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

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The University of Southern Mississippi

AN INSIGHT INTO ASYMMETRIC SYNTHESIS AND BIOORGANIC APPLICATIONS OF NOVEL C^{α}-METHYL-LYSINE, -PROLINE, -NIPECOTIC ACID ANALOGUES

by

Souvik Banerjee

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

December 2013

ABSTRACT

AN INSIGHT INTO ASYMMETRIC SYNTHESIS AND BIOORGANIC APPLICATIONS OF NOVEL C^{α}-METHYL-LYSINE, -PROLINE, -NIPECOTIC ACID ANALOGUES

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Prochiral malonic diesters consisting of a quaternary carbon center have been successfully converted into a different set of ^{t}Boc-Fmoc-$\alpha^{2,2}$-methyllysine-OH analogues through chiral malonic half-ester intermediates achieved via enzymatic (Pig Liver Esterase, PLE) hydrolysis. The selection of chiral half-ester intermediates, which vary from 1 to 6 methylene units in the side chain, are achieved in high optical purity (92% - 97% ee) and in good yields (65% - 72%). The PLE hydrolysis of malonic diesters with a variety of side chain lengths observed to obey the Jones's PLE model as evidenced from the stereochemical configurations of the resulting chiral half-esters. The optimized synthetic strategy allows the construction of both enantiomers of $\alpha^{2,2}$-methyllysine analogues, and a (*S*)-$\beta^{2,2}$-methyllysine analogue from a common synthon by straightforward exploitation of protecting groups. Two different straightforward synthetic strategies are illustrated for the synthesis of $\alpha^{2,2}$-methyllysine analogues. The described strategies should find significant usefulness in preparing novel peptide libraries with unnatural lysine analogues. A Vapreotide analogue incorporating (*S*)-$\alpha^{2,2}$-methyllysine was constructed. However, the Vapreotide analogue with (*S*)-α-methyl-α-lysine is found to lose its specific binding to somatostatin receptor subtype 2 (SSTR2). In an additional project, a stereoselective and enantiodivergent cyclization strategy for the preparation of

γ/δ-lactams is exhibited. The cyclization strategy exploits chiral malonic esters prepared from enantiomerically enriched (92% ee - 97% ee) mono esters of disubstituted malonic acid. The cyclization takes place with the selective departure of a substituted benzyl alcohol as the leaving group. A Hammett study demonstrates that the cyclization is under electronic control. The resulting γ/δ-lactam was readily converted into a novel proline/nipecotic acid analogue.

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CHAPTER I

INTRODUCTION

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

Unnatural Amino Acids (UAAs) in Peptidomimetics

UAAs have been extensively employed in peptidomimetics in order to develop a variety of inhibitors of enzymes that are responsible for disease progression.[2-6] It is evident that the presence of artificial backbones in the unnatural peptides confers a higher degree of resistance to enzymatic degradation as opposed to their natural counterparts.[4] A few years ago, Oh et al. reported that the introduction of unnatural amino acids in anti-

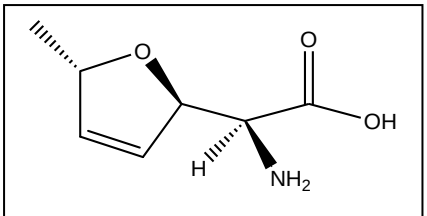
microbial peptides improves their protease resistance as much as three times without affecting their activity.[7]

Unnatural Amino Acids in Foldamers

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

Unnatural Amino Acids in Antibiotics

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



L-Furanomycine

Figure 1. L-furanomycine, a nature made nonproteinogenic amino acid.

UAAs as Building Blocks of Complex Molecular Structures

Unnatural amino acids have often served their role as the starting materials or building blocks of advanced molecules with unique biological activity.[16-18] Recently Wohlrab et al. reported total synthesis of Plusbacin A_3, a depsipeptide antibiotic, containing a number of non-proteinogenic amino acids.[17] Chandrashekhar et al. reported a concise total synthesis of Azumamide E, a marine cyclic terapeptide containing novel $\beta^{2,3}$-amino acids, that shows inhibitory activity to histone deacetylase.[19] Recently, Konno established total synthesis of three marine natural products (miraziridine A, tokaramide A, and callipeltins) containing unusual amino acids, showing strong cysteine protease inhibitor activity.[20]

UAAs as Chiral Auxiliaries and Organocatalysts

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

$C^{\alpha,\alpha}$-Disubstituted Non-Proteinogenic Amino Acids

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

Secondary Structures of the Peptides Consisting of $C^{\alpha,\alpha}$-Disubstituted Amino Acids

The secondary structures of peptides can be construed in term of torsion angles ψ, φ, ω, and the side chain conformations of the amino acids are illustrated by the torsion angles χ_1, χ_2, χ_3 (Figure 2). The torsion angle values in protein and peptides are determined through number of experimental structural data and computational simulation. In reality, it is possible to predict the effect of conformationally restricted amino acids on the outcome of the secondary structure of a peptide by evaluating the torsion angles of the conformationally rigid amino acids.[33]

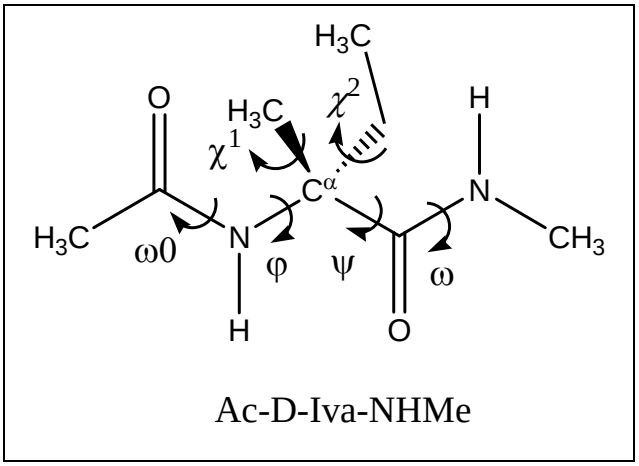


Figure 2. The backbone and side chain dihedral angles of the $C^{\alpha,\alpha}$-disubstituted amino acids.

One of the most frequently known $C^{\alpha,\alpha}$-disubstituted amino acids is α-aminoisobutyric acid (Aib, dimethylglycine, α-methyl-alanine) (Figure 3). In Aib residue, substitution of the α-hydrogen atom in alanine by a methyl group considerably restricts the available conformational space (Figure 4).[33] It is evident from a number of experimental results that Aib induces right handed (P) and left handed (M) 3_{10}-helical structures (φ, ψ = $\pm$ 60^0, $\pm$ 30^0) in a 1:1 ratio, both in solution and the crystal state.[33, 34] The reason behind the generation of two enantiomeric P and M- helices by Aib is that Aib is an achiral amino acid. To strongly emphasize, Aib residues neither induce semi-extended conformations nor extended conformations, unlike alanine which is found in both folded and extended conformations.[33]

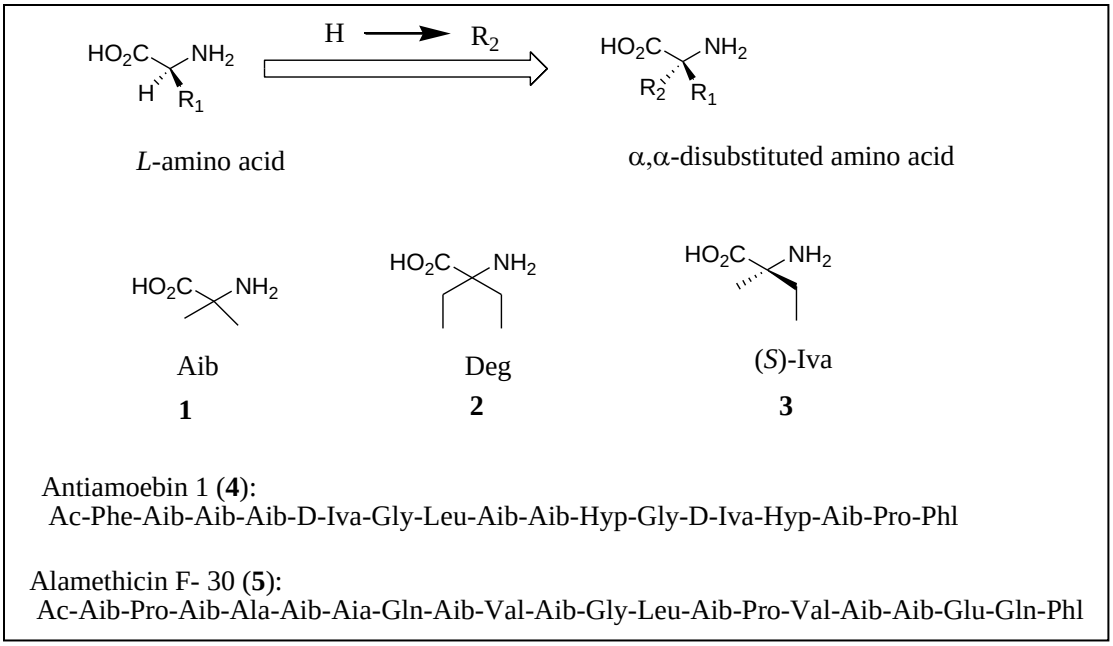


Figure 3. Structure of α,α-disubstituted amino acids and peptaibol antibiotics.

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

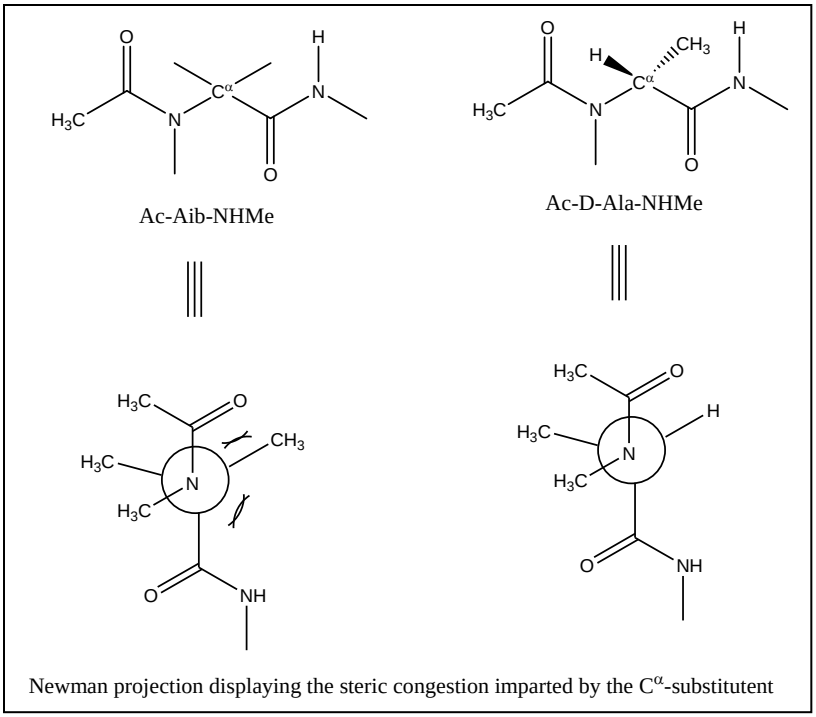


Figure 4. Newman projection exhibiting limited conformational space availability for the $C^{\alpha,\alpha}$-disubstituted amino acids.

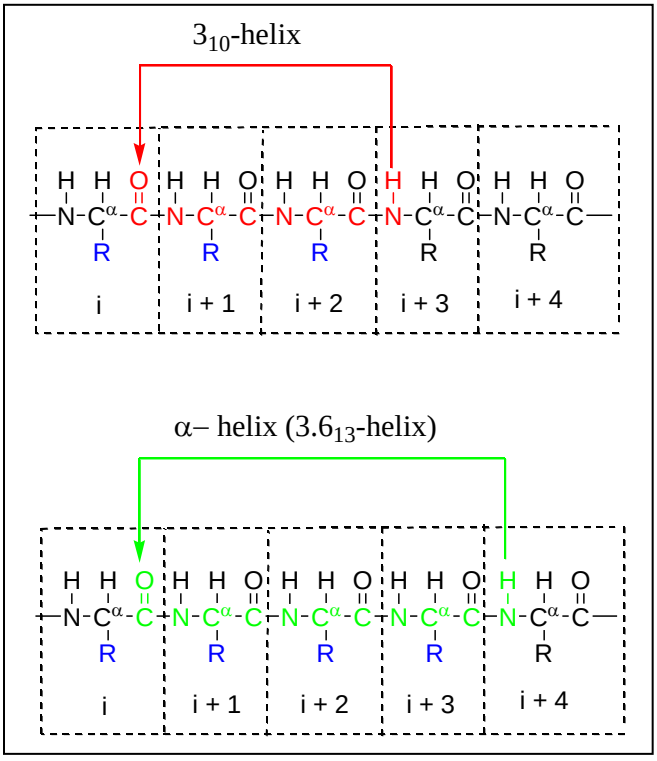


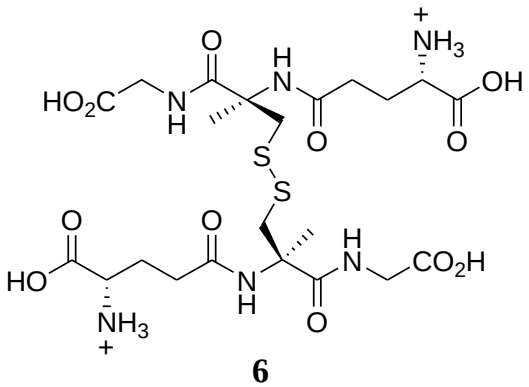
Figure 5. Hydrogen bonding pattern of 3_{10}-helix and α-helix.

$C^{\alpha,\alpha}$-Nonproteinogenic Amino Acids in Peptidomimetics

Oligopeptides consisting of naturally occurring L-α-amino acids often lead to unordered or unstable secondary structures due to the conformational flexibility of natural amino acids.[34] This is why $C^{\alpha,\alpha}$-disubstituted non-proteinogeninc amino acids have made their strong demand in the widely extended field of preparation of peptides with the interest to humanity. Natural peptaibol antibiotics, such as *anti*-amoebin, alamethicin, and zervamicin, have been found to be composed of Aib residues (Figure 3).[34] This is why α,α-disubstituted amino acids should be named as nonproteinogenic amino acids or non-coded amino acids as opposed to unnatural amino acids. Aib is one of the most widely used amino acids not only to introduce helical secondary structures into peptides but to design and synthesis of organocatalyst and drug candidates as well.[34] Substitution of the α-hydrogen atom in an L-α-amino acid, which results in $C^{\alpha,\alpha}$-disubstituted amino acid, with an alkyl moiety results in (1) improved chemical stability of the amino acids, (2) improved hydrophobicity of the amino acids, (3) constrained conformational freedom of the amino acid side chain, and (4) restricted conformation flexibility of the peptides containing them, and as a consequence enhanced metabolic stability of their peptides (Figure 1).[34]

In recent years, a variety of $C^{\alpha,\alpha}$-disubstituted nonproteinogenic amino acids have been used in the synthesis of medicinally important peptides due to their propensity to stabilize secondary structures of the peptides by introducing tremendous helix inducing potential.[29, 34-39] This helix inducing propensity is considered to be capable of stabilizing the secondary structures of the peptides by rigidifying the peptide backbone.[29, 34, 39-41] Hence, the peptides with higher conformational stability present improved resistance

against enzymatic and chemical degradations.[35, 36, 41, 42] Thus, α,α-disubstituted amino acids have very often been introduced in the peptide synthesis to confer enhanced metabolic stability to synthetic peptides (Figure 6).[3, 35-37] In addition, the remarkable helix inducing potential employed by $C^{\alpha,\alpha}$-disubstituted nonproteinogenic amino acids is believed to be responsible for the bacterial membrane destabilization effort produced by peptaibol antibiotics.[29]



6

Oxidized glutathione consisting $C^{\alpha,\alpha}$-disubstituted cysteine

D-Phe-Cys-Tyr-D-Trp-Orn-XY-Pen-Thr-NH

7

XY = Aib (**1**), Deg (**2**)

Somatostatin octreotide analogue consisting $C^{\alpha,\alpha}$-disubstituted amino acids

Figure 6. Synthetic peptides consisting of $C^{\alpha,\alpha}$-disubstituted amino acids.[35, 37]

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

$C^{\alpha,\alpha}$-disubstituted class of nonproteinogenic amino acids has been frequently employed as a pharmaceutical active agent (enzyme inhibitors or receptor antagonist) by itself or as a building block of the complex therapeutic agent.[29, 43-45] The remarkable increment in the steric congestion imparted by an additional α-substituent of $C^{\alpha,\alpha}$-disubstituted amino

acids either keeps the substrate from accessing the active site of enzyme or hinders the enzyme from initiating its catalyzing activity.[29, 36, 37] Thus, conformational constraint plays an important role in order for this class of amino acids being potent inhibitors of enzymes (reversible or irreversible).[36, 37, 46] Recently, Ilies et al. have developed two novel $C^{\alpha,\alpha}$-disubstituted amino acid analogues (Figure 7) as potent inhibitors of human arginase that is known to hydrolyze L-arginine to L-ornithine.[44]

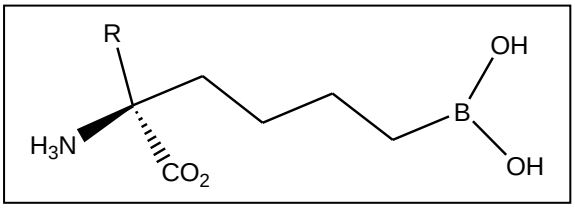


Figure 7. $C^{\alpha,\alpha}$-disubstituted amino acids as potent arginase inhibitor.[44]

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

The restricted conformational flexibility and improved metabolic stability of $C^{\alpha,\alpha}$-disubstituted amino acids make them an important constituent in complex receptor antagonist structures.[45] A few years back, a group of scientists from Meck & Co. developed a potent inhibitor of neurokinin 1 (NK1) receptor consisting of cyclic $C^{\alpha,\alpha}$-disubstituted-β-amino acid (Figure 9).[45] This group illustrates that the $C^{\alpha,\alpha}$-disubstituted amino acid framework is important for effective inhibition.[45]

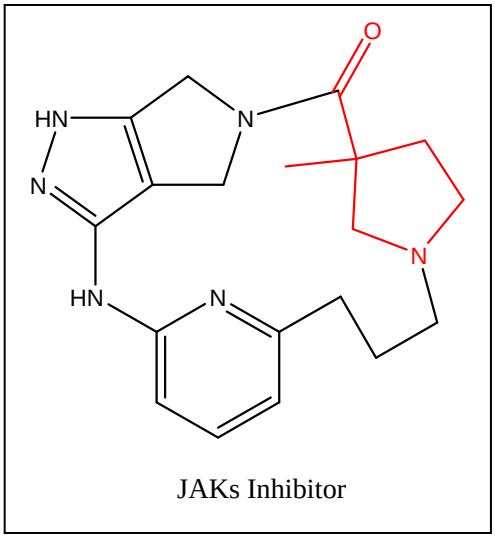


Figure 8. JAKs inhibitor consisting α-methyl-β-proline.[47]

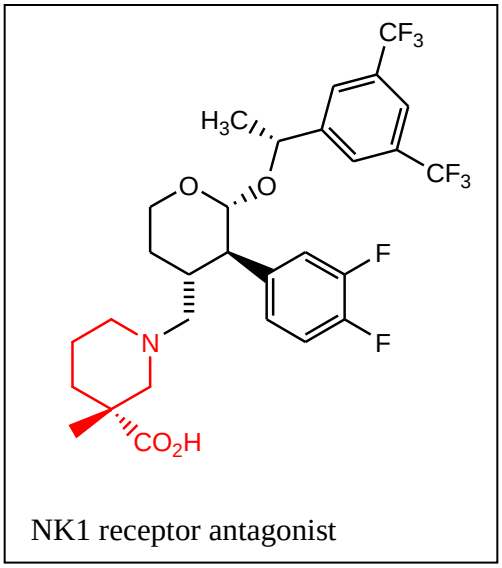


Figure 9. NK1 receptor antagonist consisting $C^{\alpha,\alpha}$-disubstituted amino acid.[45]

Recently, Novartis AG has discovered a novel small molecule potent antagonist of S1P receptor (for the treatment of diseases caused by S1P receptor modulators) consisting of constrained $C^{\alpha,\alpha}$-disubstituted amino acid as an important building block (Figure 10).[1]

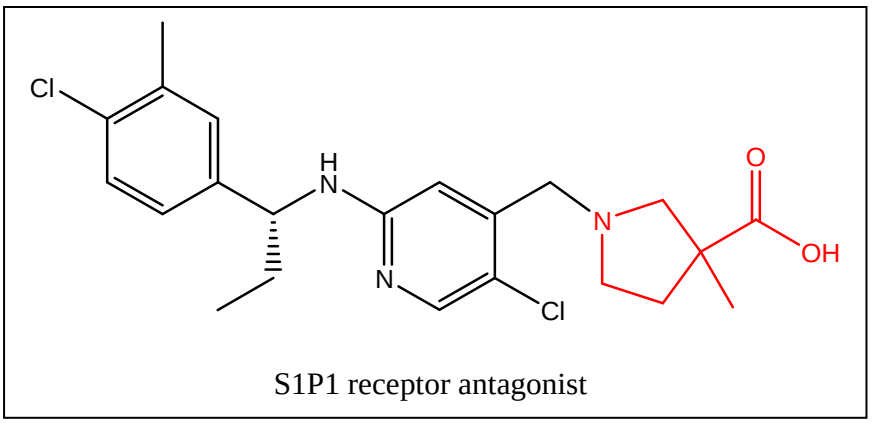
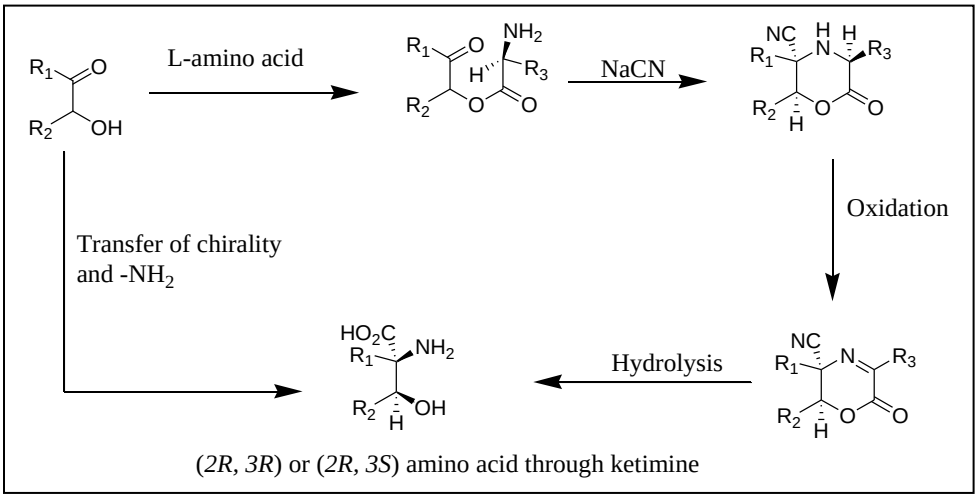


Figure 10. S1P receptor antagonist consisting C^{α}-methyl-β-proline.[1]

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

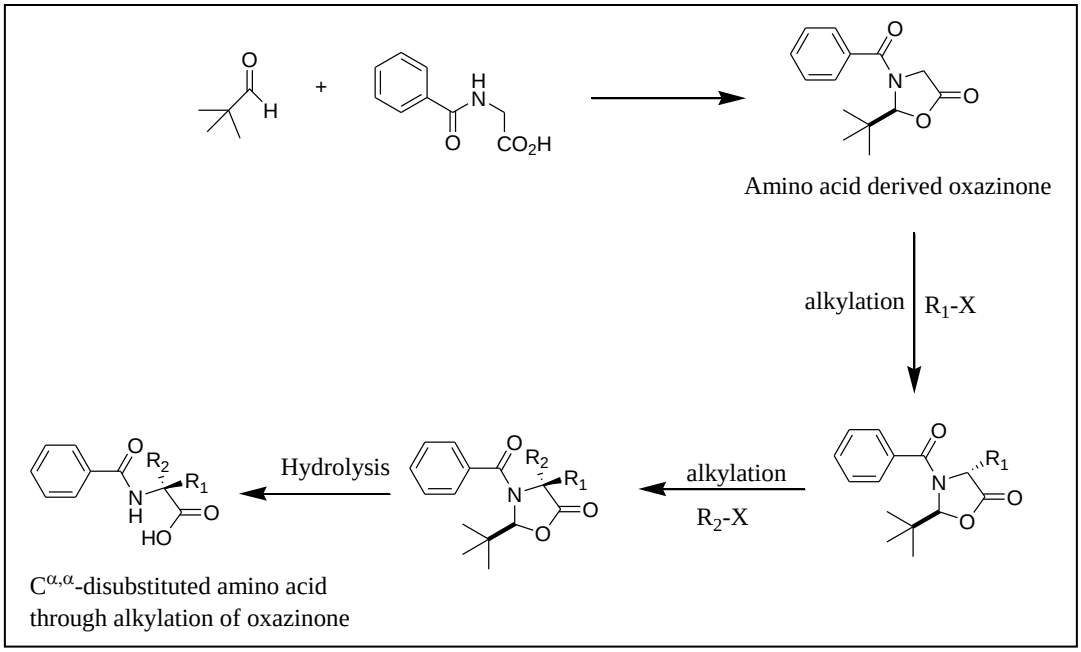
Although a number of synthetic strategies have been reported to date, synthesis of quaternary chiral centers is still one of the toughest challenges to synthetic organic chemists. Recently Vogt et al.[29] reviewed the recent widely explored synthetic strategies, which are employed to constitute quaternary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

1. Asymmetric Strecker Reaction Involving Aldimines or Ketimines or Strecker Related Reactions (Scheme 1).[28]



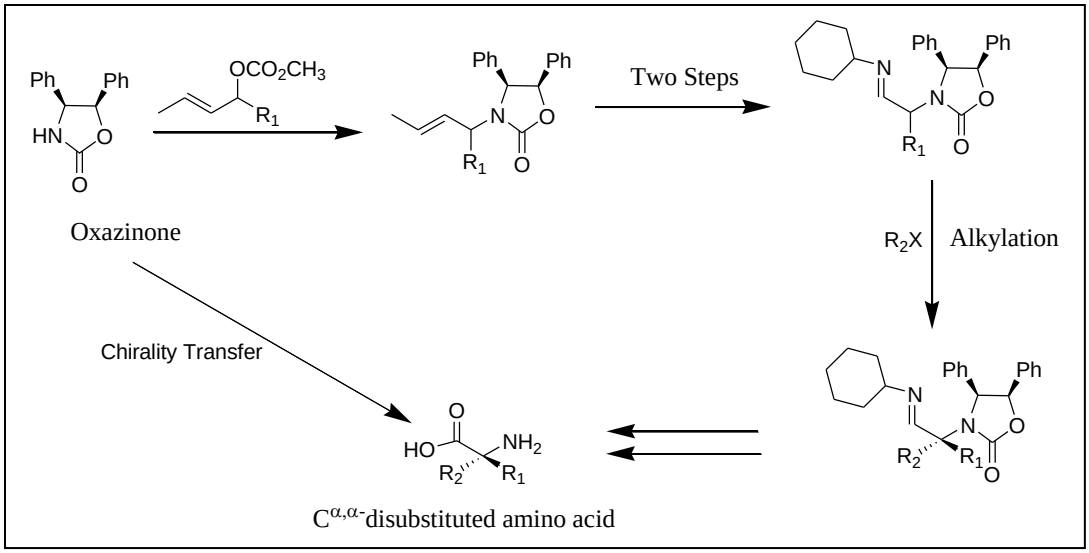
Scheme 1. Asymmetric Strecker reaction mediated trough ketimine.[28]

2. Electrophilic Alkylation of the Enolates Resulting from Oxazinones, Oxazolines, Oxazolidines, Azalactones, or Imines Derived from Amino Acids as Chiral Auxiliaries (Scheme 2).[30]



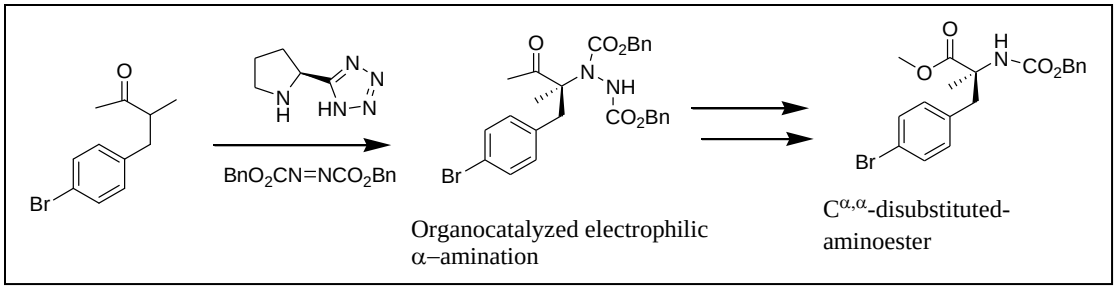
Scheme 2. Oxazinones as chiral auxiliaries.[30]

3. Electrophilic Alkylation of Chiral Imine Attached to an Oxazinone (Scheme 3).[48]



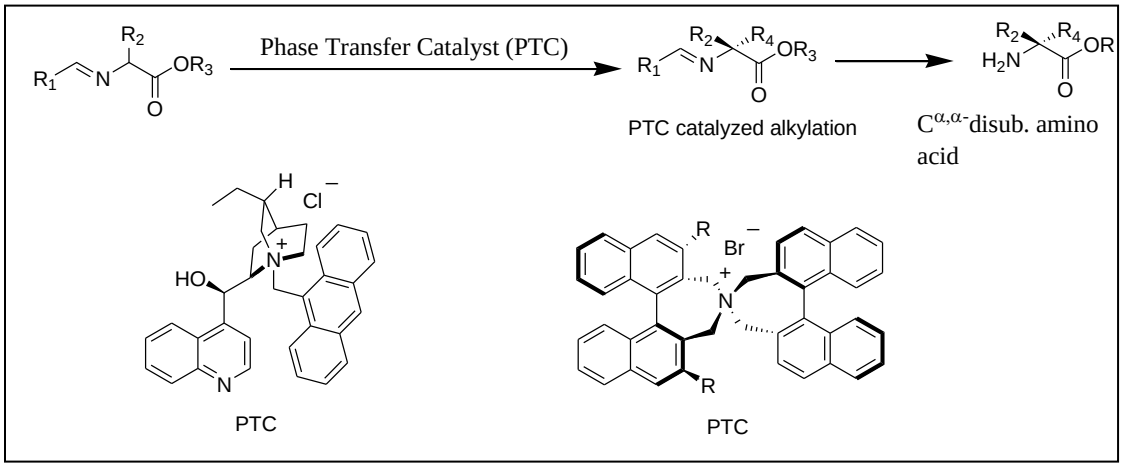
Scheme 3. Electrophilic alkylation of chiral imine.[48]

4. Organo Catalyzed Electrophilic α-Amination of the α-Substituted Carbonyl Compounds (Scheme 4).[49]



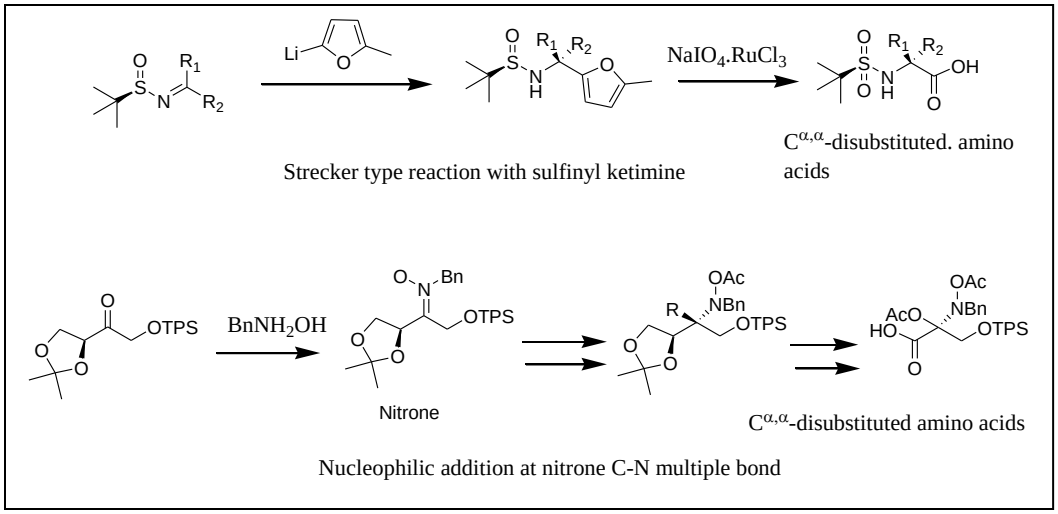
Scheme 4. Organocatalyzed electrophilic α-amination.[49]

5. Phase Transfer Catalyst Mediated Electrophilic Alkylation of Schiff Bases Derived from Amino Acids (Scheme 5).[29]



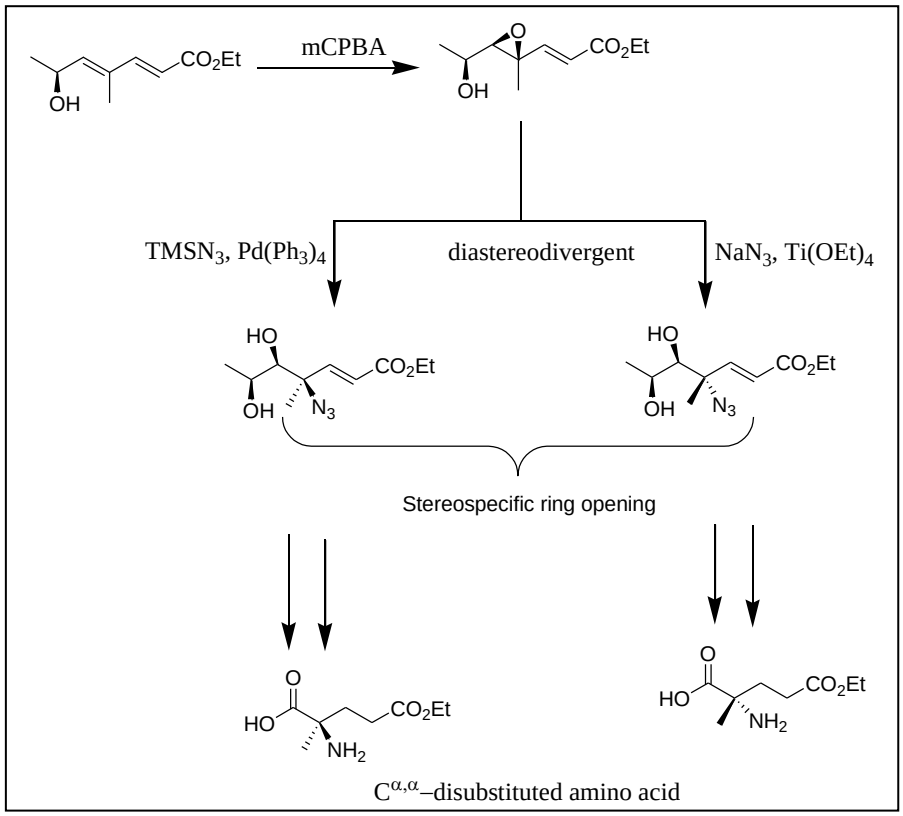
Scheme 5. PTC catalyzed electrophylic alkylation of Schiff bases.[29]

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



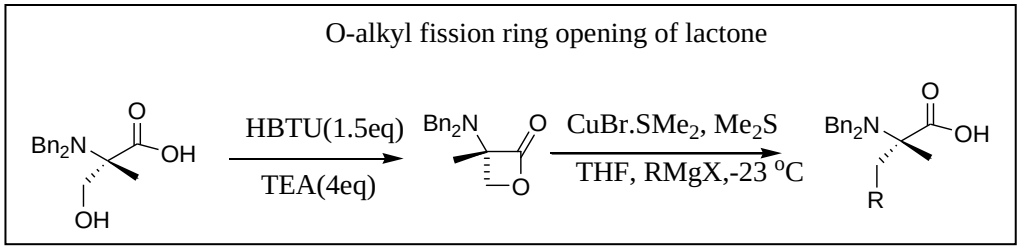
Scheme 6. Nucleophilic addition to C-N multiple bonds.[50, 51]

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

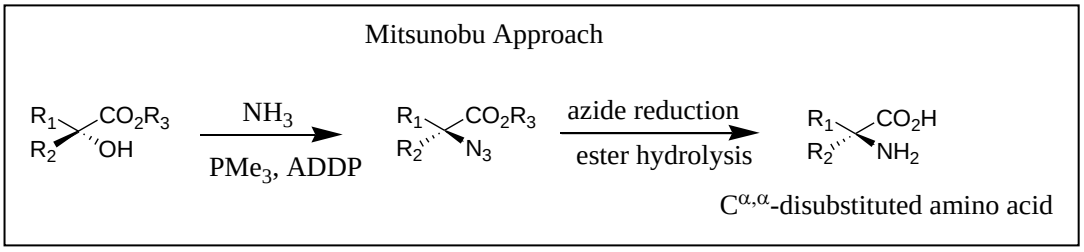


Scheme 7. Stereospecific ring opening of epoxide leading to the formation of $C^{\alpha,\alpha}$-disubstituted-amino acids.[52]

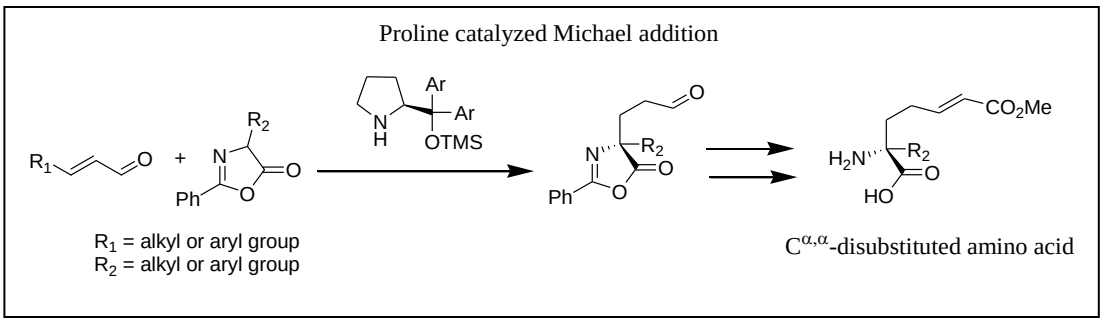
In continuation, Smith et al. presented the synthesis of several α,α-disubstituted-α-amino acids from a common intermediate employing nucleophilic "O-Alkyl Fission" ring opening of the NBn_2-α-methylserine lactone, using various organocuprates (Scheme 8).[53] Green et al. reported a novel strategy to prepare $C^{\alpha,\alpha}$-disubstituted amino acids through a Mitsunobu approach starting with optically pure α,α-disubstituted-α-hydroxy ester (Scheme 9).[54] Cabrera et al. optimized a unique strategy implicating organocatalyzed Michael addition of oxazolone enolates to the Michael acceptors (Scheme 10).[42] Recently, Hartmann et al. reported the synthesis of optically enriched α-methyl phenylglycine through *L*-proline catalyzed amination of racemic 2-arylpropionaldehydes, using DEAD and DBAD (Scheme 11).[55]



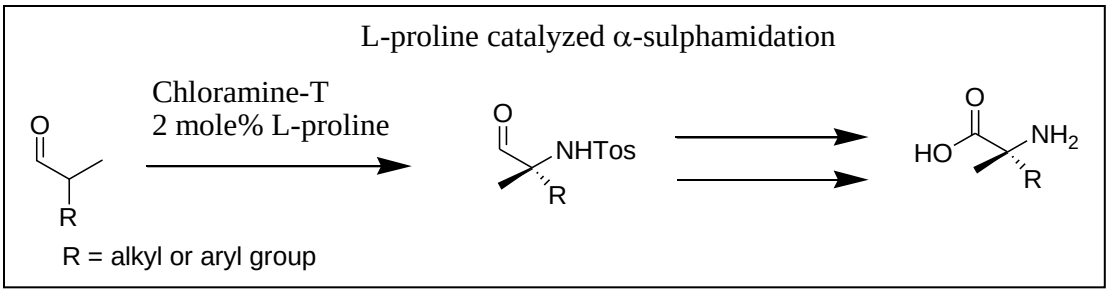
Scheme 8. O-alkyl fission ring opening of β-lactone.[53]



Scheme 9. Mitsunobu approach to prepare $C^{\alpha,\alpha}$-disubstituted amino acids.[54]



Scheme 10. Organocatalyzed Michael addition leading to $C^{\alpha,\alpha}$-disubstituted amino acid.[42]

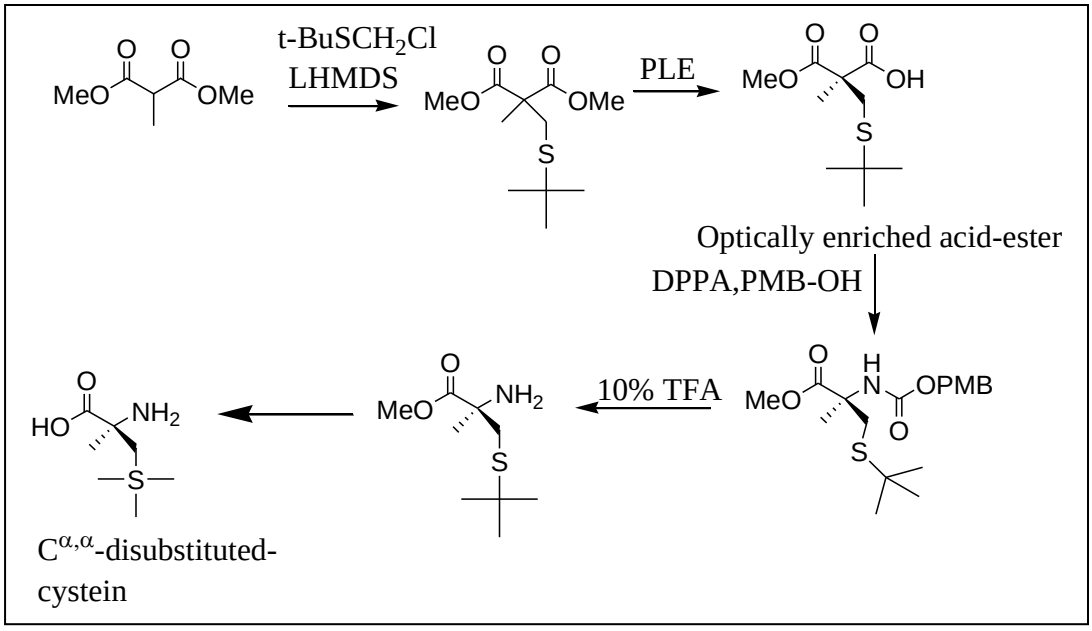


Scheme 11. L-proline catalyzed α-sulphimidation of α,α-disubstituted aldehydes.[55]

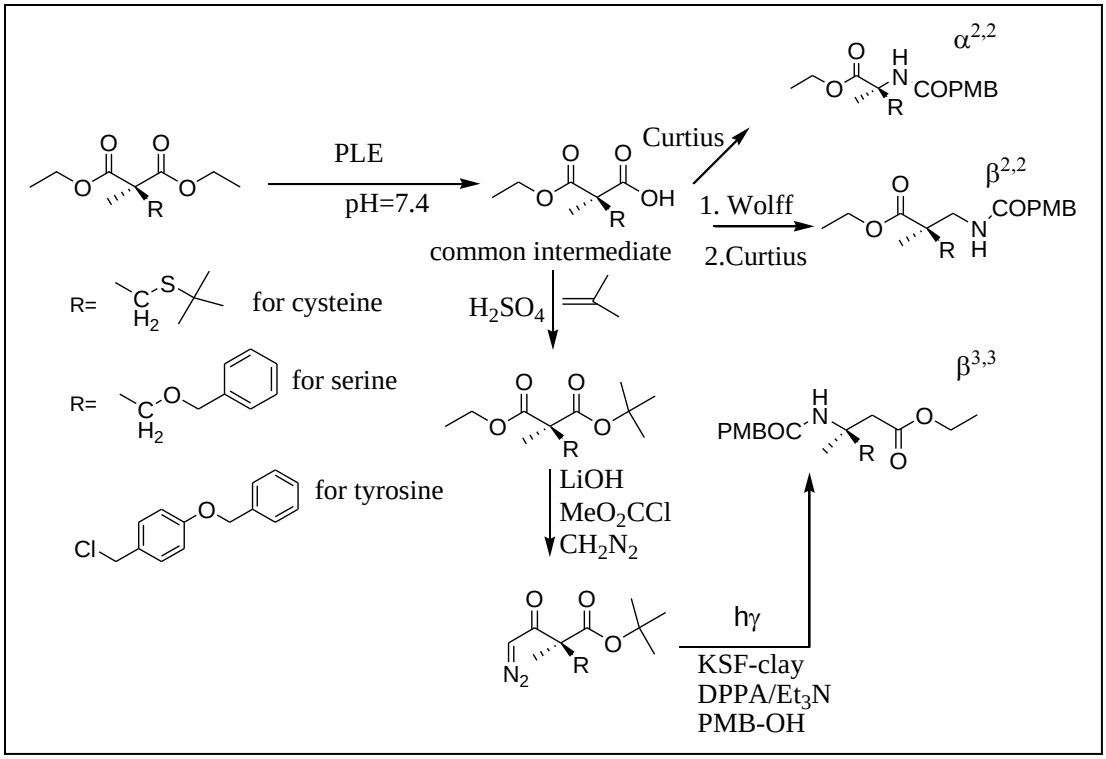
Pig Liver Esterase (PLE) Desymmetrization Approach to Prepare $C^{\alpha,\alpha}$-Disubstituted Amino Acids

Although numerous synthetic methodologies are established to construct α,α-disubstituted-α-amino acids, most often they rely on expensive chiral auxiliaries. Most of the time, different chiral auxiliaries are required to produce different stereoisomers of the same α,α-disubstituted amino acids. In addition, there are very few synthetic strategies allowing the constitution of both –α- and β- amino acid from a common synthon. This is why Pig Liver Esterase (PLE) is used. PLE is cheap and has proven its excellence in hydrolyzing a wide variety of prochiral quaternary malonic diesters to the corresponding optically enriched α,α-disubstitutedmalonic half-esters, which have been extensively employed as ideal precursors of α,α-disubstituted amino acids.[3, 32, 37, 46, 56, 57] To the best of our knowledge, Kedrowski[46] is the first to report both enantiomers of orthogonally

protected α-methyl-α-cysteine from the common optically enriched quaternary malonic half-ester, derived from PLE hydrolysis of prochiral dimethyl malonate (Scheme 12). Very recently Iosub et al.[58] has also shown the synthesis of α,α-disubstituted-α-amino acid from enantiomerically enriched PLE hydrolyzed methyl malonic half-ester . However, dimethyl-2-methyl malonate that is used for enolization is expensive compared to diethyl-2-methyl malonate. Masterson et al. have recently reported synthesis of both (*R*)- and (*S*)-$\alpha^{2,2}$-, $\beta^{2,2}$-, and $\beta^{3,3}$-Cysteine and serine analogues from the common PLE hydrolyzed optically enriched quaternary ethyl malonic half-esters with respective amino acid side chains (Scheme 13).[3] Recently, Falgner et al.[56] have also established the synthesis of both (*R*)-and (*S*)-α-methyl-trimethylsilyl alanine with excellent enantio purity starting with PLE desymmetrized ethyl malonic half-ester and the same manipulation of protecting group as Kedrowski and Masterson reported earlier.[3, 46]



Scheme 12. PLE desymmetrization approach by Kedrowski.[46]



Scheme 13. Strategy of Masterson et al.[3]

CHAPTER II

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

Background

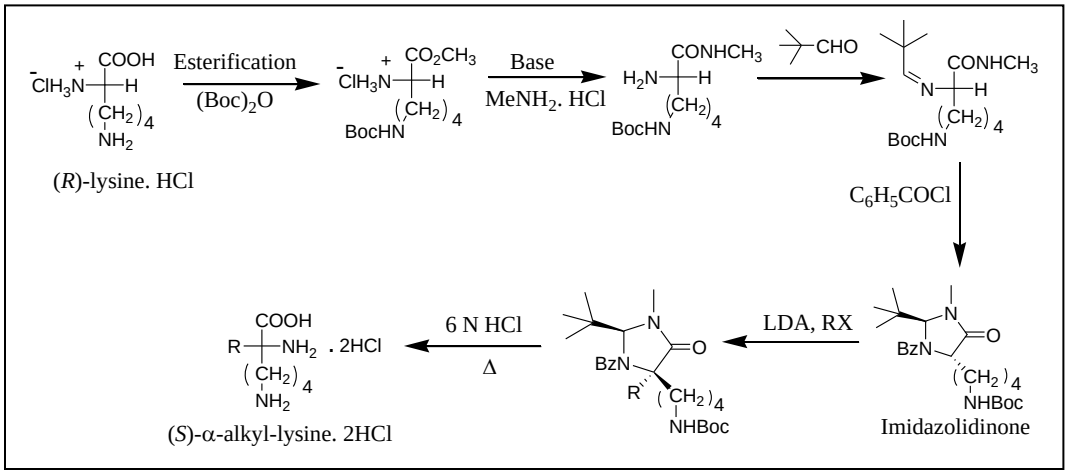
The growing importance of $C^{\alpha,\alpha}$-disubstituted class of non-proteinogenic amino acids in various fields has drawn the interest of synthetic organic chemists over the last two decades.[3, 46, 59] This class of sterically constrained amino acids, which consist of quaternary carbon center,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

congestion to keep the substrates from reaching the enzyme active site.[29, 36, 37] Thus, the above examples reveal the growing interest in preparation of the $C^{\alpha,\alpha}$-disubstituted amino acids in the medicinal chemistry community.

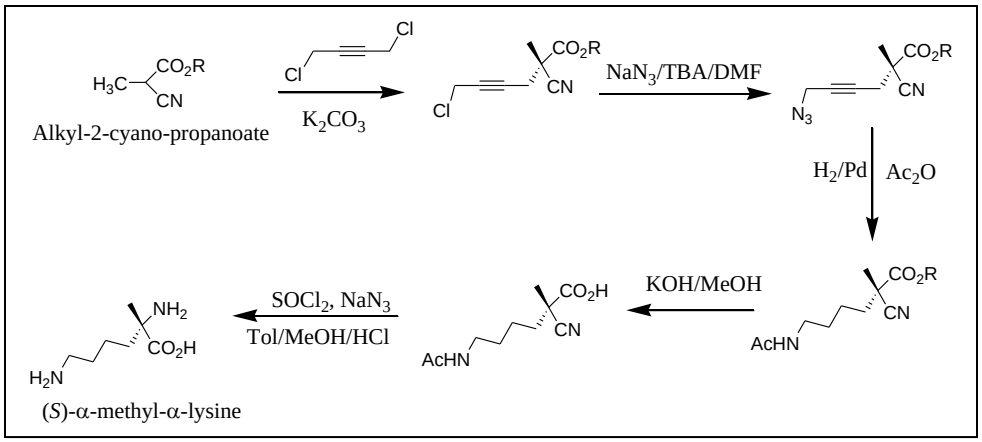
Although a number of synthetic strategies have been reported to prepare different optically enriched α,α-disubstituted amino acids, very few reports exist on the $C^{\alpha,\alpha}$-disubstituted lysine analogues.[30, 59-61] Lysine is an essential amino acid and is one of the mandatory building blocks in number of biologically active peptides for their function.[62-66] However, peptides consisting of lysine are often susceptible to degradation by serine like proteases.[67] Hence, organic chemists have been exploring more options to prepare $C^{\alpha,\alpha}$-disubstituted-lysine analogues to derive protease resistant peptides.[30] Recently, Berkowitz et al. have discovered that unlike D-lysine, α-vinyl-lysine strongly behaves as an inhibitor of lysine decarboxylase.[68, 69] Few years back, Jones et al. reported that $C^{\alpha,\alpha}$-disubstituted malonic diesters carrying lysine amino acid side chain functionality does not undergo hydrolysis with trypsin.[70]

All the above examples point to the growing interest in α,α-disubstituted lysine analogues. However, appropriate protection of two nitrogen atoms in lysine to prepare it for solid phase polypeptide synthesis has come out as a challenging task to synthetic chemists. Few years ago Seebach et al.[59] reported the synthesis of α-methyl-α-lysine analogues in free diamino form employing self-regeneration of stereo center (SRS) principle (Scheme 14). However, the Seebach strategy allows us to synthesize only α-methyl-α-lysine in low yield. Recently, Cativiela et al. reported the synthesis of (*S*)-α-methyl-α-lysine via chiral cyanopropanoate using a chiral auxiliary in ten steps, but this strategy also let us synthesize only (*S*)-α-methyl-α-lysine in unprotected form (Scheme

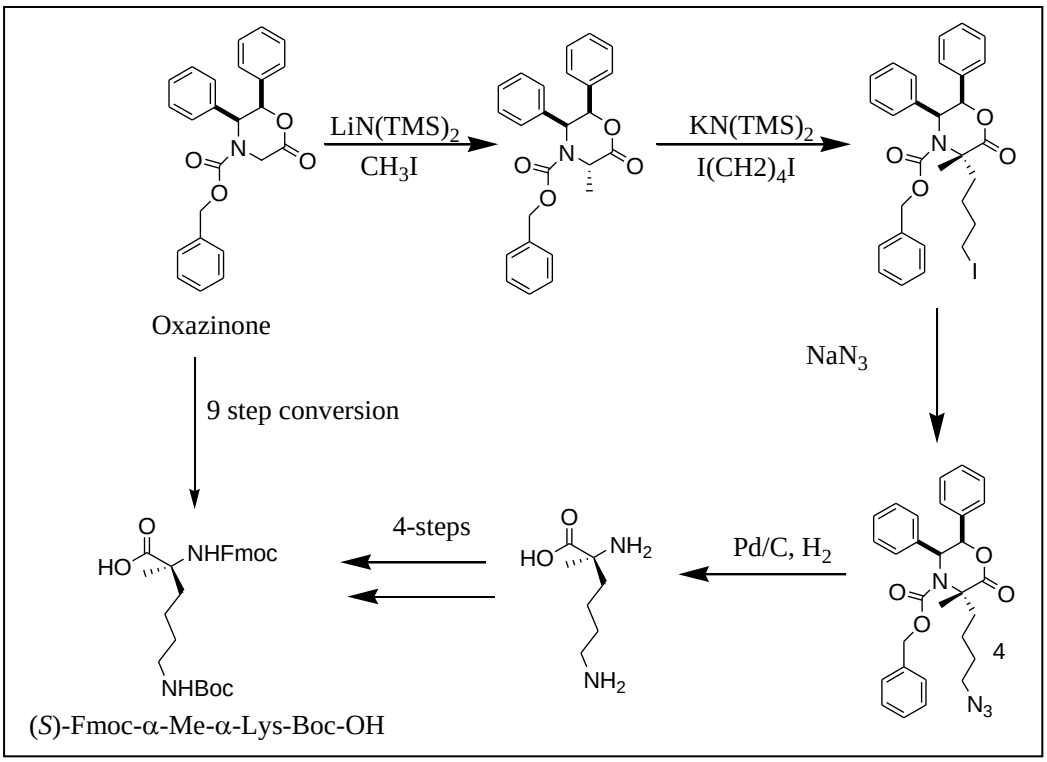
15). To the best of our knowledge Chauhan is the first to report ^{t}Boc-Fmoc protected (*S*)-α-methyl-α-lysine using William's Oxazinone as a chiral auxiliary in eight steps with overall 26% yield and 95% optical purity (Scheme 16).[30] However, this methodology needs to be using expensive chiral auxiliary, and the same auxiliary cannot be utilized to result in both (*R*)- and (*S*)-α-methyl-α-lysine derivative. In addition, this methodology requires the use of explosive azide to attain lysine side chain functionality, hence, could be dangerous in scale up batch. Moreover, this methodology has been able to obtain the final product in only 90% purity, what in addition would be problematic for the incorporation of the amino acid in to a peptide.



Scheme 14. Seebach's strategy to prepare optically enriched α,α-disubstituted-lysine.[59]



Scheme 15. Strategy of Cativiela et al to prepare (*S*)-α-methyl-α-lysine.[60]



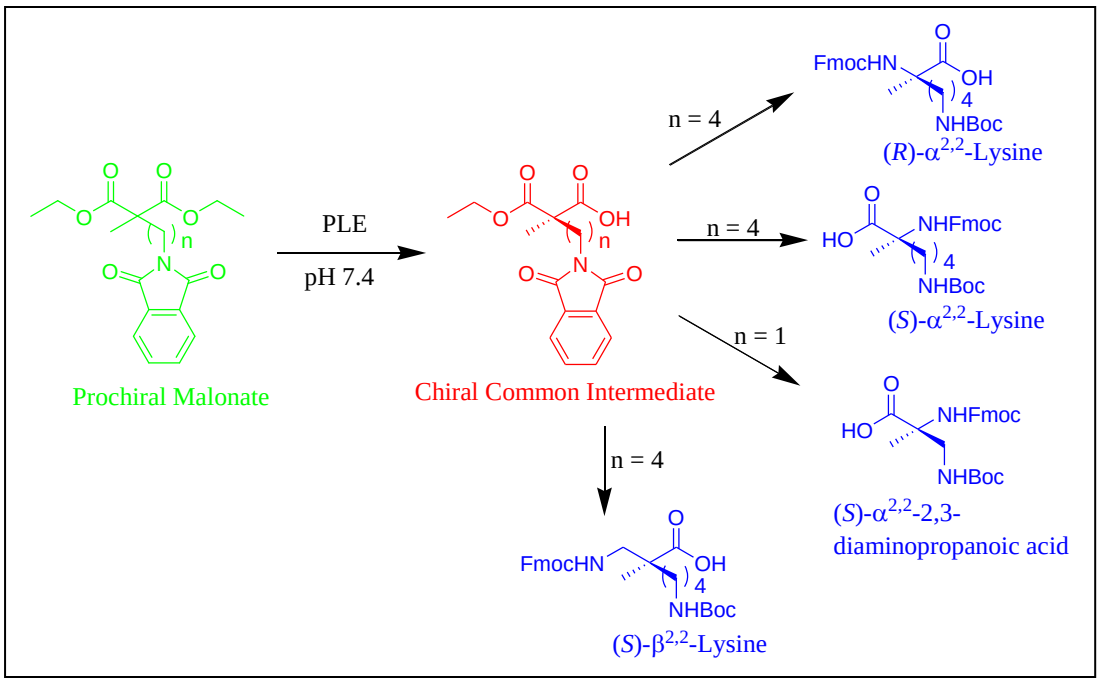
Scheme16. Chauhan's strategy to prepare protected C_{α}-methyllysine analogue.[30]

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

Hypothesis 1.

Our unique enantiodivergent synthetic strategy allows us to derive a variety of orthogonally protected $C^{\alpha,\alpha}$-disubstituted-α-/β-lysine analogues and prepare both enantiomers of C^{α}-methyl-α-lysine from Pig Liver Esterase desymmetrized optically enriched common intermediate types (Scheme 17).

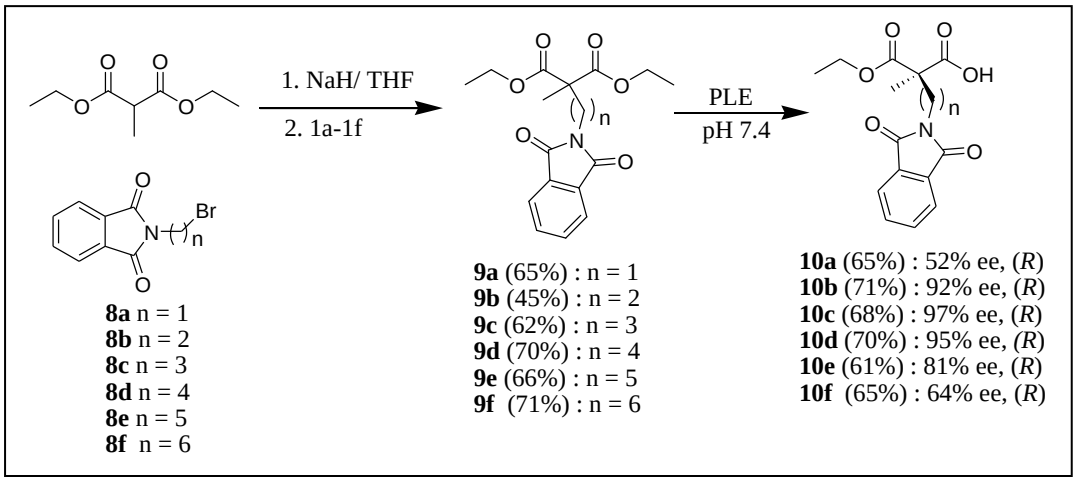
I have developed a PLE catalyzed enzymatic desymmetrized approach to prepare both orthogonally protected (*R*)- and (*S*)-α-methyl-α-lysine, orthogonally protected (*S*)-2,3-diaminopropanoic acid, and orthogonally protected (*S*)-α-methyl-$\beta^{2,2}$-lysine analogue from the optically enriched (52%~97%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



Scheme 17. General synthetic strategy to prepare diverse orthogonally protected lysine analogues from common intermediate types.

Results and Discussion

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



Scheme 18. Synthesis of optically enriched half-esters (**10a - 10f**).[73]

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

The stereochemical configuration of the major enantiomer of **10a** was determined to have the (*R*) absolute stereochemistry as shown in Scheme 19.[74] The absolute rotation of the half-ester **17**, which was obtained from the half-ester **12** by synthesis, was compared with the one obtained from **10a**. The stereochemical configuration of the major enantiomer of **10b** was determined by synthesis[75] as shown in Scheme 20. The optical activity of compound **22** was compared to literature values in order to establish the absolute configuration of **10b**. The configurations of **10c** and **10d** were determined by conversion into **24a** and **24b** as shown in Scheme 21. The optical rotations of **24a** and **24b** were compared with literature values in order to determine the stereochemical configurations of **10c** and **10d**.[59] The stereochemical configurations of **10e** and **10f** were

also determined by synthetic means as shown in Scheme 22. [76-78] The half-esters **10e** and **10f** were converted into α,α-disubstituted amino acids **28a** and **28b** to compare their absolute rotation to the literature values.[77, 78]

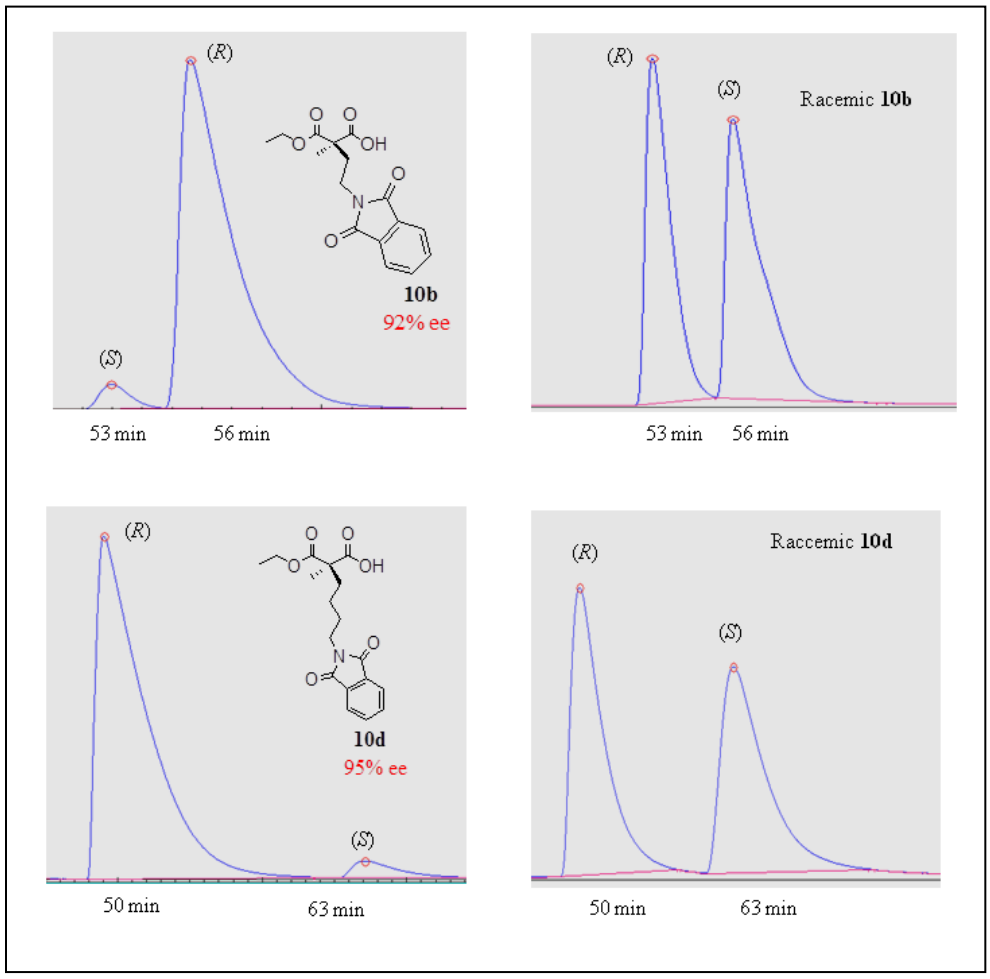
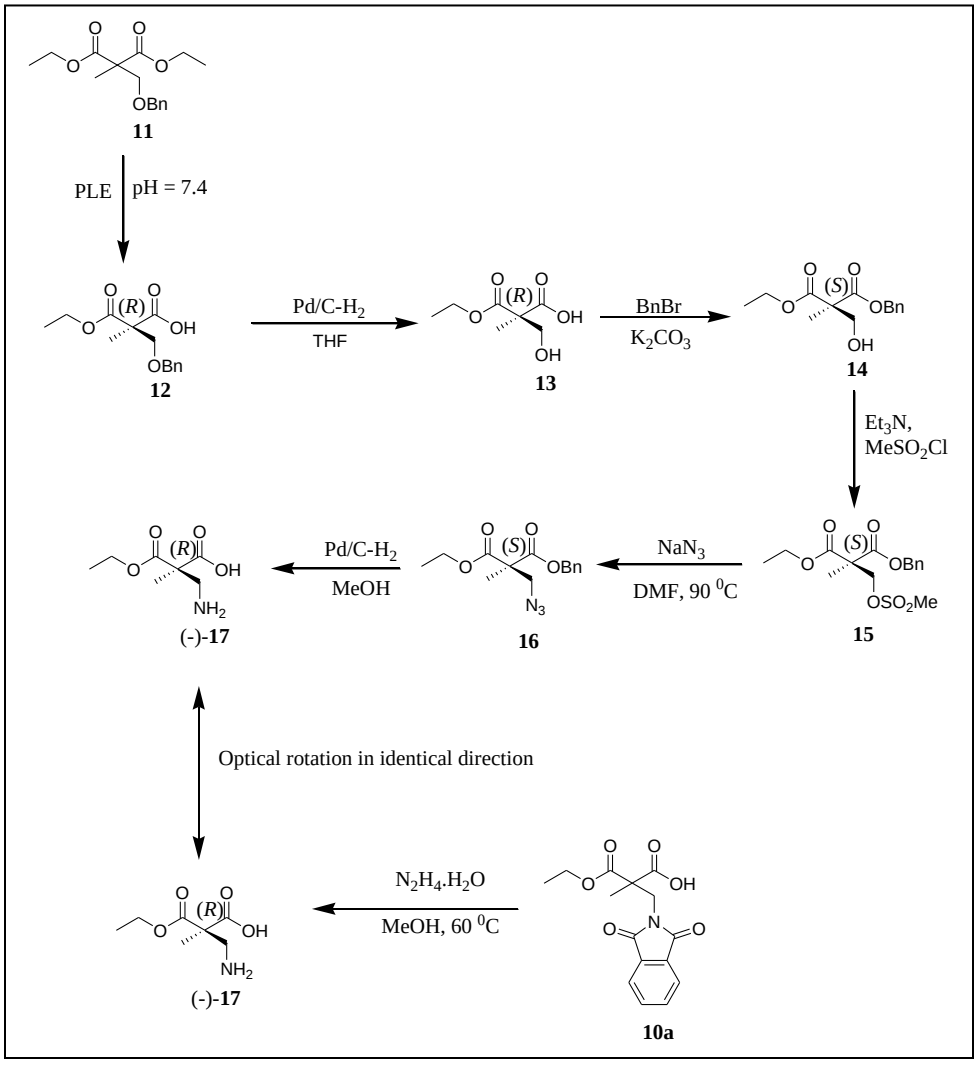
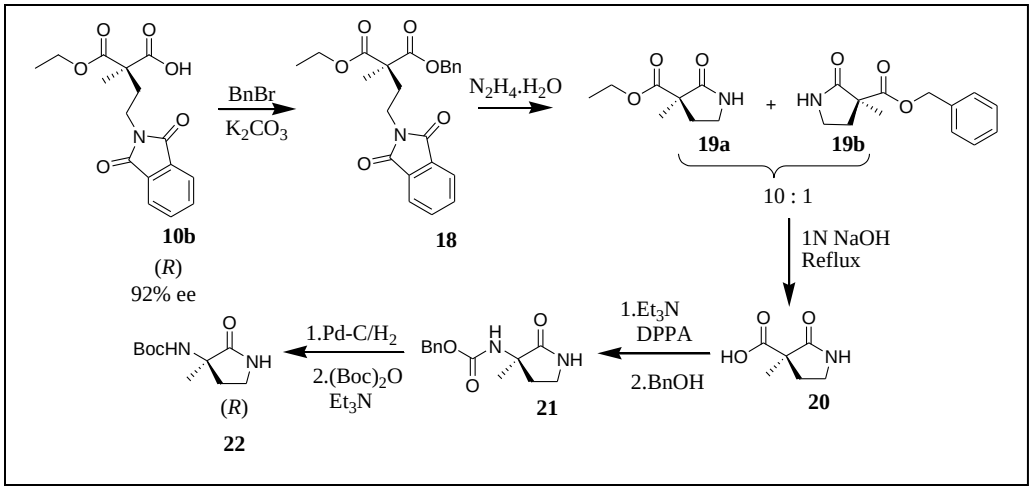


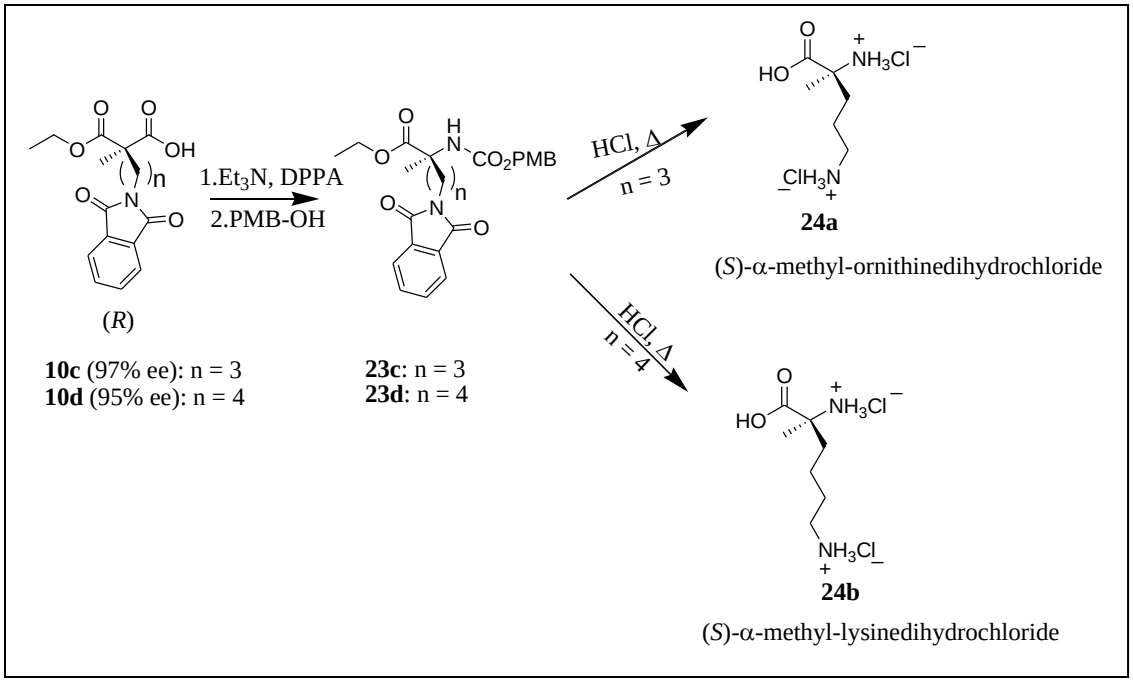
Figure 11. Chiral HPLC chromatograms of few half-esters (**10b** and **10d**).



