Université de Montréal

Substituted Azetidine-2-carboxylic Acid Synthesis

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Abstract

Azetidine-2-carboxylic acid (Aze) is the four-member homologue of proline. Aze and substituted Aze are constituents of natural and pharmacological products and biologically active compounds. The preparation of substituted Aze analogues is, however, a challenge for which few routes have been reported. In studying general methodology for the preparation of Aze derivatives, we have now focused on developing new routes for the synthesis of Aze analogues with substituents at the 3- and 4-position. These orthogonally protected amino acid-Aze chimeras have been designed to serve as tools for studying the influence of conformation on peptide activity.

In chapter 2, the synthesis of azetidine 2-carboxylic acid (Aze) analogs possessing various heteroatomic side chains at the 3-position is presented and features modification of 1-PhF-3-allyl-Aze *tert*-butyl ester (2S,3*S*)-**2.1** (PhF = 9-(9-phenylfluorenyl)). 3-Allyl-Aze **2.1** was synthesized from a sequence commencing with regioselective allylation of α-*tert*-butyl β-methyl *N*-(PhF)aspartate **2.13**, followed by selective ω-carboxylate reduction, tosylation and intramolecular *N*-alkylation. Removal of the PhF group and olefin reduction by hydrogenation followed by Fmoc protection produced nor-Leucine-Aze chimera **2.2**. Regioselective olefin hydroboration of (2S,3*S*)-**2.1** produced primary alcohol **2.23**, which was protected as a silyl ether, hydrogenated and *N*-protected to give 1-Fmoc-3-hydroxypropyl-Aze **2.26**. Enantiopure (2*S*,3*S*)-3-(3-azidopropyl)-1-Fmoc-azetidine-2-carboxylic acid *tert*-butyl ester **2.3** was prepared as a Lys-Aze chimera by activation of 3-hydroxypropyl-Aze **2.26** as a methanesulfonate and displacement with sodium azide. Moreover, orthogonally protected azetidine dicarboxylic acid **2.4** was synthesized as an α-aminoadipate-Aze chimera by oxidation of alcohol **2.26**.

Chapter 3, a study to develop new methodology for the preparation of 4-substituted Aze analogues was performed. Three approaches were examined. In the first approach novel 2,4-disustituted azetidine-3-one **3.43** was synthesized for the first time by employing an intramolecular metal carbenoid N-H insertion of α-diazo β-ketophosphonate **3.40** which was prepared from L-serine as a chiral educt. In the second approach, a S_N2' strategy has been used to synthesize 4-vinyl Aze analogues. For example, diastereomeric mixture of 4-propenyl *N*-(PhF)Aze **3.48** was synthesized by the Horner-Wadsworth-Emmons (HWE) olefination of α-*tert*-butyl-*N*-(PhF)aspartate β-aldehyde **3.42** with β-keto phosphonate **3.44**, followed by reduction of the resulting α,β-unsaturated ketone **3.45**, activation of the corresponding alcohol **3.46** as a tosylate **3.47** and intramolecular S_N2' reaction. In an attempt to synthesize 4-vinyl *N*-(PhF) Aze **3.59**, pipecolate **3.58** was formed instead of 4-substituted azetidine **3.59** during the S_N2' reaction in the presence of DMAP. Diastereomeric mixtures of 4-vinyl Aze **3.65** were produced in 11 steps from aspartic acid by application of allylglycine methyl ester **3.63** in a cross-metathesis/ intramolecular S_N2' *N*-alkylation strategy. The novel 4-substituted Aze analogues offer potential to serve as precursors for making azabicyclo[4.2.0] and [5.2.0]alkane amino acids, such as **3.11** and **3.13**. Although an alternative approach failed to produce 4-substituted Aze by intramolecular ring opening of an epoxide by nucleophilic attack of a β-amine, new amino epoxides **3.70** and **3.74** were synthesized for the first time from L-Asp as an inexpensive chiral educt. These amino epoxides may serve as intermediates for synthesis of other functionalized compounds.

We have developed new syntheses of amino acid analogues including enantiopure 3-substituted azetidine-2-carboxylic acids **2.2**, **2.3** and **2.4**, for exploring structure-activity

relationships of various biologically active peptides. Also, we have developed new methodologies for synthesizing 4-substituted Aze analogues **3.43**, **3.48**, **3.65**, which may enable syntheses of novel azabicyclo[X.Y.0]alkane derivatives. In summary, this thesis has provided new methodology for making azetidines to explore their conformational and biological properties.

Key words: azetidine 2-carboxylic acid; azetidines; amino acid; chimera; heterocycle; peptide synthesis

Sommaire

L'acide 2-azétidine carboxylique (Aze) est un homologue à quatre chaînons de la proline. Aze et Aze substitué sont des constituants de produits naturels et pharmacologiques ainsi que de composés biologiquement actifs. La préparation des analogues d'Aze substitués est, cependant, un défi pour lequel peu de voies de synthèse ont été reportées. En étudiant une méthodologie générale pour la préparation des dérivés d'Aze, nous nous sommes concentrés à développer de nouvelles voies de synthèse pour les analogues d'Aze substitués en position 3 et 4. Ces chimères d'Aze ont été élaborées pour servir d'outil dans l'étude de l'influence de la conformation dans l'activité des peptides.

Dans le chapitre 2, la synthèse d'analogues de l'acide 2-azétidine carboxylique (Aze) possédant différents hétéroatomes sur les chaînes latérales à la position 3 est présentée en plus de la modification de l'ester *tert*-butylique du 1-PhF-3-allyl-Aze (2*S*,3*S*)-**2.1** (PhF = 9-(9-phénylfluorényle)). Le 3-allyl Aze **2.1** a été synthétisé tout d'abord avec une allylation régiosélective du diester α-tert-butylique β-méthylique de l'acide *N*-(PhF)aspartique **2.13**, suivie par une réduction sélective du ω-carboxylate, tosylation et *N*-alkylation intramoléculaire. Le clivage du groupement PhF et la réduction de l'oléfine par hydrogénation suivis par une protection avec un groupement Fmoc ont produit la chimère norleucine-Aze **2.2**. Une hydroboration régiosélective de l'oléfine (2*S*,3*S*)-**2.1** a produit l'alcool primaire **2.23**, qui après protection par un groupement éther de silyle, hydrogénation et protection de l'amine a donné le 1-Fmoc-3-hydroxypropyle-Aze **2.26**. L'ester *tert*-butylique énantiopur de l'acide (2*S*,3*S*)-3-(3-azidopropyle)-1-Fmoc-azétidine-2-carboxylique **2.3** a été préparé comme une chimère de la Lys-Aze par activation du 3-hydroxypropyl-Aze **2.26** comme un méthanesulfonate et déplacement avec un azidure de

sodium. De plus, l'acide dicarboxylique **2.4** de l'azétidine protégée orthogonalement a été synthétisé comme une chimère du α-aminoadipateAze par oxydation de l'alcool **2.26**.

Dans le chapitre 3, une étude pour développer une nouvelle méthodologie pour la préparation des analogues de l'Aze substitué en position 4 a été réalisée. Trois approches ont été examinées. Dans une première approche, l'azétidine-3-one 2,4-disubstituée **3.43** a été synthétisée pour la première fois en employant une insertion intramoléculaire d'un carbénoïde métallique N-H du α-diazo β-cétophosphonate **3.40** qui a été préparé à partir de la L-sérine comme entité chirale. Dans une seconde approche, une stratégie S_N2' a été utilisée pour la synthèse d'analogues 4-vinyle Aze. Par exemple, un mélange diastéréomérique du 4-propényle *N*-(PhF)Aze **3.48** a été synthétisé par oléfination d'Horner-Wadsworth-Emmons (HWE) du α-*tert*-butyl-*N*-(PhF)aspartate β-aldéhyde **3.42** avec le β-cétophosphonate **3.44**, suivie par une réduction de la cétone α,β-insaturée résultante **3.45**, activation de l'alcool correspondant **3.46** comme un tosylate **3.47** et une réaction intramoléculaire S_N2'. Dans une tentative de synthèse du 4-vinyl *N*-(PhF) Aze **3.59**, le pipécolate **3.58** a été formé au lieu de l'azétidine 4-substituée **3.59** durant la réaction S_N2' en présence de DMAP. Un mélange diastéréomérique du 4-vinyle Aze **3.65** a été produit en 11 étapes à partir de l'acide aspartique par application de l'ester méthylique de l'allylglycine **3.63** dans une stratégie de métathèse croisée / *N*-alkylation intramoléculaire S_N2'. Ces nouveaux analogues de l'Aze substitué en position 4 peuvent servir comme précurseurs dans la synthèse des acides aminés [4.2.0] et [5.2.0] azabiycliques, tels que **3.11** et **3.13**. Bien qu'une approche alternative pour former l'Aze 4-substituée par ouverture intramoléculaire d'un époxyde par attaque nucléophile d'une β-amine ait échoué, de nouveaux amino époxydes **3.70** et **3.74** ont été synthétisés à partir du

L-Asp. Ces amino époxydes peuvent servir d'intermédiaires dans la synthèse d'autres composés fonctionnalisés.

Nous avons développé de nouvelles synthèses d'analogues d'acides aminés dont les acides 3-substitués azétidine-2-carboxyliques **2.2**, **2.3**, **2.4**, pour explorer la relation structure-activité de différents peptides biologiquement actifs. Aussi, nous avons développé de nouvelles stratégies pour synthétiser des analogues de l'Aze 4-substitués **3.43**, **3.48**, **3.65**, qui devraient être utiles dans la synthèse de nouveaux dérivés de β-lactame azabicyclique[X.Y.0]alcane.

Mots Clés: acide 2-azétidine carboxylique; azétidines; acides aminés; chimères; hétérocycles; synthèse peptidique

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Abbreviations

$[\alpha]_D$	optical rotation
aq	aqueous
Aze	Azetidine-2-carboxylic acid
Bn	Benzyl
bs	broad single
Boc	*tert*-butoxycarbonyl
DCM	dichloromethane
DMF	*N*,*N*-dimethylformamide
d	doublet
dd	doublet of doublet
DIBAL-H	Diisobutylaluminum hydride
ee	enantiomeric excess
Fmoc	9-flourenylmethyloxycarbonyl
g	gram
Glu	Glumatic acid
h	hour
HRMS	High Resolution Mass Spectrometry
IR	Infrared spectroscopy
J	coupling constant
LiHMDS	lithium hexamethyldisilazane
KHMDS	potassium hexamethyldisilazane
lit.	literature

m	multiplet
Me	methyl
mg	milligram
μM	micromolar
min	minutes
MHz	megahertz
mL	milliliter
mmol	millimole
mp	melting point
Ms	methanesulfonyl
MS	Mass spectrum
NMR	Nuclear Magnetic Resonance
Ph	phenyl
PhF	9-(9-phenylflourenyl)
ppm	parts per million
rt	room temperature
s	singlet
t	triplet
t-Bu	*tert*-butyl
TBDMS	*tert*-butyl dimethylsilyl
TBDPS	*tert*-butyl diphenylsilyl
TFA	trifluroacetic acid
THF	tetrahydrofuran

TLC	thin layer chromatography
TMS	trimethylsilyl

CHAPTER 1

Introduction

1. Peptide and peptide mimicry

1.1.1 Peptides and proteins:

A peptide is a chain of amino acids covalently linked together by amide bonds. Longer peptides (usually more than 50 residues) are called proteins. Interest in peptide science has been great due to their remarkable biological and structural properties. Table **1** lists some representative short natural peptides and their biological properties.[1,2]

Table 1 Examples of biologically active peptides.

Name	No. of residues	Biological property
Thyrotropin-releasing hormone (TRH)	3	A hormone that controls the release of another hormone (thyrotrophin) in the body, and also affects the central nervous system
Enkephalins	5	Found in the brain, these peptides are involved in the control of pain sensations.
Phalloin	7	A poisonous bicyclic peptide, found in the Deathcap toadstool
Angiotensin II	8	This peptide is a hypertensive agent used by the human body to increase blood pressure.
Oxytocin	9	A hormonal cyclic nonapeptide which, among other things, induces labour in pregnancy
Vasopressin	9	A cyclic peptide antidiuretic hormone that induces contraction of smooth muscles.
Gramicidin S	10	A cyclic decapeptide antibiotic
Somatostatin	14	A cyclic peptide hormone that inhibits the secretion of growth hormone, insulin and glucagon.

Peptides exhibit numerous activities in human physiology as neurotransmitters, neuromodulators, hormones, antibiotics, growth factors, cytokines, and antigens. They influence essentially all vital physiological processes via inter- and intracellular communication, and signal transduction mediated through various classes of receptors.[3,4] An important goal of peptide research has been to elucidate the relationship between the

three-dimensional (3D) structure and biological activity of a peptide. Such information is useful for developing therapeutic agents to control or intervene in disease states involving peptide receptors.

1.1.2 Structure of peptides:

Peptides share a common structure featuring a polyamide backbone and side chains linked to the α-carbons of the amino acid residues. The primary structure of a peptide consists of the linear amino acid sequence (Figure 1.1).[5]

The secondary structure of a peptide describes local conformations such as α helices and β strands. The formation of secondary structure in a polypeptide chain is usually contingent upon the primary structure and can be affected significantly by its environment. Secondary structure elements typically arrange themselves in motifs that give rise to higher ordered structures by the close packing of side chains from adjacent α helices or β strands or inter-residue H-bonds (Figure 1.1).[5]

Tertiary structure refers to the complete three-dimensional structure of a given protein; the spatial relationship of different secondary structures to one another within a polypeptide chain and the fold of these secondary structures into a three-dimensional form. The interactions of different domains are governed by several forces such as hydrogen bonding, hydrophobic interactions, electrostatic interactions and van der Waals forces.

Many proteins contain two or more different polypeptide chains that are held in association by the same non-covalent interactions that stabilize the tertiary structures of proteins. Proteins with multiple polypeptide chains are termed oligomeric proteins. The

structure formed by monomer-monomer interaction in an oligomeric protein is known as quaternary structure.

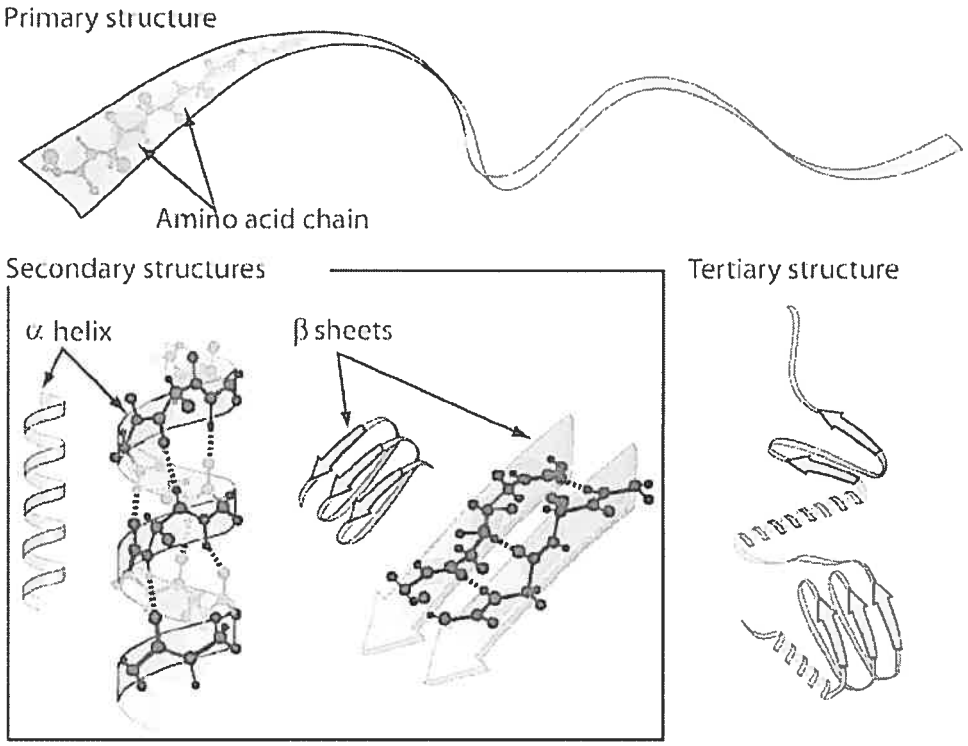


Figure 1.1: Schematic illustration of the various structural forms characteristic of proteins. The primary structure is determined by the linear sequence of amino acids. The secondary structure consists in chain segments that may have α-helical or β-sheet structure. The entire amino acid chain that forms the protein consists of connected segments of secondary structure. These come together to form the tertiary structure of the protein.[5]

1.1.3 The drawbacks of the use of peptides:

For peptide-based drug design, there are several major considerations that can limit the clinical application of peptides such as: (i) rapid degradation by many specific or nonspecific peptidases under physiological conditions; (ii) conformational flexibility which allows a peptide to bind to more than one receptor or receptor subtype leading to undesirable side effects; (iii) poor absorption and transportation because of their high molecular mass and the lack of specific delivery systems, especially for peptides which require passage across the blood-brain-barrier (BBB) to act in the central nervous system (CNS).[6-10]

1.1.4 Peptide mimicry and its importance:

A peptide mimic refers to a molecule resembling a peptide, which can act like the peptide as a ligand of a biological receptor or inhibit the effect of the natural peptide at its receptor. Peptide-based enzyme inhibitors may also be considered peptide mimics. From a medicinal chemistry point of view, peptide mimics replicate the topology of the peptide; however, they lack aspects of the peptide structure that may be drawbacks to the use of the peptide as a drug. In peptide mimics, the peptide backbone and side chain conformations may be constrained to allow a better interaction with the enzyme or receptor protein to which the peptide binds.[6, 7, 10]

1.1.5 The importance of peptidomimetics in drugs:

The use of peptidomimetics to overcome the limitations inherent in the physical characteristics of peptides has become an important strategy for improving the

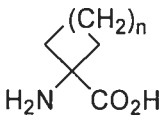
therapeutic potential of peptides. As one of the major efforts in organic chemistry, a variety of molecules have been designed to mimic the secondary structures of peptides, such as α-helices, β-turns, and β-strands. To explore structure-activity relationships (SAR) of bioactive peptides, a number of strategies have been developed that feature incorporation of conformationally constrained amino acids, modification of the peptide backbone by amide bond isosteres, cyclizations, attachment of pharmacophores to a template or scaffold, and the syntheses of non-peptide analogues.[7,10] Peptidomimetics are desired which exhibit the same biological effects as natural peptides, and at the same time, are more metabolically stable. Peptidomimetics can possess favourable attributes for drug development including, a conformationally restrained structure that may minimize binding to non-target receptors and enhance activity at the desired receptor, and improved transport properties through cellular membranes. Certain peptide mimics, such as amide isosteres,[8] retro-inverso peptides,[11] cyclic peptides[12] and non-peptidomimetics[13], have exhibited a reduced rate of degradation compared to native peptides. Peptidomimetics with improved pharmacokinetic profiles may also be designed with molecular frameworks that direct the position of the pharmacophores.[6, 14, 15]

1.1.6 Different approaches for peptide mimicry:

There are several different approaches for peptide mimicry:

1) Backbone *N*-alkylation facilitates *cis-trans* amide bond isomerization and changes the conformational space available to the φ, ψ and χ dihedral angles (Figure 1.2). *N*-Alkylation eliminates the hydrogen bonding capability of the amide bond. *N*-Methylated amino acids have been incorporated into bioactive analogues of the opioid peptides,[16]

34) segment of parathyroid hormone (PTH), several analogues of hPTH(1-11) were synthesized (Figure 1.3). The substitution of the $C_{\alpha,\alpha}$-dialkylated amino acid residue Ac_5c in hPTH(1-11) at position 1 resulted in a potent analogue with biological activity 3500-fold higher than that of native PTH(1-11) and 15-fold weaker than that of the native sequence hPTH(1-34).[26]



S-V-S-E-I-Q-L-M-H-Q-R-NH_2

hPTH(1-11)-NH_2

Figure 1.3: α-Aminocycloalkane carboxylic acid ($Ac_{n+3}c$)

3) D-Amino acid substitutions can favour formation of β-turn structures. Replacement of some or even all L-amino acids with their corresponding D-amino acids in a peptide sequence confers resistance to proteolytic degradation. Another important advantage for peptide drug development is that D-amino acid analogues have been found to be significantly less immunogenic than their L-amino acid counterparts.[27] D-Amino acid residues may stabilize a reverse β-turn (hairpin) structure, as well as increase potency, affinity and activity due to the importance of β-turn conformations for biological activity.[28]

4) Peptide amide bond isosteres may restrain the ω-dihedral angle to values 0 ° or 180°, or allow greater freedom for rotation about this bond (Figure 1.4).

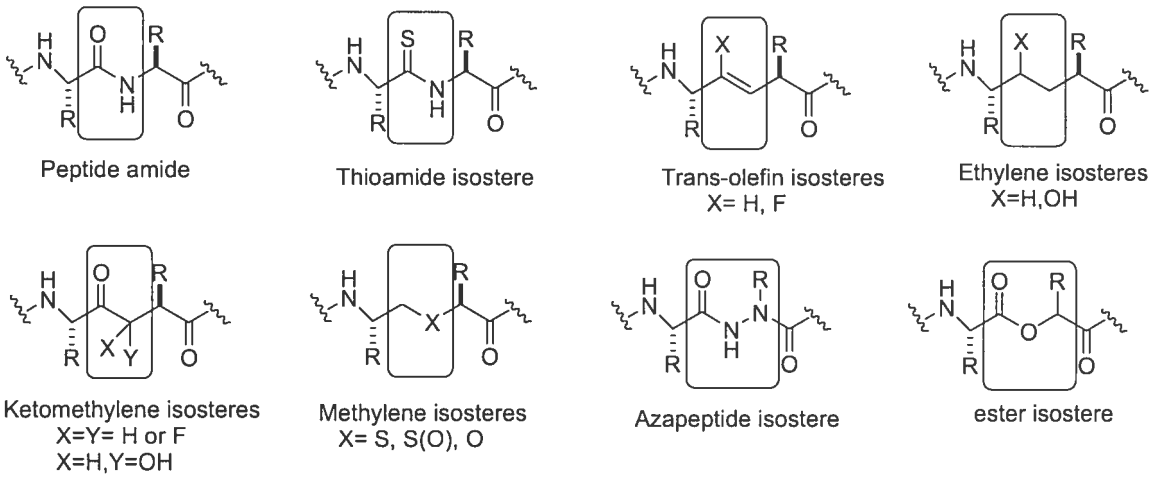


Figure 1.4: Common amide bond isosteres[8]

For example, it is possible to eliminate both a backbone H-bond acceptor (C=O) and a donor (N-H) by replacing the amide functionality with an *E*-alkene moiety (Figure 1.5).

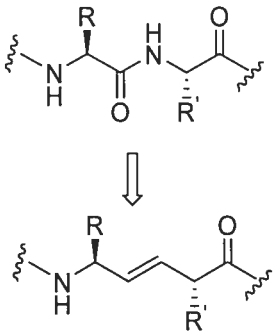


Figure 1.5: The backbone amide-to-*E*-olefin mutation eliminates both a H-bond acceptor (C=O) and a donor (N-H).

The Phe-Phe *E*-olefin dipeptide isostere (Fig. 1.6) was incorporated into the 40 residue Alzheimer's amyloid peptide, referred to as Aβ(1-40), in place of phenylalanines 19 and 20 utilizing a Boc/benzyl solid phase strategy, to examine the role of intermolecular H-bonding in the process of amyloidogenesis, that may cause Alzheimer's disease. This amide-to-*E*-alkene backbone mutation precludes amyloid formation, but not spherical

aggregate formation, providing insight into the structural requirements of amyloidogenesis.[29]

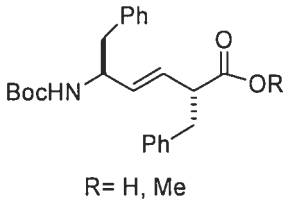


Figure 1.6: Phe-Phe *E*-olefin dipeptide isostere

5) Cyclic amino acids, such as proline, [30,31] can lower the energy differences between the *cis* and *trans*-amide isomers and bias global restrictions of the conformation of a peptide's φ and ψ dihedral angles towards forming β- or γ-turns. Proline is often found at the end of an α helix or in turns or loops. Unlike other amino acids which exist almost exclusively in the *trans*-amide form in polypeptides, proline can exist in the amide *cis*-conformation in peptides.[32] Introducing pipecolic acid, called homoproline, instead of proline into peptides is reported to introduce significant changes in biological activity and has lead to interesting model compounds for studies on peptide conformation.[33] Derivatives of pipecolic acid have also found a role as β-turn mimics[34] as well as precursors for natural product synthesis[35] and rigid analogues of prolyl amide bonds in peptides.[36]

6) Dehydroamino acids (Figure 1.7) can restrict the χ dihedral angle to value of 0° (*Z*) or 180°(*E*). For example, α,β-dehydrophenylalanine (ΔPhe) has been used as a conformationally restricted residue in the design of polypeptide helices. Dehydroamino acids generally favor the formation of β or γ-turns when placed in the (i+2) position of

the putative turn sequence. In addition, sequential placement of ΔPhe in a peptide induces repeated β-turns, that form a 3^{10} helix-like structure (Figure 1.7).[37]

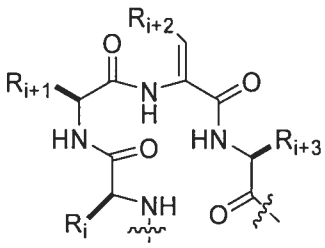
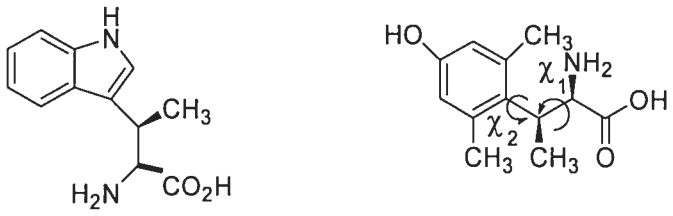


Figure 1.7: Dehydroamino acid

7) β-Alkylation has been used to constrain the χ dihedral angle, and may also effect backbone conformation. Introduction of methyl groups at the β-position of Phe and related aryl alanines has been used to orient the aromatic ring. Additional alkyl groups usually enhance the lipophilicity of the peptide analogue, and can thus help in crossing the blood-brain barrier. Figure 1.8 represents structures of some novel amino acids that are constrained in χ space.[38] For example, the constrained tyrosine derivative, (2*S*,3*S*)-2',6'-dimethyl β-methyltyrosine[39] [(2S,3S)-TMT] (Figure 1.8 b) was synthesized and incorporated into cyclic analogs of [D-Pen^2,D-Pen^5]enkephalin (DPDPE) (δ_1). Because of the methyl substitution in the tyrosine derivative (Figure 1.8 b), rotation about the χ_1 and χ_2 torsion angles was restricted about the C^{β}-C^{γ} bond. This tyrosine analogue has been used in the study of the role of side chain dynamics in peptide-receptor interactions and protein functions.[39]



a b

Figure 1.8: **a**: *Erythro* (2*S*,3*R*) β-methyltryptophan[40]; **b**: (2*R*,3*S*)-2',6'-dimethyl β-methyltyrosine[39]

In summary, incorporation of peptidomimetics into peptides can offer significant advantages for drug development. These benefits include increased bioavailability, better transport through cellular membranes, decreased rates of excretion, increased selectivity and decreased hydrolysis by peptidases. A tremendous variety of constrained amino acids have been developed to rigidify particular amino acids and peptide sequences. Control of backbone and side chain conformation by way of steric and electronic factors can be implemented through modifications of the peptide backbone. Mimetics are usually designed based on analogy to the native peptide structure, then optimized to give the best possible pharmacokinetic properties.

