An Insight Into Asymmetric Synthesis and Bioorganic Applications of Novel Cα- Methyl-Lysine, - Proline, - Nipocotic Acid Analogues

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AN INSIGHT INTO ASYMMETRIC SYNTHESIS AND BIOORGANIC APPLICATIONS OF NOVEL Cα-METHYL-LYSINE, -PROLINE, -NIPECOTIC ACID ANALOGUES

by

Souvik Banerjee

Abstract of a Dissertation
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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ABSTRACT

AN INSIGHT INTO ASYMMETRIC SYNTHESIS AND BIOORGANIC APPLICATIONS OF NOVEL Cα-METHYL-LYSINE, -PROLINE, -NIPECOTIC ACID ANALOGUES

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Prochiral malonic diesters consisting of a quaternary carbon center have been successfully converted into a different set of \( \text{Boc-Fmoc-} \alpha^{2,2}\)-methyllysine-OH analogues through chiral malonic half-ester intermediates achieved via enzymatic (Pig Liver Esterase, PLE) hydrolysis. The selection of chiral half-ester intermediates, which vary from 1 to 6 methylene units in the side chain, are achieved in high optical purity (92% - 97% ee) and in good yields (65% - 72%). The PLE hydrolysis of malonic diesters with a variety of side chain lengths observed to obey the Jones’s PLE model as evidenced from the stereochemical configurations of the resulting chiral half-esters. The optimized synthetic strategy allows the construction of both enantiomers of \( \alpha^{2,2}\)-methyllysine analogues, and a (S)-\( \beta^{2,2}\)-methyllysine analogue from a common synthon by straightforward exploitation of protecting groups. Two different straightforward synthetic strategies are illustrated for the synthesis of \( \alpha^{2,2}\)-methyllysine analogues. The described strategies should find significant usefulness in preparing novel peptide libraries with unnatural lysine analogues. A Vapreotide analogue incorporating (S)-\( \alpha^{2,2}\)-methyllysine was constructed. However, the Vapreotide analogue with (S)-\( \alpha\)-methyl-\( \alpha\)-lysine is found to lose its specific binding to somatostatin receptor subtype 2 (SSTR2). In an additional project, a stereoselective and enantiodivergent cyclization strategy for the preparation of
γ/δ-lactams is exhibited. The cyclization strategy exploits chiral malonic esters prepared from enantiomerically enriched (92% ee - 97% ee) mono esters of disubstituted malonic acid. The cyclization takes place with the selective departure of a substituted benzyl alcohol as the leaving group. A Hammett study demonstrates that the cyclization is under electronic control. The resulting γ/δ-lactam was readily converted into a novel proline/nipecotic acid analogue.
The University of Southern Mississippi

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CHAPTER I

INTRODUCTION

Over the last few decades unnatural amino acids have drawn remarkable attention from researchers in diverse fields of science due to a number of reasons. Researchers have made continuous efforts to come up with different structural motifs of unnatural amino acids and have used them extensively to constitute biologically active peptidomimetic therapeutic leads due to the ability of unusual amino acids to stabilize secondary structures of peptides. However, unnatural amino acids have not only been implicated in peptidomimetics but also in construction of enzyme inhibitors, receptor antagonists, antibacterial agents and so on. Mother nature has also created several nonnatural amino acids and presented them as therapeutic leads by themselves or as an essential constituent of complex therapeutic agent structure. One of the most important factors that has propelled the nonnatural amino acids in huge demand at diverse fields of science is their conformation rigidity. In this introduction I am going to be discussing the recent utilities of nonnatural amino acids in different scientific fields and unique approaches to synthesize them.

Unnatural Amino Acids (UAAs) in Peptidomimetics

UAAs have been extensively employed in peptidomimetics in order to develop a variety of inhibitors of enzymes that are responsible for disease progression. It is evident that the presence of artificial backbones in the unnatural peptides confers a higher degree of resistance to enzymatic degradation as opposed to their natural counterparts. A few years ago, Oh et al. reported that the introduction of unnatural amino acids in anti-
microbial peptides improves their protease resistance as much as three times without affecting their activity.\(^7\)

Unnatural Amino Acids in Foldamers

Currently, scientists are highly interested in the ability of the UAAs to introduce the tendency of acquiring specific compact conformation (tertiary structure) into synthetic peptides.\(^8\)-\(^11\) Professor Gellman introduced the term "foldamers" to describe any synthetic peptide that strongly adopts the highly stable tertiary structures in organic and aqueous solutions.\(^8\) A few years ago Seebach et al. reported that the β-peptides not only have a strong tendency to form foldamers but are highly resistant to the action of proteases as well.\(^12\) Recently, Saludes et al. reported that the α/δ-peptides form stable foldamer structures in solution and exhibit two to three orders of magnitude higher half-life than α-peptides in human blood plasma.\(^13\)

Unnatural Amino Acids in Antibiotics

Mother nature has created few classes of non-proteinogenic amino acids with anti-herbicidal and antibiotic properties.\(^14\),\(^15\) One of the most important antibiotic unusual amino acids produced by Mother Nature is Furanomycine.\(^15\) Furanomycine was extracted from metabolites of *Streptomycestreomyceticus* in 1967, and this class of unusual α-amino acids (Figure 1) suppresses the growth of number of bacterial species.\(^15\)

![L-Furanomycine](image.png)

*Figure 1.* L-furanomycine, a nature made nonproteinogenic amino acid.
UAAs as Building Blocks of Complex Molecular Structures

Unnatural amino acids have often served their role as the starting materials or building blocks of advanced molecules with unique biological activity.\textsuperscript{16-18} Recently Wohlrab et al. reported total synthesis of Plusbacin A\textsubscript{3}, a depsipeptide antibiotic, containing a number of non-proteinogenic amino acids.\textsuperscript{17} Chandrashekhar et al. reported a concise total synthesis of Azumamide E, a marine cyclic terapeptide containing novel $\beta^{2,3}$-amino acids, that shows inhibitory activity to histone deacetylase.\textsuperscript{19} Recently, Konno established total synthesis of three marine natural products (miraziridine A, tokaramide A, and callipeltins) containing unusual amino acids, showing strong cysteine protease inhibitor activity.\textsuperscript{20}

UAAs as Chiral Auxiliaries and Organocatalysts

UAAs serve as chiral auxiliary/organocatalyst by themselves or as building blocks of the complex chiral auxiliary/organocatalyst structure.\textsuperscript{21-25} A few years ago, Vicario et al. pointed out nonnatural $\alpha$-amino acids, $\beta$-amino alcohols, and related compounds that have been utilized recently as chiral auxiliaries, or catalysts in the asymmetric aldol reaction.\textsuperscript{22} Barbas et al. established unnatural proline analogues as one of the most promising catalysts for the anti-Mannich type reactions.\textsuperscript{23} Barbas et al. also reported that the non-natural pipecolic acid analogues function as strong catalysts for the syn-Mannich type reactions.\textsuperscript{26} Recently Wang et al. have discovered that the $\beta$-aminoaldehyde, which is formed as the product in syn-Mannich type reactions, could be employed as an autocatalyst to drive the asymmetric syn-Mannich type reactions.\textsuperscript{24} Lately Momami et al. have exhibited a number of hydroxyl-L-proline analogues as very promising catalysts for the asymmetric aldol, Mannich, and Michael reactions.\textsuperscript{25}
Cα,α-Disubstituted Non-Proteinogenic Amino Acids

In recent years, there has been growing interest in optically pure α,α-disubstituted-α/β-nonproteinogenic amino acids in a number of fields of science.27-29 This class of sterically constrained amino acids have mostly drawn the attention of researchers in biochemical research and drug discovery.29 The reason they greatly attract biochemical researchers to the α,α-disubstituted-quaternary amino acids is that they do not undergo in vivo racimization due to absence of the Cα-hydrogen.30 This class of sterically restricted amino acids have been witnessed to strongly stabilize secondary structures of the peptides as opposed to their Cα-substituted partners.30 This class of conformationally constrained amino acids are often found in nature either in free form or as a building block of complex natural product.31 In addition, synthesis of alkaloids or other natural products consisting of amine moiety attached to a quaternary carbon center has been known to be difficult, since effective installation of such centers is greatly challenged by the steric congestion.32

Secondary Structures of the Peptides Consisting of Cα,α-Disubstituted Amino Acids

The secondary structures of peptides can be construed in term of torsion angles ψ, ϕ, ω, and the side chain conformations of the amino acids are illustrated by the torsion angles χ1, χ2, χ3 (Figure 2). The torsion angle values in protein and peptides are determined through number of experimental structural data and computational simulation. In reality, it is possible to predict the effect of conformationally restricted amino acids on the outcome of the secondary structure of a peptide by evaluating the torsion angles of the conformationally rigid amino acids.33
One of the most frequently known $C^{\alpha,\alpha}$-disubstituted amino acids is $\alpha$-aminoisobutyric acid (Aib, dimethylglycine, $\alpha$-methyl-alanine) (Figure 3). In Aib residue, substitution of the $\alpha$-hydrogen atom in alanine by a methyl group considerably restricts the available conformational space (Figure 4). It is evident from a number of experimental results that Aib induces right handed (P) and left handed (M) $3_{10}$-helical structures ($\varphi, \psi = \pm 60^0, \pm 30^0$) in a 1:1 ratio, both in solution and the crystal state. The reason behind the generation of two enantiomeric P and M-helices by Aib is that Aib is an achiral amino acid. To strongly emphasize, Aib residues neither induce semi-extended conformations nor extended conformations, unlike alanine which is found in both folded and extended conformations.
Figure 3. Structure of α,α-disubstituted amino acids and peptaibol antibiotics.

Each 3\textsubscript{10}-helix gives rise to an intramolecular hydrogen bonded ring containing 10 atoms, and one 3\textsubscript{10}-helix turn accommodates 3 amino acid residues (Figure 5).\textsuperscript{34} On the contrary, one α-helix (3.6\textsubscript{13}-helix) gives birth to an intermolecular hydrogen bonded ring consisting of 13 atoms and each α-helix turn accommodates 3.6 amino acid residues (Figure 5).\textsuperscript{34} Thus, it is conceivable that the 3\textsubscript{10}-helix is more compact than α-helix.\textsuperscript{34} In addition to Aib (achiral C\textsuperscript{α,α}-disubstituted amino acid), intensive efforts have been made by a number of research groups to explore the conformations of homo- or hetero-peptides consisting of chiral C\textsuperscript{α,α}-disubstituted amino acids (including Cα-methyl quaternary amino acids).\textsuperscript{34} The consequences of all the precedent studies are in consensus revealing that the chiral C\textsuperscript{α,α}-disubstituted amino acids induce the 3\textsubscript{10}-helix in peptides as well. However, helical screw sense (right handedness) relies on the R or S absolute configuration at the α-carbon of chiral C\textsuperscript{α,α}-disubstituted amino acids.\textsuperscript{34}
Figure 4. Newman projection exhibiting limited conformational space availability for the $C^\alpha$-$C^\alpha$-disubstituted amino acids.

Figure 5. Hydrogen bonding pattern of $3_{10}$-helix and $\alpha$-helix.
Cα,α-Nonproteinogenic Amino Acids in Peptidomimetics

Oligopeptides consisting of naturally occurring L-α-amino acids often lead to unordered or unstable secondary structures due to the conformational flexibility of natural amino acids. This is why Cα,α-disubstituted non-proteinogenic amino acids have made their strong demand in the widely extended field of preparation of peptides with the interest to humanity. Natural peptaibol antibiotics, such as anti-amoebin, alamethicin, and zervamicin, have been found to be composed of Aib residues (Figure 3). This is why α,α-disubstituted amino acids should be named as nonproteinogenic amino acids or non-coded amino acids as opposed to unnatural amino acids. Aib is one of the most widely used amino acids not only to introduce helical secondary structures into peptides but to design and synthesis of organocatalyst and drug candidates as well.

Substitution of the α-hydrogen atom in an L-α-amino acid, which results in Cα,α-disubstituted amino acid, with an alkyl moiety results in:
1. Improved chemical stability of the amino acids,
2. Improved hydrophobicity of the amino acids,
3. Constrained conformational freedom of the amino acid side chain,
4. Restricted conformation flexibility of the peptides containing them, and as a consequence enhanced metabolic stability of their peptides (Figure 1).

In recent years, a variety of Cα,α-disubstituted nonproteinogenic amino acids have been used in the synthesis of medicinally important peptides due to their propensity to stabilize secondary structures of the peptides by introducing tremendous helix inducing potential. This helix inducing propensity is considered to be capable of stabilizing the secondary structures of the peptides by rigidifying the peptide backbone. Hence, the peptides with higher conformational stability present improved resistance
against enzymatic and chemical degradations.\textsuperscript{35, 36, 41, 42} Thus, \(\alpha,\alpha\)-disubstituted amino acids have very often been introduced in the peptide synthesis to confer enhanced metabolic stability to synthetic peptides (Figure 6).\textsuperscript{3, 35-37} In addition, the remarkable helix inducing potential employed by \(C^{\alpha,\alpha}\)-disubstituted nonproteinogenic amino acids is believed to be responsible for the bacterial membrane destabilization effort produced by peptaibol antibiotics.\textsuperscript{29}

\begin{center}
\begin{tabular}{c}
\includegraphics[width=0.5\textwidth]{figure6.png}
\end{tabular}
\end{center}

**Figure 6.** Synthetic peptides consisting of \(C^{\alpha,\alpha}\)-disubstituted amino acids.\textsuperscript{35, 37}

\(C^{\alpha,\alpha}\)-Disubstituted Amino Acids in Therapeutic Leads

\(C^{\alpha,\alpha}\)-disubstituted class of nonproteinogenic amino acids has been frequently employed as a pharmaceutical active agent (enzyme inhibitors or receptor antagonist) by itself or as a building block of the complex therapeutic agent.\textsuperscript{29, 43-45} The remarkable increment in the steric congestion imparted by an additional \(\alpha\)-substituent of \(C^{\alpha,\alpha}\)-disubstituted amino acids...
acids either keeps the substrate from accessing the active site of enzyme or hinders the enzyme from initiating its catalyzing activity.\textsuperscript{29, 36, 37} Thus, conformational constraint plays an important role in order for this class of amino acids being potent inhibitors of enzymes (reversible or irreversible).\textsuperscript{36, 37, 46} Recently, Ilies et al. have developed two novel C\textsuperscript{\alpha,\alpha}-disubstituted amino acid analogues (Figure 7) as potent inhibitors of human arginase that is known to hydrolyze L-arginine to L-ornithine.\textsuperscript{44}

![Figure 7. C\textsuperscript{\alpha,\alpha}-disubstituted amino acids as potent arginase inhibitor.\textsuperscript{44}](image)

Recently, our group has illustrated how the incorporation of C\textsuperscript{\alpha}-methyl-cysteine into glutathione inhibits glutathione reductases from cleaving the disulphide bond of oxidized glutathione GSSG (Figure 6, compound 6).\textsuperscript{36} A couple of years ago Hoffmann-La Roche AG had introduced a few potent macrocyclic inhibitors of Janus Kinases (JAKs) consisting of C\textsuperscript{\alpha,\alpha}-disubstituted amino acids (Figure 8).\textsuperscript{47} Inhibitors of JAKs are used in the treatment of cancer and inflammatory diseases.\textsuperscript{47}

The restricted conformational flexibility and improved metabolic stability of C\textsuperscript{\alpha,\alpha}-disubstituted amino acids make them an important constituent in complex receptor antagonist structures.\textsuperscript{45} A few years back, a group of scientists from Meck & Co. developed a potent inhibitor of neurokinin 1 (NK1) receptor consisting of cyclic C\textsuperscript{\alpha,\alpha}-disubstituted-\beta-amino acid (Figure 9).\textsuperscript{45} This group illustrates that the C\textsuperscript{\alpha,\alpha}-disubstituted amino acid framework is important for effective inhibition.\textsuperscript{45}
Recently, Novartis AG has discovered a novel small molecule potent antagonist of S1P receptor (for the treatment of diseases caused by S1P receptor modulators) consisting of constrained C\textsuperscript{\textalpha,\textalpha}-disubstituted amino acid as an important building block (Figure 10).\textsuperscript{1}
Figure 10. S1P receptor antagonist consisting C\(^\alpha\)-methyl-\(\beta\)-proline.\(^1\)

Current State of the Art Toward Asymmetric Synthesis of C\(^{\alpha,\alpha}\)-Disubstituted Amino Acids

Although a number of synthetic strategies have been reported to date, synthesis of quaternary chiral centers is still one of the toughest challenges to synthetic organic chemists. Recently Vogt et al.\(^29\) reviewed the recent widely explored synthetic strategies, which are employed to constitute quaternary chiral centers for the preparation of C\(^{\alpha,\alpha}\)-disubstituted amino acids as follows

1. Asymmetric Strecker Reaction Involving Aldimines or Ketimines or Strecker Related Reactions (Scheme 1).\(^{28}\)

Scheme 1. Asymmetric Strecker reaction mediated trough ketimine.\(^{28}\)
2. Electrophilic Alkylation of the Enolates Resulting from Oxazinones, Oxazolines, Oxazolidines, Azalactones, or Imines Derived from Amino Acids as Chiral Auxiliaries (Scheme 2).\(^{30}\)

![Scheme 2. Oxazinones as chiral auxiliaries.\(^{30}\)](image)

3. Electrophilic Alkylation of Chiral Imine Attached to an Oxazinone (Scheme 3).\(^{48}\)

![Scheme 3. Electrophilic alkylation of chiral imine.\(^{48}\)](image)
4. Organo Catalyzed Electrophilic $\alpha$-Amination of the $\alpha$-Substituted Carbonyl Compounds (Scheme 4).\textsuperscript{49}

\textbf{Scheme 4.} Organocatalyzed electrophilic $\alpha$-amination.\textsuperscript{49}

5. Phase Transfer Catalyst Mediated Electrophilic Alkylation of Schiff Bases Derived from Amino Acids (Scheme 5).\textsuperscript{29}

\textbf{Scheme 5.} PTC catalyzed electrophilic alkylation of Schiff bases.\textsuperscript{29}

6. Nucleophilic Addition to C-N Multiple Bond Leads to the Synthesis of C$^{\alpha,\alpha}$-Disubstituted Amino Acids (Scheme 6).\textsuperscript{50, 51}
Scheme 6. Nucleophilic addition to C-N multiple bonds.\textsuperscript{50, 51}

7. Synthesis of C\textsuperscript{\alpha,\alpha}-Disubstituted-Amino Acids through Stereospecific Ring Opening of Epoxides or Aziridines and Rearrangement Reaction (Scheme 7).\textsuperscript{52}

Scheme 7. Stereospecific ring opening of epoxide leading to the formation of C\textsuperscript{\alpha,\alpha}-disubstituted-amino acids.\textsuperscript{52}
In continuation, Smith et al. presented the synthesis of several α,α-disubstituted-α-amino acids from a common intermediate employing nucleophilic “O- Alkyl Fission” ring opening of the NBn$_2$-α-methylserine lactone, using various organocuprates (Scheme 8). Green et al. reported a novel strategy to prepare C$^{\alpha,\alpha}$-disubstituted amino acids through a Mitsunobu approach starting with optically pure α,α-disubstituted-α-hydroxy ester (Scheme 9). Cabrera et al. optimized a unique strategy implicating organocatalyzed Michael addition of oxazolone enolates to the Michael acceptors (Scheme 10). Recently, Hartmann et al. reported the synthesis of optically enriched α-methyl phenylglycine through L-proline catalyzed amination of racemic 2-arylpropionaldehydes, using DEAD and DBAD (Scheme 11).

Scheme 8. O-alkyl fission ring opening of β-lactone. 

Scheme 9. Mitsunobu approach to prepare C$^{\alpha,\alpha}$-disubstituted amino acids.
Scheme 10. Organocatalyzed Michael addition leading to $\text{C}^{\alpha,\alpha}$-disubstituted amino acid.$^{42}$

$$\text{Proline catalyzed Michael addition}$$

$$\begin{align*}
\text{R}_1\text{=alkyl or aryl group} \\
\text{R}_2=\text{alkyl or aryl group}
\end{align*}$$

Scheme 11. L-proline catalyzed $\alpha$-sulphamidation of $\alpha,\alpha$-disubstituted aldehydes.$^{55}$

Pig Liver Esterase (PLE) Desymmetrization Approach to Prepare $\text{C}^{\alpha,\alpha}$-Disubstituted Amino Acids

Although numerous synthetic methodologies are established to construct $\alpha,\alpha$-disubstituted-$\alpha$-amino acids, most often they rely on expensive chiral auxiliaries. Most of the time, different chiral auxiliaries are required to produce different stereoisomers of the same $\alpha,\alpha$-disubstituted amino acids. In addition, there are very few synthetic strategies allowing the constitution of both $-\alpha$- and $\beta$- amino acid from a common synthon. This is why Pig Liver Esterase (PLE) is used. PLE is cheap and has proven its excellence in hydrolyzing a wide variety of prochiral quaternary malonic diesters to the corresponding optically enriched $\alpha,\alpha$-disubstituted malonic half-esters, which have been extensively employed as ideal precursors of $\alpha,\alpha$-disubstituted amino acids.$^3,32,37,46,56,57$ To the best of our knowledge, Kedrowski$^{46}$ is the first to report both enantiomers of orthogonally
protected α-methyl-α-cysteine from the common optically enriched quaternary malonic half-ester, derived from PLE hydrolysis of prochiral dimethyl malonate (Scheme 12). Very recently Iosub et al.\textsuperscript{58} has also shown the synthesis of α,α-disubstituted-α-amino acid from enantiomerically enriched PLE hydrolyzed methyl malonic half-ester.

However, dimethyl-2-methyl malonate that is used for enolization is expensive compared to diethyl-2-methyl malonate. Masterson et al. have recently reported synthesis of both (R)- and (S)-α\textsuperscript{2,2}, β\textsuperscript{2,2}, and β\textsuperscript{3,3}-Cysteine and serine analogues from the common PLE hydrolyzed optically enriched quaternary ethyl malonic half-esters with respective amino acid side chains (Scheme 13).\textsuperscript{3} Recently, Falgner et al.\textsuperscript{56} have also established the synthesis of both (R)-and (S)-α-methyl-trimethylsilyl alanine with excellent enantio purity starting with PLE desymmetrized ethyl malonic half-ester and the same manipulation of protecting group as Kedrowski and Masterson reported earlier.\textsuperscript{3,46}

\begin{center}
\includegraphics[width=\textwidth]{scheme12.png}
\end{center}

\textit{Scheme 12. PLE desymmetrization approach by Kedrowski.}\textsuperscript{46}
Scheme 13. Strategy of Masterson et al.\textsuperscript{3}
CHAPTER II

PIG LIVER ESTERASE DESYMMETRIZATION APPROACH TO PREPARE DIVERSE ORTHOGONALLY PROTECTED C$^{\alpha,\alpha}$-DISUBSTITUTED LYSINE ANALOGUES

Background

The growing importance of C$^{\alpha,\alpha}$-disubstituted class of non-proteinogenic amino acids in various fields has drawn the interest of synthetic organic chemists over the last two decades.\textsuperscript{3, 46, 59} This class of sterically constrained amino acids, which consist of quaternary carbon center, have exhibited enhanced chemical stability, improved hydrophobicity, conformational inflexibility of the amino acid side chain, and thus, restricted conformational flexibility of the peptides containing them.\textsuperscript{34} This class of quaternary amino acids are of the most frequently and widely used structural motifs in peptidomimetics, since they introduce enormous helix inducing potential into the peptides to acquire stable secondary structures.\textsuperscript{3, 29, 34, 36, 37} The precedent experimental reports suggest that the peptides consisting of $\alpha,\alpha$-disubstituted amino acids achieve more stable secondary structure ($3_{10}$-helix) with rigid backbone in comparison to those containing natural amino acids.\textsuperscript{34} The propensity of C$^{\alpha,\alpha}$-disubstituted amino acids to impart profound helix inducing potential in the peptides is found to be the causative reason for the membrane destroying impact exerted by peptaibol class of peptide broad spectrum antibiotics.\textsuperscript{29, 34} This class of sterically congested amino acids has also been widely employed as enzyme inhibitors by themselves or a crucial moiety of the complex inhibitor structures.\textsuperscript{35, 36} Previous experimental evidences strongly suggests that an additional $\alpha$-substituent in C$^{\alpha,\alpha}$-disubstituted amino acids confer enough steric
congestion to keep the substrates from reaching the enzyme active site. Thus, the above examples reveal the growing interest in preparation of the $\text{C}^{\alpha,\alpha}$-disubstituted amino acids in the medicinal chemistry community.

Although a number of synthetic strategies have been reported to prepare different optically enriched $\alpha,\alpha$-disubstituted amino acids, very few reports exist on the $\text{C}^{\alpha,\alpha}$-disubstituted lysine analogues. Lysine is an essential amino acid and is one of the mandatory building blocks in number of biologically active peptides for their function. However, peptides consisting of lysine are often susceptible to degradation by serine like proteases. Hence, organic chemists have been exploring more options to prepare $\text{C}^{\alpha,\alpha}$-disubstituted-lysine analogues to derive protease resistant peptides. Recently, Berkowitz et al. have discovered that unlike D-lysine, $\alpha$-vinyl-lysine strongly behaves as an inhibitor of lysine decarboxylase. Few years back, Jones et al. reported that $\text{C}^{\alpha,\alpha}$-disubstituted malonic diesters carrying lysine amino acid side chain functionality does not undergo hydrolysis with trypsin.

All the above examples point to the growing interest in $\alpha,\alpha$-disubstituted lysine analogues. However, appropriate protection of two nitrogen atoms in lysine to prepare it for solid phase polypeptide synthesis has come out as a challenging task to synthetic chemists. Few years ago Seebach et al. reported the synthesis of $\alpha$-methyl-$\alpha$-lysine analogues in free diamino form employing self-regeneration of stereo center (SRS) principle (Scheme 14). However, the Seebach strategy allows us to synthesize only $\alpha$-methyl-$\alpha$-lysine in low yield. Recently, Cativiela et al. reported the synthesis of (S)-$\alpha$-methyl-$\alpha$-lysine via chiral cyanopropanoate using a chiral auxiliary in ten steps, but this strategy also let us synthesize only (S)-$\alpha$-methyl-$\alpha$-lysine in unprotected form (Scheme
15). To the best of our knowledge Chauhan is the first to report 1Boc-Fmoc protected (S)-α-methyl-α-lysine using William’s Oxazinone as a chiral auxiliary in eight steps with overall 26% yield and 95% optical purity (Scheme 16). However, this methodology needs to be using expensive chiral auxiliary, and the same auxiliary cannot be utilized to result in both (R)- and (S)-α-methyl-α-lysine derivative. In addition, this methodology requires the use of explosive azide to attain lysine side chain functionality, hence, could be dangerous in scale up batch. Moreover, this methodology has been able to obtain the final product in only 90% purity, what in addition would be problematic for the incorporation of the amino acid into a peptide.

