공학석사학위논문

Stereoselective synthesis of L-*threo*-*β*-hydroxy aspartate: A building block of Antibiotic Ramoplanin A_2

항생제 라모플라닌 에이2의 전구체 L-트레오-*β*-하이드록시 아스팔테이트의 입체선택적 합성

2021년 8월

서울대학교 대학원

화학생물공학부

정 경 수

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이 논문을 공학석사 학위논문으로 제출함

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정경수의 공학석사 학위논문을 인준함

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ABSTRACT

Stereoselective synthesis of L-*threo*-*β*-hydroxy aspartate: A building block of Antibiotic Ramoplanin A_2

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This paper covers the contents of the synthesis of building block of ramoplanin A_2 based on the stereoselectivity of the L-*threo*-*β*-hydroxy aspartate. Ramoplanin A_2, synthesized by Actinoplanes, is a type of glycolipodepsipeptide and exhibits clinical effects. In particular, ramoplanin A_2 plays an effective role in the treatment of vancomycin-resistant *E. faecium* (VREF) in the gastrointestinal tract by inhibiting the biosynthesis of bacterial cell wall in Gram-positive bacteria.

According to the currently published papers, ramoplanin A_2 is composed of three key subunits and can be synthesized through coupling reactions and cyclization reactions. Among them, the structure of L-*threo*-*β*-hydroxy asparagine is considered to be important in terms of stereochemistry as the backbone of the second key subunit, pentadepsipeptide.

In our group, we have studied the stereoselective synthesis of *trans*-oxazolidine. *N*-hydroxymethyl-*α*-amino aldehyde has a stable structure by shifting its equilibrium to hemiacetal and participates in the reaction. *Trans*-oxazolidine is synthesized through the reaction of *N*-hydroxymethyl-*α*-amino aldehyde with phenylsulfonyl-nitromethane, and *H*-eclipsed formation of transition state enhances the stereoselectivity of *trans*-oxazolidine structure in the ratio of 20:1.

Through this procedure, L-*threo*-*β*-hydroxy aspartate was synthesized in a total of 11 steps, 11% from D-serine. This product was synthesized to contain different protection groups on each terminal, which allows selective conversions to other form such as L-*threo*-*β*-hydroxy asparagine. Moreover, the possibility of synthesizing ramoplanin A_2 was confirmed through Yamaguchi coupling reaction with *N*-phenylalanine.

Keywords: *N*-hydroxymethyl-*α*-amino aldehyde, *trans*-oxazolidine, L-*threo*-*β*-hydroxy aspartate, stereoselectivity, Glycolipodepsipeptide, Ramoplanin A_2

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

Boc	*tert*-Bututyloxycarbonyl
Bn	Benzyl
br	broad
BuLi	Butyl lithium
conc.	Concentrated
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
DMAP	4-(Dimethylamino)pyridine
DMF	*N*, *N*-dimethylformamide
EDCl	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
eq.	equivalent
EtOAc	Ethylacetate
g	gram(s)

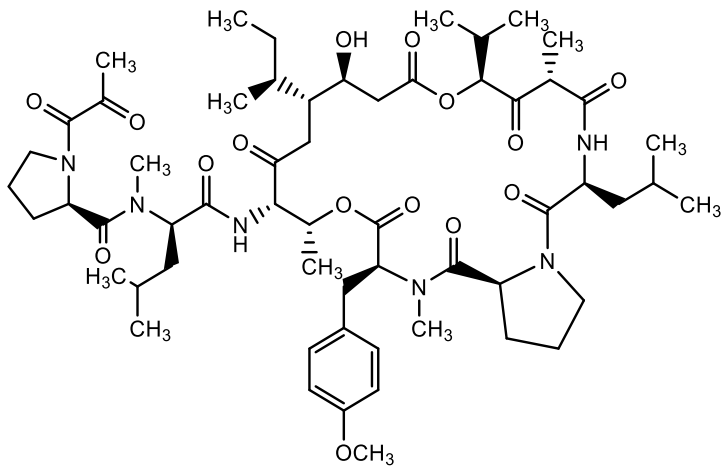
GC-MS	Gas chromatography Mass spectrometry
Hz	hertz
J	coupling constant(s)
LDA	Lithium diisopropylamide
m	multiplet
Me	methyl
MeCN	Acetonitrile
MeOH	Methanol
min	minute(s)
mg	milligram(s)
mL	milliliter(s)
mmol	millimole(s)
N	normality
NMR	nuclear magnetic resonance
Phe	phenylalanine
ppm	parts per million
quant.	quantitative
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
UV	Ultraviolet spectrum

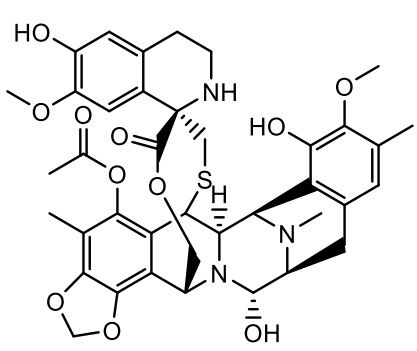
1. Introduction

1.1 Introduction of depsipeptide

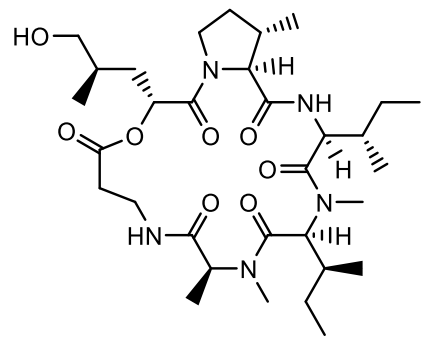
Depsipeptides are polypeptides having sequences of amino and hydroxyl carboxylic acid residues. Depsipeptides are usually in cyclic form and well known as their promising stance in biological activities, which include antibacterial, antiviral, antifungal, anticancer, anti-imflammatory, and anti-clotting or antiantherogenic properties. Many depsipeptides have their potent in clinical area, and several of them are on their clinical trials to evaluate their potentials as pharmacotherapeutic agents.[1,2] Some cyclic depsipeptides are shown in **Figure 1**. Plitidepsin, isolated from Mediterranean tunicate, is one of cyclic depsipeptides that has anticancer activity. It is currently undergoing clinical trial testing, naming as Aplidine.[3] Trabectedin is another commercialized depsipeptide with antitumor activity. It is approved by Europe and USA for clinical use in ovarian caner and soft tissue sarcomas.[4] Trichomide A is natural cyclodepsipeptide, showing effective immunosuppressive activity on activated T-cell and proinflammatory cytokines.[5] Another natural depsipeptide is Kahalalide F and it demonstrated strong cytotoxic activity against human breast cancer cells.[6] Ramoplanin A_2 is also one of depsipeptide that shows effective clinical activity.



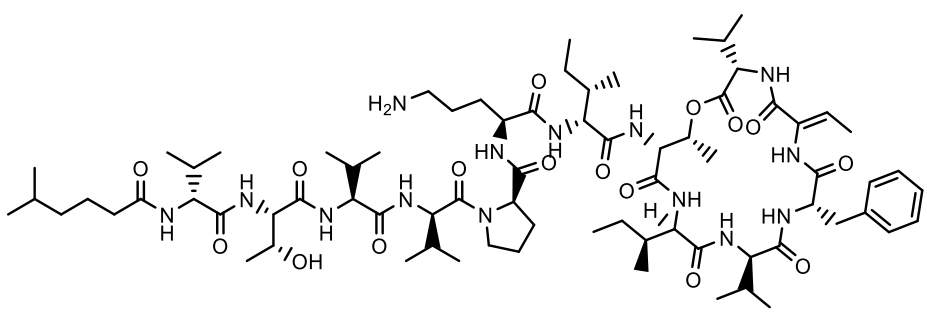
Plitidepsin



Trabectedin



Trichomide A



Kahalalide F

Figure 1. Structure of depsipeptides

1.2 Introduction of Ramoplanin A_2

Ramoplanin is known as a glycolipodepsipeptide produced by sp. ATCC 33076, a member of the genus *Actinoplanes*. Ramoplanin was originally isolated by the research laboratory of Gruppo Lepetit S.p.A. in 1984 and known to demonstrate antibacterial activity against aerobic and anaerobic Gram-positive bacteria.[7] In particular, ramoplanin A_2 has clinical effects against vancomycin-resistant strains of gram-positive organisms, such as Staphylococcus, Bacillus, and Enterococcus.[8] Ramoplanin's action as an antibacterial agent is considered to be disrupting bacterial cell wall synthesis. Researches have been ongoing on how and where it works, and to date, ramoplanin directly binding to lipid II plays a large role in its antibacterial activity. Ramoplanin A_2 is one of the ramoplanins (A_1, A_2, A_3) distinguished by the acyl group of Asn^1 *N*-terminus shown in **Figure 2**. Among those ramoplanins, ramoplanin A_2 is in clinical trials because of the abundant production quantities compared to A_1 and A_3. Total synthetic route for ramoplanin A_2 was first disclosed in 2001 by Jiang-Boger group.[10] Jiang-Boger group defined two strategically effective macro-cyclization sites. Jiang-Boger group reported synthesis of key subunits of ramoplanin A_2 and compared antimicrobial activities of derivatives. According to the report, derivatives of which macrocyclic lactone being hydrolyzed, showed inactive antimicrobial activity. Also, protecting δ-amino groups of Orn^4 and Orn^{10} with acyl groups showed the same inactive result. By the result, the contribution of lactone site and amines of Orn to clinical effects were clearly proved.

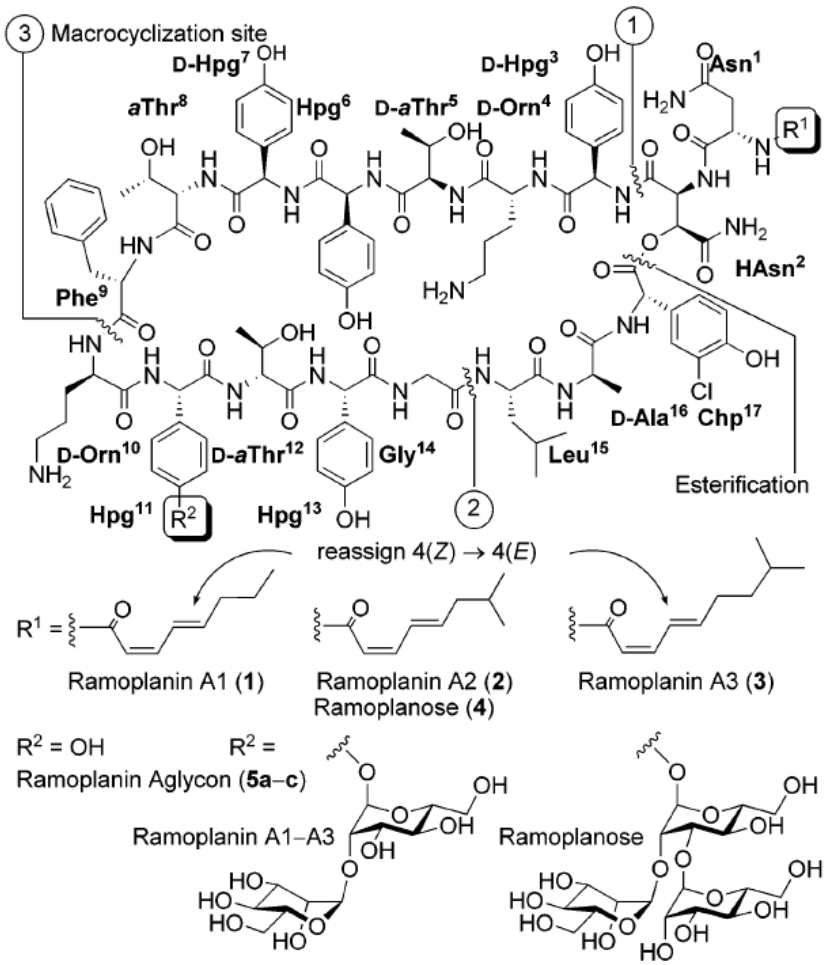


Figure 2. Key disconnection sites of subunits for the ramoplanins [10]

1.3 Introduction of buliding block of Ramoplanin A_2

Ramoplanin A_2 is composed of three key subunits, heptapeptide, pentadepsipeptide, and pentapeptide, sequentially connected through coupling reaction and cyclization, forming 49-membered depsipeptide core. (**Figure 2**.)[10] There are 20 stereoselective sites in ramoplanin A_2 and it is important to form and maintain the desired stereochemistry during the synthesis. In the second subunit, pentadepsipeptide, synthesis of *β*-hydroxyaspartic acid or *β*-hydroxyasparagine is one of the major issue. (**Figure 3**.) Some research reported on synthesizing *β*-hydroxyaspartic acid or *β*-hydroxyasparagine. (**Scheme 1**.)[11]