1.2 Cyclic amino acid chimeras

1.2.1 Proline and pipecolic acids as higher homologues of azetidine-2-carboxylic acid

As mentioned, cyclic amino acids, such as proline and its higher homologue pipecolic acid, are often used as conformationally constrained amino acids in peptide mimicry to study conformation activity relationships in peptides.[41-44] These amino acids increase conformational freedom and favour turn geometry. β-Substituted prolines and pipecolates have been also been designed to serve as amino acid chimeras on which the functional

groups of the amino acid side-chain are combined with the conformational rigidity of the cyclic amino acid residue. Several chimeras based on proline bodies have been used to study the relationship between the side-chain geometry and the bioactivity in different peptides.[45,46,32] For example, proline-amino acid chimeras have been used in place of natural amino acids in peptide-based enzyme inhibitors[47] and in peptide ligands of G-protein coupled receptors (GPCRs). In the latter case, tyrosine was replaced with either *trans*-3-(4'-hydroxyphenyl)proline (*t*-Hpp) or *cis*-3-(4'-hydroxyphenyl)proline (*c*-Hpp) in δ opioid receptor selective tetrapeptide Tyr-c[D-Cys-Phe-D-Pen]OH and displayed δ receptor binding affinity similar to the parent Tyr^1 –containing peptide (Figure 1.9).[48]

β-Substituted pipecolic acids have similarly been synthesized to serve as amino acid chimeras. For example, 3-substituted Pipecolic acid **1.2** was introduced into the potent and sst5 subtype selective somatostatin mimetic **1.1** (IC_{50} = 87 nM). The resulting analog **1.3** (IC_{50} = 41 nM) exhibited 2-fold and 25-fold binding enhancement against the somatostatin receptor subtypes sst5 and sst4, relative to lead compound **1.1**, respectively (Figure 1.9).[49]

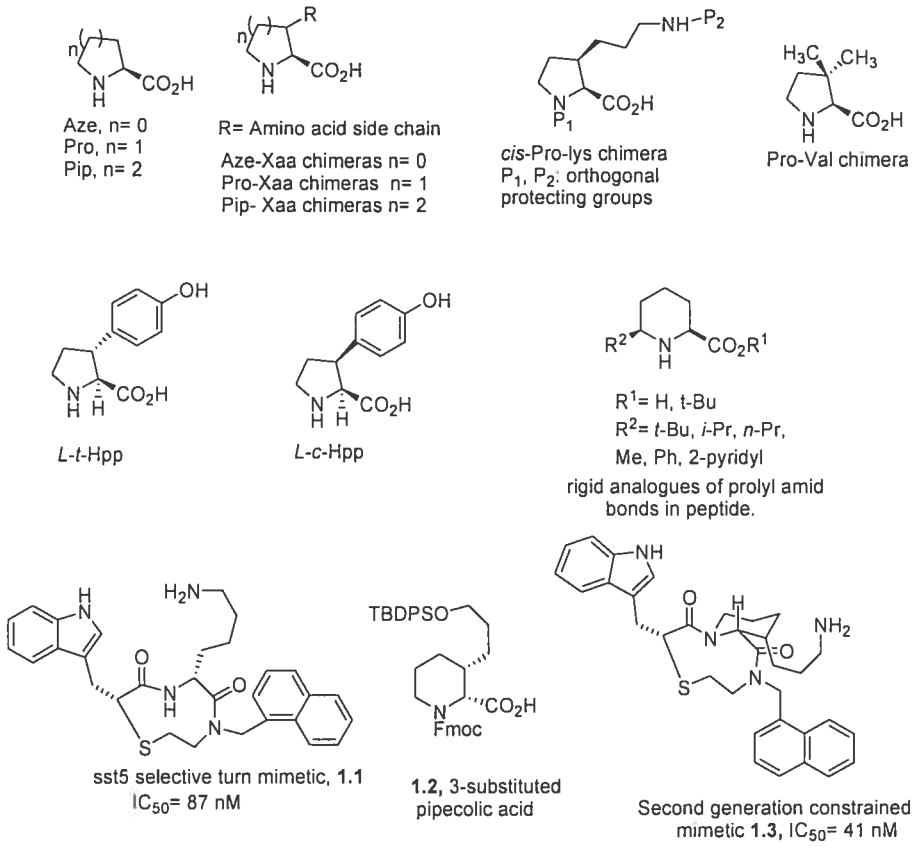


Figure 1.9: Structures of representative proline and pipecolate analogues.

1.3 Azetidine-2-carboxylic acids (Aze) and their applications

1.3.1 Applications of azetidine 2-carboxylic acid:

L-Azetidine-2-carboxylic acid (Aze) is a four member cyclic amino acid discovered by Fowden[50] and Vitanen[51] in *Convallaria majalis* and *Polgonatum offocinale*, two members of the *Liliaceae* family. Commercially available and isolated from hydrolysates of natural plants,[52] L-azetidine-2-carboxylic acid occurs in high concentrations in a variety of species.[53]

L-Aze exhibits growth inhibitory effects in many biological systems.[54] L-Aze, as a constrained amino acid, has also found applications for the modification of peptide conformation.[55-60] L-Aze has also been applied in synthesis as an additive for asymmetric

reduction of ketones,[61] Michael additions,[62] cyclopropanations,[63] and the Diels-Alder reaction.[64]

Some substituted Aze analogs are part of natural and pharmacologically interesting products such as medicanine,[65] nicotianamine,[66] mugineic acid,[67] and red-mold rice fermentation products, some of which seem to be involved in iron transport processes of certain plants.[68] L-Aze derivatives have served as active pharmaceutical ingredients, in for example, the non-opioid analgesic agent ABT-594(*R*) [69] and the thrombin inhibitor melagatran (Figure 1.10).[70]

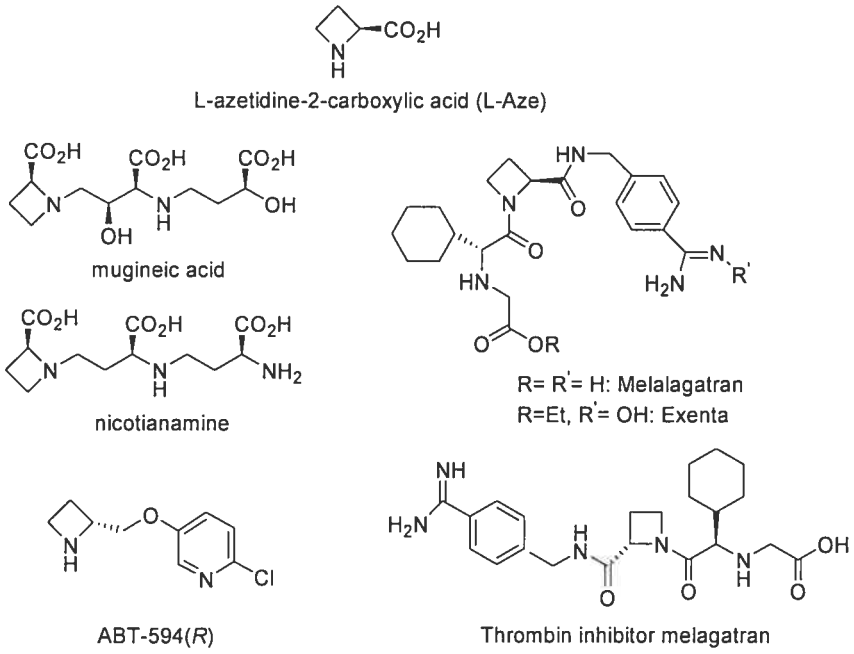


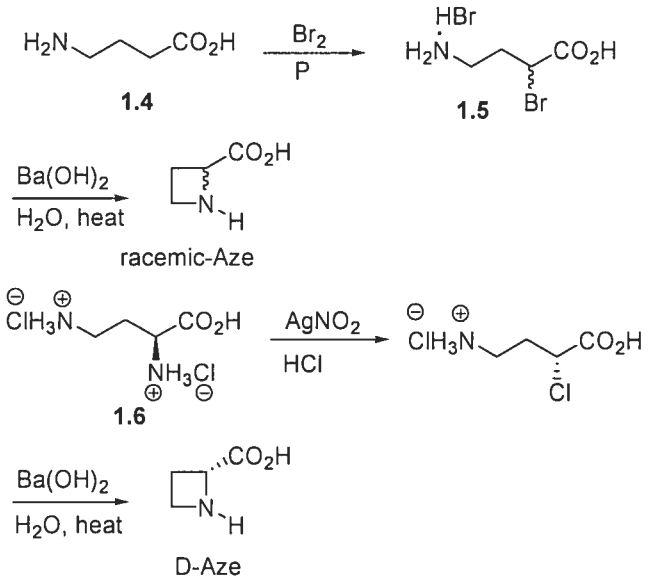
Figure 1.10: L-Aze and examples of naturally and pharmaceutically important L-azetidine-2-carboxylic acid derivatives.

1.3.2 Synthesis of azetidine 2-caboxylic acids:

Cyclic α-amino acids have been the subject of considerable research in the last decade because of the physiological activities associated with these derivatives.[41,71,72] Although a number of syntheses of cyclic amino acids such as proline and pipecolic acid have been developed, fewer methods are available for making azetidine-2-carboxylic acid derivatives.

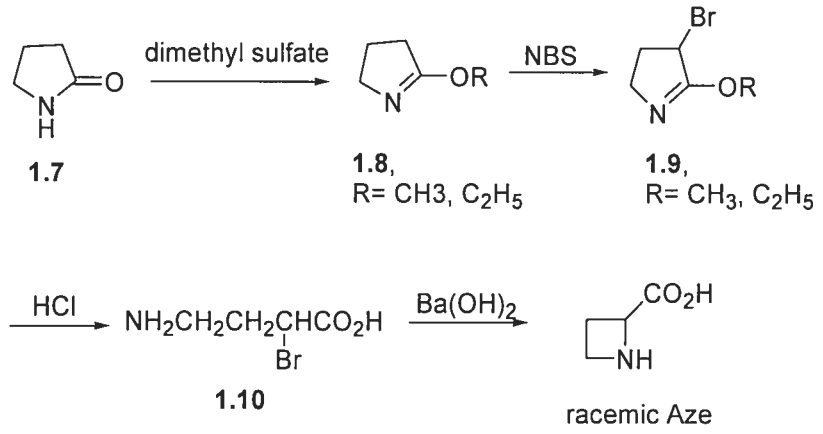
1.3.2.1 Synthesis of DL- and D-Aze starting from 2-bromo or 2-chloro-4-aminobutanoic acid:

Racemic and enantiomerically enriched Aze have been synthesized respectively from γ-aminobutyric acid **1.4** and L-α,γ-diaminobutyric acid **1.6**. Bromination of amino acid **1.4** in the presence of red phosphorous gave α-bromo derivative **1.5**. Ring annulations were effected using 0.5 N barium hydroxide in H_2O to yield (±)-Aze and optically active (+)-Aze (Scheme 1.1).[73]



Scheme 1.1: Synthesis of (±) and (+)-Aze

In addition, racemic Aze was also prepared from α-bromo- γ-amino acid **1.10** using *Z*-pyrrolidinone **1.7** as a starting material. [74]



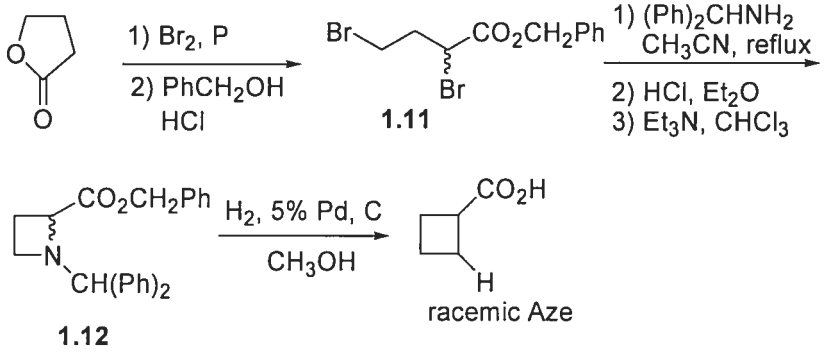
1.7 **1.8**, R= CH_3, C_2H_5 **1.9**, R= CH_3, C_2H_5

1.10 racemic Aze

Scheme 1.2: Synthesis of (±) Aze from 2-pyrrolidinone **1.7**.

1.3.2.2 Synthesis of Aze starting from γ-butyrolactone:

The synthesis of the (±)-Aze was achieved from γ-butyrolactone by a route featuring bis-alkylation of a given amine (Scheme 1.3). [75,76]



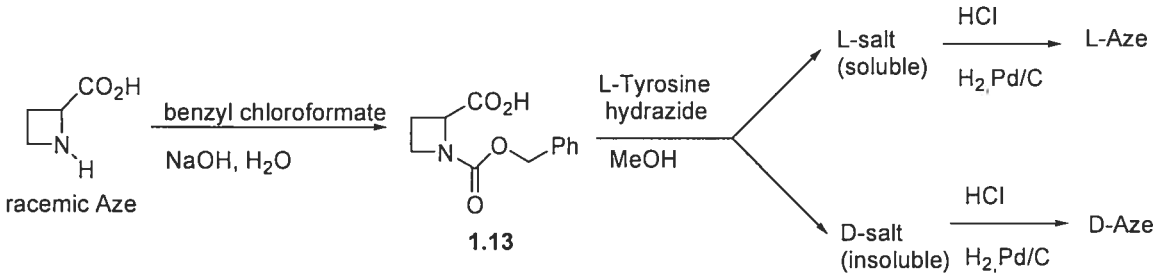
1.11

1.12 racemic Aze

Scheme 1.3: Synthesis of (±)Aze.

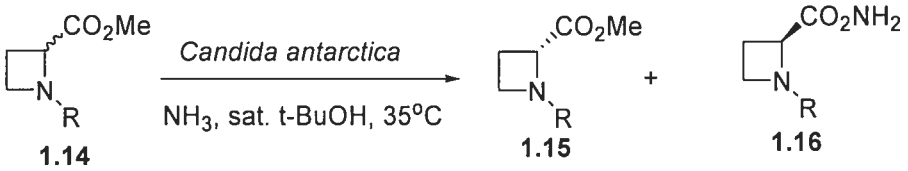
Enantiomerically enriched D- and L-Aze has been obtained by resolution (Scheme 1.4).[77] First, the DL-amino acid was converted to its *N*-carbobenzyloxy derivative **1.13**.

Treatment with L-tyrosine hydrazide in MeOH yielded diastereomeric salts, which exhibited different solubilities in methanol. [77,78]



Scheme 1.4: Resolution of DL-Aze.

Enzymatic resolution of DL-*N*-substituted Aze has also been achieved using *Candida antarctica* (Scheme 1.5), which produced carboxamide **1.16** from *S*-ester **1.14** using ammonia as the nucleophile. *R*-Ester **1.15** did not react under these conditions.[79]

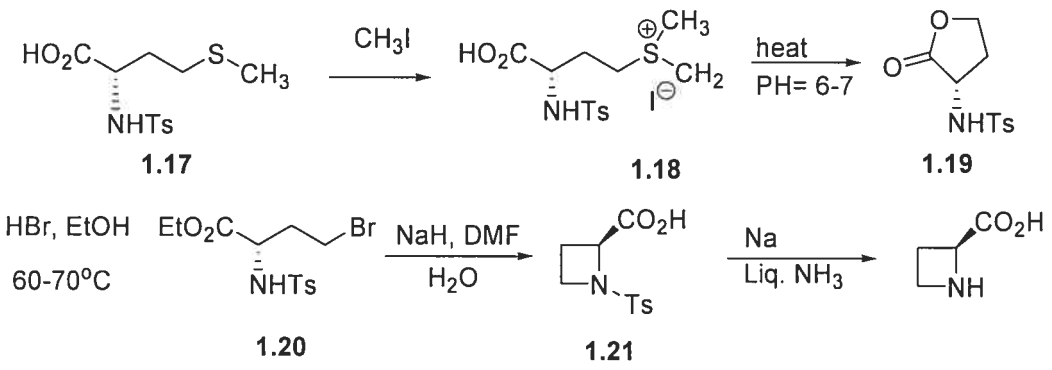


R	**1.15**	**1.16**
benzyl	99%ee	80%ee
p-methoxybenzyl	99%ee	84%ee
allyl	99%ee	
benzhydryl		97%ee

Scheme 1.5: Enzymatic resolution of *N*-substituted Aze.

1.3.2.3 Synthesis of L-Aze starting from (*S*)-*N*-toluenesulfonyl-homoserine lactone: Enantiopure L-Aze was synthesized without resolution from *N*-toluenesulfonyl-L-methionine **1.17**, which was readily converted to *N*-toluenesulfonyl-lactone **1.19**. Treatment of L-homoserine lactone **1.19** with hydrogen bromide, followed by cyclization of **1.18** with sodium hydride provided *N*-toluenesulfonyl-L-Aze **1.18**. The

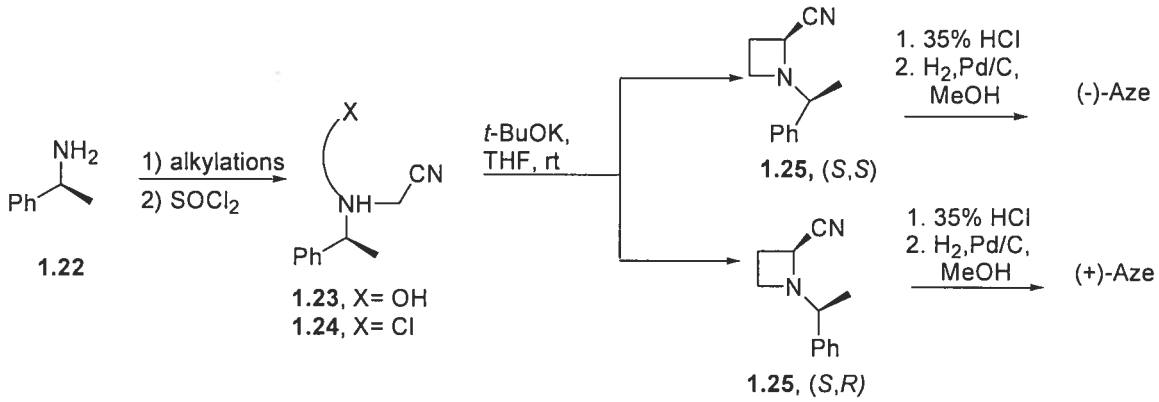
toluensulfonamide was removed using sodium in liquid ammonia to give L-Aze in 62% overall yield (Scheme 1.6).[80,81]



Scheme 1.6: Synthesis of L-Aze from homoserine lactone **1.19**.

1.3.2.4 Asymmetric synthesis of (+)- and (-)-Aze from α-methylbenzyl amine as the source of chirality:

Construction of the azetidine ring has been achieved using an intramolecular alkylation of a linear substrate derived from α-methyl benzyl amine as chiral aduct.[82] α-Methylbenzyl amine **1.22** was subjected to two consecutive alkylations with bromoethanol and bromoacetonitrile to afford the tertiary amino alcohol **1.23**. Chloride **1.24** was prepared by reaction of alcohol **1.23** with thionyl chloride in dichloromethane. A 1:1 mixture of diastereoisomers (*S*,*S*)- and (*S*,*R*)-**1.25** were synthesized by intramolecular alkyation with potassium *tert*-butoxide in THF. The diastereoisomers were separated by column chromatography. Respective hydrolysis of nitriles **1.25** with concentrated hydrochloric acid, followed by hydrogenolysis of the HCl salt gave the desired (-)- and (+)-azetidine 2-carboxylic acids (Scheme 1.7).



Scheme 1.7: Synthesis of (+)-Aze and (-)-Aze by intramolecular alkylation.

Employing benzyl α-bromo acetate, instead of bromo acetonitrile in the alkylation step, gave a similarly unselective sequence, which provided Aze in similar overall yields.

In summary, prior syntheses of Aze have been reported; however, few provide a satisfactory route to either enantiomer in practical quantities. Asymmetric syntheses have required chemical and enzymatic resolution as well as the use of a chiral auxiliary. In light of the importance of Aze and substituted Aze as constituents of biologically active compounds, the development of new methodologies for synthesizing Aze analogues remains an important field of research.

1.4 Substituted azetidine-2-carboxylic acids

Introduction:

During our research on the effect of azacycloalkane amino acids on peptide conformation, we became interested in employing β and γ substituted Aze analogues for studying secondary structure. A search of the literature revealed that there are few methods for selective and stereocontrolled introduction of alkyl substituents on the Aze

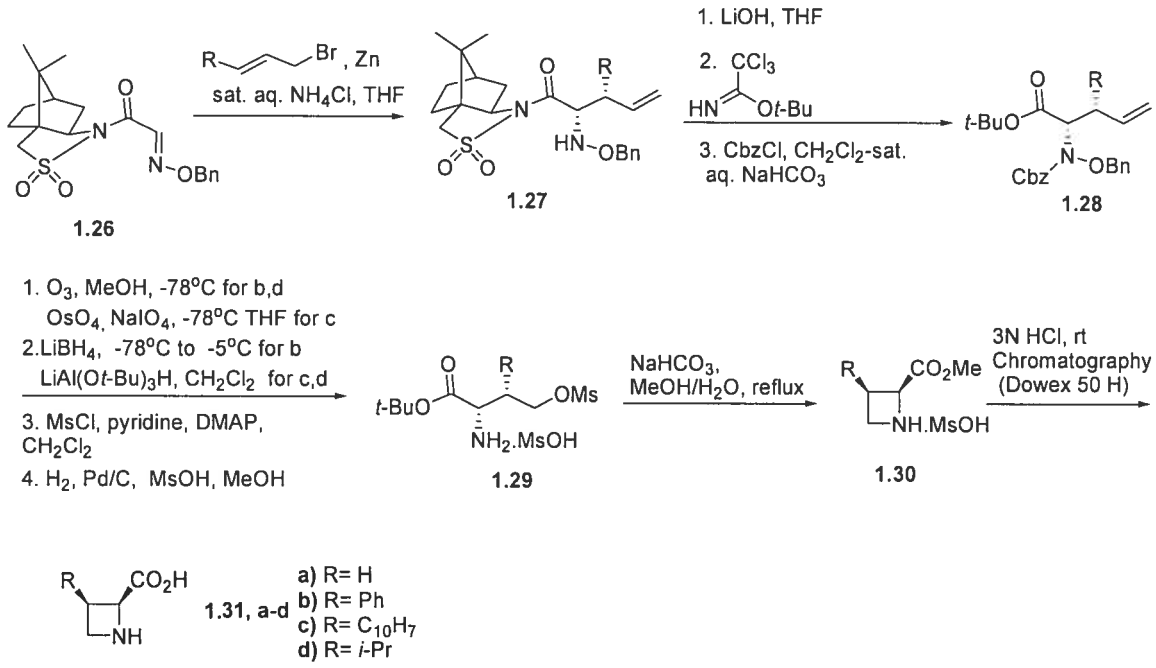
ring carbons. In spite of current interest in the synthesis and chemistry of azetidine 2-carboxylic acids, there are only a few reports on the synthesis of 3- and 4-substituted Aze analogues.

1.4.1 3-Substituted azetidine 2-carboxylic acids:

As mentioned, there are few general methods for the synthesis of substituted Aze analogues. Several representative examples are presented below which deliver 3-substituted Aze analogs in enantiopure form.

1.4.1.1 Synthesis of Phe-Aze, Nal-Aze, Leu-Aze chimeras:

Hanessian *et al.* used Oppolzer's sultam methodology for the preparation of enantiopure 3-substituted L-Aze analogs.[83] The camphor sultam derivative of glyoxalic acid O-benzyl oxime **1.26** on treatment with allylic halides and zinc dust was converted to allyl glycine **1.27**. Hydrolysis of the chiral auxiliary, esterification and *N*-protection produced *N*-benzyloxy tert-butyl amino esters **1.28**. Oxidation of the olefin followed by reduction of resulting aldehyde and mesylation provided the corresponding mesylate **1.29**. Hydrogenolysis followed by cyclization in the presence of $NaHCO_3$ gave L-Aze methyl esters **1.30 b-d** as mesylate salts. Hydrolysis of the ester with 3N HCl and purification on Dowex-$50H^+$ provided 3-aryl and 3-alkyl Aze derivatives **1.31 b-d** (Scheme 1.8). Unsubstituted Aze **1.31 a** was also prepared in a similar manner using allyl bromide.

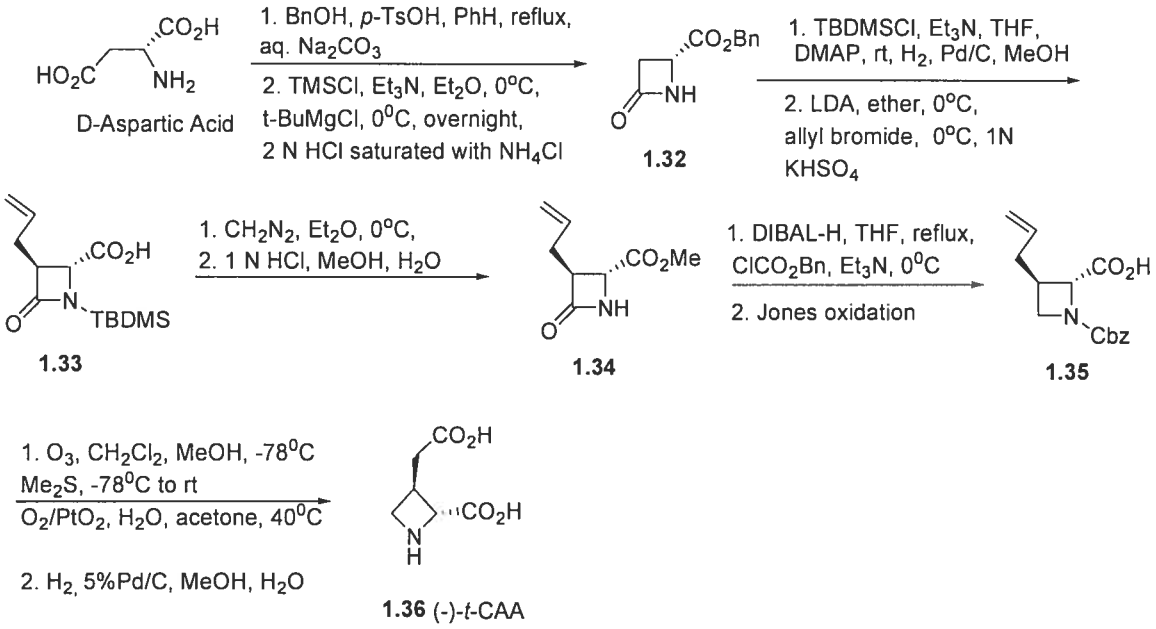


Scheme 1.8: Synthesis of Phe-Aze, Nal-Aze, and Leu-Aze chimeras

1.4.1.2 Synthesis of the Glu-Aze chimera, (-)-*trans*-2-carboxyazetidine-3-acetic acid (*t*-CAA):

3-Carboxymethyl Aze, a Glu-Aze chimera was synthesized by allylation of a β-lactam enolate derived from D-aspartic acid, subsequent olefin oxidation and lactam reduction. D-Asp was transformed via its dibenzyl ester into β-lactam **1.32**. Lactam **1.32** was converted to its *N*-silyl lactam and hydrogenated to give *N*-silyl β-lactam 4-carboxylic acid **1.33**, which was treated with two equivalents of LDA to form the corresponding dianion, which was alkylated with allyl bromide. Esterification using diazomethane and silyl group cleavage provided allyl β-lactam methyl ester **1.34**. The lactam and ester moieties were reduced simultaneously with DIBAL-H. The azetidine nitrogen was reprotected by carbobenzyloxylation, and the alcohol was oxidized to the corresponding acid. The double bond of acid **1.35** was subjected to ozonolysis, and the resulting

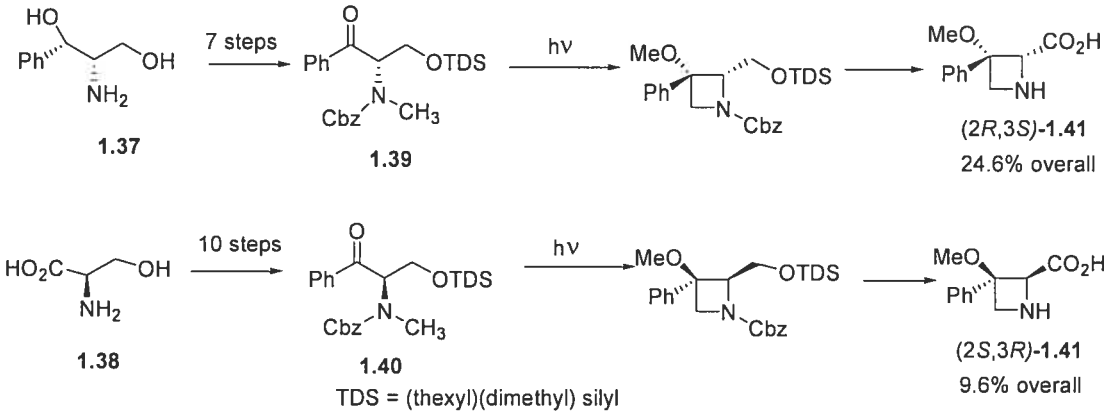
aldehyde was oxidized with O_2 /PtO_2 to afford the *N*-protected azetidine diacid. Removal of the Cbz group by hydrogenolytic cleavage gave the desired azetidine diacid **1.36** (Scheme1.9). The (+)-isomer of **1.36** was prepared in an identical fashion starting from L-aspartic acid. *t*-CAA was found to be an inhibitor of Na^+-dependent Glu uptake (5 μM) and to act as a kainic acid receptor agonist (0.7 μM). [84]



Scheme 1.9: Synthesis of (-)-*trans*-2-carboxyazetidine-3-acetic acid.

