공학석사학위논문

Stereoselective Synthesis of a Key Intermediate of Antibiotic Rakicidin A; L-*threo*-β-hydroxyaspartate

항생제 라키시딘 에이의 주요 중간체 L-트레오-β-하이드록시아스팔테이트의 입체선택적 합성

2020년 2월

서울대학교 대학원

화학생물공학부

권 현 영

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이 논문을 공학석사 학위논문으로 제출함
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Abstract

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This paper covers the contents of synthesis of building block of rakicidin A which contains L-*threo*-β-hydroxy asparagine structure. Rakicidin A is a natural compound and one of the cyclic depsipeptides. Many cyclic depsipeptides are known to have clinical effects. Especially, Rakicidin A has exhibited a unique growth inhibitory activity against chronic myelogenous leukemia (CML) stem cells and acts as an antitumor agent for selective treatment of solid tumors.

Some papers reported about the total synthesis of rakicidin A. According to the papers, rakicidin A could be synthesized by several coupling reactions of building blocks. Among them, L-*threo*-β-hydroxy asparagine is key building block in terms of stereochemistry and several papers reported about the synthesis of this structure.

Our group reported about stereoselective synthesis of *trans*-oxazolidine using *N*-hydroxymethly-*α*-amino aldehyde whose equilibrium shifted to hemi-acetal leading to a stable structure. *Trans*-oxazolidine was obtained through the reaction of *N*-hydroxymethyl-*α*-amino aldehyde and phenylsulfonylnitromethane. During this reaction, formation of *H*-eclipsed conformation could enhance stereoselectivity to *trans*-oxazolidine structure.

With this experience, L-*threo*-*β*-hydroxy aspartate was synthesized from D-serine in 11 steps 9% and optical purity was confirmed through the measurement of specific rotation of derivative of L-*threo*-*β*-hydroxyaspartate. Furthermore, the possibility of total synthesis of rakicidin A was confirmed by coupling with *N*-methylglycine.

Keywords : Desipeptide, clinical effects, *α*-amino aldehyde, *trans*-oxazolidine, L-*threo*-*β*-hydroxy aspartate, building block, rakicidin A

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LIST OF SCHEMES

LIST OF ABBREVIATIONS

Boc	*tert*-Butyloxycarbonyl
BnBr	Benzylbromide
br	broad
CSA	Camphorsulfonic acid
δ	chemical shift, ppm
d	doublet
DBU	1, 8-Diazabicyclio[5.4.0]undec-7-ene
DCM	methylene chloride
DIBAL-H	Diisobuthylaluminium hydride
DIPEA	*N*,*N*-Diisopropylethyamine
DMAP	4-(Dimethylamino)pyridine
DMF	*N*, *N*-dimethylformamide
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
eq.	equivalent
EtOAc	Ethylacetate
g	gram(s)

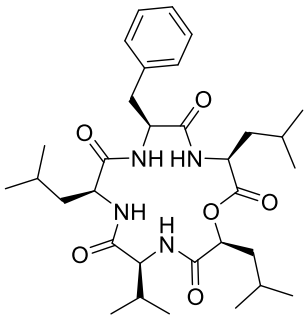
HOBt	Hydroxybenzotriazole
Hz	hertz
J	coupling constant(s)
m	multiplet
Me	methyl
MeOH	Methanol
min	minute(s)
mg	milligram(s)
mL	milliliter(s)
mmol	millimole(s)
N	normality
NMR	nuclear magnetic resonance
ppm	parts per million
s	singlet
TBAF	Tetrabutylammonium fluoride
TBS	*tert*-Buthyldimethylsilyl
TEA	Triethylamine
TEMPO	2, 2, 6, 6-Tetramethyl-1-piperidinyloxy free radical

THF	Tetrahydrofuran
TLC	Thin-layer chromatography
p-TSA	*p*-Toluenesulfonic acid

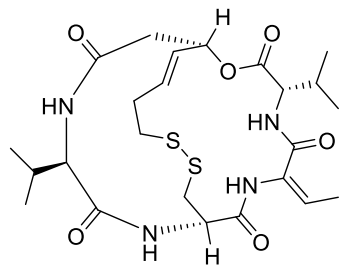
1. Introduction

1-1. Introduction of cyclic depsipeptide

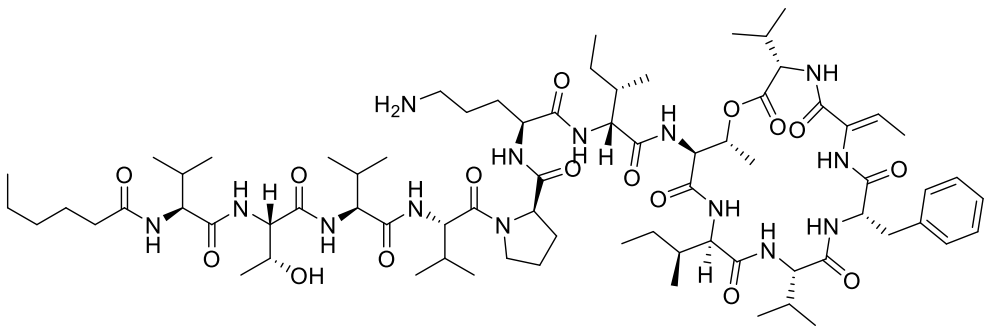
Depsipeptides are polypeptides in which the corresponding es ter groups replace one or more of its amide groups. So far, it has been known that cyclic depsipeptide has a clinical effect. Many depsipeptides, including anticancer, antiviral, antifungal, antibacterial, anti-inflammatory, and anticlotting or anti-antherogenic properties, show very promising biological activities. The potent effects of cyclic depsipeptides, especially on tumor cells, have led to several clinical trials to evaluate their potential as chemotherapeutic agents.[1,2] There are some examples of cyclic depsipeptides in **Figure. 1.** Sansalvamide A, isolated from a marine fungus, is one of cyclic depsipeptides and shows anti-tumor activity. It is reported that its derivatives also have anti-cancer activity.[3] Romidepsin is bicyclic depsipeptide and known as an anticancer agent for CTCL (cutaneous T-cell lymphoma) and PTClLs (peripheral T-cell lymphomas). Kahalalide F is one of the nature depsipeptides and KF demonstrated powerful cytotoxic activity in tumor cell.[4] Cyclosporin A (CsA) is an immunosuppressive drug and it is widely used for patients who have received organ transplantation. Rakicidin A is also one of the cyclic depsipeptides as shown in **Figure 2.**



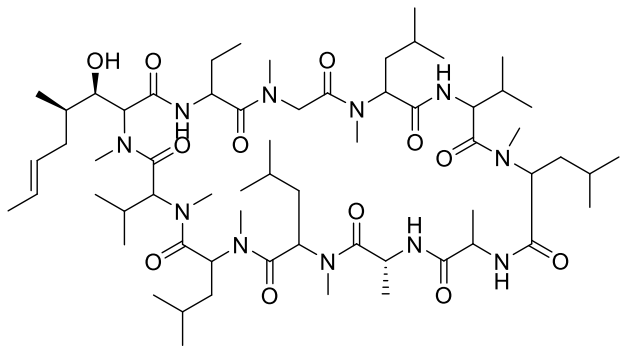
Sansalvamide A



romidepsin



Kahalalide F



Cyclosporin A

Figure 1. Structure of cyclic depsipeptides

1-2. Introduction of rakicidin A

Rakicidin A is known as a cyclic depsipeptide. Rakicidin A was isolated from broth of a *Micromonospora* in 1995 by Myers Squibb Com.[5] Rakicidin A is the one of the rakicidins (A, B, C, D, E and F) distinguished by the lipophilic side chain and the fragment of β-asparagine.[6] (Figure. 1) Rakicidins have a unique 4-amino-2, 4-pentadienolate (APD) structure. APD structure can be found in natural products such as vinylamycin, microtermolide A, and BE-43547A1.[7]

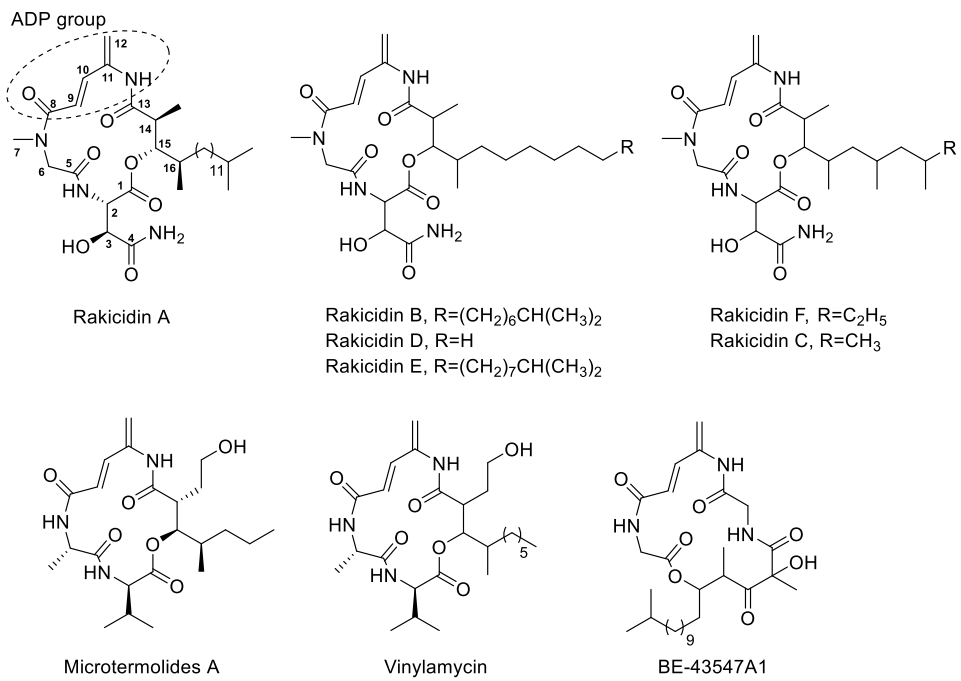


Figure 2. Structure of rakicidin A, B, C, D, E, and F, microtermolides A, vinylamycin, and BE43547A1

According to the results, rakicidin A has exhibited a unique growth inhibitory activity against chronic myelogenous leukemia (CML) stem cells. In addition, it also acts as an antitumor agent for selective treatment of solid tumors.[8]

Total synthesis methods of rakicidin A were reported by Chen group and Poulsen group. [6, 9, 10] Chen group determined 5 chiral centers (2S, 3S, 14S, 15S, 16R) as well as total synthesis of rakicidin A.[7] Chen group reported synthesis method and compared bioactivities of rakicidin A analogues with rakicidin A. For example, 4-methylester-rakicidin A (MERA) which has amine group replaced with methyl group has higher anti-cancer effect than rakicidin A. They also discovered important of ADP group and configuration for inhibitory effects. Recently, they changed many functional group in rakicidin A and tested its bioactivities.[6] As a result, it was found that the bioactivity changes depending on the substituent.

1-3. Building block of rakicidin A

There are five chiral centers (2S, 3S, 14S, 15S, 16R) in rakicidin A. Therefore, it is important that the desired stereochemistry should be formed during the total synthesis. Rakicidin A can be synthesized through coupling reaction of major building blocks. Among the building blocks, synthesizing of β-hydroxyasparagine or β-hydroxyaspartic aicd is important for synthesis of rakicidin A. (Figure 2) Several synthetic methods of β-Hydroxyasparagine and β-Hydroxyaspartic acid have been published.[7] (Scheme 1.) Chen group synthesized L-*threo*-β-hydroxyasparagine from hydroxy amino acid salt.[5] (Scheme 2.) Poulsen group reported the isolation of *threo*-(2S, 3S) enantiomer of β-hydroxyaspartic acid from the commercial racemic mixture.

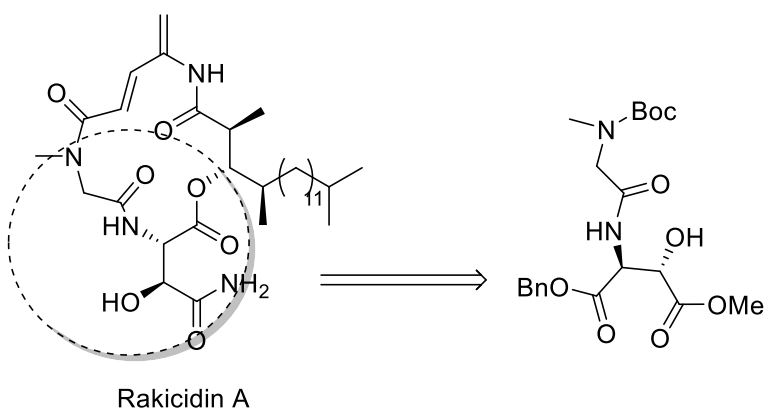
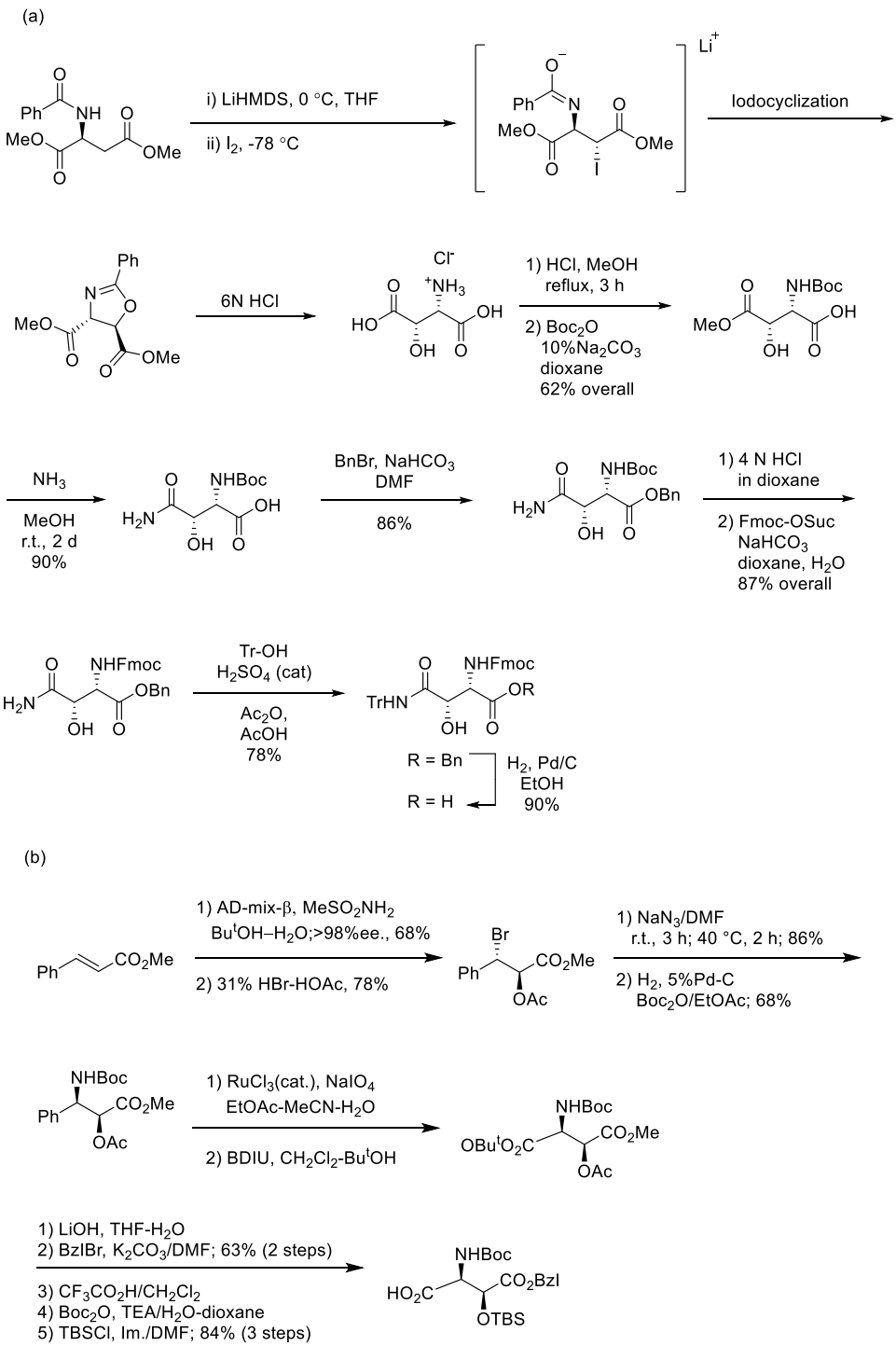
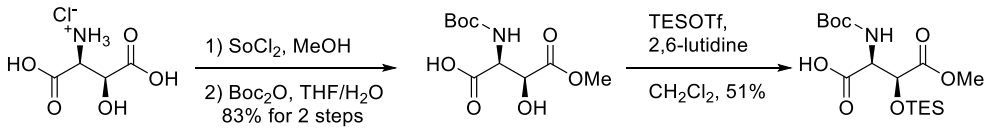