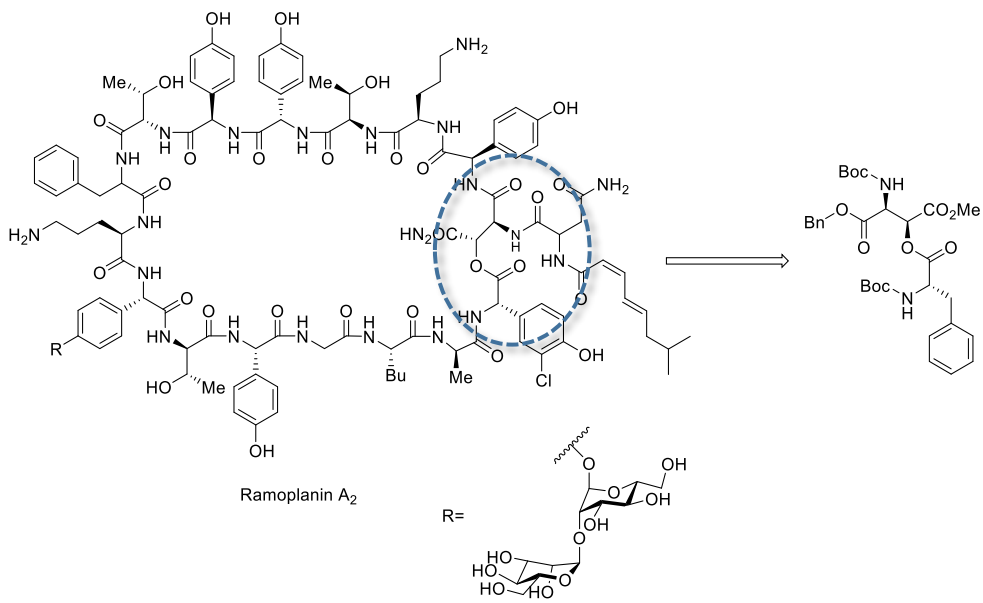
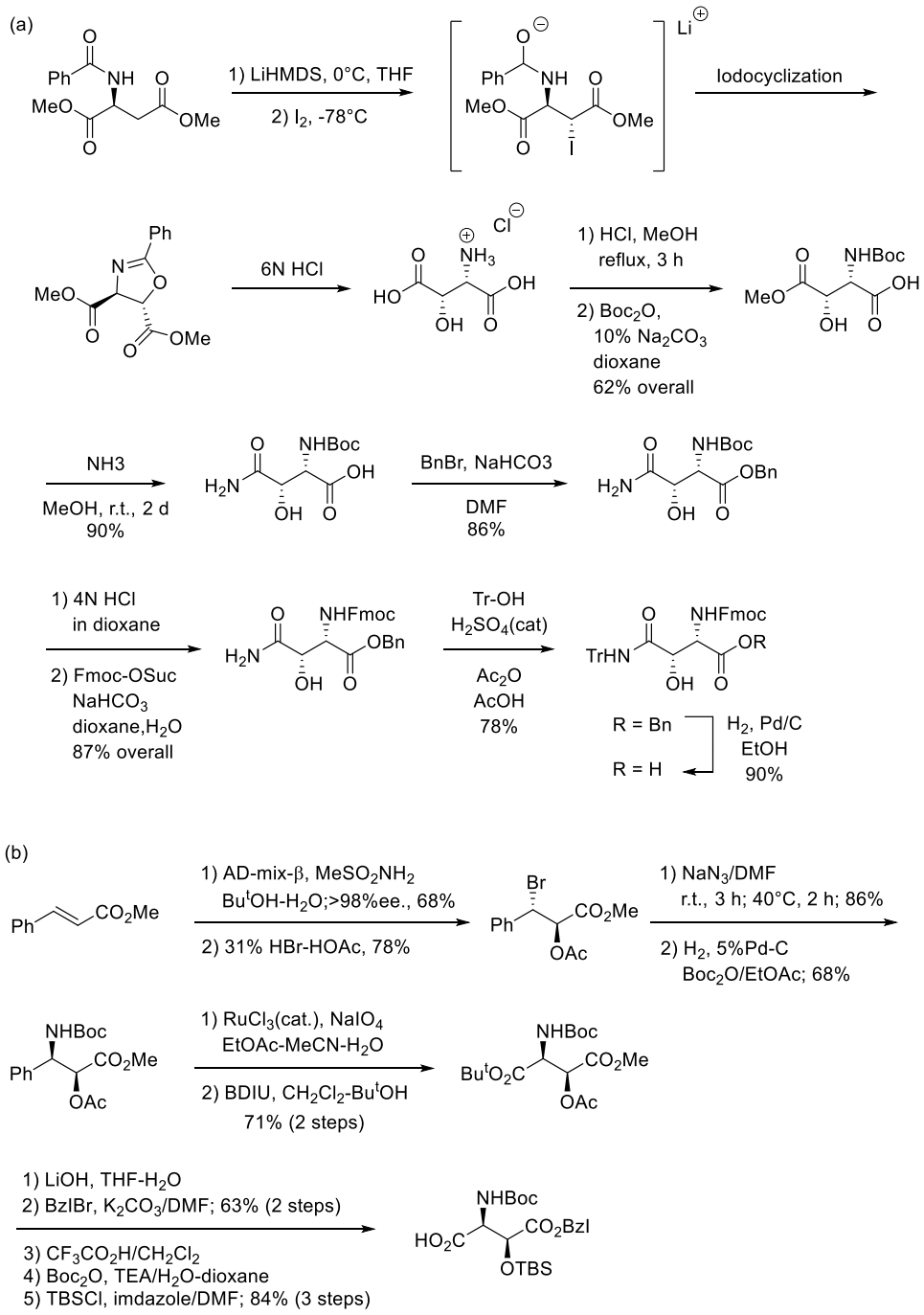
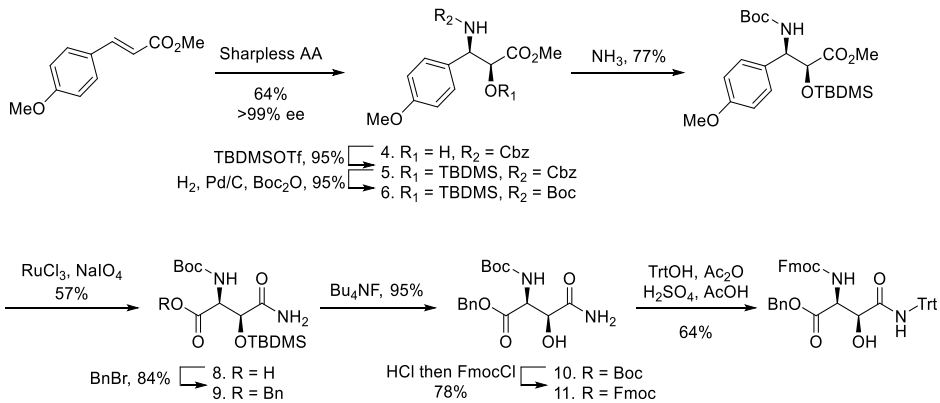


Figure 3. Structure of building block of ramoplanin A_2



Scheme 1. Synthetic routes of L-tHyAsn and L-tHyAsp reported [11]

Jiang-Boger group proposed L-*threo*-*β*-hydroxyasparagine synthesis from methyl 4-methoxycinnamate via the Sharpless asymmetric aminohydroxylation (AA) reaction. (**Scheme 2**.)[12]



Scheme 2. Synthetic route of L-tHyAsn reported by Jiang-Boger group[12]

In **Scheme 1 (a)**, hydrolyzed *trans*-oxazoline intermediate was successfully obtained through iodocyclization. Sharpless hydroxylation was used in **Scheme 1 (b)** and **Scheme 2** to control enantiomer. While synthesizing the desired stereoselective intermediates, some had problems about the high costs of which some catalysts and chiral auxiliaries used in reaction.

1.4 Introduction of *N*-protected-*α*-amino aldehyde

N-protected-α-amino aldehyde is known to have an important role as a chiral auxiliary of building block. For this reason, some researchers have focused on developing stable *N*-protected-*α*-amino aldehydes.[13] Garner aldehyde, Falorni aldehyde, Rapoport aldehyde and Reetz aldehyde are some examples of reported *N*-protected-*α*-amino aldehydes.[14]

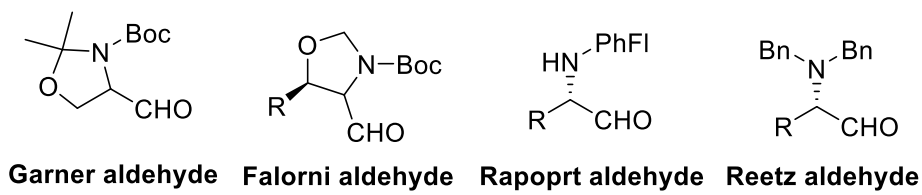
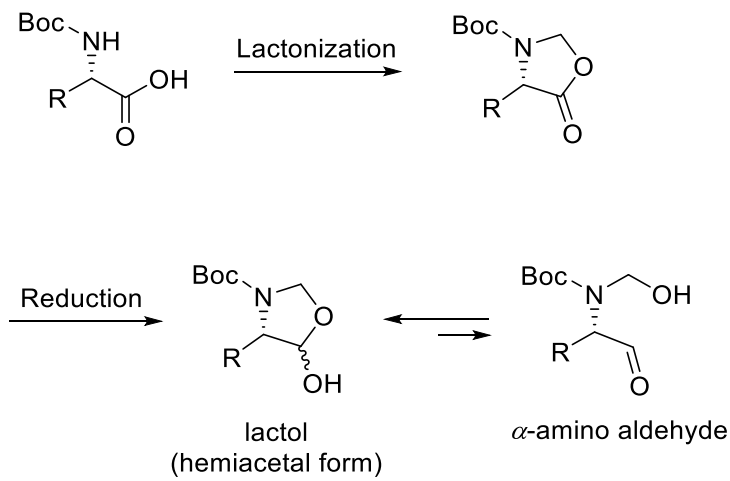


Figure 4. Previously reported *N*-protected-*α*-amino aldehydes[14]

However, each of these *α*-amino aldehydes has limitations. Limited amino acids could be applied to Garner and Falorni aldehyde, of which containing hydroxyl groups on their side chain. The high cost of protecting reagent for *N*-terminal such as PhFl was the limitation of Rapoport aldehyde. Reetz aldehyde showed some problems like incompatibility with hydrogenolysis. To deal with these limitations, saturated hemiacetal structure, synthesized by attaching *N*-hydroxymethyl group to *α*-amino aldehyde, was suggested.[15] Synthesis of *N*-protected-*α*-amino aldehydes could start from protecting *N*-terminal of commercially available amino acid. After the protection of amino acid, lactonization using paraformaldehyde and acidic catalyst, camphor-10-sulfonic acid (CSA) forms *N,O*-acetal oxazolidine ring. Then, following reduction of lactone using DIBAL-H gave *N*-protected-*α*-amino aldehyde.

The stability of this α-amino aldehyde is achieved from the equilibrium state with lactol, the hemiacetal form.

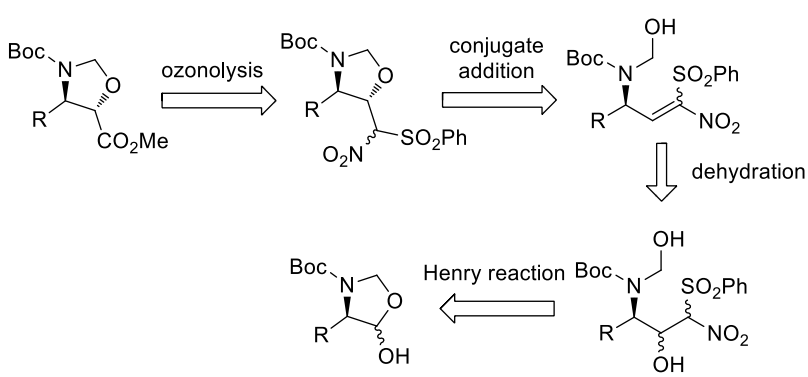


Scheme 3. Preparation of the stable α-amino aldehyde [15]

1.5 Stereoselective synthetic method toward *trans*-oxazolidine carboxylate

The structure of α-hydroxy-β-amino acids have captured the interest of researchers due to their abundance in natural compounds that are biologically active. Since it comes from biological system, we must be aware of the absolute and relative configuration of asymmetric carbon of the compound. Many synthetic methods for this structure have been reported over time. However, some methods have their limitations to solve, like sensitivity to reaction conditions, high cost of catalyst and reagents. So alternative methods for preparing α-hydroxy-β-amino acid structure are still in need.

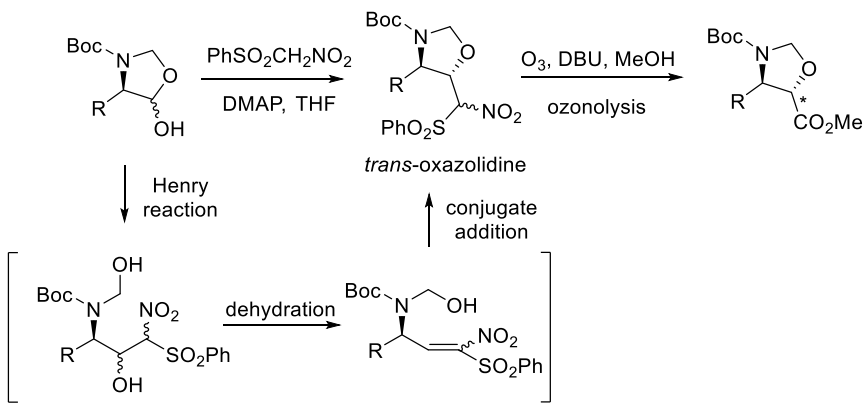
Having the insight from these limitations, our group demonstrated a new synthetic route of forming enantiomerically pure α-hydroxy-β-amino acids via *trans*-oxazolidine, without using any expensive catalyst or chiral auxiliaries.[15] Retrosynthetic strategy to *trans*-oxazolidine from lactol in shown below.



Scheme 4. Retrosynthetic strategy to the *trans*-oxazolidine methylester[15]

Phenylsulfonyl nitromethane was first to be added to the *N*-protected-*α*-amino aldehyde to introduce a new stereocenter to oxazolidine ring. Among other bases, DMAP was chosen for this reaction because it showed highest yield. No reaction was observed with other bases such as BuLi, KHMDS and carbonate base, only to recover the starting materials after the reaction. The reaction using amine based base such as TEA, DBU and *N*-methylmorpholine, was carried out in low yield as a mixture of diastereomers.

Once nitro-olefin is added to *N*-protected-*α*-amino aldehyde, three reactions are proceeded sequentially, henry reaction, the dehydration, and intramolecular conjugate addition. After the conjugate addition, ozonolysis is the next step. Reactions of *α*-amino aldehyde with nitro-olefin are described in **Scheme 5**.



