I. $A^{1,3}$ - STRAIN ENABLED RETENTION OF CHIRALITY DURING BIS-CYCLIZATION OF β-KETOAMIDES: ASYMMETRIC SYNTHESIS AND BIOACTIVITY OF SALINOSPORAMIDE A AND DERIVATIVES

II. OPTIMIZATION OF AN *ORGANIC SYNTHESES* PROCEDURE: ASYMMETRIC NUCLEOPHILE-CATALYZED ALDOL-LACTONIZATION WITH ALDEHYDE ACIDS

A Dissertation

by

HENRY NGUYEN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Chemistry

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Approved by:

Chair of Committee,	Daniel Romo
Committee Members,	Daniel Singleton
	Brian Connell
	Luis Rene Garcia
Head of Department,	David Russell

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ABSTRACT

I. $A^{1,3}$-Strain Enabled Retention of Chirality During Bis-Cyclization of β-Ketoamides: Asymmetric Synthesis and Bioactivity of Salinosporamide A and Derivatives

II. Optimization of an *Organic Syntheses:* Asymmetric Nucleophile-Catalyzed Aldol-Lactonization of Aldehyde Acids.

(December 2010)

Henry Nguyen, B. A.; M. S., Texas Woman's University

Chair of Advisory Committee: Dr. Daniel Romo

The potential of human 20S proteasome inhibitors continues to be of interest for anticancer chemotherapy and the recent FDA approval of bortezomib (Velcade) validates the proteasome as a target for cancer chemotherapy. Salinosporamide A, a marine unique bicycle [3.2.0] β-lactone-containing natural product, is not only a potent nanomolar inhibitor of the human proteasome but also active against bortezomib-resistant multiple myeloma cells. The racemic and asymmetric syntheses of salinosporamide A and derivatives were targeted.

In this dissertation, we successfully accomplished the shortest route to date with only a 9-step total synthesis of (–)-salinosporamide A. The conciseness of this strategy arises from the key bis-cyclization of a β-keto tertiary amide, amenable to gram scale, constructs both the γ-lactam and the fused-β-lactone in one operation with high enantiopurity, which was enabled by $A^{1,3}$-strain. Several derivatives were synthesized

and their inhibition activity toward chymotripsin-like, caspase-like, and trypsin-like of the human 20S proteasome was evaluated.

This dissertation also included a successfully optimized *Organic Syntheses* procedure for asymmetric synthesis of (1*S*,5*R*)-6-oxaspiro[bicyclo[3.2.0]heptane-3,2'-[1,3]dioxolan]-7-one via the nucleophile-catalyzed aldol-lactonization.

DEDICATION

In Memory of my Mother

ACKNOWLEDGEMENTS

I would like to thank my advisor Professor Daniel Romo, for his guidance and giving me such interesting and challenging projects that has continually kept me on my toes.

I would like to thank Professor Daniel Singleton and Professor Brian Connell for their insightful problem sections during my first year and throughout my doctoral program, and helpful discussions of reaction mechanism. I would also like to thank Professor Luis Garcia for his time and graciously serving on my committee.

I would like to thank all Romo group members past and present. I would especially like to thank Supakarn Chamni for her helpful discussions and being a continual source of support throughout my graduate career. I would like to thank Dr. Richard Duffy for his inspiration, discussions of chemistry, and for his friendship. I would also like to thank Dr. Seongho Oh, and Dr. Gil Ma for being a mentor during my first year, and also to my best friend Tony Lai who is an understanding, and supportive person. Also, I would like to thank Dr. Yonggang Wang, Dr. Carolyn Leverett and Gang Liu for their insightful discussion in chemistry.

Finally, I would like to thank my family, especially my mother, and sisters Kimoanh and Kimphung Nguyen, for their undying support in all my endeavors. Without them, it would have made this journey all the more difficult.

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CHAPTER I

INTRODUCTION AND THE SYNTHESES OF A NOVEL PROTEASOME INHIBITOR SALINOSPORAMIDE A

1.1. Introduction

Inhibitors of the human 20S proteasome are of continued intense interest due to their potential as anticancer therapeutics and the recent FDA approval of bortezomib (Velcade) validates the proteasome as a target for cancer chemotherapy.[1] Salinosporamides A/B (**1.1a-b**), are unique bicyclo [3.2.0] β-lactone-containing natural products isolated by Fenical and coworkers[2] from the marine actinomycete, *Salinispora tropica.* Salinosporamide A (salino A) is not only a potent nanomolar inhibitor of human proteasome, but also active against bortezomib-resistant multiple myeloma cells.[3] Salinosporamide A, which bears close structural similarities to the terrestrial metabolites, lactacystin-β-lactone (omuralide, **1.2**) derived from the prodrug lactacystin (**1.3**),[4] (Figure 1.1) is currently in Phase I human clinical studies for multiple myeloma having shown potential in mouse models toward several cancers when administered intravenously despite the potentially labile β-lactone.[5] Salinosporamide A (**1.1a**), besides superior bioactivity to its congener omuralide (**1.2**) (35 times more potent IC_{50} value)[2] , its challenging structure comprised of five contiguous stereogenic centers has been targets for total[6] and formal[7] syntheses, structure activity relationship studies,[8]

This dissertation follows the style of *Journal of the American Chemical Society.*

biosynthetic engineering,[9] and crystallographic studies with the 20S proteasome.[10] In this dissertation, we disclose our bioinspired racemic, asymmetric syntheses of salinosporamide A and derivatives. Furthermore, the bioactivity of these derivatives was assayed against the chymotripsin-like, caspase-like, and trypsin-like activities of the human proteasome. The aim of this review as described in Chapter I is to provide an overview of these syntheses and to illustrate their potential applications in drug discovery. This review will be restricted only to total syntheses of salinosporamide A.

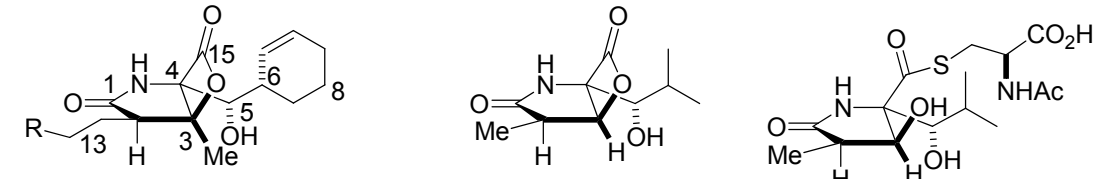


Figure 1.1. Structures of Naturally Occuring Bicyclic-β-Lactone Proteasome Inhibitors

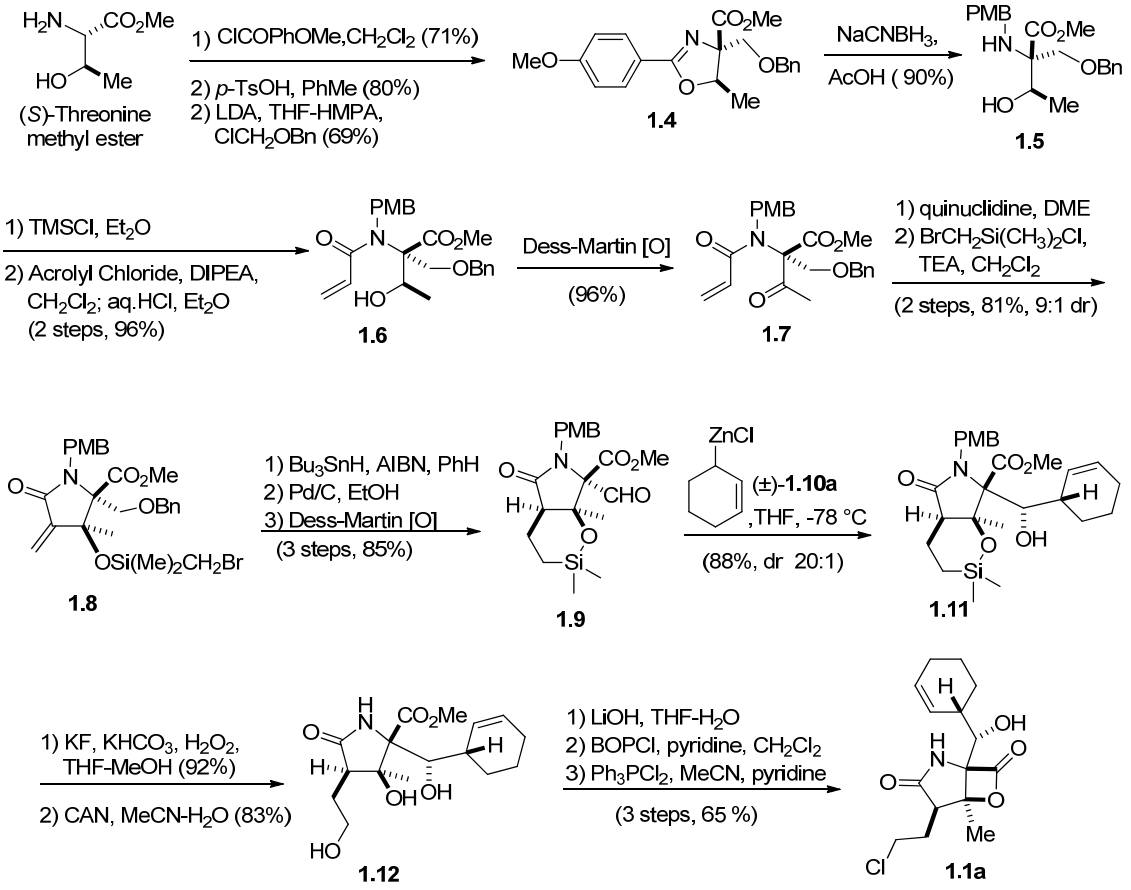
1.2. Enantioselective Synthesis of (–)-Salinosporamide A (1.1a)

1.2.1. Corey's Enantioselective Synthesis

Only one year after its structure was published, Corey and co-workers reported the first enantioselective synthesis of salinosporamide A.[6a] (*S*)-Threonine methyl ester was N-acylated with 4-methoxybenzoyl chloride, followed by *p*-TsOH catalyzed cyclization to the corresponding oxazoline, which allowed for diastereoselective alkylation with chloromethyl benzyl ether to to afford oxazoline **1.4**. Reduction of **1.4** with $NaBH_3CN$/AcOH afforded threonine derivative **1.5**. Transient TMS silylation of

1.5 was followed by acylation with acrolyl chloride and acidic work-up to give β-hydroxy amide **1.6.** Subsequent Dess-Martin periodinane oxidation of the alcohol **1.6** gave β-keto amide **1.7,** which underwent an intramolecular Baylis-Hillman reaction, and subsequent cyclization using quinuclidine as the nucleophile led to a γ-lactam (dr 9:1) and silylation provided ether **1.8**. Tributyltin hydride mediated radical cyclization delivered a bicyclic compound followed by hydrogenolysis of benzylether, and oxidation gave aldehyde **1.9**. The remaining two stereogenic centers were established by diastereoselective addition of the organozinc reagent. Cyclohexenyl zinc chloride (±)-**1.10** was reacted with aldehyde **1.9** to furnish the desired alcohol **1.11** with high stereoselectivity (dr 20:1). Tamao-Fleming oxidation of the silylether **1.11** followed by oxidative PMB deprotection to give triol **1.12.** Finally, salinosporamide A **1.1a** was obtained by saponification, lactone formation with BOPCl and chlorination in 13.7% overall yield and a longest linear sequence of 17 steps (Scheme 1.1).

Scheme 1.1. Corey's Synthesis of (–)-**1.1a** from *L*-Threonine Methyl Ester

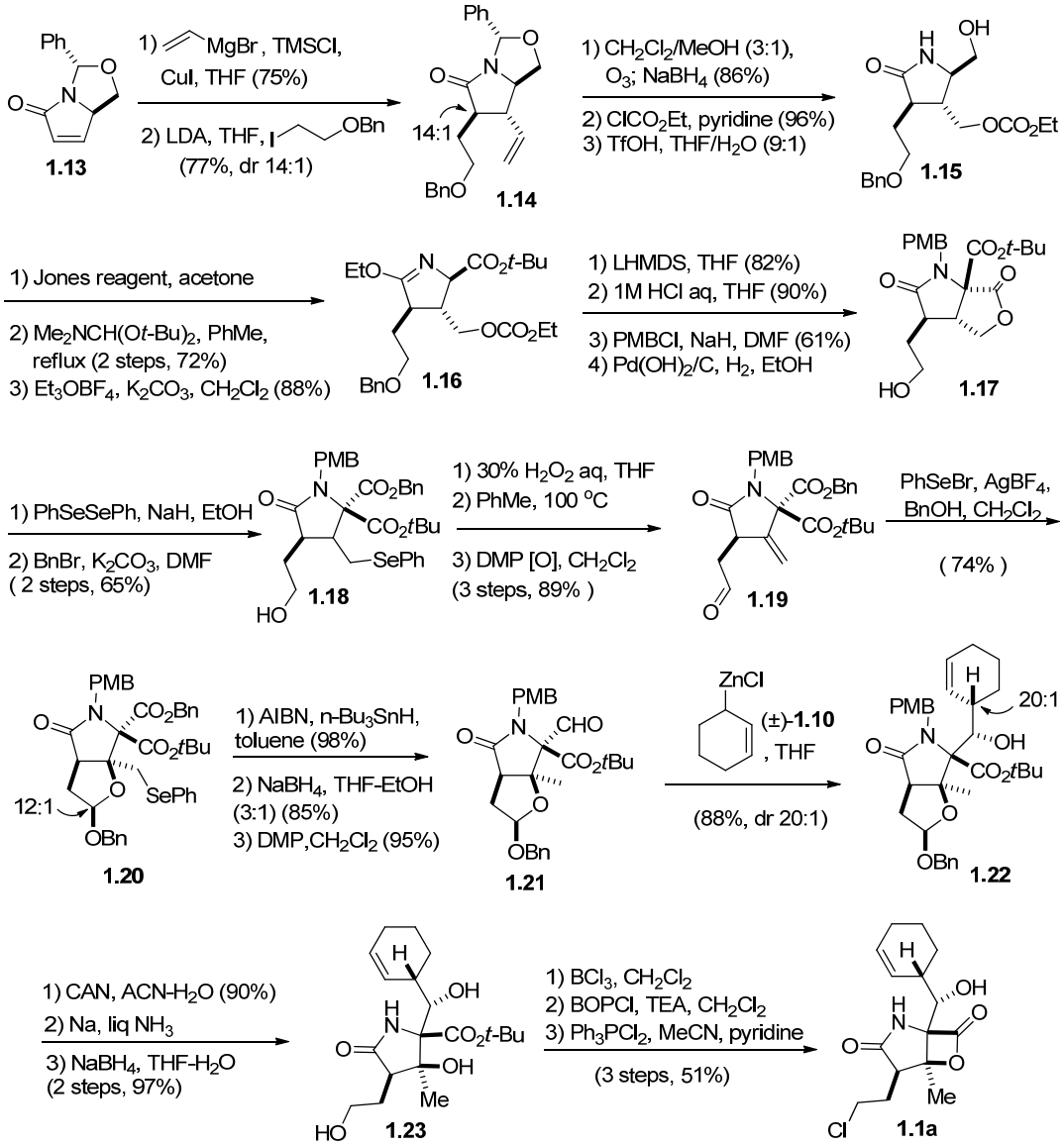


1.2.2. Danishefsky's Enantioselective Synthesis of (–)-1.1a

Danishefsky's Synthesis of (–)-**1.1a**[6c] commenced from strong facial bias of the pyroglutamate derivative **1.13** which directed the attack of vinyl cuprate from the α-face and subsequent diastereoselevtive alkylation at the C2 from its β-face to give lactam **1.14** (dr 14:1). Ozonolysis of the olefin and subsequent reduction with $NaBH_4$ gave the corresponding primary alcohol which was then advanced to the carbonate ester, followed by *N,O*-acetal cleavage to afford **1.15.** Hydroxymethyl lactam **1.15** was converted to the imidate ester **1.16** following Jones oxidation, esterification, and treatment with

Meerwein's reagent (Et_3OBF_4). Treatment of this masked lactam **1.16** with LiHMDS led to exclusive enolate formation at the C4, which underwent intramolecular acylation with ethyl carbonate to delivered γ-lactone. Restoration of the lactam moiety under acidic conditions, followed by PMB protection and subsequent removal of the benzyl protecting group by hydrogenolysis afforded γ-lactone **1.17**. Nucleophilic γ-lactone ring opening with phenyl selenium anion gave a carboxylic acid which was benzylated to deliver diester **1.18**. Subsequent selenide oxidation, elimination and Dess-Martin oxidation sequence gave aldehyde **1.19**. It was set for a key acetal-mediated cationic cyclization. Treament of **1.19** with phenylselenyl bromide and $AgBF_4$ in the presence of benzyl alcolhol gave **1.20** with complete stereocontrol at C3**.** After the benzyl ester was converted to aldehyde **1.21,** it was then treated with cyclohexenyl zinc employing Corey protocol. The reaction proceeded with excellent diastereocontrol to afford amide **1.22** (dr 20:1). Oxidative cleavage of the PMB group and reductive debenzylation gave triol **1.23**. Acid promoted cleavage of the *t*-butyl ester, followed by lactonization and chlorination to provide salinosporamide A **1.1a** (Scheme 1.2).

Scheme 1.2. Danishefsky's Synthesis of (–)-**1.1a** from Pyroglutamate Derivative

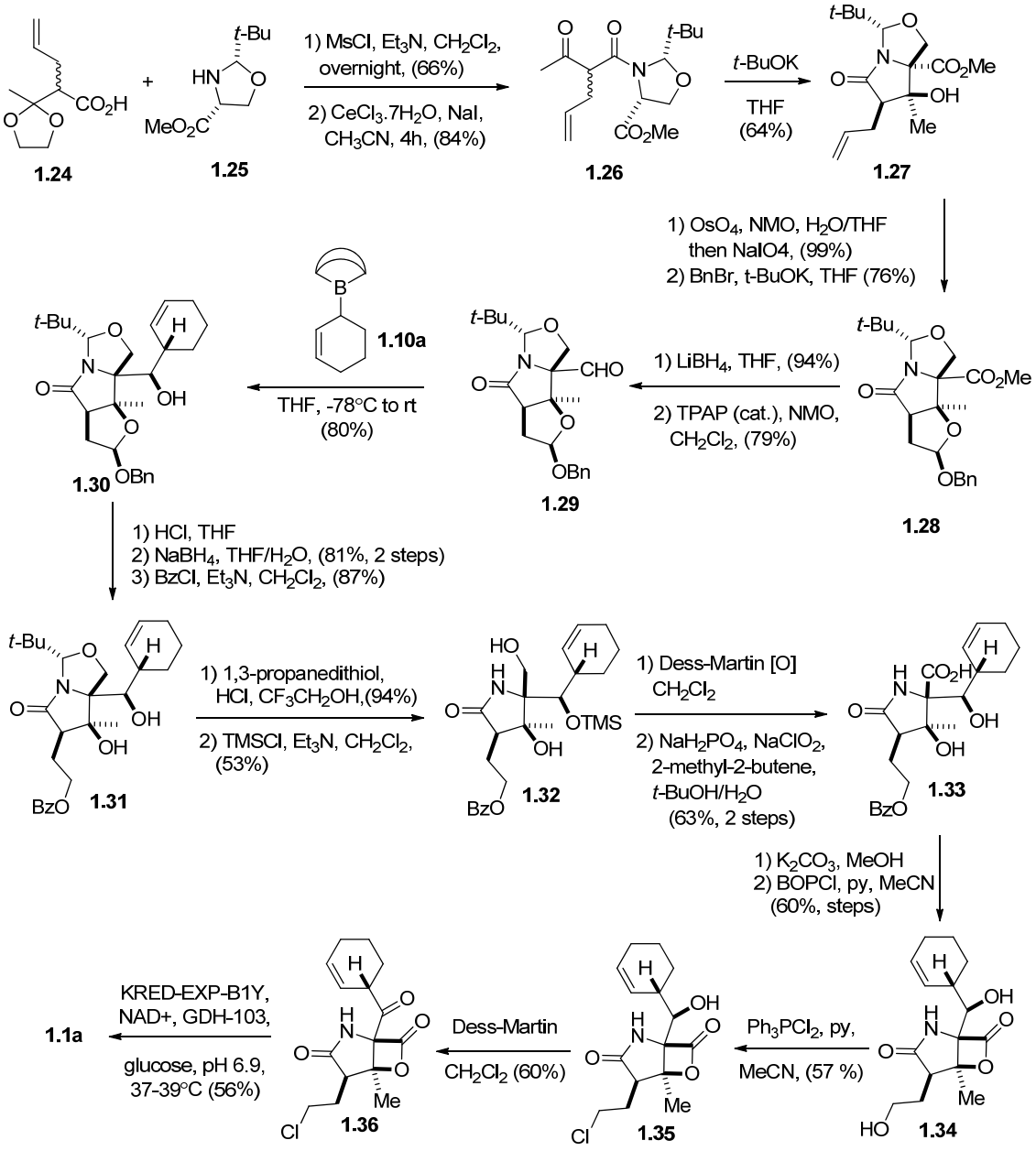


1.2.3. Nereus Enantioselective Synthesis of (–)-1.1a

Nereus Pharmaceutials synthesis of **(–)-1.1a**[6f] started with an amide coupling between carboxylic acid **1.24** and oxazolidine **1.25**. The ketone functionality was then unveiled by the use of cerium trichloride to give β-keto ester **1.26**. The next step in their

synthesis was cyclization on to the ketone to give bicycle **1.27**. An osmium catalyzed oxidative cleavage sequence was then followed by conversion of the hemiacetal to the benzyl acetal **1.28**. Then a lithium borohydride reduction of the ester to the alcohol was followed by Ley oxidation to give aldehyde **1.29**. Attachment of the cyclohexenyl moiety was accomplished by an allylborylation with **1.10a** to give alcohol **1.30**. The aldehyde was then unmasked with the use of acid and reduced to the primary alcohol which was then capped as its benzoyl ester **1.31**. The hemiaminal was then hydrolyzed with the use of 1,3-propanedithiol and acid. The secondary hydroxyl was protected with TMS to give silyl ether **1.32**. The primary alcohol was then converted to the corresponding carboxylic acid via a two step protocol. First, it was oxidized to the aldehyde by Dess-Martin periodinane, followed by Pinnick oxidation to carboxylic acid **1.33**. The completion of the β-lactone was accomplished by first removal of the benzoyl group under basic conditions and then lactonization by using BOPCl yielding the desired β-lactone **1.34**. With the primary hydroxyl already deprotected, conversion to the alkyl chloride was the next step giving alcohol **1.35**. The stereochemistry of the side chain alcohol was then corrected by a two step process. First, oxidation of the alcohol to the ketone with the Dess-Martin reagent gave ketone **1.36**. The ketone was then enzymatically reduced to give salino A (**1.1a)** (Scheme 1.3).

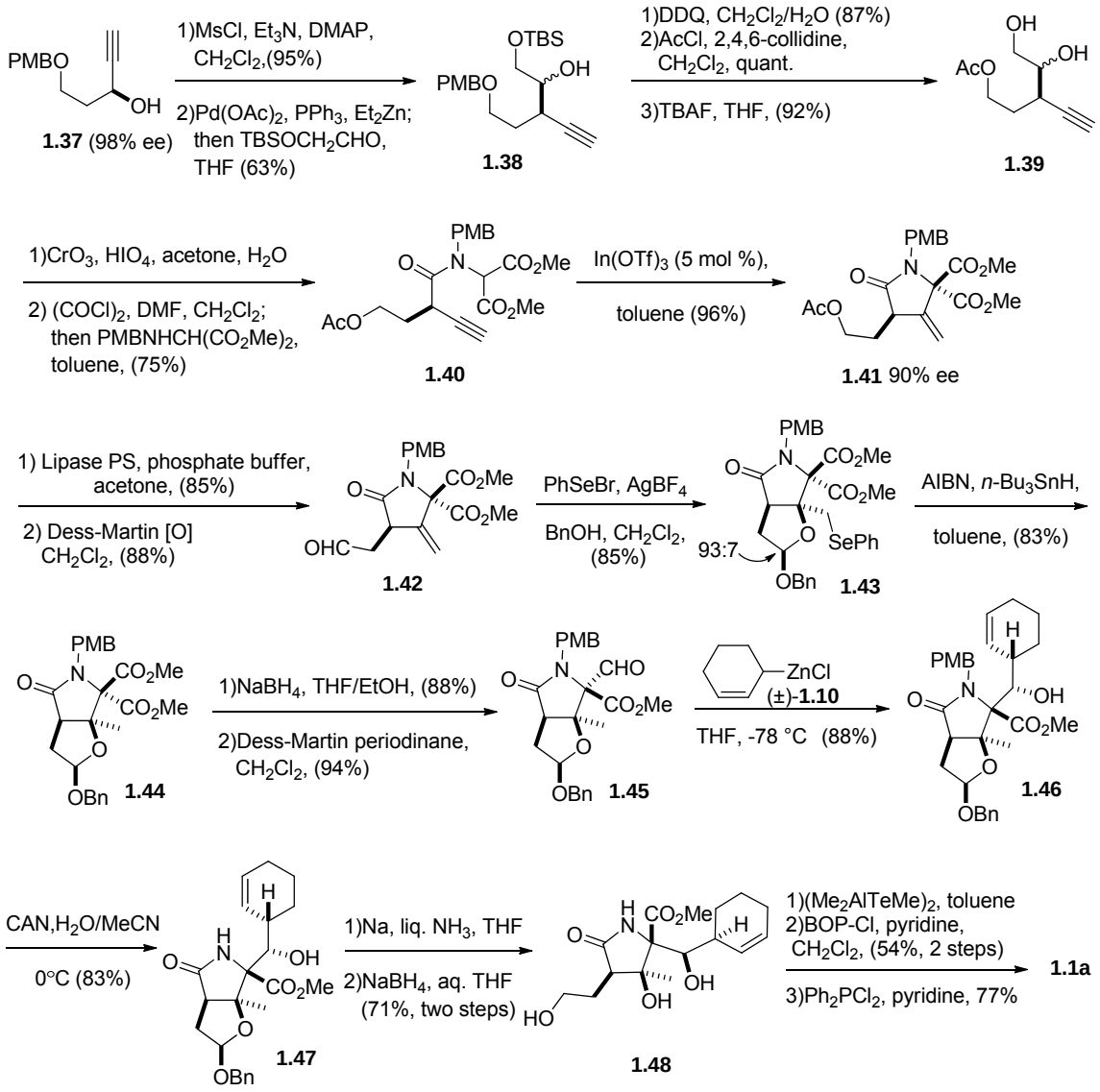
Scheme 1.3. Nereus Synthesis of (–)-**1.1a** from (*R*)-Serine



1.2.4. Hatakeyama's Enantioselective Synthesis of (–)-1.1a

Hatakeyama's synthesis of **(–)-1.1a**[6h] began with propargyl alcohol **1.37** by a palladium mediated addition to an acetal dehyde derivative to give ether **1.38**. The PMB protection group was removed with DDQ followed by selective acylation and fluoride mediated removal of the silyl group yielded diol **1.39**. Oxidative cleavage of the diol to the carboxylic acid and coupling with dimethyl 2-(4-methoxybenzylamino) malonate by way of the acid chloride gave amide **1.40**. With amide **1.40** in hand they were set to carry out their key reaction, next an indium catalyzed cyclization gave lactam **1.41**. An enzyme mediated selective hydrolysis of the acetate yielded the corresponding primary alcohol, which was then oxidized to aldhyde **1.42**. Next, cyclization of the aldehyde onto the alkene was accomplished by the action of phenyl selenium bromide in the presense of benzyl alcohol to capture the intermediate oxocarbenium ion to give acetal **1.43**. The selenium was removed via radical reduction with *n*-tribuyl tin hydride to give diester **1.44**. Selective reduction of one of the ester moieties followed by oxidation with Dess-Martin periodinane gave aldehyde **1.45**. Coupling of the cyclohexenyl moiety was accomplished using Corey's protocol to give alcohol **1.46**. The PMB group was then removed by CAN to give free lactam **1.47**. Next the benzyl group was removed using Birch conditions and the resulting lactol was reduced to triol **1.48** by sodium borohydride. The completion of the synthesis was achieved by conversion of the ester to the acid and lactonization utilizing BOPCl and after conversion of the alcohol to chloride yielded salinosporamide A (Scheme 1.4).

Scheme 1.4. Hatakeyama's Synthesis of (–)-**1.1a**

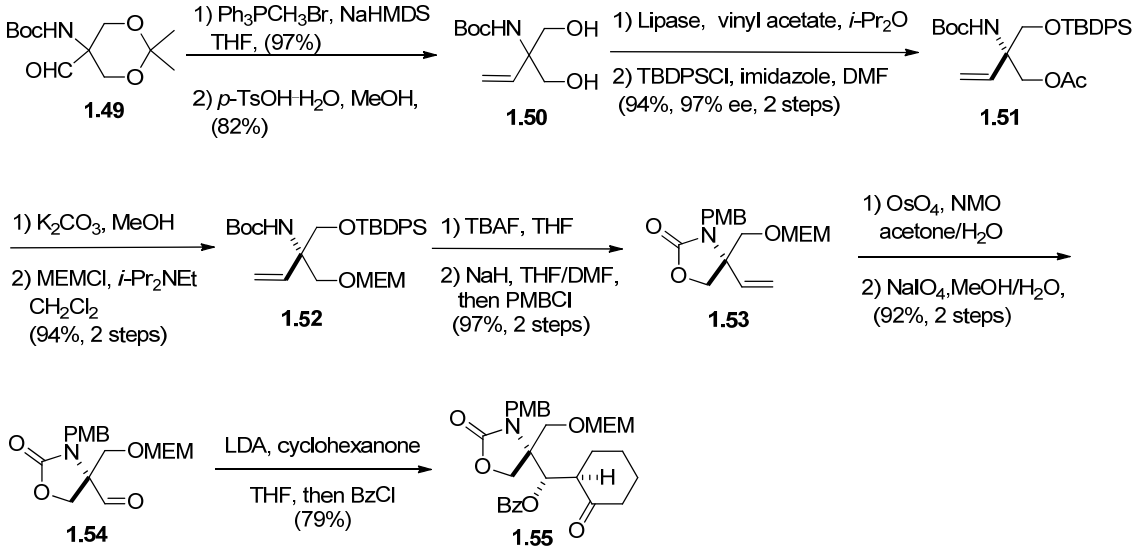


1.2.5. Omura's Enantioselective Synthesis of (–)-1.1a

Omura's synthesis of **(–)-1.1a**[6i] began with aldehyde **1.49** which underwent a Wittig reaction and subsequent deprotection to give diol **1.50**. Diol **1.50** was then converted to enantiomerically pure acetate by a lipase catalyzed acylation followed by

protection of the remaining hydroxyl group as its TBDPS ether to give acetate **1.51**. This was then followed by hydroylysis of the acetate and protection of the hydroxyl as a MEM ether **1.52**. Deprotection of the silyl ether and transcarbamoylation and PMB protection of the amine gave carbamate **1.53**. The pendent alkene of carbamate **1.53** was then dihydroxylated utilizing osmium tetraoxide, followed by cleavage of the diol under the action of sodium periodate to give aldehyde **1.54**. The next step was the installation of the cyclohexenyl side chain, this was accomplished by an aldol reaction between cyclohexanone and aldehyde **1.54** using LDA as base. The aldol product was then converted *in situ* to its corresponding benzoyl ester **1.55** (Scheme 1.5).

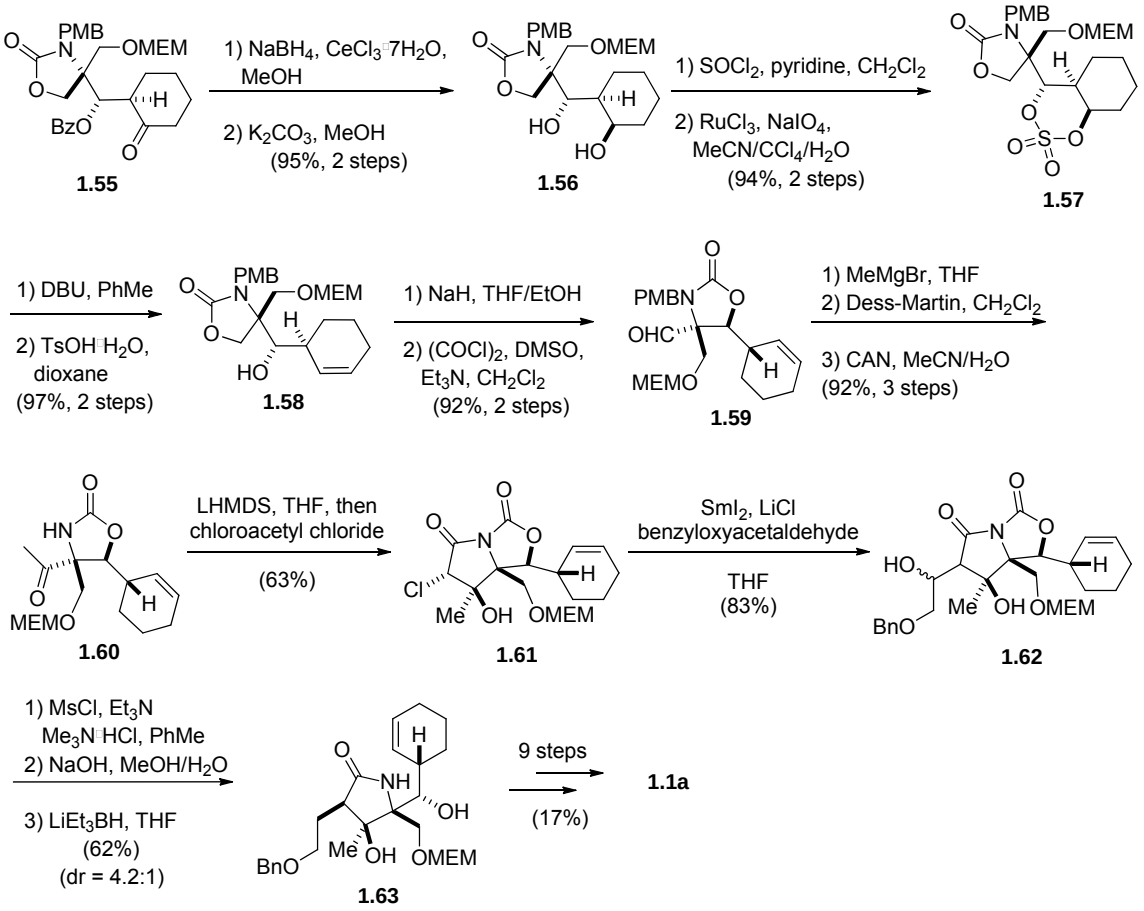
Scheme 1.5. Synthesis of Carbamate **1.55**



The next steps provided the necessary unsaturation in the cyclohexenyl side chain. First reduction of the ketone to its corresponding alcohol and hydrolysis of the

benzoyl ester gave diol **1.56**. The alkene was then installed by first conversion to the sulfinate and then to the cyclic sulfate **1.57** by oxidation with ruthenium trichloride and sodium periodate. The sulfate **1.57** was then eliminated under basic conditions which gave alcohol **1.58** after hydrolysis of the sulfate. A second transcarbamoylation was then performed followed by Swern oxidation of the primary alcohol gave aldehyde **1.59**. Methyl Grignard was then added to this aldehyde and the resulting alcohol was oxidized to the ketone followed by oxidative deprotection of the amine to give ketone **1.60**. Acylation of the nitrogen with chloroacetylchloride followed by intramolecular aldol reaction gave bicycle **1.61**. This was followed by an intermolecular Reformatsky reaction with benzyloxy-acetaldehyde under the action of samarium diiodide which gave alcohol **1.62**. Lactam **1.63** was then formed by a two step elimination of the alcohol followed by 1,4-reduction with lithium triethylborohydride. An additional nine steps yielded salinosporamide A (**1.1a**) (Scheme 1.6).

Scheme 1.6. Execution of Salinosporamide A (**1.1a**) Synthesis



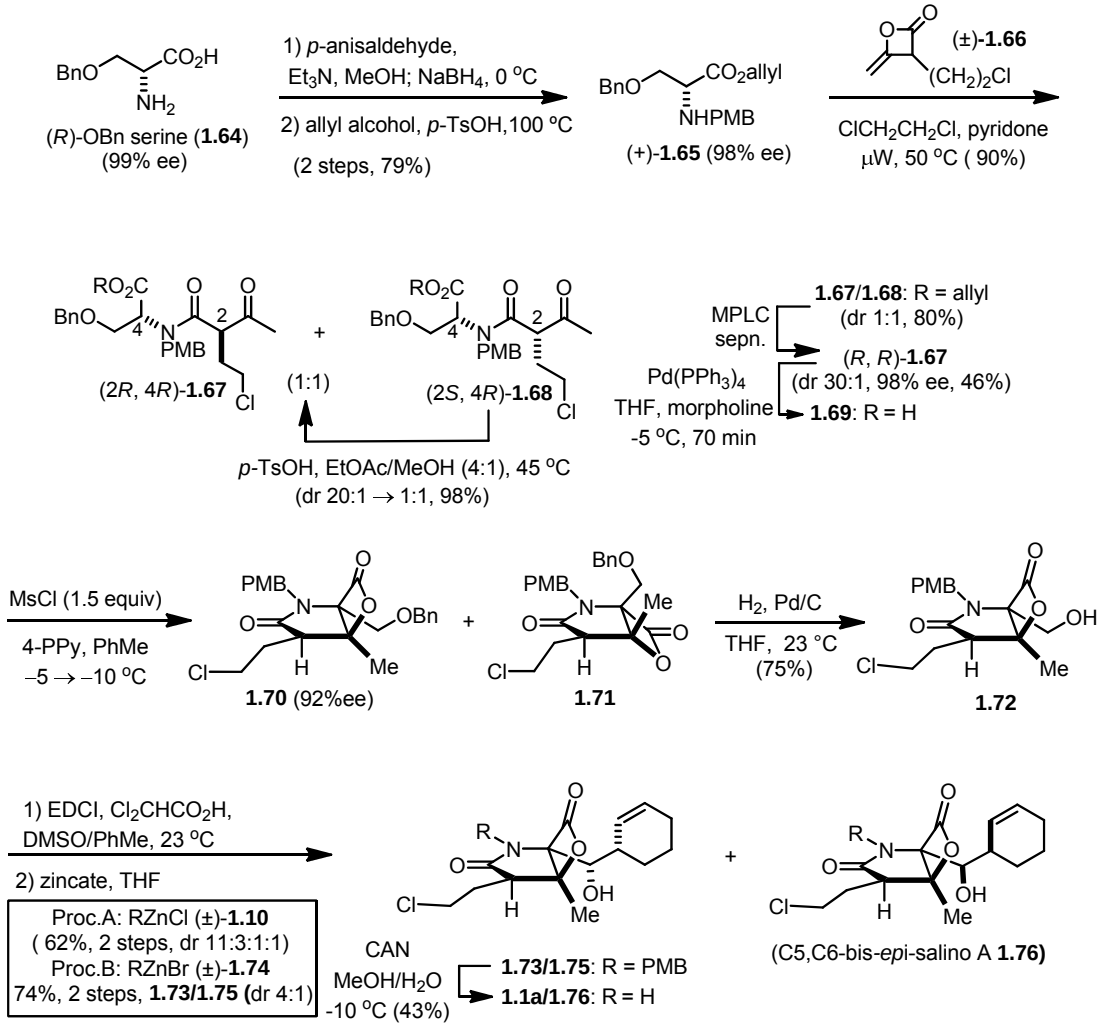
1.2.6. Romo's Enantioselective Synthesis of (–)-1.1a

In our early optimization studies, we determined that the diastereoselectivity was highly dependent on whether (*S*) or (*R*)-serine derived ketoacids were employed leading to different diastereoselectives, 1:2 vs 8:1 respectively. The synthesis of **(–)-1.1a**[6j] (Scheme 1.7) began with reductive amination of commercially available (*R*)-*O*-benzyl serine **1.64** (99% ee) with *p*-anisaldehyde and subsequent esterification provided allyl ester (**1.65**, 98% ee). Acylation of serine derivative **1.65** with unsymmetrical ketene

dimer (±)-**1.66** under microwave conditions gave diastereomeric β-ketoamides **1.67**/**1.68** (dr 1:1). At this stage of the synthesis, only the desired β-ketoamide (2*R*,4*R*)-**1.67** with high diastereomeric purity was carried out to the next step since the absolute stereochemistry at the C2 will retain in the subsequent steps. Separation of the diastereomers by MPLC provided the required (2*R*,4*R*)-**1.67** ketoamide (dr 30:1, 98% ee, 46%). Under acidic conditions, the undesired diastereomer (2*S*,4*R*)- **1.68** could be transformed to a 1:1 mixture of diastereomers via epimerization of the C2 but not the C4 stereocenter thus achieving an effective resolution of ketene dimer (±)-**1.66** and greater material throughput. Pd(0)-mediated allyl deprotection of ester (2*R*,4*R*)-**1.67** was also required to minimize epimerization of the C2 stereocenter and ultimately delivered keto acid **1.69** with negligible erosion of the diastereomeric purity. Treatment of keto acid **1.69** with acid activating agent mesyl chloride, 4-pyrrolidino pyridine and toluene constructed the γ-lactam and β-lactone **1.70/1.71** in one step with minimum loss in optical purity (92% ee) and moderate diastereoselectivity 7:1 favoring relative stereochemistry found in natural product. Importantly, this transformation could be performed on gram scale with comparable diastereoselectivity and retention of enantiopurity (52% over 2 steps, dr 5:1, 90% ee). Completion of the salino A synthesis entailed benzyl deprotection through hydrogenolysis to provide alcohol **1.72** which

could be separated at this stage from the minor diastereomer produced during the bis-cyclization process. Modified Moffatt oxidation and addition of the zinc reagent **1.10** derived from the reaction of *n*-butyllithium with cyclohexenyl tributyltin and $ZnCl_2$, following the procedure of Corey gave a mixture of C5, C6-diastereomeric alcohols (dr 11:3:1:1) in 62% yield (2 steps, Procedure A 'Proc. A') and the desired diastereomer **1.73** was the major adduct. Alternately, zinc reagent **1.74** could be prepared directly from commercially available 3-bromocyclohexene and activated zinc by the method of Knochel. Treatment of this zincate **1.74** with the aldehyde intermediate simplified product purification since tin by-products were avoided (Proc. B). Most importantly, this protocol gave only two diasteomers (dr 4:1), improved the yield to 74% (2 steps). This procedure enabled isolation of a novel salino A diastereomer **1a'** (C5, C6-bis-*epi*-salino A) whose relative stereochemistry was verified by X-ray analysis. Finally, deprotection of PMB-protected lactam **1.73** led to salino A (**1.1a**).

Scheme 1.7. Romo's Synthesis of (–)-**1.1a** from (*R*)-*O*-Benzyl Serine



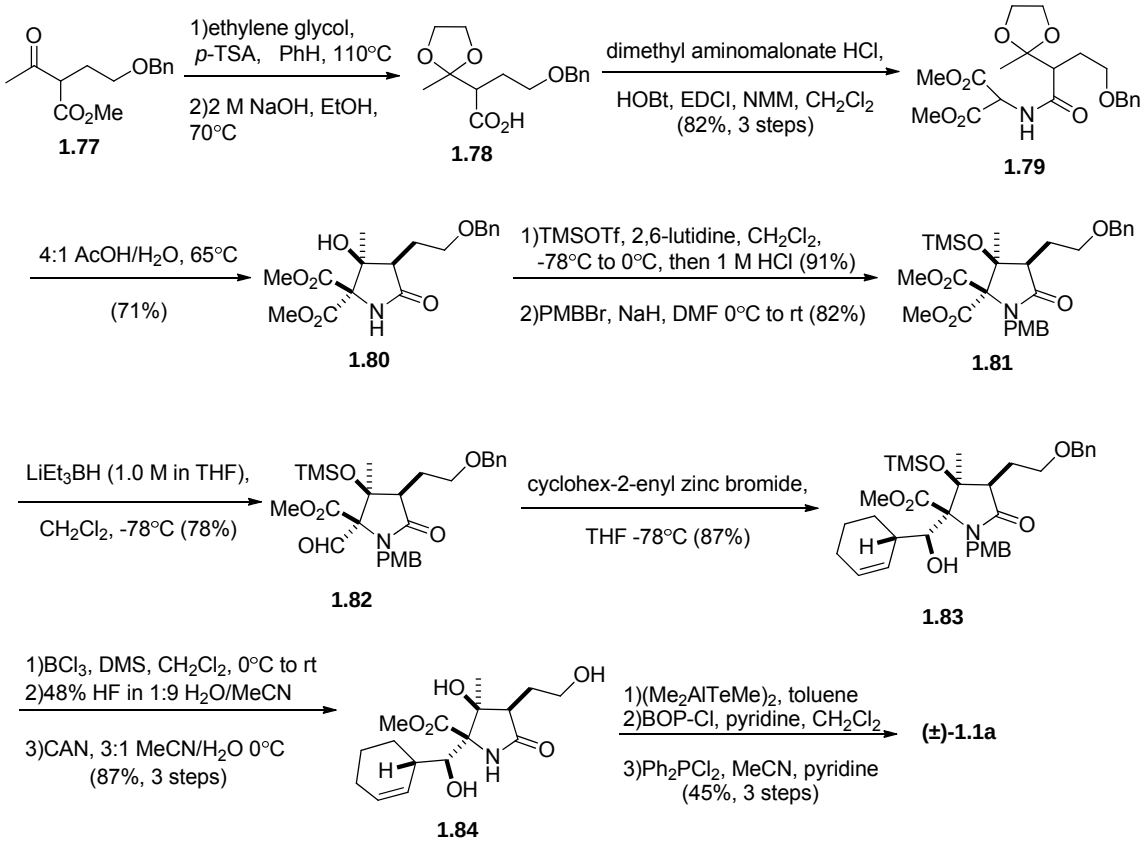
1.3. Racemic Syntheses of (±)-Salinosporamide A

1.3.1. Pattenden's Synthesis of (±)-1.1a

The Pattenden group reported a first biosynthetically inspired approach towards racemic synthesis of salino A[6g]. Pattenden's synthesis of salinosporamide A began with known β-ketoester **1.77** (Scheme 1.8). First the ketone was protected as its acetal and the ester was saponified to give acid **1.78**. Acid **1.78** was then coupled with dimethyl

amino malonate to give amide **1.79**. In order to complete the formation of the lactam ring, amide **1.79** was exposed to acidic conditions leading to lactam **1.80**. Next the tertiary hydroxyl was protected as its TMS ether and the nitrogen was protected with a PMB group to give diester **1.81**. Differentiation of the two esters was accomplished by selective reduction with lithium triethylborohydride to yield aldehyde **1.82**. The completion of the side chain was carried out by addition of cyclohex-2-enyl zinc bromide to aldehyde **1.82** to produce alcohol **1.83**. Removal of the protecting groups was accomplished by Lewis acid mediated deprotection of the benzyl ether, fluoride induced desilylation and oxidative cleavage of the PMB group gave triol **1.84**. The completion of the synthesis of salinosporamide A was carried out by cleavage of the methyl ester and β-lactonization with BOPCl and final conversion of the primary hydroxyl group to the corresponding chloride.

Scheme 1.8. Racemic Synthesis of (±)-Salinosporamide A

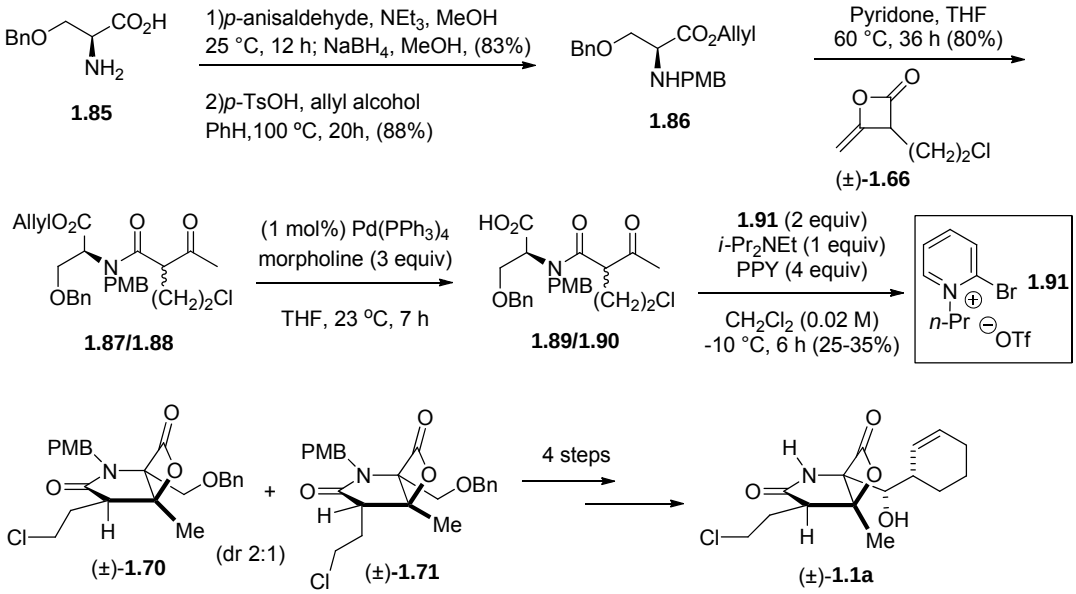


1.3.2. Romo's Synthesis of Salinosporamide A (±)-1.1a

Our bioinspired racemic synthesis of salino A[6e] commenced from reductive amination of (*S*)-*O*-benzyl serine **1.85**, followed by esterification to afford allyl ester **1.86**. The β-ketoamide substrate **1.87/1.88** was obtained from coupling of ketene heterodimer **1.66** of acetyl chloride and commercially available 4-chlorobutanoyl chloride (13%) on multigram scale by the method of Saeur, and serine derivative **1.86**. In this case, the bis-cyclization was employed both diastereomeric keto acid **1.89/1.90** following allyl deprotection to give β-lactone **(±)-1.70/1.71** in 25-35% yield (dr 2-3:1), however the relative stereochemistry of the major diastereomer corresponded to salino A

as confirmed by subsequent conversion to the natural product. Bicyclic β-lactone **(±)-1.70** was processed to racemic salino A (**1.1a**) using an identical sequence to that described for enantioselective synthesis of (–)-**1.1a**.

Scheme 1.9. Romo's Synthesis of **(±)-1.1a** from (*S*)-*O*-Benzyl Serine



1.4. Conclusion

The discovery of salinosporamide A has been a milestone in synthetic organic chemistry in a number of ways that are reflected in its biological importance and the variety and methods of its synthesis. Salinosporamide A drew attention due to its structural complexity and its potent biological activity. A fair assessment of this is the number of syntheses that have been published in its short existence in the literature.

The first enantioselective synthesis was accomplished by Corey and co-workers. This route, in addition of setting the stage for syntheses to come, featured a Baylis-

Hillman reaction in order to construct the lactam ring. Another of the features of this synthesis is the use of a radical cyclization in order to construct what would become the C2 stereocenter. Perhaps the most important feature of this synthesis, due to its use in others, is the addition of a cyclohex-2-enyl zinc reagent to C5 in high selectivity.

Following in the wake of the Corey synthesis, Danishefsky and co-workers also developed an enantioselective route to salinosporamide A. Their synthesis featured the construction of a rigid bicylic intermediate which ultimately led to excellent control in the construction of one of their key intermediate, a benzylic acetal. This bicyclic structure, similar to Corey's intermediate, also gives excellent stereocontrol with respect to the addition of the cyclohex-2enyl zinc reagent ultimately leading to the construction of salinosporamide A.

Nereus Pharmaceuticals developed a route that differed from the previous enantioselective syntheses preceeding it. The first difference is that instead of the addition of a cyclohex-2-e-nyl zinc reagent to install the side chain, they resorted to an allyl borylation. This unfortunately resulted in the incorrect stereochemistry at the C5 hydroxyl. In order to correct this, they then employed a unique oxidation and enzymatic reduction in order to provide salinosporamide A.

Hatakeyama's synthesis, while featuring an end game similar to those above, also contributed a unique reaction in their construction of Salinosporamide A. Hatakeyama employed an indium catalyzed intramolecular cyclization of a diester on to an alkyne in order to construct their key lactam. Their synthesis also featured a selective reduction of this geminal diester intermediate.

Another method of attachment of the cyclohex-2-enyl fragment was put forth by Omura and co-workers. Their method involved the aldol reaction between cyclohexanone and the oxazolidone aldehyde intermediate. This was followed by a sequence which involved conversion to the cyclic sulfate and then elimination under basic conditions to give the cyclohex-2-yl side chain. Their synthesis also featured an alternative method of attachment of the C2 side chain. This involved a Reformatsky reaction in order to attach this necessary fragment.

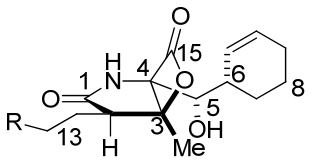
Romo and colleagues developed the shortest route to date with only a 9-step sequence. The highlight in his synthesis is the key bis-cyclization which constructs a C-C and C-O bond simultaneously to form the bicyclic core γ-lactam-β-lactone. Most importantly, in our enantioselective synthesis the application of $A^{1,3}$–strain in ketoamides to retain the optical purity at the C2 during the bis-cyclization process. The success in addition of zincate and Grignard reagent to the late stage aldehyde demonstrated the stability of the β-lactone and chloro substituent functionalities to the reaction conditions. This feature in our synthesis suggested the high potential for evaluation of P1 derivatives for their unique inhibition profiles across all three subunits CT-L, T-L, and C-L sites.[9c]

CHAPTER II

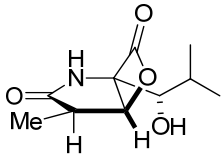
RACEMIC SYNTHESIS OF (±)-SALINOSPORAMIDE A, AND SIMPLIFIED DERIVATIVES*

2.1. Introduction

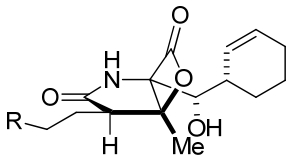
As mentioned previously, salinosporamide A (**1.1a**) besides superior bioactivity to its congener omuralide (**2.1**) (35 times more potent the IC_{50} value)[2], its challenging structure comprised of five contiguous stereogenic centers recently attracted intense interest from the synthetic chemists. Besides salino A (**1.1a**) we were also attracted to cinnabaramide A (**2.2**)[11], which showed more potent inhibition than omuralide (**2.1**), and we envisioned salinosporamide A as an ideal target for synthesis (Figure 2.1).



Salinosporamide A: ((**1.1a**), R = Cl)



Omuralide (**2.1**)
(Lactacystin-β-lactone)



Cinnabaramide A: ((**2.2**), R = $(CH_2)_3CH_3$)

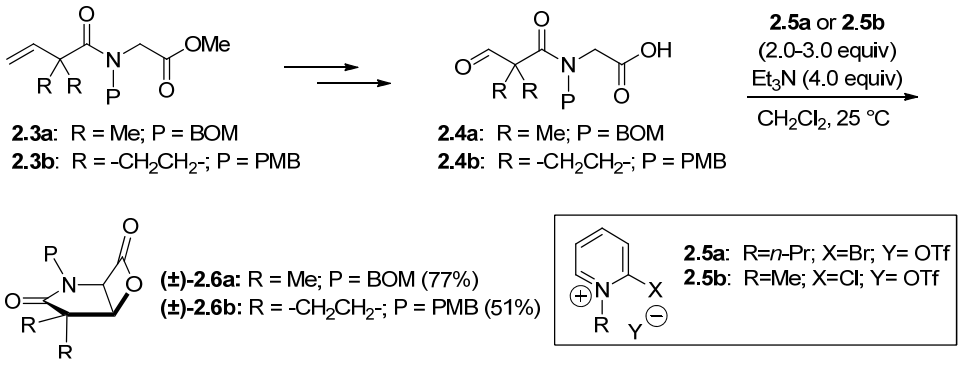
Figure 2.1. Structures of Bicyclic-β-Lactone Proteasome Inhibitors

Previously, our group reported a catalytic, asymmetric intramolecular, nucleophile -catalyzed aldol-lactonization (NCAL) process employing aldehyde acids that allows

*Reprinted with permission from American Chemical Society for "Concise Total Synthesis of (±)-Salinosporamide A, (±)-Cinnabaramide A, Derivatives via a Bis-cyclization Process: Implications for a Biosynthetic Pathway?" by Ma, G.; Nguyen, H.; Romo, D. *Org. Lett*, **2007**, *9*, 2143.

access to carbocycle-fused-β-lactones[12] and this process was recently extended to keto acid substrates.[13] This methodology was initially inspired by omuralide (**2.1**) and initial studies of employing the NCAL reaction in aldehyde acids containing such an amide functionality by Dr. Seongho Oh encountered difficulties in preparing substrates required from coupling of α,α-disubstituted acids and *N*-alkylated-α-substituted amino acids. The only accessible substrates were those derived from glycine **2.3a, 2.3b** and the addition of an α-cyclopropane moiety simplified the couplings. We were pleased to find that the intramolecular NCAL process does proceed with these substrates and provided the first bicyclic γ-lactam-fused-β-lactones **2.6a, 2.6b** via the NCAL process (Scheme 2.1). This provided an entry to simplified omuralide derivatives.

Scheme 2.1. Synthesis of Simplified Omuralide Derivatives

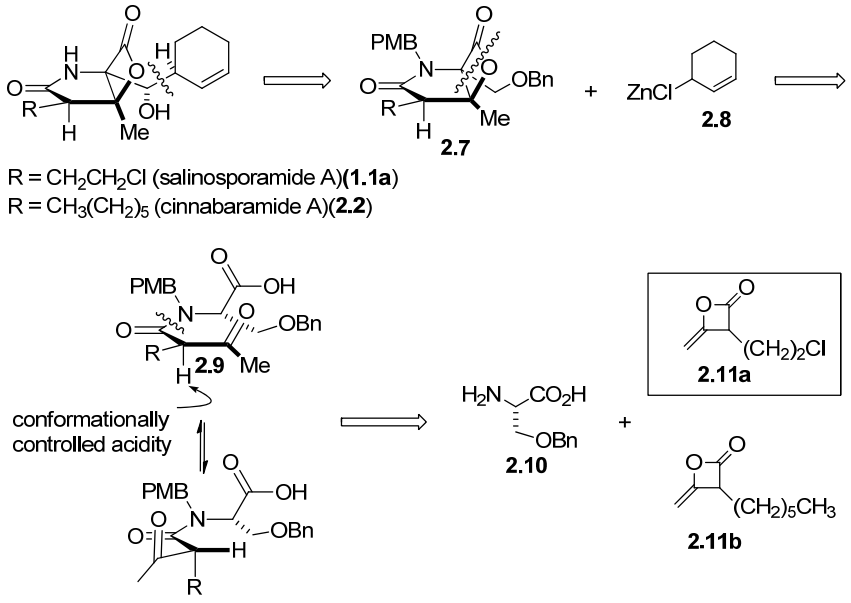


2.2. Retrosynthetic Analysis of Salinosporamide A and Cinnabaramide A

Building on our work with carbocyclic and heterocyclic fused-β-lactones, we envisioned a biomimetic synthesis of (±)-salinosporamide A[6e] that would allow a one-step route to the bicyclic β-lactones **2.8** by simultaneous formation of the C-C and C-O

bonds from a keto acid precursor **2.9** via an intramolecular bis-cyclization process. Attachment of the cyclohexenyl moiety, or other side-chains, would be possible by addition of appropriate zinc reagents **2**.**7**[14] to the aldehyde derived from benzyl ether **2**.**8**, however the success of this process and subsequent manipulations were not guaranteed given the potential reactivity of β-lactone. The keto acid substrate **2.9** could be derived from coupling of an amino acid **2.10** and a ketene dimer **2.11a**, the latter serving as a suitable latent equivalent for a β-ketoester.[15] One concern with this approach was the potential for enolization of the substrate β-ketoamide rendering the ketone non-electrophilic. However, due to the known conformationally controlled acidity of β-ketoamides due to $A^{1,3}$ strain,[16] such an approach appeared plausible (Scheme 2.2).

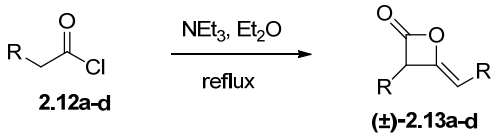
Scheme 2.2. Retrosynthetic Analysis of Salinosporamide A and Cinnabaramide A

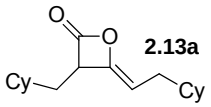
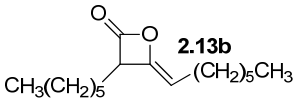
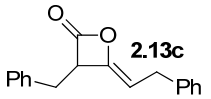
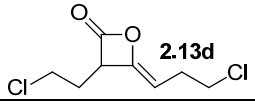


2.3. Initial Studies of Simplified C4-Unsubstituted Bicyclic β-Lactones

We first targeted simple C4-unsubstituted substrates for the bis-cyclization. Ketene homodimers **2.13(a-d)** were prepared by dimerization of acid chlorides **2.12 (a-d)**, triethylamine and reflux in ether by the method of Sauer (Table 2.1).[17] As expected, homodimers **2.13 (a-d)** were obtained in moderate to good yields (43-65%) after silica gel purification. Homodimer **2.13d** (entry 4, Table 2.1) which was chosen to test the mildness of the reaction sequences given the presence of the chloro-substituent in the required keto acid substrate leading to the most similar derivative to salinosporamide A (**1.1a**).

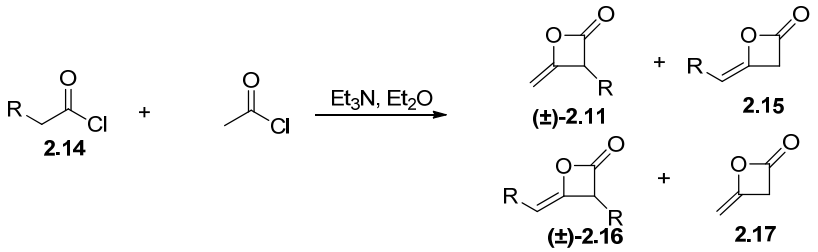
Table 2.1. Synthesis of Ketene Homodimers

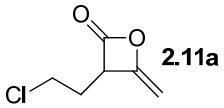
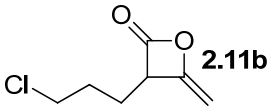
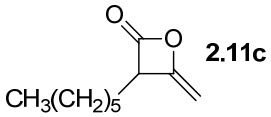


Entry	R	Dimer	% yield
1	$CyCH_2$	 **2.13a**	60
2	*n*-hexyl	 **2.13b**	65
3	$PhCH_2$	 **2.13c**	48
4	$Cl(CH_2)_2$	 **2.13d**	43

Subsequently employed (*vide infra*) heterodimers **2.11a** toward salinosporamide A and derivatives from acid chloride **2.14** and acetyl chloride (Table 2.2) however provided the expected statistical mixtures and further reductions in yields were also noted during purification leading to low absolute yields (5-13% yield, 20-52% based on a statistical, theoretical yield of 25%). The best yield was obtained from dimerization of 4-chlorobutanoyl chloride and acetyl chloride (13%, entry 1, Table 2.2) due to similar reactivity of the corresponding ketenes with products ratio (~ 1:1:1:1). Importantly, these dimerizations are readily run on multigram scale from inexpensive, commercially available acid chlorides and the desired heterodimers, despite low absolute yields, can be obtained in multigram quantities.

Table 2.2. Synthesis of Ketene Heterodimers

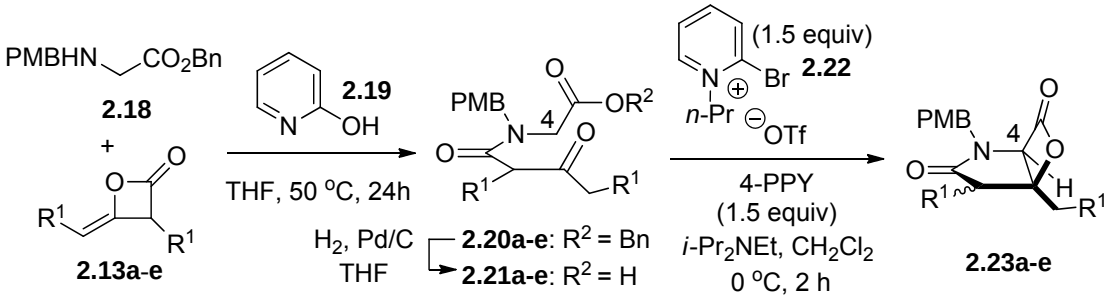


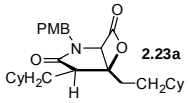
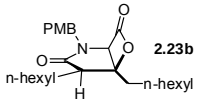
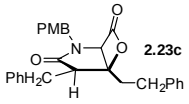
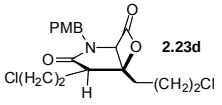
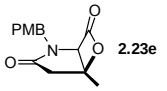
Entry	R	Dimer	% yield
1	$Cl(CH_2)_2$	 **2.11a**	13
2	$Cl(CH_2)_3$	 **2.11b**	10
3	*n*-hexyl	 **2.11c**	5

With racemic ketene homodimers **13a-e** in hand, these were coupled with *N*-PMB-glycine benzyl ester **2.18** by the method of Calter, which proceeded efficiently to provide keto acid substrates **21a-e** following hydrogenolysis of the benzylester (Table 2.3). Bis-cyclization of these ketoacids was achieved using conditions similar to those developed for carbocycles, with 4-pyrrolidinopyridine (4-PPY) as nucleophilic promoter at 23 ºC (not reported) and 0 ºC proceeded efficiently (70-93%) in most cases to give bicyclic-β-lactones **23a-e** with moderate diastereoselectivity (2.2-4:1). Ketoacid **21c** gave high diastereoselectivity (>19:1) but in reduced yield (54%; entry 3, Table 2.3), however monitoring this reaction by ^{1}H NMR at 23 ºC indicated that the high selectivity was due to selective degradation of the minor diastereomer (not shown) with prolonged reaction times. The C2-unsubstituted ketoacid **21e** gave only 25% yield (entry 5, Table

2.3) which may be due to facile enolization due to the absence of the C2-substitutent (R1), which likely leads to diminished rates of the initial aldol step. Importantly, ketoacid **21d** bearing two primary chlorides thus mimicking the substrate required for the proposed salino A synthesis also proceeded efficiently (entry 4, Table 2.3).

Table 2.3. Synthesis of Simplified, C4-Unsubstituted Bicyclic β-Lactones

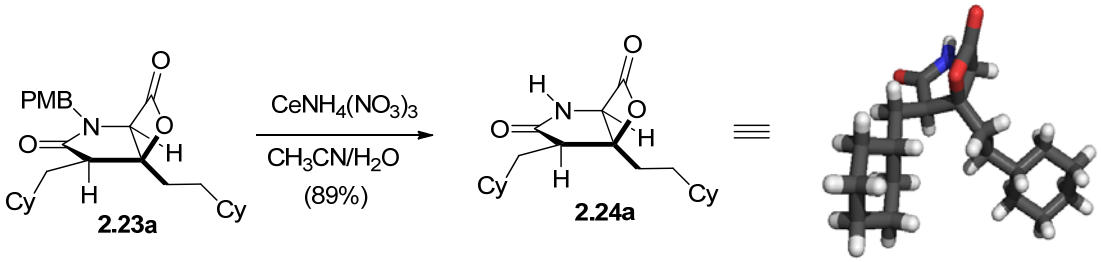


Entry	% yield (**2.21**)[a,b]	% yield (**2.23**)[b]	β-lactone	dr[c]
1	84 (**2.21a**)	93 (**2.23a**)		2.2:1
2	80 (**2.21b**)	90 (**2.23b**)		2.2:1
3	72 (**2.21c**)	85 (**2.23c**)		2.5:1 (>19:1)[d]
4	81 (**2.21d**)	70 (**2.23d**)		4:1
5	77 (**2.21e**)	25 (**2.23e**)		-

[a]Yield is for 2 steps. [b]Yields refer to isolated, purified (SiO_2) product. [c]Determined by ^{1}H NMR analysis of crude reaction mixtures. [d]Observed diastereomeric ratio (dr) if reaction is allowed to proceed at 23 °C for 36 h (54% yield). PMB = *p*-methoxybenzyl, 4-PPY = 4-pyrrolidinopyridine.

The relative stereochemistry of the major diastereomeric β-lactone **2.23a** was confirmed by X-ray analysis following cleavage of the PMB group (Scheme 2.3). Importantly, the relative stereochemistry corresponds to that found in salinosporamide.

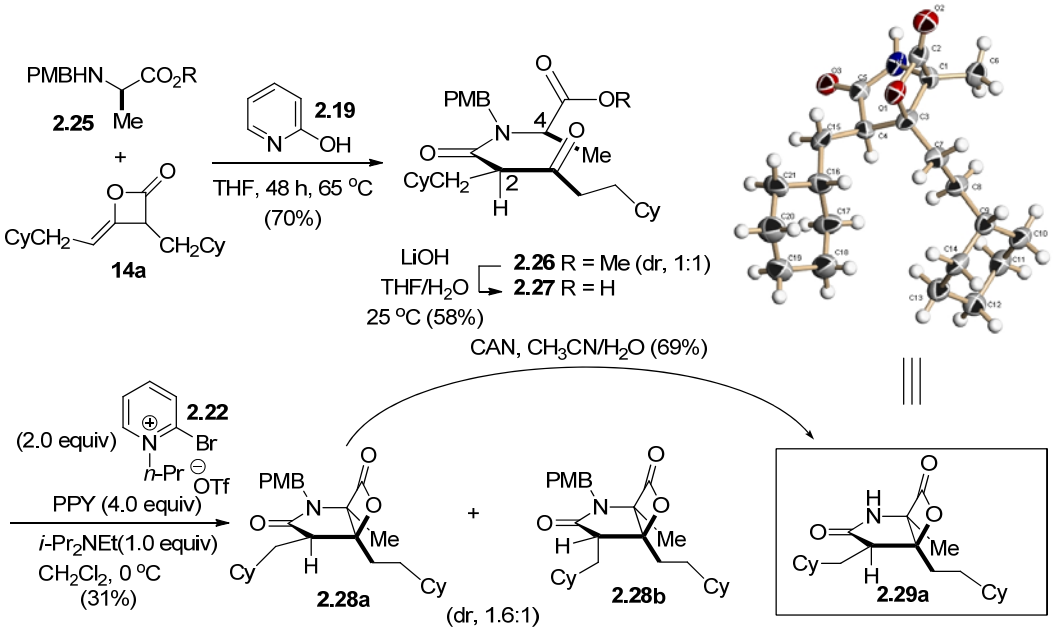
Scheme 2.3. X-ray Analysis of the C4-Unsubstited Bicyclic β-Lactone



2.4. Synthesis of C4 Substituted Salinosporamide Derivatives

Encouraged by the results with α-unsubstituted keto acid substrates (**2.21a-e**), we next studied more sterically demanding substrates derived from (*R*)-alanine. Ring opening of homodimer **2.13a** with (*R*)-*N*-PMB-alanine methyl ester **2.25** gave a 1:1 mixture of diastereomeric ketoamides **2.26** which were underwent saponification to diastereomeric keto acids **2.27** (Scheme 2.4). Pleasingly, bis-cyclization also proceeded with these substrates albeit in reduced yields (31%) to provide bicyclic β-lactone **2.28a** and **2.28b** (dr 1.6:1). The major diastereomer again possessed the relative stereochemistry found in the natural products as verified by X-ray analysis of the β-lactone **2.29** following PMB-deprotection (Scheme 2.4).

Scheme 2.4. Synthesis of Sterically Hindered C4-Substituted Derivatives

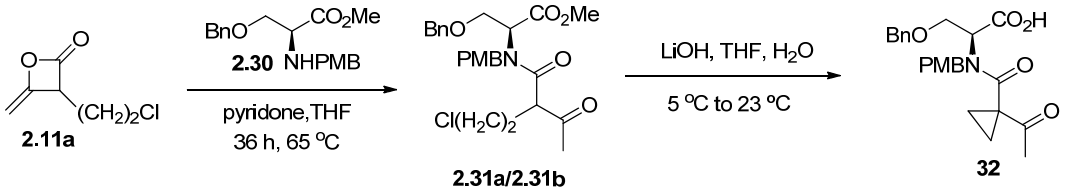


2.5. Racemic Synthesis of (±)-Salinosporamide A

To validate the mildness of this strategy, Dr. Gil Ma successfully completed the synthesis of cinnabaramide A (**2.2**).[6e] In a similar strategy, we targeted salinosporamide A **(1.1a)** bearing the required chloro-substituent in the keto acid substrate. The (*S*)-*N*-PMB-serine methyl ester **2.30** (synthesized by Dr. Gil Ma) was coupled with heteroketene dimer **(±)-2.11a**, readily available in gram quantities from heterodimerization of acetyl chloride and commercially available 4-chlorobutanoyl chloride, to provide the amides **2.31a** and **2.31b** as a mixture of two diastereomers (1:1 dr) in 84 % yield. The saponification of the methyl ester **2.31a** and **2.31b** using LiOH, however gave undesired keto acid **2.32** due to deprotonation of H_α of the ketone

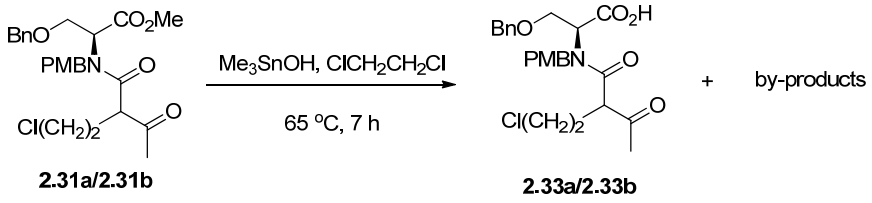
following intramolecular S_N2 displacement of chloride to afford possible cyclopropane by-product **2.32** (Scheme 2.5).

Scheme 2.5. Synthesis of Ketoacids **2.31a/2.31b**



Alternatively, hydrolysis of ester **2.31a** and **2.31b** using Me_3SnOH[18] which was employed successfully in preparation of the keto acid subtrate in synthesis of cinnabaramide A (**2.2**) also gave an irreproducible low yield of the desired keto acids **2.33a** and **2.33b** plus unknown by-products (Scheme 2.6).

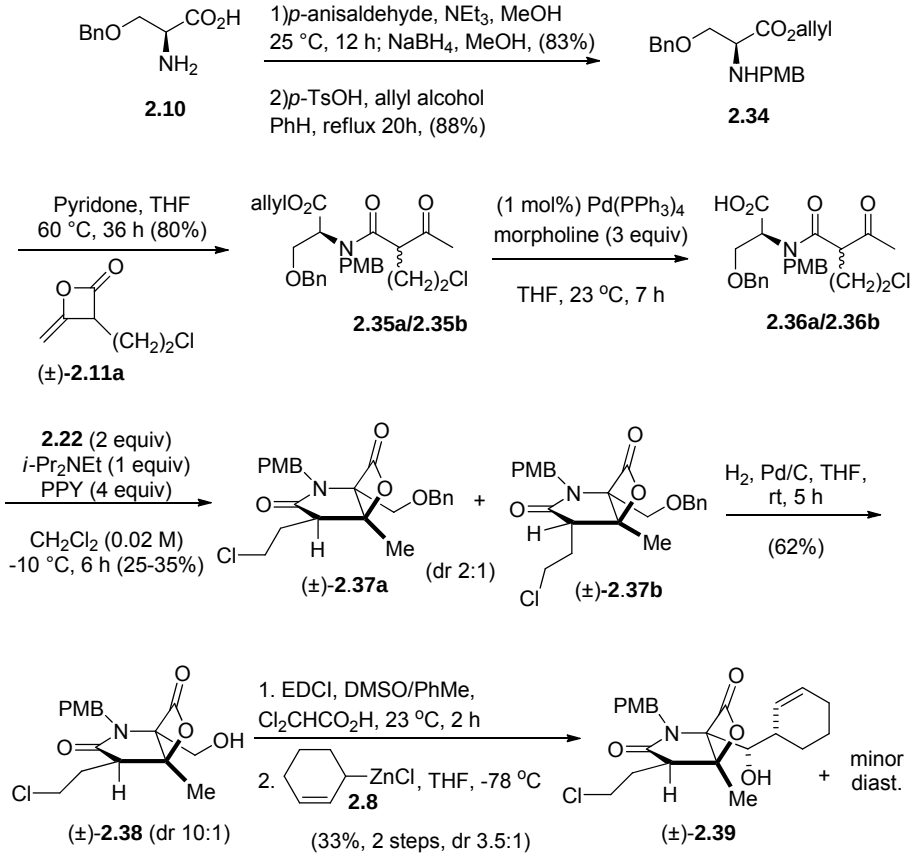
Scheme 2.6. Sn-Mediated Hydrolysis of Ketoamides **2.31a/2.31b**



Due to sensitivity of the substrate under basic conditions during saponification, instead (*S*)-*N*-PMB serine allyl ester **2.34** was prepared by reductive amination of commercially available (*S*)-*O*-benzyl serine **2.10** with *p*-anisaldehyde followed by

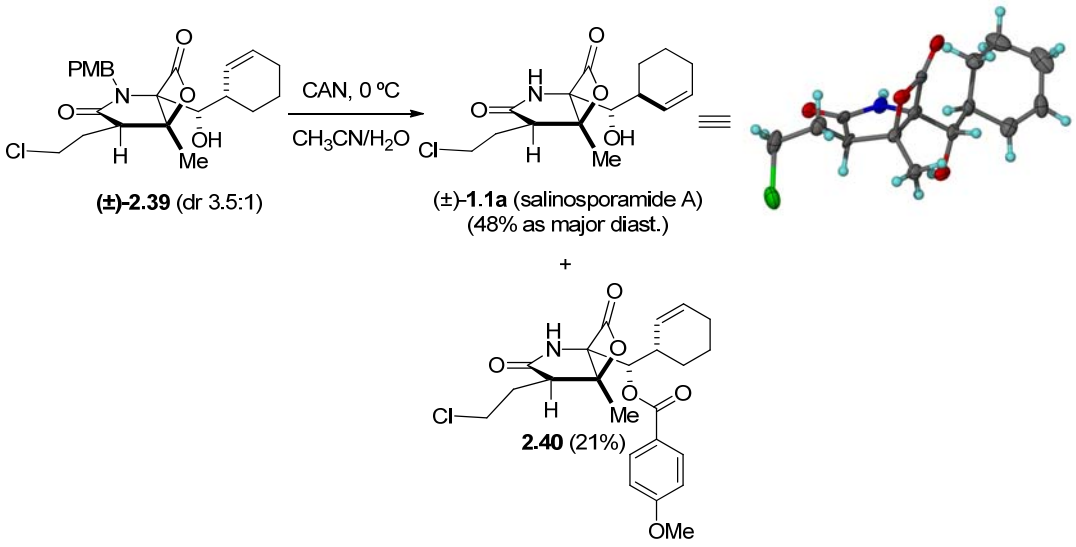
esterification with allyl alcohol. The (*S*)-serine allyl ester **2.34** was coupled with heterodimer (±)-**2.11a** to provide a keto amide mixture **2.35a/2.35b** in 80% yield (dr 1:1). The keto acids **2.36a/2.36b** were obtained from Pd-mediated allyl ester deprotection. Bis-cyclization provided β-lactones **2.37a**/**2.37b** in 25-35% yield (dr 2:1) along with multiple by-products after some optimizations on reaction conditions (by-products and optimized conditions for this reaction will be discussed in Chapter IV). Deprotection of the benzyl ether enabled enrichment of the major diastereomer alcohol **2.38** to (dr 10:1) upon MPLC purification. Parikh-Doering oxidation of the alcohol **2.38** did not give the aldehyde but decomposition of β-lactone. Finally, modified Moffatt[19] oxidation using 1-(1,3-dimethylaminopropyl)-3-ethyl carbodiimide hydro-chloride (EDCI)[20] and dichloroacetic acid[21] granted the corresponding aldehyde and recovered alcohol **2.38**. Subsequent addition of zinc reagent **2.8** applying Corey protocol gave predominantly two diastereomeric alcohols **2.39** (33% yield, dr 3.5:1, 2 steps) (Scheme 2.7).

Scheme 2.7. Synthesis of Hydroxy-β-Lactone (±)-**2.39**



Final deprotection of the PMB group using ceric ammonium nitrate (CAN) enabled isolation of diastereomerically pure *rac*-salinosporamide A (48%) along with an isolated by-product ester **2.40** resulting from C5 free alcohol. X-ray analysis of single structure correlated with the published data and the relative stereochemistry of the natural product (Scheme 2.8).

Scheme 2.8. Oxidative PMB Deprotection and Completion of the Synthesis of (±)-Salino A **(1.1a)**



2.6. Conclusion

We have succesfully developed concise synthetic routes to *rac*-salinosporamide A, and its simplified derivatives. This strategy is unique in enabling simultaneous construction of both the γ-lactam and fused-β-lactone found in these metabolites via a bis-cyclization process. The β-lactone in these systems and the chloro-substituent in salinosporamide precursors has been shown to be tolerant to several transformations which contributes to the brevity of the sequence.[22] The described bis-cyclization process points to a logical biosynthetic origin for these intriguing natural products and raises the interesting question of whether such a bis-cyclization might be involved in the biosynthesis of these natural products.[23] Further optimization of this synthetic strategy

including the low yield for the oxidation of alcohol to the corresponding aldehyde, followed by attachment of the cyclohexenyl side-chain, and oxidative PMB deprotection. Most importantly, the extension to an enantioselective strategy premised on $A^{1,3}$-strain in keto acid precursors is a great challenge.