Figure 3. Structure of building block for rakicidin A



Scheme 1. Reported synthetic routes (a) β-OH-Asn, (b) β-OH-Asp derivative.[12]

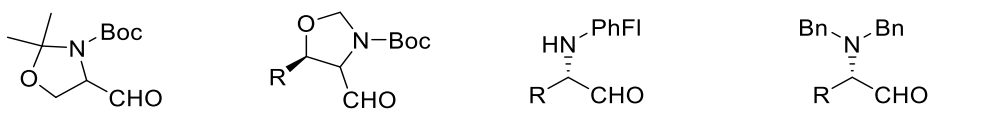


Scheme 2. Reported route for Protected L-*threo*-β-hydroxyasparagine derivative.[6]

As shown in **Scheme 1(a)**, 3-benzoylaminoaspartic acid iodocyclization provided an efficiently hydrolyzed intermediate oxazoline dicarboxylate to L-*threo*-β-hydroxyaspartic acid. In **Scheme 1(b)**, they used Sharpless hydroxylation to control enantiomer with AD-mix-β. Some methods have problem which use expensive catalyst and chiral auxiliary.

1-4. Introduction of *N*-protected-*α*-amino aldehyde

N-protected-*α*-amino aldehyde acts an important building block and chiral auxiliary. Some *α*-amino aldehydes have been reported such as Garner aldehyde and Falorni aldehyde and Rapport aldehyde and Reetz aldehyde. (Figure. 4)

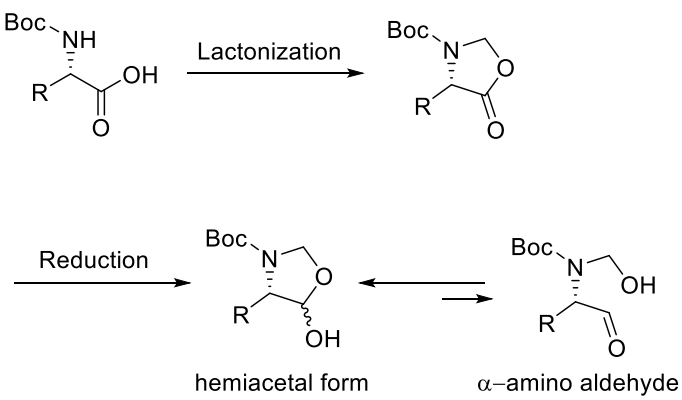


Garner aldehyde Falorni aldehyde Rapport aldehyde Reetz aldehyde

Figure 4. Reported *α*-amino aldehydes

These *α*-amino aldehydes have some limitations. Garner and Falorni aldehydes are apply to limited amino acids. Rapport aldehyde should use expensive protecting group as PhFl. In case of Reetz aldehyde is incompatible with hydrogenolysis. To solve these limitations, we used thermodynamically stable *α*-amino aldehyde. It was obtained by introducing *N*-hydroxymethyl group to *α*-amino aldehyde. *N*-hydroxymethyl-*α*-amino aldehyde could be prepared from protected amino acid. Protected amino acid was synthesized from D-serine which is commercially available amino acid. After synthesis of protected amino acid, lactonization proceeded by using

paraformaldehyde to form *N,O*-acetal oxazolidin ring. After that, stable *N*-protected-α-amino aldehyde was formed through reduction by using DIBAL-H. *N*-hydroxymethyl-α-aldehyde is in the equilibrium state with lactol.

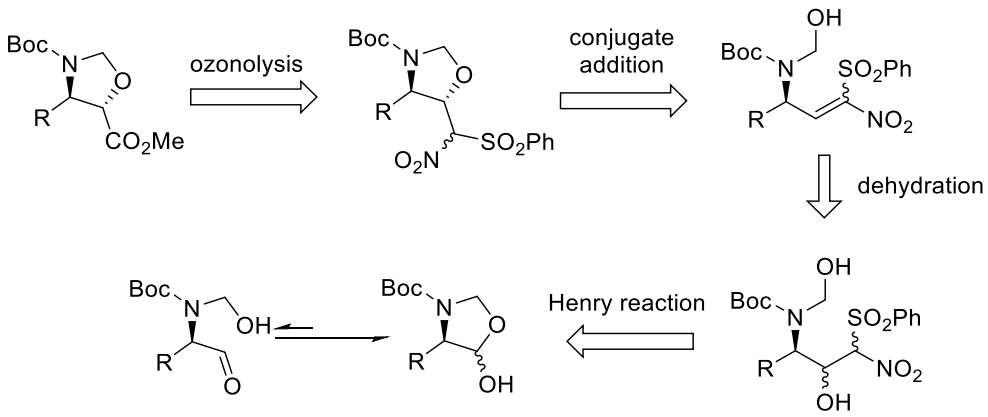


Scheme 3. Preparation of the stable α-amino aldehyde.[11]

1-5. Introduction of *trans*-oxazolidine

Our group developed stereoselective and efficient synthetic route for stable *trans*-oxazolidine methylester structure using stable *N*-hydroxymethyl-α-aldehyde.[13] *Trans*-oxazolidine struecture was used to synthesize L-*threo*-β-hydroxyaspartate Retrosynthetic strategy to synthesis *trans*-oxazolidine methylester is shown in **Scheme 4.**

Stereoselective *trans*-oxazolidine was synthesized through reaction of *N*-hydroxymethyl-α-aldehyde and phenylsulfonylnitromethane. To be specific, phenylsulfonylnitromethane was added to *N*-hydroxymethyl-α-aldehyde to introduce stereocenter in the oxazolidine ring through henry reaction first. Then dehydration, conjugate addition and ozonolysis reaction proceeded continuously. In these steps, steric hindrance is key strategy to enhance *trans*-stereo selectivity. During the conjugation addition, there are two possible conformations. One is the *H*-eclipsed conformation and the other is *R*-eclipsed conformation. The former is favored conformation which forms *trans*-oxazolidine structure. The conformations are shown in **Figure 5.**



Scheme 4. Retrosynthetic strategy to the *trans*-oxazolidine methylester[13]

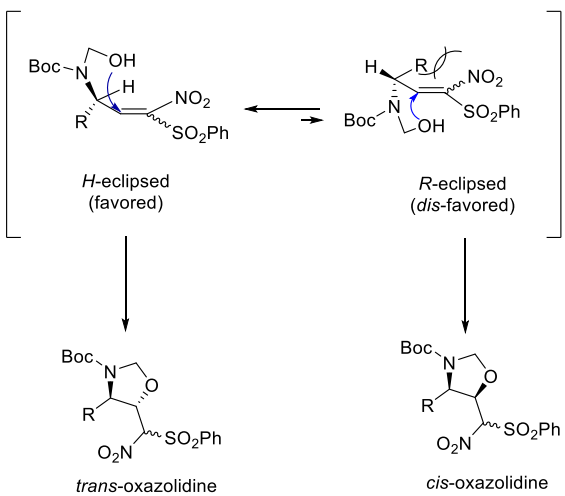
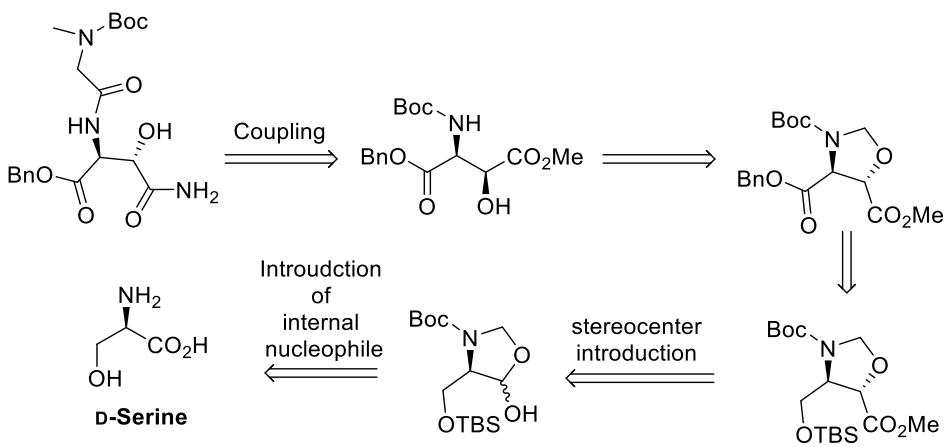


Figure 5. Possible conformations for *trans*-selectivity

2. Results and Discussion

2-1. Retro-synthetic strategy

Retro-synthetic strategy for building block of rakicidin A is shown in **scheme 5**. In retro-synthetic strategy, the important intermediates are stable *α*-amino aldehyde and *trans*-oxazolidine. These intermediates could give desired stereochemistry to building block which have *syn* conformation.

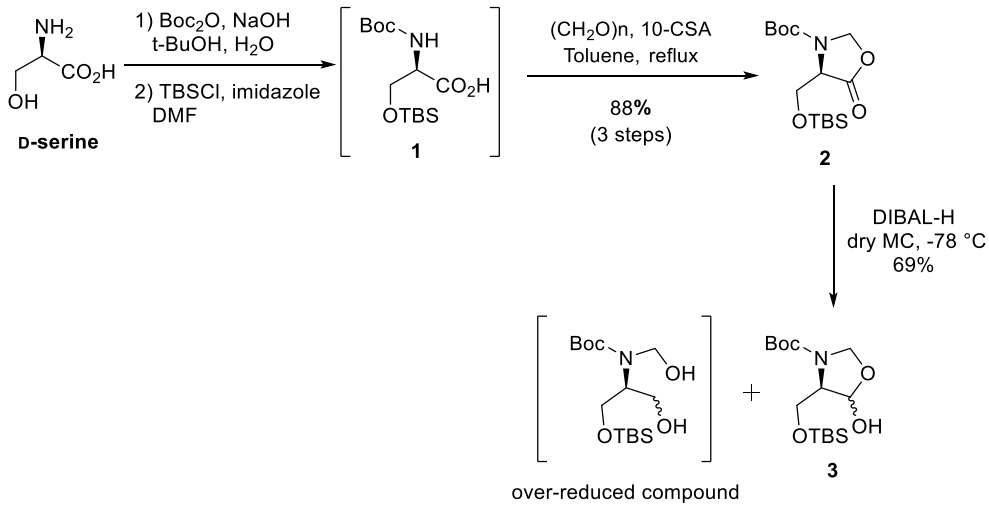


Scheme 5. Retrosynthetic strategy for building block of rakicidin A

During the reaction, sensitive functional group that could give undesired product should be protected through Boc group or TBS group. Then, de-protection reaction was carried out to synthesize target compound.

2-2. Synthesis of D-serinal from D-serine

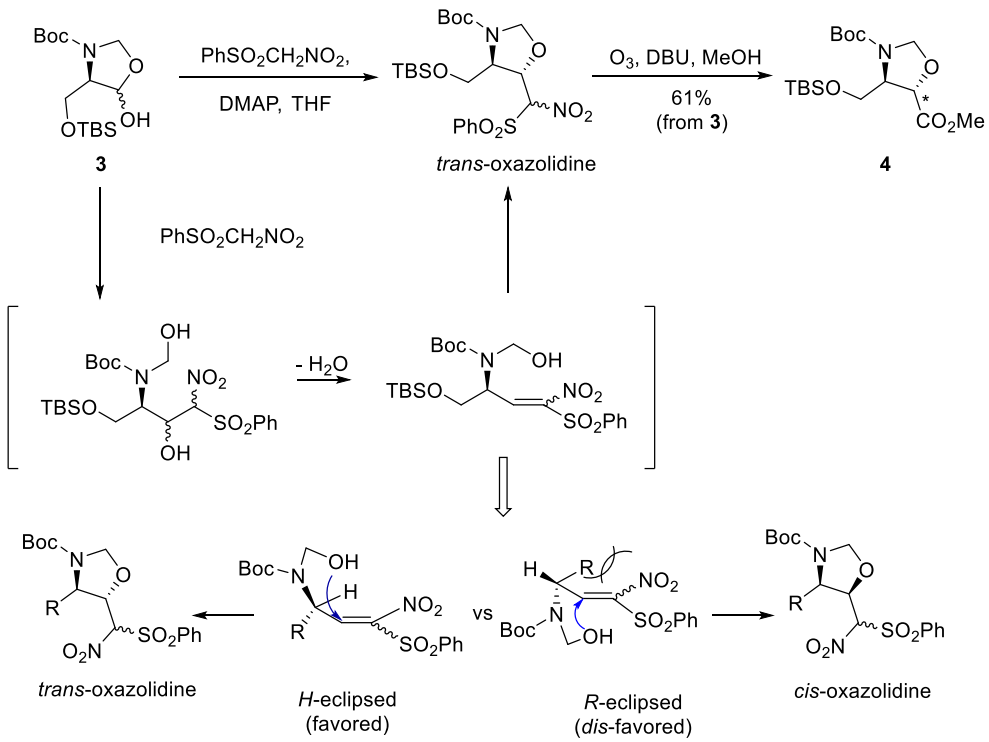
Amine functional group in D-serine was protected with Boc group by using Boc anhydride (Boc_2O) in basic condition. After reaction, remained Boc_2O was removed through Hexane washing and product was obtained by EtOAc Extraction after acidification. Because hydroxyl functional group is also sensitive to reaction, we protected this functional group with TBS group. TBSCl and imidazole are added in DMF. After that, lactonization was conducted by using paraformaldehyde and camphorsulfonic acid (CSA) as an acid catalyst. Compound **2** which purified with silica gel column chromatography was obtained upto 87% yield in 3 steps. Compound **3** was synthesized through reduction from lactone by using DIBAL-H in DCM under -78 °C condition. In this reaction, addition rate and amount of DIBAL-H are important factors. As reaction rate and amount of DIBAL-H increase, diol which is over-reduced compound was formed more than lactol which is desired product. For this reason, we controlled addition rate by using dropping funnel at -78 °C condition. After reaction, product was purified extraction and column chromatography. Through this method, compound **3** was obtained upto 69% yield. Since this compound could be racemized, it used as soon as synthesis.



