Synthesis and Catalytic Evaluation of Novel C-alpha-methyl-beta-proline Analogues, and Concise Synthetic Approach to NH-Fmoc-S-Trityl-C-alpha-Methyl Cysteine

Hari Kiran Kotapati
University of Southern Mississippi

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SYNTHESIS AND CATALYTIC EVALUATION OF NOVEL C-ALPHA-METHYL-
BETA-PROLINE ANALOGUES, AND CONCISE SYNTHETIC APPROACH
TO NH-FMOC-S-TRITYL-C-ALPHA-METHYL CYSTEINE

by

Hari Kiran Kotapati

A Dissertation
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for the Degree of Doctor of Philosophy

August 2017
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August 2017

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ABSTRACT

SYNTHESIS AND CATALYTIC EVALUATION OF NOVEL C-ALPHA-METHYL-
BETA-PROLINE ANALOGUES, AND CONCISE SYNTHETIC APPROACH TO
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August 2017

In the field of chemistry there is a growing demand for small molecule
organocatalysts such as amino acids, more specifically proline and its analogues,
which could catalyze various key chemical reactions in the synthesis of several
biologically important molecules. Even though natural proline is reported to
catalyze various chemical reactions, its use as organocatalyst is limited mainly
due to the solubility issues in the reaction media and high catalyst loadings,
which is not very ideal for bulk scale manufacturing. To address these limitations
we planned to develop unnatural analogues of proline that could catalyze the
reactions with lower catalyst loadings and expanded solvent choice. Herein we
disclose a novel synthetic route that utilizes enzyme porcine liver esterase and a
novel stereoselective cyclization strategy, developed in our lab, to prepare two
diastereomers of 5-benzyl substituted Cα-methyl-β-Proline in their optically pure
forms. These two diastereomers were tested for their activities in both Aldol and
Mannich reactions, an important class of carbon-carbon bond forming reactions
between carbonyl compounds. Among them, only one diastereomer proved to be
an excellent organocatalyst in the Mannich Reaction providing anti-selective
products with ee of up to 99%. The catalyst also catalyzed the Aldol reaction
between acetone and 4-nitrobenzaldehyde in water yielding racemic mixture of Aldol product.

PLE isoenzyme studies were conducted on two malonate diesters 4b and 4c with different groups in the side chain. Isoenzymes 1 and 2 did not show any reactivity towards both diester substrates. Isoenzyme 5 did not show reactivity with substrate 4b. Whereas isoenzymes 3 and 4 showed reversal of diastereoselectivity with the diester 4c.

A concise synthetic methodology, for the preparation of orthogonally protected Cα-Methyl cysteine has been developed. Curtius rearrangement of enantiomerically enriched malonate halfester from PLE hydrolysis followed by Fmoc protection using Titanium (IV) Isopropoxide is the key step in the synthesis of NH-Fmoc-S-Trityl-Cα-Methyl Cysteine. The synthesized amino acid is currently under investigation for its effect when incorporated into a biologically active polypeptide.
ACKNOWLEDGMENTS

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DEDICATION

I am forever indebted to my parents and grandparents for their support and encouragement through the successful completion of my Ph.D. I would like to dedicate my doctoral dissertation work to my maternal grandmother, Late Smt. V. Rukma Bai who has always been my inspiration. Also, I would like to thank my family and friends for extending their support to me.
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<tr>
<td>$[\alpha]$</td>
<td>specific rotation</td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>dielectric constant (Solvents)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>chemical shift in ppm (NMR)</td>
</tr>
<tr>
<td>$2-D$</td>
<td>two-dimensional</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
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<td>Ar</td>
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</tr>
<tr>
<td>atm</td>
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<td>de</td>
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</tr>
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</tr>
<tr>
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**et al** and others

**Fmoc**  9-fluorenlymethoxycarbonyl

**HPLC**  high-performance liquid chromatography

**HRMS**  high-resolution mass spectrometry

**IR**  infrared

**Me**  methyl

**NMR**  nuclear magnetic resonance

**NOESY**  nuclear Overhauser effect spectroscopy

**obsd**  observed

**PLE**  Porcine (Pig) Liver Esterase

**Ph**  phenyl

**i-Pr**  iso-propyl

**tert**  tertiary

**Tf**  trifluoromethanesulfonfyl (triflyl)

**TFA**  trifluoroacetic acid

**THF**  tetrahydrofuran

**TLC**  thin-layer chromatography

**TMS**  tetramethysilane (NMR)

**¹R**  retention time (Chromatography)

**Trityl (Tr)**  triphenylmethyl

**UV**  ultraviolet

---

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CHAPTER I - INTRODUCTION

In recent years, the demand for chiral molecules, preferentially one stereoisomer, has increased significantly because two thirds of current prescription drugs are chiral and the majority of them are single stereoisomers.\textsuperscript{1,2} As a result, there has been extensive ongoing research to develop efficient methodologies to produce optically pure chiral molecules in a very selective manner. Among all the strategies for synthesizing optically pure chiral molecules, asymmetric catalysis is found to be more efficient because it can produce chiral products in a quantitative yield. Asymmetric catalysis is a chemical process where a chiral catalyst promotes a stereoselective transformation of an achiral substrate to a chiral product. Stereoselective transformation is a method where achiral molecule reacts to form a disproportionate mixture of stereoisomers during the reaction process.

In asymmetric catalysis, stereoselective transformations can be achieved by several different methods that include, the use of metal catalysts, the use of chiral auxiliaries, the use of chemical reactions that involve chiral phase transfer catalysts or by employing optically active compounds that occur in nature.\textsuperscript{3} On the basis of the catalyst being used, asymmetric catalysis can be broadly classified into organo-metal catalysis and bioorganic catalysis.\textsuperscript{3} In the early days, researchers mainly focused on the asymmetric catalysis mediated by metal ion containing catalysts to carry out a wide variety of chemical transformations.\textsuperscript{4-7} However there are several disadvantages of using metal ions as catalysts in organic reactions. Most metal ion containing catalysts are very sensitive which
require them to be used under closed environments and some of them are expensive and also toxic.\textsuperscript{3,8} In order to overcome the problems with metal ions researchers have found several alternative novel bioorganic catalysts that include peptide based compounds, chiral quaternary ammonium compounds, optically active compounds isolated from natural sources, and optically active amino acids.\textsuperscript{9-13}(Figure 1.1) Among all the alternative approaches listed above, researchers have mainly focused on using optically active amino acids since they are readily available in their optically active forms and also relatively inexpensive. This field of asymmetric catalysis that involves small molecule catalysts that are free of metal atoms has emerged into a whole new field, Organocatalysis.

![Organocatalysts](image)

**Figure 1.1 Examples of few organocatalysts used in asymmetric synthesis**

**1.2 Amino acids in organocatalysis**

Naturally available amino acids are widely used as catalysts in the field of organocatalysis not only because of their ease of use but also due to the fact that they are readily available in their optically active forms at a reasonable price. In the year 2004, Pizzarello and Weber published their study on the origin of homochirality in prebiotic building blocks.\textsuperscript{14} In this study, an Aldol condensation reaction was carried out between two aldehydes to synthesize sugars, Threose.
and Erythrose using non racemic alanine and isovaline of varying enantiomeric excess. The enantiomeric excess of product sugars, in the reaction, decreased as the amino acid with lower enantiomeric excess was used as a catalyst. This study suggests the prebiotic amino acids may have played an important role in the induction of homochirality in biological molecules. In early 1980’s Julia et al reported a poly-L-alanine catalyzed epoxidation of chalcones that provided products with up to 96% enantiomeric excess.\textsuperscript{15,16} Even though the reports of amino acid catalyzed asymmetric chemical transformations exist since early 1970’s, it has been widely explored only after List and coworkers reported a novel intermolecular Aldol reaction catalyzed by L-proline in the year 2000.\textsuperscript{17}

### 1.2.1 Proline and its analogues as organocatalysts

Amino acid catalyzed carbon-carbon bond forming reactions were reported four decades ago by two different research groups. In 1971, Weichert and coworkers reported a novel Aldol reaction involving intramolecular cyclization of cyclic triketones\textsuperscript{18} by optically pure aminoacids to provide optically active chiral bicyclic compounds that were used as starting materials in the synthesis of steroids.\textsuperscript{19} Hajos and Parrish also reported L-Proline catalyzed asymmetric transformation of tricyclic ketone to an optically active intermediate that was used in the synthesis of steroids.\textsuperscript{20} (Scheme 1.1)

![Scheme 1.1 (S)-(−)-Proline catalyzed Intramolecular Aldol Cyclization reaction (Weichert et al 1971 and Hajos et al 1974)]
Proline’s use as organocatalyst has expanded to various applications in the field of chemistry since the report by List et al in the year 2000. In this work a direct asymmetric Aldol reaction was carried out using 30 mol% of L-Proline as a catalyst. Since then, Proline and its analogues have successfully been used as catalysts in the asymmetric synthesis causing a variety of chemical transformations. Enders et al reported the preparation of Ulosonic acid precursors in an asymmetric fashion using L-Proline as a catalyst. Proline catalyzed α-aminoxylation reactions are also used to prepare chiral building blocks that are used in the synthesis of several biologically active compounds. Volchkov et al used L-Proline to carry out crossed Aldol condensation of two aldehydes, a key step in the total synthesis of Amphidinolide V. An efficient enantioselective synthesis for HMG-CoA reductase inhibitors, Atorvastatin (Lipitor) and Rosuvastatin (Crestor) that involves a one pot tandem Aldol reaction, catalyzed by deoxyribose-5-phosphate aldolases, was reported by William Greenberg and co-workers. Steven Lenger reported the total synthesis of Rosuvastatin that involves the stereoselective Aldol reaction. Recently L-Proline catalyzed Aldol reaction that is a key step in the synthesis of prostaglandin, PGF\textsubscript{2α} has been reported. In this report, Aldol reaction was carried out between two succinaldehyde molecules in the presence of L-Proline catalyst to prepare a key bicyclic enal intermediate in 98% ee as shown in Scheme 1.2.
Scheme 1.2 Proline catalyzed Aldol reaction in the synthesis of prostaglandin PGF$_{2\alpha}$ (Coulthard et al)

Mannich reaction involves carbon-carbon bond formation in the reaction between carbonyl compounds and amines.$^{35}$ Mannich reaction is also used as a key tool in the synthesis of various bioactive skeletons of several compounds that possess potent anticancer, antiepileptic, antituberculotic and antimalarial properties.$^{36}$ Usually L-Proline catalyzes Mannich reactions to provide “syn” selective products. In 2005, Hayashi et al reported an efficient asymmetric synthetic strategy to prepare Nikkomycin B and BX. This strategy utilizes asymmetric Mannich reaction between furfural, propanal and an aromatic amine catalyzed by L-Proline. This reaction provided a syn Mannich intermediate that is later converted to an N-terminal amino acid moiety corresponding to Nikkomycins B and BX. (Scheme 1.3)

Scheme 1.3 Proline catalyzed asymmetric reaction in the synthesis of Nikkomycins B and BX (Hayashi et al)

L-Proline’s use as a catalyst in organic reactions is not restricted to just Aldol or Mannich reactions. L-Proline is also reported to catalyze a wide
spectrum of chemical reactions that include α-Amination\textsuperscript{23,24} and α-oxyamination\textsuperscript{25,28} of aldehydes/ketones, asymmetric Knoevenagel/Diels alder reaction.\textsuperscript{27} L-Proline is also used as a co-catalyst in the Morita-Baylis-Hillman reaction catalyzed by a peptide based catalyst.\textsuperscript{26} The above are few examples of other organic reactions catalyzed by L-Proline besides Aldol and Mannich reactions.

\textbf{1.2.1.2 Proline catalyzed asymmetric intermolecular Aldol reaction}

The Aldol reaction is one of the important classes of classic carbon-carbon bond forming reactions between two carbonyl compounds that often occurs in nature. Aldol reaction is carried out by the enzyme Aldolase in the nature. There are two types of Aldolases, Aldolase I and Aldolase II that catalyze an Aldol reaction and each one of these enzymes follow their own pathway during the reaction. During the Aldol reaction, Type I Aldolases usually proceed via an Iminium ion intermediate whereas Type II Aldolases utilize Zn\textsuperscript{2+} as a cofactor in the catalytic process.\textsuperscript{38,39}

There were no published reports on the Type I Aldolase mimicking metal free small molecule asymmetric catalyst for Aldol reaction until List et al reported the intermolecular Aldol reaction catalyzed by L-Proline.\textsuperscript{17} In this report, an Aldol reaction was carried out between aldehydes and an unmodified ketone in DMSO using 30 mol\% of catalyst. When the Aldol reaction was performed between acetone and 4-nitro benzaldehyde in DMSO, (\textit{R})-Aldol product was produced in 68\% yield and 76\% ee. (Scheme 1.4)
Scheme 1.4 L-Proline catalyzed intermolecular aldol reaction

The mechanism for Proline catalyzed intermolecular Aldol reaction was proposed by List et al. In this mechanism Proline proceeds via iminium-enamine intermediate as shown in Scheme 1.5. The mechanism for the intermolecular Aldol reaction between acetone and an aldehyde, catalyzed by L-Proline, proposed by List et al involves six steps that account for the stereoselective outcome in the reaction. Amino group of L-Proline serves as a nucleophile (Lewi’s base) and Carboxyl group acts as a Lewi’s acid during the mechanism. In the carbon-carbon bond forming step of the mechanism, the enamine attacks the carbonyl of 4-nitro benzaldehyde from the re-face. This re-facial approach is responsible for the stereoselectivity that is observed in the reaction.

Scheme 1.5 Enamine mechanism of L-Proline catalyzed asymmetric Aldol reaction
1.2.1.3 Proline catalyzed asymmetric Mannich reaction

Mannich reaction is another classic example for carbon-carbon bond formation reaction that involves an amino compound, donor carbonyl compound and an acceptor carbonyl compound. This transformation results in the class of β-amino carbonyl compounds that have a wide range of applications in the field of chemistry. A condensation reaction involving three components; ammonia, antipyrene and formaldehyde was first reported by Mannich and Kroesche in 1912.\textsuperscript{35} Enantioselective Mannich reaction, which involves a preformed enolate and a preformed imine, catalyzed by various metal based catalysts was reported in late 1990's.\textsuperscript{40-46} In the year 2001, Juhl et al reported an enantio- and diastereoselective Mannich reaction between an α-imino ester and an activated carbonyl compound using chiral Lewis acids as catalysts.\textsuperscript{47} List et al reported the first organocatalytic asymmetric three component Mannich reaction catalyzed by L-Proline.\textsuperscript{48,49} In these reports an asymmetric Mannich reaction between acetone, 4-nitrobenzaldehyde and p-anisidine using 35 mol\% L-proline as catalyst was reported. The catalyst loading studies performed by List and coworkers suggested that increased catalyst loadings of L-Proline reduced the reaction times significantly.\textsuperscript{49} Cobb et al designed proline analogues with tetrazole and acyl sulfonamide functionalities and used them in the catalysis of asymmetric Mannich, Aldol and nitro-Michael reactions.\textsuperscript{50}

The Mannich reaction that involves a preformed imine and an unmodified aldehyde catalyzed by L-Proline was reported by Barbas and coworkers in the year 2003.\textsuperscript{51} In this report, an α-imino ester was prepared and reacted with an
aldehyde in the presence of 10 mol% L-Proline to provide β substituted α-amino acid analogues with two contiguous stereo centers. Use of L-Proline as a catalyst facilitated the formation of a single diastereomer predominantly over the other in high enantiopurity. In this case L-Proline provided “syn” selective products in a diastereomeric ratio of >19:1 and an enantiomeric excess of >99%. The reaction between isovaleraldehyde and N-PMP protected α-imino ethyl glyoxylate favored the formation of “syn” product in a diastereomeric ratio of >10:1 and an enantiomeric excess of 87%. (Scheme 1.6)

Scheme 1.6 L-Proline catalyzed Mannich reaction of isovaleraldehyde with an α-imino ester (Barbas et al)\textsuperscript{51}

Mannich reactions catalyzed by various catalysts that provide “anti” selective products have also been reported.\textsuperscript{52-55} In 2006, Barbas and coworkers synthesized β-Proline or 3-Pyrrolidine carboxylic acid, an analogue of proline, and used it as a catalyst in the direct asymmetric Mannich reaction, between an unmodified aldehyde and a N-PMP protected α-imino ester.\textsuperscript{56} (Scheme 1.7) This reaction proceeded with excellent diastereo- and enantioselectivities, to provide “anti” selective products. The same catalyst was later used in asymmetric Mannich reaction between N-PMP protected α-imino ester and an unmodified ketone to produce “anti” selective products.\textsuperscript{57}
Scheme 1.7 β-Proline catalyzed anti-Mannich reaction of isovaleraldehyde with an α-imino ester (Barbas et al) \(^{56}\)

Though L-proline is known to catalyze Aldol and Mannich reactions in a stereoselective manner, its use as an organocatalyst is limited due to poor solubility in organic solvents. The efficiency of L-proline as a catalyst was found to be lowered in less polar solvents where solubility is also a concern.\(^ {12,58}\) Use of natural proline as catalyst also requires high catalyst loadings, which is not very ideal for bulk scale manufacturing. To address the limitations and challenges related to solubility and catalyst loadings of L-proline's use as catalyst, in the Aldol and Mannich reactions, we had planned to develop an unnatural analogue of proline that could catalyze the reactions with lower catalyst loadings and expanded solvent choice and still provide good stereoselectivities. Even there are few existing reports for the synthesis of C\(^\alpha\)-methyl-β-Proline, the unique approach of hydrolysis by Porcine Liver Esterase and stereoselective cyclization is more advantageous as it utilizes nature friendly enzyme to induce chirality in a very selective fashion.
CHAPTER II - SYNTHESIS OF (3R, 5S) AND (3S, 5S)-5-BENZYL-3-METHYLPYRROLIDINE-3-CARBOXYLIC ACID

2.1 Hypothesis 1

With all available tools, developed in our group, at our disposal we can synthesize both diastereomers of 5-benzyl substituted Cα-methyl-β-proline analogue in a diastereoselective manner by utilizing a chemo enzymatic synthetic strategy that involves the enzyme Porcine Liver Esterase and a selective cyclization reaction.

For the synthesis of 5-benzyl substituted Cα-methyl-β-proline analogue we chose to use relatively inexpensive and commercially available optically active L-phenylalanine as starting material. L-Phenylalanine was transformed into a branched malonate diester which was then subjected to asymmetric enzymatic hydrolysis by Porcine Liver Esterase. The diastereomerically enriched half ester was then taken to stereoselective cyclization reaction sequence to provide the desired Cα-methyl-β-proline analogue. (Scheme 2.1)

![Scheme 2.1 Synthetic strategy for 5-benzyl substituted Cα-methyl-β-proline](image-url)
2.2 Background

2.2.1.1 Synthesis of β-Proline Analogues for Mannich Reactions

L-Proline is used as a catalyst to carry out a broad range of chemical transformations that involve carbon-carbon bond formation in a stereoselective manner. However, L-Proline’s use as a catalyst in organocatalysis is limited due to the arising solubility concerns. Also high loadings of L-Proline were reportedly used in the reactions to facilitate the desired transformations. Most organic reactions, that use L-proline as catalyst, are carried out in polar solvents like water, methanol, DMSO and DMF. The efficiency of L-proline as catalyst was found to be lowered, in organic reactions involving compounds with poorer solubility in polar solvents.\textsuperscript{12,58} Hence, researchers have focused on designing unnatural analogues of proline that are more hydrophobic than natural proline and catalyze organic reactions in nonpolar organic solvents. However, synthesis of unnatural analogues of proline in an enantioselective fashion has become a challenging task. Seebach et al reported the synthesis of alpha substituted proline derivatives for the first time in 1983.\textsuperscript{59} In this work a series of α-substituted proline derivatives were synthesized from optically active proline, which underwent self-reproduction of chirality, to provide enantiopure analogues of proline. Later, Mitsumori et al designed an analogue of β-proline and reported its catalytic activity in anti-Mannich reaction.\textsuperscript{56} The synthesis of the β-Proline analogue involves 14 steps as illustrated in Scheme 2.2 and the yields obtained were moderate to excellent. The synthetic strategy utilized optically pure starting material with anti-configuration that provided access to single stereoisomer of the
β-proline analogue. The synthetic sequence involves double inversion at one stereo center that resulted in retention of the configuration.

Scheme 2.2 Synthesis of a β-Proline analogue for catalysis in anti Mannich reaction reported by Barbas et al.

An enantiodivergent strategy for the synthesis of α-methyl-β-proline analogues was reported for the first time by Banerjee et al in our group. Both enantiomers of the β-proline analogue were prepared successfully by chemoenzymatic pathway in an enantioselective fashion as shown in Scheme 2.3. The synthetic strategy reported by Banerjee et al utilized the enzyme Porcine Liver Esterase (PLE) to create an all carbon quaternary center in an enantioselective fashion. Malonate diester was subjected to asymmetric enzymatic hydrolysis by Porcine Liver Esterase (PLE) to provide enantioenriched chiral half ester in 91% ee. This chiral half ester underwent a stereoselective cyclization process in the later steps to provide a γ-lactam that was transformed into a Cα-methyl-β-proline. Both enantiomers of Cα-methyl-β-proline were prepared successfully using a novel cyclization reaction.
Scheme 2.3 PLE mediated enantioselective synthesis of C\textsuperscript{\textalpha}-methyl-\textbeta-Proline by Banerjee et al

Later, Nagata et al synthesized \textalpha-methyl-\textbeta-proline analogue and reported its catalytic activity in Mannich reactions\textsuperscript{[61]} The catalyst provided excellent enantioselectivities and \textit{anti} diastereoselectivities in the Mannich reactions involving aldehydes and cyclic ketones with preformed \textalpha-imino esters. The enantioselective synthetic strategy reported by Nagata et al utilizes a chiral phase transfer catalyst to provide an enantioenriched compound. The enantiopurity of this compound was further improved by single recrystallization using (S)-1-phenylethylamine as illustrated in Scheme 2.4.
2.3 Results and Discussion

2.3.1 Synthesis of malonate half-ester intermediates by PLE hydrolysis-

Substrate Screening

In the designed synthetic strategy, we utilized optically pure amino acids as starting materials. In the first step the amino acids Leucine and Phenylalanine were reduced to corresponding amino alcohols using NaBH\(_4\)/I\(_2\) according to the reported procedure.\(^6\) Alanine, however, had to be reduced by LiAlH\(_4\) according the reported procedure\(^6\) because isolation of L-alaninol (2a) proved to be difficult from the borohydride reaction. The amino alcohol was then reacted with phthalic anhydride using a Dean-Stark apparatus to provide N-phthalimide protected amino alcohol.\(^6\) The protected amino alcohols 2a-c, were then transformed into the respective triflate compounds 3a-c, to facilitate the SN\(_2\) chemistry with enolate of diethyl methyl malonate. Synthesis and handling of these triflate
compound 3a proved to be very difficult as the triflate was very unstable and started to decompose quickly at room temperature. The compounds 3b and 3c were prepared in decent yields but the triflate 3a was unstable resulting in a yield of 36% (Scheme 2.5).