CHAPTER III

$A^{1,3}$-STRAIN ENABLED RETENTION OF CHIRALITY DURING BIS-CYCLIZATION OF β-KETOAMIDES: ASYMMETRIC SYNTHESIS OF (–)-SALINOSPORAMIDE A*

3.1. Introduction

Building on biosynthetic considerations and *rac*-synthesis of salinosporamide A (**1.1a**), the key step in our asymmetric synthesis[6j] is a diastereoselective, nucleophile-promoted, bis-cyclization process that mandates preservation of optical purity of a potentially stereochemically labile β-keto acid substrate **3.2** imposed by $A^{1,3}$ strain (Figure 3.1). This is crucial since the C4 stereocenter in β-keto acid **3.2** is lost during the bis-cyclization process, thus the integrity of the labile C2 stereocenter dictates the optical purity of β-lactone **3.1**. This strategy delivers the bicyclic β-lactone **3.1** in a single operation from acyclic precursors and the optical purity of the β-lactone reflects the diastereomeric purity of β-keto amide **3.2**, derived from the acylation of serine derivatives and racemic ketene dimer **2.11a**.

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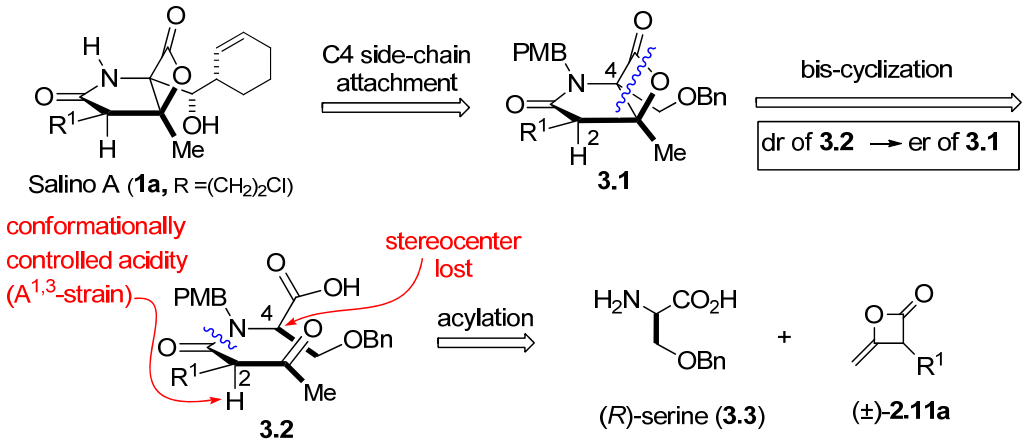


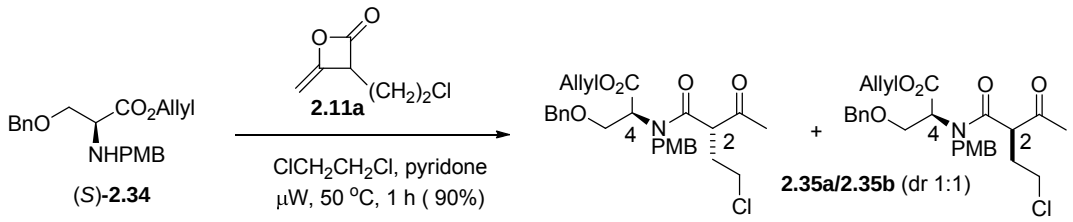
Figure 3.1. Enantioselective Strategy to Salino A

3.2. Enantioselective Syntheses of (–)-Salinosporamide A

3.2.1. Preliminary Result: Synthesis of (+)-Salinosporamide A Employing (*S*)-*O*-Benzyl Serine

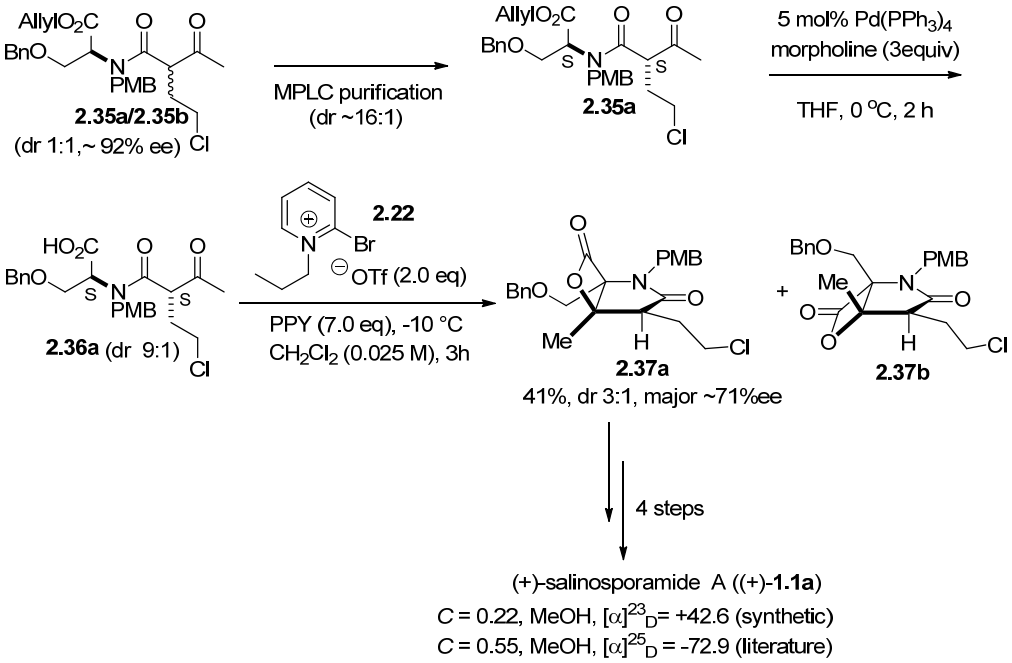
Similar to the racemic synthesis of salinosporamide A, the enantioselective synthesis commenced from reductive amination of (*S*)-*O*-benzyl serine with *p*-anisaldehyde followed by esterification with allyl alcohol to prepare *N*-PMB allyl ester (*S*)-**2.34**. Given that known potential racemization of serine derivative, milder conditions were employed; however, HPLC methods to resolve (*R*)-**2.34** and (*S*)-**2.34** were not successful after several attempts. (*S*)-serine allyl ester **2.34** was coupled with racemic heterodimer (±)-**2.11a** for a shorter reaction time using microwave in order to afford keto amide **2.35a** and **2.35b** as a mixture of two diastereomers (dr 1:1, 90% yield) (Scheme 3.1).

Scheme 3.1. Microwave Mediated Synthesis of β-Ketoamides



To access the enantioselective bis-cyclization reaction, separation of the two diastereomeric β-ketoamides employing MPLC using EtOAC/hexanes = 1:9 enabled enrichment of the higher R_f keto ester **2.35a** to dr 16:1 (~ 92% ee resolution conditions were developed by chiral HPLC). Since the absolute stereochemistry at the C2 was not determined, the higher R_f keto ester **2.35a** was the substrate of choice for a Pd mediated allyl deprotection to provide keto acid **2**.**36a** with erosion of diastereomeric ratio to 9:1. The bis-cyclization of the keto acids **2**.**36a,** when 4-PPY was used without *i*-Pr_2NEt, and modified Mukaiyama salt **2**.**22** improved the yield of β-lactones **2**.**37a**/**2**.**37b** to 41% (dr 3:1) favoring the one found in the natural product. Most importantly, enatiopurity was conserved due to $A^{1,3}$-strain as expected to provide β-lactone **2.37a** (71% ee confirmed by chiral HPLC). Subsequent conversions afforded salinosporamide A employing the synthetic strategy and same reaction conditions developed in the racemic synthesis as discussed in Chapter II. Optical rotation of final product $[\alpha]^{23}_D$ = +42.6, *c* = 0.22, MeOH versus $[\alpha]^{25}_D$ = -72.9, *c* = 0.55, MeOH of natural product indicated that the enantiomer of naturally occurring salinosporamide A had been obtained in this sequence (Scheme 3.2).

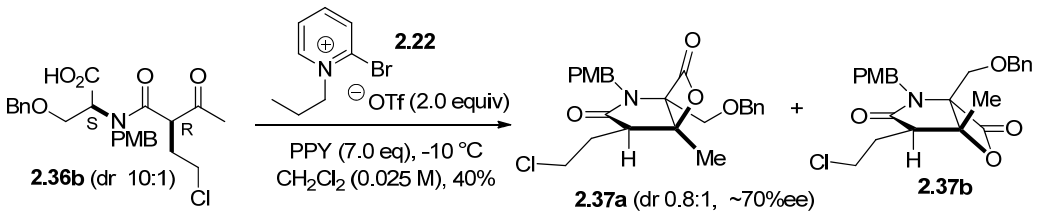
Scheme 3.2. Synthetic Execution of (+)-Salinosporamide A



3.2.2. Preliminary Result: Synthesis of (–)-Salinosporamide A Employing (*S*)-*O*-Benzyl Serine

Synthesis of (–)-salinosporamide A (**1.1a**) then utilized the lower R_f value β-ketoamide (*2R,4S*)-**2.35b** (dr 18:1) which underwent allyl deprotection to provide keto acids (*2R,4S*)-**2.36b** with a reduction of dr 10:1. Unexpectedly, the bis-cyclization of keto acid **2.36b** gave β-lactones **2.37a**/**2.37b** (40% yield, dr 0.8:1) favoring the undesired diastereomer **2.37b** (scheme 3.3).

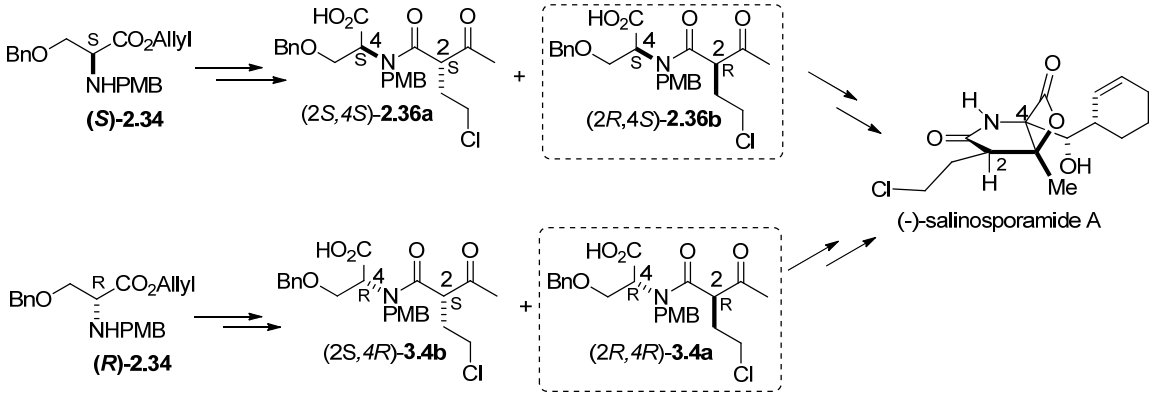
Scheme 3.3. Bis-Cyclization of β-Ketoacid **2.36b**



Subsequent conversions to the corresponding aldehyde via oxidation of alcohol after benzyl deprotection. Side chain attachment and followed by oxidative PMB deprotection to give salinosporamide A ((–)-**1.1a**) employing the same conditions developed in the racemic synthesis (Chaper II). Optical rotation of final product $[\alpha]^{23}_{D}$ = -32.1 corresponds to $[\alpha]^{25}_{D}$ = -72.9 (literature) which indicated that the natural product had been obtained in this sequence.

To approach the asymmetric synthesis of γ-lactam fused β-lactone bicyclic core of **(**–**)**-salinosporamide A, ether ketoacid (2*R*,4*S*)-**2.36b** or (2*R*,4*R*)-**3.4a** could be desired subtrates (Scheme 3.4) since the C2 center would be retained during the bis-cyclization due to $A^{1,3}$-strain. Although the C4 center is lost during the bis-cyclization, preliminary results showed diastereoselectivity highly depended on whether (*R*) or (*S*)-serine derived ketoacids were employed leading to different diastereoselectivity, 3:1 vs 0.8:1. Therefore, to address the diastereomeric selectivity of the bis-cyclization, we modified our synthetic strategy commencing from (*R*)-*O*-benzyl serine, and (2*R*,4*R*)-**3.4a** was our desired keto acid for the bis-cyclization (scheme 3.4).

Scheme 3.4. Summary of β-ketoacid Derived from (*R*) and (*S*)-*O*-Benzyl Serine

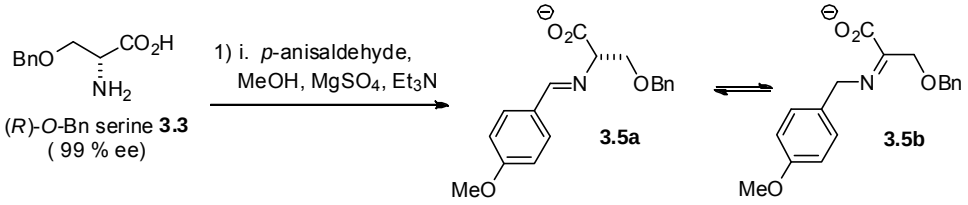


3.2.3. Enantioselective Synthesis of (–)-Salinosporamide A Employing (*R*)-*O*-Benzyl Serine

As mentioned previously, a key step in our strategy is a diastereoselective, nucleophile-promoted, bis-cyclization of β-keto acid (2*R*,4*R*)-**3.4a** (Scheme 3.4). This process delivers the bicyclic-β-lactone **2.37a** in a single operation from acyclic precursors and the optical purity of this β-lactone would reflect the diastereomeric and enatiomeric purity of β-ketoacid (2*R*,4*R*)-**3.4a.** We were set to synthesize **(*R*)-2.34** by reductive amination of (*R*)-*O*-benzyl serine **3.3** (99% ee) with *p*-anisaldehyde employing the procedure developed previously, and subsequent esterification to provide allyl ester **(*R*)-2.34**. At this point, we extensively explored HPLC conditions in conjunction with commercially available chiral columns to resolve the racemic *O*-benzyl serine allyl ester (±)**-2.34**. Fortunately, we found conditions which separated the two enantiomers showing that the enantiomeric excess of **(*R*)-2.34** had dropped to 87% ee. It is known that challenges in racemization / epimerization are in the reductive amination step could

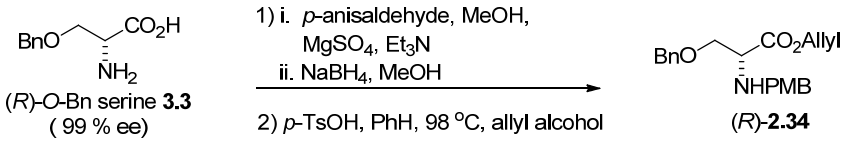
be due to imine isomerization[24] between **3.5a** and **3.5b** isomers prior to reduction to the corresponding amine (scheme 3.5).

Scheme 3.5. Challenges of Racemization in Reductive Amination Reactions



At the outset of our studies, we carefully explored reducing agents other than $NaBH_4$ such as $NaCNBH_3$, however a lower conversion was obtained (entry 1, Table 3.1). Therefore, $NaBH_4$ was the reducing agent of choice for the optimized studies. The outcome of entry 2 and 3 highlighted the importance of low temperature during the imine reduction stage to minimize racemization of the corresponding amine. Finally, conditions were found to prepare *O*-benzyl serine allyl ester (*R*)-**2.34** with minimal racemization in 79% yield (2 steps, 98% ee) (entry 5, Table 3.1).

Table 3.1. Optimization of *N*-PMB-(*R*)-Serine Allyl Ester Synthesis



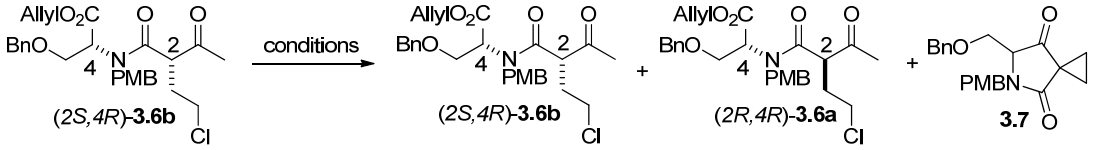
entry	conditions (step 1)	% yield (*R*)-**2.34**[a,b]	% ee (*R*)-**2.34**
1	8h, 0 ºC then $NaCNBH_3$, AcOH 0 ºC – 23 ºC, 3 h	low conversion	–
2	8h, 0 ºC then $NaBH_4$, 0 ºC – 23 ºC, 1 h	70%	87%
3	8h, 0 ºC then $NaBH_4$, 0 ºC, 2 h	48%	98%
4	8h, 0 ºC then $NaBH_4$, 0 ºC, 5 h	60%	98%
5	**8h, 0 ºC then $NaBH_4$, 0 ºC, 2 h; overnight in freezer (~ -10 ºC)**	**79%**	**98%**

[a]Yield is for 2 steps. [b]Yields refer to isolated, purified (SiO_2) product.

Acylation of serine derivative (*R*)-**2.34** (98% ee) with heterodimer (±)-**2**.**11a** under microwave conditions (50 ºC, 1 h) in place of conventional heating (50 ºC, 48 h) gave diastereomeric β-ketoamides (2*R*,4*R*)-**3.6a**/(2*S*,4*R*)-**3.6b** (dr 1:1, 90%). Most importantly, the microwave mediated coupling reaction could be run in 3-4g scale with only a slight reduction in yields (dr 1:1, 80%). Separation by MPLC using a different solvent system (95% CH_2Cl_2/EtOAc) enabled the enrichment of the required β-keto amide (2*R*,4*R*)-**3.6a** (45%, 30:1 dr, 98% ee). To demonstrate the utility of our synthetic strategy, we investigated conditions to recycle the undesired ketoamide (2*S*,4*R*)-**3.6b.** Under basic conditions, ketoamide (2*S*,4*R*)-**3.6b** (dr 23:1) could be converted to a mixture of **3.6a/3.6b** (78%, dr 1:1) along with by-product cyclopropyl ketoamide **3.7** (19%) (entry 2, Table 3.2). However, a more hindered base such as *i*-Pr_2NEt did not efficiently epimerize C2 center after 48 h at 23 ºC (entry 3, Table 3.2). Eventually, equilibrium under acidic conditions was obtained to avoid by-product **3.7**, and the

undesired diastereomer (2*S*,4*R*)-**3.6b** could be transformed to a 1:1 mixture of diastereomers via epimerization of the C2 but not the C4 stereocenter (verified by chiral HPLC) thus achieving an effective resolution of ketene dimer (±)-**2.11a** and greater material throughput (entry 4, Table 3.2).

Table 3.2. Epimerization of Undesired β-Ketoamide (2*S*,4*R*)-**3.6b**



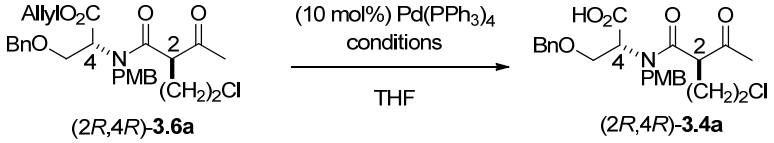
entry	β-keto amides (dr **3.6b:3.6a**)	Conditions	Reaction outcome	
			3.6b/3.6a[a]	**3.7**[a]
1	23:1	morpholine (6 equiv), CH_2Cl_2, 23 ºC, 12 h	49% (dr 1:1)	45%
2	23:1	morpholine (6 equiv), CH_2Cl_2, -10 ºC, 48 h	78% (dr 1:1)	19%
3	20:1	*i*-Pr_2NEt (6 equiv), CH_2Cl_2, 23 ºC, 48 h	dr 12:1	5%
4	20:1	*p*-TsOH, EtOAc/MeOH (4:1), 45 ºC, 48 h	98% (dr 1:1)	–

[a]Yields refer to isolated, purified (SiO_2) product.

Towards the required keto acid (2*R*,4*R*)-**3.4a** precursor for bis-cyclization, one hurdle needed to be overcome was the epimerization during deprotection of the enriched keto amide (2*R*,4*R*)-**3.6a** under mildly basic conditions as mentioned in preliminary studies of asymmetric synthesis. Previously, 3.0 equiv of morpholine was used as a nucleophilic scavenger for the allylic cation. To avoid epimerization, 1.2 equiv of morpholine was used instead of excess morpholine (entry 1 and 2).[25] Another option was employing 1,3-dimethylbarbituric acid (entry 4) as the nucleophile in place of

morpholine, conditions developed for base-labile substrates, unfortunately a reduction in diastereomeric purity was also observed.[26] Notably, ketoacid (2*R*,4*R*)-**3.4a** could be epimerized with silica gel; therefore, allyl deprotection conditions were desired that were column free. Finally, a mild and brief exposure of β-ketoamide (2*R*,4*R*)-**3.6a** (dr ~ 32:1) to a Pd(0)-mediated deprotection provided keto acid (2*R*,4*R*)-**3.4a** with similar diastereomeric purity, which was used in the next step without further purification (entry 5, Table 3.3). This condition was also applied successfully on gram scale synthesis (entry 6, Table 3.3).

Table 3.3. Pd-Meditated Allyl Deprotection Optimization



(2*R*,4*R*)-**3.6a** (2*R*,4*R*)-**3.4a**

entry	β-keto amide (2*R*,4*R*)-**3.6a** (dr)[a]	reaction conditions	β-keto acid (2*R*,4*R*)-**3.4a** (dr)[a]
1	30:1	morpholine (1.2 equiv), -5 ºC, 2 h	20:1
2	30:1	morpholine (1.2 equiv), 0 ºC, 3 h	13:1
3	26:1	Bu_3SnH (2 equiv), 0 ºC, 2 h	impure prod.
4	26:1	1,3-dimethylbarbituric acid (3 equiv), 0 ºC, 2 h	16:1
5	***32:1***	***morpholine (1.2 equiv), -5 ºC, 1 h, 0.5M***	***30:1***
6[b]	**30:1**	**morpholine (1.2 equiv), -5 ºC, 70 min, 0.5M**	**30:1**

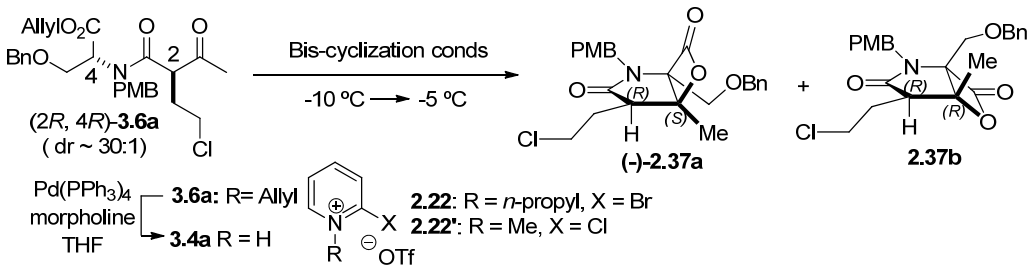
[a]Determined by ^{1}H NMR analysis of crude reaction mixtures. [b]Conditions for gram-scale synthesis.

At the outset, it was unclear whether the $A^{1,3}$-strain induced conformational bias in β-keto tertiary amides (~4 kcal mol^{-1}) would be sufficient to avoid epimerization of

this center in the time frame and under the basic conditions of the bis-cyclization. I reasoned that an understanding of reaction mechanism of the bis-cyclization would give me the insight how to optimize this key reaction, thefore mechanistic studies (which will be discussed in Chaper IV) of the bis-cyclization were performed in parallel with asymmetric synthesis. Applying bis-cyclization conditions that were recently developed in preliminary studies of the asymmetric synthesis of salino A (**1.1a)** to keto acid (2*R*,4*R*)-**3.4a** (dr 30 : 1, 98% ee) as expected led to similar yields and diastereoselectivity (40% yield, dr 2.5 : 1); however erosion of optical purity of adduct **2.37** was observed (85% ee) (Table 3.4, entry 1). In this condition, 4-PPY was used as both base and nucleophile since diisopropylethylamine showed inefficient deprotonation at the C2 stereocenter when epimerization reaction of the β-ketoamide (2*S*,4*R*)-**3.6b** was explored previously. In our earlier report, although the modified Mukaiyama salt **2.22** used above to activate the acid in conjuction with dichloromethane are critical for aldehyde acid substrate to be obtained in high yield in NCAL reaction,[12a] and later was proven to facilitate the bis-cyclization of the ketoacid substrates,[13] it took two steps to prepare. Application of recently developed optimized conditions that rendered the process more practical with the recognition of the importance of the counterion of the Mukaiyama reagent **2.22'** which required acetonitrile as solvent[12b] to ketoacid (2*R*,4*R*)-**3.4a** (dr 30 : 1, 98% ee) gave comparable yields with 5% decrease in enantiomeric access (entry 2, Table 3.4). The outcome of entries 1 and 2 showed that a less polar solvent could potentially slow down the epimerization of ketoacid (2*R*,4*R*)-**3.4a**. Since modified Mukaiyama salt **2.22** was not soluble in toluene, which was used with acetonitrile in the

bis-cyclization of ketoacid (2*R*,4*R*)-**3.4a** (dr 30:1) surprisingly improved the diastereoselectivity to 7:1 and gave high enantiopurity (92% ee), however only a 25% yield and 42% recovered ketoacid **3.4a/3.4b**. The low conversion was probably due to solubility of the Mukaiyama salt **2.22** in toluene/acetonitrile (5:1) (entry 3, Table 3.4). The yield was improved with longer reaction times, and when more acetonitrile was used but resulted in lower diastereomeric and enantiomeric ratios (entry 4, Table 3.4).

Table 3.4. Optimization of the Bis-Cyclization Reaction Conditions Employing Mukaiyama Salts as Activating Agents



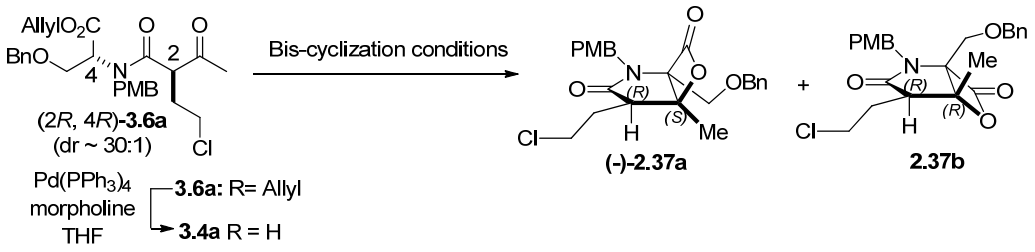
entry	Bis-cyclization conditions	**2.37a/b**[a,b]	**2.37a/b** (dr, ee)[c]	rec. **3.4a/b**[b]
1	4-PPY, **2.22**, CH_2Cl_2, 3.5 h	41%	dr 2.5:1, 85% ee	-
2	4-PPY, **2.22'**, CH_3CN, 3.5 h	40%	dr 2:1, 80% ee	-
3	4-PPY, **2.22**, PhMe/CH_3CN (5:1), 2 h	25%	dr 7:1, 92% ee	42%
4	4-PPY, **2.22**, PhMe/CH_3CN (3:1), 3.5 h	46%	dr 4:1, 85% ee	-

[a]Yield is for 2 steps (deprotection and bis-cyclization). [b]Yields refer to isolated, purified (SiO_2) product. [c] % ee refers to (–)-**2.37a.**

Encouraged by the outcome of the bis-cyclization in toluene, in that a less polar solvent might slow down epimerization at C2, we attempted to seek an alternative commercially available activating reagent, trifluoromethane sulfonic andhydride, the

reaction occured at -20 ºC with promising results of 37% yield, dr 7:1 and high enantioretention at C2 (92% ee) after 70 min. As was expected longer reaction times lead to higher yields but erosion in enatiomeric and diastereomeric ratio. Next, the bis-cyclization was explored with the less reactive activating agents such as *p*-toluene sulfonyl chloride (TsCl), mesyl chloride (MsCl), and *p*-nitrosulfonyl chloride the reaction worked well with ether MsCl (Table 3.6) or TsCl (61% yield, 85% ee, entry 4, Table 3.5). However, due to the ease of product purification MsCl was the activating agent of choice for our asymmetric synthesis.

Table 3.5. Optimization of the Bis-Cyclization Reaction Conditions Employing Commercially Available Activating Agents

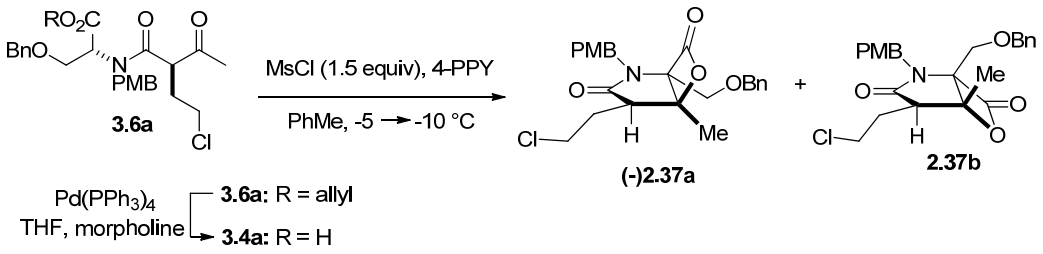


entry	bis-cyclization conditions	**2.37a/b** [a,b]	**2.37a/b** (dr, ee [c])	rec. SM [b]
1	4-PPY, $(CF_3SO_2)_2O$, PhMe, 70 min, -20 ºC	37%	dr 7:1, 92% ee	31%
2	4-PPY, $(CF_3SO_2)_2O$, PhMe, 2 h, -20 ºC	44%	dr 5:1, 90% ee	29%
3	4-PPY, TsCl, PhMe, 1.5 h, -10 ºC to -5 ºC	30%	dr 7:1, 92% ee	37%
4	4-PPY (3.5 equiv), TsCl, PhMe, 3.5 h, -10 ºC to -5 ºC	61%	dr 4:1, 85% ee	11%
5	4-PPY, $4\text{-}NO_2PhSO_2Cl$, PhMe, -10 ºC to -5 ºC, 70 min	40%	dr 5:1, 90% ee	19%

[a]Yield is for 2 steps (deprotection and bis-cyclization). [b]Yields refer to isolated, purified products. [c]%ee refers to **2.37a**.

After patient and extensive optimization of the reaction conditions for the bis-cyclization, we were pleasantly surprised to find that mesyl chloride at lower temperature and most importantly in a less polar solvent (toluene) led to a dramatic increase in both diastereoselectivity (7:1) and retained enantiopurity of bicyclic β-lactone 3a (92% ee, 35% yield, 28% recovered keto acid, Table 3.6, entry 1). In addition, a yield of 60% was obtained with longer reaction times under these conditions; however this led to reduced diastereoselectivity and enantiopurity (dr 4:1, 88% ee, Table 3.6, entry 2). Importantly, this reaction could be performed on gram scale with comparable diastereoselectivity and retention of enantiopurity (52%, dr 5:1, 90% ee, Table 3.6, entry 3).

Table 3.6. Varying Conditions for the Bis-Cyclization/Gram-Scale Synthesis Employing MsCl

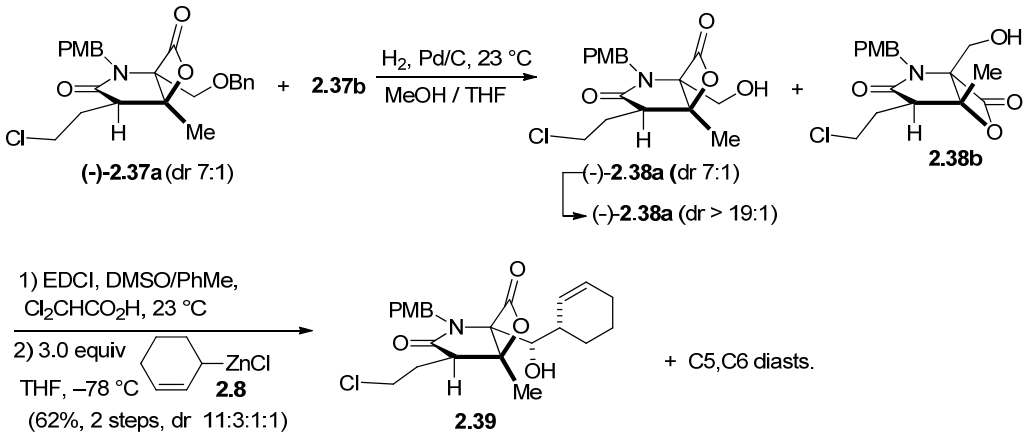


entry	time (h)	% yield[a,b]	% ee	dr	% recovered ketoacid
1	1.5	35	92	7:1	28
2	3.5	60	88	4:1	10
3[c]	3.5	52	90	5:1	11

[a]Yield is for 2 steps (deprotection and bis-cyclization). [b]Yields refer to isolated, purified β-lactones. [c]This run was performed on gram scale.

Completion of the salino A synthesis entailed hydrogenolysis of the benzyl group, which facilitated isolation of alcohol (–)-**2.38a** in 75% yield. Modified Moffatt oxidation[27] with 0.5 equiv of dicloroacetic acid granted the complete conversion to the corresponding aldehyde which was used in the subsequent step without further purification. Addition of the zinc reagent **2.8** derived from the reaction of *n*-butyllithium with the prior prepared cyclohexenyl tributyltin and $ZnCl_2$ following the procedure of Corey[6a], to the aldehyde gave a mixture of C5, C6-diastereomeric alcohols (dr 11:3:1:1) in 62% yield (2 steps, Scheme 3.6).

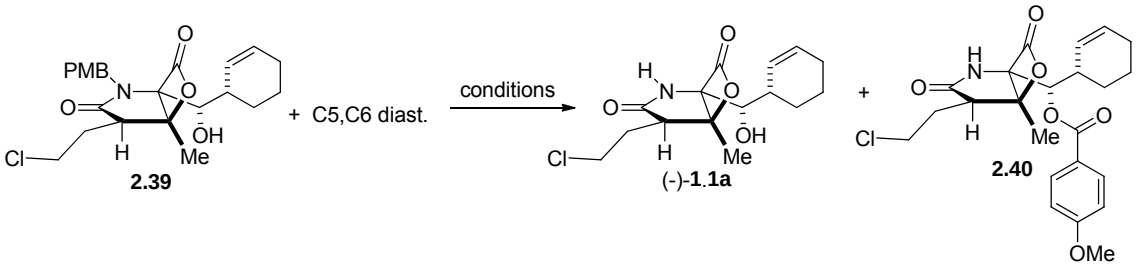
Scheme 3.6. Elaboration of the Core β-Lactone (–)-**2.38a** to PMB (–)-Salinosporamide A **2.39**.



As mentioned previously in Chapter II, the reduced yield in oxidative PMB deprotection was due to the formation of an ester by-product **2**.**40** in the presence of the C5 free alcohol. These conditions were modified by addition of excess methanol or *iso*-propanol in attempts to capture the benzylic cation intermediate. Alternatively,

employing 2,3-dichloro-5,6-dicyanobenzoquinone also gave similar result. Finally, mixture solvent MeOH/H_2O slightly improved the yield of desired product (43%, dr ~ 15:1) and reduced the formation of ester **2.40** to 11% (entry 3, Table 3.7). Pure (–)-salinosporamide A (**1.1a)** was then obtained via recrystallization (syn. $[\alpha]^{23}_D$ = -71.3, *c* = 0.39, MeOH); lit. $[\alpha]^{25}_D$ = -72.9 (*c* = 0.55, MeOH).[2]

Table 3.7. Optimization of Oxidative PMB Deprotection



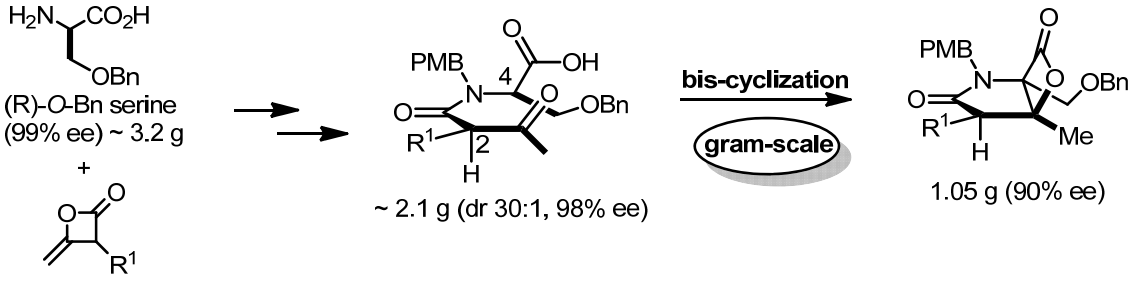
entry	PMB-lactam (dr **2.39**:C5,C6 diasts.)	Conditions	Reaction outcome	
			salino A **(–)-1.1a**	ester **2.40**
1	12:3:1:1	CAN (5 equiv), CH_3CN/H_2O, -10 ºC, 2h	34%	18%
2	11.5:3:1:1	CAN (5 equiv), *i*-PrOH/H_2O, -10 ºC, 6h	36%	19%
3	11:3:1:1	CAN (5 equiv), MeOH/H_2O, -10 ºC, 6h	43%	11%

3.3. Conclusion

In summary, we have developed a concise, 9-step enantioselective route to (–)-salinosporamide A (92% ee, 3.2%) from (*R*)-*O*-benzyl serine (99% ee). The key bis-cyclization of a β-ketoamide, amenable to gram scale, constructs both the γ-lactam and the fused-β-lactone in one operation with a high degree of stereoretention and moderate diastereoseletivity up to 7:1, which contributed to the brevity of the synthesis (Scheme

3.7). The ability to optimize the bis-cyclization in asymmetric fashion given readily epimerization at C2 center based on deep understanding and extensive studies of the mechanistic rational and how this system is operated are discussed in detail in Chaper V. Moreover, the ability of the described β-keto tertiary amide substrates to maintain stereochemical integrity by virtue of $A^{1,3}$-strain raises the intriguing question of how such stereochemical integrity is maintained with β-keto secondary amides, known salino A precursors, prior to and during related biosynthetic cyclizations or if a dynamic kinetic resolution are operative.

Scheme 3.7. Gram-Scale Synthesis toward Bicyclic Core β-Lactone.



CHAPTER IV

MECHANISTIC STUDIES OF THE KEY BIS-CYCLIZATION AND FURTHER OPTIMIZATION TOWARDS SALINOSPORAMIDE A*

4.1. Introduction

Our racemic syntheses of cinnabaramide A and salinosporamide A demonstrated the viability of our synthetic approach in which the potentially labile β-lactone and primary chloride were stable through several final steps in the synthesis including organozinc additions to the aldehyde. However, our ultimate goal is to develop an efficient, enantioselective route to a versatile salino A core (*i.e.* β-lactone **37**) amenable to the synthesis of derivatives. As mentioned earlier, mechanistic studies were explored in parallel with asymmetric synthesis of salino A. In this chapter, we set out to identify reaction conditions that would minimize formation of observed by-products. Most importantly, epimerization at the C2 stereocenter of the β-ketoacid **4.1** occurs during the biscyclization. Additionally, an optimized procedure for zincate generation by the method of Knochel was generally useful for C4 side chain addition toward the synthesis of salinosporamide A derivatives.[28]

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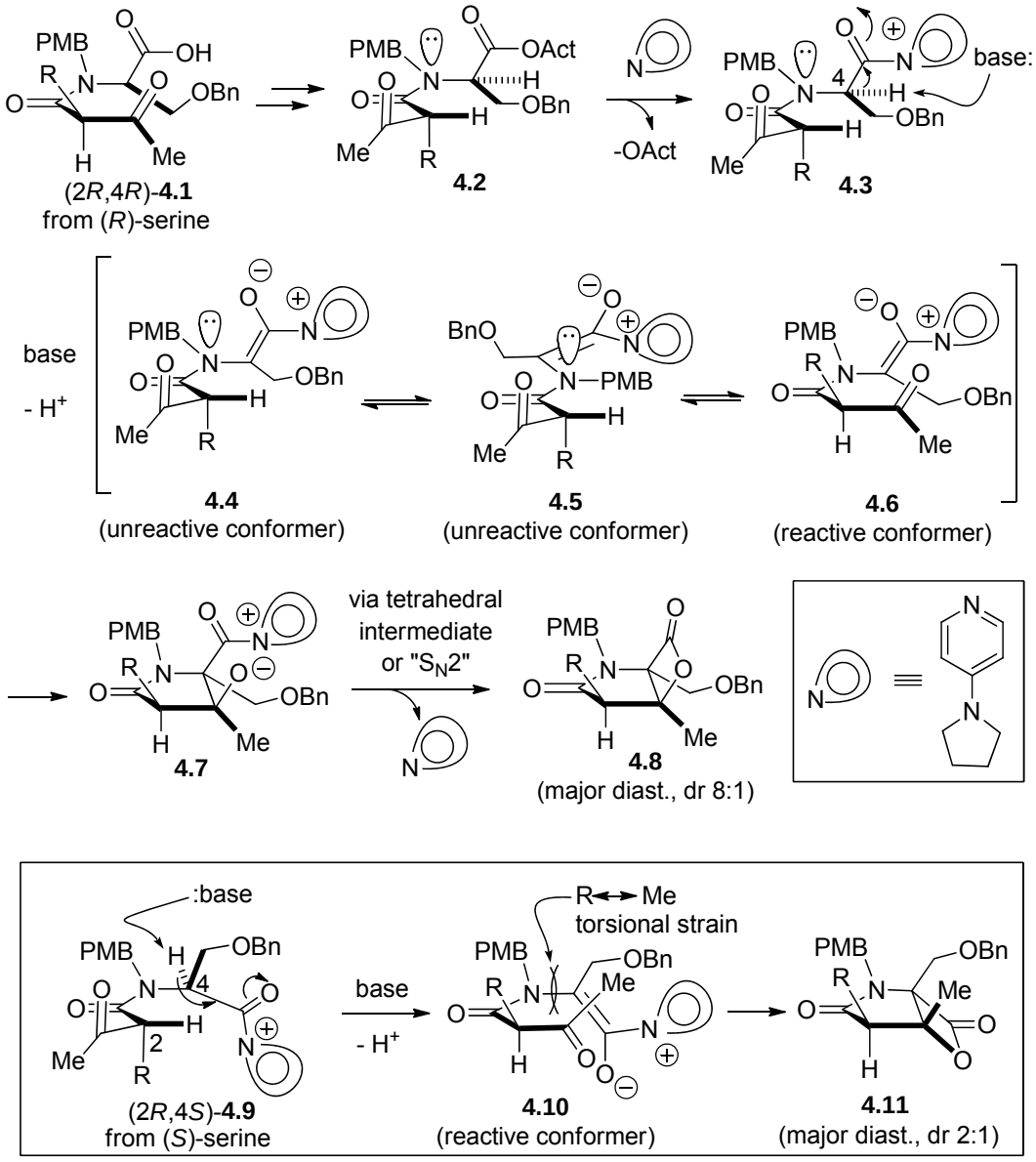
4.2. Mechanistic Studies of the Key Bis-Cyclization

Our working mechanism for this bis-cyclization invokes a nucleophile catalyzed aldol-lactonization process and follows from our previous studies with carbocyclic-fused β-lactones (Scheme 4.1).[12,13] Activation of the carboxylic acid **4.2** followed by transacylation with 4-PPY to provide acyl ammonium **4.3**, and deprotonation provides ammonium enolate **4.4**. Equilibration to the reactive conformer **4.6** enables subsequent aldol reaction leading to aldolate **4.7**. Lactonization, which may occur via a 'S_N2' process,[13,29] then delivers β-lactone **4.8**. While a [2+2] cycloaddition mechanism via an intermediate ketene has not been excluded,[30] evidence to date including reduced conversions upon varying nucleophilic promoters (*vide infra*) is suggestive of nucleophile involvement in the rate determining or prior step. Indirect evidence comes from previously reported highly enantioselective bis-cyclization routes to carbocycle-fused β-lactones employing chiral nucleophiles.[12a,13a]

Scheme 4.1. Proposed Mechanism for the Bis-Cyclization with Two Diastereomeric Ketoacid Substrates



At the outset of our studies, we recognized that several energetically favored conformers dictated by $A^{1,3}$-strain *e.g.* conformers **4.4** and **4.5**, while unproductive for aldol-lactonization since the ketone is not proximal to the ammonium enolate,[31] would

preserve the enantiopurity of the single stereocenter (C2). This would be possible since the α-proton is nearly in plane with the amide carbonyl (Scheme 4.1) in these conformations due to $A^{1,3}$-strain.[16] Furthermore, the energetically unfavorable conformer such as that represented by **4.6** is required to achieve bis-cyclization and, due to proximity and favorable formation of a 5-membered lactam ring, aldol reaction would be facile. What we did not anticipate was the impact of the C4 absolute stereochemistry on the diastereoselectivity of the bis-cyclization. In our early optimization studies, we determined that diastereoselectivity was highly dependent on whether (*S*) or (*R*)-serine derived keto acids were employed leading to different diastereoselectivities, 1:2 vs 8:1 respectively. We suggest that the moderate diastereoselectivity observed for the bis-cyclization is governed by developing torsional strain between the methyl group (C3) of the ketone and the C2 substituent (*cf.* **4.10**, R ⇔ Me) during the initial aldol reaction, leading to the preferred relative stereochemistry following lactonization (*cf.* **4.8**). Note that only a moderate preference for (*cf.* **4.6**) over the alternative (*cf.* **4.10**) is expected[32] and this is reflected in the observed diastereoselectivity for the bis-cyclization (dr 4-7:1, *vide infra*). Regarding the C4 absolute stereochemistry dependence, this is likely a result of required conformations for deprotonation imposed by the N-C4 bond rotamer of the two diastereomeric, acyl ammonium species **4.3** and **4.9** leading to ammonium enolates **4.6** and **4.10** and suggests the intriguing possibility of memory of chirality which we are currently studying in related systems. With acyl ammonium **4.3** derived from (*R*)-serine, deprotonation leads directly to a N-C4 conformer that can access reactive conformer **4.6** leading to major diastereomer **4.8**. Alternatively, deprotonation of the diastereomeric

acyl ammonium **4.9** derived from (*S*)-serine leads directly to reactive conformer **4.10** that delivers the diastereomeric β-lactone **4.11**.

In most cases, the energy of activation for a chemical reaction is greater than that for a conformational equilibrium.[33] If this had applied to our situation in which the energy needed for reactive conformer **4.6** and **4.10** to convert to product **4.8** and **4.11** respectively are higher than the energy for interconversion between **4.6** and **4.10,** diastereomeric ratio of the bis-cyclization of the two diastereomeric ketoacid would be similar (Figure 4.1).

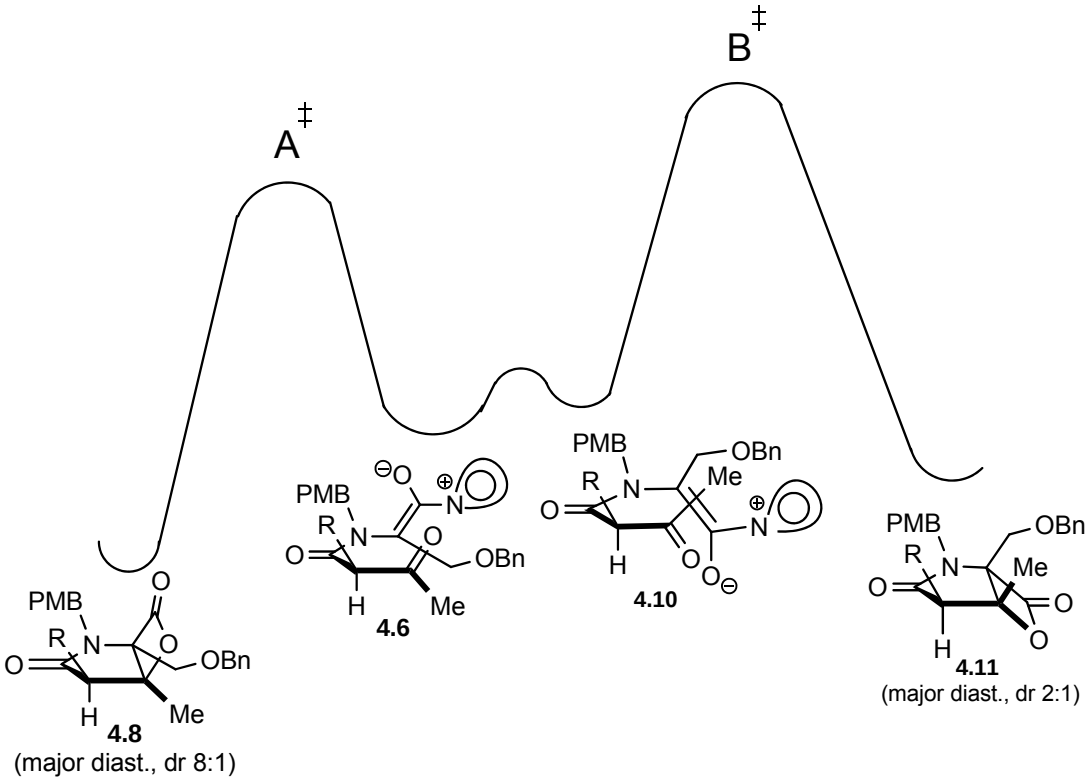


Figure 4.1. Effect of Conformation on Product Distribution in a Curtin-Hammett Situation

In our case, we assumed a rapid bis-cyclization since enantiopurity could indeed be maintained in a non-Curtin-Hammett situation, if once the higher energy reactive conformation is achieved, bis-cyclization immediately takes place. The activation energy for the intramolecular aldol of the reactive conformers **4.6** and **4.10**, followed by lactonization led to products **4.8** and **4.11** respectively might be lower than that for N-C4 bond rotation energy barrier between **4.6** and **4.10** (Figure 4.2).

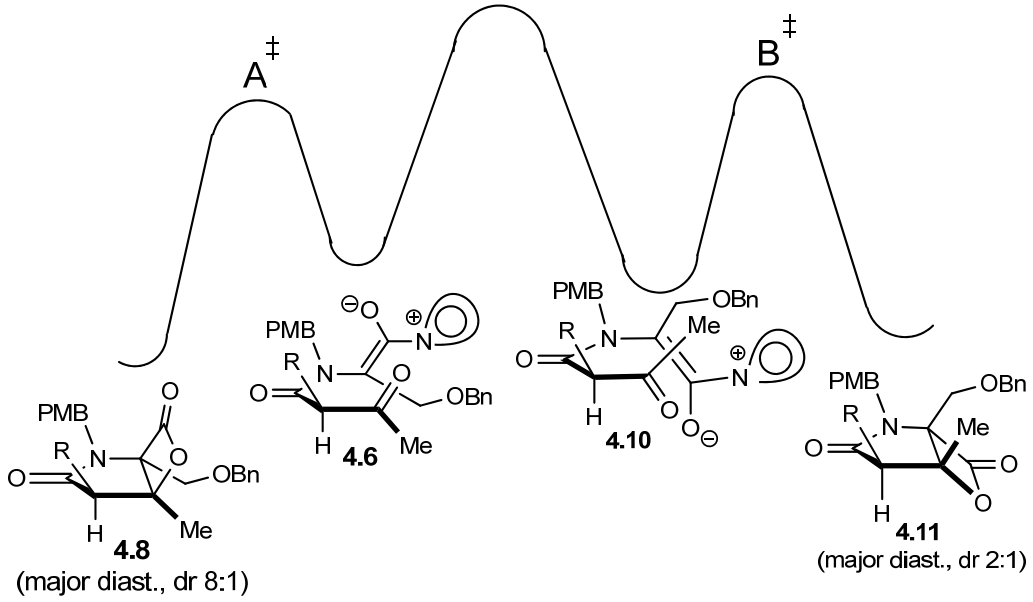
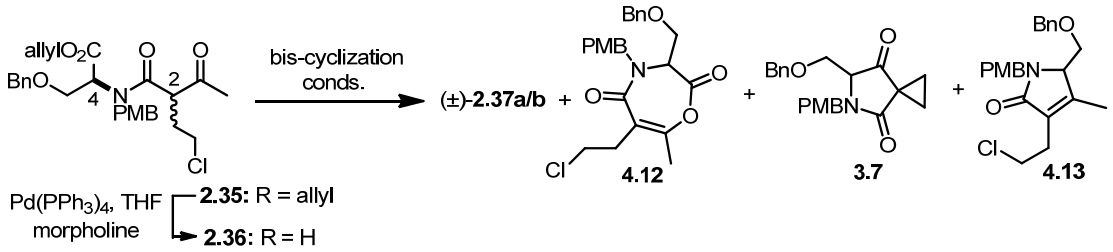


Figure 4.2. Effect of Conformation on Product Distribution in a Non-Curtin-Hammett Situation

In early optimization studies of the bis-cyclization of ketoacid **2.36** directed toward salino A, we isolated and identified three by-products; enol lactone **4.12**, cyclopropyl ketoamide **3.7**, and unsaturated lactam **4.13** (Table 4.1). Only the former

two by-products were observed when both 4-PPY and Hünig's base were employed and only low yields of β-lactones **2.37a**, **2.37b** were obtained (Table 4.1, entries 1 and 2). Enol lactone **4.12** is likely derived from intramolecular acylation of enol **4.14** of the β-ketoamide with the activated ester (Scheme 4.2). Cyclopropyl ketoamide **1.42** may be derived from a condensation reaction of enol **4.14** leading to β-diketone **4.15**, a retro-Claisen process induced by 4-PPY via **4.16**, and finally cyclization via enol **4.17** leading to the cyclopropane. We next studied the use of 4-PPY (7.0 equiv) as both base and nucleophilic promoter, which reduced the amount of by-products **3.7** and **4.12** with attendant dramatic increase in yield of β-lactone but formation of unsaturated γ-lactam **4.13** (Table 4.1, entry 3). Consideration of possible mechanisms for formation of this by-product suggested control experiments to determine if lactam **4.13** was derived from decomposition of β-lactones **2.37a**, **2.37b** via a decarboxylation pathway. Subjecting β-lactones **2.37a**, **2.37b** to 4-PPY at 23 ºC indeed led to lactam **4.13** (31%) and interestingly recovered β-lactones **2.37a**, **2.37b** with improved diastereomeric ratio (dr 5:1→9:1, Scheme 4.3). Decomposition was much slower at –10 → –5 ºC and was not observed if only Hünig's base was added to β-lactones **2.37a**, **2.37b**. This suggests a differential rate of decomposition for the two diastereomers. This may be explained by a lower barrier syn-elimination (endothermic, late transition state) for β-lactone **2.37b** due to a more accessible proton and release of torsional strain (R Me) which is absent in diastereomeric β-lactone **2.37a**. Finally, decarboxylation and protonation leads to the observed unsaturated γ-lactam **4.13**.

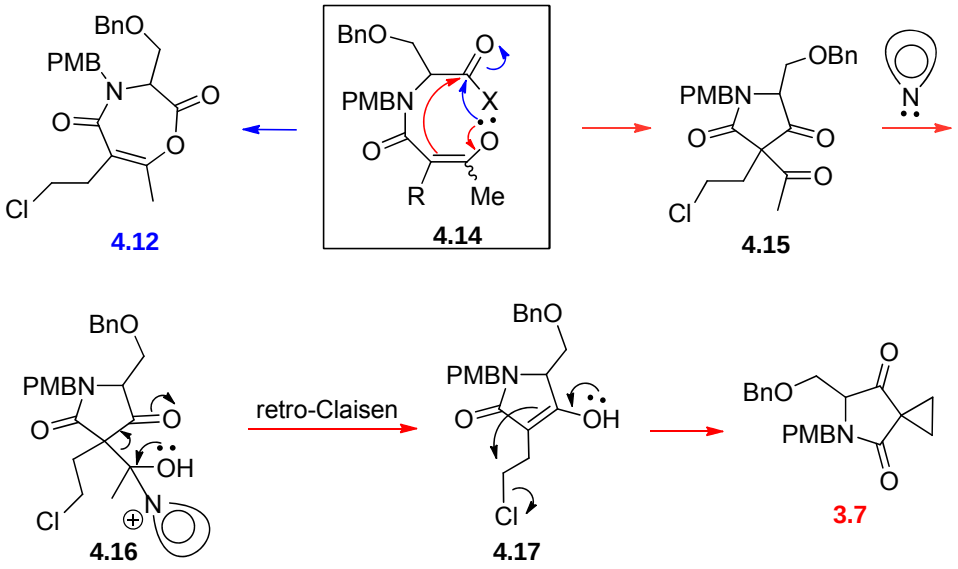
Table 4.1. Optimization of the Bis-Cyclization Leading to Bicyclic-β-Lactone (±)-**2.37**



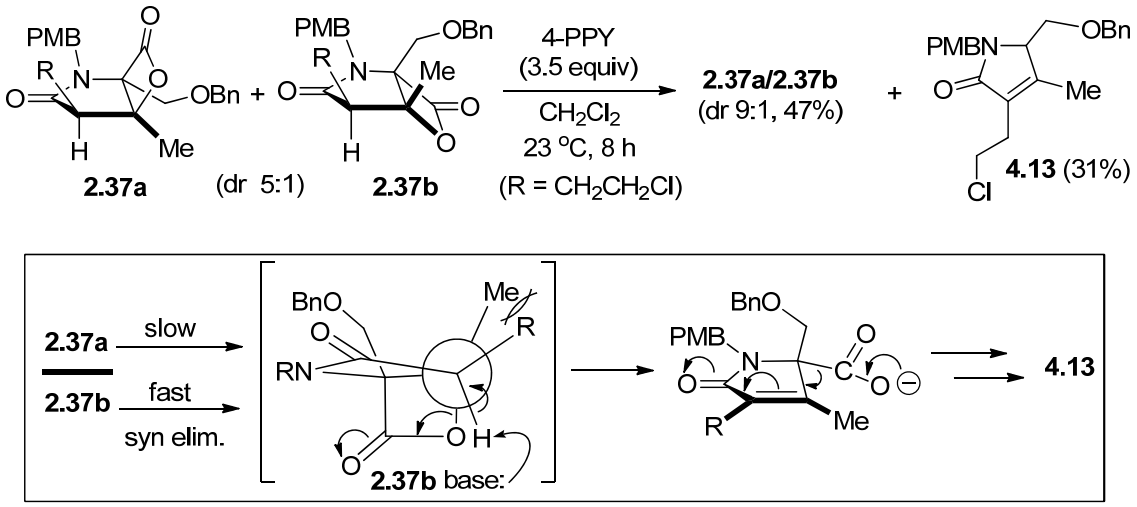
entry	reaction conditions[a] act. agent[b] (equiv)	base (equiv)	nuc.[c] (equiv)	solvent	% yield **2.37a**/**2.37b**[d]	% yield **3.7, 4.12, 4.13**[e]
1	**2.22** (1.5)	*i*-Pr_2NEt (3.0)	4-PPY (1.5)	CH_2Cl_2	10	23 (**3.7**/**4.12**)
2	**2.22** (1.5)	*i*-Pr_2NEt (1.0)	4-PPY (4.0)	CH_2Cl_2	33	25 (**3.7**/**4.12**)
3	**2.22** (1.5)	-	4-PPY (7.0)	CH_2Cl_2	42	19 (**3.7**/**4.13**)
4	MsCl (1.5)	-	4-PPY (3.5)	PhMe	61	10[f] (rec. **2.36**)
5	MsCl (1.5)	-	DMAP (3.5)	PhMe	54	8[f] (rec. **2.36**)

[a]All reactions were performed at $-5 \rightarrow -10$ °C except entry 1 which was run at 23 °C. [b]Carboxylic acid activating agent. [c]Nucleophilic promoter. [d]Yields are given for 2 steps (bis-cyclization/allyl deprotection) and refer to isolated, purified (SiO_2) products. [e]By-products were not easily separated and thus approximate yields are provided based on ^{1}H NMR (500 MHz) integration. [f]Recovered ketoacid **2.36**.

Scheme 4.2. Proposed Mechanisms for the Formation of By-Products **3.7** and **4.12**



Scheme 4.3. Differential Decomposition of β-Lactones **2.37a**/**2.37b** Leading to Unsaturated γ-Lactam **4.13**



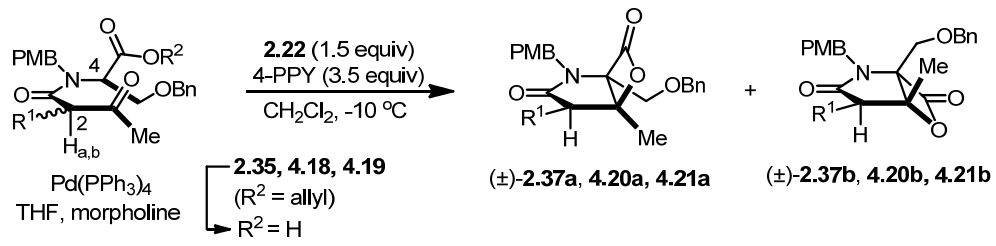
These studies highlighted the importance of 4-PPY as both nucleophilic promoter and base which required minimizing reaction time to avoid decomposition of β-lactones

and C2-deprotonation to avoid these by-products and ultimately to maintain optical purity during the bis-cyclization process. Dramatic improvements were therefore realized when alternative activating agents and most importantly a less polar solvent (*e.g.* toluene), which may slow C2-deprotonation, was employed. Use of MsCl in toluene avoided formation of all by-products and yields of the β-lactones **2.37a**/**2.37b** increased to 61% (Table 4.1, entry 4). Use of the less nucleophilic dimethylaminopyridine (DMAP) also led to β-lactone **2.37a**/**2.37b** but in slightly reduced yields 54% under similar conditions suggestive of nucleophile involvement in the rate-determining or prior step (Table 4.1, entry 5).

The impact of the chlorine atom on the acidity of the C2-proton was investigated under the original bis-cyclization conditions with a dual purpose of studying inductive effects on conversion and by-product formation and demonstrating the versatility of this synthetic strategy for C2-derivative synthesis. Ketoamide substrate **4.18** was prepared from a β-ketothioester while keto amide **4.19** was prepared from heteroketenedimer **11b**. Bis-cyclization of ketoacid derived from allyl ester **4.18** bearing a γ-siloxy group (Table 4.2, entry 2) led to similar results as from ketoamide **2.35** bearing a γ-chloro substituent (salino core shown for comparison, Table 4.2, entry 1), however the δ-chloro ketoacid resulted from keto amide **4.19** led to a significant increase in yield (Table 4.2, entry 3). The yields obtained appear to correlate with the ^{1}H NMR chemical shifts of the C2 protons of β-ketoamides **2.35**, **4.18** and **4.19** (Table 4.2, entry 1, 2 and 3). Comparision with the salino A core (Table 4.2, entry 1) shows a Δδ of 0.4 between γ- and δ-heteroatom substituted substrates, pointing to the inductive effects and attendant

increased acidity leading to by-product formation imposed by the heteroatoms. Inductive effects leading to reduced yields (70-85%) and greater by-product formation was also observed in simpler C4-unsubstituted substrates bearing α-phenyl and β-chloro C2 substituents (Table 2.3, entries 3 and 4) in comparison to other C2-alkyl substrates in that series (90-93% yield).

Table 4.2. Variation of the C2-Side Chain: Inductive Effects on Efficiency of the Bis-Cyclization Reaction



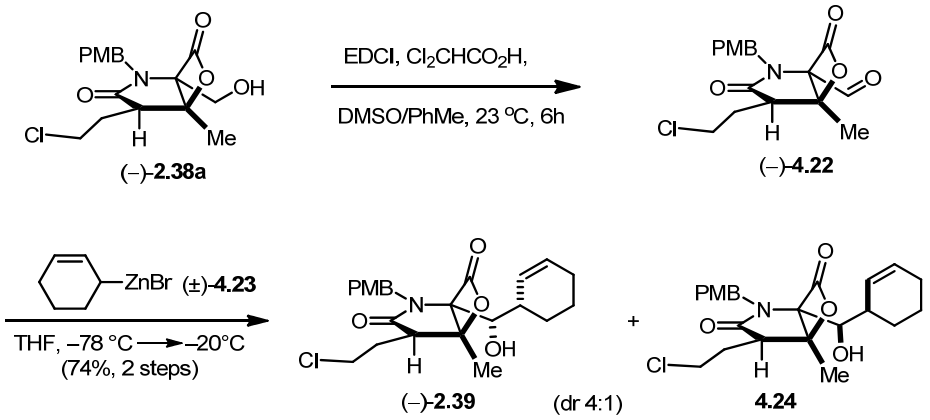
entry	R^1	β-lactones	% yield	δ H_a, H_b
1	$(CH_2)_2Cl$	**2.37a/2.37b**	40	3.92, 3.94
2	$(CH_2)_2OTBS$	**4.20a/4.20b**	42	3.92, 3.95
3	$(CH_2)_3Cl$	**4.21a/4.21b**	62	3.52, 3.57

4.3. Further Optimization towards Salinosporamide A

As mentioned earlier, introduction of the C4-sidechain in our synthetic strategy relied on the method developed by Corey.[6a] One drawback from this protocol is that it requires a prior prepared tin reagent which is inefficient for a facile C4-sidechain addition to the aldehyde **4.22** (Scheme 4.4). Alternately, zinc reagent (±)-**4.23** could be prepared directly from commercially available 3-bromocyclohexene and activated zinc by the method of Knochel,[34,6g] which was then added to aldehyde **4.22** at –78 ºC and

allowed to warm up to –20 ºC afforded only two diasteomers (dr 4:1), and improved the yield (2 steps, 74% to 81%). While side chain addition using zinc reagent **2.8**[6a] derived from the reaction of *n*-butyllithium, prior prepared cyclohexenyl tributyltin and $ZnCl_2$ following the procedure of Corey gave a mixture of C5,C6-diastereomeric alcohols (dr 11:3:1:1) in 62% yield at –78 ºC (Scheme 4.4).

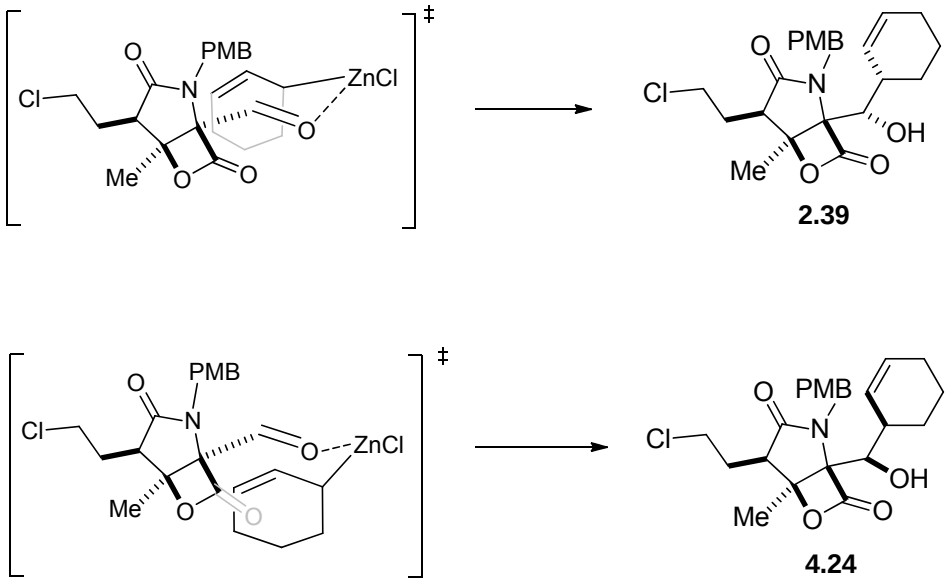
Scheme 4.4. Optimization of C4 Side Chain Addition Employing Knochel's Protocol



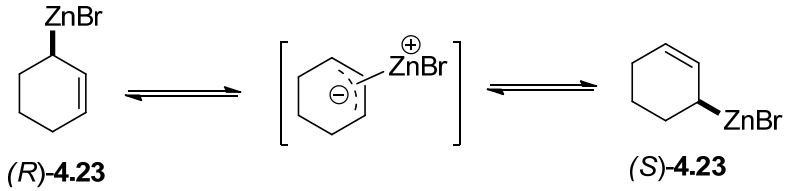
We reasoned the moderate diastereoselectivity in the latter as a result of a cyclic chair-like transition state with *syn* addition. Product **2.39** resulted from the more favorable addition of the zincate (*S*)-**4.23** to the aldehyde from the side opposite to the β-lactone due to more steric hinderance of the β-lactone than the adjacent sp^3 methyl. On the other hand, product **4.24** derived from the addition of the zincate (*R*)-**4.23** to the same side of the β-lactone is less favored due to the steric hinderance of the β-lactone. Currently, we use 2 equiv of zincate (±)-**4.23** in the reaction for complete conversion. Possibly, facial selectivity can be improved in favor the desired diastereomer if only 1

equiv of zincate is used. If racemization of the zincate is facile, dynamic kinetic resolution will facilitate the reaction with improved facial selectivity (Scheme 4.5). Zincate (*R*)-**4.23** can be equilibrated to (*S*)-**4.23** via 1,3-transposition of zinc atom (Scheme 4.6). The former reaction of zincate addition to the aldehyde following Corey's protocol, LiCl was formed in *situ* and could behave as Lewis acid which coordinated to the aldehyde resulting in an open transition state. A control experiment of C4 side chain addition was explored in which zincate (±)-**4.23** was generated following method of Knochel and LiCl was added. As expected, reaction proceeded at −78 ºC, and products were obtained as a mixture of four diasatereomers (dr 10:3:1:1).

Scheme 4.5. Facial Selectivity of Zincate Addition to the Aldehyde **4.22**

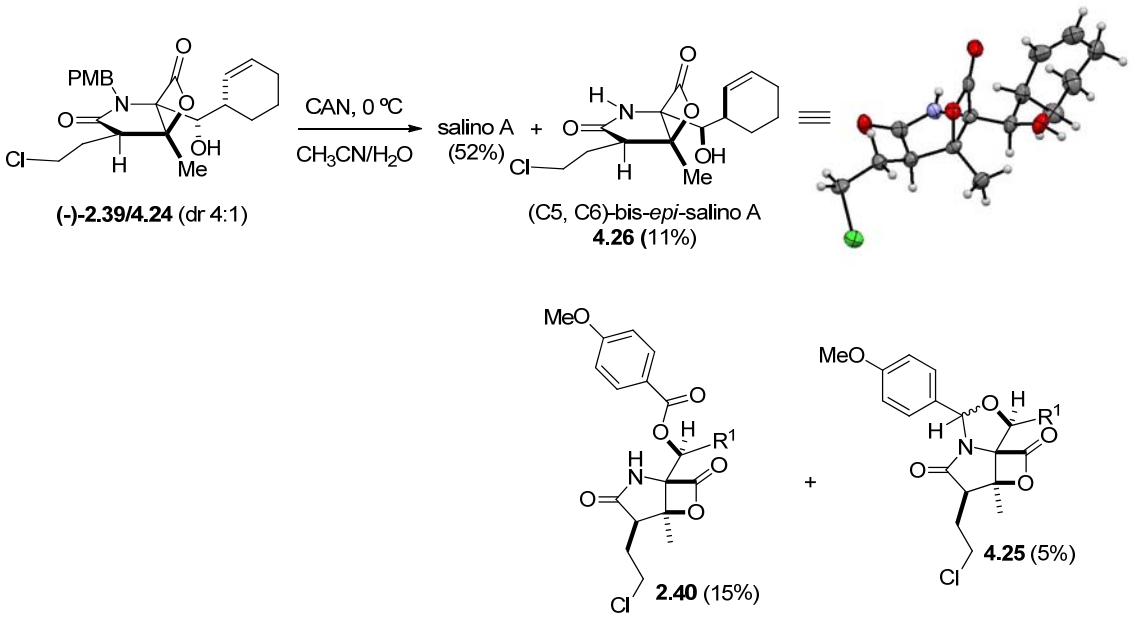


Scheme 4.6. Proposed Mechanism for Racemization of Zincate **4.23**



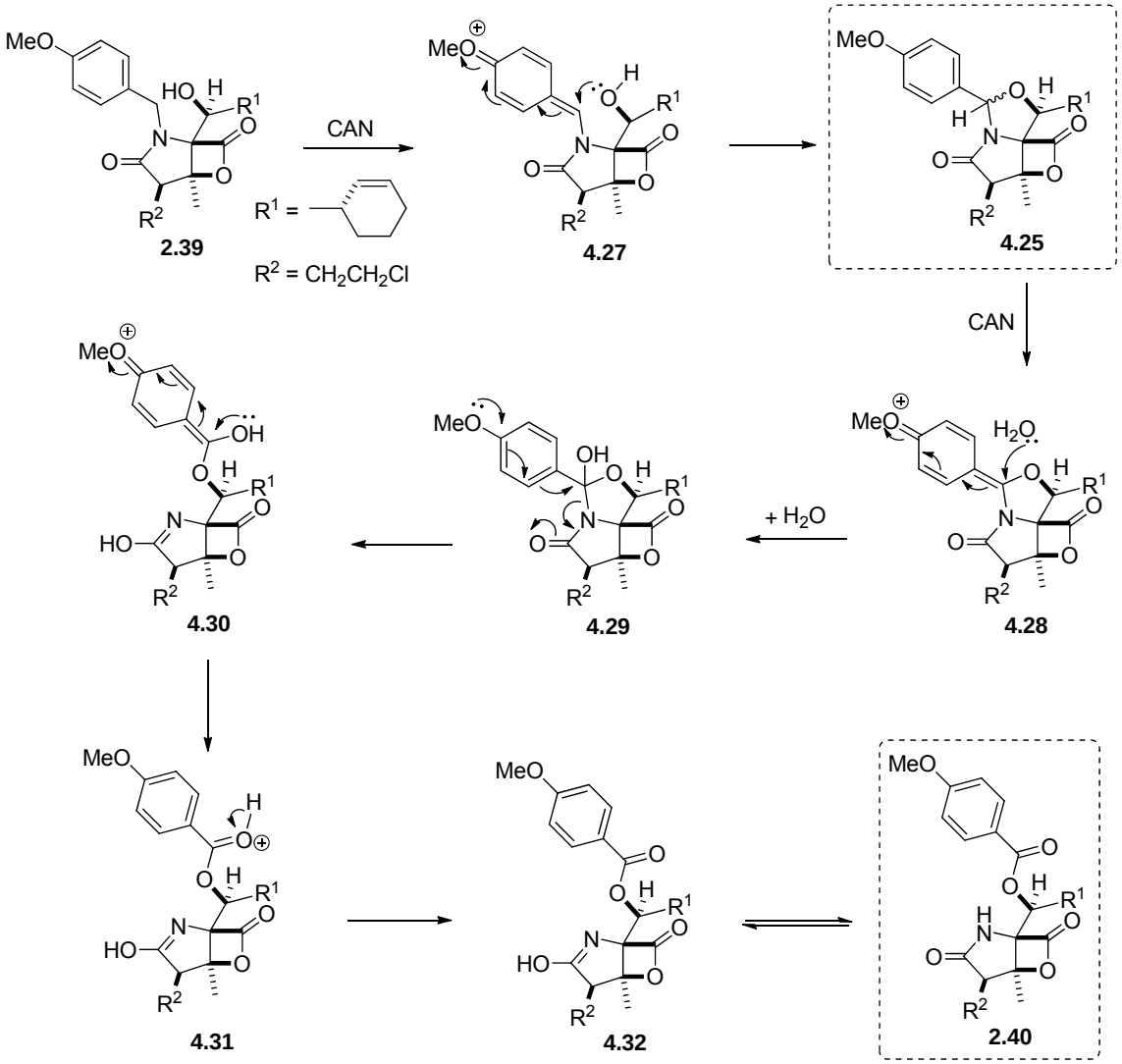
With PMB lactam (–)-**2.39**/**4.24** (dr 4:1) in hand, attempts further improvement to PMB deprotection were explored. PMB deprotection using ceric ammonium nitrate (CAN) and CH_3CN/H_2O gave salino A (**1.1a**, 51%), (C5, C6-bis-*epi*-salino A) **4.26** (12%) and isolation of the salino ester **2.40** (15%) along with a diastereomer oxazolidine **4.25** (5%) due to less complications from fewer number of products to the previous result with four diastereomers at C5, C6. The absolute stereochemistry at C5 according to PMB lactam **2.39** was tentatively assigned due to the diastereomeric ratio of **2.39**/**4.24** as 4:1. Most importantly, this procedure enabled isolation of a novel salino A diastereomer (C5, C6-bis-*epi*-salino A) **4**.**26** whose relative stereochemistry was verified by X-ray analysis (Scheme 4.7).

Scheme 4.7. Completion of Salino A Synthesis



The by-product oxazolidine **4.25** could be derived from the intramolecular trapping of benzylic cation **4.27** with the C5 alcohol. The oxazolidine **4.25** in the presence of excess CAN led to the formation of benzylic cation intermediate **4.28** which was then trapped by H_2O to deliver the intermediate **4.29**. C-N bond cleavage, followed by rearomatization, removal of the proton and the final tautomerization of the amide to afford salino ester **2.40** (Scheme 4.8).

Scheme 4.8. Proposed Mechanism for the Formation of By-products **2.40** and **4.25**



4.4. Conclusion

Optimization studies of the key bis-cyclization are described that eventually allowed for gram scale synthesis of a versatile bicyclic β-lactone with a high degree of stereoretention despite the presence of a stereochemically labile β-ketoamide. Isolation and characterization of by-products in the bis-cyclization provided information regarding

this process and various alternative reaction pathways. Also, we successfully optimized a procedure for zincate formation by the method of Knochel which greatly simplified product purification since tin by-products were avoided. Most importantly, this protocol gave only two diasteomers (dr 4:1) leading to the isolation of a novel salinosporamide A diastereomer (C5, C6-bis-*epi*-salinosporamide A, **4.26**). Additionally, mild and stable zincate conditions improved the yield (74% to 81%), and avoided the use of toxic tin reagents. The ability to generate zincates directly from its alkyl halide, and subsequent successful addition to the versatile aldehyde intermediate **4.22** further validated our synthetic strategy in variation of the C4-side chain.

CHAPTER V

SYNTHESIS AND BIOACTIVITY OF SALINOSPORAMIDE DERIVATIVES*

5.1. Introduction

Previous structure-activity relationship (SAR) studies of omuralide (**1.2**) showed that the amide, β-lactone, and hydroxyl group are essential for biological activity.[11b] Recently SAR studies of salinosporamide A (**1.1a)** have been investigated.[8] In these studies, a series of analogues were assayed for cytotoxicity, proteasome inhibition, and inhibition of NF-kB activation. These results indicated a dramatic reduction in potency in cell-based assays that accompanies the replacement of the chloroethyl group with unhalogenated substituents (Figure 5.1). Halogen exchange to Br, I and a cyclohexene ring epoxidation can be tolerated, while some stereochemical modification significantly reduces activity, for example; C2 epimer was 100 times less potent than salinosporamide A against chymotrypsin-like (CT-L) and trypsin-like (T-L) activities of the proteasome. Modifications to the cyclohexene ring gave various degrees of reduction in biological

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activity such as replacement with cyclohexyl results in 3 to 12 fold lower potency in most assays. Interestingly, epoxidation of the olefin to the (*R*,*R*) diastereomer is well tolerated in contrast to the (*S*,*S*) diastereomer which led to a 25-40 fold lower potency across the assays reported (Figure 5.1).

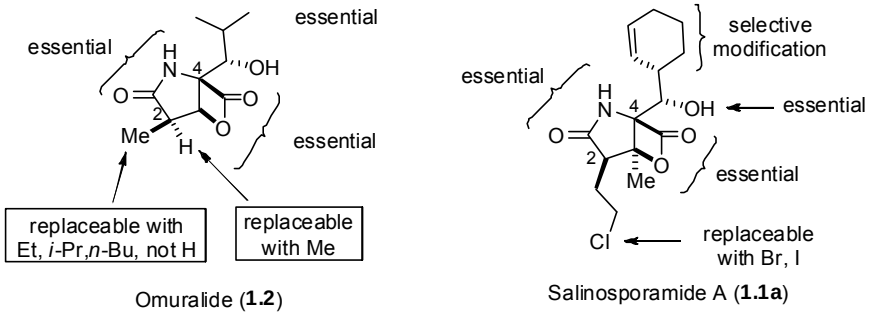


Figure 5.1. Summary of Omuralide and Salinosporamide A SAR Data

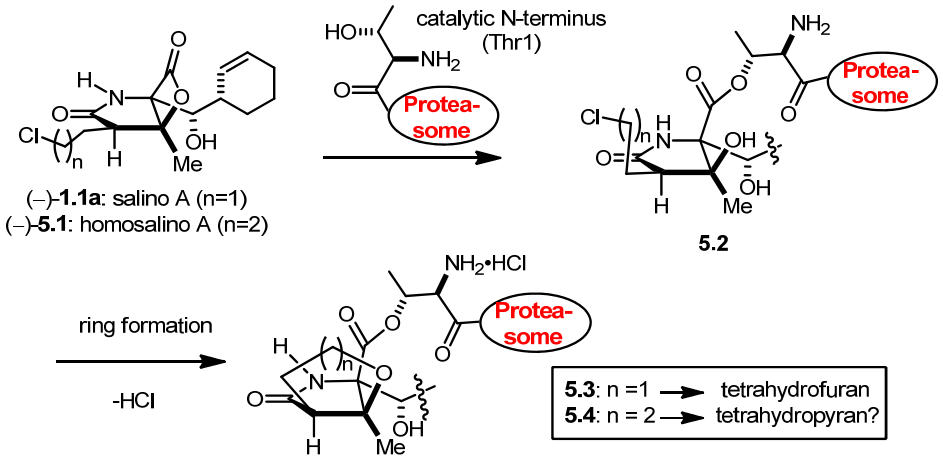
5.2. Variation of C2-Side Chain

5.2.1. Synthesis of (–)-Homosalinosporamide A

In our optimization studies described above, we altered the C2-side chain with the dual purpose of studying inductive effects (*cf.* Table 4.2, Chapter IV) and providing the one carbon homologated side-chain that could ultimately lead to (–)-homosalinosporamide A (homosalino A, **5.1**). Groll and co-workers have proposed that acylation of the proteasome by the β-lactone of salino A (**1.1a**) at the catalytic N-terminus (Thr 1) is followed by cyclization of the incipient alcohol with the pendant chloroethyl group leading to the formation of a tetrahydrofuran (**5.3**), as verified by X-ray analysis (Scheme 5.1). [10a] Thus, we became interested in the synthesis of homosalino

A (**5.1**) to determine if a tetrahydropyran (*cf.* **5.4**, n = 2) could be formed at the active site of the proteasome in analogy to the known formation of a tetrahydrofuran.

