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Synthesis of the western hemisphere of theonellamide C

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SYNTHESIS OF THE WESTERN HEMISPHERE OF THEONELLAMIDE C

A Dissertation

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To My Family…

To my parents, Jagdish and Sushila Yadav, for believing in and supporting all my life pursuits.

To my husband Prathivind Bejgum, whose unconditional love inspires me to fulfill my academic milestones.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS AND SYMBOLS

Å	angstrom
AA	amino acid
AAA	α-amino adipic acid
Aboa	(3*S*,4*S*,5*E*,7*E*)-3-amino-8-(4-bromophenyl)-4-hydroxy-6-methylocta-5,7-dienoic acid
Ac*	Acetol
Ahad	(2*S*,4*R*)-2-amino-4-hydroxyadipic acid
Alloc	allyloxycarbonyl
Apoa	(3*S*,4*S*,5*E*,7*E*)-3-amino-4-hydroxy-6-methyl-8-phenylocta-5,7-dienoic acid
BCB	*B*-bromocatecholborane
BINOL	1,1'-Bi(2-naphthol)
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BOP	benzotriazol-1-yloxytris(dimethylamino)-phosphoniumhexafluorophosphate
BORSM	based on recovered starting material
^{t}Bu	*tert*-butyl
° C	degrees Celsius
Cbz	carbobenzyloxy
CDI	carbonyldiimidazole
COSY	correlation spectroscopy
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
d.e.	diastereomeric excess
DIEA	*N,N*-diisopropylethylamine

DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DPPA	diphenylphosphoryl azide
e.e.	enantiomeric excess
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
eHyAsn	*erythro*-hydroxyasparagine
ESI	electrospray ionization
FK463	micafungin
Fmoc	9-fluorenylmethoxycarbonyl
τ-HAL	τ-histidinoalanine
HATU	*O*-(7-azabenzotriazol-1yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate
HBTU	*O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HIV	human immunodeficiency virus
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HyAsn	hydroxyasparagine
Hz	hertz
IC_{50}	half maximal inhibitory concentration
Me	methyl
MOA	mode of action
MoBY-ORF	molecular barcoded yeast open reading frame
MS	mass spectrometry
MVD1	mevalonate pyrophosphate decarboxylase
NIS	*N*-iodosuccinimide

NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
PMA	phosphomolybdic acid
ppm	parts per million
PPTS	pyridinium *p*-toluenesulfonate
PyBOP	benzotriaozol-1-yloxytri(pyrrolidino)phosphonium hexafluorophosphate
PyBroP	bromotris(pyrrolidino)phosphonium hexafluorophosphate
R_f	retention factor
rt	room temperature
s	singlet
q	quartet
SPPS	solid phase peptide synthesis
TBAF	tetra-*n*-butylammonium fluoride
TBS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Trt	triphenylmethyl
Troc	2,2,2-trichloroethoxycarbonyl
UV	ultraviolet

Standard 3 letter codes are utilized throughout the document for amino acids

ABSTRACT

Theonellamides A-F were isolated from *Theonella swinhoei* by Fusetani and co-workers. Despite considerable synthetic effort to produce theonellamide F by the Shioiri group in the early 1990's, the total synthesis of a theonellamide has yet to be reported. We report herein our efforts toward some of the required amino acid residues and construction of the western ring of theonellamide C.

We describe the synthesis of an uncoded amino acid, (2*S*,4*R*)-ɣ-hydroxy-α-amino adipic acid (Ahad), a building block for theonellamide C. We initially investigated the Corey-Lygo method for the catalytic asymmetric generation of the *C*α stereocenter. Unfortunately, the alkylation of glycine benzophenone imines with a series of electrophiles, including [(*S*)-benzyl-3-(*tert*-butyl dimethylsilyl)oxy]-4-iodobutanoate (**104a**), was unsuccessful due to low inherent reactivity and steric hindrance.

A second approach was based on MacMillan's enantioselective organocatalytic amine conjugate addition. Addition of CbzNHOTBS (**136a**) to (*E*)-benzyl-4-oxo-but-2-enoate (**149**), in the presence of catalytic (*S*)-2-diphenyl[trimethylsilyl)oxy]methylpyrrolidine (**165**) led to β-amino aldehyde (*S*)-benzyl 2-[(benzyloxycarbonyl)(*tert*-butyldimethylsilyloxy)amino-4-oxo-butanoate, (**148a**). A Wittig reaction between **148a** and methyl-(triphenylphosphoranylidene)-acetate (**142**) afforded (2*S*,4*E*)-1-benzyl-2-[(benzyloxycarbonyl)(*tert*-butyldimethylsilyloxy)-amino-6-methyl-hex-4-enedioate (**147a**). The ɣ-hydroxy group was installed intramolecularly with 4:1 *d.r.* by treatment of **147a** with TBAF. Hydrogenolysis was carried out to give unprotected Ahad.

The next challenge was to find the right combination of protecting groups for Ahad that would be compatible with the synthesis of the western hemisphere of theonellamide C. We performed model studies on α-aminoadipic acid (α-AAA) which revealed that the δ-COOMe, introduced in the Wittig reaction, was incompatible with a *C*α-acetol ester and a *N*α-Alloc

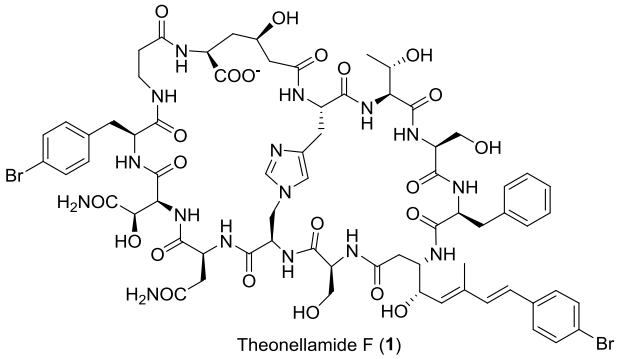
carbamate. α-AAA and Ahad bearing a δ-COO^{t}Bu group were synthesized in an analogous fashion. The Ahad building block was synthesized in 11 steps and 6.5% overall yield. In readiness for coupling; the *tert*-butyl ester was cleaved to afford the free δ-acid.

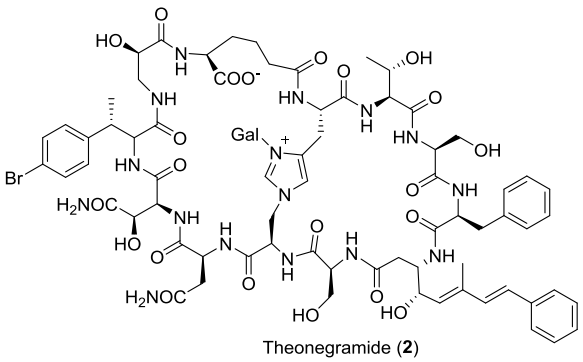
Two tetrapeptides were assembled: Boc-Asn-HyAsn(OTBS)-Phe-β-Ala-OAll (**249**) and the simplified Fmoc-Asn(Trt)-Asn(Trt)-Phe-β-Ala-OAll (**254**). Removal of the *N*-terminal Fmoc-group from **254** and coupling with a suitably protected τ-histidinoalanine acid afforded a linear hexapeptide that is a precursor to the western hemisphere of theonellamide C.

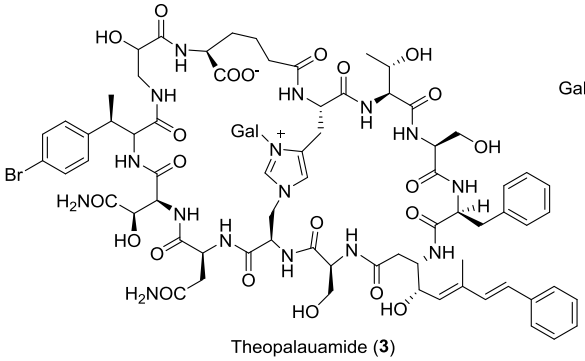
CHAPTER 1: THEONELLAMIDES A-F: INTRODUCTION

1.1 Occurrence of Theonellamides

Sponges are widely found across the world and appeared more than six million years ago. Bergmann's pioneering work in the 1950s on the isolation of sterols and novel nucleosides made sponges a target for extensive studies to isolate new substances.[1] These research activities led to the discovery of a variety of new compounds, often structurally novel and highly biologically active metabolites.[2] Sponges of the family Lithistida, which include the genera *Discodermia* and *Theonella,* are a prominent source of bioactive secondary metabolites. These secondary metabolites include macrolides such as swinholide A,[3] along with cyclic and linear peptides such as cyclotheonamides,[4] onnamides[5] and theonellamides.[6]







Gal = β-*D*-Galactose

Figure 1.1 Chemical structures of theonellamide F, theonegramide and theopalauamide.

Matsunaga *et al*. first reported the isolation and characterization of theonellamide F (**1**) (Figure 1.1) in 1989[6] and then theonellamides A-E in 1995[7] as bicyclic dodecapeptides from a *Theonella* sp. collected off Hachijo-jima Island in Japan. Subsequently, Bewley and Faulkner isolated the closely related compound theonegramide (**2**) in 1994,[8] followed by theopalauamide (**3**) in 1998[9] from the same marine species.

Early studies by Bewley and co-workers reported that a number of symbiotic bacteria live in association with *Theonella swinhoei*.[10,11] The sponge contains four cell populations: sponge cells, unicellular heterotrophic bacteria (eubacteria), unicellular cyanobacteria and filamentous heterotrophic bacteria (filaments). All populations of associated bacteria are located extracellularly. The unicellular cyanobacterium *Aphanocapsa feldmanni* has been the only species identified based on ultrastructural studies. The unicellular cyanobacteria occur only in the ectosome (cortical part of sponge) while the filamentous bacteria reside only in the endosome (membrane-bounded compartment inside the sponge). Heterotrophic eubacteria and sponge cells occur in both endosome and ectosome.

The separation of four cell populations was followed by dissociation and differential centrifugation of the cell suspension which led to cell types of >90% purity (Figure 1.2). Analysis of the four cell fractions by HPLC and ^{1}H NMR spectroscopy showed that the unicellular cyanobacteria and sponge cells lacked bioactive metabolites. The polyketide swinholide A (**4**) was present in the fraction of eubacteria and theopalauamide (**3**) occurred in the filamentous bacteria.

Theonellamides were isolated in limited quantities from nature. From 15 kilograms of sponge, Fusetani and coworkers isolated: theonegramides A (200 mg), B (19 mg), C (32 mg), D (14 mg), E (30 mg), and F (500 mg).[6,7] Therefore, collaborative investigations have focused on theonellamides A and F in a variety of assays.

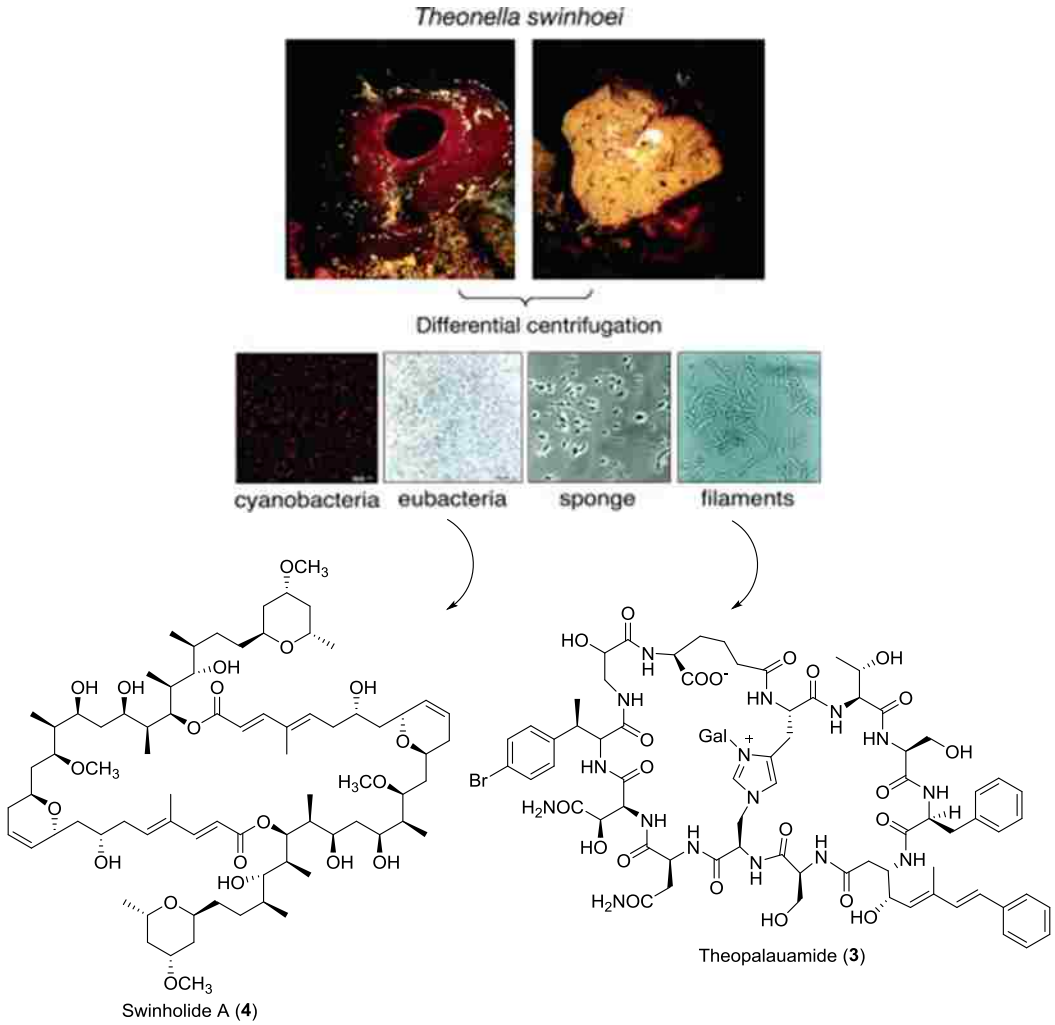


Figure 1.2 Swinholide A and theopalauamide are derived from different types of cells in *Theonella swinhoei*. Copyright 1998, John Wiley and Sons, reprinted with permission (p. 219).

1.2 Structural Characteristics of Theonellamides

Theonellamides A-F are characterized by a bridging τ-histidinoalanine (τ-HAL) residue. There are some other features common to all family members. All contain *allo*-threonine, serine and phenylalanine at positions AA1-AA3 respectively and serine, asparagine and *erythro*-β-hydroxyasparagine at positions AA5-AA7. Also, at the AA4 position there is (5*E*,7*E*)-3-amino-4-hydroxy-6-methyl-8-phenyl-5,7-octadienoic acid (Apoa) or its 4'-brominated derivative (Aboa). In position AA10 is (2*S*,4*R*)-α-amino-ɣ-hydroxy adipic acid (Ahad) or α-amino adipic acid (AAA).

There are further structural variations including glycosylation. Theonellamide A bears a β-*D*-galactose covalently linked to the imidazole π-nitrogen and isoserine at AA9 instead of β-alanine. Theonellamides D and E contain a β-*D*-arabinoside and β-*D*-galactoside respectively. There is *p*-bromophenylalanine (BPA) in theonellamides D-F and β-methyl-*p*-bromophenylalanine (BMPA) in theonellamides A and B at position AA8, whereas theonellamide C contains unmodified phenylalanine (Figure 1.3).

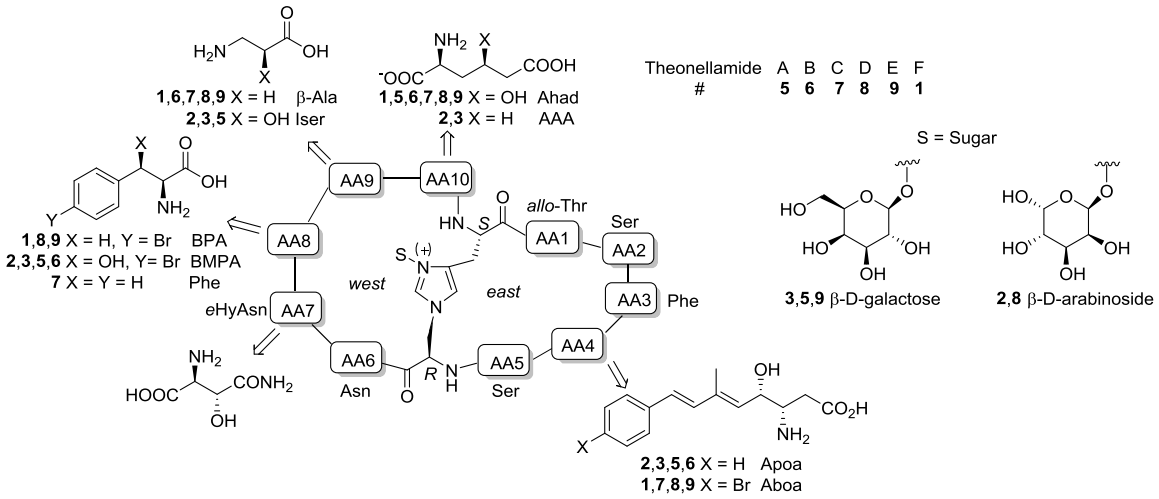


Figure 1.3 Amino acid composition of theonellamides A-F, theonegramide and theopalauamide.

Details of the structure elucidation of *e*HyAsn have been described in Douglas Wong's dissertation.[12] The origins and structure determination of Ahad will be detailed in Chapter 2.

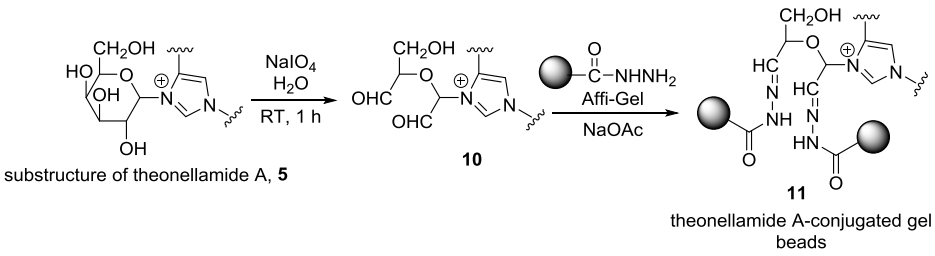
1.3 Initial Biological Studies

Initial studies by Matsunaga and co-workers on theonellamides A-F showed that they are antifungal and cytotoxic agents. They demonstrated cytotoxicity against P388 murine leukemia cells, with IC_{50} values of 5.0, 1.7, 2.5, 1.7, 0.9 and 2.7 μg/mL respectively.[7] Theonellamide E (**9**) was shown to be the most cytotoxic. Glycosylation seems to have little effect on the cytotoxicity of the theonellamides. In addition, congener F was toxic to L120

leukemia cells with an IC_{50} value of 3.2 μg/mL. Theonellamide F (**1**) also showed activity against fungi of *Candida* spp., *Trichophyton* spp., and *Aspergillus* spp.

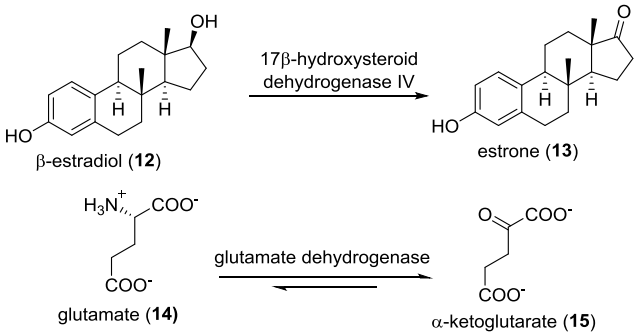
In later studies, Watabe *et al*. reported that theonellamide F induced the formation of vacuoles (fluid-filled compartments in the cytoplasm) in 3Y1 rat embryonic fibroblasts.[13,14] At higher concentrations, theonellamide F induced not only the formation of large vacuoles equivalent to the size of a cell, but also remarkable morphologic changes. The theonellamide F induced vacuoles contain an ATPase and thus maintain a slightly acidic pH. The disturbance of intralysosomal (within a lysosome) pH affects the function of lysosomal proteases that are responsible for autophagic degradation of organelles. The formation of vacuoles is a well-known marker of microbial activity that often precedes cell autophagy, although the mechanism is not well understood. Theonellamides are the first low molecular weight compounds to induce extraordinarily large vacuoles and thus are good molecular probes for studies on intracellular membrane structures.

The novelty of the molecular architecture of these theonellamides prompted Wada *et al*.[15] to probe whether they bind to proteins. They immobilized theonellamide A (**5**) to affinity gel beads and screened for binding to proteins in rabbit liver. As a prerequisite to this screening process, theonellamide A (**5**) was treated with sodium periodate, cleaving the vicinal 2,3- and 3,4-diols of the β-D-galactose and liberating aldehydic handles for the covalent attachment of the molecule to beads. Compound **10** was then reacted with hydrazine-containing Affi-Gel® beads to give TNM A-conjugated gel beads **11** (Scheme 1.1). Rabbit liver tissue extracts were exposed to the TNM A-conjugated gel beads for two hours and two major proteins were found to bind to the beads.



Scheme 1.1 Preparation of theonellamide A - conjugated gel beads.

The proteins which bound to the beads were identified as 17β-hydroxysteroid dehydrogenase IV and glutamate dehydrogenase (Scheme 1.2). 17β-Hydroxysteroid dehydrogenases mediate interconversion of estrone and estradiol and type IV of this enzyme oxidizes estradiol (**12**) to estrone (**13**). Theonellamide activates the conversion of α-ketoglutarate (**15**) from glutamate (**14**) by glutamate dehydrogenase, but has no effect on the reverse reaction.



Scheme 1.2 Function of 17β-hydroxysteroid dehydrogenase IV and glutamate dehydrogenase.

The effect of theonellamides on 17β-hydroxysteroid dehydrogenase IV might be the cause of its cytotoxicity. The studies described above, probing the origin of the metabolite, and its possible roles in biology, reflect the novelty of the molecular architecture.

1.4 More Recent Biological Studies: Chemical Genetics

A central problem in chemical biology is to determine the mode-of-action (MOA) of new compounds. Rich functional information has been obtained from scoring ~5000 viable yeast

haploid deletion mutant strains for hypersensitivity to a diverse set of compounds, a process termed chemical genetic profiling. Cells with gene deletions that are deemed hypersensitive to a specific compound can give clues about its MOA. In the yeast *Saccharomyces cerevisiae*, ~6000 potential genes have been characterized by the genome sequencing project.

With this genetic profiling method Boone *et al*.[16] found that two extracts containing natural products stichloroside (**16**) (Figure 1.4) and theopalauamide (**3**) showed very similar chemical-genetic profiles (correlation coefficient of 0.892). Thus, these compounds shared a common MOA in yeast and this illustrates that chemical-genetic profiling is an effective means of functional classification of natural product extracts. Moreover, stichloroside-resistant mutants also displayed theopalauamide resistance, suggesting that the compounds function similarly.

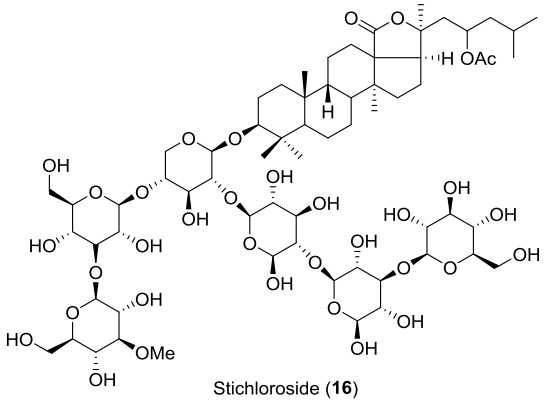
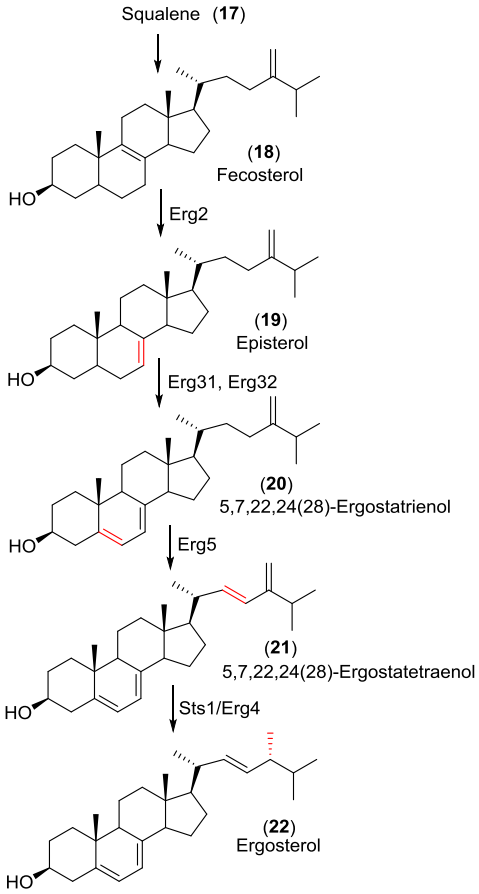


Figure 1.4 Structure of stichloroside.

There are limitations to these assays. Haploinsufficiency is the state of a diploid organism having only a single copy of a particular gene, the other copy being inactive due to mutation. An approach based on this status is unlikely to identify a drug target that is the product of an enzyme rather than the enzyme itself. Moreover, these protocols (one or the other) based upon clustering of chemical-genetic profiles are difficult to implement because they depend upon the assembly of a relatively large compendium of reference profiles. Hence, Ho *et al*.[17] developed a new strategy by generating a library of molecular barcoded yeast open

reading frames (MoBY-ORF) to clone wild-type versions of mutant, drug-resistant genes by complementation using a minimal amount of bioactive compound. Each gene of this MoBY-ORF library is controlled by its native promoter and terminator. Cloning by complementation with the MoBY-ORF library is an assay that can be carried out with any *S. cerevisiae* strain.

Using the MoBY-ORF complementation assay, they identified an essential enzyme involved in an early step of the ergosterol biosynthesis pathway: mevalonate pyrophosphate decarboxylase (MVD1).



Scheme 1.3 Ergosterol biosynthesis pathway.

A theopalauamide-resistant mutant (*theo*^{R}) was made to determine whether or not the compound targets a product of the MVD1 pathway. The *theo*^{R} mutant was partially resistant to amphotericin B (a sterol-binding antifungal compound). Experiments showed that deletion

mutants *erg2Δ*, *erg31Δ* and *erg32Δ*, which participate in the second and third steps of ergosterol biosynthesis, were resistant to theopalauamide.

To test the hypothesis that theopalauamide (**3**) and stichloroside (**16**) bind to ergosterol (**22**), exogenous ergosterol was added to cultures containing toxic levels of these compounds alongside amphotericin B (**23**), a polyene antifungal drug, and ketoconazole (**24**) (Figure 1.5), another antifungal compound that inhibits Erg11p, different ergosterol biosynthetic enzyme. Exogenous ergosterol rescued the toxicity of theopalauamide, stichloroside and amphotericin B, but had no effect on ketoconazole's toxicity. This observation suggested that, like amphotericin B, both theopalauamide and stichloroside may interact on a molecular level with ergosterol.

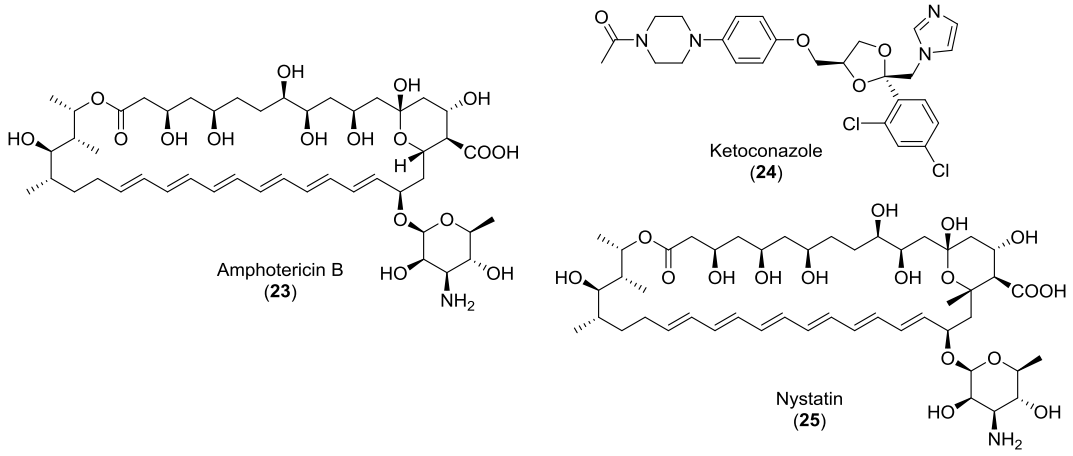


Figure 1.5 Structures of amphotericin B, ketoconzole and nystatin.

The Ho Group also studied the effect of these compounds using fluorescent markers released from phosphatidylcholine liposomes that contained various levels of ergosterol to show that theopalauamide and stichloroside interact with ergosterol. Theopalauamide (10 µg/mL) caused a maximal 30% leakage of calcein from liposomes containing 20% ergosterol in their membrane, whereas it had no effect on liposomes lacking ergosterol. Stichloroside (**16**) and nystatin (**25**) also caused ergosterol-enhanced leakage, and were more potent than theonellamide. Thus, theopalauamide, stichloroside and nystatin disrupt ergosterol-containing membranes specifically.

A similar experiment showed that fluorescently labeled theonellamide A bound to ergosterol specifically in an *in vitro* lipid-binding assay. Theonellamide A (**5**) was conjugated to AMCA to give compounds described by structure **26** (Figure 1.6). Aminomethyl coumarin acetate (AMCA) is a blue-fluorescent dye used to label antibodies, proteins and other molecules for fluorescence imaging.

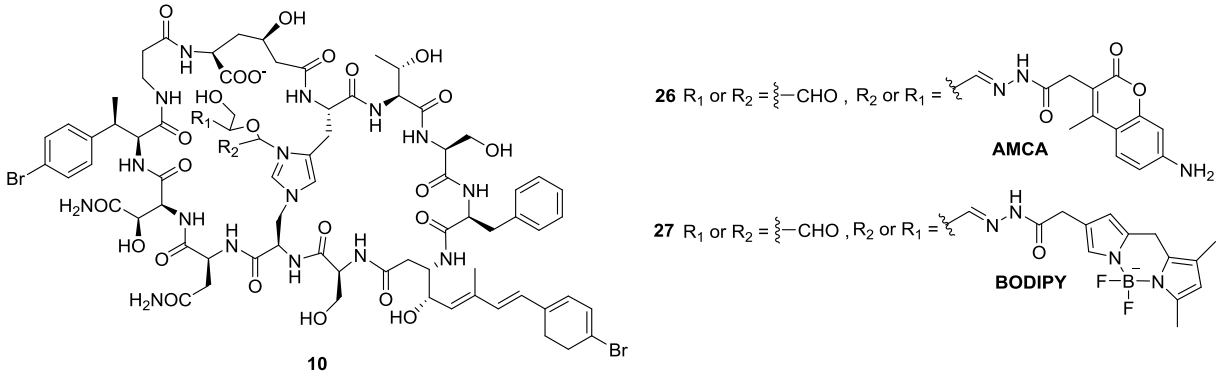


Figure 1.6 Structure of theonellamide A-fluorophore conjugates.

The theopalauamide-resistant yeast strain (*theo*^{R}) was also resistant to theonellamide A, previously established to be toxic to wild-type *S. cerevisiae*.[16,17] Ho *et al*.[17] demonstrated that fluorescently labeled theonellamide A stained the cell surface of wild-type budding yeast, but only weakly stained the plasma membrane of *theo*^{R} mutant cells. All these studies suggest that theopalauamide and theonellamide A represent a novel class of sterol-binding compound and behave similarly.

The specificity of lipid-binding properties and the mechanism of their antifungal activity remained ambiguous. In 2010 Yoshida and co-workers[18] reported on the production of theonellamide affinity beads by a photo-affinity immobilization method (similar to the earlier work by the Wada group, Scheme 1.1) and tried to identify the binding proteins from fusion yeast cells. They were unable to detect any proteins that specifically bound to theonellamide F. Therefore, they decided to search for chemical-genetic interactions using a set of strains expressing all 5000 protein-coding ORFs of the fission yeast *Schizosaccharomyces pombe*,

which might provide insights into the genes and pathways targeted by general bioactive metabolites.

They generated a chemical-genomic profile of theonellamide F using a collection of fission yeast strains in which each open reading frame is expressed under the control of an inducible promoter. Strains showing a significantly altered sensitivity compared to the control strain were selected. A total of 20 profiles were obtained and analyzed by 2D-hierarchical clustering analysis. They found a weak correlation between amphotericin B (**23**) and nystatin (**25**) (correlation = 0.18 for each of these compounds with respect to theonellamide F), implying that the MOA of theonellamide F is only partially shared with these sterol-binders. The most sensitive strain of yeast overexpressed *SPAC26A3.09*, which encodes a homolog of Rho-type GTPase activating protein Rga2, a small GTPase that plays a role in a signaling pathway of cell polarity and in 1,3-β-*D*-glucan synthesis in fission yeast. The second most sensitive strain overexpressed *pck1*, which encodes a protein kinase C homolog that regulates 1,3-β-*D*-glucan synthesis. 2D-Hierarchical clustering analysis showed a modest linkage (correlation coefficient = 0.35) between theonellamide F and FK463 (**28**) (Figure 1.7), a frontline clinical antifungal drug (common name Micafungin®) that inhibits the synthesis of 1,3-β-*D*-glucan.

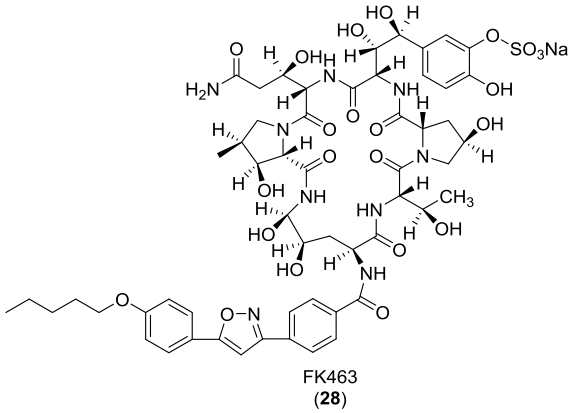


Figure 1.7 Chemical structure of FK463 (**28**).

Of the 32 genes from generated chemical-genomic profile of theonellamide F, 12 were in common with those affected by FK463, suggesting that the MOA of theonellamide F and FK463 are functionally related. They compared the morphology of cells after exposure to each drug. Cell lysis was observed at the growing ends in fungi treated with FK463, whereas theonellamide F did not lead to any signs of cell lysis. Unexpectedly, calcofluor white (Cfw) staining showed strong signals due to increased 1,3-β-*D*-glucan at the growing ends and/or the medial region of the cell treated with theonellamide F. Thus, theonellamide F appears to counteract FK463 action by enhancing 1,3-β-*D*-glucan synthesis.

Further experimental results suggested that the major pathway for 1,3-β-*D*-glucan synthesis upon theonellamide F treatment is the direct activation of Bgs1 (1,3-β-D-glucan-UDP glucosyltransferase) by Rho1. Theonellamide A (**5**) was fluorescently labeled as its TNMF-BF derivative **27** (TNMF-BF = theonellamide F-boron-dipyrromethane conjugate) by Yoshida and co-workers[18] to determine the subcellular localization of the theonellamide-binding molecule (Figure 1.6). The *in vitro* binding assay revealed that TNM-BF (**27**, Figure 1.6) recognizes ergosterol (**22**), cholesterol (**29**), cholestanol (**30**) and 5α-cholest-7-en-3β-ol (**31**) (Figure 1.8). Thus, theonellamide F recognizes 3β-hydroxysterols, a class of lipid molecules, rather than a protein.

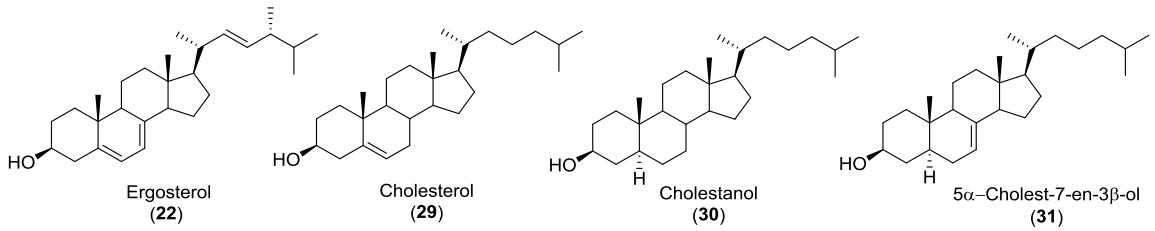


Figure 1.8 Structures of 3β-Hydroxysterols.

Mutations to genes in the ergosterol biosynthetic pathway (Scheme 1.3) have shown sensitivity to polyene antibiotics in yeast. Mutants lacking the enzymes Erg2, or simultaneous deletion of Erg31 and Erg32, displayed high tolerance to theonellamide F and a decreased

ability of the cells to bind theonellamide F. Deletion of other enzymes (Erg5, Δsts1/Erg4), resulted in slight resistance to theonellamide F. Calcein (a fluorescent dye) was added to *S. pombe* cells that had been treated with theonellamide F for nine hours. Entry of calcein over the plasma membrane was observed, suggesting that cells do not retain their membrane integrity in the presence of thenellamide F. Moreover, calcein diffusion into theonellamide F treated cells gradually increased over time and in a dose-dependent manner.

The MOA of theonellamide F is different from that of the polyene antibiotics. A typical morphological change in yeast cells after treatment with polyene antibiotics is enlargement of vacuoles whereas the vacuoles of theonellamide F-treated cells became marginally fragmented. This finding is in contrast with earlier rat fibroblast experiments where theonellamides caused the formation of extremely large vacuoles (Section 1.3). The other characteristic aspect of polyene antibiotics is their acute fungicidal effect while theonellamide F showed gradual time dependent toxicity. In agreement with Ho and co-workers, Yoshida concluded that the theonellamides represent a new class of sterol-binding agents.

In 2013, Yoshida *et al*. published new results from surface plasmon resonance (SPR) and solid-state ^{2}H NMR experiments leading them to postulate a mechanism for sterol recognition by theonellamide F in lipid bilayers.[19] Earlier studies by the same research group had evaluated the binding of amphotericin B (**23**) [20] and amphidinol[21] to palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (**32**, POPC) liposomes. They performed analogous experiments with theonellamide A, studying the interaction of theonellamide A with POPC liposomes containing ergosterol (**22**), cholesterol (**29**), or epicholesterol (**33**) (Figure 1.9). Surface plasmon resonance (SPR) revealed that the incorporation of 10 mole percent cholesterol or ergosterol into POPC membranes significantly enhances the affinity of theonellamide F for the membrane, particularly initial binding to the membrane surface. Conversely, binding of the peptide to epicholesterol (diastereomer of cholesterol, varying in configuration at C3) liposomes and pure POPC

liposomes was weak by comparison. This demonstrated the preference of the peptide for the 3β-hydroxysterol-containing membranes.

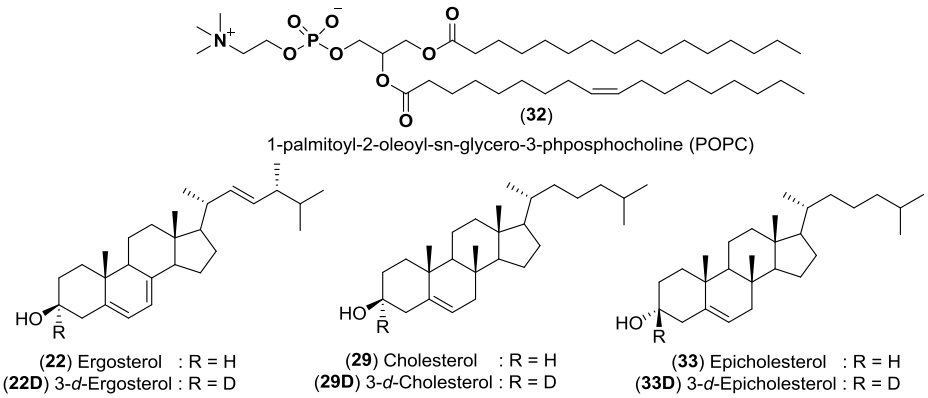


Figure 1.9 Chemical structure of POPC, cholesterol, ergosterol and epicholesterol.

Yoshida *et al.* also conducted solid-state ^{2}H NMR experiments using cholesterol, ergosterol and epicholesterol. These compounds were labeled by deuterium at C3 and incorporated into POPC liposomes in the absence and presence of theonellamide A. The axial rotation in NMR for sterol molecules in lipid bilayers undergoes fast lateral diffusion and gives quadrupolar splitting which depends on the tilt angle of the C–^{2}H bond with respect to the rotation axis and the wobbling of the molecule. Experiments conducted using 3-*d*-cholesterol (**29D**) and 3-*d*-ergosterol (**22D**) showed similar results. In the absence of peptide (Figure 1.10 **A** and **C**), a characteristic triplet peak was observed, indicating fast rotational motion of the sterol in the POPC bilayers.

However, a stark attenuation of the splitting signal was observed upon addition of the peptide (Figure 1.10 **B** and **D**), suggesting that the molecular rotation falls into an intermediate motional speed with correlation times of 10^{-4} to 10^{-5} seconds. On the contrary, the characteristic splitting didn't change much for 3-*d*-epicholesterol (**33D**) containing POPC liposomes (Figure 1.10 **E** and **F**). The ^{2}H NMR experiments showed that theonellamide A inhibits the fast rotational motion of cholesterol and ergosterol.

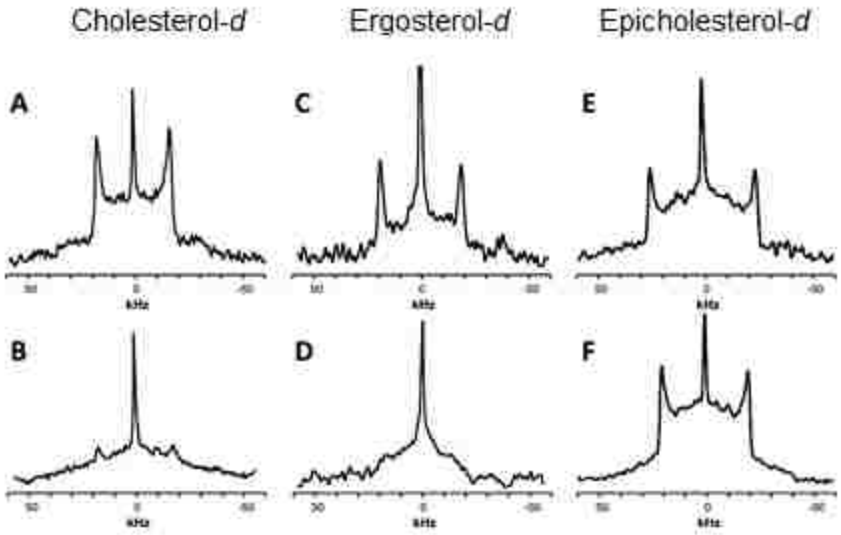


Figure 1.10 ^{2}H NMR spectra of 3-*d*-sterol incorporated into POPC bilayers in the absence (**A**, **C**, and **E**) and presence (**B**, **D**, and **F**) of TNM-A. 3-*d*-Cholesterol (**A** and **B**), 3-*d*-ergosterol (**C** and **D**), and 3-*d*-epicholesterol (**E** and **F**). Copyright 2013, ACS Publications reprinted with permission (p. 220).

On this background of intriguing biological activity, the theonellamides present a worthy target for total synthesis. They present a formidable chemical challenge that requires the asymmetric synthesis of unusual amino acids and their assembly into a demanding bicyclic array.

1.5 Previous Studies Directed Toward the Synthesis of Theonellamides

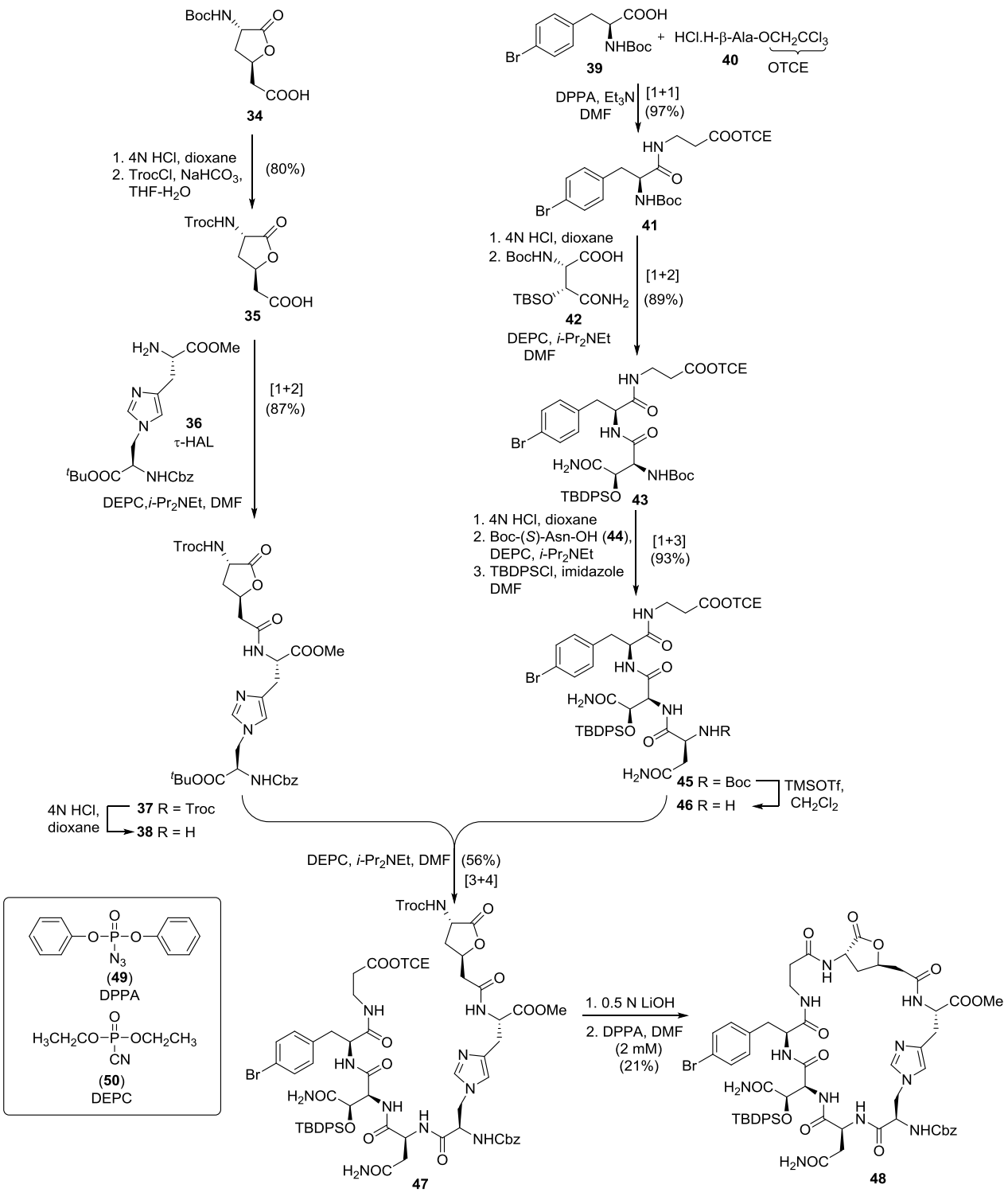
Nearly two decades ago, Tohdo, Hamada and Shioiri reported progress towards the synthesis of theonellamide F in the form of conference proceedings and communications.[22,23,24,25] The synthesis has never been completed. They formed the two rings independently but never described plans for the formation of the bicycle. They presumably targeted theonellamide F because it was the first structure to be revealed; none of the other theonellamides have been the subject of synthetic studies.

In 1994, Tohdo *et al*. published a short letter on their efforts toward the synthesis of what they termed as the northern hemisphere* of theonellamide F.[24] The synthesis of pertinent building blocks will be discussed in relevant chapters of this dissertation. The construction of the western hemisphere was carried out by a fragment condensation approach (Scheme 1.4).

Synthesis of the Ahad-HAL tripeptide is illustrated on the left hand side of Scheme 1.4. At the outset, the protecting group of the Ahad α-amine was switched from Boc (compound **34**) to Troc (compound **35**). This was done in order that both protecting groups could be removed simultaneously from linear precursor **47** prior to cyclization. The δ-COOH of Ahad lactone **35** was coupled with the free amine of HAL **36** to give tripeptide **37**. In readiness for coupling, the *tert*-butyl ester was cleaved under acidic conditions to afford acid **38**.

The remaining tetrapeptide was assembled as shown in the right hand side of Scheme 1.4. *p*-Bromophenylalanine derivative **39** was coupled with the trichloroethyl (TCE) ester of β-alanine (**40**) to give dipeptide **41**. Elongation of the peptide in the C→N terminal direction was accomplished by Boc deprotection and homologation with *e*HyAsn building block **42**, giving tripeptide **43**. Analogous deprotection and coupling with Boc-Asn-OH (**44**) was accompanied by loss of the TBS ether under acidic conditions. The β-OH of the *e*HyAsn residue was reprotected as the more robust TBDPS ether to obtain compound **45**. In readiness for coupling, the *tert*-butyloxycarbonyl group was cleaved under Lewis acidic conditions to afford amine **46**. They used diphenylphosphoryl azide [DPPA, $(C_6H_5O)_2P(O)N_3$ (**49**)] and diethyl phosphorocyanidate [DEPC, $(C_2H_5O)_2P(O)CN$) (**50**)] for the various coupling reactions. These coupling reagents were introduced by Shioiri in the 1970's.[26] In readiness for coupling, the *tert*-butyloxycarbony group was cleaved under Lewis acidic conditions to afford amine **46**.

*They drew the molecule rotated 90° relative to representations in this dissertation. Thus their references to northern and southern hemispheres correlate with our western and eastern hemispheres respectively.



Scheme 1.4 Assembly of the western hemisphere of theonellamide F.

The tripeptide acid **38** was coupled with tetrapeptide amine **46** by using DEPC. The linear precursor **47** was treated with lithium hydroxide followed by cyclization with DPPA to give the western hemisphere of theonellamide F, *viz.* compound **48**. While these coupling reagents gave reasonable results for small peptides, more hindered couplings and the key cyclization steps were low yielding. The synthesis involves 10 steps (longest linear sequence) with an overall yield of 7.9%. Moreover, they incorporated the Ahad residue into the cyclic peptide in its lactone form. We believe that the Ahad residue should be incorporated into the cyclic peptides in the open chain form and that the rigidity of the lactone may have a contributing factor to the low-yielding cyclization of the western ring in the synthetic effort of Tohdo *et al*.

1.6 Research Goals of This Dissertation

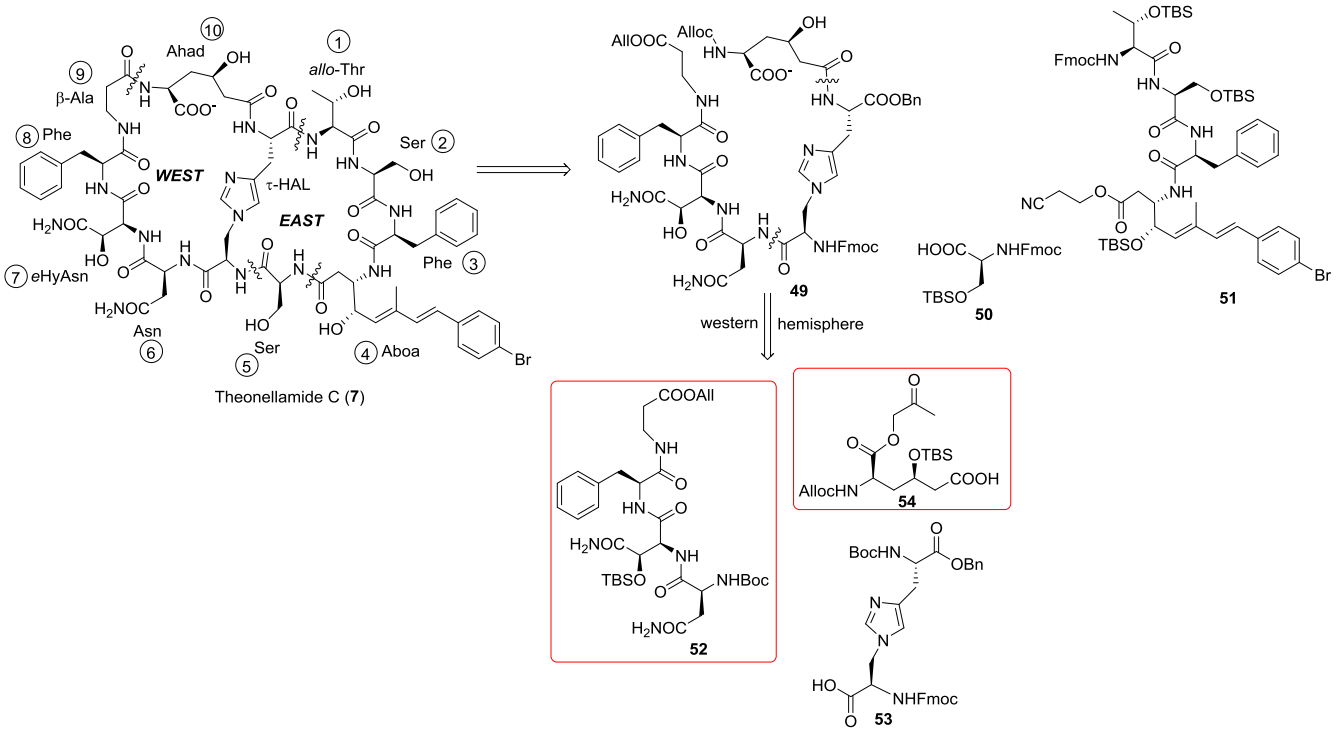
Despite considerable synthetic effort to produce theonellamide F by the Shioiri group in the early 1990s, a total synthesis of a theonellamide has yet to be reported. An efficient chemical synthesis of these compounds offers benefits such as an alternative to cultivation from natural sources and the potential to produce analogs and explore new ideas about their emerging biological role. We chose to synthesize theonellamide C (**7**) in our laboratory as it is the simplest congener to make, requiring the synthesis of the least number of amino acids, prior to fragment condensations and cyclizations.

My goal was to synthesize the western hemisphere of theonellamide C (**7**). In common with the other theonellamide congeners, theonellamide C (**7**) has ten amino acids and one *bis*-amino acid in total. The two hemispheres of theonellamide C (**7**), designated east and west, each have five amino acids and the bicycle is bridged by a τ-histidinoalanine (τ-HAL) residue.

1.7 Retrosynthetic Analysis

There are four amino acids in theonellamide C (**7**) that are not commercially available. These are Aboa ④, *e*HyAsn ⑦, Ahad ⑩, and τ-HAL. Since the major element of

conformational restraint in the molecule is the τ-HAL residue, we plan to construct this *bis*-amino acid and build on the two rings sequentially. It is desirable to perform fragment couplings with carboxy components that are not prone to epimerization at Cα; three residues provide this opportunity: β-Ala ⑨ and Ahad ⑩ in the western ring and Aboa ⑤ in the eastern ring. Capitalizing on the opportunities provided by these non-epimerizable residues, the major cyclization disconnection for the western hemisphere is between residues ⑨ and ⑩ (Scheme 1.5). This leads to cyclization precursor **49**. The deprotection of the Alloc and Allyl groups will be accomplished simuntaneously by using Pd(0) catalysys, followed by subsequent cyclization. Further disconnection of compound **49** leads to tetrapeptide **52**, τ-HAL (**53**) and Ahad (**54**) residues.



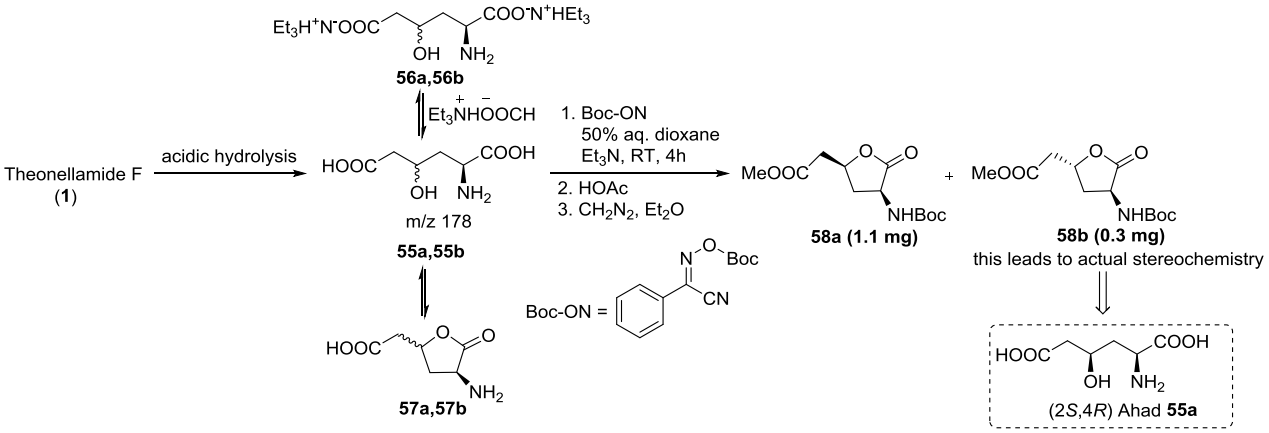
Scheme 1.5 Retrosynthesis of the western hemisphere of theonellamide C.

This dissertation will highlight the synthesis of the Ahad residue (Chapters 2, 3 and 4) and the ⑥-⑨ tetrapeptide (western hemisphere, Chapter 5) (both building blocks shown in red boxes) and peptide assembly for the western hemisphere of theonellamide C.

CHAPTER 2: INITIAL APPROACH TO (2*S*,4*R*)-α-AMINO-γ-HYDROXY ADIPIC ACID (AHAD): COREY-LYGO METHOD

2.1 Structural Determination of (2*S*,4*R*)-α-Amino-γ-Hydroxy Adipic Acid (Ahad)

Theonellamide F (**1**) showed UV maxima at 283 nm, 294 nm and 315 nm.[6] Upon acid hydrolysis with 6N HCI at 110 °C for 16 hours compound **1** generated several ninhydrin-positive spots on TLC. The hydrolysate was dissolved in triethylamine/formic acid buffer (pH 3.41) and subjected to HPLC. This HPLC analysis of the hydrolysate indicated the presence of asparagine (Asn), *allo*-threonine (*a*Thr), phenylalanine (Phe), β-alanine (β-Ala) and two residues of serine (Ser). The stereochemistry of the standard amino acid residues was determined by chiral GC-MS analysis of derivatives.



Scheme 2.1 Ahad structure elucidation from theonellamide F degradation.

Preparative ion-exchange chromatography showed a single peak corresponding to compounds **55a** and **55b** (Scheme 2.1) which eluted after β-Ala in the acid hydrolysate HPLC analysis. The $(M+H)^+$ ion at *m/z* 178 was observed in the fast atom bombardment (FAB) mass spectrum of the amino acid derivatives, suggesting that **55a** and **55b** were a diastereomeric mixture of aminohydroxyadipic acids. According to ^1H and ^{13}C NMR analysis, these diastereomeric mixtures were isolated as an equilibrium mixture of γ-lactones **57a**, **57b** and triethylammonium salts of dicarboxylic acids **56a**, **56b** formed during evaporation of

triethylammonium formate buffer. The isolated mixture was treated with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BocON) and triethylamine, followed by acidification to promote lactonization and esterification with diazomethane. The two diastereomers were separated by normal phase HPLC to afford lactones **58a** and **58b** (Scheme 2.1).

When the acidic hydrolysate was analyzed after a shorter reaction time, the (2S,4R) isomer (trans) was detected as the major isomer. The ratios of 4*S* to 4*R* isomers were 1:4, 1:2, and 1:1.5, upon acidic hydrolysis for 2, 4, and 8 hours, respectively, as analyzed by chiral GC-MS analysis. The CD spectrum of **58a**, showed a positive Cotton effect at 217 nm.[27] Therefore the configuration at C2 of both isomers was deemed to be *S*. The two diastereomers were distinguished based on the ^{1}H NMR signals for H3 and H3'. For *cis*-2,4-disubstituted ɣ-lactones, two separate signals are observed; a single signal is observed for the *trans* diastereomer (Figure 2.1). Proton NMR studies showed that the isomer **58a** appeared to be the *cis*-lactone, by comparison with the data on synthetic model compounds prepared by Stoddart and coworkers.[28]

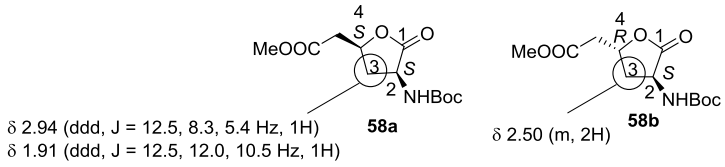
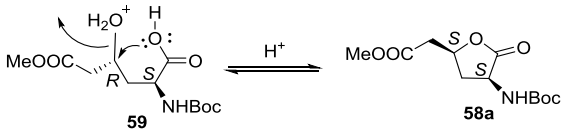


Figure 2.1 Comparison of H3 signals ^{1}H NMR of *cis*- and *trans*-disubstituted lactones.

Accordingly, the *cis*-lactone **58a** had (2*S*,4*S*) stereochemistry, while the *trans*-lactone **58b,** had the (2*S*,4*R*) stereochemistry, as deduced from the ^{1}H NMR spectrum. The C-4 hydroxyl group might have been epimerized through a backside attack of the carboxyl group during lactonization (Scheme 2.2). Moreover, equilibration to the more stable *cis*-2,4-disubstituted ɣ-lactone may have enhanced the (2*S*,4*S*) diastereomer.

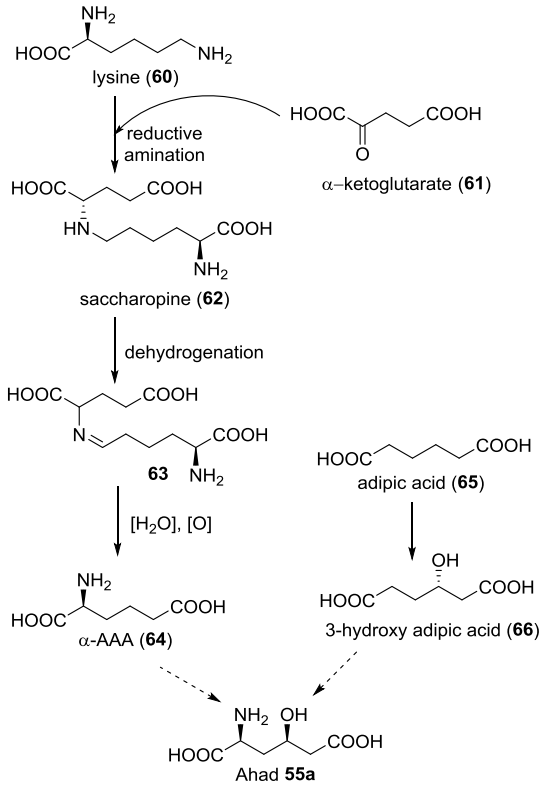


Scheme 2.2 Lactonization through backside attack of the carboxyl group at C4.

Therefore, Matsunaga and co-workers concluded that (2*S*,4*R*)-2-amino-4-hydroxyadipic acid (**55a**) was the constituent of theonellamide F (**1**), while the (2*S*,4*S*) isomer was an artifact of the isolation procedure.

2.2 Biogenesis of Ahad and α-AAA

Structure elucidation of the theonellamides showed that they contain (2*S*,4*R*)-α-amino-ɣ-hydroxy adipic acid (Ahad) (**55a**) (Schemes 2.1 and 2.3).

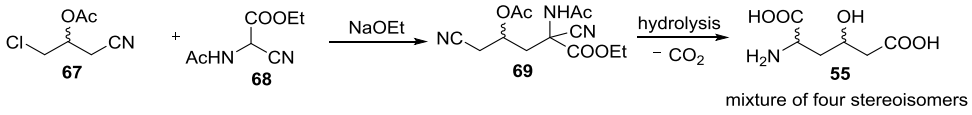


Scheme 2.3 Biogenesis of adipic acids.

Theonegramide and theopalauamide contain (2*S*)-α-amino-adipic acid (α-AAA, **64**, Scheme 2.3), which lacks the ɣ-hydroxy group. In mammals and higher plants metabolic degradation of lysine[29] gives saccharopine (**62**), formed by condensation of lysine (**60**) and α-ketoglutarate (**61**). In eukaryotes, normal metabolic degradation of lysine (**60**) gives α-AAA (**64**). Whereas, metabolic degradation of adipic acid (**65**) gives (3*R*)-3-hydroxyadipic acid (**66**) which is excreted in the urine and observed in the serum of ketoacidosis patients.[30] α-Amino-ɣ-hydroxy adipic acid (Ahad, **55a**) would appear to be of novel, or mixed, biogenesis. Aside from the appearance in theonellamides (*vide supra*), there has been only one other report of Ahad by Blass and Macheboeuf who proposed the occurrence of Ahad in cultures of *Vibrio cholera*, based on elemental analysis but without spectroscopic evidence.[31]

2.3 Previous Syntheses of (2*S*,4*R*)-ɣ-Hydroxy-α-Amino Adipic Acid (Ahad)

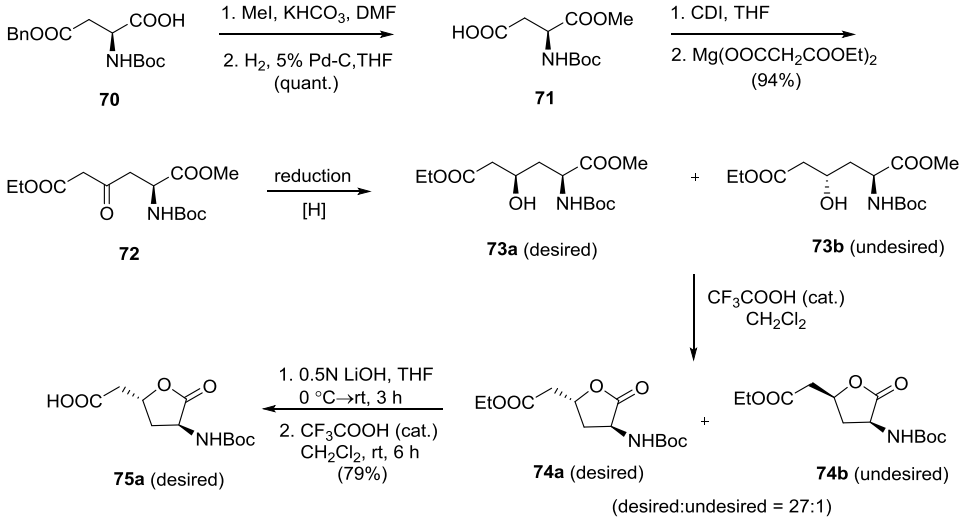
In 1957 Benoiton and co-workers synthesized γ-hydroxyglutamic acids.[32] By analogy, Kristensen *et al.* reported the synthesis of a mixture of all four streoisomers of Ahad in 1980[33] (Scheme 2.4). Condensation of the anion of ethyl acetamidocyanacetate (**68**) with 3-acetoxy-4-chlorobutyronitrile (**67**), followed by hydrolysis and decarboxylation, afforded a mixture of four isomers of **55** which was separated into racemates of the two diastereoisomers by ion-exchange chromatography and preparative high voltage electrophoresis.



Scheme 2.4 Synthesis of four stereoisomers of Ahad by Kristensen *et al.*

In 1994, Tohdo *et al*.[22,23] reported the synthesis of Ahad in optically active form (Scheme 2.5). They took commercially available *N-tert*-butoxycarbonyl-(*S*)-aspartic acid β-benzyl ester (**70**) and converted it to Boc-(*S*)-Asp-OMe (**71**). The β-carboxyl group of **71** was activated with carbonyldiimidazole (CDI) and the resulting imidazolide was reacted with the

magnesium enolate of ethyl hydrogen malonate to yield β-keto ester **72** which was reduced stereoselectively. The yield and diastereomeric excess of the reduced products **73a** and **73b** were measured following acid-catalyzed lactonization. The best result was obtained by asymmetric hydrogenation with Noyori's Ru-(*R*)-BINAP catalyst to give a mixture of **73a** and **73b** that was converted to the γ-lactones **74a** and **74b** (ratio of 27:1) by treating with a catalytic amount of trifluoroacetic acid. Hydrolysis of compound **74a** with lithium hydroxide followed by relactonization with acid gave the lactone **75a**. Tohdo *et al.* used this γ-lactone functionality as "intramolecular protection" for their Ahad building block as discussed in Chapter 1, Section 1.5.



Scheme 2.5 Synthesis of the Ahad lactone by Tohdo *et al.*

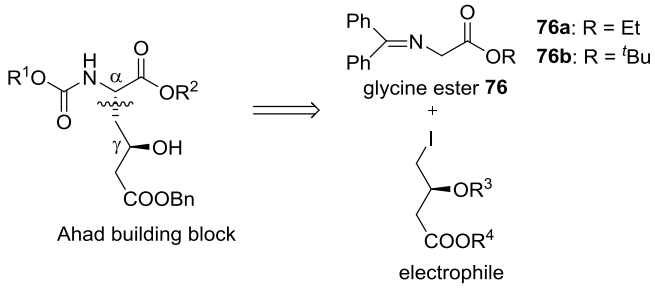
The α-carboxylic acid is not part of the peptide backbone and may be important for stabilization of an imidazolium cation in the full theonellamide structure. As described earlier, Ahad **55a** was isolated as the corresponding γ-lactone **57a** following acidic degradation of the theonellamides. The work of Benson Edagwa in the Taylor Laboratory with 4,5-dihydroxyleucine[34] alerted us to the fact that there would be a high risk of epimerization at Cα while opening the γ-lactone. Michl had shown earlier that epimerization of *N*α-acyl and *N*α-carbamoyl γ-lactones occurs through oxazolone formation.[35] On this basis, we have concerns

for the stereochemical integrity of **75a** in Scheme 2.5. For this reason, we wanted to avoid the ɣ-lactone formation moiety throughout our synthesis.

2.4 Retrosynthesis of Ahad via Alkylation of a Glycine Ester Enolate

Our Ahad building block has two stereocenters: Cα and Cɣ (Scheme 2.6). We planned to establish the Cɣ configuration in an electrophilic reaction partner and focus our attention on the Corey-Lygo method for the catalytic asymmetric generation of the Cα stereocenter. Corey and Lygo independently developed a new class of asymmetric phase-transfer catalyst derived from Cinchona alkaloids[36] and applied them to the enantioselective synthesis of α-amino acids.

Retrosynthetically, it can be imagined that an Ahad building block might be arrived at via stereoselective alkylation of the enolate of glycine derivative **76** (commercially available) with an electrophile (Scheme 2.6).



Scheme 2.6 Retrosynthesis of Ahad.

2.5 Phase-Transfer Catalysis (PTC) and the Corey Lygo Approach

2.5.1 The Concept

Phase-transfer catalysis (PTC)[37] facilitates the migration of a reactant in a heterogeneous system from one phase into another phase where a reaction can take place.[38] An inexpensive, commercially available source of effective chiral phase-transfer catalysts is the family of Cinchona alkaloids (Figure 2.2). These alkaloids can be transformed into quaternary

ammonium salt catalysts in one or two steps.[39] Furthermore, these alkaloids are available as pseudoenantiomers (quinine and quinidine, *e.g.*, **77** and **78**) that can achieve similar levels of asymmetric induction with the opposite sense of enantioselectivity.[37] An interfacial mechanism is implicated for lipophilic quaternary ammonium catalysts (Q^+X^-) when basic conditions are employed in PTC, as illustrated in Scheme 2.7. The interfacial mechanism involves formation of a contact ion pair between the quarternary ammonium cation (Q^+) and the anionic organic nucleophile (enolate, M^+) that is generated at the interface by an inorganic base. The generated quarternary ammonium cation complex (enolate, Q^+) transfers the anionic organic nucleophile into the organic phase, where it can react with the organic electrophile (R-X).[40]

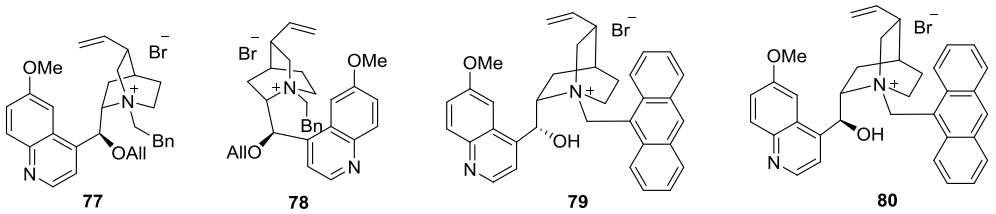
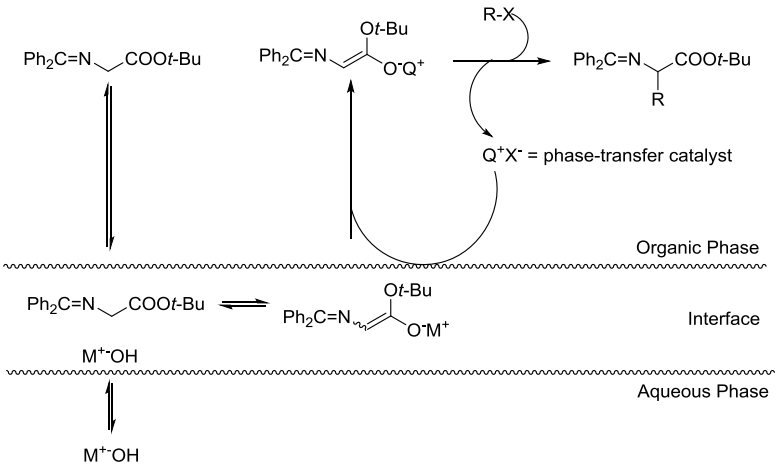


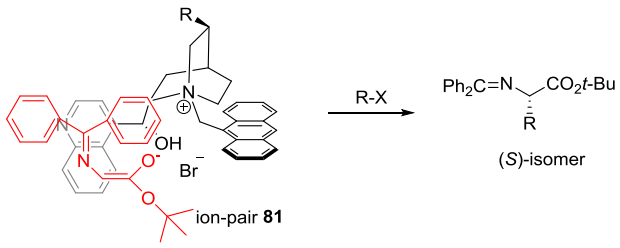
Fig 2.2 Cinchona-derived chiral phase-transfer catalysts.



Scheme 2.7 Phase transfer alkylation of imine.

One reason for the enantioselectivities obtained in the presence of quaternary ammonium salts would be reaction via an ion-pair arrangement **81** (Scheme 2.8).[36a,41] In this

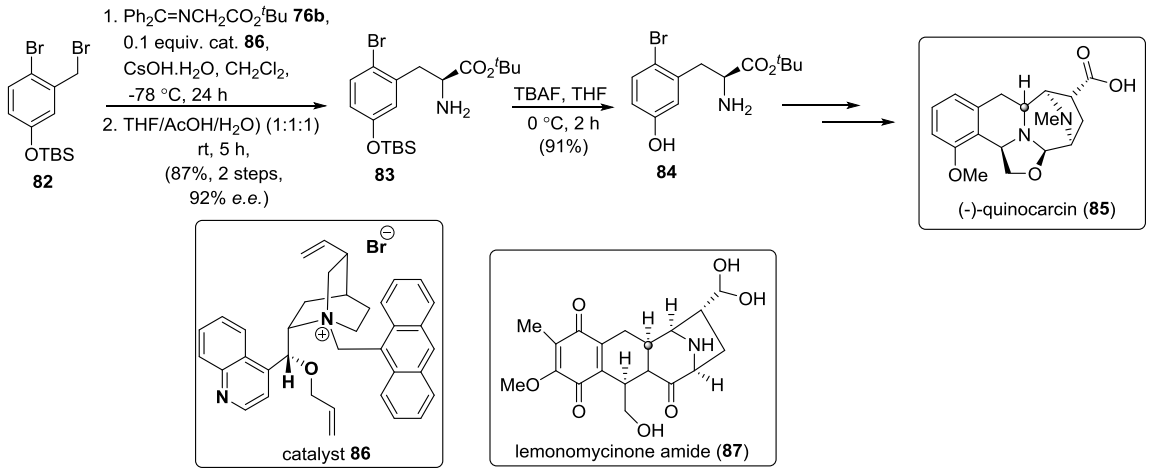
arrangement the preferential attack would occur via the Si-face as the Re-face of the enolate carbon is blocked by the quinoline ring of the quaternary ammonium salt.



Scheme 2.8 Possible ion pair arrangement that explains facial selectivity.

2.5.2 Previous Examples

Previous examples of the alkylation of esters of glycine benzophenone imine with relatively complex electrophiles encouraged us to pursue such an approach to the stereoselective formation of Cα in Ahad. Zhu and coworkers used the Corey-Lygo method for the synthesis of (-)-quinocarcin (**85**)[42] and lemonomycinone amide (**87**) (Scheme 2.9).[43]

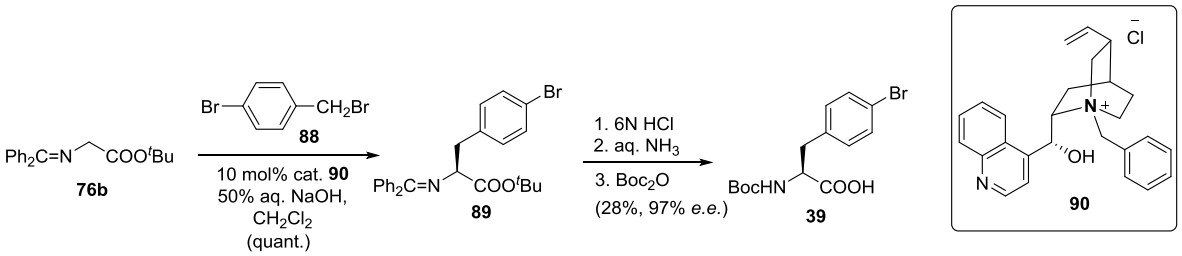


Scheme 2.9 Application of Corey-Lygo alkylation to complex molecules.

The preparation of intermediate **84** for the total synthesis of (-)-quinocarcin (**85**) is shown in Scheme 2.9. Following Corey's procedure, the enantioselective alkylation of *N*-(diphenylmethylene)glycine *tert*-butyl ester **76b** was performed with 2-bromo-5-*tert*-

butyldimethylsilyloxybenzyl bromide (**82**) in the presence of *O*-allyl-*N*-(9-anthracenylmethyl) cinchonidinium bromide (**86**), followed by chemoselective hydrolysis of the imine functionality gave the amino ester **83** in 87% yield. Cleavage of the silyl ether gave amino phenol **84** in 91% yield.

Also, Tohdo *et al.* utilized *N*q-benzylcinchonidinium chloride (**90**) as a phase transfer catalyst[44] for the preparation of *N*α-Boc-(*S*)-*p*-bromophenylalanine (**39**) which occurs in theonellamide F. The asymmetric alkylation of the Schiff base **76b** was carried out with *p*-bromobenzyl bromide (**88**) using catalyst **90** to obtain **89** (Scheme 2.10). Acid hydrolysis followed by neutralization and treatment with Boc_2O afforded the required Boc derivative **39**.

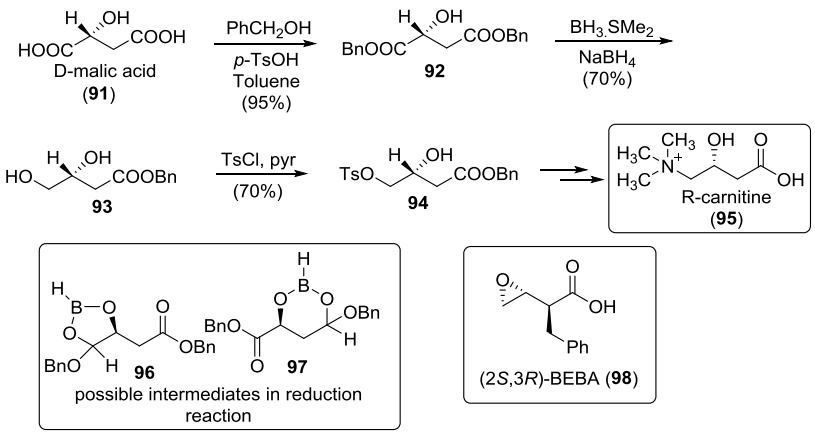


Scheme 2.10 Preparation of (*S*)-*N*α-Boc-*p*-bromophenylalanine by Tohdo *et al.*

2.6 Synthesis of an Electrophile for Ahad Synthesis

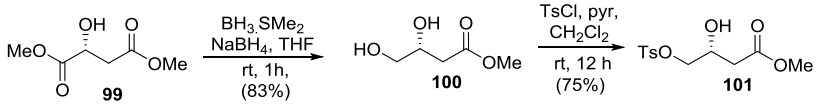
As depicted in Scheme 2.6, we required an appropriate electrophile. The initial steps in the synthesis of this electrophile were accomplished using an established procedure reported independently by Lee *et al*.[45] and Bellamy *et al*.[46] Bellamy described the synthesis of (*R*)-carnitine (**95**) according to Scheme 2.11. Starting from *D*-malic acid (**91**), the formation of dibenzyl ester **92** was performed. Chemoselective reduction of the α-hydroxyester of compound **92** was carried out by using $BH_3.SMe_2$ to obtain diol **93**. This chemoselectivity was first observed by Moriwake.[47] The reaction involves the formation of a boroxolane-type intermediate **96** (Scheme 2.11). The chemoselectivity observed in the reduction might be derived from the fact that relatively short B-O bond length in **96** vs. **97** and low entropy factor for the more feasible

formation of **96** than that of boroxane-type intermediate **97**. Monotosylation of the diol **93** led to compound **94**, a key intermediate en route to the synthesis of (*R*)-carnitine (**95**). Similar reactions were carried out by Lee *et al*. for the synthesis of (2*S,*3*R*)-2-benzyl-3,4-epoxybutanoic acid (BEBA) (**98**) (shown in a box in Scheme 2.11).[45]



Scheme 2.11 Synthetic manipulations of malic acid by Lee *et al.* and Bellamy *et al.*

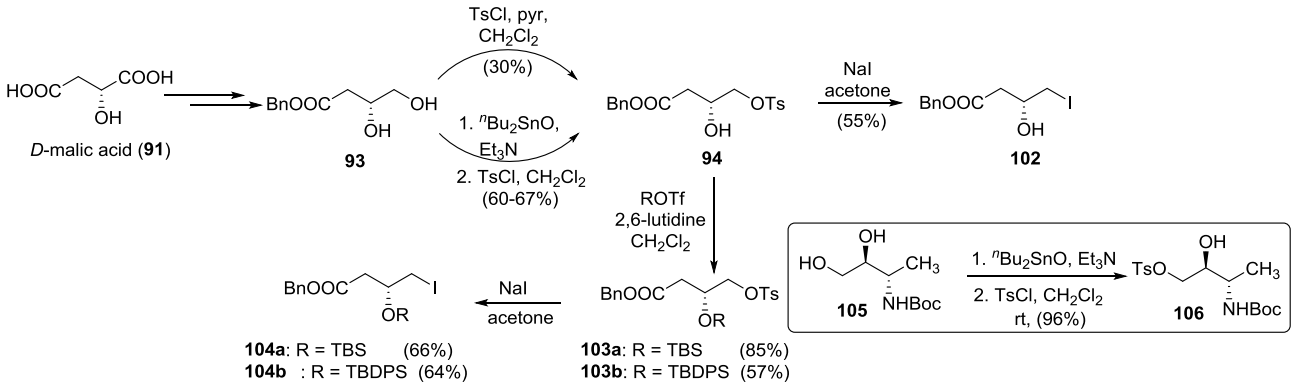
Kocienski *et al*.[43] synthesized the analogous methyl ester **101** in their efforts toward the total synthesis of (-)-lipstatin. Enantiomerically pure dimethyl (*S*)-(-)-malate (**99**) was selectively reduced to diol **100**. The primary hydroxyl function of the resultant diol **100** was converted to the tosylate **101**.[48]



Scheme 2.12 Synthesis of intermediate electrophile for total synthesis of (-)-lipstatin by Kocienski *et al.*

We repeated the reactions as shown in Scheme 2.11 using *D*-malic acid (**91**). However, we obtained only low yields of tosylate **94** under the conditions reported by Kocienski *et al*. Galvez and coworkers reported that conversion of a diol to a stannylene acetal enhanced the nucleophilicity of the oxygen, while still promoting regioselectivity (shown in a box in Scheme

2.13).[42] Diol **93** was successfully monotosylated using this strategy. Protection of the remaining secondary alcohol as both TBS ether **103a** and TBDPS (more hindered) ether **103b** was achieved. The primary tosylates **103a** and **103b** were each treated with sodium iodide/acetone to obtain another pair of electrophiles **104a** and **104b** with iodide as a good leaving group. Moreover, another less hindered electrophile was synthesized without the protection of the secondary alcohol, *viz.* **102**.