1.4.1.3 Synthesis of (2*R*,3S)- and (2*S*,3*R*)-azetidine-2-carboxylic acids 1.41:

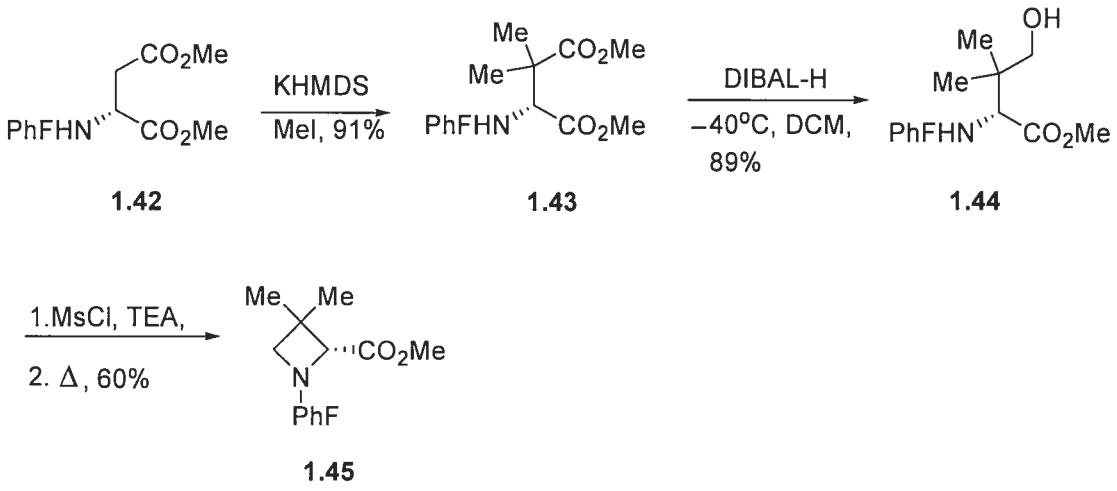
(2*R*,3*S*)- and (2*S*,3*R*)-Methoxy-3-phenylazetidine-2-carboxylic acid **1.41** were synthesized from the commercially available enantiomerically pure amino diol **1.37** and D-serine **1.38**, respectively (Scheme1.10).[85] Using a stereoselective photochemical ring closure of the amino ketone **1.39** and amino alcohol **1.40**, enantiopure (2*R*,3*S*)- and (2*S*,3*R*)- azetidines **1.41** were obtained in 71% yield.



Scheme 1.10: Synthesis of 3-substituted (2*R*,3*S*)- and (2*S*,3*R*)-Aze **1.41**.

1.4.1.4 Synthesis of the Val-Aze chimera, 3,3-dimethyl-azetidine 2-carboxylic acid :

In the course of syntheses of β,β-dimethylated amino acid building blocks, β,β-dimethyl-Aze **1.45** was synthesized from *N*-(PhF) aspartate dimethyl ester **1.42**. *N*-PhF- β,β-dimethyl D-Asp dimethyl ester **1.43** was obtained by dialkylation of dimethyl aspartate **1.42** using methyl iodide and KHMDS in THF in 91% yield. Regioselective reduction of the β-methyl carboxylate of *N*-PhF-β,β-dimethyl-D-aspartate **1.43** to β,β-dimethyl-D-homoserine **1.44** was achieved using DIBAL-H in DCM. Activation of the resulting alcohol to the corresponding mesylate, followed by intramolecular nucleophilic displacement gave β,β-dimethyl-Aze **1.45** (Scheme1.11).[86]

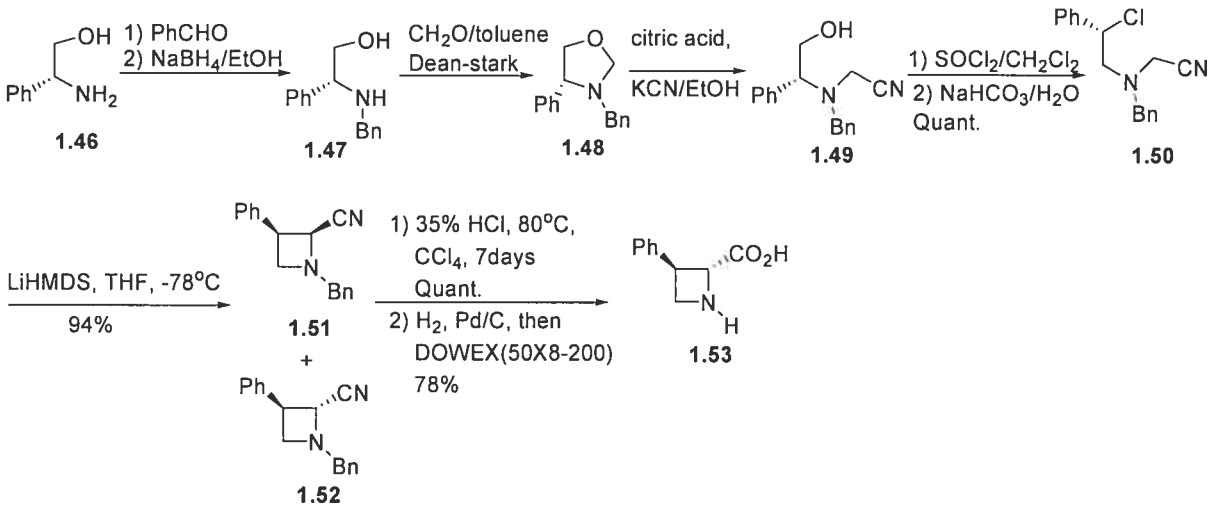


Scheme 1.11: Synthesis of β,β-dimethyl-Aze

1.4.1.5 3-Phenyl Aze from β-amino alcohol 1.46:

Enantiopure 3-phenyl Aze was synthesized from 2-cyano azetidines starting from phenylglycinol.[87] (R)-*N*-Benzyl phenylglycinol **1.49** was prepared through reductive alkylation, and then cyanomethylated in a two-step procedure involving ring opening of intermediate oxazolidines **1.48** with citric acid and KCN/EtOH. Chlorination of the cyanomethylated amino alcohol **1.49** with two equivalents of thionyl chloride in DCM at 0°C produced the rearranged chloride **1.50**. This rearrangement involves concerted nucleophilic attack of the chloride anion at the benzylic carbon of aziridinium intermediate.[88] Cyclization of chloride **1.50** was next affected by treatment with LiHMDS in THF at -78°C to afford 2-cyano azetidines **1.51** and **1.52** as a 3:7 mixture of stereoisomers at C(2). Hydrolysis of 2-cyano azetidine **1.52** by using concentrated HCl (35% wt.), followed by debenzylation of the corresponding hydrochloride salt with Pd/C,

and purification by ion exchange chromatography, yielded 3-phenyl Aze **1.53** that can be considered as a conformationally constrained analog of phenylalanine (Scheme 1.12).

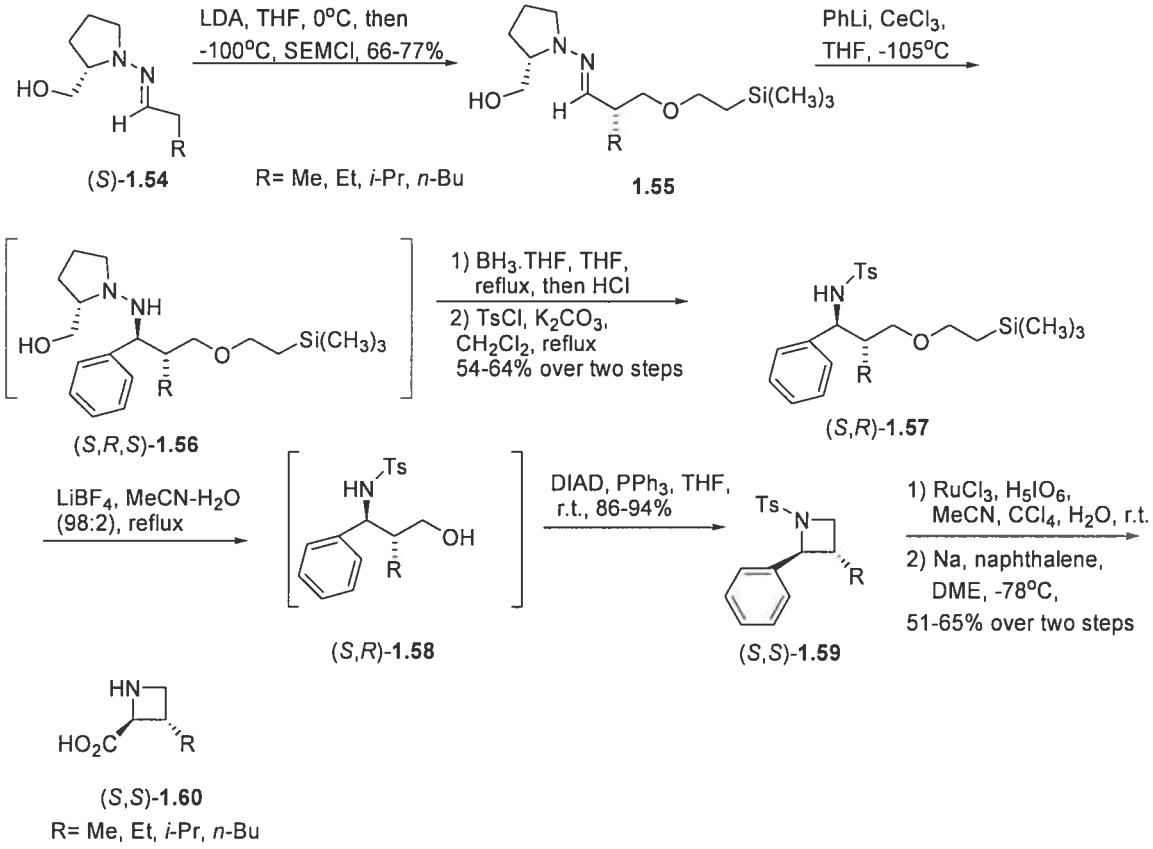


Scheme 1.12: Synthesis of enantiopure 3-phenyl Aze **1.53** as a phenylalanine chimera.

1.4.1.6 Asymmetric synthesis of 3-substituted Aze employing SAMP/RAMP methodology:

Very recently Enders *et al.* reported the preparation of substituted azetidine type α- and β-amino acids via 1,3-amino alcohols by employing SAMP/RAMP-hydrazone methodology.[89] The SAMP/RAMP hydrazones **1.54** were hydroxymethylated by α-alkylation with (2-trimethylsilylethoxy)methyl chloride (SEMCl). The phenyl substituent was introduced by nucleophilic 1,2-addition of phenylcerium reagent to the C=N double bond of hydrazones **1.55**. The hydrazines **1.56** were directly submitted to a reductive N-N bond cleavage to remove the chiral auxiliary. Tosylation of O-protected amino alcohols with toluene sulfonyl chloride in the presence of K_2CO_3 at reflux yielded N,O-protected

amino alcohol **1.57**. The amino alcohols **1.58** were cyclized under Mitsunobu conditions and the corresponding *N*-tosylated 2-phenylazetidines **1.59** were obtained as one diastereomer without racemization in good yields. Conversion of 2-phenylazetidines **1.59** to the corresponding α-amino acids was accomplished by catalytic oxidation with ruthenium tetroxide in a biphasic solvent system CH_3CN, H_2O and CCl_4. Detosylation with sodium naphthalene gave the free α-amino acid **1.60** (Scheme 1.13).[90]



Scheme1.13: Synthesis of 3-substituted Aze by employing SAMP/RAMP-hydrazone methodology.

1.4.2 Synthesis of 4-substituted azetidine-2-carboxylic acids

Introduction:

4-substituted azetidine 2-carboxylic acids with one or two carboxy groups have been found in natural products[91] and rigid glutamate analogues with metabotropic receptor activity.[84] For example, *cis*-2,4-azetidine dicarboxylic acid is a nonproteinogenic amino acid that has been reported as a neurotransmitter potentiator.[92,93] Red-mold rice is effective in decreasing blood pressure [94] and lowering plasma cholesterol levels [95] and has antibacterial activity. [96] Recently, (+)-monascumic acid **1.61** and (-)-monascumic acid **1.61** were isolated from red-mold rice (Figure 1.11).[94]

1.61, (2*S*,4*R*)or(2*R*,4*S*)
1.61, (2*R*,4*S*)or(2*S*,4*R*)

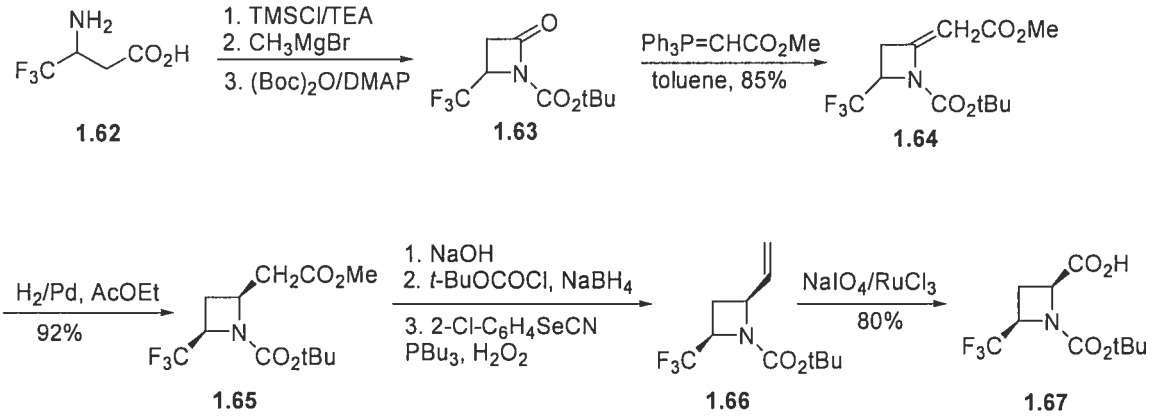
Figure 1.11: Structures of cis-2,4-azetidine dicarboxylic acid from exracts of red-mold rice.

Moreover, 2,4-disubstituted azetidines with hydroxymethyl groups have been used as chiral auxiliaries[97] and catalysts[98] for asymmetric synthesis, and have been prepared from the corresponding 2,4-azetidine dicarboxylic acids .[99]

1.4.2.1 Synthesis of 4-trifluoromethylated azetidine -2-carboxylic acid:

A diastereoselective synthesis of 4-trifluoromethylazetidine-2-carboxylic acid **1.67** was accomplished by the Wittig reaction of 4-trifluoromethyl β-lactam **1.63** followed by hydrogenation and functional group transformations.[100] 4-Trifluoromethyl-β-lactam **1.63**

was prepared from the commercially available β-amino acid **1.62**. Without purification, the lactam was treated with $(Boc)_2O$ in the presence of 10% mol DMAP to afford *N*-Boc-protected β-lactam **1.63** in 25% overall yield from acid **1.62**. The Wittig reaction with ylide and lactam **1.63** in toluene at reflux afforded enamine **1.64**. Hydrogenation of the C=C bond of enamide ester **1.64** over Pd/C in ethyl acetate afforded exclusive *cis*-2,4-substituted azetidine **1.65** in 92% yield. 4-trifluoromethylazetidin-2-yl olefin **1.66** was prepared from ester **1.65** in 3 steps by ester hydrolysis, carboxylate reduction via a mixed anhydride and elimination of the primary alcohol. Subsequent oxidation of olefin **1.66** to the carboxylic acid **1.67** was accomplished using $NaIO_4$/ $RuCl_3$ in 80% yield (Scheme 1.14).

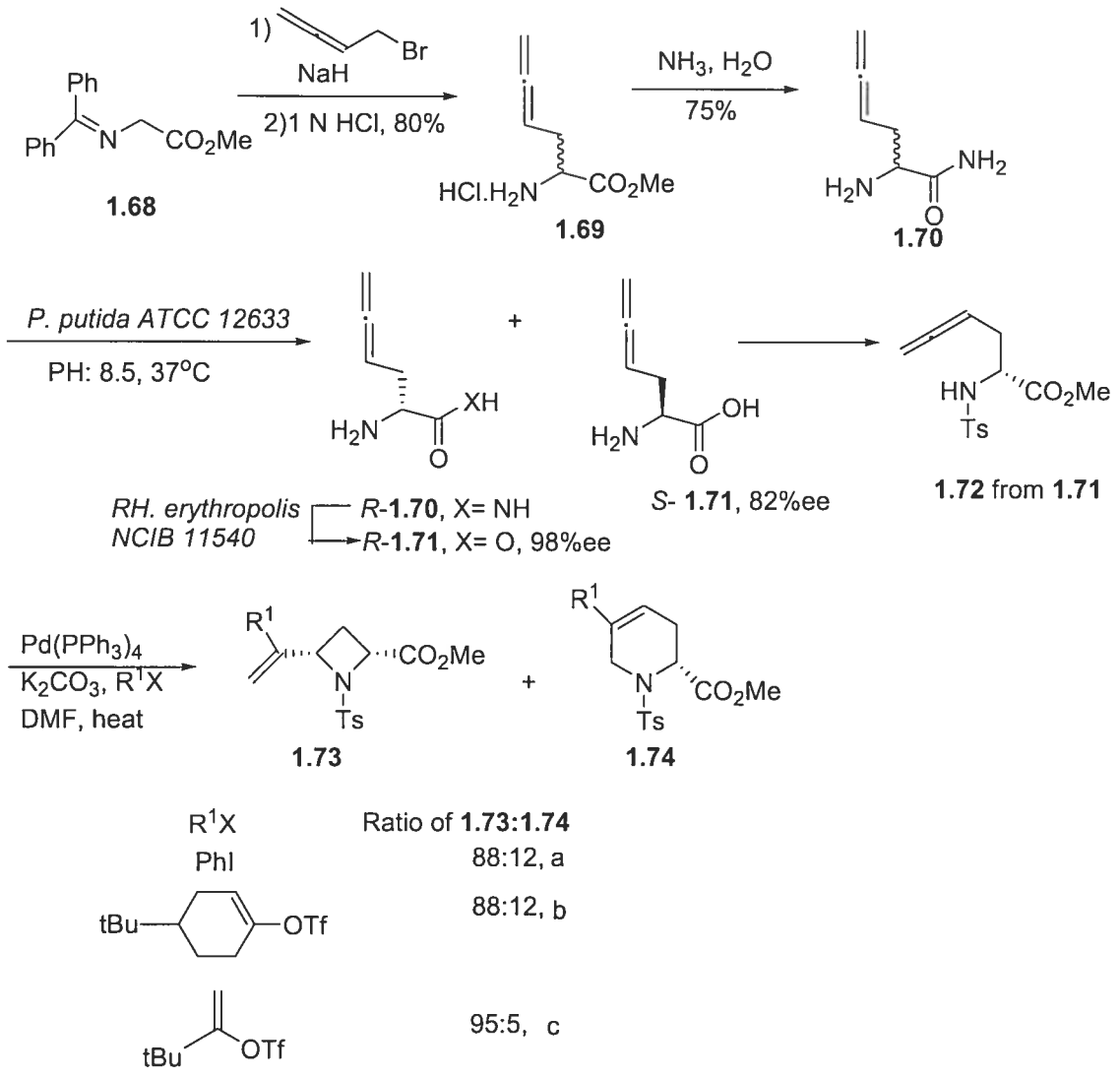


Scheme 1.14: Synthesis of *N*-Boc-4-trifluoromethyl Aze.

1.4.2.2 Selective azetidine formation via Pd-catalyzed cyclizations of allene-substituted amines and amino acids:

4-Substituted Aze analog **1.73** was obtained in enantiomerically enriched form by enzymatic resolution of ω-allenyl amino acid **1.70** followed by Pd-catalyzed ring

annulation (Scheme 1.15).[101] The hydrochloride salt of allenyl amino ester **1.69** was synthesized via alkylation of the glycine-derived ketimine **1.68** with 4-bromo-1,2-butadiene, followed by acid hydrolysis of the imine. Reaction of the ester with aqueous ammonia afforded the amino amide **1.70**. Enzymatic resolution of the (*S*)-amide **1.70** with *P.putida ATCC 12633* provided the (S)- acid **1.71** in 82% ee. Subsequently, the (*R*)-amide was hydrolyzed with *RH. erthropolis NCIB 11540* to provide (*R*)-**1.71** in 98% ee. Application of different cyclization conditions using $Pd(PPh_3)_4$, and K_2CO_3, with alkenyl or aryl halides, and triflates in DMF lead to different ratios of four and six membered rings (Scheme 1.15).

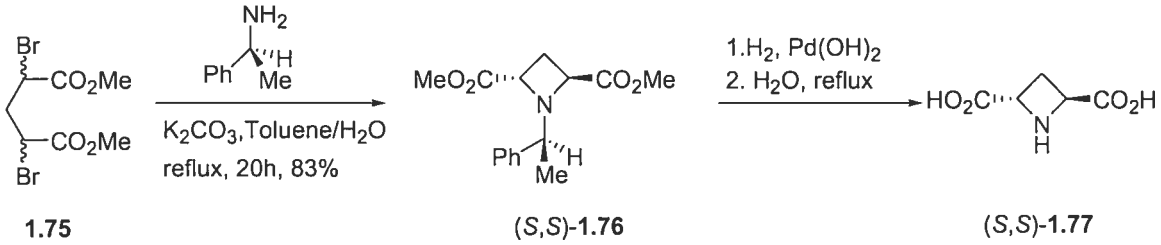


Scheme 1.15: Azetidine formation via Pd-catalyzed cyclization

1.4.2.3 Synthesis of azetidine-2,4-dicarboxylic acid

A diastereoisomeric mixture of azetidine 2,4-dicarboxylate **1.76** was synthesized by heating dimethyl 2,4-dibromopentanedioate **1.75** with (*S*)-1-phenylethylamine in toluene containing aqueous potassium carbonate (Scheme 3.3). Three components (*S*,*S*)-**1.76** (22%), (*R*,*R*)-**1.76** (22%) and cis-**1.76** (44%) were separated by flash chromatography.

Hydrogenolysis of diester (*S*,*S*)-**1.76** over palladium hydroxide on carbon followed by hydrolysis in hot water afforded (2*S*,4*S*)-azetidine-2,4-dicarboxylic acid **1.77** (Scheme 1.16).[97, 102] The (*R*,*R*)-isomer was also prepared by the same reaction sequence from diester (*R*,*R*)-**1.76**.

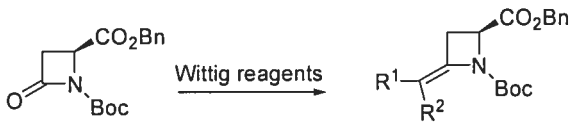


1.75 (*S*,*S*)-**1.76** (*S*,*S*)-**1.77**

Scheme 1.16: Synthesis of azetidine-2,4-dicarboxylates.

1.4.2.4 Synthesis of 4-substituted Aze via Wittig reaction of monocyclic lactams:

Substituted 2-exo-methylene azetidines were synthesized by a Wittig reaction on β-lactam **1.78** (Scheme 1.17). The resulting compounds were subjected to a range of ylides. Substituted 2-exo-methylene azetidines **1.79** were prepared using stabilized ylides. The unstabilized ylides or Horner-Emmons reagents led only to decomposition of starting material.[103]



1.78

1.79, a. R^1= CO_2Et, R^2= H, 80%
b. R^1= CO_2tBu, R^2= H, 98%
c. R^1= CN, R^2= H, 53%
d. R^1= CO_2Et, R^2= Me, 50%

Scheme 1.17: Synthesis of 4-olefin Aze via Wittig reaction of monocyclic lactams

Excluding the intensely studied β-lactams, few syntheses of substituted azetidines have been reported. The reported yields of Aze analogues have been typically poor. Methods for synthesizing substituted Aze are also limited by the availability of the starting materials and cannot claim wide applicability. Therefore, the synthesis of substituted Aze remains a challenging problem for which new methodology is required.

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CHAPTER 2

Article: Amino Acid-Azetidin Chimeras: Synthesis of Enantiopure 3-Substituted Azetidine-2-carboxylic Acids

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2.1 Abstract

Azetidine 2-carboxylic acid (Aze) analogs possessing various heteroatomic side chains at the 3-position have been synthesized by modification of 1-PhF-3-allyl-Aze *tert*-butyl ester (2S,3*S*)-**2.1** (PhF = 9-(9-phenylfluorenyl)). 3-Allyl-Aze **2.1** was synthesized from a sequence commencing with regioselective allylation of α-*tert*-butyl β-methyl *N*-(PhF)aspartate **2.13**, followed by selective ω-carboxylate reduction, tosylation and intramolecular *N*-alkylation. Removal of the PhF group and olefin reduction by hydrogenation followed by Fmoc protection produced nor-Leucine-Aze chimera **2.2**. Regioselective olefin hydroboration of (2S,3*S*)-**2.1** produced primary alcohol **2.23**, which was protected as a silyl ether, hydrogenated and N-protected to give 1-Fmoc-3-hydroxypropyl-Aze **2.26**. Enantiopure (2*S*,3*S*)-3-(3-azidopropyl)-1-Fmoc-azetidine-2-carboxylic acid *tert*-butyl ester **2.3** was prepared as a Lys-Aze chimera by activation of 3-hydroxypropyl-Aze **2.26** as a methanesulfonate and displacement with sodium azide. Moreover, orthogonally protected azetidine dicarboxylic acid **2.4** was synthesized as an α-aminoadipate-Aze chimera by oxidation of alcohol **2.26**. These orthogonally protected amino acid-Aze chimeras are designed to serve as tools for studying the influence of conformation on peptide activity.