Scheme 2.5 Synthesis of branched malonate half esters, (2R, 4S)-5a-c from by crude PLE hydrolysis

When we had attempted to do the SN2 chemistry with the mesylate, tosylate and iodide of the protected amino alcohol, instead of triflate, none of the reactions provided the substituted product. The triflate was taken immediately to the next step, reaction with diethyl methyl malonate on the same day to avoid further decomposition and side products thereof. This reaction yielded the desired malonate diesters 4a-c, with a preexisting chiral center in the side chain as shown in Scheme 2.5. In the enzymatic hydrolysis reaction, the chiral malonate diesters 4a-c were subjected to asymmetric ester hydrolysis by the Porcine Liver Esterase enzyme to afford the mixture of diastereomeric half esters 5a-c. The
reaction conditions for enzymatic hydrolysis by PLE, yields and the resulted
diastereoselectivities are tabulated in Table 2.1.

Table 2.1
PLE hydrolysis results of malonate diesters, 4a-c and co-solvent screens of 4c

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ethanol (V %)</th>
<th>CH$_2$Cl$_2$ (V %)</th>
<th>d.r (1'H NMR)</th>
<th>%Yield</th>
<th>Major Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>2.5%</td>
<td>-</td>
<td>8:1</td>
<td>75</td>
<td>(2R, 4S)-5a</td>
</tr>
<tr>
<td>4b</td>
<td>2.5%</td>
<td>-</td>
<td>6:1</td>
<td>12</td>
<td>(2R, 4S)-5b</td>
</tr>
<tr>
<td>4c</td>
<td>2.5%</td>
<td>0.8%</td>
<td>3.5:1</td>
<td>10</td>
<td>(2R, 4S)-5c</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>-</td>
<td>4.7:1</td>
<td>30</td>
<td>(2R, 4S)-5c</td>
</tr>
<tr>
<td></td>
<td>2.5%</td>
<td>0.6%</td>
<td>4.1:1</td>
<td>57</td>
<td>(2R, 4S)-5c</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>-</td>
<td>3.1:1</td>
<td>18</td>
<td>(2R, 4S)-5c</td>
</tr>
</tbody>
</table>
PLE hydrolysis reactions were carried out in phosphate buffer of pH 7.4 and 1.0N NaOH was dispensed into the reaction using an auto burette set up as the reaction progressed. The reactions were worked up and the crude products were isolated. $^1$H-NMR was obtained on the crude half esters that helped us determine the diastereomeric ratios. The PLE hydrolysis reactions involving substrates 4b and 4c appeared to be very slow and had to be stopped after 5 days and worked up. For the optimization of the PLE hydrolysis reaction conditions we chose to pursue the malonate diester substrate 4c.

The diester substrate 4c is a viscous liquid and tended to hinder proper stirring of the reaction media. The reaction conditions had to be optimized for appropriate co-solvents that would facilitate proper stirring of the reaction. To the reaction, 2.5 % ethanol was added as a co-solvent to the phosphate buffer to improve the percent diastereomeric excess of the product. In addition to ethanol, 0.6-0.8% (Volume %) methylene chloride was added along with the diester substrate, 4c as a solution. Adding methylene chloride helped the substrate to disperse as oil globules in the reaction media. Otherwise, the substrate 4c would stick to the bottom of the flask and hinder the stir bar from proper stirring. Improper stirring of the reaction buffer resulted in the lower conversions of diester substrate 4c.

In the product, (2R, 4S)-5c, the stereochemistry of the quaternary chiral center created from the PLE hydrolysis reaction was determined from the single crystal X-ray analysis data of pure diastereomer of its derivative (2S, 4S)-6c. The stereochemistry of the halfester 5b was assigned from the 2D-NOESY NMR data.
of the corresponding lactam 7b, derived via a stereoselective cyclization reaction.

The stereochemistry of the half-ester 5a derived from hydrolysis by the crude PLE enzyme is determined to be (2R, 4S). This assignment is derived from the 1H NMR shifts of the ethyl group of the ester. The protons of the methyl and methylene units in the major diastereomeric half esters (2R, 4S)-5b-c are further down field compared to those of the minor diastereomeric half-esters (2S, 4S)-5b and (2S, 4S)-5c as illustrated in the 1H NMR spectra below.

Figure 2.1 1H NMR data for stereochemistry assignment at the quaternary chiral center in half-ester 5a

2.3.2 PLE Isoenzyme studies of malonate diester substrates, 4b and 4c

For the last three decades PLE has been a valuable bio catalytic tool in organic synthesis. PLE is used in a variety of asymmetric chemical
transformations that involve different substrates providing stereoselective products very efficiently. These enzymatic hydrolysis reactions usually utilize buffer as reaction media and titrate standardized sodium hydroxide into the reaction as the reaction progresses. PLE is known to catalyze the reactions involving malonate diesters causing asymmetric hydrolysis to provide optically enriched chiral half ester synthons in a stereoselective manner. These chiral synthons are used as valuable materials for the synthesis of several biologically important compounds.

Our research group has been very successful in utilizing the enzyme PLE to prepare the optically active chiral synthons from prochiral malonate diesters. These chiral synthons were used in the preparation of several unnatural α, β, γ and δ amino acids that have a methyl substituent at the Cα position of the backbone. For our synthetic purposes we utilize the crude PLE enzyme to perform the asymmetric enzymatic hydrolysis of the malonate diesters. In some cases adding ethanol as a co-solvent significantly improved the enantioselectivity of the product in the hydrolysis reaction. Crude PLE is a mixture of at least six different isoenzymes in various proportions. Each one of these isoenzymes could provide different yields and selectivities from that of the crude enzyme.

After observing the asymmetric ester hydrolysis results by crude PLE, we looked into the isoenzymes of PLE to see if any of the isoenzymes would provide better selectivities or conversions for our synthetic purposes. For this purpose, we chose to pursue the asymmetric hydrolysis of the diethyl methyl malonate diester substrates 4b and 4c. The diesters that were chosen for our enzymatic
hydrolysis reactions have a preexisting chiral center in the side chain and also a bulky phthalimide group. To the extent of our knowledge, these substrates are the very first ones of this type to be investigated for hydrolysis by PLE. Recently, malonate diester with a preexisting chiral center has been utilized for PLE hydrolysis to create the product in a diastereoselective fashion.\textsuperscript{84}

When diesters 4b and 4c are subjected to enzymatic hydrolysis, it is possible for the reaction to produce only two compounds (2R, 4S)-5b-c and (2S, 4S)-5b-c that are diastereomers to one another. (Scheme 2.6) This is due to the fact that our starting materials are optically pure and the stereochemistry at the preexisting chiral center is already set to be “S”.

![Scheme 2.6 PLE isoenzyme hydrolysis reaction studies of malonate diesters 4b and 4c](image)

The PLE hydrolysis reactions were set up in phosphate buffer, pH 7.4 and the reactions were carried out on a titrator instrument using an auto burette set up that dispenses 1.0N NaOH into the reaction as the reaction progressed. The enzyme was added to the reaction as a suspension in 3.0 M Ammonium Sulfate. The reactions with substrate 4b were allowed to proceed for 7 days and then worked up. Among the six different isoenzymes of PLE (1-6), PLE 1, 2 and 5 did not show any conversion of diester 4b to the product. With the use of
isoenzymes there is a slight improvement in the yields of substrate 4b, however the stereoselectivity was not improved. The percent diastereomeric ratios were determined from ¹H-NMR of the crude product. The results of the isoenzyme studies are tabulated below. (Table 2.2)

Table 2.2
PLE isoenzyme studies of malonate diester substrate, 4b

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>d.r. (¹H NMR)</th>
<th>% Yield</th>
<th>Major Diastereomer</th>
<th>Minor Diastereomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLE 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLE 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLE 3</td>
<td>5.7:1</td>
<td>24</td>
<td>(2R, 4S)-5b</td>
<td>(2S, 4S)-5b</td>
</tr>
<tr>
<td>PLE 4</td>
<td>1.3:1</td>
<td>13</td>
<td>(2R, 4S)-5b</td>
<td>(2S, 4S)-5b</td>
</tr>
<tr>
<td>PLE 5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLE 6</td>
<td>11:1</td>
<td>25</td>
<td>(2R, 4S)-5b</td>
<td>(2S, 4S)-5b</td>
</tr>
<tr>
<td>Crude PLE</td>
<td>6:1</td>
<td>12</td>
<td>(2R, 4S)-5b</td>
<td>(2S, 4S)-5b</td>
</tr>
</tbody>
</table>

The reactions of substrate 4c was allowed to proceed for 5 days. The conversion with substrate 4c did not improve with any of the six isoenzymes compared to that of the crude PLE enzyme. Like with substrate 4b, PLE 1 and 2 did not show any reactivity with diester substrate 4c. To our surprise PLE isoenzymes 3 and 4 showed a reversal in the diastereoselectivity of the product half ester. However, the yields of the reactions with isoenzymes did not seem to improve at all when compared to that of the crude PLE enzyme. The reversal of selectivity was determined from the ¹H-NMR shifts of the product half ester. We found these results to be interesting because this reversal in selectivity would
help us alter the stereochemistry at quaternary chiral carbon of the half ester without any additional chemical transformations. The yields and diastereoselectivities of the PLE isoenzyme studies with malonate diester substrate 4c are tabulated below (Table 2.3)

Table 2.3
PLE isoenzyme studies of malonate diester substrate, 4c

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>d.r. (1H NMR)</th>
<th>% Yield</th>
<th>Major Diastereomer</th>
<th>Minor Diastereomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLE 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLE 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLE 3</td>
<td>3.8:1</td>
<td>30</td>
<td>(2S, 4S)-5c</td>
<td>(2R, 4S)-5c</td>
</tr>
<tr>
<td>PLE 4</td>
<td>3.4:1</td>
<td>20</td>
<td>(2S, 4S)-5c</td>
<td>(2R, 4S)-5c</td>
</tr>
<tr>
<td>PLE 5</td>
<td>4.2:1</td>
<td>13</td>
<td>(2R, 4S)-5c</td>
<td>(2S, 4S)-5c</td>
</tr>
<tr>
<td>PLE 6</td>
<td>4.1:1</td>
<td>10</td>
<td>(2R, 4S)-5c</td>
<td>(2S, 4S)-5c</td>
</tr>
<tr>
<td>Crude PLE</td>
<td>4.1:1</td>
<td>57</td>
<td>(2R, 4S)-5c</td>
<td>(2S, 4S)-5c</td>
</tr>
</tbody>
</table>

The stereochemistry of the major diastereomer of the half ester 5b was assigned from 2D-NOESY data of the lactam 7b derived through our stereoselective cyclization methodology (Scheme 2.7) followed by diastereomeric ratio improvement. The half ester from the asymmetric hydrolysis, of the malonate diester 4b, by crude PLE was utilized for this purpose.
Scheme 2.7 Synthesis of lactam (3R, 5S)-7b, via stereoselective cyclization

The product half ester 5b was transformed into 4-nitro benzyl ester 6b upon reaction with 4-nitro benzyl bromide. Compound 6b was then treated with hydrazine hydrate to cause stereoselective cyclization to provide lactam 7b. The crude lactam was then purified carefully via flash column chromatography to obtain essentially diastereopure lactam 7b. The diastereopure lactam 7b was completely characterized and the stereochemistry at the quaternary chiral center was determined to be “R” from the 2D-NOESY NMR experimental data. NOESY interactions are through space and to observe these correlations the two atoms or groups must be within 5 Å distance to each other. It is also essential that the atoms or groups must be in a restricted free rotation environment and for this purpose lactams are very useful. The key NOESY interactions that were used to assign the stereochemistry are shown in Figure 2.1.

![Diagram of lactam 7b showing NOESY interactions](image-url)

Figure 2.2 Specific 2D-NOESY correlations for compound (3R, 5S)-7b

2.3.3 Synthesis of (3R, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid

The half ester (2R, 4S)-5c, from the PLE hydrolysis reaction was converted to (3R, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid (3R, 5S)-10c, in five steps (Scheme 2.8), via the stereoselective cyclization reaction that
was reported by our group. During this synthetic sequence the diastereomeric ratio of the major product was improved to 24:1 by simple recrystallization and absolute stereochemistry at quaternary chiral center was assigned from its X-ray crystal structure.

Scheme 2.8 Synthesis of (3R, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid Malonate half ester, (2R, 4S)-5c, upon reaction with 4-nitro benzyl bromide afforded the 4-nitro benzylic ester (2S, 4S)-6c in 67% yield and a diastereomeric ratio of 24:1. During the reaction work up with diethyl ether, the major diastereomer product (2S, 4S)-6c precipitated out of the crude reaction mixture as a white solid. The improvement in the diastereomeric ratio was observed from the ¹H-NMR data. Crystals of diastereopure compound (2S, 4S)-6c were developed for X-ray crystallography analysis by solvent diffusion technique. Single X-ray crystal analysis data revealed the stereochemistry at the new quaternary chiral center to be “S”. (Figure 2.2) Based on this stereochemical assignment, the quaternary chiral center in the malonate half ester (2R, 4S)-5c from the PLE hydrolysis reaction is determined to be in “R” configuration due to
the changing priorities of the functional groups. Absolute stereochemical assignment was made relative to the preset chiral center, adjacent to the phthalimide nitrogen, which is derived from the optically pure amino acid starting material.

Figure 2.3 Single X-ray crystal structure of (S)-1-ethyl 3-(4-nitrobenzyl) 2-((S)-2-(1,3-dioxoisindolin-2-yl)-3-phenylpropyl)-2-methylmalonate, (2S, 4S)-6c

In the next step of synthetic sequence, diastereopure compound (2S, 4S)-6c was treated with hydrazine hydrate in methanol. The compound was not completely soluble in pure methanol. Hence methylene chloride was added to facilitate the solubility of the 4-nitrobenzyl ester, (2S, 4S)-6c in methanol. The reaction was heated to reflux the solvent and the reaction went to completion after 5 days. The chemistry of this reaction was previously reported by Banerjee.
et al in the synthesis of Cα-methyl-β-proline. In this reaction, hydrazine cleaves off the phthalimide resulting in the formation free amine. Under basic conditions this free amine reacts with the more electrophilic carbonyl to cause cyclization. The cyclization is controlled towards one specific carbonyl by installing more electron withdrawing 4-nitro benzyl group on the carbonyl moiety. In our case the selective cyclization happened predominantly towards the 4-nitro benzylic ester side and no trace of cyclization towards ethyl ester was observed. However, even under harsh refluxing conditions the reaction appeared to be very slow and took 5 days to go to completion. The reason for the longer reaction times in this reaction could be due to the steric congestion created by the bulky benzylic substituent adjacent to the nitrogen of phthalimide group.

The cyclization product (3R, 5S)-7c, was then reacted with benzyl bromide in the presence of sodium hydride to create an N-benzylated product according to the reported procedure by Banerjee et al. This reaction, however yielded a complex mixture of the products that included the N-benzylated product with the ethyl ester hydrolysed to acid. After our unsuccessful attempt to N-benzylate the lactam (3R, 5S)-7c, we chose to avoid the N-benzyl protection step. This approach facilitated the reduction of the lactam to amine in decent yields and at the same eliminated two steps (N-benzyl protection and deprotection) of the proposed synthetic strategy.

Upon reaction with Lawesson’s reagent, the lactam (3R, 5S)-7c, was transformed into a thio lactam (3S, 5S)-8c in 93% yield. By utilizing Raney Ni under hydrogen atmosphere, the thio lactam (3S, 5S)-8c, was then reduced to
the corresponding amino ester \((3R, 5S)-9c\) in 51% yield.\textsuperscript{68,69} In the final step the amino ester \((3R, 5S)-9c\) was dissolved in 6.0N HCl and refluxed for 24 hrs to hydrolyze the ethyl ester and provide the amino acid \((3R, 5S)-10c\) as its HCl salt in a quantitative yield. The amino acid was then purified via an ion exchange chromatography using DOWEX 50X8-200 resin to provide the amino acid \((3R, 5S)-10c\), in its zwitterionic form in a quantitative yield. The synthesis of \((3R, 5S)-5\)-benzyl-3-methylpyrrolidine-3-carboxylic acid was achieved in five steps in good overall yield. Avoiding N-benzyl protection of the lactam \((3R, 5S)-7c\), allowed for the elimination of two steps in the proposed synthetic strategy while providing reasonable yields during the process.

2.3.4 Synthesis of \((3S, 5S)-5\)-benzyl-3-methylpyrrolidine-3-carboxylic acid

After investigating the catalytic activity of the designed \(\beta\)-proline analogue, \((3R, 5S)-10c\) in Aldol and Mannich reactions we had decided to prepare the \(\beta\)-proline analogue with alternate stereochemical configuration at the quaternary chiral center. For this purpose we chose to perform a preliminary experiment where we took the diethyl methyl malonate diester substrate and treated with hydrazine hydrate in presence of methanol. Hydrazine cleaved off the phthalimide liberating the free amine. The free amine, under basic conditions went on to react with the carbonyls of both ethyl esters causing cyclization. In this reaction we had expected to see a 1:1 mixture of diastereomers. Surprisingly the reaction yielded mixture of diastereomers in a ratio of 1.3:1 with \((3R, 5S)-7c\) being the major diastereomer. (Scheme 2.9)
We have then tried resolving the diastereomers by flash column chromatography to isolate (3S, 5S)-7c so that we could proceed with the synthesis to the desired amino acid. Upon trying several solvent conditions we were able to find a solvent system that provided a reasonable resolution of the diastereomers. After flash column purification of the crude reaction mixture, the desired lactam (3S, 5S)-7c was isolated in 29% over all yield from the malonate diester substrate 4c. The product lactam (3S, 5S)-7c was identified from ¹H-NMR chemical shifts as it has different chemical shifts from its diastereomer (3R, 5S)-7c. The diastereopure lactam (3S, 5S)-7c was also identified and the stereochemistry at the quaternary chiral center on the lactam was confirmed by single X-ray crystal data analysis. X-ray quality crystals of the lactam were developed by solvent diffusion technique using ether/methylene chloride mixture.
X-ray data showed that the carbonyl of the ethyl ester and the benzyl group on the pyrrolidine core are in two different planes (Figure 2.3).

Figure 2.4 Single X-ray crystal structure of (3S, 5S)-ethyl 5-benzyl-3-methyl-2-oxopyrrolidine-3-carboxylate, (3S, 5S)-7c

The method to prepare the lactam (3S, 5S)-7c from malonate diester 4c (Scheme 2.9) provided the desired diastereomer in a decent yield of 29%, considering the fact that this method involves separation of diastereomers. We then decided to perform PLE isoenzyme studies to see if any of the six different isoenzymes would provide the desired diastereomeric half ester (2S, 4S)-5c in an asymmetric fashion. To our surprise, PLE Isoenzymes 3 and 4 provided the desired half ester in a decent diastereomeric excess. The half ester from PLE 3 hydrolysis was taken to the next steps of stereoselective cyclization methodology and then transformed into (3S, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid, (3S, 5S)-10c as shown in Scheme 2.10. The half ester (2S, 4S)-5c (d.r =
5.7:1] from PLE Isoenzyme 3 hydrolysis was converted to 4-nitro benzylic ester
(2R, 4S)-6c (d.r = 5.7:1) in 91% yield. At this point attempts were made to
improve the diastereomeric ratio and none of them were successful. The crude
product looked essentially pure and did not require any further purification.

Scheme 2.10 Synthesis of (3S, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic
acid

4-Niro benzylic ester compound, (2R, 4S)-6c, was subjected to phthalimide
cleavage by treatment with hydrazine hydrate to produce free amine. The free
amine then went to cyclize on to the carbonyl containing 4-nitro benzylic group
predominantly through a stereoselective cyclization reaction mechanism. The
crude material was then purified carefully via flash column chromatography to
further improve the diastereomeric ratio of the cyclized product (3S, 5S)-7c to
>24:1. The lactam (3S, 5S)-7c was reacted with Lawesson’s reagent to provide
thiolactam (3R, 5S)-8c in 99% yield. The thiolactam obtained was then reduced
with Raney-Ni under H2 atmosphere to provide corresponding amine (3S, 5S)-9c
in 59% yield. The amino ester (3S, 5S)-9c was refluxed in 6.0N HCl to provide
the amino acid (3S, 5S)-10c as a hydrochloride salt. The amino acid HCl salt was then subjected to ion exchange chromatography using DOWEX 50X8-200 resin to provide the amino acid in its zwitterionic form as a white solid in quantitative yield.

2.4 Experimental Methods

All anhydrous solvents were obtained by passage through a column of activated silica. Flash column chromatography was performed using SiliaFlash® P60 40-63µm (230-400 mesh) silica gel. NMR experiments were performed on a Bruker 400 MHz NMR instrument and chemical shifts were reported with reference to either TMS (for CDCl₃) or residual solvent proton peak (for CD₃OD). Infrared analysis was performed on a Thermo Nicolet nexus 470 FT-IR instrument. Melting point analysis was performed using a Thomas-Hoover “Uni-Melt” Melting Point Apparatus. High Resolution mass spectra were obtained using positive electrospray ionization on a Bruker 12 Tesla APEX -Qe FTICR-MS with an Apollo II ion source. Triflic anhydride was freshly distilled on phosphorous pentoxide under Nitrogen atmosphere before use according to the reported procedure.⁹⁰

1a, (S)-2-aminopropan-1-ol:

6.291g (165.8 mmol) of Lithium Aluminum Hydride was suspended in 125 mL of anhydrous THF in a 500 mL three neck flask at 0 °C and under N₂ atmosphere. To the above flask, 10.0g (112.0 mmol) of L-Alanine was added in portions over a period of 10 minutes. Reaction appeared to be highly exothermic and once the bubbling subsided, reaction was brought to room temperature. The
reaction mixture was heated to reflux the solvent overnight for 16 hrs. Then the reaction was cooled to room temperature and 100 mL diethyl ether was added. To this reaction mixture was added 4.7 mL water, 4.7 mL methanol followed by 14 mL water in the same order. Contents were transferred to a 500 mL beaker and the reaction flask was rinsed with methanol (25 mL). The contents in the beaker were stirred for 30 minutes. Then the reaction mixture was filtered through celite and the resulted white cake was washed with diethyl ether (6 x 30 mL). All the organic portions were dried over sodium sulfate, filtered and the solvent was removed under vacuum to provide 2.3 g (30.62 mmol) of product as yellow liquid in 27% yield. The isolation of product was very difficult from the aqueous portion of the reaction mixture. The crude product appeared to be essentially pure and the $^1$H-NMR data obtained corresponded to that of the product.$^{91}$$^1$H NMR (400 MHz, MeOD) $\delta$ 3.46 (dd, $J = 10.7$, 4.7 Hz, 1H), 3.29 (dd, $J = 10.7$, 7.3 Hz, 1H), 2.91 (dqd, $J = 13.1$, 6.5, 4.8 Hz, 1H), 1.07 – 1.02 (m, 3H).

