STEREOCHEMICAL ASSIGNMENT AND TOTAL SYNTHESIS OF AN ANTIMALARIAL LIPOPEPTIDE

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To my wife

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SUMMARY

Malaria is one of the three prime causes (together with tuberculosis and AIDS) responsible for the high mortality in this world. 300-500 Millions people suffer from the disease every year resulting in about one million deaths. In recent years, malaria is considered as a complex multisystem disorder. As more than 40% of the world's population lives in malaria endemic areas, the challenge is to understand the complexities of this disease and develop potential tools for improving the present scenario. There is also the immediate need for the discovery of cost effective drugs or vaccines to fight mainly chloroquine-resistant strains of *P. falciparum*.

The lipopeptide (**N1708**) isolated from *Streptomyces* sp. using bioassay-guided isolation by MerLion Pharmaceuticals exhibits promising activity against *Plasmodium falciparum* (IC_{50}= 0.8 μM against 3D7 strain). NMR and mass analyses suggest that this peptide contains two non-proteinogenic amino acids, one aspartic acid and a ten carbon long chain fatty acid containing a *trans*-double bond and a chiral centre. As it is a well-known problem that the half life of peptide drugs is short because of the enzymatic hydrolysis of the amide bond formed by proteinogenic amino acids, thereby we are interested to find out the configuration of these six chiral centres (one of them is quaternary) present in this lipopeptide. Synthesis and stereochemical assignment of the non-proteinogenic amino acids and rest of the fragments have been performed in this work. The non-proteinogenic amino acids have been synthesised and their absolute configuration assigned to the chiral centres with the help of Marfey's reagent. The full structure of **N1708** has been confirmed by the total synthesis of the targeted lipopeptide.

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ABBREVIATIONS AND SYMBOLS

δ	Chemical shift (in NMR spectroscopy)
^{13}C NMR	carbon nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
4Å MS	4Å molecular sieves
Ac	acetyl
ACN	acetonitrile
AD-mix α	$(DHQ)_2PHAL+K_2OsO_2(OH)_4+K_3Fe(CN)_6$
AD-mix β	$(DHQD)_2PHAL+K_2OsO_2(OH)_4+K_3Fe(CN)_6$
AIBN	2,2'-azo *bis*isobutyronitrile
AIDS	acquired immunodeficiency syndrome
aq.	aqueous
AQN	anthraquinone
Atm.	atmosphere
BAIB	bis(acetoxy)iodobenzene
Bn	benzyl
Boc	*t*-butoxycarbonyl
$(Boc)_2O$	di-*tert*-butyl dicarbonate
br	broad
BuLi	butyl lithium
Bz	benzoyl
calcd	calculated

CAN	cerium(IV) Ammonium Nitrate
Cat D	cathepsin D
cat.	catalytic
Cbz	benzyloxycarbonyl
CM	cerebral malaria
COSY	correlated spectroscopy
CSA	camphorsufonic acid
d	doublet
DCC	*N,N'*-dicyclohexylcarbodiimide
DCE	dichloroethane
DCM	dichloromethane
dd	doublet of doublets
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEPT	distortionless enhancement by polarization transfer
DHFR	dihydrofolate reductase
DHPS	deoxyhypusine synthase
$(DHQ)_2PHAL$	*bis*(dihydroquinino)phthalazine
$(DHQD)_2PHAL$	*bis*(dihydroquinidino)phthalazine
DIAD	diisopropyl azodicarboxylate
DIBALH	diisobutylaluminium hydride
DIPEA	*N,N*-diisopropylethylamine (Hünig's base)
DMAP	*N,N*-4-dimethylaminopyridine
DMBA	dimethylbenzyl acetal

DME	dimethoxyethane
DMF	*N,N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DOXP	1-deoxy-D-xylulose-5-phosphate
DPAP1	dipeptidyl aminopeptidase 1
dr	diasteromeric ratio
EA	ethyl acetate
ee	enantiomeric excess
ESI-MS	electrospray ionization mass spectrometry
Et	ethyl
FDAA	1-fluoro-2,4-dinitrophenyl-5-L-alanine amide
Fmoc	9-fluorenylmethoxycarbonyl
FP	falcipain
FV	food vacuole
GCMS	gas chromatography–mass spectrometry
h	hour
HAP	histo-aspartic protease
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HF	hydrofluoric acid
HGXPRT	hypoxanthine-guanine-xanthine

	phosphoribosyltranferase
HMBC	heteronuclear multiple bond correlation
HMDS	1,1,1,3,3,3-hexamethyldisilazane
HMPA	*N,N,N′,N′,N″,N″*-hexamethylphosphoric triamide
HMQC	heteronuclear multiple quantum coherence
HOAt	1-hydroxy-7-azabenzotriazole
HPLC	high-performance liquid chromatography
HRMS	high resolution mass spectrum
HWE	Horner-Wadsworth-Emmons
Hz	hertz
IBX	2-iodoxybenzoic acid
IC_{50}	half maximal inhibitory concentration
Im	imidazole
IR	infrared
J	coupling constant
KHMDS	potassium bis(trimethylsilyl)amide
LAH	lithium aluminum hydride
LCMS	liquid chromatography-mass spectrometry
LDA	lithium diisopropylamide
LDH	lactate dehydrogenase
LiHMDS	lithium bis(trimethylsilyl)amide
M	Molar
m	meta

m	multiplet
m/z	mass to charge ratio
mCPBA	*m*-chloroperbenzoic acid
Me	methyl
mg	milligram
mL	milliliter
mmol	millimol
Ms	methanesulfonyl
n	normal (*e.g.*, unbranched alkyl chain)
NAD^+	nicotinamide adenine dinucleotide
NaHMDS	sodium bis(trimethylsilyl)amide
NMO	*N*-methylmorpholine oxide
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser enhancement
NPP	new permeation pathway
o	ortho
O/N	overnight
p	para
PCC	pyridinium chlorochromate
Pd/C	palladium on charcoal
PDC	pyridinium dichromate
PG	protecting group
Ph	phenyl

Piv	pivaloyl
PK	protein kinase
PM	plasmepsin
PMB	*p*-methoxybenzyl
PMP	*p*-methoxyphenyl
ppm	parts per million
PPM	parasite plasma membrane
PPTS	pyridinium p-toluenesulfonate
psi	pound per square inch
PTSA	*p*-toluenesulfonic acid
Py	pyridine
q	quartet
RBC	red blood cell
R_f	retention factor
RNA	ribonucleic acid
rt	room temperature
s	singlet
S.M	starting material
SAD	Sharpless asymmetric dihydroxylation
t	triplet
t (or) *tert*	tertiary
TBAF	tetra-*n*-butylammonium fluoride
TBAI	tetra-*n*-butylammonium iodide

TBDPS	*t*-butyldiphenylsilyl
TBS	*t*-butyldimethylsilyl
TCA	tricarboxylic acid cycle
TCA	trichloroacetimidate
TEA	Triethylamine
TEMPO	(2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl
TES	triethyl silyl
TESOTf	triethylsilyl trifluoromethanesulfonate
Tf	triflate (trifluoromethanesulfonate)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TPAP	tetrapropylammonium perruthenate
Ts	*p*-toluenesulfonyl
UV	ultraviolet
μM	micromolar

PUBLICATIONS

1. *Absolute Configuration and Total Synthesis of a Novel Antimalarial Lipopeptide by the de Novo Preparation of Chiral Nonproteinogenic Amino Acids*, Shibaji K. Ghosh, Brinda Somanadhan, Kevin S.-W. Tan, Mark S. Butler, and Martin J. Lear.- *Org. Lett.* **2012**, *14*, 1560-1563.
2. *Synthesis of 2-C-Methylerythritols and 2-C-Methylthreitols via Enantiodivergent Sharpless Dihydroxylation of Trisubstituted Olefin*, Shibaji K. Ghosh, Mark S. Butler, and Martin J. Lear.- *Tetrahedron Lett.* (*in press).*

Conference Publications:

1. Shibaji K. Ghosh, Martin J. Lear; "*Stereochemical Assignment and Total Synthesis of an Anti-malarial Lipopeptide",* **The 6th Mathematics and Physical Science Graduate Congress, University of Malaya, Malaysia , 13th - 15th Dec, 2010.** *(Oral Presentation)*
2. Shibaji K. Ghosh, Brinda Somanadhan, Mark S. Butler, Martin J. Lear; *"Amino Acid Stereochemical Assignment and Total Synthesis of A Natural Anti-malarial Peptide"*, **239th ACS National Meeting, San Francisco, CA, United States, 21st - 25th Mar, 2010**.*(Poster)*
3. Shibaji K. Ghosh, Brinda Somanadhan, Mark S. Butler, Martin J. Lear; *"Synthetic Determination of the Absolute Configuration of A Natural Anti-malarial Peptide"*, **6th Singapore International Chemical Conference, Singapore, 15th – 18th Dec, 2009**. *(Poster)*
4. Shibaji Kumar Ghosh, Martin J. Lear; *"Asymmetric Synthesis of 2-C-methylerythritol and 2-C-methylthreitol in High Enantiomeric Purity"*, **Tenth Tetrahedron Symposium, Paris, France, 23rd – 26th Jun, 2009**. *(Poster)*

CHAPTER 1

Introduction

1.1 Malaria background

Malaria is one of the three prime causes (together with tuberculosis and AIDS) responsible for high mortality in this world. 300-500 Millions people suffer from the disease every year resulting in about one million deaths.[1] It is a very old parasitic disease caused by different types of *Plasmodium* species namely *P. falciparum*, *P. vivax*, *P. malariae and P. ovale*. *P. falciparum* is the most deadly one for the majority of humans. High fever, chills, headache and vomiting are the signs of malaria. Severe malaria is traditionally viewed in two pathogenic processes; destruction of red blood cells (anaemia) and cerebral malaria (CM) due to small vessels blockage in the brain by sequestered parasites. In recent years, malaria is considered as a complex multisystem disorder.[2] As more than 40% of the world's population lives in malaria endemic areas (Figure **1.1**), the challenge is to understand the complexities of this disease and to produce some potential tools for improving the present scenario. There is also the immediate need for the discovery of cost effective drugs or vaccines to fight mainly chloroquine-resistant strains of *P. falciparum.*

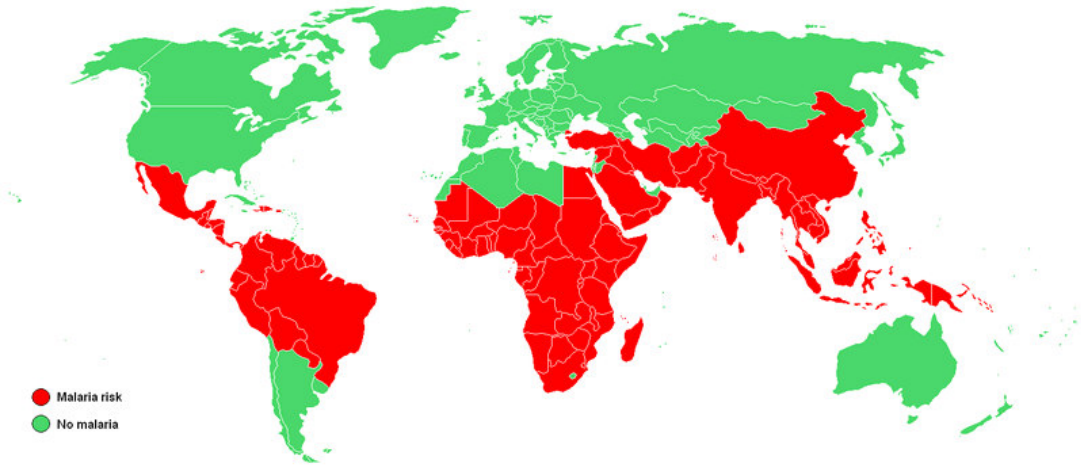


Figure 1.1: Global malaria distribution and endemicity, 2003.*

1.1.1 Life cycle of the malaria parasite

The life cycle of malarial parasites (Figure **1.2**) is distinctly divided into two hosts. The female anopheles mosquito, where the sexual cycle of the parasites takes place, is the primary host. The secondary host is the human body, which is needed for completing their asexual cycle. Sporozoites are released into the human blood stream when an infected female anopheles mosquito bites. Sporozoites first hit the human liver and begin their asexual cycle resulting in the formation of merozoites which enter the erythrocytes and grow as trophozoites in the ring stage by feeding on the host cell haemoglobin. Lysis of the erythrocyte releases merozoites that attack new erythrocytes thus completing the cycle. This whole process occurs in 48 hours and the release of merozoits from red blood cells (RBC) causes the sporadic symptoms of fever, shivering and anaemia, i.e., the characteristics of malaria. During this process, some immature trophozoites produce gametocytes. The male and female gametocytes enter into the mosquito when it bites the person carrying the parasite. After reaching the mid-gut of the mosquito, female gametocytes transform into macro-gametes whereas the male gametocytes divide into micro-gametes. Next, the male and female gametes combine to form a zygote. The zygote transforms into sporozoites through

various complex stages. Finally sporozoites reach the salivary glands and are transmitted to the human body by the bite of the mosquito.

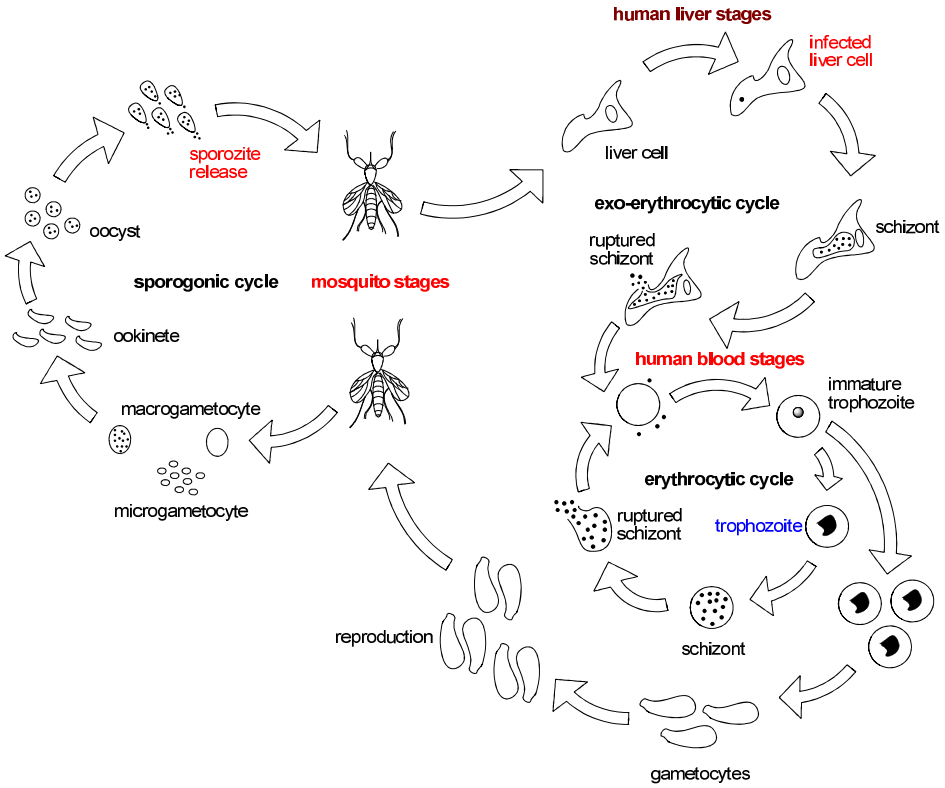


Figure 1.2: Life cycle of *Plasmodium falciparum*.[20]

1.1.2 Haemoglobin metabolism

Metabolism of haemoglobin is crucial for the survival of the malaria parasite.[3] Inside the erythrocyte the parasite breaks down the host haemoglobin to produce amino acids for parasitic protein synthesis.[4,5,6] Aspartic protease (plasmepsin) initiates the break down process and then cysteine protease (falcipain) and some other proteases are involved for proteolysis to occur optimally in the acidic food vacuole (FV). Here, it is found that plasmepsins (PM) are highly selective towards native haemoglobin and it is believed PM I and II cleave at the hinge between 33-

phenylalanine and 34-leucine which is vital for the tetrameric structure of haemoglobin. Cysteine protease is unable to detect the native haemoglobin, but it takes part to degrade denatured haemoglobin.[7] The aspartic protease can also cleave the 105-106 peptide bond in the loosely folded α chain, but not in native haemoglobin.[8,9] The metalloprotease falcilysin can only hydrolyse small peptides.[10] Dipeptidyl aminopeptidase 1 (DPAP1) is known to be involved to the hydrolysis of haemoglobin derived oligopeptides.[11] Finally, free amino acids are produced in the cytoplasm by aminopeptidases.[12] The general pathway is depicted in Figure **1.3.**[13] Degradation of haemoglobin produce considerable amounts of heme which is almost entirely oxidised from ferrous (II) to ferric (III) hematin.[14] As heme and hematin are toxic to the parasite, the released hematin is detoxified by polymerase activity to generate the crystalline insoluble polymer hemozoin.[15] Hemozoin is also well known as the malaria pigment that is microscopically visible as a characteristic feature of the disease.

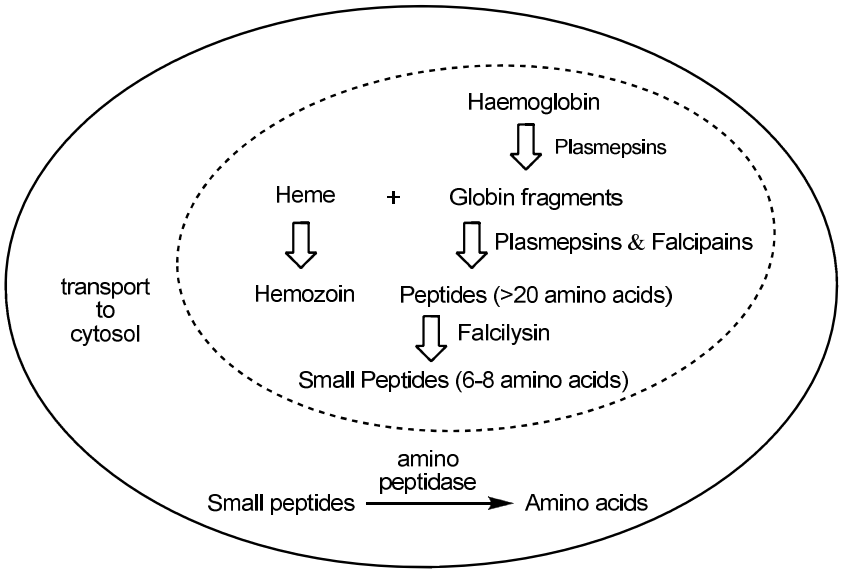


Figure 1.3: General haemoglobin catabolism pathway.

1.2 Antimalarial drugs

As the mechanisms of action of most antimalarial drugs are not clear, one popular way to categorise them is according to their activity in different stages of parasite life cycle.

1.2.1 Causal prophylaxis

These type of agents have lethal effects at the pre-erythrocytic stage of the parasite. Primaquine and malarone are currently used for that purpose. As these work at an early stage, this agents prevent the typical characteristics of malaria. Vaccines can be a very effective tool in this category in future.[16]

1.2.2 Suppressive prophylaxis

Suppressive treatments work at the erythrocytic stage. Causal prophylactic agents along with those used for chemoprophylaxis are applied when travelling in malaria endemic areas. Common suppressive prophylactic agents are chloroquine and mefloquine.

1.2.3 Clinical cure

These type of agents are involved in killing erytrocytic schizogony and prevent clinical attack. They are also called blood schizonticides. These include the 4-aminoquinolines (e.g. chloroquine), the phenanthrenes (e.g. halofrantrine), the antifolates (e.g. pyrimethamine, proguanil, dapsone and sulfadoxine), the artemisinin group (e.g. dihydroartemisinin, artesunate and artemether) and some antibiotics (e.g. tetracycline and doxycycline).

1.2.4 Radical cure

After elimination of the parasite from the bloodstream, the hypnozoites are taken out from the liver by the 14 day course of primaquine, a radical cure agent.[17]

1.2.5 Controlling transmission

Transmission of malaria via mosquito can be restricted by demolishing the gametocytes using primaquine, the artemisinins and pyrimethamine.

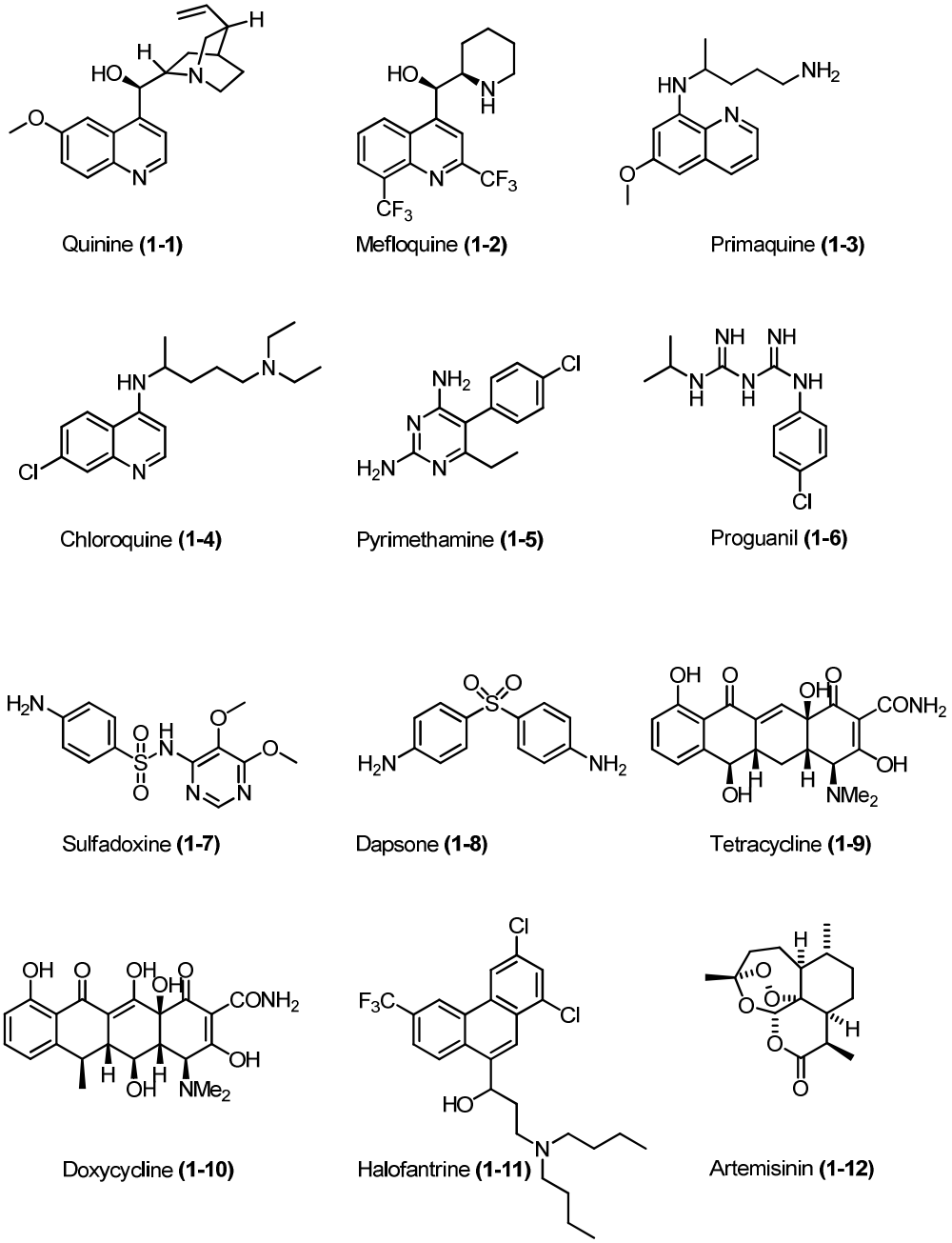


Figure 1.4: Structurally different antimalarial drugs.

Antimalarial drugs can also be classified according to their chemical structures.

1.2.6 Quinoline with secondary alcohols

Quinine and mefloquine belongs to this group. Quinine was isolated from the bark of cinchona trees whereas mefloquine (a synthetic analogue of quinine) was developed by the Walter Reed Army Institute of Research in 1970. Quinine was the only known effective drug for many years for the treatment of malaria but currently it is only used for the treatment of severe malaria due to increasing drug resistance. Although the exact modes of action of these drugs are not known, it is believed that they play a role in preventing hemezoin formation from heme.[18]

1.2.7 8-aminoquinilines

Primaquine (**1-3**) is the only marketed antimalarial drug that belongs to this group. This is a radical cure agent. Tafenoquine shows promise in a clinical trial (phase II) for the treatment of *P. vivax* in adults. The main advantage of tafenoquine is its long half-life and thereby no need to take as frequently as primaquine.[19] It is proposed that this class of drug has an effect on parasite mitochondria.

1.2.8 4-aminoquinilines

Chloroquine (**1-4**) is the main drug that belongs to this group. This highly toxic compound was considered as an antimalarial drug during the Second World War. It was the first-line treatment even ten years ago but huge parasite resistance has forced a reduction in its use. This class may also have an important role in the heme poisoning process.

1.2.9 Antifolates

This class of antimalarials works by inhibiting dihydrofolate reductase (DHFR) and deoxyhypusine synthase (DHPS). Pyrimethamine (**1-5**) and proguanil (**1-6**) are common DHFR inhibitors whereas sulfadoxine (**1-7**, sulfonamides) and dapsone (**1-8**,

sulfones) are DHPS inhibitors. Sulfadoxine and pyrimethamine are used in combination for drug therapy in some parts of Africa.[18]

1.2.10 Antibiotics

Antibiotics are used along with other antimalarial drugs. Tetracycline and doxycycline are the common drugs for this purpose.

1.2.11 Phenanthrenes

Halofantrine (**1-11**) is a popular antimalarial drug in this class. This was identified during the Second World War. This class of drug acts on blood schizonts in preventing the disease.

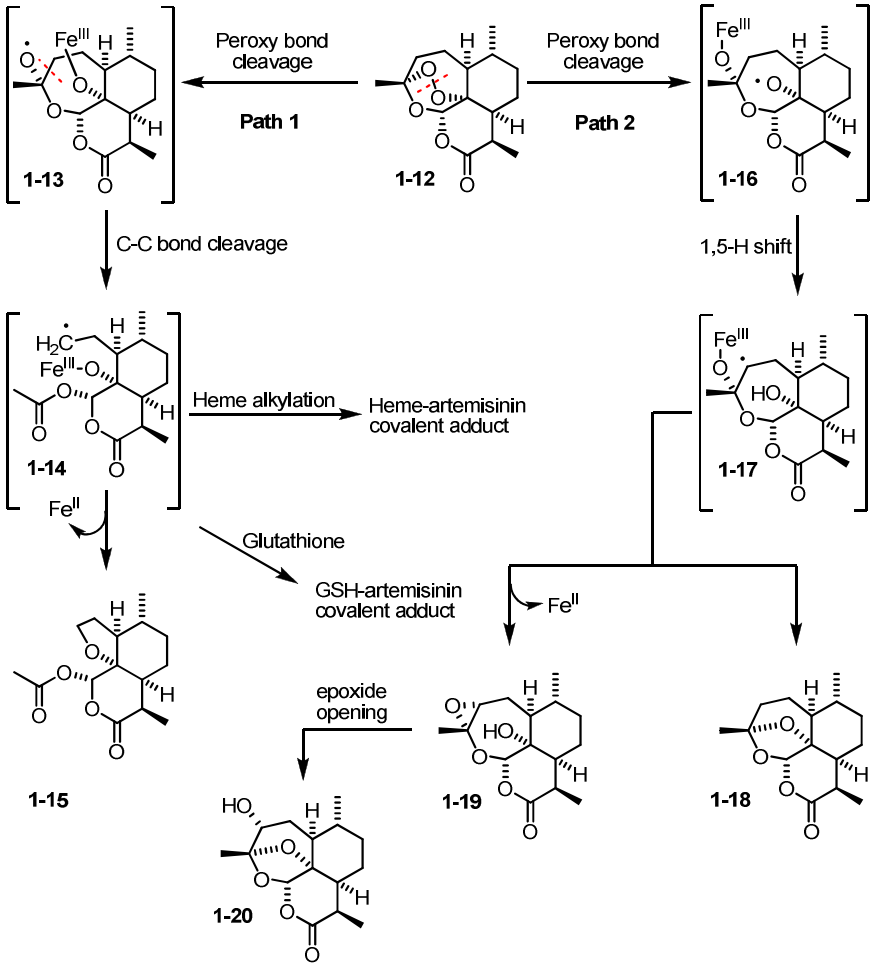
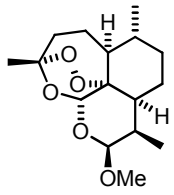


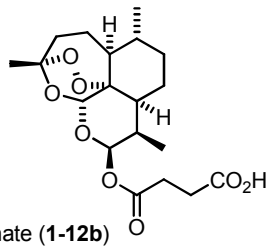
Figure 1.5: Radical mechanism of the artemisinin class of drugs.[20]

1.2.12 Artemisinins/Sesquiterpene

Artemisinin is a very useful antimalarial. Because of poor bioavailability, the semi-synthetic artemether (**1-12a**) and artesunate (**1-12b**) were developed. The artemisinin class of drugs has a unique radical mode of action (Figure **1.5**)[20] therefore drug resistance is not found significantly. However it has been reported in 2008 that some resistance is developing in western Cambodia.[21] This group of drugs are the last-line of defence for fighting against malaria.



Artemether (**1-12a**)



Artesunate (**1-12b**)

1.3 Antimalarial drug resistance

The main challenge for fighting against malaria is its emerging parasite resistance to almost all the marketed drugs to date.[22] In addition, multidrug-resistance strains of *P. falciparum* has been identified in many parts of the globe.[23] In most of the cases, the resistance comes from mutations in genes encoding the parasite drug target or influx/efflux pump that is crucial for maintaining the drug concentration at the target. The mechanism of chloroquine resistance has been studied in detail and its resistance in *P. falciparum* may be multigenic but is largely recognised to occur by mutations in genes encoding transport membrane proteins of the digestive vacuole.[24] To circumvent this problem, it is important to develop drugs with different modes of action. Presently, several combination therapies have been taken as a strategy so that the effective form of the drug can survive for a relatively longer time.[25] Some fixed

combination therapies are in developmental stage and some has been approved for clinical use.[26]

1.4 Antimalarial drug targets

As emerging drug resistance is a challenging problem for the treatment of malaria, many new approaches have been proposed. Identification of novel drug targets and design of new molecules for the known targets is one of the major research area developed in present scenario,[27] especially after releasing the genome sequence of *P. falciparum*.[28] Currently these targets can be categorised as:

a) Targets responsible for membrane transport and signalling (e.g. protein kinases and the choline transporter).

b) Enzymes involved in macromolecular and metabolite synthesis (e.g. DOXP reductoisomerase, parasite HGXPRT and lactate dehydrogenase).

c) Targets taking part in the processes occurring in the digestive vacuole (e.g. haemoglobin digestion and haem detoxification). Proteases namely plasmepsins and falcipans are the most explored in this class of targets.[29]

1.4.1 Protein kinases

Protein kinases (PKs) encoding genes in the *P. falciparum* genome have been characterised recently.[30] This study has highlighted that a classical gene identification approach is not suitable for plasmodium functional gene identification. However a reverse genetic approach has been used to address this issue. Protein kinases are believed to be involved in signal transduction processes essential for parasite growth. It has been found that PKs of *Plasmodium* and mammalian are different in their compositions and organisation of signalling pathways.[31] P*f*CPK and P*f*CPK2, the calcium-dependent protein kinases have been described in *P. falciparum*.[32] Previously

this class of enzymes has been isolated only in plants and some protozoan species. This makes the target promising as it may be significantly different from mammalian PKs.

1.4.2 Choline transporter

The malaria parasites protect themselves from the host immune system by invading RBCs. This is important for developing antimalarials because drugs must pass through multiple membranes (the red cell membrane, the parasitophorous vacuolar membrane, the parasite plasma membrane, the food vacuole membrane and the mitochondrial membrane) to access most intra-parasitic targets, depending on the site of action of the drug. It is known that malaria-infected human RBCs have better permeability than normal RBCs and show a new permeation pathway (NPP).[33] NPP may consist of single or multiple channels, which prefer anions over cations. Choline carrier activity is much (10 fold) higher in infected RBCs. The antimalarial activity of choline analogues is due the inhibition of the *de novo* synthesis of major parasite phospholipid phosphatidylcholine (PC) which is essential for supplying large amount of phospholipid in infected RBCs.[34] It is also assumed that the parasite plasma membrane (PPM) choline transporter has a significant role for killing parasite using the choline mimic compounds.[35] As infected RBCs are very much different from normal RBCs, the choline transporter becomes an interesting target for developing antimalrials.

1.4.3 DOXP reductoisomerase

1-Deoxy-D-xylulose-5-phosphate (DOXP) pathway was found as an alternative nonmevalonate pathway for the biosynthesis of isoprenoids in some bacteria, algae and plants. In this pathway, glyceraldehyde 3-phosphate and pyruvate

condensation gives DOXP, which is finally converted to 2-*C*-methyl-D-erythritol-4-phosphate by the enzyme DOXP reductoisomerase. The similarity of DOXP reductoisomerase found in *P. falciparum* suggests the existence of a nonmevalonate pathway. As this alternative pathway is absent in humans, scientists are interested in this parasite specific enzyme as a future target to combat malaria.[36]

1.4.4 Purine salvage enzyme HGXPRT

Malaria parasites in the intra-erythrocytic stage are unable to synthesise purine, thereby needing to rely on pre-formed host purine precursors. Parasites use the salvage enzyme hypoxanthine-guanine-xanthine phosphoribosyltranferase (HGXPRT) for converting purine bases (from the host) to nucleotides needed for their DNA and RNA synthesis. So, if we introduce some purine base analogues, then HGXPRT will use them to produce nucleotide which will be toxic to the parasite. One major issue in this strategy is that, this type of purine analogues should be very specific for parasite enzyme not for similar type of human enzyme HGPRT. The chlorine or nitrogen position in the purine analogues has a significant role for their specificity towards the parasite enzyme over the human enzyme. This encouraging result validates the parasite HGXPRT as a potential drug target for developing animalarials.[37]

1.4.5 Lactate dehydrogenase

Malaria parasites have to depend on glycolysis primarily for producing energy for themselves. It is known that the NAD^+ used up during glycolysis process is generated back by the fermentation of pyruvate in the cytoplasm and/or through the electron transport process occurring in the mitochondria. Unlike the mammalian cells, pyruvate does not enter the citric acid cycle (TCA) in plasmodia. Pyruvate is reduced to lactate as the end-product by a lactate dehydrogenase (LDH) catalyzed reaction. As

pyruvate is not an inhibitor of LDH, the energy production is fast which helps the rapid growth of the parasite. Plasmodial LDH is different from its human counterpart by the presence of a 5 amino acid insertion at the pyruvate binding site. This specific divergent can be explored as a potential drug target.[29]

1.4.6 Plasmepsins

The aspartic proteases in plasmodium are called plasmepsin (PM). Ten plasmepsins (PM I, II, IV, V, VI, VII, VIII, IX, X and histo-aspartic protease) are well known plasmepsins found in plasmodium parasite.[13] The exact contribution of each PM is not clear to date but PM I, II, IV, V, IX and X are involved in the erythrocytic stage whereas PM VI, VII and VIII are expressed in the exo-erythrocytic stage. It is known that PM I and II are found to be involved significantly in haemoglobin metabolism. Haemoglobin metabolism takes place only in an infected RBC. This specific event makes PM I and II very popular drug targets for antimalarials. Recently the PM IV and the histo-aspartic protease (HAP) have been found to be localized in the parasite food vacuole and shown to participate in haemoglobin digestion.[9] In addition to haemoglobin catabolism, PM II and IV are also known to be involved in rupturing the host erytrocytic membrane.[38] The main hurdle is to develop the drug, which is specific to the plasmepsin and not to the similar counterpart human aspartic protease cathepsin D (Cat D).

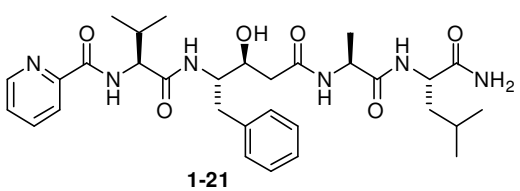
1.4.7 Falcipains

The well known cysteine proteases of *P. falciparum* are called falcipains (FP). A cysteine-histidine pair that is embedded at the catalytic centre is key to their catalytic activity. The FP-2 and FP-3 are known to be located in the food vacuole and involved in the degradation of the host haemoglobin at the early trophozoite stage

whereas FP-2 is also involved in the cleavage of erythrocyte membrane skeletal proteins, including ankyrin and protein 4.1 at the late trophozoite and schizont stages. This proteolysis of the skeleton protein causes RBCs instability thereby releasing the parasite.[39] The crystal structure of the free FP-2 and in complex with cystatin are known.[40] More recently, the crystal structures of FP-3 in complex with leupeptin have been reported.[41] These structural details of FP may help to design drugs in the future.

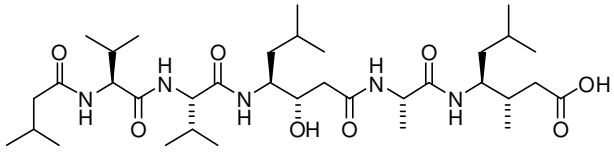
1.5 Antimalarial peptides

Several peptides are found to be active against the malaria parasite. A few very potent peptides are shown below.



1-21

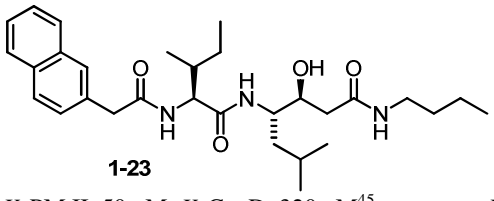
K_i PM II, 0.56 nM, K_i Cat D, 21 nM[42]



1-22

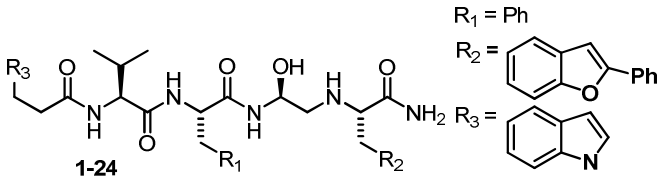
K_i PM I, 0.39 nM, K_i PM II, 0.025 nM,[9]

K_i Cat D, 0.0038 nM[43], *P. falciparum* IC_{50}, 4 μM[44]



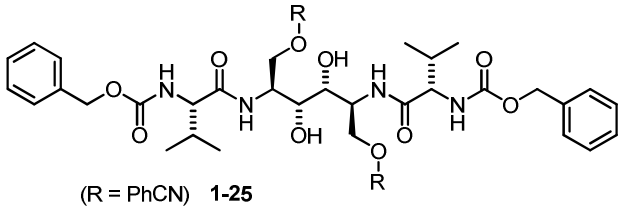
1-23

K_i PM II, 50 nM, K_i Cat D, 320 nM[45]



1-24

K_i PM I, 4.1 nM , K_i PM II, 95 nM, K_i Cat D, 180 nM[46]



(R = PhCN) **1-25**

K_i PM I, 2.7 nM , K_i PM II, 0.25 nM, K_i Cat D, 340 nM[47]

Figure 1.6: Example of antimalarial peptides.

1.6 Aims of this study

The lipopeptide (N1708) isolated from *Streptomyces* sp. using bioassay guided isolation by MerLion Pharmaceuticals exhibits promising activity against *Plasmodium falciparum* (IC_{50}= 0.8 μM against 3D7 strain). NMR and mass analysis suggests that this peptide contains two non-proteinogenic amino acids, one aspartic acid and a ten carbon long chain fatty acid with a *trans* double bond and a chiral centre. Merlion Pharmaceuticals proposed the linear structure and found that this peptide was already patented[48] as an antimalarial agent that is interestingly not active against mammalian, fungi and Gram positive bacteria cell lines. As it is a well known problem that the half life of the peptide drug is short because of the enzymatic hydrolysis of the amide bond formed by proteinogenic amino acids thereby we were interested to find out the full structure of this lipopeptide. Synthesis and stereochemical assignment of the non-proteinogenic amino acids and the rest of the fragments was performed in this work. Full structure determination has also been confirmed by the total synthesis of the complete lipopeptide.

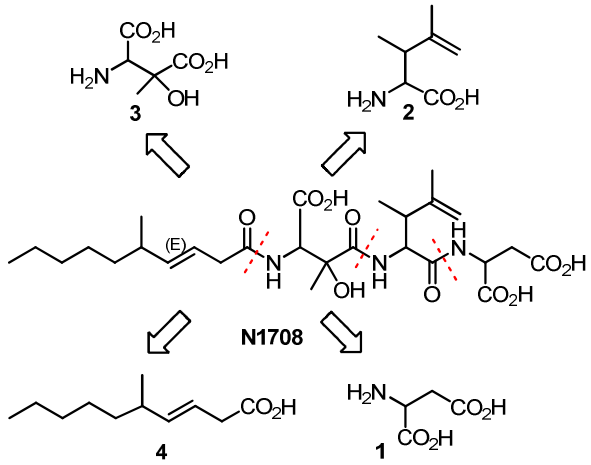


Figure 1.7: Linear structure and fragments of the isolated natural lipopeptide **N1708**.

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CHAPTER 2

Hydrolysis of the Peptide and Identification of Amino Acids

2.1 Hydrolysis of peptide N1708

We first hydrolysed the isolated natural peptide by refluxing with 6M HCl for 24 hours. This acidic reaction mixture was extracted with chloroform to obtain the fatty acid fragments (amino acids should remain in the water layer because of the amine salt formed in the acidic medium). This fraction was injected into the GCMS but we were unable to detect the fatty acid. We believed that during the hydrolytic process, the double bond of the fatty acid part reacted and formed some volatile residue. Next, we took a small part of the water layer and treated this with Marfey's reagent (ref. [16] in chapter 3) to prepare the Marfey's derivative of each amino acid residue for comparison purposes. The main water part was treated with benzyl chloroformate (CbzCl) to protect all the amine groups present in the amino acids. We chose Cbz because of its UV activity would help us separate each derivatised amino acid fragment by preparative HPLC. After isolation of each Cbz protected amino acid fragment, we ran their NMR to reconfirm the structure of the amino acid fragments. Aspartic acid **1** and disubstituted aspartic acid **3** were the same as expected. Interestingly, ^{1}H-NMR did not match with the Cbz protected isoleucine derivative residue **2**. The expected double bond peak was missing and two singlets were observed instead of one. The corresponding peak of the double bond was also missing in the ^{13}C-NMR, but a characteristic peak at 86.25 ppm (later confirmed to be a

quaternary centre) was observed. Interestingly, the ESI mass showed exactly the same mass as expected, although the NMR were dissimilar.

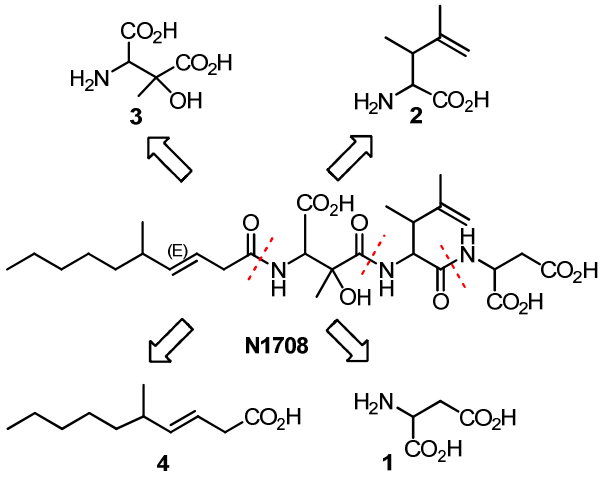


Figure 2.1: Linear structure and fragments of the isolated natural lipopetide **N1708**.

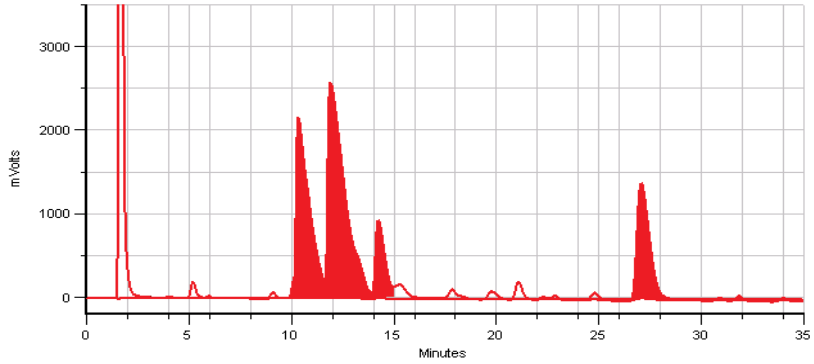


Figure 2.2: Prep HPLC chromatogram of Cbz protected amino acids from peptide **N1708**.

Preparative conditions:
Solvent A: (H_2O+0.1%HCOOH)
Solvent B: (ACN+0.1% HCOOH)
Flow rate: 15mL/minute
Column: XTerra® Prep RP18 OBD TM Column, 5µm, 19x 30mm

Analysis programme:

Time (minute)	0	3	10	40
% solvent B	0	0	10	35

These observations suggested that the double bond was converted to something which had the same mass. We proposed a 5-membered lactone. We reasoned that during the hydrolysis process double bond was protonated and generate a stable tertiary carbocation, which was trapped intramolecularly by the carboxylic acid group to form the lactone. To clarify this, we isolated the lactone (with free amine) from the acidic water layer. We thus hydrolysed the natural peptide again on a small scale. The acidic layer was basified by $NaHCO_3$ (to make the sodium salt of the free carboxylic acid so that all the other amino acids would dissolve in water) and then extracted with chloroform to obtain the lactone with the free amine. Indeed, the lactone (**2-2**) was confirmed by NMR and mass analysis. After confirming the lactone structure, we thought this cyclic structure would help us predict the relative stereochemistry of α and β carbon centres. The coupling constant was 12 Hz in the isolated lactone, which indicated a *trans* relative stereochemistry in the lactone. This relative stereochemistry was again supported by nOe experiments. Finally, it was confirmed through synthesis (next chapter).

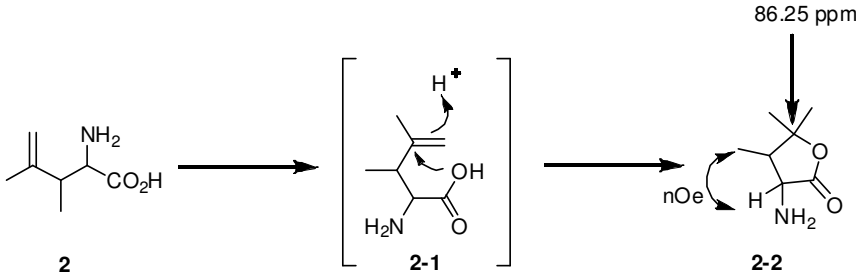
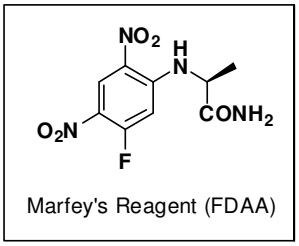


Figure 2.3: Mechanism of lactone formation.



2.2 Stereochemical assignment of the aspartic acid residue

Marfey's reagent (FDAA) was used to derivatise the pure aspartic acid (L- and D- forms) and the whole amino acid mixture obtained from peptide hydrolysis. Because of one fixed chiral centre present in Marfey's reagent, it produced a diastereomer after being coupled with the amino acid. Next, we injected all the three reaction mixtures into the LCMS and found the retention time of L-aspartic acid derivative matched with the natural amino acid residue. We thus concluded that the aspartic acid present in the peptide was L (*S* configuration).

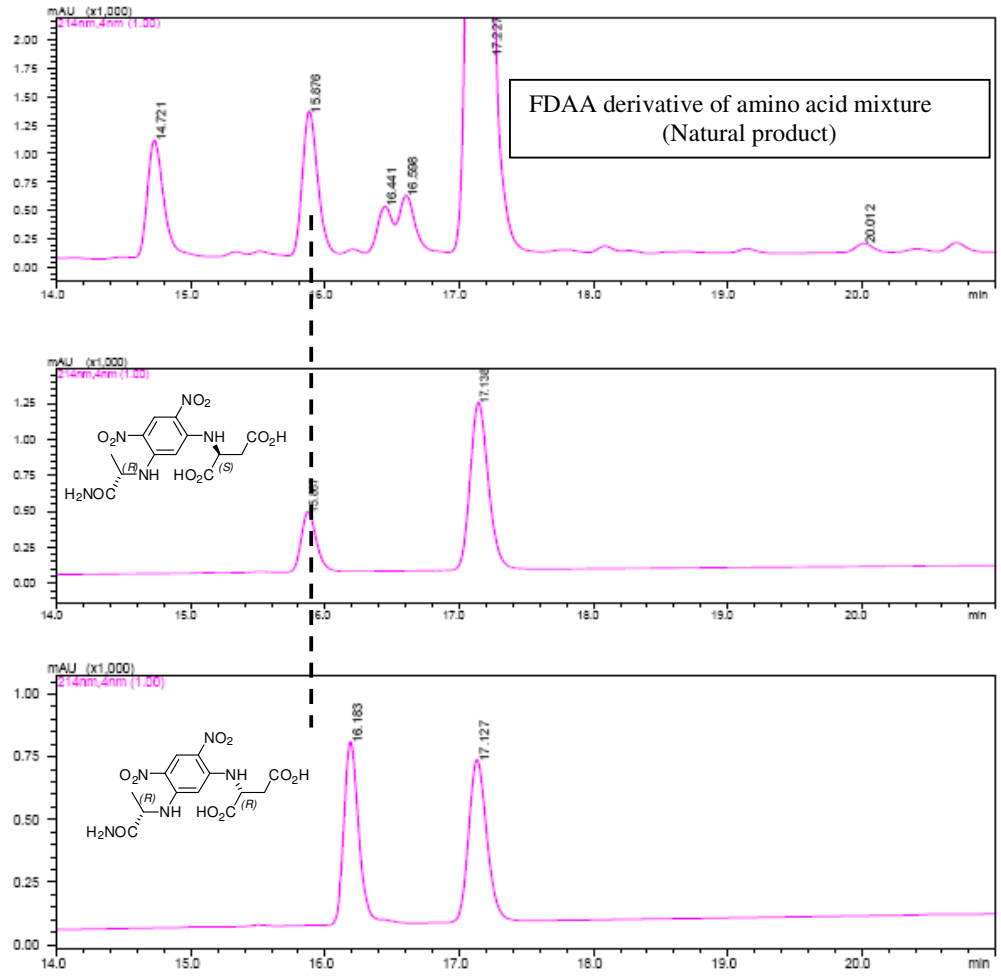


Figure 2.4: LCMS chromatogram to determine the absolute stereochemistry.

2.3 Conclusion

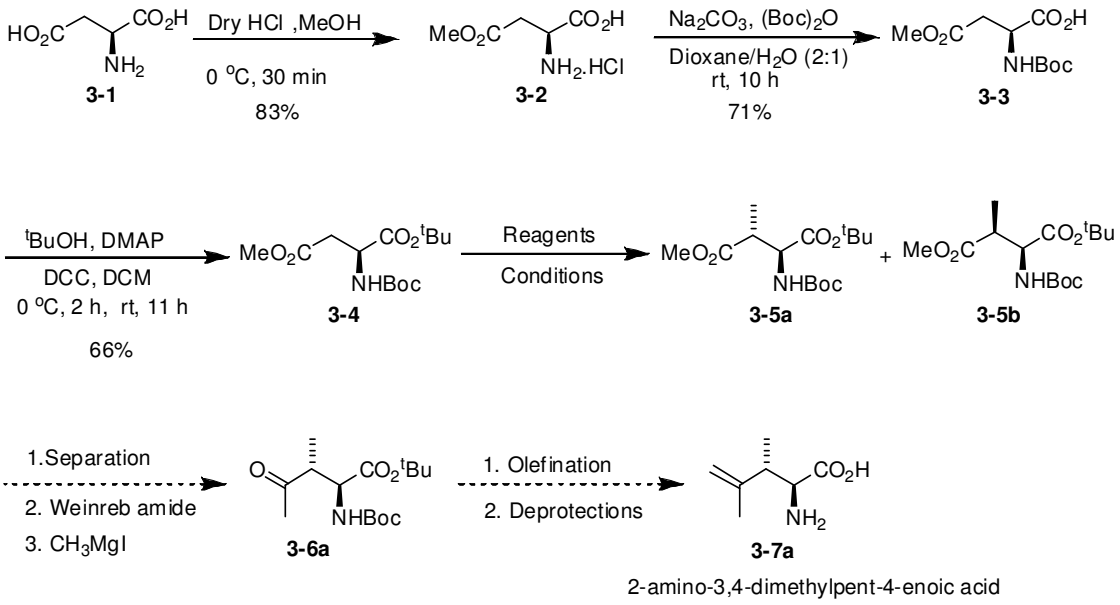
In this chapter, we reconfirmed the structure of the amino acid fragments. The relative stereochemistry of the isoleucine derivative **2** was identified. The absolute stereochemistry of aspartic acid **1** was confirmed by Marfey's reagent. We will discuss in the following chapter how we synthesised and confirmed the absolute stereochemistry of the other fragments.

CHAPTER 3

Synthesis of 2-Amino-3, 4-Dimethylpent-4-Enoic Acid

3.1 Direct methylation of aspartic acid

Having identified the linear structure of lipopeptide **N1708** and amino acid fragments, it was necessary to make a general protocol to produce the isomers of 2-amino-3,4-dimethylpent-4-enoic acid **3-7a** for determining the absolute configuration of the two chiral centers present in this fragment. We planned to use aspartic acid because generation of another chiral centre with the methyl group would give diastereomers (Scheme **3.1**). The β-carboxylic acid of aspartic acid was selectively[1] converted to its methyl ester with dry HCl. Monoester salt **3-2** was protected with $(Boc)_2O$ in basic medium in dioxane-water to give **3-3** in 71% yield.[1]



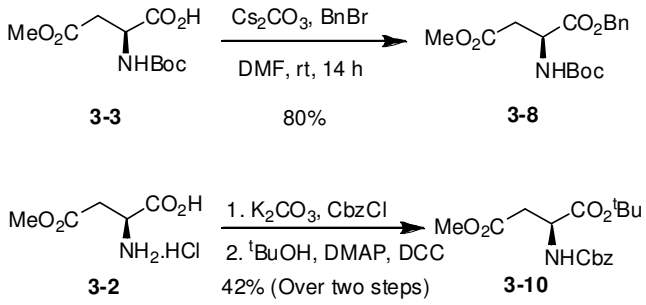
Scheme 3.1: 1st Generation synthetic plan of isoleucine derivative (**3-7a**).

The free acid group was reacted with ^{t}BuOH in the presence of DCC and DMAP in DCM to produce the corresponding tert-butyl ester **3-4**.[2] We converted two carboxylic groups with two different ester groups because according our plan we needed to convert β-carboxylic acid to the Weinreb amide. Unfortunately, we faced many problems to install the methyl group at the β-centre. Different reaction conditions[3] were used as shown in Table **3.1**.

Table 3.1: β-Methylation studies of protected aspartic acid. *Isolated yield.

Entry	Reagents	Conditions	Results*		
			3-4	**3-5a**	**3-5b**
1	NaHMDS (2.2 eq), MeI	-78 ^{o}C to -10 ^{o}C, 1 h -78 ^{o}C to -30 ^{o}C, 2 h	0%	0%	0%
2	LiHMDS (1.1 eq), BuLi (1.2 eq.), MeI	-78 ^{o}C to 0 ^{o}C, 0.5 h -78 ^{o}C to rt, 16 h	10%	15%	7%
3	LDA (2.2), MeI	-78 ^{o}C to -30 ^{o}C, 1 h -78 ^{o}C to rt, 20 h	40%	15%	0%

At this point, we thought that changing the ester and the amine protecting group might help with the methylation yield. The carboxylic group of **3-3** was thus converted to the benzyl ester.[4] Alternatively, the carboxylic and the amine group of **3-2** were converted to its tert-butylester and Cbz derivative, respectively.[5] Several reaction conditions[6] were tried to improve the yield of the β-methylated product, but all the attempts were unsuccessful in our hands (Table **3.2**).



Scheme 3.2: Changing the protecting groups.

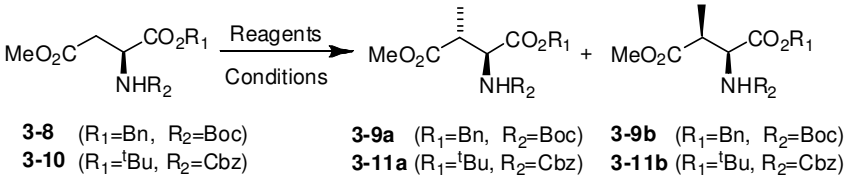
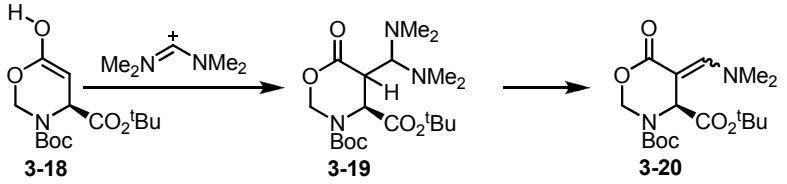


Table 3.2: Further β-methylation studies of protected aspartic acid. *Isolated yield.

Entry	Reagents	Conditions	Results*		
			3-8	**3-9a**	**3-9b**
1	LiHMDS (2.2 eq), MeI	-78 °C to -30 °C, 1 h -78 °C to rt, 12 h	70%	0%	0%
2	NaHMDS (2.2 eq), MeI	-78 °C to -30 °C, 1 h -78 °C to rt, 12 h	0%	0%	0%
			3-10	**3-11a**	**3-11b**
3	LiHMDS (2.2 eq), MeI, LiCl	-78 °C to -40 °C, 2 h -78 °C to rt, 14 h	85%	0%	0%
4	BuLi (1.2 eq), NaHMDS (1.1 eq), MeI	-78 °C to -40 °C, 2 h -78 °C to rt, 14 h	0%	0%	0%

3.2 Synthetic plan via 1, 3-oxazin-6-ones

After being unsuccessful at a direct methylation, we changed our plan and sought to follow a 2nd generation reaction sequence (Scheme **3.3**). We found a different procedure developed by Young *et al.* to install the methyl group at the α position of the terahydro-1,3-oxazin-6-ones.[7] For this purpose, the methyl ester of **3-4** was converted to carboxylic acid **3-12**, which was treated with paraformaldehyde in acidic condition to form the terahydro-1,3-oxazin-6-one **3-13**. Bredereck's reagent was used to form the β-N,N-dimethyl α,β-unsaturated lactone, which was reduced to produce the methylated 1,3-oxazin-6-ones **3-15a** and **3-15b**. It is believed that the enol **3-18** formed from 1,3-oxazin-6-ones reacted with amidinium ion to form **3-19** and then an elimination process took place to generate **3-20**. In the next steps, reduction followed by elimination produced the exomethelene group, which upon reduction by catalytic hydrogenation afforded the methylated product. Two diastereomers can be separated by careful column chromatography in moderate yield. One of them was confirmed by X-ray crystallography. We faced a problem to cleave the *N*,*O*-acetal type bonds to produce **3-16**. It was found that the reaction was sluggish in acetic acid-water medium at 45 ^{o}C. We managed to afford trace amount of **3-16** in poor yield (4%) and decided to abort this scheme at this stage because Bredereck's reagent was costly and our overall yield was too poor.



3-18 **3-19** **3-20**

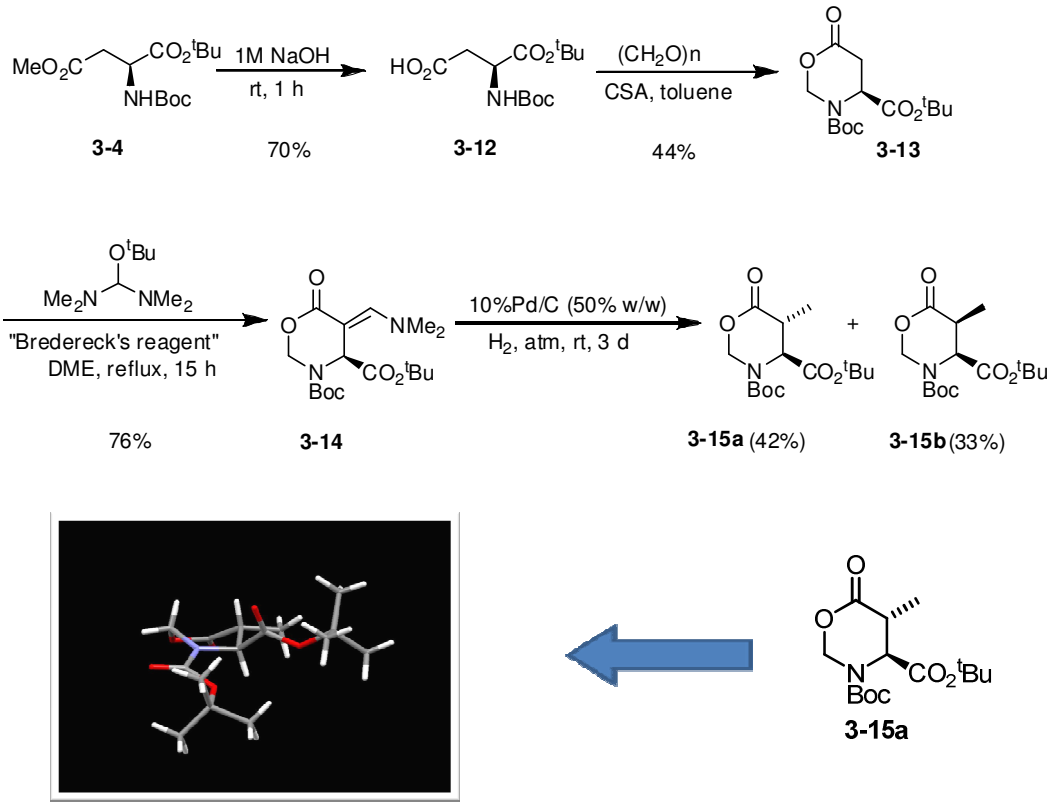
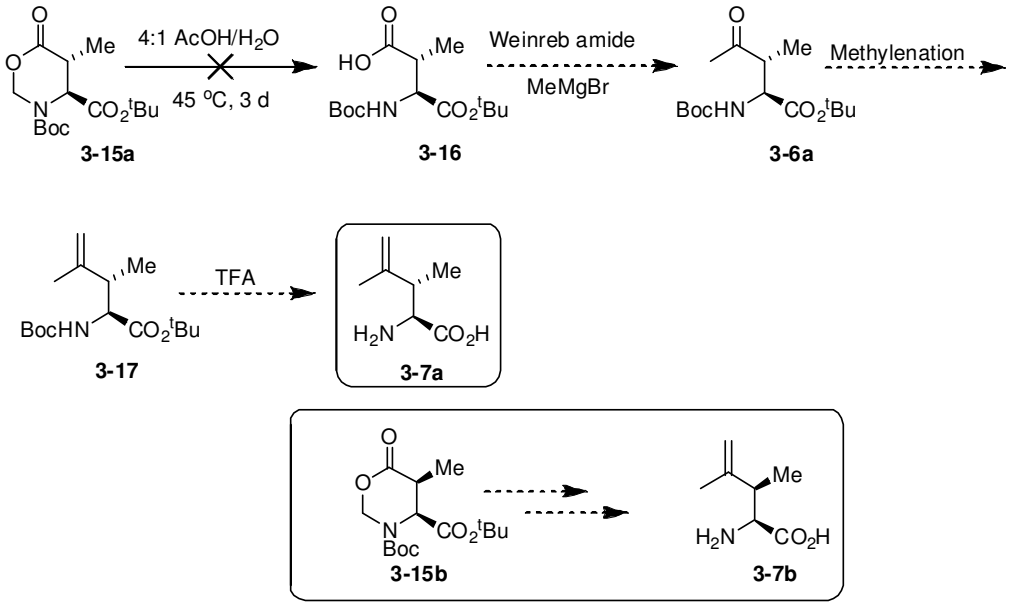


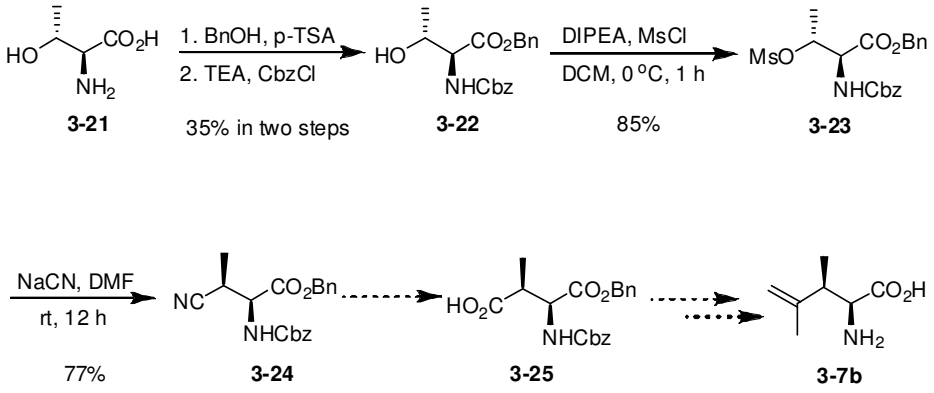
Figure 3.1: X-ray crystal.



Scheme 3.3: 2nd Generation synthetic plan of isoleucine derivative (**3-7**).

3.3 Synthetic plan with threonine

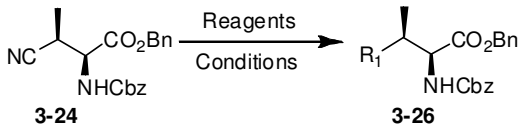
After another unsuccessful result to install the methyl group, we sought to start our synthesis with the methyl group already present in the molecule. With this vision in mind, we moved to a 3rd generation plan. Here, the acid group of the natural threonine was protected as its benzyl ester[8] and the amine with Cbz. The secondary alcohol **3-22** was converted to its mesylate so that it could be displaced by the cyanide to get the inverted cyano product **3-24**.



Scheme 3.4: 3rd Generation synthetic plan of isoleucine derivative (**3-7b**).

Our attempts to convert the cyano group to an acid or equivalent group (amide or ester) ultimately failed. We investigated a variety of acidic conditions to hydrolyse the cyano group. Sodium hydroxide in the presence of hydrogen peroxide was used to make strong nuceophilic conditions to form the acid. Strong acidic medium in MeOH at elevated temperature also failed to produce the methyl ester. Next, the cyano compound was refluxed with $Hg(OAc)_2$ in AcOH to obtain the amide.[9] This effort also failed (Table **3.3**). We did not attempt harsher basic conditions to avoid potential epimerization of the chiral centre next to the cyano group. The methyl group present

next to the cyano group might cause some steric repulsion so that neucleophiles were unable to attack the electrophilic carbon centre of the cyano group.



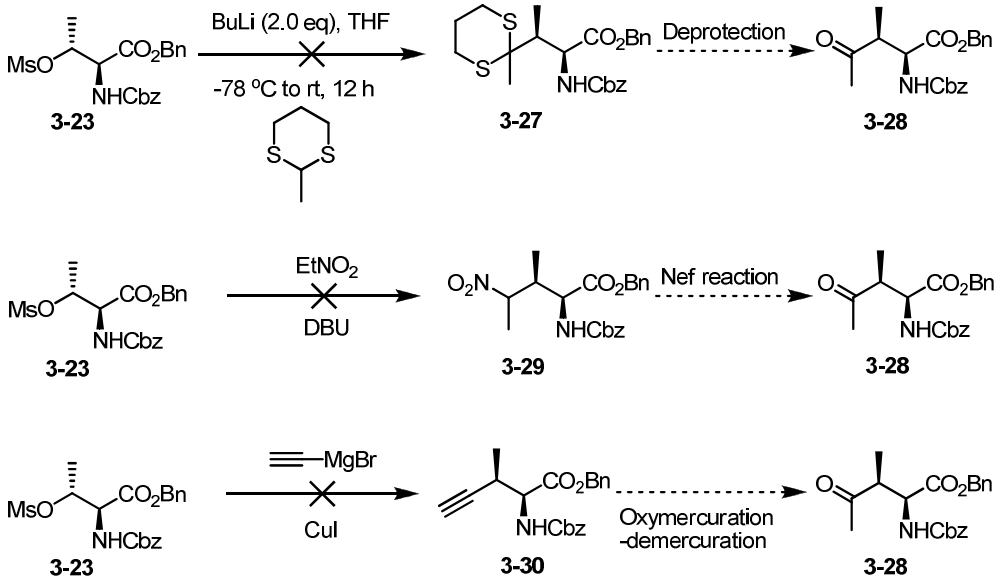
3-24 **3-26**

Table 3.3: Reaction conditions to manipulate the cyano group.

Entry	Reagents	Conditions	Results (R_1)	
1	60% aq. H_2SO_4	60 ^oC, 10 h	CO_2H	No desired Product
2	3N NaOH, 30% H_2O_2	70 ^oC to 90 ^oC (2 h)	CO_2H	
3	Conc. HCl	Reflux, 10 h	CO_2H	
4	H_2SO_4, MeOH	Reflux, 10 h	CO_2Me	
5	$Hg(OAc)_2$, AcOH	Reflux, 10 h	$CONH_2$	

Next, we realized that instead of going through the Weinreb amide we might get our desired 3-methyl-4-keto ester (e.g. **3-28**) using dithiane chemistry. For this purpose, the methyl substituted dithiane nucleophile was generated with BuLi and then the mesylate was added to the reaction mixture at low temperature to accomplish the desired **3-27**. Unfortunately, these attempts failed. On the other hand, it was planned to synthesise **3-29** using nitroethane, keeping in mind that later we could convert the nitro group to a ketone **3-28** using Nef conditions. This attempt was also unsuccessful. As the steric effect created by the adjacent methyl group might be the cause for the failure of these reactions, we wanted to introduce an alkynyl (by displacing the mesylate group with Grignard reagent in presence of CuI) since the *sp* hybridized carbon nucleophile would be linear thereby reducing steric effects. This group could then be converted to the ketone using oxymercuration-demercuration

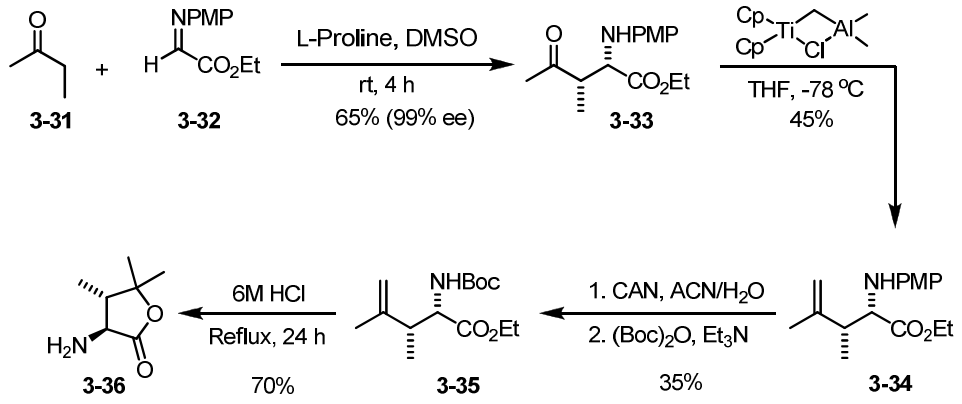
reaction conditions. This strategy also did not work in practice and produced eliminated product only.



Scheme 3.5: Attempts to replace -OMs with different nucleophiles.

3.4 Proline catalysed Mannich reaction

After several unsuccessful studies we concentrated on the L-proline catalysed asymmetric Mannich reaction developed by Barbas III.[10] We thus planned to prepare the *syn* amino acid fragment **3-33** using both D- and L- proline. This plan was attractive to us due to its flexibility and quickness to produce the desired amino acid (Scheme **3.6**).



Scheme 3.6: 4th Generation synthesis of the isoleucine derivative (**3-36**).

Imine **3-32** was prepared following the literature procedure[11] and was then reacted with ketone **3-31** to produce **3-33** in 99% ee. Various Wittig reaction conditions were tried to convert the ketone to the methelene, but all were unsuccessful presumably due to the steric effect created by the α-methyl group in this highly functionalised small molecule (Table **3.4**). Finally, we prepared **3-34** by chemo-selective olefination of ketone **3-33** in presence of ester by employing Tebbe reagent[12] at -78 °C. This reaction was standardised to accomplish an optimal yield of 45% in our hand.

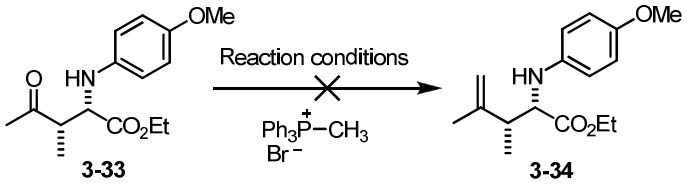


Table 3.4: Wittig reaction for the methylenation.

Entry	Reagents	Conditions	Remarks
1	NaH (2.2 eq.), DMSO	75-80 °C (45 min), rt, 16 h	S.M recovered
2	LiHMDS (2.2 eq.), THF	0 °C- rt, 15 h	S.M recovered
3	NaHMDS(2.2 eq.), THF	-78 °C (30 min), rt, 16 h	Weak MS Peak

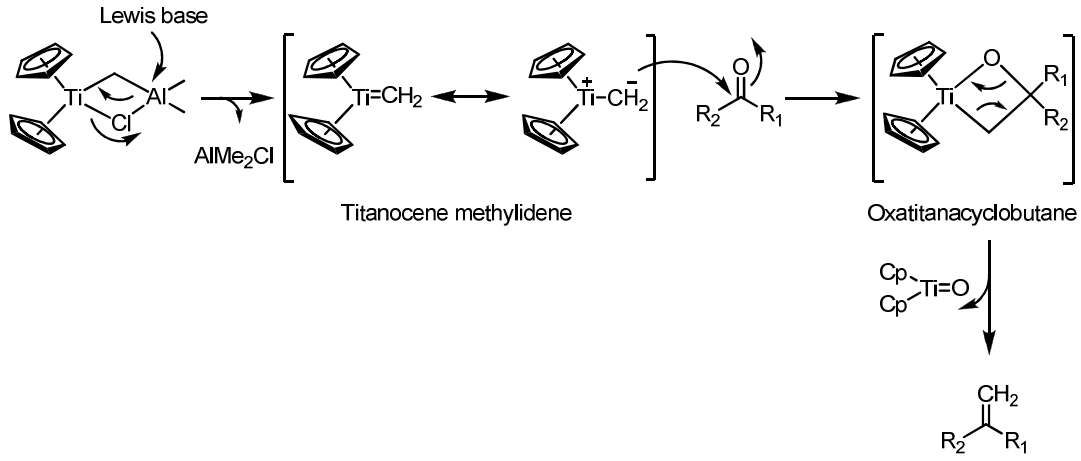
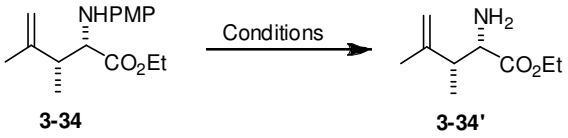


Figure 3.2: Mechanism of Tebbe olefination.



3-34 **3-34'**

Table 3.5: PMP deprotection.

Entry	Reagents	Conditions	Remarks
1	Iodosobenzene diacetate,[13] MeOH	rt, 1 h	Mass found but complex NMR
2	Trichloroisocyanuric acid,[14] H_2SO_4	rt, O/N	Mass not found
3	CAN, ACN/H_2O[15]	0 ^oC - rt, 1 h	Clean reaction (reverse addition)

Next, we just needed to deprotect the PMP group to produce the free amine. After trying several unsuccessful attempts (Table **3.5**) we obtained the desired product by reverse addition i.e., **3-34** was added dropwise to a CAN solution. The free amine was protected with Boc to furnish **3-35**. Compound **3-35** is important to us in two ways. It is a useful intermediate for the total synthesis of the target peptide and it can be used to prove our hypothesis of a cyclisation process during acidic hydrolysis of

the natural peptide. The reaction conditions as applied for peptide degradation were thus performed on **3-35** and the cyclised product **3-36** was identified and isolated. Interestingly, it was found that the coupling constant between the α and β protons was 12.0 Hz which supported our assumption that the relative stereochemistry was *trans* in the cyclic lactone **3-36**. Next, Marfey's reagent[16] was used to couple with cyclic lactone **3-35** and the reaction mixture was injected to the LCMS and found it matched exactly with the derivatised natural amino acid fragment (Figure **3.4**).

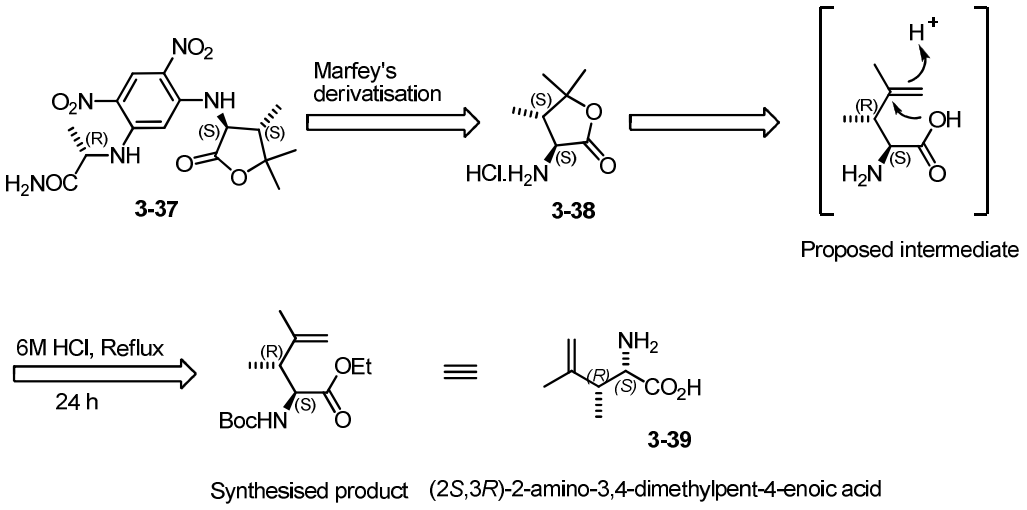


Figure 3.3: Determination of absolute configuration.

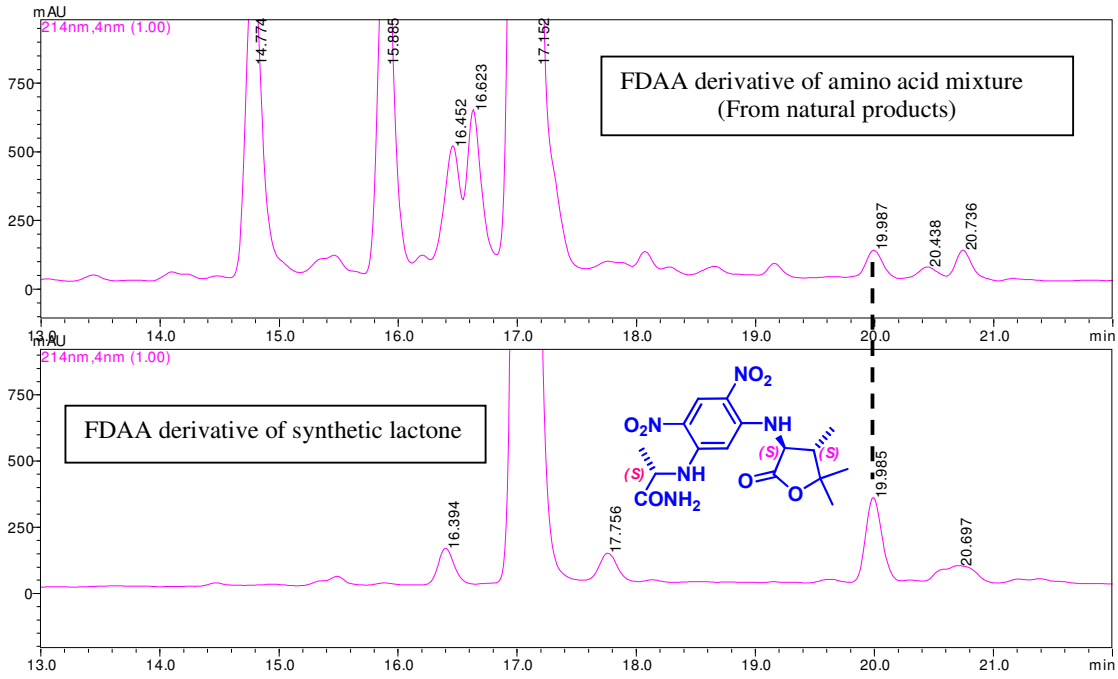


Figure 3.4: LCMS chromatogram to determine the absolute stereochemistry.

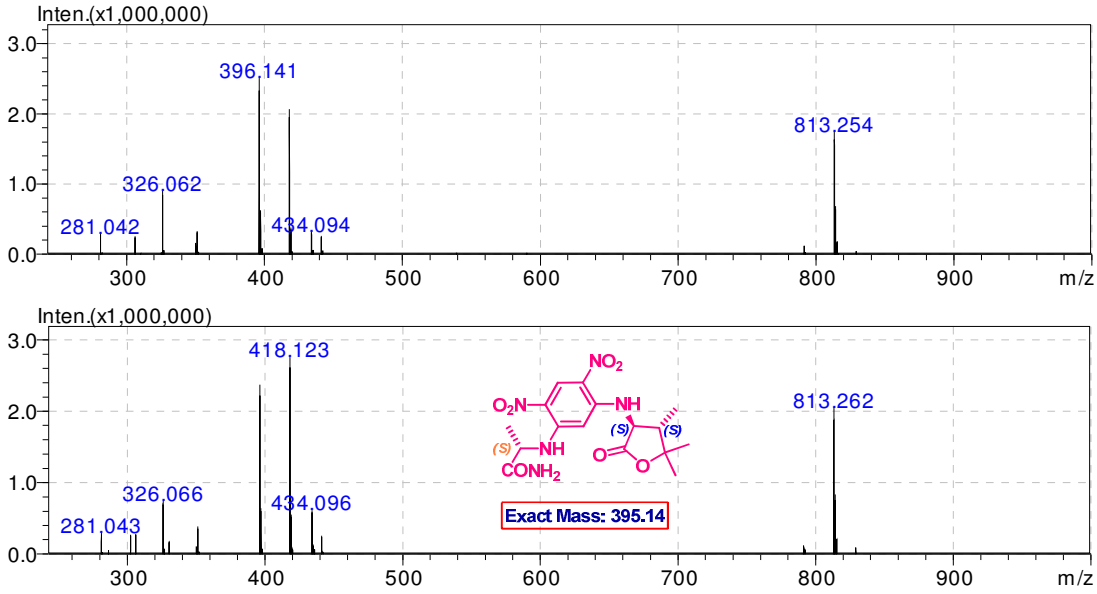


Figure 3.5: Mass chromatogram of the derivatised desired amino acid.

3.5 Conclusion

In this chapter, we discussed the successful *de novo* asymmetric synthesis of a new non-proteinogenic amino acid (2-amino-3,4-dimethylpent-4-enoic acid). After attempting several strategies, **3-36** was successfully synthesised in five steps. This confirmed our hypothesis of a cyclisation process occuring under acidic condition. From the derivatisation technique using Marfey's reagent, we established the absolute configuration of the 2-amino-3,4-dimethylpent-4-enoic acid fragment **2** of the natural product. So it was confirmed that the absolute configuration of 2-amino-3,4-dimethylpent-4-enoic acid was (2*S*, 3*R*) as **3-39**.

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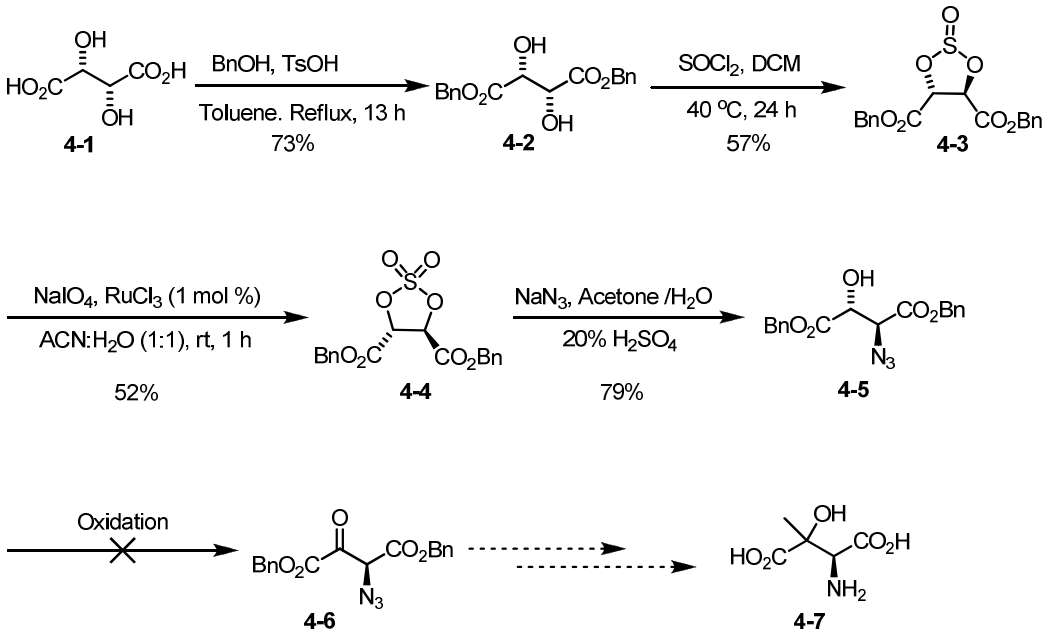
CHAPTER 4

Synthesis of 3-Amino-2-Hydroxy-2-Methylsuccinic Acid

4.1 1st Generation synthesis from L-Tartaric acid

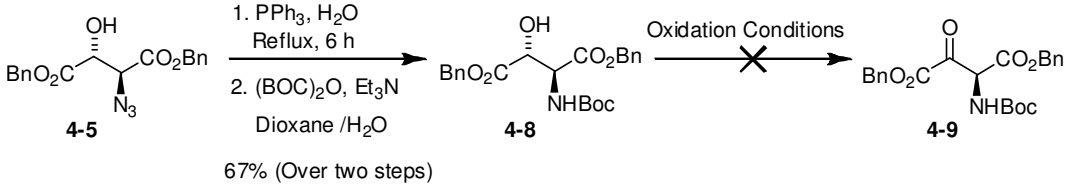
After determining the absolute stereochemistry of the two amino acid fragments, we needed to synthesise the 3-amino-2-hydroxy-2-methylsuccinic acid. As we had no idea about the absolute stereochemistry of this fragment, our synthetic plan needed to be flexible to generate all four diastreomers possible for that fragment. L-Tartaric acid **4-1** was treated with benzyl alcohol in the presence of TsOH to get the dibenzyl ester.[1] The vicinal alcohol was converted to the cyclic sulfite **4-3** with $SOCl_2$.[2] To make a good electrophilic centre at the α-carbon, **4-3** was treated with an oxidant to afford the cyclic sulfate **4-4**. The cyclic sulfate was opened up with sodium azide as the azide source to obtain the azido sulfate salt intermediate. Acidic hydrolysis of the salt generated azido-alcohol **4-5**.[3] Next, we needed to oxidise the secondary hydroxyl group to the ketone. Common oxidation methods failed most likely because the product keto-diester **4-6** was not stable. Being unsuccessful in oxidizing the azide intermediate, we thought to convert the azide to an amine under Staudinger reaction conditions[4] and protect with Boc. Again, several oxidation conditions were attempted on **4-8** but none of them were successful. At this point, it was believed that the oxidised product might not be stable. So, we changed our synthetic strategy (Scheme **4.2**).



Scheme 4.1: 1st Generation synthesis of β-disubstituted aspartic acid derivative.

Table 4.1: Oxidation of azido-alcohol.

Entry	Reagents	Conditions	Remarks
1	DMP, DCM	rt, 16 h	S.M
2	Swern oxidation	-78 °C, 4 h	Unidentified product
3	PCC	rt, 16 h	Decomposition



Scheme 4.2: Staudinger reduction of azide and Boc protection.

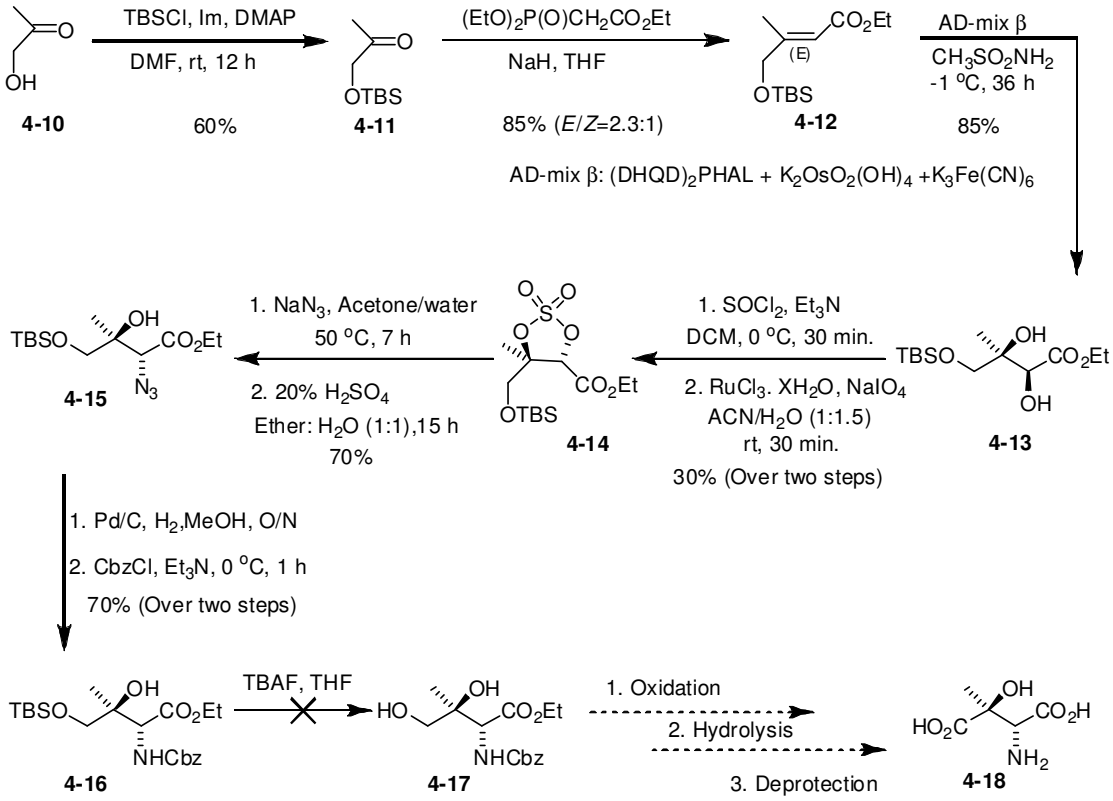
Table 4.2: Oxidation of Boc protected amino-alcohol (**4-8**).

Entry	Reagents	Conditions	Remarks
1	TEMPO, NaOCl NaBr, $NaHCO_3$, EA/Toluene/Water[5]	rt, 30 min.	Complicated TLC
2	$RuCl_3$, mCPBA[6]	rt, 2 h	S.M
3	DMP, DCM	rt, 1.5 h	Unidentified Polar spot isolated
4	Trichloroisocyanuric acid, TEMPO[7]	rt, 2 h	Unidentified Polar spot isolated
5	Swern oxidation	-78 ^oC, 4 h	S.M
6	PCC	rt, 16 h	Decomposition

4.2 2nd Generation synthesis from hydroxyacetone

In this protocol the primary alcohol group of hydroxyacetone was protected with TBS using standard reaction conditions. The HWE reaction was chosen to afford the olefin **4-12**. Though we did not get good selectivity (*E*/*Z*=2.3/1), all four diastereomers of the diol **4-13** could be formed by just changing the geometry of the double bond and the AD-mix composition (α or β) in the Sharpless dihydroxylation reaction. To standardise the scheme, the pure *E*-isomer was carefully separated (*Z*- and *E*-isomers were confirmed by nOe studies and comparing with literature[8] NMR) and treated with AD-mix β (Sharpless Asymmetric Dihydroxylation) to produce the diol **4-13**. The cyclic sulfate **4-14** was obtained readily by following a similar reaction sequence as shown in Scheme **4.1**. It was planned deliberately to synthesise **4-14** so that the cyclic sulfate can be opened with the azide regioselectively. Being α to the ester, electronically it is more eletrophilic than the β carbon (quaternary) centre of the cyclic sulfate. Steric effects also favour nucleophilic attack at the α-centre. With this

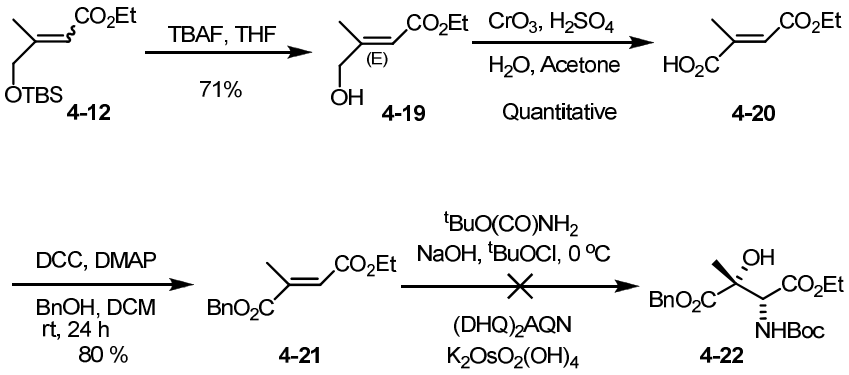
reasoning, **4-14** was treated with sodium azide in acetone/water at 50 °C for 7 h and consecutive biphasic (ether-water) acid hydrolysis yielded the azido alcohol **4-15** in 70% yield. Gratifyingly, the expected regioselective product was obtained and confirmed by ^{13}C-NMR and DEPT experiments. The azide was hydrogenated to amine, which was protected with CbzCl to afford **4-16**. Standard reaction conditions (using TBAF in THF and HF in ACN) for cleaving the TBS group were employed but were not successful and lead to complicated mixtures by TLC.



Scheme 4.3: 2nd Generation synthesis of β-disubstituted aspartic acid derivative.