Scheme 6. Preparation of D-serinal 3 from D-serine

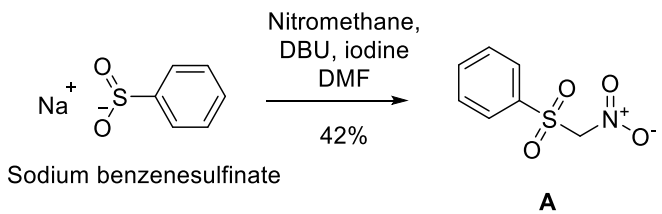
2-3. Synthesis of stereoselective *trans*-oxazolidine

The mechanism for synthesizing stereoselective *trans*-oxazolidine is shown in Table 7. According to the mechanism, *H*-eclipsed conformation is favored to synthesis trans-oxazolidine. *H*-eclipsed conformation was formed during the reaction of intramolecular conjugate addition. In this reaction, stereoselectivity of oxazolidine was enhanced through steric hindrance of nitro olefin.



Scheme 7. Preparation of *trans*-oxazolidine **4** from lactol

Phenylsulfonylnitromethane has an acidic proton due to the effects of two electron withdrawing groups. Therefore, deprotonated compound could be added to lactol. Phenylsulfonylnitromethane reagent is so expansive that we synthesized this reagent using reported procedure. [13] It was obtained by using nitromethane and sodium benzenesulfinate in 42% yield which is purified by column chromatography. Reaction scheme shown in scheme 8.[13]

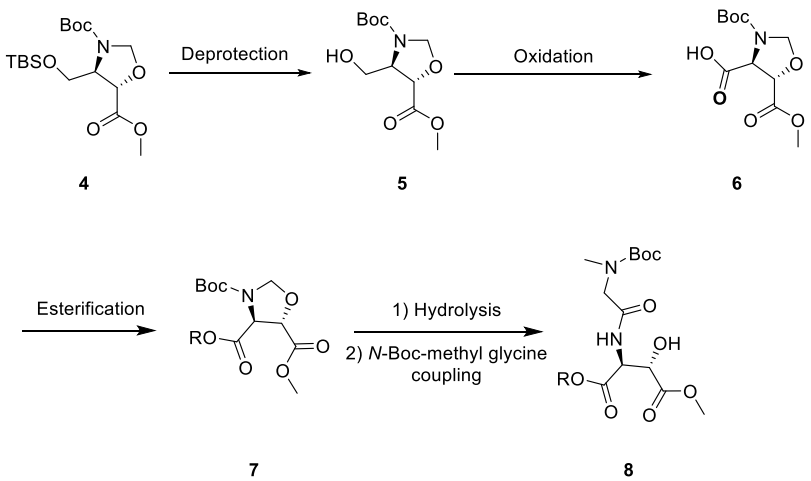


Scheme 8. Synthesis of phenylsulfonylnitromethane A

After the addition of compound A to compound 3, dehydration was occurred followed by a conjugate addition reaction. Firstly, Compound A and DMAP were added to lactol in THF. In case of the Henry reaction, reaction rate is slow since starting material exists stable lactol form more than α-amino aldehyde which is desired starting material at equilibrium state. Therefore, this reaction was carried out for 2 days. After that, solution was diluted with methanol and DBU was added at 0 ℃ and the ozonolysis reaction conducted by using an ozonizer at −78 ℃ for 15−20 min. When the reaction finished, compound 4 was obtained in 63% yield from compound 3 after purification through extraction and column chromatography.

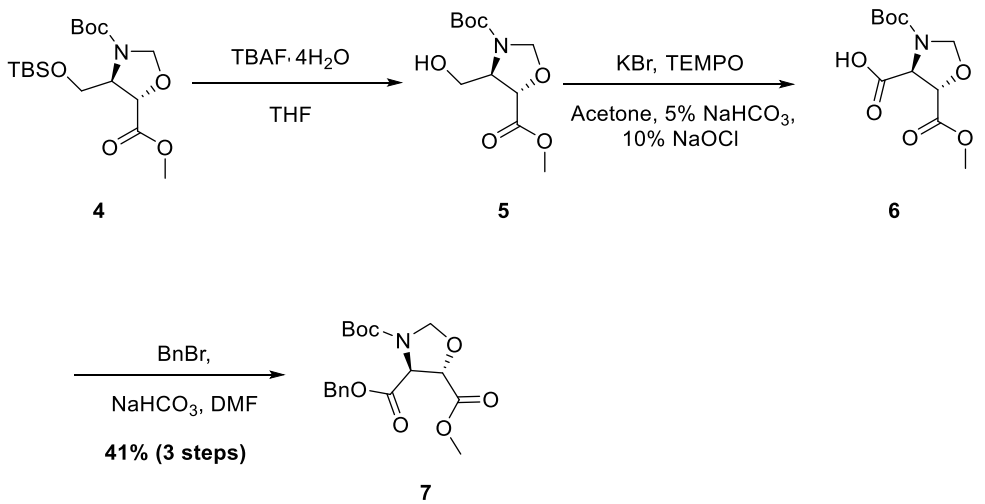
2-4. Synthesis of building block for rakicidin A

Proposed route to synthesize final product from compound **4** is shown in **scheme 9.** Following this route, compound **5** was synthesized from compound **4** through de-protection reaction of TBS group. TBAF · H_2O was added in compound **4** in THF. After that, tempo oxidation was carried out. Compound **5** was dissolved in acetone and $NaHCO_3$. Next, KBr, TEMPO and NaOCl is added at 0 ° C. If the reaction is carried out at room temperature, the methyl ester group can be unstable due to the heat generated during the NaOCl addition.



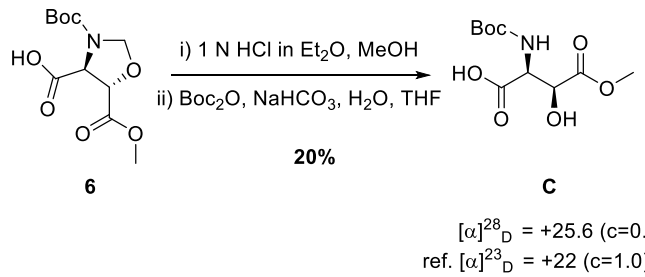
Scheme 9. Possible route for synthesis final product

On this route, benzyl group was chosen as the alkyl ester group. Therefore, compound **7** was produced in 41% yield from compound **4** by using BnBr and $NaHCO_3$. (**Scheme 10**) This compound was purified with column chromatography.



Scheme 10. Preparation of compound 7

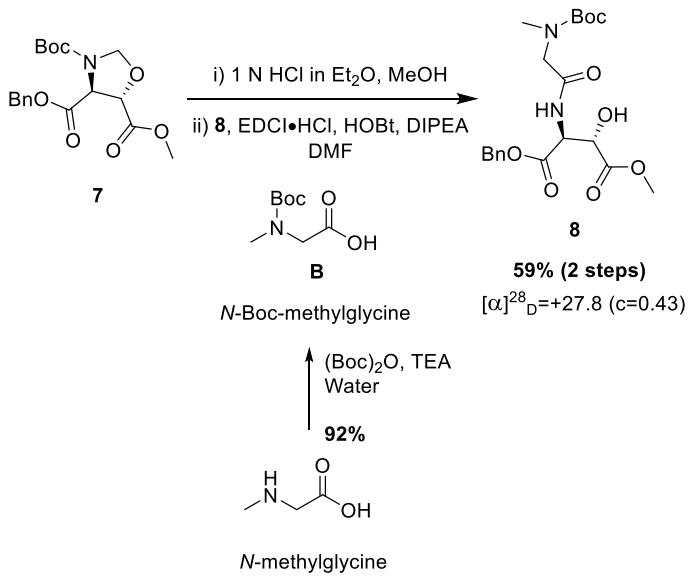
Before the coupling reaction, I confirmed stereoselectivity through synthesis of compound C of which specific rotation was reported from compound 6.[12] Therefore, compound C was synthesized from compound 6 through hydrolysis followed by Boc protection to check optical purity. (Scheme 11.) Hydrolysis was conducted by using 1 N HCl in Et_2O reagent in methanol reflux condition. Then, free amine functional group was re-protected with Boc group. Compound C was obtained from compound 6 in 20% yield in 2 steps. The specific rotation was determined by polarimeter. The value of observed specific rotation and reported one are +25.6 and +22, respectively. Since, absolute value of the observed specific rotation was higher than the reported value, the synthetic compound 6 is thought to have higher optical purity compared with to the reference.



Scheme 11. Preparation of compound C[12]

To obtain a target compound, the oxazolidine ring should be hydrolyzed to give linear structure. Hydrolysis was carried out using 1 N HCl in Et_2O reagent in methanol reflux condition. This compound was used directly without purification. Then, compound 8 could be synthesized through coupling reaction of this one and *N*-methylglycine. Since *N*-methylglycine has low solubility in organic solvent, Boc protection was conducted to free amine functional group before peptide coupling reaction. *N*-Boc-methylglycine was obtained in 92% yield. It was confirmed that the compound was almost pure through NMR data.

Therefore, coupling reaction to synthesis peptide bond of building block proceeded. Compound which have free amine group was dissolved in DMF with *N*-Boc-methylglycine followed by addition of EDCI · HCl, HOBt. After 5 minutes, DIPEA was added in solution. Then, product was extracted with Et_2O and purified with column chromatography in 59% yield in 2 steps. (Scheme 12)



Scheme 12. Preparation of final compound 8

3. Conclusion

In conclusion, stereoselective building block of rakicidin A which have L-*threo*-β-hydroxy aspartate structure was synthesized by using *trans*-oxazolidine structure. *Trans*-oxazolidine was synthesized from D-serine which is commercially available using reported method. Stereoselective *trans*-oxazolidine was synthesized through the reaction of phenylsulfonylnitromethane and *N*-hydroxymethyl-α-aldehyde. During the reaction, nitrophenylsulfonyl olefin was formed Due to steric hindrance in the transition state during the following conjugate addition reaction, the *H*-eclipsed conformation is preferred to the *R*-eclipsed conformation. Therefore, *trans*-oxazolidine could be synthesized more than *cis*-oxazolidine about 20:1. Stereoselectivity was confirmed through measurement of specific rotation of compound C which is synthesized from compound 6.

After synthesis of *trans*-oxazolidine, methylester group was converted to benzyl ester group. This step could give possibility of synthesis for total synthesis. Peptide bond of target compound was formed through coupling reaction with *N*-Boc-methylglycine. If further reactions proceed using this final product, rakicidin A could be synthesized through the intramolecular coupling reaction of desired building blocks. Based on this study, synthesis of a key intermediate has shown applicability to the synthesis of compound having L-*threo*-β-hydroxy groups as well as rakicidin A.

4. Experimental Details

4-1. General Information

General procedures

All materials were obtained from commercial suppliers and were used without further purification unless stated otherwise. DCM was distilled from calcium hydride immediately prior to use. DMF was dried with molecular sieves (4 Å). Air or moisture sensitive reactions were conducted under nitrogen atmosphere using oven-dried glassware and standard syringe/septa techniques. When the reagents are added to the crude product, the amount of the reagents are calculated based on the previous starting material. Additional purification methods are explained in detail with * mark since these procedures are inessential for next step. Reaction procedures are fundamentally described in the perspective of the convenience and efficacy. The reactions were monitored with a SiO_2 TLC plate under UV light (254 nm) and by visualization with a ninhydrin staining solution. Column chromatography was performed on silica gel 60 (70-230 mesh). Unless otherwise stated, 'H and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively in $CDCl_3$ on a Bruker AC-250 spectrometer. The ^{1}H NMR spectral data were reported as follows in ppm (δ) from the internal standard (TMS, 0.0 ppm): chemical shift (multiplicity, integration, coupling constant in Hz). The ^{1}H NMR spectra were referenced with the 7.26 resonance of $CDCl_3$ using tetramethylsilane as internal standard. The ^{13}C NMR

spectra were referenced with the 77.16 resonance of $CDCl_3$. The specific rotation was measured by using polarimeter, P-2000.

Phenylsulfonylnitromethane (A) ; To nitromethane (9.00 mL, 165.0 mmol) in DMF (180 mL), 1.1 eq. of DBU (27.42 mL, 181.8 mmol) was slowly added at 0℃, ice bath. 0.83 eq. of sodium benzenesulfinate (22.50 g, 137.1 mmol) and 0.76 eq. of iodine (31.86 g, 125.4 mmol) were also added to the cold reaction mixture and stirred for 30 min. Then warmed up to room temperature and stirred for another 1 h. After reaction complete, it was cooled down again and diluted with Na_2SO_3 aqueous solution until the color of the reaction mixture turned into bright yellow. Then slowly acidify with conc. HCl. Since large amount of the solvent DMF was used as a reaction medium, Et_2O (300 mL x 4) was selected as organic solvent and combined organic layers were washed with 0.1-0.2 N of HCl aqueous solution (300 mL x 3). The organic layer was dried over $MgSO_4$, filtered and concentrated to give the crude product as a yellowish solid. Purification was performed with silica gel column chromatography with gradient elution, HEX:EtOAc=5:1 to HEX:EtOAc=2:1 (v:v) to give a pure compound A (11.57 g, 42%) as a white powder. R_f = 0.45 (HEX:EtOAc=2:1)

A: ^{1}H NMR δ 7.97-7.97 (m, 2H), 7.80-7.76 (m, 1H), 7.66-7.62 (m, 2H), 5.65 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.75, 129.90, 129.48, 90.41,

Boc-*N*-methylglysine (B); To sarcosine (0.5 g, 5.74 mmol) in water, 3.00 eq. of TEA (2.4 mL) and 1.1 eq. of di-*tert*-butyl dicarbonate (1.45 mL 6.31 mmol) were added with stirring at the ice-bath. After

20 min at 0 °C, the mixture was warmed up to room temperature and stirred for 8 h. The mixture was concentrated with vacuum evaporation to remove *t*−BuOH and the resulting concentrate was extracted with n−hexane to remove unreacted di−*tert*−butyl dicarbonate. The aqueous phase was acidified to pH 1−2 with 2 N HCl. The resulting mixture was extracted with EtOAc (3 x 75 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give the crude product.

B : ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02−3.95(s, 2H), 2.94 (s, 3H), 1.47(s 9H); ^{13}C NMR δ (100 MHz, $CDCl_3$)175.23, 174.94, 156.51, 155.64, 80.73, 50.84, 50.34, 35.73, 28.38

N-Boc-L-*threo*-*β*-hydroxyaspartic acid−2−monomethylester (C); To a solution of 6 (163.8 mg, 1.595 mmol) in MeOH (5 mL), 1N HCl of diethyl ether solution (1.02 eq., 0.607 mL, 0.607 mmol) was added and refluxed for 12 h. After cooled down to room temperature, the reaction mixture was concentrated under reduced pressure. Then, it was diluted with THF (1.2 mL). 1.02 eq. of Boc2O (0.14 mL, 0.607 mmol), 1.05 eq. of NaHCO3 (52.5 mg, 0.625 mmol) and H2O (1.2 mL) were added to the diluted solution and stirred at room temperature for 8 h. After then, the resulting mixture was concentrated under reduced pressure and the residue was partitioned between brine (20 mL) and EtOAc (20 × 2). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification was performed with silica gel column chromatography with CH_2Cl_2:MeOH=10:1 to 5:1 (v:v) to give C (31 mg, 20%) as a colorless oil R_f = 0.2 (CH_2Cl_2:MeOH =5:1)

C : ^{1}H NMR (400 MHz, MeOD) δ 4.74(s, 1H), 4.48 (s, 1H), 3.73(s,

3H), 1.43(s, 9H); ^{13}C NMR δ (100 MHz, $CDCl_3$) 176.53, 175.07, 158.08, 81.14, 73.51, 59.61, 53.32, 29.16. $[\alpha]^{28}_D$=25.6(c=0.8, MeOH)

Specific procedure for building block of Rakicidin A

(R)-*tert*-butyl-4-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-oxooxazolidine-3-carboxylate (2) ; To D-serine (1.50 g, 14.27 mmol) in a mixture of water (2.50 mL) and *t*-BuOH (2.50 mL), 1.06 eq. of NaOH (604 mg, 15.1 mmol) and 1.03 eq. of di-*tert*-butyl dicarbonate (3.38 mL, 14.7 mmol) were added with stirring at the ice-bath. After 20 min at 0 ℃, the mixture was warmed up to room temperature and stirred for 8 h. The mixture was concentrated with vacuum evaporation to remove *t*-BuOH and the resulting concentrate was extracted with n-hexane to remove unreacted di-*tert*-butyl dicarbonate. The aqueous phase was acidified to pH 1-2 with 2 N HCl. The resulting mixture was extracted with EtOAc (3 x 75 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give the crude product, Boc-D-serine, as a colorless oil. To an ice-cold solution of Boc-D-serine (crude) in DMF (15 mL), 1.5 eq. of TBSCl (3.25 g, 21.5 mmol) and 3 eq. of imidazole (2.90 g, 42.9 mmol) were added with stirring under nitrogen atmosphere. After 30 min at 0 ℃, the mixture was warmed to room temperature and stirred for 24 h. The reaction mixture was extracted with Et_2O (2 x 100 mL) and the organic layer was acidified to pH 1-2 with 2 N aqueous HCl. Then partitioned phases were separated. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give the crude product 1 as a colorless.

To a solution of 1 (crude) in toluene (220 mL), 10 eq. of paraformaldehyde (4.3 g, 143 mmol) and 0.03 eq. of ((1S)-10-camphorsulfonic acid (CSA, 100 mg, 0.43 mmol) were added. The flask was then fitted with a Dean-Stark trap with a water-cooled condenser and the reaction mixture was heated under reflux with stirring for 45 min to remove generated water. The reaction mixture was cooled, filtered with $MgSO_4$ and under reduced pressure. Purification was done with silica gel column chromatography with gradient elution, HEX:EtOAc=8:1 to 4:1 (v:v) to give **2** (4.17 g, 88% (from D-serine in 3 steps)) as a colorless oil. R_f = 0.6 (HEX:EtOAc=2:1)

2: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52-5.41 (br d, 1H), 5.18 (d, 1H, *J*=3.8), 4.20-4.02 (br m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.83, 151.59, 81.90, 79.01, 62.28, 61.52, 58.04, 57.66, 28.40, 25.76, 18.14, -5.54

(4R)-*tert*-butyl 4-(((tert-butyldimethylsilyl)oxy)methyl)-5-hydroxyoxazolidine-3-carboxylate, D-serinal (3); To a stirred solution of 2 (2.07 g, 6.25 mmol) in dry DCM (63 mL. 0.5 M) at -78 °C, 1.0 M solution of DIBAL-H in DCM (1.5 eq., 9.4 mL, 9.4 mmol)was added dropwise under a nitrogen atmosphere. The reaction was quenched slowly by adding MeOH (14 mL). After 5 min at –78 °C, the cold solution was warmed to room temperature. The resulting reaction mixture was extract with a saturated aqueous solution of Rochelle salt (5 x 70 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification was performed with silica gel column chromatography with gradient elution, HEX:EtOAc=8:1 to HEX:EtOAc=4:1 (v:v) to

give a diastereomeric mixture of **3** (1.44 g, 69%) as a white solid. R_f = 0.5 (HEX:EtOAc=2:1)

3: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.59 (d, 1H, *J*=3.6), 5.09 (s, 1H), 4.89 (s, 1H), 3.79 (s, 2H), 3.47 (s, 1H), 2.67 (d, 1H, *J*=3.2), 1.46 (s, 9H), 0.88 (s, 9H), 0.06 (d, 6H, *J*=4.8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.73, 99.00, 80.61, 78.12, 63.47, 61.42, 28.47, 25.86, 18.20, −5.33

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(4R,5S)−3−*tert*−butyl 5−methyl 4−(((*tert*−butyldimethylsilyl)oxy)methyl)oxazolidine −3,5−dicarboxylate (4); To a−amino aldehyde 3 (1.16 g, 3.48 mmol) in THF (2 mL) 1.3 eq. of **A** (909 mg, 4.52 mmol) and 1.3 eq. of DMAP (552 mg, 4.52 mmol) were added. The reaction mixture was stirred at room temperature for 2 days with vigorous stirring until the starting material 3 disappeared. The reaction mixture was diluted with THF (3 mL) and methanol (10 mL) and 3 eq. of DBU (1.56 mL, 10.4 mmol) was added at 0 ℃ ice−bath. Then the reaction mixture was cooled to −78 ℃ and ozonolysis was done over 20 min. The resulting mixture was warmed to room temperature and concentrated under reduced pressure. After then, the residue was partitioned between EtOAc (10 mL) and an aqueous saturated solution of NH_4Cl (20 mL). The aqueous layer was extracted with EtOAc (20 mL x 2), and the combined organic layers were dried over $MgSO_4$ filtered and concentrated under reduced pressure. Purification was performed with silica gel column chromatography with gradient elution, HEX:EtOAc=8:1 to HEX:EtOAc=2:1 (v:v) to give a single stereoisomer **5** (0.797 g, 61%) as a colorless oil. R_f = 0.62 (HEX:EtOAc=2:1)

4: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.19 (br s, 1H), 4.80 (br s, 1H), 4.74

(d, 1H, *J*=3.6), 4.09 (br s, 1H), 3.79 (br m, 5H), 1.46 (s, 9H), 0.89 (s, 9H), 0.07 (d, 6H, *J*=4.8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.17, 152.40, 80.79, 80.00, 61.66, 60.11, 52.32, 28.35, 25.75, 18.12, −5.45

4−benzyl 3−(*tert*−butyl) 5−methyl (4S,5S)−oxazolidine−3,4,5−tricarboxylate (7); To a solution of 4 (619 mg, 1.65 mmol) in THF (16.5 mL), 1.1 eq. of TBAF·$4H_2O$ (475 mg, 1.82 mol) was added. The reaction mixture was stirred at room temperature until the starting material disappeared from TLC. The reaction mixture was partitioned between EtOAc (30 mL) and an aqueous saturated solution of NH4Cl (30 mL). The aqueous layer was extracted with EtOAc (30 mL x 2), and the combined organic layers were dried over $MgSO_4$ filtered and concentrated under reduced pressure. An aqueous solution of 5 wt% NaHCO3 (5 mL) and acetone (5 mL) was directly added to the stirring reaction mixture of **5** at 0 ℃. After then 0.5 eq. of KBr (25.7 mg, 0.56 mmol), 1 eq. of TEMPO (175 mg, 1.12 mmol) and an aqueous solution of 7 eq. of 5% NaOCl (15 mL, 7.84 mmol) were added to the reaction mixture. The resulting mixture was stirred for 3 h at room temperature. The resulting mixture was washed with EtO_2 (20 mL × 2), and the aqueous layers were acidified with an aqueous solution of 2 N HCl to pH 4~5. The acidified aqueous solution was partitioned between brine (20 mL) and EtOAc (20 mL × 3). The combined organic layers were dried with $MgSO_4$ filtered and concentrated under reduced pressure. The concentrated resultant was dissolved in DMF (9 mL) at 0 ℃. 2 eq. of $NaHCO_3$ (77 mg, 0.913 mmol) and 4 eq. of BnBr (0.22 mL, 1.827 mmol) were added to solution. The resulting mixture was stirred for 8 h at room

temperature. The reaction was quenched slowly by adding H_2O (3 mL). The reaction mixture was extracted with Et_2O (2 x 20 mL). The organic layers were dried with $MgSO_4$ filtered and concentrated under reduced pressure. Purification was performed with silca gel column chromatography with gradient elution, HEX:EtOAc=8:1 to HEX:EtOAc=4:1 (v:v) to give **7** (247 mg, 41% (from **4**)) as a colorleessoil, R_f = 0.5 (HEX:EtOAC=2:1)

7 ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 5H), 5.20−4.98 (br m, 3H), 5.03 (d, 1H, *J*=34.3) 4.77−4.60 (m, 2H), 3.79 (s, 3H), 1.40 (d, 9H, *J*=37.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.48, 135.23, 128.77, 128.69, 81.74, 80.05, 67.74, 60.35, 52.94, 28.29, 0.104. HR−EI−MS: m/z [M^+] called for $C_{18}H_{23}NO_7$: 365.1500; found: 365.1477

1−benzyl 4−methyl (2S,3S)−2−(2−((tert−butoxycarbonyl)(methyl)amino)acetamido)−3−hydroxysuccinate (8) ; To a solution of 8 (19 mg, 0.102 mmol) and 7 (56 mg, 0.153 mmol) in DMF (3 mL) was added EDCI・HCl (40 mg, 0.204 mmol) and HOBt (21 mg, 0.153 mmol), followed by addition of DIPEA (0.036 mL, 0.204 mmol) under argon atmosphere at 20 ºC. The reaction mixture was stirred for 4 h, and then quenched with H_2O (2 mL). The aqueous phase was extracted with Et_2O (3 × 5 mL). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel HEX:EtOAc=1:1 to HEX:EtOAc=1:2 obtain 9 (56 mg, 37%) as a colorless oil. R_f = 0.25 (HEX:EtOAc=1:1)

8: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.355(m. 5H), 5.23(s, 2H), 5.14 (d,

1H, J = 8.4), 4.74 (d, 1H, J = 5.6), 3.87 (d, 2H, J = 4), 3.79 (s, 3H), 2.89 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.32, 169.65, 168.75, 135.07, 128.82, 128375, 128.44, 81.07, 70.88, 68.05, 54.56, 53.9, 35.63, 28.37, HR-EI-MS: m/z [M^+] called for $C_{20}H_{28}N_2O_8$: 424.1800; found: 365.1848. [α]$^{28}_D$=+27.8(c=0.43, $CHCl_3$)

REFERENCES

1. Jirouta Kitagaki, Genbin Shi, Shizuka Miyauchi, Shinya Murakami and Yili Yang., *Anticancer Drugs.*, 2015, *26*, 259-71

2. C.E. Ballard, H. Yu and B. Wang., *Current Medicinal Chemistry.*, 2002, *9*, 471-498

3. Rodrigo A. Rodriguez, Po-Shen Pan, Chung-Mao Pan, Suchitra Ravula, Stephanie Lapera, Erinprit K. Singh, Thomas J. Styers, Joseph D. Brown, Julia Cajica, Emily Parry, Katerina Otrubova, and Shelli R. McAlpine., *J. Org. Chem.*, 2007, *72*, 1980-2002

4. Yajaira Suárez, Laura González, Ana Cuadrado, Maite Berciano, Miguel Lafarga, and Alberto Muñoz., *Mol Cancer Ther.*, 2003, *2*, 863–872

5. McBrien, K. D.; Berry, R. L.; Lowe, S. E.; Neddermann, K. M. Bursuker, I.; Huang, S.; Klohr, S. E.; Leet, J. E. *J. Antibiot.* 1995, *48*, 1446.

6. Jian Chen , Jingpei Li, Lingling Wu , Yan Geng, Jianming Yu, Chuanke Chong, Mengmeng Wang, Yuan Gao, Cuigai Bai b , Yahui Ding , Yue Chen, Quan Zhang. J. *European Journal of Medicinal Chemistry*, 2018, *151*, 601-627

7. Feng Sang, Yahui Ding, Jinghan Wang, Bingxia Sun, Jianlei Sun, Yan Geng, Zhang Zhang, Ke Ding, Ling-Ling Wu, Jian-Wei Liu, Cuigai Bai, Guang Yang, Quan Zhang, Lu-Yuan Li, and Yue Chen., *J. Med. Chem.* 2016, *59*, 1184-1196.

8. (a) Yamazaki, Y.; Kunimoto, S.; Ikeda, D. *Biol. Pharm. Bull.* 2007, *30*, 261-265., (b) Takeuchi, M.; Ashihara, E.; Yamazaki, Y.; Kimura, S.; Nakagawa, Y.; Tanaka, R.; Yao, H.; Nagao, R.; Hayashi, Y.; Hirai, H.; Maekawa, T. *Cancer Sci.* 2011, *102*, 591-596.

9. Feng Sang, Dongmei Li, Xiaolong Sun, Xianqiang Cao, Liang Wang, Jianlei Sun, Bingxia Sun, Lingling Wu, Guang Yang, Xiaoqian Chu, Jinghan Wang, Changming Dong, Yan Geng, Hong Jiang, Haibo Long, Sijia Chen, Guiyan Wang, Shuzhong Zhang, Quan Zhang,and Yue Chen., *J. Am. Chem. Soc.* 2014, *136*, 15787-15791

10. Michail Tsakos, Kristian M. Jacobsen, Wanwan Yu, Nikolaj L. Villadsen, Thomas B. Poulsen., *Synlett.*, 2016, *27*, 1898-1906

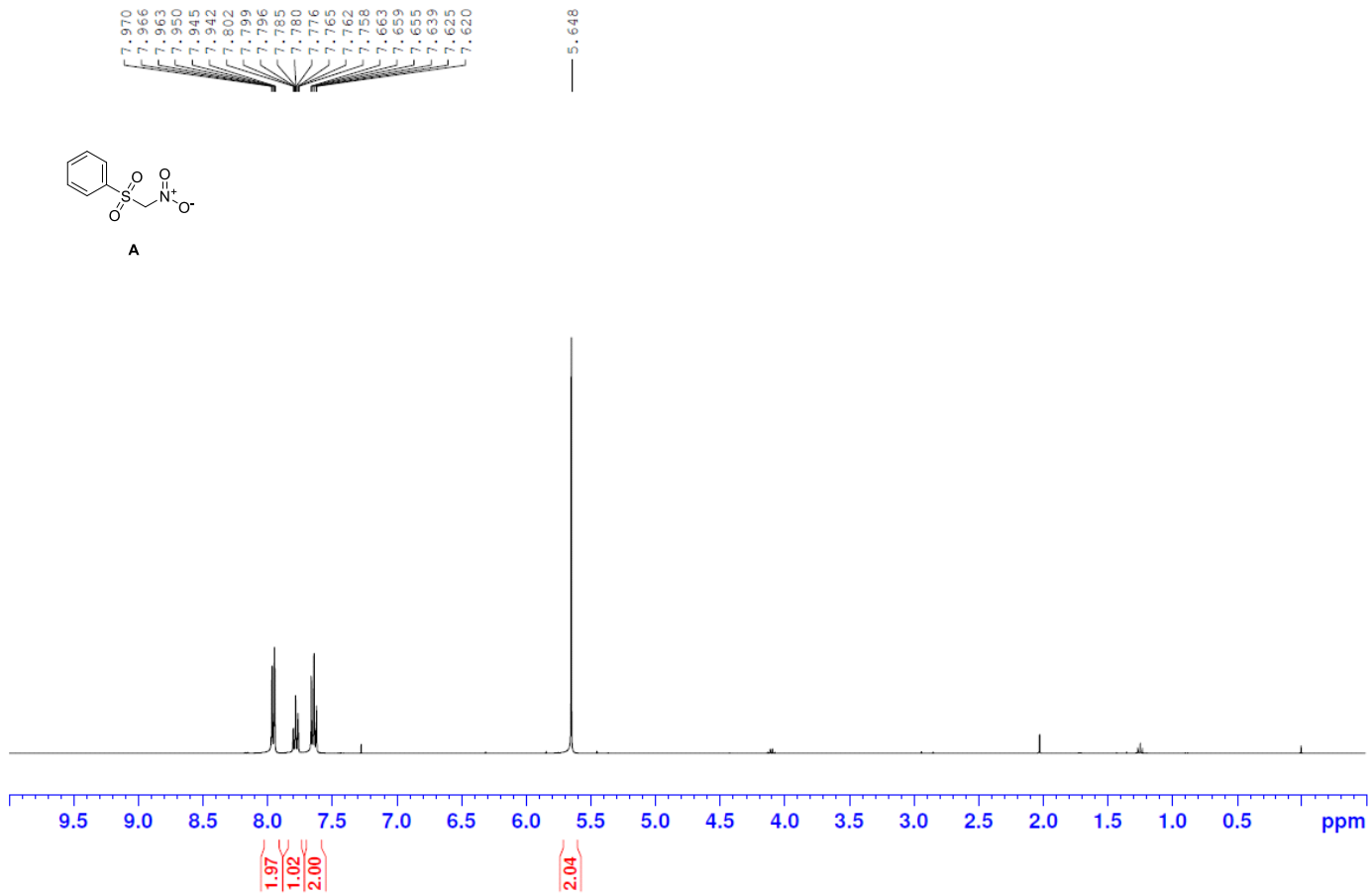
11. Michail Tsakos, Lise L. Clement, Eva S. Schaffert, Frank N. Olsen, Sebastiano Rupiani, Rasmus Djurhuus, Wanwan Yu, Kristian M. Jacobsen, Nikolaj L. Villadsen, and Thomas B. Poulsen., *Angew. Chem. Int. Ed.*, 2016, *55*, 1030 -1035