Scheme 2.13 Synthesis of electrophiles.

In conclusion, we made a series of potential electrophiles to do the alkylation from less sterically hindered **102** to more bulky group on secondary alcohol **103** and from good leaving group tosylate **94** to better leaving group iodide **104**.

2.7 Alkylation of Glycine Ester Enolates

The next step was to alkylate a glycine anion synthon with the electrophiles synthesized in Scheme 2.13. According to the literature, glycine benzophenone imine *tert*-butyl ester **76b** has typically been used in these reactions; the *tert*-butyl ester can be cleaved under the same conditions as the benzophenone imine in a single step. For our synthesis we chose the corresponding ethyl ester **76a** as it is less expensive and simple examples demonstrate that the ethyl ester is equally effective (Table 2.1).

In order to develop some experience of the asymmetric phase-transfer alkylation of imine **76** we repeated some known examples from the literature.

There are two sets of conditions employed for alkylation with some differences.

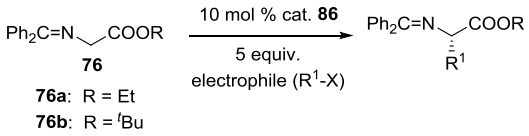
1. Corey's conditions:[36a] ten equivalents of $CsOH.H_2O$, five equivalents of electrophile, CH_2Cl_2, -60 °C. An example was shown earlier in Zhu's synthesis of (-) quinocarcin (**85**) (Scheme 2.9).[42,43]
2. Lygo's conditions:[36b,39] 50 percent aqueous KOH/NaOH, water, five equivalents of electrophile, toluene, -60 °C to room temperature. An example was shown earlier in Tohdo's synthesis of *p*-bromophenylalanine (**39**) (Scheme 2.10).

We initially investigated the alkylation of glycine benzophenone imine ethyl ester (**76a**) with allyl bromide as alkylating agent employing 10 equivalents of base (Table 2.1, Entry 1). The same reaction conditions were applied with glycine benzophenone imine *tert*-butyl ester **76b**, leading to a comparable yield of the product (Entry 2). Corey[36a] reported 97% *e.e.* for the latter alkylation. The enantioselectivity of the product from Entry 2 was determined to be 92% *e.e.* by chiral HPLC. We applied the same reaction conditions to a commercially available iodobutane, a linear aliphatic alkyl halide, and the yields from both the glycine derivatives were comparable (Entries 3 and 4). Lygo's conditions[36b] were also tested and the yield was much the same for alkylation of both the glycine enolates (Entry 5 and 6). Furthermore, we tried the Lygo conditions using a mixture of solvents (Entry 7). The low yield suggested that the ethyl ester hydrolysis was competing kinetically with enolate formation and alkylation under the strongly basic conditions.

Excited that we could achieve the alkylation in good yield under Corey's conditions, we subjected our more complex electrophile **103b** to the reaction conditions; no product was isolated (Entry 8). Therefore, we switched from the tosylate **103b**, to the iodide **104b** as a superior leaving group (Entry 9). Again no product was isolated. One reason for this could be

that the electrophile is unreactive due to the adjacent, bulky TBDPS ether. Therefore, we reduced the size of the adjacent protecting group, to the smaller TBS ether. No product was isolated from the attempted alkylation using **104a** (Entry 10). We raised the temperature to room temperature; but again no desired product was formed (Entry 11). We presume that the TBS group also provides steric hindrance to the displacement reaction. The reaction was also performed, without success, on unprotected alcohol **102** (Entry 12). Proton transfer is a potential problem here.

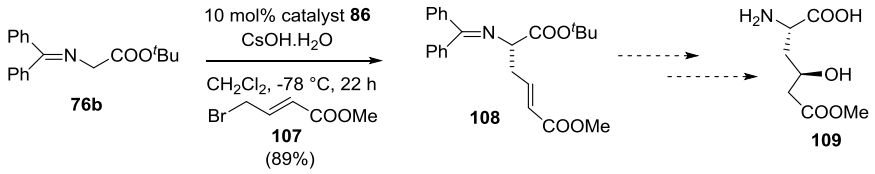
Table 2.1 Alkylations of glycine enolates.



Entry	Glycine	Electrophile	Conditions	Result
1	**76a**	CH_2=CH_2CH_2Br	$CsOH.H_2O$, CH_2Cl_2, -78→-60 °C, 22 h	95%
2	**76b**	CH_2=CH_2CH_2Br	$CsOH.H_2O$, CH_2Cl_2, -78→-60 °C, 22 h	85%
3	**76a**	$CH_3(CH_2)_3I$	$CsOH.H_2O$, CH_2Cl_2, -78→-60 °C, 22 h	54%
4	**76b**	$CH_3(CH_2)_3I$	$CsOH.H_2O$, CH_2Cl_2, -78→-60 °C, 22 h	53%
5	**76a**	$CH_3(CH_2)_3I$	50 % aq. KOH, H_2O, PhMe, RT, 24 h	50%
6	**76b**	$CH_3(CH_2)_3I$	50 % aq. KOH, H_2O, PhMe, RT, 24 h	46%
7	**76b**	$CH_3(CH_2)_3I$	50 % aq. KOH, CH_2Cl_2-PhMe (7:1), 24 h	34%
8	**76a**	**103b**	$CsOH.H_2O$, CH_2Cl_2, -78→-60 °C, 22 h	-
9	**76a**	**104b**	$CsOH.H_2O$, CH_2Cl_2, -78→-60 °C, 22 h	-
10	**76a**	**104a**	$CsOH.H_2O$, CH_2Cl_2, -78→-60 °C, 22 h	-
11	**76a**	**104a**	$CsOH.H_2O$, CH_2Cl_2, RT, 22 h	-
12	**76b**	**102**	$CsOH.H_2O$, CH_2Cl_2, -78→-60 °C, 22 h	-

Collectively, the results described show that activated alkyl halides (*e.g.,* allyl bromide in Table 2.1 and benzylic bromides in Schemes 2.9 and 2.10) are the best electrophiles for these reactions. The need to engage a five-fold excess of electrophile in these alkylations is not widely appreciated and is a drawback when synthetically valuable electrophiles are involved. Even then, the use of excess electrophile didn't afford our desired product. Also, the neighboring silyl ether provides severe steric hindrance to the displacement reaction.

We employed commercially available methyl 4-bromobut-2-enoate (**107**) as the electrophile and successful asymmetric alkylation of the glycine imine **76b** was achieved as shown in Scheme 2.14.



Scheme 2.14 Alkylation of glycine imine with an allylic bromide.

While looking for a suitable method for diastereoselective epoxidation, or conjugate addition of an *O*-nucleophilic species, we became aware of another approach that might deliver the 1,3-*syn* amino alcohol in a more reliable fashion. This will be the subject of Chapter 3.

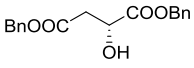
2.8 Experimental Section

2.8.1 General Methods

All reactions were performed under a dry nitrogen atmosphere unless otherwise noted. Reagents were obtained from commercial sources and used directly; exceptions are noted. Diisopropylethylamine, 2,4,6-collidine, pyridine, triethylamine and 2,6-lutidine were dried and distilled from CaH_2 and stored over KOH pellets. Methanol was distilled from Mg turnings and stored over 4 Å molecular sieves. Flash chromatography was performed using silica gel (32-63 μ) from Dynamic Absorbents Inc. Reactions were followed by TLC on precoated silica plates

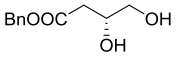
(200 µm, F-254 from Dynamic Adsorbents Inc.). The compounds were visualized by UV fluorescence or by staining with phosphomolybdic acid (PMA), cerium-ammonium-molybdate (CAM), ninhydrin, potassium permanganate, or 2,4-dinitrophenylhydrazine (2,4-DNP) stains. HPLC was performed on Waters 600E multisolvent delivery system (Waters 2487 dual λ absorbance detector). Optical rotations were recorded on JASCO P-2000 digital polarimeter. NMR spectra were recorded on a Bruker AV-400-liquid spectrometer. Proton NMR data is reported in ppm downfield from TMS as an internal standard. High resolution mass spectra were recorded using either an Agilent 6210 time-of-flight MS, or a Hitachi MS-8000 3DQ LC-ion trap mass spectrometer with electrospray ionization.

2.8.2 Procedures



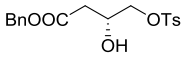
(*S*)-Dibenzyl 2-hydroxysuccinate (**92**)

Benzyl alcohol (1.50 mL, 1.61 g, 14.90 mmol, 2.00 equiv.) and *p*-toluenesulfonic acid monohydrate (14 mg, 0.074 mmol, 0.01 equiv.) were added to a solution of *D*-malic acid (**91**) (1.00 g, 7.46 mmol, 1.00 equiv.) in dry toluene (12 mL). The reaction mixture was heated under reflux with a Dean-Stark apparatus to effect the azeotropic removal of water. When no more water appeared in the distillate, the mixture was allowed to cool to rt and washed with saturated aqueous $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by flash column chromatography on silica gel with Hex:EtOAc (4:1) as eluent to give the dibenzyl ester **92** as a colorless oil (80%). R_f 0.42 (4:1 Hex-EtOAc). $[\alpha]_D^{25}$ +42.98 (*c* 2.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.80 (dd, *J* = 16.4, 6.1 Hz, 1H), 2.86 (dd, *J* = 16.4, 4.7 Hz, 1H), 3.41 (br s, 1H), 4.52 (dd, *J* = 5.9, 4.8 Hz, 1H), 5.06 (s, 2H), 5.13 (s, 2H), 7.24-7.33 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 38.8, 66.8, 67.5, 67.6, 128.3, 128.4, 128.5, 128.6, 128.7, 135.2, 135.6, 170.3, 173.2. HRMS (ESI) calcd for $C_{18}H_{19}O_5$ $(M+H)^+$ 315.1227, obsd 315.1220.



(*S*)-Benzyl 3,4-dihydroxybutanoate (**93**)

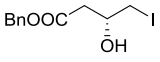
Borane-dimethylsulfide complex (175 µL, 0.350 mmol, 2.0 M solution in THF, 1.10 equiv.) was added dropwise over 15 min to a solution of (*S*)-dibenzyl-2-hydroxysuccinate (**92**) (100 mg, 0.318 mmol, 1.00 equiv.) in dry THF (1 mL) at 0 °C under N_2. After 1 h of stirring, sodium borohydride (0.6 mg, 0.016 mmol, 0.05 equiv.) was added and the mixture was stirred overnight at rt. The mixture was quenched by the addition of methanol (1.5 mL) and stirring continued for 30 min. The solvent was evaporated and the residue purified by column chromatography on silica gel (1:1 Hex-EtOAc) to obtain diol **93** as a colorless oil (41 mg, 61%). R_f 0.31 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ +18.84 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.49 (dd, *J* = 16.3, 4.3 Hz, 1H) 2.55 (dd, *J* = 16.3, 8.4 Hz, 1H), 3.35 (br s, 2H) 3.48 (dd, *J* = 11.4, 6.4 Hz, 1H) 3.62 (dd, *J* = 11.4, 3.5 Hz, 1H), 4.08-4.17 (m, 1H), 5.13 (s, 1H), 7.30-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.8, 65.7, 66.7, 68.6, 128.3, 128.4, 128.6, 135.6, 172.3. HRMS (ESI) calcd for $C_{11}H_{14}NaO_4$ $(M+Na)^+$ 233.0784, obsd 233.0793.



(*S*)-Benzyl 3-hydroxy-4-(tosyloxy)butanoate (**94**)

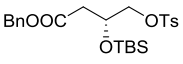
To a solution of diol **93** (200.0 mg, 0.95 mmol, 1.00 equiv.) in dry CH_2Cl_2 (2.5 mL) was added successively ^{n}Bu_2SnO (7.0 mg, 0.03 mmol, 0.03 equiv.), a solution of Et_3N (135 µL, 98.4 mg, 0.97 mmol, 1.02 equiv.) in dry CH_2Cl_2 (2.5 mL) and *p*-toluenesulfonyl chloride (190.7 mg, 0.97 mmol, 1.02 equiv.) at rt. The mixture was stirred for 6 h, then treated with brine (10 mL) The aqueous layer was extracted with CH_2Cl_2 (4 x 15 mL). The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by silica gel column chromatography (2:1 Hex-EtOAc) to obtained tosylated product **94** (222 mg, 64%), R_f 0.43 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ +7.34 (*c* 1.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.00 (br s, 1H), 2.45 (s, 3H), 2.53 (dd, *J* = 16.6, 4.8 Hz, 1H), 2.62 (dd, *J* = 16.7, 7.5 Hz, 1H), 4.04 (d, *J* = 8.3 Hz, 2H), 4.25-4.28 (m, 1 H), 5.14 (s, 2H), 7.26-7.39 (m, 7H), 7.78 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR

($CDCl_3$, 100 MHz) δ 21.7, 37.4, 66.0, 66.9, 71.9, 128.0, 128.2, 128.5, 128.7, 130.0, 132.5, 135.3, 145.2, 171.4. HRMS (ESI) calcd for $C_{18}H_{21}O_6S$ $(M+H)^+$ 365.1053, obsd 365.1043.



(*S*)-Benzyl 3-hydroxy-4-iodobutanoate (**102**)

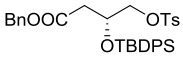
Sodium iodide (42 mg, 0.274 mmol, 2.0 equiv.) was added to a solution of (*S*)-Benzyl 3-hydroxy-4-(tosyloxy)butanoate (**94**) (50 mg, 0.137 mmol, 1.0 equiv.) in acetone (5 mL). The mixture was stirred and heated at reflux overnight. Saturated aqueous $NaHCO_3$ (4 mL) was added and the mixture extracted with diethyl ether (3 x 10 mL). The combined organic layers were washed with brine (5 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded as a brownish yellow oil **102** (24 mg, 55%). R_f 0.46 (2:1 Hex-EtOAc). $[\alpha]_D^{25}$ +12.31 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.00 (br s, 1H), 2.60 (dd, *J* = 15.4, 7.1 Hz, 1H), 2.73 (dd, *J* = 15.4, 4.9 Hz, 1H), 3.21-3.30 (m, 2H), 4.05 (app. pent, *J* = 5.5 Hz, 1H), 5.09 (d, *J* = 12.3 Hz, 1H), 5.14 (d, *J* = 12.3 Hz, 1H), 7.25-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.0, 37.8, 66.7, 128.3, 128.4, 128.6, 135.6, 172.3. HRMS (ESI) calcd for $C_{11}H_{14}IO$ $(M+H)^+$ 320.9817, obsd 320.9821.



(*S*)-Benzyl 3-[(*tert*-butyldimethylsilyl)oxy]-4-(tosyloxy)butanoate (**103a**)

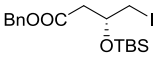
2,6-Lutidine (178 µL, 163 mg, 1.5 mmol, 3.0 equiv.) was added dropwise to a solution of (*S*)-benzyl 3-hydroxy-4-(tosyloxy)butanoate (**94**) (185 mg, 0.5 mmol, 1.0 equiv.) in dry CH_2Cl_2 (7.5 mL) under N_2 at rt. The mixture was stirred for 30 min before the dropwise addition of TBDMSOTf (140 µL, 161 mg, 0.6 mmol, 1.2 equiv.). The mixture was stirred overnight, the solvent evaporated and the residue purified by silica gel chromatography, eluting with 2:1→1:1 (Hex-EtOAc), to give TBS ether **103a** (206 mg, 85%); R_f 0.38 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ +8.01 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.02 (s, 3H), 0.03 (s, 3H), 0.81 (s, 9H), 2.45 (s, 3H), 2.49 (dd, *J* = 15.4, 6.9 Hz, 1H), 2.56 (dd, *J* = 15.4, 5.2 Hz, 1H), 3.93-4.00 (m, 2H), 4.3-4.35 (m, 1H),

5.06 (d, *J* = 12.3 Hz, 1H), 5.12 (d, *J* = 12.3 Hz, 1H), 7.32-7.38 (m, 5H), 7.77-7.79 (m, 4H), ^{13}C NMR ($CDCl_3$, 100 MHz) δ -5.1, -4.7, 17.9, 21.6, 25.6, 39.5, 66.5, 67.2, 72.3, 128.0, 128.2, 128.3, 128.6, 129.9, 132.8, 135.6, 144.9, 170.3. HRMS (ESI) calcd for $C_{24}H_{35}O_6SSi$ $(M+H)^+$ 479.1918, obsd 479.1925.



(*S*)-Benzyl 3-[(*tert*-butyldiphenylsilyl)oxy]-4-(tosyloxy)butanoate (**103b**)

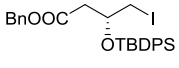
2,6-Lutidine (168 µL, 155 mg, 1.44 mmol, 3.5 equiv.) was added dropwise to a solution of (*S*)-benzyl 3-hydroxy-4-(tosyloxy)butanoate (**94**) (150 mg, 0.41 mmol, 1.0 equiv.) in dry CH_2Cl_2 (7.5 mL) under N_2 at rt. The mixture was stirred for 30 min before the dropwise addition of TBDPSOTf (320 µL, 320 mg, 0.82 mmol, 2.0 equiv.). The mixture was stirred overnight, the solvent evaporated and the residue purified by silica gel chromatography, eluting with 5:1 (Hex-EtOAc) to yield TBDPS ether **103b** (141 mg, 57%). R_f 0.62 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ +5.76 (*c* 0.75, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.98 (s, 9H), 2.41 (s, 3H), 2.54 (d, *J* = 5.9 Hz, 2H), 3.89-3.98 (m, 2H). 4.23-4.27 (m, 1H), 4.90 (d, *J* = 12.3 Hz, 1H), 4.99 (d, *J* = 12.3 Hz, 1H), 7.21-7.53 (m, 10H), 7.54-7.59 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.3, 21.8, 26.9, 39.0, 66.6, 67.9, 71.8, 127.8, 128.1, 128.4, 128.7, 129.9, 130.1, 132.9, 133.0, 133.1, 135.0, 135.7, 135.9, 136.0, 144.9, 170.2. HRMS (ESI) calcd for $C_{34}H_{39}O_6SSi$ $(M+H)^+$ 603.2231, obsd 603.2212.



(*S*)-Benzyl 3-[(*tert*-butyldimethylsilyl)oxy]-4-iodobutanoate (**104a**)

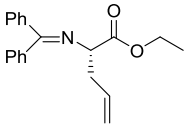
Sodium iodide (392 mg, 2.08 mmol, 8.0 equiv.) was added to a solution of (*S*)-benzyl 3-[(*tert*-butyldimethylsilyl)oxy]-4-(tosyloxy)butanoate **103a** (125 mg, 0.26 mmol, 1.0 equiv.) in acetone (5 mL). The mixture was stirred and heated at reflux overnight. Saturated aqueous $NaHCO_3$ (10 mL) was added and the mixture extracted with diethyl ether (5 x 15 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded as a brownish

yellow oil **104a** (88 mg, 78%). R_f 0.58 (2:1 Hex-EtOAc). $[\alpha]_D^{25}$ +10.01 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 0.04 (s, 3H), 0.11 (s, 3H), 0.87 (s, 9H), 2.60 (dd, *J* = 15.4, 7.1 Hz, 1H), 2.73 (dd, *J* = 15.4, 4.9 Hz, 1H), 3.21-3.30 (m, 2H), 4.05 (app. pent, *J* = 5.5 Hz, 1H), 5.09 (d, *J* = 12.3 Hz, 1H), 5.14 (d, *J* = 12.3 Hz, 1H), 7.25-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -4.9, -4.5, 12.8, 17.9, 25.7, 42.6, 66.5, 68.4, 128.3, 128.4, 128.6, 135.7, 170.7. HRMS (ESI) calcd for $C_{17}H_{28}IO_3Si$ $(M+H)^+$ 435.0847, obsd 435.0831.



(*S*)-Benzyl 3-[(*tert*-butyldiphenylsilyl)oxy]-4-iodobutanoate (**104b**)

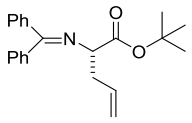
Sodium iodide (169 mg, 1.13 mmol, 8.0 equiv.) was added to a solution of (*S*)-benzyl 3-[(*tert*-butyldiphenylsilyl)oxy]-4-(tosyloxy)butanoate (**103b**) (85 mg, 0.14 mmol, 1.0 equiv.) in acetone (3.5 mL). The mixture was stirred and heated at reflux overnight. Saturated aqueous $NaHCO_3$ (7 mL) was added and the mixture extracted with diethyl ether (5 x 10 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded **104b** as a yellow oil (50 mg, 64%). R_f 0.58 (2:1 Hex-EtOAc). $[\alpha]_D^{25}$ +5.01 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.05 (s, 9H), 2.65 (dd, *J* = 15.7, 6.1 Hz, 1H), 2.75 (dd, *J* = 15.7, 5.9 Hz, 1H), 3.20 (d, *J* = 4.6 Hz, 2H), 3.92-3.98 (m, 1H), 5.65 (d, *J* = 12.4 Hz, 1H), 5.04 (d, *J* = 12.4 Hz, 1H), 7.25-7.45 (m, 10H), 7.62-7.69 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -0.0, 13.4, 19.3, 26.9, 42.2, 66.4, 68.7, 127.7, 127.8, 128.2, 128.5, 129.9, 130.0, 133.2, 135.8, 135.9, 170.4. HRMS (ESI) calcd for $C_{27}H_{32}IO_3Si$ $(M+H)^+$ 559.1160, obsd 559.1153.



Ethyl 2-[(diphenylmethylene)amino]pent-4-enoate (Table 2.1, Entry 1)

Allyl bromide (144 µL, 199.6 mg, 1.65 mmol, 5.0 equiv.) was added dropwise to a mixture of ethylglycinate benzophenone imine (**76a**) (88.2 mg, 0.330 mmol, 1.0 equiv.), *O*(9)-

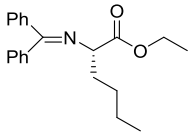
allyl-N-9-anthracenylmethylcinchonidium bromide (**86**) (20.0 mg, 0.033 mmol, 0.1 equiv.) and $CsOH.H_2O$ (554.2 mg, 3.300 mmol, 10.0 equiv.) in CH_2Cl_2 (3 mL) at -78 °C. The mixture was warmed to -60 °C and stirred vigorously for 24 h. The suspension was diluted with ether (30 mL), washed with water (2 x 10 mL), brine (20 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded ethyl 2-[(diphenylmethylene)amino]pent-4-enoate as a yellow colored oil (82 mg, 80%). R_f 0.39 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ +9.81 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (t, *J* = 7.1 Hz, 3H), 2.61 (dd, *J* = 14.0, 7.5 Hz, 1H), 2.68 (dd, *J* = 13.0, 7.0 Hz, 1H), 4.10-4.22 (m, 3H), 5.01 (d, *J* = 10.8 Hz, 1H), 5.06 (dd, *J* = 16.5, 1.38 Hz, 1H), 5.63-5.74 (m , 1H), 7.16-7.46 (m, 7H), 7.48-7.57 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2, 38.1, 60.9, 65.3, 117.5, 127.9, 128.0, 128.3, 128.5, 128.8, 132.4, 134.4, 139.6, 170.5, 171.8. HRMS (ESI) calcd for $C_{20}H_{22}NO_2$ $(M+H)^+$ 308.1620, obsd 308.1614.



tert-Butyl 2-[(diphenylmethylene)amino]pent-4-enoate (Table 2.1, Entry 2)

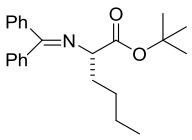
Allyl bromide (74 µL, 102.2 mg, 0.845 mmol, 5.0 equiv.) was added dropwise to a mixture of *tert*-butylglycinate benzophenone imine (**76b**) (50.0 mg, 0.170 mmol, 1.0 equiv.), *O*(9)-allyl-*N*-9-anthracenylmethylcinchonidium bromide (**86**) (10.2 mg, 0.017 mmol, 0.1 equiv.) and $CsOH.H_2O$ (283.8 mg, 1.700 mmol, 10.0 equiv.) in CH_2Cl_2 (3 mL) at -78 °C. The mixture was warmed to -60 °C and stirred vigorously for 24 h. The suspension was diluted with ether (30 mL), washed with water (2 x 10 mL), brine (20 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded *tert*-butyl 2-[(diphenylmethylene)amino]pent-4-enoate as a yellow oil (48 mg, 85%, 92% *e.e.*). The enantiomeric excess was determined by HPLC analysis using Chiralcel OD-H column (5% ^{i}PrOH-Hex, 0.5 mL/min); major enantiomer t_r = 8.7 min and minor enantiomer t_r = 17.7 min;

Detection 254 nm. R_f 0.40 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ +8.21 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9H), 2.59 (dd, *J* =14.0, 7.5 Hz, 1H), 2.67 (dd, *J* = 14.0, 6.6 Hz, 1H), 4.00 (dd, *J* = 7.4, 5.4 Hz, 1H), 5.0 (d, *J* = 10.1 Hz, 1H), 5.06 (d, *J* = 17.1 Hz, 1H), 5.67-5.78 (m, 1H), 7.16-7.56 (m, 10 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.1, 38.2, 65.9, 81.0, 117.2, 128.0, 128.3, 128.4, 128.5, 128.8, 130.1, 130.2, 132.4, 134.8, 136.7, 139.8, 170.1, 170.9. HRMS (ESI) calcd for $C_{22}H_{26}NO_2$ $(M+H)^+$ 336.1958, obsd 336.1966.



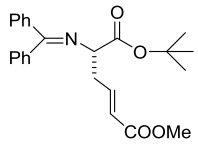
Ethyl 2-[(diphenylmethylene)amino]hexanoate (Table 2.1, Entry 3)

Iodobutane (34 µL, 54.5 mg, 0.296 mmol, 1.2 equiv.) was added dropwise to a mixture of ethylglycinate benzophenone imine (**76a**) (66.2 mg, 0.250 mmol, 1.0 equiv.), *O*(9)-allyl-N-9-anthracenylmethylcinchonidium bromide (**86**) (15.0 mg, 0.025 mmol, 0.1 equiv.) and 50% aqueous KOH (0.5 mL) in toluene (3 mL) at rt. The mixture was stirred vigorously for 24 h and extracted with EtOAc (4 x 15 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded ethyl 2-[(diphenylmethylene)amino]hexanoate as a yellow oil (40 mg, 50%). R_f 0.43 (2:1 Hex-EtOAc). $[\alpha]_D^{25}$ +20.50 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.83 (t, *J* = 7.4 Hz, 3H), 1.17-136 (m, 7H), 1.89 (dd, *J* = 15.0, 7.5 Hz, 2H), 4.04 (t, *J* = 6.6 Hz, 1H), 4.16 (t, *J* = 7.0 Hz, 2H), 7.25-7.61 (m, 6H), 7.79-7.81 (m, 4H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.8, 14.2, 19.3, 28.3, 32.9, 66.9, 127.9, 128.0, 128.3, 128.5, 128.8, 130.0, 130.2, 132.4, 137.6, 169.9, 170.2. HRMS (ESI) calcd for $C_{21}H_{26}NO_2$ $(M+H)^+$ 324.1960, obsd 324.1953.



tert-Butyl 2-[(diphenylmethylene)amino]hexanoate (Table 2.1, Entry 4)

Iodobutane (97 µL, 155.5 mg, 0.845 mmol, 5.0 equiv.) was added dropwise to a mixture of *tert*-butylglycinate benzophenone imine (**76b**) (50.0 mg, 0.170 mmol, 1.0 equiv.), *O*(9)-allyl-*N*-9-anthracenylmethylcinchonidium bromide (**86**) (10.2 mg, 0.017 mmol, 0.1 equiv.) and $CsOH.H_2O$ (283.8 mg, 1.700 mmol, 10.0 equiv.) in CH_2Cl_2 (3 mL) at -78 °C. The mixture was warmed to -60 °C and stirred vigorously for 24 h. The suspension was diluted with ether (30 mL), washed with water (2 x 10 mL), brine (20 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1) afforded *tert*-butyl 2-[(diphenylmethylene)amino]hexanoate as a yellow oil (38 mg, 51%). R_f 0.44 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ +15.01 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.92 (t, *J* = 7.4 Hz, 3H), 1.37-1.43 (m, 2H), 1.46 (s, 9H), 1.80 (pent, *J* = 7.1 Hz, 2H), 3.19 (t, *J* = 7.0 Hz, 2H), 4.12 (s, 1H), 7.17-7.41 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 6.8, 12.9, 23.6, 28.1, 35.5, 56.4, 81.0, 127.7, 128.0, 128.6, 128.7, 130.3, 136.2, 139.4, 169.8, 171.4. HRMS (ESI) calcd for $C_{23}H_{30}NO_2$ $(M+H)^+$ 352.2271, obsd 352.2282.



(*S*,*E*)-6-*tert*-butyl-1-methyl-5-[(diphenylmethylene)amino]hex-2-enedioate (**108**)

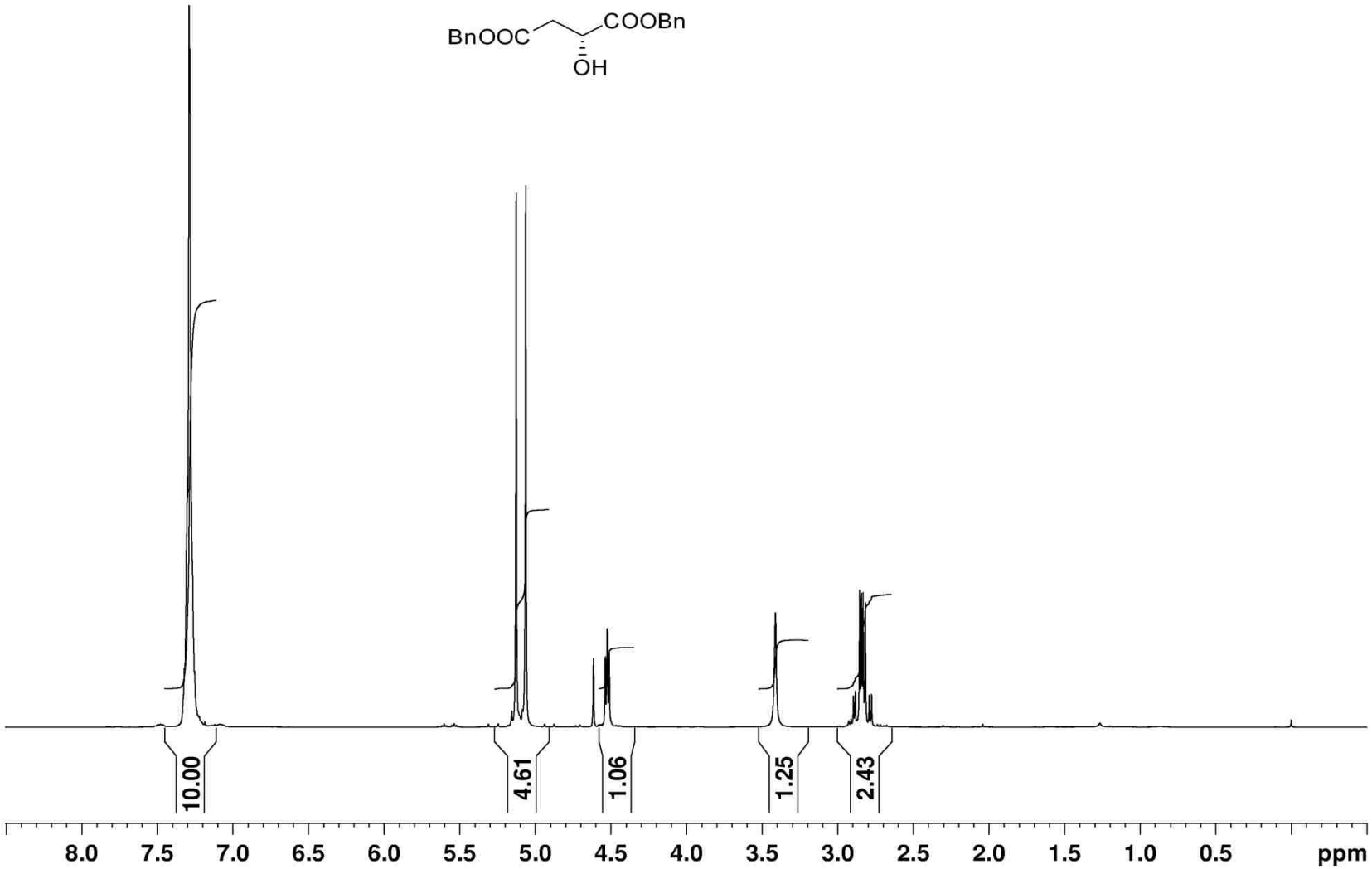
(*E*)-Methyl-4-bromobut-2-enoate (**107**) (151 mg, 0.845 mmol, 5.0 equiv.) was added dropwise to a mixture of *tert*-butylglycinate benzophenone imine (**76b**) (50 mg, 0.170 mmol, 1.0 equiv.), *O*(9)-allyl-*N*-9-anthracenylmethylcinchonidium bromide (**86**) (11 mg, 0.017 mmol, 0.1 equiv.) and $CsOH.H_2O$ (2834 mg, 1.700 mmol, 10.0 equiv.) in CH_2Cl_2 (5 mL) at -78 °C. The mixture was warmed to -60 °C and stirred vigorously for 24 h. The suspension was diluted with ether (30 mL), washed with water (2 x 10 mL), brine (20 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded compound **108** as a yellow oil (60 mg, 89%). R_f 0.30 (4:1 Hex-EtOAc). $[\alpha]_D^{25}$ +39.24 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 9H), 2.59 (dd, *J* =14.0, 7.5 Hz, 1H), 2.67 (dd, *J* = 14.0, 6.6 Hz, 1H), 3.73 (s,

3H), 4.00 (dd, *J* = 7.4, 5.4 Hz, 1H), 5.0 (d, *J* = 10.1 Hz, 1H), 5.06 (d, *J* = 17.1 Hz, 1H), 5.67-5.78 (m, 1H), 7.16-7.56 (m, 10 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.1, 38.2, 65.9, 81.0, 117.2, 128.0, 128.3, 128.4, 128.5, 128.8, 130.1, 130.2, 132.4, 134.8, 136.7, 139.8, 168.8, 170.1, 170.9. HRMS (ESI) calcd for $C_{24}H_{28}NO_4$ $(M+H)^+$ 394.2610, obsd 394.2614.

2.8.3 Spectra

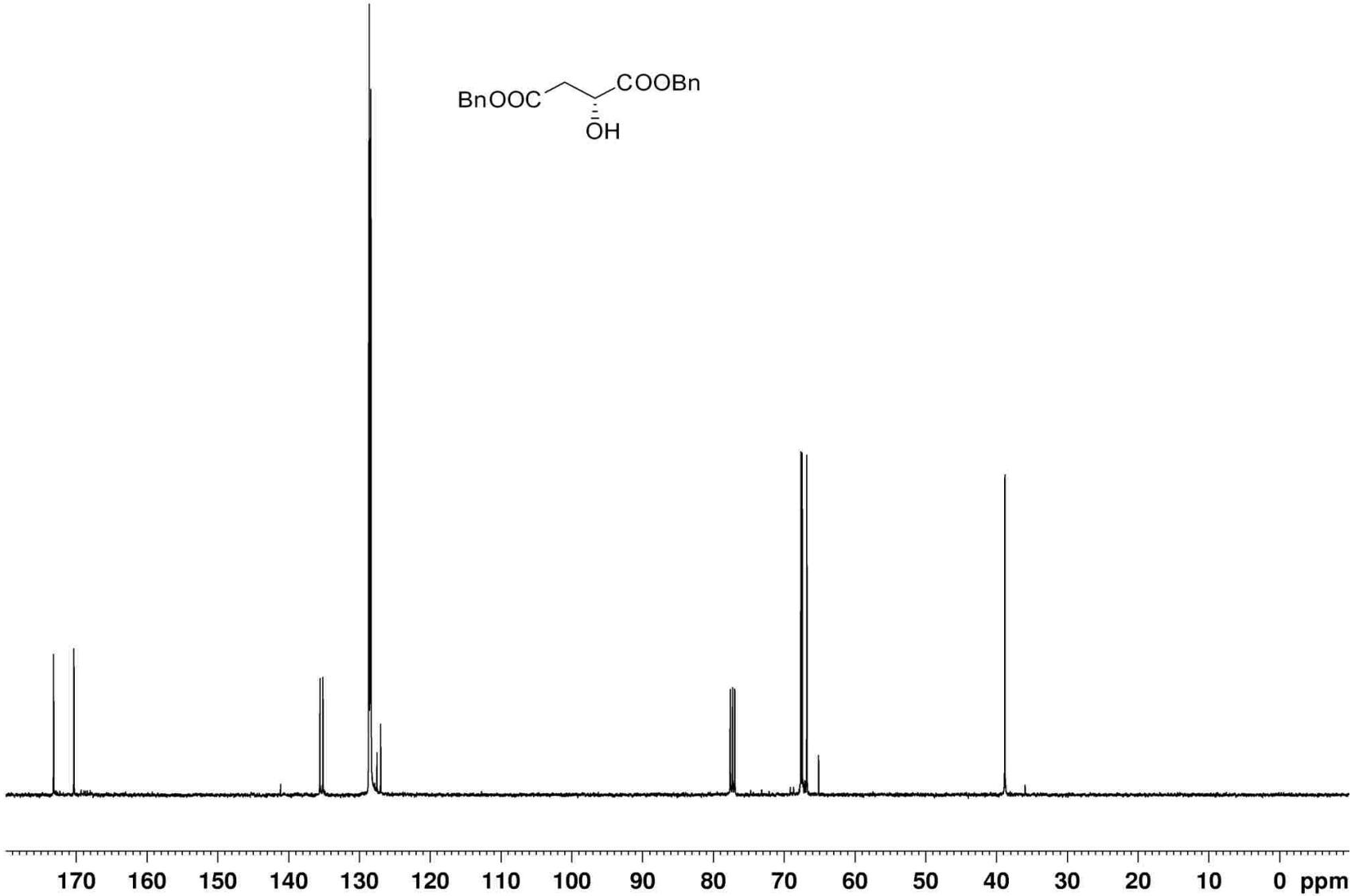
Compound **92** (Scheme 2.11) - ^{1}H NMR spectrum

SY–01–(S)–dibenzyl–2–hydroxysuccinate in CDCl3 at 400 MHz



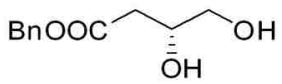
Compound **92** (Scheme 2.11) - ^{13}C NMR spectrum

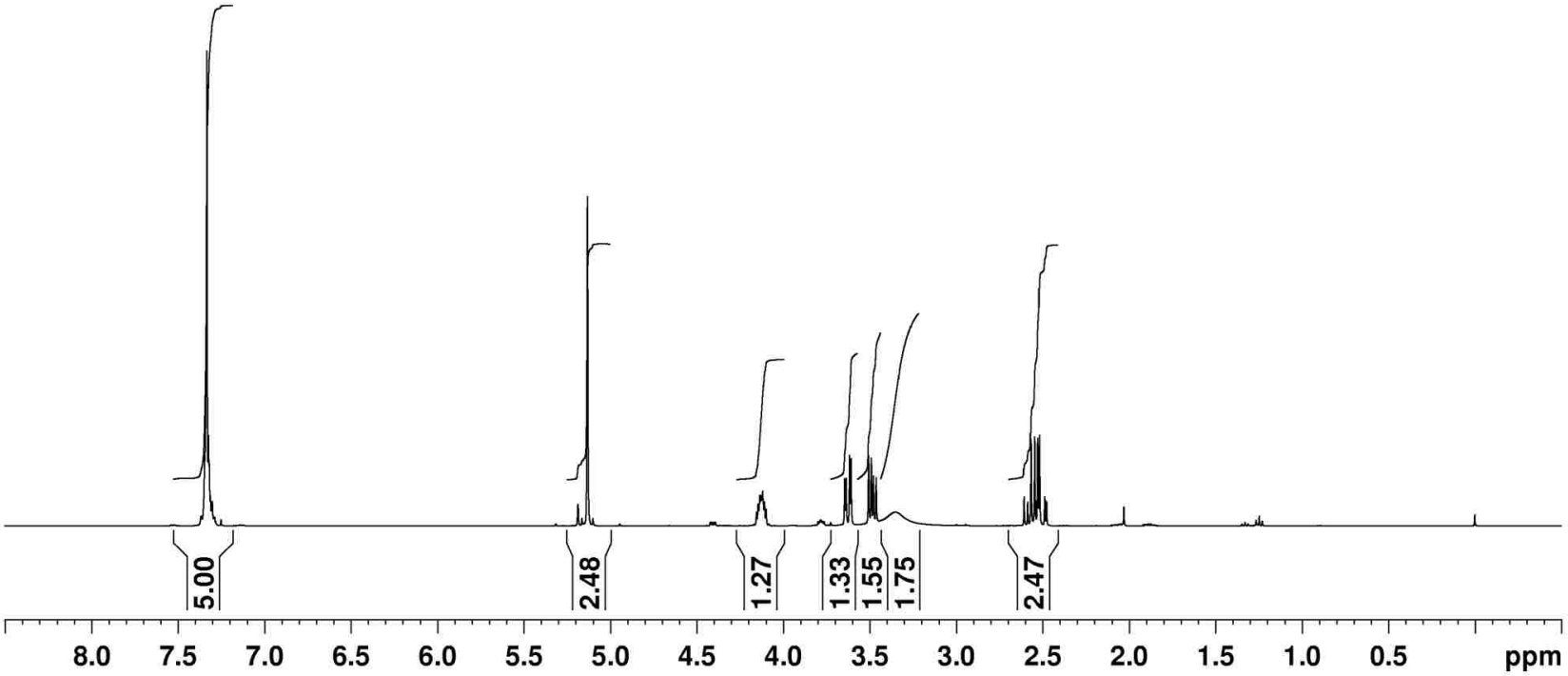
SY–01–(S)–dibenzyl–2–hydroxysuccinate in CDCl3 at 100 MHz



Compound **93** (Scheme 2.11) - ^{1}H NMR spectrum

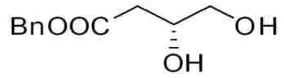
SY–01–084 in CDCl3 at 400 MHz

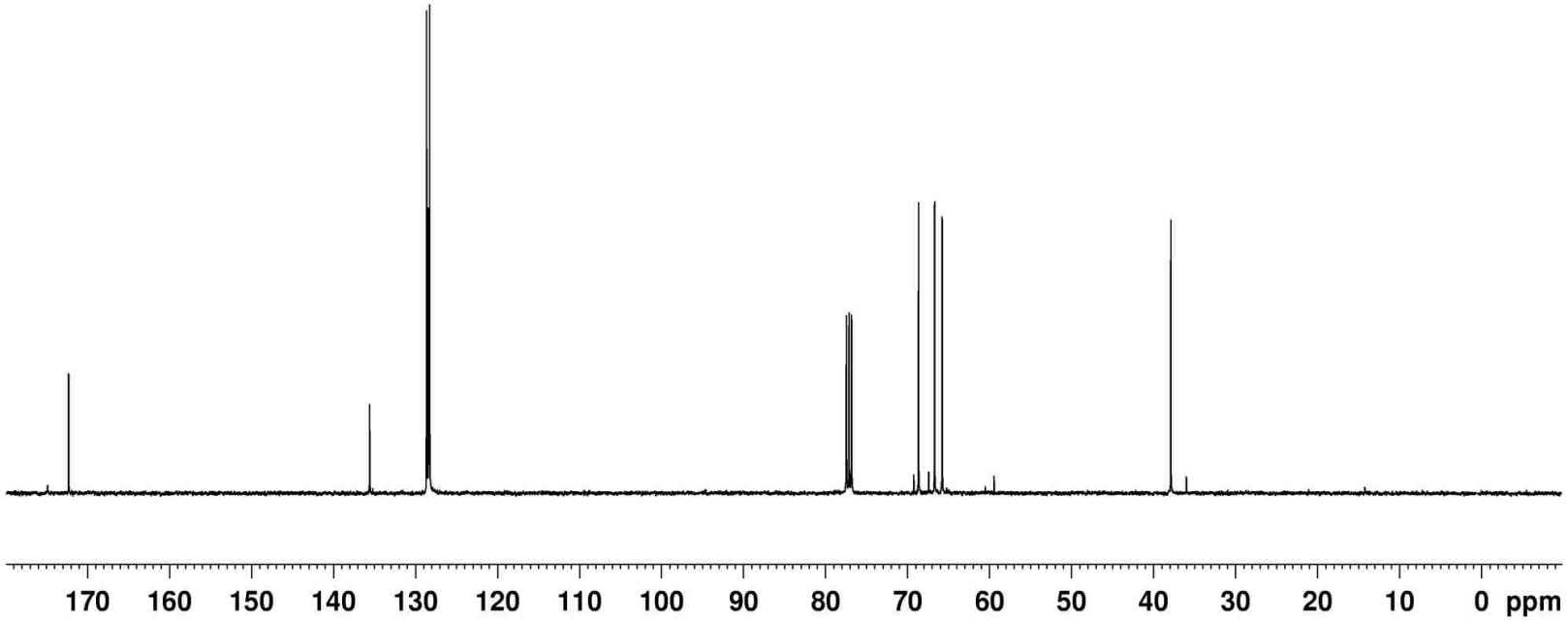




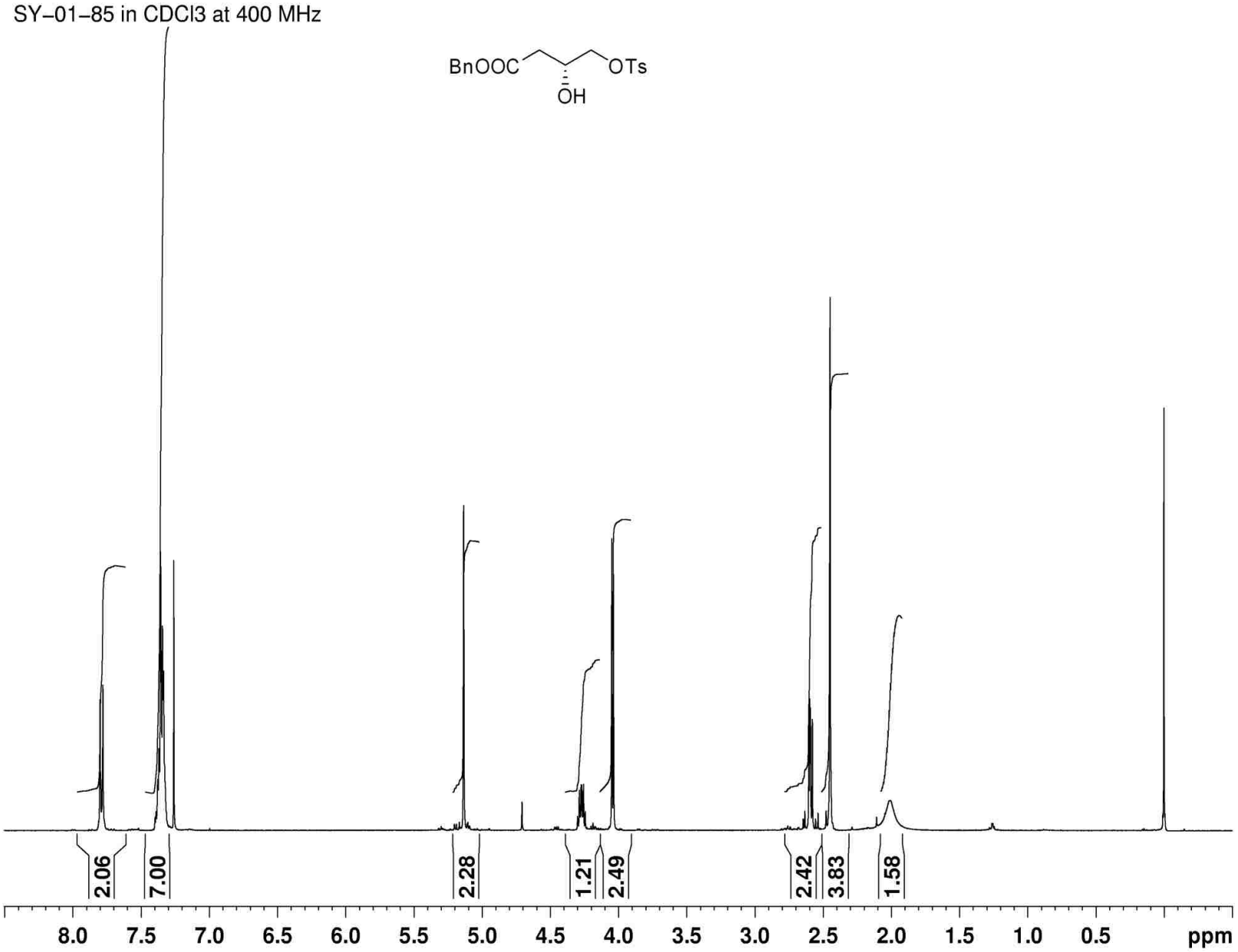
Compound **93** (Scheme 2.11) - ^{13}C NMR spectrum

SY–01–084 in CDCl3 at 100 MHz

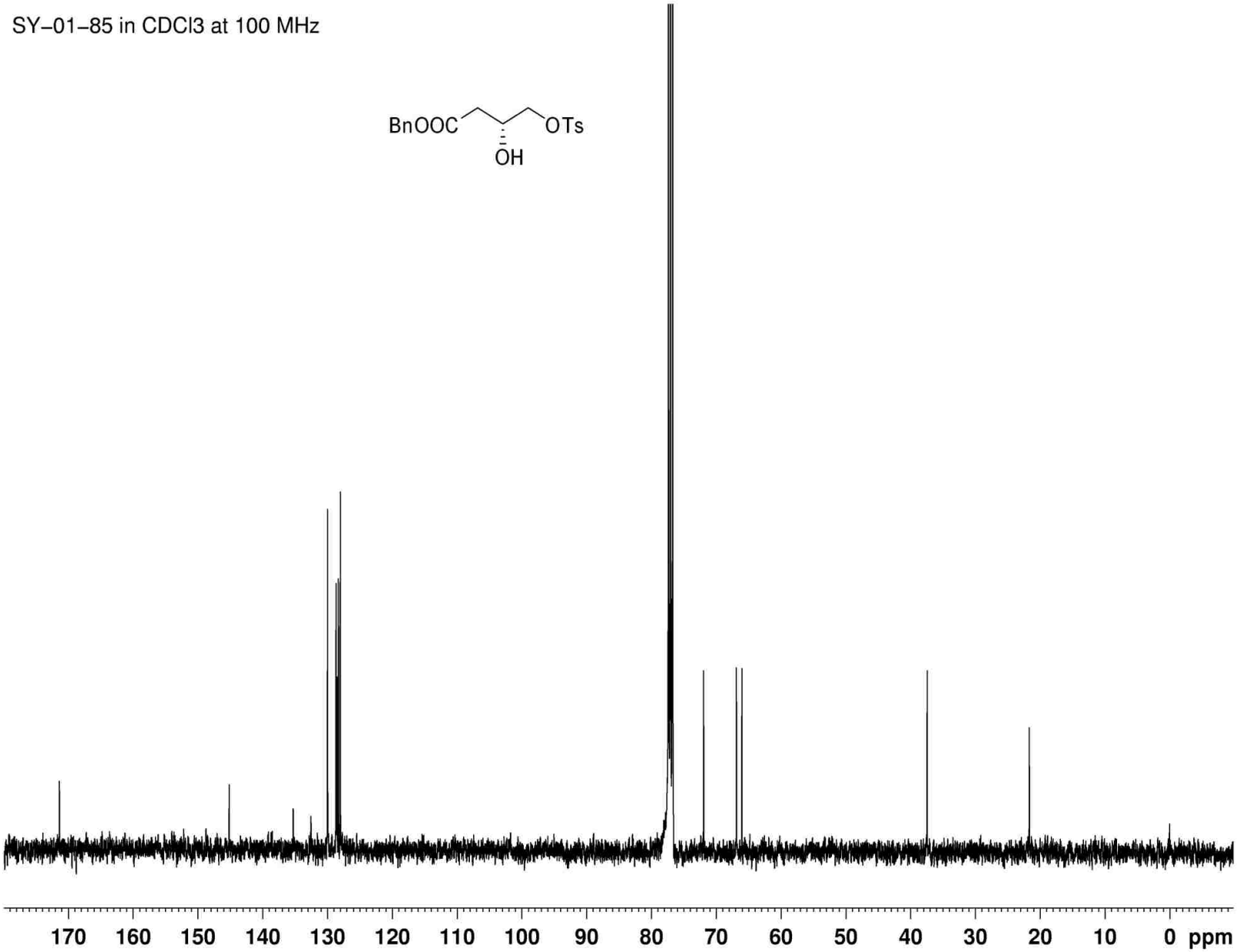




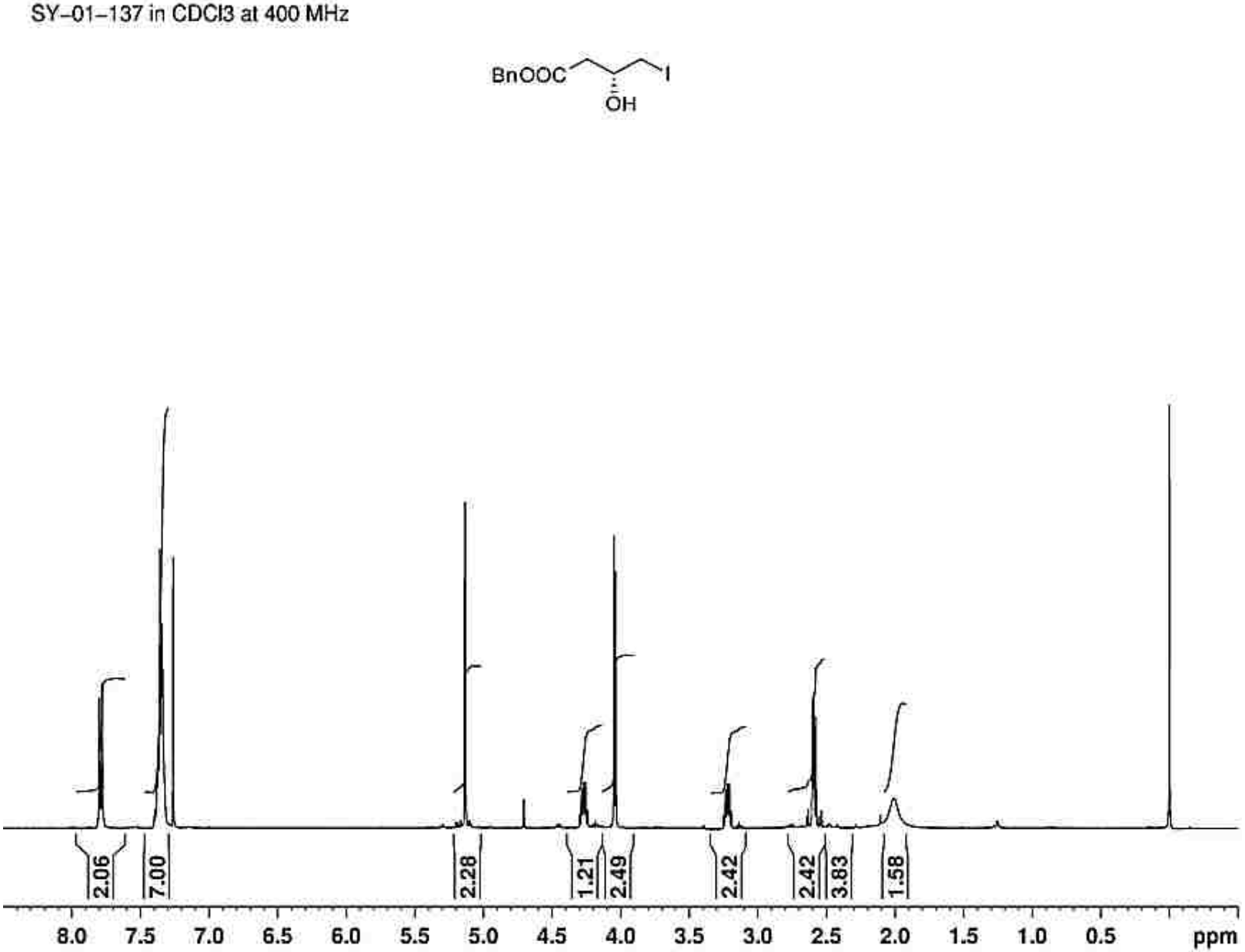
Compound **94** (Scheme 2.13) - ^{1}H NMR spectrum



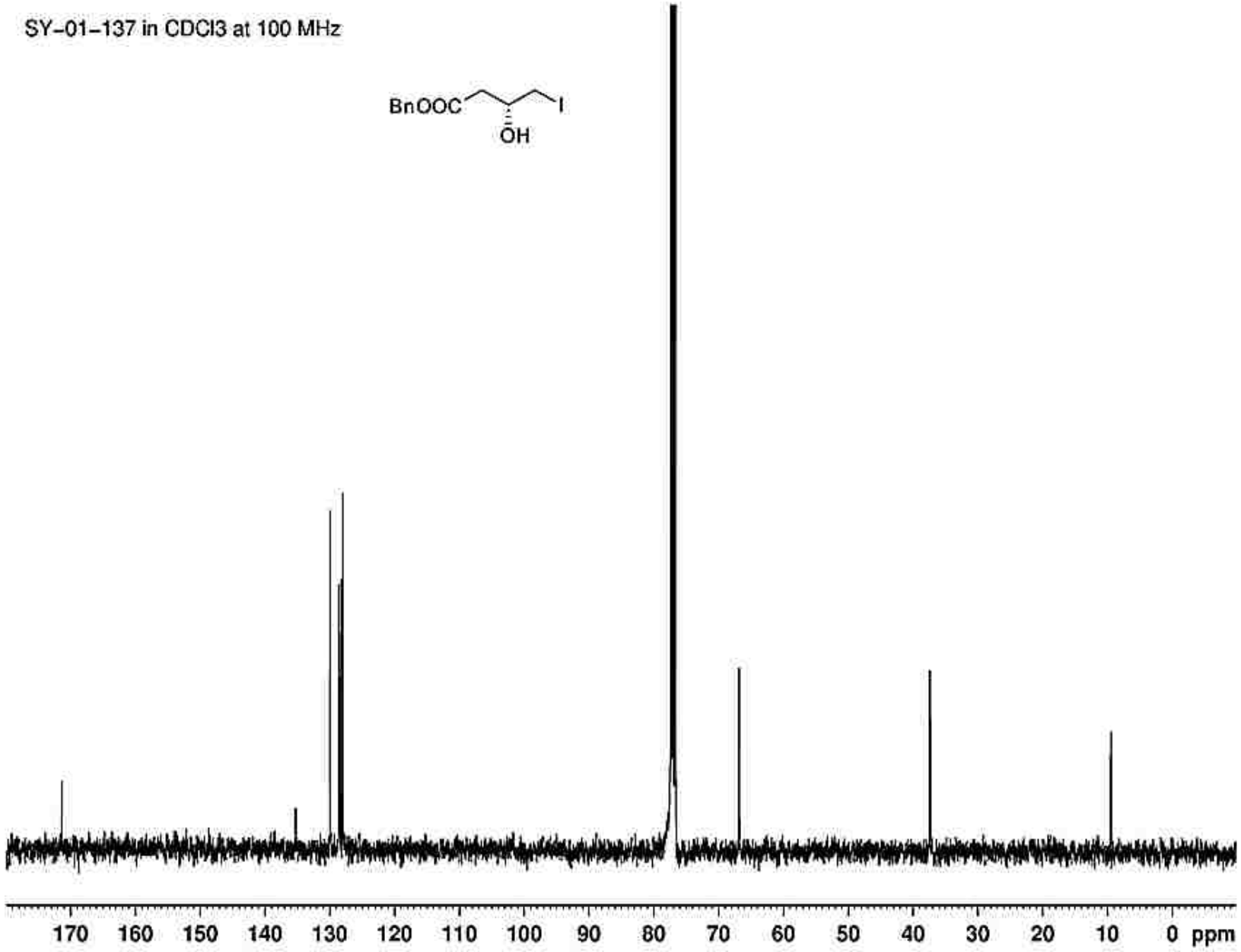
Compound **94** (Scheme 2.13) - ^{13}C NMR spectrum



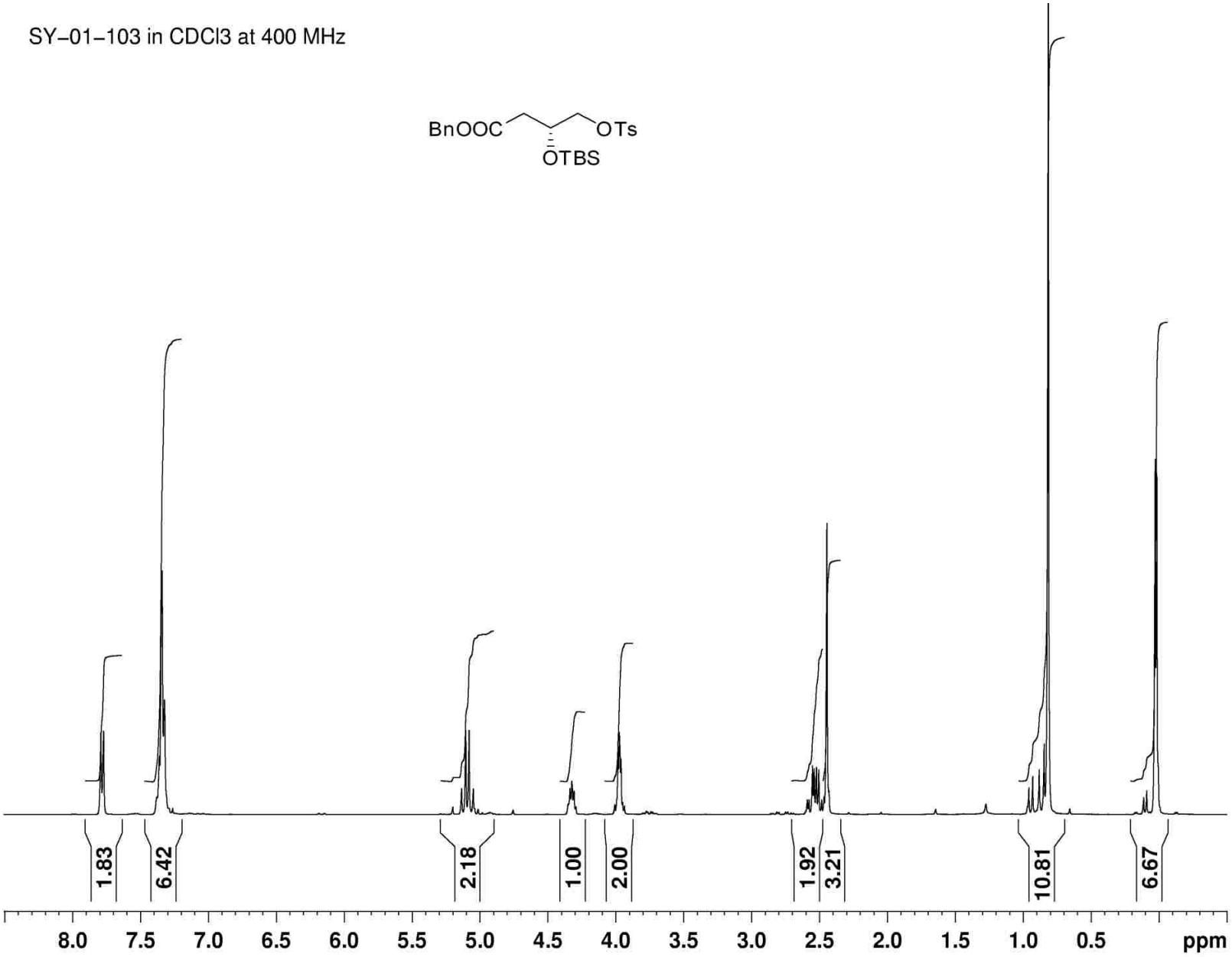
Compound **102** (Scheme 2.13) - ^{1}H NMR spectrum



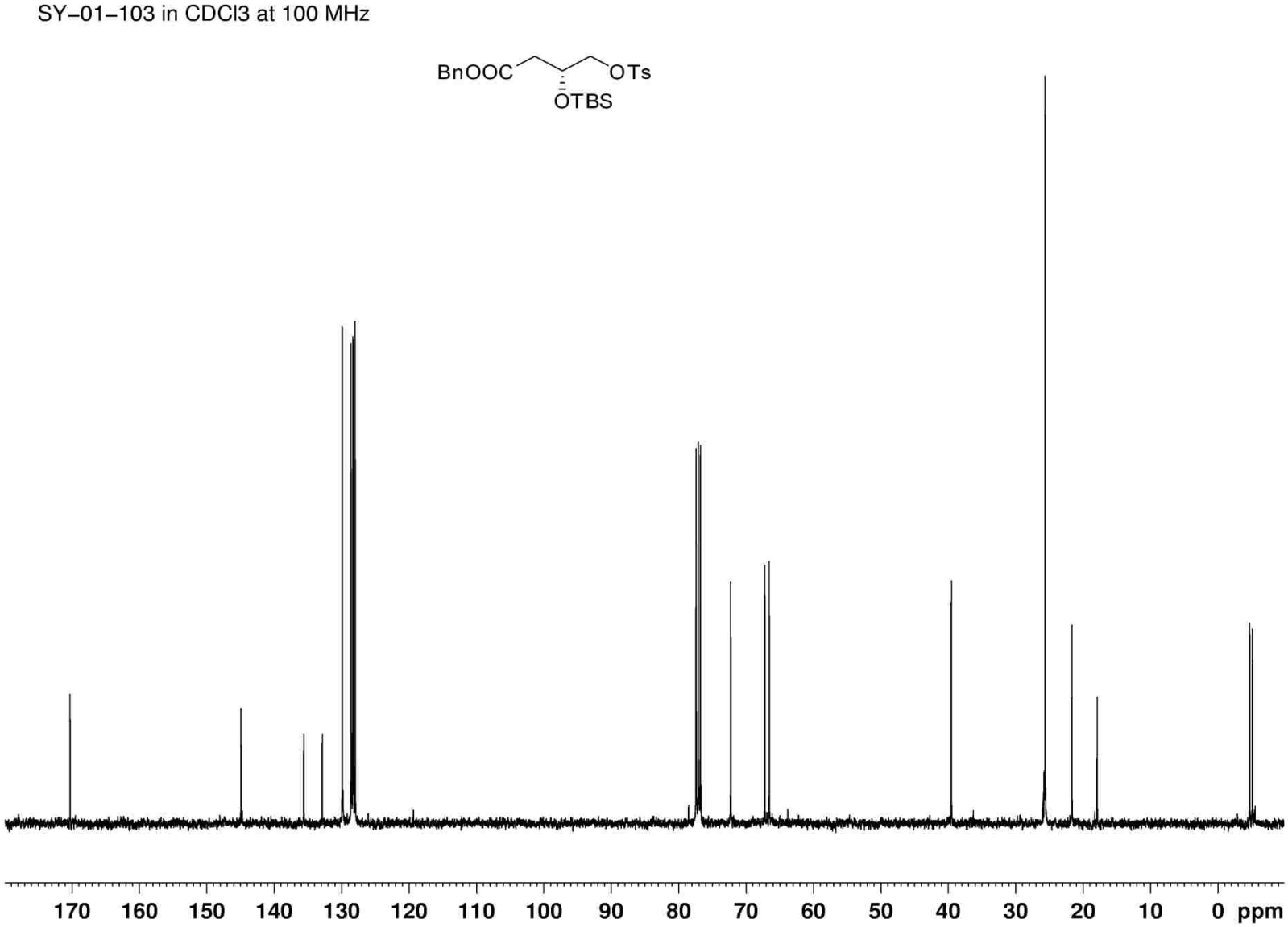
Compound **102** (Scheme 2.13) - ^{13}C NMR spectrum



Compound **103a** (Scheme 2.13) - ^{1}H NMR spectrum

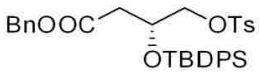


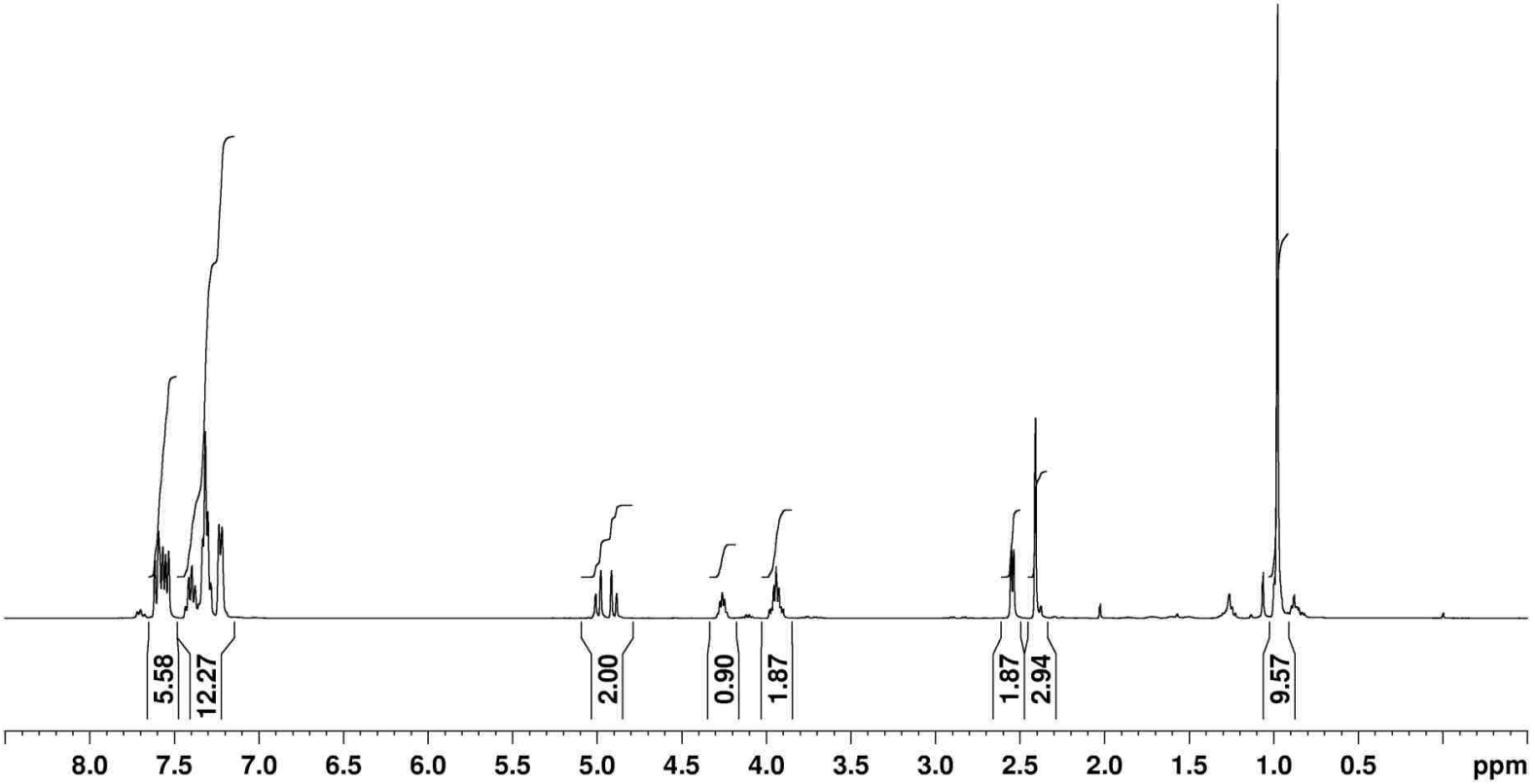
Compound **103a** (Scheme 2.13) - ^{13}C NMR spectrum



Compound **103b** (Scheme 2.13) – ^{1}H NMR spectrum

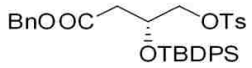
SY–01–91 in CDCl3 at 400 MHz

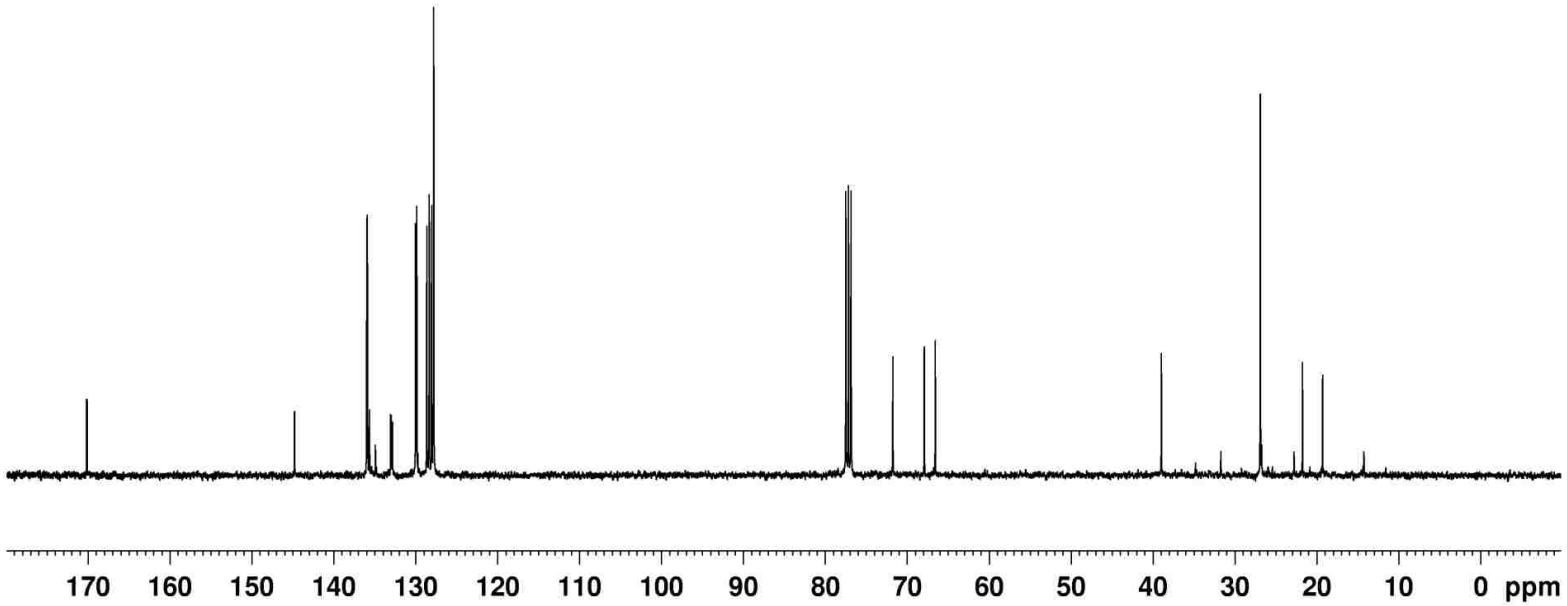




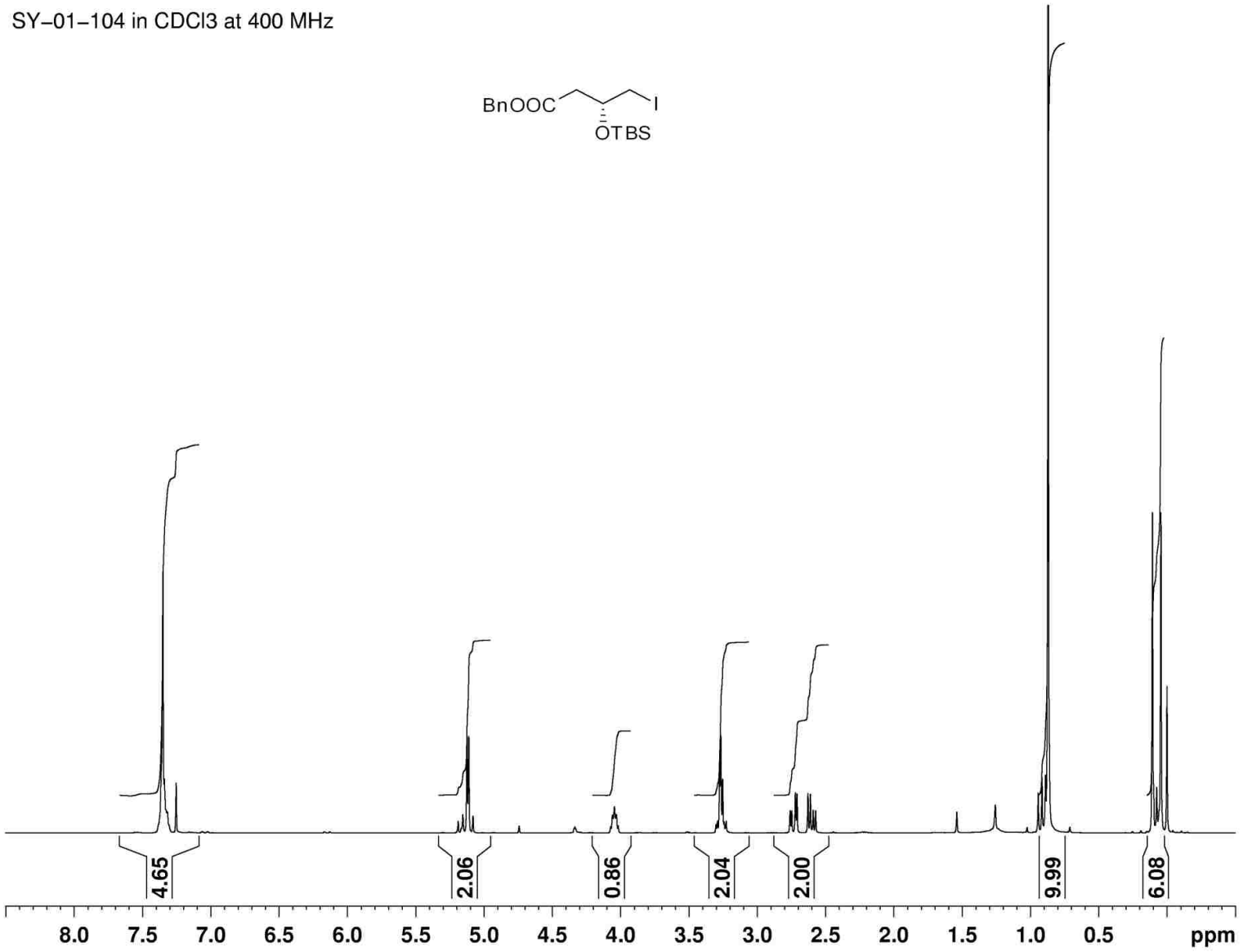
Compound **103b** (Scheme 2.13) - ^{13}C NMR spectrum