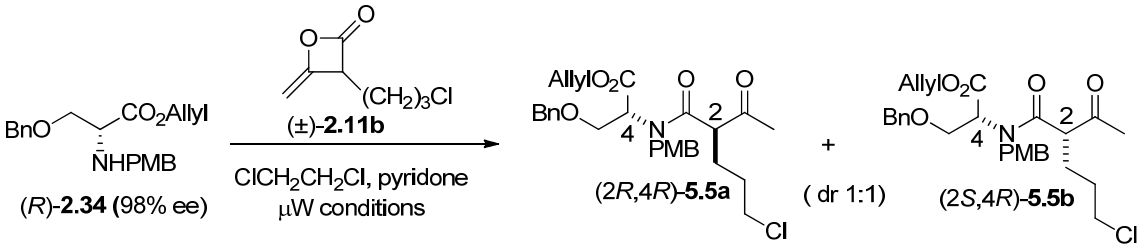
Scheme 5.1. Mechanism of Inhibition of the Proteasome (CT Site) with Salino A (**1.1a**) and Proposed for Homosalino A **5.1**



In a similar manner as described in the asymmetric synthesis of salinosporamide A (Chapter III), the enantioselective synthesis of (–)-Homosalinosporamide A began with reductive amination of (*R*)-*O*-benzyl serine (**3.3**) (99% ee) with *p*-anisaldehyde and subsequent esterification with allyl alcohol provided allyl ester (*R*)-**2.34** (98% ee) (Scheme 5.1). Acylation of serine derivative (*R*)-**2.34** with unsymmetrical ketene dimer (±)-2.**11b** employing optimized microwave conditions developed for asymmetric synthesis of salinosporamide A surprisingly lead only to 50% conversion. Prolonged reation times or elevated temperatures (entry 2, Table 5.1) resulted in higher yield but lower enantiomeric access (88% ee). As discussed previously (Chapter IV), inductive

effects only impact the acidity of the C2 proton with attendant of δ-heteroatom substituted substrates. As a result, the C4 stereocenter is more prone to epimerization under these reaction conditions. The optimized reaction (entry 3, Table 5.1) gave a mixture of two diastereomers (2*R*,4*R*)-**5.5a**/(2*S*,4*R*)-**5.5b** (80% yield, dr 1:1) and recovered (*R*)-serine allyl ester (*R*)-**2.34**. Subsequent MPLC separation of the β-keto amides (2*R*,4*R*)-**5.5a**/(2*S*,4*R*)-**5.5b** (dr 1:1) led to desired ketoamide (2*R*,4*R*)-**5.5a** (dr 25:1) after two separations with erosion of optical purity (98% to 94% ee).

Table 5.1. Microwave Asssited Ketoamide Coupling of Ketene Heterodimer and (*R*)-*O*-Benzyl Serine Allyl Ester

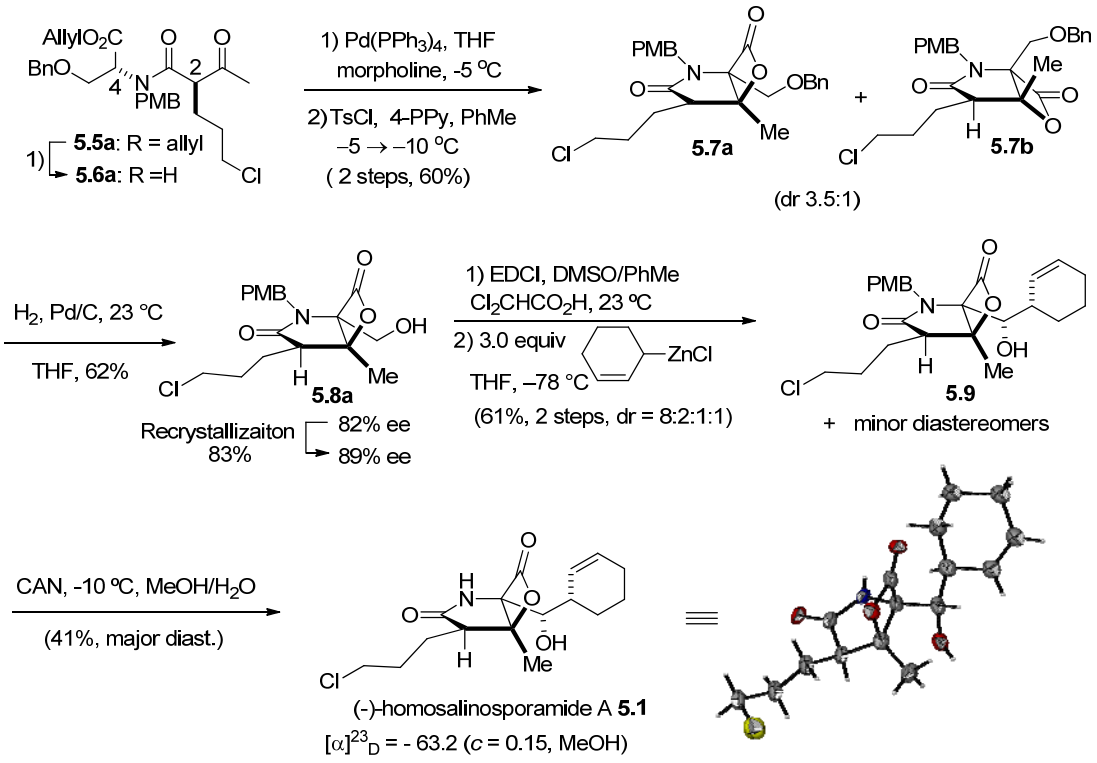


entry	microwave conditions	% yield (**5.5a/5.5b**)	% ee (*2R,4R*)-**5.5a**
1	50 °C, 120 min	~ 50% conversion	-
2	60 °C, 120 min	93%	88
3	**53 °C, 120 min**	**80%**	**94**

Pd(0)-mediated deprotection of allyl ester (2*R*,4*R*)-**5.5a** delivered keto acid **5.6a** with similar diastereomeric purity (dr ~ 24:1). Utilization of the optimized bis-cyclization conditions developed in the asymmetric synthesis of salinosporamide A with TsCl as an activating agent gave the bicyclic-β-lactones (–)-**5.7a/5.7b** in 60% yield (dr 3.5:1, 2 steps) and recovered ketoacid **5.6a/5.6b** (dr 1:1, 11%). Hydrogenolysis of the

benzyl ethers **5.7a/5.7b** (dr 3.5:1) facilitated very simple separation of the major diastereomer **5.8a** (62 %, dr > 19:1) via flash chromatography compared to salinosporamide A substrate (separation by MPLC). The enantiomeric excess of the desired alcohol (–)-**5.8a** was determined by chiral HPLC as 82% ee due to the moderate diastereromic ratio of ketoacid (2*R*,4*R*)-**5.6a** (dr ~ 24:1, 94% ee according to ketoamide **5.5a**) or epimerization had occurred at the C4 stereocenter. However, the enantiopurity of the alcohol (–)-**5.8a** could be improved by recrystallization (CH_2Cl_2/Et_2O), which removed the minor enantiomer since it had lower solubility and crystallized more readily leaving the major enantiomer in the mother liquor. One recrystallization led to an enrichment of 89 % ee for the hydroxy-β-lactone (–)-**5.8a** (83% yield), which was followed by modified Moffat oxidation to deliver the aldehyde intermediate which was used in the next step without further purification. Treatment of zinc reagent **2.8** using the method developed by Corey provived alcohol **5.9** in 61% yield (2 steps, dr 8:2:1:1). Final oxidative PMB deprotection with ceric ammonium nitrate (CAN) gave (–)-homosalinosporamide A **5.1**, which could be isolated diastereomerically pure in 41%. The structure and relative stereochemistry of **5.1** were confirmed by X-ray analysis (Scheme 5.2).

Scheme 5.2. Completion of (–)-Homosalinosporamide A **5.1** Synthesis



5.2.2. Synthesis toward Salino-Belactosin Chimera

Recently, X-ray structures of the overlay of 20S proteasome/salino A and homo CBz-Bn-belactosin C showing active site residues and distances to key groups on salino and belactosin were reported by Groll and co-workers.[10a] A chimeric structure of salino A and belactosin C **5.10**, another proteasome inhibitor synthesized in our group,[35] was targeted to probe the effect of replacing the THF forming chloroethyl group with the dipeptide found to be effective in the belactosin structures (Figure 5.2, **5.11**).

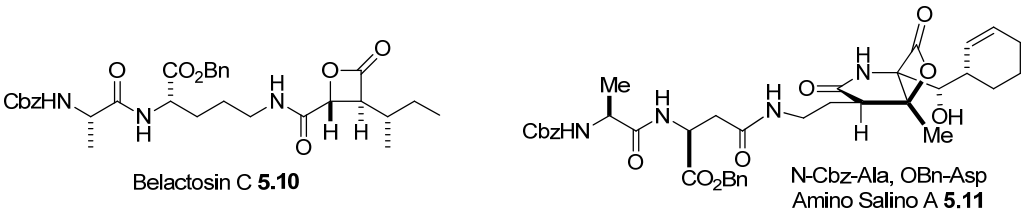
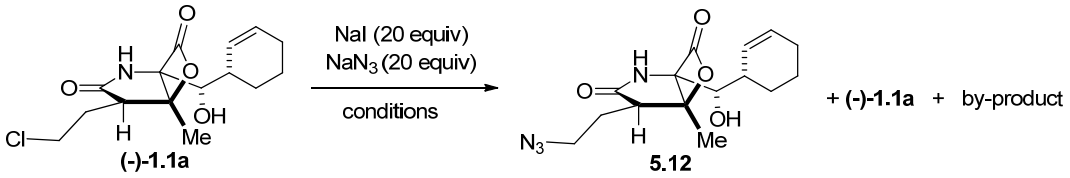


Figure 5.2. Proposed Salino A and Belactosin C Chimera

The synthesis of salino-belactosin chimera commenced from the preparation of the azido salino derivative **5.12**, which was recently described by workers at Nereus derived from the action of azide on salino A.[8a] In their synthesis, the conversion of clorosalino A (**1**.**1a**) to iodosalino A via Finkelstein reaction only gave 11% yield of product following isolation by HPLC. In our hands similar results were observed, therefore a one pot transformation of salino A (**1**.**1a**) to azidosalino **5.12** was explored under various conditions. Currently, the best reaction conditions (Table 5.3, entry 3) found to be in DMSO at 23 ºC for 48h provided azide **5.12** in 20%, along with recovered salino A (**1**.**1a**) (48%), and decomposition of β-lactone (24%).

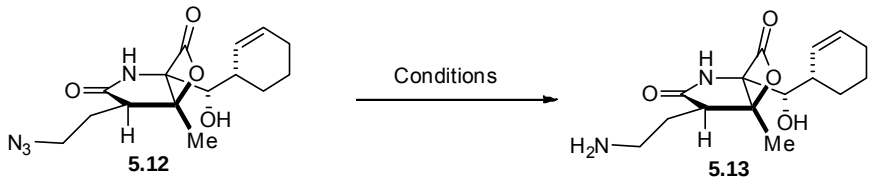
Table 5.2. Azido Salinosporamide A Synthesis



entry	reaction conditions	reaction outcome
1	Acetone, 23 ºC, 48h	Recovered SM (**1**.**1a)**
2	Acetone, 58 ºC, 48h	20% (**5.12**), 35% rec. SM (**1**.**1a)**, 28% by-product
3	DMSO, 23 ºC, 48 h	20% (**5.12**), 48% rec.SM (**1**.**1a)**, 24% by-product
4	DMSO, 23 ºC, 60 h	26% (**5.12**), 37% rec. SM (**1**.**1a)**, 32% by-product

Subsequent reduction of azide **5.12** to amino salino A **5.13** employing a Staudinger reduction with Ph_3P proved difficulties in term of separation due to the high polarity of primary amine in the presence of β-lactone **5.13** and triphenylphosphine (Ph_3PO) by-product. Alternatively, reduction of azide **5.12** applying Lindlar's catalyst gave promising results (entry 3, Table 5.3). A crude ^{1}HNMR showed a mixture of products amino salino A **5.13**, a dimer of product **5.15** and unreacted azide **5.12** as also confirmed by LC-MS analysis. However, there was over reduction (entry 2, Table 5.3) when longer reaction times were applied.

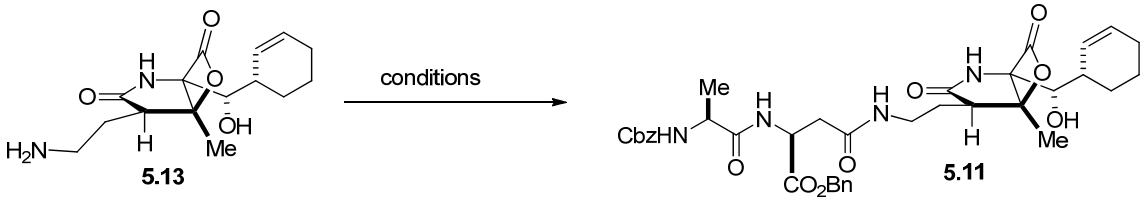
Table 5.3. Studies of Azido Salino A Reduction

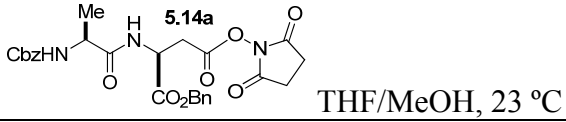


entry	reaction conditions	reaction outcome
1	PPh_3, 23 ºC, THF/H_2O, 5h	Small peak observed by IR
2	Lindlar catalyst, 23 ºC, THF, H_2, 12h	Product obtained with over reduction
3	Lindlar catalyst, 23 ºC, THF, H_2, 3h	Product used w/o further purification

Attempts to purify amino salino A **5.13** resulted in loss of product **5.13**. Therefore, reduction of azide **5.12** followed by coupling with peptide **5.14** using N-hydroxybenzotriazole, 1-(1,3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) at 23 ºC was explored. Unfortunately, the reaction did not give the desired product but instead decomposition of the β-lactone under basic condition (entry 1, Table 5.4). Therefore, coupling reaction conditions were explored using succiimide ester **5.14a**, which however did not provide the desired product **5.11**, surely dimerization of amino salino A **5.13** was detected by LC-MS (entry 2, Table 5.4).

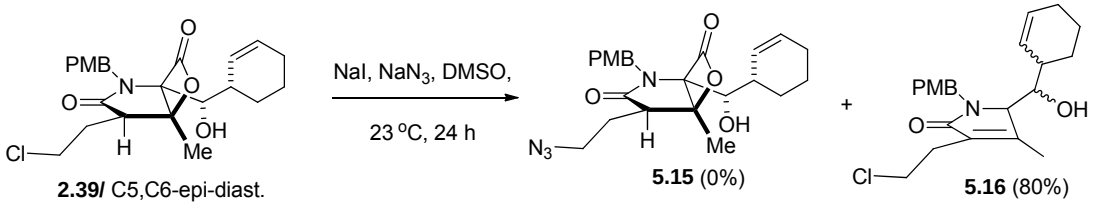
Table 5.4. Attempt Synthesis of Salino A Belactosin Chimera



entry	reaction conditions	reaction outcome
1	HOBt, EDCI, *i*-Pr_2NEt,CH_2Cl_2, 23 ºC, 6h	No desired product by LC-MS
2	 THF/MeOH, 23 ºC	No desired product by LC-MS

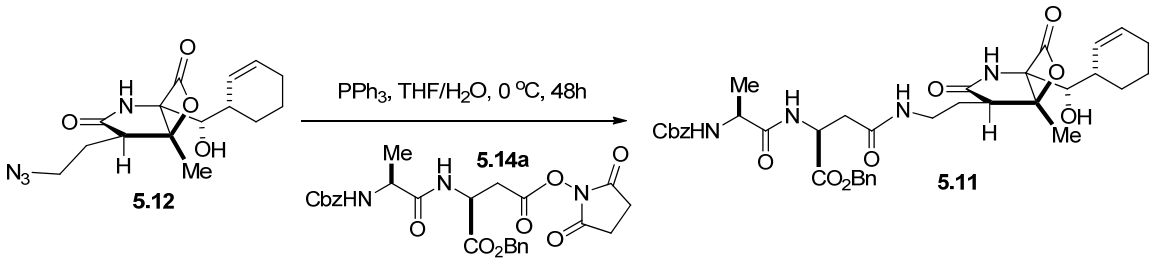
Alternatively, prior to PMB deprotection the mixture of alcohols was subjected to a Finkelstein reaction developed for natural product salino A derivatives. Unfortunately, the reaction only resulted in decomposition of the β-lactone to the unsaturated amides **5.16** (Scheme 5.3).

Scheme 5.3. Atempt Synthesis of PMB Azido Salino A **5.15**.



Since PMB salino A is less stable than the natural product salinoA under the reaction conditions, the goal was to go back to the original strategy starting from salino A. Given that amino salino A **5.13** was unstable at 23 ºC under the reaction condition, a one pot two step transformation was explored. In this event, azido salino A **5.12** (1 mg scale) was subjected to Staudinger reduction conditions in the presence of NHS-ester **5.14a** gave promising result of potential salino-belactosin chimera **5.11** by LC-MS analysis (Scheme 5.4).

Scheme 5.4. An One Pot Reduction /Amide Coupling of Azide **5.12**

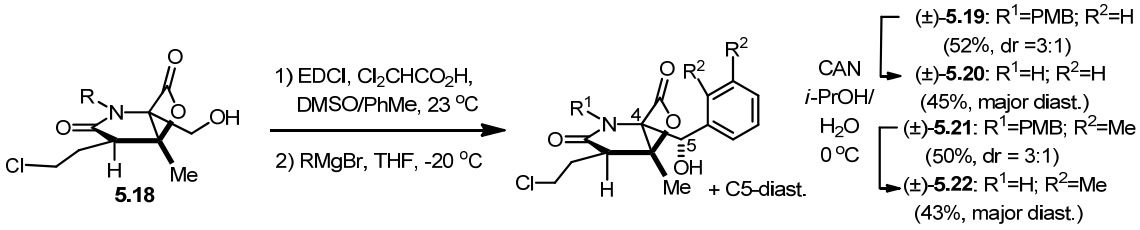


5.3. Variation of the C4-Side-Chain of the Salino Core

X-ray analysis of salino A complexes with proteasome showed a large hydrophobic pocket accommodating the cyclohexenyl group of salino A terminating in a highly basic region (lysine residues) suggests that a larger hydrophobic group including those bearing a carboxyl group could be accessed to probe this feature of the binding pocket.[10] Our synthetic strategy enables faciles variation of the C4-side chain by addition of organometallic reagents to the versatile aldehyde by addition of organozinc which has been applied successfully using Knochel protocol in the synthesis of salino A. Moreover, simple derivatives could be accessed by adddition of Grignard reagents to the

aldehyde intermediate following oxidation of the alcohol **5.18.** Oxidative PMB deprotection provided phenyl and dimethylphenyl salino derivatives **5.20** and **5.22**, respectively (dr ~3:1, Scheme 5.5). The structure of **5.20** was confirmed by X-ray analysis. However, the addtion of isopropyl magnasium chloride was unsuccessful after several attempts, probably is due to steric effects of isopropyl and the aldehyde.

Scheme 5.5. Varying the C4-Sidechain of Salino A.



5.4. Biological Studies

The derivatives synthesized were analyzed for their ability to inhibit the chymotrypsin-like (CT-L), caspase-like (C-L), and trypsin-like (T-L) activities of the 20S and 26S human proteasome in a luminogenic enzymatic assay (Table 5.5). Both velcade (entry 6) and synthetic salino A (92% ee, entry 2) were included as controls and data obtained correlates well with previous analyses. As expected based on known structure-activity relationships,[10,8] changes made to the C5-sidechain in general led to lower activity with drastic loss of activity observed for the *rac*-dimethylphenyl derivative **22**. However, changes in the C2-sidechain are more tolerable as indicated by (–)-homosalino A (**5.1**, Table 5.5, entry 5) and indeed this derivative exhibited very similar activity to the natural product with the exception of inhibition of the trypsin-like

activity toward the 20S human proteasome (~3X decrease).

Table 5.5. IC_{50} Values for Inhibition of the 20S and 26S Proteasome by Salino A Derivatives in a Luminogenic Enzymatic Assay[a]

Entry	Compound	IC_{50}		
		CT-L activity 20S/26S (nM)	C-L activity 20S/26S (nM)	T-L activity 20S/26S (nM)
1	*rac*-cinnabaramide A, (±)-**2.1c**	2.8±0.6 2.2±0.04	125±12[b] 230±24[b]	260±29[b] 412±83
2	(-)-salino A (**1.1a**) (92% ee)	0.8±0.08 2.5±1.3	111±22 156±18	39±7 37±16
3	*rac*-phenyl derivative (±)- **5.20**	29±0.7 57±20	788±50 1585±633	702±235 589±120
4	*rac*-diMePh (**5.22**)	inact. [b,c]	inact. [b,c]	inact. [b,c]
5	(-)-homosalino (**5.1**) (88% ee)	0.7±0.04 2.3±1.1	144±12 188±3	118±28 125±30
6	velcade	2.6 ±0.7 7.4±1.9	25±7 72±17	254±24 680±150

[a]IC_{50} values are the mean ± standard deviation of 4 or more experiments. [b]The average of 2 experiments. [c]Essentially inactive at >99,000 nM. (CT-L: chymotrypsin-like activity; C-L: caspase-like activity; T-L: trypsin-like activity)

5.5. Conclusion

Our synthetic strategy enables facile variation of the C4-side chain by addition of organometallic reagents to the versatile aldehyde by addition of organozinc which has been applied successfully using Knowchel protocol in the synthesis of salinosporamide A and extended to Grignard's reagent for other C4 side chain derivatives. Given the role of C4 side chain in recognizing the S1 specificity pocket of the proteasome, which largely imparts the CT-L, T-L and C-L sites with their substrate references, our synthetic strategy would quickly provide a wide range of derivatives which help reveal unique

inhibition profiles among subunits. Regard the C2 side chain variation, in particular salino belactosin chimera **5.11** we should be able to synthesize this novel compound according our preliminary result.

CHAPTER VI

OPTIMIZATION OF AN *ORGANIC SYNTHESES* PROCEDURE: ASYMMETRIC NUCLEOPHILE-CATALYZED ALDOL-LACTONIZATION WITH ALDEHYDE ACIDS*

6.1. Introduction

This procedure describes an organocatalytic, enantioselective method for the asymmetric synthesis of bicyclic β-lactones. The intramolecular nucleophile-catalyzed aldol-lactonization (NCAL) process effectively merges catalytic, asymmetric carbocycle synthesis with β-lactone synthesis leading to unique bicyclic β-lactone.[12] The demand for concise synthetic routes to optically active β-lactones continues to grow due to continued development of novel transformations of these heterocycles and a reappraisal of their utility as synthetic intermediates[36] including natural product synthesis,[37] their continued occurrence in natural products,[38] their potent activity as enzyme inhibitors[39] and their utility as activity based probes.[40] β-Lactones can be viewed as "activated aldol products" since they possess the structural features of aldol products yet they also have inherent reactivity due to ring strain (β-lactones, 22.8 kcal/mole; epoxides, 27.2 kcal/mole, Scheme 6.1).[41]

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*Reprinted with permission from *Organic Syntheses* for "Asymmetric Synthesis of (1*S*,5*R*)-6-oxaspiro[bicyclo[3.2.0]heptane-3,2'-[1,3]dioxolan]-7-one via the Nucleophile-Catalyzed Aldol-Lactonization (NCAL)" by Nguyen, H.; Oh, S.; Henry-Riyad, H.; Sepulveda, D.; Romo, D. *Org. Syn.* **2010**,*88*.

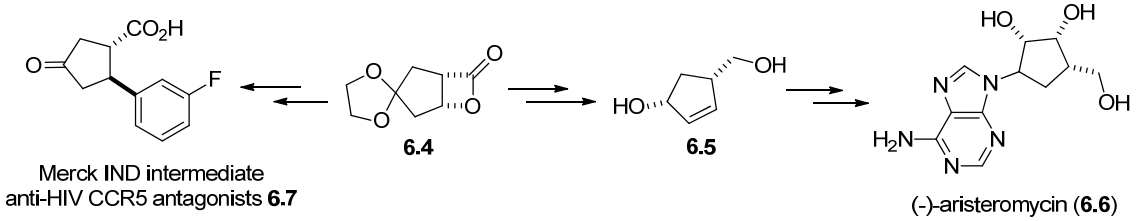
Scheme 6.1. Comparison of Structure and Reactivity of β-Lactones to Aldol Adducts and Epoxides.

aldol adduct **6.1** epoxide **6.2** β–lactone **6.3**

The aldol motif, the β-hydroxy carbonyl motif, derived from polyketide biosynthesis, is common to many natural products, therefore asymmetric methods for their synthesis have been studied extensively. However, β-lactones arguably have greater utility over simple aldol products since they can undergo divergent nucleophilic cleavage processes occurring at either the acyl-oxygen (C_2-O_1) bond or the alkyl-oxygen (C_4-O_1) bond typically driven by release of ring strain. As a result, β-lactones undergo a number of interesting and useful stereospecific transformations making them versatile intermediates for organic synthesis.[36] Despite these facts, general methods for the direct synthesis of β-lactones in optically active form lag far behind those developed for epoxides and aldol adducts. Furthermore, there is much unexploited synthetic potential of β-lactones as chiral synthetic intermediates. Consequently, our group has been engaged in the development of efficient methods for the asymmetric synthesis of β-lactones and their subsequent transformations with applications to natural product synthesis.[42] Building on our initial reports of the NCAL process,[12] we recently developed optimized conditions that render the process more practical with the recognition of the importance of the counterion of the Mukaiyama reagent.[12b] The utility

of the dioxolane bicyclic β-lactone **6.4** described in this *Organic Syntheses* procedure was demonstrated in the synthesis of cyclopentane diol **6.5**,[43] a useful intermediate in the synthesis of antiviral carbocyclic nucleosides including (-)-aristeromycin (**6.6**)[12a] and Merck IND intermediate anti-HIV CCR5 antagonists (**6.7**)[44] (Scheme 6.2)**.**

Scheme 6.2. Utility of β-lactone **6.4** toward a Formal Synthesis of (-)-Aristeromycin and Merck IND Intermediate.

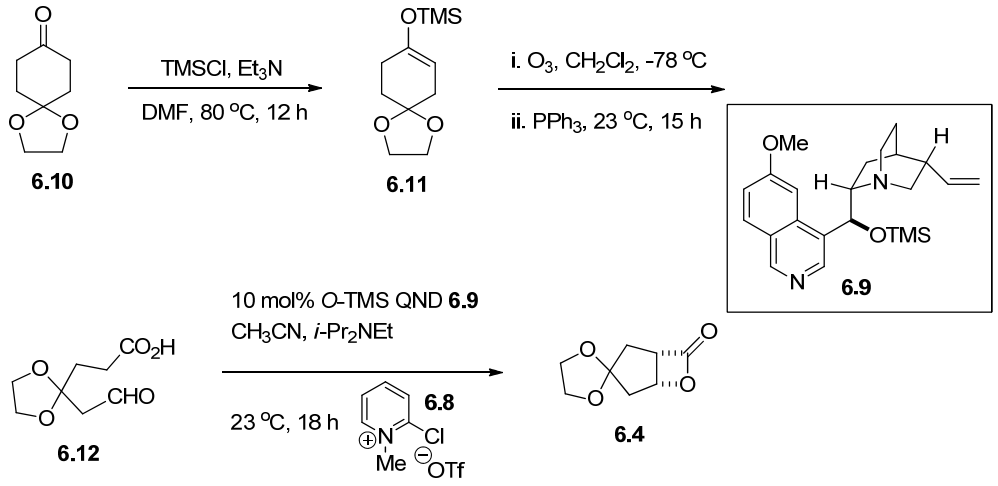


6.2. *Organic Syntheses* Procedure for the Asymmetric Synthesis of the Bicyclic β-Lactone

To demonstrate the utility of the NCAL methodology, a gram-scale procedure for preparing optically active bicyclic β-lactone **6.4** has been developed and optimized to meet standard guidelines and requirements for an *Organic Syntheses* procedure. This procedure contains a three step sequence with a total of five procedures which include one procedure for the preparation of Mukaiyama salt **6.8** (an acid activating agent), and one procedure for the synthesis of chiral catalyst *O*-TMS quinidine **6.9**. The synthesis of bicyclic β-lactone **6.4** commenced from commercially available cyclohexanone **6.10** which was *O*-silylated with TMSCl to give silyl enol ether **6.11** followed by ozonolysis to provide aldehyde acid **6.12** which underwent our asymmetric NCAL reaction using

Mukaiyama reagent **6.8** and chiral nucleophile *O*-TMS quinidine (*O*-TMSQND) **6.9** to give optically active bicyclic β-lactone **6.4** (Scheme 6.3).

Scheme 6.3. Synthesis of Optically Active Bicyclic β-Lactone **6.4** via Asymmetric NCAL Reaction



6.10 **6.11** **6.9** **6.12** **6.8** **6.4**

Ideally, an *Organic Syntheses* procedure is designed for a large-scale synthesis, easy to handle with minimum column chromatography, and reproducible results which can be followed by a chemist with only fundamental laboratory experience. Moreover, the target molecule / building block is broadly interesting. Finally, safety is also a great concern when large scale reactions are carried out.

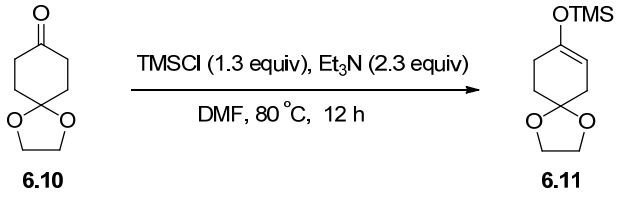
6.2.1. Synthesis of (1,4-dioxaspiro[4.5]dec-7-en-8-yloxy)trimethylsilane

Originally, the synthesis of silyl enol ether **6.11** on small scale was derived from cyclohexanone **6.10**, LDA and TMSCl.[12a] This protocol was modified to be applicable

for the gram scale synthesis using cyclohexanone **6.10** (25g)**,** TMSCl, triethylamine and *N*,*N*-dimethyl formamide. This procedure was optimized to ensure reproducibility with quantitative yield (Scheme 6.4). Notably, silyl enol ether **6.11** is acid sensitive, therefore excess Et_3N was needed for the reaction to reach completion due to the by-product triethylammonium chloride which is mildly acidic under the reactions. After the reaction was completed, an aqueous work-up was neccessary to remove triethyl ammonium hydrochloride salt (soluble in water) and majority of the non volatile and water soluble solvent (*N*,*N*-dimethyl formamide). However, to guarantee maximum product transfer, a back extraction from the aqueous layer was needed for this work-up procedure. One problem that was also recognized was that substantial amounts of starting material 1,4-cyclohexadione mono-ethylene ketal **6.10** was carried on to the last step while procedure was reproduced by a first year graduate student. This required optimization of the reaction procedure with follow up by ^{1}H NMR since the purity of product was not easily monitored by TLC due to facile desilylation of the TMS enol ether **6.11**. One of the contributors for the recovery of cyclohexanone **6.10** came from the original extensively acidic work-up procedure in order to remove excess triethylamine which had been used in the reaction. Monitoring the work-up procedure by ^{1}H NMR showed excessively acidic work-up condition (saturated ammonium chloride solution) resulted in desilylation to the corresponding ketone. However, the largest contributor came from the *N*,*N*-dimethyl formamide, a highly hydroscopic solvent, high concentrations of water in DMF resulted in incomplete reaction due to partial hydrolysis of TMSCl. Therefore, a note was added to the procedure “it is

important to use very dry *N,N*-dimethylformamide (<150 ppm of H_2O) to avoid hydrolysis of TMSCl" to be certain of reproducibility of the procedure . As a result, this work-up procedure guaranteed high product yield, purity and avoided column chromatography.

Scheme 6.4. Synthesis of Silyl Enol Ether **6.11**



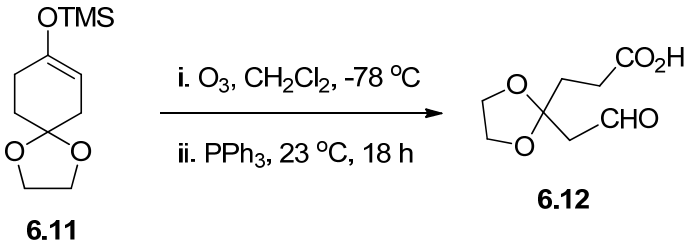
6.10 **6.11**

6.2.2. 3-(2-(2-oxoethyl)-1,3-dioxolan-2-yl) Propanoic Acid

This step of the procedure was an ozonolysis of silyl enol ether **6.11** and subsequent reductive work-up using dimethyl sulfide or triphenyl phosphine to deliver aldehyde acid **6.12** (Scheme 6.5). Original conditions for small scale ozonolysis employed dimethyl sulfide to reduce the ozonide. Product aldehyde acid **6.12** was purified by column chromatography. One hurdle that needed to be overcome was the purification procedure of aldehyde acid **6.12** had to avoid column chromatography. Since by-product was DMSO which was difficult to remove when performing on large scale reaction, and definitely could not remove by an extraction procedure due to DMSO's presence both in the organic and aqueous layers. Alternatively, the use of triphenylphosphine (PPh_3) increased the product yield but isolation of aldehyde acid **6.12** from the by-product triphenyl phosphine oxide was impossible via column

chromatography. Normally, an acid base work-up procedure is applied to purify an acid substrate. First, aldehyde acid **6.12** was converted to carboxylate salt with NaOH which was transferred to aqueous layer and triphenylphine oxide (PPh_3O) remained in organic layer. The aqueous layer was then acidified and aldehyde acid was back extracted into organic layer. Unfortunately, dioxolane functionality was unstable to the strong acid conditions necessary for back extraction. This resulted in dioxolane deprotection to the corresponding ketone. Notably, aldehyde acid **6.12** is highly water soluble, taking advantage of this property, aldehyde acid **6.12** was first dissolved in aqueous layer, and then was back extracted with dichloromethane. Multiple back extractions with dichloromethane helped recover most of aldehyde acid with high purity. The partition constant (partition coefficient) of aldehyde acid **6.12** between water and an organic solvent was the key to an effective extraction. First, aldehyde acid was dissolved in ether and most of the triphenyl phosphine precipitated which was then removed by filtration. Aldehyde acid **6.12** was then brought to the aqueous layer. This protocol worked well with this aldehyde acid whose ether has a low partition consant with. Dichloromethane on the other hand has high partition constant for **6.12** would ensure effective removal of product from water. It is also imperative that extractions of the aldehyde acid are done vigorously to guarantee efficient transfer of the aldehyde acid **6.12** to the organic phase.

Scheme 6.5. Sythesis of Aldehyde Acid **6.12** Precusor for NCAL Reaction

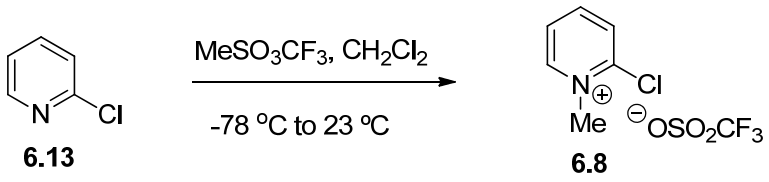


6.11 **6.12**

6.2.3. Preparation of *N*-Methyl-2-Chloropyridinium Trifluoromethane Sulfonate

Mukaiyama reagent **6.8** was prepared using 2-chloropyridine **6.13** and methyl trifluoromethane sulfonate in dichloromethane (Scheme 6.6). A detailed procedure with fully characterization data including IR, ^{1}H NMR and ^{13}C NMR spectra were provided along with the procedure.

Scheme 6.6. Preparation of Mukaiyama Reagent **6.8**



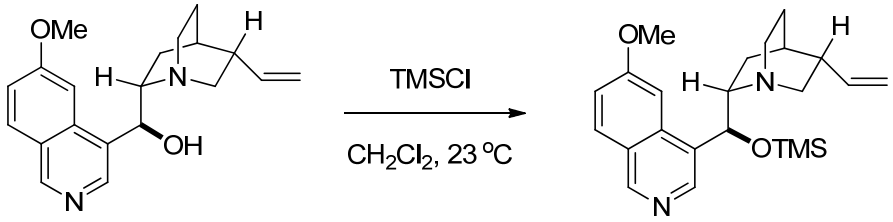
6.13 **6.8**

6.2.4. Preparation of *O*-trimethylsilylquinidine (*O*-TMS QND)[45]

A modified procedure that is described in detail was built on the previously reported protocol by Calter and co-workers and applicable for large scale synthesis. *O*-trimethylsilyl quinidine **6.9** was prepared by treating commercially available quinidine and trimethylchlorosilane in dichloromethane (Scheme 6.7). Purification via column

chromatography was found unnecessary, in this *organic syntheses* procedure the preparation protocol for *O*-TMS QND **6.9** was modified from the original. Since *O*-TMS quinidine **6.9** is a mixture of two conformers at ambient temperature but low temperature ^{1}HNMR allowed two set of data which was also submitted along with the procedure for clarity purposes.

Scheme 6.7. Preparation of *O*-TMS Quinidine **6.9**



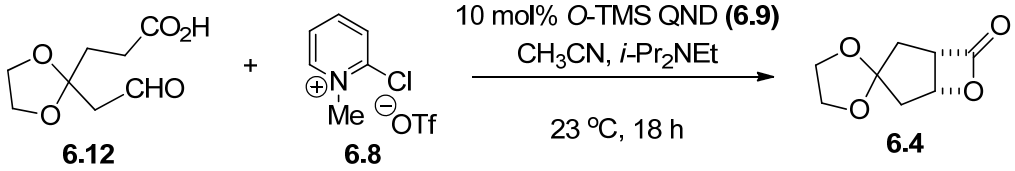
quinidine **6.14** **6.9**

6.2.5. Asymmetric Synthesis of the Bicyclic β-Lactone

Finally, the procedure for the asymmetric NCAL reaction for the formation of the bicyclic β-lactone **6.4** in large scale (~15–17g) was developed and optimized. Low reaction concentrations were originally used for NCAL reaction; however, for this procedure it was found that the reaction could be obtained in higher yield at 0.2–0.4 M as compared to 0.05 M, and complete consumption of starting aldehyde acid **6.12** even with shorter reaction times. Due to the amount of by-product such as pyridone derived from Mukaiyama reagent, and side-product formed in the reaction, a recrystallization protocol for bicyclic β-lactone **6.4** was impossible to obtain. Due to a high affinity of the product with SiO_2, β-lactone **6.4** tends to be decomposed under extended exposure to

column chromatography and found too difficult to isolate in the pure form. In this procedure, a short column was employed to remove most of by products. The semi-crude β-lactone **6.4** after column chromatography could be purified by recrystallization using 50% ethyl acetate and 50% hexanes. Finally, to determine enaniomeric excess of β-lactone **6.4**, ring opening of the β-lactone by (*D*)–(+)–α-methylbenzylamine gave two diastereomers and enantiomeric ratio of β-lactone could be determined indirectly by diasteromeric ratio of ^{1}H NMR; however, the outcome could have resulted from a kinetic resolution. A direct method was developed by chiral HPLC in place of resolution method previously reported by chiral GC using a non-commercially available chiral column: 2,3-di-*O*Ac-6-TBS ®-CD (Scheme 6.8) .

Scheme 6.8. Synthesis of Optically Active β-Lactone

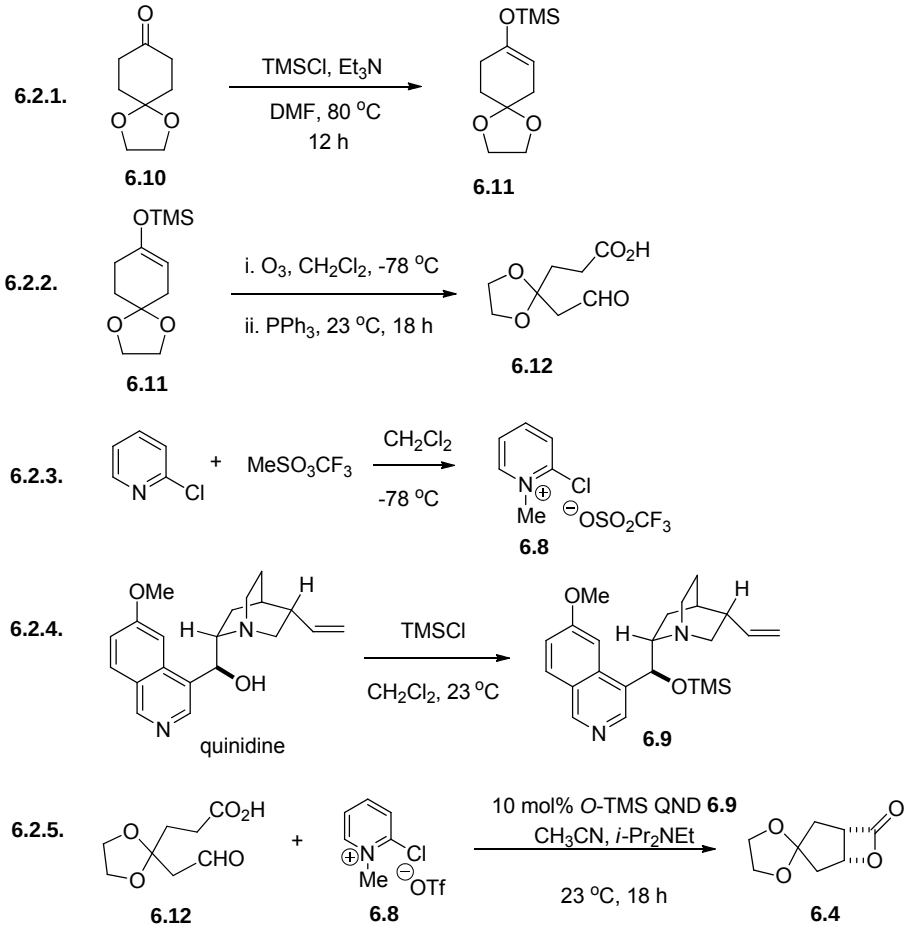


6.3. Conclusion

We successfully developed an *Organic Syntheses* procedure for the asymmetric synthesis of (1*S*,5*R*)-6-oxaspiro[bicyclo[3.2.0]heptane-3,2'-[1,3]dioxolan]-7-one via the nucleophile-catalyzed aldol-lactonization (NCAL). The advantage of this procedure was that an optically active common intermediate (~ 5g, 90% ee) was obtained from achiral starting material 25g with a three step sequence (Scheme 6.9). It was a great challenge

to complete the procedure. The work accomplished resulted from a great team effort among a postdoctoral fellow, two graduate students and a summer intern student. It took almost 4 years since the time the project has started until it actually got accepted. Although it took a lot of effort to finish this *Organic Syntheses* procedure, the reward was remarkable that the procedure we created would be handy and reliable if someone needed to synthesize the same intermediate. The most important thing was the experience that I learned from this opportunity was phenomenal. This experience gave me the idea of how process chemistry is performed in industry.

Scheme 6.9. *Organic Syntheses* Procedure for Optically Active Bicyclic β-Lactone **6.4** via Asymmetric NCAL Reaction



CHAPTER VII

CONCLUSIONS

Natural products continue to be an important proving ground for synthetic chemists due to their diverse molecular architectures and interesting biological activities. The potential of human 20S proteasome inhibitors continues to be of interest for anticancer chemotherapy and the recent FDA approval of bortezomib (Velcade) validates the proteasome as a target for cancer chemotherapy. Salino A is a unique bicyclo [3.2.0] β-lactone-containing natural product. Salino A is a potent nanomolar inhibitor of the proteasome, and is currently in Phase I human clinical studies for multiple myeloma following the finding that salino A exhibited great potential in mouse models toward several cancers when administered intravenously despite the potentially labile β-lactone.

We developed a concise, 9-step racemic synthesis of salino A from *O*-benzyl serine, then extended to enantioselective to (–)-salino A. Our strategy of (–)-salino A and derivatives made possible by the $A^{1,3}$-strain of β-keto tertiary amides which enables retention of optical purity during a key bis-cyclization process that simultaneously forms the γ-lactam and fused β-lactone core. In addtion, the key bis-cyclization of a β-ketoamide, amenable to gram scale, constructs both the γ-lactam and the fused-β-lactone in one operation leading to the brevity of the synthesis. The flexibility of the described strategy derives from the versatility of the bicyclic core enabling attachment of various C4-sidechains, even in the presence of the β-lactone, and the ability to use alternative

ketene heterodimers to vary the C2 side chain. Several derivatives including (–)-homosalino A were synthesized and their activity toward proteasome inhibition was evaluated. The ability of the described β-keto *tertiary* amide substrates to maintain stereochemical integrity by virtue of $A^{1,3}$ strain raises interesting questions regarding how such integrity is maintained with β-keto *secondary* amides, known salino A precursors, or if a dynamic kinetic resolution is operative during related biosynthetic aldol-lactonizations.

Finally, an *Organic Syntheses* procedure for the asymmetric synthesis of (1*S*,5*R*)-6-oxaspiro[bicyclo[3.2.0]heptane-3,2'-[1,3]dioxolan]-7-one via the nucleophile-catalyzed aldol-lactonization (NCAL) was successfully optimized with other colleagues.

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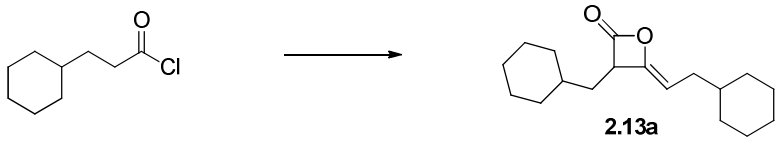
APPENDIX A

EXPERIMENTAL PROCEDURES AND SELECTED SPECTRA

General

All reactions were carried out under nitrogen atmosphere in flame-dried glassware. Dichloromethane, acetonitrile, methanol, tetrahydrofuran, and ethyl ether were purified by passage through activated molecular sieves. Hünig's base and triethylamine were distilled from potassium hydroxide prior to use. All other commercially obtained reagents were used as received. ^{1}H NMR chemical shifts are reported as δ values in ppm relative to $CDCl_3$ (7.27 ppm), C_6D_6 (7.15 ppm), C_2D_6O (2.05 ppm) and coupling constants (*J*) are reported in Hertz (Hz). Unless indicated otherwise, deuterochloroform ($CDCl_3$) served as an internal standard (77.23 ppm), C_6D_6 (128.39 ppm), C_2D_6O (29.92 ppm) for all ^{13}C spectra. Flash column chromatography was performed using 60Å Silica Gel (Silicycle, 230-400 mesh) as a stationary phase. Mass spectra were obtained at the Center for Chemical Characterization and Analysis (Texas A&M University). Thin layer chromatography (TLC) was performed using glass-backed silica gel 60_{F254} (250 μm thickness).

Procedure

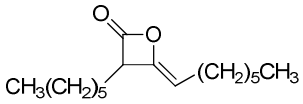


2.13a

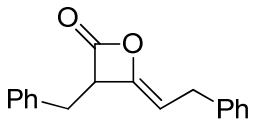
Representative Procedure for Ketene Dimerization[1] as Described for Homodimer (±)-2.13a: To a solution of 3-cyclohexyl propionyl chloride (17.5 g, 100 mmol) in Et_2O (75 mL) was added triethylamine (16.0 mL, 110 mmol) at a rate sufficient to maintain gentle refluxing.

[1] (a) Duffy, R. J.; Morris, K. A.; Romo, D. *J. Am. Chem. Soc.*, **2005**, 127, 16754-16755. (b) Purohit, V. C.; Richardson, R. D.; Smith, J. W.; Romo, D. *J. Org. Chem.* **2006**, *71*, 4549-4558.

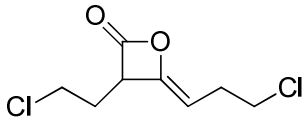
During addition of triethylamine, a white solid precipitated. After complete addition of triethylamine, the reaction mixture was refluxed for an additional 1 h, cooled to ambient temperature, and filtered through a pad of Celite and SiO_2. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (95:5 pentane:Et_2O) to afford ketene dimer (±)-2.**13a** (8.25 g, 60 %) as a colorless oil.



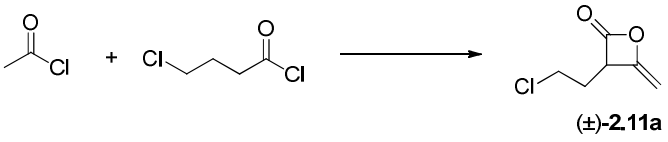
Homodimer (±)-2.13b:[1] Prepared according to the representative procedure using octanoyl chloride (5.4 g, 33 mmol) in Et_2O (25 mL) and triethylamine (5.2 mL, 37 mmol). Purification by flash chromatography on SiO_2 (95:5 pentane:Et_2O) gave ketene dimer (±)-**2.13b** (3.0 g, 65%) as a clear oil. R_f = 0.74 (20% EtOAc/hexanes); IR (neat) 1875, 1726cm^{-1}cm^{-1}; ^{1}H NMR (500 MHz, C_6D_6) δ 4.36 (dt, *J* = 1.5, 7.5 Hz, 1H), 3.33 (dt, *J* = 1.0, 7.0 Hz, 1H), 2.01-2.15 (m, 2H), 1.02-1.36 (m, 18H), 0.87 (t, *J* = 7.0 Hz, 3H), 0.84 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, C_6D_6) δ169.0, 146.5, 101.0, 54.0, 31.9, 31.7, 29.8, 29.10, 29.08, 27.6, 26.5, 25.0, 23.0, 22.8, 14.3, 14.2; LRMS (CI) Calcd. for $C_{16}H_{28}O_2$ [M+H] 253, found 253.



Homodimer (±)-2.13c:[1] Prepared according to the representative procedure using hydrocinnamoyl chloride (5.0 g, 30 mmol) in diethyl ether (25 mL) and triethylamine (4.6 mL, 33 mmol). Purification by flash chromatography on SiO_2 (95:5 pentane:Et_2O) gave ketene dimer (±)-2.**13c** (1.75 g, 46%) as a clear oil.



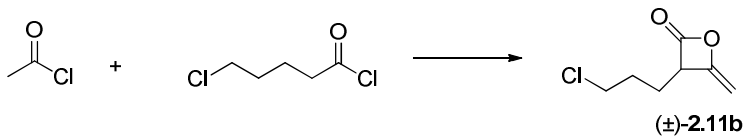
Homodimer (±)-2.13d: To a solution of 4-chlorobutyrylchloride (5.64 mL, 0.05 mol) in Et_2O (50 mL) was added triethylamine (8.4 mL, 0.06 mol) via a syringe pump at 23 ^oC for a period of 1 h. During addition of triethylamine, a salt precipitated as a white solid. After stirring for an additional 90 min, the reaction mixture was diluted with hexanes (75 mL), filtered through a pad of SiO_2 via a fritted funnel, and then the pad of SiO_2 was washed with 200 mL of 40% Et_2O/hexanes. The combined filtrates were concentrated under reduced pressure and the residue was purified by flash chromatography (95:5 pentane/Et_2O) gave ketene dimer (±)-2.**13d** (2.25 g, 43 %) as a colorless oil. R_f = 0.29 (90% pentane/ether); IR (neat) 1874, 1726cm^{-1}; ^1H NMR (500 MHz, C_6D_6) δ 4.20 (t, *J* = 7.0 Hz, 1H), 3.36 (t, *J* = 7.0 Hz, 1H), 2.98-3.01 (m, 2H), 2.92-2.96 (m, 1H), 2.86-2.90 (m, 1H), 2.09-2.21 (m, 2H), 1.30-1.45 (m, 2H); ^{13}C NMR (125 MHz, C_6D_6) δ 167.7, 147.3, 98.2, 51.7, 44.1, 41.4, 30.4, 28.5; LRMS (EI) Calcd. for $C_8H_{10}Cl_2O_2$ [M] 208, found 208. Satisfactory HRMS could not be obtained for this compound by MALDI or ESI.



(±)-2.11a

Heterodimer (±)-2.11a: To a 2-neck 500 mL round bottom flask fitted with a condenser, acetyl chloride (11.0 mL, 0.156 mol), 4-chlorobutyrylchloride (14.7 mL, 0.130 mol), and Et_2O (200 mL) was added, followed by triethylamine (43.9 mL, 0.312 mol) via a syringe pump at 23 ^oC for a period of 1 h. During addition of triethylamine, the triethylamine hydrochloride salt precipitated as a white solid. After stirring for an additional 1 h, the reaction mixture was diluted

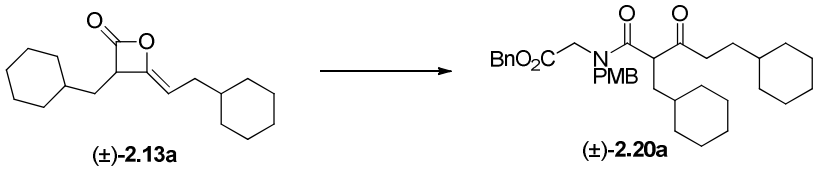
with hexanes (300 mL) and filtered through a pad of SiO_2 via a fritted funnel. The pad of SiO_2 was then washed with 300 mL of 40% Et_2O/hexanes. The combined filtrates were concentrated under reduced pressure, and the residue was purified by flash chromatography (95/5 pentane/Et_2O) to afford ketene-dimer (±)-**2.11a** (2.3 g, 13 %) as a clear oil. R_f = 0.41 (9:1 pentane/Et_2O); IR (neat) 1860, 1694 cm^{-1}; ^1H NMR (300 MHz, benzene-d_6) δ 4.41 (dd, *J* = 2.1, 4.5 Hz, 1H), 3.80 (dd, *J* = 1.5, 4.5 1H), 3.35 (t, *J* = 7.8 Hz, 1H), 2.79-2.95 (m, 2H), 1.25-1.46 (m, 2H); ^{13}C NMR (125 MHz, benzene-d_6) δ 167.4, 152.6, 85.7, 51.7, 40.9, 29.9; LRMS (CI) Calcd. for $C_6H_8ClO_2$ [M+H] 147, found 147.



(±)-**2.11b**

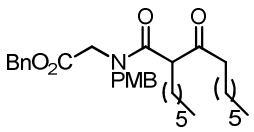
Heterodimer (±)-2.11b: To a solution of acetyl chloride (7.5 mL, 0.105 mol) and valerylchloride (10.4 mL, 0.081mol) in Et_2O (200 mL) was added triethylamine (29.6 mL, 0.211 mol) via a syringe pump at 0 ^oC for a period of 1h. During addition of triethylamine, the triethylamine hydrochloride salt precipitated as a white solid. After stirring for an additional 10 min at 0 ^oC, the reaction mixture was removed from the ice-bath and stirring was continued at 23 ^oC for 45 min. The reaction mixture was diluted with hexanes (300 mL), filtered through a pad of SiO_2 via a fritted funnel, and then the pad of SiO_2 was washed with 300 mL of 40% Et_2O/hexanes. The combined filtrates were concentrated under reduced pressure and the residue was purified by flash chromatography (95:5 pentane/Et_2O) to afford ketene dimer (±)-**2.11b** (1.3 g, 10 %) as a clear oil. R_f = 0.51 (20% EtOAc/hexanes); IR (neat) 1860, 1694 cm^{-1}; ^1H NMR (500 MHz, benzene-d_6) δ 4.43 (dd, *J* = 1.5, 4.5 Hz, 1H), 3.79 (dd, *J* = 1.5, 4.5 Hz, 1H), 2.98-3.01 (m, 1H), 2.80-2.83 (m, 2H), 1.25-1.36 (m, 1H), 1.16-1.24 (m, 3H); ^{13}C NMR (125 MHz,

benzene-d_6) δ 168.2, 153.7, 85.6, 54.1, 44.1, 29.3, 24.8; LRMS (CI) Calcd. for $C_6H_{10}ClO_2$ [M+H] 161, found 161.

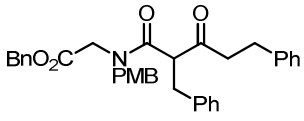


(±)-**2.13a** (±)-**2.20a**

Representative Procedure for Ring Opening of Ketene Dimers to Give β-Ketoamides as Described for β-Ketoamide (±)-2.20a: To a solution of (4-methoxy-benzylamino)-acetic acid benzyl ester (178 mg, 0.624 mmol) and 2-hydroxypyridine (59 mg, 0.624 mmol) in THF (2 mL) was added ketene dimer (±)-**2.13a** (259 mg, 0.936 mmol). The reaction mixture was stirred at 50 °C for 24h (or treated at 60 °C for 3h with microwave irradiation) and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (1:4 EtOAc/hexanes) to afford keto ester (±)-**2.20a** (303 mg, 86%) as a colorless oil and as a 2.2:1 ratio of rotamers: IR (neat) 1749, 1652 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30-7-40 (m, 5 H), 7.07-7.13 (m, 2 H), 6.81-6.89 (m, 2 H), 5.15 (s, 1.4 H), 5.14 (0.6 H), 4.73 (d, *J* = 16.5 Hz, 0.7H), 4.68 (d, *J* = 15.3 Hz, 0.3H), 4.49 (d, *J* = 15.6 Hz, 0.3H), 4.43 (d, *J* = 16.5 Hz, 0.7H), 4.27 (d, *J* = 17.1 Hz, 0.7H), 4.13 (d, *J* = 18.6 Hz, 0.3H), 3.94 (d, *J* = 17.4 Hz, 0.7H), 3.93 (d, *J* = 18.3 Hz, 0.3H), 3.78-3.83 (m, 3.7 H) 3.55 (t, *J* = 9.0 Hz, 0.3H), 2.40-2.58 (m, 2H), 0.76-1.94 (m, 26H); ^{13}C NMR were complex due to the presence of rotamers and attempted VT NMR did not lead to coalescence so these are not included; LRMS (ESI Calcd. For $C_{35}H_{47}NO_5$ [M+H] 561, found 562.

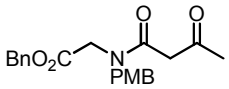


β-Ketoamide (±)-2.20b: Prepared according to the representative procedure using (4-methoxy-benzylamino)-acetic acid benzyl ester (910 mg, 3.02 mmol), 2-hydroxypyridine (304 mg, 3.02 mmol) in THF (13 mL), and ketene dimer (±)-**2.13b** (800 mg, 3.02 mmol). Purification by flash chromatography on SiO_2 (1:4 EtOAc/hexanes) gave keto ester (±)-**2.20b** (1.36 g, 82%) as a colorless oil and as a 2.2:1 ratio of rotamers: IR (neat) 1750, 1646 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.38 (m, 5H), 7.11 (d, *J* = 8.0 Hz, 0.6H), 7.08 (d, *J* = 8.5 Hz, 1.4H), 6.87 (d, *J* = 8.5 Hz, 1.4H), 6.82 (d, *J* = 8.5 Hz, 0.6H), 5.09-5.17 (m, 2H), 4.72 (d, *J* = 16.5 Hz, 0.7H), 4.64 (d, *J* = 15.0 Hz, 0.3H), 4.53 (d, *J* = 15.0 Hz, 0.3H), 4.43 (d, *J* = 16.5 Hz, 0.7H), 4.25 (d, *J* = 17.5 Hz, 0.7H), 4.13 (d, *J* = 19.0 Hz, 0.3H), 3.93 (d, *J* = 18.5 Hz, 0.3H), 3.92 (d, *J* = 17.5 Hz, 0.7H), 3.80 (s, 2.1H), 3.78 (s, 0.9H), 3.65 (t, *J* = 7.0 Hz, 0.7H), 3.40 (t, *J* = 7.0 Hz, 0.3H), 2.42-2.57 (m, 2H), 1.94-2.01 (m, 1H), 1.79-1.86 (m, 1H), 1.47-1.55 (m, 2H), 1.17-1.31 (m, 16H), 0.86-0.90 (m, 6H); ^{13}C NMR were complex due to the presence of rotamers and attempted VT NMR did not lead to coalescence so these are not included; LRMS (ESI) Calcd. for $C_{33}H_{47}NO_5$ [M+Li] 544, found 544.

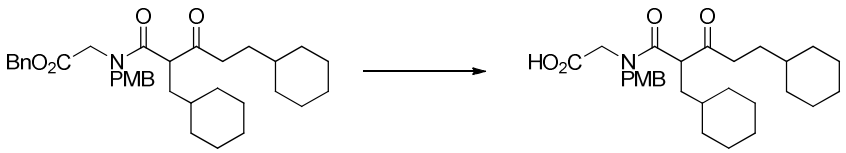


β-Ketoamide (±)-2.20c: Prepared according to the representative procedure using (4-methoxy-benzylamino)-acetic acid benzyl ester (636 mg, 2.23 mmol), 2-hydroxypyridine (212 mg, 2.23 mmol) in THF (22 mL), and ketene dimer (±)-**2.13c** (588 mg, 2.22 mmol). Purification by flash chromatography on SiO_2 (1:4 EtOAc/hexanes) gave keto ester (±)-**2.20c** (1.04 g, 85%) as a

colorless oil. 2.2:1 ratio of rotamers: IR (neat) 1745, 1642 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.08-7.38 (m, 15H), 6.98 (d, *J* = 8.5 Hz, 0.6H), 6.78 (d, *J* = 9.0 Hz, 0.6H), 6.72 (d, *J* = 8.5 Hz, 1.4H), 6.69 (d, *J* = 9.0 Hz, 1.4H), 5.14 (s, 1.4H), 5.05 (s, 0.6H), 4.77 (d, *J* = 14.5 Hz, 0.3H), 4.56 (d, *J* = 16.5 Hz, 0.7H), 4.31 (d, *J* = 17.5 Hz, 0.7H), 4.28 (d, *J* = 12.5 Hz, 0.3H), 4.18 (d, *J* = 16.5 Hz, 0.7H), 3.96 (d, *J* = 8.5 Hz, 0.3H), 3.95 (d, *J* = 9.0 Hz, 0.3H), 3.79 (s, 0.9H), 3.78 (s, 2.1H), 3.64-3.72 (m, 1.7H), 3.29 (dd, *J* = 9.0, 14.0 Hz, 0.7H), 3.16-3.24 (m, 0.6H), 3.12 (dd, *J* = 5.5, 13.5 Hz, 0.7H), 2.77-2.96 (m, 4H); ^{13}C NMR were complex due to the presence of rotamers and attempted VT NMR did not lead to coalescence so these are not included; LRMS (ESI) Calcd. for $C_{35}H_{35}NO_5$ [M+Li] 556, found 556.

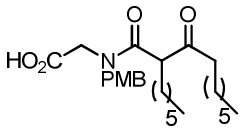


β-Ketoamide (±)-2.20e: Prepared according to the representative procedure using (4-methoxy-benzylamino)-acetic acid benzyl ester (910 mg, 3.19 mmol), 2-hydroxypyridine (304 mg, 3.20 mmol) in THF (25 mL), and commercially available ketene dimer **2.13e** (1.0 mL, 16 mmol). Purification by flash chromatography on SiO_2 (1:4 EtOAc/hexanes) gave keto ester **2.20e** (930 mg, 78%) as a colorless oil. Due to the presence of enol tautomers and amide rotamers, the NMR spectra of this compound is extremely complex and so line listing is not provided.



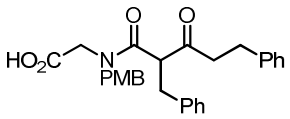
Representative Procedure for Preparation of Ketoacid Intermediate from Benzyl Ester as Described for β-Ketoacid (±)-2.21a: A racemic mixture of ketoamide benzyl ester **(±)-2.20a**

(270 mg, 0.481 mmol), and 10 wt% palladium on carbon (27 mg) in THF (10 mL) was stirred at ambient temperature for 3 h under H_2 atmosphere. The reaction mixture was filtered through a pad of Celite, and concentrated to afford keto acid (±)-**2.21a** (222 mg, 98%) as a white solid and as a 2.2:1 ratio of two rotamers: IR (neat) 1729, 1652 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.5 Hz, 0.6H), 7.09 (d, *J* = 8.5 Hz, 1.4H), 6.89 (d, *J* = 9.0 Hz, 1.4H), 6.84 (d, *J* = 9.0 Hz, 0.6H), 4.71 (d, *J* = 16.5 Hz, 0.7H), 4.68 (d, *J* = 13.5 Hz, 0.3H), 4.47 (d, *J* = 15.0 Hz, 0.3H), 4.42 (d, *J* = 16.5 Hz, 0.7H), 4.25 (d, *J* = 17.5 Hz, 0.7H), 4.13 (d, *J* = 19.0 Hz, 0.3H), 3.94 (d, *J* = 18.0 Hz, 0.3H), 3.90 (d, *J* = 17.0 Hz, 0.7H), 3.81 (s, 3H), 3.79 (dd, *J* = 1.5, 4.5 Hz, 0.7H), 3.57 (t, *J* = 7.0 Hz, 0.3H), 2.44-2.56 (m, 2H), 1.88-1.94 (m, 1H), 1.54-1.74 (m, 11H), 1.35-1.43 (m, 2H), 1.07-1.20 (m, 8H), 0.78-0.92 (m, 4H); LRMS (APCI) Calcd. for $C_{28}H_{41}NO_5$ [M–H] 470, found 470.

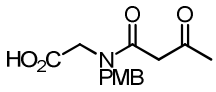


β-Ketoacid (±)-2.21b: Prepared according to the representative procedure for preparation of ketoacid intermediate from benzyl ester (±)-**2.20b** (415 mg, 0.772 mmol), 10 wt% palladium on carbon (40 mg) in THF (10 mL) afford keto acid (±)-**2.21b** (340 mg, 98%) as a colorless oil and as a 3:1 ratio of rotamers: IR (neat) 1721, 1649, 1614 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.5 Hz, 0.5H), 7.09 (d, *J* = 8.5 Hz, 1.5H), 6.89 (d, *J* = 9.0 Hz, 1.5H), 6.84 (d, *J* = 8.5 Hz, 0.5H), 4.71 (d, *J* = 16.5 Hz, 0.75H), 4.65 (d, *J* = 14.5 Hz, 0.25H), 4.51 (d, *J* = 14.5 Hz, 0.25H), 4.42 (d, *J* = 16.5 Hz, 0.75H), 4.23 (d, *J* = 17.5 Hz, 0.75H), 4.12 (d, *J* = 19.0 Hz, 0.25H), 3.94 (d, *J* = 19.0 Hz, 0.25H), 3.89 (d, *J* = 17.5 Hz, 0.75H), 3.81 (s, 2.25H), 3.79 (s, 0.75H), 3.66 (dd, *J* = 6.0, 8.0 Hz, 0.75H), 3.43 (t, *J* = 7.0 Hz, 0.25H), 2.45-2.55 (m, 2H), 1.95-2.04 (m, 1H), 1.79-1.87

(m, 1H), 1.48-1.55 (m, 2H), 1.16-1.34 (m, 16H), 0.85-0.88 (m, 6H); LRMS (ESI) Calcd. for $C_{26}H_{41}NO_5$ [M-H] 446, found 446.

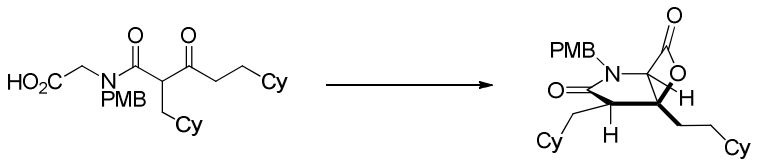


β-Ketoacid (±)-2.21c: Prepared according to the representative procedure for preparation of ketoacid intermediate from benzyl ester (±)-**2.20c** (985 mg, 1.79mmol), 10 wt% palladium on carbon (99 mg) in a mixture of solvent THF (20 mL) and MeOH (4 mL) afforded ketoacid (±)-**2.21c** (0.70g, 85%) as a white solid and as a 2.2:1 ratio of rotamers: IR (neat) 1722, 1634, 1612 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.10-7.28 (m, 10H), 6.98 (d, *J* = 8.5 Hz, 0.6H), 6.79 (d, *J* = 9.0 Hz, 0.6H), 6.73 (d, *J* = 9.0 Hz, 1.4H), 6.69 (d, *J* = 9.0 Hz, 1.4H), 4.81(d, *J* = 15.0 Hz, 0.3H), 4.50 (d, *J* = 16.5 Hz, 0.7H), 4.22 (d, *J* = 14.5 Hz, 0.3H), 4.21 (d, *J* = 17.0 Hz, 0.7H), 4.14 (d, *J* = 16.5 Hz, 0.7H), 3.96 (d, *J* = 9.0 Hz, 0.3H), 3.95 (d, *J* = 9.0 Hz, 0.3H), 3.78 (s, 0.9H), 3.77 (s, 2.1H), 3.69 (d, *J* = 17.5 Hz, 0.7H), 3.30 (dd, *J* = 9.0, 13.0 Hz, 0.7H), 3.24 (dd, *J* = 9.0, 13.0 Hz, 0.3H), 3.18 (dd, *J* = 5.0, 13.5 Hz, 0.3H), 3.12 (dd, *J* = 5.0, 13.5 Hz, 0.7H), 2.76-2.97 (m, 5H); LRMS (ESI) Calcd. for $C_{28}H_{29}NO_5$ [M-H] 458, found 458.

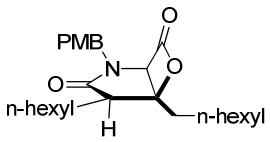


β-Ketoacid 2.21e: Prepared according to the representative procedure for preparation of ketoacid intermediate from benzyl ester **2.20e** (0.320 mg, 0.866 mmol), 10 wt% palladium on carbon (35 mg) in THF (10 mL) afford ketoacid **2.21e** (250 mg, 99%) as a colorless oil. IR (neat) 1723, 1612 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.13 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.0

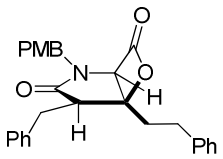
Hz, 2H), 4.51 (s, 2H), 4.05 (s, 2H), 3.81 (s, 3H), 3.68 (s, 2H), 2.30 (s, 3H) (only major peaks were assigned); LRMS (ESI) Calcd. for $C_{14}H_{17}NO_5$ [M–H] 278, found 278.



Representative Procedure for Preparation of β-Lactone via Bis-Cyclization as Described for β-Lactone, (±)-2.23a: To a suspension of *N*-propyl-2-bromo pyridinium triflate (95 mg, 0.27 mmol) and 4-pyrrolidinopyridine (40 mg, 0.27 mmol) in CH_2Cl_2 (4 mL) was added Hünig's base (63 μL, 0.36 mmol) at 0 °C. After stirring for 10 min, a solution of ketoacid **(±)-2.21a** (85 mg, 0.18 mmol) in CH_2Cl_2 (3 mL) was added via syringe pump over 1 h at 0 °C. The resulting suspension was stirred for 2 h at 0 °C. The crude reaction mixture was diluted with Et_2O (50 mL) and washed with aqueous NH_4Cl solution and brine (each 30 mL). The organic layer were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (1:10 EtOAc/hexanes) to give a mixture of two diastereomeric β-lactones (76 mg, 93%, dr 2.2:1) as a colorless oil. **(±)-2.23a** (major): R_f = 0.76 (40% EtOAc/hexanes); IR (neat) 1825, 1709 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 5.03 (d, *J* = 14.5 Hz, 1H), 4.34 (s, 1H), 4.04 (d, *J* = 14.5 Hz, 1H), 3.81 (s, 3H), 2.70 (dd, *J* = 6.0, 7.5 Hz, 1H), 1.86-1.97 (m, 2H), 1.60-1.81 (m, 11H), 1.08-1.32 (m, 10H), 0.80-1.00 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7, 166.2, 159.5, 130.0, 126.8, 114.3, 83.1, 68.2, 53.3, 45.2, 43.8, 37.3, 34.8, 33.6, 33.4, 33.2, 32.9, 32.6, 32.5, 31.2, 26.5, 26.4, 26.2, 26.1, 26.0 (2); LRMS (ESI) Calcd. for $C_{28}H_{39}NO_4$ [M+Li] 460, found 460.

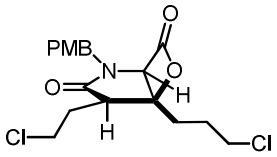


β-Lactone (±)-2.23b: Prepared according to the representative procedure for preparation of β-lactone via bis-cyclization using *N*-propyl-2-bromo pyridinium triflate (141 mg, 0.402 mmol), 4-pyrrolidinopyridine (60 mg, 0.40 mmol), Hünig's base (93 μL, 0.54 mmol), and ketoacid **(±)-2.21b** (120 mg, 0.268 mmol) in CH_2Cl_2 (11 mL). Purification by flash chromatography on SiO_2 (1:10 EtOAc/hexanes) gave a mixture of two diastereomeric β-lactones (104 mg, 90%, dr = 2.2:1). **(±)-2.23b** (major): IR (neat) 1836, 1705 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 5.04 (d, *J* = 15.0 Hz, 1H), 4.36 (s, 1H), 4.05 (d, *J* = 15.0 Hz, 1H), 3.80 (s, 3H), 2.55 (dd, *J* = 5.5, 9.0 Hz, 1H), 1.85-2.00 (m, 3H), 1.69-1.77 (m, 1H), 1.47-1.58 (m, 2H), 1.18-1.39 (m, 16H), 0.89 (t, *J* = 6.8 Hz, 3H), 0.87 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 166.3, 159.7, 130.2, 127.0, 114.5, 83.0, 68.5, 55.4, 47.4, 45.4, 35.6, 31.74, 31.68, 29.5, 29.4, 29.1, 28.0, 26.3, 24.0, 22.8, 22.7, 14.24, 14.19; LRMS (ESI) Calcd. for $C_{26}H_{39}NO_4$ [M+H] 430, found 430.



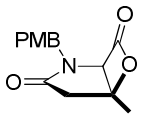
β-Lactone (±)-2.23c: Prepared according to the representative procedure for preparation of β-lactone via biscyclization using *N*-propyl-2-bromo pyridinium triflate (84.6 mg, 0.245 mmol), 4-pyrrolidinopyridine (36.2 mg, 0.245 mmol), Hünig's base (57 μL, 0.33 mmol), and ketoacid **(±)-2.21c** (75 mg, 0.16 mmol) in CH_2Cl_2 (6.5 mL). Purification by flash chromatography on SiO_2 (1:4 EtOAc/hexanes) gave two diastereomeric β-lactones (61 mg, 85%, dr = 2.5:1). **(±)-2.23c**

(major): R_f = 0.29 (20% EtOAc/hexanes); IR (neat) 1830, 1702, 1612 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.83-7.34 (m, 14H), 4.99 (d, *J* = 15.0 Hz, 1H), 4.12 (s, 1H), 4.07 (d, *J* = 14.0 Hz, 1H), 3.83 (s, 3H), 3.38 (dd, *J* = 3.0, 13.0 Hz, 1H), 2.98 (dd, *J* = 11.5, 13.0 Hz, 1H), 2.92 (dd, *J* = 3.5, 11.5 Hz, 1H), 2.34-2.43 (m, 2H), 1.63-1.79 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 166.1, 159.7, 139.3, 138.6, 130.4, 129.4, 128.9, 128.8, 128.1, 127.0, 126.7, 126.6, 114.5, 82.5, 68.8, 55.5, 49.7, 45.6, 36.2, 31.6, 30.1; LRMS (ESI) Calcd. for $C_{28}H_{27}NO_4$ [M+H] 442, found 442.

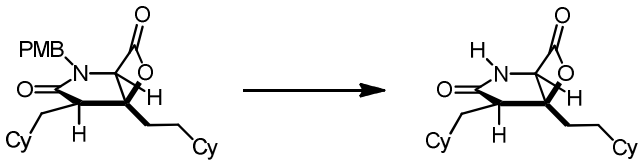


β-Lactone (±)-2.23d: To a suspension of *N*-propyl-2-bromo pyridinium triflate (130 mg, 0.371 mmol) and 4-pyrrolidinopyridine (53 mg, 0.371 mmol) in CH_2Cl_2 (6 mL) was added Hünig's base (63 μL, 0.494 mmol) at 0 °C. After stirring for 10 min, a solution of the crude ketoacid **2.21d** from hydrogenolysis (100 mg, 0.247mmol) in CH_2Cl_2 (4 mL) was added via syringe pump over 1 h at 0 °C. The resulting suspension was stirred for 2 h at 0 °C. The crude reaction mixture was diluted with Et_2O (70 mL) and washed with aqueous NH_4Cl solution and brine (30 mL of each). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (1:10 EtOAc/hexanes) to give a mixture of two diastereomeric β-lactones (67 mg, 70%, dr 4:1) as a colorless oil. **(±)-2.23d** (major): R_f = 0.66 (40% EtOAc/hexanes); IR (neat) 1832, 1702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.22 (d, *J* = 14.5 Hz, 2H), 6.90 (d, *J* = 14.5 Hz, 2H), 5.05 (d, *J* = 24.5 Hz, 1H), 4.45 (s, 1H), 4.02-4.10 (m, 1H), 4.06 (d, *J* = 24.5 Hz, 1H), 3.82 (s, 3H), 3.73-3.81 (m, 1H), 3.49-3.62 (m, 2H), 2.98 (t, *J* = 12 Hz, 1H), 2.26-2.40 (m, 1H), 2.05-2.24 (m, 3H), 1.84-1.93 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1,

165.1, 159.6, 130.1(2C), 126.3, 114.4(2C), 81.4, 68.9, 53.3, 45.3, 43.8, 43.7, 42.4, 32.4, 29.0, 26.6; LRMS (ESI) Calcd. for $C_{18}H_{22}Cl_2NO_4$ [M+H] 386, found 386.



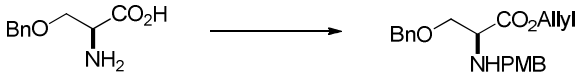
β-Lactone (±)-2.23e: Prepared according to the representative procedure for preparation of β-lactone via biscyclization using *N*-propyl-2-bromo pyridinium triflate (188 mg, 0.537 mmol), 4-pyrrolidinopyridine (79.6 mg, 0.577 mmol), Hünig's base (125 μL, 0.716 mmol), and ketoacid **2.21e** (100 mg, 0.358 mmol) in CH_2Cl_2 (14 mL). Purification by flash chromatography on SiO_2 (2:3 EtOAc/hexanes) gave β-lactone **(±)-2.23e** (23 mg, 25%). R_f = 0.14 (33% EtOAc/hexanes); IR (neat) 1836, 1702 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.4 Hz, 2H), 6.90 (d, *J* = 8.4 Hz, 2H), 5.06 (d, *J* = 14.7 Hz, 1H), 4.41 (s, 1H), 4.07 (d, *J* = 14.7 Hz, 1H), 3.82 (s, 3H), 3.05 (d, *J* = 18.9 Hz, 1H), 2.70 (d, *J* = 18.6 Hz, 1H), 1.70 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 165.8, 159.8, 130.4, 126.7, 114.5, 77.9, 71.7, 55.5, 45.5, 41.6, 22.2; LRMS (APCI) Calcd. for $C_{14}H_{15}NO_4$ [M+Li] 268, found 268.



PMB-Deprotection of β-lactone, (±)-2.24a: To a solution of **(±)-2.23a** (20 mg, 0.044 mmol) in CH_3CN (1 mL) was added an aqueous solution of CAN (123 mg, 0.225 mmol) in H_2O (0.4 mL) at 0 °C dropwise. After stirring at ambient temperature for 1 h, the reaction mixture was diluted with saturated $NaHCO_3$ (2 mL) and extracted with EtOAc (20 mL x 3). The combined organic

layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography (1:6 to 1:1 EtOAc/hexanes) to give the desired product **(±)-2.24a** (13 mg, 89%) as a white solid. A crystal suitable for X-ray analysis was obtained by slow evaporation from Et_2O with ~5% CH_2Cl_2. R_f = 0.55 (40% EtOAc/hexanes); IR (neat) 1832, 1709 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.48 (s, 1H), 4.61 (s, 1H), 2.62 (dd, *J* = 6.5, 8.5 Hz, 1H), 1.98-2.02 (m, 2H), 1.51-1.81 (m, 13H), 1.12-1.35 (m, 9H), 0.87-0.98 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.4, 166.7, 85.6, 65.2, 42.8, 37.5, 34.7, 33.7, 33.2, 33.1, 32.9, 32.7, 32.5, 31.4, 26.44, 26.37, 26.12, 26.09, 26.08, 26.0; LRMS (ESI) Calcd. for $C_{20}H_{31}NO_3$ [M+H] 334, found 334.

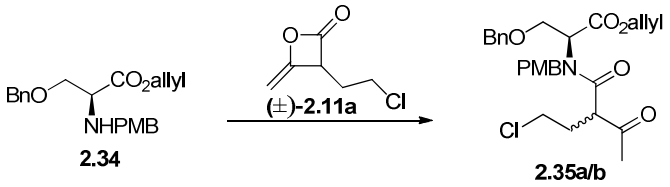
Procedure for Racemic Synthesis of (±)-Salinosporamide A



2.10 **2.34**

(*S*)-*O*-Benzyl Serine Allyl Ester 2.34: To the suspension of (*S*)-*O*-benzyl serine **2.10** (3.85 g, 19.6 mmol) and *p*-anisaldehyde (3.21 g, 23.5 mmol) in MeOH (40 mL) was added triethylamine (3.28 mL, 23.5 mmol) at ambient temperature. The resulting suspension was stirred at ambient temperature for 1 h. The resulting solution was diluted with additional MeOH (40 mL) and $NaBH_4$ (1.11 g, 29.4 mmol) was added at 0 °C portionwise. After stirring at ambient temperature for 2 h, all volatiles were removed under reduced pressure. The remained solid was dissolved in water (50 mL) and acidified to pH 2 with 1 N HCl. The precipitate white solid was filtered, washed with water (2 X 30 mL) and Et_2O (2 X 30 mL), and dried under vacuum to give *O*-benzyl-*N*-PMB serine (5.21g, 84%) as a white solid.