4.3 Sharpless Asymmetric Aminohydroxylation

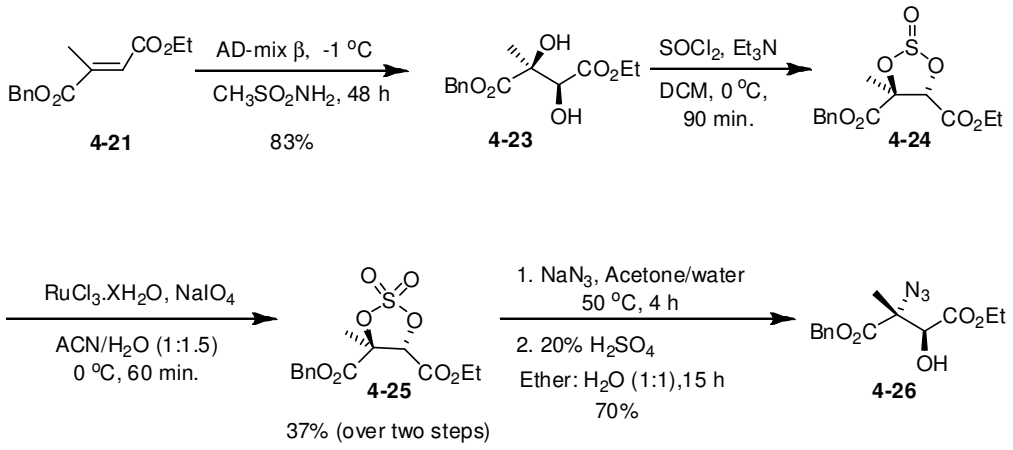
Following another strategy, the TBS protecting group of **4-12** (*E*/*Z*=2.3/1) was readily cleaved by standard reaction conditions to afford **4-19**. Deprotection of TBS group afforded the readily separable *E*-isomer **4-19** because the resulting *Z*-isomer reacted with the ester intramolecularly to form a lactone. Jones oxidation of the free alcohol **4-19** produced the corresponding acid **4-20,** which was converted to its benzyl ester by DCC coupling method. Sharpless asymmetric aminohydroxylation was tried to obtain the Boc protected amino alcohol **4-22** in a single step but this was unfruitful.



Scheme 4.4: Sharpless asymmetric aminohydroxylation strategy.

4.4 Sharpless Asymmetric Dihydroxylation

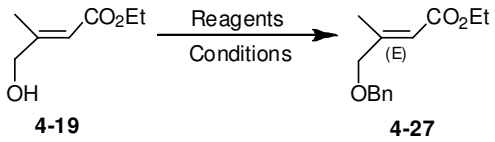
We thus moved forward with the same Sharpless cyclic sulfate strategy to produce an azido alcohol. This time, however, the wrong regioisomer **4-26** was obtained as confirmed by ^{13}C-NMR and DEPT experiments (Scheme **4.5**).



Scheme 4.5: Sharpless asymmetric dihydroxylation strategy.

4.5 Changing TBS group to benzyl group

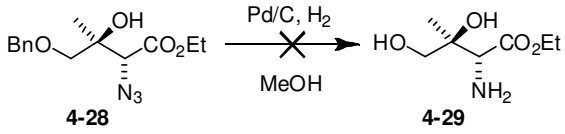
In an adoption of Scheme **4.3** the replacement of the TBS group of **4-12** with benzyl was planned such that a deprotection/reduction of the benzyl and azide groups could occur in a single step. Unfortunately, benzyl protection of the tri-substituted allylic alcohol was difficult. We tried this protection in basic medium with and without activator (TBAI) but reaction mixtures always produced complex mixture of compounds. Even neutral (Table **4.3,** entry **3**) reaction conditions were not successful. Eventually, freshly prepared benzyl 2,2,2-trichloroacetimidate (Bn-TCA) in the presence of TMS-triflate afforded the desired protected alcohol **4-27** in 50% yield. Next, following same reactions sequence as in Scheme **4.3**, **4-28** was successfully prepared. As per our previous plan we attempted reduction of the azide and deprotection of the benzyl group in a single step by using Pd/C, H_2 but the desired product **4-29** was not isolated. So it was planned to go for a stepwise reaction sequence because we suspected that even if we would get **4-29,** it would be difficult to isolate it due to its high polarity and potential water solubility.



Scheme 4.6: Benzylation of tri-substituted allylic alcohol.

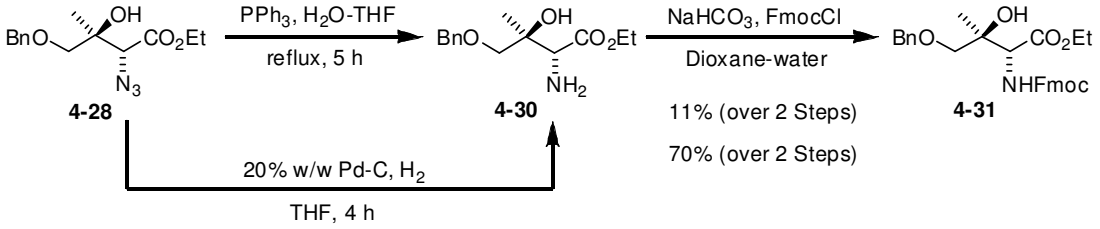
Table 4.3: Benzylation of tri-substituted allylic alcohol.

Entry	Reagents	Conditions	Remarks
1	NaH, BnBr, DMF	0 ^{o}C to rt, 30 min.	Complicated TLC
2	NaH, BnBr, TBAI, THF[9]	40 ^{o}C, 12 h	Polar spot found
3	Silver (I) Oxide, BnBr, DMF	rt, 2 h	No desired product
4	Bn-TCA, TMS-triflate, DCM[10]	rt, 3 h	50% yield

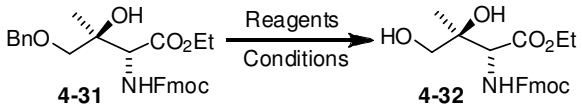


Scheme 4.7: Debenzylation and azide reduction.

Staudinger reaction[11] was performed to reduce the amine which was protected with Fmoc to get **4-31** in 11% yield in two steps (Scheme **4.8**). Later, it was found that in THF solvent the selective reduction of azide was obtained without cleaving the benzyl protecting group. Compound **4-31** was produced in 70% overall yield with this procedure.



Scheme 4.8: Reduction of azide and protection with FmocCl.



Scheme 4.9: Debenzylation in presence of Fmoc group.

Table 4.4: Debenzylation

Entry	Reagents	Conditions	Remarks
1	Pd/C, H_2, MeOH	rt, 12 h, 1 atm.	S.M and trace amount of Fmoc cleaved product
2	Pd/C, H_2, MeOH	rt, 5 h, 50 psi	Complex TLC
3	Pearlman's catalyst, H_2, THF	rt, 2 h, 1 atm.	No desired product
4	DDQ (10 eq.), DCM	40 ^oC, 72 h	No desired product
5	BCl_3, DCM[12]	-78 ^oC, 30 min	Lactone formed

It was again found difficult to deprotect the benzyl group of this substrate. In Lewis acid mediated (Table **4.4,** entry **5**) conditions, the resulting alcohol interestingly cyclised to produce a five membered lactone. To avoid the unwanted cyclisation, it was planned to synthesise the cyclic urethane, *N*,*O*-acetal,[13] primary alcohol, acetal or ketal (**4-34** to **4-37**) and then deprotect the benzyl. Our first attempt to form the cyclic urethane **4-34** was unsuccessful. After various failures to construct the *N*,*O*-acetal **4-**

35, the ester group was reduced to alcohol with the hope to form ketal **4-37.** This was also unsuccessful.

Scheme 4.10: Lactone formation.

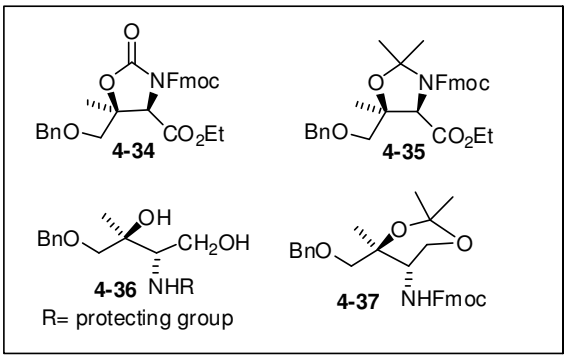
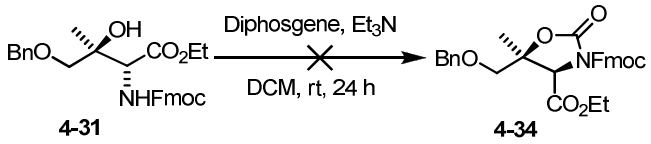
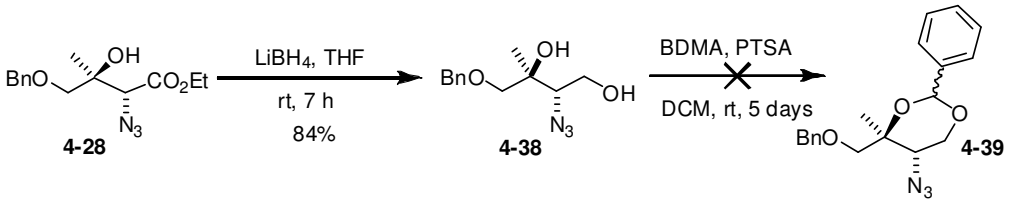


Figure 4.1: Proposed intermediates for avoiding lactone formation.



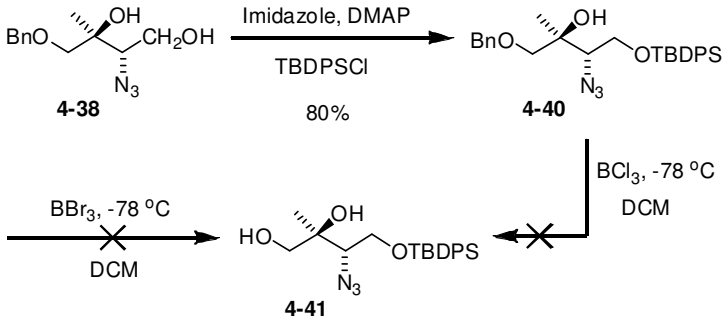
Scheme 4.11: Attempt to form cyclic urethane.

Here we thought Fmoc may create a steric problem due to its bulky size. So we planned to reduce the ester in presence of azide by $LiBH_4$ and then form the acetal using BDMA and catalytic PTSA. This plan did not give the desired product (Scheme **4.12**).



Scheme 4.12: Reduction of ester and attempt to form ketal.

The free hydroxyl group of **4-38** was then protected as TBDPS with TBDPSCl, imidazole and DMAP and then the benzyl group was attempted to be cleaved with Lewis acids,[12,14] which again was unsuccessful.

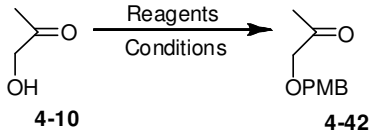


Scheme 4.13: TBDPS protection and debenzylation.

4.6 Inserting *p*-Methoxybenzyl (PMB) group

Adopting Scheme **4.3** we replaced the TBS group of **4-11** with PMB, so that the PMB group could help to improve high enantiomeric excess by the virtue of π-π interaction in the transition state[15] of Sharpless asymmetric dihydroxylation reaction and also the cleavage of PMB would be relatively easy. In this new protocol (Scheme **4.15**) we faced the problem of preparing the PMB ether of hydroxyacetone. It was found that Dudley's reagent produced the best results. After getting the PMB protected product, the azido-alcohol **4-46** was obtained following the same reactions sequence as given in Scheme **4.3**. Interestingly, it was found that Sharpless

asymmetric dihydroxylation was faster for the *E*-isomer **4-43** as compared to the *Z*-isomer. The enantiomeric excess was also high for the *E*-isomer. The absolute stereochemistry was determined through functional group manipulation of **4-44** to the tetraol (Scheme **4.16**) and compared with the optical rotation value found in the literature.[16] Unfortunately, the PMB deprotection was not as easy as planned. Instead of getting the desired product, we got mainly the benzyl ester **4-53** when DDQ was used. As this benzylic oxidation was found when tertiary alcohol was not protected, we proposed a cyclization/ring opening mechanism for the formation of **4-53**.

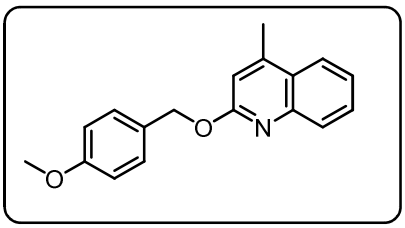


4-10 **4-42**

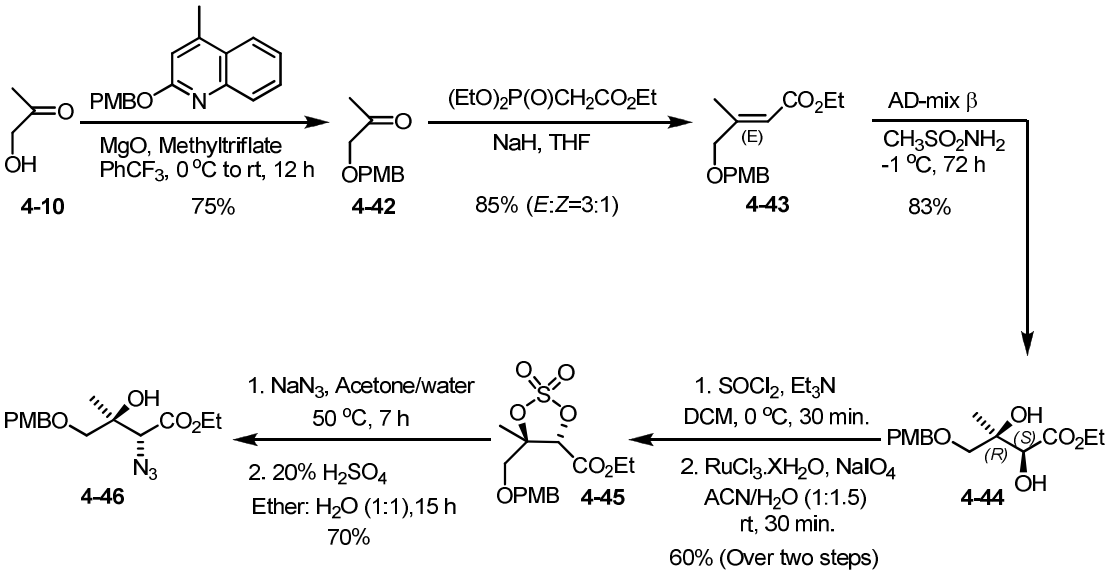
Scheme 4.14: PMB protection of hydroxyacetone.

Table 4.5: PMB protection of hydroxyacetone.

Entry	Reagents	Conditions	Remarks
1	NaH, PMBBr, DMF	0 ^{o}C to rt, 30 min.	Complicated TLC
2	NaH, PMBBr, TBAI, THF	40 ^{o}C, 12 h	Polar spot found
3	PMB-TCA (Fresh), PPTS, DCM	rt, 12 h	40% yield
4	Dudley's reagent,[17] MgO, MeOTf, $PhCF_3$	0 ^{o}C to rt, 12 h.	75% yield

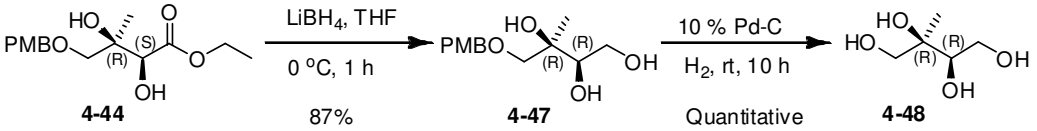


Dudley's reagent



Double bond geometry of **4-43**	AD-mix	Absolute configuration of **4-44**	%ee (%Yield)
(E)	β	(2S,3R)	98 (83)
(E)	α	(2R,3S)	95 (79)
(Z)	β	(2S,3S)	85 (84)
(Z)	α	(2R,3R)	80 (85)

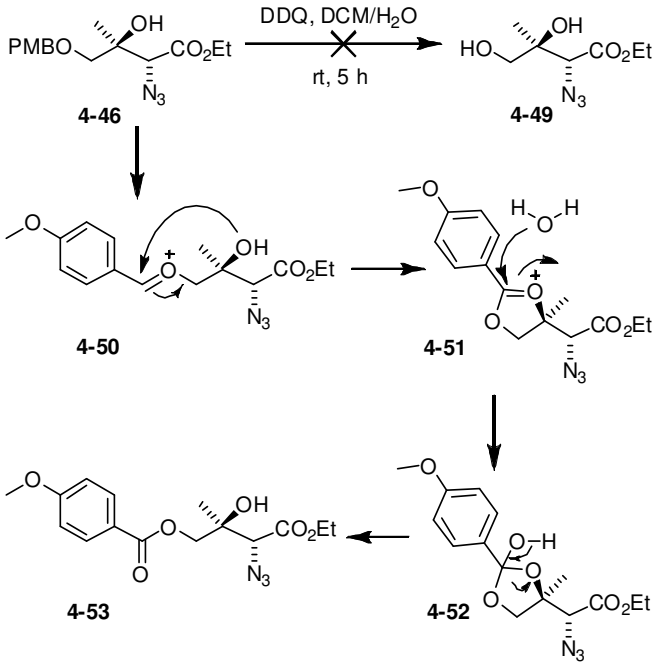
Scheme 4.15: Preparation of azido alcohol with PMB group.



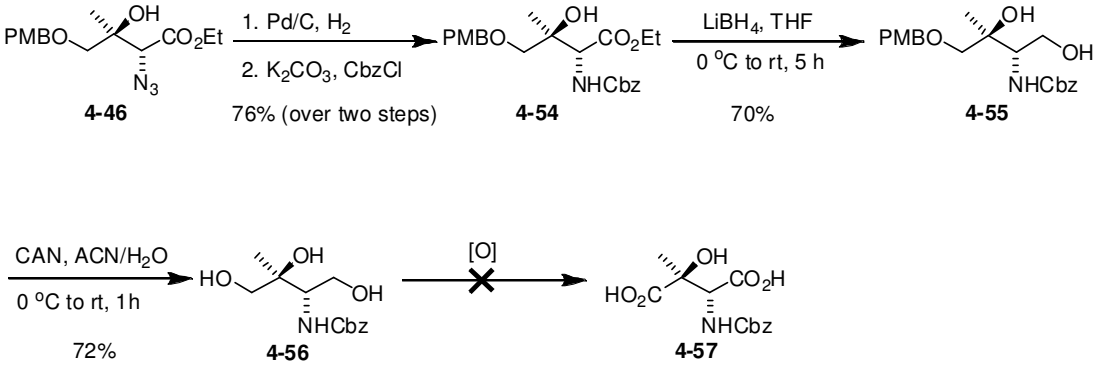
Scheme 4.16: Synthesis of tetraol for determining the absolute configuration.

Next, we thought to reduce the azide selectively to an amine and then to protect it with Cbz so that we would have more options to cleave the PMB ether. To avoid lactone formation, we reduced the ester group to the alcohol prior to PMB

depreotection. Compound **4-46** was reduced to the amine under hydrogenation conditions and protected with Cbz using standard reaction conditions to get **4-54.** $LiBH_4$ was used to produce the diol **4-55**. CAN[18] was applied to deprotect the PMB group which successfully afforded the triol **4-56**. Our attempts (PDC in DMF and Jones oxidation) to oxidise both the primary alcohols to the dicarboxylic acid were unsuccessful.

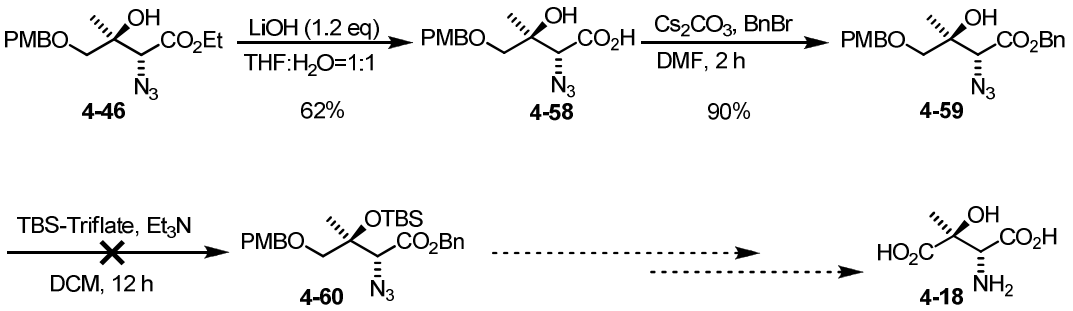


Scheme 4.17: Plausible mechanism for side product formation.



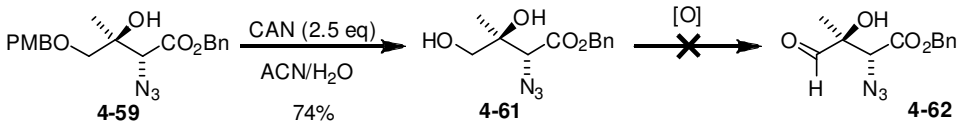
Scheme 4.18: Functional group manipulation.

We were next interested to change the ethyl ester to benzyl ester to prevent lactone formation because the benzyl ester would be less reactive towards nucleophilic oxygen and it was thought that the benzyl ester and azide would be converted to acid and amine respectively in one step. The ethyl ester **4-46** was thus hydrolysed with LiOH in THF/H_2O and then the carboxylate anion[19] of **4-58** (generated by Cs_2CO_3) was reacted with BnBr to obtain the benzyl ester **4-59**. As it was known from the previous experience that the tertiary alcohol must be protected to stop formation of the side product, the TBS group was chosen for protection. Unfortunately the TBS protection did not work because of steric effects of the neighbouring groups present in the small molecule.



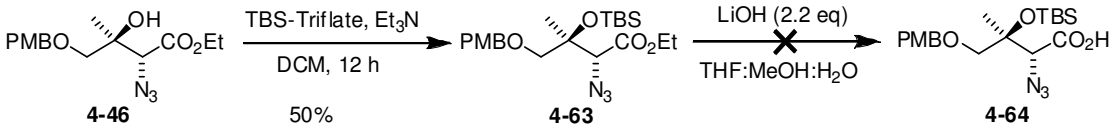
Scheme 4.19: Functional group manipulation.

After failure to protect the tertiary hydroxyl group, PMB deprotection was successfully performed with CAN, but again attempted oxidation methods (Swern,[20] Parikh-Doering,[21] DMP) forced us to abandon the Scheme **4.20**.



Scheme 4.20: Oxidation of primary alcohol.

At this point, we believed that the tertiary alcohol must be protected before oxidation of the adjacent primary alcohol. The reaction sequence was changed for this purpose. First, the TBS group was attached in **4-46** and then hydrolysis of the ethyl ester **4-63** was attempted to generate the free acid **4-64** which was planned to be converted to its benzyl ester for the above mentioned reasons. Here again, the desired compound was not generated, but instead migration of the TBS group took place between the carboxyl and hydroxyl groups to give **4-64′** (Figure **4.2**).



Scheme 4.21: Functional group manipulation.

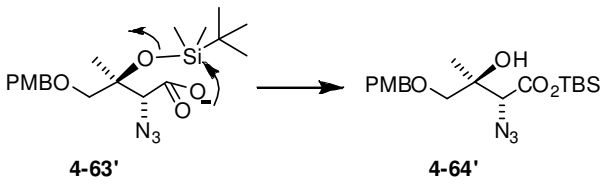
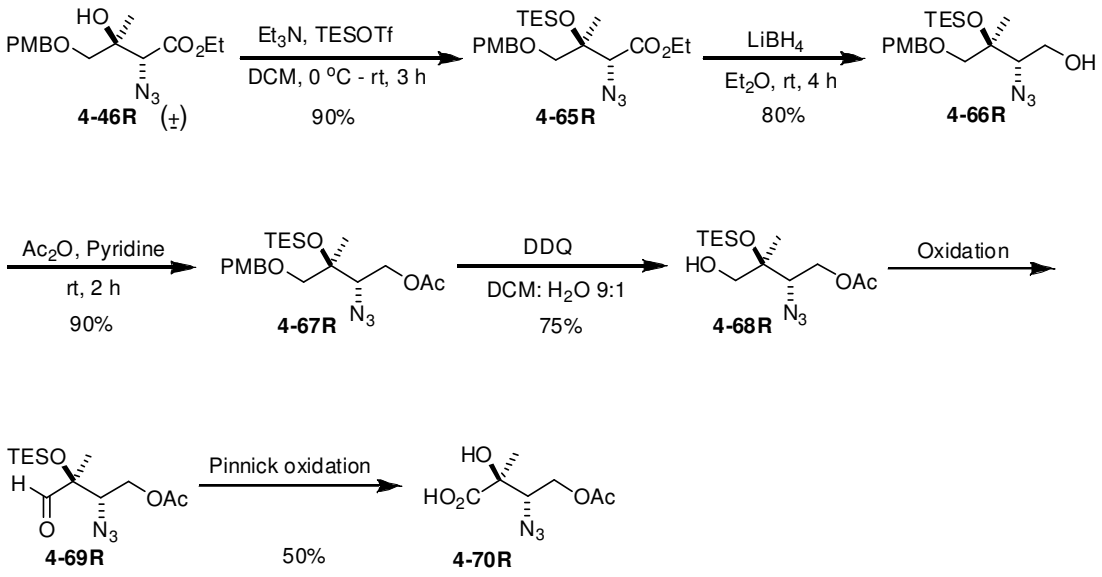


Figure 4.2: TBS migration due to high functional density in the small molecule.

At this juncture, two things seemed crucial: i) protect the tertiary alcohol before oxidation and ii) change the ethyl ester into another functional group to avoid lactone formation. To standardise the protocol, racemic intermediates were used to

reduce cost and time (Scheme **4.22**). For protection of tertiary alcohol **4-46R**, TES was chosen because it has less migratory aptitude than TBS during PMB deprotection.



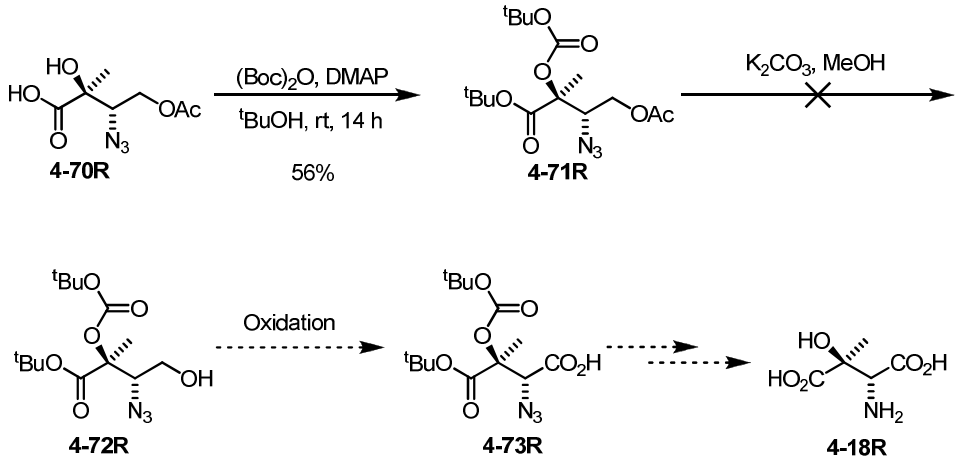
Scheme 4.22: Functional group manipulation.

Table 4.6: Oxidation studies of **4-68R**.

Entry	Reagents	Conditions	Remarks
1	PCC, DCM	rt, 14 h	Trace amount of product
2	Swern	-78 ^{o}C	S.M recovered
3	Parikh-Doering	rt, 14 h	S.M recovered
4	TPAP, NMO, DCM[22]	rt, 14 h	50% yield but not reproducible
5	TEMPO, BAIB, DCM[23]	rt, 12 h	68% yield

The TES group was put on the tertiary alcohol **4-46R** by using TESOTf (TESCl did not work because of its reduced reactivity and steric repulsion) and triethylamine (Et_3N). Under optimised conditions, it was found that the reduction of the ester **4-65R** by $LiBH_4$ in diethylether (Et_2O) produced alcohol **4-66R** in 80% isolated yield. The free alcohol **4-66R** was protected as its acetate **4-67R** and then the

PMB group was deprotected successfully to **4-68R** using DDQ.[24] Various oxidation methods were tried and finally the aldehyde **4-69R** was successfully generated under TEMPO conditions (Table **4.6**, entry **5**) which was immediately converted to the carboxylic acid **4-70R** under mild Pinnick oxidation[25] conditions. Interestingly the TES group hydrolysed off during the Pinnick oxidation step. From previous experience to allow easy handling of intermediates, it was planned to protect both the carboxylic acid and the tertiary alcohol of **4-70R**. Our attempt to protect the carboxylic acid **4-70R** as tert-butyl ester using standard conditions was unsuccessful. A relatively more efficient method[26] with $(Boc)_2O$ and DMAP was thus employed to prepare the corresponding ester. Interestingly, this reactive reagent combination not only generated the tert-butyl ester, but it also protected the tertiary alcohol as its tert-butyl carbonate **4-71R**. Surprisingly, acetate deprotection failed under K_2CO_3 in MeOH. Interestingly, the Boc group migrated to the primary alcohol very quickly when the stronger base NaOMe was applied (Figure **4.3**).



Scheme 4.23: Protection of acid and alcohol in one step.

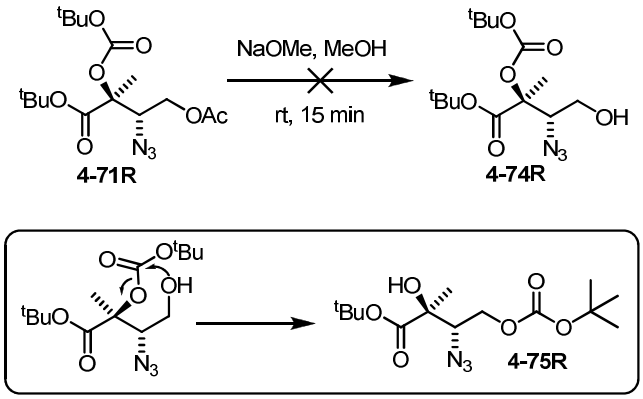
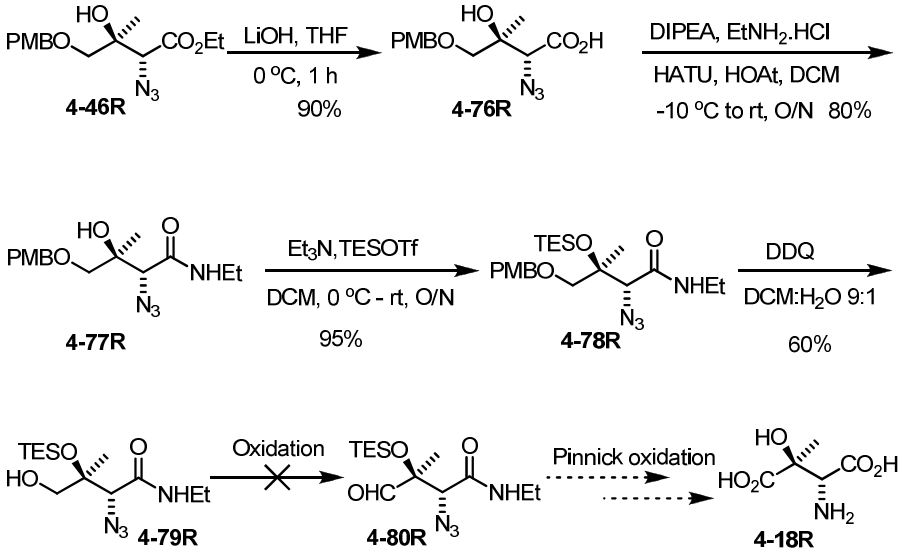


Figure 4.3: Boc migration in strong basic medium.

We next changed the ethyl ester group of **4-46R** to an amide (Scheme **4.24**) to inhibit lactone formation during PMB deprotection. For this, **4-46R** was hydrolysed to its acid **4-76R** and then converted to its ethyl amide **4-77R**. The tertiary alcohol was first TES protected before PMB deprotection by DDQ. In this case, we were happy to get the free alcohol **4-79R**. Unfortunately, various oxidation attempts were not successful to get the desired aldehyde **4-80R** (Table **4.7**).

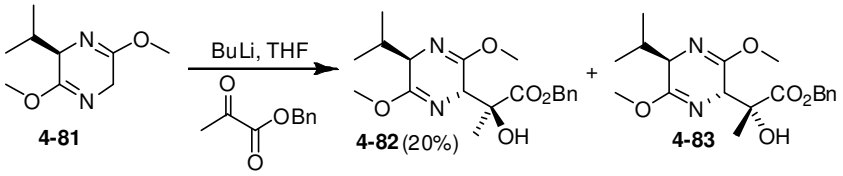


Scheme 4.24: Converting the ester group to an amide group.

Table 4.7: Oxidation studies of **4-79R**.

Entry	Reagents	Conditions	Remarks
1	PCC, DCM	rt, 14 h	Trace amount of product
2	DMP, DCM	0 ^{o}C to rt, 14 h	Complex TLC
3	Parikh-Doering	0 ^{o}C to rt, 14 h	TES group migration
4	TPAP, NMO, DCM	rt, 14 h	TES group migration
5	TEMPO, BAIB, DCM	rt, 12 h	Complex TLC

After several sets of failures in functional group manipulation to get the desired amino acid, it was necessary to search for an alternative plan to prepare the amino acid **4-18R**. In this study, we found that Schöllkopf's auxiliary[27] may be best suited for that purpose, although there are limited examples to explore the generation of quaternary centres.



Scheme 4.25: Exploring Schöllkopf auxiliary for chirality control.

The ideas behind choosing this auxiliary were as follows:

1) Potentially it is possible to generate all four isomers.
2) In a single reaction, it is possible to get two diastereomers because the newly formed chiral centre should be controlled by the chiral centre already present in the auxiliary.

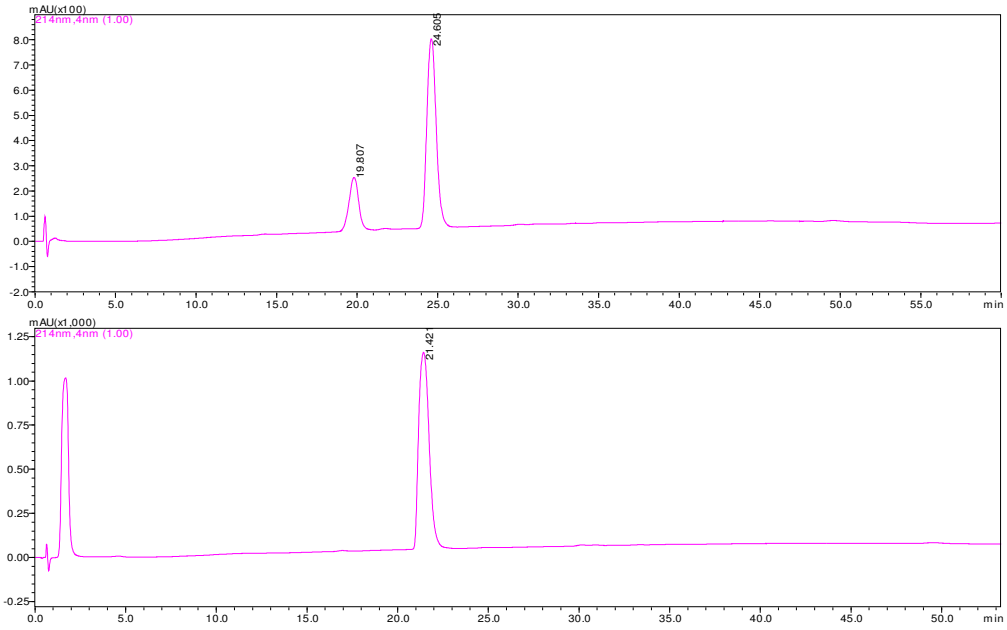
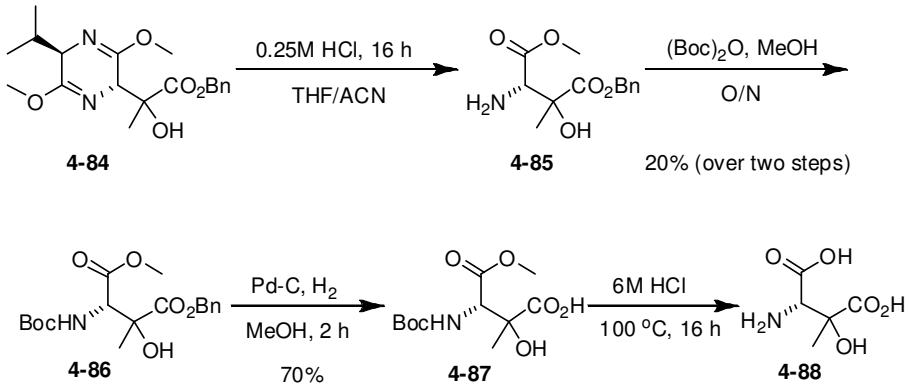


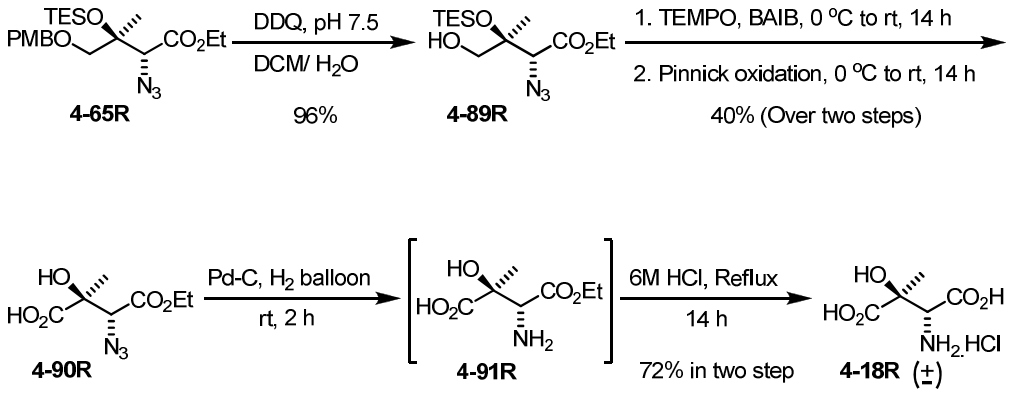
Figure 4.4: LCMS profile of column purified products (**4-82** and **4-83**) using Schöllkopf auxiliary.

In our plan, we converted pyruvic acid to its benzyl ester and that was used as the electrophile with the Schöllkopf's auxiliary **4-81** in the presence of BuLi (Scheme **4.25**). The desired coupling product was obtained but four diastereomers were found instead of two. After careful column chromatography, we obtained the major isomers **4-82** and **4-83** but one of them was contaminated with another isomer (Figure **4.4**). The absolute stereochemistry of the products was assumed from theory but we did not know which one was which stereoisomer. In the next step, the most pure fraction was subjected with 0.25M HCl to cleave the auxiliary. The resulting amine **4-85** was converted to its Boc protected form **4-86**. The yield of the reaction was poor (20%) but still we proceeded further by cleaving the benzyl ester and hydrolysing the methyl ester. Trace amounts of product **4-88** was obtained albeit in impure form by NMR.



Scheme 4.26: Cleaving of auxiliary.

Poor stereoselectivity made Schöllkopf's protocol difficult to use for determining the absolute stereochemistry of the amino acid present in the natural peptide. Returning to a previous scheme, it was thought that deprotection of the PMB of **4-65R** in buffered condition (towards neutral pH) may produce the desired free alcohol (because lactone formation was facilitated in acidic or basic medium). It was first tried using DDQ in pH 7.5 (Phosphate buffer)[28] and despite previous failures the primary alcohol **4-89R** was obtained in excellent yield. Next, BAIB and TEMPO were found to be the best combination (developed by Margarita)[23] for the problematic oxidation step to produce the aldehyde from **4-89R**. The resulting aldehyde was oxidised to acid by mild Pinnick oxidation to generate **4-90R** in moderate yield. In the Pinnick oxidation, the quaternary TES group deprotected reasonably via migration to form a silylester and hydrolysis during acidic workup. The azide reduction and ester hydrolysis were performed next to prepare the desired amino acid **4-18R** in racemic form. With the amino acid in hand, it was coupled with Marfey's reagent[29] and compared with the natural residue via LCMS.



Scheme 4.27: PMB deprotection under buffered pH 7.5.

As **4-18R** was racemic, this would clarify two aspects: if one isomer matched then we needed to synthesise one of the two *anti*-isomers but if none matched, then we should concentrate synthetic effort on its *syn*-isomers. Since none matched (Figure **4.5**), we concluded that one of the *syn* isomers would be present in the natural peptide. To synthesise the *syn*-amino acid, we needed to use the *Z*-isomer **4-92** (Scheme **4.28**). First, we chose to use AD-mix α because we knew that it gave 80% ee of diol **4-93**, so 10% of the other enantiomer would be present for comparison purposes after Marfey's derivatisation during LCMS. Thus, following the established synthetic strategy (Scheme **4.28**) we made the (2*S*, 3*S*) isomer of the desired amino acid **4-99** in 80% ee and found that the major enantiomer exactly matched with the amino acid present in the peptide (Figure **4.6**).

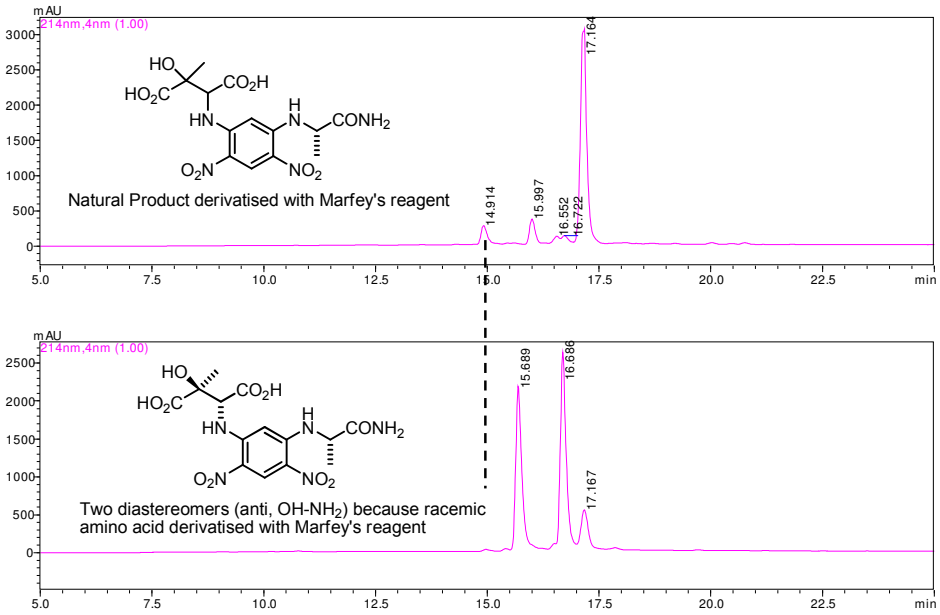
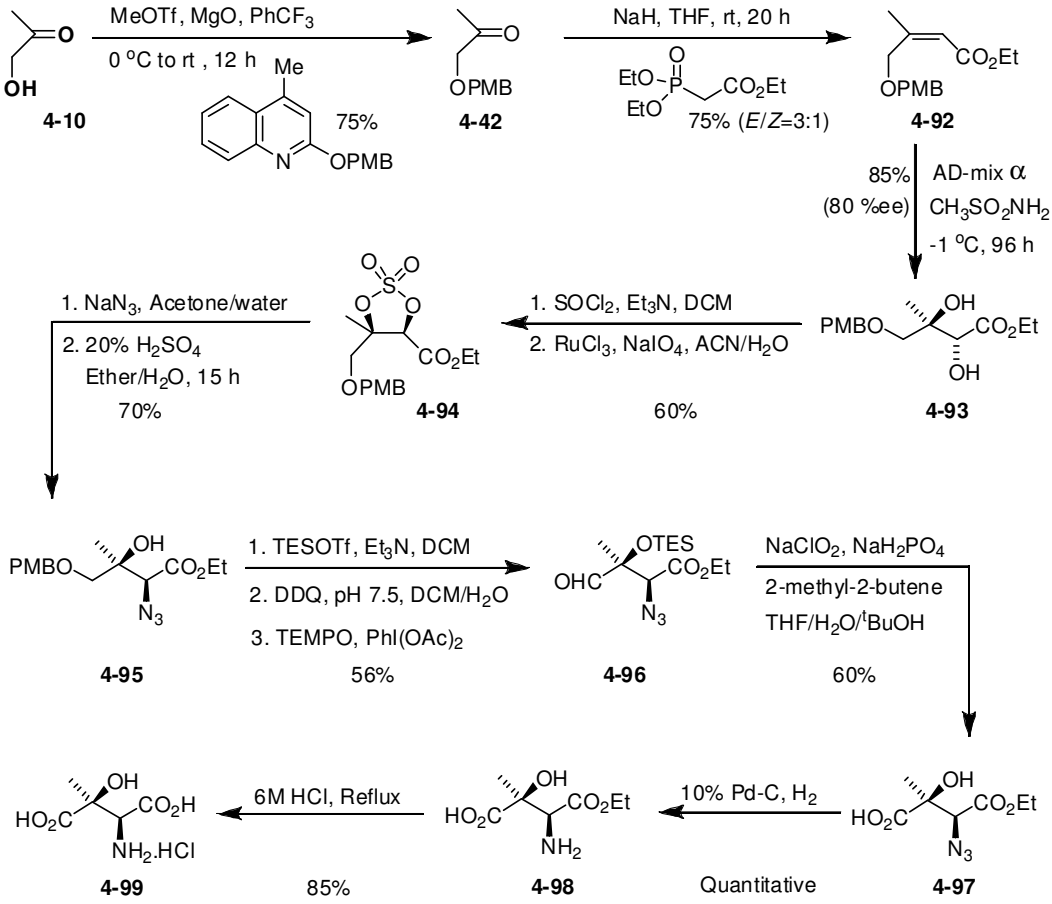


Figure 4.5: LCMS of mixture of two diastereomers.

In the conclusion, the absolute stereochemistry of the amino acid present in the peptide was determined to be (2*S*, 3*S*) as **4-99**.



Scheme 4.28: Established protocol to synthesise the desired amino acid **4-99**.

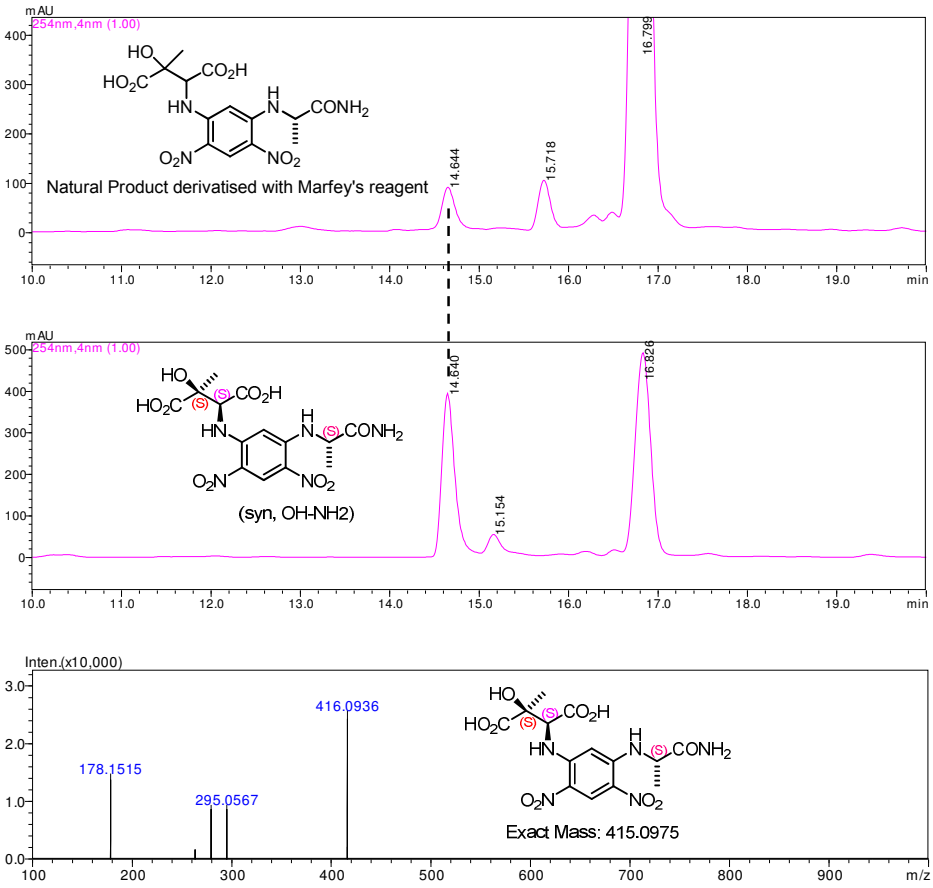
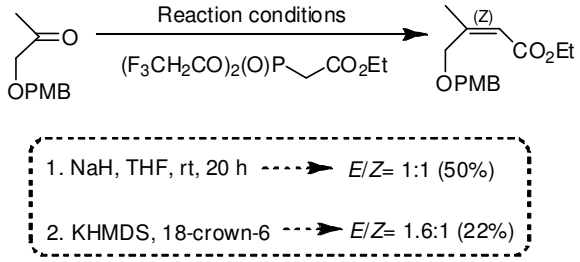


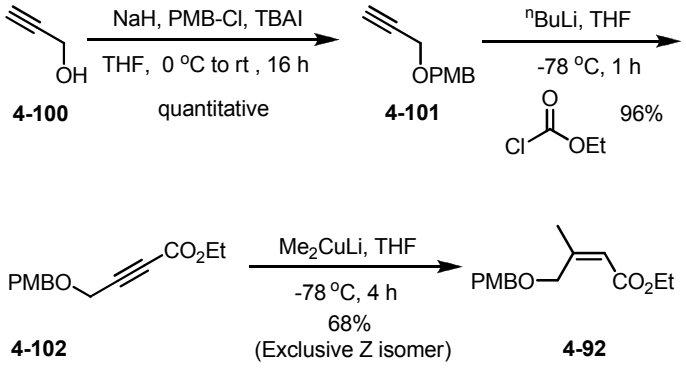
Figure 4.6: LCMS chromatogram for comparison.

After knowing the fact that we needed more of the *Z*-olefin **4-92** to prepare the specific amino acid enantiomer **4-99**, we need to improve the *Z* selectivity (Scheme **4.29**). Thus the Still-Gennari modification[30] of the HWE reaction was tried and gave moderately better selectivity, however, separation of the two isomers was still tedious and time consuming.



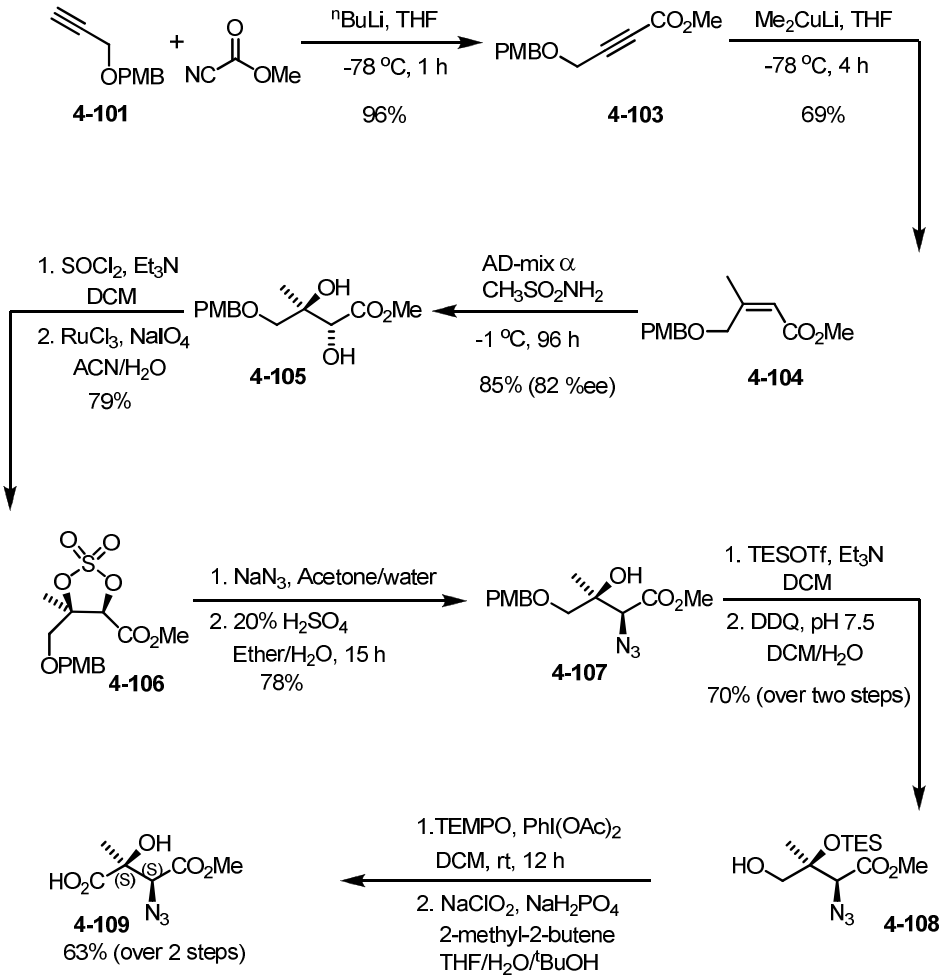
Scheme 4.29: Still-Gennari HWE modification.

It was thus decided to perform the conjugate addition of a Gilman reagent to an alkyne ester to generate the tri-substituted *Z*-olefin (Scheme **4.30**). We started with the protection of propargyl alcohol **4-100** with PMB-Cl and then the terminal alkyne **4-101** was coupled with ethyl chloroformate to prepare **4-102** in 96% yield.[31] Me_2CuLi was prepared freshly and then used for Michael addition to alkyne **4-102** at the β-carbon to get the *Z*-isomer exclusively.[32] Scaling up of this reaction was found problematic in order to maintain exclusive *Z*-selectivity. This was solved by extensive standardisation of the workup procedure, whereby double-bond isomerisation was presumed to occur.



Scheme 4.30: Stereoselective synthesis of the *Z*-isomer.

Having a good method to generate the *Z*-isomer of the ethyl ester, we next planned to synthesise the all methyl ester of the whole natural and synthetic peptide for a direct comparison without resorting to an ester hydrolysis step. We reasoned that this strategy would save one step and facilitate our aim without purification and isolation problems on small scales (the final peptide would also be highly polar and may be to some extent water soluble, so there would be a possibility to lose some material during purification and isolation).



Scheme 4.31: Final complete synthetic plan.

Finally, we reformed our whole synthetic scheme for the methyl ester version as depicted in Scheme **4.31**. In our previous Scheme **4.28**, we obtained the ethyl ester **4-97** in 1.7% overall yield in 9 steps. Here, the methyl ester **4-109** was prepared in 16% overall yield in 9 steps with better enantiomeric purity (82% ee) in the SAD reaction of methyl ester **4-104**.

4.7 Conclusion

In this chapter we described our synthetic studies to prepare all the isomers of the desired amino acid **3** and determined the absolute stereochemistry of the amino acid present in the natural peptide **N1708**. The desired amino acid precursor **4-109** was obtained in 16% overall yield in 9 steps. The absolute configuration of the amino acid **3** present in natural peptide **N1708** was (2*S*, 3*S*).

References

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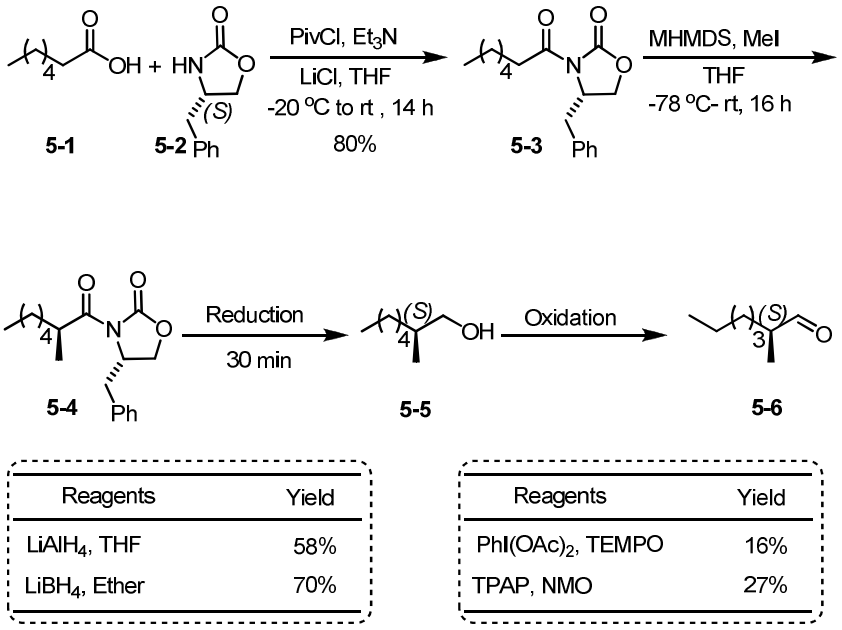
CHAPTER 5

Synthesis of 5-Methyl-$\Delta^{3,4}$ Decanoic Acid

5.1 Asymmetric methylation using Evan's chiral auxiliary

The fatty acid moiety **4** was the last fragment to be synthesised before we engaged in the total synthesis. As we did not isolate this part from natural product hydrolysis, it was required to be synthesised in both enantiomeric forms to finish the full molecule. HPLC and NMR comparison between the two diastereomers of the whole synthetic peptide and the isolated peptide would then confirm the absolute configuration of the fatty acid in the lipopeptide. For asymmetric methylation, one of the best and easy choices is to use Evan's auxiliary. Normally, Evan's auxiliary is coupled to the carboxylic acid using BuLi but this was found inefficient with heptanoic acid in our hands. We thus used a modified method to attach the auxiliary to the heptanoic acid using a mixed anhydride strategy in the presence of LiCl.[1]

The methylation[2] step was attempted using different strong bases (Table **5.1**) and LDA was found to be the best. The auxiliary was cleaved by reduction[3] of **5-4** to afford alcohol **5-5**. $LiBH_4$ in Et_2O produced better results than those reported. Oxidation of the primary alcohol was performed carefully with relatively mild methods in order to reduce the chance of a potential racemisation at the α-centre of the resultant aldehyde. Unfortunately, the isolated yield of the oxidation step was not good because of the volatility of the aldehyde **5-6**.



Scheme 5.1: 1st Generation synthetic plan of fatty acid portion.

Table 5.1: Methylation using different bases.

Entry	Reagents	Conditions	Remarks
1	NaHMDS, THF	-78 °C to rt, 16 h	48%
2	KHMDS, THF	-78 °C to rt, 16 h	53%
3	LDA, THF	-78 °C to rt, 16 h	66%

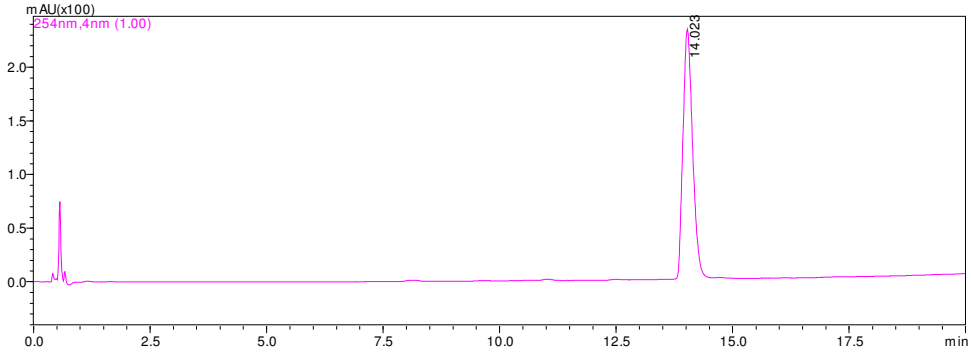
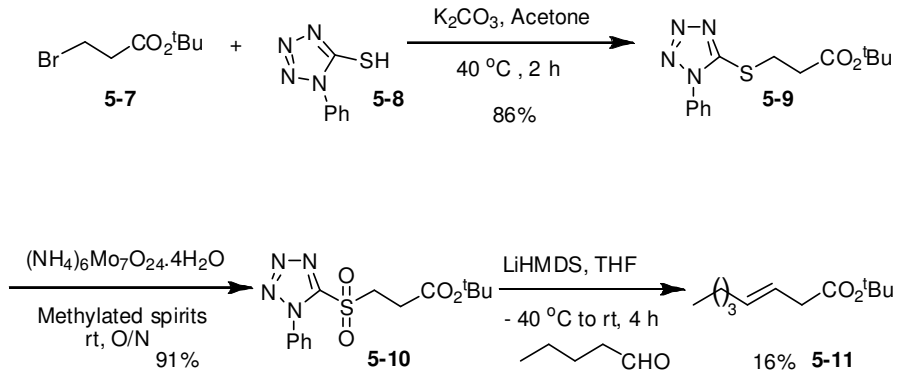


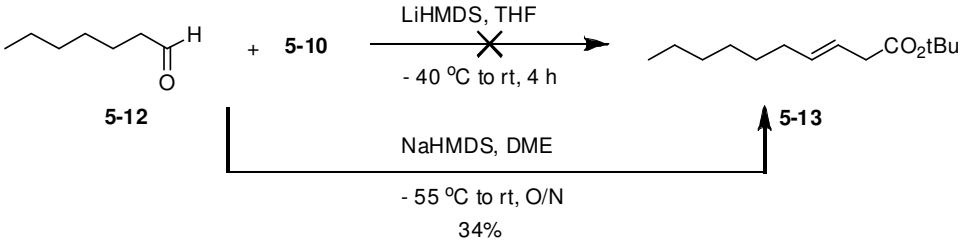
Figure 5.1: LCMS data for asymmetric methylation.



Scheme 5.2: Model study for sulfone formation and olefination reaction.

5.2 Model studies of Julia-Kocienski olefination

We targeted a fatty aldehyde unit **5-6** because it would be one of our substrates for the Kocienski modified Julia olefination reaction. The thioether **5-9** was thus synthesised by combining tert-butyl-3-bromopropanoate and tetrazol **5-8** in basic conditions.[4] Thioether **5-9** was converted to sulfone **5-10** by oxidation with hexaammonium heptamolybdate. In our model study for olefination, we first used pentanal in the presence of LiHMDS at low temperature to produce the olefin in 16% yield. We also tried with heptanal using the same reacting conditions but did not obtain the desired product at all. From the literature, NaHMDS normally produces better yields. We thus used NaHMDS and achieved a 34% yield of **5-13**. Next, we wanted to use the same reaction conditions on our targeted fatty aldehyde **5-6**. This generated trace amounts of the desired olefin product. As the yield of **5-6** was too low and the olefination reaction was not efficient, we reversed the sulfone and aldehyde counterparts. The idea behind this change was to gain ready access to the two fragments for the olefination reaction.



Scheme 5.3: Kocienski modified Julia olefination reaction with heptanal.

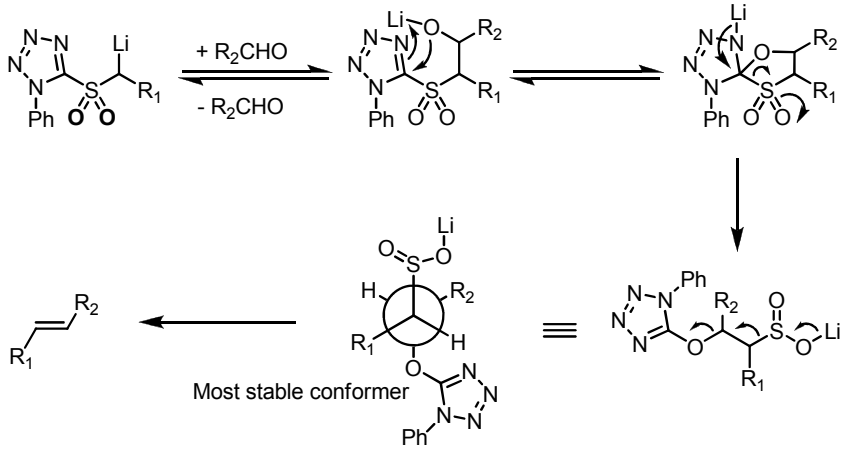
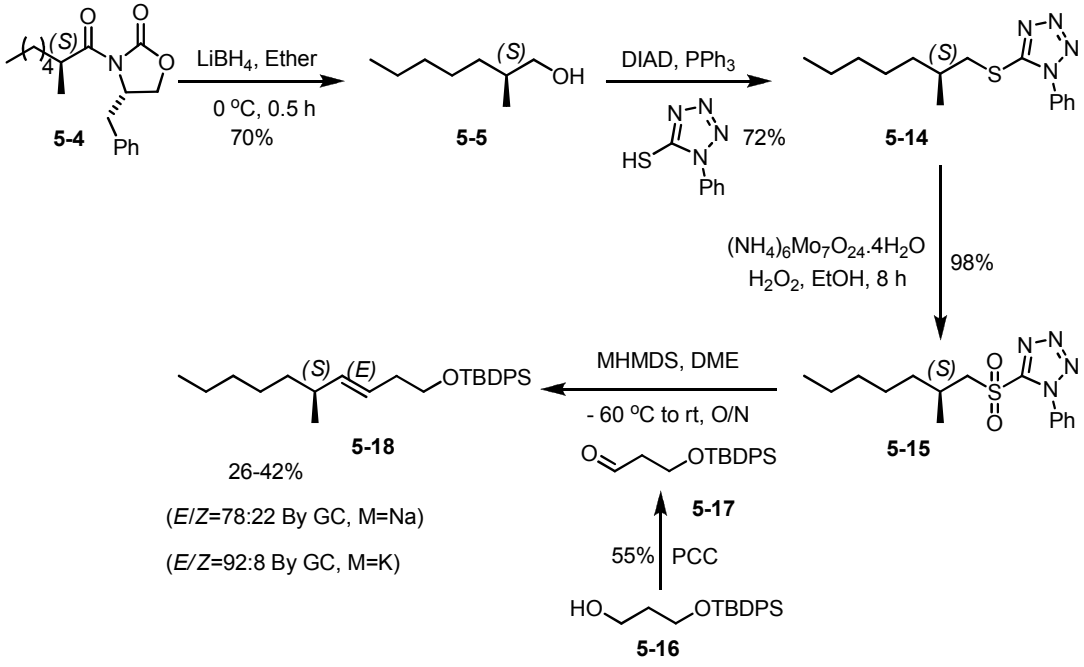


Figure 5.2: Mechanism of Julia-Kocienski olefination.

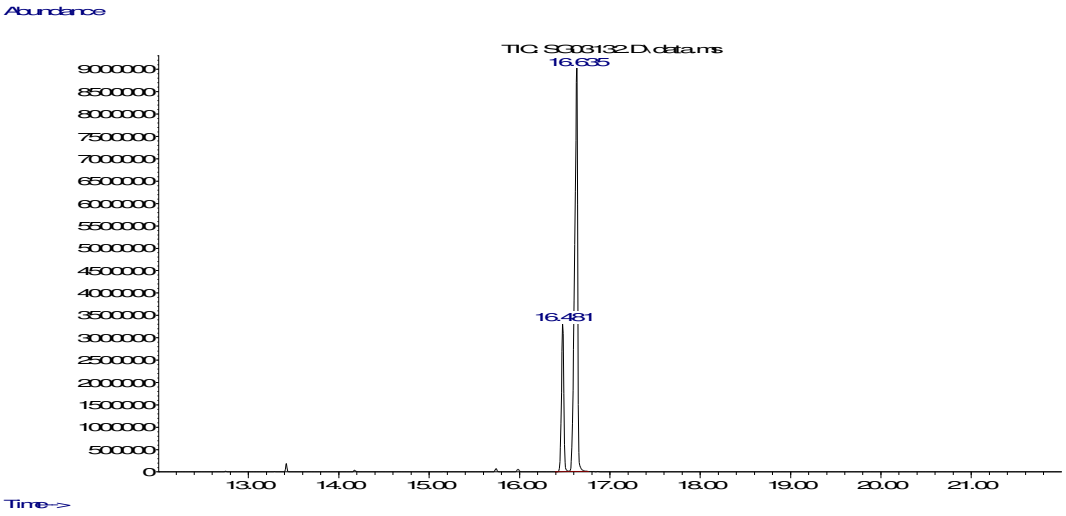
5.3 2nd Generation synthetic plan

In this new strategy, alcohol **5-5** was converted to the thioether **5-14** by Mitsunobu reaction (Scheme **5.4**). Following the same oxidation method (Scheme **5.2**), sulfone **5-15** was prepared. Using the reported method, TBDPS protected 3-carbon aldehyde **5-17** was obtained readily. We succeeded to produce reasonable amounts of the two counterparts for the olefination reaction, hopefully sufficient material to prepare enough fatty acid. Kocienski's modified[5] olefination procedures using two different bases showed that the best selectivity (*E/Z*=92:8) as measured by GCMS, was obtained using KHMDS. With **5-18** in hand, the TBDPS group was deprotected by TBAF to the alcohol **5-19**. Purposefully avoiding a stepwise oxidation sequence to

circumvent potential double bond migration to produce a more stable α, β-unsaturated aldehyde, the desired carboxylic acid was obtained directly using Jones oxidation, albeit in poor yield. While Jones reagent was added to the alcohol, considerable amount of the ester was formed; however, the reverse addition technique reduced unwanted ester formation and produced better yields (Table **5.2**, entry **3**).



Scheme 5.4: 2nd Generation synthetic plan.



Area Percent Report

Data Path : D:\1\DATA\Chemistry\Sada\
Data File : SG03132.D
Acq On : 1 Dec 2009 11:26
Operator : SWXIAO
Sample : SG03132
Misc :
ALS Vial : 2 Sample Multiplier: 1

Integration Parameters: autoint1.e
Integrator: ChemStation

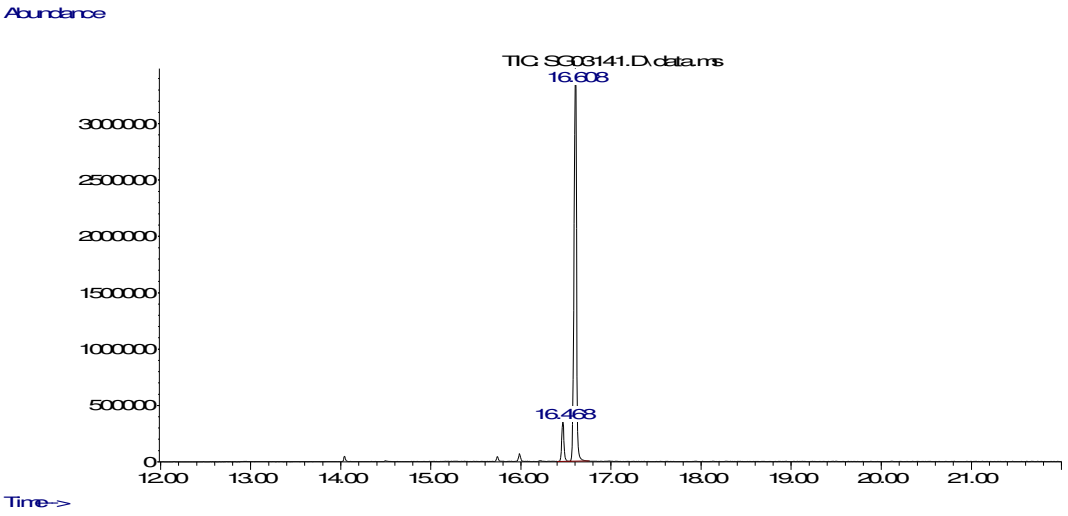
Method : C:\msdchem\1\METHODS\TLL-Quant.M
Title : Epichlorohydrin

Signal : TIC: SG03132.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	16.481	2020	2034	2045	BV	3268970	59342243	28.05%	21.907%
2	16.635	2045	2058	2077	VB	8935221	211541213	100.00%	78.093%

Sum of corrected areas: 270883457

Figure 5.3: GCMS profile of Julia-Kocienski reaction using NaHMDS.



Area Percent Report

Data Path : D:\1\DATA\Chemistry\Sada\
Data File : SG03141.D
Acq On : 23 Dec 2009 12:32
Operator : SWXIAO
Sample : SG03141
Misc :
ALS Vial : 4 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 1 Min Area: 5 % of largest Peak
Start Thrs: 0.1 Max Peaks: 100
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Tangent else baseline drop >
Peak separation: 2

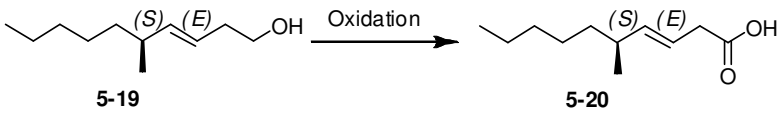
Method : C:\msdchem\1\METHODS\TLL-Quant.M
Title : Epichlorohydrin

Signal : TIC: SG03141.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	16.465	2022	2031	2040	rBB	351706	616483	9.08%	8.321%
2	16.604	2044	2053	2075	rBB	3482859	6792336	100.00%	91.679%

Sum of corrected areas: 7408819

Figure 5.4: GCMS profile of Julia-Kocienski reaction using KHMDS.



5-19 5-20

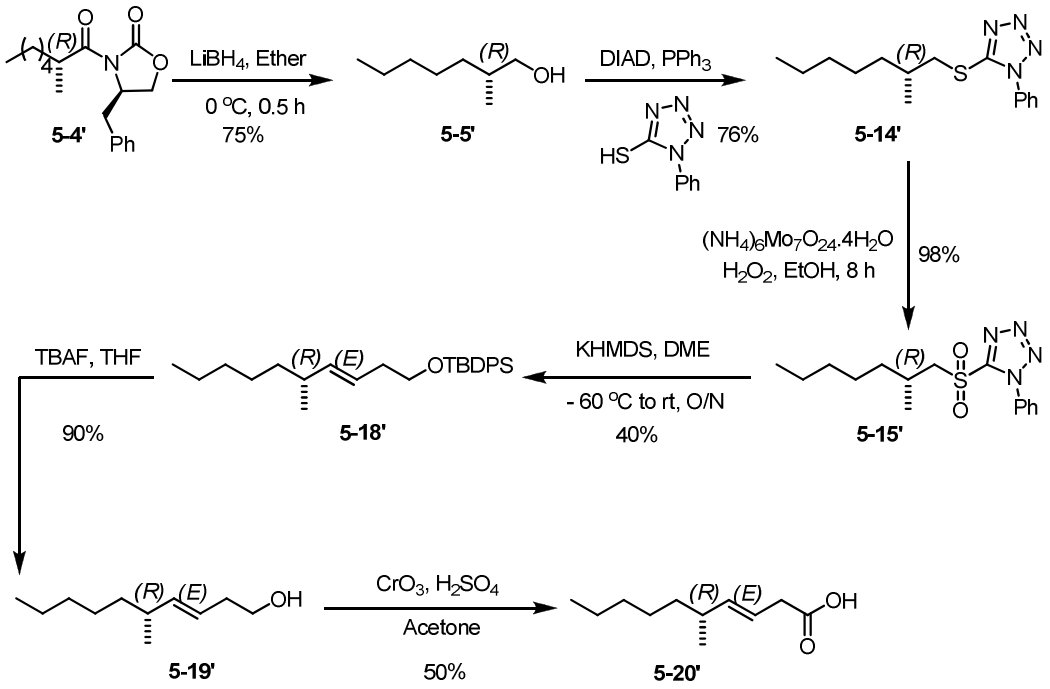
Scheme 5.5: Oxidation studies.

Table 5.2: Oxidation studies.

Entry	Reagents	Conditions	Remarks
1	TEMPO, BAIB, ACN/H_2O[6]	0 ^oC to rt, 16h	15%
2	TPAP, NMO, Acetone[7]	0 ^oC to rt, 16h	No reaction
3	CrO_3, Acetone, H_2SO_4	0 ^oC to rt, 16h	50%

5.4 Synthesis of other enantiomer of the fatty acid

We prepared the *R*-isomer of the alcohol unit **5-5′** with the help of the other enantiomer of the Evan's auxiliary by following the same procedure as established (Scheme **5.1**). Mitsunobu reaction conditions produced **5-14′** in 76% yield. The sulfone **5-15′** was obtained by oxidation with hexaammonium heptamolybdate. Next, Kocienski's modified procedure was applied to produce **5-18′**. Standard TBAF mediated conditions produced free alcohol **5-19′** followed by Jones oxidation afforded desired fatty acid **5-20′** with (*R*) configuration (Scheme **5.6**).



Scheme 5.6: Synthesis of (*R*)-fatty acid.

5.5 Conclusion

In this chapter, we successfully synthesised two enantiomers of the desired fatty acid (**5-20** and **5-20′**) for preparing the whole peptide. In this strategy, we used Evan's auxiliary-directed asymmetric methylation and Kocienski-modified Julia olefination to install the *trans* double bond present in the fatty acid.

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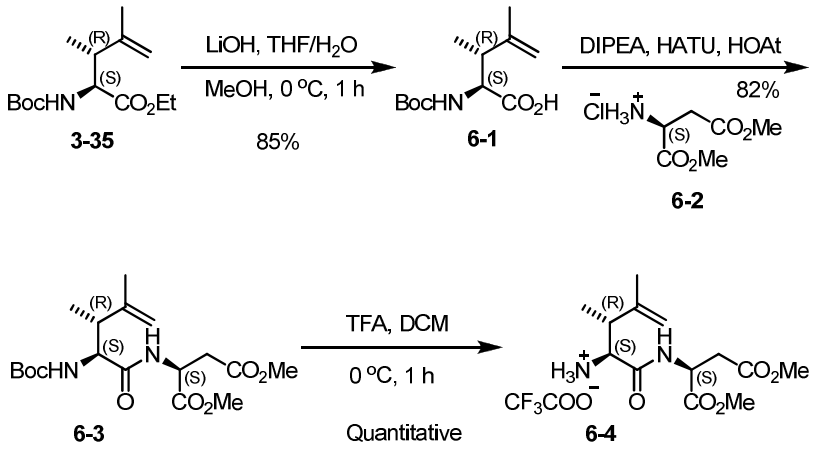
CHAPTER 6

Coupling of Fragments & Full Structural Assignment

6.1 Peptide coupling

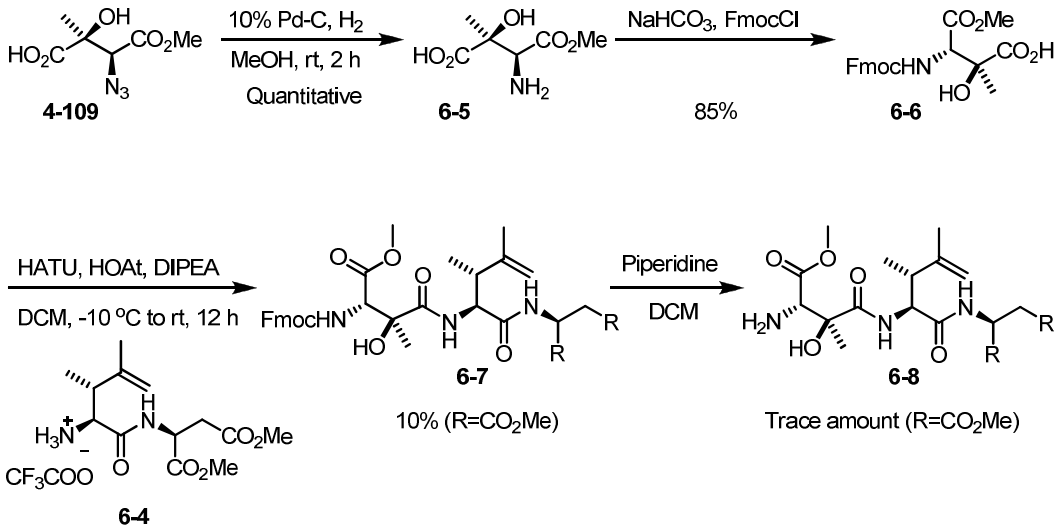
Having all fragments in hand, we needed to synthesise the whole peptide. As we were unsuccessful in isolating the fatty acid residue of the natural lipopeptide, we had to prepare two possible diastereomers to compare with the isolated natural product. To save materials, it was planned to prepare the whole peptide as its trimethyl ester. The advantages are: i) the trimethyl ester could be directly compared with the isolated natural peptide (as it is easy to convert the tricarboxylic acid to its trimethyl ester) avoiding a peptide hydrolysis step; ii) isolation and purification of the resulting triacid from the hydrolysis of the synthetic peptide might be difficult on a small scale due to its polarity at the late stage; and iii) we would obtain more characteristic proton signal which may help us to compare ^{1}H-NMR data.



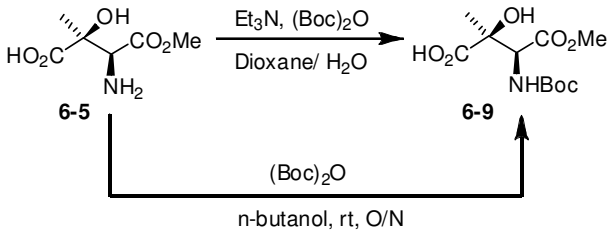
Scheme 6.1: Synthesis of dipeptide **6-4**.

It was reasoned that the sequential linear coupling of the amino acid fragments and then the fatty acid would be more a logical choice over a convergent coupling sequence because we needed to attach two enantiomers of the fatty acid portion to the common tripeptide unit to obtain the two possible structures of the final peptide. L-aspartic acid was thus converted to the dimethyl ester hydrochloride salt **6-2** using $SOCl_2$ in MeOH.[1] Compound **3-35** was hydrolysed to its free carboxylic acid to couple with **6-2** to prepare **6-3** in good yield. Deprotection of the Boc group was performed by TFA to yield the TFA salt **6-4** quantitatively, which was ready for the synthesis of the tripeptide (Scheme **6.2**).

Next, we wanted to reduce the azide of **4-109** and protect the free amine before coupling to the dipeptide. Initially we chose Fmoc because its UV activity would help to purify the tripeptide by prep HPLC on small scale. Catalytic hydrogenation followed by standard Fmoc protection[2] was performed to obtain **6-6** which was then coupled to **6-4** to generate the tripeptide **6-7**. It was found that the latter coupling reaction was inefficient and the isolated yield was about 10%. We pushed forward to get the free amine, which proceeded in a messy fashion and only trace amounts of the desired product **6-8** were isolated.

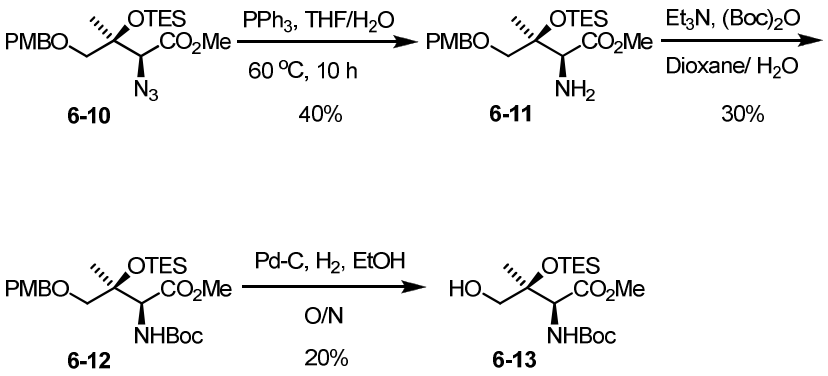


Scheme 6.2: Synthesis of tripeptide.



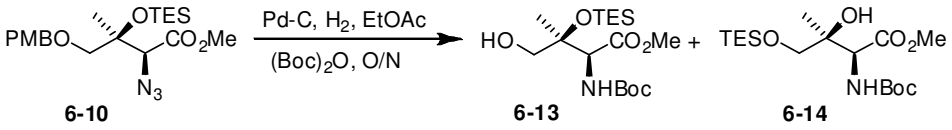
Scheme 6.3: Boc protection.

These practical limitations forced us to switch the protecting group to Boc. We used biphasic conditions as well as base free conditions (Scheme **6.3**), but none of them turned out to be clean; only 10-15% isolated yields were obtained. At this point we changed the sequence of reactions. We used Staudinger reaction to reduce the azide in the presence of PMB group to generate the free amine **6-11** and then protected with Boc, which was then subjected to hydrogenation conditions to cleave the PMB group and prepare the free alcohol **6-13**. This functional group manipulation (Scheme **6.4**) was also found to be low yielding.



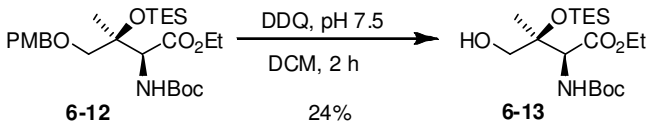
Scheme 6.4: Functional group manipulation.

As the above scheme was inefficient, we tried to improve reaction yields using other methods. We made an effort to synthesise **6-13** in one step from **6-10** using hydrogenation conditions in the presence of $(Boc)_2O$ (Scheme **6.5**).[3] Interestingly, it was noticed (by TLC monitoring) that in addition to the desired product **6-13**, considerable amounts of **6-12** and TES group migrated **6-14** were also present in the reaction mixture.



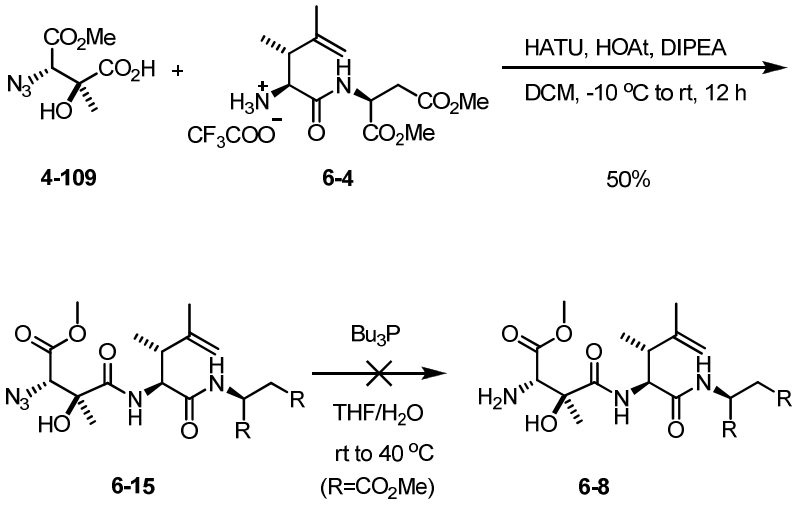
Scheme 6.5: One step procedure for azide reduction, Boc protection and PMB cleavage.

This observation indicated that azide reduction and Boc protection were faster than the PMB deprotection. In our next plan for the preparation of **6-13** the oxidative cleavage of the PMB group was attempted on **6-12** under buffered conditions[4] but again very low yields were realised, which indicated that we needed to search another reaction sequence.

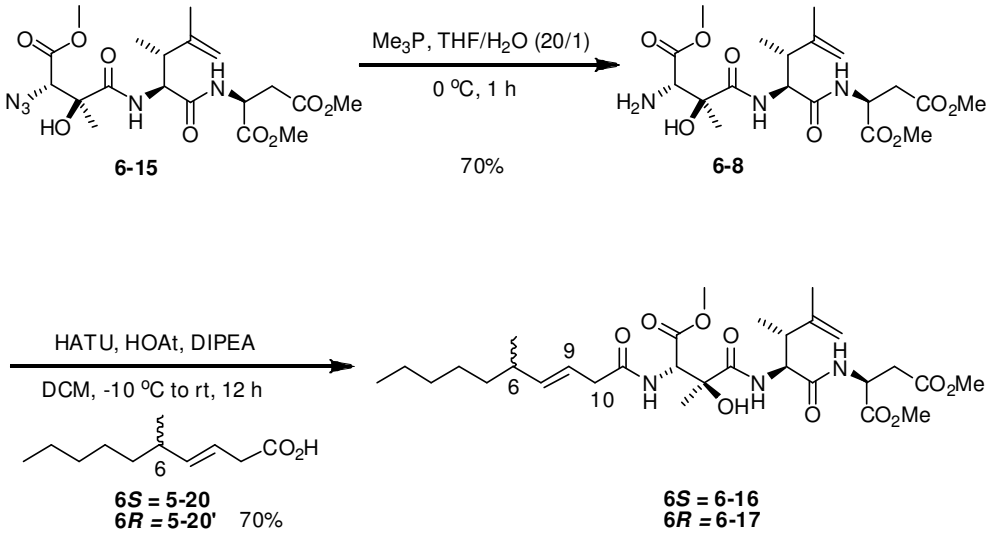


Scheme 6.6: Oxidative cleavage of PMB.

To circumvent these inefficiencies, we thought to couple the azido acid **4-109** directly with the dipeptide **6-4** to generate tripeptide **6-15** with an azide functionality and then generate the free amine by Staudinger reaction. We accomplished **6-15** in 50% isolated yield by using standardised coupling reaction conditions. As our azide was situated near to the quaternary centre, the bulky common reagent Ph_3P was reasoned to be a bad choice for the reduction of the azide present in the tripeptide. It is also known that $Ph_3P(O)$ (side product when Ph_3P is used for Staudinger reaction) could create an issue during purification. For these two reasons, we used the more reactive Bu_3P for the azide reduction, but unfortunately the azido-reduction did not occur. Eventually we used Me_3P that has been reported by the Nicolaou group for azide reduction in a complicated peptide.[5]

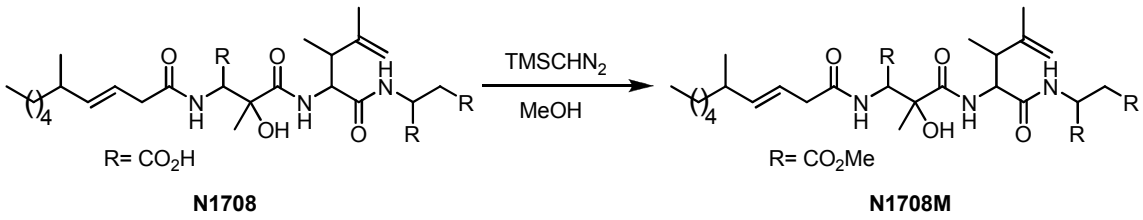


Scheme 6.7: Synthesis of azido tripeptide and Staudinger reaction.



Scheme 6.8: Final coupling and synthesis of trimethyl ester of target peptide.

Staudinger reaction using the reactive and small phosphine Me_3P was performed on **6-15** under slightly modified reaction conditions gave **6-8** in 70% yield. This free amine was then coupled with the two enantiomers of the fatty acid to generate two diastereomers. After obtaining the trimethyl ester of the natural lipopeptide, we wanted to convert our isolated natural product to its trimethyl ester derivative for comparison purpose. In this case, we used the less toxic $TMSCHN_2$ in MeOH to convert the carboxylic acid of natural product to its trimethyl ester.[6]



Scheme 6.9: Conversion of the natural product **N1708** to trimethyl ester derivative.

For the convenience of NMR analysis, we named the different trimethyl esters according to the chiral centre present in the fatty acid portion (N represents the derivatised Natural product with unknown stereochemistry).

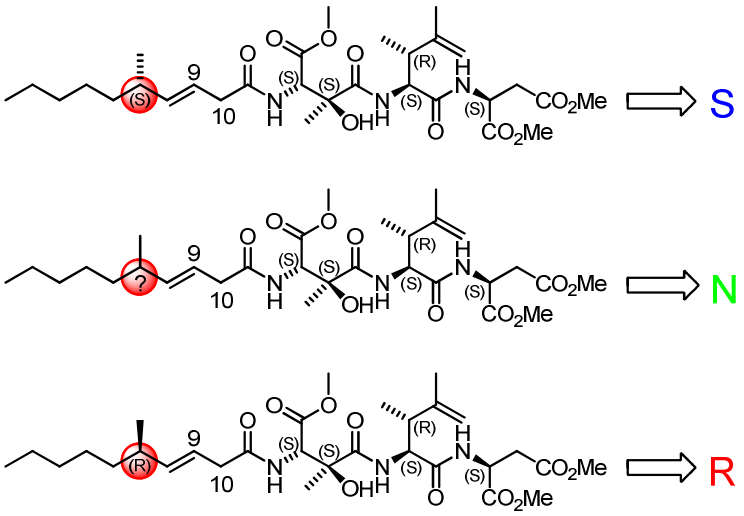


Figure 6.1: Alphabetic representation of the full peptide structure.

We next recorded the ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) of the three compounds in CD_3OD and $CDCl_3$. In methanol, no significant difference was observed in their chemical shift values but in chloroform significant differences in ^{13}C-NMR were observed.

^{13}C NMR in $CDCl_3$ in 125 MHz :

(S)	(N)	(R)
14.08	14.11	14.09
14.15	14.12	14.14
20.40	20.42	20.41
20.93	20.95	20.93
22.60	22.61	22.60
23.97	23.96	23.97
26.92	26.94	26.93
29.69	29.69	29.69
31.97	31.97	31.97
35.88	35.87	35.88
36.73	36.74	36.76
36.77	36.76	36.76
40.19	**40.19**	**40.24**
42.16	42.15	42.17
48.66	48.63	48.66
52.04	52.07	52.05
52.80	52.83	52.81
52.92	52.95	52.92
55.19	55.14	55.19
57.56	57.52	57.57
76.44	76.44	76.44
112.68	112.70	112.68
119.93	**119.91**	**119.98**
142.48	142.50	142.46
145.59	145.56	145.59
170.03	170.03	170.06
170.65	170.66	170.66
171.29	171.32	171.31
171.75	171.76	171.78
172.74	172.76	172.69
175.86	175.88	175.83

Table 6.1: Chemical shift values (δ_{ppm}).

We first plotted the differences between the two diastereomers R and S and found significant difference at C-9 and C-10 (all the carbons were assigned according to COSY, HMQC and HMBC NMR spectra) which would be expected because these

carbons are part of the fatty acid portion and are near to the chiral center of interest (Figure **6.2**).

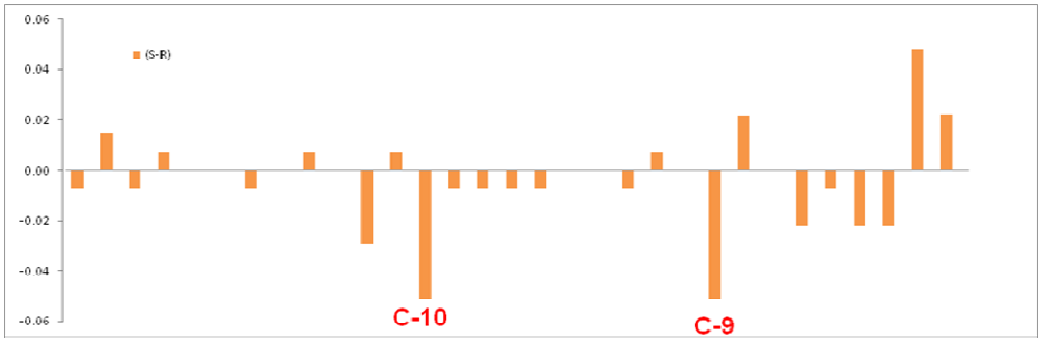


Figure 6.2: Differences of chemical shift of corresponding carbon between S and R.

Next, we plotted the differences of the corresponding carbon between N to S (in blue) and N to R (in maroon) in a single plot for easy visualisation and found the C-9 and C-10 of S being much closer in value to N as compared to R (the blue colour for C-10 is absent which means equal in value to N and the blue colour is very short compared to maroon for C-9 meaning it is close to the value of N, Figure **6.3**).