SY–01–91 in CDCl3 at 100 MHz

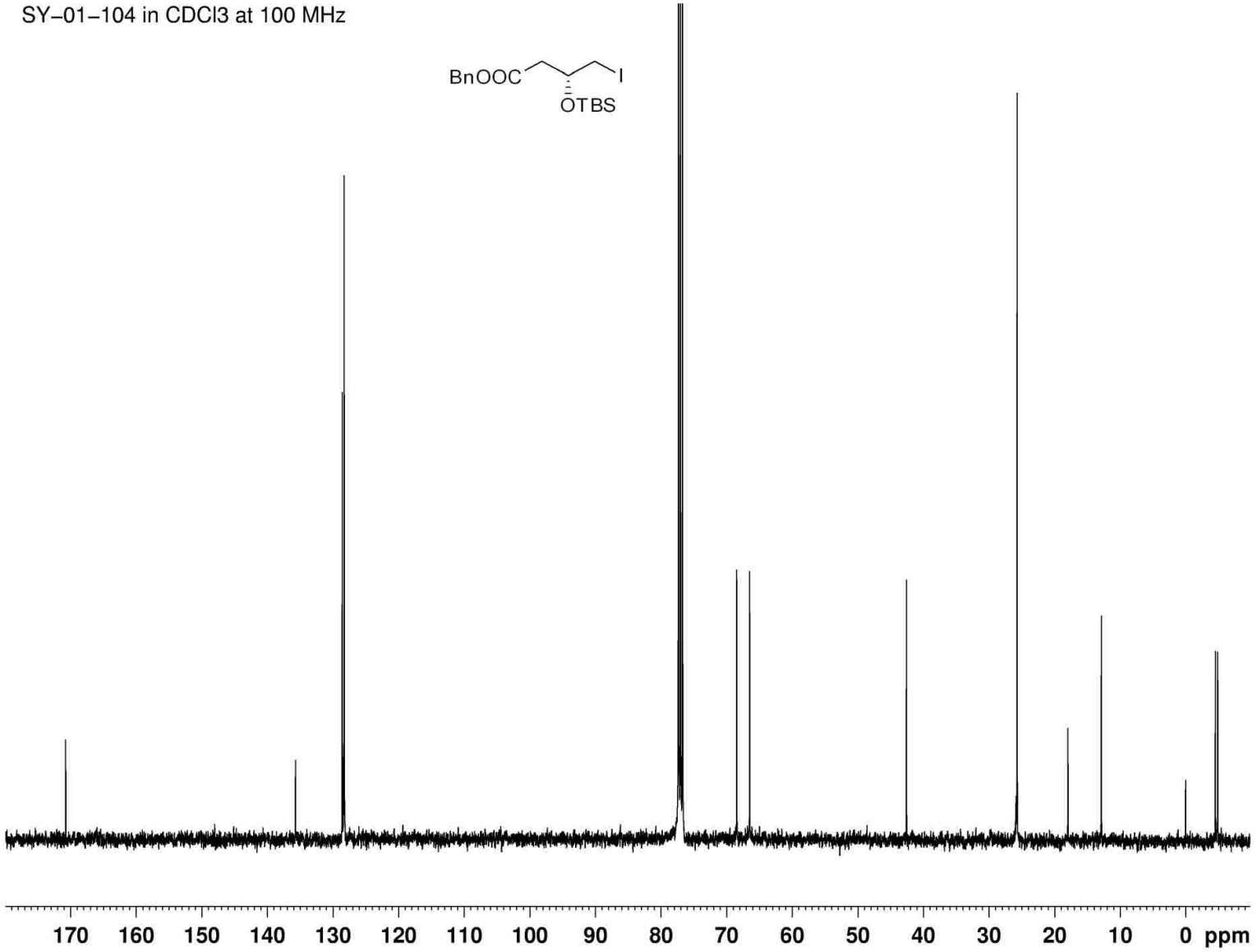




Compound **104a** (Scheme 2.13) – ^{1}H NMR spectrum

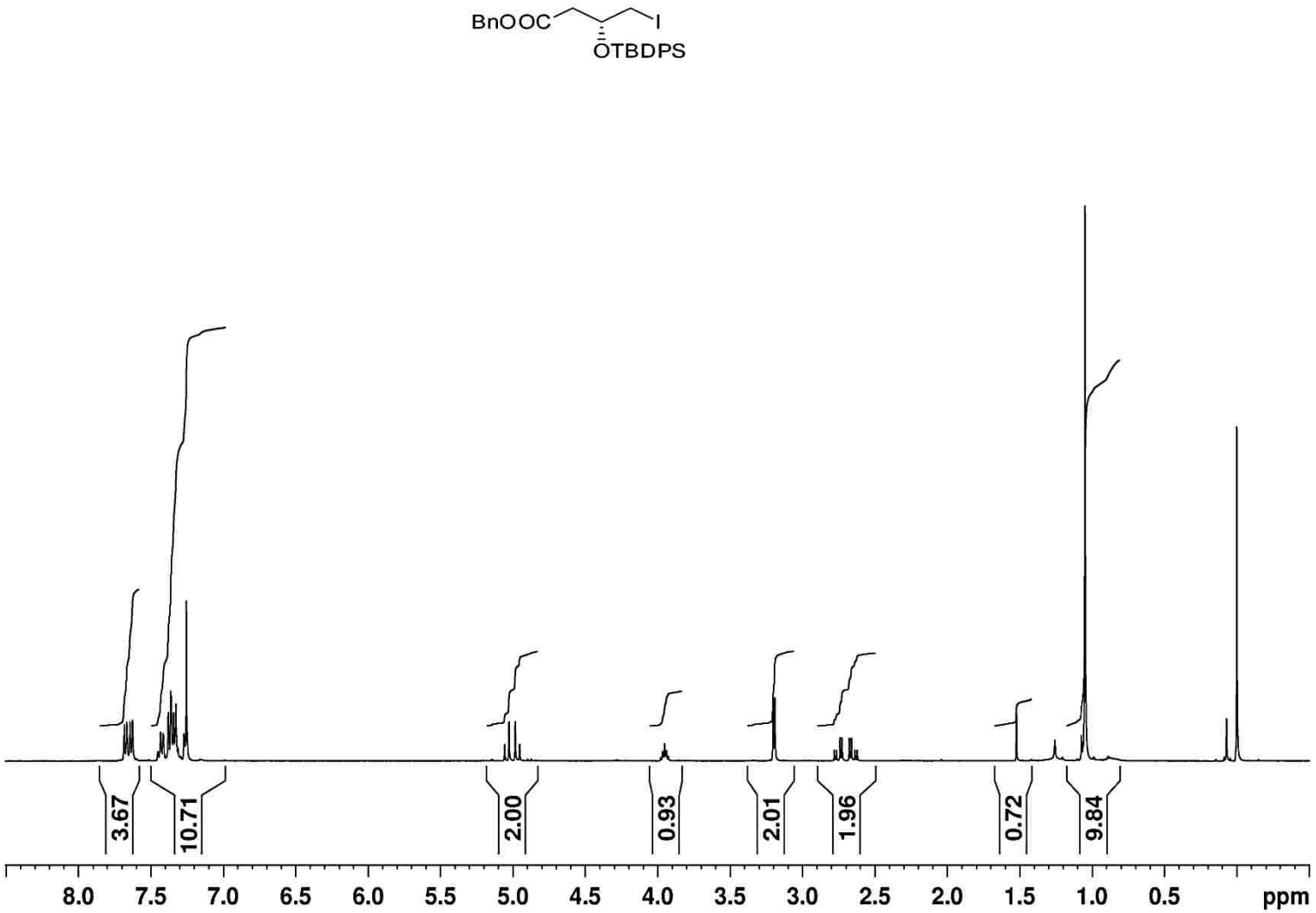


Compound **104a** (Scheme 2.13) - ^{13}C NMR spectrum



Compound **104b** (Scheme 2.13) – ^{1}H NMR spectrum

SY–01–96 in CDCl3 at 400 MHz



Compound **104b** (Scheme 2.13) – ^{13}C NMR spectrum

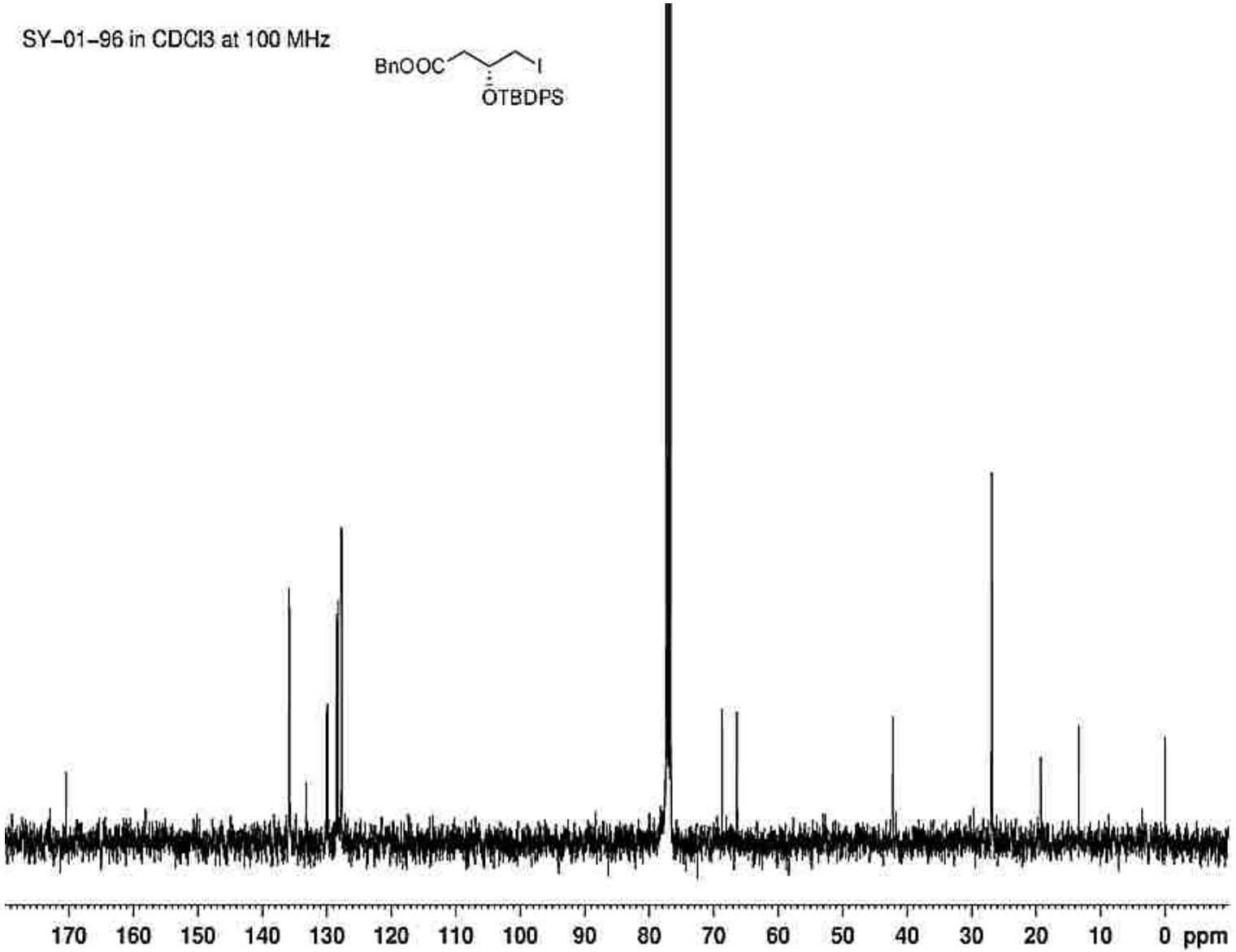


Table 2.1, Entry 1 – ^{1}H NMR spectrum

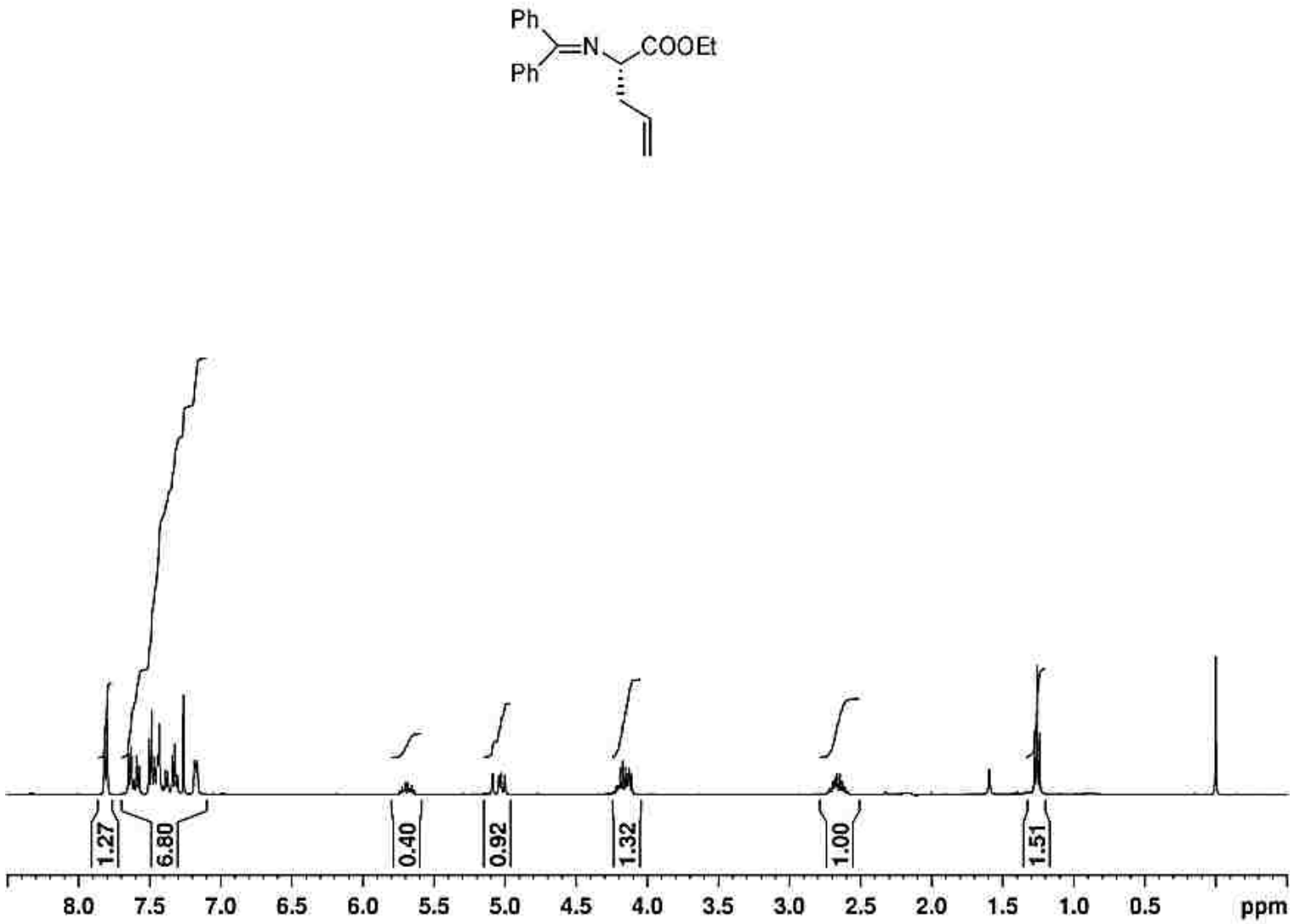


Table 2.1, Entry 1 – ^{13}C NMR spectrum

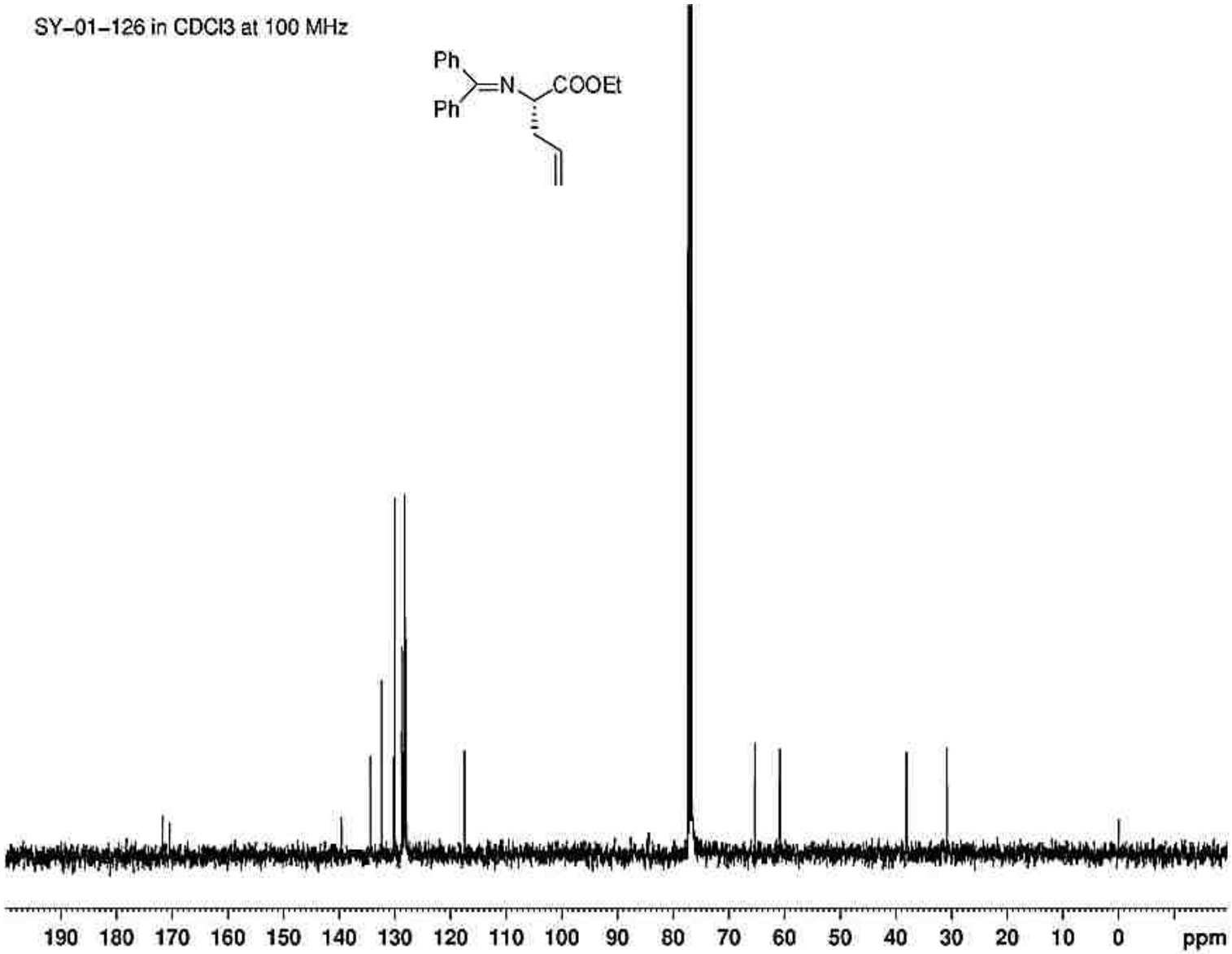


Table 2.1, Entry 2 – ^{1}H NMR spectrum

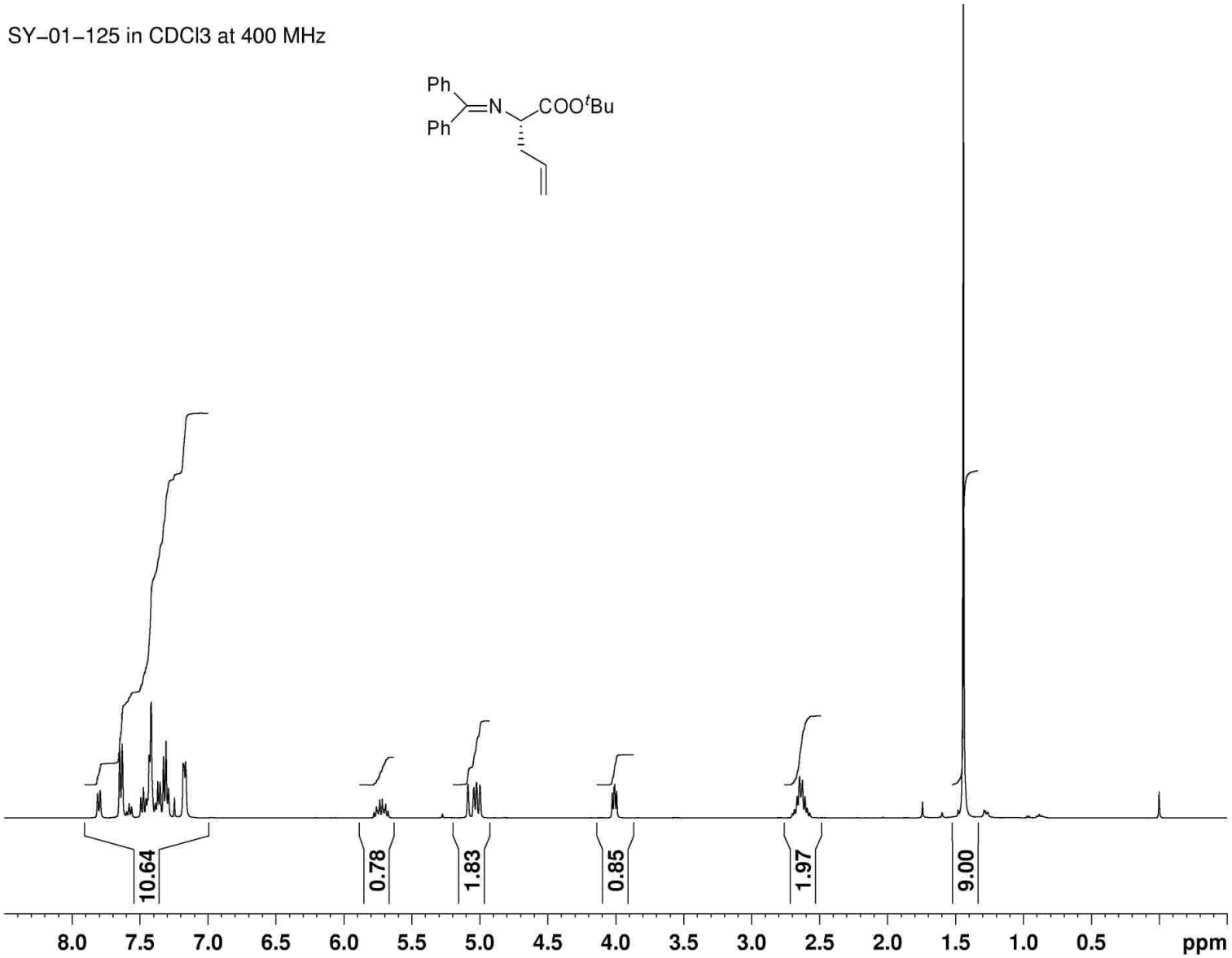


Table 2.1, Entry 2 – ^{13}C NMR spectrum

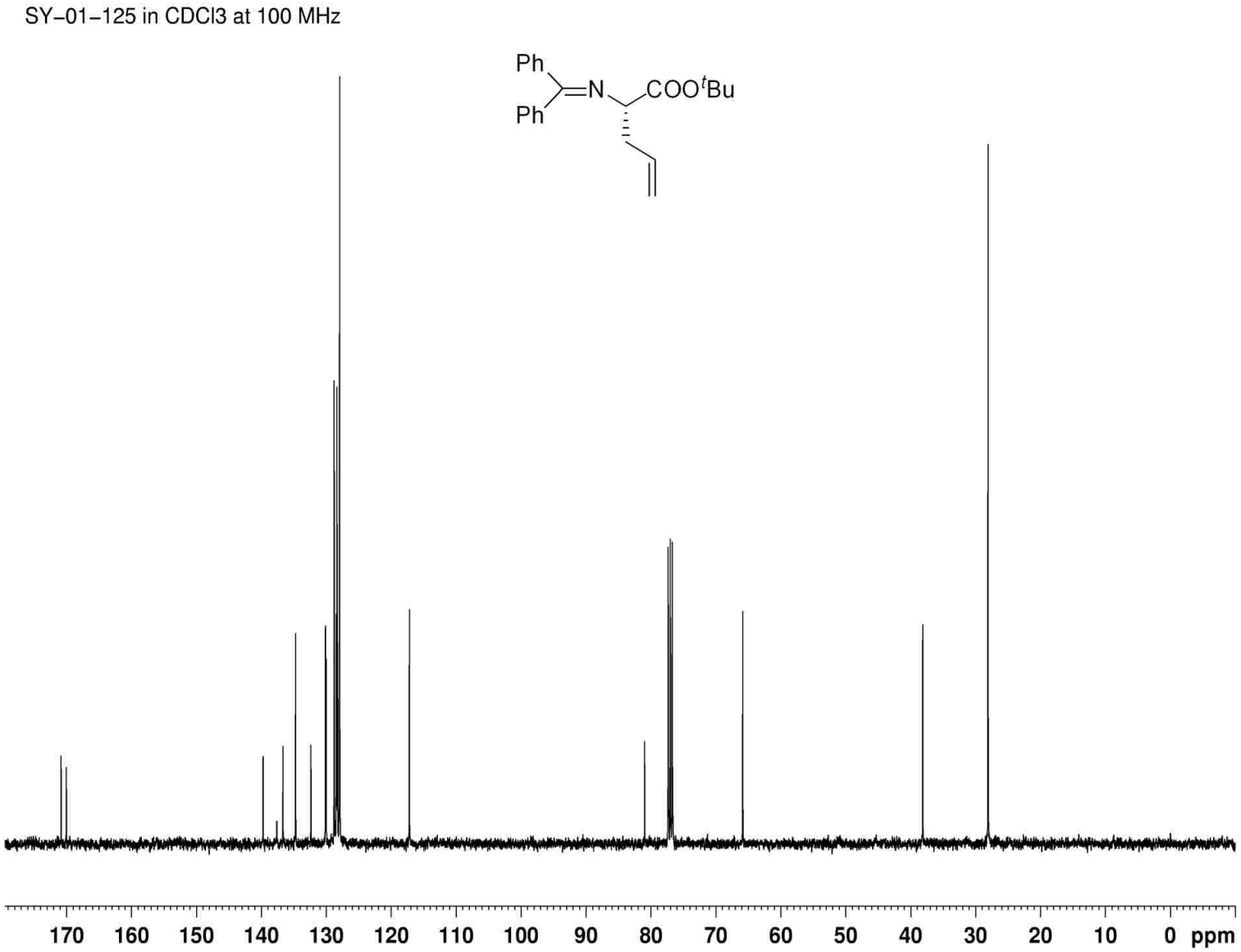
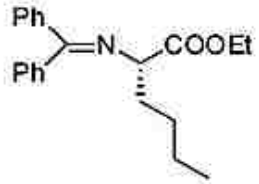


Table 2.1, Entry 3 – ^{1}H NMR spectrum

SY-01-112 in CDCl3 at 400MHz



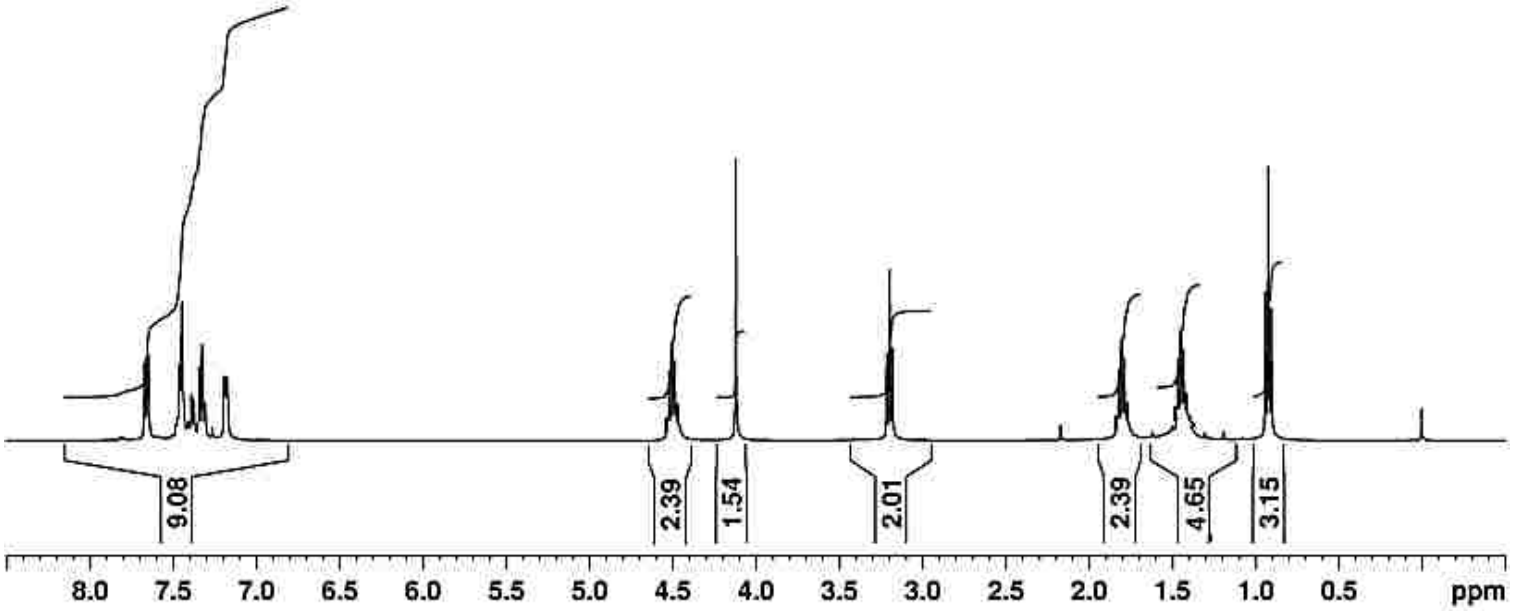


Table 2.1, Entry 3 – ^{13}C NMR spectrum

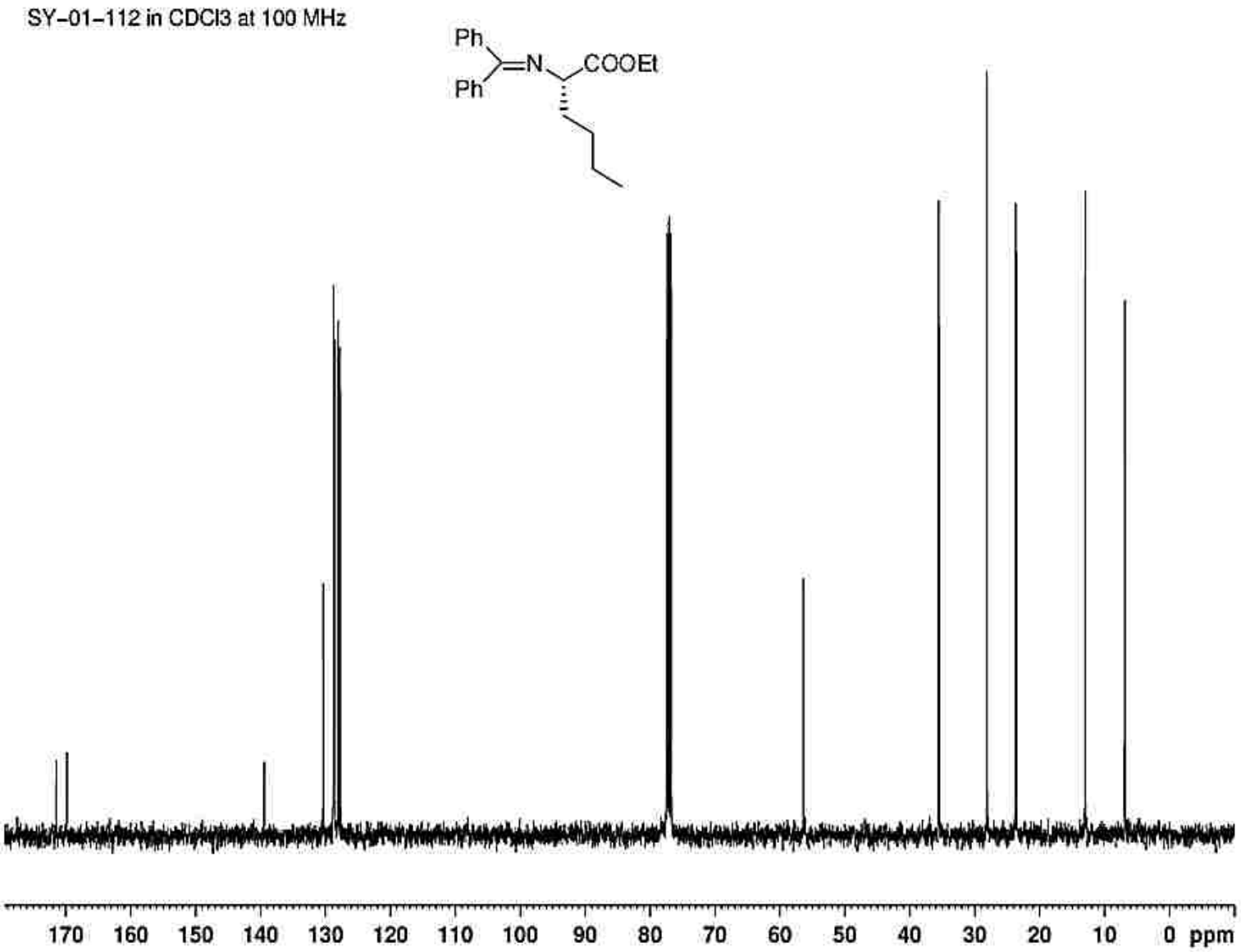


Table 2.1, Entry 4 – ^{1}H NMR spectrum

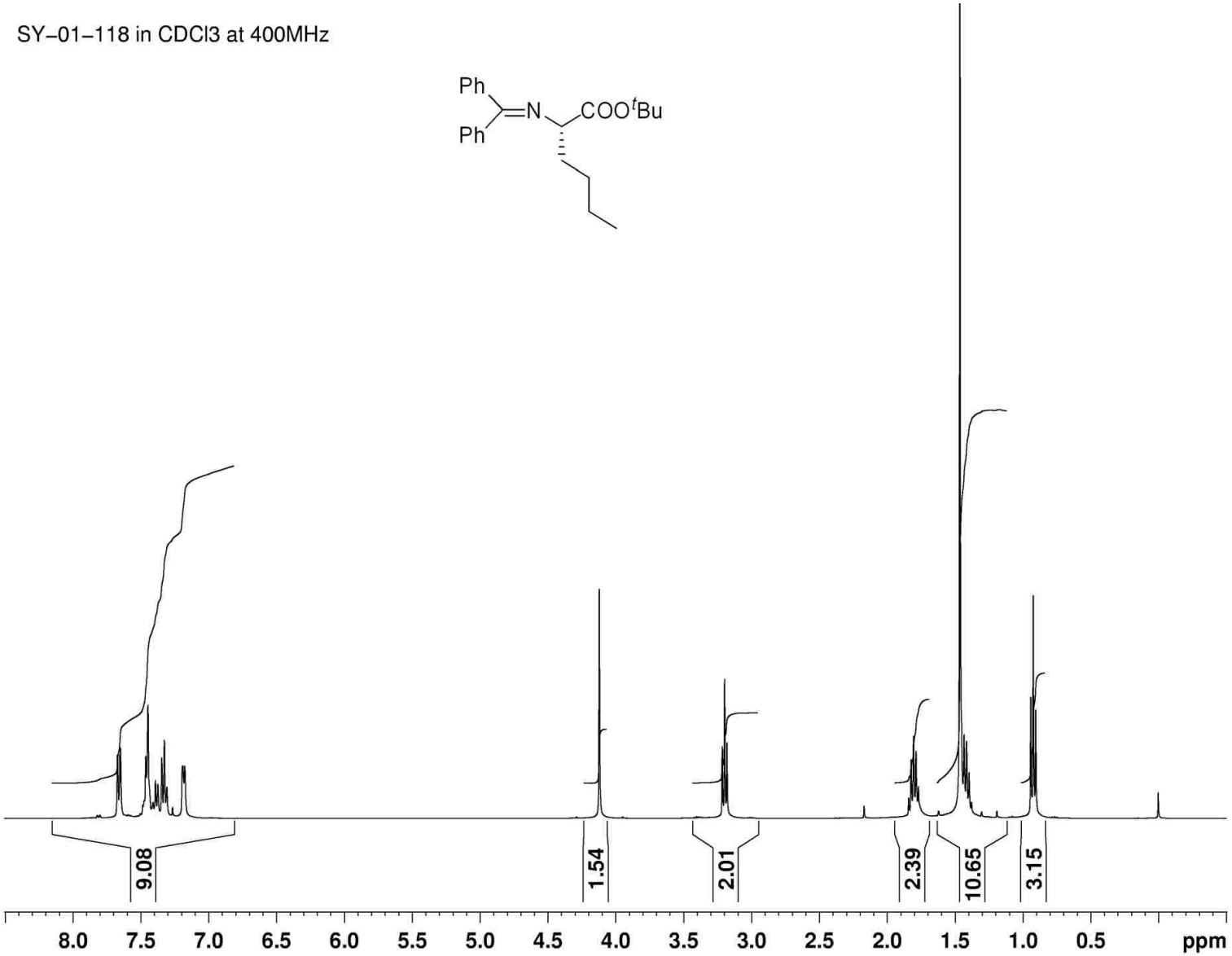
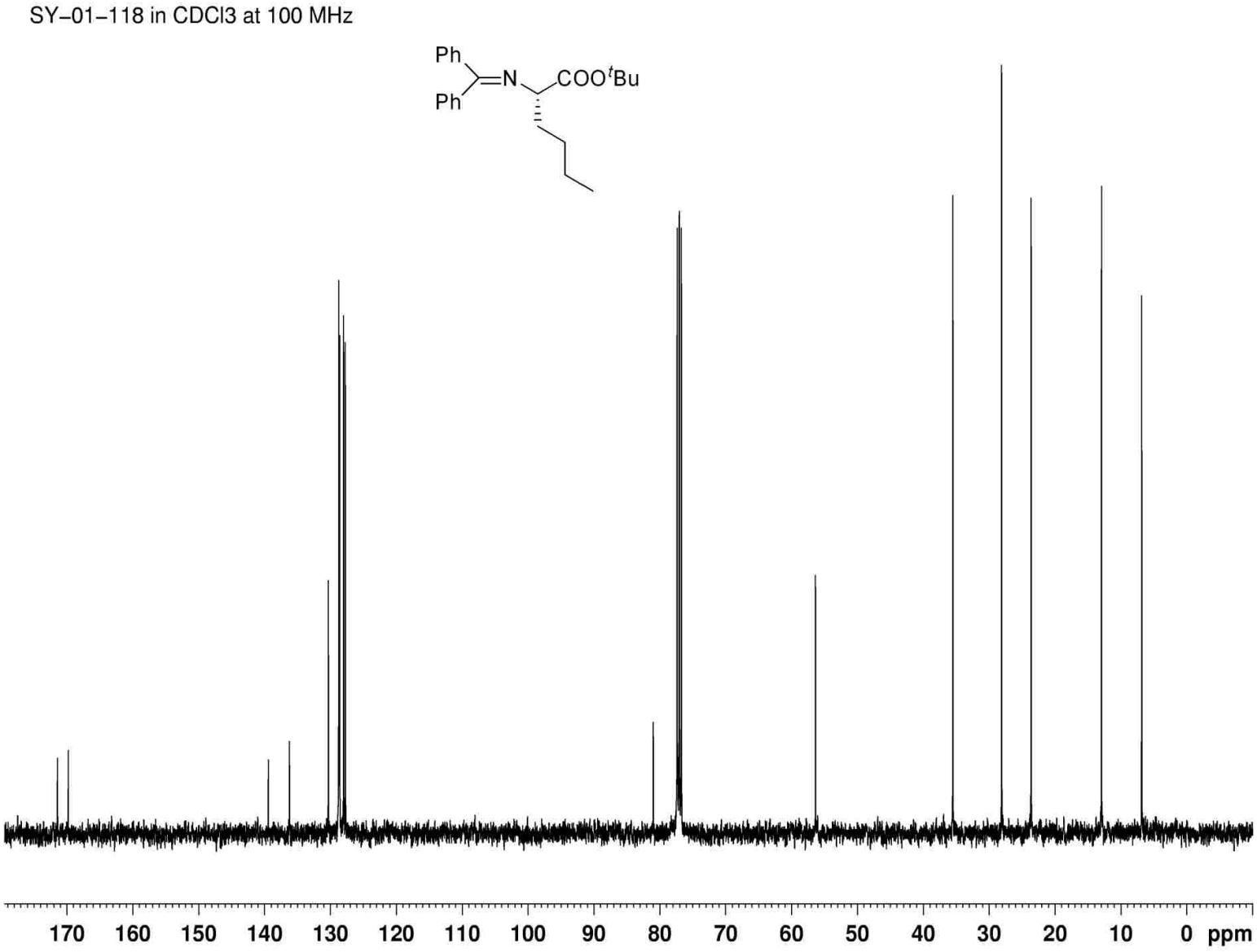
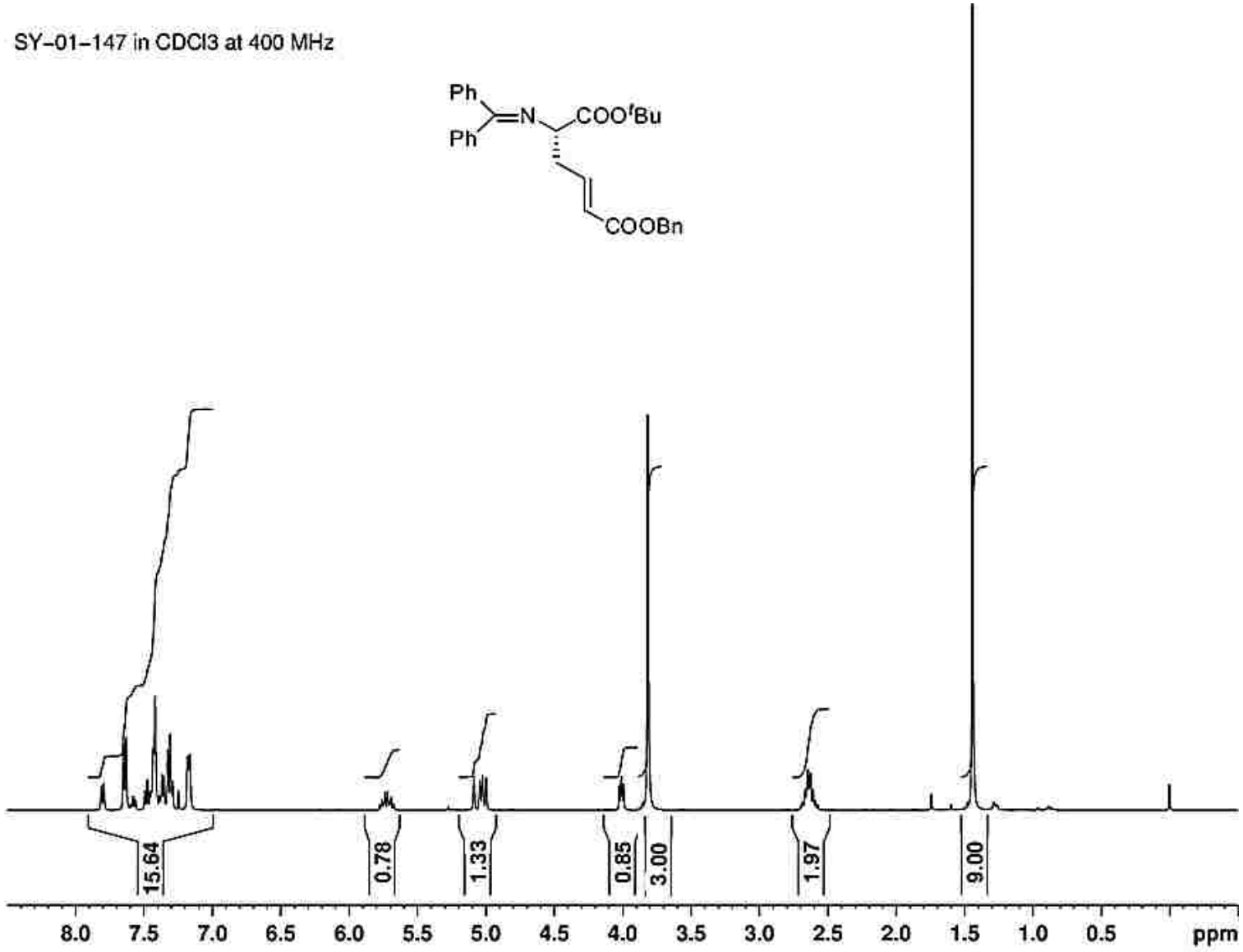


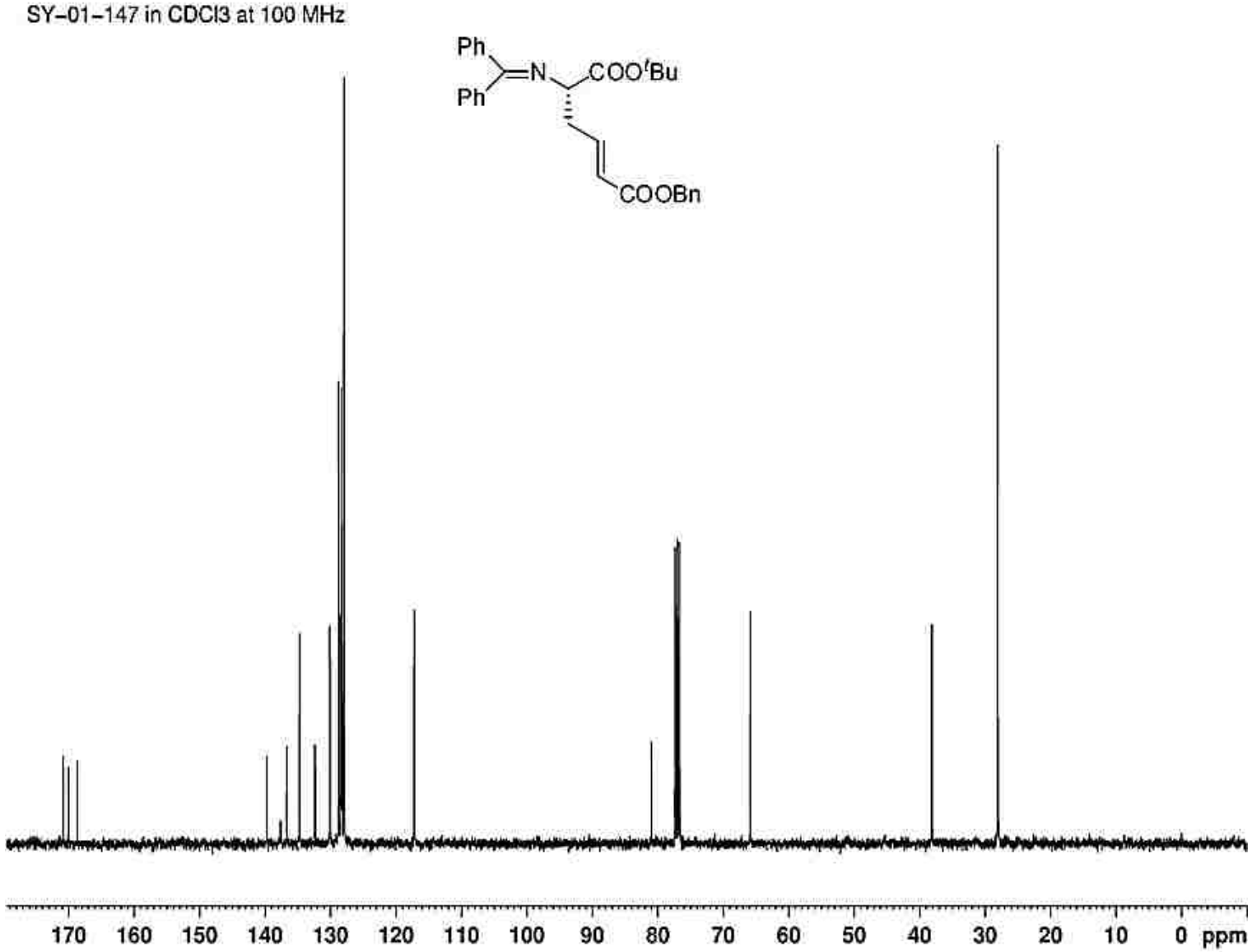
Table 2.1, Entry 4 – ^{13}C NMR spectrum



Compound **108** (Scheme 2.14) – ^{1}H NMR spectrum



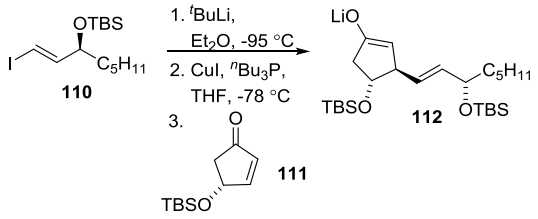
Compound **108** (Scheme 2.14) – ^{13}C NMR spectrum



CHAPTER 3: AN AMINE CONJUGATE ADDITION APPROACH TO AHAD

3.1 Conjugate Addition Reactions to Afford β-Amino Acids

Conjugate addition reactions are well known in organic chemistry. Most familiar is the conjugate addition of organometallic reagents to α,β-unsaturated carbonyl compounds for assembling structurally complex organic molecules.[49] For example, addition of the functionalized organocuprate reagent obtained from iodide **110** to the chiral cyclopentenone **111** occurred in a *trans*-selective fashion to give intermediate compound **112** (Scheme 3.1) which was used for the synthesis of prostaglandin E_2.[50,51,52]



Scheme 3.1 Preparation of intermediate for the synthesis of prostaglandin E_2 through conjugate addition.

Over the last two decades, there has been an increasing interest in functionalized β-amino acids and their derivatives[53] due to their biologically important properties and their occurrence in natural products (Figure 3.1). β-Alanine (**113**) is a β-amino acid that occurs in the western hemisphere of theonellamide C (**1**) (Figure 1.1, Chapter 1). (3*R*)-β-Leucine[54] (**114**) exists in equilibrium with (2*S*)-α-leucine and their interconversion is catalyzed by leucine-2,3-aminomutase.[55] β-Arginine is found in the natural dipeptide antibiotic TAN 1057 (**115**).[56] *N*-Acetyl-β-phenylalanine (**116**) is a substrate for a bacterial *t*RNA(Phe) peptidyltransferase.[57] β-Amino-α-phenylpropionic acid is present in the antibiotic betacine (**117**) which displays a broad spectrum of antibacterial activity[58] and its derivatives show important neurophysiological control properties.[59]

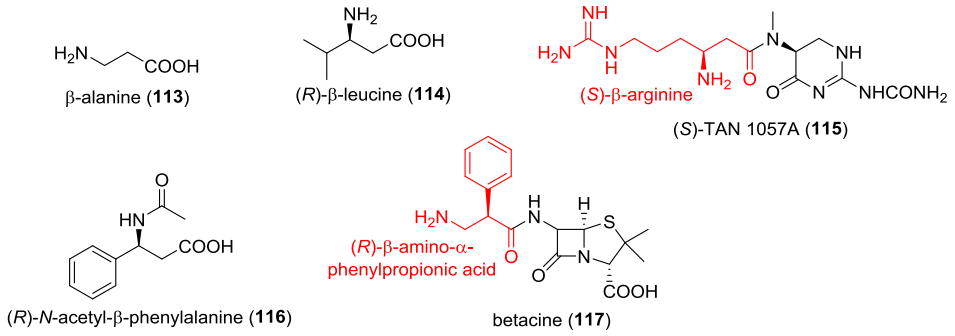
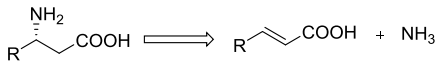


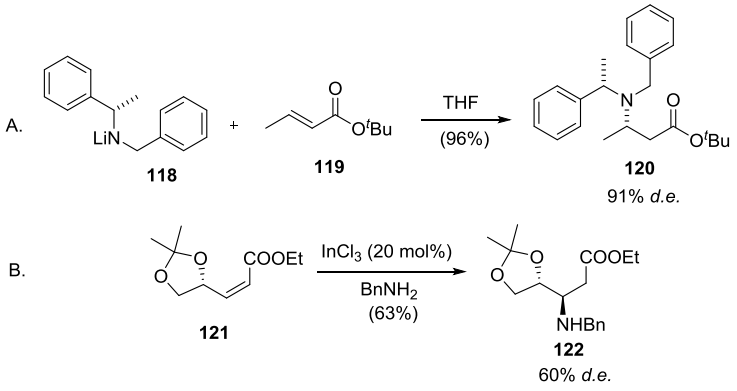
Figure 3.1 β-Amino acids (highlighted in red where they are part of a natural product).

A potentially straightforward method for the synthesis of β-amino acids is the conjugate addition of nitrogen nucleophiles to α,β-unsaturated carboxylic acid derivatives (Scheme 3.2).



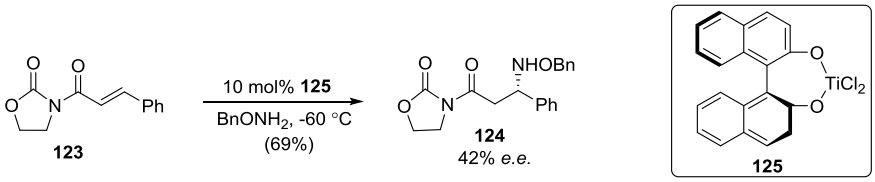
Scheme 3.2 General retrosynthesis of conjugate amine addition.

This reaction is also known as an aza-Michael reaction wherein there is 1,4-addition of a nitrogen nucleophile to the β-carbon vinylogously attached to an electron-withdrawing group.[60] There are two major approaches to achieve asymmetric induction: by using chiral auxiliaries, typically amines (Scheme 3.3-A), or enantiomerically pure starting materials, *e.g.* a chiral acceptor (Scheme 3.3-B)



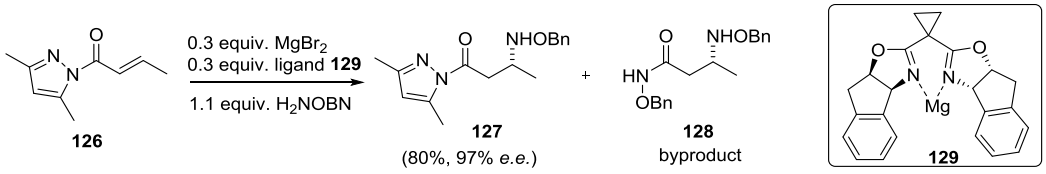
Scheme 3.3 Examples of the aza-Michael reaction.

The first example of an enantioselective conjugate amine addition was reported by Jørgensen in 1996.[61] They used a titanium BINOL catalyst **125** to catalyze the addition of benzyloxyamine ($BnONH_2$) to *N*-acyloxazolidinone **123**. *N*-Substituted hydroxylamines are more nucleophilic than *O*-substituted hydroxylamines which are useful precursors for β-amino acids.[62,62b, 63] High conversions were obtained, but enantioselectivity was moderate (up to 42% e.e.) (Scheme 3.4)



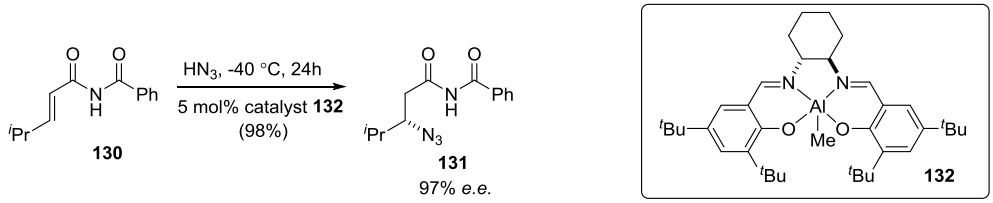
Scheme 3.4 Enantioselective conjugate amine addition using BINOL catalyst.

Sibi and co-workers have made major contributions to the field of amine conjugate additions. They developed a highly enantioselective protocol for the conjugate addition of *O*-benzylhydroxylamine to 3,5-dimethylpyrazole-derived enamide **126** using catalytic amounts of a chiral Lewis acid prepared from magnesium bromide diethyl etherate and a bisoxazoline **129**.[53] β-Amino acid derivatives, *e.g.*, **127**, were synthesized in good chemical yields and high *e.e.* The high ***e.e.***'s obtained were accounted by use of Lewis acid along with catalyst (Scheme 3.5).



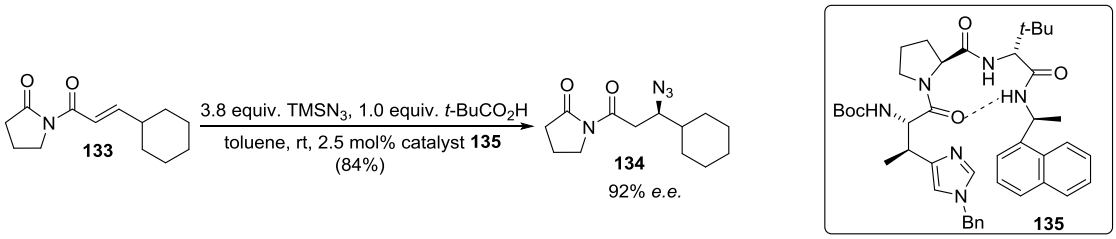
Scheme 3.5 Conjugate addition using a chiral catalyst and Lewis acid.

The use of azide as a nucleophile in conjugate addition reactions is also well known.[64] Jacobsen *et al.* demonstrated the conjugate addition of hydrazoic acid (HN_3) to α,β-unsaturated imides (*e.g.* **130**) using a chiral aluminium(III)salicylaldimine complex catalyst **132** (Scheme 3.6).[65]



Scheme 3.6 Conjugate addition using azide as nucleophile.

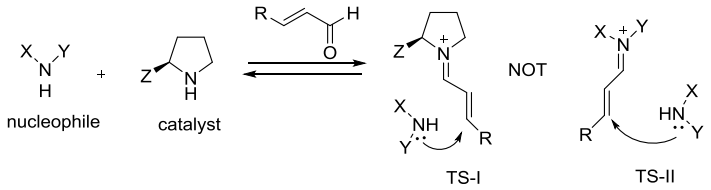
Miller and co-workers developed milder reaction conditions for azidation under metal-free conditions, in which the azide was generated from a mixture of trimethylsilyl azide ($TMSN_3$) and pivalic acid in toluene.[66,66b, 67] β-Amino acid derivative **134** was obtained in 92% *e.e.* by conjugate addition of azide to enamide **133** mediated by β-turn tripeptide **135** (Scheme 3.7).



Scheme 3.7 Preparation of β-azido derivative.

3.2 MacMillan Conjugate Addition

MacMillan and coworkers have developed organocatalysts to achieve a highly chemo- and stereoselective amine conjugate addition that is founded upon a rationally designed *N*-centered nucleophile, as depicted in Scheme 3.8. [68,69,70]



Scheme 3.8 Iminium catalyzed amination requires selective amine participation.

They showed that the rate determining step for conjugate addition reactions involving carbon and hydrido nucleophiles is the reversible formation of iminium ions from chiral amine

catalysts and α,β-unsaturated aldehydes. Moreover, the MacMillan Group illustrated a design plan for the enantioselective conjugate amination using iminium catalysis.

In their design, they stipulated that the amine required to function as a 1,4-addition nucleophile must not participate in iminium activation; this presents a racemic pathway via TS-II (Scheme 3.8). Moreover, the catalyst, a chiral secondary amine, must perform as an iminium catalyst (giving rise to TS-I) but not compete as a nucleophile. The reaction should be kinetically controlled, whereby irreversible loss of the nucleophile's proton (Nu-H) must accompany the stereodefining heteroatom addition step. More importantly, this deprotonation step removes the possibility of an equilibrium-controlled process involving a reversible *N*-addition step, a thermodynamic pathway that would demand the formation of product mixture. *N*-Silyloxycarbamates were deemed to be the best candidates for these requirements and act as as the nucleophilic component on the basis that the N−O functionality enhances nucleophilicity at the nitrogen center via the α-effect (Figure 3.2)[71] (estimated pK_a of NH in a silyloxycarbamate is 9.0 whereas pK_a of regular carbamate is nearly 13).

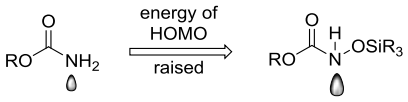
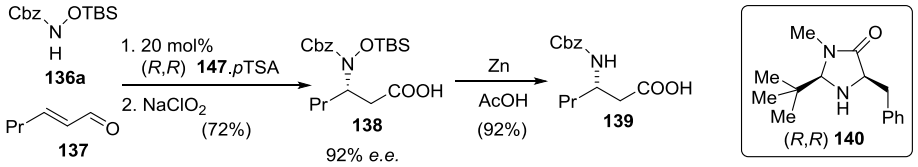


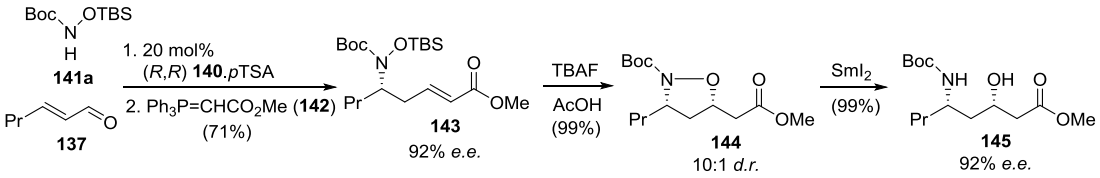
Figure 3.2 Carbamate nucleophilicity enhanced by the α-effect.

They successfully applied the organocatalytic amine addition to various substrates. For example, exposure of 2-hexenal **137** to the asymmetric amination conditions followed by oxidation provided the corresponding β-amino acid **138** with excellent enantioselectivity. The cleavage of the N-O bond was accomplished under mildly reducing conditions (Zn/AcOH) (Scheme 3.9).[72]



Scheme 3.9 Two-step synthesis of a β-amino acid.

They also showed that the amino-oxy moiety can be strategically exploited to generate 1,3-amino alcohols (Scheme 3.10). Hexenal amination was followed by *in situ* Wittig homologation to afford the unsaturated ester **143** in a single step (Scheme 3.10).



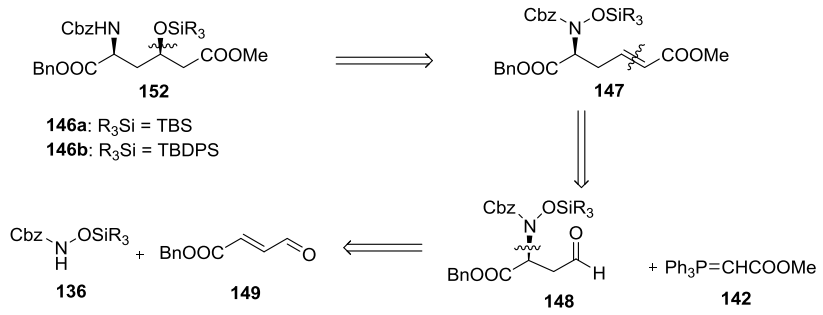
Scheme 3.10 Enantioselective synthesis of a 1,3-amino alcohol.

The amino enoate **143** was exposed to a fluoride ion source which then enabled both silyl group removal and intramolecular oxy-Michael addition to afford isoxazolidine **144** with excellent yield and diastereocontrol (99% yield, 10:1 *syn*/*anti*). The N−O bond was reduced with samarium diiodide (SmI_2) to afford the 1,3-amino alcohol **145** in high yield (70% yield over three steps).[73]

3.3 Application of the MacMillan Strategy to Ahad

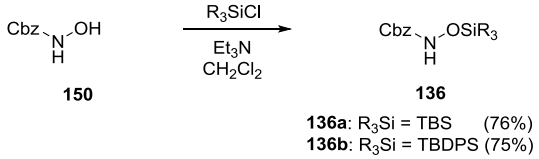
According to Scheme 3.11, a new retrosynthesis was designed for Ahad, based on MacMillan's enantioselective organocatalytic amine conjugate addition. The γ-hydroxy group would be installed intramolecularly; exposure of **147** to fluoride ion would generate an oxyanion that would add in a conjugate fashion to the α,β-unsaturated ester. Wittig homologation of aldehyde **148** would afford the α,β-unsaturated ester **147**. Conjugate addition of Cbz-NHOTBS (**136**) to a chiral iminium ion generated from aldehyde **149** would occur diastereoselectively to

generate the first stereocenter of Ahad. The stereocenter of the chiral auxiliary/catalyst is only transient.



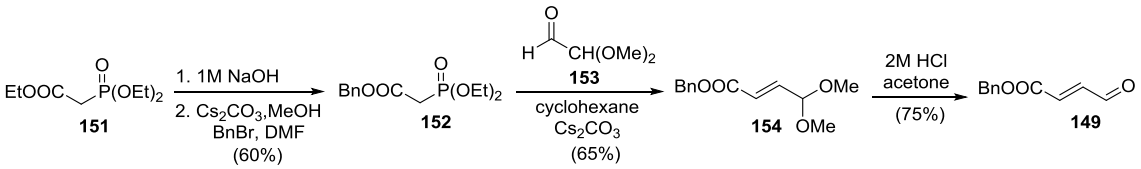
Scheme 3.11 Second generation retrosynthesis of Ahad.

The benzyl *tert*-butyldimethylsilyloxycarbamate **136a** and benzyl *tert*-butyldiphenylsilyloxycarbamate **136b** were prepared, according to the literature,[70] from commercially available benzyl hydroxycarbamate (**150**) (Scheme 3.12).



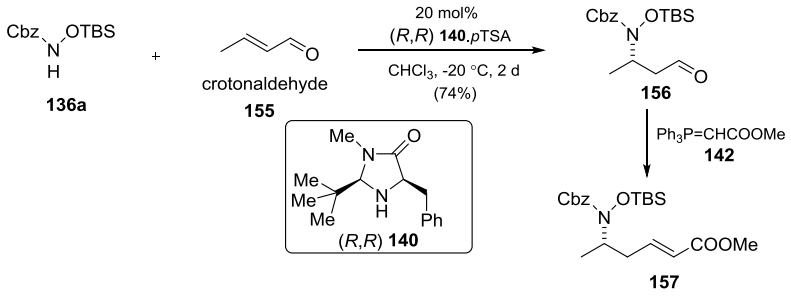
Scheme 3.12 Synthesis of silyloxy carbamate nucleophiles.

α,β-Unsaturated aldehyde **149** was synthesized according to Scheme 3.13. Diethyl(benzyloxycarbonylmethyl)phosphonate **152** was prepared by hydrolysis of the carboxylate ester of triethylphosphonoacetate (**151**) and conversion to the benzyl ester. Compound **154** was prepared via a Horner-Emmons reaction in a biphasic reaction mixture of cyclohexane and an aqueous solution of glyoxal dimethyl acetal (**153**).[74,75] Hydrolysis of the dimethyl acetal in compound **154** was attempted with various reagents: 10 mole percent *p*-toluenesulfonic acid in acetone,[74,75] 10 mole percent iodine in acetone[76] and 25 percent aqueous acetic acid.[77] The best result was obtained with HCl in acetone[78] which led to the formation of aldehyde **149** (Scheme 3.13).



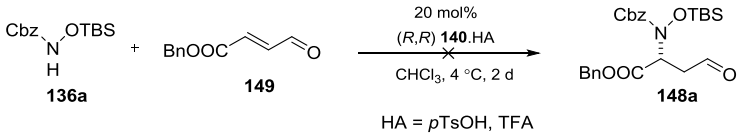
Scheme 3.13 Synthesis of aldehyde **149**

In order to develop some experience of the enantioselective organocatalytic amine conjugate addition, we repeated a known example from the literature.[70] We investigated the exposure of crotonaldehyde (**155**) to benzyl *tert*-butyldimethylsilyloxycarbamate (**136a**) in the presence of the (*R*,*R*)-imidazolidinone catalyst **140** with *p*-toluenesulfonic acid (Scheme 3.14). This provided the β-amino aldehyde product **156** that was subjected to Wittig homologation to afford the unsaturated ester **157**. Although we recognized that we would need the (*S,S*)-catalyst to give the (2*S*,4*R*) stereochemistry of Ahad, we began investigations with the (*R,R*)-catalyst **140** because it was on-hand.



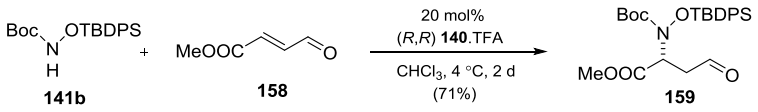
Scheme 3.14 Enantioselective organocatalytic conjugate amination.

MacMillan's 2006 communication stated that variation in the carbamate (Boc, Cbz, Fmoc) and trialkylsilyl (TBS, TBDPS) groups is possible. Therefore, we tried the conjugate addition of benzyl *tert*-butyldimethylsilyloxycarbamate **136a** to aldehyde **149** under their standard reactions conditions. Unfortunately, no product **148a** was isolated (Scheme 3.15).



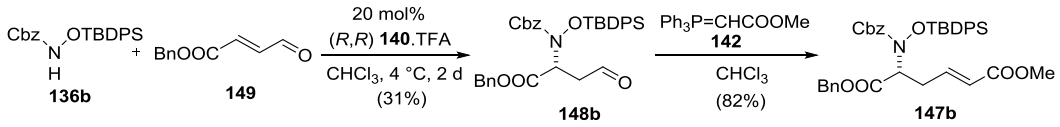
Scheme 3.15 Attempted synthesis of β-amino aldehyde **148a**.

While the scope for variation in reaction components was emphasized in their paper, we note with interest that the conjugate addition of *tert*-butyl-(*tert*-butyldiphenylsilyloxy)carbamate (**141b**) with methyl 3-formoylacrylate (**158**) was reported in the presence of a stronger acid co-catalyst (trifluoroacetic acid, TFA) (Scheme 3.16). A more hindered/stable silyloxycarbamate and elevated reaction temperature afforded compound **159**. This example is particularly relevant to our similarly electron-deficient electrophile.



Scheme 3.16 Conjugate amine addition to electron deficient electrophile.

Therefore, we switched from the *tert*-butyldimethylsilyl group (TBS), to the *tert*-butyldiphenylsilyl (TBDPS) protecting group for our hydroxylamine nucleophile. We presume that the TBS group is less stable than the TBDPS protecting group in the presence of a strong acid (TFA). The conjugate addition of compound **136b** to compound **149** was performed using the TFA salt of (*R*,*R*) chiral amine catalyst **140** (Scheme 3.17). This afforded compound **148b** in 31% yield. These addition reactions were impossible to follow by TLC because the intermediate iminium species was hydrolyzed on silica gel but progress can be monitored by ^{1}H NMR (Figure 3.3). The product of conjugate addition showed the disappearance of olefinic H1 and H2 and appearance of Hα and Hβ on sp^{3} C. Wittig reaction of aldehyde **148b** with methyl-(triphenylphosphoranylidene)-acetate (**142**) afforded unsaturated ester **147b** (Scheme 3.17).



Scheme 3.17 Synthesis of α,β-unsaturated ester **147b**.

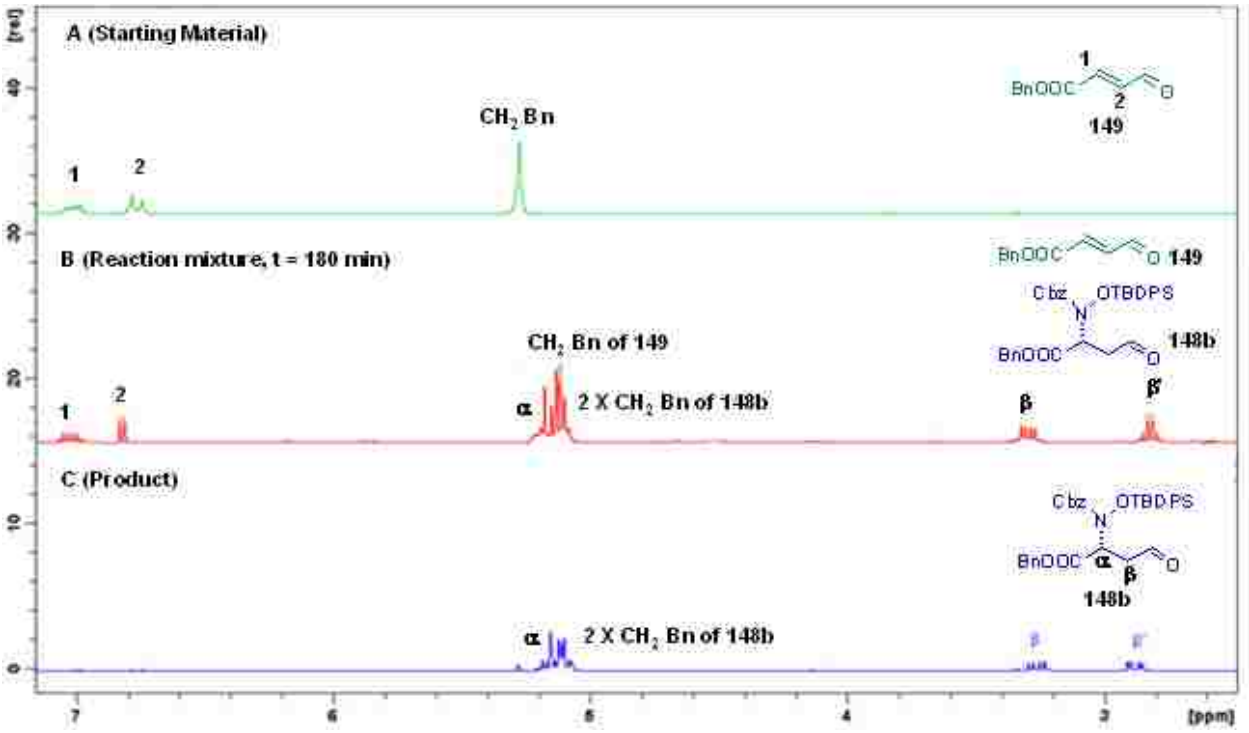
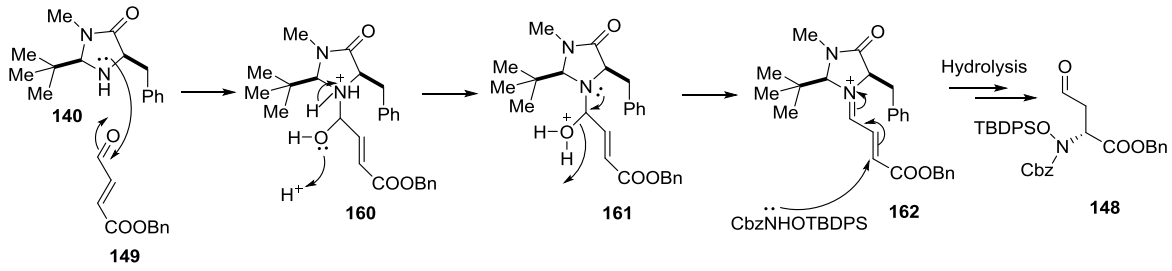


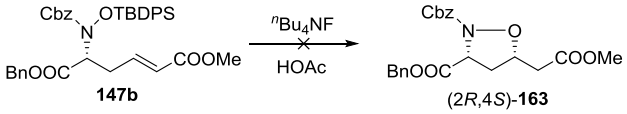
Figure 3.3 Monitoring the conjugate addition reaction by ^{1}H NMR.

In terms of the mechanism of the reaction, the lone pair on the nitrogen attacks the carbonyl carbon of the aldehyde to form the complex **160**. Protonation of the aminal facilitates the elimination of water. The intermediate iminium ion species **162** is planar and the amine nucleophile will come from the opposite face of the ^{t}Bu and Bn groups flanking the iminium ion nitrogen to give the desired product after hydrolysis (Scheme 3.18).



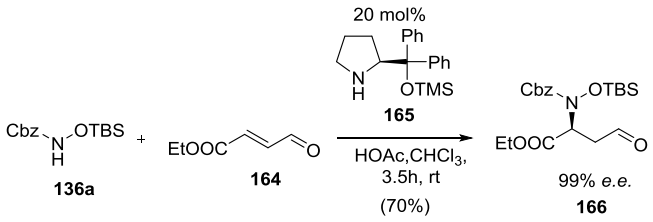
Scheme 3.18 Mechanism of stereoselctive conjugate addition.

Treatment of compound **147b** with *tetra*-*n*-butylammonium fluoride (TBAF) in the presence of acetic acid removed the TBDPS ether but did not give the cyclized product (2*R*,4*S*)-**163** (Scheme 3.19). The low yield of the conjugate addition product and subsequent difficulties in achieving the intramolecular oxy-Michael addition to afford the isoxazolidine led us to investigate other organocatalysts which are commercially available and may meet our requirements.



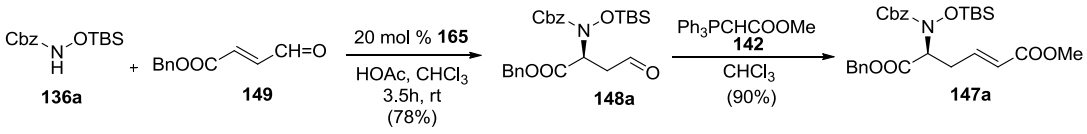
Scheme 3.19 Attempted synthesis of isoxazolidine.

In 2007, Cordova *et al.*[79] used a chiral pyrrolidine-based catalyst **165** for these types of conjugate addition reactions. They reported that simple proline-derived compounds catalyze highly enantioselective conjugate additions of protected *N*-silyloxycarbamates to α,β-unsaturated aldehydes. The reaction gives access to the β-amino aldehydes in good yields and 82–99% *e.e.* Reactions were conveniently performed at room temperature (Scheme 3.20).



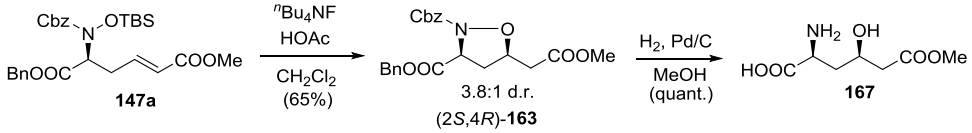
Scheme 3.20 Enantioselective conjugate amine addition using a pyrrolidine-based catalyst.

Therefore, we investigated the conjugate addition of CbzNHOTBS **136a** to α,β-unsaturated aldehyde **149** with catalyst **165**. We obtained our first desired stereocenter as 2*S*. This catalyst gave us a better yield of the conjugate addition product **148a** compared with the MacMillan catalyst **140**. Compound **148a** was then subjected to a Wittig reaction to obtain **147a** (Scheme 3.21).



Scheme 3.21 Synthesis of α,β-unsaturated ester **147a**.

Treatment of compound **147a** with fluoride ion facilitated silyl group removal with concomitant, intramolecular oxy-Michael addition to afford isooxazolidine (2*S*,4*R*)-**163**. Initially, we attempted the reaction with 1.5 equivalents each of tetrabutylammonium fluoride solution and acetic acid, but no product was observed. Switching to tetrabutylammonium fluoride solution containing 5 percent water gave a 65% yield of the product. Compound (2*S*,4*R*)-**163** was then subjected to hydrogenolysis[80] to afford unprotected Ahad **167** (Scheme 3.22).



Scheme 3.22 Synthesis of unprotected Ahad.

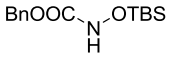
The next challenge was to find the right combination of protecting groups for Ahad that would be compatible with the synthesis of the western hemisphere of theonellamide C. Chapter 4 will address this issue of protecting group strategy and will reveal that the δ-COOMe, introduced in the Wittig reaction, was incompatible with other protecting groups on Ahad.

3.4 Experimental Section

3.4.1 General Methods

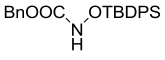
The general methods are as described in Chapter 2.

3.4.2 Procedures



Benzyl (*tert*-butyldimethylsilyl)oxycarbamate (**136a**)

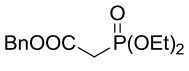
Triethylamine (249 µL, 181 mg, 1.79 mmol, 1.2 equiv.) was added to a solution of benzyl-*N*-hydroxycarbamate (250 mg, 1.49 mmol, 1.0 equiv.) in dry CH_2Cl_2 (5 mL) under N_2. The mixture was cooled to 0 °C and TBSCl (270 mg, 1.79 mmol, 1.2 equiv.) was added. The reaction mixture was warmed to rt and stirred overnight. The mixture was washed with water (5 mL) and brine (5 mL) and the organic layer was dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 4:1→1:2) afforded **136a** as a clear oil (321 mg, 77%). R_f 0.66 (2:1 Hex-EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.15 (s, 6H), 0.94 (s, 9H), 5.16 (s, 2H), 7.02 (s, 1H), 7.30-7.35 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -5.8, 18.0, 25.9, 67.6, 128.3, 128.4, 128.5, 135.7, 158.6. HRMS (ESI) calcd for $C_{14}H_{24}NO_3Si$ $(M+H)^+$ 282.1520, obsd 282.1516.



Benzyl *tert*-butyldiphenylsilyloxycarbamate (**136b**)

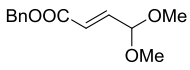
Triethylamine (0.83 mL, 0.605 g, 5.98 mmol, 2.0 equiv.) was added to a solution of benzyl-*N*-hydroxycarbamate (0.500 g, 2.99 mmol, 1.0 equiv.) in dry CH_2Cl_2 (15 mL) under N_2. The mixture was cooled to 0 °C and TBDPSCl (1.55 mL, 1.644 g, 5.98 mmol, 2.0 equiv.) was added. The reaction mixture was warmed to rt and stirred overnight. The mixture was washed with water (30 mL) and brine (25 mL) and the organic layer was dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 8:1→5:1) afforded as **136b** a

clear oil (910 mg, 75%). R_f 0.39 (2:1 Hex-EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.12 (s, 9H), 5.07 (s, 2H), 6.97 (s, 1H), 7.24-7.45 (m, 11H), 7.70-7.73 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.1, 26.9, 67.6, 127.7, 127.9, 128.4, 128.6, 129.7, 130.3, 131.7, 134.9, 135.6, 135.8, 157.9. HRMS (ESI) calcd for $C_{24}H_{27}NNaO_3Si$ $(M+Na)^+$ 428.1652, obsd 428.1643.