1b, (S)-2-amino-4-methylpentan-1-ol:

Reduction of L-Leucine was performed using sodium borohydride and iodine and the reaction was worked up according to the procedure reported for 1c. Materials used for this reaction are L-leucine (3.0 g, 22.87 mmol), sodium borohydride (2.16g, 57.175 mmol), iodine (5.96g, 23.5 mmol) and anhydrous THF (40 mL). 2.43 g (20.75 mmol) of crude product was isolated after the work up as a clear viscous liquid in 91% yield. $^1$H-NMR data of this crude material suggested that compound is pure.$^{93}$$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 3.70 – 3.59 (m,
1H), 3.33 (dd, J = 10.9, 8.0 Hz, 1H), 3.02 (qd, J = 7.1, 3.7 Hz, 1H), 2.96 – 2.85 (m, 4H), 1.78 – 1.63 (m, 1H), 1.26 (t, J = 7.1 Hz, 2H), 0.98 – 0.86 (m, 7H).

**1c, (S)-2-amino-3-phenylpropan-1-ol:**

22.10 g (0.5842 mol) of sodium borohydride was suspended in 800 mL of anhydrous THF in a 2.0 L three neck flask at 0 °C under N₂ atmosphere. The reaction flask was connected to an overhead stirrer and also an addition funnel. 40.00 g (0.2421 mol) of L-phenylalanine was added to the above flask in portions over a 10 minute period. Then 61.96 g (0.2441 mol) of iodine was dissolved in 150 mL anhydrous THF and added to the above reaction flask at 0 °C via an addition funnel over a period of 3 hrs. Reaction mixture was stirred at room temperature for 45 minutes and then heated to reflux the solvent over the night. After refluxing for 18 hrs the reaction mixture was cooled to room temperature and methanol (approximately 50 mL) was added slowly to the flask under ice until the reaction mixture became clear. Reaction mixture was allowed to stir for 30 minutes at room temperature. Then the solvent was removed under vacuum to provide a white slurry. This slurry was dissolved in 200 mL of 20% KOH and the resulting solution was stirred for 4.5 hrs at room temperature under N₂ atmosphere. Then the organic contents were extracted with dichloromethane (4 x 200 mL). All organic portions were combined, dried over magnesium sulfate, filtered and the solvent was removed under vacuum to provide crude product. 22.365g (0.148 mol) of the pure product was recrystallized from toluene as a white solid in 61% yield. \(^1\)HNMR data of the product matches to that of the product.\(^{92}\) \(^1\)H NMR (400 MHz, CDCl₃) δ 7.35 – 7.27 (m, 2H), 7.26 – 7.16 (m, 3H),
3.64 (dd, J = 10.5, 3.9 Hz, 1H), 3.38 (dd, J = 10.5, 7.2 Hz, 1H), 3.20 – 3.05 (m, 1H), 2.80 (dd, J = 13.5, 5.3 Hz, 1H), 2.53 (dd, J = 13.5, 8.6 Hz, 1H), 1.65 (s, 1H).

**2a, (S)-2-(1-hydroxypropan-2-yl)isoindoline-1,3-dione:**

1.0 g (13.31 mmol) of 2a was placed in a single neck round bottom flask. 15 mL of freshly distilled toluene was added to this flask and contents were stirred for 5 minutes. 0.186 mL (1.331 mmol) of triethylamine was added and contents were continued to stir for another 10 minutes under N$_2$ atmosphere at room temperature. Then 1.971 g (13.31 mmol) of phthalic anhydride was added to the above flask. The reaction flask was connected to a Dean-Stark apparatus and the reaction flask was heated to 135 °C using an oil bath set up for 3 hrs. Then the reaction mixture was cooled and 20 mL of ethyl acetate was added to it. At this point the reaction mixture was dark brown colored. The organic portion was washed with 5% sodium bicarbonate (3 x 20 mL), 1.0M HCl (2 x 15 mL), Water (2 x 15 mL) and saturated solution of sodium chloride (2 x 20 mL) in the same order. The organic portion was dried over magnesium sulfate, filtered and the solvent was removed under vacuum to provide crude material as a light yellow solid. The crude material was purified by flash column chromatography (30% ethyl acetate/70% chloroform, R$_f$ = 0.37) to provide 1.58g (7.70 mmol) of pure product in 58% yield. $^1$H and $^{13}$C NMR data corresponded to that of the product.$^{94}$ $^1$H NMR (400 MHz, CDCl$_3$) δ 7.91 – 7.64 (m, 4H), 4.51 (pd, J = 7.2, 3.7 Hz, 1H), 4.03 (ddd, J = 11.8, 8.8, 7.5 Hz, 1H), 3.89 (dt, J = 11.8, 3.8 Hz, 1H), 2.72 (dd, J = 8.8, 3.9 Hz, 1H), 1.45 (d, J = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 168.96, 134.10, 131.93, 123.32, 64.29, 49.38, 14.79.
2b, (S)-2-(1-hydroxy-4-methylpentan-2-yl) isoindoline-1,3-dione:

Reaction was set up and worked up according to the procedure reported for 2a. Materials used for this reaction are 1b (2.43 g, 20.74 mmol), phthalic anhydride (3.07 g, 20.74 mmol), triethylamine (0.3 mL) and freshly distilled toluene (30 mL). 4.04 g (79%) of crude product isolated was analyzed by 1H NMR and determined to be pure enough to proceed to the next step. 1H NMR (400 MHz, CDCl₃) δ 7.87 – 7.79 (m, 2H), 7.76 – 7.68 (m, 2H), 4.53 – 4.41 (m, 1H), 4.04 (dt, J = 11.6, 7.5 Hz, 1H), 3.86 (dd, J = 11.6, 3.1 Hz, 1H), 2.58 (d, J = 4.8 Hz, 1H), 2.06 – 1.91 (m, 1H), 1.59 – 1.46 (m, 2H), 0.98 – 0.90 (m, 6H).

2c, (S)-2-(1-hydroxy-3-phenylpropan-2-yl)isoindoline-1,3-dione:

Reaction was set up and worked up according to the procedure reported for 2a. Materials used for this reaction are 1a (4.0 g, 26.45 mmol), phthalic anhydride (3.90 g, 26.33 mmol), triethylamine (0.37 mL) and freshly distilled toluene (50 mL). This reaction afforded 6.15g (21.86 mmol) of product which is essentially pure in 83% yield. NMR analysis was performed and the data corresponds to that of product. 1H NMR (400 MHz, CDCl₃) δ 7.83 – 7.65 (m, 4H), 7.24 – 7.11 (m, 5H), 4.64 (ddd, J = 15.1, 8.0, 3.5 Hz, 1H), 4.14 – 4.00 (m, 1H), 3.94 (dd, J = 11.9, 3.4 Hz, 1H), 3.22 (t, J = 7.0 Hz, 2H), 2.77 (s, 1H). 13C NMR (101 MHz, CDCl₃) δ 168.95, 137.41, 134.10, 131.66, 129.08, 128.54, 126.69, 123.34, 62.90, 55.24, 34.82.

3a, (S)-2-(1,3-dioxoisooindolin-2-yl)propyl trifluoromethanesulfonate:

0.760 g (3.70 mmol) of 2a, was placed in a 100 mL three neck flask under N₂ atmosphere along with 20 mL of anhydrous methylene chloride. The reaction
flask was cooled to -25 °C and 0.592 mL (4.25 mmol) of triethylamine was added to it. The contents of the flask were allowed to stir for 10 minutes. Then 0.72 mL (1.198 g, 4.25 mmol) of freshly distilled triflic anhydride was added as a solution in 8 mL anhydrous methylene chloride to the above reaction flask under N₂ atmosphere at -25 °C. Reaction was continued to stir under these conditions for one hour. Then solvent was removed under vacuum to provide crude material as orange colored syrup. The crude reaction mixture was purified by a silica gel plug chromatography using methylene chloride/hexanes (50/50) as solvent to provide 0.440 g of pure product in 35% yield. ¹H NMR was obtained on the pure product and it matches to that of the product. The product was very unstable and started to decompose at room temperature. Hence it was taken to the next step immediately on the same day. ¹H NMR (300 MHz, CDCl₃) δ 7.94 – 7.70 (m, 4H), 5.09 (t, J = 9.7 Hz, 1H), 4.87 – 4.72 (m, 1H), 4.67 (dd, J = 10.1, 5.1 Hz, 1H), 1.54 (d, J = 7.1 Hz, 3H).

3b, (S)-2-(1,3-dioxoisindolin-2-yl)-4-methylpentyl trifluoromethanesulfonate:

4.776 g (19.31 mmol) of 2b was dissolved in 25 mL of anhydrous methylene chloride in a 250 mL three neck round bottom flask under N₂ at 0 °C. 2.7 mL of triethylamine was added to the reaction flask and reaction was continued to stir for 15 minutes. Freshly distilled triflic anhydride (5.994 g, 21.245 mmol) was added to the above reaction flask, slowly in a dropwise fashion, as a solution in 30 mL anhydrous methylene chloride via an addition funnel at 0 °C and under N₂ atmosphere. After the addition was complete, the reaction was continued to stir for one hour at 0 °C under inert atmosphere. The reaction
mixture was then passed through a silica plug under vacuum and the silica bed was rinsed with additional anhydrous methylene chloride. The solvent was then removed under vacuum to provide 6.64g (17.5 mmol) of product as clear liquid, that is essentially pure as determined from $^1$H NMR. (%yield = 91%) The compound 3b appeared to be unstable as it started to decompose upon standing at room temperature. Hence it was taken on to the next step immediately on the same day to avoid further decomposition.$^1$H NMR (400 MHz, CDCl$_3$) δ 7.93 – 7.82 (m, 2H), 7.81 – 7.73 (m, 2H), 5.07 (t, $J = 10.0$ Hz, 1H), 4.78 – 4.69 (m, 1H), 4.64 (dd, $J = 10.3$, 4.6 Hz, 1H), 2.13 (ddd, $J = 13.3$, 10.7, 4.1 Hz, 1H), 1.57 – 1.40 (m, 2H), 0.95 (dd, $J = 8.8$, 6.3 Hz, 6H).

3c, (S)-2-(1,3-dioxoisoinolin-2-yl)-3-phenylpropyl trifluoromethanesulfonate: 

Reaction was set up and worked up according to the procedure reported for 3b. The materials used in this reaction were alcohol 2c (20.0 g, 71.09 mmol), freshly distilled triflic anhydride (22.065g, 78.20 mmol), triethylamine (9.92 mL, 71.09 mmol) and anhydrous methylene chloride (330 mL). After purification by silica gel plug chromatography 24.65 g (59.64 mmol) of essentially pure product was obtained in 84% yield. $^1$H NMR data was obtained on the product and it matched to that of the product.$^9$ $^1$H NMR (400 MHz, CDCl$_3$) δ 7.84 – 7.66 (m, 4H), 7.28 – 7.13 (m, 5H), 5.17 (t, $J = 9.9$ Hz, 1H), 4.93 (tdd, $J = 9.5$, 6.9, 4.6 Hz, 1H), 4.71 (dd, $J = 10.4$, 4.6 Hz, 1H), 3.25 (ddd, $J = 20.8$, 13.9, 8.2 Hz, 2H).

4a, (S)-diethyl 2-(2-(1,3-dioxoisoinolin-2-yl) propyl)-2-methylmalonate: 

0.144 g (6.02 mmol) of NaH (60% by weight in mineral oil) was placed in a 50 mL, three neck round bottom flask at 0 °C under N$_2$ atmosphere. NaH was
washed with pentane (2 X 2 mL) to remove mineral oil and pentane layers were
decanted. 4 mL anhydrous THF was added to the reaction flask and contents
were stirred for 5 minutes. 0.523 g (3.01 mmol) of diethyl methylmalonate was
added to the above reaction flask slowly in a dropwise manner using a gas tight
syringe as a solution in 3 mL anhydrous THF. The reaction contents were stirred
for 10 minutes at 0 °C under N₂ atmosphere and then brought to room
temperature. Reaction mixture was continued to stir for 30 minutes at room
temperature under N₂ atmosphere. The above enolate, under N₂ atmosphere,
was carefully dripped into a 50 mL single neck round bottom flask containing
0.460 g (1.36 mmol) of 3a dissolved in 7mL of anhydrous THF at 0 °C using a
Teflon canula. The drip rate was controlled by the Nitrogen flow to the reaction
flask. The reaction mixture was allowed to stir at 0 °C for one hour, then brought
to room temperature and left to stir over night at room temperature under N₂
atmosphere. After stirring overnight, solvent was removed on the rotovapor and
20 mL water was added to the crude reaction mixture. Organic contents were
extracted with diethyl ether (3 x 20 mL). All the ether portions were combined and
washed with water (3 x 25 mL) followed by brine (1 x 25 mL). Ether portion was
then dried over MgSO₄ and solvent was removed under vacuum to afford crude
product which was later purified by gradient flash column chromatography (10%
ethyl acetate/90% hexanes to 15% ethyl acetate/85% hexanes) to provide 0.180
g (0.500 mmol) of pure 4a as light yellow liquid in 37% yield. IR (cm⁻¹): 2927,
1727, 1707. [α]D²⁷ = -29.0 (c = 0.93, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.89 –
7.61 (m, 4H), 4.63 – 4.46 (m, 1H), 4.24 – 4.04 (m, 2H), 3.92 – 3.61 (m, 2H), 3.00
(dd, J = 15.0, 10.3 Hz, 1H), 2.21 (dd, J = 15.0, 3.5 Hz, 1H), 1.50 (d, J = 7.0 Hz, 3H), 1.47 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H), 1.10 (t, J = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 172.16, 171.55, 168.01, 133.91, 131.97, 123.04, 61.47, 61.26, 52.62, 43.18, 38.34, 20.14, 19.57, 13.95, 13.72. HRMS (C$_{19}$H$_{23}$NO$_6$Na$^+$) calcd = 384.141759 m/z, obsd = 384.141321 m/z.

4b, (S)-diethyl 2-(2-(1, 3-dioxoisindolin-2-yl)-4-methylpentyl)-2-methylmalonate:

Reaction was set up and worked up according to the procedure reported for 4a. Materials used in this reaction are triflate 3b (6.64g, 17.5 mmol), diethyl methyl malonate (3.961 g, 22.75 mmol), sodium hydride (0.655g, 27.3 mmol) and anhydrous THF (60 mL). 3.10 g (7.68 mmol) of pure diester was recrystallized from cold diethyl ether as a clear crystalline solid in 44% yield. M.P. = 62-65 °C. [α]$_D$ = -38.8 (c = 1.0, CHCl$_3$). IR (cm$^{-1}$): 2956, 1770, 1705. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.95 – 7.61 (m, 4H), 4.48 (tdd, J = 10.5, 4.7, 3.0 Hz, 1H), 4.13 (qq, J = 10.8, 7.1 Hz, 2H), 3.74 (ddq, J = 66.3, 10.8, 7.1 Hz, 2H), 2.97 (dd, J = 15.1, 10.5 Hz, 1H), 2.25 – 2.09 (m, 2H), 1.48 (d, J = 6.6 Hz, 3H), 1.46 – 1.33 (m, 2H), 1.21 (t, J = 7.1 Hz, 3H), 1.09 (t, J = 7.1 Hz, 3H), 0.89 (dd, J = 15.4, 6.3 Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 172.19, 171.60, 168.33, 133.90, 132.17, 131.55, 123.41, 122.72, 61.45, 61.22, 52.63, 45.85, 42.50, 37.54, 25.06, 23.11, 21.79, 19.63, 13.96, 13.70. HRMS (C$_{22}$H$_{29}$NO$_6$Na$^+$) calcd = 426.188709 m/z, obsd = 426.188385 m/z.

4c, (S)-diethyl 2-(2-(1, 3-dioxoisindolin-2-yl)-3-phenylpropyl)-2-methylmalonate:

Reaction was set up and worked up according to the procedure reported for 4a. Crude product was purified by flash column chromatography (20% ethyl acetate /
80% hexanes, $R_f = 0.18$). Pure product was analyzed by $^1$H NMR and the data matches to that of the product reported in literature. $^6$ $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.84 – 7.54 (m, 4H), 7.21 – 7.02 (m, 5H), 4.73 – 4.57 (m, 1H), 4.22 – 4.00 (m, 2H), 3.89 – 3.58 (m, 2H), 3.42 – 3.29 (m, 1H), 3.15 – 2.99 (m, 2H), 2.32 (dd, $J = 15.1, 2.9$ Hz, 1H), 1.44 (d, $J = 7.8$ Hz, 3H), 1.23 – 1.18 (m, 3H), 1.09 (t, $J = 7.1$ Hz, 3H). HRMS (C$_{25}$H$_{27}$NO$_6$Na$^+$) calcd = 460.173059 m/z, obsd = 460.172227 m/z

(2$R$, 4$S$)-5a, (2$R$, 4$S$)-4-(1,3-dioxoisindolin-2-yl)-2-(ethoxycarbonyl)-2-methylpentanoic acid:

120 mg (0.332 mmol) of 4a, dissolved in 1.5 mL ethanol, was placed in a 100 mL beaker containing 60 mL phosphate buffer of pH 7.4. Then, 0.120g (18 units/mg) of crude Porcine Liver Esterase (PLE) enzyme was added to the above reaction beaker and the reaction was continued to stir vigorously. The pH of the reaction was maintained at 7.4 using an auto titrator that was set to deliver 1.0 M NaOH as the reaction progressed. After 16 hrs, the reaction went to completion. The pH of the reaction was brought up to 9.0 and a quick extraction was performed with ethyl acetate (1 x 30 mL). The aqueous portion was filtered through a celite bed under vacuum to remove the enzyme and then acidified to pH 2.0 using 12M HCl. The organics were extracted with diethyl ether (6 x 30 mL). The resulting organic layers were combined and washed with brine (1 x 100 mL), dried over MgSO$_4$, filtered and solvent was removed under vacuum to provide 0.083g (0.249 mmol) of 5a in 75% yield and 78% de. IR (cm$^{-1}$): 2983, 1701. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.11 (dd, $J = 85.0, 80.6$ Hz, 1H), 7.83 – 7.64
(m, 4H), 4.53 (dq, \( J = 10.3, 6.9, 3.4 \text{ Hz}, 1\text{H} \)), 4.17 – 3.97 (m, 2H), 2.91 (dd, \( J = 15.0, 9.9 \text{ Hz}, 1\text{H} \)), 2.25 (dd, \( J = 15.0, 3.4 \text{ Hz}, 1\text{H} \)), 1.50 (t, \( J = 5.6 \text{ Hz}, 3\text{H} \)), 1.45 (s, 3H), 1.22 (t, \( J = 7.1 \text{ Hz}, 3\text{H} \)). \(^{13}\text{C NMR (101 MHz, CDCl}_3\) \( \delta 176.41, 172.38, 168.16, 134.01, 131.80, 123.21, 62.01, 52.55, 43.49, 38.98, 20.41, 20.09, 13.82.\)

HRMS (C\(_{17}\)H\(_{19}\)NO\(_6\)Na\(^+\)) calcd = 356.110458 m/z, obsd = 356.109945 m/z.

(2R, 4S)-5b, (2R, 4S)-4-(1, 3-dioxoisooindolin-2-yl)-2-(ethoxycarbonyl)-2, 6-dimethylheptanoic acid:

Reaction was set up according to the procedure reported for 5a. 0.3g (0.744 mmol) of 4b in 2.0 mL ethanol, 80 mL of phosphate buffer, 19mg of Crude PLE(18 units/mg) were used in this reaction. Reaction was stopped after 5 days and worked up. During the work up 60% of the unreacted starting material 4b, was recovered from the reaction. The work up procedure is similar to that reported for 5a, except for extractions were performed using dichloromethane instead of diethyl ether. After the work up 40 mg (0.106 mmol) of (2R, 4S)-5b was obtained as a clear liquid in 14% yield. The diastereomeric ratio was determined to be 6:1 from \(^1\text{H NMR of the crude product. Flash column purification of the crude product did not improve the diastereomeric ratio as the mixture of diastereomers seemed to be inseparable. IR (cm}^{-1}\): 2958, 1770, 1704.\(^1\text{H NMR (400 MHz, CDCl}_3\) \( \delta 8.50 (s, 1\text{H} \)), 7.89 – 7.65 (m, 4\text{H} \)), 4.45 (ddd, \( J = 10.2, 6.9, 4.0 \text{ Hz}, 1\text{H} \)), 4.21 – 4.02 (m, 2\text{H} \)), 2.85 (dd, \( J = 15.0, 10.1 \text{ Hz}, 1\text{H} \)), 2.24 – 2.12 (m, 2\text{H} \)), 2.02 (dd, \( J = 15.0, 2.6 \text{ Hz}, 1\text{H} \)), 1.50 – 1.29 (m, 5\text{H} \)), 1.23 (t, \( J = 7.1 \text{ Hz}, 3\text{H} \)), 0.92 (ddd, \( J = 19.0, 8.6, 4.5 \text{ Hz}, 6\text{H} \)). \(^{13}\text{C NMR (101 MHz, CDCl}_3\) \( \delta 176.57, 172.58, 168.37, 134.16, 123.58, 123.15, 62.15, 52.60, 46.24, 60\).
42.53, 38.32, 25.15, 23.21, 21.85, 20.59, 13.94. HRMS (C_{20}H_{25}NO_{6}S Na^+) calcd = 398.157409 m/z, obsd = 398.157033 m/z.

(2R, 4S)-5c, (2R, 4S)-4-(1,3-dioxoisindolin-2-yl)-2-(ethoxycarbonyl)-2-methyl-5-phenylpentanoic acid:

Reaction was set up according to the procedure reported for 5a. 2.38g (5.45 mmol) of 4c in 6.25 mL ethanol and 2.2 mL CH_2Cl_2, 280 mL of phosphate buffer, 120 mg of Crude PLE (18 units/mg) were used in this reaction. Reaction was stopped after 7 days and worked up. The work up procedure is similar to that reported for 5a, except for extractions were performed using dichloromethane instead of diethyl ether. After the work up 1.442 g (3.52 mmol) of (2R, 4S)-5c was obtained as a clear hygroscopic liquid in 65% yield. The diastereomeric ratio was determined to be 4.1:1 from ^1H NMR of the crude product. Flash column purification of the crude product did not improve the diastereomeric ratio, as the mixture of diastereomers seemed to be inseparable.