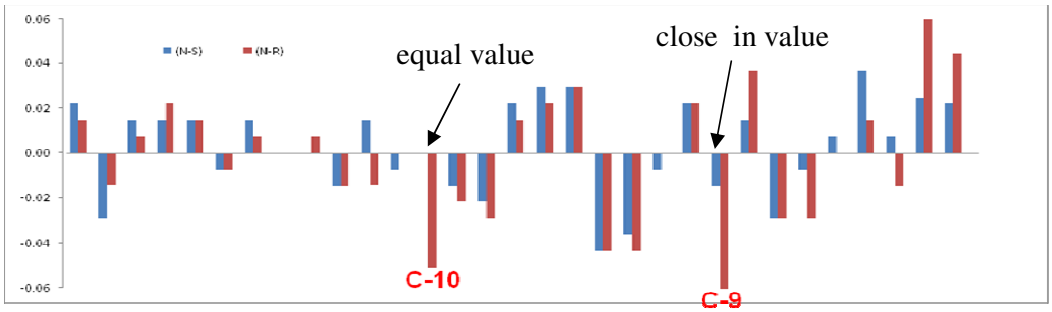


Figure 6.3: Comparison of differences in ^{13}C NMR (in $CDCl_3$) among N, S and R forms of lipopeptide **N1708**.

The LCMS (10% to 100% ACN/H_2O) for all three components were found to display similar retention times (ΔRT for S was 0.01 min. whereas ΔRT for R was 0.05 min.). The colour code representation and LCMS data gave us confidence to conclude primarily that the S diastereomer is closer to the natural form N.

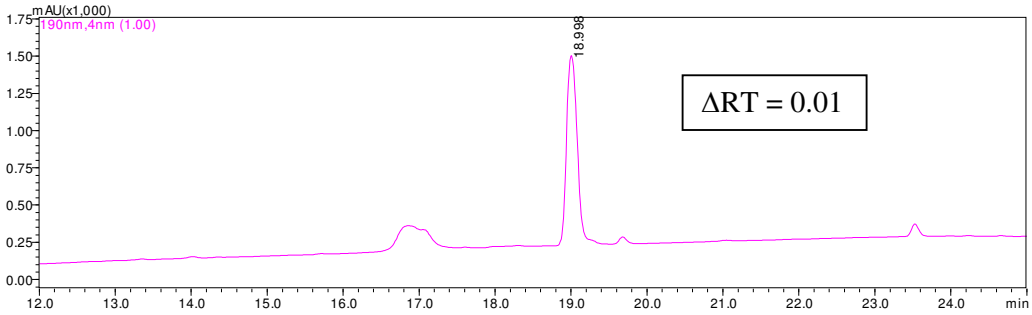


Figure 6.4: LCMS Chromatogram of S.

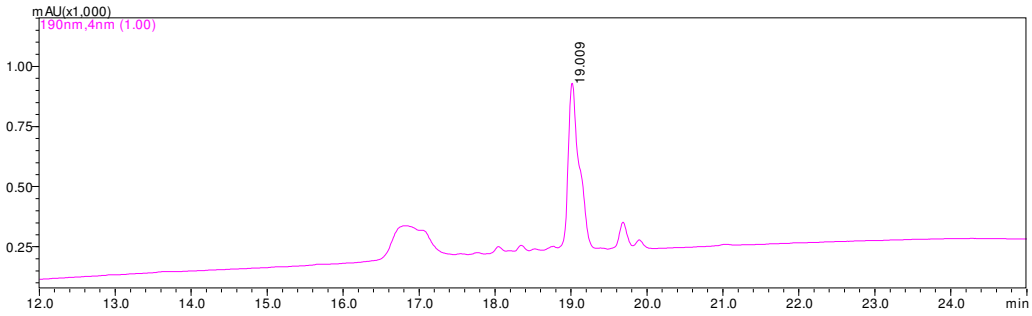


Figure 6.5: LCMS Chromatogram of N.

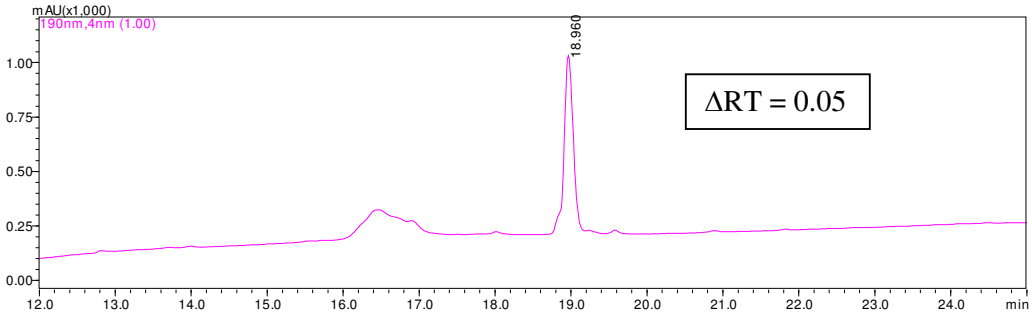
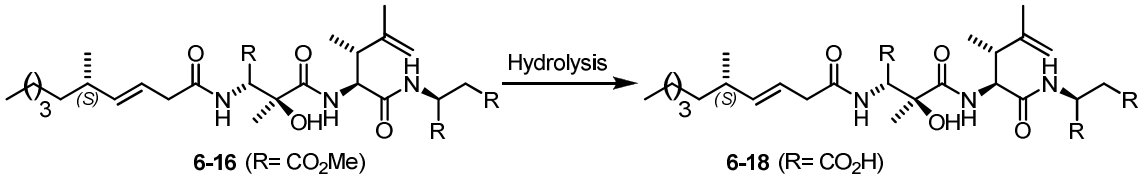


Figure 6.6: LCMS Chromatogram of R.

We measured the optical rotations {$[\alpha]^{25}_D$ = +31.6 (c = 0.18) for N, $[\alpha]^{25}_D$ = +18.8 (c = 0.25) for S and $[\alpha]^{25}_D$ = -23.1 (c = 0.22) for R} which confirmed the (*S*)

configuration of the fatty acid. Finally, we tried to hydrolyse **6-16** with Bu_3SnOH at 80 ^oC for 48 h; however ESI mass indicated the diacid as the major product. We believed that the poor solubility in DCE might be the factor for not getting the fully hydrolysed triacid. With this assumption, the crude diacid was dissolved in THF/H_2O/MeOH and treated with LiOH at 0 ^oC and allowed to stir for 12 h and **6-18** was obtained. The NMR data and optical rotation value of **6-18** ($[\alpha]^{25}_D$ = -20.0 (c = 0.1) for **6-18** and $[\alpha]^{25}_D$ = -23.7 (c = 0.3) for isolated natural lipopeptide **N1708**) were in accordance with the isolated natural product **N1708**.



Scheme 6.10: Hydrolysis to get target lipopeptide.

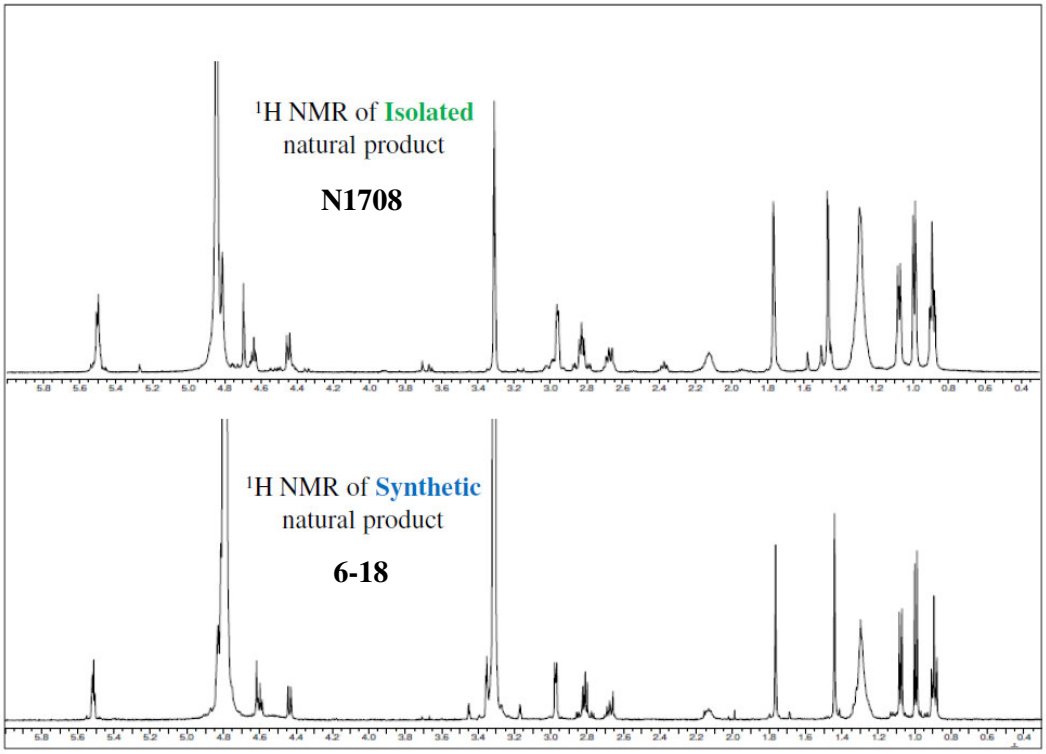


Figure 6.7: ^{1}H-NMR (500 MHz) comparison in CD_3OD.

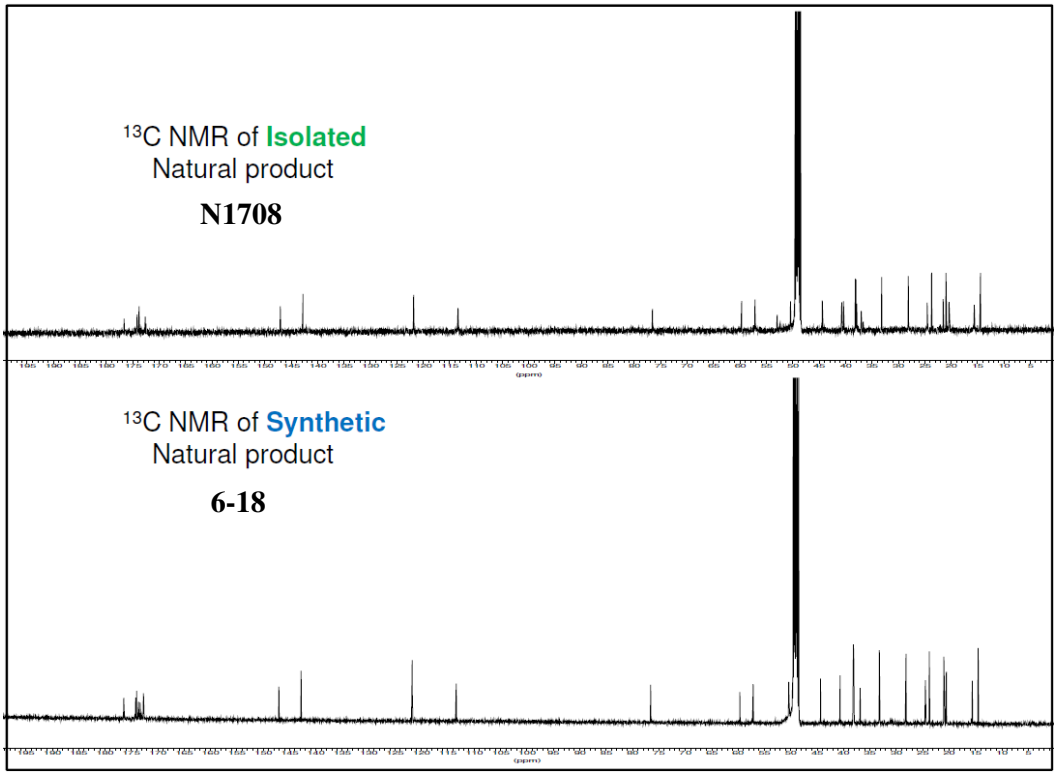


Figure 6.8: ^{13}C-NMR (125 MHz) comparison in CD_3OD.

6.2 Conclusion

In summary, we established the absolute stereochemistry of each amino acid fragment of the lipopeptide **N1708** individually and then through total synthesis of two possible diastereomers confirmed the absolute stereochemistry of the fatty acid part and hence the natural product.

References

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Experimental Section

General Techniques.

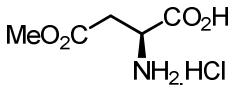
Unless stated otherwise, all non-aqueous reactions were performed in flame-dried round bottom flasks under an inert argon atmosphere with freshly distilled dry solvents under anhydrous conditions. Tetrahydrofuran (THF) was distilled over sodium/benzophenone. Dichloromethane (CH_2Cl_2) was distilled over CaH_2. Commercial reagents were purchased from Sigma Aldrich, Fluka, Merck, Alfa Aesar or Strem Chemicals, and used as received without further purification. 4Å molecular sieves were activated by heating in a furnace at 300 °C for 20 h before storing in a dry dessicator, which would be heated at 200 ºC under high vacuum for 15-20 min immediately prior to use. Yields refer to chromatographically and spectroscopically homogeneous materials, unless otherwise stated. Reaction progress was monitored by analytical thin layer chromatography (TLC) with 0.25 mm E. Merck pre-coated silica gel plates (60F-254) using UV light (254 nm) as visualising agent, and ceric ammonium molybdate, KMnO4 or ninhydrin as developing stains. Flash chromatography was performed on silica gel 60 (0.040 – 0.063 mm) purchased from SiliCycle or ACME Research Support. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX500 (500 MHz) and Bruker ACF300 (300 MHz) NMR spectrometer at ambient atmosphere. 2D NMR was performed on a Bruker AMX500 (500 MHz) NMR spectrometer. The deuterated solvents used were $CDCl_3$ and CD_3OD. Chemical shifts are reported in parts per million (ppm), and residual undeuterated solvent peaks were used as internal reference: proton (δ 7.26), carbon (δ 77.0) for $CDCl_3$ and proton (δ 3.31), carbon (δ 49.0) for CD_3OD. ^{1}H NMR coupling constants (*J*) are reported in Hertz (Hz), and multiplicities are presented as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), and br (broad). Low

resolution mass spectra were obtained on a Finnigan/MAT LCQ spectrometer in ESI mode. High resolution ESI mass spectra were obtained on a Bruker micrOTOF-Q II. Shimadzu LCMS-IT-TOF spectrometer was used for comparing retention time. Mass samples were dissolved in CH_3OH (HPLC Grade), unless otherwise stated. Samples for infra-red measurements were prepared as thin films neat or in CH_2Cl_2 solution spread on NaCl cells, and spectra were recorded on a IRPrestige-21 Shimadzu FTIR spectrometer. Optical rotations were recorded on a Jasco DIP-1000 polarimeter with a sodium lamp of wavelength 589 nm. Enantiomeric excess was determined by chiral-phase HPLC analysis on Shimadzu LC-10AT using the indicated chiral column.

Chapter 2:

General procedure for the preparation of Marfey's derivative: To a solution of the amino acid (1.5 mg) in H_2O (500 μL) were added FDAA (50 μL, 1 mg/100 μL acetone) and $NaHCO_3$ (50 μL, 1M solution) at rt. The reaction mixture was heated at 40 ^oC for 1 h and quenched with 2M HCl at rt. The solvent was evaporated under reduced pressure to obtain a residue which was dissolved in MeOH/H_2O (1:1) before LCMS analysis.

Chapter 3:

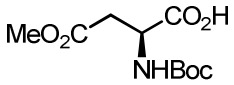


3-2

Preparation of compound 3-2: To a solution of dry HCl (2.2 g, 60.1 mmol) in dry MeOH (40 mL) was added compound **3-1** (5.0 g, 37.56 mmol) slowly at 0 ^oC and the reaction mixture was stirred at that temperature. After 30 minute, Et_2O was added slowly until slight white precipitate appeared and the reaction mixture was kept in the fridge to allow full precipitation. The reaction mixture was taken out from the fridge

after one hour and then filtered off. The residue was recrystallised from EtOH/ Et_2O to obtain pure mono ester **3-2** (5.7 g, 83%) as a white crystalline solid.

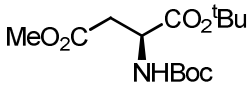
^{1}H NMR (DMSO, 300 MHz): δ 8.62 (3H, br), 4.16 (1H, t, *J* = 5.8 Hz), 3.64 (3H, s), 2.99-2.97 (2H, dd, *J* = 6.0, 1.7 Hz).



3-3

Preparation of compound 3-3: To a solution of **3-2** (4.6 g, 25.1 mmol) in dioxane/water (75 mL, 2:1) was added Na_2CO_3 (5.3 g, 50.2 mmol) slowly at 0 ^oC and the reaction mixture was stirred for 10 minute. $(Boc)_2O$ (6.34 mL, 27.6 mmol) was added to the reaction mixture at 0 ^oC and stirred for 10 h at rt. After completion of the reaction, it was acidified with a saturated $NaHSO_4$ solution to pH 2.5 and extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded pure **3-3** (4.4 g, 71%) as a viscous material.

R_f = 0.25 (TLC, 10% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.57 (1H, d, *J* = 8.2 Hz), 4.63-4.60 (1H, m), 3.70 (3H, s), 3.01-3.00 (1H, dd, *J* = 6.2, 1.8 Hz), 2.88-2.82 (1H, dd, *J* = 6.2, 1.8 Hz), 1.44 (9H, s).

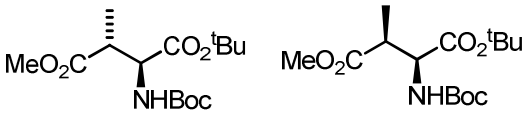


3-4

Preparation of compound 3-4: To a solution of **3-3** (1.0 g, 4.04 mmol) in CH_2Cl_2 (10 mL) were added DCC (1.0 g, 4.84 mmol), ^{t}BuOH (0.4 mL, 4.44 mmol) and DMAP (40 mg, 0.32 mmol) at 0 ^oC and the reaction mixture was stirred for 11 h at rt. After completion of the reaction, it was extracted with Et_2O and the organic layer

was washed with brine and then dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified by column chromatography over silica gel (7.0% EtOAc/hexane) to obtain pure **3-4** (800 mg, 66%) as a colourless oil.

R_f = 0.4 (TLC, 20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.42 (1H, br), 4.45-4.42 (1H, m), 3.68 (3H, s), 2.97-2.90 (1H, dd, *J* = 11.5, 5.1 Hz), 2.79-2.72 (1H, dd, *J* = 11.5, 5.1 Hz), 1.45-1.43 (18H, s).

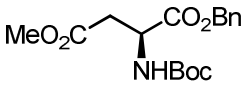


3-5a **3-5b**

Preparation of compound 3-5: To a solution of **3-4** (100 mg, 0.33 mmol) in freshly dried THF (1.0 mL) were added LiHMDS (0.37 mL, 1.0 M soln. in THF, 0.37 mmol) and BuLi (0.25 mL, 1.6 M soln. in THF, 0.40 mmol) at -78 ^oC and the reaction mixture was allowed to warm to rt slowly over the period of 30 minute. The reaction mixture was again cooled back to -78 ^oC and MeI (83 μL, 1.32 mmol) was added dropwise. The reaction mixture was stirred for 16 h before quenching with dropwise addition of a saturated NH_4Cl solution at -78 ^oC. It was then extracted with Et_2O and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed *in vacuo* to get the crude material which was carefully purified by column chromatography over silica gel (3% EtOAc/hexane) to obtain **3-5a** (16 mg, 15%) and **3-5b** (7 mg, 7%) as colourless oil.

R_f = 0.4 (TLC, 15% EtOAc/hexane) for **3-5a**; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.35 (1H, d, *J* = 8.7 Hz), 4.50-4.46 (1H, m), 3.68 (3H, s), 3.16-3.12 (1H, m), 1.46 (9H, s), 1.45 (9H, s), 1.19 (3H, d, *J* = 7.2 Hz).

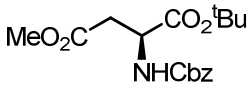
R_f = 0.35 (TLC, 15% EtOAc/hexane) for **3-5b**; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.22 (1H, d, *J* = 7.6 Hz), 4.51 (1H, m), 3.70 (4H, s), 3.13-2.92 (1H, m), 1.46 (9H, s), 1.44 (9H, s), 1.19 (3H, d, *J* = 7.2 Hz).



3-8

Preparation of compound 3-8: To a solution of **3-3** (0.5 g, 2.02 mmol) in dry DMF (6 mL) were added Cs_2CO_3 (1.32 g, 4.04 mmol) and BnBr (0.73 mL, 6.06 mmol) at 0 ^oC and the reaction mixture was stirred for 14 h at rt. After completion of the reaction, it was extracted with Et_2O and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified by column chromatography over silica gel (10% EtOAc/hexane) to obtain pure **3-8** (550 mg, 80%) as a colourless oil.

R_f = 0.4 (TLC, 20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.34 (5H, s), 5.51 (1H, d, *J* = 8.2 Hz), 5.21 (1H, d, *J* = 12.3 Hz), 5.15 (1H, d, *J* = 12.3 Hz), 4.63-4.60 (1H, m), 3.62 (3H, s), 3.06-2.98 (1H, dd, *J* = 17.3, 4.8 Hz), 2.86-2.78 (1H, dd, *J* = 17.3, 4.8 Hz), 1.43 (9H, s).

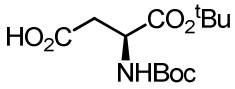


3-10

Preparation of compound 3-10: To a solution of **3-2** (1.0 g, 5.45 mmol) in Et_2O/water (24 mL, 1:3) were added K_2CO_3 (1.05 g, 7.63 mmol) and benzyl chloroformate (1.08 mL, 7.63 mmol) at 0 ^oC and the reaction mixture was stirred for 4 h at rt. After completion of the reaction, it was acidified with 1M HCl and then extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded pure Cbz protected

free acid which was converted to **3-10** (780 mg, 42% over two steps) as a colourless oil by following the same procedure described above for **3-4**.

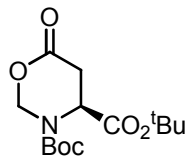
R_f = 0.35 (TLC, 15% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.36-7.32 (5H, m), 5.71 (1H, d, *J* = 7.9 Hz), 5.11 (2H, s), 4.54-4.48 (1H, m), 3.67 (3H, s), 3.01-2.94 (1H, dd, *J* = 16.8, 4.4 Hz), 2.84-2.77 (1H, dd, *J* = 16.8, 4.4 Hz), 1.44 (9H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.11, 169.49, 155.89, 136.22, 128.47, 128.11, 128.04, 82.55, 66.95, 51.82, 50.93, 36.78, 27.80.



3-12

Preparation of compound 3-12: To a solution of **3-4** (0.66 g, 2.18 mmol) in acetone/water (30 mL, 4:1) was added NaOH (130 mg, 3.26 mmol) slowly at 0 ^oC and the reaction mixture was stirred for 1 h. After completion of the reaction, it was acidified with 5% aq. $NaHSO_4$ solution up to pH 3.0. The reaction mixture was extracted with EtOAc and the organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded pure **3-12** (440 mg, 70%) as a viscous material.

R_f = 0.25 (TLC, 10% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.47 (1H, d, *J* = 7.0 Hz), 4.45 (1H, br), 3.02-2.78 (2H, m), 1.45 (18H, s).

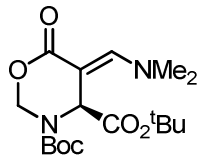


3-13

Preparation of compound 3-13: To a solution of **3-12** (0.50 g, 1.73 mmol) in dry toluene (10 mL) were added paraformaldehyde (245 mg), 4Å MS (200 mg) and

CSA (80 mg, 0.35 mmol) and then the reaction mixture was heated at 90 ^{o}C for 3 h. As the reaction completed, it was extracted with EtOAc and the organic layer was washed with sat. $NaHCO_3$ solution and brine. The collected organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified by column chromatography over silica gel (10% EtOAc/hexane) to obtain pure **3-13** (230 mg, 44%) as a white solid.

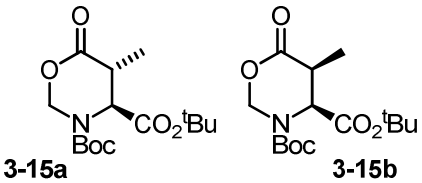
R_f = 0.45 (TLC, 20 % EtOAc/hexane); IR (KBr): ν_{max} =2983, 2932, 1748, 1717, 1480, 1409, 1245, 1156, 1005 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.87-5.72 (1H, m), 5.19 (1H, d, *J* = 8.7 Hz), 4.59-4.43 (1H, m), 3.0 (1H, m), 2.82-2.73 (1H, dd, *J*= 10.8, 16.0 Hz), 1.45 (18H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.18, 152.34, 82.71, 72.50, 52.63, 32.06, 27.95, 27.76; (ESI-MS): *m/z* $[M+Na]^+$ 324.0.



3-14

Preparation of compound 3-14: To a solution of **3-13** (0.40 g, 1.33 mmol) in dry DME (12 mL) was added *tert*-butoxybis(dimethylamino) methane (Bredereck's reagent) (0.8 mL, 3.98 mmol). A solution was heated to reflux for 15 h and the solvent was removed *in vacuo*. The residue was purified by column chromatography over silica gel (40 % EtOAc/hexane) to obtain **3-14** (360 mg, 76%) as a yellow viscous material.

R_f = 0.30 (TLC, 50% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.68 (1H, s), 5.72 (1H, s), 5.48 (1H, d, *J* = 9.6 Hz), 5.26 (1H, d, *J* = 9.6 Hz), 3.16 (6H, s), 1.48 (9H, s), 1.45 (9H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.77, 152.10, 86.01, 82.29, 72.29, 71.81, 53.04, 43.35, 28.06, 27.74; (ESI-MS): *m/z* $[M+Na]^+$ 379.0.

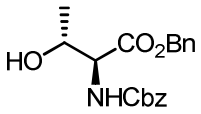


3-15a **3-15b**

Preparation of compound 3-15a and 3-15b: To a solution of **3-14** (0.35 g, 0.98 mmol) in EtOAc (15 mL) was added 10% palladium on carbon (175 mg, 50% w/w). The reaction was stirred under hydrogen atmosphere (1 atm) for 3 days at rt and filtered through Celite. The solvent was removed *in vacuo* and the residue was purified by column chromatography over silica gel (10% to 15% EtOAc/hexane) to obtain **3-15a** (130 mg, 42%, crystallized from Et_2O/Hexane, mp 80 ^oC) and **3-15b** (100 mg, 33%, mp 90 ^oC) as white solids.

R_f = 0.50 (TLC, 20% EtOAc/hexane) for **3-15a**; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.94-5.75 (1H, m), 5.17-5.14 (1H, m), 4.05-3.90 (1H, m), 2.95-2.85 (1H, m), 1.50 (9H, s), 1.46 (9H, s), 1.33 (3H, d, J = 6.0 Hz).

R_f = 0.25 (TLC, 20% EtOAc/hexane) for **3-15b**; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.91-5.73 (1H, m), 5.17 (1H, t, J = 10.2 Hz), 4.30 (1H, m), 3.07-3.03 (1H, m), 1.45 (18H, s), 1.23 (3H, d, J = 6.9 Hz); (ESI-MS): *m/z* $[M+Na]^+$ 338.0.

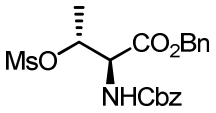


3-22

Preparation of compound 3-22: To a solution of **3-21** (1.0 g, 8.4 mmol) in benzene (75 mL) were added pTSA (1.76 g, 9.24 mmol) and benzyl alcohol (11.8 mL, 109.24 mmol) and then the reaction mixture was refluxed for 25 h. After evaporation of the solvent *in vacuo*, the residue was dissolved in EtOAc and the organic layer was washed with sat. $NaHCO_3$ solution and brine. The collected organic layer was dried

over Na_2SO_4. The solvent was removed under reduced pressure to get the benzyl ester (800 mg) which was dissolved in THF (10 mL) and treated with benzyl chloroformate (0.58 mL, 4.0 mmol) in presence of Et_3N (0.64 mL, 4.6 mmol) at 0 ^oC. The reaction mixture was then allowed to stir at rt for 1 h. THF was removed after completion of the reaction and the residue was then dissolved in EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified by column chromatography over silica gel (25% EtOAc/hexane) to obtain pure **3-22** (1.0 g, 35% over two steps) as a white solid.

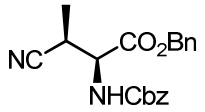
R_f = 0.30 (TLC, 50% EtOAc/hexane); ^1H NMR ($CDCl_3$, 500 MHz): δ 7.35 (10H, br), 5.54 (1H, br), 5.23 (1H, d, *J* = 6.9 Hz), 5.19 (1H, d, *J* = 6.9 Hz), 5.13 (2H, s), 4.39-4.37 (2H, m), 1.24 (3H, d, *J* = 6.3 Hz).



3-23

Preparation of compound 3-23: To a solution of **3-22** (0.30 g, 0.88 mmol) in dry CH_2Cl_2 (5 mL) were added DIPEA (180 μL, 1.05 mmol) and MsCl (80 μL, 0.96 mmol) at 0 ^oC and the reaction mixture was stirred for 1 h. As the reaction completed, it was extracted with EtOAc and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified by column chromatography over silica gel (15% EtOAc/hexane) to obtain pure **3-23** (300 mg, 85%) as a colourless oil.

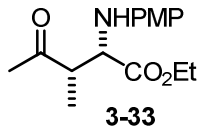
R_f = 0.4 (TLC, 70% Et_2O/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.40-7.33 (10H, m), 5.56 (1H, d, *J* = 9.5 Hz), 5.31-5.13 (5H, m), 4.62-4.58 (1H, m), 2.78 (3H, s), 1.48 (3H, d, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 168.93, 156.40, 135.85, 134.69, 128.79, 128.71, 128.66, 128.54, 128.05, 78.01, 68.12, 67.43, 58.02, 38.22, 18.35; (ESI-MS): *m/z* $[M+Na]^+$ 444.0.



3-24

Preparation of compound 3-24: To a solution of **3-23** (0.30 g, 0.74 mmol) in anhydrous DMF (3 mL) was added NaCN (44 mg, 0.89 mmol) at 0 ^{o}C and the reaction mixture was stirred at rt for 12 h. As the reaction completed, it was extracted with Et_2O and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified by column chromatography over silica gel (20% EtOAc/hexane) to obtain pure **3-24** (200 mg, 77%) as a colourless oil.

R_f = 0.5 (TLC, 50% EtOAc/hexane); IR (neat): ν_{max} = 3331, 3036, 2952, 2248, 1960, 1722, 1523, 1456, 1058, 744, 710 cm^{-}1; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.37-7.35 (10H, m), 5.57-5.55 (1H, m), 5.27 (1H, d, *J* = 12.0 Hz), 5.23 (1H, d, *J* = 12.0 Hz), 5.14 (2H, s), 4.63-4.61 (1H, m), 3.30 (1H, br), 1.33 (3H, d, *J* = 5.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 168.66, 156.11, 135.73, 134.46, 128.88, 128.77, 128.64, 128.62, 128.41, 128.14, 119.10, 68.40, 67.65, 55.68, 29.61, 14.66; (ESI-MS): *m/z* [M+Na]$^{+}$ 375.1.



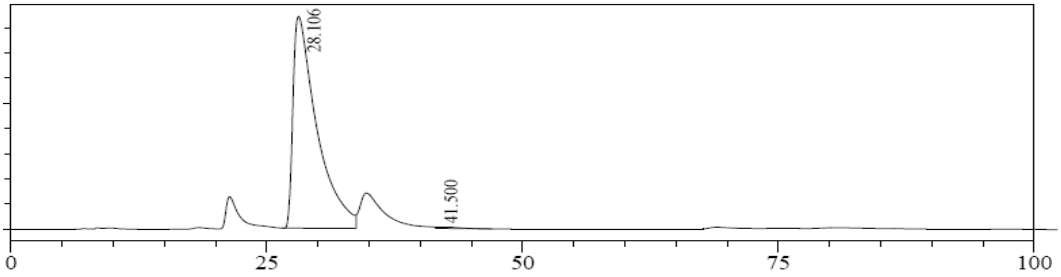
3-33

Preparation of compound 3-33: To a solution of ethyl glyoxylate (2.0 g, 19.59 mmol) in dry DCM (20 mL) were added p-anisidine (2.41 g, 19.59 mmol) and 4Å MS (4.5 g) at rt and the reaction mixture was allowed to stir for 3 h at that temperature. After completion of the reaction (checked by TLC), it was passed through a silica plug with a 15% EtOAc/hexane mixture to get the crude imine intermediate (3.8 g). To a solution of ethyl methyl ketone (36.7 mL, 412.59 mmol) and L-Proline

(739 mg, 6.42 mmol) in DMSO (42 mL) was added the crude imine slowly at 0 ^{o}C and the reaction mixture was stirred for 4 h at rt. Upon completion of the reaction (checked by TLC) half saturated NH_4Cl solution was added at 0 ^{o}C and the reaction mixture was extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was carefully purified over silica gel (15% EtOAc/hexane) to obtain **3-33** (3.3 g, 65%, 99% ee,) as a brown oil. The enantiomeric excess was determined through HPLC analysis with Chiralpak column (0.46 cm ϕx25 cm) using hexanes/2-propanol (90:10) at a flow rate of 1.0 mL/min; detection UV 254 nm.

R_f = 0.4 (TLC, 15% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.77 (2H, d, J = 8.9 Hz), 6.64 (2H, d, J = 8.9 Hz), 4.31 (1H, br), 4.19-4.12 (2H, m), 3.86 (1H, br), 3.74 (3H, s), 3.04-2.97 (1H, m), 2.22 (3H, s), 1.26-1.18 (6H, m).

L-Proline catalysed reaction:



Peak#	Ret. Time	Area	Height	Area %	Height %
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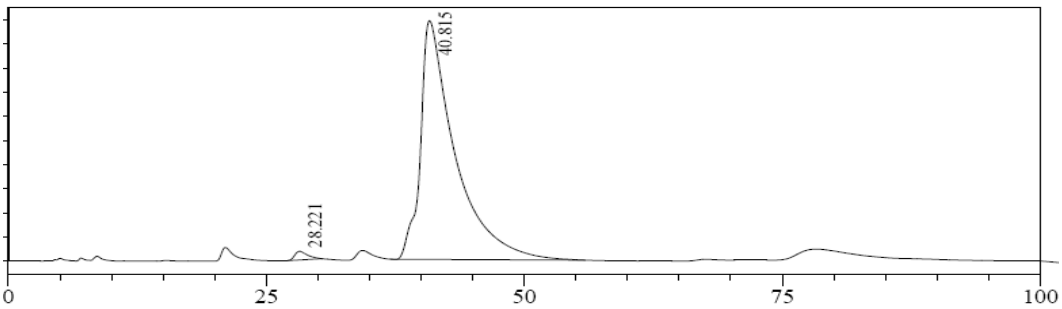
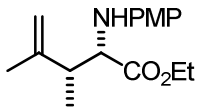
2	41.500	1712040	10078	0.624	0.596
Total		274244996	1690611	100.000	100.000

D-Proline catalysed reaction:



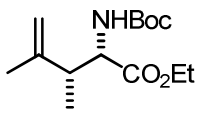
Peak#	Ret. Time	Area	Height	Area %	Height %
1	28.221	1557069	17930	1.330	3.497
2	40.815	115524596	494775	98.670	96.503
Total		117081664	512705	100.000	100.000



3-34

Preparation of compound 3-34: To a solution of compound **3-33** (565 mg, 2.02 mmol) in dry THF (10 mL) was added Tebbe reagent (4.45 mL, 2.2 mmol) slowly at -78 ^{o}C and the reaction mixture was stirred at that temperature for 2 h and then slowly warmed up to rt and allowed to stir for 12 h. THF (5 mL) was added to the reaction mixture and then 2.5 mL of 5% NaOH solution was added at -15 ^{o}C. After stirring for 10 minute, it was warmed up to rt and filtered through short silica plug and washed with Et_2O. The collected filtrate was extracted with Et_2O and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was carefully purified over silica gel (7.5% EtOAc/hexane) to afford **3-34** (252 mg, 45%) as a yellow oil.

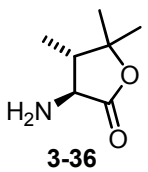
R_f = 0.5 (TLC, 15% EtOAc/hexane); $[\alpha]_D^{25}$ = -90.2 (*c* = 2.0, $CHCl_3$); IR (thin film): ν_{max} = 3373, 2979, 2832, 1729, 1516, 1369, 1241, 1182, 1036, 895, 821 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.76 (2H, d, *J* = 8.9 Hz), 6.60 (2H, d, *J* = 8.9 Hz), 4.82 (2H, s), 4.15-4.07 (2H, m), 3.91 (1H, d, *J* = 7.6 Hz), 3.79 (1H, br), 3.73 (3H, s), 2.66-2.57 (1H, m), 1.78 (3H, s), 1.22-1.18 (6H, m); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.71, 152.78, 145.94, 141.41, 115.29, 114.83, 112.84, 61.69, 60.67, 55.70, 44.70, 19.52, 15.43, 14.18; HRMS (ESI): *m/z* calcd for $C_{16}H_{23}O_3NNa^+$ $[M+Na]^+$ 300.1570, found 300.1579.



3-35

Preparation of compound 3-35: To a solution of ceric ammonium nitrate (CAN) (643.3 mg, 0.47 mmol) in H_2O (10 mL) was added compound **3-34** (130 mg, 1.2 mmol) in acetonitrile (5 mL) dropwise at 0 ^oC and the reaction mixture was stirred at that temperature for 45 minute. The reaction mixture was diluted with Et_2O and 1M HCl (5 mL) was added and then it was extracted with Et_2O. The aqueous layer was basified with sat. $NaHCO_3$ solution and extracted with EtOAc. EtOAc layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was dissolved in Dioxane/H_2O (3.0 mL/1.0 mL). To this solution were added Et_3N (68 μL, 0.5 mmol) and $(Boc)_2O$ (142 μL, 0.62 mmol) at 0 ^oC and the reaction mixture was stirred at rt for 12 h. The reaction mixture was extracted with EtOAc and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified over silica gel (7.5% EtOAc/hexane) to afford **3-35** (80 mg, 63%) as a colourless oil.

R_f = 0.6 (TLC, 15% EtOAc/hexane); $[\alpha]_D^{25}$ = +16.2 (c = 1.25, $CHCl_3$); IR (thin film): ν_{max} = 3360, 2979, 2935, 1720, 1504, 1367, 1344, 1249, 1165, 1027 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 4.98-4.89 (1H, br), 4.79 (2H, d, J = 18.0 Hz), 4.40-4.37 (1H, m), 4.20-4.14 (2H, m), 2.58-2.55 (1H, m), 1.78 (3H, s), 1.43 (9H, s), 1.26 (3H, t, J = 7.6 Hz), 1.05 (3H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.29, 155.49, 145.56, 112.46, 79.75, 61.05, 56.18, 43.87, 28.29, 20.13, 14.41, 14.16; HRMS (ESI): *m/z* calcd for $C_{14}H_{25}O_4NNa^+$ $[M+Na]^+$ 294.1676, found 294.1672.

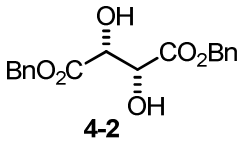


3-36

Preparation of compound 3-36: A suspension of **3-35** (5 mg**,** 0.02 mmol) in 1 mL 6M HCl was refluxed for 24 h. The reaction mixture was basified with sat. $NaHCO_3$ solution and then extracted with $CHCl_3$ to obtain pure **3-36** (1.5 mg, 61%) as a colourless material.

R_f = 0.3 (TLC, 20% MeOH/ CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.31 (1H, d, *J* = 12.4 Hz), 1.99-1.92 (1H, m), 1.44 (3H, s), 1.26 (3H, s), 1.16 (3H, d, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 177.75, 84.36, 57.31, 48.97, 27.32, 22.01, 12.32; (ESI-MS): *m/z* $[M+H]^+$ 143.9.

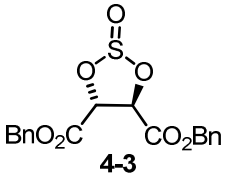
Chapter 4:



4-2

Preparation of compound 4-2: To a solution of compound **4-1** (6.0 g, 40.0 mmol) in toluene (120 mL) were added benzyl alcohol (12.41 mL, 120.0 mmol) and TsOH (96 mg, 0.5 mmol) and then the reaction mixture was refluxed for 13 h. The solvent was removed under reduced pressure to get the crude material which was dissolved in Et_2O and washed with sat. $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 and removed *in vacuo* to obtain a viscous material. 5% Et_2O/hexane was added to dissolve the residue and then cooled down to -20 ^{o}C. A white solid was formed after 20 minute which was filtered to afford **4-2** (9.6 g, 73%) as a white solid (mp 52 ^{o}C) .

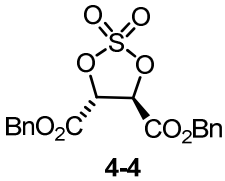
R_f = 0.25 (TLC, 25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.36 (10H, s), 5.30 (2H, d, *J* = 12.2 Hz), 5.25 (2H, d, *J* = 12.2 Hz), 4.62 (2H, s), 2.90 (2H, br).



4-3

Preparation of compound 4-3: To a solution of compound **4-2** (1.0 g, 3.03 mmol) in dry CH_2Cl_2 (3 mL) were added Et_3N (0.64 mL, 4.55 mmol) and $SOCl_2$ (264 μL, 3.64 mmol) at 0 ^oC and the reaction mixture was stirred for 1 h. After completion of the reaction, the solvent was evaporated and the residue was extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified by column chromatography over silica gel (10% EtOAc/hexane) to obtain **4-3** (650 mg, 57%) as a colourless oil.

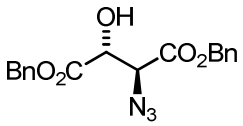
R_f = 0.45 (TLC, 25% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.39 (10H, s), 5.77 (1H, d, J = 4.3 Hz), 5.30-5.26 (5H, m); (ESI-MS): *m/z* $[M+Na]^+$ 399.0.



4-4

Preparation of compound 4-4: To a solution of compound **4-3** (650 mg, 1.73 mmol) in ACN (3 mL) were added $RuCl_3.XH_2O$ (6.0 mg), $NaIO_4$ (416 mg, 1.95 mmol) and H_2O (4.5 mL) at 0 ^oC and the reaction mixture was stirred for 1 h. After completion of the reaction, the solvent was evaporated and the residue was extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain the crude material which was purified by column chromatography over silica gel (15% EtOAc/hexane) to obtain **4-4** (352 mg, 52%) as a colourless oil.

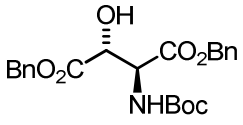
R_f = 0.4 (TLC, 25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.42-7.41 (10H, br), 5.51 (2H, s), 5.35 (4H, s); (ESI-MS): *m/z* $[M-H]^-$ 391.0.



4-5

Preparation of compound 4-5: To a solution of compound **4-4** (220 mg, 0.56 mmol) in acetone/H_2O (4.0 mL/1.0 mL) was added NaN_3 (73 mg, 1.12 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 1 h at rt. After consumption of the starting material (checked by TLC) acetone was evaporated *in vacuo* and the residue was dissolved in Et_2O. 20% H_2SO_4 (2.3 mL) was added carefully at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. Et_2O was used for extraction and the organic layer was washed with brine solution, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (20% EtOAc/hexane) to obtain **4-5** (156 mg, 79%) as a colourless oil.

R_f = 0.5 (TLC, 25% EtOAc/hexane); IR (neat): ν_{max} = 3481, 2115, 1749, 1735, 1267, 1213, 1111, 696 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.35-7.29 (10H, m), 5.15-5.02 (4H, m), 4.70-4.67 (1H, m), 4.36 (1H, d, *J* = 2.6 Hz), 3.26 (1H, d, *J* = 4.9 Hz); (ESI-MS): *m/z* $[M+Na]^+$ 378.1.

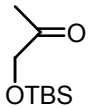


4-8

Preparation of compound 4-8: To a solution of compound **4-5** (300 mg, 0.85 mmol) in THF (10 mL) were added PPh_3 (444 mg, 1.69 mmol) and H_2O (100 μL) and then the reaction mixture was refluxed for 6 h. The solvent was removed under reduced pressure to get the crude free amine which was dissolved in Dioxane/H_2O (9

mL/3 mL). To this mixture were added Et_3N (173 µL, 1.21 mmol) and $(Boc)_2O$ (174 µL, 0.76 mmol) at 0 ^oC and the reaction mixture was stirred the reaction mixture for 12 h at rt. The reaction mixture was extracted with EtOAc and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified over silica gel (20% EtOAc/hexane) to afford **4-8** (242 mg, 67% over two steps) as a colourless oil.

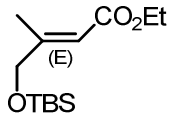
R_f = 0.35 (TLC, 25% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.36-7.23 (10H, m), 5.47 (1H, d, *J* = 7.6 Hz), 5.11-4.85 (5H, m), 4.55 (1H, s), 1.43 (9H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.44, 168.62, 155.73, 134.80, 134.72, 128.86, 128.79, 128.74, 128.69, 80.71, 72.29, 68.11, 68.02, 57.16, 28.35; (ESI-MS): *m/z* $[M+Na]^+$ 452.1.



4-11

Preparation of compound 4-11: To a solution of compound **4-10** (1.4 g, 18.92 mmol) in DMF (20 mL) were added imidazole (1.8 g, 26.5 mmol), DMAP (116 mg, 0.95 mmol) and TBSCl (4.3 mL, 24.6 mmol) at 0 ^oC and the reaction mixture was stirred at rt for 12 h. After completion of the reaction, H_2O (100 mL) was added and the reaction mixture was extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified over silica gel (2% EtOAc/hexane) to afford **4-11** (2.15 g, 60%) as a colourless oil.

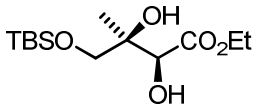
R_f = 0.5 (TLC, 5% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 4.12 (2H, s), 2.14 (3H, s), 0.90 (9H, s), 0.07 (6H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 209.03, 69.49, 25.69, 18.20, -5.60.



4-12

Preparation of compound 4-12: To a suspension of NaH (244.6 mg, 10.2 mmol) (washed with hexane prior to the reaction) in dry THF (20 mL) was added triethyl phosphonoacetate (2.0 g, 8.95 mmol) in dry THF (10 mL) slowly at 0 ^{o}C and after complete addition, the mixture was allowed to stir for 1 h at rt. To this reaction flask compound **4-11** (1.37 g, 7.28 mmol) in 5 mL dry THF was added slowly at 0 ^{o}C and the reaction mixture was stirred for 20 h at rt. The reaction was quenched by careful dropwise addition of H_2O at 0 ^{o}C until a clear solution appeared and then it was extracted with Et_2O twice. Combined extract was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude mixture. Careful purification (0.5% to 1.5% EtOAc/hexane) over silica gel column chromatography afforded **4-12** (1.6 g, 85%, *E/Z* = 2.3:1) as a colourless oil.

R_f = 0.7 (TLC, 5% Et_2O/hexane); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.97 (1H, d, *J* = 1.9 Hz), 4.17-4.11 (2H, m), 4.09 (2H, d, *J* = 1.3 Hz), 2.03 (3H, s), 1.27 (3H, t, *J* = 7.0 Hz), 0.90 (9H, s), 0.07 (6H, s).

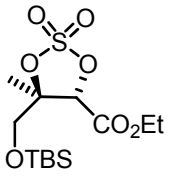


4-13

Preparation of compound 4-13: To a solution of compound **4-12** (160 mg, 0.62 mmol) in ^{t}BuOH/H_2O (1:1, 6 mL) were added AD-mix β (870 mg, 1.4 g/mmol) and methanesulfonamide (62 mg, 100 mg/mmol) at -1 ^{o}C and the reaction mixture was allowed to stir for 96 h at that temperature. The reaction was quenched by addition of Na_2SO_3 (918 mg, 1.48 mg/mmol) and stirred for 1 h at rt until it became colourless. CH_2Cl_2 was used for extraction and the organic layer was washed with brine, dried

over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (15% EtOAc/hexane) to obtain pure **4-13 (**154 mg, 85%) as a colourless oil.

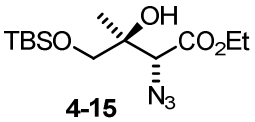
R_f = 0.25 (TLC, 20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.27-4.16 (2H, m), 4.05 (1H, s), 3.67 (1H, d, *J* = 9.7 Hz), 3.47 (1H, d, *J* = 9.7 Hz), 3.34 (1H, br), 3.11 (1H, br), 1.30 (3H, t, *J* = 7.2 Hz), 1.19 (3H, s), 0.88 (9H, s), 0.05 (6H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.11, 73.96, 73.86, 67.38, 61.69, 25.81, 21.17, 18.32, 14.09, -5.6; (ESI-MS): *m/z* $[M+H]^+$ 293.0, $[M-H]^-$ 291.1.



4-14

Preparation of compound 4-14: To a solution of compound **4-13** (400 mg, 1.37 mmol) in CH_2Cl_2 (10 mL) were added Et_3N (384 μL, 2.74 mmol) and $SOCl_2$ (200 μL, 2.74 mmol) carefully at 0 ^oC and the reaction mixture was allowed to stir for 30 minute at that temperature. After consumption of the starting material (checked by TLC), CH_2Cl_2 was evaporated *in vacuo* and the cyclic sulphite was eluted through silica plug with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated at reduced pressure to obtain the crude product which was dissolved in CH_3CN (10 mL). To this solution were added $RuCl_3$ (2 mg), $NaIO_4$ (440 mg, 2.06 mmol) and H_2O (15 mL) at 0 ^oC and the reaction mixture was allowed to stir for 30 minute at that temperature. The reaction mixture was diluted with Et_2O and extracted. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (10% EtOAc/hexane) to afford **4-14** (146 mg, 30% over two steps) as a colourless oil.

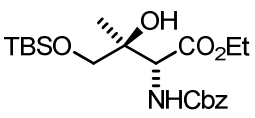
R_f = 0.35 (TLC, 15% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.43 (1H, s), 4.36 (2H, q, *J* = 7.2 Hz), 3.91 (1H, d, *J* = 12.0 Hz), 3.80 (1H, d, *J* = 12.0 Hz), 1.56 (3H, s), 1.35 (3H, t, *J* = 7.1 Hz), 0.92 (9H, s), 0.12 (3H, s), 0.11 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.08, 92.99, 78.87, 65.02, 62.89, 25.66, 18.21, 17.72, 14.05, -5.44, -5.67; (ESI-MS): *m/z* $[M+Na]^+$ 377.0, $[M-H]^-$ 353.4.



4-15

Preparation of compound 4-15: To a solution of compound **4-14** (250 mg, 0.70 mmol) in acetone/H_2O (8 mL/ 2 mL) was added NaN_3 (230 mg, 3.5 mmol) and the reaction mixture was heated at 50 ^{o}C for 8 h. After consumption of the starting material (checked by TLC), acetone was evaporated *in vacuo* and the residue was dissolved in Et_2O. 20% H_2SO_4 (3 mL) was added carefully at 0 ^{o}C and the reaction mixture was allowed to stir for 12 h at rt. Et_2O was used for extraction and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (5% EtOAc/hexane) to afford **4-15** (168 mg, 70%) as a colourless oil.

R_f = 0.5 (TLC, 10% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.37-4.25 (2H, m), 3.98 (1H, s), 3.64 (1H, d, *J* = 10.2 Hz), 3.42 (1H, d, *J* = 10.2 Hz), 3.10 (1H, s), 1.33 (3H, t, *J* = 7.2 Hz), 1.21 (3H, s), 0.91 (9H, s), 0.09 (6H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.28, 74.59, 67.85, 65.11, 61.85, 25.82, 19.80, 18.29, 14.15, -5.49, -5.54; (ESI-MS): *m/z* $[M+Na]^+$ 340.1.

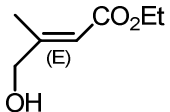


4-16

Preparation of compound 4-16: To a solution of compound **4-15** (55 mg, 0.17 mmol) in MeOH (5 mL) was added 10% Pd/C (10 mg) and the reaction mixture was

stirred over night under hydrogen atmosphere. The amine was eluted with MeOH through a celite pad. To a solution of the crude amine in THF (2 mL) were added Et_3N (26 μL, 0.19 mmol) and CbzCl (23 μL, 0.16 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 1 h at rt. After completion of the reaction, it was extracted with EtOAc and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (15% EtOAc/hexane) to afford **4-16** (51 mg, 70% over two steps) as a colourless oil.

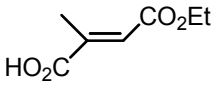
R_f = 0.3 (TLC, 20% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.35-7.30 (5H, m), 5.80 (1H, br), 5.12-5.07 (2H, m), 4.38-4.18 (3H, m), 3.66 (1H, d, *J* = 10.0 Hz), 3.43 (1H, d, *J* = 10.0 Hz), 1.17 (3H, s), 0.91 (9H, s), 0.06 (6H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.09, 156.00, 136.24, 128.46, 128.09, 127.95, 72.94, 68.24, 67.03, 61.37, 59.74, 25.80, 21.44, 18.21, 14.04, -5.60, -5.65; (ESI-MS): *m/z* $[M+Na]^+$ 448.2.



4-19

Preparation of compound 4-19: To a solution of compound **4-12** (50 mg, 0.19 mmol) in dry THF (2 mL) was added TBAF (200 μL, 0.22 mmol) at 0 ^oC and the reaction mixture was stirred for 30 minute. The reaction mixture was concentrated under reduced pressure to get the crude product which was purified over silica gel (8% EtOAc/hexane) to afford **4-19** (20 mg, 71%) as a colourless oil.

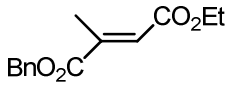
R_f = 0.3 (TLC, 15% EtOAc/hexane); ^1H NMR ($CDCl_3$, 500 MHz): δ 5.97-5.96 (1H, m), 4.18-4.12 (4H, m), 2.07 (3H, s), 1.27 (3H, t, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.89, 157.26, 113.70, 67.01, 59.74, 15.55, 14.24.



4-20

Preparation of compound 4-20: To a solution of compound **4-19** (20 mg, 0.14 mmol) in acetone/H_2O (1 mL/ 0.1 mL) was added CrO_3 (32 mg, 0.32 mmol) at 0 ^oC and the reaction mixture was stirred for 90 minute. The reaction mixture was concentrated under reduced pressure to get the crude product which was purified over silica gel (30% EtOAc/hexane) to afford **4-20** (20 mg, quantitative) as a colourless oil.

R_f = 0.35 (TLC, 50% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 9.13 (1H, br), 6.89 (1H, d, *J* = 1.3 Hz), 4.24 (2H, q, *J* = 7.2 Hz), 2.28 (3H, s), 1.32 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.36, 165.66, 142.36, 128.78, 60.85, 14.13, 13.88.

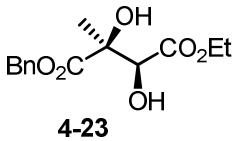


4-21

Preparation of compound 4-21: To a solution of **4-20** (20 mg, 0.13 mmol) in CH_2Cl_2 (1 mL) were added DCC (32 mg, 0.15 mmol), BnOH (15 μL, 0.14 mmol) and DMAP (1.5 mg, 0.01 mmol) at 0 ^oC and the reaction mixture was stirred the reaction mixture for 12 h at rt. After completion of the reaction, it was extracted with Et_2O and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified by column chromatography over silica gel (4.0% EtOAc/hexane) to obtain pure **4-21** (25 mg, 80%) as a colourless oil.

R_f = 0.6 (TLC, 15% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.39-7.36 (5H, m), 6.81 (1H, d, *J* = 1.5 Hz), 5.23 (2H, s), 4.26-4.17 (2H, m), 2.31 (3H, d, *J* = 0.5 Hz),

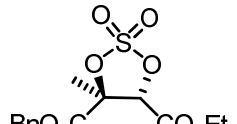
1.30 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.95, 165.83, 143.34, 135.46, 128.62, 128.41, 128.23, 127.13, 67.27, 60.64, 14.30, 14.14.



4-23

Preparation of compound 4-23: To a solution of compound **4-21** (50 mg, 0.20 mmol) in ^{t}BuOH/H_2O (1:1, 3 mL) were added AD-mix β (280 mg, 1.4 g/mmol) and methanesulfonamide (20 mg, 100 mg/mmol) at -1 ^{o}C and the reaction mixture was allowed to stir for 48 h at that temperature. The reaction was quenched by addition of Na_2SO_3 (300 mg, 1.48 mg/mmol) and stirred for 1 h at rt until it became colourless. CH_2Cl_2 was used for extraction and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (25% EtOAc/hexane) to obtain pure **4-23 (**47 mg, 83%) as a colourless oil.

R_f = 0.25 (TLC, 25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.39-7.33 (5H, m), 5.25 (2H, d, *J* = 1.8 Hz), 4.36-4.25 (3H, m), 3.66 (1H, br), 3.40 (1H, br), 1.53 (3H, s), 1.31 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.14, 171.17, 135.02, 128.61, 128.50, 128.12, 76.93, 75.02, 68.01, 62.20, 21.87, 14.11; (ESI-MS): *m/z* $[M+Na]^+$ 305.1.

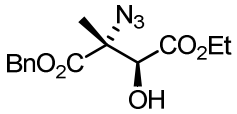


4-25

Preparation of compound 4-25: To a solution of compound **4-23** (50 mg, 0.18 mmol) in CH_2Cl_2 (1 mL) were added Et_3N (50 µL, 0.36 mmol) and $SOCl_2$ (26 µL, 0.36 mmol) carefully at 0 ^{o}C and the reaction mixture was allowed to stir for 30 minute at

that temperature. After consumption of the starting material (checked by TLC), CH_2Cl_2 was evaporated *in vacuo* and the cyclic sulphite was eluted through silica plug with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to obtain the crude product which was dissolved in CH_3CN (1 mL). To this solution were added $RuCl_3$ (1 mg), $NaIO_4$ (54 mg, 0.25 mmol) and H_2O (1.5 mL) at 0 ^oC and the reaction mixture was allowed to stir for 30 minute at that temperature. The reaction mixture was diluted with Et_2O and extracted. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (10% EtOAc/hexane) to afford **4-25** (24 mg, 37% over two steps) as a colourless oil.

R_f = 0.4 (TLC, 20% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.41-7.37 (5H, m), 5.68 (1H, s), 5.32 (2H, s), 4.33 (2H, q, J = 7.2 Hz), 1.73 (3H, s), 1.31 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.31, 163.20, 133.94, 128.98, 128.76, 128.43, 86.92, 80.55, 69.32, 63.29, 19.15, 13.95; (ESI-MS): *m/z* $[M+Na]^+$ 367.0.

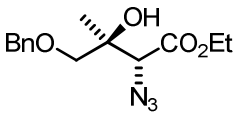


4-26

Preparation of compound 4-26: To a solution of compound **4-25** (50 mg, 0.15 mmol) in acetone/H_2O (4 mL/1 mL) was added NaN_3 (48 mg, 0.75 mmol) and the reaction mixture was heated at 50 ^oC for 4 h. After consumption of the starting material (checked by TLC), acetone was evaporated *in vacuo* and the residue was dissolved in Et_2O. 20% H_2SO_4 (1 mL) was added carefully at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. Et_2O was used for extraction and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced

pressure to get the crude product which was purified over silica gel (10% EtOAc/hexane) to afford **4-26** (31 mg, 70%) as a colourless oil.

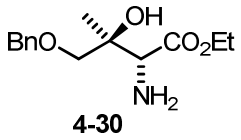
R_f = 0.3 (TLC, 20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38 (5H, s), 5.23 (2H, s), 4.51 (1H, s), 4.22-4.07 (2H, m), 3.18 (1H, br), 1.69 (3H, s), 1.22 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.09, 170.37, 134.88, 128.64, 128.61, 128.40, 73.82, 68.86, 67.86, 62.68, 19.64, 13.87; (ESI-MS): *m/z* $[M+NH_4]^+$ 325.0, $[M+Na]^+$ 330.0



4-28

Preparation of compound 4-28: To a solution of cyclic sulphate of benzyl protected analogue of **4-14** (1.25 g, 3.79 mmol) in acetone/H_2O (100 mL/25 mL) was added NaN_3 (1.43 g, 22 mmol) and the reaction mixture was heated at 50 ^{o}C for 7 h. After consumption of the starting material (checked by TLC), acetone was evaporated *in vacuo* and the residue was dissolved in Et_2O. 20% H_2SO_4 (16 mL) was added carefully at 0 ^{o}C and the reaction mixture was allowed to stir for 12 h at rt. Et_2O was used for extraction and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (7% EtOAc/hexane) to afford **4-28** (800 mg, 73%) as a colourless oil.

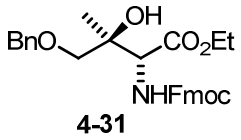
R_f = 0.4 (TLC, 20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.39-7.28 (5H, m), 4.58 (1H, d, *J* = 11.9 Hz), 4.53 (1H, d, *J* = 11.9 Hz), 4.27-4.16 (2H, m), 4.04 (1H, s), 3.52 (1H, d, *J* = 9.6 Hz), 3.36 (1H, d, *J* = 9.6 Hz), 3.27 (1H, s), 1.31-1.24 (6H, m); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.30, 137.48, 128.41, 127.86, 127.75, 74.55, 74.31, 73.55, 65.43, 61.88, 20.59, 14.05; (ESI-MS): *m/z* $[M+Na]^+$ 316.2.



4-30

Preparation of compound 4-30: To a solution of compound **4-28** (60 mg, 0.20 mmol) in THF (2 mL) was added 10% Pd/C (12 mg) and the reaction mixture was stirred under hydrogen atmosphere for 4 h. The amine was eluted with MeOH through a celite pad. The solvent was concentrated under reduced pressure to get the crude product which was purified over silica gel (15% MeOH/CH_2Cl_2) to afford **4-30** (54 mg, quantitative) as a brown oil.

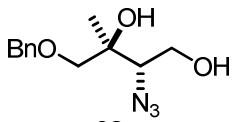
R_f = 0.2 (TLC, 20% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29-7.19 (5H, m), 4.44 (1H, d, *J* = 12.0 Hz), 4.39 (1H, d, *J* = 12.0 Hz), 4.09-3.98 (2H, m), 3.40-3.37 (2H, m), 3.25 (1H, d, *J* = 9.4 Hz), 1.21 (3H, s), 1.15 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.05, 137.96, 128.39, 128.31, 127.79, 127.67, 127.64, 75.07, 73.51, 72.71, 61.01, 60.11, 21.63, 14.07; (ESI-MS): *m/z* $[M+H]^+$ 268.1, $[M+Na]^+$ 290.1.



4-31

Preparation of compound 4-31: To a solution of compound **4-30** (20 mg, 0.08 mmol) in Dioxane/H_2O (0.5 mL/0.2 mL) were added $NaHCO_3$ (10 mg, 0.11 mmol) and FmocCl (20 mg, 0.08 mmol) at 0 ^oC and the reaction mixture was stirred at rt for 12 h. The reaction mixture was extracted with EtOAc and the organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified over silica gel (25% EtOAc/hexane) to afford **4-31** (25 mg, 70%) as a colourless oil.

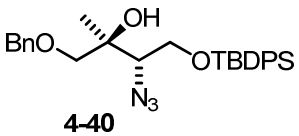
R_f = 0.2 (TLC, 50% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.76 (2H, d, *J* = 7.6 Hz), 7.58 (2H, d, *J* = 7.6 Hz), 7.43-7.28 (10H, m), 5.80 (1H, br), 4.54 (2H, d, *J* = 4.3 Hz), 4.41-4.39 (3H, m), 4.25-4.05 (3H, m), 3.55 (1H, d, *J* = 9.4 Hz), 3.40 (1H, d, *J* = 9.4 Hz), 3.13 (1H, br), 1.30-1.20 (6H, m); (ESI-MS): *m/z* $[M+Na]^+$ 512.3.



4-38

Preparation of compound 4-38: To a solution of compound **4-28** (250 mg, 0.68 mmol) in dry THF (5 mL) was added $LiBH_4$ (30 mg, 1.36 mmol) portionwise at 0 ^oC and the reaction mixture was allowed to stir for 7 h. After complete consumption of the starting material MeOH was added slowly at 0 ^oC until a clear solution was formed and then the solvent was evaporated to get the crude material. 1M HCl was added to the crude viscous material and then extracted with EtOAc. The organic layer was dried over Na_2SO_4, concentrated under reduced pressure to get the crude product which was purified over silica gel (20% EtOAc/hexane) to afford pure **4-38** (180 mg, 84%) as a colourless oil.

R_f = 0.2 (TLC, 25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.39-7.32 (5H, m), 4.58 (2H, s), 4.01-3.96 (1H, m), 3.79-3.68 (2H, m), 3.55 (1H, d, *J* = 9.4 Hz), 3.33 (1H, d, *J* = 9.4 Hz), 2.81 (1H, br), 2.45 (1H, br), 1.14 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 137.32, 128.48, 127.97, 127.85, 74.90, 74.17, 73.51, 66.88, 61.96, 19.71; (ESI-MS): *m/z* $[M+Na]^+$ 274.1.

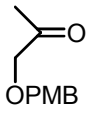


4-40

Preparation of compound 4-40: To a solution of compound **4-38** (18 mg, 0.07 mmol) in DMF (1 mL) were added imidazole (7 mg, 0.1 mmol), DMAP (1 mg) and

TBDPSCl (25 μL, 0.09 mmol) at 0 ^{o}C and the reaction mixture was stirred at rt for 12 h. After completion of the reaction, H_2O (10 mL) was added and the reaction mixture was extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified over silica gel (7% EtOAc/hexane) to afford **4-40** (28 mg, 80%) as a colourless oil.

R_f = 0.4 (TLC, 15% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.64-7.59 (5H, m), 7.36-7.17 (10H, m), 4.42 (2H, s), 3.99-3.95 (1H, dd, *J* = 10.5, 3.2 Hz), 3.78-3.72 (1H, dd, *J* = 10.6, 8.3 Hz), 3.63-3.59 (1H, dd, *J* = 8.4, 3.0 Hz), 3.41 (1H, d, *J* = 9.2 Hz), 3.12 (1H, d, *J* = 9.2 Hz), 2.60 (1H, s), 1.00 (9H, s), 0.93 (3H, s); (ESI-MS): *m/z* $[M+Na]^+$ 512.2.

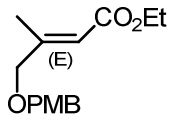


4-42

Preparation of compound 4-42: To a solution of hydroxyacetone (100 mg, 1.35 mmol) and 2-PMBO-lepidine or Dudley's reagent (764 mg, 2.70 mmol) in trifluorotoluene (10 mL) was added dried MgO (109 mg, 2.7 mmol) and then the reaction mixture was cooled to 0 ^{o}C. To this cold solution was added methyl triflate (308 μL, 2.7 mmol) slowly and the reaction mixture was warmed to rt and allowed to stir for 12 h at that temperature. After completion of the reaction, it was diluted with EtOAc and decanted away from MgO. The collected organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was purified over silica gel (4% EtOAc/hexane) to afford **4-42** (196 mg, 75%) as a colourless oil.

R_f = 0.3 (TLC, 10% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32-7.29 (2H, dd, *J* = 6.8, 2.0 Hz), 6.93-6.90 (2H, dd, *J* = 6.8, 2.0 Hz), 4.54 (2H, s), 4.04 (2H, s), 3.83

(3H, s), 2.17 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 206.68, 159.40, 129.49, 129.13, 113.81, 74.88, 72.85, 55.16, 26.30.



4-43

Preparation of compound 4-43: To the suspension of NaH (35 mg, 1.44 mmol) (washed with Hexane prior to the reaction) in dry THF was added triethyl phosphonoacetate slowly (0.25 mL, 1.24 mmol) at 0 ^{o}C and after complete addition, the reaction mixture was allowed to stirred for 1 h at rt. To this reaction flask compound **4-42** (196 mg, 1.0 mmol) was added slowly at 0 ^{o}C and stirred for 20 h at rt. The reaction was quenched by very careful dropwise addition of water at 0 ^{o}C until a clear solution appeared and then it was extracted with Et_2O twice. The combined organic extract was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude mixture. Careful purification over silica gel column chromatography (0.5% to 1.5% EtOAc/hexane) produced **4-43** (203 mg, 75%, *E/Z* = 3:1) as a colourless oil.

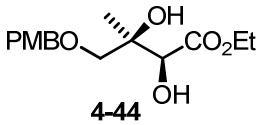
(*E*)-isomer:

R_f = 0.6 (TLC, 5% Et_2O/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.27 (2H, d, *J* = 6.9 Hz), 6.89 (2H, d, *J* = 6.9 Hz), 5.99 (1H, s), 4.46 (2H, s), 4.17 (2H, q, *J* = 7.6 Hz), 3.96 (2H, s), 3.81 (3H, s), 2.10 (3H, s), 1.28 (3H, t, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 166.70, 159.32, 154.61, 129.87, 129.30, 115.27, 113.86, 73.83, 72.19, 59.67, 55.27, 15.85, 14.3.

(*Z*)-isomer:

R_f = 0.65 (TLC, 5% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.26 (2H, d, *J* = 8.2 Hz), 6.87 (2H, d, *J* = 8.2 Hz), 5.75 (1H, s), 4.62 (2H, s), 4.44 (2H, s), 4.12 (2H, q, *J* = 7.6 Hz), 3.80 (3H, s), 1.99 (3H, s), 1.26 (3H, t, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 75

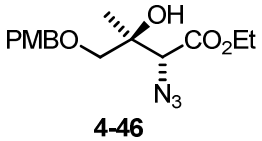
MHz): δ 165.93, 159.18, 157.03, 130.36, 129.65, 129.26, 117.24, 113.75, 72.34, 69.01, 59.77, 55.23, 21.69, 14.23.



4-44

Preparation of compound 4-44: To a solution of compound **4-43** (80 mg, 0.30 mmol) in ^{t}BuOH/H_2O (1:1, 8 mL) were added AD-mix β (420 mg, 1.4 g/mmol) and methanesulfonamide (30 mg, 100 mg/mmol) at -1 ^{o}C and the reaction mixture was allowed to stir for 72 h at that temperature. The reaction was quenched by addition of Na_2SO_3 (444 mg, 1.48 mg/mmol) and stirred for 1 h at rt until it became colourless. CH_2Cl_2 was used for extraction and the organic layer was washed with 2M NaOH, brine solution, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (20% EtOAc/hexane) to afford **4-44** (75 mg, 83%) as a colourless oil.

R_f = 0.35 (TLC, 25% EtOAc/hexane); $[\alpha]_D^{24}$ = +19.5 (c = 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.22 (2H, d, *J* = 8.8 Hz), 6.86 (2H, d, *J* = 8.2 Hz), 4.40 (2H, d, *J* = 5.1 Hz), 4.12-4.10 (1H, m), 4.05-4.03 (2H, m), 3.78 (3H, s), 3.53 (2H, d, *J* = 9.5 Hz), 3.40 (1H, s), 3.35 (1H, d, *J* = 9.5 Hz), 1.23 (3H, s), 1.18 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.15, 159.08, 129.35, 113.48, 73.86, 73.70, 73.26, 73.02, 61.57, 54.99, 21.53, 13.77; (ESI-MS): *m/z* $[M+Na]^+$ 321.1.

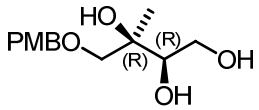


4-46

Preparation of compound 4-46: To a solution of compound **4-45** (1.1 g, 3.0 mmol) in acetone/H_2O (100 mL/25 mL) was added NaN_3 (1.4 g, 21.6 mmol) and the reaction mixture was heated at 50 ^{o}C for 7 h. After consumption of the starting

material (checked by TLC), acetone was evaporated *in vacuo* and the residue was dissolved in Et_2O. 20% H_2SO_4 (15 mL) was added carefully at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. Et_2O was used for extraction and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (15% EtOAc/hexane) to afford **4-46** (690 mg, 70%) as a colourless oil.

R_f = 0.5 (TLC, 25% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.24 (2H, d, *J* = 8.6 Hz), 6.89 (2H, d, *J* = 8.6 Hz), 4.50 (1H, d, *J* = 11.5 Hz), 4.45 (1H, d, *J* = 11.5 Hz), 4.26-4.15 (2H, m), 4.02 (1H, s), 3.81 (3H, s), 3.47 (1H, d, *J* = 9.6 Hz), 3.33 (1H, d, *J* = 9.6 Hz), 3.25 (1H, s), 1.29 (3H, t, *J* = 7.2 Hz), 1.23 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.30, 159.35, 129.55, 129.41, 113.79, 74.27, 74.19, 73.16, 65.40, 61.85, 55.22, 20.53, 14.04.

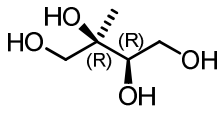


4-47

Preparation of compound 4-47: To a solution of compound **4-44** (100 mg, 0.34 mmol) in dry THF (1.5 mL) was added $LiBH_4$ (11.6 mg, 0.51 mmol) portionwise at 0 ^oC and the reaction mixture was allowed to stir for 1h. After complete consumption of the starting material MeOH was added slowly at 0 ^oC until a clear solution appeared and then it was evaporated to get the crude material. 1M HCl was added to the viscous material and it was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (40% EtOAc/hexane) to afford **4-47** (75 mg, 87%) as a colourless oil.

R_f = 0.3 (TLC, 50% EtOAc/hexane); $[\alpha]_D^{24}$ = +23.4 (c = 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.22 (2H, d, *J* = 9.0 Hz), 6.86 (2H, d, *J* = 9.0 Hz), 4.49 (2H, d, *J*

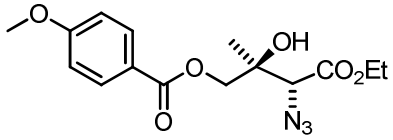
= 11.7 Hz), 4.44 (2H, d, *J* = 11.7 Hz), 3.78 (3H, s), 3.72-3.64 (4H, m), 3.43 (2H, d, *J* = 9.0 Hz), 3.38 (2H, d, *J* = 9.0 Hz), 3.27 (1H, s), 1.14 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.35, 129.39, 113.86, 75.75, 74.82, 73.35, 73.33, 62.72, 55.21, 21.00; (ESI-MS): *m/z* $[M+Na]^+$ 279.1, $[M-H]^-$ 255.1.



4-48

Preparation of compound 4-48: To an ethanolic (2 mL) solution of compound **4-47** (50 mg, 0.20 mmol) was added 10% Pd-C (15 mg) carefully under argon atmosphere. The reaction mixture was connected to vacuum to remove oxygen from the flask and purged with H_2 gas. The reaction mixture was allowed to stir for 10 h under H_2 atmosphere (1 atm.) at rt. The reaction mixture was filtered through celite and washed with EtOH. The solvent was evaporated to dryness and then chloroform was added to dissolve the impurity and decanted away to afford **4-48** (26 mg, quantitative) as a viscous material.

R_f = 0.2 (TLC, EtOAc); $[\alpha]_D^{24}$ = +9.0 (c = 1.1, MeOH); ^{1}H NMR (CD_3OD, 300 MHz): δ 3.74 (1H, m), 3.62-3.44 (4H, m), 1.16 (3H, s); ^{13}C NMR (CD_3OD, 75 MHz): δ 76.70, 74.90, 68.11, 63.68, 21.35; (ESI-MS): *m/z* $[M+Na]^+$ 159.0, $[M-H]^-$ 135.1.

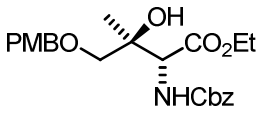


4-53

Preparation of compound 4-53: To a solution of compound **4-46** (15 mg, 0.05 mmol) in CH_2Cl_2/H_2O (1 mL/ 0.1 mL) was added DDQ (13 mg, 0.06 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 5 h at rt. After completion of the

reaction, it was directly transferred into silica gel column and purified with 15% EtOAc/hexane to obtain **4-53** (8 mg, 50%) as a colourless oil.

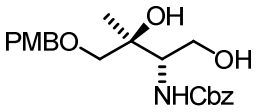
R_f = 0.3 (TLC, 25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.00 (2H, d, *J* = 9.0 Hz), 6.93 (2H, d, *J* = 9.0 Hz), 4.34-4.22 (4H, m), 4.05 (1H, s), 3.87 (3H, s), 3.41 (1H, s), 1.38 (3H, s), 1.30 (3H, t, *J* = 7.0 Hz); (ESI-MS): *m/z* $[M+Na]^+$ 360.2.



4-54

Preparation of compound 4-54: To a solution of compound **4-46** (300 mg, 0.93 mmol) in THF (5 mL) was added 10% Pd/C (90 mg) and the reaction mixture was stirred under hydrogen atmosphere (1 atm.) for 4 h. The amine was eluted with MeOH through celite pad. To a solution of the crude amine in Et_2O/H_2O (3 mL/3 mL) were added K_2CO_3 (150 mg, 1.1 mmol) and CbZCl (160 μL, 1.1 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. After completion of the reaction, it was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (15% EtOAc/hexane) to afford **4-54** (305 mg, 76% over two steps) as a colourless oil.

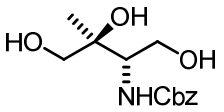
R_f = 0.4(TLC, 25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.35-7.32 (5H, m), 7.23 (2H, d, *J* = 8.5 Hz), 6.88-6.85 (2H, m), 5.79 (1H, d, *J* = 9.0 Hz), 5.11 (2H, s), 4.45-4.35 (3H, m), 4.13-4.06 (2H, m), 3.80 (3H, s), 3.49 (1H, d, *J* = 9.5 Hz), 3.33 (1H, d, *J* = 9.5 Hz), 3.09 (1H, s), 1.25-1.21 (6H, m); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.94, 159.26, 136.15, 129.41, 129.25, 128.40, 128.05, 127.94, 113.73, 74.54, 73.15, 72.81, 66.97, 61.29, 59.77, 55.15, 21.88, 13.92.



4-55

Preparation of compound 4-55: To a solution of compound **4-54** (60 mg, 0.14 mmol) in dry THF (1.5 mL) was added $LiBH_4$ (5 mg, 0.21 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 5 h. After complete consumption of the starting material MeOH was added slowly at 0 ^oC until a clear solution appeared and then it was evaporated to get the crude material. 1M HCl was added to the viscous material and then extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (40% EtOAc/hexane) to afford **4-55** (38 mg, 70%) as a colourless oil.

R_f = 0.2 (TLC, 60% EtOAc/hexane); ^1H NMR ($CDCl_3$, 500 MHz): δ 7.37-7.29 (5H, m), 7.22 (2H, d, *J* = 8.5 Hz), 6.87 (2H, d, *J* = 8.5 Hz), 5.74 (1H, d, *J* = 8.2 Hz), 5.10 (2H, s), 4.46 (2H, s), 3.83-3.79 (4H, m), 3.73-3.68 (2H, m), 3.43-3.37 (2H, m), 3.14 (1H, br), 1.23 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 159.39, 157.19, 136.27, 129.42, 129.28, 128.46, 128.09, 127.99, 113.87, 74.56, 74.31, 73.32, 66.95, 62.61, 57.57, 55.19, 23.03; (ESI-MS): *m/z* $[M-H]^-$ 387.9.

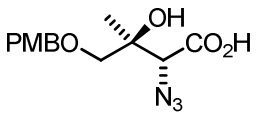


4-56

Preparation of compound 4-56: To a solution of compound **4-55** (20 mg, 0.05 mmol) in ACN/H_2O (1 mL/0.1 mL) was added CAN (55 mg, 0.1 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 1 h. After complete consumption of the starting material, it was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was

purified over silica gel (80% EtOAc/hexane) to afford **4-56** (10 mg, 72%) as a colourless oil.

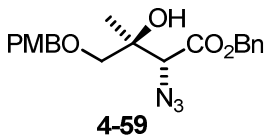
R_f = 0.2 (TLC, EtOAc); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.37-7.33 (5H, m), 5.41 (1H, d, *J* = 8.8 Hz), 5.11 (2H, s), 3.88-3.86 (2H, m), 3.74-3.70 (1H, m), 3.59 (1H, d, *J* = 12.0 Hz), 3.31 (1H, d, *J* = 12.0 Hz), 3.15 (1H, br), 2.70 (1H, br), 1.14 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 157.70, 135.97, 128.58, 128.31, 128.09, 74.51, 67.55, 67.36, 61.55, 56.05, 20.05; (ESI-MS): *m/z* $[M-H]^-$ 267.9.



4-58

Preparation of compound 4-58: To a solution of compound **4-46** (90 mg, 0.28 mmol) in THF/H_2O (2 mL/2 mL) was added LiOH (10 mg, 0.42 mmol) at 0 ^{o}C and the reaction mixture was allowed to stir for 2 h at that temperature. After completion of the reaction (checked by TLC), it was extracted with Et_2O. The aqueous layer was acidified with 10% $NaHSO_4$ to pH 2-3 and then extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain **4-58** (50 mg, 62%) as a colourless oil.

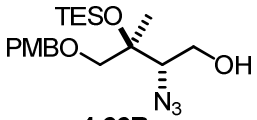
R_f = 0.25 (TLC, 15% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.26 (2H, d, *J* = 8.6 Hz), 6.89 (2H, d, *J* = 8.6 Hz), 6.24 (2H, br), 4.53 (1H, d, *J* = 11.4 Hz), 4.48 (1H, d, *J* = 11.4 Hz), 4.19 (1H, s), 3.80 (3H, s), 3.54 (1H, d, *J* = 9.6 Hz), 3.32 (1H, d, *J* = 9.6 Hz), 1.20 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.73, 159.48, 129.61, 129.47, 129.09, 113.93, 113.81, 74.32, 73.32, 73.17, 64.83, 55.25, 19.50; (ESI-MS): *m/z* $[M-H]^-$ 294.1.



4-59

Preparation of compound 4-59: To a solution of **4-58** (50 mg, 0.17 mmol) in dry DMF (1 mL) were added Cs_2CO_3 (66 mg, 0.2 mmol) and BnBr (41 μL, 0.34 mmol) at 0 ^oC and the reaction mixture was stirred for 2 h at rt. After completion of the reaction, it was extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude material which was carefully purified by column chromatography over silica gel (12% EtOAc/hexane) to obtain pure **4-59** (59 mg, 90%) as a colourless oil.

R_f = 0.45 (TLC, 20% EtOAc/hexane); ^1H NMR ($CDCl_3$, 500 MHz): δ 7.36-7.33 (5H, m) 7.20 (2H, d, *J* = 8.2 Hz), 6.86 (2H, d, *J* = 8.2 Hz), 5.19 (1H, d, *J* = 12.0 Hz), 5.15 (1H, d, *J* = 12.0 Hz), 4.46-4.40 (2H, m), 4.04 (1H, s), 3.80 (3H, s), 3.46 (1H, d, *J* = 10.1 Hz), 3.30 (1H, d, *J* = 10.1 Hz), 3.16 (1H, s) 1.22 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.18, 159.42, 134.92, 129.46, 128.63, 128.56, 128.43, 113.86, 74.45, 74.24, 73.20, 67.55, 65.55, 55.27, 20.63; (ESI-MS): *m/z* $[M+Na]^+$ 408.0.



4-66R

Preparation of compound 4-66R: To a solution of compound **4-65R** (110 mg, 0.25 mmol) in dry Et_2O (2 mL) was added $LiBH_4$ (44 mg, 2.0 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 2 h. After complete consumption of the starting material MeOH was added slowly at 0 ^oC until a clear solution appeared and then it was evaporated to get the crude material. 1M HCl was added to the viscous material and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (6% EtOAc/hexane) to afford **4-66R** (80 mg, 80%) as a colourless oil.

R_f = 0.5 (TLC, 15% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.23 (2H, d, *J* = 8.5 Hz), 6.88 (2H, d, *J* = 8.5 Hz), 4.49 (1H, d, *J* = 11.5 Hz), 4.41 (1H, d, *J* = 11.5 Hz), 3.82-3.77 (4H, m), 3.48-3.44 (2H, m), 3.32 (1H, d, *J* = 9.3 Hz), 2.51-2.50 (1H, m), 1.26 (3H, s), 0.94 (9H, t, *J* = 4.2 Hz), 0.59 (6H, q, *J* = 7.7 Hz).

4-67R

Preparation of compound 4-67R: To a solution of compound **4-66R** (140 mg, 0.35 mmol) in pyridine (4 mL) was added Ac_2O (216 μL, 2.2 mmol) at 0 ^{o}C and the reaction mixture was allowed to stir for 2 h. After complete consumption of the starting material, 10% citric acid was added and it was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (8% EtOAc/hexane) to afford **4-67R** (140 mg, 90%) as a colourless oil.

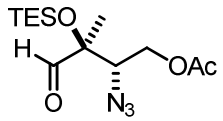
R_f = 0.65 (TLC, 15% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.25 (2H, d, *J* = 9.0 Hz), 6.88 (2H, d, *J* = 9.0 Hz), 4.48-4.39 (3H, m), 4.17-4.10 (1H, m), 3.81 (3H, s), 3.77 (1H, d, *J* = 2.4 Hz), 3.36 (1H, d, *J* = 9.6 Hz), 3.32 (1H, d, *J* = 9.6 Hz), 2.10 (3H, s), 1.18 (3H, s), 0.92 (9H, t, *J* = 7.7 Hz), 0.57 (6H, q, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.82, 159.29, 129.52, 113.74, 76.23, 74.76, 72.97, 65.72, 64.15, 55.24, 21.87, 20.81, 6.94, 6.44.

4-68R

Preparation of compound 4-68R: To a solution of compound **4-67R** (120 mg, 0.27 mmol) in CH_2Cl_2/H_2O (3.6 mL/ 0.4 mL) was added DDQ (156 mg, 0.67 mmol) at 0 ^{o}C and the reaction mixture was allowed to stir for 2 h at rt. After completion of the

reaction, it was directly transferred into a silica gel column and purified with 15% EtOAc/hexane to obtain **4-68R** (65 mg, 75%) as a colourless oil.

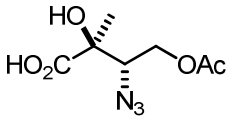
R_f = 0.35 (TLC, 20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.52-4.48 (1H, dd, *J* = 11.5, 2.6 Hz), 4.19-4.12 (1H, dd, *J* = 11.3, 9.7 Hz), 3.82-3.78 (1H, dd, *J* = 9.7, 2.6 Hz), 3.58 (1H, d, *J* = 11.3 Hz), 3.43 (1H, d, *J* = 11.3 Hz), 2.11 (3H, s), 1.17 (3H, s), 0.95 (9H, t, *J* = 7.7 Hz), 0.62 (6H, q, *J* = 8.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.85, 76.83, 68.16, 64.82, 64.29, 20.75, 20.49, 6.90, 6.55.



4-69R

Preparation of compound 4-69R: To a solution of compound **4-68R** (30 mg, 0.1 mmol) in dry CH_2Cl_2 (2 mL) were added TEMPO (3 mg, 0.02 mmol) and diacetoxyiodobenzene (77 mg, 0.25 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. CH_2Cl_2 was concentrated and directly transferred into a silica gel column and purified with 3% EtOAc/hexane to obtain **4-69R** (22 mg, 68%) as a colourless oil.

R_f = 0.65 (TLC, 10% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.61 (1H, s), 4.30-4.17 (2H, m), 3.69-3.65 (1H, m), 2.08 (3H, s), 1.37 (3H, s), 0.96 (9H, t, *J* = 7.7 Hz), 0.66 (6H, q, *J* = 8.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 202.00, 170.32, 81.16, 65.59, 62.61, 20.66, 20.38, 6.82, 6.47.

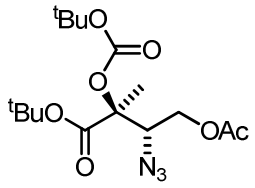


4-70R

Preparation of compound 4-70R: To a solution of compound **4-69R** (25 mg, 0.08 mmol) in THF/H_2O/^{t}BuOH (1.2 mL/1.2 mL/0.3 mL) were added 2-methyl-2-

butene (500 μL), $NaClO_2$ (14 mg, 0.16 mmol) and NaH_2PO_4 (38 mg, 0.32 mmol) in 1 mL H_2O at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. THF was removed under reduced pressure and 5 mL 1M HCl was added and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the residue which was dissolved in 2 mL sat. $NaHCO_3$ solution and extracted with Et_2O. The aqueous layer was acidified with 2 mL 3M HCl and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to obtain **4-70R** (9 mg, 50%) as a colourless oil.

R_f = 0.2 (TLC, 15% MeOH/CH_2Cl_2); ^1H NMR (CD_3OD, 300 MHz): δ 4.28 (2H, d, *J* = 6.6 Hz), 3.84 (1H, t, *J* = 6.6 Hz), 2.07 (3H, s), 1.47 (3H, s); (ESI-MS): *m/z* $[M-H]^-$ 216.1.

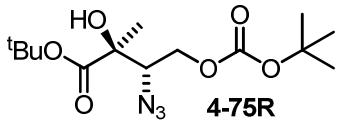


4-71R

Preparation of compound 4-71R: To a solution of compound **4-70R** (25 mg, 0.12 mmol) in ^{t}BuOH (1 mL) were added DMAP (2 mg) and $(Boc)_2O$ (110 mg, 0.48 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 14 h at rt. The reaction mixture was directly transferred into a silica gel column and purified with 4% EtOAc/hexane to obtain **4-71R** (24 mg, 56%) as a colourless oil.

R_f = 0.6 (TLC, 15% EtOAc/hexane); ^1H NMR (CD_3OD, 500 MHz): δ 4.46-4.43 (1H, dd, *J* = 6.8, 1.5 Hz), 4.16-4.12 (1H, dd, *J* = 6.8, 5.7 Hz), 4.06-4.04 (1H, dd, *J* = 5.7, 1.5 Hz), 2.11 (3H, s), 1.56 (3H, s), 1.50 (9H, s), 1.47 (9H, s); ^{13}C NMR ($CDCl_3$, 125

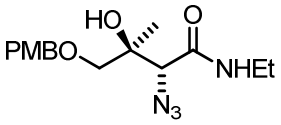
MHz): δ 170.49, 168.34, 151.29, 83.18, 82.97, 81.51, 64.51, 63.41, 27.76, 27.65, 20.68, 17.29; (ESI-MS): *m/z* $[M+Na]^+$ 395.9.



4-75R

Preparation of compound 4-75R: To a solution of compound **4-71R** (13 mg, 0.03 mmol) in MeOH (1 mL) was added NaOMe (2 mg) at 0 ^oC and the reaction mixture was allowed to stir for 15 min. The reaction mixture was concentrated, transferred into a silica gel column and purified with 4% EtOAc/hexane to obtain **4-75R** (8 mg, 70%) as a colourless oil.

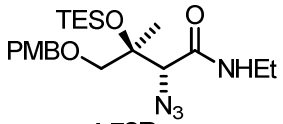
R_f = 0.5 (TLC, 15% EtOAc/hexane); ^1H NMR ($CDCl_3$, 500 MHz): δ 4.26-4.23 (1H, dd, *J* = 12.0, 4.0 Hz), 4.21-4.17 (1H, dd, *J* = 12.0, 8.5 Hz), 3.74-3.71 (1H, dd, *J* = 8.5, 4.0 Hz), 1.52 (9H, s), 1.49 (9H, s), 1.46 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.18, 153.01, 84.18, 82.77, 75.73, 66.36, 65.43, 27.83, 27.70, 23.71.



4-77R

Preparation of compound 4-77R: To a solution of compound **4-76R** (200 mg, 0.67 mmol) in dry CH_2Cl_2 (20 mL) were added DIPEA (234 μL, 1.34 mmol), ethylamine hydrochloride salt (83 mg, 1.01 mmol), HATU (300 mg, 0.8 mmol) and HOAt (9.5 mg, 0.08 mmol) at -10 ^oC and the reaction mixture was allowed to stir for 12 h at rt. The reaction was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (20% EtOAc/hexane) to afford **4-77R** (175 mg, 80%) as a colourless oil.

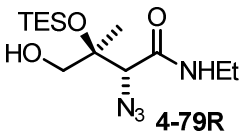
R_f = 0.35 (TLC, 25% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.27 (2H, d, *J* = 8.6 Hz), 6.89 (2H, d, *J* = 8.6 Hz), 6.72 (1H, br), 4.72 (1H, s), 4.54 (2H, s), 4.29 (1H, s), 3.80 (3H, s), 3.50-3.30 (4H, m), 1.17 (3H, t, *J* = 7.2 Hz), 1.06 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 129.58, 113.78, 74.92, 74.58, 73.21, 65.42, 55.26, 34.41, 19.94, 14.64; (ESI-MS): *m/z* $[M+Na]^+$ 345.0, $[M-H]^-$ 321.0.



4-78R

Preparation of compound 4-78R: To a solution of compound **4-77R** (200 mg, 0.62 mmol) in dry CH_2Cl_2 (5 mL) were added Et_3N (0.69 mL, 4.97 mmol) and TESOTf (0.70 mL, 3.10 mmol) slowly at 0 ^oC and the reaction mixture was allowed to stir over night at rt. CH_2Cl_2 was used for extraction and washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (10% EtOAc/hexane) to afford **4-78R** (257 mg, 95%) as a colourless oil.

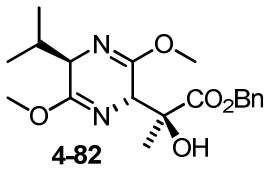
R_f = 0.5 (TLC, 20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.24 (2H, d, *J* = 9.0 Hz), 6.87 (2H, d, *J* = 9.0 Hz), 4.45 (1H, d, *J* = 11.5 Hz), 4.41 (1H, d, *J* = 11.5 Hz), 4.00 (1H, s), 3.80 (3H, s), 3.44 (1H, d, *J* = 9.0 Hz), 3.36 (1H, d, *J* = 9.0 Hz), 3.30-3.21 (2H, m), 1.33 (3H, s), 1.10 (3H, t, *J* = 7.5 Hz), 0.95 (9H, t, *J* = 7.5 Hz), 0.61 (6H, q, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.59, 159.25, 129.87, 129.44, 113.71, 77.32, 74.53, 72.99, 69.24, 55.24, 34.24, 22.51, 14.59, 6.88, 6.40.



4-79R

Preparation of compound 4-79R: To a solution of compound **4-78R** (100 mg, 0.23 mmol) in CH_2Cl_2/H_2O (9 mL/1 mL) was added DDQ (110 mg, 0.46 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 2 h at rt. After completion of the reaction, it was directly transferred into a silica gel column and purified with 15% EtOAc/hexane to obtain **4-79R** (43 mg, 60%) as a colourless oil.

R_f = 0.35 (TLC, 25% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 6.33 (1H, br), 4.03 (1H, s), 3.55 (1H, d, *J* = 11.4 Hz), 3.46 (1H, d, *J* = 11.4 Hz), 3.38-3.27 (2H, m), 1.42 (3H, s), 1.17 (3H, t, *J* = 7.2 Hz), 0.95 (9H, t, *J* = 7.5 Hz), 0.61 (6H, q, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 168.34, 77.84, 70.48, 68.92, 34.48, 23.28, 14.58, 6.90, 6.62; (ESI-MS): *m/z* $[M+H]^+$ 317.1, $[M+Na]^+$ 339.0, $[M-H]^-$ 315.0.