Key words: azetidine 2-carboxylic acid; azetidines; amino acid; chimera; heterocycle; peptide synthesis

2.2 Introduction

Conformationally constrained amino acids have been used as tools for studying peptide secondary structure and for developing peptide-derived pharmaceutical agents.[1-4] In particular, cyclic amino acids such as proline and its higher homologue pipecolic acid have been used to restrict the phi and psi dihedral angles of the peptide backbone to induce turn geometry.[5,6] Furthermore, the introduction of β-substituents onto proline and pipecolate has been used to create amino acid chimeras, which can restrict the side-chain orientations and backbone geometry of specific residues within a peptide to study the relationship between conformation and activity. For example, proline-amino acid chimeras have been used in place of natural amino acids in peptides to provide a better understanding of the bioactive conformations of G-protein coupled receptor (GPCR) ligands [7-10] and peptide-based enzyme inhibitors. [11]

Azetidine 2-carboxylic acid (Aze) has been used less often as a probe for studying peptide chemistry and biology. Aze has been incorporated *in vivo* and *in vitro* into cellular proteins of *Escherichia coli* [12] as well as in chick embryo[13] for studies on collagen biosynthesis, [14] in which replacement of Pro in collagen with as little as 4% of Aze was sufficient to destabilize triple-helix bundle formation. The study of Aze in polypeptides such as poly-L-Aze, has shown that peptides containing Aze are more flexible than the corresponding sequences containing Pro and that the tendency for β-bend formation is higher for Gly-Aze than for Gly-Pro dipeptides. [15-18] Introduction of Aze into a peptide containing three consecutive proline residues in a linear sequence perturbed the normal proline peptide secondary structure and the *cis-trans* amide isomer

equilibrium in Boc-(L-Pro)$_3$-L-Aze-Opcp (pcp = pentachlorophenyl).[19] Replacement of Aze for proline in peptide analogs has also altered receptor selectivity. [20]

A component of biologically and pharmacologically important natural products and synthetic compounds, Aze is found in the polyoxin group of antifungal antibiotics, [21] nicotianamine, [22] mugineic acid [23] and red-mold rice fermentation products. [24] Among substituted azetidine carboxylic acid analogs, *cis*-azetidine-2,4-dicarboxylic acid has been reported as a neurotransmitter potentiator [25,26] and *trans*-2-carboxyazetidine-3-acetic acid (t-CAA) has been used as a rigid glutamic acid analog exhibiting effects such as inhibition of Na^+-dependent Glu uptake and neuroexcitatory activity at the kainate receptor sub-type. [26]

In the context of our research on the effects of azacycloalkane amino acids on peptide conformation, we became interested in employing β-alkylazetidines as azetidine-amino acid chimeras for studying secondary structure. In light of its importance as a constituent of biologically active compounds, the development of methodology for synthesizing Aze analogs is merited; however, relative to proline and pipecolate analogs the synthesis of alkyl-substituted azetidine 2-carboxylic acid derivatives has received less attention. Few methods have offered the potential for selective and stereocontrolled introduction of alkyl substituents at the ring carbons. Earlier syntheses of racemic 2-azetidine carboxylic acids by resolution and by a series of transformations starting from homoserine have been reviewed.[27-29] Azetidine-2-carboxylic acids have been synthesized bearing substituents at the 1-[30-37], 2- [38-42], 3- [21,43-50] and 4-positions,[51] as well as with multiple substitution patterns. [52-55]

Synthesis of the azetidine ring is typically achieved by intramolecular cyclization of the open-chain compounds to form the C-N bond. [27,56] At the time of our investigation, we were aware of five methods for the synthesis of 3-substituted azetidine-2-carboxylates in enantiopure form. For example, 3-substituted L-azetidine 2-carboxylic acids possessing phenyl, naphthyl and isopropyl substituents at the 3-position were synthesized by Hanessian and co-workers using a general route featuring diastreoselective zinc-mediated additions of substituted allylic halides to the camphor sultam derivative of glyoxalic acid O-benzyl oxime, followed by a series of transformations to convert the linear α-amino pentenamide product into the 3-aryl and 3-alkyl Aze derivatives **2.5-2.7** (Fig. 2.1).[48] Both *cis*- and *trans*-polyoximic acids were also synthesized by the Hanessian lab starting from D-serine as a chiral educt. [21,46] In addition, 3-acetic acid (*t*-CAA) and 3-propionic acid analogs **2.8** and **2.9** of Aze have been synthesized by allylation of a β-lactam enolate derived from D-aspartic acid and subsequent olefin oxidations and lactam reduction. [26]

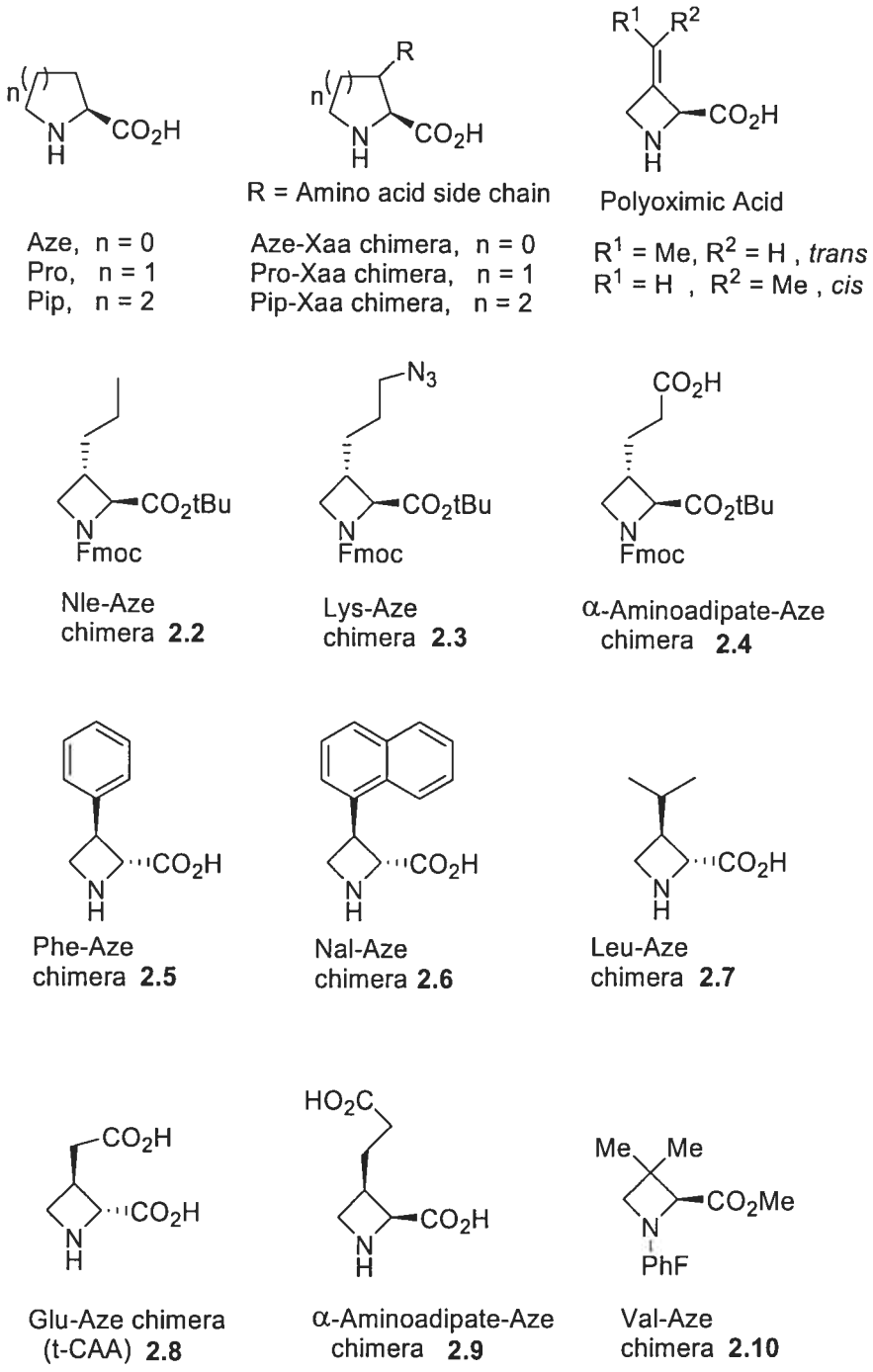


Figure 2.1: Representative Aze-Xaa amino acid chimeras.

Among the strategies for making 3-substituted Aze analogs, the synthesis of 3,3-dimethyl-Aze **2.10** by Goodman seemed practical,[47] because it employed N-

(PhF)aspartate dimethyl ester **2.11**, which could be prepared by regioselective alkylation using methodology introduced by Rapoport for the installation of various side-chain groups (57). 3,3-Dimethyl-Aze **2.10** was prepared from β,β-dimethyl aspartate **2.11** by regioselective reduction of the β-methyl carboxylate, activation of the resulting alcohol and intramolecular nucleophilic displacement (Fig. 2.2).[47]

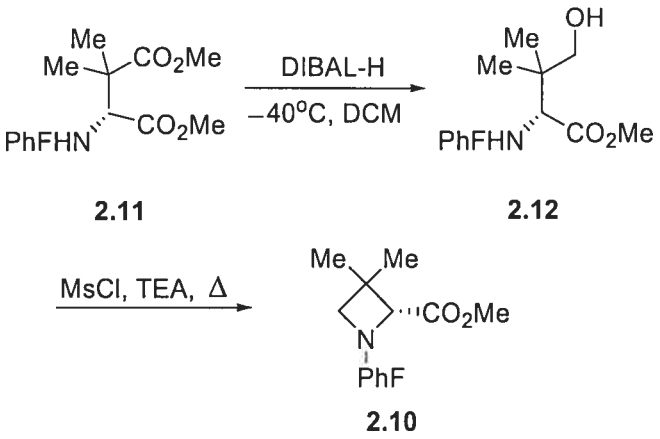


Figure 2.2: Synthesis of β,β-dimethyl-Aze by Goodman.[47]

Azetidine 2-carboxylic acids possessing heteroatomic side-chain substituents at the 3-position were thus targeted, using α-*tert*-butyl β-methyl *N*-(PhF)aspartate **2.13** as a common intermediate. Regioselective enolization and alkylation of aspartate **2.13** was considered for introducing allyl substituents stereoselectively at the β-position.[57,58] (2*S*,3*S*)- and (2*S*,3*R*)-3-Allyl 1-PhF-Aze *tert*-butyl ester **2.1** were synthesized in 4 steps from β-allyl aspartate **2.19** by a route featuring selective ω-carboxylate reduction and intramolecular *N*-alkylation (Fig. 2.4). (2*S*,3*S*)-Olefin **2.1** was subsequently used as a common intermediate for the synthesis of a series of azetidine-amino acid chimeras involving hydrogenation or hydroboration of the olefin to provide constrained nor-leucine-Aze chimera **2.2** and 3-hydroxypropyl-azetidine-2-carboxylate **2.23**, respectively

(Fig. 2.6). Conversion of PhF-azetidine **2.23** into its Fmoc counterpart **2.26**, activation of the hydroxyl group and displacement with azide gave Lys-Aze chimera **2.3** (Fig. 2.7). Oxidation of alcohol **2.26** provided α-aminoadipate-Aze chimera **2.4**.

2.3 Results and discussion:

α-*tert*-Butyl β-methyl *N*-(PhF)aspartate **2.13** was synthesized in three steps on a 15 g scale from L-aspartic acid according to a literature method. [59] The parent amino acid, Aze was first prepared to compare the reactivity of dialkyl, monoalkyl and compounds lacking a substituent at the β-position during the cyclization step. ω-Ester reduction with DIBAL-H in tetrahydrofuran (THF) at −40 °C provided the corresponding homoserine **2.14** without lactone formation (Fig. 2.3).[59] Homoserine **2.14** was then converted to the corresponding tosylate **2.15** in 72% yield by using toluenesulfonyl chloride and pyridine in dichloromethane (DCM). Under these conditions, concurrent intramolecular ring closure occurred to provide azetidine **2.16** as an additional product in 16% yield after chromatography. In our hands, the intramolecular displacement was best effected using Cs_2CO_3 in acetonitrile; Na_2CO_3 in acetonitrile gave a slower reaction and lower yield. Fmoc-Aze-OtBu **2.18** was synthesized in two steps and 77% yield from N-PhF-Aze **2.16**. Hydrogenation of **2.16** at 8 atm of H_2 using palladium-on-carbon as catalyst in 9:1 EtOH: AcOH gave the amino ester which was isolated as its HCl salt. The Fmoc group was then installed using FmocOSu and sodium bicarbonate in acetone/H_2O at room temperature.

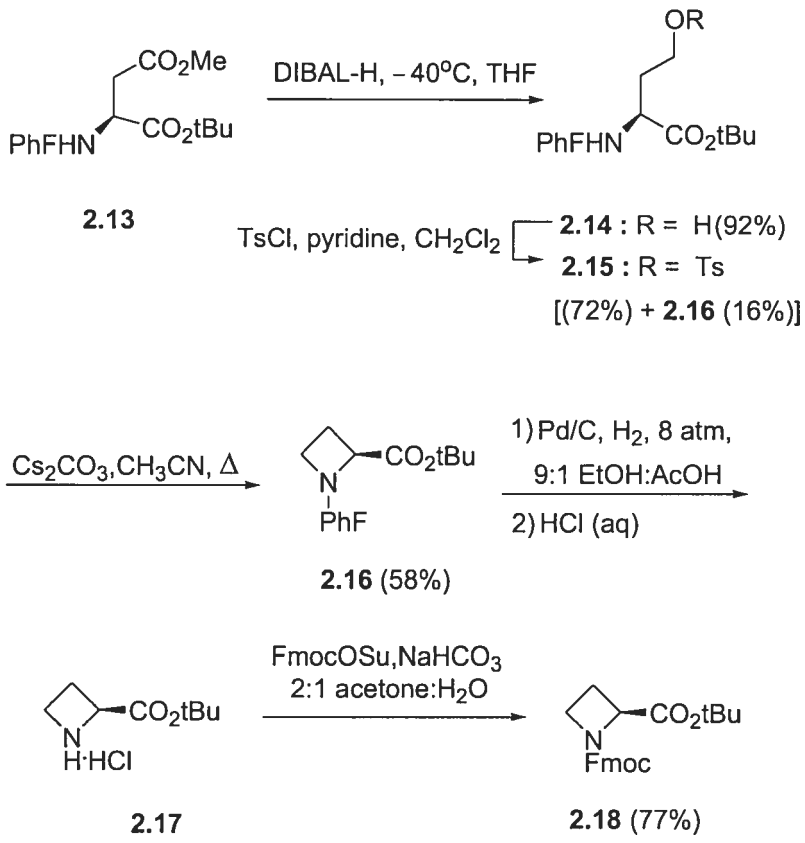


Figure 2.3: Synthesis of Fmoc-Aze-OtBu **2.18** from aspartate **2.13**.

3-Allyl-1-(PhF)-azetidine-2-carboxylic acid *tert*-butyl esters **2.1** were synthesized in a similar manner using β-allyl aspartate **2.19** (Fig. 2.4). Regioselective alkylation of α-*tert*-butyl β-methyl *N*-(PhF)aspartate **2.13** was achieved using sodium bis(trimethylsilyl)amide (NaHMDS, 1M in THF) followed by reaction with allyl iodide, which produced a 3 : 2 mixture of diastereomers **2.19** in 96% yield after chromatography. The unseparated mixture of diastereomers were reduced with DIBAL-H in THF at –40 °C to provide homoserine diastereomers (2*S*,3*R*)- and (2*S*,3*S*)-**2.20** in 91% yield after chromatography. The mixture of diastereomeric alcohols was separated by performing a second chromatography using toluene-isopropylether (8 : 2) as eluant. Tosylation and intramolecular displacement of (2*S*,3*R*)- and (2*S*,3*S*)-**2.20** were performed as described

for the parent system using toluenesulfonyl chloride and pyridine in DCM and afforded the corresponding tosylates **2.21**, which were then heated with Cs_2CO_3 in CH_3CN to provide (2*S*,3*R*)- and (2*S*,3*S*)-azetidine **2.1,** respectively.

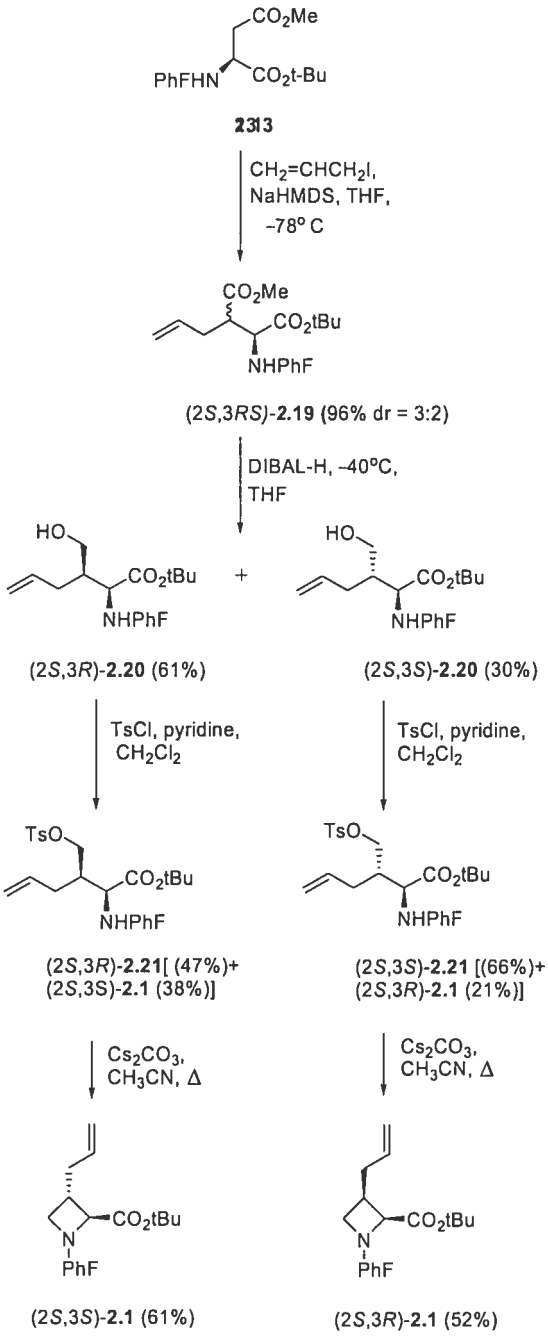
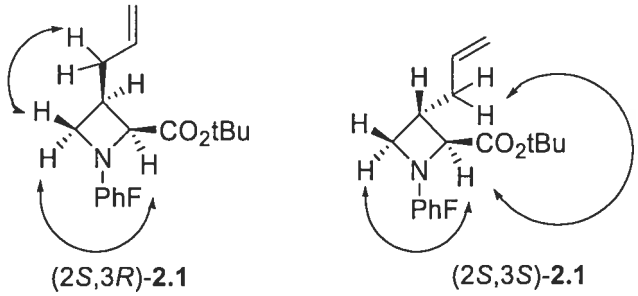


Figure 2.4: Synthesis of 3-Allyl 1-PhF-Aze (2*S*,3*S*)- and (2*S*,3*R*)- **2.1**

The configurations of the (2*S*,3*R*)- and (2*S*,3*S*)-3-allyl azetidines **2.1** from cyclization of the (2*S*,3*S*)- and (2*S*,3*R*)- tosylate diastereomers, respectively, were assigned based on their NOESY spectra in $CHCl_3$ (Fig. 2.5). Transfer of magnetization between the α-hydrogen and the allylic methylene protons indicated their *cis* relationship in (2*S*,3*S*)-**2.1**. The *trans* relationship between the allyl group and α-proton in (2*S*,3*R*)-**2.1** was indicated by their respective NOEs with the two different γ-protons.



(2*S*,3*R*)-**2.1** (2*S*,3*S*)-**2.1**

Figure 2.5: Long distance NOE correlations for the stereochemical assignments of (2*S*,3*R*)- and (2*S*,3*S*)-3-allyl-1-PhF-azetidine-2-carboxylates **2.1** (characteristic transfers of magnetization are written with double-tipped arrows).

Orthogonally protected nor-leucine-Aze chimera **2.2,** suitable for peptide synthesis, was prepared in two steps and 58% yield from *β*-allyl *N*-(PhF)Aze **2.1** (Fig. 2.6). Hydrogenation at 6 atm using palladium-on-carbon as catalyst in 9 : 1 EtOH : AcOH effected double bond reduction and cleavage of the phenylfluorenyl group. Installation of the Fmoc group was then accomplished using FmocOSu and sodium bicarbonate in acetone/H_2O at room temperature.

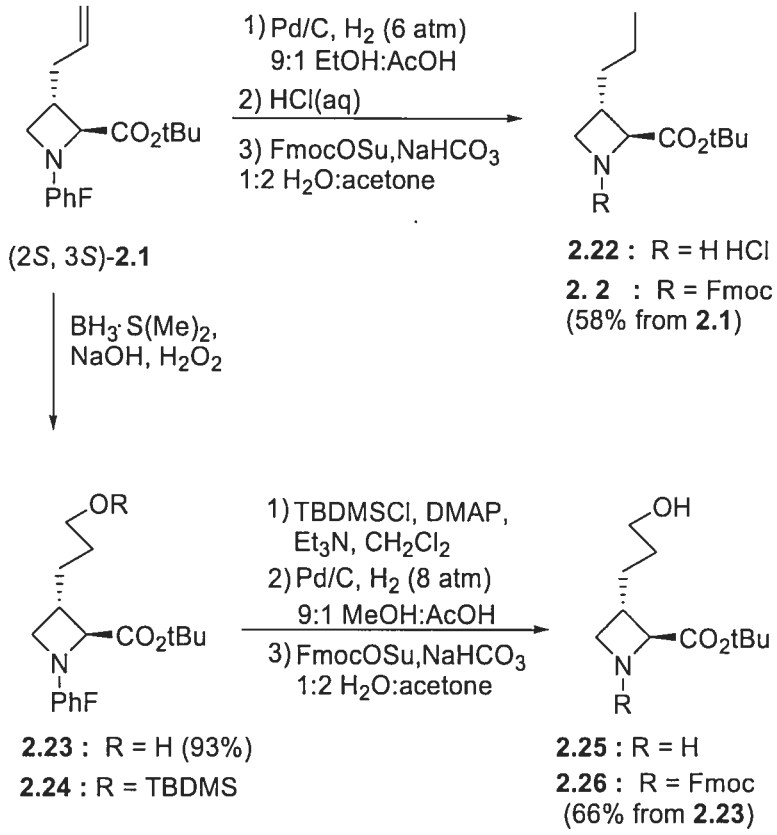


Figure 2.6: Reduction and hydroboration of olefin **2.1**; synthesis of Nle-Aze chimera **2.2**.

The elaboration of olefin **2.1** into a variety of heteroatom-containing side-chains was initiated by hydroboration and conversion to the corresponding 3-hydroxypropyl-Aze **2.23** (Fig. 2.6). Earlier attempts to prepare the primary alcohol using disiamyl borane [60] and 9-BBN in THF, [61] followed by treatment with NaOH and H_2O_2, were unsuccessful and starting material was recovered. Employing BH_3 as a less sterically bulky borane in THF produced a mixture of primary and secondary alcohols in low yield. Selective formation of primary alcohol **2.23** was achieved in 93% yield using borane dimethyl sulfide complex in THF followed by oxidative work-up. [62]

The exchange of the nitrogen protecting group from PhF to Fmoc was examined to later

facilitate oxidative and peptide chemistry. Several solvent systems were examined for the hydrogenolysis of primary alcohol **2.23** to remove the PhF group, such as 9 : 1 MeOH : AcOH, 9 : 1 EtOH : AcOH, and THF, using palladium-on-carbon under hydrogen atmosphere. However, unsatisfactory results were obtained. On the contrary, treatment of 3-hydroxypropyl-Aze **2.23** with TBDMSCl in the presence of DMAP and triethylamine gave 3-siloxypropyl-Aze **2.24**, which underwent hydrogenolytic cleavage of the PhF group in 9 : 1 MeOH : AcOH using palladium-on-carbon under 8 atm of H_2. 1-Fmoc-3-Hydroxypropyl-Aze **26** was then prepared by treatment of the hydrogenation mixture with $NaHCO_3$ and FmocOSu in acetone/H_2O at room temperature for 24 h, work-up and chromatography on silica gel.

Activation of *N*-Fmoc-3-hydroxypropyl **2.26** using methanesulfonyl chloride and triethylamine in dichloromethane gave methanesulfonate **2.27** in 80% yield after purification by chromatography (Fig. 2.7). Nucleophilic displacement of **2.27** with sodium azide in dimethylformamide (DMF) at 80 °C furnished azide **2.3** in 62% yield. α-Amino adipate-Aze chimera **2.4** was finally synthesized in 90% yield by oxidation of 3-hydroxypropyl-Aze **2.26** with a cocktail of $NaClO_2$, NaOCl and TEMPO in CH_3CN. [63]

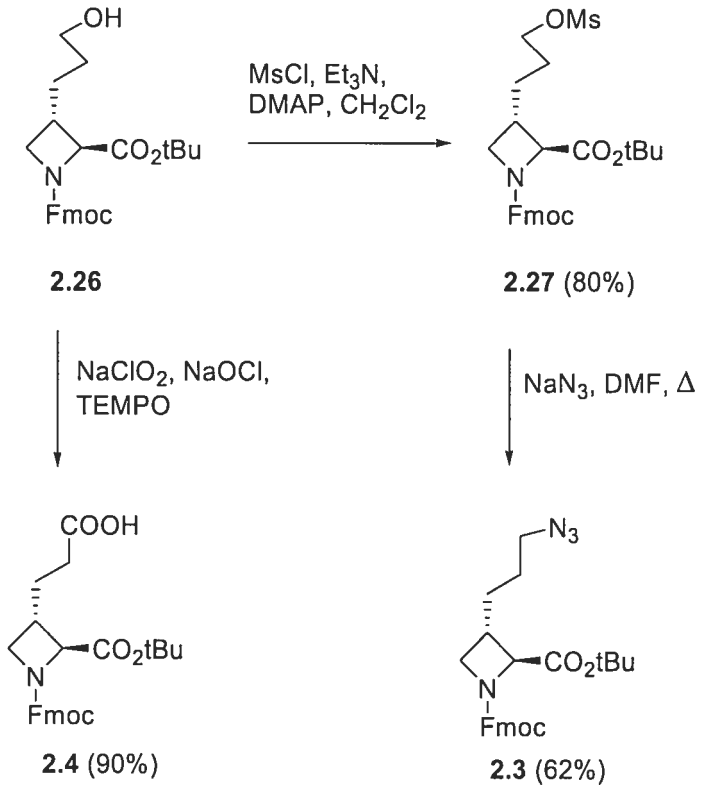


Figure 2.7: Synthesis of Lys-Aze and α-Amonoadipate-Aze chimeras **2.3** and **2.4**.

2.4 Conclusion:

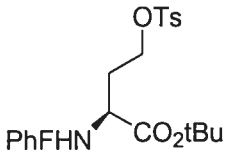
A series of enantiopure amino acid-Aze chimeras possessing heteroatomic side-chains have been synthesized starting from aspartate diester **2.13** as an inexpensive chiral educt. β-Allylation of diester **2.13** followed by ω-ester reduction, tosylation and intramolecular cyclization gave 3-allyl-1-PhF-Aze **2.1**. Olefin reduction and Fmoc-protection provided nor-leucine-Aze chimera **2.2**. Olefin hydroboration afforded the corresponding 3-hydroxypropyl-Aze **2.23** which was converted respectively to Lys-Aze and α-aminoadipate-Aze chimeras **2.3** and **2.4** by routes featuring alcohol activation and displacement with azide ion and TEMPO-catalysed oxidation. We are currently exploring the introduction of these amino acid-Aze chimeras into peptides to study structure activity relationships.

2.5 Experimental Section

General. Unless otherwise noted, all reactions were run under nitrogen atmosphere and distilled solvents were transferred by syringe. Anhydrous tetrahydrofuran (THF), dichloromethane (DCM), acetonitrile (CH_3CN) and dimethylformaide (DMF) were obtained from solvent filteration systems. Triethylamine (Et_3N) and pyridine were distilled from ninhydrin and CaH_2. Final reaction mixture solutions were dried over Na_2SO_4 and rotary-evaporated under vacuum. Melting points are uncorrected. Mass spectral data, HRMS/ESMS, were obtained by the Université de Montréal Mass Spectrometry facility. Unless otherwise noted, ^1H NMR (300/400 MHz) and ^{13}C NMR (75/100 MHz) spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm (δ

units) downfield of internal tetramethylsilane ($(CH_3)_4Si$) or residual solvent: $CHCl_3$ and MeOH. Coupling constants are reported in hertz (Hz). Chemical shifts of aromatic and vinylic carbons are not reported for the ^{13}C NMR spectra of PhF containing compounds. Analytical thin-layer chromatography (TLC) was performed using aluminum-backed silica plates coated with a 0.2 mm thickness of silica gel 60 F_{254} (Merck KGaA Germany). Chromatography was performed using Kieselgel 60 (230-400 mesh).

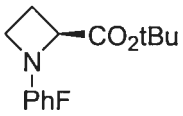
(2S)-*tert*-Butyl 2-[*N*-(PhF)Amino]-4-(toluenesulfonyloxy)-butyrate (2.15):



To a magnetically stirred solution of alcohol **2.14** (500 mg, 1.2 mmol, 100 mol %, prepared according to reference 59) and dry pyridine (1.2 ml) in dry CH_2Cl_2 (12 ml) at 0 °C, solid p-toluenesulfonyl chloride (693 mg, 3.6 mmol, 300 mol %) was added in one portion. The mixture was stirred at 0 °C for 2 h and then at room temperature for 24h. The reaction mixture was poured into ice water and extracted with ethyl acetate (3 × 20 ml). The organic phases were combined, washed with brine, dried ($MgSO_4$) and evaporated to a residue that was chromatographed using 5% EtOAc in hexanes as eluant. First to elute was azetidine **2.16** (77 mg, 16% yield) as a solid: m.p. 156.5-157.5 °C, spectral properties were identical to those reported below. Next to elute was tosylate **2.15** (496 mg, 72% yield) as white crystals: m.p. 106-107 °C; $[\alpha]^{20}_D$ –184.3° (*c* 0.01; $CHCl_3$). ^{1}HNMR δ: 1.21 (s,9 H), 1.51-1.62 (m,1 H); 1.72-1.82 (m,1 H); 2.47 (s,3 H); 2.52-2.55 (t,

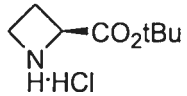
J = 5 Hz, 1 H); 3.09 (s, 1 H); 4.03-4.10 (dd, J = 13.16; 6.68 Hz, 1 H), 4.21-4.23 (dd, J = 16.1; 7.1 Hz, 1 H); 7.20-7.85 (m, 17 H). ^{13}C NMR δ 22.1; 28.1; 34.4; 53.1; 68.0; 73.2; 81.7; 174.44. ESMS m/z 592.1 $(M+Na)^+$.