**Scheme 14.** Seebach’s strategy to prepare optically enriched α,α-disubstituted-lysine.59

**Scheme 15.** Strategy of Cativiela et al to prepare (S)-α-methyl-α-lysine.60
Scheme 16. Chauhan’s strategy to prepare protected C$_{\alpha}$-methyllysine analogue.$^{30}$

Hence, all the previously reported synthetic strategies to prepare C$_{\alpha,\alpha}$-disubstituted lysine analogues indicate that there is a lack of a synthetic strategy that is capable of deriving diverse orthogonally protected C$_{\alpha,\alpha}$-lysine analogues from common intermediate types. To the best of our knowledge, Masterson et al. is the first group to report diverse cysteine and serine analogues from a common intermediate type without using expensive chiral auxiliaries (Scheme 13).$^{3}$ Hence, based on the previous success of our group we have drawn our first hypothesis, which is as follows:

**Hypothesis 1.**

Our unique enantiodivergent synthetic strategy allows us to derive a variety of orthogonally protected C$_{\alpha,\alpha}$-disubstituted-$\alpha$/-$\beta$-lysine analogues and prepare both enantiomers of C$_{\alpha}$-methyl-$\alpha$-lysine from Pig Liver Esterase desymmetrized optically enriched common intermediate types (Scheme 17).
I have developed a PLE catalyzed enzymatic desymmetrized approach to prepare both orthogonally protected (R)- and (S)-α-methyl-α-lysine, orthogonally protected (S)-2,3-diaminopropanoic acid, and orthogonally protected (S)-α-methyl-β²,²-lysine analogue from the optically enriched (52%~97% ee) half-esters (common intermediate types). The optimized synthetic strategy is convenient and flexible, allowing for the alteration of the side chain of lysine analogues from one to six methylene units, construct both enantiomers of an α²,²-methyllysine analogue from the same common intermediate. In continuation, the same synthetic strategy allows to homologate the (S)-α²,²-methyllysine to the relevant (S)-β²,²-methyllysine. I have optimized two different synthetic strategies to obtain orthogonally protected α²,²-lysine analogues from chiral malonic half-esters in good yield. One of the synthetic strategies consists of seven steps (long path) and the other consists of total three steps (Short Path) from PLE derived chiral malonic half-ester with lysine side chain functionality. The short path, which I have recently achieved, is the most concise strategy to date. Scheme 17 illustrates the convergent strategy to synthesize diverse Cα-methyl lysine analogues from a common synthon. I altered the side chain length of lysine in order to explore the effect of chain length in PLE hydrolysis ⁷¹, ⁷².
Results and Discussion

Synthesis of optically enriched half-ester intermediates: The prochiral malonic diesters (9a-f) were prepared by electrophilic alkylation of diethyl-2-methylmalonate with the appropriate N-(bromoalkyl)-phthalimide as shown in Scheme 18. The consequential diesters, with the exception of 9b, were purified and isolated in good yield (65% - 71%). The poor yield of 9b was due to the dehydrohalogenation of 8b as evidenced by isolation of significant quantities of alkene (51%). Compounds 9a-9f were subjected to enzymatic hydrolysis using crude PLE at pH 7.4. The hydrolysis resulted in enantiomerically enriched half-esters 10a-10f in good isolated yields as shown in Scheme 18. Interestingly, PLE was observed to provide 10a-f predominantly of the (R)-enantiomer with significant optical activity in all cases.
Scheme 18. Synthesis of optically enriched half-esters (10a - 10f). \(^{73}\)

Half-esters 10a-10f were successfully resolved employing chiral HPLC techniques and the enantioselectivity was determined by integration of the relevant chromatographic peaks (Figure 11). The chiral HPLC chromatograms of the half-esters were matched to those of racemic standards of 10a-10f prepared by standard non-enzymatic strategy.

The stereochemical configuration of the major enantiomer of 10a was determined to have the (R) absolute stereochemistry as shown in Scheme 19. \(^{74}\) The absolute rotation of the half-ester 17, which was obtained from the half-ester 12 by synthesis, was compared with the one obtained from 10a. The stereochemical configuration of the major enantiomer of 10b was determined by synthesis \(^{75}\) as shown in Scheme 20. The optical activity of compound 22 was compared to literature values in order to establish the absolute configuration of 10b. The configurations of 10c and 10d were determined by conversion into 24a and 24b as shown in Scheme 21. The optical rotations of 24a and 24b were compared with literature values in order to determine the stereochemical configurations of 10c and 10d. \(^{59}\) The stereochemical configurations of 10e and 10f were
also determined by synthetic means as shown in Scheme 22. The half-esters 10e and 10f were converted into α,α-disubstituted amino acids 28a and 28b to compare their absolute rotation to the literature values.

![Chiral HPLC chromatograms](image)

Figure 11. Chiral HPLC chromatograms of few half-esters (10b and 10d).
Scheme 19. Absolute configuration of 10a.\textsuperscript{74}

Scheme 20. Absolute configuration of 10b.\textsuperscript{73}
Scheme 21. Absolute stereochemical configuration of 10c and 10d.\textsuperscript{73}

\[ \text{(R)} \]

10c (97% ee): n = 3
10d (95% ee): n = 4

23c: n = 3
23d: n = 4

\[ (S)-\text{α-methyl-ornithinedihydrochloride} \]

\[ \text{(S)-α-methyl-lysinedihydrochloride} \]

Scheme 22. Absolute stereochemical configuration of 10e and 10f.\textsuperscript{73}

\[ \text{(R)} \]

10e (81% ee): n = 5
10f (64% ee): n = 6

25a: n = 5
25b: n = 6

26a: n = 5
26b: n = 6

\[ (S)-\text{α-hexyl-alanine} \]

\[ (S)-\text{α-pentyl-alanine} \]
Insight into PLE Biocatalytic Hydrolysis of the Prochiral Diesters (9a-9f)

It is evident from the Figure 12 and Figure 13 that the PLE hydrolysis of diesters 9a-9f obey the Jones Active Site Model (JASM) (Figure 13). JASM is a 3D model, which is not shown here. PLE hydrolysis of diester 9c results in the highest level of optical purity. I hypothesize that the size of the side chain of 9c closely matches with the size of the large hydrophobic pocket (H_L) in the JASM. The other prochiral diesters having relative disparity of size with the large hydrophobic pocket of the JASM results in diminished enantioselectivity with respect to 9c.

Figure 12. PLE hydrolysis assay of 10a-10f.

Figure 13. Jones active site model for Pig Liver Esterase.
Conversion of half-esters (10a-10f) into Moz protected carbamates: The acid-esters (10a-10f) were subjected to the Curtius rearrangement resulting in Moz-protected (S)-α²,²-carbamates (23a-23f) in good isolated yields as shown in Scheme 23. Compounds 23a-23f can be taken into consideration as fully protected non-proteinogenic amino acids.

Scheme 23. Conversion of the half-esters (10a-10f) into fully protected amino acids.
Synthesis of Orthogonally Protected (S)-Fmoc-α^{2,2}-Lysine-Boc-OH (Long Path)

Scheme 24. Synthesis of orthogonally protected (S)-α^{2,2}-lysine analogue.

Scheme 24 illustrate the synthesis of (S)-α^{2,2}-Fmoc-lysine-Boc-OH (34) in seven steps starting with the optically enriched 10d with good isolated yield. In the first step the chiral half-ester (10d) was subject to a Curtius rearrangement producing the Moz protected α-amino ester (23d) in good isolated yield. The carbamate (23d) was then treated with TFA in methylene chloride in order to chemoselectively deprotect the Moz group leading to free α-amine (29). Compound 29 was then subject to dibenzylation using excess BnBr and K₂CO₃ under reflux for 48 hours preparing dibenzylated-α-amine (30). The simple base hydrolysis of 30 using 8N NaOH/EtOH over 72 hours resulted in saponification of the ethyl ester and deprotection of the phthalimido group in a single step leading to free amino acid (31) in good yield. The free amine (31) was then selectively
protected with the Boc group using Boc anhydride and NaHCO$_3$ in a H$_2$O/Dioxane system producing 32 in good isolated yield. The chemoselective hydrogenolysis of 32 led to the α-free amino acid 33 in nearly quantitative yield. The free α-amino acid (33) was reprotected with Fmoc group leading to tBoc, fmoc protected (S)-α$_{2,2}$-Lysine analogue (34) with good reproducible isolated yields in overall seven steps from the chiral half-ester.

*Synthesis of (S)-Fmoc-α$_{2,2}$-Lysine-Boc-OH (Short Path)*

![Scheme 25: Short path synthesis of (S)-Fmoc-α$_{2,2}$-lysine-Boc-OH analogue.](image)

*Scheme 25. Short path synthesis of (S)-Fmoc-α$_{2,2}$-lysine-Boc-OH analogue.*

The short path synthetic strategy (Scheme 25) leads to the tBoc-Fmoc-(S)-α$_{2,2}$-lysine (34) in three steps starting with optically enriched 10d. The bulky quaternary chiral half-ester (10d) was directly converted to the Fmoc protected carbamate (35) by Ti (IV) isopropoxide catalyzed Curtius rearrangement with good isolated yield. The acid
hydrolysis, that was employed in 5N HCl/dioxane system under reflux, of the carbamate (35) hydrolyzed both phthalimido group and ethyl ester leading to the free amino acid (36), as evident by ESI-MS. The crude free amino acid (36) was undertaken for re-protection of the side chain amine by the Boc group without further purification. Eventually, the 'Boc-Fmoc-(S)-α^{2,2}-lysine-OH (34) is obtained in three steps. Hence, the short path synthetic strategy is the most concise way to synthesize 'Boc-Fmoc-(S)-α^{2,2}-Lysine-OH.\(^{30}\)

**Preparation of (R)-Fmoc-α^{2,2}-Lysine-Boc-OH Analogue**

![Scheme 26](image)

**(a):** isobutylene, H\(_2\)SO\(_4\), RT (b): N\(_2\)H\(_4\), H\(_2\)O, MeOH, reflux (c): LiOH, EtOH/H\(_2\)O, RT (d): (Boc)\(_2\)O, NaHCO\(_3\), (e): 1. Et\(_3\)N, DPPA 2. 9-fluorenylmethanol, Ti (iv) isopropoxide (cat), reflux (f): 1. TFA 2. (Boc)\(_2\)O, NaHCO\(_3\)

**Scheme 26. Synthesis of (R)-Fmoc-α^{2,2}-Lysine-Boc-OH.**\(^{73}\)

In order to achieve the synthesis of 42, the chiral half-ester 10d was turned into the mixed diester 37.\(^3\),\(^{56}\) Compound 37 was subjected to selective deprotection of the phthalimide group producing 38. Compound 38 was saponified resulting in 39 in
excellent yield. Amino acid 39 was transformed into 40 using standard reaction conditions. The sterically restricted carboxylic acid 40 was then converted into Fmoc protected α-amino ester 41 in good yield employing Curtius rearrangement using DPPA and 9-fluorenymethanol in presence of catalytic Ti (IV) isopropoxide. However, the chemoselective deprotection of the tert-butyl ester in the presence of Boc did not work out in our hands using known literature procedures. This failure is attributed to the inaccessibility of the sterically hindered ester by those reagents. Hence, we had to treat the amino ester 41 with TFA to deprotect both the tert-butyl and Boc groups followed by treatment with (Boc)₂O. However, this deprotection and reprotection was a one pot strategy that gave birth to the (R)-α²,²-2-methyllysine analogue 42 in eight steps in reasonable overall yield (30%).

**Synthesis of Orthogonally Protected (S)-α²,²-2,3-Diaminopropanoic Acid**

Scheme 27 describes the synthesis of orthogonaly protected (S)-α²,²-2,3-diaminopropanoic acid (49) in eight steps starting with optically enriched half-ester 10a in good isolated yield. At first the chiral half-ester (10a) was subjected to a Curtius rearrangement resulting in the Moz protected α-amino ester (23a). The carbamate (23a) was then allowed to react with TFA to chemoselectively deprotect the Moz group resulting in 43. Compound 43 was then allowed to undergo a dibenzylation using excess BnBr and K₂CO₃ at solvent reflux for 48 hours leading to 44. However, standard base hydrolysis failed to drive the deprotection of the phthalimide group along with ester saponification in a single step starting with the α-dibenzylated aminoester (44). This failure was considered to be due to the closeness of phthalimido group to the bulky quaternary center. However, the chemoselective cleavage of the phthalimide of the
dibenzylated amino ester (44) employing hydrazine resolved the problem providing access to 45. Saponification of 45 results in the desired 46. The free amino acid (46) was then taken up for a selective installation of Boc group using Boc anhydride and NaHCO₃ in H₂O/Dioxane system producing 47 in good isolated yield. The selective hydrogenolysis of 47 resulted in the α-free amino acid 48 in near quantitative yield. The free α-amino acid (48) was reprotected with the Fmoc group leading to tBoc, Fmoc protected amino acid analogue (49) in total ten steps in 14% overall yield.

Scheme 27. Synthesis of orthogonally protected (S)-α^{2,2}-2,3-diaminopropanoic acid.
Based on precedent literature, Nadir et al.\textsuperscript{61} is the first group to report the 2,3-diaminopropanoic acid in the free form (unprotected form of 49), which is not convenient in terms of solid phase peptide synthesis (SPPS). This success allowed us to synthesize (S)-2,3-diaminopropanoic acid appropriate for SPPS in eight steps starting with optically enriched chiral half ester 10a. Additionally, we have recently presented that the optical purity of the acid-ester (10a) could be further enhanced to 95\% ee by substituting the crude PLE with PLE Isoenzyme 1, and 2\% EtOH as a co-solvent in the biocatalytic hydrolysis of 9a.\textsuperscript{74} Hence, this established synthetic strategy is capable of providing access to (S)-2,3-diaminopropanoic acid in high optical purity, and in properly protected form for SPPS.

*Synthesis of (S)-Fmoc-β\textsuperscript{2,2}-Methyllysine-Boc-OH*

![Scheme 28. Synthesis of orthogonally protected (S)-β\textsuperscript{2,2}-methyllysine analogue.\textsuperscript{73}](image-url)
Scheme 28 describes the synthesis of orthogonally protected \((S)\)-\(\beta^{2,2}\)-methyllysine (53) in eight steps starting with optically pure half-ester 3d. The chiral half-ester (40), that was achieved from 3d following Scheme 8, was transformed into diazoketone (50) using standard procedures. The diazoketone (50) was then taken for photolysis resulting in the \(\gamma\)-keto acid (51). The \(\gamma\)-keto acid (51) was then allowed to undergo a Curtius rearrangement leading to the the Fmoc protected \(\beta\)-amino ester (52). \(\beta\)-amino ester 52 was eventually converted into the \(^1\)Boc-Fmoc-(\(S\))-\(\beta^{2,2}\)-methyllysine (53) using well established procedures.

Conclusions

I have optimized two convenient straightforward synthetic strategies to derive a variety of orthogonally protected \(\alpha^{2,2}\), and \(\beta^{2,2}\)-methyllysine analogues mediated through inexpensive PLE hydrolysis derived acid-ester intermediates. This developed technique does not necessarily require expensive chiral auxiliaries and reagents to introduce the needed chiral quaternary carbon center. In addition, this enantiodivergent methodology permits to prepare both D and L- isomers of the orthogonally protected \(\alpha^{2,2}\)-lysine-OH starting with the enantiomerically enriched common synthon by simple manipulation of the protecting groups. To the best of my knowledge, this is the first time synthesis of such diverse lysine analogues were made possible in properly protected form through a common and simple synthetic strategy. Additionally, Scheme 22 has made available the previously difficult to synthesize \(\alpha,\alpha\)-disubstituted amino acids containing hydrophobic side chain in moderate to high % ee via a straightforward reductive deamination procedure.
Experimental

General Methods: THF, CH₂Cl₂, and DMF were dried by passage through a column of activated alumina. All reagents were used as received from commercial sources unless otherwise stated. Melting points were determined in open capillary tubes and are uncorrected. P-60 silica gel was used to conduct flash chromatography. Silica pre-coated TLC plates were used to perform TLC analysis. Normal phase pre-coated silica rotors were chosen to perform radial chromatography. NMR was obtained using 400 MHz Bruker or 300 MHz Varian instruments. MS was obtained using ESI/FTICR-MS, and low resolution MS was obtained by ESI/ion trap. IR was conducted on Thermo Nicolet nexus 470 FT-IR instrument. Pig Liver Esterase (PLE) is the commercially available crude preparation.

General Experimental Procedure for the Synthesis of Malonate Esters (9a-9f)

A 250 mL roundbottom 3-neck flask fitted with a nitrogen inlet, an addition funnel, and a reflux condenser was charged with 1.2 eq. of NaH (60% dispersion in mineral oil), a stirbar, and 100 mL of dry THF. The resulting suspension was cooled to 0 °C in an icebath. A 50 mL solution of diethyl-2-methylmalonate (1eq) in THF was added over 30 min with stirring. The reaction mixture was then allowed to stir for 60 min at room temperature. A 100 mL solution of N-(bromoalkyl)-phthalimide (1eq) in THF was added over 30 min with stirring. The reacton mixture was then heated to reflux solvent for 12 h. The solution was cooled to RT, diluted with ether (300 mL), washed twice with 1 N HCl, washed with brine and dried over MgSO₄. The resulting suspension was then filtered and the solvent was evaporated "in vacuo". The resulting yellowish liquid was purified by flash chromatography.
Diethyl 2-(N-Methylphthalimido)-2-Methyl Malonate (9a)

9a was prepared following the general procedure for the formation of diester (9a-9f) with 10g (57.4 mmol) diethyl-2-methylmalonate. The resulting yellowish liquid was purified by flash chromatography (30:70 EtOAc/Hexanes), giving the pure product as a white solid (12.5g, 65%).[^74] Rf 0.54 (30:70 EtOAc/hexanes), MP= 89 °C. ^1H-NMR (300 MHz, CDCl3): 7.84 (m, 2H), 7.73 (m 2H), 4.27 (s, 2H), 4.26 (q, 4H, J= 7 Hz), 1.41 (s, 3H), 1.30 (t, 6H, J=7 Hz), ^13C-NMR (75 MHz, CDCl3): 14.0, 18.3, 42.0, 54.0, 62.0, 123.0, 132.0, 134.7, 168.5, 170.5. HRMS (C_{17}H_{19}NO_{6}Na^+): calculated = 356.1104, found = 356.1100.

Diethyl 2-(N-Ethylphthalimido)-2-Methyl Malonate (9b)

9b was prepared following the general procedure for the formation of diester (9a-9f) with 10g (57.4 mmol) diethyl-2-methylmalonate, 14.6g (57.4 mmol) N-(bromoethyl)phthalimide, and 2.74g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (18:82 EtOAc/Hexanes), giving the pure product as a white solid (9g, 45%).[^84]

Diethyl 2-(N-Propylphthalimido)-2-Methyl Malonate (9c)

9c was prepared following the general procedure for the formation of diester (9a-9f) with 10g (57.4 mmol) diethyl-2-methylmalonate, 15.4g (57.4 mmol) N-(bromopropyl)-phthalimide and 2.74g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (30:70 EtOAc/Hexanes), giving the pure product as a colorless liquid (12.8g, 62%). Rf = 0.36 (30% EtOAc/Hexanes). IR (cm⁻¹) = 2981, 1772, 1705, 1614. ^1H-NMR (CDCl3, 400 MHz): δ 7.84 (m, 2H), 7.72 (m, 2H), 4.16 (q, 4H, J = 7Hz), 3.69 (t, 2H, J = 7Hz), 1.91 (m, 2H), 1.67 (m, 2H), 1.39 (s, 3H), 1.23 (t, 6H,
$J = 7\text{Hz}$). $^{13}\text{C}$- NMR (CDCl$_3$, 100 MHz): $\delta$ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 53.0, 38.0, 32.5, 23.3, 20.0, 14.0. HRMS (C$_{10}$H$_{23}$NO$_6$Na$^+$) calculated = 384.1423, found = 384.1406.

*Diethyl 2-(N-Butylphthalimido)-2-Methyl Malonate (9d)*

9d was prepared following the general procedure for the formation of diester (9a-9f) with 10g (57.4 mmol) diethyl-2-methylmalonate, 16.2g (57.4 mmol) N-(bromobutyl)-phthalimide and 2.74g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (30:70 EtOAc/Hexanes), giving the pure product as a white solid (15.2g, 70%). $R_f = 0.40$ (30% EtOAc/Hexanes). IR (cm$^{-1}$) = 2950, 1702. MP = 48$^\circ$C.

$^1$H- NMR (CDCl$_3$, 300 MHz): $\delta$ 7.85 (m, 2H), 7.71 (m, 2H), 4.17 (q, 4H, $J = 7\text{Hz}$), 3.67 (t, 2H, $J = 7\text{Hz}$), 1.90 (m, 2H), 1.69 (m, 2H), 1.39 (s, 3H), 1.27 (m, 8H). $^{13}\text{C}$-NMR (CDCl$_3$, 75 MHz): $\delta$ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 53.0, 38.0, 35.0, 29.0, 22.0, 20.0, 14.0. HRMS (C$_{20}$H$_{25}$NO$_6$Na$^+$) calculated = 398.1574, found = 398.1573.

*Diethyl 2-(N-Pentylphthalimido)-2-Methyl Malonate (9e)*

9e was prepared following the general procedure for the formation of diester (9a-9f) with 10g (57.4 mmol) diethyl-2-methylmalonate, 17g (57.4 mmol) N-(bromopentyl)-phthalimide and 2.74g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (30:70 EtOAc/Hexanes), giving the pure product as a colorless liquid (14.8g, 66%). $R_f = 0.42$ (30% EtOAc/Hexanes). IR (cm$^{-1}$) = 2938, 1772, 1706. $^1$H- NMR (CDCl$_3$, 300 MHz): $\delta$ 7.83 (m, 2H), 7.71 (m, 2H), 4.17 (q, 4H, $J = 7\text{Hz}$), 3.68 (t, 2H, $J = 7\text{Hz}$), 1.82 (m, 2H), 1.67 (m, 2H), 1.30 (m, 13H). $^{13}\text{C}$-NMR (CDCl$_3$, 75 MHz): $\delta$ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 54.0, 38.2, 35.2, 28.5, 27.0, 24.0, 20.0, 14.0. HRMS (C$_{21}$H$_{27}$NO$_6$Na$^+$) calculated = 412.1730, found = 412.1728.
Diethyl 2-(N-Hexylyphthalimido)-2-Methyl Malonate (9f)

9f was prepared following the general procedure for the formation of diester (9a-9f) with 10g (57.4 mmol) diethyl-2-methylmalonate, 17.8g (57.4 mmol) N-(bromopentyl)-phthalimide and 2.74g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (40:60 Et₂O/Hexanes), giving the pure product as a colorless liquid (16.5g, 71%). R<sub>f</sub> = 0.44 (30% EtOAc/Hexanes). IR (cm<sup>-1</sup>) = 2940, 1702.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz): δ 7.83 (m, 2H), 7.71 (m, 2H), 4.16 (m, 4H), 3.67 (m, 2H), 1.84 (m, 2H), 1.66 (m, 2H), 1.36 (m, 7H), 1.24 (m, 8H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz): δ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 54.0, 38.0, 35.0, 29.0, 28.0, 27.0, 24.0, 20.0, 14.0.

HRMS (C<sub>21</sub>H<sub>27</sub>NO<sub>6</sub>Na<sup>+</sup>) calculated = 426.1893, found = 426.1894.

General Experimental Procedure for the Formation of Chiral Half-Esters (10a-10f)

10g (1 eq) of the appropriate malonate (9a-9f) was dispersed in 1000 mL of rapidly stirring phosphate buffer (0.1N, pH 7.4) containing 2% (vol/vol)EtOH as a cosolvent. The pH was maintained using an autotitrator set to maintain a pH of 7.4 and titrate to a volume of 1 eq NaOH (1.06M). PLE (27 units/mg, 90 units per mmol of the substrate) was added and the titration was started. The hydrolysis proceeded for 1-6 days depending on substrate. The reaction was stopped when 1 eq. of NaOH was added. The reaction mixture was extracted three times with 500 mL of Et₂O. The aqueous layer was then acidified to pH = 1 using 12 M HCl, extracted eight times with Et₂O. The organic layer was dried over MgSO₄, filtered, and concentrated in vacuo.

(R)-2-(N-Methylphthalimido)-3-Ethoxy-2-Methyl-3-Oxopropanoic Acid (10a)

10a was prepared following the general procedure for the formation of half-esters (10a-10f) with 10g (30 mmol) of 9a. An amount of 6g (65%) of 10a was obtained as a
white solid in 52% ee.\textsuperscript{74} MP = 148 °C. R\textsubscript{f} = 0.31 (40:60 EtOAc/hexanes). \textsuperscript{1}H-NMR (300 MHz, CDCl\textsubscript{3}): 9.63 (s, 1H), 7.86 (m, 2H), 7.74 (m, 2H), 4.29 (m, 4H), 1.48 (s, 3H), 1.34 (t, 3H, J = 7 Hz), \textsuperscript{13}C-NMR (75 MHz, CD\textsubscript{3}OD) 14.0, 19.0, 42.0, 53.0, 62.0, 124.0, 133.0, 135.0, 170.0, 174.0, 175.0. HRMS (C\textsubscript{15}H\textsubscript{15}NO\textsubscript{6}Na\textsuperscript{+}) calculated = 328.0791, found = 328.0787. The % ee was determined by chiral HPLC (Chiralcel OJ-H, 305 nm, 5% iPrOH/hexane) Rt\textsubscript{(S)} = 33 min, Rt\textsubscript{(R)} = 47.8 min (52% ee). [\alpha]\textsubscript{D}\textsuperscript{22} = +5.2 (c = 1, MeOH).

(R)-2-(N-ethylphthalimido)-3-ethoxy-2-methyl-3-oxopropanoic acid (10b): 10b was prepared following the general procedure for the formation of half-esters (10a-10f) with 10g (29 mmol) of 9b. An amount of 7.2g (71%) of 10b was obtained as a white solid in 92% ee.\textsuperscript{84}

(R)-2-(N-Propylphthalimido)-3-Ethoxy-2-Methyl-3-Oxopropanoic Acid (10c)

10c was prepared following the general procedure for the formation of half-esters (10a-10f) with 10g (28 mmol) of 9c. The resulting half-ester was purified by flash chromatography (40:60 EtOAc/Hexanes) giving the product as a colorless liquid (6.4g, 68%). The % ee was determined to be 97% by chiral HPLC (Diacel Chiralpak OJ-H, 4% iPrOH/Hexanes, flow rate = 1 mL/min, λ = 305 nm) Rt\textsubscript{(S)} = 54.9 min (Area = 130.13), Rt\textsubscript{(R)} = 58.8min (Area = 7770.41). R\textsubscript{f} = 0.22 (40% EtOAc/Hexanes). IR (cm\textsuperscript{-1}) = 2983, 2937, 1773, 1747, 1697. [\alpha]\textsubscript{D}\textsuperscript{24} = + 5.8 (c = 2, MeOH). \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 400 MHz): δ 7.85 (m, 2H), 7.73 (m, 2H), 4.21 (q, 2H, J = 7Hz), 3.71 (t, 2H, J = 7Hz), 1.93 (m, 2H), 1.71 (m, 2H), 1.45 (s, 3H), 1.25 (t, 3H, J = 7Hz). \textsuperscript{13}C-NMR (CDCl\textsubscript{3}, 100 MHz): δ 176.0, 172.0, 168.0, 134.0, 132.0, 123.0, 62.0, 53.0, 38.0, 33.0, 24.0, 20.0, 14.0. HRMS (C\textsubscript{16}H\textsubscript{17}NO\textsubscript{6}Na\textsuperscript{+}) calculated = 356.3256, found = 356.3253.
(R)-2-(N-Butylphthalimido)-3-Ethoxy-2-Methyl-3-Oxoproanoic Acid (10d)

10d was prepared following the general procedure for the formation of half-esters (10a-10f) with 10g (27 mmol) of 9d. The resulting half-ester was purified by flash chromatography (40:60 EtOAc/Hexanes) giving the pure product as a white solid (6.6g, 70%). The % ee was determined to be 95% by chiral HPLC (Diacel Chiralpak OJ-H, 4% iPrOH/Hexanes, flow rate = 1mL/min, λ = 305 nm) Rt(S) = 63.0 min (Area = 325.24), Rt(R) = 49.6 min (Area = 11659.65). Rf = 0.24 (40% EtOAc/Hexane). IR (cm⁻¹) = 3250, 2943, 1718, 1696. MP = 63.0°C. \([\alpha]_D^{23} = +3.3 (c = 1, \text{CH}_2\text{Cl}_2), \] ¹H-NMR (CDCl₃, 300 MHz): \(\delta 9.82 (bs, 1H), 7.82 (m, 2H), 7.73 (m, 2H), 4.21 (q, 2H, J = 7Hz), 3.71 (t, 2H, J = 7Hz), 1.93 (m, 2H), 1.69 (m, 2H), 1.45 (s, 3H), 1.35 (m, 2H), 1.26 (t, 3H, J = 7Hz), 13C-NMR (CDCl₃, 100 MHz): \(\delta 176.0, 174.0, 169.2, 135.3, 133.2, 124.0, 62.0, 54.3, 39.0, 36.0, 30.0, 23.0, 20.0, 14.0. \) HRMS (C₁₆H₁₇NO₆Na⁺) calculated = 370.1261, found = 370.1256.