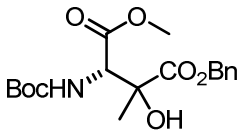


4-82

Preparation of compound 4-82: To a solution of compound **4-81** (50 mg, 0.27 mmol) in dry THF (2 mL) was added a 1.8 M solution of *n*-BuLi in cyclohexane (159 μL, 0.29 mmol) at -78 ^oC and the reaction mixture was stirred for 30 minute. After 30 minute, benzylpyruvate (58 mg, 0.32 mmol) in THF (1 mL) was added dropwise to the reaction mixture at -78 ^oC and the mixture was stirred for 4 h. The reaction mixture was quenched with sat. NH_4Cl solution -78 ^oC and then extracted with Et_2O. The extract was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (4% EtOAc/hexane) to afford **4-82** (20 mg, 20%) as a colourless oil.

R_f = 0.4 (TLC, 15% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.41-7.33 (5H, m), 5.30 (2H, s), 5.24 (1H, d, *J* = 11.2 Hz), 4.36 (1H, d, *J* = 3.5 Hz), 3.94 (1H, t, *J* =

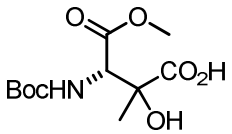
3.5 Hz), 3.70 (3H, s), 3.53 (3H, s), 3.50 (1H, s), 2.35-2.25 (1H, m), 1.55 (3H, s), 1.05 (3H, d, J = 6.0 Hz), 0.64 (3H, d, J = 6.9 Hz); (ESI-MS): *m/z* $[M+H]^+$ 363.1, $[M+Na]^+$ 385.1.



4-86

Preparation of compound 4-86: To a solution of compound **4-82** (20 mg, 0.06 mmol) in THF/ACN (0.5 mL/1.0 mL) was added 0.25N HCl (550 μL) at 0 ^oC and the reaction mixture was stirred for 12 h. The solvent was evaporated and the residue was dissolved in MeOH (0.5 mL). $(Boc)_2O$ (19 μL, 0.09 mmol) was added and the mixture was stirred over night. The reaction mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (10% EtOAc/hexane) to afford **4-86** (4 mg, 20% over two steps) as a colourless oil.

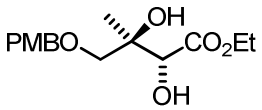
R_f = 0.4 (TLC, 20% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.37 (5H, br), 5.30 (1H, d, J = 11.0 Hz), 5.24 (2H, s), 4.81 (1H, d, J = 10.2 Hz), 3.56 (3H, s), 3.42 (1H, s), 1.45 (12H, s); (ESI-MS): *m/z* $[M+Na]^+$ 390.0.



4-87

Preparation of compound 4-87: To a solution of compound **4-86** (4 mg, 0.01 mmol) in MeOH (1 mL) was added 10% Pd/C (5 mg) and the reaction mixture was stirred under hydrogen atmosphere (1 atm.) for 2 h. The product was eluted with MeOH through celite pad. The solvent was concentrated under reduced pressure to afford **4-87** (2 mg, 70%) as a viscous material.

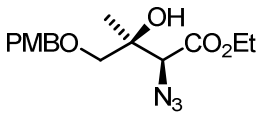
R_f = 0.3 (TLC, 15% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.40 (1H, br), 4.78 (1H, br), 3.74 (3H, s), 1.46 (12H, s); (ESI-MS): *m/z* $[M+Na]^+$ 300.0, $[M-H]^-$ 275.9.



4-93

Preparation of compound 4-93: To a solution of compound **4-92** (270 mg, 1.02 mmol) in ^{t}BuOH/H_2O (10 mL/10 mL) were added AD-mix α (1.4 g, 1.4 g/mmol) and methanesulfonamide (100 mg, 100 mg/mmol) at -1 ^{o}C and the reaction mixture was allowed to stir for 96 h at that temperature. The reaction was quenched by addition of Na_2SO_3 (1.5 g, 1.48 mg/mmol) and stirred for 1 h at rt until it became colourless. CH_2Cl_2 was used for extraction and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel to afford **4-93** (260 mg, 85%) as a colourless oil.

R_f = 0.2 (TLC, 20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.24 (2H, d, *J* = 8.6 Hz), 6.86 (2H, d, *J* = 8.6 Hz), 4.44 (2H, s), 4.20-4.09 (3H, m), 3.8 (3H, s), 3.47 (1H, d, *J* = 9.4 Hz), 3.37 (1H, d, *J* = 9.4 Hz), 3.18 (1H, br), 1.27-1.23 (6H, m); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.84, 159.23, 129.65, 129.33, 113.69, 75.17, 74.28, 73.19, 73.14, 61.58, 55.16, 20.62, 13.99; HRMS (ESI): *m/z* calcd for $C_{15}H_{22}O_6Na^+$ $[M+Na]^+$ 321.1309, found 321.1314.

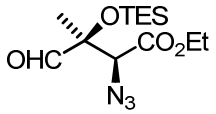


4-95

Preparation of compound 4-95: To a solution of compound **4-94** (1.1 g, 3.0 mmol) in acetone/H_2O (100 mL/25 mL) was added NaN_3 (1.4 g, 21.6 mmol) and the reaction mixture was heated at 50 ^{o}C for 7 h. After consumption of the starting

material (checked by TLC), acetone was evaporated *in vacuo* and the residue was dissolved in Et_2O. 20% H_2SO_4 (15 mL) was added carefully at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. Et_2O was used for extraction and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (15% EtOAc/hexane) to afford **4-95** (690 mg, 70%) as a colourless oil.

R_f = 0.45 (TLC, 25% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.25-7.22 (2H, m), 6.89-6.86 (2H, m), 4.44 (2H, s), 4.14 (2H, q, *J* = 7.0 Hz), 4.02 (1H, s), 3.80 (3H, s), 3.47 (1H, d, *J* = 9.0 Hz), 3.41-3.38 (2H, m), 1.27-1.22 (6H, m); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 168.91, 159.33, 129.65, 129.47, 113.73, 74.32, 73.74, 73.18, 66.52, 61.88, 55.22, 22.25, 13.98; (ESI-MS): *m/z* $[M+Na]^+$ 346.0.

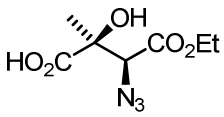


4-96

Preparation of compound 4-96: To a solution of compound **4-95** (400 mg, 1.23 mmol) in dry CH_2Cl_2 (10 mL) were added Et_3N (686 μL, 4.95 mmol) and TESOTf (700 μL, 3.08 mmol) slowly at 0 ^oC and the reaction mixture was allowed to stir for 2 h. at that temperature. CH_2Cl_2 was added and extracted. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel to get pure 450 mg (1.03 mmol) of protected compound and then it was dissolved in CH_2Cl_2 (9 mL). To this solution were added 850 μL phosphate buffer (pH 7.5) and DDQ (583 mg, 2.6 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 3 h at rt. After completion of reaction, the reaction mixture was directly transferred into a silica gel column and

purified with 7.5% EtOAc/hexane to obtained 308 mg of alcohol. To a solution of the alcohol (308 mg, 0.97 mmol) in dry CH_2Cl_2 (24 mL) were added TEMPO (30 mg, 0.2 mmol) and diacetoxyiodobenzene (940 mg, 2.91 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. CH_2Cl_2 was concentrated and directly transferred into a silica gel column and purified with 3% EtOAc/hexane to obtain **4-96** (218 mg, 56% over three steps) as a colourless oil.

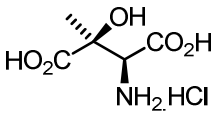
R_f = 0.6 (TLC, 10% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 9.64 (1H, s), 4.26 (2H, q, *J* = 7.0 Hz), 3.86 (1H, s), 1.41 (3H, s), 1.32 (3H, t, *J* = 7.2 Hz), 0.96 (9H, t, *J* = 7.9 Hz), 0.64 (6H, q, *J* = 7.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 200.39, 167.25, 81.74, 67.20, 62.14, 20.16, 14.05, 6.71, 6.35.



4-97

Preparation of compound 4-97: To a solution of compound **4-96** (36 mg, 0.12 mmol) in THF/H_2O/^{t}BuOH (1.6 mL/1.6 mL/0.4 mL) were added 2-methyl-2-butene (800 μL), $NaClO_2$ (21 mg, 0.23 mmol) and NaH_2PO_4 (55 mg, 0.46 mmol) in 1 mL H_2O at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. THF was removed under reduced pressure and 5 mL 1M HCl was added and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to obtain the residue which was dissolved in 2 mL sat. $NaHCO_3$ solution and extracted with Et_2O. The aqueous layer was acidified with 2 mL 3M HCl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to obtain **4-97** (15 mg, 60%) as a colourless oil.

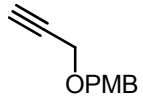
R_f = 0.2 (TLC, 15% MeOH/CH_2Cl_2); ^{1}H NMR (CD_3OD, 300 MHz): δ 4.30-4.26 (2H, m), 4.08 (1H, s), 1.47 (3H, s), 1.33 (3H, t, J = 6.9 Hz); ^{13}C NMR (CD_3OD, 75 MHz): δ 177.33, 169.63, 78.35, 67.96, 62.88, 23.79, 14.46.



4-99

Preparation of compound 4-99: To a solution of compound **4-97** (15 mg, 0.07 mmol) in MeOH (2 mL) was added 10% Pd/C (9 mg) and the reaction mixture was stirred under hydrogen atmosphere (1 atm.) for 2 h. The amine was eluted with MeOH through celite pad. The solvent was concentrated under reduced pressure to get the crude product which was dissolved in 6M HCl (5 mL) and refluxed for 24 h to afford **4-99** (11 mg, 85% over two steps) as a viscous material.

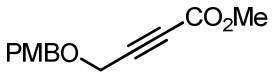
^{1}H NMR (D_2O, 300 MHz): δ 4.25 (1H, s), 1.50 (3H, s).



4-101

Preparation of compound 4-101: To a solution of compound **4-100** (0.5 mL, 8.9 mmol) in THF (22 mL) was added NaH (385 mg, 16.0 mmol) at 0 ^{o}C and the reaction mixture was stirred for 15 minute. Next, *p*-methoxybenzyl chloride (1.8 mL, 13.4 mmol) and TBAI (430 mg, 1.15 mmol) were added to the reaction mixture at 0 ^{o}C and it was stirred for 16 h at rt. The reaction was quenched carefully with dropwise addition of sat. NH_4Cl solution and then extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was concentrated under reduced pressure to get the crude product which was purified over silica gel (5% EtOAc/hexane) to afford **4-101** (1.5 g, quantitative) as a colourless oil.

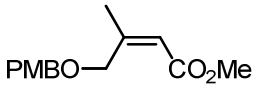
R_f = 0.5 (TLC, 10% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29 (2H, d, *J* = 8.7 Hz), 6.90 (2H, d, *J* = 8.7 Hz), 4.55 (2H, s), 4.14 (2H, d, *J* = 2.4 Hz), 3.81 (3H, s), 2.47 (1H, t, *J* = 2.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.36, 129.70, 129.27, 113.78, 79.73, 74.43, 71.06, 56.60, 55.17.



4-103

Preparation of compound 4-103: To a solution of compound **4-101** (330 mg, 1.88 mmol) in dry THF (6 mL) was added a *n*-BuLi (1.0 mL, 2.0 M solution in cyclohexane, 2.0 mmol) at -78 ^{o}C and the reaction mixture was stirred for 30 minute. After 30 minute, methyl cyanoformate (240 μL, 2.82 mmol) was added dropwise to the reaction mixture at -78 ^{o}C and it was stirred for 1 h at that temperature. It was then warmed to -40 ^{o}C and stirred for 15 minute. The reaction mixture was quenched with sat. NH_4Cl solution at -40 ^{o}C and then extracted with Et_2O. The extract was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (7% EtOAc/hexane) to afford **4-103** (420 mg, 96%) as a colourless oil.

R_f = 0.35 (TLC, 10% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.29 (2H, d, *J* = 8.9 Hz), 6.90 (2H, d, *J* = 8.9 Hz), 4.56 (2H, s), 4.27 (2H, s), 3.82 (3H, s), 3.81 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 159.62, 153.58, 129.88, 128.76, 113.95, 83.80, 77.88, 71.68, 56.33, 55.27, 52.78.

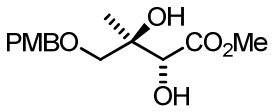


4-104

Preparation of compound 4-104: To the suspension of CuI (640 mg, 3.36 mmol) in dry THF (10 mL) was added MeLi (3.35 mL, 6.70 mmol) at 0 ^{o}C and the

reaction mixture was allowed to stir for 30 minute at that temperature. To this colourless solution was added **4-103** (555 mg, 2.24 mmol) drop wise at -78 ^{o}C and stirred at that temperature for 4 h. To this reaction mixture was added 16 mL sat. NH_4Cl solution drop wise and after complete addition it was kept at -78 ^{o}C for 10 minute. The reaction mixture was then warmed up slowly to rt. The precipitate was filtered and the filtrate was extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to get the crude product which was purified over silica gel (1.5% EtOAc/hexane) to obtain **4-104** (400 mg, 69%, exclusive *Z*-isomer) as a colourless oil.

R_f = 0.7 (TLC, 5% EtOAc/hexane); IR (thin film): ν_{max} = 2952, 2838, 1716, 1613, 1515, 1444, 1363, 1248, 1153, 1035, 1032, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.25 (2H, d, *J* = 8.2 Hz), 6.86 (2H, d, *J* = 8.2 Hz), 5.74 (1H, s), 4.61 (2H, s), 4.43 (2H, s), 3.78 (3H, s), 3.66 (3H, s), 1.98 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.29, 159.18, 157.47, 130.32, 129.23, 116.70, 113.74, 72.35, 68.99, 55.20, 50.97, 21.69; HRMS (ESI): *m/z* calcd for $C_{14}H_{18}O_4Na^+$ $[M+Na]^+$ 273.1097, found 273.1106.

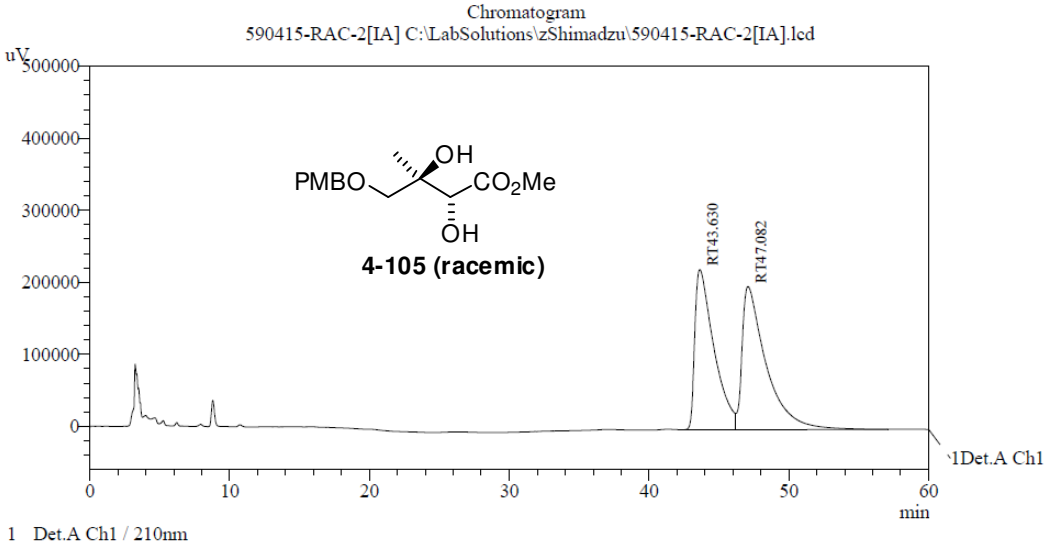


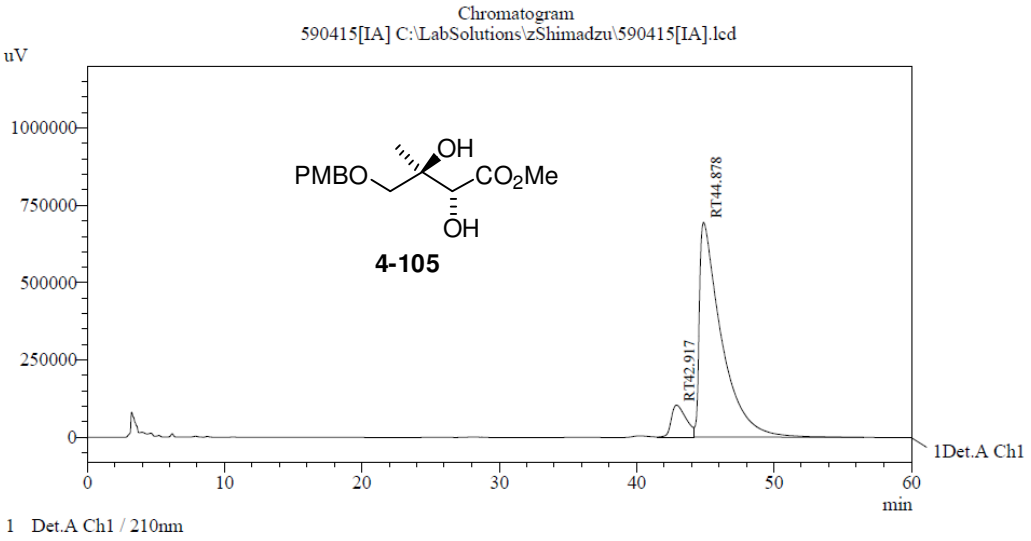
4-105

Preparation of compound 4-105: To a solution of compound **4-104** (80 mg, 0.30 mmol) in ^{t}BuOH/H_2O (1:1, 8 mL) were added AD-mix α (420 mg, 1.4 g/mmol) and methanesulfonamide (30 mg, 100 mg/mmol) at -1 ^{o}C and the reaction mixture was allowed to stir for 96 h at that temperature. The reaction was quenched by addition of Na_2SO_3 (444 mg, 1.48 mg/mmol) and stirred for 1 h at rt until it became colourless. CH_2Cl_2 was used for extraction and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which

was purified over silica gel (20% EtOAc/hexane) to obtain **4-105** (75 mg, 85%) as a colourless oil. The enantiomeric excess was determined through HPLC analysis with Chiralpak-IA column (0.46 cm ϕx25 cm) using hexanes/2-propanol (90:10) at a flow rate of 1.0 mL/min; detection UV 210 nm; 82 % *ee*.

R_f = 0.3 (TLC, 25% EtOAc/hexane); $[\alpha]_D^{25}$ = -16.2 (*c* = 4.0, $CHCl_3$); IR (thin film): ν_{max} = 3473, 2954, 2863, 1728, 1613, 1515, 1248, 1174, 1091, 1032 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.23 (2H, d, *J* = 8.9 Hz), 6.88 (2H, d, *J* = 8.9 Hz), 4.43 (2H, s), 4.10 (1H, s), 3.80 (3H, s), 3.70 (3H, s), 3.47 (1H, d, *J* = 9.5 Hz), 3.37 (1H, d, *J* = 8.9 Hz), 1.22 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.39, 159.35, 129.44, 113.80, 75.26, 74.31, 73.32, 73.18, 55.25, 52.39, 20.65; HRMS (ESI): *m/z* calcd for $C_{14}H_{20}O_6Na^+$ $[M+Na]^+$ 307.1152, found 307.1159.





PeakTable

Detector A Ch1 210nm

Peak#	Name	Ret. Time	Area	Height	Area %	Units
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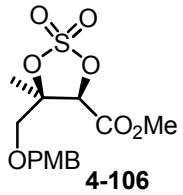
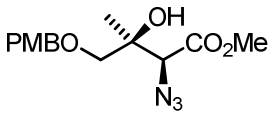


4-106

Preparation of compound 4-106: To a solution of compound **4-105** (835 mg, 2.94 mmol) in CH_2Cl_2 (20 mL) were added Et_3N (1.03 mL, 7.35 mmol) and $SOCl_2$ (540 µL, 7.35 mmol) carefully at 0 ^oC and the reaction mixture was allowed to stir for 30 minute at that temperature. After consumption of the starting material (checked by TLC), CH_2Cl_2 was evaporated *in vacuo* and the resulting cyclic sulphite was eluted with Et_2O through silica plug. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was dissolved in CH_3CN (14 mL). To this solution were added $RuCl_3$ (2 mg), $NaIO_4$ (1.13 g, 5.3 mmol), and H_2O (18 mL) at 0 ^oC and the reaction mixture was allowed to stir for 30 minute at that temperature. The reaction mixture was diluted with Et_2O and

extracted. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (20% EtOAc/hexane) to afford **4-106** (800 mg, 79%) as a colourless oil.

R_f = 0.45 (TLC, 20% EtOAc/hexane); $[\alpha]_D^{25}$ = +34.3 (*c* = 4.2, $CHCl_3$); IR (thin film): ν_{max} = 2957, 2875, 1766, 1742, 1612, 1516, 1440, 1418, 1302, 1249, 1215, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.21 (2H, d, *J* = 8.7 Hz), 6.88 (2H, d, *J* = 8.7 Hz), 4.99 (1H, s), 4.46 (1H, d, *J* = 11.5 Hz), 4.39 (1H, d, *J* = 11.5 Hz), 3.80 (3H, s), 3.77 (1H, d, *J* = 10.4 Hz), 3.70 (3H, s), 3.59 (1H, d, *J* = 10.4 Hz), 1.75 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.65, 159.50, 129.38, 128.72, 113.85, 91.87, 81.56, 73.35, 69.89, 55.26, 53.02, 29.68, 22.15; HRMS (ESI): *m/z* calcd for $C_{14}H_{18}O_8SNa^+$ $[M+Na]^+$ 369.0615, found 369.0624.

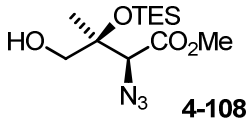


4-107

Preparation of compound 4-107: To a solution of compound **4-106** (800 mg, 2.31 mmol) in acetone/H_2O (56 mL/14 mL) was added NaN_3 (900 mg, 13.87 mmol) and the reaction mixture was heated at 50 ^oC for 9 h. After consumption of the starting material (checked by TLC), acetone was evaporated *in vacuo* and the residue was dissolved in Et_2O. 20% H_2SO_4 (10 mL) was added carefully at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. Et_2O was used for extraction and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (15% EtOAc/hexane) to afford **4-107** (560 mg, 78%) as a colourless oil.

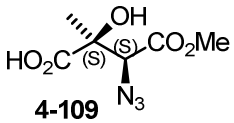
R_f = 0.48 (TLC, 20% EtOAc/hexane); $[\alpha]_D^{25}$ = -34.4 (*c* = 4.5, $CHCl_3$); IR (thin film): ν_{max} = 3512, 2909, 2864, 2114, 1733, 1613, 1514, 1248, 1207, 1175, 1094, 1032, 821 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.23 (2H, d, *J* = 8.7 Hz), 6.88 (2H, d, *J* = 8.7 Hz),

4.43 (2H, s), 4.02 (1H, s), 3.80 (3H, s), 3.66 (3H, s), 3.47 (1H, d, J = 9.4 Hz), 3.40 (1H, s), 3.36 (1H, d, J = 9.4 Hz), 1.26 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.43, 159.39, 129.62, 129.58, 113.77, 74.39, 73.70, 73.29, 66.71, 55.27, 52.52, 22.29; HRMS (ESI): *m/z* calcd for $C_{14}H_{19}O_5N_3Na^+$ $[M+Na]^+$ 332.1217, found 332.1229.



4-108

Preparation of compound 4-108: To a solution of compound **4-107** (560 mg, 1.81 mmol) in CH_2Cl_2 (20 mL) were added Et_3N (1.02 mL, 7.25 mmol) and TESOTf (1.02 mL, 4.53 mmol) slowly at 0 ^oC and the reaction mixture was allowed to stir for 45 minute at that temperature. CH_2Cl_2 was used for extraction and was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel to get pure 680 mg (1.61 mmol) of protected compound. To a solution of the TES protected compound in CH_2Cl_2 (30 mL) were added 6.1 mL phosphate buffer (pH 7.5) and DDQ (912 mg, 4.02 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 3 h at rt. After completion of the reaction, the reaction mixture was directly transferred into a silica gel column and purified with 7.5% EtOAc/hexane to obtain **4-108** (268 mg, 70% over two steps) as a colourless oil. R_f = 0.5 (TLC, 20% EtOAc/hexane); $[\alpha]_D^{25}$ = +15.6 (c = 1.0, $CHCl_3$); IR (thin film): ν_{max} = 3440, 2956, 2878, 2114, 1747, 1458, 1436, 1277, 1243, 1174, 1057, 1010, 746 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.94 (1H, s), 3.79 (3H, s), 3.65-3.57 (1H, dd, J = 11.4, 7.6 Hz), 3.56-3.53 (1H, dd, J = 11.4, 7.6 Hz), 2.00-1.98 (1H, m), 1.38 (3H, s), 0.97 (9H, t, J = 8.2 Hz), 0.64 (6H, q, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.20, 78.31, 68.40, 67.38, 52.40, 21.93, 6.83, 6.58; HRMS (ESI): *m/z* calcd for $C_{12}H_{26}O_4N_3Si$ $[M+H]^+$ 304.1693, found 304.2841.

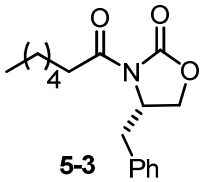


4-109

Preparation of compound 4-109: To a solution of compound **4-108** (240 mg, 0.8 mmol) in dry CH_2Cl_2 (25 mL) were added TEMPO (25 mg, 0.16 mmol) and diacetoxyiodobenzene (773 mg, 2.4 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 10 h at rt. CH_2Cl_2 was concentrated and directly transferred into a silica gel column and purified with 3% EtOAc/hexane to obtain 168 mg (70%) of aldehyde which was again dissolved in THF/H_2O/^{t}BuOH (12 mL/12 mL/2.4 mL) and to this solution were added 4.8 mL 2-methyl-2-butene, $NaClO_2$(160 mg, 1.8 mmol) and NaH_2PO_4 (400 mg, 3.34 mmol) in 6 mL H_2O at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. THF was removed under reduced pressure and 50 mL 1M HCl was added and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the residue which was dissolved in 12 mL sat. $NaHCO_3$ solution and extracted with Et_2O. The aqueous layer was acidified with 10 mL 3M HCl and then extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to obtain **4-109** (101 mg, 63% over two steps) as a colourless oil.

R_f = 0.2 (TLC, 5% CH_3OH/CH_2Cl_2); $[\alpha]_D^{25}$ = -44.1 (c = 2.0, $CHCl_3$); IR (thin film): ν_{max} = 3490, 2959, 2117, 1730, 1438, 1263, 1215, 1182, 1106, 1013, 847 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 4.16 (1H, s), 3.88 (3H, s), 1.57 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 176.61, 167.99, 66.37, 53.03, 23.05; HRMS (ESI): *m/z* calcd for $C_6H_8O_5N_3$ $[M-H]^-$ 202.0469, found 202.0473.

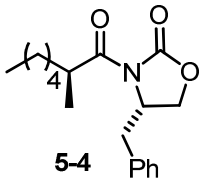
Chapter 5:



5-3

Preparation of compound 5-3: To a solution of heptanoic acid (1.0 g, 7.7 mmol) in THF (30 mL) were added Et_3N (3.2 mL, 23.1 mmol) and pivaloyl chloride (1.2 mL, 8.5 mmol) at -20 ^oC and the reaction mixture was stirred for 1 h. Lithium chloride (500 mg, 11.8 mmol) and (S)-4-benzyl-2-oxazolidinone (1.3 g, 7.4 mmol) were added to the reaction mixture and it was allowed to stir for 14 h at rt. The reaction was quenched by addition of 0.2M HCl (10 mL), and THF was removed *in vacuo*. The residue was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (5% EtOAc/hexane) to afford **5-3** (1.78 g, 80%) as a colourless oil.

R_f = 0.45 (TLC, 10% EtOAc/hexane); ^1H NMR ($CDCl_3$, 300 MHz): δ 7.40-7.24 (5H, m), 4.75-4.68 (1H, m), 4.27-4.18 (2H, m), 3.33 (1H, dd, *J* = 13.2, 3.0 Hz), 3.07-2.77 (3H, m), 1.78-1.69 (2H, m), 1.45-1.36 (6H, m), 0.96 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.43, 153.44, 135.33, 129.40, 128.93, 127.31, 66.13, 55.13, 37.93, 35.52, 31.54, 28.78, 24.22, 22.49, 14.00; (ESI-MS): *m/z* $[M+Na]^+$ 312.1.

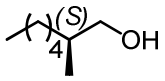


5-4

Preparation of compound 5-4: To a stirred solution of *N*,*N*-diisopropylamine (0.76 mL, 5.40 mmol) in dry THF (5 mL) was added *n*-BuLi (4.2 mL, 1.3M solution in hexane, 5.4 mmol) at 0°C and the reaction mixture was stirred for 35 minute. This

freshly prepared LDA was added to a solution of compound **5-3** (1.04 g, 3.6 mmol) in dry THF (10 mL) dropwise at -78 ^{o}C and then the mixture was allowed to stir for 1 h. After 1 h, MeI (1.4 mL, 21.6 mmol) was added dropwise at -78 ^{o}C to the reaction mixture and it was stirred for 16 h. The reaction mixture was quenched with sat. NH_4Cl solution and extracted with Et_2O. The extract was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (5% EtOAc/hexane) to afford **5-4** (716 mg, 66%) as a colourless oil.

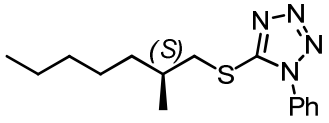
R_f = 0.35 (TLC, 10% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.28-7.20 (5H, m), 4.71-4.65 (1H, m), 4.23-4.15 (2H, m), 3.74-3.67 (1H, m), 3.26 (1H, dd, *J* = 13.2, 3.0 Hz), 2.80-2.72 (1H, m), 1.78-1.71 (1H, m), 1.42-1.21 (10H, m), 0.88 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 177.36, 153.06, 135.35, 129.44, 128.92, 127.32, 65.99, 55.35, 37.92, 37.70, 33.37, 31.81, 29.57, 26.91, 22.51, 17.34, 14.00.



5-5

Preparation of compound 5-5: To a solution of compound **5-4** (716 mg, 2.36 mmol) in dry Et_2O (10 mL) was added $LiBH_4$ (155 mg, 7.08 mmol) at 0 ^{o}C and the reaction mixture was allowed to stir for 30 minute at rt. MeOH was added slowly to quench and after getting the clear solution, the solvent was evaporated and the residue was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (10% EtOAc/hexane) to afford **5-5** (215mg, 70%) as a colourless oil.

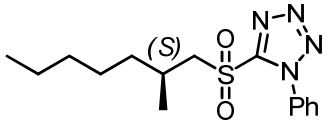
R_f = 0.35 (TLC, 20% EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.51-3.45 (1H, m), 3.41-3.35 (1H, m), 1.76 (1H, s), 1.61-1.55 (1H, m), 1.39-1.23 (7H, m), 1.11-1.05 (1H, m), 0.90-0.84 (6H, m); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 68.39, 35.74, 33.09, 32.12, 26.62, 22.62, 16.55, 14.05.



5-14

Preparation of compound 5-14: To a solution of compound **5-5** (1.0 g, 7.7 mmol) in dry THF (80 mL) were added PPh_3 (2.83 g, 10.77 mmol), 1-phenyl-1H-tetrazole-5-thiol (1.9 g, 10.77 mmol) and DIAD (2.16 mL, 10.77 mmol) at 0 ^{o}C and the reaction mixture was allowed to stir for 8 h at rt. THF was concentrated and directly transferred into a silica gel column and purified with 5% EtOAc/hexane to obtain **5-14** (1.6 g, 76%) as a colourless oil**.**

R_f = 0.6 (TLC, 15% EtOAc/hexane); $[\alpha]_D^{25}$ = +1.4 (*c* = 1.84, $CHCl_3$); IR (thin film): ν_{max} = 2957, 2927, 2856, 1600, 1500, 1386, 1240, 760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.54-7.51 (4H, m), 5.24-5.21 (1H, m), 3.47-3.43 (1H, dd, *J* = 12.6, 5.7 Hz), 3.27-3.23 (1H, m), 1.93-1.90 (1H, m), 1.49-1.34 (2H, m), 1.31-1.22 (7H, m), 1.03-1.01 (3H, d, *J* = 6.3 Hz), 0.87 (3H, t, *J* = 6.95 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 154.72, 133.77, 130.00, 129.71, 123.85, 74.29, 40.53, 35.84, 32.91, 31.86, 26.42, 22.52, 21.54, 19.05, 13.98; HRMS (ESI): *m/z* calcd for $C_{15}H_{22}N_4SNa^+$ $[M+Na]^+$ 313.1457, found 313.1455.

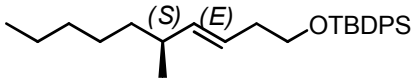


5-15

Preparation of compound 5-15: To a solution of compound **5-14** (1.02 g, 3.52 mmol) in absolute EtOH (60 mL) was added $(NH_4)_6Mo_7O_{24}.4H_2O$ (870 mg, 0.7 mmol)

dissolved in 19 mL H_2O_2 at 0 ^oC and the reaction mixture was allowed to stir for 8 h at rt. The solvent was evaporated and then the residue was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (7.5% EtOAc/hexane) to obtain **5-15** (1.1 g, 98%) as a colourless oil..

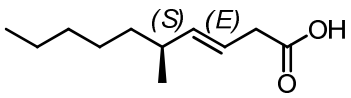
R_f = 0.38 (TLC, 15% EtOAc/hexane); $[\alpha]_D^{25}$ = -8.3 (*c* = 1.75, $CHCl_3$); IR (thin film): ν_{max} = 2957, 2930, 2859, 1497, 1339, 1153, 762 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.70-7.66 (2H, m), 7.62-7.56 (3H, m), 3.84-3.77 (1H, dd, *J* = 14.5, 4.9 Hz), 3.61-3.54 (1H, dd, *J* = 14.5, 8.07 Hz), 2.36-2.30 (1H, m), 1.57-1.50 (1H, m), 1.38-1.28 (7H, m), 1.15 (3H, d, *J* = 6.8 Hz), 0.87 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 154.08, 133.07, 131.40, 129.63, 125.13, 61.84, 36.49, 31.57, 28.22, 25.93, 22.46, 19.68, 13.95; HRMS (ESI): *m/z* calcd for $C_{15}H_{22}O_2N_4SNa^+$ $[M+Na]^+$ 345.1356, found 345.1348.



5-18

Preparation of compound 5-18: To a solution of compound **5-15** (200 mg, 0.62 mmol) in dry DME (4 mL) was added KHMDS (1.4 mL, 0.5M in toluene, 0.7 mmol) drop wise at -60 ^oC and the reaction mixture was stirred at that temperature for 1 h. 3-(tert-butyldiphenylsilyloxy)propanal (290 mg, 0.92 mmol) in DME (4 mL) was added slowly at -60 ^oC and the reaction mixture was allowed to stir for 12 h. To this reaction mixture 2 mL H_2O was added and it was vigorously stirred for 1 h. The reaction mixture was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (1.5% EtOAc/hexane) to obtain **5-18** (89 mg, 35%) as a colourless oil.

R_f = 0.8 (TLC, 5% EtOAc/hexane); $[\alpha]_D^{25}$ = +13.5 (*c* = 0.6, $CHCl_3$); IR (thin film): ν_{max} = 2957, 2929, 2857, 1738, 1472, 1429, 1112, 969, 823, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.68 (4H, d, *J* = 5.8 Hz), 7.42-7.35 (6H, m), 5.37-5.34 (2H, m), 3.68 (2H, t, *J* = 6.6 Hz), 2.29-2.23 (2H, m), 2.05 (1H, m), 1.44-1.25 (10H, m), 1.05 (9H, s), 0.97 (3H, d, *J* = 8.0 Hz), 0.87 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 138.66, 135.60, 134.13, 129.48, 127.55, 124.51, 64.12, 37.07, 36.73, 36.08, 32.02, 26.96, 26.85, 22.63, 20.68, 19.23, 14.10; GCMS: [M-^{t}Bu] 351.2.



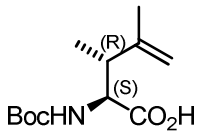
5-20

Preparation of compound 5-20: To a solution of compound **5-18** (89 mg, 0.22 mmol) in dry THF (2 mL) was added TBAF (335 μL, 0.33 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 2 h at rt. The reaction was quenched with sat. NH_4Cl solution and THF was evaporated and the residue was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (10% EtOAc/hexane) to get 26 mg (70%) of the corresponding alcohol. To a solution of CrO_3 (100 mg, 1.0 mmol) in 1 mL 3M H_2SO_4 was added the resulting alcohol (obtained from first step) in 2 mL acetone at 0 ^oC and the reaction mixture was allowed to stir for 3 h at rt. The reaction mixture was quenched with EtOH and filtered through filter paper. The filtrate was concentrated and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (25% EtOAc/hexane) to afford **5-20** (20 mg, 72%) as colourless oil.

R_f = 0.3 (TLC, 50% EtOAc/hexane); $[\alpha]_D^{25}$ = +14.0 (*c* = 2.0, $CHCl_3$); IR (thin film): ν_{max} = 2957, 2927, 2855, 1713, 1703, 1462, 1248, 969, 805, 725 cm^{-1}; ^1H NMR

($CDCl_3$, 500 MHz): δ 5.48-5.46 (2H, m), 3.07 (2H, d, *J* = 5.0 Hz), 1.29 (8H, br), 0.96 (3H, d, *J* = 8.0 Hz), 0.86 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 176.66, 141.34, 118.95, 37.55, 36.77, 36.61, 31.93, 26.86, 22.61, 20.32, 14.05; HRMS (ESI): *m/z* calcd for $C_{11}H_{19}O_2$ $[M-H]^-$ 183.1391, found 183.1389.

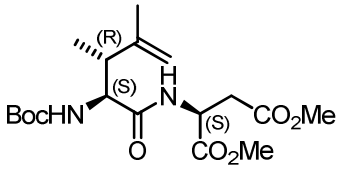
Chapter 6:



6-1

Preparation of compound 6-1: To a solution of compound **3-35** (110 mg, 0.41 mmol) in THF/H_2O/MeOH (2 mL/2 mL/0.4 mL) was added LiOH (11 mg, 0.45 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 2 h at that temperature. After completion of the reaction (checked by TLC), the reaction mixture was extracted with Et_2O. The aqueous layer was acidified with 10% $NaHSO_4$ to pH 2-3 and then extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford **6-1** (102 mg, 85%) as colourless oil.

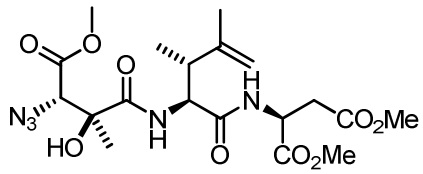
R_f = 0.2 (TLC, 5% CH_3OH/CH_2Cl_2); $[\alpha]_D^{25}$ = +17.8 (*c* = 0.64, $CHCl_3$); IR (thin film): ν_{max} = 3324, 2977, 2363, 1734, 1507, 1163 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 4.98 (1H, br), 4.81 (2H, d, *J* = 35.0 Hz), 4.45 (1H, s), 2.65 (1H, br), 1.78 (3H, s), 1.42 (9H, s), 1.06 (3H, d, *J* = 6.95 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 176.91, 155.67, 145.31, 112.52, 80.07, 55.99, 42.94, 28.24, 20.57, 13.99; HRMS (ESI): *m/z* calcd for $C_{12}H_{20}O_4N$ $[M-H]^-$ 242.1398, found 242.1398.



6-3

Preparation of compound 6-3: To a solution of compound **6-1** (60 mg, 0.25 mmol) in dry CH_2Cl_2 (2 mL) were added DIPEA (87 μL, 0.5 mmol), dimethylasparate hydrochloride salt (73 mg, 0.37 mmol), HATU (113 mg, 0.3 mmol) and HOAt (3.5 mg, 0.03 mmol) at -10 ^oC and the reaction mixture was allowed to stir for 12 h at rt. The reaction was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (35% EtOAc/hexane) to afford **6-3** (77 mg, 82%) as a colourless oil.

R_f = 0.3 (TLC, 40% EtOAc/hexane); $[\alpha]_D^{25}$ = +22.4 (c = 0.8, $CHCl_3$); IR (thin film): ν_{max} = 3317, 3256, 3077, 2979, 1747, 1682, 1655, 1652, 1557, 1519, 1434, 1367, 1303, 1168, 1013 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 6.98 (1H, br), 4.87 (2H, s), 4.77 (2H, s), 4.20 (1H, br), 3.74 (3H, s), 3.67 (3H, s), 3.00 (1H, dd, J = 17.6, 4.4 Hz), 2.86 (1H, dd, J = 17.6, 4.4 Hz), 2.68 (1H, t, J = 6.9), 1.77 (3H, s), 1.42 (9H, s), 0.99 (3H, d, J = 6.9); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.21, 170.72, 155.63, 145.96, 112.34, 80.13, 57.04, 52.66, 51.91, 48.68, 42.39, 35.98, 28.19, 21.08, 13.84; HRMS (ESI): *m/z* calcd for $C_{18}H_{30}O_7N_2Na^+$ $[M+Na]^+$ 409.1945, found 409.1953.

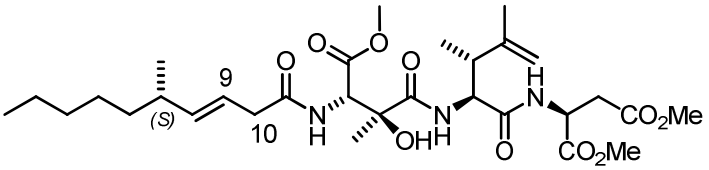


6-15

Preparation of compound 6-15: To a solution of compound **6-3** (30 mg, 0.08 mmol) in dry CH_2Cl_2 (1 mL) was added TFA (500 μL) at 0 ^oC and the reaction mixture was allowed to stir for 2 h at rt. CH_2Cl_2 was concentrated under reduced pressure to get the dry TFA salt which was dissolved in dry CH_2Cl_2 (1 mL). To this solution were added DIPEA (34 μL, 0.2 mmol), compound **4-109** (16 mg, 0.08 mmol)

in CH_2Cl_2 (1 mL), HATU (60 mg, 0.16 mmol) and HOAt (5 mg, 0.04 mmol) at -10 ^oC and the reaction mixture was allowed to stir for 12 h at rt. The reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (65% EtOAc/hexane) to afford **6-15** (18 mg, 50% over two steps) as a white solid.

R_f = 0.3 (TLC, 50% EtOAc/hexane); $[\alpha]_D^{25}$ = -34.7 (*c* = 1.0, $CHCl_3$); IR (thin film): ν_{max} = 3305, 2106, 1744, 1738, 1652, 1544, 1438, 1299, 1133, 1002, 912, 856, 732 cm^{-1}; H NMR ($CDCl_3$, 500 MHz): δ 7.19 (1H, d, *J* = 8.8 Hz), 6.99 (1H, d, *J* = 7.6 Hz), 4.91 (1H, s), 4.84 (1H, s), 4.80-4.78 (1H, m), 4.57-4.54 (1H, m), 4.23 (1H, s), 3.90 (1H, s), 3.85 (3H, s), 3.75 (3H, s), 3.69 (3H, s), 2.98 (1H, dd, *J* = 17.3, 4.4 Hz), 2.88 (1H, dd, *J* = 17.3, 4.4 Hz), 2.80-2.77 (1H, m), 1.8 (3H, s), 1.48 (3H, s), 1.08 (3H, d, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.62, 171.24, 170.67, 170.46, 169.28, 145.60, 112.59, 65.84, 55.53, 52.90, 52.76, 52.03, 48.71, 42.14, 38.58, 35.85, 29.66, 23.92, 20.86, 14.11; HRMS (ESI): *m/z* calcd for $C_{19}H_{29}O_9N_5Na^+$ $[M+Na]^+$ 494.1857, found 494.1851.

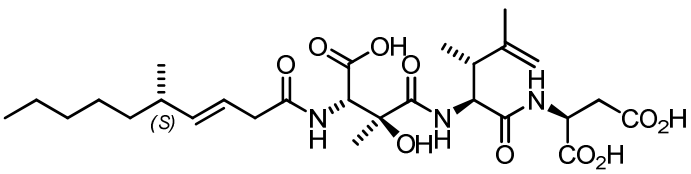


6-16

Preparation of compound 6-16: To a solution of compound **6-15** (20 mg, 0.04 mmol) in THF/H_2O (3 mL/150 μL) was added Me_3P (300 μL, 1.0M in THF) at 0 ^oC and the reaction mixture was allowed to stir for 1 h. After complete consumption of the starting material, THF was removed *in vacuo* and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced

pressure to get the crude product which was purified over silica gel (5% MeOH/CH_2Cl_2) to afford 13 mg (0.03 mmol) of corresponding amine which was dissolved in dry CH_2Cl_2 (3 mL). To this solution were added DIPEA (15 μL, 0.09 mmol), compound **5-20** (8 mg, 0.04 mmol) in CH_2Cl_2 (2 mL), HATU (35 mg, 0.09 mmol) and HOAt (2 mg, 0.02 mmol) at -10 ^oC and the reaction mixture was allowed to stir for 12 h at rt. The reaction was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified over silica gel (50% EtOAc/hexane) to obtain **6-16** (10 mg, 40% over two steps) as a colourless oil.

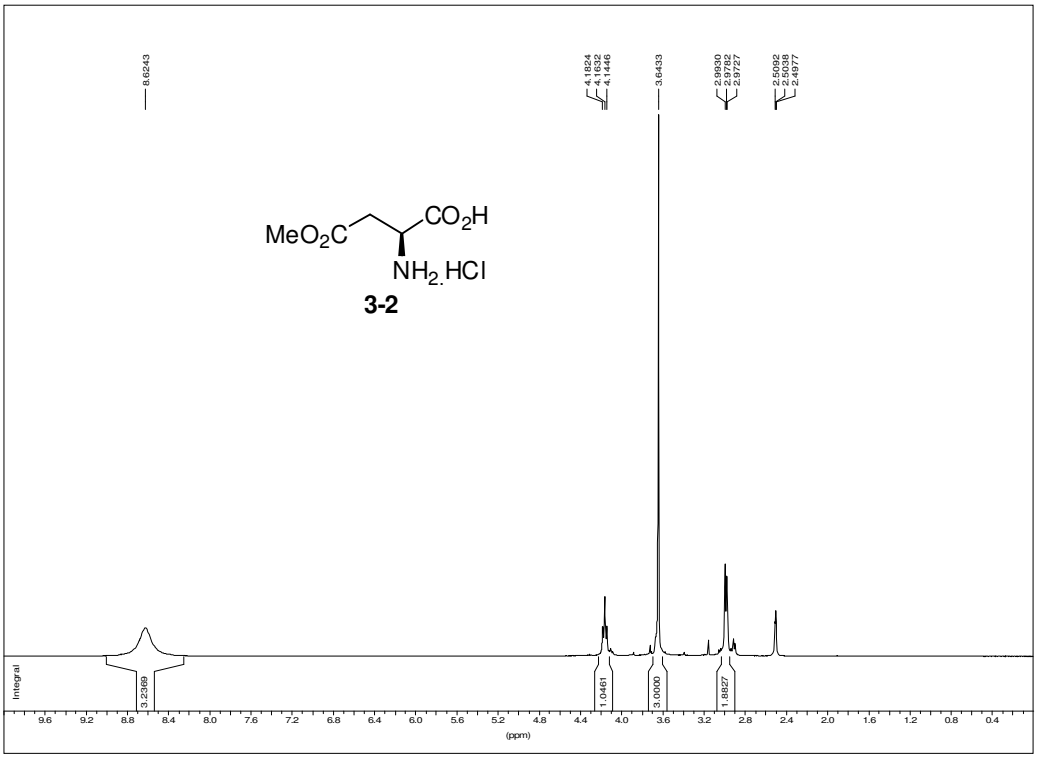
R_f = 0.38 (TLC, 40% EtOAc/hexane); $[\alpha]_D^{25}$ = +18.8 (*c* = 0. 25, $CHCl_3$); IR (thin film): ν_{max} = 3289, 2957, 2921, 2850, 1748, 1729, 1654, 1647, 1527, 1287, 1210, 1107 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.13 (1H, d, *J* = 8.8 Hz), 7.08 (1H, d, *J* = 8.2 Hz), 6.88 (1H, d, *J* = 8.2 Hz), 5.55 (2H, m), 4.9 (1H, s), 4.84 (3H, m), 4.77 (1H, m), 4.43 (1H, m), 3.75 (3H, s), 3.72 (3H, s), 3.68 (3H, s), 3.0 (3H, m), 2.86 (1H, dd, *J* = 17.3, 4.4 Hz), 2.75 (1H, t, *J* = 7 Hz), 2.16-2.13 (1H, m), 1.76 (3H, s), 1.46 (3H, s), 1.29 (8H, br), 1.04 (3H, d, *J* = 6.95 Hz), 0.97 (3H, d, *J* = 7.0 Hz), 0.87 (3H, t, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.86, 172.74, 171.75, 171.29, 170.65, 170.03, 145.59, 142.48, 119.93, 112.68, 76.44, 57.56, 55.19, 52.92, 52.80, 52.04, 48.66, 42.16, 40.19, 36.77, 36.73, 35.88, 31.97, 29.69, 26.92, 23.97, 22.60, 20.93, 20.40, 14.15, 14.08; HRMS (ESI): *m/z* calcd for $C_{30}H_{49}O_{10}N_3Na^+$ $[M+Na]^+$ 634.3310, found 634.3291.



6-18

Preparation of compound 6-18: To a solution of **6-16** (3 mg, 0.005 mmol) in $ClCH_2CH_2Cl$ (2 mL) was added Me_3SnOH (27 mg, 0.15 mmol) and the reaction mixture was heated at 80 ^oC for 48 h. ESI mass was checked for the crude reaction mixture and found diacid as major product. The solvent was evaporated and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was dissolved in THF/H_2O/MeOH (1 mL/1 mL/0.2 mL). To this solution was added LiOH (10 mg, 0.42 mmol) at 0 ^oC and the reaction mixture was allowed to stir for 12 h at rt. The reaction was acidified with 3M HCl and extracted with EtOAc The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to get the crude product which was purified by prep. HPLC (30-100% ACN/H_2O) to afford **6-18** (1.3 mg, 46%) as colourless oil.

R_f = 0.1 (TLC, 20% CH_3OH/CH_2Cl_2); $[\alpha]_D^{25}$ = -20.0 (*c* = 0.1, CH_3OH); IR (thin film): ν_{max} = 2927, 2855, 1733, 1656, 1521, 1195 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz): δ 5.52-5.50 (2H, m), 4.61-4.60 (2H, m), 4.43 (1H, d, *J* = 6.9 Hz), 2.97 (2H, d, *J* = 6.9 Hz), 2.81 (2H, t, *J* = 7.0 Hz), 2.67-2.65 (2H, m), 2.13 (1H, m), 1.76 (3H, s), 1.44 (3H, s), 1.29 (8H, br), 1.07 (3H, d, *J* = 6.9 Hz), 0.97 (3H, d, *J* = 6.9 Hz), 0.89 (3H, t, *J* = 7.0 Hz); ^{13}C NMR (CD_3OD, 125 MHz): δ 176.35, 174.13, 173.95, 173.59, 173.28, 172.66, 147.01, 142.79, 121.74, 113.38, 76.51, 57.12, 50.33, 44.33, 40.63, 38.09, 38.00, 36.83, 33.15, 28.10, 24.45, 23.67, 20.95, 20.45, 15.55, 14.43; HRMS (ESI): *m/z* calcd for $C_{27}H_{42}O_{10}N_3[M-H]^-$ 568.2876, found 568.2868.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CO2H
NH2.HCl
3-2
Integral
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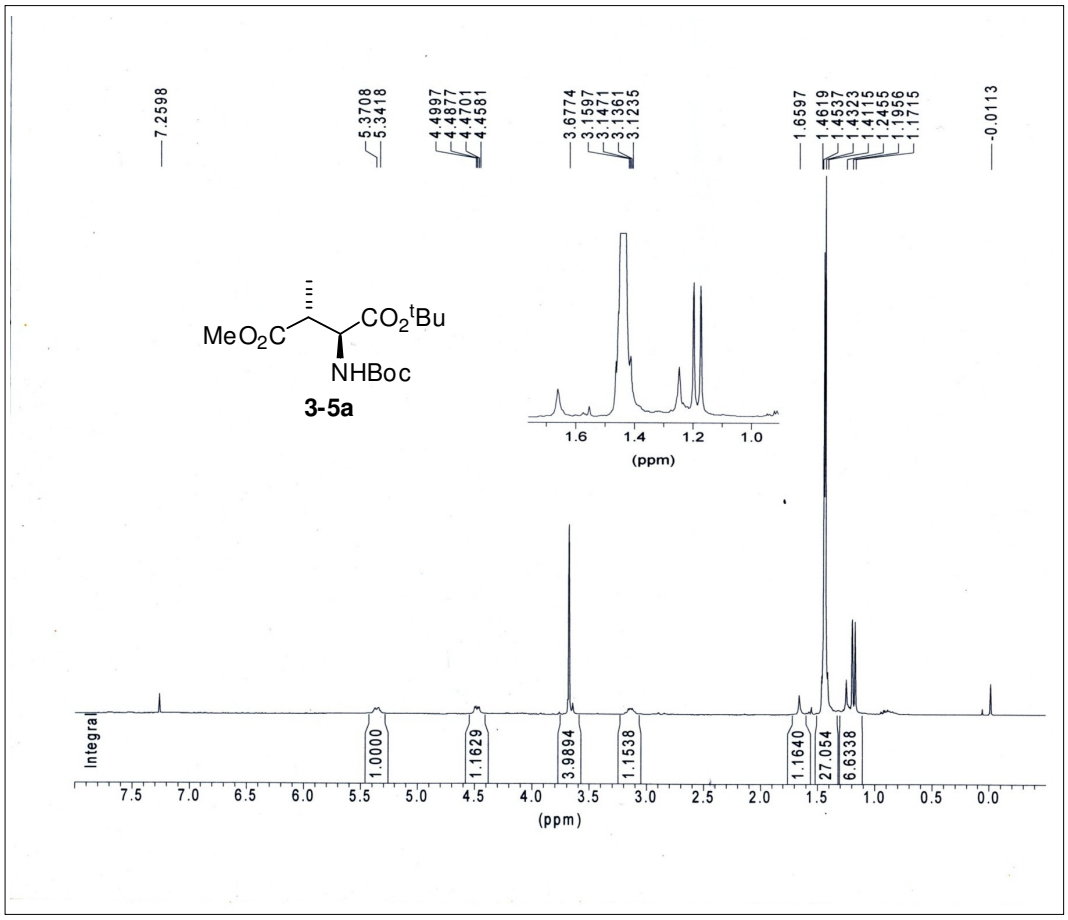
1.8827
(ppm)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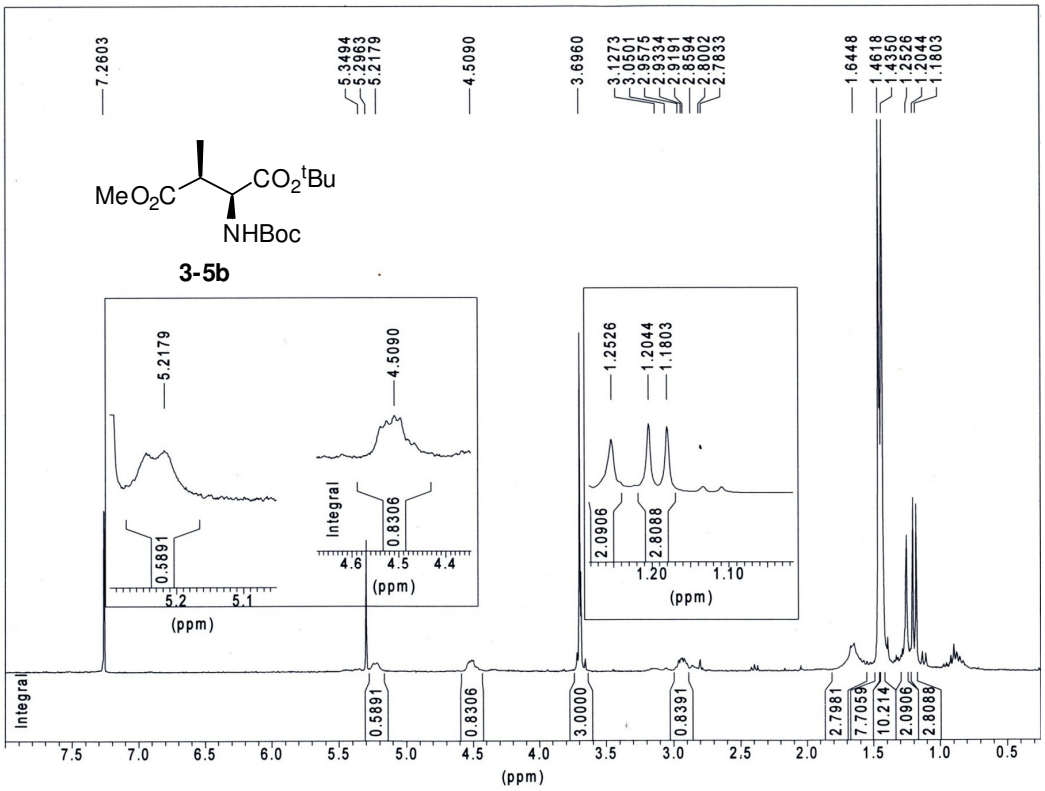
(ppm)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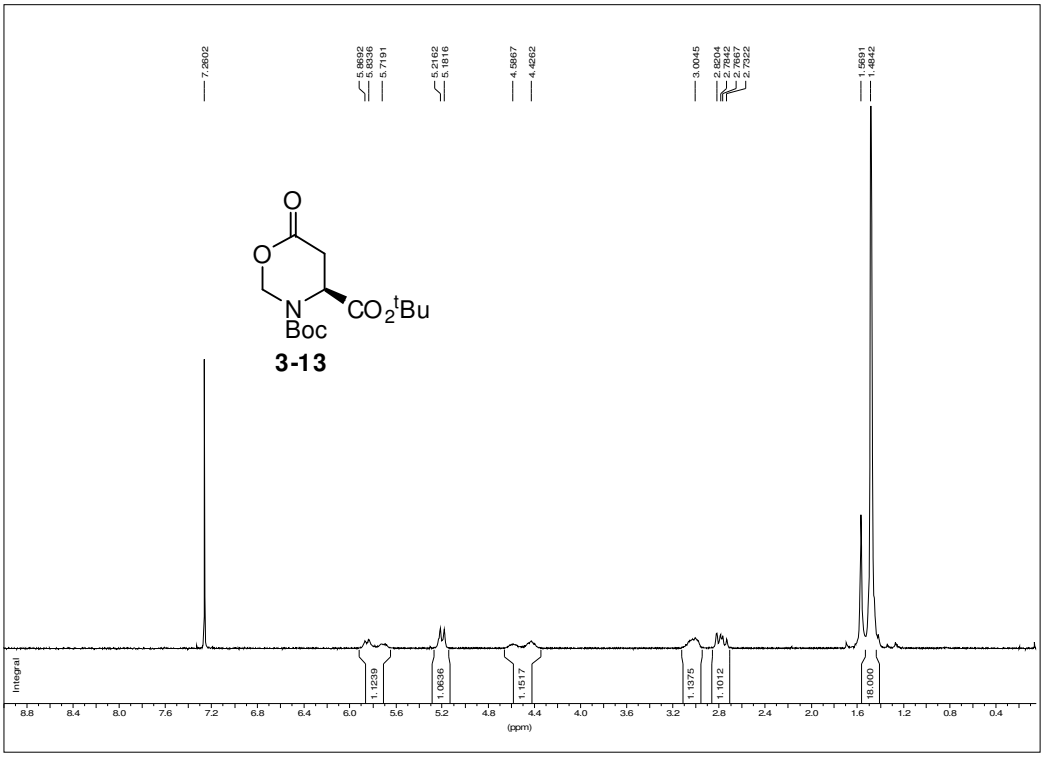
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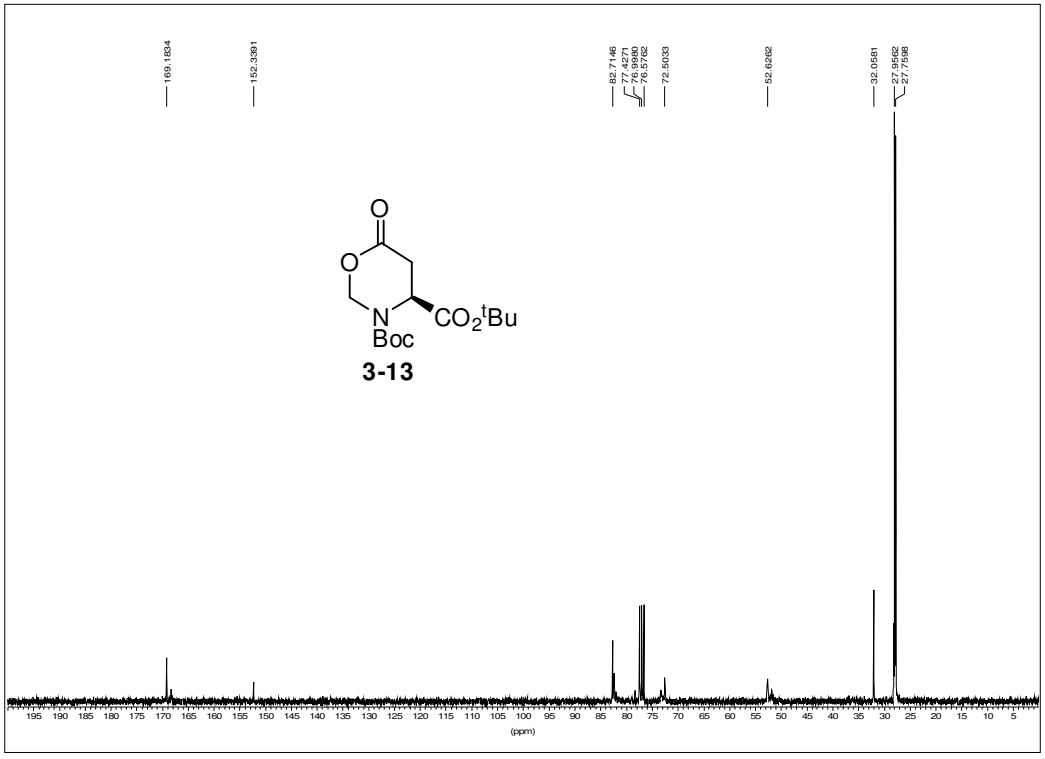
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N
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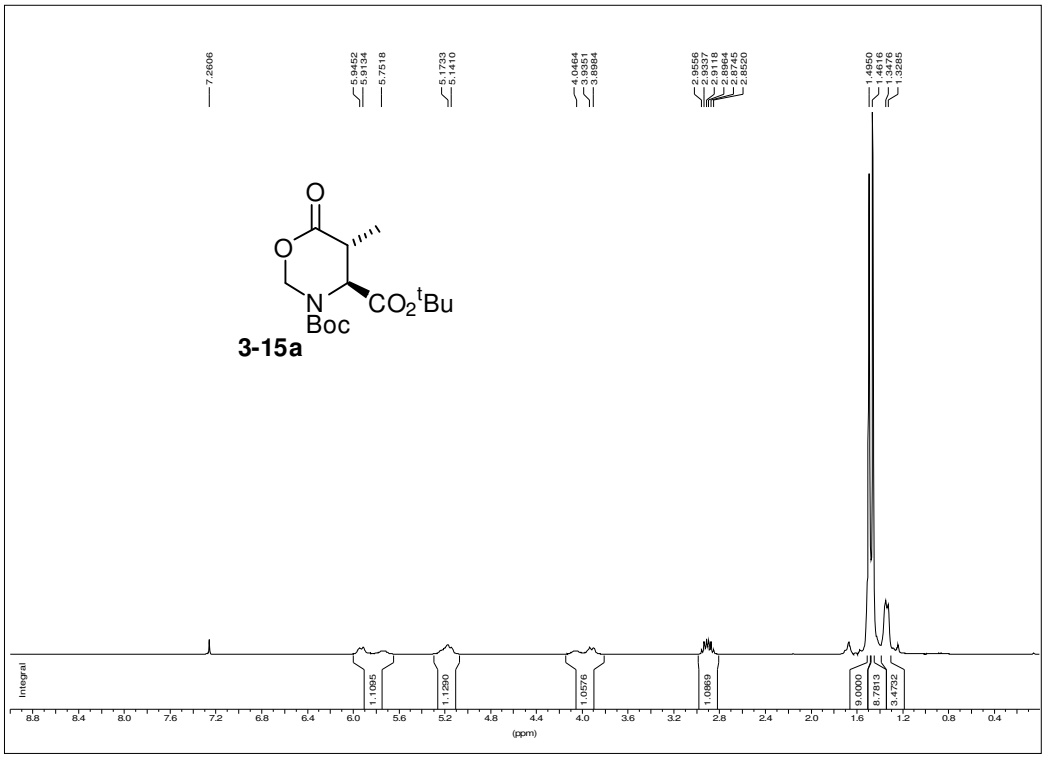
3-13
(ppm)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1.4959
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O
N
Boc
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Integral
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9.0000
8.7913
3.4752
(ppm)

Table 1. Crystal data and structure refinement for **3-15a**.

Identification code	7468	
Empirical formula	C15 H25 N O6	
Formula weight	315.36	
Temperature	223(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 6.4285(4) Å	α= 79.7320(10)°.
	b = 10.4987(6) Å	β= 77.9400(10)°.
	c = 13.6098(8) Å	γ = 74.7870(10)°.
Volume	859.34(9) $Å^3$	
Z	2	
Density (calculated)	1.219 Mg/m^3	
Absorption coefficient	0.094 mm^{-1}	
F(000)	340	
Crystal size	0.60 x 0.60 x 0.50 mm^3	
Theta range for data collection	2.03 to 27.47°.	
Index ranges	-8<=h<=8, -13<=k<=13, -17<=l<=17	
Reflections collected	11336	
Independent reflections	3928 [R(int) = 0.0232]	
Completeness to theta = 27.47°	99.7 %	
Absorption correction	Sadabs,(Sheldrick 2001)	
Max. and min. transmission	0.9546 and 0.9459	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3928 / 0 / 206	
Goodness-of-fit on F^2	1.079	
Final R indices [I>2sigma(I)]	R1 = 0.0573, wR2 = 0.1674	
R indices (all data)	R1 = 0.0675, wR2 = 0.1761	
Largest diff. peak and hole	0.483 and -0.342 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for 7468. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
N(1)	-1326(2)	3934(1)	2664(1)	48(1)
O(1)	-1061(2)	5634(1)	3506(1)	63(1)
O(2)	2055(2)	5486(1)	3989(1)	70(1)
O(3)	-698(2)	3105(1)	1223(1)	55(1)
O(4)	-3857(3)	4633(2)	1630(1)	86(1)
O(5)	2570(2)	840(1)	2782(1)	52(1)
O(6)	-1031(2)	1244(1)	3386(1)	68(1)
C(1)	-2486(3)	4852(2)	3365(2)	57(1)
C(2)	832(3)	4921(2)	3792(1)	51(1)
C(3)	1215(3)	3425(2)	3856(1)	45(1)
C(4)	744(2)	3061(1)	2884(1)	40(1)
C(5)	-2121(3)	3949(2)	1807(1)	54(1)
C(6)	-1270(3)	2775(2)	317(1)	58(1)
C(7)	-1654(6)	3991(3)	-460(2)	94(1)
C(8)	-3220(5)	2171(3)	633(2)	99(1)
C(9)	767(5)	1781(3)	-57(2)	93(1)
C(10)	613(2)	1605(2)	3047(1)	41(1)
C(11)	3021(3)	-637(2)	2982(1)	58(1)
C(12)	2387(5)	-1094(2)	4080(2)	90(1)
C(13)	5491(5)	-1004(3)	2714(4)	151(2)
C(14)	1878(8)	-1131(3)	2347(3)	128(1)
C(15)	3468(3)	2723(2)	4089(2)	71(1)

Table 3. Bond lengths [Å] and angles [°] for 7468.

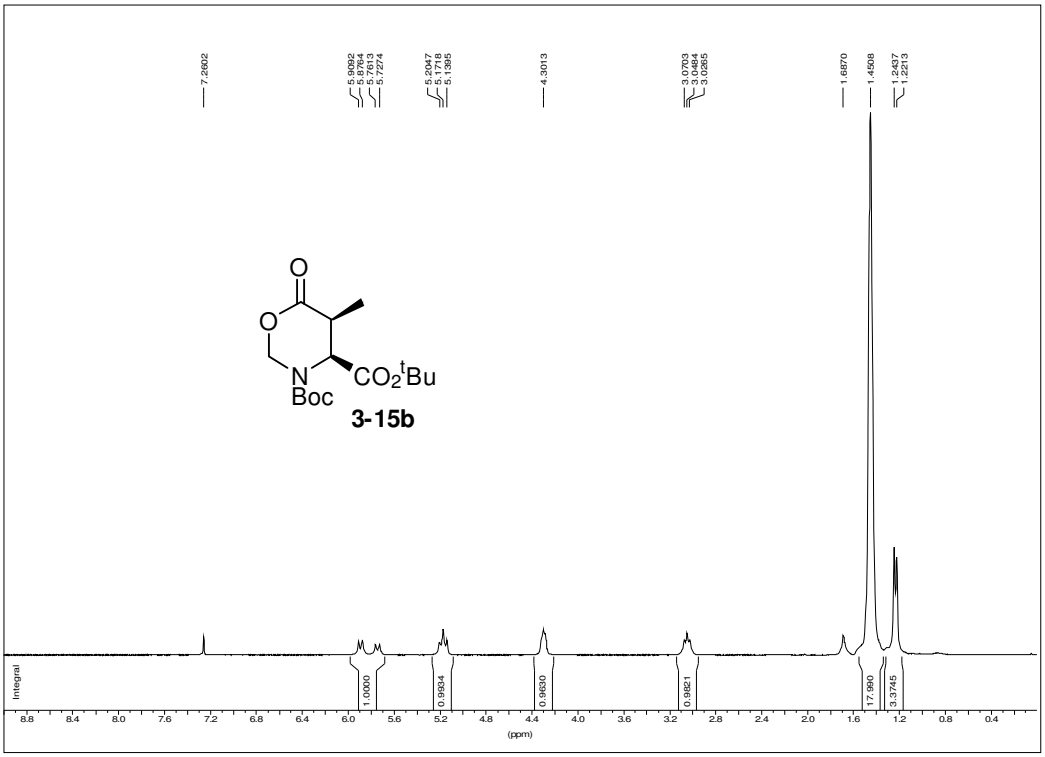
N(1)-C(5)	1.365(2)
N(1)-C(1)	1.431(2)
N(1)-C(4)	1.4591(18)
O(1)-C(2)	1.345(2)
O(1)-C(1)	1.440(2)
O(2)-C(2)	1.196(2)
O(3)-C(5)	1.332(2)
O(3)-C(6)	1.4777(19)
O(4)-C(5)	1.204(2)
O(5)-C(10)	1.3199(18)
O(5)-C(11)	1.4862(19)
O(6)-C(10)	1.1910(19)
C(2)-C(3)	1.513(2)
C(3)-C(15)	1.513(2)
C(3)-C(4)	1.548(2)
C(4)-C(10)	1.527(2)
C(6)-C(8)	1.501(3)
C(6)-C(9)	1.502(3)
C(6)-C(7)	1.507(3)
C(11)-C(14)	1.477(4)
C(11)-C(12)	1.491(3)
C(11)-C(13)	1.513(3)
C(5)-N(1)-C(1)	119.69(14)
C(5)-N(1)-C(4)	122.85(13)
C(1)-N(1)-C(4)	117.38(13)
C(2)-O(1)-C(1)	114.77(13)
C(5)-O(3)-C(6)	121.27(14)
C(10)-O(5)-C(11)	122.37(13)
N(1)-C(1)-O(1)	109.45(15)
O(2)-C(2)-O(1)	119.36(15)
O(2)-C(2)-C(3)	124.99(17)
O(1)-C(2)-C(3)	115.64(14)
C(15)-C(3)-C(2)	111.50(15)
C(15)-C(3)-C(4)	113.39(13)
C(2)-C(3)-C(4)	110.00(13)

N(1)-C(4)-C(10)	110.51(12)
N(1)-C(4)-C(3)	108.05(11)
C(10)-C(4)-C(3)	109.82(11)
O(4)-C(5)-O(3)	126.69(16)
O(4)-C(5)-N(1)	123.62(16)
O(3)-C(5)-N(1)	109.68(14)
O(3)-C(6)-C(8)	109.13(16)
O(3)-C(6)-C(9)	101.78(15)
C(8)-C(6)-C(9)	112.0(2)
O(3)-C(6)-C(7)	110.54(16)
C(8)-C(6)-C(7)	112.4(2)
C(9)-C(6)-C(7)	110.5(2)
O(6)-C(10)-O(5)	126.46(14)
O(6)-C(10)-C(4)	123.59(14)
O(5)-C(10)-C(4)	109.94(12)
C(14)-C(11)-O(5)	109.57(16)
C(14)-C(11)-C(12)	111.7(2)
O(5)-C(11)-C(12)	110.95(15)
C(14)-C(11)-C(13)	114.6(3)
O(5)-C(11)-C(13)	101.11(16)
C(12)-C(11)-C(13)	108.4(3)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for 7468. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
N(1)	52(1)	41(1)	48(1)	-12(1)	-16(1)	5(1)
O(1)	72(1)	40(1)	78(1)	-20(1)	-21(1)	-1(1)
O(2)	77(1)	61(1)	83(1)	-26(1)	-11(1)	-26(1)
O(3)	66(1)	56(1)	43(1)	-12(1)	-20(1)	-2(1)
O(4)	81(1)	89(1)	84(1)	-29(1)	-47(1)	26(1)
O(5)	50(1)	34(1)	63(1)	-7(1)	-1(1)	-2(1)
O(6)	48(1)	51(1)	100(1)	-5(1)	-4(1)	-12(1)
C(1)	53(1)	50(1)	67(1)	-24(1)	-16(1)	5(1)
C(2)	60(1)	45(1)	48(1)	-16(1)	-5(1)	-13(1)
C(3)	51(1)	42(1)	43(1)	-10(1)	-11(1)	-8(1)
C(4)	44(1)	36(1)	38(1)	-7(1)	-8(1)	-3(1)
C(5)	62(1)	48(1)	51(1)	-7(1)	-21(1)	0(1)
C(6)	74(1)	63(1)	44(1)	-10(1)	-19(1)	-18(1)
C(7)	144(2)	93(2)	57(1)	7(1)	-45(1)	-36(2)
C(8)	112(2)	124(2)	84(2)	-33(2)	-5(2)	-64(2)
C(9)	101(2)	110(2)	69(1)	-44(1)	-22(1)	1(2)
C(10)	45(1)	39(1)	39(1)	-7(1)	-10(1)	-5(1)
C(11)	70(1)	32(1)	63(1)	-6(1)	-3(1)	-1(1)
C(12)	138(2)	53(1)	68(1)	6(1)	-30(1)	-5(1)
C(13)	89(2)	54(1)	251(5)	1(2)	41(2)	17(1)
C(14)	242(4)	50(1)	115(2)	-20(1)	-95(3)	-15(2)
C(15)	70(1)	64(1)	89(1)	-26(1)	-41(1)	1(1)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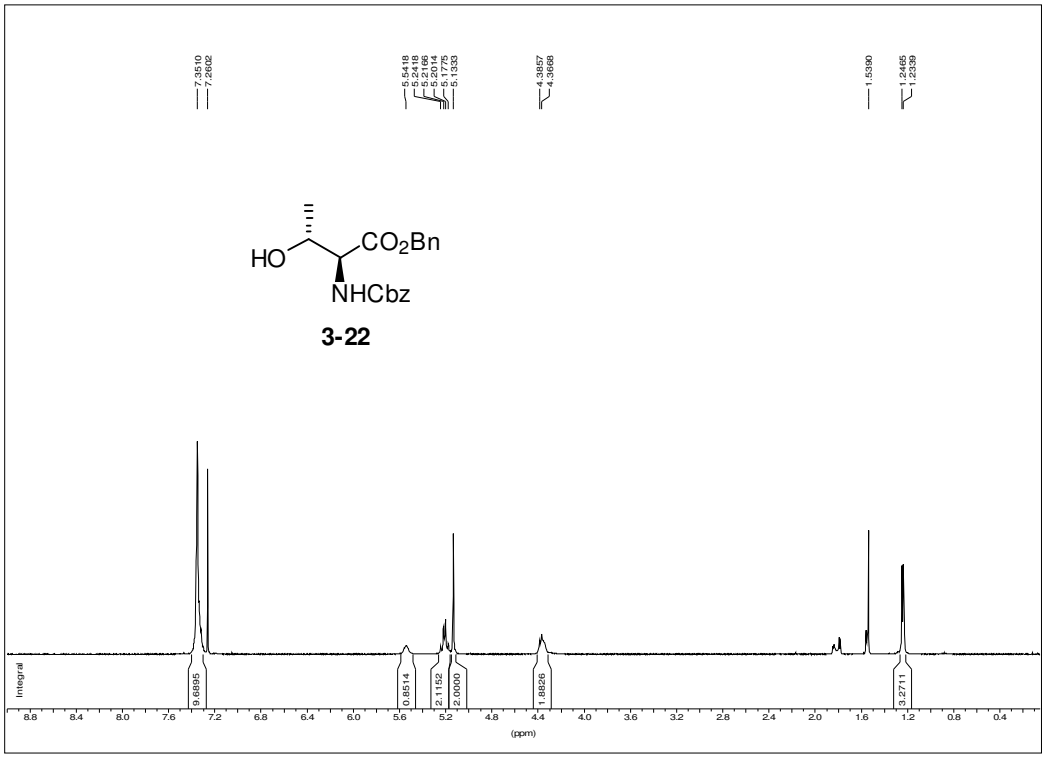
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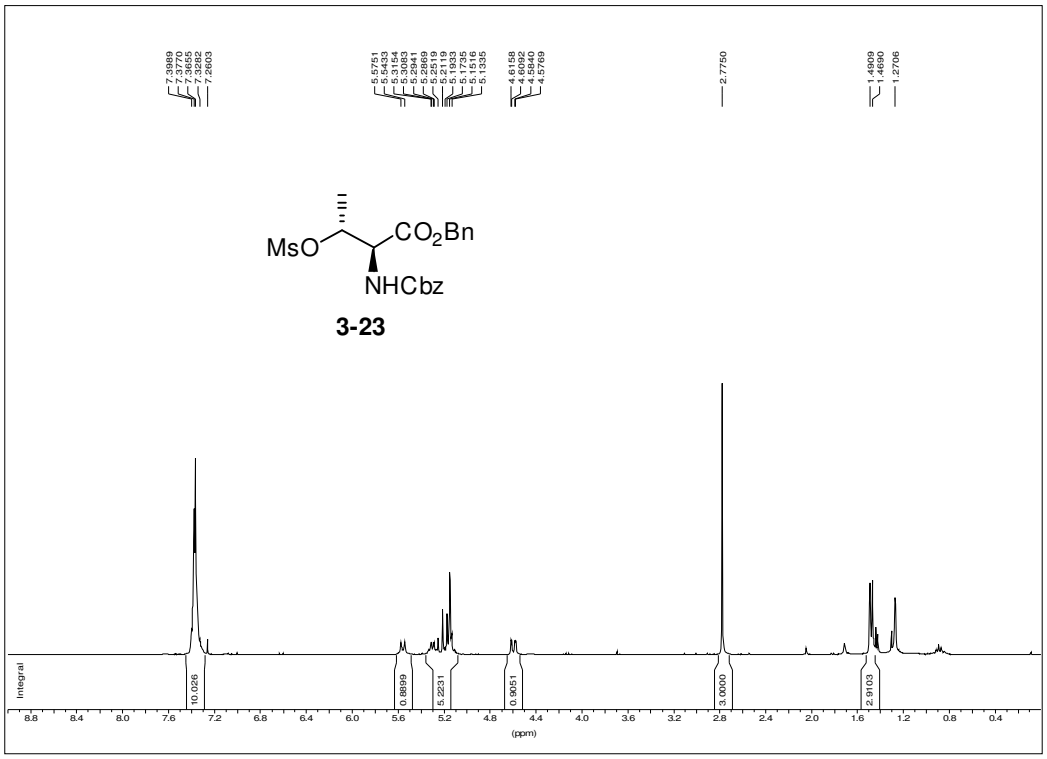
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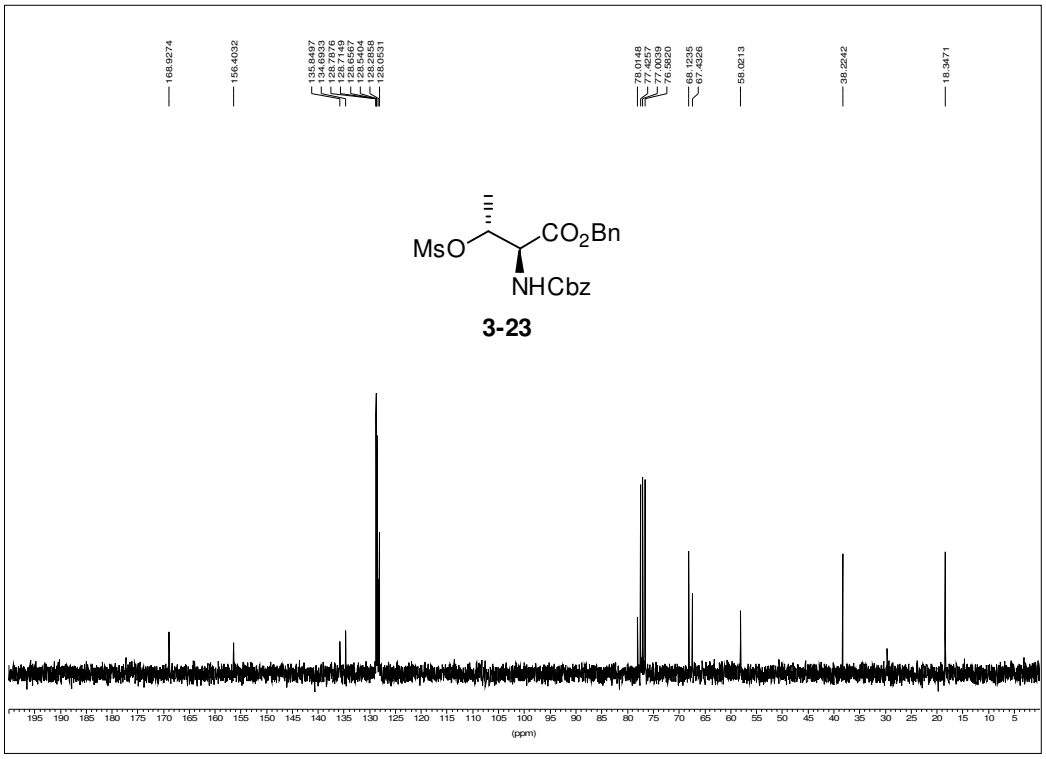
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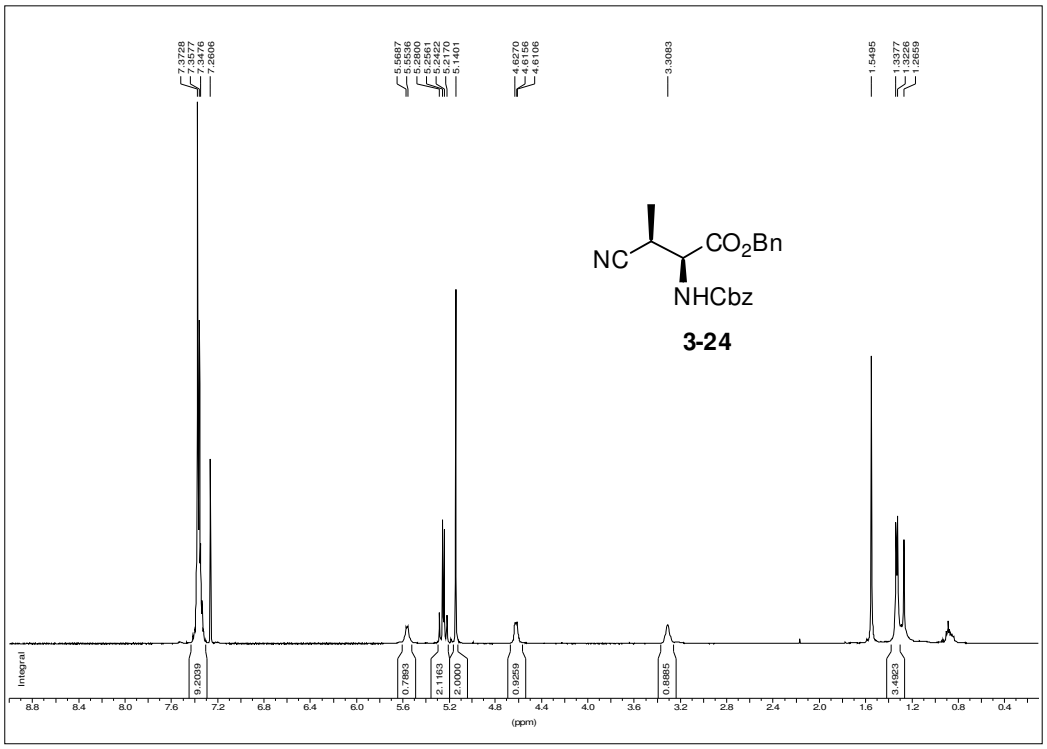
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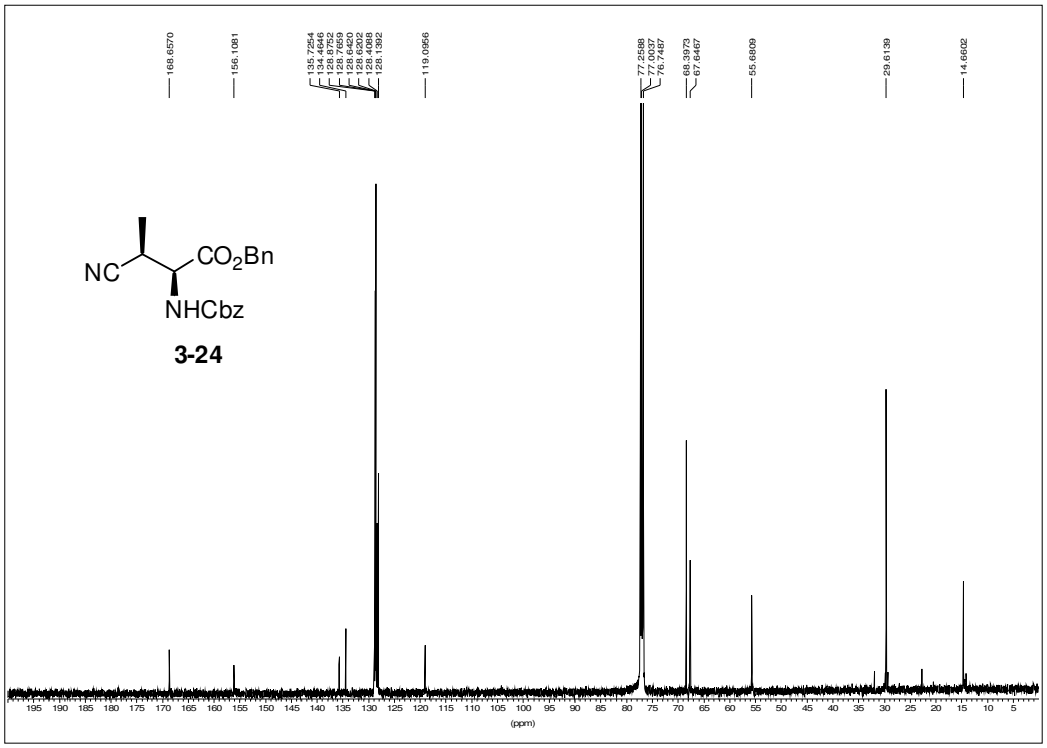
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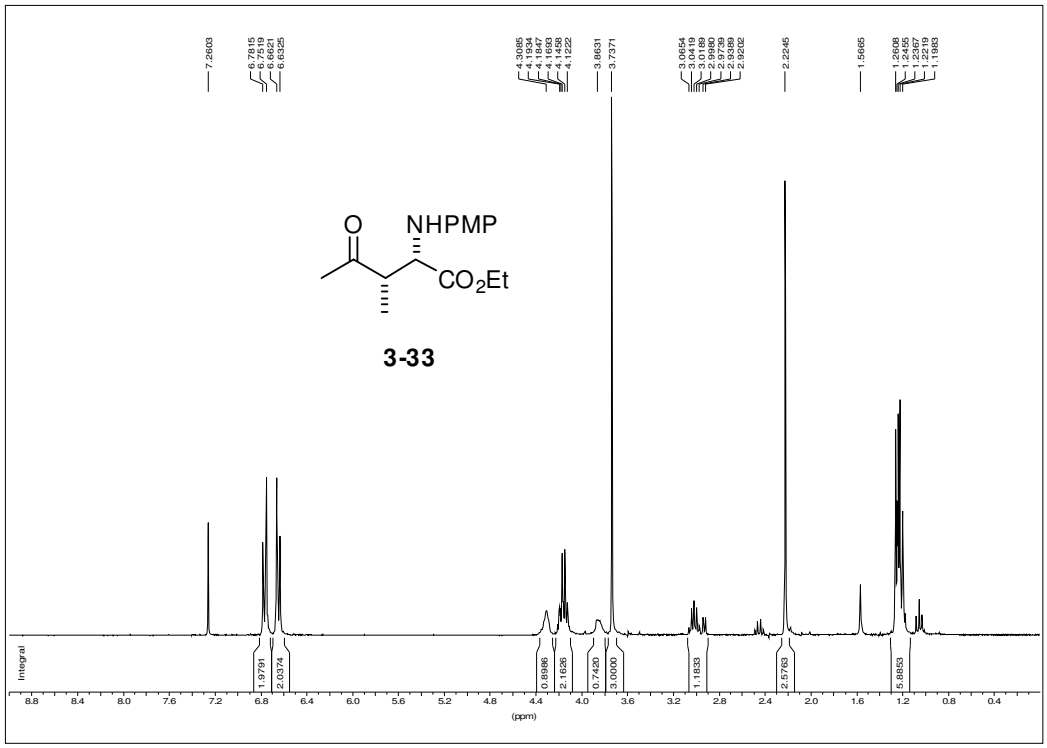
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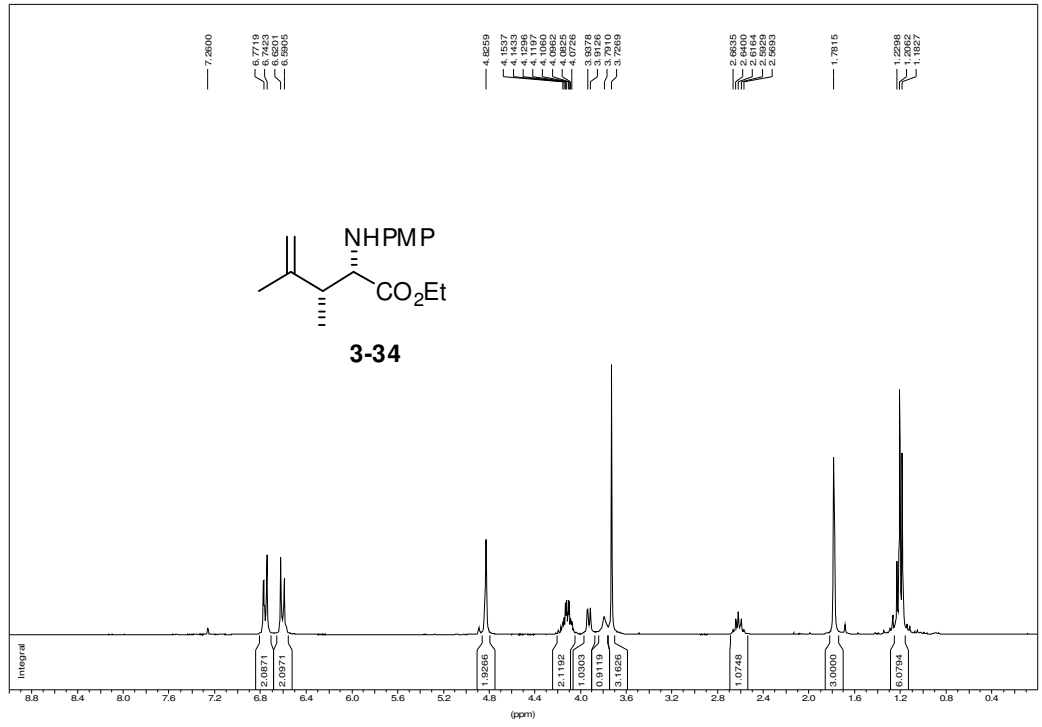
3-33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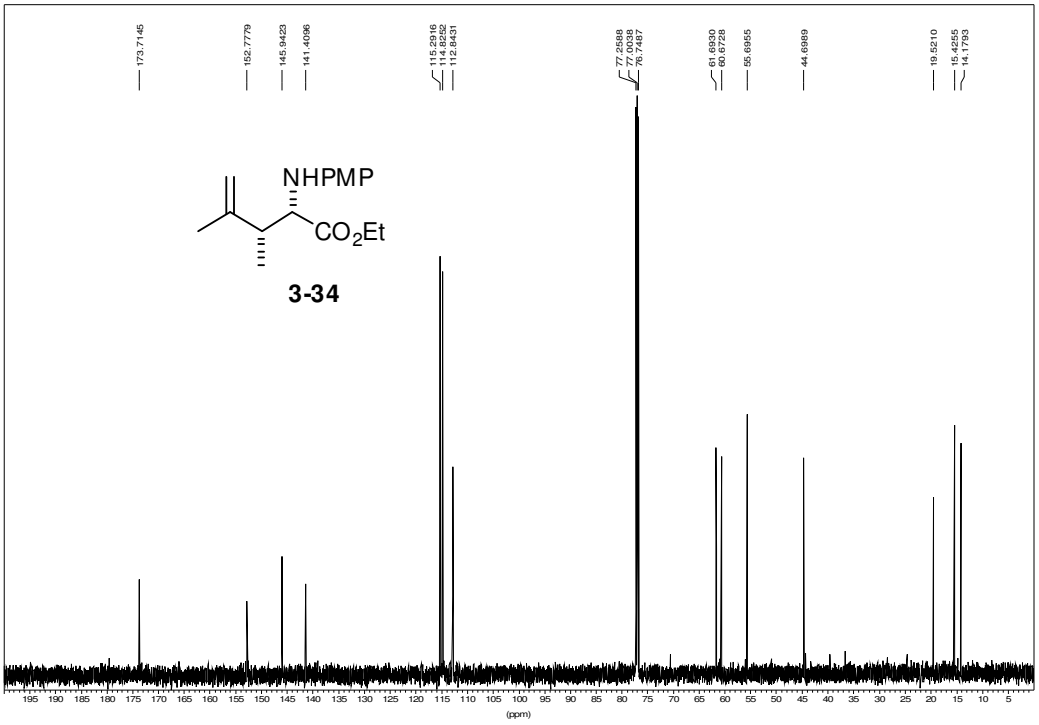
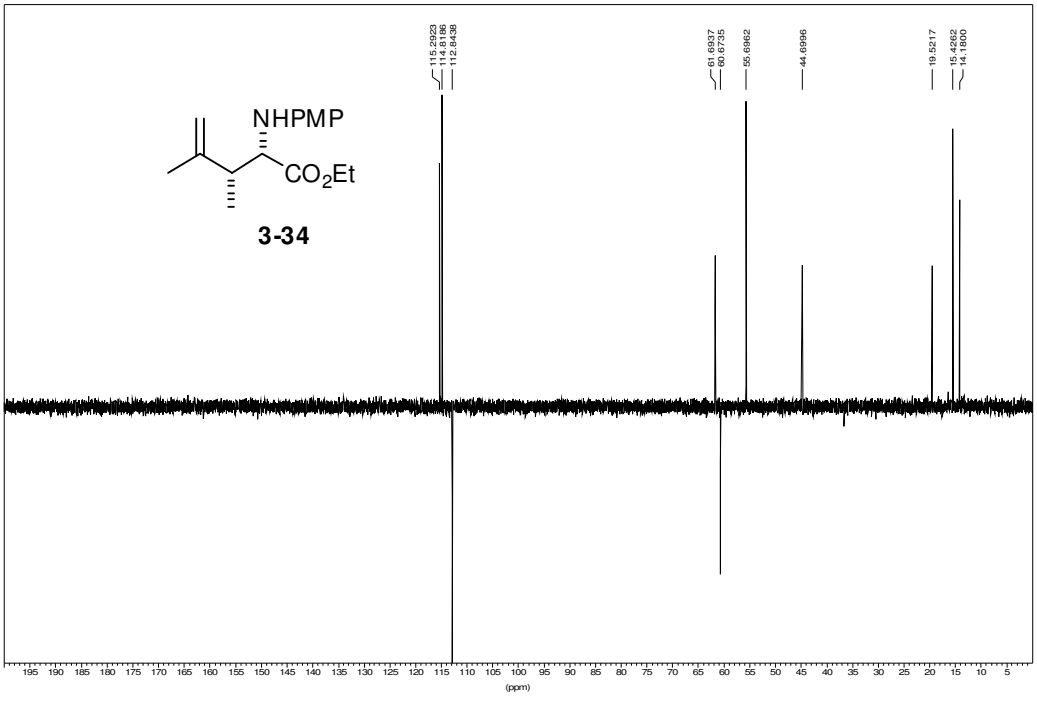
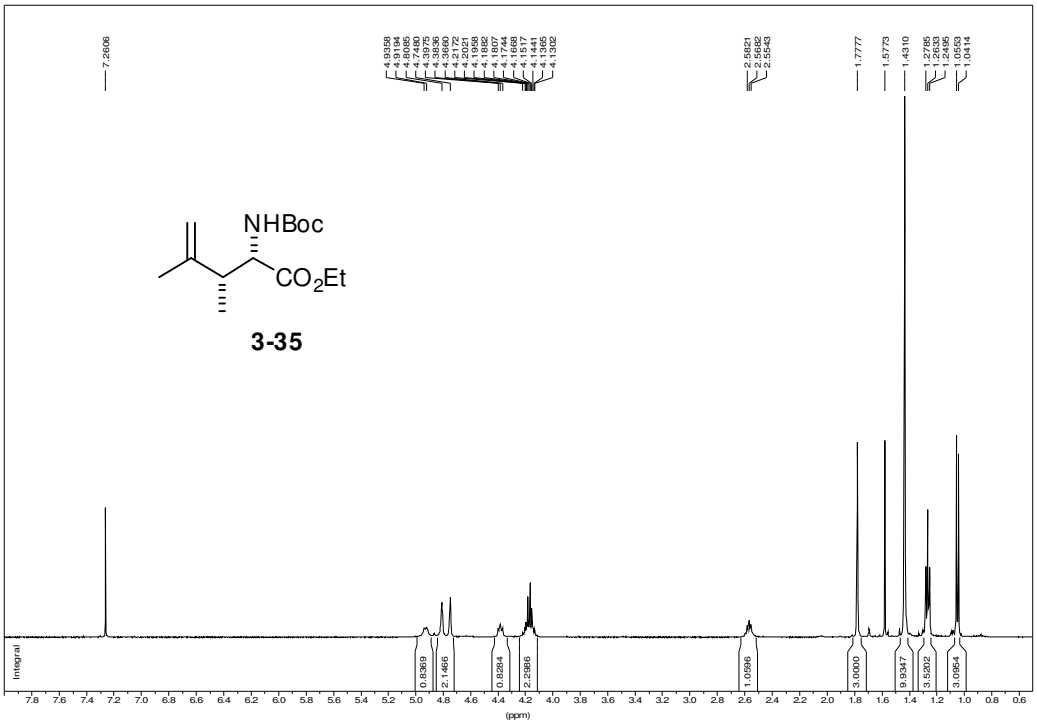
2.5929
2.5693
1.7815
1.2598
1.2452
1.1827
NHPMP
CO2Et
Integral
2.0871
2.0971
1.9266
2.1192
1.0303
0.9119
3.1626
1.0748
3.0000
6.0794
(ppm)