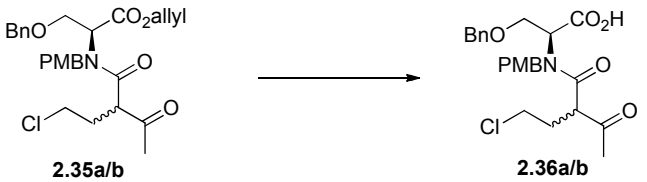
The suspension of *O*-benzyl-*N*-PMB serine (12.8 g, 40.6 mmol) and *p*-TsOH (9.65 g, 50.8 mmol) in allyl alcohol (30 mL) and benzene (100 mL) was stirred at reflux with a Dean-Stark apparatus until the calculated amount of water had been collected. The resulting solution was concentrated in *vacuo*, re-suspended in 5% aqueous $NaHCO_3$ (100 mL), the pH was adjusted to 9.0 with 1 M NaOH, and the product was extracted with Et_2O:EtOAc (1:1, 100 mL x 3). The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by flash chromatography (1:6 EtOAc/hexanes) to give the desired allyl ester **2.34** (12.7 g, 88%) as a yellow oil. R_f = 0.61 (33% EtOAc/hexanes); IR (neat) 1738, 1612 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.25-7.35 (m, 7 H), 6.90 (d, *J* = 8.5 Hz, 2H), 5.87-5.95 (m, 1 H), 5.22-5.35 (m, 2 H), 4.69 (dt, *J* = 1.2, 5.7 Hz, 2H), 4.58 (d, *J* = 12.3 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 3.89 (d, *J* = 12.6 Hz, 1H), 3.82 (s, 3H), 3.70-3.82 (m, 2H), 3.71 (d, *J* = 13.2 Hz, 1H), 3.57 (t, *J* = 4.8 Hz, 1H), 2.28 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 158.9, 138.1, 132.2, 131.9, 129.8 (2C), 128.6 (2C), 127.9, 127.8(2C), 118.7, 114.0 (2C), 73.4, 71.3, 65.7, 60.6, 55.5, 51.6; LRMS (ESI) Calcd. for $C_{21}H_{26}NO_4$ [M+H] 356, found 356.



β-Ketoamide 2.35a/2.35b: Prepared according to the representative procedure for ring opening of hetero-ketene dimers using allyl ester **2.34** (1.92 g, 5.40 mmol), 2-hydroxypyridine (642 mg, 6.75 mmol) in THF (14 mL), and ketene dimer (±)-**2.11a** (990 mg, 6.75 mmol). The reaction mixture was stirred at 60 °C for 36 h and purification by flash chromatography on SiO_2 (1:4

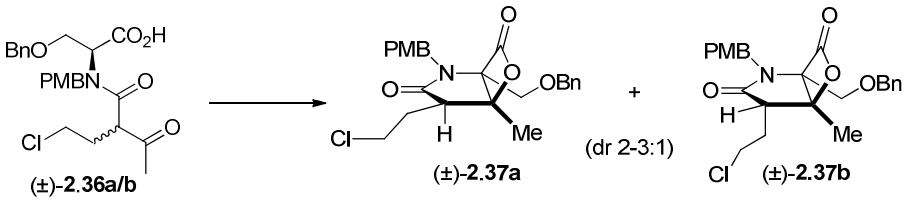
EtOAc:Hexanes) gave a mixture of two diastereomers **2.35a/2.35b** (2.17 g, 80%, dr 1:1) as a colorless oil.

2.35a: R_f = 0.58 (40% EtOAc/Hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18-7.36 (m, 7H), 6.87 (d, *J* = 8.0 Hz, 2H), 5.85-5.93 (m, 1H), 5.24-5.33 (m, 2H), 4.82 (d, *J* = 16.5 Hz, 1H), 4.66 (d, *J* = 17.0 Hz, 1H), 4.59-4.61 (m, 2H), 4.50 (dd, *J* = 4.0, 8.5 Hz, 1H), 4.47 (d, *J* = 11.5 Hz, 1H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.08 (dd, *J* = 8.5, 10.0 Hz, 1H), 4.01 (dd, *J* = 3.5, 10.0 Hz, 1H), 3.93 (dd, *J* = 5.5, 8.5 Hz, 1H), 3.81 (s, 3H), 3.46-3.58 (m, 2H), 2.34-2.43 (m,1H), 2.17-2.24 (m, 1H), 2.11 (s, 3H); LRMS (APCI) Calcd. for $C_{27}H_{32}ClNO_6$ [M+H] 502, found 502. **2.35b:** R_f = 0.50 (40% EtOAc/Hexanes); IR (neat) 1738, 1642, 1613 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.36 (m, 7H), 6.89 (d, *J* = 8.5 Hz, 2H), 5.86-5.94 (m, 1H), 5.24-5.33 (m, 2H), 4.88 (d, *J* = 16.5 Hz, 1H), 4.69 (d, *J* = 17.0 Hz, 1H), 4.57-4.66 (m, 3H), 4.50 (d, *J* = 11.5 Hz, 1H), 4.45 (d, *J* = 11.5 Hz, 1H), 4.03-4.06 (m, 2H), 3.92 (t, *J* = 7.0 Hz, 1H), 3.82 (s, 3H), 3.57 (t, *J* = 6.0 Hz, 2H), 2.34-2.41 (m, 1H), 2.15-2.21 (m, 1H), 1.97 (s, 3H); ^{13}C (125 MHz, $CDCl_3$) δ 202.5, 170.9, 168.5, 159.5, 137.8, 131.8, 128.8, 128.7, 128.6, 128.0, 127.9, 119.1, 114.4, 73.6, 68.5, 66.3, 60.3, 55.5, 53.7, 52.7, 43.3, 31.9, 28.7; LRMS (APCI) Calcd. for $C_{27}H_{32}ClNO_6$ [M+H] 502, found 502.

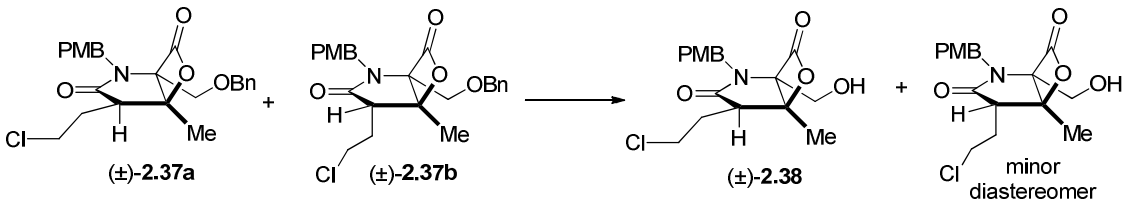


β-Keto Acid 2.36a/b: To a solution of allyl ester **2.35a/b** (1.24 g, 2.47 mmol) in THF (20 mL) was added morpholine (646 mg, 7.41 mmol) and $Pd(PPh_3)_4$ (29 mg, 0.025 mmol) at ambient temperature. The reaction mixture was stirred at ambient temperature for 3 h and diluted with Et_2O (200 mL). The organic layer was washed with 0.2 N HCl and brine, dried over $MgSO_4$ and

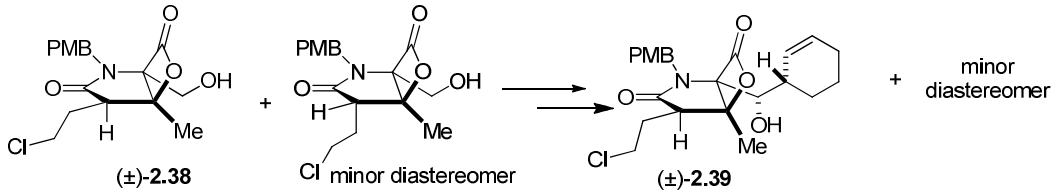
concentrated. The residue was purified by flash chromatography on SiO_2 (15:85 acetone/dichloromethane) to give acid **2.36a/b** (620 mg, 75%). Data for **2.36a**: IR (neat) 1721, 1639cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20-7.40 (m, 7H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.82 (d, *J* = 16.5 Hz, 1H), 4.70 (d, *J* = 16.5 Hz, 1H), 4.48 (s, 2H), 4.41-4.45 (m, 1H), 4.00-4.10 (m, 3H), 3.84 (s, 3H), 3.50-3.65 (m, 2H), 2.20-2.50 (m, 2H), 2.13 (s, 3H); LRMS (ESI) Calcd. for $C_{24}H_{28}ClNO_6$ [M–H] 460, found 460.



Benzyloxy-β-Lactone (±)-2.37: Prepared according to the representative procedure for bis-cyclization process using *N*-propyl-2-bromo pyridinium triflate (273 mg, 0.789 mmol), 4-pyrrolidinopyridine (223 mg, 1.56 mmol), Hünig's base (70 μL, 0.39 mmol), and ketoacid **2.36a/b** (180 mg, 0.390 mmol) in CH_2Cl_2 (15 mL). Purification by flash chromatography (SiO_2, 10% EtOAc/hexanes) gave a mixture of two diastereomeric β-lactones **2.37a** and **2.37b** (59 mg, 34 %, dr = 2:1, 500 MHz ^{1}H NMR). Data for (±)-**2.37a:** R_f = 0.32 (20% EtOAc/hexanes); IR (neat) 1830, 1703 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.36 (m, 3H), 7.13-7.15 (m, 4H), 6.80 (d, *J* = 8.5 Hz, 2H), 4.73 (d, *J* = 15.5 Hz, 1H), 4.31 (d, *J* = 15.5 Hz, 1H), 4.17 (d, *J* = 12.0 Hz, 1H), 4.13 (d, *J* = 11.5 Hz, 1H), 4.01 (ddd, *J* = 5.0, 7.5, 12.5 Hz, 1H), 3.77-3.81 (m, 1H), 3.77 (s, 3H), 3.73 (d, *J* = 11.5 Hz, 1H), 3.57 (d, *J* = 11.5 Hz, 1H), 2.91 (t, *J* = 7.5 Hz, 1H), 2.31-2.38 (m, 1H), 2.10-2.16 (m, 1H), 1.72 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8, 166.1, 159.2, 136.4, 129.2, 128.6, 128.5, 128.2, 128.0, 113.9, 83.4, 79.3, 73.5, 61.6, 55.2, 45.0, 44.3, 42.5, 28.4, 19.2; LRMS (ESI) Calcd. for $C_{24}H_{26}ClNO_5$ [M+H] 444, found 444.

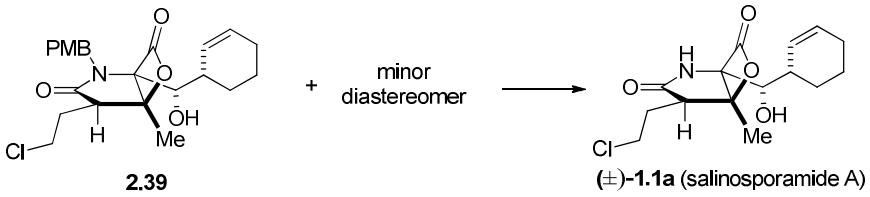


Hydroxy-β-Lactone (±)-2.38: Prepared according to the representative procedure for debenzylation using the mixture of β-lactones (38 mg, 0.13 mmol, dr 6:1) and 10 wt% palladium on carbon (10 mg) in THF (5 mL) at ambient temperature for 5 h under H_2 atmosphere. Purification by flash chromatography (1:40 EtOAc/CH_2Cl_2) gave the desired alcohol (±)-**2.38** along with the minor diastereomer (29.9 mg, 98%, dr 6:1) as a waxy solid. Further purification allowed enrichment to ~10-19:1 dr (500 MHz ^{1}H NMR). (±)-2.**38**: R_f = 0.29 (4.8% EtOAc/CH_2Cl_2); IR (neat) 3449, 1831, 1687 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 5.13 (d, *J* = 15.0 Hz, 1H), 4.06 (d, *J* = 15.5 Hz, 1H), 4.03 (ddd, *J* = 5.5, 7.5, 12.5 Hz, 1H), 3.92 (dd, *J* = 9.0, 13.5 Hz, 1H), 3.85 (dd, *J* = 4.5, 13.5 Hz, 1H), 3.80 (s, 3H), 3.78-3.82 (m, 1H), 2.94 (t, *J* = 7.0 Hz, 1H), 2.32-2.38 (m, 1H), 2.01-2.18 (m, 1H), 1.77 (s, 3H), 0.86 (dd, *J* = 5.0, 9.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 166.7, 159.6, 129.0, 128.7, 114.7, 83.6, 80.2, 55.3, 55.1, 44.9, 44.1, 42.4, 28.4, 19.1; LRMS (ESI) Calcd. for $C_{17}H_{20}ClNO_5$ [M+H] 354, found 354.



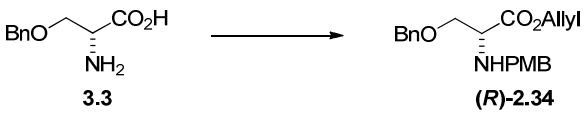
***N*-PMB-Salinosporamide A (±)-2.39:** To a solution of diastereomeric alcohols, (±)-2.**38** plus minor diastereomer (29 mg, 0.082 mmol, dr >10:1), in DMSO/toluene (0.8 mL/0.8 mL) was added EDCI (79 mg, 0.41 mmol), followed by dichloroacetic acid (14 μL, 0.16 mmol) at ambient temperature. The reaction mixture was stirred at ambient temperature for 2 h and diluted with EtOAc (50 mL). The organic layer was washed with 0.1 N HCl, and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was used for the next step without further purification due to some instability of resulting aldehyde to column chromatography.

A solution of tri-*n*-butyl-2-cyclohexenyltin (140 mg, 0.377 mmol) in THF (0.7 mL) was treated with *n*-BuLi (2.5 M in hexanes, 133 μL, 0.333 mmol) at –78 °C. After 30 min, $ZnCl_2$ (0.5 M in THF, 0.77 mL, 0.39 mmol) was added and following an additional 30 min, a solution of the crude aldehyde in THF (1.3 mL) was slowly added to the freshly prepared zinc reagent **2.8**. The resulting mixture was stirred at –78 °C for 2.5 h, quenched with water and diluted with EtOAc (50 mL). The organic layer was washed with saturated NH_4Cl and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (1:6 EtOAc/hexanes) to give a mixture of predominantly two diastereomers (12 mg, 33%, dr 3.5:1 + trace minor diasts., 500 MHz ^{1}H NMR) as a colorless oil which was carried directly to the next step without further characterization. The major diastereomer **2.39** was confirmed to possess the correct relative stereochemistry following subsequent conversion to salinosporamide A (below): R_f = 0.64 (40% EtOAc/Hexanes); IR (neat) 3467, 1828, 1692 cm^{-1}; LRMS (ESI) Calcd. for $C_{23}H_{28}ClNO_5$ [M+Li] 440, found 440.



***Rac*-Salinosporamide A ((±)-1.1a):** To a mixture of diastereomer (±)-**2.39** (10 mg, 0.023 mmol, dr 3.5:1) in CH_3CN (0.1 mL) was added an aqueous solution of CAN (63 mg, 0.12 mmol) in H_2O (25 μL) at 0 °C. After stirring at 0 °C for 2 h, the reaction mixture was diluted with EtOAc (25 mL) and washed with brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (1:10 to 1:4 EtOAc/CH_2Cl_2) providing diastereomerically pure salinosporamide A (±)-**1.1a** (3.5 mg, 49%) as a white solid (dr >19:1, 500 MHz ^{1}H NMR). A crystal suitable for X-ray analysis was obtained by slow evaporation from CH_2Cl_2 with ~5% CH_3CN: R_f = 0.09 (5% EtOAc/CH_2Cl_2); IR (neat) 3413, 1821, 1700 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ 10.63 (s, 1H), 6.42 (d, *J* = 10.5 Hz, 1H), 5.86-5.90 (m, 1H), 4.26 (t, *J* = 9.0 Hz, 1H), 4.13 (dt, *J* = 7.5, 10.5 Hz, 1H), 4.02 (dt, *J* = 7.0, 10.5 Hz, 1H), 3.18 (t, *J* = 7.0 Hz, 1H), 2.82-2.89 (m, 1H), 2.45-2.52 (m, 1H), 2.27-2.36 (m, 2H), 2.07 (s, 3H), 1.89-1.95 (m, 2H), 1.66-1.72 (m, 1H), 1.35-1.40 (m, 1H) 1H was overlapped with H_2O; ^{13}C NMR (125 MHz, pyridine-d_5) δ 176.9, 169.4, 129.1, 128.7, 86.3, 80.4, 71.0, 46.2, 43.3, 39.3, 29.0, 26.5, 25.4, 21.7, 20.0; LRMS (ESI) Calcd. for $C_{15}H_{20}ClNO_4$ [M+Li] 314, found 314.

Procedure for Asymmetric Synthesis of (–)-**Salinosporamide A**

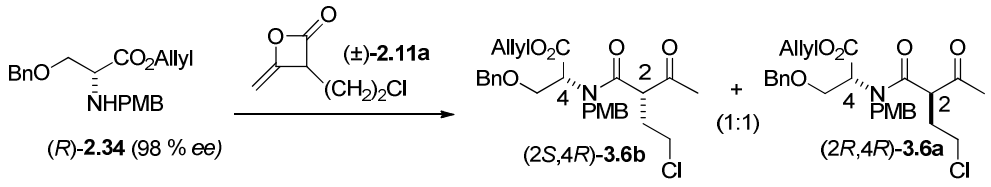


(*R*)-*O*-Benzyl Serine Allyl Ester (*R*)-2.34: To a suspension of (*R*)-*O*-benzyl-serine **3.3** (4.35 g, 22.3 mmol) in distilled MeOH (80 mL) was added triethylamine (3.76 mL, 26.8 mmol) and *p*-

anisaldehyde (4.55 g, 33.4 mmol) at 23 ^{o}C. The resulting suspension was stirred until the solution became homogeneous (~ 30 min). The solution was then cooled to 0 ^{o}C, followed by addition of anhydrous $MgSO_4$ (13.4 g, 112 mmol). After 7 h, the $MgSO_4$ was filtered via fritted funnel and washed with MeOH (80 mL). The combined filtrate was cooled to 0 ^{o}C for 15 min and then $NaBH_4$ (1.11 g, 29.4 mmol) was added portionwise. After stirring at 0 ^{o}C for 2 h, the solidified reaction mixture was left in a freezer (~ –10 ^{o}C) for 12 h. All volatiles were removed under reduced pressure and the remaining solid was resuspended in water (50 mL) and acidified to pH 3 with 2 N HCl. The precipitated white solid was filtered via a Büchner funnel, washed with ice-cold water (2 x 30 mL) and ice-cold Et_2O (2 x 30 mL), and dried under vacuum to give *O*-benzyl-*N*-PMB serine (6.80 g, 97 %) as a white solid.

To *O*-benzyl-*N*-PMB serine (6.80 g, 21.6 mmol) and *p*-TsOH (4.93 g, 25.9 mmol) was added allyl alcohol (20 mL) and benzene (40 mL). The solution was stirred at reflux (~ 100 ^{o}C) with a Dean-Stark apparatus until the calculated amount of water had been collected (~8 h). The resulting solution was concentrated, resuspended in 5% aqueous $NaHCO_3$ (120 mL), and extracted with EtOAc (500 mL). The pH was adjusted to 10.0 (until pH of aqueous solution maintained at 10 after extraction) with 2 M NaOH solution. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography (1:6 EtOAc/hexanes) to give the desired allyl ester (*R*)-**2.34** (6.30 g, 82%) as a yellow oil. R_f = 0.61 (33% EtOAc/hexanes); $[\alpha]^{23}_{D}$ = + 20.6 (*c* = 1.8, $CHCl_3$); IR (neat) 1738, 1612 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25-7.35 (m, 7 H), 6.90 (d, *J* = 8.5 Hz, 2H), 5.87-5.95 (m, 1 H), 5.22-5.35 (m, 2 H), 4.69 (dt, *J* = 1.2, 5.7 Hz, 2H), 4.58 (d, *J* = 12.3 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 3.89 (d, *J* = 12.6 Hz, 1H), 3.82 (s, 3H), 3.70-3.82 (m, 2H), 3.71 (d, *J* = 13.2 Hz, 1H), 3.57 (t, *J* = 4.8 Hz, 1H), 2.28 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 158.9,

138.1, 132.2, 131.9, 129.8 (2C), 128.6 (2C), 127.9, 127.8(2C), 118.7, 114.0 (2C), 73.4, 71.3, 65.7, 60.6, 55.5, 51.6; HRMS (ESI) Calcd. for $C_{21}H_{26}NO_4$ [M+H] 356.1862, found 356.1858. Enantiomeric excess was determined to be 98% by chiral HPLC (CHIRALPAK IA, 250 x 4.6 mm (L x I.D.), solvent (isocratic) 95:5 hexanes/2-propanol, flow rate 1.0 mL/min, λ = 230 nm). Retention times: (*S*)-serine derivative 15.97 min; (*R*)-serine derivative 22.34 min.

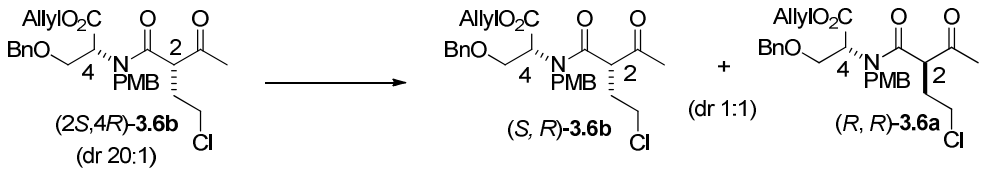


β-Ketoamides, 3.6a/3.6b. To a 10 mL microwave vessel containing (*R*)-*O*-benzyl serine allyl ester (*R*)**-2.34** (356 mg, 1.00 mmol) was added ketene-dimer (±)-**2.11a** (191 mg, 1.30 mmol), 2-hydroxypyridine (124 mg, 1.30 mmol) and dichloroethane (3.5 mL). The reaction mixture was stirred at 23 ^{o}C until the solution turned transparent. The reaction vessel was heated to 50 ^{o}C and irradiated in the microwave at 100 W for 2 h (same scale reaction was repeated for 5 times). The combined reaction mixtures were removed by means of a rotary evaporator, and the residue was purified by a short column (1:4 EtOAc/hexanes) to afford a 1:1 mixture of diastereomeric keto amides (2*R*,4*R*)-**3.6a/**(2*S*,4*R*)-**3.6b** (2.26 g, 90%) as a colorless oil. (2*R*,4*R*)-**3.6a**: R_f = 0.57 and (2*S*,4*R*)-**3.6b**: R_f = 0.51 (5:95 EtOAc/CH_2Cl_2); the first MPLC separation (5:95 EtOAc/CH_2Cl_2) gave 850 mg of (2*R*,4*R*)-**3.6a** (30:1 dr, 38%), second MPLC separation gave additional 0.16 g (28:1 dr, 7%). Data for (2*R*,4*R*)-**3.6a** diastereomer (45:1 dr, 98% ee): $[\alpha]^{23}_D$ = + 66.1 (c = 1.0, $CHCl_3$); IR (neat) 1739, 1645 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) for major rotamer δ 7.22-7.36 (m, 7H), 6.87 (d, *J* = 9 Hz, 2 H), 5.85-5.93 (m, 1H), 4.82 (d, *J* = 16.5 Hz, 1H), 4.66 (d, *J* = 16.5 Hz, 1H), 4.59-4.61 (m, 2H), 4.50 (dd, *J* = 4.0, 8.5 Hz, 1H), 4.47 (d, *J* = 12 Hz, 1H), 4.44 (d, *J* = 12 Hz, 1H), 4.08 (dd, *J* = 8.5, 10.0 Hz, 1H), 4.01 (dd, *J* = 3.5, 10.0 Hz, 1H), 3.93 (dd, *J* = 5.5, 8.5

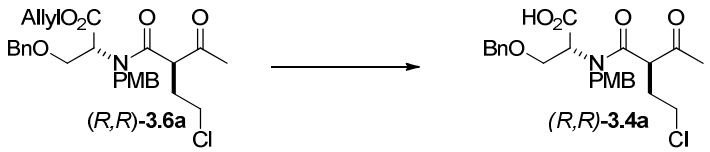
Hz, 1H), 3.81 (s, 3H), 3.46-3.58 (m, 2H), 2.34-2.43 (m,1H), 2.17-2.24 (m, 1H), 2.11 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for major rotamer δ 203.3, 169.9, 168.5, 164.9, 159.5, 137.8, 131.8, 128.9, 128.6, 128.0, 127.9, 119.1, 114.3, 73.9, 68.6, 66.3, 60.1, 55.5, 54.8, 52.6, 42.9, 32.1, 28.0; HRMS (ESI) Calcd. for $C_{27}H_{32}ClNO_6Li$ [M+Li] 508.2078, found 508.2073. Enantiomeric excess was determined by chiral HPLC (Chiralpak IA, 250 x 4.6 mm (L x I.D.), solvent (isocratic) 90:10 hexanes/2-propanol, flow rate 1.0 mL/min, wavelength λ = 230 nm). Retention times: (2*R*, 4*R*)-**3.6a** 19.34 min; *ent*-**3.6a**(2*S*, 4*S*): 21.09 min.

β-Ketoamide (2*R*,4*R*)-**3.6a/**(2*S*,4*R*)-**3.6b** (gram-scale synthesis)**.** To an 80 mL microwave vessel containing (*R*)-*O*-benzyl serine allyl ester (*R*)-**2.34** (3.56 g, 0.01 mol) was added ketene dimer (±)-**2.11a** (1.61 g, 0.011 mmol), 2-hydroxypyridine (1.05 g, 0.011 mmol) and dichloroethane (35 mL). The reaction mixture was stirred at 23 ^{o}C until the solution turned transparent. The reaction vessel was heated to 48 ^{o}C and irradiated in the microwave at 100 W for 2 h (same scale reaction was repeated one more time). The reaction mixture was concentrated under reduced pressure, and the residue was purified by a short SiO_2 column (95:5 DCM/EtOAc) to afford a 1:1 mixture of diastereomeric keto amides (2*R*,4*R*)-**3.6a/**(2*S*,4*R*)-**3.6b** (8.02 g, 80%) as a colorless oil. Two sequential separations by MPLC (SiO_2, 5:95 EtOAc/CH_2Cl_2) gave 2.30 g of (2*R*,4*R*)-**3.6a** (32:1 dr). Data for (2*R*,4*R*)-**3.6a** (45:1 dr, 98% ee): $[\alpha]^{23}_D$ = + 66.1 (*c* = 1.0, $CHCl_3$); IR (neat) 1739, 1645 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) for major rotamer δ 7.22-7.36 (m, 7H), 6.87 (d, *J* = 9 Hz, 2 H), 5.85-5.93 (m, 1H), 5.24-5.33 (m, 2H), 4.82 (d, *J* = 16.5 Hz, 1H), 4.66 (d, *J* = 16.5 Hz, 1H), 4.59-4.61 (m, 2H), 4.50 (dd, *J* = 4.0, 8.5 Hz, 1H), 4.47 (d, *J* = 12 Hz, 1H), 4.44 (d, *J* = 12 Hz, 1H), 4.08 (dd, *J* = 8.5, 10.0 Hz, 1H), 4.01 (dd, *J* = 3.5, 10.0 Hz, 1H), 3.93 (dd, *J* = 5.5, 8.5 Hz, 1H), 3.81 (s, 3H), 3.46-3.58 (m, 2H), 2.34-2.43 (m,1H), 2.17-2.24 (m, 1H), 2.11 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for major rotamer δ 203.3, 169.9, 168.5, 164.9, 159.5, 137.8, 131.8,

128.9 (2C), 128.6 (2C), 128.0, 127.9 (2C), 119.1, 114.3 (2C), 73.9, 68.6, 66.3, 60.1, 55.5, 54.8, 52.6, 42.9, 32.1, 28.0; HRMS (ESI) Calcd. for $C_{27}H_{32}ClNO_6Li$ [M+Li] 508.2078, found 508.2073. Enantiomeric excess was determined by chiral HPLC (Chiralpak IA, 250 x 4.6 mm (L x I.D.), solvent (isocratic) 90:10 hexanes/2-propanol, flow rate 1.0 mL/min, λ = 230 nm). Retention times: (2*R*, 4*R*)-**3.6a** 19.34 min; *ent*-**3.6a**(2*S*, 4*S*): 21.09 min.



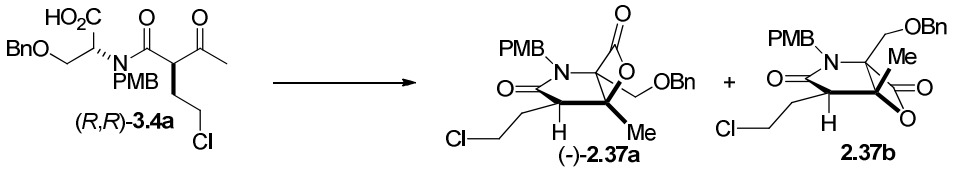
Epimerization of β-ketoamide 3.6b. To a solution of ketoamide (2*S*,4*R*)-**3.6b** (0.30 g, 0.598 mmol, ~ 20:1 dr) in 10 mL of EtOAc/MeOH (4:1) was added TsOH (137 mg, 0.718 mmol) and the solution was heated to 45 ^{o}C for 48 h. After cooling to room temperature, the reaction mixture was diluted with Et_2O (150 mL), H_2O (100 mL) was added and the pH of the aqueous layer was adjusted to ~10 using a 0.1 M NaOH solution. After extraction, the layers were separated and the organic layer was washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to deliver 0.295 g (98 %) of a 1:1 mixture of ketoamides **3.6a /3.6b** which could be repurified by MPLC to increase material throughput of the desired diastereomer **3.6a**. HPLC analysis of (2*R*,4*R*)-ketoamide **3.6a** verified that epimerization only occurred at the β-ketoamide and not the α-amino acid position under these conditions.



β-Ketoacid (2*R*,4*R*)-3.4a. To a solution of ketoamide (2*R*,4*R*)-**3.6a** (0.85 g, 1.69 mmol, dr ~ 30:1) in THF (34.0 mL) at -5 ^{o}C (ice and saturated NaCl solution) was added $Pd(PPh_3)_4$ (195

mg, 0.169 mmol), and which was immediately followed by morpholine (0.177 ml, 2.03 mmol). The reaction mixture was stirred at -5 ^{o}C for 1 h and diluted with ice cold Et_2O (500 mL). The organic layer was acidified to pH 3 with 0.025 N HCl. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude ketoacid (2*R*,4*R*)-**3.4a** (29:1 dr according to 500 MHz ^{1}H NMR) was used in the subsequent step without further purification. (Note: longer reaction leads to epimerization). Data for (2*R*,4*R*)-**3.4a:** ^{1}H NMR (500 MHz, $CDCl_3$) for major rotamer δ 7.25-7.36 (m, 7H), 6.88 (d, *J* = 9 Hz, 2 H), 4.80 (d, *J* = 16.5 Hz, 1H), 4.66 (d, *J* = 16.5 Hz, 1H), 4.48 (d, *J* = 12 Hz, 1H), 4.45 (d, *J* = 12 Hz, 1H), 4.43 (dd, *J* = 5, 7.5 Hz, 1H), 4.03 (ddd, *J* = 5, 7.5,10 Hz, 2H), 3.97 (dd, *J* = 5.5, 8.5 Hz, 1H), 3.81 (s, 3H), 3.75-3.78 (m, 2H), 3.54-3.59 (m, 1H), 3.48 (ddd, *J* = 5, 8.5, 11.5 Hz, 1H), , 2.39-2.46 (m,1H), 2.19-2.26 (m, 1H), 2.12 (s, 3H).

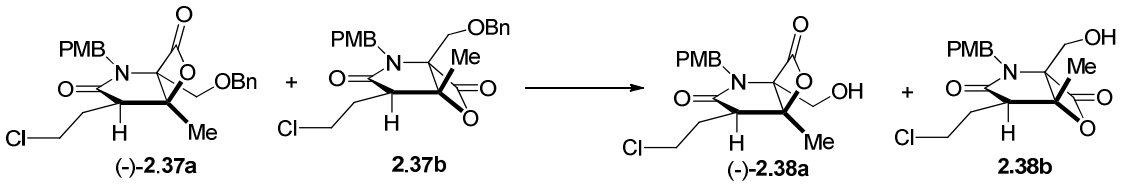
β-Ketoacid (2*R*,4*R*)-3.4a (gram-scale synthesis). To a solution of ketoamide (*R*,*R*)- **3.6a** (2.30 g, 4.55 mmol, ~ 32:1 dr) in THF (91.0 mL) at -5 ^{o}C (ice and saturated NaCl solution) was added $Pd(PPh_3)_4$ (526 mg, 0.455 mmol), followed by immediate addition of morpholine (0.475 ml, 5.46 mmol). The reaction mixture was stirred at -5 ^{o}C for 70 min and diluted with ice cold Et_2O (800 mL). A 0.02 N HCl solution was added until the pH was measured to be ~3. The layers were separated and the organic layer was washed with brine (400 mL), dried over $MgSO_4$ and concentrated. The crude ketoacid (*R*,*R*)-**3.4a** (~ 32:1 dr according to 500 MHz ^{1}H NMR) was used in the subsequent step without further purification. (Note: longer reaction time led to epimerization).



Benzyloxy-β-lactone (–)-2.37a/2.37b. To a solution of 4-pyrrolidinopyridine (1.21g, 8.45 mmol) in toluene (46 mL) at –10 °C was added MsCl (0.20 mL, 2.54 mmol). Immediately, a solution of freshly synthesized ketoacid (*R*,*R*)-**3.4a** (1.69 mmol) in toluene (12 mL) was added to the resulting suspension via syringe pump over 30 min. After 50 min, the reaction mixture was diluted with ice-cold Et_2O (400 mL) and washed with 20 % $CuSO_4$ solution (2 x 200 mL) to remove excess 4-pyrrolidinopyridine and then washed with water (2 x 200 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. Immediately, the residue was purified by flash chromatography (1:9 → 3:7 EtOAc/hexanes) to give a mixture of two co-eluting, inseparable β-lactones **(–)-2.37a/2.37b** (260 mg, 35%, 7:1 dr, 500 MHz ^{1}H NMR) as a colorless oil and recovered ketoacid (36%, 2:1 dr). R_f = 0.36 (20% EtOAc/hexanes). Data for **(–)-2.37a:** IR (neat) 1830, 1703 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.36 (m, 3H), 7.13-7.15 (m, 4H), 6.80 (d, *J* = 8.5 Hz, 2H), 4.73 (d, *J* = 15.5 Hz, 1H), 4.31 (d, *J* = 15.5 Hz, 1H), 4.17 (d, *J* = 12.0 Hz, 1H), 4.13 (d, *J* = 11.5 Hz, 1H), 4.01 (ddd, *J* = 5.0, 7.5, 12.5 Hz, 1H), 3.77-3.81 (m, 1H), 3.77 (s, 3H), 3.73 (d, *J* = 11.5 Hz, 1H), 3.57 (d, *J* = 11.5 Hz, 1H), 2.91 (t, *J* = 7.5 Hz, 1H), 2.31-2.38 (m, 1H), 2.10-2.16 (m, 1H), 1.72 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8, 166.1, 159.2, 136.4, 129.2 (2C), 128.6, 128.5 (2C), 128.2, 128.0 (2C), 113.9 (2C), 83.4, 79.3, 73.5, 61.6, 55.2, 45.0, 44.3, 42.5, 28.4, 19.2; LRMS (ESI) Calcd. for $C_{24}H_{27}ClNO_5$ [M+H] 444, found 444. Enantiomeric excess of (-)-**32** was determined to be 92% by chiral HPLC (CHIRALPAK IA, 250 x 4.6 mm (L x I.D.), solvent (isocratic) 87:13 hexanes/2-propanol, flow rate 1.0 mL/min, λ = 230 nm). Retention times: **(–)-2.37a**: 13.68 min; **(+)-2.37a:** 16.12 min.

Benzyloxy-β-lactone (–)-2.37a/2.37b (gram-scale synthesis). To a solution of 4-pyrrolidinopyridine (2.60 g, 18.2 mmol, 4.0 equiv) in toluene (84 mL) at -5 °C (ice and saturated

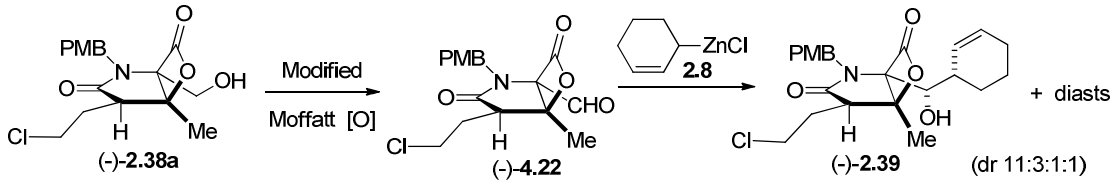
NaCl solution) was added MsCl (0.53 mL, 6.83 mmol, 1.5 equiv). Immediately, a solution of freshly prepared ketoacid (*R*,*R*)-**3.4a** (4.55 mmol) in toluene (25 mL) was added to the resulting suspension via syringe pump over 45 min and 5 mL of additional toluene were used to ensure complete transfer. After 3 h, the reaction mixture was diluted with ice cold Et_2O (700 mL) and washed with 20% $CuSO_4$ solution (500 mL) to remove most of the 4-pyrrolidinopyridine, saturated NH_4Cl (500 mL), and then washed with water (2 x 500 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (1:9 → 3:7 EtOAc/hexanes) to give a mixture of two inseparable β-lactones **(–)-2.37a/2.37b** (1.05 g, 52%, 5:1 dr, 500 MHz ^{1}H NMR) as a yellow oil and recovered ketoacid (10 %, 4:1 dr). R_f = 0.36 (20% EtOAc/hexanes). Enantiomeric excess of **(–)-2.37a** was determined to be 90%. The diastereomeric β-lactones were carried directly forward to the deprotection at which point they could be separated.



Representative Procedure for Debenzylation as Described for Hydroxy-β-Lactone (–)-2.38a/2.38b. To a mixture of β-lactones **(–)-2.37a** and **2.37b** (260 mg, 0.586 mmol, dr 7:1) in THF was added palladium on carbon (52 mg, 20 wt%). After evacuating twice by aspirator vacuum, and refilling with H_2, a balloon of H_2 was attached to the flask and the heterogenous solution was stirred vigorously at 23 ^oC for 12 h. The reaction mixture was then diluted with Et_2O, and dried over $MgSO_4$. The organics were filtered through a pad of Celite, concentrated, and purified by MPLC (SiO_2, 5:95 EtOAc/CH_2Cl_2) to give the desired alcohol (–)-**2.38a** in 75%

yield (155 mg, dr >19:1, 92% ee) as a waxy solid. Data for (–)-**2.38a**: R_f = 0.29 (5:95 EtOAc/CH_2Cl_2); $[\alpha]^{23}_D$ = – 67.0 (*c* = 0.95, $CHCl_3$). IR (neat) 3449, 1831, 1687 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 5.13 (d, *J* = 15.0 Hz, 1H), 4.06 (d, *J* = 15.5 Hz, 1H), 4.03 (ddd, *J* = 5.5, 7.5, 12.5 Hz, 1H), 3.92 (dd, *J* = 9.0, 13.5 Hz, 1H), 3.85 (dd, *J* = 4.5, 13.5 Hz, 1H), 3.80 (s, 3H), 3.78-3.82 (m, 1H), 2.94 (t, *J* = 7.0 Hz, 1H), 2.32-2.38 (m, 1H), 2.01-2.18 (m, 1H), 1.77 (s, 3H), 0.86 (dd, *J* = 5.0, 9.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 166.7, 159.6, 129.0(2C), 128.7, 114.7(2C), 83.6, 80.2, 55.3, 55.1, 44.9, 44.1, 42.4, 28.4, 19.1; LRMS (ESI) Calcd. for $C_{17}H_{21}ClNO_5$ [M+H] 354, found 354.

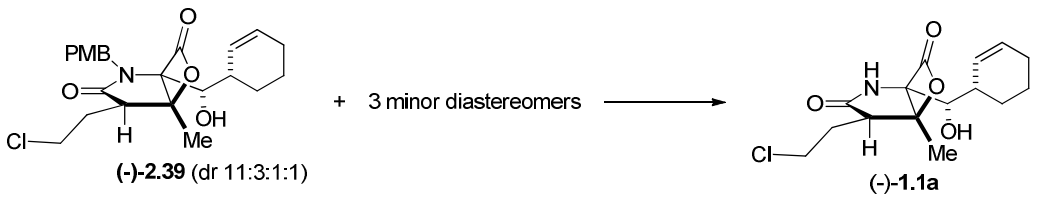
Procedure A for Zincate Addition:



***N*-PMB-salinosporamide A (–)-2.39.** To a solution of alcohol (–)-**2.38a** (155 mg, 0.440 mmol, dr >19:1) in DMSO/toluene (2.2 mL/2.2 mL) was added EDCI (424 mg, 2.20 mmol), and dichloroacetic acid (18 μL, 0.22 mmol) at 23 °C. The reaction mixture was stirred for 5 h, and diluted with EtOAc (150 mL). The reaction mixture was acidified using 0.1 N HCl to pH 3. The organic layer was then washed with brine (100 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was used for the next step without further purification due to some instability of the resulting aldehyde to column chromatography. For characterization purpose, aldehyde **(–)-4.22** was isolated via flash column chromatography (5:95 EtOAc/CH_2Cl_2) as a clear oil. Data for (–)-**4.22**: R_f = 0.35 (5:95 EtOAc/CH_2Cl_2); IR (neat) 1839, 1712 (br) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.73 (s, 1H), 7.28 (d, *J* = 8.5 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 5.13 (d, *J* =

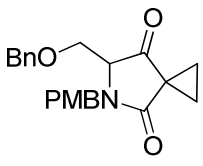
14.5 Hz, 1H), 4.12 (d, *J* = 14.5 Hz, 1H), 4.03 (ddd, *J* = 4.5, 7.5, 11.5 Hz, 1H), 3.81 (s, 3H), 3.74-3.80 (m, 1H), 3.08 (t, *J* = 7.0 Hz, 1H), 2.34-2.41 (m, 1H), 2.11-2.18 (m, 1H), 1.61 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.5, 174.2, 162.3, 160.4, 131.4(2C), 126.2, 114.9(2C), 84.7, 82.8, 55.5, 46.1, 45.1, 42.3, 28.6, 20.1; MS could not be obtained for this compound due to instability of β-lactone.

A solution of tri-*n*-butyl-2-cyclohexenyltin (490 mg, 1.32 mmol) in THF (2.0 mL) was treated with *n*-BuLi (2.5 M in hexanes, 512 μL, 1.28 mmol) at –78 °C. After 30 min, $ZnCl_2$ (0.5 M in THF, 2.62 mL, 1.41 mmol) was added and after an additional 30 min, a solution of the crude aldehyde in THF (1.5 mL) was slowly added to the freshly prepared zinc reagent **2.8**. The resulting mixture was stirred at –78 °C for 4 h, quenched with water and diluted with Et_2O (150 mL). Saturated NH_4Cl was added until a pH of 7 was achieved. The layers were separated and then the organic layer was washed with brine (100 mL). The filtrate was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (95:5 → 85:5 EtOAc/hexanes) to give a mixture of four inseparable diastereomers (100 mg, 62 %, dr = 11:3:1:1 according to 500 MHz ^{1}H NMR) as a colorless oil, which was carried directly to the next step without further purification. The major diastereomer **(–)-2.39** was confirmed to possess the correct relative stereochemistry following subsequent conversion to salinosporamide A.



(-)-2.39 (dr 11:3:1:1) **(-)-1.1a**

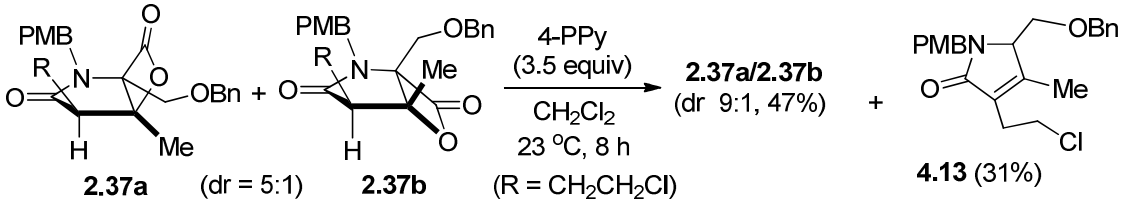
Representative Procedure for PMB-Deprotection as Described for (–)-salinosporamide A ((–)-1.1a): To a methanolic solution (1.2 mL) containing the mixture of alcohol (–)-**2.39** (100 mg, 0.23 mmol) and other diastereomers from the previous step, was added an aqueous solution of CAN (1.26 g, 2.3 mmol) in H_2O (0.6 mL) at 0 °C dropwise. After stirring at 0 °C for 6 h, the reaction mixture was diluted with EtOAc (100 mL), washed with saturated solution of $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (5:95 → 15:85 EtOAc/CH_2Cl_2) to give salinosporamide A (–)-**1.1a** (31 mg, 43 %) as a white solid (dr ~ 15:1, 500 MHz ^{1}H NMR). Further purification was accomplished by recrystallization involving slow evaporation from 95:5 CH_2Cl_2/acetone to provide (–)-**1.1a** (28 mg, 90 %, dr > 19:1): R_f = 0.50 (33 % EtOAc/hexanes); m.p. 152-156 ^{o}C, lit. 169-171 ^{o}C; $[\alpha]^{23}_D$ = – 71.3 (*c* = 0.39, MeOH); lit. $[\alpha]^{25}_D$ = – 72.9 (*c* = 0.55, MeOH); IR (neat) 3346, 1820, 1698 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ 10.69 (s, 1H), 7.63 (d, *J* = 9.0 Hz, 1H), 6.45 (d, *J* = 10.0 Hz, 1H), 5.89-5.93 (m, 1H), 4.28 (t, *J* = 9.0 Hz, 1H), 4.16 (dt, *J* = 7.0, 11.0 Hz, 1H), 4.05 (dt, *J* = 7.0, 11.0 Hz, 1H), 3.21 (t, *J* = 7.0 Hz, 1H), 2.84-2.91 (m, 1H), 2.48-2.55 (m, 1H), 2.30-2.39 (m, 2H), 2.10 (s, 3H), 1.91-1.98 (m, 2H), 1.70-1.73 (m, 2H), 1.35-1.42 (m, 1H); ^{13}C NMR (125 MHz, pyridine-d_5) δ 176.9, 169.4, 129.1, 128.7, 86.3, 80.4, 71.0, 46.2, 43.3, 39.3, 29.0, 26.5, 25.4, 21.7, 20.0; HRMS (ESI) Calcd. for $C_{15}H_{21}ClNO_4$ [M+H] 314.1161, found 314.1162.



3.7

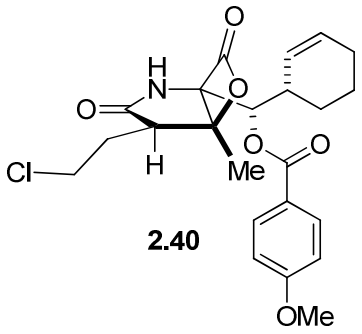
Cyclopropyl Ketoamide 3.7: R_f = 0.29 (95% DCM/EtOAc); IR (neat) 1759, 1697 cm^{-1}; (500 MHz, $CDCl_3$) δ 7.30-7.38 (m, 3H), 7.24-7.26 (m, 2H), 7.15 (d, *J* = 9Hz, 2H), 6.83 (d, *J* = 9Hz,

2H), 5.18 (d, *J* = 14.5 Hz, 1H), 4.46 (d, *J* = 12 Hz, 1H), 4.37 (d, *J* = 12 Hz, 1H), 4.08 (d, *J* = 14.5 Hz, 1H), 3.87 (t, *J* = 3.0 Hz, 1H), 3.79 (s, 3H), 3.74 (dd, *J* = 10, 3 Hz, 1H), 3.70 (dd, *J* = 10, 3 Hz, 1H), 1.76 (d, *J* = 9.5 Hz, 1H), 1.73 (d, *J* = 9.5 Hz, 1H), 1.64 (dd, *J* = 9.5, 5 Hz, 1H), 1.56(dd, *J* = 9.5, 5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.0, 172.8, 159.5, 137.7, 129.9 (2C), 128.7 (2C), 128.2 (2C), 127.9 (2C), 114.4(2C), 73.5, 66.8, 65.2, 55.6, 44.0, 32.0, 21.7, 20.8; HRMS (ESI) Calcd. for $C_{22}H_{23}NO_4Li$ [M+Li] 372.1787, found 372.1786.



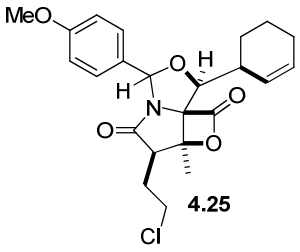
Decomposition of β-lactones 2.37a/2.37b. To a mixture of β-lactones (16 mg, 0.036 mmol, dr 5:1) in 1.5 mL CH_2Cl_2 was added 4-pyrrolidinopyridine (18 mg, 0.126 mmol) at 23 °C. After 8 h, the reaction mixture was diluted with ether (25 mL) and washed with 20 % $CuSO_4$ solution (20 mL) to remove the majority of the 4-pyrrolidinopyridine and this was followed by washing with saturated NH_4Cl (20 mL), and then water (2 x 20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (1:9 → 3:7 EtOAc/hexanes) to give a mixture of recovered β-lactones **2.37a/2.37b** (7.5 mg, 47%, 9:1 dr) and unsaturated γ-lactam **4.13** (4.5 mg, 31%): R_f = 0.26 (95% DCM/EtOAc); IR (neat) 1682, 1513 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.38 (m, 2H), 7.31-7.33 (m, 1H), 7.26-7.28 (m, 2H), 7.10 (d, *J* = 9Hz, 2H), 6.80 (d, *J* = 9Hz, 2H), 5.03 (d, *J* = 15 Hz, 1H), 4.45 (d, *J* = 12 Hz, 1H), 4.41 (d, *J* = 12 Hz, 1H), 4.17 (d, *J* = 15 Hz, 1H), 3.85 (t, *J* = 4.5, 1H), 3.73-3.81 (m, 2H), 3.78 (s, 3H), 3.66 (dd, *J* = 10, 4 Hz, 1H), 3.53 (dd, *J* = 10, 5 Hz, 1H), 2.75-2.78 (m, 2H), 1.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 159.1, 151.2, 137.8, 130.2, 129.9, 129.6 (2C), 128.7 (2C),

128.2, 128.0 (2C), 114.2(2C), 73.6, 68.7, 63.0, 55.5, 44.1, 43.4, 27.9, 12.9; HRMS (ESI) Calcd. for $C_{23}H_{27}ClNO_3$ [M+H] 400.1679, found 400.1683.



2.40

Salino Ester 2.40. IR (neat) 1833, 1712cm^{-1}; (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 11Hz, 2H), 6.95 (d, *J* = 11Hz, 2H), 6.47 (s, 1H), 5.91-5.94 (m, 1H), 5.60-5.62 (m, 1H), 5.48 (d, *J* = 5.5 Hz, 1H), 3.94 (ddd, *J* = 7.5, 6.0, 5.5 Hz, 1H), 3.88 (s, 3H), 3.76 (ddd, *J* = 7.0, 6.0, 5.0 Hz, 1H), 2.79 (t, *J* = 7 Hz, 1H), 2.74-2.78 (m, 1H), 2.26-2.32 (m, 1H), 2.08-2.15 (m, 1H), 2.01-2.05 (m, 2H), 1.88-1.94 (m, 1H), 1.77-1.84 (m, 1H), 1.76 (s, 3H), 1.65-1.72 (m, 1H), 1.57-1.63 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 166.7, 165.6, 164.6, 132.4 (2C), 132.1, 124.5, 121.2, 114.5 (2C), 84.5, 70.5, 56.0, 44.7, 42.5, 37.5, 30.1, 28.5, 26.7, 25.1, 21.3, 20.2; HRMS (ESI) Calcd. for $C_{23}H_{26}ClNO_6$ [M+H] 448.1527, found 448.1429.

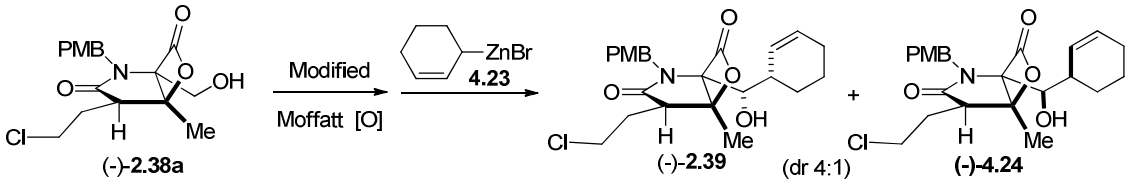


4.25

Salino Oxazolidine 4.25. IR (neat) 1830, 1723cm^{-1}; (500 MHz, $CDCl_3$) δ 7.26-7.33 (m, 2H), 6.92-6.94 (m, 2H), 5.93 (s, 1H), 5.91 (d, *J* = 13.5 Hz ,1H), 5.84-5.88 (m, 1H), 4.18 (d, *J* = 10.0 Hz, 1H), 3.88 (ddd, *J* = 11.5, 6.5, 5.0 Hz, 1H), 3.83 (s, 3H), 3.68 (ddd, *J* = 11.5, 6.5, 5.0 Hz, 1H),

2.95 (t, *J* = 6.5 Hz, 1H), 2.88-2.93 (m, 1H), 2.14-2.27 (m, 2H), 2.03-2.08 (m, 2H), 1.80-1.86 (m, 2H), 1.82 (s, 3H), 1.60-1.66 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 168.2, 161.2, 130.1, 129.3(2C), 125.4, 114.3 (2C), 89.5, 85.2, 83.0, 80.3, 55.6, 50.3, 42.4, 36.8, 30.1, 29.5, 26.5, 25.3, 21.3, 21.2; HRMS (ESI) Calcd. for $C_{23}H_{26}ClNO_5Li$ [M+Li] 438.0910, found 438.1861.

Procedure B for Zincate Addition:

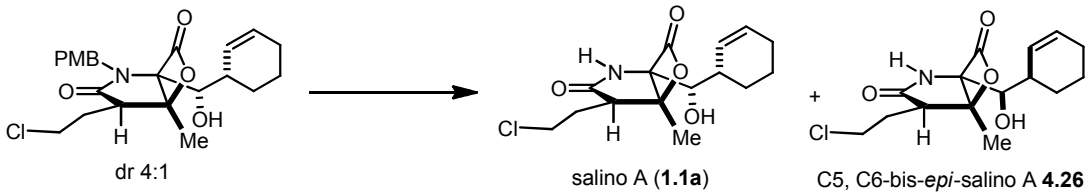


Procedure for Modified Moffatt Oxidation and Zincate Formation/Addition as Described for *N*-PMB-salinosporamide A (–)-2.39/4.24. To a solution of alcohol (–)-**2.38a** (42 mg, 0.119mmol, dr >19:1) in DMSO/toluene (0.9mL/0.9mL) was added EDCI (0.114mg, 0.595mmol), anddichloroacetic acid (5 mL, 0.060 mmol) at 23 ^{o}C. The reaction mixture was stirred for 6 h, and diluted with EtOAc (100 mL). The reaction mixture was acidified using 0.1 N HCl to pH 3. The organic layer was then washed with brine (60 mL), dried over MgSO4, filtered, and concentrated. The crude aldehyde was used for the next step without further purification due to some instability of the aldehyde **(–)-4.22** observed upon flash column chromatography.

Preparation of stock solution of zincate **4.23**: Zinc dust (1.64 g, 25.0 mmol, Baker, 20 mesh) was activated by the addition of an HCl solution (5 mL, 1.0 M) in a 25 mL round-bottomed flask. After stirring vigorously for 15 min at 23 ºC, the acid solution was decanted and the zinc was repeatedly washed with dry THF (3 X 5 mL). the zinc was then dried under high vacuum for 2 h. Addition of dry THF (5 mL) was followed by slow addition of 3-bromo-

cyclohexene (0.806g, 5.0 mmol, Acros) over 15 min. The resulting mixture was stirred at 23 ºC for 18 h, and then transferred via syringe to a dry centrifuge tube to remove the excess zinc suspension (10 min, 80 rpm). The resulting clear solution was then utilized directly, following titration as described below, being careful not to disturb the pellet at the bottom during transfers. The concentration of the zincate **4.23** solution was determined as follows: A stock solution of iodine in THF was prepared by addition of iodine (254 mg, 1.0 mmol) in 10 mL of THF leading to a 100 mM solution. A portion (500 μL) of this iodine solution was placed into a dry 5 mL round-bottomed flask equipped with a magnetic stir bar. The zincate solution, after being centrifuged, was added dropwise into the iodine stock solution until the red color disappeared. Using this procedure, the prepared zincate solution was determined to be ~200 μM and was used (in excess) directly in the following reaction.

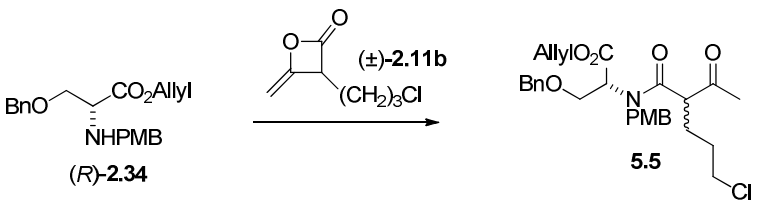
To a solution of the crude aldehyde (~0.119 mmol) in THF (1.0 mL) at –78 °C was added ~1.2 mL (0.238 mmol, ~200 μM in THF, ~2.0 equiv) of zincate **4.23** via syringe pump over 30 min. The resulting mixture was slowly warmed up to – 20 ºC for 1 h, quenched with water and diluted with Et_2O (120 mL). Saturated NH_4Cl was added until a pH of 7 was achieved. The layers were separated and then the organic layer was washed with brine (80 mL). The filtrate was dried over MgSO4, filtered, and concentrated. The residue was purified by flash chromatography (95:5 → 85:5 EtOAc/hexanes) to give a mixture of two inseparable diastereomers **(**–)-**2.39/**(–)-**4.24 (**38 mg, 74%, dr = 4:1 according to 500 MHz ^{1}H NMR) as a colorless oil, which was carried directly to the next step without further purification.



Salinosporamide A (–)-1.1a, and C5, C6-Bis-*Epi*-Salinosporamide A (–)-4.26: To a methanolic solution (0.8 mL) containing the mixture of alcohols (–)-**2.39a/4.24** (38 mg, 0.088 mmol) was added an aqueous solution of CAN (0.240 g, 0.44 mmol) in H_2O (0.2 mL) at 0 °C dropwise. After stirring at 0 °C for 6 h, the reaction mixture was diluted with EtOAc (100 mL), washed with saturated solution of $NaHCO_3$, and brine. The organic layer was dried over MgSO4, filtered, and concentrated. The residue was purified by flash chromatography (5:95 → 15:85 EtOAc/CH_2Cl_2) to give salinosporamide A (–)-**1.1a** (14 mg, 51 %, dr > 19:1, 500 MHz ^{1}H NMR) as a white solid in addition to a novel diastereomeric salinosporamide A **4.26** (3.3 mg, 12%, dr > 19:1, 500 MHz ^{1}H NMR).

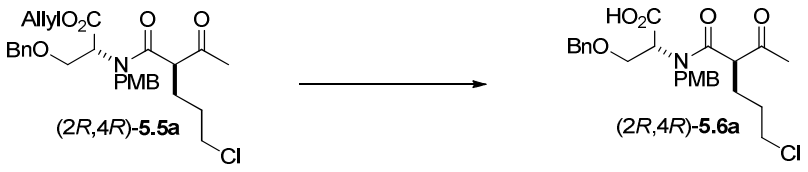
Data for minor diastereomer **4.26**: R*f* = 0.56 (33 % EtOAc/CH_2Cl_2); IR (neat) 2931, 1830, 1702 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.28 (s, 1H), 6.10 – 6.14 (m, 1H), 5.85 – 5.88 (m, 1H), 3.99 (ddd, *J* = 5.0, 7.5, 12.5 Hz, 1H), 3.83 (dd, *J* = 7.5, 9.0 Hz, 1H), 3.78 (ddd, *J* = 5.0, 7.5, 12.5 Hz, 1H), 2.83 (t, *J* = 7.5 Hz, 1H), 2.48-2.50 (m, 1H), 2.27-2.34 (m, 1H), 2.11 – 2.18 (m, 1H), 2.06-2.08 (m, 2H), 1.98 (d, *J* = 9 Hz, 1H), 1.80 – 1.91 (m, 2H), 1.87 (s, 3H), 1.60 – 1.67 (m, 2H); 13C NMR (125 MHz, $CDCl_3$) δ 175.8, 165.0, 135.2, 123.5, 85.4, 78.7, 71.8, 44.9, 42.7, 38.4, 28.5 26.6, 25.2, 21.8, 19.4; HRMS (MALDI) Calcd. for $C_{15}H_{20}ClNO_4Na$ [M+Na] 336.0973, found 336.0957.

Procedure for Asymmetric Synthesis of (–)-Homosalinosporamide A



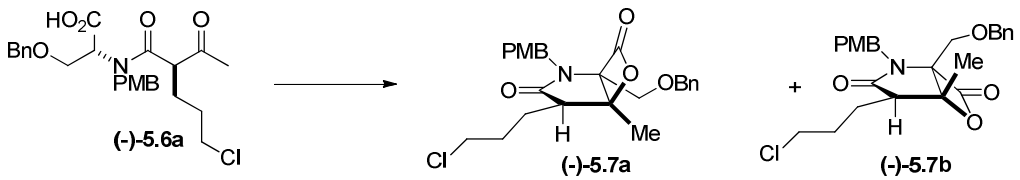
β-Ketoamide (-)-5.5: To a 10 mL microwave vessel containing (*R*)-*O*-benzyl serine allyl ester (*R*)**-2.34** (356 mg, 1.00 mmol) was added ketene-dimer (±)-**2.11b** (177 mg, 1.1 mmol), 2-hydroxypyridine (105 mg, 1.1 mmol) and dichloroethane (3.5 mL). The reaction mixture was stirred at 23 ^{o}C until the solution turned transparent. The reaction vessel was heated to 53 ^{o}C and irradiated in the microwave at 100 W for 2 h (same scale reaction was repeated for 3 times). The combined reaction mixtures were removed by means of a rotary evaporator, and the residue was purified by a short column (95:5 DCM/EtOAc) to afford a 1:1 mixture of diastereomeric keto amides **5.5a/5.5b** (1.21g, 80%) as a colorless oil. MPLC separation (5:95 EtOAc/CH_2Cl_2) gave 350 mg of (2*R*,4*R*)-**5.5a** (25:1 dr, 29%)**.** Enantiomeric excess was determined to be 94% ee by chiral HPLC (Chiralpak IA, 250 x 4.6 mm (L x I.D.), solvent (isocratic) 87:13 hexanes/2-propanol, flow rate 1.0 mL/min, wavelength λ = 230 nm). Retention times: (*R*, *R*)-**5.5a** 12.92 min; (*S*, *S*)-**5.5a** 16.52 min.

(2*R*,4*R*)-**5.5a:** R_f = 0.74 (5:95 EtOAc/DCM); IR (neat) 1738, 1646, 1613 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) for major rotamer δ 7.21-7.35 (m, 7H), 6.87 (d, *J* = 8.0 Hz, 2H), 5.85-5.93 (m, 1H), 5.24-5.33 (m, 2H), 4.82 (d, *J* = 17.0 Hz, 1H), 4.66 (d, *J* = 17.0 Hz, 1H), 4.59-4.61 (m, 3H), 4.44 (d, *J* = 12.0 Hz, 1H), 4.41 (d, *J* = 12.0 Hz, 1H), 4.05 (dd, *J* = 8.0, 10.0 Hz, 1H), 3.98 (dd, *J* = 3.5, 10.0 Hz, 1H), 3.81 (s, 3H), 3.53 (dd, *J* = 3.5, 8.0 Hz, 1H), 3.36-3.44 (m, 2H), , 2.15 (s, 3H), 2.04-2.11 (m, 1H), 1.84-1.91 (m, 1H), 1.67-1.76 (m,1H), 1.53-1.59 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) for major rotamer δ 204.5, 170.2, 168.5, 164.9, 159.4, 137.8, 131.8, 128.6, 128.2, 128.0, 127.8, 119.1, 114.4, 73.6, 68.5, 66.3, 60.1, 57.9, 55.5, 52.0, 44.5, 30.4, 27.3, 26.9; LRMS (ESI) Calcd. for $C_{28}H_{35}ClNO_6$ [M+H] 516, found 516.



β-Ketoacid (-)-(2*R*,4*R*)-5.6a:

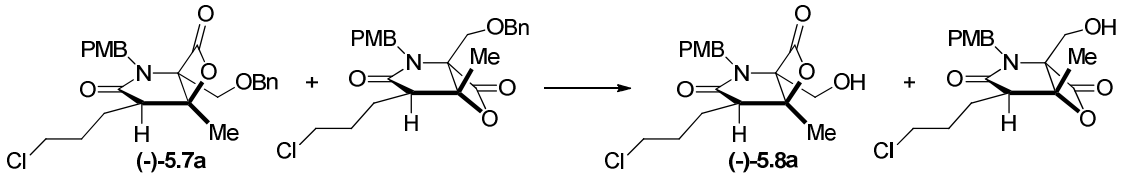
To a solution of ketoamides (-)-(*R,R*)**5.5a** (350 mg, 0.678 mmol, 25:1 dr) in THF (13.6 mL) at -5 ^{o}C (ice and saturated NaCl solution), was added $Pd(PPh_3)_4$ (78 mg, 0.068 mmol), and immediately followed by morpholine (0.071 mL, 0.814 mmol). The reaction mixture was stirred at -5 ^{o}C for 1 h and diluted with ice-cold Et_2O (500 mL). The organic layer was washed with 0.05 N HCl to pH 3 and brine, dried over $MgSO_4$, filtered and concentrated. The crude ketoacid (*R,R*)-**5.6a** (24:1 dr according to 500 MHz ^{1}H NMR) was used in the subsequent step without further purification.



Benzyloxy-β-lactone (-)-5.7a/(-)-5.7b: to a solution of 4-pyrrolidinopyridine (386 mg, 2.71 mmol) and (194 mg, 1.02 mmol) TsCl in toluene (16 mL) at -5 ^{o}C (ice and saturated NaCl solution), freshly synthesized ketoacid **5.6a** in toluene (7 mL) was added via syringe pump for a period of 30 min. The resulting suspension was stirred for 3.5 h. The reaction mixture was diluted with ice-cold Et_2O (400 mL) and washed with 20 % $CuSO_4$ solution (150 mL x 2) to remove excess 4-pyrrolidinopyridine and then washed with water (150 mL x 2). The organic

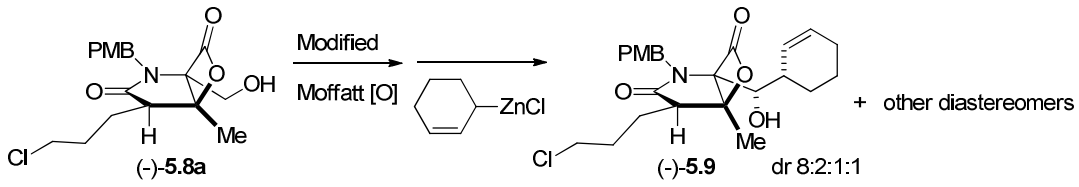
layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (20% EtOAc/hexanes), which gave a mixture of two diastereomeric β-lactones **(-)-5.7a/5.7b** (186 mg, 60 % for 2 steps, dr = 3.5:1 based on 500 MHz ^{1}H NMR).

(-)-**5.7a** (major): R_f = 0.35 (30% EtOAc/hexanes); IR (neat) 1827, 1700 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.62 (m, 3H), 7.13-7.16 (m, 4H), 6.79-6.81 (m, 2H), 4.72 (d, *J* = 15.5 Hz, 1H), 4.33 (d, *J* = 15.5 Hz, 1H), 4.14 (s, 3H), 3.77 (s, 3H), 3.74 (d, *J* = 11.0 Hz, 1H), 3.59 (d, *J* = 11.0 Hz, 1H), 3.58-3.64 (m, 2H), 2.54 (t, *J* = 7.0 Hz, 1H), 2.16-2.24 (m, 1H), 1.97-2.11 (m, 1H), 1.87-1.94(m, 2H), 1.72 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 166.4, 159.3, 136.7, 129.5, 128.9, 128.7, 128.4, 128.2, 114.1, 83.8, 79.3, 73.7, 61.8, 55.5, 47.9, 44.8, 44.5, 30.6, 23.0, 19.9; HRMS (ESI) Calcd. for $C_{25}H_{28}ClNO_5Na$ [M+Na] 480.1554, found 480.1559.



Hydroxy-β-Lactone (-)-5.8a: prepared according to the representative procedure for debenzylation using the mixture of β-lactones **5.7a/5.7b** (186 mg, 0.407 mmol, dr 3.5:1) and 20 wt% palladium on carbon (38 mg) in THF (10 mL) at 23 ^{o}C for 12 h under H_2 atmosphere. Purification by flash chromatography (5:95 EtOAc/CH_2Cl_2) gave the desired alcohol (-)-**5.8a** (93 mg, 62 %) along with the minor diastereomer as a white solid. Enantiomeric excess was determined to be 82 % ee by chiral HPLC (Chiralcel OD, 250 x 4.6 mm (L x I.D.), solvent (isocratic) 87:13 hexanes/2-propanol, flow rate 1.0 mL/min, wavelength λ = 230 nm). Retention times: (-)-**5.8a** 19.72 min; (+)-**5.8a** 28.76 min. Enantiomeric excess of the desired alcohol was enriched by recrystallization with DCM/ether with mother liquor of 89 %ee.

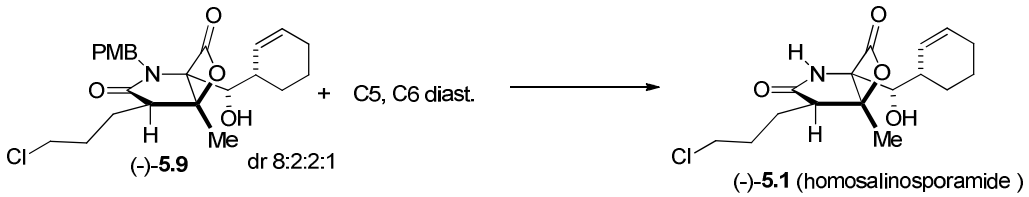
(-)-**5.8a**: R_f = 0.26 (5% EtOAc/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 5.13 (d, *J* = 15.5 Hz, 1H), 4.07 (d, *J* = 15.5 Hz, 1H), 3.92 (dd, *J* = 9.0, 14.0 Hz, 1H), 3.86 (dd, *J* = 4.5, 13.5 Hz, 1H), 3.81 (s, 3H), 3.61-3.64 (m, 2H), 2.56 (t, *J* = 7.0 Hz, 1H), 2.17-2.26 (m, 1H), 1.98-2.12 (m, 2H), 1.89-1.96 (m, 1H), 1.77 (s, 3H), 0.86 (dd, *J* = 4.5, 9.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.5, 167.0, 159.7, 129.2, 129.1, 114.9, 84.0, 80.3, 55.5, 55.3, 47.9, 44.7, 44.3, 30.5, 23.0, 19.8; LRMS (ESI) Calcd. for $C_{17}H_{20}ClNO_5$ [M+H] 368, found 368.



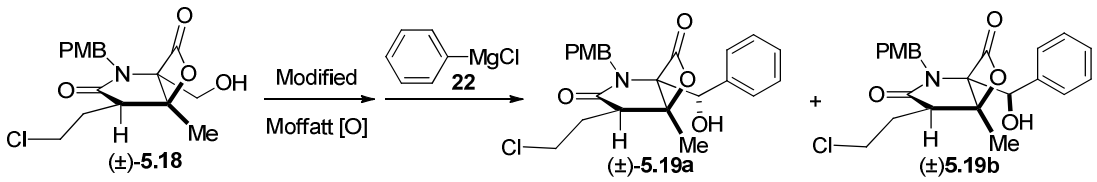
OH-β-Lactone (-)-5.9: to the alcohol (-)-**5.8a** (77 mg, 0.210 mmol), and EDCI (403 mg, 2.10 mmol), was added DMSO/toluene (1.5 mL/1.5 mL), followed by dichloroacetic acid (9 μL, 0.105 mmol). The reaction mixture was stirred at 23 ^{o}C for 6h and diluted with Et_2O (150 mL). The organic layer was acidified to pH 3 with 0.1 N HCl, and washed with brine. The organic was then dried over $MgSO_4$, filtered, and concentrated. The residue was used for the next step without further purification due to some instability of resulting aldehyde to SiO_2.

A solution of tri-*n*-butyl-2-cyclohexenyltin (234 mg, 0.630 mmol) in THF (1.5 mL) was treated with *n*-BuLi (2.05M in hexanes, 297 μL, 0.609 mmol) at –78 °C. After 30 min, $ZnCl_2$ (0.5 M in THF, 1.34 mL, 0.672 mmol) was added and after an additional 30 min, a solution of the crude aldehyde in THF (1.0 mL) was slowly added to the freshly prepared zinc reagent **2.8**. The resulting mixture was stirred at –78 °C for 5 h, quenched with water and diluted with EtOAc (150 mL). The organic layer was washed with saturated NH_4Cl until pH 7 and brine (100 mL).

The filtrate was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (95:5 → 85:5 EtOAc/hexanes) to give a mixture of four diastereomers (58 mg, 61%, dr = 8:2:1:1 according to 500 MHz ^{1}H NMR) as a colorless oil, which was carried directly to the next step without further purification.

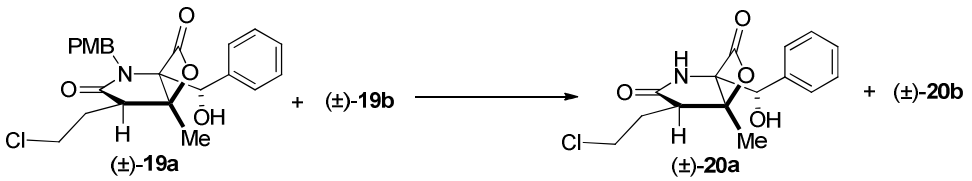


Homosalinosporamide A (-)-5.1: to a solution of alcohol (-)-**5.9** (58 mg, 0.127 mmol), along with other diastereomers from the previous step, in MeOH (0.9 mL) was added an aqueous solution of CAN (694 mg, 1.27 mmol) in H_2O (0.3 mL) at -10 °C dropwise. After stirring at -10 °C for 6 h, the reaction mixture was diluted with EtOAc (100 mL), washed with saturated solution of $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (5:95 to 15:85 EtOAc/CH_2Cl_2) to give homosalinosporamide A (-)-**5.1** (17 mg, 41%) as a white solid (dr >19:1, 500 MHz ^{1}H NMR): R_f = 0.36 (40% EtOAc/hexanes); $[\alpha]^{23}_D$ = - 63.2 (c = 0.15, MeOH); IR (neat) 3360, 1821, 1697 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ 10.58 (s, 1H), 6.46 (d, *J* = 10.0 Hz, 1H), 5.89-5.93 (m, 1H), 4.28 (d, *J* = 9.0 Hz, 1H), 3.63 (dt, *J* = 2.0, 6.5 Hz, 2H), 2.93 (t, *J* = 7.0, 1H), 2.86-2.90 (m, 1H), 2.28-2.37 (m, 2H), 2.12-2.25 (m, 1H), 2.07 (s, 3H), 1.93-1.97 (m, 2H), 1.68-1.75 (m, 2H), 1.35-1.41 (m, 3H); ^{13}C NMR (125 MHz, C_3D_6O) δ 176.7, 169.1, 129.4, 128.8, 86.7, 79.8, 71.2, 48.2, 45.8, 39.4, 31.4, 26.6, 25.8, 23.3, 22.1, 20.4; LRMS (ESI) Calcd. for $C_{16}H_{23}ClNO_4$ [M+1] 328.132, found 328.134.



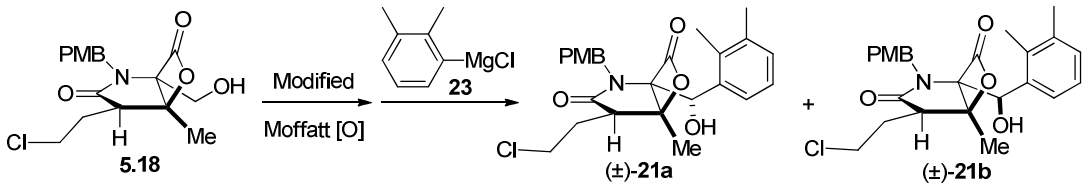
PMB-salino A derivative, (±)-19a/19b. Aldehyde was prepared according to the representative modified Moffatt oxidation using the alcohol (±)-**5.18** (80 mg, 0.218 mmol), in DMSO/toluene (1.2 mL/1.2 mL), and addition of EDCI (208 mg, 1.09 mmol), followed by dichloroacetic acid (10 µL, 0.109 mmol) at 23 ^{o}C for 5 h. The crude aldehyde was used in the subsequent step without further purification.

To a solution of aldehyde (0.109 mmol) in THF at -78 ^{o}C, Grignard reagent **22** (0.164 mmol) in THF was added slowly down the side of the flask. The reaction mixture was warmed to 0 ^{o}C for 10 min, quenched with NH_4Cl saturated solution, and diluted with ether (100 mL). The organic layer was then washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (95:5 → 85:15 EtOAc/hexanes) to provide mixture of (±)-**19a/19b** (22.5 mg, 48 %, dr = 3:1 according to 500 MHz ^{1}H NMR) as a colorless oil which was carried directly to the next step without further purification.

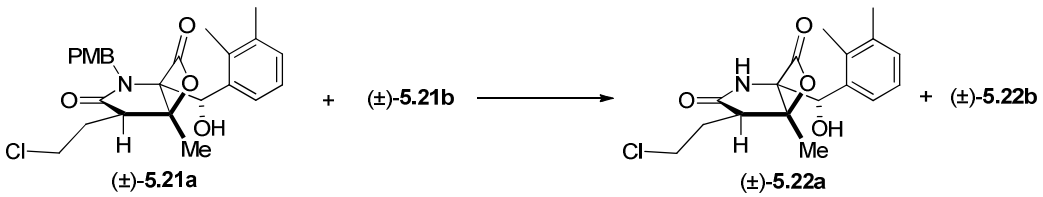


Salino A derivative (±)-20a. Prepared according to the representative procedure for PMB deprotection using a mixture of alcohol (±)-**19a/19b** (22.5 mg, 0.052 mmol, dr = 3:1) in *i*-PrOH (0.3 mL) was added an aqueous solution of CAN (285 mg, 0.52 mmol) in H_2O (0.1 mL) at 0 °C dropwise. The residue was purified by flash chromatography (5:95 to 15:85 EtOAc/CH_2Cl_2) to

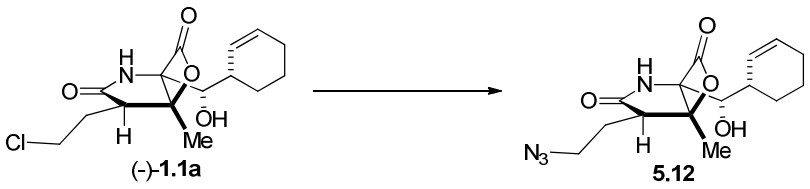
give (±)-**20a** (7.2 mg, 45 %, dr >19:1) as a white solid: R_f = 0.48 (5:95 EtOAc/CH_2Cl_2); IR (neat) 3354, 1829, 1705 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.41-7.51 (m, 5H), 5.81 (s, 1H), 5.32 (d, *J* = 4.5 Hz, 1H), 3.98 (ddd, *J* = 5.0, 7.5, 11.0 Hz, 1H), 3.78 (ddd, *J* = 4.5, 7.5, 11.0 Hz, 1H), 2.92 (t, *J* = 7.0 Hz, 1H), 2.26-2.33 (m, 1H), 2.24 (d, *J* = 4.5 Hz, 1H), 2.09-2.16 (m, 1H), 1.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ and C_3D_6O) δ 175.3, 167.3, 137.2, 128.8, 128.7, 126.4, 85.7, 77.8, 68.1, 45.1, 42.3, 28.0, 19.6; HRMS (ESI) Calcd. for $C_{15}H_{17}ClNO_4$ [M+H] 310.0848, found 310.0858.



PMB-Salino A Derivative (±)-5.21a/b: To a solution of aldehyde (0.109 mmol) in THF at -78 ^oC, Grignard reagent **23** (0.164 mmol) in THF was added slowly down the side of the flask. The reaction mixture was then warmed up to 0 ^oC for 10 min, quenched with NH_4Cl saturated solution, and diluted with ether (100 mL). The organic layer was then washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (95:5 → 85:15 EtOAc/hexanes) to provide mixture of (±)-**5.21a/b** (22.0 mg, 44 %, dr = 3:1 according to 500 MHz ^1H NMR) as a colorless oil, which was carried directly to the next step without further purification.