(R)-2-(N-Pentylphthalimido)-3-Ethoxy-2-Methyl-3-Oxoproanoic Acid (10e)

10e was prepared following the general procedure for the formation of half-esters (10a-10f) with 10g (26 mmol) of 9e. The resulting half-ester was purified by flash chromatography (40:60 EtOAc/Hexanes) giving the pure product as a colorless liquid (5.8g, 61%). The % ee was determined to be 81% by chiral HPLC (Diacel Chiralpak AD-H, 3% iPrOH/Hexanes, flow rate = 1 mL/min, λ = 305 nm) Rt(S) = 114.60 min (Area = 1591.84), Rt(R) = 78.75 min (Area = 15497.54). Rf = 0.28 (40% EtOAc/Hexanes). IR (cm⁻¹) = 3250, 2938, 1770, 1700. \([\alpha]_D^{24} = +3.2 (c = 2, \text{CHCl}_3), \] ¹H-NMR (CDCl₃, 300 MHz): \(\delta 7.83 (m, 2H), 7.72 (m, 2H), 4.21 (q, 2H, J = 7.19), 3.68 (t, 2H, J = 7 Hz), 1.86 (m, 2H), 1.69 (m, 2H), 1.44 (s, 3H), 1.34 (m, 4H), 1.27 (t, 3H, J = 7 Hz), 13C-NMR (CDCl₃, 75
MHz): δ 178.0, 172.0, 168.4, 134.0, 132.0, 123.0, 123.0, 123.0, 61.4, 54.0, 38.0, 35.5, 28.3, 27.2, 24.0, 20.0, 14.0. HRMS (Cl$_9$H$_{23}$NO$_6$Na$^+$) calculated = 384.1417, found = 384.1413.

(R)-2-(N-Hexylphthalimido)-3-Ethoxy-2-Methyl-3-Oxopropanoic Acid (10f)

10f was prepared following the general procedure for the formation of half-esters (10a-10f) with 10g (25 mmol) of 9f. The resulting half-ester was purified by flash chromatography (40:60 EtOAc/Hexanes) giving the pure product as a colorless liquid (6.12g, 61%). The % ee was determined to be 64% by chiral HPLC (Diacel Chiralpk OJ-H, 3% iPrOH/Hexanes, flow rate = 1 mL/min, λ = 305 nm) Rt$_{(R)}$ = 57.7 min (Area = 13605958), Rt$_{(S)}$ = 71.7min (Area = 2954776). [α]$_D^{24}$ = + 2.05 (c = 2, CHCl$_3$), $^1$H-NMR (CDCl$_3$, 400 MHz): δ 7.83 (m, 2H), 7.72 (m, 2H), 4.21 (q, 2H, $J$ = 7.19), 3.68 (t, 2H, $J$ = 7.12), 1.86 (m, 2H), 1.69 (m, 2H), 1.44 (s, 3H), 1.34 (m, 4H), 1.27 (t, 3H, $J$ = 7.15), $^{13}$C-NMR (CDCl$_3$, 100 MHz): δ 177.0, 173.0, 168.4, 134.0, 132.0, 123.0, 123.0, 61.3, 53.0, 38.0, 35.5, 29.2, 28.2, 26.3, 24.0, 20.0, 14.0. HRMS (C$_{20}$H$_{25}$NO$_6$Na$^+$) calculated = 398.1574, found = 398.1572.

(R)-2-(Ethoxycarbonyl)-3-Hydroxy-2-Methylpropanoic Acid (13)

A 100 mL roundbottom flask was charged with a stirbar, 400 mg of 10% Pd/C, 4.0g (15 mmol) of (R)-12, and 50 mL of THF.$^{85}$ The resulting suspension was stirred rapidly under a hydrogen atmosphere (atmospheric pressure) for 5 h. The reaction mixture was then filtered through a Celite® bed and the filtrate was evaporated to give 2.5 g (14.2 mmol, 95%) of 13 as a yellow viscous liquid. The characterization data matched with the literature values.$^{86}$
(S)-1-Benzyl-3-Ethyl-2-(Hydroxymethyl)-2-Methylmalonate (14)

A 100 mL roundbottom flask was charged with a stirbar, 2.3g of 13 (13 mmol), and 20 mL of DMF. The flask was placed under a nitrogen atmosphere and cooled to 0 °C with stirring and 2.15 g of K₂CO₃ (15.6 mmol) was added. Benzyl bromide (1.96g, 11.5 mmol) was added drop wise to the stirring suspension. The suspension was allowed to stir overnight at room temperature. The suspension was then diluted with 100 mL of diethyl ether and the organic layer was washed three times with water. The organic layer was then dried over MgSO₄, filtered, and evaporated under reduced pressure. The crude liquid was then purified by flash chromatography (40% EtOAc/Hexane) giving 2.7 g (10.1 mmol, 88%) of 14 as a viscous liquid. Rₛ = 0.5 (40% EtOAc/Hexane). ¹H-NMR (300 MHz, CDCl₃): 7.33 (5H, m), 5.2 (2H, s), 4.16 (2H, q, J = 7Hz), 3.87 (2H, s), 2.92 (1H, bs), 1.47 (3H, s), 1.18 (3H, t, J = 7Hz). ¹³C-NMR (75 MHz, CDCl₃): 172.0, 135.0, 128.8, 128.6, 128.3, 67.3, 66.9, 62.0, 56.0, 17.8, 14.0. IR (cm⁻¹): 3528, 2983, 1721. HRMS (C₁₄H₁₈O₅Na⁺) calculated= 289.1098, found = 555.2197 (C₁₄H₁₈O₅)₂Na⁺.

(S)-Benzylethyl-2-Methylsulfonylmethyl-2-Methyl Malonate (15)

A 100 mL sealed tube was charged with a stir bar, 2.6g of 14 (9.7 mmol), and 20 mL of DMF. Et₃N (1.2g, 11.7 mmol) was then added drop wise and the solution was allowed to stir for 5 min. followed by the rapid addition of 1.34g (11.7 mmol) of methanesulfonyl chloride. The tube was tightly sealed and allowed to stir at room temperature for 6 h. The tube was opened and the reaction mixture was diluted with 100 mL of diethyl ether. The organic layer was washed three times with water, dried over MgSO₄, filtered, and evaporated under reduced pressure to give 2.7 g (7.8 mmol, 80%) of
15 as colorless liquid. Due to the potential reactivity of 15, it was used in the next step without further purification.

\textbf{(S)-1-Benzyl-3-Ethyl-2-(Azidomethyl)-2-Methylmalonate (16)}

A 100 mL sealed tube was charged with a stirbar, 20 mL of DMF, and 2.7g of 15 (7.8 mmol). The solution was sparged for 5 min. with dry nitrogen gas and then 1g (15.7 mmol) of NaN\textsubscript{3} was added. The tube was sealed and allowed to stir at 90 °C for 12 h. The reaction mixture was cooled and diluted with 100 mL of diethyl ether. The resulting organic layer was washed three times with water, dried over MgSO\textsubscript{4}, and evaporated under reduced pressure. The crude product was purified by column chromatography (30% EtOAc/Hexane) giving 0.8 g (2.7 mmol, 35%) of 10 as a pale yellow liquid. R\textsubscript{f} = 0.74 (30% EtOAc/Hexane), \([\alpha]^{24}_D = -1.61\) (c = 2.86, CHCl\textsubscript{3}), \(^1\)H-NMR (300 MHz, CDCl\textsubscript{3}): 7.35 (m, 5H), 5.20 (s, 2H), 4.16 (q, 2H, \(J = 6\) Hz), 3.75 (s, 2H), 1.52 (s, 3H), 1.17 (t, 3H, \(J = 7\) Hz), \(^{13}\)C-NMR (75 MHz, CDCl\textsubscript{3}): 14.0, 18.0, 54.0, 56.0, 62.0, 68.0, 129.0, 136.0, 170.0. IR (cm\textsuperscript{-1}): 1727, 2104. HRMS (C\textsubscript{14}H\textsubscript{17}O\textsubscript{4}N\textsubscript{3}Na\textsuperscript{+}) calculated = 314.2923, found = 605.2329 (C\textsubscript{14}H\textsubscript{17}N\textsubscript{3}O\textsubscript{4}Na\textsuperscript{2+}).

\textbf{(R)-2-(Ethoxycarbonyl)-3-Amino-2-Methylpropanoic Acid (17)}

A 50 mL roundbottom flask was charged with 100 mg of 10% Pd/C, 10 mL of THF, 0.5g (1.7 mmol) of 16, and a stir bar. The roundbottom flask was fitted with a septum and a balloon filled with hydrogen was attached to the flask via a needle. The mixture was stirred vigorously under a hydrogen atmosphere for 12 h. The reaction vessel was then opened and the contents were filtered through a pad of Celite\textsuperscript{®} and the filter cake was washed with additional THF. The filtrate was evaporated under reduced pressure at room temperature giving 0.27 g (1.5 mmol, 88%) of 17 as a white solid. R\textsubscript{f} =
0.2 (10% MeOH/CH$_2$Cl$_2$), MP = 114 °C, $\left[\alpha\right]^{D}_{D} = -4.16 \ (c = 1.25, \text{MeOH})$. $^1$H-NMR (300 MHz, CDCl$_3$): 4.19 (m, 2H), 3.25 (d, 1H, J= 14 Hz), 3.03 (d, 1H, J= 14Hz), 1.47 (s, 3H), 1.27 (t, 3H, J= 7 Hz). $^{13}$C-NMR (75 MHz, CDCl$_3$): 13.0, 19.0, 44.0, 52.0, 61.0, 173.0, 175.0. IR (cm$^{-1}$): 1582, 3245. HRMS (C$_7$H$_{13}$NO$_4$Na$^+$) calculated = 198.1824, found = 373.1579 (C$_7$H$_{13}$NO$_4$)$_2$Na$^+$).

(R)-2-(Ethoxycarbonyl)-3-Amino-2-Methylpropanoic Acid (17) from 10a

A 50 mL round bottom flask was charged with 0.5g (1.6 mmol) of 10a, 20 mL of MeOH, 0.058g (1.8 mmol) of hydrazine hydrate (35% in water), and a stir bar. The flask was fitted with a reflux condenser and the reaction mixture was heated to 60 °C. The reaction was allowed to proceed for 6 h. The reaction mixture was allowed to cool and then filtered. The filtrate was evaporated under reduced pressure and the resulting residue was purified by column chromatography (8% MeOH/CHCl$_3$) to give 0.15g (0.86 mmol, 54%) of 17 as a white solid. $\left[\alpha\right]^{D}_{D} = -2.4 \ (c = 1.25, \text{MeOH})$. The characterization data matched with 17 that was prepared from 16 as indicated above.

(S)-1-Benzyl-3-Ethyl-2-Methyl-2-(2-(1,3-Dioxoisoindolin-2-Yl)Malonate (18)

A 250 mL round bottom flask was charged with 10g of 10b (31 mmol), 4.3 g of K$_2$CO$_3$ (31 mmol), 100 mL of anhydrous DMF, and a stirbar. A solution of 4.8g benzyl bromide (28 mmol) in 20 mL anhydrous DMF was slowly added over 15 minutes. The reaction was allowed to stir approximately 12 hr. under a nitrogen atmosphere. The reaction mixture was then diluted with 100 mL of water and the resulting mixture was washed with Et$_2$O (3 x 100 mL). The combined ether layer was washed with water (5 x 100 mL), washed with brine (2 x 100 mL), dried over MgSO$_4$, and the solvent was removed under reduced pressure. The product was purified by flash chromatography.
(40% Et₂O/Hexanes) providing 11g of 18 (27 mmol, 96%) as a colorless liquid. R₇ = 0.2 (40% Et₂O/Hexanes). [α]²⁴ = -3.08 (c = 1, CHCl₃). IR (cm⁻¹): 2980, 1773, 1708. ¹H-NMR (CDCl₃, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.33 (m, 5H), 5.15 (m, 2H), 4.10 (m, 2H), 3.74 (m, 2H), 2.28 (m, 2H), 1.56 (s, 3H), 1.16 (t, 3H, J = 7 Hz). ¹³C-NMR (CDCl₃, 100 MHz): δ 171.4, 171.3, 168.0, 135.5, 134.0, 132.0, 128.5, 128.3, 128.1, 123.0, 67.0, 61.0, 52.0, 33.8, 33.8, 20.0, 14.0. HRMS (C₂₃H₂₃NO₆Na⁺): calculated = 432.1417, found = 432.1406.

**Synthesis of (R)-Ethyl-3-Methyl-2-Oxopyrrolidine-3-Carboxylate (19a)**

A volume of 930 µL (10.2 mmol) 35% hydrazine in water was added to a solution of 3.8g (9.3 mmol) of 18 in 50 mL MeOH. The mixture was heated to reflux solvent overnight. A white precipitate was observed within an hour of reflux. The reaction mixture was allowed to cool to RT, and the resulting mixture was filtered. The filtrate was evaporated under reduced pressure. The resulting residue was taken up in CH₂Cl₂ and washed with water. The organic layer was dried over MgSO₄, evaporated under reduced pressure, and purified by column chromatography using 30% Hexanes/EtOAc giving 1.2 g of a 10:1 mixture of 19a:19b as a white solid. The mixture was recrystallized in cold Et₂O giving 1g (6 mmol, 64.5%) of pure 19a as white crystals. R₇ (5) = 0.31 (30% Hexanes/EtOAc). MP = 63 °C. [α]²³ = +19.0 (c = 2, MeOH). IR (cm⁻¹): 3245, 2985, 1726, 1698, 1660. ¹H-NMR (CDCl₃, 400 MHz): δ 7.06 (bs, 1H), 4.20 (m, 2H), 3.47 (m, 1H), 3.36 (m, 1H), 2.64 (m, 1H), 2.02 (m, 1H), 1.45 (s, 3H), 1.28 (t, 3H, J = 7 Hz). ¹³C-NMR (CDCl₃, 100 MHz): δ 177.0, 172.0, 61.0, 51.0, 40.0, 34.0, 20.0, 14.0. HRMS (C₈H₁₃NO₃Na⁺): calculated = 194.0788, found = 194.0795.
(R)-3-Methyl-2-Oxopyrrolidine-3-Carboxylic Acid (20)

An amount of 1.6g (9.4 mmol) of 19a was dissolved in 15 mL ethanol. A volume of 7 mL 1N NaOH was added to the reaction mixture. The solution was brought to reflux solvent for an hour. The solution was cooled and acidified with HCl to pH 4. The water layer was concentrated at 35 °C under high vacuum. A volume of 10 mL MeOH was added to the residue and stirred for 5 min. The MeOH layer was decanted from the remaining solid and concentrated in vacuo giving 1g of 20 (6.9 mmol, 73%) as a white solid. MP = 155 °C. IR (cm⁻¹) = 3363, 3368, 2975, 2906, 1749, 1722, 1704, 1636, 1485. Rf = 0.17 (5% MeOH/ CH₂Cl₂). ¹H-NMR (CD₃OD, 400 MHz): δ 3.35 (m, 1H), 3.25 (m, 1H), 2.49 (m, 1H), 1.95 (m, 1H), 1.27 (s, 3H). ¹³C-NMR (CD₃OD, 100 MHz): δ 179.6, 175.7, 52.1, 40.7, 35.0, 20.3. ESI-MS (C₆H₁₀NO₃)⁺ = 143.1, observed = 143.2.

Benzyl (R)-3-Methyl-2-Oxopyrrolidin-3-Ylcarbamate (21)

An amount of 1.77g (12.4 mmol) of 20 was dissolved in 50 mL of dry dichloroethane. A volume of 3.6 mL (26 mmol) Et₃N was added followed by 3.1 mL (13.6 mmol) diphenylphosphorylazide (DPPA). The solution was allowed to stir for 2 hrs at RT and then heated to reflux solvent for 2 hr. A volume of 1.8 mL (17.4 mmol) benzyl alcohol was then added and the solution was allowed to reflux solvent over night. The dichloroethane layer was concentrated in vacuo and the residue was purified by flash chromatography (40% EtOAc/Hexanes) giving 1.97g of 21 as a white wax (7.9 mmol, 64%). Rf = 0.10 (40% EtOAc/Hexanes). IR (cm⁻¹) = 3225, 1725, 1693, 1657, 1536. ¹H-NMR (CDCl₃, 400 MHz): δ 7.33 (m, 5H), 6.75 (bs, 1H), 5.55 (bs, 1H), 5.06 (m, 2H), 3.34 (m, 2H), 2.52 (m, 1H), 2.31 (m, 1H), 1.40 (s, 3H). ¹³C-NMR (CDCl₃, 100 MHz): δ
178.2, 155.2, 136.2, 128.7, 128.3, 128.2, 66.7, 57.2, 39.0, 34.8, 22.3. HRMS 
(C_{13}H_{16}N_{2}O_{3}Na^{+}) = 271.1053, observed = 271.1047.

(R)-Tert-Butyl-3-Methyl-2-Oxopyrrolidin-3-Ylcarbamate (22)

An amount of 1.6 g (6.4 mmol) of 21 was dissolved in 25 mL MeOH in a pressure bottle. An amount of 0.16g Pd-C (10%) was added to the reaction mixture. The reaction mixture was allowed to shake under 20 psi H_{2} pressure for 12 hr. The MeOH layer was filtered off through a Celite bed. The filtrate was concentrated "in vacuo" giving 0.66g of the free amine (5.8 mmol), which was then dissolved in 20 mL THF. A volume of 1.7mL (11.6 mmol) Et_{3}N was added to the reaction mixture. A solution of 1.5g (BOC)_{2}O (6.9 mmol) in 10 mL THF was added to the reaction mixture drop wise. The reaction mixture was allowed to stir over night at RT. The THF was concentrated and the resulting residue was extracted with Et_{2}O and water. The ether layer was concentrated and the residue was rinsed with hexane giving 0.83g of the 22 (3.9 mmol, 61% over two steps) as a white solid. The characterization of 22 complied with the literature.\[\gamma\] = -16 (c = 0.35, CHCl_{3}).

General Experimental Procedure for the Formation of Carbamates (23a-23f)

An amount of 10g (1eq) of the appropriate chiral half-ester (10a-10f) was dissolved in 50 mL dichloroethane in a 500 mL round bottom flask with a stirbar under a N_{2} atmosphere. A measured volume of Et_{3}N (2.1 eq) and diphenylphosphorylazide (DPPA) (1.1eq) was added to the solution and the solution was allowed to stir at RT for 90 min. At this point the reaction was heated to reflux solvent for 2 hrs. A measured volume of para-methoxybenzyl alcohol (PMB-OH) (1.4 eq) was added to the reaction mixture and the reaction was continued to reflux solvent for 12 hrs. The reaction was
cooled and diluted with CH$_2$Cl$_2$, filtered through a silica bed (1” bed in a Buchner funnel) and evaporated. The resulting residue was purified by flash chromatography (40% EtOAC/Hexanes) giving the pure product as a white wax or colorless viscous oil.

4-Methoxybenzyl-(S)-2-(Ethoxycarbonyl)-1-(1,3-Dioxoisoindolin-2-Yl)Propan-2-Ylcarbamate (23a)

23a was prepared following the general synthetic procedure for the formation of car bamates (23a-23f). An amount of 11.7g (26.5 mmol, 79%) of product was obtained as a white wax. R$_f$ = 0.25 (40% EtOAc/Hexanes). IR (cm$^{-1}$) = 3368, 2958, 1774, 1708, 1612. [$\alpha$]$^D_{23}$ = - 1.2 (c = 1.2, CHCl$_3$). $^1$H-NMR (CDCl$_3$, 400 MHz): $\delta$ 7.84 (m, 2H), 7.73 (bs, 2H), 7.31 (d, 2H, J = 9 Hz), 6.87 (d, 2H, 9 Hz), 6 (bs, 1H), 5 (q, 2H, J = 12 Hz), 4.18 (m, 2H), 4.12 (s, 2H), 3.8 (s, 3H), 1.65 (S, 3H), 1.25 (m, 3H). $^{13}$C-NMR (CDCl$_3$, 100MHz): $\delta$ 171.0, 168.5, 159.0, 155.0, 134.0, 132.0, 130.0, 128.4, 123.0, 113.3, 66.0, 62.0, 60.2, 55.0, 43.4, 20.0, 14.0. HRMS (C$_{23}$H$_{24}$N$_2$O$_7$Na$^+$) calculated = 463.1476, found = 463.1469.

4-Methoxybenzyl-(S)-2-(Ethoxycarbonyl)-4-(1,3-Dioxoisoindolin-2-Yl)Butan-2-Ylcarbamate (23b)

23b was prepared following the general synthetic procedure for the formation of car bamates (23a-23f). An amount of 12g (26.4 mmol, 84%) of product was obtained as a colorless viscous oil. R$_f$ = 0.29 (35% EtOAc/Hexanes). IR (cm$^{-1}$) = 3353, 2952, 1771, 1704, 1612. [$\alpha$]$^D_{23}$ = + 11.3 (c =1, CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 400 MHz): $\delta$ 7.81 (m, 2H), 7.69 (m, 2H), 7.28 (m, 2H), 6.87 (m, 2H), 5.78 (bs, 1H), 4.94 (m, 2H), 4.07 (m, 2H), 3.80 (s, 3H), 3.67 (m, 2H), 2.57 (bm, 1H), 2.36 (m, 1H), 1.60 (s, 3H), 1.12 (t, 3H, J = 7Hz). $^{13}$C-NMR (CDCl$_3$, 100MHz): $\delta$ 173.0, 168.0, 159.0, 154.0, 134.0, 132.0, 130.0, 128.6,
123.0, 114.0, 66.0, 62.0, 58.0, 55.0, 34.0, 33.6, 24.0, 14.0. HRMS (C_{24}H_{26}N_{2}O_{7}Na^+) calculated = 477.1638, found = 477.1635.

4-Methoxybenzyl-(S)-2-(Ethoxycarbonyl)-5-(1,3-Dioxoisoindolin-2-Yl)Pentan-2-Ylcarbamate(23c)

23c was prepared following the general synthetic procedure for the formation of carbamates (23a-23f). An amount of 11.8g (25.2 mmole, 84%) of product was obtained as a colorless viscous oil. R_f = 0.27 (35% EtOAc/Hexane). IR (cm^{-1}) = 3359, 2939, 1770, 1702, 1612, [\alpha]^D_{23} = - 6.0 (c = 0.8, CH_2Cl_2). ^1H-NMR (CDCl_3, 400 MHz): δ 7.82 (m, 2H), 7.70 (m, 2H), 7.28 (m, 2H), 6.88 (m, 2H), 5.63 (bs, 1H), 4.95 (s, 2H), 4.16 (m, 2H), 3.79 (s, 3H), 3.65 (m, 2H), 2.26 (m, 1H), 1.84 (m, 1H), 1.69 (m, 1H), 1.54 (s, 3H), 1.48 (m, 1H), 1.21 (t, 3H, J = 7Hz). ^13C-NMR (CDCl_3, 100MHz): δ 174, 168, 160, 154, 134, 132, 130, 128, 123, 114, 66, 62, 60, 55, 38, 34, 23.5, 23.4, 14. HRMS (C_{25}H_{28}N_{2}O_{7}Na^+) calculated = 491.1789, found = 491.1782.

4-Methoxybenzyl-(S)-2-(Ethoxycarbonyl)-6-(1,3-Dioxoisoindolin-2-Yl)Hexan-2-Ylcarbamate(23d)

23d was prepared following the general synthetic procedure for the formation of carbamates (23a-23f). An amount of 11.5g (24 mmol, 83%) of product was obtained as a colorless viscous oil. R_f = 0.1 (35% EtOAc/Hexanes). IR (cm^{-1}) = 3360, 2958, 1768, 1701, 1612. [\alpha]^D_{23} = - 1.5 (c = 1.5, CHCl_3). ^1H-NMR (CDCl_3, 400 MHz): δ 7.82 (m, 2H), 7.69 (m, 2H), 7.30 (m, 2H), 6.88 (m, 2H), 5.62 (bs, 1H), 4.99 (s, 2H), 4.17 (m, 2H), 3.8 (s, 3H), 3.63 (t, 2H, J = 7Hz), 2.17 (m, 1H), 1.83 (m, 1H), 1.65 (m, 2H), 1.56 (s, 3H), 1.33 (m, 1H), 1.23 (t, 3H, J = 7Hz), 1.12 (m, 1H). ^13C-NMR (CDCl_3, 100MHz): δ 174.0, 168.0, 159.5, 154.6, 134.0, 132.1, 130.0, 128.7, 123.2, 114.0, 66.2, 62.0, 60.0, 55.3, 38.0,
36.0, 28.4, 23.4, 21.4, 14.1. HRMS \( (C_{26}H_{30}N_2O_7Na^+) \) calculated = 505.1945, found = 505.1930.

4-Methoxybenzyl-(S)-2-(Ethoxycarbonyl)-7-(1,3-Dioxoisindolin-2-Yl)Heptan-2-Ylcarbamate(23e)

23e was prepared following the general synthetic procedure for the formation of carbamates (23a-23f). An amount of 11.5g (23 mmol, 82%) of product was obtained as a colorless viscous oil. \( R_f = 0.33 \) (35% EtOAc/Hexanes), IR (cm\(^{-1}\)) = 3367, 2938, 1770, 1703, 1612. \( [\alpha]_D^{23} = + 1.4 \) (c = 1, CH\(_2\)Cl\(_2\)). \(^1\)H-NMR (CDCl\(_3\), 400 MHz): \( \delta \) 7.83 (m, 2H), 7.70 (m, 2H), 7.28 (d, 2H, \( J = 9Hz \)), 6.87 (m, 2H, \( J = 9Hz \)), 5.63 (bs, 1H), 4.99 (s, 2H), 4.18 (m, 2H), 3.79 (s, 3H), 3.64 (t, 2H, \( J = 7Hz \)), 2.12 (bm, 1H), 1.76 (m, 1H), 1.64 (m, 2H), 1.55 (s, 3H), 1.27 (m, 7H). \(^1^3\)C-NMR (CDCl\(_3\), 100MHz): \( \delta \) 174.0, 168.0, 159.0, 155.0, 134.0, 132.0, 130.0, 129.0, 123.0, 114.0, 66.0, 61.0, 60.0, 55.0, 38.0, 36.0, 28.0, 27.0, 24.0, 23.5, 14.0. HRMS \( (C_{27}H_{32}N_2O_7Na^+) \) calculated = 519.2102, found = 519.2095.

4-Methoxybenzyl-(S)-2-(Ethoxycarbonyl)-8-(1,3-Dioxoisindolin-2-Yl)Octan-2-Ylcarbamate(23f)

23f was prepared following the general synthetic procedure for the formation of carbamates (23a-23f). An amount of 11.3g (22 mmol, 83%) of product was obtained as colorless viscous oil. \( R_f = 0.34 \) (35% EtOAc/Hexanes). IR (cm\(^{-1}\)) = 3366, 2936, 2859, 1770, 1703, 1612. \( [\alpha]_D^{23} = - 1.7 \) (c = 1.4, CH\(_2\)Cl\(_2\)). \(^1\)H-NMR (CDCl\(_3\), 400 MHz): \( \delta \) 7.74 (m, 2H), 7.62 (m, 2H), 7.22 (d, 2H, \( J = 7Hz \)), 6.80 (d, 2H, \( J = 8Hz \)), 5.51 (bs, 1H), 4.91 (s, 2H), 4.11 (m, 2H), 3.73 (s, 3H), 3.58 (t, 2H, \( J = 7Hz \)), 2.04 (m, 1H), 1.67 (m, 1H), 1.56 (m, 2H), 1.47 (s, 3H), 1.20 (m, 8H), 0.98 (bm, 1H). \(^1^3\)C-NMR (CDCl\(_3\), 100MHz): \( \delta \) 173.0,
167.0, 157.0, 154.0, 133.0, 131.0, 129.0, 128.0, 122.0, 113.0, 65.0, 60.0, 59.0, 54.0, 37.0,
36.0, 28.0, 27.0, 26.0, 23.0, 22.0, 13.0.

HRMS (C_{28}H_{34}N_{2}O_{7}Na^{+}) calculated = 533.2258, found = 533.2251.