Benzyl 2-(diethoxyphosphoryl)acetate (**152**)

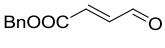
Triethylphosphonoacetate (**151**) (500 µL, 560 mg, 2.49 mmol, 1.00 equiv.) was dissolved in 1M NaOH (1 mL, 2.49 mmol, 1.0 equiv.) and stirred at 60 °C overnight. The ethanol formed in the reaction was evaporated and aqueous layer was acidified to pH 2 with 10% $KHSO_4$. The aqueous layer was extracted with CH_2Cl_2 (4 x 15 mL) and dried over $MgSO_4$, filtered and concentrated to give the carboxylic acid (373 mg) which was dissolved in dry MeOH (5 mL). Cesium carbonate (310 mg, 0.95 mmol, 0.50 equiv.) was added and the mixture stirred for 2 h under N_2. The mixture was concentrated, dissolved in DMF (5 mL) and cooled to 0 °C. Benzyl bromide (274 µL, 391 mg, 2.28 mmol, 1.20 equiv.) was added, the mixture warmed to rt, stirred overnight, diluted with EtOAc (10 mL), washed with water (15 mL) and brine (15 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (EtOAc-Hex 1:1→2:1) afforded **152** as a colorless oil (326 mg, 60%). R_f 0.53 (2:1 EtOAc-Hex). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (t, J = 7.1 Hz, 6H), 2.99 (d, $^3J_{H\text{-}P}$ = 21.5 Hz, 2H), 4.09-4.16 (m, 4H), 5.18 (s, 2H), 7.30-7.40 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.3 (^3J = 16.3 Hz), 34.4 ($^1J_{C\text{-}P}$ = 134.2 Hz), 62.7 (^2J = 6.3 Hz), 67.3, 128.3, 128.4, 128.5, 135.4, 165.6. HRMS (ESI, -ve) calcd for $C_{13}H_{18}O_5P$ $(M-H)^-$ 285.0897, obsd 285.0897.



(2*E*)-Benzyl 4,4-dimethoxybut-2-enoate (**154**)

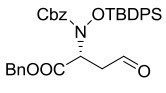
Cesium carbonate (2.20 g, 6.75 mmol, 1.5 equiv.) was added to a solution of benzyl 2-(diethoxyphosphoryl)acetate **152** (1.28 g, 4.5 mmol, 1.0 equiv.) in cyclohexane (20 mL). The

suspension was heated to 65 °C and then 2,2-dimethoxyacetaldehyde (**153**) (15.6 mL of a 60 wt.% solution in H_2O, 937 mg, 9.0 mmol, 2.0 equiv.) was added. The biphasic mixture became clear pale yellow and stirred for 2 h at 65 °C. Saturated aqueous NH_4Cl (25 mL) was added and the mixture was extracted with EtOAc (4 x 25 mL). The combined organic layers were washed with brine (25 mL), dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 2:1) afforded compound **154** as a colorless oil (947 mg, 89%). R_f 0.56 (1:1 Hex-EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.32 (s, 6H), 4.94 (d, *J* = 3.0 Hz, 1H), 5.19 (s, 2H), 6.19 (d, *J* = 15.9 Hz, 1 H), 6.81 (dd, *J* = 15.8, 3.9 Hz, 1H), 7.30-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.8, 66.4, 100.5, 124.4, 128.2, 128.3, 128.6, 135.8, 143.2, 165.6. HRMS (ESI) calcd for $C_{13}H_{17}O_4$ $(M+H)^+$ 237.1160, obsd 237.1153.



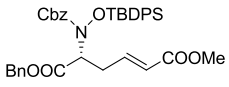
(2*E*)-Benzyl 4-oxobut-2-enoate (**149**)

A solution of (2*E*)-benzyl 4,4-dimethoxybut-2-enoate **154** (150 mg, 0.635 mmol) in acetone (30 mL) was treated with 2M HCl (0.3 mL) and stirred at rt for 2 d. The acetone was evaporated and residue was treated with water (5 mL) and extracted with $CHCl_3$ (3 x 15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (Hex-EtOAc 2:1) afforded aldehyde **149** as a colorless oil (90 mg, 75%). R_f 0.46 (1:1 Hex-EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.26 (s, 2H), 6.74 (d, *J* = 15.9 Hz, 1H), 6.98 (dd, *J* = 15.9, 7.6 Hz, 1H), 7.32-7.39 (m, 5H), 9.72 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 67.5, 128.5, 128.7, 135.0, 140.0, 140.6, 164.7, 192.43. HRMS (ESI) calcd for $C_{11}H_{11}O_3$ $(M+H)^+$ 191.0712, obsd 191.0719.



(*R*)-Benzyl-2-[(benzyloxycarbonyl)(tert-butyldiphenylsilyloxy)amino]-4-oxobutanoate (**148b**)

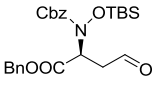
A solution of (2*R*,5*R*)-2-*tert*-butyl-5-benzyl-3-methylimidazolidine-4-one (**140**) (12.8 mg, 0.052 mmol, 0.2 equiv.) in $CHCl_3$ (0.75 mL) was treated with trifluoroacetic acid (4.0 µL, 5.9 mg, 0.052 mmol, 0.2 equiv.) and stirred for 10 min at 4 °C. The aldehyde **149** (50.0 mg, 0.260 mmol, 1.0 equiv.) was added, followed by the addition of the silyloxycarbamate nucleophile **136b** (105.0 mg, 0.26 mmol, 1.0 equiv.) in one portion, stirred for 48 h at 4 °C and the reaction mixture was then filtered through a plug of silica, eluted with Et_2O then concentrated. Purification by silica gel chromatography (Hex-EtOAc 5:1) provided compound **148b** as a clear oil (35 mg, 31%). R_f 0.32 (4:1 Hex-EtOAc). $[\alpha]_D^{25}$ +32.72 (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.04 (s, 9H), 2.50 (dd, *J* = 17.9, 5.2 Hz, 1H), 3.11 (ddd, *J* = 17.9, 8.0, 1.1 Hz, 1H), 4.80 (d, *J* = 12.0 Hz, 1H), 4.88 (d, *J* = 12.0 Hz, 1H), 4.98 (d, *J* = 12.4 Hz, 1H), 5.02-5.05 (m, 2H) 7.05 (dd, *J* = 7.8, 1.4 Hz, 2H), 7.22-7.42 (m, 14H), 7.57 (d, *J* = 7.5 Hz, 4H), 9.42 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.5, 27.0, 42.8, 60.3, 67.3, 68.3, 127.5, 127.6, 128.1, 128.2, 128.3, 128.4, 128.5, 128.7, 130.1, 130.2, 131.7, 132.0, 135.1, 135.2, 136.3, 158.7, 168.9, 197.9. HRMS (ESI) calcd for $C_{35}H_{38}NO_6Si$ $(M+H)^+$ 596.2712, obsd 596.2719.



(*R*,*E*)-6-benzyl 1-methyl 5-[(benzyloxycarbonyl)(*tert*-butyldiphenylsilyloxy)amino]hex-2-enedioate (**147b**)

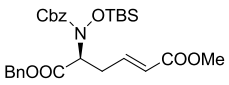
A solution of (*R*)-benzyl 2-[(benzyloxycarbonyl)(*tert*-butyldiphenylsilyloxy)amino]-4-oxobutanoate **148b** (20.0 mg, 0.034 mmol, 1.0 equiv.) in chloroform (1 mL) was treated with methyl (triphenylphosphorylidine)acetate (**142**) (16.8 mg, 0.050 mmol, 1.5 equiv.) at 0 °C and allowed to warm to rt. The reaction mixture was stirred for 20 h, concentrated to a viscous oil, and the triphenylphosphine oxide byproduct was precipitated by the addition of Et_2O. The solid was removed by filtration and the filtrate concentrated. Flash chromatography on silica gel (Hex-EtOAc 8:1→4:1) afforded an α,β-unsaturated ester **147b** as a colorless oil (18 mg, 82%). R_f 0.31 (4:1 Hex-EtOAc). $[\alpha]_D^{25}$ +38.12 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.01 (s, 9H), 2.68-2.84 (m, 2H), 3.69 (s, 3H), 4.59 (dd, *J* = 8.4, 6.3 Hz, 1H), 4.71 (d, *J* = 12.0 Hz, 1 H), 4.82

(d, *J* = 12.0 Hz, 1H), 4.86 (d, *J* = 12.4 Hz, 1H), 5.04 (d, *J* = 12.4 Hz, 1H), 5.65 (d, *J* = 15.7 Hz, 1H), 6.64 (dt, *J* = 22.8, 7.2 Hz, 1H), 7.02 (d, *J* = 6.6 Hz, 2H), 7.12-7.42 (m, 15H), 7.56 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.5, 26.9, 31.4, 51.4, 64.3, 67.1, 68.2, 123.5, 127.4, 127.5, 128.2, 128.3, 128.5, 128.7, 129.9, 130.1, 131.8, 132.3, 135.2, 135.3, 136.2, 136.3, 144.1, 158.9, 166.4, 168.9. HRMS (ESI) calcd for $C_{38}H_{42}NO_7Si$ $(M+H)^+$ 652.2725, obsd 652.2712.



(*S*)-benzyl-2-{[(benzyloxy)carbonyl)][(*tert*-butyldimethylsilyl)oxy]amino}-4-oxobutanoate (**148a**)

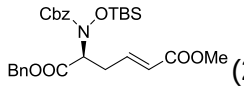
A solution of benzyl (*tert*-butyldimethylsilyl)oxycarbamate **136a** (207.0 mg, 0.736 mmol, 1.4 equiv.) in chloroform (1 mL) was treated with (*S*)-2-{diphenyl[(trimethylsilyl)oxy]methyl}pyrrolidine (**165**) (34.0 mg, 0.105 mmol, 0.2 equiv) and acetic acid (9 µL, 9.4 mg, 0.105 mmol, 0.2 equiv.) and stirred for 10 min at rt. Benzyl 4-oxo-but-2-enoate (**149**) (100.0 mg, 0.525 mmol, 1.0 equiv.) was added and the reaction mixture was stirred for 3.5 h at rt, concentrated and the residue was subjected to silica gel chromatography (Hex-EtOAc 5:1) and provided compound **148a** as a colorless oil (239 mg, 69%). R_f 0.30 (2:1 Hex-EtOAc). $[\alpha]_D^{25}$ -13.5 (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 3H), 0.12 (s, 3H), 0.87 (s, 9H), 2.87 (dd, *J* = 17.7, 5.8 Hz, 1H), 3.26 (dd, *J* = 17.7, 5.7 Hz, 1H), 5.07-5.18 (m, 5H), 7.26-7.40 (m, 10H), 9.81 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -4.8, -4.7, 18.1, 25.8, 42.7, 60.1, 67.5, 68.6, 128.1, 128.3, 128.5, 128.5, 128.6, 135.3, 159.2, 169.1, 198.2. HRMS (ESI) calcd for $C_{25}H_{34}NO_6Si$ $(M+H)^+$ 472.215, obsd 472.2139.



(2*S*,4*E*)-6-benzyl-1-methyl-5-{[(benzyloxy)carbonyl][(*tert*-butyldimethylsilyl)oxy]-amino}hex-2-enedioate (**147a**)

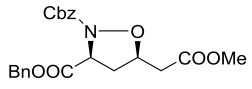
A solution of (*S*)-benzyl 2-{[(benzyloxy)carbonyl][(*tert*-butyldimethylsilyl)oxy]amino}-4-oxobutanoate (**148a**) (137.0 mg, 0.290 mmol, 1.0 equiv.) in chloroform (2.5 mL) was treated

with methyl (triphenylphosphorylidine)acetate (**142**) (164.0 mg, 0.435 mmol, 1.5 equiv.) at 0 °C and allowed to warm to rt. The reaction mixture was stirred for 1.5 h and concentrated to a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded the α,β-unsaturated ester as colorless oil **147a** (140 mg, 91%). R_f 0.30 (2:1 Hex-EtOAc); $[\alpha]_D^{25}$ -41.2 (*c* 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.07 (s, 3H), 0.09 (s, 3H), 0.85 (s, 9H), 2.78-2.93 (m, 2H), 3.73 (s, 3H), 4.64 (dd, *J* = 8.9, 6.1 Hz, 1H), 5.07 (d, *J* = 12.0 Hz, 1H), 5.10 (d, *J* = 12.2 Hz, 1H), 5.12 (d, *J* = 12.0 Hz, 1H), 5.16 (d, *J* = 12.0 Hz, 1H), 5.94 (d, *J* = 15.7 Hz, 1H), 6.97 (dt, *J* = 15.7, 7.0 Hz, 1H), 7.26-7.40 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -4.8, -4.7, 18.1, 25.8, 31.1, 51.5, 64.2, 67.2, 68.5, 123.6, 128.2, 128.3, 128.4, 128.5, 128.5, 128.6, 135.2, 144.3, 168.8. HRMS (ESI) calcd for $C_{28}H_{38}NO_7Si$ $(M+H)^+$ 528.2412, obsd 528.2420.



(2*S*,4*E*)-1-Benzyl-6-methyl2-{[(benzyloxy)carbonyl][(*tert*-butyldimethylsilyl)oxy]amino}hex-5-enedioate (One–pot procedure) (**147a**)

A solution of benzyl (*tert*-butyldimethylsilyl)oxycarbamate (**136a**) (305.00 mg, 1.05 mmol, 1.4 equiv.) in chloroform (1 mL) was treated with (*S*)-2-{diphenyl[(trimethylsilyl)oxy]methyl}pyrrolidine (**165**) (50.35 mg, 0.15 mmol, 0.2 equiv), followed by the addition of acetic acid (10 µL, 9.28 mg, 0.15 mmol, 0.2 equiv.) and stirred for 10 min at rt. Benzyl 4-oxo-but-2-enoate (**149**) (147.20 mg, 0.77 mmol, 1.0 equiv.) was added and the reaction mixture was stirred for 3.5 h at rt. Upon completion (determined by TLC), methyl (triphenylphosphorylidine)acetate (**142**) (357.00 mg, 0.75 mmol, 1.5 equiv.) was added in one portion. The reaction mixture was stirred for 2.5 h and then concentrated to a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 5:1) afforded the α,β-unsaturated ester as colorless oil **147a** (280 mg, 71%). R_f 0.30 (2:1 Hex-EtOAc). NMR data was identical to that obtained via the two-step procedure.

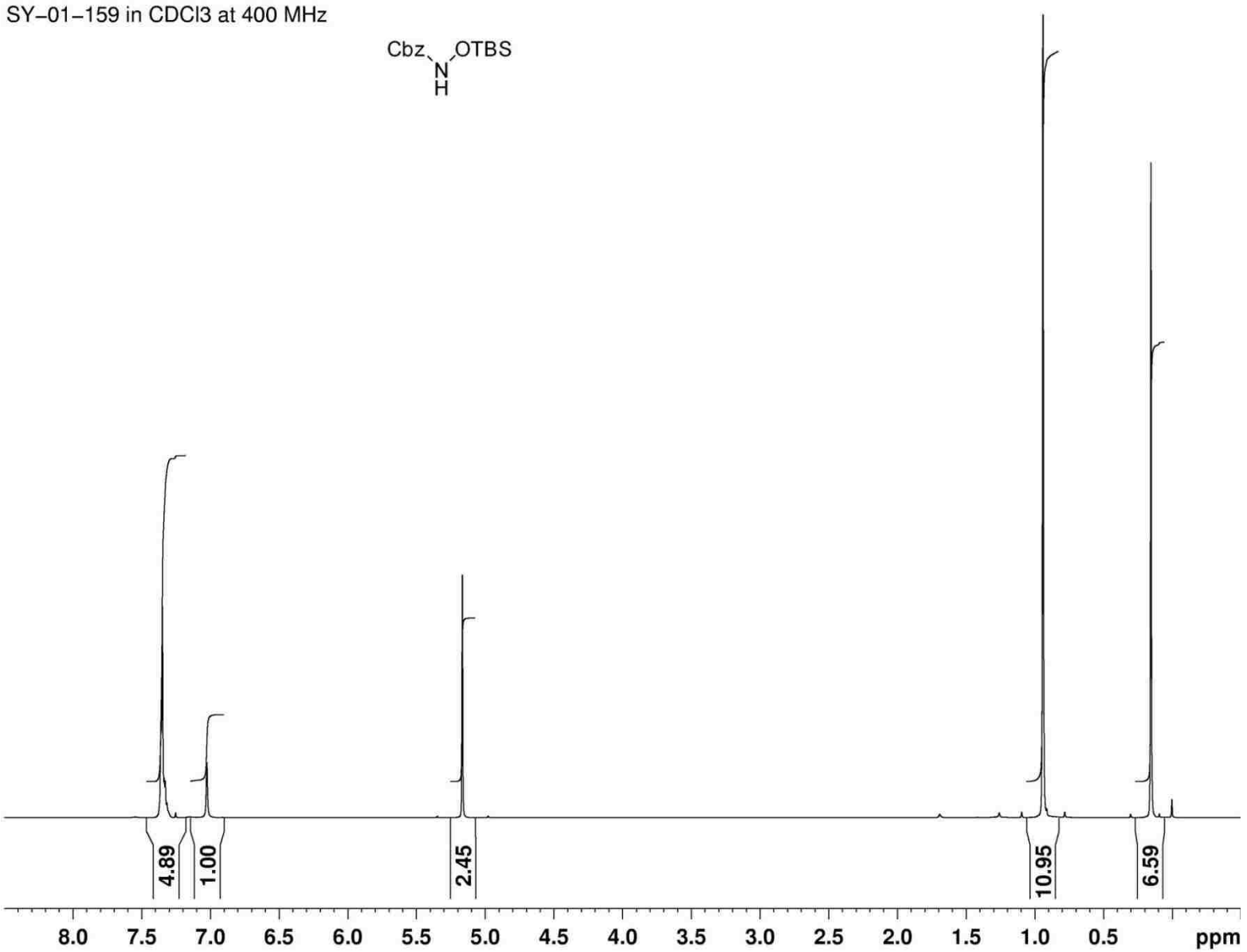


(2*S*,4*R*)-Dibenzyl 2-(6-methoxy-2-oxoethyl)isoxazolidine-1,2-dicarboxylate (**163**)

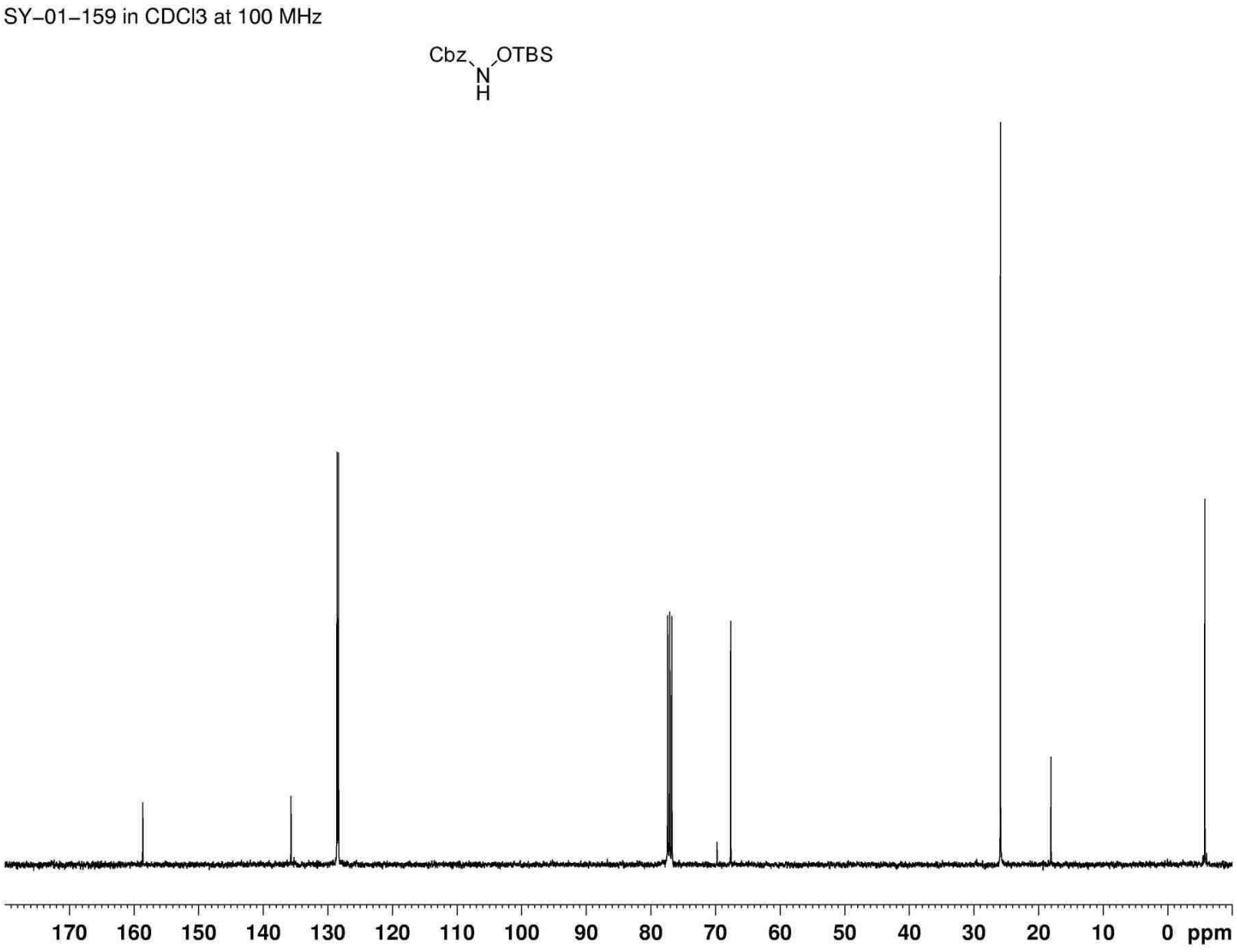
To a solution of (2*S*,5*E*)-6-benzyl 1-methyl 5-[{(benzyloxy)carbonyl}{(*tert*-butyldimethylsilyl)oxy}amino]hex-2-enedioate (**147a**) (105 mg, 0.198 mmol, 1.0 equiv.) in $CHCl_3$ (1 mL) was added acetic acid (17 µL, 17 mg, 0.297 mmol, 1.5 equiv.). The mixture was cooled to 0 °C and TBAF (297 µL, 1 M in THF with 5% H_2O, 0.297 mmol, 1.5 equiv.) was added. The reaction was allowed to stir at 0 °C for 4 d. Reaction was monitored by TLC and upon completion, the reaction mixture was concentrated and applied directly to a flash column, eluting with 4:1→2:1 (Hex-EtOAc), to obtain (3*S*,5*R*)-dibenzyl 5-(2-methoxy-2-oxoethyl)isoxazolidine-2,3-dicarboxylate **163b** as a colorless oil (50 mg, 65%); R_f 0.23 (2:1 Hex-EtOAc). $[\alpha]_D^{25}$ -33.9 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.23 (ddt, *J* = 12.8, 7.9, 4.8 Hz, 1H), 2.60 (dd, *J* = 16.4, 7.8 Hz, 1H), 2.83-2.93 (m, 2H), 3.68 (s, 3H), 4.35 (app. p, *J* = 7.1 Hz, 1H), 4.88 (dd, *J* = 9.5, 5.7 Hz, 1H), 5.19 (d, *J* = 2.4 Hz, 2 H), 5.21 (s, 2H), 7.33-7.36 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 36.9, 37.9, 52.0, 60.6, 67.4, 68.4, 128.1, 128.2, 128.4, 128.5, 128.5, 128.6, 135.2, 135.5, 156.9, 170.0. HRMS (ESI) calcd for $C_{22}H_{24}NO_7$ $(M+H)^+$ 414.1547, obsd 414.1548.

3.4.3 Spectra

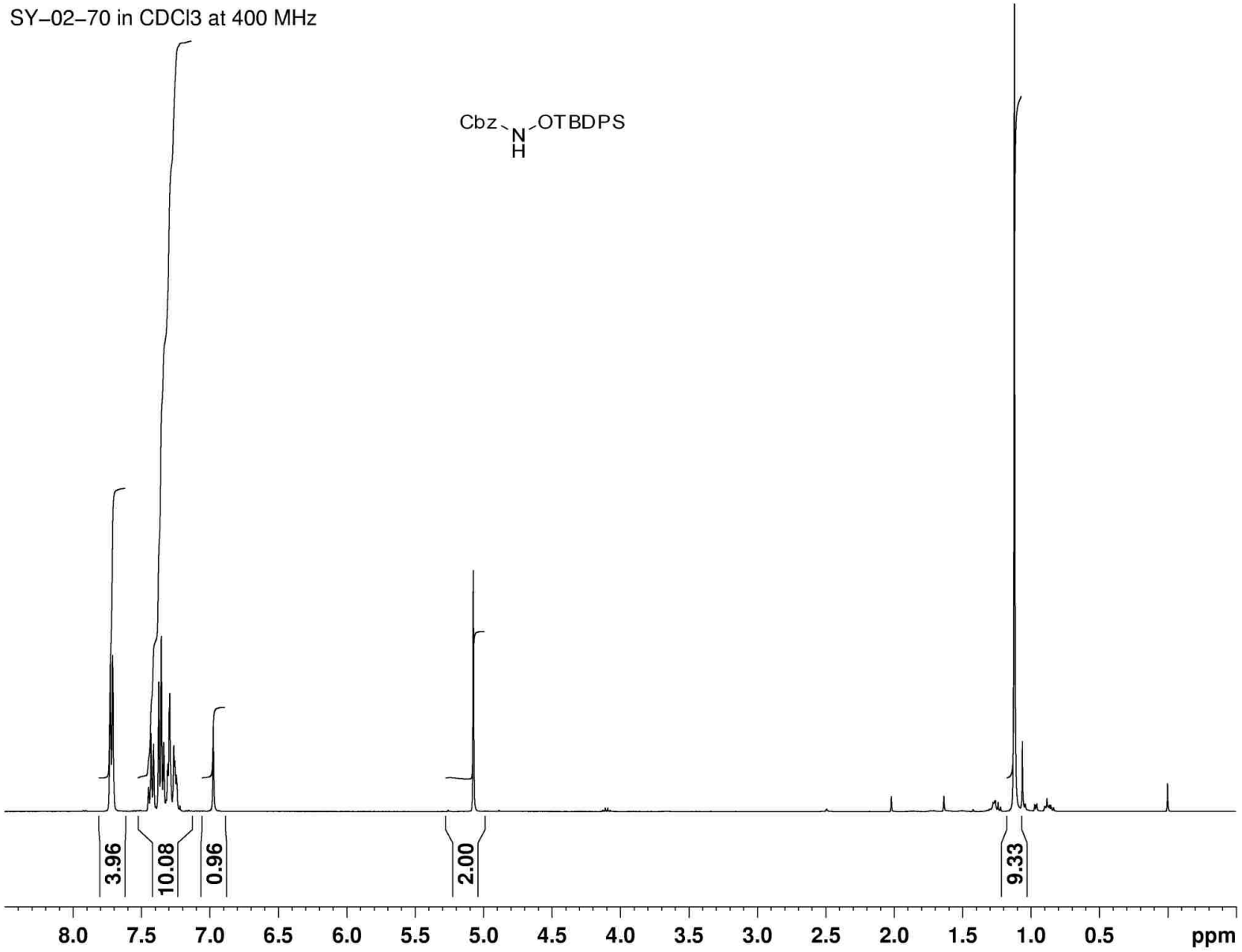
Compound **136a** (Scheme 3.12) - ^{1}H NMR spectrum



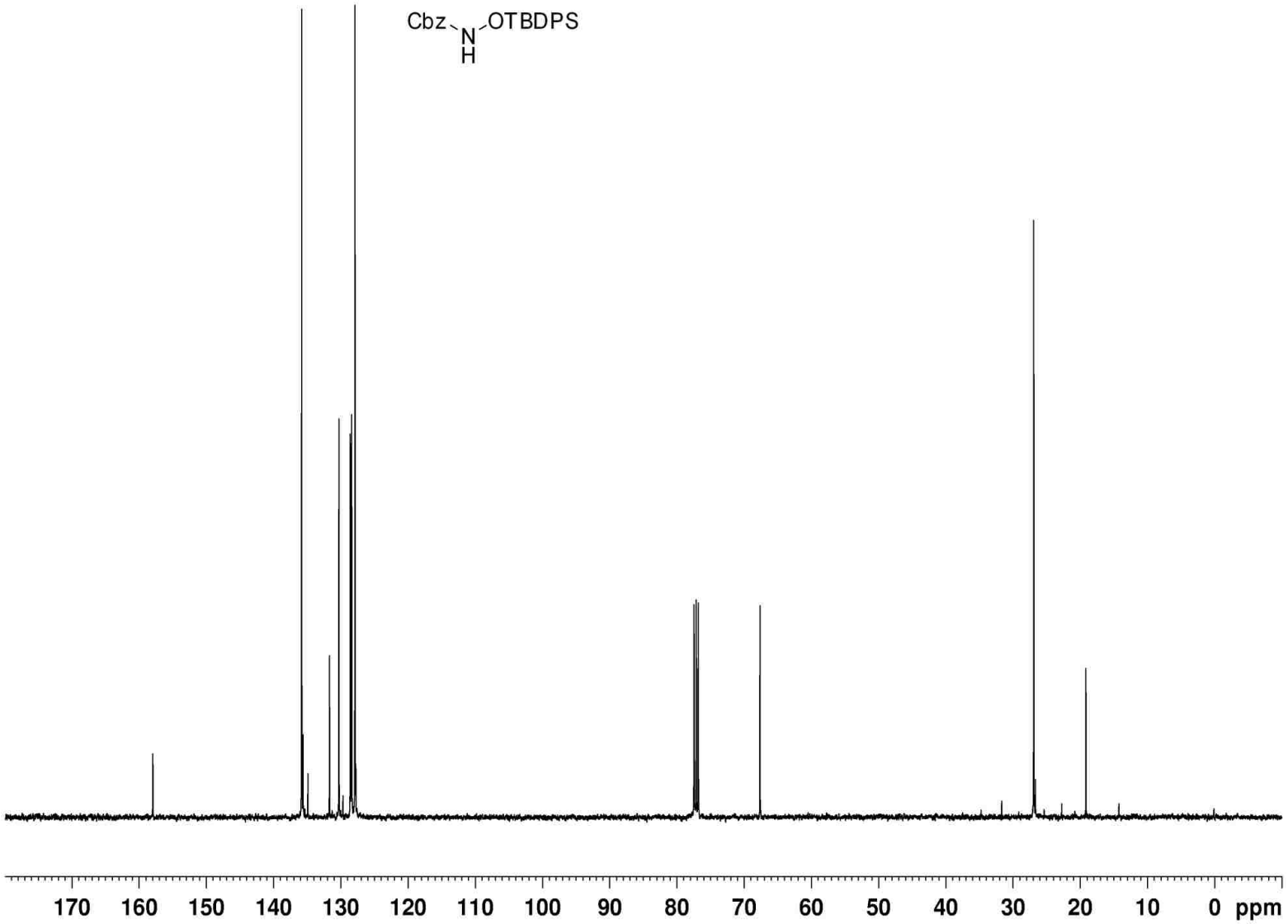
Compound **136a** (Scheme 3.12) - ^{13}C NMR spectrum



Compound **136b** (Scheme 3.12) - ^{1}H NMR spectrum

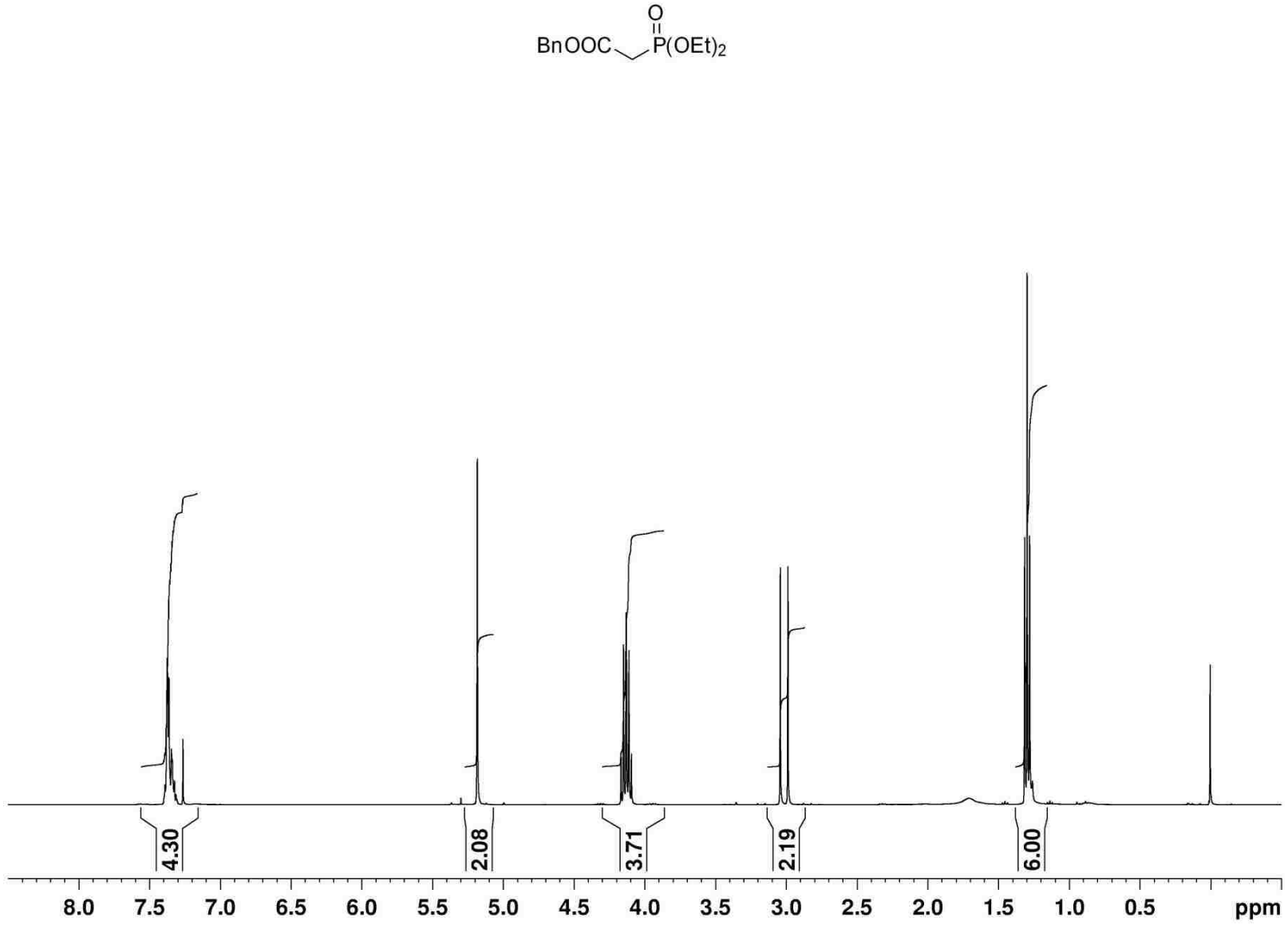


Compound **136b** (Scheme 3.12) – ^{13}C NMR spectrum

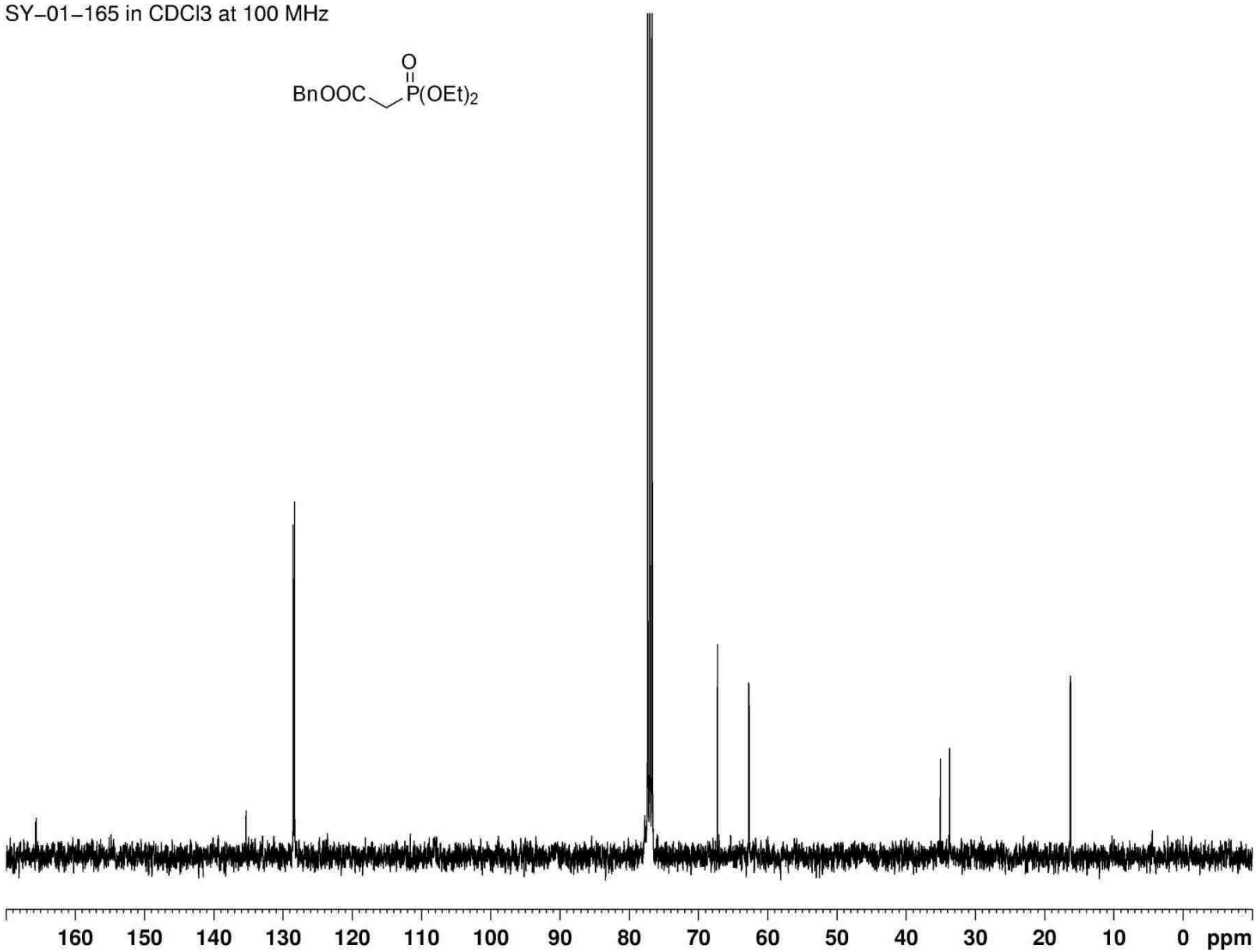


Compound **152** (Scheme 3.13) - ^{1}H NMR spectrum

SY–01–165 in CDCl3 at 400 MHz

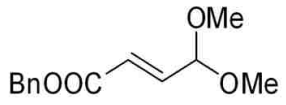


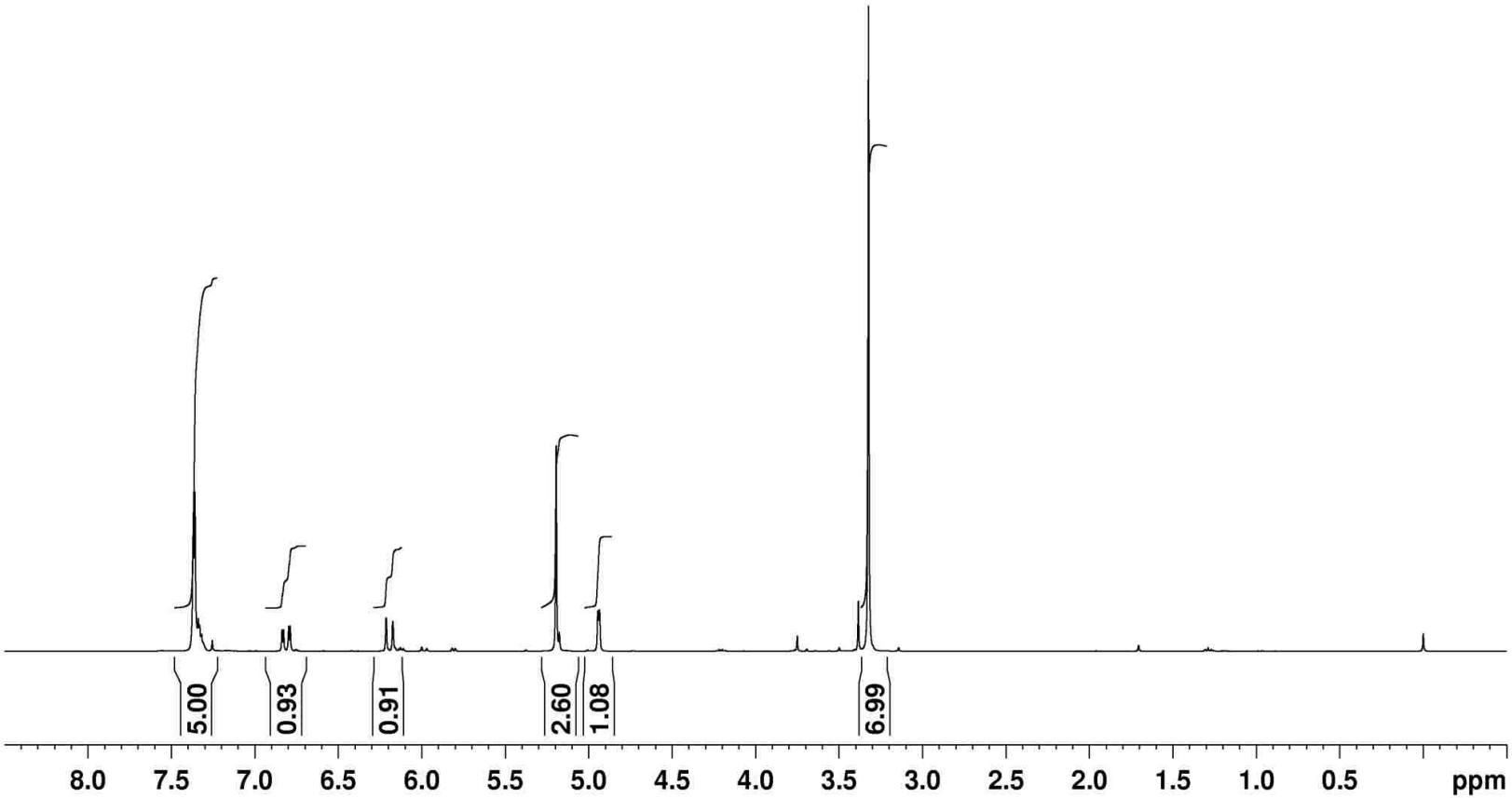
Compound **152** (Scheme 3.13) - ^{13}C NMR spectrum



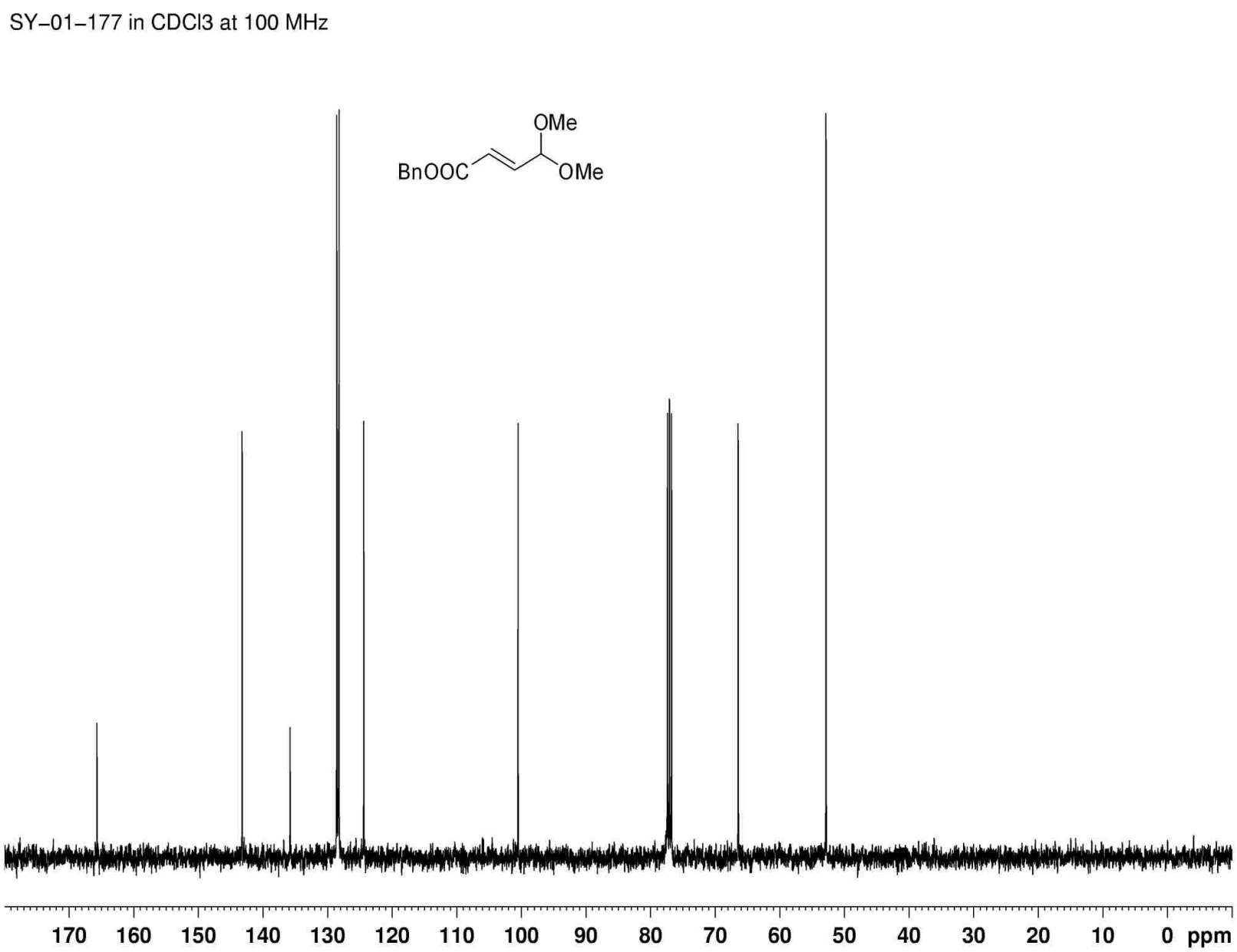
Compound **154** (Scheme 3.13) - ^{1}H NMR spectrum

SY–01–177 in CDCl3 at 400 MHz



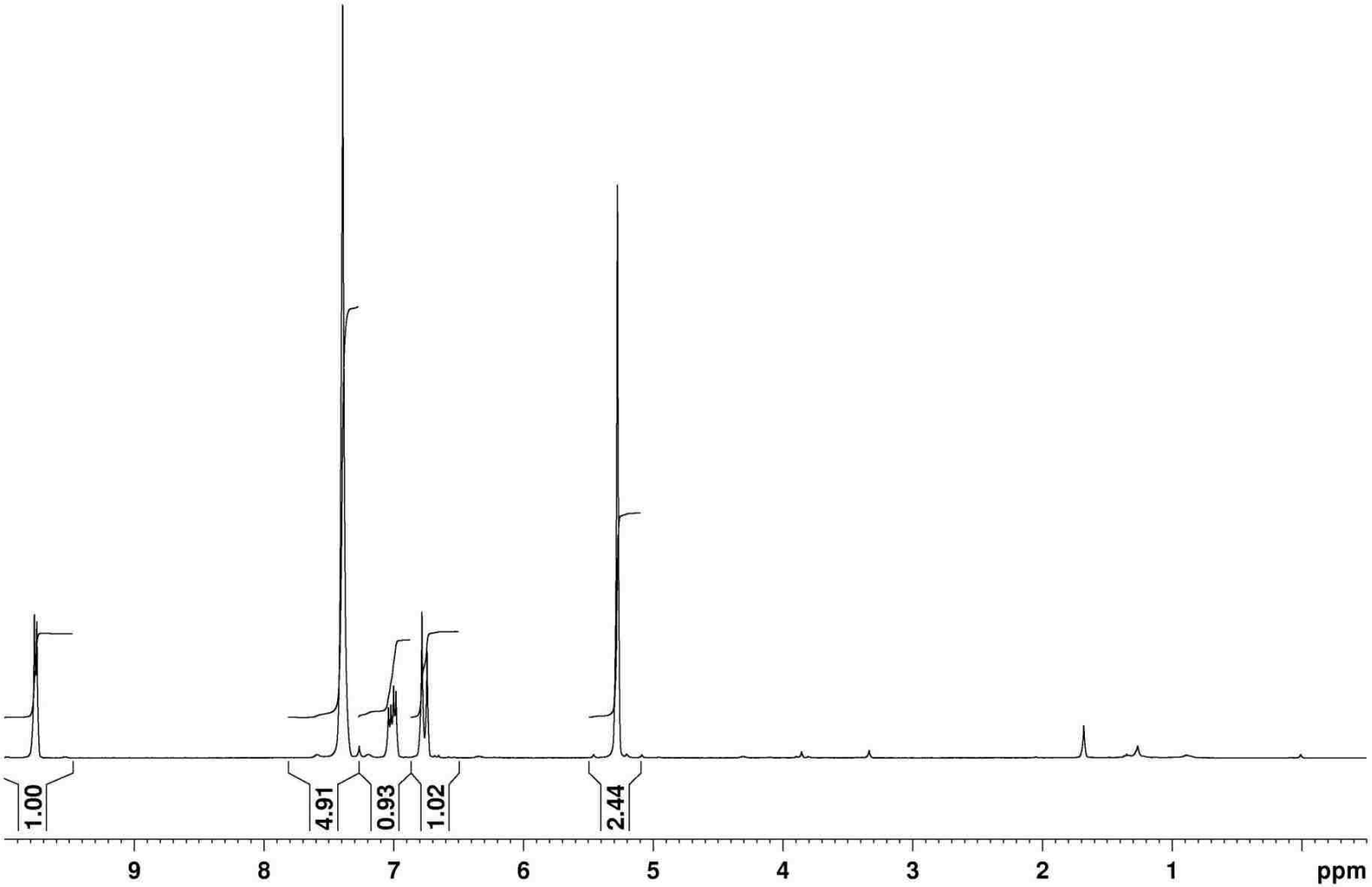


Compound **154** (Scheme 3.13) - ^{13}C NMR spectrum

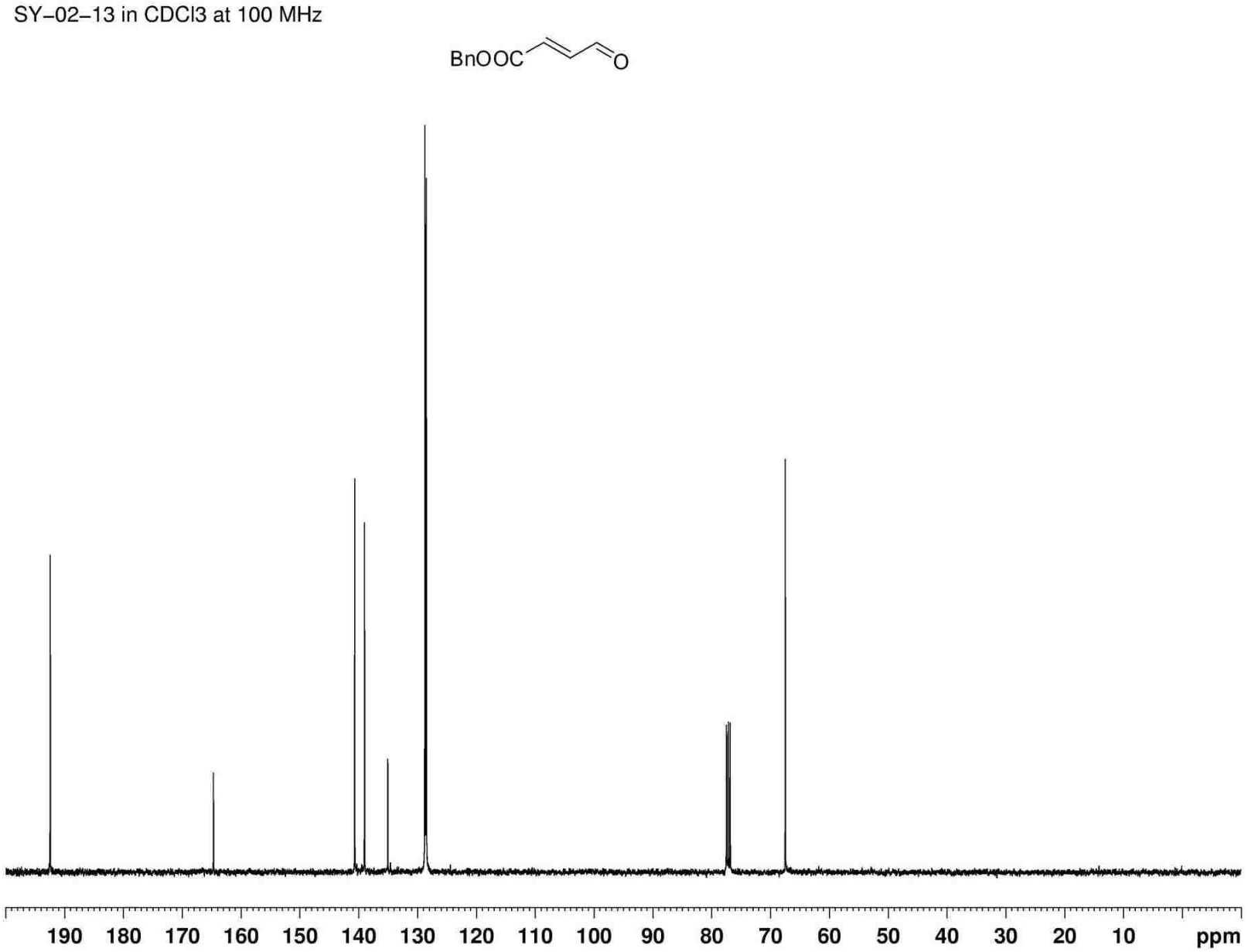


Compound **149** (Scheme 3.13) - ^{1}H NMR spectrum

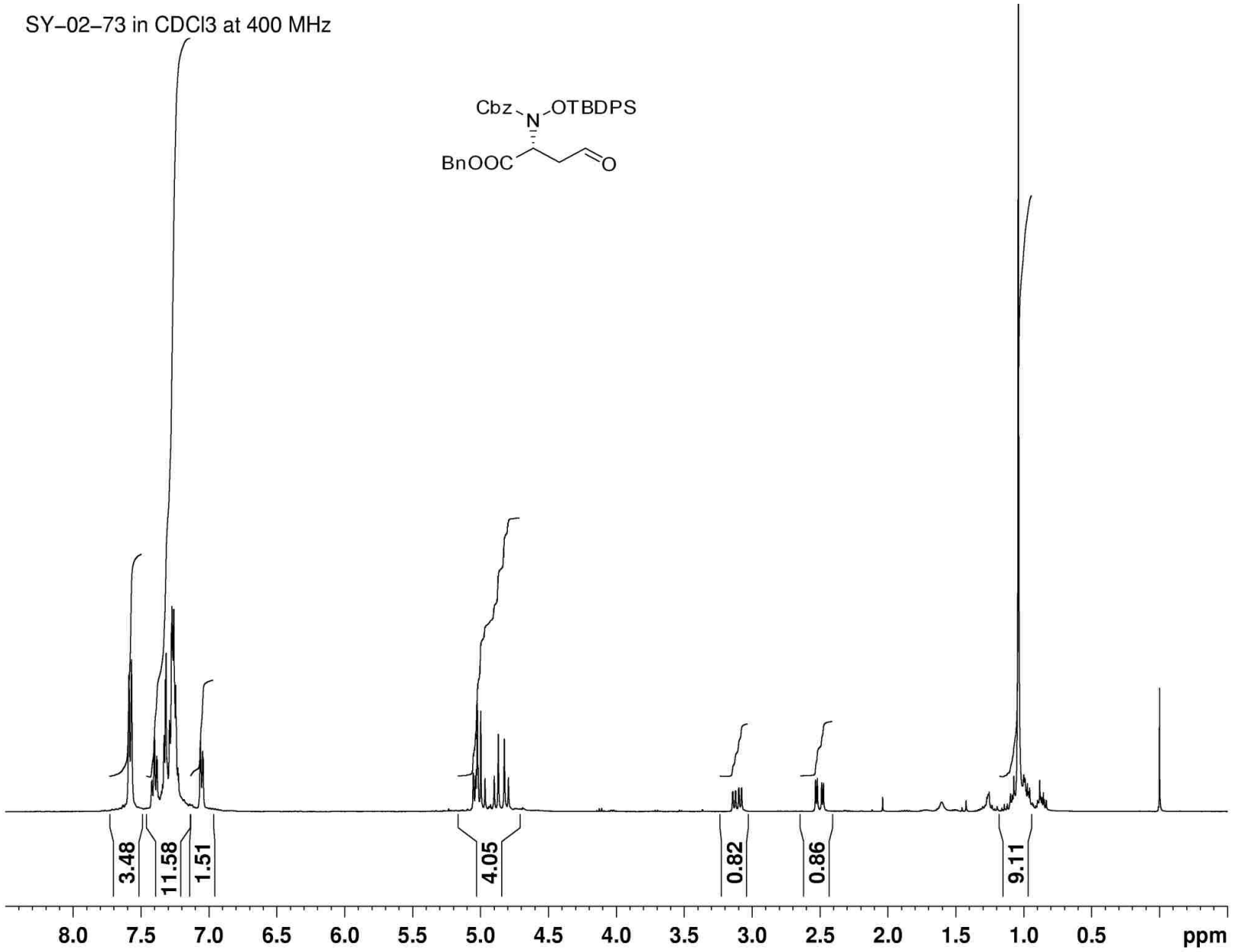
SY–02–13 in CDCl3 at 400 MHz



Compound **149** (Scheme 3.13) - ^{13}C NMR spectrum

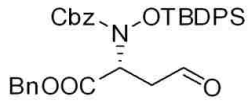


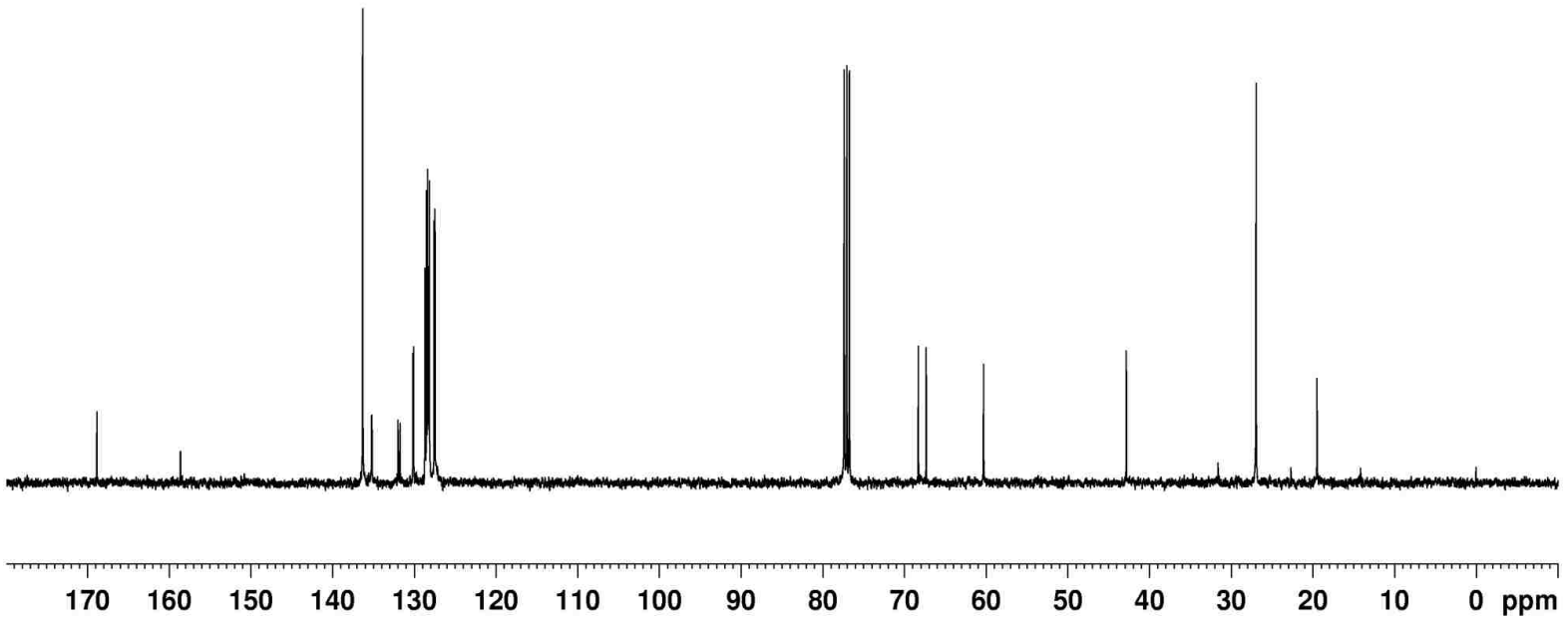
Compound **148b** (Scheme 3.17) - ^{1}H NMR spectrum



Compound **148b** (Scheme 3.17) - ^{13}C NMR spectrum

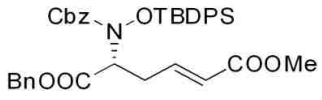
SY–02–73 in CDCl3 at 100 MHz

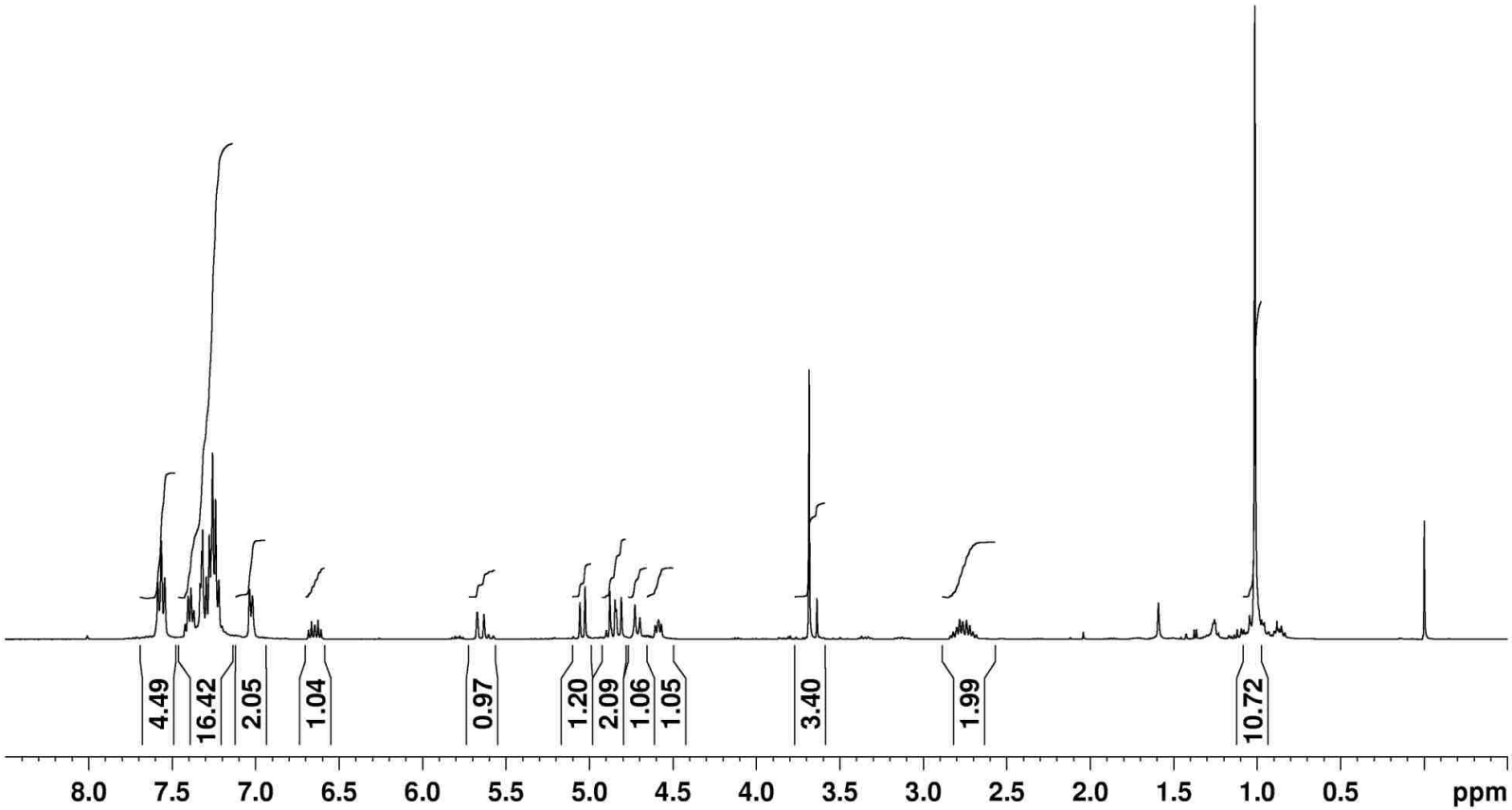




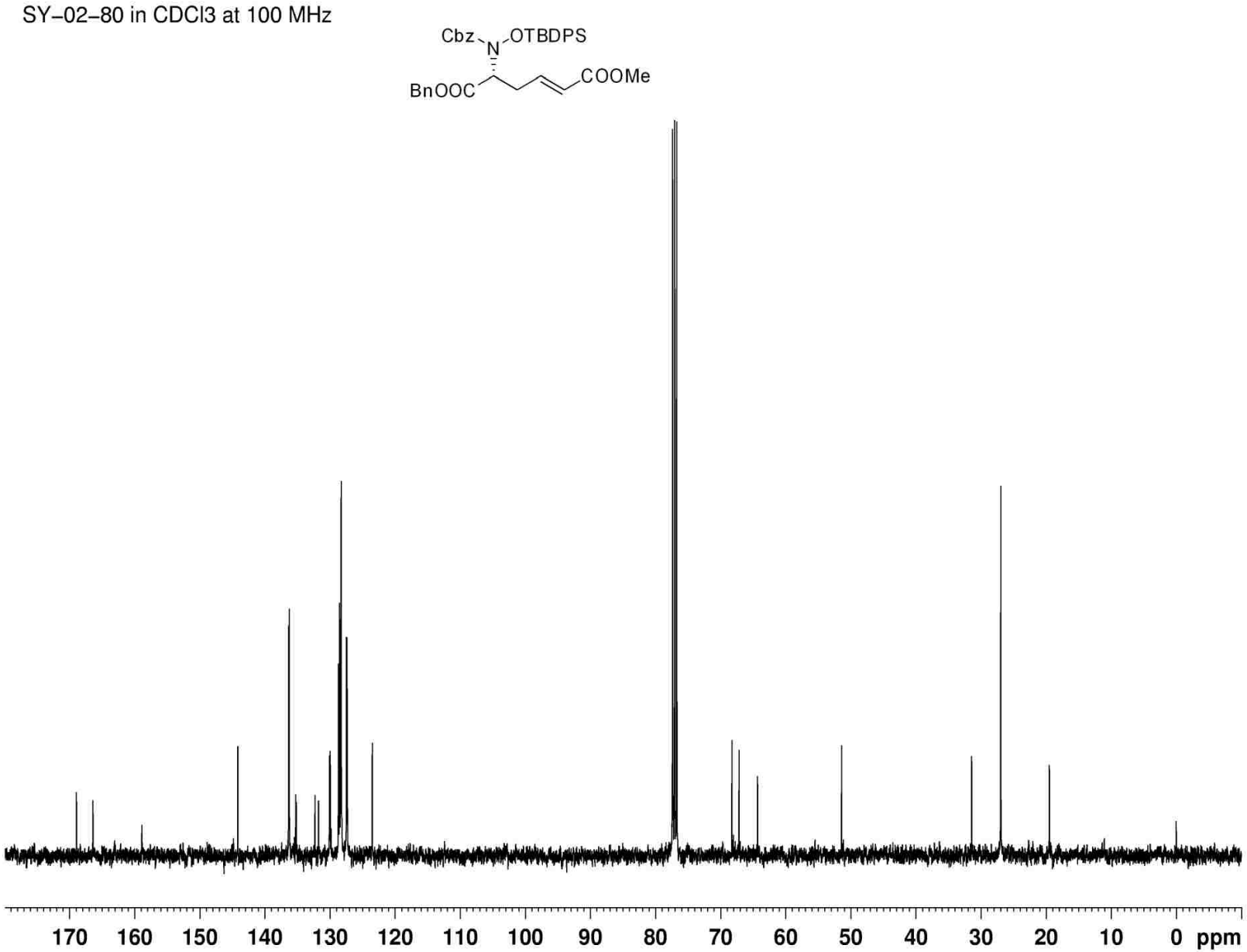
Compound **147b** (Scheme 3.17) - ^{1}H NMR spectrum

SY–02–80 in CDCl3 at 400 MHz



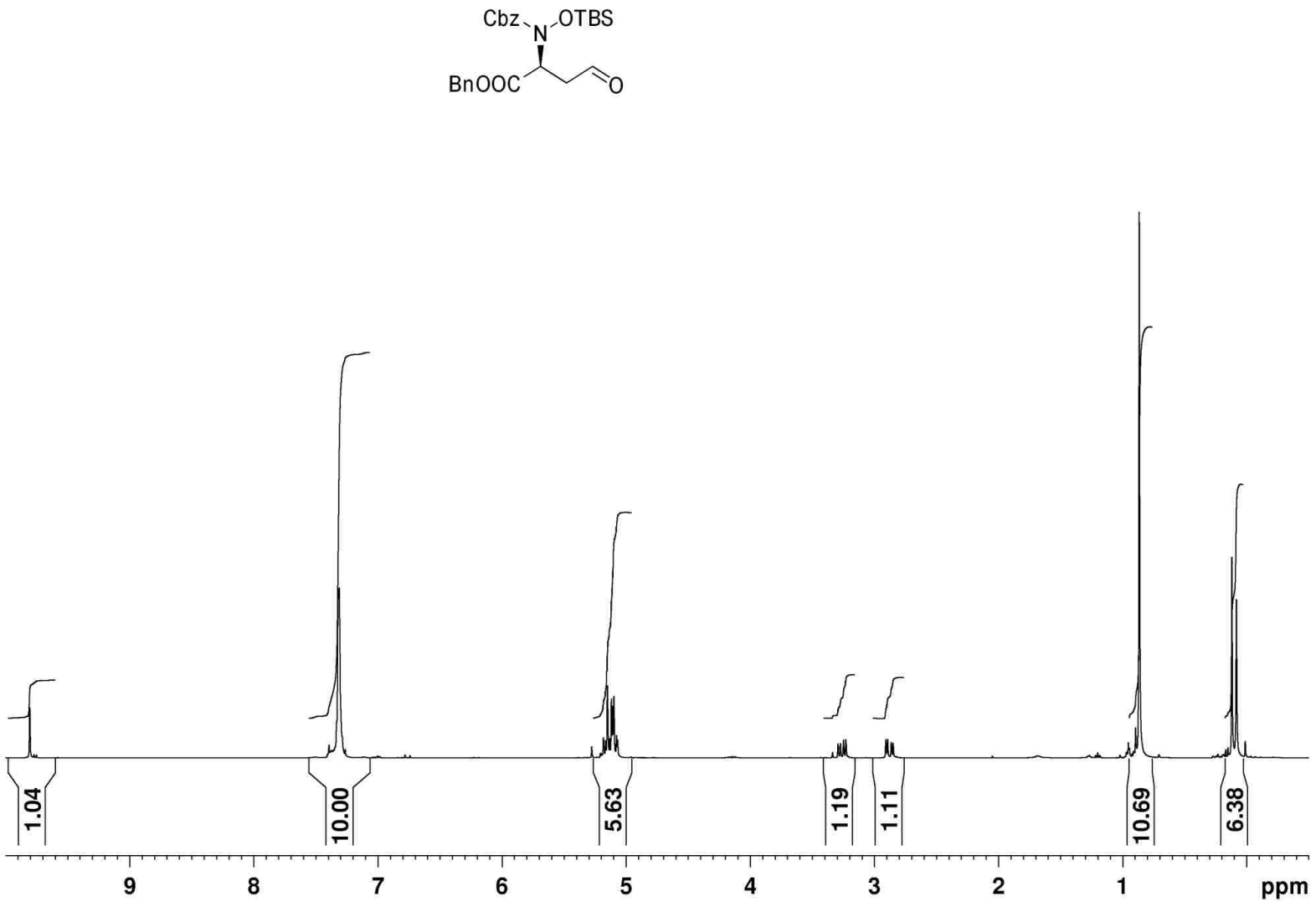


Compound **147b** (Scheme 3.17) – ^{13}C NMR spectrum



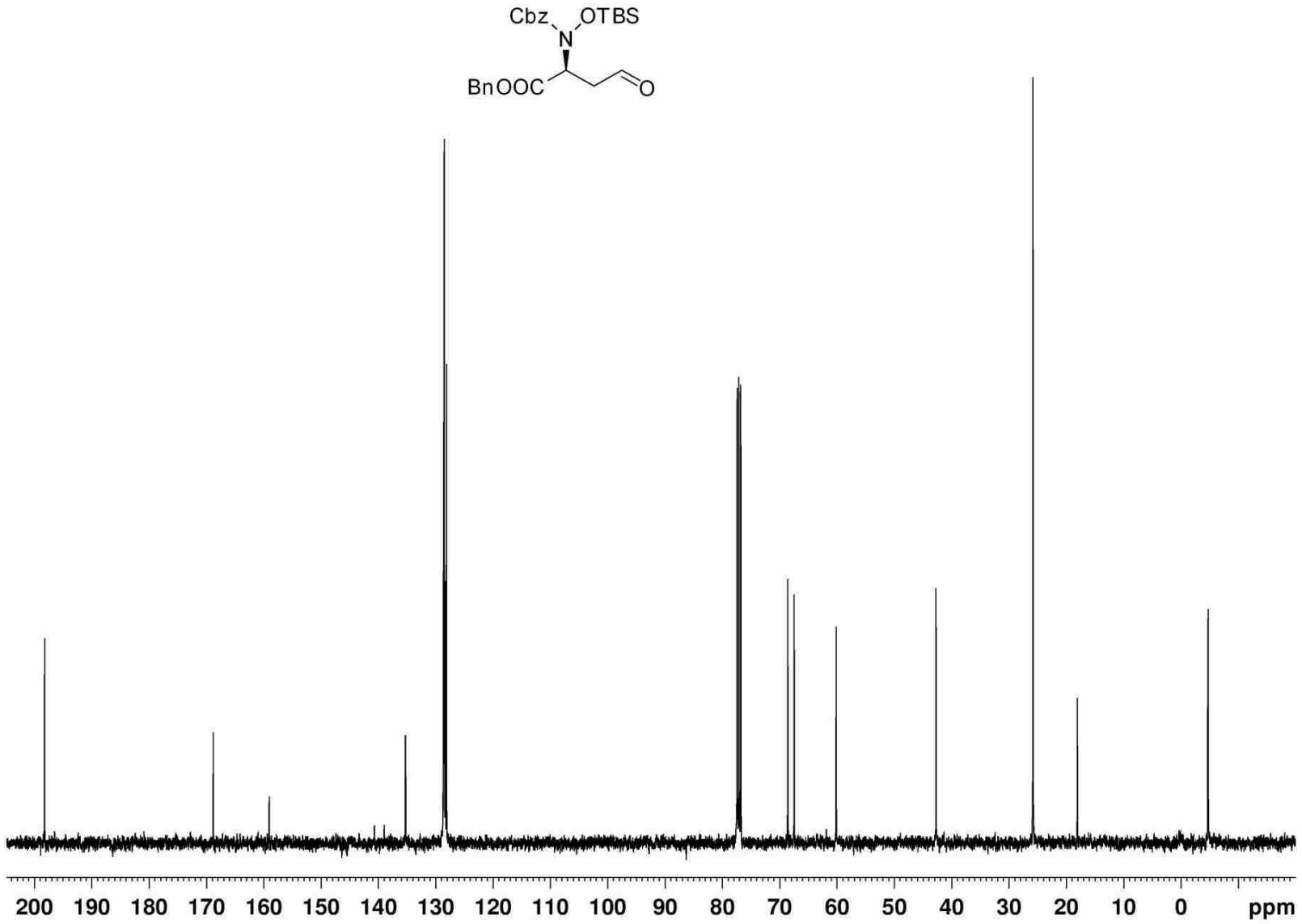
Compound **148a** (Scheme 3.21) - ^{1}H NMR spectrum

SY–03–17 in CDCl3 at 400 MHz



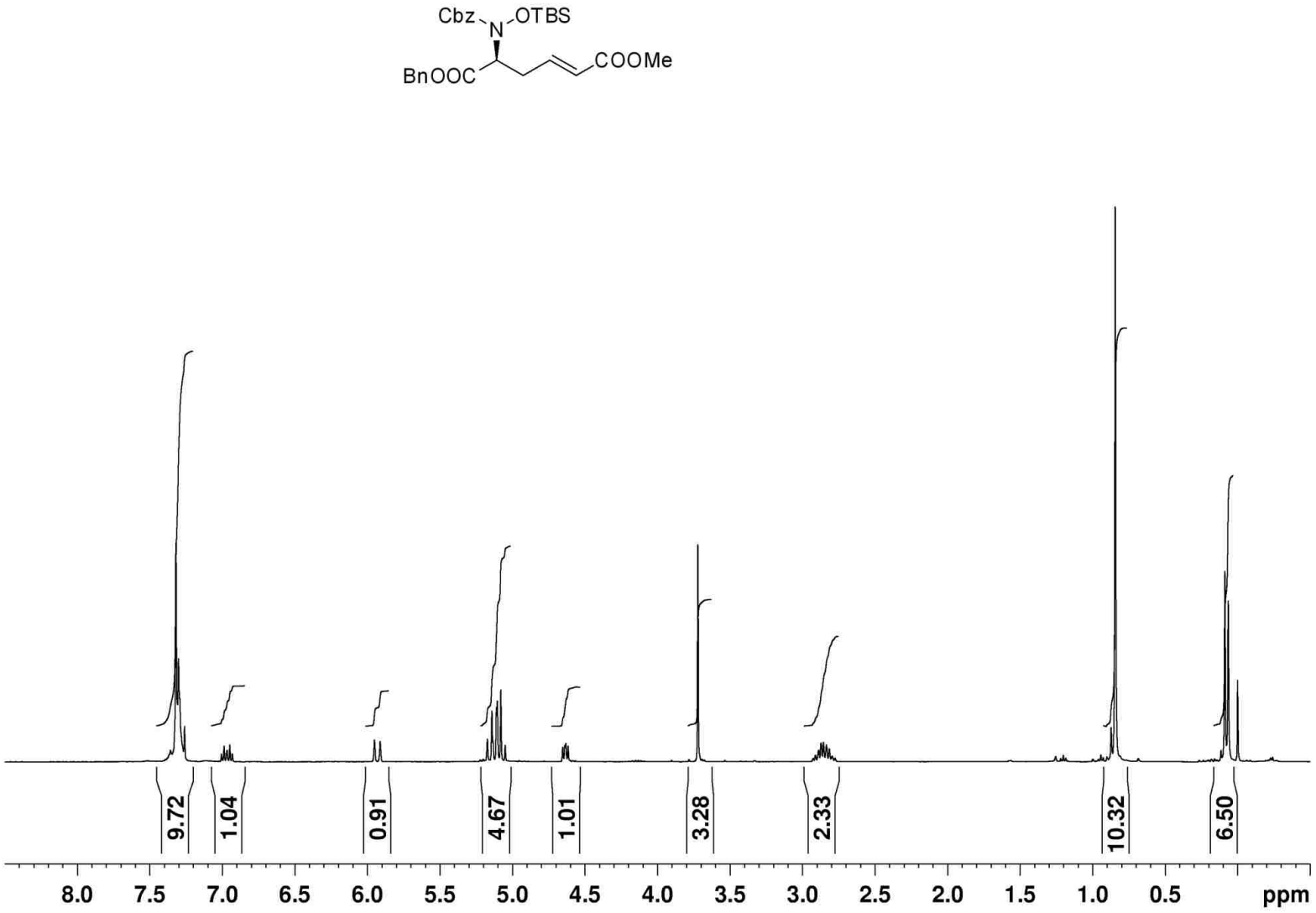
Compound **148a** (Scheme 3.21) – ^{13}C NMR spectrum