(2S)-*tert*-Butyl 1-(PhF)-azetidine-2-carboxylate (2.16):



A vigorously stirred mixture of tosylate **2.15** (500 mg, 0.877 mmol, 100 mol%), and powdered Cs_2CO_3 (430 mg, 1.31 mmol, 150 mol%) in 10 ml of acetonitrile was heated at reflux under argon for 19 h and evaporated. The residue was treated with ice-water (10 ml), and extracted with EtOAc (3 × 20 ml). The combined organic phases were washed with brine, dried ($MgSO_4$) and evaporated to a residue that was purified by chromatography using an eluant of 3% EtOAc in hexanes. Azetidine **2.16** (202 mg, 58% yield) was obtained as white crystals: m.p. 156.5-157.5 °C. $[\alpha]^{20}_D$ 38.5° (*c* 0.01, $CHCl_3$). ^{1}H NMR δ 1.13 (s, 9 H); 1.72-1.82 (m, 1 H); 2.03-2.15 (m, 1 H); 3.10-3.18 (dd, J = 15.6; 8.2 Hz, 1 H), 3.25-3.36 (m, 2 H); 7.13-7.68 (m, 13 H). ^{13}C NMR δ 20.8; 28.1; 46.7; 60.8; 76.5; 80.3; 172.4. ESMS m/z 398.1 $(M+H)^+$. HRMS calcd. for $C_{27}H_{27}NO_2$ $(M+H)^+$ 398.21146, found 398.21153.

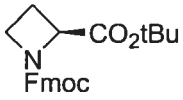
Azetidine-2-carboxylic Acid *tert*-Butyl Ester Hydrochloride (2.17):



A solution of PhF-Aze **2.16** (322 mg, 0.81 mmol, 100 mol%) in 30 ml of EtOH:HOAc

(9:1) was transferred into a hydrogenation apparatus and treated with palladium-on-carbon (32 mg, 10 wt %). The pressure bottle was filled, vented, and refilled four times with hydrogen. The reaction mixture was stirred for 24 h at 6 atm of H_2, filtered onto a plug of Celite^R 521(Aldrich USA), and washed thoroughly with EtOH. The filtrate was treated with 1ml of 1N HCl and the volatiles were evaporated to give a crude residue (125 mg) that was used directly in the Fmoc protection without purification. ^{1}H NMR of crude **2.17**: δ 1.41 (s, 9 H); 2.56 (s, 1 H); 2.77 (s, 1 H); 4.15 (d, J = 37.2 Hz, 2 H); 4.75 (s, 1 H); 5.00 (s, 1 H). ^{13}C NMR δ 22.9; 27.9; 43.5; 57.5; 84.5; 166.9. ESMS m/z 157.9 $(M)^+$

***tert*-Butyl 1-(Fmoc)-Azetidine-2-carboxylate (2.18):**



Fmoc-Aze **2.18** (295 mg, in 77% yield) was synthesized from Aze **2.17** (147 mg, 0.93 mmol) using the general Fmoc protection protocol described below and purified by chromatography using an eluant of 3-11% EtOAc in hexanes. Evaporation of the collected fractions gave **2.18**: $[\alpha]^{20}_D$ –78.9 ° (*c* 0.03, $CHCl_3$). ^{1}H NMR δ 1.50 (s, 9 H); 2.17-2.25 (m,1 H); 2.53-2.62 (m, 1 H); 4.00 (d, J = 6.21 Hz, 1 H); 4.10-4.38 (m, 4 H); 4.64 (d, J = 4.6, Hz, 1 H); 7.30-7.77 (m, 8 H). ^{13}C NMR δ 20.8; 28.0; 47.2; 50.3; 65.1; 67.3; 81.8; 119.9; 125.2; 127.0; 127.7; 141.4; 144.5; 155.8; 170.3. ESMS m/z 402.1 $(M+Na)^+$. HRMS calcd. for $C_{23}H_{25}NO_4$ $(M+Na)^+$ 402.16758, found 402.16822.

α- *tert*-Butyl β-Methyl β-Allylaspartate (2.19):

Diester **2.13** (11.16 g, 25.16 mmol, 100 mol% prepared according to reference 59) was dissolved in 230 ml of dry THF, cooled in an acetone/dry ice bath to −78 °C, treated dropwise with NaHMDS (26.4 ml of a 1M solution in THF, 105 mol%), stirred at −78°C for 1h, treated with allyl iodide (4.6 ml, 50.3 mmol, 200 mol%), and stirred for 2h. The cold reaction mixture was quenched with methanol (25 ml) and 1M NaH_2PO_4 (60 ml), allowed to warm to room temperature, and extracted (3 × 100 ml) with ethyl acetate. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated to a residue that was chromatographed using 3-5% EtOAc in hexanes as eluant. Evaporation of the collected fractions gave a mixture of two diastereomers in a 3:2 ratio as assessed by measurement of the diastereomeric methyl ester singlets in the NMR spectrum (11.63 g, 95% yield). ^{1}H NMR for the major diastereomer: δ 1.26 (s, 9 H); 2.09-2.17 (m, 1 H); 2.43-2.67 (m, 2 H); 2.87- 2.96 (m, 1 H); 3.27- 3.37 (d, J = 9.4 Hz, 1 H); 3.70 (s, 3 H); 5.00-5.06 (m, 2 H); 5.66-5.75 (m, 1 H); 7.24-7.74 (m, 13 H). ^{13}C NMR δ 28.0; 32.5; 50.8; 51.5; 57.1; 57.9; 72.9; 77.6; 81.5; 172.9, 173.0. ESMS m/z 484.1 $(M)^+$. HRMS calcd. for $C_{31}H_{33}NO_4$ $(M+H)^+$ 484.24824, found 484.24852.

(2*S*,3*S*)- and (2*S*,3*R*)-3-Hydroxymethyl-2-[(PhF)amino]-hex-5-enoic Acid *tert*-Butyl Ester (2.20):

To a stirred solution of alkylated diester **2.19** (11.63, 24.06 mmol, 100 mol%) in THF (484 ml) at -40 °C, DIBAL-H (72.2 ml of a 1M solution in THF, 300 mol %) was added and stirring was continued for 3h. The mixture was treated with acetone (15 ml) to quench excess hydride, diluted with methanol (50 ml), allowed to warm to room

temperature and evaporated to a residue that was dissolved in ether (500 ml) and washed with NaOH 1M (3 × 150 ml). The combined organic layers were washed with brine, dried, filtered, and evaporated. The residue was first chromatographed with 5-10% EtOAc in hexanes to provide a mixture of diastereomers, that were separated by performing another column using toluene-isopropropylether (8:2) as eluant.

Minor diastereomer (2*S*,3*S*)-**2.20** (3.4 g, 30% yield) eluted first: $[\alpha]^{20}_{D}$ –327.3° (*c* 0.01, $CHCl_3$). ^{1}H NMR δ 1.25 (s, 9 H); 1.62-1.66 (m, 1 H); 1.96-2.05 (m, 1 H); 2.17-2.25 (m, 1 H); 2.79 (d, *J* = 3.2 Hz, 1 H); 3.55 (s, 1 H); 3.49 (dd, *J* = 11.1; 8.0 Hz; 1 H); 3.63 (dd, *J* = 11.0; 4.3 Hz, 1 H); 4.89-5.03 (m, 2 H); 5.60-5.7 (m, 1 H); 7.23-7.71 (m, 13 H). ^{13}C NMR δ 27.9; 30.4; 43.9; 57.3; 60.9; 62.9; 73.1; 81.1; 173.7.

Major diastereomer (2*S*,3*R*)-**2.20** (6.7 g, 61%) eluted second: $[\alpha]^{20}_{D}$ –227.3° (*c* 0.01, $CHCl_3$). ^{1}H NMR δ 1.11 (s, 9 H); 1.58-1.66 (m, 1 H); 1.69-1.85 (m, 2 H); 2.49 (d, *J* = 6.1 Hz, 1 H); 3.41 (dd, *J* = 11.3; 7.1 Hz, 1 H); 3.63 (dd, *J* = 11.3; 2.8 Hz, 1 H); 4.68-4.79 (m, 2 H); 5.35-5.46 (m,1 H); 7.11-7.61 (m,13 H). ^{13}C NMR δ 28.0; 33.1; 44.2; 59.3; 64.1; 73.1; 77.4; 81.6; 174.5. ESMS m/z 478.0 $(M+Na)^+$. HRMS calcd. for $C_{30}H_{33}NO_3$ $(M+H)^+$ 456.25332, found 456.25338.

(2*S*,3*R*)-2-[*N*-(PhF)Amino]-3-(toluenesulfonyloxymethyl)-hex-5-enoic Acid *tert*-Butyl Ester (2.21):

A magnetically stirred solution of (2*S*,3*R*)-alcohol **2.20** (4.41 g, 9.65 mmol, 100 mol%) and dry pyridine (9.6 ml) in dry CH_2Cl_2 (96 ml) was cooled to 0 °C, treated with solid toluenesulfonyl chloride (5.56 g, 28.9 mmol), stirred at 0 °C for 2 h and at room

temperature for 24h, and poured into ice water. The mixture was extracted with ethyl acetate (3 × 100 ml). The combined organic phase was washed with brine, dried ($MgSO_4$) and evaporated to a residue that was chromatographed using 5-10% EtOAc in hexanes as eluant. First to elute was (2*S*,3*S*)-3-allyl azetidine **2.1** (1.06 g, 38% yield) as a solid: m.p. 112.3-113.3 °C, spectral properties were identical to those reported below. Second to elute was (2*S*,3*R*)-tosylate **2.21** (3.18 g, 47% yield): a clear oil $[\alpha]^{20}_D$ –282.8° (*c* 0.01, $CHCl_3$). ^{1}H NMR δ 1.13 (s, 9 H); 1.65 (s, 1 H); 1.95 (dd, *J* = 5.5; 4.7 Hz, 2 H); 2.33 (s, 3 H); 2.52 (s, 1 H); 3.87-3.92 (m, 2 H); 4.71 (d, *J* = 12.8 Hz, 2H); 5.10-5.30 (m, 1 H); 7.05-7.67 (m, 17 H). ^{13}C NMR δ 21.7; 27.9; 31.5; 43.6; 55.2; 70.5; 73.0; 81.5; 173.3.

(2*S*,3*S*)-2-[*N*-(PhF)Amino]-3-(toluenesulfonyloxymethyl)-hex-5-enoic Acid *tert*-Butyl Ester (2.21):

(2*S*,3*S*)-Tosylate was synthesized from (2*S*,3*S*)-alcohol **2.20** (222 mg, 0.486 mmol, 100 mol%) using the same procedure as that described above The residue was chromatographed with 5-10% EtOAc in hexanes as eluant. First to elute was (2*S*,3*R*)-3-allyl-1-(PhF)-azetidine-2-carboxylic acid *tert*-butyl ester **2.1** (45 mg, 21%) as white crystals: m.p. 128.5-129.5 °C, spectral properties were identical to those reported below. Second to elute was (2*S*,3S)-tosylate **2.21** (197 mg, 66% yield): a clear oil $[\alpha]^{20}_D$ –146.4° (*c* 0.01, $CHCl_3$). ^{1}H NMR δ 1.25 (s, 9 H); 1.83 (s, 1 H); 2.11-2.14 (m, 2 H); 2.51(s, 3 H); 2.63 (s, 1 H); 3.22 (s, 1 H); 4.02-4.11 (m, 2 H); 4.88 (d, *J* = 13.2 Hz, 2 H); 5.34-5.40 (m, 1 H); 7.22-7.84 (m, 17 H). ^{13}C NMR 21.6; 27.8; 31.5; 43.6; 55.1; 70.4; 73.0; 81,4; 173.2. ESMS m/z 610.4 $(M+H)^+$.

(2*S*,3*S*)-3-Allyl-1-(PhF)-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.1):

A vigorously stirred mixture of (2*S*,3*R*)-tosylate **2.21** (698 mg, 1.14 mmol, 100 mol%) and powdered Cs_2CO_3 (560 mg, 1.71 mmol, 150 mol%) in 10 ml of acetonitrile was heated at reflux under argon for 19 h, cooled and evaporated to a residue, which was poured into ice water (15 ml) and extracted with EtOAc (3 × 20 ml). The combined organic phases were washed with brine, dried ($MgSO_4$) and evaporated to a foam. Chromatography using 3-5% EtOAc in hexanes as eluent provided (2*S*,3*S*)-3-allyl-1-(PhF)-azetidine-2-carboxylic acid *tert*-butyl ester **2.1** (340 mg, 68 % yield) as white crystals: m.p. 112.3-113.3 °C. $[\alpha]^{20}_D$ 95.1° (*c* 0.01, $CHCl_3$). ^{1}H NMR δ 1.25 (s, 9 H); 1.92-2.00 (m, 2 H); 2.54-2.62 (m,1 H); 2.99 (t, *J* = 7.3 Hz, 1 H); 3.13 (d, *J* = 7.0 Hz, 1 H); 3.58 (t, *J* = 7.3 Hz, 1 H); 4.89 (d, *J* = 14.7 Hz, 2 H); 5.50-5.60 (m, 1 H); 7,22-7.80 (m, 13 H). ^{13}C NMR δ 27.8; 33.9; 36.9; 52.3; 66.1; 76.1; 79.8; 171.7. HRMS calcd. For $C_{30}H_{31}NO_2$ $(M+Na)^+$ 460.22470 found 460.22582.

(2*S*,3*R*)-3-Allyl-1-(PhF)-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.1):

(2*S*,3*R*)-Azetidine **2.1** was synthesized from (2*S*,3*S*)-tosylate **2.21** (214 mg, 100 mmol) using the same procedure as that described above to provide (2*S*,3*R*)-3-allyl-1-(PhF)-azetidine-2-carboxylic acid *tert*-butyl ester **2.1** (80 mg, 52% yield) as white crystals: m.p. 128.5-129.5 °C; $[\alpha]^{20}_D$ 127.6° (*c* 0.01, $CHCl_3$); ^{1}H NMR δ 1.21 (s, 9 H); 2.18-2.26 (m, 1 H); 2.32-2.40 (m, 1 H); 2.47-2.55 (m, 1 H); 3.24 (dd, *J* = 7.5; 3.7 Hz, 1 H), 3.38 (d, *J* = 9.2 Hz, 1 H); 3.50 (t, *J* = 8.7 Hz, 1 H); 4.95-5.02 (m, 2 H); 5.61-5.71 (m, 1 H); 7.14-7.76 (m, 13 H). ^{13}C NMR δ 28.1; 30.6; 34.1; 51.0; 61.6; 76.0; 80.3; 170.2.

(2*S*,3*S*)-3-Propyl-azetidine-2-carboxylic Acid *tert*-Butyl Ester Hydrochloride (2.22):

A solution of (2*S*,3*S*)-**2.1** (110 mg, 0.25 mmol, 100 mol%) in 10 ml of EtOH : HOAc (9 :

1) was transferred into a hydrogenation apparatus and treated with palladium-on-carbon (20 mg, 10 wt %). The pressure bottle was filled, vented, and refilled four times with 6 atm of H_2. The reaction mixture was stirred overnight (24 h), filtered onto a plug of Celite^R 521(Aldrich USA), and washed thoroughly with EtOH. The filtrate was treated with 5 drops of 1N HCl and the volatiles were evaporated to give a crude residue of **2.22** (50 mg) as the hydrochloride salt that was used directly for the Fmoc protection: ^{1}H NMR (CD_3OD, 400 MHz) δ 0.85 (t, J = 7.3 Hz, 3 H); 1.14-1.29 (m, 2 H); 1.45 (s, 9 H); 1.49-1.63 (m, 2 H), 3.06-3.11 (m, 1 H), 3.48-3.58 (m, 1 H), 4.02 (dd, J = 19.7; 9.2 Hz, 1 H); 4.85 (d, J = 9.6 Hz, 1 H). ^{13}C NMR δ 13.6; 19.6; 28.0; 35.1; 37.1; 48.7; 62.9; 84.6; 166.7. ESMS m/z 200.0 $(M+H)^+$.

Typical procedure for Fmoc protection of azetidine derivatives. (2*S*,3*S*)-3-Propyl-1-(Fmoc)-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.2):

Amine **2.22** (50 mg, 0.25 mmol, 100 mol% from above) in 1 ml of acetone/H_2O (2 : 1) was treated with FmocOSu (101.69, 0.30 mmol, 120 mol%) and $NaHCO_3$ (25.2, 0.30 mmol, 120 mol %), stirred overnight, treated with an additional 120 mol% of $NaHCO_3$, stirred overnight and evaporated to a reduced solution that was diluted with water and washed with Et_2O (4 × 2 ml). The aqueous phase was acidified to pH = 3 and extracted with EtOAc (3 × 3 ml). The combined organic phases were washed with brine, dried and evaporated to a residue that was purified by chromatography using 3-7% EtOAc in hexanes as eluant. Evaporation of the collected fractions gave **2.2** (51.9 mg, 58% yield) as a foam: $[\alpha]^{20}_D$ –58.6 ° (c 0.01, $CHCl_3$). ^{1}H NMR δ 0.85 (t, J = 2.8 Hz, 3 H); 1.18-1.22 (m, 2 H); 1.43 (s, 9 H); 1.46-1.52 (m, 1 H); 2.81-2.87 (m, 1 H); 3.72 (bm, 1 H); 3.67-4.31

(m, 5 H); 4.62 (s, 1 H); 7.21-7.69 (m, 8 H); ^{13}C NMR δ 13.9; 20.2; 28.1; 31.7; 32.9; 47.3; 53.4; 54.1; 67.3; 82.2; 120.0; 125.3; 127.1; 127.7; 141.3; 144.2; 156.0; 168.7. ESMS m/z 444.1 $(M+Na)^+$. HRMS calcd. For $C_{26}H_{31}NO_4$ $(M+H)^+$ 422.23258, found 422.23242.

(2*S*,3*S*)-3-(3-Hydroxypropyl)-1-(PhF)-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.23):

Borane methyl sulfide complex (25.98 mg, 0.342 mmol, 300 mol%) was added to a solution of 3-allyl-azetidine **2.1** (50 mg, 0.114 mmol, 100 mol %) in anhydrous THF (1 ml) at 0° C under argon, stirred for 3 h and treated dropwise with 3N NaOH (0.342 ml, 900 mol %) which resulted in a vigorous evolution of gas. Hydrogen peroxide (35% aqueous, 0.119 ml, 900 mol %) was added to the mixture, which was stirred for 2 h, poured into water (12 ml) and extracted with EtOAc (4 ×10 ml). The combined organic phases were dried, filtered, and concentrated *in vacuo* to a residue that was chromatographed using $CHCl_3$:CH_3OH 97:3 as eluant to provide primary alcohol **2.23** (48.5 mg, 93% yield) as a light white solid: m.p. 214.6-215.6 °C. $[\alpha]^{20}_D$ 88.0 (*c* 0.01, $CHCl_3$:MeOH, 3:1). ^{1}H NMR ($CDCl_3$:CD_3OD, 3:1, 400 MHz) δ 1.09-1.24 (m, 13 H); 2.32 (dd, J = 14.0; 7.0 Hz, 1 H); 2.90 (t, J = 6.9 Hz, 2 H); 3.32-3.36 (m, 2 H); 3.47 (t, J = 7.2 Hz,1 H); 7.10-7.70 (m, 13 H). ^{13}C NMR ($CDCl_3$:CD_3OD, 3:1, 400 MHz) δ 27.4; 29.0; 29.4; 34.8; 52.6; 61.4; 66.4; 76.0; 80.3; 172.7. ESMS m/z 456.1 $(M+H)^+$. HRMS calcd. For $C_{30}H_{33}NO_3$ $(M+H)^+$ 456.25332, found 456.25396.

(2*S*,3*S*)-3-(-3-*tert*-Butyldimethylsilanyloxypropyl)-1-(PhF)-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.24):

A solution of alcohol **2.23** (500 mg, 1.09 mmol, 100 mol%) in 100 ml of CH_2Cl_2 at room temperature was treated with TBDMSCl (496 mg, 3.29 mmol, 300 mol%), DMAP (4 mol %) and triethylamine (444 mg, 4.39 mmol, 400 mol %) and stirred for 24 h. The residue was dissolved in EtOAc and washed with 1M KH_2PO_4 (50 ml). The aqueous phase was extracted with EtOAc (2 × 40 ml). The combined organic phases were dried and evaporated to a crude residue of **2.24** (600 mg) that was used directly in the next step. ESMS m/z 570.2 $(M+H)^+$.

(2*S*,3*S*)-3-(3-Hydroxypropyl)-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.25):

A hydrogenation vessel containing a solution of silanyloxypropyl azetidine **2.24** (120 mg, 0.21 mmol, 100 mol% from above) in 20 ml of MeOH:AcOH (9:1) was charged with 12 mg of palladium-on-carbon (10 wt %) and stirred for 24 h under 8 atm of hydrogen. The reaction mixture was filtered through Celite™ and washed with EtOAc (30 ml) and MeOH (20ml). The filtrate was evaporated to dryness. The residue was triturated with hexane (3 × 10 ml). The remaining solid was used without additional purification. ^{1}H NMR of crude **2.25**: δ 1.44 (s, 9 H); 1.45-1.61 (m, 2 H); 1.74-1.82 (m, 2 H); 2.81 (dd, J = 15.3; 7.7 Hz, 1 H); 3.57 (m, 3 H); 3.87 (t, J = 8.8 Hz, 1 H); 4.33 (d, J = 7.0 Hz, 1 H). ^{13}C NMR δ 28.0; 29.5, 30.0, 30.9, 48.9, 61.8, 63.2, 83.4, 169.3; ESMS m/z 216.0 $(M+H)^+$.

(2*S*,3*S*)-3-(3-Hydroxypropyl)-1-Fmoc-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.26):

(2*S*,3*S*)-1-Fmoc-3-(3-Hydroxypropyl)-Aze **2.26** (120 mg, 66% yield from **2.23**) was

synthesized from crude (2*S*,3*S*)-3-(3-hydroxypropyl)-Aze **2.25** (90 mg, 0.418 mmol) using the general Fmoc protection protocol described above: ^{1}H NMR δ 1.44 (s, 9 H); 1.50-1.66 (m, 2 H), 1.73 (dd, *J* = 14.7; 7.4 Hz, 2 H); 2.10 (s,1 H); 3.54 (dd, *J* = 8.0; 4.1 Hz, 1 H); 3.62 (t, *J* = 6.1 Hz, 2 H); 4.05-4.35 (m, 6 H); 7.25-7.73 (m, 8 H). ^{13}C NMR δ 14.2; 28.0; 28.2; 29.6; 30.3; 35.0; 47.2; 62.0; 67.3; 81.9; 119.9; 125.2; 127.1; 127.7; 141.3; 144.0; 156.1; 170.0. ESMS m/z 437.9 $(M)^{+}$.

(2*S*,3*S*)-3-(3-Methanesulfonyloxypropyl)-1-Fmoc-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.27):

A solution of alcohol **2.26** (61 mg, 0.133 mmol, 100 mol %) in 11 ml of CH_2Cl_2 at 0 °C was treated with DMAP (1.63 mg, 10 mol%), Et_3N (0.055 ml, 0.399 mmol, 300 mol%) and methanesulfonyl chloride (0.041 ml, 0.532 mmol, 400 mol %). The ice bath was removed and the mixture was stirred for 5h at room temperature when TLC showed no remaining starting material (R_f = 0.19, 30% EtOAc in hexanes). The mixture was partitioned between EtOAc (45 ml) and water (15 ml). The organic layer was washed with 2N HCl (10 ml), 5% $NaHCO_3$ (10 ml) and water, dried and evaporated to a residue that was purified by chromatography using 30-35% EtOAc in hexanes as eluant. Evaporation of the collected fractions provide an oil (57 mg, 80 % yield) of **2.27**: ^{1}H NMR δ 1.42 (s, 9 H); 1.782-1.88 (m, 4 H); 2.48-2.52 (m, 1 H); 3.02 (dd, *J* = 8.0; 2.7 Hz, 3 H), 3.57 (dd, *J* = 8.0; 2.7 Hz, 1 H); 4.08-4.37 (m, 7 H); 7.28-7.76 (m, 8 H). ^{13}C NMR δ 14.3; 26.5; 28.1; 28.2; 29.9; 34.6; 37.5; 47.3; 67.3; 69.0; 82.1; 120.0; 125.3; 127.1; 127.8; 141.3; 143.9; 156.1; 169.7. ESMS m/z 538.1 $(M+Na)^{+}$.

(2*S*,3*S*)-3-(3-Azidopropyl)-1-Fmoc-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.3):

Methansulfonate **2.27** (42 mg, 0.08 mmol, 100 mol%) was dissolved in DMF (5 ml), and treated with NaN_3 (26.32 mg, 0.405 mmol, 500 mol%), stirred at 80°C for 8h, cooled and partitioned between EtOAc (12 ml) and water (6 ml). The organic layer was washed with 2N HCl (5 ml), 5 % $NaHCO_3$ (5 ml) and water (5 ml), dried and evaporated. The residue was chromatographed using 3-5% EtOAc in hexanes as eluant. Evaporation of the collected fractions gave azide **2.3** as white foam (23 mg, 62% yield): IR cm^{-1} 2098.44. ^1H NMR δ 1.30 (s, 9 H); 1.46-1.64 (m, 5 H); 2.50-2.60 (m, 2 H); 2.74 (dd, J = 11.7; 6.8 Hz, 1 H); 2.96 (dd, J = 11.6; 7.6 Hz, 1 H), 3.22 (dd, J = 12.5; 6.3 Hz, 2 H); 3.52 (t, J = 6.3 Hz,1 H); 3.80 (t, J = 7.1 Hz, 1 H); 7.18-7.68 (m, 8 H). ^{13}C NMR δ 26.5; 28.1; 31.4; 35.0; 46.1; 51.3; 58.5; 63.1; 72.8; 81.1; 119.7; 125.6; 126.8; 127.2; 141.1; 146.2, 171.9. ESMS m/z 419.1 $(M- 43)^+$.

(2*S*,3*S*)-3-(2-Carboxyethyl)-1-Fmoc-azetidine-2-carboxylic Acid *tert*-Butyl Ester (2.4):

A mixture of alcohol **2.26** (0.114 mmol, 100 mol%), TEMPO (1.23 mg), CH_3CN (0.57 ml) and sodium phosphate buffer (0.427 ml, 0.67 M, pH = 6.7) was heated to 35 °C, treated with sodium chlorite ($NaClO_2$, 6 mg, 80%, 0.228 mmol in 0.114 ml H_2O), stirred 5 min and treated with dilute bleach (0.0014 ml, 10% NaOCl diluted into 0.057 ml). The mixture was stirred at 35 °C for 24 h when TLC showed no starting material (R_f = 0.30, 4% MeOH in $CHCl_3$). The pH was adjusted to 8.0 with 2.0 N NaOH (~0.136 ml). The reaction was quenched by pouring into cold (0 °C) Na_2SO_3 solution (0.57 ml, 0.5 M) maintaining the bath temperature below 20 °C. The pH of the aqueous layer was between 8.5-9.0. After stirring for 0.5 h at room temperature, diethyl ether (2 ml) was added. The

organic layer was separated and discarded. The aqueous layer was treated with EtOAc (5 ml) and acidified with 2.0 N HCl (~0.5 ml) to pH 3-4. The organic layer was separated, washed with water (2 × 5 ml) and brine (1.5 ml), and concentrated to a solid that was recrystalyzed from EtOAc:petroleum ether (2:10). Dicarboxylate **2.4** (47 mg, 90 % yield) was isolated as white crystals: m.p. 61.5-62.5 °C. $[\alpha]^{20}_{D}$ –56.3° (*c* 0.01, $CHCl_3$). ^{1}H NMR δ 1.48 (s, 9 H); 1.97-2.04 (m, 2 H); 2.34-2.49 (m, 2 H); 2.50-2.57 (m, 1 H); 3.53 (dd, J = 8.0; 2.6 Hz, 1 H); 4.11-4.21 (m, 3 H); 4.39 (d, J = 7.0 Hz, 2 H); 7.28-7.74 (m, 8 H). ^{13}C NMR δ 28.0; 28.2; 28.7; 31.0; 34.5; 47.3; 52.6; 67.4; 82.2; 120.0; 125.2; 127.1; 127.8; 141.4; 144.0; 156.2; 169.6; 177.8. ESMS m/z 474.2 $(M+Na)^+$. HRMS calcd. For $C_{26}H_{29}NO_6$ $(M+H)^+$ 452.20676 found 452.20700.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **2.1-2.4** and **2.15-2.23**, **2.25-2.27** and NOESY spectra of **2.1**.