**Synthesis of (S)-2- Methyl-Ornithinedihydrochloride (24a)**

A volume of 30 mL 6N HCl solution was added to 1g of 23c (2.1 mmol) in a
round bottom flask. The reaction mixture was heated to reflux solvent for 24 hr. The
aqueous layer was evaporated to dryness under reduced pressure. The resulting gummy
solid was triturated with EtOAc multiple times leading to 0.4g (1.8 mmol, 86%) of 24a as
a white solid. All the characterization data of the product complied with the literature.\(^{59}\) \([\alpha]_{D}^{24} = +6.86 (c = 0.7, 4N HCl).\)

**Synthesis of (S)-2- Methyl-Lysinedihydrochloride (24b)**

A volume of 30 mL 6N HCl solution was added in 1g of 23d (2.1 mmol) in a
round bottom flask. The reaction mixture was heated to reflux solvent for 24 hr. The
aqueous layer was evaporated to dryness under reduced pressure. The resulting gummy
solid was triturated with EtOAc multiple times leading to 0.36g (1.5 mmol, 71%) of 24b as
a white solid. All the characterization data of the product complied with the
literature.\(^{59}\) \([\alpha]_{D}^{24} = +7.25 (c = 1, 4N HCl).\)

**General Synthetic Procedure for the Formation 25a, and 25b**

An amount of 10e/10f (1 equivalent) was dissolved in DMF under N\(_2\). A
calculated amount of K\(_2\)CO\(_3\) (1.2 equivalent) was added to the solution. A measured
volume of benzyl bromide (0.95 equivalents) was added to the reaction mixture. The
reaction was allowed to stir over night under N\(_2\). Water was added to the reaction mixture,
and the aqueous layer was extracted with Et\(_2\)O (3 x 50 mL). The combined ether layer
was given a water wash (10 x 50 mL). The Et₂O layer was dried over MgSO₄, concentrated, and the residue was purified by flash chromatography (40% Et₂O/Hexanes) giving the product as a colorless oil.

(S)-1-Benzyl-3-Ethyl-2-Methyl-2-(5-(1,3-Dioxoisooindolin-2-Yl)Pentyl)Malonate (25a)

25a was synthesized following the general synthetic procedure for the formation of 25a/25b using 5g (14 mmol) of 10e. An amount of 5.2g of 25a (11.5 mmol, 82%) was obtained as a colorless viscous oil after purification (40% Et₂O/Hexanes). Rₜ = 0.16 (40% Et₂O/Hexanes). IR (cm⁻¹) = 2938, 1770, 1700. ¹H-NMR (CDCl₃, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.32 (m, 5H), 5.15 (m, 2H), 4.11 (q, 2H, J = 7Hz), 3.64 (t, 2H, J = 7Hz), 1.85 (m, 2H), 1.64 (m, 2H), 1.41 (s, 3H), 1.28 (m, 4H), 1.15 (t, 3H, J = 7Hz). ¹³C-NMR (CDCl₃, 100 MHz): δ 172.2, 172.0, 168.4, 136.0, 134.0, 132.2, 128.4, 128.2, 128.0, 123.2, 66.6, 61.1, 54.0, 38.0, 35.4, 28.2, 27.0, 24.0, 20.0, 14. HRMS (C₂₆H₂₉NO₆Na⁺) calculated = 474.1887, observed = 474.1905.

(S)-1-Benzyl-3-Ethyl-2-Methyl-2-(6-(1,3-Dioxoisooindolin-2-Yl)Hexyl)Malonate (25b)

25b was synthesized following the general synthetic procedure for the formation of 25a/25b using 5g (13.3 mmol) of 10f. An amount of 5.3g of 25b (11.4 mmol, 86%) was obtained as a colorless viscous oil after purification (40% Et₂O/Hexanes). Rₜ = 0.30 (40% Et₂O/Hexanes). IR (cm⁻¹) = 2936, 1770, 1706. ¹H-NMR (CDCl₃, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.32 (m, 5H), 5.15 (m, 2H), 4.11 (q, 2H, J = 7Hz), 3.64 (t, 2H, J = 7Hz), 1.85 (m, 2H), 1.64 (m, 2H), 1.41 (s, 3H), 1.30 (m, 4H), 1.17 (m, 5H). ¹³C-NMR (CDCl₃, 100 MHz): δ 172.3, 172.1, 168.4, 136.0, 134.0, 132.2, 128.5, 128.2, 128.0, 123.2, 66.7, 61.1, 54.0, 38.0, 35.5, 29.4, 28.5, 26.5, 24.2, 20.0, 14. HRMS (C₂₇H₃₁NO₆Na⁺) calculated = 488.2043, observed = 488.2030.
General Synthetic Procedure for the Formation of 26a and 26b

A measured amount of 25a/25b (1 equivalent) was dissolved in methanol. A calculated amount of 35% N₂H₄.H₂O in water (1.2 equivalents) was added to the reaction mixture. The reaction mixture was heated to reflux solvent for 6 hr. The reaction mixture was cooled to RT and the white precipitate was filtered off. The MeOH layer was concentrated "in vacuo" and the gummy solid was taken up in CH₂Cl₂ leading to more white precipitate. The white precipitate is again removed by filtration and the CH₂Cl₂ layer was again concentrated "in vacuo" giving pure product as colorless oil.

(S)-1-Benzyl-3-Ethyl-2-(5-Aminopentyl)-2-Methylmalonate (26a)

26a was prepared from 25a following the general synthetic procedure for the formation of 26a/26b using 5g of 25a (11 mmol). An amount of 3.3g (10.3 mmol, 94%) of 26a was obtained as a colorless viscous oil. Rₓ = 0.12 (3% MeOH/CH₂Cl₂). IR (cm⁻¹) = 3100, 3000, 2938, 1724. ¹H-NMR (CDCl₃, 400 MHz): δ 7.32 (m, 5H), 5.15 (m, 2H), 4.11 (q, 2H, J = 7Hz), 2.65 (t, 2H, J = 7Hz), 1.87 (t, 2H, J = 8Hz), 1.61 (bs, 2H), 1.41 (m, 5H), 1.24 (m, 7H). ¹³C-NMR (CDCl₃, 100 MHz): δ 172.3, 172.2, 136.0, 128.5, 128.2, 128.0, 67, 61.2, 54.0, 42.0, 35.4, 33.5, 27.0, 24.0, 20.0, 14.0. HRMS (C₁₈H₂₁NO₄Na⁺) calculated = 344.1832, observed = 344.1823.

(S)-1-Benzyl-3-Ethyl-2-(6-Aminohexyl)-2-Methylmalonate (26b)

26b was prepared from 25b following the general synthetic procedure for the formation of 26a/26b using 5g of 25b (10.7 mmol). An amount of 3g (8.9 mmol, 83%) of 26b was obtained as colorless viscous oil. Rₓ = 0.14 (3% MeOH/CH₂Cl₂). IR (cm⁻¹) = 3300, 2932, 1726. ¹H-NMR (CDCl₃, 400 MHz): δ 7.25 (m, 5H), 5.07 (m, 2H), 4.04 (q, 2H, J = 7Hz), 2.68 (bs, 2H), 2.61 (t, 2H, J = 7Hz), 1.77 (t, 2H, J = 7Hz), 1.33 (m, 5H),
1.14 (m, 9H). $^{13}$C-NMR (CDCl$_3$, 100 MHz): δ 171.3, 171.2, 135.0, 127.5, 127.2, 127.0, 66.0, 60.1, 53.0, 41.0, 34.5, 32.0, 28.5, 25.5, 23.1, 19.0, 13.0. HRMS (C19H29NO4Na$^+$) calculated = 358.1988, observed = 358.1983.

**General Synthetic Procedure for the Formation of 27a and 27b**

27a/27b were synthesized from 26a/26b following a literature procedure.$^{87}$ A measured amount of 26a/26b (1 equivalent) was dissolved in 24 mL 2:1 2.5M NaOH/EtOH mixture. The solution was cooled to 0 °C and a measured amount of NH$_2$OSO$_3$H (2 equivalent) was added to the solution. The solution was stirred at 0 °C for 35 minutes. At that point an additional amount of NH$_2$OSO$_3$H (1 equivalent) and 5 mL 2.5 M NaOH were added to the reaction mixture. The reaction was allowed to stir at 0 °C for another 90 minutes and then allowed to warm to RT overnight. The reaction mixture was acidified to pH 1. The aqueous layer was extracted with Et$_2$O (3 x 50 mL). The combined organic layer was washed with brine, dried over MgSO$_4$, concentrated "in vacuo", and purified in 1:1 Et$_2$O/Hexanes giving the product as a colorless oil.

(R) - 2-(Ethoxycarbonyl)-2-Methylheptanoic Acid (27a)

27a was prepared from 26a following the general synthetic procedure of making 27a/27b using 3g of 26a (9.3 mmol). An amount of 1.2g of 27a was obtained (5.5 mmol, 59%) after purification. R$_f$ = 0.49 (50% Et$_2$O/Hexanes). IR (cm$^{-1}$) = 2956, 2930, 2871, 1705. $\left[\alpha\right]_D^{25}$ = + 3.15 (c = 2, CH$_2$Cl$_2$). $^1$H-NMR (CDCl$_3$, 400 MHz): δ 10.38 (s, 1H), 4.21 (q, 2H, $J$ = 7Hz), 1.87 (m, 2H), 1.44 (s, 3H), 1.27 (m, 9H), 0.88 (t, 3H, $J$ = 7Hz). $^{13}$C-NMR (CDCl$_3$, 100 MHz): δ 178.0, 172.5, 61.5, 53.6, 35.7, 32.0, 24.0, 22.3, 20.0, 14.0, 13.9. HRMS (C$_{11}$H$_{20}$O$_4$Na$^+$) calculated = 239.1255, observed = 239.1253.
(R)- 2-((Ethoxycarbonyl)-2-Methyloctanoic Acid (27b)

27b was prepared from 26b following the general synthetic procedure of making 27a/27b using 3g of 26b (8.9 mmol). An amount of 1.3g pure 27b was obtained (5.6 mmol, 63%) after purification. 

IR (cm$^{-1}$) = 2955, 2927, 2858, 1705. 

$\alpha$ = 2.2 (c = 1, CH$_2$Cl$_2$). 

$^1$H-NMR (CDCl$_3$, 400MHz): $\delta$ 4.21 (q, 2H, $J = 7$Hz), 1.87 (m, 2H), 1.44 (s, 3H), 1.28 (m, 11H), 0.88 (t, 3H, $J = 7$Hz). 

$^{13}$C-NMR (CDCl$_3$, 100MHz): $\delta$ 178.0, 172.6, 61.6, 53.6, 35.8, 31.4, 29.4, 24.2, 22.6, 20.0, 14.1. 

HRMS (C$_{12}$H$_{22}$O$_4$Na$^+$) calculated = 253.1410, observed = 253.1409.

General Synthetic Procedure for the Formation of 28a and 28b

A measured amount of 27a/27b (1 equivalent) was dissolved in 3 mL of H$_2$O, and 1 mL of acetone was added to the solution. A solution of Et$_3$N (1.2 equivalent) in 1 mL acetone was added to the reaction mixture drop wise followed by a solution of methylchloroformate (1.55 equivalent) in 1 mL acetone. The reaction was allowed to stir for 30 minutes at RT. A solution of NaN$_3$ (1.6 equivalent) in 3 mL H$_2$O was added to the reaction mixture and the mixtures was stirred for 2hrs. The reaction mixture was then poured into 25 mL of ice cold water. The water layer was extracted with ether (3 x 50 mL). The combined ether layer was dried over MgSO$_4$, concentrated "in vacuo" giving the acylazide as colorless oil. The acylazide was dissolved in toluene and heated to reflux solvent for 2 hrs. The toluene was concentrated "in vacuo" giving the isocyanate as yellowish oil. 

A volume of 10 mL 4M HCl was added to the isocyanate and the mixture was heated to reflux solvent for 4 hrs. The water layer was concentrated under reduced pressure giving the (S)-α-alkyl-alaninehydrochloride as a pale yellowish solid. The 28a/28b HCl salt was then dissolved in MeOH and NaHCO$_3$ was added portion wise to
neutralize it to (S)-α-alkyl-alanine (28a/28b). The MeOH layer was filtered and concentrated giving 28a/28b as a white solid.

*Synthesis of (S)-α-Pentylalanine (28a)*

28a was prepared following the general synthetic procedure for the formation of 28a/28b using 1g of 27a (5 mmol). An amount of 0.5g (3 mmol, 60%) of (S)-α-pentylalanine (28a) was obtained as a white solid after neutralization. All the characterization data of 28a complied with the literature.78, 88 [α]_D^{25} = + 4.1 (c = 1, MeOH).

*Synthesis of (S)-α-Hexylalanine (28b)*

28b was prepared following the general synthetic procedure for the formation of 28a/28b using 1g of 27b (4.6 mmol). An amount of 0.55g (3.4 mmol, 74%) of (S)-α-pentylalanine (28b) was obtained as a white solid after neutralization. All the characterization data of 28b complied with the literature.78, 88 [α]_D^{25} = + 6.7 (c = 0.15, MeOH).

*Synthesis of (S)-Ethyl 2-Amino-2-Methyl-6-(1,3-Dioxoisoindolin-2-Yl)Hexanoate (29)*

An amount of 10gm (21 mmol) 23d was dissolved in 60 mL of methylene chloride. A volume of 10 mL TFA was added. The solution was stirred over 1 h. The solution turned dark purple. A volume of 100 mL H_2O was added to the solution. The methylene chloride layer was extracted, washed with NaHCO_3 solution, washed with H_2O, dried over MgSO_4. The crude was purified by flash chromatography (5% MeOH/CH_2Cl_2) giving 6.2gm (19.4mmoles, 92%) of the pure product as a white wax.

TLC R_f = 0.35 (5% MeOH/CH_2Cl_2), 1H NMR (CDCl_3, 400MHz) 7.81(m, 2H), 7.71(m, 2H), 4.15(q, 2H, J= 7Hz), 3.67(t, 2H, J= 7Hz), 1.67(m, 6H), 1.39(m, 1H), 1.31(s, 3H),
1.25(m, 4H), $^{13}$C NMR (CDCl$_3$, 400MHz) 177, 168, 134, 132, 123, 61, 57, 40, 37, 28, 26, 21, 14.. HRMS (C$_{17}$H$_{22}$N$_2$O$_4$Na$^+$) calculated = 341.1477, observed = 341.1464.

**Synthesis of (S)-Ethyl 2-(dibenzylamino)-2-methyl-6-((1,3-dioxoisindolin-2-yl)hexanoate (30)**

An amount of 5g (16 mmol) 29 was dissolved in 60 mL of distilled acetonitrile in a 250 mL three necked flask under N$_2$ atmosphere. An amount of 13.3gm (96 mmol) of K$_2$CO$_3$ was added with stirring. A volume of 9.5 mL (80 mmol) of BnBr was added drop wise. The reaction was brought to reflux for 12 hrs. The reaction mixture was diluted with 50 mL of H$_2$O. The solution was extracted with ether three times. The ether layer was washed with H$_2$O, washed with brine, dried over MgSO$_4$ and evaporated. The crude was then purified by flash chromatography (30% EtOAc/Hexane), giving 3.84 g (7.7 mmoles, 48%) of the pure product as white solid. TLC R$_f$ = 0.61 (30% EtOAc/Hexane), MP = 91°C. $^1$H NMR (CDCl$_3$, 300MHz) 7.82(m, 2H), 7.69(m, 2H), 7.15(m, 10H), 4.13(m, 2H), 3.80(m, 4H), 3.61(t, 2H, J=7Hz), 1.79(m, 2H), 1.52(m, 3H), 1.29(m, 7H).

$^{13}$C NMR (CDCl$_3$, 75MHz) 175, 169, 142, 134, 132, 128.6, 128, 127, 123, 67, 61, 55, 38, 37, 29, 21.7, 21.6, 15. HRMS (C$_{31}$H$_{34}$N$_2$O$_4$Na$^+$) calculated = 521.2416, observed = 521.2398.

**Synthesis of (S)-6-Amino-2-(Dibenzylamino)-2-Methylhexanoic Acid (31)**

An amount of 3.5g (10.3 mmoles) 30 was dissolved in 15mL EtOH in a 250mL single neck round bottom falsk with a stir bar. A volume of 100mL 8N NaOH solution was added to it and the resulting reaction mixture was brought to reflux over 48 hrs. The water layer was acidified to pH 2, evaporated out under reduced pressure, and triturated with MeOH. The MeOH layer was neutralized by solid NaHCO$_3$, evaporated out under
reduced pressure giving 2.8g (8.5mmoles, 82%) pure 31 as white wax, which was taken for next step without further purification. $^1$H NMR (CD$_3$OD, 300MHz): 7.26(bs, 10H), 4.34(m, 4H), 2.81(bs, 2H), 1.90(m, 2H), 1.54(m, 6H), 1.24(b, 1H).

*Synthesis of (S)-2-(Dibenzylamino)-6-(Tert-Butyloxycarbonylamino)-2-Methylhexanoic Acid (32)*

A solution of 1gm 31 (1eq) in 10mL water was placed in 50mL round bottom flask. A measured amount of NaHCO$_3$ (2eq) was added with stirring. The solution was cooled down to 0°C under ice. A solution of (Boc)$_2$O (1.4eq) in 10mL 1,4 dioxane was added dropwise. The reaction was continued to stir at 0°C over an hr. The reaction was then allowed to come back to RT and continued to stir over the night. The reaction was diluted with 15mL H$_2$O, acidified to pH4 by NaHSO$_4$, extracted with Et$_2$O twice. The combined ether layer was washed with water (5 X 30mL), washed with brine, dried over MgSO$_4$, and evaporated out. The crude was chromatographed (40% EtOAc/hexanes), giving 1.14g (2.6 mmol, 87%) pure 32 as white solid. TLC $R_f$ = 0.31 (40% EtOAc/Hexane). MP = 57°C. $^1$H NMR (CDCl$_3$, 400MHz) 7.27(m, 10H), 4.51(bs, 1H), 3.89(m, 4H), 3 (bs, 2H), 1.68(m, 2H), 1.39(m, 16H). $^{13}$C NMR (CDCl$_3$, 100MHz) 175, 156, 137, 128.8, 128.6, 128, 79, 71, 55, 40, 37, 30, 29, 22, 18. HRMS (C$_{26}$H$_{36}$N$_2$O$_4$Na$^+$) calculated = 463.2573, observed = 463.2562.

*(S)-2-Amino-6-(Tert-Butyloxycarbonylamino)-2-Methylhexanoic Acid (33)*

A solution of 1g 32 (1eq) in 25mL MeOH was placed in a pershaker bottle. An amount of 0.2gm (20% by weight) Pd-C was added to the bottle. The reaction was continued to shake at 25psi over 12 hrs at room temperature. The reaction mixture was filtered through selite bed. The filtrate was evaporated giving the pure product. An amount
of 0.55gm (2.1 mmoles, 91%) product was obtained. The product was confirmed by $^1$H NMR and HRMS. However, $^{13}$C NMR showed up an extra peak. The product was taken for next step without further purification. $R_f = 0.21$ (5% MeOH/CH$_2$Cl$_2$), IR (cm$^{-1}$), $^1$H NMR (CD$_3$OD, 400MHz): 3.03(t, 2H, J=7Hz), 1.45(m, 18H). HRMS (C$_{12}$H$_{24}$N$_2$O$_4$Na$^+$) calculated = 283.1634, observed = 283.1628.

**Synthesis of (S)-tBoc-Fmoc-α-Methyl-α-Lysine-OH (34)**

An amount of NaHCO$_3$ (2eq) was added to a solution of 0.5gm 33 (1eq) in 15mL water with stirring. The solution was cooled down to 0 $^\circ$C under ice. A solution of Fmoc-Osu (1.5 eq) in 15mL 1,4-dioxane was added to the reaction mixture over 20min. The reaction was continued to stir at 0 $^\circ$C over an hr and at ambient temperature over 12 hrs. At that point the reaction was diluted with 30mL of water, acidified to pH 4 with 4M HCl, extracted (3X50mL) with Et$_2$O. The combined ether layer was given brine wash, dried over MgSO$_4$, and evaporated out at reduced pressure giving crude as light yellowish oil. The crude product was purified by radial chromatography using 5% MeOH/CH$_2$Cl$_2$, giving 0.78g pure 34 (1.62 mmol, 85%) as white solid. $R_f = 0.33$ (5% CH$_2$Cl$_2$/MeOH), IR (cm$^{-1}$) = 3350, 2941, 1681, 1504, $[^\alpha]_D^{22} = +14.4$ (C=1, CHCl$_3$). MP = 95 $^\circ$C $^1$H NMR (CD$_3$OD, 400MHz) 7.69(d, 2H, J=8Hz), 7.55(d, 2H, J=8Hz), 7.28(t, 2H, J=8Hz), 7.21(t, 2H, J=8Hz), 4.22(d, 2H, J=7Hz), 4.11(t, 1H, J=7Hz), 2.92(t, 2H, J=7Hz), 1.77(bs, 2H), 1.33(m, 16H). $^{13}$C NMR (CD$_3$OD,MHz) 176,157, 155, 144, 143.9, 141, 127, 126.7, 125, 119, 78, 66, 59, 40, 36, 29, 27, 21, 20. HRMS (C$_{27}$H$_{34}$N$_2$O$_6$Na$^+$) calculated = 505.2309, observed = 505.2296.
Synthesis of (9H-Fluoren-9-Yl)-Methyl-(S)-2-(Ethoxycarbonyl)-6-(1,3-Dioxoisooindolin-2-Yl)Hexan-2-Ylcarbamate (35)

A volume of 320 µL Et₃N (2.3 mmol) was added to a solution of 0.7g (1.9 mmol) 10d in 25 mL dichloroethane under a N₂ atmosphere. A volume of 460 µL DPPA (2 mmol) was added to the reaction mixture. The mixture was allowed to stir at RT for 2 hrs. The mixture was then heated to reflux solvent for 3 hrs. The reaction was cooled and washed with saturated NH₄Cl solution. The organic layer was dried over MgSO₄, filtered, and evaporated under reduced pressure giving the crude isocyanate. The isocyanate was dissolved in dry toluene under a N₂ atmosphere. An amount of 0.75g (3.8 mmol) 9-fluorenylmethanol and a volume of 66 µL Ti (IV) isopropoxide was added to the solution. The mixture was heated to 80°C for 12 hrs. The mixture was cooled and the toluene was evaporated under reduced pressure giving the crude product. The residue was purified by chromatography (10% Hexanes/CH₂Cl₂), giving 0.95g 35 (1.75 mmol, 92%) as a white solid. R₁ = 0.29 (10% Hexanes/CH₂Cl₂). IR (cm⁻¹) = 3365, 2940, 1769, 1704, 1613, 1504. MP = 81°C, [α]D²² = -14.3 (c = 1, CHCl₃). ¹H-NMR (CDCl₃, 400MHz): δ 7.78 (m, 4H), 7.64 (m, 4H), 7.40 (t, 2H, J = 7Hz), 7.30 (t, 2H, J = 7Hz), 5.72 (bs, 1H), 4.35 (bm, 2H), 4.20 (bm, 3H), 3.65 (bm, 2H), 2.21 (bm, 1H), 1.85 (bm, 1H), 1.60 (m, 5H), 1.35 (bm, 1H), 1.24 (bm, 3H), 1.13 (bm, 1H). ¹³C-NMR (CDCl₃, 100MHz): δ 174.0, 168.0, 144.0, 141.0, 134.0, 132.0, 128.0, 127.0, 125.0, 123.0, 120.0, 66.0, 62.0, 60.0, 47.0, 37.5, 36.0, 28.0, 23.4, 21.0, 14.0. HRMS (C₃₂H₃₂N₂O₆Na⁺) calculated = 563.2152, observed = 563.2144.

Fluorenylmethyloxyxycarbonylamino)-2-Methylhexanoic Acid.HCl (36)

36 was synthesized from 35 following a literature published procedure. An amount of 0.9g (1.7 mmol) 35 was dissolved in 12 mL 1, 4-dioxane. A volume of 12 mL
5N HCl was added to the solution. The solution was heated to reflux solvent for 24 hrs. At which time the reaction was found to be completed by ESI-MS. The solution was concentrated under reduced pressure and the residue was taken for the next step without further purification.

*Synthesis of (S)-1Boc-Fmoc-α-Methyl-α-Lysine-OH (34)*

An amount of 0.25g NaHCO$_3$ (3 mmol) was added to a solution of 0.6g of 36 (~1.5 mmol) in 15 mL of water with stirring. The solution was cooled to 0°C. A solution of 0.65g (Boc)$_2$O (3 mmol) in 15 mL 1,4-dioxane was added to the reaction mixture over 20 min. The reaction was allowed to stir at 0°C for an hour and then at ambient temperature for 12 hrs. The mixture was given pentane wash to remove excess (Boc)$_2$O. The reaction mixture was diluted with 30 mL of water, acidified to pH 4 with 2M HCl, and extracted (3 x 50 mL) with Et$_2$O. The combined ether layer was washed with brine, dried over MgSO$_4$, evaporated under reduced pressure giving the crude product as light yellow oil. The residue was purified by radial chromatography using 5% MeOH/CH$_2$Cl$_2$ giving 0.78g (1.6 mmol, 94% over two steps) of product as a white solid. $R_f = 0.33$ (5% MeOH/CH$_2$Cl$_2$). IR (cm$^{-1}$) = 3350, 2941, 1681, 1504. MP = 95°C. $[\alpha]_D^{22} = +14.4$ (c = 1, CHCl$_3$), $^1$H-NMR (CD$_3$OD, 400MHz): δ 7.69 (d, 2H, $J = 8$Hz), 7.55 (d, 2H, $J = 8$Hz), 7.28 (t, 2H, $J = 8$Hz), 7.21 (t, 2H, $J = 8$Hz), 4.22 (d, 2H, $J = 7$Hz), 4.11 (t, 1H, $J = 7$Hz), 2.92 (t, 2H, $J = 7$Hz), 1.77 (bs, 2H), 1.33 (m, 16H). $^{13}$C-NMR (CD$_3$OD, 100 MHz): 176.0, 157.0, 155.0, 144.0, 143.9, 141.0, 127.0, 126.7.0, 125.0, 119.0, 78.0, 66.0, 59.0, 40.0, 36.0, 29.0, 27.0, 21.0, 20.0. HRMS (C$_{27}$H$_{34}$N$_2$O$_6$Na$^+$) calculated = 505.2309, observed = 505.2296.
Synthesis of (S)-1-Tert-Butyl 3-Ethyl 2-[4-(1,3-Dioxoisoindolin-2-Yl)Butyl]-2-Methylmalonate (37)

A volume of 3 mL conc. H$_2$SO$_4$ was added to a solution of 10g 10d (29 mmol) in 100 mL CH$_2$Cl$_2$ in a 250 mL sealed tube. The solution was cooled to -7 °C in an ice salt bath. A volume of 50 mL of condensed isobutylene was added to the solution. The tube was capped tightly and allowed to stir over night at RT. The tube was uncapped and allowed to stir for 2 hrs at ambient pressure to allow the excess isobutylene to evaporate. The solution was diluted with CH$_2$Cl$_2$ and gently washed three times with 1N NaOH (50 mL). The CH$_2$Cl$_2$ layer was dried over MgSO$_4$, evaporated under reduced pressure, and purified by chromatography (40% EtOAc/Hexanes), giving 10.8g of product (26.7 mmol, 92%) as a colorless liquid. R$_f$ = 0.60 (40% EtOAc/Hexanes), IR (cm$^{-1}$) = 2977, 2937, 1771, 1707. $^1$H-NMR (CDCl$_3$, 400MHz): δ 7.84 (m, 2H), 7.71 (m, 2H), 4.16 (q, 2H, J = 7Hz), 3.68 (t, 2H, J = 7Hz), 1.85 (m, 2H), 1.69 (m, 2H), 1.42 (s, 9H), 1.28 (m, 8H). $^{13}$C-NMR (CDCl$_3$, 100MHz): δ 172.0, 171.0, 168.0, 134.0, 132.0, 123.0, 81.0, 61.0, 54.0, 38.0, 35.0, 29.0, 28.0, 22.0, 20.0, 14.0. HRMS (C$_{22}$H$_{29}$NO$_6$Na$^+$) calculated = 426.1887, observed = 426.1873.