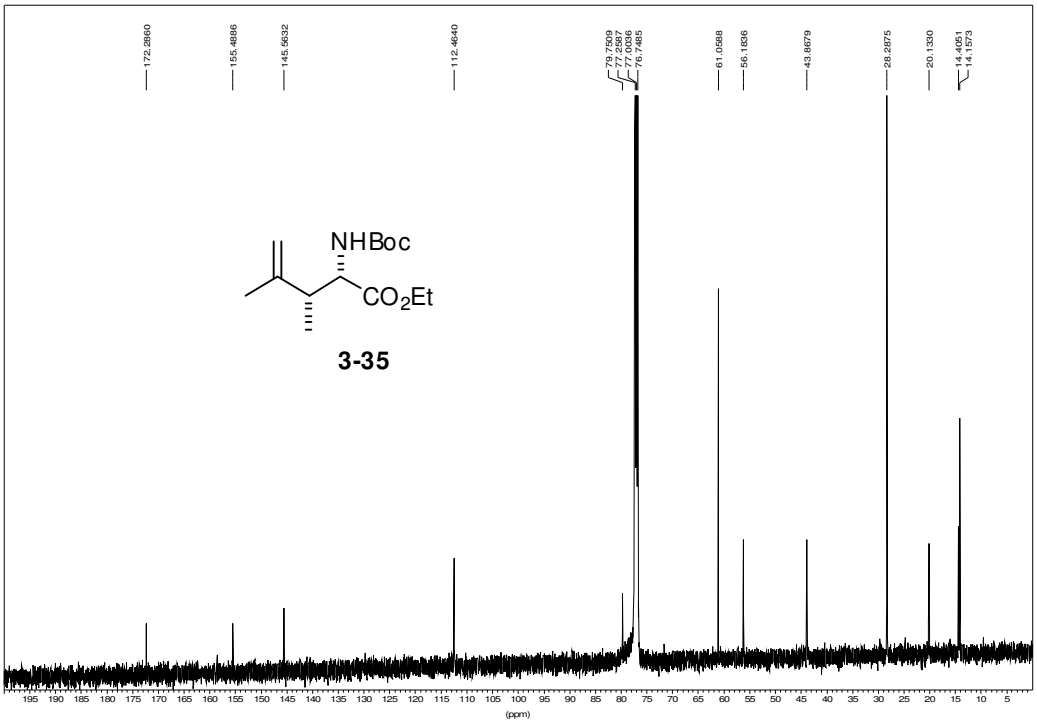
3-34



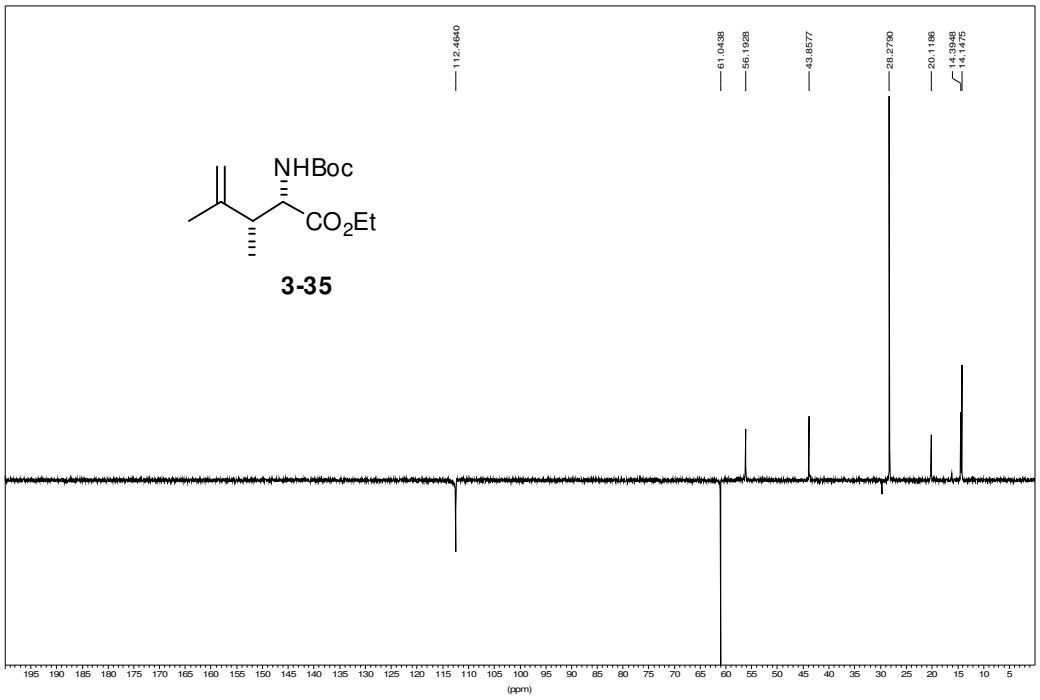
DEPT135 AMX500

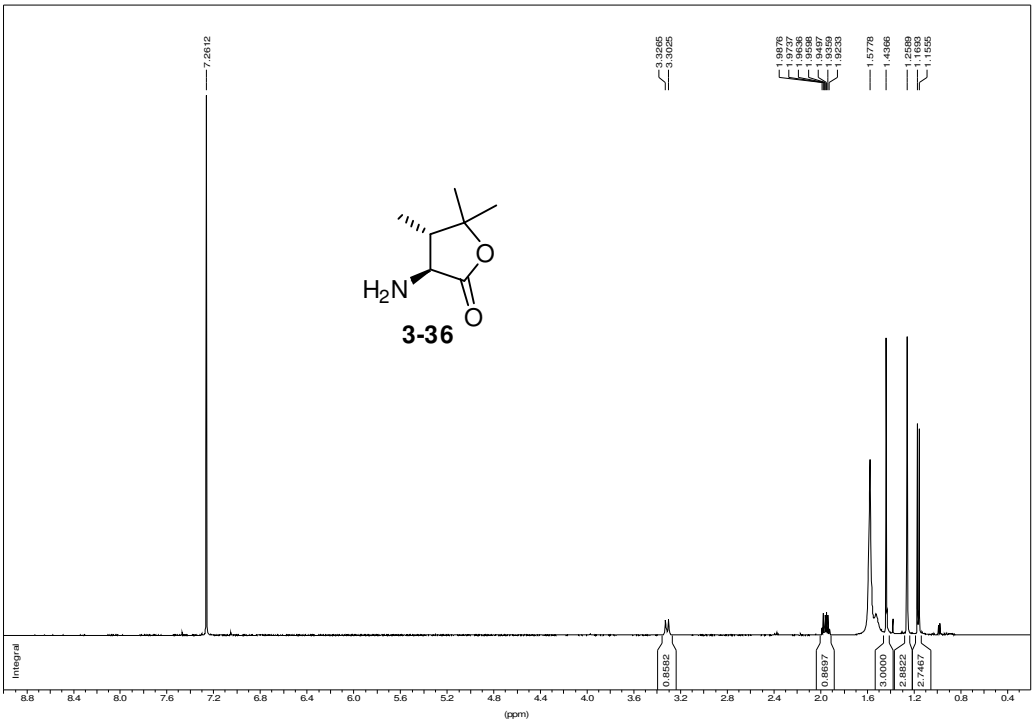


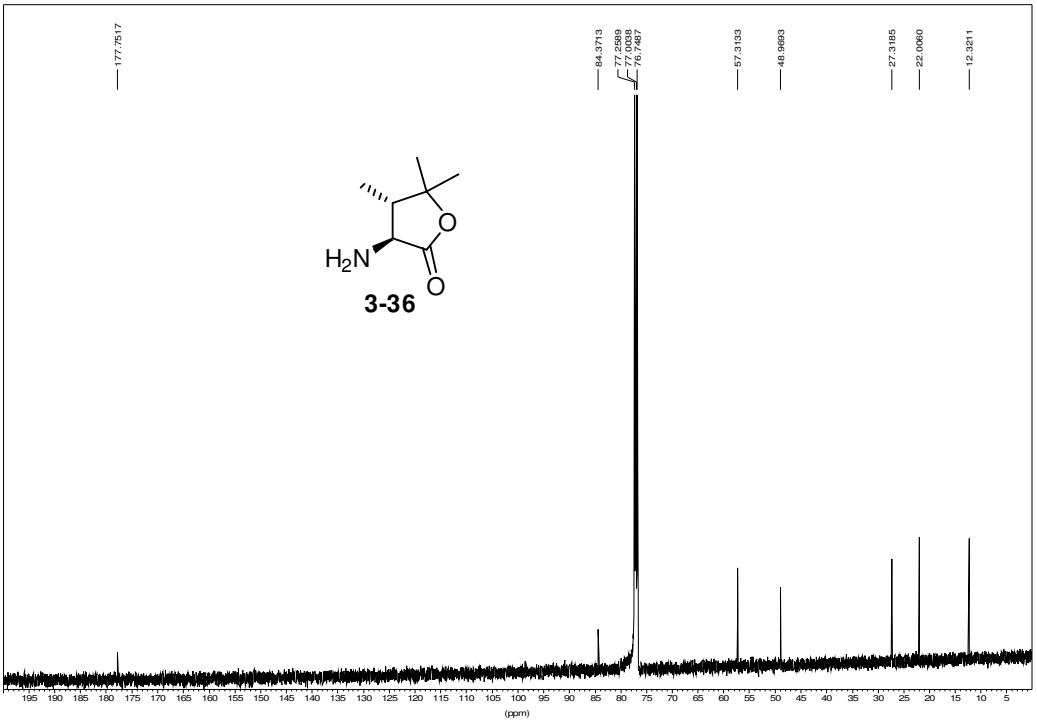

NHBoc
CO2Et
3-35

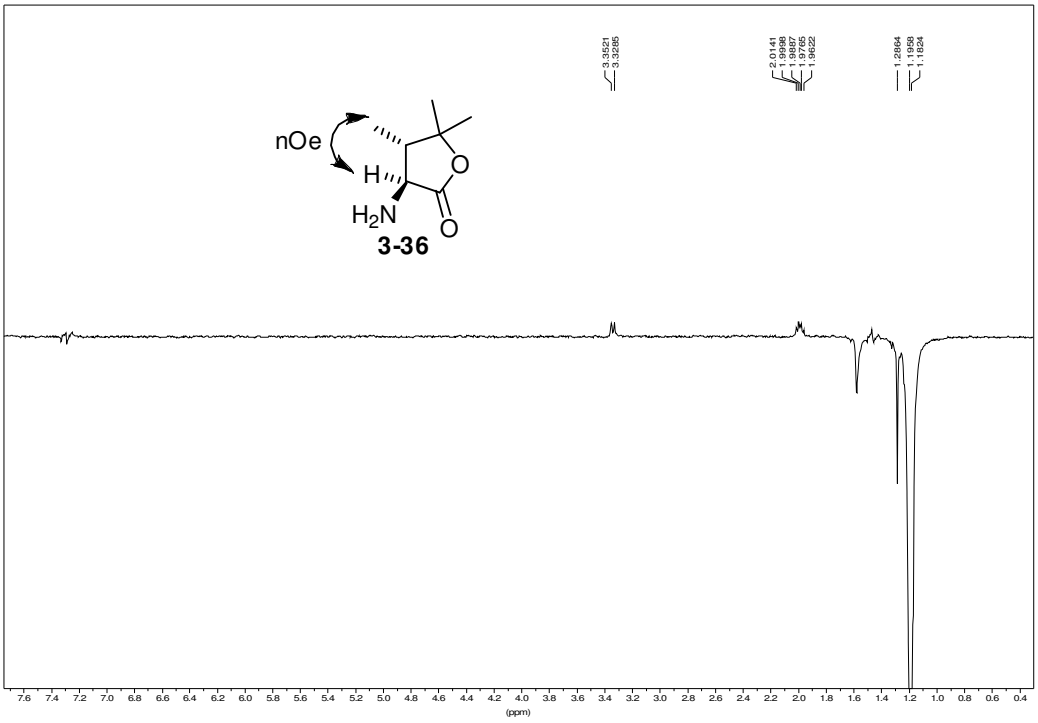

NHBoc
CO2Et
3-35

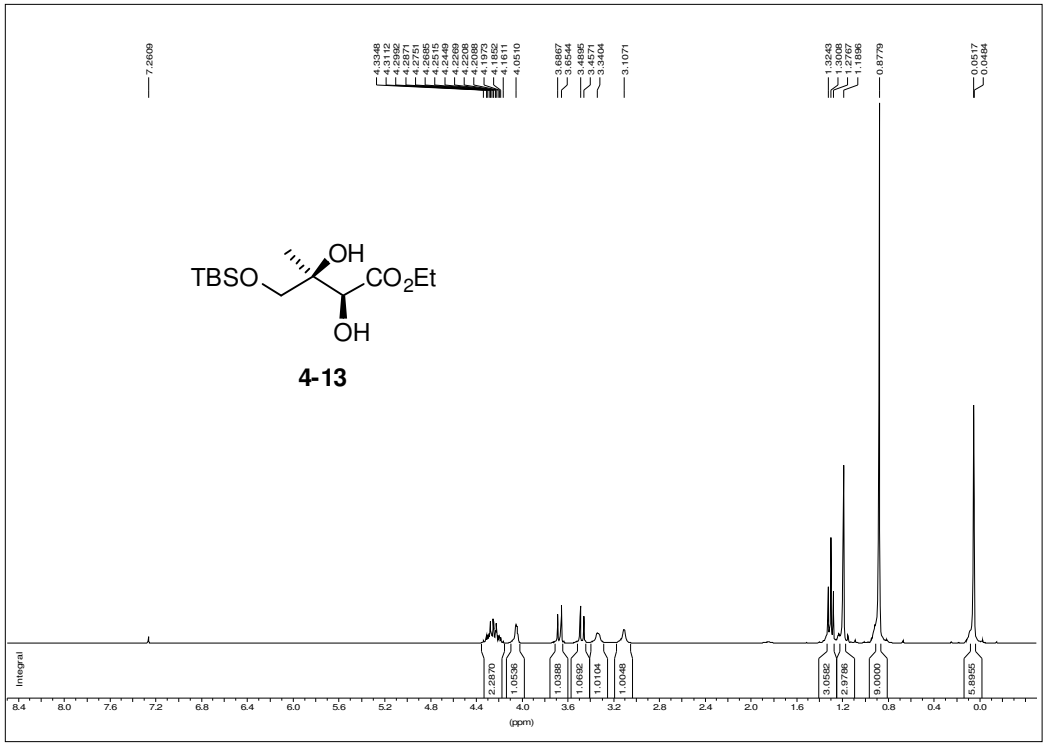
DEPT135 AC300

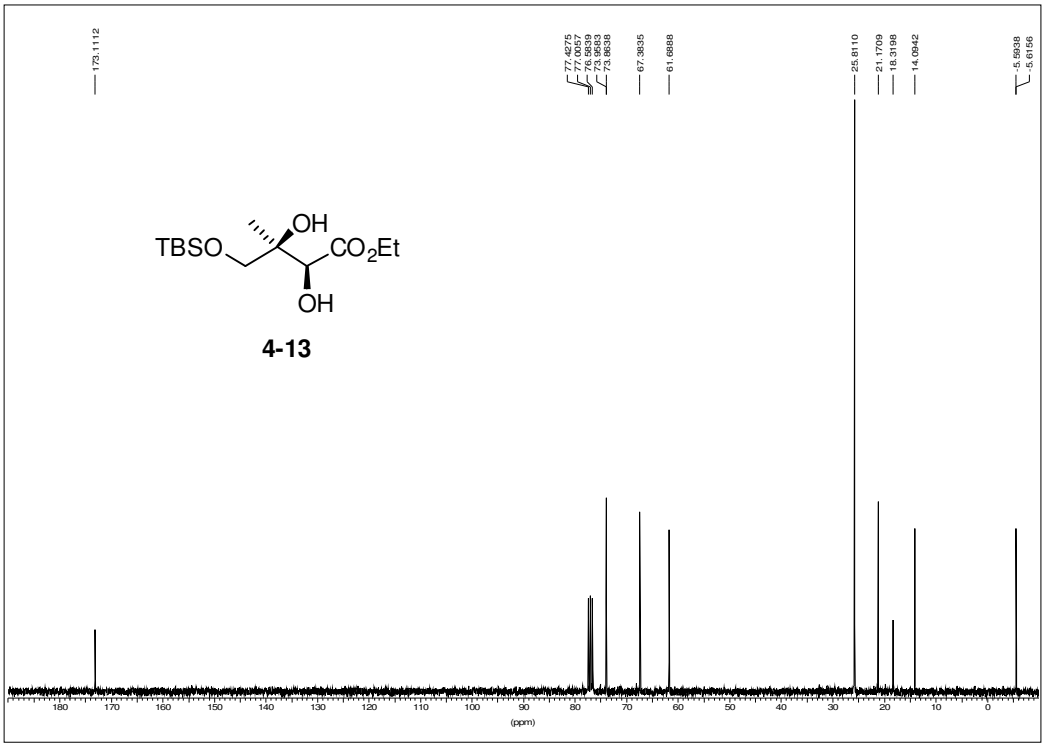


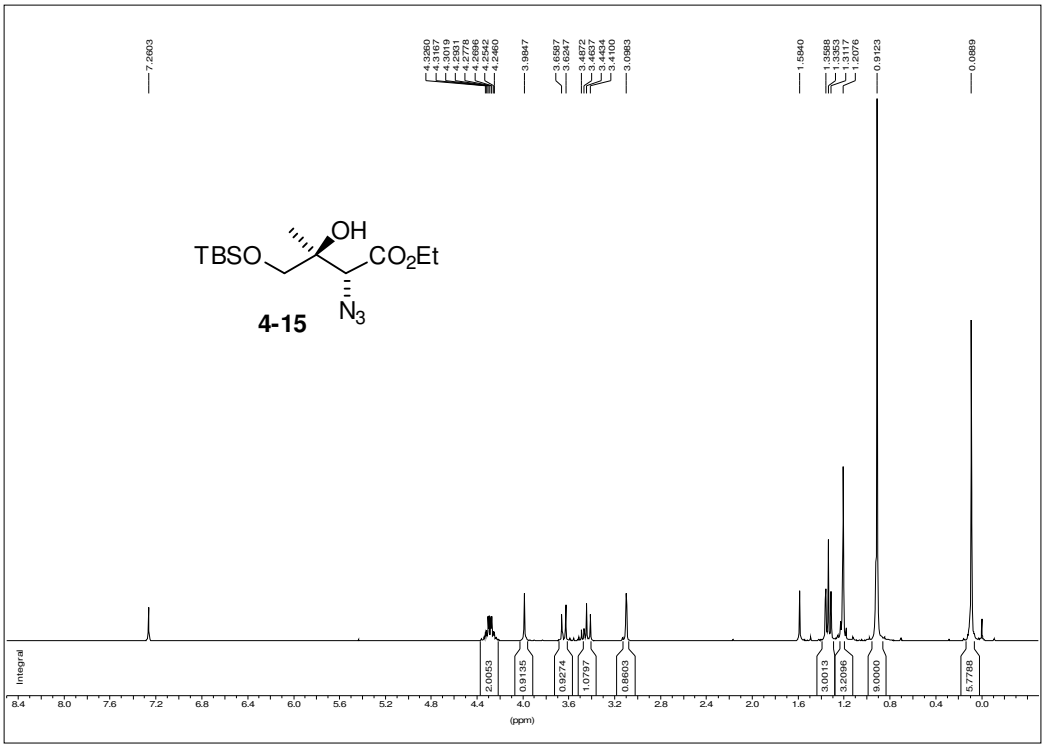


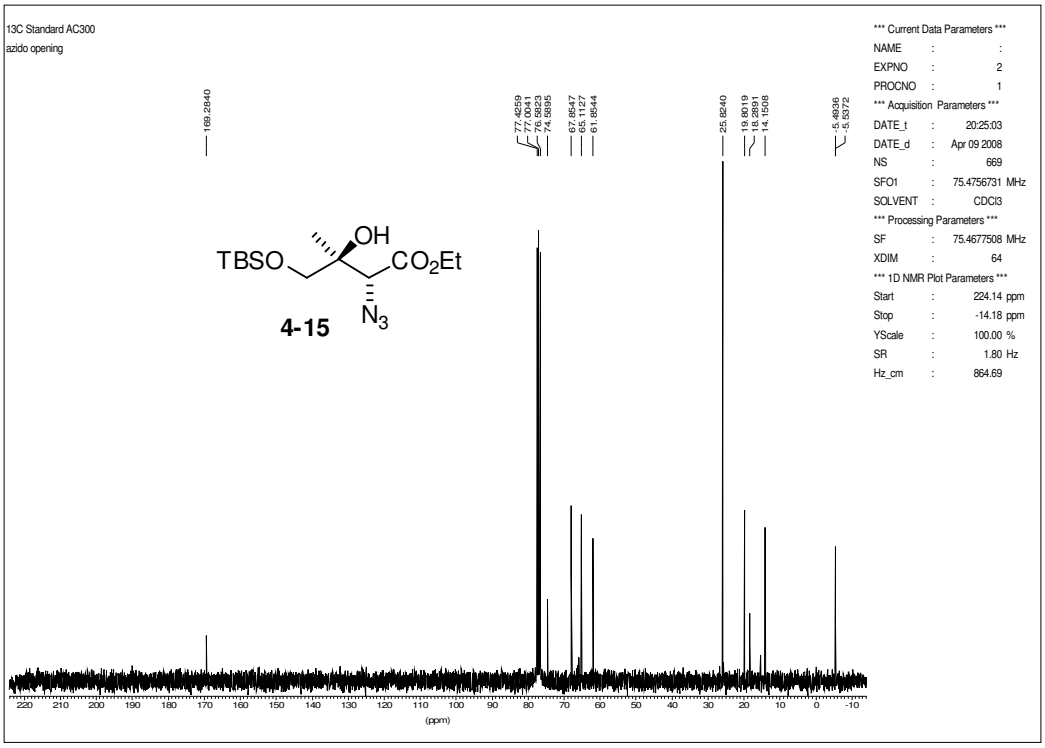

177.7517
84.3713
77.2589
77.0038
76.7487
57.3133
48.9693
27.3185
22.0060
12.3211
O
H_2N
O
3-36
(ppm)

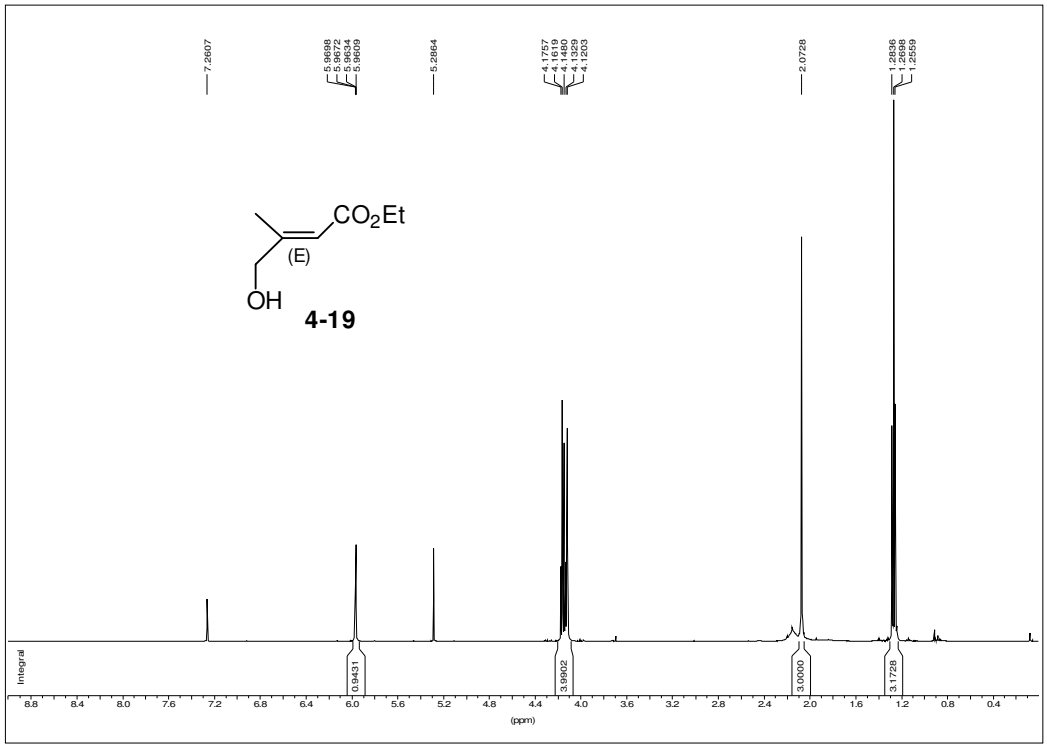

3.3521
3.3285
2.0141
1.9998
1.9867
1.9765
1.9622
1.2864
1.1958
1.1824
nOe
H
O
H_2N
O
3-36
(ppm)

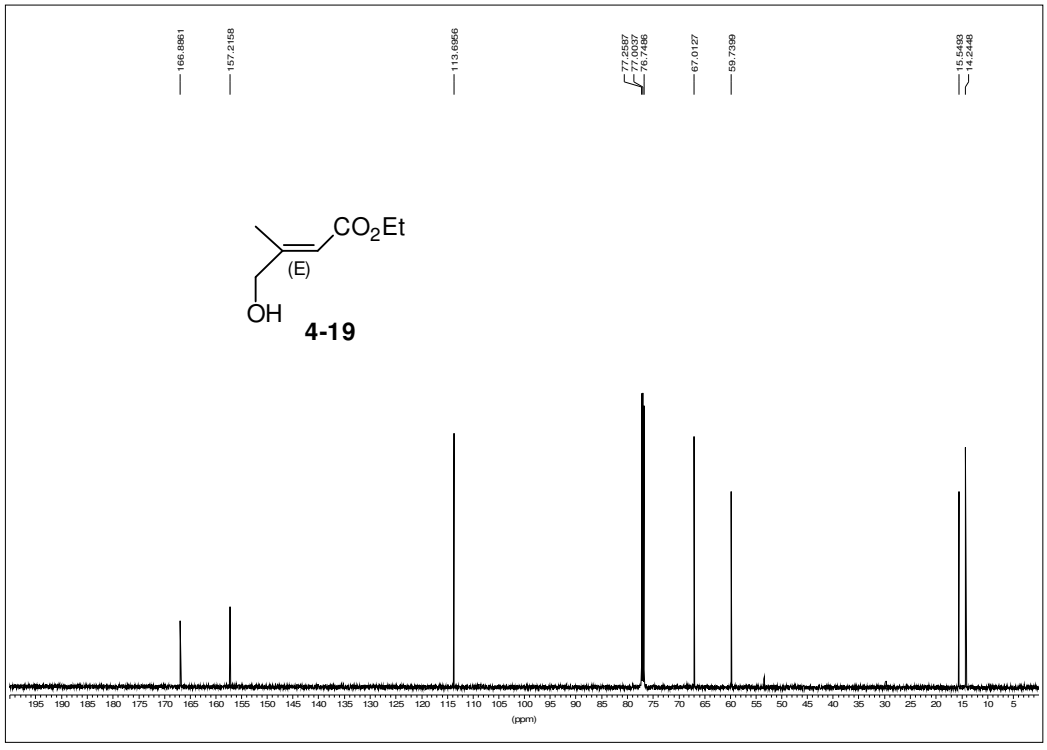

7.2609
4.3148
4.3112
4.2992
4.2871
4.2751
4.2685
4.2515
4.2449
4.2269
4.2208
4.2098
4.1975
4.1852
4.1611
4.0510
3.6967
3.6844
3.4895
3.4571
3.3404
3.1071
1.3243
1.3008
1.2767
1.1896
0.8779
0.0517
0.0484
OH
TBSO
CO2Et
OH
4-13
Integral
2.2870
1.0536
1.0388
1.0692
1.0104
1.0048
3.0582
2.9786
9.0000
5.8955
(ppm)

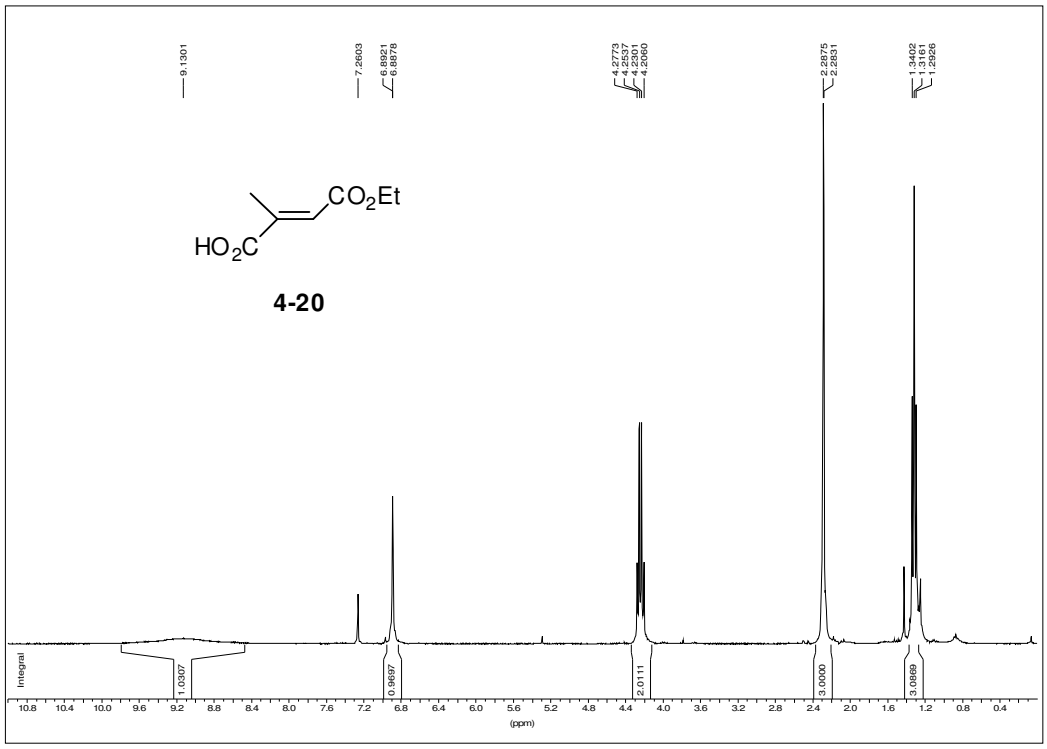

173.1112
77.4275
77.0057
76.5839
73.9633
73.8638
67.3835
61.6888
25.8110
21.1709
18.3198
14.0942
-5.5938
-5.6156
OH
TBSO
CO2Et
OH
4-13
(ppm)

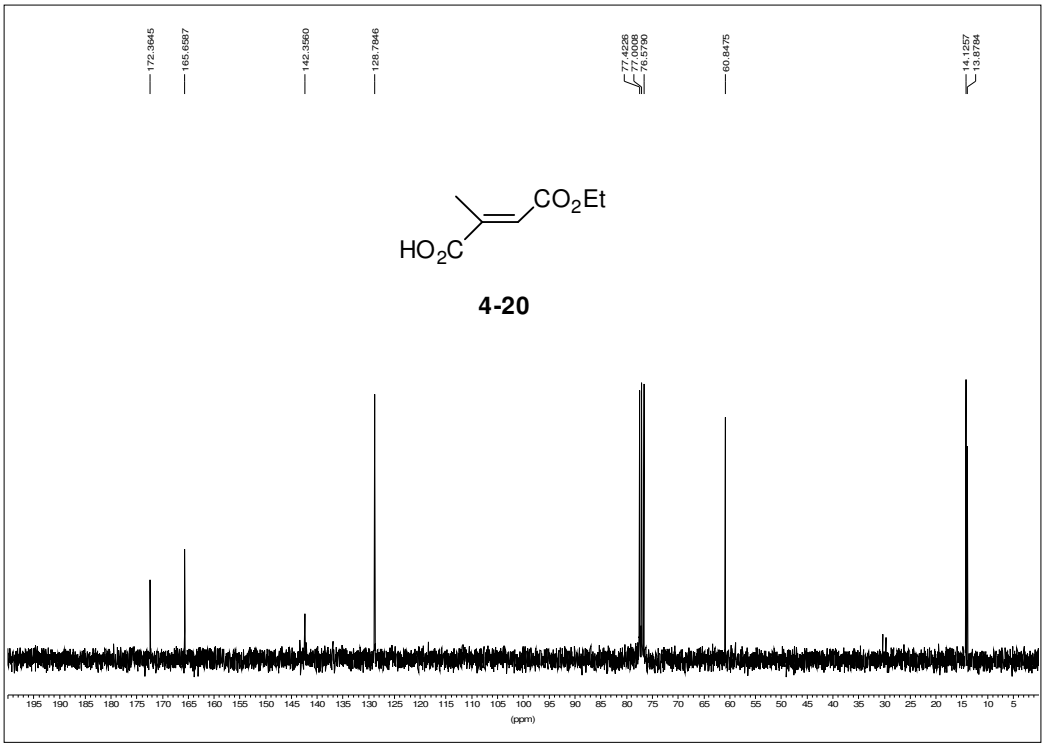

7.2603
4.3380
4.3167
4.3009
4.2931
4.2778
4.2686
4.2542
4.2460
3.9947
3.6597
3.6247
3.4872
3.4687
3.4454
3.4100
3.0983
1.5940
1.5588
1.3353
1.3117
1.2076
0.9123
0.0889
TBSO
OH
CO2Et
N3
4-15
Integral
2.0053
0.9135
0.9274
1.0797
0.8603
3.0013
3.2096
9.0000
5.7788
(ppm)

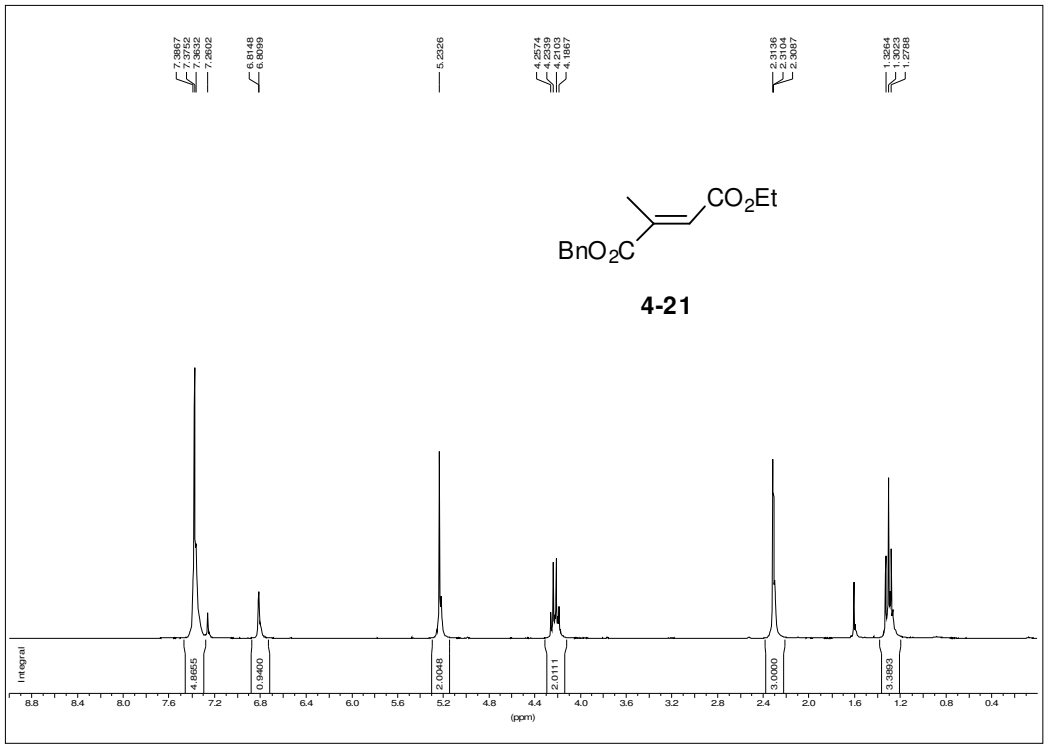

13C Standard AC300
azido opening
169.2940
77.4059
77.0041
76.5823
74.5895
67.8547
65.1127
61.8544
25.8240
18.4019
14.1508
-5.4836
-5.5372
TBSO
OH
CO2Et
N3
4-15
*** Current Data Parameters ***
NAME :
EXPNO : 2
PROCNO : 1
*** Acquisition Parameters ***
DATE_t : 20:25:03
DATE_d : Apr 09 2008
NS : 669
SFO1 : 75.4756731 MHz
SOLVENT : CDCl3
*** Processing Parameters ***
SF : 75.4677508 MHz
XDIM : 64
*** 1D NMR Plot Parameters ***
Start : 224.14 ppm
Stop : -14.18 ppm
YScale : 100.00 %
SR : 1.80 Hz
Hz_cm : 864.69
(ppm)

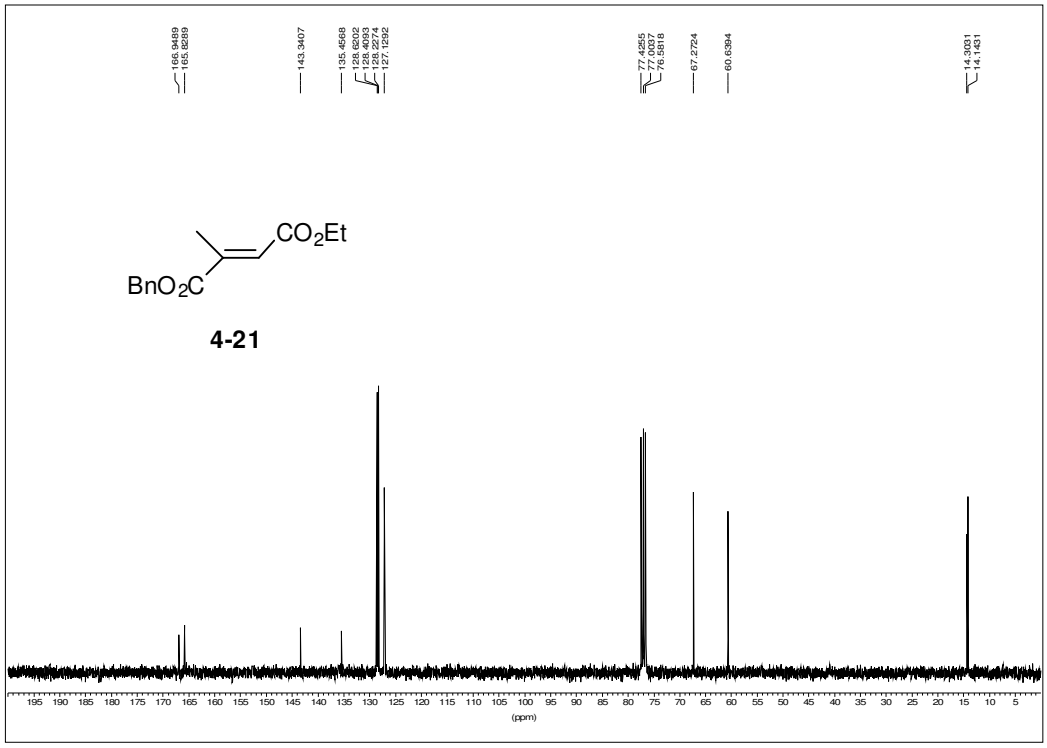

7.2607
5.9698
5.9672
5.9644
5.9609
5.2864
4.1757
4.1619
4.1480
4.1339
4.1203
2.0728
1.2836
1.2698
1.2559
CO2Et
(E)
OH
4-19
Integral
0.9431
3.9902
3.0000
3.1728
(ppm)

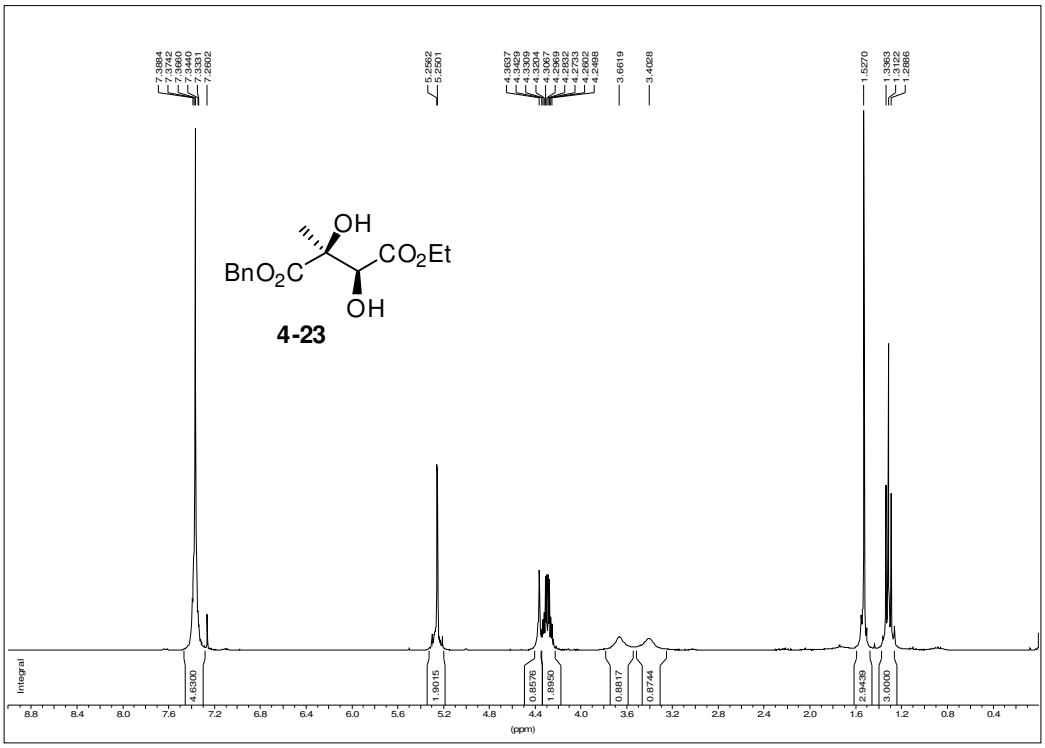

166.8861
157.2158
113.6956
77.2587
77.0037
76.7486
67.0127
59.7399
15.5493
14.2448
CO2Et
(E)
OH
4-19
(ppm)

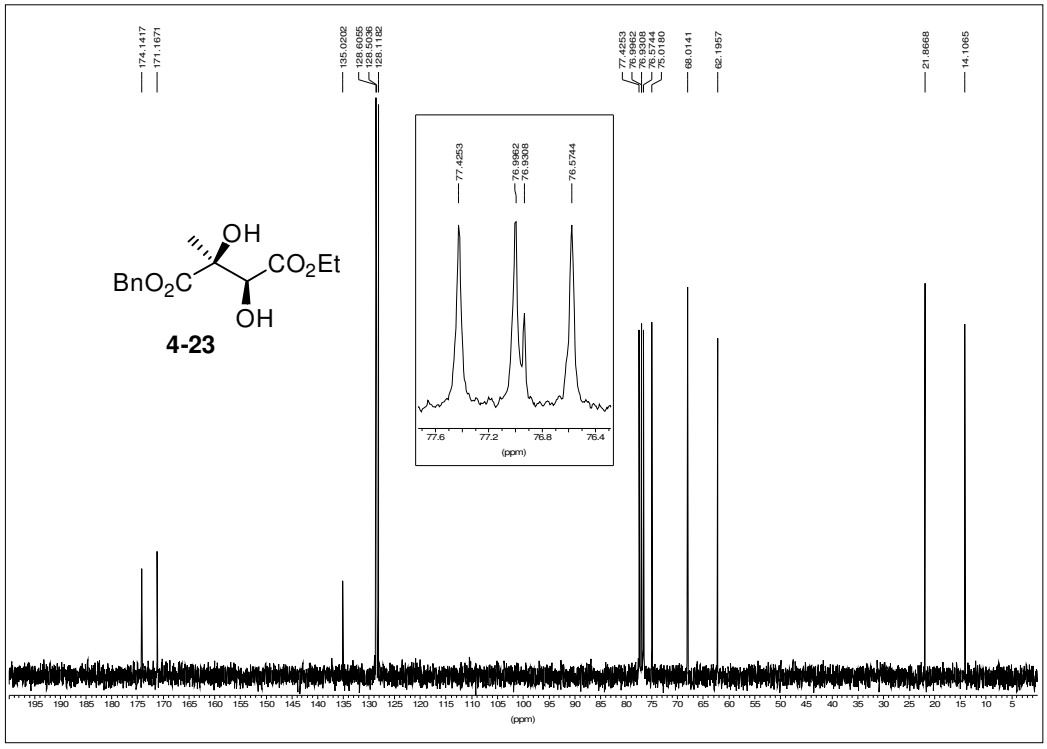

9.1301
7.2603
6.8921
6.8878
4.2773
4.2537
4.2301
4.2060
2.2875
2.2831
1.3402
1.3161
1.2926
CO2Et
HO2C
4-20
Integral
1.0307
0.9697
2.0111
3.0000
3.0869
(ppm)

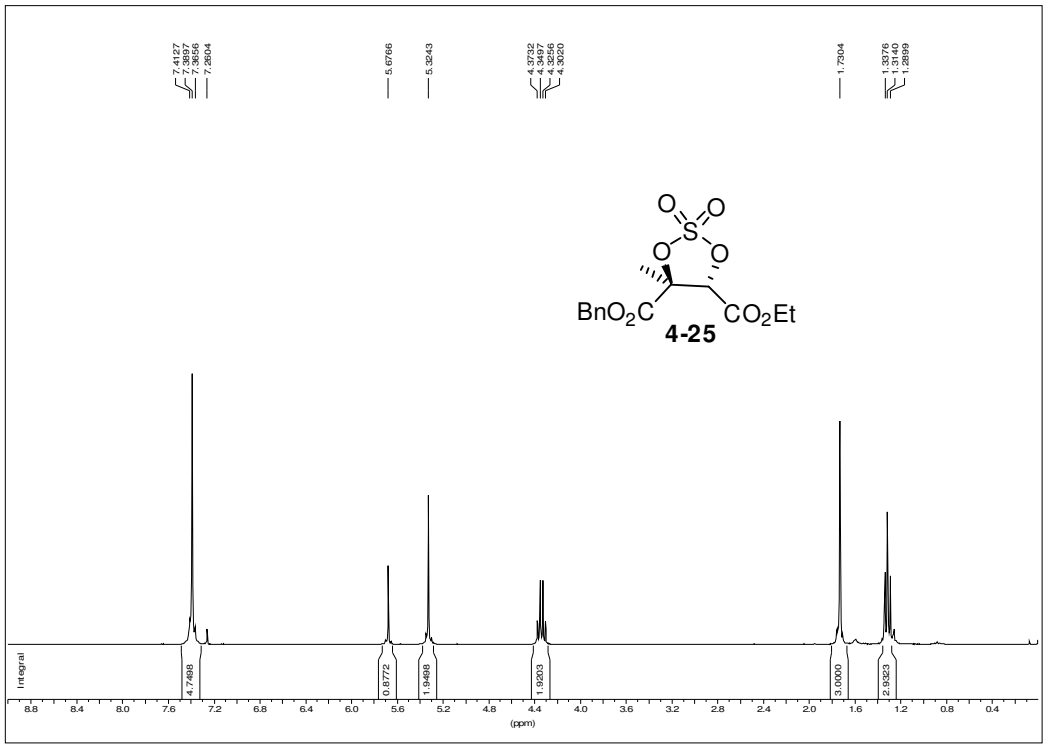

172.3645
165.6587
142.3560
128.7946
77.4236
77.0008
76.5790
60.8475
14.1257
13.8794
CO2Et
HO2C
4-20
(ppm)

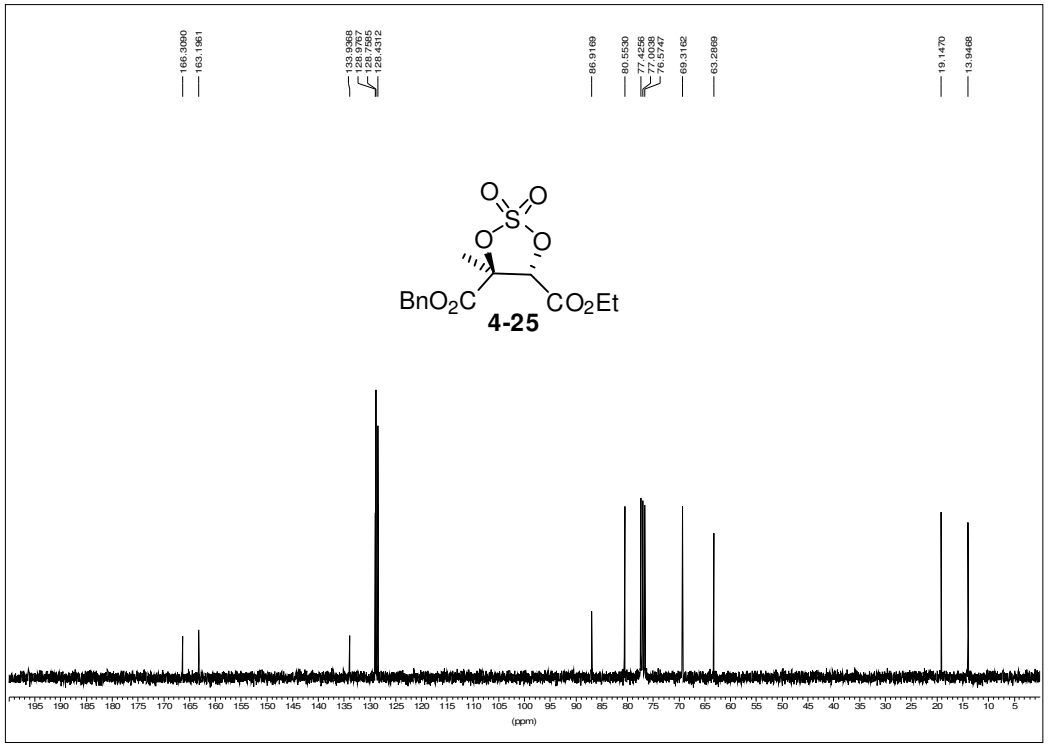

7.3867
7.3752
7.3632
7.2602
6.8148
6.8099
5.2326
4.2574
4.2339
4.2103
4.1867
2.3136
2.3014
2.3097
1.3264
1.3023
1.2782
CO2Et
BnO2C
4-21
Integral
4.8655
0.9400
2.0048
2.0111
3.0000
3.3983
(ppm)

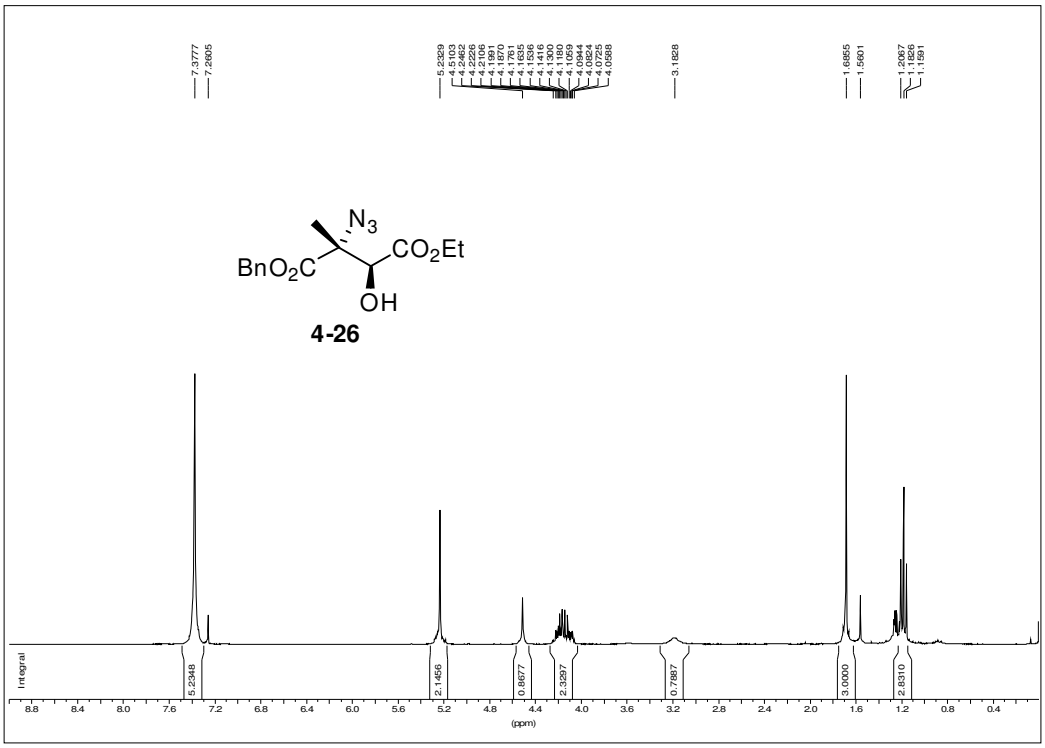

166.9489
165.6289
143.3407
135.4568
128.6202
128.4093
128.2274
127.1292
77.4255
77.0037
76.5818
67.2724
60.6394
14.3031
14.1451
CO2Et
BnO2C
4-21
(ppm)

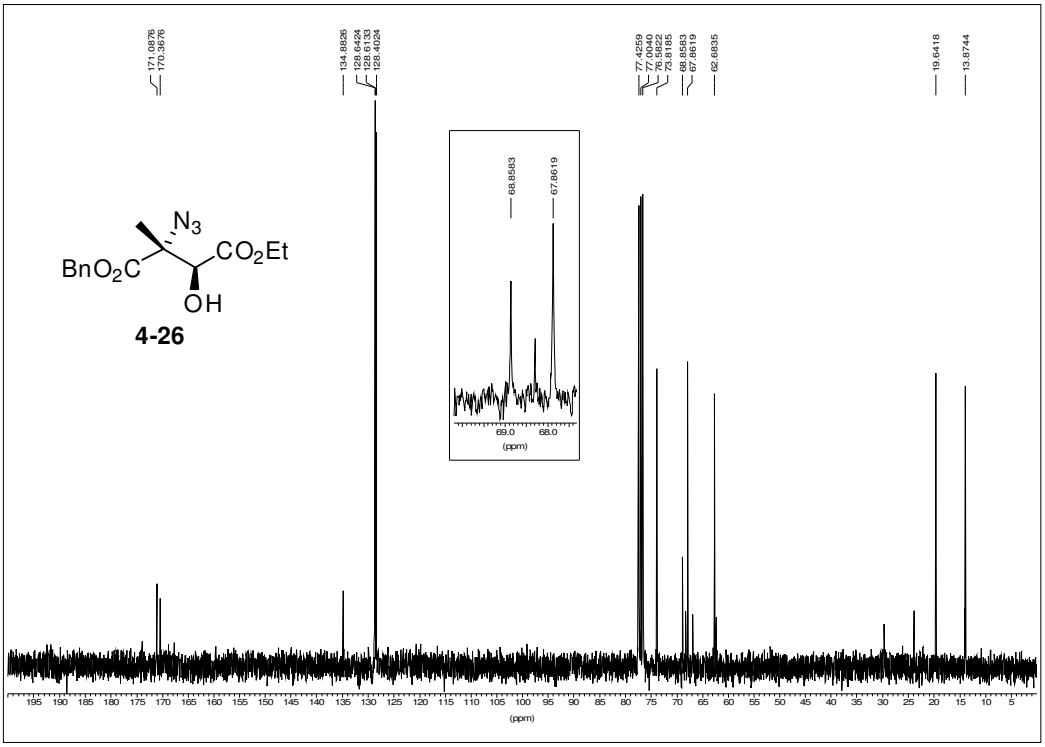

7.3884
7.3742
7.3440
7.3331
7.2952
5.2562
5.2501
4.3637
4.3479
4.3309
4.3204
4.3067
4.2928
4.2832
4.2703
4.2562
4.2498
3.6619
3.4028
1.5270
1.3363
1.3232
1.2986
OH
BnO2C
CO2Et
OH
4-23
Integral
4.6300
1.9015
0.8576
1.8950
0.8817
0.8744
2.9439
3.0000
(ppm)

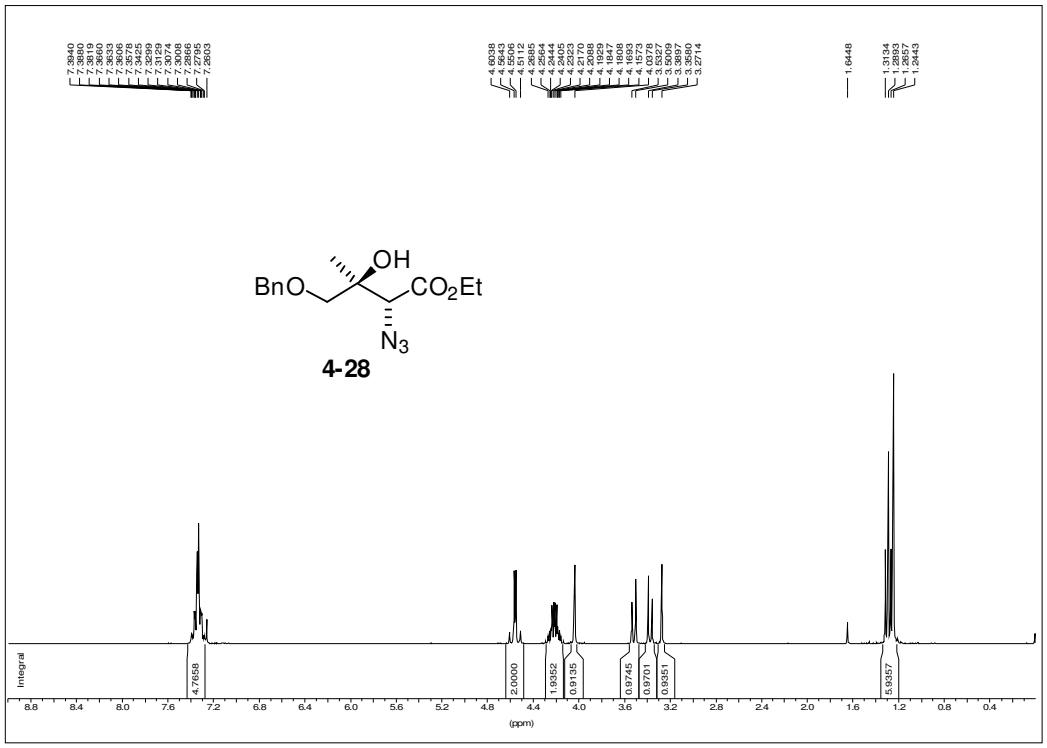

174.1417
171.1671
135.0202
128.6055
128.5036
128.1182
77.4253
76.9962
76.9308
76.5744
75.0180
68.0141
62.1957
21.8668
14.1065
OH
BnO2C
CO2Et
OH
4-23
77.4253
76.9962
76.9308
76.5744
77.6
77.2
76.8
76.4
(ppm)
(ppm)

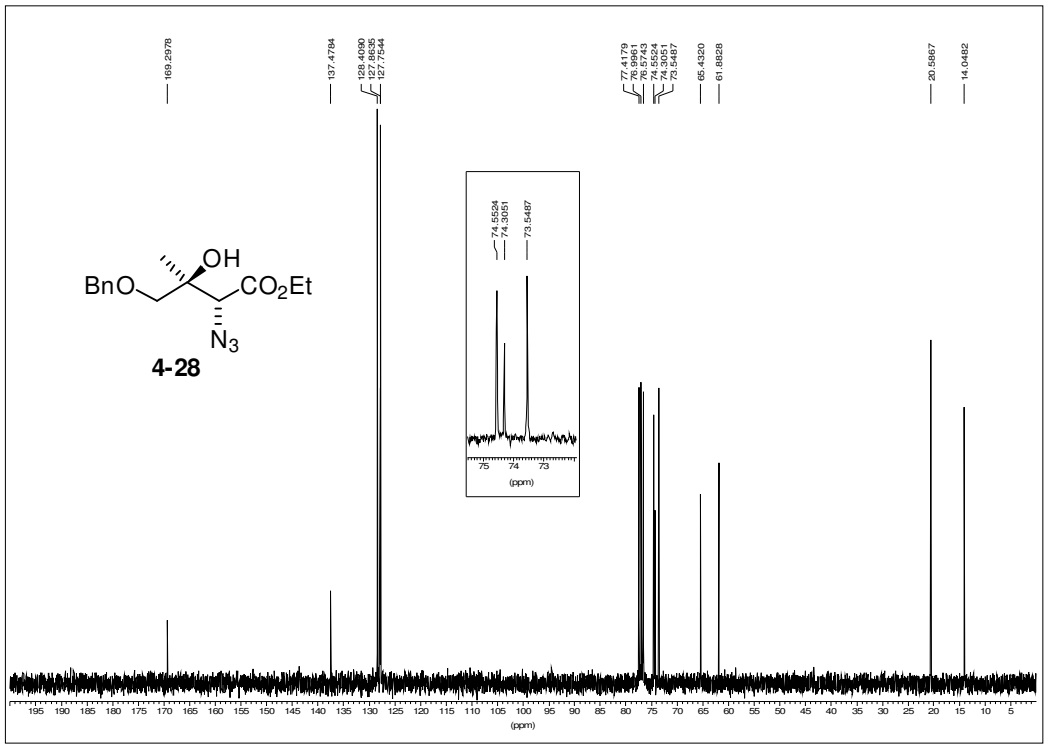

7.4127
7.3897
7.3656
7.2804
5.6766
5.3243
4.3732
4.3497
4.3256
4.3020
1.7304
1.3376
1.3140
1.2899
O
O
S
O
O
BnO2C
CO2Et
4-25
Integral
4.7498
0.8772
1.9488
1.9203
3.0000
2.9323
8.8 8.4 8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4
(ppm)


166.3090
163.1961
133.9368
128.9767
128.7585
128.4312
86.9169
80.5530
77.4256
77.0038
76.5747
69.3162
63.2869
19.1470
13.9468
O
O
S
O
O
BnO2C
CO2Et
4-25
195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5
(ppm)


7.3777
7.2605
5.2329
4.5103
4.2952
4.2252
4.2106
4.1991
4.1830
4.1781
4.1635
4.1536
4.1416
4.1300
4.1159
4.1059
4.0944
4.0824
4.0725
4.0598
3.1828
1.6855
1.5601
1.2067
1.1826
1.1561
N3
BnO2C
CO2Et
OH
4-26
Integral
5.2348
2.1456
0.8677
2.3297
0.7887
3.0000
2.8310
(ppm)


171.0976
170.3676
134.8826
128.6424
128.6133
128.4024
77.4259
77.0040
76.5822
73.1145
68.8583
67.8619
62.6835
19.6418
13.8744
68.8583
67.8619
69.0
68.0
(ppm)
N3
BnO2C
CO2Et
OH
4-26
(ppm)


BnO
OH
CO2Et
N3
4-28
Integral
4.7658
2.0000
1.9352
0.9135
0.9745
0.9701
0.9351
5.9357
(ppm)


BnO
OH
CO2Et
N3
4-28
169.2978
137.4784
128.4090
127.8635
127.7544
77.4179
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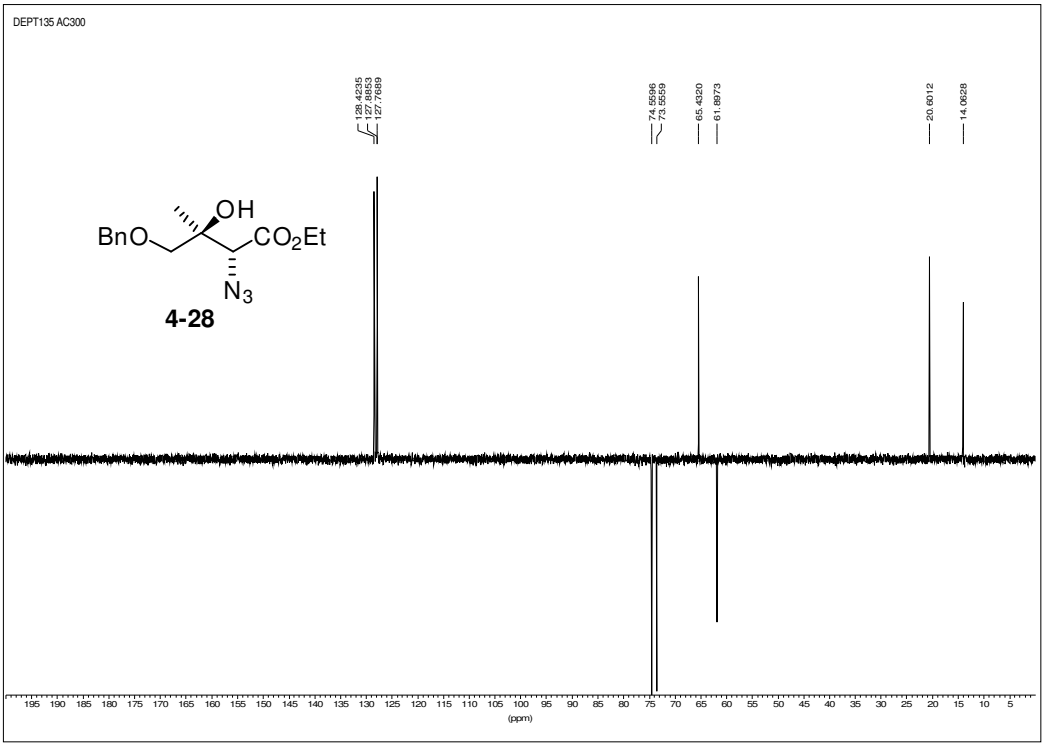
65.4320
61.8828
20.5867
14.0482
(ppm)


DEPT135 AC300
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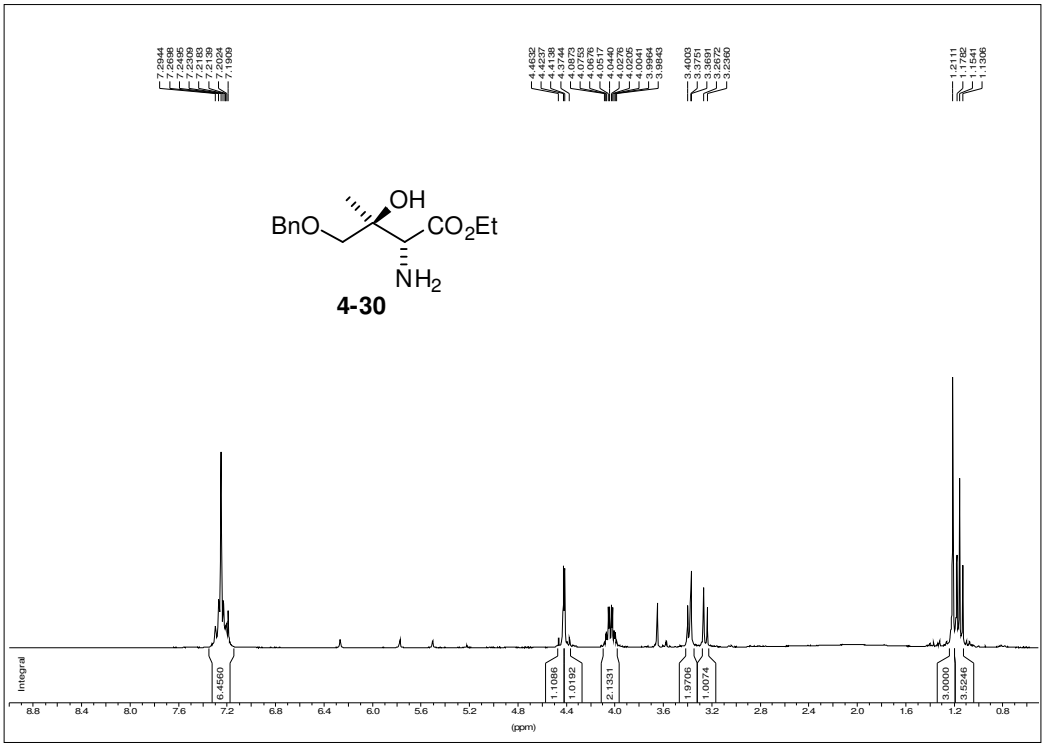
127.8853
127.7689
74.5596
73.5559
65.4320
61.8973
20.6012
14.0628
OH
BnO
CO2Et
N3
4-28
(ppm)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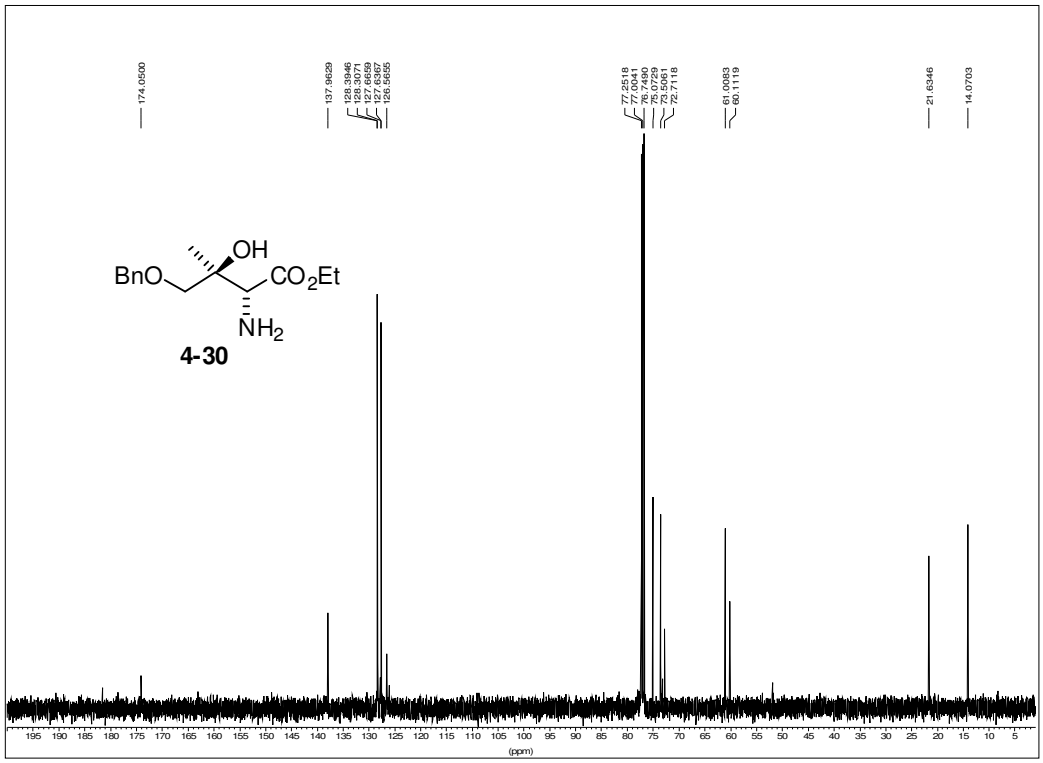
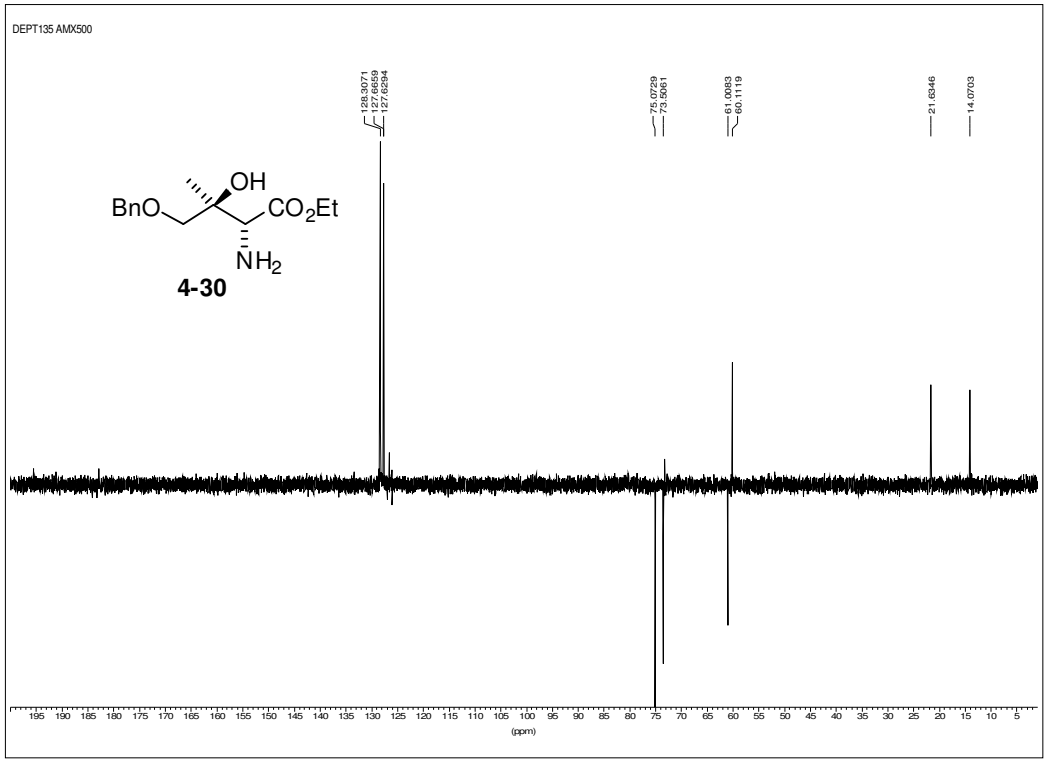
7.2698
7.2465
7.2309
7.2183
7.2039
7.2024
7.1909
4.4692
4.4237
4.4138
4.3744
4.0952
4.0753
4.0676
4.0657
4.0440
4.0276
4.0095
4.0041
3.9964
3.9843
3.4093
3.3751
3.3691
3.2672
3.2360
1.2111
1.1782
1.1541
1.1306
OH
BnO
CO2Et
NH2
4-30
Integral
6.4560
1.1086
1.0192
2.1331
1.9706
1.0074
3.0000
3.5246
(ppm)


174.0500
137.9629
128.3946
128.3071
127.6659
127.6287
126.5935
77.2518
77.0041
76.7490
75.0729
73.5061
72.7118
61.0093
60.1119
21.6346
14.0703
OH
BnO
CO2Et
NH2
4-30
(ppm)


DEPT135 AMX500
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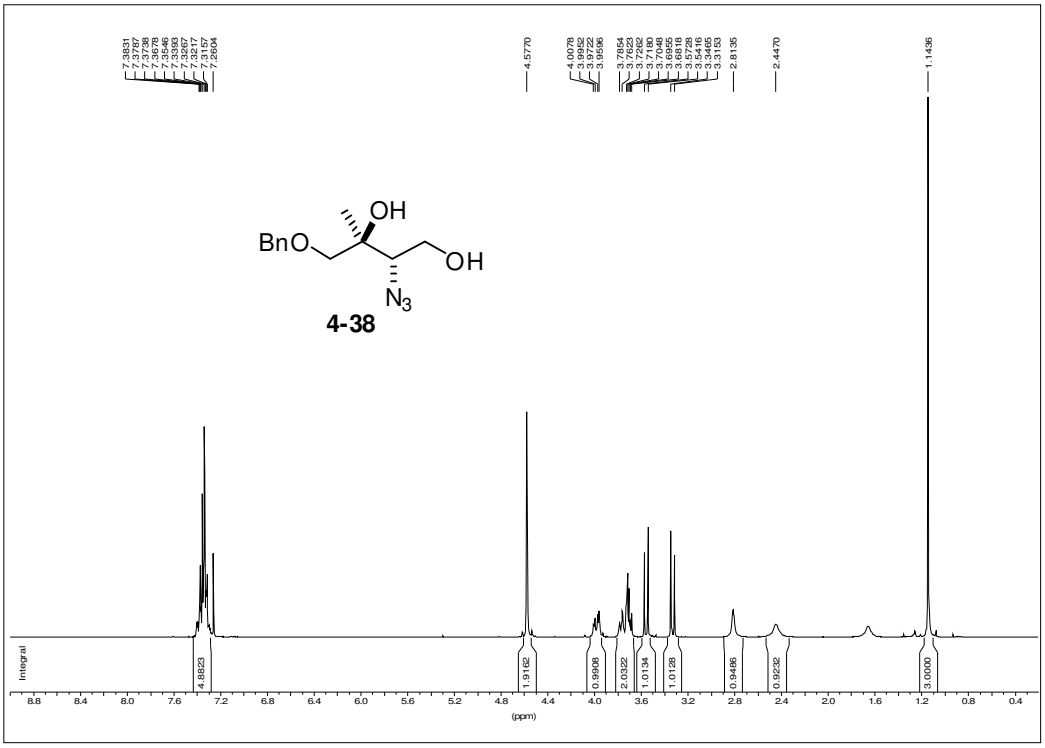
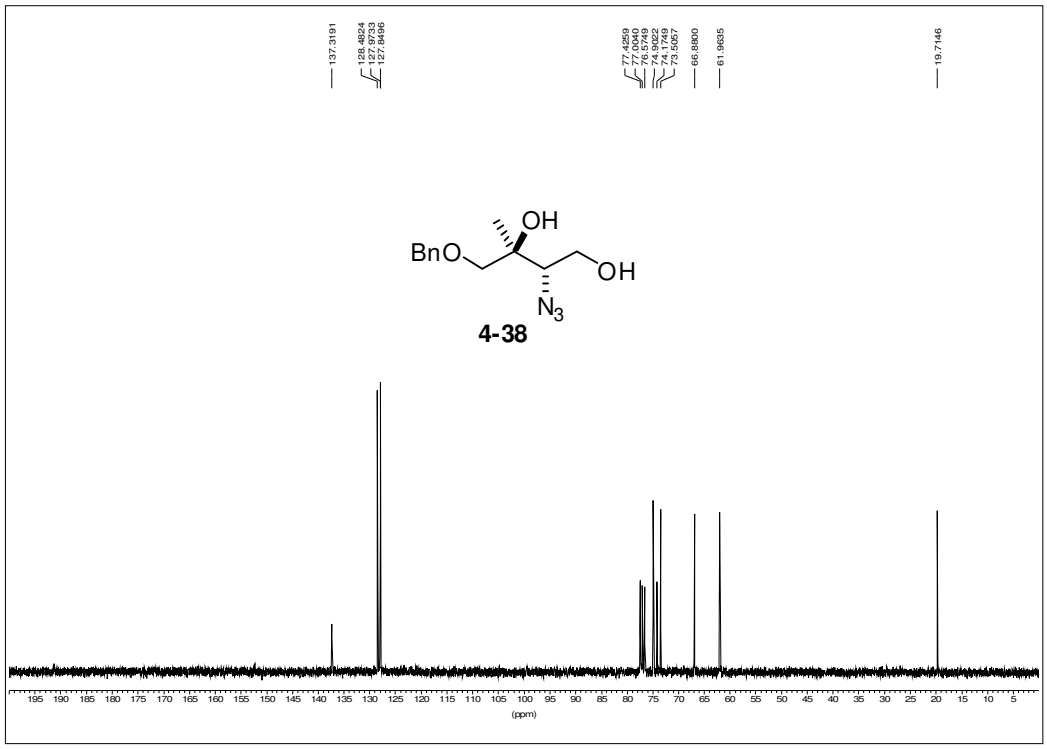
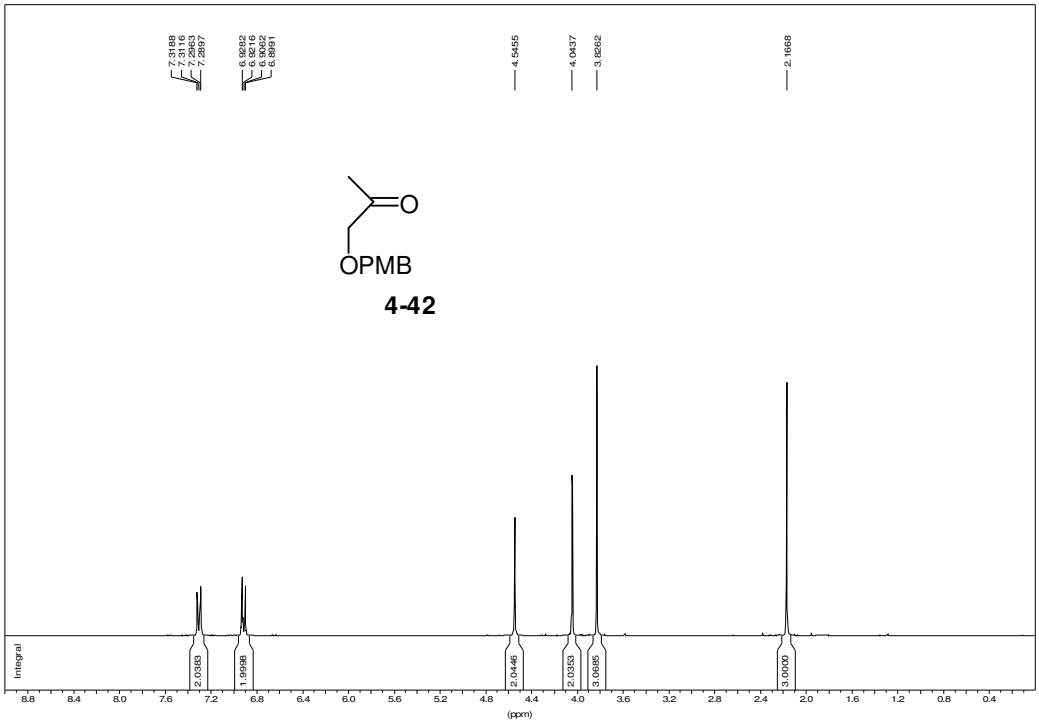
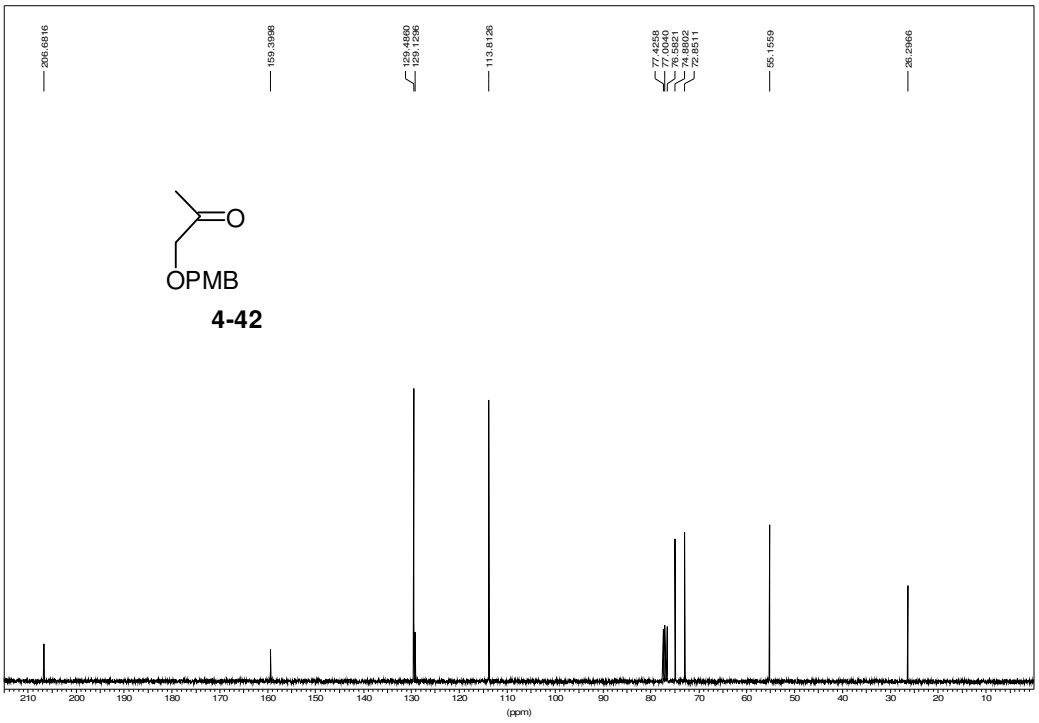
127.6659
127.6294
75.0729
73.5061
61.0093
60.1119
21.6346
14.0703
OH
BnO
CO2Et
NH2
4-30
(ppm)


7.3831
7.3787
7.3738
7.3678
7.3546
7.3393
7.3267
7.3217
7.3157
7.2604
4.5770
4.0078
3.9952
3.9722
3.9598
3.7854
3.7653
3.7282
3.7190
3.7046
3.6955
3.6818
3.5728
3.5446
3.3465
3.3153
2.8135
2.4470
1.1436
OH
BnO
OH
N3
4-38
Integral
4.8823
1.9162
0.9998
2.0322
1.0134
1.0128
0.9486
0.9232
3.0000
(ppm)

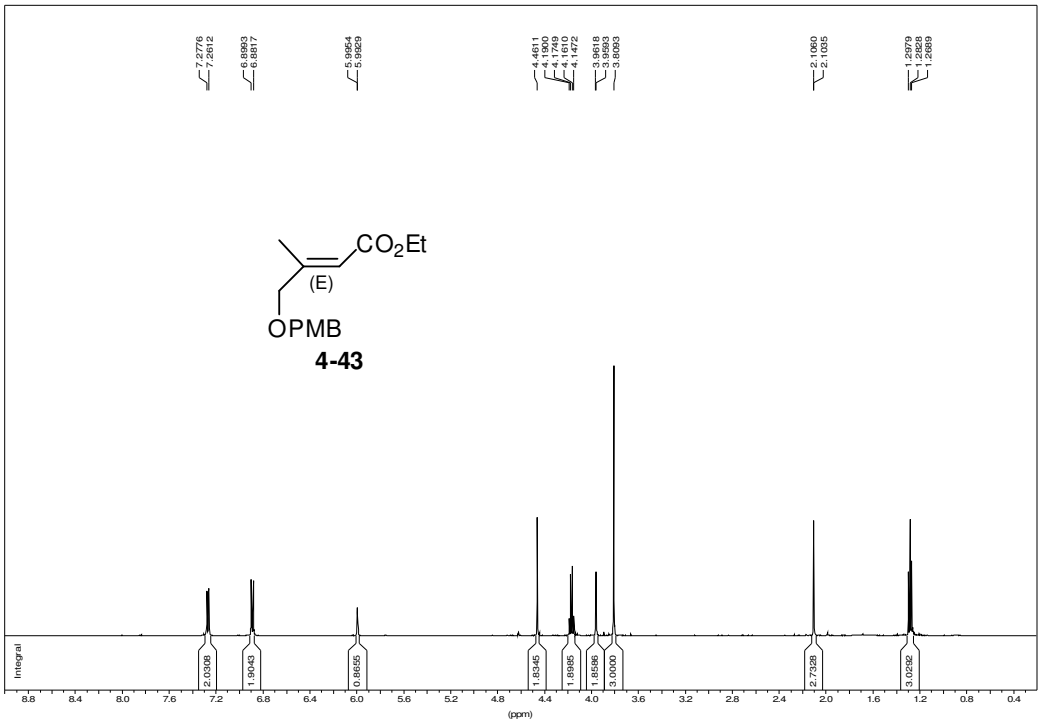

137.3191
128.4824
127.9733
127.8496
77.4259
77.0040
76.5749
74.9662
74.1749
73.5057
66.8800
61.9635
19.7146
OH
BnO
OH
N3
4-38
(ppm)

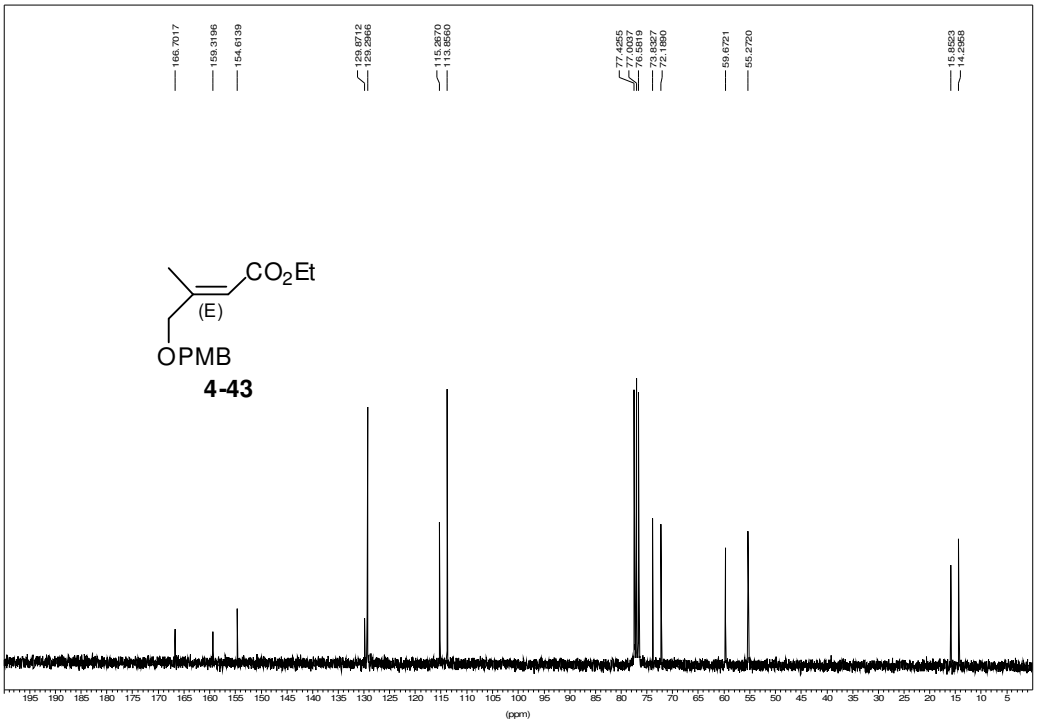

7.3188
7.3116
7.2863
7.2697
6.9282
6.9216
6.9062
6.8991
4.5455
4.0437
3.8262
2.1668
O
OPMB
Integral
2.0383
1.9998
2.0446
2.0353
3.0585
3.0000
(ppm)