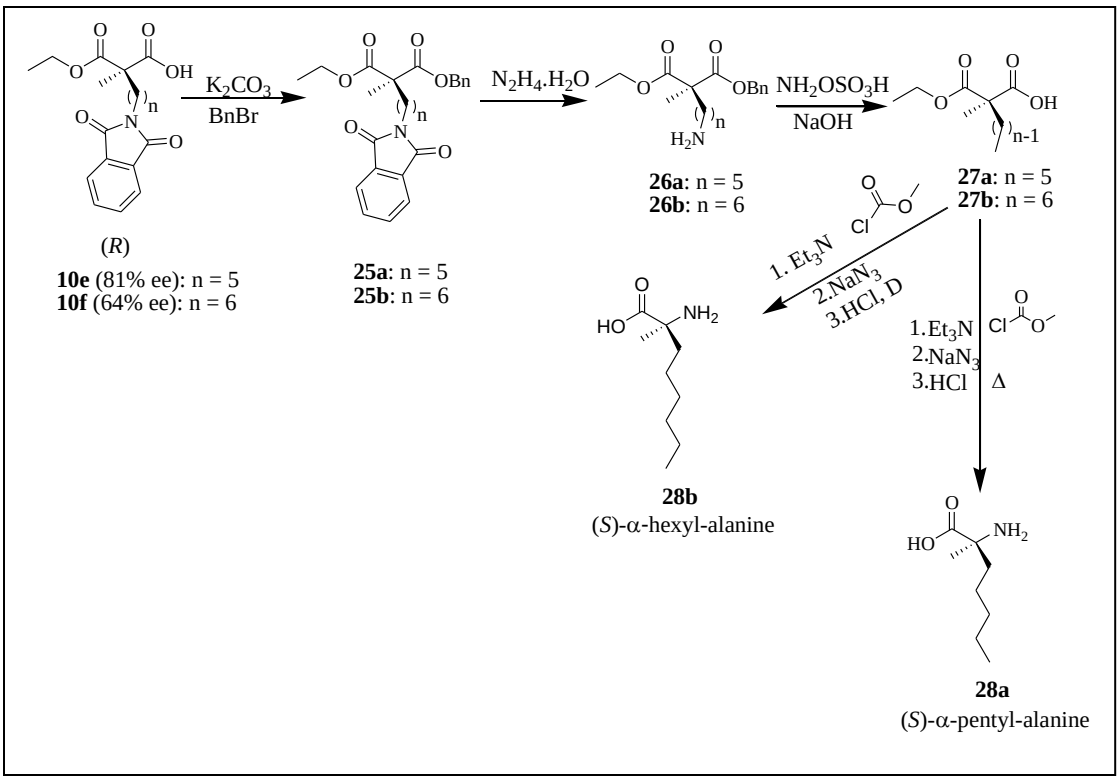
Scheme 19. Absolute configuration of **10a**.[74]



Scheme 20. Absolute configuration of **10b**.[73]



Scheme 21. Absolute stereochemical configuration of **10c** and **10d**.[73]



Scheme 22. Absolute stereochemical configuration of **10e** and **10f**.[73]

*Insight into PLE Biocatalytic Hydrolysis of the Prochiral Diesters (**9a-9f**)*

It is evident from the Figure 12 and Figure 13 that the PLE hydrolysis of diesters **9a-9f** obey the Jones Active Site Model (JASM) (Figure 13).[72] JASM is a 3D model, which is not shown here.[72] PLE hydrolysis of diester **9c** results in the highest level of optical purity. I hypothesize that the size of the side chain of **9c** closely matches with the size of the large hydrophobic pocket (H_L) in the JASM. The other prochiral diesters having relative disparity of size with the large hydrophobic pocket of the JASM results in diminished enantioselectivity with respect to **9c**.

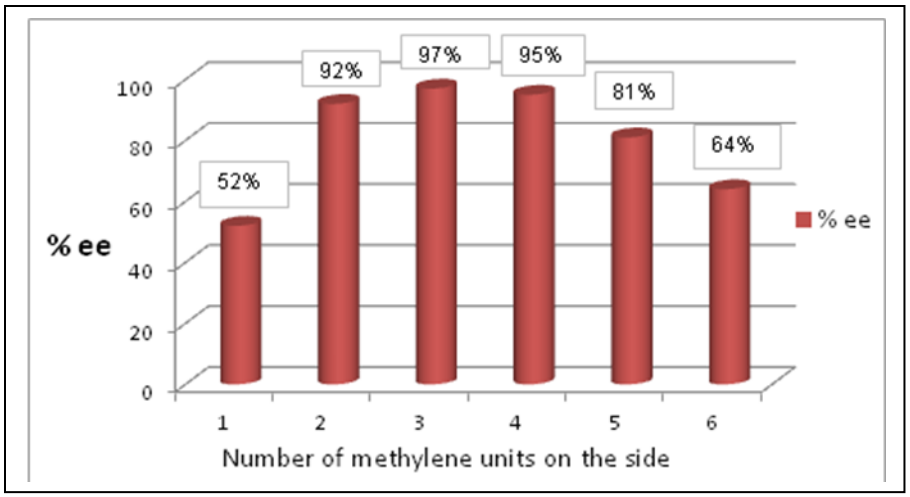


Figure 12. PLE hydrolysis assay of **10a-10f**.

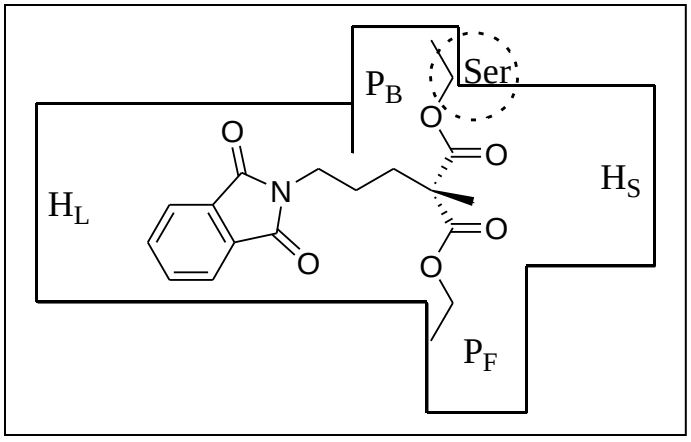
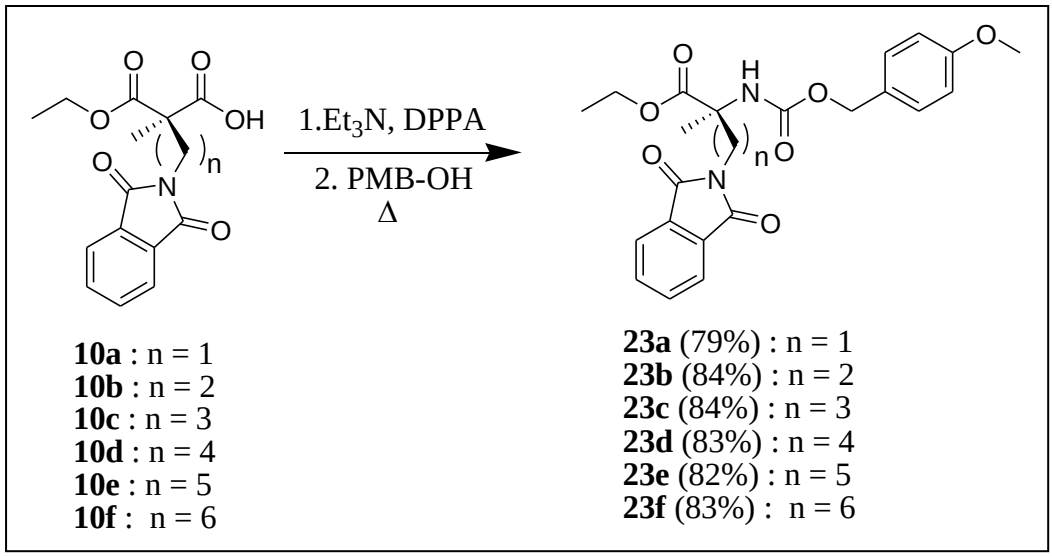


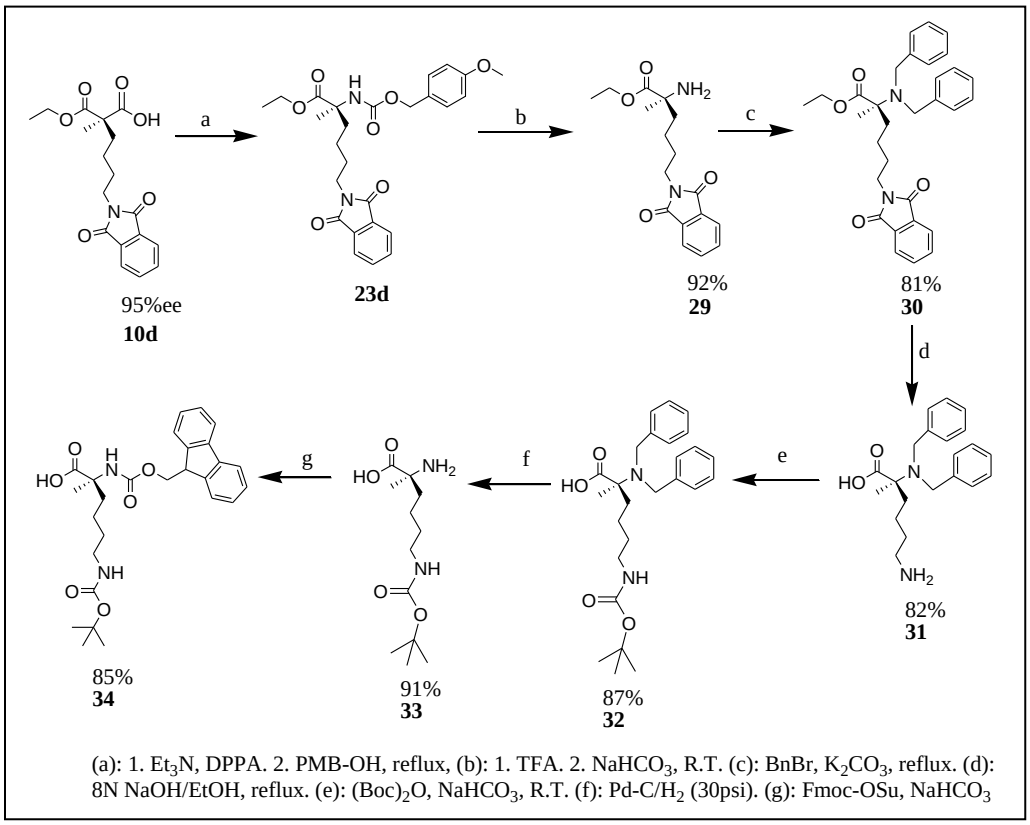
Figure 13. Jones active site model for Pig Liver Esterase.

Conversion of half-esters (10a-10f) into Moz protected carbamates: The acid-esters (**10a-10f**) were subjected to the Curtius rearrangement resulting in Moz-protected (*S*)-$\alpha^{2,2}$-carbamates (**23a-23f)** in good isolated yields as shown in Scheme 23. Compounds **23a-23f** can be taken into consideration as fully protected non-proteinogenic amino acids.



Scheme 23. Conversion of the half-esters (**10a-10f**) into fully protected amino acids.[73]

Synthesis of Orthogonally Protected (S)-Fmoc-α^{2,2}-Lysine-Boc-OH (Long Path)

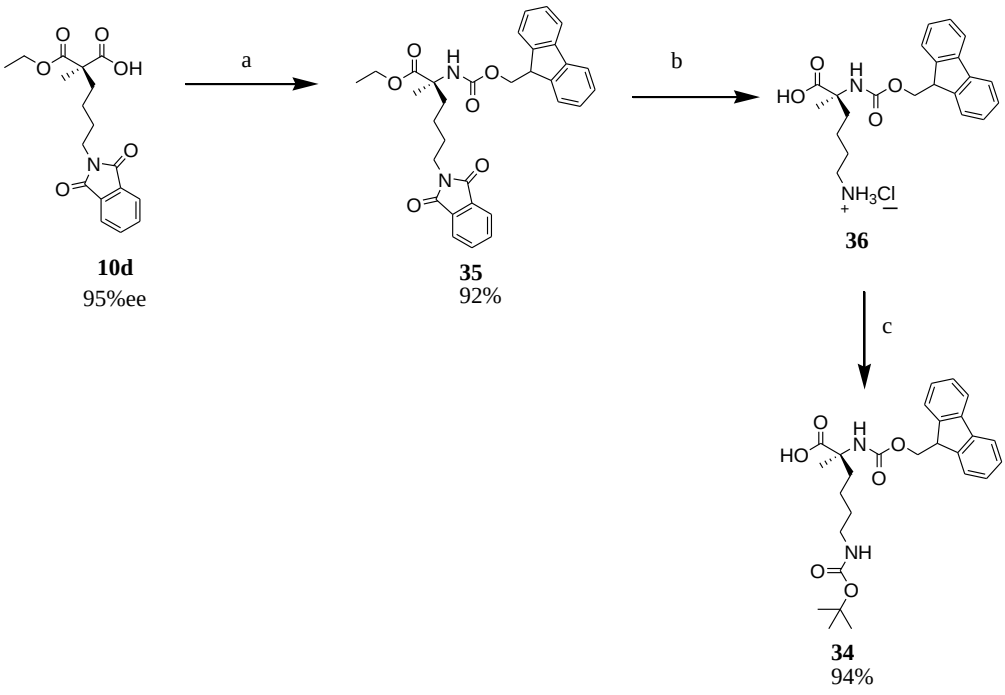


Scheme 24. Synthesis of orthogonally protected (*S*)-α^{2,2}-lysine analogue.

Scheme24 illustrate the synthesis of (*S*)-α^{2,2}-Fmoc-lysine-Boc-OH (**34**) in seven steps starting with the optically enriched **10d** with good isolated yield. In the first step the chiral half-ester (**10d**) was subject to a Curtius rearrangemt producing the Moz protected α-amino ester (**23d**) in good isolated yield. The carbamate (**23d**) was then treated with TFA in methylene chloride in order to chemoselectively deprotect the Moz group leading to free α-amine (**29**).[46] Compound **29** was then subject to dibenzylation using excess BnBr and K_2CO_3 under reflux for 48 hours preparing dibenzylated-α-amine(**30**).[53] The simple base hydrolysis of **30** using 8N NaOH/EtOH over 72 hours resulted in saponification of the ethyl ester and deprotection of the phthalimido group in a single step leading to free amino acid (**31**) in good yield. The free amine (**31**) was then selectively

protected with the Boc group using Boc anhydride and $NaHCO_3$ in a H_2O/Dioxane system producing **32** in good isolated yield. The chemoselective hydrogenolysis of **32** led to the α-free amino acid **33** in nearly quantitative yield. The free α-amino acid (**33**) was reprotected with Fmoc group leading to tBoc, fmoc protected (*S*)-$\alpha^{2,2}$-Lysine analogue (**34**) with good reproducible isolated yields in overall seven steps from the chiral half-ester.

Synthesis of (S)-Fmoc-$\alpha^{2,2}$-Lysine-Boc-OH (Short Path)



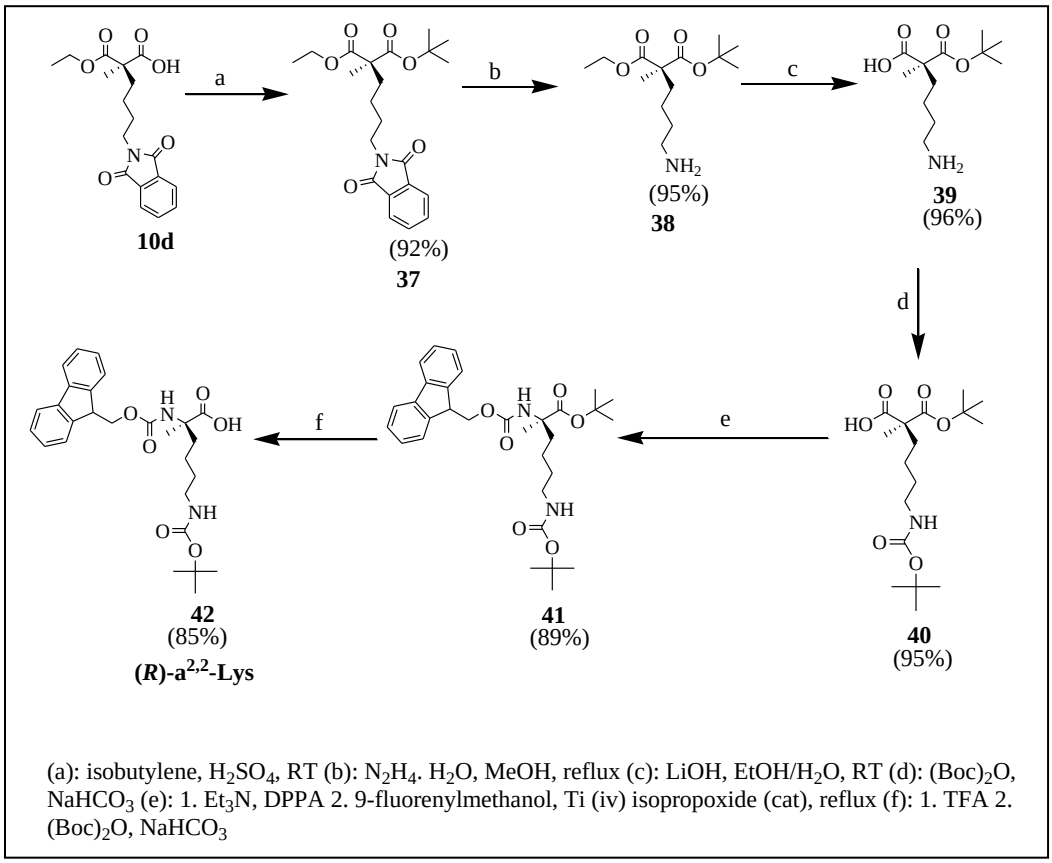
(a): 1. DPPA, Et_3N, 2. 9-fluorenylmethanol, Ti (IV) isopropoxide, reflux. (b): 5N HCl/dioxane, reflux. (c): $(Boc)_2O$, $NaHCO_3$

Scheme 25. Short path synthesis of (*S*)-Fmoc-$\alpha^{2,2}$-lysine-Boc-OH analogue.[73]

The short path synthetic strategy (Scheme 25) leads to the ^{t}Boc-Fmoc-(*S*)-$\alpha^{2,2}$-lysine (**34**) in three steps starting with optically enriched **10d**. The bulky quaternary chiral half-ester (**10d**) was directly converted to the Fmoc protected carbamate (**35**) by Ti (IV) isopropoxide catalyzed Curtius rearrangement with good isolated yield.[79] The acid

hydrolysis, that was employed in 5N HCl/dioxane system under reflux, of the carbamate (**35**) hydrolyzed both pthalimido group and ethyl ester leading to the free amino acid (**36)**, as evident by ESI-MS.[80] The crude free amino acid (**36**) was undertaken for reprotection of the side chain amine by the Boc group without further purification. Eventually, the ^{t}Boc-Fmoc-(*S*)-$\alpha^{2,2}$-lysine-OH (**34**) is obtained in three steps. Hence, the short path synthetic strategy is the most concise way to synthesize ^{t}Boc-Fmoc-(S)-$\alpha^{2,2}$-Lysine-OH.[30]

Preparation of (R)-Fmoc-$\alpha^{2,2}$-Lysine-Boc-OH Analogue



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

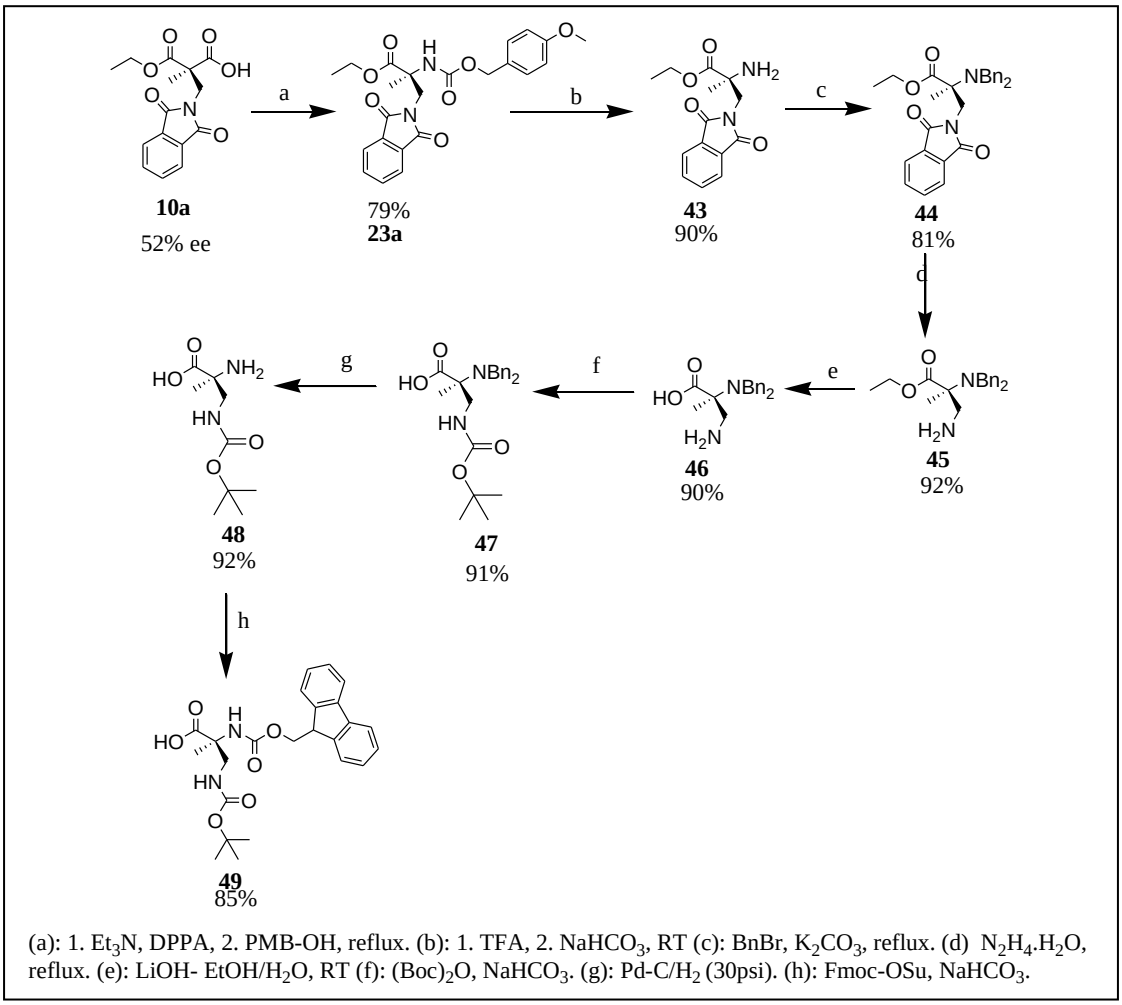
In order to achieve the synthesis of **42**, the chiral half-ester **10d** was turned into the mixed diester **37**.[3, 56] Compound **37** was subjected to selective deprotection of the phthalimide group producing **38**. Compound **38** was saponified resulting in **39** in

excellent yield. Amino acid **39** was transformed into **40** using standard reaction conditions. The sterically restricted carboxylic acid **40** was then converted into Fmoc protected α-amino ester **41** in good yield employing Curtius rearrangement using DPPA and 9-fluorenylmethanol in presence of catalytic Ti (IV) isopropoxide.[79] However, the chemoselective deprotection of the *tert*-butyl ester in the presence of Boc did not work out in our hands using known literature procedures.[81-83] This failure is attributed to the inaccessibility of the sterically hindered ester by those reagents. Hence, we had to treat the amino ester **41** with TFA to deprotect both the *tert*-butyl and Boc groups followed by treatment with $(Boc)_2O$. However, this deprotection and reprotection was a one pot strategy that gave birth to the (*R*)-$\alpha^{2,2}$-methyllysine analogue **42** in eight steps in reasonable overall yield (30%).

Synthesis of Orthogonally Protected (S)-$\alpha^{2,2}$-2,3-Diaminopropanoic Acid

Scheme 27 describes the synthesis of orthogonaly protected (*S*)-$\alpha^{2,2}$-2,3-diaminopropanoic acid (**49**) in eight steps starting with optically enriched half-ester **10a** in good isolated yield. At first the chiral half-ester (**10a**) was subjected to a Curtius rearrangement resulting in the Moz protected α-amino ester (**23a**). The carbamate (**23a**) was then allowed to react with TFA to chemoselectively deprotect the Moz group resulting in **43**.[46] Compound **43** was then allowed to undergo a dibenzylation using excess BnBr and K_2CO_3 at solvent reflux for 48 hours leading to **44**.[53] However, standard base hydrolysis failed to drive the deprotection of the phthalimide group along with ester saponification in a single step starting with the α-dibenzylated aminoester (**44**). This failure was considered to be due to the closeness of phthalimido group to the bulky quaternary center. However, the chemoselective cleavage of the phthalimide of the

dibenzylated amino ester (**44**) employing hydrazine resolved the problem providing access to **45**. Saponification of **45** results in the desired **46**. The free amino acid (**46**) was then taken up for a selective installation of Boc group using Boc anhydride and $NaHCO_3$ in H_2O/Dioxane system producing **47** in good isolated yield. The selective hydrogenolysis of **47** resulted in the α-free amino acid **48** in near quantitative yield. The free α-amino acid (**48**) was reprotected with the Fmoc group leading to ^{t}Boc, Fmoc protected amino acid analogue (**49**) in total ten steps in 14% overall yield.

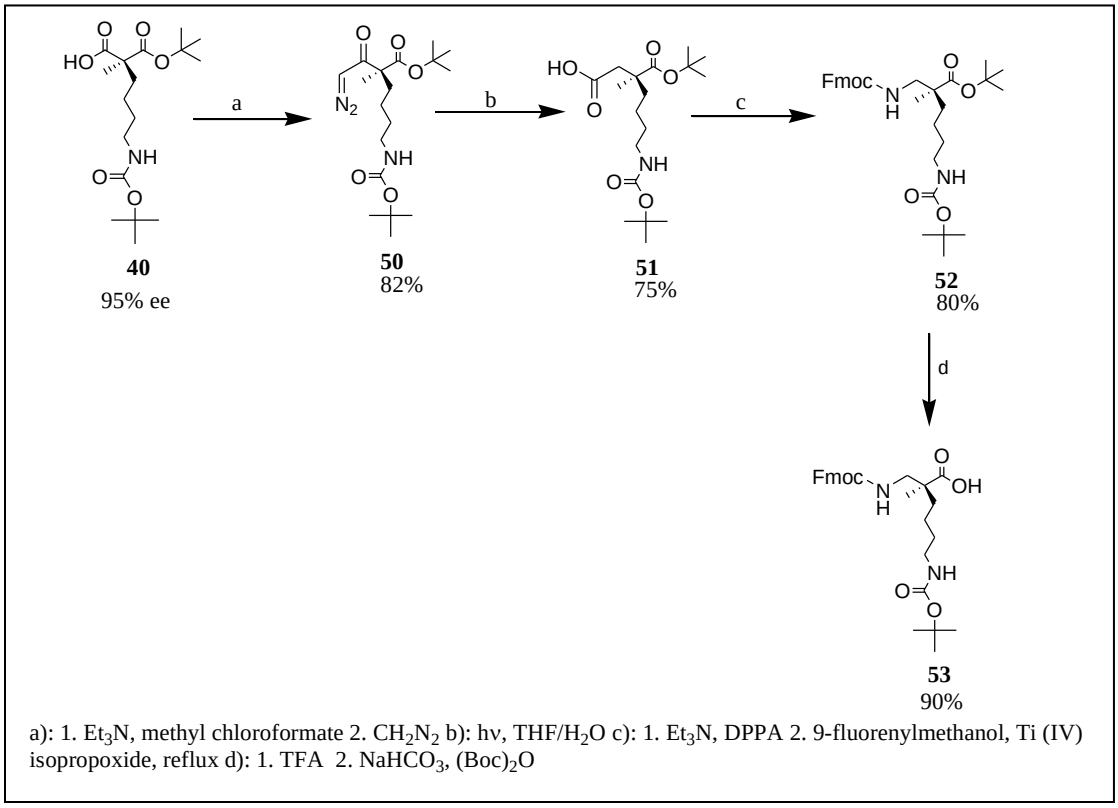


(a): 1. Et_3N, DPPA, 2. PMB-OH, reflux. (b): 1. TFA, 2. $NaHCO_3$, RT (c): BnBr, K_2CO_3, reflux. (d) $N_2H_4.H_2O$, reflux. (e): LiOH- EtOH/H_2O, RT (f): $(Boc)_2O$, $NaHCO_3$. (g): Pd-C/H_2 (30psi). (h): Fmoc-OSu, $NaHCO_3$.

Scheme 27. Synthesis of orthogonally protected (*S*)-α[2,2]-2,3-diaminopropanoic acid.[73]

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

Synthesis of (S)-Fmoc-β^{2,2}-Methyllysine-Boc-OH



Scheme 28. Synthesis of orthogonally protected (*S*)-β^{2,2}-methyllysine analogue.[73]

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

Conclusions

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

Experimental

General Methods: THF, CH_2Cl_2, and DMF were dried by passage through a column of activated alumina. All reagents were used as received from commercial sources unless otherwise stated. Melting points were determined in open capillary tubes and are uncorrected. P-60 silica gel was used to conduct flash chromatography. Silica pre-coated TLC plates were used to perform TLC analysis. Normal phase pre-coated silica rotors were chosen to perform radial chromatography. NMR was obtained using 400 MHz Bruker or 300 MHz Varian instruments. MS was obtained using ESI/FTICR-MS, and low resolution MS was obtained by ESI/ion trap. IR was conducted on Thermo Nicolet nexus 470 FT-IR instrument. Pig Liver Esterase (PLE) is the commercially available crude preparation.

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

A 250 mL roundbottom 3-neck flask fitted with a nitrogen inlet, an addition funnel, and a reflux condenser was charged with 1.2 eq. of NaH (60% dispersion in mineral oil), a stirbar, and 100 mL of dry THF. The resulting suspension was cooled to 0 ^{o}C in an icebath. A 50 mL solution of diethyl-2-methylmalonate (1eq) in THF was added over 30 min with stirring. The reaction mixture was then allowed to stir for 60 min at room temperature. A 100 mL solution of *N*-(bromoalkyl)-phthalimide (1eq) in THF was added over 30 min with stirring. The reacton mixture was then heated to reflux solvent for 12 h. The solution was cooled to RT, diluted with ether (300 mL), washed twice with 1 N HCl, washed with brine and dried over $MgSO_4$. The resulting suspension was then filtered and the solvent was evaporated "*in vacuo*". The resulting yellowish liquid was purified by flash chromatography.

*Diethyl 2-(N-Methylphthalimido)-2-Methyl Malonate (**9a**)*

9a was prepared following the general procedure for the formation of diester (**9a-9f**) with 10g (57.4 mmol) diethyl-2-methylmalonate. The resulting yellowish liquid was purified by flash chromatography (30:70 EtOAc/Hexanes), giving the pure product as a white solid (12.5g, 65%).[74] R_f 0.54 (30:70 EtOAc/hexanes), MP= 89 ^oC. ^1H-NMR (300 MHz, $CDCl_3$): 7.84 (m, 2H), 7.73 (m 2H), 4.27 (s, 2H), 4.26 (q, 4H, J= 7 Hz), 1.41 (s, 3H), 1.30 (t, 6H, J=7 Hz), ^{13}C-NMR (75 MHz, $CDCl_3$): 14.0, 18.3, 42.0, 54.0, 62.0, 123.0, 132.0, 134.7, 168.5, 170.5. HRMS ($C_{17}H_{19}NO_6Na^+$): calculated = 356.1104, found = 356.1100.

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

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

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

9c was prepared following the general procedure for the formation of diester (**9a-9f**) with 10g (57.4 mmol) diethyl-2-methylmalonate, 15.4g (57.4 mmol) *N*-(bromopropyl)-phthalimide and 2.74g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (30:70 EtOAc/Hexanes), giving the pure product as a colorless liquid (12.8g, 62%). R_f = 0.36 (30% EtOAc/Hexanes). IR (cm^{-1}) = 2981, 1772, 1705, 1614. ^1H-NMR ($CDCl_3$, 400 MHz): δ 7.84 (m, 2H), 7.72 (m, 2H), 4.16 (q, 4H, *J* = 7Hz), 3.69 (t, 2H, *J* = 7Hz), 1.91 (m, 2H), 1.67 (m, 2H), 1.39 (s, 3H), 1.23 (t, 6H,

J = 7Hz). ^{13}C- NMR ($CDCl_3$, 100 MHz): δ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 53.0, 38.0, 32.5, 23.3, 20.0, 14.0. HRMS ($C_{19}H_{23}NO_6Na^+$) calculated = 384.1423, found = 384.1406.

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

9d was prepared following the general procedure for the formation of diester (**9a-9f**) with 10g (57.4 mmol) diethyl-2-methylmalonate, 16.2g (57.4 mmol) *N*-(bromobutyl)-phthalimide and 2.74g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (30:70 EtOAc/Hexanes), giving the pure product as a white solid (15.2g, 70%). R_f = 0.40 (30% EtOAc/Hexanes). IR (cm-1) = 2950, 1702. MP = 48 ^{0}C. ^{1}H- NMR ($CDCl_3$, 300 MHz): δ 7.85 (m, 2H), 7.71 (m, 2H), 4.17 (q, 4H, *J* = 7Hz), 3.67 (t, 2H, *J* = 7Hz), 1.90 (m, 2H), 1.69 (m, 2H), 1.39 (s, 3H), 1.27 (m, 8H). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 53.0, 38.0, 35.0, 29.0, 22.0, 20.0, 14.0. HRMS ($C_{20}H_{25}NO_6Na^+$) calculated = 398.1574, found = 398.1573.

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

9e was prepared following the general procedure for the formation of diester (**9a-9f**) with 10g (57.4 mmol) diethyl-2-methylmalonate, 17g (57.4 mmol) *N*-(bromopentyl)-phthalimide and 2.74g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (30:70 EtOAc/Hexanes), giving the pure product as a colorless liquid (14.8g, 66%). R_f = 0.42 (30% EtOAc/Hexanes). IR (cm^{-1}) = 2938, 1772, 1706. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.83 (m, 2H), 7.71 (m, 2H), 4.17 (q, 4H, *J* = 7Hz), 3.68 (t, 2H, *J* = 7Hz), 1.82 (m, 2H), 1.67 (m, 2H), 1.30 (m, 13H). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 54.0, 38.2, 35.2, 28.5, 27.0, 24.0, 20.0, 14.0. HRMS ($C_{21}H_{27}NO_6Na^+$) calculated = 412.1730, found = 412.1728.

*Diethyl 2-(N-Hexylphthalimido)-2-Methyl Malonate (**9f**)*

9f was prepared following the general procedure for the formation of diester (**9a-9f**) with 10g (57.4 mmol) diethyl-2-methylmalonate, 17.8g (57.4 mmol) *N*-(bromopentyl)-phthalimide and 2.74g (68.9 mmol) NaH. The resulting yellowish liquid was purified by flash chromatography (40:60 Et_2O/Hexanes), giving the pure product as a colorless liquid (16.5g, 71%). R_f = 0.44 (30% EtOAc/Hexanes). IR (cm^{-1}) = 2940, 1702. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.71 (m, 2H), 4.16 (m, 4H), 3.67 (m, 2H), 1.84 (m, 2H), 1.66 (m, 2H), 1.36 (m, 7H), 1.24 (m, 8H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.0, 168.0, 134.0, 132.0, 123.0, 61.0, 54.0, 38.0, 35.0, 29.0, 28.0, 27.0, 24.0, 20.0, 14.0. HRMS ($C_{21}H_{27}NO_6Na^+$) calculated = 426.1893, found = 426.1894.

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

10g (1 eq) of the appropriate malonate (**9a-9f**) was dispersed in 1000 mL of rapidly stirring phosphate buffer (0.1N, pH 7.4) containing 2% (vol/vol)EtOH as a cosolvent. The pH was maintained using an autotitrator set to maintain a pH of 7.4 and titrate to a volume of 1 eq NaOH (1.06M). PLE (27 units/mg, 90 units per mmol of the substrate) was added and the titration was started. The hydrolysis proceeded for 1-6 days depending on substrate. The reaction was stopped when 1 eq. of NaOH was added. The reaction mixture was extracted three times with 500 mL of Et_2O. The aqueous layer was then acidified to pH = 1 using 12 M HCl, extracted eight times with Et_2O. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in va*cuo.

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

10a was prepared following the general procedure for the formation of half-esters (**10a-10f**) with 10g (30 mmol) of **9a**. An amount of 6g (65%) of **10a** was obtained as a

white solid in 52% ee.[74] MP = 148 ^{o}C. R_f = 0.31 (40:60 EtOAc/hexanes). ^{1}H-NMR (300 MHz, $CDCl_3$): 9.63 (s, 1H), 7.86 (m, 2H), 7.74 (m, 2H), 4.29 (m, 4H), 1.48 (s, 3H), 1.34 (t, 3H, *J*= 7 Hz), ^{13}C- NMR (75 MHz, CD_3OD) 14.0, 19.0, 42.0, 53.0, 62.0, 124.0, 133.0, 135.0, 170.0, 174.0, 175.0. HRMS ($C_{15}H_{15}NO_6Na^+$) calculated = 328.0791, found = 328.0787. The % ee was determined by chiral HPLC (Chiracel OJ-H, 305 nm, 5% Ipr-OH/hexane) $Rt_{(S)}$ = 33 min, $Rt_{(R)}$ = 47.8 min (52% ee). $[\propto]_D^{22}$ = +5.2 (c = 1, MeOH).

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

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

10c was prepared following the general procedure for the formation of half-esters (**10a-10f**) with 10g (28 mmol) of **9c**. The resulting half-ester was purified by flash chromatography (40:60 EtOAc/Hexanes) giving the product as a colorless liquid (6.4g, 68%). The % ee was determined to be 97% by chiral HPLC (Diacel Chiralpak OJ-H, 4% *i*PrOH/Hexanes, flow rate = 1 mL/min, λ = 305 nm) $Rt_{(S)}$ = 54.9 min (Area = 130.13), $Rt_{(R)}$ = 58.8min (Area = 7770.41). R_f = 0.22 (40% EtOAc/Hexanes). IR (cm^{-1}) = 2983, 2937, 1773, 1747, 1697. $[\alpha]_D^{24}$ = + 5.8 (c = 2, MeOH). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.85 (m, 2H), 7.73 (m, 2H), 4.21 (q, 2H, *J* = 7Hz), 3.71 (t, 2H, *J* = 7Hz), 1.93 (m, 2H), 1.71 (m, 2H), 1.45 (s, 3H), 1.26 (t, 3H, *J* = 7Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 176.0, 172.0, 168.0, 134.0, 132.0, 123.0, 62.0, 53.0, 38.0, 33.0, 24.0, 20.0, 14.0. HRMS ($C_{16}H_{17}NO_6Na^+$) calculated = 356.3256, found = 356.3253.

*(R)-2-(N-Butylphthalimido)-3-Ethoxy-2-Methyl-3-Oxopropanoic Acid (**10d**)*

10d was prepared following the general procedure for the formation of half-esters (**10a-10f**) with 10g (27 mmol) of **9d**, The resulting half-ester was purified by flash chromatography (40:60 EtOAc/Hexanes) giving the pure product as a white solid (6.6g, 70%). The % ee was determined to be 95% by chiral HPLC (Diacel Chiralpak OJ-H, 4% iPrOH/Hexanes, flow rate = 1mL/min, λ = 305 nm) $Rt_{(S)}$ = 63.0 min (Area = 325.24), $Rt_{(R)}$ = 49.6 min (Area = 11659.65). R_f = 0.24 (40% EtOAc/Hexane). IR (cm^{-1}) = 3250, 2943, 1718, 1696. MP = 63 ^{0}C. $[\alpha]_D^{23}$= + 3.3 (c = 1, CH_2Cl_2), ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.82 (bs, 1H), 7.82 (m, 2H), 7.73 (m, 2H), 4.21 (q, 2H, *J* = 7Hz), 3.71 (t, 2H, *J* = 7Hz), 1.93 (m, 2H), 1.69 (m, 2H), 1.45 (s, 3H), 1.35 (m, 2H), 1.26 (t, 3H, *J* = 7Hz),^{13}C-NMR (CD_3Cl_3, 100 MHz): δ 176.0, 174.0, 169.2, 135.3, 133.2, 124.0, 62.0, 54.3, 39.0, 36.0, 30.0, 23.0, 20.0, 14.0. HRMS ($C_{16}H_{17}NO_6Na^+$) calculated = 370.1261, found = 370.1256.

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

10e was prepared following the general procedure for the formation of half-esters (**10a-10f**) with 10g (26 mmol) of **9e**. The resulting half-ester was purified by flash chromatography (40:60 EtOAc/Hexanes) giving the pure product as a colorless liquid (5.8g, 61%). The % ee was determined to be 81% by chiral HPLC (Diacel Chiralpak AD-H, 3% *i*PrOH/Hexanes, flow rate = 1 mL/min, λ = 305 nm) $Rt_{(S)}$ = 114.60 min (Area = 1591.84), $Rt_{(R)}$ = 78.75min (Area = 15497.54). R_f = 0.28 (40% EtOAc/Hexanes). IR (cm^{-1}) = 3250, 2938, 1770, 1700. $[\alpha]_D^{24}$ = + 3.2 (c = 2, $CHCl_3$), ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.83 (m, 2H), 7.72 (m, 2H), 4.21 (q, 2H, *J* = 7.19), 3.68 (t, 2H, *J* = 7 Hz), 1.86 (m, 2H), 1.69 (m, 2H), 1.44 (s, 3H), 1.34 (m, 4H), 1.27 (t, 3H, *J* = 7 Hz), ^{13}C-NMR ($CDCl_3$, 75

MHz): δ 178.0, 172.0, 168.4, 134.0, 132.0, 123.0, 61.4, 54.0, 38.0, 35.5, 28.3, 27.2, 24.0, 20.0, 14.0. HRMS ($C1_9H_{23}NO_6Na^+$) calculated = 384.1417, found = 384.1413.

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

10f was prepared following the general procedure for the formation of half-esters (**10a-10f**) with 10g (25 mmol) of **9f**. The resulting half-ester was purified by flash chromatography (40:60 EtOAc/Hexanes) giving the pure product as a colorless liquid (6.12g, 61%). The % ee was determined to be 64% by chiral HPLC (Diacel Chiralpk OJ-H, 3% *i*PrOH/Hexanes, flow rate = 1 mL/min, λ = 305 nm) $Rt_{(R)}$ = 57.7 min (Area = 13605958), $Rt_{(S)}$ = 71.7min (Area = 2954776). $[\alpha]_D^{24}$ = + 2.05 (c = 2, $CHCl_3$), ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.72 (m, 2H), 4.21 (q, 2H, *J* = 7.19), 3.68 (t, 2H, *J* = 7.12), 1.86 (m, 2H), 1.69 (m, 2H), 1.44 (s, 3H), 1.34 (m, 4H), 1.27 (t, 3H, *J* = 7.15), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 177.0, 173.0, 168.4, 134.0, 132.0, 123.0, 61.3, 53.0, 38.0, 35.5, 29.2, 28.2, 26.3, 24.0, 20.0, 14.0. HRMS ($C_{20}H_{25}NO_6Na^+$) calculated = 398.1574, found = 398.1572.

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

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

*(S)-1-Benzyl-3-Ethyl-2-(Hydroxymethyl)-2-Methylmalonate (**14**)*

A 100 mL roundbottom flask was charged with a stirbar, 2.3g of **13** (13 mmol), and 20 mL of DMF. The flask was placed under a nitrogen atmosphere and cooled to 0 ^{o}C with stirring and 2.15 g of K_2CO_3 (15.6 mmol) was added. Benzyl bromide (1.96g, 11.5 mmol) was added drop wise to the stirring suspension. The suspension was allowed to stir overnight at room temperature. The suspension was then diluted with 100 mL of diethyl ether and the organic layer was washed three times with water. The organic layer was then dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude liquid was then purified by flash chromatography (40% EtOAc/Hexane) giving 2.7 g (10.1 mmol, 88%) of **14** as a viscous liquid. R_f = 0.5 (40% EtOAc/Hexane). ^{1}H-NMR (300 MHz, $CDCl_3$): 7.33 (5H, m), 5.2 (2H, s), 4.16 (2H, q, *J* = 7Hz), 3.87 (2H, s), 2.92 (1H, bs), 1.47 (3H, s), 1.18 (3H, t, *J* = 7Hz). ^{13}C-NMR (75 MHz, $CDCl_3$): 172.0, 135.0, 128.8, 128.6, 128.3, 67.3, 66.9, 62.0, 56.0, 17.8, 14.0. IR (cm^{-1}): 3528, 2983, 1721. HRMS ($C_{14}H_{18}O_5Na^+$) calculated= 289.1098, found = 555.2197 $(C_{14}H_{18}O_5)_2Na^+$).

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

A 100 mL sealed tube was charged with a stir bar, 2.6g of **14** (9.7 mmol), and 20 mL of DMF. Et_3N (1.2g, 11.7 mmol) was then added drop wise and the solution was allowed to stir for 5 min. followed by the rapid addition of 1.34g (11.7 mmol) of methanesulfonyl chloride. The tube was tightly sealed and allowed to stir at room temperature for 6 h. The tube was opened and the reaction mixture was diluted with 100 mL of diethyl ether. The organic layer was washed three times with water, dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give 2.7 g (7.8 mmol, 80%) of

15 as colorless liquid. Due to the potential reactivity of **15**, it was used in the next step without further purification.

*(S)- 1-Benzyl-3-Ethyl-2-(Azidomethyl)-2-Methylmalonate (**16**)*

A 100 mL sealed tube was charged with a stirbar, 20 mL of DMF, and 2.7g of **15** (7.8 mmol). The solution was sparged for 5 min. with dry nitrogen gas and then 1g (15.7 mmol) of NaN_3 was added. The tube was sealed and allowed to stir at 90 ^oC for 12 h. The reaction mixture was cooled and diluted with 100 mL of diethyl ether. The resulting organic layer was washed three times with water, dried over $MgSO_4$, and evaporated under reduced pressure. The crude product was purified by column chromatography (30% EtOAc/Hexane) giving 0.8 g (2.7 mmol, 35%) of **10** as a pale yellow liquid. R_f = 0.74 (30% EtOAc/Hexane), $[\alpha]_D^{24} = -1.61$ (c = 2.86, $CHCl_3$), ^{1}H-NMR (300 MHz, $CDCl_3$): 7.35 (m, 5H), 5.20 (s, 2H), 4.16 (q, 2H, *J* = 6 Hz), 3.75 (s, 2H), 1.52 (s, 3H), 1.17 (t, 3H, *J* = 7 Hz), ^{13}C-NMR (75 MHz, $CDCl_3$): 14.0, 18.0, 54.0, 56.0, 62.0, 68.0, 129.0, 136.0, 170.0. IR (cm^{-1}):1727, 2104. HRMS ($C_{14}H_{17}O_4N_3Na^+$) calculated = 314.2923, found = 605.2329 $(C_{14}H_{17}N_3O_4)_2Na^+$).

*(R)-2-(Ethoxycarbonyl)-3-Amino-2-Methylpropanoic Acid (**17**)*

A 50 mL roundbottom flask was charged with 100 mg of 10% Pd/C, 10 mL of THF, 0.5g (1.7 mmol) of **16**, and a stir bar. The roundbottom flask was fitted with a septum and a balloon filled with hydrogen was attached to the flask via a needle. The mixture was stirred vigorously under a hydrogen atmosphere for 12 h. The reaction vessel was then opened and the contents were filtered through a pad of Celite® and the filter cake was washed with additional THF. The filtrate was evaporated under reduced pressure at room temperature giving 0.27 g (1.5 mmol, 88%) of **17** as a white solid. R_f =

0.2 (10% MeOH/CH_2Cl_2), MP = 114 ^oC, $[\alpha]_D^{22}$ = - 4.16 (c = 1.25, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$): 4.19 (m, 2H), 3.25 (d, 1H, *J*= 14 Hz), 3.03 (d, 1H, *J*= 14Hz), 1.47 (s, 3H), 1.27 (t, 3H, *J*= 7 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$): 13.0, 19.0, 44.0, 52.0, 61.0, 173.0, 175.0. IR (cm^{-1}): 1582, 3245. HRMS ($C_7H_{13}NO_4Na^+$) calculated = 198.1824, found = 373.1579 $(C_7H_{13}NO_4)_2Na^+$).

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

A 50 mL round bottom flask was charged with 0.5g (1.6 mmol) of **10a**, 20 mL of MeOH, 0.058g (1.8 mmol) of hydrazine hydrate (35% in water), and a stir bar. The flask was fitted with a reflux condenser and the reaction mixture was heated to 60 ^oC. The reaction was allowed to proceed for 6 h. The reaction mixture was allowed to cool and then filtered. The filtrate was evaporated under reduced pressure and the resulting residue was purified by column chromatography (8% MeOH/$CHCl_3$) to give 0.15g (0.86 mmol, 54%) of **17** as a white solid. $[\alpha]_D^{22}$= - 2.4 (c = 1.25, MeOH). The characterization data matched with **17** that was prepared from **16** as indicated above.

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

A 250 mL round bottom flask was charged with 10g of **10b** (31 mmol), 4.3 g of K_2CO_3 (31 mmol), 100 mL of anhydrous DMF, and a stirbar. A solution of 4.8g benzyl bromide (28 mmol) in 20 mL anhydrous DMF was slowly added over 15 minutes. The reaction was allowed to stir approximately 12 hr. under a nitrogen atmosphere. The reaction mixture was then diluted with 100 mL of water and the resulting mixture was washed with Et_2O (3 x 100 mL). The combined ether layer was washed with water (5 x 100 mL), washed with brine (2 x 100 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified by flash chromatography

(40% Et_2O/Hexanes) providing 11g of **18** (27 mmol, 96%) as a colorless liquid. R_f = 0.2 (40% Et_2O/Hexanes). $[\alpha]_D^{24}$ = -3.08 (c = 1, $CHCl_3$). IR (cm^{-1}): 2980, 1773, 1708. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.33 (m, 5H), 5.15 (m, 2H), 4.10 (m, 2H), 3.74 (m, 2H), 2.28 (m, 2H), 1.56 (s, 3H), 1.16 (t, 3H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.4, 171.3, 168.0, 135.5, 134.0, 132.0, 128.5, 128.3, 128.1, 123.0, 67.0, 61.0, 52.0, 33.8, 33.8, 20.0, 14.0. HRMS ($C_{23}H_{23}NO_6Na^+$): calculated = 432.1417, found = 432.1406.

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

A volume of 930 µL (10.2 mmol) 35% hydrazine in water was added to a solution of 3.8g (9.3 mmol) of **18** in 50 mL MeOH. The mixture was heated to reflux solvent overnight. A white precipitate was observed within an hour of reflux. The reaction mixture was allowed to cool to RT, and the resulting mixture was filtered. The filtrate was evaporated under reduced pressure. The resulting residue was taken up in CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, evaporated under reduced pressure, and purified by column chromatography using 30% Hexanes/EtOAc giving 1.2 g of a 10:1 mixture of **19a:19b** as a white solid. The mixture was recrystallized in cold Et_2O giving 1g (6 mmol, 64.5%) of pure **19a** as white crystals. R_f (**5**) = 0.31 (30% Hexanes/EtOAc). MP = 63 ^{0}C. $[\alpha]_D^{23}$= +19.0 (c = 2, MeOH). IR (cm^{-1}): 3245, 2985, 1726, 1698, 1660. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.06 (bs, 1H), 4.20 (m, 2H), 3.47 (m, 1H), 3.36 (m, 1H), 2.64 (m, 1H), 2.02 (m, 1H), 1.45 (s, 3H), 1.28 (t, 3H, *J* = 7 Hz), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 177.0, 172.0, 61.0, 51.0, 40.0, 34.0, 20.0, 14.0. HRMS ($C_8H_{13}NO_3Na^+$): calculated = 194.0788, found = 194.0795.

*(R)–3-Methyl-2-Oxopyrrolidine-3-Carboxylic Acid (**20**)*

An amount of 1.6g (9.4 mmol) of **19a** was dissolved in 15 mL ethanol. A volume of 7 mL 1N NaOH was added to the reaction mixture. The solution was brought to reflux solvent for an hour. The solution was cooled and acidified with HCl to pH 4. The water layer was concentrated at 35 ^{0}C under high vacuum. A volume of 10 mL MeOH was added to the residue and stirred for 5 min. The MeOH layer was decanted from the remaining solid and concentrated in vacuo giving 1g of **20** (6.9 mmol, 73%) as a white solid. MP = 155 ^{0}C. IR (cm^{-1}) = 3363, 3368, 2975, 2906, 1749, 1722, 1704, 1636, 1485. R_f = 0.17 (5% MeOH/ CH_2Cl_2). ^{1}H-NMR (CD_3OD, 400 MHz): δ 3.35 (m, 1H), 3.25 (m, 1H), 2.49 (m, 1H), 1.95 (m, 1H), 1.27 (s, 3H). ^{13}C-NMR (CD_3OD, 100 MHz): δ 179.6, 175.7, 52.1, 40.7, 35.0, 20.3. ESI-MS $(C_6H_{10}NO_3)^+$ = 143.1, observed = 143.2.

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

An amount of 1.77g (12.4 mmol) of **20** was dissolved in 50 mL of dry dichloroethane. A volume of 3.6 mL (26 mmol) Et_3N was added followed by 3.1 mL (13.6 mmol) diphenylphosphorylazide (DPPA). The solution was allowed to stir for 2 hrs at RT and then heated to reflux solvent for 2 hr. A volume of 1.8 mL (17.4 mmol) benzyl alcohol was then added and the solution was allowed to reflux solvent over night. The dichloroethane layer was concentrated in vacuo and the residue was purified by flash chromatography (40% EtOAc/Hexanes) giving 1.97g of **21** as a white wax (7.9 mmol, 64%). R_f = 0.10 (40% EtOAc/Hexanes). IR (cm^{-1}) = 3225, 1725, 1693, 1657, 1536. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.33 (m, 5H), 6.75 (bs, 1H), 5.55 (bs, 1H), 5.06 (m, 2H), 3.34 (m, 2H), 2.52 (m, 1H), 2.31 (m, 1H), 1.40 (s, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ

178.2, 155.2, 136.2, 128.7, 128.3, 128.2, 66.7, 57.2, 39.0, 34.8, 22.3. HRMS ($C_{13}H_{16}N_2O_3Na^+$) = 271.1053, observed = 271.1047.

*(R)- Tert-Butyl- 3- Methyl- 2-Ooxopyrrolidin-3- Ylcarbamate (**22**)*

An amount of 1.6 g (6.4 mmol) of **21** was dissolved in 25 mL MeOH in a pressure bottle. An amount of 0.16g Pd-C (10%) was added to the reaction mixture. The reaction mixture was allowed to shake under 20 psi H_2 pressure for 12 hr. The MeOH layer was filtered off through a Celite bed. The filtrate was concentrated "*in vacuo*" giving 0.66g of the free amine (5.8 mmol), which was then dissolved in 20 mL THF. A volume of 1.7mL (11.6 mmol) Et_3N was added to the reaction mixture. A solution of 1.5g $(BOC)_2O$ (6.9 mmol) in 10 mL THF was added to the reaction mixture drop wise. The reaction mixture was allowed to stir over night at RT. The THF was concentrated and the resulting residue was extracted with Et_2O and water. The ether layer was concentrated and the residue was rinsed with hexane giving 0.83g of the **22** (3.9 mmol, 61% over two steps) as a white solid. The characterization of **22** complied with the literature.[75] $[\propto]_D^{22}$ = -16 (c = 0.35, $CHCl_3$).

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

An amount of 10g (1eq) of the appropriate chiral half-ester (**10a-10f**) was dissolved in 50 mL dichloroethane in a 500 mL round bottom flask with a stirbar under a N_2 atmosphere. A measured volume of Et_3N (2.1 eq) and diphenylphosphorylazide (DPPA) (1.1eq) was added to the solution and the solution was allowed to stir at RT for 90 min. At this point the reaction was heated to reflux solvent for 2 hrs. A measured volume of para-methoxybenzyl alcohol (PMB-OH) (1.4 eq) was added to the reaction mixture and the reaction was continued to reflux solvent for 12 hrs. The reaction was

cooled and diluted with CH_2Cl_2, filtered through a silica bed (1" bed in a Buchner funnel) and evaporated. The resulting residue was purified by flash chromatography (40% EtOAC/Hexanes) giving the pure product as a white wax or colorless viscous oil.

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

23a was prepared following the general synthetic procedure for the formation of carbamates (**23a-23f**). An amount of 11.7g (26.5 mmol, 79%) of product was obtained as a white wax. R_f = 0.25 (40% EtOAc/Hexanes). IR (cm^{-1}) = 3368, 2958, 1774, 1708, 1612. $[\propto]_D^{23}$ = - 1.2 (c = 1.2, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.84 (m, 2H), 7.73 (bs, 2H), 7.31 (d, 2H, *J* = 9 Hz), 6.87 (d, 2H, 9 Hz), 6 (bs, 1H), 5 (q, 2H, *J* = 12 Hz), 4.18 (m, 2H), 4.12 (s, 2H), 3.8 (s, 3H), 1.65 (S, 3H), 1.25 (m, 3H). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 171.0, 168.5, 159.0, 155.0, 134.0, 132.0, 130.0, 128.4, 123.0, 113.3, 66.0, 62.0, 60.2, 55.0, 43.4, 20.0, 14.0. HRMS ($C_{23}H_{24}N_2O_7Na^+$) calculated = 463.1476, found = 463.1469.

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

23b was prepared following the general synthetic procedure for the formation of carbamates (**23a-23f**). An amount of 12g (26.4 mmol, 84%) of product was obtained as a colorless viscous oil. R_f = 0.29 (35% EtOAc/Hexanes). IR (cm^{-1}) = 3353, 2952, 1771, 1704, 1612. $[\propto]_D^{23}$ = + 11.3 (c =1, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.81 (m, 2H), 7.69 (m, 2H), 7.28 (m, 2H), 6.87 (m, 2H), 5.78 (bs, 1H), 4.94 (m, 2H), 4.07 (m, 2H), 3.80 (s, 3H), 3.67 (m, 2H), 2.57 (bm, 1H), 2.36 (m, 1H), 1.60 (s, 3H), 1.12 (t, 3H, *J* = 7Hz), ^{13}C-NMR ($CDCl_3$, 100MHz): δ 173.0, 168.0, 159.0, 154.0, 134.0, 132.0, 130.0, 128.6,

123.0, 114.0, 66.0, 62.0, 58.0, 55.0, 34.0, 33.6, 24.0, 14.0. HRMS ($C_{24}H_{26}N_2O_7Na^+$) calculated = 477.1638, found = 477.1635.

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

23c was prepared following the general synthetic procedure for the formation of carbamates (**23a-23f**). An amount of 11.8g (25.2 mmole, 84%) of product was obtained as a colorless viscous oil. R_f = 0.27 (35% EtOAc/Hexane). IR (cm^{-1}) = 3359, 2939, 1770, 1702, 1612. $[\propto]_D^{23}$ = - 6.0 (c = 0.8, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.82 (m, 2H), 7.70 (m, 2H), 7.28 (m, 2H), 6.88 (m, 2H), 5.63 (bs, 1H), 4.95 (s, 2H), 4.16 (m, 2H), 3.79 (s, 3H), 3.65 (m, 2H), 2.26 (m, 1H), 1.84 (m, 1H), 1.69 (m, 1H), 1.54 (s, 3H), 1.48 (m, 1H), 1.21 (t, 3H, *J* = 7Hz). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 174, 168, 160, 154, 134, 132, 130, 128, 123, 114, 66, 62, 60, 55, 38, 34, 23.5, 23.4, 14. HRMS ($C_{25}H_{28}N_2O7Na^+$) calculated = 491.1789, found = 491.1782.

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

23d was prepared following the general synthetic procedure for the formation of carbamates (**23a-23f**). An amount of 11.5g (24 mmol, 83%) of product was obtained as a colorless viscous oil. R_f = 0.31 (35% EtOAc/Hexanes). IR (cm^{-1}) = 3360, 2958, 1768, 1701, 1612. $[\propto]_D^{23}$ = - 1.5 (c = 1.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.82 (m, 2H), 7.69 (m, 2H), 7.30 (m, 2H), 6.88 (m, 2H), 5.62 (bs, 1H), 4.99 (s, 2H), 4.17 (m, 2H), 3.8 (s, 3H), 3.63 (t, 2H, *J* = 7Hz), 2.17 (m, 1H), 1.83 (m, 1H), 1.65 (m, 2H), 1.56 (s, 3H), 1.33 (m, 1H), 1.23 (t, 3H, *J* = 7Hz), 1.12 (m, 1H). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 174.0, 168.0, 159.5, 154.6, 134.0, 132.1, 130.0, 128.7, 123.2, 114.0, 66.2, 62.0, 60.0, 55.3, 38.0,

36.0, 28.4, 23.4, 21.4, 14.1. HRMS ($C_{26}H_{30}N_2O_7Na^+$) calculated = 505.1945, found = 505.1930.

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

23e was prepared following the general synthetic procedure for the formation of carbamates (**23a-23f**). An amount of 11.5g (23 mmol, 82%) of product was obtained as a colorless viscous oil. R_f = 0.33 (35% EtOAc/Hexanes), IR (cm^{-1}) = 3367, 2938, 1770, 1703, 1612. $[\propto]_D^{23}$ = + 1.4 (c = 1, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.28 (d, 2H, *J* = 9Hz), 6.87 (m, 2H, *J* = 9Hz), 5.63 (bs, 1H), 4.99 (s, 2H), 4.18 (m, 2H), 3.79 (s, 3H), 3.64 (t, 2H, *J* = 7Hz), 2.12 (bm, 1H), 1.76 (m, 1H), 1.64 (m, 2H), 1.55 (s, 3H), 1.27 (m, 7H). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 174.0, 168.0, 159.0, 155.0, 134.0, 132.0, 130.0, 129.0, 123.0, 114.0, 66.0, 61.0, 60.0, 55.0, 38.0, 36.0, 28.0, 27.0, 24.0, 23.5, 14.0. HRMS ($C_{27}H_{32}N_2O7Na^+$) calculated = 519.2102, found = 519.2095.

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

23f was prepared following the general synthetic procedure for the formation of carbamates (**23a-23f**). An amount of 11.3g (22 mmol, 83%) of product was obtained as colorless viscous oil. R_f = 0.34 (35% EtOAc/Hexanes). IR (cm^{-1}) = 3366, 2936, 2859, 1770, 1703, 1612. $[\propto]_D^{23}$ = - 1.7 (c = 1.4, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.74 (m, 2H), 7.62 (m, 2H), 7.22 (d, 2H, *J* = 7Hz), 6.80 (d, 2H, *J* = 8Hz), 5.51 (bs, 1H), 4.91 (s, 2H), 4.11 (m, 2H), 3.73 (s, 3H), 3.58 (t, 2H, *J* = 7Hz), 2.04 (m, 1H), 1.67 (m, 1H), 1.56 (m, 2H), 1.47 (s, 3H), 1.20 (m, 8H), 0.98 (bm, 1H). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 173.0,

167.0, 157.0, 154.0, 133.0, 131.0, 129.0, 128.0, 122.0, 113.0, 65.0, 60.0, 59.0, 54.0, 37.0, 36.0, 28.0, 27.0, 26.0, 23.0, 22.0, 13.0. HRMS ($C_{28}H_{34}N_2O_7Na^+$) calculated = 533.2258, found = 533.2251.

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

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

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

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

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

An amount of **10e**/**10f** (1 equivalent) was dissolved in DMF under N_2. A calculated amount of K_2CO_3 (1.2 equivalent) was added to the solution. A measured volume of benzyl bromide (0.95 equivalents) was added to the reaction mixture. The reaction was allowed to stir over night under N_2. Water was added to the reaction mixture, and the aqueous layer was extracted with Et_2O (3 x 50 mL). The combined ether layer

was given a water wash (10 x 50 mL). The Et_2O layer was dried over $MgSO_4$, concentrated, and the residue was purified by flash chromatography (40% Et_2O/ Hexanes) giving the product as a colorless oil.

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

25a was synthesized following the general synthetic procedure for the formation of **25a/25b** using 5g (14 mmol) of **10e**. An amount of 5.2g of **25a** (11.5 mmol, 82%) was obtained as a colorless viscous oil after purification (40% Et_2O/Hexanes). R_f = 0.16 (40% Et_2O/Hexanes). IR (cm^{-1}) = 2938, 1770, 1700. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.32 (m, 5H), 5.15 (m, 2H), 4.11 (q, 2H, *J* = 7Hz), 3.64 (t, 2H, *J* = 7Hz), 1.85 (m, 2H), 1.64 (m, 2H), 1.41 (s, 3H), 1.28 (m, 4H), 1.15 (t, 3H, *J* = 7Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.2, 172.0, 168.4, 136.0, 134.0, 132.2, 128.4, 128.2, 128.0, 123.2, 66.6, 61.1, 54.0, 38.0, 35.4, 28.2, 27.0, 24.0, 20.0, 14. HRMS ($C_{26}H_{29}NO_6Na^+$) calculated = 474.1887, observed = 474.1905.

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

25b was synthesized following the general synthetic procedure for the formation of **25a/25b** using 5g (13.3 mmol) of **10f**. An amount of 5.3g of **25b** (11.4 mmol, 86%) was obtained as a colorless viscous oil after purification (40% Et_2O/Hexanes). R_f = 0.30 (40% Et_2O/Hexanes). IR (cm^{-1}) = 2936, 1771, 1706. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 2H), 7.70 (m, 2H), 7.32 (m, 5H), 5.15 (m, 2H), 4.11 (q, 2H, *J* = 7Hz), 3.64 (t, 2H, *J* = 7Hz), 1.85 (m, 2H), 1.64 (m, 2H), 1.41 (s, 3H), 1.30 (m, 4H), 1.17 (m, 5H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.3, 172.1, 168.4, 136.0, 134.0, 132.2, 128.5, 128.2, 128.0, 123.2, 66.7, 61.1, 54.0, 38.0, 35.5, 29.4, 28.5, 26.5, 24.2, 20.0, 14. HRMS ($C_{27}H_{31}NO_6Na^+$) calculated = 488.2043, observed = 488.2030.

*General Synthetic Procedure for the Formation of **26a** and **26b***

A measured amount of **25a/25b** (1 equivalent) was dissolved in methanol. A calculated amount of 35% $N_2H_4.H_2O$ in water (1.2 equivalents) was added to the reaction mixture. The reaction mixture was heated to reflux solvent for 6 hr. The reaction mixture was cooled to RT and the white precipitate was filtered off. The MeOH layer was concentrated "*in vacuo*" and the gummy solid was taken up in CH_2Cl_2 leading to more white precipitate. The white precipitate is again removed by filtration and the CH_2Cl_2 layer was again concentrated "*in vacuo*" giving pure product as colorless oil.

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

26a was prepared from **25a** following the general synthetic procedure for the formation of **26a/26b** using 5g of **25a** (11 mmol). An amount of 3.3g (10.3 mmol, 94%) of **26a** was obtained as a colorless viscous oil. R_f = 0.12 (3% MeOH/CH_2Cl_2). IR (cm^{-1}) = 3100, 3000, 2938, 1724. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.32 (m, 5H), 5.15 (m, 2H), 4.11 (q, 2H, *J* = 7Hz), 2.65 (t, 2H, *J* = 7Hz), 1.87 (t, 2H, *J* = 8Hz), 1.61 (bs, 2H), 1.41 (m, 5H), 1.24 (m, 7H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 172.3, 172.2, 136.0, 128.5, 128.2, 128.0, 67, 61.2, 54.0, 42.0, 35.4, 33.5, 27.0, 24.0, 20.0, 14.0. HRMS ($C_{18}H_{27}NO_4Na^+$) calculated = 344.1832, observed = 344.1823.

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

26b was prepared from **25b** following the general synthetic procedure for the formation of **26a/26b** using 5g of **25b** (10.7 mmol). An amount of 3g (8.9 mmol, 83%) of **26b** was obtained as colorless viscous oil. R_f = 0.14 (3% MeOH/CH_2Cl_2). IR (cm^{-1}) = 3300, 2932, 1726. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.25 (m, 5H), 5.07 (m, 2H), 4.04 (q, 2H, *J* = 7Hz), 2.68 (bs, 2H), 2.61 (t, 2H, *J* = 7Hz), 1.77 (t, 2H, *J* = 7Hz), 1.33 (m, 5H),

1.14 (m, 9H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 171.3, 171.2, 135.0, 127.5, 127.2, 127.0, 66.0, 60.1, 53.0, 41.0, 34.5, 32.0, 28.5, 25.5, 23.1, 19.0, 13.0. HRMS ($C19H29NO4Na^+$) calculated = 358.1988, observed = 358.1983.

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

27a/27b were synthesized from **26a/26b** following a literature procedure.[87] A measured amount of **26a/26b** (1 equivalent) was dissolved in 24 mL 2:1 2.5M NaOH/EtOH mixture. The solution was cooled to 0 ^{0}C and a measured amount of NH_2OSO_3H (2 equivalent) was added to the solution. The solution was stirred at 0 ^{0}C for 35 minutes. At that point an additional amount of NH_2OSO_3H (1 equivalent) and 5 mL 2.5 M NaOH were added to the reaction mixture. The reaction was allowed to stir at 0 ^{0}C for another 90 minutes and then allowed to warm to RT overnight. The reaction mixture was acidified to pH 1. The aqueous layer was extracted with Et_2O (3 x 50 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, concentrated "*in vacuo*", and purified in 1:1 Et_2O/Hexanes giving the product as a colorless oil.