Synthesis of (S)-1-Tert-Butyl 3-Ethyl 2-(4-Aminobutyl)-2-Methylmalonate (38)

A volume of 2.8 mL (31.4 mmol) N$_2$H$_4$H$_2$O (35% in H$_2$O) was added in a solution of 10.5g 37 (26 mmol) in 60 mL MeOH. The solution was heated to reflux solvent for 6 hrs. The reaction mixture was found to turn turbid and a white precipitate formed within 2 hrs of reflux. The reaction was monitored by TLC. The reaction was cooled to RT and the MeOH was removed "in vacuo". The residue was taken up in CH$_2$Cl$_2$ and the white precipitate was filtered off. The CH$_2$Cl$_2$ was evaporated under
reduced pressure, giving 6.75g (24.7 mmol, 95%) of 38 as a colorless oil. Rf = 0.16 (5% MeOH/CH₂Cl₂), IR (cm⁻¹) = 3395, 2977, 2934, 2867, 1723, 1654. ¹H-NMR (CDCl₃, 400MHz): δ 4.17 (q, 2H, J = 7Hz), 2.70 (t, 2H, J = 7Hz), 1.82 (m, 2H), 1.53 (bs, 2H), 1.45 (m, 11H), 1.35 (s, 3H), 1.27 (m, 5H). ¹³C-NMR (CDCl₃, 100MHz): δ 172.0, 171.0, 81.0, 61.0, 54.0, 42.0, 35.0, 34.0, 28.0, 21.0, 19.0, 14.0. HRMS (C₁₄H₂₇NO₄Na⁺) calculated = 296.1832, observed = 296.1828.

Synthesis of (S)-2-(Tert-Butyloxycarbonyl)-6-Amino-2-Methylhexanoic Acid (39)

An amount of 1.76g LiOH (73.5 mmol) was added in a solution of 6.7g 38 (24.5 mmol) in 30 mL of 3:7 EtOH/H₂O mixture. The solution was allowed to stir for 48 hrs at RT. The solvents were evaporated under reduced pressure upon completion as determined by TLC (5% MeOH/CH₂Cl₂). The residue was triturated with MeOH to precipitate excess LiOH. The MeOH layer was evaporated under reduced pressure giving 5.76g of 20 (96%, 23.5 mmol) as a white wax. Rf = 0.10 (5% MeOH/CH₂Cl₂). IR (cm⁻¹) = 3297, 2961, 2937, 1541, 1448. ¹H-NMR (CD₃OD, 400MHz): δ 2.64 (t, 2H, J = 7Hz), 1.79 (m, 2H), 1.46 (m, 11H), 1.29 (m, 5H). ¹³C-NMR (CD₃OD, 100MHz): δ 178.0, 175.0, 80.0, 56.0, 41.0, 36.0, 33.0, 26.0, 21.0, 20.0. ESI-MS (C₁₂H₂₃NO₄Na⁺) calculated 268.3, observed 268.2.

Synthesis of (S)-2-(Tert-Butyloxycarbonyl)-6-(Tert-Butyloxycarbonylamino)-2-Methylhexanoic Acid (40)

An amount of 3.9g NaHCO₃ (46.5 mmol) was added to a solution of 5.7g of 39 (23.2 mmol) in 20 mL H₂O. The solution was cooled to 0°C. A solution of 6g of (Boc)₂O (28 mmol) in 20 mL 1,4-dioxane was added drop wise to the reaction mixture. The reaction was allowed to stir at 0°C for an hr and then brought to RT. The reaction was
allowed to stir at RT for 12 hrs. The reaction mixture was extracted with pentane. The aqueous layer was acidified to pH 4 using 2N HCl and extracted three times with Et₂O (50 mL). The combined ether layer was dried over MgSO₄, evaporated under reduced pressure, and purified by chromatography (40% EtOAc/Hexanes), giving 7.6g (22 mmol, 95%) of 40 as a colorless viscous oil. Rf = 0.54 (40% EtOAc/Hexanes), IR (cm⁻¹) = 3380, 2976, 2934, 1706, 1522. ¹H-NMR (CDCl₃, 400MHz): δ 4.61 (bs, 1H), 3.12 (bm, 2H), 1.84 (m, 2H), 1.46 (m, 23H), 1.29 (m, 2H). ¹³C-NMR (CDCl₃, 100MHz): δ 177.0, 172.0, 156.0, 82.0, 79.0, 54.0, 40.0, 35.0, 30.0, 28.4, 27.8, 22.0, 20.0. HRMS (C₁₇H₃₁NO₆Na⁺) calculated = 368.2043, observed = 368.2035.

Synthesis of (R)-Tert-Butyl-2-(9-Fluorenymethylamino)·6-(Tert-Butyloxycarbonylamino)-2-Methylhexanoate (41)

A volume of 2.7 mL of Et₃N (19.4 mmol) was added to a solution of 5.6g 40 (16.2 mmol) in 60 mL dichloroethane under a N₂ atmosphere. A volume of 3.9 mL (17.3 mmol) of DPPA was added to the reaction mixture. The mixture was allowed to stir at RT for 2 hrs. The mixture was heated to reflux solvent for 3 hrs. The reaction was cooled, and the organic layer was extracted with saturated NH₄Cl solution, dried over MgSO₄ and evaporated under reduced pressure giving the isocyanate. The isocyanate was taken up in dry toluene under a N₂ atmosphere. An amount of 6.4g (32.4 mmol) 9-fluorenymethanol was added to the solution along with 300 µL of Ti (IV) isopropoxide. The solution was heated to 80°C over night. The reaction was cooled, and the organic layer was evaporated under reduced pressure. The residue was then purified by chromatography (CH₂Cl₂) giving 7.75g (14.4 mmol, 89%) of 41 as colorless wax. An amount of 50mg of 41 was further purified by reversed phase HPLC (40% CH₃CN/H₂O to 100% CH₃CN in
15 min at 262 nm, Rf = 16.6 min), giving 35mg of pure 41 as a colorless oil. Rf = 0.57 (CH2Cl2), IR (cm\(^{-1}\)) = 3359, 2975, 2931, 1707, 1516. \(^1\)H-NMR (CDCl3, 400MHz): \(\delta\) 7.76 (d, 2H, J=8Hz), 7.61 (d, 2H, J = 8Hz), 7.40 (t, 2H, J = 7Hz), 7.32 (t, 2H, J = 7Hz), 5.82 (bs, 1H), 4.47 (m, 2H), 4.18 (t, 1H, J = 7Hz), 4.02 (bs, 1H), 3.07 (bm, 2H), 2.23 (bm, 1H), 1.74 (bm, 1H), 1.45 (m, 25H). \(^{13}\)C-NMR (CDCl3, 100 MHz): \(\delta\) 173.2, 156.03, 154.3, 143.9, 141.4, 127.6, 127.0, 125.1, 120.0, 82.2, 79.2, 66.3, 60.1, 47.2, 40.1, 35.8, 29.7, 28.3, 27.9, 23.7, 21.2. HRMS (C\(_{31}\)H\(_{42}\)N\(_2\)O\(_6\)Na\(^+\)) calculated = 561.2935, observed = 561.2924.

**Synthesis of (R)-\(^1\)Boc-Fmoc-\(\alpha\)-Methyl-\(\alpha\)-Lysine-OH (42)**

An amount of 2g (3.7 mmol) of 41 was dissolved in 20 mL of 1:1 TFA/CH\(_2\)Cl\(_2\). The solution was allowed to stir for 12 hrs at RT under a N\(_2\) atmosphere. The reaction was monitored by TLC (5% MeOH/CH\(_2\)Cl\(_2\)) and ESI-mass spectrometry for completion. At which point the TFA/CH\(_2\)Cl\(_2\) layer was evaporated under reduced pressure giving free amino acid. The residue was taken up in 15 mL of H\(_2\)O and 0.76g (9 mmol) of NaHCO\(_3\) was added to the solution slowly to control the effervescence. The mixture was cooled to 0 \(^0\)C. A solution of 0.96g (4.4 mmol) (Boc)\(_2\)O in 15 mL 1,4-dioxane was added to the mixture slowly at 0 \(^0\)C. The reaction was allowed to stir at 0 \(^0\)C for an hour. The reaction was then allowed to warm to RT and stir for 12 hrs. The reaction mixture was extracted with pentane to remove excess (Boc)\(_2\)O. The aqueous layer was then acidified to pH 4 with 2N HCl, and extracted three times with Et\(_2\)O (50 mL). The combined ether layer was dried over MgSO\(_4\), evaporated under reduced pressure, and purified by chromatography (5% MeOH/CH\(_2\)Cl\(_2\)), giving 1.52g (3.15 mmol, 85% over two steps) of 42 as a white solid similar 34. All characterization data of 42 complied with the data for
The polarimetry reading confirmed 42 as the enantiomer to 34. $[\alpha]^{22}_D = -11.5$ (c = 1, CHCl$_3$).

**Synthesis of (S)-Ethyl 2-Amino-2-((1,3-Dioxoisindolin-2-Yl) Methyl) Propanoate (43)**

An amount of 10g (23 mmol) 23a was dissolved in 60 mL of methylene chloride and 10 mL TFA was added. The solution was stirred for 1 hr. The solution became dark purple in color. A volume of 100 mL H$_2$O was added to the solution and the organic layer was washed with NaHCO$_3$ solution, washed with H$_2$O, and dried over MgSO$_4$. The residue was purified by flash chromatography (5% MeOH/CH$_2$Cl$_2$), giving 5.7g (20.6mmol, 90%) of 43 as a white wax. $R_f = 0.64$ (5% MeOH/CH$_2$Cl$_2$), IR (cm$^{-1}$) = 3391, 3325, 3000, 2959, 1770, 1731, 1704, 1557. $^1$H-NMR (CDCl$_3$, 400 MHz): $\delta$ 7.85 (m, 2H), 7.73 (m, 2H), 4.20 (m, 2H), 3.91 (m, 2H), 1.77 (bs, 2H), 1.41 (s, 3H), 1.29 (t, 3H, $J = 7$ Hz). $^{13}$C-NMR (CDCl$_3$, 100 MHz): $\delta$ 175.0, 169.0, 134.0, 132.0, 123.0, 61.0, 58.0, 46.0, 24.0, 14.0. HRMS (C$_{14}$H$_{16}$N$_2$O$_4$Na) calculated = 299.1002, observed = 299.1002.

**Synthesis of (S)-Ethyl 2-(Dibenzylamino)-2-[(1,3-Dioxoisindolin-2-Yl)Methyl]Propanoate (44)**

An amount of 4.2g (15.2 mmol) of 43 was dissolved in 60 mL of distilled acetonitrile in a 250 mL three necked flask under a N$_2$ atmosphere. An amount of 12.6g (91.2 mmol) of K$_2$CO$_3$ was added with stirring. A volume of 9 mL (76 mmol) of BnBr was added drop wise. The reaction mixture was heated to reflux solvent for 12 hrs. The reaction mixture was diluted with 50 mL of H$_2$O and the solution was extracted with ether three times. The ether layer was washed with H$_2$O, washed with brine, dried over MgSO$_4$, and evaporated. The residue was then purified by flash chromatography (30% EtOAc/Hexanes) giving 5.6g (12.3 mmol, 81%) of 44 as a white solid. $R_f = 0.59$ (30%
EtOAc/Hexanes), IR (cm$^{-1}$) = 2970, 1770, 1713, 1620. MP = 83$^\circ$C. $^1$H-NMR (CDCl$_3$, 400MHz): δ 7.83 (m, 2H), 7.71 (m, 2H), 7.34 (d, 4H, $J = 8$Hz), 7.12 (m, 6H), 4.23 (q, 2H, $J = 7$ Hz), 3.95 (m, 6H), 1.35 (m, 6H). $^{13}$C-NMR (CDCl$_3$, 100MHz): δ 174.0, 168.0, 141.0, 134.0, 132.0, 128.3, 128.0, 126.0, 123.0, 68.0, 61.0, 55.0, 44.0, 19.0, 14.0. HRMS (C$_{28}$H$_{28}$N$_2$O$_4$Na$^+$) = 479.1941, observed = 479.1938.

(S)-Ethyl-3-Amino-2-(Dibenzylamino)-2-Methylpropanoate (45)

An amount of 3.2 g (7 mmol) of 44 was dissolved in 20 mL of (8:2) MeOH and CH$_2$Cl$_2$. A volume of 1.7 mL (21 mmol) of N$_2$H$_4$ (35% in H$_2$O) was added. The solution was heated to reflux solvent for 3 hrs. The formation of a white precipitate indicated the completion of the reaction. The reaction mixture was filtered and the filtrate was evaporated giving 2g (6.5 mmol, 92%) of 45 as a yellowish oil. R$_f$ = 0.65 (5% MeOH/CH$_2$Cl$_2$). IR (cm$^{-1}$) = 2979, 1717, 1601. $^1$H-NMR (CDCl$_3$, 400MHz): δ 7.21 (m, 10H), 4.16 (m, 2H), 3.84 (m, 4H), 2.95 (s, 2H), 1.35 (s, 3H), 1.31 (t, 3H, $J = 7$ Hz), 1.20 (bs, 2H), $^{13}$C-NMR (CDCl$_3$, 100MHz): δ 174.0, 141.0, 128.4, 128.0, 126.0, 69.0, 60.0, 55.0, 48.0, 20.0, 14.0. HRMS (C$_{20}$H$_{26}$N$_2$O$_2$Na$^+$) calculated = 349.1886, observed = 349.1873.

(S)-Ethyl-3-Amino-2-(Dibenzylamino)-2-Methylpropanoicacid (46)

An amount of 1.41 g (4.3 mmol) of 45 was dissolved in 25 mL of EtOH. An amount of 0.52 g (12.9 mmol) of well crushed NaOH pellets were added. The solution was heated to reflux solvent for 4 hrs. The EtOH layer was acidfied to pH 2, evaporated to dryness under high vacuum, and triturated with MeOH. The MeOH layer was neutralized with solid NaHCO$_3$, filtered, and evaporated under reduced pressure giving 1.16g (3.9 mmol, 90%) of 46 as yellowish wax. $^1$H-NMR (CD$_3$OD, 400MHz): δ 7.18 (m,
10H), 3.91 (m, 4H), 2.83 (m, 2H), 1.87 (s, 3H). $^{13}$C-NMR (CD$_3$OD, 100MHz): δ 182.0, 144.0, 129.6, 129.0, 127.0, 70.0, 56.0, 22.0. HRMS (C$_{18}$H$_{22}$N$_2$O$_2$Na$^+$) calculated = 321.1573, observed = 321.1573.

**Synthesis of (S)-2-(Dibenzylamino)-3-(Tert-Butyloxycarbonylamino)-2-Methylpropanoic Acid (47)**

A solution of 1g of 46 (3.4 mmol) in 10 mL water was placed in a 50 mL round bottom flask. An amount of 0.56g (6.7 mmol) of NaHCO$_3$ (2eq) was added with stirring. The solution was cooled to 0°C. A solution of 0.98g (4.7 mmol) (Boc)$_2$O (1.4eq) in 10 mL 1,4 dioxane was added drop wise. The reaction was allowed to stir at 0°C for an hour. The reaction was then allowed to warm to RT overnight. The reaction mixture was diluted with 15 mL H$_2$O, acidified to pH 4 with NaHSO$_4$, and extracted twice with Et$_2$O. The combined ether layer was washed with water (5 x 30 mL), washed with brine, dried over MgSO$_4$, and evaporated. The residue was purified by chromatography (40% EtOAc/Hexanes), giving 1.2g (3 mmol, 91%) of 47 as a white solid. $R_f = 0.28$ (40% EtOAc/Hexane). IR (cm$^{-1}$) = 2977, 1698, 1494. MP = 58°C. $^1$H-NMR (CDCl$_3$, 400 MHz): δ 7.21 (m, 10H), 5.44 (bs, 1H), 4.09 (m, 4H), 3.64 (m, 2H), 1.42 (m, 12H). $^{13}$C-NMR (CDCl$_3$, 100 MHz): δ 175.0, 156.0, 137.0, 128.8, 128.5, 127.7, 79.0, 71.0, 55.0, 44.0, 28.0, 20.0. HRMS [C$_{23}$H$_{30}$N$_2$O$_4$Na$^+$] calculated = 421.2097, observed = 421.2094.

**(S)-2-Amino-3-(Tert-Butyloxycarbonylamino)-2-Methylpropanoic Acid (48)**

A solution of 1g (2.5 mmol) of 47 in 25 mL MeOH was placed in a pressure bottle. An amount of 0.2g (20% by weight) of Pd-C was added to the bottle. The solution was placed on a Parr shaker hydrogenation apparatus and allowed to shake with 30 psi hydrogen gas for 12 hrs. The reaction mixture was filtered through a Celite bed to remove
the catalyst. The filtrate was evaporated giving 48. An amount of 0.5 g (2.3 mmole, 92%) of 48 was obtained as a white wax. $R_f = 0.3$ (5% MeOH/CH$_2$Cl$_2$). IR (cm$^{-1}$) = 2977, 1701, 1602, 1508. MP = 204 0°C. $^1$H-NMR (CD$_3$OD, 400 MHz): $\delta$ 3.44 (s, 2H), 1.47 (m, 12H). $^13$C-NMR (CD$_3$OD, 100 MHz): $\delta$ 174.0, 158.0, 79.0, 61.0, 46.0, 27.0, 19.0. HRMS (C$_9$H$_{18}$N$_2$O$_4$Na$^+$) calculated = 241.1159, 241.1158.

**Synthesis of (S)-2-(9-Fluorenylmethyloxycarbonylamino)-3-(Tert-Butyloxy carbonylamino)-2-Methylpropanoic Acid (49)**

An amount of 0.35 g of NaHCO$_3$ (4.1 mmol) was added to a solution of 0.45 g 29 (2.1 mmol) in 15 mL water with stirring. The solution was cooled 0°C. A solution of 1.1 g Fmoc-Osu (3.2 mmol) in 15 mL 1, 4-dioxane was added to the reaction mixture over 20 min. The reaction was allowed to stir at 0°C for an hour and then at ambient temperature for 12 hrs. At that point the reaction was diluted with 30 mL of water, acidified to pH 4 with 4M HCl, extracted (3 x 50 mL) with Et$_2$O. The combined ether layer was washed with brine, dried over MgSO$_4$, and evaporated under reduced pressure. The residue was purified by radial chromatography using 5% MeOH/CH$_2$Cl$_2$ giving 0.86 g (1.96 mmol, 85%) of 49 as a white solid. $R_f = 0.41$ (5% MeOH/CH$_2$Cl$_2$), IR (cm$^{-1}$) = 3317, 2974, 1694, 1513. MP = 82 0°C. $[\alpha]_D^{22} = -10.5$ (c = 1, CHCl$_3$), $^1$H-NMR (CD$_3$OD, 400 MHz): $\delta$ 7.80 (d, 2H, $J = 7$ Hz), 7.68 (d, 2H, $J = 7$ Hz), 7.39 (t, 2H, $J = 7$ Hz), 7.31 (t, 2H, $J = 7$ Hz), 4.31 (bs, 2H), 4.22 (t, 1H, $J = 7$ Hz), 3.55 (m, 2H), 1.44 (s, 12H). $^13$C-NMR (CD$_3$OD, 100 MHz): $\delta$ 159.0, 157.0, 145.4, 145.3, 143.0, 129.0, 128.0, 126.4, 126.3, 121.0, 80.0, 68.0, 55.0, 46.0, 28.0, 21.0. HRMS (C$_{24}$H$_{28}$N$_2$O$_6$Na$^+$) calculated = 463.1839, observed = 463.1835.
Synthesis of (S)-Tert-Butyl 2-Tert-Butyloxyaminobutyl-4-Diazo-2-Methyl-3-Oxobutanoate (50)

Acid 40 (3g, 8.7 mmol) was dissolved in 10 mL THF and cooled to -25 °C. A measured 1 equivalent of Et₃N (1.2 mL, 8.7 mmol), and 1.05 equivalents of ClCO₂Me (710 µL, 9.1 mmol) was added drop wise to the THF solution. The mixture was stirred for 2 hrs. giving rise to the mixed anhydride, which was taken immediately for the next step. The resulting white suspension of the mixed anhydride was allowed to warm to 0 °C and a solution of dry diazomethane (2 equivalent, 17.4 mmol) in Et₂O was carefully added. The reaction mixture was allowed to stir for 12 hrs. in the dark at 0 °C. Excess diazomethane was removed by passing N₂ through the solution for 30 mins. The reaction mixture was then diluted with Et₂O, washed with saturated NaHCO₃, saturated NH₄Cl, and brine. The organic layer was dried over MgSO₄, and concentrated under reduced pressure. The resulting residue was purified by chromatography (1:1 Et₂O/Hexanes), giving 2.62g (7.1 mmol, 82%) of 50 as a clear yellowish oil. Rᵣ = 0.42 (1:1 Et₂O/Hexanes). IR (cm⁻¹) = 3381, 2976, 2934, 2110, 1704, 1517. ^1H-NMR (CDCl₃, 400 MHz): δ 5.41 (s, 1H), 4.58 (bs, 1H), 3.09 (bm, 2H), 1.85 (m, 1H), 1.71 (m, 1H), 1.45 (m, 21H), 1.28 (m, 4H). HRMS (C₁₈H₃₁N₃O₅Na⁺) 392.2156, observed = 392.2153.

(S)-3-Tert-Butyloxyaminobutyl-4-Tert-Butyloxy-3-Methyl-4-Oxobutanoic Acid (51)

An amount of 2.5g 50 (6.8 mmol) was dissolved in 15 mL 3:7 H₂O/THF in a 50 mL round bottom flask. The flask was purged with N₂ and the resulting solution was photolyzed with a Hanovia lamp (500 W) at a distance of approximately 10 cm. The photolysis was allowed to proceed for 48 hrs. At that point the reaction was found to be completed as evident by TLC. The clear and colorless solution was concentrated under
reduced pressure and the water layer was extracted three times with Et$_2$O. The combined Et$_2$O layer was washed with brine, dried over MgSO$_4$, and evaporated under reduced pressure. The residue was purified by chromatography (30% EtOAc/Hexanes), giving 1.83g of 51 (5.1 mmol, 75%) as a yellowish oil. The $^1$H-NMR and the HRMS is highly indicative of the product. Hence, the product was taken for the next step without further purification. $R_f = 0.18$ (30% EtOAc/Hexanes), IR (cm$^{-1}$) = 3364, 2977, 2936, 1709, 1521. $^1$H-NMR (CDCl$_3$, 400 MHz): $\delta$ 4.54 (bs, 1H), 3.10 (bm, 2H), 2.73 (m, 1H), 2.38 (m, 1H), 1.55 (m, 22H), 1.20 (m, 5H). $^{13}$C-NMR (CDCl$_3$, 100 MHz): $\delta$ 176.2, 175.2, 155.9, 80.6, 72.4, 44.4, 42.4, 40.1, 38.8, 30.0, 28.4, 27.8, 21.8, 21.4. HRMS (C$_{18}$H$_{33}$NO$_6$Na$^+$) calculated = 382.220, observed = 382.2196.

$(S)$-Tert-Butyl-2-Tert-Butyloxycarbonylaminobutyl-3-(9-
Fluorenylmethyloxycarbonylaminobutyl)-2-Methylpropanoate (52)

A volume of 0.79 mL Et$_3$N was added to a solution of 1.7g of 51 (4.7 mmol) in 25 mL dichloroethane under N$_2$ atmosphere. A volume of 1.2 mL (5.2 mmol) DPPA was added to the reaction mixture. The reaction was allowed to stir at RT for 2 hrs. The mixture was heated to reflux solvent for 3 hrs. The mixture was cooled and the organic layer was extracted with saturated NH$_4$Cl solution, dried over MgSO$_4$, and evaporated under reduced pressure giving the isocyanate. The isocyanate was taken up in dry toluene under N$_2$ atmosphere. An amount of 1.84g (9.4 mmol) 9-fluorenylmethanol was added to the solution along with 100 μL of Ti (IV) isopropoxide. The reaction was heated to 80°C overnight. The reaction was cooled and the organic layer was evaporated under reduced pressure. The residue was then purified by chromatography (CH$_2$Cl$_2$ to 3% MeOH/CH$_2$Cl$_2$), giving 2g 52 (4 mmol, 80%) as sticky light yellowish wax. The
product was found too sticky to dry the solvent all the way. It was characterized by \(^1\)H-NMR and HRMS. The product was taken for the next step without further attempt to purify it. \(R_f = 0.78\) (3% MeOH/CH\(_2\)Cl\(_2\)). IR (cm\(^{-1}\)) = 3340, 2975, 2933, 1756, 1688, 1513. \(^1\)H-NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.76 (d, 2H, \(J = 7\)Hz), 7.59 (d, 2H, \(J = 7\)Hz), 7.39 (t, 2H, \(J = 7\)Hz), 7.30 (t, 2H, \(J = 7\)Hz), 4.60 (bm, 2H, \(J = 7\)Hz), 4.37 (m, 2H), 4.22 (t, 1H, \(J = 7\)Hz), 3.38 (m, 1H), 3.24 (m, 1H), 3.09 (m, 2H), 1.46 (bm, 6H), 1.44 (bm, 21H). HRMS (C\(_{32}\)H\(_{44}\)N\(_2\)O\(_6\)Na\(^+\)) calculated = 575.3091, observed = 575.3083.

**Synthesis of (S)-Fmoc-\(\alpha\)-Methyl-\(\beta^2\)-Lysine-Boc-OH (53)**

An amount of 1.5g of 52 (2.7 mmol) was dissolved in 20 mL of 1:1 TFA/CH\(_2\)Cl\(_2\). The solution was allowed to stir for 12 hrs at RT under N\(_2\) atmosphere. The reaction was monitored by TLC (5% MeOH/CH\(_2\)Cl\(_2\)) and ESI-mass spectrometry for the completion. At which point the TFA/CH\(_2\)Cl\(_2\) layer was evaporated under reduced pressure giving free amino acid. The residue was taken up in 15 mL of H\(_2\)O and 0.54g (6.5 mmol) NaHCO\(_3\) was added to the solution slowly to control the effervescence. The mixture was cooled to 0 \(^0\)C. A solution of 0.71g (Boc)\(_2\)O (3.2 mmol) in 15 mL 1,4-dioxane was added to the mixture slowly at 0 \(^0\)C. The reaction was allowed to stir at 0 \(^0\)C for an hour. The reaction was then allowed to warm to RT and stir for 12 hrs. The reaction mixture was extracted with pentane to remove excess (Boc)\(_2\)O. The aqueous layer was then acidified to pH 4 with 2N HCl, extracted three times with Et\(_2\)O (50 mL). The combines ether layer was dried over MgSO\(_4\), evaporated under reduced pressure, purified by chromatography (5% MeOH/CH\(_2\)Cl\(_2\)), giving 1.21g of 53 (2.44 mmol, 90% over two steps) as a white solid after purification by flash chromatography (CH\(_2\)Cl\(_2\) to 5% MeOH/CH\(_2\)Cl\(_2\)). \(R_f = 0.50\) (5% MeOH/CH\(_2\)Cl\(_2\)). IR (cm\(^{-1}\)) = 3338, 2940, 1693, 1518. \([\alpha]_D^{24} = -6.0\) (c = 0.7, CHCl\(_3\)). MP
= 73 °C. $^1$H-NMR (CD$_3$OD, 400 MHz): $\delta$ 7.81 (d, 2H, $J = 7$Hz), 7.66 (d, 2H, $J = 7$Hz), 7.40 (t, 2H, $J = 7$Hz), 7.32 (t, 2H, $J = 7$Hz), 4.36 (m, 2H), 6.23 (m, 1H), 3.03 (t, 2H, $J = 7$Hz), 1.61 (m, 1H), 1.44 (m, 12H), 1.30 (m, 2H), 1.13 (s, 3H). $^1$H-NMR (CDCl$_3$, 400 MHz): $\delta$ 7.75 (d, 2H, $J = 7$Hz), 7.58 (m, 2H), 7.38 (t, 2H, $J = 7$Hz), 7.30 (t, 2H, $J = 7$Hz), 6.37 (bm, 1H), 5.42 (bm, 1H), 4.58 (m, 1H), 4.35 (m, 1H), 4.21 (m, 1H), 3.36 (m, 2H), 3.09 (bm, 2H), 1.41 (m, 18H). $^{13}$C-NMR (CDCl$_3$, 100 MHz): $\delta$ 179.5, 156.9, 156.2, 143.6, 141.2, 127.7, 127.1, 125.1, 120.0, 79.3, 66.8, 47.3, 46.8, 40.0, 36.7, 36.0, 30.4, 28.4, 21.3, 20.4. HRMS (C$_{28}$H$_{36}$N$_2$O$_6$Na$^+$) calculated = 519.2965, observed = 519.2459.
CHAPTER III

SYNTHESIS AND BIOLOGICAL EVALUATION OF A VAPREOTIDE
(SOMATOSTATIN ANALOGUE) CONTAINING α-METHYL-α-LYSINE

Background

Small Peptides Containing Lysine and Their Importance

Several small peptides containing lysine have been found to be medically very important. Some of them are: Dermaseptin, AGG01, Stichodactyla toxin, Crotamine, Neurotensin, Bombesin, Cholecystokinin, and Somatostatin

Somatostatin

Somatostatin (somatotropin release inhibiting factor or SST) was discovered as a hypothalamic neurohormone, which inhibits growth factor secretion. SST was detected both in the central and peripheral nervous system, and in peripheral tissues where it plays many different roles. In periphery the endocrine pancreas and gut are the main sources of SST.