12. (a) Aikob-Hydmroxyasparagine ari Guzmán-Martínez, Michael S. VanNieuwenhze., *SYNLETT.*, 2007, *10*, 1513-1516., (b) Jingen Deng, Yasumasa Hamada, and Takayuki Shioiri., *J. Am. Chem. Soc.*, 1995, 117, 7824-7825

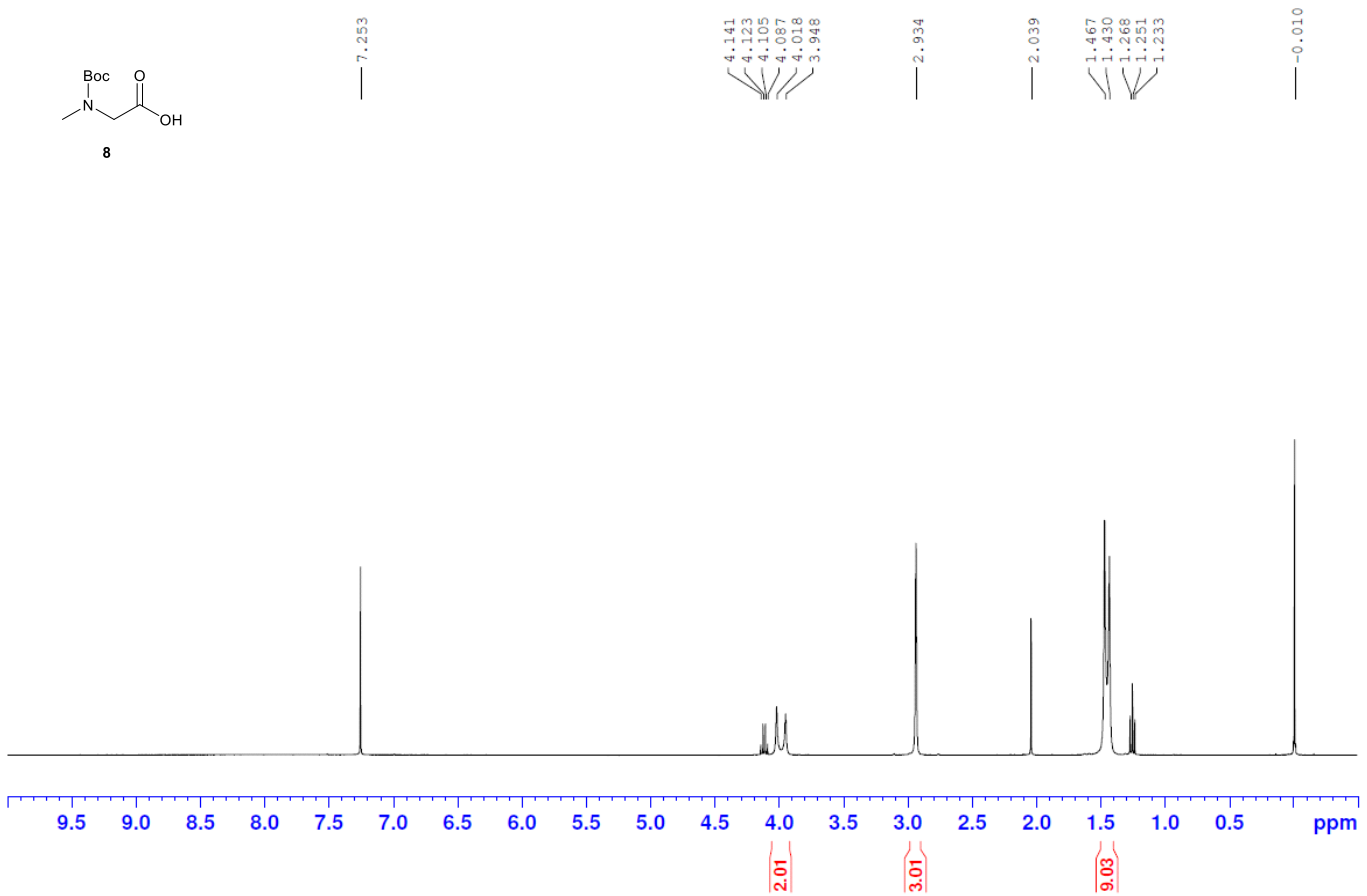
13. (a) Lee, B. thesis, Seoul National University, 2017., (b) Youngran Seo, Hyeonjeong Kim, Da Won Chae, Young Gyu Kim., *Tetrahedron: Asymmetry.*, 2014, 25, 625., (c) Dongwon Yoo, Joon Seok Oh, Dong-Whal Lee, Young Gyu Kim., *J. Org. Chem.*, 2003, 68, 2979

APPENDICES

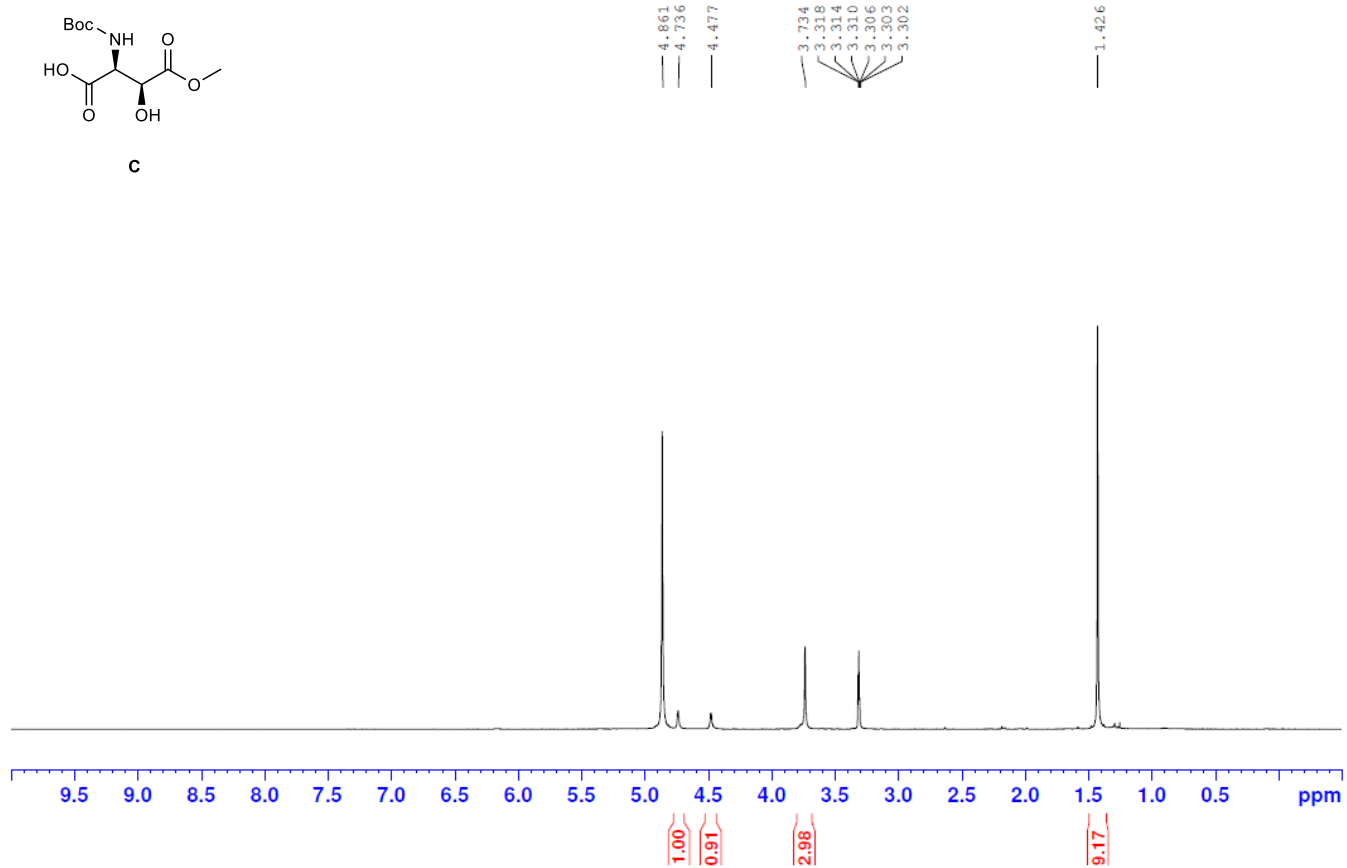
List of ^{1}H NMR Spectra of Selected Compounds



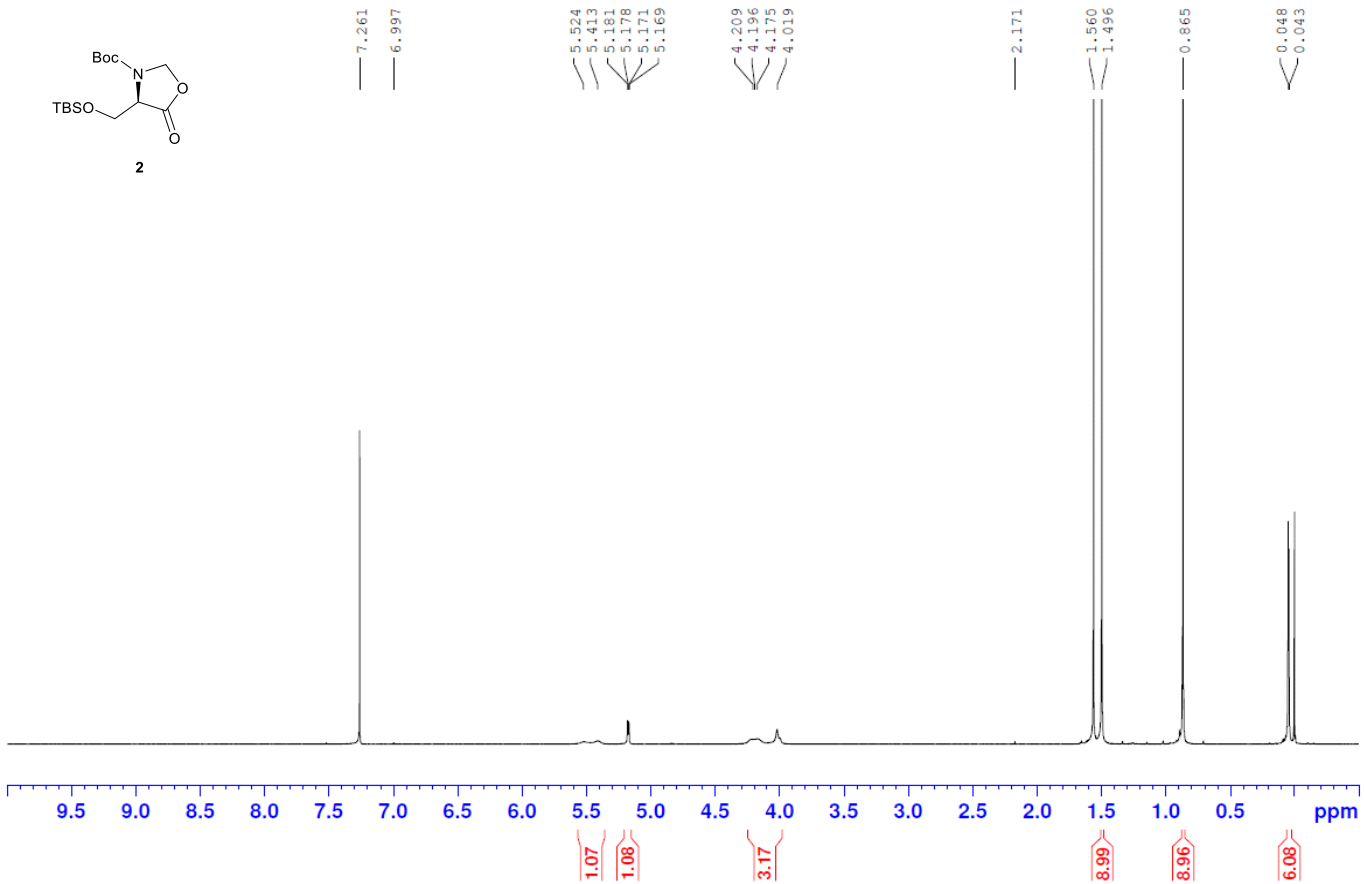
1. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of **A**