SY–03–17 in CDCl3 at 100 MHz

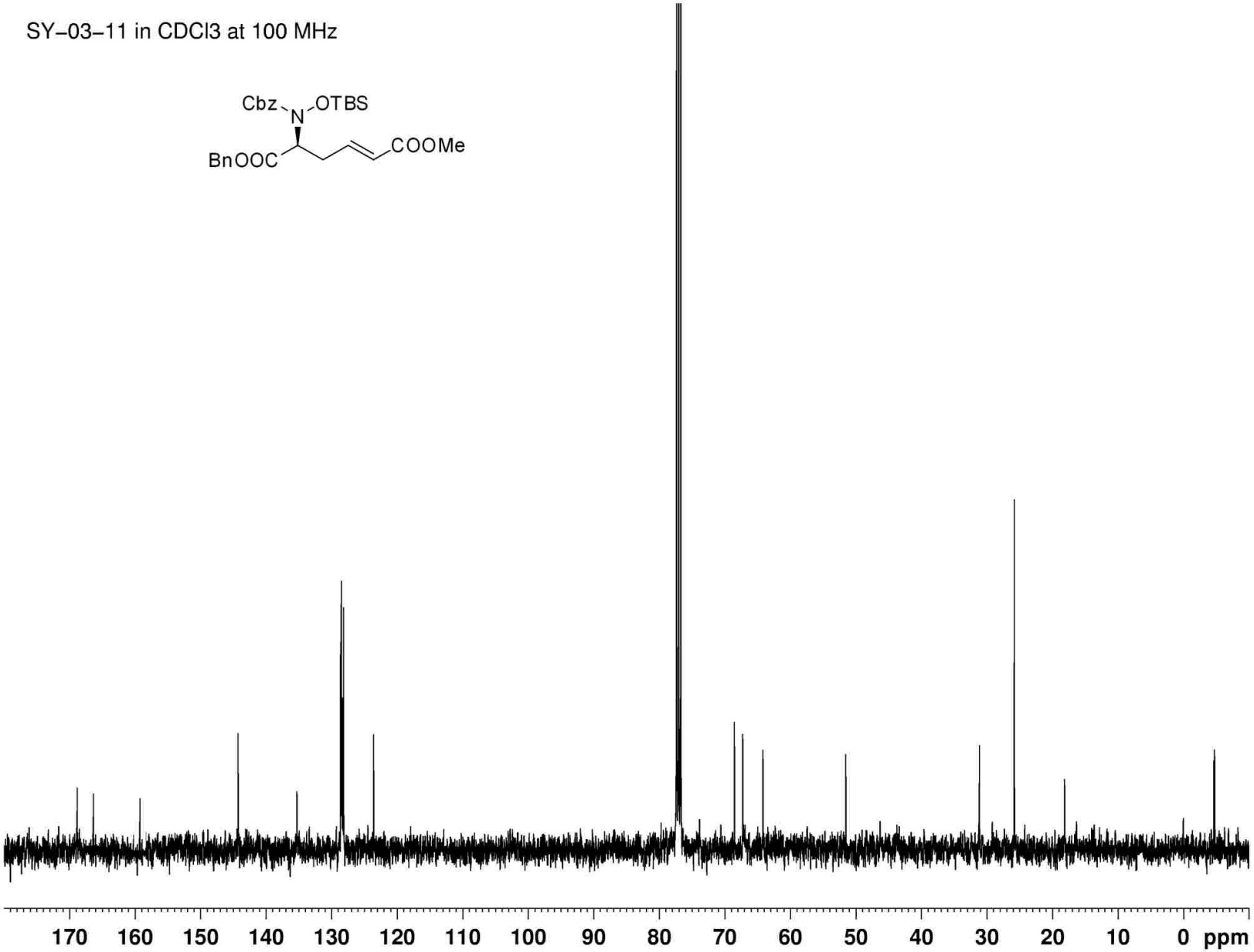


Compound **147a** (Scheme 3.21) - ^{1}H NMR spectrum

SY–03–11 in CDCl3 at 400 MHz

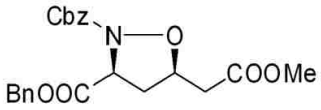


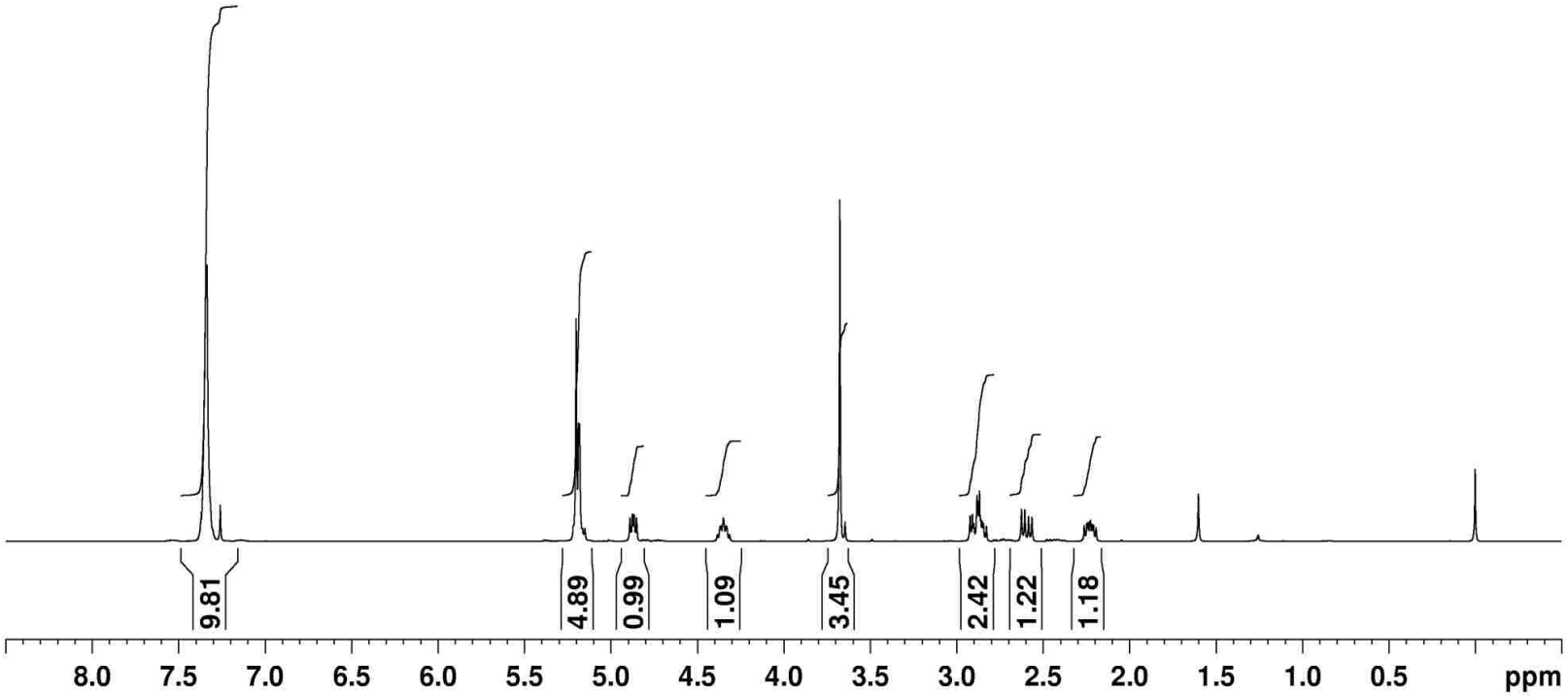
Compound **147a** (Scheme 3.21) - ^{13}C NMR spectrum



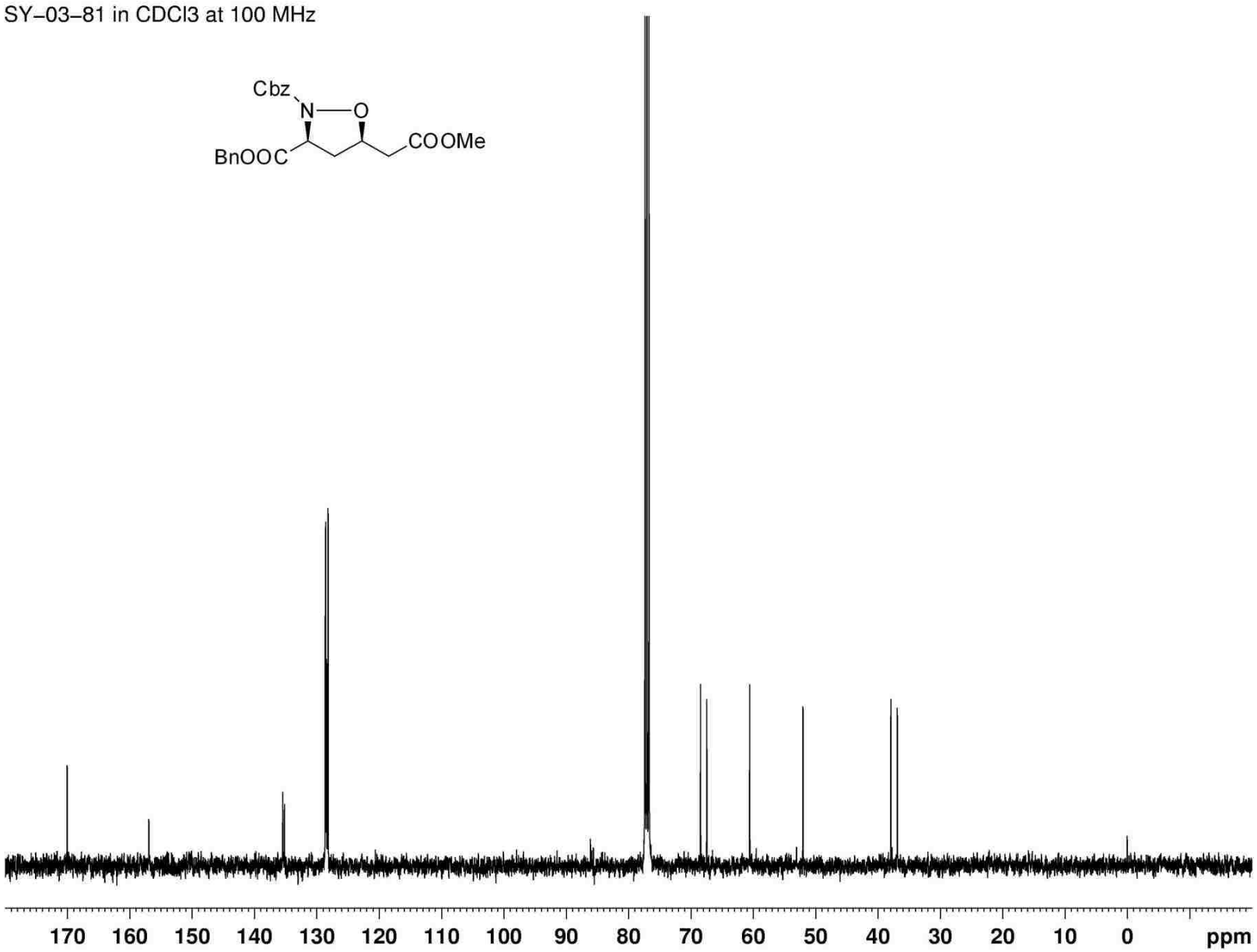
Compound **163** (Scheme 3.22) - ^{1}H NMR spectrum

SY–03–81 in CDCl3 at 400 MHz





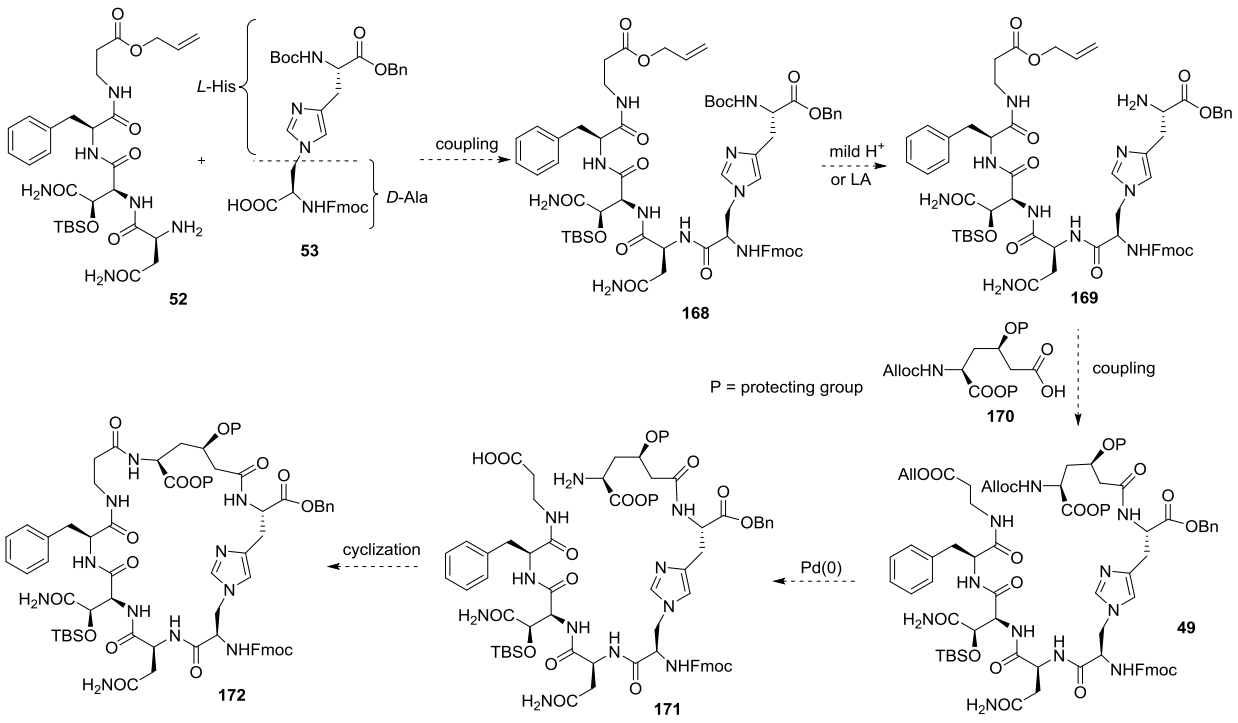
Compound **163** (Scheme 3.22) - ^{13}C NMR spectrum



CHAPTER 4: PROTECTING GROUP STRATEGY FOR (2*S*,4*R*)-α-AMINO-γ-HYDROXY ADIPIC ACID (AHAD) AND α-AMINO ADIPIC ACID (α-AAA)

4.1 The Protecting Group Challenge

A major challenge in the synthesis of the Ahad building block was selection of the right combination of protecting groups that would be compatible with the total synthesis of theonellamide C. The proposed assembly of the western hemisphere is illustrated in Scheme 4.1. The amino terminus of tetrapeptide **52** will first be coupled to the *D*-Ala carboxylic acid of the τ-histidinoalanine residue **53**. Following appropriate deprotections, the free δ-acid of the Ahad building block **170** will be coupled with the *L*-His primary amine of **169**, thereby leading to cyclization precursor **49**. Our goal is to deblock the *C*-terminal allyl group and the *N*-terminal allyloxycarbonyl group simultaneously with Pd(0), and then cyclize to obtain the western hemisphere of theonellamide C (Scheme 4.1).



Scheme 4.1 Proposed assembly of the western hemisphere of theonellamide C.

A successful protecting group strategy for the Ahad building block requires (Figure 4.1):

1. *N*α-protection as an allyloxy carbonyl (Alloc) carbamate will enable deprotection by Pd(0) concomitantly with the allyl ester as a prelude to cyclization.
2. The *C*α-COOH is not part of the macrocycle and therefore requires "permanent" protection. Our intent is for all side chain protecting groups to be fluoride-labile.
3. The *C*ɣ-OH (side chain) also requires permanent protection, *viz.*, fluoride lability.
4. The *C*δ-COOH protecting group should be removed under conditions where all other protecting groups are inert, to enable conjugation to τ-HAL.

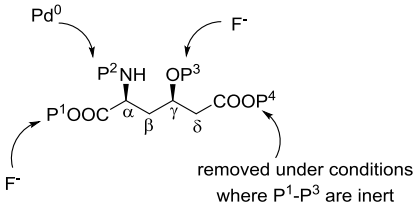


Figure 4.1 Schematic representation of protecting group requirements for Ahad residue.

These challenges prompted us to use α-amino adipic acid (α-AAA) **64** as a model system. The difference between Ahad and α-amino adipic acid (Figure 4.2) is that the former is γ-hydroxylated; the latter appears in other congeners of the theonellamides. Moreover, α-AAA is commercially available and we can work initially with racemic α-AAA (US$64.20/g) and penultimately *L*-α-AAA (US$171.50/g).

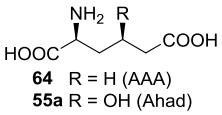
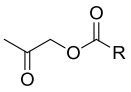


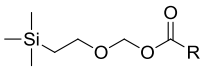
Figure 4.2 Structure of amino adipic acid and Ahad.

We investigated the acetol ester[81,82] (Ac*) and the 2-(trimethylsilyl)ethoxymethyl (SEM) ester[83,84] as candidates for protecting the α-carboxylic acid. Both these protecting groups have been found to be stable to hydrogenolytic and acidolytic conditions normally used in peptide

synthesis. Fluoride ion treatment has been demonstrated to cleave acetol and SEM esters (Figure 4.3), compatible with a global side chain deprotection of the TBS ether in the final stages of theonellamide synthesis.



Acetol ester

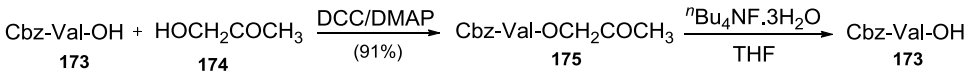


SEM ester

Figure 4.3 Structure of acetol and SEM ester protecting groups.

4.2 Acetol Esters

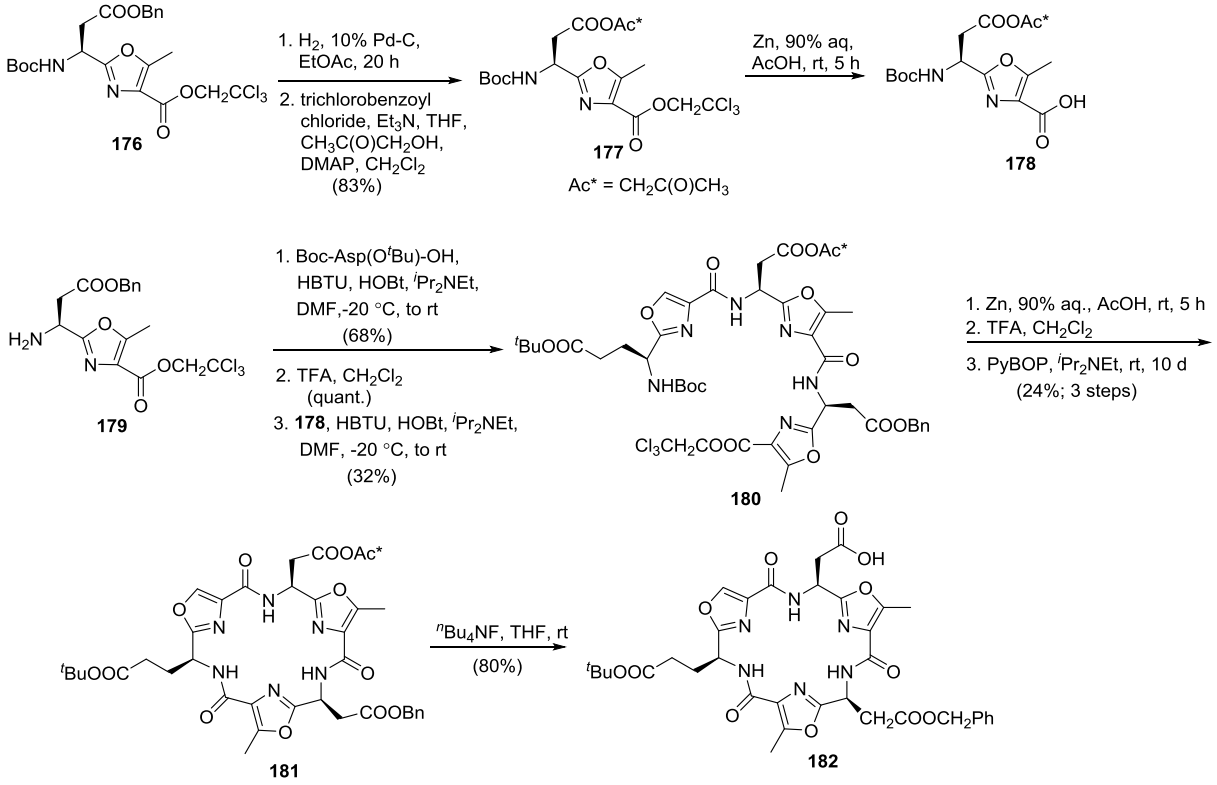
Kundu introduced the use of acetol esters to peptide synthesis in 1992.[81] He synthesized several *N*-protected amino acid acetol esters with acetol using DCC/DMAP and showed that the acetol esters were stable to acidolytic (HCl/dioxane, TFA/CH_2Cl_2) and hydrogenolytic (H_2, Pd-C) conditions routinely used in peptide synthesis for the removal of Boc and Cbz groups respectively. The deprotection of an acetol ester can be carried out using tetrabutylammonium fluoride solution in tetrahydrofuran (THF) at room temperature (Scheme 4.2).



Scheme 4.2 Synthesis and deprotection of acetol esters.

Acetol esters have not seen much utilization. An exception was in 2001, when Rebek[82] used an acetol ester in the synthesis of the marine cyclopeptide dendroamide C (Scheme 4.3) and differentiated three acids (benzyl, *tert*-butyl and acetol) from each other. First, the acetol ester **177** was prepared by removing the benzyl ester from **176** via hydrogenolysis and re-protecting the free acid with acetol using the Yamaguchi method. After removal of the trichloroethyl (TCE) ester, compound **178** was then incorporated into protected linear tripeptide **180**. Deblocking of the trichloroethyl ester afforded the free acid and deprotection of the *N*-

terminus under acidic conditions afforded the linear precursor. Cyclization was performed in DMF with PyBOP activation in the presence of diisopropylethylamine, yielding the orthogonally protected triester **181** in 24% yield from protected linear trimer **180**. The acetol ester was removed using TBAF to obtain free acid **182** (Scheme 4.3), without affecting the benzyl and *tert*-butyl esters.

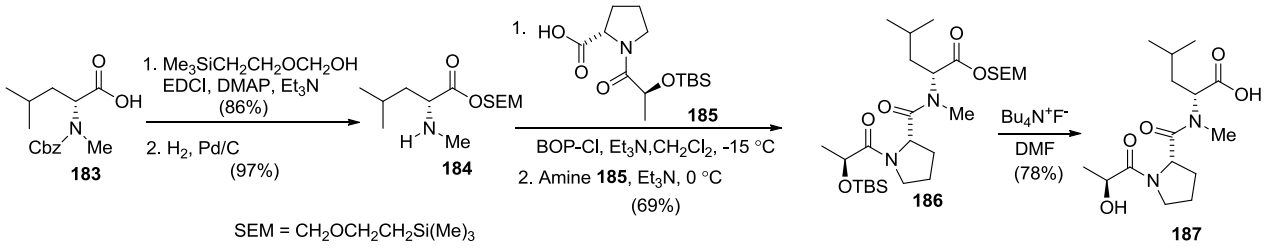


Scheme 4.3 Use of acetol ester by Rebek *et al*. in dendroamide C synthesis.

4.3 SEM Esters

Joullié *et al.* used the SEM ester as a protecting group in their total synthesis of didemnins A, B, and C.[84] The synthesis of a fragment of didemnin B is illustrated in Scheme 4.4. The acid of Cbz-*N*-methyl-*D*-leucine-OH (**183**) was protected as its 2-(trimethylsilyl)ethyl ester via a carbodiimide-mediated coupling with trimethylsilylethanol. After removal of the Cbz group by hydrogenolysis, coupling of the *N*-methyl-*D*-leucine trimethylsilylethyl ester **184** and the protected lactyl proline acid **185** was achieved by premixing *bis*(2-oxo-3-

oxazolidinyl)phosphonic chloride (BOP-CI) and triethylamine with the acid **185**, and adding a solution of the secondary amine **184** and base to the solution of activated species. Simultaneous removal of the two silyl groups was effected with tetrabutylammonium fluoride in DMF to afford compound **187** (Scheme 4.4).

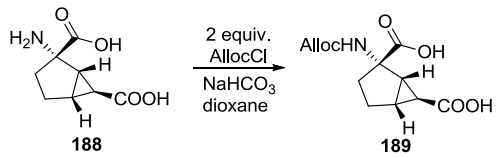


Scheme 4.4 SEM ester in the synthesis of didemnins A, B and C.

4.4 Synthesis of α-AAA-α-Esters

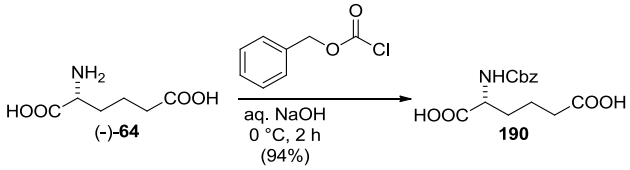
4.4.1 Precedents for *N*-Protection

Herin and coworkers protected (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (**188**) as its allyloxycarbamate.[85] They used two equivalents of allyl chloroformate in the reaction.



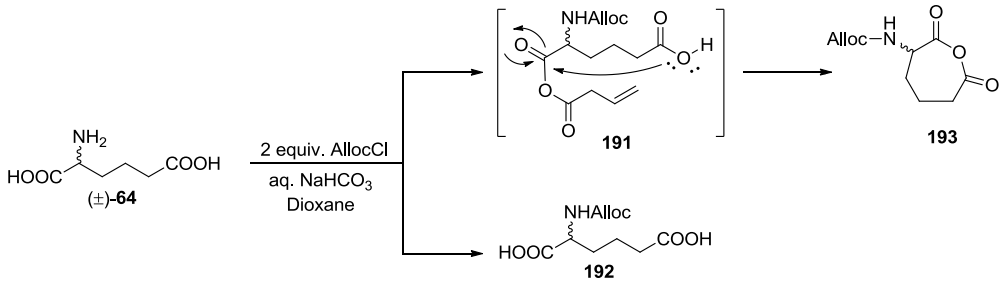
Scheme 4.5 Protection of amino group as an Alloc carbamate.

Later on we came across a procedure by Hachisako *et al*.[86] where they protected the *L*-2-aminoadipic acid (-)-(**64**) as its benzyloxycarbamate (Scheme 4.6).



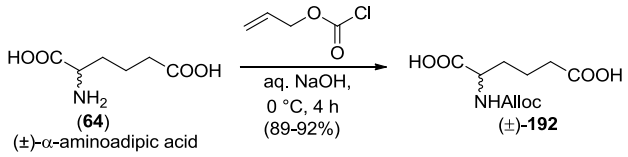
Scheme 4.6 Cbz protection of α-AAA.

Initially we followed the procedure by Herin and coworkers for protection of the primary amine in (±)-2-aminoadipic acid (±)-**64** as its allyloxycarbamate, but product was obtained in low yield. We got a mixture of two species, one was more polar and corresponded to the product **192** and the other compound was less polar and we suspected that the cyclic anhydride **193** was forming with the use of two equivalents of allylchloroformate (Scheme 4.7).



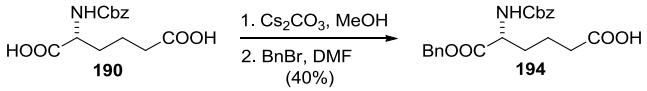
Scheme 4.7 Cyclic anhydride formation in preparation of an *N*α-Alloc carbamate.

Our next attempt at Alloc-protection of (±)-64 was by analogy to the procedure for the synthesis of *N*α-Cbz adipic acid by Hachisako with slight modifications. The reaction time was changed from two hours to four hours at 0 °C and after the work-up, the aqueous layer was extracted thoroughly several times with ethyl acetate to afford compound (±)-**192** in good yield (Scheme 4.8).



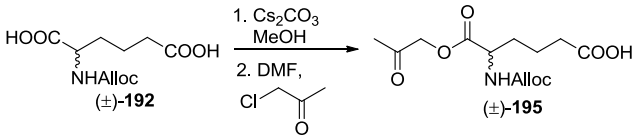
Scheme 4.8 Protection of amino group as an Alloc carbamate.

Baldwin and coworkers have demonstrated that it is possible to selectively esterify the α-carboxylic acid of α-amino adipic acid (Scheme 4.9), since its pKa is about one unit lower than the δ-carboxylic acid.[87]



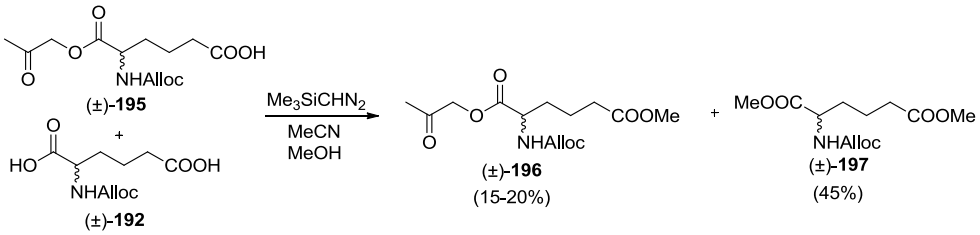
Scheme 4.9 Regioselective esterification of *C*α-COOH by Baldwin *et al.*

The synthesis of the acetol ester is illustrated in Scheme 4.10. Regioselective esterification of the α-acid in compound **192** was attempted by forming the mono-cesium salt and then reacting with chloroacetone.



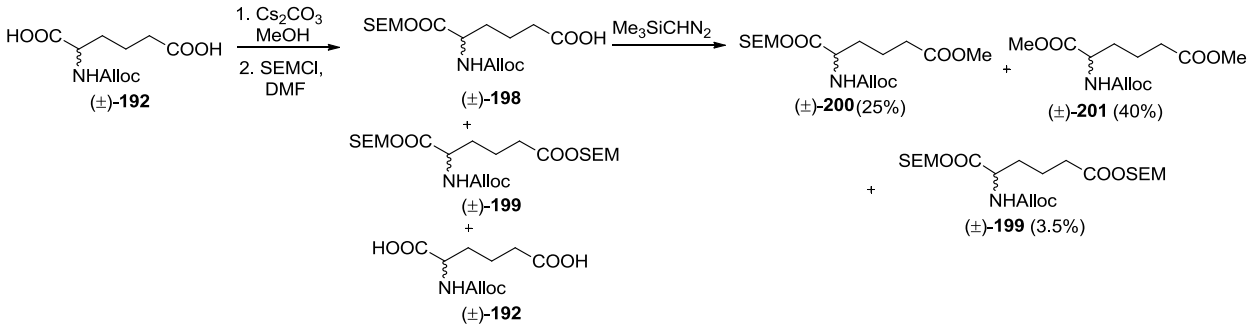
Scheme 4.10 Protection of *C*α-COOH as acetol ester.

The product mixture containing compound **195** was subjected, without any purification, to methyl ester formation using an excess of trimethylsilyldiazomethane. The overall yield, after purification by silica gel chromatography, was low because of poor regioselectivity during formation of the first ester and the lack of purification following the previous two steps. The major product obtained after purification on silica gel chromatography was in fact the dimethyl ester **197**, not the desired compound **196** (Scheme 4.11).



Scheme 4.11 Synthesis of fully protected α-AAA.

The SEM group was investigated in an analogous series of reactions to the acetol protecting group (Scheme 4.12).

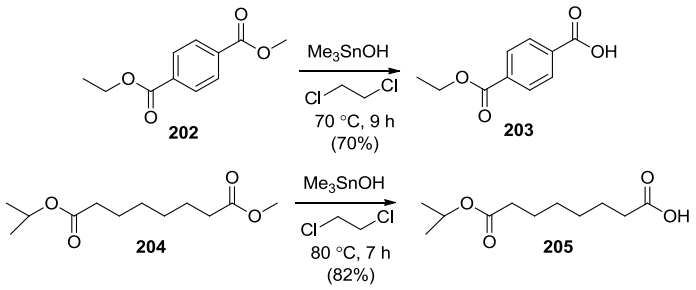


Scheme 4.12 Mixture of products in synthesis of fully protected α-AAA.

Despite the poor overall yields, compounds **196** and **200** were available in reasonable quantities to explore chemoselective liberation of δ-COOH.

4.5 Liberation of δ-COOH from Protected α-AAA Derivatives

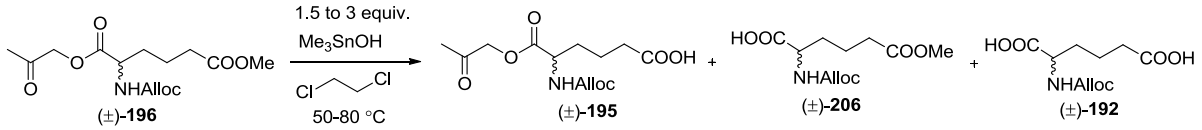
Nicolaou and coworkers had reported a mild and selective method for the hydrolysis of methyl esters with trimethyltin hydroxide.[88] They showed that the methyl ester is hydrolyzed preferentially, even in the presence of ethyl and isopropyl esters, in good yield, for both aromatic and aliphatic systems (Scheme 4.13).



Scheme 4.13 Selective hydrolysis of methyl esters with trimethyltin hydroxide.

We therefore hoped that these reaction conditions would be selective in our α-AAA derivatives in which the α-COOH ester is a substituted ethyl ester in each case. We thus attempted to hydrolyze the methyl ester in the presence of the acetol ester protecting group. We

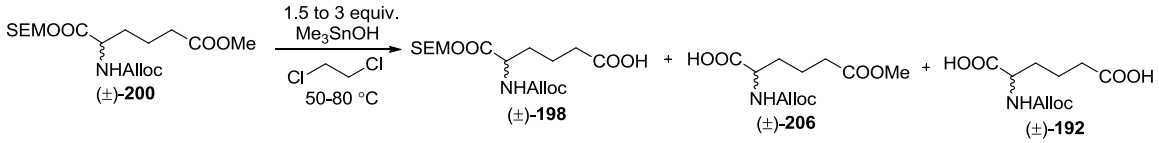
tried several small-scale reactions, varying the reaction temperature and the stoichiometry of trimethyltin hydroxide*;* but the desired product was invariably obtained in very low yield (Scheme 4.14).



Scheme 4.14 Mixture of products after hydrolysis of methyl ester in compound **196**.

The ^{1}H NMR spectrum of the product mixture showed retention of the methyl ester functionality. Analysis of the reaction mixture by LCMS showed that four species were present (Table 4.1). The major peak corresponded to acetol deprotection (compound **206**). The other minor peaks corresponded to starting material **196**, the product of methyl ester hydrolysis **195**, and fully deprotected diacid **192**. The distribution of compounds is summarized in Table 4.1.

Likewise, treatment of compound **200** with trimethyltin hydroxide led to cleavage of the SEM ester in preference to the methyl ester (Scheme 4.15). Analysis of the product mixture by LCMS showed a major peak corresponding to SEM ester deprotection (compound **206**). The other minor peaks corresponded to starting material **200**, desired product **198**, and fully deprotected diacid **192** (Table 4.1).



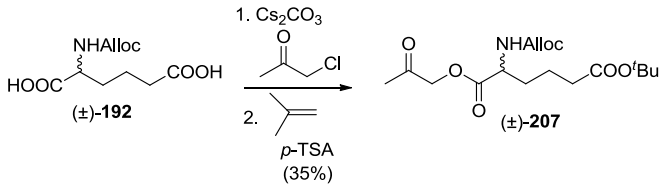
Scheme 4.15 Mixture of products obtained after treatment of diester (±)-**200** with trimethyltin hydroxide.

Table 4.1 Composition of product mixture, following hydrolysis by trimethyltin hydroxide, as determined by LCMS.

Compound	Ac*	SEM
Fully protected starting material	**196** (11.5%)	**200** (2.7%)
δ-COOMe cleavage	**195** (12.5%)	**198** (18.1%)
α-COOR cleavage	**206** (69.5%)	**206** (77.7%)
Diacid (double cleavage)	**192** (6.5%)	**192** (1.6%)

In summary, both SEM and acetol esters were cleaved in preference to methyl esters upon hydrolysis using trimethyltin hydroxide. After consideration of this problem and assessing potential substitutes for the methyl ester, we decided to pursue a *tert*-butyl ester. The δ-*tert*-butyl ester ought to be removed under acidic conditions which should not affect the other protecting groups of the Ahad building block.

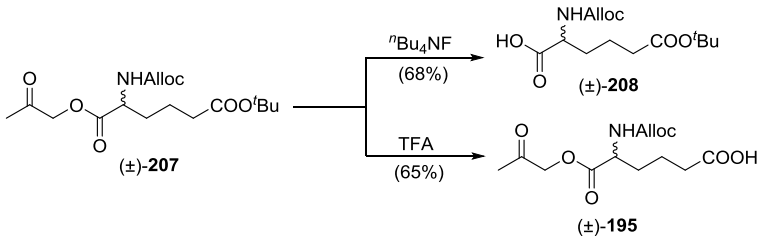
Using racemic α-AAA, the synthesis of the building block was repeated with a *tert*-butyl ester for racemic adipic acid. Compound **192** was protected as its α-acetol ester and then subjected to isobutylene under acidic conditions to form 6-*tert*-butyl 1-(2-oxopropyl)-2-[{(allyloxy)carbonyl}amino]hexanedioate (**207**) (Scheme 4.16).



Scheme 4.16 Synthesis of α-AAA with δ-COO^{t}Bu ester.

As expected, the cleavage of the *tert*-butyl ester was effected using TFA without any harm to the acetol or Alloc protecting groups (Scheme 4.17). Chemoselective deprotection of

the acetol ester was also demonstrated using TBAF. Conversely, we observed some cleavage of the SEM ester under acidic conditions in the reaction; therefore, we gave preference to the acetol ester.



Scheme 4.17 Orthogonal deprotection of acetol and *tert*-butyl esters.

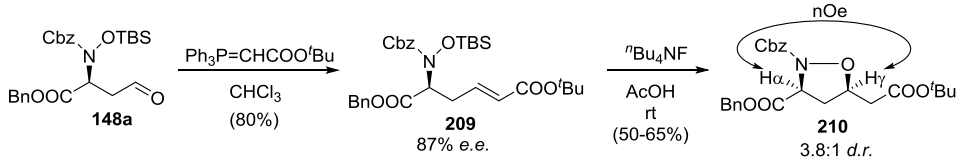
L-2-Aminoadipic acid was subjected to these series of reactions to obtain α-*tert*-butyl-δ-(2-oxopropyl)-*L*-*N*-allyloxycarbonyl-2-aminoadipate.

4.6 Synthesis of Ahad Building Block

Following these model studies, the next task was to make the Ahad precursor with a δ-*tert*-butyl ester instead of a methyl ester as described in Chapter 3. As shown in Scheme 4.18, the analogous reactions were performed with the *tert*-butyl ester protecting group being introduced using the commercially available phosphorane during the Wittig reaction to produce **209**. At this time we measured the enantiomeric purity of compound **209** by chiral HPLC and obtained 87.4% *e.e*. Treatment of compound **209** with tetrabutylammonium fluoride solution with 5% water in tetrahydrofuran facilitated silyl group removal but didn't cyclize to give compound **210**. Increasing the temperature from 0 °C to room temperature had no impact. We added cesium carbonate to the reaction mixture after two days to make the solution more basic. Addition of cesium carbonate did facilitate the cyclization but the yield was very low.

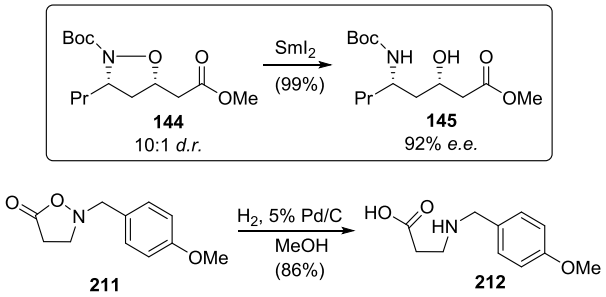
We treated compound **209** with tetrabutylammonium fluoride solution in 1M THF with acetic acid at room temperature and obtained the major *syn* isooxazolidine **210** in good yield with a 3.8:1.0 d.r. (Scheme 4.18). The major diastereomer was confirmed to have *syn* relative

stereochemistry by a NOESY experiment which showed a cross peak between α and γ protons (5.6% nOe). The *syn* diastereomer could be further purified by normal phase HPLC, although in practice the minor diastereomer was gradually removed during chromatography following the next few reaction steps.



Scheme 4.18 Synthesis of isooxazolidine precursor to Ahad.

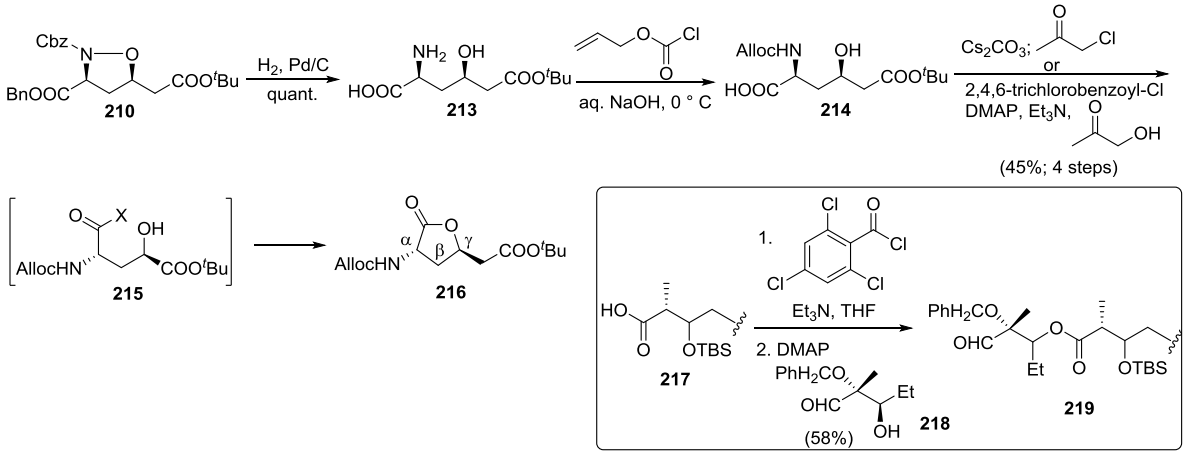
While MacMillan and co-workers had employed samarium diiodide (Chapter 3, Section 3.2) to reductively cleave the N-O bond in a compound analogous to **210** (shown in a box in Scheme 4.19),[70] Sibi and coworkers showed that exposure to standard hydrogenolytic conditions also effectively cleaves N-O bonds (Scheme 4.19).[80]



Scheme 4.19 Precedents for cleavage of N-O bond.

We anticipated that exposure of **210** to standard hydrogenolysis conditions would simultaneously remove the Cbz and benzyl ester protecting groups, along with cleavage of the N-O bond. Hydrogenolysis was performed to give compound **213** ready for introduction of a suitable suite of protecting groups. The free amine was protected as its allyloxycarbamate by analogy to the procedure for racemic α-AAA. Esterification of the α-acid was attempted by the addition of half an equivalent of cesium carbonate, followed by chloroacetone. The major

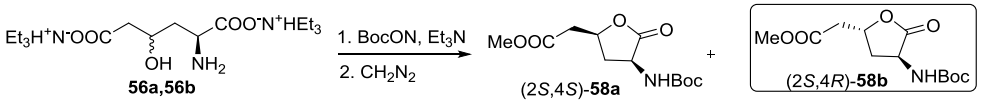
product isolated from the reaction mixture was lactone **216** (Scheme 4.20). We also attempted coupling of the α-acid to acetol in the presence of activating agents, for instance under Yamaguchi conditions (precedent shown in a box in Scheme 4.20).[89] The only product isolated from various reaction conditions was the lactone **216**. Activated acyl species, including the acetol ester, were susceptible to nucleophilic attack by the ɣ-OH, mandating that we protect the secondary alcohol before the carboxylic acid.



Scheme 4.20 Attempted synthesis of Ahad.

As we neared the completion of our Ahad synthesis we sought an approach to correlate our amino acid with that obtained from degradation of the theonellamides. In 1989, Matsunaga *et al.* deduced the structure of Ahad with the aid of NMR studies as described in Chapter 2, Section 2.1.

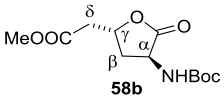
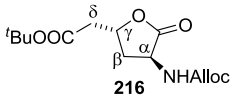
They treated a 1:2 mixture of triethylammonium salts of 2-amino-4-hydroxyadipic acids with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BocON) followed by methylation and normal phase HPLC separation afforded methylated Boc-lactone **58b**. They found out that the minor *trans*-lactone that has (2*S*,4*R*) stereochemistry is the native constituent of theonellamide C.



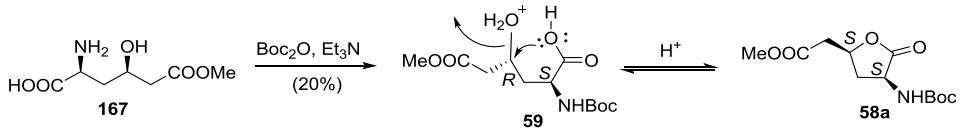
Scheme 4.21 Lactone formation reported by Matsunaga *et al.*

We compared the NMR data of Matsunaga's lactone **58b** with the undesired product isolated from α-acid esterification of Ahad precursor (Scheme 4.20). Nuclear magnetic resonance data for compound **216** showed resemblance to that of the (2*S*,4*R*) lactone **58b**. Moreover lactone **216** afforded further confirmation of the (2*S*,4*R*) stereochemistry since the Hβ protons gave rise to a multiplet at 2.4-2.8 ppm, consistent with a *trans*-1,4-disubstituted ɣ-lactone (Table 4.2).[28]

Table 4.2. NMR data of lactones **58b** and **216**

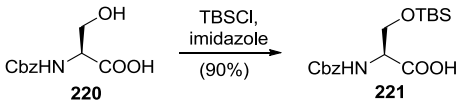
 **58b** Matsunaga *et al.*	 **216** Yadav and Taylor
δ 5.01 (m, 1NH, 1Hα)	δ 5.22-5.30 (m, 1NH, 1Hα)
δ 4.38 (ddd, *J* = 6.4, 9.8, 9.8 Hz, 1Hɣ)	δ 4.48 (dd, *J* = 16.1, 9.3 Hz, 1Hɣ)
δ 2.73 (m, 2Hδ)	δ 2.38-2.79 (m, 2Hδ & 1Hβ)
δ 2.50 (m, 2Hβ)	δ 2.14-2.17 (m, 1Hβ)

Indeed, Matsunaga *et al.* stated that the C-4 hydroxyl group might have been epimerized through a backside attack of the carboxyl group during lactonization. Moreover, equilibration to the more stable *cis*-2,4-disubstituted ɣ-lactone may have enhanced the amount of (2*S*,4*S*) diastereomer. We also observed the same results during the lactone formation of (2*S*,4*R*) compound **167** (Scheme 4.22). The NMR experiments showed that we also obtained the more stable (2*S*,4*S*) *cis*-lactone **58a**.



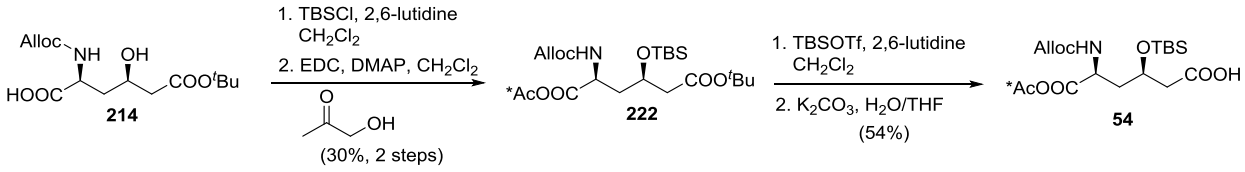
Scheme 4.22 Preparation of ɣ-lactone **58a** by Yadav and Taylor.

Lajoie and coworkers had shown that a free alcohol can be masked with TBSCl in the presence of a free acid.[90] They protected the alcohol of the Cbz-protected serine **220** as a TBS ether with TBSCl (Scheme 4.23).



Scheme 4.23 Protection of alcohol as TBS ether.

We attempted the protection of the alcohol in compound **214** with stoichiometric quantities of TBSCl and 2,6-lutidine. Carbodiimide-mediated coupling of the resulting crude acid with acetol gave rise to compound **222**.[91] Chemoselective cleavage of the *tert*-butyl ester with *tert*-butyldimethylsilyl triflate was carried out in readiness for coupling to the *L*-His amino group of τ-HAL in the western hemisphere of theonellamide C (Scheme 4.24).[92]



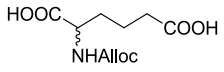
Scheme 4.24 Synthesis of orthogonally protected Ahad.

4.7 Experimental Section

4.7.1 General Methods

The general methodas are as described in Chapter 2.

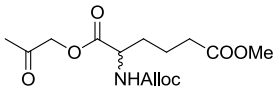
4.7.2 Procedures



2-(Allyloxycarbonylamino)adipic acid (**192**)

(±)-α-Aminoadipic acid (**64**) (250 mg, 1.55 mmol, 1.0 equiv.) was added to a solution of sodium hydroxide (223 mg, 5.60 mmol, 3.6 equiv.) in water (12.5 mL) at 0 °C. Allyl chloroformate (247 µL, 281 mg, 2.35 mmol, 1.5 equiv.) was added dropwise over 30 min and stirring continued for 4 h in an ice bath. The mixture was washed with diethyl ether (20 mL). The aqueous layer was acidified to pH 2 by the addition of conc. HCl and extracted with EtOAc (4 x 20 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated to give (±)-2-(Allyloxycarbonylamino)adipic acid (**192**) (350 mg, 92%). R_f 0.29 (6:4:1 $CHCl_3$-MeOH-H_2O). ^{1}H NMR (CD_3OD, 400 MHz) δ 1.67-1.74 (m, 3H), 1.87-190 (m, 1H), 2.33 (t, *J* = 6.8 Hz, 2H), 4.14 (dd, *J* = 8.0, 4.4 Hz, 1H), 4.54 (d, *J* = 5.3 Hz, 2H), 4.97 (br s, 1H), 5.18 (dd, *J* = 10.5, 1.4 Hz, 1H), 5.31 (dd, *J* = 17.2, 1.5 Hz, 1H), 5.93 (ddt, *J* = 15.9, 10.6, 5.3 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 19.4, 29.1, 31.2, 52.0, 63.5, 114.5, 131.3, 155.6, 172.7, 174.0. HRMS (ESI) calcd for $C_{10}H_{15}NNaO_6$ $(M+Na)^+$ 268.0792, obsd 268.0786.

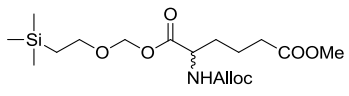
(*L*)-*N*-Allyloxycarbonyl-2-amino-adipic acid was prepared using *L*-2-aminoadipic acid (100 mg) according to the same procedure to give (-)-**193** (130 mg, 89%). $[\alpha]_D^{25}$ -7.82 (*c* 1.0, MeOH).



2-(Allyloxycarbonylamino)-6-methyl-1-(2-oxopropyl)-adipate (**196**)

Cesium carbonate (166 mg, 0.51 mmol, 0.5 equiv.) was added to a solution of (±)-*N*-allyloxycarbonyl-2-amino-adipic acid (**192**) (250 mg, 1.02 mmol, 1.0 equiv.) in dry MeOH (1 mL). The mixture was stirred for 2 h under N_2 at rt. The mixture was concentrated, dissolved in DMF (2 mL) and cooled to 0 °C. Chloroacetone (97 µL, 113 mg, 1.22 mmol, 1.2 equiv.) was added,

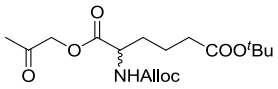
the mixture warmed to rt, stirred overnight, diluted with H_2O (15 mL) and extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and and concentrated to give the mixture containing acid 5-[{(allyloxy)carbonyl}amino]-6-oxo-6-(2-oxopropoxy)hexanoic acid as a colorless oil (300 mg) that was dissolved in acetonitrile (3 mL) and methanol (300 µL). Trimethylsilyldiazomethane (498 µL, 2 M solution in Et_2O, 0.99 mmol, 1.1 equiv.) added dropwise and stirred for 2.5 h at rt under N_2. The mixture was concentrated and applied to a flash column. Elution with a gradient of 3:1→1:1 (Hex-EtOAc) gave 2-(Allyloxycarbonylamino)-6-methyl-1-(2-oxopropyl)-adipate (**196**) as a colorless oil (64 mg, 20%). R_f 0.40 (2:1 EtOAc-Hex). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.73-1.82 (m, 3H), 1.97-2.02 (m, 1H), 2.15 (s, 3H), 2.37 (t, *J* = 6.4 Hz, 2H), 3.66 (s, 3H), 4.42-4.46 (m, 1H), 4.56 (d, *J* = 5.5 Hz, 2H), 4.63 (d, *J* = 16.8 Hz, 1H), 4.88 (d, *J* = 16.8 Hz, 1H), 5.21 (dd, *J* = 10.4, 1.3 Hz, 2H), 5.28-5.35 (m, 2H), 5.90 (ddt, *J* = 16.1, 10.5, 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.5, 26.0, 31.7, 33.3, 51.6, 53.5, 65.9, 68.7, 117.9, 132.5, 155.8, 171.7, 173.5, 200.6. HRMS (ESI) calcd for $C_{14}H_{21}NNaO_7$ $(M+Na)^+$ 338.1216, obsd 338.1220.



2-(Allyloxycarbonylamino)-6-methyl-1-(2-trimethylsilyl-ethoxy-methyl)-adpate (**200**)

Cesium carbonate (166 mg, 0.51 mmol, 0.5 equiv.) was added to a solution of (±)-*N*-allyloxycarbonyl-2-amino-adipic acid (**192**) (250 mg, 1.02 mmol, 1.0 equiv.) in dry MeOH (1 mL). The mixture was stirred for 2 h under N_2 at rt. The mixture was concentrated, the residue dissolved in DMF (2 mL) and cooled to 0 °C. 2-(Trimethylsilyl)ethoxymethyl chloride (217 µL, 204 mg, 1.22 mmol, 1.2 equiv.) was added, the mixture warmed to rt, stirred overnight, diluted with H_2O (15 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give a mixture containing acid 5-[{(allyloxy)carbonyl}amino]-6-oxo-6-[{(2-(trimethylsilyl)ethoxy}methoxy]hexanoic acid as a

colorless oil (370 mg) that was dissolved in acetonitrile (4 mL) and methanol (400 µL). Trimethylsilyldiazomethane (541 µL, 2 M solution in Et_2O, 1.08 mmol, 1.1 equiv.) added dropwise and the mixture stirred for 2.5 h at rt under N_2. The mixture was concentrated and applied to a flash column. Elution with a gradient of 3:1→1:1 (Hex-EtOAc) gave 2-(Allyloxycarbonylamino)-6-methyl-1-(2-trimethylsilyl-ethoxy-methyl)-adpate (**200**) as a colorless oil (99 mg, 25%). R_f 0.49 (2:1 EtOAc-Hex). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.01 (s, 9H) 0.95 (app.t, *J* = 8.4 Hz, 2H), 1.68-1.73 (m, 3H), 1.87-1.92 (m, 1H), 2.31-2.38 (m, 2H), 3.65 (s, 3H), 3.66-3.73 (m, 2H), 4.35-4.38 (m, 1H), 4.56 (d, *J* = 5.4 Hz, 2H), 5.20 (dd, *J* = 10.4, 1.3 Hz, 2H), 5.26-5.36 (m, 3H), 5.90 (ddt, *J* = 16.1, 10.6, 5.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -1.5, 18.0, 20.4, 20.6, 31.8, 33.3, 33.5, 51.6, 52.4, 53.5, 53.6, 65.8, 67.9, 68.2, 88.9, 89.9, 117.8, 132.6, 155.8, 171.9, 172.7, 173.3. HRMS (ESI) calcd for $C_{17}H_{31}NNaO_7Si$ $(M+Na)^+$ 412.1762, obsd 412.1748.

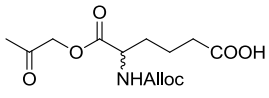


2-(Allyloxycarbonylamino)-6-(*tert*-butyl)-1-(2-oxo-propyl)-adipate (**207**)

Cesium carbonate (332 mg, 1.02 mmol, 0.5 equiv.) was added to a solution of (±)-*N*-allyloxycarbonyl-2-amino-adipic acid (**192**) (500 mg, 2.04 mmol, 1.0 equiv.) in dry MeOH (4 mL). The mixture stirred for 2 h under N_2 at rt. The mixture was concentrated, dissolved in DMF (5 mL) and cooled to 0 °C. Chloroacetone (194 µL, 226 mg, 2.44 mmol, 1.2 equiv.) was added, the mixture warmed to rt, stirred overnight, diluted with H_2O (30 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over $MgSO_4$, filtered and and concentrated to give acid 5-[{(allyloxy)carbonyl}amino]-6-oxo-6-(2-oxopropoxy)hexanoic acid as a colorless oil (500 mg) that was dissolved in CH_2Cl_2 (10 mL) and added *p*-TsOH (663 mg, 3.49 mmol, 2.1 equiv.). The reaction mixture was added to condensed isobutylene (10 mL) and stirred for 3d at rt. The mixture was diluted with 20 mL CH_2Cl_2 and added sat. sol. of $NaHCO_3$ (25 mL), extracted the organic layer. The remaining aqueous layer was washed with CH_2Cl_2 (2 x 30 mL).

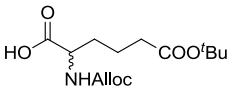
The combined organic layers were dried over $MgSO_4$, filtered, concentrated and applied to a flash column. Elution with a gradient of 3:1→1:1 (Hex-EtOAc) gave 2-(allyloxycarbonylamino)-6-(*tert*-butyl)-1-(2-oxo-propyl)-adipate (**207**) as a colorless oil (198 mg, 34%). R_f 0.32 (2:1 EtOAc-Hex). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (s, 9H), 1.64-1.79 (m, 2H), 1.91-2.02 (m, 2H), 2.14 (s, 3H), 2.25 (t, *J* = 6.9 Hz, 2H), 4.42 (dd, *J* = 12.4, 7.4 Hz, 1H), 4.55 (d, *J* = 5.4 Hz, 2H), 4.62 (d, *J* = 16.8 Hz, 1H), 4.75 (d, *J* = 16.8 Hz, 1H), 5.19 (dd, *J* = 10.4, 1.2 Hz, 1H), 5.26-5.39 (m, 1H), 5.89 (ddt, *J* = 16.2, 10.6, 5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.7, 26.0, 28.1, 31.6, 34.7, 53.4, 65.9, 68.7, 80.4, 117.8, 132.6, 155.8, 171.8, 172.4. HRMS (ESI) calcd for $C_{17}H_{28}NO_7$ $(M+H)^+$ 358.1820, obsd 358.1826.

(*L*)-*N*-allyloxycarbonyl-2-amino-adipic acid (50 mg) was subjected to the same procedure to give α-*tert*-Butyl δ-(2-oxopropyl)-(*L*)-*N*-allyloxycarbonyl-2-aminoadipate (-)-**207** (20 mg, 30%). $[\alpha]_D^{25}$ -7.75 (*c* 1.0, MeOH).



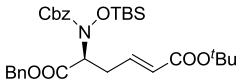
2-(Allyloxycarbonylamino)-1-(2-oxopropyl)-adipic acid (**195**)

To a solution of 2-(allyloxycarbonylamino)-6-(*tert*-butyl)-1-(2-oxo-propyl)-adipate (**207**) (25 mg, 0.069 mmol, 1.0 equiv.) in dry CH_2Cl_2 (2 mL) was added trifluoroacetic acid (2 mL) at 0 °C and allowed to stir at 0 °C for 1 h. The reaction mixture was warmed to rt and stirred overnight, concentrated and applied to a flash column. Elution with a gradient of 3:1→4:1 (EtOAc-Hex) gave 2-(Allyloxycarbonylamino)-1-(2-oxopropyl)-adipic acid (**195**) (15 mg, 65 %). R_f 0.23 (3:1 EtOAc-Hex). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.20-1.29 (m, 2H), 1.78-1.84 (m, 2H), 2.17 (s, 3H), 2.43 (t, *J* = 7.1 Hz, 2H), 4.43-4.50 (m, 1H), 4.58 (d, *J* = 5.0 Hz, 2H), 4.65 (d, *J* = 16.8 Hz, 1H), 4.80 (d, *J* = 16.8 Hz, 1H), 5.22 (dd, *J* = 10.4, 1.1 Hz, 1H), 5.29 (d, *J* = 2.6 Hz, 1H), 5.34 (s, 1H), 5.91 (ddt, *J* = 16.2, 10.7, 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.4, 26.0, 31.7, 32.9, 53.4, 66.0, 68.7, 118.0, 132.5, 155.9, 171.6, 176.6. HRMS (ESI) calcd for $C_{13}H_{19}NNaO_7$ $(M+Na)^+$ 324.1054, obsd 324.1054.



2-(Allyloxycarbonylamino)-6-(*tert*-butyl)adipate (**208**)

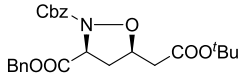
To a solution of α-*tert*-butyl δ-(2-oxopropyl) *N*-allyloxycarbonyl-2-aminoadipate (**207**) (20 mg, 0.056 mmol, 1.0 equiv.) in dry THF (1 mL) was added TBAF (111 µL, 1 M in THF, 0.112 mmol, 2.0 equiv.) at 0 °C and allowed to stir at 0 °C for 1 h. The reaction mixture was warmed to rt and stirred overnight, concentrated and applied to a flash column. Elution with a gradient of 3:1→4:1 (EtOAc-Hex) gave 2-(Allyloxycarbonylamino)-6-(tert-butyl)adipate (**208**) (11 mg, 68 %). R_f 0.25 (2:1 EtOAc-Hex). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.41 (s, 9H), 1.64-1.79 (m, 2H), 1.91-2.02 (m, 2H), 2.25 (t, *J* = 6.9 Hz, 2H), 4.42 (dd, *J* = 12.4, 7.4 Hz, 1H), 4.55 (d, *J* = 5.4 Hz, 2H), 5.19 (dd, *J* = 10.4, 1.2 Hz, 1H), 5.26-5.39 (m, 1H), 5.89 (ddt, *J* = 16.2, 10.6, 5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.7, 28.1, 31.6, 34.7, 53.4, 65.9, 80.4, 117.8, 132.6, 155.8, 171.8, 172.4. HRMS (ESI) calcd for $C_{14}H_{23}NNaO_6$ $(M+Na)^+$ 324.2020, obsd 324.2026.



(2*S*,4*E*)-1-Benzyl-2-[(benzyloxycarbonyl)(*tert*-butyldimethylsilyloxy-amino]-6-*tert*-butyl-hex-4-enedioate (One–pot procedure) (**209**)

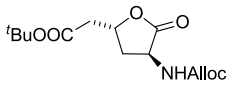
A solution of benzyl (*tert*-butyldimethylsilyl)oxycarbamate (**136a**) (250.0 mg, 0.88 mmol, 1.4 equiv.) in chloroform (1 mL) was treated with (*S*)-2-{diphenyl[(trimethylsilyl)oxy]methyl}pyrrolidine (**165**) (41.0 mg, 0.12 mmol, 0.2 equiv), followed by the addition of acetic acid (7 µL, 7.5 mg, 0.12 mmol, 0.2 equiv.) and stirred for 10 min at rt. Benzyl 4-oxo-but-2-enoate (**149**) (121.0 mg, 0.63 mmol, 1.0 equiv.) was added and the reaction mixture was stirred for 3.5 h at rt. Upon completion (determined by TLC), *tert*-butyl-2-(triphenylphosphoranylidene)acetate (237.0 mg, 0.63 mmol, 1.0 equiv.) was added in one portion. The reaction mixture was stirred for 1.5 h and then concentrated to a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 12:1) afforded the α,β-unsaturated ester as colorless oil **209** (354 mg, 70%, 87.4% *e.e.*). The enantiomeric excess was determined by HPLC analysis

using Chiralcel OD-H column (5% ^{i}PrOH-Hex, 0.5 mL/min); major enantiomer t_r = 20.1 min and minor enantiomer t_r = 36.4 min; Detection 218 nm. R_f 0.30 (2:1 Hex-EtOAc). $[\alpha]_D^{25}$ -37.2 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 3H), 0.10 (s, 3H), 0.85 (s, 9H), 1.47 (s, 9H), 2.76-2.91 (m, 2H), 4.57 (dd, *J* = 8.7, 6.2 Hz, 1H), 5.06-5.18 (m, 4H), 5.84 (d, *J* = 15.6 Hz, 1H), 6.86 (dt, *J* = 7.0, 15.6 Hz, 1H), 7.26-7.36 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -4.8, -4.7, 18.1, 25.8, 28.1, 31.2, 64.5, 67.2, 68.4, 80.3, 125.8, 128.1, 128.2, 128.4, 128.5, 128.5, 128.6, 135.3, 142.6, 159.1, 165.4, 168.9. HRMS (ESI) calcd for $C_{31}H_{44}NO_7Si$ $(M+H)^+$ 570.2412, obsd 570.2420.



(3*S*,5*R*)-benzy-5-[(*tert*-butoxycarbonyl)methyl]-isooxazolidine-2-carboxylate (**210**)

To a solution of (2*S*,4*E*)-1-benzyl-6-*tert*-butyl-2-[{(benzyloxy)carbonyl}{(*tert*-butyldimethylsilyl)oxy}amino]hex-2-enedioate (**209**) (500.0 mg, 0.88 mmol, 1.0 equiv.) in CH_2Cl_2 (3 mL) was added acetic acid (0.078 mL, 78.9 mg, 1.32 mmol, 1.5 equiv.). The mixture was cooled to 0 °C and TBAF (1.3 mL, 1 M in THF, 1.32 mmol, 1.5 equiv.) was added and allowed to stir at 0 °C for 30 min. The reaction mixture was warmed to rt and stirred for 2 days. Reaction was monitored by TLC and upon completion, the reaction mixture was concentrated and applied directly to a flash column, eluting with 4:1 (Hex-EtOAc), to obtain (2*S*,4*R*)-dibenzyl 2-{6-(*tert*-butoxy)-oxoethyl}isoxazolidine-2,3-dicarboxylate **210** (344 mg, 55%). R_f 0.25 (2:1 Hex-EtOAc). $[\alpha]_D^{25}$ -23.25 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9H), 2.20 (ddt, *J* = 12.7, 9.1, 5.9 Hz, 1 H), 2.48 (dd, *J* = 16.0, 8.0 Hz, 1H), 2.77-2.88 (m, 2H), 4.24-4.31 (m, 1H), 4.86 (dd, *J* = 9.6, 5.9 Hz, 1H), 5.16 (d, *J* = 4.9 Hz, 1 H), 5.18 (d, *J* = 2.0 Hz, 1 H), 5.19 (d, *J* = 1.0 Hz, 1 H), 5.22 (d, *J* = 4.0 Hz, 1 H), 7.26-7.37 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.0, 38.0, 38.4, 60.6, 67.4, 68.4, 81.5, 128.2, 128.3, 128.4, 128.5, 128.6, 135.2, 135.5, 168.8, 170.1. HRMS (ESI) calcd for $C_{25}H_{30}NO_7$ $(M+H)^+$ 456.5547, obsd 456.5548.



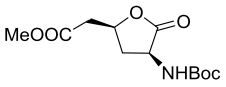
(3*S*,5*R*)-3-(Allyloxycarbonylamino)-5-[(*tert*-butoxycarbonyl)-methyl]-2-oxotetrahydrofuran (**216**)

(2S,4R)-2-Amino-6-tert-butyl-4-hydroxyadipic acid (**213**): (3*S*,5*R*)-benzy-5-[(*tert*-butoxycarbonyl)methyl]-isooxazolidine-2-carboxylate (**210**) (100 mg, 0.219 mmol, 1.0 equiv.) was dissolved in dry MeOH (2 mL) and 10 % Pd-C (93 mg, 0.879 mmol, 4.0 equiv.) was added. The suspension was stirred at rt overnight under an atmosphere of H_2. The reaction mixture was filtered through Celite® and the filtrate concentrated to give the α-amino-carboxylic acid **213** as a colorless oil (40 mg). R_f 0.56 (6:4:1 $CHCl_3$-MeOH-H_2O).

(2S,4R)-2-(Allyloxycarbonylamino)-6-tert-butyl-4-hydroxy-adipic acid (**214**): The amino acid was dissolved in a solution of sodium hydroxide (32 mg, 0.788 mmol, 3.6 equiv.) in water (2 mL) at 0 °C and followed by dropwise addition of allyl chloroformate (28 µL, 32 mg, 0.263 mmol, 1.2 equiv.) at 0 °C. The mixture was stirred an additional 1 h at 0 °C and then warmed to RT and stirred overnight. The reaction mixture was washed with diethyl ether (5 mL) to remove unreacted allylchloroformate. The aqueous layer was cooled to 0 °C and then acidified to ~pH 4 by the dropwise addition of 0.5 M HCl. The aqueous layer was extracted with EtOAc (5x10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to obtain 6-*tert*-butyl-*N*-allyloxycarbonyl-(2*S*,4*R*)-2-amino-4-hydroxy-α-oxohexanoic acid (**214**). R_f 0.54 (9:1 CH_2Cl_2-MeOH)

(3S,5R)-3-(Allyloxycarbonylamino)-5-[(tert-butoxycarbonyl)-methyl]-2-oxotetrahydrofuran (**216**): Cesium carbonate (36 mg, 0.109 mmol, 0.5 equiv.) was added to a solution of acid **214** in dry MeOH (4 mL). The mixture stirred for 2 h under N_2 at rt. The mixture was concentrated, dissolved in DMF (5 mL) and cooled to 0 °C. Chloroacetone (21 µL, 24 mg, 0.263 mmol, 1.2 equiv.) was added, the mixture warmed to rt, stirred overnight, diluted with H_2O (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered

and and concentrated to give a colorless oil that was dissolved in CH_2Cl_2 (2mL) and added 2,6-Lutidine (254 µL, 235 mg, 2.190 mmol, 10.0 equiv.) and TBSOTf (402 µL, 463 mg, 1.752 mmol, 8.0 equiv.). Reaction mixture was stirred and concentrated to remove the CH_2Cl_2. Water (2 mL) was added to the reaction mixture which was then cooled to 0 °C and acidified to ~pH 4 by the dropwise addition of 0.5 M HCl. The acidic solution was then extracted with EtOAc (4 X 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and then concentrated to afford a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 3:1) afforded the compound **216** as colorless oil (57 mg, 45% over 4 steps). R_f 0.54 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ -8.47 (*c* 2.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45 (s, 9H), 2.14-2.17 (m, 1H), 2.38-2.79 (m, 3H), 4.48 (dd, *J* = 16.1, 9.3 Hz, 1H), 4.58 (d, *J* = 5.3 Hz, 2H), 4.98 (dd, *J* = 16.1, 9.3 Hz, 1H), 5.22 (app. d, *J* = 10.4 Hz, 1H), 5.30 (app. d, *J* = 16.4 Hz, 1H), 5.46 (d, *J* = 5.7 Hz, 1H), 5.90 (ddt, *J* = 16.4, 10.8, 5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.0, 33.6, 40.6, 49.5, 66.2, 74.2, 81.9, 118.2, 132.3, 155.9, 168.5, 174.6. HRMS (ESI) calcd for $C_{14}H_{21}NNaO_6$ $(M+Na)^+$ 322.1261, obsd 322.1270.

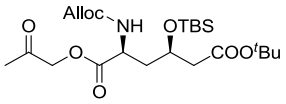


(3*S*,5*R*)-3-(*tert*-butyl-carbamate)-5-[(methoxycarbonyl)-methyl]-2-oxo-tetrahydrofuran (**58a**)

Pd/C (10% w/w, 16 mg) was added to a solution of (2*S*,4*R*)-1-benzyl 2-*tert* butyl-2-(6-methoxy-5-oxoethyl) isoxazolidine-4,5-dicarboxylate **163b** (20 mg, 0.053 mmol, 1.0 equiv.) in dry MeOH (1ml). The vessel was evacuated and then opened up to an atmosphere of hydrogen and stirred overnight. The mixture was filtered through Celite™, washing well with MeOH (~25 mL). The filtrate was concentrated to afford acid **167**, dissolved in CH_2Cl_2 (2 mL), followed by addition of EDC (30 mg, 0.158 mmol, 3.0 equiv.) and DMAP (2 mg, 0.016 mmol, 0.3 equiv.). Reaction mixture was stirred overnight at rt under N_2 atmosphere and then concentrated to afford a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 3:1) afforded lactone **58a** (3 mg, 20%). R_f 0.29 (2:1 Hex-EtOAc). ^{1}H NMR* ($CDCl_3$, 400 MHz) δ 1.50 (s, 9H), 2.20 (ddd, *J* =

12.6, 8.7, 5.6 Hz, 1H), 2.62 (dd, *J* = 16.4, 7.8 Hz, 1H), 2.81-2.86 (m, 1H), 2.90 (dd, *J* = 16.4, 5.6 Hz, 1H), 3.70 (s, 3H), 4.33 (app. pent., *J* = 7.2 Hz, 1H), 4.75 (dd, *J* = 9.5, 5.6 Hz, 1H)); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 28.1, 37.1, 38.0, 52.0, 61.8, 82.8, 156.3, 170.3, 170.6. HRMS (ESI) calcd for $C_{12}H_{18}NO_6$ $(M-H)^+$ 272.1140, obsd 272.1162.

*^{1}H NMR spectra of the title compound contaminated with ethyl acetate.



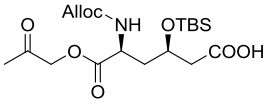
(2*S*,4*R*)-2-(Allyloxycarbonylamino)-6-(*tert*-butyl)-4-(*tert*-butyldimethylsilyloxy)-1-(2-oxopropyl)-adipate (**222**)

(2S,4R)-2-Amino-6-tert-butyl-4-hydroxyadipic acid (**213**): (3*S*,5*R*)-benzy-5-[(*tert*-butoxycarbonyl)methyl]-isooxazolidine-2-carboxylate (**210**) (80 mg, 0.176 mmol, 1.0 equiv.) was dissolved in dry MeOH (2 mL) and 10 % Pd-C (75 mg, 0.702 mmol, 4.0 equiv.) was added. The suspension was stirred at rt overnight under an atmosphere of H_2. The reaction mixture was filtered through Celite® and the filtrate concentrated to give the α-amino-carboxylic acid **213** as a colorless oil (40 mg). R_f 0.56 (6:4:1 $CHCl_3$-MeOH-H_2O).

(2S,4R)-2-(Allyloxycarbonylamino)-6-tert-butyl-4-hydroxy-adipic acid (**214**): The amino acid **213** was dissolved in 10% aqueous Na_2CO_3 solution (1.5 mL) and cooled to 0 °C in ice bath. Dioxane (1.5 mL) was added, followed by dropwise addition of allyl chloroformate (22 µL, 25 mg, 0.211 mmol, 1.2 equiv.) at 0 °C. The mixture was stirred an additional 1 h at 0 °C and then warmed to RT and stirred overnight. The reaction mixture was washed with diethyl ether (5 mL) to remove unreacted allylchloroformate. The aqueous layer was cooled to 0 °C and then acidified to ~pH 4 by the dropwise addition of 0.5 M HCl. The aqueous layer was extracted with EtOAc (5x10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to obtain compound **214**. R_f 0.54 (9:1 CH_2Cl_2-MeOH)

(2S,4R)-2-(Allyloxycarbonylamino)-6-tert-butyl-4-(tert-butyl dimethylsilyloxy)-adipic acid: 2,6-Lutidine (51 µL, 47 mg, 0.44 mmol, 2.5 equiv.) and TBSCl (66 mg, 0.44 mmol, 2.5 equiv.). were added sequentially to a solution of acid **214** in dry CH_2Cl_2 (2 mL) at rt under N_2. Reaction mixture was stirred and concentrated to remove the CH_2Cl_2. Water (2 mL) was added to the reaction mixture which was then cooled to 0 °C and acidified to ~pH 4 by the dropwise addition of 0.5 M HCl. The acidic solution was then extracted with EtOAc (4 X 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give the carboxylic acid that was used directly in the next step without further purification. R_f 0.61 (95:5 CH_2Cl_2-MeOH).

(2S,4R)-2-(Allyloxycarbonylamino)-6-(tert-butyl)-4-(tert-butyldimethylsilyloxy)-1-(2-oxopropyl)-adipate (**222**): The carboxylic acid was dissolved in dry CH_2Cl_2 (2 mL) and cooled to 0 ° C under N_2 atmosphere. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) (EDC) (68 mg, 0.352 mmol, 2.0 equiv.) was added, followed by DMAP (6 mg, 0.0528 mmol, 0.3 equiv.) and acetol (36 µL, 39 mg, 0.528 mmol, 3.0 equiv.) at 0 °C. The reaction mixture was warmed to rt, stirred overnight and then concentrated to afford a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 3:1) afforded the compound **222** as colorless oil (25 mg, 30% over 4 steps). R_f 0.60 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ -13.25 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 3H), 0.09 (s, 3H), 0.89 (s, 9H), 1.45 (s, 9H), 1.93-1.99 (m, 1H), 2.17 (s, 3H), 2.30-2.51 (m, 3H), 4.29 (app. pent. *J* = 5.9 Hz, 1H), 4.50 (ddd, *J* = 12.4, 7.7, 4.8 Hz, 1H), 4.57-4.78 (m, 4H), 5.20 (d, *J* = 10.5 Hz, 1H), 5.30 (d, *J* = 16.0 Hz, 1H), 5.62 (d, *J* = 7.1 Hz, 1H), 5.90 (ddt, *J* = 16.0, 10.7, 5.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -4.6, -4.7, 17.9, 26.1, 27.9, 28.1, 38.3, 43.1, 51.4, 65.9. 67.2, 68.7, 80.9, 117.7, 132.7, 155.9, 171.7, 173.5, 201.0. HRMS (ESI) calcd for $C_{23}H_{42}NO_8Si$ $(M+H)^+$ 488.2674, obsd 488.2684.



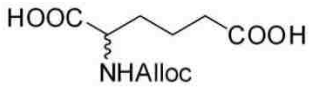
(2*S*,4*R*)-2-(Allyloxycarbonylamino)-4-(*tert*-butyldimethylsilyloxy)-1-(2-oxopropyl)-6- adipic acid (**54**)

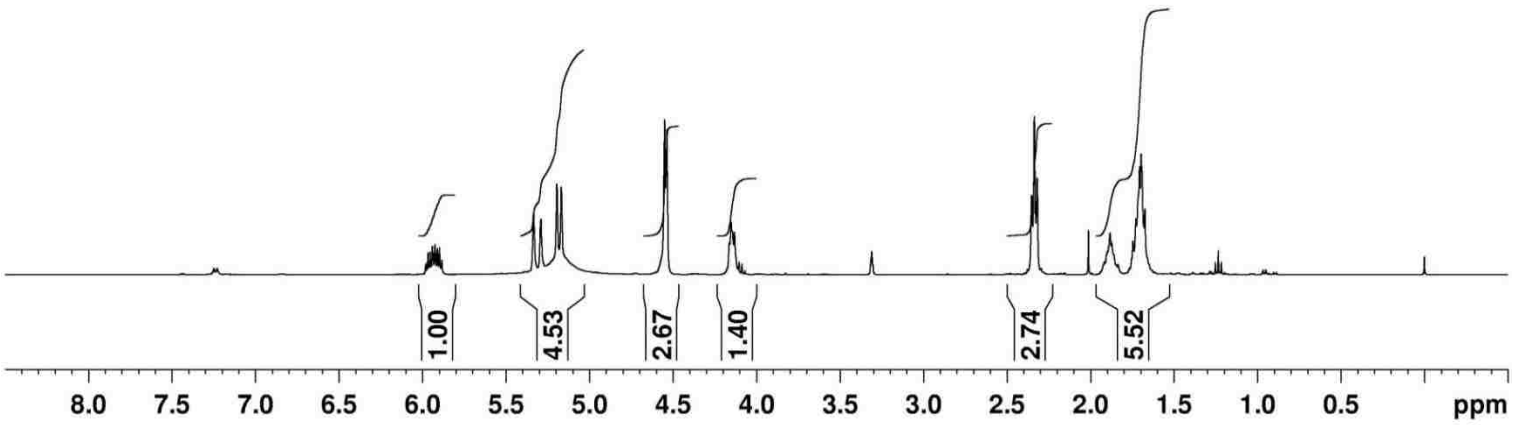
2,6-Lutidine (107 µL, 99 mg, 0.924 mmol, 18.0 equiv.) was added dropwise to a solution of compound **222** (25 mg, 0.051 mmol, 1.0 equiv.) in dry CH_2Cl_2 (2 mL) under N_2 at rt. The mixture was cooled to 0 °C and stirred for 5 min before the dropwise addition of TBSOTf (117 µL, 135 mg, 0.510 mmol, 10.0 equiv.). Reaction mixture was warmed to rt and stirred for 6 h. A solution of potassium carbonate (8 mg, 0.061 mmol, 1.2 equiv.) in 10% H_2O:THF (1 mL) was added and reaction mixture stirred for overnight at rt. The reaction mixture was concentrated to remove the CH_2Cl_2 and then acidified with saturated aqueous $NaHSO_4$ to ~pH 4. Water (2 mL) was added to the reaction mixture. The acidic solution was then extracted with EtOAc (5 X 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and then concentrated to afford a viscous oil. Flash chromatography on silica gel (Hex-EtOAc 1:2) afforded the compound **54** as colorless oil (12 mg, 54%). R_f 0.34 (1:2 Hex-EtOAc). $[\alpha]_D^{25}$ -10.25 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 3H), 0.09 (s, 3H), 0.89 (s, 9H), 1.93-1.99 (m, 1H), 2.17 (s, 3H), 2.30-2.51 (m, 3H), 4.29 (app. pent. *J* = 5.9 Hz, 1H), 4.50 (ddd, *J* = 12.4, 7.7, 4.8 Hz, 1H), 4.57-4.78 (m, 4H), 5.20 (d, *J* = 10.5 Hz, 1H), 5.30 (d, *J* = 16.0 Hz, 1H), 5.62 (d, *J* = 7.1 Hz, 1H), 5.90 (ddt, *J* = 16.0, 10.7, 5.3 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -2.4, 27.5, 33.3, 43.1, 45.4, 49.4, 65.9. 71.2, 73.7, 117.7, 132.7, 155.9, 173.7, 175.1, 201.0. HRMS (ESI) calcd for $C_{19}H_{34}NO_8Si$ $(M+H)^+$ 432.2084, obsd 432.2078.