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

27a was prepared from **26a** following the general synthetic procedure of making **27a/27b** using 3g of **26a** (9.3 mmol). An amount of 1.2g of **27a** was obtained (5.5 mmol, 59%) after purification. R_f = 0.49 (50% Et_2O/Hexanes). IR (cm^{-1}) = 2956, 2930, 2871, 1705. $[\alpha]_D^{25}$ = + 3.15 (c = 2, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 10.38 (s, 1H), 4.21 (q, 2H, *J* = 7Hz), 1.87 (m, 2H), 1.44 (s, 3H), 1.27 (m, 9H), 0.88 (t, 3H, *J* = 7Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 178.0, 172.5, 61.5, 53.6, 35.7, 32.0, 24.0, 22.3, 20.0, 14.0, 13.9. HRMS ($C_{11}H_{20}O_4Na^+$) calculated = 239.1255, observed = 239.1253.

*(R)- 2-(Ethoxycarbonyl)-2-Methyloctanoic Acid (**27b**)*

27b was prepared from **26b** following the general synthetic procedure of making **27a/27b** using 3g of **26b** (8.9 mmol). An amount of 1.3g pure **27b** was obtained (5.6 mmol, 63%) after purification. R_f = 0.51 (50% Et_2O/Hexanes). IR (cm^{-1}) = 2955, 2927, 2858, 1705. $[\alpha]_D^{22}$ = + 2.2 (c = 1, CH_2Cl_2). ^{1}H-NMR ($CDCl_3$, 400MHz): δ 4.21 (q, 2H, *J* = 7Hz), 1.87 (m, 2H), 1.44 (s, 3H), 1.28 (m, 11H), 0.88 (t, 3H, *J* = 7Hz). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 178.0, 172.6, 61.6, 53.6, 35.8, 31.4, 29.4, 24.2, 22.6, 20.0, 14.1. 14.0. HRMS ($C_{12}H_{22}O_4Na^+$) calculated = 253.1410, observed = 253.1409.

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

A measured amount of **27a/27b** (1 equivalent) was dissolved in 3 mL of H_2O, and 1 mL of acetone was added to the solution. A solution of Et_3N (1.2 equivalent) in 1 mL acetone was added to the reaction mixture drop wise followed by a solution of methylchloroformate (1.55 equivalent) in 1 mL acetone. The reaction was allowed to stir for 30 minutes at RT. A solution of NaN_3 (1.6 equivalent) in 3 mL H_2O was added to the reaction mixture and the mixtures was stirred for 2hrs. The reaction mixture was then poured into 25 mL of ice cold water. The water layer was extracted with ether (3 x 50 mL). The combined ether layer was dried over $MgSO_4$, concentrated "*in vacuo*" giving the acylazide as colorless oil. The acylazide was dissolved in toluene and heated to reflux solvent for 2 hrs. The toluene was concentrated "*in vacuo*" giving the isocyanate as yellowish oil. A volume of 10 mL 4M HCl was added to the isocyanate and the mixture was heated to reflux solvent for 4 hrs. The water layer was concentrated under reduced pressure giving the (*S*)-α-alkyl-alaninehydrochloride as a pale yellowish solid. The **28a/28b** HCl salt was then dissolved in MeOH and $NaHCO_3$ was added portion wise to

neutralize it to (S)-α-alkyl-alanine (**28a**/**28b**). The MeOH layer was filtered and concentrated giving **28a**/**28b** as a white solid.

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

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

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

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

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

An amount of 10gm (21 mmol) **23d** was dissolved in 60 mL of methylene chloride. A volume of 10 mL TFA was added. The solution was stirred over 1 h. The solution turned dark purple. A volume of 100 mL H_2O was added to the solution. The methylene chloride layer was extracted, washed with $NaHCO_3$ solution, washed with H_2O, dried over $MgSO_4$. The crude was purified by flash chromatography (5% MeOH/CH_2Cl_2) giving 6.2gm (19.4mmoles, 92%) of the pure product as a white wax. TLC R_f = 0.35 (5% MeOH/CH_2Cl_2), ^{1}H NMR ($CDCl_3$, 400MHz) 7.81(m, 2H), 7.71(m, 2H), 4.15(q, 2H, J= 7Hz), 3.67(t, 2H, J= 7Hz), 1.67(m, 6H), 1.39(m, 1H), 1.31(s, 3H),

1.25(m, 4H)., ^{13}C NMR ($CDCl_3$, 400MHz) 177, 168, 134, 132, 123, 61, 57, 40, 37, 28, 26, 21, 14.. HRMS ($C_{17}H_{22}N_2O_4Na^+$) calculated = 341.1477, observed = 341.1464.

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

An amount of 5g (16 mmol) **29** was dissolved in 60 mL of distilled acetonitrile in a 250 mL three necked flask under N_2 atmosphere. An amount of 13.3gm (96 mmol) of K_2CO_3 was added with stirring. A volume of 9.5 mL (80 mmol) of BnBr was added drop wise. The reaction was brought to reflux for 12 hrs. The reaction mixture was diluted with 50 mL of H_2O. The solution was extracted with ether three times. The ether layer was washed with H_2O, washed with brine, dried over $MgSO_4$ and evaporated. The crude was then purified by flash chromatography (30% EtOAc/Hexane), giving 3.84 g (7.7 mmoles, 48%) of the pure product as white solid. TLC R_f = 0.61 (30% EtOAc/Hexane), MP = 91^oC. ^1H NMR ($CDCl_3$, 300MHz) 7.82(m, 2H), 7.69(m, 2H), 7.15(m, 10H), 4.13(m, 2H), 3.80(m, 4H), 3.61(t, 2H, J=7Hz), 1.79(m, 2H), 1.52(m, 3H), 1.29(m, 7H). ^{13}C NMR ($CDCl_3$, 75MHz) 175, 169, 142, 134, 132, 128.6, 128, 127, 123, 67, 61, 55, 38, 37, 29, 21.7, 21.6, 15. HRMS ($C_{31}H_{34}N_2O_4Na^+$) calculated = 521.2416, observed = 521.2398.

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

An amount of 3.5g (10.3 mmoles) **30** was dissolved in 15mL EtOH in a 250mL single neck round bottom falsk with a stir bar. A volume of 100mL 8N NaOH solution was added to it and the resulting reaction mixture was brought to reflux over 48 hrs. The water layer was acidified to pH 2, evaporated out under reduced pressure, and triturated with MeOH. The MeOH layer was neutralized by solid $NaHCO_3$, evaporated out under

reduced pressure giving 2.8g (8.5mmoles, 82%) pure **31** as white wax, which was taken for next step without further purification. ^{1}H NMR (CD_3OD, 300MHz): 7.26(bs, 10H), 4.34(m, 4H), 2.81(bs, 2H), 1.90(m, 2H), 1.54(m, 6H), 1.24(b, 1H).

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

A solution of 1gm **31** (1eq) in 10mL water was placed in 50mL round bottom flask. A measured amount of $NaHCO_3$ (2eq) was added with stirring. The solution was cooled down to 0^{O}C under ice. A solution of $(Boc)_2O$ (1.4eq) in 10mL 1,4 dioxane was added dropwise. The reaction was continued to stir at 0^{O}C over an hr. The reaction was then allowed to come back to RT and continued to stir over the night. The reaction was diluted with 15mL H_2O, acidified to pH4 by $NaHSO_4$, extracted with Et_2O twice. The combined ether layer was washed with water (5 X 30mL), washed with brine, dried over $MgSO_4$, and evaporated out. The crude was cromatographed (40% EtOAc/hexanes), giving 1.14g (2.6 mmol, 87%) pure **32** as white solid. TLC R_f = 0.31 (40% EtOAc/Hexane). MP = 57^{o}C. ^{1}H NMR ($CDCl_3$, 400MHz) 7.27(m, 10H), 4.51(bs, 1H), 3.89(m, 4H), 3 (bs, 2H), 1.68(m, 2H), 1.39(m, 16H). ^{13}C NMR ($CDCl_3$, 100MHz) 175, 156, 137, 128.8, 128.6, 128, 79, 71, 55, 40, 37, 30, 29, 22, 18. HRMS ($C_{26}H_{36}N_2O_4Na^{+}$) calculated = 463.2573, observed = 463.2562.

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

A solution of 1g **32** (1eq) in 25mL MeOH was placed in a pershaker bottle. An amount of 0.2gm (20% by weight) Pd-C was added to the bottle. The reaction was continued to shake at 25psi over 12 hrs at room temperature. The reaction mixture was filtered through selite bed. The filtrate was evaported giving the pure product. An amount

of 0.55gm (2.1 mmoles, 91%) product was obtained. The product was confirmed by ^{1}H NMR and HRMS. However, ^{13}C NMR showed up an extra peak. The product was taken for next step without further purification. R_f = 0.21 (5% MeOH/CH_2Cl_2) , IR (cm^{-1}), ^{1}H NMR (CD3OD, 400MHz): 3.03(t, 2H, J=7Hz), 1.45(m, 18H). HRMS ($C_{12}H_{24}N_2O_4Na^+$) calculated = 283.1634, observed = 283.1628.

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

An amount of $NaHCO_3$ (2eq) was added to a solution of 0.5gm **33** (1eq) in 15mL water with stirring. The solution was cooled down to 0 ^{O}C under ice. A solution of Fmoc-Osu (1.5 eq) in 15mL 1,4-dioxane was added to the reaction mixture over 20min. The reaction was continued to stir at 0 ^{O}C over an hr and at ambient temperature over 12 hrs. At that point the reaction was diluted with 30mL of water, acidified to pH 4 with 4M HCl, extracted (3X50mL) with Et_2O. The combined ether layer was given brine wash, dried over $MgSO_4$, and evaporated out at reduced pressure giving crude as light yellowish oil. The crude product was purified by radial chromatography using 5% MeOH/CH_2Cl_2, giving 0.78g pure **34** (1.62 mmol, 85%) as white solid. R_f = 0.33 (5% CH_2Cl_2/MeOH), IR (cm^{-1}) = 3350, 2941, 1681, 1504, $[\alpha]_D^{22}$ = +14.4 (C=1, $CHCl_3$). MP = 95 ^{0}C ^{1}H NMR (CD_3OD, 400MHz) 7.69(d, 2H, J=8Hz), 7.55(d, 2H, J=8Hz), 7.28(t, 2H, J=8Hz), 7.21(t, 2H, J=8Hz), 4.22(d, 2H, J=7Hz), 4.11(t, 1H, J=7Hz), 2.92(t, 2H, J=7Hz), 1.77(bs, 2H), 1.33(m, 16H). ^{13}C NMR (CD_3OD,MHz) 176,157, 155, 144, 143.9, 141, 127, 126.7, 125, 119, 78, 66, 59, 40, 36, 29, 27, 21, 20. HRMS ($C_{27}H_{34}N_2O_6Na^+$) calculated = 505.2309, observed = 505.2296.

*Synthesis of (9H-Fluoren-9-Yl)-Methyl-(S)-2-(Ethoxycarbonyl)-6-(1,3-Dioxoisoindolin-2-Yl)Hexan-2-Ylcarbamate (**35**)*

A volume of 320 µL Et_3N (2.3 mmol) was added to a solution of 0.7g (1.9 mmol) **10d** in 25 mL dichloroethane under a N_2 atmosphere. A volume of 460 µL DPPA (2 mmol) was added to the reaction mixture. The mixture was allowed to stir at RT for 2 hrs. The mixture was then heated to reflux solvent for 3 hrs. The reaction was cooled and washed with saturated NH_4Cl solution. The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure giving the crude isocyanate. The isocyanate was dissolved in dry toluene under a N_2 atmosphere. An amount of 0.75g (3.8 mmol) 9-fluorenylmethanol and a volume of 66 µL Ti (IV) isopropoxide was added to the solution. The mixture was heated to 80 ^{0}C for 12 hrs. The mixture was cooled and the toluene was evaporated under reduced pressure giving the crude product. The residue was purified by chromatography (10% Hexanes/CH_2Cl_2), giving 0.95g **35** (1.75 mmol, 92%) as a white solid. R_f = 0.29 (10% Hexanes/CH_2Cl_2). IR (cm^{-1}) = 3365, 2940, 1769, 1704, 1613, 1504. MP = 81 ^{0}C, $[\alpha]_D^{22}$ = -14.3 (c = 1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400MHz): δ 7.78 (m, 4H), 7.64 (m, 4H), 7.40(t, 2H, *J* = 7Hz), 7.32 (t, 2H, *J* = 7Hz), 5.72 (bs, 1H), 4.35 (bm, 2H), 4.20 (bm, 3H), 3.65 (bm, 2H), 2.21 (bm, 1H), 1.85 (bm, 1H), 1.60 (m, 5H), 1.35 (bm, 1H), 1.24 (bm, 3H), 1.13 (bm, 1H). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 174.0, 168.0, 144.0, 141.0, 134.0, 132.0, 128.0, 127.0, 125.0, 123.0, 120.0, 66.0, 62.0, 60.0, 47.0, 37.5, 36.0, 28.0, 23.4, 21.0, 14.0. HRMS ($C_{32}H_{32}N_2O_6Na^+$) calculated = 563.2152, observed = 563.2144.

*Fluorenylmethyloxycarbonylamino)-2-Methylhexanoic Acid.HCl (**36**)*

36 was synthesized from **35** following a literature published procedure.[89] An amount of 0.9g (1.7 mmol) **35** was dissolved in 12 mL 1, 4-dioxane. A volume of 12 mL

5N HCl was added to the solution. The solution was heated to reflux solvent for 24 hrs. At which time the reaction was found to be completed by ESI-MS. The solution was concentrated under reduced pressure and the residue was taken for the next step without further purification.

*Synthesis of (S)-^{t}Boc-Fmoc-α-Methyl-α-Lysine-OH (**34**)*

An amount of 0.25g $NaHCO_3$ (3 mmol) was added to a solution of 0.6g of **36** (~1.5 mmol) in 15 mL of water with stirring. The solution was cooled to 0 ^{0}C. A solution of 0.65g $(Boc)_2O$ (3 mmol) in 15 mL 1, 4-dioxane was added to the reaction mixture over 20 min. The reaction was allowed to stir at 0 ^{0}C for an hour and then at ambient temperature for 12 hrs. The mixture was given pentane wash to remove excess $(Boc)_2O$. The reaction mixture was diluted with 30 mL of water, acidified to pH 4 with 2M HCl, and extracted (3 x 50 mL) with Et_2O. The combined ether layer was washed with brine, dried over $MgSO_4$, evaporated under reduced pressure giving the crude product as light yellow oil. The residue was purified by radial chromatography using 5% MeOH/CH_2Cl_2 giving 0.78g (1.6 mmol, 94% over two steps) of product as a white solid. R_f = 0.33 (5% MeOH/CH_2Cl_2). IR (cm^{-1}) = 3350, 2941, 1681, 1504. MP = 95 ^{0}C. $[\alpha]_D^{22}$ = +14.4 (c = 1, $CHCl_3$), ^{1}H-NMR (CD_3OD, 400MHz): δ 7.69 (d, 2H, *J* = 8Hz), 7.55 (d, 2H, *J* = 8Hz), 7.28 (t, 2H, *J* = 8Hz), 7.21 (t, 2H, *J* = 8Hz), 4.22 (d, 2H, *J* = 7Hz), 4.11 (t, 1H, *J* = 7Hz), 2.92 (t, 2H, *J* = 7Hz), 1.77 (bs, 2H), 1.33 (m, 16H). ^{13}C-NMR (CD_3OD, 100 MHz): 176.0, 157.0, 155.0, 144.0, 143.9, 141.0, 127.0, 126.7.0, 125.0, 119.0, 78.0, 66.0, 59.0, 40.0, 36.0, 29.0, 27.0, 21.0, 20.0. HRMS ($C_{27}H_{34}N_2O_6Na^+$) calculated = 505.2309, observed = 505.2296.

*Synthesis of (S)-1-Tert-Butyl 3-Ethyl 2-[4-(1,3-Dioxoisoindolin-2-Yl)Butyl]-2-Methylmalonate (**37**)*

A volume of 3 mL conc. H_2SO_4 was added to a solution of 10g **10d** (29 mmol) in 100 mL CH_2Cl_2 in a 250 mL sealed tube. The solution was cooled to -7 ^{0}C in an ice salt bath. A volume of 50 mL of condensed isobutylene was added to the solution. The tube was capped tightly and allowed to stir over night at RT. The tube was uncapped and allowed to stir for 2 hrs at ambient pressure to allow the excess isobutylene to evaporate. The solution was diluted with CH_2Cl_2 and gently washed three times with 1N NaOH (50 mL). The CH_2Cl_2 layer was dried over $MgSO_4$, evaporated under reduced pressure, and purified by chromatography (40% EtOAC/Hexanes), giving 10.8g of product (26.7 mmol, 92%) as a colorless liquid. R_f = 0.60 (40% EtOAc/Hexanes), IR (cm^{-1}) = 2977, 2937, 1771, 1707. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 7.84 (m, 2H), 7.71 (m, 2H), 4.16 (q, 2H, *J* = 7Hz), 3.68 (t, 2H, *J* = 7Hz), 1.85 (m, 2H), 1.69 (m, 2H), 1.42 (s, 9H), 1.28 (m, 8H). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 172.0, 171.0, 168.0, 134.0, 132.0, 123.0, 81.0, 61.0, 54.0, 38.0, 35.0, 29.0, 28.0, 22.0, 20.0, 14.0. HRMS ($C_{22}H_{29}NO_6Na^+$) calculated = 426.1887, observed = 426.1873.

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

A volume of 2.8 mL (31.4 mmol) $N_2H_4.H_2O$ (35% in H_2O) was added in a solution of 10.5g **37** (26 mmol) in 60 mL MeOH. The solution was heated to reflux solvent for 6 hrs. The reaction mixture was found to turn turbid and a white precipitate formed within 2 hrs of reflux. The reaction was monitored by TLC. The reaction was cooled to RT and the MeOH was removed "*in vacuo*". The residue was taken up in CH_2Cl_2 and the white precipitate was filtered off. The CH_2Cl_2 was evaporated under

reduced pressure, giving 6.75g (24.7 mmol, 95%) of **38** as a colorless oil. R_f = 0.16 (5% MeOH/CH_2Cl_2), IR (cm^{-1}) = 3395, 2977, 2934, 2867, 1723, 1654. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 4.17 (q, 2H, *J* = 7Hz), 2.70 (t, 2H, *J* = 7Hz), 1.82 (m, 2H), 1.53 (bs, 2H), 1.45 (m, 11H), 1.35 (s, 3H), 1.27 (m, 5H). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 172.0, 171.0, 81.0, 61.0, 54.0, 42.0, 35.0, 34.0, 28.0, 21.0, 19.0, 14.0. HRMS ($C_{14}H_{27}NO_4Na^+$) calculated = 296.1832, observed = 296.1828.

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

An amount of 1.76g LiOH (73.5 mmol) was added in a solution of 6.7g **38** (24.5 mmol) in 30 mL of 3:7 EtOH/H_2O mixture. The solution was allowed to stir for 48 hrs at RT. The solvents were evaporated under reduced pressure upon completion as determined by TLC (5% MeOH/CH_2Cl_2). The residue was triturated with MeOH to precipitate excess LiOH. The MeOH layer was evaporated under reduced pressure giving 5.76g of **20** (96%, 23.5 mmol) as a white wax. R_f = 0.10 (5% MeOH/CH_2Cl_2). IR (cm^{-1}) = 3297, 2961, 2937, 1541, 1448. ^{1}H-NMR (CD_3OD, 400MHz): δ 2.64 (t, 2H, *J* = 7Hz), 1.79 (m, 2H), 1.46 (m, 11H), 1.29 (m, 5H). ^{13}C-NMR (CD_3OD, 100MHz): δ 178.0, 175.0, 80.0, 56.0, 41.0, 36.0, 33.0, 26.0, 21.0, 20.0. ESI-MS ($C_{12}H_{23}NO_4Na^+$) calculated 268.3, observed 268.2.

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

An amount of 3.9g $NaHCO_3$ (46.5 mmol) was added to a solution of 5.7g of **39** (23.2 mmol) in 20 mL H_2O. The solution was cooled to 0 ^{0}C. A solution of 6g of $(Boc)_2O$ (28 mmol) in 20 mL 1,4-dioxane was added drop wise to the reaction mixture. The reaction was allowed to stir at 0 ^{0}C for an hr and then brought to RT. The reaction was

allowed to stir at RT for 12 hrs. The reaction mixture was extracted with pentane. The aqueous layer was acidified to pH 4 using 2N HCl and extracted three times with Et_2O (50 mL). The combined ether layer was dried over $MgSO_4$, evaporated under reduced pressure, and purified by chromatography (40% EtOAc/Hexanes), giving 7.6g (22 mmol, 95%) of **40** as a colorless viscous oil. R_f = 0.54 (40% EtOAc/ Hexanes), IR (cm^{-1}) = 3380, 2976, 2934, 1706, 1522. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 4.61 (bs, 1H), 3.12 (bm, 2H), 1.84 (m, 2H), 1.46 (m, 23H), 1.29 (m, 2H). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 177.0, 172.0, 156.0, 82.0, 79.0, 54.0, 40.0, 35.0, 30.0, 28.4, 27.8, 22.0, 20.0. HRMS ($C_{17}H_{31}NO_6Na^+$) calculated = 368.2043, observed = 368.2035.

*Synthesis of (R)-Tert-Butyl-2-(9-Fluorenylmethylamino)-6-(Tert-Butyloxycarbonylamino)-2-Methylhexanoate (**41**)*

A volume of 2.7 mL of Et_3N (19.4 mmol) was added to a solution of 5.6g **40** (16.2 mmol) in 60 mL dichloroethane under a N_2 atmosphere. A volume of 3.9 mL (17.3 mmol) of DPPA was added to the reaction mixture. The mixture was allowed to stir at RT for 2 hrs. The mixture was heated to reflux solvent for 3 hrs. The reaction was cooled, and the organic layer was extracted with saturated NH_4Cl solution, dried over $MgSO_4$ and evaporated under reduced pressure giving the isocyanate. The isocyanate was taken up in dry toluene under a N_2 atmosphere. An amount of 6.4g (32.4 mmol) 9-fluorenylmethanol was added to the solution along with 300 µL of Ti (IV) isopropoxide. The solution was heated to 80 ^{0}C over night. The reaction was cooled, and the organic layer was evaporated under reduced pressure. The residue was then purified by chromatography (CH_2Cl_2) giving 7.75g (14.4 mmol, 89%) of **41** as colorless wax. An amount of 50mg of **41** was further purified by reversed phase HPLC (40% CH_3CN/H_2O to 100% CH_3CN in

15 min at 262 nm, R_t = 16.6 min), giving 35mg of pure **41** as a colorless oil. R_f = 0.57 (CH_2Cl_2), IR (cm^{-1}) = 3359, 2975, 2931, 1707, 1516. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 7.76 (d, 2H, J=8Hz), 7.61 (d, 2H, *J* = 8Hz), 7.40 (t, 2H, *J* = 7Hz), 7.32 (t, 2H, *J* = 7Hz), 5.82 (bs, 1H), 4.47 (m, 2H), 4.18 (t, 1H, *J* = 7Hz), 4.02 (bs, 1H), 3.07 (bm, 2H), 2.23 (bm, 1H), 1.74 (bm, 1H), 1.45 (m, 25H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 173.2, 156.03, 154.3, 143.9, 141.4, 127.6, 127.0, 125.1, 120.0, 82.2, 79.2, 66.3, 60.1, 47.2, 40.1, 35.8, 29.7, 28.3, 27.9, 23.7, 21.2. HRMS ($C_{31}H_{42}N_2O_6Na^+$) calculated = 561.2935, observed = 561.2924.

*Synthesis of (R)-^{t}Boc-Fmoc-α-Methyl-α-Lysine-OH (**42**)*

An amount of 2g (3.7 mmol) of **41** was dissolved in 20 mL of 1:1 TFA/CH_2Cl_2. The solution was allowed to stir for 12 hrs at RT under a N_2 atmosphere. The reaction was monitored by TLC (5% MeOH/CH_2Cl_2) and ESI-mass spectrometry for completion. At which point the TFA/CH_2Cl_2 layer was evaporated under reduced pressure giving free amino acid. The residue was taken up in 15 mL of H_2O and 0.76g (9 mmol) of $NaHCO_3$ was added to the solution slowly to control the effervescence. The mixture was cooled to 0 ^{O}C. A solution of 0.96g (4.4 mmol) $(Boc)_2O$ in 15 mL 1,4-dioxane was added to the mixture slowly at 0 ^{O}C. The reaction was allowed to stir at 0 ^{0}C for an hour. The reaction was then allowed to warm to RT and stir for 12 hrs. The reaction mixture was extracted with pentane to remove excess $(Boc)_2O$. The aqueous layer was then acidified to pH 4 with 2N HCl, and extracted three times with Et_2O (50 mL). The combined ether layer was dried over $MgSO_4$, evaporated under reduced pressure, and purified by chromatography (5% MeOH/CH_2Cl_2), giving 1.52g (3.15 mmol, 85% over two steps) of **42** as a white solid similar **34**. All characterization data of **42** complied with the data for

34. The polarimetry reading confirmed **42** as the enantiomer to **34**. $[\alpha]_D^{22}$ = -11.5 (c = 1, $CHCl_3$).

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

An amount of 10g (23 mmol) **23a** was dissolved in 60 mL of methylene chloride and 10 mL TFA was added. The solution was stirred for 1 hr. The solution became dark purple in color. A volume of 100 mL H_2O was added to the solution and the organic layer was washed with $NaHCO_3$ solution, washed with H_2O, and dried over $MgSO_4$. The residue was purified by flash chromatography (5% MeOH/CH_2Cl_2), giving 5.7g (20.6mmol, 90%) of **43** as a white wax. R_f = 0.64 (5% MeOH/CH_2Cl_2), IR (cm^{-1}). = 3391, 3325, 3000, 2959, 1770, 1731, 1704, 1557. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.85 (m, 2H), 7.73 (m, 2H), 4.20 (m, 2H), 3.91 (m, 2H), 1.77 (bs, 2H), 1.41 (s, 3H), 1.29 (t, 3H, *J* = 7 Hz), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 175.0, 169.0, 134.0, 132.0, 123.0, 61.0, 58.0, 46.0, 24.0, 14.0. HRMS ($C_{14}H_{16}N_2O_4Na^+$) calculated = 299.1002, observed = 299.1002.

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

An amount of 4.2g (15.2 mmol) of **43** was dissolved in 60 mL of distilled acetonitrile in a 250 mL three necked flask under a N_2 atmosphere. An amount of 12.6g (91.2 mmol) of K_2CO_3 was added with stirring. A volume of 9 mL (76 mmol) of BnBr was added drop wise. The reaction mixture was heated to reflux solvent for 12 hrs. The reaction mixture was diluted with 50 mL of H_2O and the solution was extracted with ether three times. The ether layer was washed with H_2O, washed with brine, dried over $MgSO_4$, and evaporated. The residue was then purified by flash chromatography (30% EtOAc/Hexanes) giving 5.6g (12.3 mmol, 81%) of **44** as a white solid. R_f = 0.59 (30%

EtOAc/Hexanes), IR (cm^{-1}) = 2970, 1770, 1713, 1620. MP = 83 ^{0}C. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 7.83 (m, 2H), 7.71 (m, 2H), 7.34 (d, 4H, *J* = 8Hz), 7.12 (m, 6H), 4.23 (q, 2H, *J* = 7 Hz), 3.95 (m, 6H), 1.35 (m, 6H). ^{13}C-NMR ($CDCl_3$, 100MHz): δ 174.0, 168.0, 141.0, 134.0, 132.0, 128.3, 128.0, 126.0, 123.0, 68.0, 61.0, 55.0, 44.0, 19.0, 14.0. HRMS ($C_{28}H_{28}N_2O4Na^+$) = 479.1941, observed = 479.1938.

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

An amount of 3.2 g (7 mmol) of **44** was dissolved in 20 mL of (8:2) MeOH and CH_2Cl_2 . A volume of 1.7 mL (21 mmol) of N_2H_4 (35% in H_2O) was added. The solution was heated to reflux solvent for 3 hrs. The formation of a white precipitate indicated the completion of the reaction. The reaction mixture was filtered and the filtrate was evaporated giving 2g (6.5 mmol, 92%) of **45** as a yellowish oil. R_f = 0.65 (5% MeOH/CH_2Cl_2). IR (cm^{-1}) = 2979, 1717, 1601. ^{1}H-NMR ($CDCl_3$, 400MHz): δ 7.21 (m, 10H), 4.16 (m, 2H), 3.84 (m, 4H), 2.95 (s, 2H), 1.35 (s, 3H), 1.31 (t, 3H, *J* = 7 Hz), 1.20 (bs, 2H), ^{13}C-NMR ($CDCl_3$, 100MHz): δ 174.0, 141.0, 128.4, 128.0, 126.0, 69.0, 60.0, 55.0, 48.0, 20.0, 14.0. HRMS ($C_{20}H_{26}N_2O_2Na^+$) calculated = 349.1886, observed = 349.1873.

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

An amount of 1.41 g (4.3 mmol) of **45** was dissolved in 25 mL of EtOH. An amount of 0.52 g (12.9 mmol) of well crushed NaOH pellets were added. The solution was heated to reflux solvent for 4 hrs. The EtOH layer was acidfied to pH 2, evaporated to dryness under high vacuum, and triturated with MeOH. The MeOH layer was neutralized with solid $NaHCO_3$, filtered, and evaporated under reduced pressure giving 1.16g (3.9 mmol, 90%) of **46** as yellowish wax. ^{1}H-NMR (CD_3OD, 400MHz): δ 7.18 (m,

10H), 3.91 (m, 4H), 2.83 (m, 2H), 1.87 (s, 3H). ^{13}C-NMR (CD_3OD, 100MHz): δ 182.0, 144.0, 129.6, 129.0, 127.0, 70.0, 56.0, 22.0. HRMS ($C_{18}H_{22}N_2O_2Na^+$) calculated = 321.1573, observed = 321.1573.

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

A solution of 1g of **46** (3.4 mmol) in 10 mL water was placed in a 50 mL round bottom flask. An amount of 0.56g (6.7 mmol) of $NaHCO_3$ (2eq) was added with stirring. The solution was cooled to 0 ^{0}C. A solution of 0.98g (4.7 mmol) $(Boc)_2O$ (1.4eq) in 10 mL 1,4 dioxane was added drop wise. The reaction was allowed to stir at 0 ^{0}C for an hour. The reaction was then allowed to warm to RT overnight. The reaction mixture was diluted with 15 mL H_2O, acidified to pH 4 with $NaHSO_4$, and extracted twice with Et_2O. The combined ether layer was washed with water (5 x 30 mL), washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography (40% EtOAc/Hexanes), giving 1.2g (3 mmol, 91%) of **47** as a white solid. R_f = 0.28 (40% EtOAc/Hexane). IR (cm^{-1}) = 2977, 1698, 1494. MP = 58 ^{0}C. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.21 (m, 10H), 5.44 (bs, 1H), 4.09 (m, 4H), 3.64 (m, 2H), 1.42 (m, 12H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 175.0, 156.0, 137.0, 128.8, 128.5, 127.7, 79.0, 71.0, 55.0, 44.0, 28.0, 20.0. HRMS [$C_{23}H_{30}N_2O_4Na^+$] calculated = 421.2097, observed = 421.2094.

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

A solution of 1g (2.5 mmol) of **47** in 25 mL MeOH was placed in a pressure bottle. An amount of 0.2g (20% by weight) of Pd-C was added to the bottle. The solution was placed on a Parr shaker hydrogenation apparatus and allowed to shake with 30 psi hydrogen gas for 12 hrs. The reaction mixture was filtered through a Celite bed to remove

the catalyst. The filtrate was evaporated giving **48**. An amount of 0.5g (2.3 mmole, 92%) of **48** was obtained as a white wax. R_f = 0.3 (5% MeOH/CH_2Cl_2). IR (cm^{-1}) = 2977, 1701, 1602, 1508. MP = 204 ^{0}C. ^{1}H-NMR (CD_3OD, 400MHz): δ 3.44 (s, 2H), 1.47 (m, 12H). ^{13}C-NMR (CD_3OD, 100MHz): δ 174.0, 158.0, 79.0, 61.0, 46.0, 27.0, 19.0. HRMS ($C_9H_{18}N_2O_4Na^+$) calculated = 241.1159, 241.1158.

*Synthesis of (S)-2-(9-Fluorenylmethyloxycarbonylamine)-3-(Tert-Butyloxycarbonylamino)-2-Methylpropanoic Acid (**49**)*

An amount of 0.35g of $NaHCO_3$ (4.1 mmol) was added to a solution of 0.45g **29** (2.1 mmol) in 15 mL water with stirring. The solution was cooled 0 ^{0}C. A solution of 1.1g Fmoc-Osu (3.2 mmol) in 15 mL 1, 4-dioxane was added to the reaction mixture over 20 min. The reaction was allowed to stir at 0 ^{0}C for an hour and then at ambient temperature for 12 hrs. At that point the reaction was diluted with 30 mL of water, acidified to pH 4 with 4M HCl, extracted (3 x 50 mL) with Et_2O. The combined ether layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by radial chromatography using 5% MeOH/CH_2Cl_2 giving 0.86g (1.96 mmol, 85%) of **49** as a white solid. R_f = 0.41 (5% MeOH/CH_2Cl_2), IR (cm^{-1}) = 3317, 2974, 1694, 1513. MP = 82 ^{0}C. $[\alpha]_D^{22}$ = -10.5 (c = 1, $CHCl_3$), ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.80 (d, 2H, *J* = 7 Hz), 7.68 (d, 2H, *J* = 7 Hz), 7.39 (t, 2H, *J* = 7 Hz), 7.31 (t, 2H, *J* = 7 Hz), 4.31 (bs, 2H), 4.22 (t, 1H, *J* = 7 Hz), 3.55 (m, 2H), 1.44 (s, 12H). ^{13}C-NMR (CD_3OD, 100 MHz): δ 159.0, 157.0, 145.4, 145.3, 143.0, 129.0, 128.0, 126.4, 126.3, 121.0, 80.0, 68.0, 55.0, 46.0, 28.0, 21.0. HRMS ($C_{24}H_{28}N_2O_6Na^+$) calculated = 463.1839, observed = 463.1835.

*Synthesis of (S)-Tert-Butyl 2-Tert-Butyloxyaminobutyl-4-Diazo-2-Methyl-3-Oxobutanoate (**50**)*

Acid **40** (3g, 8.7 mmol) was dissolved in 10 mL THF and cooled to -25 ^{0}C. A measured 1 equivalent of Et_3N (1.2 mL, 8.7 mmol), and 1.05 equivalents of $ClCO_2Me$ (710 µL, 9.1 mmol) was added drop wise to the THF solution. The mixture was stirred for 2 hrs. giving rise to the mixed anhydride, which was taken immediately for the next step. The resulting white suspension of the mixed anhydride was allowed to warm to 0 ^{0}C and a solution of dry diazomethane (2 equivalent, 17.4 mmol) in Et_2O was carefully added. The reaction mixture was allowed to stir for 12 hrs. in the dark at 0 ^{0}C. Excess diazomethane was removed by passing N_2 through the solution for 30 mins. The reaction mixture was then diluted with Et_2O, washed with saturated $NaHCO_3$, saturated NH_4Cl, and brine. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure. The resulting residue was purified by chromatography (1:1 Et_2O/Hexanes), giving 2.62g (7.1 mmol, 82%) of **50** as a clear yellowish oil. R_f = 0.42 (1:1 Et_2O/Hexanes). IR (cm^{-1}) = 3381, 2976, 2934, 2110, 1704, 1517. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.41 (s, 1H), 4.58 (bs, 1H), 3.09 (bm, 2H), 1.85 (m, 1H), 1.71 (m, 1H), 1.45 (m, 21H), 1.28 (m, 4H). HRMS ($C_{18}H_{31}N_3O_5Na^+$) 392.2156, observed = 392.2153.

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

An amount of 2.5g **50** (6.8 mmol) was dissolved in 15 mL 3:7 H_2O/THF in a 50 mL round bottom flask. The flask was purged with N_2 and the resulting solution was photolyzed with a Hanovia lamp (500 W) at a distance of approximately 10 cm. The photolysis was allowed to proceed for 48 hrs. At that point the reaction was found to be completed as evident by TLC. The clear and colorless solution was concentrated under

reduced pressure and the water layer was extracted three times with Et_2O. The combined Et_2O layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by chromatography (30% EtOAc/Hexanes), giving 1.83g of **51** (5.1 mmol, 75%) as a yellowish oil. The ^{1}H-NMR and the HRMS is highly indicative of the product. Hence, the product was taken for the next step without further purification. R_f = 0.18 (30% EtOAc/Hexanes), IR (cm^{-1}) = 3364, 2977, 2936, 1709, 1521. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.54 (bs, 1H), 3.10 (bm, 2H), 2.73 (m, 1H), 2.38 (m, 1H), 1.55 (m, 22H), 1.20 (m, 5H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 176.2, 175.2, 155.9, 80.6, 72.4, 44.4, 42.4, 40.1, 38.8, 30.0, 28.4, 27.8, 21.8, 21.4. HRMS ($C_{18}H_{33}NO_6Na^+$) calculated = 382.2200, observed = 382.2196.

*(S)-Tert-Butyl-2-Tert-Butyloxycarbonylaminobutyl-3-(9-Fluorenylmethyloxycarbonylamino)-2-Methylpropanoate (**52**)*

A volume of 0.79 mL Et_3N was added to a solution of 1.7g of **51** (4.7 mmol) in 25 mL dichloroethane under N_2 atmosphere. A volume of 1.2 mL (5.2 mmol) DPPA was added to the reaction mixture. The reaction was allowed to stir at RT for 2 hrs. The mixture was heated to reflux solvent for 3 hrs. The mixture was cooled and the organic layer was extracted with saturated NH_4Cl solution, dried over $MgSO_4$, and evaporated under reduced pressure giving the icocyanate. The icocyanate was taken up in dry toluene under N_2 atmosphere. An amount of 1.84g (9.4 mmol) 9-fluorenylmethanol was added to the solution along with 100 μL of Ti (IV) isopropoxide. The reaction was heated to 80 ^{0}C overnight. The reaction was cooled and the organic layer was evaporated under reduced pressure. The residue was then purified by chromatography (CH_2Cl_2 to 3% MeOH/CH_2Cl_2), giving 2g **52** (4 mmol, 80%) as sticky light yeollowish wax. The

product was found too sticky to dry the solvent all the way. It was characterized by ^{1}H-NMR and HRMS. The product was taken for the next step without further attempt to purify it. R_f = 0.78 (3% MeOH/CH_2Cl_2). IR (cm^{-1}) = 3340, 2975, 2933, 1756, 1688, 1513. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.76 (d, 2H, *J* = 7Hz), 7.59 (d, 2H, *J* = 7Hz), 7.39 (t, 2H, *J* = 7Hz), 7.30 (t, 2H, *J* = 7Hz), 4.60 (bm, 2H, *J* = 7Hz), 4.37 (m, 2H), 4.22 (t, 1H, *J* = 7Hz), 3.38 (m, 1H), 3.24 (m, 1H), 3.09 (m, 2H), 1.46 (bm, 6H), 1.44 (bm, 21H). HRMS ($C_{32}H_{44}N_2O_6Na^+$) calculated = 575.3091, observed = 575.3083.

*Synthesis of (S)-Fmoc-α-Methyl-β^{2,2}-Lysine-Boc-OH (**53**)*

An amount of 1.5g of **52** (2.7 mmol) was dissolved in 20 mL of 1:1 TFA/CH_2Cl_2. The solution was allowed to stir for 12 hrs at RT under N_2 atmosphere. The reaction was monitored by TLC (5% MeOH/CH_2Cl_2) and ESI-mass spectrometry for the completion. At which point the TFA/CH_2Cl_2 layer was evaporated under reduced pressure giving free amino acid. The residue was taken up in 15 mL of H_2O and 0.54g (6.5 mmol) $NaHCO_3$ was added to the solution slowly to control the effervescence. The mixture was cooled to 0 ^{0}C. A solution of 0.71g $(Boc)_2O$ (3.2 mmol) in 15 mL 1,4-dioxane was added to the mixture slowly at 0 ^{0}C. The reaction was allowed to stir at 0 ^{0}C for an hour. The reaction was then allowed to warm to RT and stir for 12 hrs. The reaction mixture was extracted with pentane to remove excess $(Boc)_2O$. The aqueous layer was then acidified to pH 4 with 2N HCl, extracted three times with Et_2O (50 mL). The combines ether layer was dried over MgSO4, evaporated under reduced pressure, purified by chromatography (5% MeOH/CH_2Cl_2), giving 1.21g of **53** (2.44 mmol, 90% over two steps) as a white solid after purification by flash chromatography (CH_2Cl_2 to 5% MeOH/CH_2Cl_2). R_f = 0.50 (5% MeOH/CH_2Cl_2). IR (cm^{-1}) = 3338, 2940, 1693, 1518. $[\alpha]_D^{23}$ = - 6.0 (c = 0.7, $CHCl_3$). MP

= 73 ^{0}C. ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.81 (d, 2H, *J* = 7Hz), 7.66 (d, 2H, *J* = 7Hz), 7.40 (t, 2H, *J* = 7Hz), 7.32 (t, 2H, *J* = 7Hz), 4.36 (m, 2H), 6.23 (m, 1H), 3.03 (t, 2H, *J* = 7Hz), 1.61 (m, 1H), 1.44 (m, 12H), 1.30 (m, 2H), 1.13 (s, 3H). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.75 (d, 2H, *J* = 7Hz), 7.58 (m, 2H), 7.38 (t, 2H, *J* = 7Hz), 7.30 (t, 2H, *J* = 7Hz), 6.37 (bm, 1H), 5.42 (bm, 1H), 4.58 (m, 1H), 4.35 (m, 1H), 4.21 (m, 1H), 3.36 (m, 2H), 3.09 (bm, 2H), 1.41 (m, 18H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 179.5, 156.9, 156.2, 143.6, 141.2, 127.7, 127.1, 125.1, 120.0, 79.3, 66.8, 47.3, 46.8, 40.0, 36.7, 36.0, 30.4, 28.4, 21.3, 20.4. HRMS ($C_{28}H_{36}N_2O_6Na^{+}$) calculated = 519.2965, observed = 519.2459.

CHAPTER III

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

Background

Small Peptides Containing Lysine and Their Importance

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

Somatostatin

Somatostatin (somatotropin release inhibiting factor or SST) was discovered as a hypothalamic neurohormone, which inhibits growth factor secretion.[90-92] SST was detected both in the central and peripheral nervous system, and in peripheral tissues where it plays many different roles.[90-92] In periphery the endocrine pancreas and gut are the main sources of SST .[92-95]

SST has several functions[92, 95, 96] which are as follows:

1. Inhibition of endocrine and exocrine secretion.
2. Modulation of neurotransmission.
3. Motor and cognitive functions.
4. Inhibition of intestinal motility.
5. Absorption of nutrients and ions.
6. Vascular contractility and cell proliferation.
7. Inhibition of proliferation of several tumor and normal cells.

Naturally occurring somatostatin is available in two molecular forms: a tetradecapeptide (SST-14) and a 28-amino acid peptide (SST-28) containing the amino acid sequence of SST-14, N-terminally extended by 14 amino acid residues as shown in Figure 14.[92, 95] The diverse action of SST peptides are mediated through interaction with 5 different SST receptors (SSTRs) expressed by variety of normal and malignant tissues.[95] The rationale behind evaluation SST as an anticancer drug is the dual character of SST analogues to inhibit hormone release and cell growth.[92, 95]

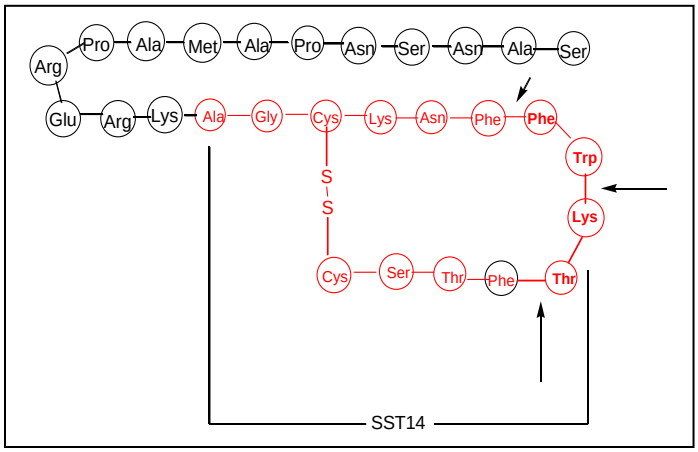


Figure 14. Structures of SST14 and SST28. The enzymatic cleavage sites on SST14 are shown with arrows.

Somatostatin Receptors and Their Lligands/Tissue Expression

The biological effects of somatostatin are executed by binding to five distinct high-affinity G Protein coupled receptors (SSTR1-SSTR5) over expressed on the cell surface.[95, 97] These SST receptors exhibit a high degree of resemblance but differ mainly at their amino and carboxy terminal segments. This disparity is attributed to their specificity in ligand binding and intracellular signaling.[92, 95] The up regulation of SSTR subtypes has been evidenced in human tissues, diverse tumor tissues and cell lines at mRNA level and protein level with SSTR subtype specific ligands.[98] In addition to the

normal tissues, SSTRs have also been witnessed to be over expressed by gastroenteropancreatic (GEP) tumors, carcinoid tumors, small-cell lung tumors, prostate carcinoma, breast carcinoma, renal carcinoma, frequently nervous system tumors, and medullary carcinoma of the thyroid.[95, 98] Most of the time, cancer cells express more than one SST subtypes with SSTR2 most frequently followed by SSTR1, SSTR3, SSTR4, and SSTR5.[98] It has also been observed that there is disparity in the SSTR subtype expression pattern in different tumor types, within the same type of tumors, and even in each patient.[99] Multiple SSTR subtype protein expression has also been reported in medullary carcinoma of thyroid.[100] A summary of the present knowledge on SSTR subtype mRNA and protein expression in tumor cells is given in the table 1 below.[100]

Table 1

Expression of SSTR subtypes in several tumor cells.[98]

Subtype	Cancer cell over expressing SSTRs
SSTR1	Prostate carcinomas, sarcomas, GEP tumors, phaeochromocytomas.
SSTR2	Neuroblastomas, meningiomas, medulloblastomas, breast carcinomas, lymphomas, renal carcinomas, small cell lung carcinomas, hepatocarcinomas, pituitary adenomas, GEP tumors, phaeo chromocytomas, paraganglomas etc.
SSTR3	Pituitary adenomas
SSTR4	-
SSTR5	GH-screening pituitary adenomas

Function of SSTR Subtypes and Receptor Binding Specificity of SST Analogues

Receptors are the binding sites for peptides. One of the most common characteristics of SSTRs is that they stimulate rapid internalization of receptor-agonist complex into the cell upon binding of agonist to the SSTRs overexpressed on the tumor cells.[101] The internalization of SSTR-agonist complex may take place upon binding of either endogenous agonist or exogenous agonist to the SSTRs.[101] The mechanism of higher degree of internalization of SSTR-somatostatin radioligand complex into the tumors overexpressing SSTRs is often employed to effectively and specifically accumulate radio activity into the tumor cells.[101] The accumulated somatostatin radioligands permit successful tumor imaging in patients as well as targeted chemotherapy.[101] The 5 receptor subtypes (SSTR1-5) bind with the naturally occurring peptides (SST-14 & SST28) with low nanomolar affinity.[95, 98] Out of five SSTR subtypes, only SSTR5 shows a 10 fold higher affinity for SST28.[98] Short synthetic peptides have been observed to bind strongly to 3 of 5 SSTR Subtypes (SSTR 2, SSTR3, and SSTR5) as opposed to SST14.[98] For instance, octapeptide analogues of SST, which are currently in use in clinical studies as antineoplastic agents (figure 15), are more selective than SST14.[98]

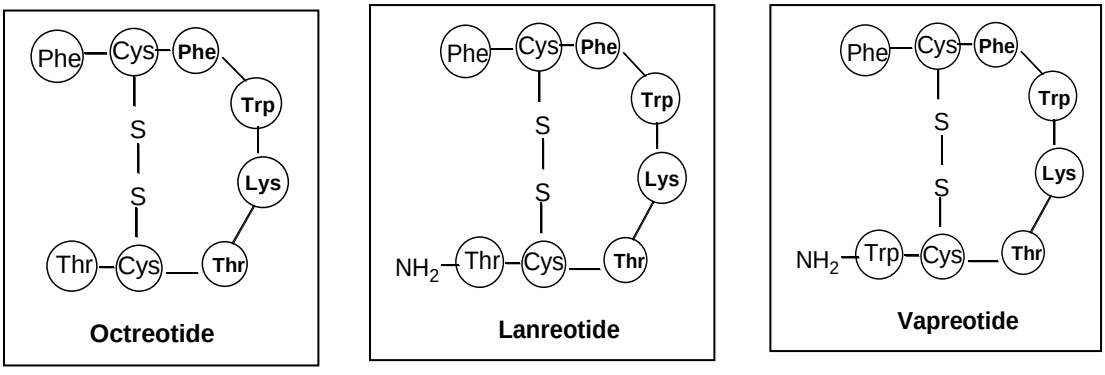


Figure15. SST octapeptide analogues.

Octapeptides (Figure 14) show very low affinity for SSTR1 and SSTR4 (> 1000 nM), moderate affinity for SSTR3 (225 nM) and high affinity for SSTR2 and SSTR5 (2.8 ~ 9.9 nM).[98, 102] The hexapeptide, Seglitide, exhibits a similar pattern of binding selectivity and potency.[100] However, some of the tumors overexpress predominantly SSTR1 or SSTR4 but lack in SSTR2 and SSTR5.[98] Lately, there have been many efforts to prepare diverse SST analogues, that differ in ring sizes, with selective binding affinity for SSTR1 and SSTR4.[103, 104] Additionally, there has been much research on development of SST receptor subtype-selective analogues, both as peptides and nonpeptides.[98, 102, 105] There are three reasons behind this type of research:

1. Evaluation of the distribution of various receptor subtype proteins in tissue.
2. Determination of the specific biological effects mediated by the various subtypes.
3. Design of new drugs for specific therapeutic strategies.

Mechanism of the Antiproliferative Activity of SST Octapeptide Analogue

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

Indirect Effect

Indirect effect consequences from the inhibition of several growth promoting horones and growth factors that stimulates the growth of a variety types of cancers.[98] For instance, it is witnessed that IGF-1, which is excreted by hepatocytes either by dependent or independent mechanism, is a growth promoter of a number of tumors that express IGF-1 receptors.[98] Octreotide was found to downregulate the serum IGF-1 level either by inhibiting GF secretion, or by directly inhibiting IGF-1 gene expression, or by rising circulating IGF-1 binding proteins.[98] This IGF-1 suppression strategy has been found to

be very effective in the treatment of IGF-1 dependent tumors such as GH screening pituitary adenomas and to a lesser extent breast, lung and prostate tumors.[98] Several other growth factors or hormones, which are known to play an important role in tumor growth, are being regulated either by naturally occurring somatostatin or SST analogues, as for example, gastrin, insulin, glucagon epidermal growth factor, and transforming growth factor-alpha.[98]

Direct Effect

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

In comparison to naturally occurring SST (SST14 and SST28), short synthetic SST analogues have been found to be even more efficient due to high receptor binding affinity and selective binding to the receptors.[95, 98] Out of several short SST analogues, octapeptide analogues (Figure 14) are being used frequently as drugs to suppress

cancers.[95] Out of three synthetic octapeptides (Figure 14), the octreotide is used most frequently due to its high binding affinity to SSTR2 and SSTR5.

Application of Somatostatin Octapeptide Analogues in the Clinic

At present, there is wide spread application of somatostatin octapeptide analogues in treatment of various types of cancers.[106] Some of important of them are noted below.

Carcinoid Tumor

Octreotides and lanreotides are permitted in most countries to treat the hormonal symptoms in the patients with carcinoids. Endocrine cells give rise to the carcinoids.[98] They generally arise in the ileum and metastasize to liver resulting in so called "carcinoid syndrome" (flushing, diarrhea, cardiac vascular lesions).[98] Some carcinoids may appear in the non-gastrointestinal origin such as lung or ovary. Recent studies suggest that octreotide treatment results in a substantial improvement of hormonal symptoms in more than 90% of the patients with carcinoids.[98]

Endocrine Pancreatic Tumors

Another application of Octreotide in the field of oncology is its use in the treatment of hormonal hypersecretion associated with endocrine pancreatic tumors.[98] Octreotide has been well explored in the treatment of insulinomous, gastrinomas, VIP-oms, glucagonomas, and somatostatinomas with variable effects on symptoms in spite of its capability of lowering plasma concentrations of marker peptide in most patients.[95, 98, 107] In some cases, VIP-omas cannot be cured either by surgery or by chemotherapy, and Octreotide is the only choice of treatment approved by Food and Drug Administration.[98] Improvement of symptoms and biochemical processes have been observed in more than

80% of the patients.[98] Octreotide has been found to be beneficial for 50% of the patients with insulinomas and 90% of the patients with gastrinomas.[98]

Pituitary Tumors

The best way to treat pituitary tumors is surgical removal of the tumors. However, it is not always possible to remove them by surgery because of their extension to pituitary and super pituitary areas. In this situation, Octreotide is the treatment of choice to reduce the hormone hyper-secretion.[95,98] Recent observations show that the octreotide administered to acromegatic patients stimulated rapid and remarkable clinical improvement by lowering the GH levels to less than 5μg/L in half of the patients.[95, 98] The size of the pituitary adenoma has been observed to reduce by more than 20% in half of the patients on octreotide treatment.[98]

Other Cancers

Octreotide has also been proposed for other cancerous cells based on the SSTR expressions, for example: pancreatic tumors, hepatocellular carcinoma, small cell lung cancer, breast cancer, Prostate cancer, non-neurodocrine solid tumors.[95, 98, 103, 104]

Radiolabeled Somatostatin Octapeptide Analogues and Their Applications

It is always very difficult to determine the localization of neuroendocrine tumors in patient bodies by standard techniques such as ultra sonography or computed tomography, since those tumors are very small in size. One alternative approach is to visualize the SSTRs on the neuroendocrine tumors.[98] This approach is very promising and viable, because SST radiopharmaceuticals are ssuitable for scintigraphy and are available.[98] Moreover, the presence of SSTRs on the endocrine tumors with high density made this imaging possible.[98] The first radioactive SST analogue used for tumor imaging

was I^{123}-TYR^3-Octreotide.[98] Later alternative labeling procedures and radioisotopes were explored to minimize several problems, as for example, halogenations of I123-TYR^3, or non specific uptake, etc.[98] More recently, various novel chelators are being attached to the N-terminus of the SST analogues for labeling with radio-metals, which were designed and studies in the clinic.[108] A list of the recent chelators is given below in Figure 16.

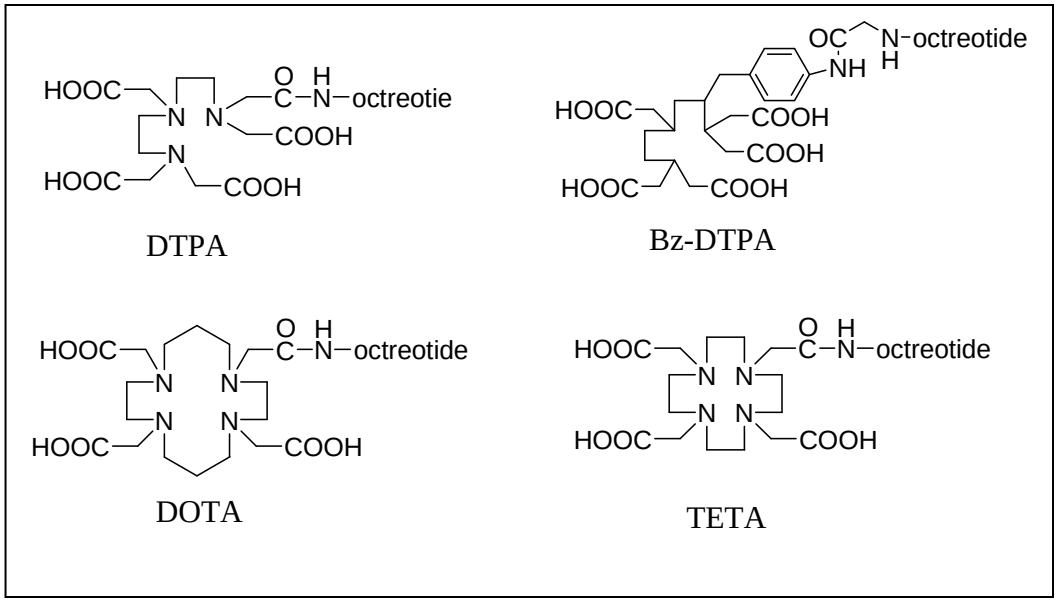


Figure 16. Somatostatin analogues attached to chelators.

Targeted Chemotherapy

Another application of SST in oncology is its use in targeted chemotherapy.[98] Just like targeted radiotherapy, SSTR targeted chemotherapy seems to be a promising approach in the treatment of SSTR expressing malignant sites.[95, 98] More over SSTR targeted chemotherapy has been found to be more efficient either from the anti-cancer drug alone or from the SST analogues alone.[95] It was already proven when nude mice bearing xe-nographts of MIA-paca-2 human pancreatic cancer cells were treated with this combination, a significant reduction in tumor size was reported as compare to anticancer drug alone or the SST analogues alone.[95] More recently, another compound

was synthesized by a different group and that was a complex made of Octreotide conjugated to Taxol. This complex has been found to induce apoptosis in MCF-7 cells in SSTRs selective way by making the conjugate less toxic than free Taxol itself.[98] Hence, to summarize, there are widespread applications of somatostatin analogues in the field of oncology. Short half life period (2-3minutes) of Somatostatin analogues (due to proteolytic degradation) is the main problem.[98] Short synthetic octapeptides (Octreotide, Lanreotide and Vapreotide) have 45 times higher half life than SST14.[98] However, octapeptide treatment require further improvement in half-lives of the SST analogues.[98] This problem has become a major impediment for frequent clinical use of SST analogues. Since the last decade, there has been much focus on the improvement of somatostatin analogues to make it more potent to proteolytic degradation.[35, 109] Work has been carrying out by several research groups to give birth to a new protease resistant SST analogue.

Instability and Amelioration of Somatostatine Analogues

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

analogues, because they have much higher affinity and selectivity to the SSTRs compare to the SST-14 and SST-28.[101, 103, 104, 110, 111]

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

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

transient abdominal cramps, flatulence, diarrhea, delay of insulin release in response to meal, formation of gallstones, etc.[98] Extensive research still needs to be done to improve the half life period of somatostatin analogues.[98]

Current State of Work on Developing Protease Resistant Somatostatin

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

Site Directed Mutagenesis and Its Limitation

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

Peptidomimetic

This technique could incorporate sterically congested unusual amino acids in protease specific cut sites in peptides of interest.[100] The concept is to either block the

active site of protease enzymes or slow down the catalytic activity (peptide bond degradation) of protease enzymes. Synthesis of the more protease resistant SST analogues should not be the only thing to consider, but they should have to have higher binding affinity and selectivity to the SSTRs as well.[100] Recently, there has been much research on the synthesis of unnatural α, β, γ- unusual lysine and Trp analogues to make SST analogues more stable and more resistant towards proteolytic degradation.[30, 113-117] The rationale behind incorporating sterically constrained unnatural amino acids into SST analogues is to induce remarkable propensity in the SST analogues to form stable helical secondary structures.[34] It has been observed that peptides consisting of β and γ-amino acids can adopt a stable secondary structure in solution with a few as four to six residues.[118] Small peptides of four to six γ-amino acid residues form stable helical secondary structure in solution and in solid state.[119] The improved secondary structure is witnessed to provide remarkable stability towards proteolytic degradation as compared to naturally occurring α-peptide controls. [115, 118, 119] A few years ago, Rajeswaran et al. reported that the introduction of *N*-methyl-lysine in somatostatin analogues is tolerated and retains the binding affinity to SSTR2.[120] To the best of my knowledge, Prasad et al. is the first to report SST octapeptides consisting of $C^{\alpha,\alpha}$-disubstituted-amino acid analogues retain binding affinity to SSTR2 and exhibit improved stability in blood plasma.[35] Based on the precedent literatures, I hypothesize:

Hypothesis 2.

Substitution of naturally occuring lysine with conformationally constrained (*S*)-C^{α}-methyl-α-lysine would retain specific binding affinity of the SST octapeptides for SSTRs.

Results and Discussion

To test our hypothesis, I chose Vapreotide (one of the SST octepeptides). Vapreotide® is a widely studied somatostatin analogue with anti-neoplastic properties. Vapreotide has a higher binding affinity to somatostatin receptor subtype 2 (SSTR2) than native somatostatin.[98, 121] However, Vapreotide is prone to degradation at the Lys-Val bond by serine proteases (Trypsin, Plasmin, Plasma Kallikrein, etc.).[67] We have made an effort to prepare a Vapreotide analogue **54** (Figure 17) replacing naturally occurring lysine with our (*S*)-$\alpha^{2,2}$-methyllysine (**34**) analogue in order to study the specific binding of **54** to SSTR2. The vapreotide analogue was synthesized using properly protected (*S*)-$\alpha^{2,2}$-methyllysine analogue (**34**). The Vapreotide analogue (**54**) was 99% pure as determined by HPLC.

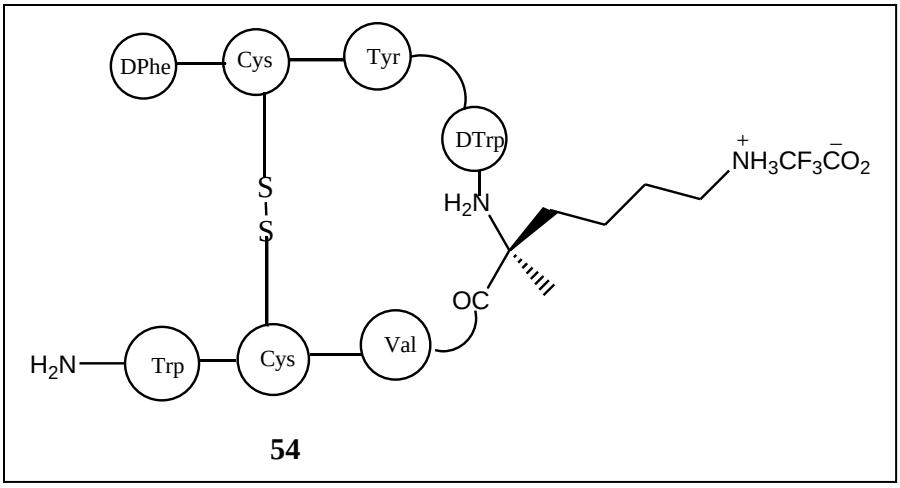


Figure 17. Vapreotide analogue consisting of C^{α}-methyl-α-lysine

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

Conclusions

A simple switch from naturally occurring lysine to $C^{\alpha,\alpha}$-disubstituted lysine diminishes the specific binding of the Vapreotide analogue (**54**) to SSTR2. I suspect that the loss of specific binding for SSTR2 is attributed to conformational changes of the **54** ring resulting from the introduction of conformationally constrained $C^{\alpha,\alpha}$-disubstituted lysine.[120, 122]

Experimental

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

Binding of the Vapreotide analogue (**54**) was conducted against IMR 32 human neuroblastoma cells. These cells over express SSTR2 receptors. In order to perform the binding assays, four groups of triplicate wells were studied (n = 12 total). Each well contained 500,000 IMR 32 cells in 2 mL of media. These wells also contained 100,000 counts of ^{111}In-pentetreotide. Three wells were competed with 10^{-6} M octreotide and three wells were competed with **54**. All 12 wells were incubated at 37 ^{0}C for 20 hours. Cells were harvested, washed and counted in a gamma counter. However, gamma counter result revealed that the Vapreotide analogue **54** has no specific binding for SSTR2 (Table 2).

Specific Binding of Vapreotide Analogue (54) against IMR 32 Cells

Table 2 illustrates the specific binding experiments of Vapreotide analogue (**54**) against IMR 32 human neuroblastoma cells that are known to over express SSTR2.[107] In the binding assay ^{111}In-Pentetreotide, which is known to effectively bind to SSTR2, was used as the radio ligand (Hot ligand).[73] In addition, Octreotide acetate, which is known to have high selectivity for SSTR2, was used as a positive control (Cold ligand 1) to compete with the ^{111}In-Pentetreotide.[107] The Vapreotide analogue (**54**, Cold ligand 2) was allowed to compete with the ^{111}In-Petetreotide as well (Cold ligand 2). Cells were harvested, washed, and counted in gamma counter to determine the quantity of the ^{111}In-Pentetreotide (CPM) bound to the cells. The specific binding of each cold ligand was determined from the equation below based on the amount of ^{111}In-Pentetreotide bound to the IMR 32 cells as obtained from the gamma counter.

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

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

Table 2

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

Competitor	CPM (Hot)	CPM (Hot + Cold)	CPM Specific binding (Hot – Hot + Cold)
Octreotide Acetate (Cold 1)	2591 3096 3238	317 332 287	2663.0
Mean CPM	2975	312	
Standard Deviation	340	22.9	
Vapreotide (Cold 2) (**35, Figure 2**) with (*S*)-α-methyl-α-lysine	3272 4062 3574	4284 3415 3444	-78.3
Mean CPM	3636	3714.3	
Standard Deviation	398.6	493.6	
Background CPM	40		
Radio Ligand Used	^{111}In-Pentetreotide (Hot)		

CHAPTER IV

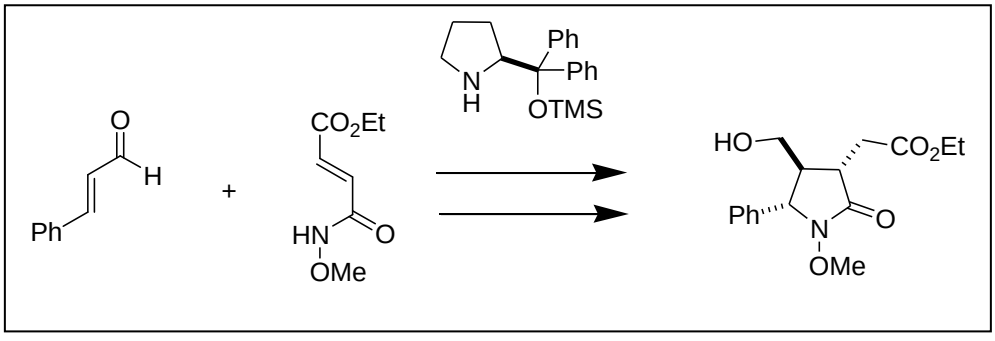
STEREOSELECTIVE CYCLIZATION STRATEGY TO PREPARE γ-/δ- LACTAMS AND THEIR USE IN THE PREPARATION OF PROLINE AND NIPECOTIC ACID ANALOGUES

Background

Lactams are one of the most significant classes of amides. Lactams are frequently obtained as building blocks in a variety of biologically active molecules and are employed very often as intermediates in preparation of diverse materials.[123, 124] Amongst the lactam family, chiral γ-, and δ-lactams have significance, since they provide access to the optically enriched γ-/δ-amino acids, chiral pyrrolidine, and chiral piperidine analogues.[123, 125] In continuation, this class of compounds has been utilized in peptidomimetics.[126] A number of synthetic strategies are established to prepare a variety of γ-, and δ-lactams from the readily accessible precursors.[123, 124, 127-129]

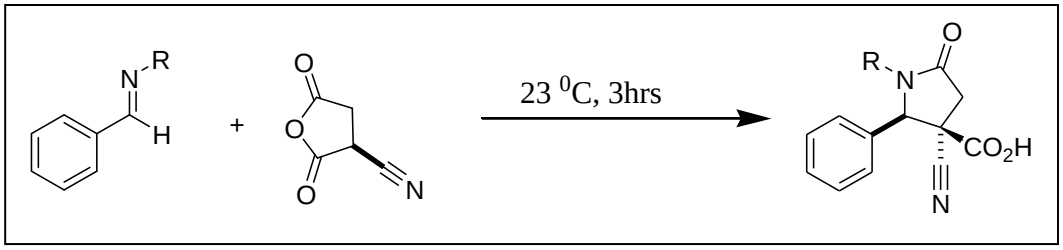
Current State of the Art to Prepare γ-/δ-Lactams

Over the last few years, researchers have explored a number of ways that led to asymmetric synthesis of γ-lactams. Lately, Yokosaka et al. reported an aza-Michael cascade reaction strategy to prepare a number of γ-lactam derivatives (Scheme 29).[130]



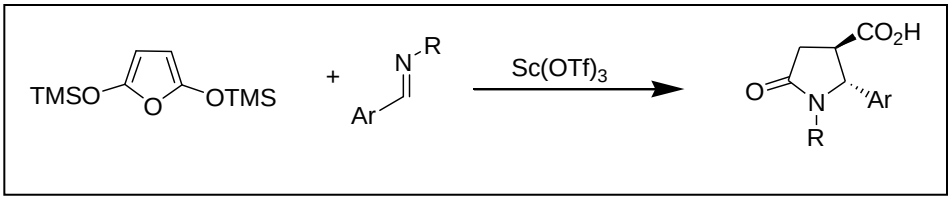
Scheme 29. Strategy of Yokosaka et al.

Recently, Darlene et al. reported a cycloaddition strategy to derive a number of γ-lactam analogues through malic anhydride derivatives. [131]



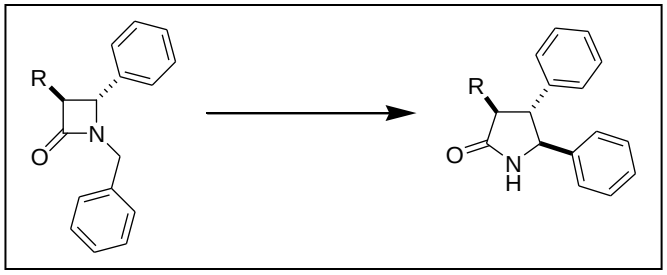
Scheme 30. Strategy of Darlene et al.

A few years ago, Pohmakotr et al. had shown that the Mukaiyama-Aldol reaction provides access to asymmetric synthesis of a variety of γ-lactams (Scheme 31).[132]



Scheme 31. Strategy of Pohmakotr et al.

Park et al. exhibited that the β-lactam rings could be expanded to the corresponding γ-lactam derivatives.[133]

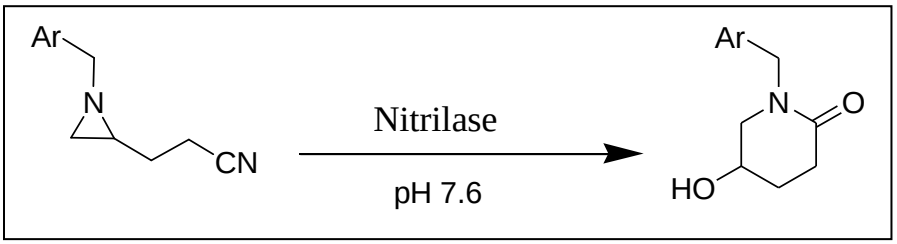


Scheme 32. Strategy of Park et al.