Salino A Derivative (±)-5.22a/b: Prepared according to the representative procedure PMB deprotection using a mixture of alcohol (±)-**5.21a/b** (22.0 mg, 0.048 mmol, dr = 3:1) in *i*-PrOH (0.3 mL) was added an aqueous solution of CAN (263 mg, 0.48 mmol) in H_2O (0.1 mL) at 0 °C dropwise. The residue was purified by flash chromatography (5:95 → 15:85 EtOAc/CH_2Cl_2) to give (±)-**5.22a** (6.9 mg, 43 %, dr >19:1) as a white solid: R_f = 0.55 (4:6 EtOAc/Hexanes); IR (neat) 3350, 1825, 1702 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, *J* = 7.5, 1H), 7.21-7-28 (m, 2H), 5.44 (s, 1H), 5.24 (d, *J* = 4.5 Hz, 1H), 3.98 (ddd, *J* = 5.0, 7.0, 11.5 Hz, 1H), 3.78 (ddd, *J* = 5.0, 8.0, 11.0 Hz, 1H), 2.91 (t, *J* = 7.0 Hz, 1H), 2.33 (s, 3H), 2.26-2.32 (m, 1H), 2.27 (s, 3H), 2.14-2.21 (m, 1H), 2.16 (d, *J* = 4.5 Hz, 1H), 2.0 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ and C_3D_6O) δ 174.9, 167.6, 138.0, 135.9, 134.7, 131.3, 127.2, 124.8, 84.5, 78.8, 67.6, 45.3, 42.3, 28.2, 21.0, 19.1, 15.2; LRMS (ESI) Calcd. for $C_{17}H_{20}ClNO_4Na$ [M+Na] 360, found 360.



Azido salino A (–)-5.12: to the salino A (–)-**1.1a** (11 mg, 0.035 mmol), NaI (105 mg, 0.70 mmol), and NaN_3 (403 mg, 2.10 mmol), was added DMSO (1.0 mL). The reaction mixture was stirred at 23 ^oC for 48h and diluted with EtOAc (30 mL). The reaction mixture was washed with H_2O (15 mL) and the aqueous layer was back extracted with EtOAc (15 mL). The combined extracts were washed with brine (15 mL), dried over anhydrous $MgSO_4$, filtered via fritted

funnel, and concentrated down under reduced pressure. The residue was purified by flash chromatography (5:95 → 15:85 EtOAc/CH_2Cl_2) to give (-)-**5.12**(2.6 mg, 23 %, dr >19:1) as a white solid: R_f = 0.35 (15:85 EtOAc/CH_2Cl_2) and recovered salino A **1.1a** (4.4 mg, 40%); Data for **5.12:** IR (neat) 2096, 1821, 1699 cm^{-1}; ^1H NMR (500 MHz, C_3D_6O) δ 8.08 (s, 1H), 5.93–5.96 (m, 1H), 5.77–7.81(m, 1H), 4.52 (d, *J* = 8.5 Hz, 1H), 3.89 (t, *J* =8.5 Hz, 1H), 3.71 (t, *J* =7 Hz, 2H), 2.63 (dd, *J* = 7.0, 8.0 Hz, 1H), 2.44-2.50 (m, 2H), 1.92-2.01 (m, 1H), 1.87(s, 3H), 1.75–1.82 (m, 2H), 1.46–1.53 (m, 2H), 1.36–1.45 (m, 2H).

Organic Syntheses **Procedure: Asymmetric Nucleophile-Catalyzed Aldol-Lactonization with Aldehyde Acids**

A. (1,4-dioxaspiro[4.5]dec-7-en-8-yloxy)trimethylsilane (**6.11**). An oven dried 500 mL, two-necked, round-bottomed flask is equipped with a large stir bar (Note 2) and one neck is fitted with a 50-mL pressure-equalizing addition funnel fitted with a nitrogen inlet and the second neck is sealed with a rubber septum. The reaction flask is charged with solid 1,4-cyclohexanedione mono-ethylene ketal (25 g, 160 mmol, 1 equiv) and then *N,N*-dimethylformamide (Note 3) (100 mL) is added to dissolve the solid. Triethylamine (52.0 mL, 368 mmol, 2.3 equiv) is added via syringe to the reaction vessel. Freshly distilled trimethylsilyl chloride (26.7 mL, 208 mmol, 1.3 equiv) is added to the addition funnel and then dispensed dropwise into the reaction vessel over a period of ~15 minutes. After the addition is complete, the addition funnel is replaced with a condenser and the reaction mixture is then heated in an oil bath at 80 ^oC (external temperature) for 12 h (Note 4). On cooling to ambient temperature (23 ^oC), the reaction mixture is quenched by addition of cold H_2O (150 mL, cooled in an ice bath for 15 min), the mixture is diluted with hexanes (300 mL), and the mixture is transferred to a 1000

mL separatory funnel. The aqueous layer is removed and back-extracted with hexanes (2 x 100 mL). The combined hexane extracts iswashed with saturated $NaHCO_3$ solution (1 x 100 mL), saturated NH_4Cl (2 x 150 mL), and then brine (1 x 150 mL). The organic layer is dried over anhydrous $MgSO_4$ (25 g) for 15 min and then concentrated on a rotary evaporator (25 mm Hg, 30 ^oC). Further concentration under reduced pressure (0.5 mm Hg, ambient temperature, 23 ^oC) delivers 31-33 g (85-90 %) of the silyl enol ether **6.11** (Note 5) as a dark yellow oil, which is of sufficient purity for use in the subsequent step without further purification.

B. 3-(2-(2-oxoethyl)-1,3-dioxolan-2-yl) propanoic acid (**6.12**). To a 1000 mL one-neck, round-bottomed flask containing the crude silyl enol ether (32 g, 140 mmol, 1.0 equiv) is added 500 mL of CH_2Cl_2. The reaction mixture is then cooled to –78 ^oC (acetone/dry ice bath) with stirring for 15 min. Stirring is stopped and ozone is bubbled through the solution with a large gas sparge tube (40-60 micrometer) for ~1-2 h until the blue color persists (Note 6). A stream of nitrogen is then bubbled through the solution mixture until the blue color disappears (~ 1 h). Solid triphenylphosphine (37.1 g, 141 mmol, 1.01 equiv) is added with stirring, and the reaction is allowed to warm gradually to ambient temperature (23 ^oC) and stirred for ~18 h until the ozonide test provides a negative result (Note 7). The solvent is removed by means of a rotary evaporator (25 mm Hg, 30 ^oC). Residual CH_2Cl_2 is removed under reduced pressure at 0.5 mm Hg for 12 h to give a yellow slurry (Note 8). Diethyl ether (80 mL) is added and the solid/liquid mixture is stirred vigorously for 30 min and the solid is then filtered off by vacuum filtration using a Buchner funnel fitted with filter paper (Whatmann #1, 70 mm). The solid is rinsed with ice cold ether (2 x15 mL). The ether filtrates are combined and transferred to a separatory funnel (250 mL) and extracted vigorously with water (4 x 80 mL) (Note 9). To a 500 mL Erlenmeyer

flask containing the combined aqueous extracts (~250 mL), solid NaCl (70 g) is then added with stirring and additional NaCl was added until the solution is saturated. The aqueous solution is transferred to a 500 mL separatory funnel and extracted with hexanes (2 x 100 mL) to remove unreacted cyclohexane dione monoketal from step A (Note 4). The aqueous solution is then transferred to a clean 1000 mL separatory funnel and extracted vigorously with CH_2Cl_2 (3 x 150 mL). The aqueous solution is saturated again with additional sodium chloride (5 g) and extracted vigorously again with CH_2Cl_2 (3 x 150 mL). The combined CH_2Cl_2 extracts are dried over anhydrous $MgSO_4$ (50 g) for 15 min, filtered using a Buchner funnel equipped with filter paper (Whatman #1, 70 mm), and concentrated by rotary evaporation (25 mm Hg, 30 ^oC) to deliver a light yellow oil. Further concentration under high vacuum (0.5 mm Hg, 23 ^oC) provides 15-17 g (57-65%) of the aldehyde acid as a light yellow solid, which is of sufficient purity for use in the subsequent step without further purification (Notes 10 and 11).

C. Preparation of *N*-methyl-2-chloropyridinium trifluoromethane sulfonate (**6.8**):

A 1000 mL round-bottomed flask, equipped with a magnetic stirring bar and a rubber septum, is flame dried under a stream of nitrogen, and cooled to ambient temperature. Following addition of 250 mL of CH_2Cl_2 and then 2-chloropyridine (13.1 mL, 139 mmol, 1 equiv) the flask is cooled in a dry ice/acetone bath (-78 ^oC) under nitrogen. Methyl trifluoromethane sulfonate (25 g, 153 mmol, 1.1 equiv) is then added slowly via syringe over 10 minutes through the septum down the side of the flask to allow cooling prior to mixing with the bulk solvent. The cold bath is removed and the reaction mixture is allowed to warm slowly to ambient temperature (23 ^oC)

and stirred an additional 12 h leading to a white precipitate. The reaction mixture is concentrated by rotary evaporation (25 mm Hg, 30 ^{o}C) to deliver a white solid. Toluene (120 mL) is added to further induce precipitation, and after swirling under nitrogen for ~10 minutes, the toluene/CH_2Cl_2 mixture is removed from the solids via cannula under N_2 pressure. The white solid is dried under reduced pressure at 0.5 mmHg for 12 h at 23 ^{o}C to afford 37.6-38.4 g (91-93%) of *N*-methyl-2-chloropyridinium trifluoromethane sulfonate **6.8** as a white solid (mp. 147-148º C) (Note 12).

D. Preparation of *O*-trimethylsilylquinidine (**6.9**, *O*-TMS QND): A one-neck, 250 mL round-bottomed flask, equipped with a mechanical stirrer, is flame dried with a stream of nitrogen and left to cool down to ambient temperature for 10 min. Quinidine (4.0 g, 12.4 mmol, 1 equiv) is added to 100 mL CH_2Cl_2 and then distilled trimethylsilyl chloride (1.9 mL, 15 mmol, 1.2 equiv) is added slowly via syringe pump over 15 min. The resulting solution is stirred at ambient temperature for 24 h and then partioned between 100 mL CH_2Cl_2 and 100 mL $NaHCO_3$ in a separatory funnel. The layers are separated, and the aqueous layer is extracted with two 50 mL portions of CH_2Cl_2. The combined organic extracts are dried over $MgSO_4$, filtered, and concentrated by rotary evaporation ((25 mm Hg, 30 ^{o}C). The residue is then left to dry under reduced pressure at 0.5 mmHg for 12 h at 23 ^{o}C to afford 3.80-3.95 g (77-80% yield) of *O*-trimethylsilylquinidine (**6.9**) as a clear oil which was of sufficient purity for use without purification (Note 13).

E. *(*1*R*, 5*S*)-6-oxaspiro[bicyclo[3.2.0]heptane-3,2'-[1,3]dioxolan]-7-one (**6.4**). *N*-methyl-2-chloropyridinium trifluoromethane sulfonate (**6.8**) (Note 12) (33.2 g, 119.6 mmol, 1.5 equiv) is weighed out in a one-neck, oven-dried 1000 mL round-bottomed flask. A stir bar is added and the flask is purged with nitrogen and then charged with 240 mL dry acetonitrile. The catalyst *O*-

TMS QND (**6.9**) (3.16 g, 7.97 mmol, 0.1 equiv) (Note 13) is dissolved in 100 mL dry acetonitrile at 23 ^{o}C and transferred to the reaction flask via cannula with nitrogen pressure which is followed by addition of freshly distilled Hünig's base via syringe (35.4 mL, 199.3 mmol, 2.5 equiv). The reaction mixture is stirred for 10 min and then a solution of 3-(2-(2-oxoethyl)-1,3-dioxolan-2-yl) propanoic acid (**6.12**) (15 g, 79.7 mmol, 1.0 equiv) in 60 mL of dry CH_3CN is added via syringe pump over ~1 h (30 mL syringe, ~1 mL/min, syringe loaded twice). The reaction mixture changes from yellow to a dark red color during the addition of aldehyde acid. The reaction mixture is stirred for an additional 18 h at ambient temperature and reaction progress is monitored by disappearance of aldehyde acid **6.12** and formation of β-lactone **6.4** by TLC analysis (R_f 0.29 and 0.52, respectively in 60 % EtOAc/hexanes, $KMnO_4$ stain). When judged complete, the reaction mixture is concentrated by rotary evaporation and then by high vacuum (0.5 mm Hg, 30 ^{o}C) for 3 h to provide a dark brown, viscous oil (~ 75 g). The reaction mixture is diluted with 25 mL of CH_2Cl_2 and loaded onto a silica gel plug (188 g, 2.5 g silica gel per gram of crude product, 10 cm diameter flash column) pre-eluted with 50% EtOAc:hexanes using a long stem funnel to carefully deliver the crude mixture onto the silica gel surface (washing with 5 mL CH_2Cl_2 and then 10 mL 50% EtOAc:hexanes). The product is quickly eluted with 50% EtOAc:hexanes (2000 mL). Forty fractions (~45 mL in 50 mL test tubes) are collected and fractions 7–35 contained the majority of the β-lactone (R_f = 0.52 (50% EtOAc:hexanes; $KMnO_4$). The fractions are combined and concentrated by rotary evaporation (25 mm Hg, 30 ^{o}C) and then under high vacuum (0.5 mm Hg at 23 ^{o}C) to deliver 7.5-7.8 g of semi-crude β-lactone. Recrystallization (Note 14) from EtOAc:hexanes gives a total of 4.6–4.8 g (34 - 35%) of bicyclic-β-lactone (Note 15). Enantiomeric excess is deteremined by chiral HPLC analysis (Note 16).

2. Notes

1. 1,4-Cyclohexanedione mono-ethylene ketal (Aldrich, 97%), PPh_3 (Acros, 99%), 2-chloropyridine (Aldrich, 99%), methyl trifluoromethanesulfonate (Acros, 96%), quinidine (Acros, 99%), NEt_3 (Acros, 99%), TMSCl (Acros, 98%), *N,N*-dimethylformamide (Aldrich, 99.9%), acetonitrile (Aldrich, 99.8%), *N, N*-diisopropylethyl amine base (Hünig's base, Acros, 98%), $MgSO_4$ (EMD, powder 98%), sodium bicarbonate (EMD, Powder), dichloromethane (EMD, 99.8%), NaCl (EMD, crystals, 99%), ammonium chloride (EMD, 99.5%), hexanes (Fisher, 98.5%), diethylether (EMD, 99%), silica gel (Silicycle, 230-400 mesh), KI (EM, 99%). Triethylamine, trimethylsilyl chloride, and Hünig's base were freshly distilled over CaH_2 prior to use. *N,N*-Dimethylformamide, dichloromethane and acetonitrile were dried through activated alumina using a converted MBraun System.

2. A football-shaped stir bar is ideal since copious amounts of Et_3N•HCl formed during the course of the reaction makes stirring difficult.

3. It is important to use very dry *N*,*N*-dimethylformamide (<150 ppm of H_2O) to avoid hydrolysis of TMSCl.

4. While it is best to have all starting material converted to the silyl enol ether, reaction progress is monitored by aliquot NMR not by TLC due to facile desilylation of the TMS enol ether **6.11**. Generally, ensuring high quality and dryness of reagents/solvents and using specified reaction times ensured complete reaction. Any residual cyclohexanone is not deleterious to the subsequent ozonolysis and can be removed in the next step during extraction.

5. Data for silyl enol ether **6.11**: R_f = 0.65 (20% EtOAc/hexanes); IR (neat) 1673 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.15 (s, 9 H), 1.76 (t, *J* = 6.5 Hz, 2 H), 2.18-2.20 (m, 4 H), 3.93-3.97 (m, 4

H), 4.68-4.71 (m, 1 H); ^{13}C (125 MHz, $CDCl_3$) δ 149.9, 107.8, 100.8, 64.5, 34.1, 31.3, 28.7, 0.44; MS (EI) m/z calc. for $C_{11}H_{20}O_3$ (M+1): 228.12. Found: 228.12.

6. The time required for excess ozone to be added is of course dependent on the O_2 pressure and ozone generation efficiency. We used a Welsbash Ozonizer (total pressure: 7-10 psi; O_2/ozone pressure: 3.0-4.5 psi).

7. The ozonide test is performed by adding 0.1 g of potassium iodide to 1.0 mL of glacial acetic acid and ~1.0 mL of the ozonolysis reaction mixture in a 5 mL round-bottomed flask. A brown mixture indicates the presence of ozonide. Blanks must always be prepared; 0.1 g of KI added to 1.0 mL CH_2Cl_2 and 1.0 mL of glacial acetic acid. The test solution has a very short shelf life and will naturally result in high blank values if stored for any length of time. *Caution: Care must be excercised in transferring and manipulating the ozonide reaction mixture due to the known instability of ozonides. It is preferable to perform the quenching step in the same fume hood and avoid transferring the reaction mixture.* A yellow solution is a negative result and suggests it is safe to proceed to work up.

8. The majority of the CH_2Cl_2 must be removed at this step to minimize retention of aldehyde acid in the organic layer during the first stage of the extraction procedure.

9. It is imperative that extractions of the aldehyde acid are done very vigorously to ensure efficient transfer of the aldehyde acid to the aqueous phase. However, patience is then required to allow for separation of the layers due to emulsion formation. A copper wire loop was used to assist in breakdown of the emulsion.

10. It is best to use the aldehyde acid soon after drying otherwise it should be stored in the freezer (-5 ^{o}C) under N_2 to avoid oxidation to the diacid. Data for aldehyde acid: R_f = 0.29 (60% EtOAc:hexanes); IR (thin film) 1791, 1731 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.11 (t, J = 7.5,

2 H), 2.45 (t, *J* = 7.5 Hz, 2 H), 2.69 (d, *J* = 3 Hz, 2 H), 4.03 (s, 4 H), 9.73 (t, *J* = 3 Hz, 1 H), ^{13}C (75 MHz, $CDCl_3$) δ 200.0, 179.0, 108.3, 65.2, 50.6, 32.9, 28.2; MS (EI) m/z calc. for $C_8H_{11}O_5$ (M-H): 187.1. Found: 187.1.

11. A minor product formed during the ozonolysis was identified to be the corresponding diacid formed by over oxidation and this is mostly removed during the extraction procedure.

12. The pyridinium salt is somewhat hygroscopic and moisture sensitive and thus should be weighed out and transferred rapidly. IR (solid) 3092, 1616, 1252 cm^{-1}; ^{1}H NMR (500 MHz, CD_3CN) δ 4.40 (s, 3H), 8.05 (dt, *J* = 8.0, 1.5, 1H), 8.23 (dd, *J* = 8.0, 1.5 Hz, 1 H), 8.79 (dt, *J* = 8.0, 1.5 Hz, 1 H), 8.92 (dd, *J* = 8.0, 1.5 Hz, 1 H); ^{13}C NMR (125 MHz, CD_3CN) δ 149.0, 148.9, 148.3, 130.9, 127.3, 122.0 (q, *J* = 319 Hz), 48.6.

13. Characterization data: IR (thin film) 3076, 2945, 1622, 1510 cm^{-1}); This compound is a mixture of two conformers at ambient temperature but low temperature ^{1}H NMR at -40 ^{o}C allowed assignment of one major conformer (500 MHz, -40 ^{o}C, $CDCl_3$) δ 0.06 (S, 9 H), 0.76-0.82 (m, 1 H), 1.31-1.53 (m, 2 H), 1.68 (br s, 1 H), 2.12 (t, 1 H), 2.21 (m, 1H), 2.76-2.99 (m, 4H), 3.39 (dd, *J* = 12.0, 8.0 Hz, 1H), 3.94 (s, 3H), 5.04 (d, *J* = 9.0 Hz, 1H), 5.06 (d, *J* = 17.5 Hz, 1H), 5.66 (br s, 1H), 6.10 (apparent quint, *J* = 9.0 Hz, 1H), 7.08(br s, 1H), 7.38 (dd, *J* = 2.5 and 9.0 Hz, 1H), 7.56 (d, *J* = 4.5 Hz, 1H), 8.02 (d, *J* = 9.0 Hz, 1H), 8.75 (d, *J* = 4.5, 1H); ^{13}C NMR was taken at ambient temperature. ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.3, 147.5, 147.3, 144.1, 140.7, 131.7, 126.0, 121.5, 118.5, 114.3, 100.3, 73.2, 60.1, 55.6, 50.3, 49.6, 40.1, 28.2, 26.3, 19.6, 0.03.

14. Recrystallization is performed in a 50 mL Erlenmeyer flask by addition of 20 mL hot EtOAc to the dried, semi-crude β-lactone. A stir bar is added to the flask and then heated with stirring to ~50 ^{o}C on a hot plate to dissolve the solid. Hexanes are added to the hot solution (~16 mL) with

continued heating until the solution turns cloudy. On cooling to ambient temperature for ~1 h, crystals form and the flask is then placed in an ice-bath for an additional 30 min. The crystals are collected by rapid vacuum filtration and washed quickly with an ice-cold mixture of 50 % EtOAc:hexanes (~ 10 mL) to give 4.0-4.2 g (30 – 31 %) of the bicyclic-β-lactone as light yellow crystals. A second crop is collected by removal of ~1/3 of the mother liquor and placing the solution in a freezer (-10 ^{o}C) overnight to provide an additional 0.5 -0.6 g (4–5 %) as darker yellow crystals.

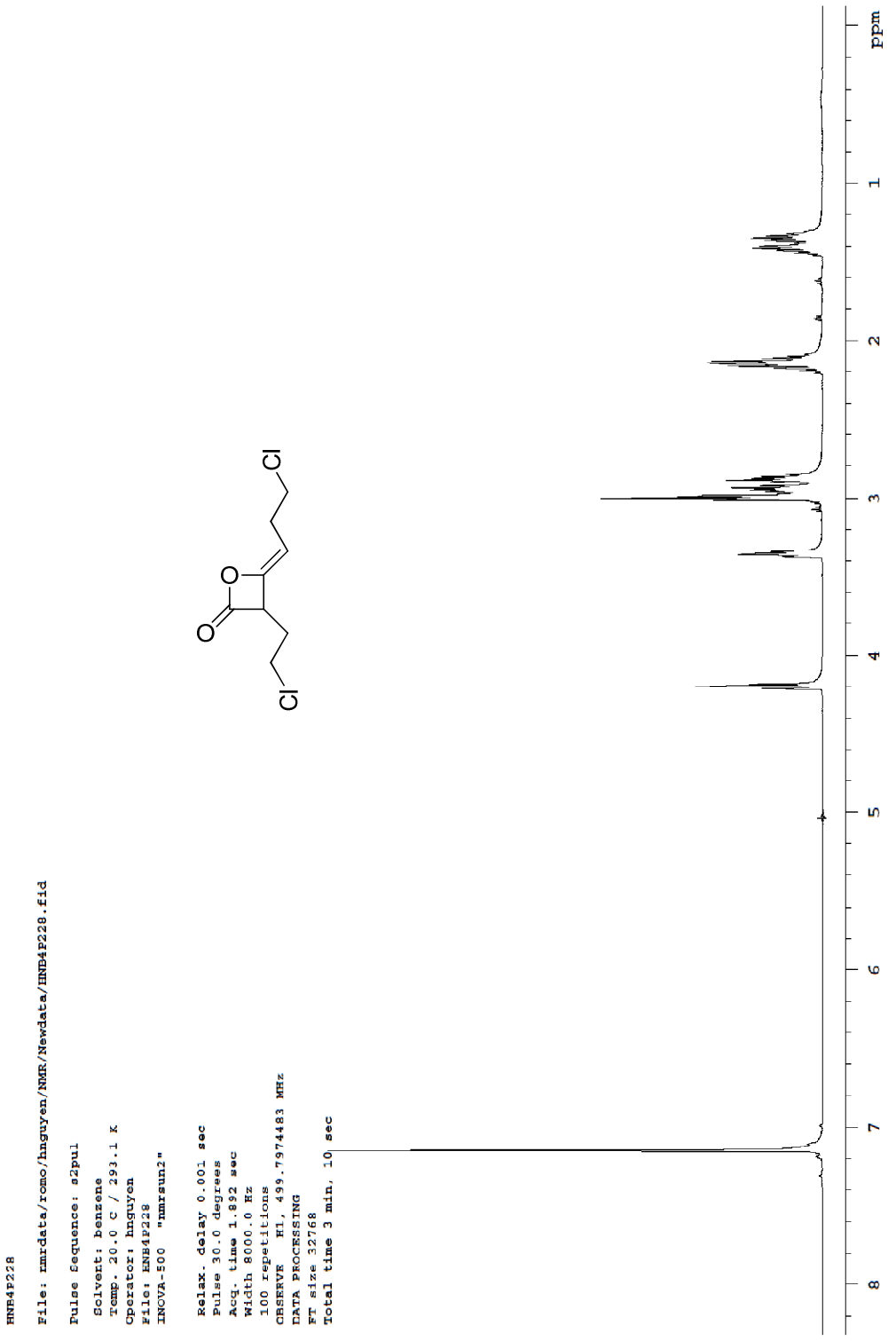
15. Higher yields of β-lactone could be achieved by using aldehyde acid purified by flash chromatrography, however loss on the column led to further reduction of the overall yield. Characterization data for (1*R*,5*S*)-6-oxaspiro[bicyclo[3.2.0]heptane-3,2'-[1,3]dioxolan]-7-one (**6.4**): R_f = 0.52 (60% EtOAc/hexanes); m.p. 103-104 ºC; $[\alpha]_D^{25}$ + 62.5 (*c* 0.95, $CHCl_3$); IR (thin film) 1821 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.03 (dd, *J* = 8.5, 14 Hz, 1 H), 2.08 (dd, *J* = 5.0, 15.5 Hz, 1 H), 2.32 (ddd, *J* = 0.5, 1.0, 14 Hz, 1 H), 2.38 (dd, *J* = 1.5, 15.5 Hz, 1 H), 3.88 – 3.94 (m, 2 H), 3.99 – 4.09 (m, 3 H), 5.00 (t, *J* = 5 Hz, 1 H) ; ^{13}C NMR (75 MHz, $CDCl_3$) Anal. Calcd. For $C_{18}H_{10}O_4$: C, 56.47; H, 5.92. Found: C, 56.67; H, 5.91.

16. Enantiomeric excess was determined to be 86-88% by chiral HPLC. Conditions: Chiralcel OD, 250 x 4.6 mm (L x I.D.), solvent (isocratic) 85% hexanes, 15% 2-propanol, flow rate 1.0 mL/min, wavelength λ = 220 nm. Retention times were: (1R, 5S)-β-lactone, 18.06 min; (1S, 5R)-β-lactone 22.96 min. An alternative method was developed previously using chiral GC with a non-commercially available chiral column: 2,3-di-OAc-6-TBS-CD and showed 92% ee with *O*-Ac quinidine as chiral promoter.

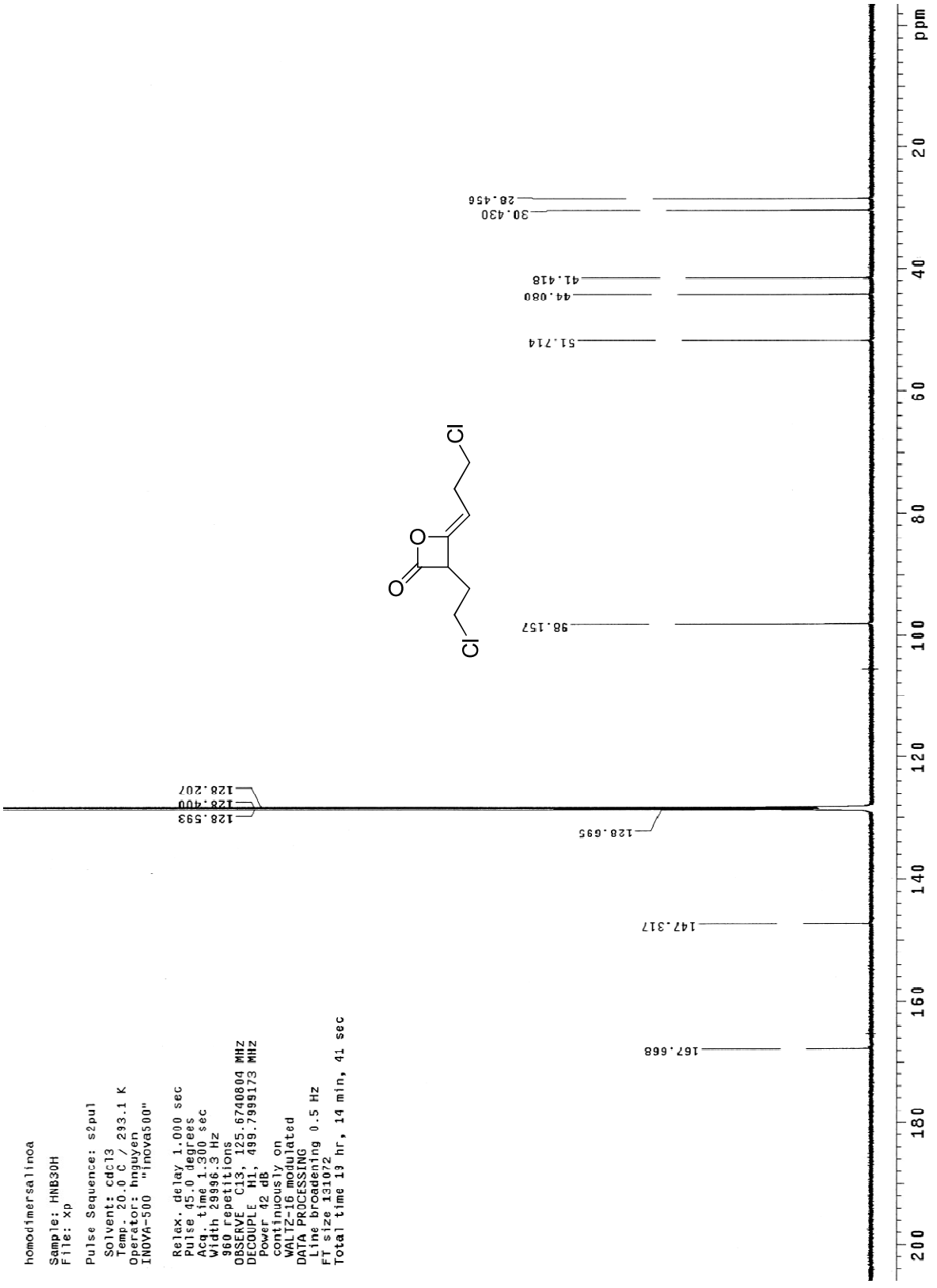
Waste Disposal Information and Hazards

All toxic materials were disposed of in accordance with "Prudent Practices in the Laboratory"; National Academy Press; Washington, DC, 1995.

Caution: Care must be excercised in transferring and manipulating the ozonide reaction mixture due to the known instability of ozonides. It is best to perform the ozonide reduction step in the same fume hood and avoid transferring and manipulation of the reaction mixture. A yellow solution following is a negative result and suggests it is safe to proceed to work up.



^{1}H NMR of homodimer (±)-**2.13d** (500 MHz, C_6D_6)



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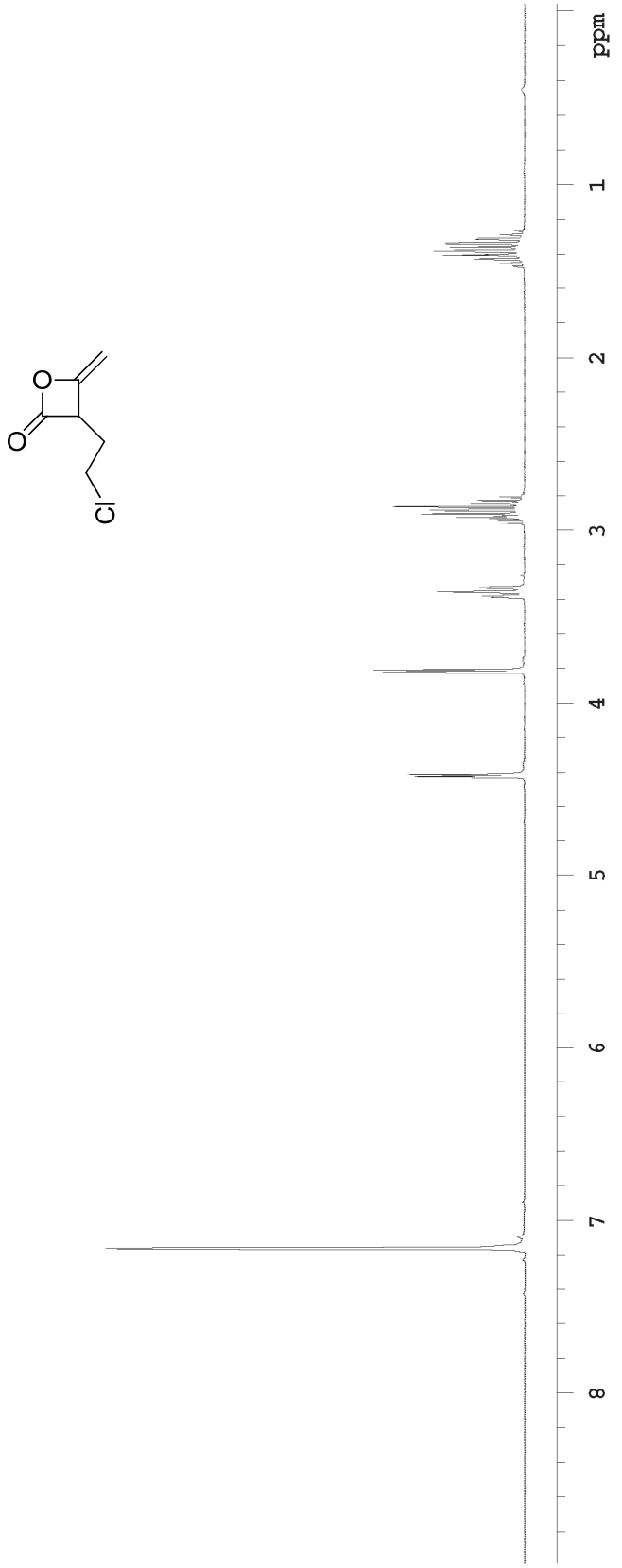
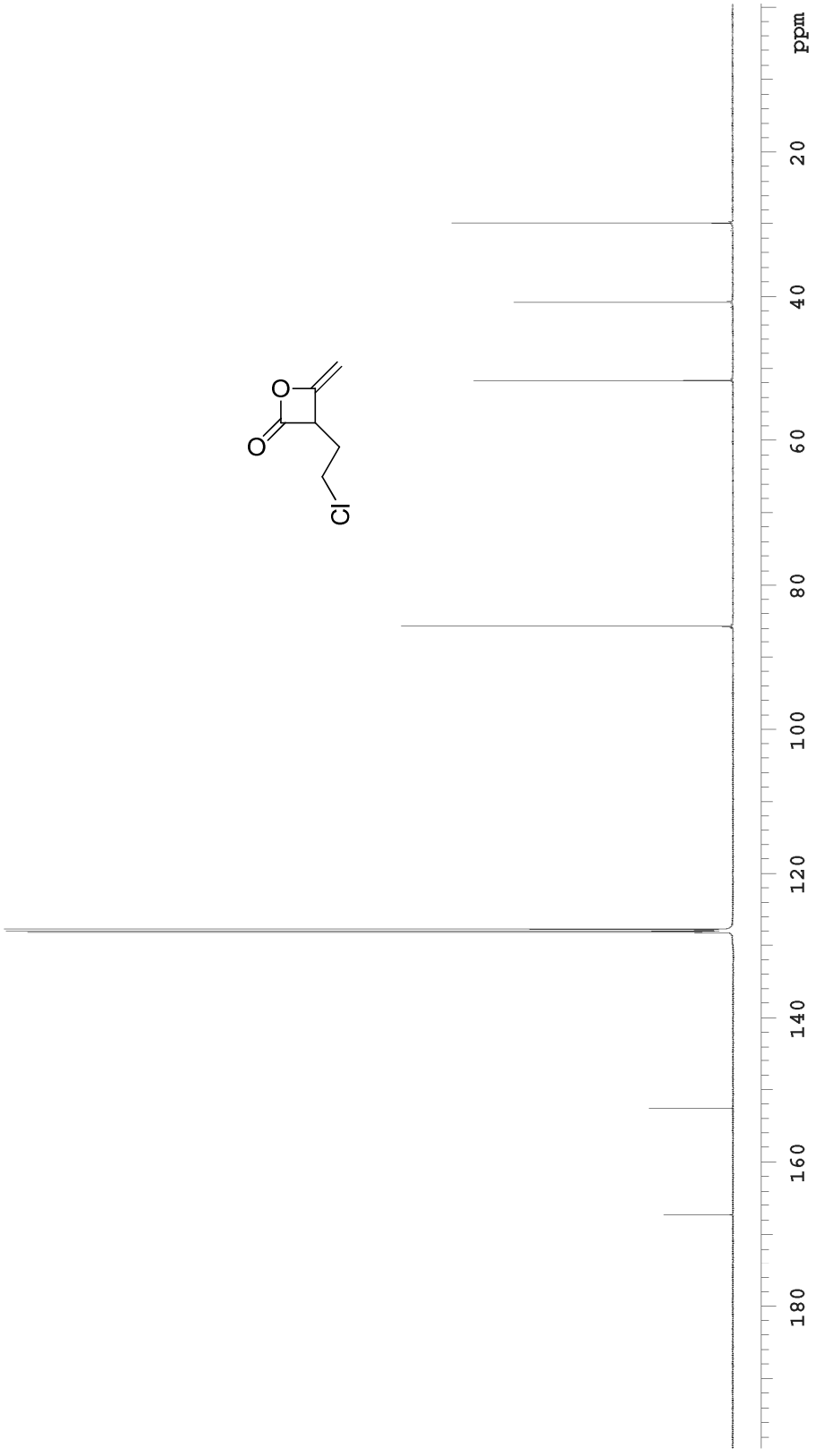


^{1}H NMR of heterodimer (±)-**2.11a** (500 MHz, C_6D_6)



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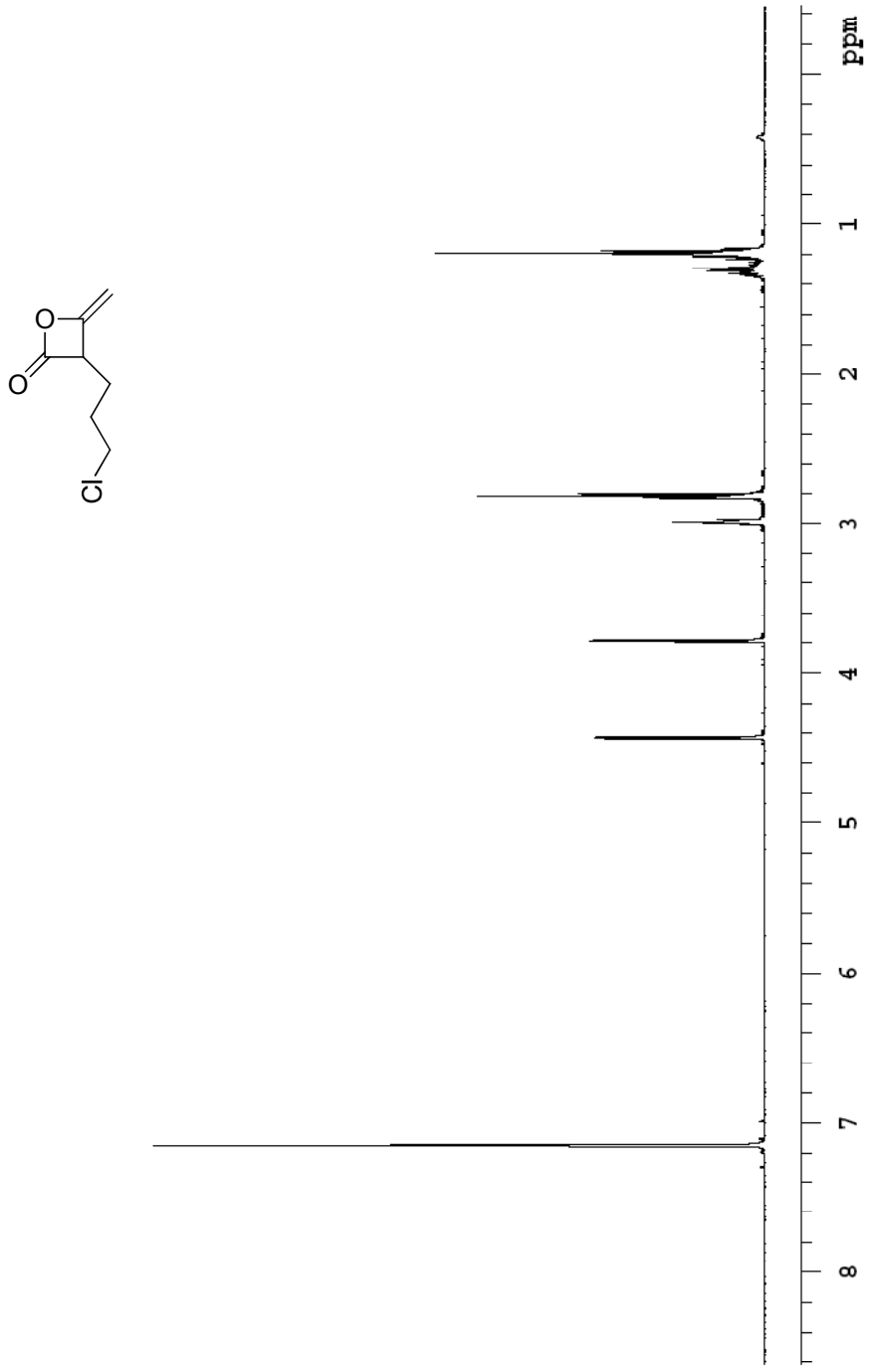
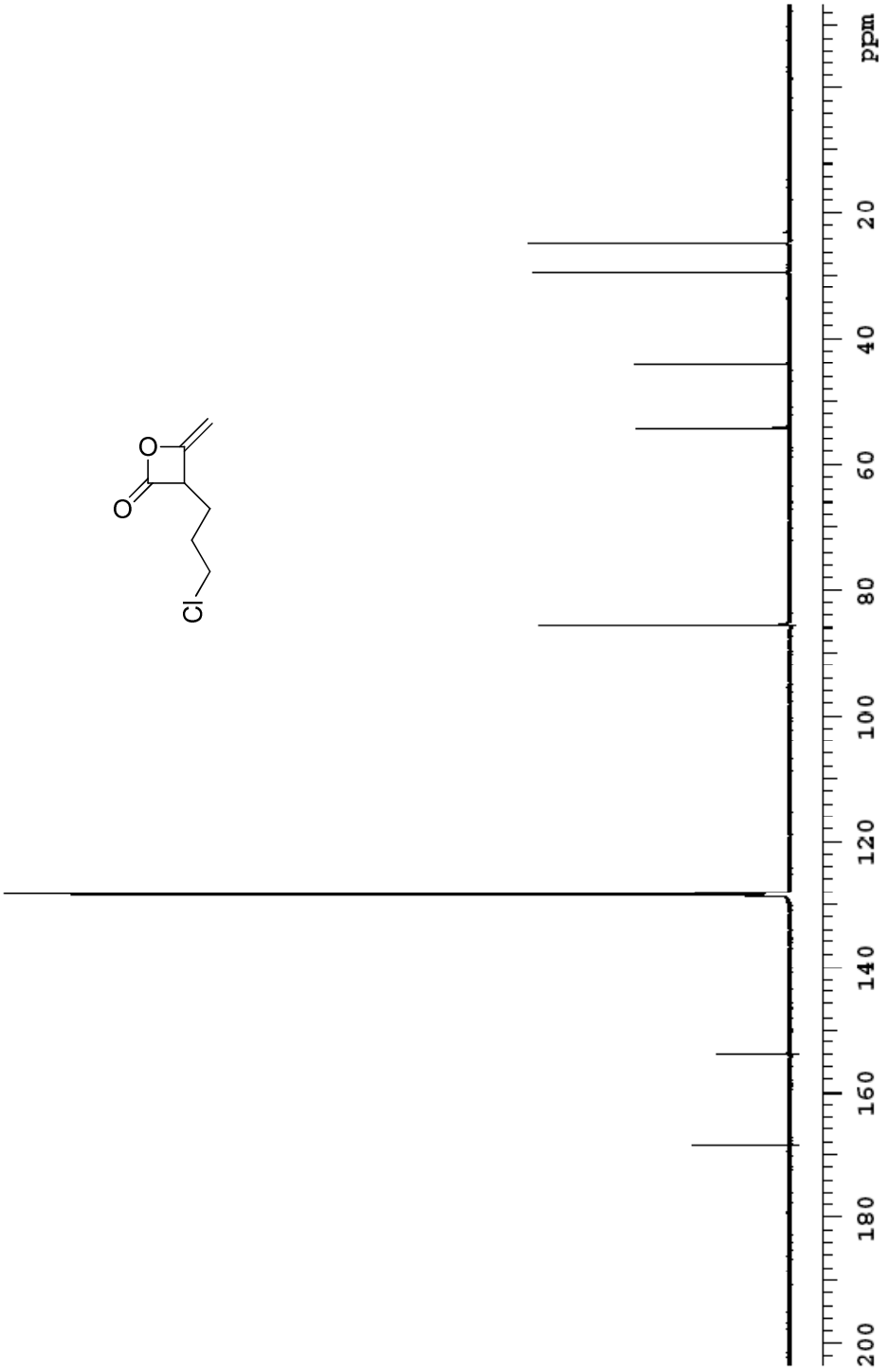


^{1}H NMR of heterodimer (±)-**2.11b** (500 MHz, C_6D_6)



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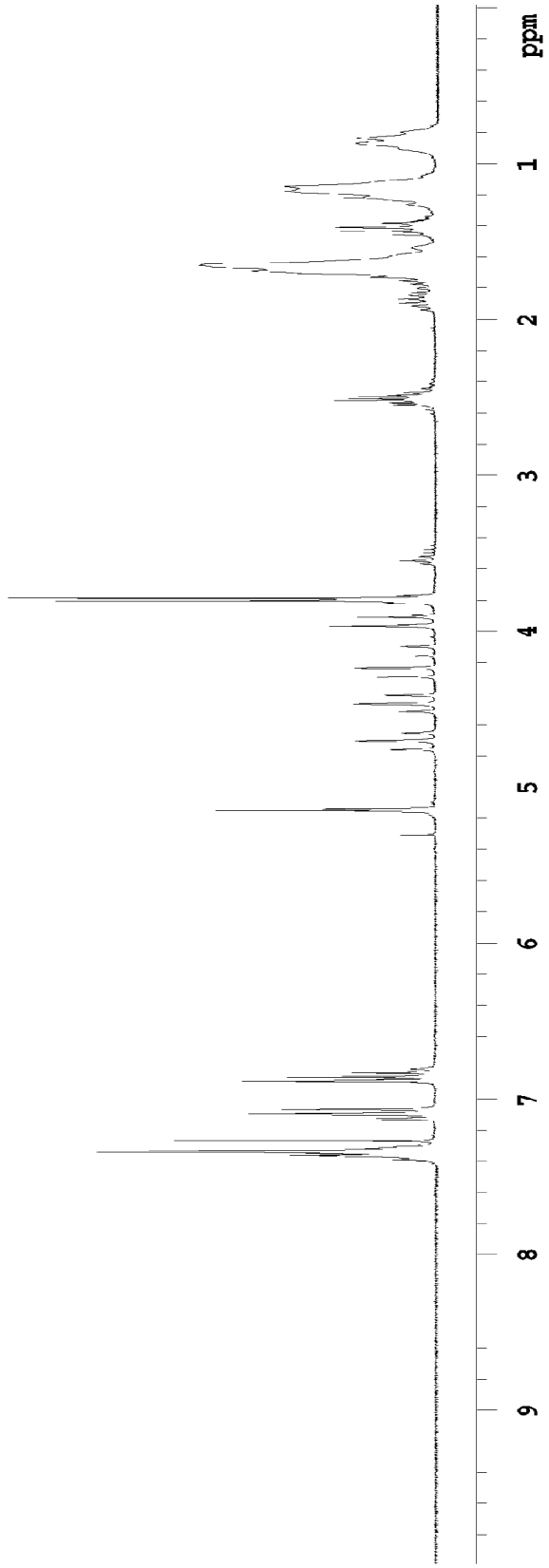
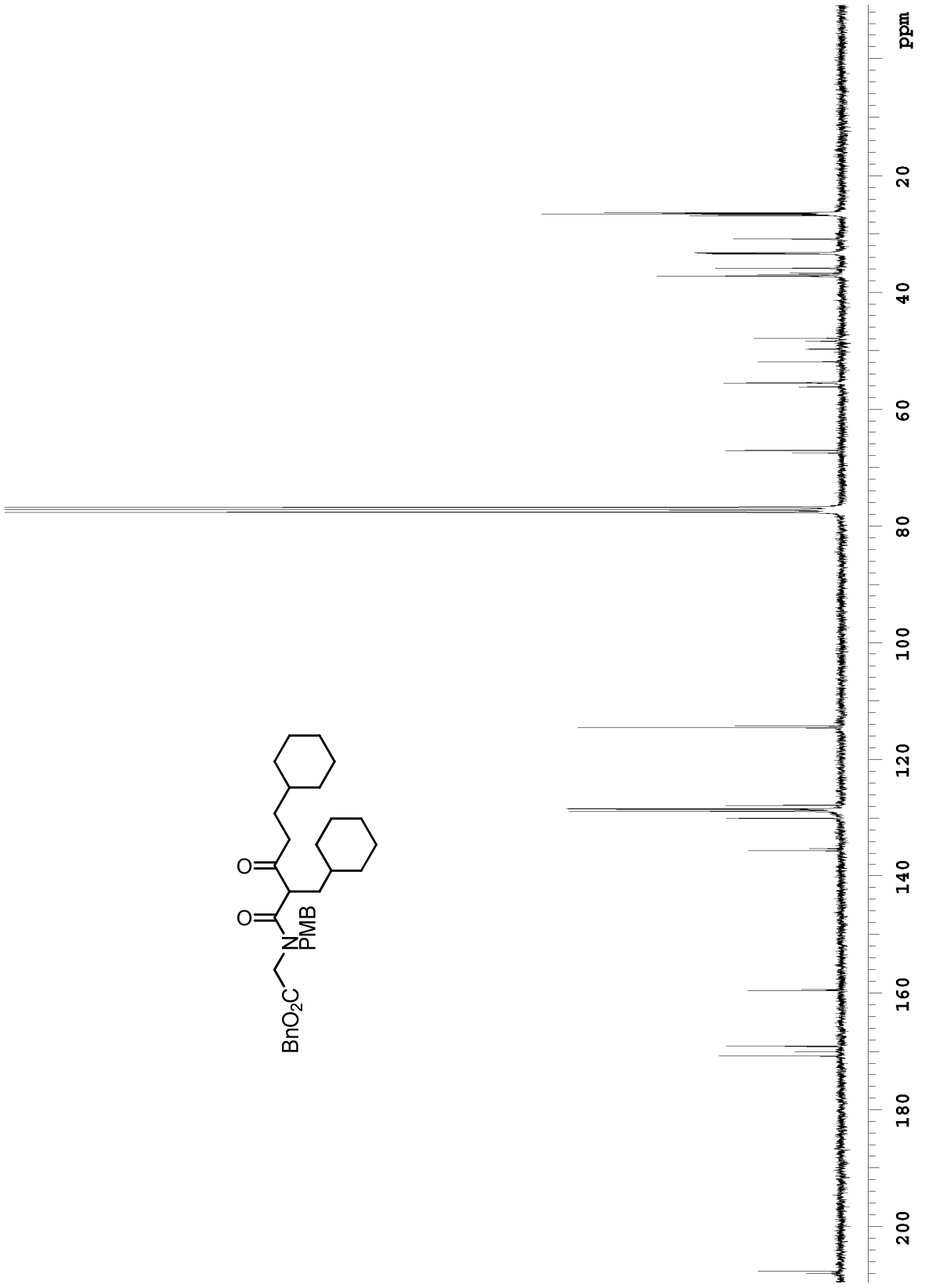


^{1}H NMR of ketoamide (±)-**2.20a** (500 MHz, $CDCl_3$)



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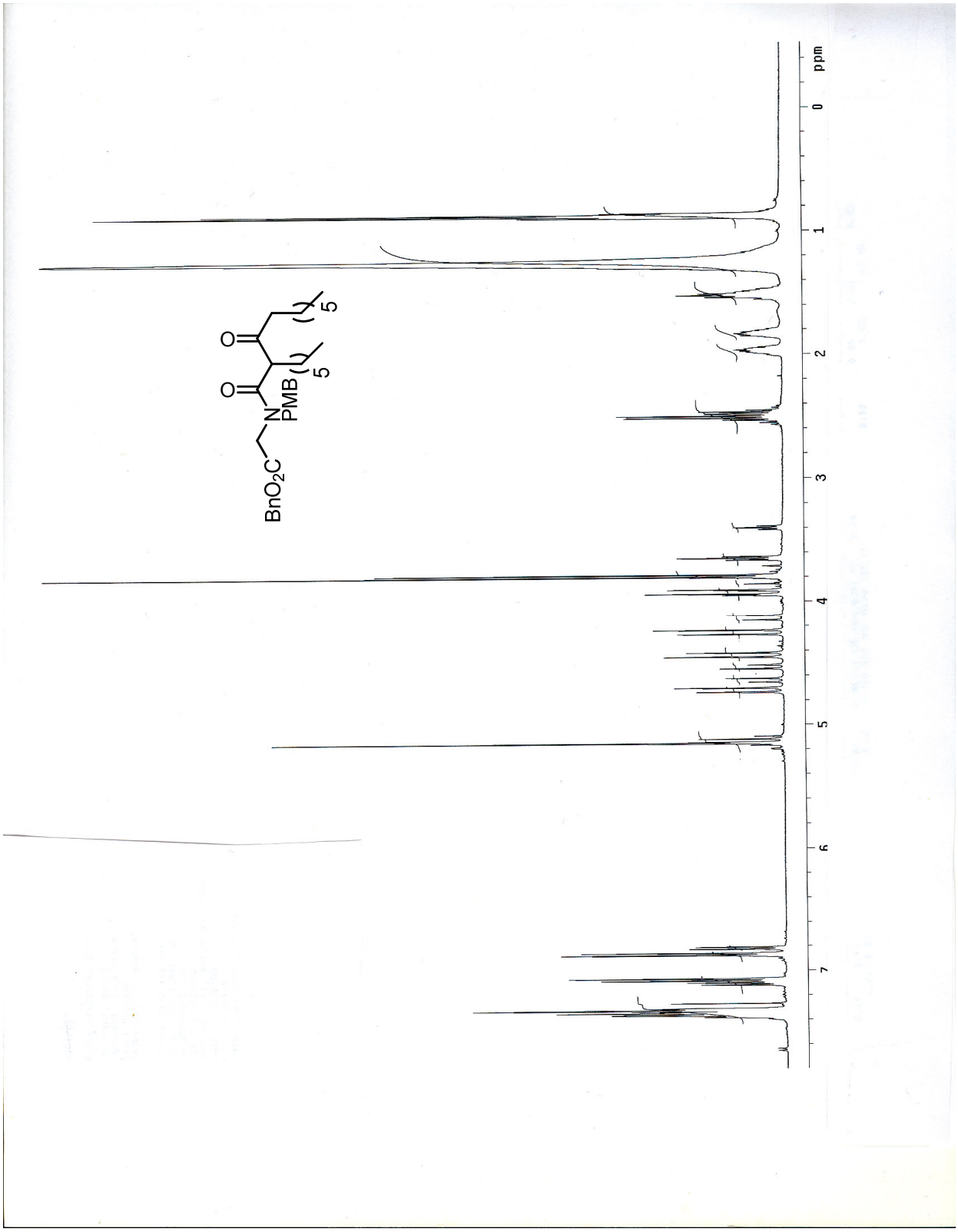

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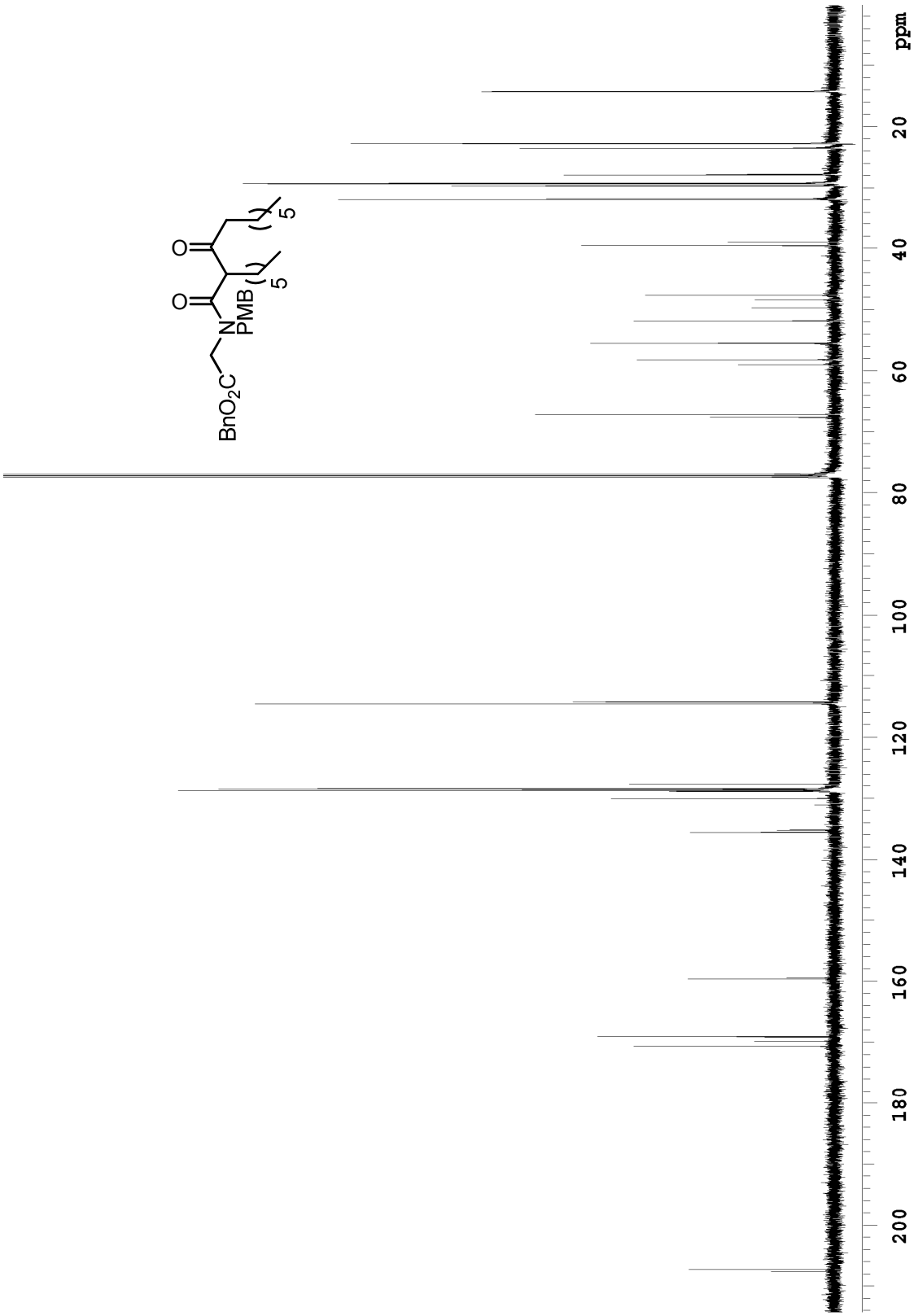

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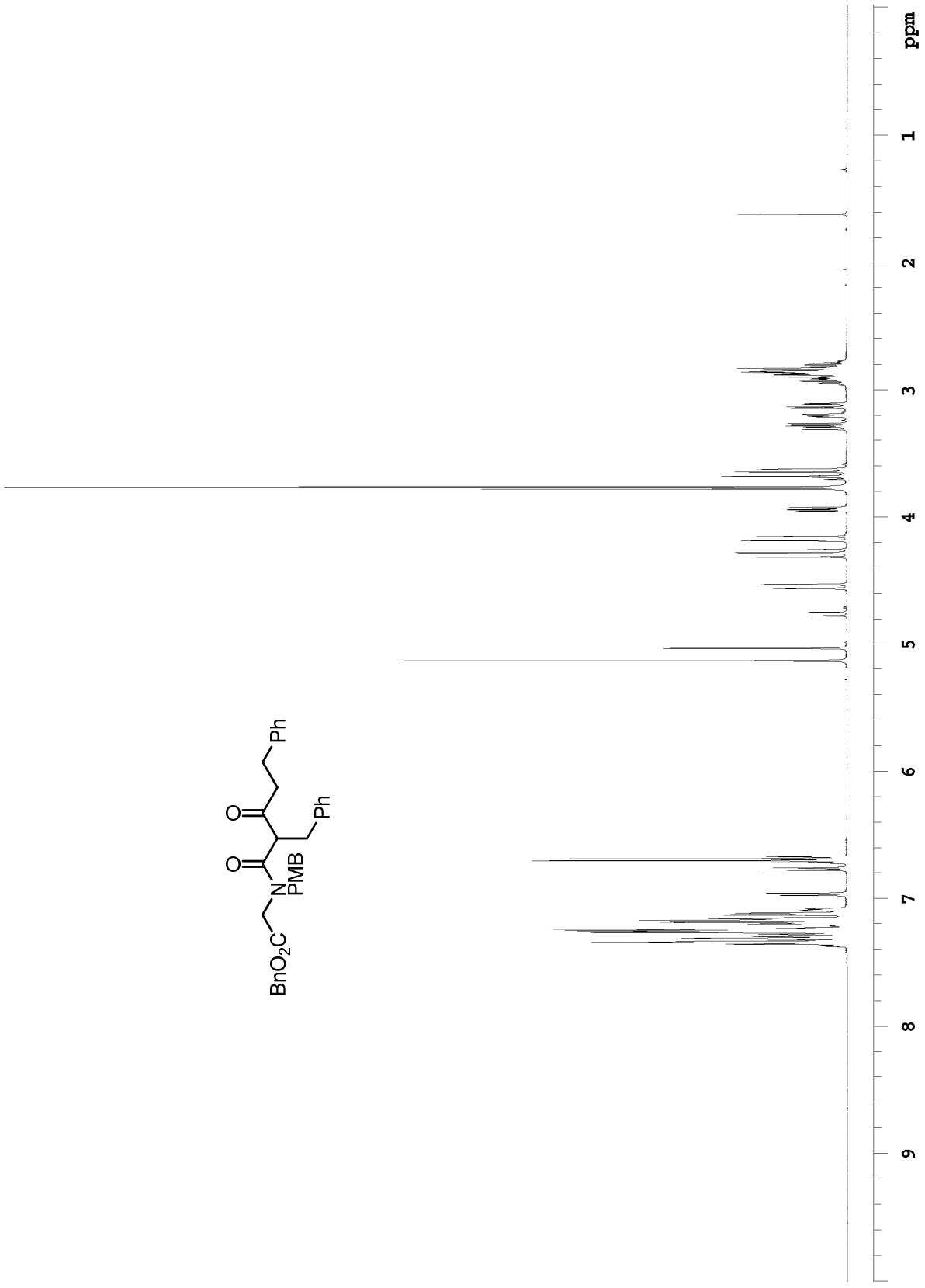

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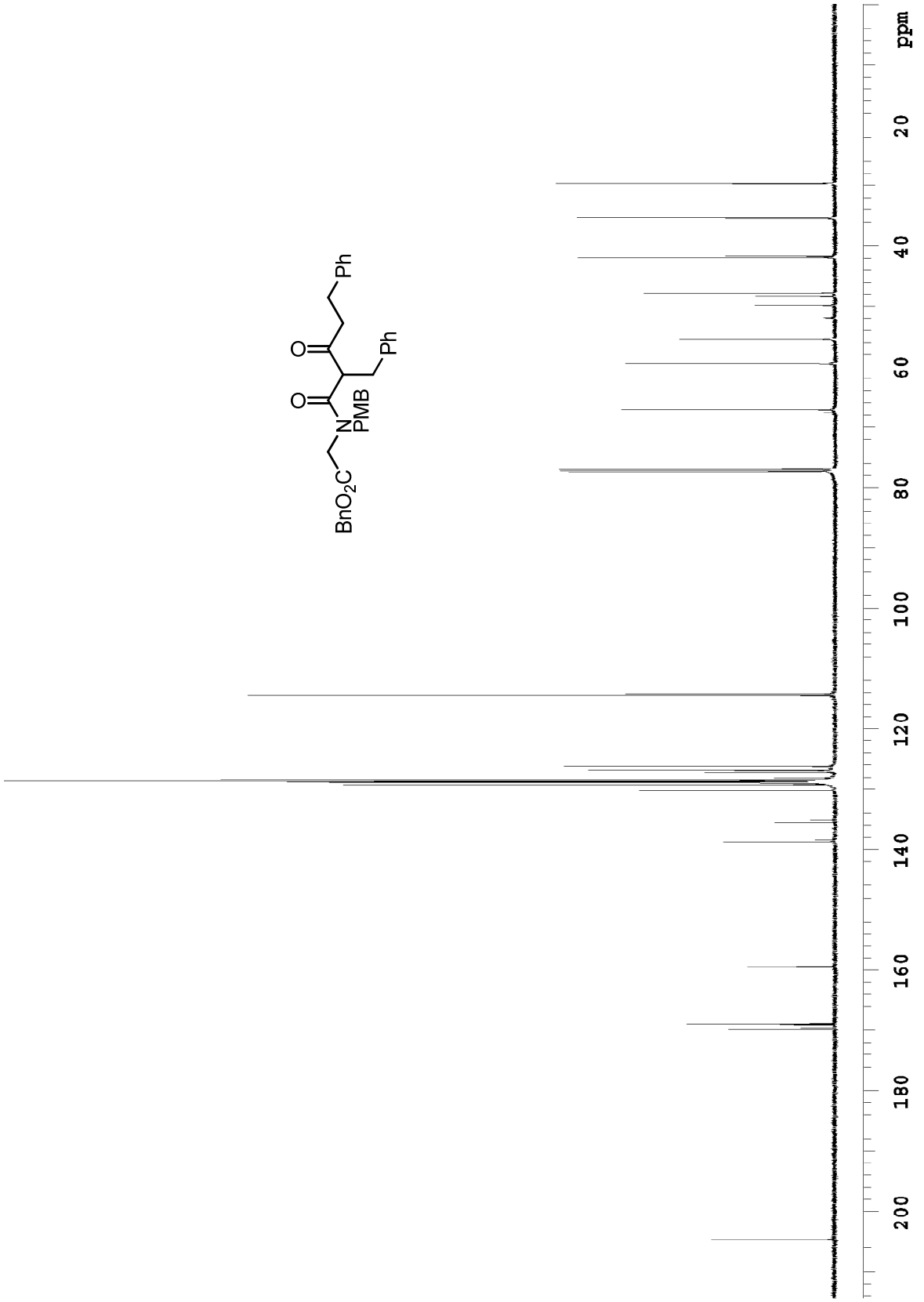

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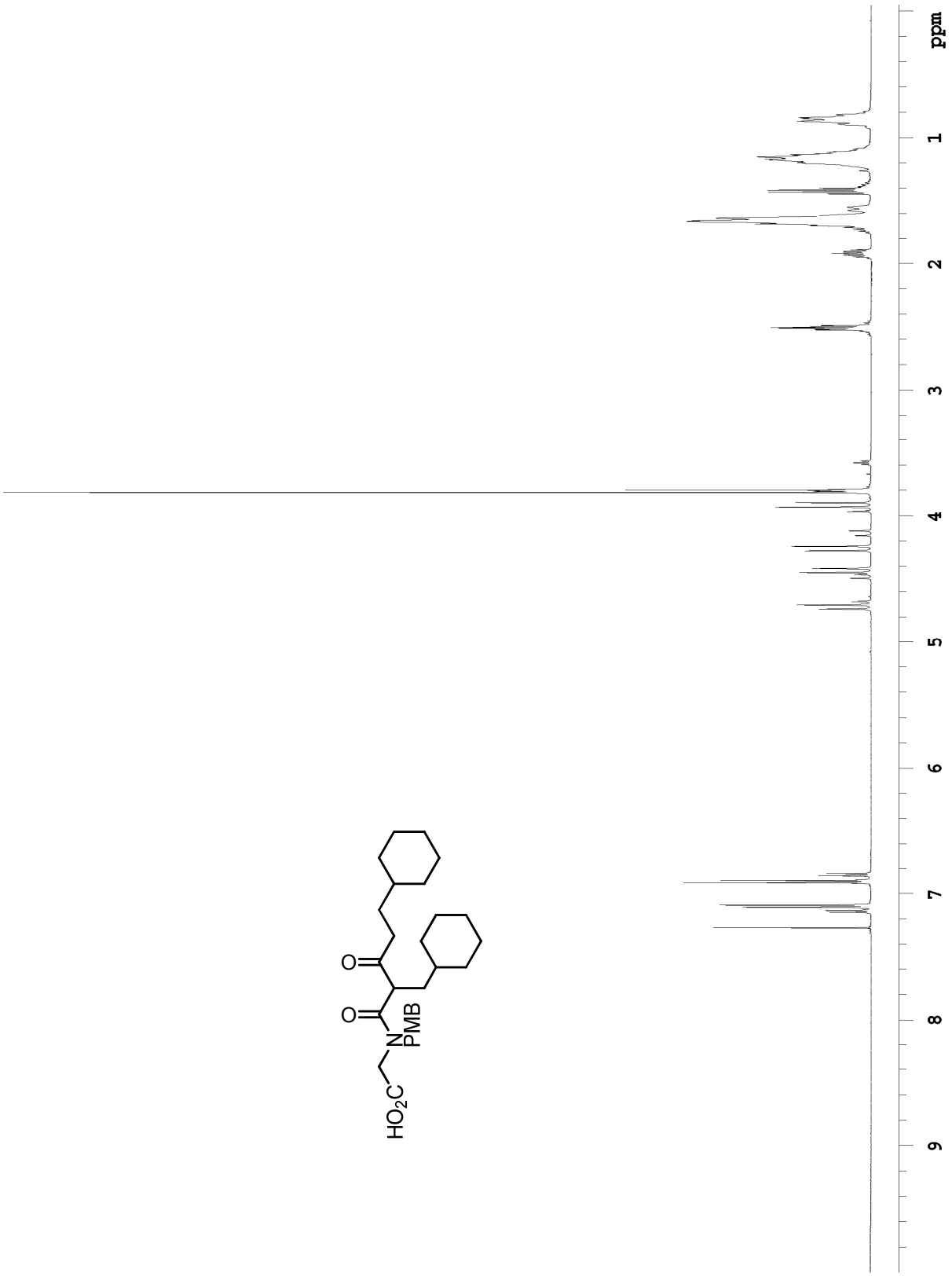
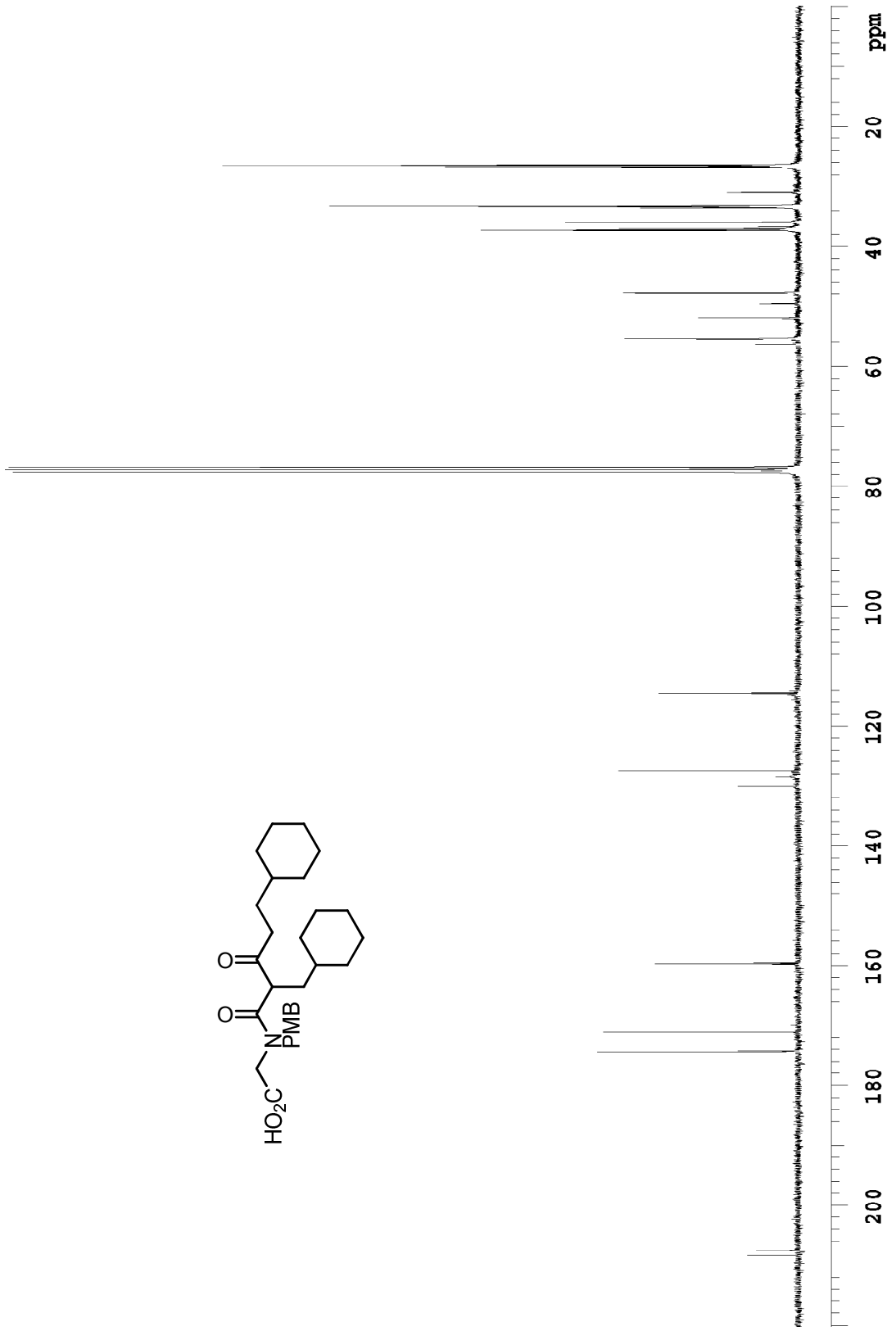


^{1}H NMR of ketoacid (±)-**2.22a** (500 MHz, $CDCl_3$)



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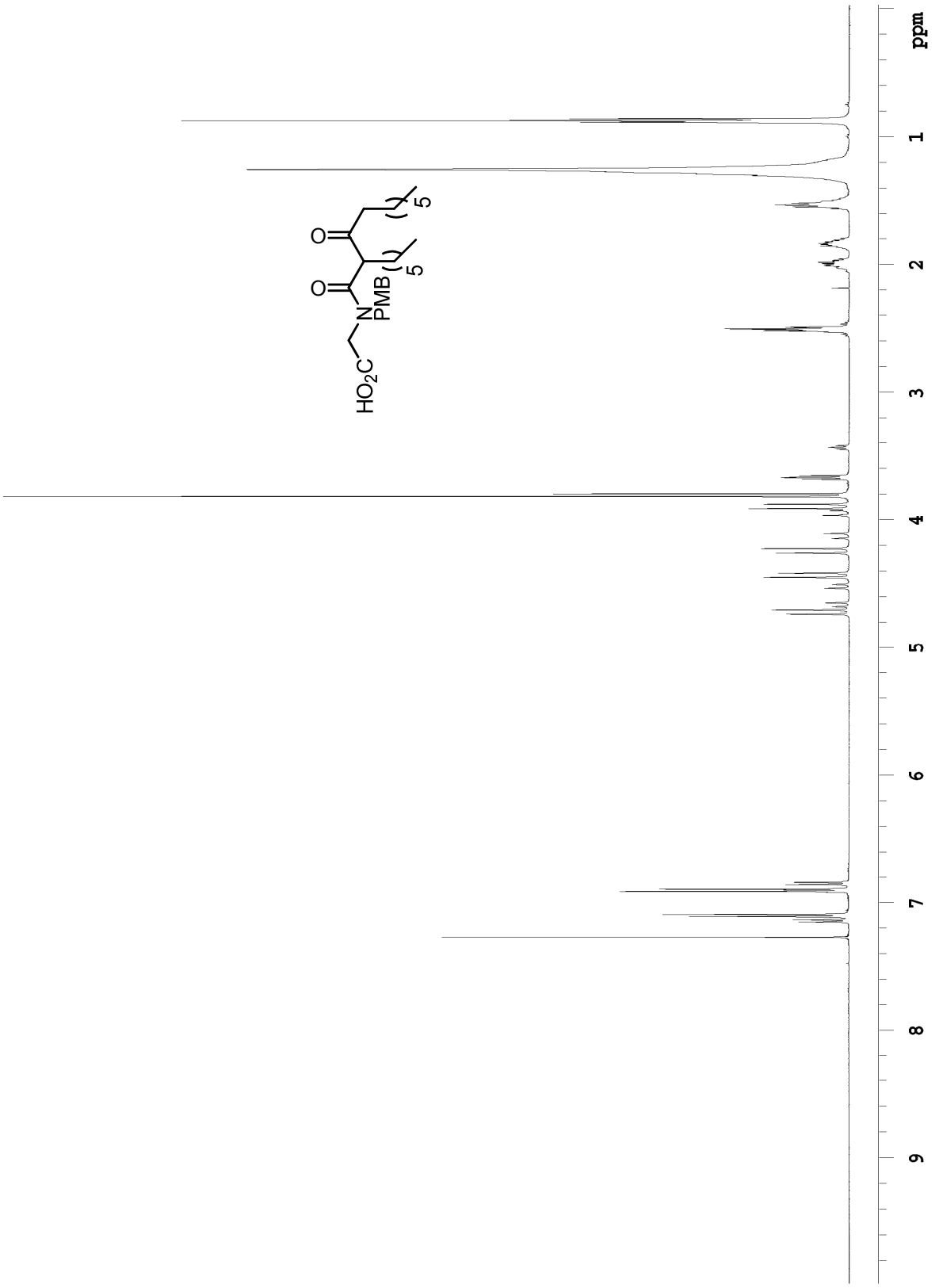
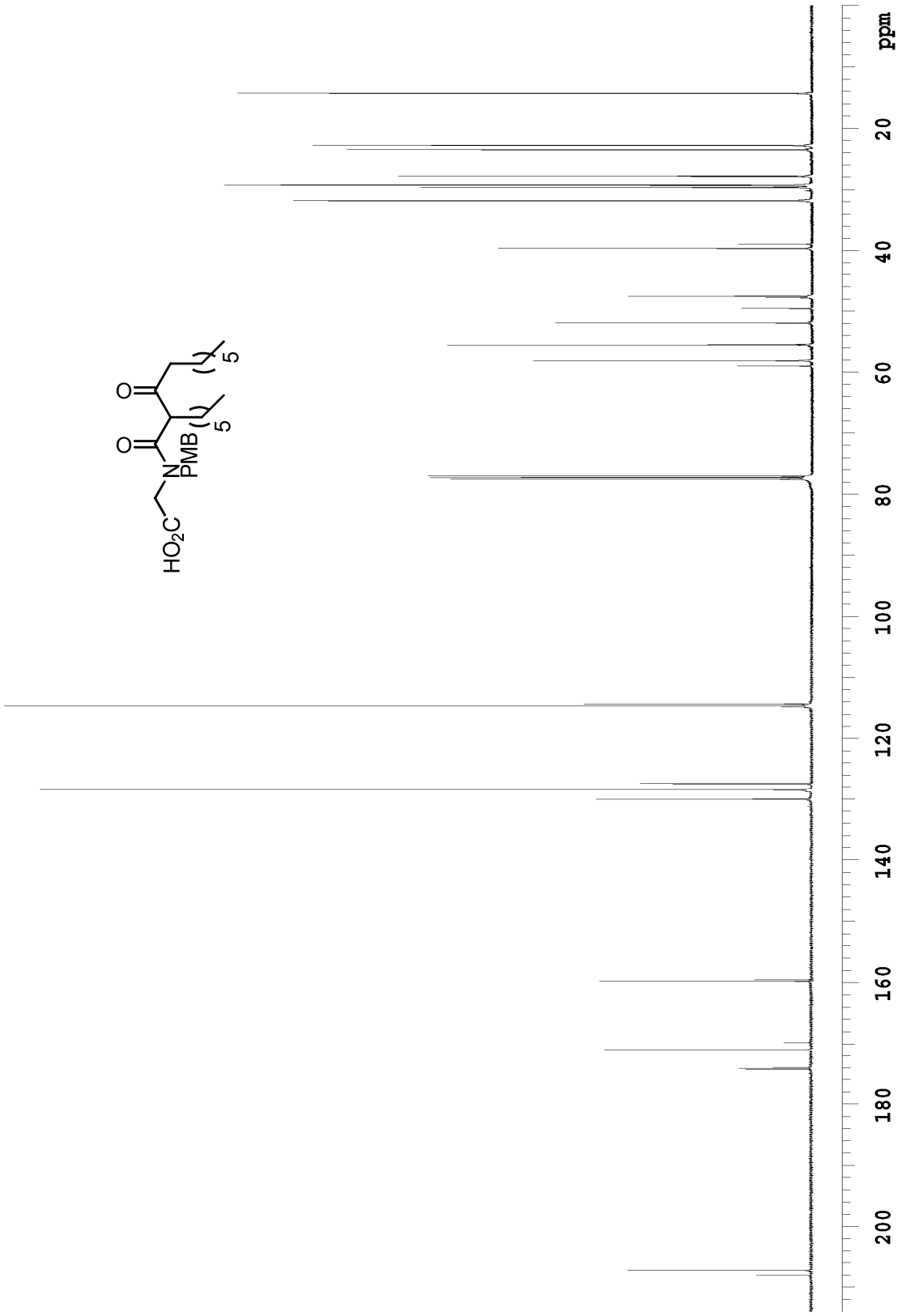