4.7.3 Spectra

Compound **192** (Scheme 4.8) - ^{1}H NMR spectrum

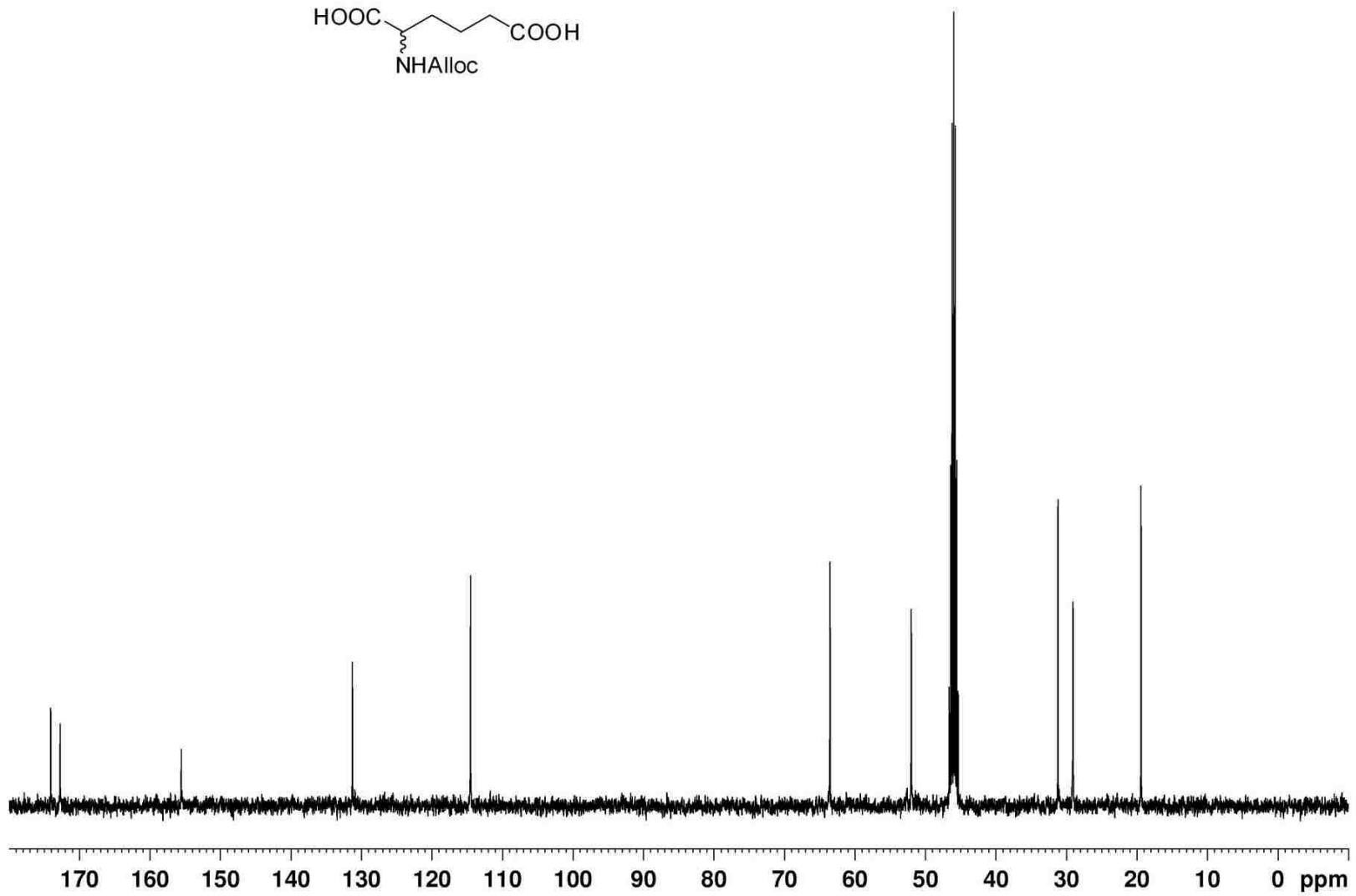
SY–03–25 in MeOD at 400 MHz





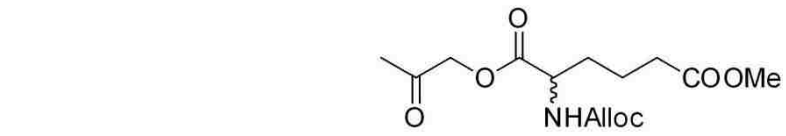
Compound **192** (Scheme 4.8) - ^{13}C NMR spectrum

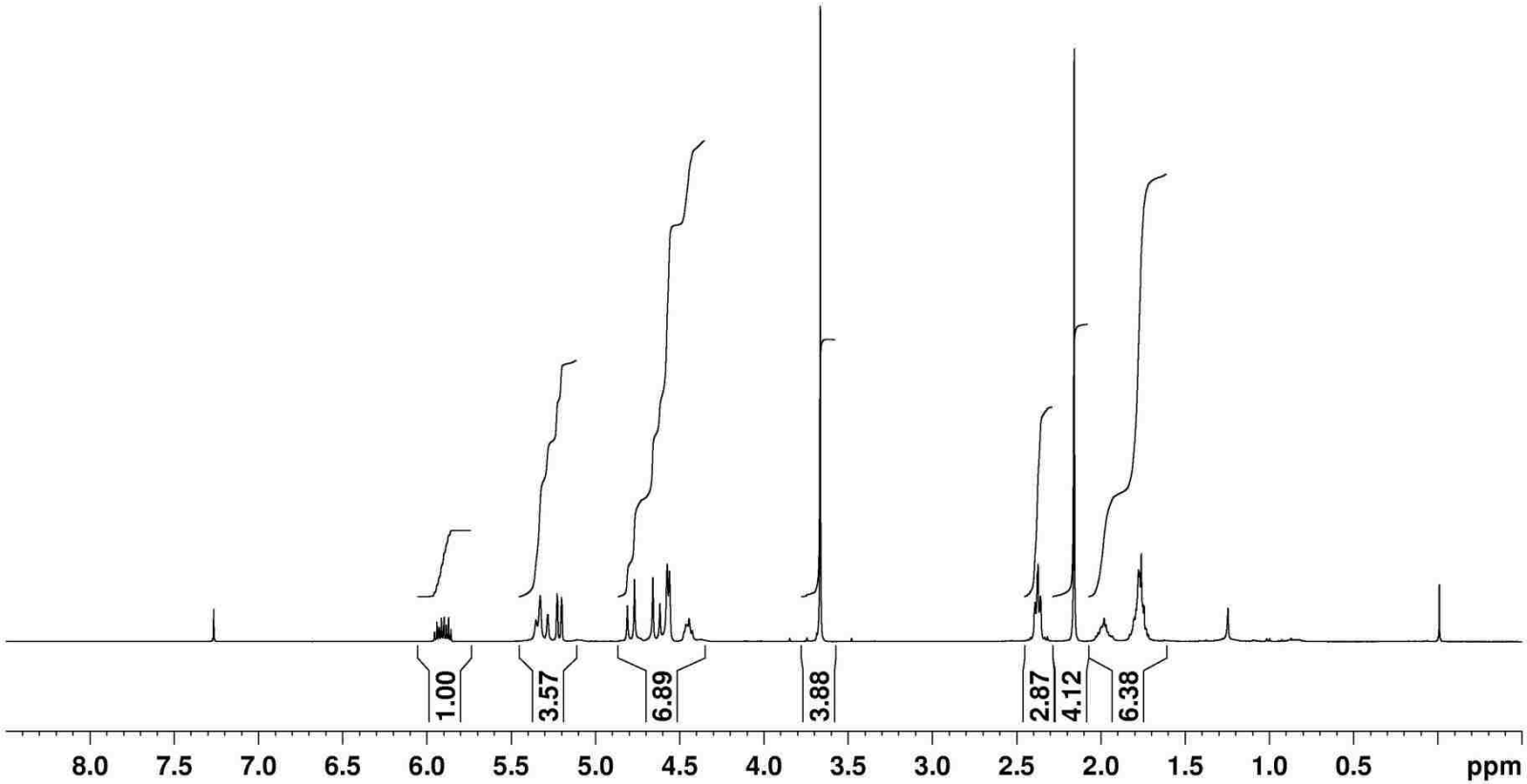
SY–03–25 in MeOD at 100 MHz



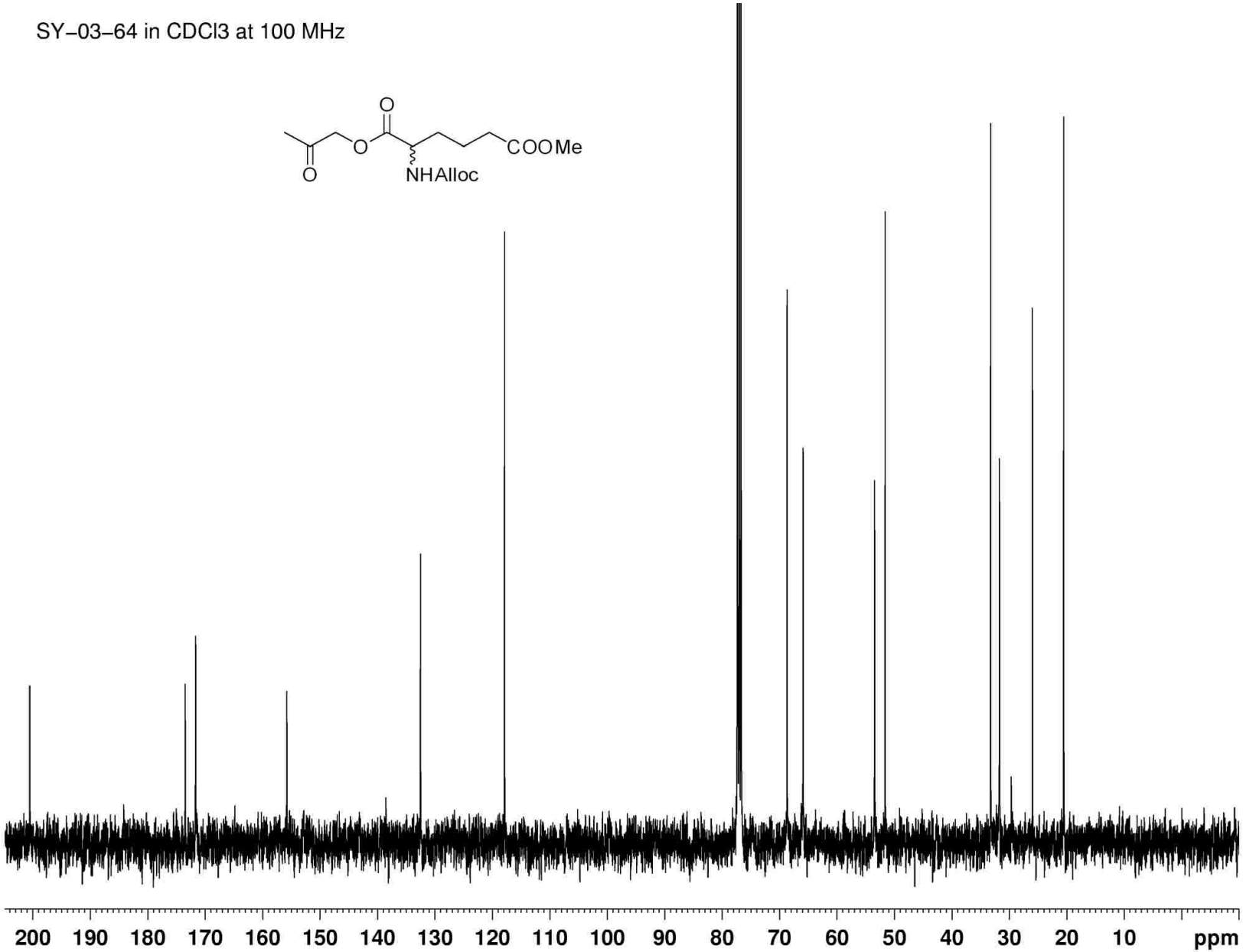
Compound **196** (Scheme 4.11) – ^{1}H NMR spectrum

SY–03–64 in CDCl3 at 400 MHz

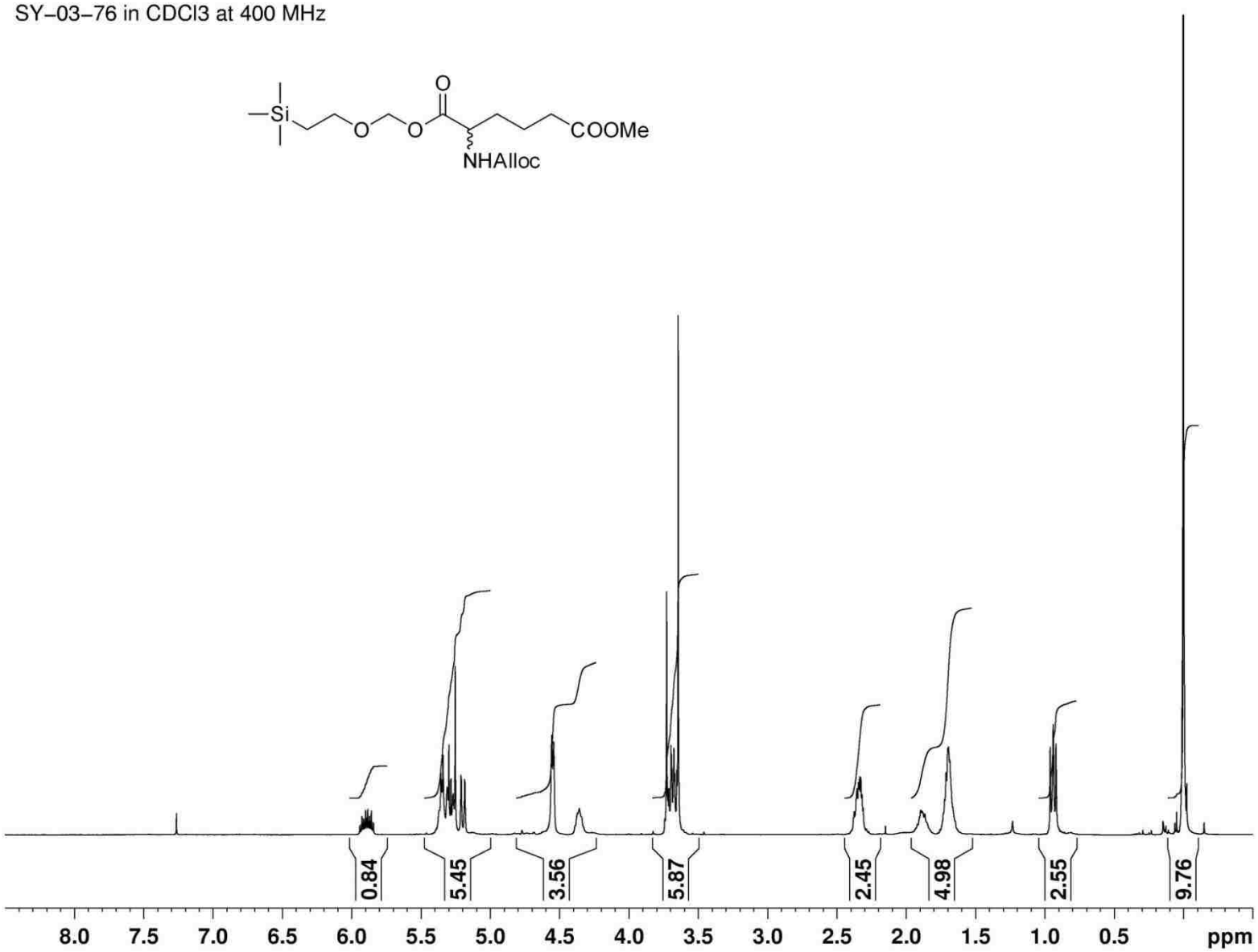




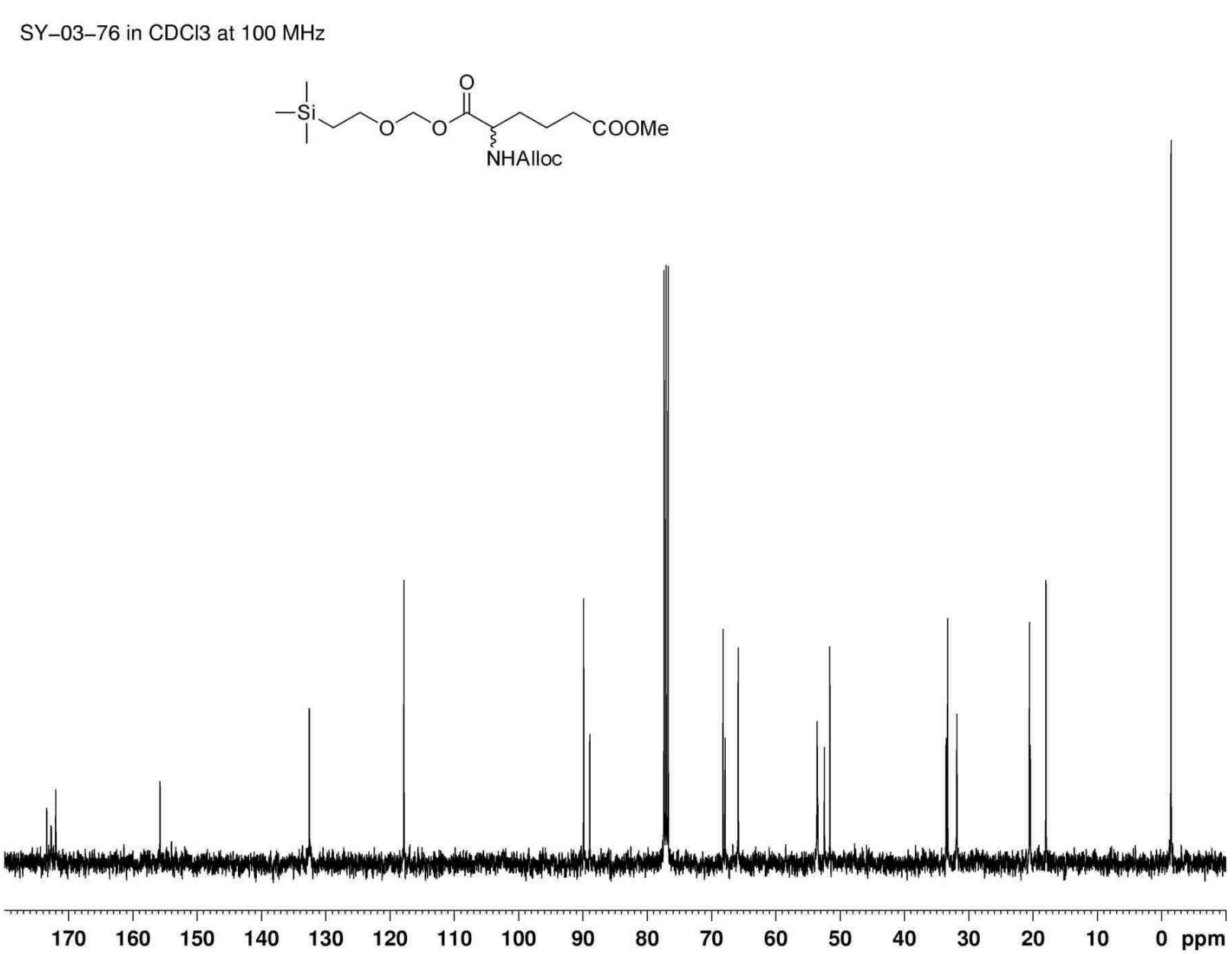
Compound **196** (Scheme 4.11) - ^{13}C NMR spectrum



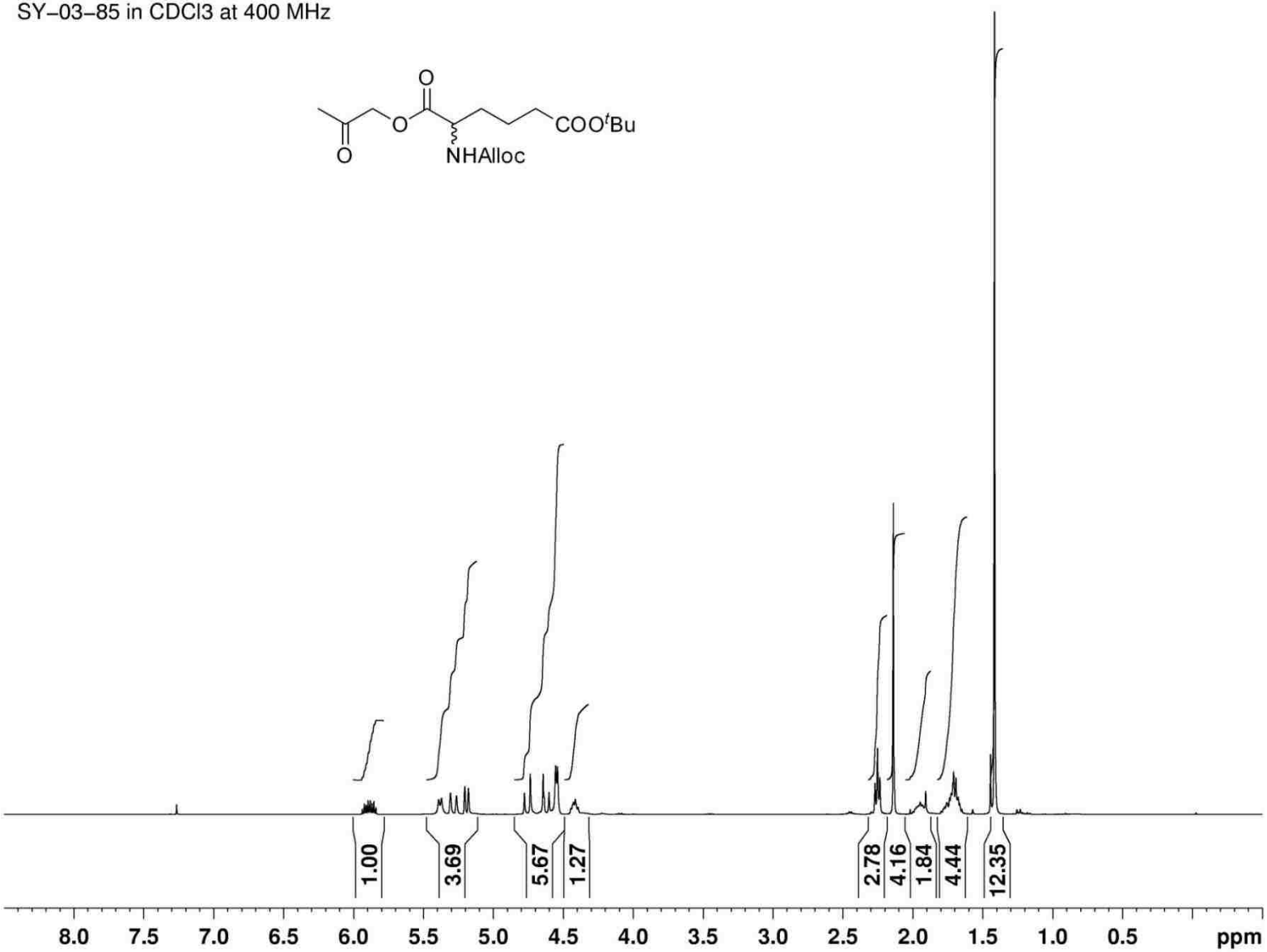
Compound **200** (Scheme 4.12) – ^{1}H NMR spectrum



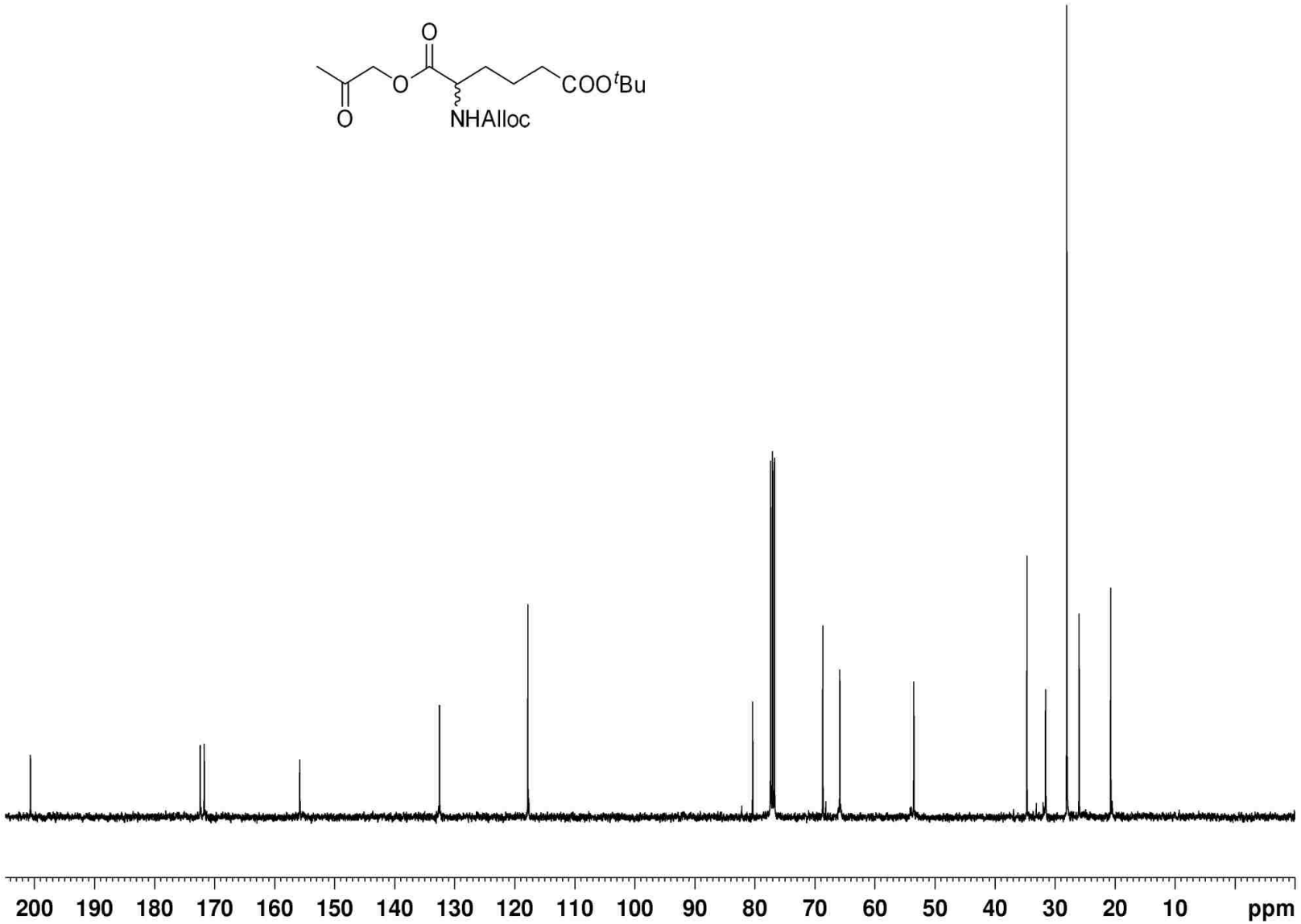
Compound **200** (Scheme 4.12) - ^{13}C NMR spectrum



Compound **207** (Scheme 4.16) – ^{1}H NMR spectrum

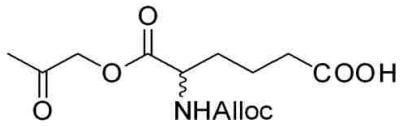


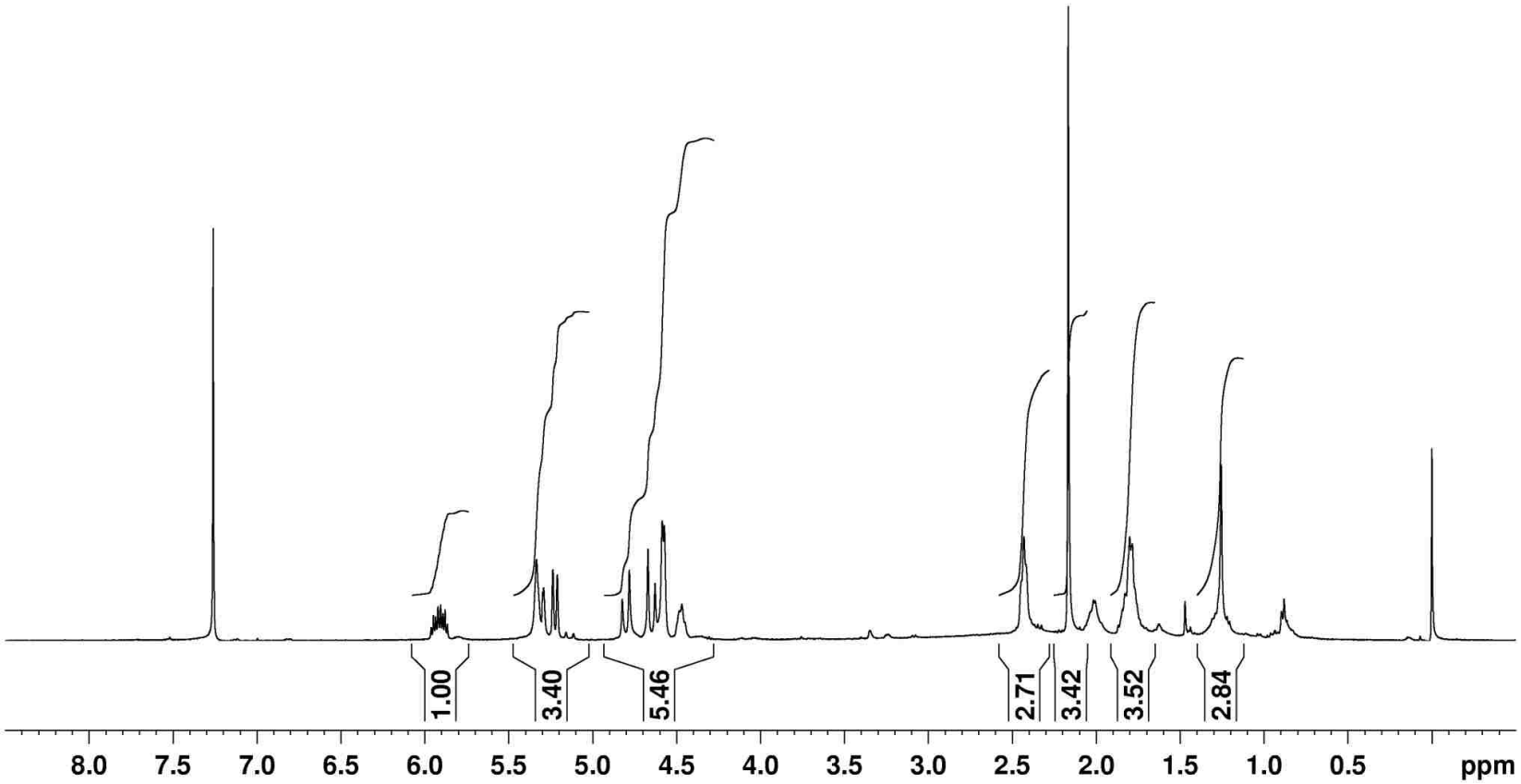
Compound **207** (Scheme 4.16) - ^{13}C NMR spectrum



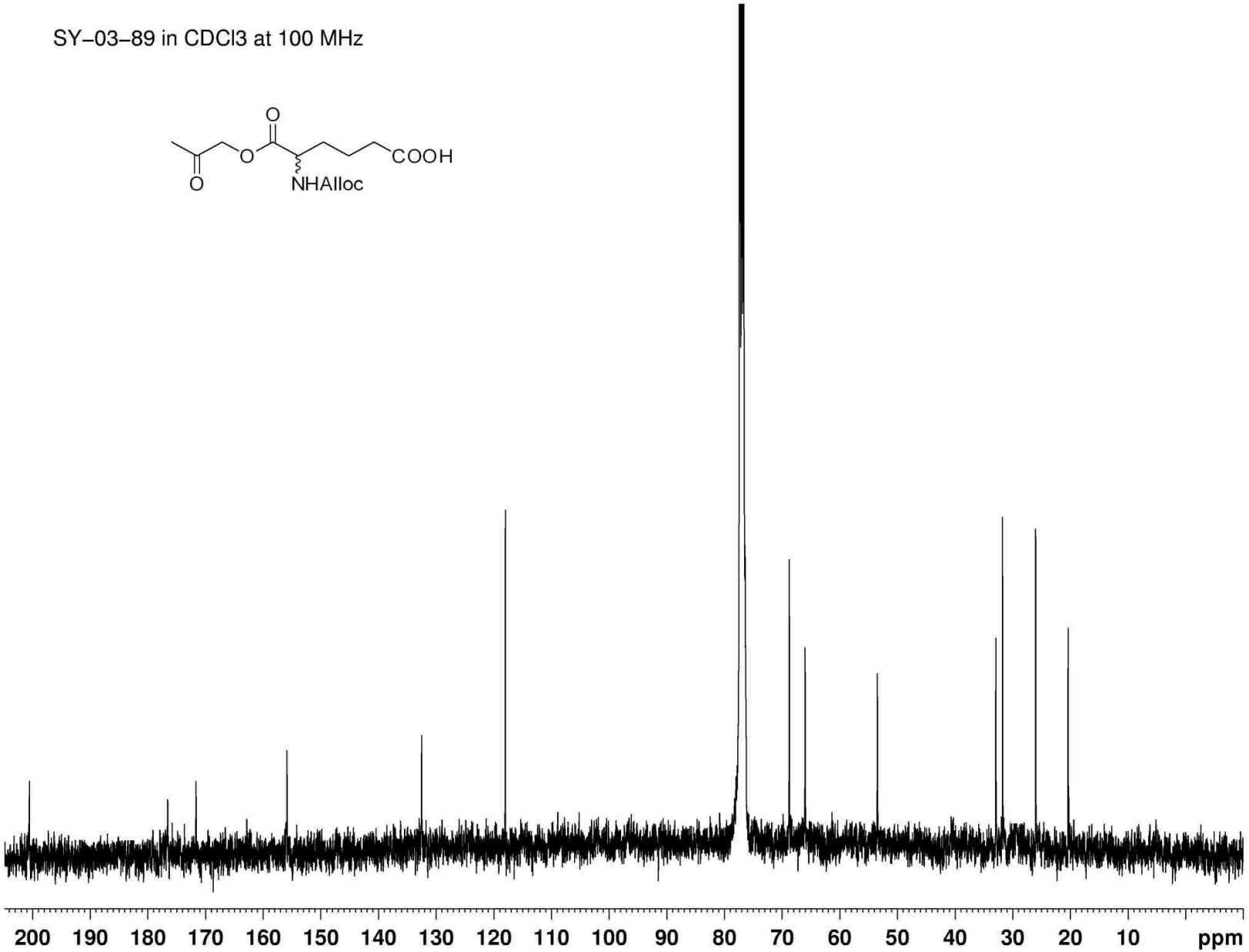
Compound **195** (Scheme 4.17) – ^{1}H NMR spectrum

SY–03–89 in CDCl3 at 400 MHz

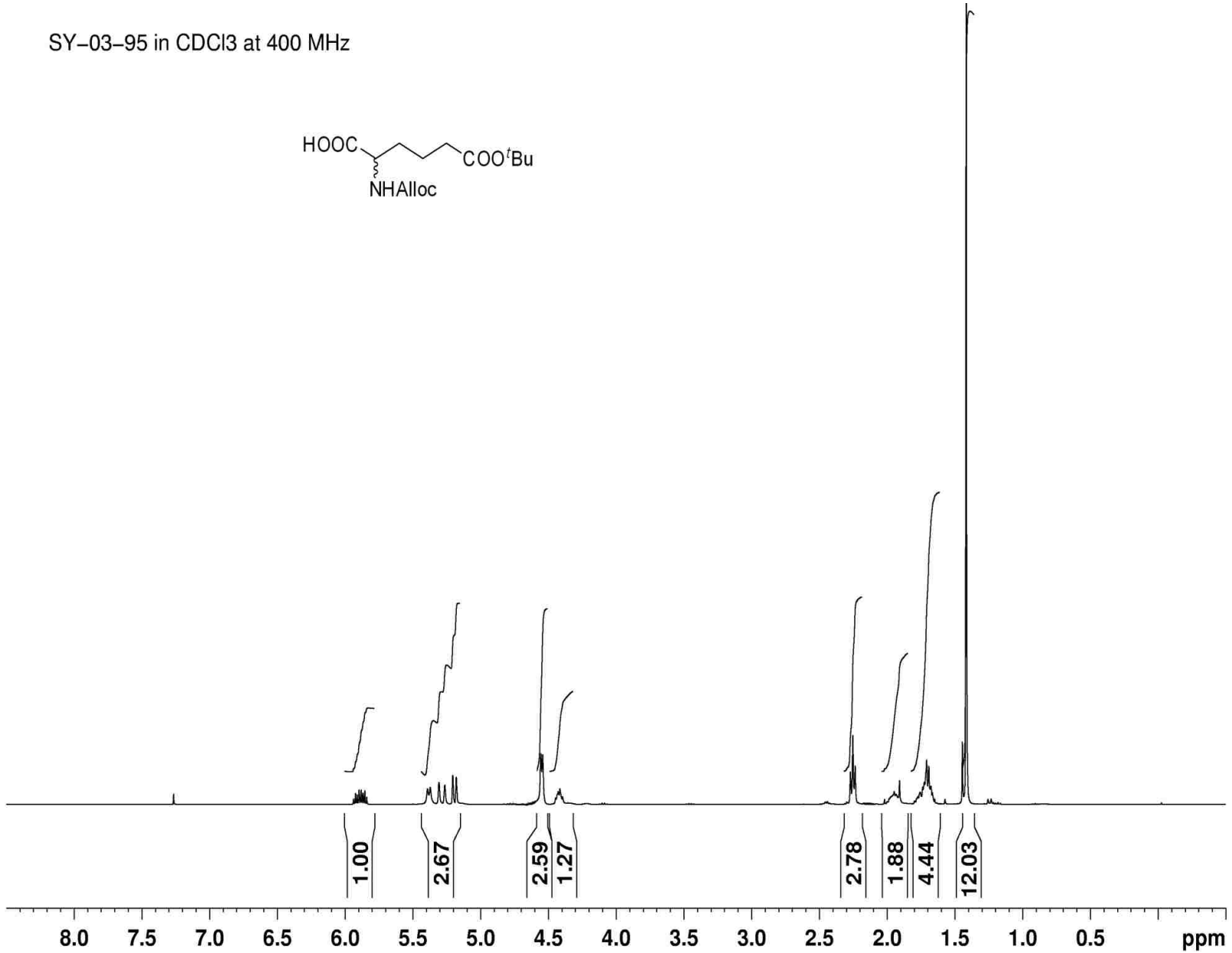




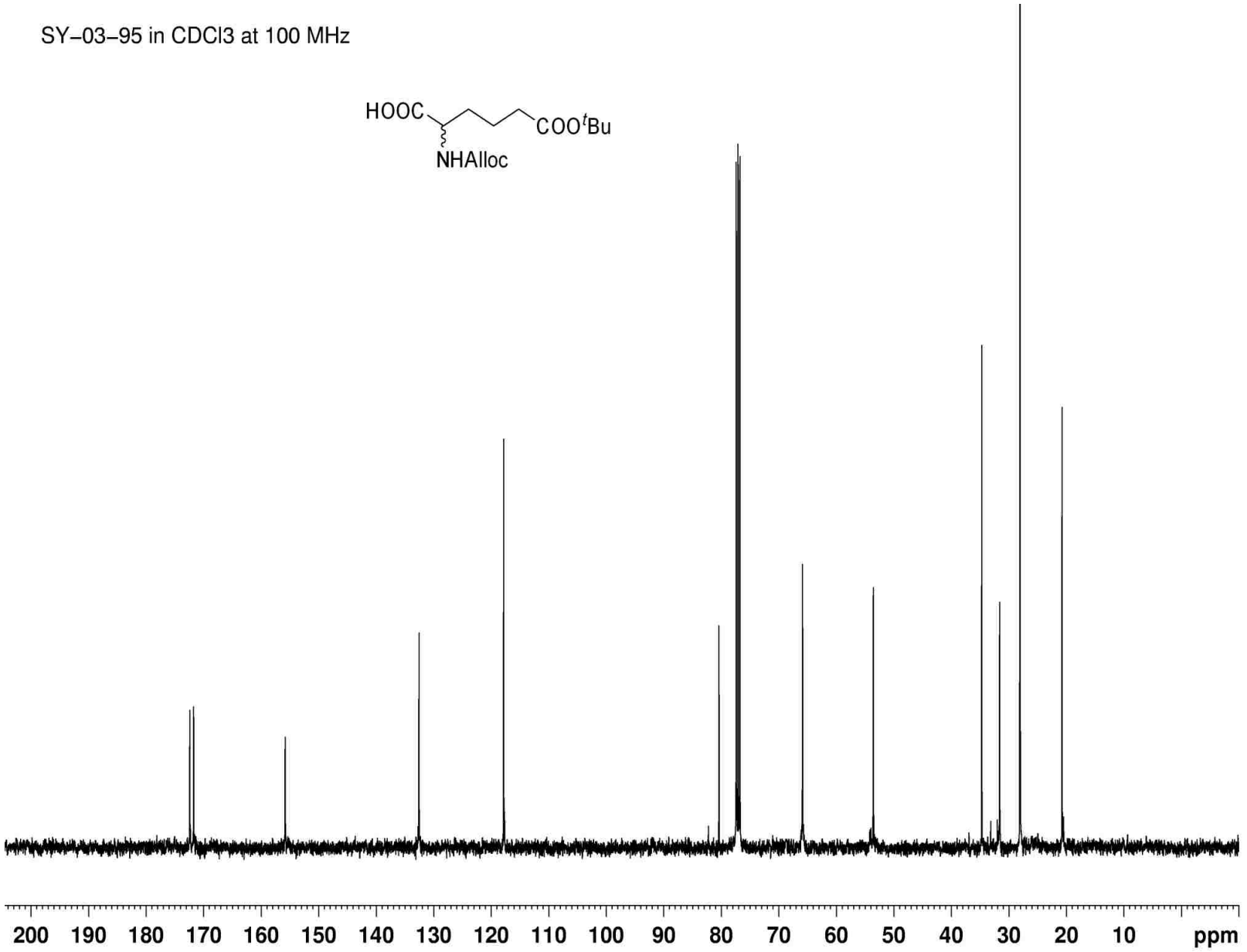
Compound **195** (Scheme 4.17) – ^{13}C NMR spectrum



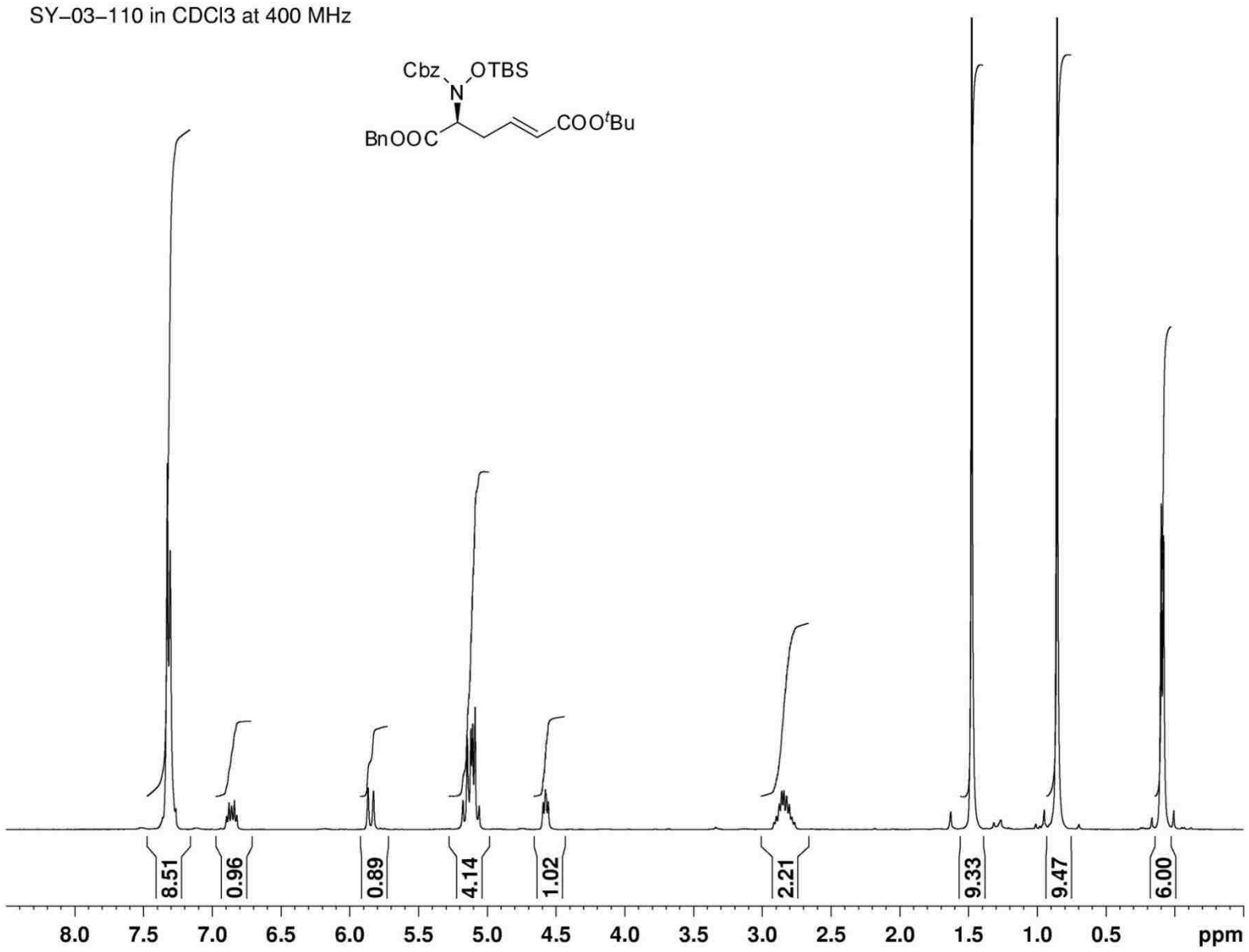
Compound **208** (Scheme 4.17) – ^{1}H NMR spectrum



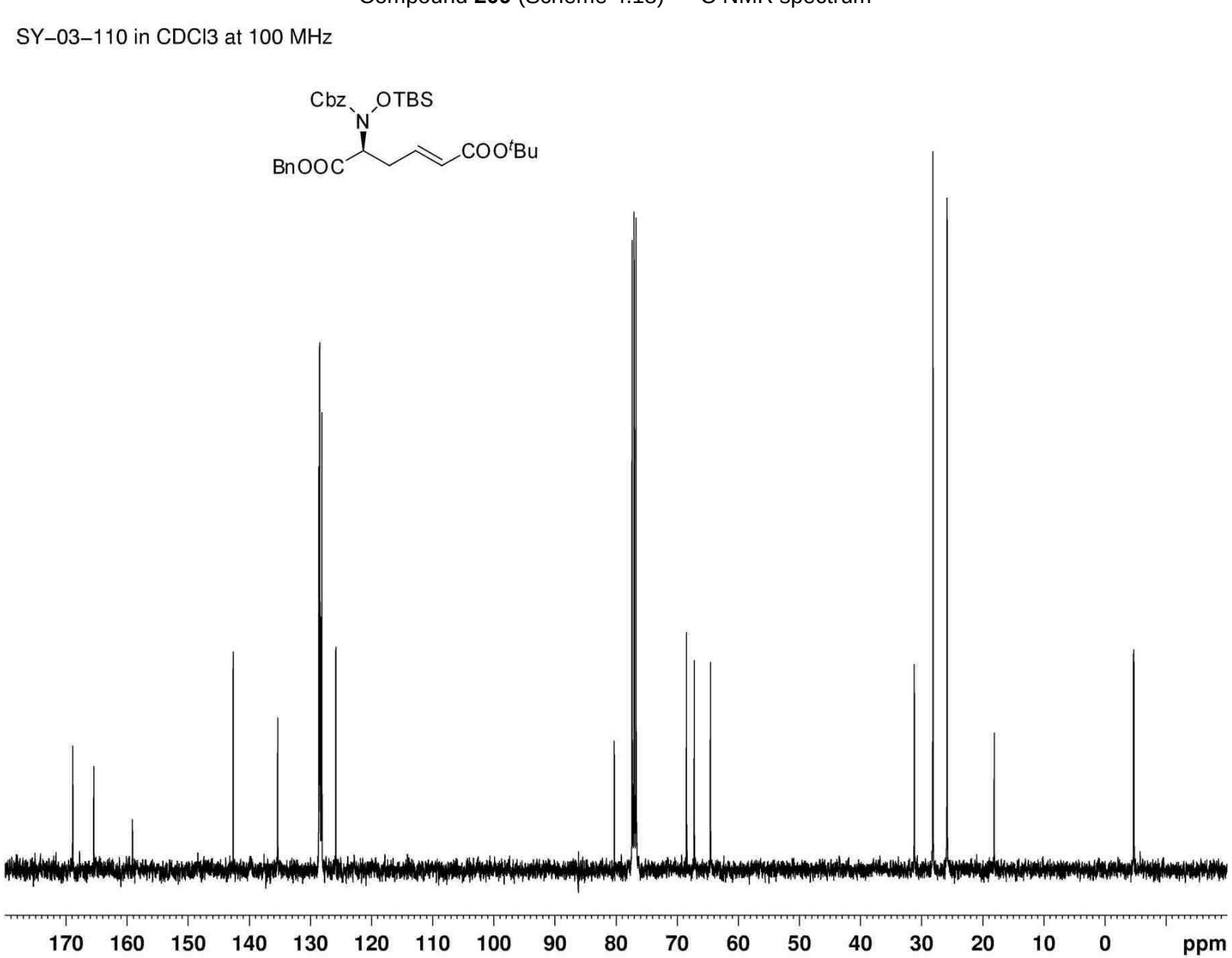
Compound **208** (Scheme 4.17) – ^{13}C NMR spectrum



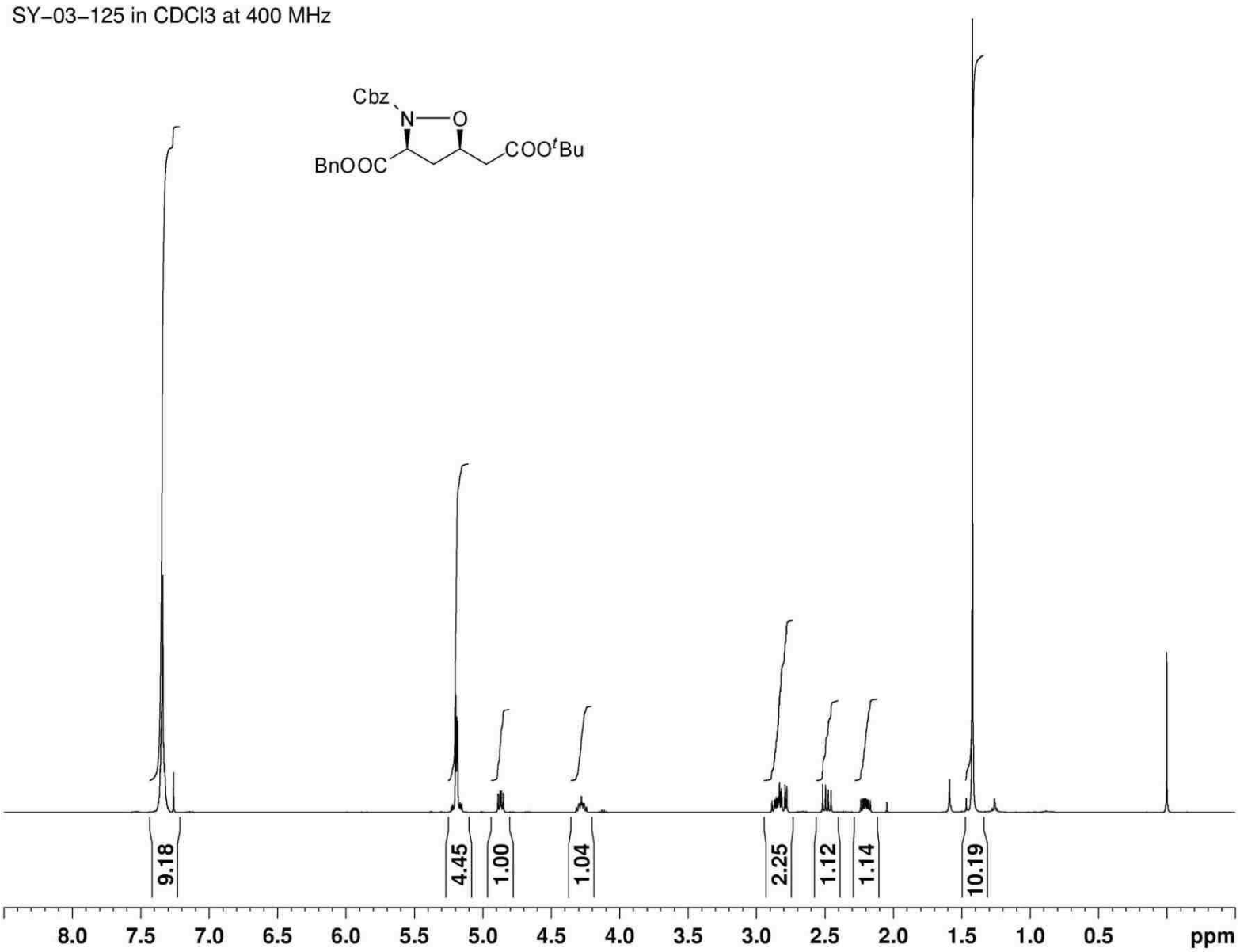
Compound **209** (Scheme 4.18) – ^{1}H NMR spectrum



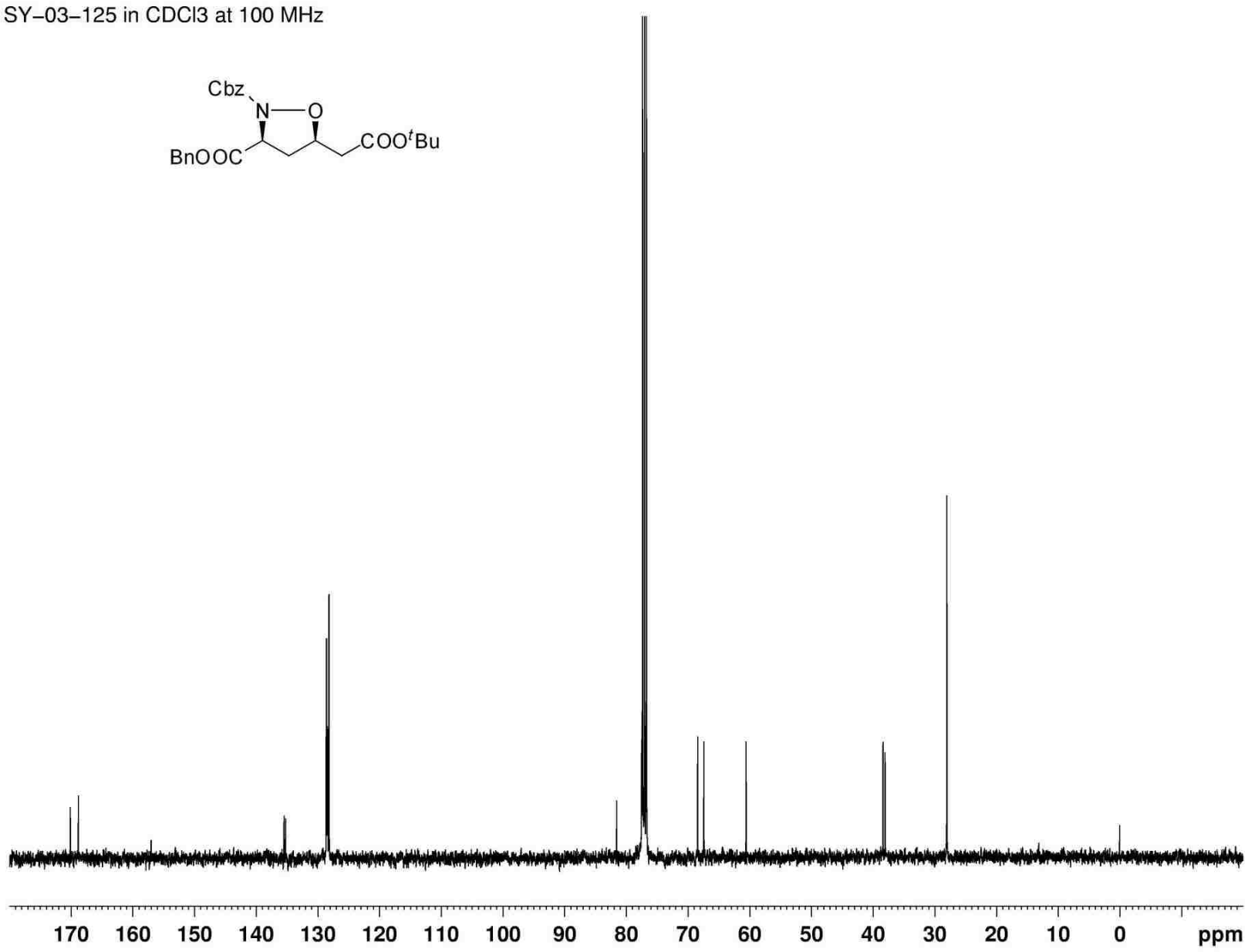
Compound **209** (Scheme 4.18) - ^{13}C NMR spectrum



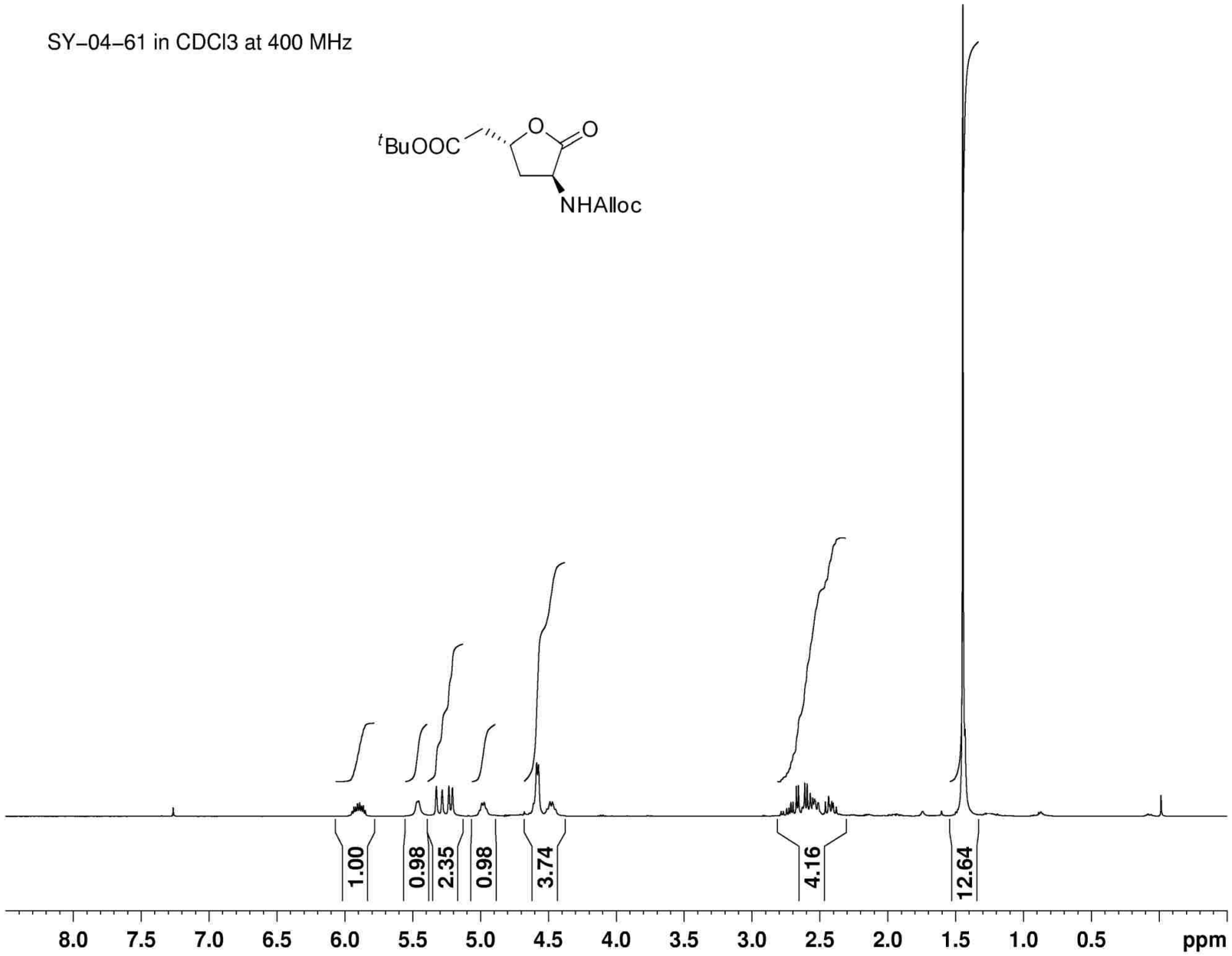
Compound **210** (Scheme 4.18) – ^{1}H NMR spectrum



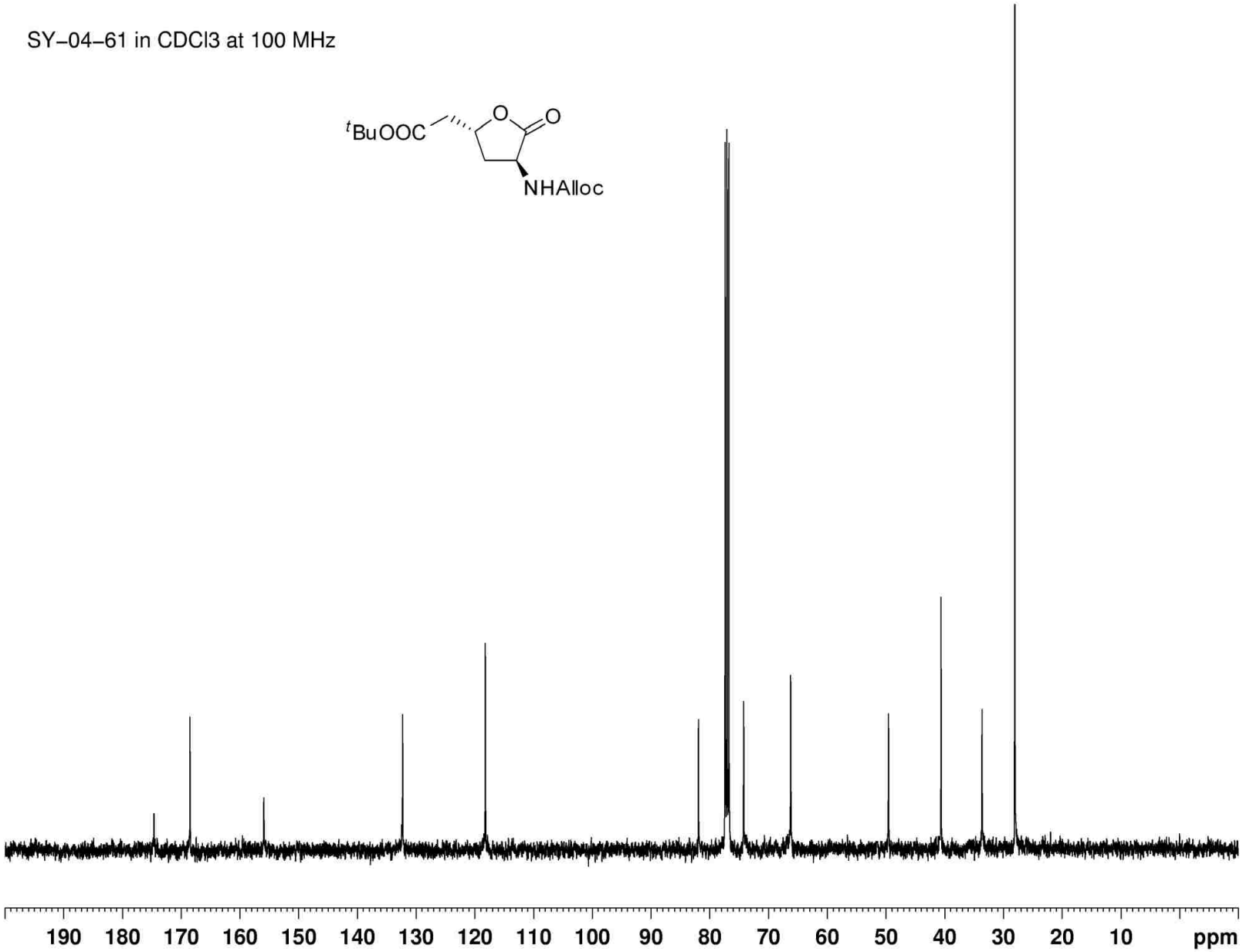
Compound **210** (Scheme 4.18) - ^{13}C NMR spectrum



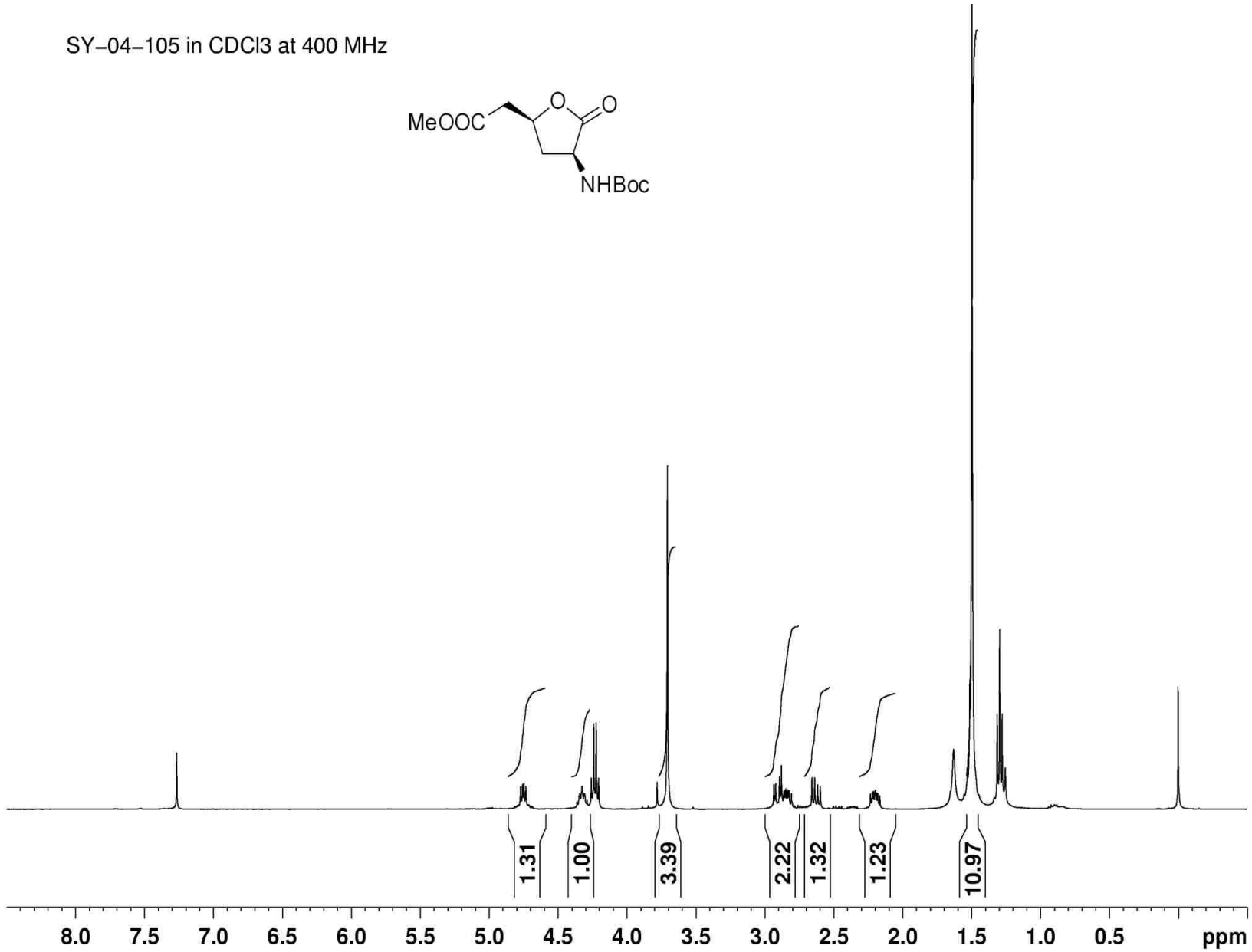
Compound **216** (Scheme 4.20) – ^{1}H NMR spectrum



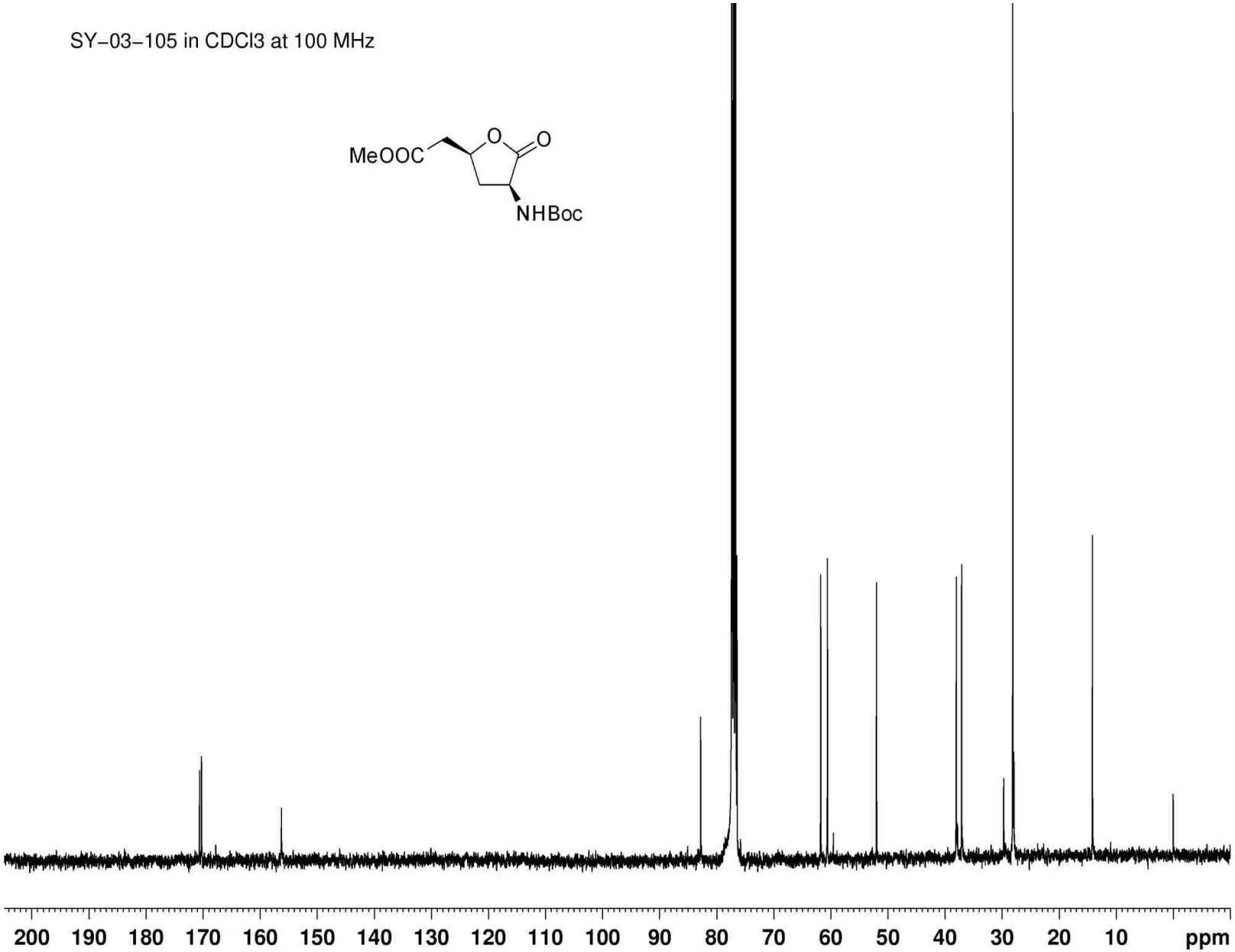
Compound **216** (Scheme 4.20) – ^{13}C NMR spectrum



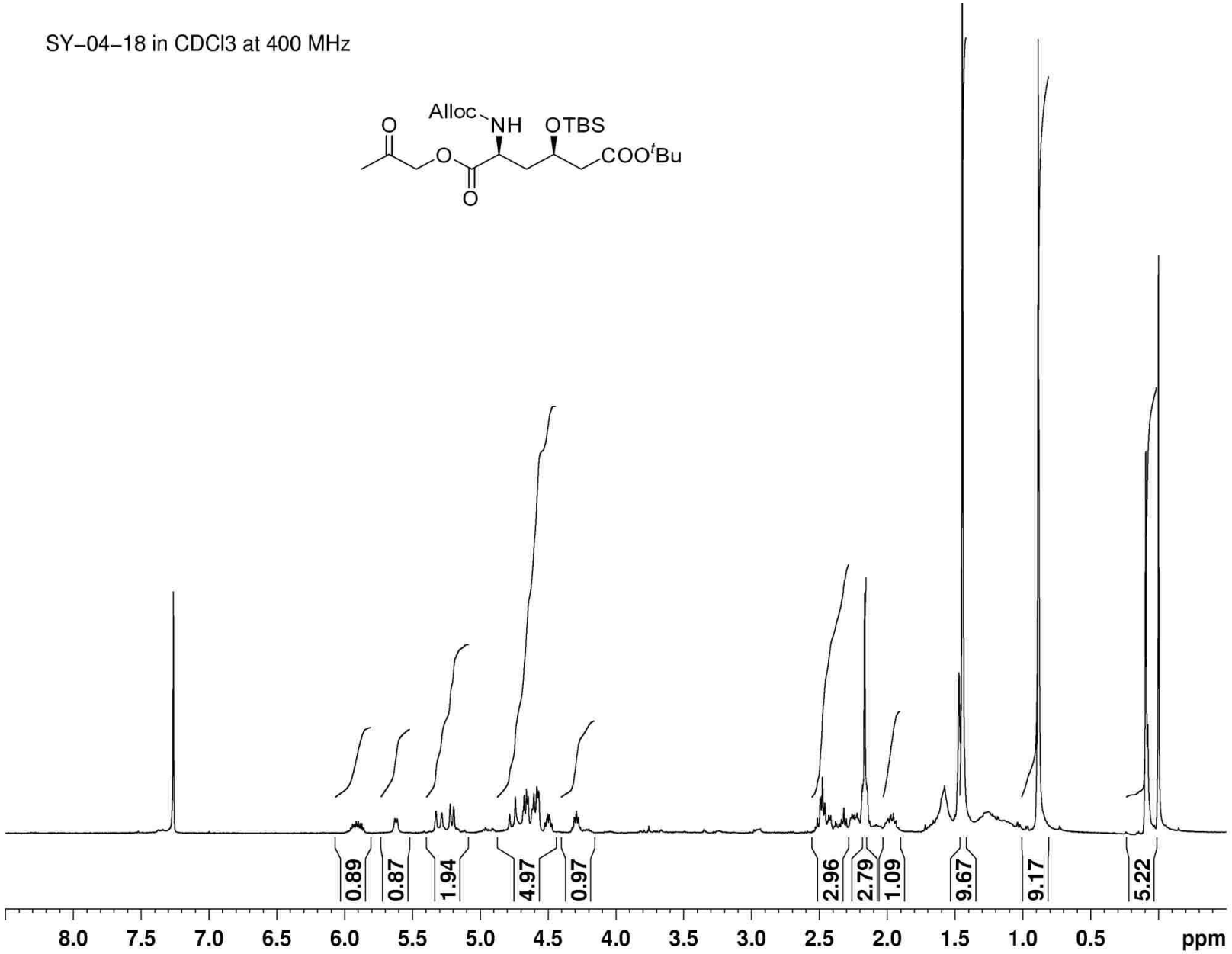
Compound **58a** (Scheme 4.22) – ^{1}H NMR spectrum



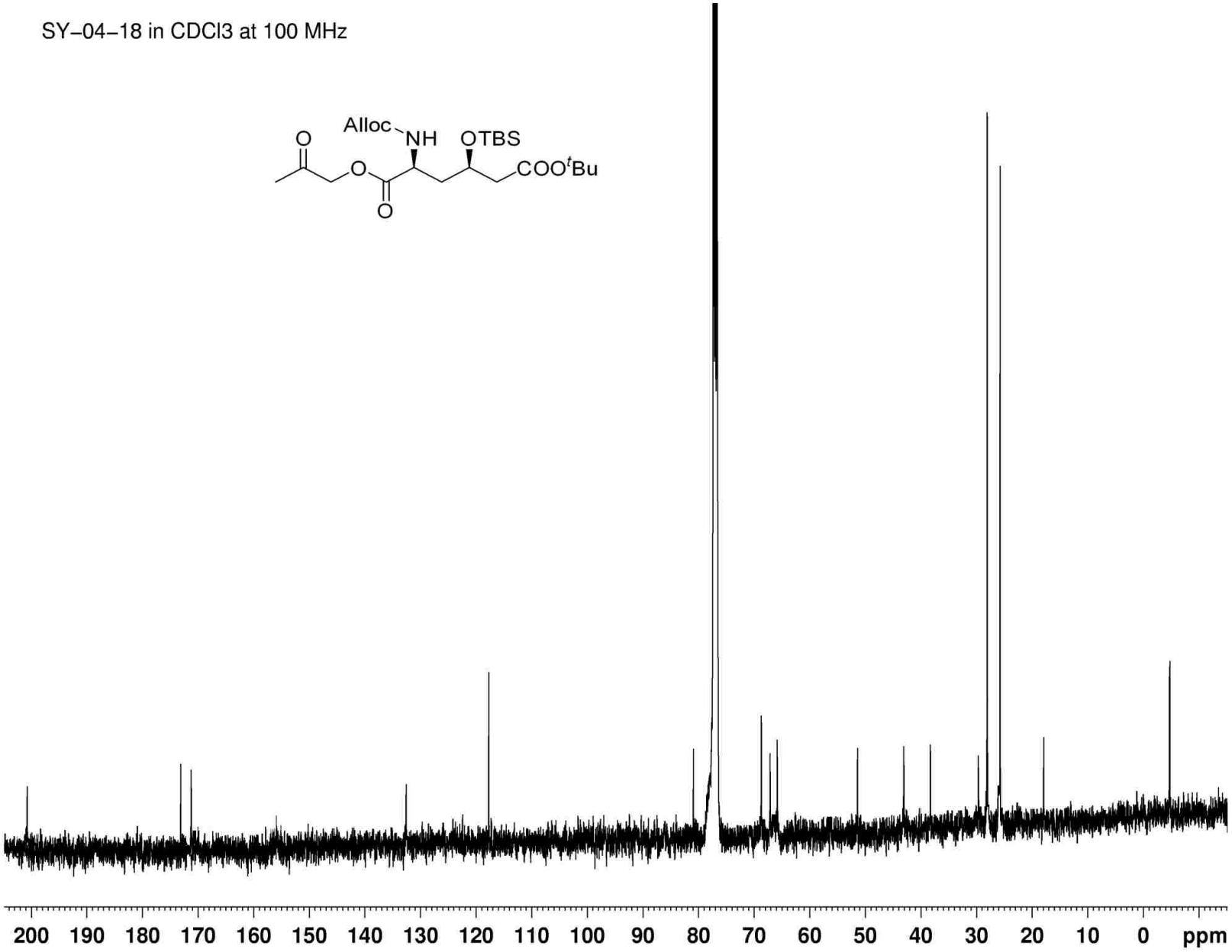
Compound **58a** (Scheme 4.22) – ^{13}C NMR spectrum



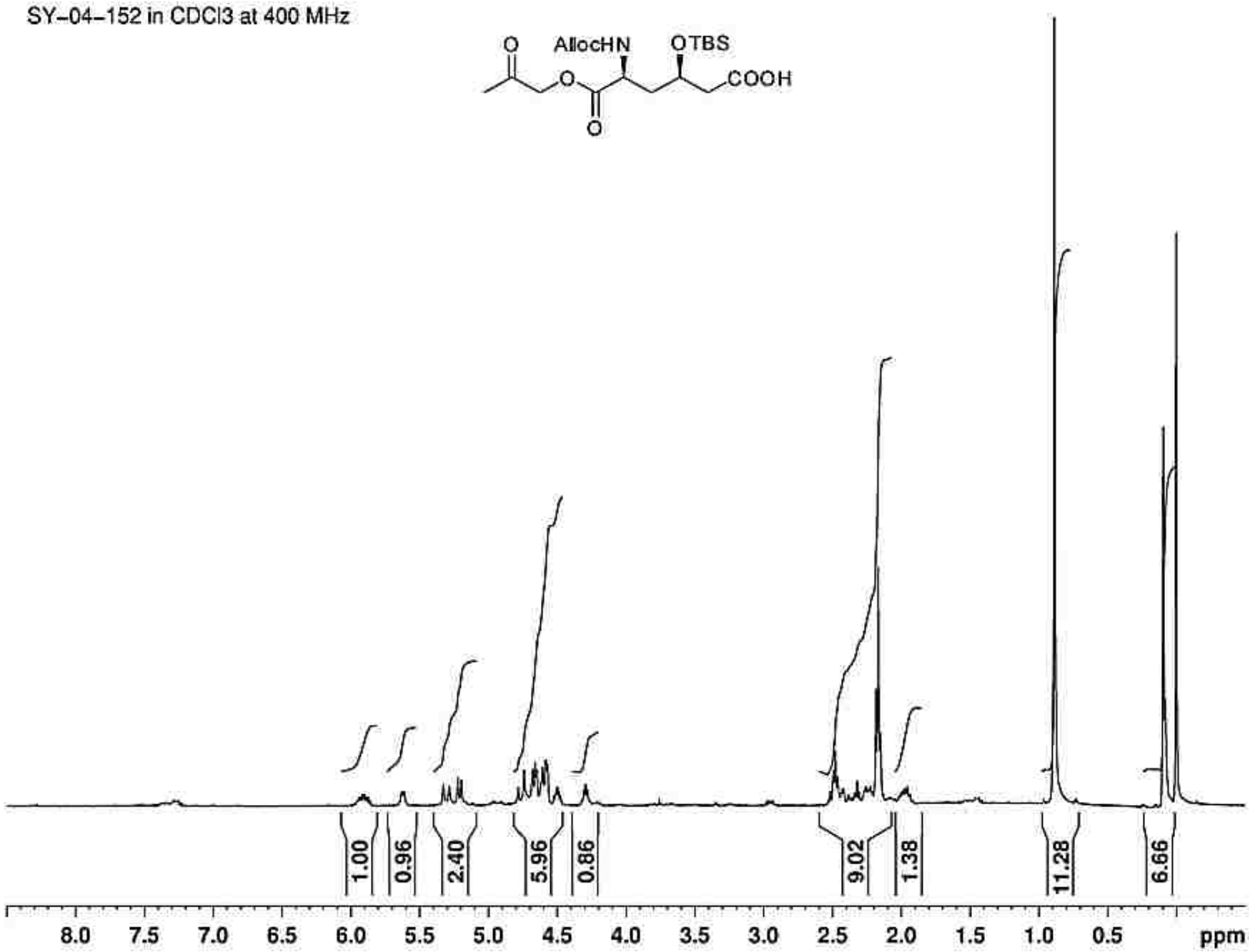
Compound **222** (Scheme 4.24) – ^{1}H NMR spectrum



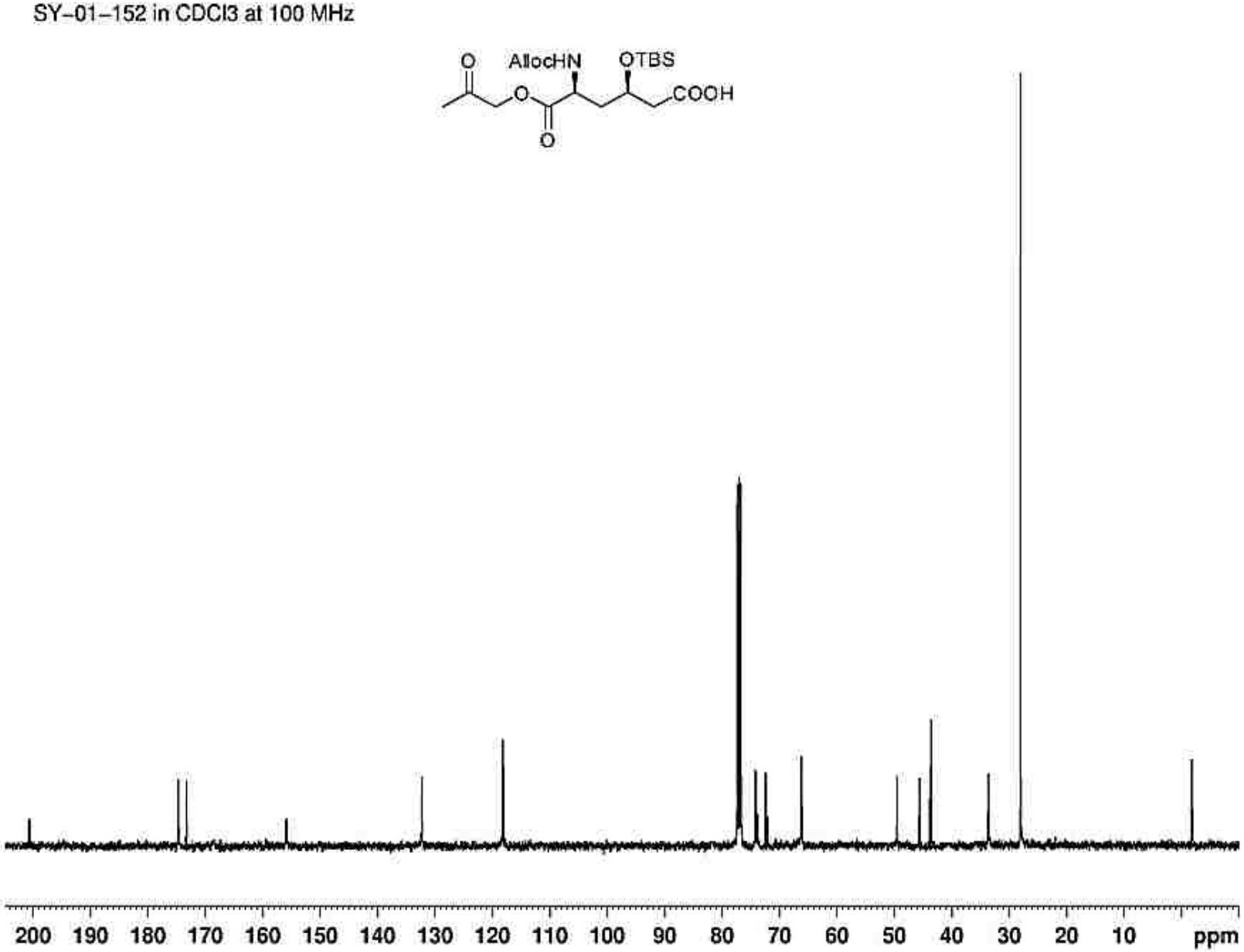
Compound **222** (Scheme 4.24) – ^{13}C NMR spectrum



Compound **54** (Scheme 4.24) – ^{1}H NMR spectrum



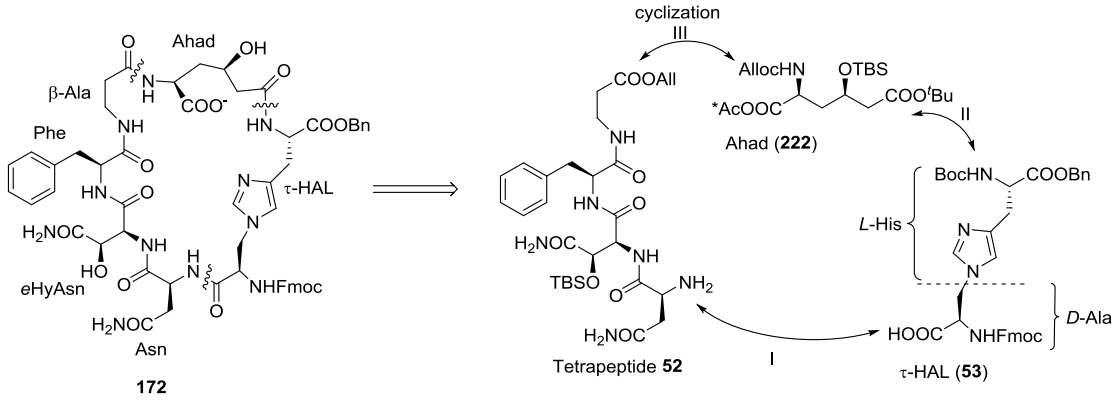
Compound **54** (Scheme 4.24) – ^{13}C NMR spectrum



CHAPTER 5: PEPTIDE FRAGMENT ASSEMBLY AND CYCLIZATION OF THE WESTERN HEMISPHER OF THEONELLAMIDE C

5.1 Retrosynthesis for the Western Hemisphere of Theonellamide C

Revisiting our retrosynthetic analysis from Chapter 1, recall that our approach to the western hemisphere of theonellamide C involves major disconnections between τ-HAL/Asn, Ahad/β-Ala and τ-HAL/Ahad residues (Scheme 5.1). This leads to three building blocks: tetrapeptide **52**, τ-HAL **53** and Ahad **222**. In a forward sense, the initial peptide bond to be formed will be between the *D*-Ala acid component of τ-HAL and the amino-terminus of the tetrapeptide (I), followed by a condensation of the *L*-His amine component of τ-HAL with δ-acid of Ahad (II). Deallylation of the two termini will be accomplished simultaneously prior to cyclization of the amino-terminus of Ahad **222** with the carboxyl-terminus of tetrapeptide **52** (III). The site chosen for cyclization is ideal, since the β-Ala residue of the tetrapeptide is not susceptible to racemization during peptide bond formation.