Scheme 5. Reaction mechanism for *trans*-oxazolidine formation

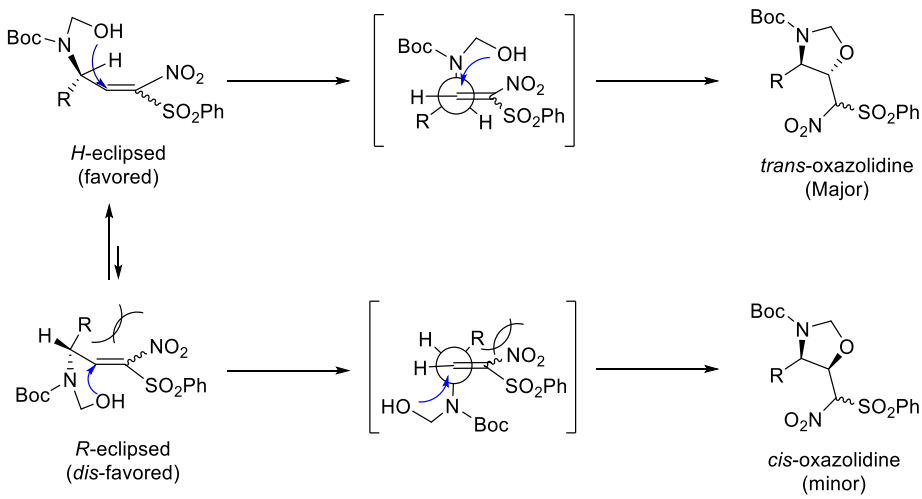


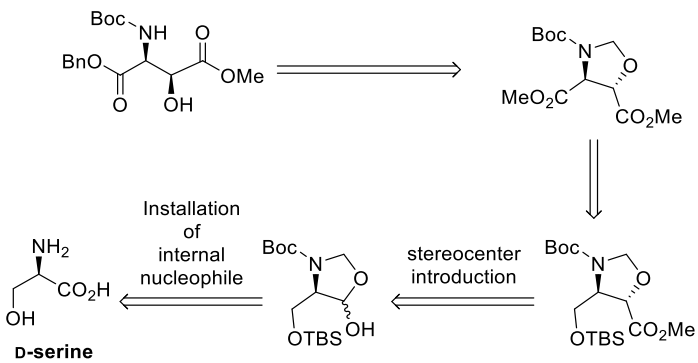
Figure 5. Possible transition state to enhance *trans*-selectivity

The reason for the achievement of high *trans*-stereoselectivity was found in the intramolecular conjugate addition. In this step, there are two possible transition state shown in **Figure 5**. According to the structures shown above, *H*-eclipsed conformation has less steric hindrance compared to *R*-eclipsed one during the transition state. Due to the steric effect, the former conformation, which gives *trans*-oxazolidine structure, is preferred.

2. Results and Discussion

2.1 Retrosynthesis of building block of Ramoplanin A_2

Retrosynthetic strategy for building block of Ramoplanin A_2 is given in **Scheme 6**. In this retrosynthetic strategy, D-serine was chosen to have proper side chain, hydroxymethyl group. The key intermediates are stable *α*-amino aldehyde and trans-oxazolidine, for which they could give the desired *syn* conformation of amino and hydroxyl group of target compound.



Scheme 6. Retrosynthetic strategy for building block of Ramoplanin A_2

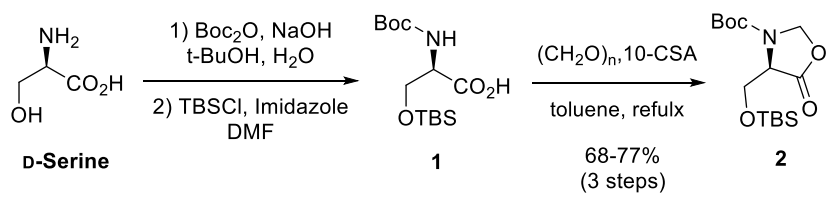
In the course of this scheme, some functional groups required protection reaction, such as Boc group or TBS group to prevent undesired product. Then, deprotection was proceeded throughout the reaction to give target compound.

2.2 Formation of D-serinal

Boc protection was performed to protect amine functional group of D-serine with Boc anhydride (Boc_2O) under basic condition. Almost pure product was obtained after removing Boc_2O through Hexane washing and acidic aqueous phase (pH 1-2)/EtOAc extraction.

Due to sensitive activity of hydroxyl group in the starting material, TBSCl was treated for the protection of this functional group. The protection was carried out in DMF solvent system with imidazole under N_2 atmosphere.

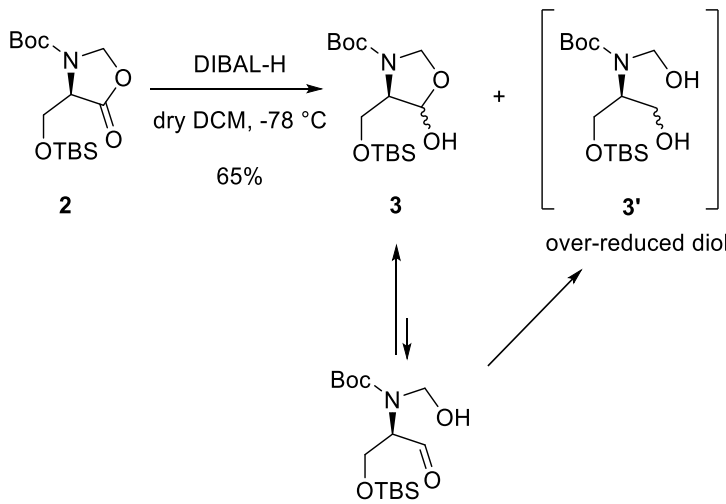
After the protections of D-serine, addition of paraformaldehyde and camphor-10-sulfonic acid (CSA) under reflux condition lead to lactonization. Through purification with silica gel column chromatography, compound **2** was obtained around 68 - 77% yield in 3 steps.



Scheme 7. Preparation of *N*-Boc-*O*-TBS-protected oxazolidinone **2**

Synthesis of compound **3** was carried out with DIBAL-H in anhydride DCM, the reduction form of compound **2**, hemi-acetal conformation of *N*-hydroxymethyl-*α*-amino aldehyde. The key point of this reaction was rate and amount of DIBAL-H added. Over reduction formation diol **3'**, the undesired product, could be carried out if the second reduction proceed on compound **3**. The ratio of compound **3'** to

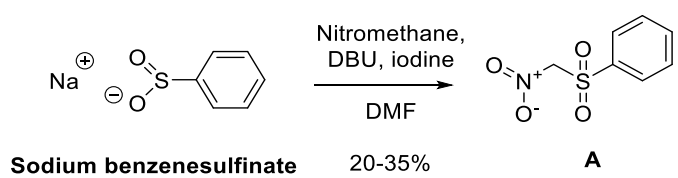
compound **3** increases as the addition rate of DIBAL-H increases. To prevent the over reduction, the reaction was carried out under -78℃ to control the reaction rate and DIBAL-H was added in droplet using glass syringe to treat reactive anhydrous DIBAL-H. After the reduction, separation of compound **2** and **3'** from compound **3** was possible using silica gel chromatography. As a result, the yield of compound **3** was up to 65% yield. Due to the possibility of racemization, the next process should be conducted right away, or it should be kept at the deep-freezer (-70℃).



Scheme 8. Preparation of D-serinal **3**

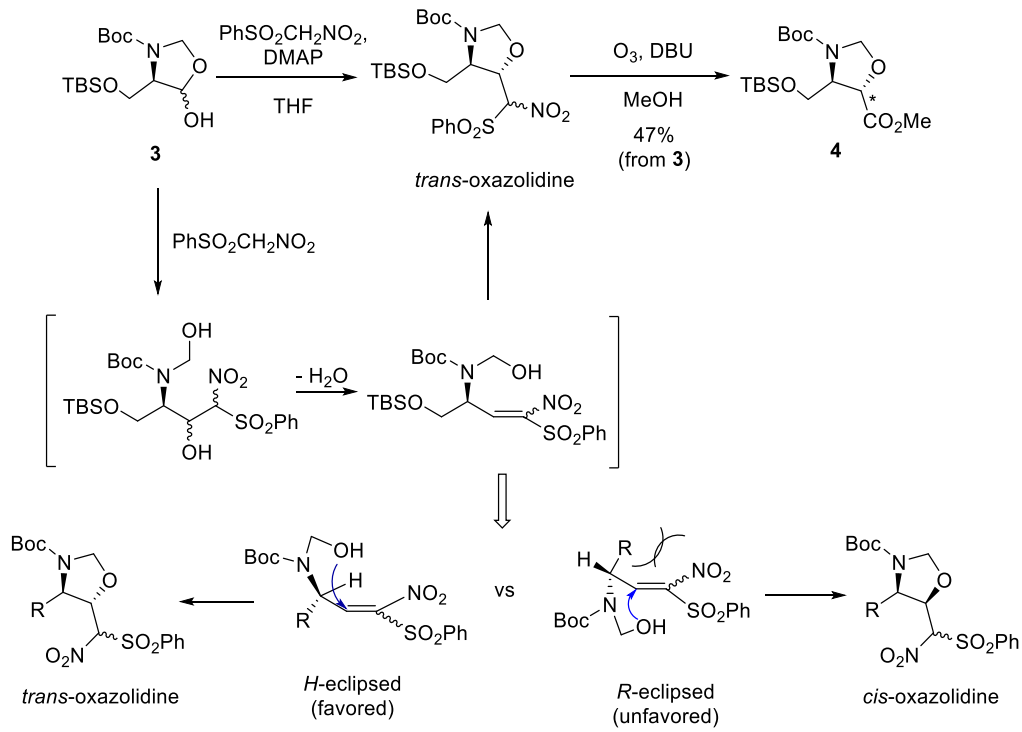
2.3 Formation of stereoselective *trans*-oxazolidine

Synthesis of *trans*-oxazolidine is the important process in enhancing stereoselectivity of L-*threo*-*β*-hydroxy aspartate backbone. For that, efficient nitro-olefin adduct should be added to the starting material. Phenylsulfonyl nitromethane, compound **A**, having active acidic proton due to two electron withdrawing groups, was suitable for the nitro-aldol condensation. However, the cost of this reagent was too high, we synthesized the reagent using the reported procedure with sodium benzenesulfinate and nitromethane. Following the reaction scheme shown in **Scheme 9**, compound **A** was obtained in 20-35% yield with purification using silica gel column chromatography.



Scheme 9. Preparation of phenylsulfonyl nitromethane **A**

The overall mechanism is shown in **Scheme 10**. Dehydration and intramolecular conjugate addition reaction were followed after the addition of compound **A** to D-serinal **3**. During the intramolecular conjugate addition reaction, there are two possible transition state. The enhanced stereoselectivity is due to the favored transition state formation, *H*-eclipsed conformation. In *R*-eclipsed conformation, steric hindrance with nitro-olefin caused the less preferred transition state.

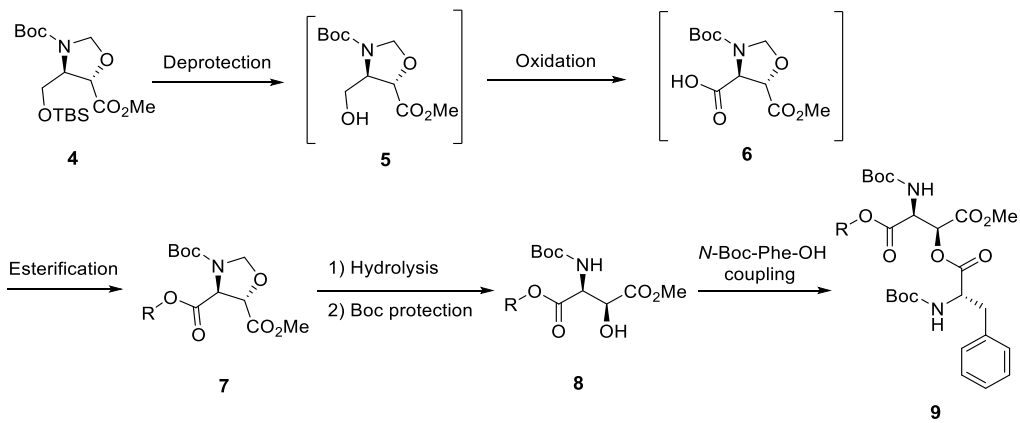


Scheme 10. Preparation of *trans*-oxazolidine **4**

Compound **A** and DMAP were added to the starting material **3** in THF. The starting material exists mostly in lactol form at equilibrium state, more stable conformation compared to the α-amino aldehyde. Since the desired form of the following reaction, Henry reaction, is the α-amino aldehyde form, the reaction rate is very slow compared to the other reactions in this scheme, dehydration and conjugate addition reaction. For this reason, the reaction was conducted for 3 days. Then, solution was diluted with MeOH and DBU at 0℃, followed by ozonolysis under -78℃ using ozonizer for 15-20 minutes. Pure compound **4** was obtained up to 47% yield with purification using extraction and column chromatography.

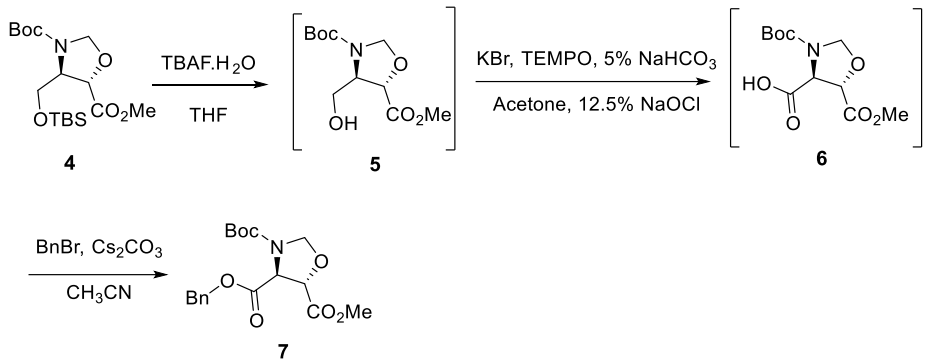
2.4 Formation of building block for Ramoplanin A_2

Synthesis of building block for Ramoplanin A_2 is shown in **Scheme 11**. Following this scheme, deprotection reaction of TBS group was carried out from compound **4** in THF with addition of TBAF·H_2O, obtaining compound **5**. Without purification, oxidation reaction of compound **5** was carried out using the oxidation reagent, TEMPO. Addition of KBr and TEMPO to compound **5** in acetone and 5% $NaHCO_3$ at 0℃ is the first step to oxidation. Then 12.5% NaOCl addition followed. The reaction needed to be kept at 0℃, since the heat from adding NaOCl could make methyl ester group unstable.