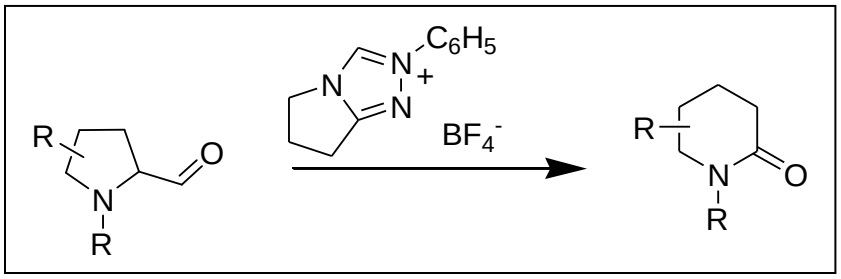
Brenner at al. reported that reduction of optically enriched 4-nitro-carboxylic acid derivatives followed by cyclization provides access to γ-lactam derivatives.[134] In addition, a number of methods have been optimized to construct optically enriched γ-lactam

derivatives employing chiral auxiliaries,[127, 134] enantiomerically enriched starting materials,[135] and organocatalysts.[130, 135]

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

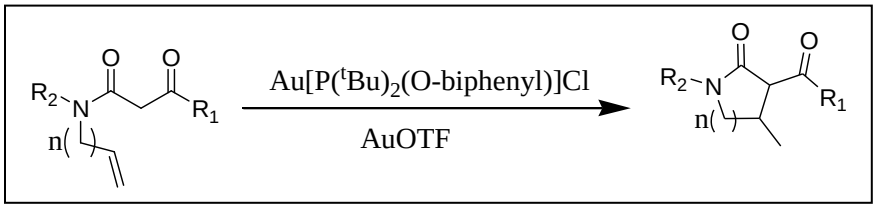


Scheme 33. Chemoenzymatic transformation of aziridines into δ-lactams.



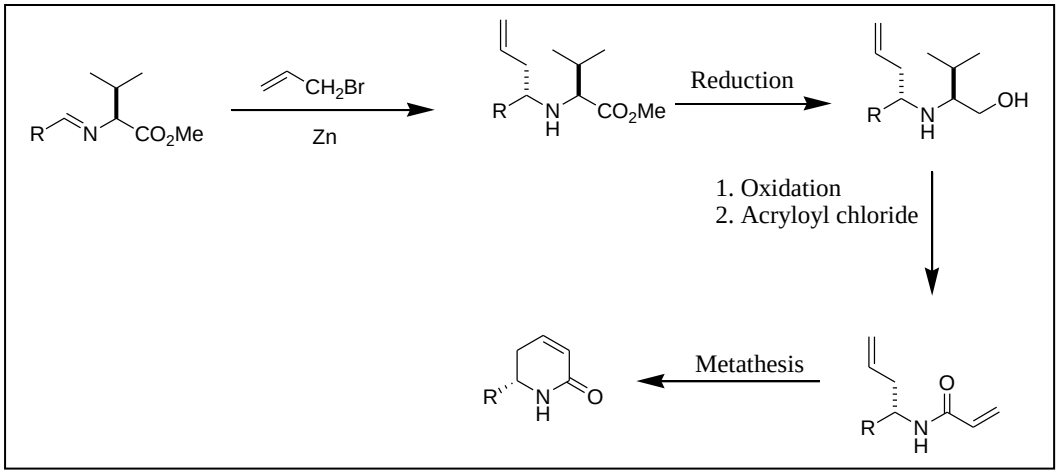
Scheme 34. Synthesis of δ-lactams by ring expansion.

Zhou et al. reported a gold catalyzed highly regioselective cyclization strategy deriving a variety of γ-, and δ-lactams (Scheme 35).[139]



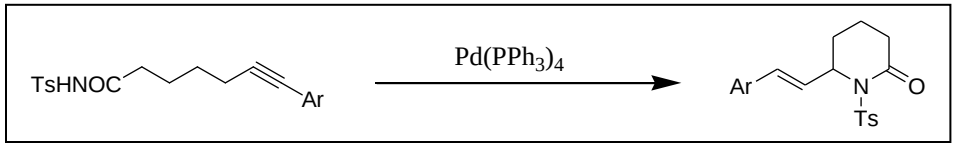
Scheme 35. Conversion of N-alkenyl-β-ketoamides to corresponding γ-/δ-lactams.

Fiorelli et al. reported a novel ring closing metathesis mediated preparation of δ-lactams (Scheme 36).[140]



Scheme 36. Preparation of δ-lactams through ring closing metathesis.

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

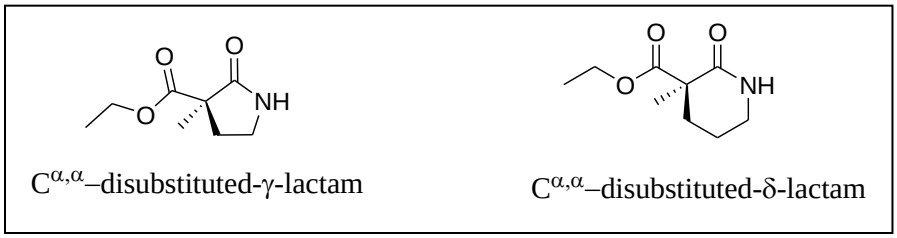


Scheme 37. Intramolecular hydroamidation of amidoalkynes.

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

To summarize, a number of methods have been developed to achieve diverse γ-, and δ-lactams derivatives. However, there is limited scope to provide access to optically pure γ-, and δ-lactam derivatives.[123, 140] To the best of our knowledge, none of the synthetic strategies provide access to both enantiomers of a chiral γ-/δ-lactam. In addition,

very few synthetic strategies are available to obtain $C^{\alpha,\alpha}$-disubstituted-γ-, and δ-lactams.[123, 124]

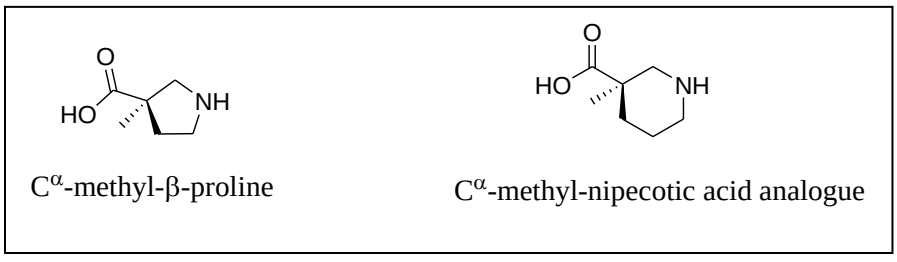


$C^{\alpha,\alpha}$–disubstituted-γ-lactam

$C^{\alpha,\alpha}$–disubstituted-δ-lactam

Figure 18. $C^{\alpha,\alpha}$-disubstituted-γ-/δ-lactams.

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



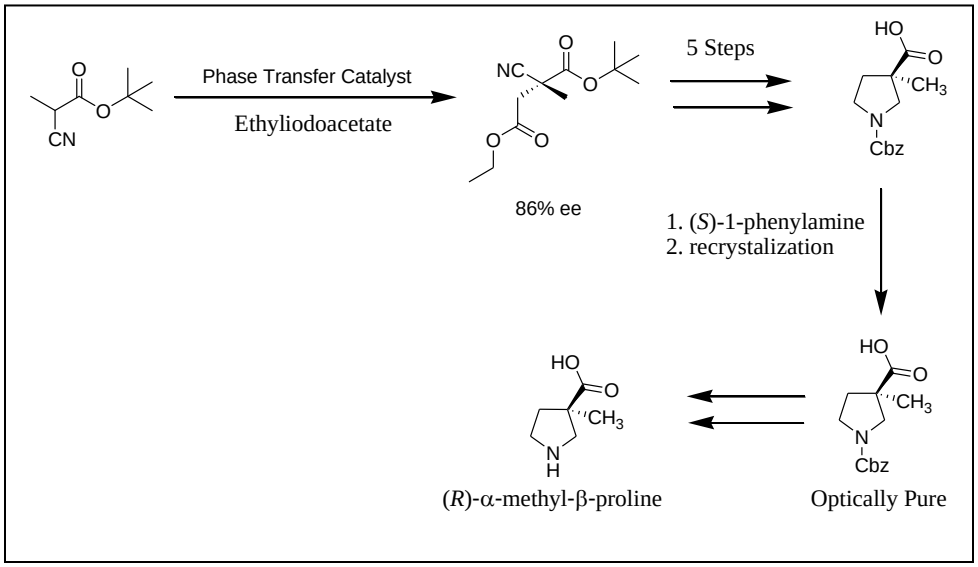
C^{α}-methyl-β-proline

C^{α}-methyl-nipecotic acid analogue

Figure 19. Proline and nipecotic acid analogues.

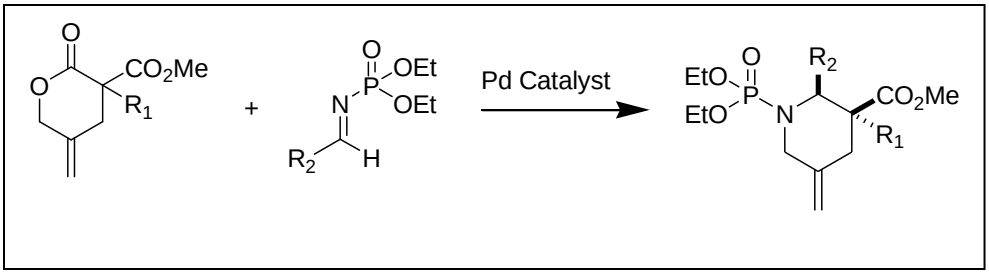
Current State of Art to Prepare C^{α}-Methyl-β-Proline, and C^{α}-Methyl-Nipecotic Acid Analogue

Over the last few years, several research groups in academia and in industry have explored a number of methods to prepare C^{α}-methyl-β-proline, and C^{α}-methyl-nipecotic acid analogues.[1, 45, 47] However, the number of ways known to prepare these analogues is still limited. Recently, Nagata et al. reported a phase transfer catalyst mediated strategy to prepare γ-lactam and its transformation into a C^{α}-methyl-β-proline analogue (Scheme 38).[144] However, their method was not able to produce the β-proline analogue in high optical purity. Hence, they had to use chiral amine to resolve other enantiomer. This group has also exhibited the potent catalytic activity of C^{α}-methyl-β-proline analogues in anti-Mannich type reactions.[144]



Scheme 38. Strategy of Nagata et al. to prepare C^{α}-methyl-β-proline.

A few years back, Shintani et al. reported a Pd catalyzed decarboxylative cyclization strategy to prepare chiral $C^{\alpha,\alpha}$-disubstituted nipecotic acid analogues (Scheme 39).

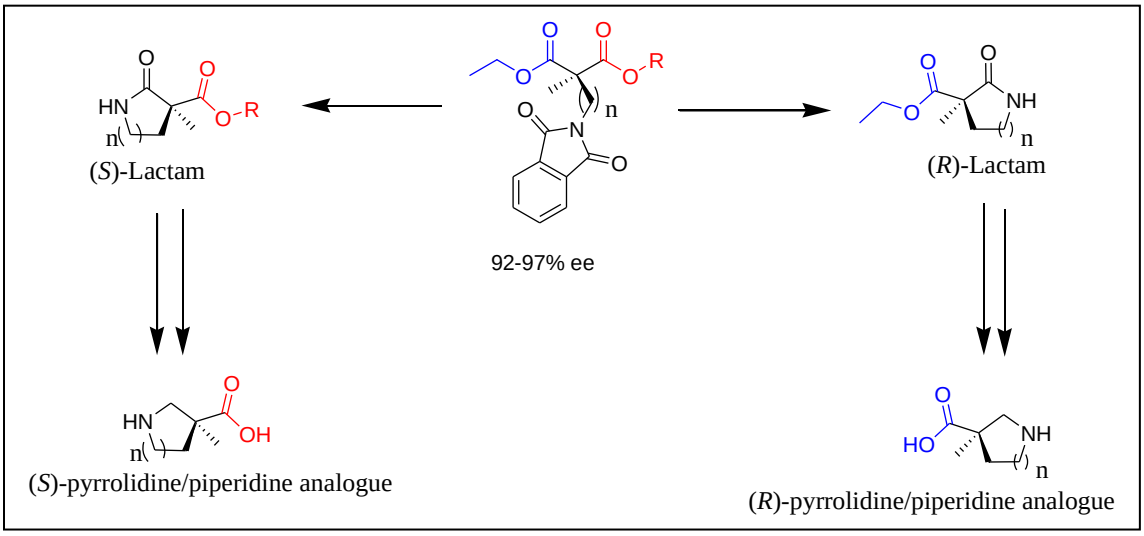


Scheme 39. Synthesis of $C^{\alpha,\alpha}$-disubstituted nipecotic acid analogues through decarboxylative cyclization.

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

Hypothesis 3.

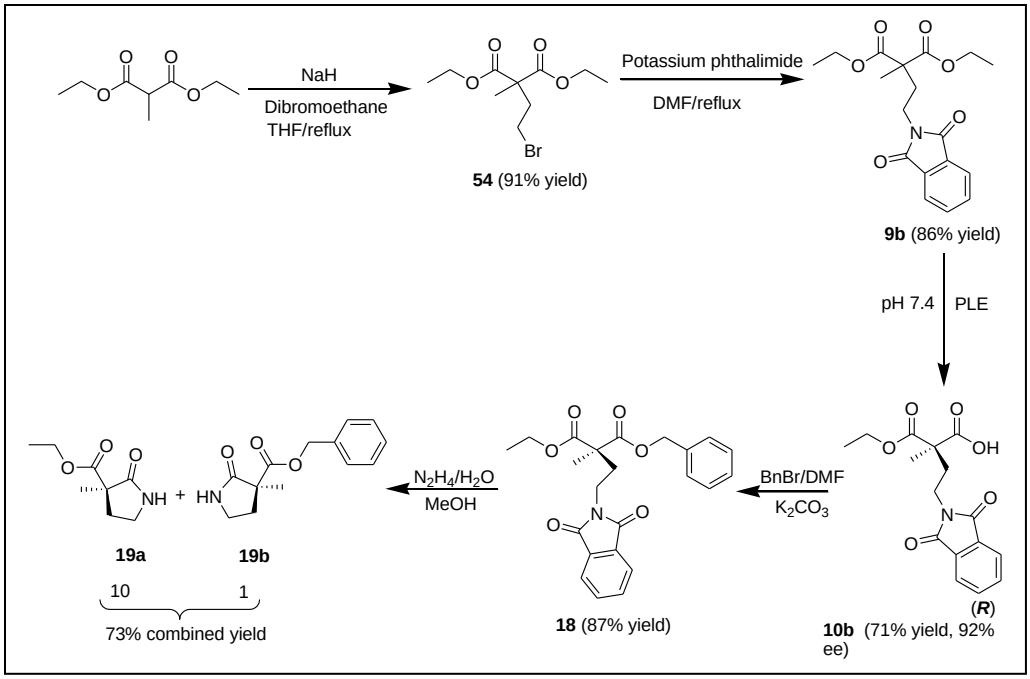
Optically enriched unsymmetrical malonic-diesters could be stereoselectively cyclized to the relevant $C^{\alpha,\alpha}$-disubstituted-γ-/δ-lactams (Scheme 39). A Hammett study should be able to provide insight into electronic activation of one of the ester groups towards nucleophilic attack. The consequential γ-/δ-lactams could be further chemoselectively reduced to the corresponding C^{α}-methyl-β-proline/C^{α}-methyl-nipecotic acid analogues (Scheme 40).



Scheme 40. Stereoselective cyclization strategy.

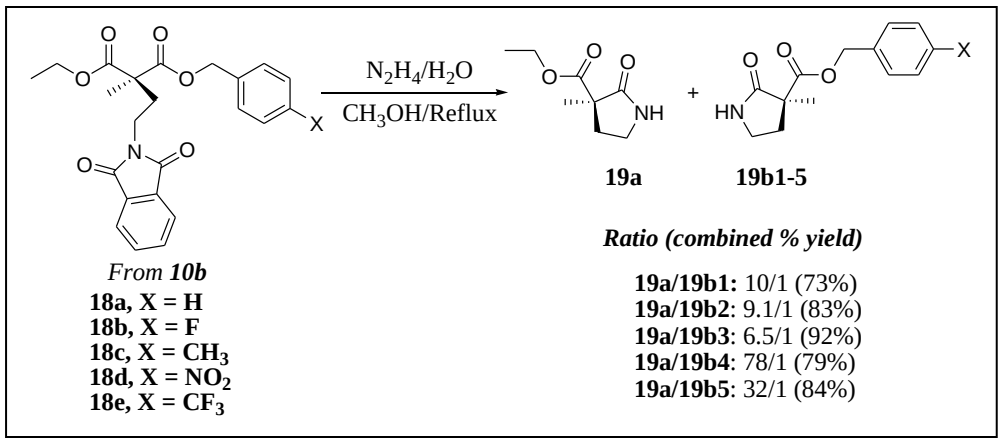
Results and Discussion

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



Scheme 41. Stereoselective cyclization to prepare γ-lactam.

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



Scheme 42. Cyclization using various benzyl esters.

The introduction of substituents on the para position allows us to construct a Hammett plot providing insight into the electronic factors regulating the selectivity of the cyclization. I hypothesized that the σ_p constants[146] would provide insight into the electronic activation of the benzyl ester toward nucleophilic attack. Figure 20 illustrates the results of the Hammett analysis with a strong correlation of product ratio to σ_p. The positive slope clearly indicates that electron withdrawing substituents favor cyclization to γ-lactam **19a** over γ-lactam **19b**. This illustrates that benzyl esters with para electron withdrawing substituents activate the carbonyl toward nucleophilic attack by the 1^o-amine resulting in selective formation of **19a**.

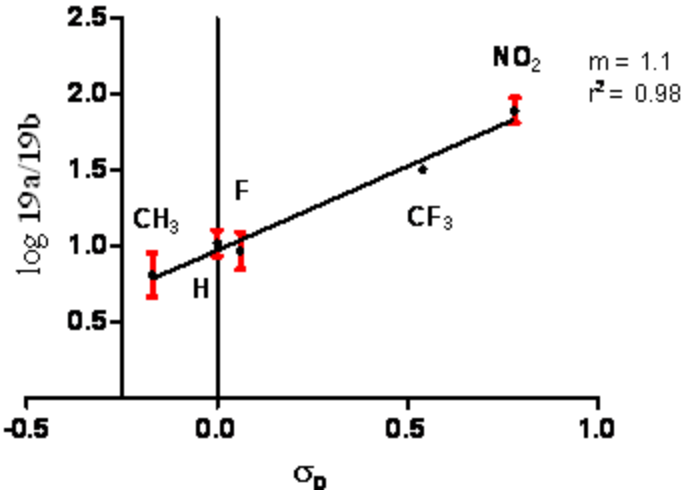
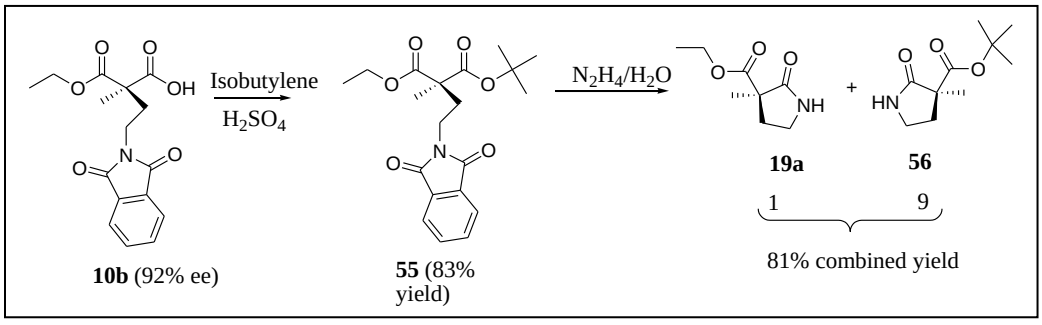


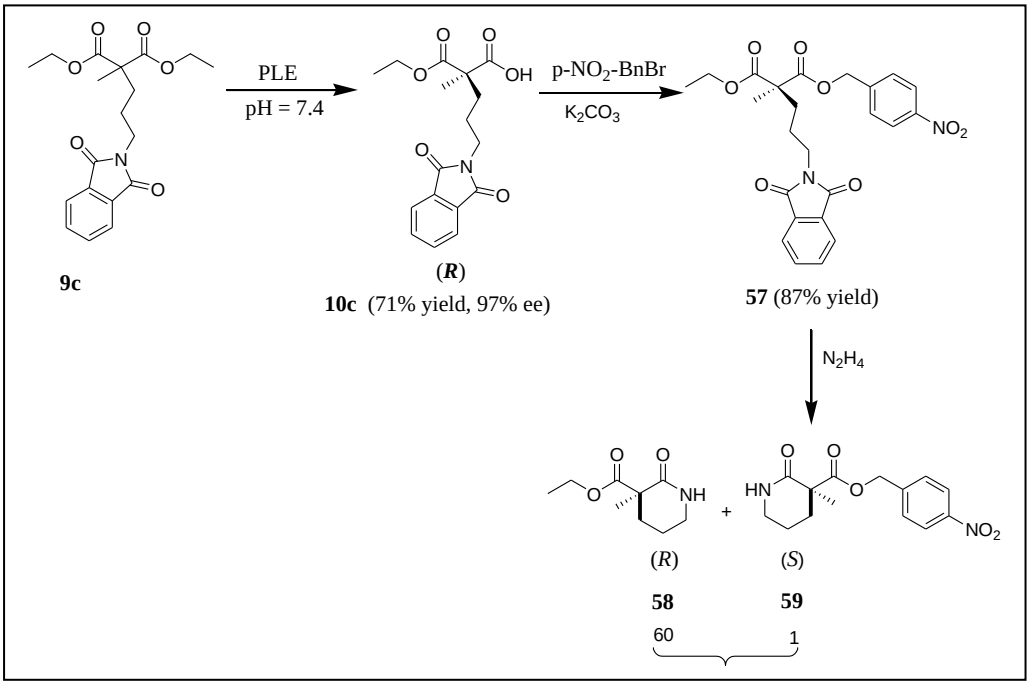
Figure 20. Hammett Plot.

I wanted to further extend the stereoselective cyclization concept to provide **19b** as the major product. The possibility of being able to obtain **19b** as the major product would allow for a potentially useful enantiodivergent cyclization strategy. The results of the Hammett study point that it would be difficult to achieve high selectivity in the formation of **19b** just by exploring electronic factors on the benzyl ester. I prepared diester **55** from **10b** that would introduce steric hindrance (Scheme 43). The ethyl ester should behave as the better electrophile from a steric congestion standpoint leading to the stereoselective cyclization to **56**. Product **56** is an analogue of **19b** and has the same absolute stereochemistry as **19b**.



Scheme 43. Selective cyclization leading to (*S*)-γ-lactam **56**.

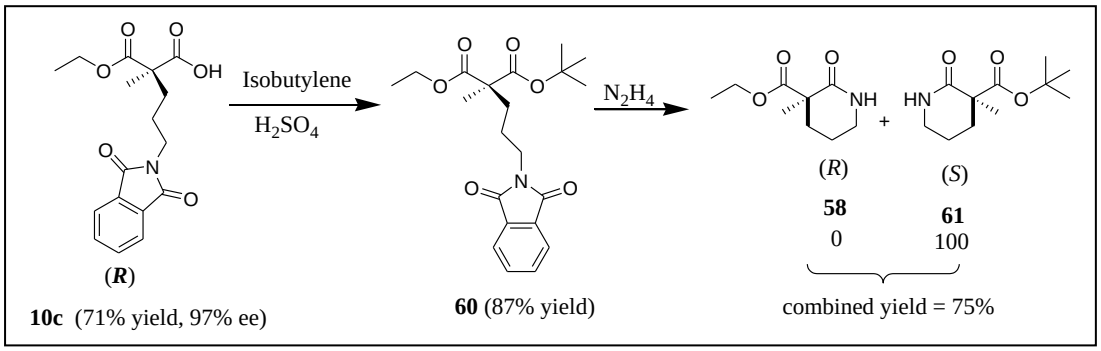
Upon achievement of (*R*)-, and (*S*)-γ-lactams, I wanted to further utilize the optimized method to obtain chiral δ-lactams. Upon close inspection of half-ester **10c**, it is conceivable that unsymmetrical diesters of **10c** could be stereoselectively cyclized to the δ-lactams. Upon treatment of **57**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



Scheme 44. Stereoselective cyclization leading to δ-lactam.

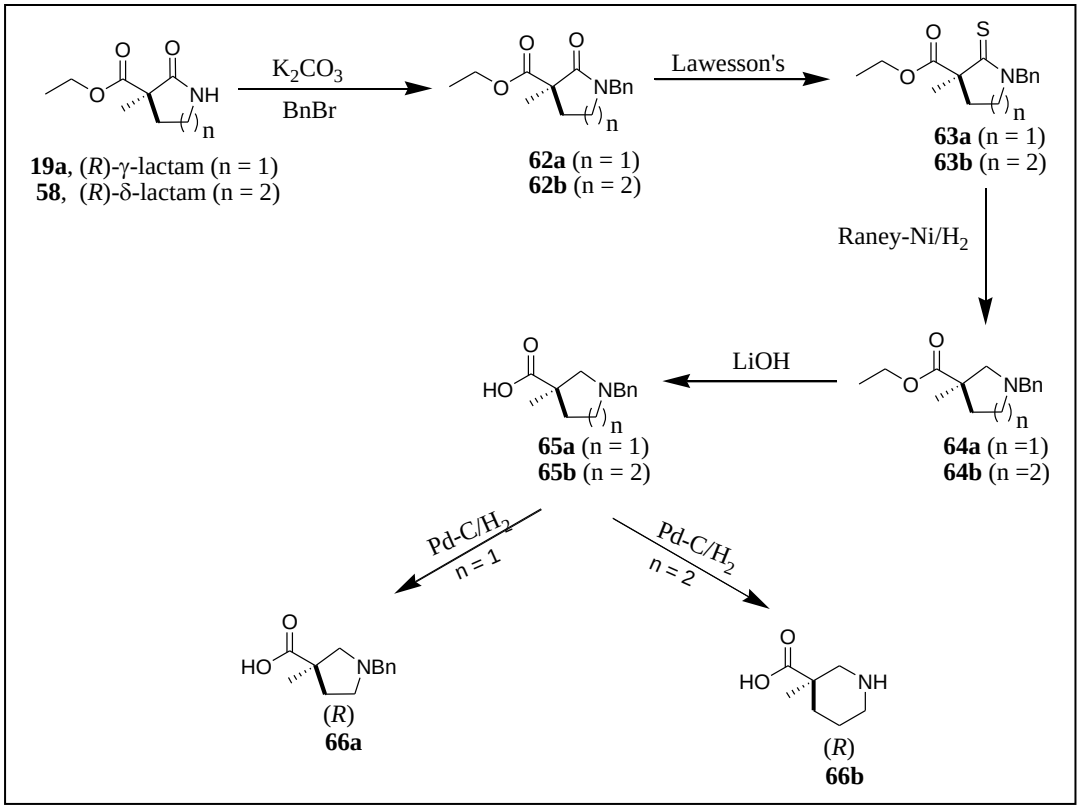
At this point, **59** was wanted to be achieved as the major isomer in order to be able to develop an enantiodivergent strategy to provide access to chiral δ-lactams. Hence, the half-ester **60** was synthesized as shown in Scheme 44 following the synthetic strategy to prepare (S)-γ-lactam **56** as predominant isomer (Scheme 42). Interestingly, upon

treatment of **60** with hydrazine hydrate a stereospecific cyclization took place providing the δ-lactam **61** as only isomer (Scheme 45).



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

We wanted to display the potential utility of the γ-/δ--lactams prepared above as precursors to unnatural amino acids. Upon inspection of **19a**, it is conceivable that a β-proline analogue[147-153] could be readily prepared by reduction of the lactam to a 2^{o}-amine. Similarly, **58** could be readily converted reduced into a nipecotic acid analogue.[45] I made attempt of a direct reduction of the lactam with various reducing agents and conditions that are known to reduce lactams to amines.[154, 155] However, all attempts at direct reduction of the lactam resulted in reduction of both the ester and lactam functional groups providing a complex mixture of products. The synthetic approach shown in Scheme 46 was used to overcome the over-reduction problem and ultimately provide the unprotected β-proline /nipecotic acid analogue in a reasonable overall yield.

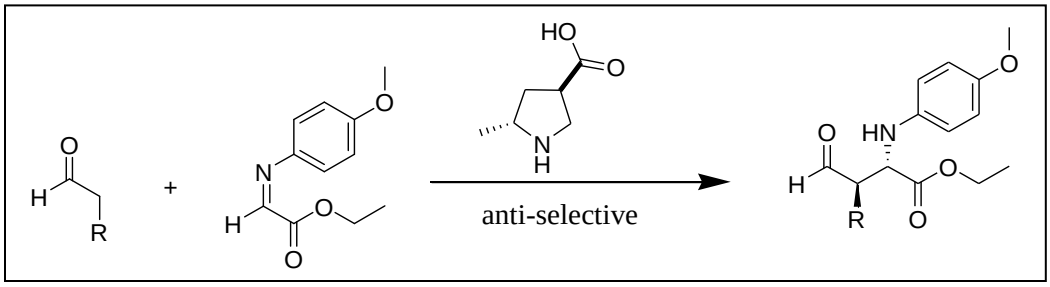


Scheme 46. Synthesis of proline and nipecotic acid analogue.

Compounds **19a nad 58** were treated with NaH and benzyl bromide to provide the resulting **62a** nad **62b** in good yield (68% - 75%). The lactams of **62a**/**62b** were converted in good yield (80% - 85%) to thiolactam **63a**/**63b** using Lawesson reagent.[156] The thiolactams were then easily reduced to the 2^0-amines **64a**/**64b** by hydrogenation over Raney nickel catalyst in good yield. The amines **64a**/**64b** were then subjected to saponification giving **65a/65b** in 78% - 85% yield. Amino acids **65a/65b** were then converted to the α-methyl-β-proline analogue (**66a**) and α-methyl-nipecotic acid analogue (**66b**) in excellent yield (91% to 95%) by hydrogenation over Pd/C catalyst. The overall yield of **66a** from **19a** is 28% over the five straightforward steps shown in Scheme 45. In continuation, the overall yield of **66b** from **58** is 45% over the five straightforward steps as shown in Scheme 45.

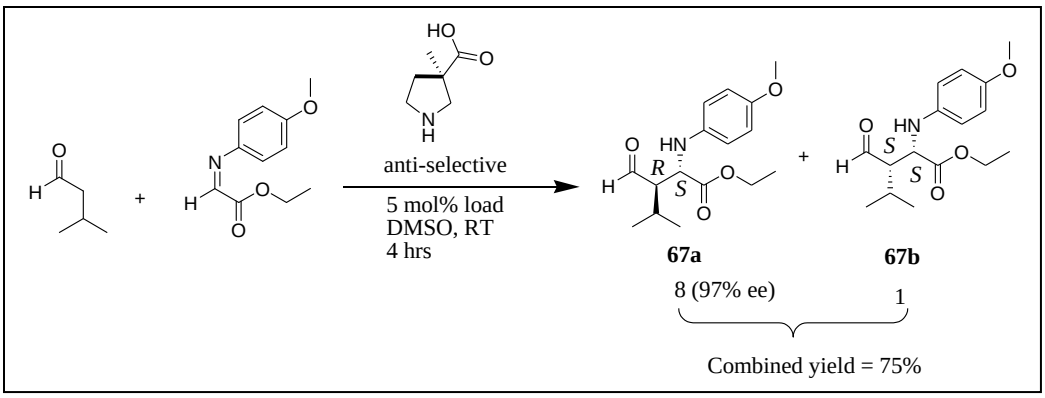
C^{α}-Methyl-β-Proline as an Organocatalyst in Anti-Mannich Type Reactions

Proline and its derivatives have been frequently used as organocatalysts in Aldol, Mannich, and Michael type reactions over the last decade.[25, 123, 144, 157] This class of catalysts have proven their excellence in asymmetric synthesis of new C-C bond.[144, 157] One of the biggest advantages to use this set of catalysts is the ease of handling as opposed to metal-catalysts.[157] In most cases, this class of catalysts is easily isolated from the crude mixture by simple water extraction at the end of the reaction.[144] In recent years, Barbas et al. exhibited a number of β-proline and nipecotic acid analogues as potent catalysts in anti-Mannich type reactions (Scheme 47).[23, 158] This set of catalysts are observed to predominantly produce the anti-Mannich isomers in excellent diastereomeric ratio and enantiopurity.[23]



Scheme 47. β-proline analogue in anti-Mannich type reactions.

However, there was no report until recently utilizing $C^{\alpha,\alpha}$-disubstituted-β-proline in anti-Mannich type reactions.[144] Hence, we have made an effort to explore the α-methyl-β-proline analogue (**66a**) made in our hand in Mannich type reactions (Scheme 47). We have observed that the β-proline analogue (**66a**) is a strong anti-selective catalyst producing the anti-Mannich product **67a** as the predominant stereoisomer in 8: 1 diastereomeric ratio and 97% ee (Scheme 48, Figure 21). The combined yield was 75%.



Scheme 48. C$^{\alpha}$-methyl-β-proline catalyze anti-Mannich type reactions.

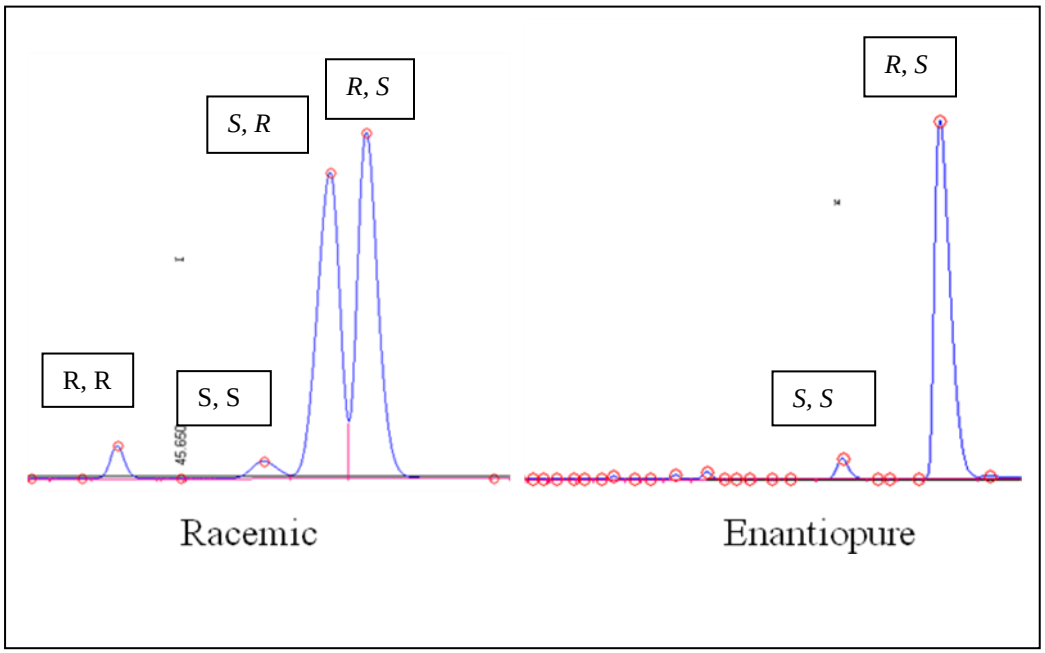
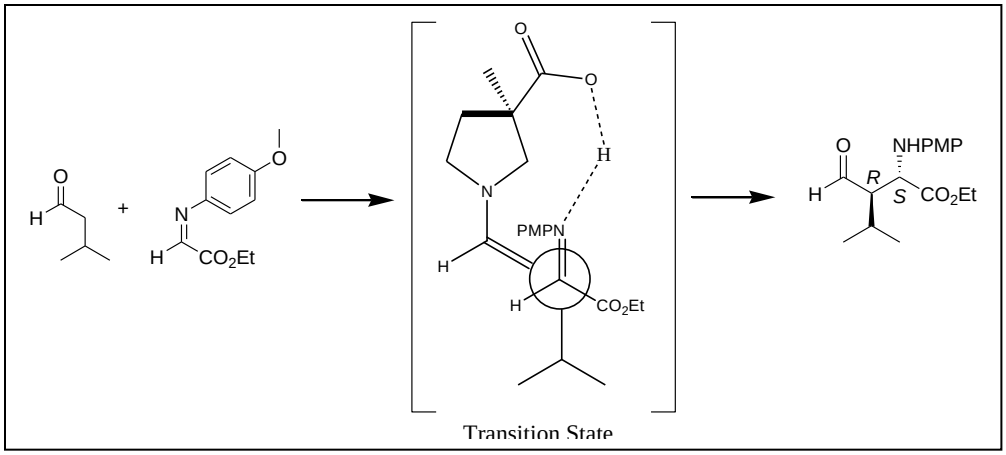


Figure 21. Chiral-HPLC chromatogram of racemic and optically pure anti-Mannich reaction.

Based on the results obtained, we have proposed the plausible transition state of the anti-Mannich type reactions employing α-methyl-β-proline (Scheme 49).



Scheme 49. Transition state of anti-Mannich type reaction.

Conclusions

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

Experimental

*General Procedure for the Synthesis of **18a-18e***

A 250 mL roundbottom flask was charged with 9.9 g of **10b** (31 mmol), 4.3 g of K_2CO_3 (31 mmol), 100 mL of anhydrous DMF, and a stirbar. A solution of the appropriately substituted benzyl bromide (28 mmol) in 20 mL of anhydrous DMF was slowly added over 15 minutes. The reaction was allowed to stir approximately 12 hr

under a nitrogen atmosphere. The reaction mixture was then diluted with 100 mL of water and the resulting mixture was washed with Et_2O (3 x 100 mL). The combined ether layer was washed with water (5 x 100 mL), washed with brine (2 x 100 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was isolated by flash chromatography (40% Et_2O/hexanes).

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

18a was synthesized following the general synthetic procedure for the preparation of 18a-**18e**. An amount of 11 g of product (27 mmol, 87%) was obtained after flash chromatography purification as a colorless liquid. R_f = 0.2 (40% Et_2O/hexanes). $[\alpha]_D^{24}$ = -3.08 (c = 1, $CHCl_3$). IR (cm^{-1}): 2980, 1773, 1708. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.83 (m, 2H), 7.70 (m, 2H), 7.33 (m, 5H), 5.15 (m, 2H), 4.10 (m, 2H), 3.74 (m, 2H), 2.28 (m, 2H), 1.56 (s, 3H), 1.16 (t, 3H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 171.4, 171.3, 168.0, 135.5, 134.0, 132.0, 128.5, 128.3, 128.1, 123.0, 67.0, 61.0, 52.0, 33.8, 33.8, 20.0, 14.0. HRMS [$C_{23}H_{23}NO_6Na^+$]: calcd = 432.1417, found = 432.1406.

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

18b was synthesized following the general synthetic procedure for the preparation of **18a-18e**. An amount of 9.95 g of product (23.3 mmol, 75%) was obtained after purification as colorless liquid. R_f = 0.25 (40% Et_2O/hexanes). $[\alpha]_D^{24}$= -3.7 (c = 1, $CHCl_3$). IR (cm^{-1}): 2984, 1773, 1708. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.83 (m, 2H), 7.71 (m, 2H), 7.33 (m, 2H), 7.03 (m, 2H), 5.11 (m, 2H), 4.10 (m, 2H), 3.72 (m, 2H), 2.26 (m, 2H), 1.55 (s, 3H), 1.16 (t, 3H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 171.4, 171.3, 163.0 (d, ^1J =

250 Hz), 134.0, 132.0, 131.0 (d, ^{4}J =3.6 Hz), 130.0 (d, ^{3}J = 8.5 Hz), 123.0, 115.0 (d, ^{2}J =22 Hz), 123.0, 66.0, 62.0, 52.0, 33.8, 33.7, 20.0, 14.0. HRMS [$C_{23}H_{22}FNO_6Na^{+}$]: calcd = 450.1323, found = 450.1312.

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

18c was synthesized following the general synthetic procedure for the preparation of **18a-18e**. An amount of 9.4 g of product (22.3 mmol, 72%) was obtained after purification as colorless liquid. R_f = 0.24 (40% Et_2O/hexanes). $[\alpha]_D^{24}$= -5.3 (c = 1, $CHCl_3$). IR (cm^{-1}): 2983, 1773, 1708. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.83 (m, 2H), 7.70 (m, 2H), 7.22 (d, 2H, *J* = 8 Hz), 7.14 (d, 2H, *J* = 8 Hz), 5.11 (m, 2H), 4.10 (m, 2H), 3.73 (m, 2H), 2.34 (s, 3H), 2.26 (m, 2H), 1.55 (s, 3H), 1.16 (t, 3H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 171.4, 171.3, 168.0, 138.0, 134.0, 132.5, 132.0, 129.0, 128.0, 123.0, 67.0, 62.0, 52.0, 34.0, 21.0, 20.0, 14.0. HRMS [$C_{24}H_{25}NO_6Na^{+}$]: calcd = 446.1574, found = 446.1565.

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

18d was synthesized following the general synthetic procedure for the preparation of **18a-18e**. An amount of 10.27 g of product (22.6 mmol, 73%) was obtained after purification as a white solid. mp = 65 ^{O}C. R_f = 0.1 (40% Et_2O/hexanes). $[\alpha]_D^{24}$= + 1.4 (c = 1, $CHCl_3$). IR (cm^{-1}): 2983, 1773, 1729, 1707, 1517. ^{1}H-NMR ($CDCl_3$, 400 MHz): 8.23 (d, 2H, *J* = 8 Hz), 7.83 (m, 2H), 7.72 (m, 2H), 7.52 (d, 2H, *J* = 8 Hz), 5.25 (m, 2H), 4.16 (m, 2H), 3.74 (m, 2H), 2.29 (m, 2H), 1.59 (s, 3H), 1.21 (t, 3H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 171.2, 171.1, 168.0, 148.0, 143.0, 134.0, 132.0, 128.0, 124.0, 123.0,

66.0, 62.0, 52.0, 34.0, 33.8, 20, 14. HRMS [$C_{23}H_{22}N_2O_8Na^+$]: calcd = 477.1268, found = 477.1266.

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

4e was synthesized following the general synthetic procedure for the preparation of **18a-18e**. An amount of 10.8 g of product (22.6 mmol, 87%) was obtained after purification as a colorless liquid. R_f = 0.26 (40% Et_2O/hexanes). $[\alpha]_D^{24}$= -8.33 (c = 3, $CHCl_3$). IR (cm^{-1}): 2983, 1773, 1709. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.83 (m, 2H), 7.71 (m, 2H), 7.62 (d, 2H, *J* = 8 Hz), 7.46 (d, 2H, *J* = 8 Hz), 5.20 (s, 2H), 4.12 (m, 2H), 3.73 (m, 2H), 2.29 (m, 2H), 1.57 (s, 3H), 1.17 (t, 3H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): 171.3, 171.2, 168.0, 139.0, 134.0, 132.0, 130.0 (q, *J* = 33 Hz), 128.0, 125.0 (q, *J* = 4 Hz), 123.0, 122.0, 66.0, 62.0, 52.0, 34.0, 33.8, 20.0, 14.0. HRMS [$C_{24}H_{22}F_3NO_6Na^+$]: calcd = 500.1291, found = 500.1278.

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

A volume of 930 µL (10.2 mmol) 35% hydrazine in water was added to a solution of 3.8 g (9.3 mmol) **18a** in 50 mL MeOH. The mixture was heated to reflux solvent overnight. A white precipitate was observed within an hour of reflux. The reaction mixture was allowed to cool to RT, and the resulting mixture was filtered. The filtrate was evaporated under reduced pressure. The resulting residue was taken up in CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, evaporated under reduced pressure, and purified by column chromatography, using 30% hexanes/EtOAc giving 1.2 g of a 10:1 mixture of **19a**:**19b** as a white solid. The mixture was further recrystallized in cold Et_2O giving 1 g (6 mmol, 64.5%) of pure **19a** as white crystals. R_f

(**19a**) = 0.31 (30% hexanes/EtOAc). mp = 63 ^{O}C. $[\alpha]_D^{23}$= +19.0 (c = 2, MeOH). IR (cm^{-1}): 3245, 2985, 1726, 1698, 1660. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.06 (bs, 1H), 4.20 (m, 2H), 3.47 (m, 1H), 3.36 (m, 1H), 2.64 (m, 1H), 2.02 (m, 1H), 1.45 (s, 3H), 1.28 (t, 3H, *J* = 7 Hz), ^{13}C-NMR ($CDCl_3$, 100 MHz): 177.0, 172.0, 61.0, 51.0, 40.0, 34.0, 20.0, 14.0. HRMS [$C_8H_{13}NO_3Na^+$]: calcd = 194.0788, found = 194.0795.

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

A volume of 600 µL conc. H_2SO_4 was added to a solution of 2 g of **10b** (6 mmol) in 30 mL CH_2Cl_2 in a 100 mL sealed tube. The solution was cooled to -7 ^{o}C. A volume of 6 mL condensed isobutylene was added to the solution. The tube was sealed tightly and allowed to stir overnight at RT. The tube was uncapped and allowed to stir for 2hrs. at ambient pressure to allow excess isobutylene to evaporate. The solution was diluted with 30 mL of CH_2Cl_2 and gently washed three times with 1N NaOH (50 mL). The CH_2Cl_2 layer was dried over $MgSO_4$, evaporated under reduced pressure, and chromatographed (40% EtOAc/hexanes), giving 1.8 g (5 mmol, 83%) of **55** as colorless viscous liquid. The viscosity of the material made removal of residual solvent impractical and **55** was utilized in the subsequent reaction without further purification. R_f = 0.51 (40% EtOAc/hexanes). IR (cm^{-1}): 2979, 1774, 1709. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.84 (m, 2H), 7.71 (m, 2H), 4.17 (m, 2H), 3.72 (m, 2H), 2.19 (m, 2H), 1.50 (s, 3H), 1.48 (s, 9H), 1.28 (t, 3H, *J* = 7 Hz). ^{13}C NMR ($CDCl_3$, 100MHz) 172.0, 170.5, 168.0, 134.0, 132.2, 123.3, 82.0, 61.0, 53.0, 34.0, 33.8, 28.0, 20.0, 14.0. HRMS [$C_{20}H_{25}NO_6Na^+$]: Calcd = 398.1574, found = 398.1568.

*Synthesis of (S)-Tert-Butyl-3-Methyl-2-Oxopyrrolidine-3-Carboxylate (**56**)*

A volume of 398 µL (4.4 mmol) 35% hydrazine in water was added to a solution of 1.5 g (4 mmol) **55** in 25 mL MeOH. The mixture was heated to reflux solvent overnight. A white precipitate was observed within an hour of reflux. The reaction mixture was allowed to cool to RT and the solution was filtered. The filtrate was evaporated under reduced pressure and taken up in CH_2Cl_2. The resulting mixture was washed with water and the organic layer was dried over $MgSO_4$, evaporated under reduced pressure, and chromatographed using 30% hexanes/EtOAc giving 0.62 g of a 9:1 mixture of **56**:**19a** as a white solid. The mixture was further recrystallized in cold Et_2O giving 0.52 g (2.6 mmol, 65%) pure **56** as a white solid. R_f (**56**) = 0.27 (30% hexanes/EtOAc). mp = 130 ^{O}C. IR (cm^{-1}): 3255, 2970, 1727, 1688, 1660. $[\alpha]_D^{23}$= -14.3 (c = 1, CH_2Cl_2). ^{1}H- NMR ($CDCl_3$, 400 MHz): 6.44 (bs, 1H), 3.45 (m, 1H), 3.33 (m, 1H), 2.55 (m, 1H), 2.0 (m, 1H), 1.46 (s, 9H), 1.39 (s, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 177.0, 171.0, 81.0, 51.0, 40.0, 34.0, 28.0, 20.0. HRMS [$C_{10}H_{17}NO_3Na^+$]: calcd = 222.1101, found = 222.1097.

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

A 250 mL round-bottom flask was charged with 6.41 g of **10c** (19 mmol), 2.62 g of K_2CO_3 (19 mmol), 75 mL of anhydrous DMF, and a stirbar. A solution of the 4-nitrobenzyl bromide (17.1 mmol) in 20 mL of anhydrous DMF was slowly added over 15 min. The reaction was allowed to stir approximately 12 hrs. under a nitrogen atmosphere. The reaction mixture was then diluted with 100 mL of water, and the resulting mixture was washed with Et_2O (3 × 150 mL). The combined ether layer was washed with water

(8 × 150 mL) and brine (2 × 100 mL) and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was isolated by flash chromatography (30% EtOAc/hexanes), giving 801g (17.4 mmol, 87%) pure **57** as white solid. R_f = 0.1 (30% EtOAc/hexanes). MP = 66 ^{0}C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.19 (m, 2H), 7.81 (m, 2H), 7.72 (m, 2H), 7.46 (m, 2H), 5.22 (m, 2H), 4.15 (q, 2H, J = 7 Hz), 3.68 (t, 2H, J = 7 Hz), 1.95 (m, 2H), 1.65 (m, 2H), 1.44 (s, 3H), 1.18 (t, 3H, J = 7 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.5, 168.2, 148.7, 134.0, 132.0, 128.2, 124.0, 123.3, 65.2, 61.4, 58.5, 53.0, 37.8, 33.0, 24.0, 20.0, 18.2, 13.6. ESI-MS [$C_{24}H_{24}N_2O_8Na^+$] = 468.4, observed = 468.3.

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

A volume of 1.8 mL (20 mmol) 35% hydrazine in water was added to a solution of 5.1 g (11 mmol) of **57** in 50 mL of MeOH. The mixture was heated to reflux overnight. A white precipitate was observed within 1 hr of reflux. The reaction mixture was allowed to cool to rt, and the resulting mixture was filtered through an HPLC filter. The filtrate was evaporated under reduced pressure. The resulting residue was taken up in CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, evaporated under reduced pressure, and purified by column chromatography using 60% hexanes/EtOAc giving 1.5g (8.3 mmol, 75%) of **58** as a white solid. R_f = 0.35 (30% EtOAc/hexanes). MP = 65 ^{0}C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.25 (bs, 1H), 4.2 (m, 2H), 3.36 (m, 2H), 2.26 (m, 1H), 1.84 (m, 2H), 1.73 (m, 1H), 1.50 (s, 3H), 1.27 (t, 3H, J = 7 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.6, 172.0, 61.2, 50.3, 42.3, 33.0, 22.4, 90.4, 14.0. ESI-MS [$C_9H_{15}NO_3Na^+$] = 208.1, observed = 208.1.

*Synthesis of (S)-1-Tert-Butyl-3-Ethyl-2-Methyl-2-[3-(1,3-Dioxoisoindolin-2Yl)Propyl]Malonate (**60**)*

A volume of 600 µL conc. H_2SO_4 was added to a solution of 2 g of **10c** (6 mmol) in 30 mL CH_2Cl_2 in a 100 mL sealed tube. The solution was cooled to -7 ^oC. A volume of 6 mL condensed isobutylene was added to the solution. The tube was sealed tightly and allowed to stir overnight at RT. The tube was uncapped and allowed to stir for 2hrs at ambient pressure to allow excess isobutylene to evaporate. The solution was diluted with 30 mL of CH_2Cl_2 and gently washed three times with 1N NaOH (50 mL). The CH_2Cl_2 layer was dried over $MgSO_4$, evaporated under reduced pressure, and chromatographed (40% EtOAc/hexanes), giving 2 g (5.1 mmol, 87%) of **60** as white solid. R_f = 0.53 (40% EtOAc/hexanes). $[\alpha]_D^{23}$ = - 5.2. IR (cm^{-1}): 2979, 1774, 1709. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.84 (m, 2H), 7.71 (m, 2H), 4.18 (m, 2H), 3.74 (m, 2H), 1.91 (m, 2H), 1.61 (m, 2H), 1.48 (s, 9H), 1.39 (s, 3H), 1.28 (t, 3H, *J* = 7 Hz). ^{13}C NMR ($CDCl_3$, 100MHz) 172.3, 171.03, 168.3, 134.0, 132.1, 123.2, 81.5, 61.1, 54.0, 38.1, 34.7, 28.0, 25.3, 20.0, 14.0. ESI-MS [$C_{21}H_{27}NO_6Na^+$]: Calcd = 412.4, found = 412.3.

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

A volume of 398 µL (4.4 mmol) 35% hydrazine in water was added to a solution of 1.5 g (4 mmol) **60** in 25 mL MeOH. The mixture was heated to reflux solvent overnight. A white precipitate was observed within an hr of reflux. The reaction mixture was allowed to cool to RT and the solution was filtered. The filtrate was evaporated under reduced pressure and taken up in CH_2Cl_2. The resulting mixture was washed with water and the organic layer was dried over $MgSO_4$, evaporated under reduced pressure, and chromatographed using 30% hexanes/EtOAc giving 0.62 g of **61** as a white solid. R_f

(**61**) = 0.27 (30% hexanes/EtOAc). Mp = 130 ^{0}C. IR (cm^{-1}): 3255, 2970, 1727, 1688, 1660. $[\alpha]_D^{23}$= -16.2 (c = 1, CH_2Cl_2). ^{1}H- NMR ($CDCl_3$, 400 MHz): 6.28 (bs, 1H), 3.61 (m, 2H), 2.2 (m, 1H), 1.8 (m, 2H), 1.61 (m, 1H), 1.42 (s, 12H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 174.2, 173.9, 83.0, 51.0, 43.0, 34.0, 28.0, 20.0, 14.0. ESI-MS [$C_{11}H_{19}NO_3Na^+$]: calcd = 236.3, found = 236.2.

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

A solution of 3.6 g (22 mmol) of **19a** in 20 mL anhydrous THF was added slowly to a suspension of 0.62 g NaH (26 mmol) in 10 mL THF at 0 ^{O}C under a N_2 atmosphere. The reaction mixture was allowed to stir 5 minutes. A volume of 3.3 mL (4.75 g, 24 mmol) BnBr was added drop wise to the reaction mixture at 0 ^{O}C. The reaction mixture was allowed to stir for 10 minutes at 0 ^{O}C and then allowed to warm to RT. The reaction was continued for 1 hr at RT. A volume of 15 mL dry DMF was added to the reaction mixture and continued to stir for 2 hrs. The reaction mixture was poured into 25 mL of H_2O. The water layer was extracted with Et_2O (3 X 50 mL). The combined ether layer was washed with water (5 X 30 mL), dried over $MgSO_4$, evaporated under reduced pressure, and chromatographed (gradient, 15%-20% EtOAc/hexanes) giving 3.8 g (15 mmol, 68%) of **62a** as a colorless oil. 1H NMR evidenced that the product contains some trans-esterified compound as well. The mixture was utilized in the next step without further purification. R_f = 0.27 (20% EtOAc/hexanes). IR (cm^{-1}): 2979, 1735, 1685. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.29 (m, 7.5H), 5.16 (m, 0.5H), 4.6 (m, 1.25H), 4.36 (m, 1.27H), 4.17 (m, 2H), 3.33 (m, 1.26H), 3.17 (m, 1.25H), 2.46 (m, 1.25H), 1.89 (m, 1.27H), 1.50 (s, 0.76H), 1.47 (s, 3H), 1.23 (t, 3H, *J* =7 Hz). ESI-MS [$C_{15}H_{20}NO_3$, **62a**]$^+$ calcd = 261.3, found = 262.2.

*Synthesis of (S)-Ethyl-1-Benzyl-3-Methyl-2-Oxopiperidine-3-Carboxylate (**62b**)*

A solution of 0.3 g (1.6 mmol) of **58** in 10 mL of anhydrous THF was added slowly to a suspension of 0.046 g NaH (1.92 mmol) in 10 mL of THF at 0 °C under a N_2 atmosphere. The reaction mixture was allowed to stir for 5 min. A volume of 210 µL (1.76 mmol) of BnBr was added dropwise to the reaction mixture at 0 °C. The reaction mixture was allowed to stir for 10 min at 0 °C and then allowed to warm to rt. The reaction was continued for 1hr at rt. A volume of 6 mL of dry DMF was added to the reaction mixture, which continued to stir for 2 hrs. The reaction mixture was poured into 15 mL of H_2O. The water layer was extracted with Et_2O (3 × 25 mL). The combined ether layer was washed with water (3 × 10 mL), dried over $MgSO_4$, evaporated under reduced pressure, and chromatographed (gradient, 15−20% EtOAc/hexanes) giving 0.36g (1.3 mmol, 81%) as a colorless oil. R_f = 0.3 (20% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.29 (m, 5H), 5.0 (m, 1H), 4.2 (m, 3H), 3.24 (m, 2H), 2.23 (m, 1H), 1.79 (m, 3H), 1.54 (s, 3H), 1.29 (t, 3H, J = 7 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.6, 169.3, 137.2, 128.5, 127.8, 127.3, 61.4, 50.7, 50.4, 47.3, 33.4, 22.7, 19.4, 14.2. ESI-MS [$C_{16}H_{21}NO_3Na^+$] = 298.3, observed = 298.2.

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

An amount of 5.3 g (13 mmol) Lawesson's reagent was added to a solution of 3.8 g (15 mmol) of **62a** mixture in 20 mL anhydrous toluene under a N_2 atmosphere. The reaction mixture was heated to 95 ^{O}C and stirred over night. The toluene layer was evaporated under reduced pressure and the residue was chromatographed (20% EtOAc/hexanes) giving 3.3 g (12 mmol, 80%) of **63a** as a colorless oil. The conversion of lactam (**62a**) to the corresponding thiolactam (**63a**) was confirmed by comparing the ^{13}C-

NMR chemical shift of the lactam (**62a**) carbonyl carbon (172 ppm) to the thiolactam (**63a**) carbonyl carbon (202 ppm). R_f = 0.35 (20% EtOAc/hexanes). IR (cm^{-1}): 2980, 1733, 1505, 1449. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.29 (m, 7.3H), 5.18 (m, 2H), 4.82 (m, 1.23H), 4.16 (q, 2H, *J* = 7 Hz), 3.70 (m, 1.24H), 3.51 (m, 1.25H), 2.51(m, 1.23H), 1.92 (m, 1.32H), 1.61 (s, 0.68H), 1.58 (s, 3H), 1.21 (t, 3H, *J* = 7 Hz). ESI-MS [$C_{15}H_{20}NO_2S$, **10a**]$^+$ calcd = 277.4, found = 278.1.

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

A 1.7 g (4.2 mmol) portion of Lawesson's reagent was added to a solution of 1.3 g (4.7 mmol) of **62b** in 20 mL of anhydrous toluene under a N_2 atmosphere. The reaction mixture was heated to 95 °C and stirred over 12 h. The reaction completion was verified by TLC (20% EtOAc/hexanes). The toluene layer was evaporated under reduced pressure, and the residue was chromatographed (20% EtOAc/hexanes) giving 0.97 g (3.3 mmol, 80%) as colorless oil. The conversion of lactam **62b** to the corresponding thiolactam **63b** was confirmed by comparing the ^{13}C NMR chemical shift of the lactam **62b** carbonyl carbon (173.6 ppm) to the thiolactam (**63b**) carbonyl carbon (202.5 ppm). Rf = 0.3 (20% EtOAc/hexanes). $[\infty]_D^{22}$ = +75.2 (c = 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.31 (m, 5H), 5.69 (m, 1H), 5.04 (m, 1H), 4.23 (m, 2H), 3.43 (m, 2H), 2.28 (m, 1H), 2.01 (m, 1H), 1.83 (m, 2H), 1.75 (s, 3H), 1.30 (t, 3H, J = 7 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 202.5, 173.6, 135.0, 129.0, 127.7, 127.5, 61.5, 57.7, 55.5, 50.4, 32.1, 27.8, 19.4, 14.0. ESI-MS [$C_{16}H_{21}NO_2SNa^+$] calculated = 314.4, observed = 314.3.

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

An amount of 0.5 g of **63a** was dissolved in 15 mL of 4:1 THF/EtOH. An amount of 0.05 g Raney-Ni slurry in water (10% by weight) was added to the solution. The

solution was stirred vigorously under a H_2 atmosphere for 4 hrs, at which point the reaction was found to be complete by TLC. The mixture was filtered through a Celite bed, and the filtrate was evaporated under reduced pressure. The product was purified by radial chromatography using 20% hexanes/CH_2Cl_2 to give 0.25 g (1.01 mmol, 72%) of **64a** as a colorless liquid. R_f = 0.32 (20% hexanes/CH_2Cl_2). IR (cm^{-1}): 2974, 2790, 1725, 1452. $[\alpha]_D^{24}$ = -8 (c = 1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, 400 MHz)(**11a**): 7.3 (m, 5H), 4.13 (q, 2H, *J* = 7 Hz), 3.61 (m, 2H), 2.94 (d, 1H, *J* = 9 Hz), 2.64 (m, 2H), 2.41 (m, 2H), 1.65 (m, 1H), 1.33 (s, 3H), 1.25 (t, 3H, *J* = 7 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz)(**11a**): 177.0, 139.0, 128.5, 128.2, 127.0, 64.0, 61.0, 60.0, 54.0, 48.0, 36.0, 25.0, 14.0. HRMS [$C_{15}H_{21}NO_2Na^+$]: calcd = 270.1464, found = 270.1463.

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

A 0.8 g portion of **63b (**2.7 mmol**)** was dissolved in 20 mL of 4:1 THF/EtOH. A 0.16 g portion of Raney-Ni slurry in water (20% by weight) was added to the solution. The solution was stirred vigorously under a H_2 atmosphere for 6 hrs, at which point the reaction was found to be half-complete by TLC (10% hexanes/CH_2Cl_2) . The mixture was continued to stir under H_2 atmosphere another 6 hrs. The mixture was found to be completed via TLC to give 0.54 g (2 mmol, 78%) of **63b** as a colorless liquid. R_f = 0.35 (20% heanes/CH_2Cl_2). $[\infty]_D^{21}$ = + 11.8 (c = 1, CH_2Cl_2). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.29 (m, 5H), 4.43 (m, 2H), 3.52 (m, 1H), 3.40 (m, 1H), 2.97 (bm, 1H), 2.58 (bm, 1H), 2.02 (m, 3H), 1.73 (m, 1H), 1.59 (m, 1H), 1.20 (m, 4H), 1.13 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): 176.5, 138.2, 128.8, 127.0, 63.1, 62.0, 60.2, 54.0, 43.1, 33.2, 24.0, 23.0, 14.0. ESI-MS [$C_{16}H_{23}NO_2Na^+$] calculated = 284.3, observed = 284.3.

*Synthesis of (R)-1-Benzyl-3-Methylpyrrolidine-3-Carboxylic Acid (**65a**)*

An amount of 0.13 g LiOH (6 mmol) was added to a solution of 0.46 g (2 mmol) **64a** in 12 mL 3:2 H_2O/EtOH. The reaction was stirred at RT for 24hrs and determined to be complete by TLC. The mixture was acidified to pH 3 (4N HCl), and the water layer was evaporated under reduced pressure giving a colorless gummy residue. The gummy residue was triturated with MeOH multiple times and the MeOH fractions were dried over $MgSO_4$. The solvent was removed under reduced pressure giving 0.34 g (1.56 mmol, 78%) of **12** as a colorless liquid. R_f = 0.13 (5% MeOH/CH_2Cl_2). IR (cm^{-1}): 3371(broad), 2946, 2615, 1712, 1455. $[\alpha]_D^{23}$ = -11.3 (c = 1, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz): 7.58 (m, 2H), 7.50 (m, 3H), 4.44 (m, 2H), 3.87 (d, 1H, *J* = 12 Hz), 3.51 (m, 2H), 3.21 (d, 1H, *J* = 12 Hz), 2.58 (m, 1H), 2.09 (m, 1H), 1.47 (s, 3H). ^{13}C-NMR (CD_3OD, 100 MHz): 176.0, 130.4, 130.2, 129.7, 129.0, 61.0, 58.0, 53.0, 35.0, 22.0. HRMS [$C_{13}H_{17}NO_2Na^+$]: calcd = 242.1151, found = 242.1145.

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

A 0.125 g portion of crushed LiOH powder (5.2 mmol) was added to a solution of 0.46 g (1.7 mmol) of **64b** in 20 mL of 3:2 H_2O/EtOH. The reaction was stirred at rt overnight. The reaction was determined to be complete by TLC (5% MeOH/CH_2Cl_2). The mixture was acidified to pH 3 (10% HCl), and the water layer was evaporated under reduced pressure giving a colorless gummy residue. The gummy residue was triturated with 10% MeOH/CH_2Cl_2 (20 mL x 20), and the MeOH fractions were dried over $MgSO_4$. The solvent was removed under reduced pressure giving 0.36 g (1.56 mmol, 88%) of **65b** as a white solid as verified by TLC and staining with bromocresol green. Rf = 0.15 (5% MeOH/CH_2Cl_2). $[\infty]_D^{22}$ = + 19.0 (c = 1, MeOH). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.52

(m, 5H), 4.50 (m, 1H), 4.17 (m, 1H), 3.60 (m, 1H), 3.40 (m, 1H), 3.10 (m, 1H), 2.80 (m, 1H), 2.20 (m, 1H), 1.98 (m, 1H), 1.80 (m, 1H), 1.54 (m, 1H), 1.2 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 178.1, 132.0, 131.2, 130.4, 130.3, 62.1, 57.7, 55.0, 43.4, 33.0, 24.1, 22.1. ESI-MS [$C_{14}H_{19}NO_2Na^+$] = 256.3, observed = 256.3.

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

An amount of 0.3 g (2.3 mmol) of **65a** was dissolved in 15 mL MeOH and added to 0.03g Pd/C (10% by weight). The solution was allowed to stir over night under a H_2 atmosphere at RT. The resulting mixture was filtered through Celite and the filtrate was evaporated under reduced pressure giving 0.27 g (2.1 mmol, 91%) of **66a** as a white solid. mp = 98 ^{O}C. IR (cm^{-1}): 3392(broad), 3177, 2877, 1704. $[\alpha]_D^{24}$= -20.4 (c = 0.33, MeOH). ^{1}H-NMR (CD_3OD, 400 MHz): 3.78 (m, 1H), 3.48 (m, 1H), 3.38 (m, 1H), 3.10 (d, 1H, *J* = 12 Hz), 2.49 (m, 1H), 2.01 (m, 1H), 1.45 (s, 3H). ^{13}C-NMR (CD_3OD, 100 MHz): 176.0, 53.0, 48.5, 45.0, 35.0, 21.0. HRMS [$(C_6H_{11}NO_2)_2Na^+$]: calcd = 281.1472, found = 281.1468.

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

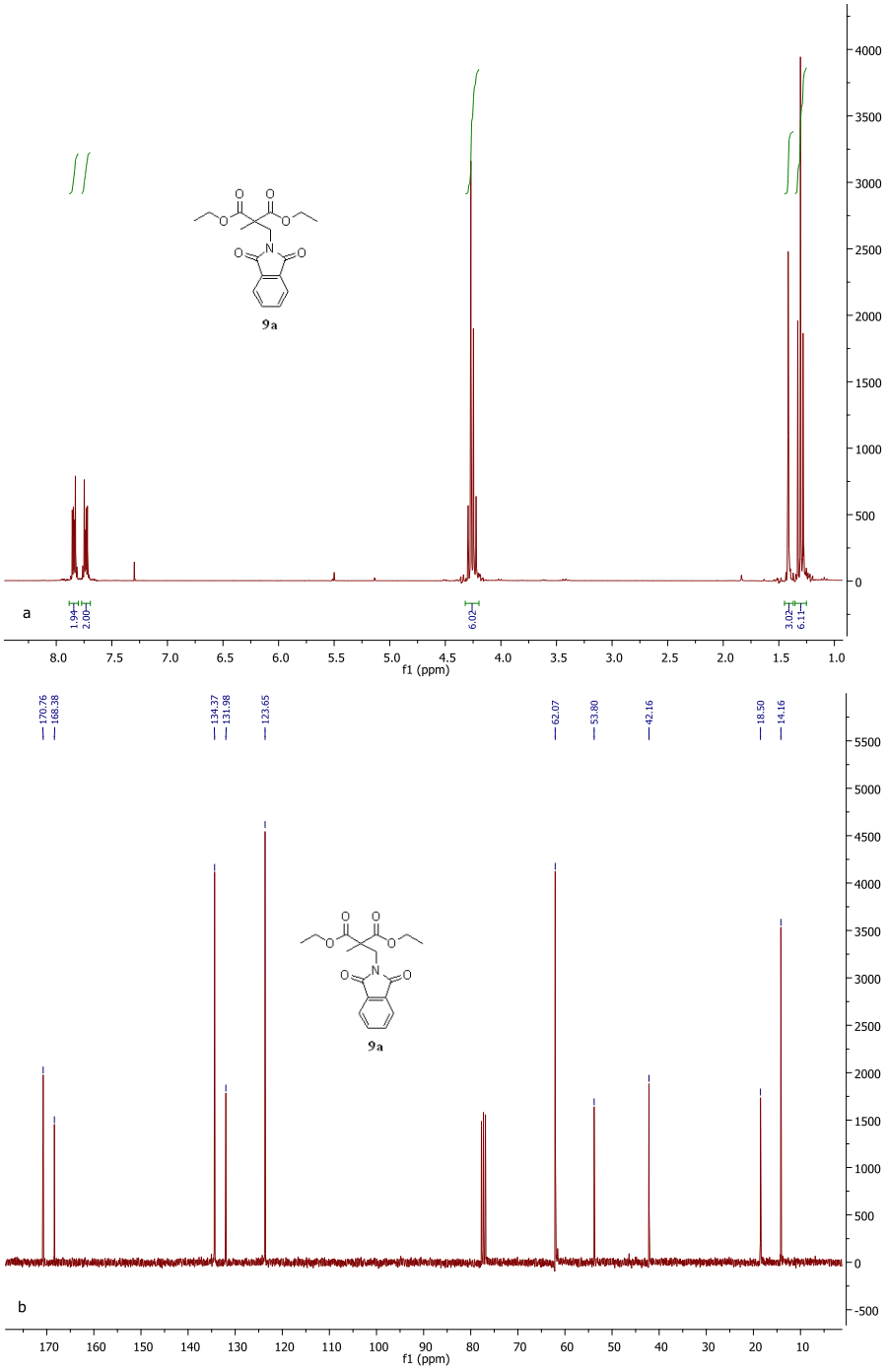
A 0.3 g (1.3 mmol) portion of **65b** was dissolved in 15 mL of MeOH and added to 0.06 g Pd/C (20% by weight). The solution was allowed to stir overnight under a H_2 atmosphere at rt. The resulting mixture was filtered through Celite, and the filtrate was evaporated under reduced pressure giving 0.185 g (1.29 mmol, 93%) of **66b** as a white solid. ^{1}H NMR (CD_3OD, 400 MHz): δ 3.53 (m, 1H), 3.28 (m, 1H), 2.97 (m, 1H), 2.83 (m, 1H), 2.19 (m, 1H), 1.88 (m, 1H), 1.69 (m, 1H), 1.58 (m, 1H), 1.27 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 178.5, 50.10, 44.3, 41.4, 33.5, 23.5, 21.0. ESI-MS [$C_7H_{13}NO_2Na^+$] = 166.2, observed = 166.3.