^{1}H NMR of ketoacid (±)-**2.22b** (500 MHz, $CDCl_3$)



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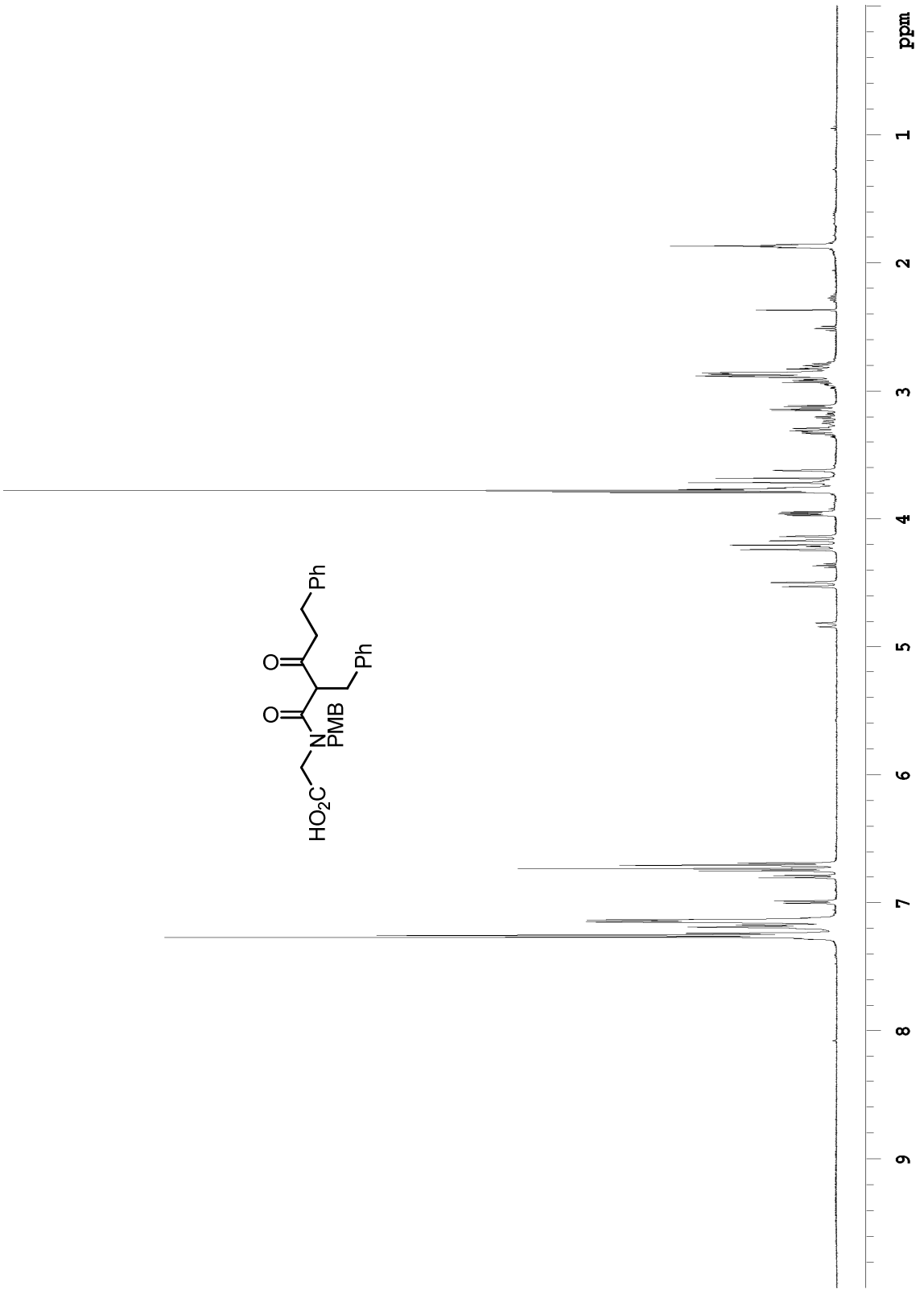

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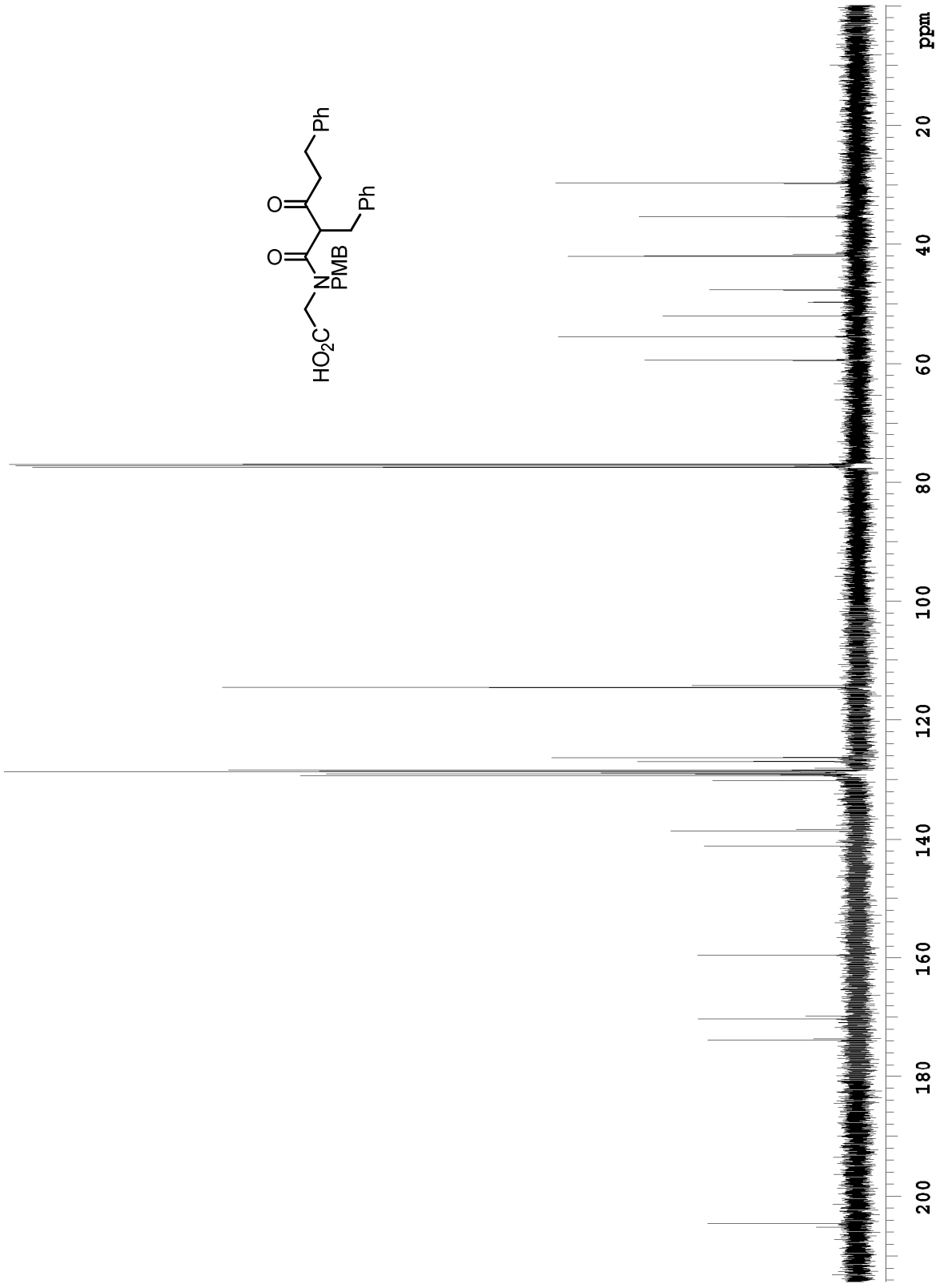

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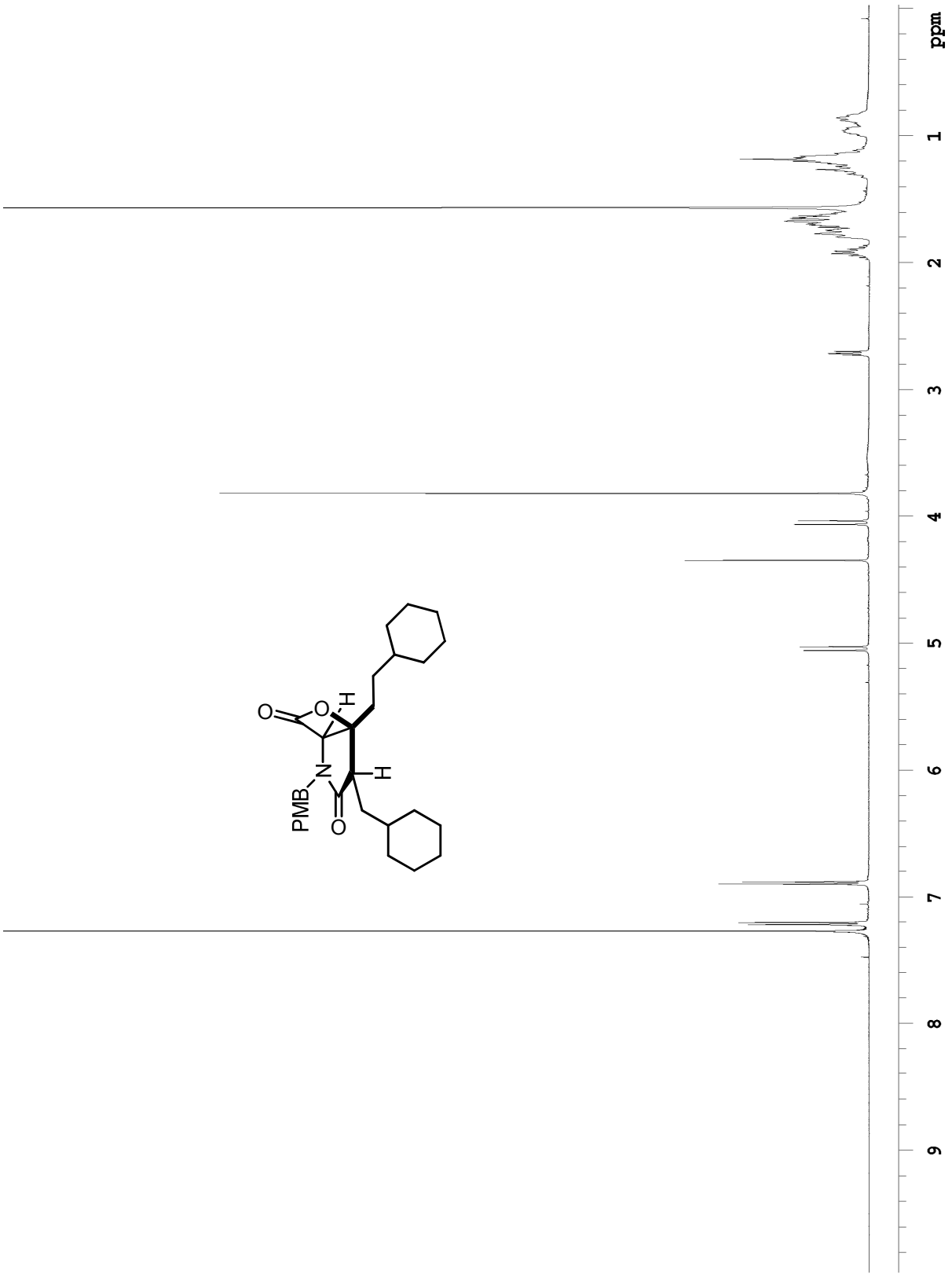
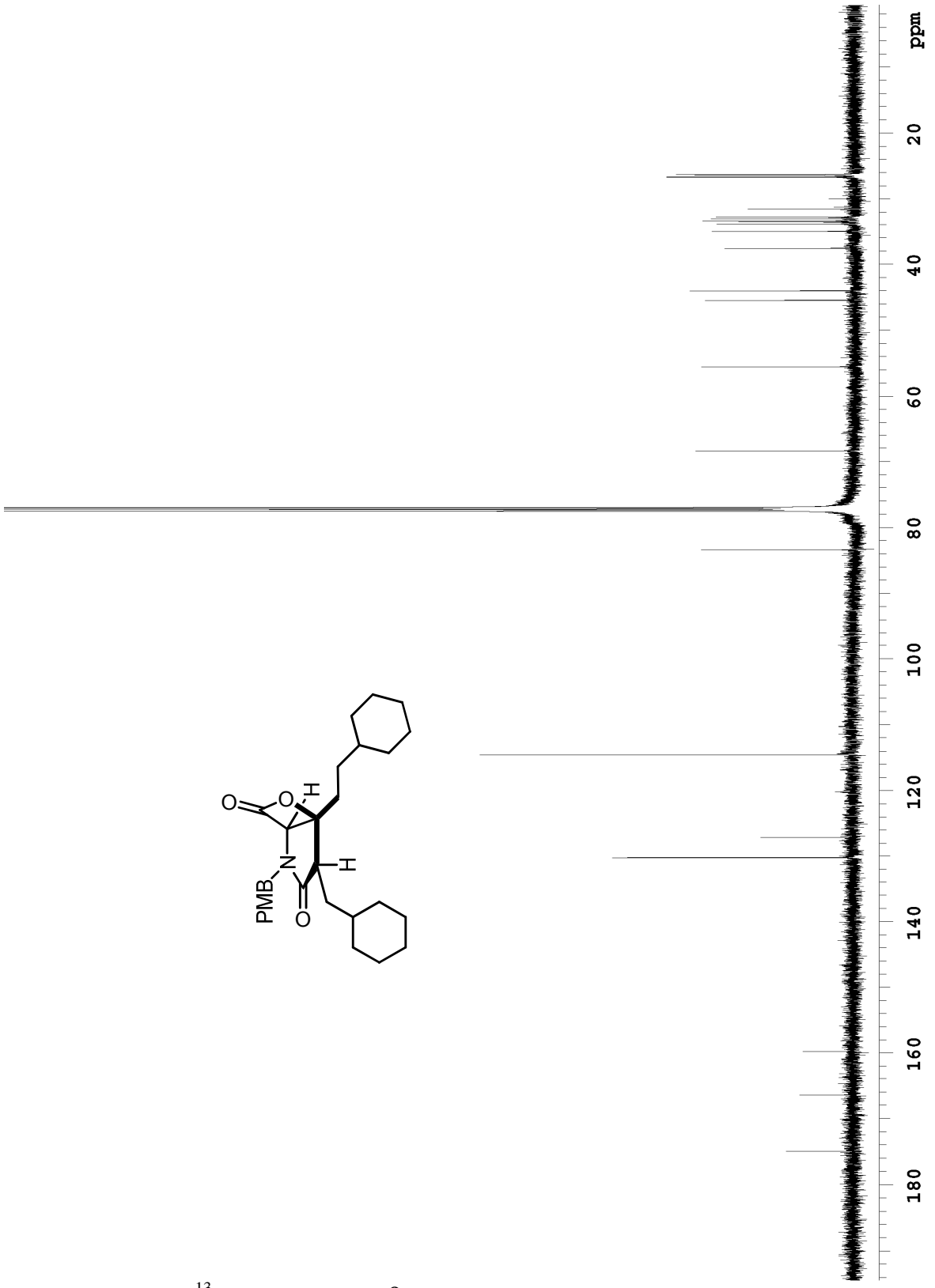


^{1}H NMR of PMB-β-lactone (±)-2.23a (500 MHz, $CDCl_3$)



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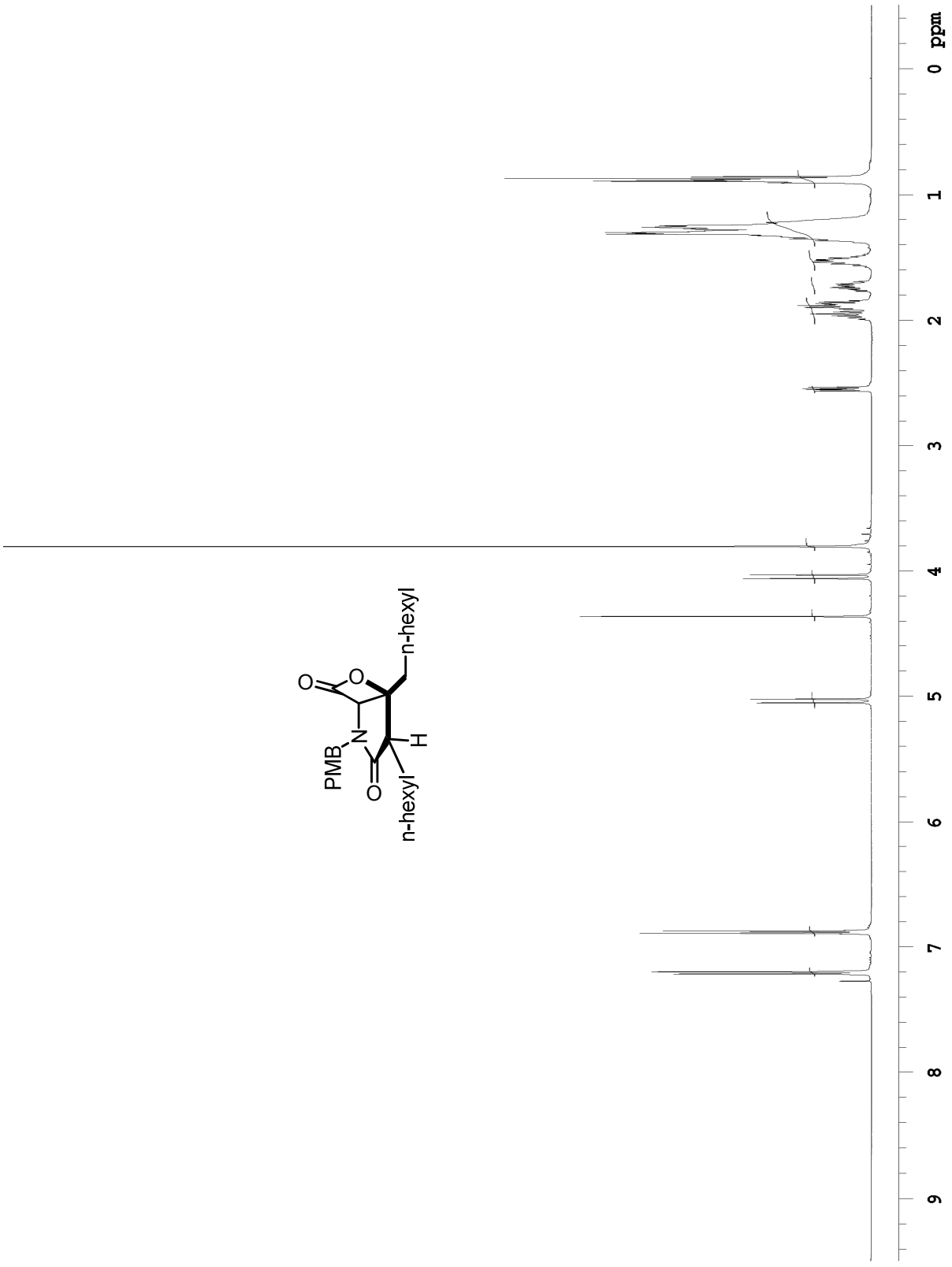

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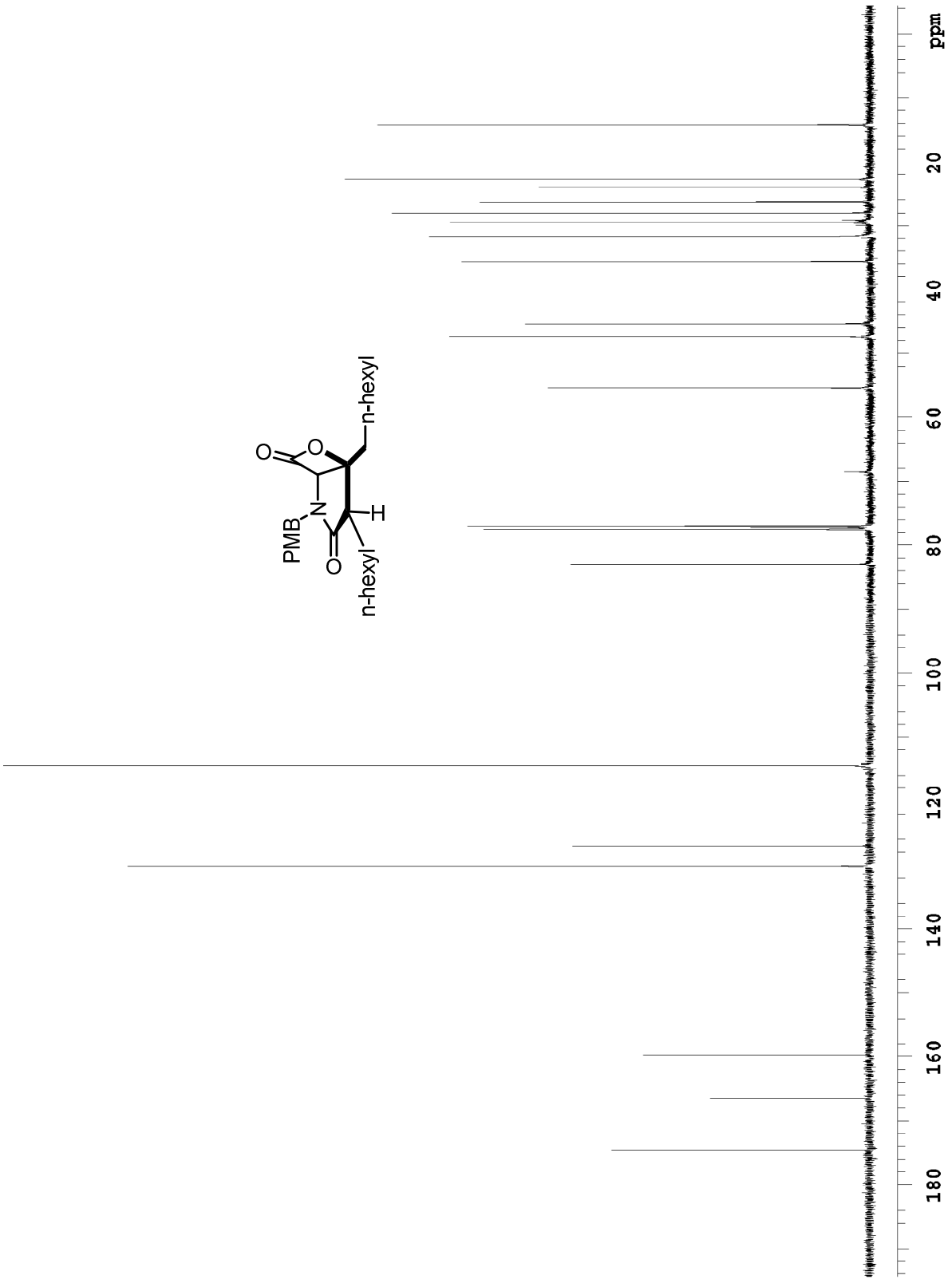
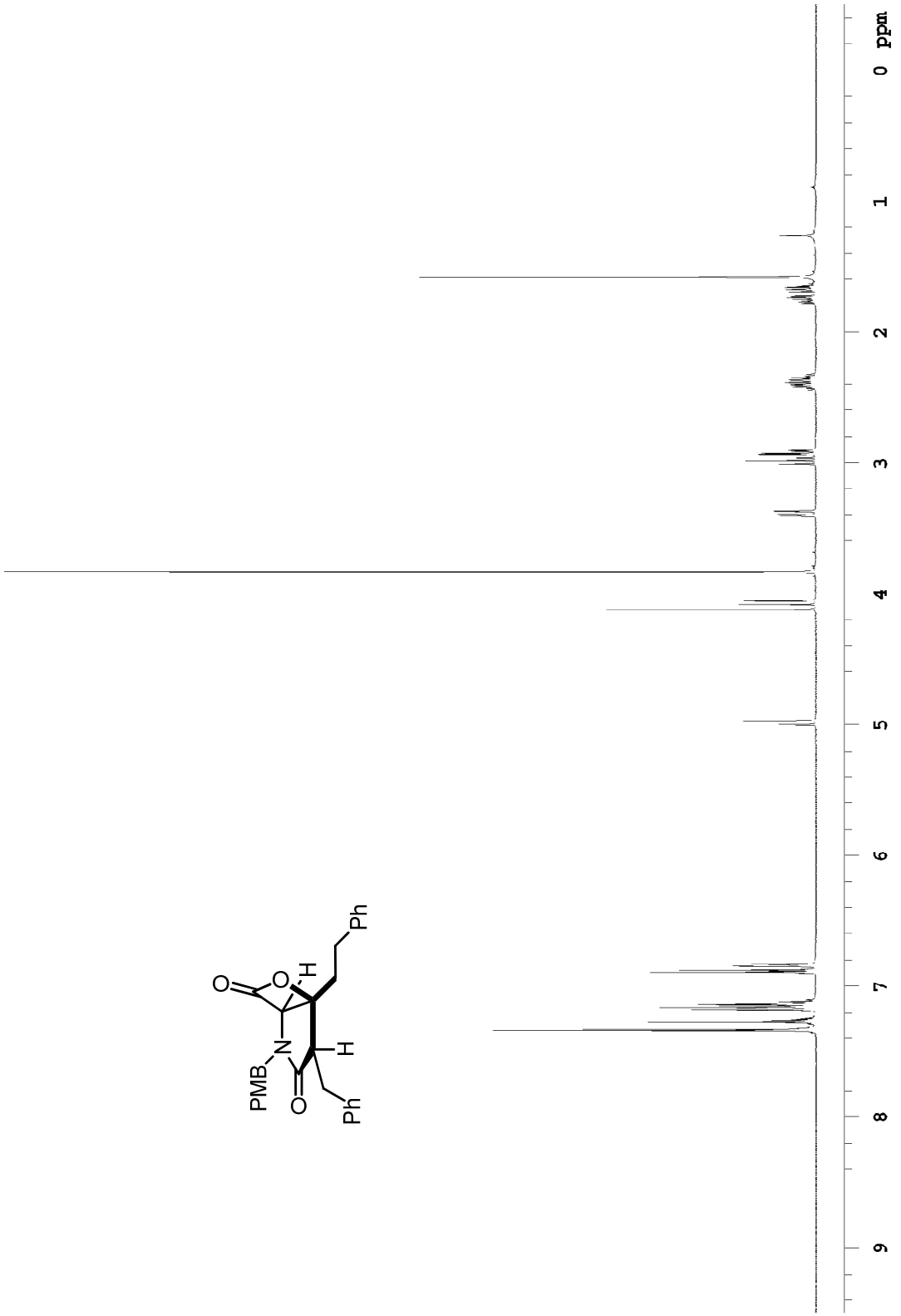
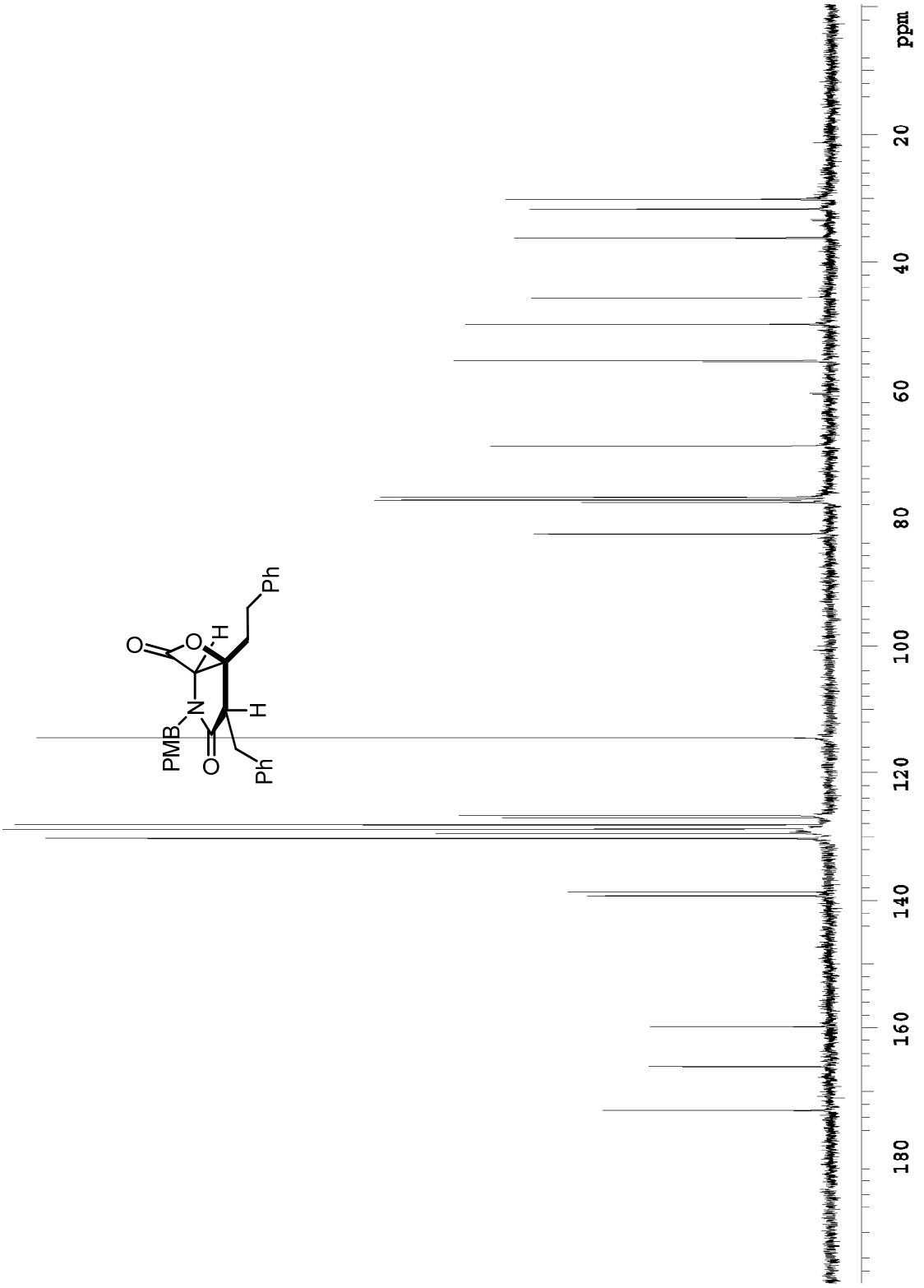


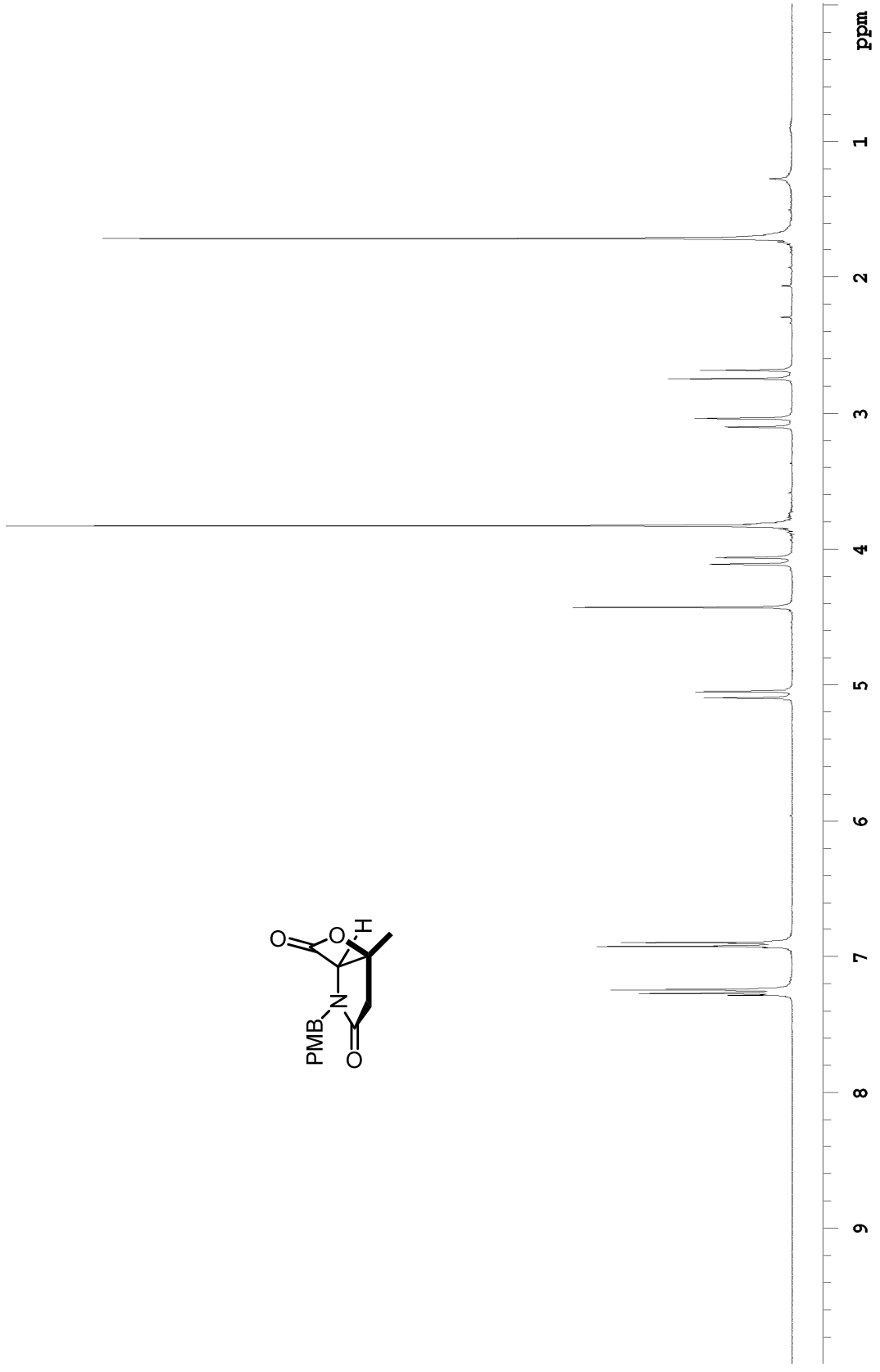
^{13}C NMR of PMB-β-lactone (±)-**2.23b** (500 MHz, $CDCl_3$)



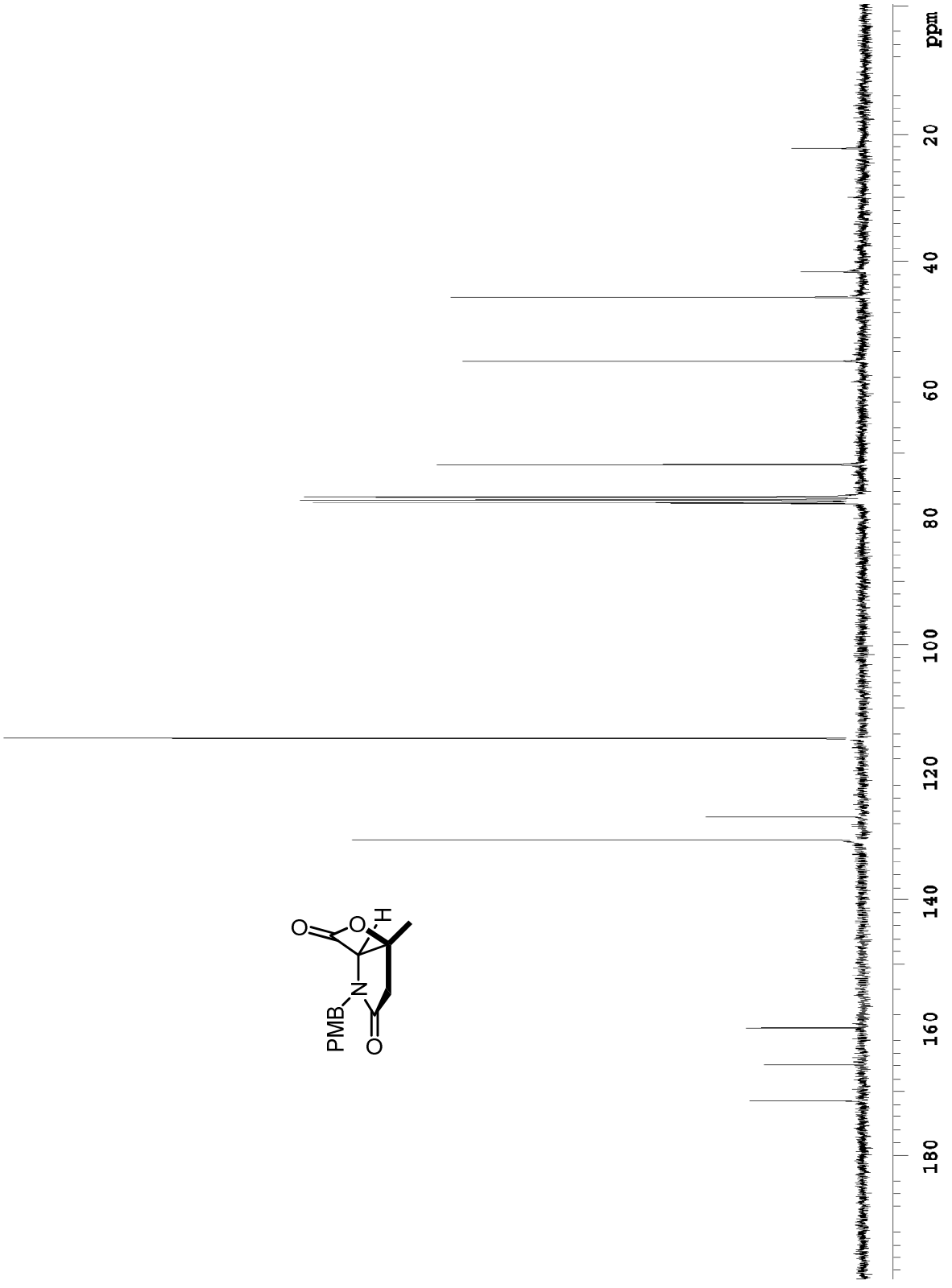
^{1}H NMR of PMB-β-lactone, (±)-**2.23c** (500 MHz, $CDCl_3$)



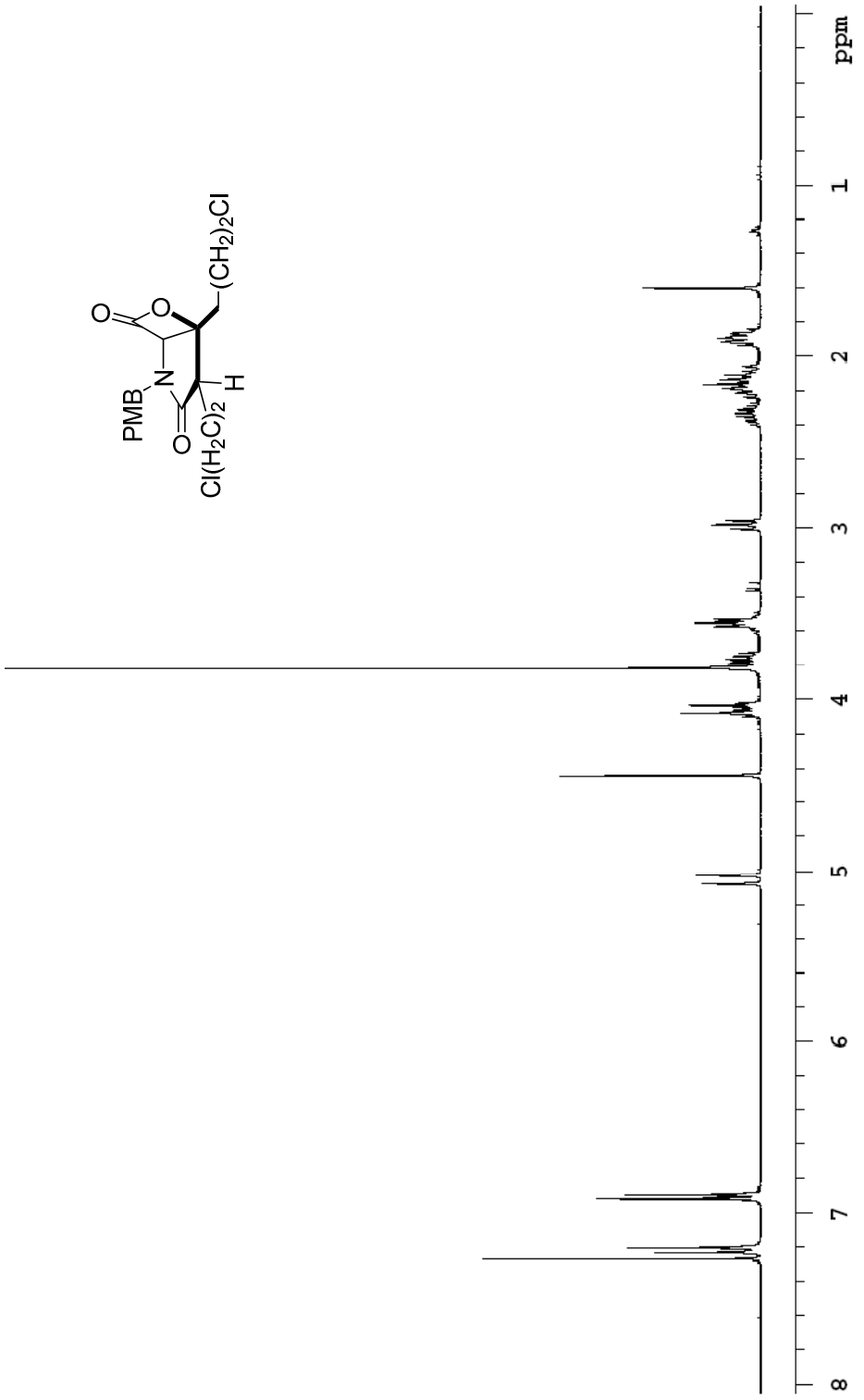
^{13}C NMR of PMB-β-lactone (±)-**2.23c** (500 MHz, $CDCl_3$)


ppm
1
2
3
4
5
6
7
8
9
O
O
H
N
PMB
O

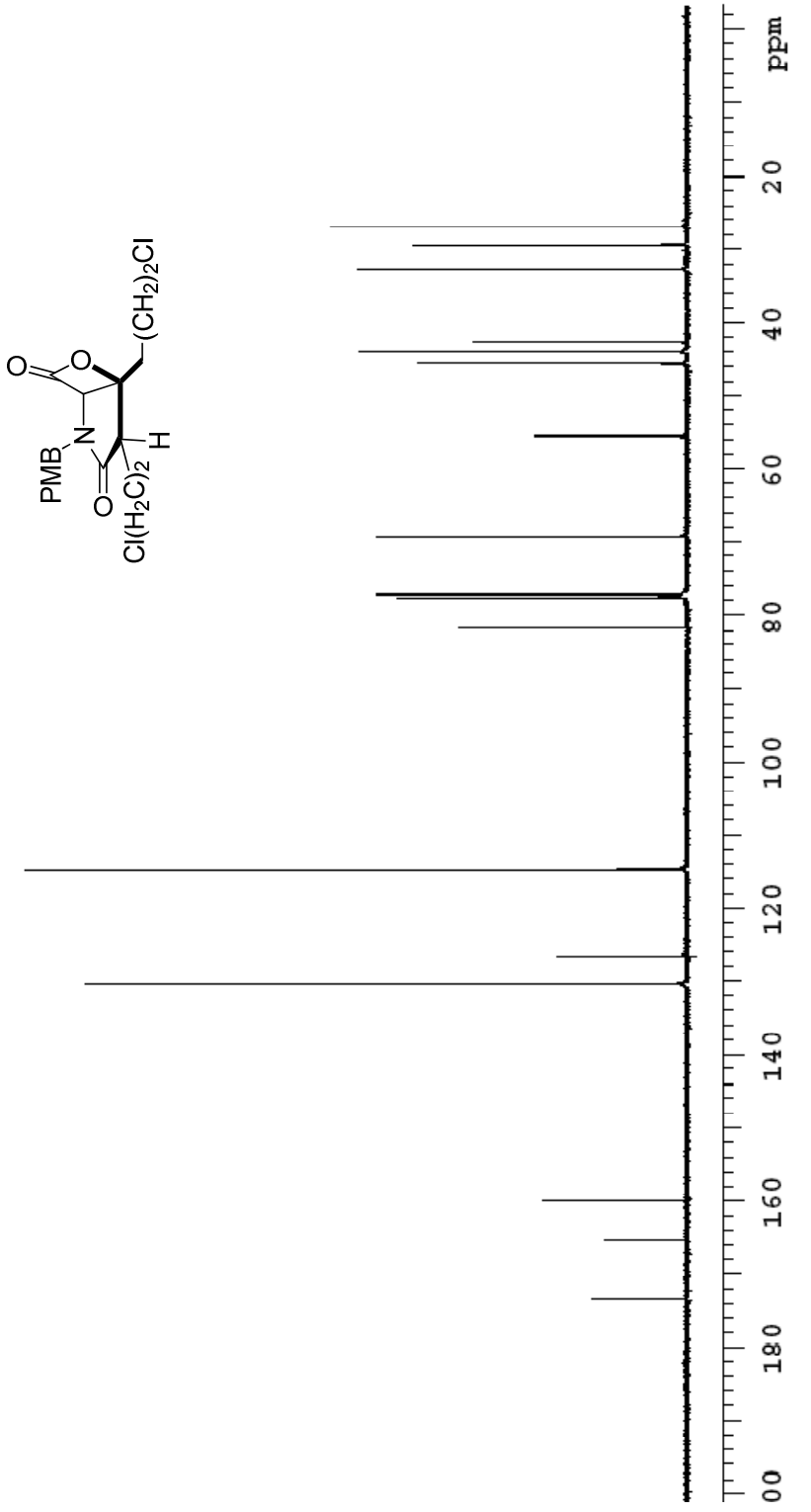
^{1}H NMR of PMB-β-lactone (±)-**23e** (500 MHz, $CDCl_3$)



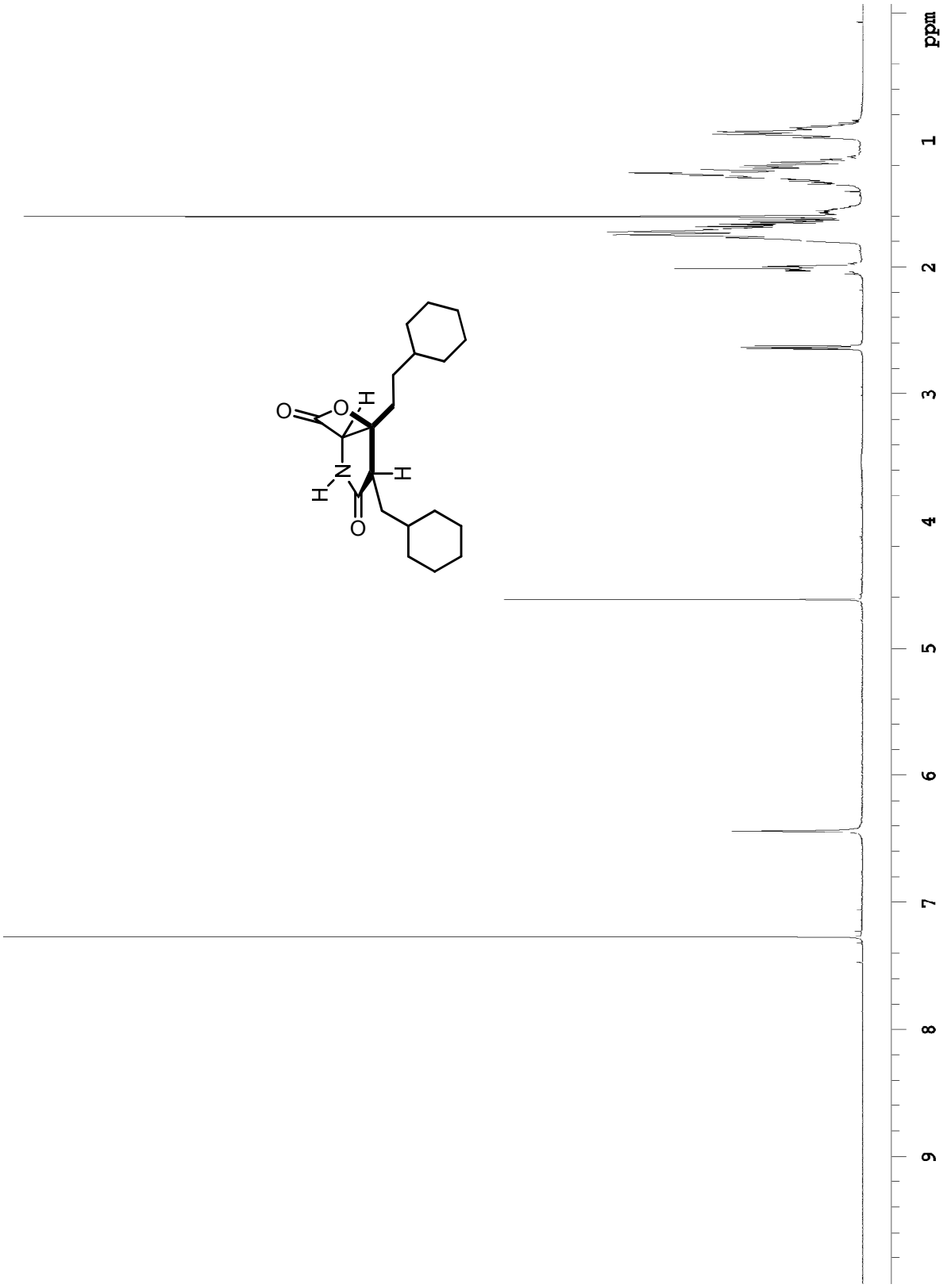
^{13}C NMR of β-lactone, (±)-**2.23e** (500 MHz, $CDCl_3$)



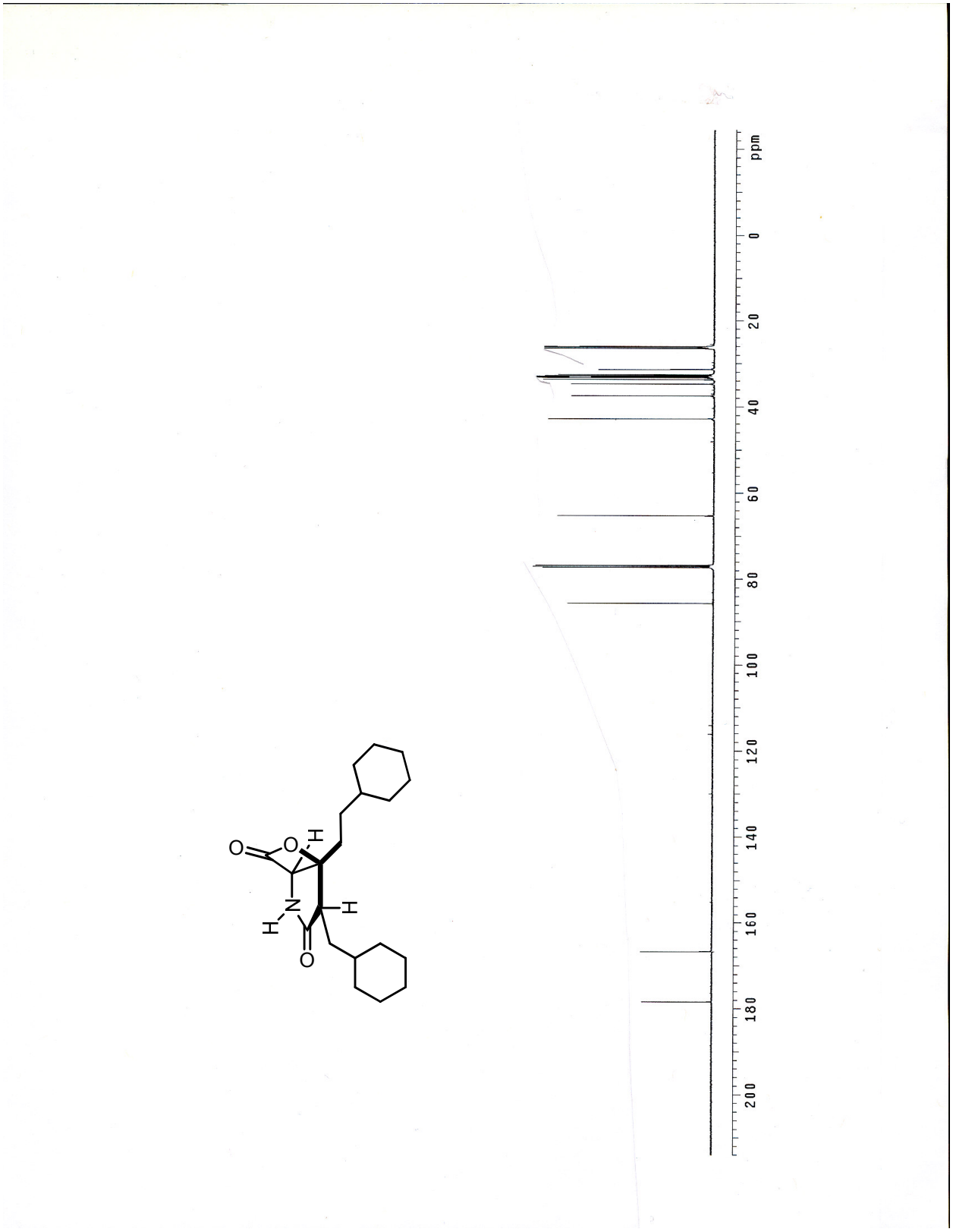
^{1}H NMR of PMB-β-lactone, (±)-**2.23d** (500 MHz, $CDCl_3$)



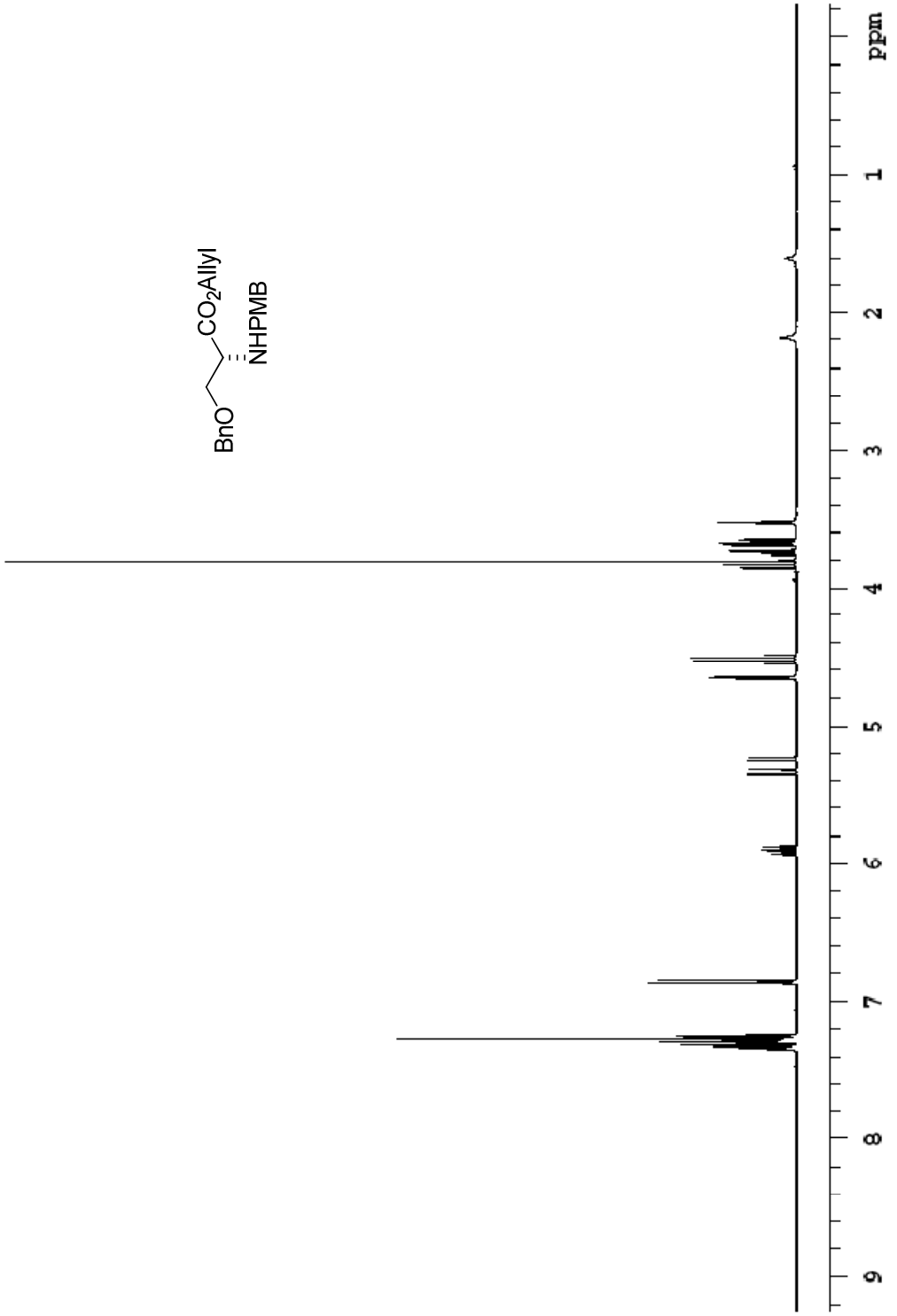
^{13}C NMR of PMB-β-lactone, (±)-**2.23d** (500 MHz, $CDCl_3$)



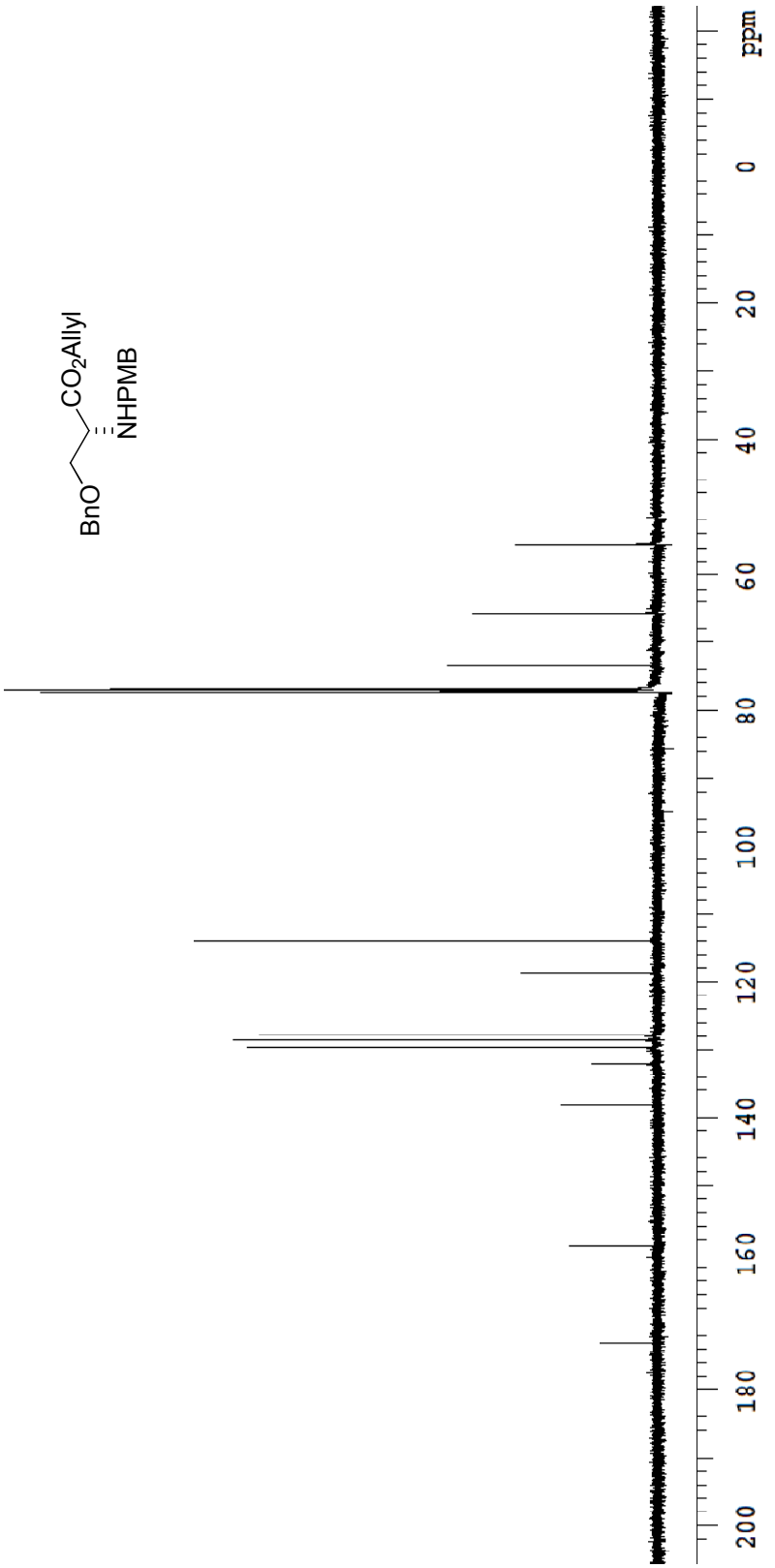
^{1}H NMR of β-lactone, (±)-**2.24a** (500 MHz, $CDCl_3$)



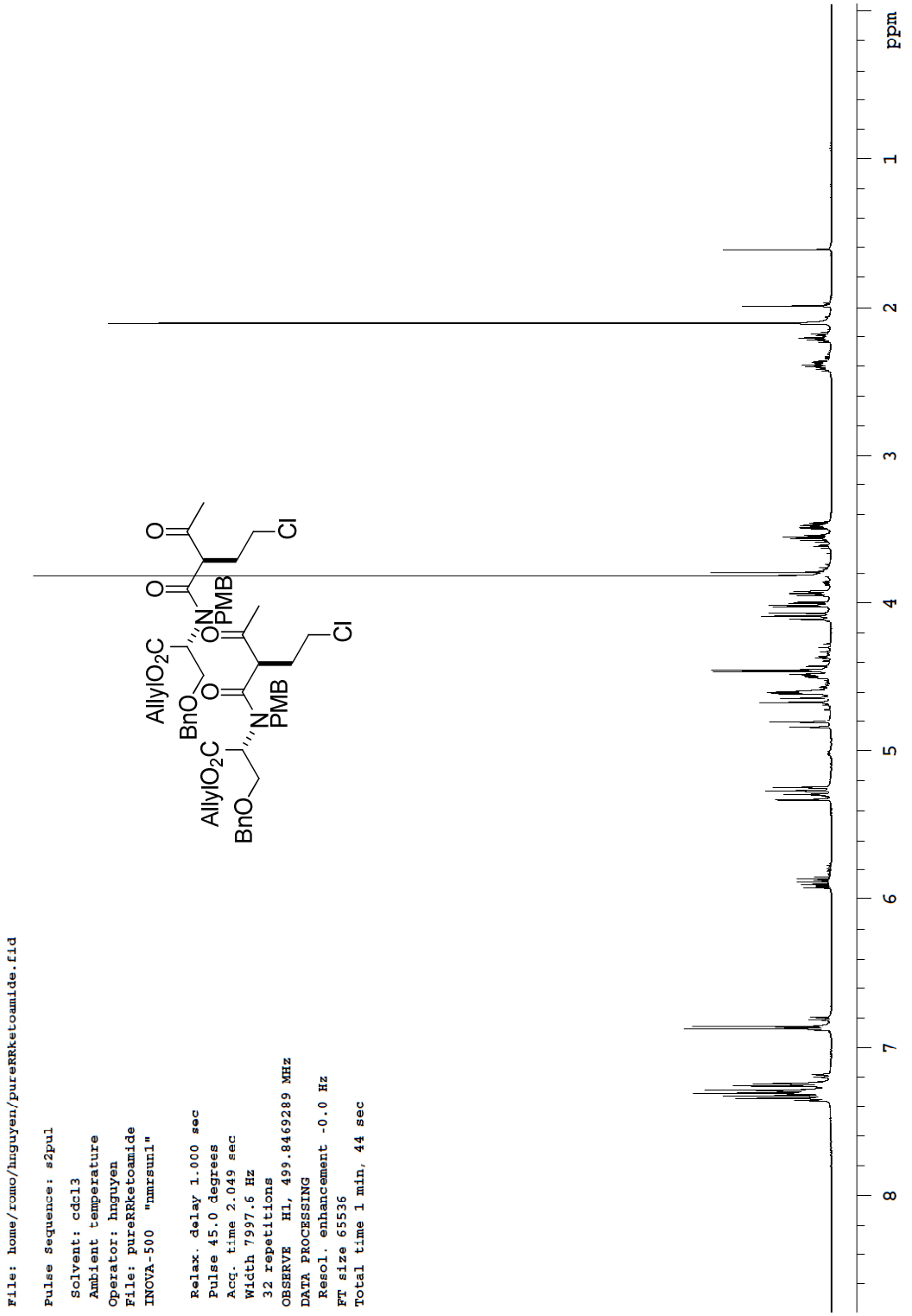
^{13}C NMR of β-lactone, (±)-**2.24a** (500 MHz, $CDCl_3$)



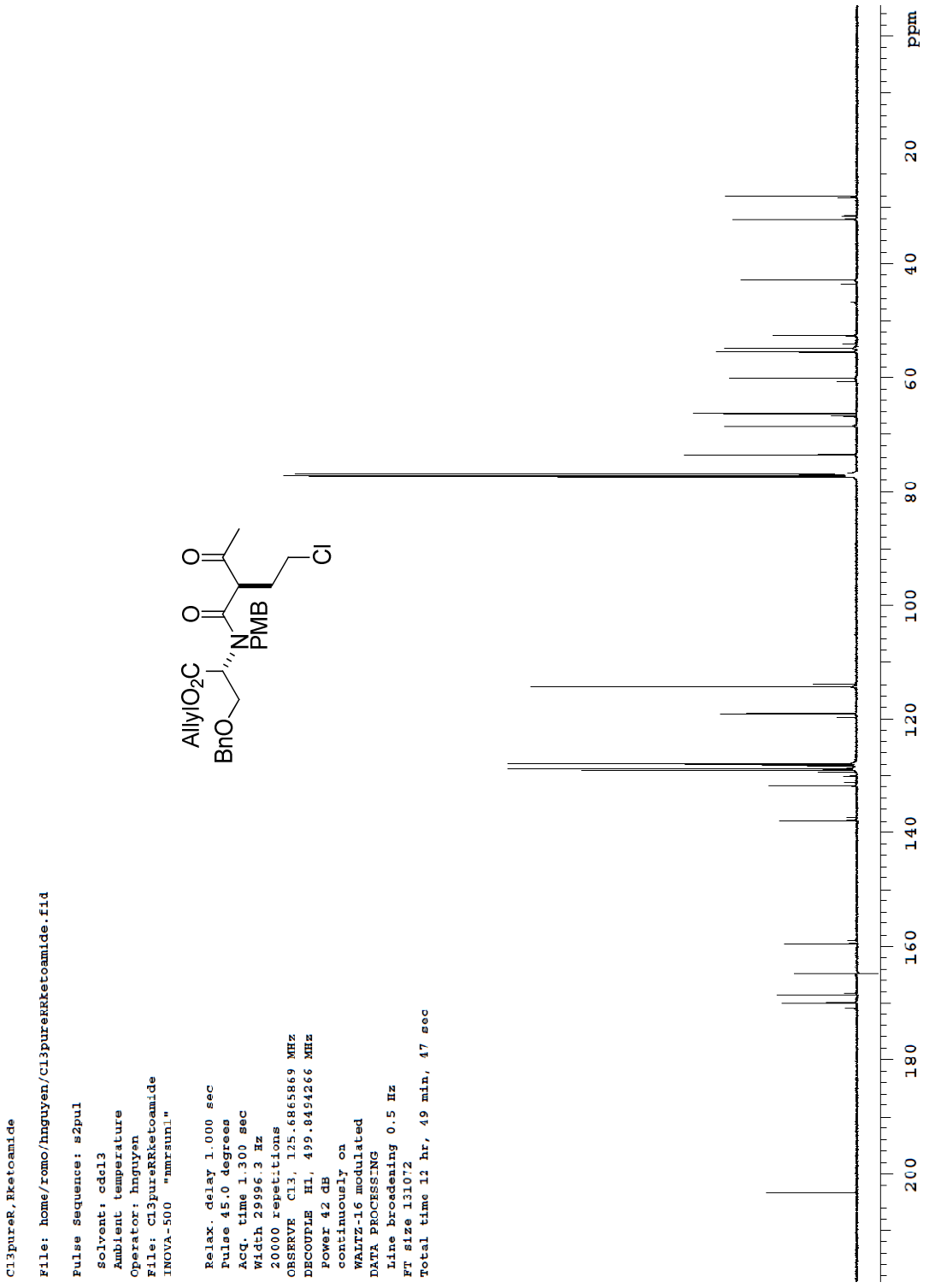
^{1}H NMR of (*R*)-O-benzyl serine allyl ester **2.34** (500 MHz, $CDCl_3$)



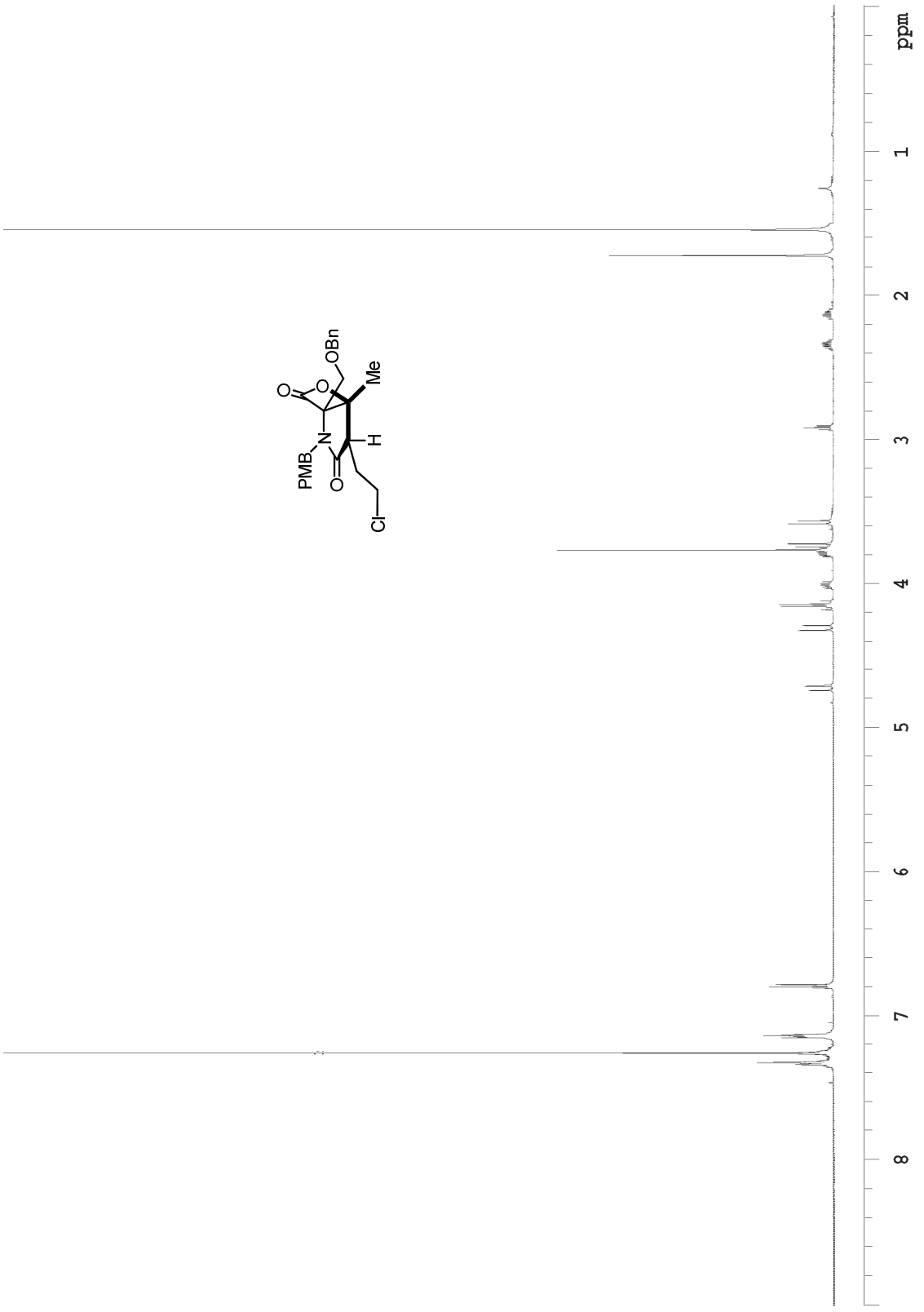
^{13}C NMR of (*R*)-O-benzyl serine allyl ester **2.34** (500 MHz, $CDCl_3$)



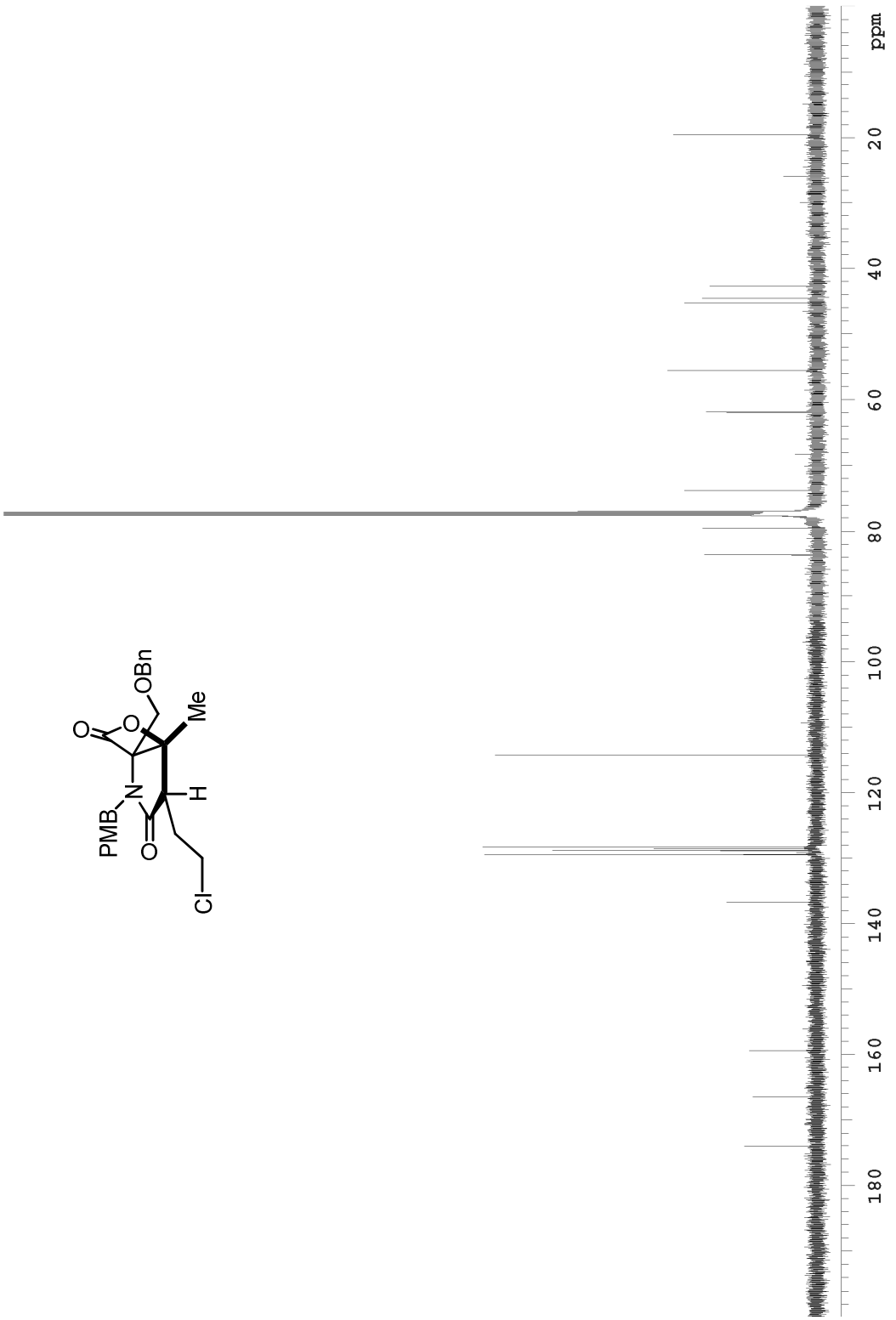
^{1}H NMR of ketoamide (2*R*,4*R*)-**3.6a** (500 MHz, $CDCl_3$)



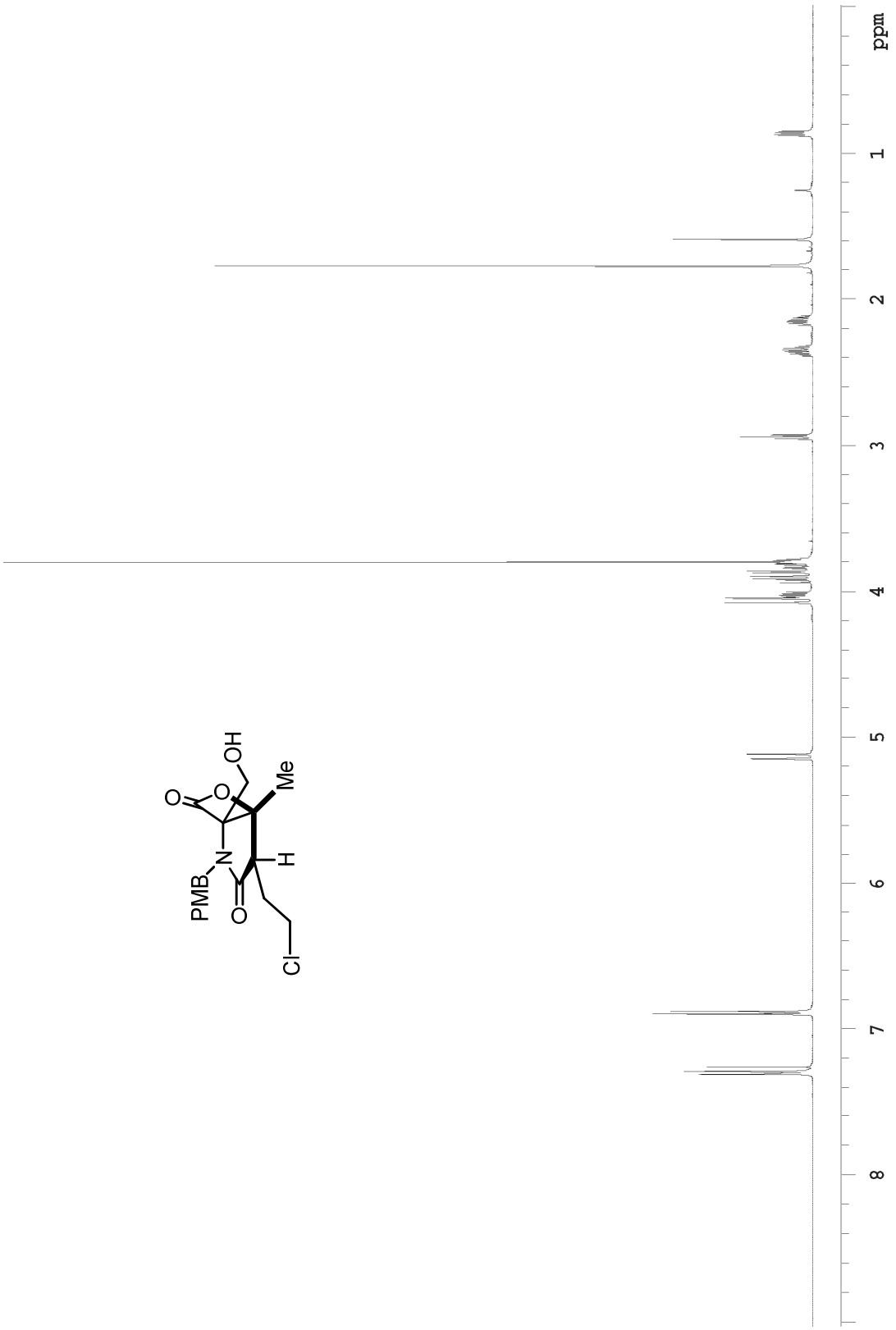
^{13}C NMR of ketoamide (2*R*,4*R*)-**3.6a** (500 MHz, $CDCl_3$)