Scheme 11. Possible synthetic route for final product **9**

Then we used BnBr to add benzyl group in esterification process. BnBr and Cs_2Co_3 was used for esterification, producing compound **7** in 40% yield from compound **4** with purification using MeCN washing and silica gel column chromatography. (**Scheme 12**)

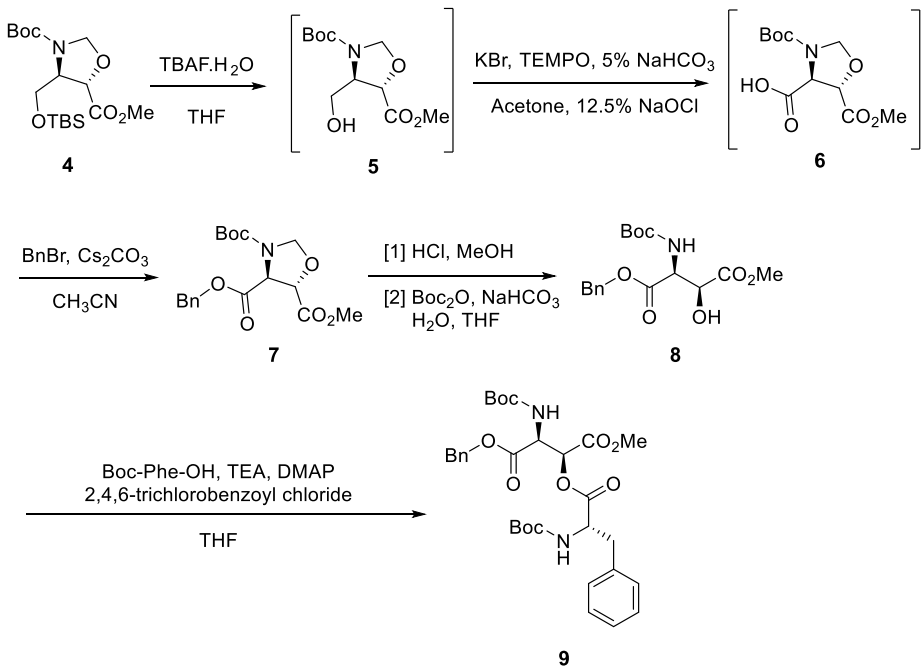


Scheme 12. Preparation of compound **7**

To get the backbone structure of building block, linear structure was required. For that, ring opening with hydrolysis with 1 N HCl in Et_2O reagent was carried out. Compound **7** was dissolved in MeOH and after the addition of 1 N HCl, the reaction was conducted under reflux condition. Because the Boc protection group is deprotected under acidic condition, protection free amine group with Boc_2O was the next step of hydrolysis. Pure compound **8** was obtained up to 81% yield in 2 steps with following purification, EtOAc washing and column chromatography.

Coupling *N*-Boc-phenylalanine to the compound 8 was the final step for the process. 2,4,6-trichlorobenzoyl chloride was added to *N*-Boc-phenylalanine in THF with TEA and stirred for 10 minutes. During this process, TEA activates the carboxylic group of *N*-Boc-phenylalanine. This activated carboxylate is added to the carbonyl part of 2,4,6-trichlorobenzoyl chloride, forming anhydride structure. Then, compound **8** and DMAP is added. Reaction between this anhydride structure and DMAP enhances the electrophilicity of reagent, *N*-Boc-phenylalanine. This highly electrophilic agent is attacked by alcohol of compound **8**, forming ester bond. Purification using extraction and silica gel chromatography gave clear target compound **9** in 80% yield.

The total synthesis of final compound **9** from compound **4** is shown in **Scheme 13**.



Scheme 13. Total synthesis of final compound **9** from **4**

3. Conclusion

In this paper, the stereoselective backbone of building block of Ramoplanin A_2, which has L-*threo*-*β*-hydroxy aspartate structure was synthesized through concise synthetic route. We synthesized stereoselective backbone without any chiral auxiliaries or catalysts starting from D-serine, commercially available amino acid. The key step of our synthesis was forming *trans*-oxazolidine structure. Through reaction of *N*-hydroxymethyl-*α*-amino aldehyde and phenylsulfonyl nitromethane, we obtained the key intermediate, *trans*-oxazolidine **4**. In this reaction, the steric hindrance of nitro olefin during intramolecular conjugate addition reaction enhanced the stereoselectivity, making *H*-eclipsed conformation more favorable than *R*-eclipsed conformation. For this reason, *trans*-oxazolidine was preferred over *cis*-oxazolidine in the ratio of about 20:1. [15]

After synthesizing trans-oxazolidine structure, esterification and benzylation was conducted. Especially, *N*-phenylalanine coupling reaction with hydroxyl group showed possibility of synthesis for depsipeptides. Having L-*threo*-*β*-hydroxy asparagine as a backbone, coupling reaction with proper *N*-protected amino acids can give desired depsipeptide as a building block. If further reactions are carried out, Ramoplanin A_2 can be synthesized through coupling and cyclization of building blocks. In conclusion, synthesizing the building block of Ramoplanin has shown potential of total synthesis of depsipeptides having L-*threo*-*β*-hydroxy group as a backbone.

4. Experimental details

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. 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 ^{I3}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 $CDCl_3$.

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 = 8:1 to 2:1 (v:v) to give a pure compound **R** (6.494 g, 20%) as a white powder. R_f = 0.45 (HEX:EtOAc = 2:1)

A: ^{1}H NMR δ 7.99-7.97 (m, 2H), 7.82-7.80 (m, 1H), 7.68-7.64 (m, 2H), 5.60 (s, 2H); ^{13}C NMR δ 135.94, 135.67, 129.79, 129.45, 90.26

Specific procedure for building block of Ramoplanin

(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), and 1.06 eq. of NaOH (605 mg, 15.1 mmol) were added with stirring at the ice-bath. After 20 min at 0 °C, the mixture was warmed up to room temperature and 1.03 eq. of di-*tert*-butyl dicarbonate (3.38 g, 14.7 mmol) was added, then stirred for 8 h. The mixture was extracted with n-hexane to remove unreacted di-*tert*-butyl dicarbonate and concentrated with vacuum evaporation to remove *t*-BuOH. The aqueous phase of concentrated mixture was acidified to pH 1-2 with 2 N HCl and 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 (3.23 g, 21.4 mmol) and 3 eq. of imidazole (2.91 g, 42.8 mmol) were added with stirring under nitrogen atmosphere. After 30 h at 0 °C, the mixture was warmed to room temperature and stirred for 24 h. The reaction mixture was extracted with Et2O (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 oil. R_f = 0.6 (DCM:MeOH = 10:1) To a solution of **1** (crude) in toluene (40 mL), 10 eq. of paraformaldehyde (4.3 g, 143 mmol) and 0.03 eq. of ((1*S*)-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** (3.41 g, 72% (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 5.53-5.41 (br d, 1H), 5.18 (d, 1H, *J*=3.6) , 4.23-4.02 (br m, 3H), 1.49 (s, 9H), 0.87 (s, 9H), 0.04 (s, 6H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.85, 151.46, 81.82, 78.92, 62.17, 61.40, 57.99, 57.52, 28.29, 25.64, 18. 02, -5.65

(4*R*)-*tert*-butyl-4-(((*tert*-butyldimethylsilyl)oxy)methyl)-5-hydroxyoxazolidine-3-carboxylate, D-serinal (3); To a stirred solution of **2** (2.65 g, 7.98 mmol) in dry DCM (80 mL. 0.1 M) at -78 °C, 1.0 M solution of DIBAL-H in DCM (1.5 eq., 12.0 mL, 11.96 mmol)was added dropwise under a nitrogen atmosphere. The reaction was quenched slowly by adding cold 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 4:1 (v:v) to give a diastereomeric mixture of **3** (1.30 g, 49%) as a white solid. R_f = 0.5 (HEX:EtOA c= 2:1)

3: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.59 (d, 1H, *J*=3.6), 5.09 (s, 1H), 4.90 (s, 1H), 3.80 (s, 2H), 3.47 (s, 1H), 2.65 (d, 1H, *J*=2.8), 1.47 (s, 9H), 0.89 (s, 9H), 0.06 (d, 6H, *J*=5.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.52, 99.05, 80.54, 78.29, 63.41, 61.3

0, 28.43, 25.80, 18.17, -5.37

(4*R*,5S)-3-*tert*-butyl 5-methyl 4-(((*tert*-butyldimethylsilyl)oxy)methyl) oxazolidine-3,5-dicarboxylate (4); To a-amino aldehyde **3** (0.86 g, 2.59 mmol) in THF (1.3 mL) 1.3 eq. of **A** (678 mg, 3.37 mmol) and 1.3 eq. of DMAP (412 mg, 3.37 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 (1.09 g, 7.77 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 × 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 2:1 (v:v) to give a single stereoisomer **4** (0.45 g, 47%) as a colorless oil. R_f = 0.62 (HEX:EtOAc =2:1)

4: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.19-5.17 (br s, 1H), 4.79 (br s, 1H), 4.73 (d, 1H, *J*=3.6), 4.10-4.00 (br s, 1H), 3.79 (br m, 5H), 1.46 (s, 9H), 0.89 (s, 9 H), 0.07 (d, 6H, *J*=4.4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.33, 143.18 80.53, 77.33, 63.41, 60.34, 50.88, 28.42, 25.80, 18.17, -5.37

(4S,5S)-4-benzyl 3-(*tert*-butyl) 5-methyl oxazolidine-3,4,5-tricarboxylate (7); To a solution of **4** (435 mg, 1.16 mmol) in THF (1.2 mL), 1.1 eq. of TBAF·H_2O (333 mg, 1.27 mmol) was added. The reaction mixture was stirred at room temperature until the starting material disappeared from TLC. The reaction mixture was partitioned between EtOAc (30mL) and an aqueous saturated solution of NH_4Cl (30mL). The aqueous layer was extracted with EtOAc (30 mL × 2), and the combined organic layers were dried over $MgSO_4$ filtered and concentrated under reduced pressure. An aqueous solution of 5 wt% $NaHCO_3$ (5 mL) and acetone (5 mL) was directly added to the stirring reaction mixture of **5** at 0 ℃. After then 0.25 eq. of KBr (34.5 mg, 0.29 mmol), 1.5 eq. of TEMPO (271 mg, 1.74 mmol) and an aqueous solution of 7.7 eq. of 12.5% NaOCl (5.3 mL, 8.92 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 and aqueous solution of 2N 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 MeCN (4 mL) at room temperature. 1.5 eq. of BnBr (0.2 mL, 1.74 mmol) and 1.5 eq. of Cs_2CO_3 (566 mg, 1.74 mmol) were added to solution. The resulting mixture was stirred for 8 h at room temperature. The reaction was quenched slowly by adding $NaHCO_3$ (4 ml). The reaction mixture was extracted with EtOAc (20 mL × 2) after condensation. The organic layer were dried with $MgSO_4$ filtered and concentrated under reduced pressure. Purification was performed with silica gel column chromatography with gradient elution, HEX:EtOAc = 8:1 to 4:1 (v:v) to give **7** (168 mg, 40% (from 4)) as a colorless oil.

R_f = 0.5 (HEX:EtOAc=2:1)

7: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 5H), 5.20 (br s, 1H), 5.09-4.99 (d, 1H, *J*=40), 4.77-4.59 (br s, 2H), 3.79 (s, 3H), 1.40 (d, 9H, *J*=41.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.75, 99.18, 80.71, 78.36, 63.61, 61.49, 28.57, 25.94, 18.30, -5.24

(2S,3S)-1-benzyl 4-methyl 2-((*tert*-butoxycarbonyl)amino)-3-hydroxysuccinate (8); To a solution of 7 (168 mg, 0.46 mmol) in MeOH (6 mL), 1.02 eq. of 1N HCl of diethyl ether solution (0.5 mL, 0.47 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 (1.5 ml). 1.02 eq. of Boc_2O (0.11 mL, 0.47 mmol), 1.05 eq. of $NaHCO_3$ (41 mg, 0.48 mmol) and H_2O (1.5 mL) was added to the diluted solution and stirred at room temperature for 8 h. After then, the mixture was partitioned between brine (20 ml) and EtOAc (20 mL × 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 HEX:EtOAc = 8:1 to 4:1 (v:v) to give 8 (80 mg, 49%) as a colorless oil. Rf = 0.2 (HEX:EtOAc = 2:1)

8: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 5H), 5.25 (br s, 1H), 5.24 (s, 2H), 4.83-4.71 (br m, 3H), 3.81 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.79, 128.63 125.51, 77.01, 67.97, 34.23, 28.15, 25.61, 21.18

(2S,3S)-1-benzyl 4-methyl 3-(((tert-butoxycarbonyl)-L-phenylalanyl)oxy)-2-((tert-butoxycarbonyl)amino)succinate (9); To 1.0 eq. of Boc-L-phenylalanine (25 mg, 0.094 mmol) in THF (3.5 ml), 1.05 eq. of 2,4,6-trichlorobenzoyl chloride (0.015 ml, 0.099 mmol) and 2.0eq. of TEA (0.03 ml, 0.20 mmol) was added and stirred for 10 minutes. Then a solution of 8 (1.05 eq., 35 mg, 0.10 mmol) and 1.0 eq. of DMAP (12 mg, 0.094 mmol) was added and stirred for 3 h at room temperature. The reaction was quenched slowly by adding $NaHCO_3$ (4 ml). The mixture was extracted with Et_2O (20 mL × 2). The organic layer was dried with $MgSO_4$ filtered and concentrated under reduced pressure. Purification was performed with silica gel column chromatography with gradient elution, HEX:EtOAc = 4:1 to 2:1 (v:v) to give 9 (56 mg, 80%) as a colorless oil. Rf = 0.5 (HEX:EtOAC = 2:1)

9: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.29 (br m, 7H), 5.57 (br s, 1H), 5.20-5.01 (br m, 4H), 4.88 (br s, 1H), 4.47 (m, 1H), 3.74 (s, 3H), 3.17-3.02 (m, 2H) 1.43 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.85 168.27, 167.99, 155.15, 136.15, 134.85, 128.68, 80.62, 77.04, 72.89, 69.51, 60.36, 54.02, 53.84, 52.92, 37.90, 28.27, 14.20