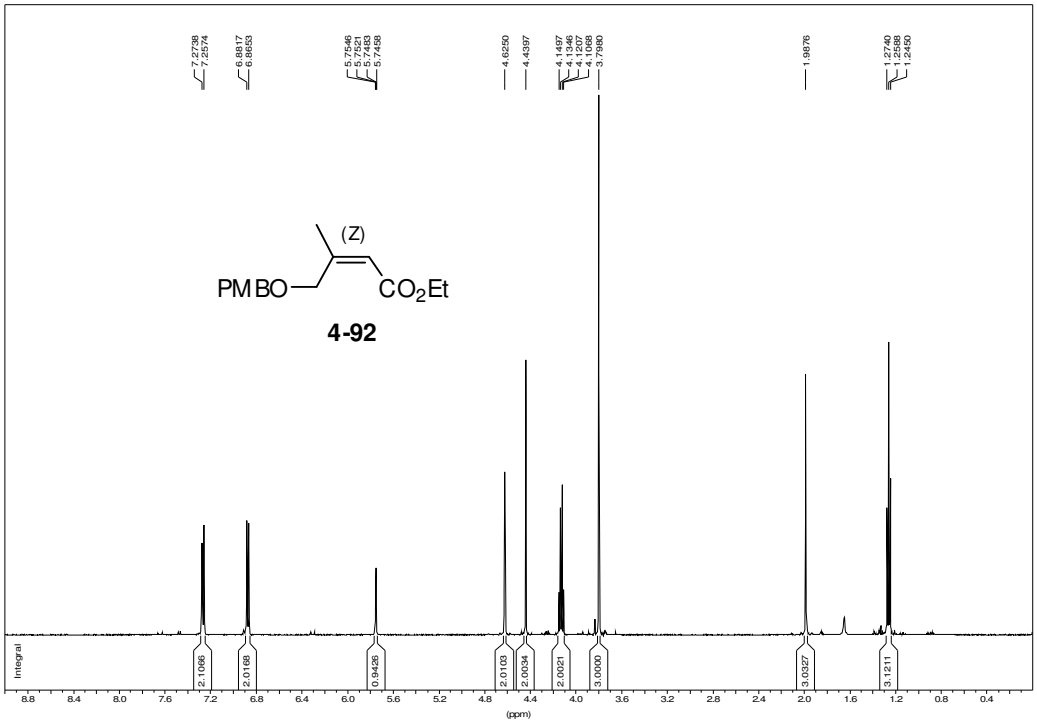
4-42

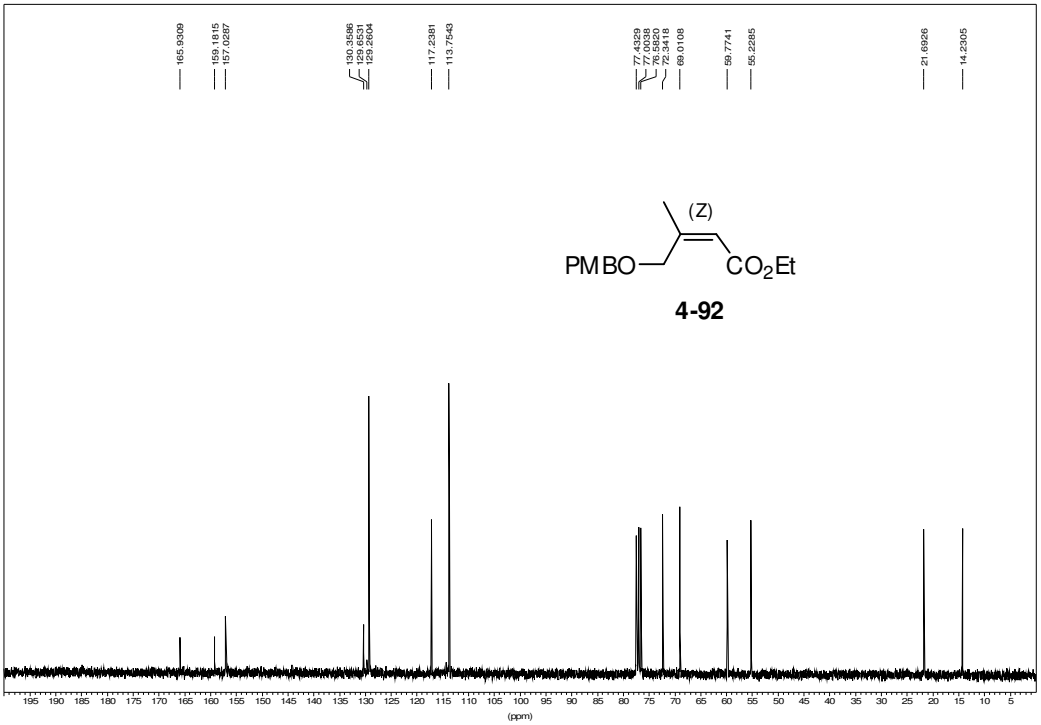

206.6816
159.3998
129.4860
129.1296
113.8126
77.4258
77.0040
76.5821
74.8802
72.8511
55.1559
26.2966
O
OPMB
(ppm)

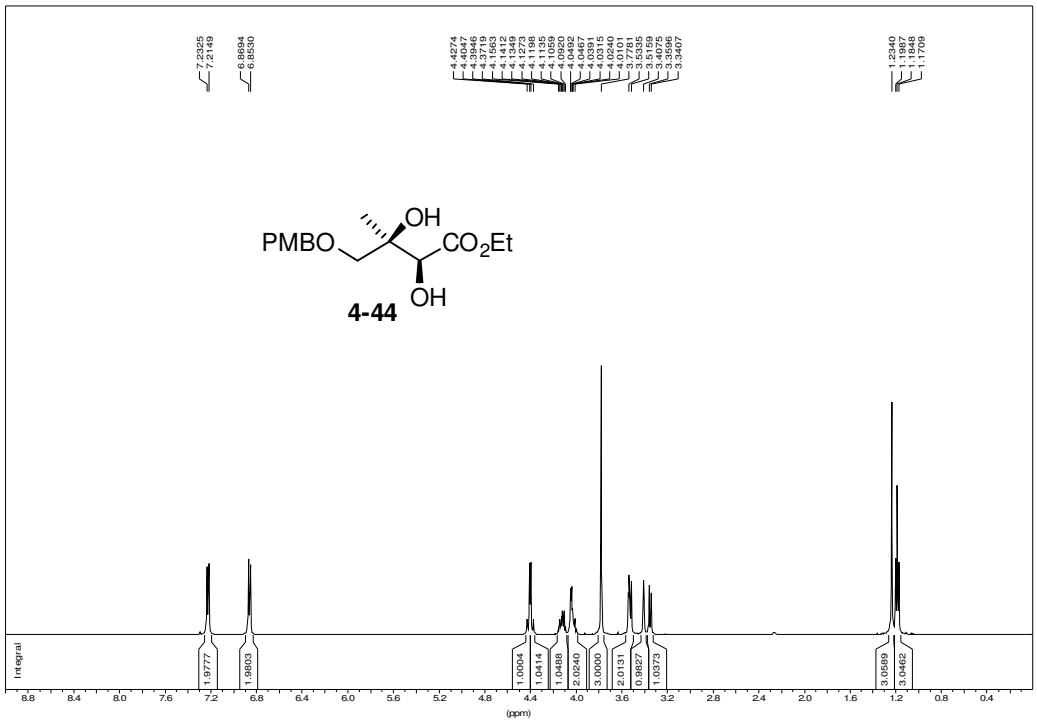
4-42

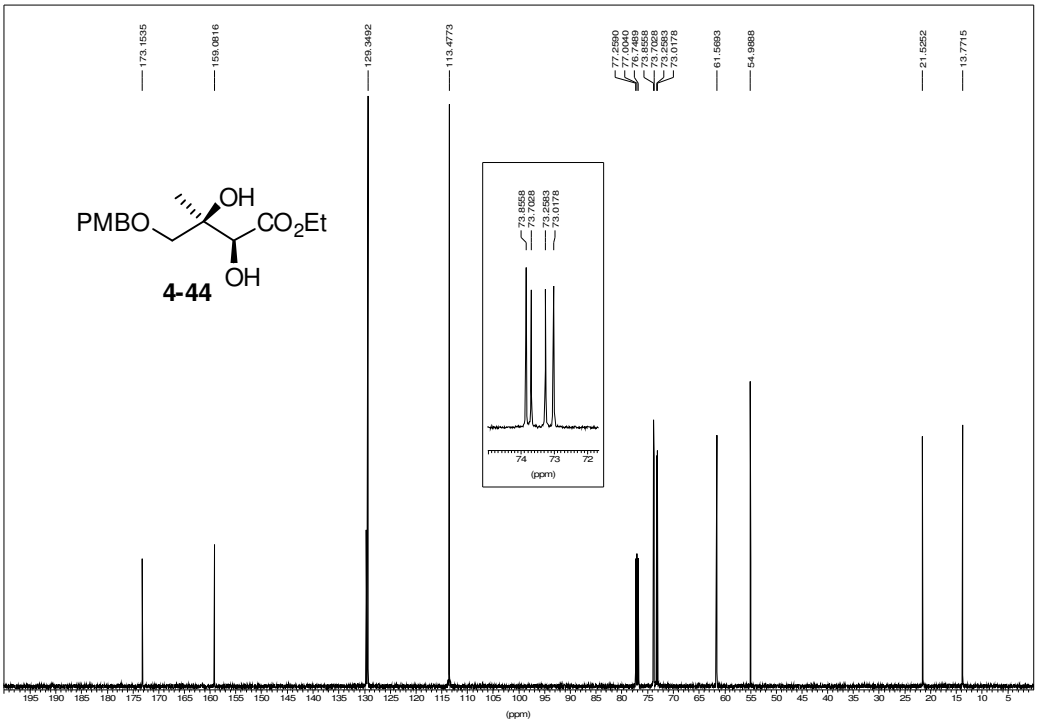

7.2776
7.2612
6.8993
6.8817
5.9954
5.9929
4.4611
4.1500
4.1358
4.1610
4.1472
3.9618
3.9593
3.8093
2.1080
2.1035
1.5979
1.2938
1.2889
CO2Et
(E)
OPMB
4-43
Integral
2.0398
1.9043
0.9855
1.8345
1.8985
1.8586
3.0000
2.7328
3.0282
(ppm)


166.7017
159.3196
154.6139
129.9712
129.2866
115.2870
113.8560
77.4255
77.0037
76.5819
73.1897
72.1890
59.6721
55.2720
15.8523
14.2956
CO2Et
(E)
OPMB
4-43
(ppm)

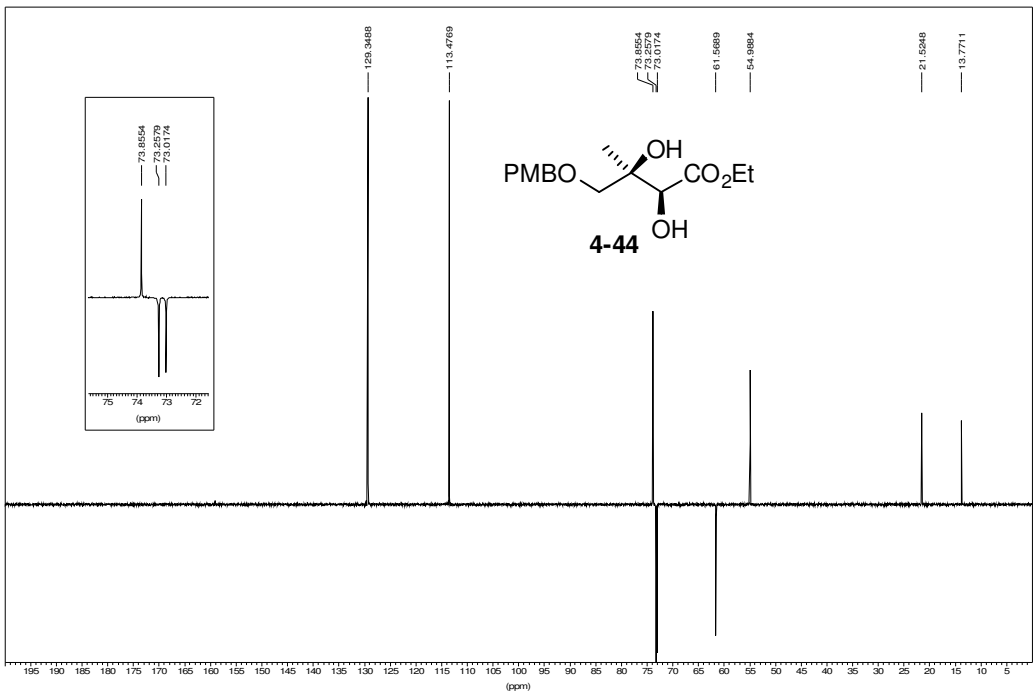

7.2738
7.2574
6.8817
6.8653
5.7546
5.7521
5.7483
5.7458
4.6250
4.4397
4.1497
4.1346
4.1207
4.1068
3.7980
1.9876
1.2740
1.2588
1.2450
(Z)
PMBO
CO2Et
4-92
Integral
2.1066
2.0188
0.9436
2.0103
2.0034
2.0021
3.0000
3.0327
3.1211
(ppm)

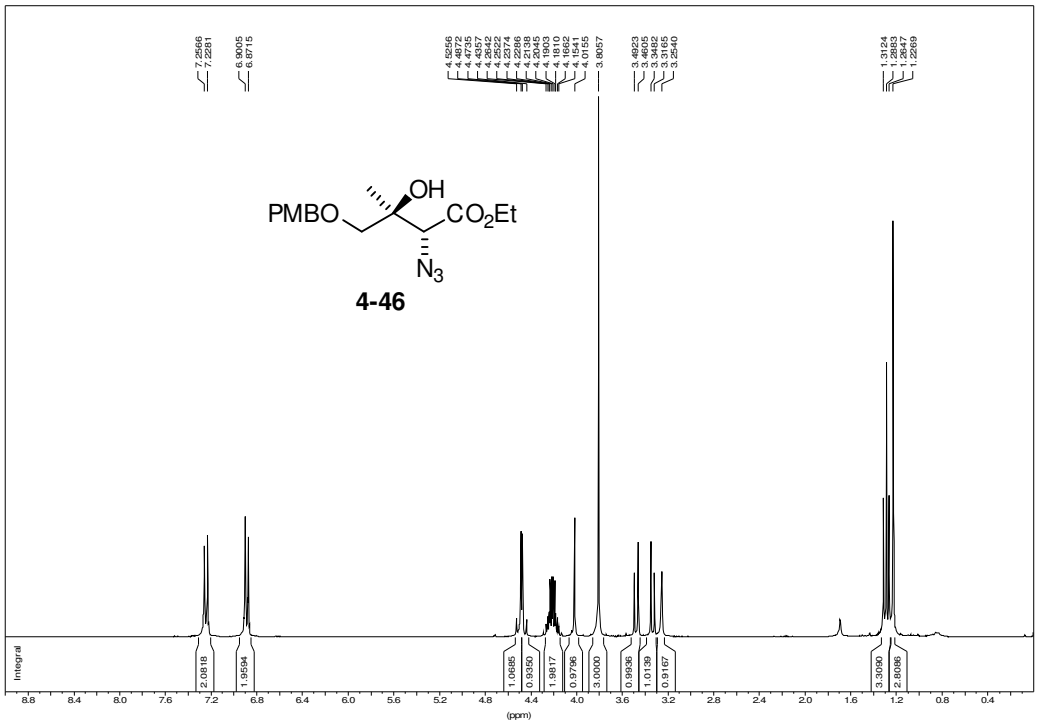

165.9309
159.1815
157.0287
130.3586
129.6531
129.2934
117.2381
113.7543
77.4329
77.0038
76.5820
72.3415
69.0108
59.7741
55.2285
21.6926
14.2305
(Z)
PMBO
CO2Et
4-92
(ppm)

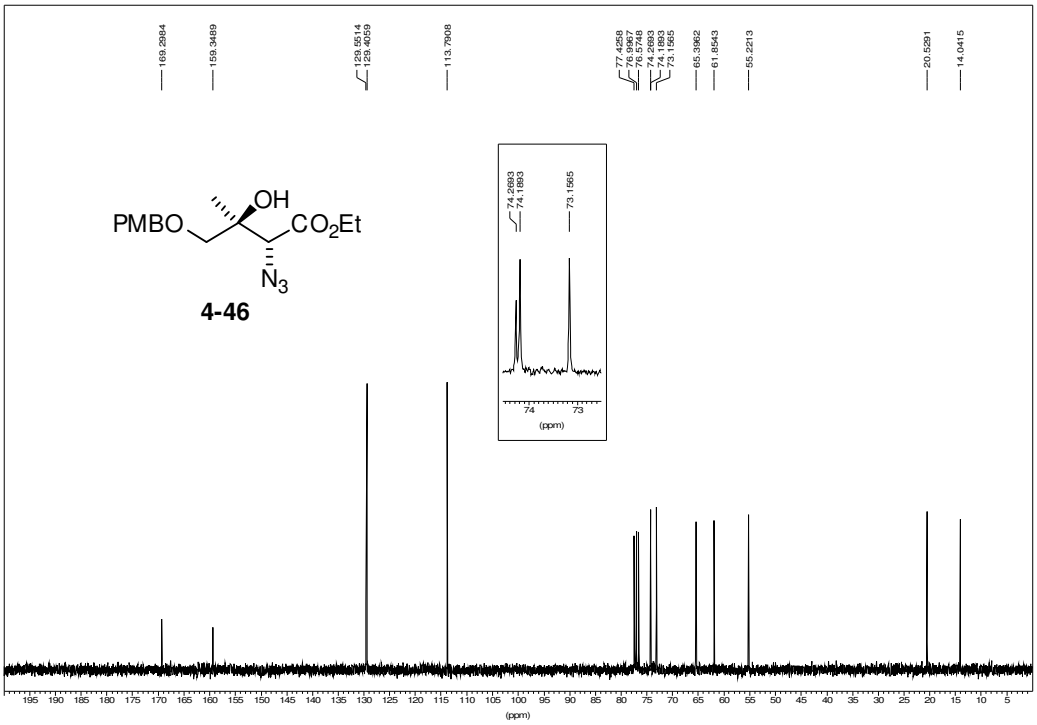

PMBO
OH
CO2Et
OH
4-44
Integral
(ppm)

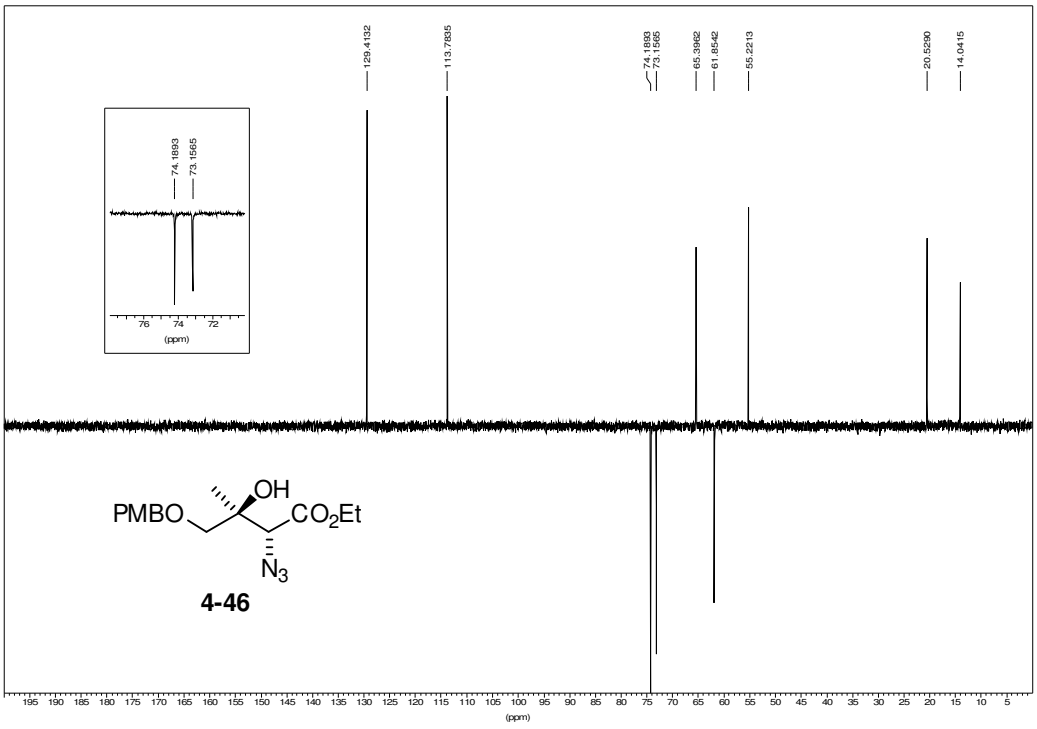

PMBO
OH
CO2Et
OH
4-44
(ppm)

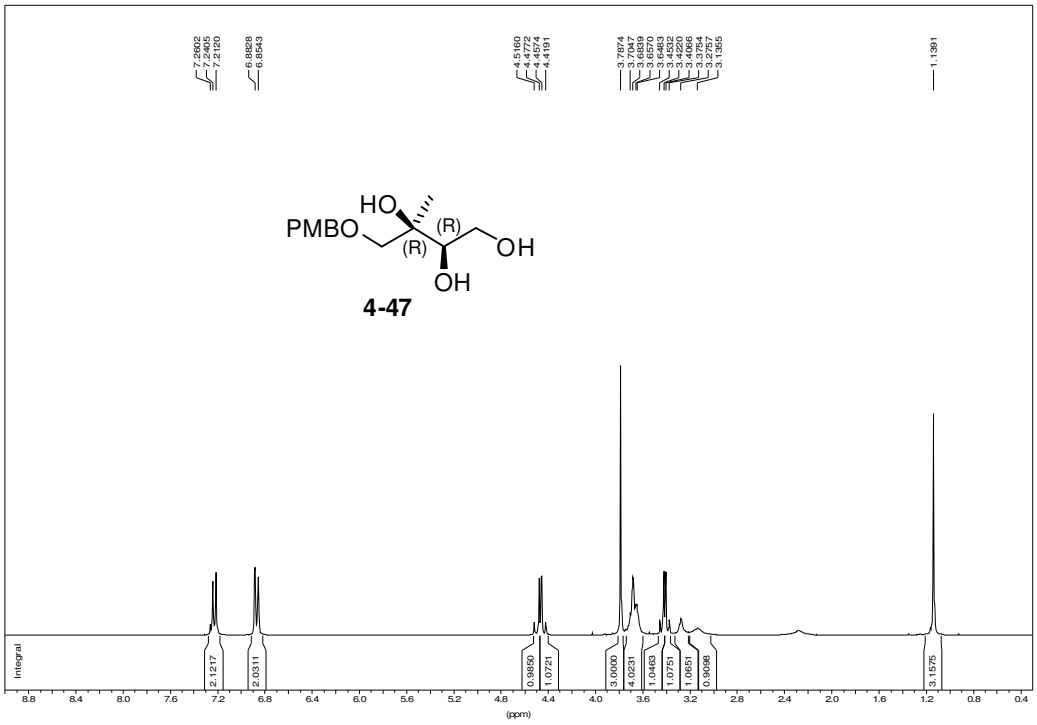
DEPT135 AMX500

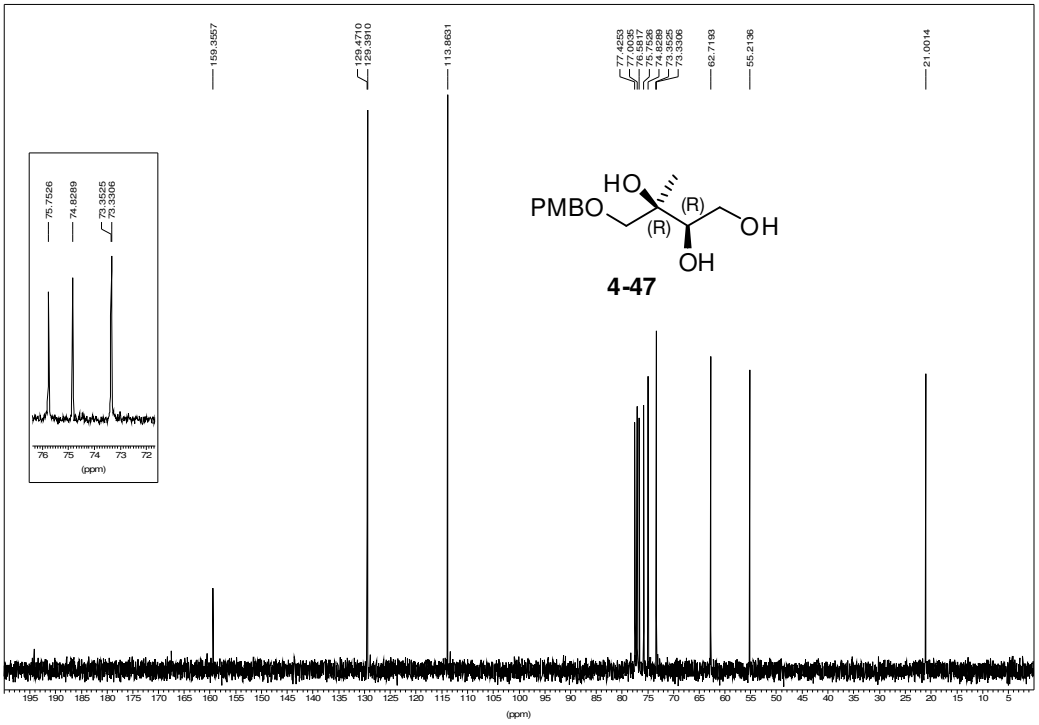




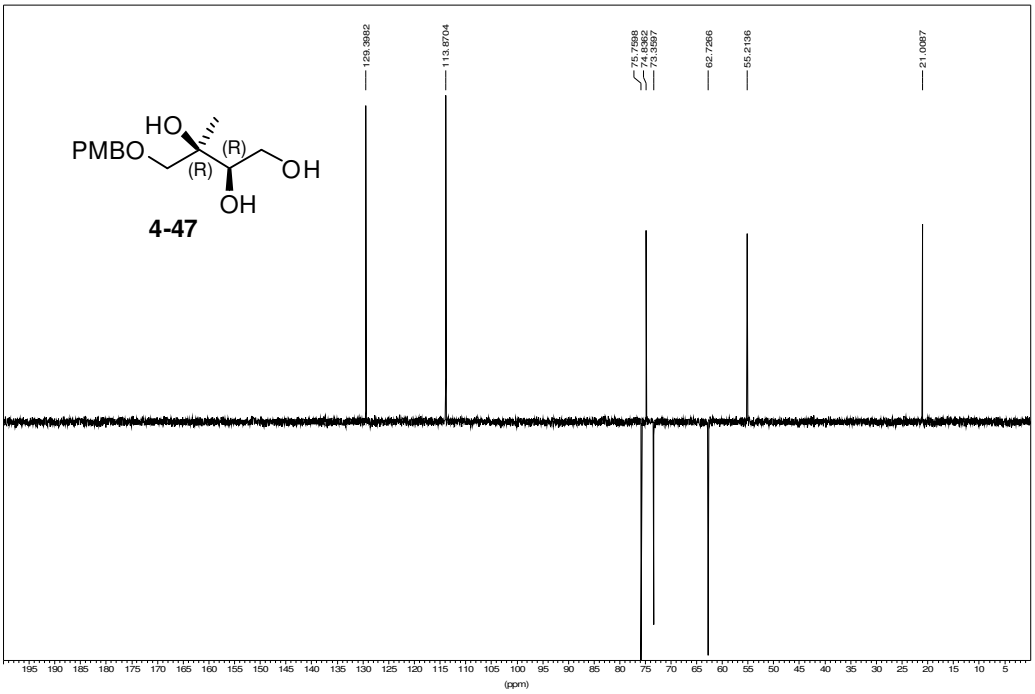

169.2984
159.3489
129.5514
129.4059
113.7908
77.4258
76.9967
76.5748
74.2993
74.1893
73.1565
65.3962
61.8543
55.2213
20.5291
14.0415
74.2993
74.1893
73.1565
74
73
(ppm)
OH
PMBO
CO2Et
N3
4-46
(ppm)


129.4132
113.7835
74.1893
73.1565
65.3982
61.8542
55.2213
20.5290
14.0415
74.1893
73.1565
76
74
72
(ppm)
OH
PMBO
CO2Et
N3
4-46
(ppm)

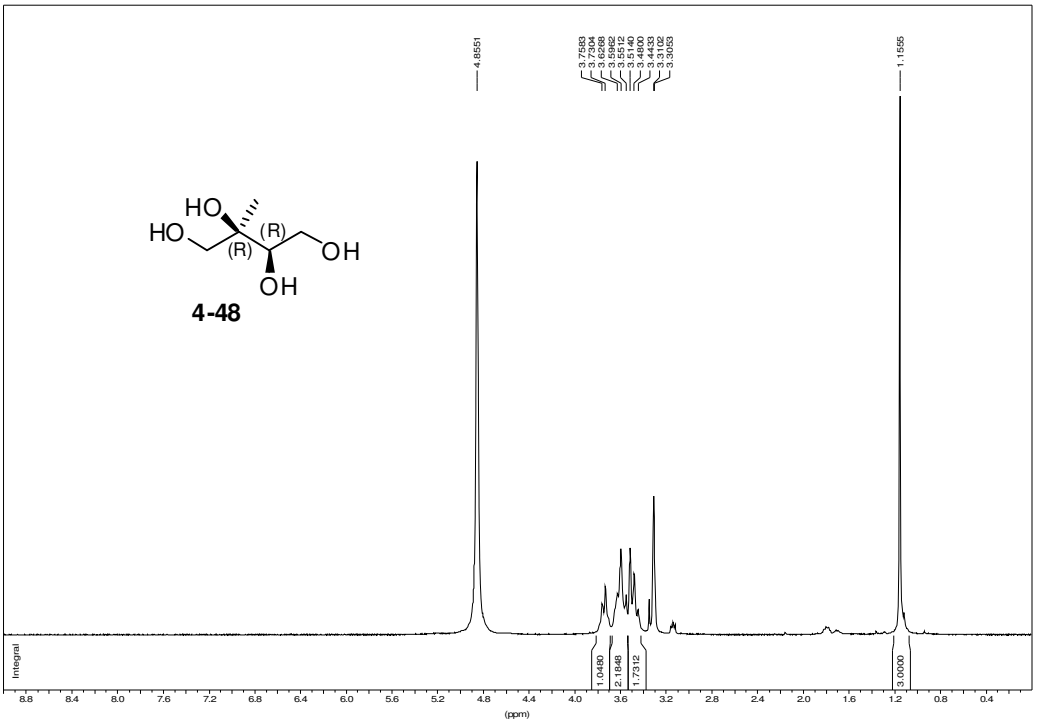

7.2602
7.2405
7.2160
6.8828
6.8543
4.5160
4.4772
4.4574
4.4191
3.7874
3.7047
3.6859
3.6570
3.6483
3.4552
3.4260
3.4066
3.3754
3.3757
3.1355
1.1391
HO
PMBO
(R)
(R)
OH
OH
4-47
Integral
2.1217
2.0311
0.9850
1.0721
3.0000
4.0231
1.0463
1.0751
1.0651
0.9098
3.1575
(ppm)


159.3557
129.4710
129.3910
113.8631
77.4253
77.0035
76.5817
75.7526
74.8289
73.3525
73.3306
62.7193
55.2136
21.0014
HO
PMBO
(R)
(R)
OH
OH
4-47
(ppm)

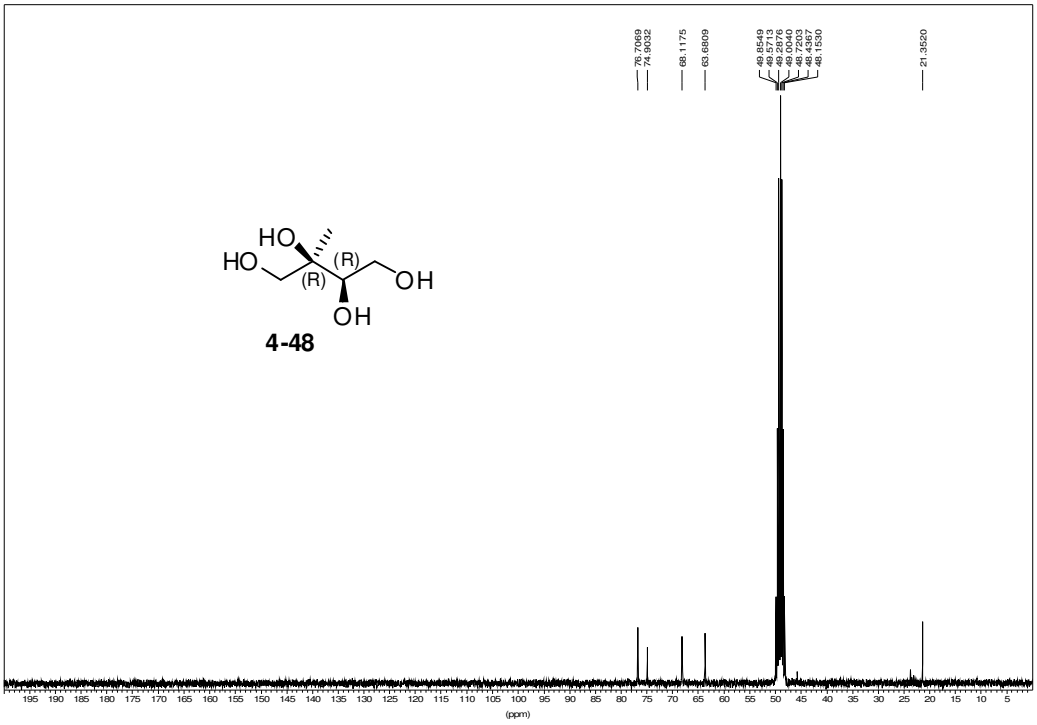
DEPT135 AC300

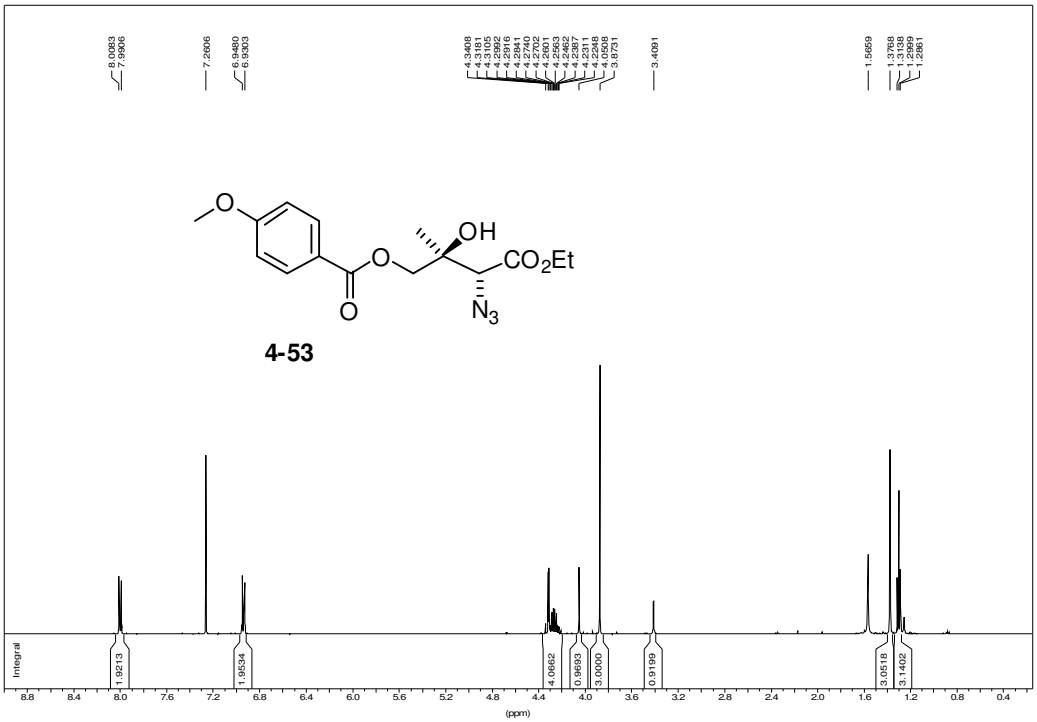


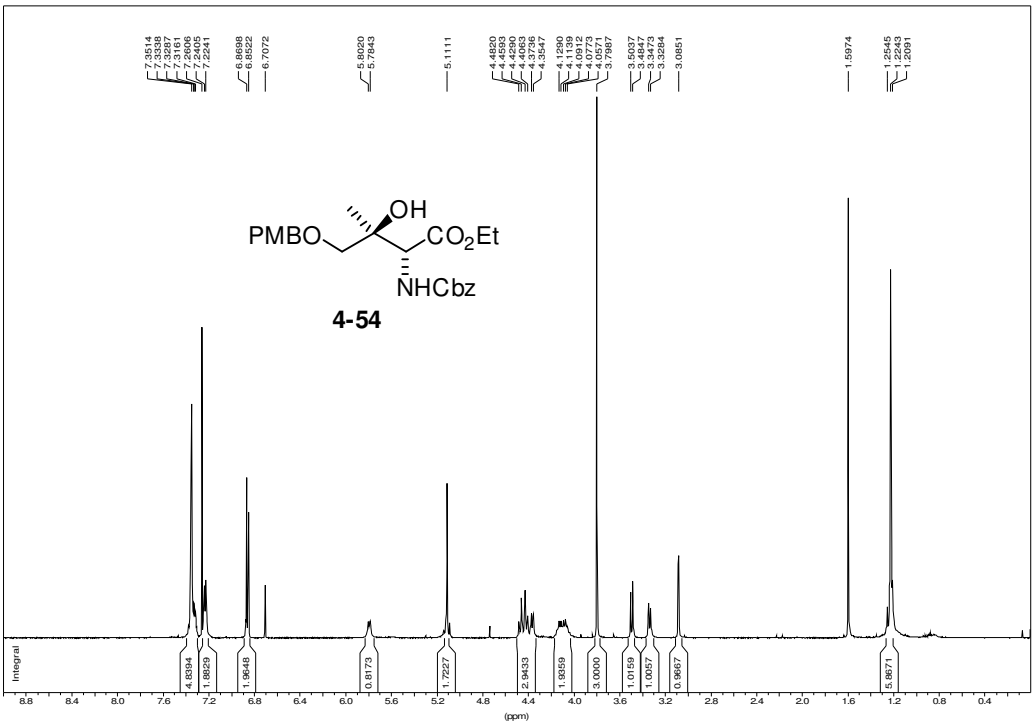
4-47

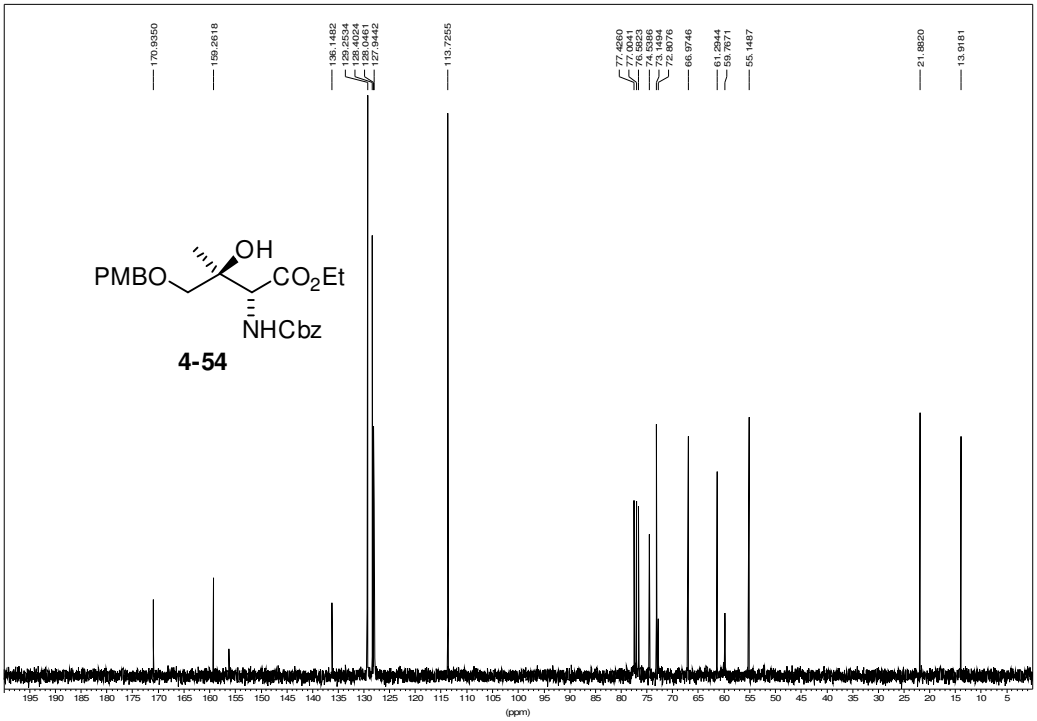


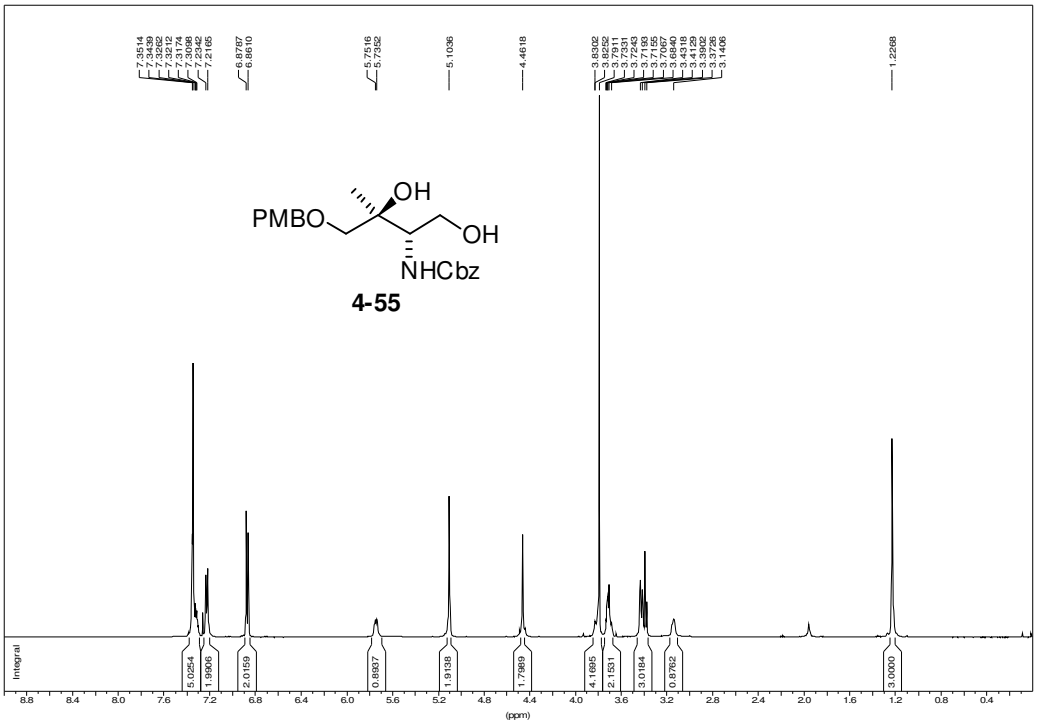
4-48

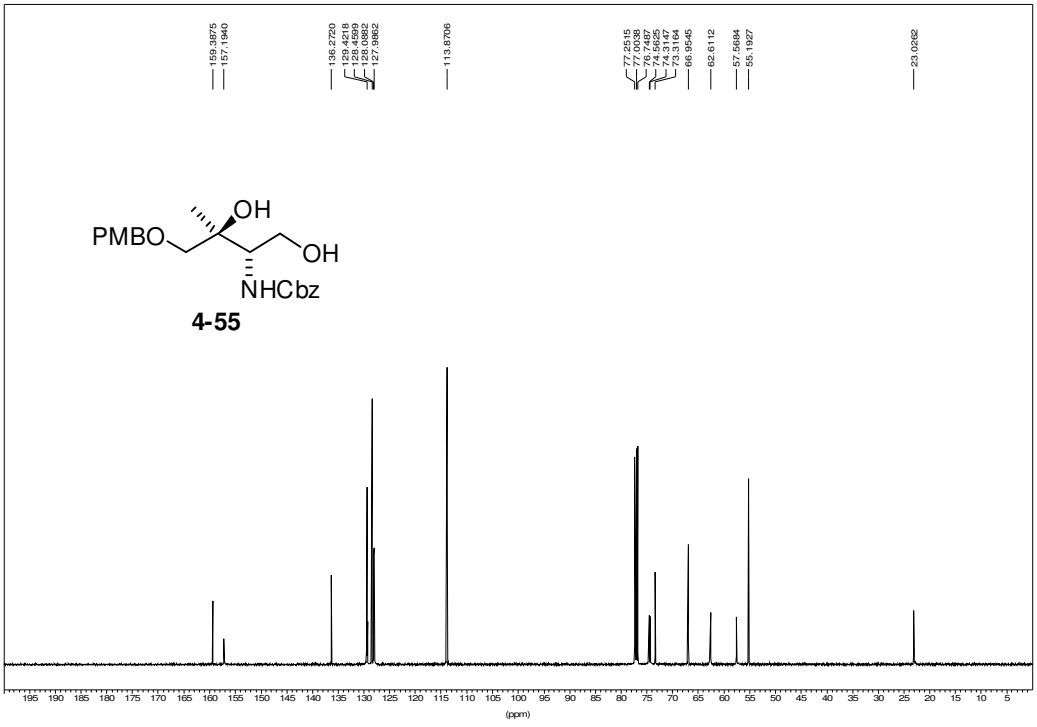

HO
HO
(R)
(R)
OH
OH
4-48
78.7069
74.9032
68.1175
63.6809
49.8549
49.5713
49.2876
49.0040
48.7203
48.4367
48.1530
21.3520
(ppm)

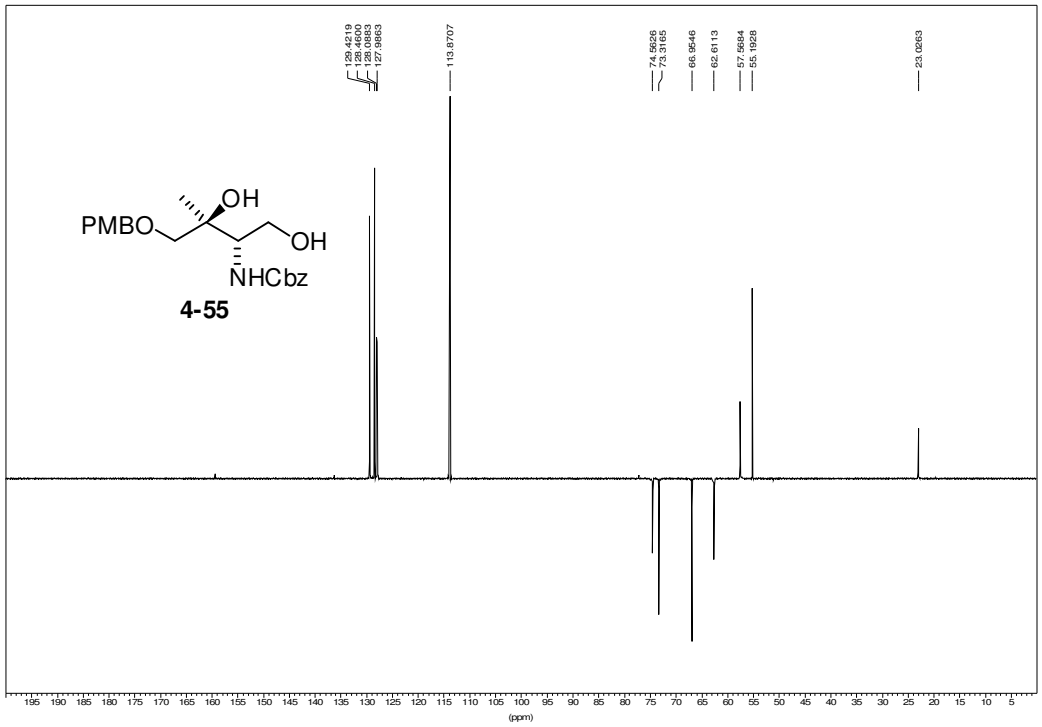

8.0083
7.9906
7.2606
6.9480
6.9303
4.3408
4.3181
4.3105
4.2992
4.2916
4.2841
4.2720
4.2702
4.2601
4.2583
4.2482
4.2387
4.2311
4.2248
4.0508
3.8731
3.4091
1.5659
1.3788
1.3138
1.2999
1.2861
O
OH
CO2Et
O
O
N3
4-53
Integral
1.9213
1.9534
4.0662
0.9693
3.0000
0.9199
3.0518
3.1402
(ppm)

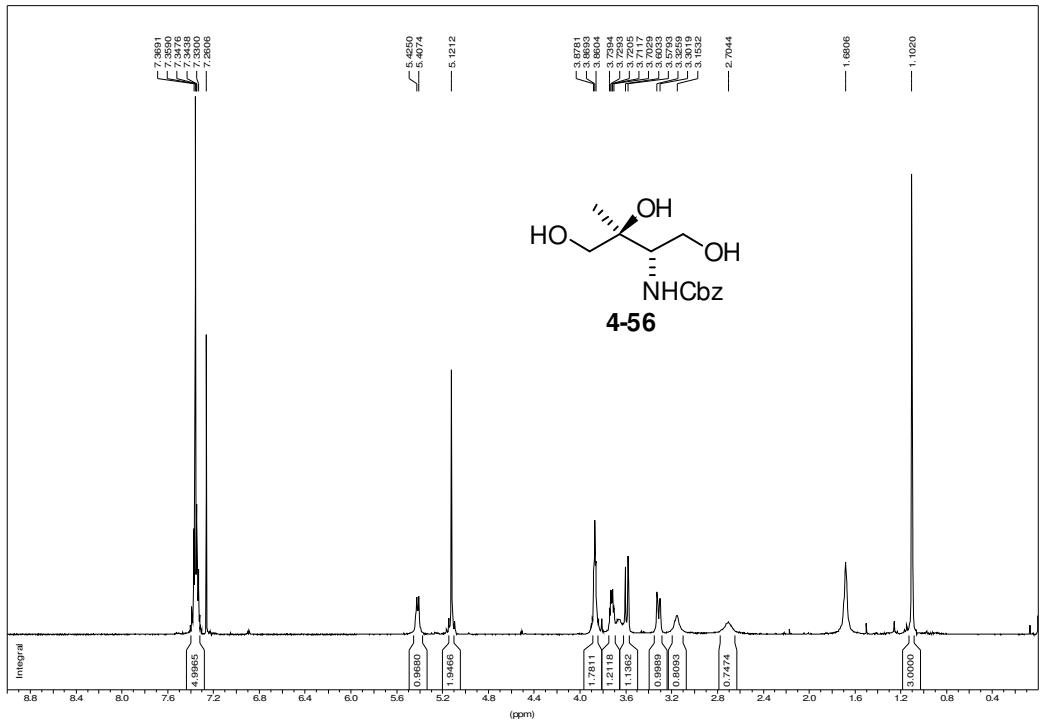

PMBO
OH
CO2Et
NHCbz
4-54
Integral
(ppm)

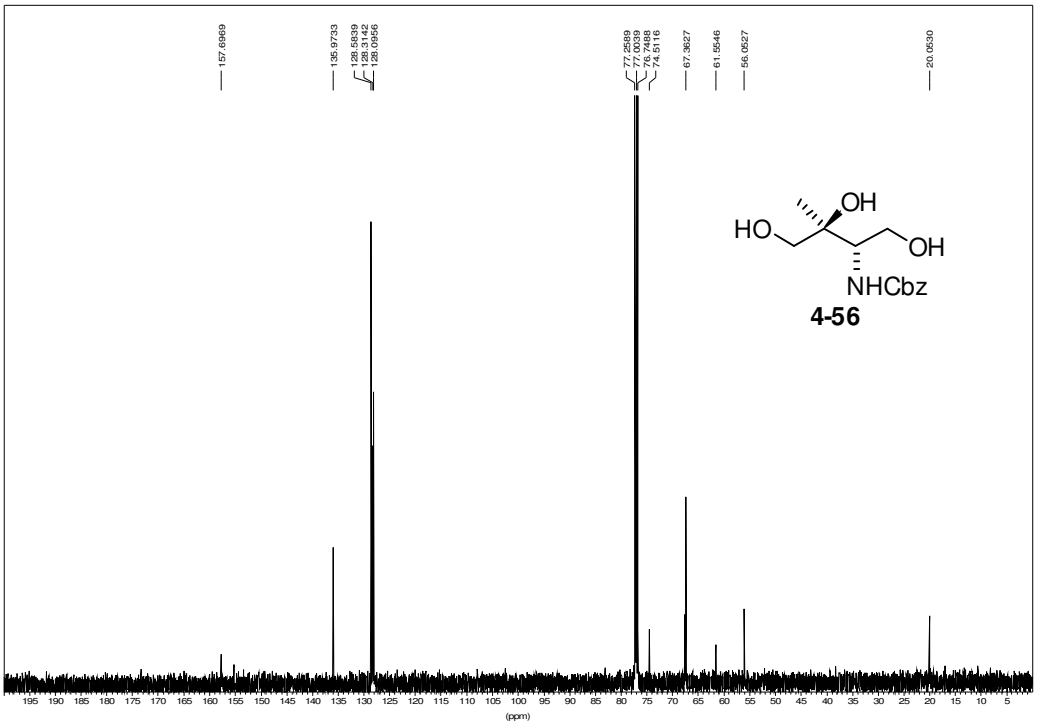

PMBO
OH
CO2Et
NHCbz
4-54
(ppm)

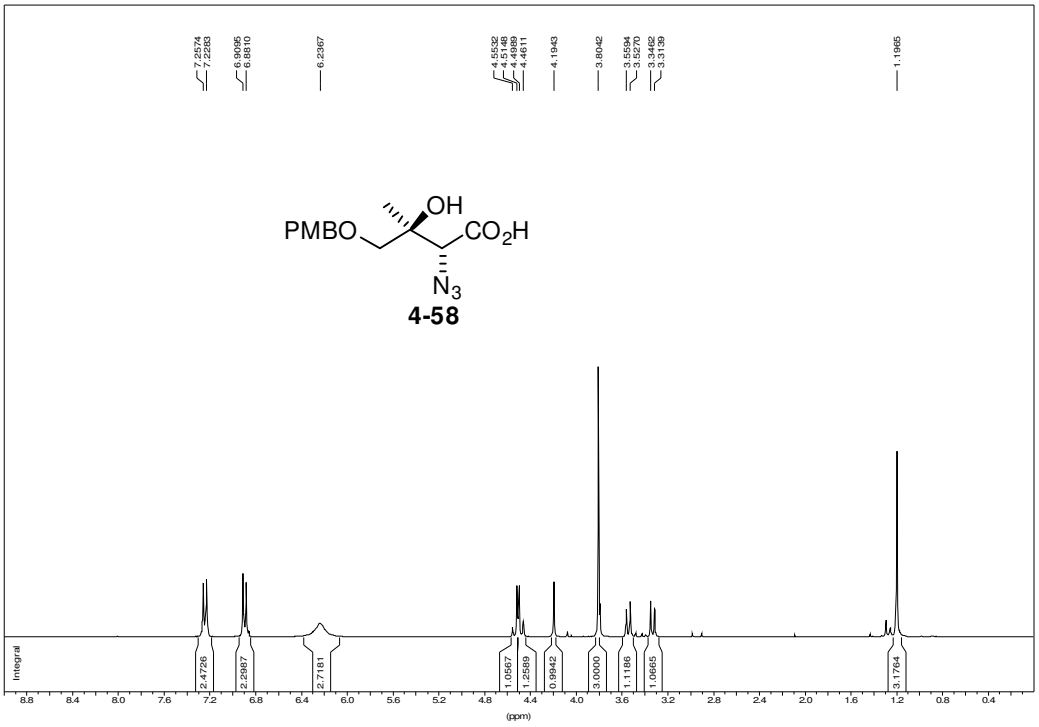

PMBO
OH
OH
NHCbz
4-55

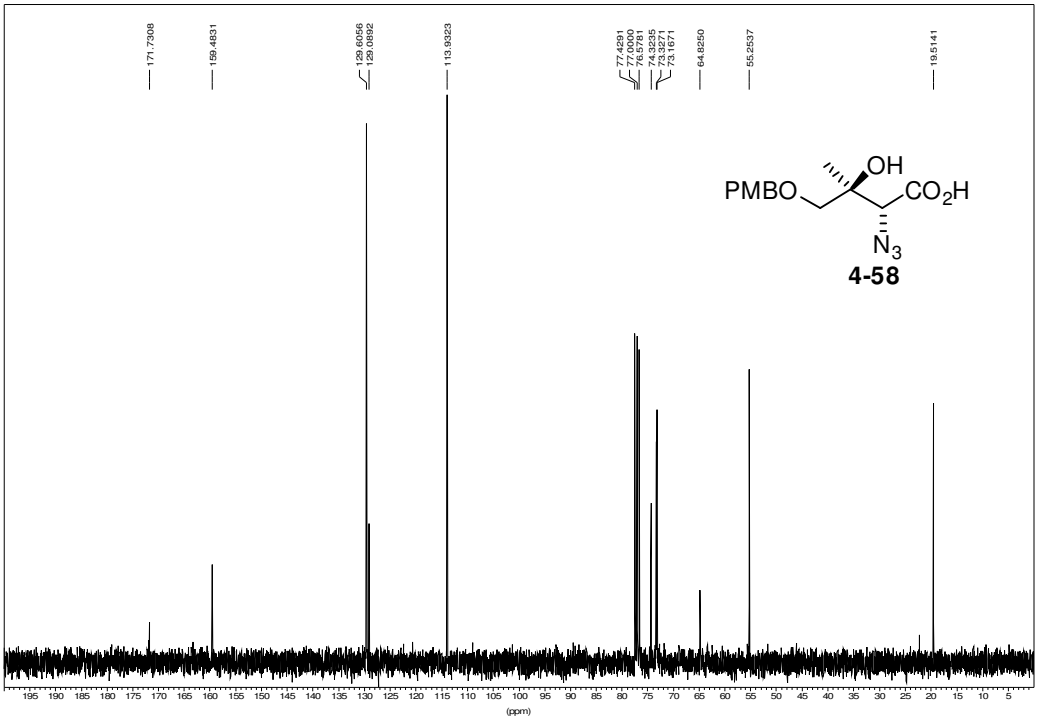

PMBO
OH
OH
NHCbz
4-55

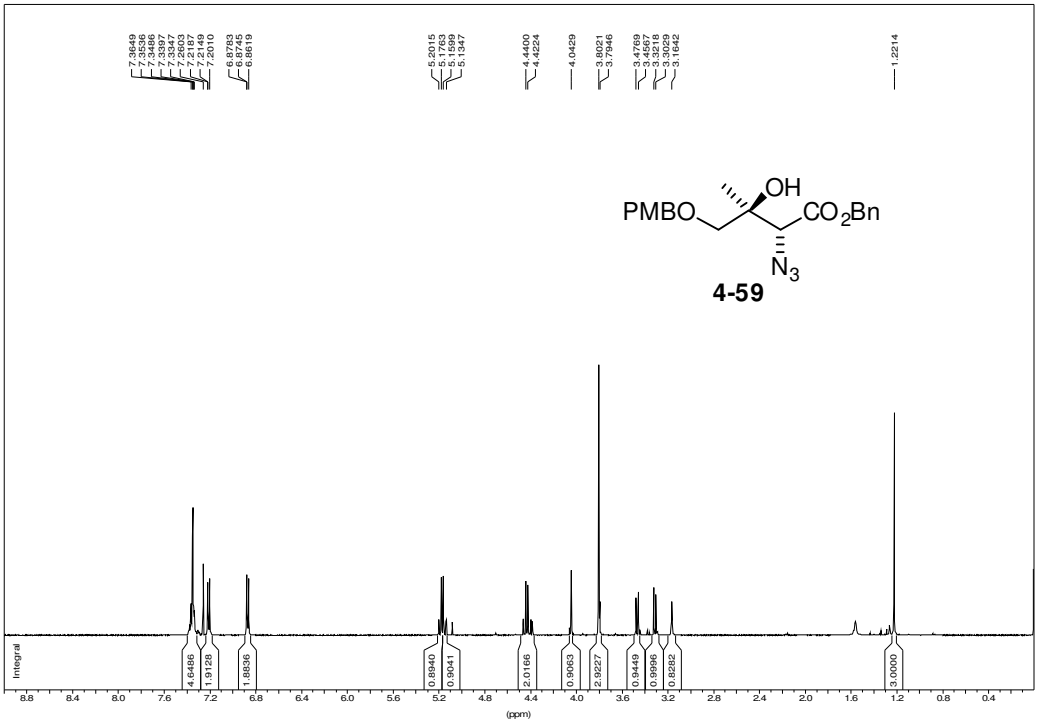

129.4219
128.4600
128.0883
127.9863
113.8707
74.5626
73.3165
66.9546
62.6113
57.5684
55.1928
23.0263
OH
PMBO
OH
NHCbz
4-55
(ppm)

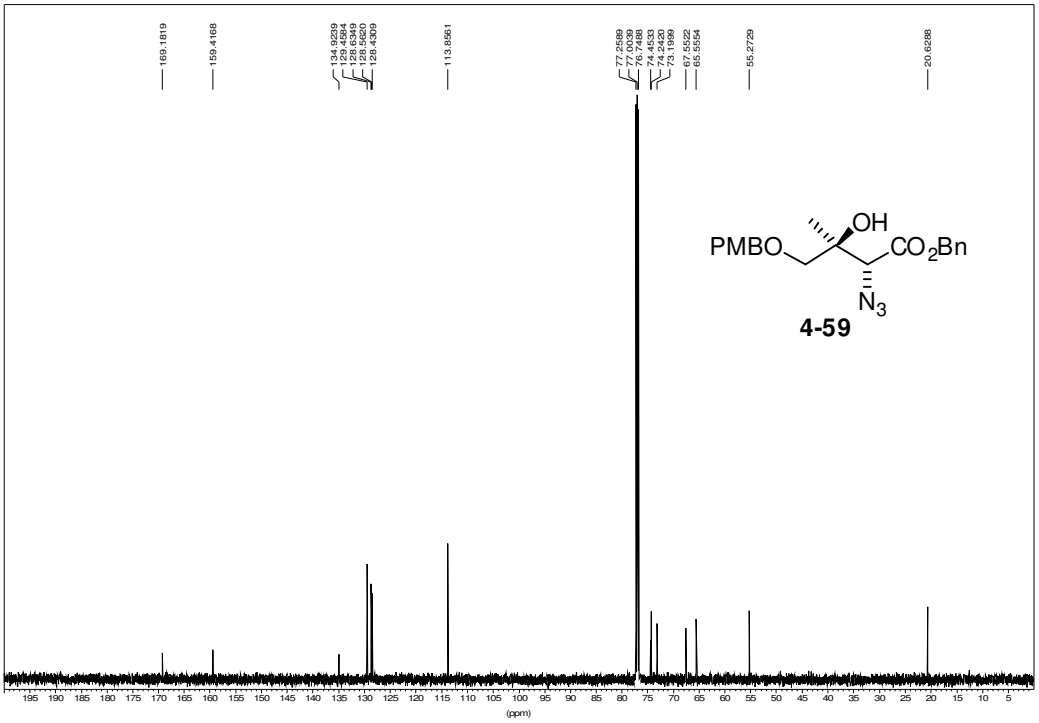

7.3691
7.3590
7.3476
7.3438
7.3300
7.2806
5.4250
5.4074
5.1212
3.8781
3.8693
3.8644
3.7394
3.7293
3.7205
3.7117
3.7029
3.6033
3.5793
3.3259
3.3019
3.1532
2.7044
1.6806
1.1020
OH
HO
OH
NHCbz
4-56
Integral
4.9985
0.9680
1.9466
1.7811
1.2118
1.1362
0.9989
0.8093
0.7474
3.0000
(ppm)

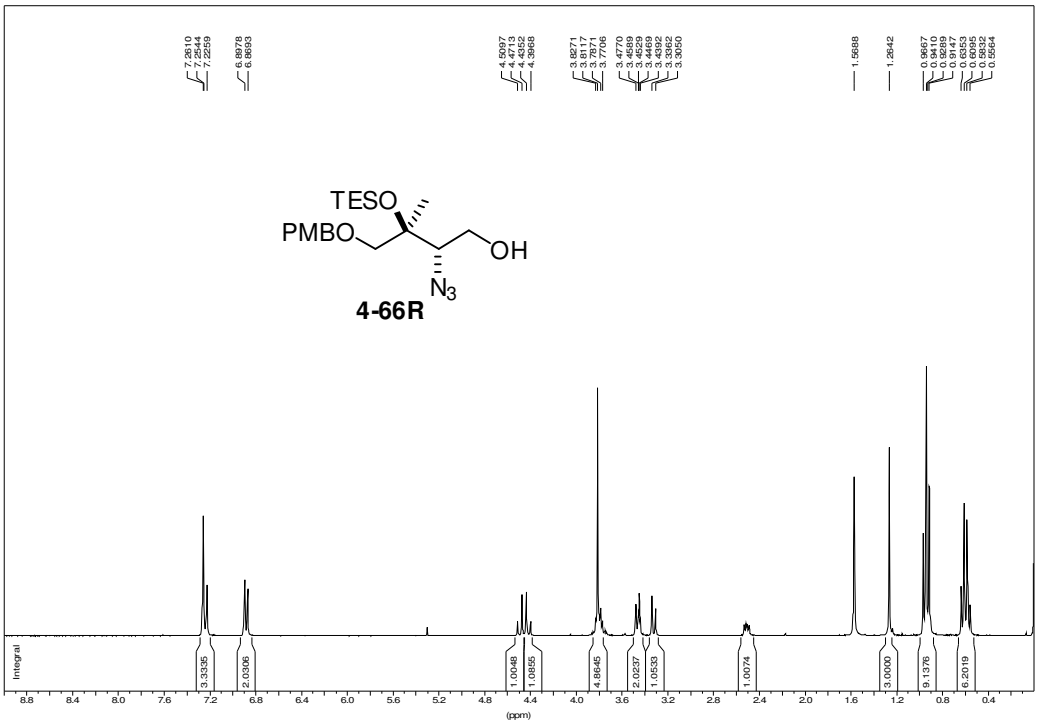

157.6969
135.9733
129.5839
129.3142
129.0956
77.2589
77.0063
76.7488
74.5116
67.3627
61.5546
56.0527
20.0530
OH
HO
OH
NHCbz
4-56
(ppm)

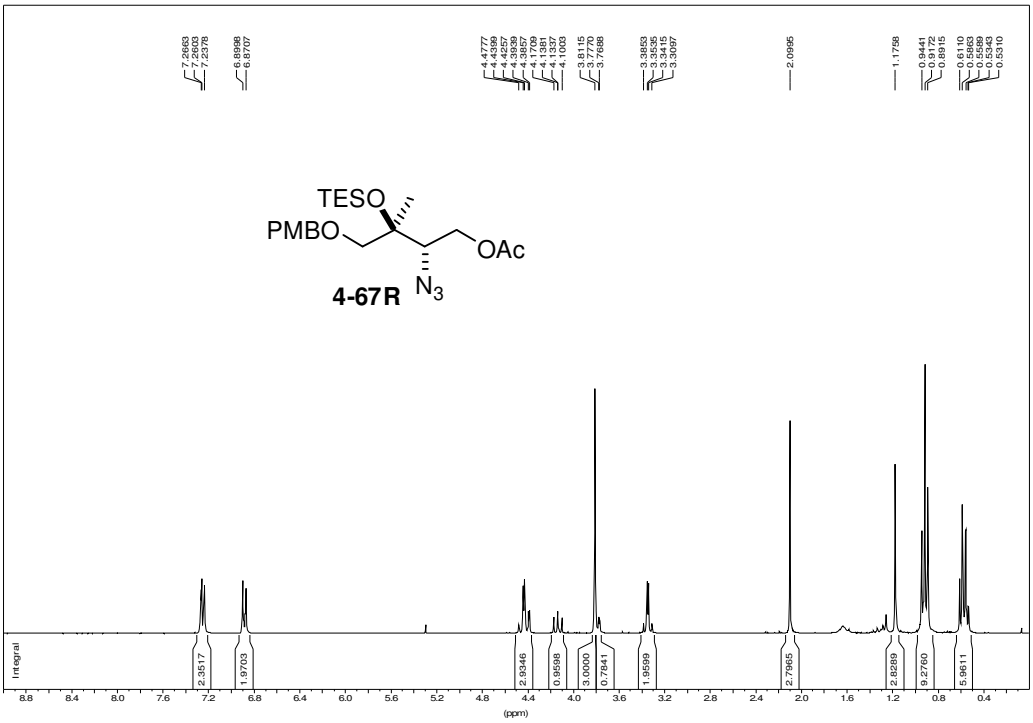

7.2574
7.2283
6.9095
6.8810
6.2367
4.5532
4.5148
4.4899
4.4611
4.1943
3.8042
3.5594
3.5270
3.3462
3.3139
1.1965
OH
PMBO
CO2H
N3
4-58
Integral
2.4726
2.2987
2.7181
1.0567
1.2589
0.9942
3.0000
1.1186
1.0665
3.1764
(ppm)

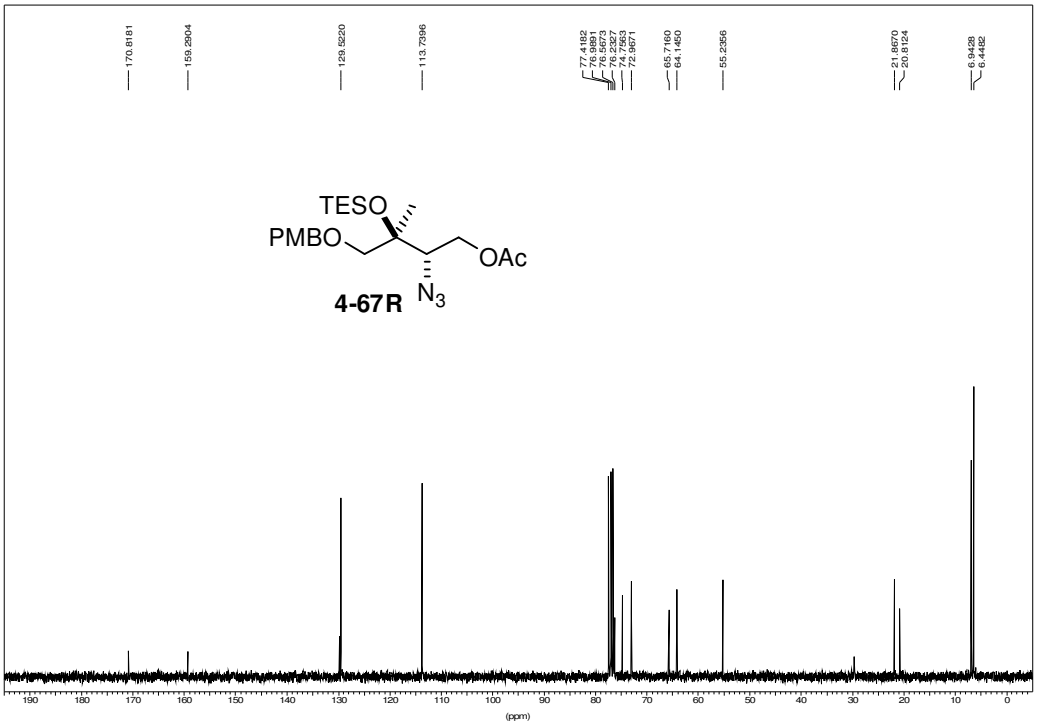

171.7308
159.4831
129.6056
129.0092
113.9323
77.4291
77.0000
76.5781
74.3095
73.3271
73.1671
64.8250
55.2537
19.5141
OH
PMBO
CO2H
N3
4-58

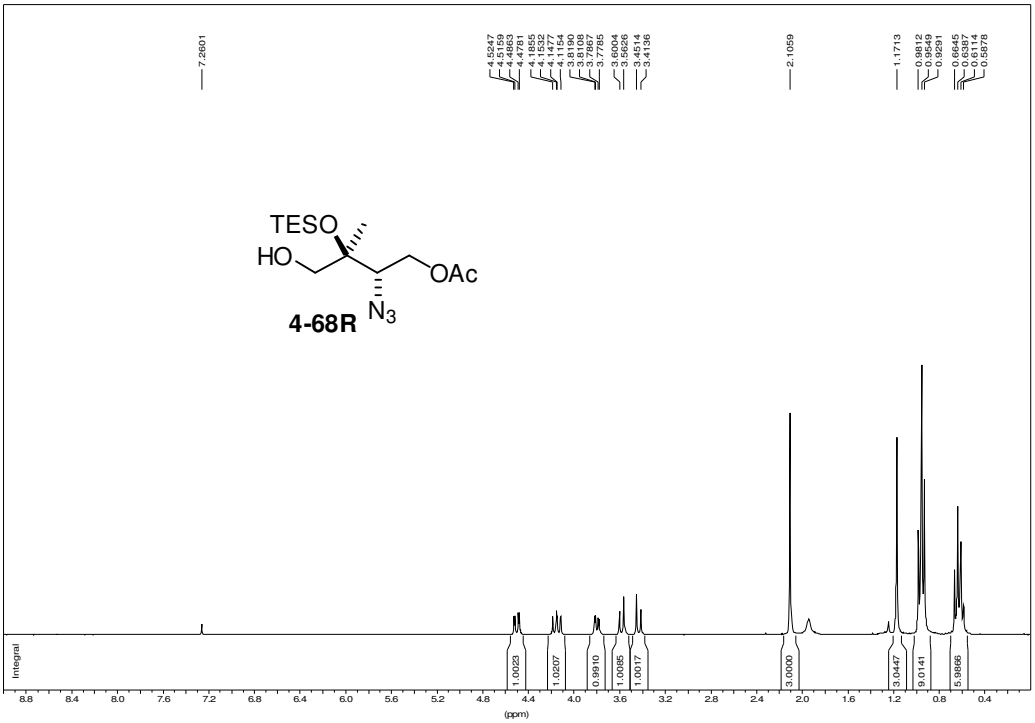

7.3649
7.3536
7.3486
7.3397
7.3347
7.2663
7.2437
7.2149
7.2010
6.8783
6.8745
6.8619
5.2015
5.1763
5.1599
5.1347
4.4400
4.4224
4.0429
3.8021
3.7946
3.4769
3.4567
3.3218
3.3059
3.1642
1.2214
OH
PMBO
CO2Bn
N3
4-59
Integral
4.6486
1.9128
1.8836
0.8940
0.9041
2.0166
0.9063
2.9227
0.9449
0.9996
0.8282
3.0000

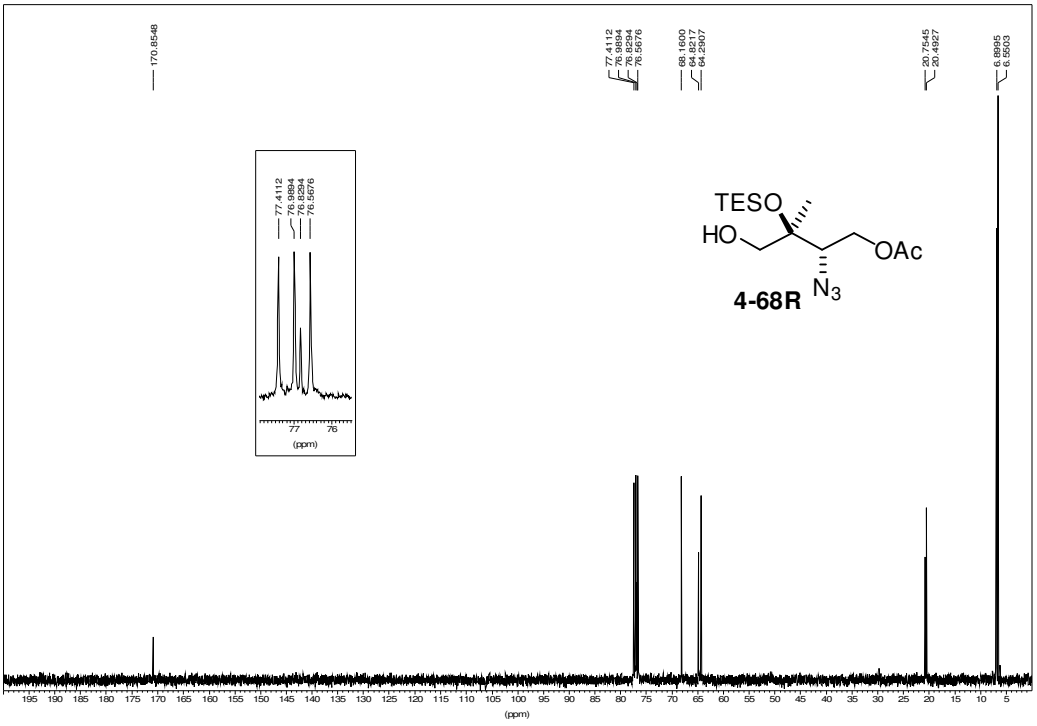

169.1619
159.4166
134.9239
129.4964
128.6349
128.5620
128.4309
113.8561
77.2589
77.0039
76.7488
74.4553
74.2420
73.1999
67.5522
65.5554
55.2729
20.6288
PMBO
OH
CO2Bn
N3
4-59
(ppm)

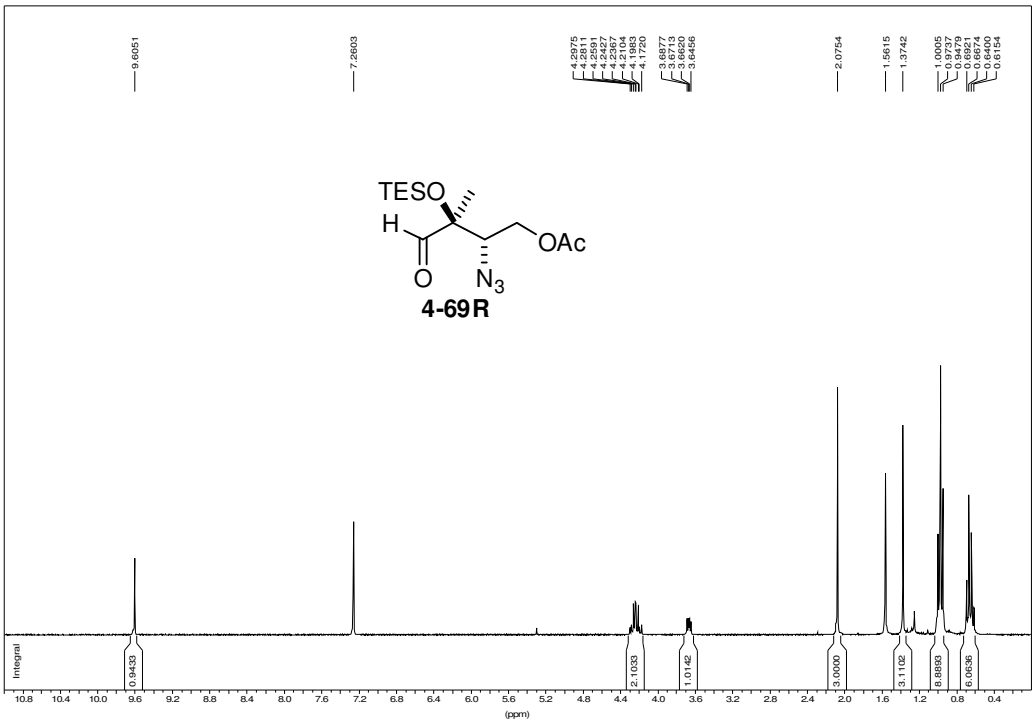

7.2610
7.2544
7.2259
6.8978
6.8693
4.5097
4.4753
4.4252
4.3968
3.8271
3.8117
3.7970
3.7706
3.4770
3.4589
3.4459
3.4469
3.4392
3.3862
3.3650
1.5688
1.2642
0.9667
0.9419
0.9289
0.9147
0.6353
0.6055
0.5832
0.5564
TESO
PMBO
OH
N3
4-66R
Integral
3.3335
2.0306
1.0048
1.0855
4.8645
2.0237
1.0533
1.0074
3.0000
9.1376
6.2019
(ppm)

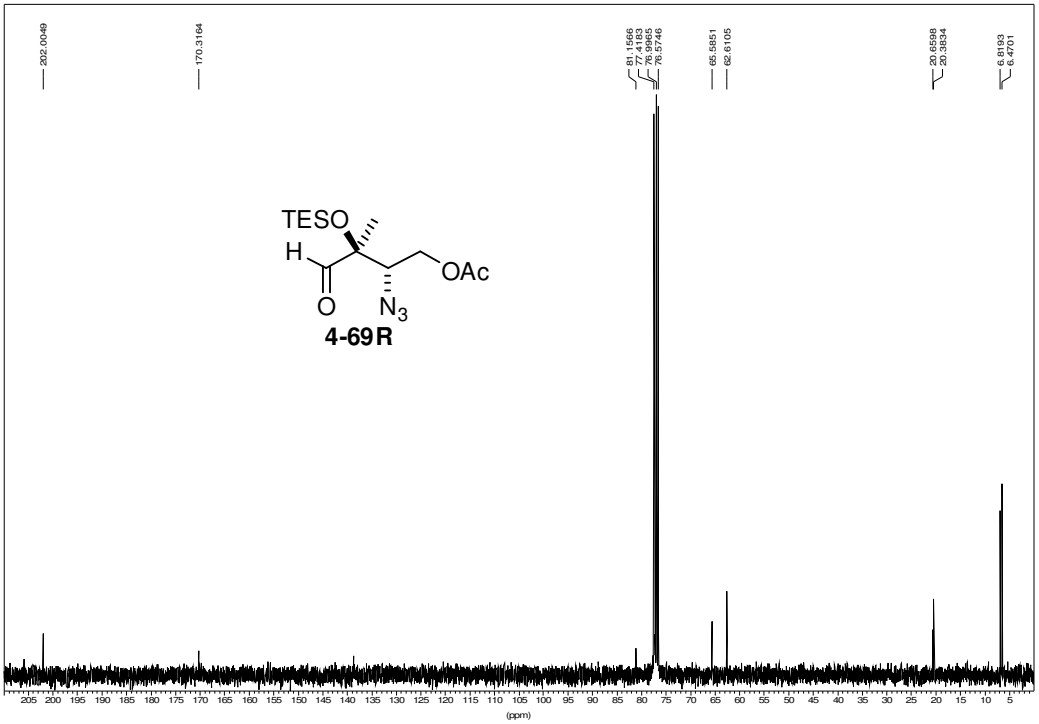

7.2663
7.2603
7.2378
6.8998
6.8707
4.4777
4.4499
4.4257
4.3839
4.3555
4.1709
4.1381
4.1037
4.1003
3.8115
3.7770
3.7688
3.3853
3.3535
3.3415
3.3097
2.0995
1.1798
0.9441
0.9172
0.8915
0.6110
0.5585
0.5589
0.5343
0.5310
TESO
PMBO
OAc
N3
4-67R
Integral
2.3517
1.9703
2.9346
0.9598
3.0000
0.7841
1.9599
2.7965
2.8289
9.2760
5.9611
(ppm)

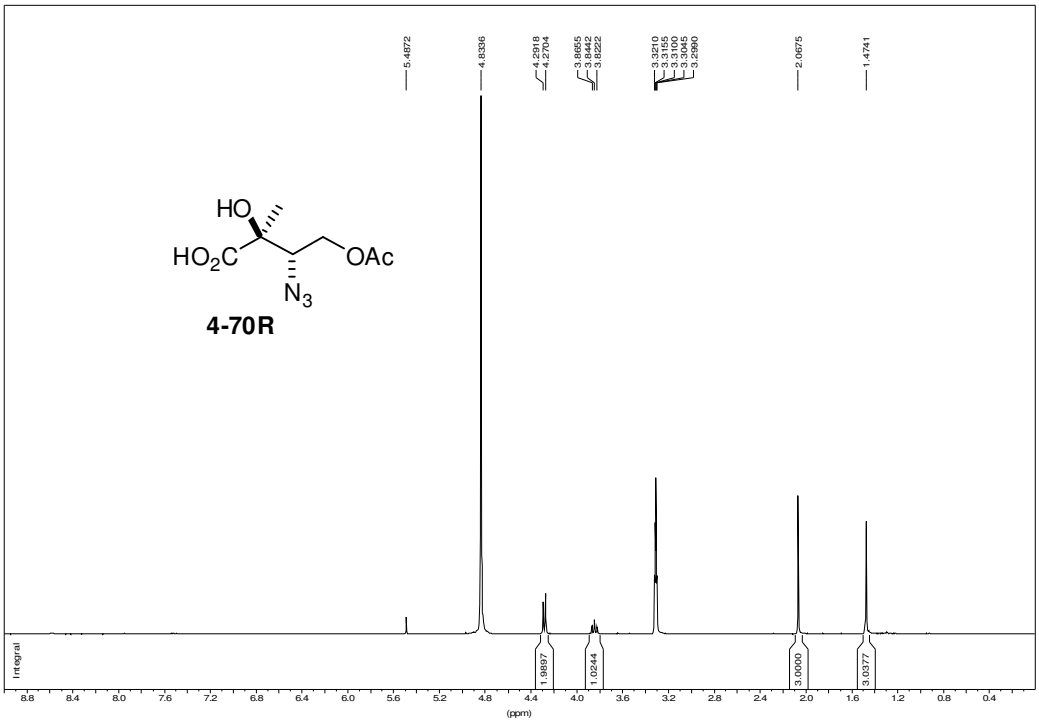

170.8181
159.2904
129.5220
113.7396
77.4182
76.9981
76.5673
76.2367
74.3963
72.9671
65.7160
64.1450
55.2356
21.8670
20.8124
6.9428
6.4482
TESO
PMBO
OAc
N3
4-67R
(ppm)

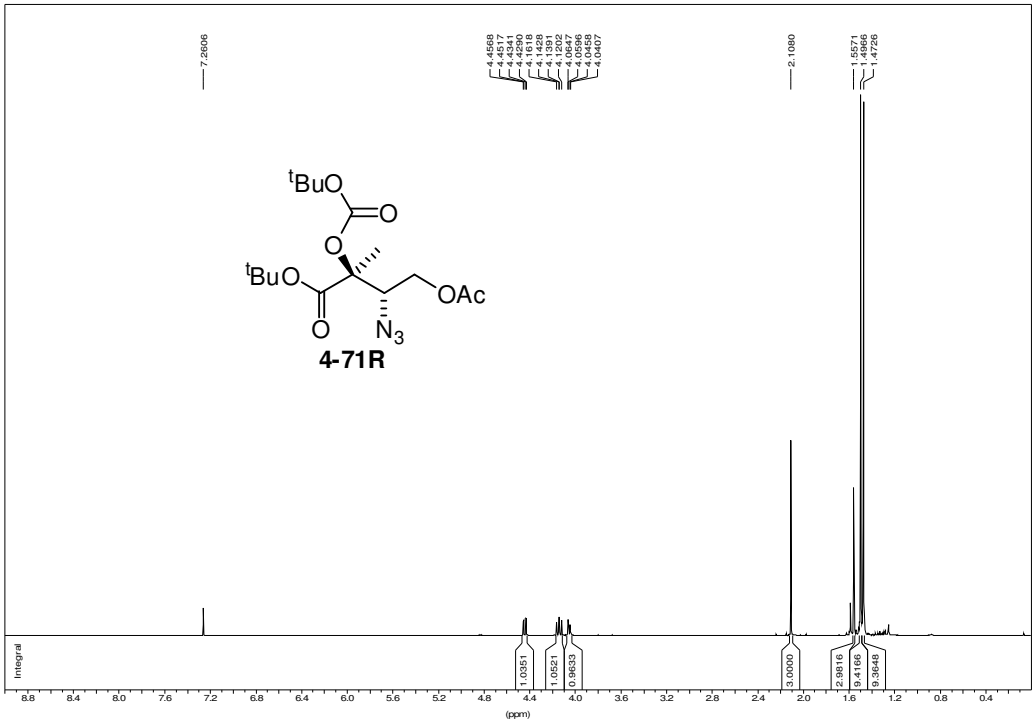

TESO
HO
OAc
N3
4-68R
Integral
(ppm)

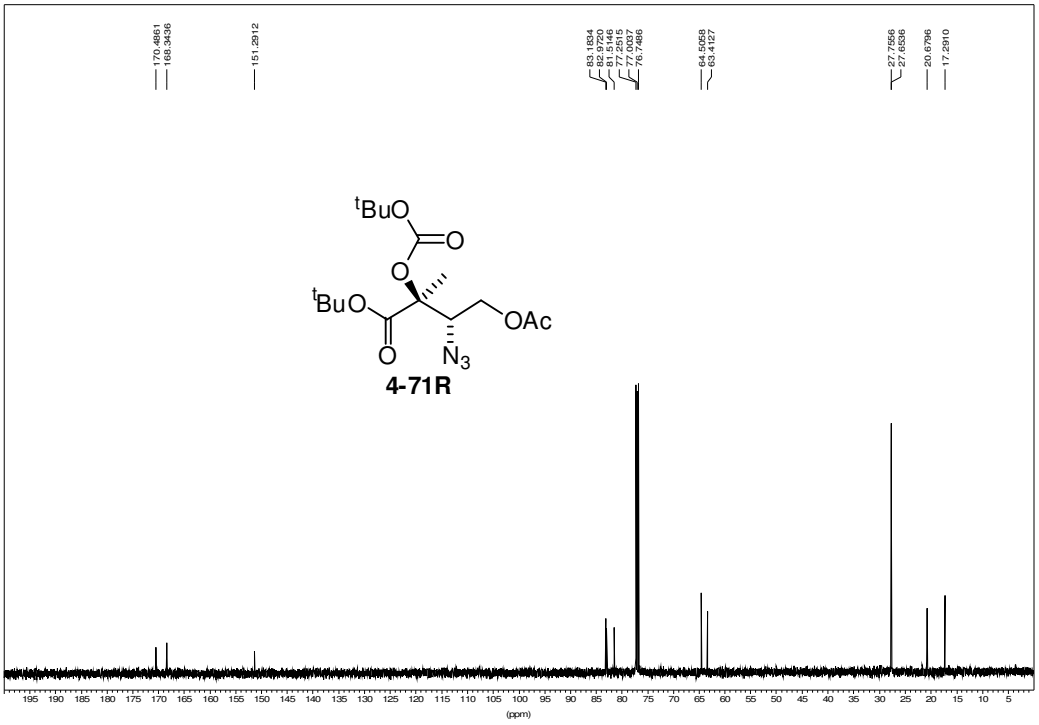

TESO
HO
OAc
N3
4-68R
(ppm)

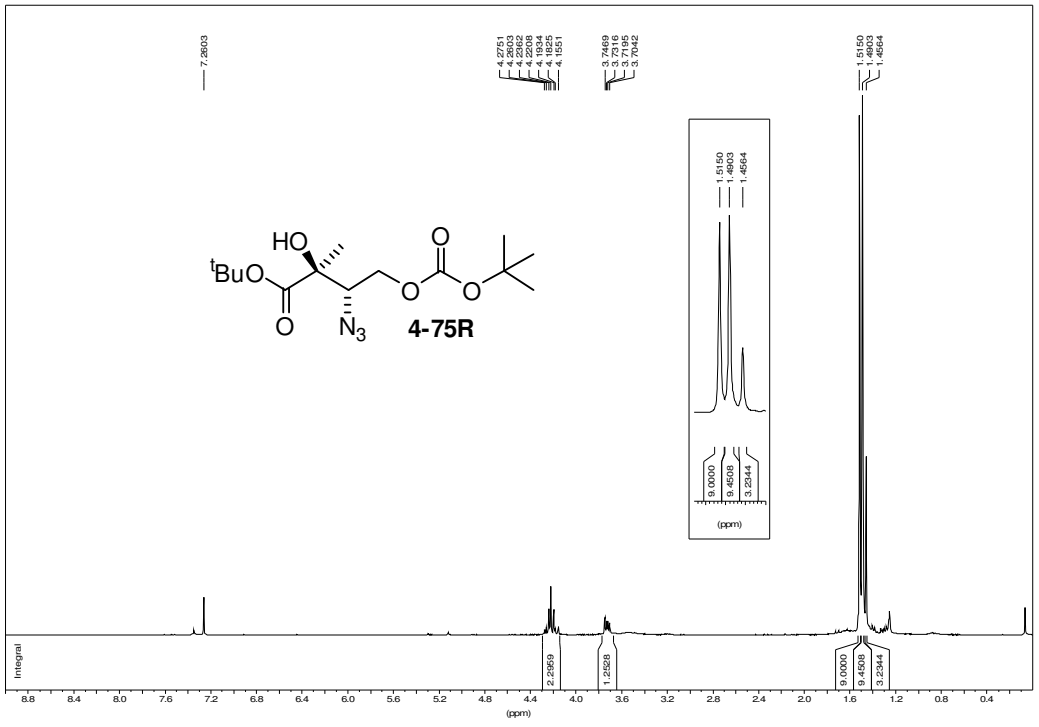

9.6051
7.2603
4.2975
4.2811
4.2581
4.2427
4.2387
4.2164
4.1983
4.1720
3.6877
3.6713
3.6620
3.6456
2.0754
1.5615
1.3742
1.0005
0.9737
0.9579
0.6921
0.6674
0.6490
0.6154
TESO
H
O
OAc
N3
4-69R
Integral
0.9433
2.1033
1.0142
3.0000
3.1102
8.8893
6.0636
(ppm)

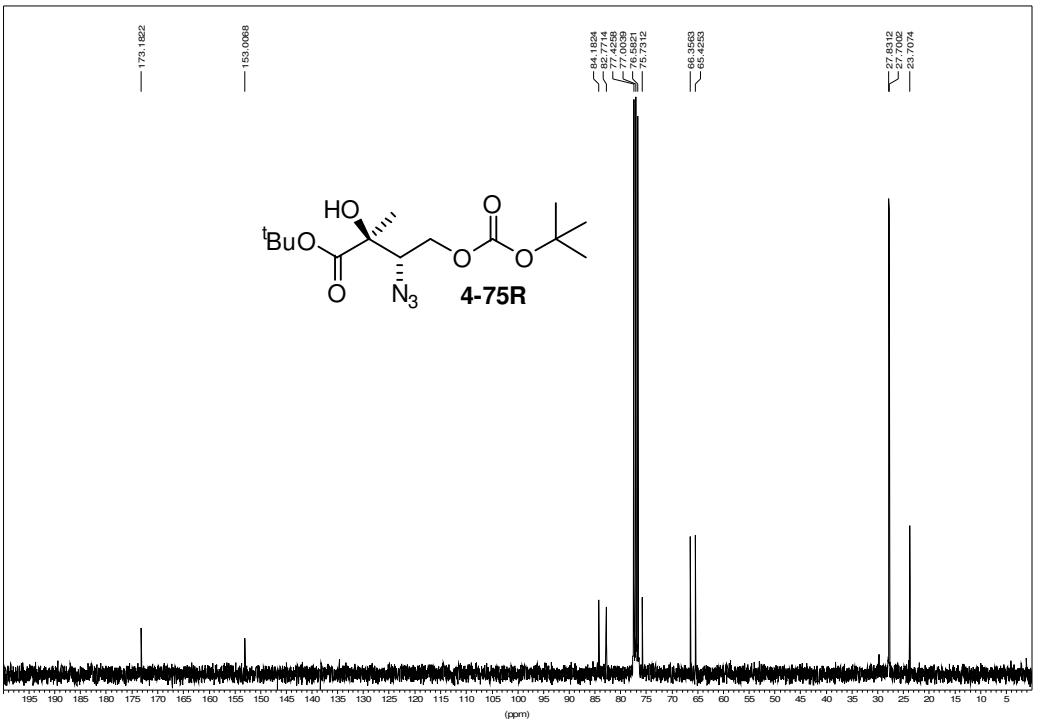

202.0049
170.3164
81.1566
77.4163
76.9965
76.5746
65.5851
62.6105
20.6598
20.3834
6.8193
6.4701
TESO
H
O
OAc
N3
4-69R
(ppm)