SST has several functions, which are as follows:

1. Inhibition of endocrine and exocrine secretion.
3. Motor and cognitive functions.
4. Inhibition of intestinal motility.
5. Absorption of nutrients and ions.
6. Vascular contractility and cell proliferation.
7. Inhibition of proliferation of several tumor and normal cells.
Naturally occurring somatostatin is available in two molecular forms: a tetradecapeptide (SST-14) and a 28-amino acid peptide (SST-28) containing the amino acid sequence of SST-14, N-terminally extended by 14 amino acid residues as shown in Figure 14. The diverse action of SST peptides are mediated through interaction with 5 different SST receptors (SSTRs) expressed by variety of normal and malignant tissues. The rationale behind evaluation SST as an anticancer drug is the dual character of SST analogues to inhibit hormone release and cell growth.

![Figure 14. Structures of SST14 and SST28. The enzymatic cleavage sites on SST14 are shown with arrows.](image)

**Somatostatin Receptors and Their Ligands/Tissue Expression**

The biological effects of somatostatin are executed by binding to five distinct high-affinity G Protein coupled receptors (SSTR1-SSTR5) over expressed on the cell surface. These SST receptors exhibit a high degree of resemblance but differ mainly at their amino and carboxy terminal segments. This disparity is attributed to their specificity in ligand binding and intracellular signaling. The up regulation of SSTR subtypes has been evidenced in human tissues, diverse tumor tissues and cell lines at mRNA level and protein level with SSTR subtype specific ligands. In addition to the
normal tissues, SSTRs have also been witnessed to be over expressed by
gastroenteropancreatic (GEP) tumors, carcinoid tumors, small-cell lung tumors, prostate
carcinoma, breast carcinoma, renal carcinoma, frequently nervous system tumors, and
medullary carcinoma of the thyroid.\textsuperscript{95, 98} Most of the time, cancer cells express more than
one SST subtypes with SSTR2 most frequently followed by SSTR1, SSTR3, SSTR4, and
SSTR5.\textsuperscript{98} It has also been observed that there is disparity in the SSTR subtype expression
pattern in different tumor types, within the same type of tumors, and even in each
patient.\textsuperscript{99} Multiple SSTR subtype protein expression has also been reported in medullary
carcinoma of thyroid.\textsuperscript{100} A summary of the present knowledge on SSTR subtype mRNA
and protein expression in tumor cells is given in the table 1 below.\textsuperscript{100}

Table 1

\textit{Expression of SSTR subtypes in several tumor cells.}\textsuperscript{98}

\begin{tabular}{|l|l|}
\hline
Subtype & Cancer cell over expressing SSTRs \\
\hline
SSTR1 & Prostate carcinomas, sarcomas, GEP tumors, phaeochromocytomas. \\
\hline
SSTR2 & Neuroblastomas, meningiomas, medulloblastomas, breast carcinomas, lymphomas, renal carcinomas, small cell lung carcinomas, hepatocarcinomas, pituitary adenomas, GEP tumors, phaeochromocytomas, paraganglomas etc. \\
\hline
SSTR3 & Pituitary adenomas \\
\hline
SSTR4 & - \\
\hline
SSTR5 & GH-screening pituitary adenomas \\
\hline
\end{tabular}
Function of SSTR Subtypes and Receptor Binding Specificity of SST Analogues

Receptors are the binding sites for peptides. One of the most common characteristics of SSTRs is that they stimulate rapid internalization of receptor-agonist complex into the cell upon binding of agonist to the SSTRs overexpressed on the tumor cells. The internalization of SSTR-agonist complex may take place upon binding of either endogenous agonist or exogenous agonist to the SSTRs. The mechanism of higher degree of internalization of SSTR-somatostatin radioligand complex into the tumors overexpressing SSTRs is often employed to effectively and specifically accumulate radioactivity into the tumor cells. The accumulated somatostatin radioligands permit successful tumor imaging in patients as well as targeted chemotherapy. The 5 receptor subtypes (SSTR1-5) bind with the naturally occurring peptides (SST-14 & SST28) with low nanomolar affinity. Out of five SSTR subtypes, only SSTR5 shows a 10 fold higher affinity for SST28. Short synthetic peptides have been observed to bind strongly to 3 of 5 SSTR Subtypes (SSTR 2, SSTR3, and SSTR5) as opposed to SST14. For instance, octapeptide analogues of SST, which are currently in use in clinical studies as antineoplastic agents (figure 15), are more selective than SST14.

Figure 15. SST octapeptide analogues.
Octapeptides (Figure 14) show very low affinity for SSTR1 and SSTR4 (> 1000 nM), moderate affinity for SSTR3 (225 nM) and high affinity for SSTR2 and SSTR5 (2.8 to 9.9 nM). The hexapeptide, Seglitide, exhibits a similar pattern of binding selectivity and potency. However, some of the tumors overexpress predominantly SSTR1 or SSTR4 but lack in SSTR2 and SSTR5. Lately, there have been many efforts to prepare diverse SST analogues, that differ in ring sizes, with selective binding affinity for SSTR1 and SSTR4. Additionally, there has been much research on development of SST receptor subtype-selective analogues, both as peptides and nonpeptides.

There are three reasons behind this type of research:

1. Evaluation of the distribution of various receptor subtype proteins in tissue.
2. Determination of the specific biological effects mediated by the various subtypes.

Mechanism of the Antiproliferative Activity of SST Octapeptide Analogue

SST octapeptide analogues could influence the tumor cell growth either by indirect effect or by direct effect.

Indirect Effect

Indirect effect consequences from the inhibition of several growth promoting hormones and growth factors that stimulates the growth of a variety types of cancers. For instance, it is witnessed that IGF-1, which is excreted by hepatocytes either by dependent or independent mechanism, is a growth promoter of a number of tumors that express IGF-1 receptors. Octreotide was found to downregulate the serum IGF-1 level either by inhibiting GF secretion, or by directly inhibiting IGF-1 gene expression, or by rising circulating IGF-1 binding proteins. This IGF-1 suppression strategy has been found to
be very effective in the treatment of IGF-1 dependent tumors such as GH screening pituitary adenomas and to a lesser extent breast, lung and prostate tumors.\textsuperscript{98} Several other growth factors or hormones, which are known to play an important role in tumor growth, are being regulated either by naturally occurring somatostatin or SST analogues, as for example, gastrin, insulin, glucagon epidermal growth factor, and transforming growth factor-alpha.\textsuperscript{98}

**Direct Effect**

As mentioned earlier, a number of cancer cells overexpress SSTRs.\textsuperscript{98} Some of them often express more than one SST subtypes.\textsuperscript{95, 98} For instance, SSTR expressions have been recognized in human primary colorectal carcinomas, small cell lung carcinoma, breast cancer, renal cell carcinoma, and malignant lymphoma.\textsuperscript{98} A direct inhibitory effect of SST and its analogues have been evidenced for these tumor cell lines.\textsuperscript{98} Hence, precedent experimental results indicates that somatostatin can directly interact with the blood vessels of the tumor cells through specific receptors.\textsuperscript{98, 103, 104} The antiproliferative activity of SST may consequence either from the blockage of mitogenic growth factor signal or from the induction of apoptosis, depending on the SSTR subtype or the target cell.\textsuperscript{98} Octreotide and vapreotide have been found to inhibit both serum and insulin driven proliferation of NIHT3/CHO cell transfected with SSTR2 via stimulation of the tyrosine phosphate.\textsuperscript{98}

In comparison to naturally occurring SST (SST14 and SST28), short synthetic SST analogues have been found to be even more efficient due to high receptor binding affinity and selective binding to the receptors.\textsuperscript{95, 98} Out of several short SST analogues, octapeptide analogues (Figure 14) are being used frequently as drugs to suppress
out of three synthetic octapeptides (Figure 14), the octreotide is used most frequently due to its high binding affinity to SSTR2 and SSTR5.

**Application of Somatostatin Octapeptide Analogues in the Clinic**

At present, there is widespread application of somatostatin octapeptide analogues in treatment of various types of cancers. Some of important of them are noted below.

**Carcinoid Tumor**

Octreotides and lanreotides are permitted in most countries to treat the hormonal symptoms in the patients with carcinoids. Endocrine cells give rise to the carcinoids. They generally arise in the ileum and metastasize to liver resulting in so called “carcinoid syndrome” (flushing, diarrhea, cardiac vascular lesions). Some carcinoids may appear in the non-gastrointestinal origin such as lung or ovary. Recent studies suggest that octreotide treatment results in a substantial improvement of hormonal symptoms in more than 90% of the patients with carcinoids.

**Endocrine Pancreatic Tumors**

Another application of Octreotide in the field of oncology is its use in the treatment of hormonal hypersecretion associated with endocrine pancreatic tumors. Octreotide has been well explored in the treatment of insulinomous, gastrinomas, VIP-omas, glucagonomas, and somatostatinomas with variable effects on symptoms in spite of its capability of lowering plasma concentrations of marker peptide in most patients. In some cases, VIP-omas cannot be cured either by surgery or by chemotherapy, and Octreotide is the only choice of treatment approved by Food and Drug Administration. Improvement of symptoms and biochemical processes have been observed in more than
80% of the patients. Octreotide has been found to be beneficial for 50% of the patients with insulinomas and 90% of the patients with gastrinomas.

*Pituitary Tumors*

The best way to treat pituitary tumors is surgical removal of the tumors. However, it is not always possible to remove them by surgery because of their extension to pituitary and super pituitary areas. In this situation, Octreotide is the treatment of choice to reduce the hormone hyper-secretion. Recent observations show that the octreotide administered to acromegatic patients stimulated rapid and remarkable clinical improvement by lowering the GH levels to less than 5μg/L in half of the patients. The size of the pituitary adenoma has been observed to reduce by more than 20% in half of the patients on octreotide treatment.

*Other Cancers*

Octreotide has also been proposed for other cancerous cells based on the SSTR expressions, for example: pancreatic tumors, hepatocellular carcinoma, small cell lung cancer, breast cancer, Prostate cancer, non-neurodocrine solid tumors.

*Radiolabeled Somatostatin Octapeptide Analogues and Their Applications*

It is always very difficult to determine the localization of neuroendocrine tumors in patient bodies by standard techniques such as ultra sonography or computed tomography, since those tumors are very small in size. One alternative approach is to visualize the SSTRs on the neuroendocrine tumors. This approach is very promising and viable, because SST radiopharmaceuticals are suitable for scintigraphy and are available. Moreover, the presence of SSTRs on the endocrine tumors with high density made this imaging possible. The first radioactive SST analogue used for tumor imaging
was $^{123}\text{-TYR}^3$-Octreotide. It was later determined that alternative labeling procedures and radioisotopes were explored to minimize several problems, as for example, halogenations of $^{123}\text{-TYR}^3$, or non specific uptake, etc. More recently, various novel chelators are being attached to the N-terminus of the SST analogues for labeling with radio-metals, which were designed and studies in the clinic. A list of the recent chelators is given below in Figure 16.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{somatostatin_analogues_chelators}
\caption{Somatostatin analogues attached to chelators.}
\end{figure}

**Targeted Chemotherapy**

Another application of SST in oncology is its use in targeted chemotherapy. Just like targeted radiotherapy, SSTR targeted chemotherapy seems to be a promising approach in the treatment of SSTR expressing malignant sites. More over SSTR targeted chemotherapy has been found to be more efficient either from the anti-cancer drug alone or from the SST analogues alone. It was already proven when nude mice bearing xenografts of MIA-paca-2 human pancreatic cancer cells were treated with this combination, a significant reduction in tumor size was reported as compare to anticancer drug alone or the SST analogues alone. More recently, another compound
was synthesized by a different group and that was a complex made of Octreotide conjugated to Taxol. This complex has been found to induce apoptosis in MCF-7 cells in SSTRs selective way by making the conjugate less toxic than free Taxol itself.\textsuperscript{98} Hence, to summarize, there are widespread applications of somatostatin analogues in the field of oncology. Short half life period (2-3 minutes) of Somatostatin analogues (due to proteolytic degradation) is the main problem.\textsuperscript{98} Short synthetic octapeptides (Octreotide, Lanreotide and Vapreotide) have 45 times higher half life than SST14.\textsuperscript{98} However, octapeptide treatment require further improvement in half-lives of the SST analogues.\textsuperscript{98} This problem has become a major impediment for frequent clinical use of SST analogues. Since the last decade, there has been much focus on the improvement of somatostatin analogues to make it more potent to proteolytic degradation.\textsuperscript{35, 109} Work has been carrying out by several research groups to give birth to a new protease resistant SST analogue.

\textit{Instability and Amelioration of Somatostatine Analogues}

Both naturally occurring somatostatins (SST-14 and SST-28) are extremely sensitive to peptidases, and they rapidly degrade due to cleavage of peptide bonds by several types of peptidases present in most tissues (Trypsin, Plasmin, Plasma Kallikrein).\textsuperscript{35, 98, 109} The naturally occurring SST-14 contains at least four sites susceptible to proteolytic degradation.\textsuperscript{98} Out of these four sites, Lys\textsuperscript{9}-Thr\textsuperscript{10} bond is more important because cleavage of this bond results in loss of agonist action.\textsuperscript{98} In most of the cases, trypsin plays its role in cleaving the bond next to lysine.\textsuperscript{67} In accordance with the recent data, it would be rational to work on the improvement of the octapeptide somatostatin
analogenues, because they have much higher affinity and selectivity to the SSTRs compare to the SST-14 and SST-28\textsuperscript{101, 103, 104, 110, 111}.

**Identification of the Amino Acids Essential for the Biological Activity of SST**

Several systematic experiments have been carried out by replacing residues of SST by Ala, which is called alanine scan. Alanine scan has revealed that residues in the position 7-10 are very important for the activity of SST, whereas the N-terminal amino acids (Ala-Gly) are less important.\textsuperscript{98} Among all of them, Lys9 is most important since cleavage of Lys-Thr bond leads to loss of SST agonist action.\textsuperscript{98} It has also been found that the octapeptide analogues with the sequence of Phe\textsuperscript{7}-D-Trp\textsuperscript{8}-Lys\textsuperscript{9}-Thr\textsuperscript{10} are more efficient in receptor binding compare to other Octapeptide analogues.\textsuperscript{92, 98} For instance, octreotide with the above sequence and C-terminal amino alcohol have been found to be long lasting in blood plasma and 45-70 times more potent than naturally occurring SST in prohibition of GH secretion.\textsuperscript{92, 98} Octreotide has also been found to suppress the insulin and glucagon secretion to a lesser extent than GH secretion.\textsuperscript{98} Hence, the above mentioned evidences suggest that Octreotide is very specific for the inhibition of GH secretion. Prolonged studies and observations have proven octapeptides with much higher affinity, specific receptor binding ability and most importantly more stability to almost all sorts of proteases.\textsuperscript{98} Though the stability of these octapeptides is not yet high enough to minimize their doses of administration and cut down the astronomical amount of expenses to the common and poor patients around the word.\textsuperscript{98} In most of the cases, common patients cannot afford the long term treatment with these ocrapeptides as they are required to administer in higher amounts frequently for their low stability towards the proteases.\textsuperscript{98} Moreover higher doses give rise to many of the side effects like nausea,
transient abdominal cramps, flatulence, diarrhea, delay of insulin release in response to meal, formation of gallstones, etc.\textsuperscript{98} Extensive research still needs to be done to improve the half life period of somatostatin analogues.\textsuperscript{98}

\textit{Current State of Work on Developing Protease Resistant Somatostatin}

There has been extensive research on developing novel protease resistant SST analogues and emphasis is on the Lys-Thr bond. This bond is very important with respect to the receptor binding and enzymatic activity of SST.

\textit{Site Directed Mutagenesis and Its Limitation}

Scientists thought of replacing either Lys or Trp with other amino acids, and this is where the concept of site-directed mutagenesis comes into play.\textsuperscript{98} Recently, there has been much research on the site directed mutagenesis because it is a powerful tool in selectively replacing any amino acid in the peptide of interest with any other natural amino acid.\textsuperscript{100} The absolute site specificity and replacement with well characterized structures are the strength of this technique.\textsuperscript{100} The down side of this technique is that the Lys or Trp must be replaced with other naturally occurring amino acids. Furthermore, in the loop of SST, both Trp and Lys are very much essential in terms of receptor binding by salt bridge interaction.\textsuperscript{112} This is where the concept of unnatural amino acids arose. Unnatural amino acids are chemical modification of the naturally occurring amino acids. The problem is natural amino acids cannot be replaced with unnatural Trp or Lys with the help of site directed mutagenesis.

\textit{Peptidomimetic}

This technique could incorporate sterically congested unusual amino acids in protease specific cut sites in peptides of interest.\textsuperscript{100} The concept is to either block the
active site of protease enzymes or slow down the catalytic activity (peptide bond degradation) of protease enzymes. Synthesis of the more protease resistant SST analogues should not be the only thing to consider, but they should have to have higher binding affinity and selectivity to the SSTRs as well. Recently, there has been much research on the synthesis of unnatural α, β, γ- unusual lysine and Trp analogues to make SST analogues more stable and more resistant towards proteolytic degradation. The rationale behind incorporating sterically constrained unnatural amino acids into SST analogues is to induce remarkable propensity in the SST analogues to form stable helical secondary structures. It has been observed that peptides consisting of β and γ-amino acids can adopt a stable secondary structure in solution with a few as four to six residues. Small peptides of four to six γ-amino acid residues form stable helical secondary structure in solution and in solid state. The improved secondary structure is witnessed to provide remarkable stability towards proteolytic degradation as compared to naturally occurring α-peptide controls. A few years ago, Rajeswaran et al. reported that the introduction of N-methyl-lysine in somatostatin analogues is tolerated and retains the binding affinity to SSTR2. To the best of my knowledge, Prasad et al. is the first to report SST octapeptides consisting of Cα,α-disubstituted-amino acid analogues retain binding affinity to SSTR2 and exhibit improved stability in blood plasma. Based on the precedent literatures, I hypothesize:

Hypothesis 2.

Substitution of naturally occurring lysine with conformationally constrained (S)-Cα-methyl-α-lysine would retain specific binding affinity of the SST octapeptides for SSTRs.
Results and Discussion

To test our hypothesis, I chose Vapreotide (one of the SST octapeptides). Vapreotide is a widely studied somatostatin analogue with anti-neoplastic properties. Vapreotide has a higher binding affinity to somatostatin receptor subtype 2 (SSTR2) than native somatostatin. However, Vapreotide is prone to degradation at the Lys-Val bond by serine proteases (Trypsin, Plasmin, Plasma Kallikrein, etc.). We have made an effort to prepare a Vapreotide analogue replacing naturally occurring lysine with our (S)-α-2,2-methyllysine analogue in order to study the specific binding of to SSTR2. The vapreotide analogue was synthesized using properly protected (S)-α-2,2-methyllysine analogue. The Vapreotide analogue was 99% pure as determined by HPLC.

Specific binding studies of the vapreotide analogue were conducted against the IMR 32 human neuroblastoma cells. However, it was observed that the vapreotide analogue showed no specific binding (Table 2) to SSTR2.
Conclusions

A simple switch from naturally occurring lysine to Cα,α-disubstituted lysine diminishes the specific binding of the Vapreotide analogue (54) to SSTR2. I suspect that the loss of specific binding for SSTR2 is attributed to conformational changes of the 54 ring resulting from the introduction of conformationally constrained Cα,α-disubstituted lysine.120,122

Experimental

Synthesis and specific binding of Vapreotide analogue (54) against IMR 32 Cell Line: The Vapreotide analogue (54) was synthesized in collaboration with New England Peptide (Gardner, MA), using properly protected (S)-α2,2-methyllysine analogue (34) prepared in our laboratories as described in chapter II. The Vapreotide analogue was determined to be 99% pure by HPLC.

Binding of the Vapreotide analogue (54) was conducted against IMR 32 human neuroblastoma cells. These cells over express SSTR2 receptors. In order to perform the binding assays, four groups of triplicate wells were studied (n = 12 total). Each well contained 500,000 IMR 32 cells in 2 mL of media. These wells also contained 100,000 counts of 111In-pentetreotide. Three wells were competed with 10^-6 M octreotide and three wells were competed with 54. All 12 wells were incubated at 37°C for 20 hours. Cells were harvested, washed and counted in a gamma counter. However, gamma counter result revealed that the Vapreotide analogue 54 has no specific binding for SSTR2 (Table 2).
**Specific Binding of Vapreotide Analogue (54) against IMR 32 Cells**

Table 2 illustrates the specific binding experiments of Vapreotide analogue (54) against IMR 32 human neuroblastoma cells that are known to over express SSTR2. In the binding assay $^{111}$In-Pentetreotide, which is known to effectively bind to SSTR2, was used as the radio ligand (Hot ligand). In addition, Octreotide acetate, which is known to have high selectivity for SSTR2, was used as a positive control (Cold ligand 1) to compete with the $^{111}$In-Pentetreotide. The Vapreotide analogue (54, Cold ligand 2) was allowed to compete with the $^{111}$In-Pentetreotide as well (Cold ligand 2). Cells were harvested, washed, and counted in gamma counter to determine the quantity of the $^{111}$In-Pentetreotide (CPM) bound to the cells. The specific binding of each cold ligand was determined from the equation below based on the amount of $^{111}$In-Pentetreotide bound to the IMR 32 cells as obtained from the gamma counter.

Specific binding of Octreotide Acetate in CPM (cold ligand 1) = competitive binding of $^{111}$In-Pentetreotide and Octreotide Acetate in CPM (Hot + Cold 1) – binding of $^{111}$In-Pentetreotide in CPM (Hot).

Specific Binding of Vapreotide analogue (35, cold ligand 2) = competitive binding of $^{111}$In-Pentetreotide and Vapreotide (35) in CPM (Hot + Cold 2) – binding of $^{111}$In-Pentetreotide in CPM (Hot).
Table 2

Specific binding assay of the Vapreotide (54) against IMR 32 cell line.

<table>
<thead>
<tr>
<th>Competitor</th>
<th>CPM (Hot)</th>
<th>CPM (Hot + Cold)</th>
<th>CPM Specific binding (Hot – Hot + Cold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octreotide Acetate (Cold 1)</td>
<td>2591</td>
<td>317</td>
<td>2663.0</td>
</tr>
<tr>
<td></td>
<td>3096</td>
<td>332</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3238</td>
<td>287</td>
<td></td>
</tr>
<tr>
<td>Mean CPM</td>
<td>2975</td>
<td>312</td>
<td></td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>340</td>
<td>22.9</td>
<td></td>
</tr>
<tr>
<td>Vapreotide (Cold 2) (35, Figure 2) with (S)-α-methyl-α-lysine</td>
<td>3272</td>
<td>4284</td>
<td>-78.3</td>
</tr>
<tr>
<td></td>
<td>4062</td>
<td>3415</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3574</td>
<td>3444</td>
<td></td>
</tr>
<tr>
<td>Mean CPM</td>
<td>3636</td>
<td>3714.3</td>
<td></td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>398.6</td>
<td>493.6</td>
<td></td>
</tr>
<tr>
<td>Background CPM</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radio Ligand Used</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>111In-Pentetreotide (Hot)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER IV

STEREOSELECTIVE CYCLIZATION STRATEGY TO PREPARE γ-/δ- LACTAMS
AND THEIR USE IN THE PREPARATION OF PROLINE AND NIPECOTIC
ACID ANALOGUES

Background

Lactams are one of the most significant classes of amides. Lactams are frequently obtained as building blocks in a variety of biologically active molecules and are employed very often as intermediates in preparation of diverse materials. In the lactam family, chiral γ-, and δ-lactams have significance, since they provide access to the optically enriched γ-/δ-amino acids, chiral pyrrolidine, and chiral piperidine analogues. In continuation, this class of compounds has been utilized in peptidomimetics. A number of synthetic strategies are established to prepare a variety of γ-, and δ-lactams from the readily accessible precursors.

Current State of the Art to Prepare γ-/δ-Lactams

Over the last few years, researchers have explored a number of ways that led to asymmetric synthesis of γ-lactams. Lately, Yokosaka et al. reported an aza-Michael cascade reaction strategy to prepare a number of γ-lactam derivatives (Scheme 29).
Recently, Darlene et al. reported a cycloaddition strategy to derive a number of \( \gamma \)-lactam analogues through malic anhydride derivatives.\(^{131}\)

\[
\begin{align*}
\text{Ph-N=H} & + \text{O} \text{O} \text{N} \text{N} \text{H} + \text{O} \text{O} \text{N} \text{N} \text{H} & \rightarrow & \text{Ph-N=CO}_{2}\text{H}
\end{align*}
\]

\textit{Scheme 30. Strategy of Darlene et al.}

A few years ago, Pohmakotr et al. had shown that the Mukaiyama-Aldol reaction provides access to asymmetric synthesis of a variety of \( \gamma \)-lactams (Scheme 31).\(^{132}\)

\[
\begin{align*}
\text{TMSO-} & \text{O} \text{O} \text{TMS} + \text{N} \text{R} \text{Ar} \text{I} & \rightarrow & \text{N} \text{R} \text{Ar} \text{CO}_{2}\text{H}
\end{align*}
\]

\textit{Scheme 31. Strategy of Pohmakotr et al.}

Park et al. exhibited that the \( \beta \)-lactam rings could be expanded to the corresponding \( \gamma \)-lactam derivatives.\(^{133}\)

\[
\begin{align*}
\text{Ar} \text{C} & \text{O}_{2}\text{H}
\end{align*}
\]

\textit{Scheme 32. Strategy of Park et al.}

Brenner et al. reported that reduction of optically enriched 4-nitro-carboxylic acid derivatives followed by cyclization provides access to \( \gamma \)-lactam derivatives.\(^{134}\) In addition, a number of methods have been optimized to construct optically enriched \( \gamma \)-lactam
derivatives employing chiral auxiliaries,\textsuperscript{127,134} enantiomerically enriched starting materials,\textsuperscript{135} and organocatalysts.\textsuperscript{130,135}

Similar to \(\gamma\)-lactams, \(\delta\)-lactams have also drawn considerable attention over the last decade.\textsuperscript{124,128,129,136,137} Lately, Vervisch et al. reported nitrilase enzyme catalyzed transformation of cyanoaziridines to relevant \(\delta\)-lactam derivatives (Scheme 33).\textsuperscript{128} Recently, Thai et al reported a novel NHC catalyzed ring expansion reaction providing access to \(\delta\)-lactam derivatives (Scheme 34).\textsuperscript{138}

\textbf{Scheme 33.} Chemoenzymatic transformation of aziridines into \(\delta\)-lactams.

\textbf{Scheme 34.} Synthesis of \(\delta\)-lactams by ring expansion.

Zhou et al. reported a gold catalyzed highly regioselective cyclization strategy deriving a variety of \(\gamma\)-, and \(\delta\)-lactams (Scheme 35).\textsuperscript{139}

\textbf{Scheme 35.} Conversion of N-alkenyl-\(\beta\)-ketoamides to corresponding \(\gamma\)-/\(\delta\)-lactams.
Fiorelli et al. reported a novel ring closing metathesis mediated preparation of δ-lactams (Scheme 36).\textsuperscript{140}

\textbf{Scheme 36.} Preparation of δ-lactams through ring closing metathesis.

Patil et al. reported a novel strategy to prepare δ-lactam derivatives through intramolecular hydroamidation of amidoalkynes (Scheme 37).\textsuperscript{141}

\textbf{Scheme 37.} Intramolecular hydroamidation of amidoalkynes.