IR (cm⁻¹): 2983, 1772, 1704. ^1H NMR (400 MHz, CDCl_3) δ 7.85 – 7.51 (m, 4H), 7.23 – 7.00 (m, 5H), 4.68 – 4.53 (m, 1H), 4.15 – 3.95 (m, 2H), 3.33 (dt, J = 9.2, 8.1 Hz, 1H), 3.14 – 2.92 (m, 2H), 2.34 (dd, J = 15.0, 2.7 Hz, 1H), 1.45 – 1.40 (m, 3H), 1.23 – 1.18 (m, 3H). HRMS (C_{23}H_{23}NO_{6}Na^+) calcd = 432.141759 m/z, obsd = 432.141484 m/z.

(2S, 4S)-6b, (S)-1-ethyl 3-(4-nitrobenzyl) 2-((S)-2-(1, 3-dioxoisindolin-2-yl)-4-methylpentyl)-2-methylmalonate:

0.160g (0.426 mmol) of (2R, 4S)-5b (d.r = 6:1) was dissolved in 4 mL anhydrous DMF in a 100 mL, three neck flask at room temperature and under N₂
atmosphere. 0.059g (0.426 mmol) of K$_2$CO$_3$ was added to the above flask and the contents were stirred for 10 minutes. Then, 0.092g (0.426 mmol) of 4-nitrobenzyl bromide was added in a dropwise manner as a solution in 2 mL anhydrous DMF to the above reaction flask. Then the reaction mixture was allowed to stir overnight at room temperature and under N$_2$ atmosphere. 6 mL water was added to the reaction flask and the organic contents were extracted with diethyl ether (4 x 6 mL). All organic portions were combined and washed with water (7 x 6 mL) followed by brine (3 x 6 mL). The organic portion was dried over MgSO$_4$ and the solvent was removed under vacuum to provide crude product. Crude compound was purified by flash column chromatography (10% ethyl acetate / 90% hexanes) to provide 0.129 g (0.253 mmol) of pure (2$S$, 4$S$)-6b in 59% Yield. ([R] = 0.38, 30% ethyl acetate / 70% hexanes) Our attempts to resolve diastereomers, to improve diastereomeric ratio, via flash column purification were not successful at this point. IR (cm$^{-1}$): 2957, 1729, 1705. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.21 – 8.11 (m, 2H), 7.87 – 7.66 (m, 4H), 7.39 (dd, $J$ = 9.0, 2.1 Hz, 2H), 4.83 (dd, $J$ = 74.9, 13.5 Hz, 2H), 4.58 – 4.42 (m, 1H), 4.07 (q, $J$ = 7.1 Hz, 2H), 3.10 – 2.97 (m, 1H), 2.28 – 2.09 (m, 2H), 1.53 (d, $J$ = 6.0 Hz, 3H), 1.50 – 1.34 (m, 2H), 1.12 (t, $J$ = 7.1 Hz, 3H), 0.89 (dt, $J$ = 12.8, 6.4 Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 171.72, 171.26, 168.39, 167.28, 147.69, 142.48, 134.04, 128.23, 123.66, 65.40, 61.74, 52.86, 45.80, 42.52, 37.68, 25.04, 23.09, 21.75, 19.70, 13.90. HRMS (C$_{27}$H$_{30}$N$_2$O$_8$ Na$^+$) calcd = 533.189437 m/z, obsd = 533.189437 m/z.
(2S, 4S)-6c, (S)-1-ethyl 3-(4-nitrobenzyl) 2-((S)-2-(1, 3-dioxoisooindolin-2-yl)-3-phenylpropyl)-2-methylmalonate:

1.506g (3.68 mmol) of (2R, 4S)-5c was dissolved in 25 mL anhydrous DMF in a 100 mL, three neck flask at room temperature and under N2 atmosphere. 0.875g (4.05 mmol) of K2CO3 was added to the above flask and the contents were stirred for 10 minutes. Then, 0.560g (4.05 mmol) of 4-nitrobenzyl bromide was added in a dropwise manner as a solution in 5 mL anhydrous DMF to the above reaction flask. Then the reaction mixture was allowed to stir overnight at room temperature and under N2 atmosphere. After stirring for 18 hrs overnight, 40 mL water was added to the reaction flask and the organic contents were extracted with CH2Cl2 (4 x 50 mL). All organic portions were combined and washed with water (6 x 100 mL) followed by brine (1 x 150 mL). The organic portion was dried over MgSO4 and the solvent was removed under vacuum to provide crude product as viscous yellow liquid. Pure (2S, 4S)-6c was crystallized using ice cold diethyl ether in 67% yield. The diastereomeric ratio of the crystallized product was determined to be 24:1 from the 1H NMR data. M.P. = 125 °C. [α]D24 = -118.1 (c = 1.0, CHCl3). IR (cm⁻¹): 2940, 1726, 1707. 1H NMR (400 MHz, CDCl3) δ 8.21 – 8.12 (m, 2H), 7.78 – 7.54 (m, 4H), 7.38 (d, J = 8.8 Hz, 2H), 7.21 – 7.03 (m, 5H), 4.92 (d, J = 13.4 Hz, 1H), 4.73 (d, J = 13.4 Hz, 1H), 4.71 – 4.62 (m, 1H), 4.12 – 3.99 (m, 2H), 3.35 (dd, J = 13.7, 10.0 Hz, 1H), 3.19 – 3.06 (m, 2H), 2.35 (dd, J = 15.1, 2.9 Hz, 1H), 1.49 (d, J = 6.5 Hz, 3H), 1.10 (t, J = 7.1 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 171.60, 171.16, 147.71, 142.42, 137.31, 133.95, 128.94, 128.48, 128.24, 126.73, 123.68, 65.43, 61.78, 52.79,
49.06, 39.61, 36.82, 19.70, 13.88. HRMS (C_{36}H_{28}N_{2}O_{8} Na+) calcd = 567.173787 m/z, obsd = 567.173350 m/z.

(3R, 5S)-7b, (3R, 5S)-ethyl 5-isobutyl-3-methyl-2-oxopyrrolidine-3-carboxylate:

0.120g (0.242 mmol) of (2S, 4S)-6b (d.r = 6:1) was dissolved in a solvent mixture of 4 mL methanol and 2 mL methylene chloride in a 25 mL single neck round bottom flask. 0.1 mL of hydrazine hydrate (35% in water) was added to the reaction flask and reaction mixture was heated to reflux the solvent. Reaction was monitored by TLC and also pH of the reaction was maintained between 8.0 and 9.0 by using litmus paper. After stirring for 24 hrs and additional 0.060 mL of hydrazine hydrate was added to the reaction to maintain the basic pH. Reaction was continued to stir for another 17 hrs (total reaction time of 41 hrs) and then cooled to room temperature. The white precipitate was washed with methylene chloride (5 x 6 mL) and the contents were filtered via a micro column to remove any white precipitate. The solvent was then removed under vacuum to provide crude material. The crude product was taken up in methylene chloride and the organic portion was washed with water (1 x 20 mL) followed by brine (1 x 20 mL). Organic portion was then dried over magnesium sulfate, filtered and the solvent was removed under vacuum. The resulted crude product was carefully purified via gradient flash column chromatography (45% ethyl acetate/55% hexanes to 80% ethyl acetate/20% hexanes) to provide 14 mg (0.062 mmol) of diastereopure (3R, 5S)-7b in 26% yield. Rf = 0.11(80%EtOAc/20% Hexanes) The improvement in diastereopurity was confirmed from the 1H NMR data analysis. 2D-NOESY experiment was performed on this diastereopure lactam and the
stereochemistry at the quaternary chiral carbon was determined to be “R”. \([\alpha]_D^{24} = +6.1 \ (c = 0.3, \text{CH}_2\text{Cl}_2). IR \ (\text{cm}^{-1}): 3218, 2957, 2872, 1737, 1701. \]  

\[^1\text{H} \text{NMR} \ (400 \ \text{MHz, CDCl}_3) \ \delta \ 6.26 \ (s, 1H), \ 4.28 – 4.14 \ (m, 2H), \ 3.69 \ (p, J = 7.0 \ Hz, 1H), \ 2.33 – 2.24 \ (m, 1H), \ 2.13 \ (dd, J = 12.8, 7.0 \ Hz, 1H), \ 1.64 \ (tt, J = 13.1, 6.6 \ Hz, 1H), \ 1.58 – 1.48 \ (m, 1H), \ 1.47 – 1.44 \ (m, 3H), \ 1.39 \ (ddd, J = 11.8, 7.3, 6.5 \ Hz, 1H), \ 1.32 – 1.24 \ (m, 3H), \ 0.93 \ (dt, J = 6.7, 3.3 \ Hz, 6H). \]  

\[^{13}\text{C} \text{NMR} \ (101 \ \text{MHz, CDCl}_3) \ \delta \ 176.37, \ 172.57, \ 61.56, \ 51.48, \ 49.60, \ 45.58, \ 40.27, \ 25.38, \ 22.82, \ 22.48, \ 20.26, \ 14.08. \]  

HRMS \ (C_{12}H_{21}NO_3H^+) \ \text{calcd} \ = 228.159420 \ m/z, \ \text{obsd} \ = 228.159218 \ m/z. \]  

(3R, 5S)-7c, (3R, 5S)-ethyl 5-benzyl-3-methyl-2-oxopyrrolidine-3-carboxylate:  

0.310g (0.56 mmol) of (2S, 4S)-6c was dissolved in 8 mL methanol and 5 mL CH₂Cl₂ and placed in a 50 mL single neck round bottom flask connected to a reflux condenser. Contents were stirred for 5 minutes and then 0.150 mL of hydrazine hydrate (35% w/v) was added to the reaction. After stirring for 10 minutes at room temperature, the reaction mixture was heated to reflux the solvent for 5 days, at which time the reaction had gone to completion. Reaction was monitored by TLC. The pH of the reaction was maintained between 8.0 and 9.0 by adding additional hydrazine hydrate (0.110 mL additional volume) over the course of reaction. Reaction was cooled to room temperature and a white fluffy solid precipitated out of the reaction mixture. The white solid was filtered off and the solvent was removed under vacuum to provide crude material. Crude material was then dissolved in 25 mL of CH₂Cl₂ and washed with water (1 x 20 mL) followed by brine (1 x 20 mL). Organic portion was dried over MgSO₄, filtered and solvent was removed under vacuum. The resulting material was
purified by gradient column chromatography (20% ethyl acetate/80% hexanes to 80% ethyl acetate/20% hexanes) to provide 0.103 g (0.394 mmol) of pure (3R, 5S)-7c as light yellowish crystalline solid in 70% yield. Rf = 0.18 (80% ethyl acetate/20% hexanes), M.P. = 78-80 °C. [α]D^25 = -29.4 (c = 1.0, CHCl₃). IR (cm⁻¹): 3202, 3087, 2986, 2939, 1729, 1688. ¹H NMR (400 MHz, CDCl₃) δ 7.33 (ddd, J = 7.5, 4.4, 1.3 Hz, 2H), 7.29 – 7.23 (m, 1H), 7.22 – 7.16 (m, 2H), 5.81 (s, 1H), 4.29 – 4.20 (m, 2H), 3.89 – 3.78 (m, 1H), 2.86 (ddd, J = 21.8, 13.4, 7.1 Hz, 2H), 2.46 (dd, J = 13.2, 6.4 Hz, 1H), 2.14 (dd, J = 13.2, 7.4 Hz, 1H), 1.45 (d, J = 2.9 Hz, 3H), 1.32 (t, J = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 175.84, 172.45, 137.45, 128.97, 128.92, 127.01, 61.71, 52.87, 51.49, 42.78, 39.40, 20.52, 14.13. HRMS (C₁₅H₁₉NO₃ Na⁺) calcd = 284.125715 m/z, obsd = 284.125416 m/z.

(3S, 5S)-8c, (3S, 5S)-ethyl 5-benzyl-3-methyl-2-thioxopyrrolidine-3-carboxylate:

0.906g (3.46 mmol) of (3R, 5S)-7c was placed in a 100 mL single neck round bottom flask and dissolved in 20 mL of freshly distilled toluene. Then 0.716g (1.77 mmol) of Lawesson’s reagent was added to the above flask and the reaction mixture was heated to 105 °C for 2 hrs under N₂ atmosphere using oil bath set up. Reaction mixture was cooled to room temperature and solvent was removed under vacuum. Diethyl ether (25 mL) was added to the above crude material and the organics were washed with water (2 x 15 mL) followed by brine (1 x 20 mL). Ether portion was dried over MgSO₄, filtered and solvent was removed under vacuum to afford crude product as viscous yellow liquid. Purification by gradient flash column chromatography (25% ethyl acetate/75% hexanes, Rf = 0.36) yielded 0.830g (2.996 mmol) of pure (3S, 5S)-8c as a clear
viscous liquid in 87% yield. $\alpha_\text{D}^{22} = -114.3$ ($c = 1.0$, CHCl$_3$). IR (cm$^{-1}$): 3155, 2979, 2932, 1732. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.39 (s, 1H), 7.42 – 7.13 (m, 5H), 4.24 (q, $J = 7.1$ Hz, 2H), 4.13 (p, $J = 7.3$ Hz, 1H), 3.05 – 2.90 (m, 2H), 2.54 (dd, $J = 13.0$, 7.3 Hz, 1H), 2.21 (dd, $J = 13.0$, 7.4 Hz, 1H), 1.51 (s, 3H), 1.31 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 206.08, 171.97, 136.69, 129.01, 128.96, 127.18, 61.83, 61.42, 60.90, 41.32, 40.64, 23.25, 14.05. HRMS (C$_{15}$H$_{19}$NO$_2$S Na$^+$) calcd = 300.102871 m/z, obsd = 300.103024 m/z.

(3R, 5S)-9c, (3R, 5S)-ethyl 5-benzyl-3-methylpyrrolidine-3-carboxylate:

To a 50 mL three neck flask, 2.5 mL Raney Nickel slurry in water was added. Raney Nickel was washed with water (3 x 5 mL), methanol (3 x 5 mL) and THF (3 x 5 mL) in the same order. 6 mL THF was added to the above flask and hydrogen gas bubbled through the reaction for 30 minutes. Then 0.102g (0.368 mmol) of (3S, 5S)-8c was added as solution in 2mL THF and the reaction was allowed to stir, under H$_2$ atmosphere for 24 hrs at which time it went to completion. Raney-Ni was filtered off on the celite bed and solvent was removed under vacuum. Crude material was then purified by gradient flash column chromatography using 40% hexanes/60%ethyl acetate to 100% ethyl Acetate, $R_f = 0.16$ (40% hexanes/60% ethyl acetate treated with NH$_4$OH) (Solvent was treated with 30% NH$_4$OH in water and decanted prior to use. 150 mL of NH$_4$OH mixed with 500 mL of 40% hexanes / 60% ethyl acetate) to afford 0.048g (0.194 mmol) of pure (3R, 5S)-9c as light yellow liquid in 53% yield. $\alpha_\text{D}^{22} = +60.1$ ($c = 1.0$, CHCl$_3$). IR (cm$^{-1}$): 3027, 2975, 2933, 2872, 1721. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.33 – 7.16 (m, 5H), 4.18 – 4.08 (m, 2H), 3.45 (d, $J = 11.6$ Hz, 1H), 3.42 – 3.32
(m, 1H), 2.88 (dd, J = 13.4, 6.6 Hz, 1H), 2.74 (dt, J = 13.4, 4.9 Hz, 1H), 2.62 (d, J = 11.6 Hz, 1H), 2.03 – 1.88 (m, 2H), 1.71 (dd, J = 12.9, 6.8 Hz, 1H), 1.29 – 1.20 (m, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 177.79, 139.50, 129.00, 128.41, 126.21, 60.91, 60.68, 58.48, 49.93, 44.21, 41.71, 23.67, 14.19. HRMS (C$_{15}$H$_{21}$NO$_2$ H$^+$) calcd = 248.164505 m/z, obsd = 248.164259 m/z.

(3R, 5S)-10c, (3R, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid:

To a one neck 100 mL round bottom flask containing 0.130g (0.53 mmol) of (3R, 5S)-9c, 24 mL of 6.0 N HCl was added and the solvent was heated to reflux. Reaction went to completion after refluxing for 24 hrs. At this point reaction mixture was cooled and solvent was removed under high vacuum to afford 0.136g (0.53 mmol) of (3R, 5S)-10c hydrochloride salt as white solid in quantitative yield. The crude product was then loaded onto DOWEX 50X8-200 resin, to remove chloride salts, and the product was eluted using 15% NH$_4$OH in water. Fractions were collected and solvent was removed under high vacuum to provide (3R, 5S)-10c in its zwitterionic form as light brown hygroscopic solid.

M.P. (HCl salt) = 175-176 °C. [α]$_D^{23}$ (HCl salt) = -16.8 (c = 1.0, MeOH). IR (cm$^{-1}$): 2937, 1732. $^1$H NMR (400 MHz, MeOD) δ 7.32 – 7.17 (m, 5H), 3.62 – 3.44 (m, 2H), 2.96 (dd, J = 13.4, 6.7 Hz, 1H), 2.82 (dt, J = 13.4, 6.7 Hz, 1H), 2.64 (t, J = 11.0 Hz, 1H), 2.07 (dd, J = 13.0, 8.9 Hz, 1H), 1.74 (dt, J = 13.9, 7.0 Hz, 1H), 1.26 (s, 3H). $^{13}$C NMR (101 MHz, MeOD) δ 184.89, 140.04, 130.19, 129.77, 127.72, 62.24, 57.97, 52.06, 44.93, 41.69, 25.05. HRMS (C$_{13}$H$_{17}$NO$_2$Na$^+$) calcd = 242.115150 m/z, obsd = 242.114982 m/z.
(2S, 4S)-5c, (2S, 4S)-4-(1, 3-dioxoisindolin-2-yl)-2-(ethoxycarbonyl)-2-methyl-5-phenylpentanoic acid:

This reaction was performed using PLE isoenzyme 3 (250 units/mmol diester). Reaction was set up and worked up according to the procedure reported for (2R, 4S)-5c. Reaction yielded the product half ester (2S, 4S)-5c in 30% yield and up to 50% of the unreacted starting material was recovered during the workup. \(^1\)H NMR was obtained on the crude product and the diastereomeric ratio was determined to be 4:1. IR (cm\(^{-1}\)): 2985, 1772, 1708. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.04 (s, 1H), 7.82 – 7.58 (m, 5H), 7.20 – 7.11 (m, 5H), 4.71 – 4.59 (m, 1H), 3.90 – 3.61 (m, 2H), 3.40 – 3.31 (m, 1H), 3.10 (ddd, \(J = 13.7, 8.4, 2.3\) Hz, 2H), 2.25 (dd, \(J = 15.0, 2.5\) Hz, 1H), 1.47 (s, 3H), 1.10 (t, \(J = 7.1\) Hz, 3H). \(^13\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 176.94, 171.50, 168.49, 137.40, 133.94, 131.57, 128.94, 128.45, 126.68, 123.14, 61.77, 52.40, 50.44, 49.04, 45.90, 39.49, 36.74, 20.00, 13.83, 13.58. HRMS (C\(_{23}\)H\(_{23}\)NO\(_6\)Na\(^+\)) calcd = 432.141759 m/z, obsd = 432.141203 m/z.

(2R, 4S)-6c, (R)-1-ethyl 3-(4-nitrobenzyl) 2-((S)-2-(1, 3-dioxoisindolin-2-yl)-3-phenylpropyl) - 2-methylmalonate:

Reaction was set up and worked up according to the procedure reported for (2S, 4S)-6c. Materials used in this reaction are; Half ester (2S, 4S)-5c (1.146g, 2.8 mmol), 4-nitrobenzylbromide (0.605g, 2.8 mmol), potassium carbonate (0.387g, 2.8 mmol) and anhydrous DMF (30 mL). This reaction provided 1.40g (2.56 mmol) of the 4-nitrobenzylester (d.r = 4:1) in 91% yield. \(^1\)H NMR of the crude product was obtained and the data suggested that the product was essentially pure. Hence it was taken to the next step without any further

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puriﬁcation. IR (cm⁻¹): 2982, 1728, 1706. ¹H NMR (400 MHz, CDCl₃) δ 8.19 (d, J = 8.8 Hz, 2H), 7.79 – 7.60 (m, 4H), 7.45 (d, J = 8.7 Hz, 2H), 7.19 – 7.06 (m, 5H), 5.25 – 5.13 (m, 2H), 4.95 – 4.72 (m, 1H), 4.71 – 4.61 (m, 1H), 4.10 – 4.02 (m, 1H), 3.85 – 3.64 (m, 2H), 3.35 (dt, J = 13.7, 9.2 Hz, 1H), 3.16 – 3.05 (m, 2H), 2.36 (dt, J = 15.0, 2.8 Hz, 1H), 1.53 – 1.45 (m, 3H), 1.11 (t, J = 7.1 Hz, 1H), 1.03 (t, J = 7.1 Hz, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 171.60, 171.11, 167.71, 147.73, 142.65, 137.35, 133.95, 128.93, 128.45, 128.27, 128.24, 126.70, 123.76, 123.66, 65.56, 61.60, 52.77, 49.05, 39.55, 36.79, 19.75, 13.66. HRMS (C₃₀H₂₈N₂O₈Na⁺) calcd = 567.173787 m/z, obsd = 567.173239 m/z.

(3S, 5S)-7c, (3S, 5S)-ethyl 5-benzyl-3-methyl-2-oxopyrrolidine-3-carboxylate:

The title compound (3S, 5S)-7c was prepared from two different compounds for our synthetic purposes. In the method 1, the 4-nitro benzylic ester (2R, 4S)-6c was used in a stereoselective cyclization procedure. In the method 2 the malonate diester 4c was treated with hydrazine hydrate in a non-selective cyclization methodology.