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CHAPTER 3

Synthesis of 2,4-substituted azetidin-3-ones and 4-substituted azetidine 2-carboxylic acids

3.1 Introduction:

Azabicyclo[X.Y.0]alkane amino acids are constrained dipeptide mimics that can serve as conformationally fixed surrogates of peptide turn secondary strucrtures.[1,2] In these bicyclic structures, three dihedral angles (ω, ψ and ϕ) are restricted (Figure 3.1). Incorporating these constrained scaffolds into peptides can give useful information about the bioactive conformation of the parent peptides. Enhanced activity and metabolic stability have also resulted upon incorporation of azabicyclo[X.Y.0]alkane amino acids.[1,2]

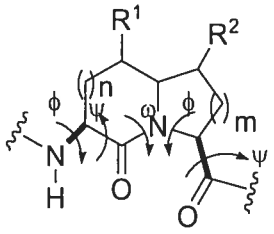
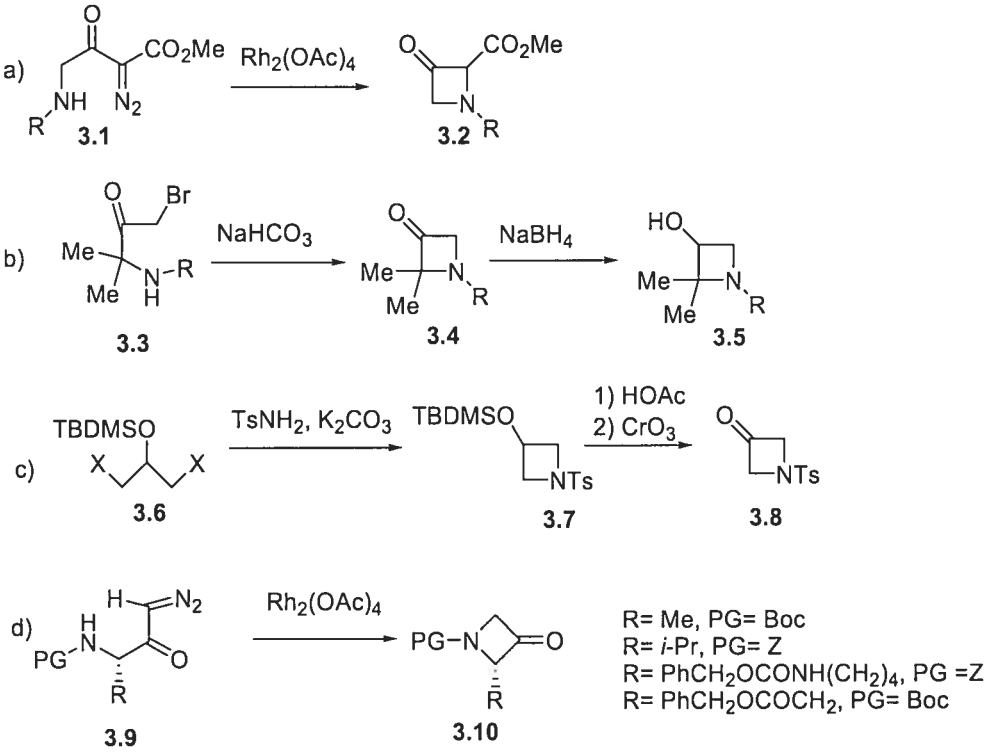


Figure 3.1: Dihedral angles constrained by an azabicyclo[X.Y.0]alkane amino acid in a peptide.

As components of β-lactams, fused azetidines represent one of the most important classes of antibiotics, and a large number of β-lactam-derivatives have been synthesized and tested for biological activity.[3] Due to increasing resistance of bacteria to popularly used antibiotics (*e.g.* penicillin), there is a need for new medications that show antibiotic activity. One approach is to use β-lactam derivatives which cannot be metabolized by the bacteria. In this respect, molecular modeling studies have proposed that the hybridization characteristics of the nitrogen in bicyclic azetidines such as fused γ-lactam-azetidine analogues can approximate the pyramidal distortions of the nitrogen atom observed in

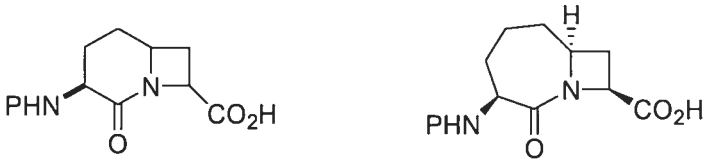
penicillin.[4] Azetidin-3-ones are non-hydrolyzable β-lactam isosteres which cannot be metabolized by bacteria and they are stable to lactam cleaving enzymes.[5]

To the best of our knowledge only a few synthetic routes to azetidin-3-ones have been published. The intramolecular carbene-insertion reaction was studied by Rapoport and co-workers using 4-amino-2-diazo-3-oxobutanoates **3.1** (Scheme 3.1, a).[6] Alternatively, ring closure was also achieved using 1-amino-3-bromopropan-2-one **3.3** in an intramolecular displacement in the presence of $NaHCO_3$ (Scheme 3.1, b).[7] Azetidin-3-ol **3.7** was synthesized using 1,3-dihalopropan-2-ol **3.6** and toluene-4-sulfonamide in 50-68% yield. Deprotection and oxidation of silylether **3.7** using CrO_3 provided the corresponding azetidin-3-ones **3.8** (Scheme 3.1, c).[8] Using amino acids as starting materials, a series of 2-substituted azetidin-3-ones **3.10** have been synthesized by application of metal carbenoid insertion reactions of diazoketones **3.9** (Scheme 3.1, d).[5,9]



Scheme 3.1: Reported synthesis of azetidin-3-ones

acid **3.12** and azabicyclo [5.2.0] alkane amino acid **3.13** (Figure 3.3). These fused azetidines are dipeptide surrogates that offer potential for developing new antibiotics.



azabicyclo [4.2.0]alkane amino acid **3.12**

azabicyclo [5.2.0] alkane amino acid **3.13**

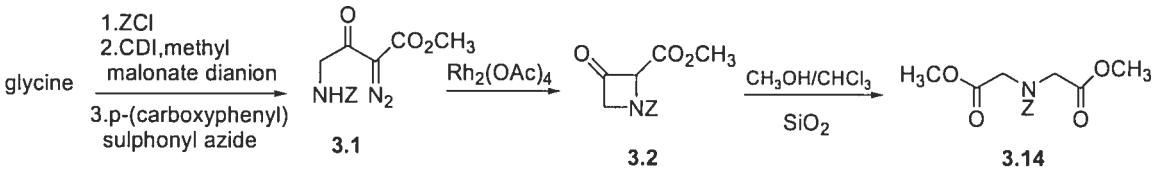
P= protecting group

Figure 3.3: Fused azetidine targets

3.2 RESULTS AND DISCUSSION

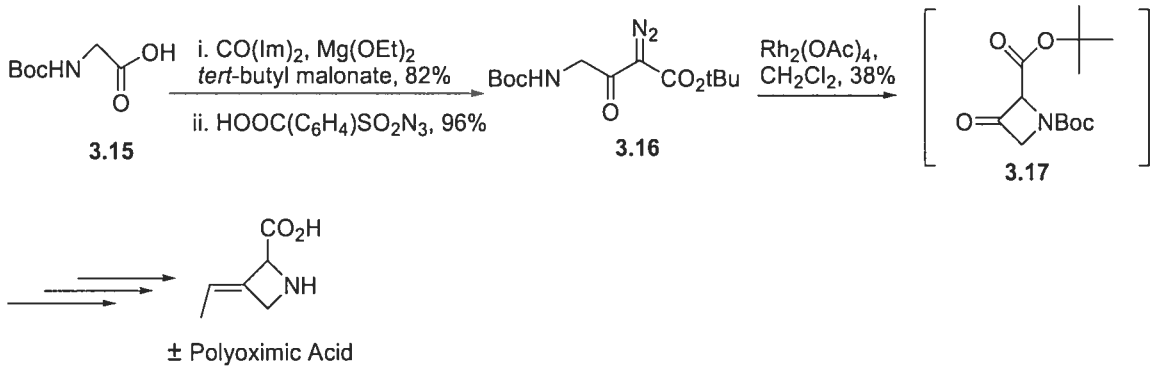
3.2.1 Study of the synthesis of 4-substituted azetidine-3-ones by carbenoid insertion into the γ-amino N-H bond of α-diazo β-diketone and β-ketoester substrates.

Metal carbene chemistry has been widely used as an efficient tool in organic chemistry. Although the rhodium catalyzed N-H insertion reactions of diazoketones have been frequently employed in heterocycle synthesis,[11] few examples of this intramolecular cyclization have been reported for forming azetidin-3-one rings. [6,11-14] For example, 2-carboxy-3-oxo azetidine **3.2** was prepared from glycine; however, its isolation by column chromatography using methanol in the eluent led to acid catalyzed ring opening and formation of diester **3.14** (Scheme 3.2).



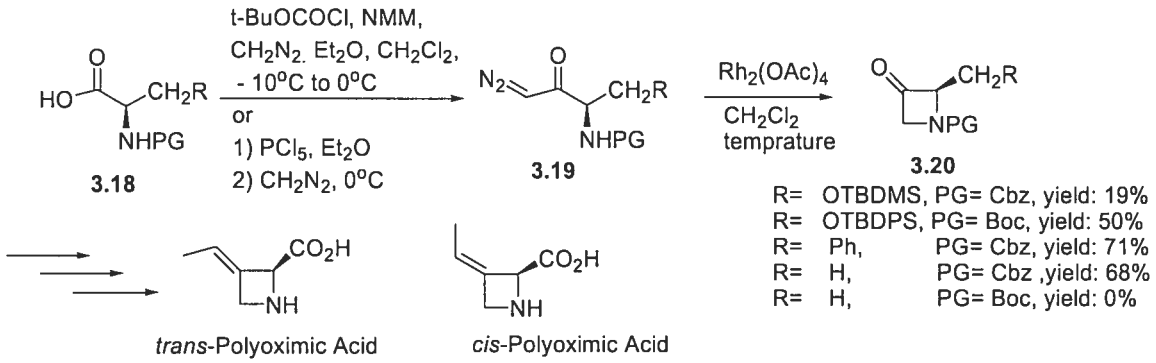
Scheme 3.2: Synthesis of 2-carboxy-3-oxoazetidine **3.2** from α-diazo β-ketoester. [6]

2-Substituted azetidin-3-ones have been synthesized and used in the total synthesis of racemic *trans*-polyoximic acid from *N*-Boc glycine as starting material by application of the carbenoid insertion to N-H bond methodology .[12]



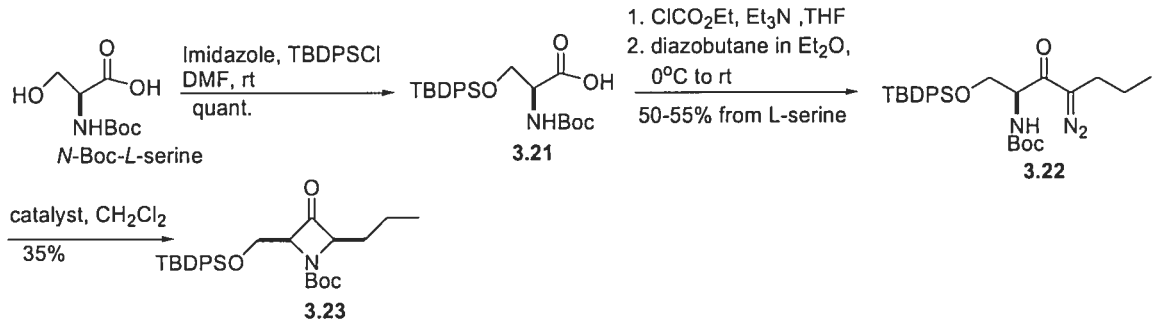
Scheme 3.3: Synthesis of racemic polyoximic acid using 2-substituted azetidinone **3.17**.[12]

α-Amino acids provided an excellent starting point for the synthesis of 2-substituted azetidine-3-ones **3.20** in enantiopure form. Application of serine in this method developed by Hanessian and co-workers led to the synthesis of polyoximic acid. [9,13]



Scheme 3.4: Synthesis of enantiopure azetidin-3-one **3.20** from *N*-protected D-serine **3.18**.[12,13]

Similarly, 2,4-dialkyl azetidin-3-one **3.23** has been synthesized from Boc-L-serine in 35% yield. The key step was based on a rhodium carbenoid N-H insertion of α'-dialkyl-α-diazoketone **3.22** (Scheme 3.5).[14]



Scheme 3.5: Synthesis of 2,4-dialkyl azetidine-3-one **3.23**. [14]

With this precedent in hand, carbenoid insertion into the γ-amino N-H bond was pursued using diazo β-diketone and α-diazo β-ketoester substrates.

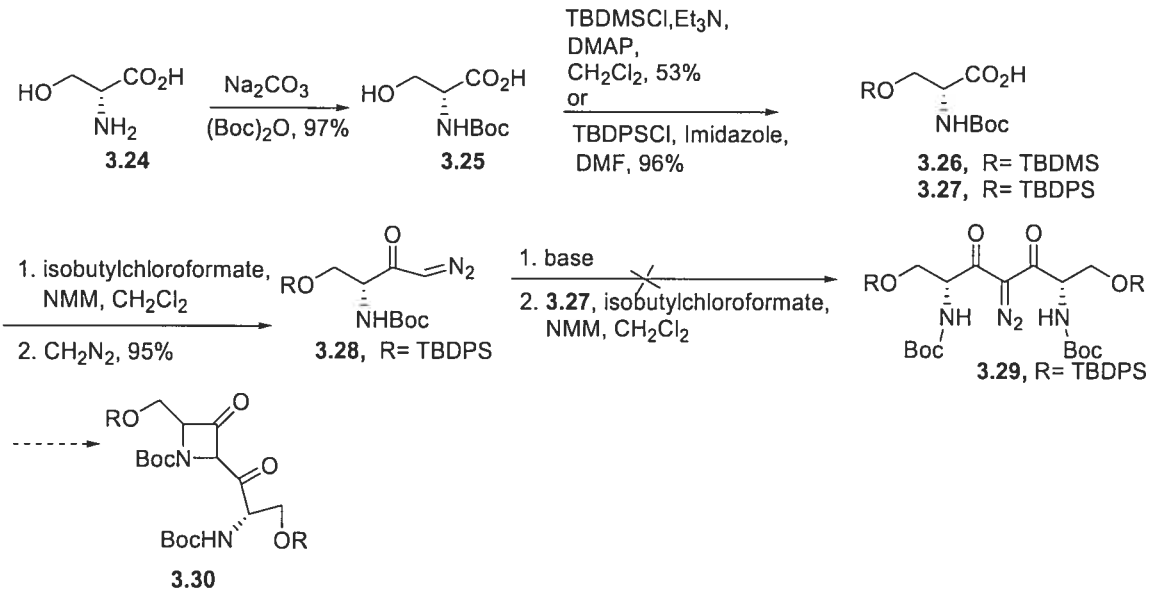
3.2.1.1 Attempted synthesis of 4-substituted azetidine-3-one 3.30 via diazo insertion:

As mentioned previously, 2,4-disubstituted azetidin-3-one **3.23** was synthesized from the acylation of diazobutane with the mixed anhydride derived from protected L-serine **3.21** , followed by metal carbenoid N-H insertion (Scheme 3.3).[14] Inspired by this result, we conceived the synthesis of 4-substituted azetidine-3-one **3.30** from the corresponding symmetric α-diazo dione **3.29**.

First, *N*-Boc-L-serine O-*tert*-butyldiphenylsilylether **3.27** was prepared from L-serine as previously reported.[11,12] Acylation of L-serine with $(Boc)_2O$ and Na_2CO_3 in 1:2 water:dioxane, followed by treatment with TBDPSCl in the presence of imidazole in DMF gave serine derivative **3.27** in 96% yield. Carboxylic acid **3.27** was activated with isobutylchloroformate and *N*-methylmorpholine (NMM) in dichloromethane and then reacted with an ethereal solution of diazomethane to provide α-diazoketones **3.28**. This diazoketone was purified by column chromatography and exhibited NMR and IR spectra that were consistent with the literature.[13] Attempts for the synthesis of α-diazo dione **3.29** by acylation of α-diazoketone **3.28** with the mixed anhydride derived from protected serine **3.27** using various bases (*n*-BuLi and diisopropylamine, Et_3N, NMM) in different solvents (THF, CH_2Cl_2) at different temperatures (rt, -20, -40, -78, -90 °C) did not meet with success and led to complicated mixtures which consisted of several products (Scheme 3.6).

In an earlier attempt, *N*-Boc-L-serine O-*tert*-butyldimethylsilylether **3.26** was prepared; however, as noted in the literature,[13] the TBDMS analogs were less stable than their TBDPS counterparts. For example, activation of acid **3.26** followed by acylation of

diazomethane and chromatography over silica gel (Scheme 3.6) led to the formation of multiple unidentified materials.

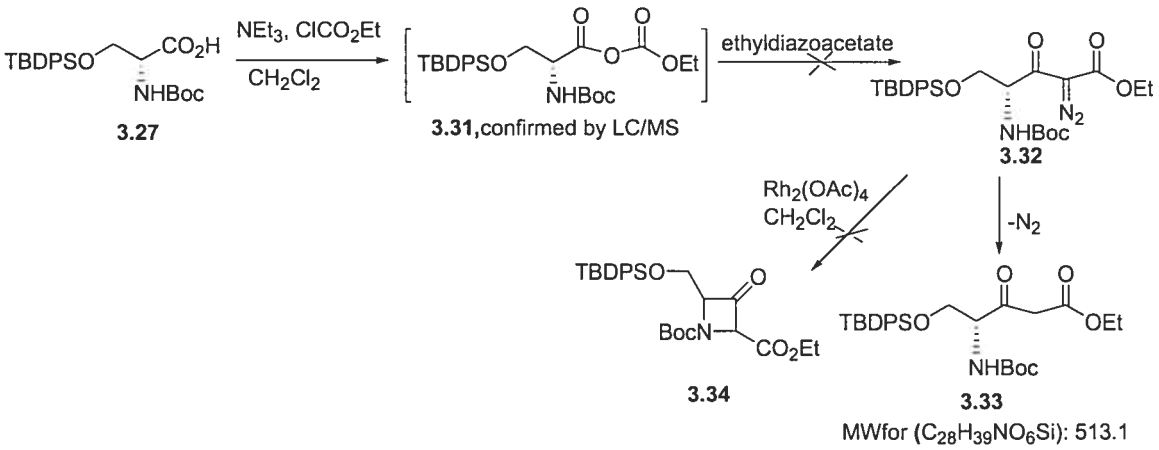


Scheme 3.6: Attempted synthesis of 4-substituted 3-azetidinone **3.30**.

3.2.1.2 Attempted synthesis of 4-substituted azetidine-3-one 3.34 via diazo insertion using ethyldiazoacetate:

In another effort, *N*-Boc-serine-O-silylether **3.27** was reacted with ethylchloroformate and a tertiary amine to afford a mixed anhydride intermediate **3.31**, which was directly reacted with ethyl diazoacetate. After purification by column chromatography, this reaction led to the formation of a side product that was studied by spectroscopic methods: ^{1}H NMR indicated 39 protons, LC/MS analysis indicated for $C_{28}H_{40}NO_6Si$ $(M+H)^+$ m/e:514.1, and in the IR spectrum, no signal in the desired region for N=N functional group was observed. Based on this evidence, the desired compound **3.32** was presumed to have lost N_2 during purification by column chromatography, and β-keto ester **3.33** was formed (Scheme 3.7).

In another attempt, the residue was directly used without purification (crude **3.32**) for the cyclizations step using $Rh_2(OAC)_4$ in CH_2Cl_2 but according to TLC and LC/MS analyses, several unidentified compounds were produced (Scheme 3.7).

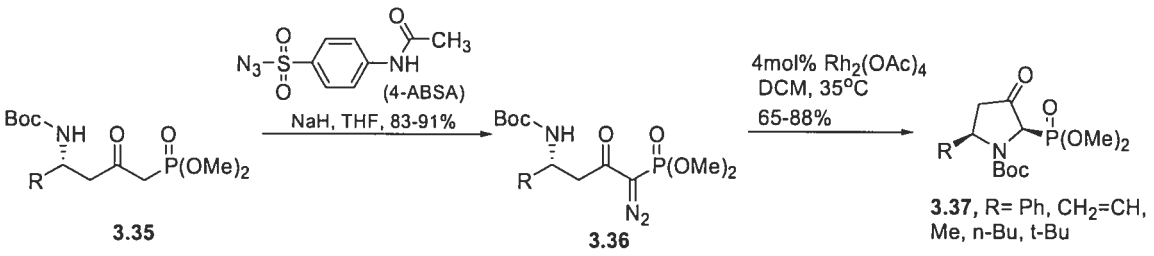


Scheme 3.7: Attempted synthesis of *N*-Boc 2,4-disubstituted azetidinone **3.34**.

In the light of the problems associated with the synthesis of α-diazo compounds **3.29** and **3.32** and their subsequent ring annulations, we began to study of an alternative approach to provide α-diazo keto compounds such as **3.40** for the synthesis of desired 3-azetidinone.

3.2.1.3 Synthesis of 4-substituted azetidine-3-one 3.43 by metal carbenoid N-H insertion on γ-amino β-ketophosphonate 3.39:

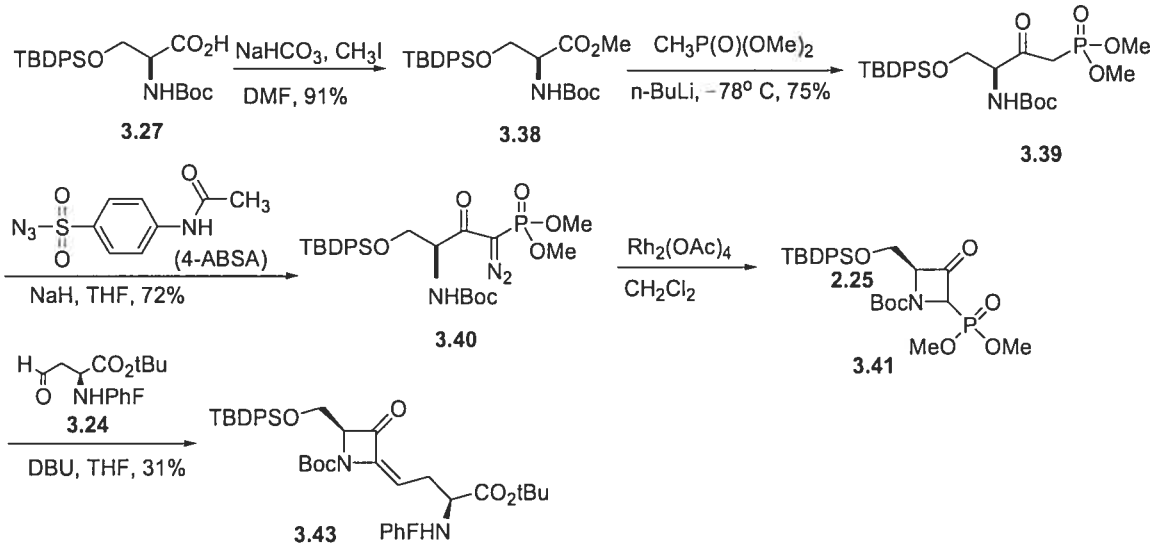
Intramolecular N-H insertion reactions have yielded 5-substituted pyrrolidine 2-phosphonate **3.37** from α-diazo β-keto phosphonate **3.36** (Scheme 3.8). [15]



Scheme 3.8: Synthesis of 3-oxo pyrrolidine phosphonate **3.37** from δ-amino α-diazo β-ketophosphonate **3.36**.

Inspired by this example for making 2,5-disubstituted pyrrolidines, we investigated a complimentary route to make 2,4-disubstituted azetidines. β-Keto α-diazo phosphonate **3.40** was derived from protected L-serine **3.38**. Esterification of *N*-(Boc)-serine-O-*tert*-butyldiphenyl silyl ether **3.27** with methyl iodide and sodium bicarbonate in DMF gave L-serine methyl ester **3.38** in 91% yield after chromatography. β-Ketophosphonate **3.39** was synthesized from methyl ester **3.38** by acylation of the lithium anion of dimethyl methyl phosphonate, produced with *n*-BuLi in THF. The diazo transfer to β-ketophosphonate **3.39** was accomplished using 4-acetamido benzene sulfonyl azide (4-ABSA) and sodium hydride as a base in THF to furnish α-diazo β-ketophosphonate **3.40** in 72% yield. Diazo derivative **3.40** was heated at reflux with $Rh_2(OAc)_4$ in CH_2Cl_2 to provide *N*-Boc azetidine-2-dimethoxy phosphonate **3.41**. Attempts to purify **3.41** by column chromatography were unsuccessful and the product decomposed; however, azetidine 2-phosphonate **3.41** could be directly condensed with aldehyde **3.42** in a Wittig reaction using DBU in THF to provide azetidine **3.43** in 31% yield from **3.22** (Scheme 3.9) as a mixture of two *cis* / *trans* olefin isomers (according LC/MS) in a 3:1 ratio as assessed by measurement of the triplet signals at δ 4.78 and 4.73 ppm in the ^{1}H NMR spectrum. The structure of 2,4-disubstituted **3.43** has been characterized by ^{13}C NMR, ^{1}H

NMR and HRMS analyses. The ^{13}C NMR spectrum showed three carbonyl groups, with a signal for ketone at δ 190.5 ppm, along with the carbonyl of Boc group at δ 152.3 ppm and carbonyl of the *t*-butylester at δ 174.6 ppm. The formation of a double bond was confirmed by ^{1}H NMR which showed the presence of the vinyl proton at δ 5.58 ppm for the major olefin isomer. Further structural confirmation of **3.43** was accomplished by HRMS calculated for $C_{52}H_{59}N_2O_6Si$ $(M+H)^+$ 835.41369, indicated the mass of 835.41484.



Scheme 3.9: Synthesis of 2,4-substituted azetidinone **3.43** via β-ketophosphonate **3.39.**

Elaboration of **3.43** toward the bicyclic azetidine analogue **3.12** via reduction of the carbonyl group of azetidinone **3.43** to the corresponding alcohol using Luche conditions or $NaBH_4$ or $NaCNBH_3$/HOAc, as well as the reduction of the double bond using Pd/C in MeOH gave only recovered starting material.

In conclusion, 2,4-disubstituted azetidin-3-one **3.43** was synthesized for the first time in 7 steps from L-serine via intramolecular metal carbenoid N-H insertion of α-diazo β-

ketophosphonate **3.40**. This method for the synthesis of substituted azetidin-3-ones may be useful for the preparation of other azetidine heterocycles.