References

1. Suarez-Jimenez G-M.; Burgos-Hernandez A.; Ezquerra-Brauer J-M., *Mar. Drugs*, **2012**, *10*, 963

2. Rangel M.; José Correia de Santana C.; Pinheiro A.; dos Anjos L.; Barth T.; Rodrigues Pires Júnior O.; Fontes W.; Castro M. S., *Current Protein and Peptide Science*, **2017**, *18*, 72

3. (a) Broggini M.; Marchini S. V.; Galliera E.; Borsotti P.; Taraboletti G.; Erba E.; Sironi M.; Jimeno J.; Faircloth G. T.; Giavazzi R.; D'Incalci M., *Leukemia*, **2003**, *17*, 52

   (b) Orafaie A.; Bahrami A. R.; Martin M. M., *Nanomedicine*, **2021**, *16*, 415

4. D'Incalci M.; Badri N.; Galmarini C. M.; and Allavena P., *British Journal of Caner*, **2014**, *111*, 646

5. Wang X.; Zhang A.; Gao J.; Chen W.; Wang S.; Wu X.; Shen Y.; Ke Y.; Hua Z.; Tan R.; Sun Y.; Xu Q., *Journal of Investigative Dermatology*, **2014**, *134*, 2737

6. Suárez Y.; González L.; Cuadrado A.; Berciano M.; Lafarga M.; Muñoz A., *Mol. Cancer Ther*., **2003**, *2*, 863

7. (a) Cavalleri B.; Pagani H.; Volpe G.; Selva E.; Parenti F., *J.Antibiot*., **1984**, *37*, 309

(b) Farver D. K.; Hedge D. D.; Lee S. C., *Annals of Pharmacotheraphy*, **2005**, *39*, 863

8. (a) Montecalvo M. J., *Antimicrob. Chem.*, **2003**, *51*, iii31

(b) Collins, L. A.; Eliopoulos, G. M.; Wennersten, C. B.; Ferraro, M. J.; Moellering, R. C., *Antimicrob. Agent Chemother.*, **1993**, *37*, 1364

9. Walker S.; Chen L.; Hu Y.; Rew Y.; Shin D.; Boger D. L., *Chem. Rev.*, **2005**, *105*, 449

10. (a) Jiang, W.; Wanner, J.; Lee, R. J.; Bounaud, P.-Y.; Boger, D. L.; *J. Am. Chem. Soc*., **2003**, *125*, 1877

(b) Boger, D. L., *Med. Res. Rev.*, **2001**, *21*, 356

11. (a) Guzmán-Martínez A.; VanNieuwenhze M. S., *Synlett.*, **2007**, *10*, 1513, Cardillo G.; Gentilucci L.; Tolomelli A.; Tomasini C., *Synlett.*, **1999**, *11*, 1727

(b) Deng J.; Hamada Y.; Shioiri T., *J. Am. Chem. Soc*., **1995**, *117*, 7824

12. Boger D.L.; Lee, R. J.; Bounaud, P.-Y.; Meier P., *J. Org. Chem*., **2000**, *65*, 6770

13. (a) Jurczak J.; Gorlebiowski A., *Chem. Rev.*, **1989**, *89*, 149

(b) Fisher, L. E.; Muchowski J. M., *Org. Prep. Proced. Int.*, **1990**, *22*, 399

14. (a) Garner P.; Park J. M., *J. Org. Chem.*, **1987**, *52*, 2361

(b) Falorni M.; Conti S.; Giacomelli G.; Cossu S.; Soccolini F., *Tetrahedron: Asymmetry*, **1995**, *6*, 287

(c) Lubell W.; Rapoport H., *J. Org. Chem.*, **1989**, *54*, 3824

(d) Reetz M. T., *Angew. Chem. Int. Edit.*, **1991**, *30*, 1531

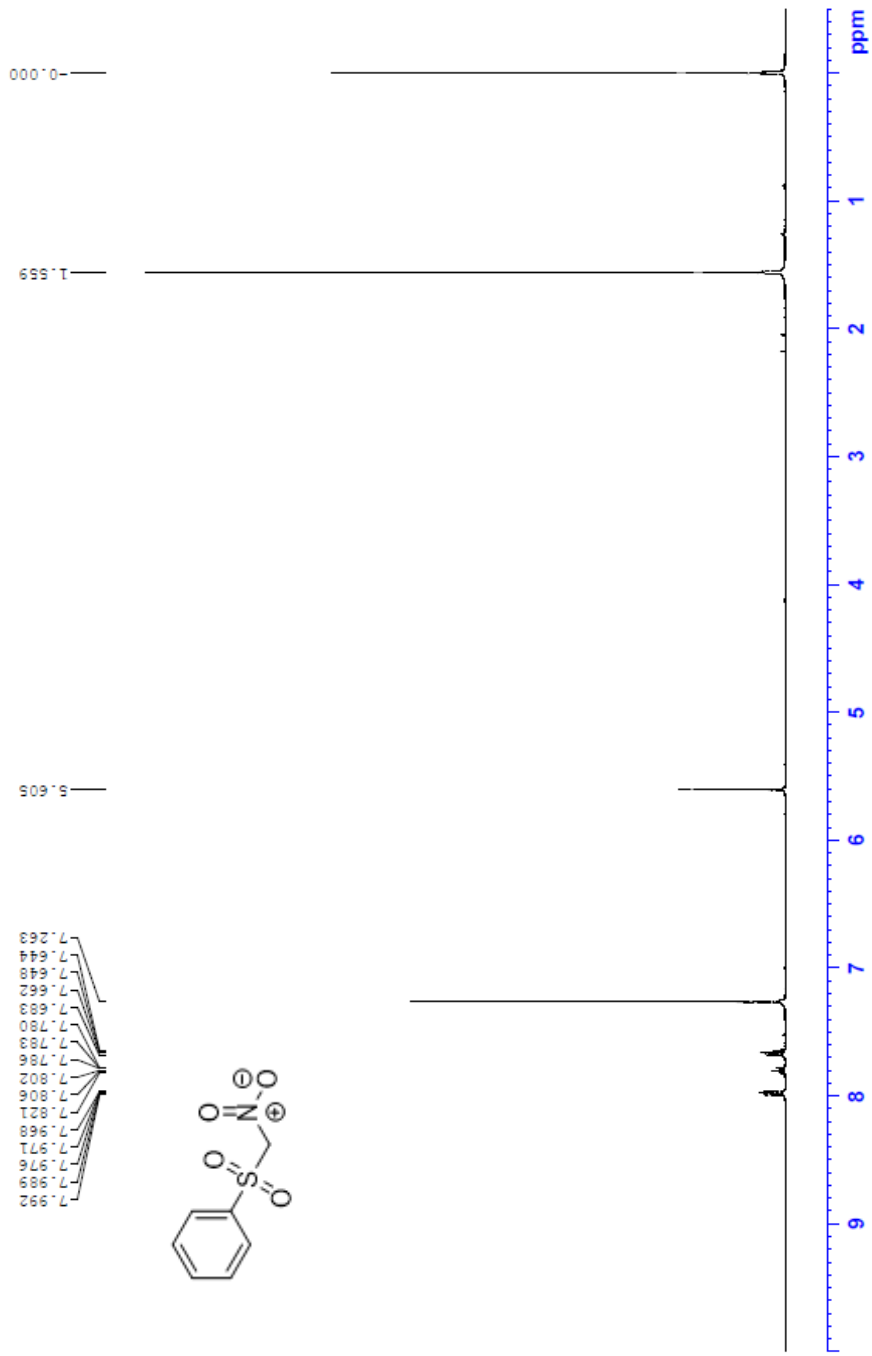
15. (a) Hyun S.; Kim Y., *Tetrahedron Letters*, **1998**, *39*, 4299

(b) Yoo D.; Oh J.; Lee D.; Kim Y., *J. Org. Chem.*, **2003**, *68*, 2979

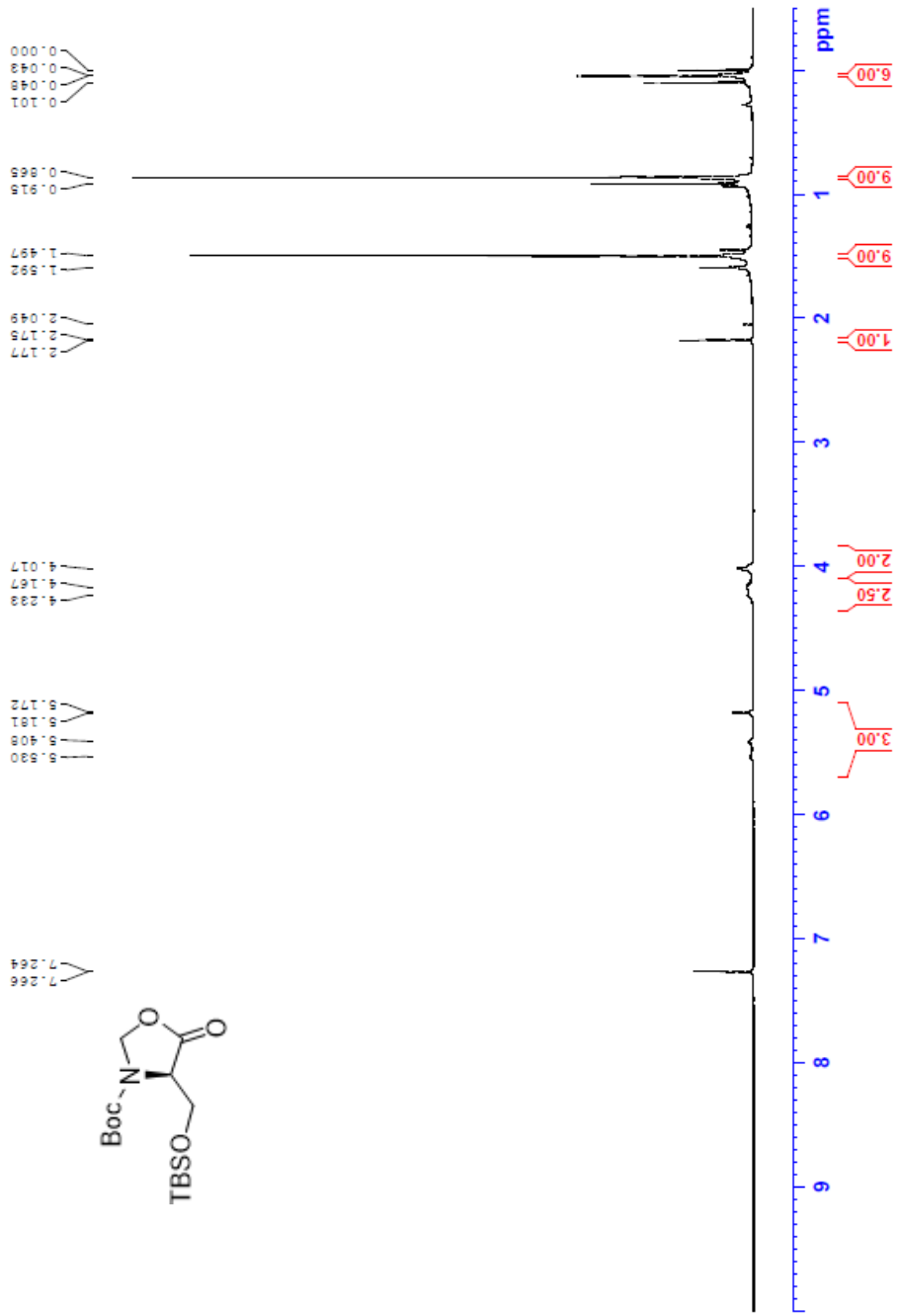
(c) Seo Y.; Kim H.; Chae D.; Kim Y., *Tetrahedron: Asymmetry*, **2014**, *25*, 625

APPENDICES

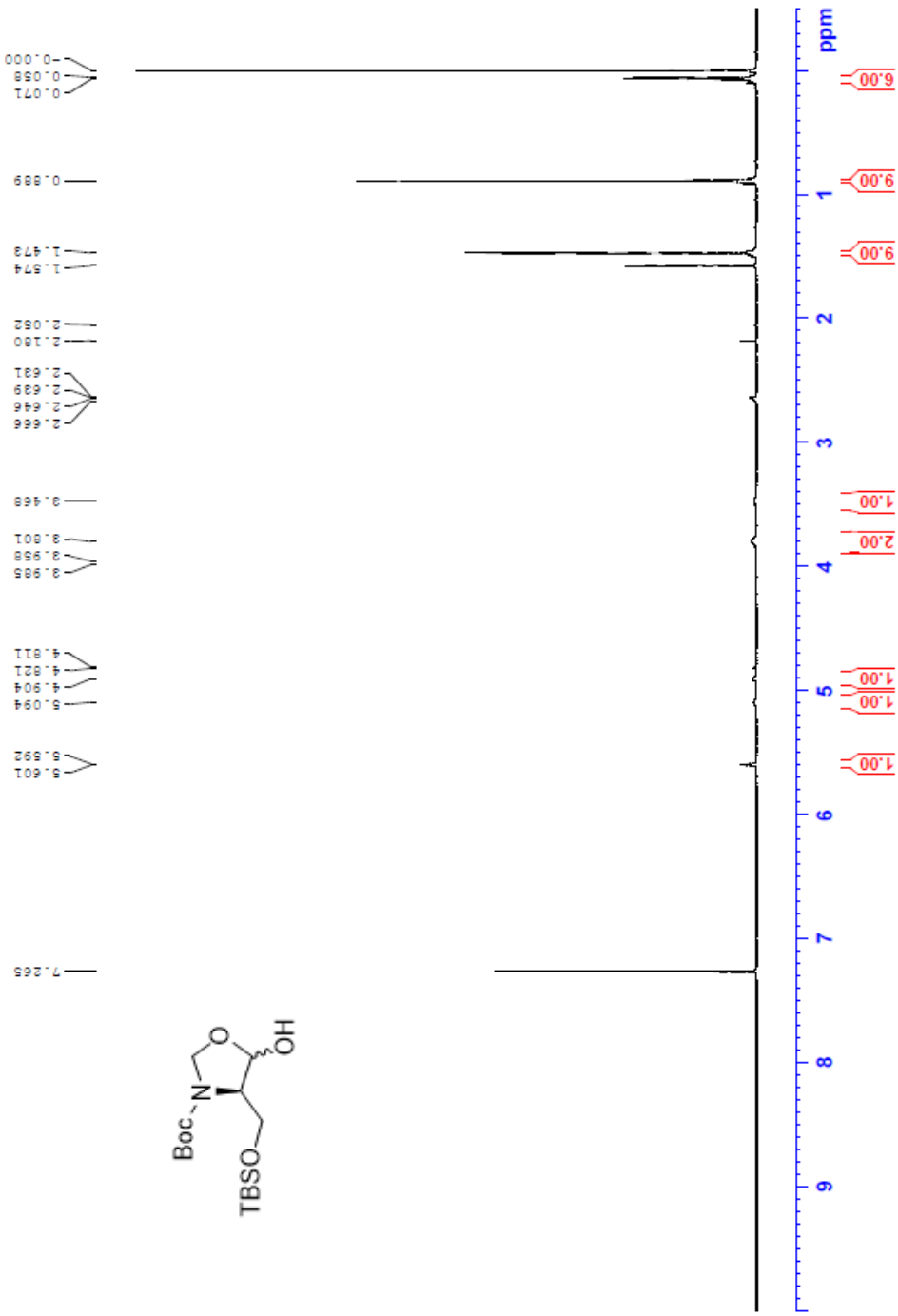
List of ^{1}H NMR Spectra of Selected Compounds