**Method 1** [From (2R, 4S)-6c]: Reaction was set up and worked up according to the procedure reported for (3R, 5S)-7c. The reaction was reﬂuxed for 2 days and then worked up. Materials used in this reaction are (2R, 4S)-6c (1.273g, 2.47 mmol), N₂H₄·H₂O (1.14 mL), methanol (12 mL) and methylene chloride (8 mL). Crude product was puriﬁed carefully by flash column chromatography (40% ethyl acetate/60% hexanes, Rf = 0.3) to cause the separation of diastereomers and provide 0.200g (0.766 mmol) of diastereopure (3S, 5S)-7c in 31% yield.
**Method 2 (From 4c):** 1.775 g (4.05 mmol) of diester 4c was dissolved in 15 mL methanol and 15 mL methylene chloride mixture in a 100 mL single neck round bottom flask. To this flask was added 1.48 mL of hydrazine monohydrate (35% in water) and the reaction mixture was heated to reflux the solvent. Reaction was monitored by TLC and it went to completion after 5 days. Additional 0.6 mL of hydrazine hydrate was added after refluxing for 4 days to maintain the pH between 8.0 and 9.0. Reaction mixture was cooled to room temperature, filtered under vacuum and the solvent was removed on the rotovaporo. To the resulting organic portion, 30 mL methylene chloride was added and the contents were filtered again to remove any remaining white solid. The white solid was washed methylene chloride (3 x 20 mL) to retrieve any organic material. All the methylene chloride portions were combined and the solvent was removed under vacuum to provide crude product, a mixture of diastereomers of 7C, as viscous light yellow liquid. The crude material was then purified carefully by flash column chromatography (40% ethyl acetate/ 60% hexanes, Rf = 0.3) to provide the 0.300g (1.15 mmol) of diastereopure (3S, 5S)-7c in 29% yield. M.P = 87-89 °C. [α]D23 = -47.6 (c = 1.0, CH2Cl2). IR (cm⁻¹): 3206, 2960, 2936, 2881, 1704, 1675. ¹H NMR (400 MHz, CDCl₃) δ 7.40 – 7.10 (m, 5H), 5.77 (s, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.99 (tt, J = 8.4, 6.6 Hz, 1H), 2.90 (dd, J = 13.5, 5.3 Hz, 1H), 2.69 (ddd, J = 19.5, 13.3, 7.6 Hz, 2H), 1.70 (dt, J = 15.4, 7.7 Hz, 1H), 1.46 (s, 3H), 1.25 (t, J = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 175.20, 172.12, 137.26, 128.93, 128.90, 127.07, 61.64, 53.14, 51.92, 43.13, 40.88, 21.03, 14.08. HRMS (C₁₅H₁₉NO₃H⁺) calcd = 262.143770 m/z, obsd = 262.143493 m/z.
(3R, 5S)-8c, (3R, 5S)-ethyl 5-benzyl-3-methyl-2-thioxopyrrolidine-3-carboxylate:

0.171g (0.654 mmol) of (3S, 5S)-7c was dissolved in 8 mL of freshly distilled toluene in a single neck 25 mL round bottom flask with a stir bar. To the flask, 0.133 g (0.327 mmol) of Lawesson’s reagent was added and the reaction mixture was heated to 95 °C for 1.5 hrs under N₂ atmosphere at which time the reaction went to completion. Reaction mixture was then cooled to room temperature and solvent was removed under vacuum. Diethyl ether (15 mL) was added to the above crude material and the organics were washed with water (2 x 10mL) followed by brine (1 x 15 mL). Ether portion was dried over MgSO₄, filtered and solvent was removed under vacuum. Flash column chromatography (30% ethyl acetate / 70% hexanes, Rᵣ = 0.38) of the crude material yielded 0.180g (0.649mmol) of pure (3R, 5S)-8c in 99% yield as a white solid. M.P. = 129-131 °C. [α]ᴰ²³ = -72.4 (c = 1.0, CH₂Cl₂). IR (cm⁻¹): 3350, 2985, 2931, 1722.

¹H NMR (400 MHz, CDCl₃) δ 7.90 (s, 1H), 7.40 – 7.31 (m, 2H), 7.31 – 7.27 (m, 1H), 7.23 – 7.17 (m, 2H), 4.36 – 4.25 (m, 1H), 4.20 – 4.13 (m, 2H), 2.95 (dd, J = 13.7, 5.6 Hz, 1H), 2.77 (dt, J = 13.7, 7.8 Hz, 2H), 1.79 (dd, J = 13.2, 8.6 Hz, 1H), 1.57 (s, 3H), 1.25 (t, J = 7.1 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 205.54, 171.69, 136.74, 129.23, 128.98, 127.43, 61.91, 61.08, 60.75, 42.19, 41.98, 24.13, 14.12. HRMS (C₁₅H₁₉NO₂S Na⁺) calcd = 300.102871 m/z, obsd = 300.102969 m/z.

(3S, 5S)-9c, (3R, 5S)-ethyl 5-benzyl-3-methylpyrrolidine-3-carboxylate:

To a 25 mL three neck flask, 2.0 mL of Raney Nickel slurry in water was added. Raney Nickel was washed with water (2 x 5 mL), methanol (2 x 5 mL) and
THF (2 x 5 mL) in the same order. 4 mL THF was added to the above flask and the hydrogen gas was bubbled through the solution for 30 minutes. Then 0.160g (0.577 mmol) of (3R, 5S)-8c was added to the above flask, as a solution in 3 mL THF and 1mL ethanol. The reaction contents were allowed to stir, under H2 atmosphere, for 6 hrs at which time the reaction went to completion. Raney-Ni was filtered off on the celite bed and solvent was removed under vacuum to provide crude material as light greenish yellow liquid. Crude material was then purified by gradient flash column chromatography, using 40% ethyl acetate/60% hexanes to 100% ethyl acetate (Solvent was treated with NH4OH and then decanted prior to use as reported for (3R, 5S)-9c) to afford 0.084g (0.340 mmol) of pure (3S, 5S)-9c as light yellow liquid in 59% yield. Rf = 0.38 (40% ethyl acetate/60% hexanes treated with NH4OH) [α]D23 = -9.8 (c = 1.0, CHCl3). IR (cm−1): 3026, 2974, 2930, 2872, 1721. 1H NMR (400 MHz, CDCl3) δ 7.32 – 7.25 (m, 2H), 7.19 (dd, J = 10.0, 4.2 Hz, 3H), 4.12 (q, J = 7.1 Hz, 2H), 3.44 (dq, J = 13.9, 6.9 Hz, 1H), 3.27 (d, J = 11.1 Hz, 1H), 2.84 – 2.69 (m, 3H), 2.41 (dd, J = 13.0, 6.9 Hz, 1H), 2.05 – 1.92 (m, 1H), 1.31 (d, J = 2.3 Hz, 3H), 1.30 – 1.26 (m, 1H), 1.23 (t, J = 7.1 Hz, 3H). 13C NMR (101 MHz, CDCl3) δ 177.34, 139.66, 129.02, 128.40, 126.17, 60.88, 60.64, 57.38, 50.10, 44.14, 42.57, 24.51, 14.17. HRMS (C15H21NO2 H+) calcd = 248.164505 m/z, obsd = 248.164453 m/z.

(3S, 5S)-10c, (3S, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid:

To a one neck 25 mL round bottom flask containing 0.062g (0.25 mmol) of (3S, 5S)-9c, 6 mL of 6.0 N HCl was added and the solvent was heated to reflux. Reaction went to completion after refluxing for 24 hrs. At this point reaction
mixture was cooled and solvent was removed under high vacuum to afford 0.063g (0.247 mmol) of \((3R, 5S)\)-10c hydrochloride salt as white solid in 99 % yield. The crude product was then loaded onto DOWEX 50X8-200 resin, to remove chloride salts, and the product was eluted using 15% NH₄OH. Fractions were collected and solvent was removed under high vacuum to provide \((3S, 5S)\)-10c in its zwitterionic form as white solid. M.P. = 207-209 °C. \([\alpha]_D^{23}\) (HCl Salt) = -9.1 (c = 1.0, MeOH). IR (cm\(^{-1}\))::2967, 2930, 1628. \(^1\)H NMR (400 MHz, D\(_2\)O) \(\delta\) 7.44 – 7.30 (m, 5H), 3.88 – 3.77 (m, 1H), 3.67 (d, \(J = 11.8\) Hz, 1H), 3.14 – 2.93 (m, 3H), 2.54 (dd, \(J = 13.4, 6.4\) Hz, 1H), 1.66 (dd, \(J = 13.4, 11.3\) Hz, 1H), 1.33 (s, 3H). \(^1^3\)C NMR (101 MHz, D\(_2\)O) \(\delta\) 182.89, 136.89, 128.89, 128.99, 128.90, 127.23, 61.47, 54.11, 50.46, 42.11, 37.93, 23.37. HRMS (C\(_{13}\)H\(_{17}\)NO\(_2\)H\(^+\)) calcd = 220.133205 m/z, obsd = 220.132985 m/z.
2.5 X-ray Crystal Data

2.5.1 Crystal data for (2$S$, 4$S$)-6c
Table 1. Crystal data and structure refinement for (2S, 4S)-6c.

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$R1 = \sum ||F_o^2 - |F_c|^2|| / \sum |F_o|$
Table 2. Atomic coordinates and equivalent isotropic displacement parameters for (2S, 4S)-6c. U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$ tensor.

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2.5.2 Crystal data for (3\textit{S}, 5\textit{S})-7c
Table 1. Crystal data and structure refinement for (3S, 5S)-7c.

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<td>Density (calculated)</td>
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<td>Absorption coefficient</td>
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<td>Absorption correction</td>
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<td>Independent reflections</td>
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<tr>
<td>R(F obsd data)</td>
<td>R&lt;sub&gt;1&lt;/sub&gt; = 0.0291</td>
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<td>Goodness-of-fit on F&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>Observed data [I &gt; 2σ(I)]</td>
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<tr>
<td>Largest and mean shift / s.u.</td>
<td>0.000 and 0.000</td>
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<tr>
<td>Largest diff. peak and hole</td>
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</table>

\[
\begin{align*}
\text{wR}_2 & = \left\{ \frac{\sum \left[ w(F_o^2 - F_c^2)^2 \right]}{\sum \left[ w(F_o^2)^2 \right]} \right\}^{1/2} \\
R_1 & = \frac{\sum |F_o| - |F_c||}{\sum |F_o|}
\end{align*}
\]
Table 2. Atomic coordinates and equivalent isotropic displacement parameters for (3S, 5S)-7c. U(eq) is defined as one third of the trace of the orthogonalized $U_{ij}$

tensor.

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<thead>
<tr>
<th></th>
<th>x</th>
<th>y</th>
<th>z</th>
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<td>0.47883(11)</td>
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<td>0.65107(11)</td>
<td>0.0221(3)</td>
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CHAPTER III - CATALYTIC EVALUATION OF DESIGNED 5-BENZYL-3-METHYLPYRROLIDINE-3-CARBOXYLIC ACID IN ALDOL AND MANNICH REACTIONS

3.1 Hypothesis 2:

The designed 5-benzyl-Cα-methyl-β-proline analogue will be an efficient organocatalyst in asymmetric anti-Mannich reactions and also in Aldol reactions.

3.2 Background

Aldol and Mannich reactions are both very important reactions in the field of organic chemistry as they involve the carbon-carbon bond formation under mild reaction conditions. Aldol reaction occurs between two carbonyl compounds whereas the Mannich reaction is between amines and carbonyl compounds. Both Aldol and Mannich reactions are very well known to be catalyzed by L-proline.

3.2.1 Mannich Reaction

In organic chemistry, the Mannich reaction is an important carbon-carbon bond forming reaction primarily between carbonyl compounds and amines. However, a modified Mannich reaction is utilized in Eschenmoser methenylation reaction for the synthesis of α-methenylated carbonyl compounds.\textsuperscript{97-99} In Mannich reaction, an activated carbonyl compound (aldehyde or ketone) is condensed with an amine (usually primary or secondary) and an enolizable carbonyl compound (aldehyde or ketone) to form amino alkylated products. In Mannich reactions catalyzed by metal catalysts or other organocatalysts (including L-Proline), products formed are syn selective.\textsuperscript{7,21,100} For the first time, List reported a Mannich reaction catalyzed by L-Proline that yielded syn selective...
Mannich product. β-Proline and its analogues catalyze the asymmetric Mannich reaction to provide *anti* selective Mannich products. Barbas et al reported *anti* selective asymmetric Mannich reaction with a designed β-proline analogue as catalyst. 

(R)-Cα-Methyl-β-proline designed by our group was tested for its catalytic activity in the Mannich reaction by Dr. Banerjee. However these results were not published by our group. The Mannich reaction was performed using isovaleraldehyde and p-methoxy phenyl (PMP) protected imine (Scheme 16). (R)-Cα-Methyl-β-proline showed good “*anti*” selectivity with a diastereomeric ratio of 8:1 (*anti*: *syn*) and an enantiomeric excess of 97%. The catalyst loading for the asymmetric Mannich reaction was 5% and the reaction time was 4 hrs. The reaction was performed in DMSO and the overall yield of the reaction was found to be 75%. These results suggested that (R)-Cα-methyl-β-proline is an efficient catalyst in the asymmetric Mannich reaction with good “*anti*” selectivity. The catalytic activity of (R)-Cα-methyl-β-proline was also independently verified by Nagata and coworkers and their data supports our results. However our synthetic strategy for (R)-α-methyl-β-proline is far superior to the strategy reported by Nagata et al. With the preliminary data supporting the efficiency of Cα-methyl-β-proline as a catalyst in the asymmetric Mannich reaction, we want to expand the scope of β-proline analogues as catalysts not only to the Mannich reaction but also to the Aldol reaction. We hypothesized that:

(i) Substituents on the pyrrolidine ring could improve the hydrophobic character of the catalyst that will facilitate organic reactions in relatively
non-polar organic solvents. This would provide expanded solvent choice for the organocatalytic reactions which in turn expands the substrate scope.

(ii) Cα-Methyl-β-proline analogue will catalyze asymmetric Mannich reactions in non-polar organic solvents with good anti stereoselectivities.

(iii) The steric hindrance caused by the benzyl substituent at position 5 on the pyrrolidine ring will be responsible for the formation of stereoselective products in the Aldol reaction as the Aldol reaction does not involve a strong hydrogen bond acceptor like an Imine in the Mannich reaction.

3.2.2 Aldol reaction

The designed β-proline analogue was tested for its activity in the Aldol reaction between acetone and 4-nitro benzaldehyde as shown in Scheme 3.1. The reaction shown in Scheme 3.1 was the first intermolecular aldol addition reaction reported to be catalyzed by L-proline. We chose this reaction to test the catalytic activity of our designed β-proline analogue as this has been the gold standard reaction in the field of enamine catalysis by L-proline since it was first reported by List et al.

![Aldol Product]

Scheme 3.1 Aldol reaction between acetone and 4-nitrobenzaldehyde
In direct aldol reactions proline and its derivatives follow enamine mechanism to provide enantioselective products. Based on the mechanism of enamine catalysis by proline analogues, we proposed a transition state model (Figure 3.1) for the asymmetric Aldol reaction catalyzed by (3S, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid, (3S, 5S)-10c. In the transition state (Figure 3) of the Aldol reaction (Scheme 3.1), we hypothesized the enamine’s nucleophilic attack on 4-nitro benzaldehyde is from the “re” face (Figure 3.1).

Figure 3.1 Predicted transition state model for the Aldol reaction catalyzed by (3S, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid, (3S, 5S)-10c

After examining the transition state model, we proposed two reasons for the “re” facial attack of the enamine on 4-nitrobenzaldehyde.

(i) In the transition state, the enamine is aligned in such a way that it is away from the benzylic substituent at position 5 on the pyrrolidine ring as shown in Figure 3.1.

(ii) The bulky benzyl group on pyrrolidine ring also favors the approach of 4-nitrobenzaldehyde towards enamine exclusively from the plane in which carboxylic acid is present. As shown in Figure 3.1, the cyclic bonded framework involves weak hydrogen bond interaction between
the carbonyl group of 4-nitro benzaldehyde and hydrogen of carboxylic acid. Having a substituent at position 5 on pyrrolidine ring would promote the re facial attack of enamine on the aldehyde, but not the weak hydrogen bond itself that promotes the attack.

3.3 Results and Discussion:

3.3.1 Mannich Reaction

3.3.1.1 Mannich Reaction catalyzed by (3R, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid, (3R, 5S)-10c:

Mannich reaction was performed between isovaleraldehyde and a prepared N-PMP protected α-imino ester as shown in Scheme 3.2. The α-imino ester was prepared fresh from ethyl glyoxylate and p-anisidine before use according to the reported procedure.50

![Scheme 3.2 Mannich reaction between isovaleraldehyde and α-imino ester](image)

We have performed the above Mannich reaction with L-proline catalyst and we used as reference to compare our results to. We have tested both β-proline analogues that were designed by us in the Mannich reaction. We have chosen to perform these reactions in methylene chloride (ε = 9.08) which is relatively non polar compared to DMSO (ε = 47), that was used for L-proline. The Mannich reaction when performed with 20 mol% of L-proline as catalyst in
DMSO, provided syn-Mannich product in 96% yield. The reaction time for this reaction was observed to be 4 hrs. The diastereomeric ratio of the product was determined from $^1$H NMR analysis and it was found to be to be 24:1 (syn: anti). The enantiomeric excess of the syn-Mannich product was determined to be 97% and it was determined by HPLC using chiral stationary phase. (Table 3.1)

Table 3.1

Mannich reaction between isovaleraldehyde and α-imino ester catalyzed by L-proline and designed β-proline analogue, (3R, 5S)-10c

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Loading</th>
<th>Solvent (Dielectric Constant, $\varepsilon$)</th>
<th>Time</th>
<th>Yield$^a$</th>
<th>d.r$^b$</th>
<th>%ee$^c$</th>
<th>Major Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-proline</td>
<td>20 mol%</td>
<td>DMSO ($\varepsilon = 47$)</td>
<td>4h, RT</td>
<td>96%</td>
<td>24:1</td>
<td>97%</td>
<td>(2S,3S)-syn-13</td>
</tr>
<tr>
<td>(3R, 5S)-10c</td>
<td>5 mol%</td>
<td>DMSO ($\varepsilon = 47$)</td>
<td>6h, RT</td>
<td>79%</td>
<td>4:1</td>
<td>9%</td>
<td>(2R,3S)-anti-13</td>
</tr>
<tr>
<td></td>
<td>5 mol%</td>
<td>$i$-PrOH:CH$_2$Cl$<em>2$ 1:1 ($\varepsilon</em>{i-PrOH} = 18.3$)</td>
<td>2h, RT</td>
<td>82%</td>
<td>19:1</td>
<td>11%</td>
<td>(2S,3R)-anti-13</td>
</tr>
<tr>
<td></td>
<td>5 mol%</td>
<td>CH$_2$Cl$_2$ ($\varepsilon = 9.08$)</td>
<td>5h, RT</td>
<td>82%</td>
<td>19:1</td>
<td>32%</td>
<td>(2S,3R)-anti-13</td>
</tr>
</tbody>
</table>

$^a$ % yield calculated from the product isolated after flash column chromatography

$^b$ Diastereomeric ratios determined from $^1$H NMR of crude product

$^c$ % ee determined from HPLC analysis using chiral stationary phase
When the Mannich reaction was performed using 5 mol% of \((3R, 5S)\)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid (10C) in DMSO, the reaction went to completion in 6 hrs yielding 79% of product in a d.r. of 4:1 (anti: syn). However, the enantiomeric excess of the anti-Mannich product was observed to be 9% ee.

At this point we thought the solubility of the catalyst might be an issue because the catalyst, in its zwitterionic form, was not soluble in any solvents. Finally we found that the catalyst was completely soluble in 1:1 mixture of \(i\)-PrOH: CH\(_2\)Cl\(_2\). Under these solvent conditions and 5 mol% catalyst loading, the reaction went to completion within 2 hrs providing 82% of the product in a d.r. of 19:1 (anti: syn). However, the %ee of the anti-Mannich product did not show any significant improvement, providing 11% ee.

We have repeated the Mannich reaction using methylene chloride as solvent and 5 mol% catalyst. The catalyst was not soluble in the solvent by itself, but after the addition of isovaleraldehyde and stirring for few minutes, the catalyst appeared to be completely soluble in the reaction mixture. This could possibly be due to the formation of enamine between catalyst and isovaleraldehyde. In methylene chloride the reaction went to completion after 5 hrs, yielding 82% of product. This reaction yielded anti-Mannich product in a d.r. of 19:1. The % ee of anti-product in the reaction showed significant improvement from the previous two reactions providing 32% ee. After observing the poor enantioselectivities of anti-Mannich products in the Mannich reactions catalyzed by the designed \(\beta\)-proline analogue \((3R, 5S)\)-10c, we proposed a transition state model that would explain for the enantioselective outcomes in the performed Mannich reaction.
The transition state model is shown in Figure 3.2, is based on the enamine catalysis mechanism by L-proline.

According to enamine mechanism, in the transition state of Mannich reaction catalyzed by \((3R, 5S)-10c\), the enamine formed between catalyst's amine and isovaleraldehyde attacks the preformed imine. This attack should be facilitated by the carboxylic acid group of the catalyst. The carboxylic acid’s proton serves as a hydrogen bond donor to the imine, which is a very strong hydrogen bond acceptor. This strong hydrogen bond interaction should facilitate selective attack of enamine on particular face of the imine. As a result of this selective attack of enamine on the imine, the products are formed in an enantioselective fashion.

If we consider the transition state of Mannich reaction, catalyzed by \((3R, 5S)-10c\) both benzyl substituent of amino acid and carboxylic acid group are in the same plane. When the incoming imine is approaching the enamine, it should
be directed by carboxylic acid of the amino acid. In this particular case, as shown in Figure 3.2, the benzyl group of the catalyst that is in the same plane as carboxylic acid is creating lot of steric congestion. We believe that as a result of this steric repulsion between the N-PMP group of imine and the benzyl substituent of the catalyst, the hydrogen bonding interaction between carboxylic acid and the imine may have been diminished. Since there is no factor, controlling the selective attack of enamine on the imine, the products obtained would usually be racemic.

Interestingly, (3R, 5S)-10c catalyzed Mannich reaction showed some enantioselectivity in the anti-Mannich product when a relatively non-polar solvent (CH$_2$Cl$_2$) was used. When the reaction was performed in DMSO ($\varepsilon = 47$), the (2$R$, 3$S$)-anti-13 Mannich product ($d.r = 4:1$) was formed in 9% ee. When the reaction was performed in a 1:1 mixture of 2-propanol/CH$_2$Cl$_2$, (2$S$, 3$R$)-anti-13 Mannich product, ($d.r = 19:1$) was formed in 11% ee. Improvement in diastereomeric excess was observed in this case and a switch in the enantioselectivity of the anti-Mannich product was observed. When the reaction was performed in CH$_2$Cl$_2$ ($\varepsilon = 9.08$), (2$S$, 3$R$)-anti-13 Mannich product, ($d.r = 19:1$) was produced in 33% ee. Although the observed enantiomeric excess is not very significant, it is interesting that the enantioselectivity of the Mannich reaction catalyzed by (3$R$, 5$S$)-10c is dependent on the nature of solvent used in the reaction.
3.3.1.2 Mannich Reaction catalyzed by (3S, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid, (3S, 5S)-10c:

We had performed the Mannich reaction (Scheme 17) by using the designed catalyst, (3S, 5S)-10c. The designed amino acid (3S, 5S)-10c provided “anti” selective Mannich products in excellent diastereoselectivities. (Table 3.2) The reactions were performed in methylene chloride solvent and at room temperature. We were able to carry out two reactions at different catalyst loadings. The diastereoselectivities of the Mannich products were determined from $^1$H NMR and the enantioselectivities were determined from HPLC analysis using a chiral stationary phase.

