *g*HMBC (600 MHz, MeOH-d_4) spectrum of Compound

25

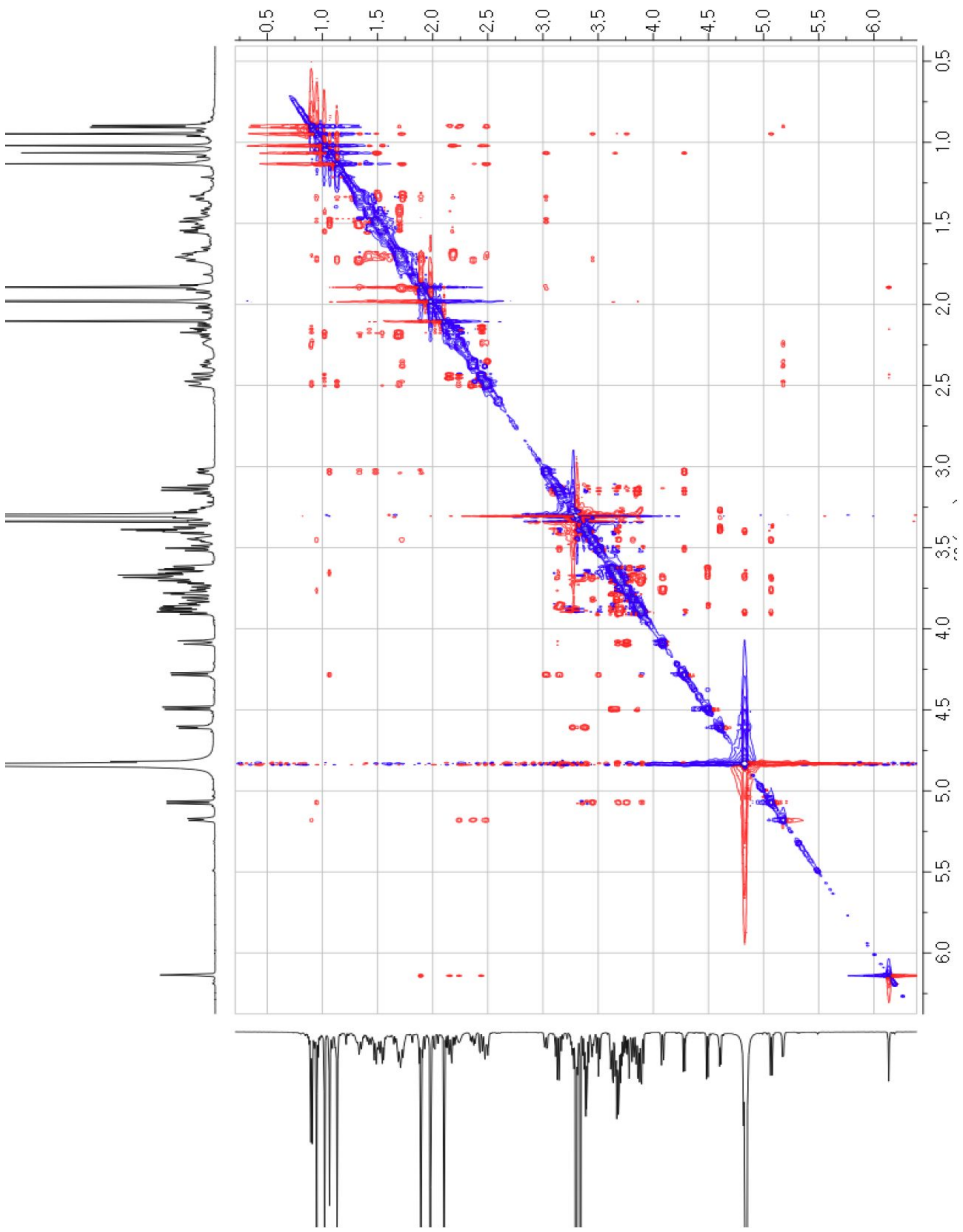

Figure A56. The ROESY (600 MHz, MeOH-d_4) spectrum of Compound

25

APPENDIX B:
Supporting Information

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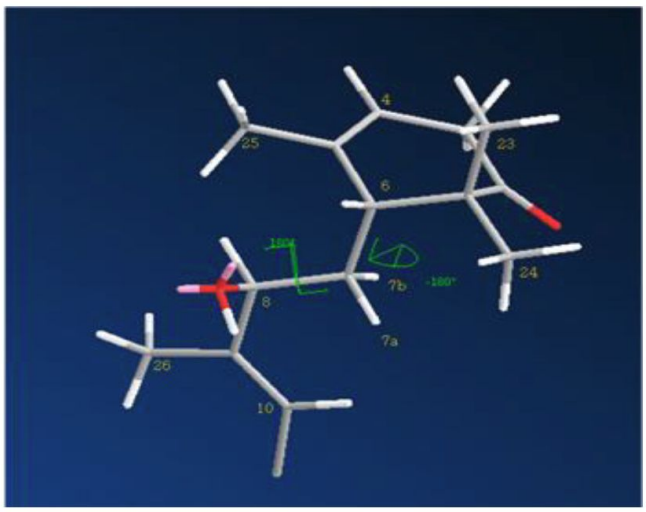
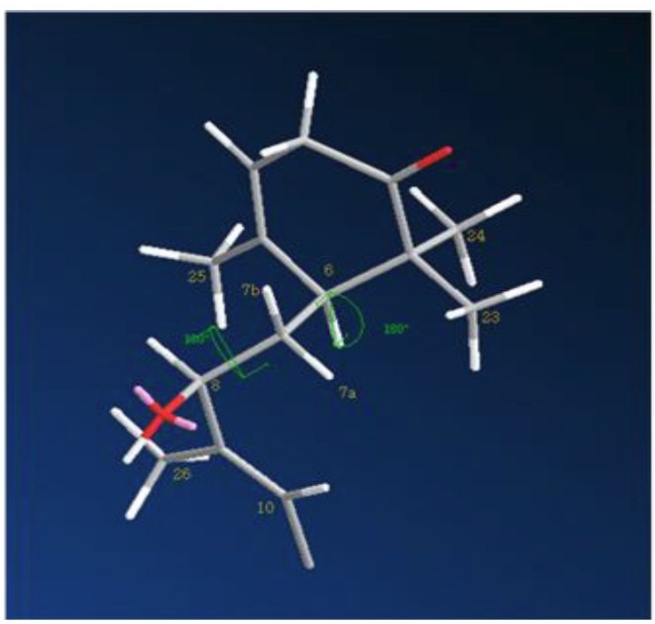
B1. Biological Assays Methods