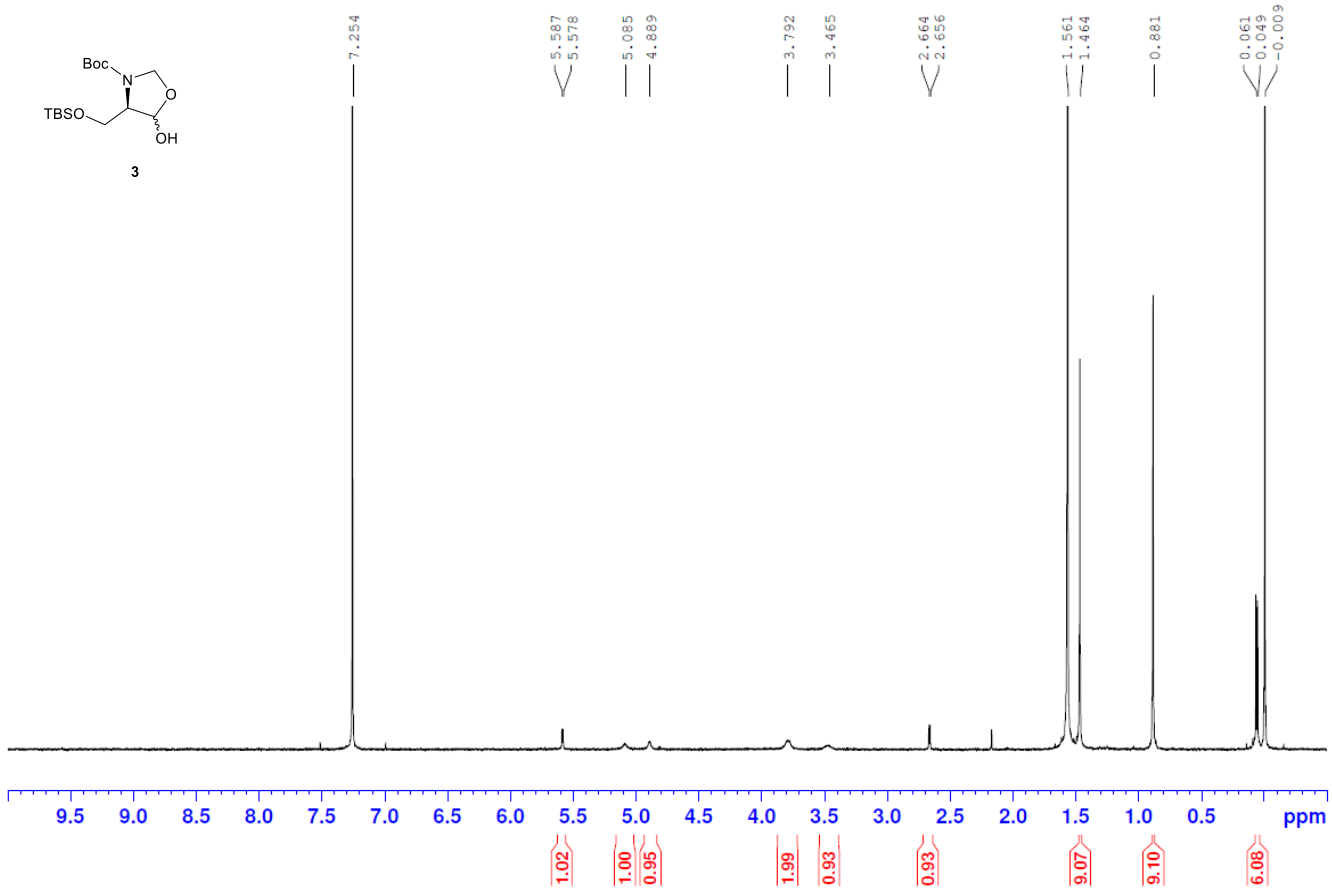
2. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of **B**



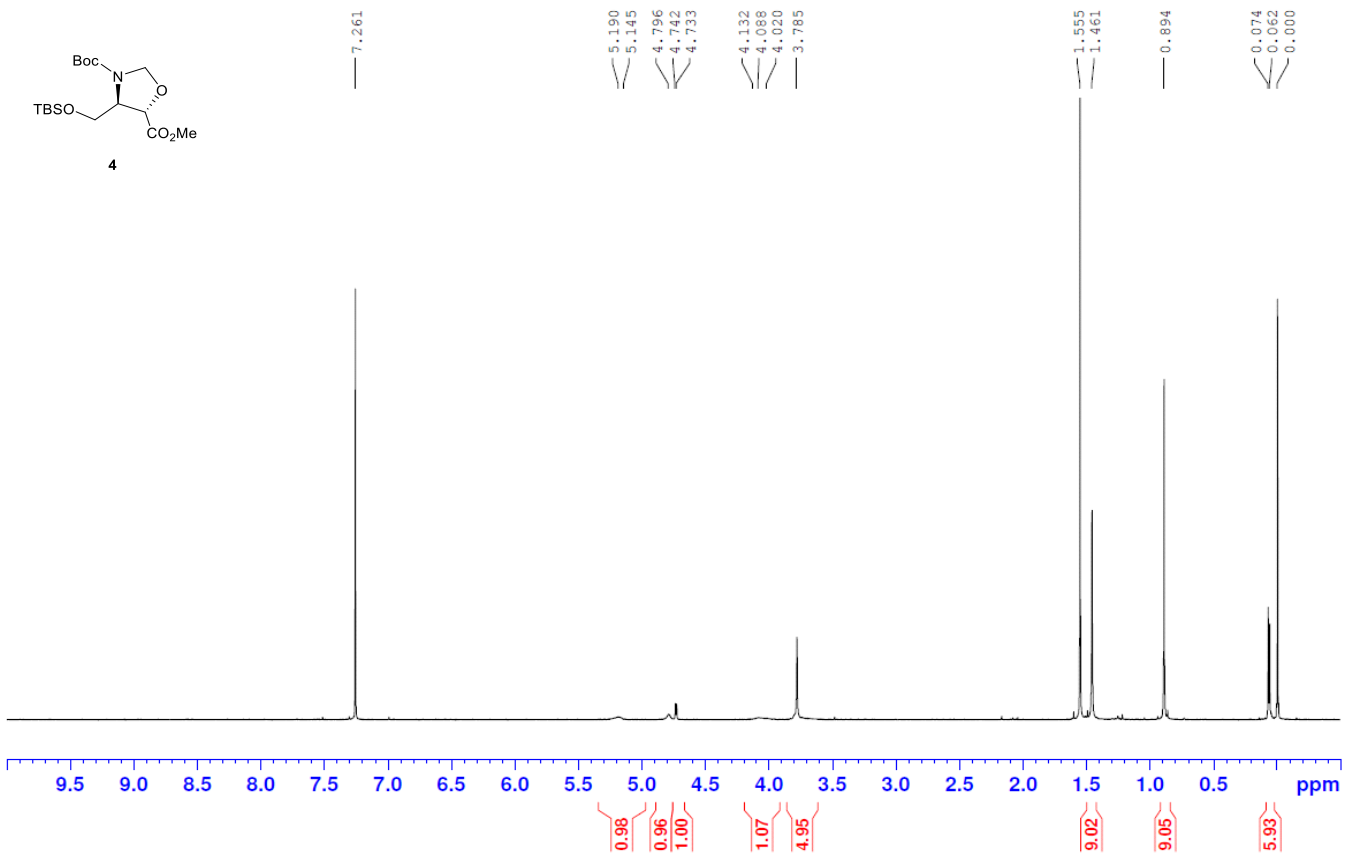
3. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of C



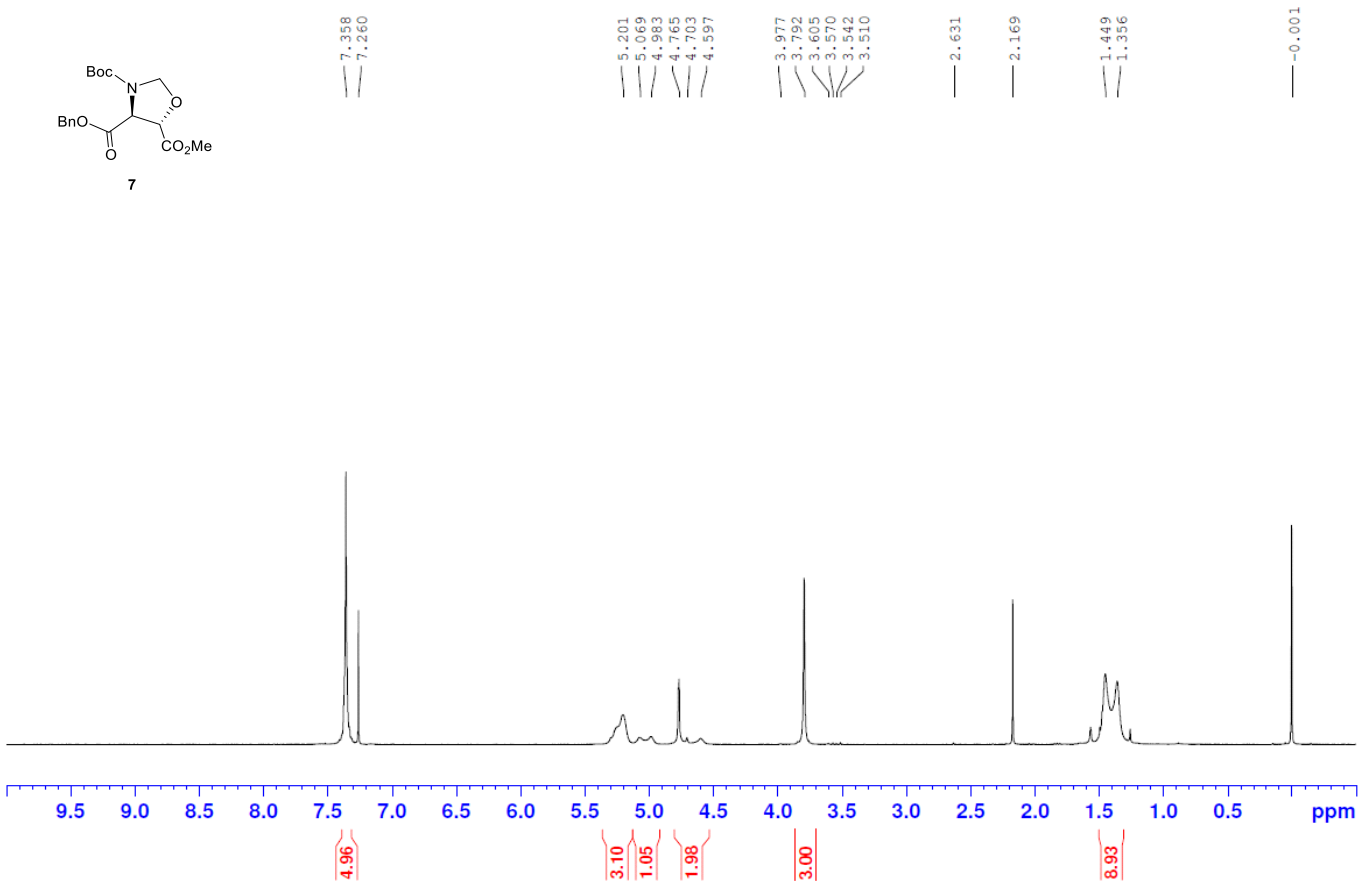
4. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of **2**



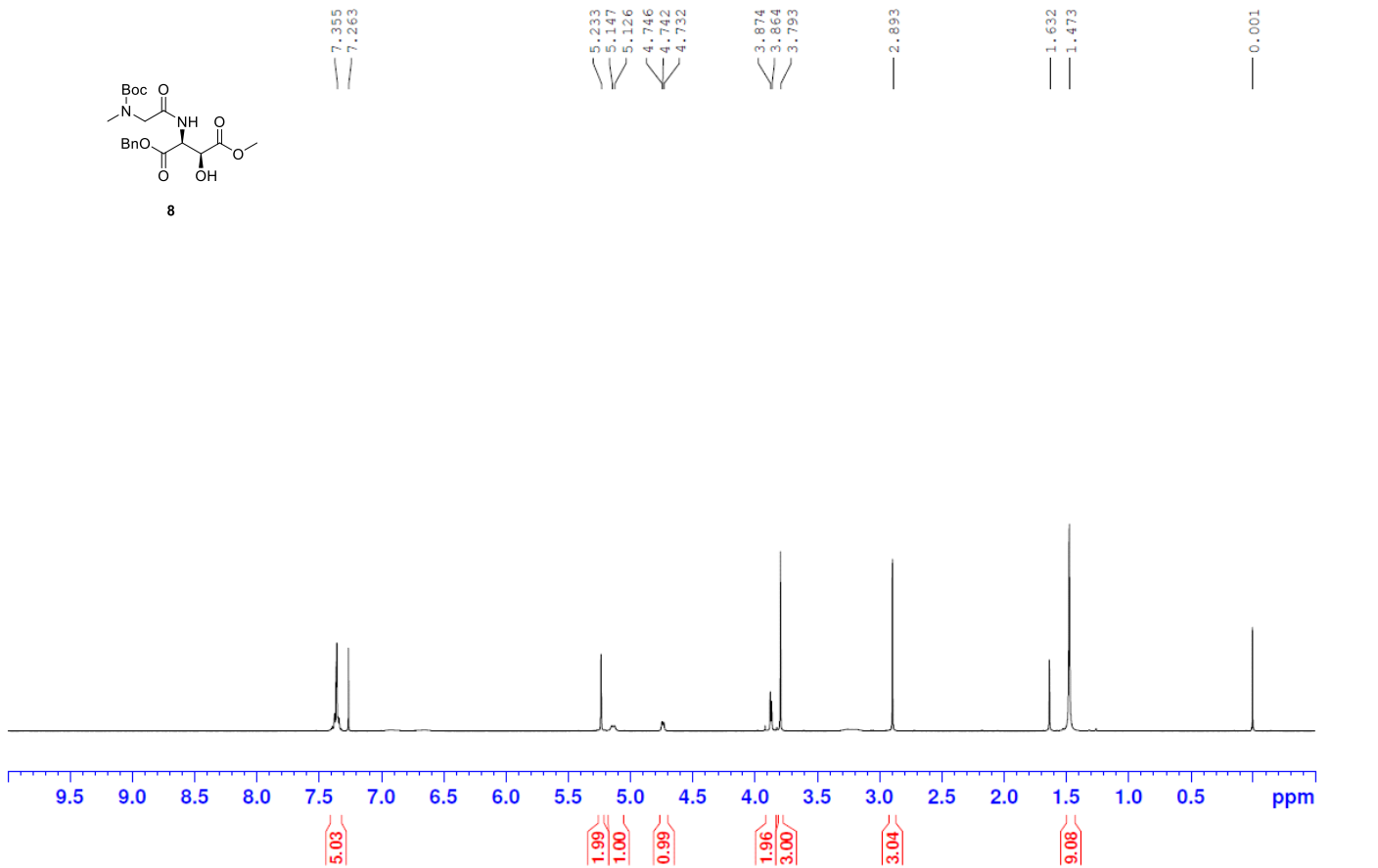
5. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of **3**



6. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of **4**



7. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of **7**



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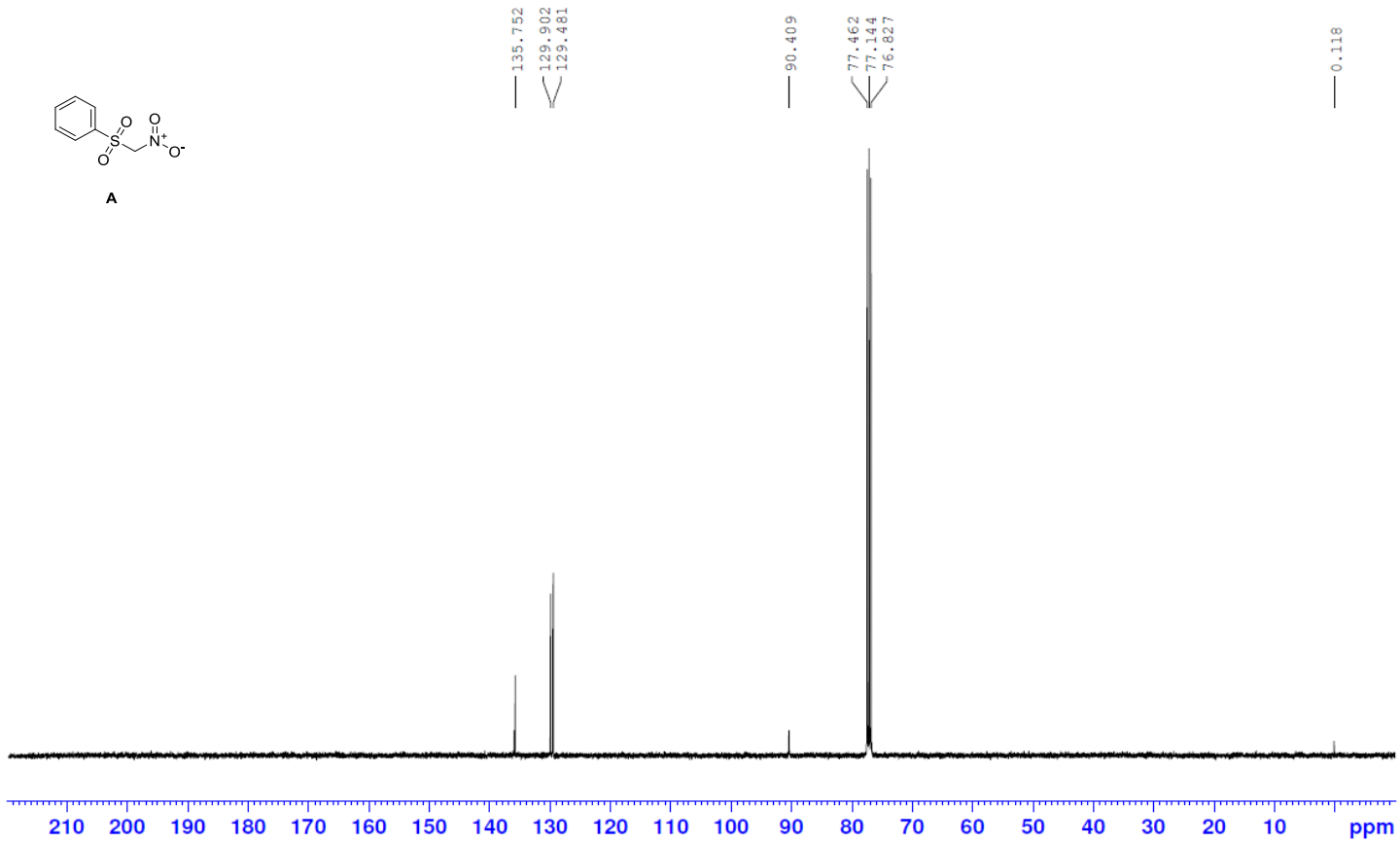
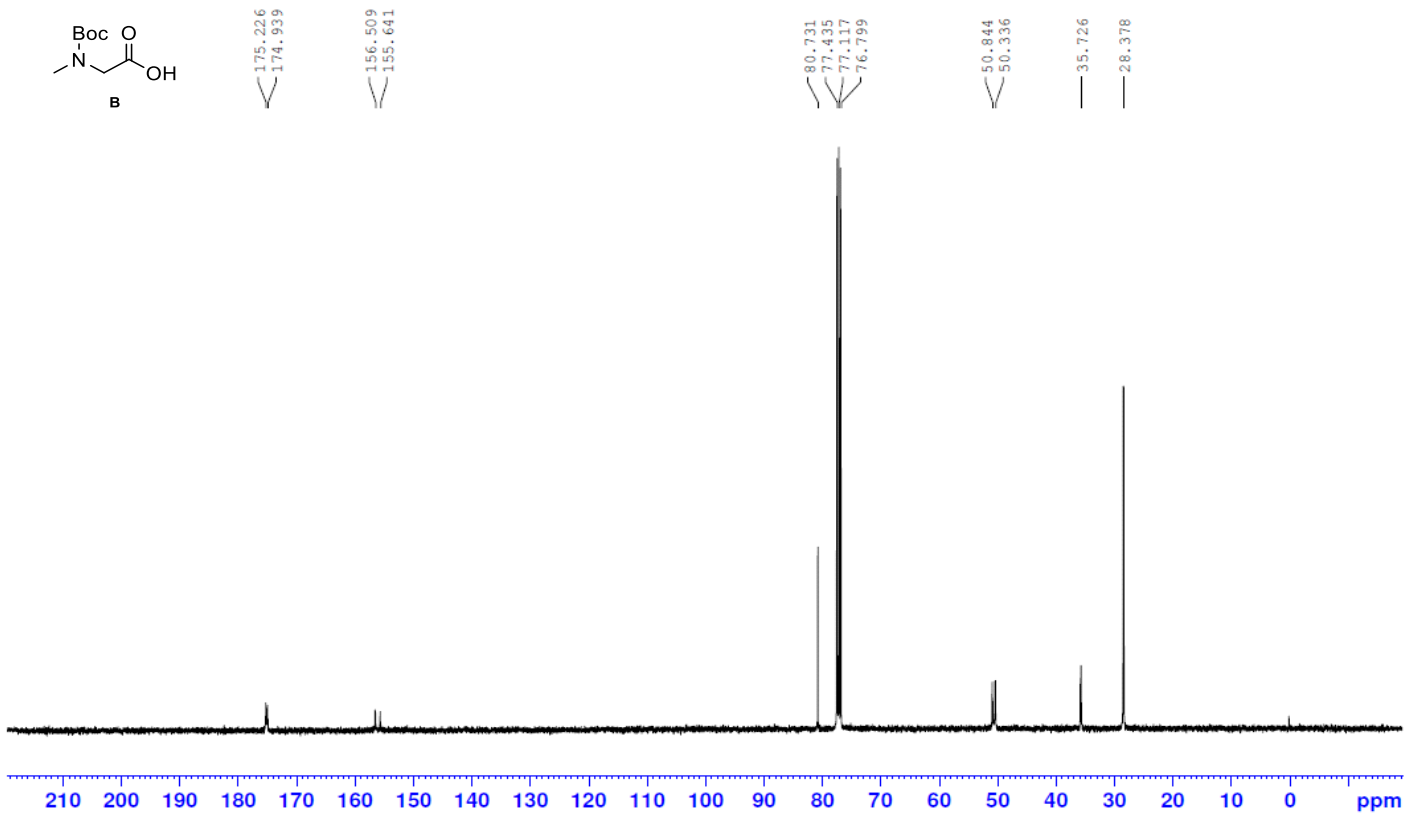
8. 400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of **8**

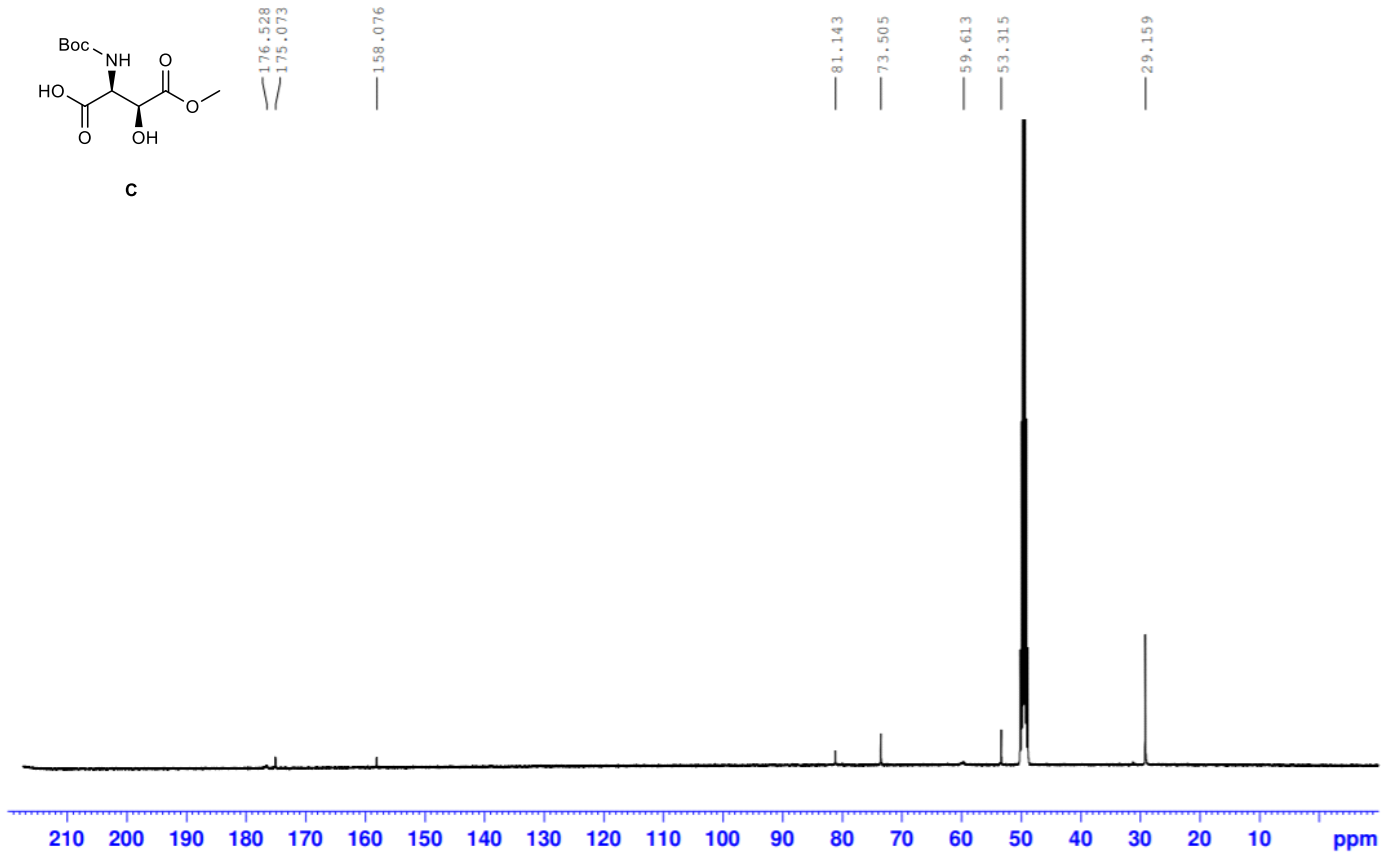
List of ^{13}C NMR Spectra of Selected Compounds



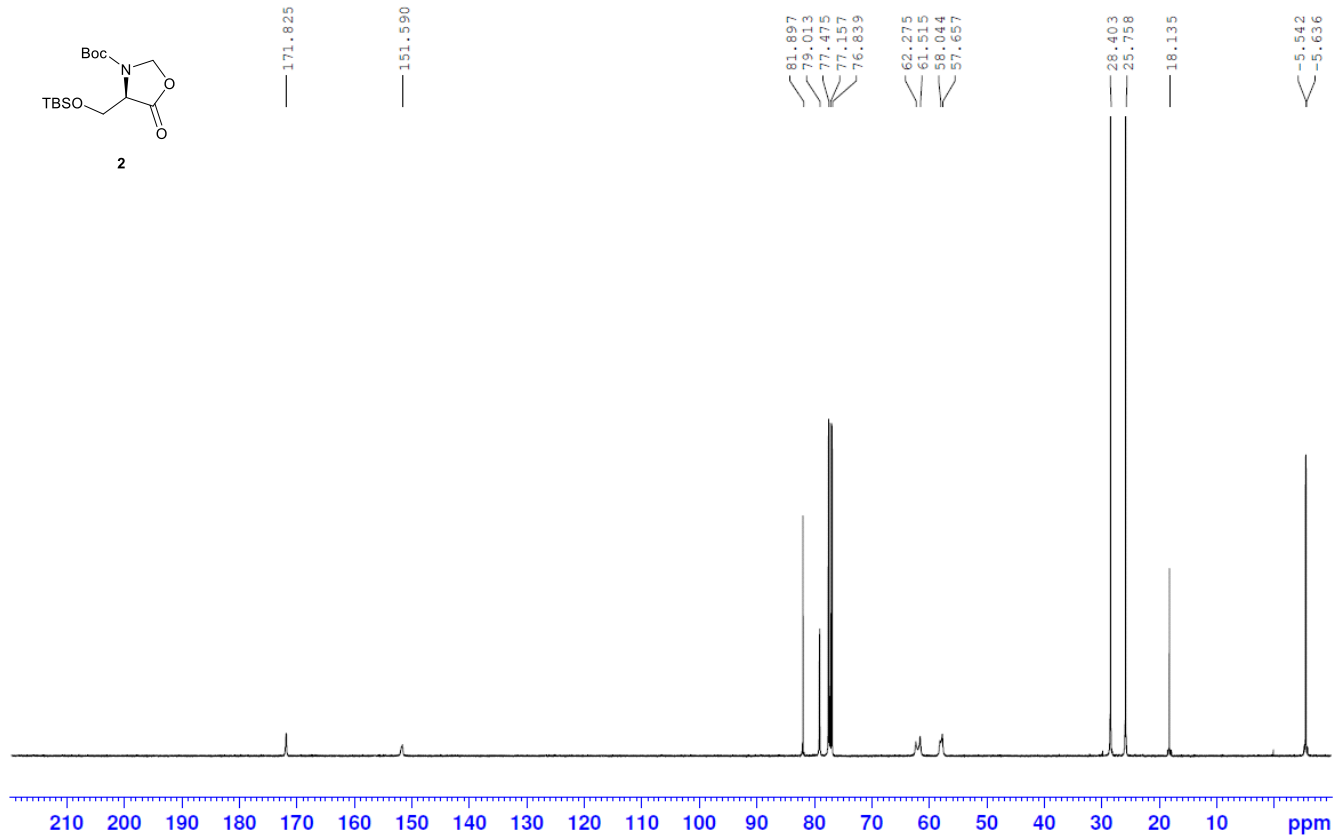
1. 100 Hz ^{13}C NMR Spectrum ($CDCl_3$) of **A**



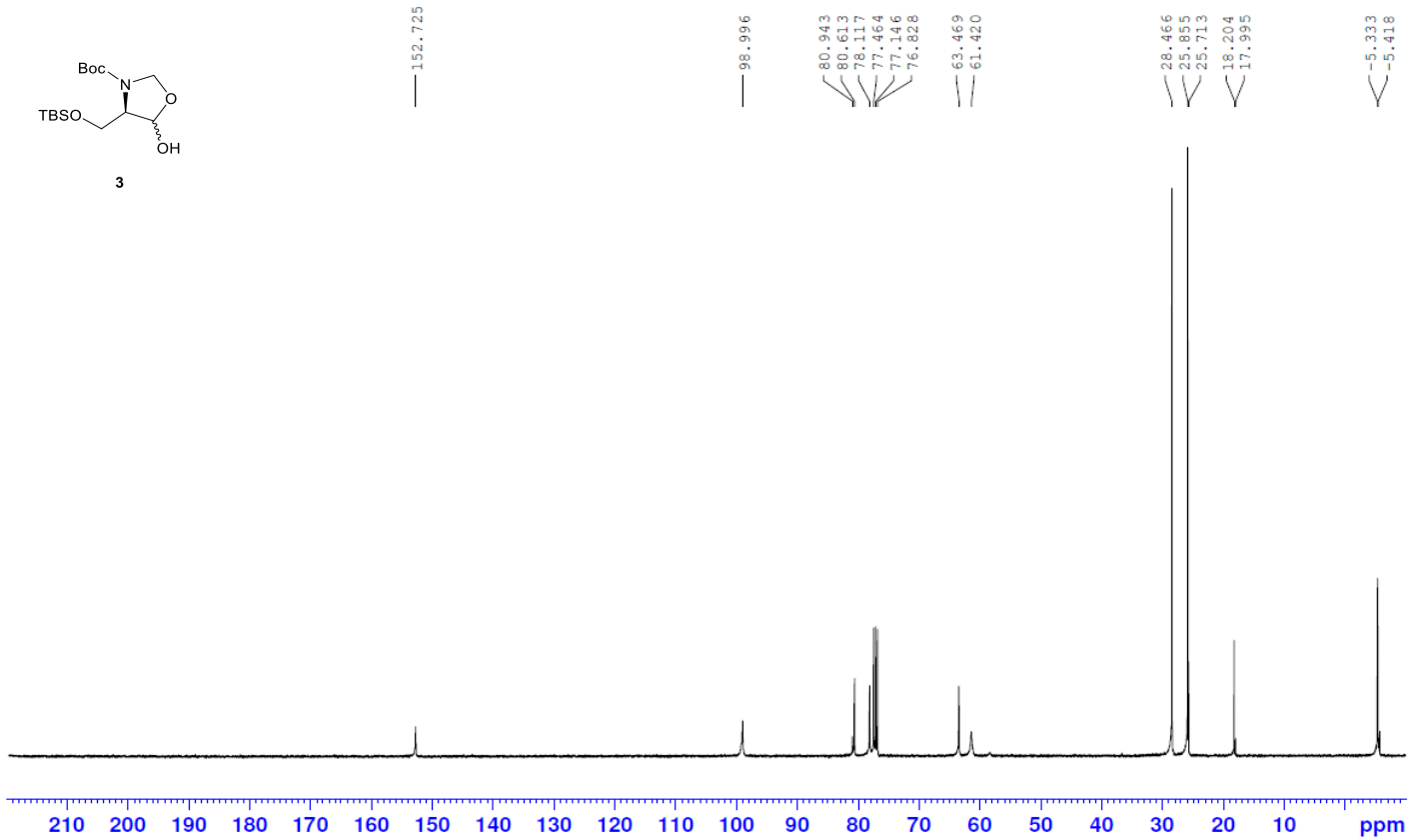
2. 100 Hz ^{13}C NMR Spectrum ($CDCl_3$) of **B**