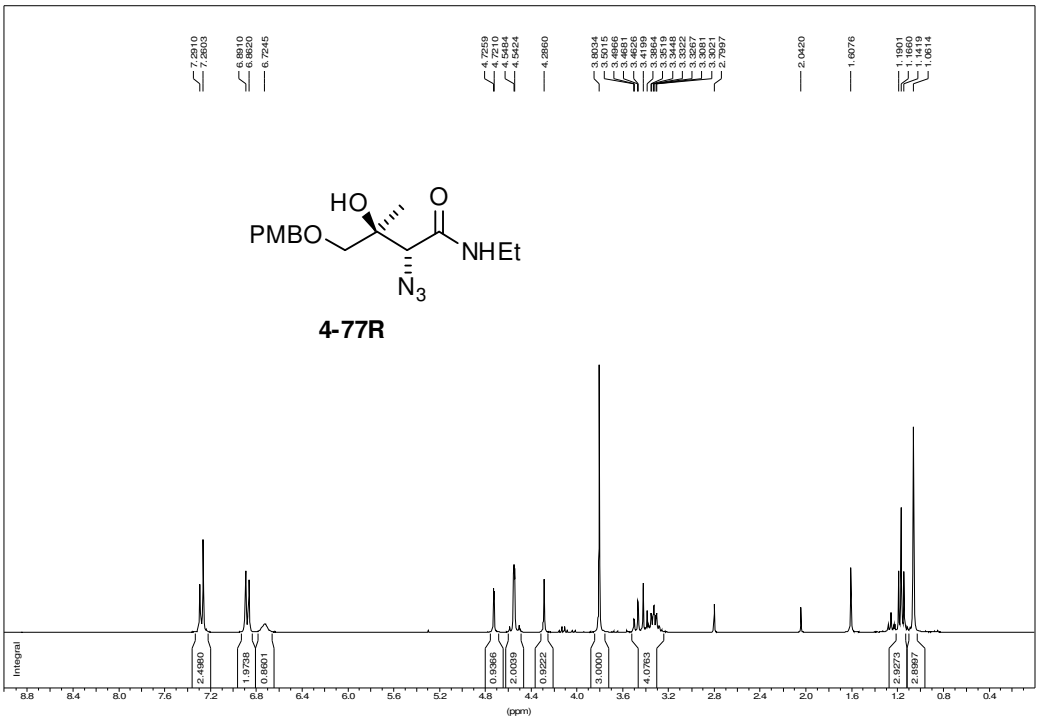

5.4972
4.8336
4.2918
4.2704
3.8655
3.8442
3.8222
3.3210
3.3155
3.3100
3.3045
3.2980
2.0675
1.4741
HO
HO2C
OAc
N3
4-70R
Integral
1.9897
1.0244
3.0000
3.0377
(ppm)

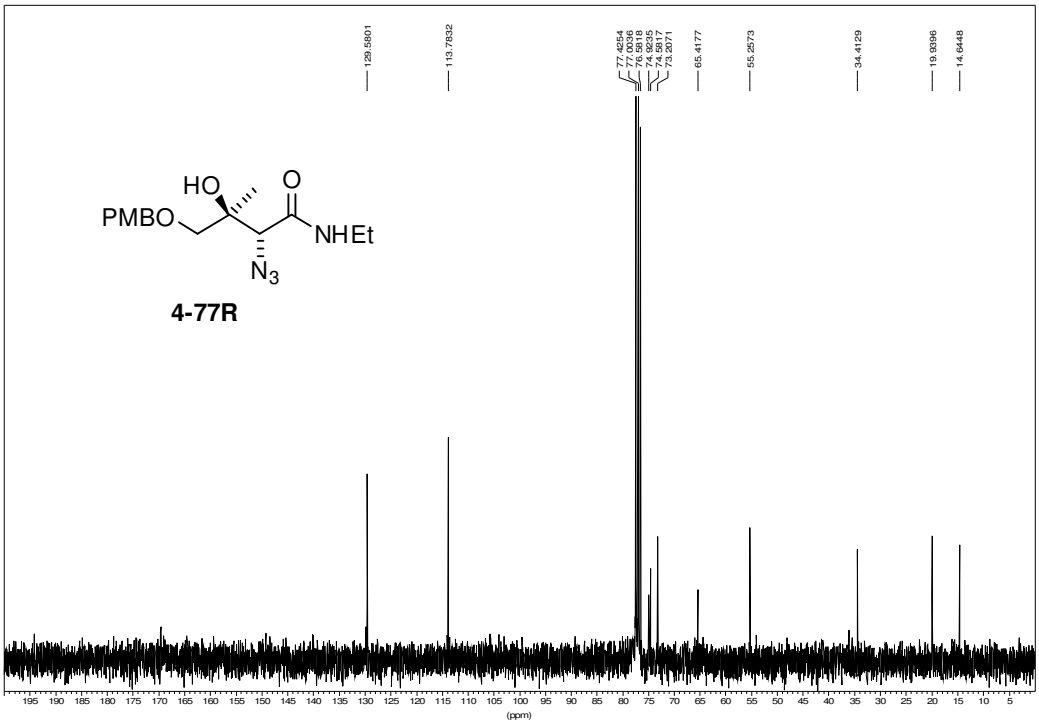

7.2606
4.4568
4.4517
4.4464
4.4290
4.1618
4.1459
4.1391
4.1202
4.0697
4.0596
4.0458
4.0407
2.1060
1.5571
1.4966
1.4726
tBuO
O
tBuO
OAc
N3
4-71R
Integral
1.0351
1.0521
0.9633
3.0000
2.9816
9.4166
9.3644
(ppm)

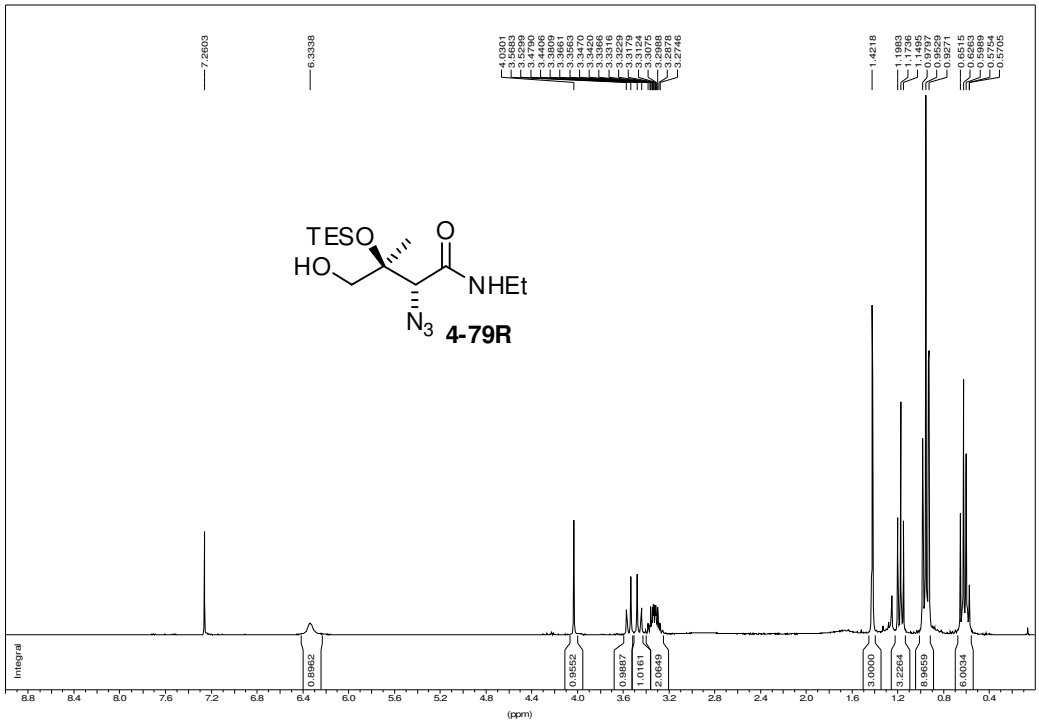

170.4861
168.3436
151.2912
83.1834
82.9720
81.5146
77.2515
77.0037
76.7486
64.5058
63.4127
27.7556
27.6586
20.6796
17.2910
tBuO
O
O
tBuO
OAc
O
N3
4-71R
(ppm)

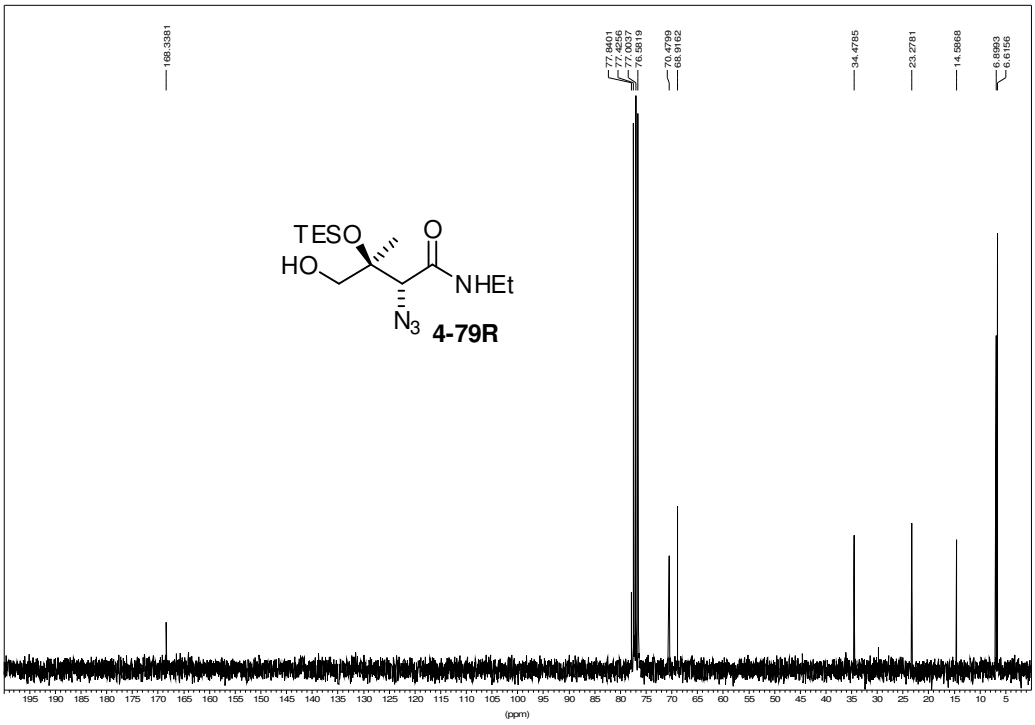

7.2603
4.2751
4.2503
4.2362
4.2208
4.1984
4.1825
4.1551
3.7469
3.7316
3.7195
3.7042
1.5150
1.4903
1.4564
HO
tBuO
O
O
O
O
N3
4-75R
9.0000
9.4508
3.2344
(ppm)
Integral
2.2969
1.2528
9.0000
9.4508
3.2344
(ppm)

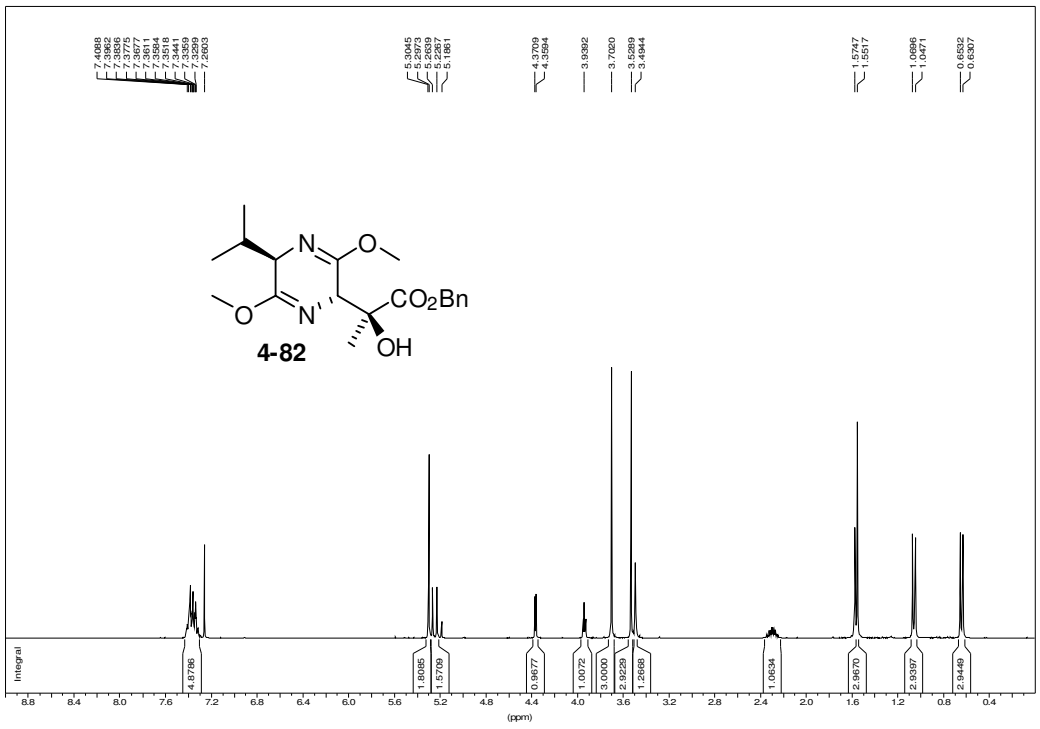

173.1822
153.0068
84.1924
82.7714
77.4258
77.0039
76.5821
75.7312
66.3563
65.4253
27.6312
27.7032
23.7074
HO
tBuO
O
O
O
O
N3
4-75R
(ppm)

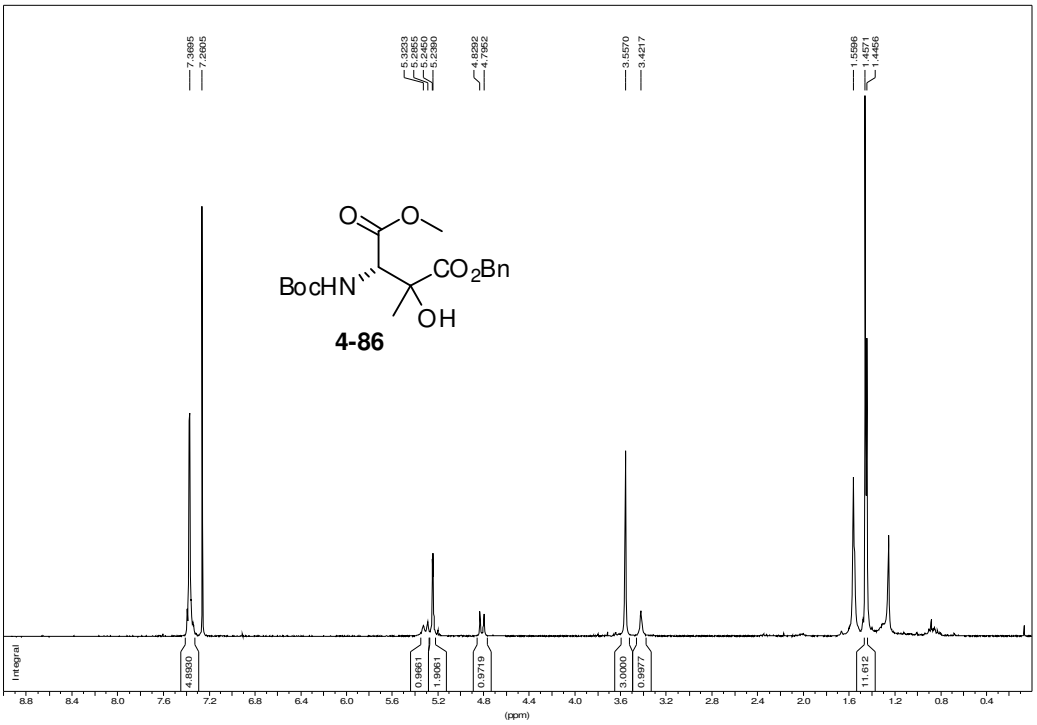

7.2910
7.2605
6.8910
6.8620
6.7245
4.7259
4.7210
4.5484
4.5424
4.2860
3.8034
3.5015
3.4866
3.4661
3.4626
3.4199
3.3984
3.3519
3.3448
3.3322
3.3057
3.3081
3.3021
2.7997
2.0420
1.6076
1.1901
1.1660
1.1419
1.0614
HO
O
PMBO
NHEt
N3
4-77R
Integral
2.4980
1.9738
0.8601
0.9366
2.0039
0.9222
3.0000
4.0763
2.9273
2.8997
(ppm)

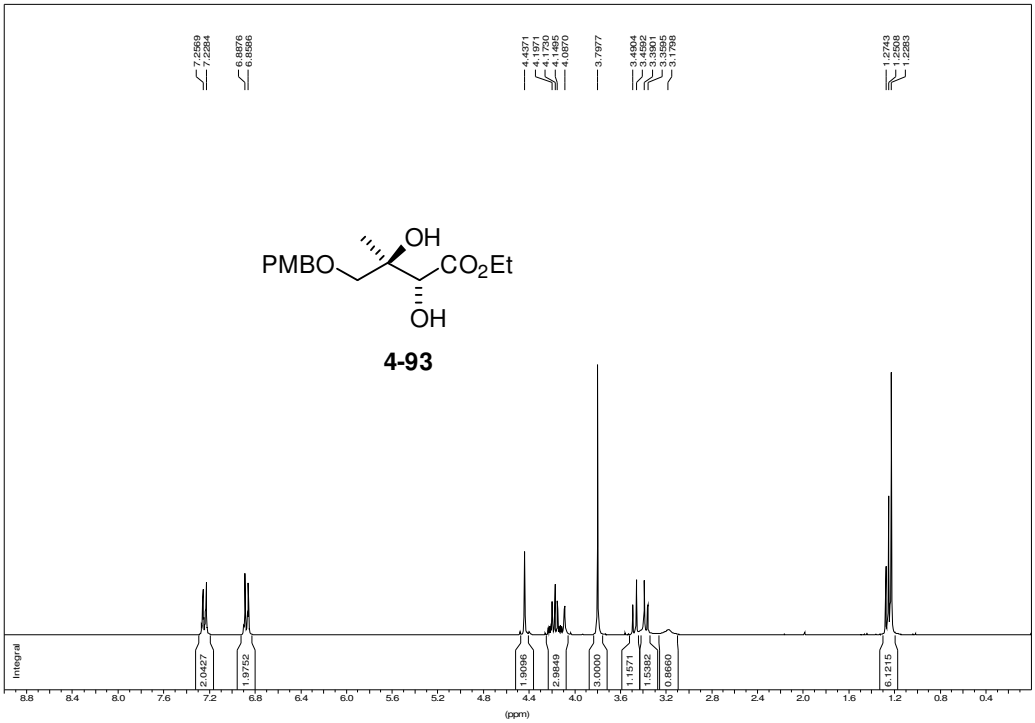

129.5801
113.7832
77.4254
77.0036
76.5818
74.5835
74.5817
73.2071
65.4177
55.2573
34.4129
19.9396
14.6448
HO
O
PMBO
NHEt
N3
4-77R

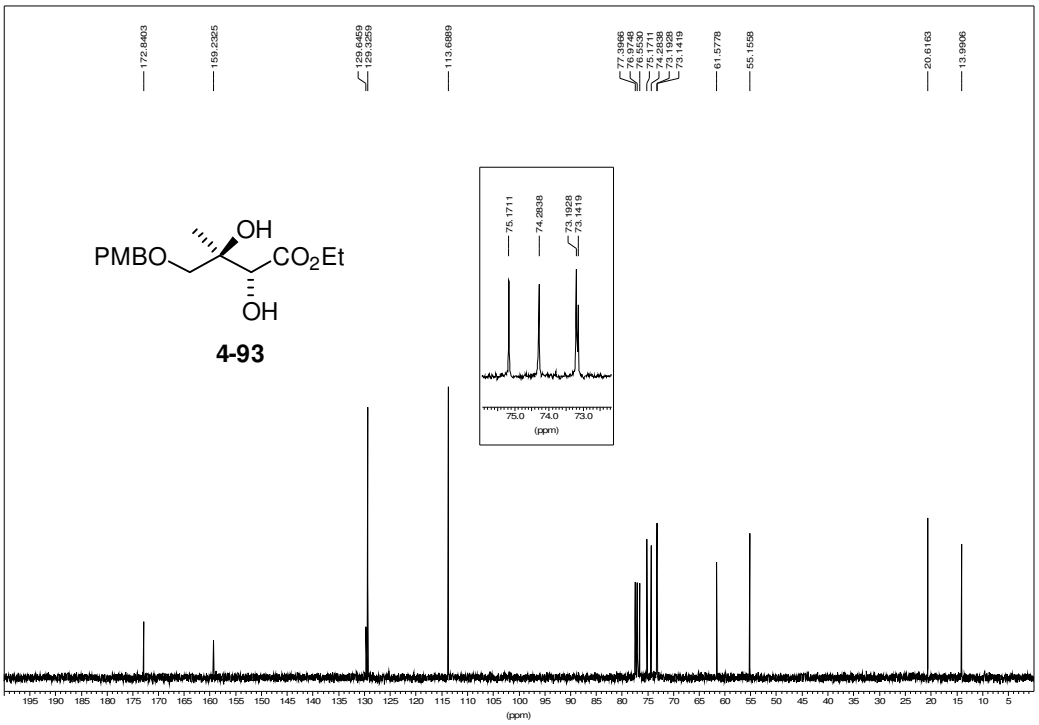

7.2603
6.3338
4.0301
3.5299
3.4790
3.3661
3.3420
3.3179
3.2988
3.2978
3.2746
1.4218
1.1983
1.1736
1.1496
0.9797
0.9529
0.9271
0.6215
0.6053
0.5989
0.5754
0.5705
TESO
HO
O
NHEt
N3
4-79R
Integral
0.8952
0.9552
0.9857
1.0161
2.0649
3.0000
3.2264
8.9659
6.0034


168.3381
77.8401
77.4256
77.0037
76.5819
70.4799
68.9782
34.4785
23.2781
14.5868
6.8993
6.6156
TESO
HO
O
NHEt
N3
4-79R

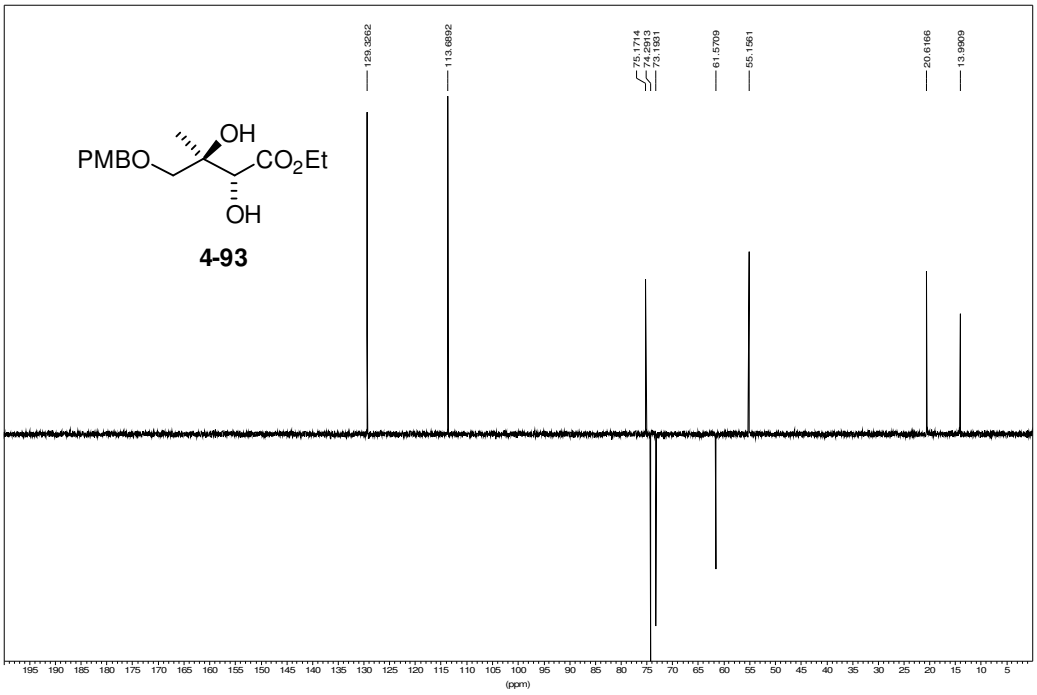

7.4088
7.3962
7.3836
7.3775
7.3677
7.3631
7.3584
7.3518
7.3441
7.3359
7.3299
7.2603
5.3045
5.2973
5.2659
5.2267
5.1861
4.3709
4.3594
3.9382
3.7020
3.5289
3.4944
1.5747
1.5517
1.0696
1.0471
0.6532
0.6307
N
O
O
N
CO2Bn
OH
4-82
Integral
4.8786
1.8085
1.5709
0.9877
1.0072
3.0000
2.8228
1.2668
1.0834
2.9670
2.9397
2.9446

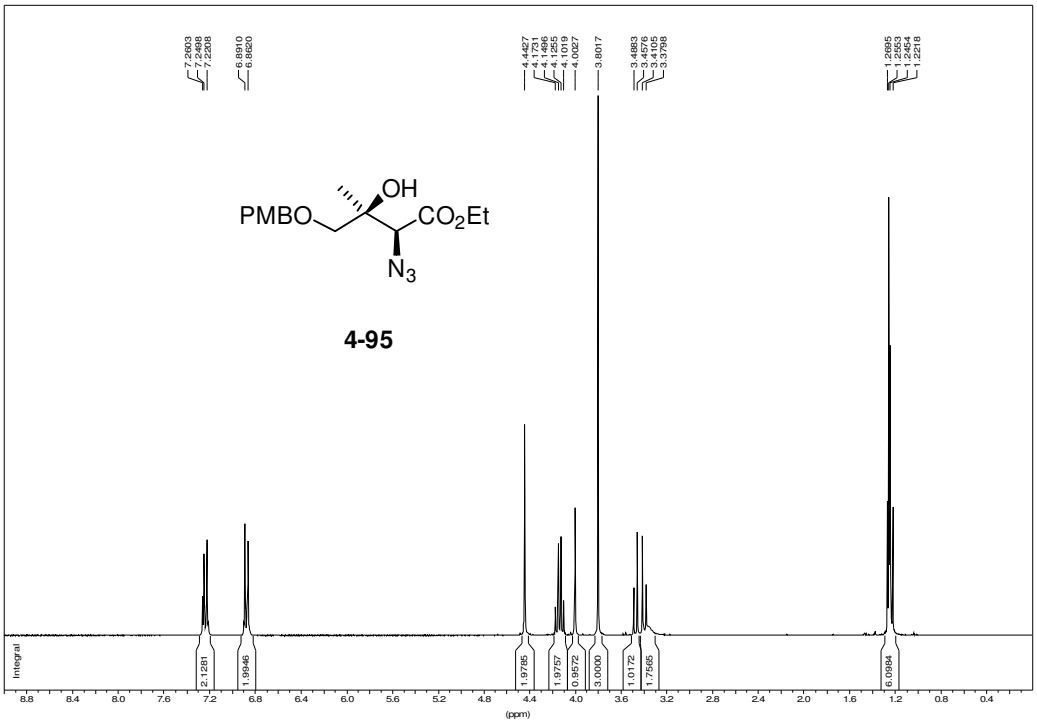

7.3685
7.2605
5.3233
5.2955
5.2640
5.2390
4.8292
4.7962
3.5570
3.4217
1.5596
1.4571
1.4456
O
O
CO2Bn
BocHN
OH
4-86
Integral
4.9330
0.9661
1.9061
0.9719
3.0000
0.9977
11.612
(ppm)


7.2569
7.2284
6.8876
6.8566
4.4371
4.1971
4.1730
4.1495
4.0970
3.7977
3.4904
3.4692
3.3901
3.3595
3.1798
1.2743
1.2508
1.2283
OH
PMBO
CO2Et
OH
4-93
Integral
2.0427
1.9752
1.9096
2.9849
3.0000
1.1571
1.5382
0.8660
6.1215
(ppm)



DEPT135 AC300

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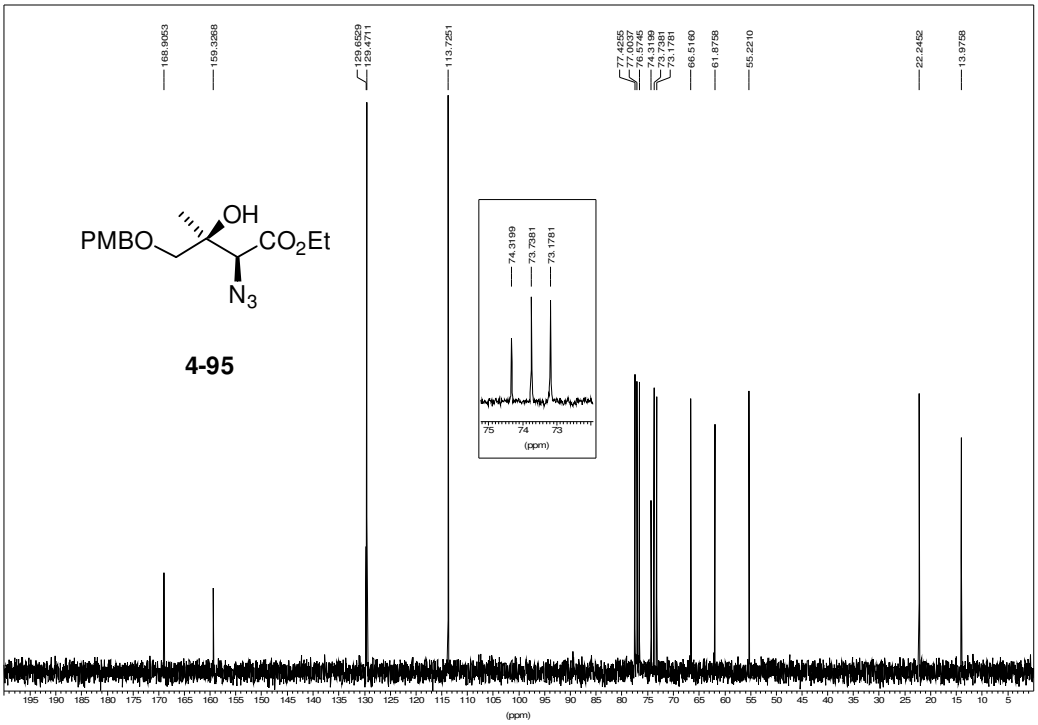
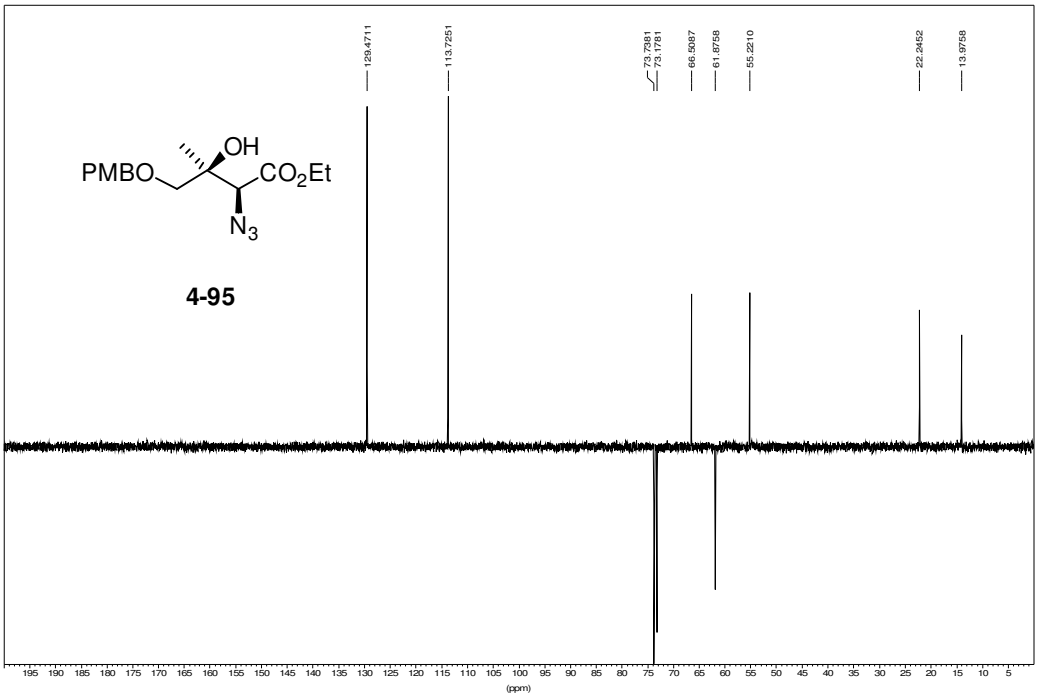
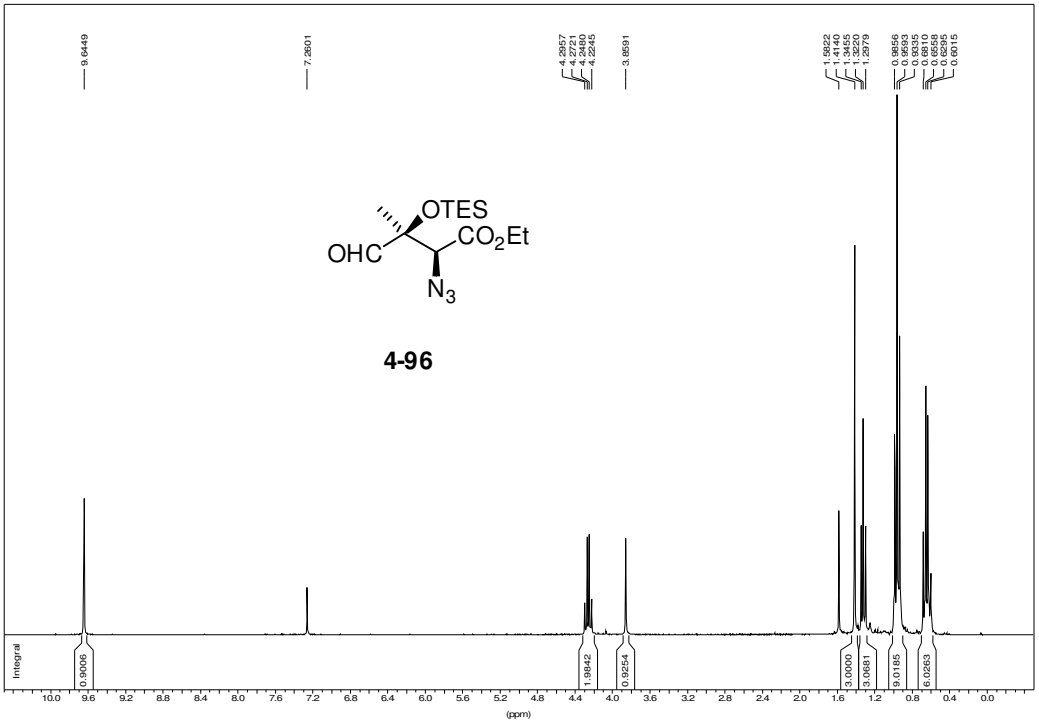
7.2498
7.2208
6.8910
6.8620
4.4427
4.1731
4.1496
4.1255
4.1019
4.0027
3.8017
3.4883
3.4576
3.4105
3.3708
1.2695
1.2553
1.2454
1.2218
OH
PMBO
CO2Et
N3
4-95
Integral
2.1281
1.9946
1.9785
1.9757
0.9572
3.0000
1.0172
1.7565
6.0984
(ppm)


168.9053
159.3358
129.6529
129.4711
113.7251
77.4255
77.0037
76.5746
74.3199
73.7381
73.1781
66.5160
61.8758
55.2210
22.2452
13.9758
OH
PMBO
CO2Et
N3
4-95
74.3199
73.7381
73.1781
(ppm)

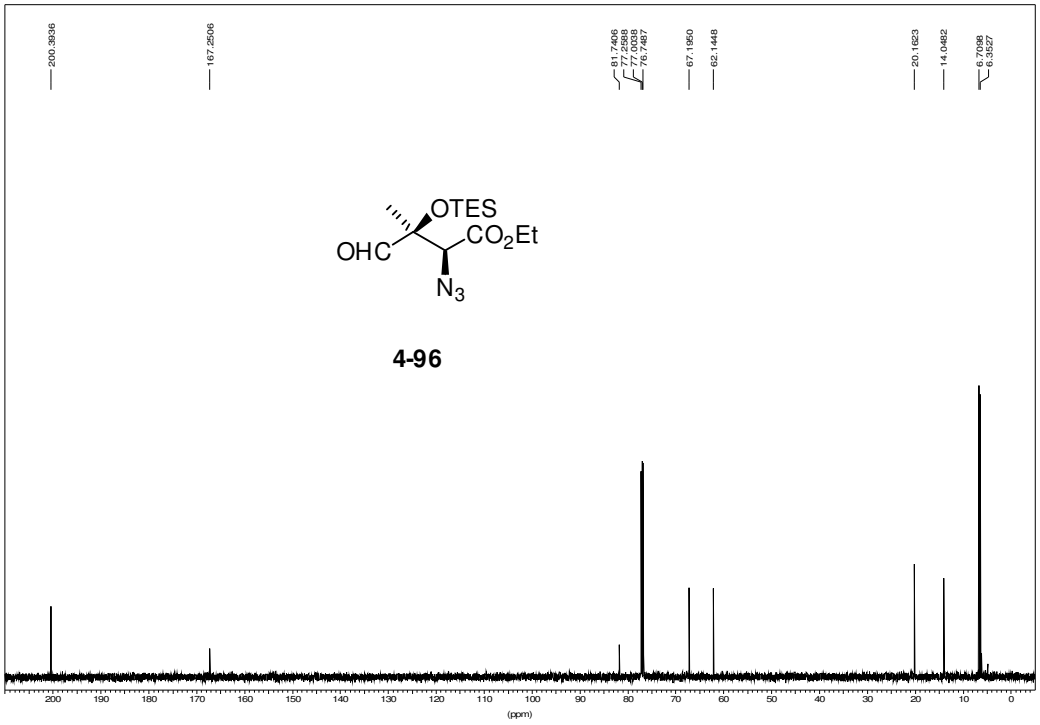
DEPT135 AC300



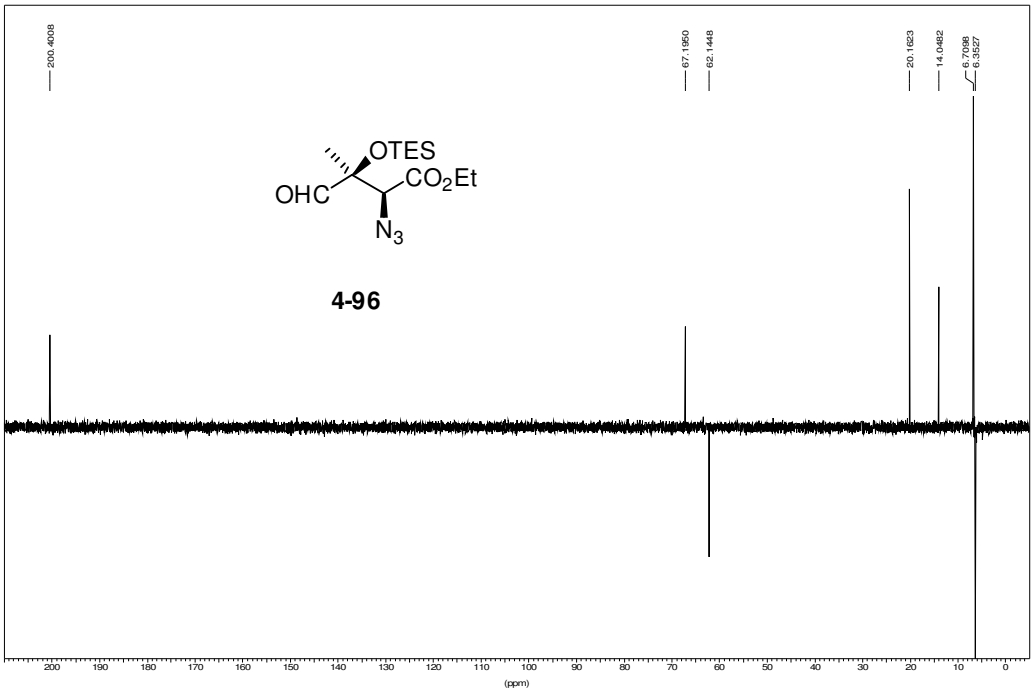
4-95

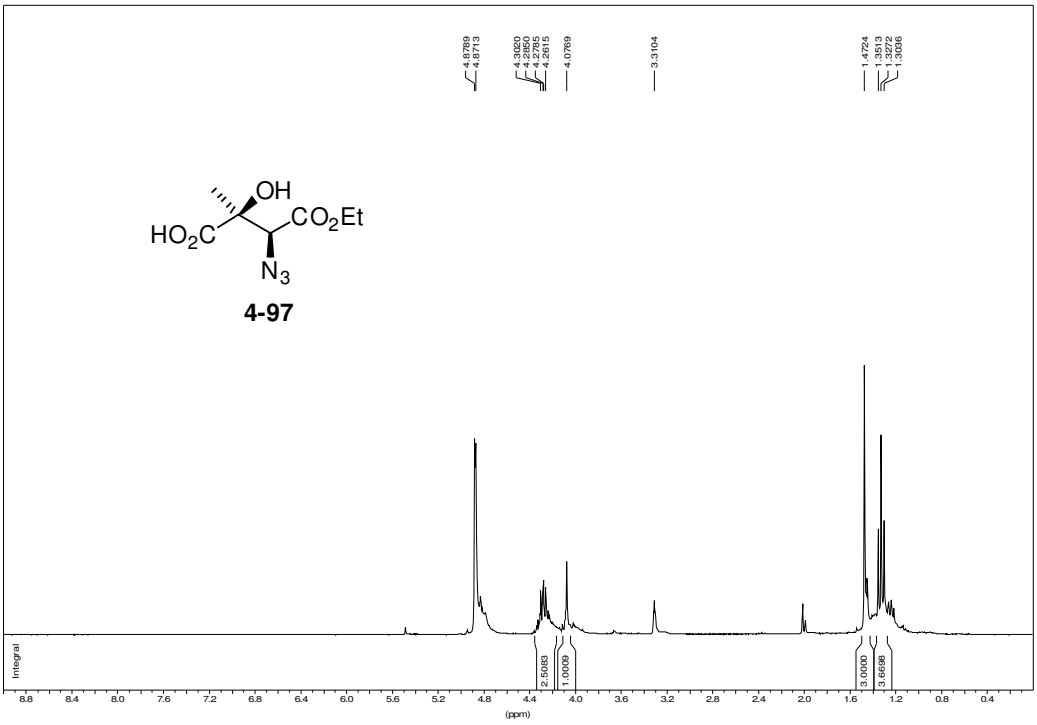


4-96

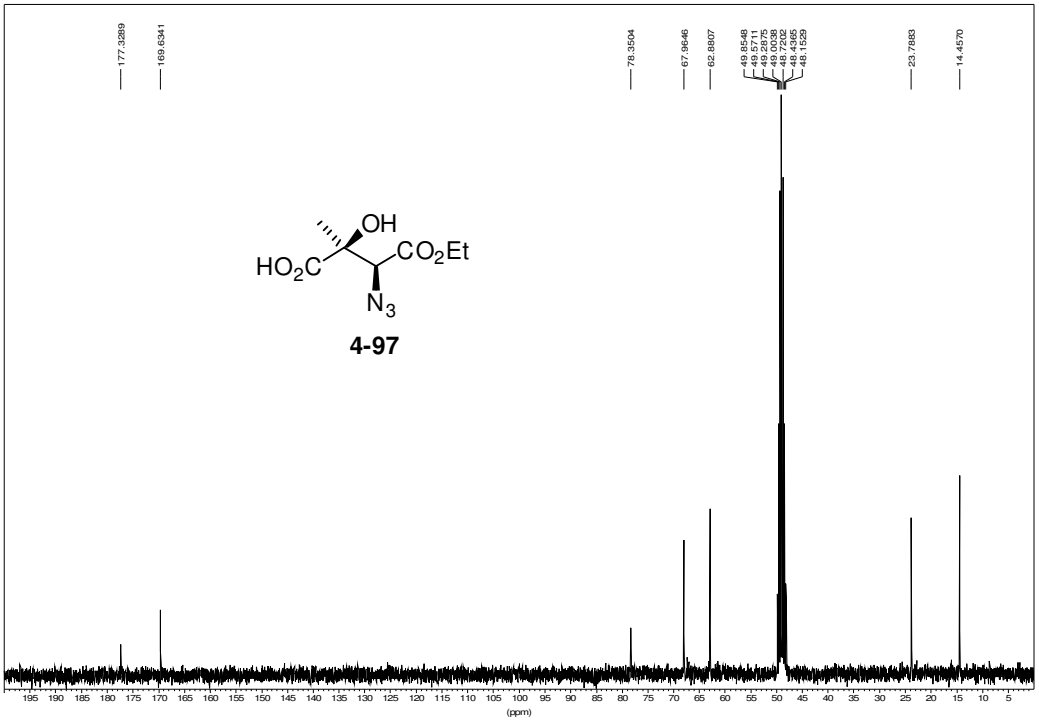


DEPT135 AMX500



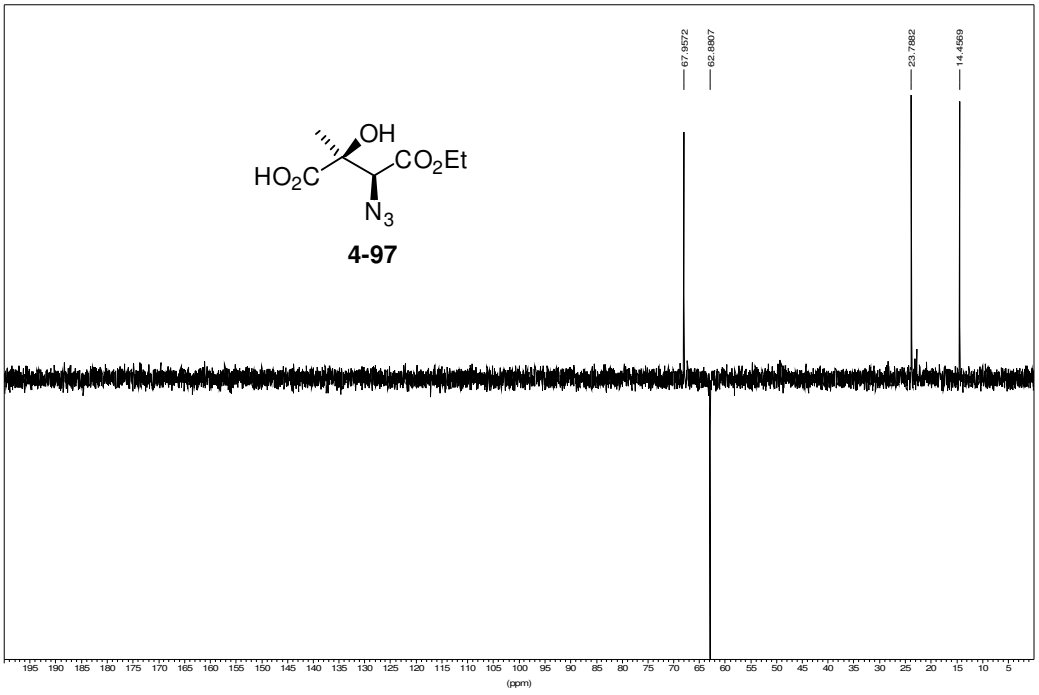

4.8789
4.8713
4.3020
4.2860
4.2705
4.2615
4.0769
3.3104
1.4724
1.3513
1.3372
1.3006
OH
HO2C
CO2Et
N3
Integral
2.5083
1.0008
3.0000
3.6698
(ppm)

4-97

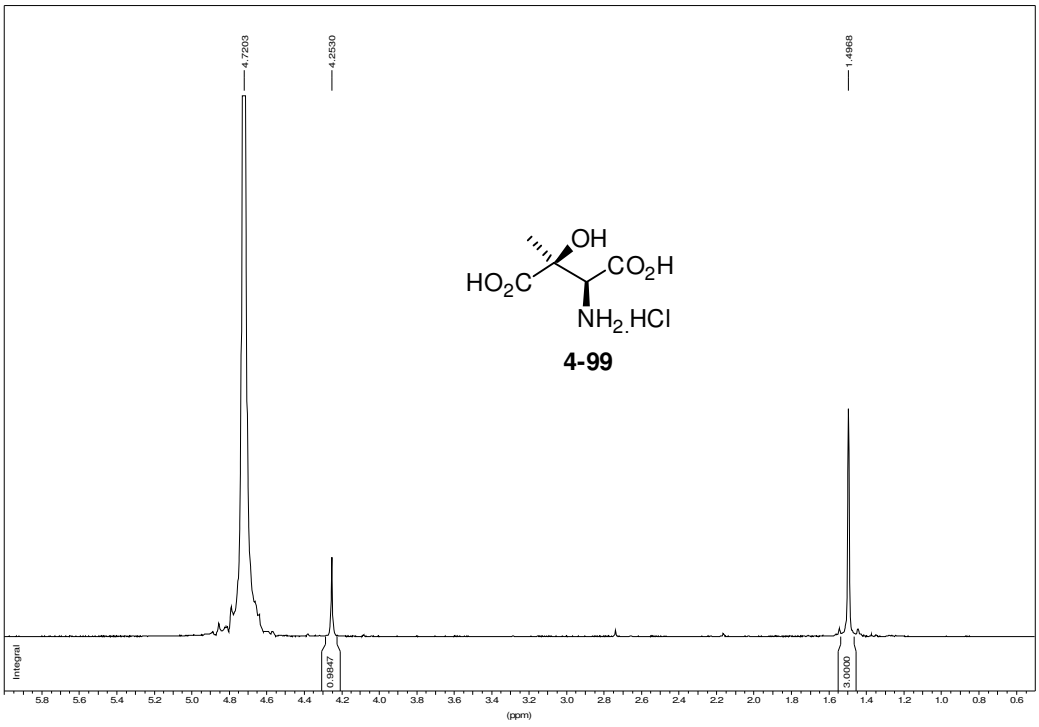

177.3289
169.6341
78.3504
67.9646
62.8807
49.8548
49.5711
49.2875
49.0038
48.7202
48.4365
48.1529
23.7883
14.4570
OH
HO2C
CO2Et
N3
(ppm)

4-97

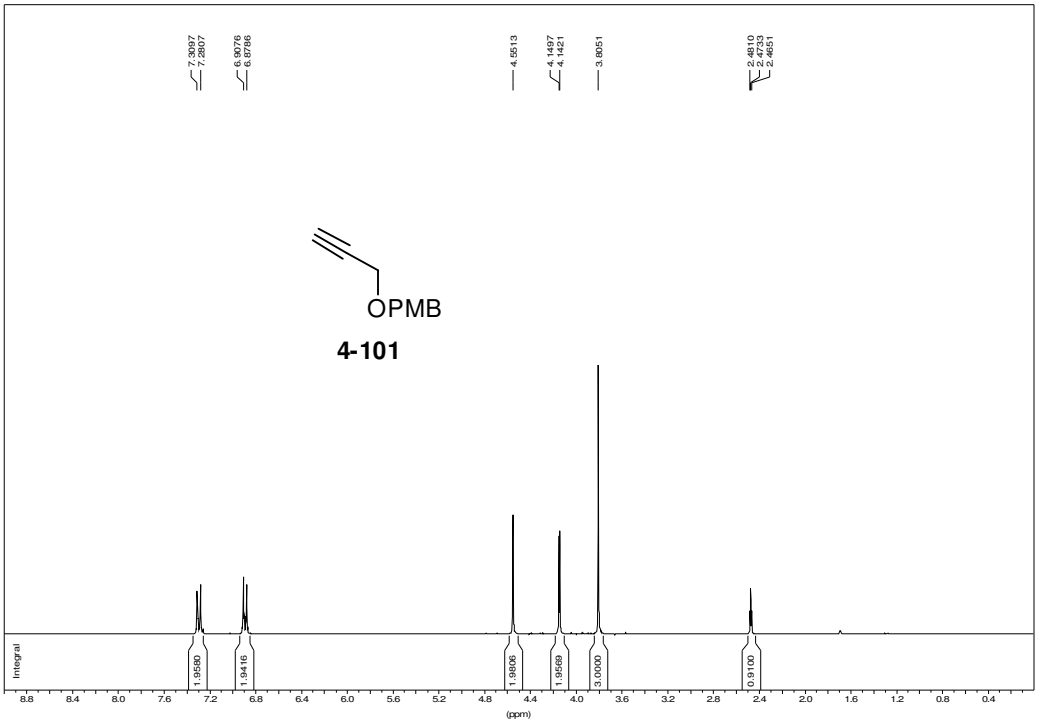
DEPT135 AC300

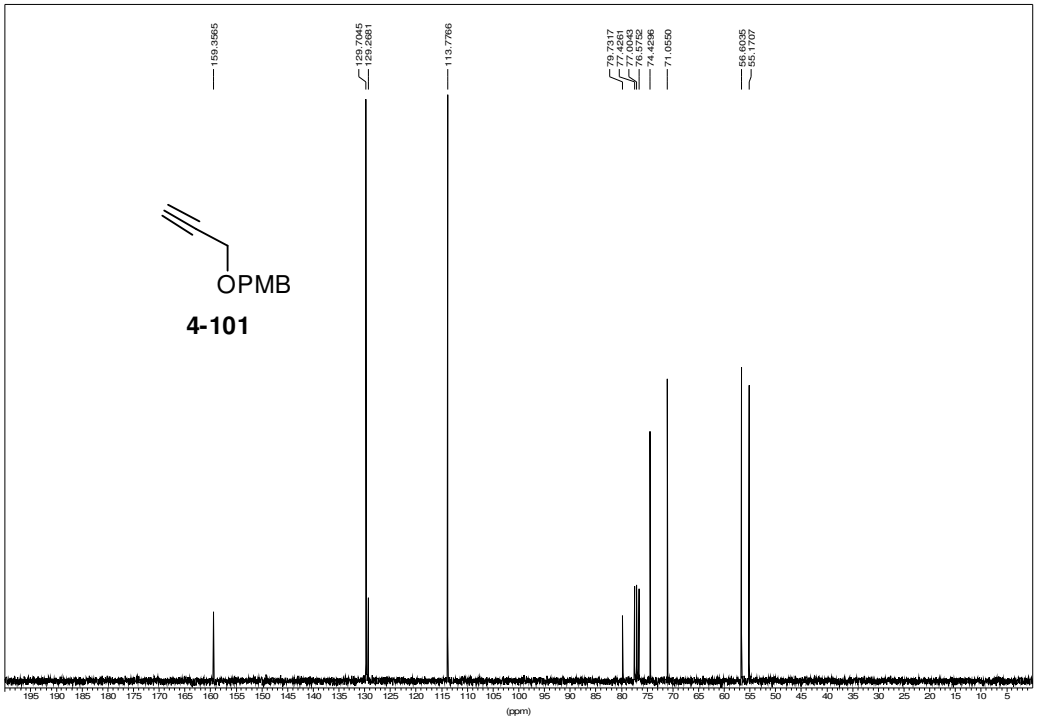


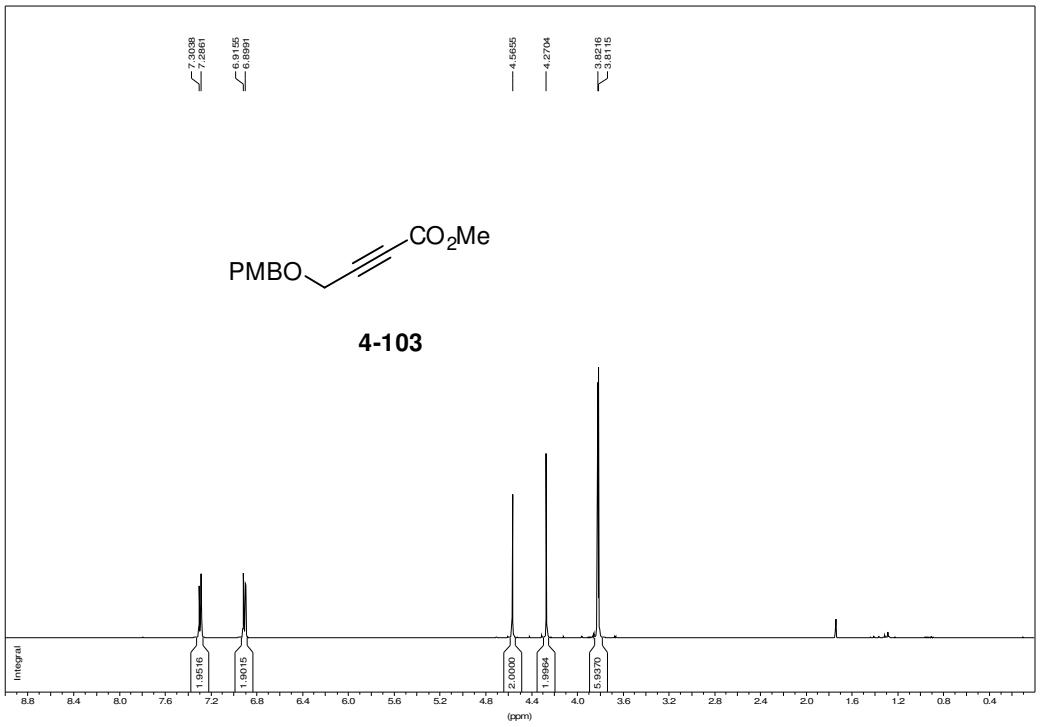
4-97



4-99


7.3097
7.2877
6.9076
6.8786
4.5513
4.1497
4.1421
3.8051
2.4810
2.4733
2.4651
OPMB
4-101
Integral
1.9580
1.9416
1.9806
1.9569
3.0000
0.9100
(ppm)


159.3565
129.7045
129.2081
113.7766
79.7317
77.4261
77.0043
76.5792
74.4286
71.0550
66.6035
55.1707
OPMB
4-101
(ppm)


7.3038
7.2861
6.9155
6.8991
4.5665
4.2704
3.8216
3.8115
PMBO
CO2Me
4-103
Integral
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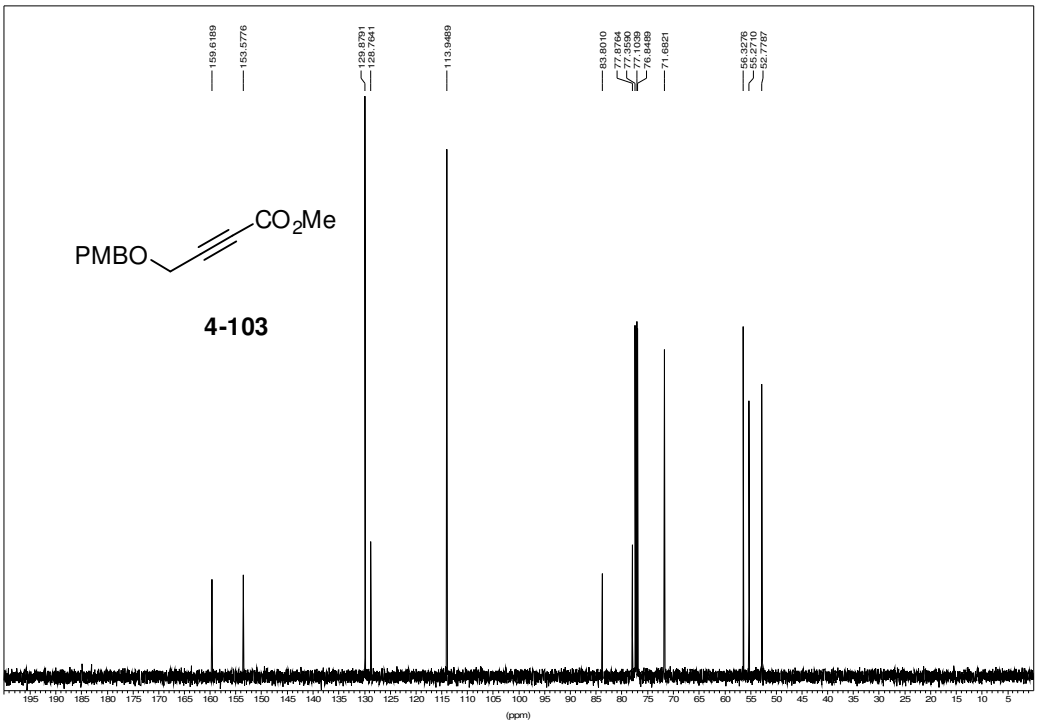
1.9015
2.0000
1.9964
5.9370
(ppm)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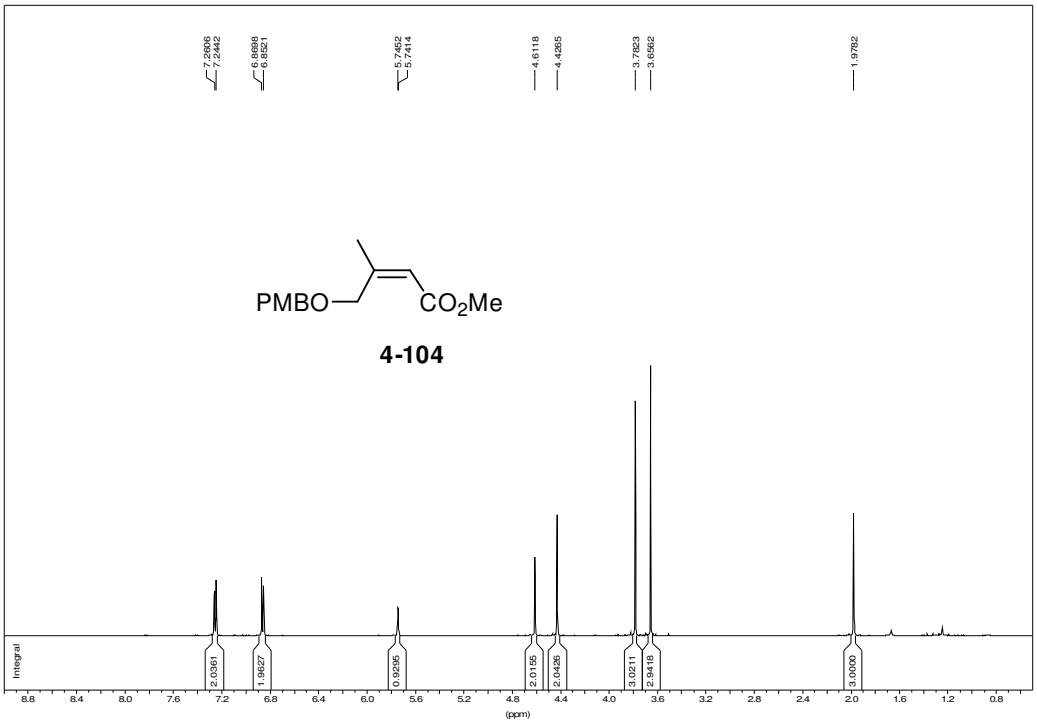
129.8791
129.7641
113.9489
83.8010
77.8764
77.3590
77.1053
76.8489
71.6821
56.3276
55.2710
52.7767
PMBO
CO2Me
4-103
(ppm)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7.2442
6.8698
6.8521
5.7452
5.7414
4.6118
4.4265
3.7823
3.6562
1.9782
PMBO
CO2Me
4-104
Integral
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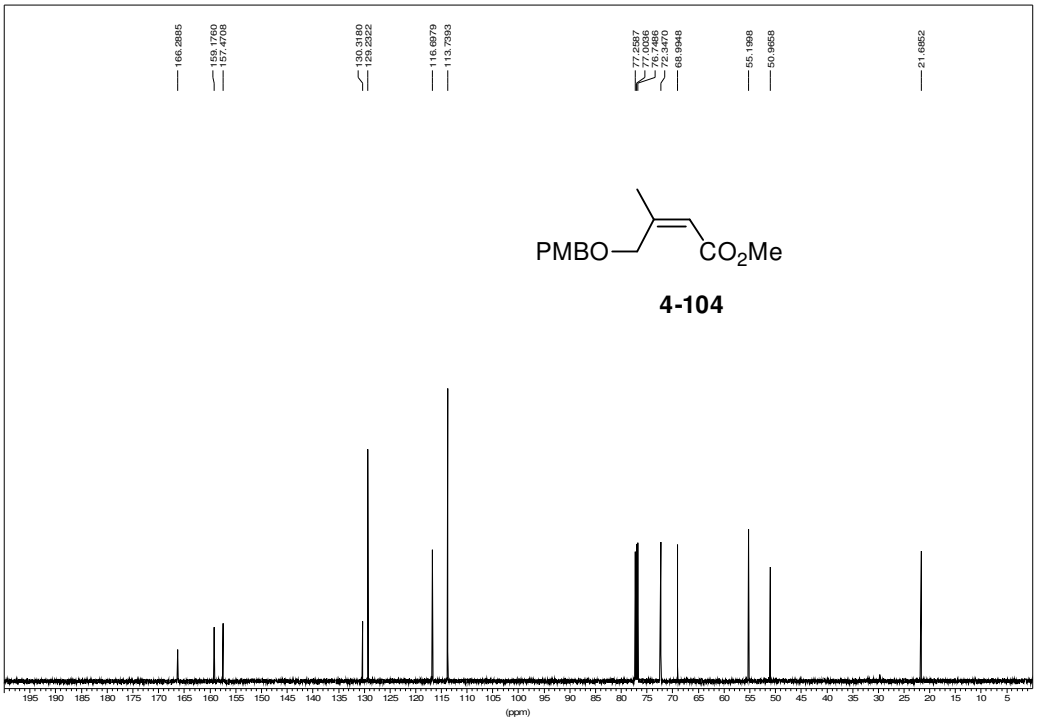
2.0436
3.0211
2.9416
3.0000
(ppm)


166.2885
159.1760
157.4708
130.3190
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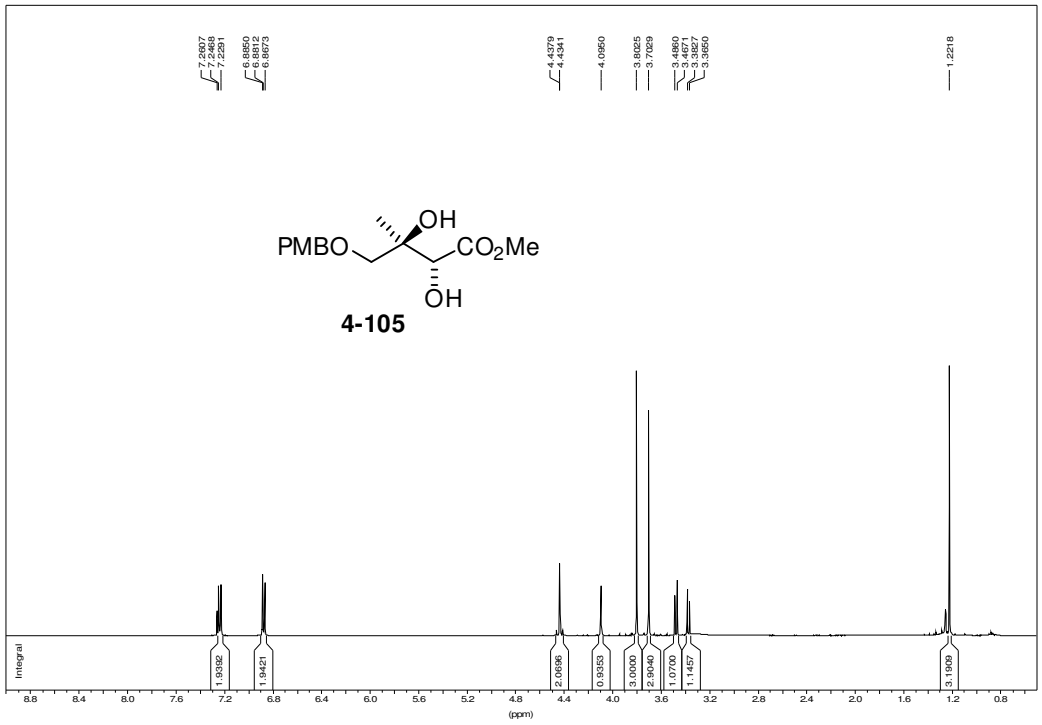
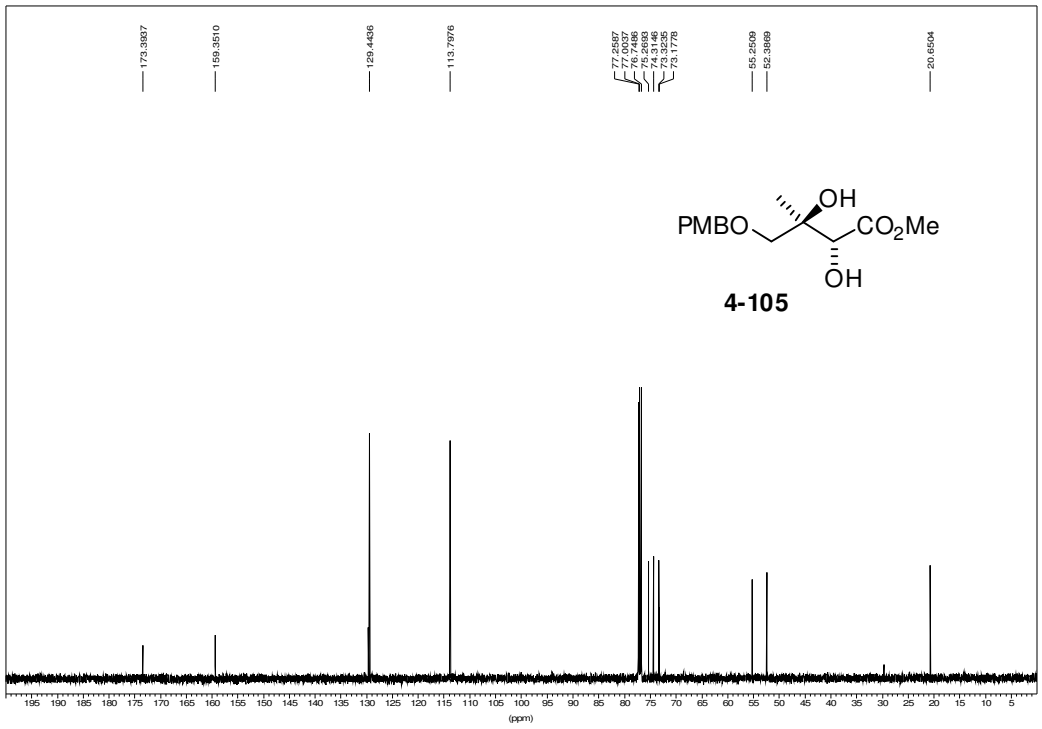
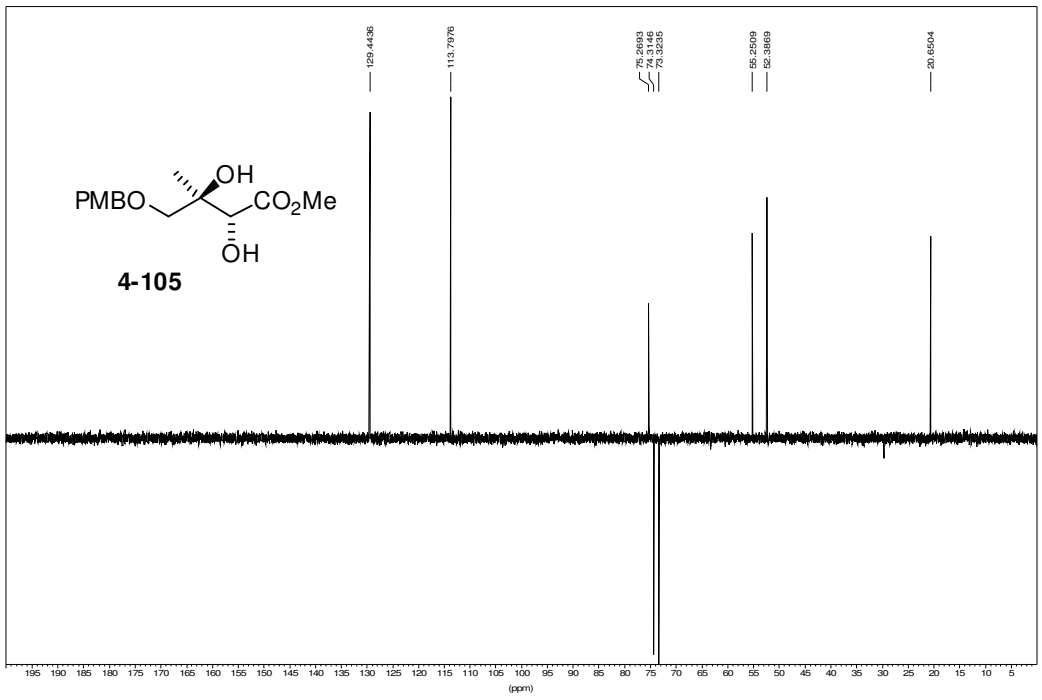
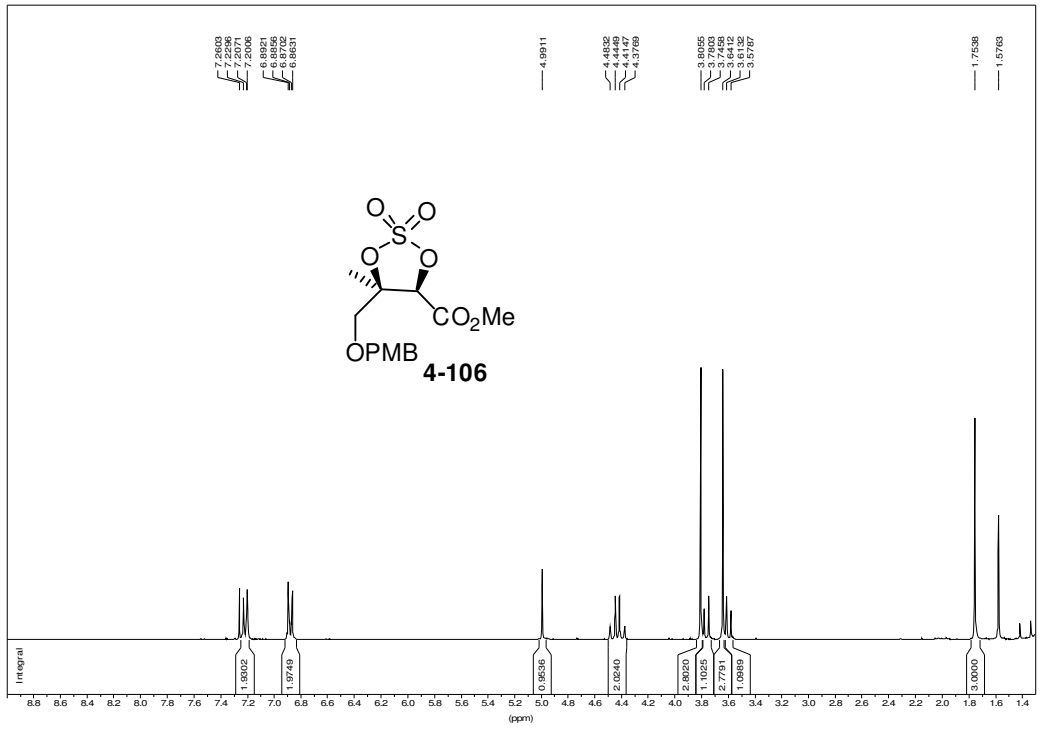
77.0036
76.7486
72.3470
68.9948
55.1998
50.9658
21.6852
PMBO
CO2Me
4-104
(ppm)

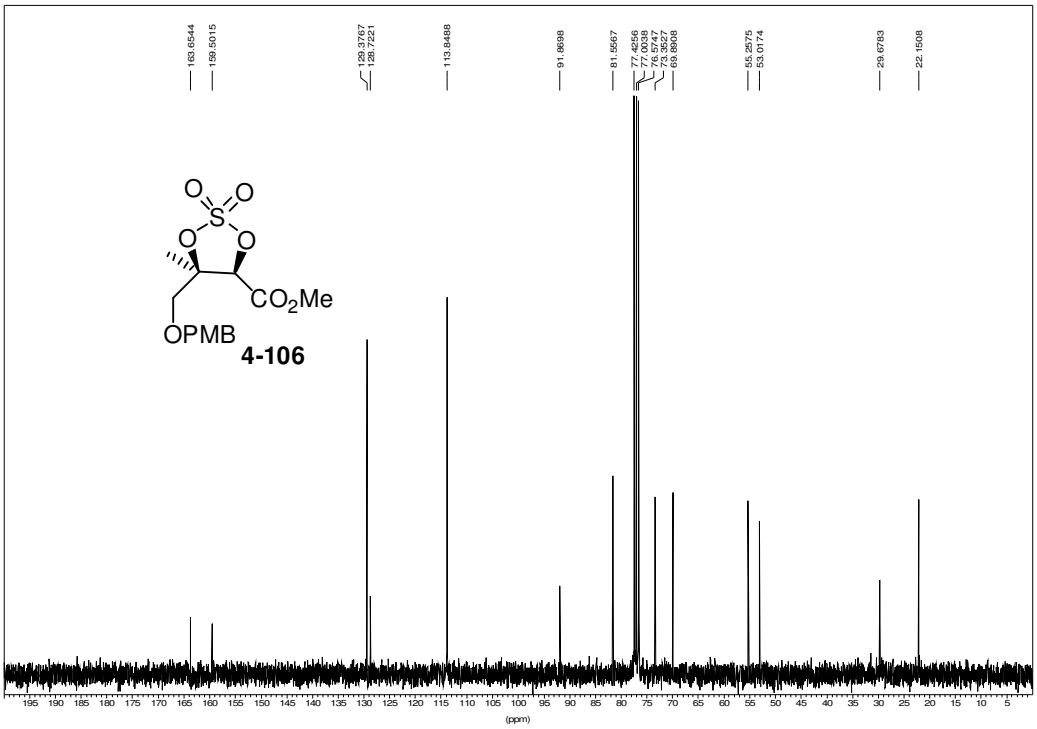

7.2607
7.2468
7.2291
6.8850
6.8812
6.8673
4.4379
4.4341
4.0960
3.8025
3.7029
3.4980
3.4871
3.3957
3.3650
1.2218
OH
PMBO
CO2Me
OH
4-105
Integral
1.9392
1.9421
2.0696
0.9353
3.0000
2.9040
1.0700
1.1457
3.1969
(ppm)

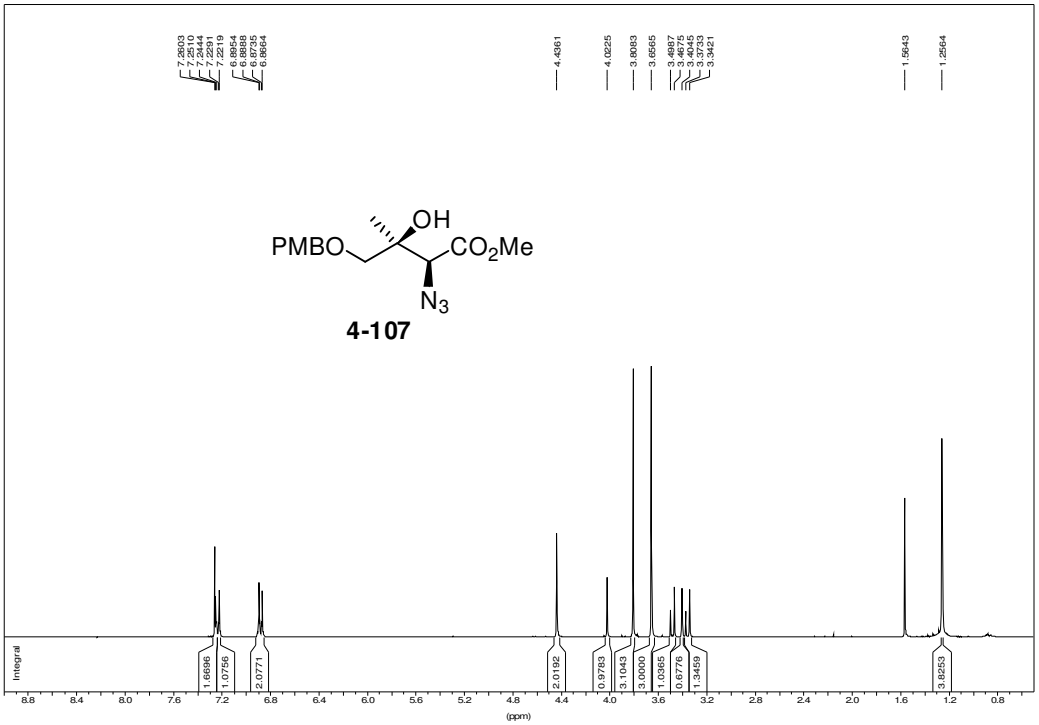

173.3937
159.3510
129.4436
113.7976
77.2587
77.0057
76.7486
75.2693
74.3146
73.3235
73.1778
55.2509
52.3869
20.6504
OH
PMBO
CO2Me
OH
4-105
(ppm)

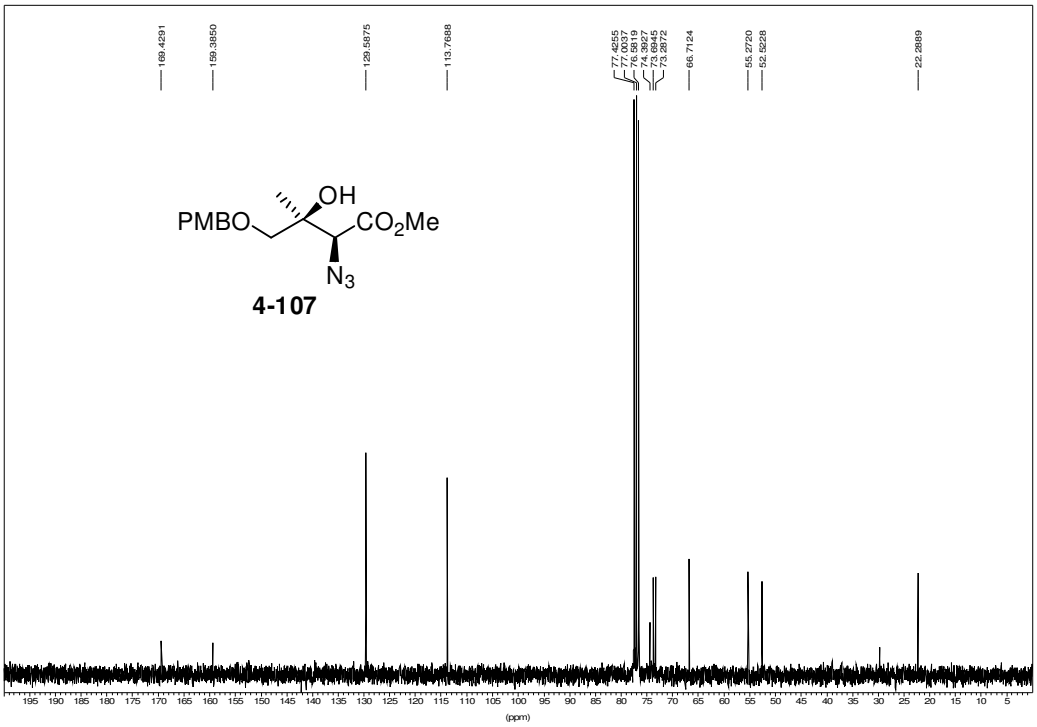
DEPT135 AMX500



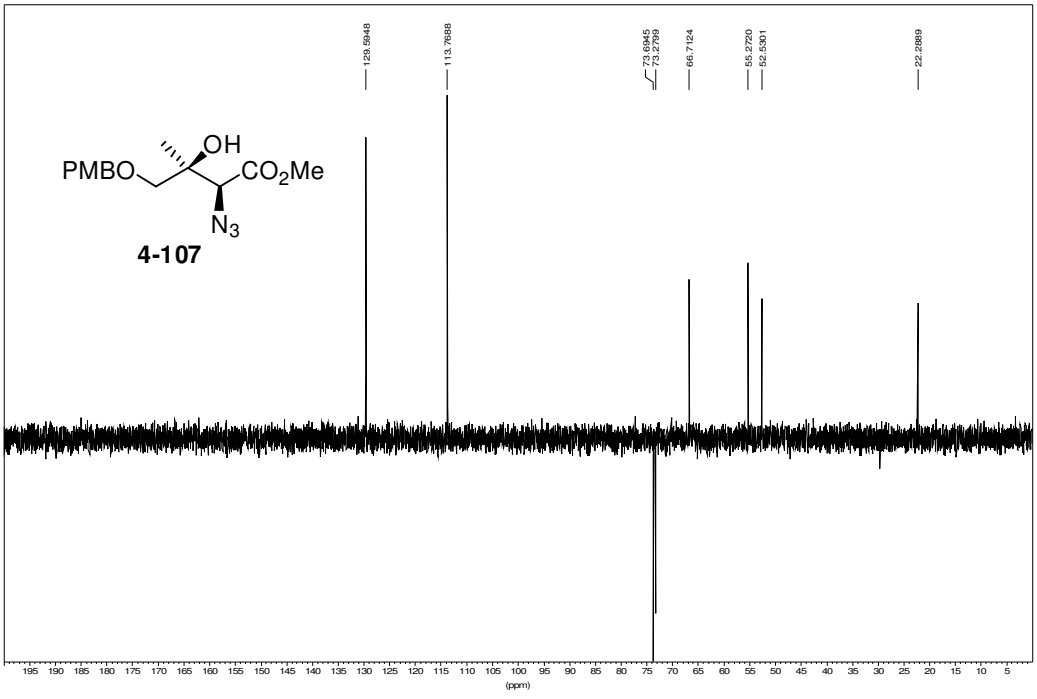


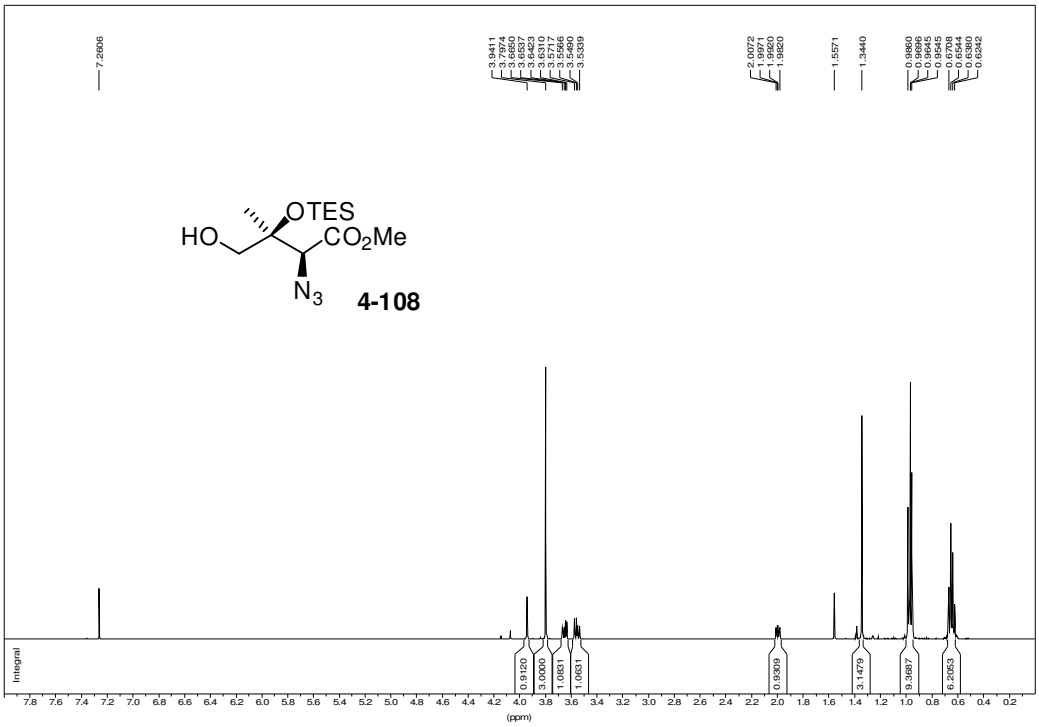

163.6544
159.5015
129.3767
128.7221
113.8488
91.8698
81.5567
77.4256
77.0038
76.5747
73.3557
69.8908
55.2575
53.0174
29.6783
22.1508
O
S
O
O
O
CO2Me
OPMB
4-106
(ppm)

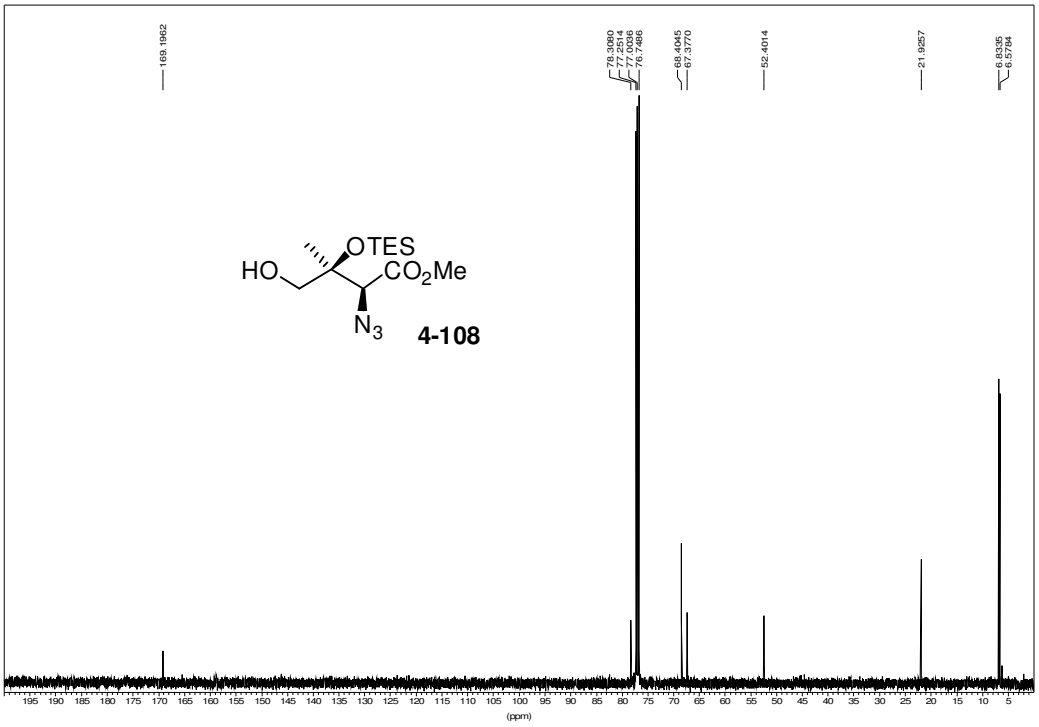

7.2803
7.2610
7.2444
7.2291
7.2219
6.8894
6.8864
6.8735
6.8664
4.4361
4.0225
3.8083
3.6565
3.4987
3.4675
3.4045
3.3733
3.3421
1.5643
1.2564
OH
PMBO
CO2Me
N3
4-107
Integral
1.6696
1.0756
2.0771
2.0192
0.9783
3.1043
3.0000
1.0365
0.6776
1.3459
3.8253
(ppm)