Synthesis of (2R, 3S)-Ethyl-3-Formyl-2-(4-Methoxyphenylamino)-4-Methylpentanoate ***(67a)***

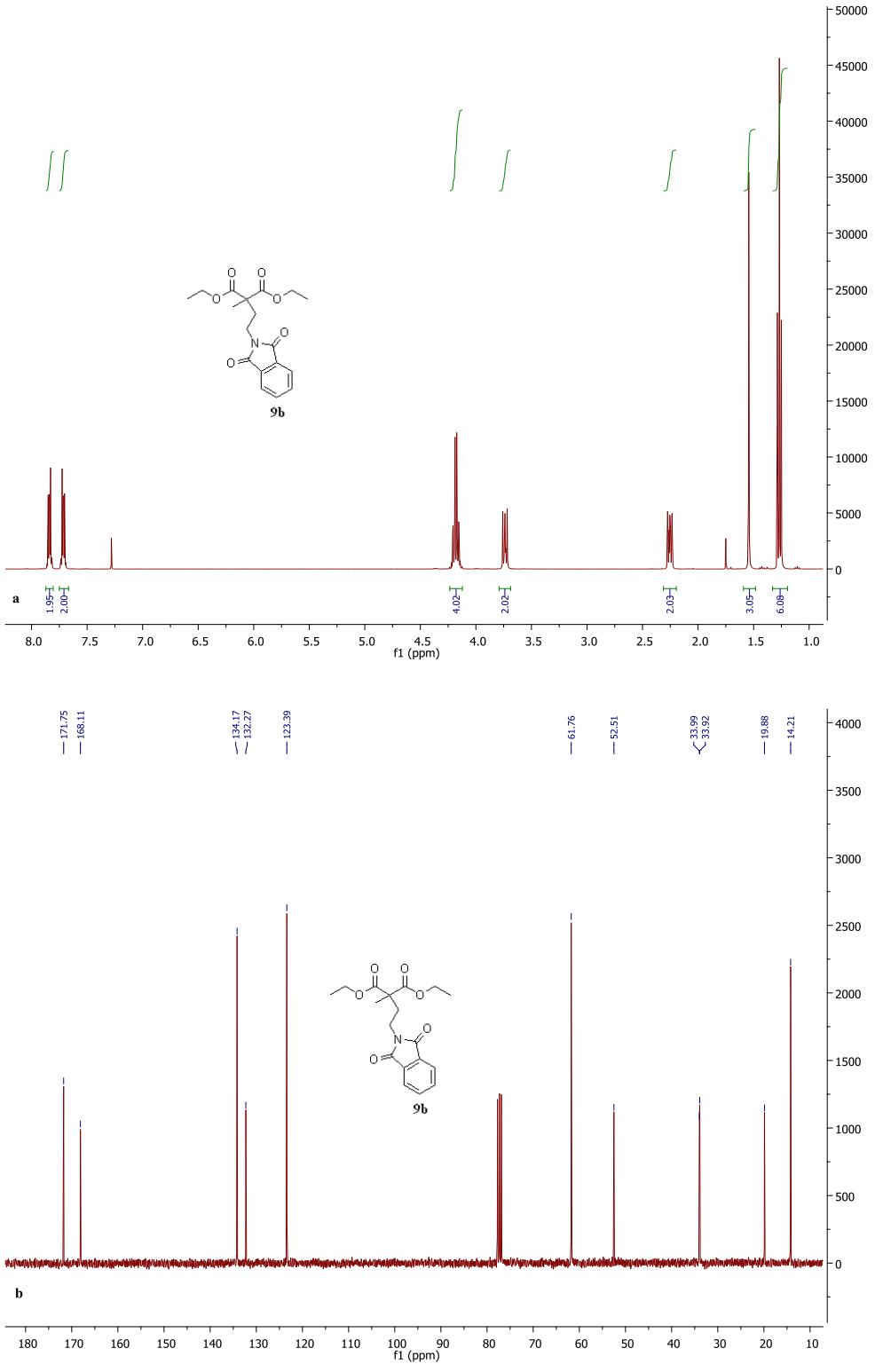
This compound was synthesized following literature reported procedure. [23] All characterizations complied with those reported in literature.[144] HPLC (Diacel Chiral Pack OJ-H, hexane/i-PrOH = 99:1, flow rate = 1mL/min, λ = 254 nm); t_{anti} (major) = 58.20 min, t_{anti} (minor) = 55.78 min, t_{syn} (major) = 51.31 min, t_{syn} (minor) = 41.28 min.

APPENDIXES

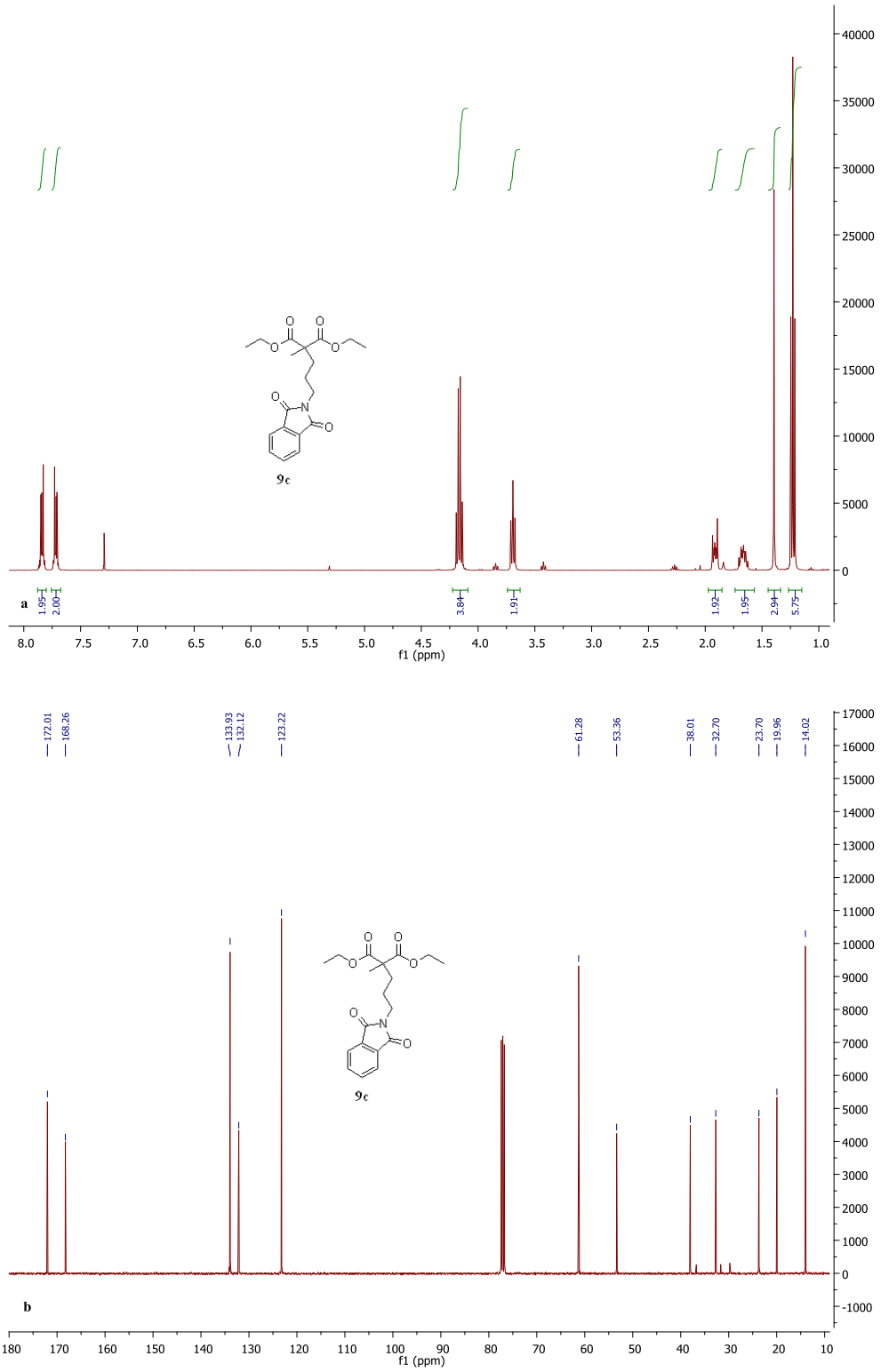
NMR Spectra



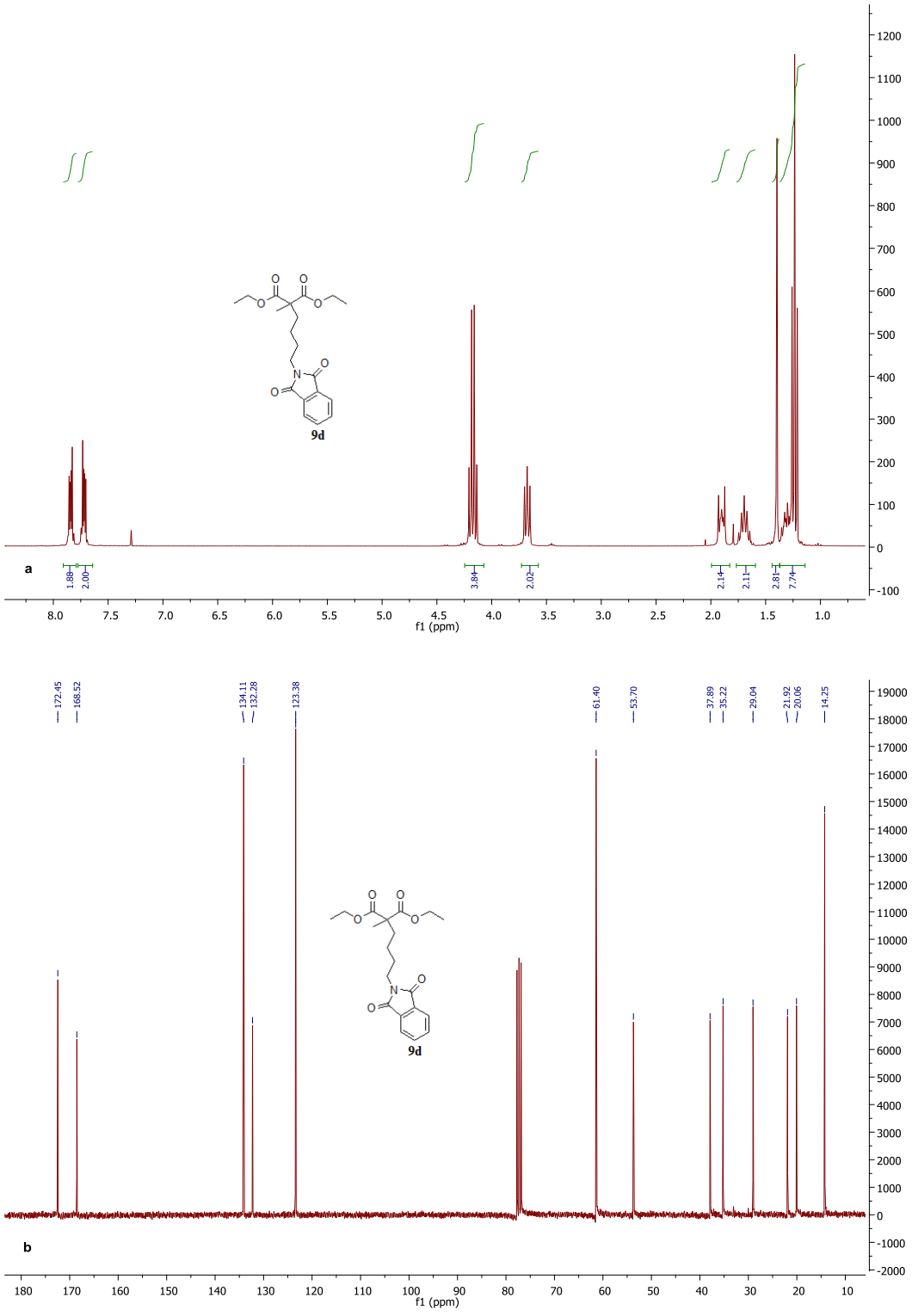
A.1.**a**) ^{1}H NMR of **9a**, b) ^{13}C NMR of **9a**



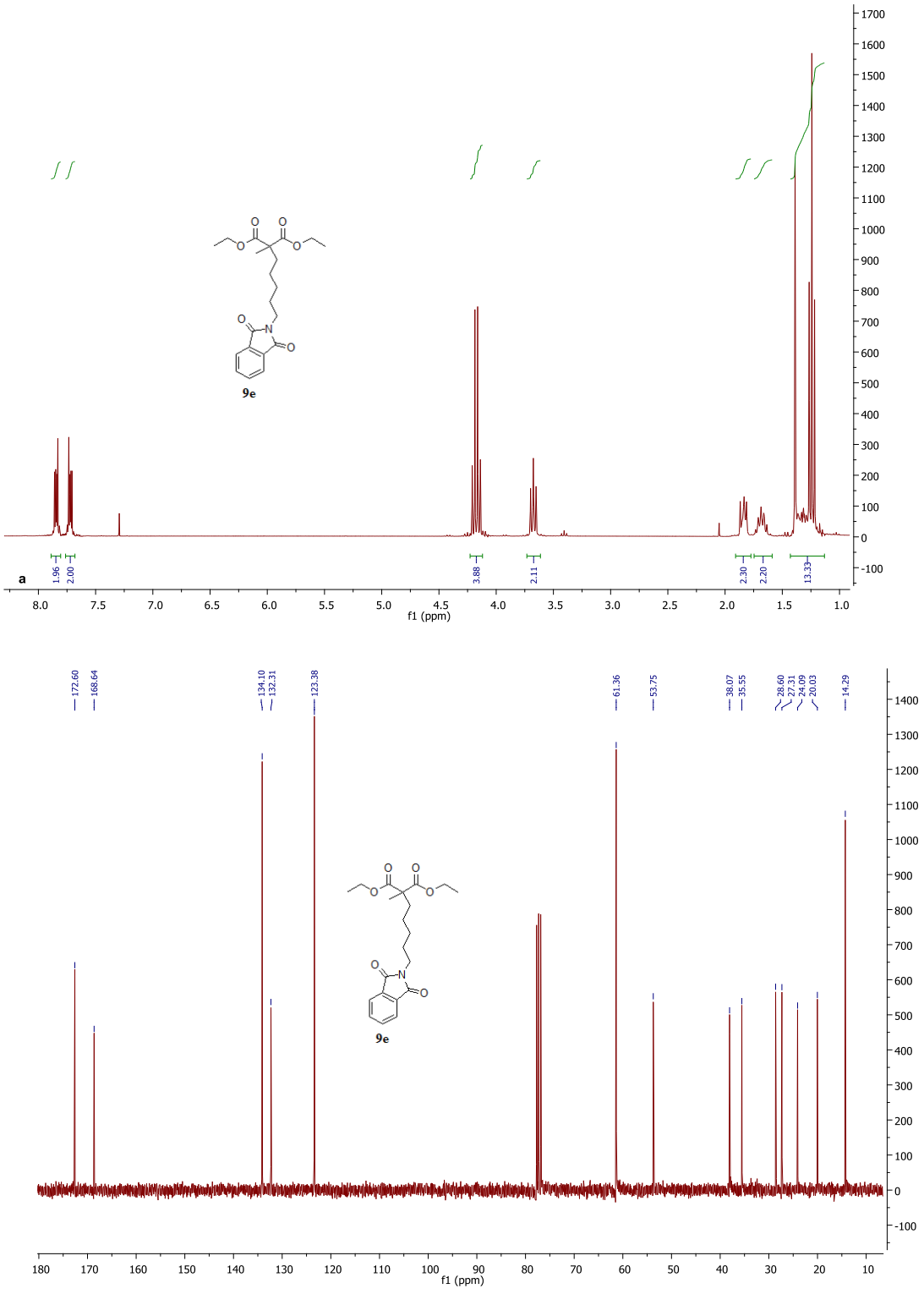
A.2. a) ^{1}H NMR of **9b**, b) ^{13}C NMR of **9b**



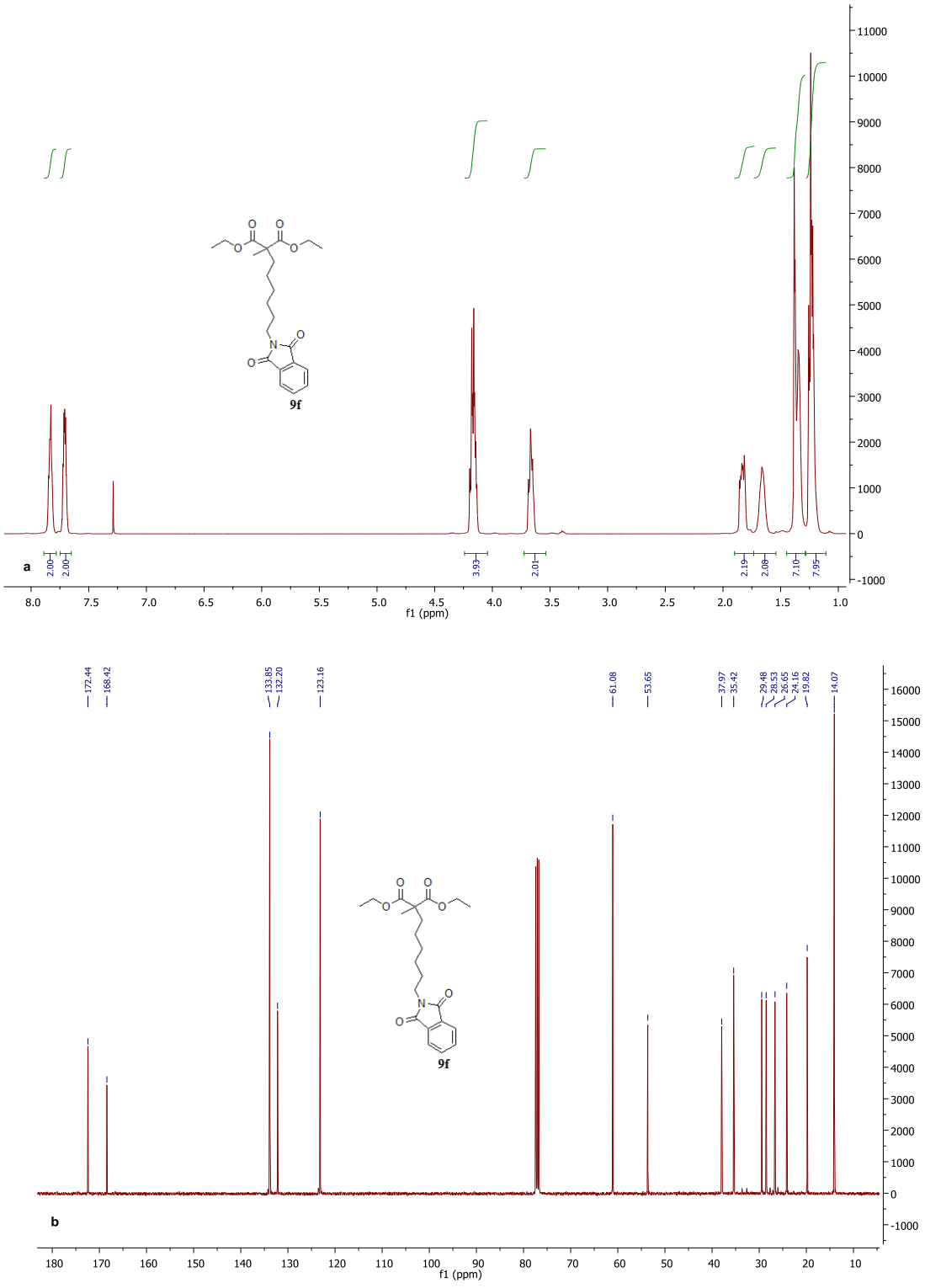
A.3.a) ^{1}H NMR of **9c**, b) ^{13}C NMR of **9c**



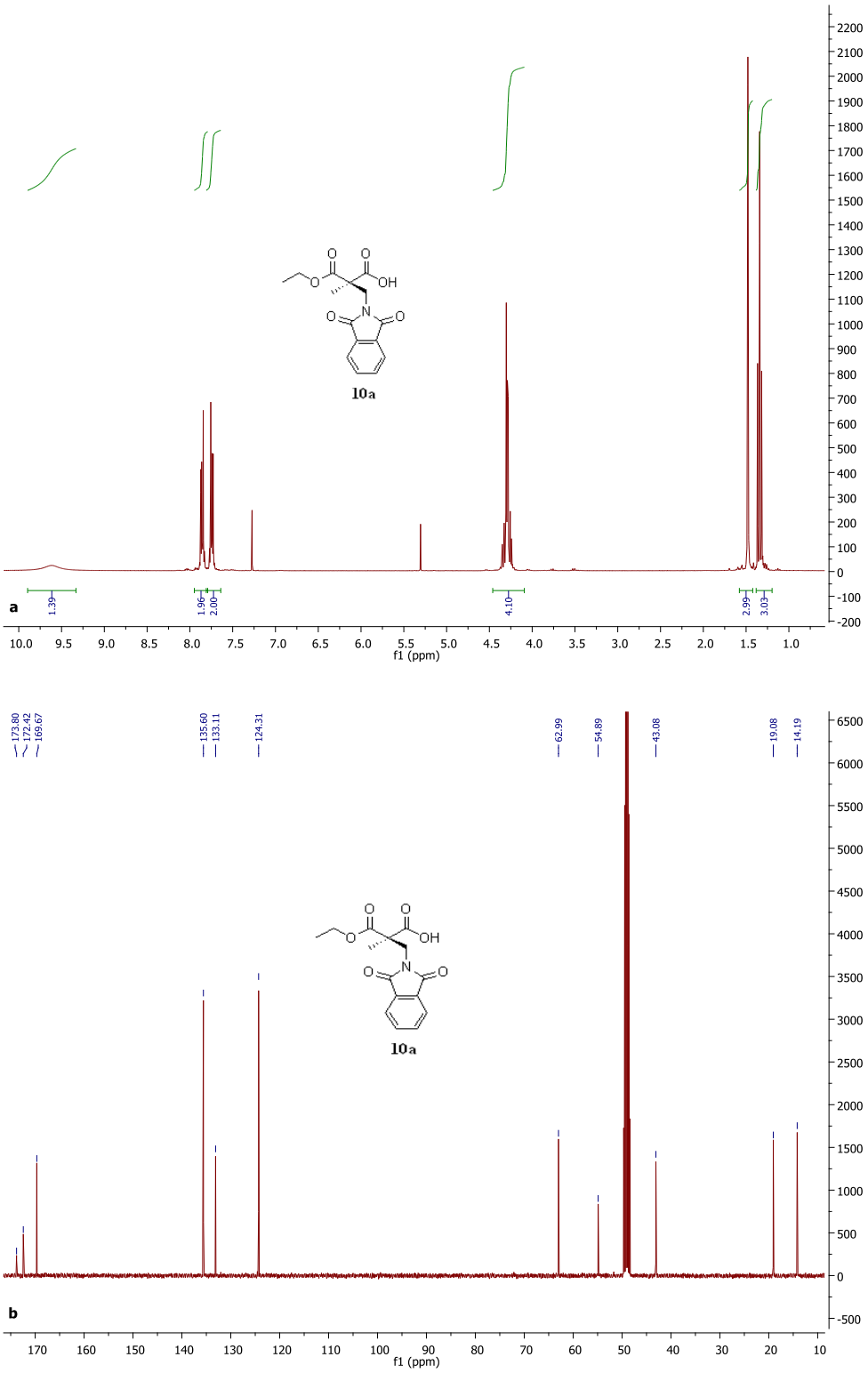
A. 4. a) ^{1}H NMR of **9d**, b) ^{13}C NMR of **9d**



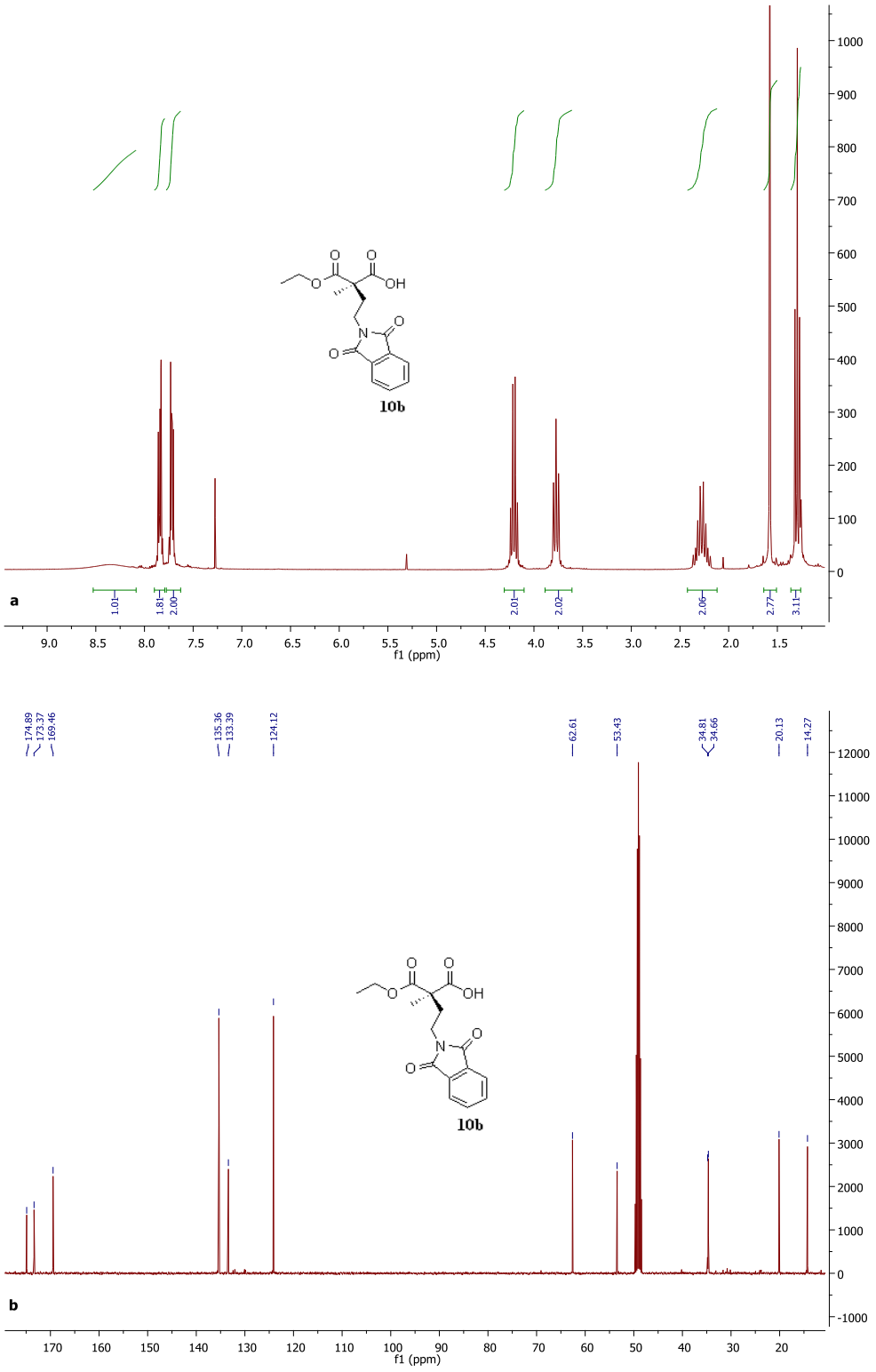
A. 5. a) ^{1}H NMR of **9e**, b) ^{13}C NMR of **9e**



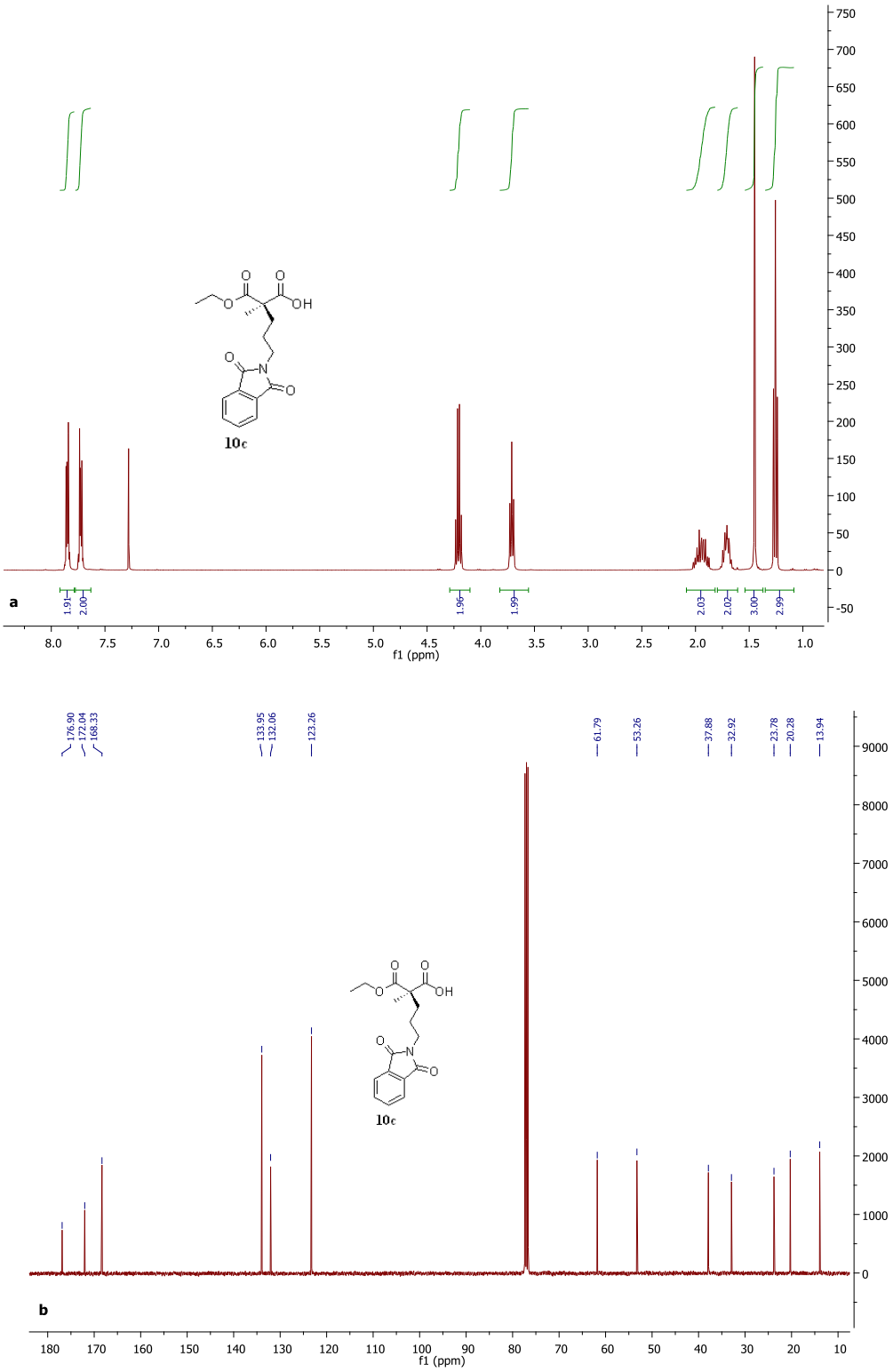
A. 6. a) ^{1}H NMR of **9f**, b) ^{13}C NMR of **9f**



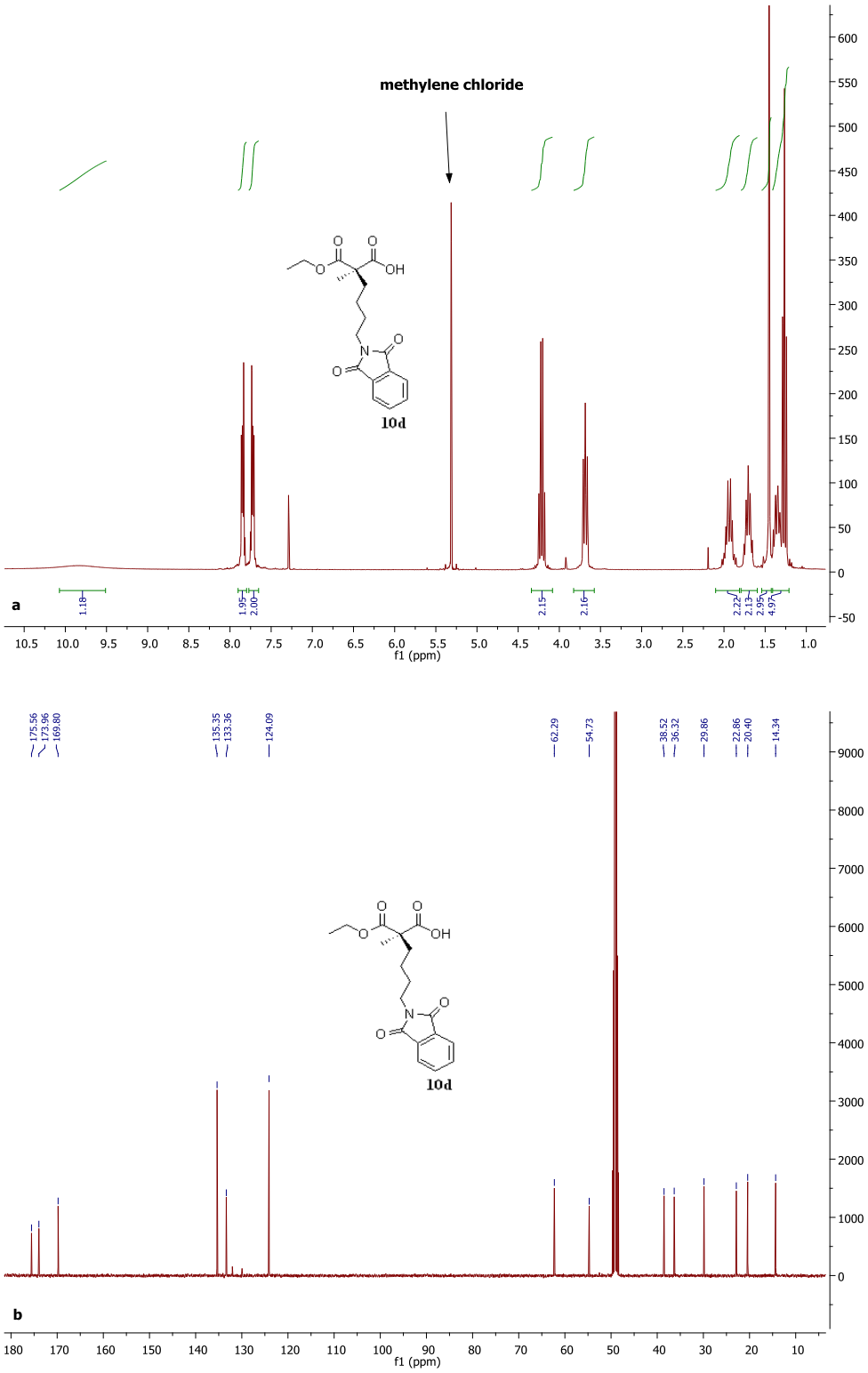
A. 7. a) ^{1}H NMR of **10a**, b) ^{13}C NMR of **10a**



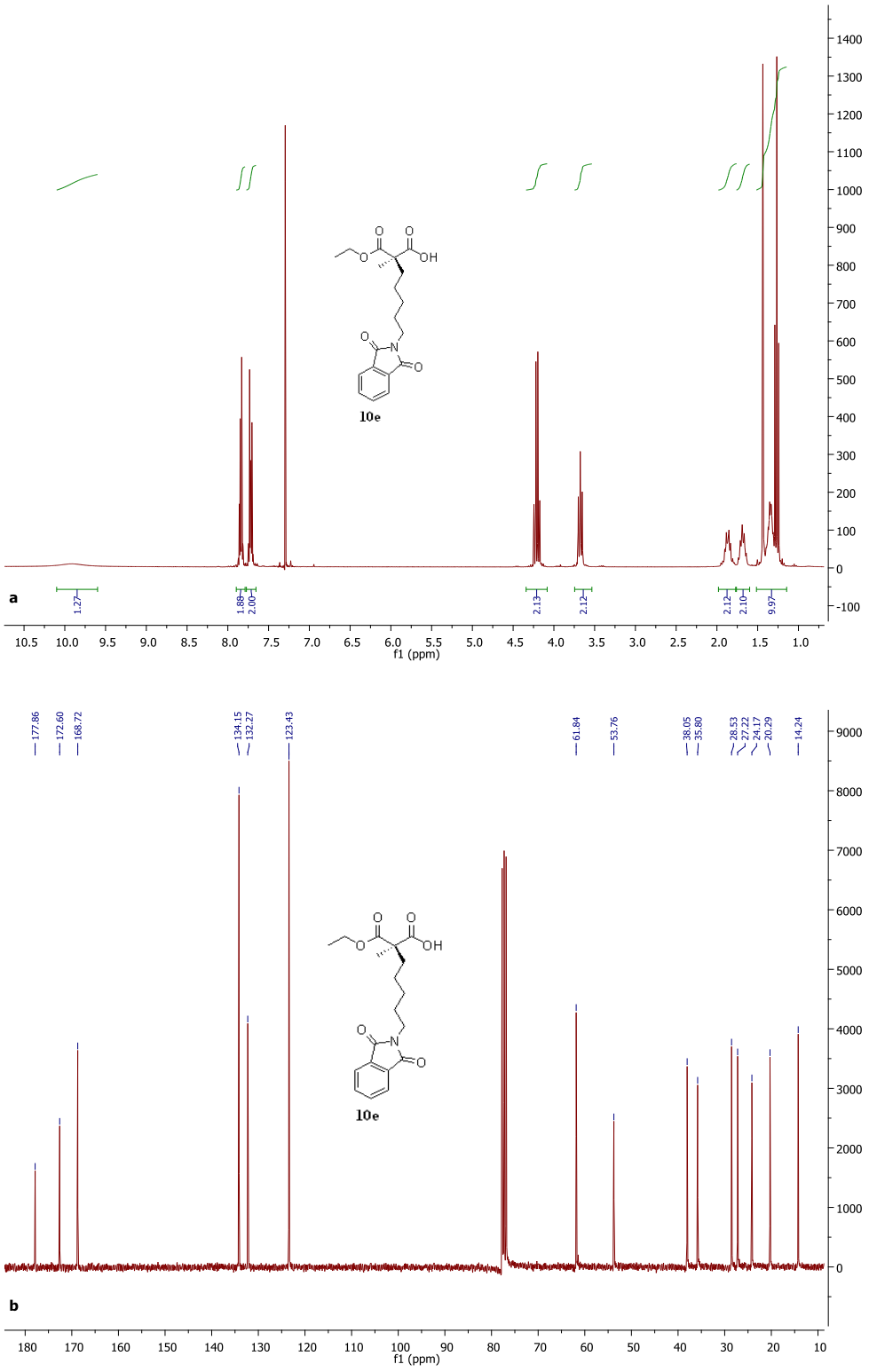
A. 8. a) ^{1}H NMR of **10b**, b) ^{13}C NMR of **10b**



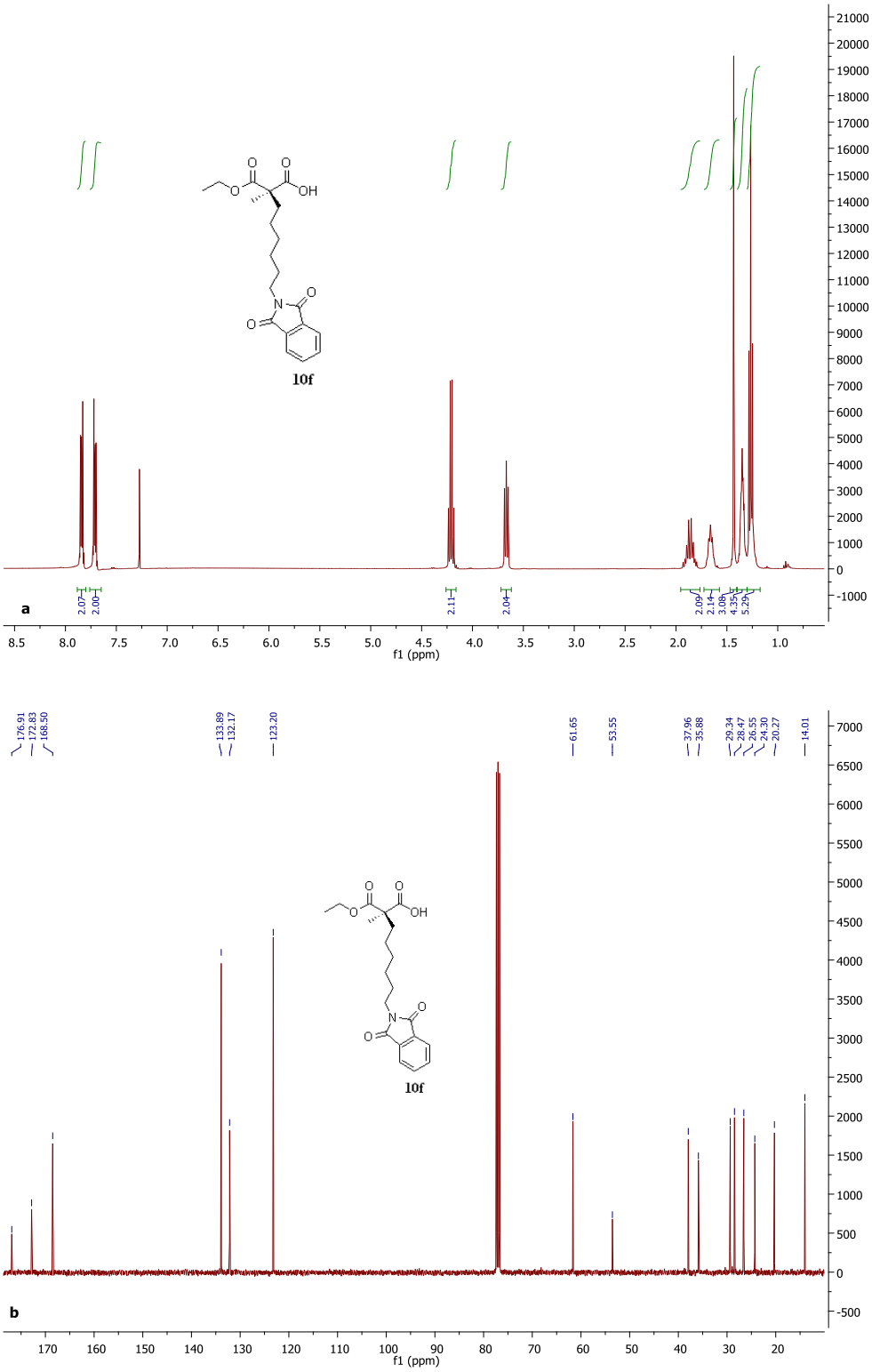
A. 9. a) ^{1}H NMR of **10c**, b) ^{13}C NMR of **10c**



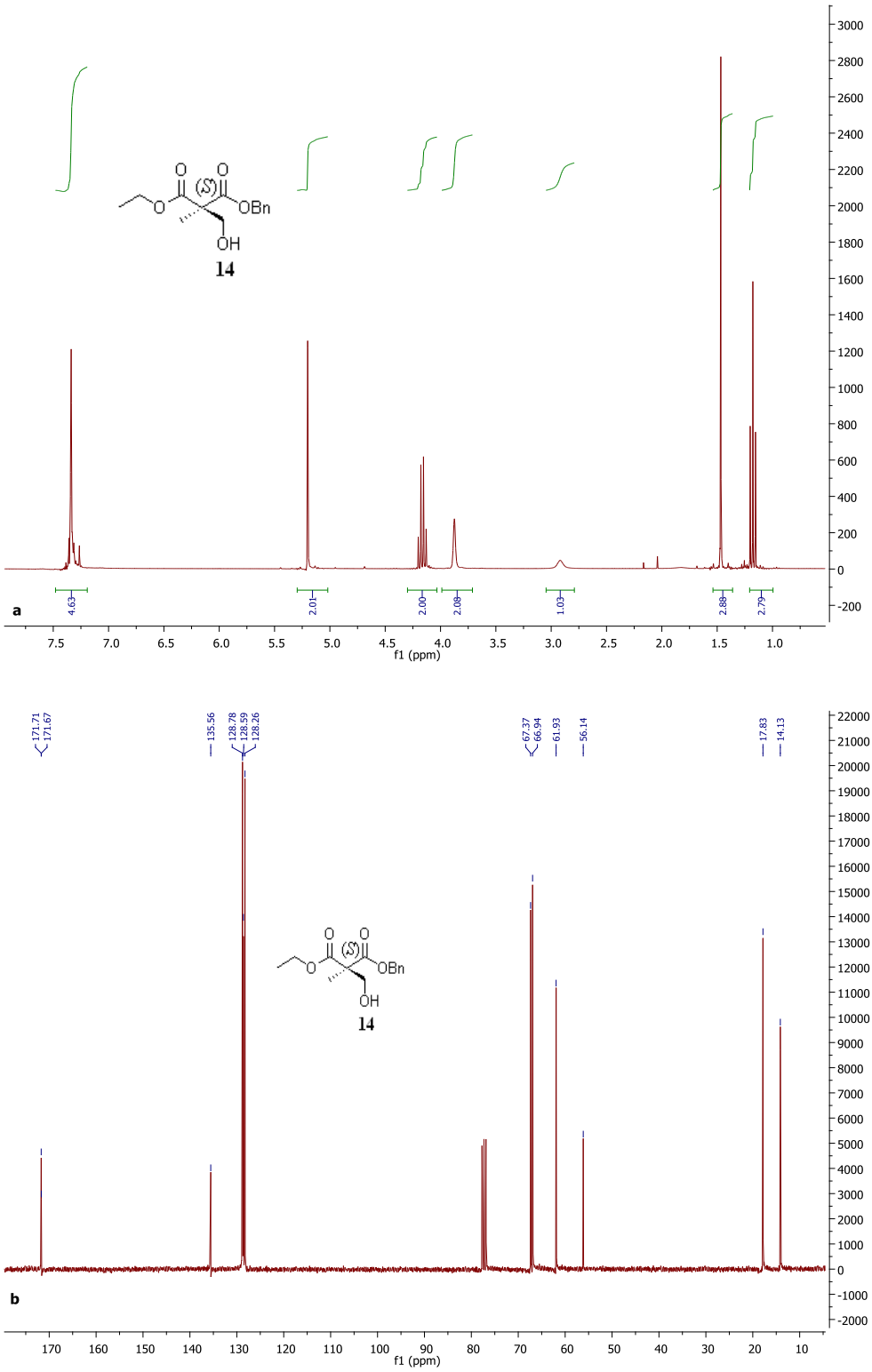
A. 10. a) ^{1}H NMR of **10d**, b) ^{13}C NMR of **10d**



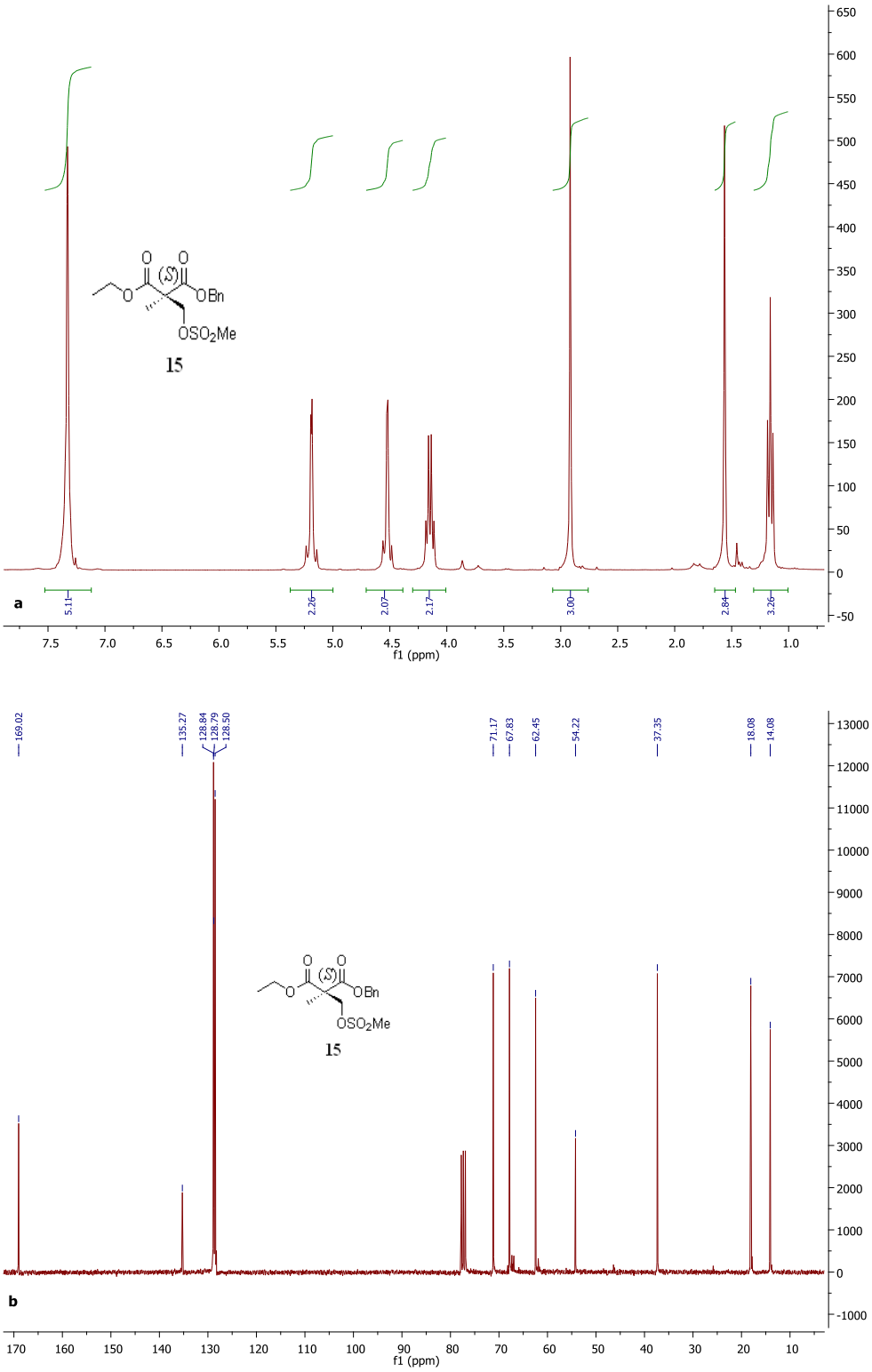
A. 11. a) ^{1}H NMR of **10e**, b) ^{13}C NMR of **10e**



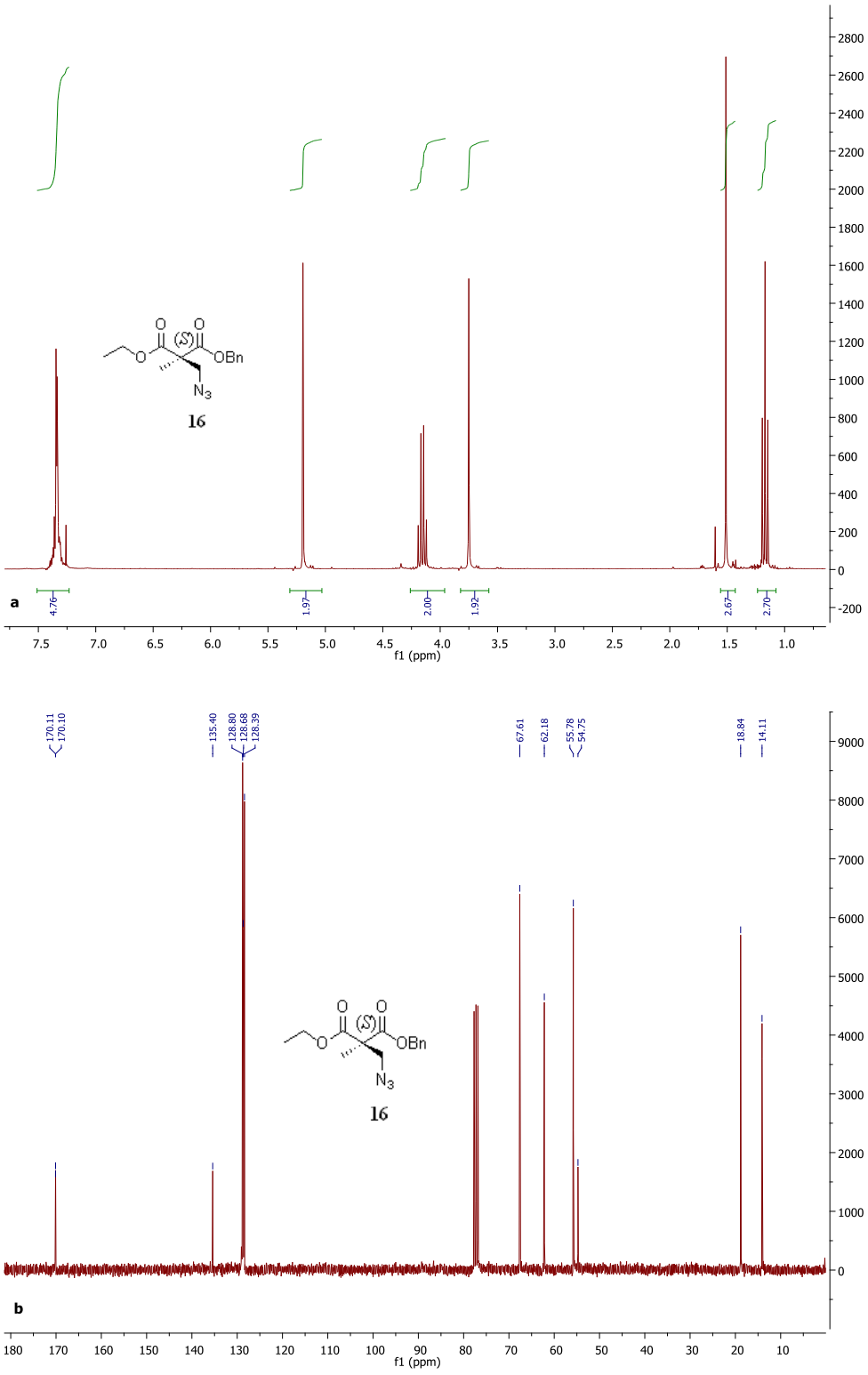
A. 12. a) ^{1}H NMR of **10f**, b) ^{13}C NMR of **10f**



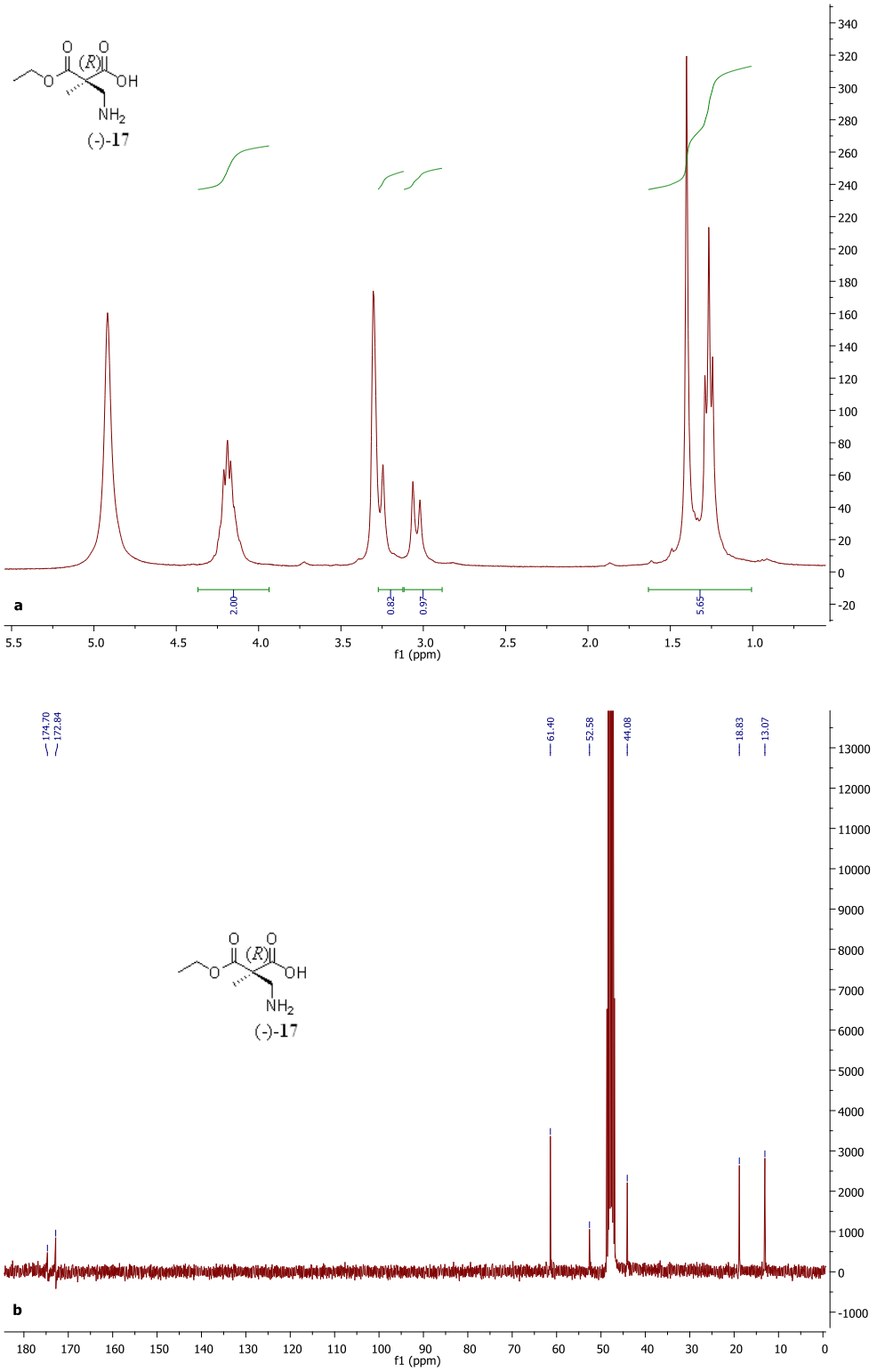
A. 13. a) ^{1}H NMR of **14**, b) ^{13}C NMR of **14**



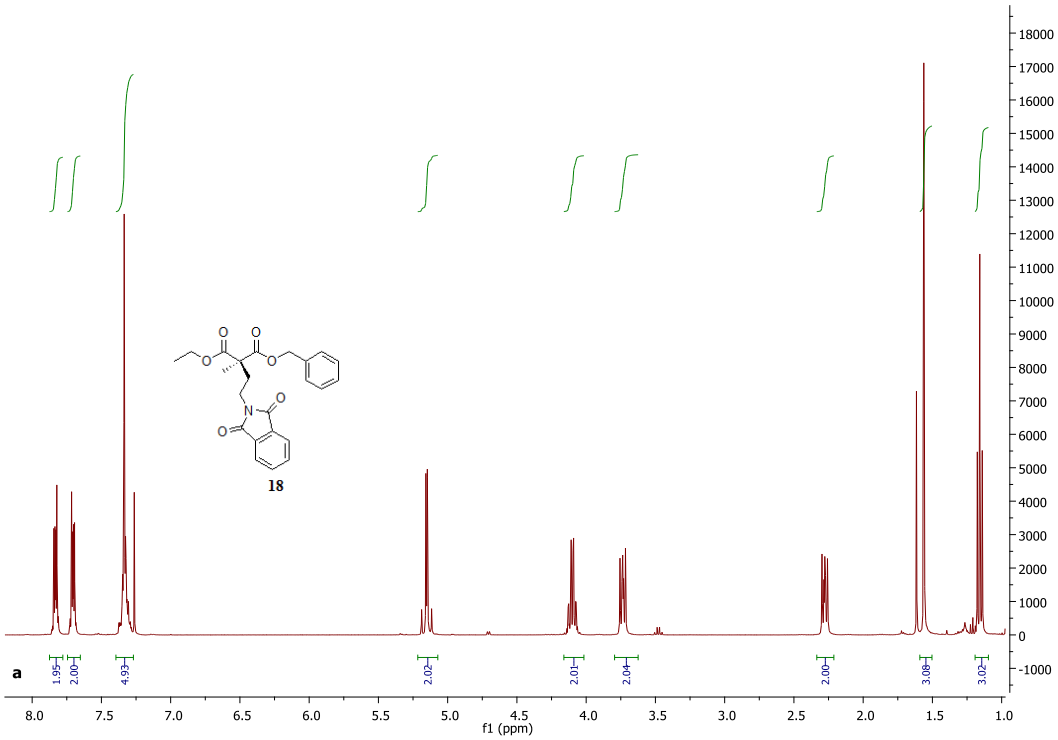
A. 14. a) ^{1}H NMR of **15**, b) ^{13}C NMR of **15**

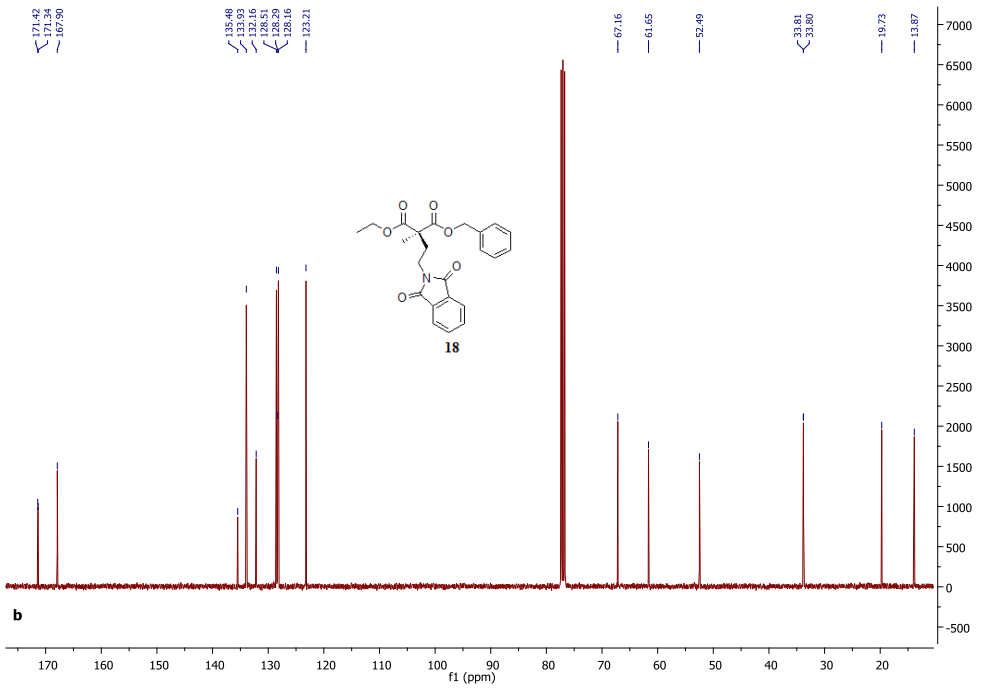


A. **15**. a) ^{1}H NMR of **16**, b) ^{13}C NMR of **16**

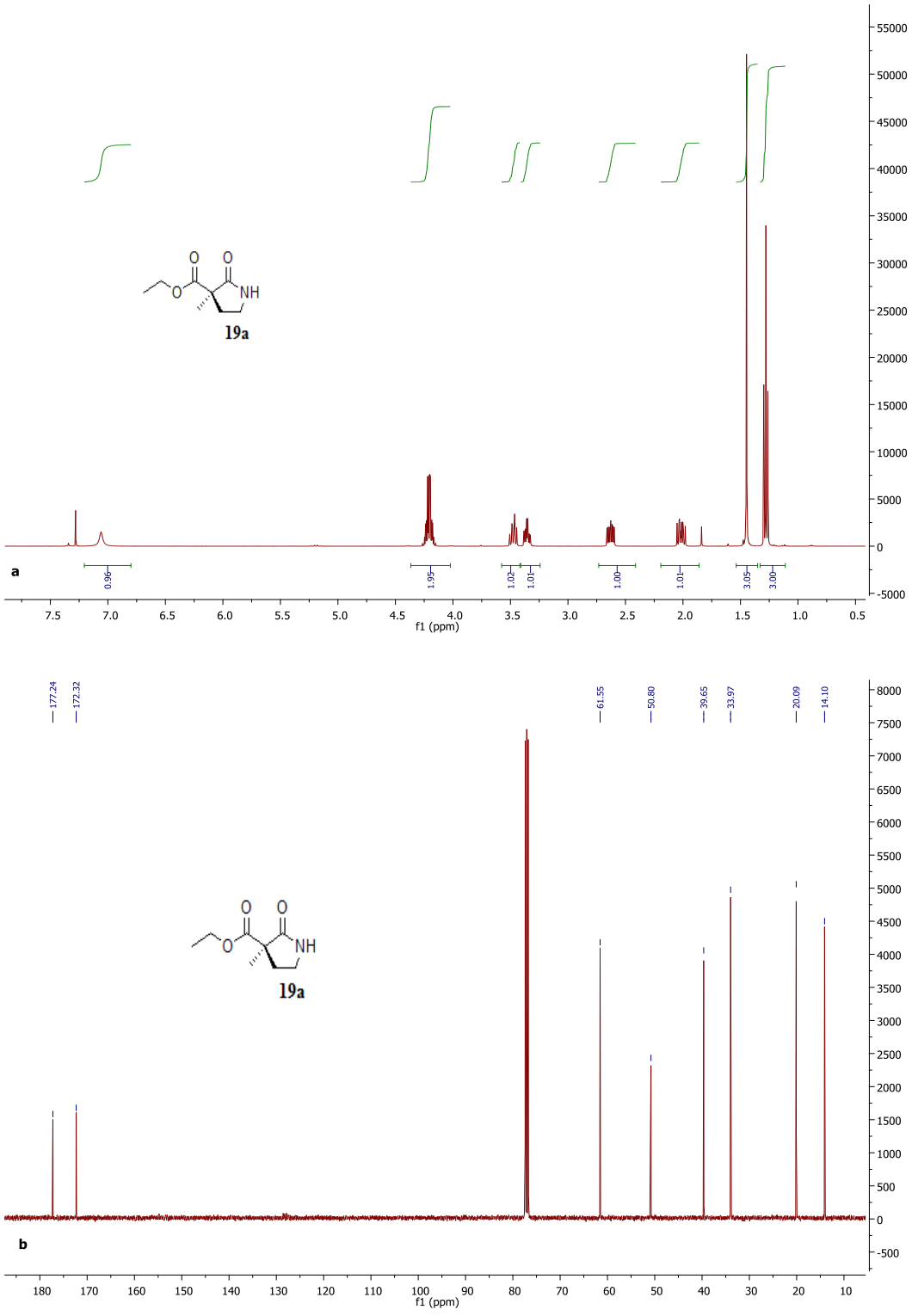


A. 16. a) ^{1}H NMR of **17**, b) ^{13}C NMR of **17**

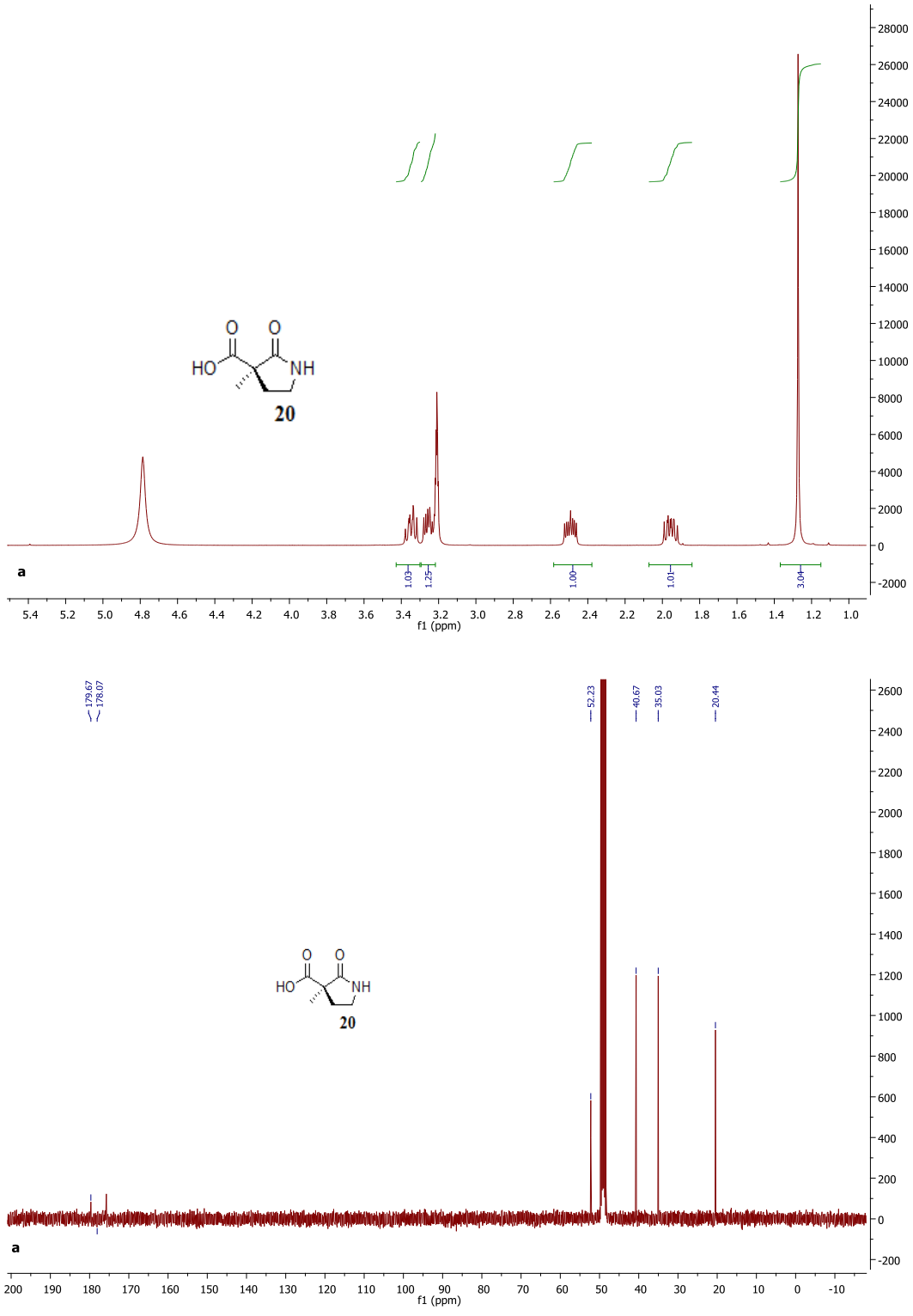




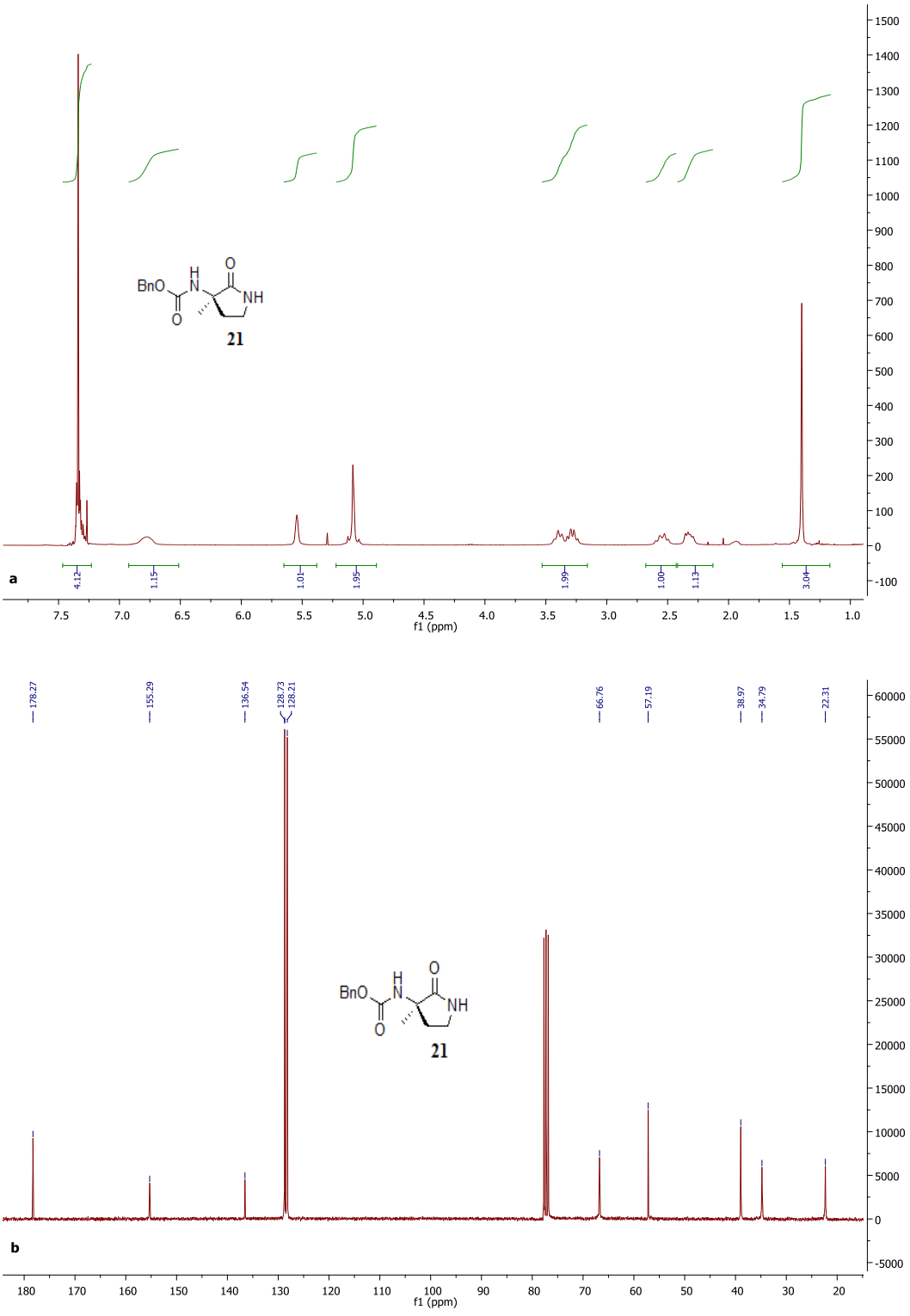
A. 17. a) ^{1}H NMR of **18**, b) ^{13}C NMR of **18**