3.2.2 Synthesis of 4-substituted Aze analogues by intramolecular S_N2' reaction:

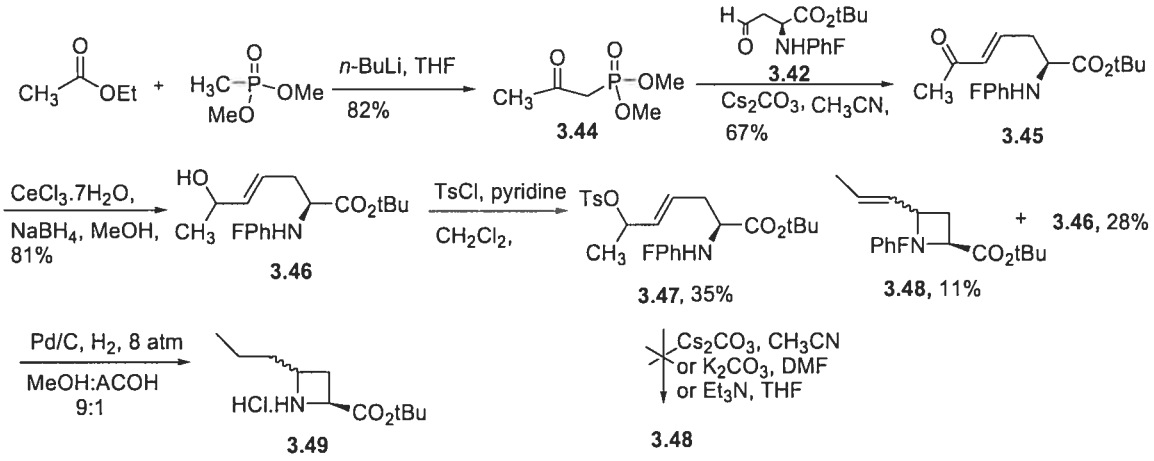
Although intramolecular rhodium catalyzed insertion of diazo ketones into N-H bonds did provide 4-substituted azetidine **3.43**, the development of more efficient methodology for making these challenging heterocycles was pursued and an alternative strategy was explored.

3.2.2.1 Synthesis of 4-propenyl Aze 3.48 from allylic alcohol 3.46 by S_N2' displacement:

To our knowledge, there was no precedent in the literature of using intramolecular cyclization via S_N2' reaction to form an azetidine ring, hence, a methodological study was carried out at first on simpler models to explore the appropriate conditions.

Allylic tosylate **3.47** was prepared as a model for exploring the S_N2' reaction. Treatment of ethyl acetate with the lithium anion of dimethylmethylphosphonate in THF, afforded α-keto phosphonate **3.44** after distillation (bp 76-79 ^{o}C/3 mmHg) in 82% yield. Aspartate β-aldehyde **3.42** was treated with **3.44** in the presence of Cs_2CO_3 in CH_3CN to provide β,γ-unsaturated ketone **3.45** in 67% yield. Reduction of ketone **3.45** with $CeCl_3.7H_2O$ and $NaBH_4$ in MeOH produced allylic alcohol **3.46** as a mixture of two diastereomers in a 4:1 ratio (confirmed by LC/MS) after purification by column chromatography. Activation of alcohol **3.46** to the corresponding tosylate **3.47** was performed using

toluenesulfonylchloride and pyridine in CH_2Cl_2. The products were purified by column chromatography using a gradient eluent of 3-10% EtOAc in hexanes and gave three fractions. The first fraction was an inseparable mixture of diastereomers and olefin isomers of azetidine **3.48** in 11% yield, and the second fraction was tosylate **3.47** in 35% yield as an inseparable mixture of diastereomers and olefin isomers. Also, alcohol **3.46** was recovered as third fraction in 28% yield after chromatography (Scheme 3.10). Attempts to prepare azetidine **3.48** from tosylate **3.47** using different bases (Cs_2CO_3, K_2CO_3, Et_3N) in different solvents (CH_3CN, DMF, THF) were unsuccessful and starting material was recovered.



Scheme 3.10: Synthesis of 4-propenyl Aze **3.48** from allylic alcohol **3.46**.

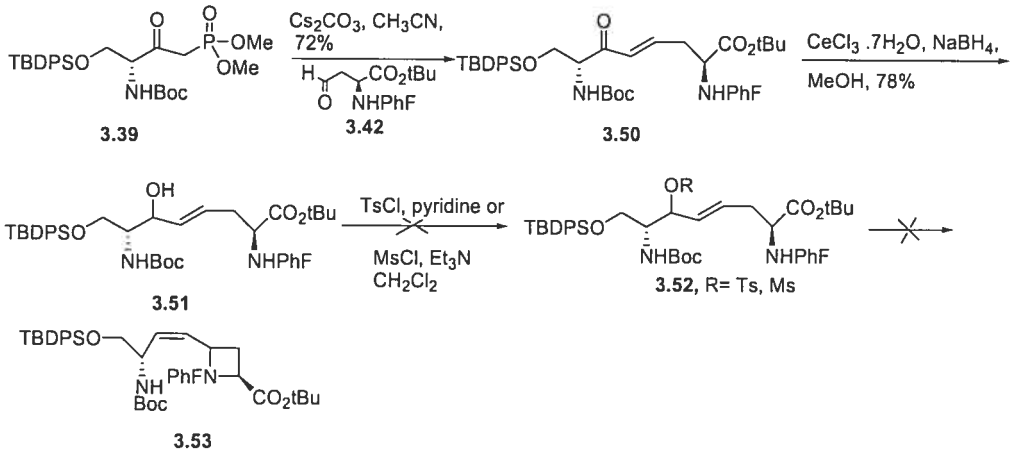
^{1}H NMR of azetidine **3.48**, showed a complicated spectrum that contained the mixture of two diastereomers as well as *Z* and *E* olefin isomers, which caused difficulties during spectral assignment.

For further evidence of the formation of propenyl azetidine **3.48**, hydrogenation was performed at 8 atm using palladium-on-carbon as catalyst in 9:1 MeOH:AcOH. This caused double bond reduction and cleavage of the phenylfluorenyl group. 4-Propyl

azetidine **3.49** was purified by HPLC which provided a mixture of two inseparable diastereomers of 4- propyl Aze **3.49** in a 3:2 ratio as assessed by the measurement of the diastereomeric multiplet signals at δ 3.51 and 3.33 ppm in the ^{1}H NMR spectrum. The exact mass of 4-propyl Aze **3.49** was confirmed by HRMS. (calcd. 200.16451 for $C_{11}H_{22}NO_2$ $(M+H)^+$, found 200.16358).

3.2.2.2 Attempted synthesis of 4-substituted Aze 3.53 from allylic alcohol 3.51 by S_N2' reaction:

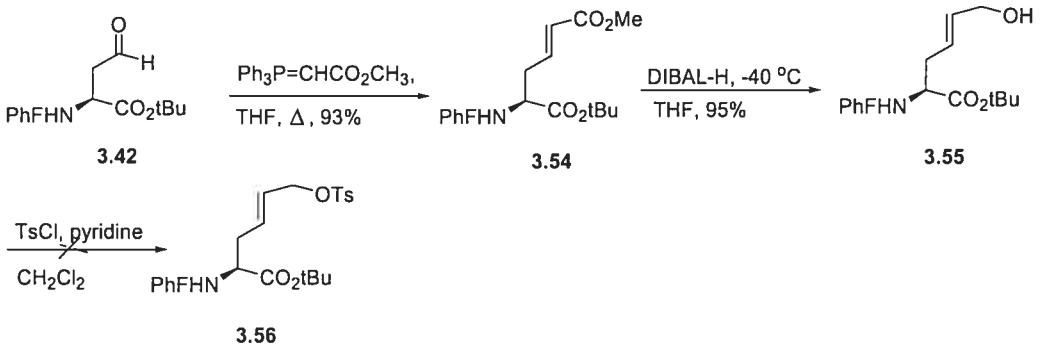
To further probe the intramolecular S_N2' methodology, allylic alcohol **3.51** was prepared from β-keto phosphonate **3.39**. For the synthesis of 4-substituted Aze **3.53**, activation of alcohol **3.51** followed by cyclizations via S_N2' reaction was examined. The Wittig reaction of aldehyde **3.42** with serine-derived β-keto phosphonate **3.39** using Cs_2CO_3 in CH_3CN gave α,β-unsaturated ketone **3.50** (72% yield). Reduction of **3.50** using $CeCl_3.7H_2O$ and $NaBH_4$ in MeOH gave secondary alcohol **3.51** as a mixture of diastereomers (78% yield), which were separated by column chromatography. The structure of secondary alcohol **3.51** was established by ^{1}H NMR and ^{13}C NMR. The formation of the *E*-isomer was confirmed by proton NMR which showed a vicinal coupling constant of 15.7 Hz for the vinyl protons. Attempts to activate alcohol **3.51** as the corresponding tosylate and mesylate, using toluenesulfonyl chloride and pyridine as base in CH_2Cl_2 or methanesulfonyl chloride and Et_3N as base in CH_2Cl_2 respectively, were however unsuccessful and only starting material was recovered (Scheme 3.11).



Scheme 3.11: Attempted synthesis of 4-substituted Aze **3.53** starting from serine phosphonate **3.39.**

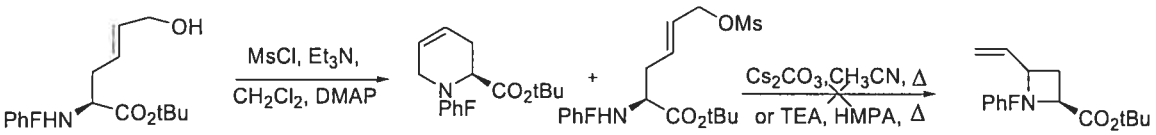
3.2.2.3 Attempted synthesis of 4-vinyl Aze 3.59 from allylic alcohol 3.55:

Preparation of 4-substituted Aze **3.59** was examined by the synthesis of allylic mesylate **3.57**. Dehydro amino adipate **3.54** was made as previously described in reference 16. Reduction of the ω-ester of diester **3.54** using DIBAL-H in THF at -40 °C provided the corresponding alcohol **3.55** in 95% yield, after purification by chromatography. Attempts at activation of alcohol **3.55** to the corresponding tosylate using toluene chloride and pyridine in CH_2Cl_2 were, however, unsuccessful and starting material was recovered (Scheme 3.12).



Scheme 3.12: Attempted synthesis of the tosylate derivative of alcohol **3.55.**

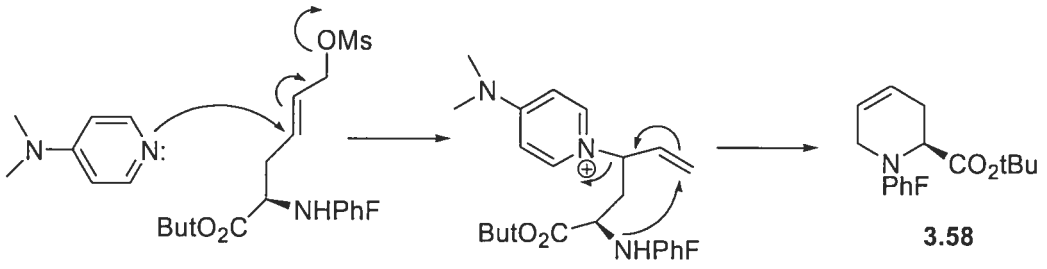
Alcohol **3.55** was thus converted to the corresponding mesylate **3.57** by using methanesulfonyl chloride with Et_3N as base and DMAP as catalyst in dichloromethane (DCM). Under these conditions, mesylate **3.57** and pipecolate **3.58** were obtained in 43% and 38% respective yields after separation by column chromatography. The next issue was the synthesis of azetidine **3.59.** Attempts to prepare azetidine **3.59** from mesylate **3.57** using Cs_2CO_3 in acetonitrile were unsuccessful and starting material was recovered. Employing triethylamine and HMPA at reflux led to the formation of several unidentified compounds (Scheme 3.13).



3.55 **3.58, 38%** **3.57, 43%** **3.59**

Scheme 3.13: Synthesis of pipecolate **3.58** from allyl alcohol **3.55** on route to mesylate **3.57.**

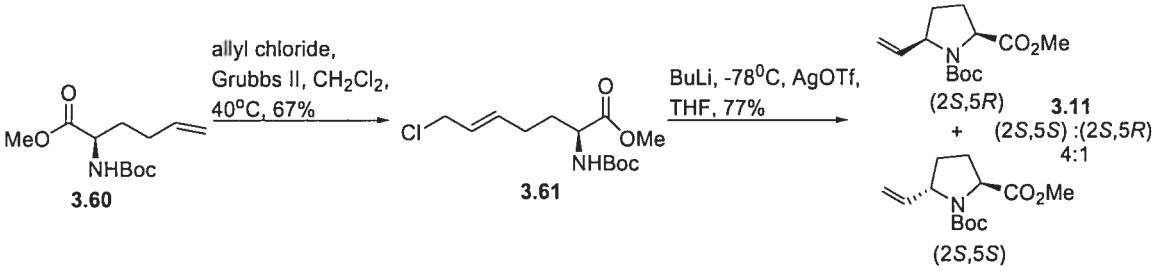
The structure of pipecolate **3.58** was assigned using NMR spectroscopy. In particular, the ^{13}C-DEPT spectrum of **3.58** in chloroform indicated the presence of two methylene groups at δ 38.76 and 45.45 ppm, and there was no methylene carbon in the vinyl region which was consistent with the formation of pipecolate **3.58** instead of azetidine **3.59**. Azetidine **3.59** was not detected in these experiments. A mechanism for the formation of pipecolate **3.58** may involve S_N2' like nucleophilic attack of the DMAP to the vinylic carbon followed by displacement of the mesylate group. The intramolecular *N*-alkylation of the corresponding intermediate via an alternative S_N2' of gave pipecolate **3.85** (Scheme 3.14).



Scheme 3.14: Possible mechanism for the intramolecular *N*-alkylation leading to pipecolate **3.58**.

3.2.2.4 Synthesis of 4-vinyl Aze 3.65 via S_N2' reaction of allylic chloride 3.64:

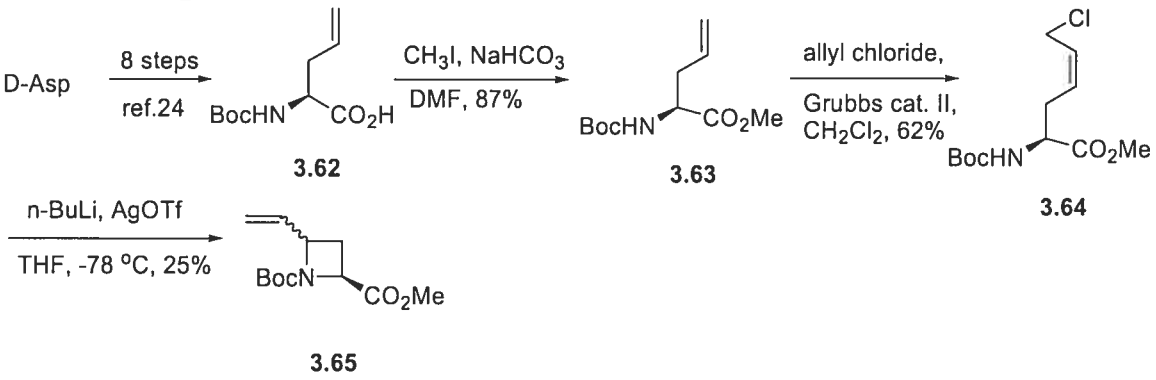
The S_N2' intramolecular *N*-alkylation to synthesize piperidines, pyrrolidines and 1,3-disubstituted 1,2,3,4-tetrahydroquinolines, has been performed with various reagents and catalysts, including complexes of Pd(0)[17] and Pd(II),[18] and Ag(I) salts.[19] For example, a 4:1 mixture of *cis*- and *trans*-2-carboxy-5-vinylpyrrolidines was synthesized in 77% yield using Ag(I) to carry out the intramolecular S_N2' *N*-alkylation[10] of 2-*tert*-butoxycarbonyloxy-7-chlorohept-5-enoic acid methyl ester **3.61** (Scheme 3.15).



Scheme 3.15: Synthesis of 2–carboxy-5-vinylpyrrolidine **3.11** using AgOTf in S_N2' alkylation.[10]

The extension of this methodology was thus pursued for the synthesis of 4-vinyl Aze. *N*-(Boc)allylglycine **3.62** was prepared according to the literature protocol,[20,19] and

esterified with CH_3I and sodium bicarbonate in DMF to afford methyl allylglycinate **3.63** as a clear oil in 87% yield (Scheme 3.16).



Scheme 3.16: Synthesis of vinyl azetidine **3.65**.

For the synthesis of azetidine **3.65**, our next objective was the introduction of the appropriate leaving group and subsequent intramolecular *N*-alkylation. Reaction of olefin **3.63** with a solution of allyl chloride and Grubbs second generation catalyst in CH_2Cl_2 gave allyl chloride **3.64** in 62% yields. Formation of the vinyl azetidine **3.65** occurred in low yield (25%) on reaction of allyl chloride **3.64** with silver triflate and *n*-BuLi in THF (Scheme 3.16). ^{1}H NMR of vinyl azetidine **3.65** showed the presence of two diastereomers and two amide rotamers in the vinylic region that caused difficulties during spectral assignment. ^{1}H NMR temperature studies of vinyl azetidine **3.65** in $CHCl_3$ at 25 °C in the vinylic region exhibited two *trans* and two *cis* protons in a 5.7:1 ratio as assessed by measurement of the *cis* doublet signals at δ 5.38 and 5.18 ppm. Also, ^{1}H NMR examination of **3.65** in $CHCl_3$ at -55 °C resulted in an increase of this ratio to 2.9:1 due to restricted rotation about the Boc group (Figure 3.4). Moreover, it was noted that the singlet *tert*-butyl group was particularly broad (δ 1.45-1.50 ppm at 25 °C and 1.39-

1.52 ppm at -55 °C) which suggested the presence of mixtures of diastereomers and carbamate *cis*- and *trans*-rotamers. The formation of vinyl azetidine **3.65** was confirmed by ^{13}C NMR and HRMS, in which a ^{13}C NMR study showed the presence of vinylic methylene groups between δ 113.2-117.3 ppm and HRMS indicated the mass of 242.10159 for $C_{12}H_{20}NO_4$ $(M+H)^+$.

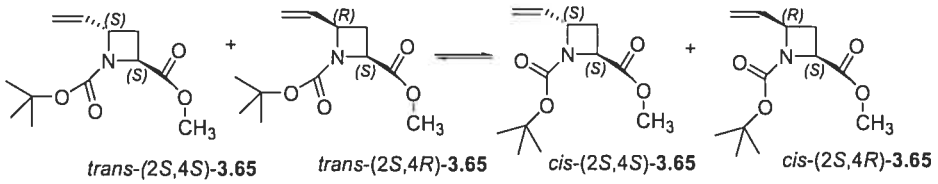


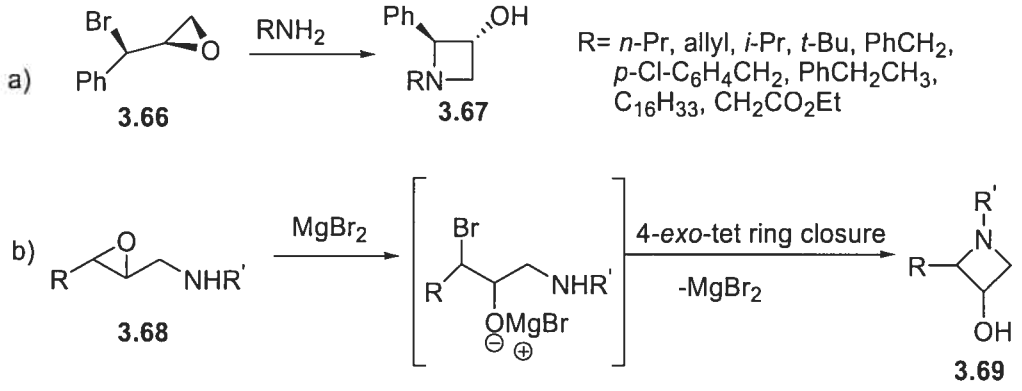
Figure 3.4: Suggested diastereomers and the carbamate *cis* and *trans*-isomers of *N*-Boc 4-vinyl azetidine 2-methyl ester **3.65**.

To further characterize azetidine **3.65**, attempts were made to remove the Boc group; however, decomposition of the azetidine ring was observed by both TLC and LC/MS analysis, when employing protic acids such as TFA in CH_2Cl_2, 4M HCl in dioxane, and HCl (g) in CH_2Cl_2. When a Lewis acid was employed, only starting material was recovered on treatment of azetidine **3.65** with $ZnBr_2$ in CH_2Cl_2 to remove the Boc group.[22] Furthermore, attempts to elaborate 4-vinyl azetidine **3.65** by transformations of the olefin such as conversion to the corresponding primary alcohol using hydroboration-oxidation, or saturation by hydrogenation using Pd/C in MeOH:HOAc at 4 atm, as well as the transfer the methyl ester to the corresponding ethyl ester using a solution of sodium ethoxide 0.04 M in ethanol, all failed.

In summary, a mixture of two diastereomers of 4-vinyl Aze **3.65** was synthesized in 11 steps from aspartic acid by application of allylglycine methyl ester **3.63** in a cross-metathesis / intramolecular S_N2' *N*-alkylation strategy.

3.2.3 Attempts to synthesize of 4-substituted Aze 3.71 via intramolecular nucleophilic ring opening of epoxide 3.70:

Little is known about the formation of azetidine rings by the nucleophilic ring opening of epoxides with amine nucleophiles. Although, several (*R*,*S*)-1-alkyl-3-hydroxy-2-phenylazetidines **3.67** were synthesized by reaction of (*R*,*R*)-2-(1-bromobenzyl) oxirane **3.66** with aliphatic primary amines (Scheme 3.17 a).[23] 3-Hydroxy azetidine **3.69** has also been made by an *exo-tet* type cyclization and double inversion in two sequential S_N2 reactions on epoxyamine **3.68** (Scheme 3.17 b).[24]



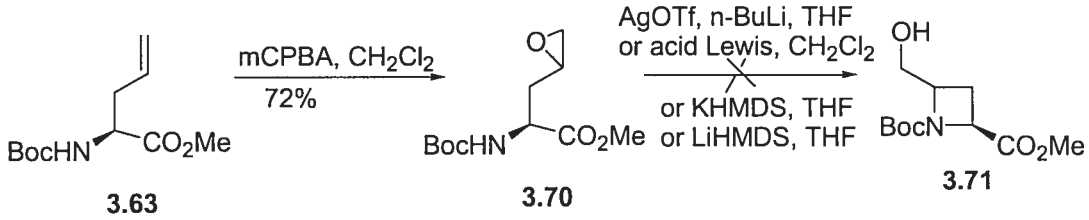
Scheme 3.17: Synthesis of azetidine ring from epoxide.

High ring strain makes epoxides reactive to nucleophilic attack.[25,26] We envisioned that a method based on intramolecular ring opening of the epoxide by an amine or amide could be used to prepare 4-substituted Aze. The synthesis of epoxide **3.70** and **3.74** from L-aspartic acid was thus explored in another attempt at azetidine ring formation.

3.2.3.1 Synthesis of epoxide 3.70 from L-aspartic acid:

Methyl allylglycinate **3.63** was synthesized as described in section 2.2.3 and reacted with meta-chloroperbenzoic acid (*m*CPBA) in CH_2Cl_2 to give epoxides **3.70** as a 4:1 mixture of two diastereomers (according to LC/MS) in 72% yield after purification by column chromatography.

With epoxides **3.70** in hand, we tried different conditions for intramolecular nucleophilic attack using Lewis acids, such as AgOTf, CuI, $Cu(OTf)_2$, and $TiCl_4$, without base or by employing various bases (*n*-BuLi in THF, NaHMDS or LiHMDS in THF); formation of azetidine **3.71** was never observed, and in all cases starting material was recovered according to TLC and LC/MS analysis (Scheme 3.18).

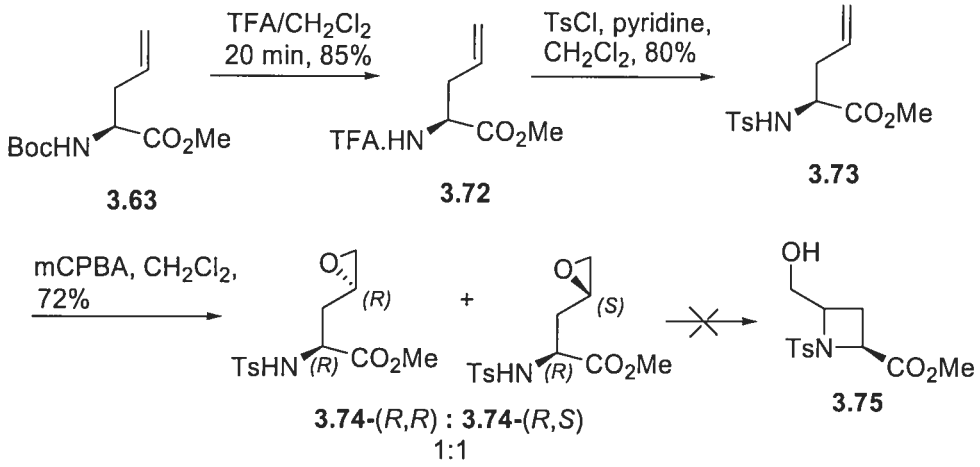


Scheme 3.18: Attempts to synthesize 4-substituted Aze **3.71** from epoxide **3.70**.

3.2.3.2 Synthesis of epoxide 3.74 from L-aspartic acid

The poor reactivity of the Boc protected nitrogen may be due to resonance with the carbonyl of the carbamate reducing the nucleophilicity of the nitrogen. Replacement of the Boc protecting group with a toluenesulfonamide was examined in order to increase the nucleophilicity of the nitrogen and favor *N*-alkylation. Deprotection of *N*-Boc allylglycine methyl ester **3.63** using 50:50 TFA/CH_2Cl_2 for 20 min provided allyglycine methyl ester **3.72** in 85% yield. Protection of the nitrogen using *p*-toluene sulfonylchloride and pyridine as base in CH_2Cl_2 gave *N*-toluenesulfonyl allylglycine **3.73**

in 80% yield after purification by column chromatography. Epoxidation of *N*-toluenesulfonyl glycine **3.74** was accomplished using *m*-chloroperbenzoic acid (*m*CPBA) in CH_2Cl_2 to produce *N*-toluenesulfonyl glycine epoxide **3.74** in 72% yield after purification as a mixture of two diastereomers in a 1:1 ratio (Scheme 3.19), as assessed by measurement of the diastereomeric methyl ester singlets at δ 3.55 and 3.57 ppm in the ^{1}H NMR spectrum.



Scheme 3.19: Synthesis of epoxide **3.74** from olefin **3.63**.

Different conditions were explored to favour the formation of azetidine ring **3.75** via intramolecular nucleophilic attack and ring opening of epoxide **3.74**. Attempts to prepare azetidine ring **3.75** using AgOTf as Lewis acid with *n*-BuLi in THF failed. Also employing other Lewis acid such as CuI, $Cu(OTf)_2$, $TiCl_4$ without base in CH_2Cl_2 only gave starting material. Similarly, only starting material was recovered after treatment of epoxide **3.74** with KHMDS and 18-crown-6 ether in THF. Employing two equivalents of either KHMDS or LiHMDS in THF led to the formation of an undesired compound which was analyzed after purification of the crude by column chromatography by LC/MS and ^{1}H NMR. According to these spectral data, *p*-toluenesulfinic acid was formed due to

the deprotonation of C-α in the epoxide **3.74** by LiHMDS or KHMDS followed by cleavage of the N-S bond.

3.3 Conclusion

In the context of our research on general methods for making 4-substituted Aze analogues, three different methodologies were pursued.

We have developed a new process for synthesizing 2,4-disubstituted azetidin-3-one **3.43** by employing intramolecular metal carbenoid N-H insertion of α-diazo β-ketophosphonate **3.40** in 7 steps from L-serine. This method for the synthesis of substituted azetidin-3-ones may be useful for the preparation of other azetidine heterocycles.

In an investigation of the synthesis of 4-substituted Aze analogues via S_N2' approach, a mixture of 4-propenyl *N*-(PhF)Aze **3.48** was synthesized by the Horner-Wadsworth-Emmons (HWE) olefination of α-*tert*-butyl-*N*-(PhF)aspartate β-aldehyde **3.42** with β-keto phosphonate **3.44**. Reduction of the resulting α,β-unsaturated ketone **3.45** followed by activation of the corresponding alcohol **3.46** as the tosylate derivative **3.47** gave a diastereomeric mixture of propenyl Aze **3.48** in low yield.