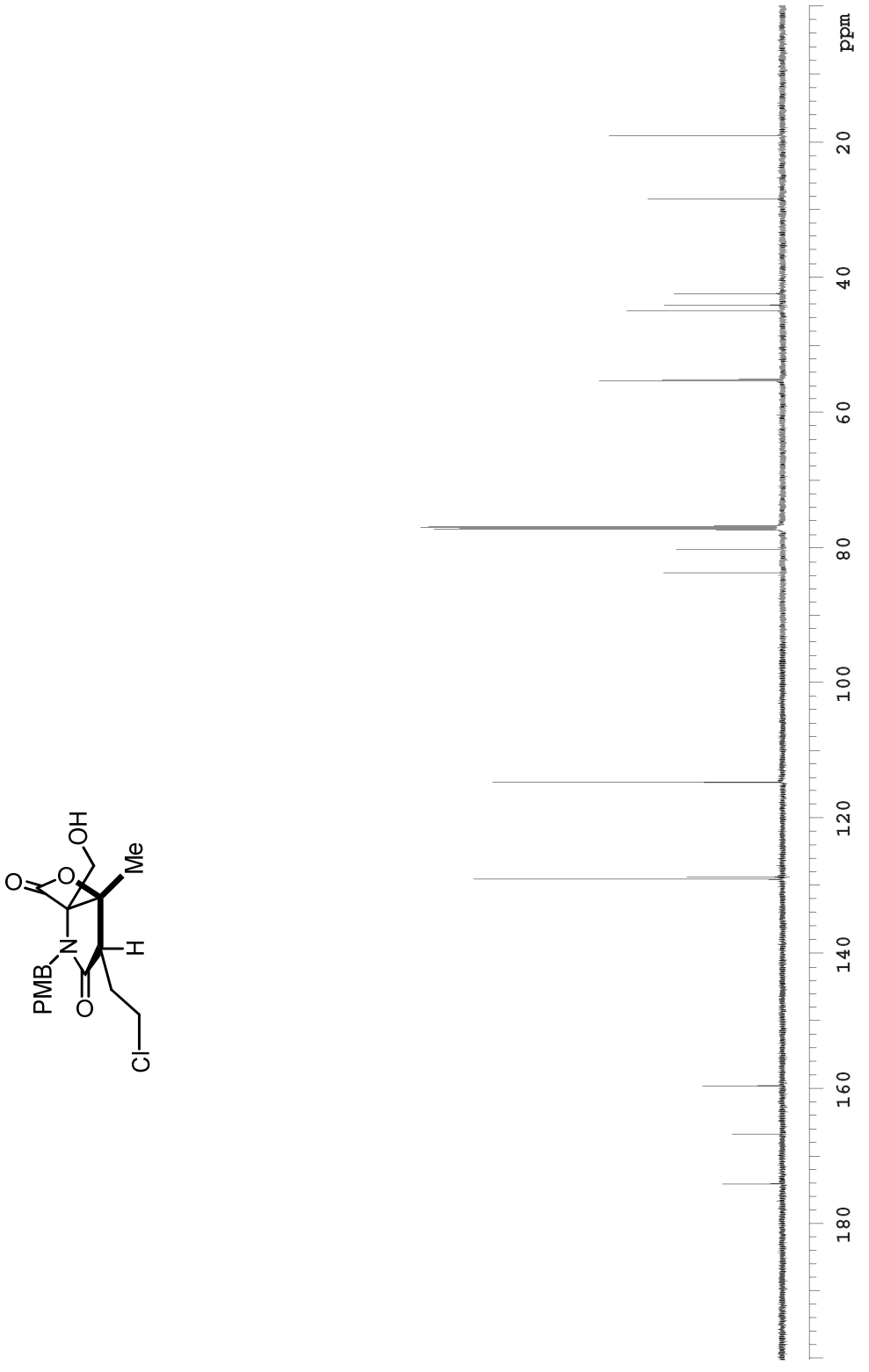
^{1}H NMR of OBn-β-lactone (–)-**2.37a** (500 MHz, $CDCl_3$)



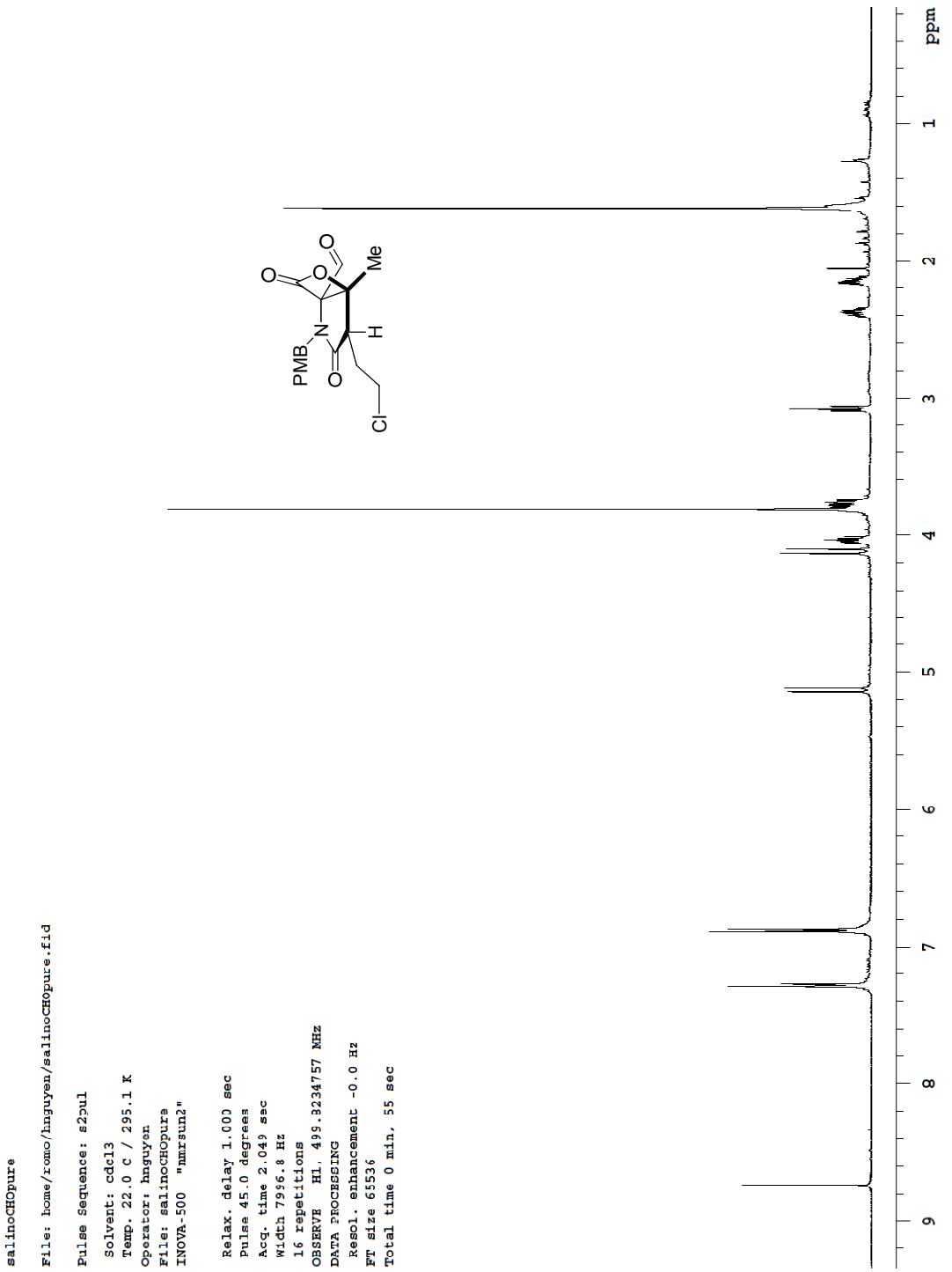
^{13}C NMR of OBn-β-lactone (–)-**2.37a** (500 MHz, $CDCl_3$)



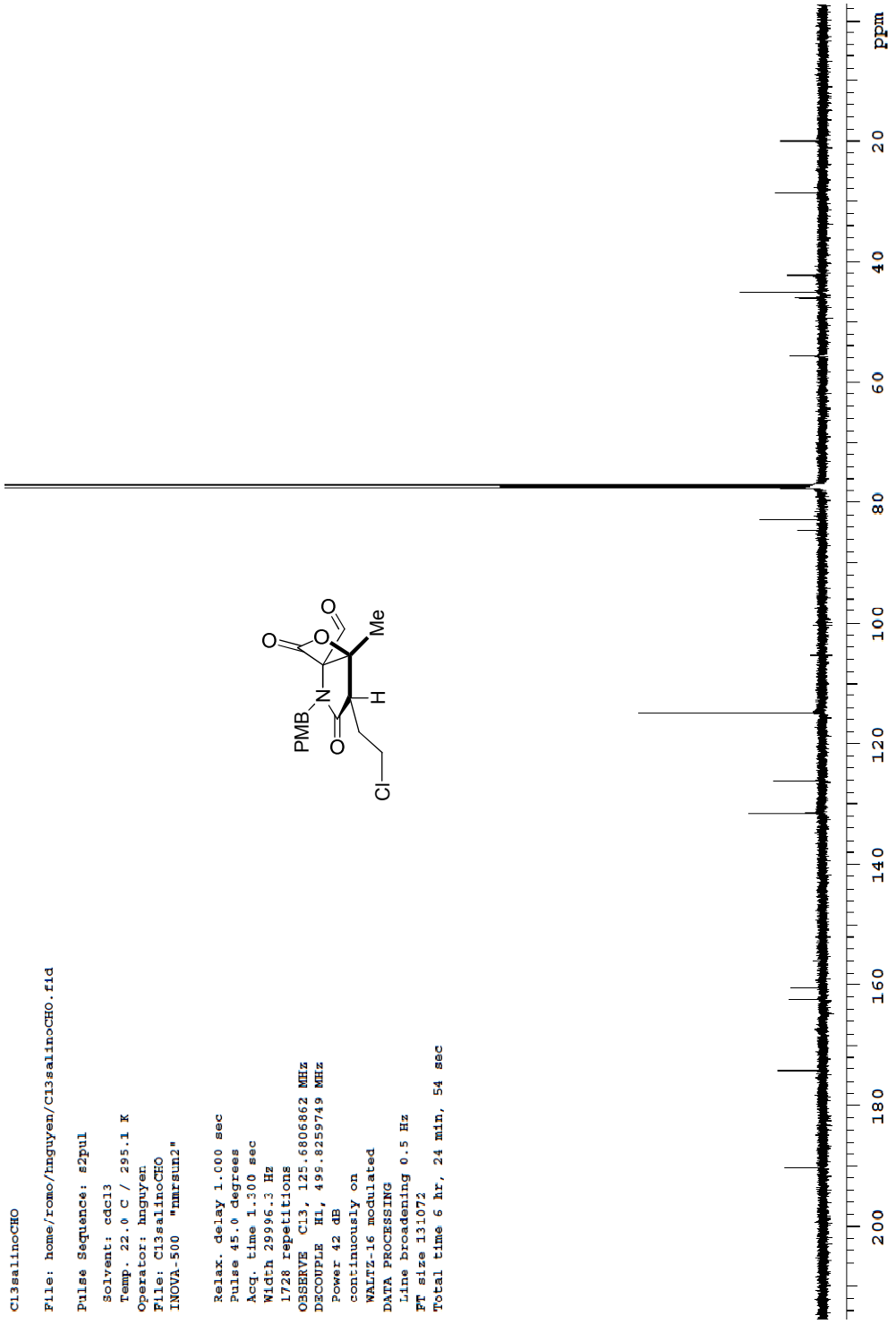
^{1}H NMR of OH-β-lactone (–)-**2.38a** (500 MHz, $CDCl_3$)



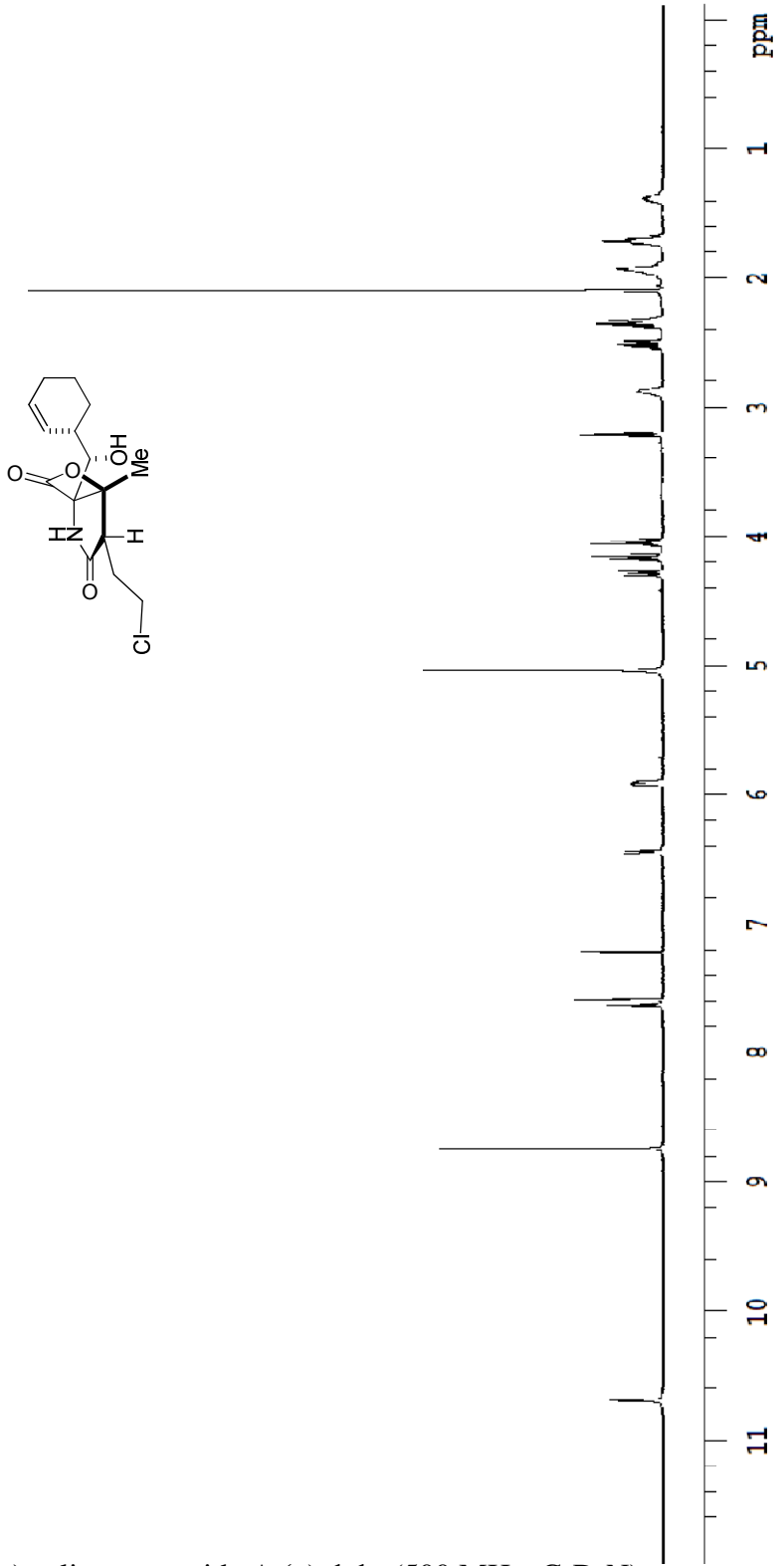
^{13}C NMR of OH-β-lactone (–)-**2.38a** (500 MHz, $CDCl_3$)



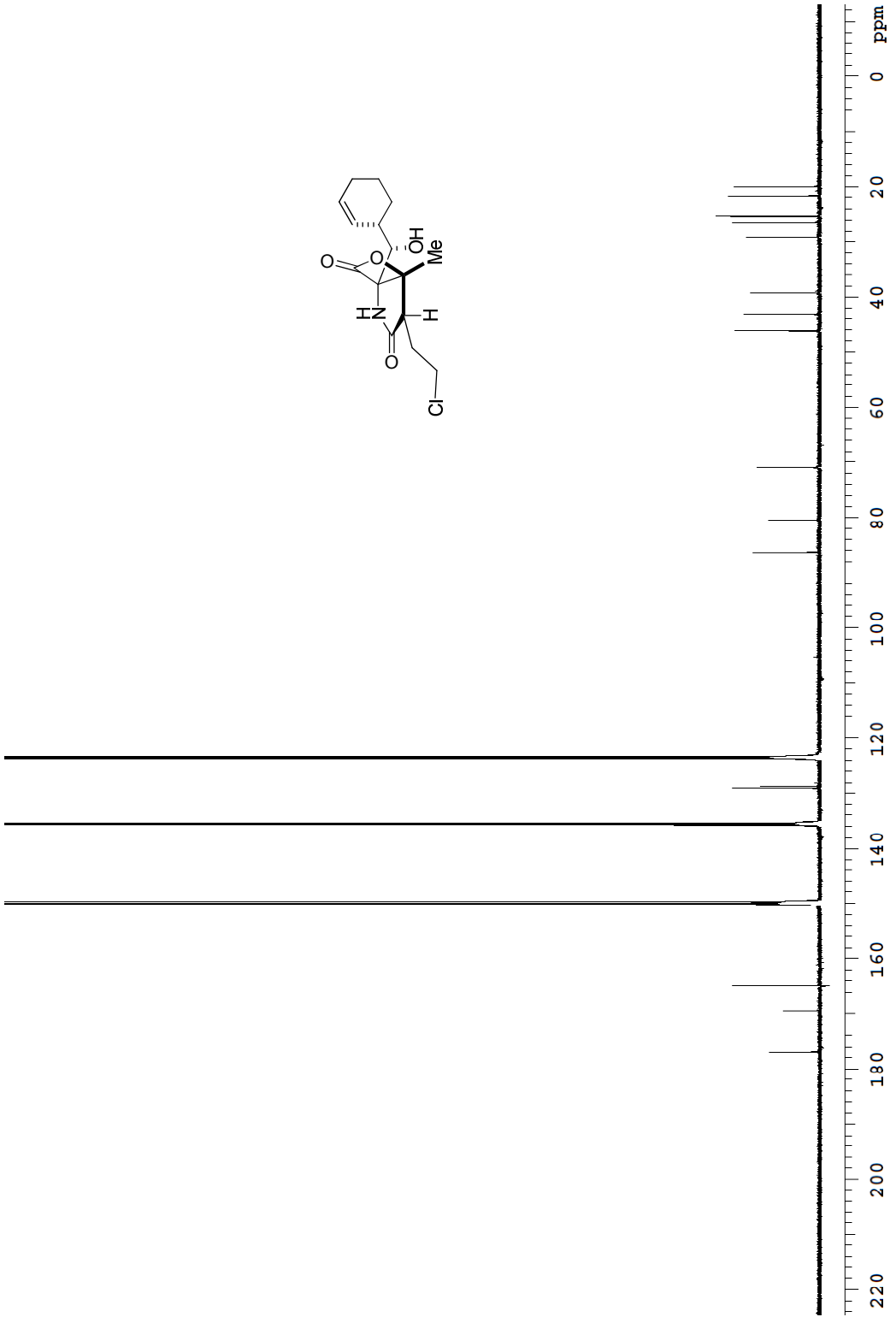
^{1}H NMR of aldehyde (–)-**4.22** (500 MHz, $CDCl_3$)



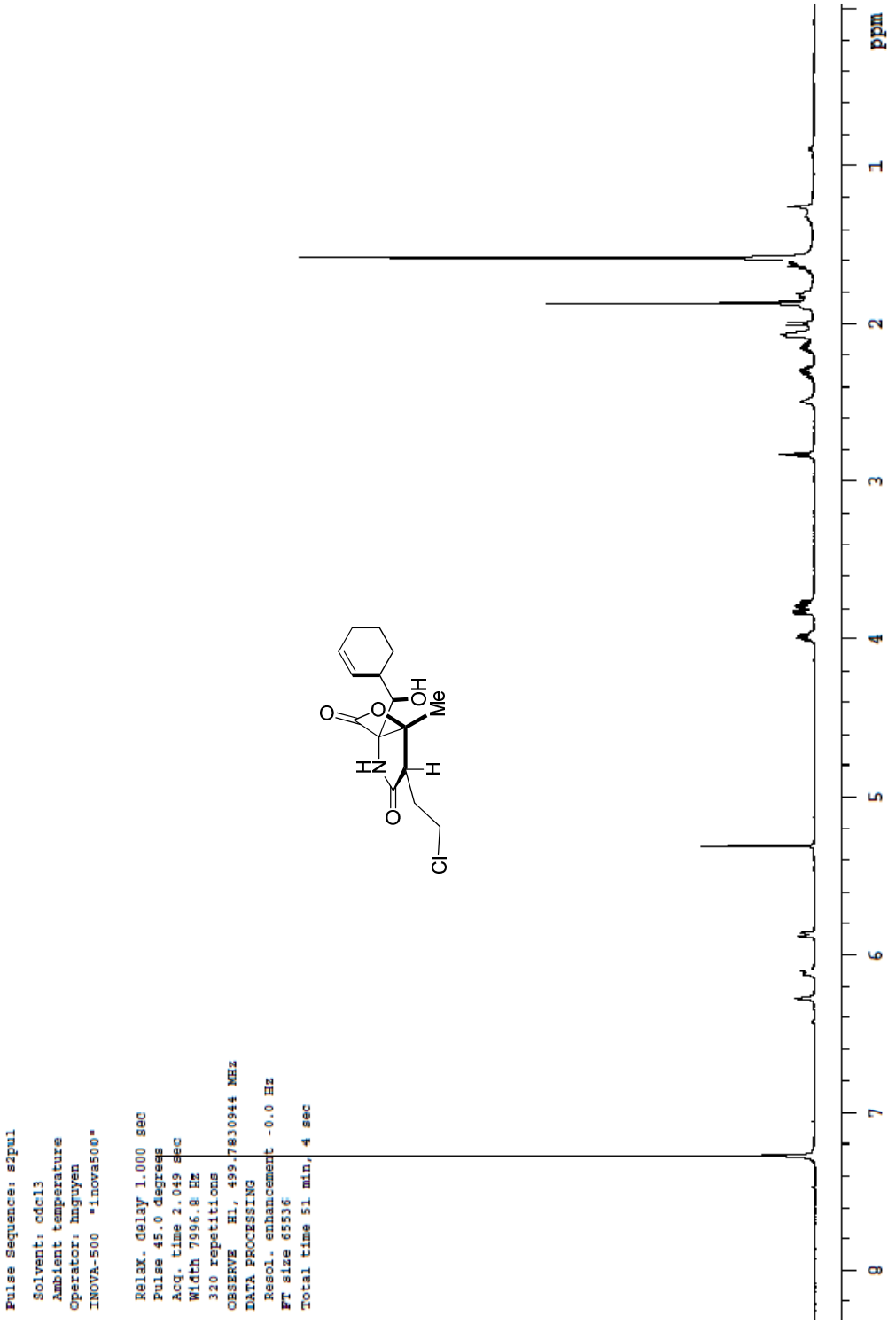
^{13}C NMR of aldehyde (–)-**2.44** (500 MHz, $CDCl_3$)



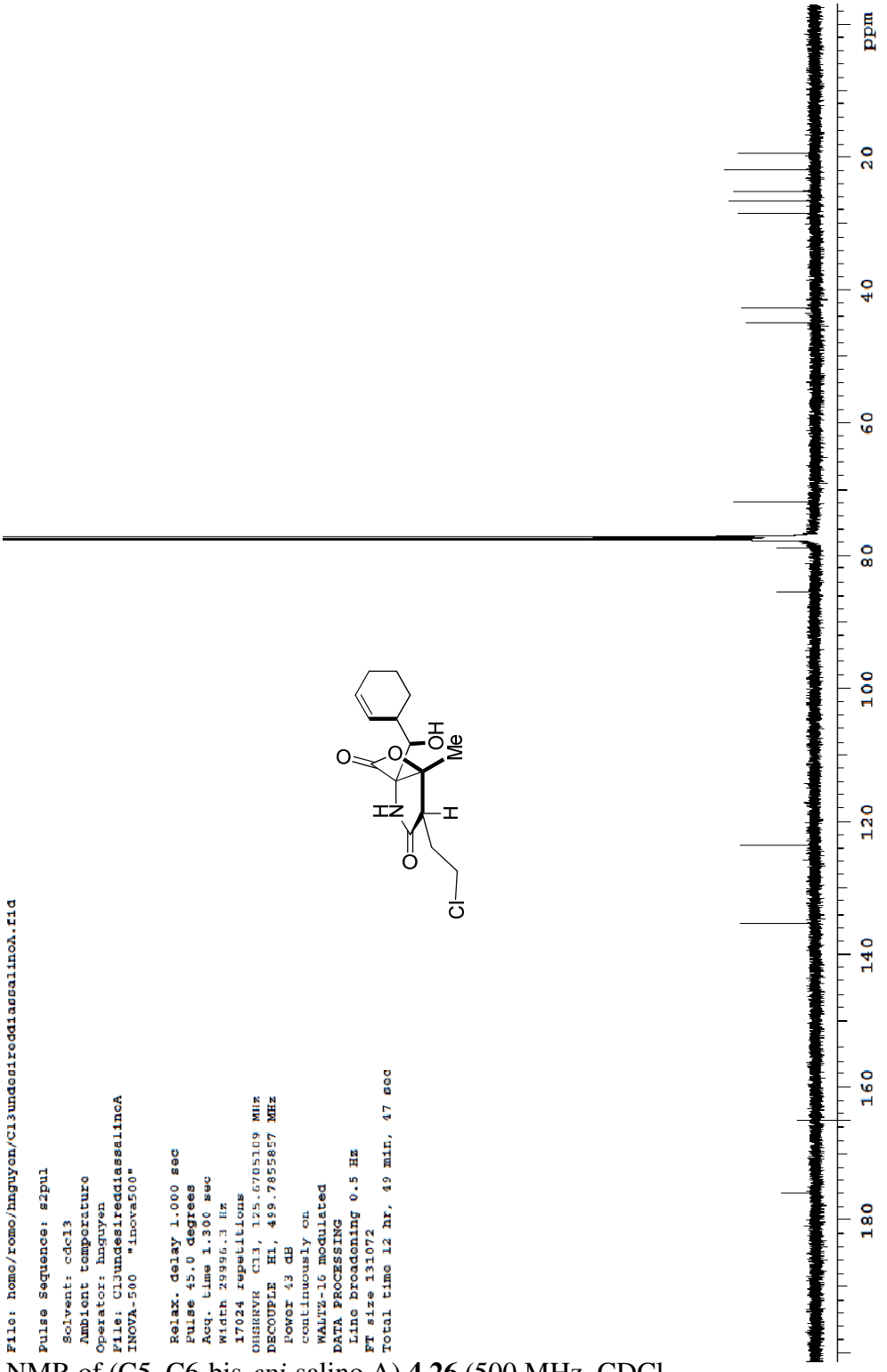
^{1}H NMR of (–)-salinosporamide A (–)-**1.1a** (500 MHz, C_5D_5N)



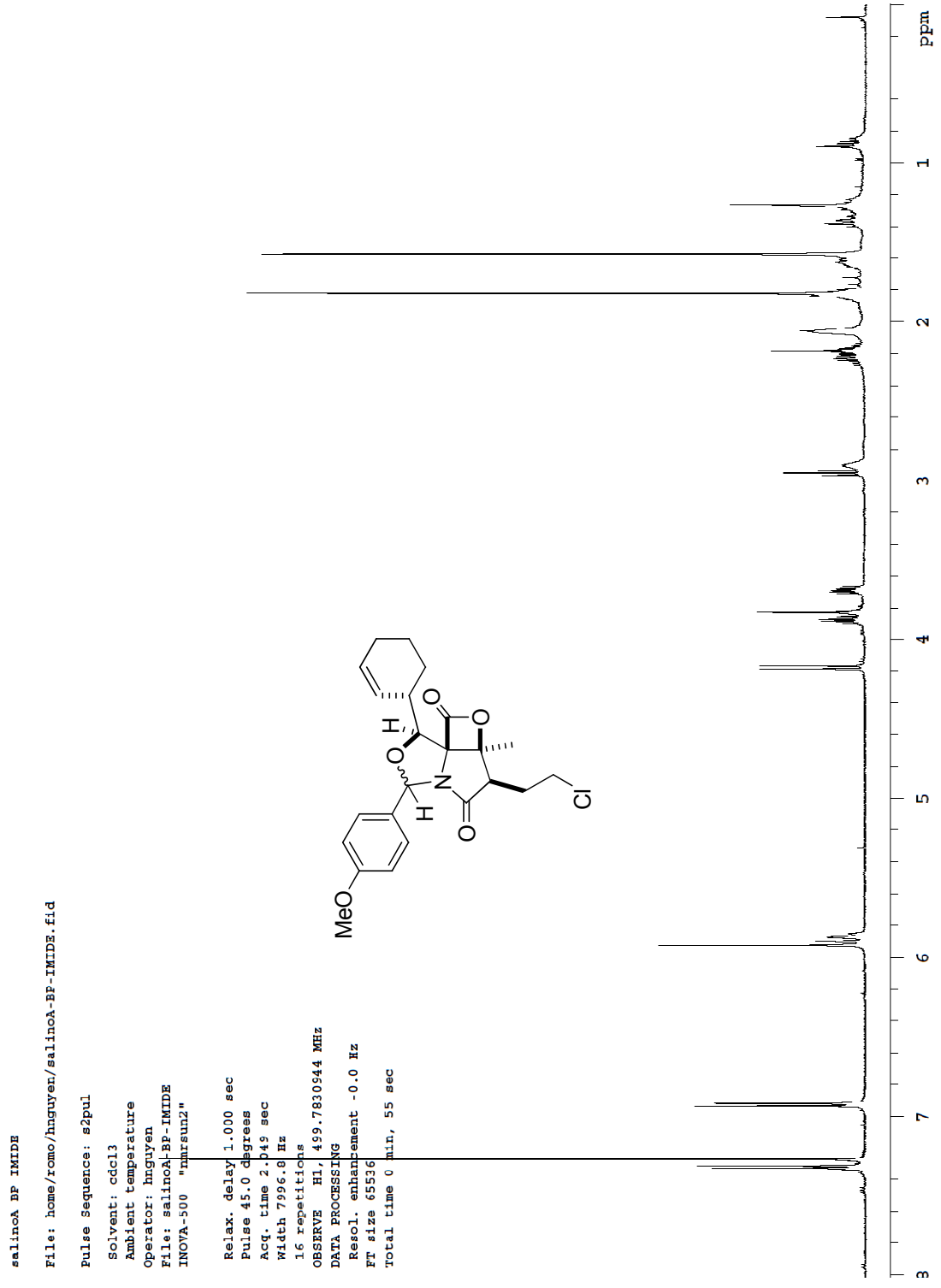
^{13}C NMR of (–)-salinosporamide A (–)-**1.1a** (500 MHz, C_5D_5N)



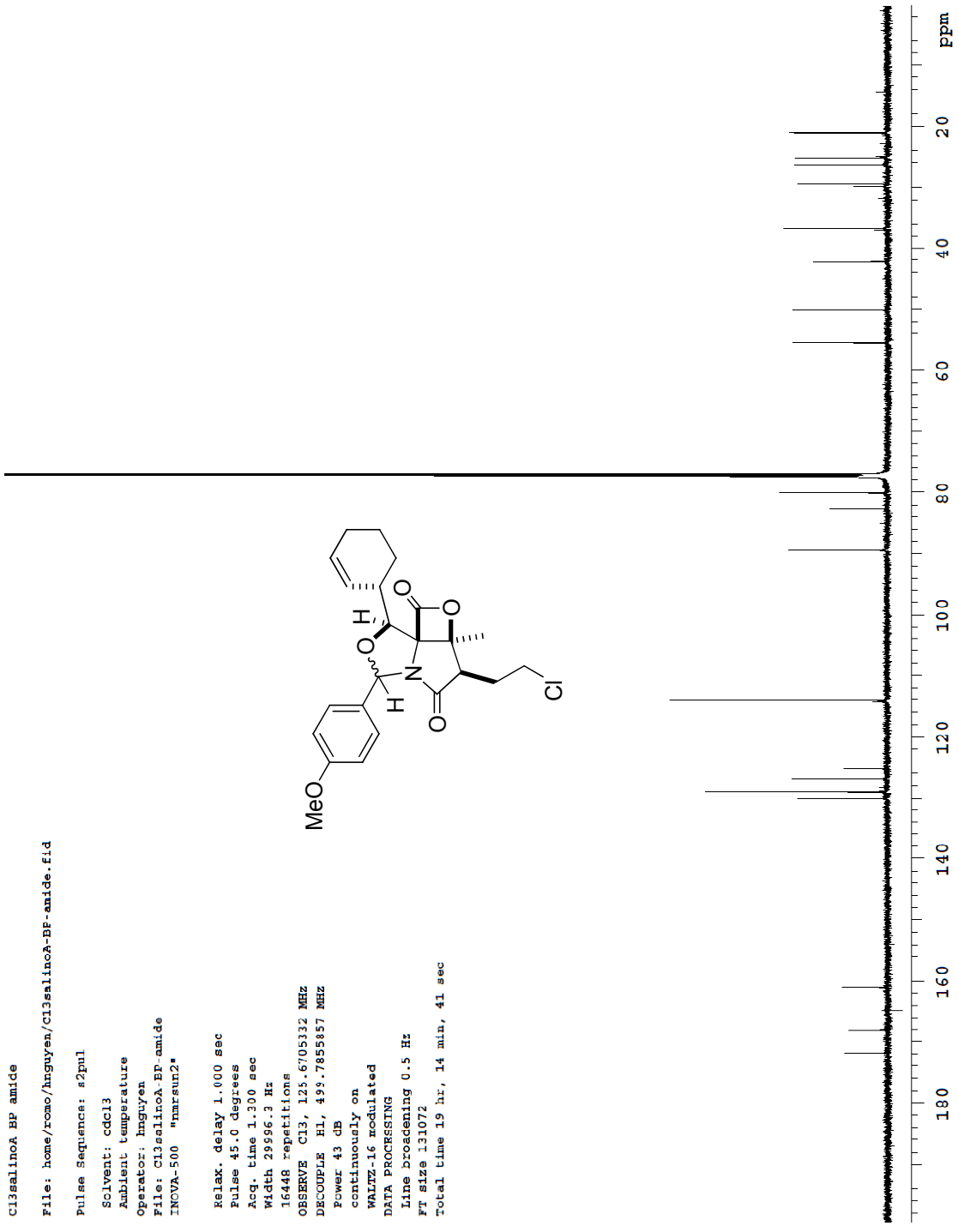
^{1}H NMR of (C5, C6-bis-*epi*-salino A) **4.26** (500 MHz, $CDCl_3$)



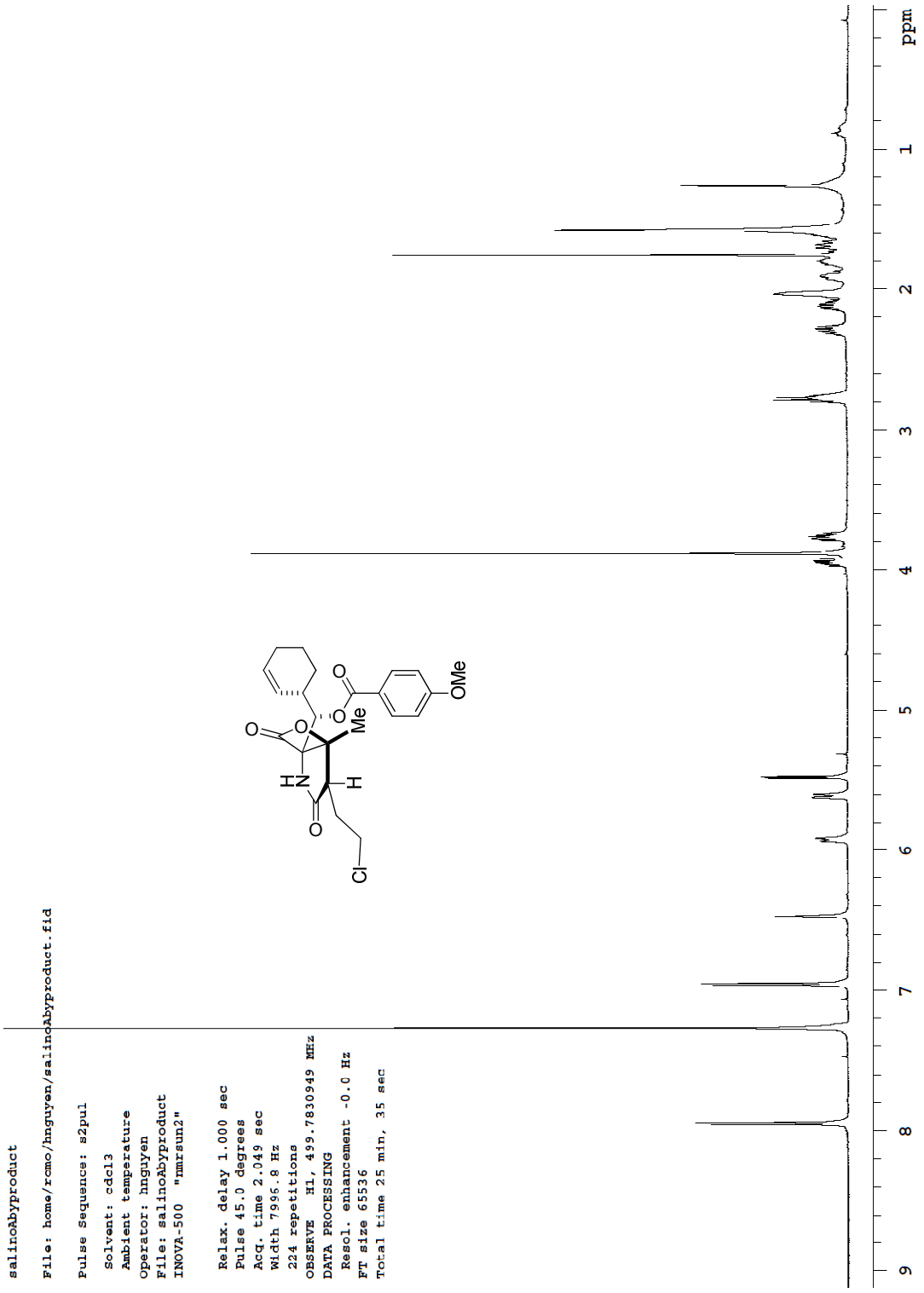
^{13}C NMR of (C5, C6-bis-*epi*-salino A) **4.26** (500 MHz, $CDCl_3$



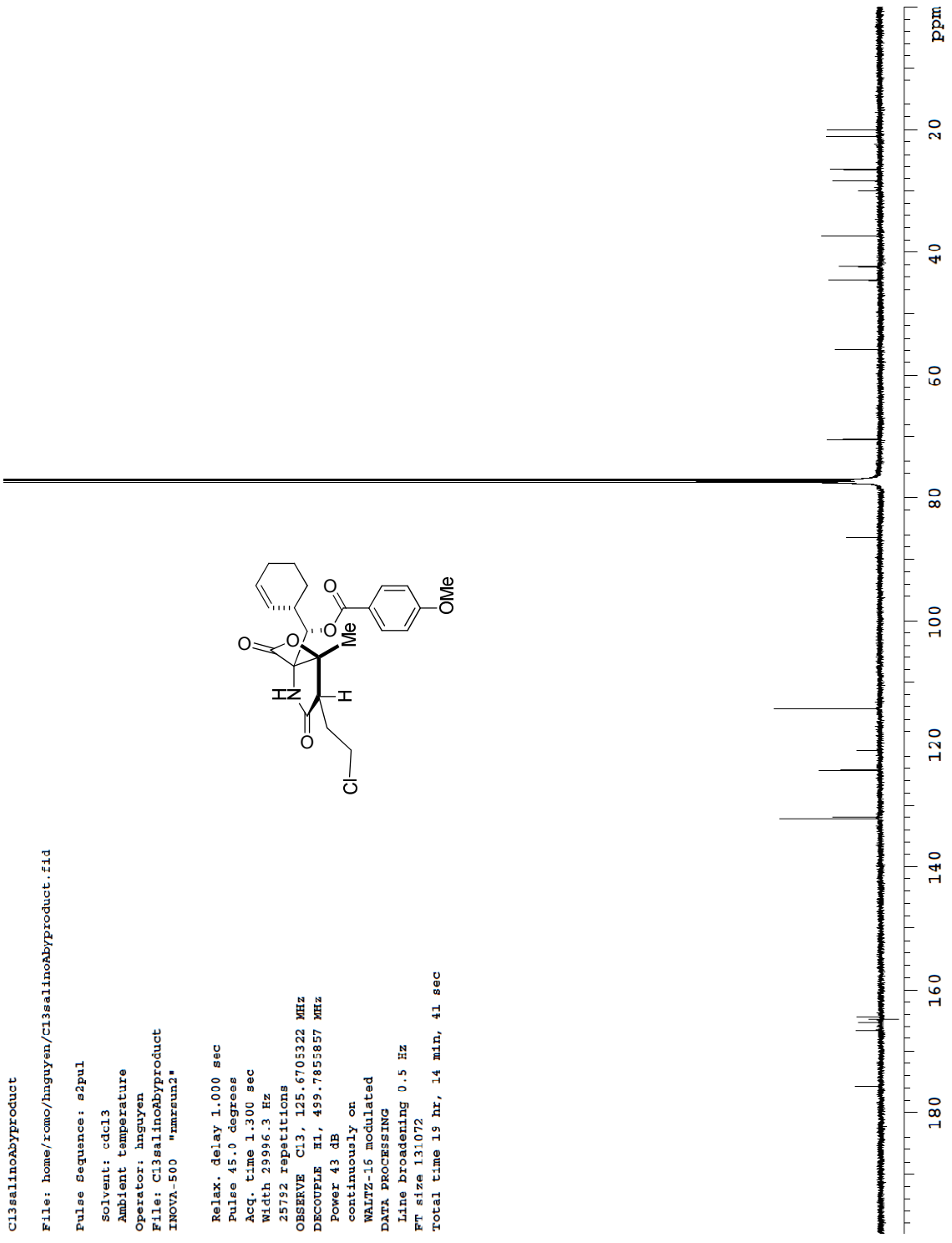
^{1}H NMR of oxazolidine by-product **4.25** (500 MHz, $CDCl_3$)



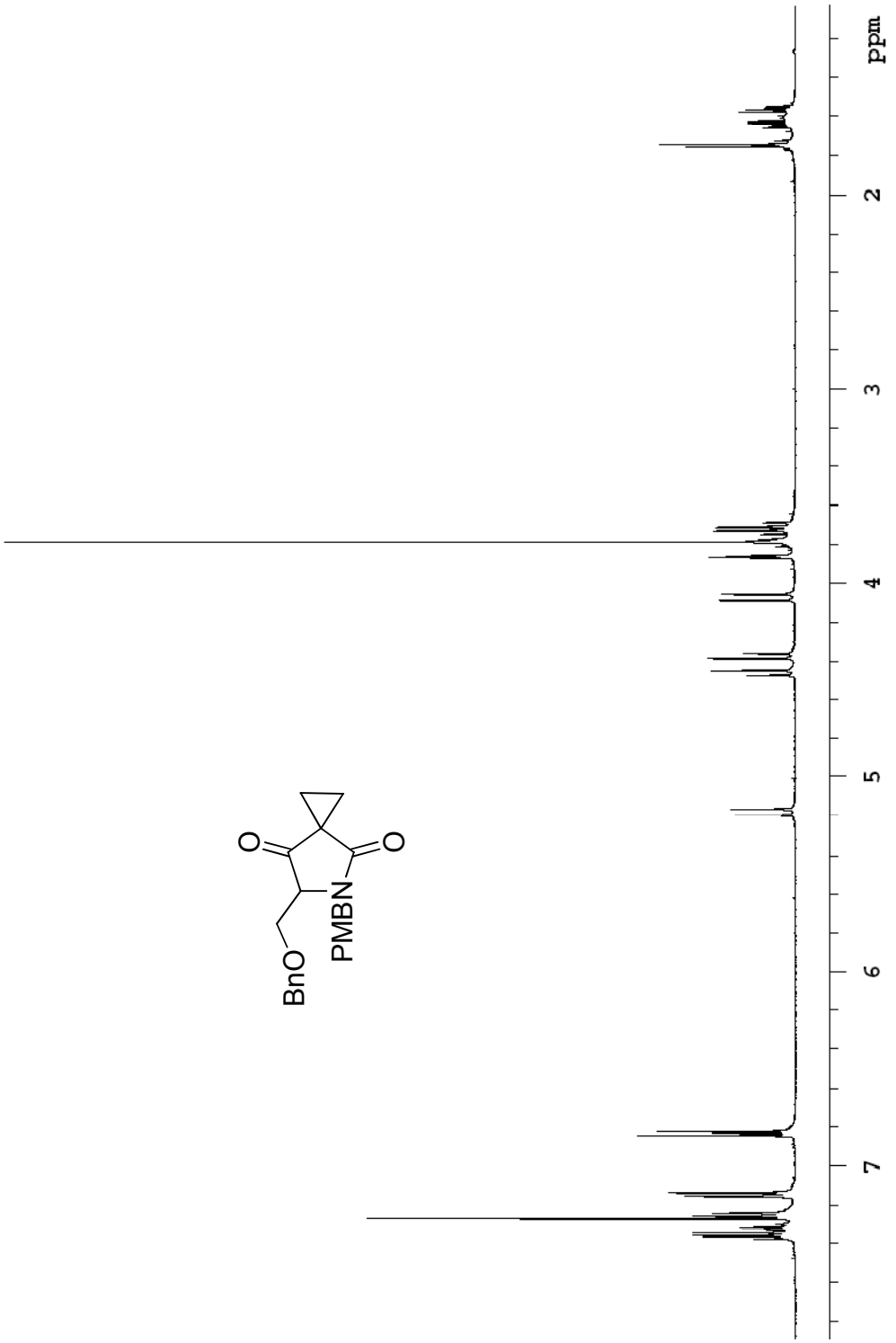
^{13}C NMR of oxazolidine by-product **4.25** (500 MHz, $CDCl_3$)



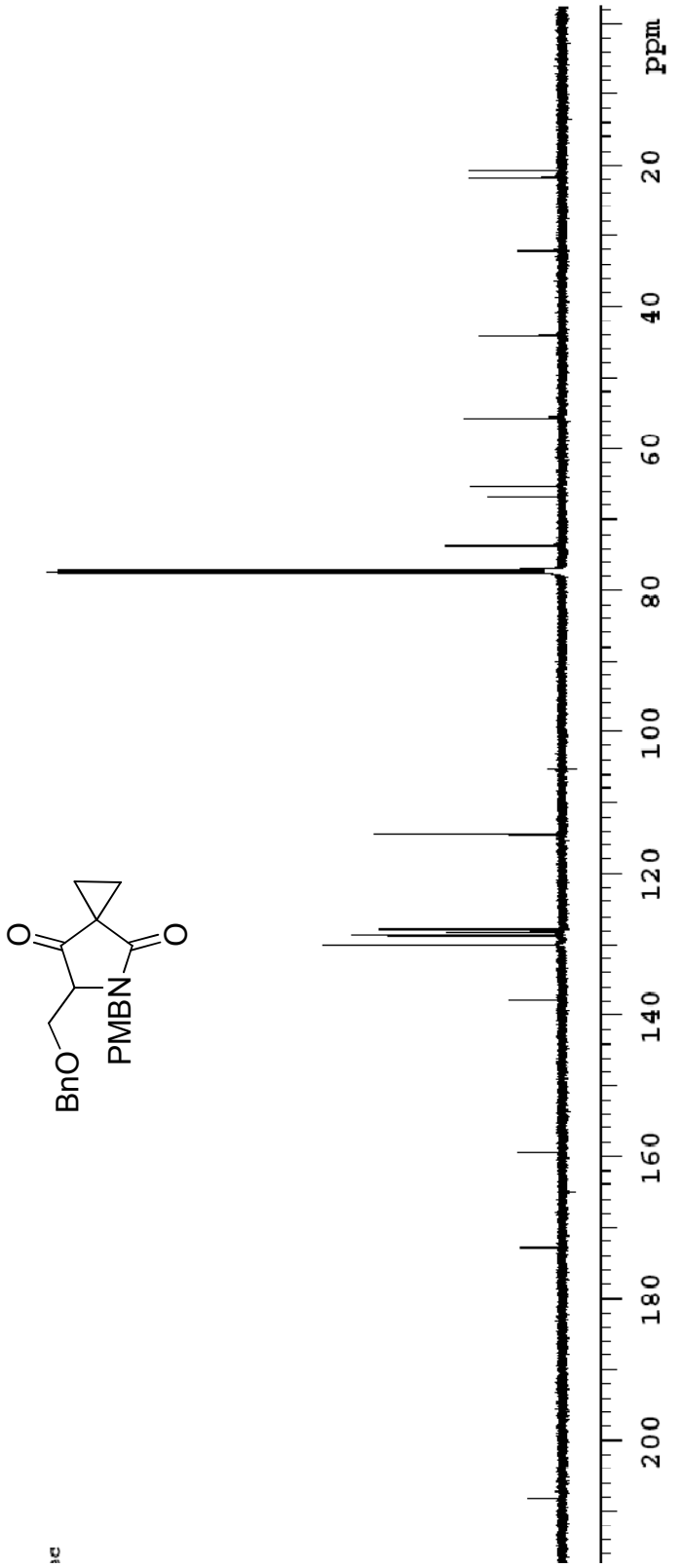
^{1}H NMR of salino ester by-product **2.40** (500 MHz, $CDCl_3$)



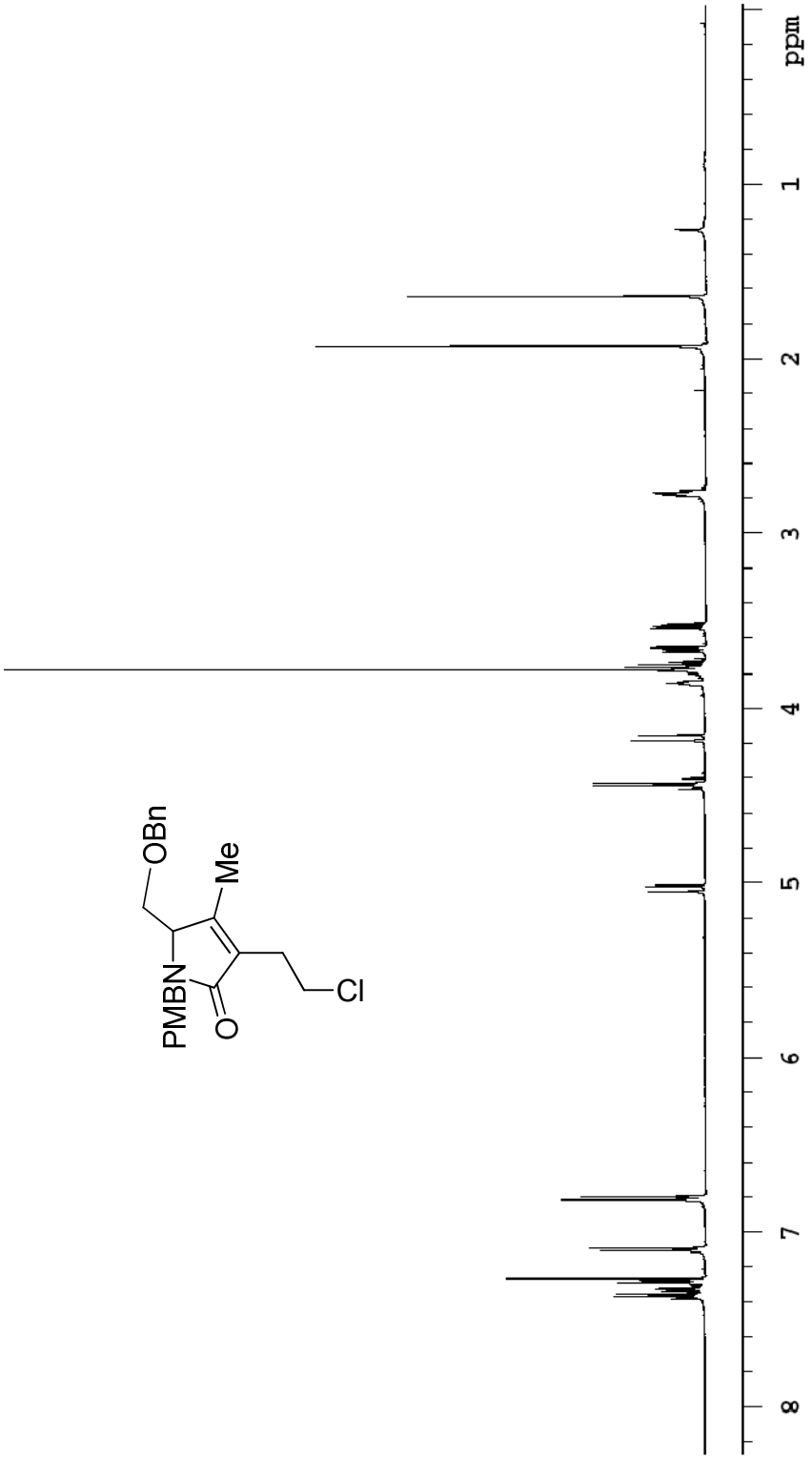
^{13}C NMR of salino ester by-product **2.40** (500 MHz, $CDCl_3$)



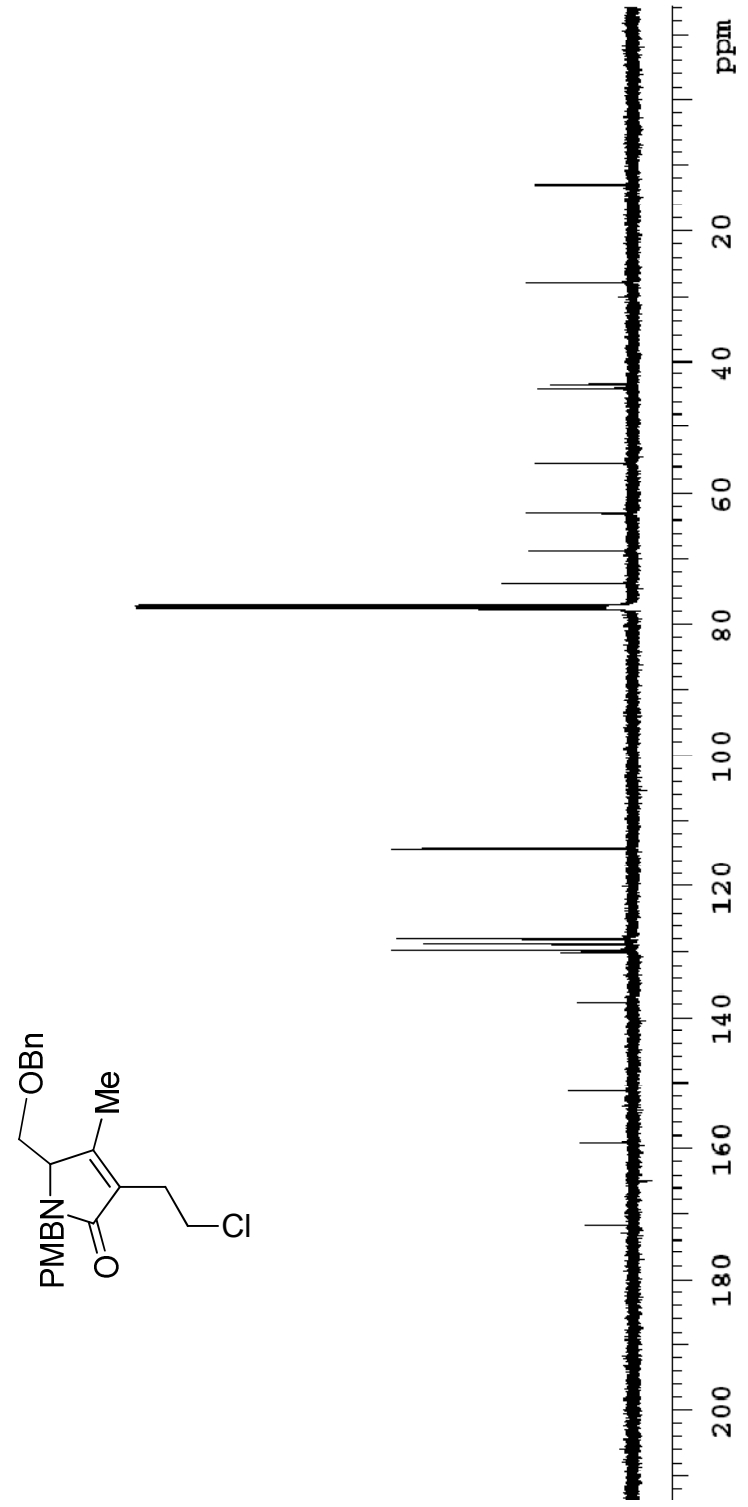
^{1}H NMR of cyclopropane by-product **3.7** (500 MHz, $CDCl_3$)



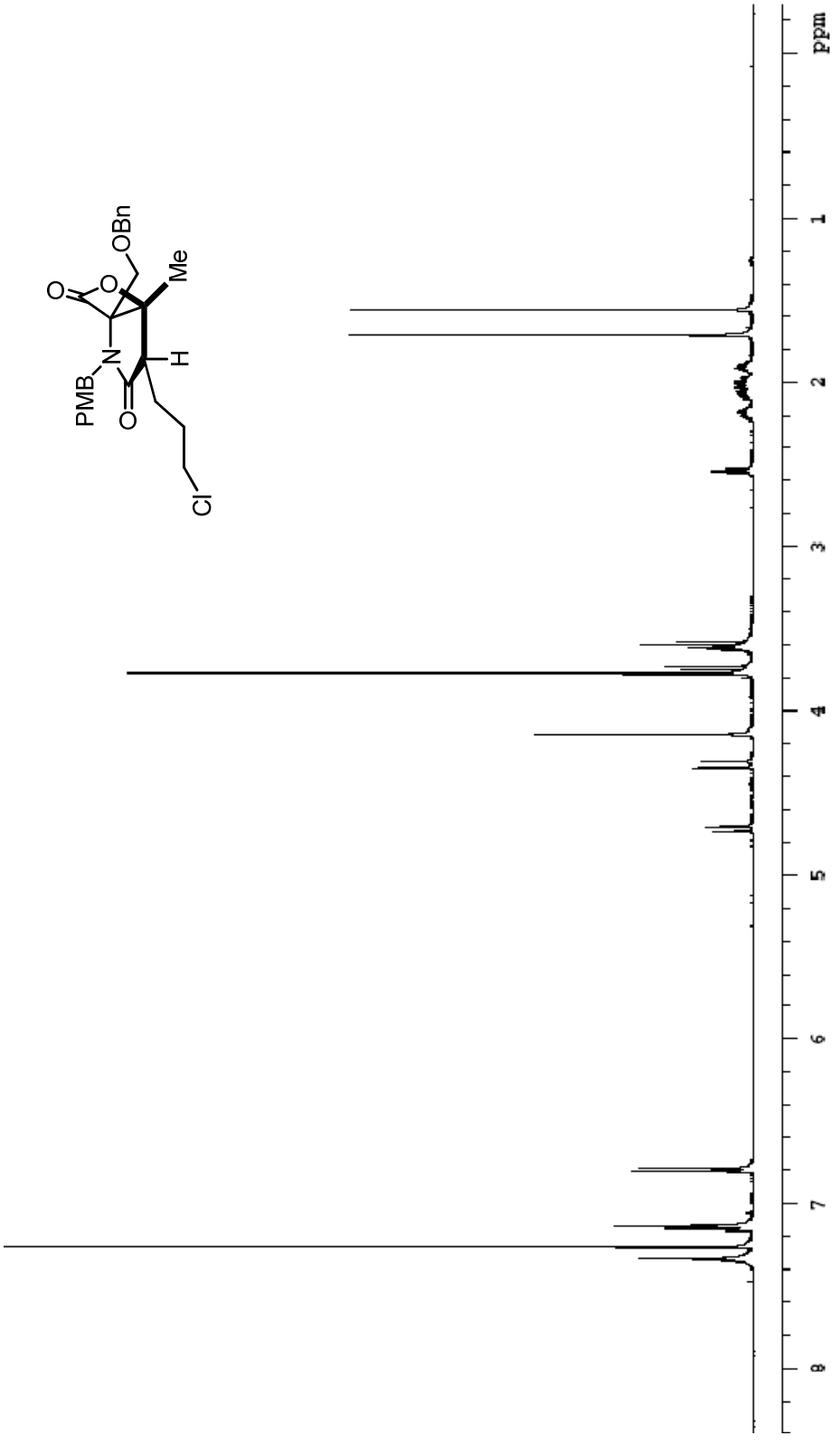
^{13}C NMR of cyclopropane by-product **3.7** (500 MHz, $CDCl_3$)



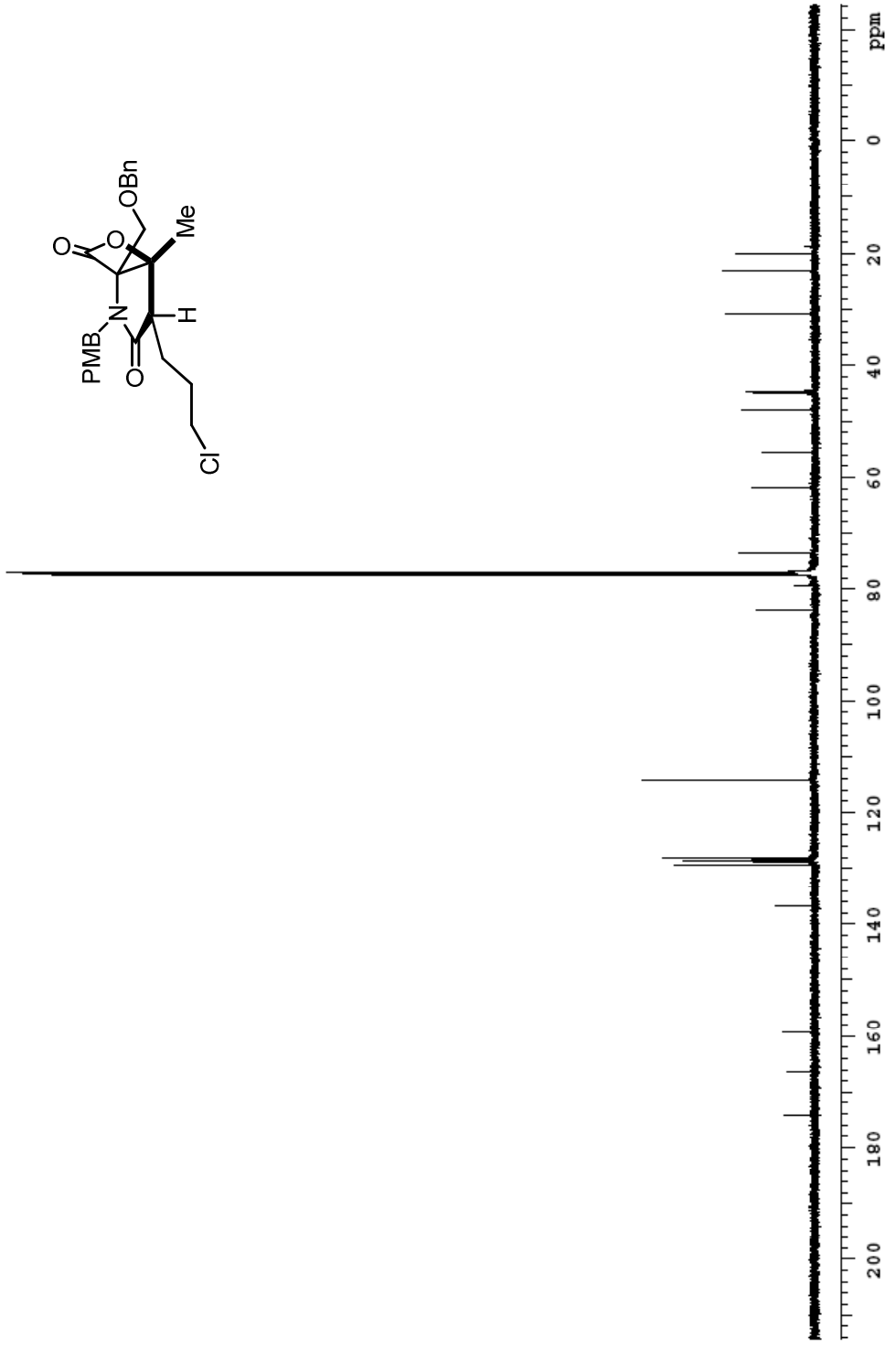
^{1}H NMR of unsaturated lactam **4.13** (500 MHz, $CDCl_3$)



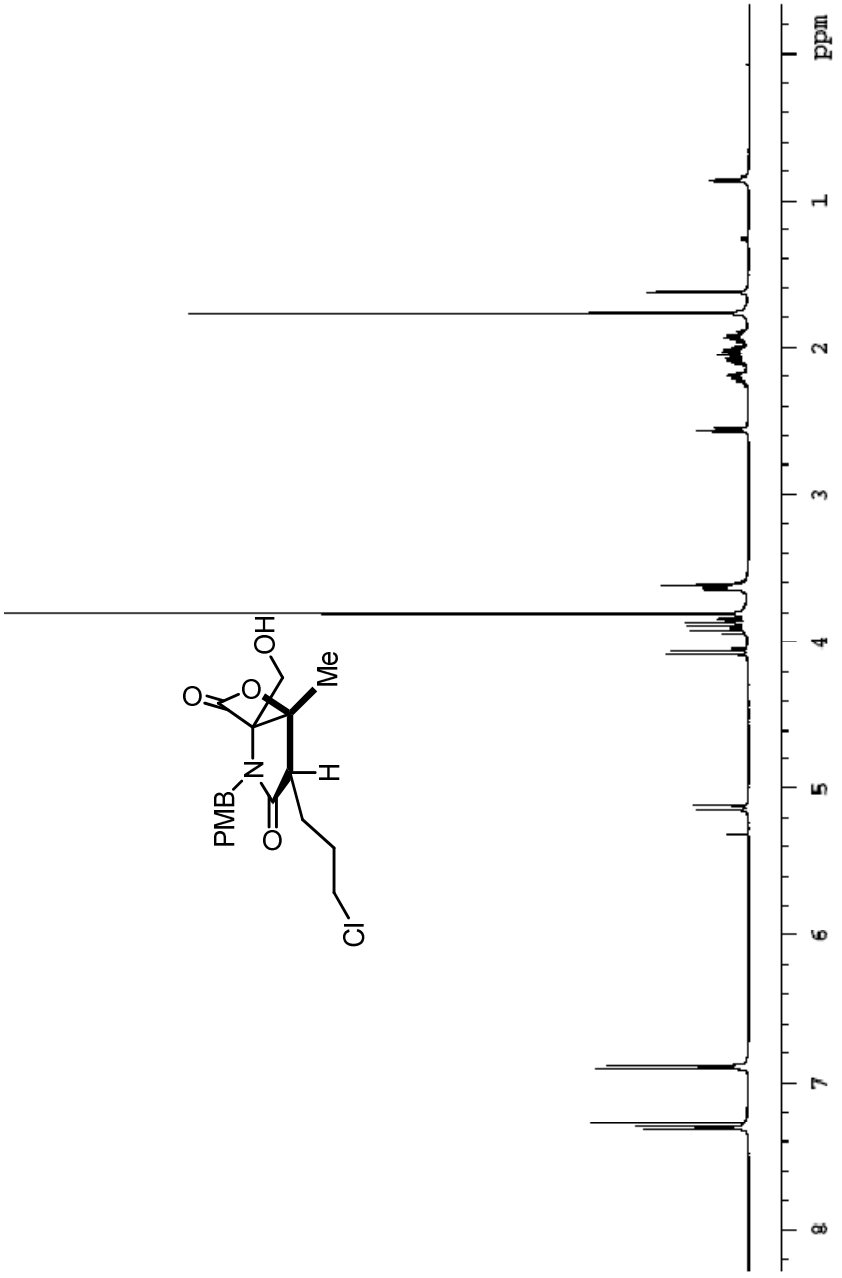
^{13}C NMR of unsaturated lactam **4.13** (500 MHz, $CDCl_3$)



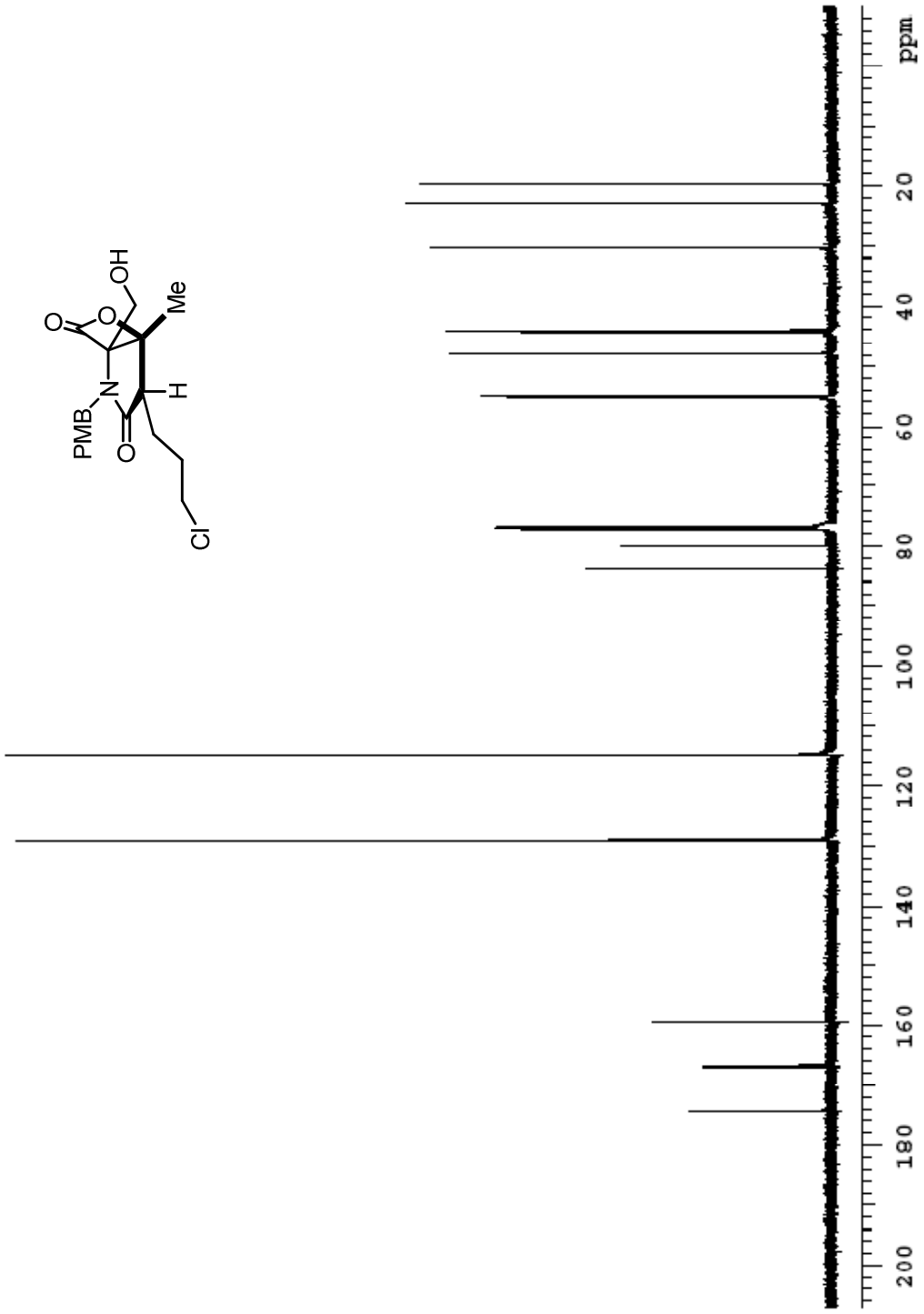
^{1}H NMR of OBn-β-lactone **5.7a** (500 MHz, $CDCl_3$)



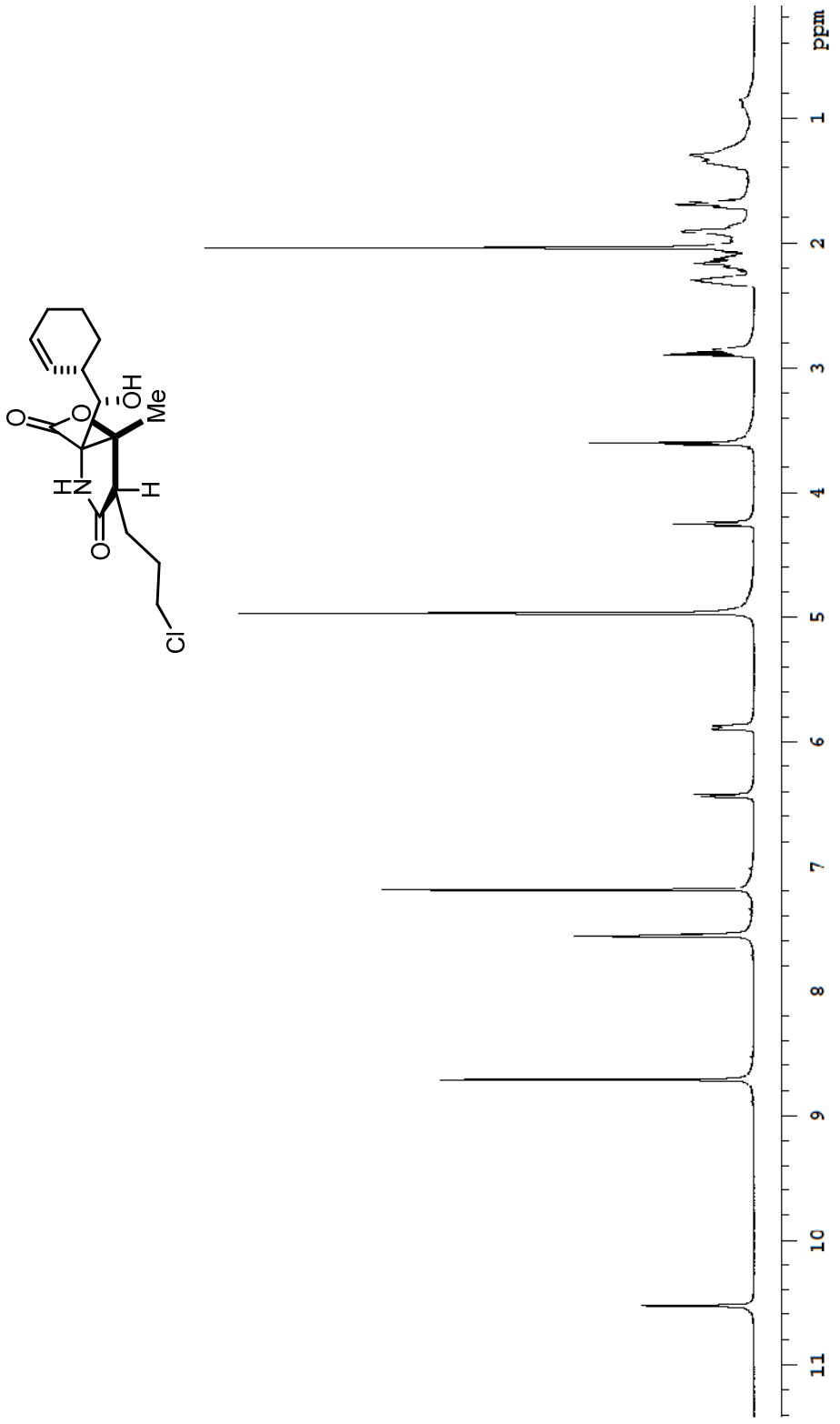
^{13}C NMR of OBn-β-lactone **5.7a** (500 MHz, $CDCl_3$)



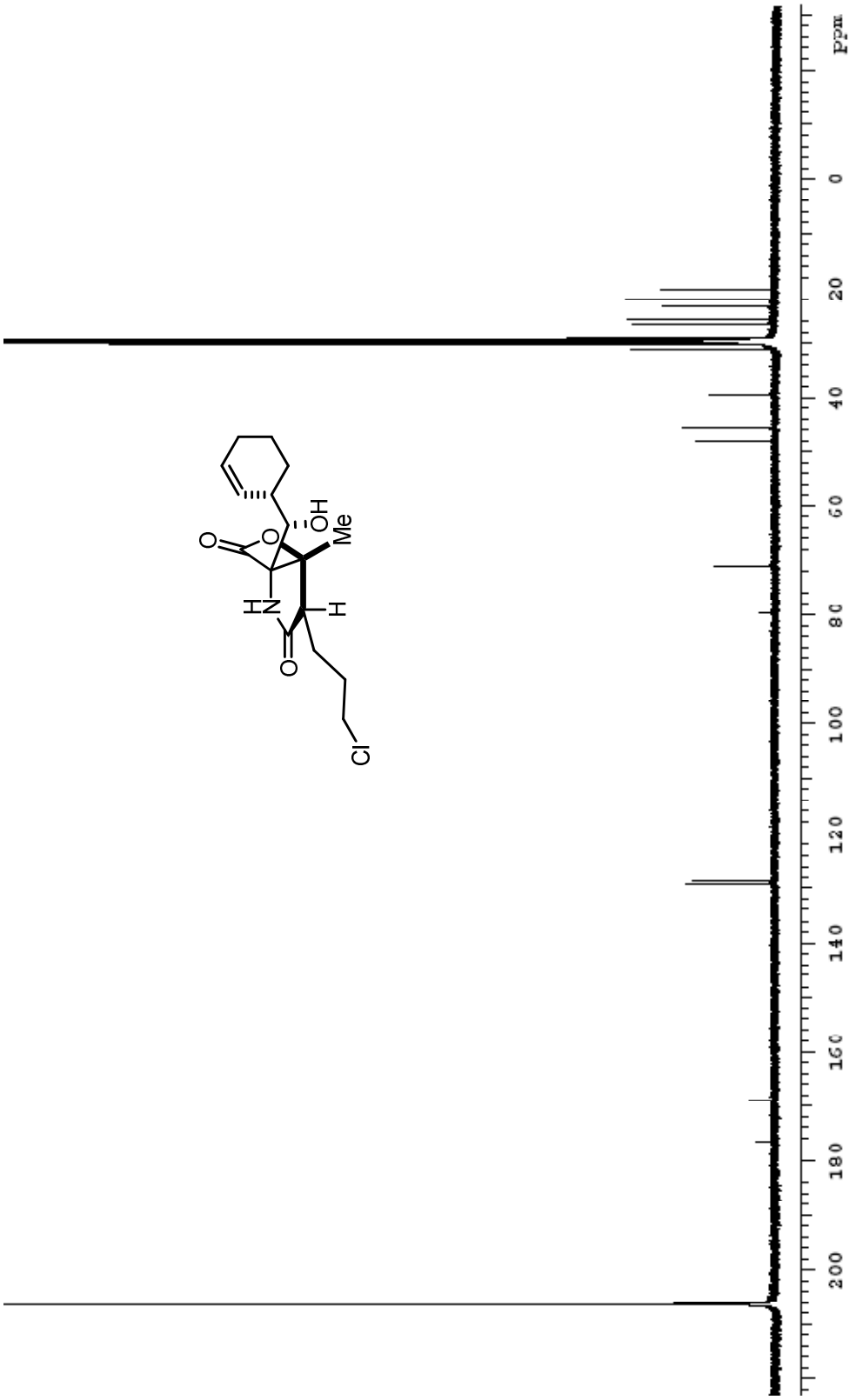
^{1}H NMR of OH-β-lactone **5.8a** (500 MHz, $CDCl_3$)



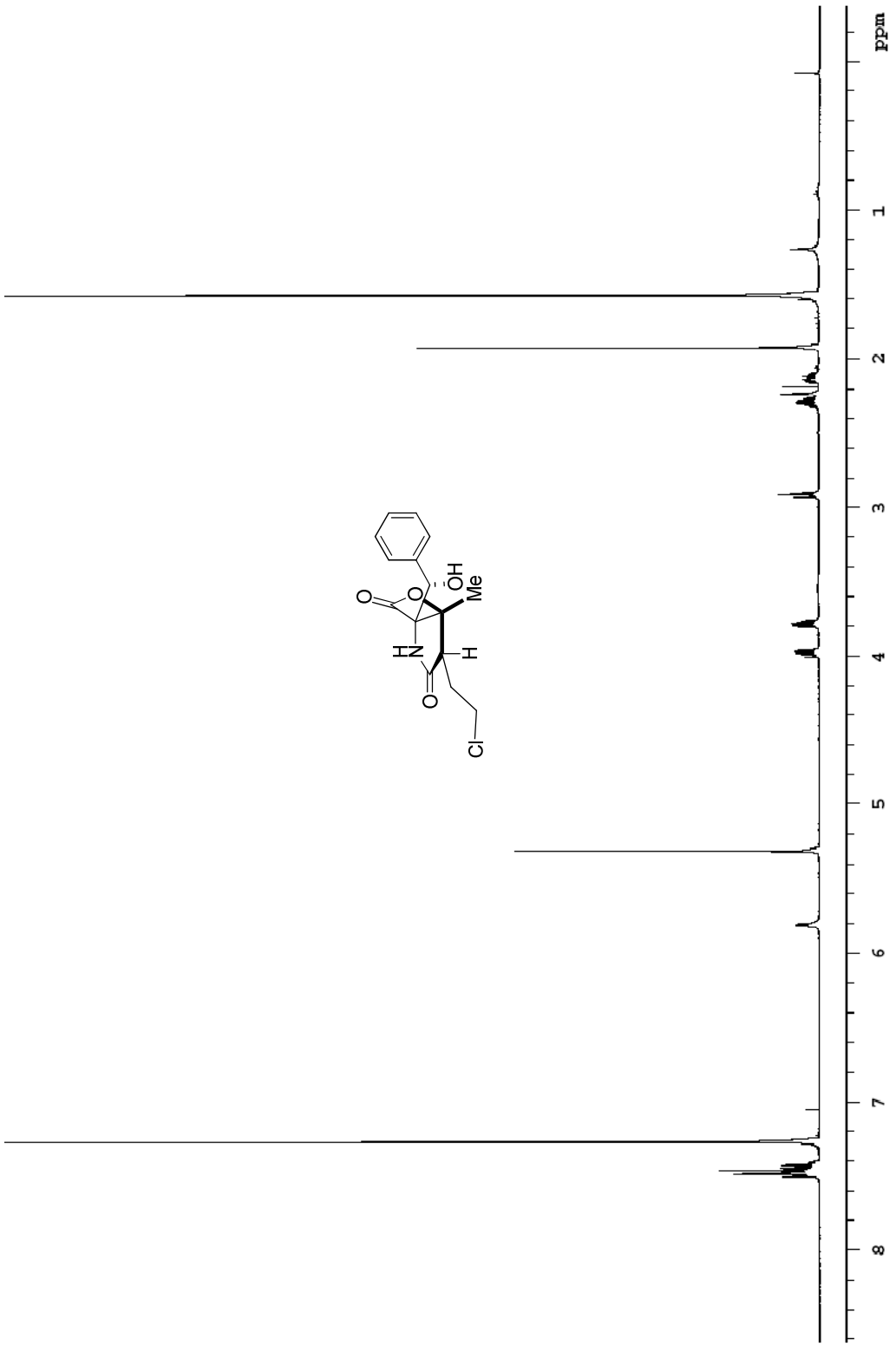
^{13}C NMR of OH-β-lactone **5.8a** (500 MHz, $CDCl_3$)