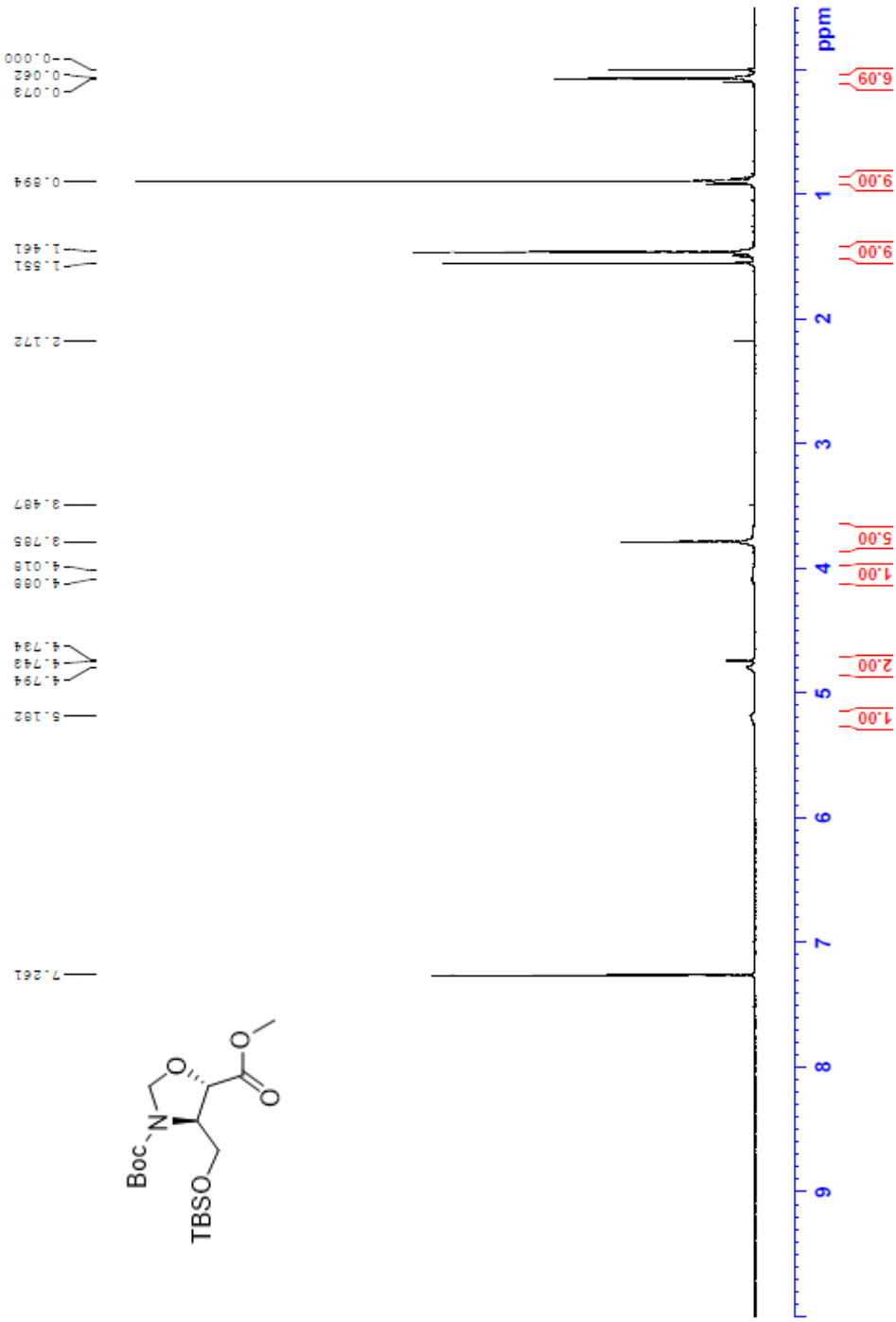
400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **A**



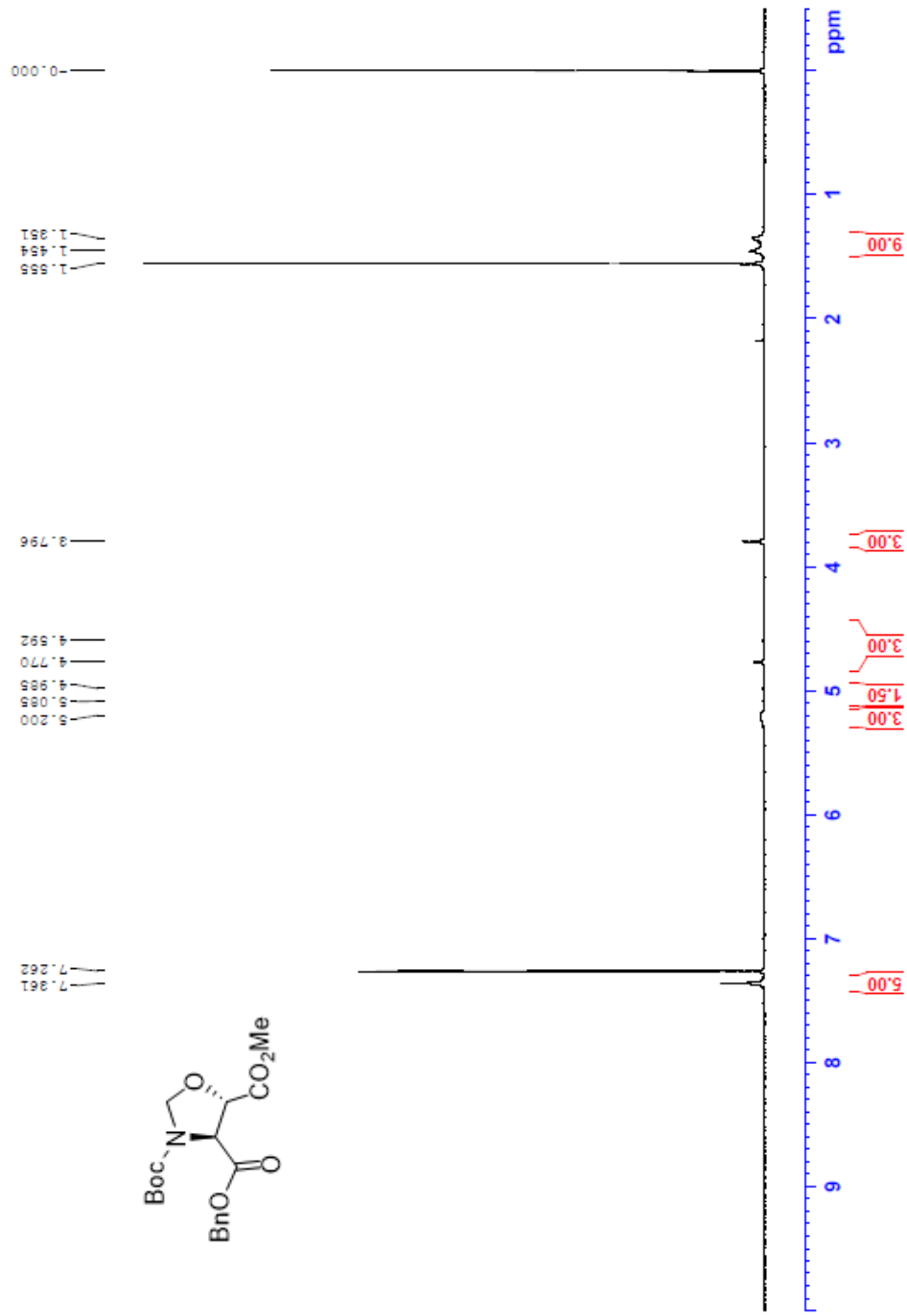
400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **2**



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



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



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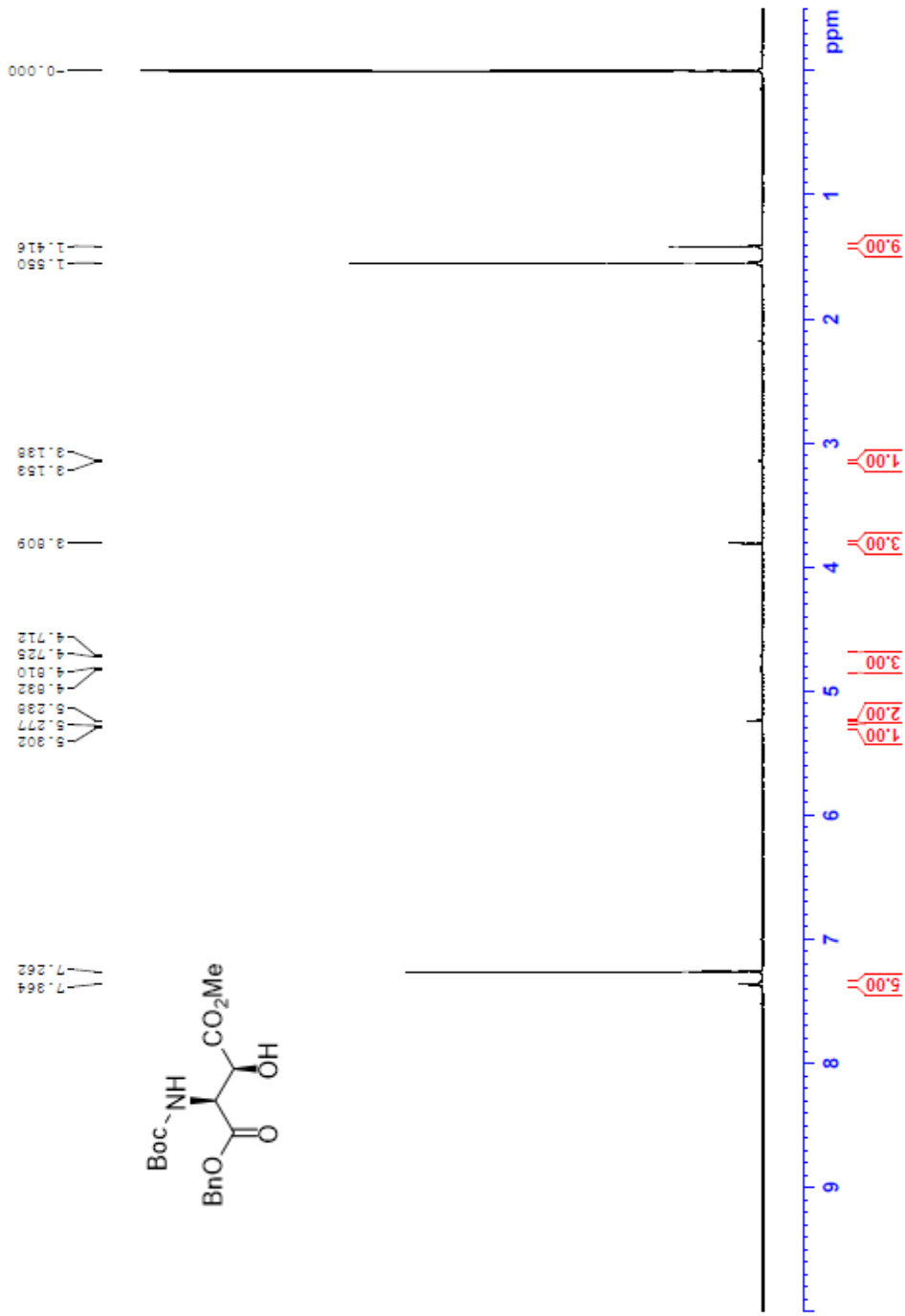