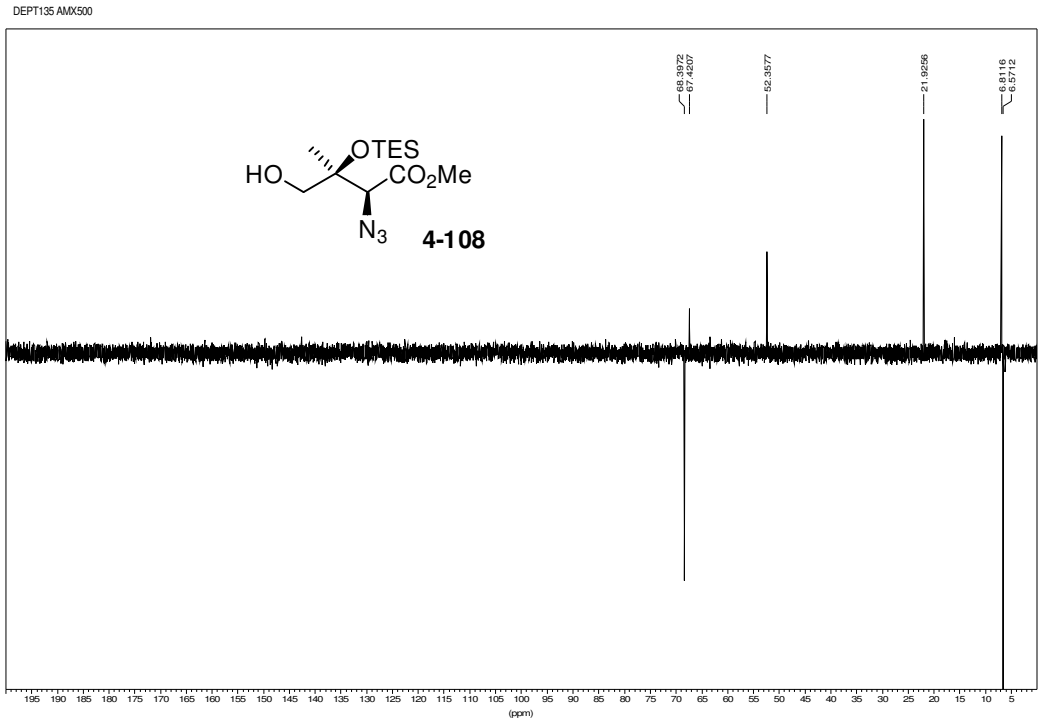


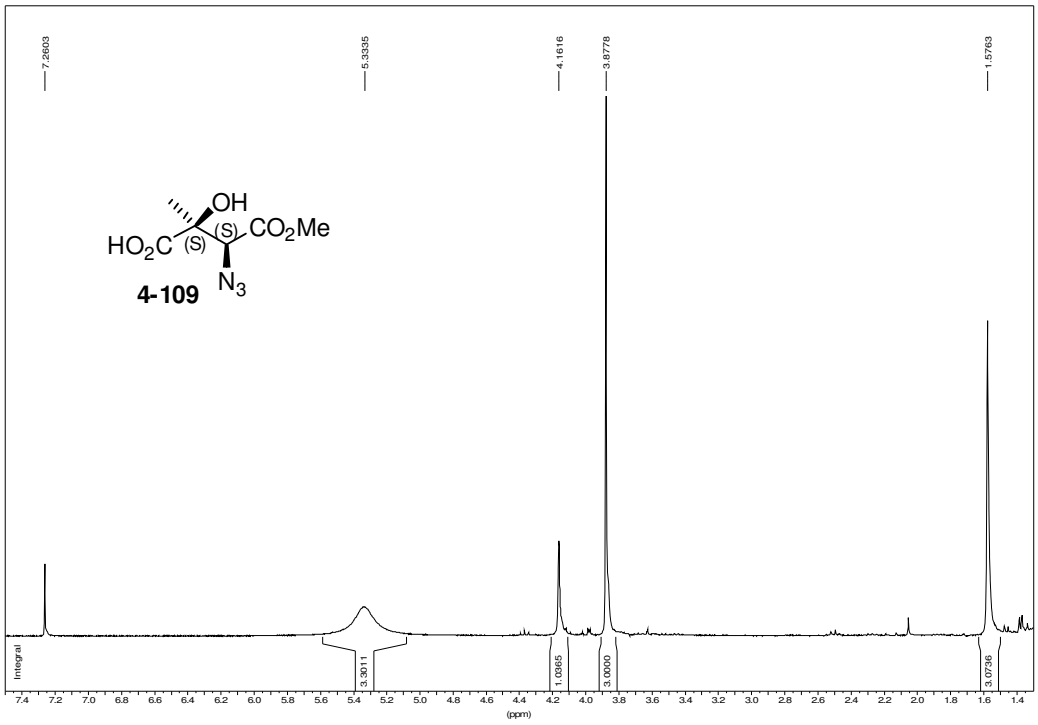
DEPT135 AC300

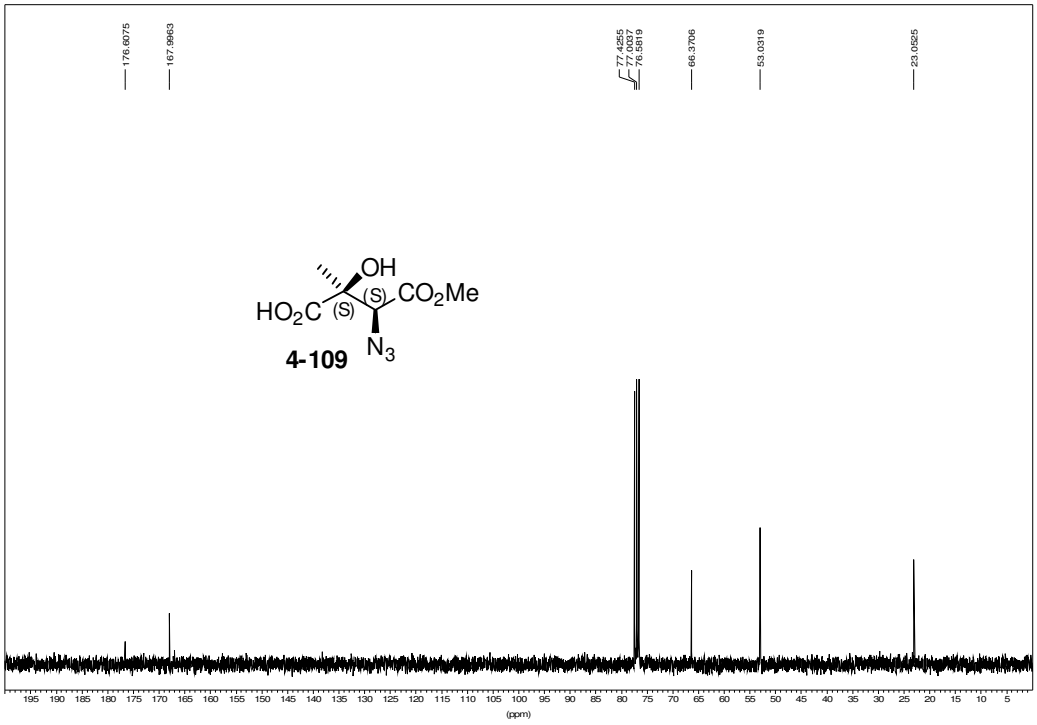


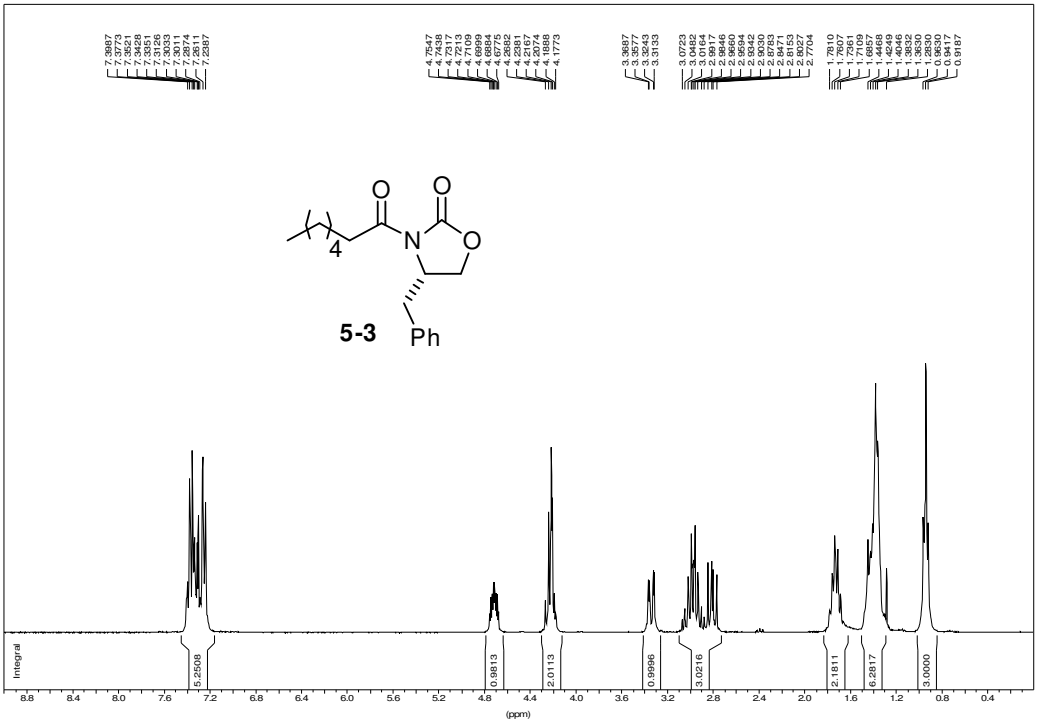

7.2606
3.9411
3.7974
3.6950
3.6537
3.6423
3.6310
3.5717
3.5586
3.5460
3.5359
2.0072
1.9971
1.9920
1.9820
1.5571
1.3440
0.9880
0.9866
0.9545
0.6708
0.6644
0.6380
0.6242
OTES
HO
CO2Me
N3
4-108
Integral
0.9120
3.0000
1.0831
1.0631
0.9309
3.1479
9.3687
6.2053
(ppm)

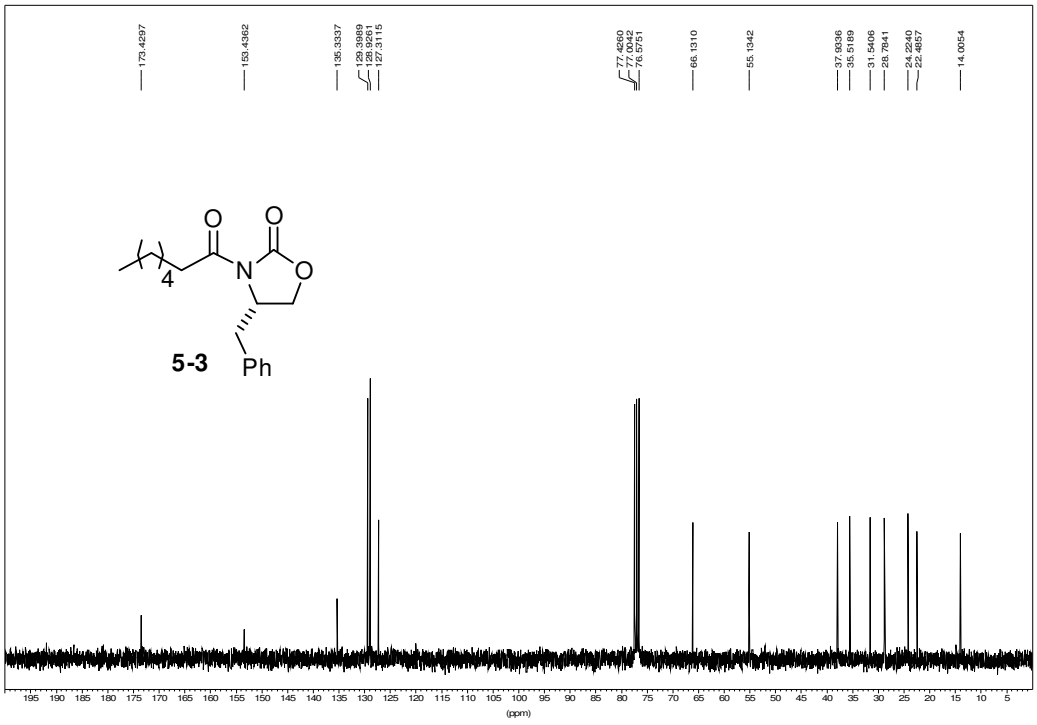

169.1962
79.3090
77.2514
77.0036
76.7486
68.4045
67.3770
52.4014
21.9257
6.8335
6.5784
OTES
HO
CO2Me
N3
4-108
(ppm)


DEPT135 AMX500
68.3972
67.4207
52.3577
21.9256
6.8116
6.5712
OTES
HO
CO2Me
N3
4-108
(ppm)

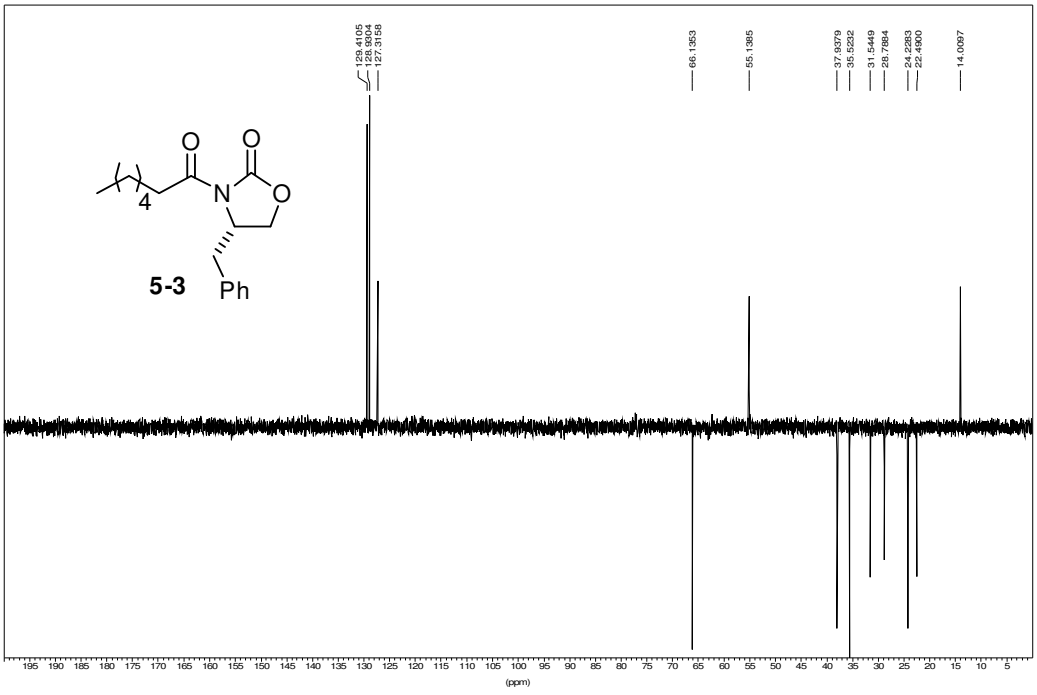

7.2603
5.3335
4.1616
3.8778
1.5763
OH
HO2C
(S)
(S)
CO2Me
N3
4-109
Integral
3.3011
1.0365
3.0000
3.0736
(ppm)

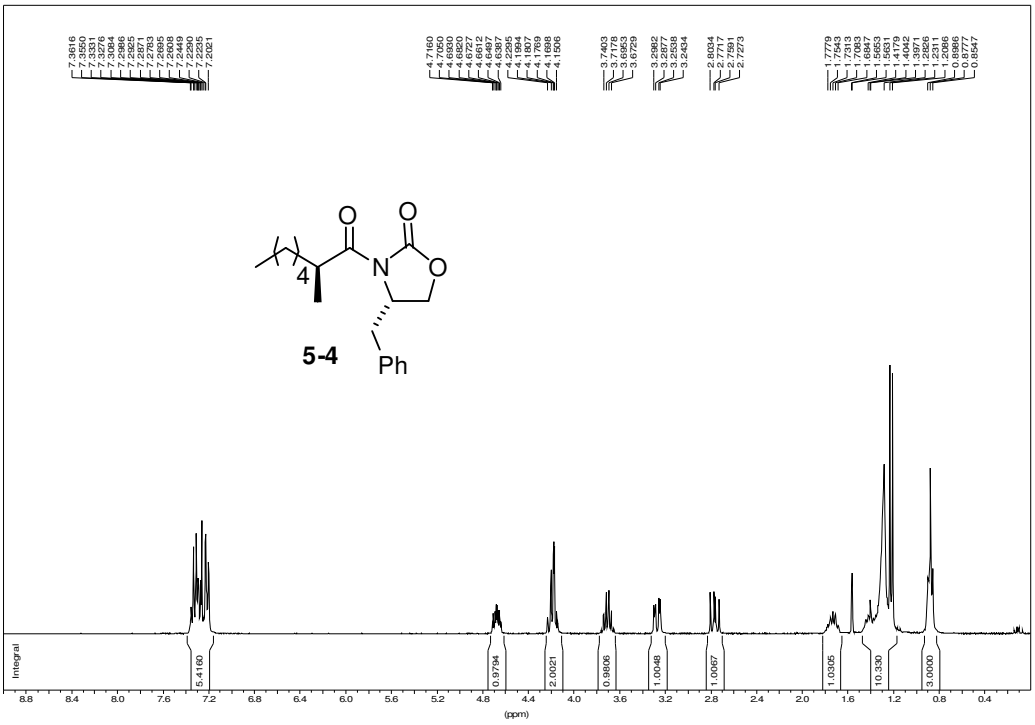

176.6075
167.9963
77.4255
77.0037
76.5819
66.3706
53.0319
23.0525
OH
HO2C (S) (S) CO2Me
N3
4-109
(ppm)

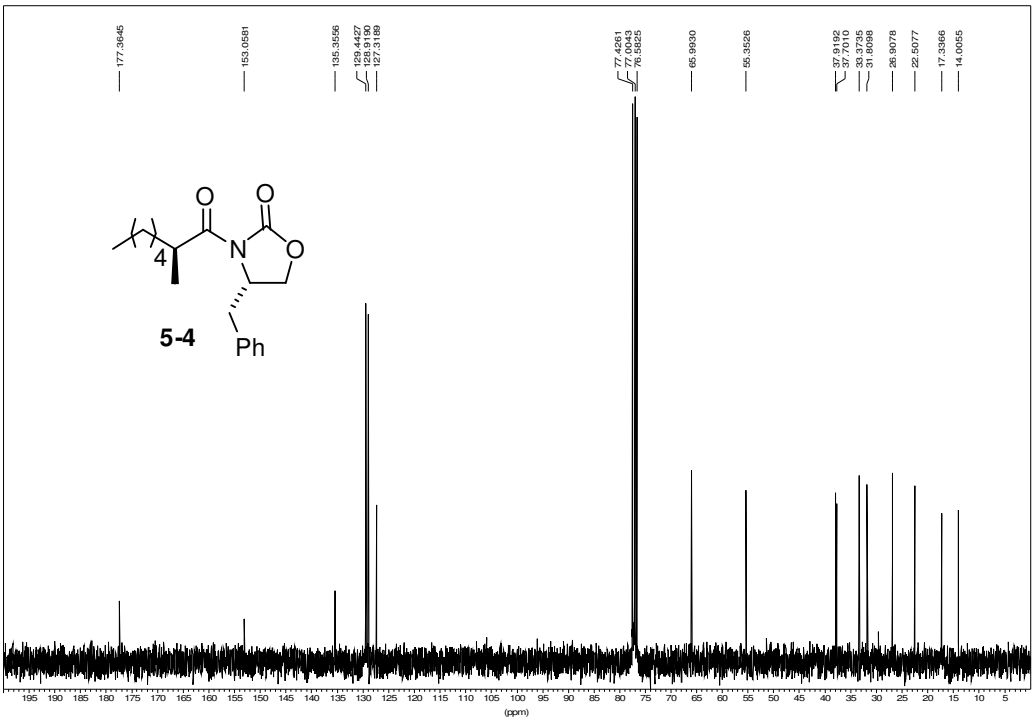

O
O
4
N
O
Ph
5-3
Integral
5.2508
0.9813
2.0113
0.9996
3.0216
2.1811
6.2817
3.0000
(ppm)



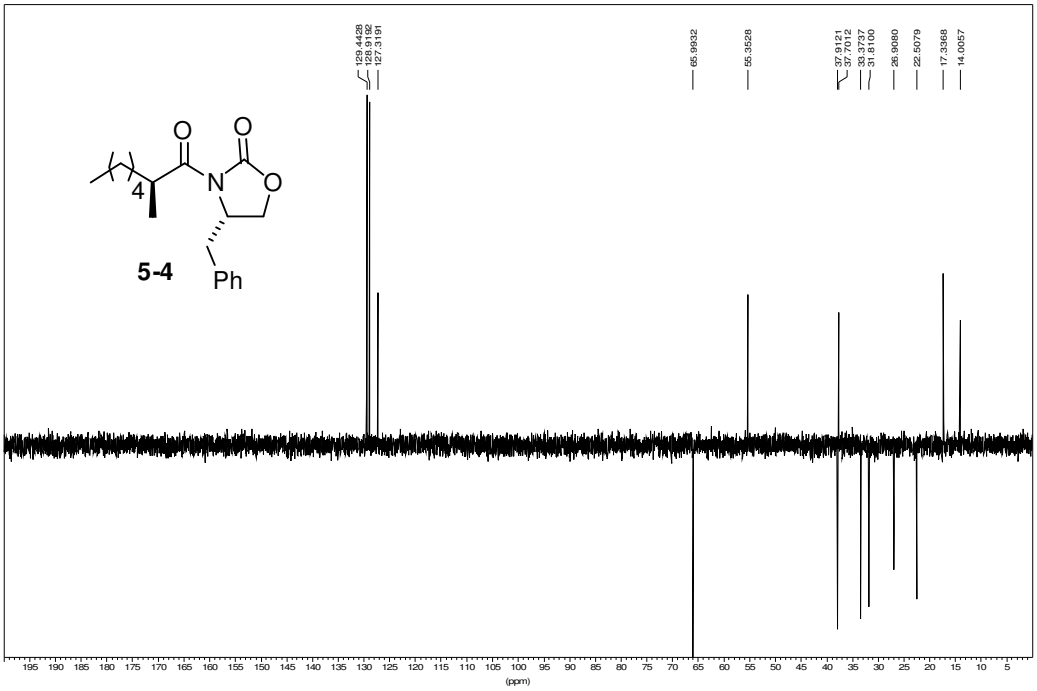
DEPT135 AC300



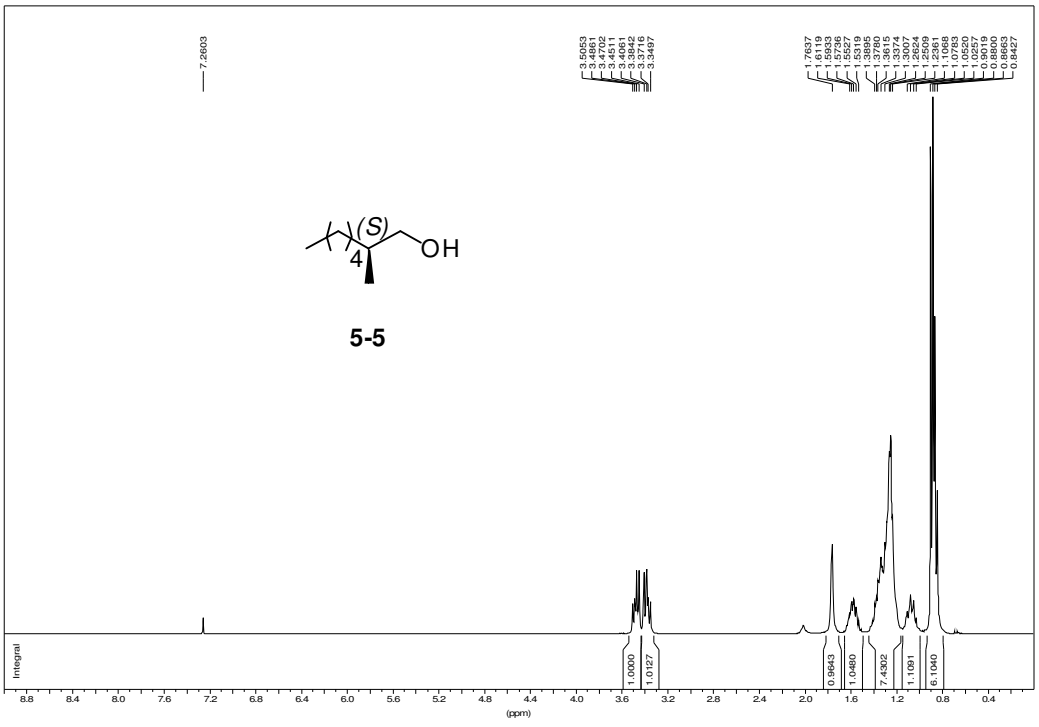

5-4
Ph
Integral
(ppm)


5-4
Ph
(ppm)

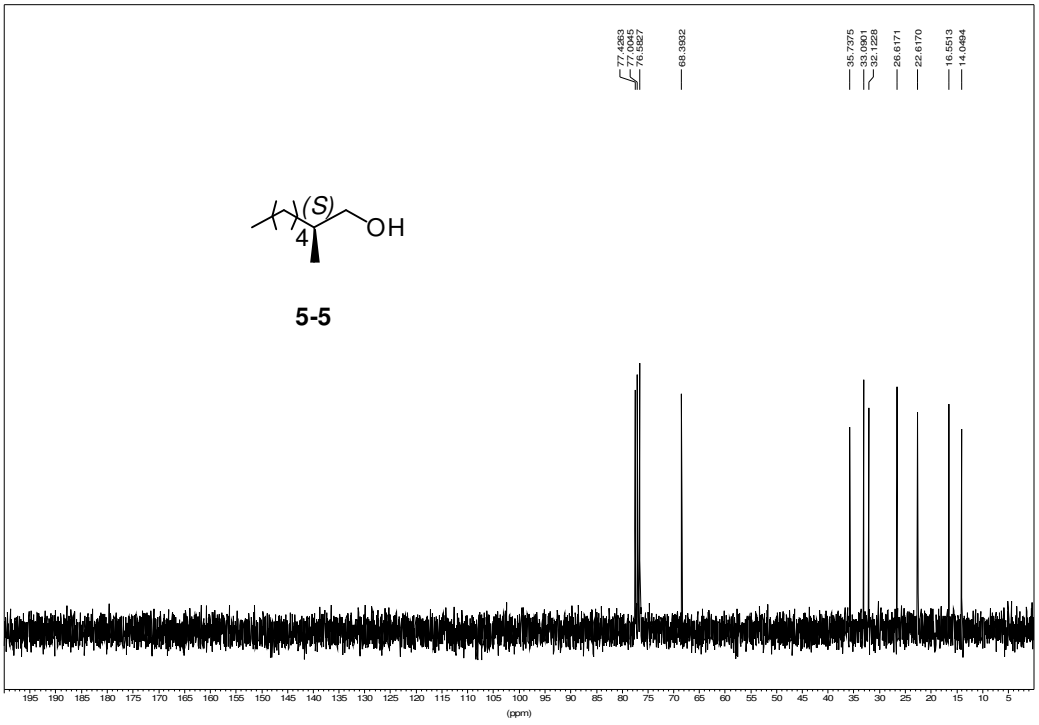
DEPT135 AC300



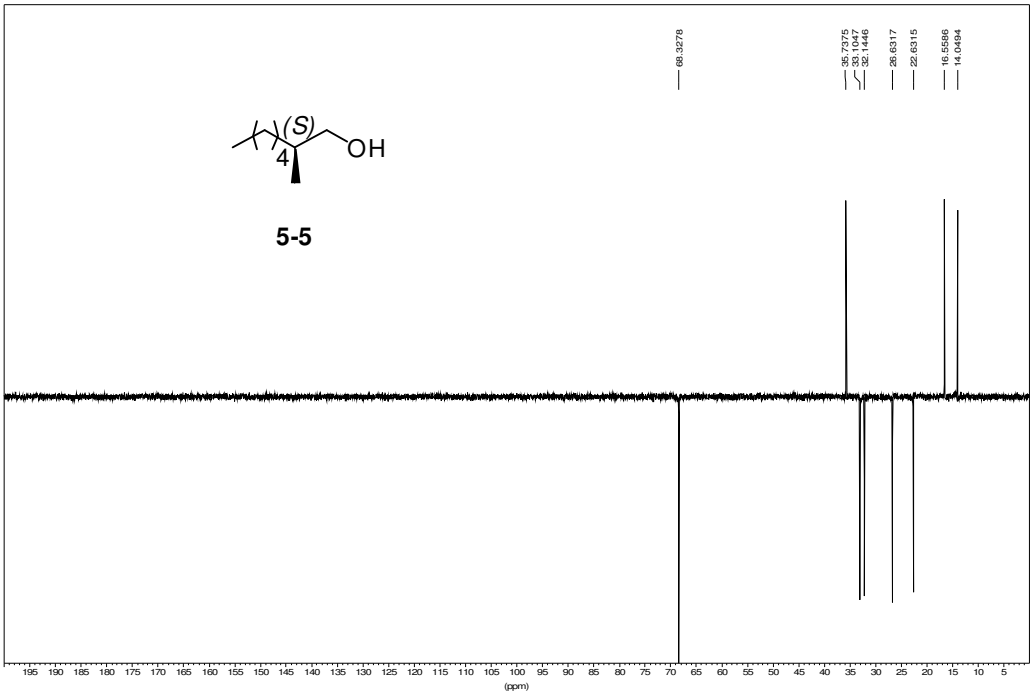
5-4

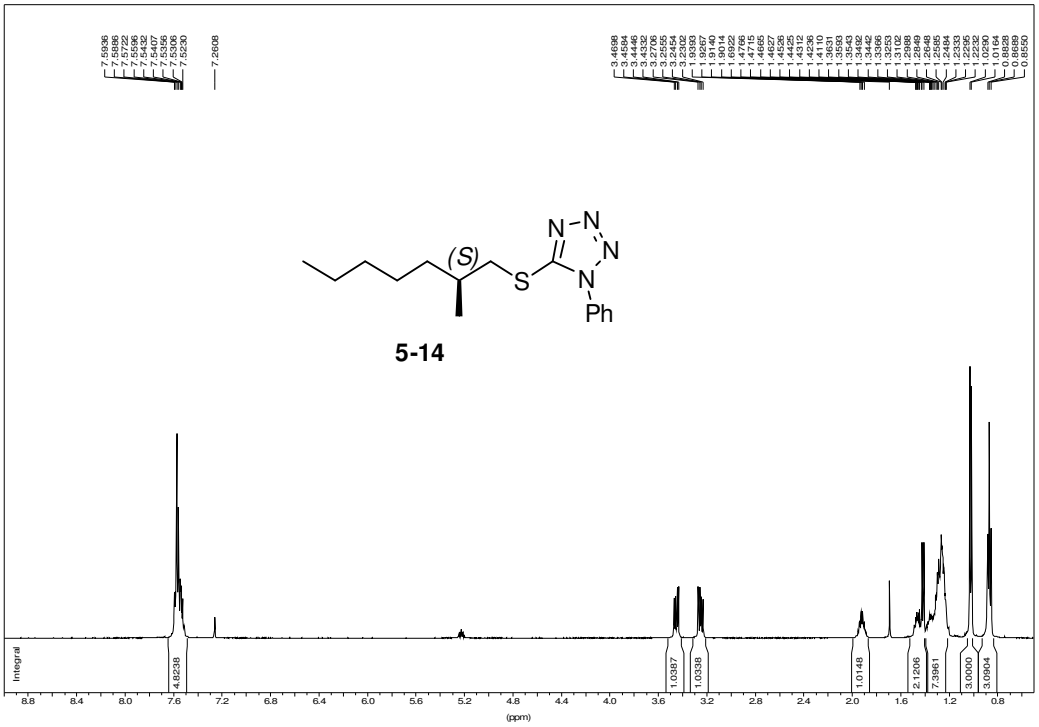


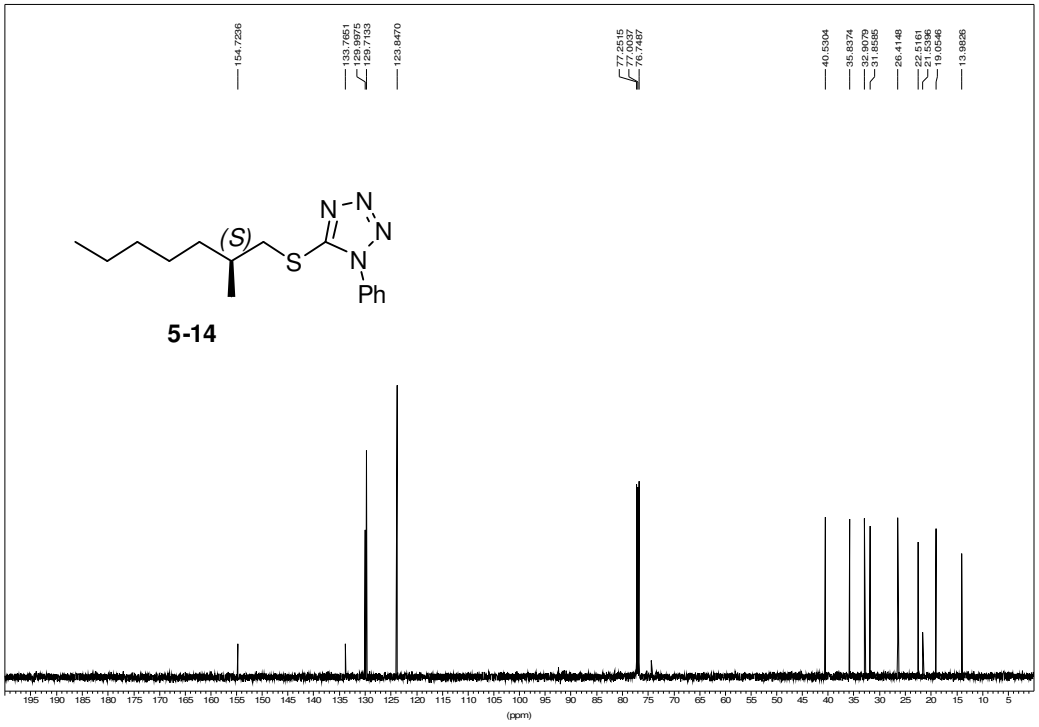
5-5

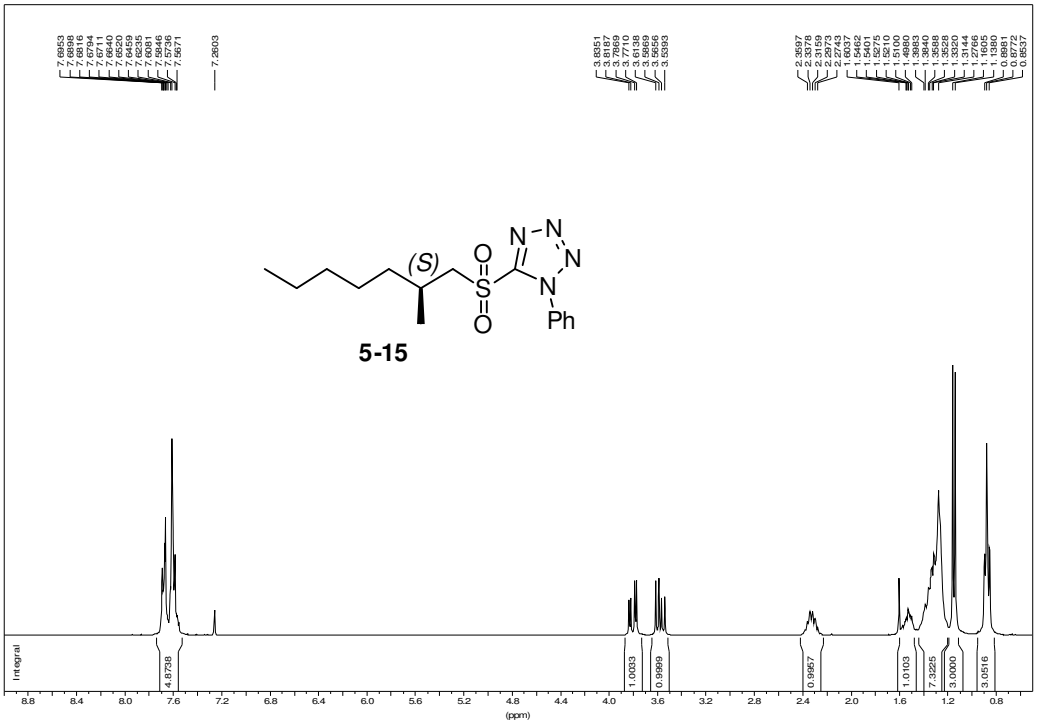


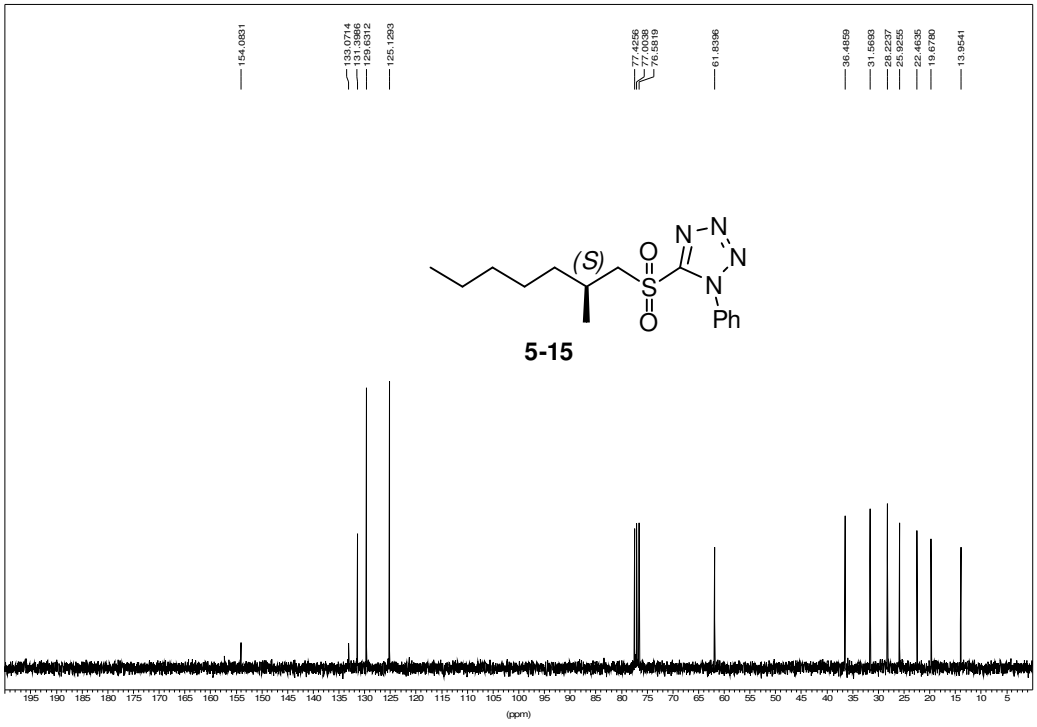
DEPT135 AC300

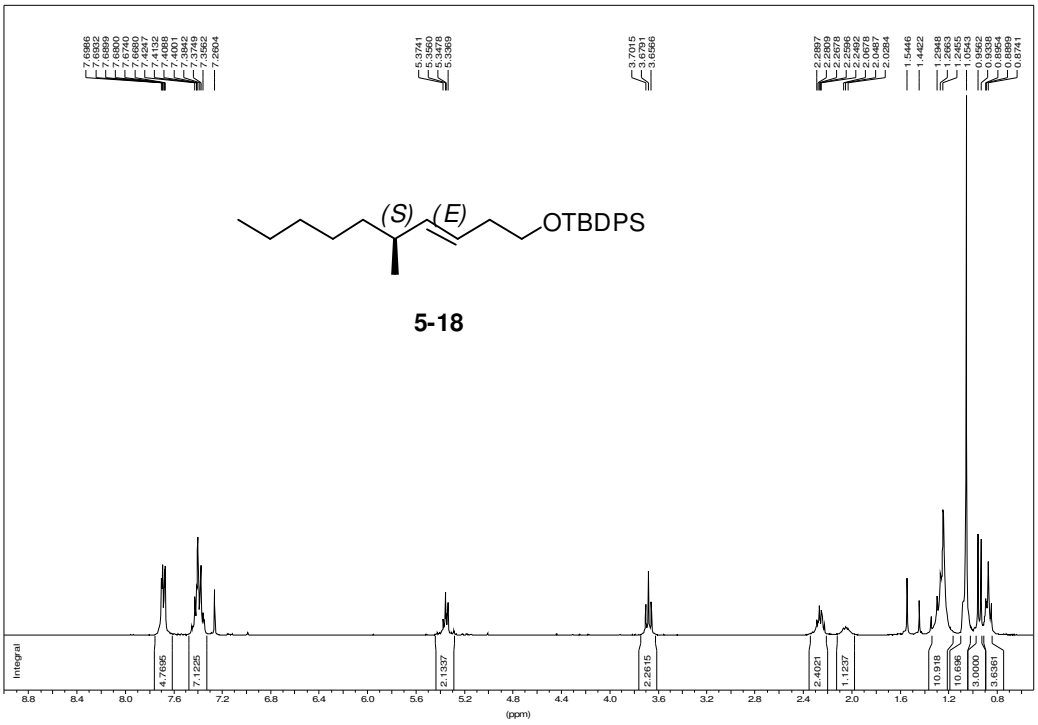



7.5936
7.5886
7.5722
7.5586
7.5432
7.5407
7.5356
7.5306
7.5230
7.2608
3.4698
3.4544
3.4446
3.4332
3.2706
3.2555
3.2454
3.2302
3.1938
1.9267
1.9140
1.8914
1.6922
1.4766
1.4715
1.4665
1.4627
1.4526
1.4465
1.4312
1.4236
1.4110
1.3631
1.3593
1.3542
1.3442
1.3365
1.3253
1.3102
1.2988
1.2849
1.2648
1.2585
1.2484
1.2333
1.2295
1.2232
1.2032
1.0164
0.8828
0.8689
0.8590
N
N
N
N
(S)
S
Ph
5-14
Integral
4.8238
1.0387
1.0338
1.0148
2.1206
7.3961
3.0000
3.0904
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
(ppm)


154.7236
133.7951
129.9955
129.7133
123.8470
77.2515
77.0037
76.7487
40.5304
35.8374
32.9079
31.8586
26.4148
22.5161
21.5396
19.0546
13.9926
N
N
N
N
(S)
S
Ph
5-14
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
(ppm)


7.6953
7.6898
7.6816
7.6784
7.6754
7.6711
7.6640
7.6600
7.6459
7.6235
7.6091
7.5946
7.5736
7.5671
7.2603
3.8351
3.8197
3.7869
3.7710
3.6158
3.5989
3.5956
3.5393
2.3597
2.3378
2.3159
2.2973
2.2743
1.6627
1.5462
1.5401
1.5275
1.5215
1.5100
1.4980
1.4843
1.3840
1.3588
1.3458
1.3320
1.3144
1.2726
1.1605
1.1380
0.8991
0.8727
0.8537
(S)
N
N
N
O
S
O
N
Ph
5-15
Integral
4.8738
1.0033
0.9999
0.9957
1.0103
7.9225
3.0000
3.0516
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
(ppm)


154.0831
133.0714
131.3986
129.6312
125.1293
77.4256
77.0038
76.5819
61.8396
36.4859
31.5693
28.2237
25.9255
22.4635
19.6780
13.9541
(S)
N
N
N
O
S
O
N
Ph
5-15
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
(ppm)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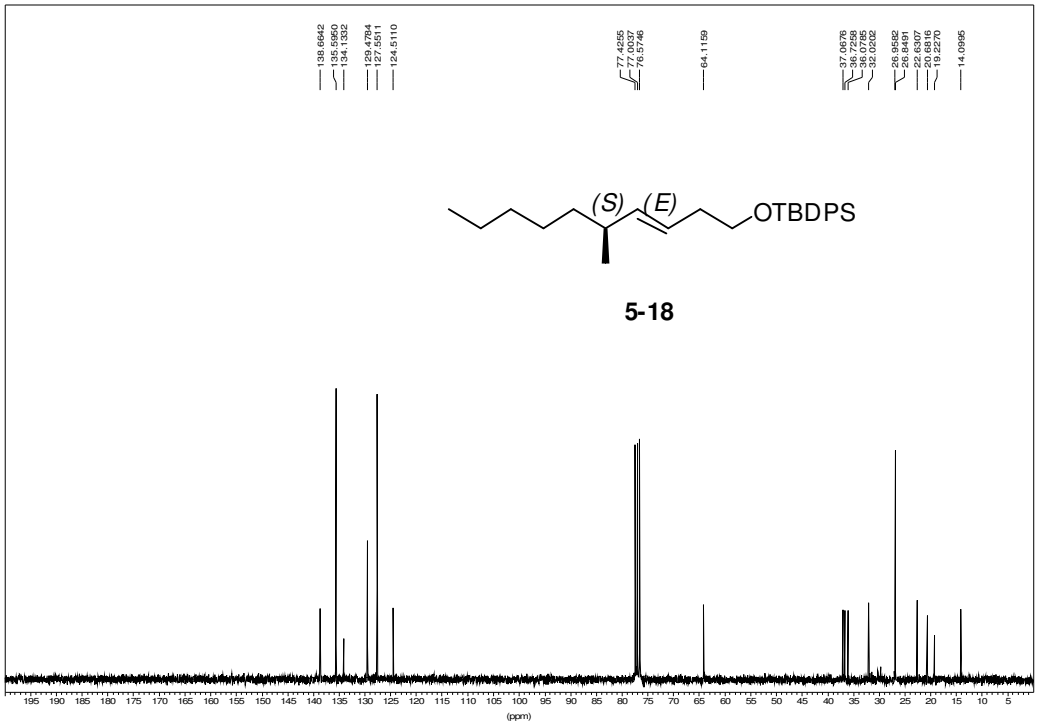
7.6932
7.6888
7.6800
7.6740
7.6690
7.4247
7.4132
7.4088
7.4041
7.3942
7.3749
7.2952
7.2604
5.3741
5.3600
5.3478
5.3369
3.7015
3.6791
3.6566
2.2897
2.2809
2.2678
2.2546
2.2492
2.0677
2.0284
1.5446
1.4422
1.2948
1.2683
1.2455
1.0543
0.9562
0.9238
0.8954
0.8899
0.8741
(S)
(E)
OTBDPS
5-18
Integral
4.7685
7.1225
2.1337
2.2615
2.4021
1.1237
10.918
10.696
3.0000
3.6361
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
(ppm)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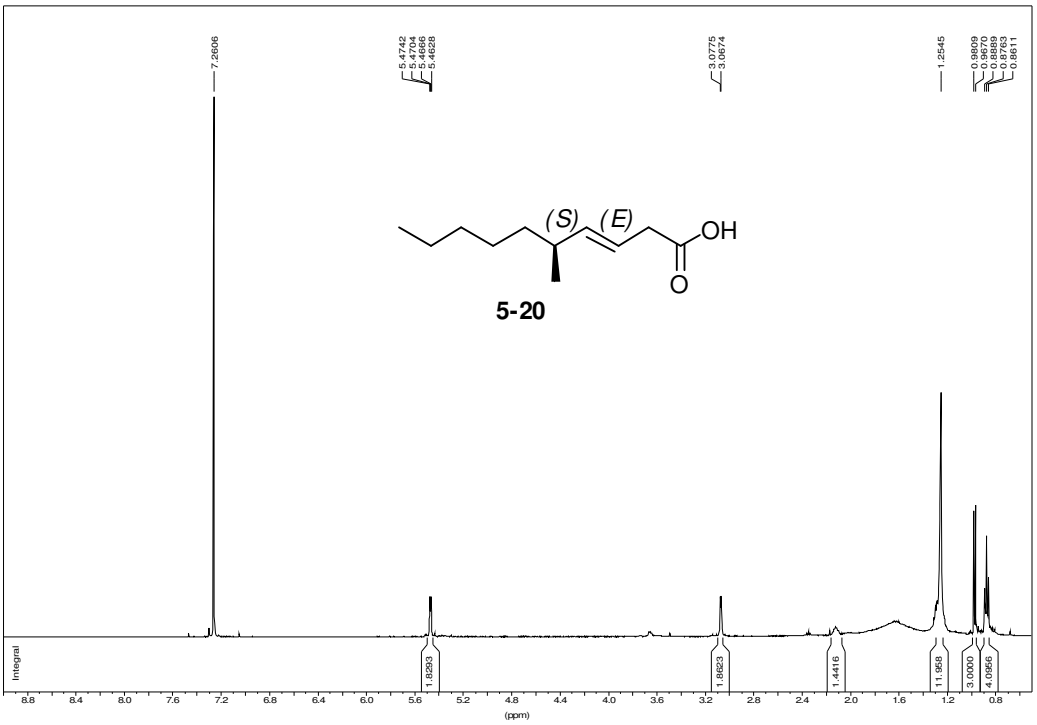
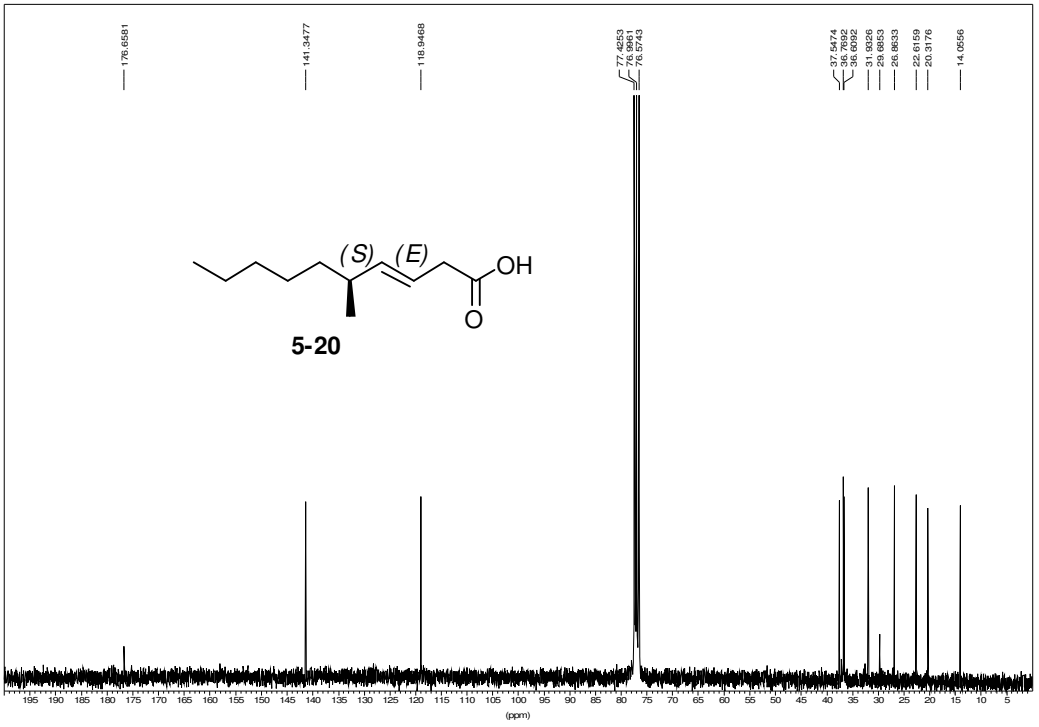
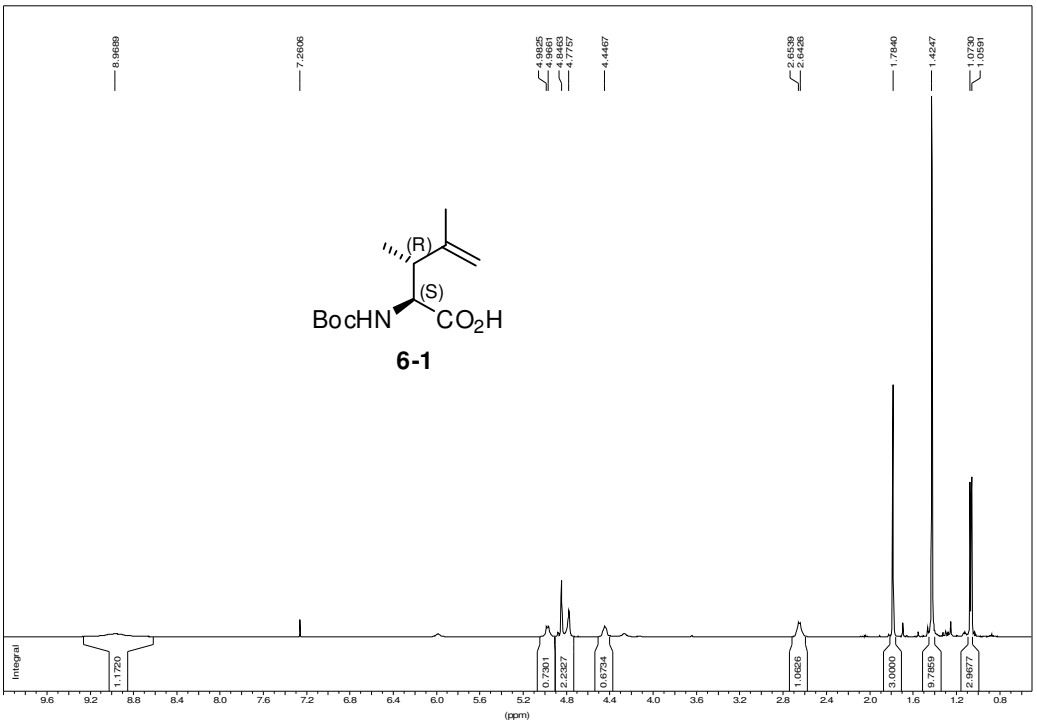
134.1532
129.4784
127.5511
124.5110
77.4255
77.0037
76.5746
64.1159
37.0676
36.7298
36.0705
32.0202
26.9582
26.8441
22.6307
20.6816
19.2270
14.0995
(S)
(E)
OTBDPS
5-18
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
(ppm)


7.2606
5.4742
5.4704
5.4666
5.4628
3.0775
3.0674
1.2545
0.9809
0.9670
0.8889
0.8763
0.8611
(S)
(E)
OH
O
5-20
Integral
1.8293
1.8623
1.4416
11.968
3.0000
4.0956
(ppm)


176.6581
141.3477
118.9468
77.4253
76.9961
76.5743
37.5474
36.7692
36.6092
31.9326
29.6853
26.8633
22.6159
20.3176
14.0556
(S)
(E)
OH
O
5-20
(ppm)


8.9689
7.2606
4.9825
4.9561
4.8463
4.7757
4.4467
2.6539
2.6426
1.7840
1.4247
1.0730
1.0591
(R)
(S)
BocHN
CO2H
6-1
Integral
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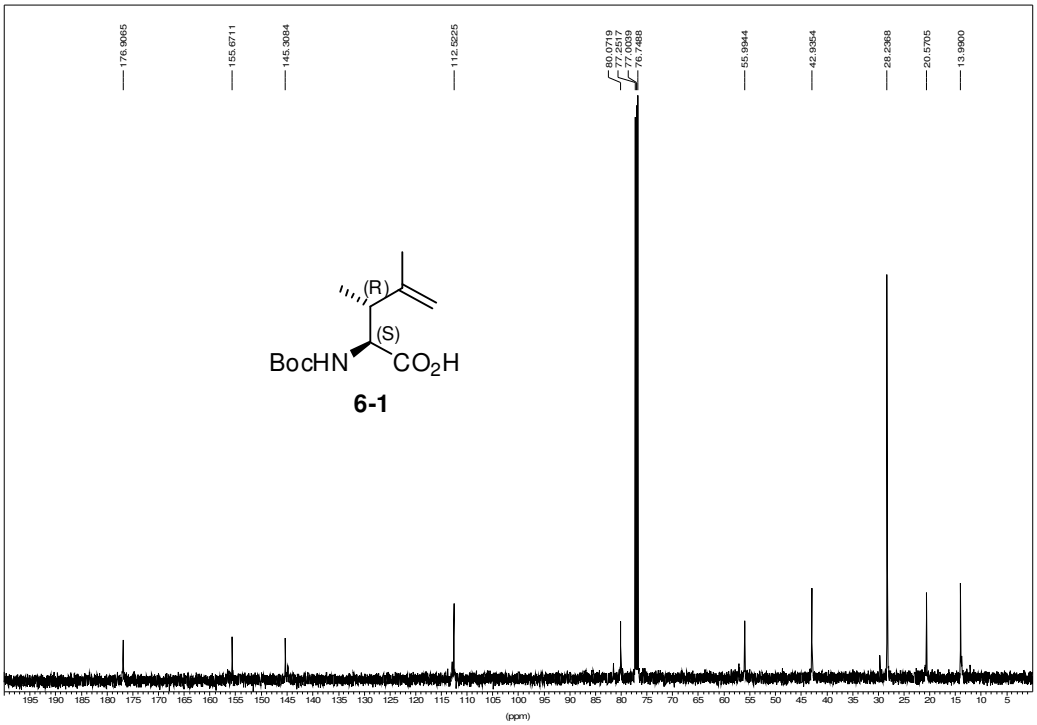
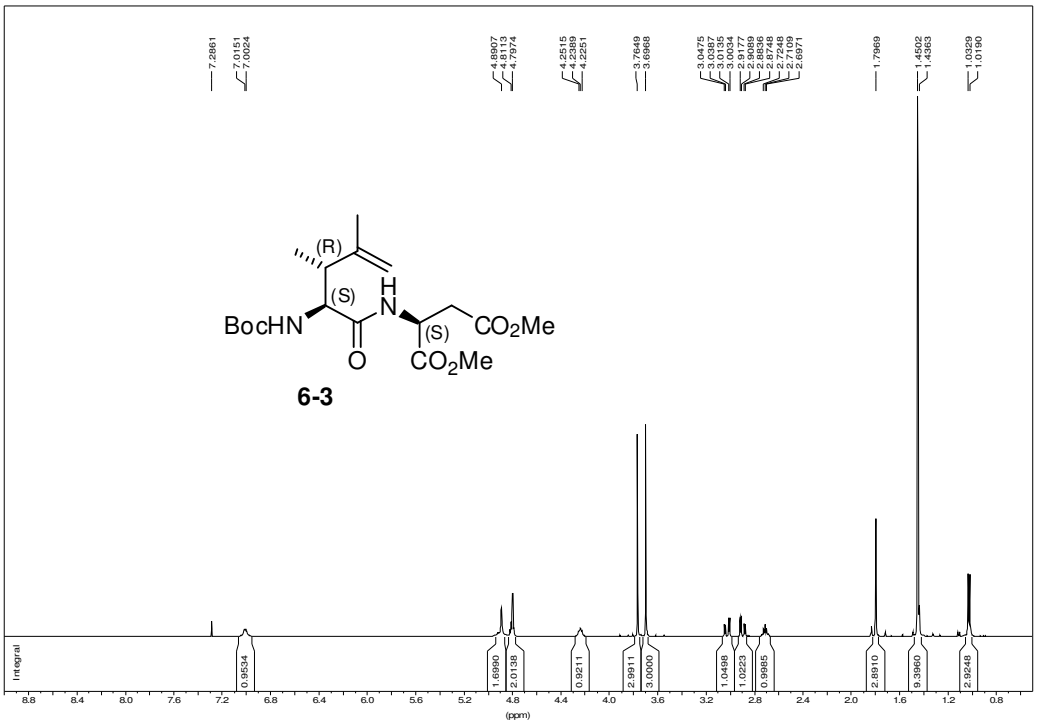
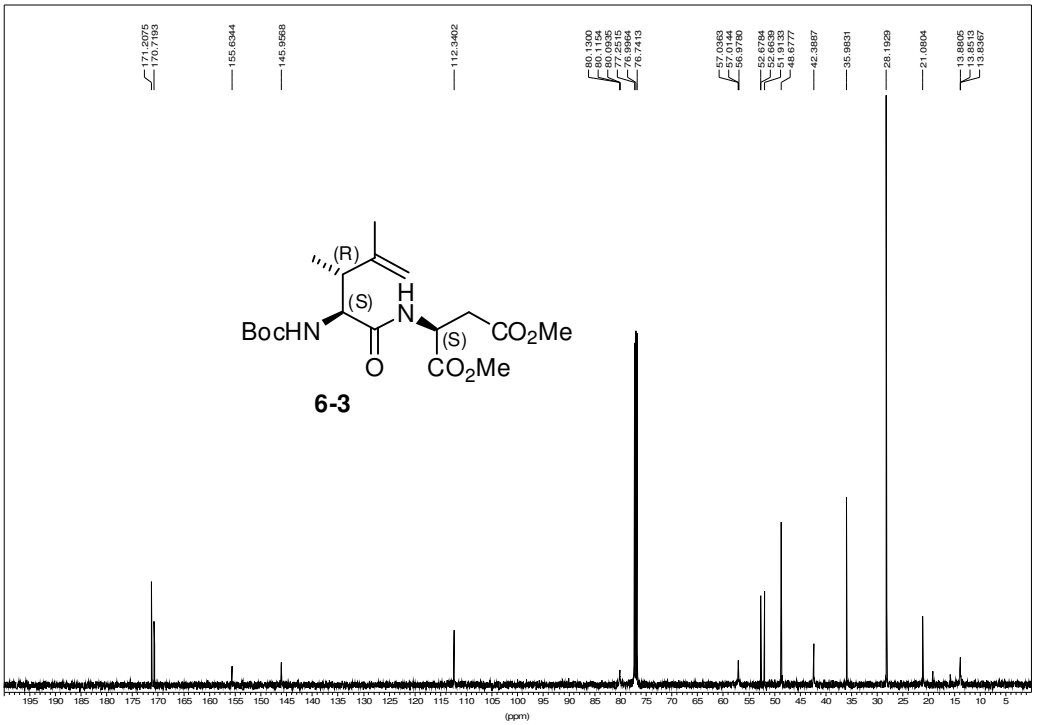
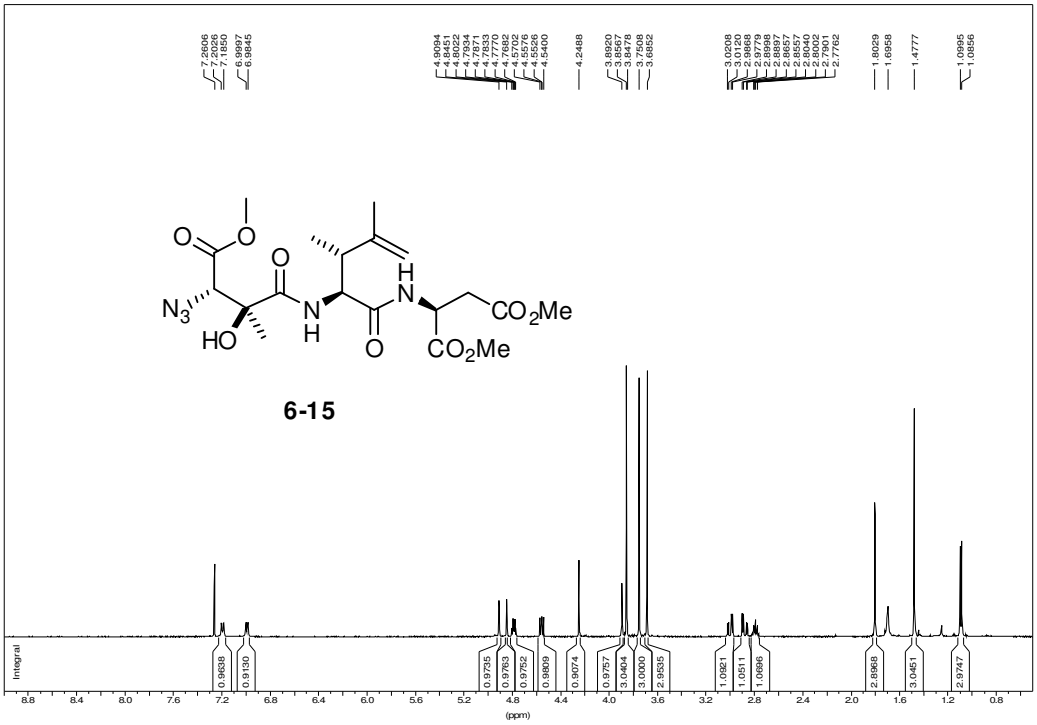
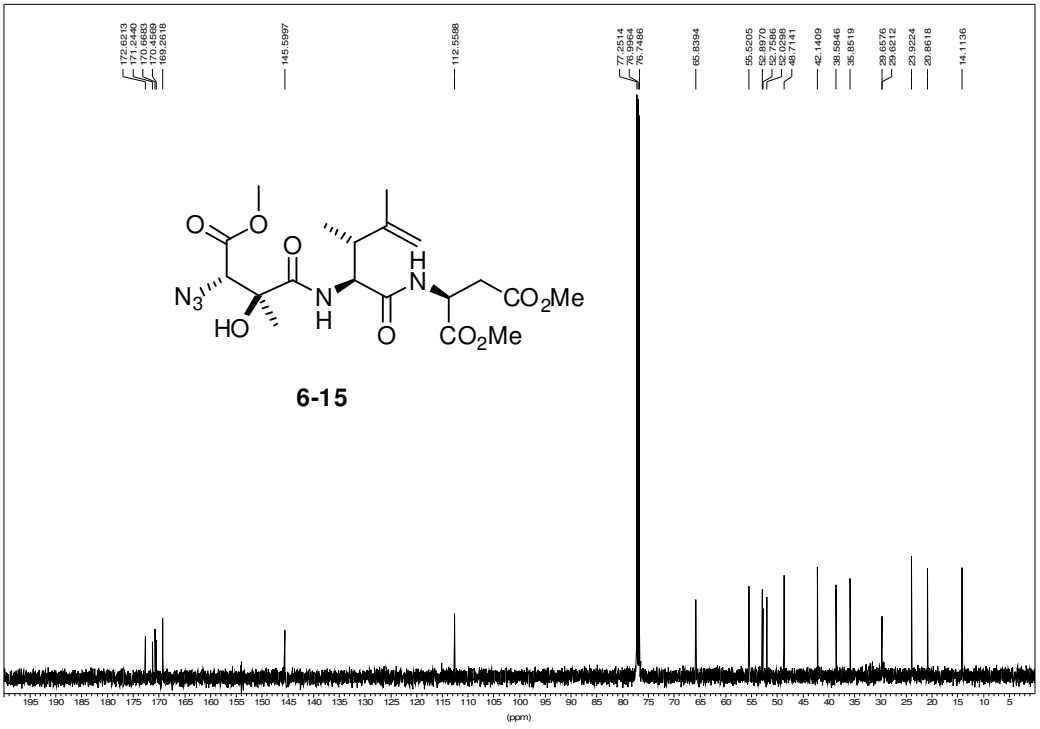
0.7301
2.2327
0.6734
1.0826
3.0000
9.7859
2.9677
(ppm)

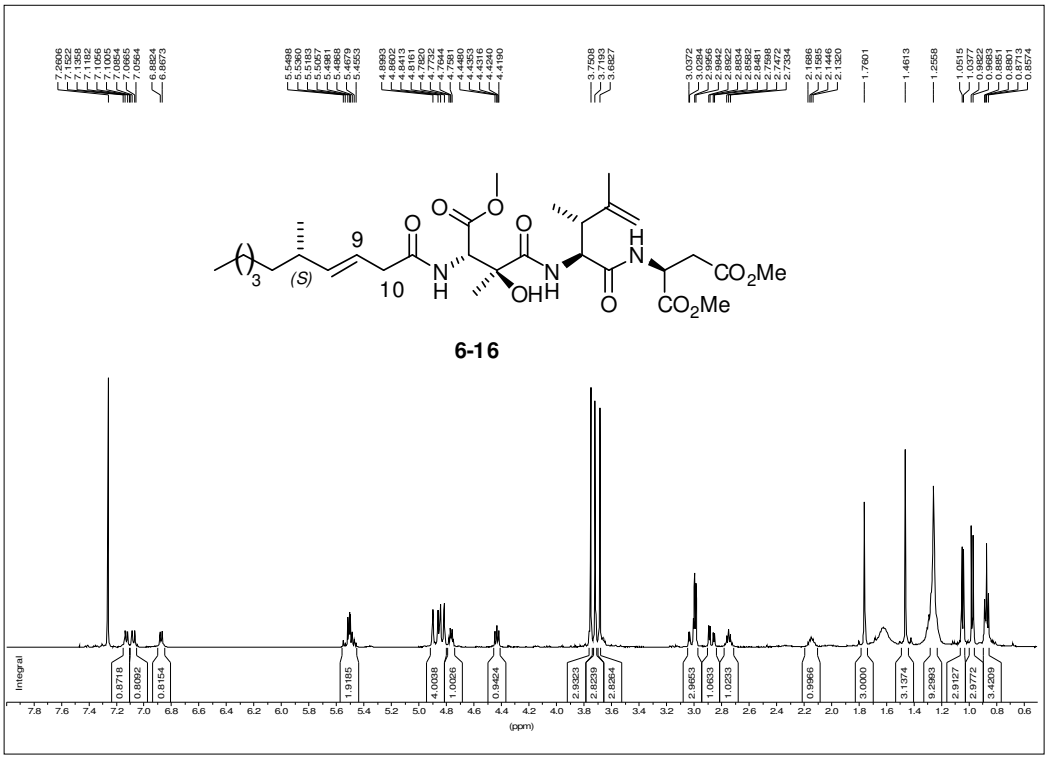

176.9065
155.6711
145.3084
112.5225
80.0719
77.2517
77.0039
76.7488
55.9944
42.9354
28.2368
20.5705
13.9900
(R)
(S)
BocHN
CO2H
6-1
(ppm)

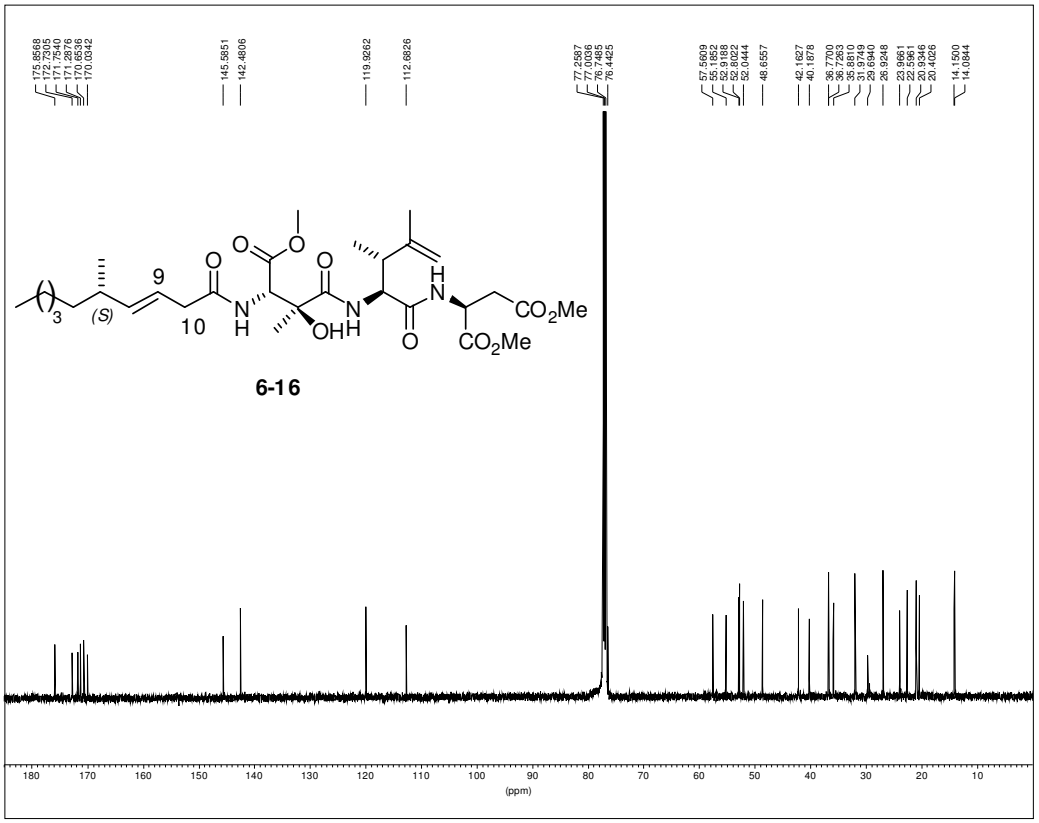

7.2861
7.0151
7.0024
4.8907
4.8113
4.7974
4.2515
4.2389
4.2251
3.7649
3.6968
3.0475
3.0387
3.0155
3.0034
2.9177
2.8969
2.8838
2.8748
2.7248
2.7059
2.6971
1.7969
1.4502
1.4363
1.0329
1.0190
(R)
(S)
H
N
BocHN
(S)
CO2Me
O
CO2Me
6-3
Integral
0.9534
1.6990
2.0138
0.9211
2.9911
3.0000
1.0498
1.0223
0.9985
2.8910
9.3960
2.9248
(ppm)


171.2075
170.7193
155.6344
145.9568
112.3402
80.1300
80.1154
80.0965
77.2515
76.9964
76.7413
57.0363
57.0144
56.9780
52.6784
52.6639
51.9133
48.6777
42.3887
35.9831
28.1929
21.0804
13.8805
13.8513
13.8367
(R)
(S)
H
N
BocHN
(S)
CO2Me
O
CO2Me
6-3
(ppm)

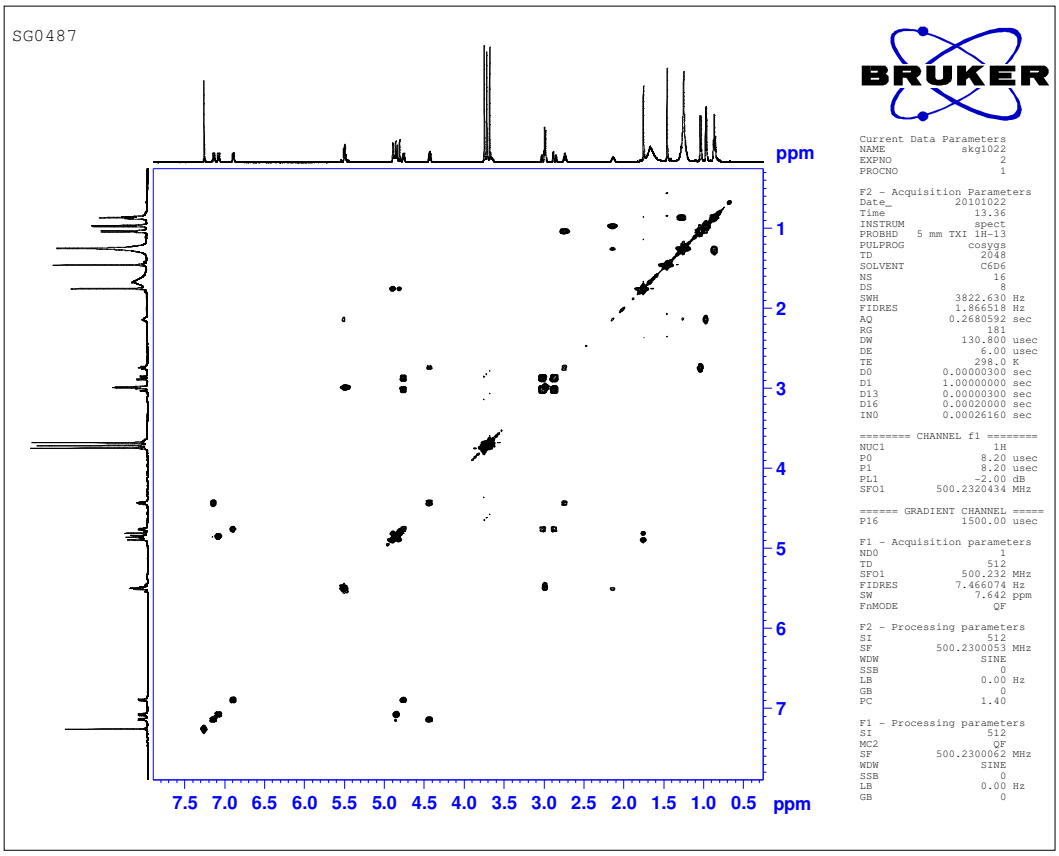

7.2606
7.2026
7.1850
6.9997
6.9845
4.9094
4.8451
4.8020
4.7934
4.7871
4.7833
4.7770
4.7682
4.5702
4.5576
4.5526
4.5400
4.2488
3.8920
3.8567
3.8478
3.7808
3.6852
3.0208
3.0120
3.0045
2.9779
2.8998
2.8897
2.8657
2.8557
2.8440
2.8002
2.7901
2.7762
1.8029
1.6958
1.4777
1.0995
1.0856
O
O
O
N3
HO
N
H
O
H
N
CO2Me
CO2Me
6-15
Integral
0.9638
0.9130
0.9725
0.9763
0.9752
0.9809
0.9074
0.9757
3.0464
3.0000
2.9535
1.0921
1.0511
1.0696
2.8968
3.0451
2.9747
(ppm)


172.6213
171.2440
170.6683
170.4589
169.2618
145.5997
112.5588
77.2514
76.9984
76.7486
65.8394
55.5205
52.8970
52.7586
52.0298
48.7141
42.1409
38.5846
35.8519
29.6576
29.6212
23.9224
20.8618
14.1136
O
O
O
N3
HO
N
H
O
H
N
CO2Me
CO2Me
6-15
(ppm)

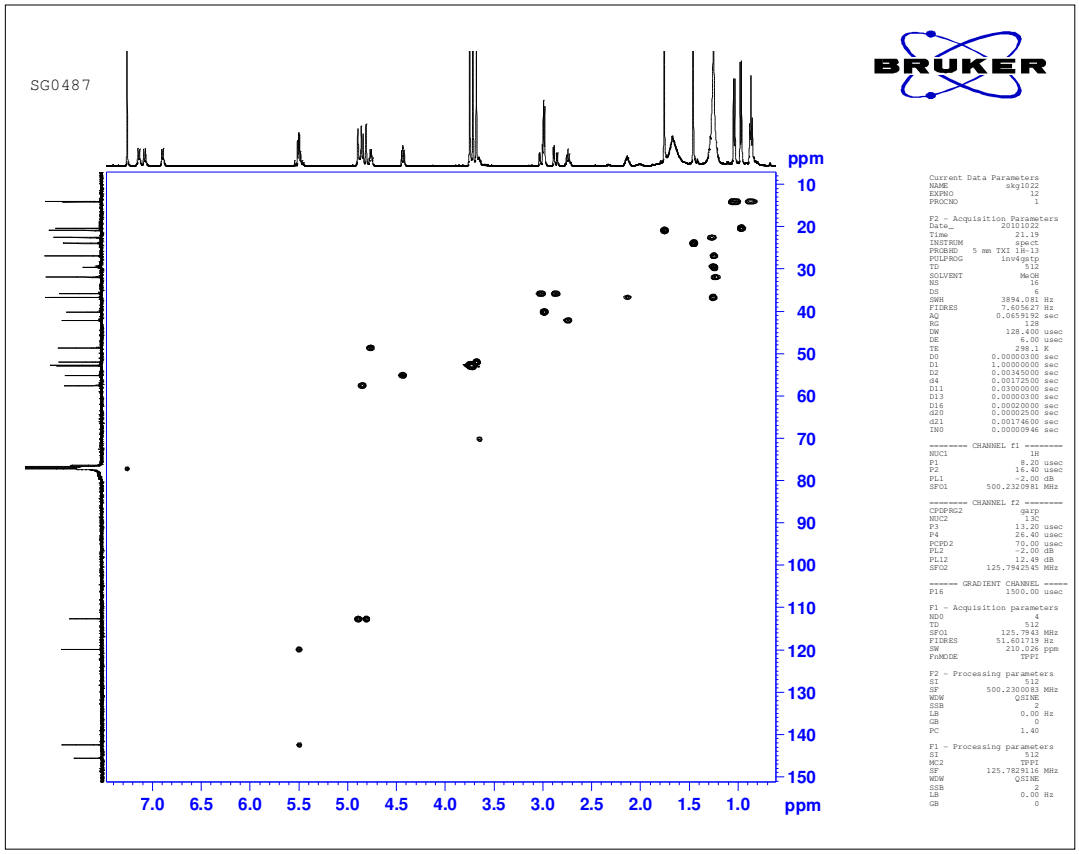

6-16
Integral
(ppm)


6-16
(ppm)

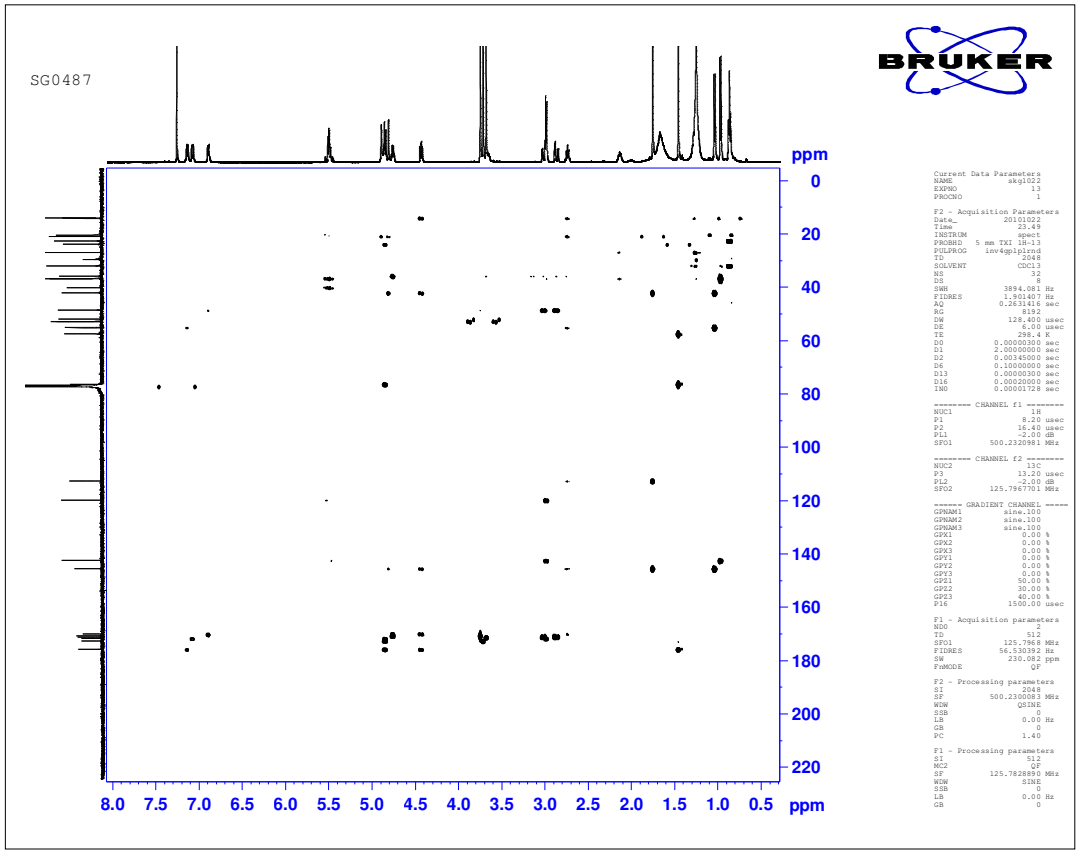
2D NMR Experiment (COSY) of compound **6-16**.

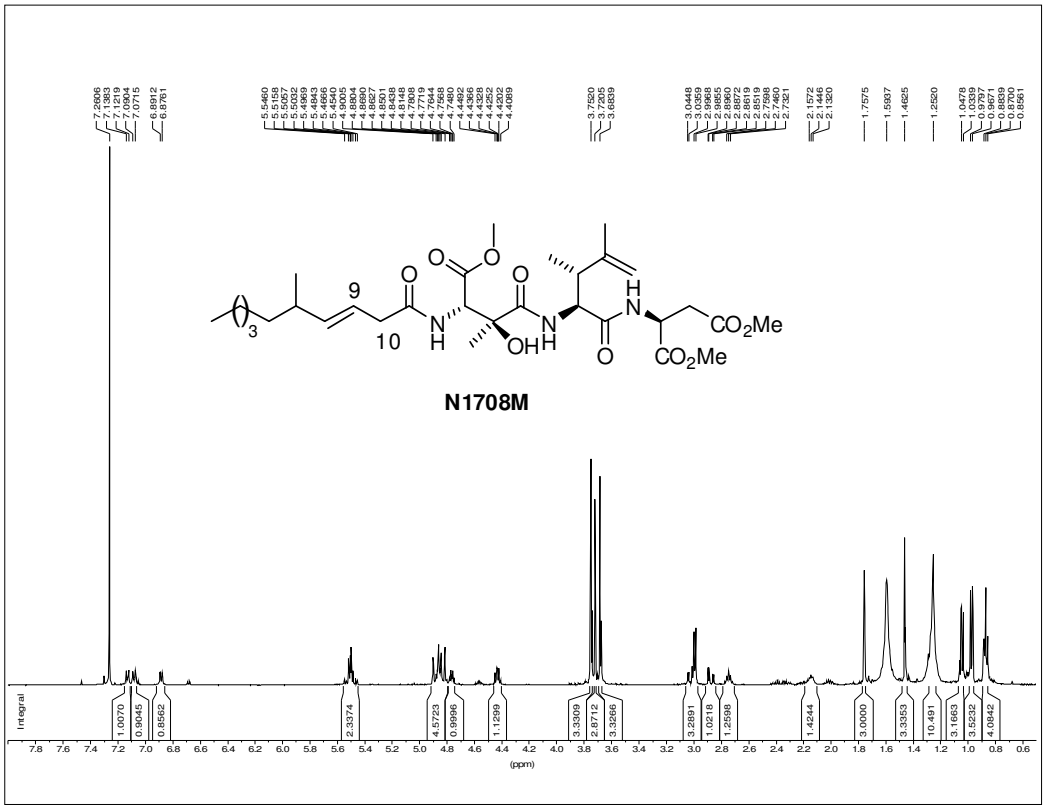


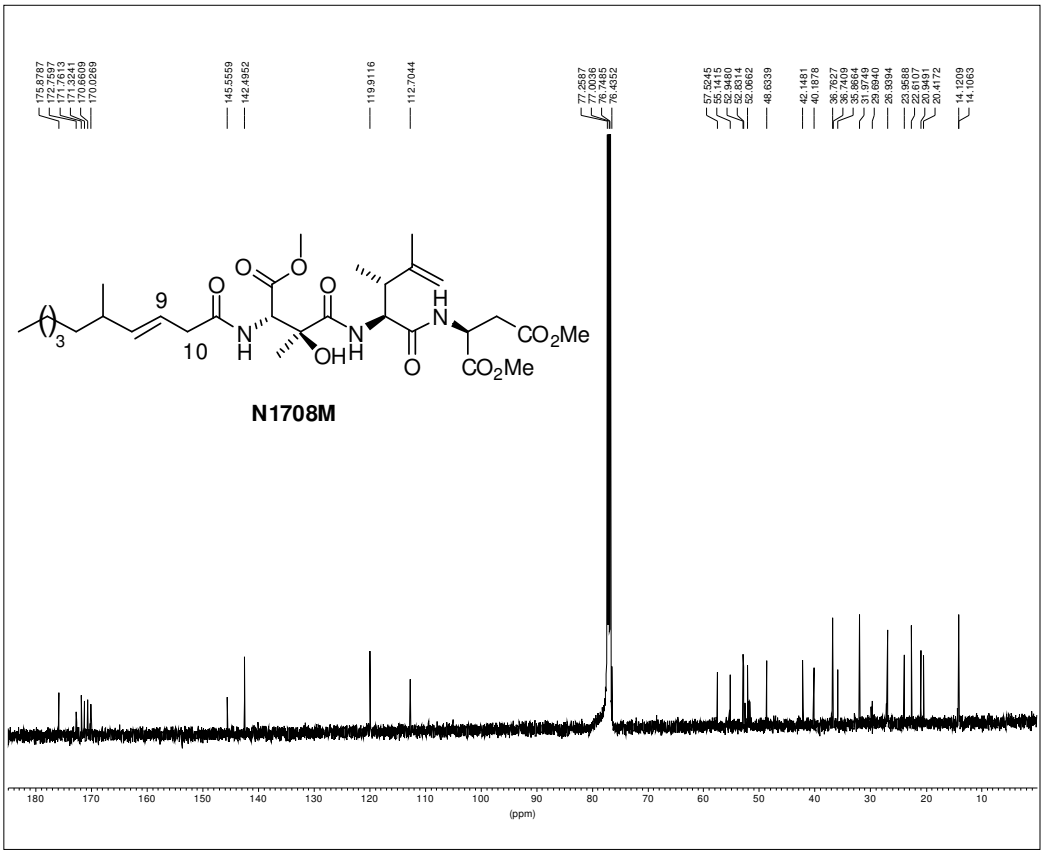
2D NMR Experiment (HMQC) of compound **6-16**.

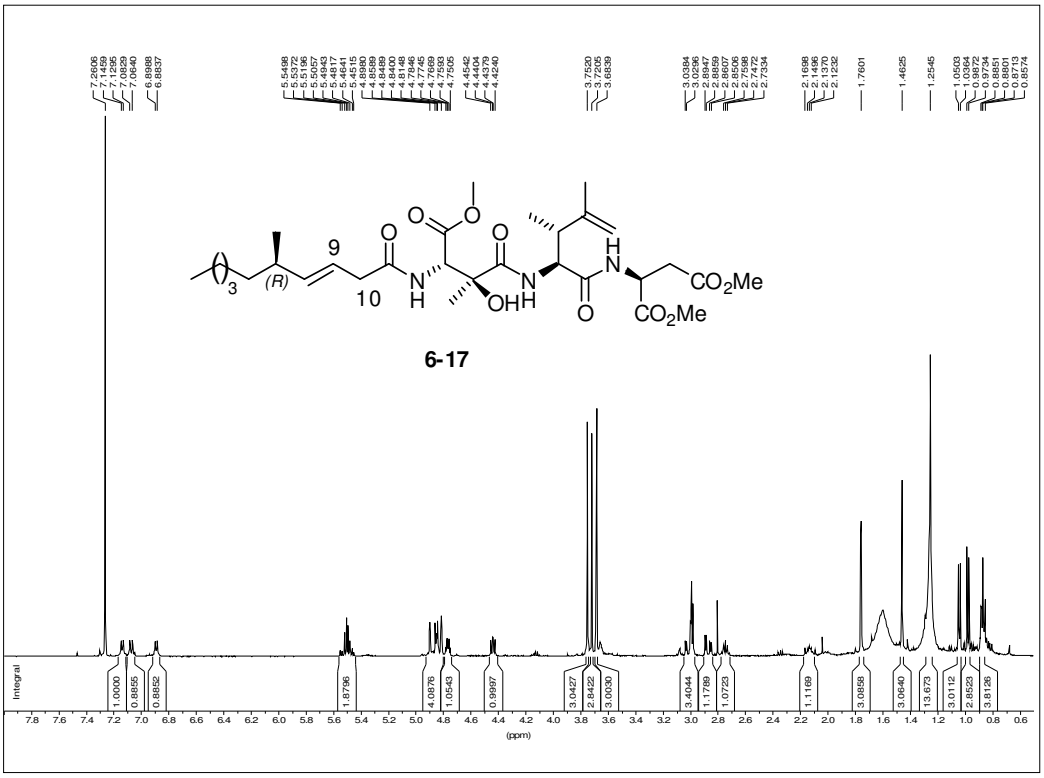


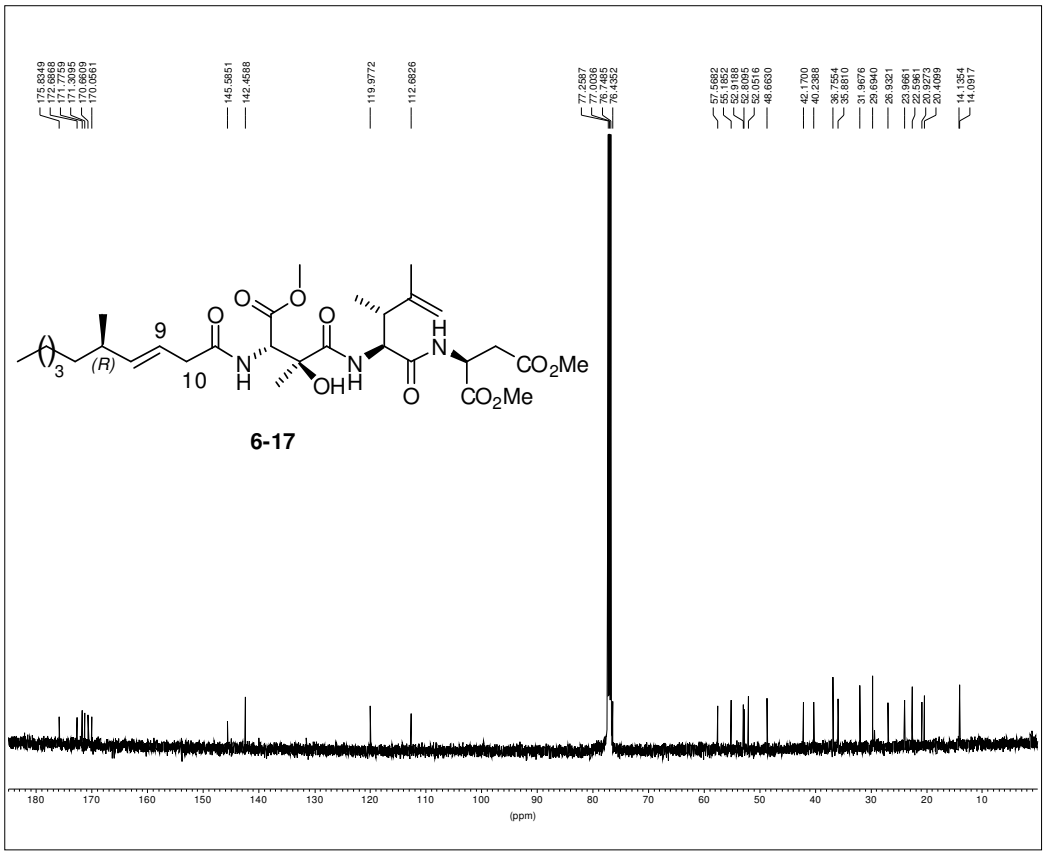
2D NMR Experiment (HMBC) of compound **6-16**.

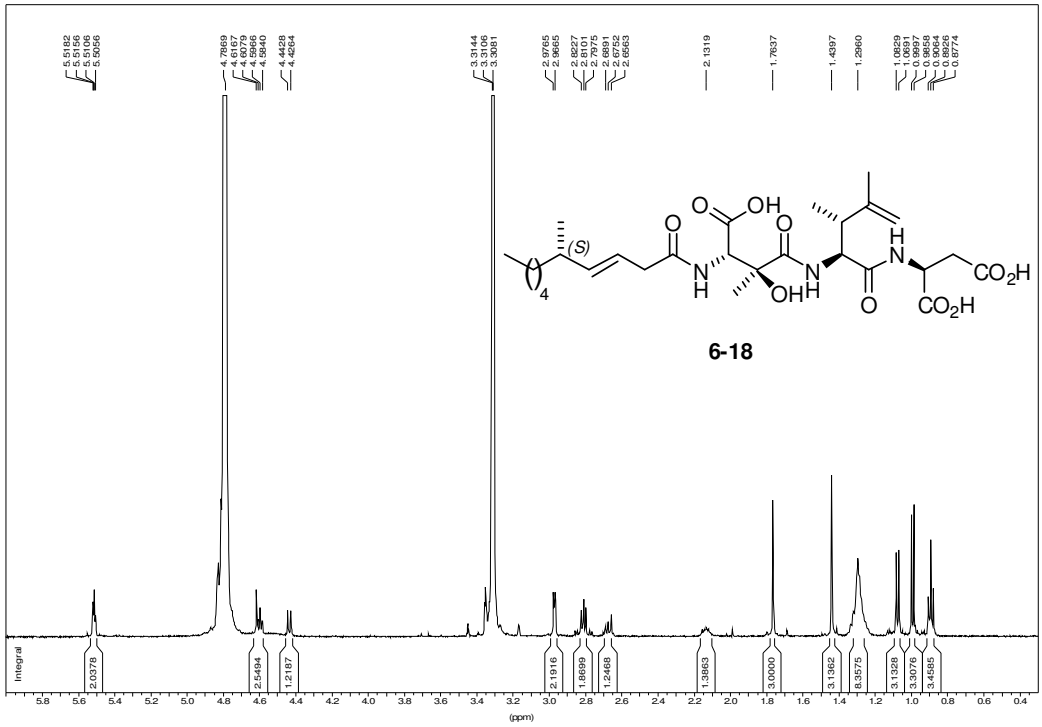


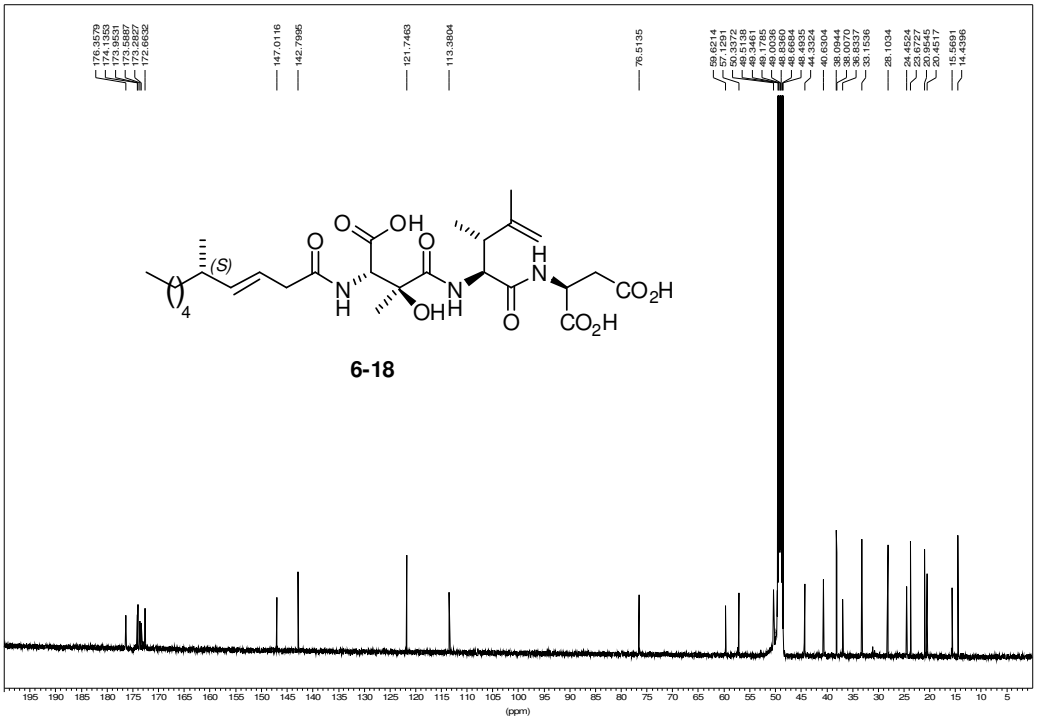

N1708M


N1708M


6-17
Integral
(ppm)


6-17
(ppm)


6-18


6-18