Table 3.2

Mannich reaction between isovaleraldehyde and α-imino ester catalyzed by L-proline and designed β-proline analogue, (3S, 5S)-10c

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Loading</th>
<th>Solvent</th>
<th>Time</th>
<th>Yielda</th>
<th>d.rb</th>
<th>%ee  c</th>
<th>Major Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Proline</td>
<td>20 mol%</td>
<td>DMSO</td>
<td>4h, RT</td>
<td>96%</td>
<td>24:1 (syn)</td>
<td>97%</td>
<td>(2S,3S)-syn-13</td>
</tr>
<tr>
<td>(3S, 5S)-10c</td>
<td>1 mol%</td>
<td>CH$_2$Cl$_2$</td>
<td>2.5h, RT</td>
<td>85%</td>
<td>24:1 (anti)</td>
<td>98%</td>
<td>(2R,3S)-anti-13</td>
</tr>
<tr>
<td></td>
<td>5 mol%</td>
<td>CH$_2$Cl$_2$</td>
<td>2.5h, RT</td>
<td>92%</td>
<td>24:1 (anti)</td>
<td>99%</td>
<td>(2R,3S)-anti-13</td>
</tr>
</tbody>
</table>

a) % yield calculated from the product isolated after flash column chromatography
b) Diastereomeric ratios determined from $^1$H NMR of crude product
c) % ee determined from HPLC analysis using chiral stationary phase
Mannich reaction when performed with 5 mol% catalyst loading in methylene chloride at room temperature, the reaction went to completion in 2.5 hrs. The reaction yielded *anti*-Mannich product in 92% yield and a diastereomeric ratio of 24:1 as determined from $^1$H NMR. The enantiomeric excess of the *anti*-Mannich products was observed to be 99% as determined by HPLC analysis. We have then repeated the reaction with catalyst (3S, 5S)-10c at 1mol% loading. The reaction was carried out in methylene chloride at room temperature and it went to completion in 2.5 hrs. This reaction was worked up and the product was isolated in 85% yield. This reaction also provided *anti* selective Mannich products in a d.r. of 24:1. The enantiomeric excess of the *anti*-Mannich product was observed to be 98%. Even at 1 mol% loading, the catalyst (3S, 5S)-10c retained its efficiency in providing stereoselective products. To explain the observed enantioselectivity in the Mannich reaction, we proposed a transition state model based on the enamine mechanism. (Figure 3.3)

![Proposed transition state model for the Mannich reaction catalyzed by designed analogue (3S, 5S)-10c](image)

**PMP** = p-methoxy phenyl

Figure 3.3 Proposed transition state model for the Mannich reaction catalyzed by designed analogue (3S, 5S)-10c
In the transition state of Mannich Reaction enamine’s selective attack on the imine is facilitated by strong hydrogen bonding interaction between carboxylic acid moiety of the catalyst and the imine as shown in Figure 3.3. The enamine formed during the reaction will be in a particular conformation as shown in Figure 3.3. The bulky benzyl substituent on the pyrrolidine ring blocks the back side face of enamine forcing the imine to approach the enamine from the top face. When this happens, the carboxylic acid group of the catalyst that is shown to be projecting out of the plane forms a hydrogen bond with the imine and holds it in the least energy conformation (anti) to avoid steric hindrance. As a result of this hydrogen bonding interaction, the enamine attacks the imine from one face. This selective facial attack yields enantioselective products in the reaction.

3.3.2 Aldol Reaction catalyzed by (3S, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid, (3S, 5S)-10c:

We performed the Aldol reaction between acetone and 4-nitrobenzaldehyde using L-proline and (3S, 5S)-5-benzyl-3-methylpyrrolidine-3-carboxylic acid as a catalysts. The Aldol reaction with L-proline was used as reference to our studies. The reaction using the designed amino acid (3S, 5S)-10c did not proceed at all when the reaction was performed in acetone or DMSO. The reaction was monitored for up to 3 days by TLC and also mini work ups were performed at regular intervals to see if there was any progress. Upon careful observation of the reaction mixture, we could still see the catalyst insoluble in the solution. We thought that the solubility of the catalyst may be the reason for the poor reactivity. Then we have performed the reaction using D$_2$O as solvent and
The reaction went to completion within a couple of hours.\textsuperscript{106} The reaction was worked up and pure Aldol product was isolate in 86\% yield after flash column chromatography. However, this reaction yielded racemic mixture of the Aldol product. (Table 3.3) HPLC using chiral stationary phase was performed to determine the \% enantiomeric excess of the Aldol product.

Table 3.3
Catalytic studies of Aldol reaction between acetone and 4-nitrobenzaldehyde

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Solvent</th>
<th>Loadings</th>
<th>Time</th>
<th>Yield$^a$</th>
<th>% ee$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(20 vol% acetone)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-proline</td>
<td>DMSO</td>
<td>30 mol%</td>
<td>3 hrs.</td>
<td>72%</td>
<td>72%</td>
</tr>
<tr>
<td>(3S, 5S)-10c</td>
<td>DMSO</td>
<td>6 mol%</td>
<td>3 days</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(3S, 5S)-10c</td>
<td>D$_2$O</td>
<td>1 mol%</td>
<td>1.5 hrs.</td>
<td>86%</td>
<td>Racemic</td>
</tr>
<tr>
<td>(3S, 5S)-10c</td>
<td>CD$_2$Cl$_2$ (4 vol% Water)</td>
<td>1 mol%</td>
<td>3 days</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ \% yield calculated from the product isolated after flash column chromatography

$^b$ \% ee determined from HPLC analysis using chiral stationary phase

The Aldol reaction between acetone and 4-nitro benzaldehyde using the designed β-proline analogue (3S, 5S)-10c did not proceed at all when performed in solvents such as DMSO or methylene chloride-D$_2$. Reaction went to
completion within 2 hrs when D$_2$O was used as a solvent, providing racemic mixture of aldol product. After observing the experimental results of the Aldol reaction catalyzed by (3S, 5S)-10c, we reexamined our hypothesized transition state for the reaction.

We hypothesized that, in the Aldol reaction catalyzed by the designed β-proline analogue (3S, 5S)-10c the selective facial attack of the enamine on 4-nitrobenzaldehyde would be facilitated by the steric hindrance created by the benzyl group at position 5 of the pyrrolidine ring. Since a strong hydrogen bond acceptor is absent in the incoming aldehyde, the carboxylic acid of the amino acid (3S, 5S)-10c, could not direct the aldehyde via a hydrogen bonding interaction as seen with L-Proline where a stable six membered ring formation is observed in the transition state.$^{17}$ In the designed β-proline analogue (3S, 5S)-10c the benzylic substituent and the carboxylic acid moiety are in the opposite planes According to our hypothesis, the bulky benzyl group on the amino acid, (3S, 5S)-10c should block one face of enamine and force 4-nitro benzaldehyde to approach the enamine only from the opposite plane in which carboxylic acid group is present. The carboxylic acid should then provide a weak hydrogen bond and direct the aldehyde towards the enamine facilitating selective facial attack that would result in the formation of enantioselective products. However, the results of Aldol reaction between acetone and 4-nitrobenzaldehyde catalyzed by (3S, 5S)-10c suggests that the steric hindrance created by the benzyl group is not resulting in the selective attack of enamine on the aldehyde.
When the reaction was performed in less polar solvents such as DMSO or methylene chloride the reaction showed no progress even after prolonged reaction times (up to 3 days) but when water was used as solvent, the reaction went to completion within a couple of hours providing racemic mixture of Aldol product. After examining the reaction mechanism we were able to come up with a possible reason for the observed poor results with water as solvent.

We speculate that the weak hydrogen bonding interaction between carboxylic acid and the aldehyde is not taking place. In the L-proline catalyzed Aldol reaction the selective facial attack of enamine on the aldehyde is facilitated by weak hydrogen bond that is a part of stable six membered ring. With the designed β-proline amino acid (3S, 5S)-10c, in the transition state the cyclic hydrogen bonded framework formation may not be taking place. If the weak hydrogen bonding interaction between the aldehyde and carboxylic acid group is absent in the transition state, the enamine’s attack on the aldehyde might not be selective. The aldehyde approaches from both planes towards the enamine that would result in both re and si-facial selectivities and as a result, the reaction provides a racemic mixture of products.

3.4 Experimental Methods

All anhydrous solvents were obtained by passage through a column of activated silica. Flash column chromatography was performed using SiliaFlash® P60 40-63µm (230-400 mesh) silica gel. NMR experiments were performed on a Bruker 400 MHz NMR instrument and chemical shifts were reported with reference to TMS (for CDCl₃). All the organocatalytic reactions were performed in
a 5 mL sealed reaction tube at room temperature. The Imine for Mannich reactions was readily prepared from ethyl glyoxylate and p-anisidine before use.\textsuperscript{107}

3.4.1 Typical procedure for Mannich reactions:

\begin{align*}
\text{PMP} \quad & \quad \text{H} \quad \text{CO}_2\text{Et} \quad \text{+} \quad \text{H} \quad \text{C} \quad \text{O} \quad \text{H} \\
11 \quad & \quad \text{Catalyst} \quad \longrightarrow \quad \text{H} \quad \text{C} \quad \text{O}_2\text{Et} \quad \text{+} \quad \text{H} \quad \text{C} \quad \text{O}_2\text{Et} \\
\text{PMP} = p\text{-methoxy phenyl} \\
\end{align*}

0.207 g (1.0 mmol) of the preformed imine (11) was weighed and added to a 5 mL sealed tube containing 2 mL anhydrous methylene chloride. Then appropriate amount of catalyst was weighed and added to the reaction vessel followed by 0.173 g (2.0 mmol) of isovaleraldehyde (12) as a solution in 2.0 mL of anhydrous methylene chloride. The reaction tube was tightly sealed and left to stir at room temperature. The reaction was monitored by TLC for the disappearance of Imine, 11. Once all the starting material had disappeared, the reaction mixture was washed with saturated NaCl (1 x 5 mL). The organic portion was dried over MgSO\textsubscript{4}, filtered and the solvent was removed under vacuum to afford crude product. Crude product was analyzed by \textsuperscript{1}H NMR to determine diastereomeric ratio of the Mannich product. The crude product was then purified immediately by flash column chromatography (20% ethyl acetate/80% hexanes) to provide pure product. The % yield of the reaction was determined from the pure product isolated in the reaction. The pure product was immediately analyzed by the HPLC using chiral stationary phase to determine the percent
enantiomeric excess. The data of the products was compared to the reported to the literature and determined to be consistent.\textsuperscript{50,51,101}

\textit{Compound 11, Imine for Mannich reaction}:\textsuperscript{107}

To a 100 mL sealed tube 3.00g of molecular sieves, 4 Å were added and a stir bar was placed in the sealed tube. 0.992g (8.0 mmol) of p-anisidine was weighed and added to the above sealed tube. 25 mL of anhydrous methylene chloride was added to the reaction tube and the contents were stirred for 5 minutes. 3.3g (16.0mmol) of ethyl glyoxylate (50\% Vol/Vol in toluene) was added to the reaction tube and the tube was tightly sealed. The contents of the reaction tube were left to stir for 3 hrs at room temperature. Then the reaction mixture was filtered under vacuum to remove molecular sieves and the solvent was removed under vacuum to provide crude product as brownish –yellow liquid. Crude product was then purified by flash column chromatography (30\%ethyl acetate/70\% hexanes)/4\%triethylamine to provide 1.49 g (7.19 mmol) pure product as light yellow liquid in 90\%yield. The $^1$H NMR data is consistent with the product as reported.\textsuperscript{50,107} $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.94 (s, 1H), 7.40 – 7.33 (m, 2H), 6.97 – 6.90 (m, 2H), 4.42 (q, $J = 7.1$ Hz, 2H), 3.84 (s, 3H), 1.41 (t, $J = 7.1$ Hz, 3H).

(2S, 3S)-syn-13, (2S,3S)-ethyl 3-formyl-2-(4-methoxyphenylamino)-4-methylpentanoate:

In the Mannich reaction, (2S, 3S)-syn-13 product was prepared by using L-proline as catalyst in 20 mol\% loading in DMSO. The reaction was set up worked up according to the common procedure reported for the Mannich reaction.
between preformed α-imino ester and isovaleraldehyde. The product was characterized by $^1$H NMR and chiral stationary phase HPLC analysis. HPLC was performed using Daicel Chiralpak AS-H column (4.6mm x 250mm, 5 µ). $^1$H NMR (400 MHz, CDCl$_3$) δ 9.78 (d, $J$ = 3.0 Hz, 1H), 6.81 – 6.73 (m, 2H), 6.70 – 6.62 (m, 2H), 4.32 (dd, $J$ = 10.3, 6.8 Hz, 1H), 4.20 – 4.11 (m, 2H), 3.85 (d, $J$ = 10.3 Hz, 1H), 3.74 (s, 3H), 2.55 (td, $J$ = 7.0, 3.1 Hz, 1H), 2.31 (dq, $J$ = 13.9, 6.9 Hz, 1H), 1.22 (td, $J$ = 7.0, 3.1 Hz, 3H), 1.17 (d, $J$ = 6.9 Hz, 3H), 1.03 (d, $J$ = 6.8 Hz, 3H).

HPLC ($\lambda_{\text{max}}$ = 254nm, flow rate = 1.0 mL/min, 1% i-PrOH / 99% Hexanes.): $^1$R (major enantiomer, (2S, 3S)-syn-13) = 32.2 min. $^1$R (minor enantiomer, (2R, 3R)-syn-13) = 66.8 min.$^{56}$

(2R, 3S)-anti-13, (2R,3S)-ethyl 3-formyl-2-(4-methoxyphenylamino)-4-methylpentanoate:

In the Mannich reaction, (2R, 3S)-anti-13 product was prepared by using the designed catalyst. The reaction was set up and worked up according to the common procedure reported for the Mannich reaction between preformed α-imino ester and isovaleraldehyde. The product was characterized by $^1$H NMR and chiral stationary phase HPLC analysis. HPLC was performed using Daicel Chiralpak AS-H column (4.6mm x 250mm, 5 µ).$^1$H NMR (400 MHz, CDCl$_3$) δ 9.74 (t, $J$ = 3.4 Hz, 1H), 6.80 – 6.73 (m, 2H), 6.70 – 6.62 (m, 2H), 4.39 – 4.30 (m, 1H), 4.20 – 4.12 (m, 2H), 3.93 (d, $J$ = 13.4 Hz, 1H), 3.74 (s, 3H), 2.59 (dddd, $J$ = 13.3, 9.9, 6.7, 3.2 Hz, 1H), 2.16 – 2.05 (m, 1H), 1.23 – 1.18 (m, 3H), 1.14 – 1.10 (m, 3H), 1.07 (d, $J$ = 6.9 Hz, 3H). HPLC ($\lambda_{\text{max}}$ = 254nm, flow rate = 1.0 mL/min, 1% i-
PrOH / 99% Hexanes.): \( \text{tR} \) (major enantiomer, (2R, 3S)-anti-13) = 56.2 min. \( \text{tR} \) (minor enantiomer, (2S, 3R)-anti-13) = 29.1 min.

(2S, 3R)-anti-13, (2S, 3R)-ethyl 3-formyl-2-(4-methoxyphenylamino)-4-methylpentanoate:

In the Mannich reaction, (2S, 3R)-anti-13 product was prepared by using the designed catalyst. The reaction was set up worked up according to the common procedure reported for the Mannich reaction between preformed \( \alpha \)-imino ester and isovaleraldehyde. The product was characterized by \( ^1 \)H NMR and chiral stationary phase HPLC analysis. HPLC was performed using Daicel Chiralpak AS-H column (4.6mm x 250mm, 5 µ).

\( ^1 \)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 9.75 (d, \( J = 3.4 \) Hz, 1H), 6.81 – 6.73 (m, 2H), 6.71 – 6.62 (m, 2H), 4.33 (t, \( J = 13.5 \) Hz, 1H), 4.19 – 4.13 (m, 2H), 3.94 (t, \( J = 11.5 \) Hz, 1H), 3.74 (s, 3H), 2.60 (ddd, \( J = 7.7, 6.2, 3.4 \) Hz, 1H), 2.10 (tt, \( J = 10.9, 5.5 \) Hz, 1H), 1.23 – 1.17 (m, 3H), 1.13 (t, \( J = 6.1 \) Hz, 3H), 1.08 (d, \( J = 6.9 \) Hz, 3H). HPLC (\( \lambda_{\text{max}} = 254 \) nm, flow rate = 1.0mL/min, 1% i-PrOH / 99% Hexanes.): \( \text{tR} \) (major enantiomer, (2S, 3R)-anti-13) = 24.9 min. \( \text{tR} \) (minor enantiomer, (2R, 3S)-anti-13) = 53 min.

3.4.2 Typical procedure for Aldol reactions

All the reactions were carried out in a 5 mL sealed tube. Catalyst was weighed and placed in the 5 mL sealed tube and 3.2 mL of appropriate solvent was added to it. Then 0.8 mL of Acetone was added to the above reaction vessel. The sealed tube was tightly capped and the contents were allowed to stir for 15 minutes at room temperature. Then 4-nitro benzaldehyde was added to the sealed tube and the reaction tube was tightly sealed and the reaction was left
to stir at room temperature. Once the reaction had gone to completion (TLC analysis), it was worked up by adding 10 mL of saturated ammonium chloride and the organic contents were extracted using ethyl acetate (2 x 15 mL). All organic portions were combined and dried over magnesium sulfate. Then magnesium sulfate was filtered off and the solvent was removed under vacuum to provide crude product as cherry red colored viscous liquid. The crude product was purified by gradient flash column chromatography (30% Ethyl acetate / 70% Hexanes to 50% Ethyl acetate / 50% Hexanes). The pure Aldol product was isolated as a light yellow crystalline solid and the % yield of the reaction was calculated from the amount of pure product isolated after flash column purification. The pure Aldol product was characterized by $^1$H NMR and chiral stationary phase HPLC analysis.$^{12,17}$

(R)-Aldol Product, (R)-4-hydroxy-4-(4-nitrophenyl) butan-2-one:

Reaction was set up and worked up according to the above reported procedure for the Aldol reaction. Reaction catalyzed by L-Proline was performed and the data obtained was used as a reference to compare our results to. Pure product was analyzed by $^1$H NMR and HPLC to determine purity and percent enantiomeric excess. HPLC was carried out using Daicel Chiralpak AS-H column (4.6mm x 250mm, 5 µ). $^1$H NMR (400 MHz, CDCl$_3$) δ 8.22 (d, $J = 8.7$ Hz, 2H), 7.54 (d, $J = 8.7$ Hz, 2H), 5.31 – 5.23 (m, 1H), 3.56 (d, $J = 3.1$ Hz, 1H), 2.92 – 2.79 (m, 2H), 2.22 (s, 3H). HPLC ($\lambda_{max} = 280$nm, flow rate = 1.0 mL/min, 15% i-PrOH / 85% Hexanes.): Retention time of (R)-Aldol product is 24.4 min. and for (S)-Aldol product it is 34.3 min.
CHAPTER IV  ENZYME MEDIATED CONCISE SYNTHESIS OF NH-FMOC-S-TRITYL-Cα-METHYL CYSTEINE

4.1 Hypothesis 3:

A short, chiral auxiliary free synthetic route can be developed to prepare both enantiomers of orthogonally protected NH-Fmoc-S-Trityl-Cα-Methyl Cysteine using the enzyme Porcine Liver Esterase.

4.2 Background

4.2.1 Cysteines and analogues in peptide chemistry:

There has been a growing demand for the orthogonally protected amino acids in the area of peptide synthesis. This is mainly due to the fact that the orthogonally protected amino acids can be used conveniently in the solid phase peptide synthesis as the protecting group removal can be performed under mild reaction conditions without affecting other groups on the peptide chain. Cysteines are widely used in the field of peptide chemistry due to their ability to form disulfide bonds that result in the folding of peptides. Cysteines with a methyl substituent at Cα-methyl position are widely used in the peptide chemistry in the place of natural cysteine. The amino acids with Cα-methyl substituent when incorporated into polypeptides restrict the conformational mobility of the peptide back bone that results in enhanced resistance to various chemical and enzymatic degradations. Moreover the Cα-methyl cysteine analogues are used as key components in the synthesis of various biologically potent compounds such as Largazoles, Mirabzoles, Tantazoles, Thiangazoles and their derivatives. In the recent work reported by Burlina et al, Cα-methyl
cysteine’s ability to form in situ thioesters was utilized in the process of protein ligation.\textsuperscript{128}

4.2.2 Synthesis of C\textsuperscript{α}-methyl cysteine:

C\textsuperscript{α}-methyl cysteines have been reported to be widely used in peptide synthesis and there are various reported synthetic procedures to prepare them in their protected forms.\textsuperscript{129-136} Pattendan et al reported a stereoselective alkylation procedure for the synthesis of various C\textsuperscript{α}-alkylated cysteines by utilizing (\textit{R})-cysteine methyl ester as the starting material.\textsuperscript{133} Kedrowski reported the synthesis of protected S-\textit{tert}-butyl protected C\textsuperscript{α}-methyl cysteine methyl ester using an enzyme mediated asymmetric hydrolysis as a key step in the process.\textsuperscript{132} Later Masterson et al reported an improved method for the preparation of the C\textsuperscript{α}-methyl cysteine and used it for the solution phase synthesis of a glutathione analogue.\textsuperscript{80} Both procedures, reported by Kedrowski and Masterson utilize Porcine Liver Esterase to cause asymmetric transformation of malonate diester into a chiral malonate half-ester in a very selective fashion in an enantiomeric excess of >90%.

Singh et al published a method for enantioselective synthesis of C\textsuperscript{α}-methyl cysteine in orthogonally protected form that is suitable for solid phase peptide synthesis.\textsuperscript{131} The procedure reported by Singh et al utilizes chiral auxiliaries \textit{S} and \textit{R}-Camphorsultam to carry out a stereoselective alkylation reaction that resulted in the formation of a quaternary chiral center as shown in Scheme 4.1. The protecting groups on the amine and thiol groups of cysteine were installed in the later steps of the synthetic strategy.
Scheme 4.1 Synthesis of (S)-NH-Fmoc-S-Trityl-Cα-methyl cysteine (Singh et al)

We have designed a synthetic route to the orthogonally protected α-methyl cysteine amino acid that utilizes Porcine Liver Esterase to prepare enantioenriched half esters and the results of the work have been published.\textsuperscript{137}

4.3 Results and Discussion:

Our initial approach to the orthogonally protected cysteine amino acid is as shown in Scheme 4.2. This method involves preparation of malonate diester with an S-trityl side chain and then subjecting it to asymmetric hydrolysis by PLE. For the preparation of malonate diester 15, Triphenylmethanethiol was treated with sodium hydride followed by addition of anhydrous methylene chloride to create alkylating agent 14. The chloride 14, was then reacted with the enolate of dimethyl methyl malonate to provide the diester 15. When the diester 15 was subjected to enzymatic hydrolysis by PLE, the reaction did not proceed. We hypothesized that this is due to the trityl group in the side chain being too large to fit in the active site of PLE.
Scheme 4.2 Attempted PLE hydrolysis of malonate diester with trityl group

After our unsuccessful attempt to prepare the target cysteine amino acid, we decided to utilize the already established strategy\textsuperscript{79,80} to synthesize the enantioenriched chiral malonate half ester intermediates that can be transformed to C$^\alpha$-methyl cysteine amino acid analogues.