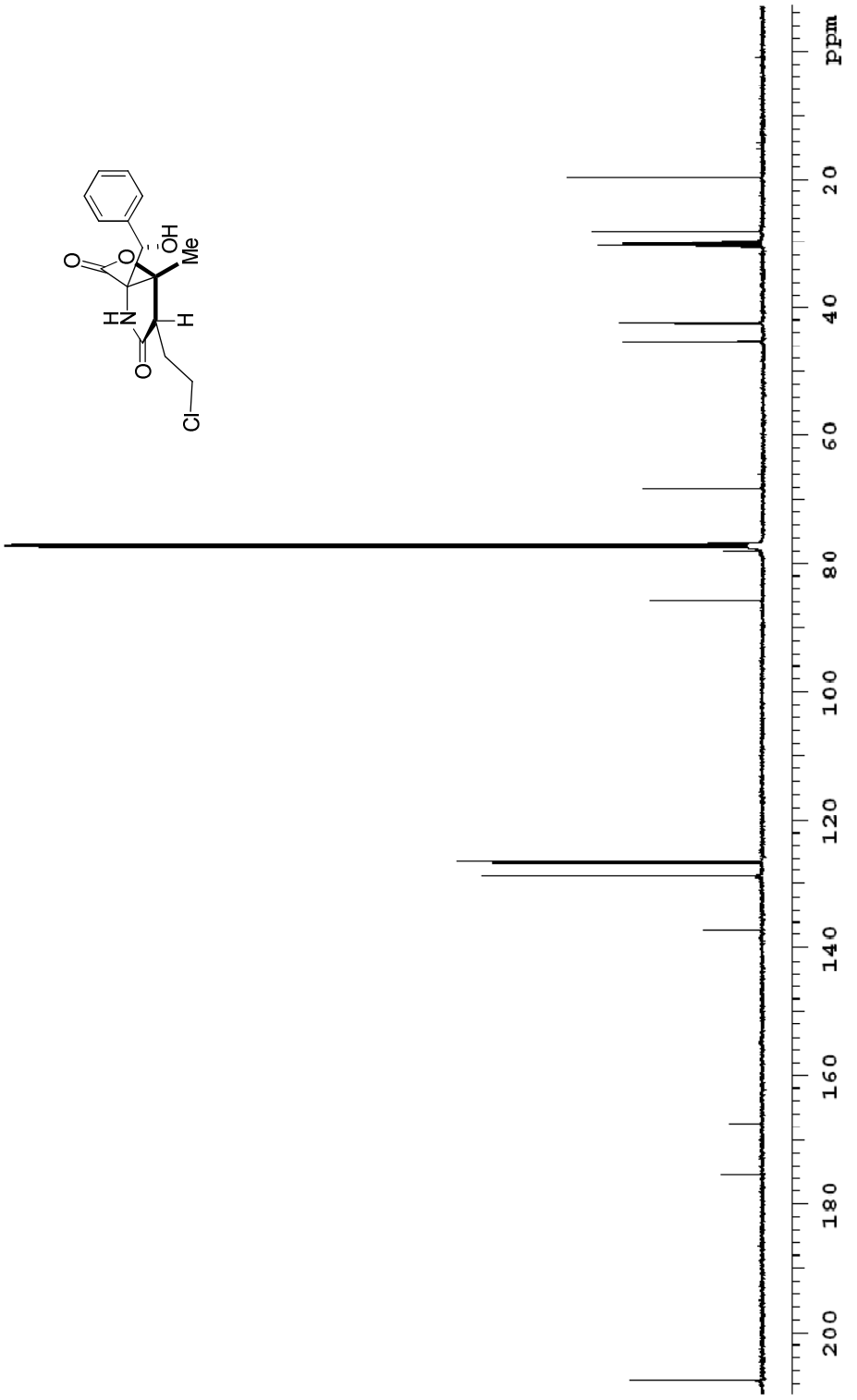
^{1}H NMR of (–)-homosalinosporamide A (–)-**5.1** (500 MHz, C_5D_5N)



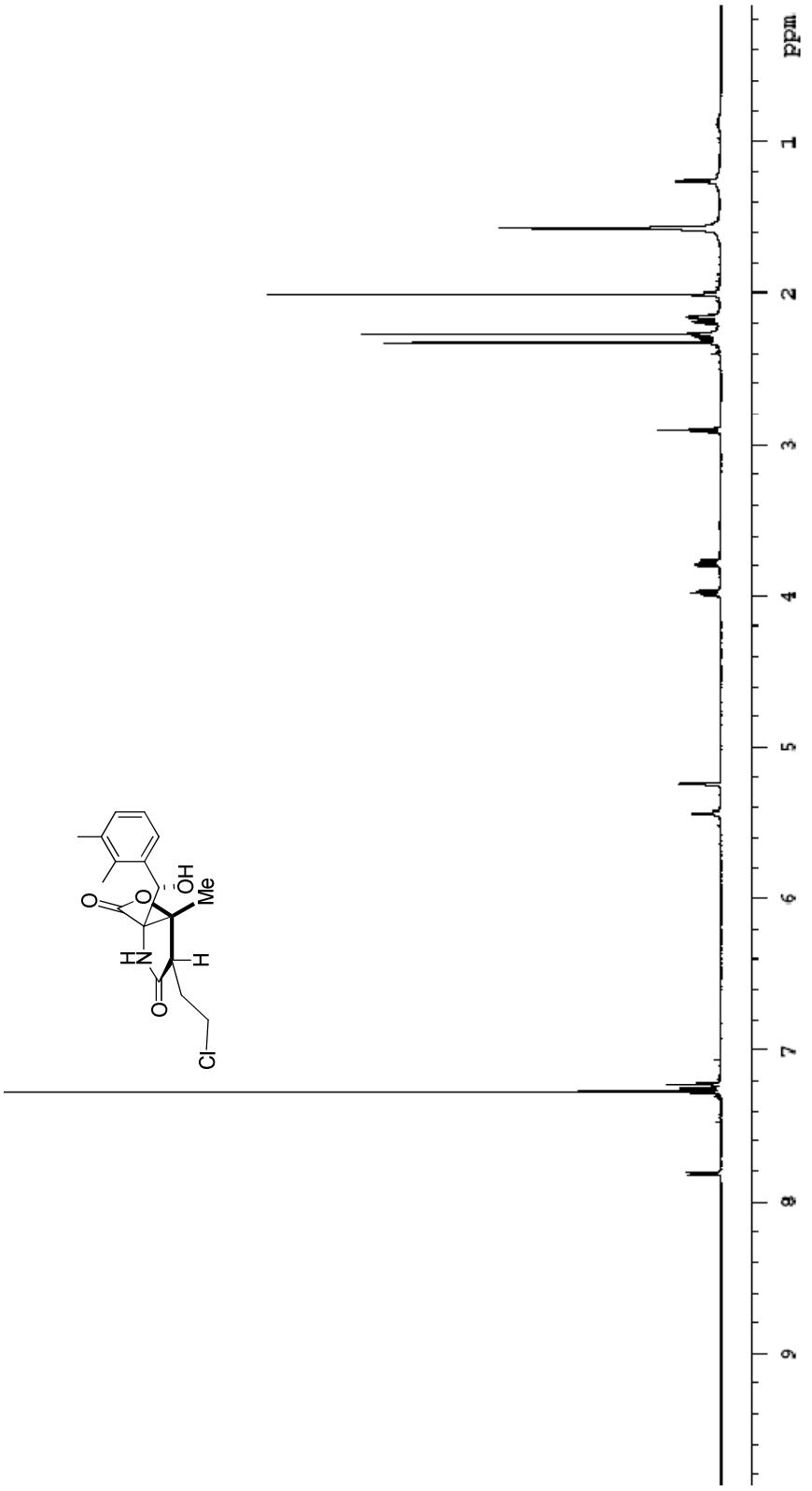
^{13}C NMR of (–)-homosalinosporamide A (–)-**5.1** (500 MHz, C_5D_5N)



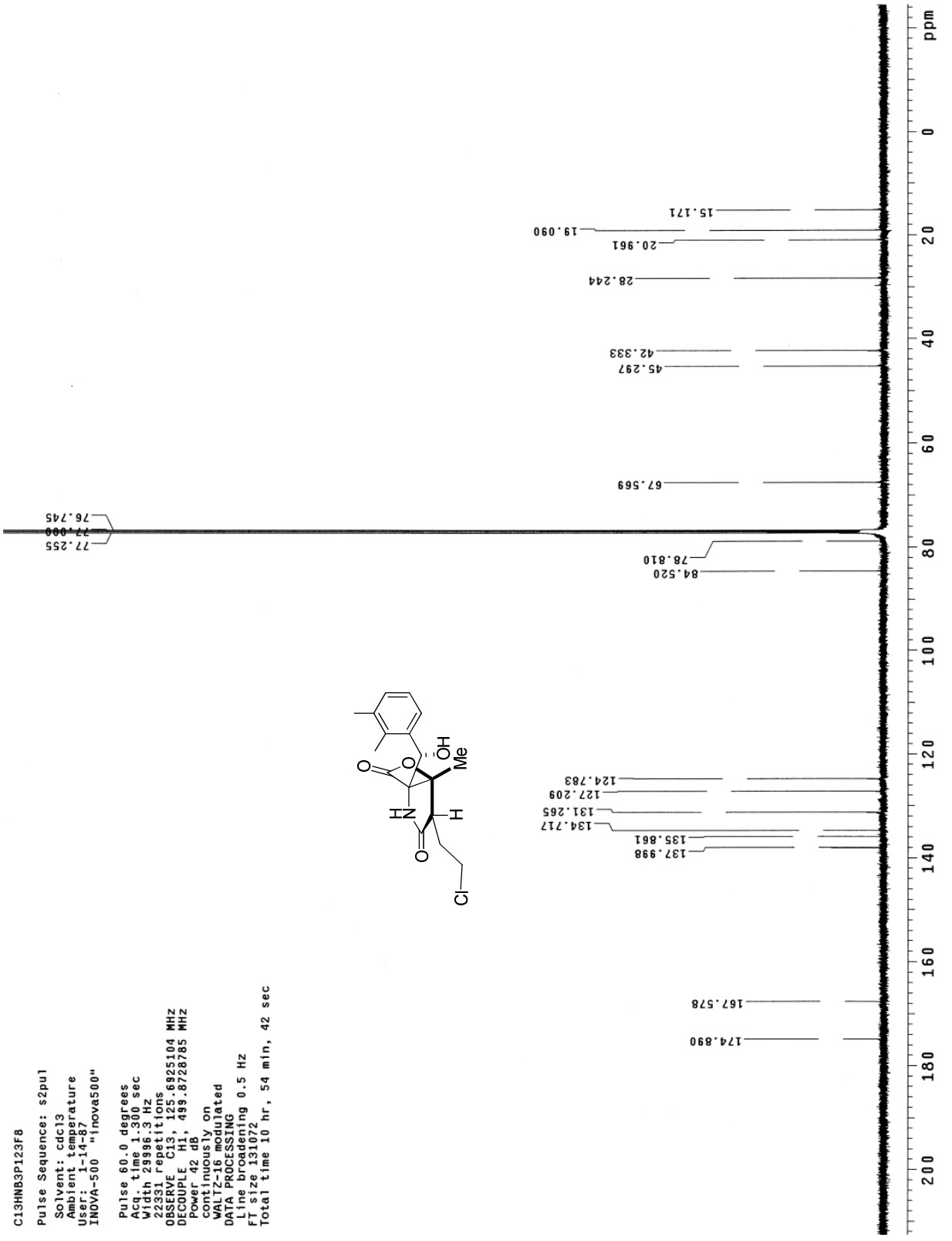
^{1}H NMR of (±)-salino A derivative (±)-**20** (500 MHz, $CDCl_3$)



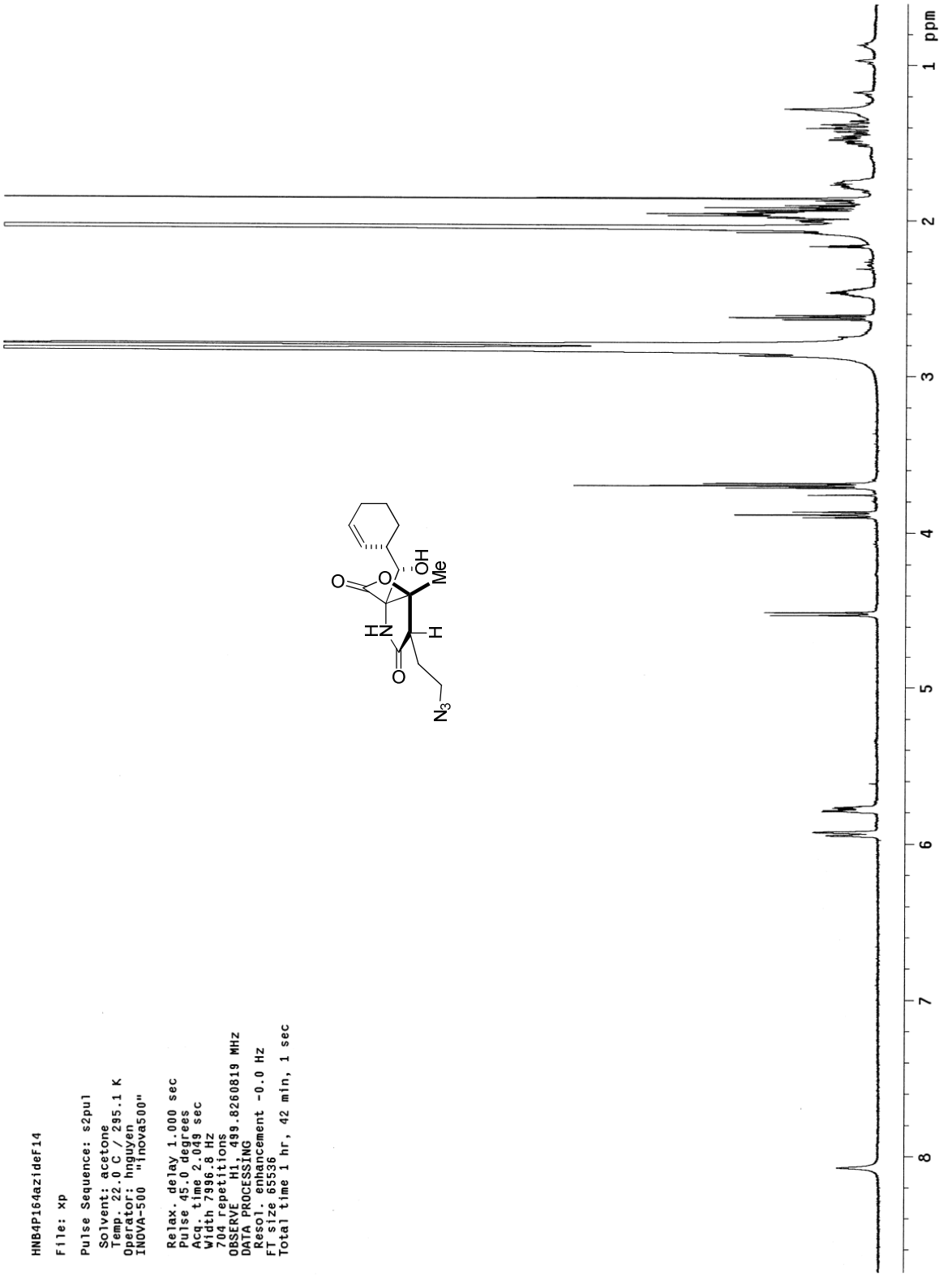
^{13}C NMR of (±)-salino A derivative, (±)-**20** (125 MHz, $CDCl_3$ and C_3D_6O)



^{1}H NMR of (±)-salino A derivative, (±)-**22** (500 MHz, $CDCl_3$)

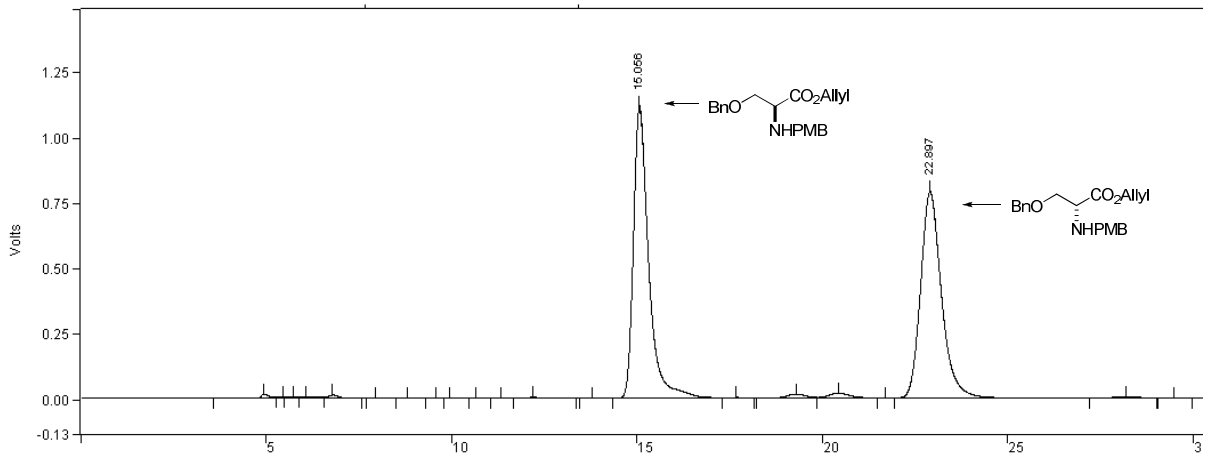


^{13}C NMR of (±)-salino A derivative, (±)-**22** (500 MHz, $CDCl_3$)

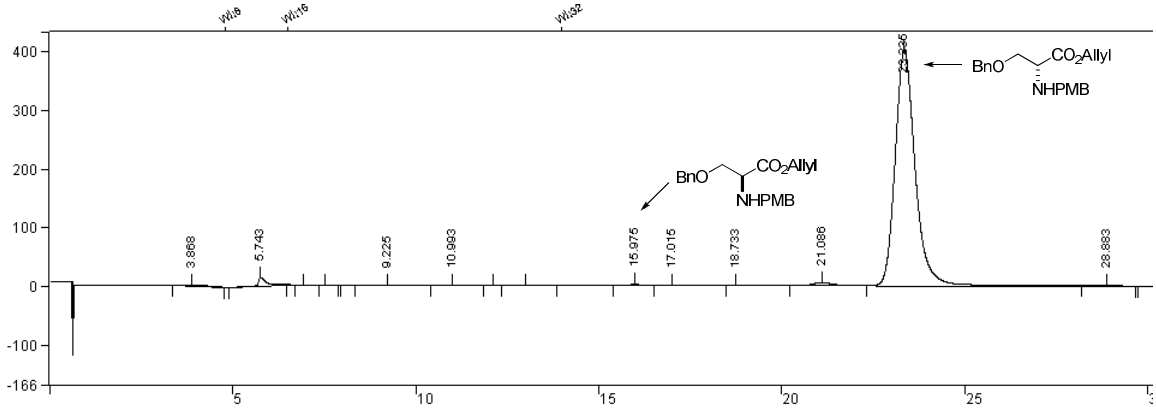


^{1}H NMR of (±)-azido-salino A derivative, (±)-**5.12** (500 MHz, C_3D_6O)

Determination of Optical Purity of (*D*)-Serine by HPLC (CHIRALPAK IA)

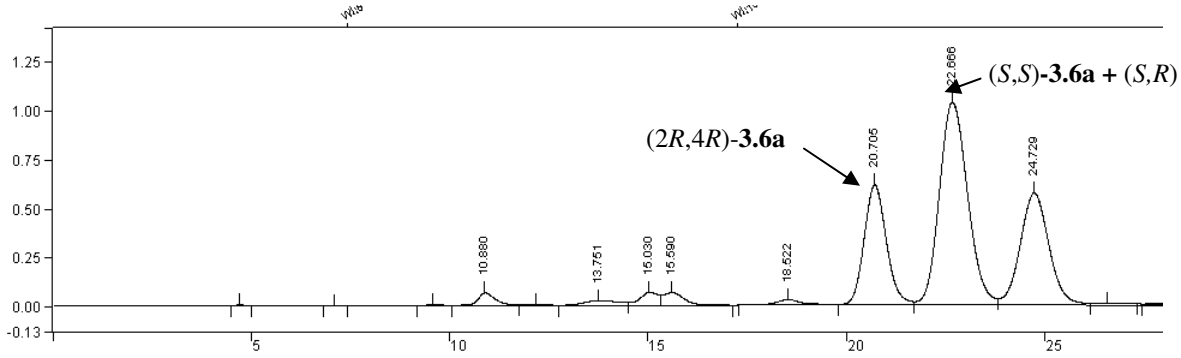


Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		50.8963	15.056	0.000	32333826	VV	24.3	
2		49.1037	22.897	0.000	31195020	VB	34.8	
Totals:		100.0000		0.000	63528846			

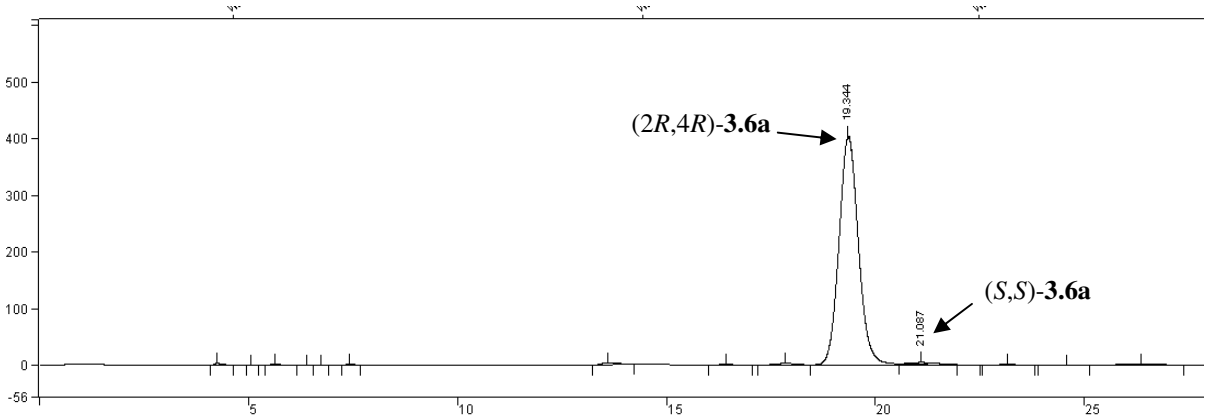


Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		0.4558	3.868	0.000	75796	BB	21.1	
2		1.1642	5.743	0.000	193622	BB	14.5	
3		0.3121	9.225	0.000	51897	BV	55.7	
4		0.2912	10.993	0.000	48425	VP	15.1	
5		0.3036	15.975	0.000	50491	BV	26.4	
6		0.4087	17.015	0.000	67975	VV	52.6	
7		0.3253	18.733	0.000	54103	VV	49.4	
8		1.6436	21.086	0.000	273349	VV	29.8	
9		93.6909	23.335	0.000	15581641	VP	33.6	
10		0.3449	28.883	0.000	57363	TS	0.0	
11		0.6537	31.478	0.000	108708	PV	0.0	
12		0.4060	32.946	0.000	67527	VB	0.0	
Totals:		100.0000		0.000	16630897			

Determination of Optical Purity of (2*R*,4*R*)-β-Ketoamide by HPLC (CHIRALPAK IA)

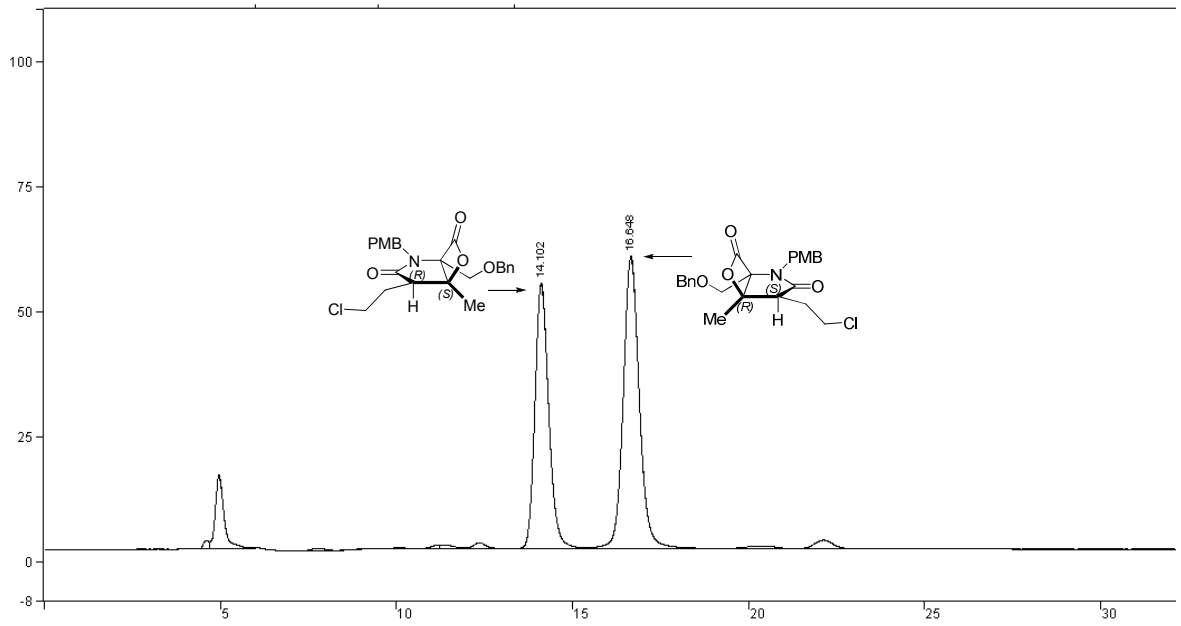


Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		1.5248	10.880	0.000	1948910	VV	25.9	
2		1.1338	13.751	0.000	1449136	VV	0.0	
3		1.6503	15.030	0.000	2109397	VV	31.0	
4		1.8826	15.590	0.000	2406279	VB	50.5	
5		1.1183	18.522	0.000	1429367	BV	38.2	
6		18.9703	20.705	0.000	24246934	VV	35.9	
7		40.4567	22.666	0.000	51709932	VV	46.1	
8		24.7207	24.729	0.000	31596874	VV	46.0	
9		3.3943	30.161	0.000	4338403	TF	0.0	
10		1.7261	31.231	0.000	2206181	TF	0.0	
11		3.4222	33.213	0.000	4374092	VB	55.4	
Totals:		100.0001		0.000	127815505			

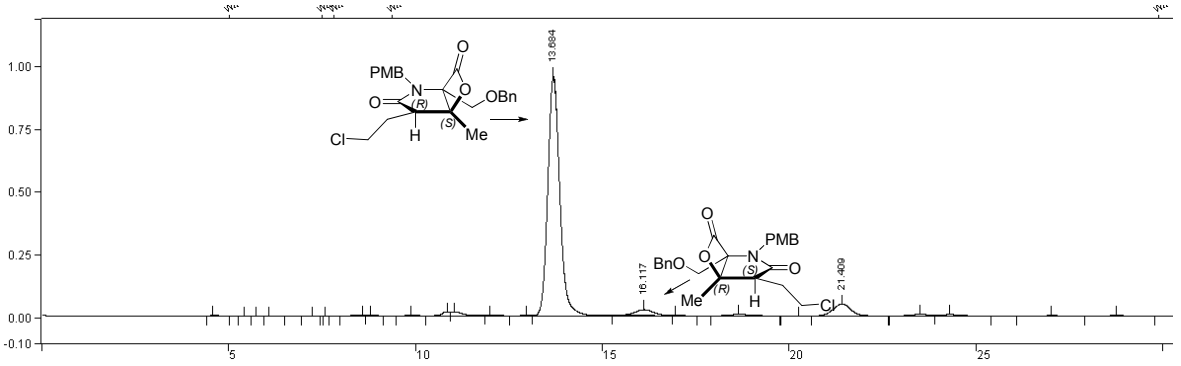


Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		99.0706	19.344	0.000	13123981	VB	29.3	
2		0.9294	21.087	0.000	123121	TS	0.0	
Totals:		100.0000		0.000	13247102			

Determination of Optical Purity of Bicyclic-β-Lactone by HPLC (CHIRALPAK IA)

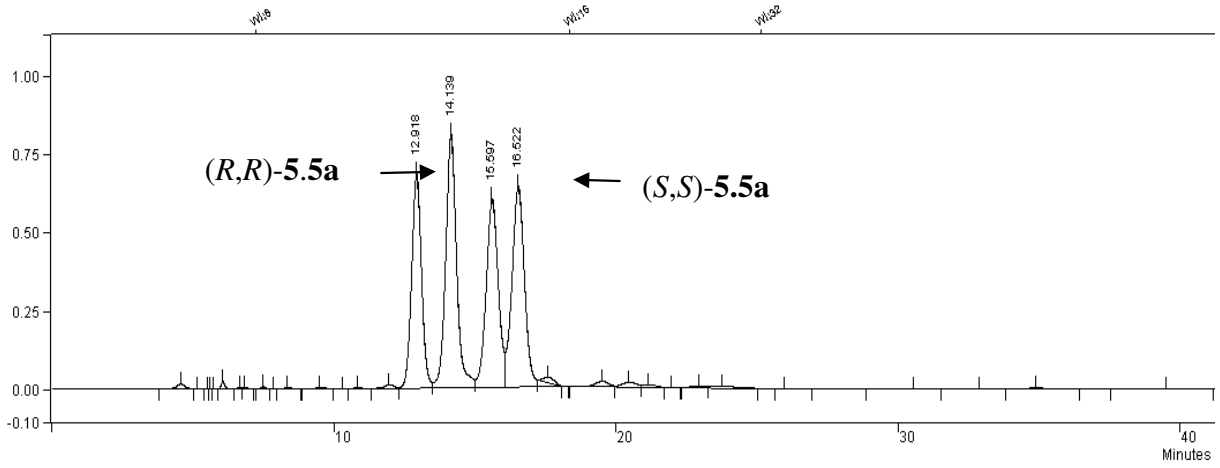


Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		43.7507	14.102	0.000	1445281	BV	24.6	
2		56.2493	16.648	0.000	1858169	VB	28.2	
Totals:		100.0000		0.000	3303450			

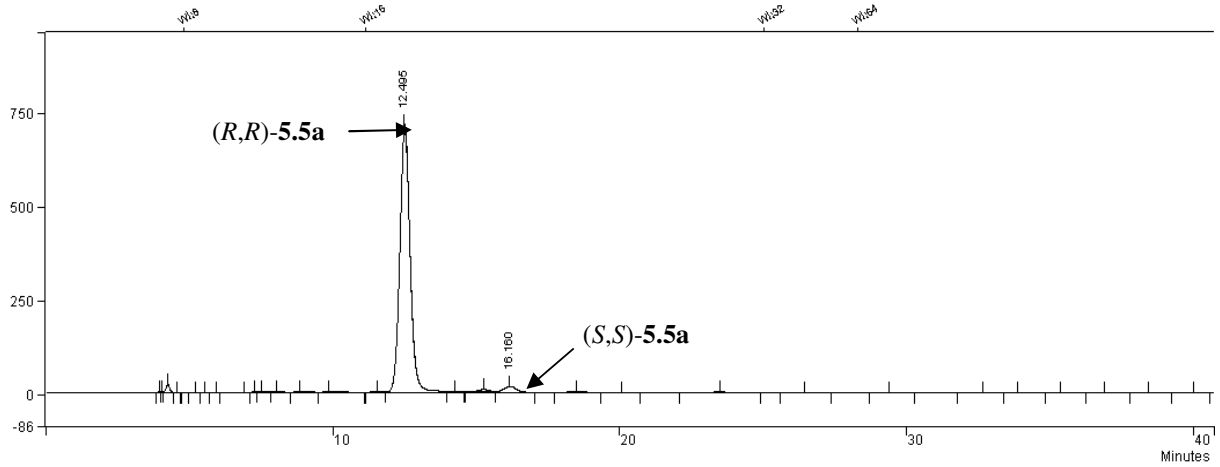


Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		89.7037	13.684	0.000	21537478	VB	20.0	
2		3.6752	16.117	0.000	882407	TF	0.0	
3		6.6211	21.409	0.000	1589704	TF	0.0	
Totals:		100.0000		0.000	24009589			

Determination of Optical Purity of (*R*,*R*)-β-Ketoamide by HPLC (CHIRALPAK IA)

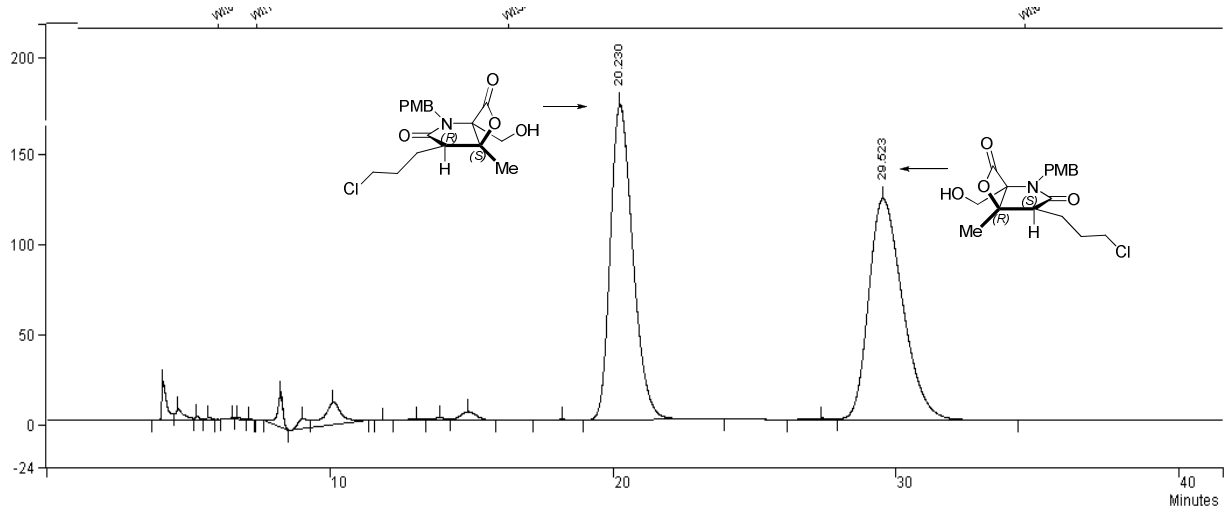


Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		21.6266	12.918	0.000	16565099	VV	22.2	
2		28.2930	14.139	0.000	21671300	VV	23.2	
3		23.4216	15.597	0.000	17940036	VV	27.5	
4		26.6588	16.522	0.000	20419554	VB	28.2	
	Totals:	100.0000		0.000	76595989			

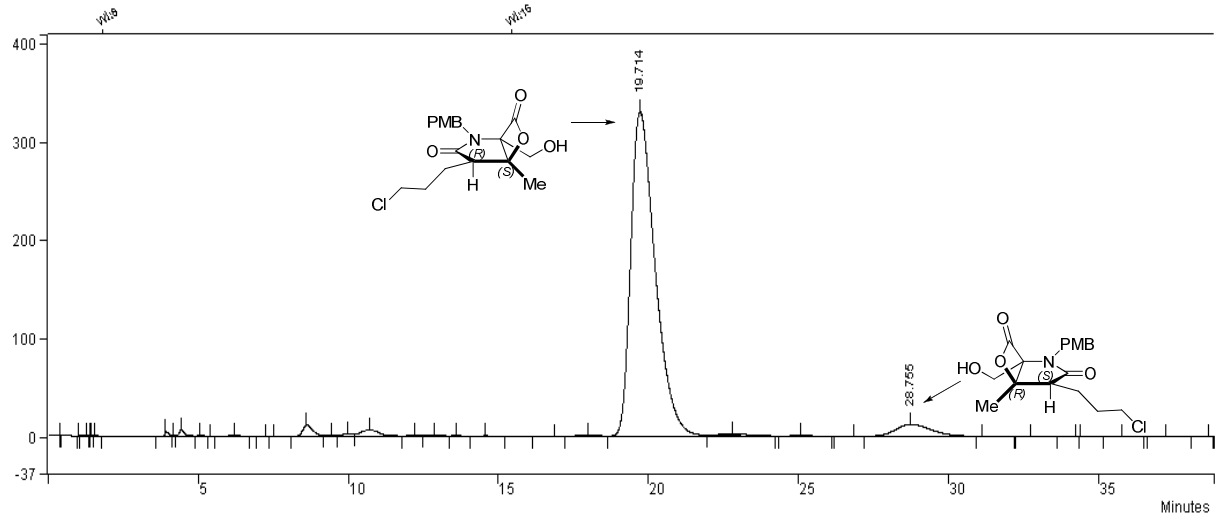


Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		97.5160	12.495	0.000	17921656	VP	22.2	
2		2.4840	16.160	0.000	456514	TF	0.0	
	Totals:	100.0000		0.000	18378170			

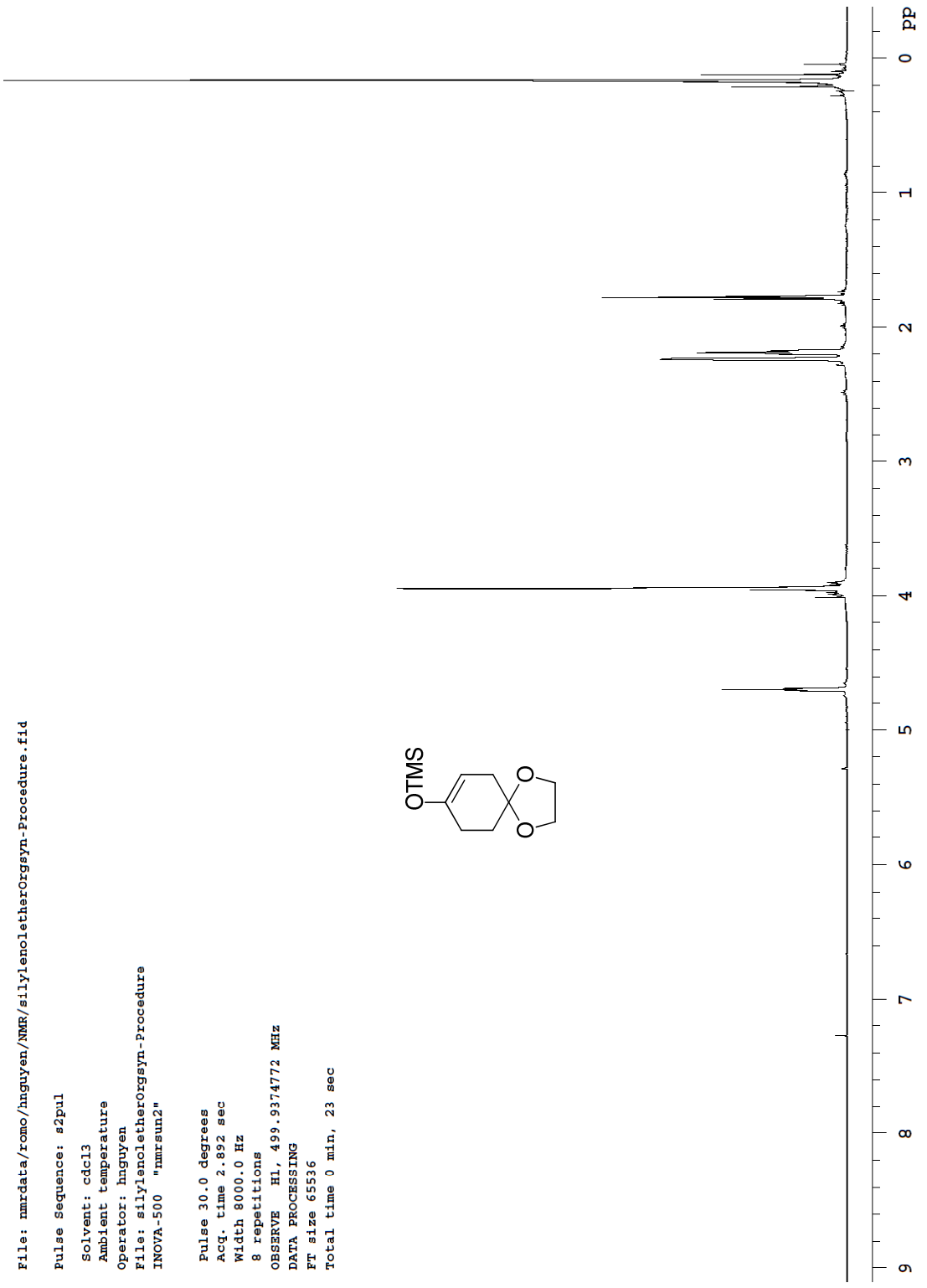
Determination of Optical Purity of Bicyclic-β-Lactone by HPLC (CHIRALCEL OD)



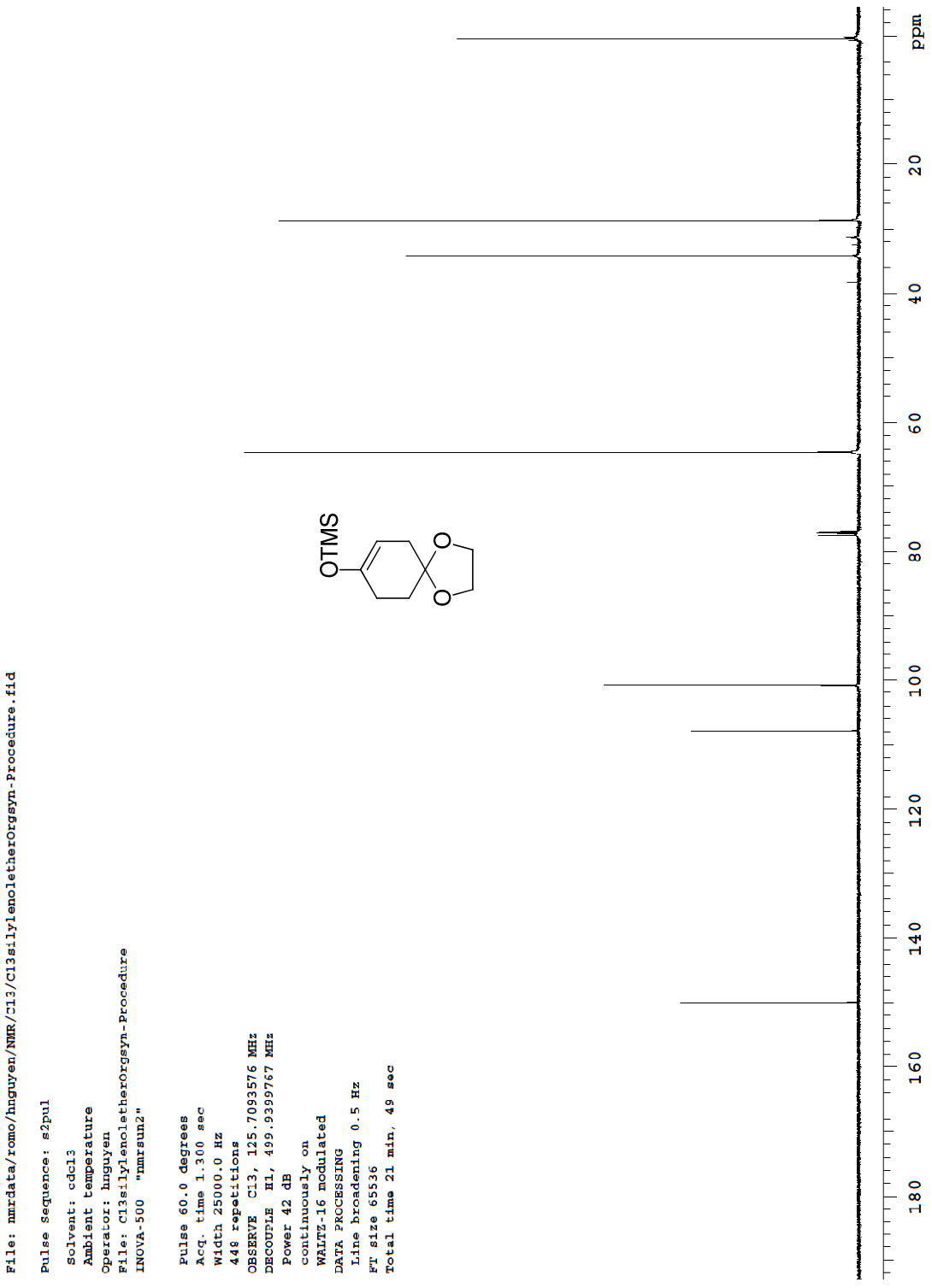
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		48.5806	20.230	0.000	9675606	PB	50.9	
2		51.4194	29.523	0.000	10240997	VB	76.8	
Totals:		100.0000		0.000	19916603			



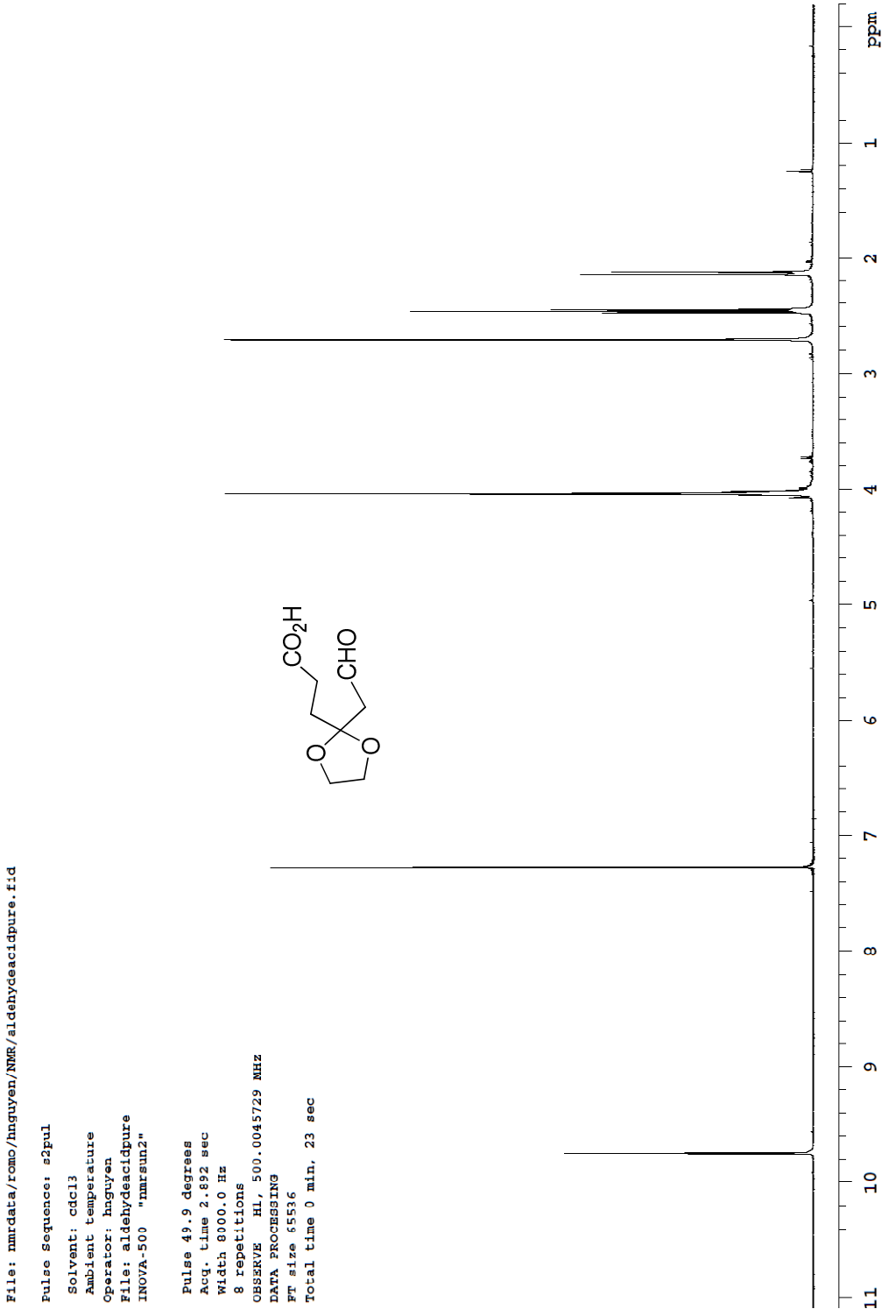
Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		94.9080	19.714	0.000	18868924	VP	51.6	
2		5.0920	28.755	0.000	1012350	TF	0.0	
Totals:		100.0000		0.000	19881274			



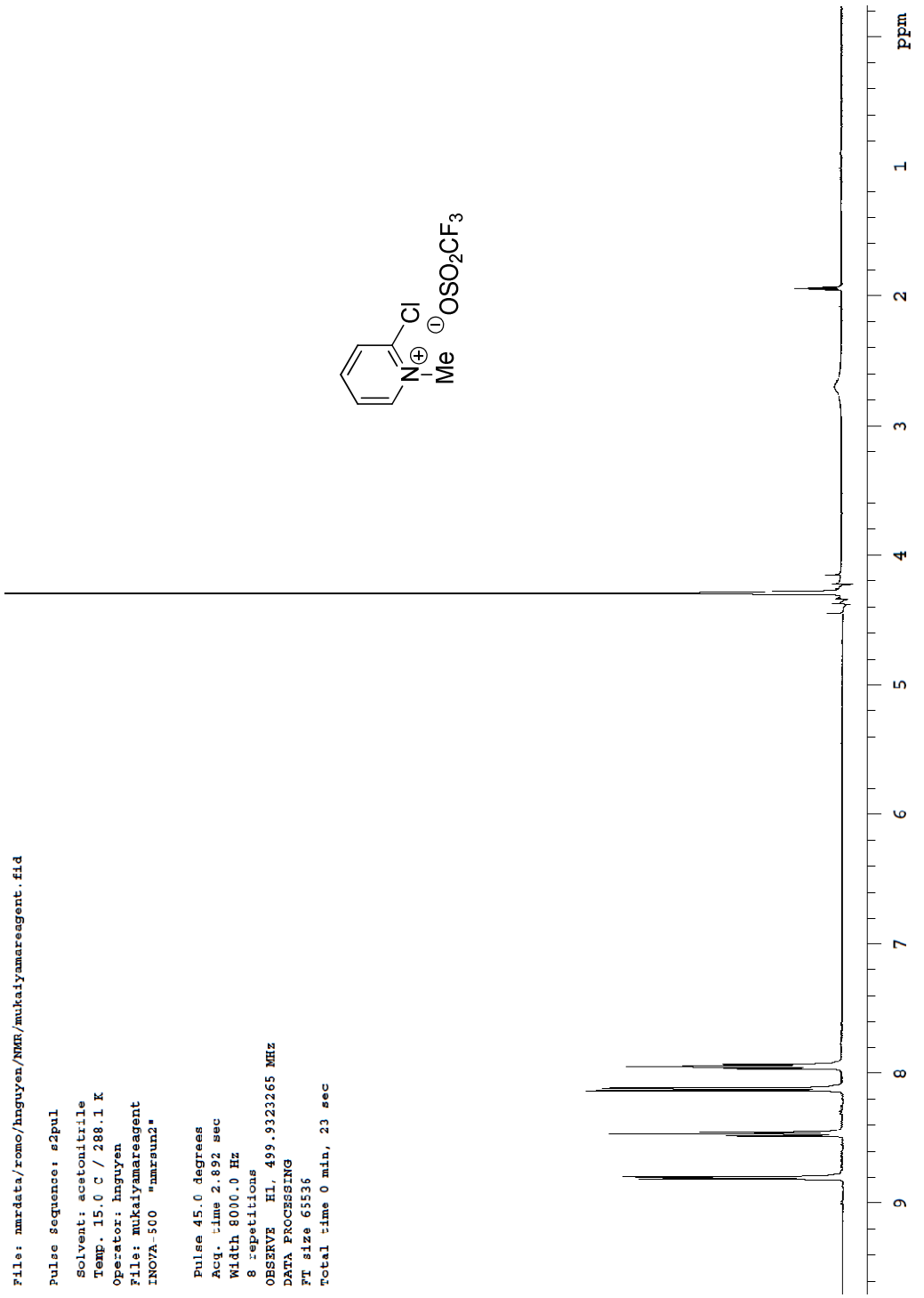
^{1}H NMR of silyl enol ether **6.11** (500 MHz, $CDCl_3$)



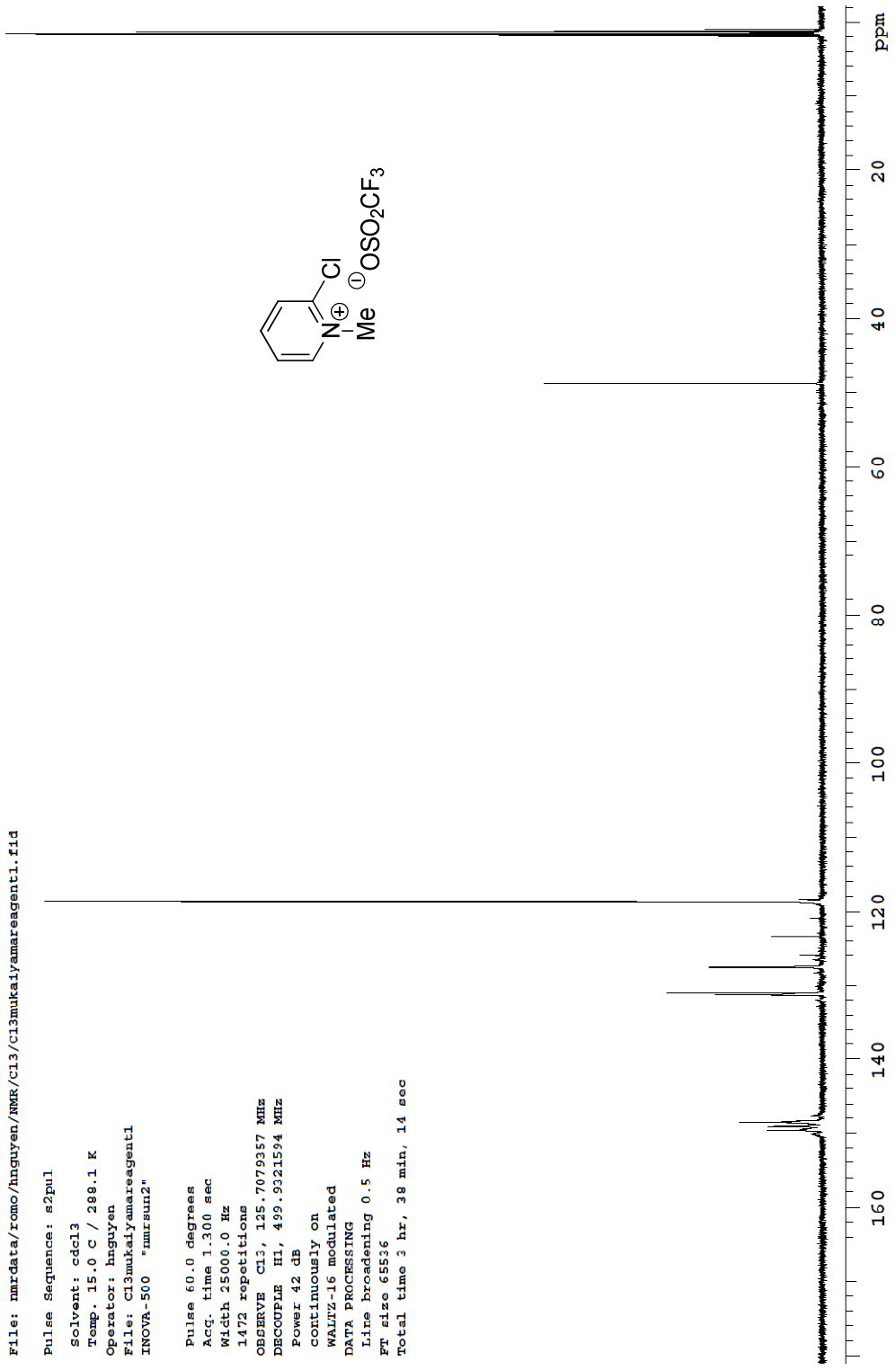
^{13}C NMR of silyl enol ether **6.11** (500 MHz, $CDCl_3$)



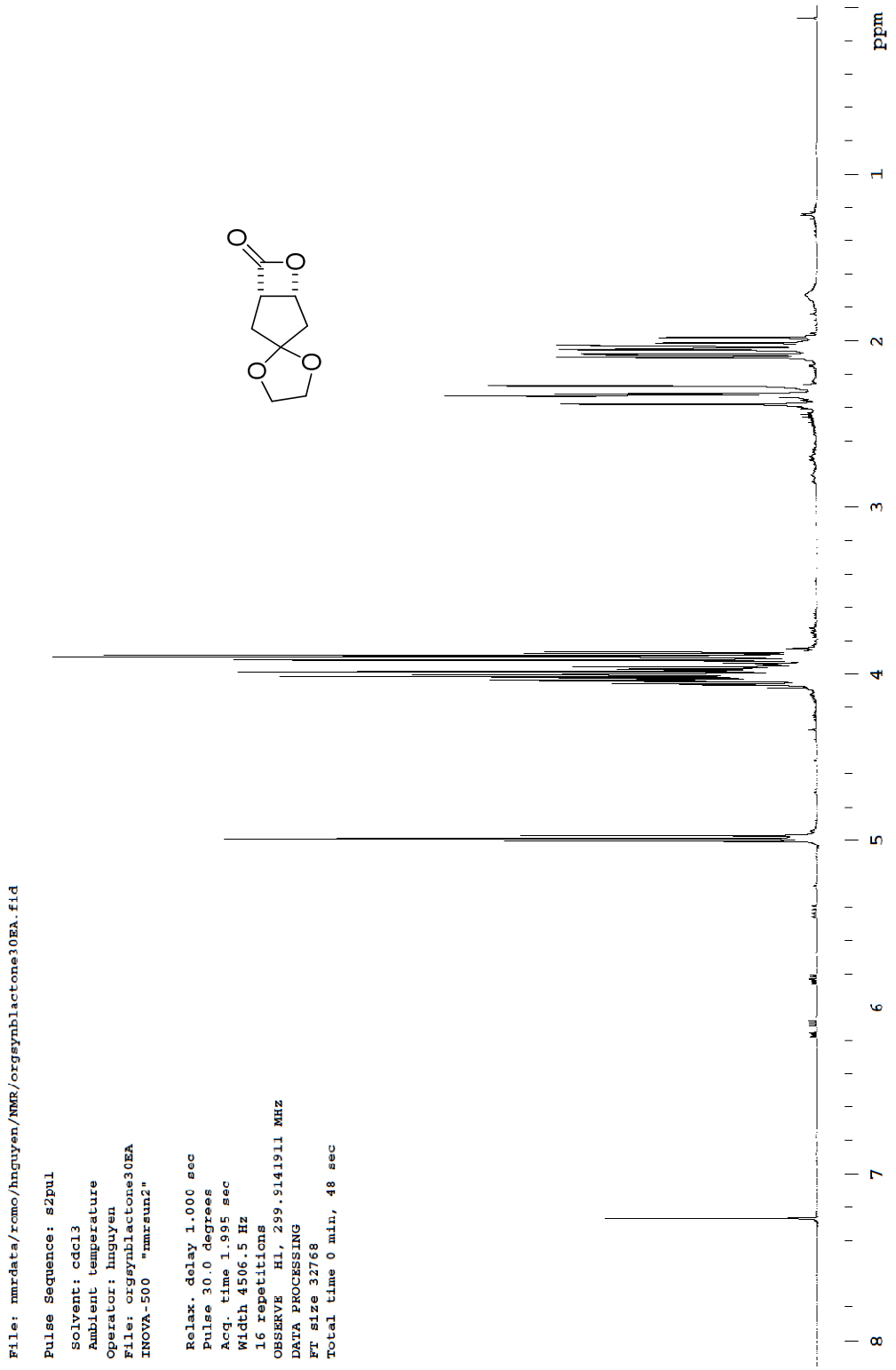
^{1}H NMR of aldehyde acid **6.12** (500 MHz, $CDCl_3$)



^{1}H NMR o Mukaiyama salt **6.8** (500 MHz, CD_3CN)

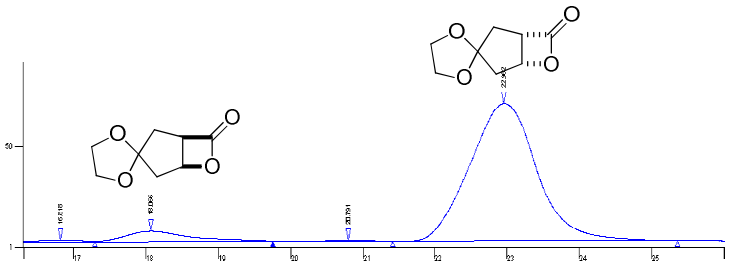


^{1}H NMR of Mukaiyama salt **6.8** (500 MHz, CD_3CN)



^{1}H NMR of β-lactone **6.4** (500 MHz, $CDCl_3$)

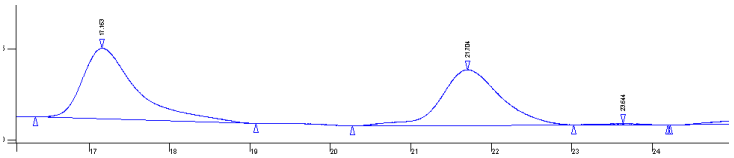
Optically Active β-lactone 6.4



Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		8.0270	3.828	0.000	885172	BV	22.7	
2		10.2218	4.415	0.000	1127212	VB	37.1	
3		0.0743	5.230	0.000	8194	TS	0.0	
4		2.3167	6.232	0.000	255469	BV	23.0	
5		18.9247	7.526	0.000	2086916	VB	7.5	
6		13.7019	11.218	0.000	1510969	BB	0.0	
7		2.1631	13.506	0.000	238533	BP	39.9	
8		0.1071	15.201	0.000	11807	TS	0.0	
9		0.2954	16.818	0.000	32570	PV	47.7	
10		2.8788	18.066	0.000	317460	VP	52.5	
11		0.2107	20.791	0.000	23237	PV	57.6	
12		39.2926	22.962	0.000	4332988	VB	59.0	
13		1.5421	27.445	0.000	170052	BV	113.7	
14		0.2439	29.607	0.000	26901	VB	76.6	
	Totals:	100.0001		0.000	11027480			

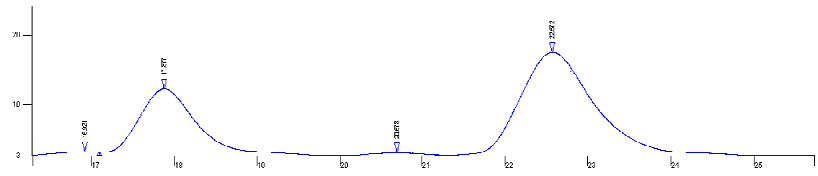
β-lactone **6.4**: er 93:7, 86% ee.

Racemic



Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		21.5809	3.598	0.000	454783	BB	13.9	
2		1.6834	4.320	0.000	35476	TS	0.0	
3		23.1696	7.216	0.000	488263	BP	41.3	
4		11.2665	8.246	0.000	237424	PV	11.2	
5		20.1235	10.436	0.000	424072	VB	0.0	
6		0.1364	11.549	0.000	2875	BV	25.9	
7		0.3004	12.307	0.000	6331	VB	20.1	
8		0.9598	13.663	0.000	20226	BV	22.4	
9		3.0626	14.760	0.000	64539	VB	45.0	
10		8.6500	17.163	0.000	182285	BB	37.2	
11		7.5646	21.704	0.000	159413	BP	45.5	
12		0.1248	23.644	0.000	2629	TS	0.0	
13		0.3638	25.109	0.000	7666	PV	0.0	
14		0.6618	26.355	0.000	13946	VV	0.0	
15		0.3519	27.290	0.000	7416	VB	0.0	
	Totals:	100.0000		0.000	2107344			

Mixed ~1:1; Racemic and Optically Active β-lactone 5



APPENDIX B

SINGLE CRYSTAL X-RAY DATA FOR β-LACTONE 5.20

Table 1. Crystal data and structure refinement for dr69.

Identification code	dr69	
Empirical formula	C15 H16 Cl N O4	
Formula weight	309.74	
Temperature	110(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P2(1)/n	
Unit cell dimensions	a = 15.516(3) Å	α = 90°.
	b = 11.0290(13) Å	β = 91.007(10)°.
	c = 16.725(2) Å	γ = 90°.
Volume	2861.5(7) $Å^3$	
Z	8	
Density (calculated)	1.438 Mg/m^3	
Absorption coefficient	2.513 mm^{-1}	
F(000)	1296	
Crystal size	0.16 x 0.06 x 0.02 mm^3	
Theta range for data collection	3.85 to 59.98°.	
Index ranges	-17<=h<=16, -12<=k<=12, -18<=l<=18	
Reflections collected	48543	
Independent reflections	4192 [R(int) = 0.1021]	
Completeness to theta = 59.98°	98.5 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9515 and 0.6893	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4192 / 0 / 397	
Goodness-of-fit on F^2	1.000	
Final R indices [I>2sigma(I)]	R1 = 0.0425, wR2 = 0.0962	
R indices (all data)	R1 = 0.0707, wR2 = 0.1038	
Largest diff. peak and hole	0.300 and -0.349 e.$Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3)
for dr69. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Cl(1A)	1106(1)	-5828(1)	10834(1)	45(1)
O(1A)	-243(1)	-2015(2)	12379(1)	24(1)
N(1A)	-1298(1)	-4188(2)	12846(1)	19(1)
C(1A)	-1418(2)	-3011(2)	12475(1)	18(1)
O(2A)	-927(1)	-1427(2)	13507(1)	26(1)
C(2A)	-690(2)	-2935(2)	11850(2)	19(1)
O(3A)	-346(1)	-5786(2)	12859(1)	25(1)
C(3A)	-212(2)	-4152(2)	11878(2)	20(1)
O(4A)	-2519(1)	-3622(2)	11610(1)	33(1)
C(4A)	-608(2)	-4813(2)	12581(1)	19(1)
C(5A)	-894(2)	-2022(2)	12911(2)	22(1)
C(6A)	-857(2)	-2407(2)	11043(2)	27(1)
C(7A)	769(2)	-4051(2)	11930(2)	24(1)
C(8A)	1243(2)	-5237(2)	11830(2)	30(1)
C(9A)	-2345(2)	-2739(2)	12212(2)	22(1)
C(10A)	-2968(2)	-2775(2)	12901(2)	23(1)
C(11A)	-3015(2)	-1791(3)	13416(2)	34(1)
C(12A)	-3586(2)	-1818(3)	14053(2)	47(1)
C(13A)	-4109(2)	-2809(4)	14154(2)	52(1)
C(14A)	-4075(2)	-3777(3)	13640(2)	48(1)
C(15A)	-3496(2)	-3773(3)	13019(2)	32(1)
Cl(1B)	8752(1)	755(1)	8654(1)	36(1)
Cl(1')	8964(11)	-462(13)	9328(8)	36(5)
O(1B)	5641(1)	-1435(2)	8968(1)	23(1)
N(1B)	6059(2)	-1655(2)	10728(1)	18(1)
C(1B)	5627(2)	-2324(2)	10102(1)	17(1)
O(2B)	4380(1)	-1002(2)	9605(1)	26(1)

C(2B)	6259(2)	-2285(2)	9386(2)	19(1)
O(3B)	7255(1)	-451(2)	10926(1)	22(1)
C(3B)	7058(2)	-1618(2)	9692(1)	21(1)
O(4B)	5976(1)	-4138(2)	10743(1)	24(1)
C(4B)	6816(2)	-1149(2)	10508(2)	20(1)
C(5B)	5060(2)	-1502(2)	9574(2)	20(1)
C(6B)	6414(2)	-3383(2)	8882(2)	25(1)
C(7B)	7394(2)	-632(2)	9127(2)	28(1)
C(8B)	8297(3)	-249(3)	9371(2)	41(1)
C(9B)	5258(2)	-3535(2)	10372(2)	19(1)
C(10B)	4488(2)	-3395(2)	10902(2)	21(1)
C(11B)	3668(2)	-3258(2)	10553(2)	26(1)
C(12B)	2958(2)	-3072(2)	11029(2)	33(1)
C(13B)	3057(2)	-3041(3)	11852(2)	34(1)
C(14B)	3859(2)	-3208(2)	12201(2)	31(1)
C(15B)	4575(2)	-3379(2)	11725(2)	28(1)

APPENDIX C

SINGLE CRYSTAL X-RAY DATA FOR HOMOSALINOSPORAMIDE 5.1

Table 1. Crystal data and structure refinement for dr72.

Identification code	dr72	
Empirical formula	C64 H87 Cl4 N4 O16	
Formula weight	1310.18	
Temperature	383(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 9.694(3) Å	α = 90°.
	b = 25.624(9) Å	β = 93.571(13)°.
	c = 13.118(4) Å	γ= 90°.
Volume	3252.2(18) $Å^3$	
Z	2	
Density (calculated)	1.338 Mg/m^3	
Absorption coefficient	2.234 mm^{-1}	
F(000)	1390	
Crystal size	0.06 x 0.01 x 0.01 mm^3	
Theta range for data collection	3.38 to 59.99°.	
Index ranges	-10<=h<=10, -28<=k<=28, -14<=l<=14	
Reflections collected	24861	
Independent reflections	9114 [R(int) = 0.2299]	
Completeness to theta = 59.99°	99.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9780 and 0.8776	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	9114 / 649 / 832	
Goodness-of-fit on F^2	1.006	
Final R indices [I>2sigma(I)]	R1 = 0.0821, wR2 = 0.1159	
R indices (all data)	R1 = 0.1842, wR2 = 0.1448	
Absolute structure parameter	0.06(3)	
Largest diff. peak and hole	0.284 and -0.296 e.$Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3)
for dr72. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Cl(1A)	3360(3)	10437(1)	5607(2)	55(1)
O(1A)	2006(6)	8479(3)	4120(5)	32(2)
O(2A)	884(6)	7690(3)	4039(5)	33(2)
O(3A)	4478(7)	8279(3)	6756(5)	34(2)
O(4A)	5595(6)	7654(3)	3488(5)	35(2)
N(1A)	3965(7)	7801(4)	5327(5)	26(2)
C(1A)	4240(10)	8696(4)	5055(7)	31(2)
C(2A)	3562(10)	8467(4)	4073(7)	31(2)
C(3A)	3487(10)	7860(4)	4255(7)	28(2)
C(4A)	4258(10)	8244(5)	5858(7)	29(2)
C(5A)	3544(10)	9202(4)	5440(8)	35(2)
C(6A)	4519(10)	9494(4)	6162(8)	38(2)
C(7A)	3856(11)	9977(4)	6611(7)	42(3)
C(8A)	3968(10)	8690(4)	3092(7)	38(3)
C(9A)	1876(11)	7948(4)	4110(7)	31(2)
C(10A)	4187(9)	7501(4)	3531(8)	31(2)
C(11A)	4141(10)	6907(5)	3862(8)	36(2)
C(12A)	2729(10)	6721(4)	4148(8)	41(2)
C(13A)	2740(12)	6138(4)	4342(8)	43(2)
C(14A)	2980(11)	5854(5)	3286(8)	46(2)
C(15A)	4086(11)	6120(5)	2722(8)	44(2)
C(16A)	4611(11)	6579(4)	3001(8)	39(2)
Cl(1B)	3309(3)	4719(1)	7335(2)	50(1)
O(1B)	1962(7)	6670(3)	8818(5)	36(2)
O(2B)	836(7)	7460(3)	8795(5)	41(2)
O(3B)	4565(6)	6863(3)	6501(5)	31(2)
O(4B)	5578(6)	7446(3)	9919(4)	30(2)

N(1B)	4003(8)	7346(4)	7878(5)	30(2)
C(1B)	4223(10)	6445(4)	8136(7)	32(2)
C(2B)	3522(10)	6665(5)	9045(8)	34(2)
C(3B)	3479(10)	7287(4)	8909(7)	32(2)
C(4B)	4306(10)	6903(5)	7378(8)	30(2)
C(5B)	3584(11)	5949(4)	7667(8)	35(2)
C(6B)	4657(11)	5664(4)	7099(8)	42(2)
C(7B)	4084(11)	5172(5)	6496(8)	47(3)
C(8B)	3860(10)	6439(4)	10089(7)	36(3)
C(9B)	1882(11)	7204(4)	8861(8)	33(2)
C(10B)	4209(10)	7615(4)	9697(7)	32(2)
C(11B)	4273(10)	8199(4)	9422(7)	35(2)
C(12B)	2855(11)	8426(4)	9040(8)	41(2)
C(13B)	3030(12)	9030(5)	8899(8)	47(2)
C(14B)	3465(11)	9303(5)	9907(8)	47(2)
C(15B)	4486(11)	9010(5)	10527(8)	44(2)
C(16B)	4859(10)	8532(5)	10314(8)	40(2)
Cl(1C)	-1744(3)	5091(1)	7955(3)	68(1)
O(1C)	324(6)	7028(3)	6236(5)	33(2)
O(2C)	1526(6)	7769(3)	6612(5)	34(2)
O(3C)	-2716(7)	7141(3)	8461(5)	40(2)
O(4C)	-3046(6)	7981(3)	5323(5)	36(2)
N(1C)	-1726(7)	7671(4)	7303(6)	30(2)
C(1C)	-1999(10)	6777(4)	6828(7)	33(2)
C(2C)	-1183(10)	7046(5)	6022(7)	31(2)
C(3C)	-1104(10)	7657(5)	6350(7)	31(2)
C(4C)	-2233(11)	7215(5)	7638(8)	33(2)
C(5C)	-1309(11)	6312(4)	7355(8)	38(2)
C(6C)	-1058(11)	5875(4)	6657(8)	45(2)
C(7C)	-478(11)	5358(5)	7208(8)	51(3)
C(8C)	-1560(10)	6906(4)	4897(7)	36(3)
C(9C)	485(10)	7539(4)	6456(7)	30(2)
C(10C)	-1608(9)	8087(4)	5591(7)	33(2)
C(11C)	-1540(10)	8640(5)	6026(8)	37(2)
C(12C)	-171(10)	8786(4)	6585(8)	42(2)

C(13C)	-216(12)	9378(5)	6832(8)	50(2)
C(14C)	-307(11)	9687(5)	5869(8)	51(2)
C(15C)	-1300(11)	9478(5)	5118(8)	42(2)
C(16C)	-1847(3)	9002(1)	5173(2)	39(2)
Cl(1D)	-2943(3)	10079(1)	8771(2)	67(2)
Cl(1')	-3541(3)	10078(1)	8951(2)	78(4)
O(1D)	714(3)	8300(1)	11716(2)	38(2)
O(2D)	1752(3)	7529(1)	11395(2)	40(2)
O(3D)	-1689(3)	8423(1)	8945(2)	36(2)
O(4D)	-2968(3)	7395(1)	11761(2)	32(2)
N(1D)	-1219(3)	7783(1)	10144(2)	30(2)
C(1D)	-1370(11)	8644(4)	10729(8)	37(2)
C(2D)	-838(10)	8302(5)	11638(8)	34(2)
C(3D)	-816(10)	7731(4)	11228(7)	30(2)
C(4D)	-1428(10)	8292(4)	9810(8)	32(2)
C(5D)	-545(12)	9154(5)	10621(9)	51(2)
C(6D)	-1087(13)	9513(5)	9869(10)	67(3)
C(7D)	-2470(13)	9606(5)	9677(10)	78(3)
C(8D)	-1418(10)	8440(4)	12649(7)	41(3)
C(9D)	754(10)	7788(5)	11419(7)	34(2)
C(10D)	-1535(11)	7303(4)	11762(8)	35(2)
C(11D)	-1154(13)	6768(5)	11398(9)	49(2)
C(12D)	-1200(20)	6370(8)	12368(14)	51(2)
C(13D)	-1010(20)	5816(8)	12094(15)	54(2)
C(14D)	-2020(20)	5638(8)	11098(15)	54(2)
C(15D)	-2320(20)	6040(8)	10416(15)	53(3)
C(16D)	-1745(19)	6546(6)	10560(15)	50(2)
C(12')	-2060(30)	6365(11)	11910(20)	52(3)
C(13')	-1820(30)	5783(11)	11780(20)	54(3)
C(14')	-610(30)	5640(10)	11180(20)	54(3)
C(15')	-270(30)	6033(10)	10460(20)	51(3)
C(16')	-150(30)	6542(9)	10780(20)	49(3)

APPENDIX D

SINGLE CRYSTAL X-RAY DATA FOR C5, C6-BIS-*EPI*-SALINOSPORAMIDE A

Table 1. Crystal data and structure refinement for DRB_HN_100927_G_SALINO_A_MINOR.

Identification code	twin5	
Empirical formula	C15 H20 Cl N O4	
Formula weight	313.77	
Temperature	110(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 10.6099(7) Å	α = 90°.
	b = 10.7142(7) Å	β = 105.557(4)°.
	c = 13.7817(8) Å	γ = 90°.
Volume	1509.26(17) $Å^3$	
Z	4	
Density (calculated)	1.381 Mg/m^3	
Absorption coefficient	2.383 mm^{-1}	
F(000)	664	
Crystal size	0.12 x 0.05 x 0.02 mm^3	
Theta range for data collection	3.33 to 59.98°.	
Index ranges	-11<=h<=11, -11<=k<=11, -15<=l<=15	
Reflections collected	9026	
Independent reflections	9031 [R(int) = 0.0000]	
Completeness to theta = 59.98°	98.1 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9539 and 0.7630	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	9031 / 1 / 384	
Goodness-of-fit on F^2	1.060	
Final R indices [I>2sigma(I)]	R1 = 0.0537, wR2 = 0.1120	
R indices (all data)	R1 = 0.0844, wR2 = 0.1229	

Absolute structure parameter	0.020(15)
Largest diff. peak and hole	0.291 and -0.311 e.Å^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for DRB_HN_100927_G_SALINO_A_MINOR. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Cl(1)	3978(1)	9644(1)	3791(1)	40(1)
Cl(2)	-168(1)	5544(1)	10015(1)	61(1)
C(1)	3584(3)	7049(3)	6308(3)	26(1)
C(2)	4235(3)	8087(3)	5862(2)	28(1)
C(3)	5180(4)	8684(3)	6773(3)	27(1)
C(4)	4859(3)	8116(3)	7725(2)	24(1)
C(5)	5435(4)	10060(3)	6728(3)	36(1)
C(6)	6239(4)	7564(3)	7944(3)	28(1)
C(7)	4796(4)	7613(3)	5018(3)	34(1)
C(8)	5267(4)	8595(3)	4411(3)	34(1)
C(9)	4501(3)	8967(3)	8496(2)	22(1)
C(10)	3934(3)	8335(3)	9279(2)	25(1)
C(11)	4940(4)	7508(3)	9954(3)	30(1)
C(12)	5137(4)	7461(4)	10945(3)	36(1)
C(13)	4355(4)	8166(4)	11513(3)	39(1)
C(14)	3128(4)	8698(4)	10804(3)	39(1)
C(15)	3435(4)	9296(3)	9905(3)	31(1)
C(16)	1818(4)	4741(4)	7907(3)	30(1)
C(17)	865(3)	4060(3)	8375(3)	28(1)
C(18)	419(3)	2960(3)	7688(2)	26(1)
C(19)	901(3)	3176(3)	6723(3)	26(1)
C(20)	-949(3)	2492(4)	7608(3)	36(1)
C(21)	1781(4)	2034(3)	7110(3)	29(1)
C(22)	1387(4)	3769(3)	9492(3)	30(1)
C(23)	1441(3)	4905(4)	10171(3)	37(1)
C(24)	-51(3)	3124(3)	5695(3)	29(1)

C(25)	505(4)	3430(3)	4788(3)	35(1)
C(26)	1247(4)	2328(4)	4507(3)	47(1)
C(27)	1114(4)	1959(4)	3573(3)	46(1)
C(28)	296(4)	2637(4)	2667(3)	42(1)
C(29)	-122(5)	3900(4)	2939(3)	53(1)
C(30)	-579(4)	3827(4)	3886(3)	39(1)
N(1)	3887(3)	7156(2)	7310(2)	24(1)
N(2)	1670(3)	4318(2)	6958(2)	29(1)
O(1)	2862(2)	6226(2)	5817(2)	31(1)
O(2)	6462(2)	8004(2)	7064(2)	32(1)
O(3)	6968(2)	6931(2)	8555(2)	35(1)
O(4)	5681(2)	9616(2)	8947(2)	28(1)
O(5)	2580(2)	5570(2)	8319(2)	34(1)
O(6)	1383(2)	1895(2)	7965(2)	30(1)
O(7)	2596(2)	1437(2)	6871(2)	37(1)
O(8)	-553(2)	1873(2)	5626(2)	36(1)

VITA

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