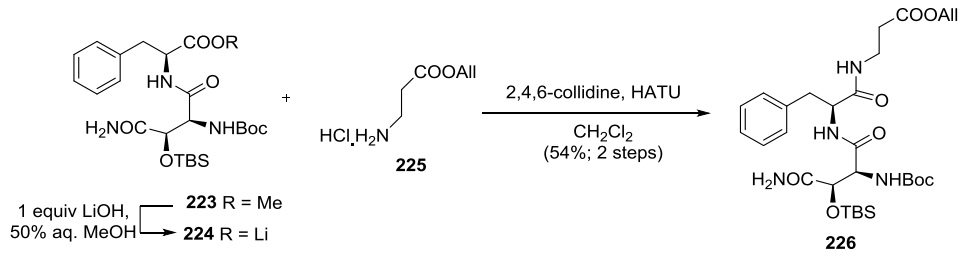
Scheme 5.1 Western hemisphere of theonellamide C: Retrosynthesis.

5.2 Formation of a Dipeptide Containing *erythro*-HydroxyAsparagine (*e*HyAsn)

The synthesis of tetrapeptide **52** required the preparation of an *erythro*-hydroxyasparagine-phenylalanine (*e*HyAsn-Phe) dipeptide. Douglas Wong had made Boc-

*e*HyAsn-Phe-OMe (**223**).[93] His approach built directly on Boger's synthesis of the *t*HyAsn diastereomer.[94,95]

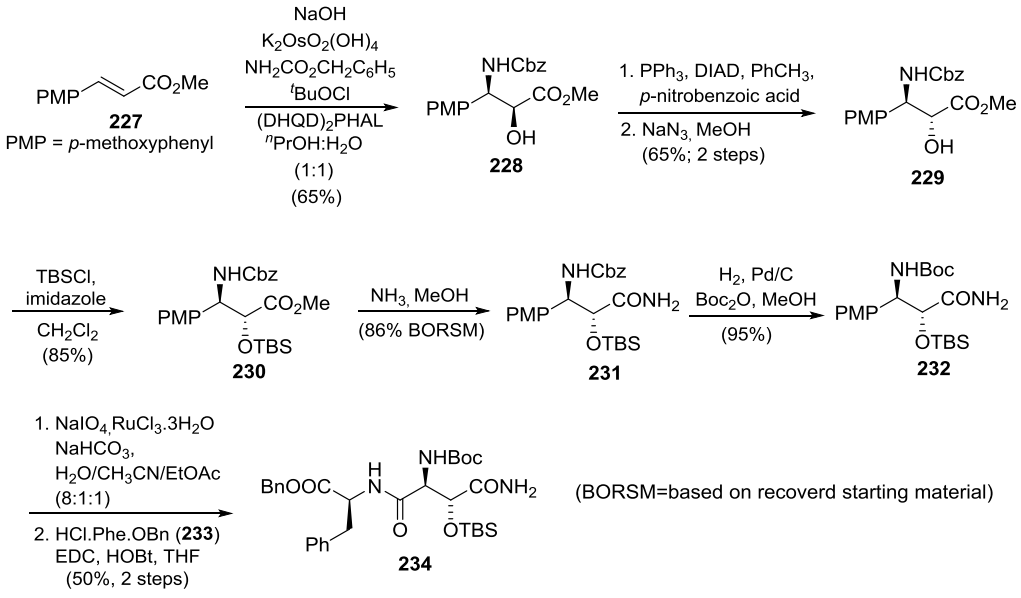
With dipeptide **223** in-hand, we sought to assemble the western fragment of theonellamide C. The methyl ester of dipeptide **223** was hydrolyzed with lithium hydroxide (Scheme 5.2). Unfortunately, the TBS group was lost on acidification, during work-up. We therefore hydrolyzed the ester with a single equivalent of LiOH and freezedried the mixture to give the lithium salt **224**. The hydrochloride salt of β-alanine allyl ester (**225**) was prepared according to an analogous preparation of the corresponding benzyl ester.[96] The lithium salt **224** was coupled with **225**. Poor yields led us to an alternative *C*-terminal protecting group that could be removed without byproducts or salt formation.



Scheme 5.2 Formation of dipeptide.

We needed more material to form the new dipeptide **234** containing a benzyl ester at the carboxyl end (Scheme 5.3). Therefore we repeated Wong's synthesis of the dipeptide, with some modifications, to obtain useful quantities. Sharpless aminohydroxylation of the double bond in methyl *p*-methoxycinnamate (**227**) proceeded as described previously to give **228** except that **228** did not precipitate from the reaction mixture as described. Flash chromatography was required for isolation. We then inverted the configuration at the stereogenic center bearing the OH substituent to give **229** by performing a Mitsunobu reaction with *p*-nitrobenzoate as the nucleophile. Wong had reported tetrahydrofuran (THF) as the solvent for this reaction and subjected the crude product mixture to the cleavage of the ester. In

the current work no desired product was isolated following this protocol. We then performed the Mitsunobu reaction with toluene as solvent. The reaction was monitored with TLC which showed appearance of a less polar product. The *p*-nitrobenzoate ester was cleaved by azidolysis to obtain a 65% yield of inverted alcohol **229**. The secondary alcohol was protected as its TBS ether using TBSCl and the methyl ester was ammonolyzed to generate the side chain amide, *viz*. compound **231**. We swapped the Cbz group for the *tert*-butyl carbamate to give **232**. The oxidative cleavage of the electron-rich aromatic ring was performed to obtain the carboxylic acid that was coupled with phenylalanine benzyl ester hydrochloride (**233**) to give dipeptide **234** (Scheme 5.3). Detailed discussion of these reactions is given in the dissertation of Douglas Wong (Chapter 2).[12]



Scheme 5.3 Synthesis of a dipeptide containing *erythro*-hydroxyasparagine.

While performing the coupling reaction between acid **232** and amine **233** we were cognizant of side reactions with the unprotected side chain of asparagine.

5.3 Side Reactions of the Unprotected Side Chain Amide of Asparagine

Asparagine (abbreviated as Asn or N, **237** and **238**) is a non-essential amino acid which is coded for by AAU and AAC in mRNA. There is one carboxamide group on the side chain with one amino and one carboxyl group on the alpha carbon atom. Hence it can be considered an amide of aspartic acid (**235** and **236**). Asparagine is a neutral, polar, uncharged amino acid under any biologically relevant pH conditions.

The amide group of asparagine can be slowly hydrolyzed to the carboxyl group to form aspartic acid. This conversion is related to the molecular basis of aging.

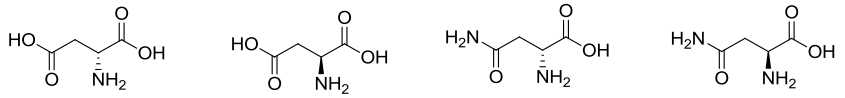
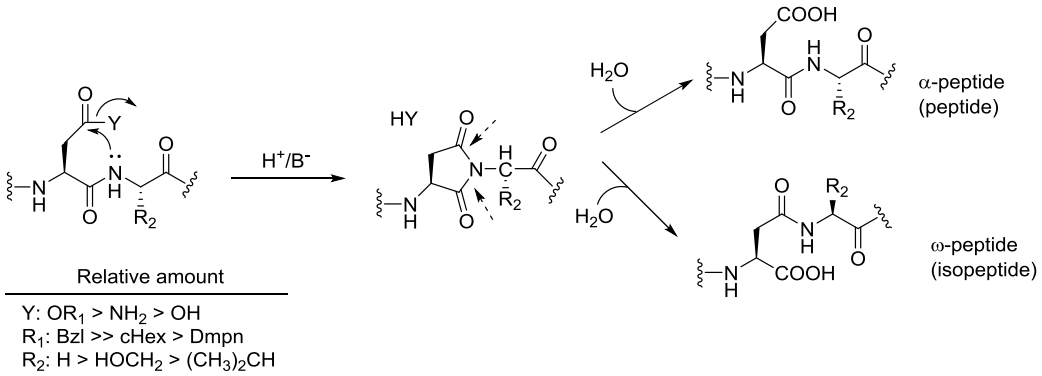


Figure 5.1 Chemical structures of *D*- and *L*-aspartic acid and *D*- and *L*-asparagine.

The carboxamido groups of asparagine should be suitably blocked during activation of the residues to prevent cyclization via the following two side reactions.

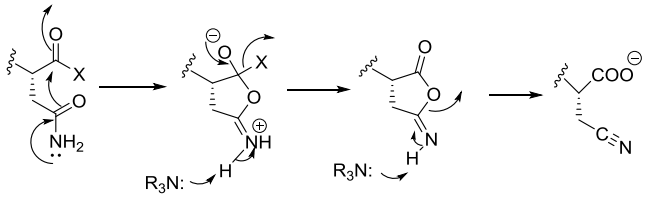
1. Aspartimide Formation: This is a general problem for peptides incorporating asparagine and the likelihood depends on the sequence and situation. Treatment of aspartyl/glutamyl peptides with acid or base can lead to a rearrangement through a cyclic imide intermediate and produces a mixture of two peptides (Scheme 5.4).[97] The aspartimide is more stable than the glutamimide, and more ω-peptide (isopeptide) is formed than α-peptide. The attack of the amide nitrogen at the side chain acyl carbon is influenced by the nature of Y and the nature of the residue located at *C*-terminal to the susceptible residue. The reaction occurs easily when the Y is a good leaving group and less readily when unsubstituted (Y = OH). The problem is solved by ester groups that are severely hindered. Bodanszky *et al*. showed that the amount of imide formed decreases in the order OBzl>>OcHex>ODpn.[98] [Dmpn = 2,4-dimethylpent-3-yl]



Scheme 5.4 Imide formation from a peptide sequence followed by generation of two peptide chains.

One way of reducing this side reaction in the case of Asn is substitution on the carboxamido group which increases the solubility of derivatives in organic solvents and eliminates the side reactions. The carboxamide group does not undergo acylation or alkylation during chain assembly.

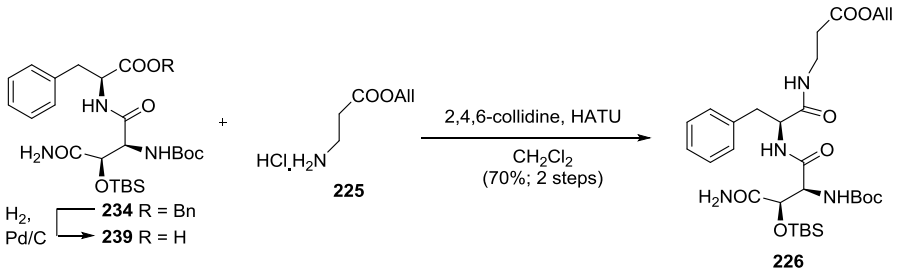
2. Isoaspartimide formation upon activation of α-COOH of Asn. Upon activation of the Cα carboxylic acid, the unprotected side chain of asparagine can undergo isoaspartimide formation, followed by dehydration to give the β-cyanoalanine derivative (Scheme 5.5). This occurs prominently when coupling with phosphonium salts. The cyano group does not interfere with couplings, so the dehydrated residue is incorporated into the peptide. The dehydration is completely reversed by HF, partly reversed by TFA and very effectively suppressed for carbodiimide- or HBTU-mediated reactions by the presence of one equivalent of 1-hydroxybenzotriazole (HOBt). The role of this additive is to serve as a superior proton donor, relative to the NH of the isoaspartimide. Thus the formation of the BtO^- anion occurs in preference to dehydration to generate a nitrile.



Scheme 5.5 Mechanism of β-cyanoalanine formation.

5.4 Formation of Tetrapeptide

The benzyl ester of dipeptide **234** was subjected to hydrogenolysis to give acid **239** that was coupled to **225**. The tripeptide was formed under conditions proven to minimize epimerization of Cα of the Phe residue.[99] This approach now gave a satisfactory yield of tripeptide **226** (Scheme 5.6).



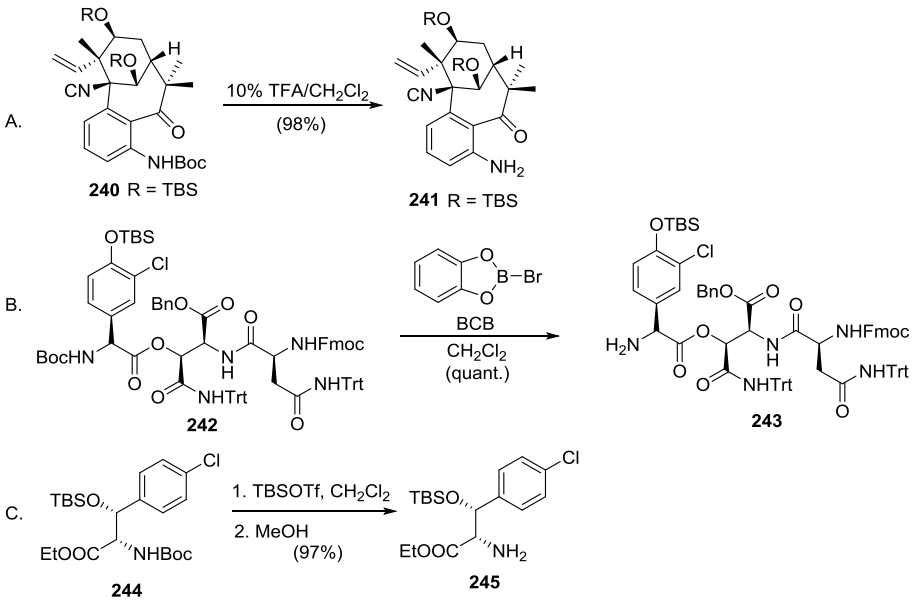
Scheme 5.6 Formation of tripeptide.

5.4.1 Deprotection of α-Amine

Before elongating **226** in the *N*-terminal direction with a suitably protected asparagine residue, it was necessary to remove the *tert*-butyl carbamate in the presence of the *tert*-butyldimethylsilyl ether (OTBS). This turned out to be challenging and several known methods were investigated.

Greshock and Funk used 10% trifluoroacetic acid (TFA) in dichloromethane to remove the Boc group from intermediate compound **240** for the synthesis of welwistatin (Scheme 5.7-A).[100] Boger *et al*.[94] achieved the deprotection of the Boc group, in the presence of TBS ethers, and trityl amides in complex peptides, with *B*-bromocatecholborane (BCB) (Scheme 5.7-B).[101]

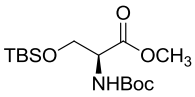
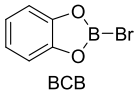
Pearson and Cui[102] used TBSOTf for the removal of a Boc group (Scheme 5.7-C). Removal of Boc via TBS-carbamates was first introduced by Sakaitani and Ohfune.[103]



Scheme 5.7 Examples of Boc group deprotection in the presence of TBS ether.

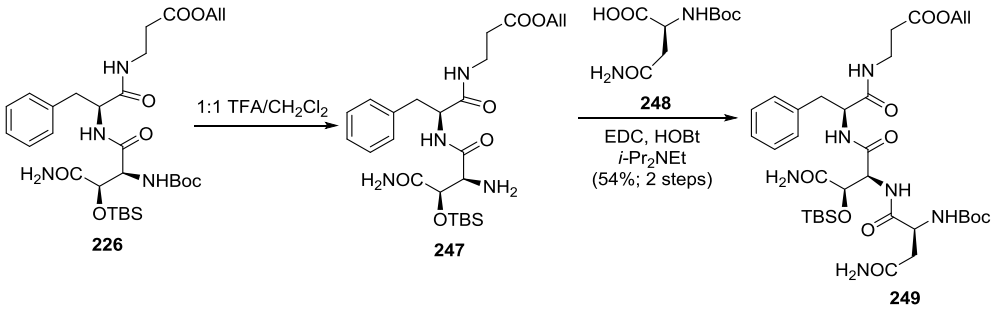
Preliminary results from attempted deprotection of tripeptide **226** by treatment of trifluoroacetic acid (TFA) gave a mixture of starting material and a compound that arises from double deprotection of both TBS and Boc groups. We used Boc-Ser(OTBS)-OMe (**246**) as a model system for the deprotection of Boc in presence of TBS ether. Unfortunately, when BCB was applied to both model and real systems, no desired product was isolated. The strategy of chemoselective transformation of an amino protecting group, via a *tert*-butyldimethylsilyl carbamate and cleaving it with TBAF worked well on a model system (**246**) but failed on the real system. Finally, treatment of compound **226** with an increased amount of trifluoroacetic acid in dichloromethane (1:1 TFA /CH_2Cl_2) resulted in amine **247** (Table 5.1, Scheme 5.8).

Table 5.1 Deprotection of *N*-terminus

Boc Protected Amine	Reaction Conditions	Results
 **246**	 , CH_2Cl_2	Recovered starting material.
246	TBSOTf, CH_2Cl_2	Product obtained in 45% yield after methanolysis and flash chromatography.
246	50% TFA in CH_2Cl_2	1 h treatment with TFA at 0 °C, product was obtained in 98% yield after flash chromatography.
226	TBSOTf, CH_2Cl_2	No desired product was isolated
226	50% TFA in CH_2Cl_2	1 h treatment with TFA at 0 °C, reaction progress was monitored by TLC (6:4:1 $CHCl_3$:MeOH:H_2O). TFA salt of free amine was taken in to the next coupling reaction without further purification.

5.4.2. Elongation to Tetrapeptide

Formation of tetrapeptide **249** was carried out using asparagine derivative **248**, EDC and hydroxybenzotriazole *(*HOBt) (Scheme 5.8).

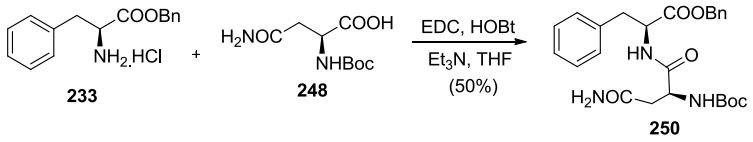


Scheme 5.8 Preparation of a tetrapeptide.

5.5 Model Studies for Cyclization

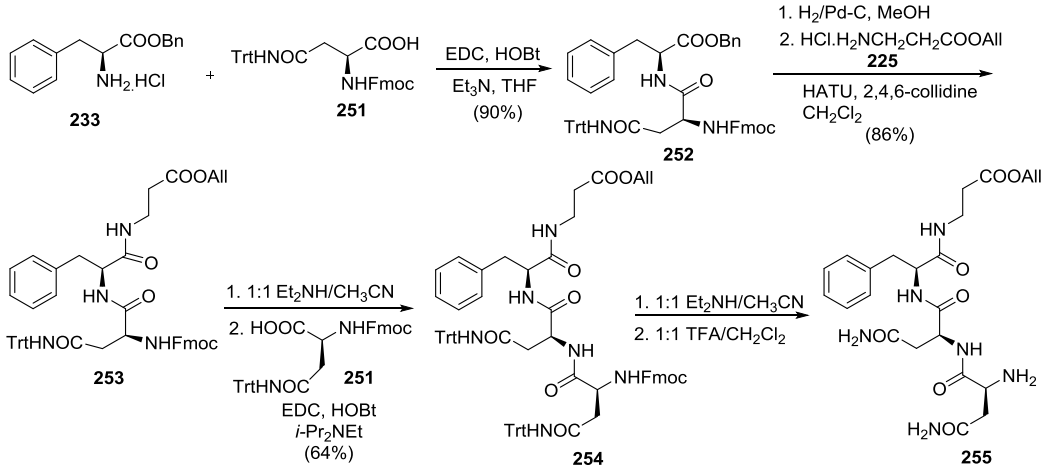
Before investigating fragment condensations with valuable tetrapeptide **249** containing *e*HyAsn, a model de-oxy tetrapeptide **252** was prepared, substituting regular Asn for HyAsn. We

started with the formation of dipeptide **250** (Scheme 5.9) using commercially available asparagine building block **248** and phenylalanine **233** in order to rapidly assemble model tetrapeptide **250**. We obtained a poor yield of the product because of the high polarity and difficulty in isolation and purification.



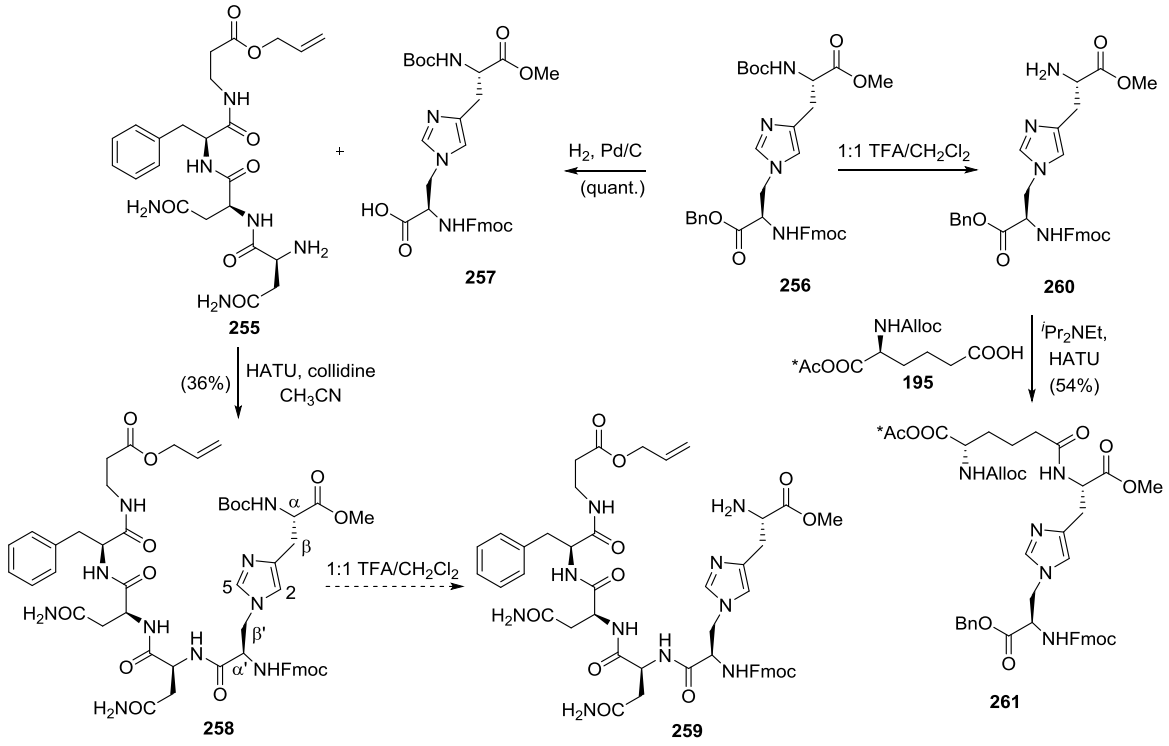
Scheme 5.9 Formation of a model dipeptide containing Phe and Asn.

Hence we started with Fmoc-Asn(Trt)-OH (**251**) in place of asparagine with an unprotected side chain, and coupled with phenylalanine benzyl ester hydrochloride (**233**) (Scheme 5.10). The dipeptide was then subjected to hydrogenolysis to obtain the free acid and coupled to β-Ala-OAll to form tripeptide **253**. Next, deblocking of Fmoc was achieved by diethylamine and then coupled to Fmoc-Asn(Trt)-OH to form the tetrapeptide **254**. It was then subjected to Fmoc cleavage with diethylamine and flash chromatography was performed to isolate the product. The trityl groups were removed with 1:1 TFA/CH_2Cl_2 to obtain free amine **255**.



Scheme 5.10 Preparation of a model tetrapeptide.

In collaboration with Chyree Batton, the next step was to couple the τ-histidinoalanine residue with the tetrapeptide assembled above. Hydrogenolysis was carried out to deblock the benzyl ester in τ-HAL **256** to obtain **257** (Scheme 5.11). The free amine **255** was coupled to τ-HAL derivative **257**, which afforded **258**. The next step is to deprotect the *L*-His amine of τ-HAL using TFA to give amine 259.



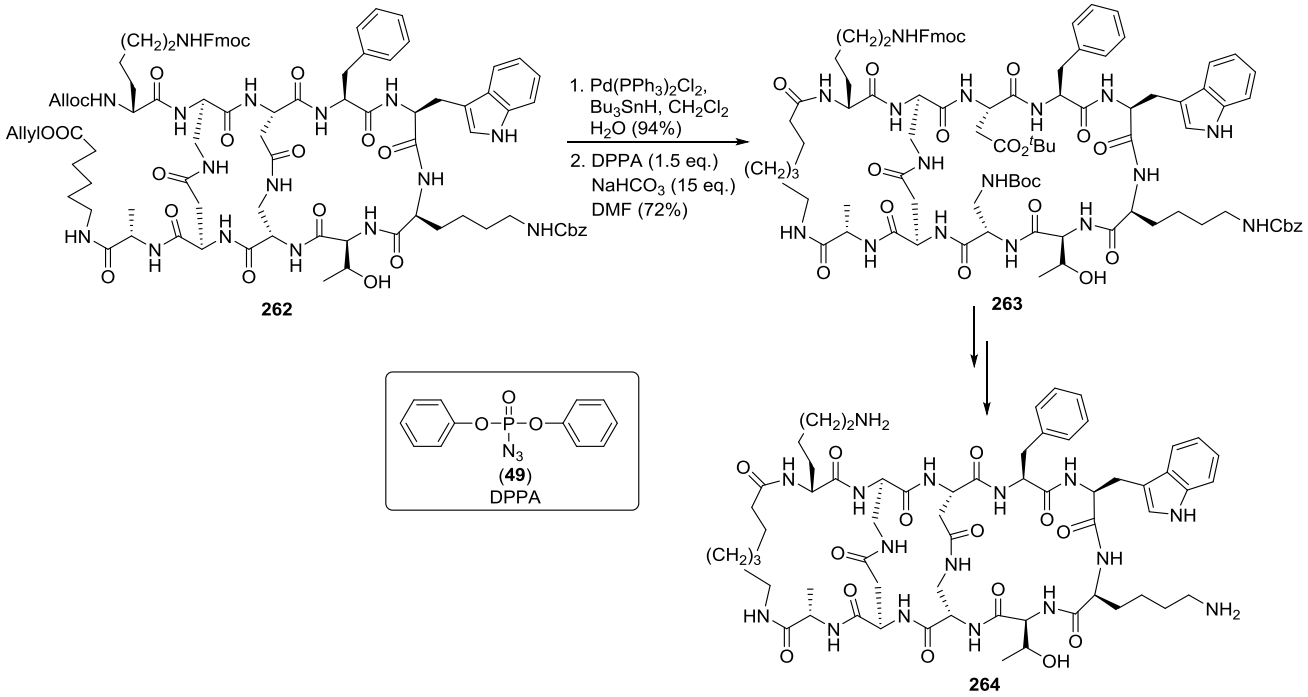
Scheme 5.11 Coupling of model tetrapeptide and τ-HAL derivative.

Proton NMR of compound **258** provided evidence for the successful coupling of the two fragments. While the 2.5-5.0 ppm region of the spectrum was complex, integration was consistent with the structure of **258** and a strong singlet at 3.8 ppm was assigned to the methyl ester. Another strong singlet appeared at 1.4 ppm for the Boc group. The allyl ester of the β-Ala residue gave rise to characteristic signals for the three olefinic protons in the 5-6 ppm region. An ion was observed at m/z 1065.4655, consistent with (M+H) for the molecular formula of **258** ($C_{53}H_{65}N_{10}O_{14}$).

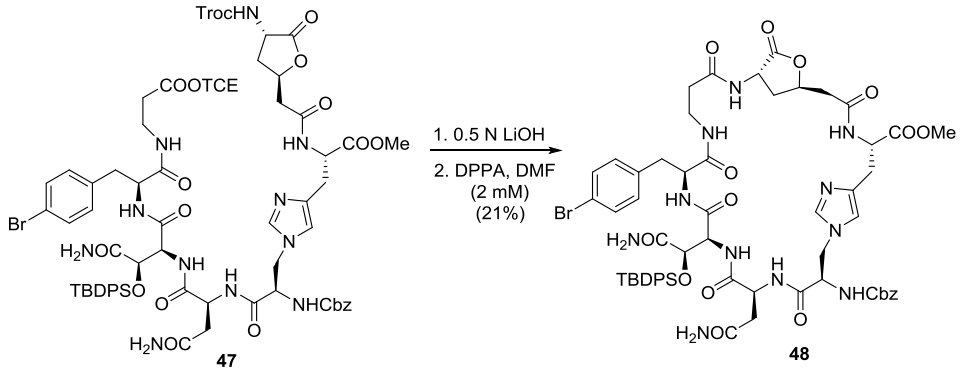
In a parallel experiment, we coupled τ-HAL with of α-amino adipic acid derivative **195** (Scheme 5.11). Removal of the Boc group from τ-HAL was carried out using TFA. The free amine **260** was condensed with the acid of α-AAA **195** which afforded **261**. Proton NMR of compound **261** provided evidence for the successful coupling of the two fragments. A strong singlet at 3.7 ppm was assigned to the methyl ester. Another strong singlet appeared at 1.8 ppm for the CH_3 group of the aceto moiety. Hβs were resonated as multiplet at 2.8-3.0. Hαs were appeared in the region of 4.1-4.8 ppm. Benzyl ester protons were characteristically observed at 5.2 ppm. The allyl ester of the NHAlloc group gave rise to characteristic signals for the three olefinic protons in the 5-6 ppm region. Imidazole H5 and H2 were observed at 7.2 and 6.4 ppm. Fmoc protons were observed in the aromatic region (7.3-7.4 ppm). An ion was observed at m/z 751.3841, consistent with (M+H) for the molecular formula of **261** ($C_{41}H_{45}N_5O_9$).

5.6 Precedent for Pairs of Palladium-Labile Protecting Groups

Hirschmann *et al.* synthesized homodetic tricyclic peptide **264** to study the conformation of the hormone somatostatin (SRIF).[104] To execute consecutive ring closures in a controlled manner, they paired carboxyl and amine protecting groups that could be simultaneously and selectively removed. They chose Alloc/Allyl as one pair of amine/carboxy protecting group partners. Deprotection of the Alloc and Allyl group was accomplished simultaneously in 94% yield using Pd(0) as catalyst and tributyltin hydride (Bu_3SnH) as the allyl acceptor (Scheme 5.12). Subsequent cyclization was carried out using diphenylphosphoryl azide [DPPA, $(C_6H_5O)_2P(O)N_3$, **49**]. Diphenylphosphoryl azide (**49**, DPPA) was also used by Tohdo *et al.* for ring closure of the western hemisphere of theonellamide F (Scheme 5.13).[24] However, their protecting groups removed prior to cyclization were not allyl-based.

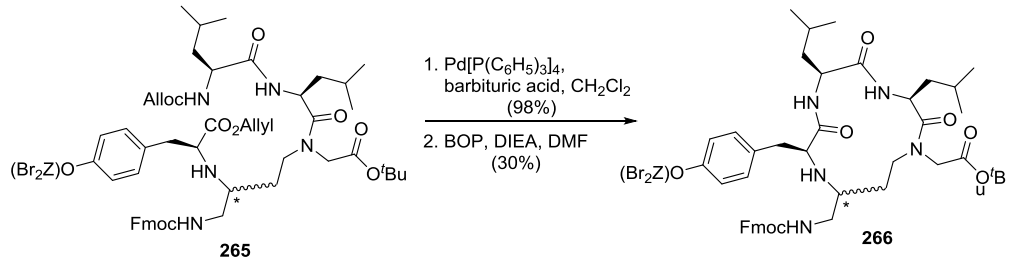


Scheme 5.12 Removal of Allyl and Alloc groups by Pd(0), cyclization and formation of the homodetic tricyclic peptide.



Scheme 5.13 Cyclization of the western hemisphere of theonellamide F by Tohdo et al.[23]

Vernall *et al.* synthesized an α-helical mimetic **266** for the 8-12 sequence of galanin, a peptide with anti-cancer and anti-diabetic properties.[105] In the final stages of their synthesis, they treated a mixture of diastereomers of **265** with Pd(0) for simultaneous deprotection of the Allyl/Alloc protecting groups and used barbituric acid as the allyl acceptor, followed by BOP-mediated cyclization to obtain cyclized product **266** as a mixture of diastereomers (Scheme 5.14).

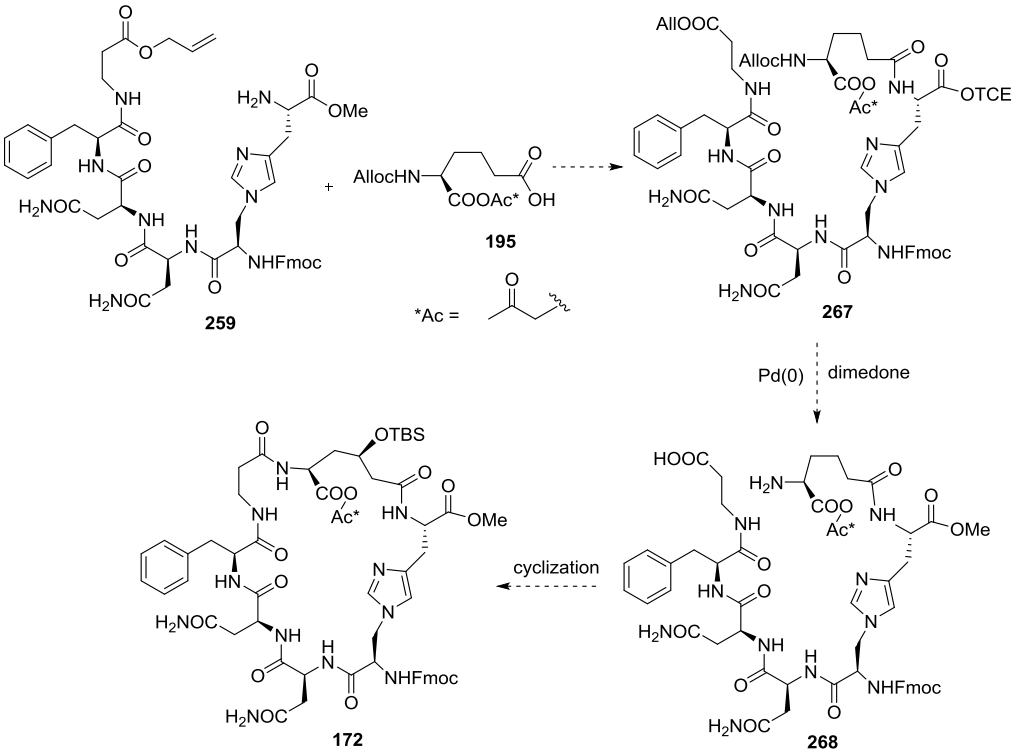


Scheme 5.14 Synthesis of cyclic pentapeptide.

5.7 Future Work

5.7.1 Formation of Western Ring

Coupling of the free γ-acid of α-AAA building block **195** (Scheme 5.15) with the primary amine **259** should lead to cyclization precursor **267**. Treatment of **267** with Pd(0) will promote simultaneous removal of the *C*-terminal allyl group and the *N*-terminal allyloxycarbonyl group to give amino acid **268.**

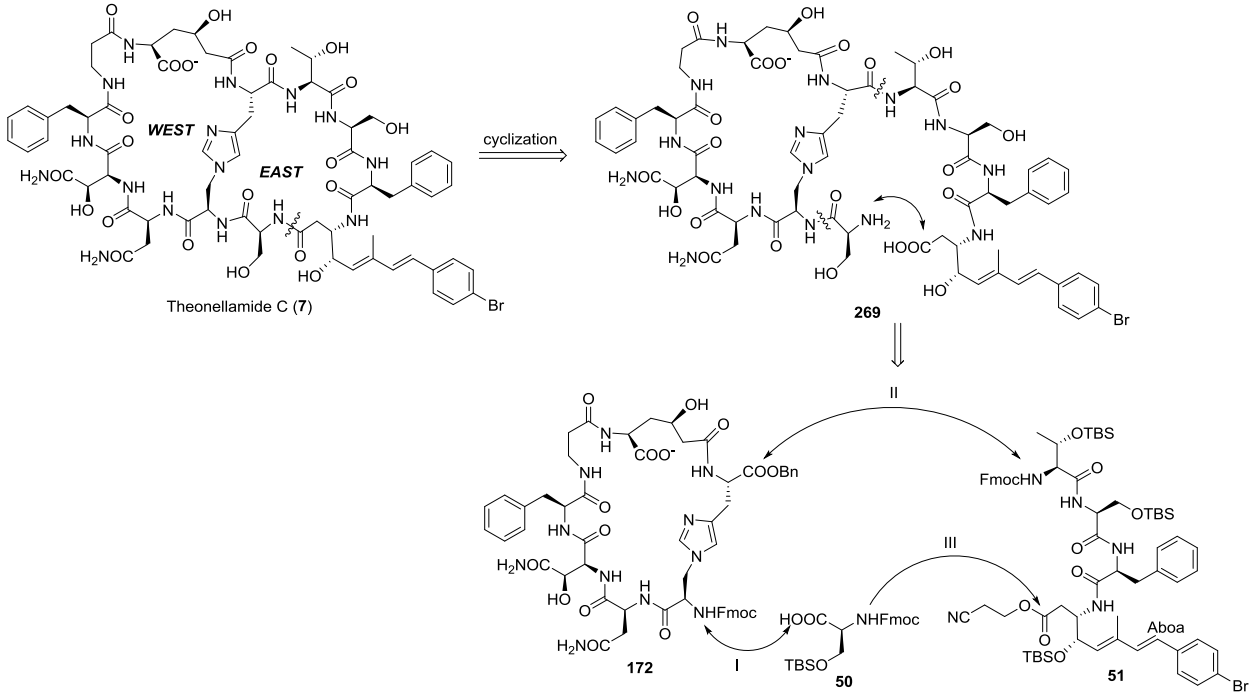


Scheme 5.15 Formation of the western hemisphere of theonellamide C.

Cyclization will result in the western hemisphere of theonellamide, compound **172**. The model system presented enables us to optimize the reaction conditions for the coupling and cyclization. Moreover, this approach will provide simplified analogs for SARs studies (Section 5.7.3).

5.7.2 Completion of Theonellamide C

The primary goal is to synthesize theonellamide C (**7**). In a forward sense, the western hemisphere will be constructed first followed by coupling of the *D*-Ala amine component of τ-HAL with a serine residue (I) (Scheme 5.16).



Scheme 5.16 Formation of theonellamide C.

The next peptide bond to be formed (II) will be between the *L*-His acid component of τ-HAL and the amino terminus of the eastern hemisphere tetrapeptide which will lead to cyclization precursor **269**. The amine component of serine and acid of Aboa will undergo HATU-mediated

cyclization under high dilution conditions (III). The site chosen for cyclization is ideal, since the Aboa residue is not susceptible to racemization during peptide bond formation. Global desilylation will be achieved by treating with TBAF as shown by Benson Edagwa's[106] work toward the synthesis of allovirodin and analogs.

5.7.3 Structure Activity Relationships (SARs) of Theonellamides

Synthesis of the western and eastern hemisphere represent penultimate goals. The plan for formation of the western hemisphere was shown earlier in Section 5.1 and the plan for synthesis of the eastern hemisphere is the subject of Douglas Wong's dissertation.[12] Each ring will be tested separately for antifungal activity. With the exception of the bridging τ-HAL residue, all other residues can be substituted for using commercially available building blocks to produce a bicyclic structure with a ring size consistent with the theonellamides (Figure 5.2).

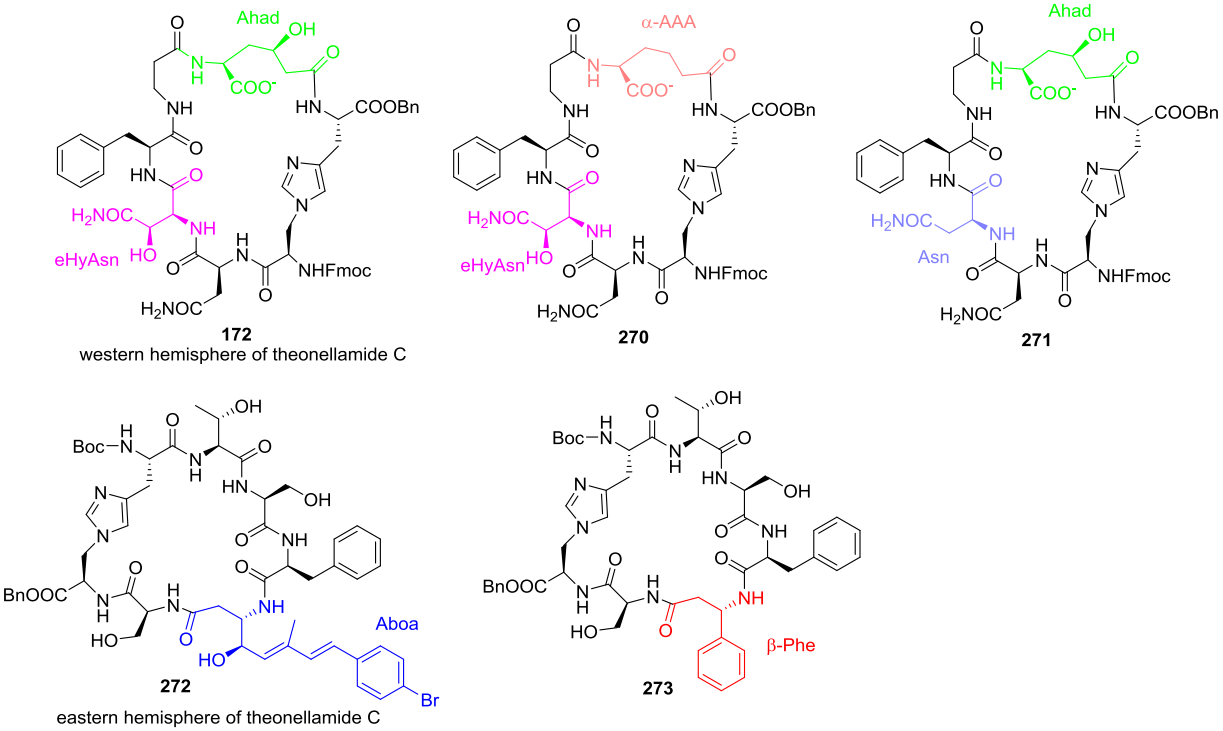
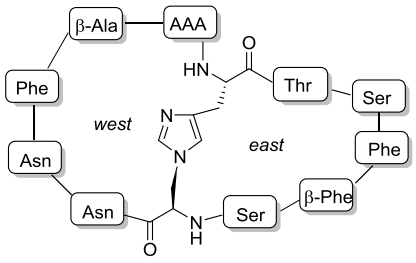


Figure 5.2 Macrocycles related to the western and eastern hemisphere with same ring size.

There are two amino acids in the western hemisphere that are not commercially available, *e*HyAsn (purple) and Ahad (green). Synthesis of the western hemisphere by replacing one amino acid at a time with a commercially available amino acid could elucidate the importance of each modified residue (**270** & **271**, Figure 5.2). The modified eastern hemisphere contains commercially available *allo*-threonine, serine, phenylalanine, and β-phenylalanine (**273**, Figure 5.2). The tetrapeptide will be coupled with the τ-HAL-serine tripeptide. Each hemisphere analog will be tested for their biological activity to reveal whether or not they independently demonstrate antifungal activity. In the end, both hemispheres will be coupled together to form the bicycle.

The identification of a minimal chemical structure that will result in potent antifungal activity could be theonellamide X (**274**, Figure 5.3), an unnatural analog in which all the substitutions proposed above are incorporated simultaneously. Our on going synthetic efforts are necessary to probe the structure activity relationship of the theonellamides.



Theonellamide X (**274**)

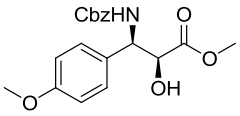
Figure 5.3 Chemical composition of Theonellamide X.

5.8 Experimental Section

5.8.1 General Methods

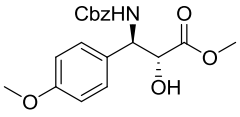
The general methods are as described in Chapter 2. NMR spectra were recorded on a Bruker AV-400 or a Bruker AV Nanobay-400 liquid spectrometer.

5.8.2 Procedures



(2*S*,3*R*)-Methyl 3-(benzyloxycarbonylamino)-2-hydroxy-3-(4-methoxyphenyl)propanoate (**228**)

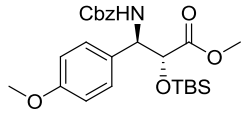
Benzyl carbamate (9.05 g, 60.0 mmol, 2.30 equiv.) was dissolved in *n*-PrOH (70 mL). A solution of NaOH (2.44 g, 61.0 mmol, 2.35 equiv.) in H_2O (110 mL) was added and the resulting solution stirred for 10 min. Freshly prepared *tert*-butyl hypochlorite[107] (6.90 mL, 6.62 g, 61.0 mmol, 2.35 equiv.) was added dropwise and the resulting solution stirred for an additional 10 min. $(DHQD)_2PHAL$ (0.83 g, 1.3 mmol, 5 mol %) in *n*-PrOH (40 mL) was added and the reaction vessel immersed in a water bath at ambient temperature and stirred for 5 min. (*E*)-Methyl 3-(4-methoxyphenyl)acrylate **227** (5.0 g, 26.0 mmol, 1.0 equiv.) was added as a solid all at once, followed immediately by $K_2OsO_2(OH)_4$ (0.38 g, 1.0 mmol, 4 mol %). The reaction mixture was stirred for 3 h at 0 ˚C. The reaction mixture was evaporated and the residue subjected to flash chromatography (Hex-EtOAc 3:1) to afford compound **228** as a colorless solid (6.35 g, 64%). R_f 0.32 (Hex-EtOAc 1:1). $[\alpha]_D^{25}$ -5.9 (*c* 1.0, $CHCl_3$. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.18 (br s, 1H), 3.80 (s, 6H), 4.45 (s, 1H), 5.07 (dd, *J* = 17.8, 12.0 Hz, 2H), 5.21 (d, *J* = 9.1 Hz, 1H), 5.65 (d, *J* = 9.3 Hz, 1H), 6.88 (d, *J* = 8.7 Hz, 2H), 7.25-7.35 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 53.1, 55.3, 56.0, 67.0, 73.5, 114.1, 128.0, 128.1, 128.2, 128.5, 131.0, 136.3, 155.7, 159.2, 173.3; HRMS (ESI) calcd for $C_{19}H_{20}NO_6$ $(M-H)^+$ 358.1496, obsd 358.1489.



(2*R*,3*R*)-Methyl 3-(benzyloxycarbonylamino)-2-hydroxy-3-(4-methoxyphenyl)propanoate (**229**)

p-Nitrobenzoic acid (855 mg, 5.12 mmol, 5.5 equiv.) was added to a solution of (2*S*,3*R*)-methyl 3-(benzyloxycarbonylamino)-2-hydroxy-3-(4-methoxyphenyl)propanoate **228** (500 mg, 0.93 mmol, 1.0 equiv.) in dry toluene (10 mL). Triphenylphosphine (1.43 g, 5.12 mmol, 5.5

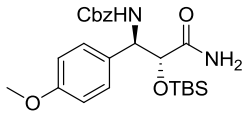
equiv.) was added under N_2 and reaction mixture was cooled to 0 °C. Diisopropyl azodicarboxylate (1 mL, 1.04 g, 5.12 mmol, 5.5 equiv.) was slowly added via syringe. Upon completion of the addition, the ice bath was removed and the reaction mixture stirred at room temperature for 24 h. The reaction mixture was concentrated and the residue dissolved in anhydrous MeOH (10 mL). Sodium azide (302 mg, 4.65 mmol, 5.0 equiv.) was added and the mixture heated at 45 ˚C for 18 h. The solvent was removed under reduced pressure and the product isolated from the residue by flash chromatography (Hex-EtOAc, 3:1) to give **229** as a pale yellow solid (325 mg, 65%). R_f 0.32 (1:1 Hex-EtOAc). $[\alpha]_D^{25}$ -20.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.94 (br s, 1H), 3.70 (s, 3H), 3.78 (s, 3H), 4.59 (dd, *J* = 6.5, 3.3 Hz, 1H), 5.11 (d, *J* = 12.2 Hz, 1H), 5.11 (d, *J* = 12.2 Hz, 1H), 5.10-5.13 (m, 1H), 5.80 (d, *J* = 8.4 Hz, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 7.18 (d, *J* = 12.2 Hz, 2H), 7.24-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.7, 55.2, 56.4, 67.0, 73.1, 113.9, 128.2, 128.5, 128.6, 136.3, 155.5, 159.5, 172.3; HRMS (ESI) calcd for $C_{19}H_{20}NO_6$ $(M-H)^+$ 358.1296, obsd 358.1295.



(2*R*,3*R*)-Methyl-3-(benzyloxycarbonylamino)-2-(*tert*-butyldimethylsilyloxy)-3-(4-methoxyphenyl)propanoate (**230**)

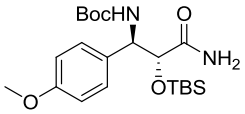
Imidazole (954 mg, 13.0 mmol, 15.0 equiv.) was added to a solution of (2*R*,3*R*)-methyl 3-(benzyloxycarbonylamino)-2-hydroxy-3-(4-methoxyphenyl)propanoate **229** (400 mg, 1.1 mmol, 1.0 equiv.) in dry CH_2Cl_2 (5 mL). The mixture was stirred for 10 min before addition of TBSCl (1700 mg, 11.0 mmol, 10.0 equiv.). The reaction mixture was stirred for 17 h at rt. The reaction mixture was diluted with ethyl acetate (25 mL) and concentrated. Flash chromatography on silica gel (Hex-EtOAc, 2:1) afforded the TBS ether **230** as a clear oil (448 mg, 85%). R_f 0.24 (4:1 Hex-EtOAc). $[\alpha]_D^{25.0}$ -16.8 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.03 (s, 3H), 0.07 (s, 3H), 0.89 (s, 9H), 3.53 (s, 3H), 3.74 (s, 3H), 4.59 (d, *J* = 3.9 Hz, 1H), 5.02-5.12 (m, 3H), 5.53 (d, *J* = 8.0 Hz, 1H), 6.79 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 7.9 Hz, 2H), 7.21-

7.32 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -5.5, -5.1, 18.3, 25.7, 51.6, 55.2, 57.0, 66.8, 74.3, 113.7, 128.1, 128.5, 128.9, 129.6, 136.4, 155.5, 159.3, 171.4; HRMS (ESI) calcd for $C_{25}H_{36}NO_6Si$ $(M+H)^+$ 474.2106, obsd 474.2114.



Benzyl (1*R*, 2*R*)-3-amino-2-(*tert*-butyldimethylsilyloxy)-1-(4-methoxyphenyl)-3-oxopropylcarbamate (**231**)

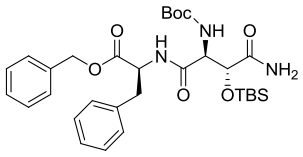
Ammonia gas was bubbled through a solution of (2*R*,3*R*)-methyl 3-(benzyloxycarbonylamino)-2-(*tert*-butyldimethylsilyloxy)-3-(4-methoxyphenyl)propanoate (**230**) (373 mg, 0.78 mmol) in dry MeOH (20 mL) at 0 ˚C. The reaction vessel was stoppered and the mixture stirred for 7 d at room temperature. The solution was cooled to 0 ˚C and resaturated with ammonia gas. The reaction vessel was stoppered and the mixture stirred for 7 d at room temperature. The solvent was removed under reduced pressure. Flash chromatography (Hex-EtOAc, 3:1) led to recovery of the methyl ester (72 mg, 20%) and isolation of the primary amide **231** as a colorless foam (239 mg, 66%). R_f 0.29 (1:1 Hex-EtOAc). $[\alpha]^{26.5}_D$ +3.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ -0.02 (s, 3H), 0.01 (s, 3H), 0.92 (s, 9H), 3.77 (s, 3H), 4.43 (d, *J* = 3.7 Hz, 1H), 4.95-5.08 (m, 3H), 5.61 (d, *J* = 6.1 Hz, 1H), 6.03 (s, 1H), 6.09 (s, 1H), 6.82 (d, *J* = 8.1 Hz, 2H), 7.23 (d, *J* = 7.8 Hz, 2H), 7.31-7.33 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -5.5, -5.4, 18.0, 25.7, 55.2, 57.6, 66.8, 89.9, 113.6, 128.0, 128.1, 128.4, 129.1, 129.7, 136.4, 155.5, 159.3, 171.4; HRMS (ESI) calcd for $C_{24}H_{35}N_2O_5Si$ $(M+H)^+$ 459.2309, obsd 459.2313.



tert-Butyl (1*R*,2*R*)-3-amino-2-(*tert*-butyldimethylsilyloxy)-1-(4-methoxyphenyl)-3-oxopropylcarbamate (**232**)

A solution of benzyl (1*R*,2*R*)-3-amino-2-(*tert*-butyldimethylsilyloxy)-1-(4-methoxyphenyl)-3-oxopropylcarbamate **231** (850 mg, 2.1 mmol, 1.00 equiv.) and Boc_2O (443 mg, 2.3 mmol, 1.12 equiv.) in CH_3OH (20 mL) was treated with 10% Pd-C (45 mg). The reaction mixture was stirred

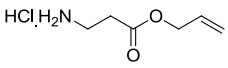
under H_2 (1 atm) at 25 °C overnight. The Pd-C was removed by filtration through Celite®, and the filtrate was concentrated. Flash chromatography (Hex-EtOAc, 2:1) provided **232** as a colorless foam (745 mg, 95%). R_f 0.30 (1:1 EtOAc-Hex). $[\alpha]^{26.5}_D$ 14.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.04 (s, 6H), 0.93 (s, 9H), 1.40 (s, 9H), 3.76 (s, 3H), 4.40 (s, 1H), 4.91 (app s, 1H), 5.35 (app s, 1H), 6.03 (s, 1H), 6.51 (s, 1H), 6.81 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -5.5, -5.3, 18.0, 25.7, 28.3, 55.1, 57.2, 76.0, 79.4, 113.5, 129.1, 129.9, 154.7, 159.1, 174.4. HRMS (ESI) calcd for $C_{21}H_{37}N_2O_5Si$ $(M+H)^+$ 425.2129, obsd 425.2135.



(*S*)-Benzyl-2-((2*S*,3*R*)-4-amino-2-(*tert*-butoxycarbonylamino)-3-(*tert*-butyldimethylsilyloxy)-4-oxobutanamido)-3-phenylpropanoate (**234**)

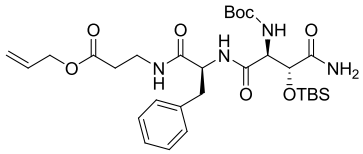
A solution of sodium periodate (5.504 g, 26 mmol, 18.1 equiv) in water (130 mL) was added, all at once, to a solution of *tert*-butyl (1*R*,2*R*)-3-amino-2-(*tert*-butyldimethylsilyloxy)-1-(4-methoxyphenyl)-3-oxopropylcarbamate **232** (604 mg, 1.4 mmol, 1.00 equiv) in a mixture of EtOAc (16 mL) and MeCN (16 mL). The biphasic mixture was stirred by an overhead mechanical stirrer for 1 h at RT. Ruthenium trichloride trihydrate (60 mg, 0.3 mmol, 0.2 mol %) and sodium bicarbonate (464 mg, 5.5 mmol, 3.90 equiv.) were add sequentially, as solids, to give a black, biphasic mixture, that was stirred at RT overnight, during which time the mixture became orange, then yellow and eventually colorless and milky. The mixture was diluted with sat'd aq. $NaHCO_3$ (240 mL) and washed with CH_2Cl_2 (160 mL). The organic layer was back-extracted with sat'd aq. $NaHCO_3$ (240 mL). The combined aqueous layers were acidified at 0 °C with 10 % aq. HCl (~150 mL) to pH 2.5 and then extracted with EtOAc (12 X 250 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give acid (2*S*,3*R*)-4-amino-2-(*tert*-butoxycarbonylamino)-3-(*tert*-butyldimethylsilyloxy)-4-oxobutanoic acid

as a brown foam (456 mg) that was dissolved in THF (22 mL) and cooled to 0 °C under N_2. Phenylalanine benzyl ester hydrochloride (**233**) (368 mg, 1.26 mmol, 1.00 equiv.) was added as a solid, followed by Et_3N (350 µL, 255 mg, 2.52 mmol, 2.00 equiv.), EDC (253 mg, 132 mmol, 1.05 equiv.) and HOBt (256 mg, 1.89 mmol, 1.50 equiv.). After 20 min the ice bath was removed, the mixture warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with a gradient of 2.5:1.0→1.0:2.0 (Hex-EtOAc) yielded dipeptide **234** as a colorless foam (415 mg, 50% over 2 steps). R_f 0.53 (1:1 EtOAc-Hex). $[\alpha]_D^{25}$ +25.7 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.08 (s, 3H), 0.12 (s, 3H), 0.90 (s, 9H), 1.43 (s, 9H), 3.00-3.18 (m, 2H), 4.61-4.67 (m, 2H), 4.84 (dd, *J* = 13.8, 6.2 Hz, 1H), 5.07 (s, 2H), 5.47 (d, *J* = 6.7 Hz, 1H), 5.84 (br s, 1H), 6.46 (br s, 1H), 6.66 (d, *J* = 7.7 Hz, 1H), 7.04-7.16 (m, 2H), 7.18-7.28 (m, 5H), 7.33-7.35 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ -5.3, -5.1, 18.0, 25.6, 28.3, 37.9, 53.5, 53.5, 57.3, 67.2, 67.2, 69.6, 74.1, 80.1, 85.4, 127.1, 128.5, 128.6, 129.3, 135.1, 135.6, 154.8, 167.4, 170.8, 174.2. HRMS (ESI) calcd for $C_{31}H_{46}N_3O_7Si$ $(M+H)^+$ 600.3100, obsd 600.3118.



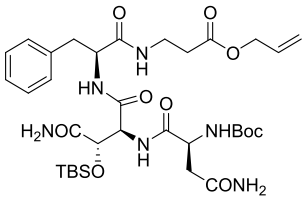
β-Alanine allyl ester hydrochloride (**225**)

β-Alanine (502 g, 5.6 mmol, 1.0 equiv.), allyl alcohol (3.4 mL, 2.9 g, 27.0 mmol, 4.8 equiv.) and chlorotrimethylsilane (1.2 mL, 1.03 g, 9.4 mmol, 1.6 equiv.) were heated at 100 °C for 4 h. The reaction mixture was cooled to RT and poured onto diethyl ether (115 mL) and stirred at 0 °C for 24 h. The precipitate was collected by filtration. The allyl protected β-alanine **225** was obtained as its hydrochloride salt (800 mg, 86%). m.p. 48-51 °C, R_f 0.38 (9:1 DCM-MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.96 (t, *J* = 6.7 Hz, 2H), 3.38 (t, *J* = 6.7, 2H), 4.63 (d, *J* = 5.8 Hz, 2H), 5.24 (dd, *J*= 10.4, 1.1 Hz, 1H), 5.33 (dd, *J* = 17.2, 1.4), 5.86-5.96 (m, 1H), 8.2 (br, 2H); ^{13}C NMR ($CDCl_3$, 100MHz) δ 31.3, 35.7, 65.9, 118.8, 131.7, 170.9; HRMS (ESI) calcd for $C_6H_{11}NO_2$ $(M+H)^+$ 130.0868, obsd 130.0863.



Boc-HyAsn(OTBS)-Phe-β-Ala-OAll (**226**)

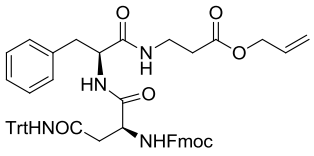
To a solution of dipeptide benzylester (**234**) (100 mg, 0.16 mmol, 1.0 equiv.) in dry MeOH (6 mL) was added 10 % Pd-C (9 mg, 0.8 mmol, 0.5 equiv.). The suspension was stirred at RT overnight under H_2 gas (1 atm). The reaction mixture was filtered through Celite® to remove the Pd-C. The solution was concentrated to give the carboxylic acid as a colorless oil (85 mg) that was used directly in the next step without further purification. The intermediate carboxylic acid (85 mg, 0.166 mmol, 1.05 equiv.) was dissolved in DCM (6 mL) and cooled to 0 °C under N_2. β-Alanine allyl ester hydrochloride (**225**) (27 mg, 0.159 mmol, 1.00 equiv.) was added as a solid followed by 2,4,6-collidine (43 µL, 39 mg, 0.318 mmol, 2.00 equiv.) and HATU (64 mg, 0.166 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of DCM. Elution with a gradient of 2:1→1:2 (Hex-EtOAc) gave tripeptide **226** as a colorless oil (85 mg, 85%), R_f 0.42 (2:1 EtOAc-Hex). $[\alpha]_D^{25}$ +8.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.13 (s, 3H), 0.15 (s, 3H), 0.91 (s, 9H), 1.43 (s, 9H), 2.35-2.52 (m, 2H), 2.96 (t, *J* = 6.7 Hz, 2H), 2.99 (dd, *J* = 13.6, 7.0 Hz, 1H), 3.18 (dd, *J* = 13.6, 6.1 Hz, 1H), 3.34-3.47 (m, 2H), 4.54 (d, *J* = 9.9 Hz, 2H), 4.50-4.64 (m, 3H), 5.25 (dd, *J* = 10.4, 1.3 Hz, 1H), 5.30 (dd, *J* = 17.2, 1.3 Hz, 1H), 5.38 br d, *J* = 6.3 Hz, 1H), 5.84-5.94 (m, 1H), 6.45 (br, 1H), 6.54 (br, 1H), 6.60 (br, 1H), 7.17-7.30 (m, 5H); ^{13}C NMR ($CDCl_3$, 100MHz) δ -5.3, -5.0, 18.0, 25.7, 28.3, 33.7, 34.8, 37.8, 54.8, 57.7, 65.4, 74.0, 80.3, 118.6, 127.0, 128.7, 129.3, 131.8, 136.4, 155.0, 167.7, 170.2, 172.0, 174.0; HRMS (ESI) calcd for $C_{30}H_{48}N_4O_8Si$ $(M+H)^+$ 621.3328, obsd 621.3334.



Boc-Asn-HyAsn(OTBS)-Phe-β-Ala-OAll (**249**)

To a solution of tripeptide **226** (100 mg, 0.161 mmol, 1.00 equiv.) in dry CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL). The reaction mixture was stirred for 1 hr at 0 °C and then concentrated to give the free amine **247** as a colorless oil (84 mg) that was used directly in the next step without further purification.

The intermediate amine **247** (84 mg, 0.161 mmol, 1.00 equiv.) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C under N_2. *N*α-Boc-*L*-asparagine (**248**) (39 mg, 0.169 mmol, 1.05 equiv.) was added as a solid followed by diisopropylethylamine (56 µL, 42 mg, 0.322 mmol, 2.0 equiv.) and HATU (64 mg, 0.169 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of DCM. Elution with a gradient of 95:5→9:1 (CH_2Cl_2-MeOH) gave tetrapeptide **249** as a colorless oil (64 mg, 54%), R_f 0.42 (2:1 EtOAc-Hex). $[\alpha]_D^{25}$ +16.2 (*c* 1.0, MeOH). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.13 (s, 3H), 0.15 (s, 3H), 0.91 (s, 9H), 1.43 (s, 9H), 2.36 (dd, J = 13.6, 7.0 Hz, 2H), 2.41-2.52 (m, 2H), 2.96 (t, *J* = 6.7 Hz, 2H), 2.99 (dd, *J* = 13.6, 7.0 Hz, 1H), 3.18 (dd, *J* = 13.6, 6.1 Hz, 1H), 3.34-3.47 (m, 2H), 4.54 (d, *J* = 9.9 Hz, 2H), 4.50-4.64 (m, 3H), 5.25 (dd, *J* = 10.4, 1.3 Hz, 1H), 5.30 (dd, *J* = 17.2, 1.3 Hz, 1H), 5.38 br d, *J* = 6.3 Hz, 1H), 5.84-5.94 (m, 1H), 7.17-7.30 (m, 5H); ^{13}C NMR (CD_3OD, 100MHz) δ -5.3, -5.0, 25.7, 28.3, 30.5, 33.7, 34.8, 37.8, 38.1, 52.8, 55.7, 57.7, 65.4, 74.0, 80.3, 118.6, 127.0, 128.7, 129.3, 131.8, 136.4, 155.0, 167.7, 170.2, 172.0, 173.4, 173.5, 174.0; HRMS (ESI) calcd for $C_{34}H_{55}N_6O_{10}Si$ $(M+H)^+$ 735.3228, obsd 735.3234.



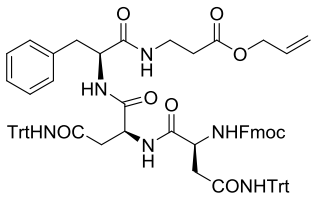
Fmoc-Asn(Trt)-Phe-β-Ala-OAll (**253**)

Fmoc-Asn(Trt)-Phe-OBn (**252**): *N*α-Fmoc-*N*-β-trityl-*L*-asparagine (**251**) (194 mg, 0.34 mmol, 1.00 equiv.) was dissolved in dry CH_2Cl_2 (2 mL) and cooled to 0 °C under N_2. Phenylalanine benzyl ester hydrochloride (100 mg, 0.34 mmol, 1.00 equiv.) was added as a solid, followed by Et_3N (95 µL, 69 mg, 0.68 mmol, 2.00 equiv.), EDC (69 mg, 0.36 mmol, 1.05 equiv.) and HOBt (69 mg, 0.51 mmol, 1.50 equiv.). After 20 min the ice bath was removed, the mixture warmed to RT and stirred overnight. The mixture was filtered through a pad of silica, washing well with 98:2 CH_2Cl_2-MeOH and concentrated to provide Fmoc-Asn(Trt)-Phe-OBn (**252**).

The dipeptide **252** was dissolved in dry MeOH (2 mL) and 10 % Pd-C (18 mg, 0.34 mmol, 1.0 equiv.) was added. The suspension was stirred at RT overnight under an atmosphere of H_2. The reaction mixture was filtered through Celite® and the filtrate concentrated to give the carboxylic acid as a colorless oil (85 mg) that was used directly in the next step without further purification.

The intermediate carboxylic acid (140 mg, 0.188 mmol, 1.05 equiv.) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C under N_2. β-Alanine allyl ester hydrochloride (**225**) (30 mg, 0.179 mmol, 1.00 equiv.) was added as a solid followed by 2,4,6-collidine (48 µL, 43 mg, 0.358 mmol, 2.00 equiv.) and HATU (71 mg, 0.188 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with a gradient of 98:2→95:5 CH_2Cl_2-MeOH yielded tripeptide **253** as a colorless foam (152 mg, 55% over 3 steps). R_f 0.50 (9:1 CH_2Cl_2-MeOH). $[\alpha]_D^{25}$ -5.2 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.22-2.34 (m, 2H), 2.76 (dd, *J* = 15.3, 6.2 Hz, 1H), 2.80-3.09 (m, 3H), 3.25 (sept., *J* = 7.7 Hz, 2H), 4.17 (t, *J* = 6.8 Hz, 1H),

4.22-4.52 (m, 4H), 4.49 (d, *J* = 5.1 Hz, 2H), 4.56 (q, *J* = 6.6 Hz, 1H), 5.23 (dd, *J* = 10.4, 1.1 Hz, 1H), 5.28 (dd, *J* = 17.2, 1.4 Hz, 1H), 5.87 (ddt, *J* = 16.2, 10.7, 5.5 Hz, 1H), 6.25 (d, *J* = 6.7 Hz, 1H), 6.60 (t, *J* = 5.9 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 7.06-7.34 (m, 23H), 7.41 (t, *J* = 7.0 Hz, 2H), 7.56 (d, *J* = 6.7 Hz, 2H), 7.76 (d, *J* = 5.9 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.7, 33.6, 34.9, 37.6, 38.2, 47.1, 51.8, 54.7, 65.2, 67.4, 70.9, 118.4, 120.0, 125.1, 126.9, 127.1, 127.2, 127.8, 128.0, 128.6, 129.2, 132.0, 136.5, 141.3, 143.7, 144.2, 170.1, 170.5, 171.6. HRMS (ESI) calcd for $C_{53}H_{51}N_4O_7$ $(M+H)^+$ 855.3752, obsd 855.3752.

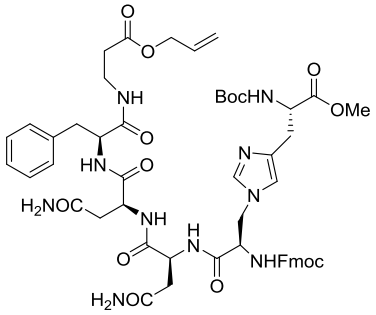


Fmoc-Asn(Trt)-Asn(Trt)-Phe-β-Ala-OAll (**254**)

To a solution of tripeptide **253** (80 mg, 0.094 mmol, 1.00 equiv.) in dry acetonitrile (1 mL) was added Et_2NH (1 mL). The reaction mixture was stirred at RT for 2 h and then concentrated to give the free amine as a colorless solid (80 mg) that was used directly in the next step without further purification.

The intermediate amine (59 mg, 0.094 mmol, 1.00 equiv.) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C under N_2. *N*α-Fmoc-*N*-β-trityl-*L*-asparagine (**251**) (58 mg, 0.098 mmol, 1.05 equiv.) was added as a solid followed by 2,4,6-collidine (27 µL, 25 mg, 0.207 mmol, 2.2 equiv.) and HATU (37 mg, 0.098 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to RT and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with 98:2 (CH_2Cl_2-MeOH) gave tetrapeptide **254** as a colorless foam (91 mg, 64%), R_f 0.52 (9:1 CH_2Cl_2-MeOH). $[\alpha]_D^{25}$ -18.8 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.28-2.34 (m, 1H), 2.72 (dd, *J* = 15.7, 6.0 Hz, 2H), 2.85 (dd, *J* = 15.4, 3.8 Hz, 2H), 3.00 (d, *J* = 5.4 Hz, 2H), 3.30 (sept., 6.4 Hz, 2H), 4.22-4.27 (m, 1H), 4.50 (d, *J* = 4.6Hz, 2H), 4.58 (dd, *J* = 11.8, 7.7 Hz, 2H), 5.22-5.27 (m, 1H), 5.32 (dd, *J* = 15.9, 5.7 Hz, 1H), 5.87

(ddt, *J* = 16.3, 10.7, 5.4 Hz, 1H), 6.55 (t, *J* = 6.4 Hz, 1H), 6.89 (d, *J* = 7.4 Hz, 1H), 7.03-7.30 (m, 39H), 7.56 (d, *J* = 6.7 Hz, 2H), 7.76 (d, *J* = 5.9 Hz, 2H); 8.34 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.2, 33.6, 34.9, 37.4, 37.5, 45.5, 47.1, 48.5, 50.8, 54.8, 65.3, 70.9, 118.4, 126.9, 127.1, 127.2, 128.0, 128.5, 128.7, 128.8, 129.3, 132.0, 136.6, 142.3, 143.1, 144.1, 168.8. 169.6. 170.0, 170.2, 172.3. HRMS (ESI) calcd for $C_{76}H_{71}N_6O_9$ $(M+H)^+$ 1212.4025, obsd 1212.4006.

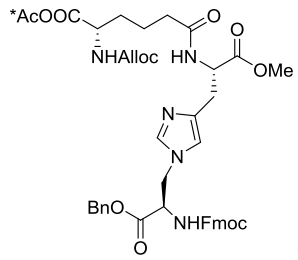


Boc-τ-HAL-Asn-Asn-Phe-β-Ala-OAll (**258**)

H.Asn-Asn-Phe-β-Ala-OAll **255**: To a solution of tetrapeptide **254** (20 mg, 0.016 mmol, 1.00 equiv.) in dry acetonitrile (1 mL) was added Et_2NH (1 mL). The reaction mixture was stirred at rt for 2 h and then concentrated to give the amine as a colorless solid (16 mg) that was dissolved in dry CH_2Cl_2 (0.3 mL) and cooled to 0 °C under N_2. Trifluoroacetic acid (0.3 mL) was added dropwise. The reaction mixture was stirred for 1 h at 0 °C. The mixture was concentrated three times from toluene (1 mL each time), then diluted with MeOH (3 mL) and neutralized by the addition of solid Na_2CO_3 to ~pH 7. The reaction mixture was filtered through a pad of silica gel with a gradient of 9:1→4:1 (CH_2Cl_2-MeOH) to afford tetrapeptide amine **255** (8 mg).

τ-HAL-COOH (**257**): The τ-HAL derivative **256** (11 mg, 0.016 mmol, 1.0 equiv.) was dissolved in dry MeOH (2 mL) and 10 % Pd-C (2 mg, 0.016 mmol, 1.0 equiv.) was added. The suspension was stirred at RT overnight under an atmosphere of H_2. The reaction mixture was filtered through Celite® and the filtrate concentrated to give the carboxylic acid **257** as a colorless oil (9 mg) that was used directly in the next step without further purification.

The intermediate amine **255** (8 mg, 0.0158 mmol, 1.00 equiv.) was dissolved in CH_2Cl_2 (1.5 mL) and cooled to 0 °C under N_2. τ-HAL-COOH **257** (9 mg, 0.016 mmol, 1.05 equiv.) was added as a solution in CH_2Cl_2 (1.5 mL) followed by collidine (2 µL, 1.8 mg, 0.322 mmol, 2.0 equiv.) and HATU (6 mg, 0.016 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to rt and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with a gradient of 95:5→9:1 (CH_2Cl_2-MeOH) gave hexapeptide **258** as a colorless oil (6 mg, 36%). R_f 0.35 (9:1 CH_2Cl_2-MeOH). HRMS (ESI) calcd for $C_{53}H_{64}N_{10}O_{14}$ $(M+H)^+$ 1065.4676, obsd 1065.4655.



τ-HAL-*N*α(Alloc)-*C*α(Ac*)-AAA (**261**)

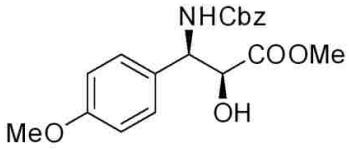
To a solution of τ-HAL **256** (10 mg, 0.015 mmol, 1.00 equiv.) in dry CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL). The reaction mixture was stirred for 1 h at 0 °C and then concentrated three times from toluene to give the free amine **260** as a colorless oil (8.5 mg) that was used directly in the next step without further purification.