4.3.2 Synthesis of (\textit{R}) NH-Fmoc-S-Trityl-C$^\alpha$-Methyl Cysteine-OH:

Malonate diester 15, was prepared and subjected asymmetric enzymatic hydrolysis by PLE according to the reported procedure.\textsuperscript{80,132} This reaction provided the product half ester in 77% yield and 94% ee. The enantioenriched chiral half ester 16 was subjected to a Curtius rearrangement in the presence of diphenyl phosphoryl azide and triethyl amine to provide the crude isocyanate. (Scheme 4.3) The crude isocyanate was isolated after a quick work up and reacted with 9-fluorenyl methanol in the presence of Titanium (IV) Isopropoxide. This reaction afforded the Fmoc protected amine\textsuperscript{81,138} in 46% yield, from the half ester 16. The amino ester 17, derived from the Curtius reaction was subjected to ester cleavage under refluxing acid conditions. To our surprise the NH-Fmoc group and S-\textit{tert}-butyl groups remained intact under the harsh acid reflux
conditions of the reaction. Even though there are reports that suggest the S-tert-butyl groups can be cleaved under refluxing acid conditions, this was not observed in this case.$^{130,134}$

For the deprotection of the S-tert-butyl group, we utilized a Mercury (II) Acetate / TFA / anisole cocktail. This reaction was carried out at room temperature and the resulting mercurial species were washed with ice cold ether to remove all the organic impurities. The resulting grey precipitate was taken up in methanol and H$_2$S gas was bubbled through the solution for 30 minutes to reduce the mercurial species. The resulting solution was centrifuged to precipitate mercury sulfide and the solution was decanted. This decanted solution was then passed through a 2.0µ HPLC syringe filter to remove any traces of mercury that were still present after centrifugation. The free thiol compound produced was taken to the next step immediately without any intermediate purification. In the next step the free thiol from the S-tert-butyl deprotection step was reacted with triphenyl methanol in the presence of TFA to provide the $(R)$ NH-Fmoc-S-Trityl-$\alpha$-Methyl Cysteine-OH $(R)$-19, in 59% yield, over two steps from 18. (Scheme 4.3)
Scheme 4.3 Synthesis of \((R)\) NH-Fmoc-S-Trityl-Cα-Methyl Cysteine-OH

The orthogonally protected amino acid was purified by reverse phase HPLC. Chiral HPLC analysis was performed on this purified product and the enantiomeric excess was found to be 94%. Upon further purification by recrystallization in acetonitrile/water mixture, the protected Cysteine amino acid \((R)-19\) was isolated in >99% enantiomeric excess. The improvement in the % ee was determined from the HPLC analysis using chiral stationary phase.

4.3.3 Synthesis of \((S)\) NH-Fmoc-S-Trityl-Cα-Methyl Cysteine-OH:

Malonate half ester 16 from PLE hydrolysis was transformed into tert-butyl half ester 20 according to the reported procedure.\textsuperscript{132} Half ester 20 was then subjected to a Curtius rearrangement using diphenylphosphorylazide and triethylamine to provide isocyanate. (Scheme 4.4) The crude isocyanate was isolated and quickly taken to next step, reaction with 9-Fluorenylmethanol in the presence of Titanium (IV) Isopropoxide. This reaction yielded NH-Fmoc protected
amine compound 21 in 51 % yield over two steps. In the next step both CO₂-tert-butyl and S-tert-butyl groups were cleaved when compound 21 was treated with Mercury (II) Acetate / Anisole / TFA cocktail followed by the bubbling of H₂S gas. The work up procedure for the mercurial is similar to that of the compound 18. The S-tert-butyl deprotected NH-Fmoc-Cα-methyl-Cys-OH was isolated and reacted immediately with triphenylmethanol in the presence of TFA to provide (S) NH-Fmoc-S-Trityl-Cα-Methyl Cysteine-OH, (S)-19 in 82% yield. The compound (S)-19 was analyzed by chiral stationary phase analysis and the % ee was determined to be 92%. This compound was further recrystallized from the acetonitrile/water mixture to provide essentially enantiopure (S)-19 in >99% ee.

Scheme 4.4 Synthesis of (S) NH-Fmoc-S-Trityl-Cα-Methyl Cysteine-OH

4.4 Experimental Methods

4.4.1 General experimental information

All anhydrous solvents were obtained by passage through a column of activated silica. Flash column chromatography was performed using SiliaFlash® P60 40-63μm (230-400 mesh) silica gel. NMR experiments were performed on a Bruker 400 MHz NMR instrument and chemical shifts were reported with reference to either TMS (for CDCl₃) or residual solvent proton peak (for CD₃OD).
Infra-Red analysis was performed on a Thermo Nicolet nexus 470 FT-IR instrument. Melting point analysis was performed using Thomas-Hoover “Uni-Melt” Melting Point Apparatus. High Resolution mass spectra were obtained using positive electrospray ionization on a Bruker 12 Tesla APEX -Qe FTICR-MS with an Apollo II ion source. The % ee of the final product was determined by chiral HPLC analysis.

*Chiral stationary phase HPLC:* Chiral HPLC was performed using a Chiralpak AD-H column (4.6mm x 250mm, 5 µ), $\lambda_{\text{max}} = 256$nm, flow rate = 1.0 mL/min, 10% i-PrOH / 90% hexanes. Retention times for R enantiomer = 7 min and S enantiomer = 9 min.

*Reverse Phase HPLC:* Reverse phase HPLC was carried out on Vydac C18 column (250mm x 10mm, 10µ) using a linear solvent gradient, 95% Solvent A / 5% Solvent B to 5% Solvent A / 95% Solvent B in 45 min. ($\lambda_{\text{max}} = 256$nm, flow rate = 5 mL/min). Retention time of the product is 35 min.

Solvent A = Water (1% TFA)  
Solvent B = Acetonitrile (1% TFA)

14, *(Chloromethyl)(Trityl) sulfane:*

1.605g (0.4015mol) of NaH was placed in a 1000 mL, three neck round bottom flask under N$_2$ atmosphere at 0 °C. NaH was washed twice with pentane to remove the mineral oil. Then 100 mL of anhydrous THF was added to the above reaction flask containing the NaH. The contents were stirred for 5 minutes and 10 g (0.0362mol) of Triphenylmethanethiol was added slowly to the reaction flask. The reaction mixture was continued to stir at 0 °C under N$_2$ atmosphere for
5 minutes. Then 500 mL of anhydrous dichloromethane was added to the flask and the resulting mixture was stirred overnight and became cloudy. The resulting mixture was filtered and washed with water (1 x 300 mL) followed by saturated NaCl (1 x 300 mL). The organic layer was dried over MgSO$_4$, filtered, and the solvent was removed under vacuum to provide 10.02g (0.031mol) of 14 as a light yellow colored solid in 86% yield. M.P. = 101-102 °C. IR (cm$^{-1}$) 3055, 2922. $^1$H-NMR (400 MHz, CDCl$_3$) δ 7.45 – 7.36 (m, 6H), 7.27 (m, 6H), 7.22 (m, 3H), 4.28 (s, 2H). $^{13}$C-NMR (100 MHz, CDCl$_3$) δ 143.86, 129.94, 128.22, 127.27, 69.42, 48.16.

15, Dimethyl 2-methyl-2-(tritylthiomethyl) malonate:

0.150 g (6.22 mmol) NaH (60% dispersion in mineral oil) was placed in a 100 mL, three neck round bottom flask at 0 °C under a N$_2$ atmosphere. The NaH was washed with pentane (2 x 5 mL), to remove mineral oil and pentane layers were decanted. 10 mL of anhydrous THF was added to the reaction flask and the contents were stirred for 5 minutes. Then 0.542g (3.11 mmol) of diethylmethylmalonate was added, as a solution in 5 mL of anhydrous THF, to the above reaction flask slowly in a dropwise manner using a gas tight syringe. The resulting mixture was stirred for 30 minutes at 0 °C under a N$_2$ atmosphere. The mixture was brought to room temperature and allowed to stir for another 30 minutes. Then 1.01 g (3.11 mmol) of 14 was added to the reaction flask, as a solution in 7 mL of anhydrous THF, using a gas tight syringe. The reaction was allowed to stir at room temperature for 5 minutes and then the reaction mixture was heated to reflux solvent for 24 hrs. The resulting mixture was cooled to room
temperature and poured onto ice. The mixture was extracted with diethyl ether (3 x 35 mL). The ether portions were combined, dried over MgSO₄ and the solvent was removed under vacuum to provide crude product as a viscous yellow liquid. The crude material was purified by flash column chromatography (7% ethyl acetate/93% hexanes; Rf = 0.22) to provide 0.75 g (1.62 mmol) of 15 as white crystalline solid in 52% yield. M.P. = 116-117 °C. IR (cm⁻¹) 3055, 2976, 1726, 1750. ¹H-NMR (400 MHz, CDCl₃) δ 7.44 – 7.41 (m, 5H), 7.30 – 7.23 (m, 7H), 7.22 – 7.18 (m, 3H), 4.20 – 4.10 (m, 4H), 2.62 (s, 2H), 1.32 (s, 3H), 1.21 (t, J = 7.1 Hz, 6H). ¹³C-NMR (100 MHz, CDCl₃) δ 170.92, 144.45, 129.60, 127.87, 126.69, 66.55, 61.53, 53.42, 36.65, 19.51, 13.97.

16, (R)-2-(tert-butylthiomethyl)-3-methoxy-2-methyl-3-oxopropanoic acid:

Compound 16 was prepared via asymmetric enzymatic hydrolysis using crude PLE according to the reported procedure. ⁷⁹, ¹³² 1.0 L phosphate buffer of pH 7.4 was placed in a 2.0 L beaker. 53.0 g (213.42 mmol) of malonate diester 15 was added along with 5 mL of ethanol (0.5 vol %) to the above beaker under constant stirring. Then 1.07g (18 units/mg, 90 units/mmol of diester) of crude Porcine Liver Esterase (PLE) enzyme was added to the above reaction beaker and the reaction was continued to stir vigorously. The pH of the reaction was maintained at 7.4 using an auto titrator that was set to deliver 1.0 M NaOH as the reaction progressed. The reaction went to completion within 2 days. The pH of the reaction was brought up to 9.0 and a quick extraction was performed with ethyl acetate (1 x300 mL). The aqueous portion was filtered through a celite bed under vacuum to remove the enzyme and then acidified to pH 2.0 using 12M
HCl. The organics were extracted with diethyl ether (4 x 500 mL). The resulting organic layers were combined and washed with brine (1 x 600 mL), dried over MgSO₄, filtered and solvent was removed under vacuum to provide 38.6g (165 mmol) of essentially pure 16 in 77% yield and 94% ee (determined from chiral phase HPLC of compound I prior to recrystallization). Proton and carbon NMR data matches to that of the literature.79,132

17, (R)-Methyl 2-(((9H-fluoren-9-yl) methoxy) carbonylamino)-3-(tert-butylthio)-2-methylpropanoate:

To a 100 mL, three neck round bottom flask under a N₂ atmosphere, 2.343 g (10.00 mmol) of chiral half ester 16 was added as a solution in 40 mL anhydrous 1,2-dichloroethane. 1.67 mL (12 mmol) of triethylamine was added and the mixture was stirred for 10 min. at room temperature. Then 2.94 g (10.68 mmol) of diphenylphosphorylazide was added and the reaction mixture was allowed to stir at room temperature under N₂ for 2 hrs. The resulting mixture was then heated to solvent reflux for 4 hrs at which point it was determined that the azide had been completely consumed (IR analysis). The reaction mixture was cooled to room temperature and washed quickly with saturated NH₄Cl solution (1 x 40 mL). The organic portion was dried over MgSO₄, filtered and solvent was removed under vacuum to afford crude isocyanate as a murky brownish oil. The crude isocyanate was placed immediately in a 100 mL one neck round bottom flask, under a N₂ atmosphere, as a solution in 40 mL of freshly distilled toluene. To the reaction flask containing crude isocyanate, 2.55 g (13 mmol) of 9-Fluorenemethanol was added and the contents were allowed to stir for 5 min.
Then 0.190 mL (0.630 mmol) of Titanium (IV) Isopropoxide was added to the reaction flask and the reaction mixture was heated to 83 °C using an oil bath. The resulting mixture was allowed to heat for 15 hrs under a N₂ atmosphere. The mixture was cooled to room temperature and the solvent was removed under vacuum to provide crude product. This crude product was taken up in diethyl ether (25 mL) and washed with water (2 x 20 mL) followed by saturated NaCl (1 x 25 mL). The organic layer was dried over MgSO₄ and the solvent was removed under vacuum to provide a viscous yellow oil. The crude product was then purified by flash column chromatography (30% diethyl ether / 70% hexanes; Rᵢ = 0.24) to provide 1.97g (4.61 mmol) of NH-Fmoc S-tert-butyl Cys-OMe (17) as a clear viscous oil in 46% yield. [α]D⁰²³ = +4.3 (c = 1.0, CHCl₃). IR (cm⁻¹) 3350, 2957, 1717. ¹H-NMR (400 MHz, CDCl₃) δ 7.74 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.2 Hz, 2H), 7.37 (t, J = 7.4 Hz, 2H), 7.29 (t, J = 7.4 Hz, 2H), 5.92 (s, 1H), 4.28 (m, 3H), 3.79 (s, 3H), 3.37 (d, J = 11.7 Hz, 1H), 3.05 (d, J = 11.4 Hz, 1H), 1.64 (s, 3H), 1.27 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃) δ 173.52, 154.55, 143.93, 143.85, 141.24, 127.63, 127.03, 125.18, 119.91, 66.74, 59.78, 52.90, 47.09, 42.30, 34.72, 30.79, 23.48. HRMS (C₂₄H₂₉NO₄S Na⁺) calcd = 450.170950 m/z, obsd = 450.170593 m/z.

**18, (R)-2-(((9H-fluoren-9-yl) methoxy) carbonylamino)-3-(tert-butythio)-2-methylpropanoic acid:**

0.440g (1.03 mmol) of NH-Fmoc S-tert-butyl Cα-Me-Cys-OMe (17) was dissolved in 15 mL of 1, 4-dioxane and transferred to a 100 mL three neck round bottom flask. Then 15 mL of 5.0N HCl was added and the flask was connected to
a reflux condenser and placed under a N₂ atmosphere. The mixture was stirred at room temperature for 10 minutes and then heated to reflux solvent for 18 hrs. at which time the reaction had gone to completion. The mixture was cooled to room temperature and 25 mL of water was added. The organic contents were extracted with methylene chloride (3 x 25 mL). The organic portions were combined and washed with water (6 x 50 mL) followed by saturated NaCl (2 x 50 mL). The organic layer was dried over MgSO₄, filtered and the solvent was removed under vacuum to provide **18** as a white solid in quantitative yield. M.P. = 65-68 °C. [α]₀²⁴ = + 2.0 (c = 1.0, CH₂Cl₂). IR (cm⁻¹) 2959, 1705, ¹H-NMR (400 MHz, CDCl₃) δ 7.76 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.2 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.3 Hz, 2H), 6.99 (bs, 1H), 5.76 (s, 1H), 4.27 (m, 3H), 3.31 (d, J = 10.9 Hz, 1H), 3.14 (d, J = 6.9 Hz, 1H), 1.69 (s, 3H), 1.29 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃) δ 177.87, 155.13, 144.07, 144.01, 141.50, 127.90, 127.30, 125.39, 120.17, 67.23, 59.68, 47.31, 42.72, 34.86, 31.03, 23.65. HRMS (C₂₃H₂₇NO₄S Na⁺) calcd = 436.155300 m/z, obsd = 436.155127 m/z.

(R)-**19**, (R)-2-(((9H-fluoren-9-yl) methoxy) carbamoylamino)-2-methyl-3-(tritylthio) propanoic acid:

**Step 1: S-tert-butyl deprotection**

0.582 g (1.41 mmol) of compound **18** was placed in a 100 mL one neck round bottom flask and 20 mL of trifluoroacetic acid (TFA) was added. The mixture was stirred for 5 min and then 0.86 mL (7.9 mmol) of anisole was added. The mixture was stirred for 5 minutes and 1.10 g (3.4 mmol) of mercury (II) acetate was added. The flask was tightly sealed with a glass stopper and allowed
to stir for 3 hrs. at room temperature. After stirring for 3 hrs the TFA was removed under vacuum to provide the crude product as a brown syrup. Then 10 mL of cold diethyl ether was added to the flask, resulting in a gray precipitate, to remove the organic impurities and the ether layer was removed. The gray precipitate was washed with cold diethyl ether two additional times and the ether layers were decanted. \( \text{N}_2 \) gas was blown down on the gray precipitate for 15 min. to remove traces of diethyl ether. The gray precipitate was then suspended in 20 mL of methanol and \( \text{H}_2\text{S} \) was bubbled through the methanolic solution for 30 min. \( \text{H}_2\text{S} \) gas was generated by dripping conc. \( \text{H}_2\text{SO}_4 \) dropwise to \( \text{Na}_2\text{S} \) at 0 °C under a \( \text{N}_2 \) stream directed into the reaction flask using a Teflon cannula. A black precipitate immediately fell out of the solution indicating the formation of mercuric sulfide. After 30 min. of passing \( \text{H}_2\text{S} \) gas through the solution, the cannula was removed and \( \text{N}_2 \) gas was bubbled through the reaction for 15 min. to remove remaining \( \text{H}_2\text{S} \). The reaction mixture was centrifuged to precipitate mercuric sulfide. The clear supernatant liquid was removed and passed through a 2.0µ HPLC syringe filter to remove remaining fine particulate. The solvent was removed under vacuum to provide 0.400g of NH-Fmoc, Cα-Me-Cys-OH as a light brownish solid which was taken immediately to the tritylation procedure.

**Step 2: S-Tritylation**

0.400g (1.12 mmol) of NH-Fmoc, Cα-Me-Cys-OH, from the previous step, was dissolved in 20 mL of trifluoroacetic acid in a 100 mL, one neck round bottom flask. The mixture was allowed to stir for 5 min. and then 0.292 g (1.12 mmol) of triphenylmethanol was added to the reaction flask in one portion. The
reaction flask was sealed tightly with a glass stopper and allowed to stir for 21 hrs at room temperature. The solvent was removed under vacuum to provide the crude product as a brown syrup. The crude compound was dissolved in methylene chloride (20 mL) and washed with water (2 x 20 mL) followed by brine (1 x 20 mL). The organic layer was dried over MgSO₄, filtered and solvent was removed to provide crude product as viscous light brown liquid. Crude material was later purified by gradient flash column chromatography (20% ethyl acetate/80% hexanes to 100% ethyl acetate) to provide product, I, as white solid in 59% yield. 62 mg of the pure product was subjected to further purification by reverse phase HPLC using a Vydac C18 (250 x 10 mm, 10 μ) column according to the reported method. The product was further recrystallized (74% recovery) from acetonitrile/water to provide amino acid, I, in >99% ee. M.P. = 186-188 °C (Lit. 182-183 °C). [α]D^23 = +45.1 (c = 0.25, CH₃OH) (Lit. [α]D^24 = +31.9, c = 0.25, CH₃OH). IR (cm⁻¹) 3056, 1709. ^1H-NMR (400 MHz, MeOD) δ 7.77 (d, J = 7.5 Hz, 2H), 7.70 (d, J = 7.0 Hz, 2H), 7.36 (dd, J = 13.3, 7.5 Hz, 8H), 7.27 – 7.12 (m, 12H), 4.34 – 4.22 (m, 3H), 3.13 (d, J = 11.6 Hz, 1H), 2.62 (d, J = 11.6 Hz, 1H), 1.32 (s, 3H). ^13C-NMR (100 MHz, MeOD) δ 176.54, 156.90, 146.14, 145.28, 145.22, 142.52, 130.74, 128.83, 128.74, 128.20, 127.73, 126.40, 120.87, 67.91, 67.22, 59.68, 38.81, 23.69. HRMS (C₃₈H₃₃NO₄S Na⁺) calcd = 622.202250 m/z, obsd = 622.201790 m/z.

20, (S)-3-tert-butoxy-2-(tert-butylthiomethyl)-2-methyl-3-oxopropanoic acid:

Compound 20 was prepared according to the reported procedure from chiral half ester 16.
21, (S)-Tert-butyl 2-(((9H-fluoren-9-yl) methoxy) carbonylamino)-3-( tert-butylthio)-2-methylpropanoate:

To a 100 mL, three neck round bottom flask under a N₂ atmosphere, 2.0 g (7.23 mmol) of chiral tert-butyl half ester 20 was added as a solution in 35 mL of anhydrous 1,2-dichloroethane. 1.21 mL (8.68 mmol) of triethylamine was added to the flask and the mixture was stirred for 10 min. at room temperature. 1.67 mL (7.73 mmol) of diphenylphosphorylazide was added to the reaction flask and the reaction mixture was allowed to stir at room temperature under a N₂ atmosphere for 2 hrs. The mixture was then heated to reflux solvent for 2.5 hrs under a N₂ atmosphere at which time the azide had been completely consumed. At this point the reaction mixture was cooled to room temperature and washed quickly with saturated NH₄Cl solution (1 x 40 mL). The organic portion was dried over MgSO₄, filtered and the solvent was removed under vacuum to afford crude isocyanate. The crude isocyanate was dissolved in 30 mL of freshly distilled toluene and transferred immediately to a 100 mL one neck round bottom flask under a N₂ atmosphere. To the reaction flask containing crude isocyanate, 1.84 g (9.4 mmol) of 9-Fluorenemethanol was added and contents were allowed to stir for 5 min. Then 0.136 mL (0.46 mmol) of titanium (IV) isopropoxide was added to the reaction flask and the reaction mixture was heated to 83 °C using an oil bath. The mixture was allowed to heat over night under a N₂ atmosphere. The mixture was cooled to room temperature and the solvent was removed under vacuum to provide crude product. This crude product was taken up in diethyl ether (25 mL) and washed with water (2 x 20 mL) followed by saturated NaCl (1 x 25 mL). The
organic layer was dried over MgSO₄ and the solvent was removed under vacuum to provide the crude product as a viscous yellow oil. The crude product was then purified by flash column chromatography (10% diethyl ether / 90% hexanes; Rf = 0.24) to provide 1.73g (3.68 mmol) of pure (S)-NH-Fmoc S-tert-butyl Cys-OMe (10) as a clear viscous liquid in 51% yield. [α]D²³ = +5.6 (c= 1.0, CHCl₃). IR (cm⁻¹) 3416, 2974, 1716. ¹H-NMR (400 MHz, CDCl₃) δ 7.75 (d, J = 7.5 Hz, 2H), 7.63 (t, J = 6.4 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.30 (t, J = 7.4 Hz, 2H), 5.95 (s, 1H), 4.44 – 4.18 (m, 3H), 3.44 (d, J = 11.8 Hz, 1H), 2.98 (d, J = 11.8 Hz, 1H), 1.66 (s, 3H), 1.50 (s, 9H), 1.29 (s, 9H). ¹³C-NMR (100 MHz, CDCl₃) δ 172.32, 154.66, 144.28, 144.13, 141.46, 141.44, 127.81, 127.23, 125.52, 125.43, 120.09, 82.80, 66.85, 60.09, 47.35, 42.28, 34.67, 31.07, 28.07, 23.91. HRMS (C₂₇H₃₅NO₄S Na⁺) calcd = 492.217900 m/z, obsd = 492.217584 m/z.