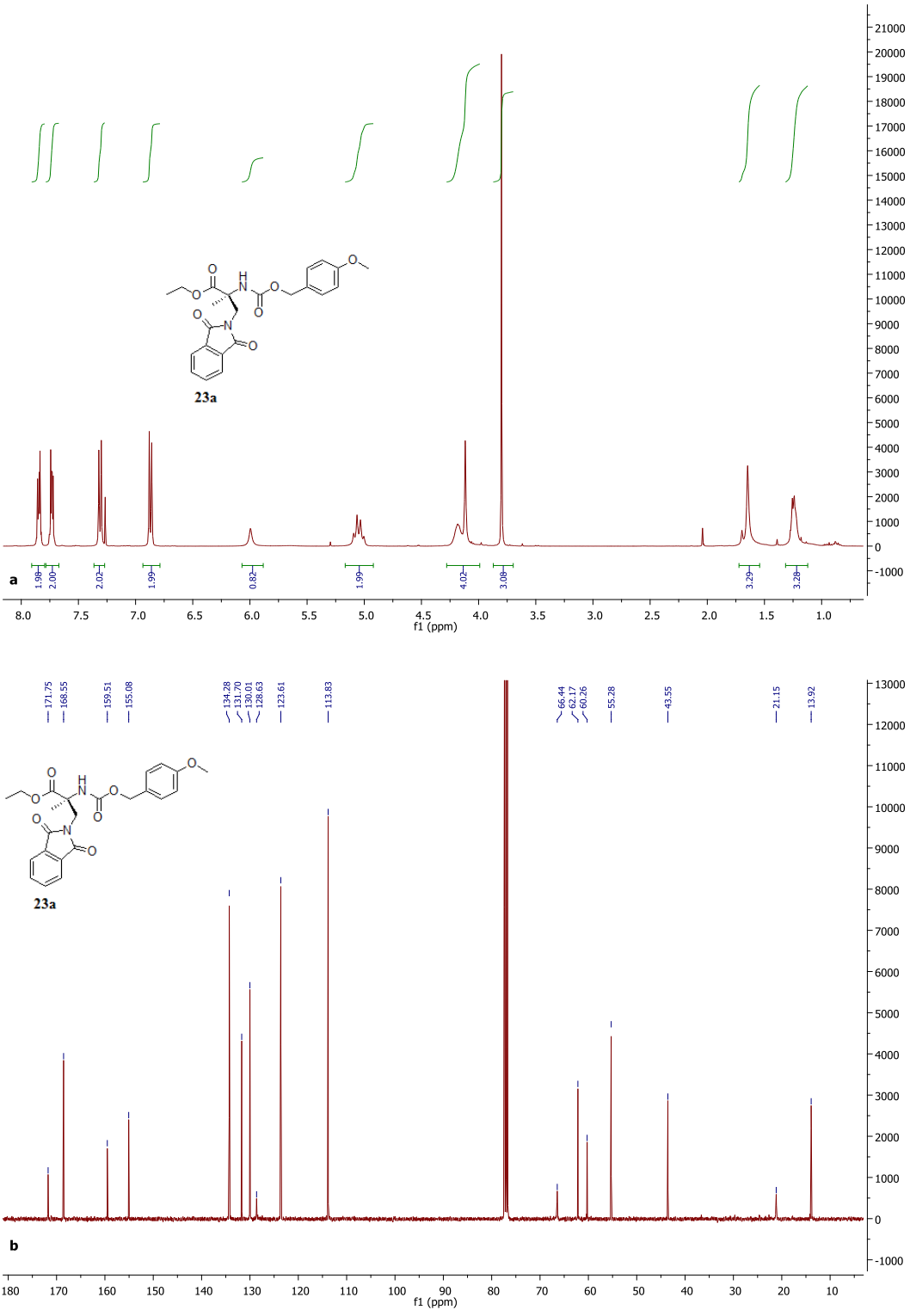
A. 18. a) ^{1}H NMR of **19a**, b) ^{13}C NMR of **19b**



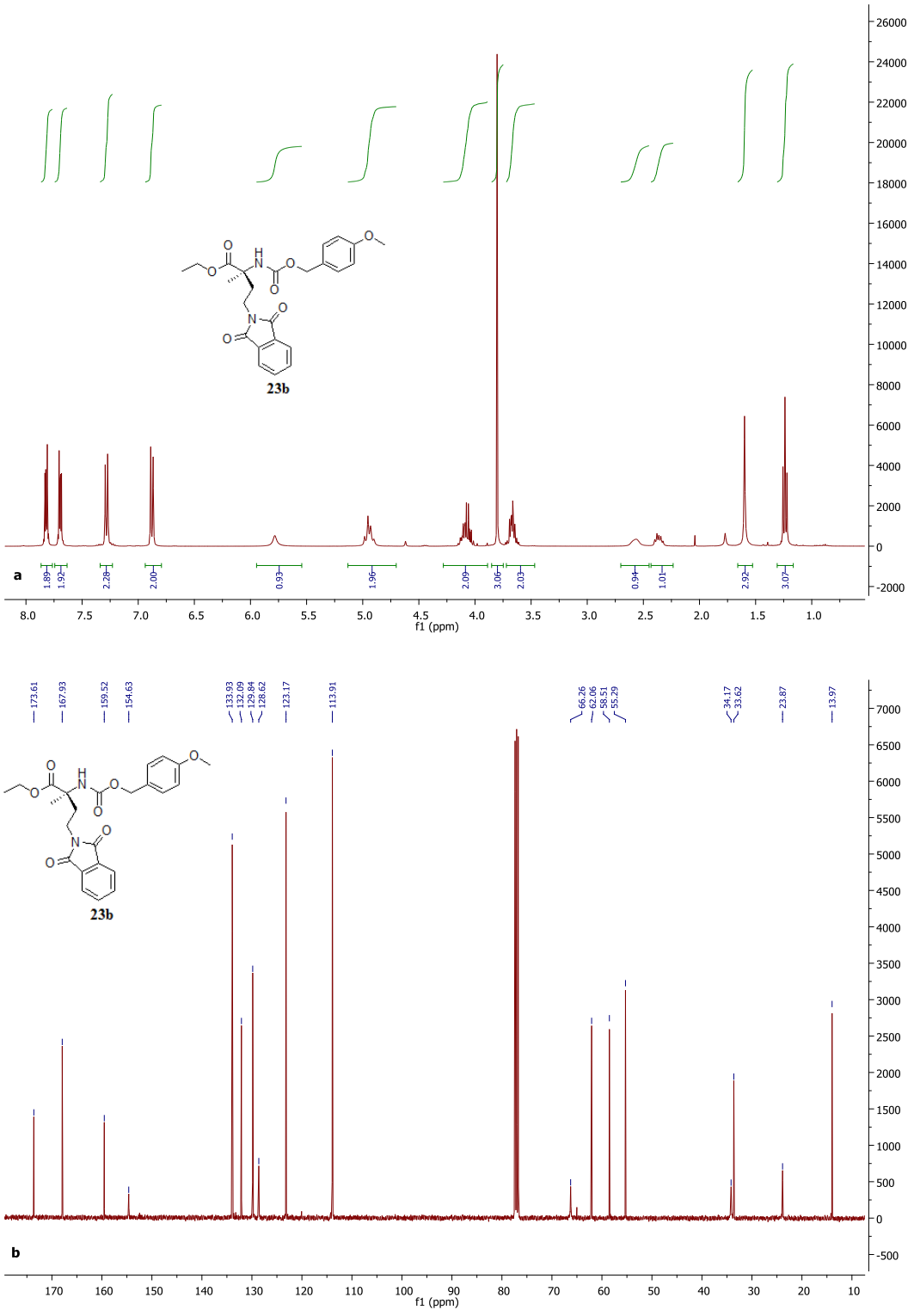
A. 18. a) ^{1}H NMR of **20**, b) ^{13}C NMR of **20**



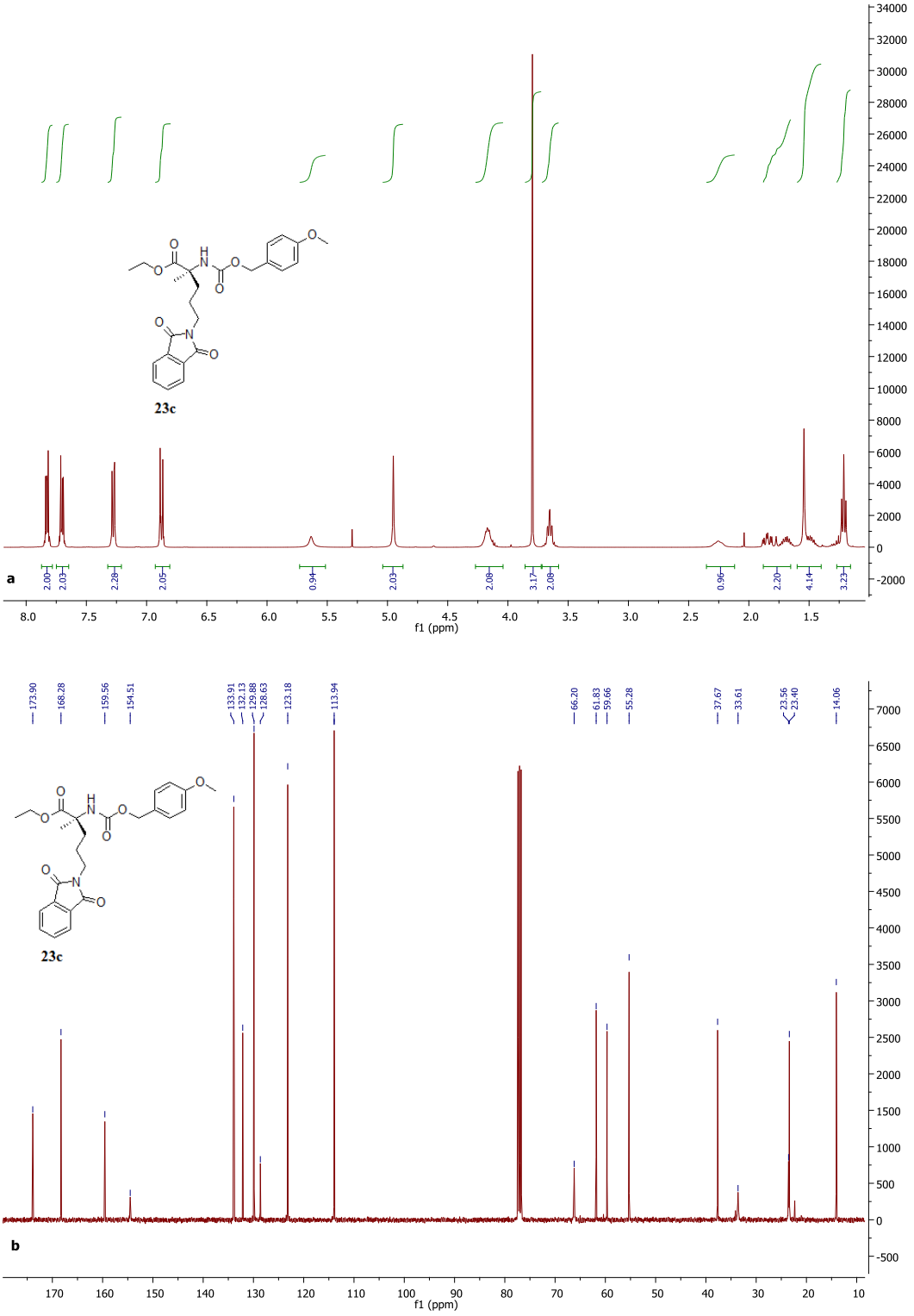
A. 19. a) ^{1}H NMR of **21**, b) ^{13}C NMR of **21**



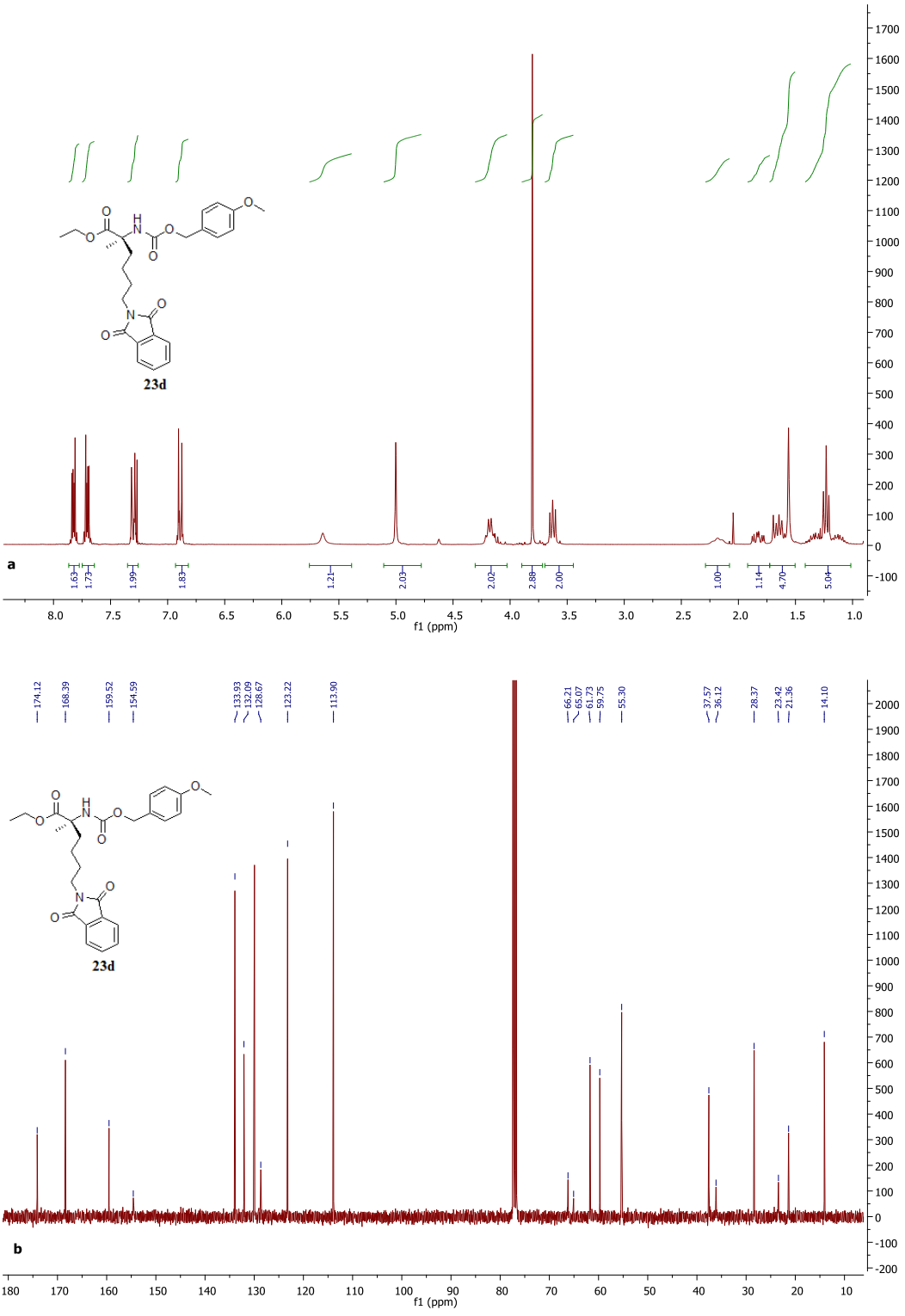
A. 20. a) ^{1}H NMR of **23a**, b) ^{13}C NMR of **23a**



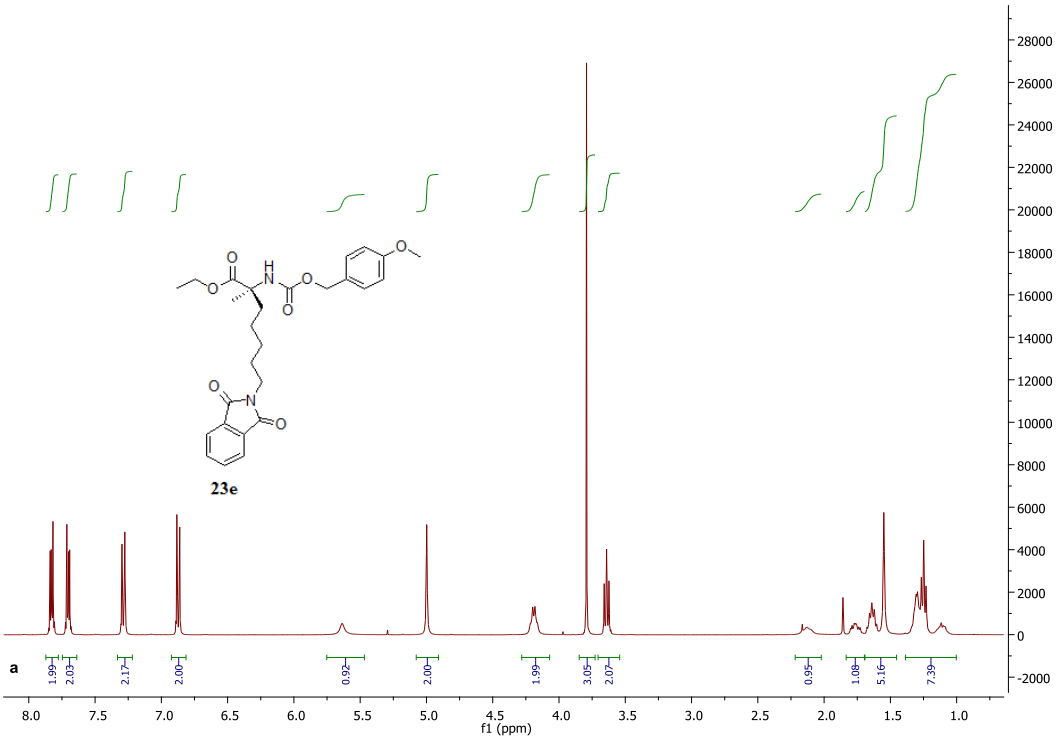
A. 21. a) 1H NMR of **23b,** b) 13C NMR of **23b**

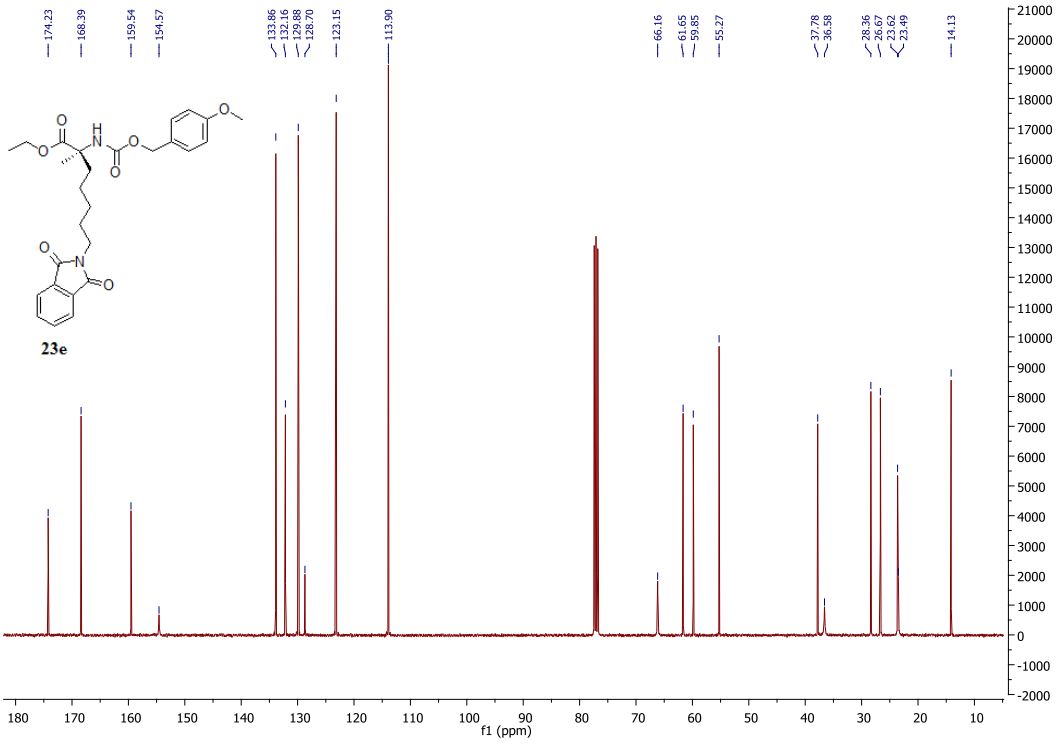


A. 22. a) 1H NMR of **23c,** b) 13C NMR of **23c**

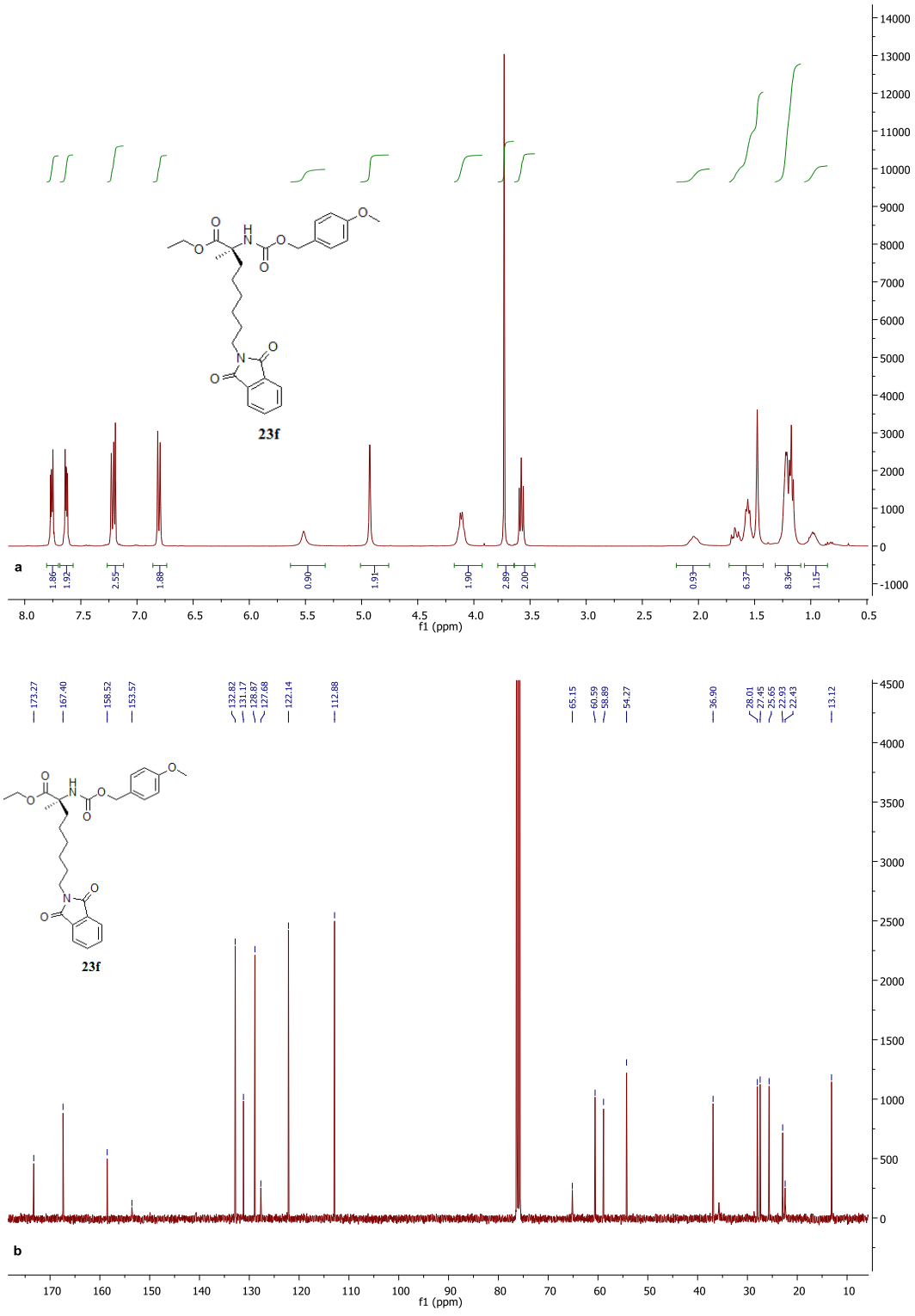


A. 23. a) ^{1}H NMR of **23d**, b) ^{13}C NMR of **23d**

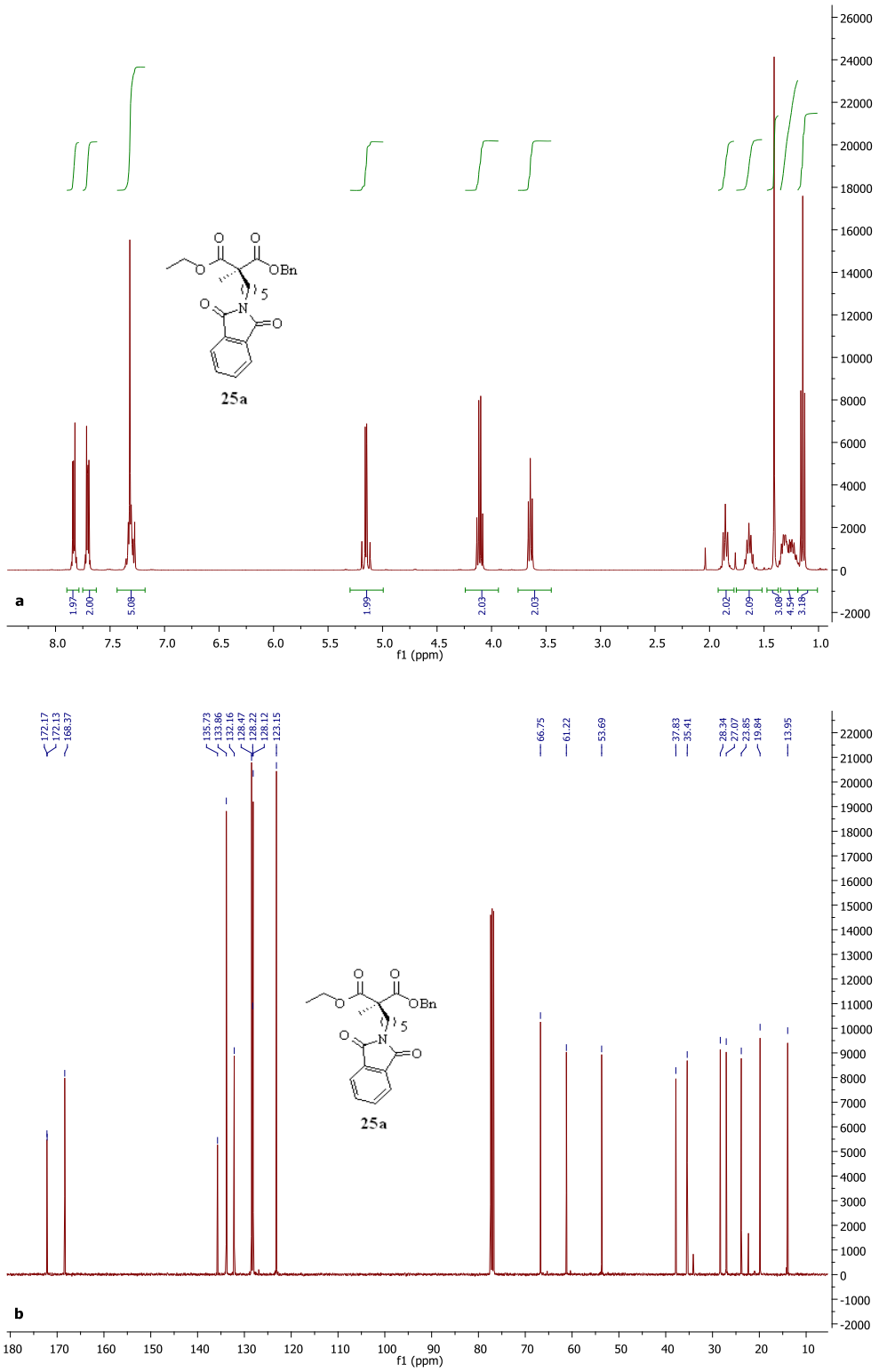




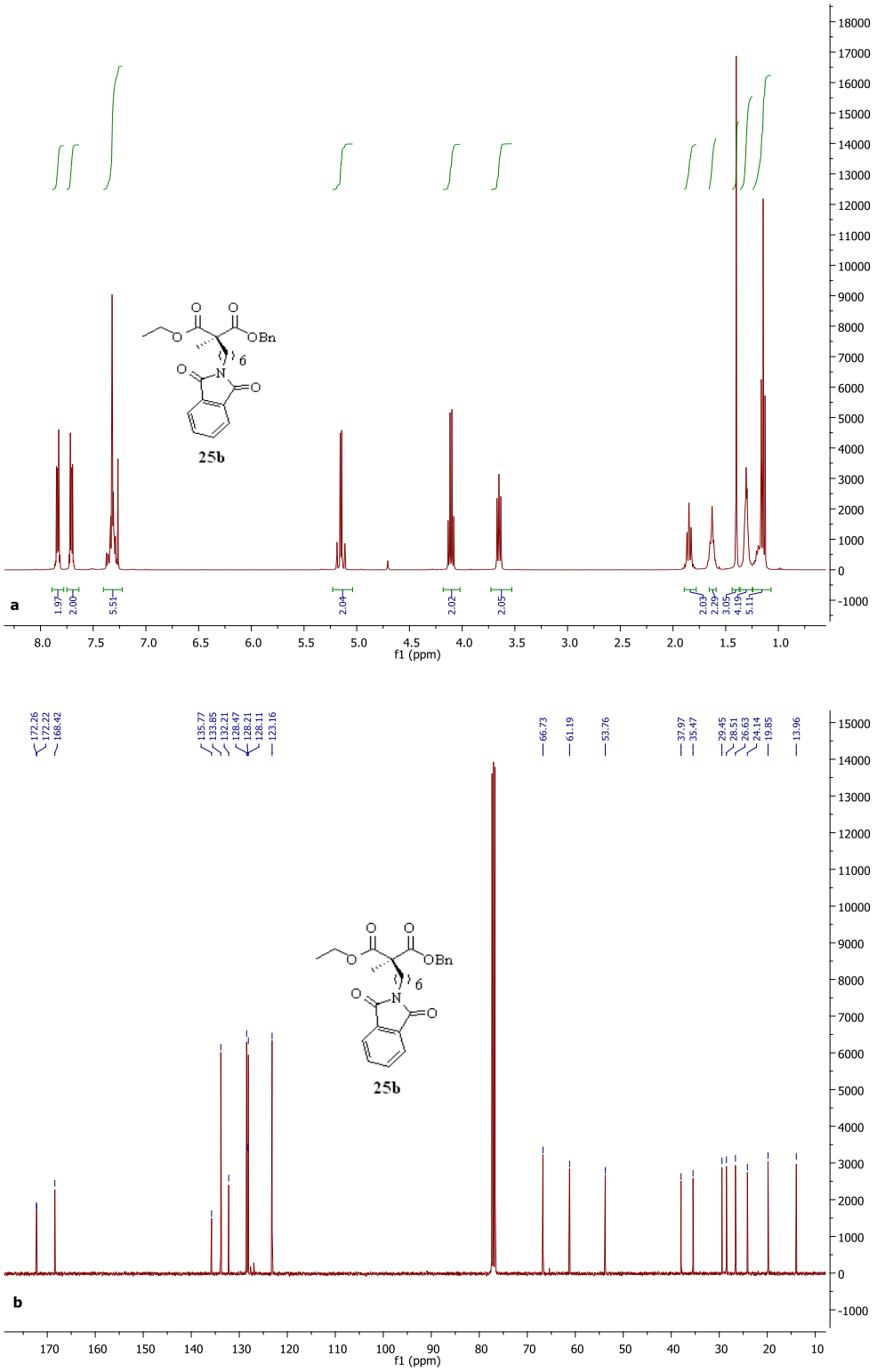
A. 24. a) ^{1}H NMR of **23e**, b) ^{13}C NMR of **23e**



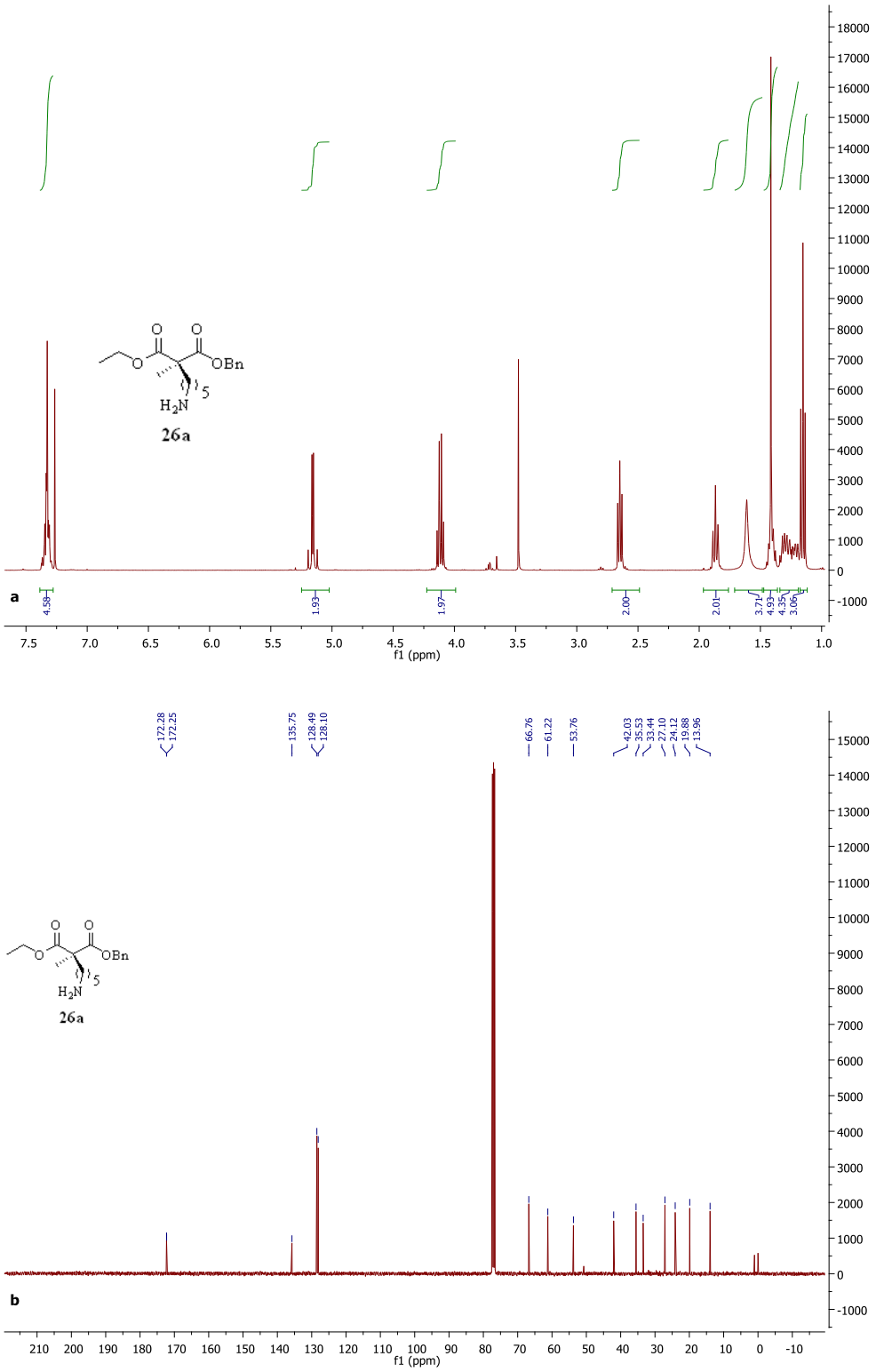
A. 25. a) ^{1}H NMR of **23f**, b) ^{13}C NMR of **23f**



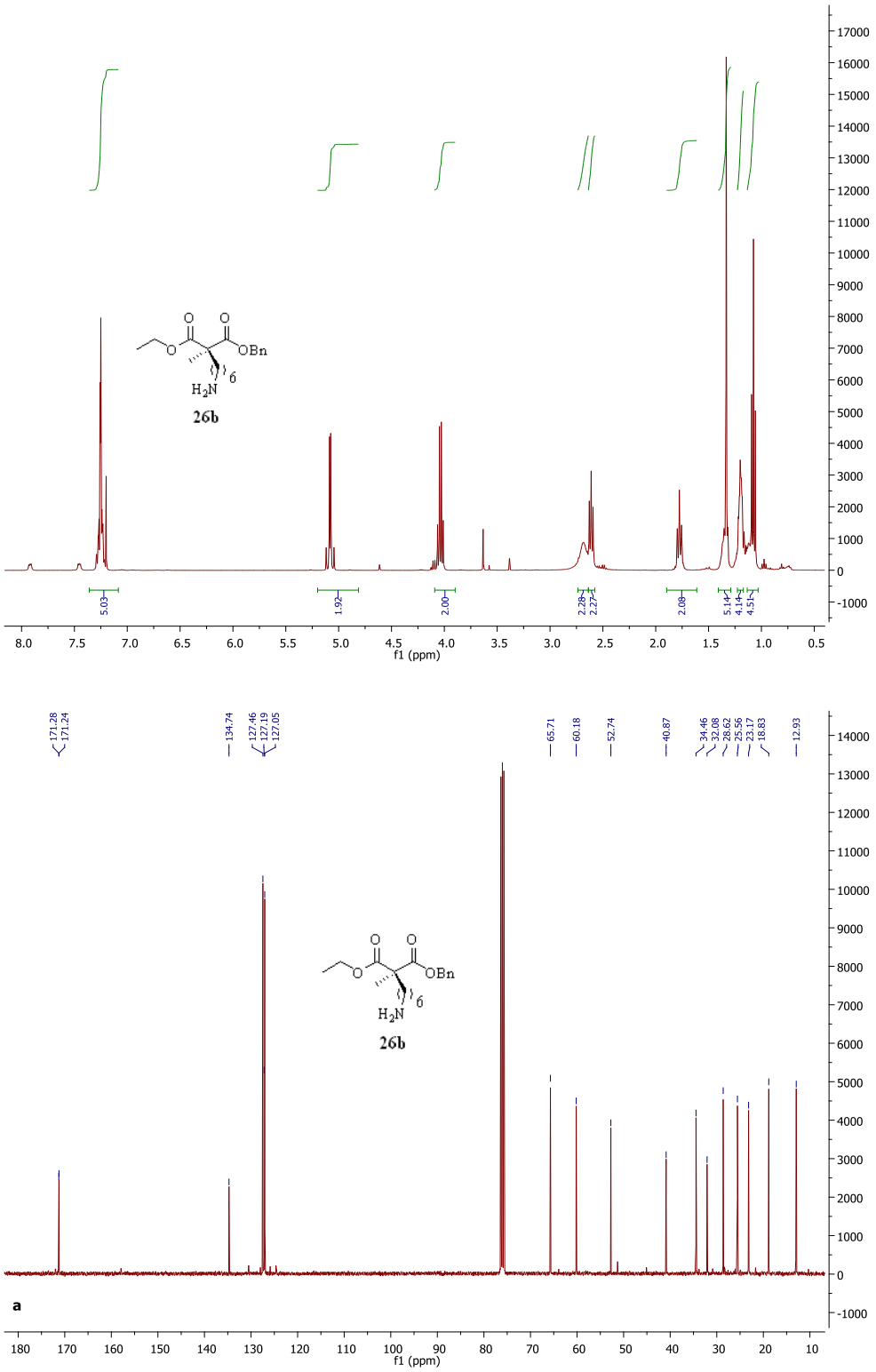
A .26. a) ^{1}H NMR of **25a**, b) ^{13}C NMR of **25a**



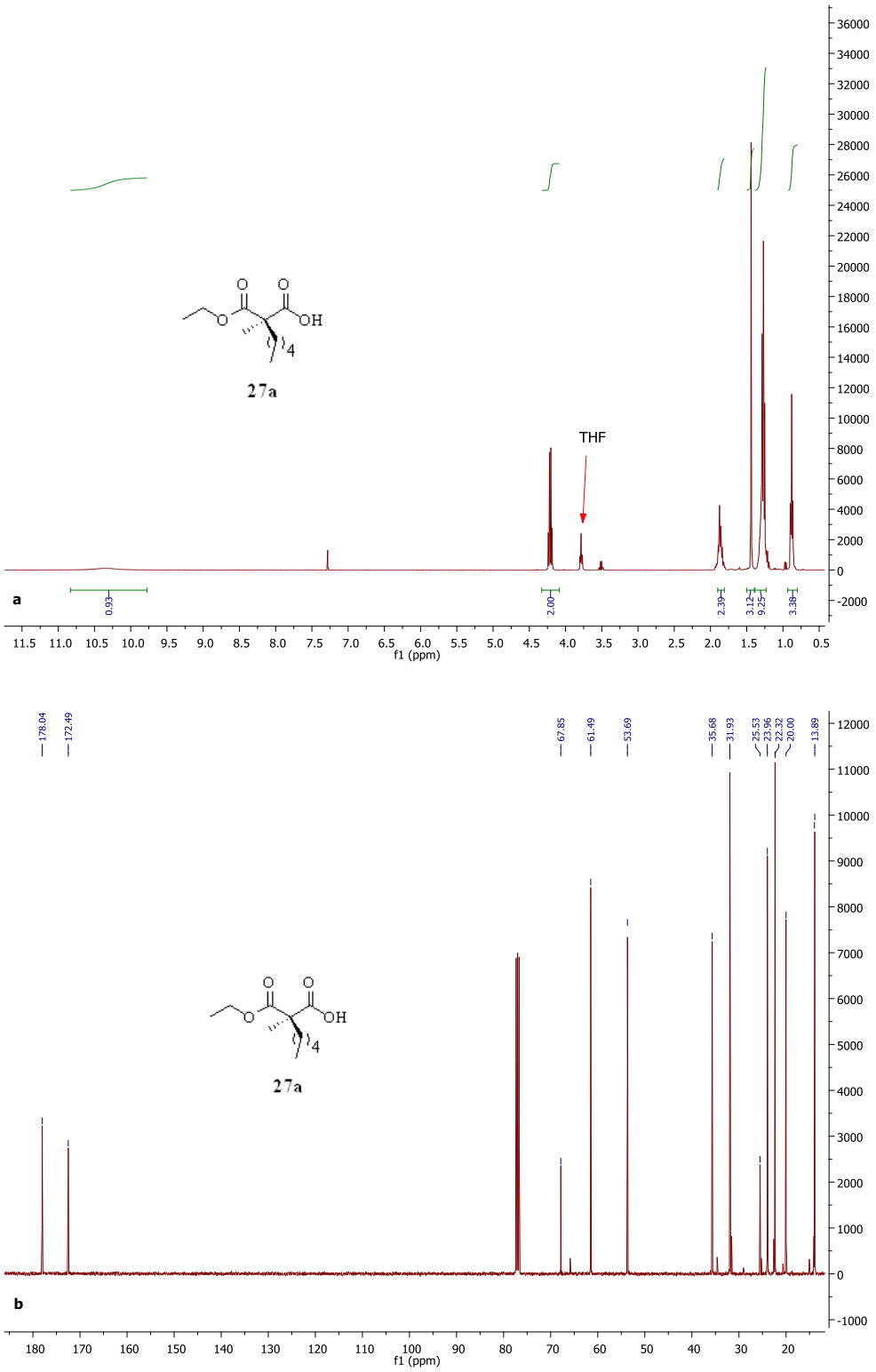
A . 27. a) ^{1}H NMR of **25b**, b) ^{13}C NMR of **25b**



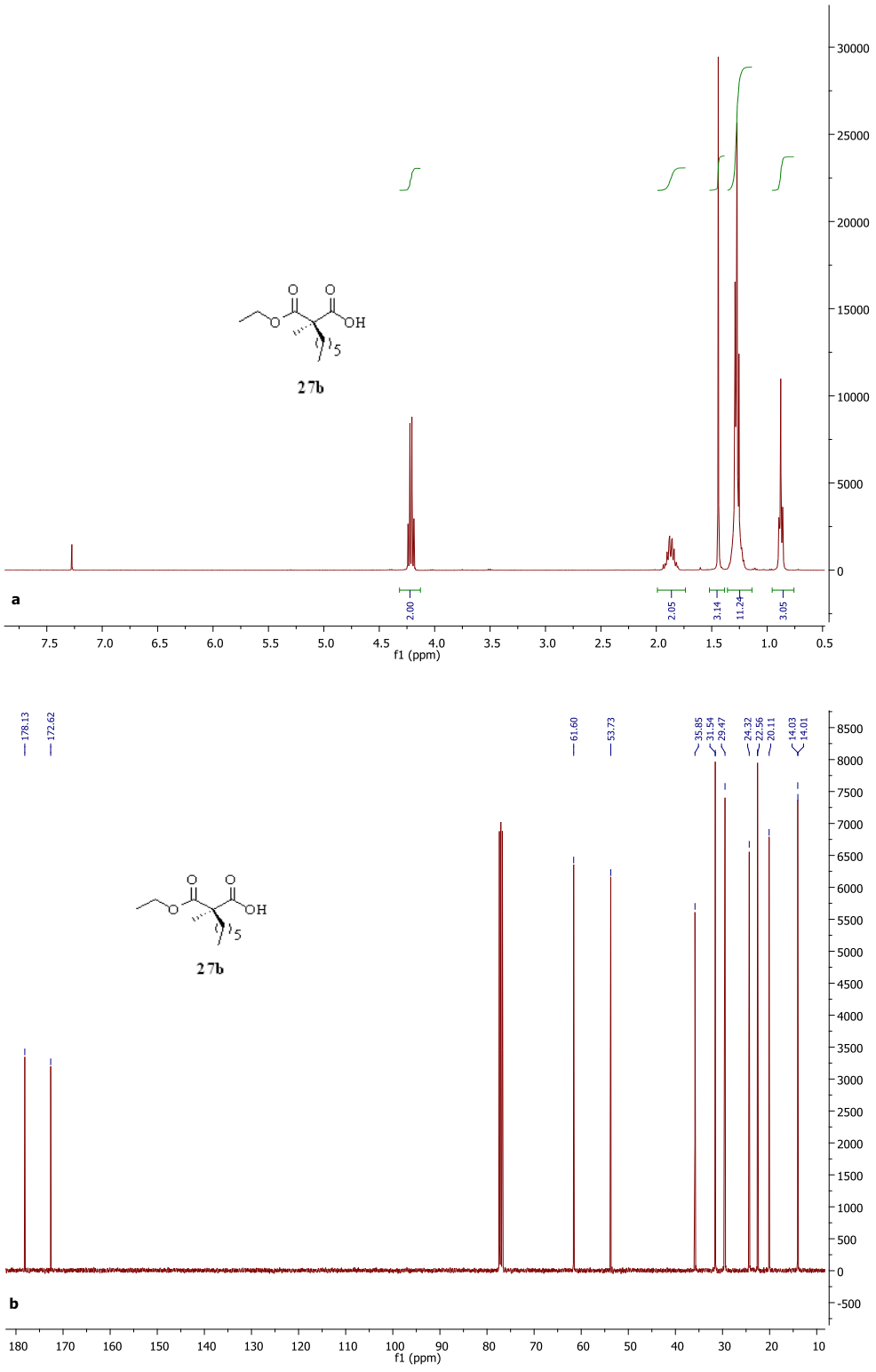
A .28. a) ^{1}H NMR of **26a**, b) ^{13}C NMR of **26a**



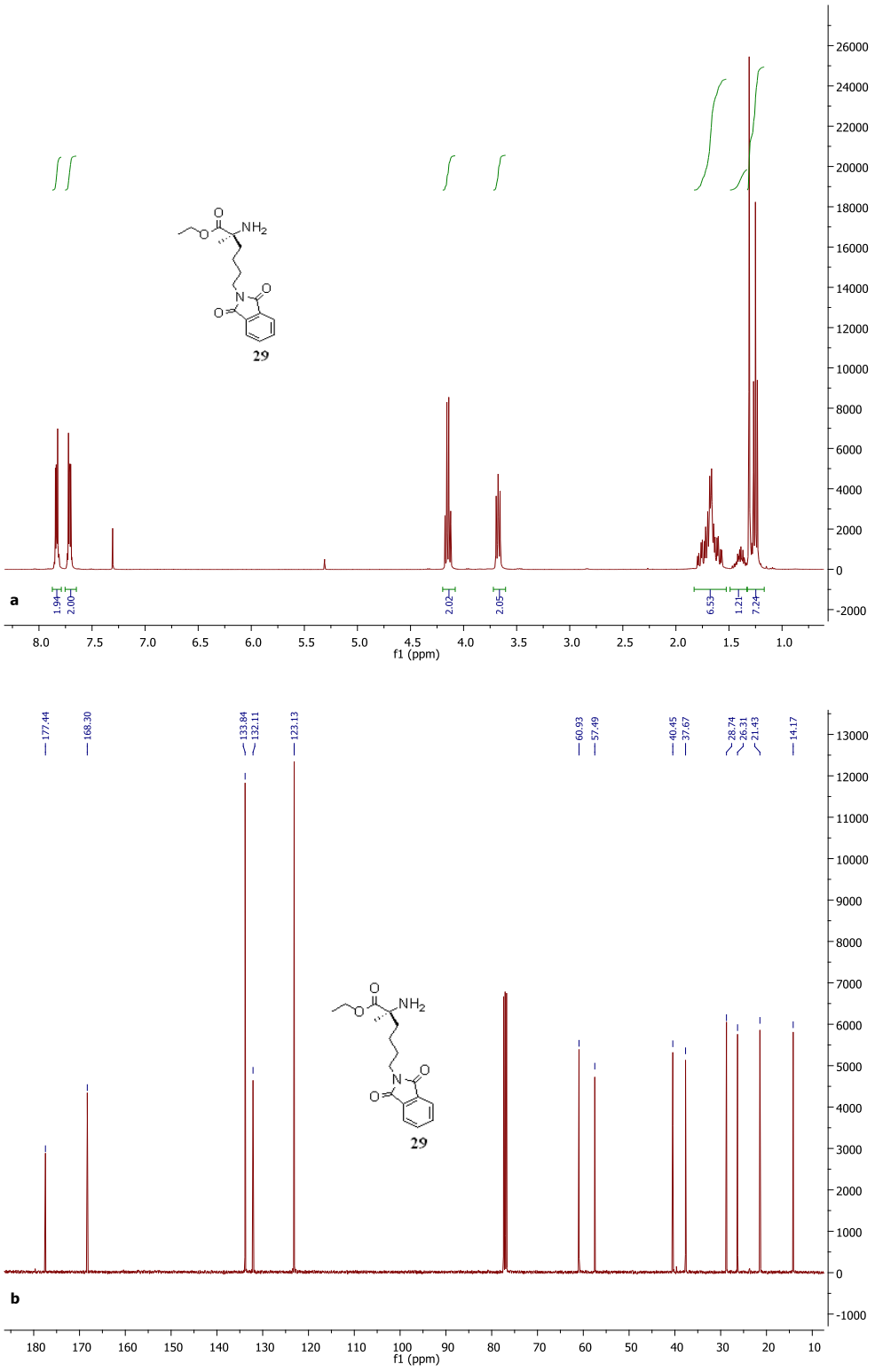
A .29. a) ^{1}H NMR of **26b**, b) ^{13}C NMR of **26b**



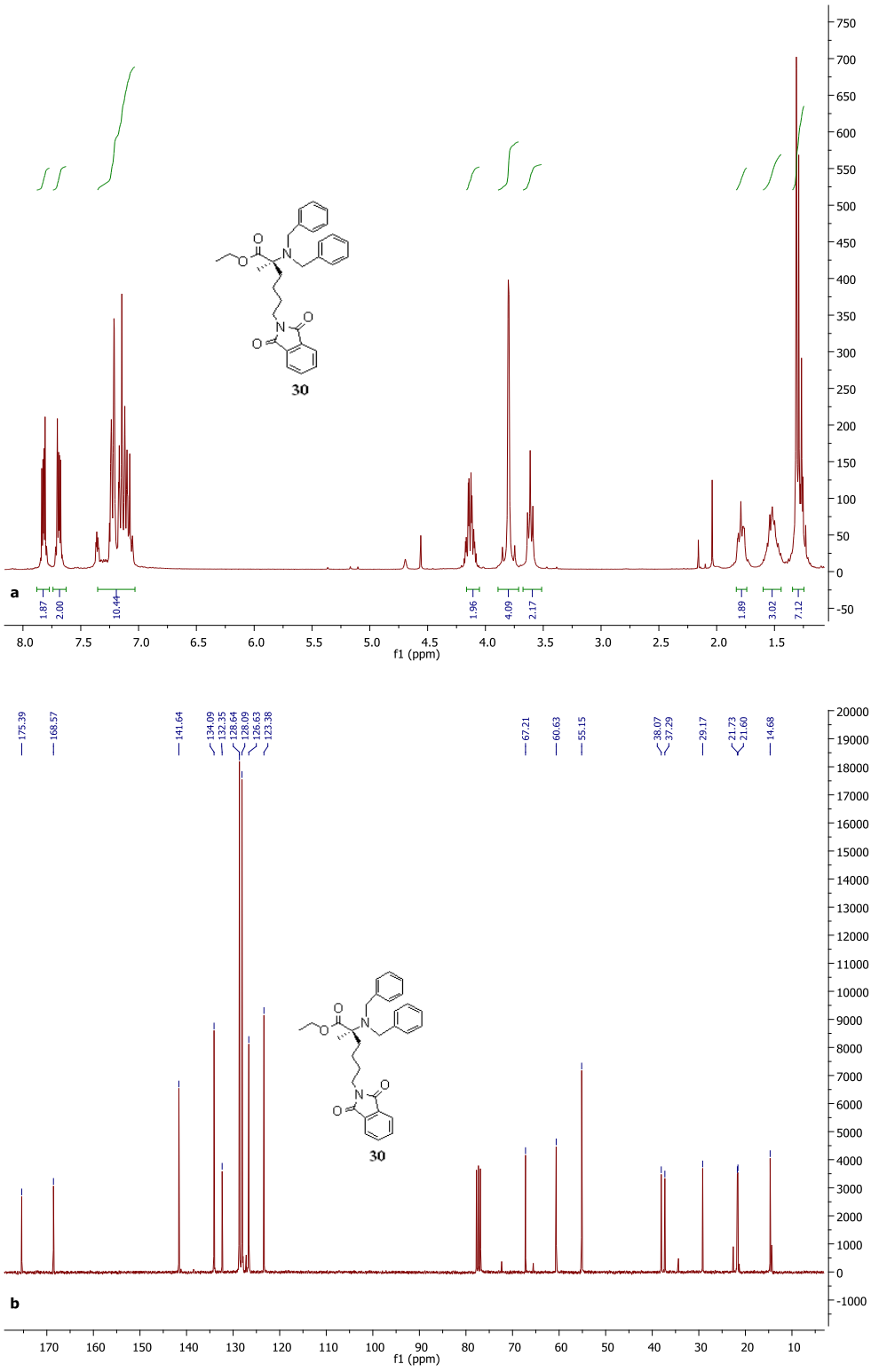
A .30. a) ^{1}H NMR of **27a**, b) ^{13}C NMR of **27a**



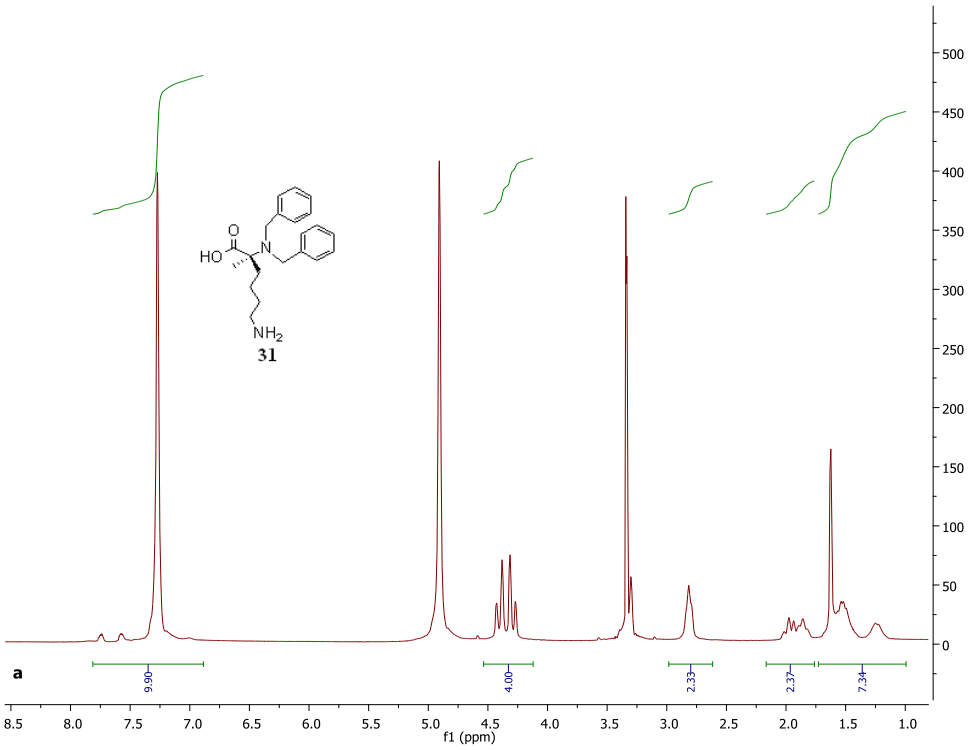
A.31. a) ^{1}H NMR of **27b**, b) ^{13}C NMR of **27b**



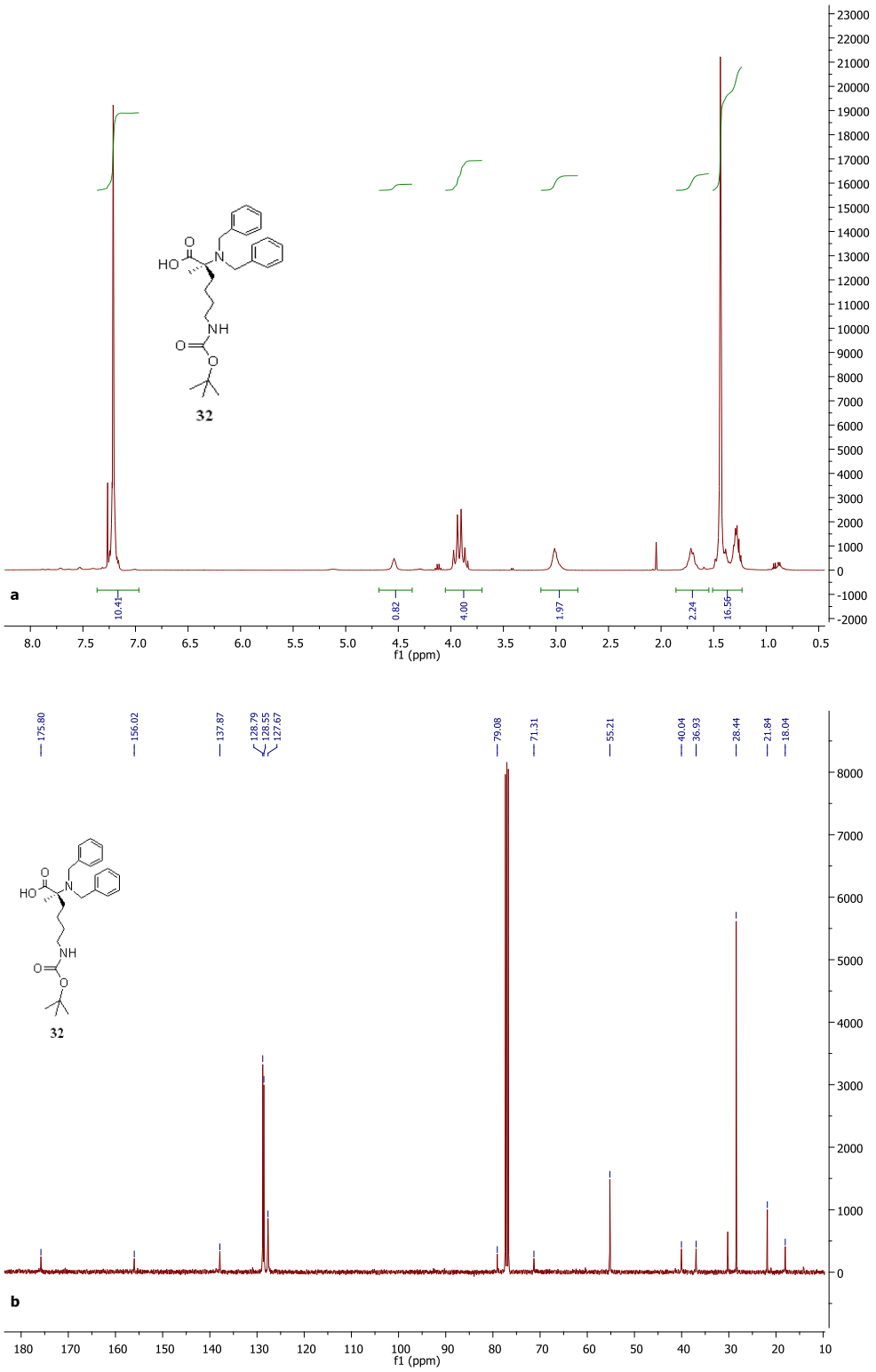
A .32. a) ^{1}H NMR of **29**, b) ^{13}C NMR of **29**



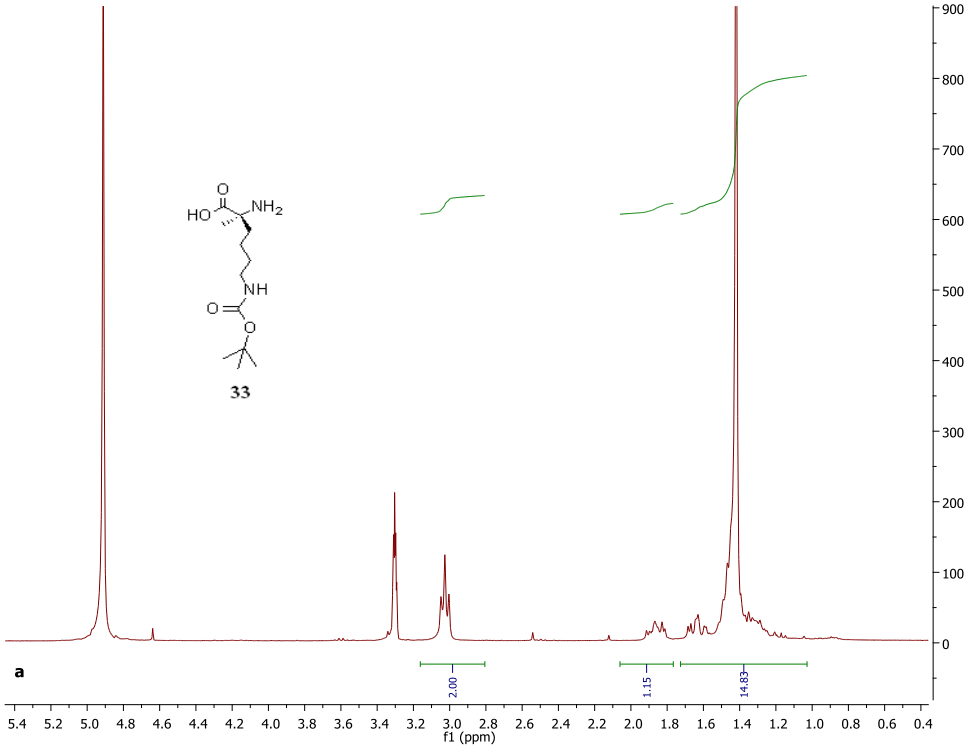
A .33. a) ^{1}H NMR of **30**, b) ^{13}C NMR of **30**



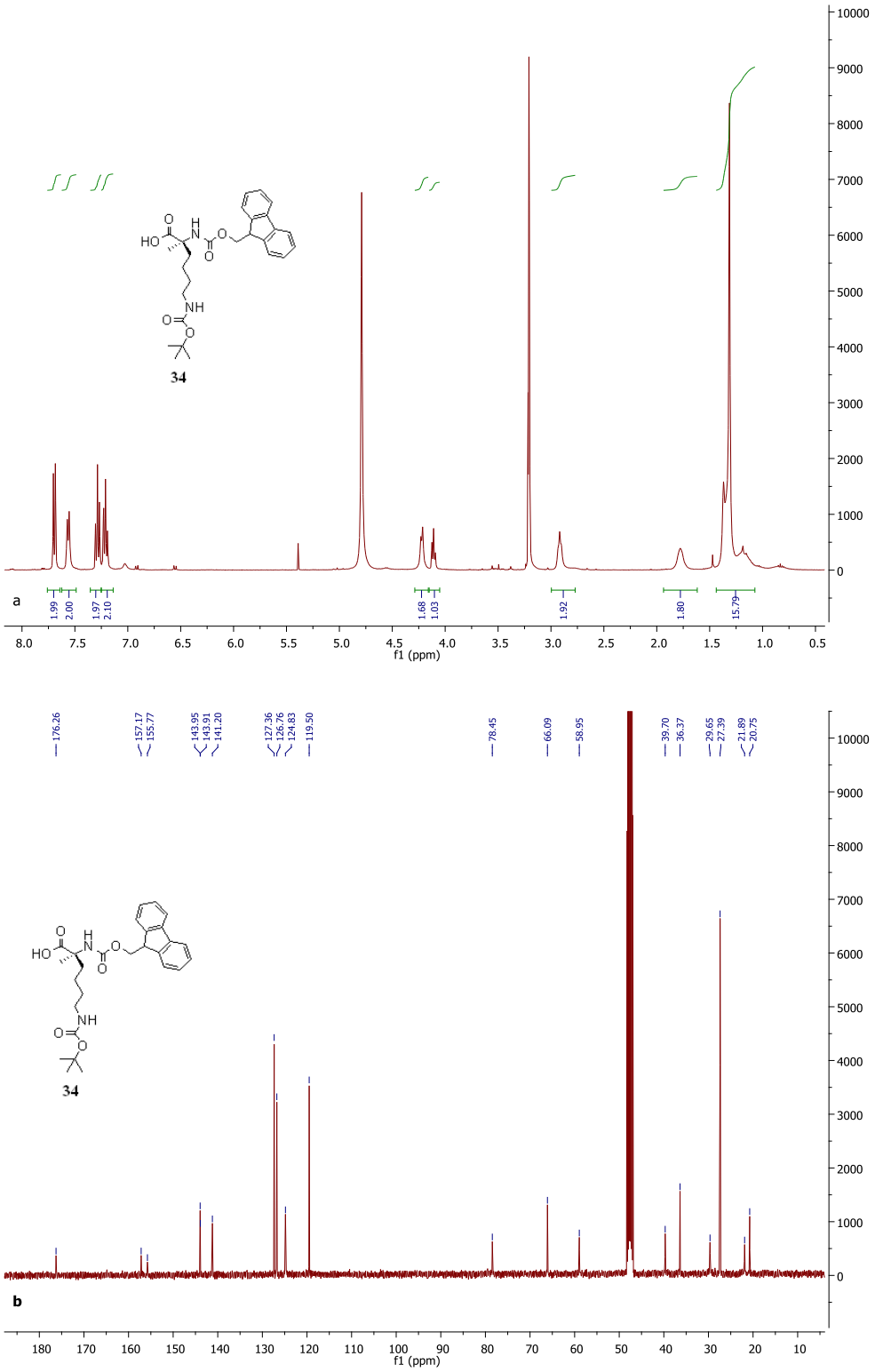
A .34. a) ^{1}H NMR of **31**



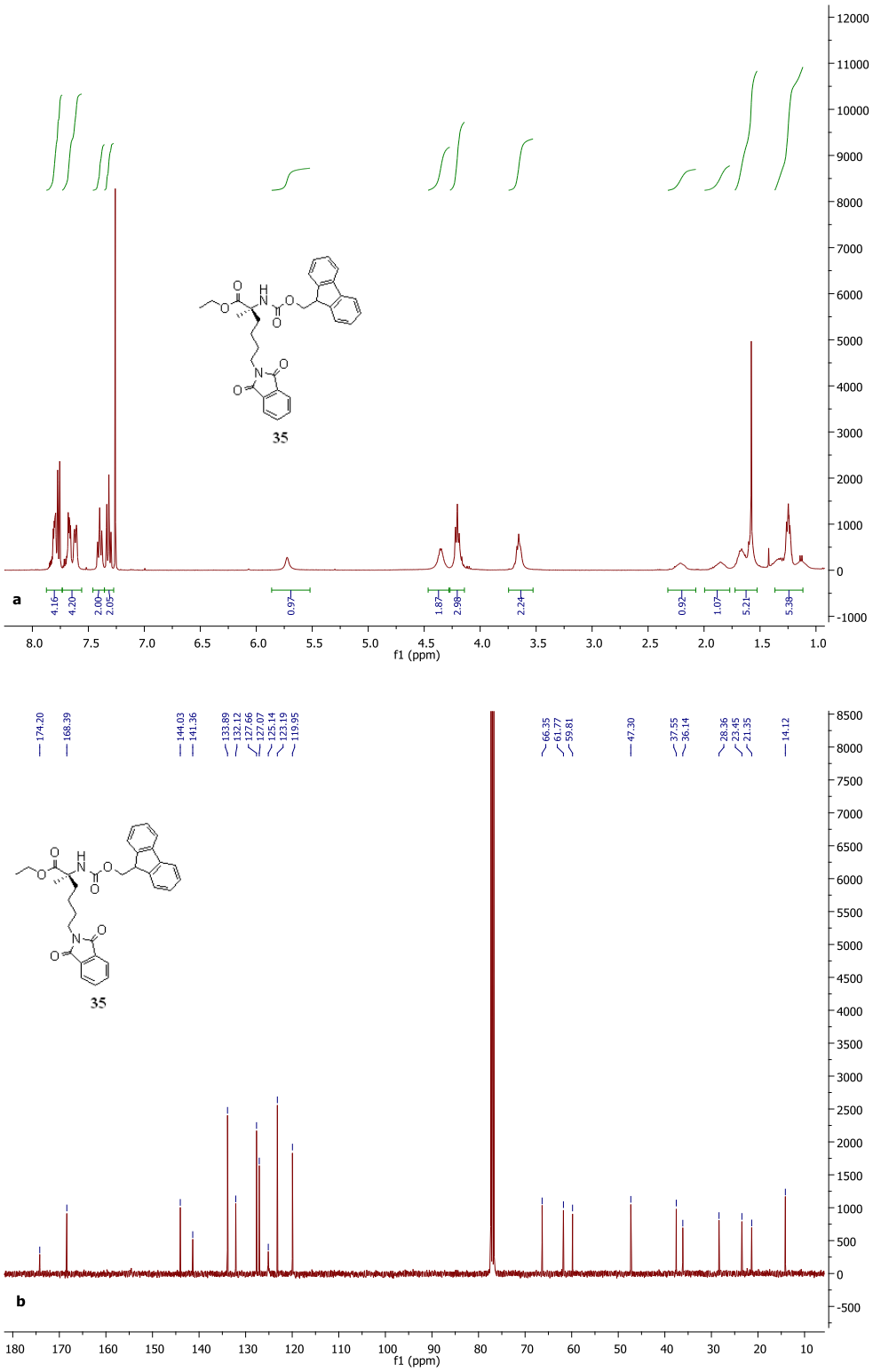
A .35. a) ^{1}H NMR of **32**, b) ^{13}C NMR of **32**