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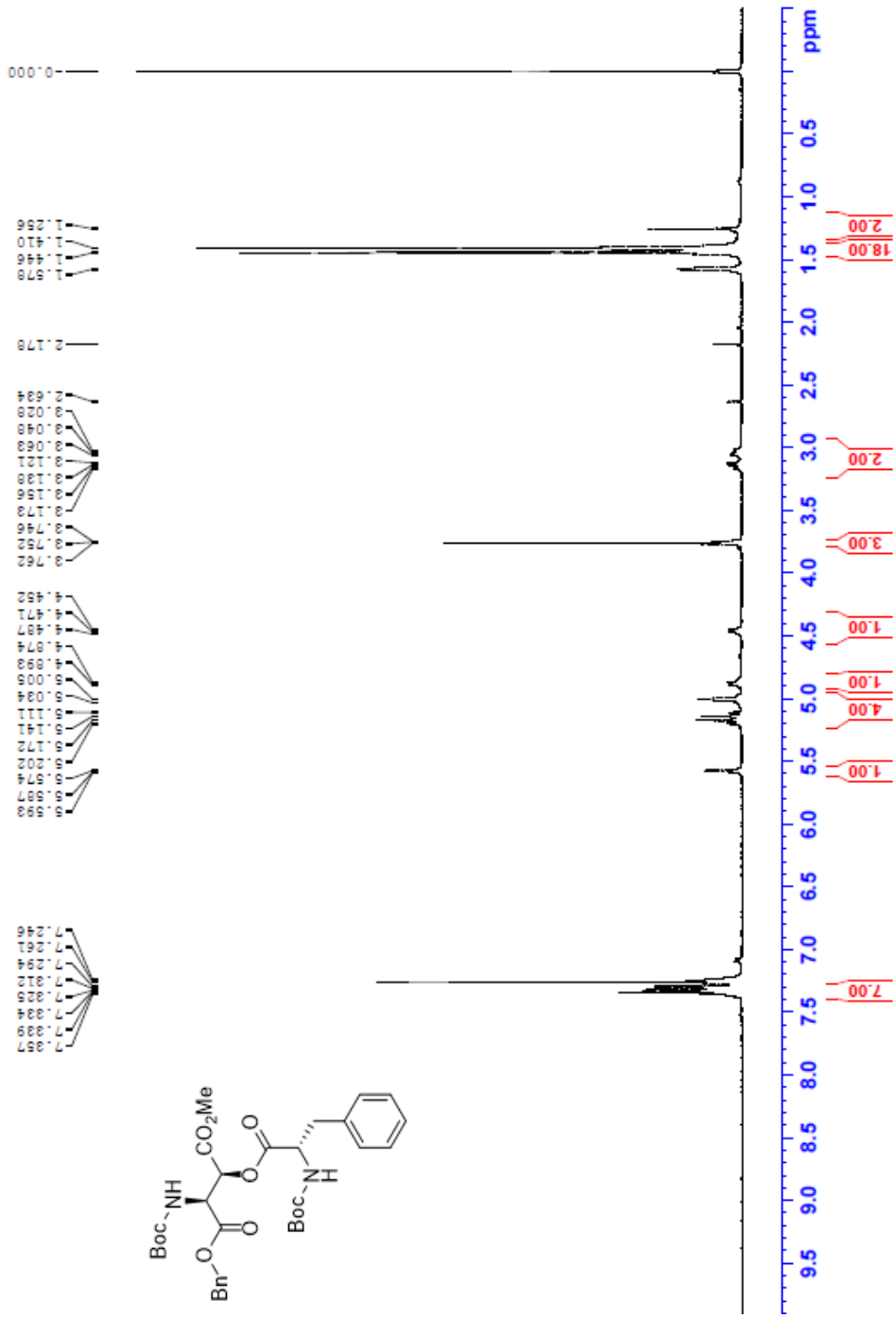
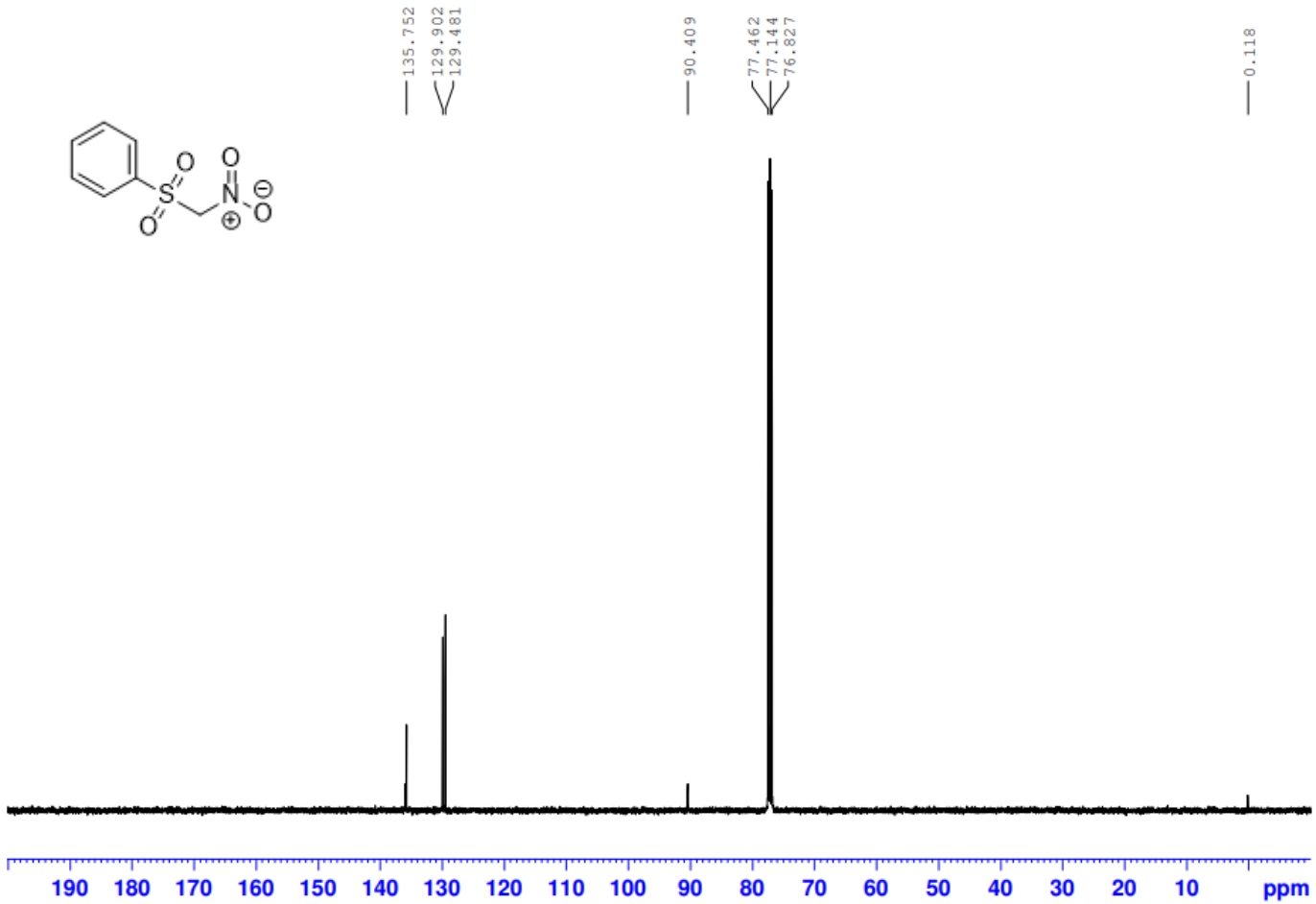
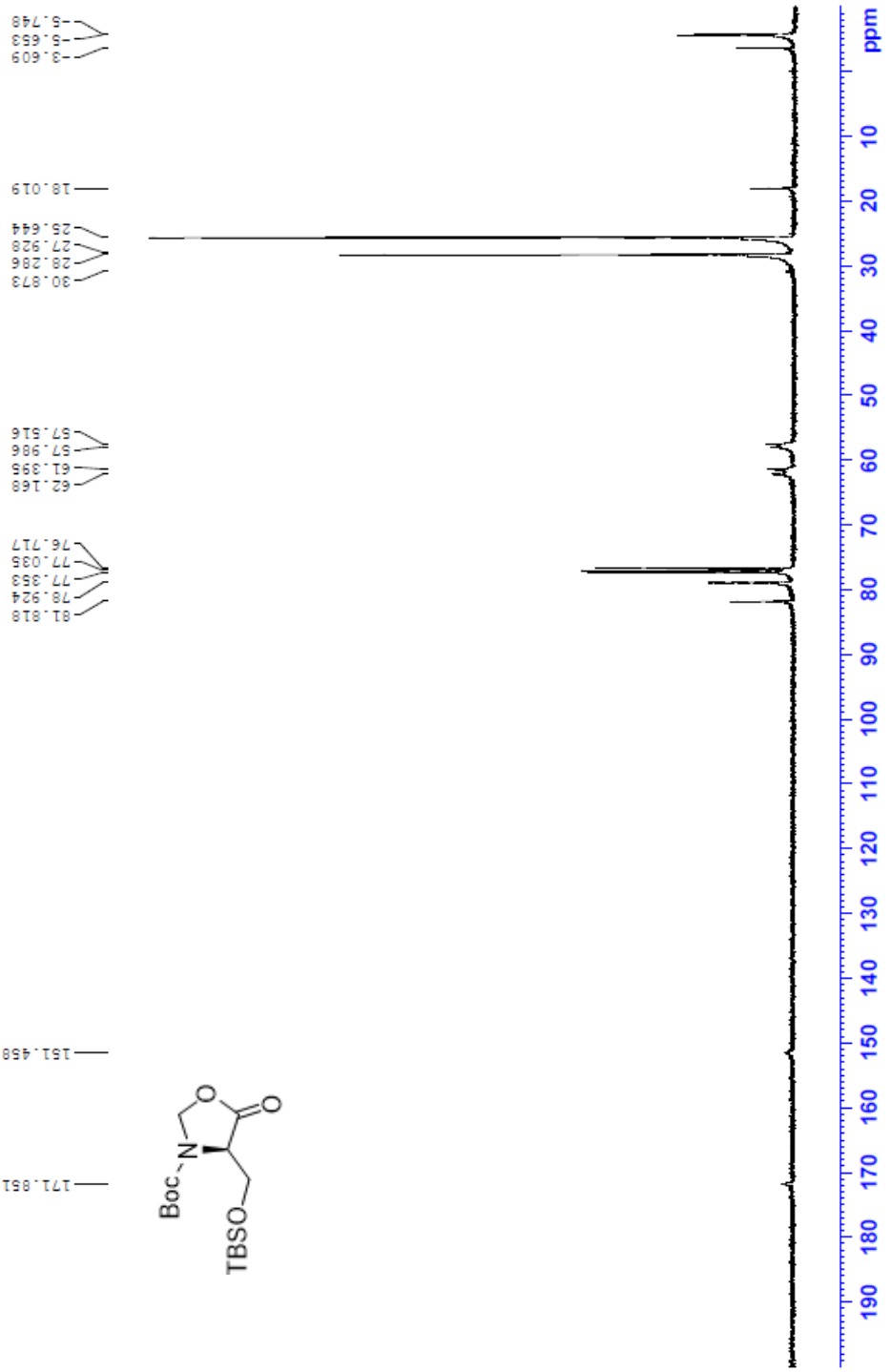