(S)-19, (S)-2-(((9H-fluoren-9-yl) methoxy) carbonylamo)-2-methyl-3-(tritylthio) propanoic acid:

Step 1: S-tert-butyl deprotection

0.68g (1.45mmol) of compound 10, 0.924 g of mercury (II) acetate, 0.335 mL of anisole and 40 mL of TFA. Tert-butyl deprotection procedure is same as that reported for I. In this reaction, both S-tert-butyl and CO₂-tert-butyl groups were cleaved in one step to provide NH-Fmoc- Cα-Me-Cys-OH in quantitative yield.

Step 2: S-Trityl Protection

0.225g (0.63mmol) of NH-Fmoc- Cα-Me-Cys-OH was reacted with 0.158 g (0.6mmol) of triphenylmethanol in 20 mL of TFA according to the procedure.
reported for S-Tritylation in the synthesis of compound I. After flash column purification, 0.295 g (0.492 mmol) of II was isolated in 82% yield. This compound was further recrystallized (84% recovery) from acetonitrile/water to provide pure amino acid, II, in >99% ee as a white solid. M.P. = 186-189 °C (Lit.\textsuperscript{131} 192 °C). IR (cm\textsuperscript{-1}) = 3053, 1713. [α]\textsubscript{D}\textsuperscript{23} = -45.1 (c = 0.15, CH\textsubscript{3}OH). \textsuperscript{1}H-NMR (400 MHz, MeOD) δ 7.76 (d, J = 7.5 Hz, 2H), 7.68 (d, J = 6.8 Hz, 2H), 7.35 (dd, J = 16.2, 7.6 Hz, 8H), 7.17 (m, 12H), 4.29 (dd, J = 18.4, 7.6 Hz, 3H), 3.14 (d, J = 11.6 Hz, 1H), 2.62 (d, J = 11.6 Hz, 1H), 1.32 (s, 3H). \textsuperscript{13}C-NMR (100 MHz, MeOD) δ 176.50, 156.89, 146.11, 145.25, 145.19, 142.50, 130.72, 128.83, 128.74, 128.19, 127.73, 126.39, 120.87, 67.90, 67.23, 59.65, 38.79, 23.68. HRMS (C\textsubscript{38}H\textsubscript{33}NO\textsubscript{4}S Na\textsuperscript{+}) calcd = 622.202250 m/z, obsd = 622.201790 m/z.

**Note:** In the compounds containing Fmoc group, magnetically inequivalent aromatic carbons of the Fmoc group resulted in additional \textsuperscript{13}C resonances in the aromatic region of the spectra.\textsuperscript{139,140}
CHAPTER V - CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Conclusions

A diastereoselective synthetic methodology for \((3R, 5S)-5\)-benzyl-3-methyl-\(\beta\)-proline \((3R, 5S)-10c\) and its diastereomer \((3S, 5S)-10c\) has been developed. In this synthetic strategy the enzyme, Porcine Liver Esterase has served as a valuable catalyst to cause diastereoselective transformations of novel branched and chiral malonate diesters.

Methods for the preparation of malonate diesters, \(4a-c\), with highly branched side chains and a preexisting chiral center were developed. The method utilizes optically pure and relatively inexpensive amino acids as the starting materials. The diester substrates \((4a-c)\) derived from the amino acids were subjected to asymmetric hydrolysis by PLE to cause diastereoselective transformations. This unique approach of having a preset chiral center in the diester molecule eliminated the concern of producing enantiomers during PLE hydrolysis reaction as the reaction provided two compounds that are diastereomers to one another. The diastereoselectivities in the half esters produced from PLE hydrolysis were determined the \(^1\)H NMR analysis of the crude product. This helped us avoid methods such as HPLC using a chiral stationary phase, which could be more expensive and also time consuming.

Substrates \(4a-c\) underwent hydrolysis by crude PLE enzyme to provide mixture of diastereomeric half esters. The initial reactions with diester substrates \(4b\) and \(4c\) did not show good conversions. Cosolvent screening was performed
with substrates 4b and 4c to investigate optimum conditions that provided decent conversions and selectivities in the asymmetric hydrolysis with crude PLE. There was no significant improvement in the conversion of compound 4b under different cosolvent conditions.

PLE isoenzyme studies were also performed on the malonate diester substrates 4b and 4c to improve the conversions in the hydrolysis reaction. PLE isoenzymes 1, 2 and 5 did not show any reactivity with diester substrate 4b. There was slight improvement in the conversion of diester 4b with isoenzymes 3 and 6. However this improvement cannot be considered as significant as the yields on the reactions were under 30%. PLE isoenzymes 1 and 2 did not show any reactivity towards substrate 4c. Reversal of diastereoselectivity was observed in the case of substrate 4c with PLE isoenzymes 3 and 4. We have taken advantage of this selectivity reversal to synthesize the second diastereomer of the 5-benzyl substituted Cα-methyl-β-proline, (3S, 5S)-10c.

We have investigated the stereoselective cyclization reaction of two substrates 6b and 6c that provided the novel γ-lactams 7b and 7c. The cyclization reaction, however, was a very slow process and we speculate that this is due to the steric congestion caused by the bulky substituent adjacent to the nitrogen of phthalimide group. The novel γ-lactams derived from stereoselective cyclization reaction was then transformed into 5-benzyl substituted Cα-methyl-β-proline analogues (3R, 5S) and (3S, 5S)-10c.

The prepared amino acid analogues (3R, 5S)-10c and (3S, 5S)-10c were tested for their catalytic properties in the Mannich reaction between
isovaleraldehyde and a preformed α-imino ester. Amino acid (3S, 5S)-10c catalyzed the Mannich reaction very efficiently providing excellent diastereo and enantio selectivities at 1% catalyst loadings when the reaction was performed in relatively non polar solvent, methylene chloride. Amino acid (3R, 5S)-10c, when used in the Mannich reaction, we saw good diastereoselectivities towards the anti-Mannich Products. However, the enantioselectivities in the reaction were observed to be poor due to steric crowding in the transition state. Solvent screening in the Mannich reaction using amino acid (3R, 5S)-10c suggested that there is significant improvement in the enantioselectivities when the reaction was performed in relatively non polar methylene chloride as compared to DMSO. This solvent dependence on enantioselectivities needs to be further investigated.

The compound (3S, 5S)-10c was also tested for its activity in Aldol reaction between acetone and 4-nitro benzaldehyde. There was no progress in the reaction when performed in solvents such as DMSO or methylene chloride. However the reaction went to completion within two hours, when the reaction was performed in water yielding racemic mixture of products. The designed amino acid (3S, 5S)-10c is a very efficient catalyst in the anti-Mannich reaction between isovaleraldehyde and a preformed N-PMP protected α-imino ester.

A short and convenient synthesis for the preparation of orthogonally protected NH-Fmoc-S-Trityl, Cα-methyl cysteine suitable for solid phase peptide synthesis was developed. This methodology involves the preparation of enantioenriched chiral malonate half-esters from asymmetric bio catalytic hydrolysis with the enzyme Porcine Liver esterase. Curtius rearrangement of the
half ester followed by Fmoc protection of amine in the presence of Titanium (IV) Isopropoxide in a single step was the crucial step in the process. A mild and quick S-tert-butyl deprotection was performed using Mercury (II) Acetate. A method to improve the enantiopurity of the orthogonally protected Ca-methyl cysteine by recrystallization has been developed. This optically pure and orthogonally protected amino acid is utilized in solid phase peptide synthesis for biological studies by our collaborators at University of Vermont and Virginia Commonwealth University.

5.2 Future Directions

The yields of PLE hydrolysis of the novel branched malonate diesters can be improved further. The reaction can be performed in the phosphate buffer with a significant excess of organic solvents such as 20% DMSO to see if there is any improvements in the conversions without affecting the diastereoselectivity of the reaction. The hydrazine hydrolysis reaction of malonate diester 4c that showed slight preference in cyclization towards one ethyl ester over the other needs to be investigated to understand the role of bulky benzyl substituent on the observed selective cyclization.

The designed amino acid (3S, 5S)-10c can be tested for its use as catalyst in the Mannich reaction with various substrates. The designed amino acid can also be investigated for its catalysis in other reactions such as Michael reaction. Computational studies can be performed on the Aldol reaction between acetone and 4-nitro benzaldehyde catalyzed by the synthesized amino acid (3S, 5S)-10c to investigate the observed poor outcomes of the reaction.
APPENDIX A

A.1 $^1$H and $^{13}$C NMR Spectra

Figure A.1 $^1$H and $^{13}$C NMR Spectra of compound 2a
Figure A.2 $^1$H NMR Spectrum of compound 2b
Figure A.3 $^1$H and $^{13}$C NMR Spectra of compound 2c
Figure A.4 $^1$H NMR Spectrum of compound 3a

Figure A.5 $^1$H NMR Spectrum of compound 3b
Figure A.6 $^1$H NMR Spectrum of compound $3c$
Figure A.7 $^1$H and $^{13}$C NMR Spectra of compound 4a
Figure A.8 $^1$H and $^{13}$C NMR Spectra of compound (2R, 4S)-5a
Figure A.9 $^1$H and $^{13}$C NMR Spectra of compound 4b
Figure A.10 $^1$H and $^{13}$C NMR Spectra of compound (2R, 4S)-5b
Figure A.11 $^1$H NMR Spectrum of compound 4c

Figure A.12 $^1$H NMR Spectrum of compound (2R, 4S)-5c
Figure A.13 $^1$H NMR Spectrum of compound (2$R$, 4$S$)-5c

Figure A.14 $^1$H NMR Spectrum of compound (2$R$, 4$S$)-5c
Figure A.15 $^1$H NMR Spectrum of compound (2$R$, 4$S$)-5c
Figure A.16 $^1$H and $^{13}$C NMR Spectra of compound (2S, 4S)-6b
Figure A.17 $^1$H and $^{13}$C NMR Spectra of compound (2S, 4S)-6c
Figure A.18 $^1$H and $^{13}$C NMR Spectra of compound (3R, 5S)-7b
Figure A.19 $^1$H and $^{13}$C NMR Spectra of compound (3$R$, 5$S$)-7c
Figure A.20 $^1$H and $^{13}$C NMR Spectra of compound (3S, 5S)-8c
Figure A.21 $^1$H and $^{13}$C NMR Spectra of compound (3$R$, 5$S$)-9c
Figure A.22 $^1$H and $^{13}$C NMR Spectra of compound (3R, 5S)-10c
Figure A.23 ¹H and ¹³C NMR Spectra of compound (3S, 5S)-7c
Figure A.24 $^1$H and $^{13}$C NMR Spectra of compound (3R, 5S)-8c
Figure A.25 $^1$H and $^{13}$C NMR Spectra of compound (3S, 5S)-9c
Figure A.26 $^1$H and $^{13}$C NMR Spectra of compound (3S, 5S)-10c
A.1.2 2-D NMR Experiments of (3R, 5S)-7b

Figure A.27 (3R, 5S)-7b showing proton assignments for 2-D NMR analysis

Figure A.28 1H HOMO COSY NMR Spectrum of compound (3R, 5S)-7b
Figure A.29 Observed Key 2D-NOESY correlations in compound (3R, 5S)-7b

Figure A.30 2-D NOESY NMR spectrum of compound (3R, 5S)-7b
A.2 $^1$H NMR Data of PLE isoenzyme Studies

A.2.1 $^1$H NMR Data - PLE Isoenzyme Reactions of substrate 4c

Figure A.31 $^1$H NMR Data of half ester from PLE 3 hydrolysis of diester 4c
Figure A.32 $^1$H NMR Data of half ester from PLE 4 hydrolysis of diester 4c

Figure A.33 $^1$H NMR Data of half ester from PLE 5 hydrolysis of diester 4c
Figure A.34 $^1$H NMR Data of half ester from PLE 6 hydrolysis of diester 4c
A.2.2 $^1$H NMR Data - PLE Isoenzyme Reactions of substrate 4b

Figure A.35 $^1$H NMR Data of half ester from PLE 3 hydrolysis of diester 4b

Figure A.36 $^1$H NMR Data of half ester from PLE 4 hydrolysis of diester 4b
Figure A.37 $^1$H NMR Data of half ester from PLE 6 hydrolysis of diester 4b
A.3 Mannich Reaction- $^1$H NMR and HPLC Data

A.3.1 $^1$H NMR Data

**Reaction conditions:**

**Catalyst (mol %):** L-proline (20 mol %)

**Reaction Solvent:** Anhydrous DMSO

**Diastereomeric Ratio:** 23:1 (syn)

Figure A.38 $^1$H NMR of (2S, 3S)-syn-13 Mannich product
Figure A.39 $^1$H NMR of (2$R$, 3$S$)-anti-13 Mannich product

**Reaction conditions:**

**Catalyst (mol %):** (3$R$, 5$S$)-10c (5 mol %)

**Reaction Solvent:** Anhydrous DMSO

**Diastereomeric Ratio:** 4:1 (anti)
Figure A.40 $^1$H NMR of (2S, 3R)-anti-13 Mannich product

**Reaction conditions:**

*Catalyst (mol %):* (3R, 5S)-10c (5 mol %)

*Reaction Solvent:* 2-Propanol: methylene chloride (1:1)

*Diastereomeric Ratio:* 22:1(*anti*)
Figure A.41 $^1$H NMR of (2S, 3R)-anti-$13$ Mannich product

**Reaction conditions:**

* Catalyst (mol %): (3R, 5S)-$10c$ (5 mol %)  
* Reaction Solvent: Methylene Chloride  
* Diastereomeric Ratio: 18:1 (anti)
Figure A.42 $^1$H NMR of (2R, 3S)-anti-13 Mannich product

**Reaction conditions:**

*Catalyst (mol %):* (3S, 5S)-10c (5 mol %)

*Reaction Solvent:* Methylene Chloride

*Diastereomeric Ratio:* 23:1(anti)
Figure A.43 $^1$H NMR of $(2R, 3S)$-anti-13 Mannich product

**Reaction conditions:**

**Catalyst (mol %):** $(3S, 5S)$-10c (1 mol %)

**Reaction Solvent:** Methylene chloride

**Diastereomeric Ratio:** 23:1 (syn)
### A.3.2 HPLC Data

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% enantiomeric excess [(2S, 3S)-syn-13] = 97%

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Figure A.44 HPLC of Mannich reaction by L-Proline (DMSO, 20 mol %)
Figure A.45 HPLC of Mannich reaction by (3R, 5S)-10c (DMSO, 5 mol %)

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% enantiomeric excess [(2R, 3S)-anti-13] = 9%
Figure A.46 HPLC of Mannich reaction by (3R, 5S)-10c (2-PrOH /CH$_2$Cl$_2$, 5 %)

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Min.)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2S,3R)-anti-13</td>
<td>25.683</td>
<td>23213.2350</td>
<td>377.139</td>
</tr>
<tr>
<td>(2S,3S)-syn-13</td>
<td>35.483</td>
<td>976.6740</td>
<td>13.276</td>
</tr>
<tr>
<td>(2R,3S)-anti-13</td>
<td>55.500</td>
<td>18418.0645</td>
<td>143.297</td>
</tr>
<tr>
<td>(2R,3R)-syn-13</td>
<td>62.866</td>
<td>989.4590</td>
<td>7.802</td>
</tr>
</tbody>
</table>

% enantiomeric excess [(2S, 3R)-anti-13] = 11%
Figure A.47 HPLC of Mannich reaction by (3R, 5S)-10c (CH2Cl2, 5 mol %)

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Min)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2S,3R)-anti-13</td>
<td>24.916</td>
<td>61760.3780</td>
<td>745.585</td>
</tr>
<tr>
<td>(2S,3S)-syn-13</td>
<td>34.366</td>
<td>2047.8290</td>
<td>27.859</td>
</tr>
<tr>
<td>(2R,3S)-anti-13</td>
<td>53.050</td>
<td>31665.3630</td>
<td>184.096</td>
</tr>
<tr>
<td>(2R,3R)-syn-13</td>
<td>63.983</td>
<td>1945.4390</td>
<td>9.099</td>
</tr>
</tbody>
</table>

% enantiomeric excess [(2S, 3R)-anti-13] = 32%
Figure A.48 HPLC of Mannich reaction by (3S, 5S)-10c (CH$_2$Cl$_2$, 5 mol %)

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Min)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2S,3R)-anti-13</td>
<td>29.116</td>
<td>810.1120</td>
<td>11.668</td>
</tr>
<tr>
<td>(2S,3S)-syn-13</td>
<td>38.416</td>
<td>1110.7530</td>
<td>13.839</td>
</tr>
<tr>
<td>(2R,3S)-anti-13</td>
<td>56.183</td>
<td>139337.5780</td>
<td>490.823</td>
</tr>
<tr>
<td>(2R,3R)-syn-13</td>
<td>70.783</td>
<td>4404.7575</td>
<td>13.001</td>
</tr>
</tbody>
</table>

% enantiomeric excess [(2R, 3S)-anti-13] = 99%
Figure A.49 HPLC of Mannich reaction by (3$S$, 5$S$)-10c (CH$_2$Cl$_2$, 1 mol %)

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Min.)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2$S$,3$R$)-anti-13</td>
<td>27.916</td>
<td>851.8245</td>
<td>13.523</td>
</tr>
<tr>
<td>(2$S$,3$S$)-syn-13</td>
<td>37.883</td>
<td>655.8450</td>
<td>7.777</td>
</tr>
<tr>
<td>(2$R$,3$S$)-anti-13</td>
<td>60.616</td>
<td>87811.9000</td>
<td>306.578</td>
</tr>
<tr>
<td>(2$R$,3$R$)-syn-13</td>
<td>77.433</td>
<td>2600.3070</td>
<td>13.218</td>
</tr>
</tbody>
</table>

% enantiomeric excess [(2$R$, 3$S$)-anti-13] = 98%
A.4 Aldol Reaction-¹H NMR and HPLC Data

Figure A.50 ¹H NMR of the Aldol Addition Product
Figure A.51 HPLC of Aldol reaction by L-proline, (DMSO, 1 mol %)

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Time)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-Aldol (Major)</td>
<td>24.4</td>
<td>115814.6770</td>
<td>619.142</td>
</tr>
<tr>
<td>(S)-Aldol (Minor)</td>
<td>36.2</td>
<td>20153.4885</td>
<td>88.884</td>
</tr>
</tbody>
</table>

% enantiomeric excess = 70% (R)-Aldol Product
Figure A.52 HPLC of Aldol Reaction by \((3S, 5S)-10c\) (D\(_2\)O, 10 mol %)

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Time)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>((R))-Aldol</td>
<td>24.4</td>
<td>63875.0665</td>
<td>619.142</td>
</tr>
<tr>
<td>((S))-Aldol</td>
<td>34.3</td>
<td>63088.0130</td>
<td>88.884</td>
</tr>
</tbody>
</table>

\% enantiomeric excess = 0.6\% (Racemic)
A.5 NMR and HPLC Data of NH-Fmoc S-trityl-Cα-methyl cysteine

A.5.1 $^1$H NMR Data

Figure A.53 $^1$H and $^{13}$C NMR data of compound 14
Figure A.54 $^1$H and $^{13}$C NMR data of compound 15
Figure A.55 $^1$H and $^{13}$C NMR data of compound 17
Figure A.56 $^1$H and $^{13}$C NMR data of compound 18
Figure A.57 $^1$H and $^{13}$C NMR data of compound (R)-19
Figure A.58 $^1$H and $^{13}$C NMR data of compound 21
Figure A.59 $^1$H and $^{13}$C NMR data of compound (S)-19
A.5.2 HPLC Data

Figure A.60 HPLC of (R)-19 prior to recrystallization

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Min)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-19 (Major)</td>
<td>6.983</td>
<td>31805.42</td>
<td>1642.371</td>
</tr>
<tr>
<td>(S)-19 (Minor)</td>
<td>9.066</td>
<td>927.342</td>
<td>29.953</td>
</tr>
</tbody>
</table>

Enantiomeric Excess = 94%
Figure A.61 HPLC of (R)-19 after recrystallization

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Min)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-19 (Major)</td>
<td>6.4</td>
<td>42529.43</td>
<td>2311.819</td>
</tr>
</tbody>
</table>

*Minor Enantiomer-Not Observed

Enantiomeric Excess = >99%
Figure A.62  HPLC of \((S)-19\) prior to recrystallization

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Min)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>((R)-19)</td>
<td>7.2</td>
<td>4161.98</td>
<td>203.036</td>
</tr>
<tr>
<td>((S)-19)</td>
<td>8.816</td>
<td>100143.3</td>
<td>2949.468</td>
</tr>
</tbody>
</table>

Enantiomeric Excess = 92%
Figure A.63 HPLC of (S)-19 after recrystallization

<table>
<thead>
<tr>
<th>Component</th>
<th>Retention (Time)</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-19 (Minor)</td>
<td>7.35</td>
<td>23.185</td>
<td>0.832</td>
</tr>
<tr>
<td>(S)-19 (Major)</td>
<td>8.966</td>
<td>39545.41</td>
<td>1372.462</td>
</tr>
</tbody>
</table>

Enantiomeric Excess = >99%
Figure A.64 Reverse Phase HPLC of Compound \((R)-19\)

Retention time of pure \((R)-19 = 35.5\) Min \(^{131}\)
REFERENCES


(13) List, B. Synlett 2001, 1675.


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(64) Sani, M.; Fossati, G.; Huguenot, F.; Zanda, M. Angew. Chem., Int. Ed. 2007, 46, 3526.


(80) Masterson, D. S.; Kedrowski, B. L.; Blair, A. *Synlett* 2010, 2941.


(91) Sigma-Aldrich; (S)-(+)2-Amino-1-propanol.

(92) Sigma-Aldrich; (S)(-)-2-Amino-3-phenyl-1-propanol.

(93) Sigma-Aldrich; (S)(+)-Leucinol.