In addition to these, a number of synthetic strategies have been developed to provide access to chiral δ-lactams, utilizing aza-Diels Alder reactions\textsuperscript{142} and metal catalyzed C-H bond amination.\textsuperscript{143}

To summarize, a number of methods have been developed to achieve diverse γ-, and δ-lactams derivatives. However, there is limited scope to provide access to optically pure γ-, and δ-lactam derivatives.\textsuperscript{123,140} To the best of our knowledge, none of the synthetic strategies provide access to both enantiomers of a chiral γ-/δ-lactam. In addition,
very few synthetic strategies are available to obtain $\text{C}^{\alpha,\alpha}$-disubstituted-$\gamma$-, and $\delta$-lactams.\textsuperscript{123,124}

![C$^{\alpha,\alpha}$–disubstituted-$\gamma$-lactam and C$^{\alpha,\alpha}$–disubstituted-$\delta$-lactam](image)

**Figure 18.** C$^{\alpha,\alpha}$-disubstituted-$\gamma$-/$\delta$-lactams.

The family of C$^{\alpha,\alpha}$-disubstituted-$\gamma$-/$\delta$-lactams (Figure 18) have significant importance, as they are frequently found as building blocks of the biologically important complex natural product structures, and they provide access to chiral C$^{\alpha,\alpha}$-disubstituted pyrrolidine and piperidine analogues.\textsuperscript{123,124} Among the class of C$^{\alpha,\alpha}$-disubstituted-pyrrolidine/piperidine derivatives, C$^{\alpha}$-methyl-$\beta$-proline, and C$^{\alpha}$-methyl-nipecotic acid analogues (Figure 19) have been frequently utilized in peptidomimetics, in natural product synthesis, in the synthesis of a variety of enzyme inhibitors, as GABA reuptake inhibitors, in the synthesis of a number of receptor antagonists, and as organocatalysts.\textsuperscript{1,45,47,124,144,145} Hence, the above examples point that there is growing demand for the preparation of chiral C$^{\alpha,\alpha}$-disubstituted-$\gamma$-/$\delta$-lactams due to their potential use in the preparation of optically pure $\alpha$-methyl-$\beta$-proline, and $\alpha$-methyl-nipecotic acid analogues.

![C$^{\alpha}$-methyl-$\beta$-proline and C$^{\alpha}$-methyl-nipecotic acid analogue](image)

**Figure 19.** Proline and nipecotic acid analogues.
Current State of Art to Prepare $C^\alpha$-Methyl-$\beta$-Proline, and $C^\alpha$-Methyl-Nipecotic Acid Analogue

Over the last few years, several research groups in academia and in industry have explored a number of methods to prepare $C^\alpha$-methyl-$\beta$-proline, and $C^\alpha$-methyl-nipecotic acid analogues. However, the number of ways known to prepare these analogues is still limited. Recently, Nagata et al. reported a phase transfer catalyst mediated strategy to prepare $\gamma$-lactam and its transformation into a $C^\alpha$-methyl-$\beta$-proline analogue (Scheme 38). However, their method was not able to produce the $\beta$-proline analogue in high optical purity. Hence, they had to use chiral amine to resolve other enantiomer. This group has also exhibited the potent catalytic activity of $C^\alpha$-methyl-$\beta$-proline analogues in anti-Mannich type reactions.

![Scheme 38](image)

Scheme 38. Strategy of Nagata et al. to prepare $C^\alpha$-methyl-$\beta$-proline.

A few years back, Shintani et al. reported a Pd catalyzed decarboxylative cyclization strategy to prepare chiral $C^\alpha,\alpha$-disubstituted nipecotic acid analogues (Scheme 39).
Years ago, Huffman et al. discovered a potent NK1 receptor antagonist that consists of chiral C\(^\alpha\)-methyl-nipecotic acid as an essential building block (Figure 9, Page 13).\(^{45}\) However, this group was not able to come up with the asymmetric synthetic strategy to prepare C\(^\alpha\)-methyl-nipecotic acid analogue.\(^{45}\) Hence, they had to resolve two enantiomers to use the one to their interest.\(^{45}\) Therefore, there are a limited number of options to prepare C\(^\alpha\)-methyl-\(\beta\)-proline and C\(^\alpha\)-methyl-nipecotic acid analogues. In addition, none of the synthetic strategies could derive both enantiomers of C\(^\alpha\)-methyl-\(\beta\)-proline and C\(^\alpha\)-methyl-nipecotic acid from a common intermediate. Recently, I have observed that the optically enriched unsymmetrical malonic esters could be readily converted into C\(^{\alpha,\alpha}\)-disubstituted-\(\gamma\)-lactam (Scheme 39). Based on the precedent literatures and my recent success we hypothesize:

Hypothesis 3.

Optically enriched unsymmetrical malonic-diesters could be stereoselectively cyclized to the relevant C\(^{\alpha,\alpha}\)-disubstituted-\(\gamma\)-/\(\delta\)-lactams (Scheme 39). A Hammett study should be able to provide insight into electronic activation of one of the ester groups towards nucleophilic attack. The consequential \(\gamma\)-/\(\delta\)-lactams could be further chemoselectively reduced to the corresponding C\(^\alpha\)-methyl-\(\beta\)-proline/C\(^\alpha\)-methyl-nipecotic acid analogues (Scheme 40).
We had to prepare compound 18 from 10b in our attempt to determine the absolute stereochemistry of 10b by synthetic means (Scheme 41). Upon treatment of 18 with hydrazine a cyclization took place resulting in compounds 19a and 19b as illustrated in Scheme 40. Interestingly, the 19a/19b ratio was 10:1 as determined by $^1$H-NMR resulting in a selective cyclization that favored ring closure by attack at the benzyl ester over the ethyl ester. The cyclization selectivity results in an enantioselective cyclization strategy as 19a has the $R$-absolute stereochemistry and 19b has the S-absolute stereochemistry.

*Scheme 40. Stereoselective cyclization strategy.*

Results and Discussion

We had to prepare compound 18 from 10b in our attempt to determine the absolute stereochemistry of 10b by synthetic means (Scheme 41). Upon treatment of 18 with hydrazine a cyclization took place resulting in compounds 19a and 19b as illustrated in Scheme 40. Interestingly, the 19a/19b ratio was 10:1 as determined by $^1$H-NMR resulting in a selective cyclization that favored ring closure by attack at the benzyl ester over the ethyl ester. The cyclization selectivity results in an enantioselective cyclization strategy as 19a has the $R$-absolute stereochemistry and 19b has the S-absolute stereochemistry.
Scheme 41. Stereoselective cyclization to prepare γ-lactam.

The option to control the cyclization reaction employing such a straightforward set of synthetic manipulations could prove useful in the enantioselective preparation of γ-lactams from simple half-ester starting materials such as 10b. The 10:1 selectivity was exciting keeping in mind that both esters are relatively open toward nucleophilic attack by the amine nucleophile. In an attempt to better understand the factors controlling the selectivity of the cyclization I performed a series of cyclization experiments where substituents were introduced on the para position of the aromatic ring of the benzyl ester (Scheme 42).
Scheme 42. Cyclization using various benzyl esters.

The introduction of substituents on the para position allows us to construct a Hammett plot providing insight into the electronic factors regulating the selectivity of the cyclization. I hypothesized that the $\sigma_p$ constants would provide insight into the electronic activation of the benzyl ester toward nucleophilic attack. Figure 20 illustrates the results of the Hammett analysis with a strong correlation of product ratio to $\sigma_p$. The positive slope clearly indicates that electron withdrawing substituents favor cyclization to $\gamma$-lactam 19a over $\gamma$-lactam 19b. This illustrates that benzyl esters with para electron withdrawing substituents activate the carbonyl toward nucleophilic attack by the $1^\circ$-amine resulting in selective formation of 19a.
I wanted to further extend the stereoselective cyclization concept to provide 19b as the major product. The possibility of being able to obtain 19b as the major product would allow for a potentially useful enantiodivergent cyclization strategy. The results of the Hammett study point that it would be difficult to achieve high selectivity in the formation of 19b just by exploring electronic factors on the benzyl ester. I prepared diester 55 from 10b that would introduce steric hindrance (Scheme 43). The ethyl ester should behave as the better electrophile from a steric congestion standpoint leading to the stereoselective cyclization to 56. Product 56 is an analogue of 19b and has the same absolute stereochemistry as 19b.

Scheme 43. Selective cyclization leading to (S)-γ-lactam 56.
Upon achievement of \((R)\)-, and \((S)\)-\(\gamma\)-lactams, I wanted to further utilize the optimized method to obtain chiral \(\delta\)-lactams. Upon close inspection of half-ester 10c, it is conceivable that unsymmetrical diesters of 10c could be stereoselectively cyclized to the \(\delta\)-lactams. Upon treatment of 57 with hydrazine, the similar cyclization took place resulting in compounds 58 and 59 as illustrated in Scheme 44. Interestingly, the \(\text{58/59}\) ratio was 60:1 as determined by \(^1\)H-NMR resulting in a selective cyclization that favored ring closure by attack at the \(p\)-nitro-benzyl ester over the ethyl ester. The cyclization selectivity results in the same enantioselective cyclization strategy as Scheme 40, since 58 has the \(R\)-absolute stereochemistry and 59 has the \(S\)-absolute stereochemistry.

\[ \text{Scheme 44. Stereoselective cyclization leading to } \delta\text{-lactam.} \]

At this point, 59 was wanted to be achieved as the major isomer in order to be able to develop an enantiodivergent strategy to provide access to chiral \(\delta\)-lactams. Hence, the half-ester 60 was synthesized as shown in Scheme 44 following the synthetic strategy to prepare (S)-\(\gamma\)-lactam 56 as predominant isomer (Scheme 42). Interestingly, upon
treatment of 60 with hydrazine hydrate a stereospecific cyclization took place providing the δ-lactam 61 as only isomer (Scheme 45).

**Scheme 45. Stereospecific synthesis of (S)-δ-lactam.**

We wanted to display the potential utility of the γ-/δ--lactams prepared above as precursors to unnatural amino acids. Upon inspection of 19a, it is conceivable that a β-proline analogue could be readily prepared by reduction of the lactam to a 2<sup>ο</sup>-amine. Similarly, 58 could be readily converted reduced into a nipecotic acid analogue. I made attempt of a direct reduction of the lactam with various reducing agents and conditions that are known to reduce lactams to amines. However, all attempts at direct reduction of the lactam resulted in reduction of both the ester and lactam functional groups providing a complex mixture of products. The synthetic approach shown in Scheme 46 was used to overcome the over-reduction problem and ultimately provide the unprotected β-proline/nipecotic acid analogue in a reasonable overall yield.
Compounds 19a and 58 were treated with NaH and benzyl bromide to provide the resulting 62a and 62b in good yield (68% - 75%). The lactams of 62a/62b were converted in good yield (80% - 85%) to thiolactam 63a/63b using Lawesson reagent. The thiolactams were then easily reduced to the 2\alpha-aminess 64a/64b by hydrogenation over Raney nickel catalyst in good yield. The amines 64a/64b were then subjected to saponification giving 65a/65b in 78% - 85% yield. Amino acids 65a/65b were then converted to the α-methyl-β-proline analogue (66a) and α-methyl-nipeotic acid analogue (66b) in excellent yield (91% to 95%) by hydrogenation over Pd/C catalyst. The overall yield of 66a from 19a is 28% over the five straightforward steps shown in Scheme 45. In continuation, the overall yield of 66b from 58 is 45% over the five straightforward steps as shown in Scheme 45.
Proline and its derivatives have been frequently used as organocatalysts in Aldol, Mannich, and Michael type reactions over the last decade. This class of catalysts have proven their excellence in asymmetric synthesis of new C-C bond. One of the biggest advantages to use this set of catalysts is the ease of handling as opposed to metal-catalysts. In most cases, this class of catalysts is easily isolated from the crude mixture by simple water extraction at the end of the reaction. In recent years, Barbas et al. exhibited a number of β-proline and nipecotic acid analogues as potent catalysts in anti-Mannich type reactions (Scheme 47). This set of catalysts are observed to predominantly produce the anti-Mannich isomers in excellent diastereomeric ratio and enantiopurity.

However, there was no report until recently utilizing Cα,α-disubstituted-β-proline in anti-Mannich type reactions. Hence, we have made an effort to explore the α-methyl-β-proline analogue (66a) made in our hand in Mannich type reactions (Scheme 47). We have observed that the β-proline analogue (66a) is a strong anti-selective catalyst producing the anti-Mannich product 67a as the predominant stereoisomer in 8:1 diastereomeric ratio and 97% ee (Scheme 48, Figure 21). The combined yield was 75%.
Scheme 48. $\alpha$-methyl-\(\beta\)-proline catalyze anti-Mannich type reactions.

Figure 21. Chiral-HPLC chromatogram of racemic and optically pure anti-Mannich reaction.

Based on the results obtained, we have proposed the plausible transition state of the anti-Mannich type reactions employing $\alpha$-methyl-\(\beta\)-proline (Scheme 49).
Scheme 49. Transition state of anti-Mannich type reaction.

Conclusions

I have shown that enantioselective γ-/δ-lactam formation is possible from 10b/10c with careful choice of benzyl ester. The highest level of selectivity was noticed using 18d containing a para nitro substituent on the benzyl ester. The cyclization selectivity has a strong correlation to $\sigma_p$ as established in the Hammett study. The positive slope of the Hammett plot indicates that electron withdrawing substituents in the para position of the benzyl ester activate the benzyl ester carbonyl toward electrophilic attack. I have confirmed that γ-/δ-lactam 19a/58 can be readily converted into 66a/66b providing straightforward access to a new class of proline and nipecotic acid analogue. I have also demonstrated 66a as a strong catalyst for anti-Mannich selective catalyst.

Experimental

General Procedure for the Synthesis of 18a-18e

A 250 mL roundbottom flask was charged with 9.9 g of 10b (31 mmol), 4.3 g of K$_2$CO$_3$ (31 mmol), 100 mL of anhydrous DMF, and a stirbar. A solution of the appropriately substituted benzyl bromide (28 mmol) in 20 mL of anhydrous DMF was slowly added over 15 minutes. The reaction was allowed to stir approximately 12 hr
under a nitrogen atmosphere. The reaction mixture was then diluted with 100 mL of water and the resulting mixture was washed with Et₂O (3 x 100 mL). The combined ether layer was washed with water (5 x 100 mL), washed with brine (2 x 100 mL), dried over MgSO₄, and the solvent was removed under reduced pressure. The product was isolated by flash chromatography (40% Et₂O/hexanes).

*Synthesis of (S)-1-Benzyl-3-Ethyl-2-Methyl-[2-(1,3-Dioxoisoindolin-2-Yl)Ethyl]Malonate (18a)*

18a was synthesized following the general synthetic procedure for the preparation of 18a-18e. An amount of 11 g of product (27 mmol, 87%) was obtained after flash chromatography purification as a colorless liquid. Rᵣ = 0.2 (40% Et₂O/hexanes). [α]²⁴_D = -3.08 (c = 1, CHCl₃). IR (cm⁻¹): 2980, 1773, 1708. ¹H-NMR (CDCl₃, 400 MHz): 7.83 (m, 2H), 7.70 (m, 2H), 7.33 (m, 5H), 5.15 (m, 2H), 4.10 (m, 2H), 3.74 (m, 2H), 2.28 (m, 2H), 1.56 (s, 3H), 1.16 (t, 3H, J = 7 Hz). ¹³C-NMR (CDCl₃, 100 MHz): 171.4, 171.3, 168.0, 135.5, 134.0, 132.0, 128.5, 128.3, 128.1, 123.0, 67.0, 61.0, 52.0, 33.8, 33.8, 20.0, 14.0. HRMS [C₂₃H₂₃NO₆Na⁺]: calcd = 432.1417, found = 432.1406.

*Synthesis of (S)-1-(4-Fluorobenzyl)-3-Ethyl-2-Methyl-[2-(1,3-Dioxoisoindolin-2-Yl)Ethyl]Malonate (18b)*

18b was synthesized following the general synthetic procedure for the preparation of 18a-18e. An amount of 9.95 g of product (23.3 mmol, 75%) was obtained after purification as colorless liquid. Rᵣ = 0.25 (40% Et₂O/hexanes). [α]²⁴_D = -3.7 (c = 1, CHCl₃). IR (cm⁻¹): 2984, 1773, 1708. ¹H-NMR (CDCl₃, 400 MHz): 7.83 (m, 2H), 7.71 (m, 2H), 7.33 (m, 2H), 7.03 (m, 2H), 5.11 (m, 2H), 4.10 (m, 2H), 3.72 (m, 2H), 2.26 (m, 2H), 1.55 (s, 3H), 1.16 (t, 3H, J = 7 Hz). ¹³C-NMR (CDCl₃, 100 MHz): 171.4, 171.3, 163.0 (d, ¹J =
250 Hz), 134.0, 132.0, 131.0 (d, $^4J$ = 3.6 Hz), 130.0 (d, $^3J$ = 8.5 Hz), 123.0, 115.0 (d, $^2J$ = 22 Hz), 123.0, 66.0, 62.0, 52.0, 33.8, 33.7, 20.0, 14.0. HRMS [C$_{23}$H$_{22}$FNO$_6$Na$^+$]: calcd = 450.1323, found = 450.1312.

**Synthesis of (S)-1-(4-Methylbenzyl)-3-Ethyl-2-Methyl-[2-(1,3-Dioisoindolin-2Yl)Ethyl]Malonate (18c)**

18c was synthesized following the general synthetic procedure for the preparation of 18a-18e. An amount of 9.4 g of product (22.3 mmol, 72%) was obtained after purification as colorless liquid. $R_f$ = 0.24 (40% Et$_2$O/hexanes). $[\alpha]_D^{24}$ = -5.3 (c = 1, CHCl$_3$). IR (cm$^{-1}$): 2983, 1773, 1708. $^1$H-NMR (CDCl$_3$, 400 MHz): 7.83 (m, 2H), 7.70 (m, 2H), 7.22 (d, 2H, $J$ = 8 Hz), 7.14 (d, 2H, $J$ = 8 Hz), 5.11 (m, 2H), 4.10 (m, 2H), 3.73 (m, 2H), 2.34 (s, 3H), 2.26 (m, 2H), 1.55 (s, 3H), 1.16 (t, 3H, $J$ = 7 Hz). $^{13}$C-NMR (CDCl$_3$, 100 MHz): 171.4, 171.3, 168.0, 138.0, 134.0, 132.5, 132.0, 129.0, 128.0, 123.0, 67.0, 62.0, 52.0, 34.0, 21.0, 20.0, 14.0. HRMS [C$_{24}$H$_{25}$NO$_6$Na$^+$]: calcd = 446.1574, found = 446.1565.

**Synthesis of (S)-1-(4-Nitrobenzyl)-3-Ethyl-2-Methyl-[2-(1,3-Dioisoindolin-2Yl)Ethyl]Malonate (18d)**

18d was synthesized following the general synthetic procedure for the preparation of 18a-18e. An amount of 10.27 g of product (22.6 mmol, 73%) was obtained after purification as a white solid. mp = 65 °C. $R_f$ = 0.1 (40% Et$_2$O/hexanes). $[\alpha]_D^{24}$ = + 1.4 (c = 1, CHCl$_3$). IR (cm$^{-1}$): 2983, 1773, 1729, 1707, 1517. $^1$H-NMR (CDCl$_3$, 400 MHz): 8.23 (d, 2H, $J$ = 8 Hz), 7.83 (m, 2H), 7.72 (m, 2H), 7.52 (d, 2H, $J$ = 8 Hz), 5.25 (m, 2H), 4.16 (m, 2H), 3.74 (m, 2H), 2.29 (m, 2H), 1.59 (s, 3H), 1.21 (t, 3H, $J$ = 7 Hz). $^{13}$C-NMR (CDCl$_3$, 100 MHz): 171.2, 171.1, 168.0, 148.0, 143.0, 134.0, 132.0, 128.0, 124.0, 123.0,
66.0, 62.0, 52.0, 34.0, 33.8, 20, 14. HRMS [C\textsubscript{23}H\textsubscript{22}N\textsubscript{2}O\textsubscript{8}Na\textsuperscript{+}]: calcd = 477.1268, found = 477.1266.

*Synthesis of (S)-1-(4-Trifluoromethylbenzyl)-3-Ethyl-2-Methyl-[2-(1,3-Dioxoisooindolin-2Yl)Ethyl]Malonate (18e)*

4e was synthesized following the general synthetic procedure for the preparation of 18a-18e. An amount of 10.8 g of product (22.6 mmol, 87%) was obtained after purification as a colorless liquid. $R_f = 0.26$ (40% Et\textsubscript{2}O/hexanes). $[\alpha]_{D}^{24} = -8.33$ (c = 3, CHCl\textsubscript{3}). IR (cm\textsuperscript{-1}): 2983, 1773, 1709. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 400 MHz): 7.83 (m, 2H), 7.71 (m, 2H), 7.62 (d, 2H, $J = 8$ Hz), 7.46 (d, 2H, $J = 8$ Hz), 5.20 (s, 2H), 4.12 (m, 2H), 3.73 (m, 2H), 2.29 (m, 2H), 1.57 (s, 3H), 1.17 (t, 3H, $J = 7$ Hz). \textsuperscript{13}C-NMR (CDCl\textsubscript{3}, 100 MHz): 171.3, 171.2, 168.0, 139.0, 134.0, 132.0, 130.0 (q, $J = 33$ Hz), 128.0, 125.0 (q, $J = 4$ Hz), 123.0, 122.0, 66.0, 62.0, 52.0, 34.0, 33.8, 20.0, 14.0. HRMS [C\textsubscript{24}H\textsubscript{23}F\textsubscript{3}NO\textsubscript{6}Na\textsuperscript{+}]: calcd = 500.1291, found = 500.1278.

*Synthesis of (R)-Ethyl-3-Methyl-2-Oxopyrrolidine-3-Carboxylate (19a)*

A volume of 930 µL (10.2 mmol) 35% hydrazine in water was added to a solution of 3.8 g (9.3 mmol) 18a in 50 mL MeOH. The mixture was heated to reflux solvent overnight. A white precipitate was observed within an hour of reflux. The reaction mixture was allowed to cool to RT, and the resulting mixture was filtered. The filtrate was evaporated under reduced pressure. The resulting residue was taken up in CH\textsubscript{2}Cl\textsubscript{2} and washed with water. The organic layer was dried over MgSO\textsubscript{4}, evaporated under reduced pressure, and purified by column chromatography, using 30% hexanes/EtOAc giving 1.2 g of a 10:1 mixture of 19a:19b as a white solid. The mixture was further recrystallized in cold Et\textsubscript{2}O giving 1 g (6 mmol, 64.5%) of pure 19a as white crystals. $R_f$
\((19a) = 0.31\) (30% hexanes/EtOAc). mp = 63 °C. \([\alpha]_D^{23} = +19.0\) (c = 2, MeOH). IR (cm\(^{-1}\)): 3245, 2985, 1726, 1698, 1660. \(^1\)H-NMR (CDCl\(_3\), 400 MHz): 7.06 (bs, 1H), 4.20 (m, 2H), 3.47 (m, 1H), 3.36 (m, 1H), 2.64 (m, 1H), 2.02 (m, 1H), 1.45 (s, 3H), 1.28 (t, 3H, \(J = 7\) Hz), \(^13\)C-NMR (CDCl\(_3\), 100 MHz): 177.0, 172.0, 61.0, 51.0, 40.0, 34.0, 20.0, 14.0.

HRMS [C\(_8\)H\(_{13}\)NO\(_3\)Na\(^+\)]: calcd = 194.0788, found = 194.0795.

*Synthesis of (S)-1-Tert-Butyl-3-Ethyl-2-Methyl-[2-(1,3-Dioxoindolin-2Yl)Ethyl] Malonate (55)*

A volume of 600 µL conc. H\(_2\)SO\(_4\) was added to a solution of 2 g of 10b (6 mmol) in 30 mL CH\(_2\)Cl\(_2\) in a 100 mL sealed tube. The solution was cooled to -7 °C. A volume of 6 mL condensed isobutylene was added to the solution. The tube was sealed tightly and allowed to stir overnight at RT. The tube was uncapped and allowed to stir for 2hrs. at ambient pressure to allow excess isobutylene to evaporate. The solution was diluted with 30 mL of CH\(_2\)Cl\(_2\) and gently washed three times with 1N NaOH (50 mL). The CH\(_2\)Cl\(_2\) layer was dried over MgSO\(_4\), evaporated under reduced pressure, and chromatographed (40% EtOAc/hexanes), giving 1.8 g (5 mmol, 83%) of 55 as colorless viscous liquid. The viscosity of the material made removal of residual solvent impractical and 55 was utilized in the subsequent reaction without further purification. \(R_f = 0.51\) (40% EtOAc/hexanes).

IR (cm\(^{-1}\)): 2979, 1774, 1709. \(^1\)H-NMR (CDCl\(_3\), 400 MHz): 7.84 (m, 2H), 7.71 (m, 2H), 4.17 (m, 2H), 3.72 (m, 2H), 2.19 (m, 2H), 1.50 (s, 3H), 1.48 (s, 3H), 1.28 (t, 3H, \(J = 7\) Hz). \(^13\)C NMR (CDCl\(_3\), 100MHz) 172.0, 170.5, 168.0, 134.0, 132.2, 123.3, 82.0, 61.0, 53.0, 34.0, 33.8, 28.0, 20.0, 14.0. HRMS [C\(_{20}\)H\(_{25}\)NO\(_6\)Na\(^+\)]: Calcd = 398.1574, found = 398.1568.
Synthesis of (S)-Tert-Butyl-3-Methyl-2-Oxopyrrolidine-3-Carboxylate (56)

A volume of 398 µL (4.4 mmol) 35% hydrazine in water was added to a solution of 1.5 g (4 mmol) 55 in 25 mL MeOH. The mixture was heated to reflux solvent overnight. A white precipitate was observed within an hour of reflux. The reaction mixture was allowed to cool to RT and the solution was filtered. The filtrate was evaporated under reduced pressure and taken up in CH₂Cl₂. The resulting mixture was washed with water and the organic layer was dried over MgSO₄, evaporated under reduced pressure, and chromatographed using 30% hexanes/EtOAc giving 0.62 g of a 9:1 mixture of 56:19a as a white solid. The mixture was further recrystallized in cold Et₂O giving 0.52 g (2.6 mmol, 65%) pure 56 as a white solid. Rᵣ(56) = 0.27 (30% hexanes/EtOAc). mp = 130 °C. IR (cm⁻¹): 3255, 2970, 1727, 1688, 1660. [α]D^23 = -14.3 (c = 1, CH₂Cl₂). ¹H- NMR (CDCl₃, 400 MHz): 6.44 (bs, 1H), 3.45 (m, 1H), 3.33 (m, 1H), 2.55 (m, 1H), 2.0 (m, 1H), 1.46 (s, 9H), 1.39 (s, 3H). ¹³C-NMR (CDCl₃, 100 MHz): 177.0, 171.0, 81.0, 51.0, 40.0, 34.0, 28.0, 20.0. HRMS [C₁₀H₁₇NO₃Na⁺]: calcd = 222.1101, found = 222.1097.

Synthesis of (S)-1-(4-Nitrobenzyl)-3-Ethyl-2-Methyl-2-(3-(1,3-Dioxoisoindolin-2-Yl)Propyl)Malonate (57)

A 250 mL round-bottom flask was charged with 6.41 g of 10c (19 mmol), 2.62 g of K₂CO₃ (19 mmol), 75 mL of anhydrous DMF, and a stirbar. A solution of the 4-nitrobenzyl bromide (17.1 mmol) in 20 mL of anhydrous DMF was slowly added over 15 min. The reaction was allowed to stir approximately 12 hrs. under a nitrogen atmosphere. The reaction mixture was then diluted with 100 mL of water, and the resulting mixture was washed with Et₂O (3 × 150 mL). The combined ether layer was washed with water
(8 × 150 mL) and brine (2 × 100 mL) and dried over MgSO₄, and the solvent was removed under reduced pressure. The product was isolated by flash chromatography (30% EtOAc/hexanes), giving 801g (17.4 mmol, 87%) pure 57 as white solid. Rᶠ = 0.1 (30% EtOAc/hexanes). MP = 66 °C. ¹H NMR (CDCl₃, 400 MHz): δ 8.19 (m, 2H), 7.81 (m, 2H), 7.72 (m, 2H), 7.46 (m, 2H), 5.22 (m, 2H), 4.15 (q, 2H, J = 7 Hz), 3.68 (t, 2H, J = 7 Hz), 1.95 (m, 2H), 1.65 (m, 2H), 1.44 (s, 3H), 1.18 (t, 3H, J = 7 Hz). ¹³C NMR (CDCl₃, 100 MHz): δ 171.5, 168.2, 148.7, 134.0, 132.0, 128.2, 124.0, 123.3, 65.2, 61.4, 58.5, 53.0, 37.8, 33.0, 24.0, 20.0, 18.2, 13.6. ESI-MS [C₂₄H₂₄N₂O₈Na⁺] = 468.4, observed = 468.3.