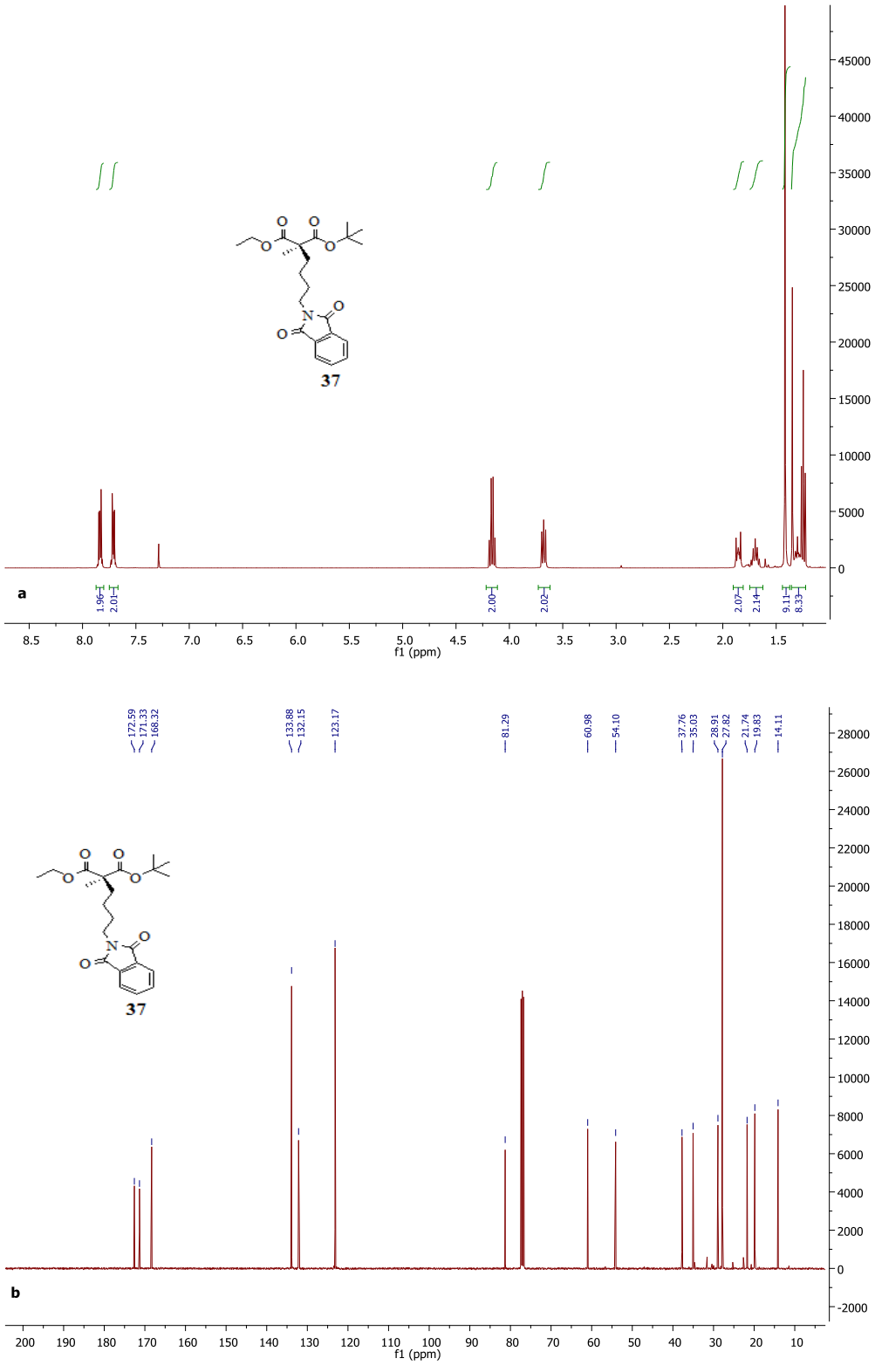
A.36. a) ^{1}H NMR of **33**



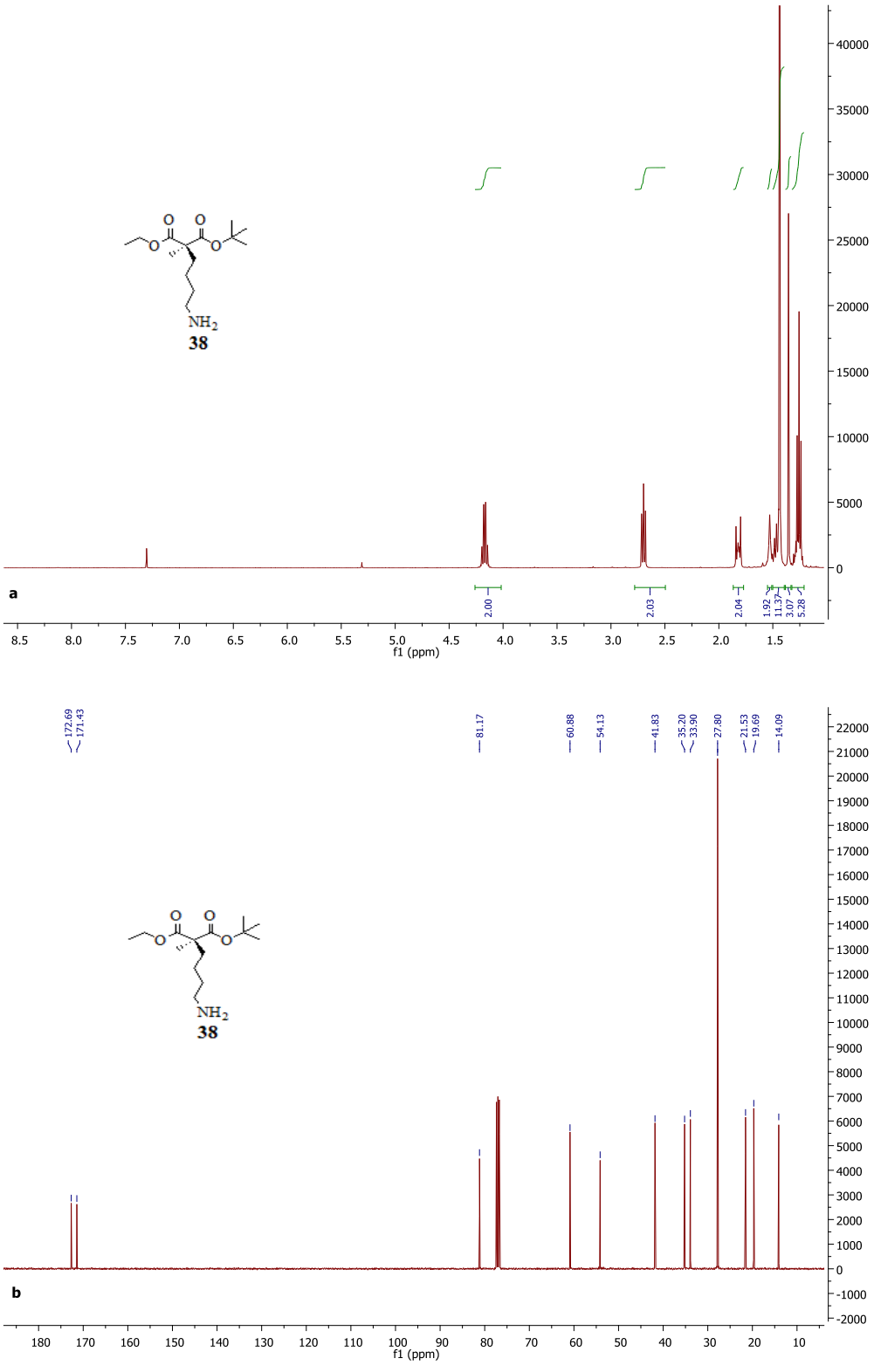
A.37. a) ^{1}H NMR of **34**, b) ^{13}C NMR of **34**



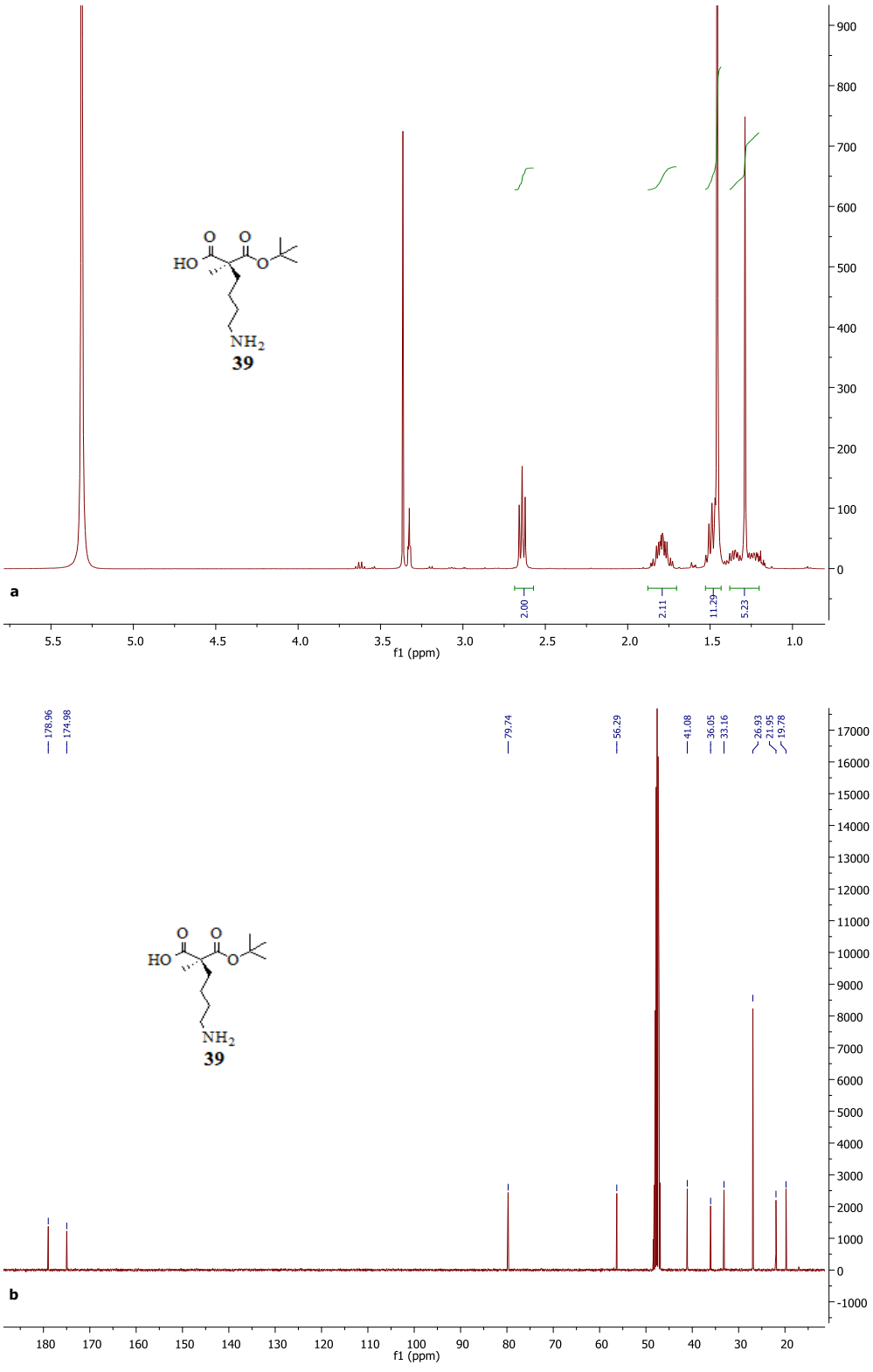
A.38. a) ^{1}H NMR of **35**, b) ^{13}C NMR of **35**



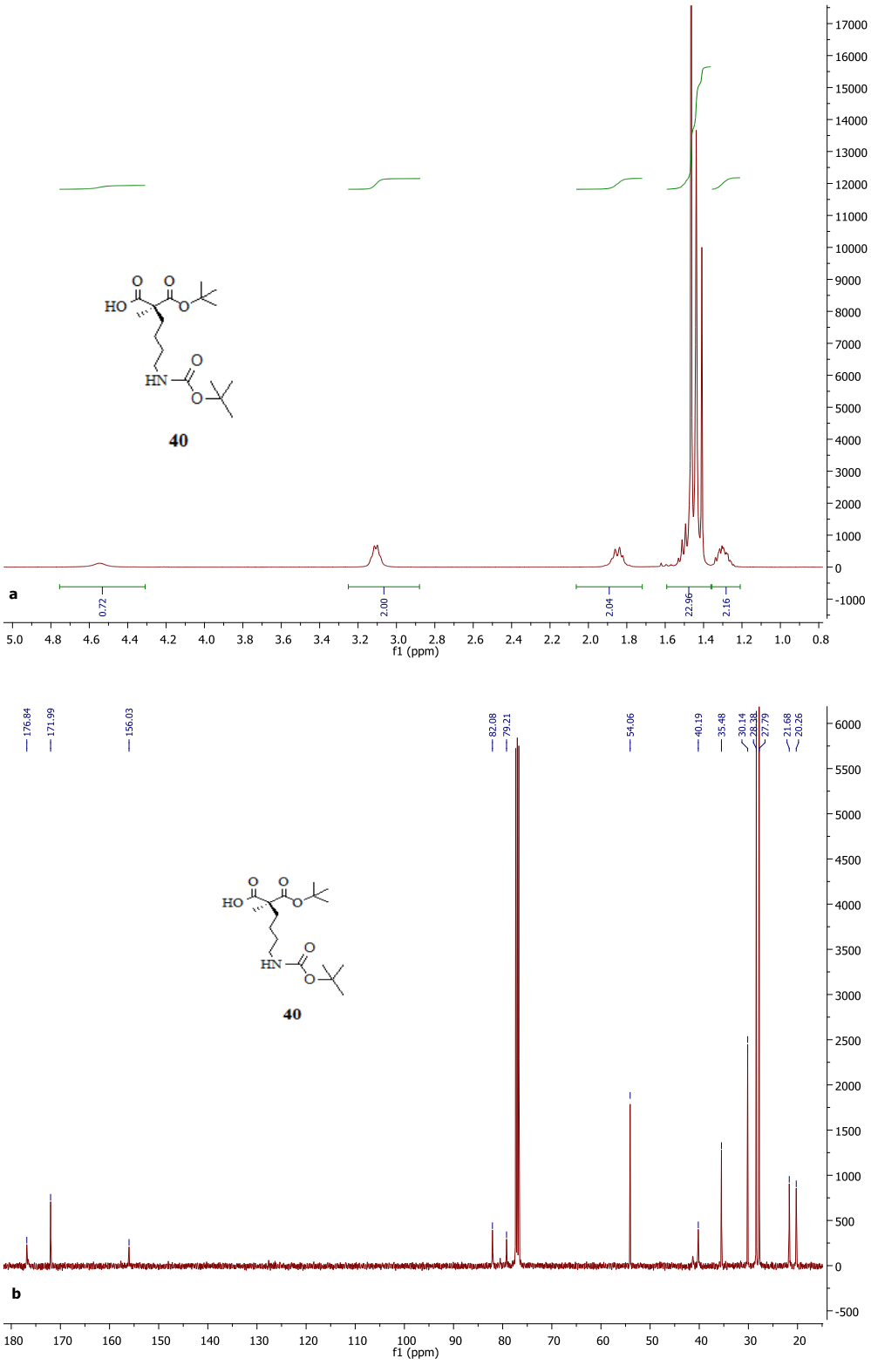
A. 39. a) ^{1}H NMR of **37**, b) ^{13}C NMR of **37**



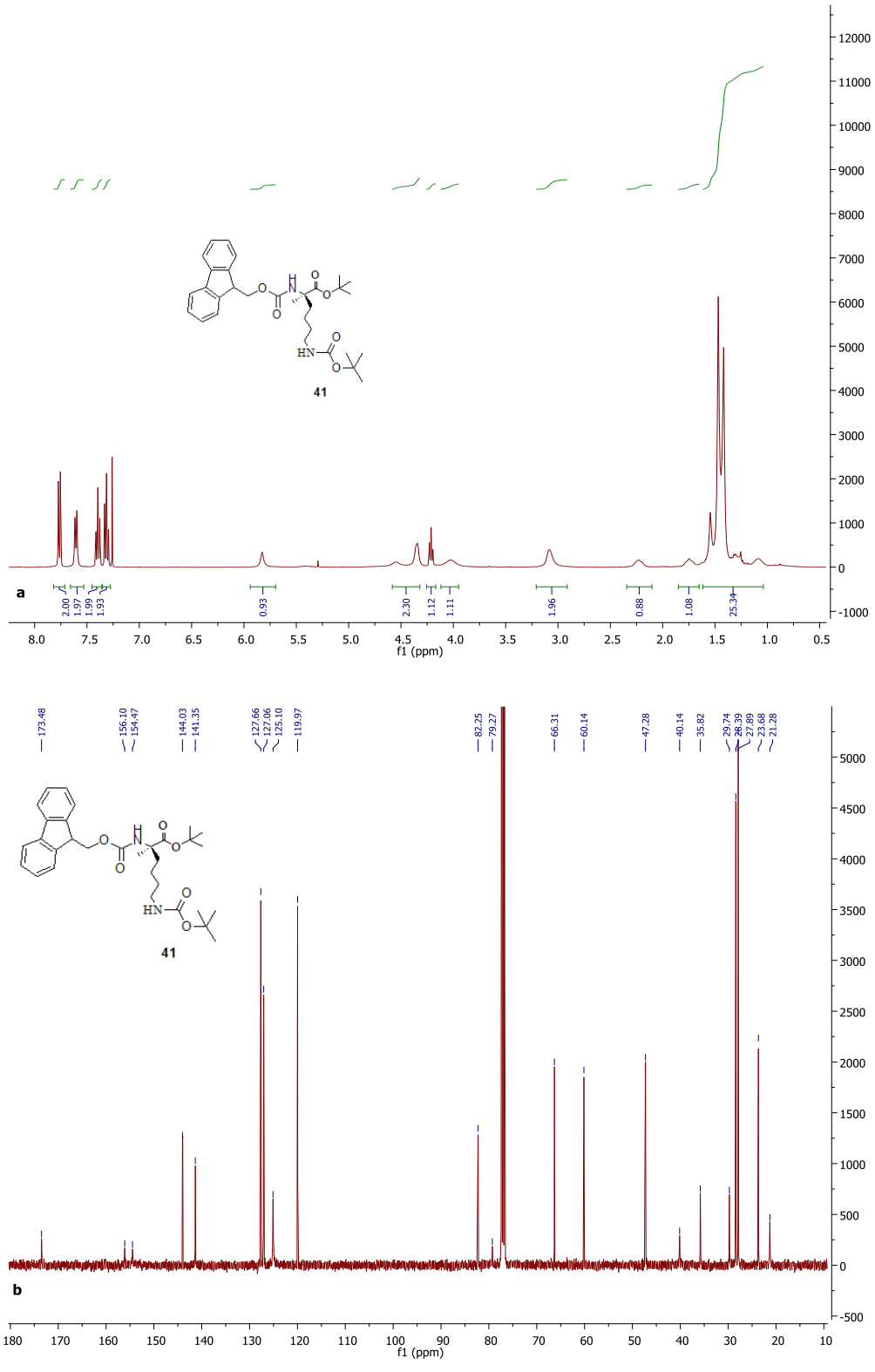
A.40. a) ^{1}H NMR of **38**, b) ^{13}C NMR of **38**



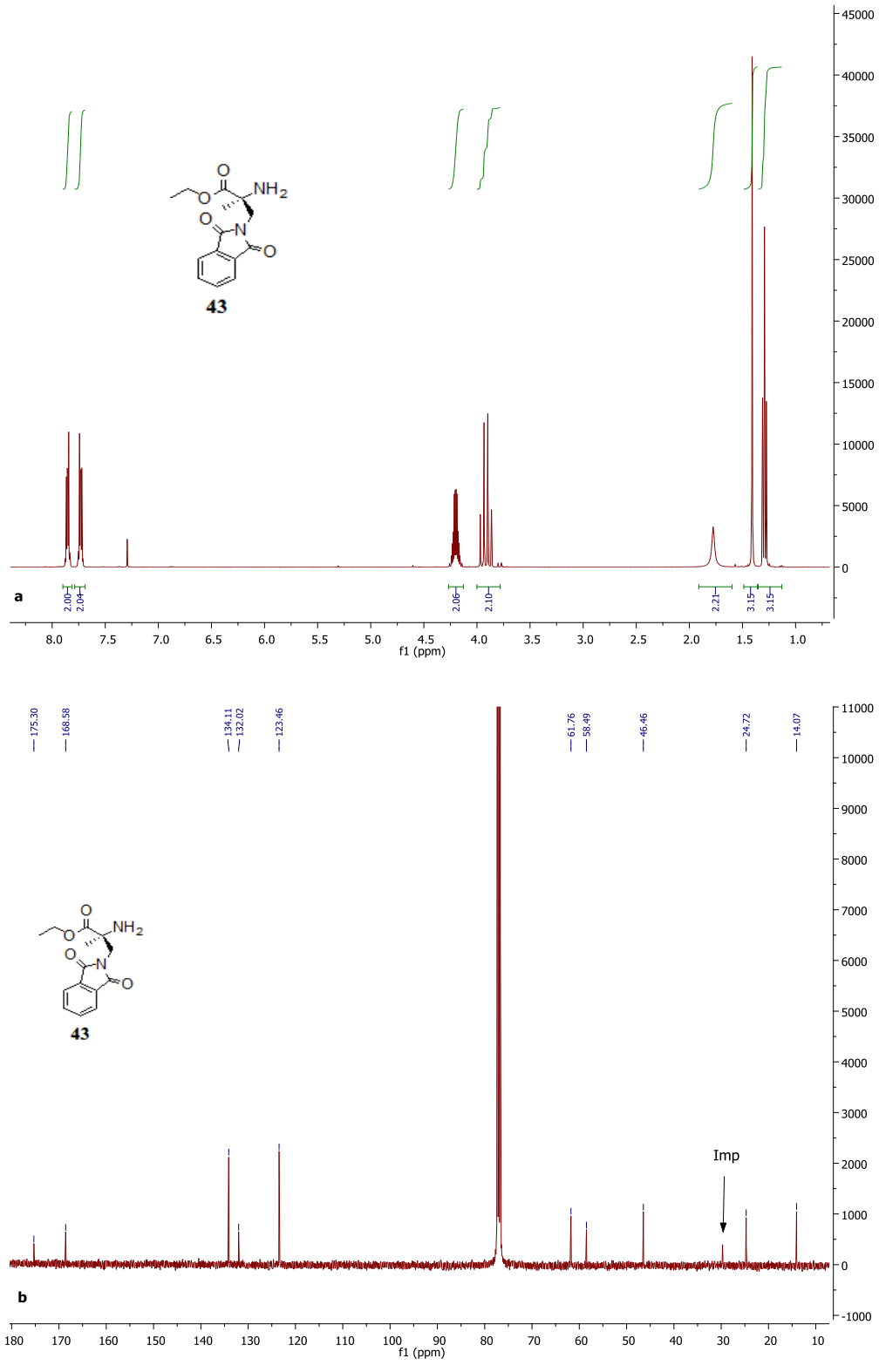
A.41. a) ^{1}H NMR of **39**, b) ^{13}C NMR of **39**



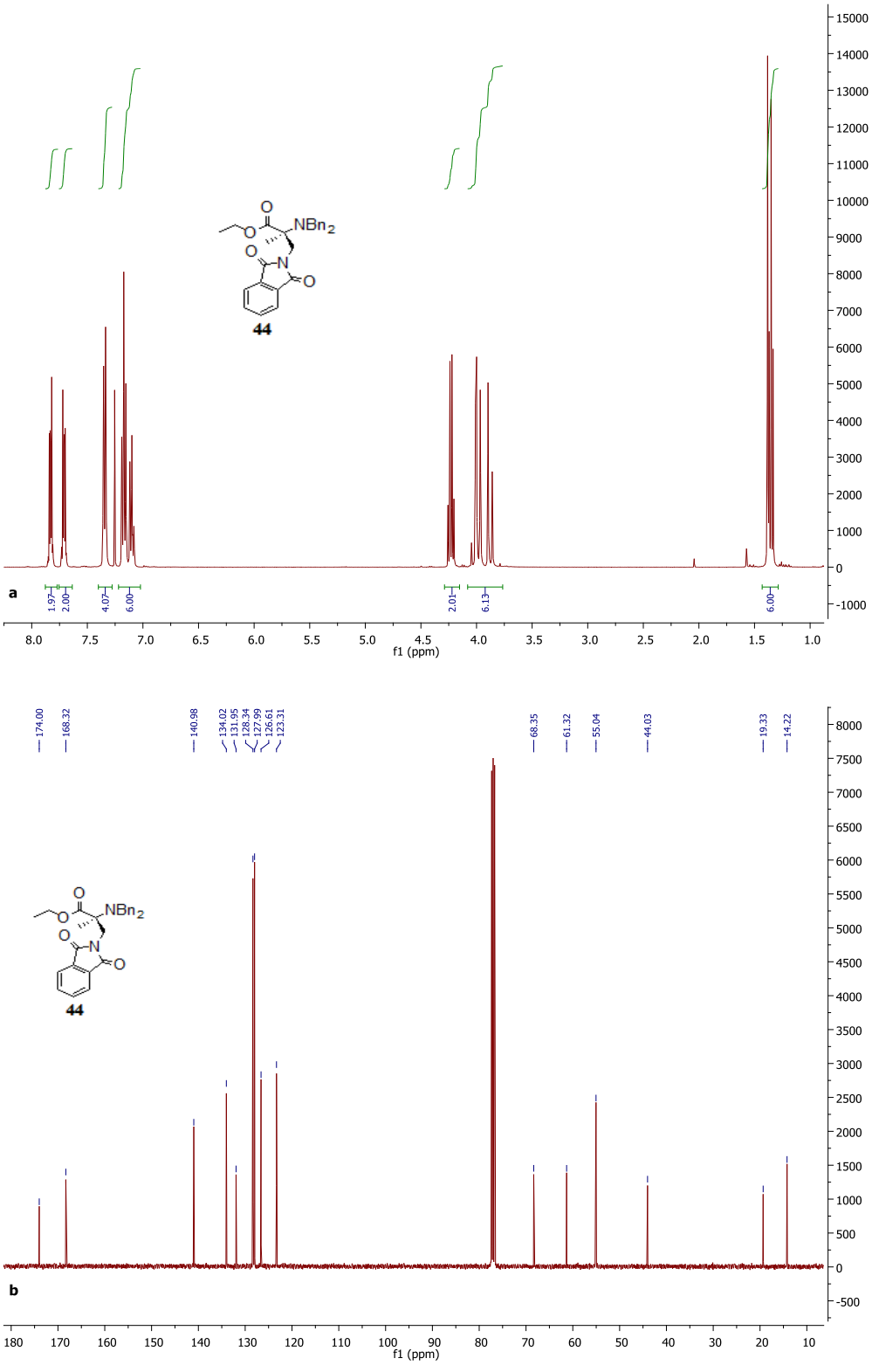
A.42. a) ^{1}H NMR of **40**, b) ^{13}C NMR of **40**



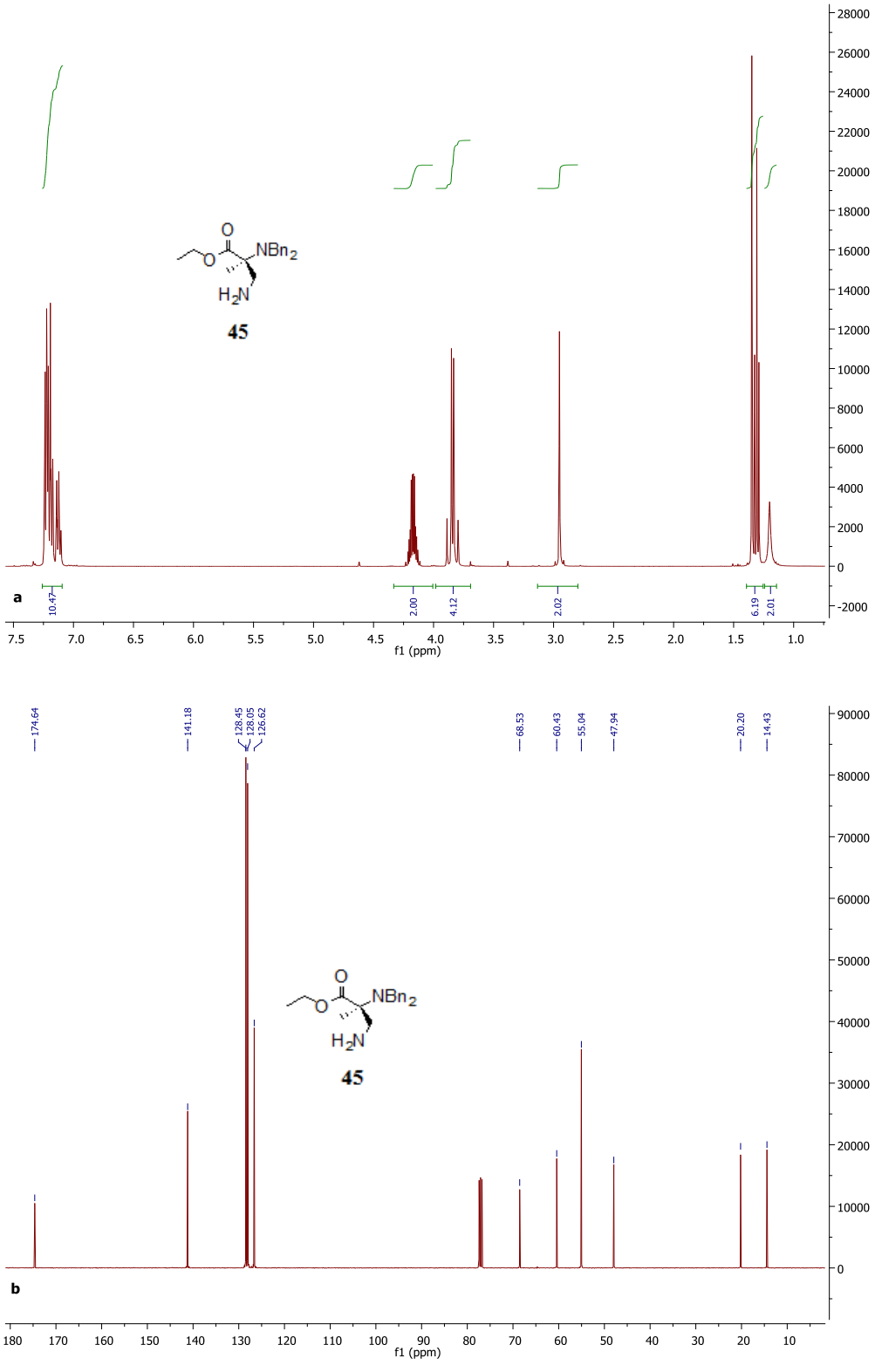
A.43. a) ^{1}H NMR of **41**, b) ^{13}C NMR of **41**



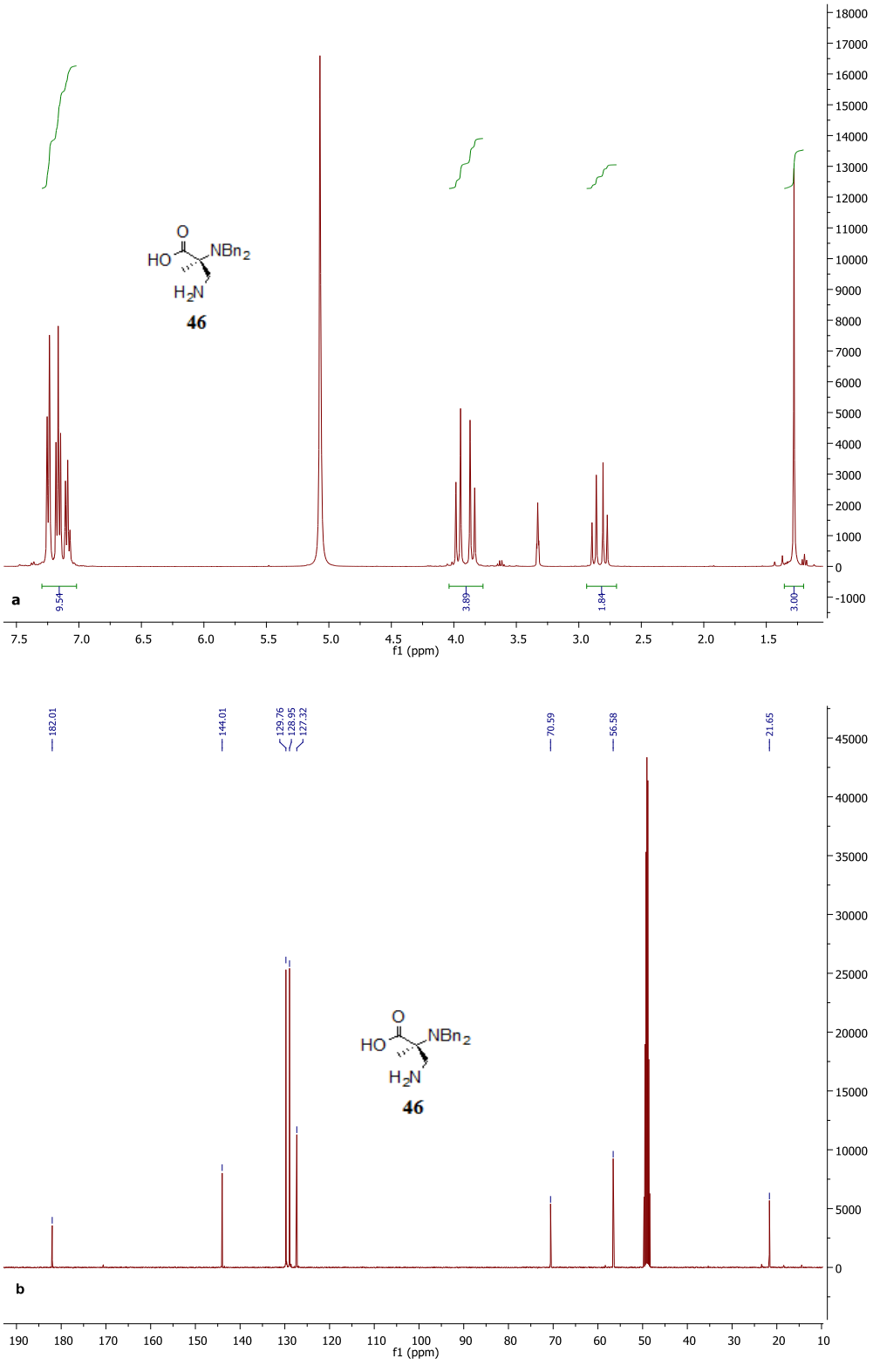
A. 44. a) ^{1}H NMR of **43**, b) ^{13}C NMR of **43**



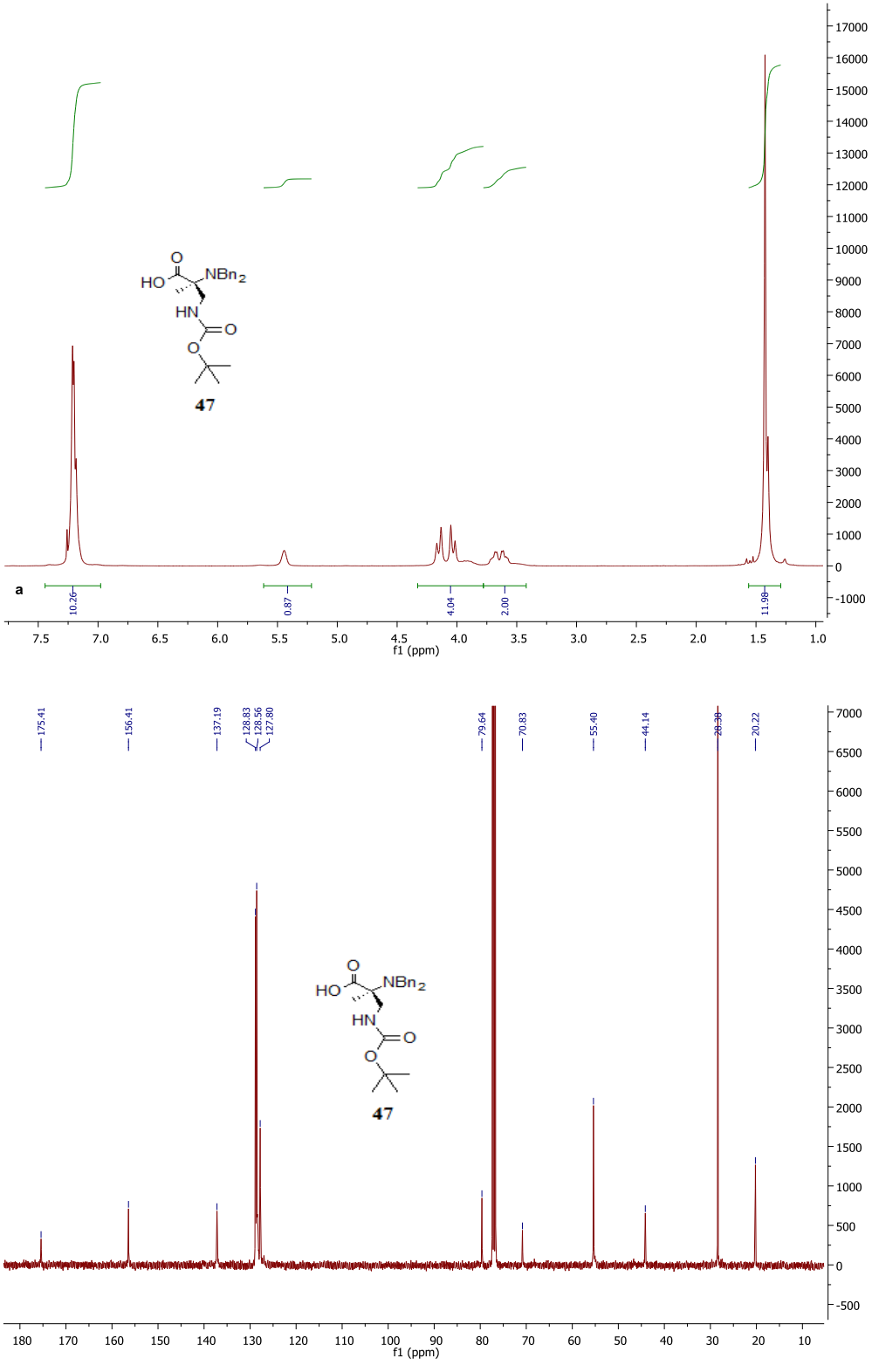
A.45. a) ^{1}H NMR of **44**, b) ^{13}C NMR of **44**



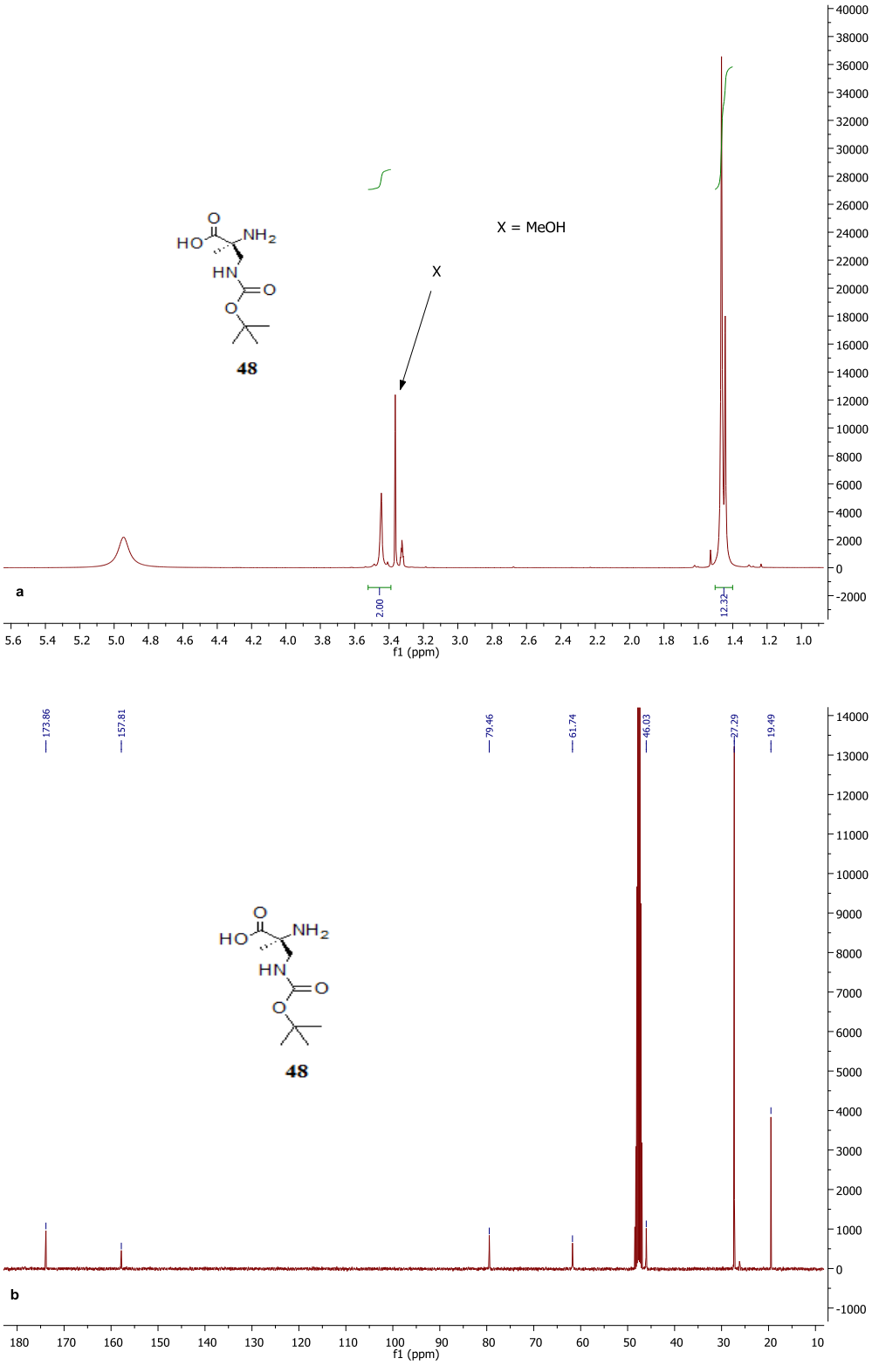
A. 46. a) ^{1}H NMR of **45**, b) ^{13}C NMR of **45**



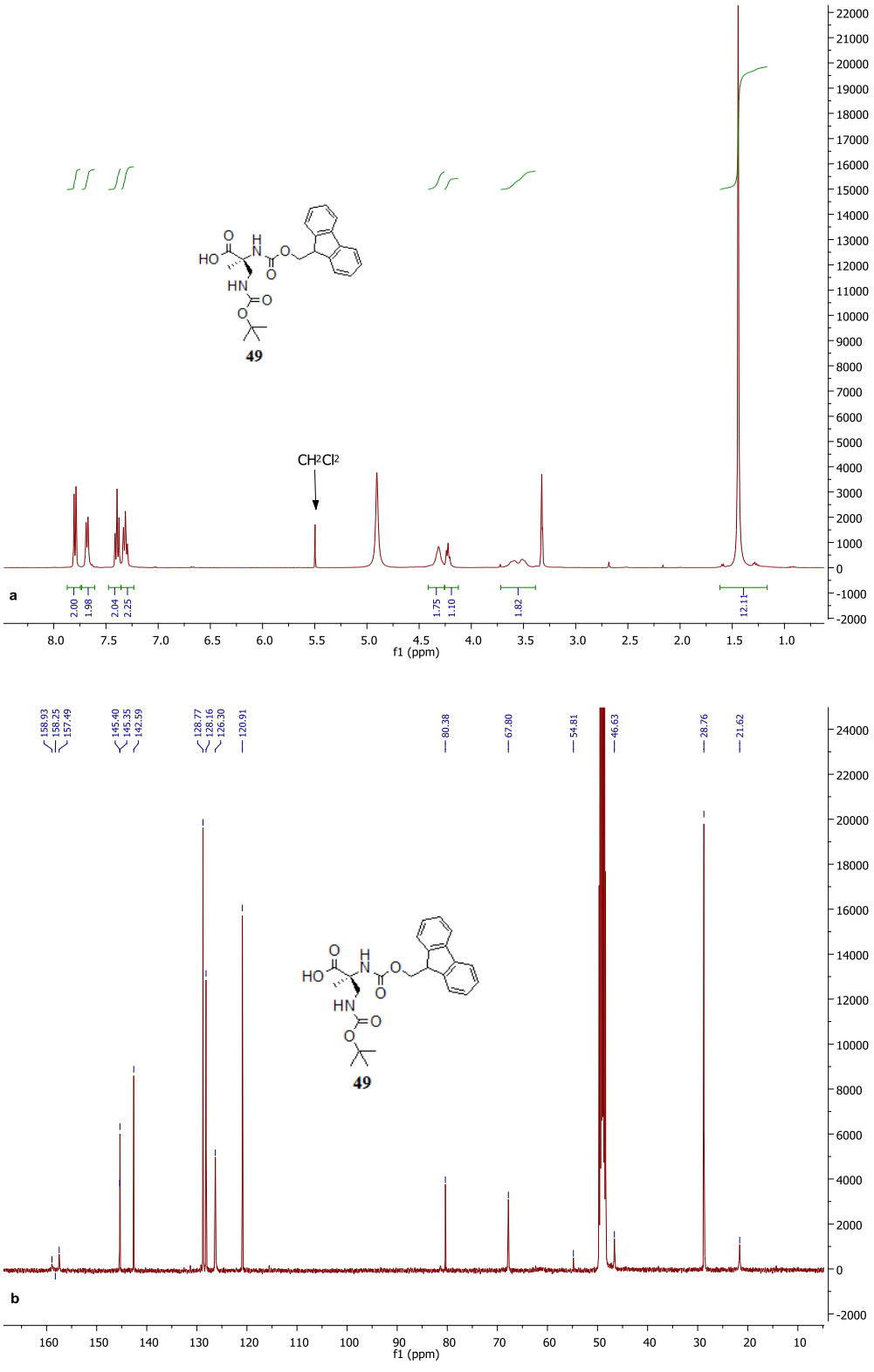
A.47. a) ^{1}H NMR of **46**, b) ^{13}C NMR of **46**



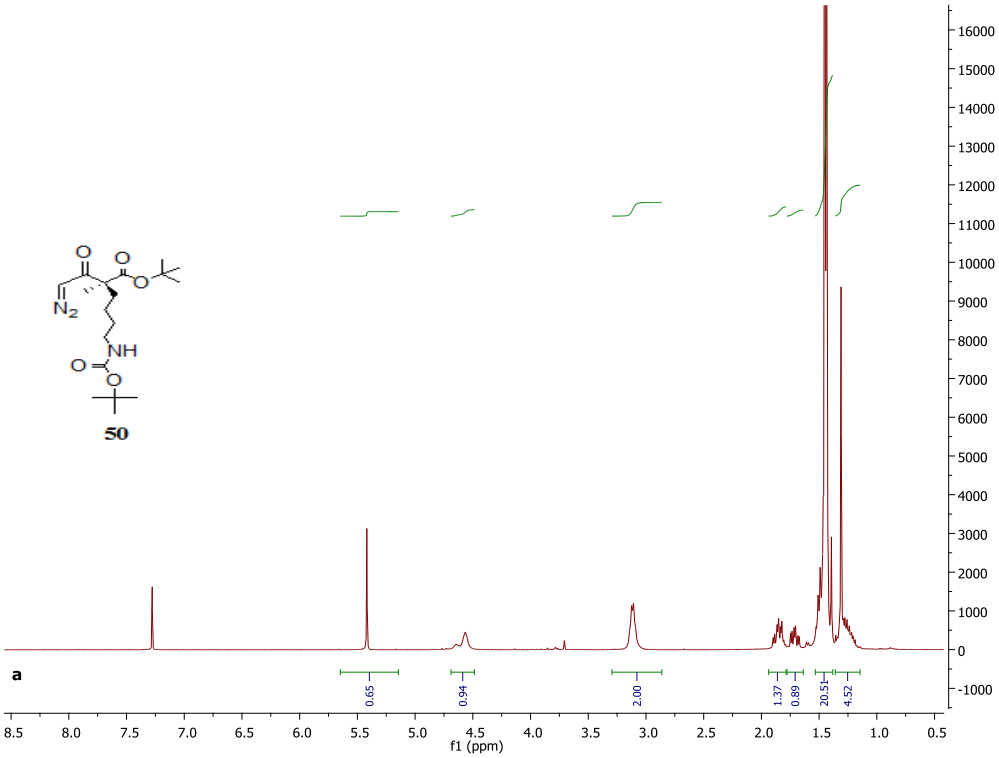
A.48. a) ^{1}H NMR of **47**, b) ^{13}C NMR of **47**



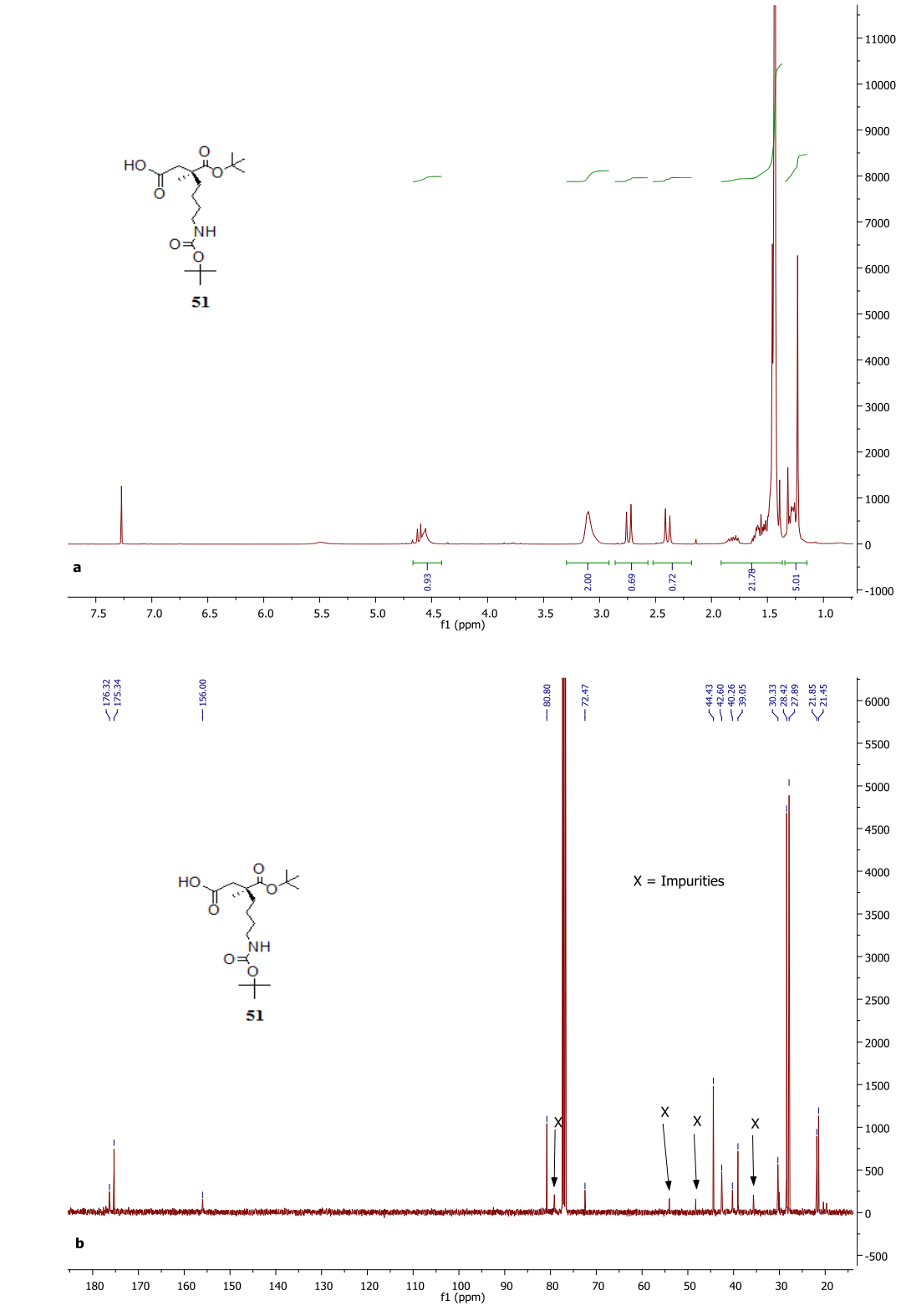
A.49. a) ^{1}H NMR of **48**, b) ^{13}C NMR of **48**



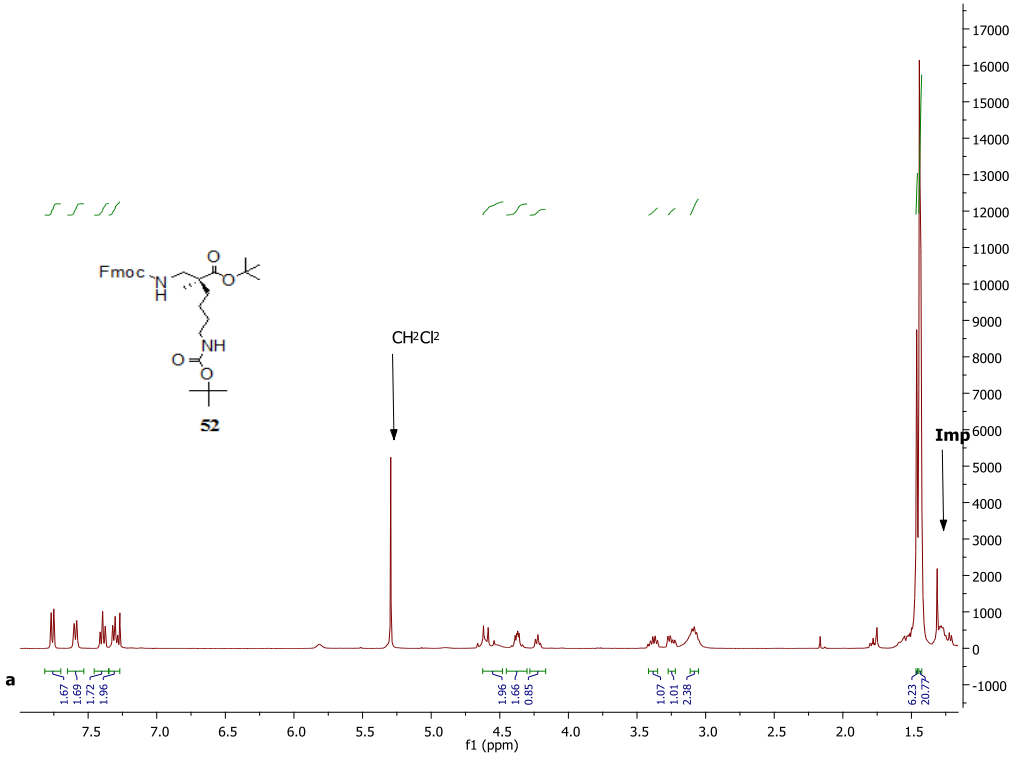
A.50. a) ^{1}H NMR of **49**, b) ^{13}C NMR of **49**



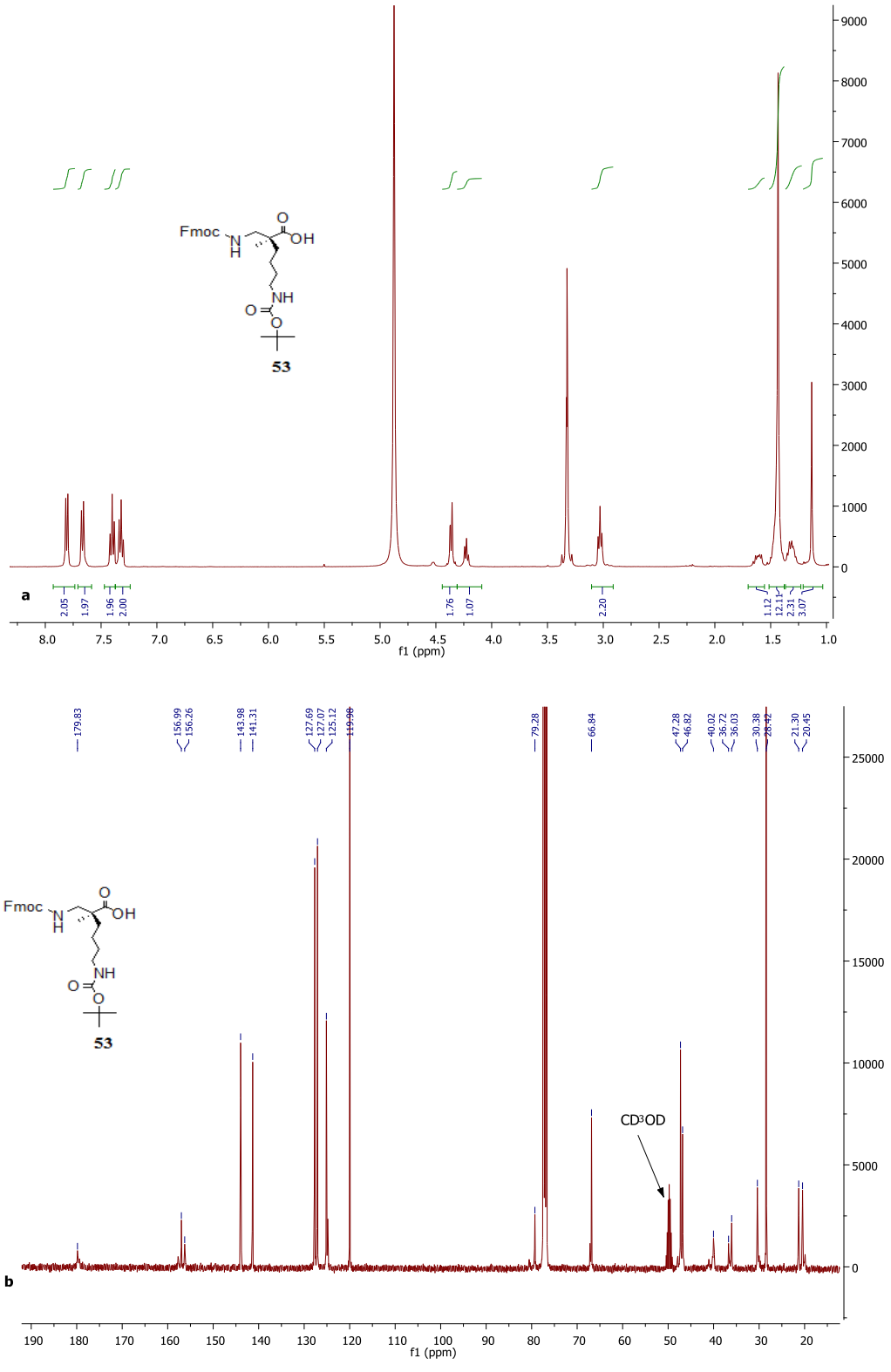
A.51. a) ^{1}H NMR of **50**



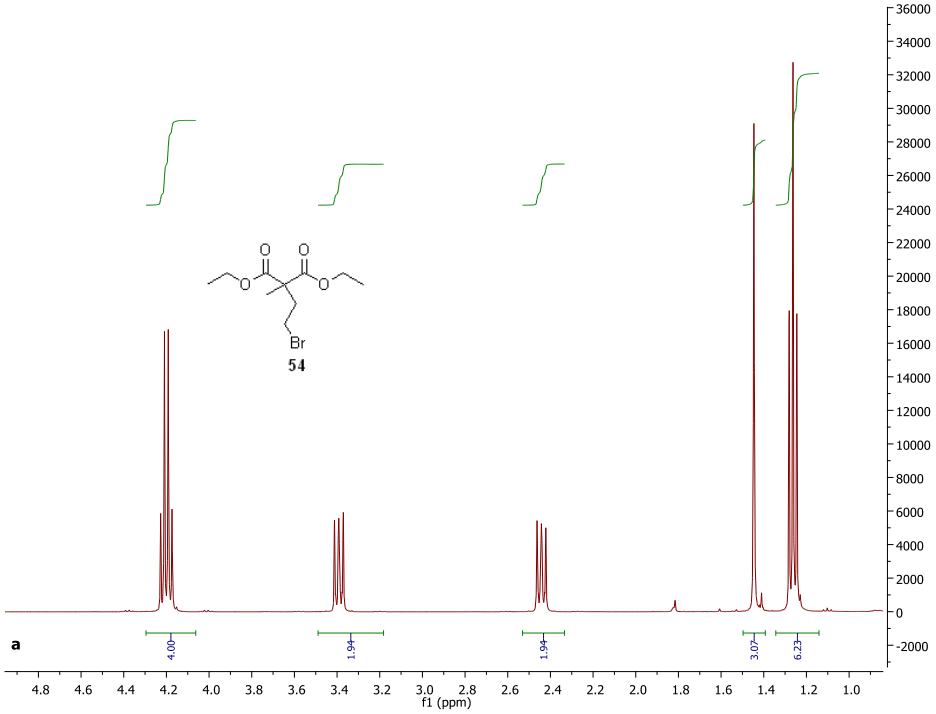
A.52. a) ^{1}H NMR of **51**, b) ^{13}C NMR of **51**

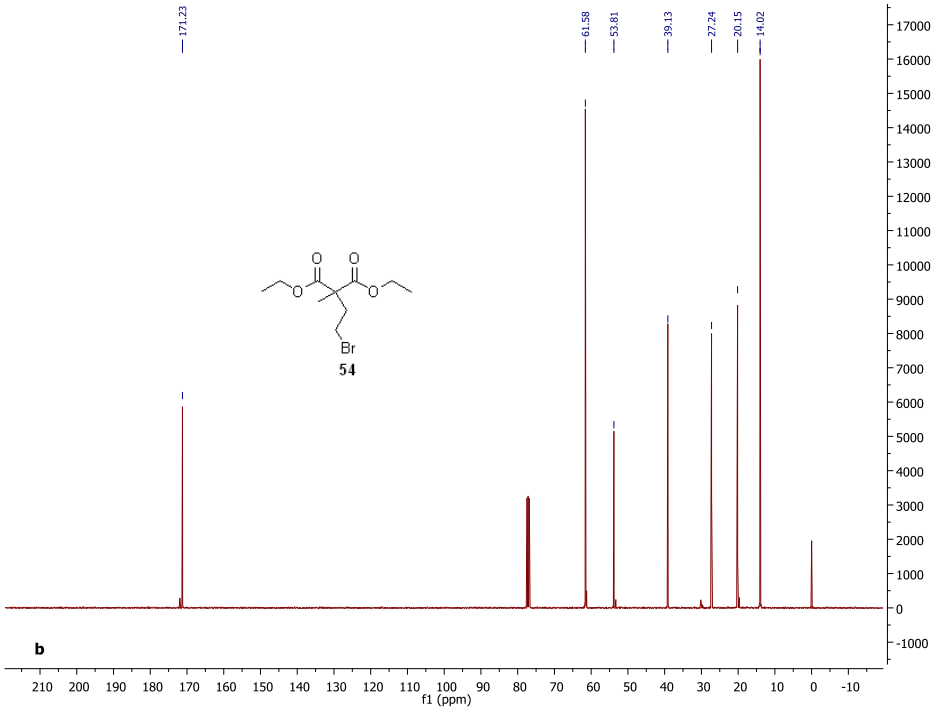


A.53. a) ^{1}H NMR of **52**

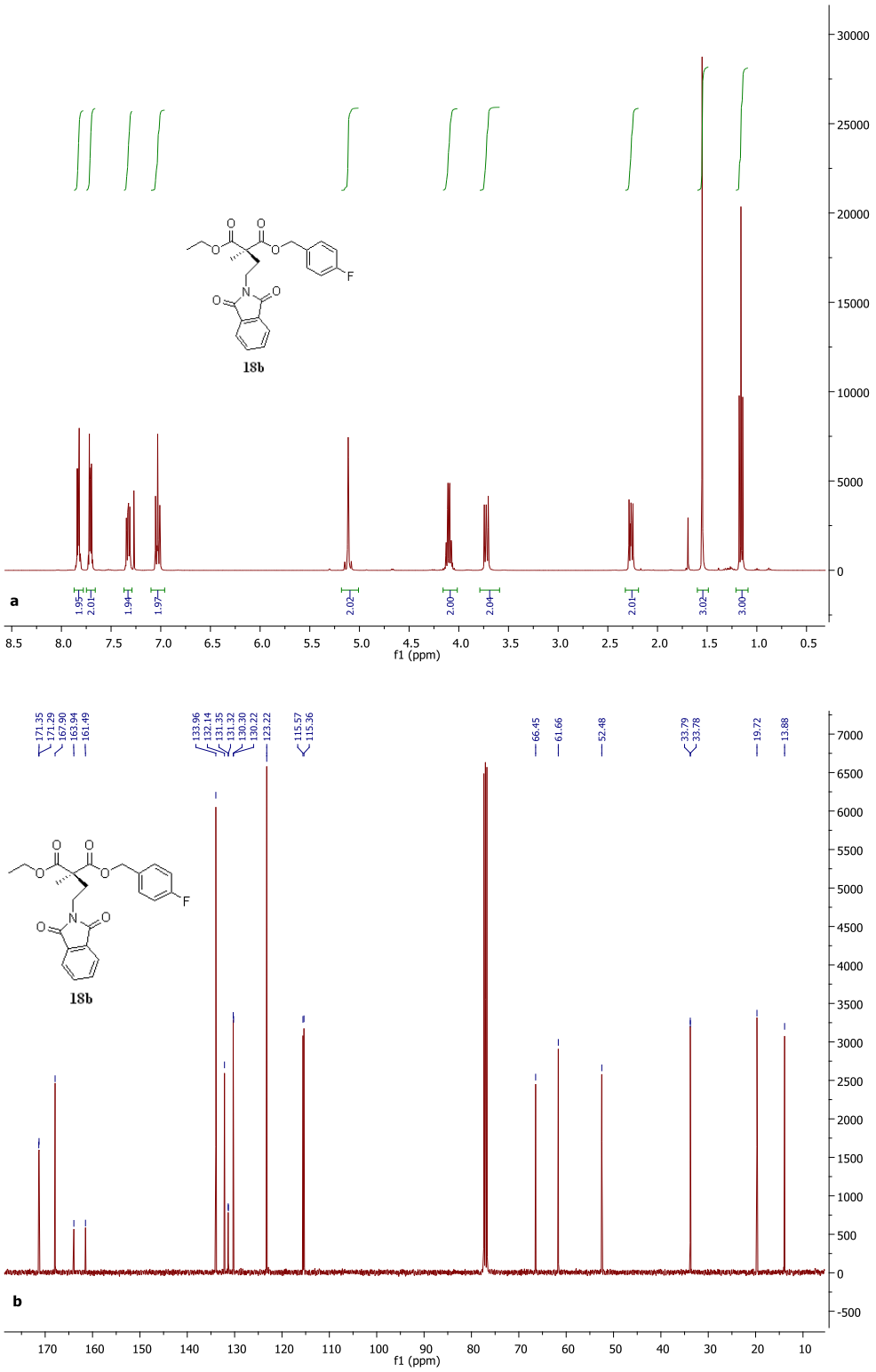


A.54. a) ^{1}H NMR of **53**, b) ^{13}C NMR of **53**

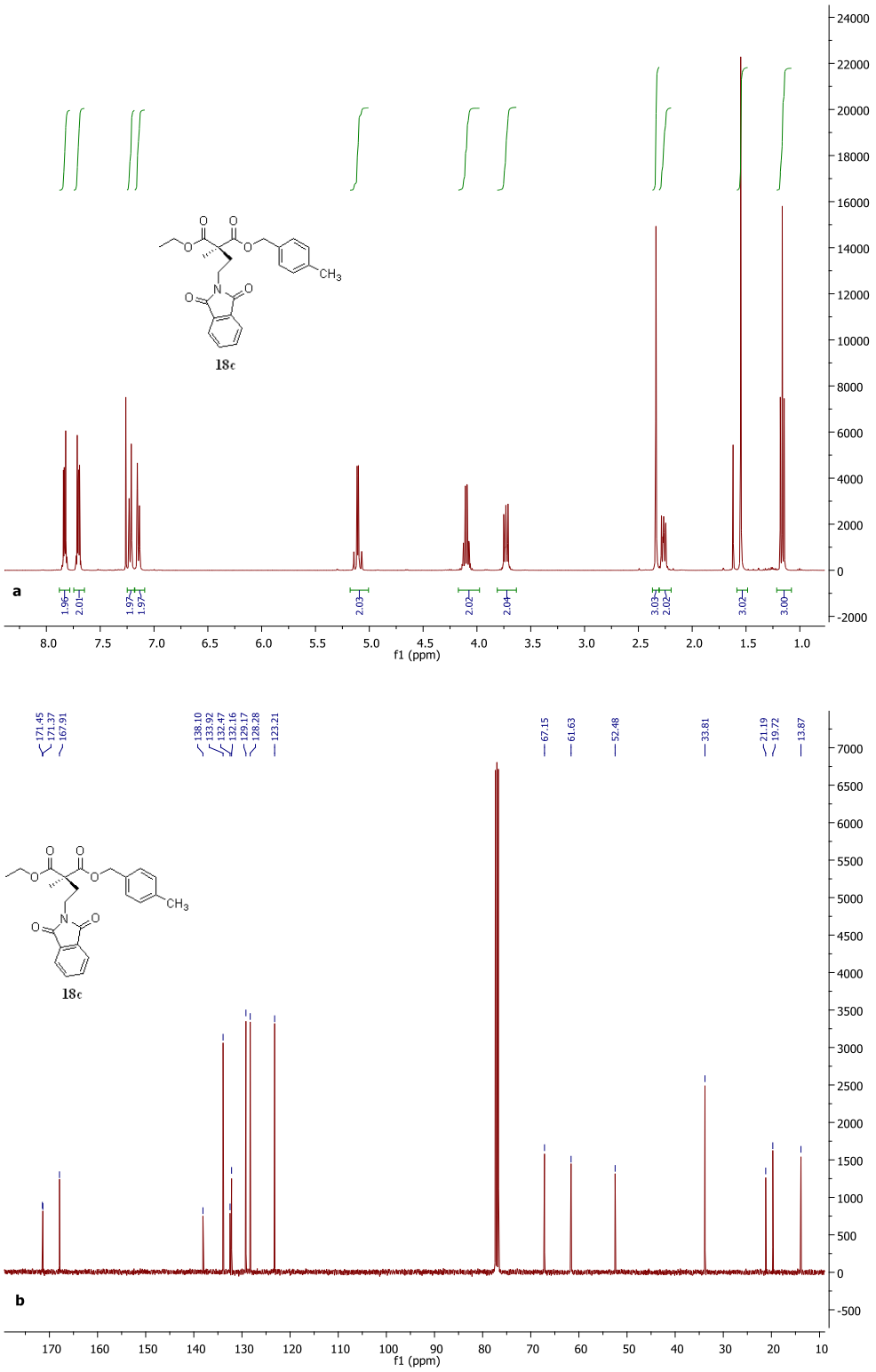




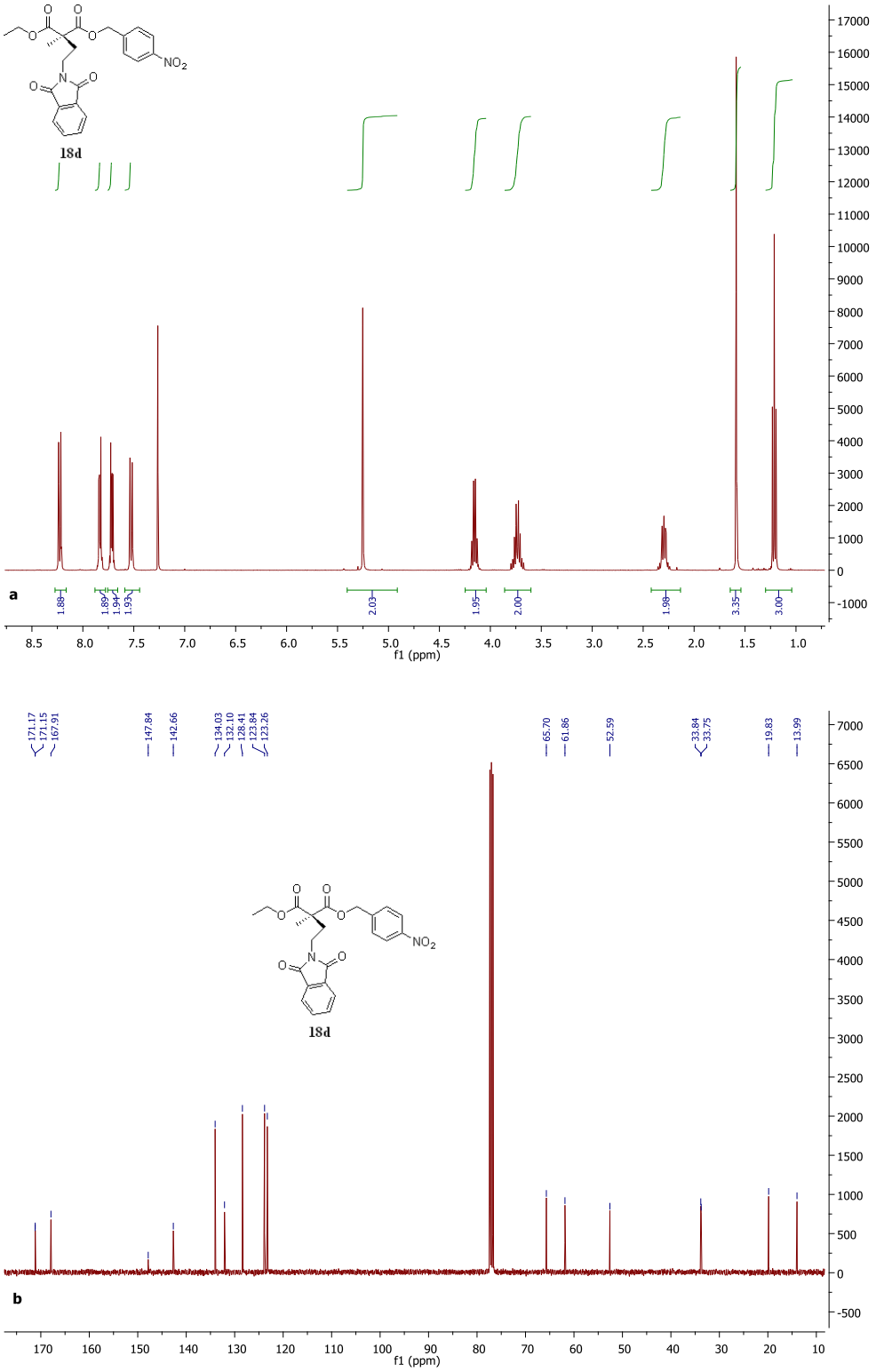
A.55. a) ^{1}H NMR of **54**, b) ^{13}C NMR of **54**