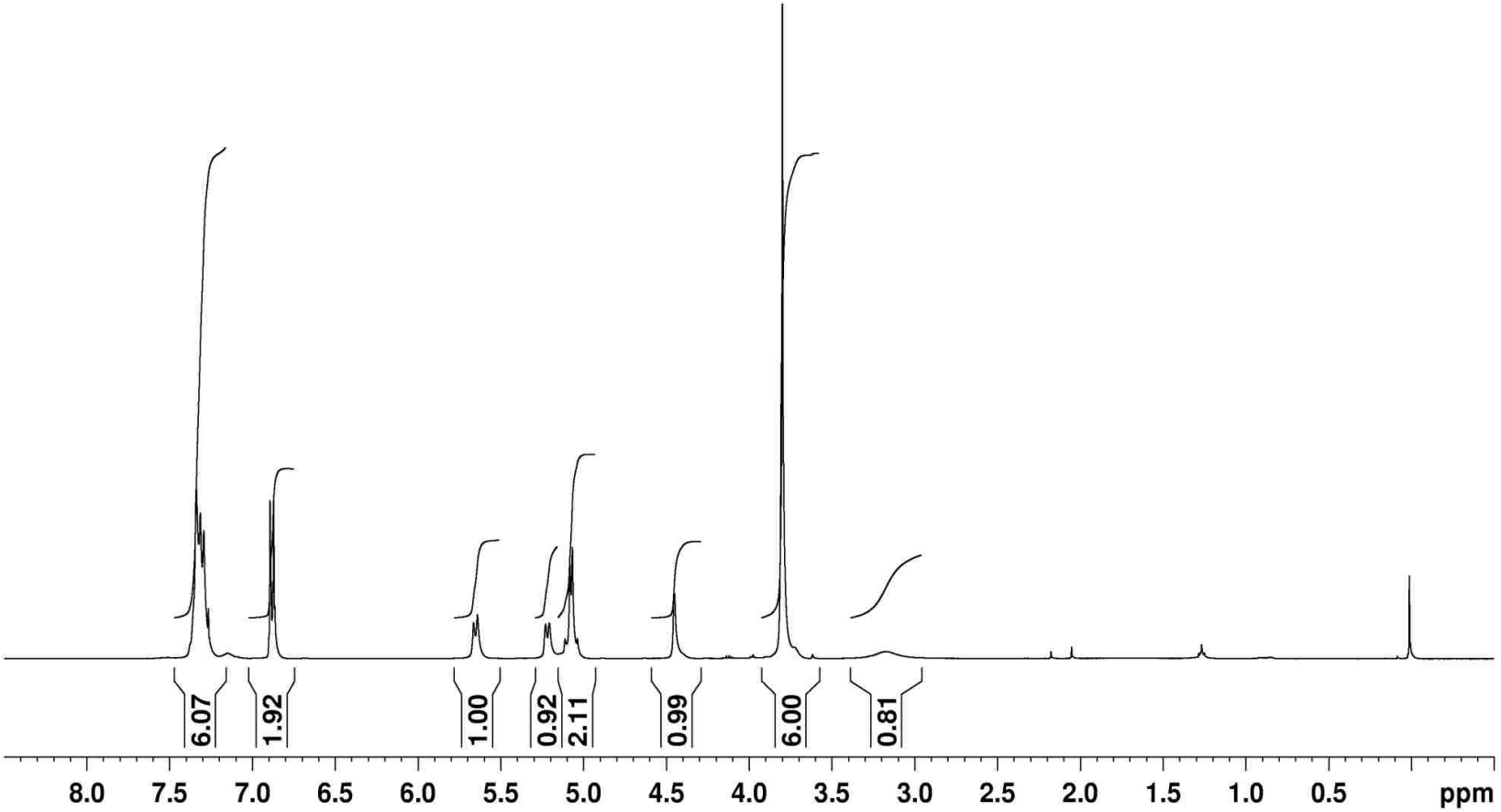
The intermediate amine **260** (8.5 mg, 0.015 mmol, 1.00 equiv.) was dissolved in CH_2Cl_2 (1.5 mL) and cooled to 0 °C under N_2. Free acid **195** (5 mg, 0.015 mmol, 1.00 equiv.) was added as a solution in CH_2Cl_2 (1.5 mL) followed by collidine (4 µL, 3.5 mg, 0.030 mmol, 2.0 equiv.) and HATU (6 mg, 0.016 mmol, 1.05 equiv.). After 20 min, the mixture was warmed to rt and stirred overnight. The mixture was concentrated and applied to a flash column in a minimum volume of CH_2Cl_2. Elution with a gradient of 95:5→9:1 (CH_2Cl_2-MeOH) gave tripeptide **261** as a colorless oil (6 mg, 54%). R_f 0.43 (95:5 CH_2Cl_2-MeOH). HRMS (ESI) calcd for $C_{41}H_{45}N_5O_9$ $(M+H)^+$ 751.3852, obsd 751.3841.

5.8.3 Spectra

Compound **228** (Scheme 5.3) – ^{1}H NMR spectrum

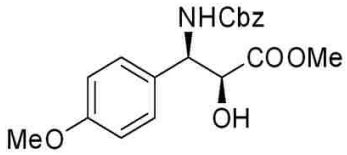
SY–04–140 in CDCl3 at 400 MHz

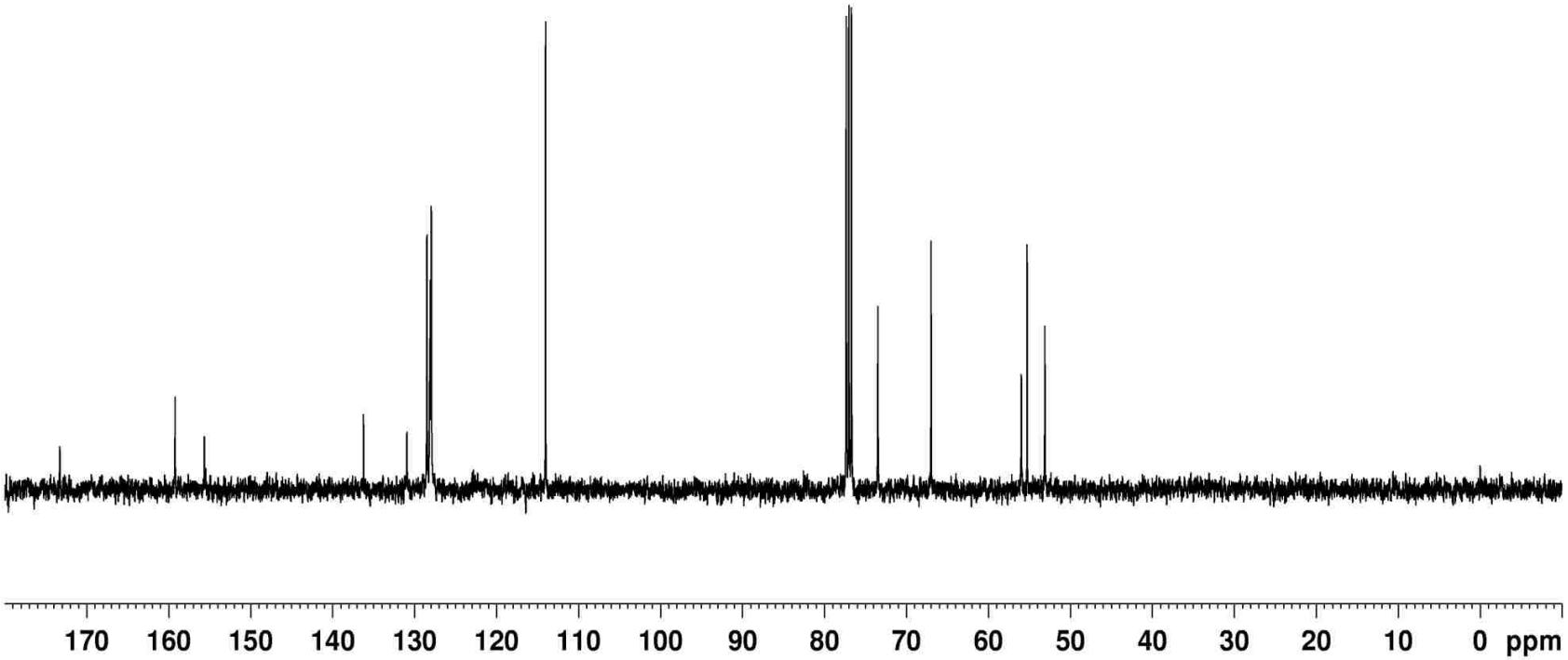




Compound **228** (Scheme 5.3) – ^{13}C NMR spectrum

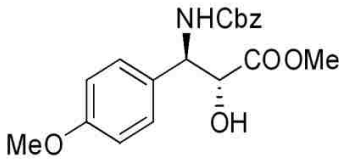
SY–04–140 in CDCl3 at 100 MHz

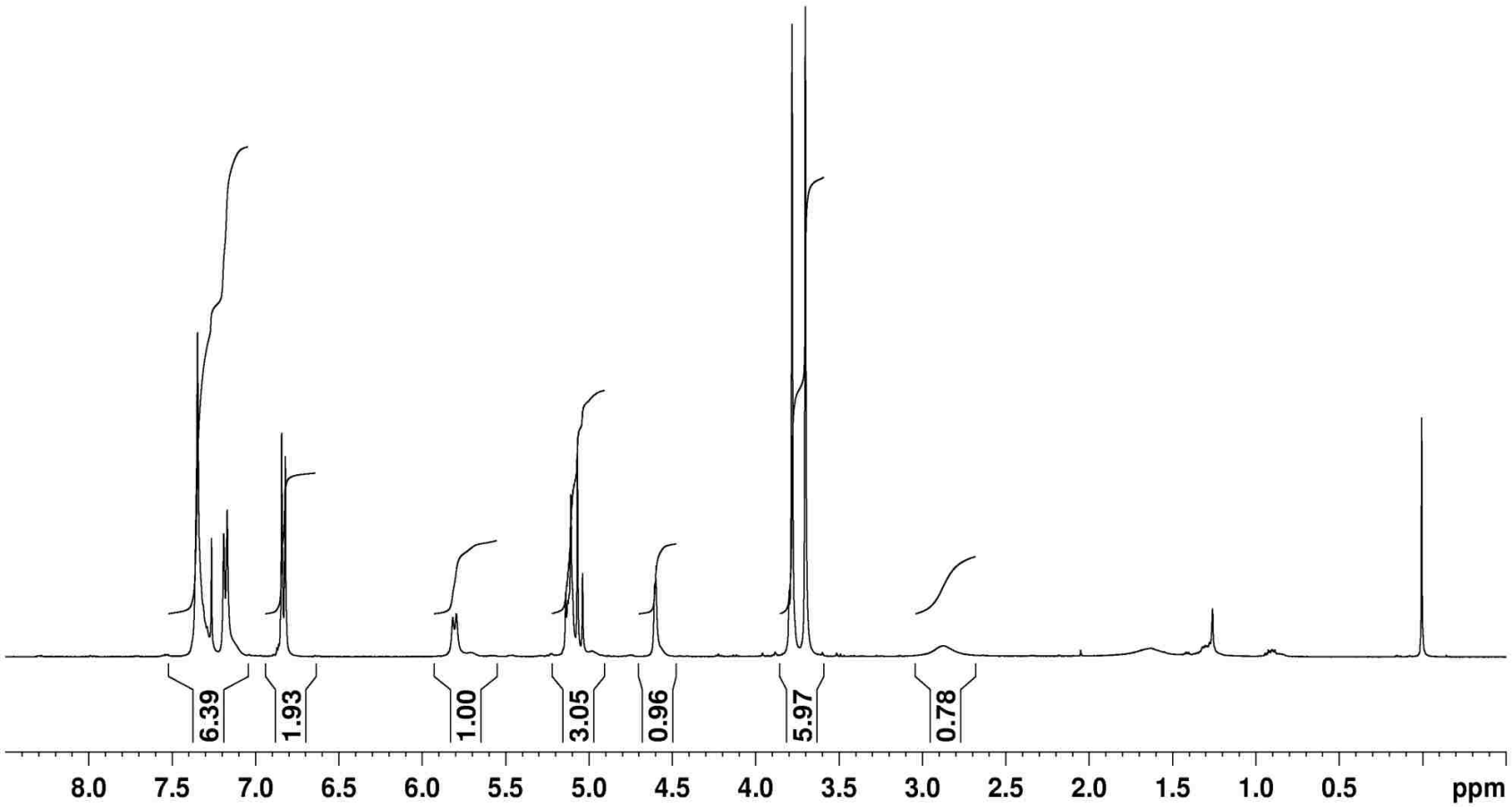




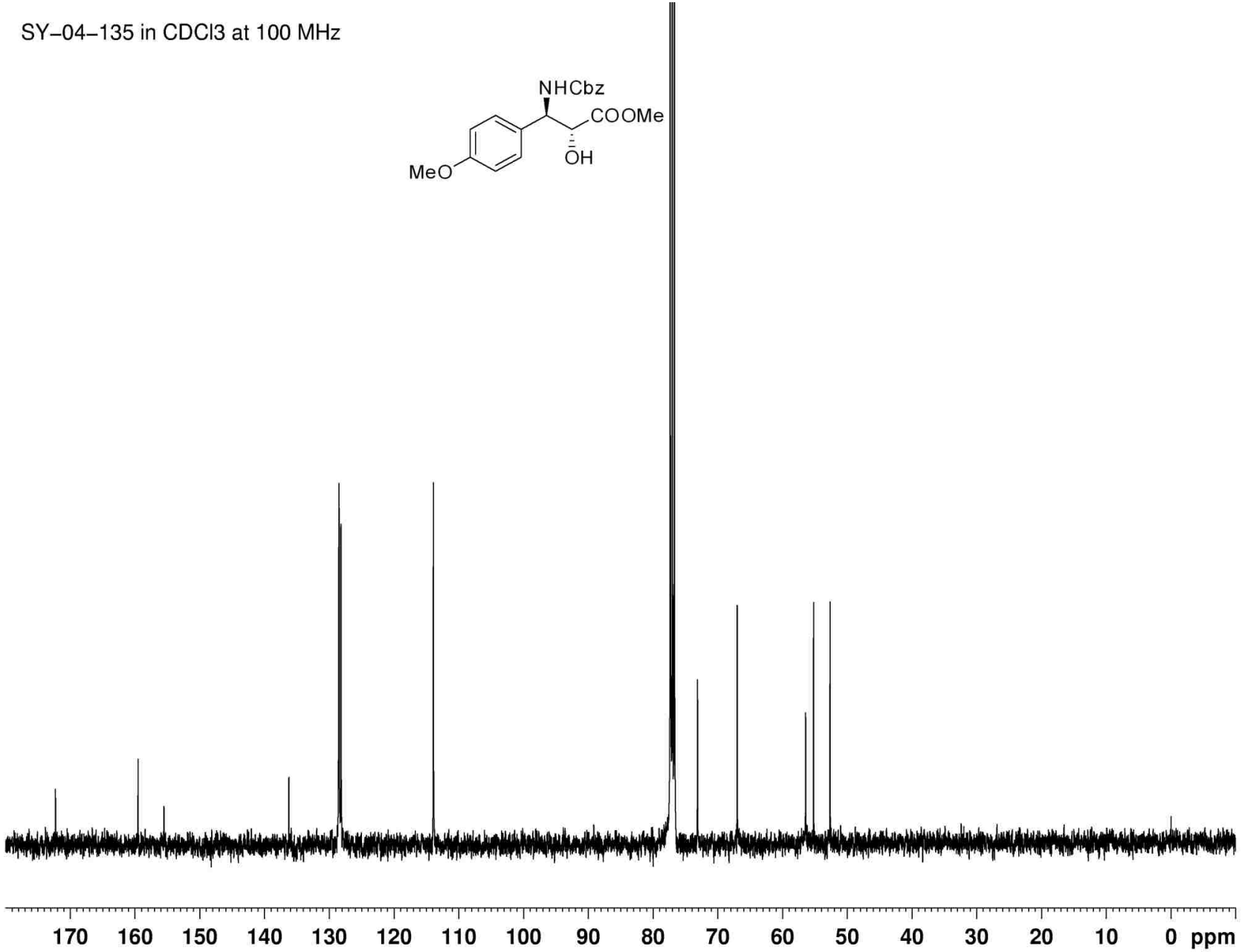
Compound **229** (Scheme 5.3) – ^{1}H NMR spectrum

SY–04–135 in CDCl3 at 400 MHz



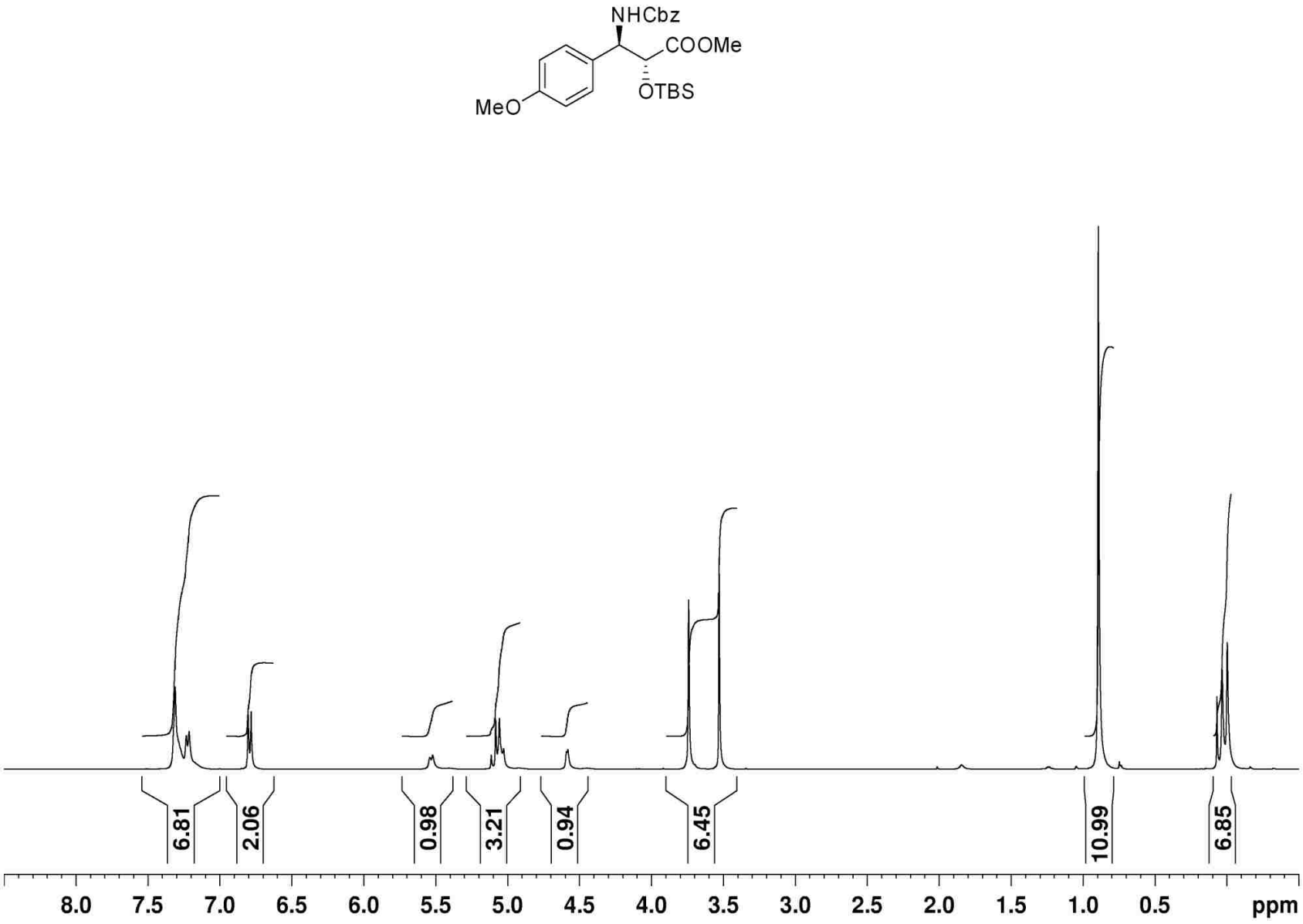


Compound **229** (Scheme 5.3) – ^{13}C NMR spectrum



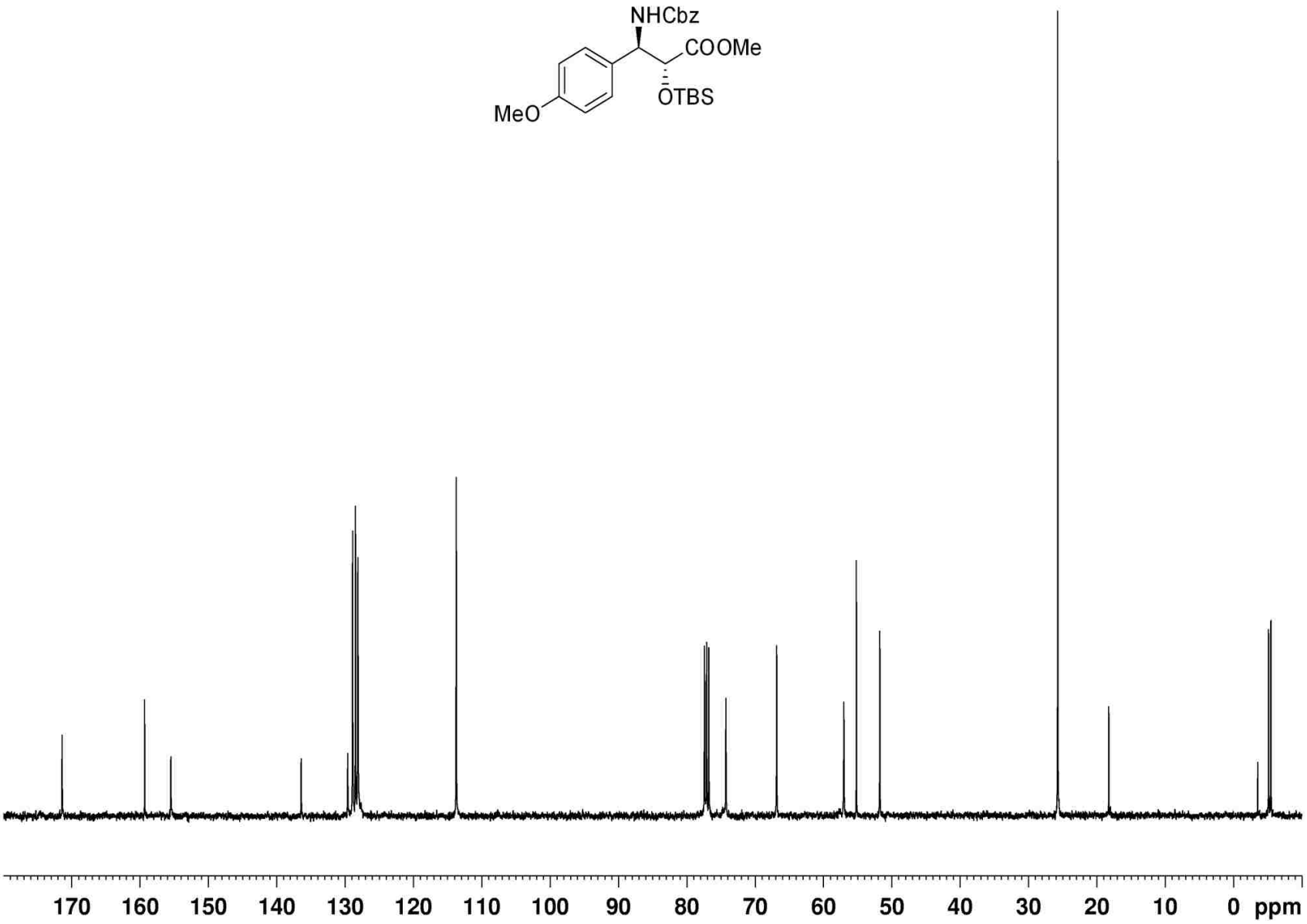
Compound **230** (Scheme 5.3) – ^{1}H NMR spectrum

SY–04–136 in CDCl3 at 400 MHz

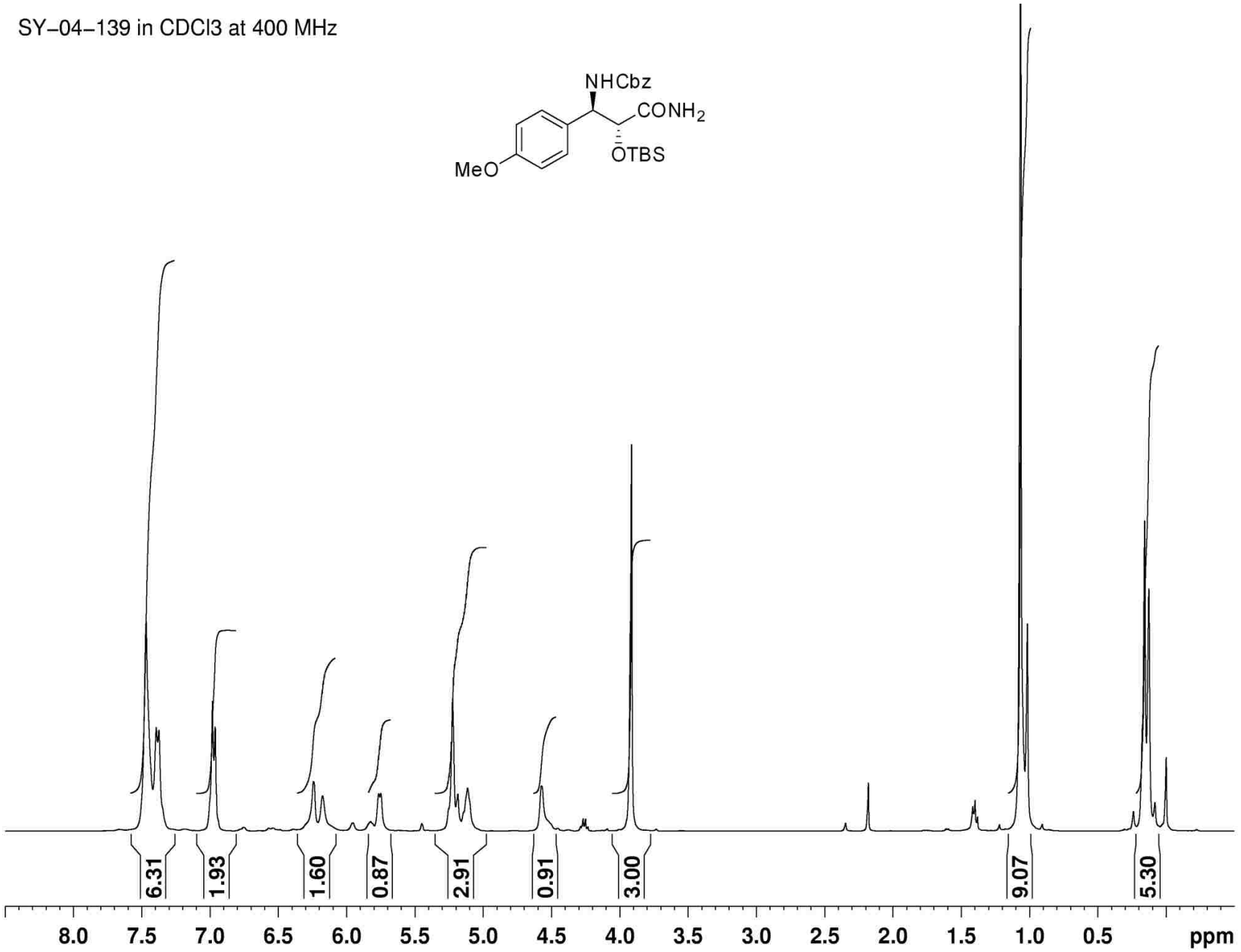


Compound **230** (Scheme 5.3) – ^{13}C NMR spectrum

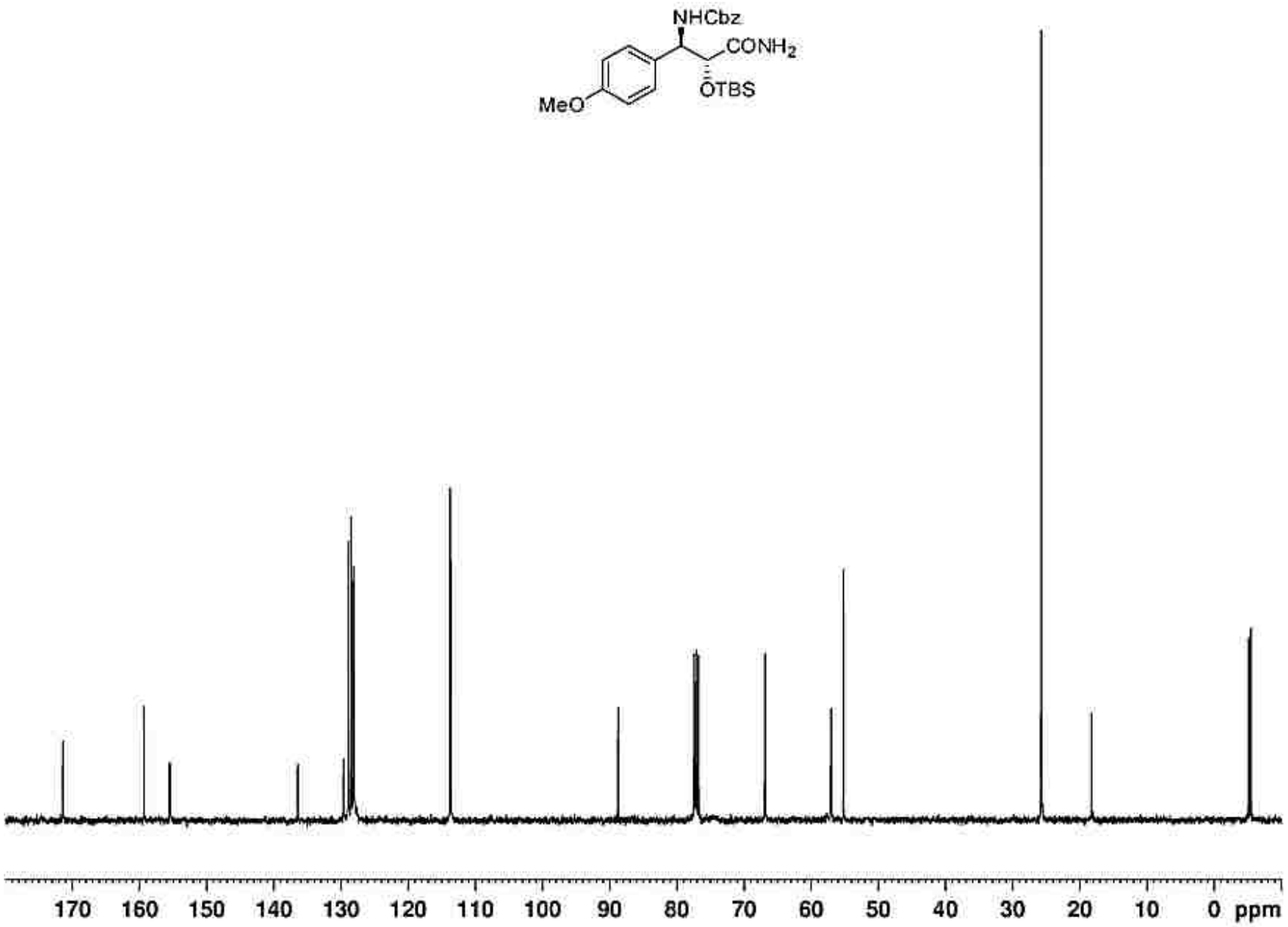
SY–04–136 in CDCl3 at 100 MHz



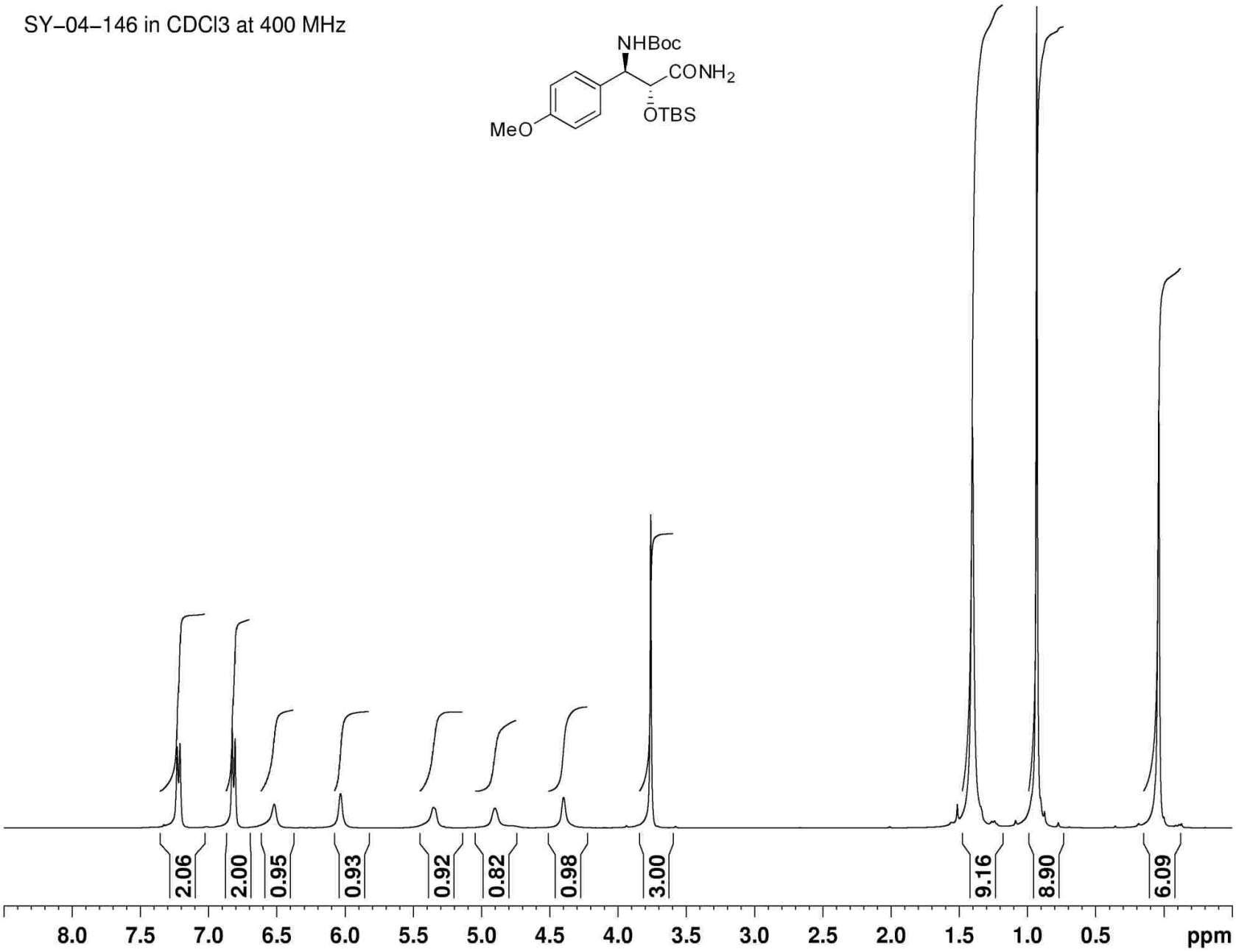
Compound **231** (Scheme 5.3) – ^{1}H NMR spectrum



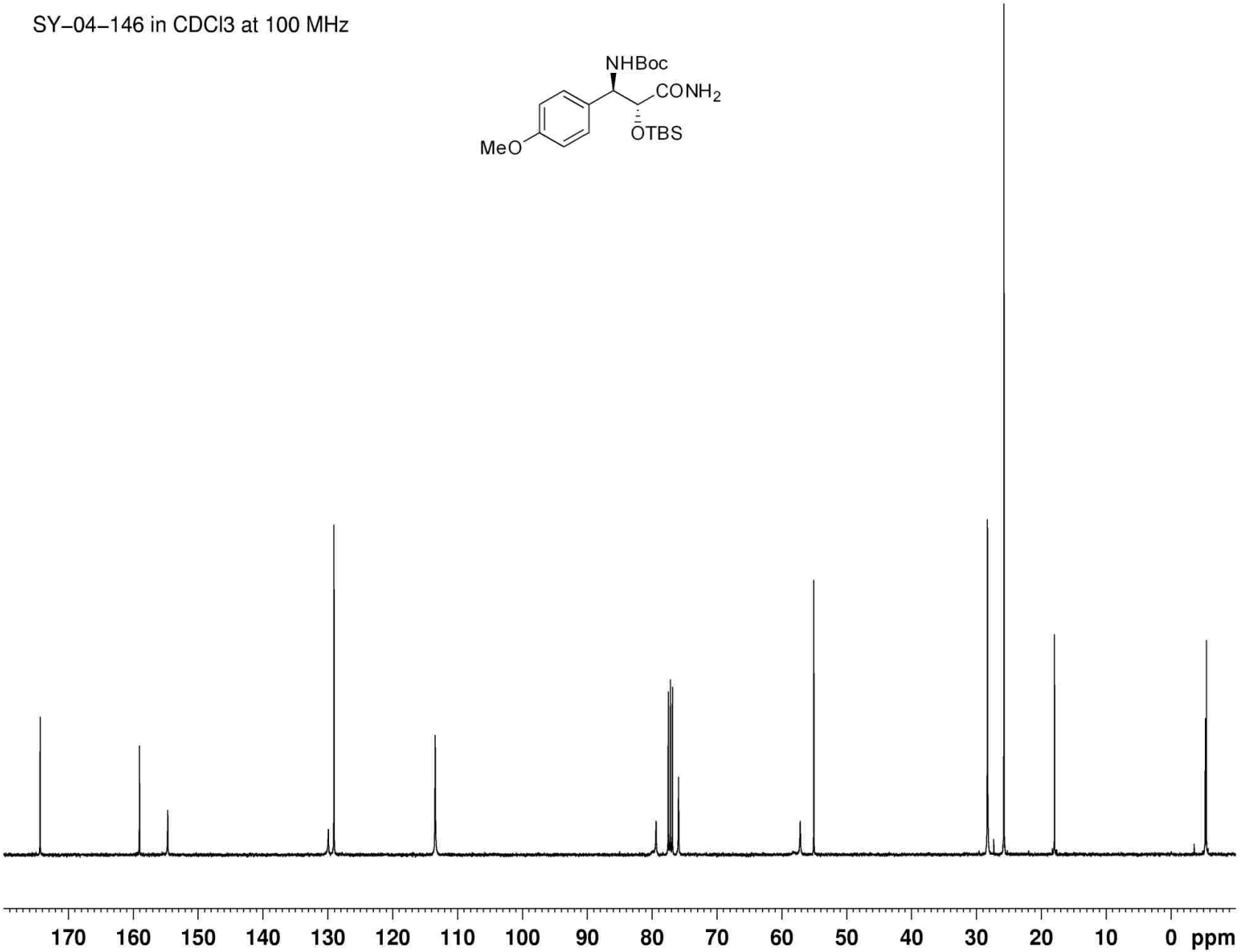
Compound **231** (Scheme 5.3) – ^{13}C NMR spectrum



Compound **232** (Scheme 5.3) – ^{1}H NMR spectrum

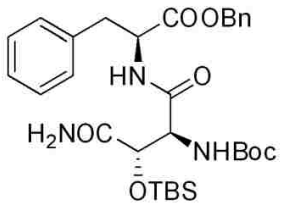


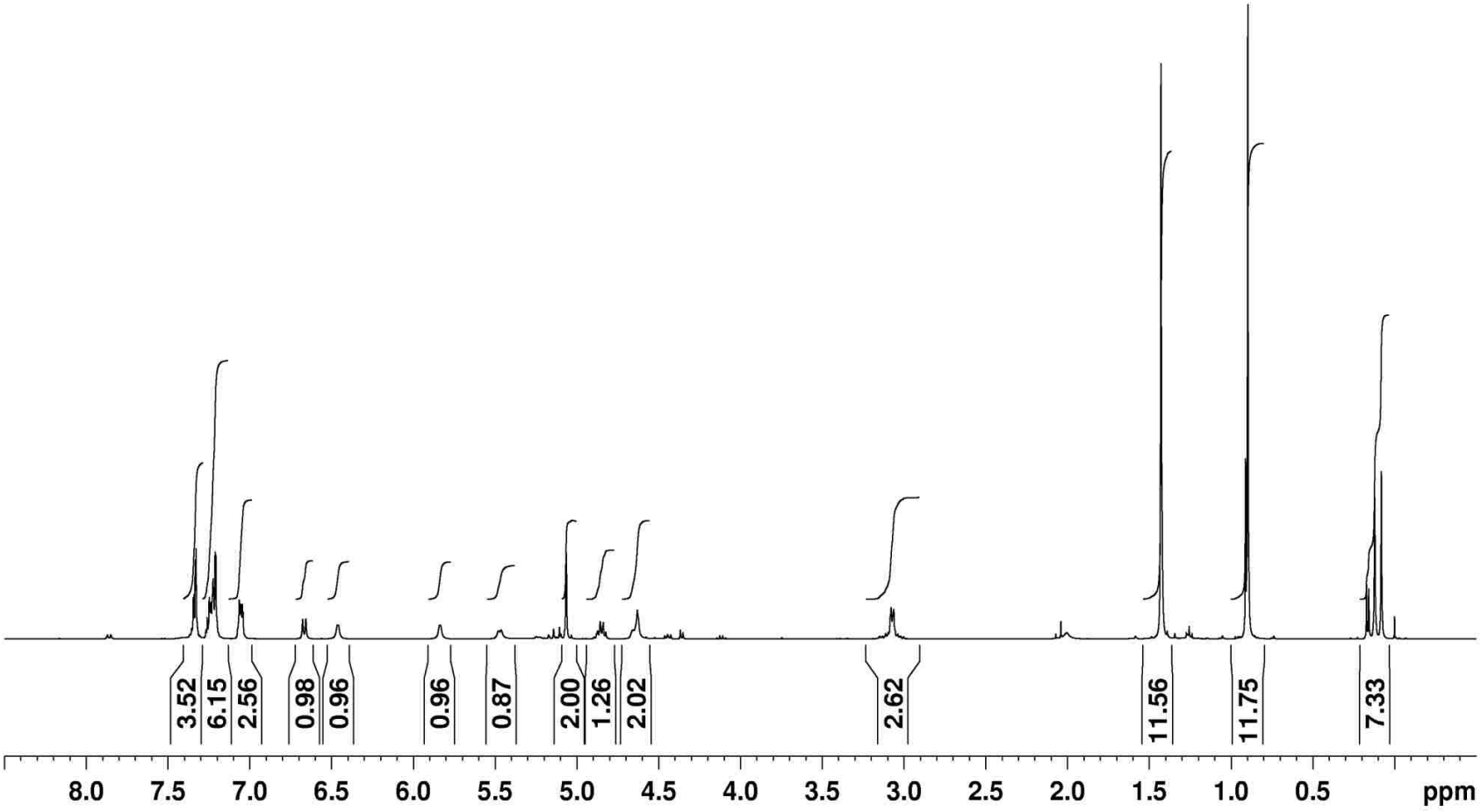
Compound **232** (Scheme 5.3) – ^{13}C NMR spectrum



Compound **234** (Scheme 5.3) – ^{13}C NMR spectrum

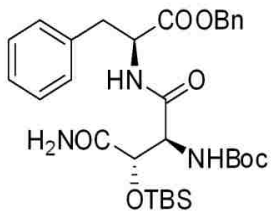
SY–01–129 in CDCl3 at 400 MHz

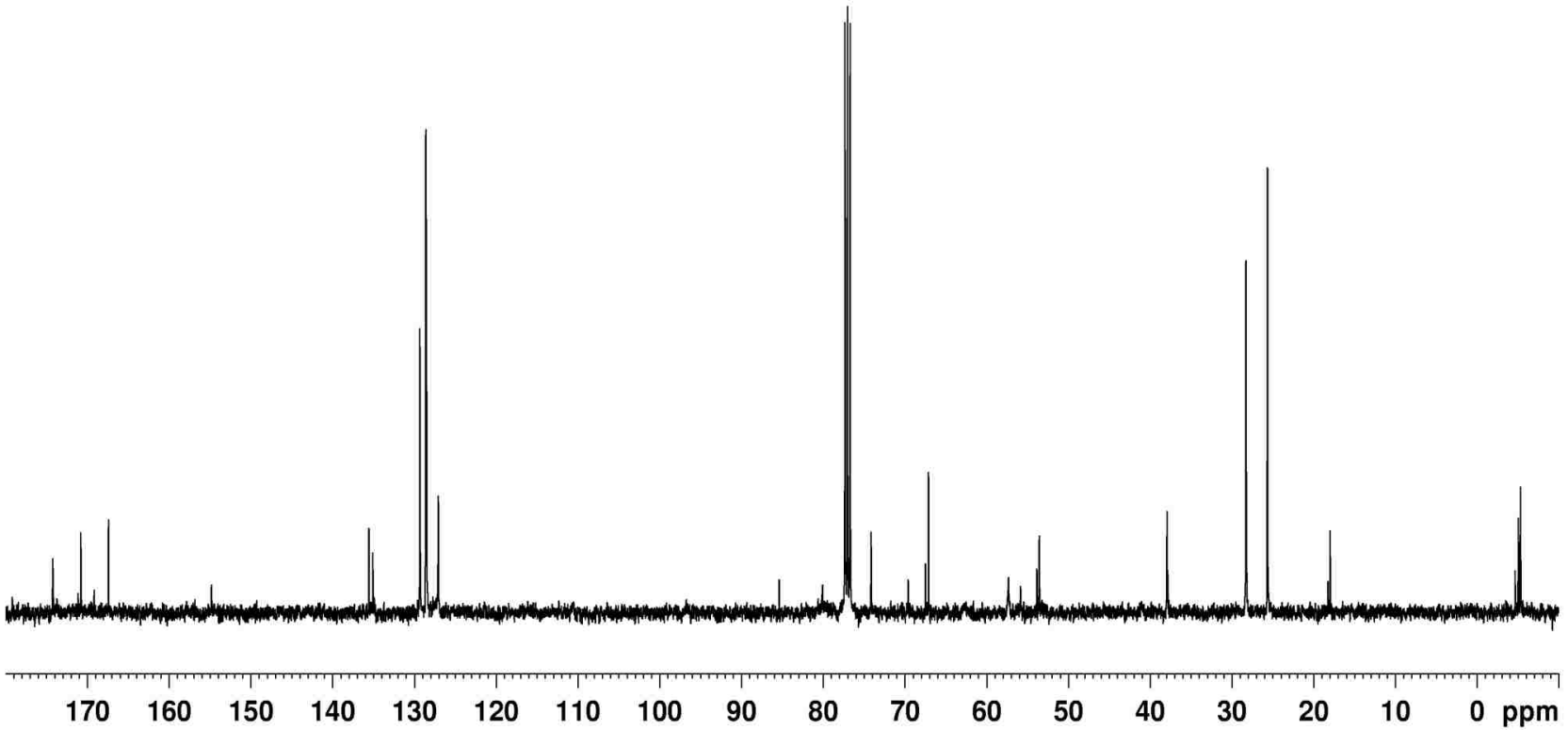




Compound **234** (Scheme 5.3) – ^{13}C NMR spectrum

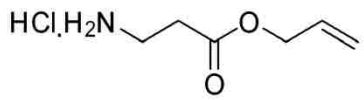
SY–01–129 in CDCl3 at 100 MHz

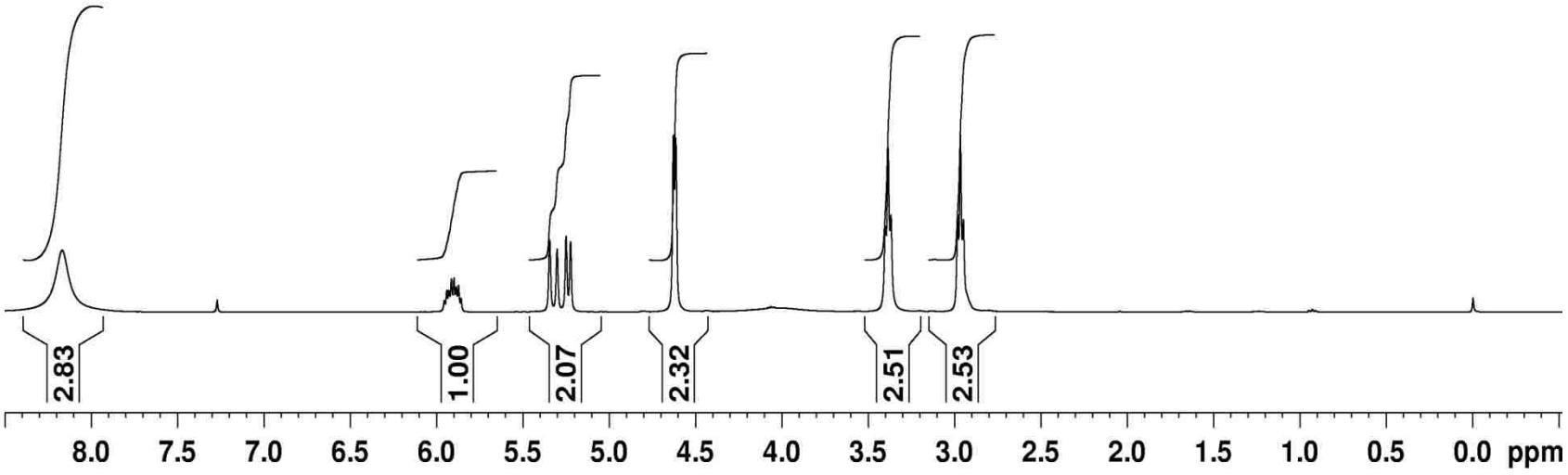




Compound **225** (Scheme 5.6) – ^{1}H NMR spectrum

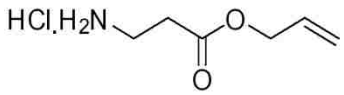
SY–01–40 in CDCl3 at 400 MHz

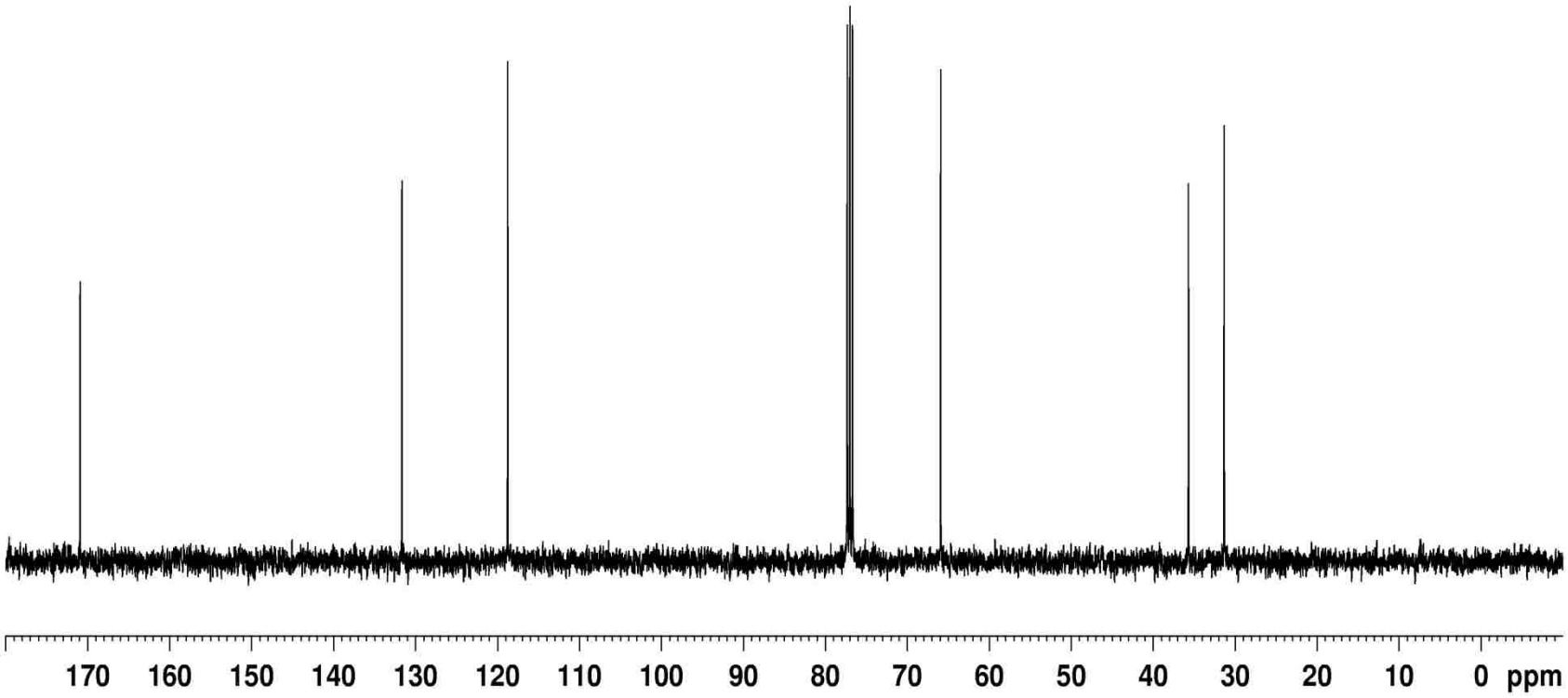




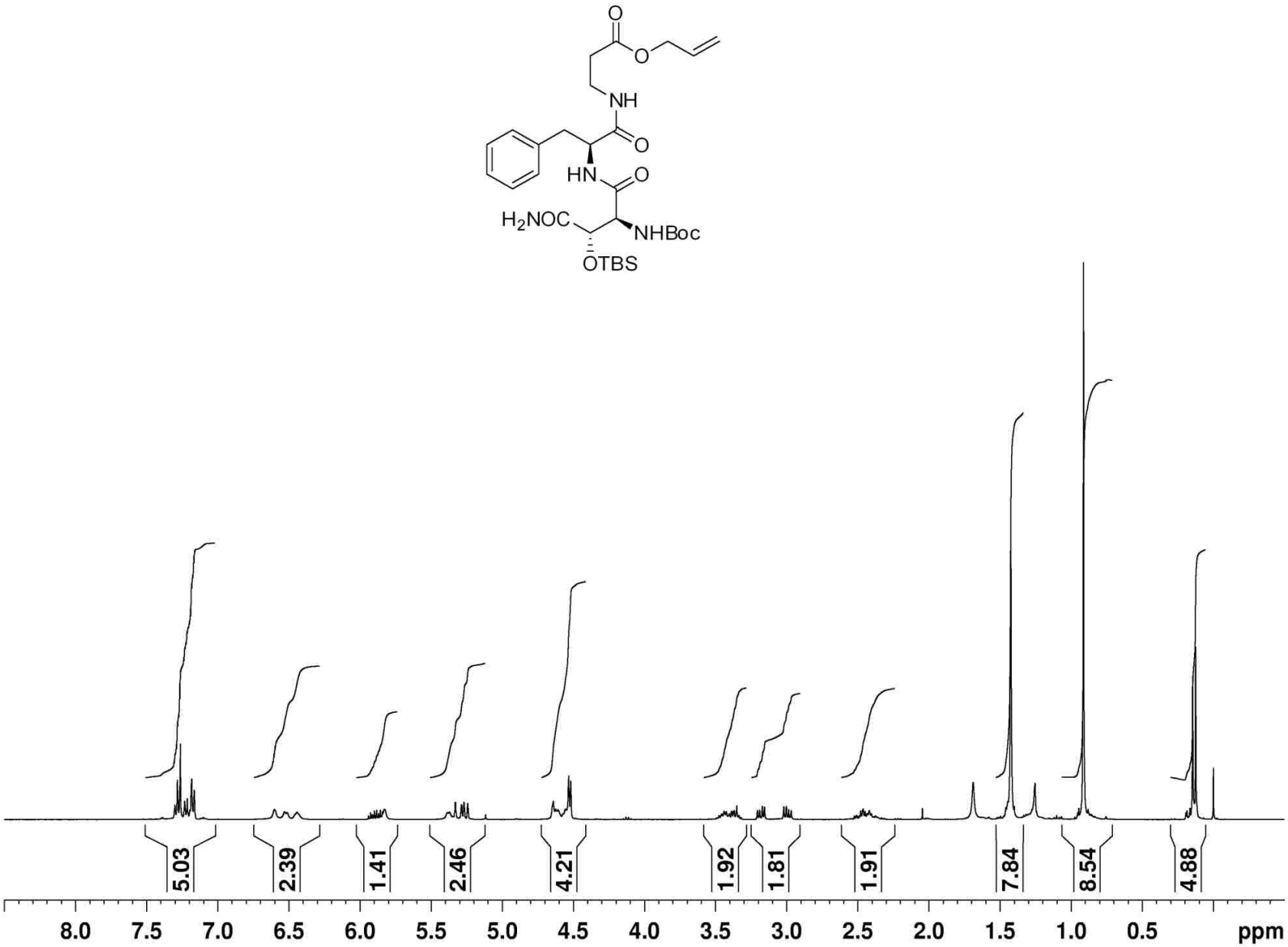
Compound **225** (Scheme 5.6) – ^{13}C NMR spectrum

SY–01–40 in CDCL3 at 100 MHz

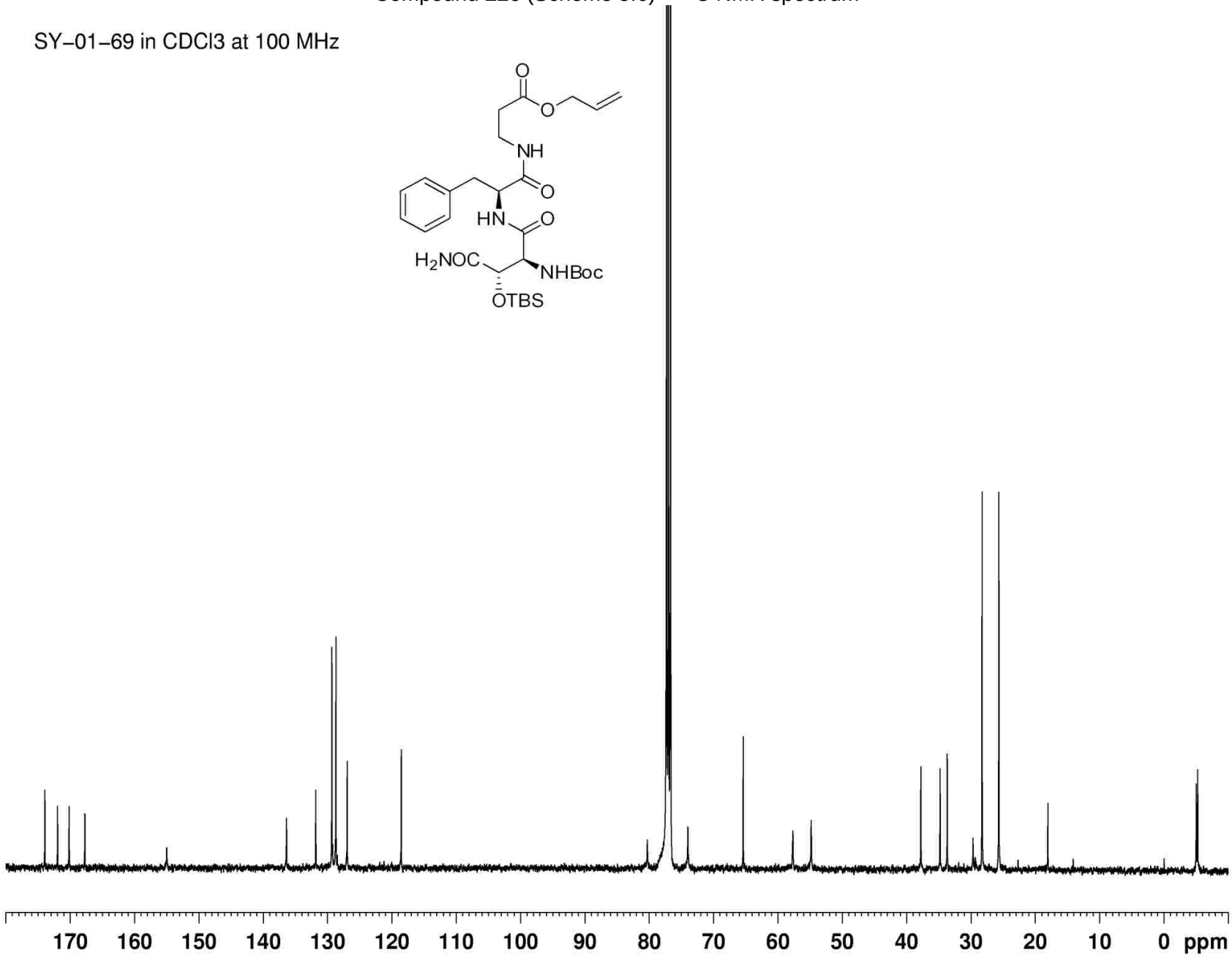




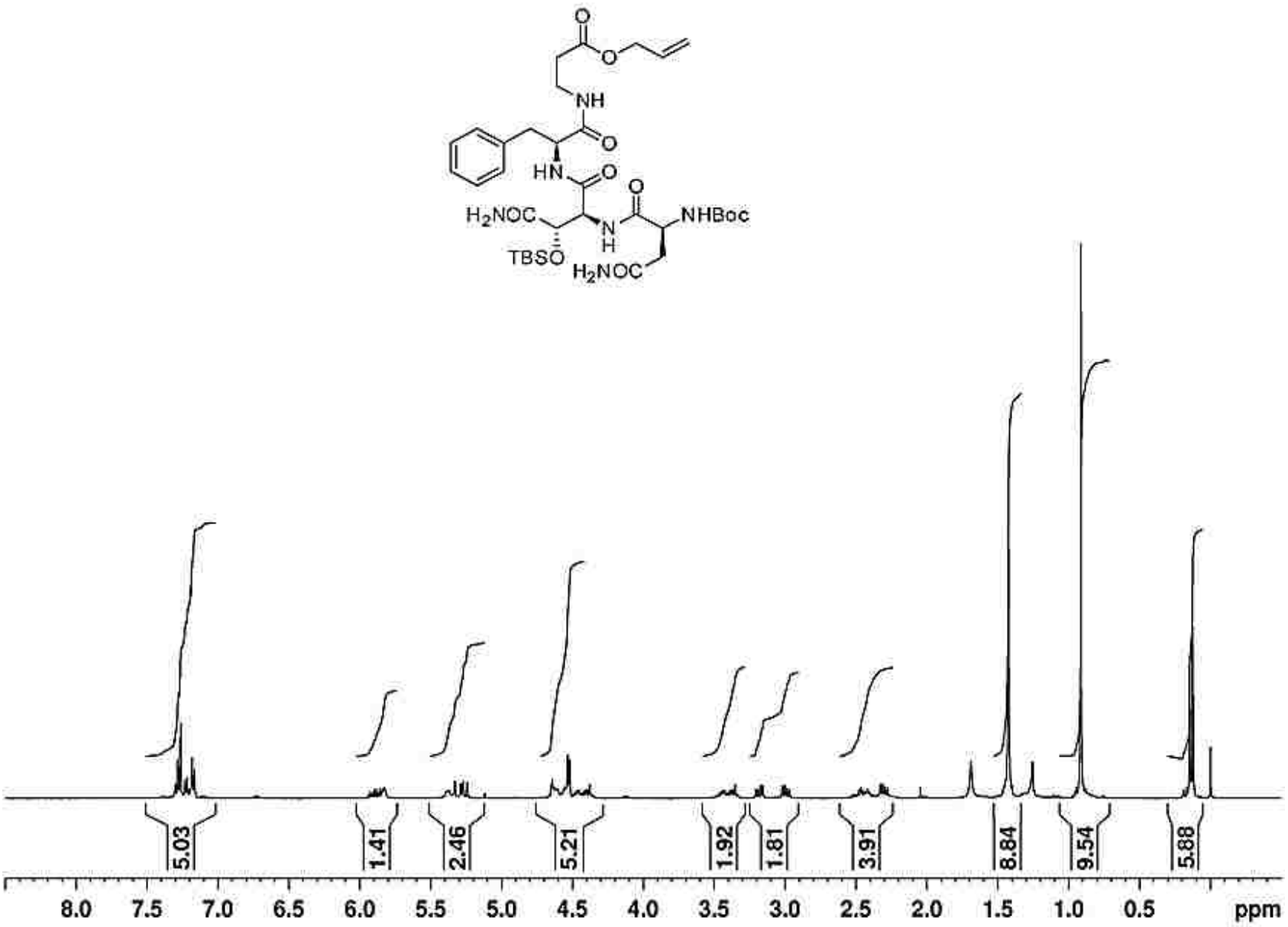
Compound **226** (Scheme 5.6) – ^{1}H NMR spectrum



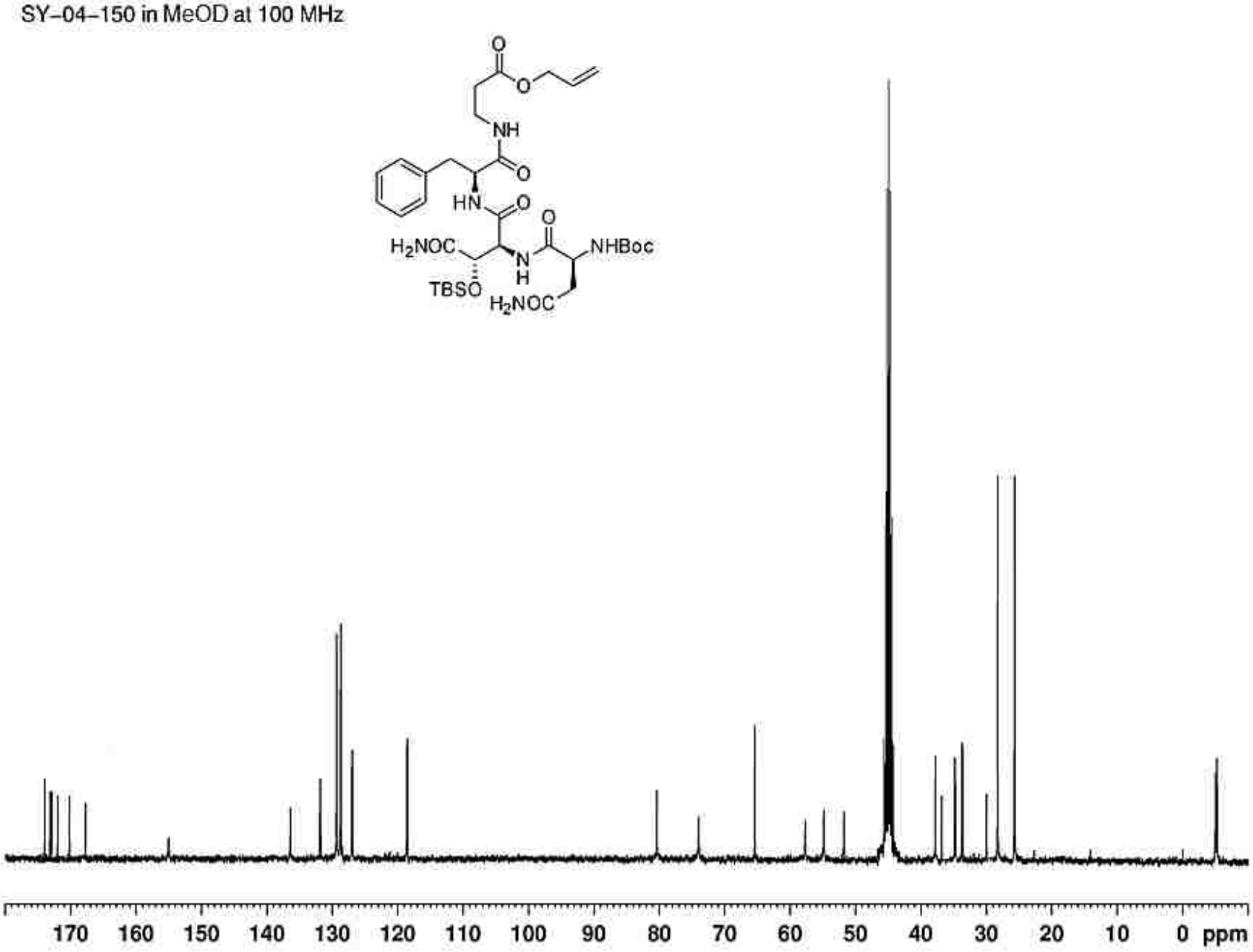
Compound **226** (Scheme 5.6) – ^{13}C NMR spectrum



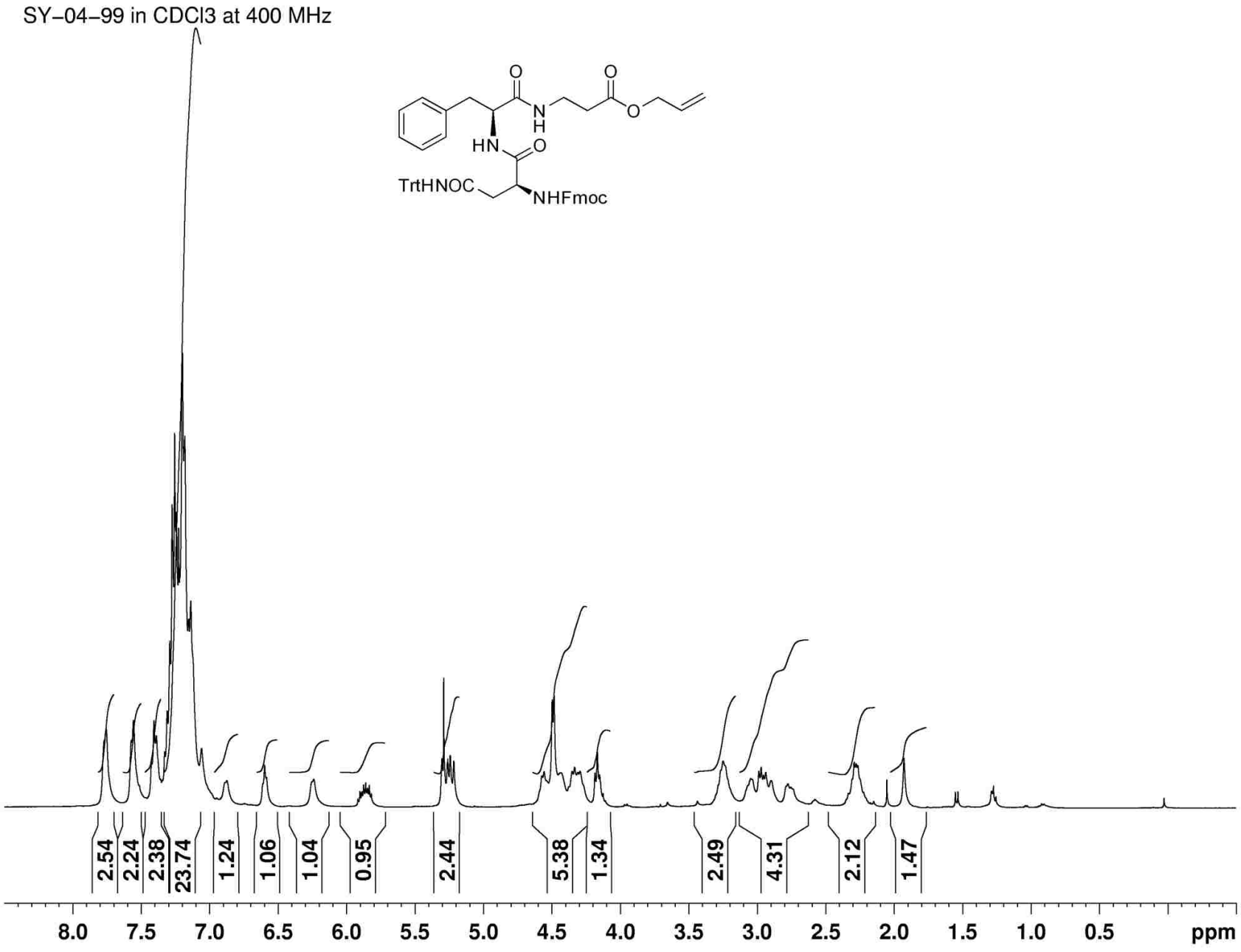
Compound **249** (Scheme 5.8) – ^{1}H NMR spectrum



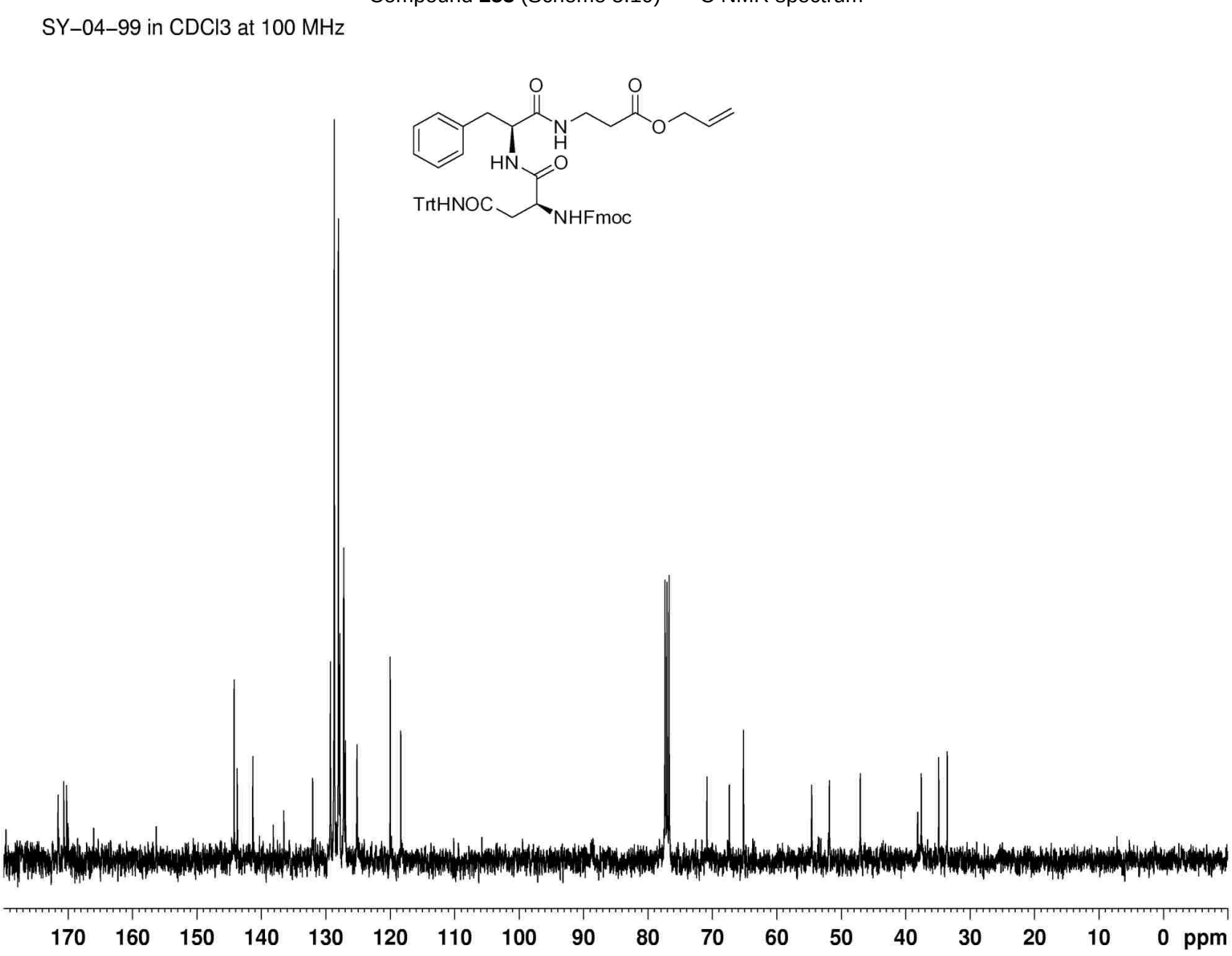
Compound **249** (Scheme 5.8) – ^{1}H NMR spectrum



Compound **253** (Scheme 5.10) – ^{1}H NMR spectrum

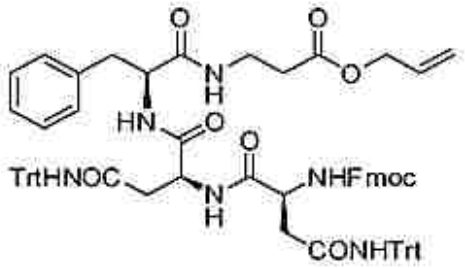


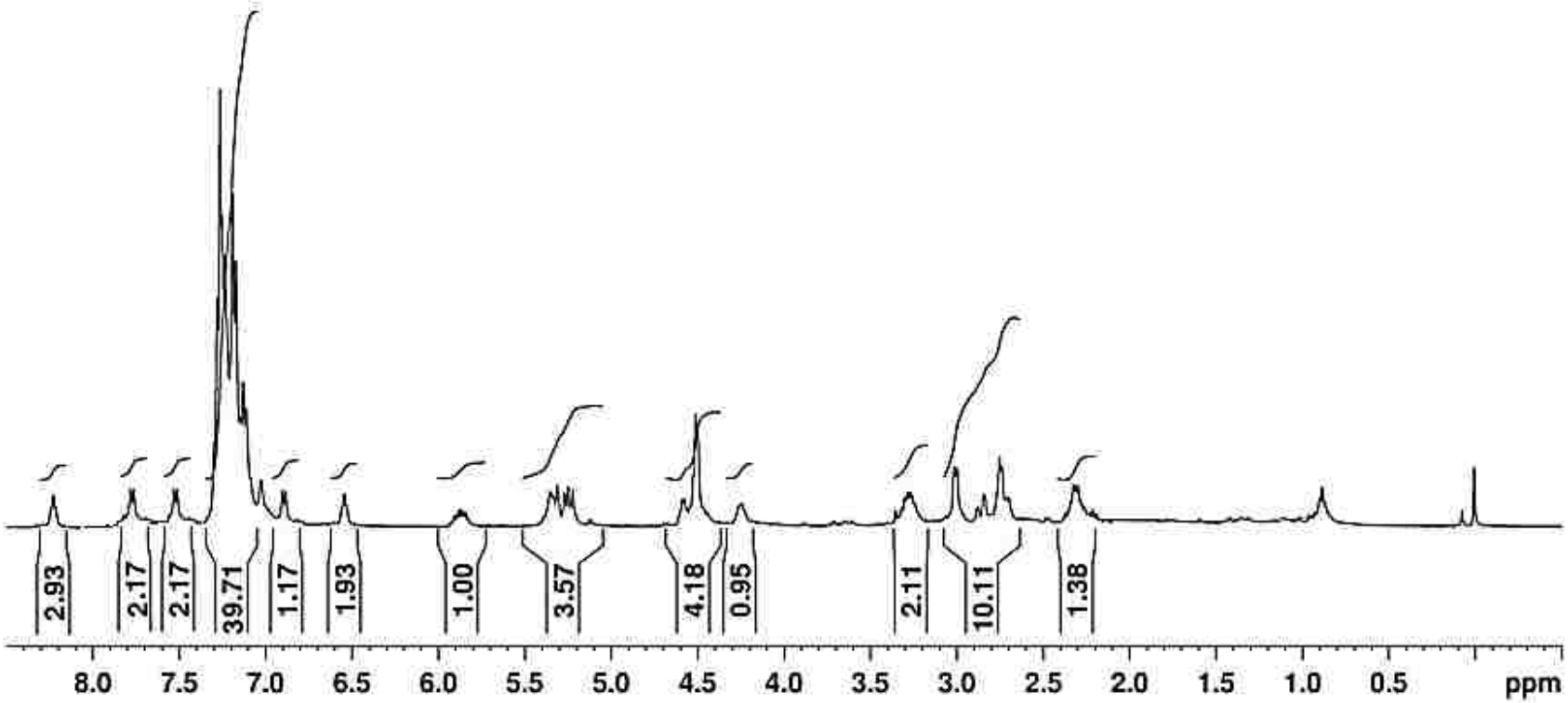
Compound **253** (Scheme 5.10) – ^{13}C NMR spectrum



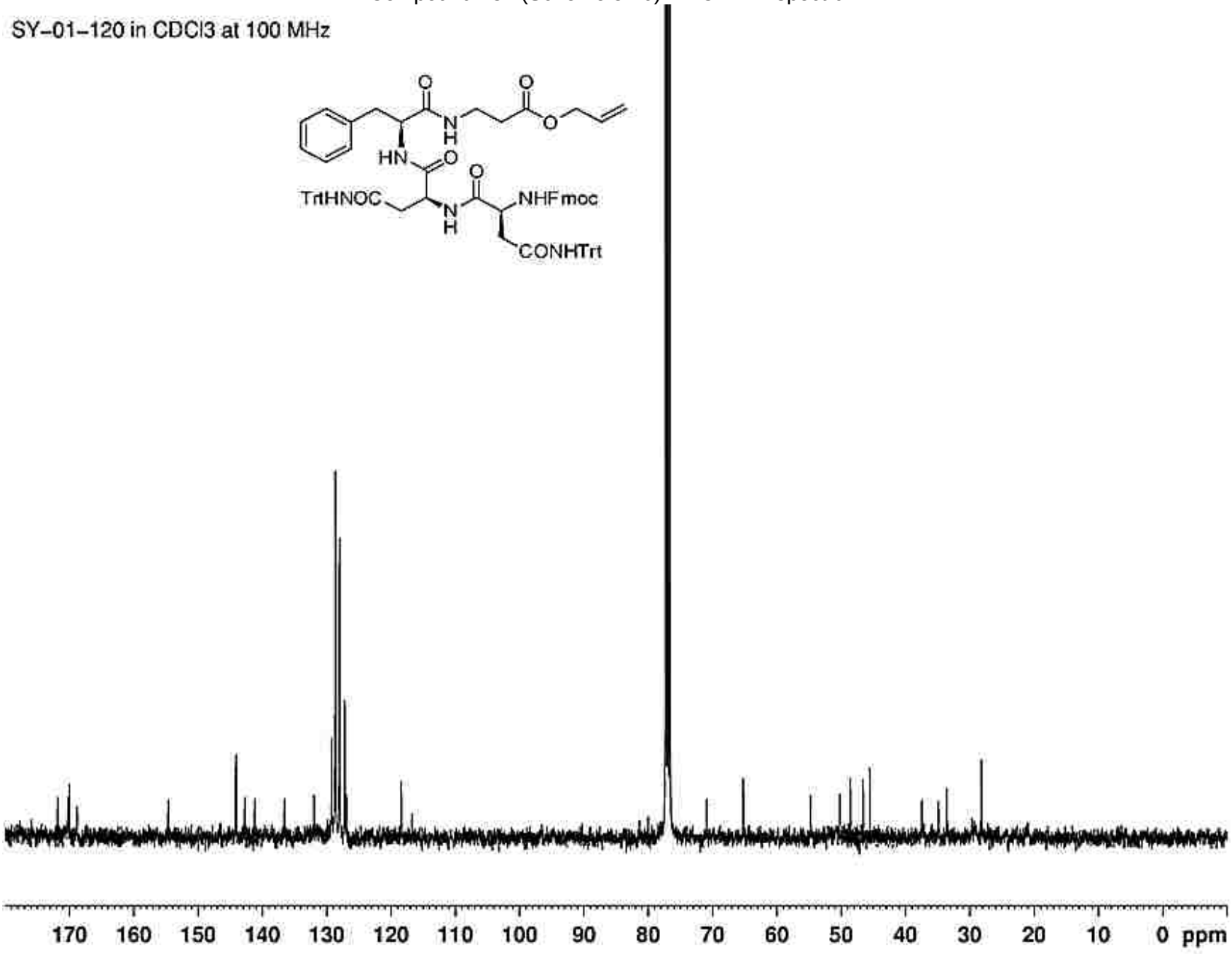
Compound **254** (Scheme 5.10) – ^{1}H NMR spectrum

SY–04–120 in CDCl3 at 400 MHz





Compound **254** (Scheme 5.10) – ^{13}C NMR spectrum



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APPENDIX: LETTERS OF PERMISSION

Figure 1.2 - Page 3

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Figure 1.8 - Page 14

Title: Interaction between the Marine Sponge Cyclic Peptide Theonellamide A and Sterols in Lipid Bilayers As Viewed by Surface Plasmon Resonance and Solid-State 2H Nuclear Magnetic Resonance

Author: Rafael Atillo Espiritu, Nobuaki Matsumori, Michio Murata, Shinichi Nishimura, Hideaki Kakeya, Shigeki Matsunaga, and Minoru Yoshida

Publication: Biochemistry

Publisher: American Chemical Society

Date: Apr 1, 2013

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