Cytotoxicity assays, antimicrobial assays, and isocitrate lyase, sortase A, and Na^+/K^+-ATPase inhibion assays were performed as described previously. For the cytotoxicity test, an MTT viability assay was performed as previously described with slight modifications. MTT was first prepared as a stock solution of 5 mg/mL in phosphate buffered saline (PBS, pH 7.2) and was filtered. At the ent of the treatment period (24 h, 48 h, and 72 h), with three different test drug concentrations in triplicate, MTT solution (20 μL) was added to each well and then incubated for 4 h at 37 °C; then solubilizing buffer (10% sodium dodecyl sulfate dissolved in 0.01 N HCl, 100 μL) was added to each well. After overnight incubation, the 96-well plate was read by an enzyme-linked immunosorbent assay (ELISA) reader at 570 nm for absorbance to determine the cell (A549 cell line and/or K562) viability. The viable cells produced a dark blue formazan product, whereas no such staining was formed by dead cells. The LC_{50} value was defined as the concentration that resulted in a 50% decrease in cell viability compared to that of control reations in the absence of an inhibitor. The values (mean ± SD) were calculated from the dose-response curves of three concentrations of each test sample in three independent experiments (n = 3)

B2. The modeling study of Compound 4 (CambridgeSoft, Chem3D® Pro 11.0)



*6R**, *8S** configuration model



*6S**, *8S** configuration medel

B3. The pictures of sponge *Lipastrotethya* sp.

국문초록

열대 해면 *Lipastrotethya* sp. 유래의 생리활성 Terpenoids 와 Saponins 에 대한 연구

서울대학교 대학원
약학과
천연물과학 전공
이 정 호

화학적·분광학적 분석방법과 생리활성 측정을 통해 선별된 열대해면 *Lipastrotethya* sp. 에서 추출한 유기물을 다양한 크로마토그래피 법으로 분리 및 정제하여 25개의 순수한 물질들을 얻었다.

각각의 물질들은 분광학 실험들을 통해 14종의 신규물질을 구조결정 하였으며 11종의 기지물질을 구조동정하였다. 입체구조 결정을 규명하기위해 화학적 실험과 분광학적 실험을 통한 자료분석 등 다양한 방법이 시도되었다. 분리된 물질들은 7종의 pouoside 계열과 5종의 그에 상응하는 sapogenin 계열, 그리고 13종의 sarasinoside 계열인 것으로 밝혀졌다. 분리된 물질들은 세포독성검사(K562 human erythroleukemia cell, A549 lung carcinoma cell), 항미생물검사(그람양성박테리아, 그람음성박테리아, 병원성진균), 효소저해검사(Na^+/K^+ ATPase, Isocitrate lyase, Sortase A) 등이 실시되

었고 일부 물질의 경우 주목할만한 세포독성과 Na^+/K^+ ATPase 저해활성이 있는 것으로 나타났다.

주요어 : 해면, 해양천연물, 천연물구조결정, 생리활성
학번 : 2010-21716

Publication List

1. “Triterpene galactosides of the pouoside class and corresponding aglycones from the sponge *Lipastrotethya* sp.” J.-H. Lee, K. H. Jang, Y.-J. Lee, H.-S. Lee, C. J. Sim, K.-B. Oh, and J. Shin, *J. Nat. Prod.* **2011**, *74*, 2563-2570.

2. “Nortriterpene glycosides of the sarasinoside class from the sponge *Lipastrotethya* sp.” J.-H. Lee, J.-e. Jeon, Y.-J. Lee, H.-S. Lee, C. J. Sim, K.-B. Oh, and J. Shin, *J. Nat. Prod.* **2012**, *75*, 1365-1372.

3. “New polyaromatic metabolites from a marine-derived fungus *Penicillium* sp.” E. Julianti, J.-H. Lee, L. Liao, W. Park, S. Park, D.-C. Oh, K.-B. Oh, J. Shin, *Org. lett*. **2013**, *15*, 1286-1289.

4. “Sortase A inhibitory metabolites from the roots of *Pulsatilla koreana*” S. Lee, I.-H. Song, J.-H. Lee, W.-Y. Yang, K.-B. Oh, J. Shin, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 44-48.

5. “Penicillipyrones A and B, meroterpenoids from a marine-derived

Penicillium sp. fungus" L. Liao, J.-H. Lee, M. You, T. J. Choi, W. Park, S. K. Lee, D.-C. Oh, K.-B. Oh, J. Shin, *J. Nat. Prod.* **2014**, *77*, 406-410.