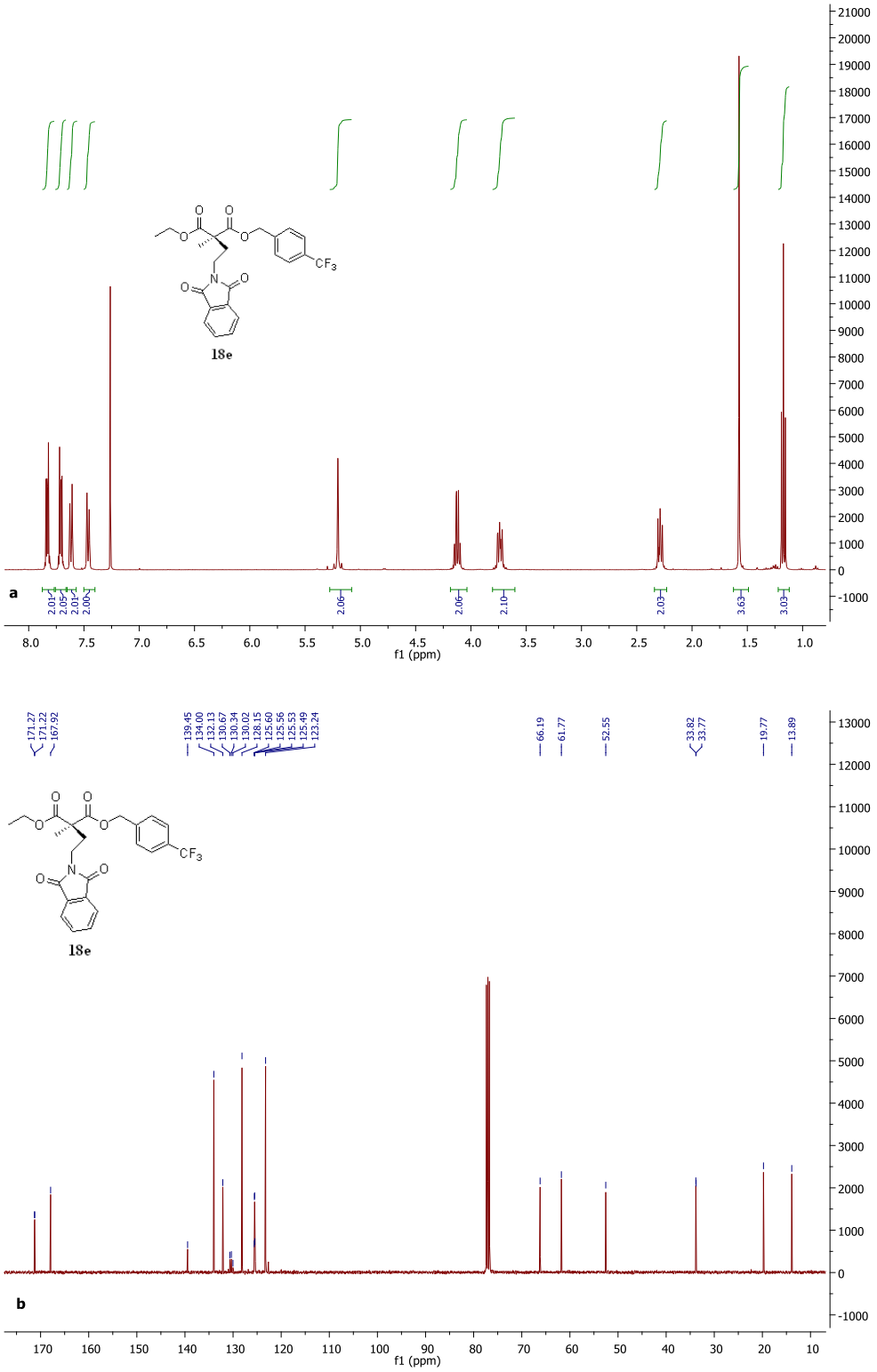
A.56. a) ^{1}H NMR of **18b**, b) ^{13}C NMR of **18b**



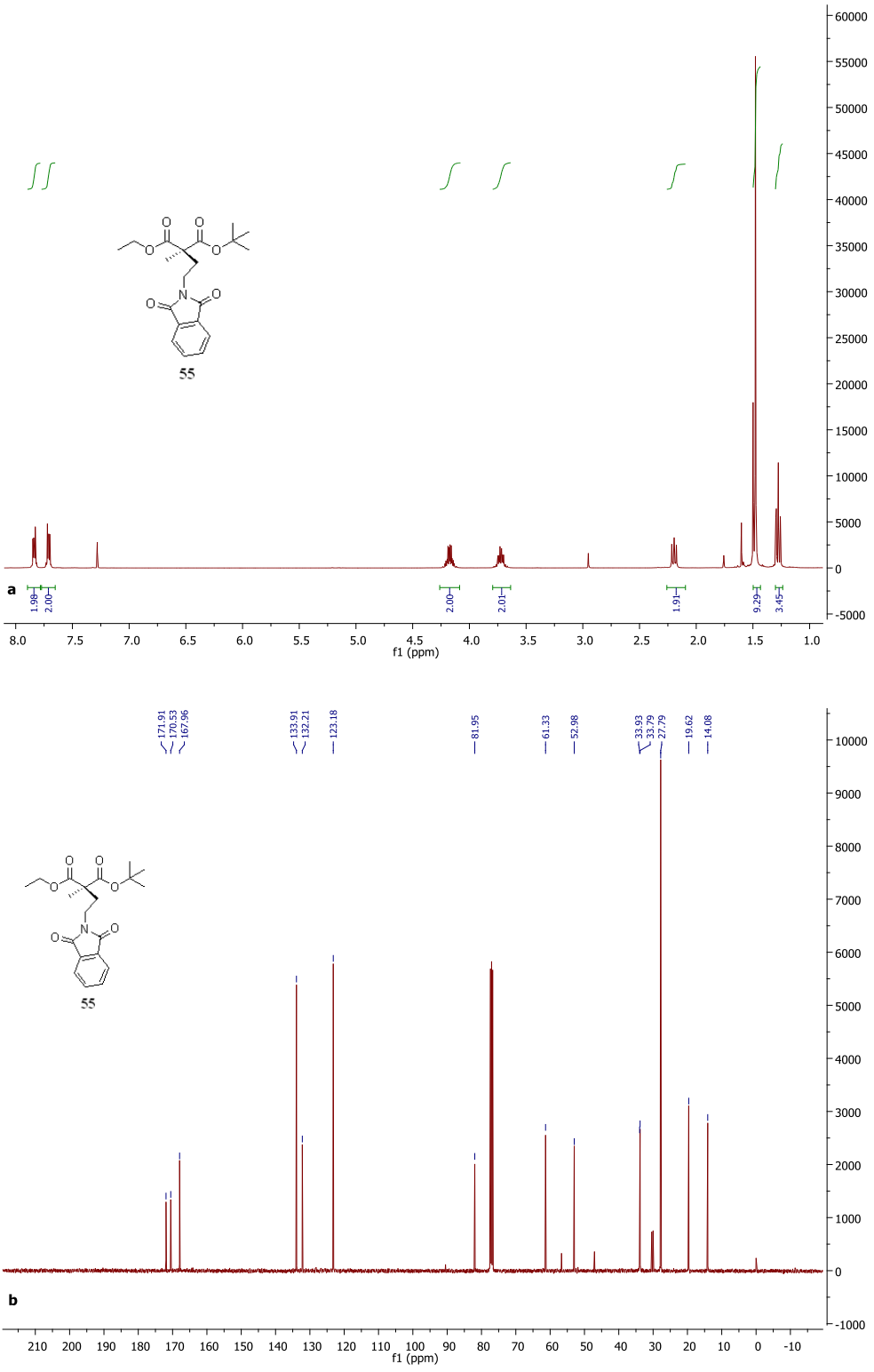
A.57. a) ^{1}H NMR of **18c**, b) ^{13}C NMR of **18c**



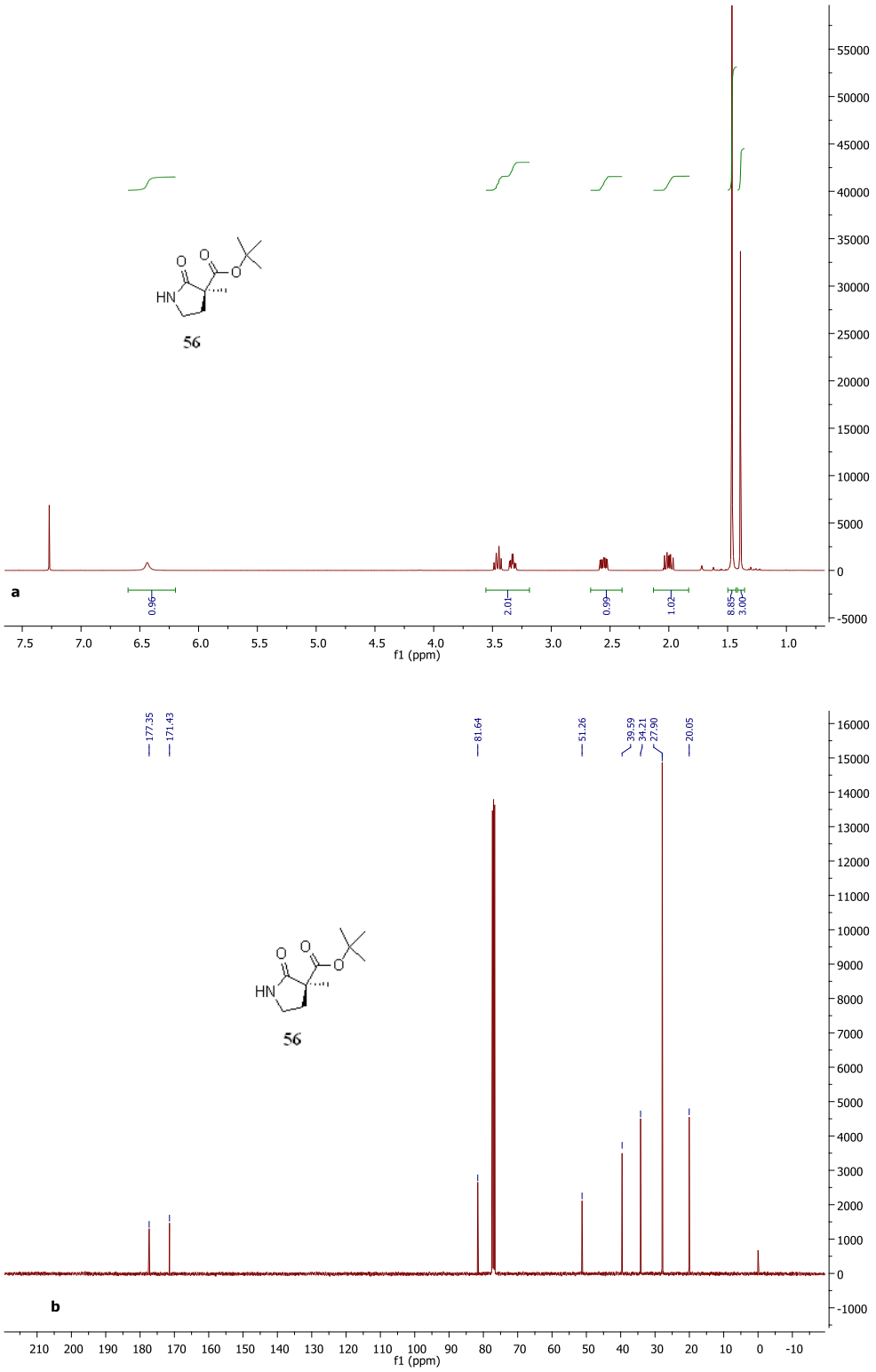
A.58. a) ^{1}H NMR of **18d**, b) ^{13}C NMR of **18d**



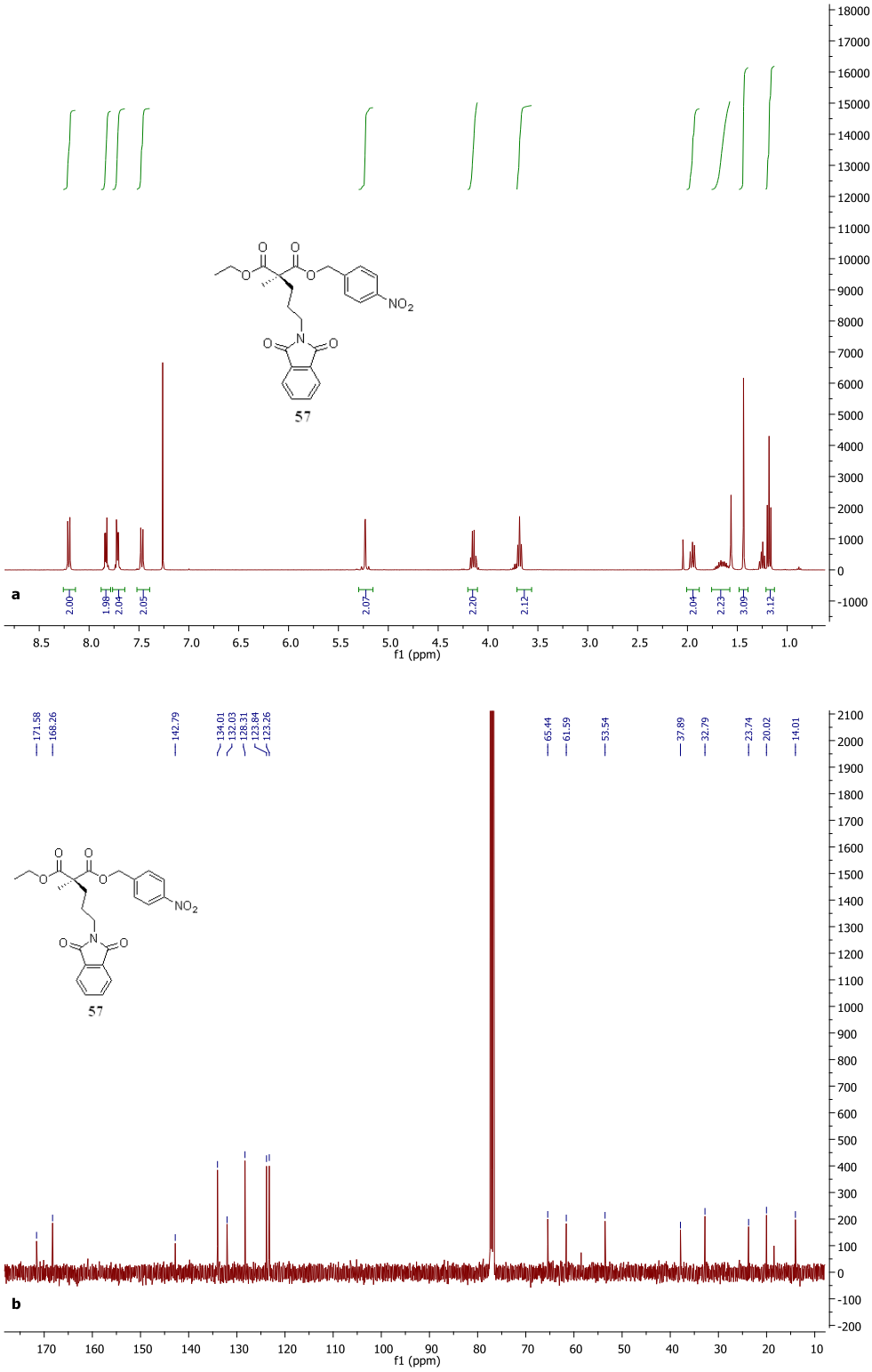
A.59. a) ^{1}H NMR of **18e**, b) ^{13}C NMR of **18e**



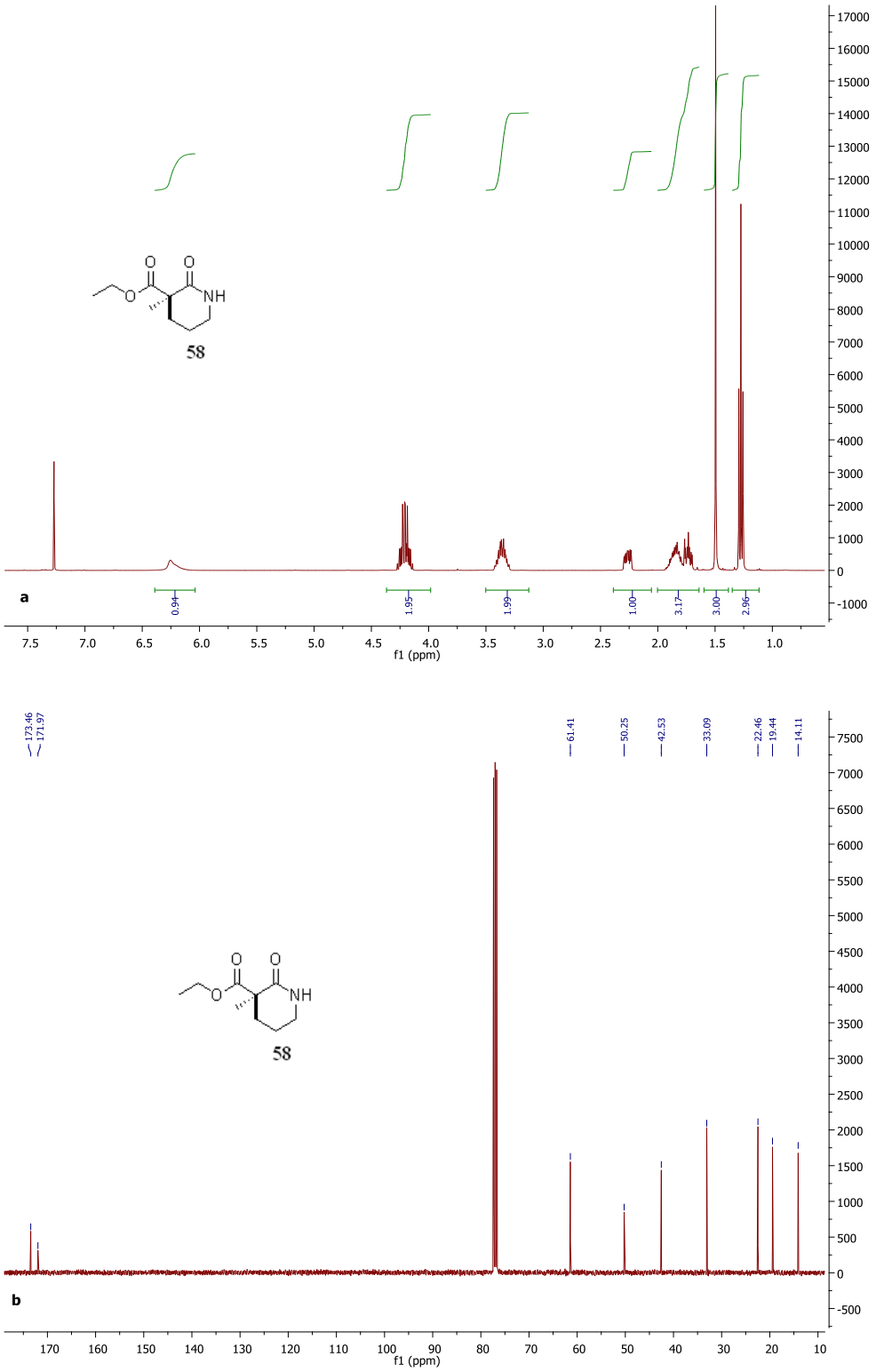
A.60. a) ^{1}H NMR of **55**, b) ^{13}C NMR of **55**



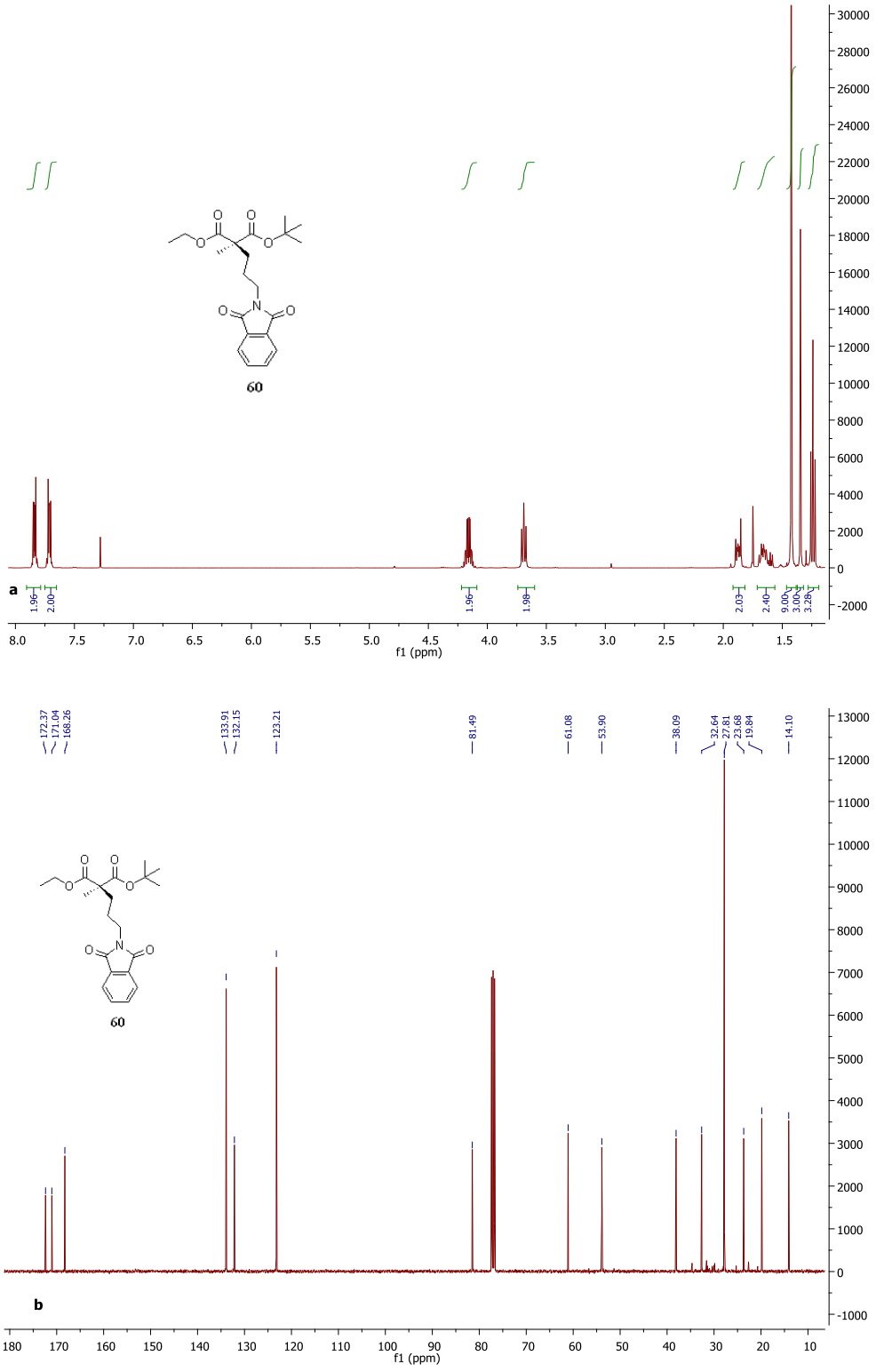
A.61. a) ^{1}H NMR of **56**, b) ^{13}C NMR of **56**



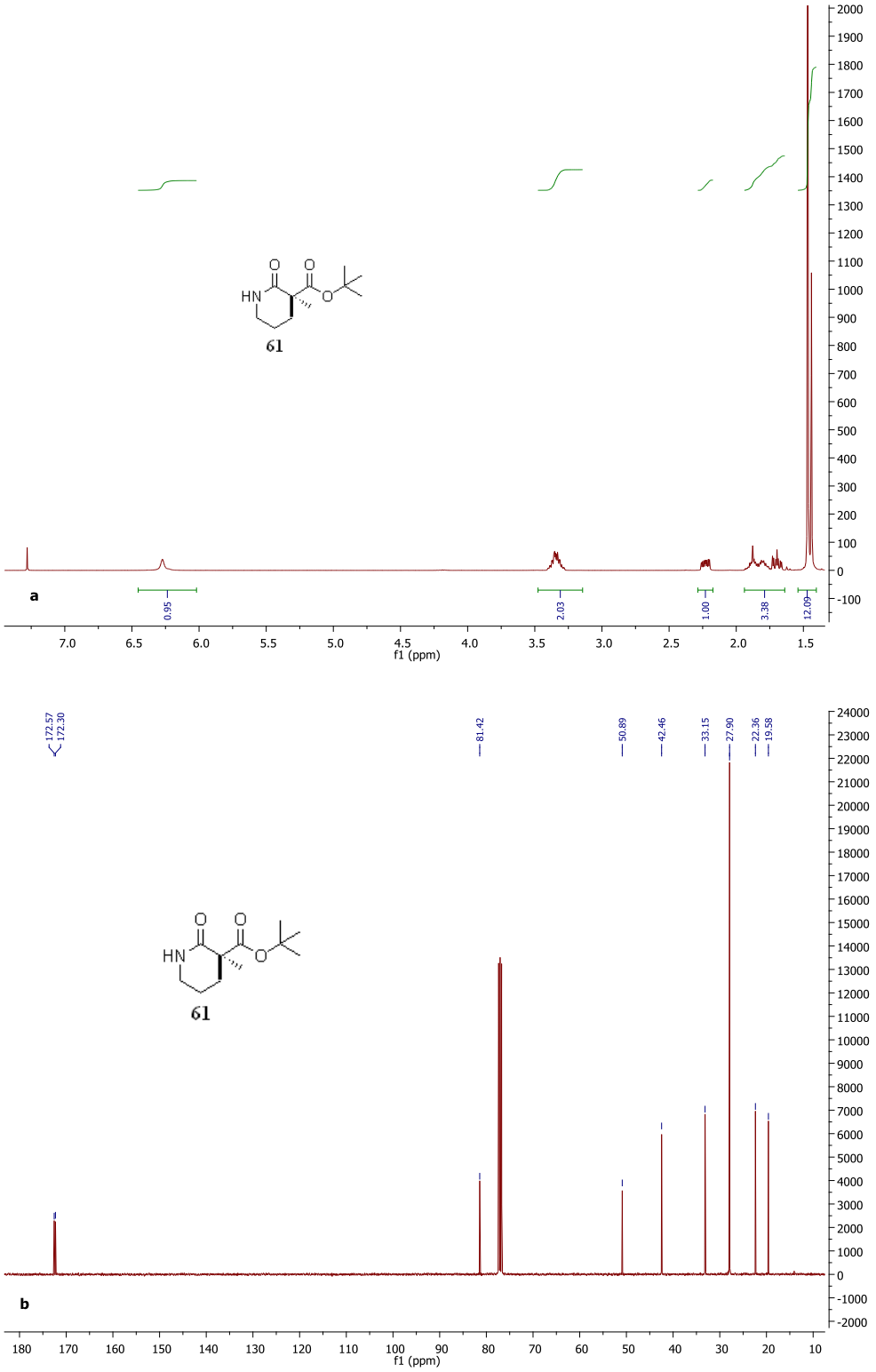
A.62. a) ^{1}H NMR of **57**, b) ^{13}C NMR of **57**



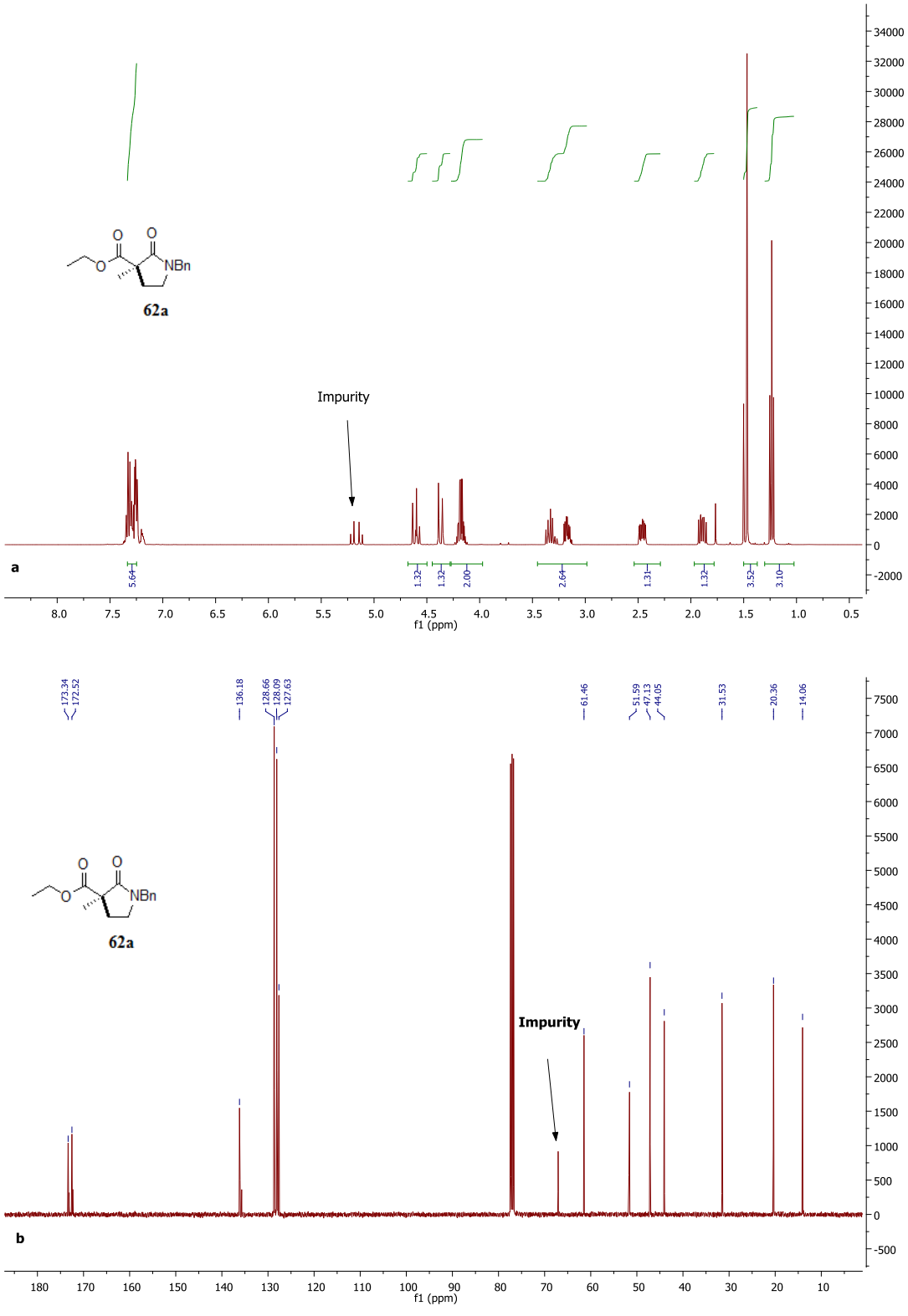
A.63. a) ^{1}H NMR of **58**, b) ^{13}C NMR of **58**



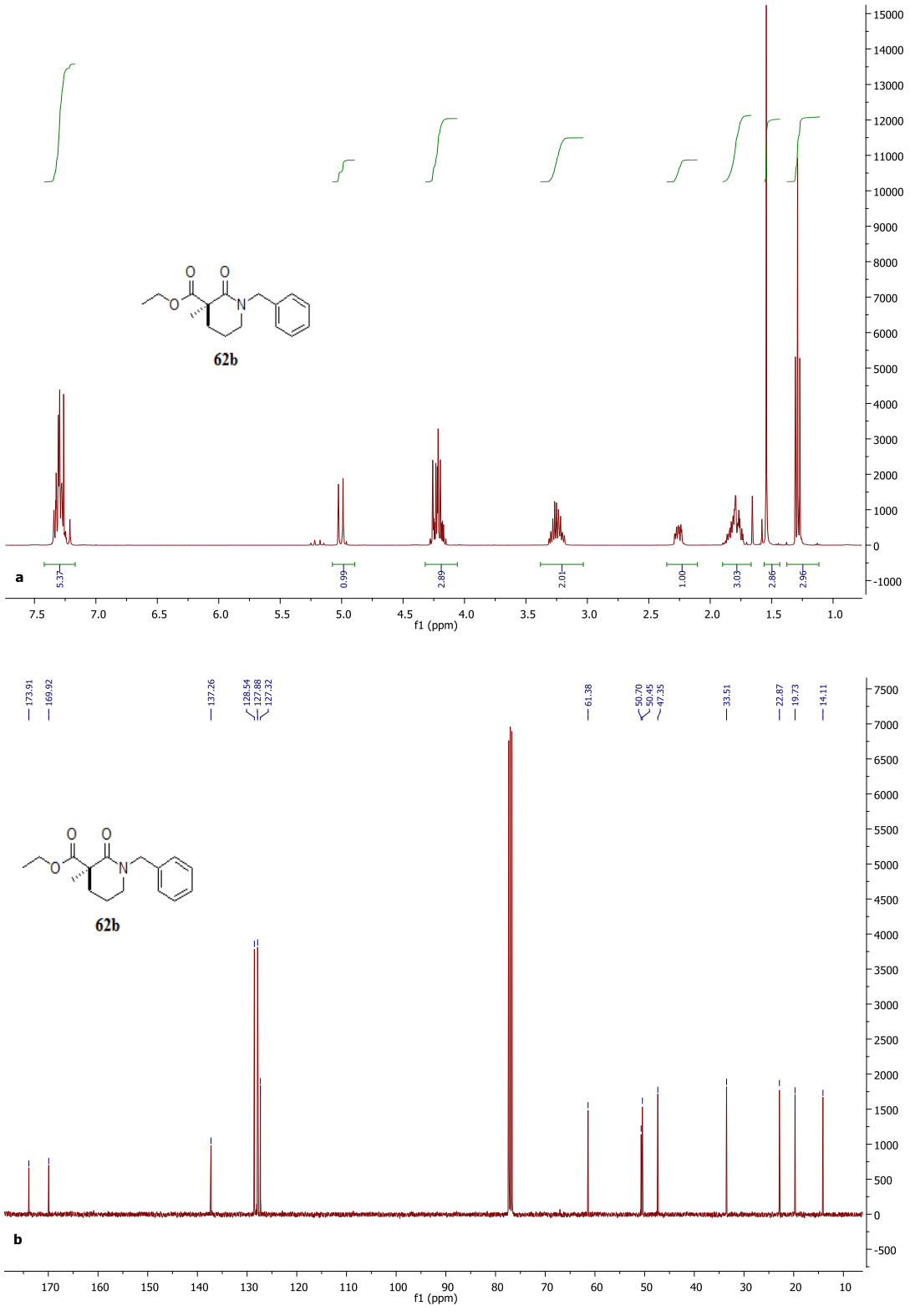
A.64. a) ^{1}H NMR of **60**, b) ^{13}C NMR of **60**



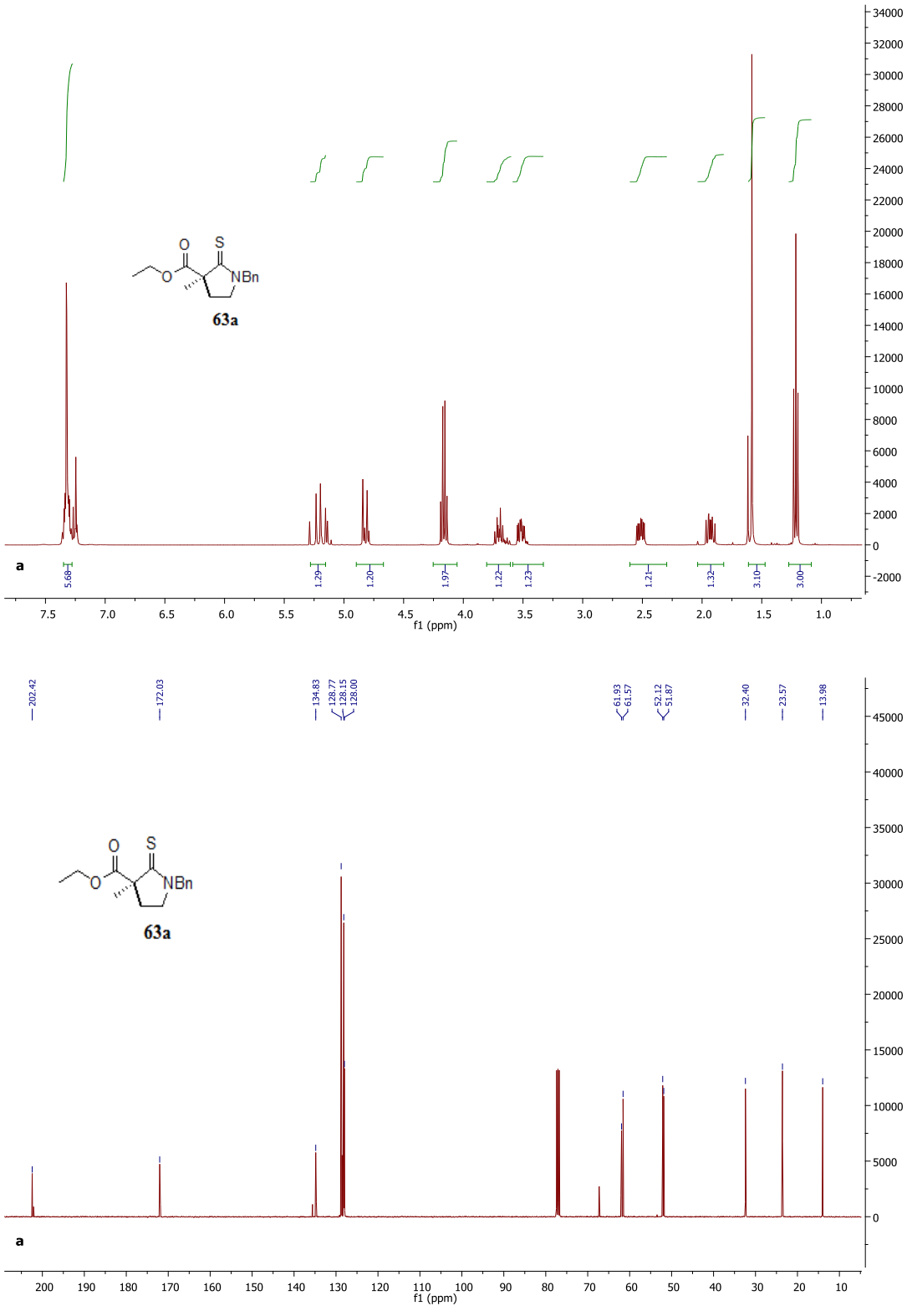
A.65. a) ^{1}H NMR of **61**, b) ^{13}C NMR of **61**



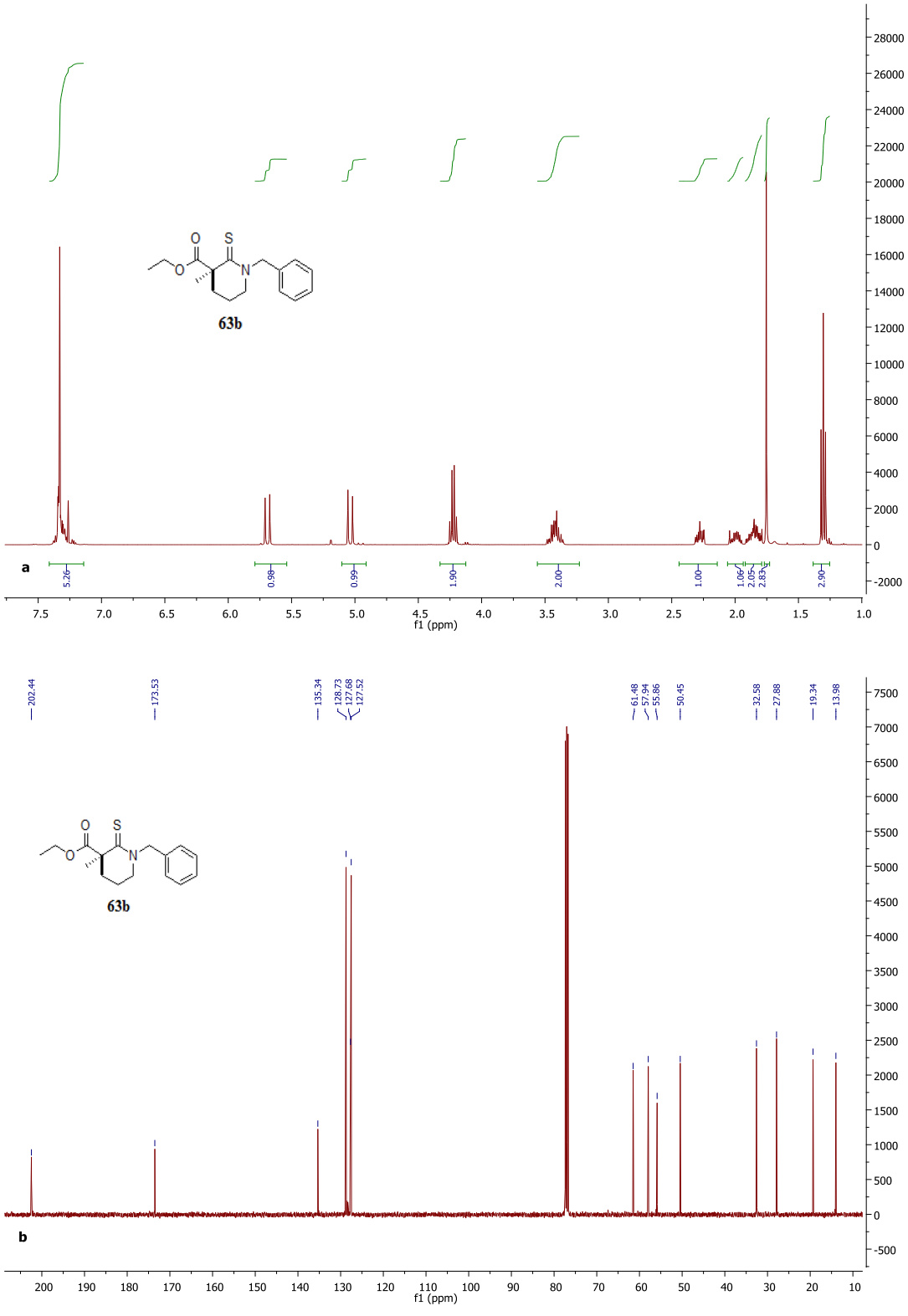
A.66. a) ^{1}H NMR of **62a**, b)^{13}C NMR of **62a**



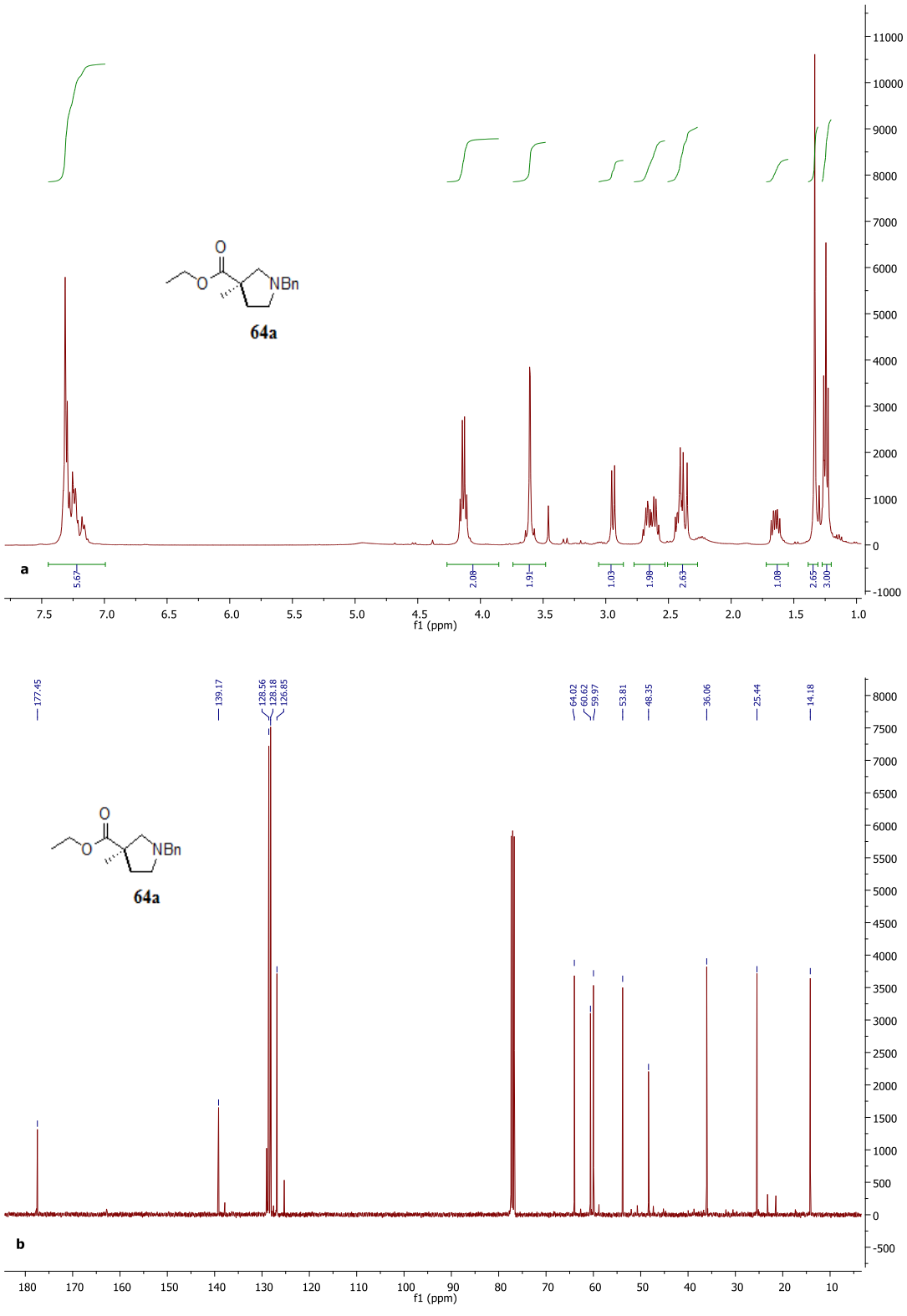
A.67. a) ^{1}H NMR of **62b**, b) ^{13}C NMR of **62b**



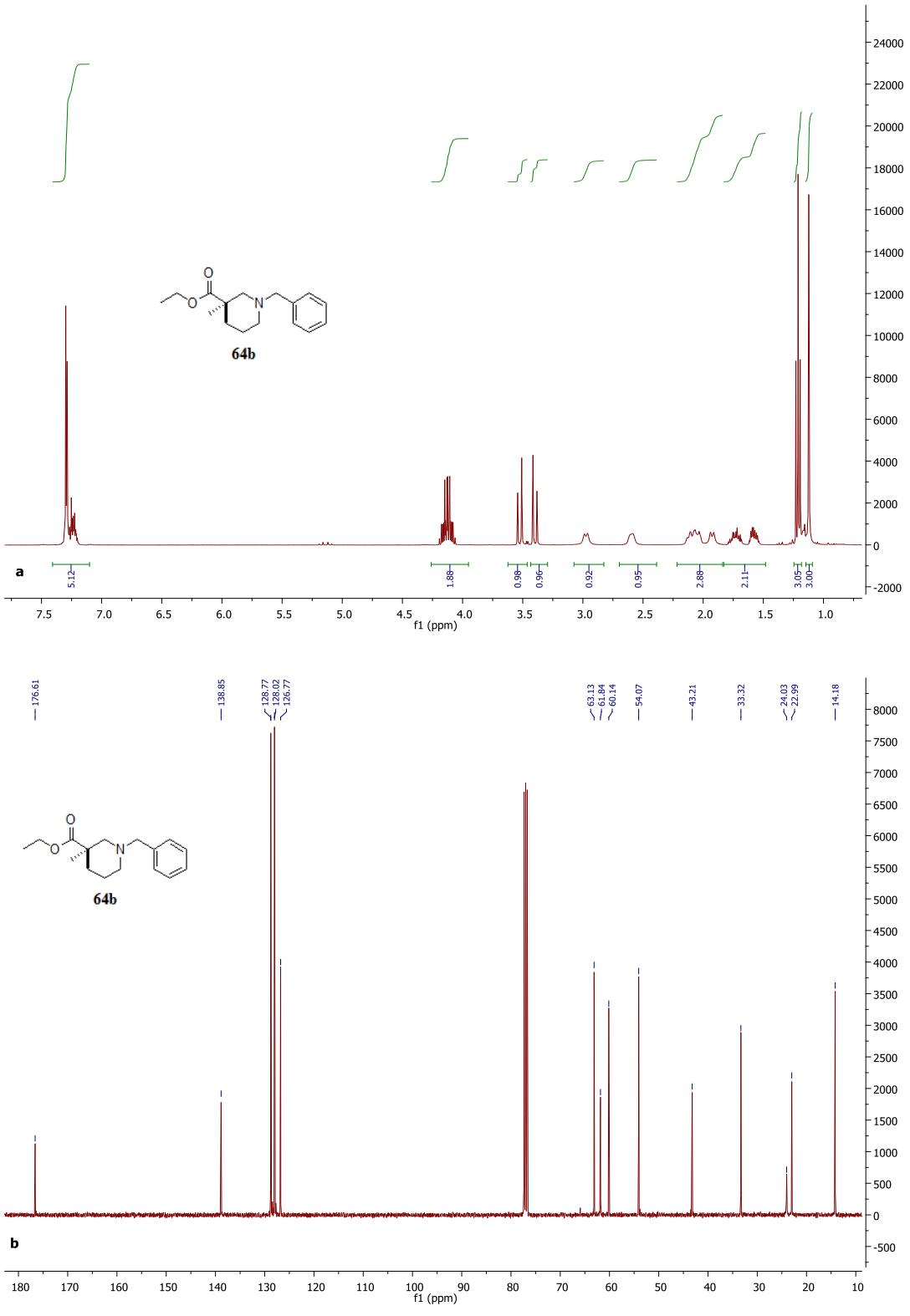
A.68. a) ^{1}H NMR of **63a**, b) ^{13}C NMR of **63a**



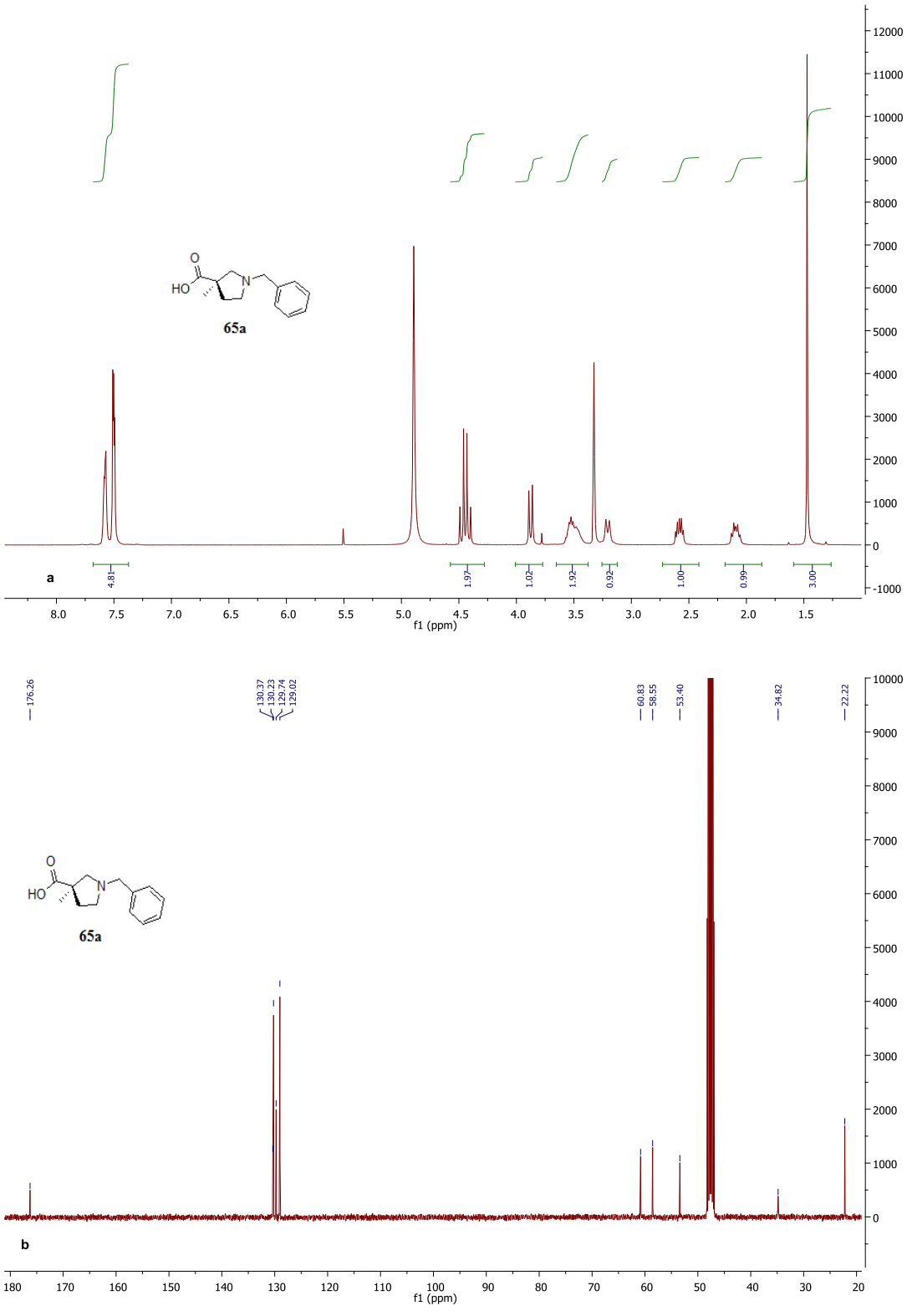
A.69. a) ^{1}H NMR of **63b**, b)^{13}C NMR of **63b**



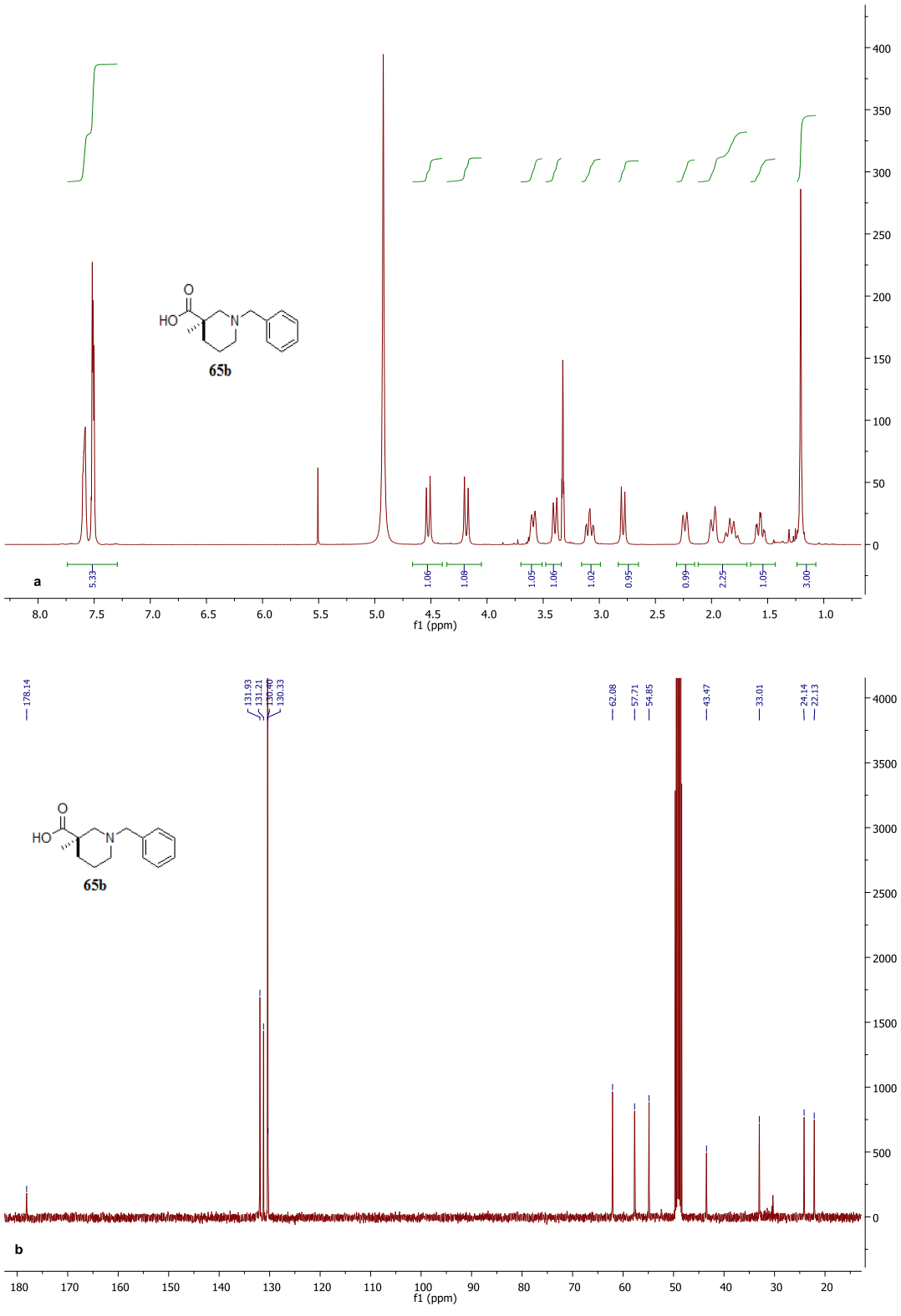
A.70. a) ^{1}H NMR of **64a**, b) ^{13}C NMR of **64a**



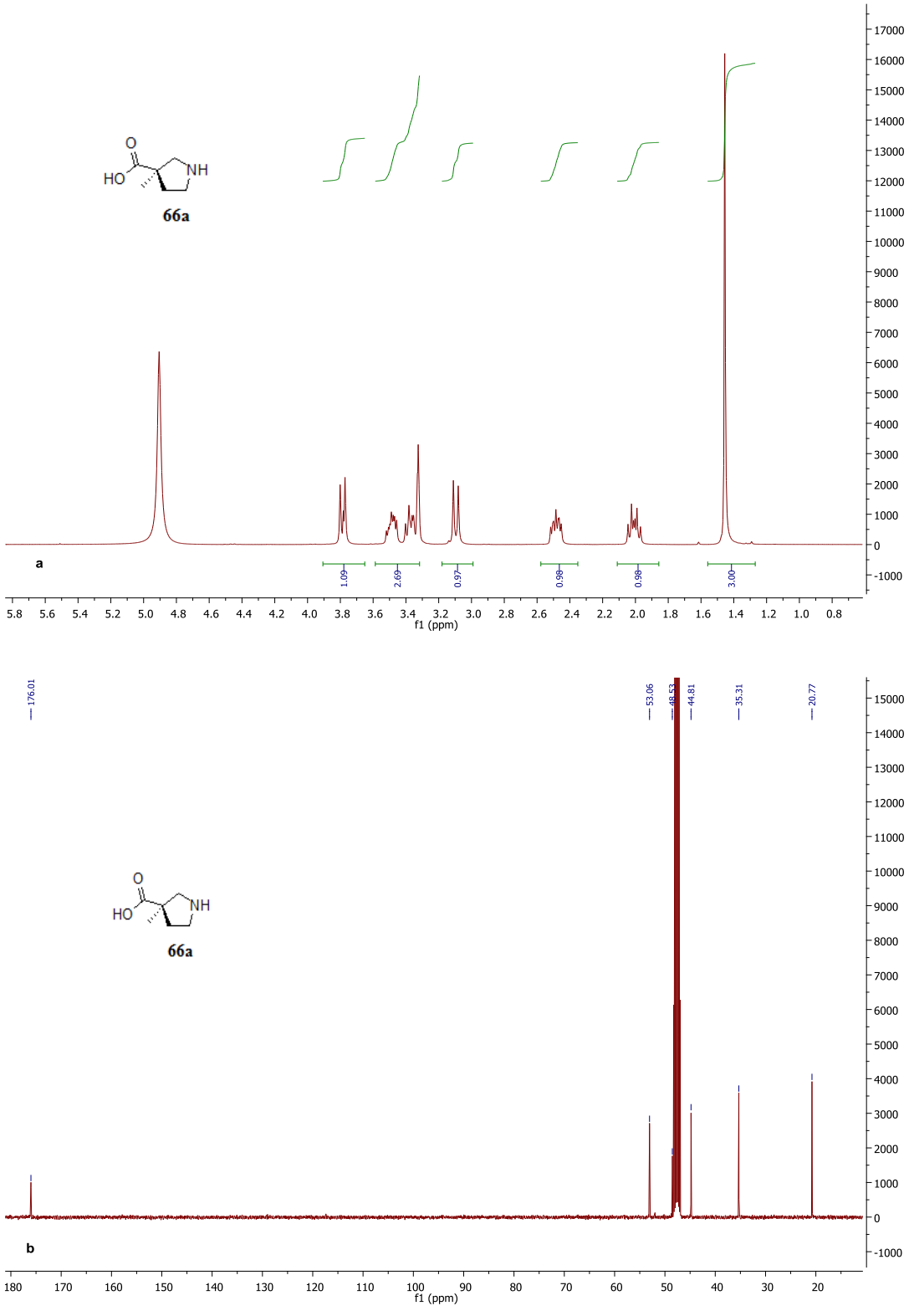
A.71. a) ^{1}H NMR of **64b**, b) ^{13}C NMR of **64b**



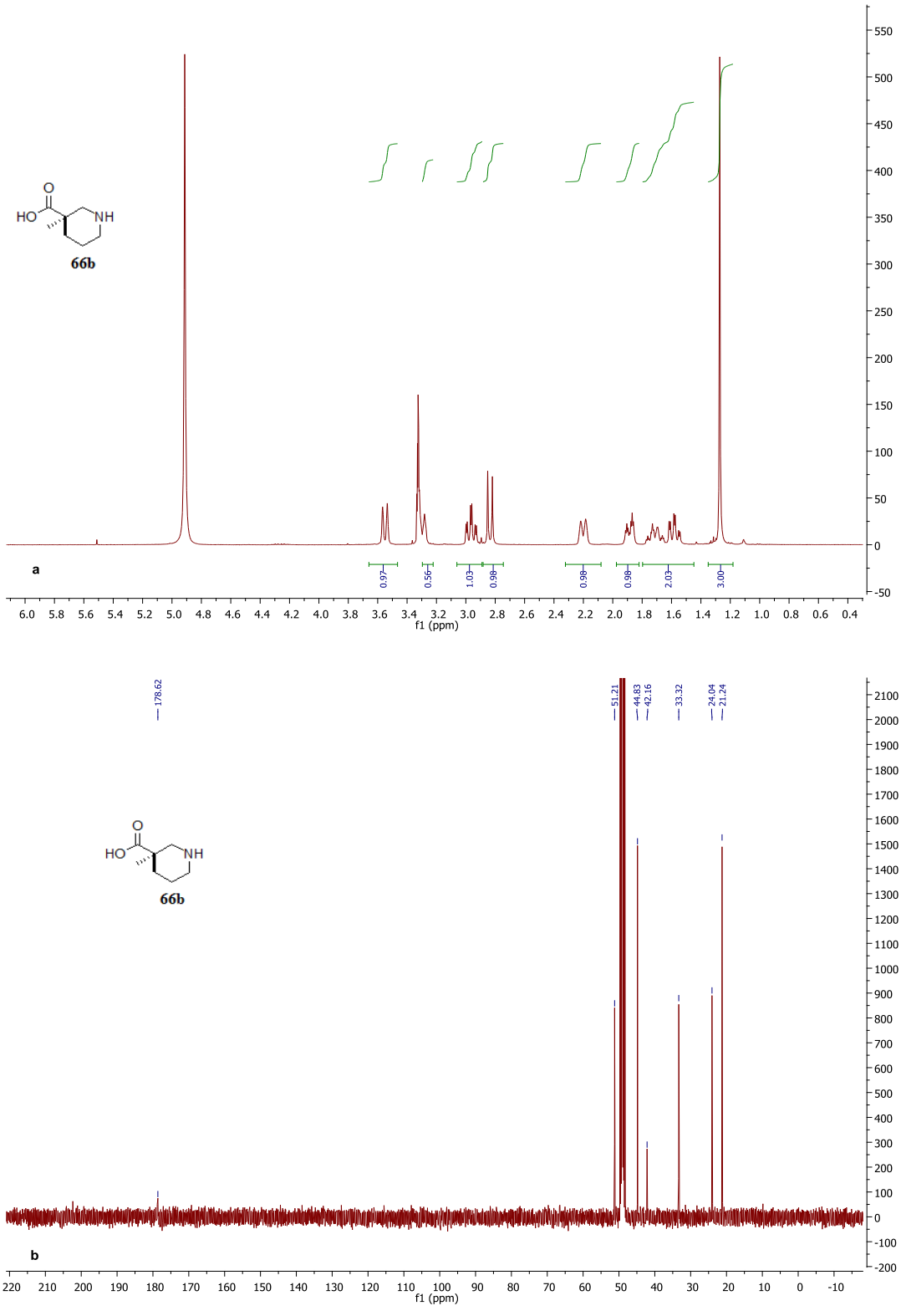
A.72. a) ^{1}H NMR of **65a**, b) ^{13}C NMR of **65a**



A.73. a) ^{1}H NMR of **65b**, b) ^{13}C NMR of **65b**



A.74. a) ^{1}H NMR of **66a**, b) ^{13}C NMR of **66a**



A.75. a) ^{1}H NMR of **66b**, b) ^{13}C NMR of **66b**.

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