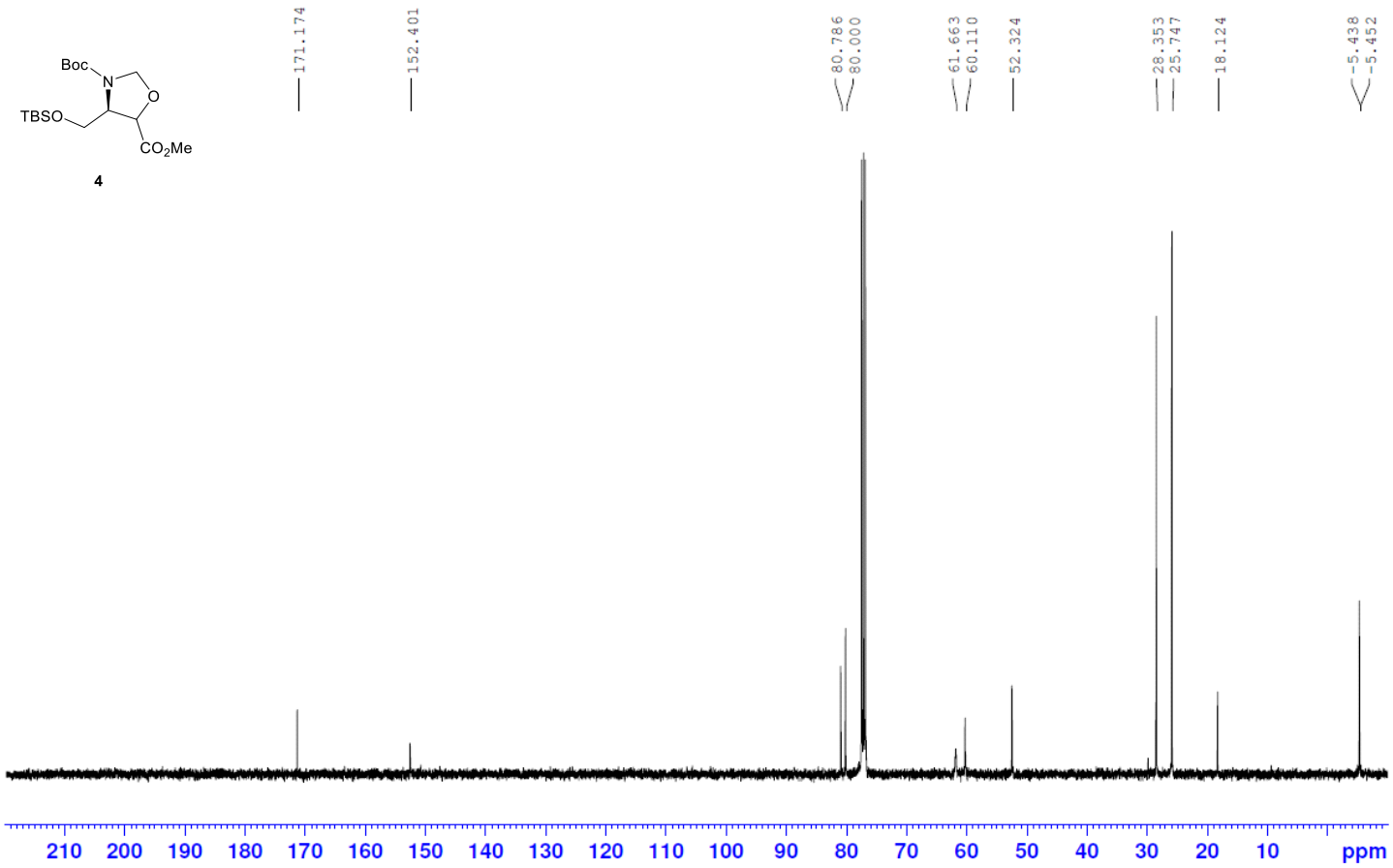
3. 100 Hz ^{13}C NMR Spectrum ($CDCl_3$) of **C**



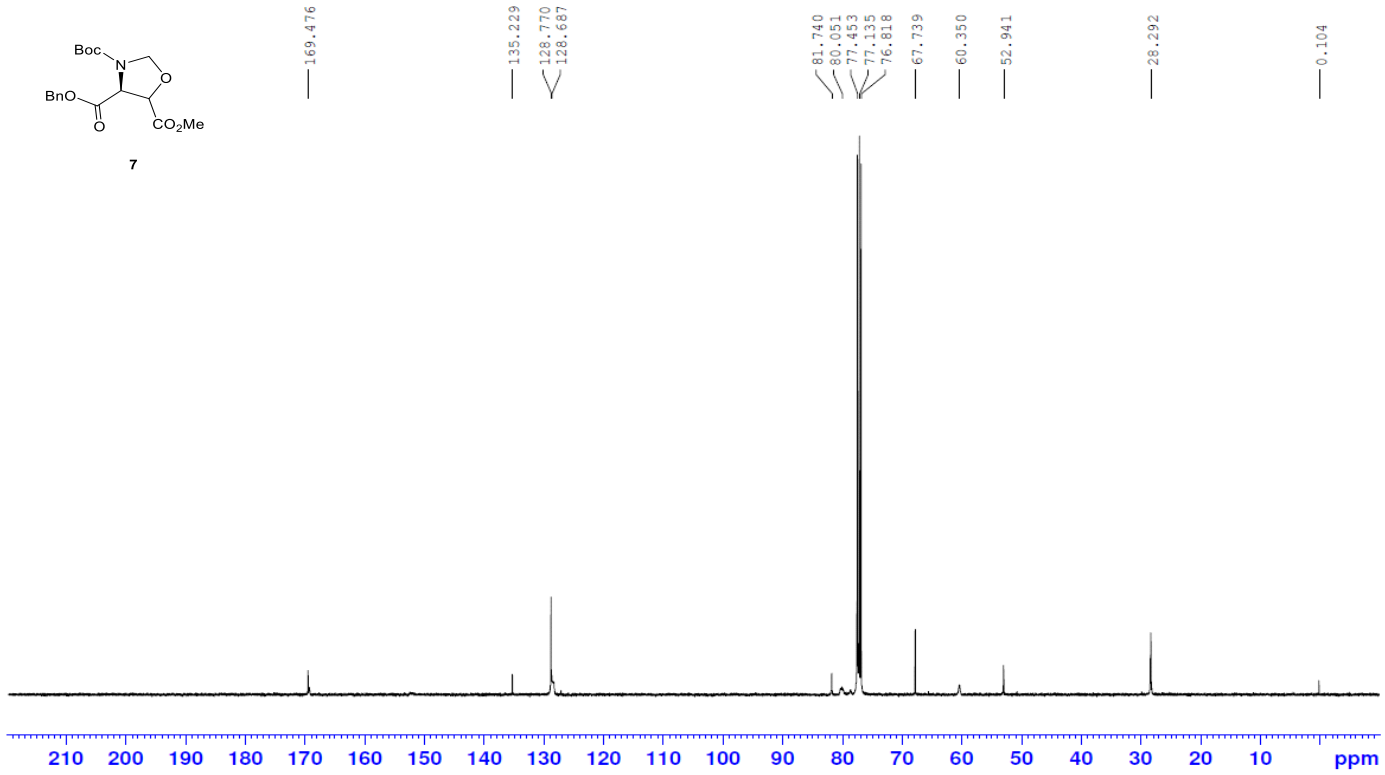
4. 100 Hz ^{13}C NMR Spectrum ($CDCl_3$) of **2**



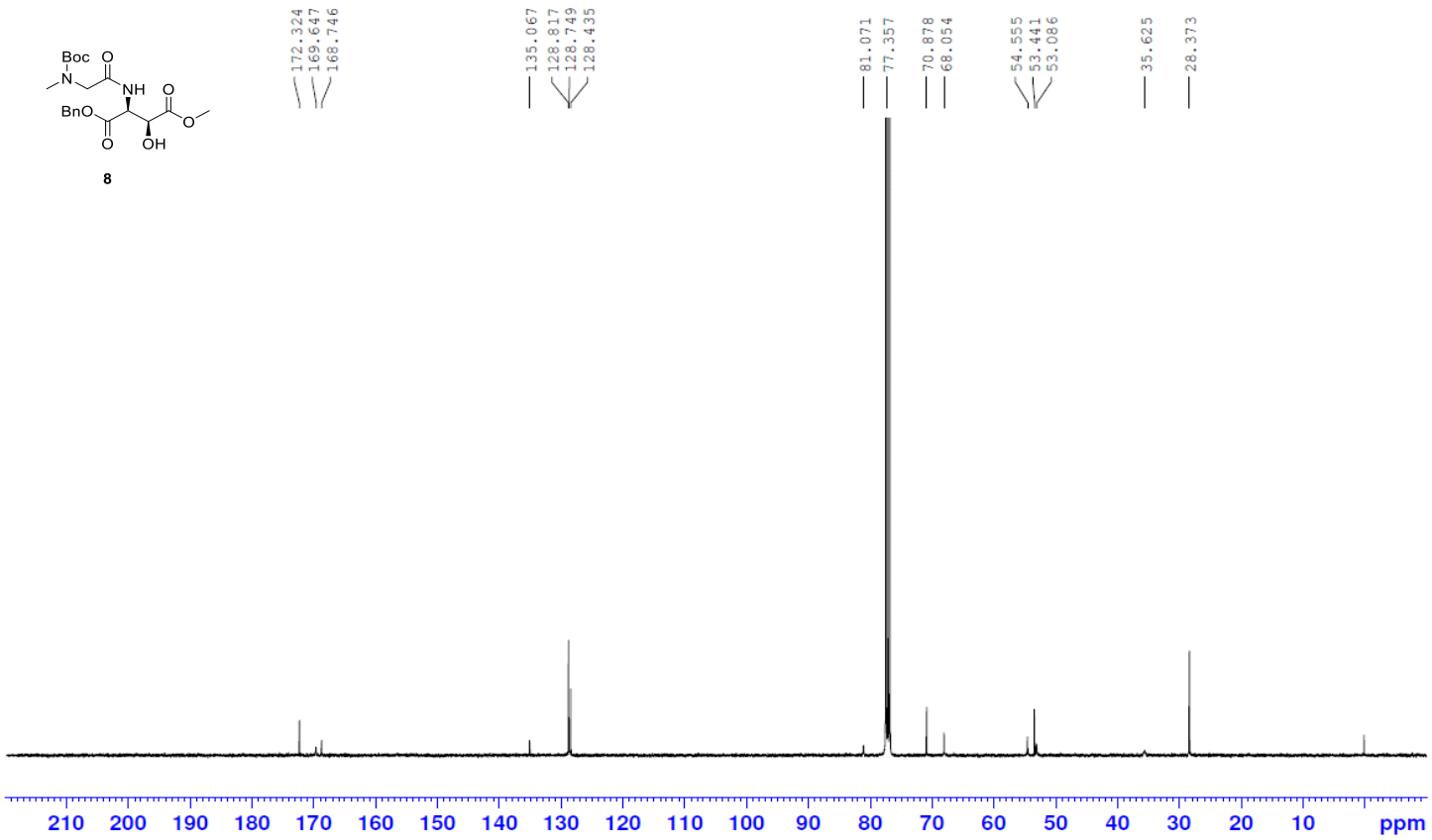
5. 100 Hz ^{13}C NMR Spectrum ($CDCl_3$) of **3**



6. 100 Hz ^{13}C NMR Spectrum ($CDCl_3$) of **4**



7. 100 Hz ^{13}C NMR Spectrum ($CDCl_3$) of **7**



8. 100 Hz ^{13}C NMR Spectrum ($CDCl_3$) of **8**

Abstract in Korean

본 논문은 L-트레오-β-하이드록시 아스파라진구조를 가지는 라키시딘 에이 전구체의 입체선택적 합성에 관한 내용을 다루고 있다. 라키시딘 에이는 천연물로 고리형 뎁시펩타이드들 중의 하나이다. 많은 고리형 뎁시펩타이드의 경우 임상효과가 있다고 알려져 있다. 특히, 라키시딘 에이는 만성 골수성 백혈병 줄기세포에 대해 성장억제활성을 나타내고 고형암을 선택적으로 치료할 수 있는 항암제의 역할을 한다.

라키시딘 에이의 전합성에 관한 몇몇 논문들이 보고 되어 있으며, 보고된 논문들에 따르면, 라키시딘 에이는 전구체들의 커플링 반응을 통해 합성할 수 있다. 그 중, L-트레오-β-하이드록시 아스파라진은 입체화학측면에서 중요한 전구체이며 이 구조 합성에 관한 몇몇 논문이 보고되어 있다.

우리 그룹에서는 헤미 아세탈로 평형이 이동하여 안정한 구조의 *N*-하이드록시메틸 -α- 아미노 알데하이드를 사용한 트랜스-옥사 졸리딘의 입체 선택적 합성에 대해 보고한 적이 있다. 트랜스-옥사졸리딘은 *N*-하이드록시메틸-α-아미노 알데하이드와 페닐설포닐나이트로 메테인의 반응을 통해 합성할 수 있다. 반응 중 형성되는 *H*-가리움형태를 통해 트랜스-옥사졸리딘으로의 입체 선택성이 강화된다.

이를 이용하여 D-세린으로부터 L-트레오-β-하이드록시 아스팔테이트를 총 11스텝, 9%로 합성하였고, L-트레오-β-하이드록시 아스팔테이트 유도체의 광회전도 값 측정을 통해 광학순도를 확인하였다. 또한, *N*-메틸글라이신과 커플링 반응으로 라키시딘 에이의 합성가능성을 보여주었다.

주요어 : 뎁시펩타이드, 임상효과, α-아미노 알데하이드, 트랜스-옥사졸리딘, L-트레오-β-하이드록시아스팔테이트, 전구체, 라키시딘 에이

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