400 MHz ^{1}H NMR Spectrum ($CDCl_3$) of compound **9**

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



100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **A**



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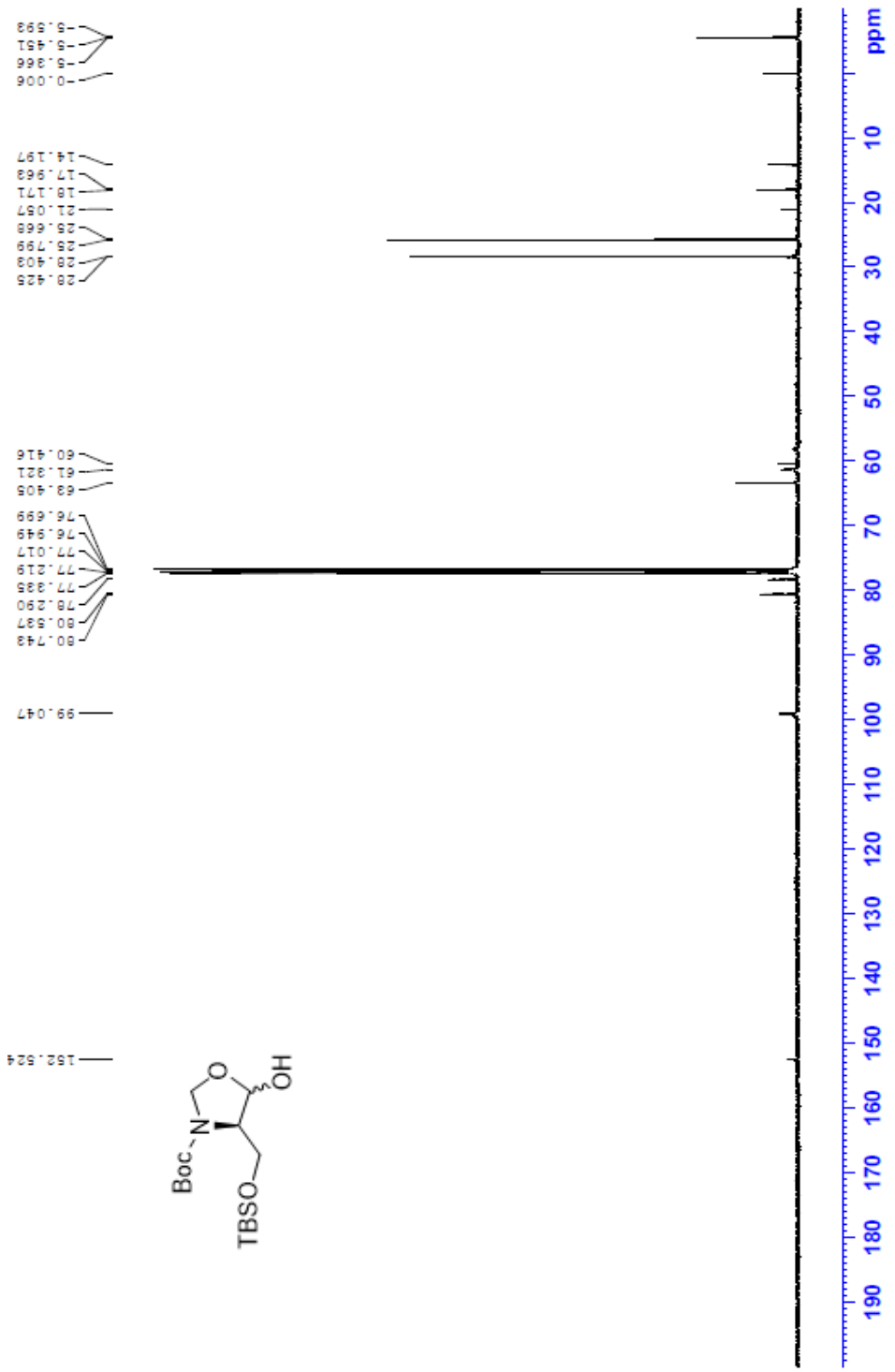
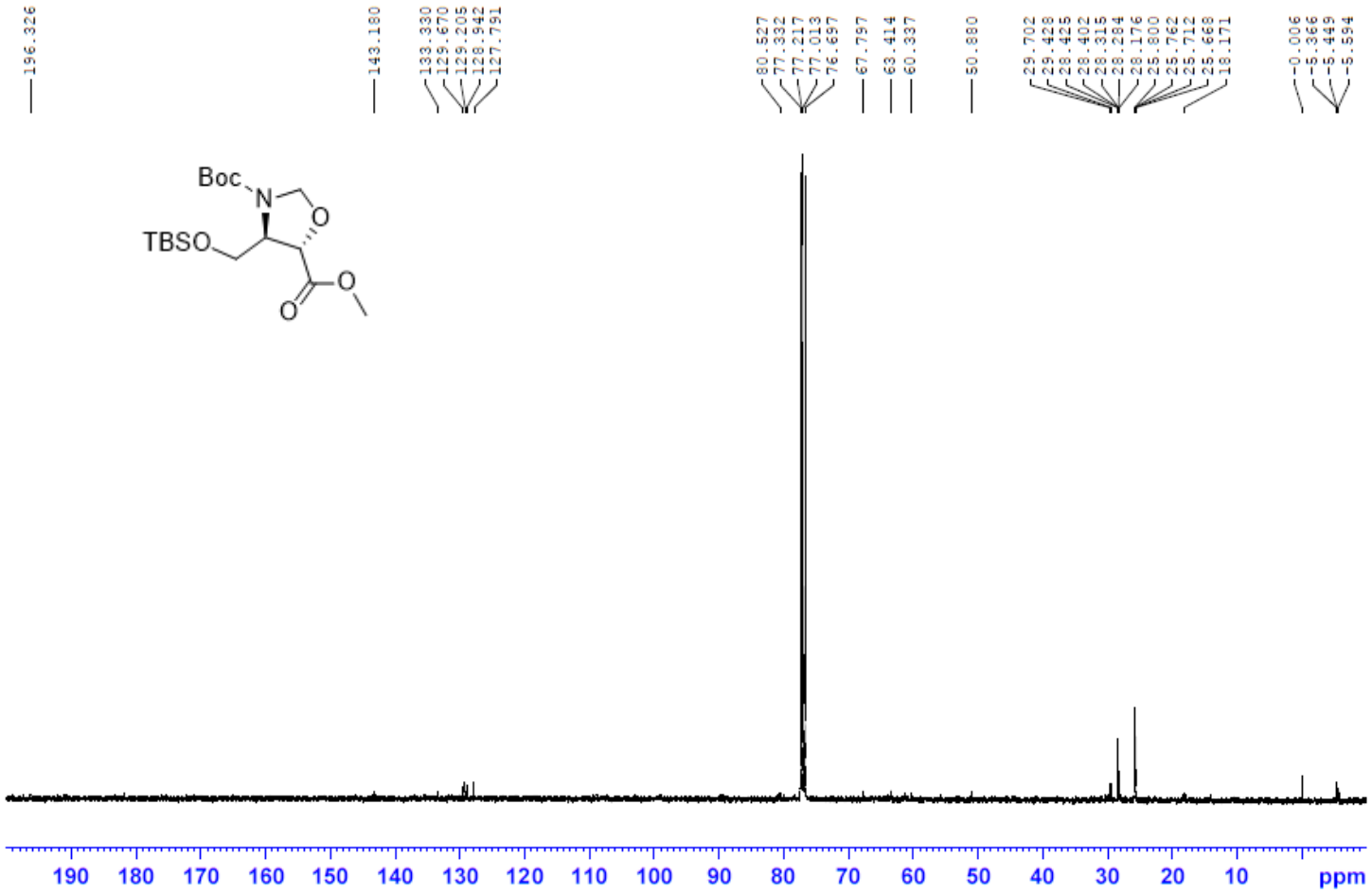
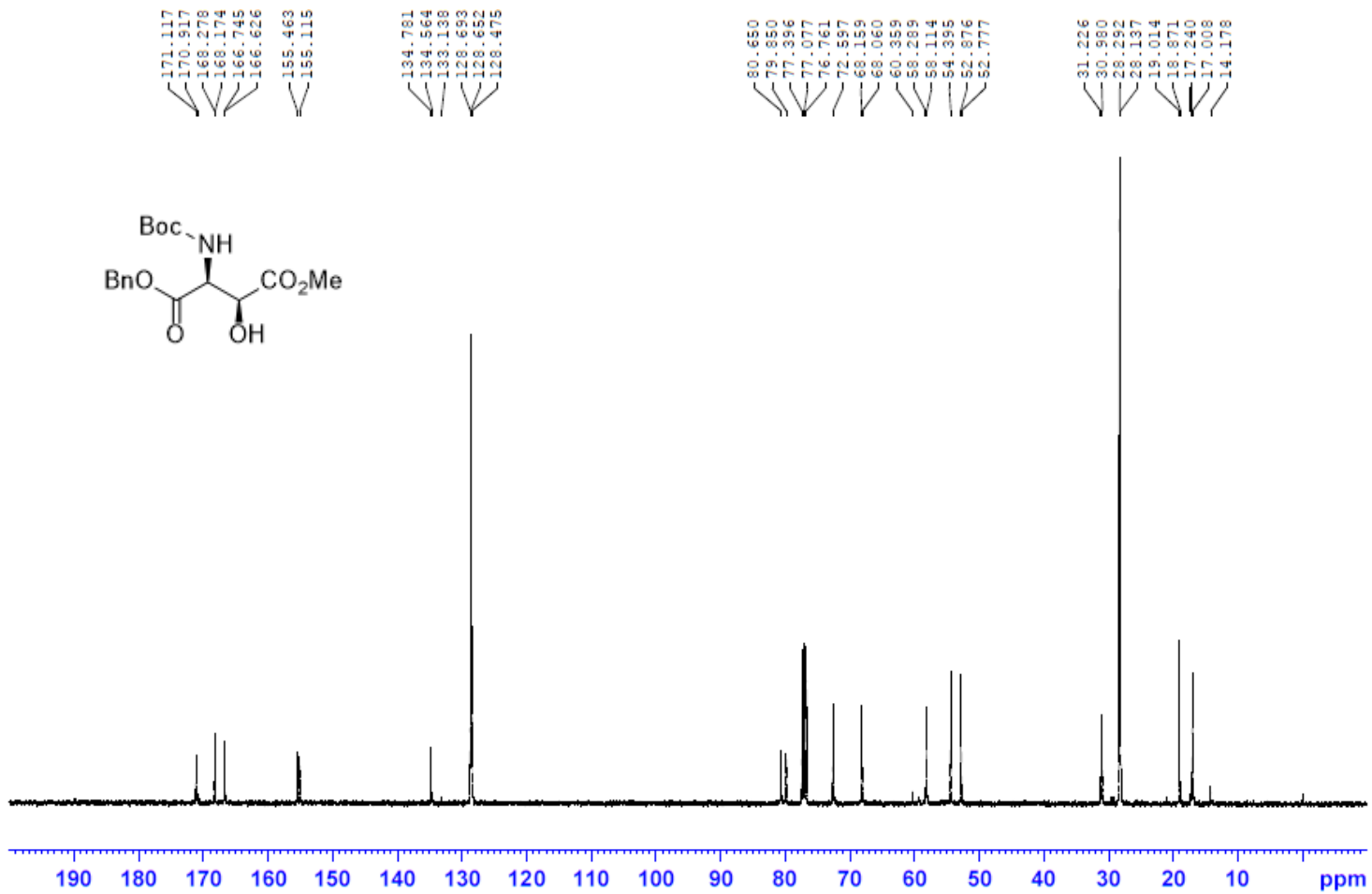


100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **3**

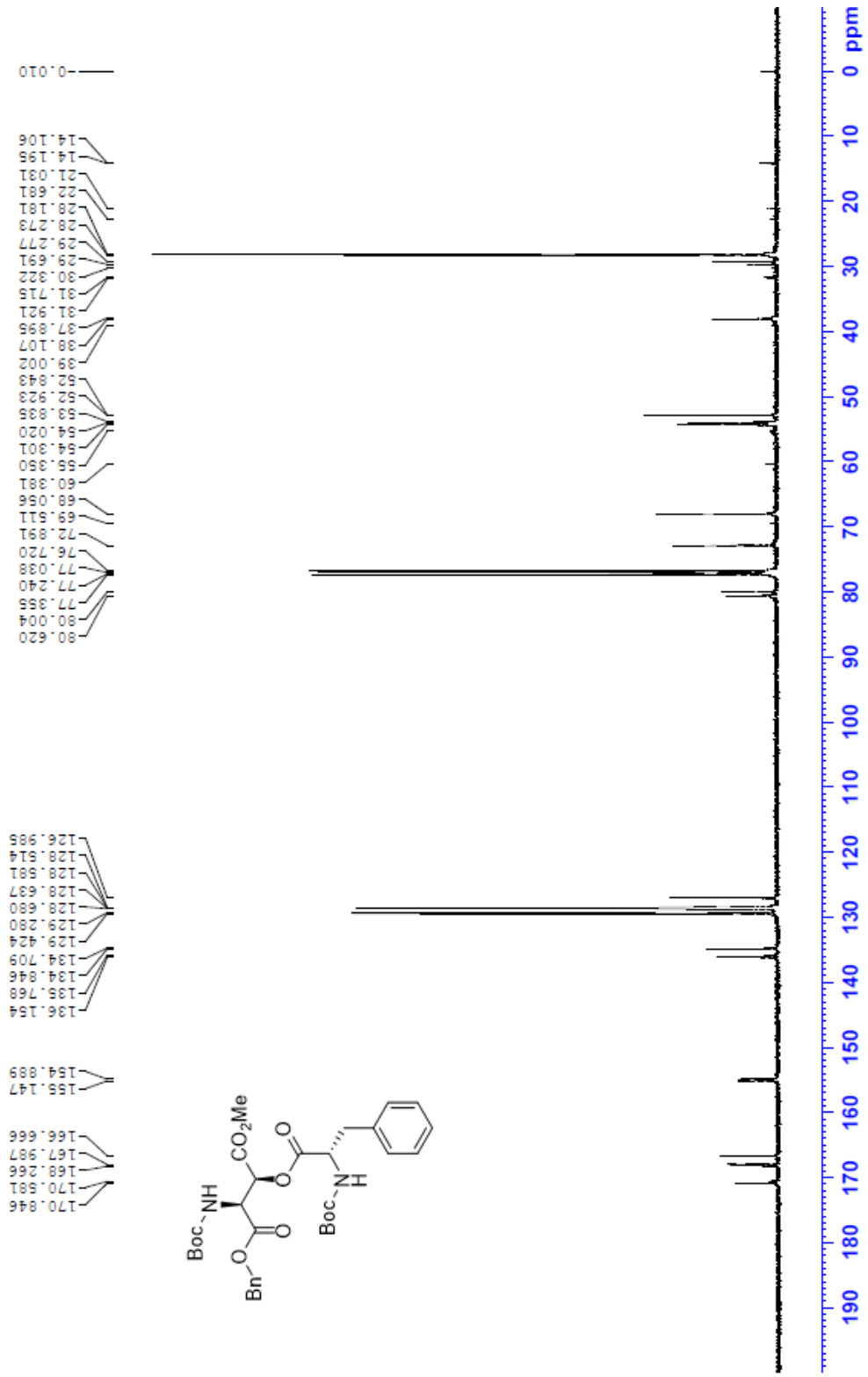


100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **4**

100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **7**



100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **8**



100 MHz ^{13}C NMR Spectrum ($CDCl_3$) of compound **9**

ABSTRACT IN KOREAN

본 논문은 L-트레오-β-하이드록시 아스파라진 구조의 입체선택적 특징을 기본으로 하는 라모플라닌 에이2의 전구체의 합성에 관한 내용을 다루고 있다. 악티노플라네스에 의해 합성되는 라모플라닌 에이2는 당지질 뎁시펩타이드의 한 종류로 임상효과를 나타낸다. 특히 라모플라닌 에이2는 그람 양성 박테리아에서 박테리아의 세포벽 생합성을 저해하여 소화기관에 존재하는 반코마이신 내성 장구균의 처리에 효과적인 역할을 한다.

현재 발표된 논문에 따르면 라모플라닌 에이2는 3개의 소단위체로 구성되어 커플링 반응과 고리화 반응을 통해 합성할 수 있다고 한다. 그 중 L-트레오-β-하이드록시 아스파라진 구조는 두 번째 주요 소단위인 펜타뎁시펩타이드의 뼈대로써 입체화학적 측면에서 중요하게 여겨진다.

우리 그룹은 트랜스-옥사졸리딘 구조를 형성할 때 일어날 수 있는 입체선택적 합성 방법에 대해 보고한 평형이 이동하여 안정한 구조를 가지고 반응에 참여한다. 트랜스-옥사졸리딘은 *N*-하이드록시메틸-α-아미노 알데하이드와 페닐설포닐나이트로메테인의 반응을 통해 합성되고, 전이상태에서의 *H*-가리움 형태로 인한 입체장애로 인해 트랜스-옥사졸리딘의 입체 선택성이 20:1의 비율로 높아진다.

이를 통하여 D-세린으로부터 L-트레오-β-하이드록시 아스팔테이트를 총 11단계, 11%로 합성하였다. 이 합성물은 각 말단이 서로 다른 종류의 보호기로 보호되어 L-트레오-β-하이드록시 아스파라진과 같은 형태로의 선택적 전환이 용이하게 합성되었다. 또한, *N*-페닐알라닌과의 야마구치 커플링 반응을 통한 뎁시펩타이드의 형성으로 라모플라닌 에이2의 합성 가능성을 확인하였다.

주요어 : 뎁시펩타이드, α-아미노 알데하이드, 트랜스-옥사졸리딘, L-트레오-β-하이드록시 아스팔테이트, 입체선택적, 라모플라닌 에이2

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