**Synthesis of (R)-Ethyl 3-Methyl-2-Oxopiperidine-3-Carboxylate (58)**

A volume of 1.8 mL (20 mmol) 35% hydrazine in water was added to a solution of 5.1 g (11 mmol) of 57 in 50 mL of MeOH. The mixture was heated to reflux overnight. A white precipitate was observed within 1 hr of reflux. The reaction mixture was allowed to cool to rt, and the resulting mixture was filtered through an HPLC filter. The filtrate was evaporated under reduced pressure. The resulting residue was taken up in CH₂Cl₂ and washed with water. The organic layer was dried over MgSO₄, evaporated under reduced pressure, and purified by column chromatography using 60% hexanes/EtOAc giving 1.5g (8.3 mmol, 75%) of 58 as a white solid. Rᶠ = 0.35 (30% EtOAc/hexanes). MP = 65 °C. ¹H NMR (CDCl₃, 400 MHz): δ 6.25 (bs, 1H), 4.2 (m, 2H), 3.36 (m, 2H), 2.26 (m, 1H), 1.84 (m, 2H), 1.73 (m, 1H), 1.50 (s, 3H), 1.27 (t, 3H, J = 7 Hz). ¹³C NMR (CDCl₃, 100 MHz): δ 173.6, 172.0, 61.2, 50.3, 42.3, 33.0, 22.4, 90.4, 14.0. ESI-MS [C₉H₁₅NO₃Na⁺] = 208.1, observed = 208.1.
Synthesis of (S)-1-Tert-Butyl-3-Ethyl-2-Methyl-2-[3-(1,3-Dioxoisindolin-2Yl)Propyl]Malonate (60)

A volume of 600 µL conc. H$_2$SO$_4$ was added to a solution of 2 g of 10c (6 mmol) in 30 mL CH$_2$Cl$_2$ in a 100 mL sealed tube. The solution was cooled to -7 °C. A volume of 6 mL condensed isobutylene was added to the solution. The tube was sealed tightly and allowed to stir overnight at RT. The tube was uncapped and allowed to stir for 2hrs at ambient pressure to allow excess isobutylene to evaporate. The solution was diluted with 30 mL of CH$_2$Cl$_2$ and gently washed three times with 1N NaOH (50 mL). The CH$_2$Cl$_2$ layer was dried over MgSO$_4$, evaporated under reduced pressure, and chromatographed (40% EtOAc/hexanes), giving 2 g (5.1 mmol, 87%) of 60 as white solid. $R_f$ = 0.53 (40% EtOAc/hexanes). $[\alpha]_D^{23}$ = - 5.2. IR (cm$^{-1}$): 2979, 1774, 1709. $^1$H-NMR (CDCl$_3$, 400 MHz): 7.84 (m, 2H), 7.71 (m, 2H), 4.18 (m, 2H), 3.74 (m, 2H), 1.91 (m, 2H), 1.61 (m, 2H), 1.48 (s, 9H), 1.39 (s, 3H), 1.28 (t, 3H, $J$ = 7 Hz). $^{13}$C NMR (CDCl$_3$, 100MHz) 172.3, 171.03, 168.3, 134.0, 132.1, 123.2, 81.5, 61.1, 54.0, 38.1, 34.7, 28.0, 25.3, 20.0, 14.0. ESI-MS [C$_{21}$H$_{27}$NO$_6$Na$^+$]: Calcd = 412.4, found = 412.3.

Synthesis of (S)-Tert-Butyl-3-Methyl-2-Oxpiperidine-3-Carboxylate (61)

A volume of 398 µL (4.4 mmol) 35% hydrazine in water was added to a solution of 1.5 g (4 mmol) 60 in 25 mL MeOH. The mixture was heated to reflux solvent overnight. A white precipitate was observed within an hr of reflux. The reaction mixture was allowed to cool to RT and the solution was filtered. The filtrate was evaporated under reduced pressure and taken up in CH$_2$Cl$_2$. The resulting mixture was washed with water and the organic layer was dried over MgSO$_4$, evaporated under reduced pressure, and chromatographed using 30% hexanes/EtOAc giving 0.62 g of 61 as a white solid. $R_f$
(61) = 0.27 (30% hexanes/EtOAc). Mp = 130 °C. IR (cm⁻¹): 3255, 2970, 1727, 1688, 1660. [α]D²³ = -16.2 (c = 1, CH₂Cl₂). ¹H-NMR (CDCl₃, 400 MHz): 6.28 (bs, 1H), 3.61 (m, 2H), 2.2 (m, 1H), 1.8 (m, 2H), 1.61 (m, 1H), 1.42 (s, 12H). ¹³C-NMR (CDCl₃, 100 MHz): 174.2, 173.9, 83.0, 51.0, 43.0, 34.0, 28.0, 20.0, 14.0. ESI-MS [C₁₁H₁₉NO₃Na⁺]: calcd = 236.3, found = 236.2.

**Synthesis of (R)-Ethyl 1-Benzyl-3-Methyl-2-Oxopyrrolidine-3-Carboxylate (62a)**

A solution of 3.6 g (22 mmol) of 19a in 20 mL anhydrous THF was added slowly to a suspension of 0.62 g NaH (26 mmol) in 10 mL THF at 0 °C under a N₂ atmosphere. The reaction mixture was allowed to stir 5 minutes. A volume of 3.3 mL (4.75 g, 24 mmol) BnBr was added drop wise to the reaction mixture at 0 °C. The reaction mixture was allowed to stir for 10 minutes at 0 °C and then allowed to warm to RT. The reaction was continued for 1 hr at RT. A volume of 15 mL dry DMF was added to the reaction mixture and continued to stir for 2 hrs. The reaction mixture was poured into 25 mL of H₂O. The water layer was extracted with Et₂O (3 X 50 mL). The combined ether layer was washed with water (5 X 30 mL), dried over MgSO₄, evaporated under reduced pressure, and chromatographed (gradient, 15%-20% EtOAc/hexanes) giving 3.8 g (15 mmol, 68%) of 62a as a colorless oil. ¹H NMR evidenced that the product contains some trans-esterified compound as well. The mixture was utilized in the next step without further purification. Rₓ = 0.27 (20% EtOAc/hexanes). IR (cm⁻¹): 2979, 1735, 1685. ¹H-NMR (CDCl₃, 400 MHz): 7.29 (m, 7.5H), 5.16 (m, 0.5H), 4.6 (m, 1.25H), 4.36 (m, 1.27H), 4.17 (m, 2H), 3.33 (m, 1.26H), 3.17 (m, 1.25H), 2.46 (m, 1.25H), 1.89 (m, 1.27H), 1.50 (s, 0.76H), 1.47 (s, 3H), 1.23 (t, 3H, J =7 Hz). ESI-MS [C₁₅H₂₀NO₃, 62a]⁺ calcd = 261.3, found = 262.2.
Synthesis of (S)-Ethyl-1-Benzyl-3-Methyl-2-Oxopiperidine-3-Carboxylate (62b)

A solution of 0.3 g (1.6 mmol) of 58 in 10 mL of anhydrous THF was added slowly to a suspension of 0.046 g NaH (1.92 mmol) in 10 mL of THF at 0 °C under a N₂ atmosphere. The reaction mixture was allowed to stir for 5 min. A volume of 210 µL (1.76 mmol) of BnBr was added dropwise to the reaction mixture at 0 °C. The reaction mixture was allowed to stir for 10 min at 0 °C and then allowed to warm to rt. The reaction was continued for 1 hr at rt. A volume of 6 mL of dry DMF was added to the reaction mixture, which continued to stir for 2 hrs. The reaction mixture was poured into 15 mL of H₂O. The water layer was extracted with Et₂O (3 × 25 mL). The combined ether layer was washed with water (3 × 10 mL), dried over MgSO₄, evaporated under reduced pressure, and chromatographed (gradient, 15–20% EtOAc/hexanes) giving 0.36 g (1.3 mmol, 81%) as a colorless oil. Rf = 0.3 (20% EtOAc/hexanes). ¹H NMR (CDCl₃, 400 MHz): δ 7.29 (m, 5H), 5.0 (m, 1H), 4.2 (m, 3H), 3.24 (m, 2H), 2.23 (m, 1H), 1.79 (m, 3H), 1.54 (s, 3H), 1.29 (t, 3H, J = 7 Hz). ¹³C NMR (CDCl₃, 100 MHz): δ 173.6, 169.3, 137.2, 128.5, 127.8, 127.3, 61.4, 50.7, 50.4, 47.3, 33.4, 22.7, 19.4, 14.2. ESI-MS [C₁₆H₂₁NO₃Na⁺] = 298.3, observed = 298.2.

Synthesis of (S)-Ethyl 1-Benzyl-3-Methyl-2-Thioxopyridine-3-Carboxylate (63a)

An amount of 5.3 g (13 mmol) Lawesson’s reagent was added to a solution of 3.8 g (15 mmol) of 62a mixture in 20 mL anhydrous toluene under a N₂ atmosphere. The reaction mixture was heated to 95 °C and stirred over night. The toluene layer was evaporated under reduced pressure and the residue was chromatographed (20% EtOAc/hexanes) giving 3.3 g (12 mmol, 80%) of 63a as a colorless oil. The conversion of lactam (62a) to the corresponding thiolactam (63a) was confirmed by comparing the ¹³C-
NMR chemical shift of the lactam (62a) carbonyl carbon (172 ppm) to the thiolactam (63a) carbonyl carbon (202 ppm). Rf = 0.35 (20% EtOAc/hexanes). IR (cm\(^{-1}\)): 2980, 1733, 1505, 1449. \(^1\)H-NMR (CDCl\(_3\), 400 MHz): 7.29 (m, 7.3H), 5.18 (m, 2H), 4.82 (m, 1.23H), 4.16 (q, 2H, \(J = 7\) Hz), 3.70 (m, 1.24H), 3.51 (m, 1.25H), 2.51 (m, 1.23H), 1.92 (m, 1.32H), 1.61 (s, 0.68H), 1.58 (s, 3H), 1.21 (t, 3H, \(J = 7\) Hz). ESI-MS \([\text{C}_{15}\text{H}_{20}\text{NO}_2\text{S}, 10a]^+\) calcd = 277.4, found = 278.1.

**Synthesis of (S)-Ethyl 1-Benzyl-3-Methyl-2-Thioxopiperidine-3-Carboxylate (63b)**

A 1.7 g (4.2 mmol) portion of Lawesson’s reagent was added to a solution of 1.3 g (4.7 mmol) of 62b in 20 mL of anhydrous toluene under a N\(_2\) atmosphere. The reaction mixture was heated to 95 °C and stirred over 12 h. The reaction completion was verified by TLC (20% EtOAc/hexanes). The toluene layer was evaporated under reduced pressure, and the residue was chromatographed (20% EtOAc/hexanes) giving 0.97 g (3.3 mmol, 80%) as colorless oil. The conversion of lactam 62b to the corresponding thiolactam 63b was confirmed by comparing the \(^{13}\)C NMR chemical shift of the lactam 62b carbonyl carbon (173.6 ppm) to the thiolactam (63b) carbonyl carbon (202.5 ppm). Rf = 0.3 (20% EtOAc/hexanes). [\(\alpha\)]\(^{22}\)\(_D\) = +75.2 (c = 1, CH\(_2\)Cl\(_2\)). \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.31 (m, 5H), 5.69 (m, 1H), 5.04 (m, 1H), 4.23 (m, 2H), 3.43 (m, 2H), 2.28 (m, 1H), 2.01 (m, 1H), 1.83 (m, 2H), 1.75 (s, 3H), 1.30 (t, 3H, \(J = 7\) Hz). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 202.5, 173.6, 135.0, 129.0, 127.7, 127.5, 61.5, 57.7, 55.5, 50.4, 32.1, 27.8, 19.4, 14.0. ESI-MS \([\text{C}_{16}\text{H}_{21}\text{NO}_2\text{SNa}^+\)] calculated = 314.4, observed = 314.3.

**Synthesis of (R)-Ethyl 1-Benzyl-3-Methylpyrrolidine-3-Carboxylate (64a)**

An amount of 0.5 g of 63a was dissolved in 15 mL of 4:1 THF/EtOH. An amount of 0.05 g Raney-Ni slurry in water (10% by weight) was added to the solution. The
solution was stirred vigorously under a H\(_2\) atmosphere for 4 hrs, at which point the reaction was found to be complete by TLC. The mixture was filtered through a Celite bed, and the filtrate was evaporated under reduced pressure. The product was purified by radial chromatography using 20\% hexanes/CH\(_2\)Cl\(_2\) to give 0.25 g (1.01 mmol, 72\%) of \(64\text{a}\) as a colorless liquid. \(R_f = 0.32\) (20\% hexanes/CH\(_2\)Cl\(_2\)). IR (cm\(^{-1}\)): 2974, 2790, 1725, 1452. \([\alpha]_{D}^{24} = -8\) (c = 1, CHCl\(_3\)). \(^1\)H-NMR (CDCl\(_3\), 400 MHz)(\(1\text{a}\)): 7.3 (m, 5H), 4.13 (q, 2H, \(J = 7\) Hz), 3.61 (m, 2H), 2.94 (d, 1H, \(J = 9\) Hz), 2.64 (m, 2H), 2.41 (m, 2H), 1.65 (m, 1H), 1.33 (s, 3H), 1.25 (t, 3H, \(J = 7\) Hz). \(^{13}\)C-NMR (CDCl\(_3\), 100 MHz)(\(1\text{a}\)): 177.0, 139.0, 128.5, 128.2, 127.0, 64.0, 61.0, 60.0, 54.0, 48.0, 36.0, 25.0, 14.0. HRMS [C\(_{15}\)H\(_{21}\)NO\(_2\)Na\(^+\)] calculated = 270.1464, found = 270.1463.

**Synthesis of (R)-Ethyl 1-Benzyl-3-Methylpiperidine-3-Carboxylate (64b)**

A 0.8 g portion of \(63\text{b}\) (2.7 mmol) was dissolved in 20 mL of 4:1 THF/EtOH. A 0.16 g portion of Raney-Ni slurry in water (20\% by weight) was added to the solution. The solution was stirred vigorously under a H\(_2\) atmosphere for 6 hrs, at which point the reaction was found to be half-complete by TLC (10\% hexanes/CH\(_2\)Cl\(_2\)). The mixture was continued to stir under H\(_2\) atmosphere another 6 hrs. The mixture was found to be completed via TLC to give 0.54 g (2 mmol, 78\%) of \(63\text{b}\) as a colorless liquid. \(R_f = 0.35\) (20\% hexanes/CH\(_2\)Cl\(_2\)). \([\alpha]_{D}^{21} = +11.8\) (c = 1, CHCl\(_3\)). \(^1\)H NMR (CDCl\(_3\), 400 MHz): 7.29 (m, 5H), 4.43 (m, 2H), 3.52 (m, 1H), 3.40 (m, 1H), 2.97 (bm, 1H), 2.58 (bm, 1H), 2.02 (m, 3H), 1.73 (m, 1H), 1.59 (m, 1H), 1.20 (m, 4H), 1.13 (s, 3H). \(^{13}\)C NMR (CDCl\(_3\), 100 MHz): 176.5, 138.2, 128.8, 127.0, 63.1, 62.0, 60.2, 54.0, 43.1, 33.2, 24.0, 23.0, 14.0. ESI-MS [C\(_{16}\)H\(_{23}\)NO\(_2\)Na\(^+\)] calculated = 284.3, observed = 284.3.
Synthesis of (R)-1-Benzyl-3-Methylpyrrolidine-3-Carboxylic Acid (65a)

An amount of 0.13 g LiOH (6 mmol) was added to a solution of 0.46 g (2 mmol) 64a in 12 mL 3:2 H₂O/EtOH. The reaction was stirred at RT for 24hrs and determined to be complete by TLC. The mixture was acidified to pH 3 (4N HCl), and the water layer was evaporated under reduced pressure giving a colorless gummy residue. The gummy residue was triturated with MeOH multiple times and the MeOH fractions were dried over MgSO₄. The solvent was removed under reduced pressure giving 0.34 g (1.56 mmol, 78%) of 12 as a colorless liquid. Rₘ = 0.13 (5% MeOH/CH₂Cl₂). IR (cm⁻¹): 3371 (broad), 2946, 2615, 1712, 1455. \([\alpha]^{23}_D = -11.3\) (c = 1, MeOH). ¹H-NMR (CD₃OD, 400 MHz): 7.58 (m, 2H), 7.50 (m, 3H), 4.44 (m, 2H), 3.87 (d, 1H, J = 12 Hz), 3.51 (m, 2H), 3.21 (d, 1H, J = 12 Hz), 2.58 (m, 1H), 2.09 (m, 1H), 1.47 (s, 3H). ¹³C-NMR (CD₃OD, 100 MHz): 176.0, 130.4, 130.2, 129.7, 129.0, 61.0, 58.0, 53.0, 35.0, 22.0. HRMS [C₁₃H₁₇NO₂Na⁺]: calcd = 242.1151, found = 242.1145.

Synthesis of (R)-1-Benzyl-3-Methylpiperidine-3-Carboxylic Acid (65b)

A 0.125 g portion of crushed LiOH powder (5.2 mmol) was added to a solution of 0.46 g (1.7 mmol) of 64b in 20 mL of 3:2 H₂O/EtOH. The reaction was stirred at rt overnight. The reaction was determined to be complete by TLC (5% MeOH/CH₂Cl₂). The mixture was acidified to pH 3 (10% HCl), and the water layer was evaporated under reduced pressure giving a colorless gummy residue. The gummy residue was triturated with 10% MeOH/CH₂Cl₂ (20 mL x 20), and the MeOH fractions were dried over MgSO₄. The solvent was removed under reduced pressure giving 0.36 g (1.56 mmol, 88%) of 65b as a white solid as verified by TLC and staining with bromocresol green. Rₘ = 0.15 (5% MeOH/CH₂Cl₂). \([\alpha]^{22}_D = + 19.0\) (c = 1, MeOH). ¹H NMR (CD₃OD, 400 MHz): δ 7.52
\(\text{H}, 5\text{H}), 4.50 \text{ (m, 1H), 4.17 (m, 1H), 3.60 (m, 1H), 3.40 (m, 1H), 3.10 (m, 1H), 2.80 (m, 1H), 2.20 (m, 1H), 1.98 (m, 1H), 1.80 (m, 1H), 1.54 (m, 1H), 1.2 (s, 3H).} \) \(^{13}\text{C NMR (CDCl}_3, 100 \text{ MHz): } \delta 178.1, 132.0, 131.2, 130.4, 130.3, 62.1, 57.7, 55.0, 43.4, 33.0, 24.1, 22.1. \) \(\text{ESI-MS } [\text{C}_{14}\text{H}_{19}\text{NO}_2\text{Na}^+] = 256.3, \text{ observed } = 256.3.\) 

**Synthesis of (R)-3-Methylpyrrolidine-3-Carboxylic Acid (66a)**

An amount of 0.3 g (2.3 mmol) of 65a was dissolved in 15 mL MeOH and added to 0.03g Pd/C (10% by weight). The solution was allowed to stir over night under a H\textsubscript{2} atmosphere at RT. The resulting mixture was filtered through Celite and the filtrate was evaporated under reduced pressure giving 0.27 g (2.1 mmol, 91%) of 66a as a white solid. mp = 98 \textdegree C. IR (cm\(^{-1}\)): 3392(broad), 3177, 2877, 1704. \([\alpha]_D^{24}=-20.4 \text{ (c } 0.33, \text{ MeOH}).\) \(^1\text{H-NMR (CD}_3\text{OD, 400 MHz): } \delta 3.78 \text{ (m, 1H), 3.48 (m, 1H), 3.38 (m, 1H), 3.10 (d, 1H, } J = 12 \text{ Hz), 2.49 (m, 1H), 2.01 (m, 1H), 1.45 (s, 3H).} \) \(^{13}\text{C NMR (CD}_3\text{OD, 100 MHz): } 176.0, 53.0, 48.5, 45.0, 35.0, 21.0. \) HRMS [(C\textsubscript{6}H\textsubscript{11}NO\textsubscript{2}Na\textsuperscript{+}]: calcd = 281.1472, found = 281.1468.

**Synthesis of (R)-3-Methylpiperidine-3-Carboxylic Acid (66b)**

A 0.3 g (1.3 mmol) portion of 65b was dissolved in 15 mL of MeOH and added to 0.06 g Pd/C (20% by weight). The solution was allowed to stir overnight under a H\textsubscript{2} atmosphere at rt. The resulting mixture was filtered through Celite, and the filtrate was evaporated under reduced pressure giving 0.185 g (1.29 mmol, 93%) of 66b as a white solid. \(^1\text{H NMR (CD}_3\text{OD, 400 MHz): } \delta 3.53 \text{ (m, 1H), 3.28 (m, 1H), 2.97 (m, 1H), 2.83 (m, 1H), 2.19 (m, 1H), 1.88 (m, 1H), 1.69 (m, 1H), 1.58 (m, 1H), 1.27 (s, 3H).} \) \(^{13}\text{C NMR (CDCl}_3, 100 \text{ MHz): } \delta 178.5, 50.10, 44.3, 41.4, 33.5, 23.5, 21.0. \) ESI-MS [C\textsubscript{7}H\textsubscript{13}NO\textsubscript{2}Na\textsuperscript{+}] = 166.2, observed = 166.3.
Synthesis of (2R, 3S)-Ethyl-3-Formyl-2-(4-Methoxyphenylamino)-4-Methylopentanoate (67a)

This compound was synthesized following literature reported procedure. All characterizations complied with those reported in literature. HPLC (Diacel Chiral Pack OJ-H, hexane/i-PrOH = 99:1, flow rate = 1mL/min, λ = 254 nm); $t_{\text{anti}}$ (major) = 58.20 min, $t_{\text{anti}}$ (minor) = 55.78 min, $t_{\text{syn}}$ (major) = 51.31 min, $t_{\text{syn}}$ (minor) = 41.28 min.
NMR Spectra

A.1.a) $^1$H NMR of 9a, b) $^{13}$C NMR of 9a
A.2. a) $^1$H NMR of 9b, b) $^{13}$C NMR of 9b
A.3.a) $^1$H NMR of 9c, b) $^{13}$C NMR of 9c
A. 4. a) $^1$H NMR of 9d, b) $^{13}$C NMR of 9d
A. 5. a) $^1$H NMR of 9e, b) $^{13}$C NMR of 9e
A. 6. a) $^1$H NMR of 9f, b) $^{13}$C NMR of 9f
A. 7. a) $^1$H NMR of 10a, b) $^{13}$C NMR of 10a
A. 8. a) $^1$H NMR of 10b, b) $^{13}$C NMR of 10b
A. 9. a) $^1$H NMR of 10c, b) $^{13}$C NMR of 10c
A. 10. a) \(^1\)H NMR of 10d, b) \(^{13}\)C NMR of 10d
A. 11. a) $^1$H NMR of 10e, b) $^{13}$C NMR of 10e
A. 12. a) $^1$H NMR of 10f, b) $^{13}$C NMR of 10f
A. 13. a) $^1$H NMR of 14, b) $^{13}$C NMR of 14
A. 14. a) $^1$H NMR of 15, b) $^{13}$C NMR of 15
A. 15. a) $^1$H NMR of 16, b) $^{13}$C NMR of 16
A. 16. a) $^1$H NMR of 17, b) $^{13}$C NMR of 17
A. 17. a) $^1$H NMR of 18, b) $^{13}$C NMR of 18
A. 18. a) $^1$H NMR of 19a, b) $^{13}$C NMR of 19b
A. 18. a) $^1$H NMR of 20, b) $^{13}$C NMR of 20
A. 19. a) $^1$H NMR of 21, b) $^{13}$C NMR of 21
A. 20. a) $^1$H NMR of 23a, b) $^{13}$C NMR of 23a
A. 21. a) 1H NMR of 23b, b) 13C NMR of 23b
A. 22. a) 1H NMR of 23c, b) 13C NMR of 23c
A. 23. a) $^1$H NMR of 23d, b) $^{13}$C NMR of 23d
A. 24. a) $^1$H NMR of 23e, b) $^{13}$C NMR of 23e
A. 25. a) $^1$H NMR of 23f, b) $^{13}$C NMR of 23f
A. 26. a) $^1$H NMR of 25a, b) $^{13}$C NMR of 25a
A. 27. a) $^1$H NMR of 25b, b) $^{13}$C NMR of 25b
A.28. a) $^1$H NMR of 26a, b) $^{13}$C NMR of 26a
A.29. a) $^1$H NMR of 26b, b) $^{13}$C NMR of 26b
A .30. a) $^1$H NMR of 27a, b) $^{13}$C NMR of 27a
A.31. a) $^1$H NMR of 27b, b) $^{13}$C NMR of 27b
A.32. a) $^1$H NMR of 29, b) $^{13}$C NMR of 29
A.33. a) $^1$H NMR of 30, b) $^{13}$C NMR of 30
A.34. a) $^1$H NMR of 31
A.35. a) $^1$H NMR of 32,  b) $^{13}$C NMR of 32
A.36. a) $^1$H NMR of 33
A.37. a) $^1$H NMR of 34, b) $^{13}$C NMR of 34
A.38. a) $^1$H NMR of 35, b) $^{13}$C NMR of 35
A. 39. a) $^1$H NMR of 37, b) $^{13}$C NMR of 37
A.40. a) $^1$H NMR of 38, b) $^{13}$C NMR of 38
A.41. a) $^1$H NMR of 39, b) $^{13}$C NMR of 39
A.42. a) $^1$H NMR of 40, b) $^{13}$C NMR of 40
A.43. a) $^1$H NMR of 41, b) $^{13}$C NMR of 41
A. 44. a) $^1$H NMR of 43, b) $^{13}$C NMR of 43
A.45. a) $^1$H NMR of 44, b) $^{13}$C NMR of 44
A. 46. a) $^1$H NMR of 45, b) $^{13}$C NMR of 45
A.47. a) $^1$H NMR of 46, b) $^{13}$C NMR of 46
A.48. a) $^1$H NMR of 47, b) $^{13}$C NMR of 47
A.49. a) $^1$H NMR of 48, b) $^{13}$C NMR of 48
A.50. a) $^1$H NMR of 49, b) $^{13}$C NMR of 49
A.51. a) $^1$H NMR of 50
A.52. a) $^1$H NMR of 51, b) $^{13}$C NMR of 51
A.53. a) $^1$H NMR of 52
A.54. a) $^1$H NMR of 53, b) $^{13}$C NMR of 53
A.55. a) $^1$H NMR of 54, b) $^{13}$C NMR of 54
A.56. a) $^1$H NMR of 18b, b) $^{13}$C NMR of 18b
A.57. a) $^1$H NMR of 18c, b) $^{13}$C NMR of 18c
A.58. a) $^1$H NMR of 18d, b) $^{13}$C NMR of 18d
A.59. a) $^1$H NMR of 18e, b) $^{13}$C NMR of 18e
A.60. a) $^1$H NMR of 55, b) $^{13}$C NMR of 55
A.61. a) $^1$H NMR of 56, b) $^{13}$C NMR of 56
A.62. a) $^1$H NMR of 57, b) $^{13}$C NMR of 57
A.63. a) $^1$H NMR of 58, b) $^{13}$C NMR of 58
A.64. a) $^1$H NMR of 60, b) $^{13}$C NMR of 60
A.65. a) $^1$H NMR of 61, b) $^{13}$C NMR of 61
A.66. a) $^1$H NMR of 62a, b) $^{13}$C NMR of 62a
A.67. a) $^1$H NMR of 62b, b) $^{13}$C NMR of 62b
A.68. a) $^1$H NMR of 63a, b) $^{13}$C NMR of 63a
A.69. a) $^1$H NMR of 63b, b) $^{13}$C NMR of 63b
A.70. a) $^1$H NMR of 64a, b) $^{13}$C NMR of 64a
A.71. a) $^1$H NMR of 64b, b) $^{13}$C NMR of 64b
A.72. a) $^1$H NMR of 65a, b) $^{13}$C NMR of 65a
A.73. a) $^1$H NMR of 65b, b) $^{13}$C NMR of 65b
A.74. a) $^1$H NMR of 66a, b) $^{13}$C NMR of 66a
A.75. a) $^1$H NMR of 66b, b) $^{13}$C NMR of 66b.
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