In an attempt to synthesize of 4-vinyl *N*-(PhF) Aze **3.59** via S_N2' strategy starting from dehydro amino adipate **3.54**, the novel pipecolate **3.58** was formed instead of the 4-substituted azetidine **3.59**. One possible mechanism involves a S_N2' like nucleophilic attack of the DMAP to the vinylic carbon of mesylate derivative **3.57** followed by mesylate group displacement. Subsequent intramolecular *N*-alkylation of corresponding intermediate via an alternative S_N2' reaction gave pipecolate **3.58**.

Also, a mixture of 4-vinyl Aze analogue **3.65** was synthesized from L-Asp in 11 steps in low yield using Ag(I) to effect the intramolecular S_N2' *N*-alkylation. 4-vinyl Aze analogue **3.65** can serve as a precursor for the preparation of fused azabicyclo[5.2.0]alkane amino acid **3.13**, a novel β-turn mimic.

Although an investigation failed to produce 4-substituted Aze by intramolecular ring opening of an epoxide by nucleophilic attack of a β-amine, new amino epoxides **3.70**, **3.74** were synthesized for the first time from L-Asp as an inexpensive chiral educt. These amino epoxides may serve as intermediates for a variety of highly functionalized compounds.

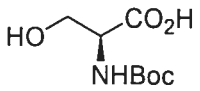
3.4 Experimental procedure

3.4.1 General Information

Unless otherwise noted, all reactions were run under nitrogen atmosphere and distilled solvents were transferred by syringe. Anhydrous tetrahydrofuran (THF), dichloromethane (DCM), acetonitrile (CH_3CN) and dimethylformaide (DMF) were obtained from solvent filtration systems. Triethylamine (Et_3N) and pyridine were distilled from ninhydrin and CaH_2. Final reaction mixture solutions were dried over Na_2SO_4 or $MgSO_4$ and rotary-evaporated under vacuum. Melting points are uncorrected. Mass spectral data, HRMS/ESMS, were obtained by the Université de Montréal Mass Spectrometry facility. Unless otherwise noted, ^1H NMR (300/400 MHz) and ^{13}C NMR (75/100 MHz) spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm (δ units) downfield of internal tetramethylsilane ($(CH_3)_4Si$) or residual solvent ($CHCl_3$ and MeOH). Coupling constants are reported in hertz (Hz). Chemical shifts of aromatic and vinylic carbons are not reported for the ^{13}C NMR spectra of PhF containing compounds. Analytical thin-layer chromatography (TLC) was performed using aluminum-backed silica plates coated with a 0.2 mm thickness of silica gel 60 F_{254} (Merck KGaA Germany). Chromatography was performed using Kieselgel 60 (230-400 mesh).

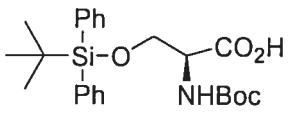
3.4.2 Study of Carbenoid insertion into the γ-amino NH bond using diazo β-diketone and α-diazo β-ketoester substrates

(2*S*)-*N*-Boc serine 3.25:



A solution of serine **3.24** (1.05 g, 10 mmol, 100 mol%) in a mixture of dioxane (20 mL) and water (10 mL) was treated with a solution of Na_2CO_3 (1.16 g, 11 mmol, 110 mol%) in 10 mL of water, cooled in ice-water bath and treated with di-*tert*-butyl pyrocarbonate (2.4 g, 11 mmol, 110 mol%). The bath was removed, and stirring was continued at room temperature for 4.5 h. The solution was concentrated in *vacuo* to about 10-15 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (30 mL) and acidified with a dilute solution of $KHSO_4$ to pH 2-3. The aqueous phase was extracted with ethyl acetate (3 x 10 mL). The combined organic phase was washed with water (2 x 30 mL), dried over anhydrous Na_2SO_4 and evaporated to give *N*-(Boc)-serine **3.25** as a solid (1.90 g Yield 97%). mp 90-91 °C , lit. 91 °C; $[\alpha]^{20}_D$ -3.13 (*c* 2, CH_3CO_2H), lit.[27] $[\alpha]^{20}_D$ -3.5 ± 0.5° (*c* 2, CH_3CO_2H).

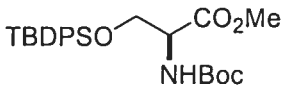
(2*S*)-2-*tert*-Butoxycarbonylamino-3-(tert-butyl-diphenyl-silanyloxy)-propionic Acid 3.27:



A solution of *N*-Boc serine **3.25** (1.5 g, 7.3 mmol, 100 mol%) in 1.8 mL of DMF at room temperature was treated with TBDPSCl (2.4 g, 8.76 mmol, 120 mol%) and imidazole (1.24 g, 18.25 mmol, 250 mol%), stirred for 36 h, treated with water (20 mL) and

extracted with ethyl acetate (3 x 100 mL), the organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was purified by chromatography eluting with 30-60% EtOAc in hexanes as eluent. Evaporation of the collected fractions provided silyl ether **3.10** as a solid (3.10 g, 96 % yield). mp 150-152 °C $[\alpha]^{20}_D$ -13.4 ° (*c*1.02, $CHCl_3$) for *D*-serine lit,[12] mp 147-149 °C $[\alpha]^{20}_D$ +12.3° (*c* 0.18, $CHCl_3$) for *L*-serine; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (s, 9H); 1.34 (s, 9 H); 3.77 (d, *J* = 3.8 Hz, 1H); 4.01 (d, *J* = 9, 5 Hz, 1H); 4.32 (d, *J* = 11.4 Hz, 1H); 5.30 (d, *J* = 11.5 Hz, 1H); 7.29-7.56 (m, 10 H). ESMS For $C_{24}H_{33}NO_5Si$ $(M)^+$ 443.2 HRMS calcd. For $C_{24}H_{34}NO_5Si$ $(M+H)^+$ 444.22008, found 444.22031.

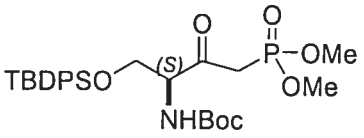
(2*S*)-2-*tert*-Butoxycarbonylamino-3-(*tert*-butyl-diphenyl-silanyloxy)-propionic Acid Methyl Ester 3.38:



To a suspension of protected amino acid **3.27** (443.0mg, 1mmol, 100 mol%) and sodium hydrogen carbonate (168 mg, 2mmol, 200mol%) in dimethylformamide (DMF, 5 mL), methyl iodide (705 mg, 5 mmol, 500 mol%) in 5 mL of DMF was added at room temperature. The mixture was allowed to react for 24 h, then water (2 mL) was added and the mixture was extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with water, dried with Na_2SO_4, evaporated to a small volume, and finally the product was purified by column chromatography using 10:90 ethyl acetate in hexanes as eluent. Evaporation of the collected fractions provided an oil (435 mg, 91% yield) of methyl ester **3.38**: $[\alpha]^{20}_D$ +15.66° (*c* 0.44, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.01 (s, 9H); 1.43 (s, 9 H); 3.69 (s, 3H); 3.86 (dd, *J* = 9.9, 7.1 Hz, 1H); 4.04 (d, *J* = 9.9 Hz, 1H);

4.37 (m, 1H); 7.31-7.57 (m, 10 H). ^{13}C NMR δ 14.2; 21.1; 29.4; 53.1; 61.9; 64.7; 74.9; (127.9-135.6); 158.2; 179.4. HRMS calcd. For $C_{25}H_{36}NO_5Si$ $(M+H)^+$ 458.23573, found 458.23586.

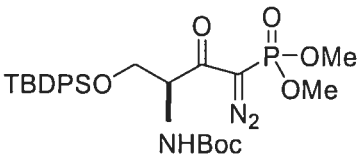
(3*S*)-[3-*tert*-Butoxycarbonylamino-4-(*tert*-butyl-diphenyl-silanyloxy)-2-oxo-butyl]-Phosphonic Acid Dimethyl Ester 3.39:



In a 50 mL round-bottom flask equipped with a magnetic stirring bar and rubber septum, under argon, a solution of dimethyl methyl phosphonate (569.2 mg, 4.6 mmol, 500 mol%) in THF (13 mL) was placed. The mixture was cooled to -78 °C and treated by syringe with *n*-BuLi (2.23 mL, 4.5 mmol, 500 mol% solution in cyclohexane). After 15 min the mixture was transferred to a 50 mL round bottom flask containing a -78°C solution of methyl ester **3.38** (435 mg, 0.92 mmol, 100 mol%) in THF (16 mL). After stirring at -78°C for 2 h, the solution was quenched by addition of saturated NH_4Cl (4 mL), warmed to room temperature, diluted with H_2O (1.5 mL) and extracted with Et_2O (10 mL) and EtOAc (2 x 10 mL). The combined organic phases were washed with brine (5 mL), dried ($MgSO_4$) and concentrated. The residue was dissolved in $CHCl_3$ (10 mL), filtered, and concentrated to give a yellow oil, that was purified by column chromatography over silica gel using 80-100% EtOAc in hexanes as eluant. Evaporation of the collected fractions provided an oil (374 mg, 75% yield) of beta-keto phosphonate **3.39**: $[\alpha]^{20}_D$ +35.8° (*c* 0.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.96 (s, 9H); 1.36 (s, 9 H); 3.67 (d, J = 10.5 Hz, 6H); 3.86 (dd, J = 10.6, 3.7 Hz, 2H); 4.02 (m, 2H);

4.46(m, 1H); 7.29-7.56 (m, 10 H). ^{13}C NMR δ 14.2; 19.3; 26.8; 28.4; 53.1 ; 61.9; 63.7; 79.9; (127.9-135.6); 155.2; 199.4 HRMS calcd. For $C_{27}H_{41}NO_7PSi$ $(M+H)^+$ 550.23844, found 550.23864.

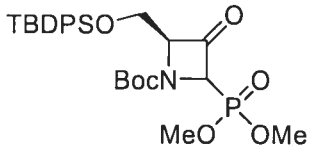
(3*S*)-[3-*tert*-Butoxycarbonylamino-4-(*tert*-butyl-diphenyl-silanyloxy)-1-diazo-2-oxo-butyl]-Phosphonic Acid Dimethyl Ester 3.40:



In a 50 mL round bottom flask equipped with a magnetic stirring bar, under argon, NaH (60% in mineral oil, 48.4 mg, 1.27 mmol) was washed with petroleum ether (2 x 5 mL). After removal of the petroleum ether by syringe, the flask was placed under vacuum for 2 min before THF (5.3 mL) was introduced by syringe. A solution of beta-keto phosphonate **3.39** (335 mg, 0.609 mmol) and 4-acetamidobenzenesulfonyl azide (4-ABSA) (144.8 mg, 0.602 mmol) in 13.5 mL THF was transferred via *cannula* quickly at room temperature to the round-bottom flask containing the NaH in THF. After stirring for 1.5 h, the reaction mixture was quenched by the addition of saturated aqueous NH_4Cl (2.6 mL), and extracted with Et_2O (20 mL) and EtOAc (2 x 20 mL). The combined organic phases were washed with brine (5 mL), dried and concentrated. The residue was purified by flash column chromatography over silica gel by using 35-50% EtOAc in hexanes as eluent. Evaporation of the collected fractions provided α -diazo phosphonate **3.40** as an oil (240 mg, 72% yield): $[\alpha]^{20}_D$ +20.97° (*c* 0.12, $CHCl_3$); IR ($CHCl_3$) ν_{max} (cm^{-1}) 2957, 2118.52 (=N_2); 1715, 1661, 1269 (P=O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.03

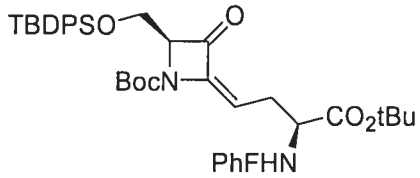
(s, 9H); 1.42 (s, 9 H); 3.70 (dd, J = 25.8, 11.9 Hz, 6H); 3.87 (m, 2H); 4.74 (m, 1H); 5.44 (d, J = 8.3 Hz, 1H); 7.35-7.59 (m, 10 H). ^{13}C NMR δ 19.4; 26.9; 28.5; 54.0 ; 57.66; 64.0; 80.2; (128.0-135.7); 155.3; 190.3 HRMS calcd. For $C_{27}H_{39}N_3O_7PSi$ $(M+H)^+$ 576.22894, found 576.22841.

(2*S*)-*N*-(Boc)-2-(*tert*-Butyl-diphenyl-silanyoxymethyl)-4-(dimethoxy-phosphonyl)-3-Azetidinone 3.41:



In a 25mL round bottom flask equipped with a magnetic stirring bar and reflux condenser, under argon, was added α-diazo phosphonate **3.39** (70 mg, 0.121 mmol) and rhodium (II) acetate dimmer (2.2 mg, 0.004 mmol) in CH_2Cl_2 (3.5 mL). The mixture was stirred for 48 h at 35°C. The solvent was evaporated. The residue was treated with H_2O (4 mL) and extracted into EtOAc (2 x 5 mL). The combined organic phase was washed with brine (5 mL), dried, and concentrated to yellow oil 46 mg of **3.41**, which was used directly in the next step. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.03 (s, 9H); 1.46 (s, 9 H); 3.75 (dd, J = 9, 6 Hz, 3H); 3.77 (d, J = 12 Hz, 3H); 4.08 (d, J = 9 Hz, 2H); 5.21 (m, 1H); 5.30 (m, 2H); 7.28-7.71 (m, 10 H). ^{13}C NMR δ 19.4; 26.9; 28.5; 54.0; 57.6; 64.0; 72.4; 80.2; (128.0-135.7); 155.3; 190.3. ESMS for $C_{27}H_{38}NO_7PSi$ $(M+Na)^+$ 570.0.

(2*R*,4*R*)-2-[3-*tert*-Butoxycarbonyl-3-(9-phenyl-9H-flouren-9-ylamino)-propylidene]-4-(*tert*-butyl-diphenyl-silanyloxymethyl)-3-Azetidinone-1-Carboxylic Acid *tert*-Butyl Ester 3.43:

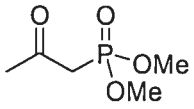


In a 25 mL, single neck round bottom flask equipped with a magnetic stirring bar and rubber septum, under argon, azetidine phosphonate **3.41** (55 mg, 0.1 mmol, 100 mol%) was dissolved in 3.2 mL of THF, cooled to 0 °C, treated with aldehyde **3.42** (41.3 mg, 0.1 mmol, 100mol%, prepared according to reference 28) followed by DBU (41.3mg, 0.2 mmol, 200mol%), stirred for 2.5 h and quenched by addition of saturated aqueous NH_4Cl (1 mL). The solution was extracted with Et_2O (25 mL). The combined organic phases were washed with brine (2 mL), dried, and concentrated. The residue was purified by chromatography eluting with 5-10% EtOAc in hexanes as eluent. Evaporation of the collected fractions provided α,β-unsaturated ketone **3.43** a mixture of two *cis- trans* olefin isomers (according LC/MS) in a 3:1 ratio as assessed by measurement of the triplet signals at δ 4.78 and 4.73 ppm in the ^{1}H NMR spectrum, as a white foam (25.9 mg, 31% yield); $[\alpha]^{20}_D$ –24.7° (*c* 0.02, $CHCl_3$); for the major olefin isomer: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.98 (s, 9H); 1.19 (s, 9 H); 1.43 (s, 9H); 2,64-2.68 (m, 2H); 3.22-3.32 (bs, 1H); 4.12-4.19 (m, 2H); 4.78 (t, J = 3.1 Hz, 1H); 5.58 (t, J = 5.8 Hz, 1H); 7.20-7.65 (m, 23H). For the major olefin isomer: ^{13}C NMR δ 19.6; 26.8; 28.1; 28.5; 30.0; 33.5; 55.7; 60.1; 73.3; 81.1; 82.3; (119.9-149.4); 152.3; 174.6; 190.5.

For the minor olefin isomer: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.01(s, 9H); 1.20 (s, 9 H); 1.47 (s, 9H); 2.38-2.47 (m, 2H); 2.81-3.00 (bs, 1H); 3.97-4.05 (m, 2H); 4.73 (t, J = 2.3 Hz, 1H); 5.53 (t, J = 5.3 Hz, 1H); 7.20-7.65 (m, 23H). HRMS calcd. For $C_{52}H_{58}N_2O_6Si$ $(M+H)^+$ 835.41369, found 835.41484.

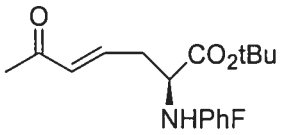
3.4.3 Synthesis of 4-substituted Aze via S_N2'

3.4.3.1 Synthesis of 4-propenyl Aze 3.48 from allylic alcohol 3.46 by S_N2' displacement (2-Oxo-propyl)-Phosphonic Acid Dimethyl Ester 3.44:



In a 250 mL flask was placed dimethyl methyl phosphonate (6.2 g, 50 mmol, 250 mol%) dissolved in THF (50 mL). The mixture was cooled to -78 °C, treated with *n*-BuLi (20 mmol, 20 mL, 250 mol%, an 2.5 M solution in cyclohexane), stirred 30 min and treated dropwise with ethyl acetate (1.762 g, 20 mmol, 100mol%). After stirring for 3 h at -78 °C, the reaction was quenched with 50 mL NaH_2PO_4, and warmed to room temperature. The mixture was treated with brine. The phases were separated. The aqueous phase was washed with EtOAc (5 x 50 mL), dried and evaporated. The residue was purified by distillation (bp 76-79 °C/3mm) to give *β*-keto phosphonate **3.44** (2.8 g, 82% yield); bp 78-80 ° C/3mmHg lit.[29] bp 76-79 °C/3mmHg; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.09 (s, 3H); 2.87 (s, 1H); 2.94 (s, 1H); 3.54(s, 3H); 3.58 (s, 3H). ESMS For $C_5H_{12}O_4P$ $(M+H)^+$ 167.0.

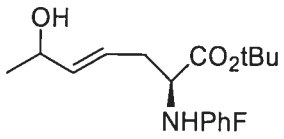
(2*S*)-6-Oxo-2-(9-phenyl-9H-fluoren-9-ylamino)-hept-4-enoic Acid *tert*-Butyl Ester 3.45:



To a stirred solution of *β*-keto phosphonate **3.44** (200 mg, 1.2 mmol, 100 mol%) in CH_3CN (4.3 mL), Cs_2CO_3 (410.7 mg, 1.2 mL, 105 mol%) was added. The suspension

was stirred for 30 minutes at r.t., cooled to 0 °C, treated with a solution of aldehyde **3.42** (497.4, 1.2 mmol, 100 mol%, prepared according to reference 28) in CH_3CN (4.3 mL), stirred at r.t. for 3.5 h, diluted with EtOAc (15 mL) and quenched with NaH_2PO_4 (3 mL, 1M). The layers were separated. The aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic phases were washed with brine, dried and evaporated to give a yellowish oil that was chromatographed using a gradient of 10-14% EtOAc in hexanes to afford α,β-unsaturated ketone **3.45** as an oil (366 mg, 67% yield). $[\alpha]^{20}_D$ −12.74° (*c* 0.60, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (s, 9H); 2.28 (s, 3H); 2.29-2.38 (m, 2H); 2.72 (t, J = 4.4 Hz, 1H); 3.31(s, 1H); 6.05 (d, J = 16 Hz, 1H); 6.72-6.80 (m, 1H); 7.20-7.74 (m, 13H). ^{13}C NMR δ 26.8; 28.0; 39.1; 55.7; 73.1; 81.4; (120.0-149.3); 174.1; 198.5. ESMS calcd. For $C_{30}H_{31}NO_3Na(M+Na)^+$ 476.2. HRMS calcd. For $C_{30}H_{32}NO_3$ $(M+H)^+$454.23767, found 454.23682.

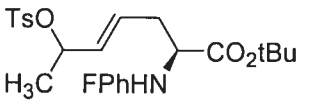
(2*S*)-6-Hydroxy-2-(9-phenyl-9H-fluoren-9-ylamino)-hept-4-enoic Acid *tert*-Butyl Ester 3.46:



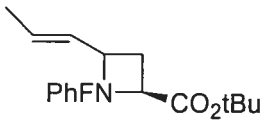
Ketone **3.45** (100 mg, 0.22 mmol, 100 mol%) was dissolved in 7.0 mL of a 0.4 M $CeCl_3.7H_2O$ methanol solution, cooled to -15 °C, treated slowly with $NaBH_4$ (12.5 mg, 0.33 mmol, 150 mol%) and stirred for 2h at -15 °C. The reaction was quenched with H_2O (0.5 mL), and extracted with Et_2O (2 x 5 mL) and EtOAc (2 x 5 mL). The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated to give a residue that was purified by column chromatography using an eluent of 15% EtOAc in

hexanes to furnish the alcohol **3.46** (82 mg, 81% yield) as a mixture of two diastereomers in a 4:1 ratio (confirmed by LC/MS). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.17 (s, 9H); 1.27 (d, J = 4.8 Hz, 3H); 2,13 (dd, J = 13.4, 6.7 Hz, 2H); 2.60 (dd, J = 12.7, 5.8 Hz, 1H); 3.1 (bs, 1H); 4.25 (dd, J = 14.2, 6.2 Hz, 1H); 5.45-5.55 (m, 1H); 5.59-5.72 (m, 1H); 7.21-7.67 (m, 13H). ^{13}C NMR δ 23.44;(23.49); 28.2; (38.84); 38.89; 56.4; (56.5); 68.9; (69.0); 73.2; 80.9; (120.0-149.7); 174.8. $(M+H)^+$ HRMS calcd. For $C_{30}H_{33}NO_3$ $(M+H)^+$ 456.25332, found 456.25336.

(2*S*)-2-(9-Phenyl-9H-fluren-9-ylamino)-6-(toluene-4-sulfonyloxy)-hept-4-enoic Acid *tert*-Butyl Ester 3.47 and *N*-(PhF) 3-Propyenyl Azetidine 2-*tert*-Butyl Ester 3.48:



3.47, 35%



3.48, 11%

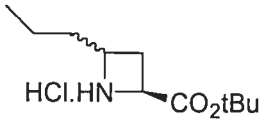
To a magnetically stirred solution of alcohol **3.46** (188 mg, 0.41 mmol 100 mol%) in 1.3 mL of CH_2Cl_2 and 1.7 mL of pyridine cooled at 0 °C was added solid *p*-toluene sulfonyl chloride (237.8 mg, 1.23 mmol, 300 mol%) in one portion. The mixture was stirred at 0 °C for 2h and 22 h at r.t. The mixture was evaporated and was poured into ice water, and extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with brine, dried over MgSO4, filtered and concentrated. The organic solutions were combined and purified by column chromatography over silica gel using 3-15% EtOAc in hexanes as eluent. Evaporation of the collected fractions gave three fractions: 57 mg of starting material **3.46** (28%), and 21 mg of propenyl azetidine **3.48** (11%) and 77 mg of tosylate **3.47** (30%). Attempts for further purification of **3.47** and **3.48** were unsuccessful

and the ^{1}H NMR and ^{13}C NMR were complicated due to existence of two diastereomers and two olefin isomers and showed multiple signals for some protons and carbons in tosylate **3.47** and propenyl azetidine **3.48**.

Tosylate **3.47** was isolated as a 3:2 mixture of diastereomers as assessed by measurement of the diastereomeric doublet signals at δ 1.45 and 1.57 ppm in the ^{1}H NMR spectrum.: $CDCl_3$, 400 MHz) δ 1.15 (s, 9H); 1.45 (d, J = 4.8 Hz, 3H); 1.49 (d, J = 6.7 Hz, 3H); 2.06 (t, J = 6.1 Hz, 1H); 2.82-3.12 (m, 1H); 3.18 (bs, 1H); 4.62 (t, J = 7.6 Hz, 1H); 4.81 (d, J = 6.7 Hz, 1H); 5.31-5.56 (m, 2H); 7.11-7.67 (m, 17H). ^{13}C NMR δ 19.1; (19.6); 21.2; (21.9); 30.2; 35.9; 36.8; 56.7; 66.2; (66.9); 71.3; (71.7); 126.2-149.4; 174.3; (174.8). ESMS m/z $C_{37}H_{40}NO_5S$ $(M+H)^+$ 610.2.

Azetidine **3.48** was isolated as a 7:5 mixture of diastereomers as assessed by measurement of the diastereomeric triplets at δ 3.43 and 3.25 ppm in the ^{1}H NMR spectrum. The ^{1}H NMR and ^{13}C NMR in the vinylic region were complicated by olefin isomers and two diastereomers and showed multiple signals for some carbons. Spectral data for the major diastereomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (s, 9H); 1.27 (d, J = 3.6 Hz, 3H); 1.97-2.10 (m, 2H); 2.51 (t, J = 7.6 Hz, 1H); 2.99 (s, 1H); 3.43 (t, J = 10.9 Hz, 1H); 4.89-5.04 (2d, J = 13.2, 22.9 Hz 1H total); 5.45-5.64 (m, 1H); 7.12-7.64 (m, 13H); for the minor isomer ^{1}H NMR δ 1.11 (s, 9H); 1.45 (d, J = 3.6 Hz, 3H); 1.77-1.95 (m, 2H); 2.51 (t, J = 7.6 Hz, 1H); 2.99 (s, 1H); 3.24 (t, J = 10.4 Hz, 1H); 4.69-4.81 (m, 1H); 4.89-5.04 (2d, J = 13.2, 22.9 Hz 1H total); 5.31-5.39 (m, 1H); 7.12-7.64 (m, 13H); ^{13}C NMR δ 17.0; 20.71;(20.78); 27.2; (27.4); 54.8; 55.1; (55.4); 72.3; 77.2; (119.0-140.1), 173.5; 173.6. HRMS calcd. For $C_{30}H_{32}NO_2$ $(M+H)^+$ 438.4327, found 438.5218.

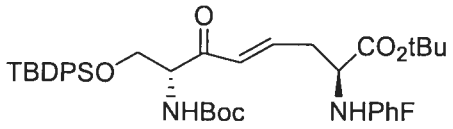
4-Propyl-Azetidine-2-Carboxylic Acid *tert*-Butyl Ester 3.49:



A solution of **3.48** (25 mg, 0.06 mmol, 100 mol %) in 2 mL of MeOH:HOAc (9:1) was transferred into a hydrogenation apparatus and treated with palladium-on-carbon (2 mg, 10 wt %). The pressure bottle was filled, vented, and refilled four times with 8 atm of H_2. The reaction mixture was stirred (24 h), filtered onto a plug of Celite^R 521(Aldrich USA), and washed thoroughly with MeOH. The filtrate was treated with 2 drops of 1N HCl and the volatiles were evaporated to give a crude residue of **3.49** (10.53 mg, 93% yield) as the hydrochloride salt which was purified by HPLC (HPLC, Prevail C18 5 μ, gradient 80-10% A:0.1 TFA in H_2O, B: 20-90% 0.1 TFA in CH_3CN; RT=12.5 min) recovery yield 56% of Azetidine **3.49** was observed to be a 3:2 ratio of diastereomers as assessed by measurement of the diastereomeric multiplet signals at δ 3.51 and 3.33 ppm in the ^1H NMR spectrum. Spectral data for the major diastereomer: ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88-095 (m, 3H); 1.19 (s, 9H); 1.33 (m, 2H); 1.82 -2.00 (m, 2 H), 2.53-2.60 (m, 1H), 3.08 (s, 1 H), 3.49-3.53 (m, 1H); 4.74-4.90 (m, 1H). For the minor isomer: ^1H NMR 0.88-095 (m, 3H); 1.26 (s, 9H); 1.58 (m, 2H); 2.10-2.23 (m, 2H), 2.53-2.60 (m, 1H), 3.08 (s, 1H), 3.32-3.34 (m, 1H); 4.58-4.60 (m, 1H). ^{13}C NMR δ 13.8; 19.8; (20.3); 28.2; 35.3; 37.1; (37.8); 48.5; (49.0); 62.9; (63.4); 84.6; (85.3); 166.6; (167.1). HRMS calcd. 200.16451 For $C_{11}H_{22}NO_2$ $(M+H)^+$, found 200.16358.

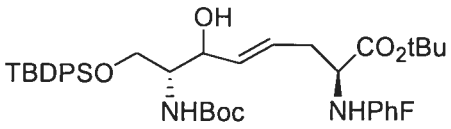
3.4.3.2 Attempted synthesis of 4-substituted Aze 3.53 from allylic alcohol 3.51 by S_N2' reaction

(2*S*,7*R*)-7-*tert*-Butoxycarbonylamino-8-(*tert*-butyl-diphenyl-silanyloxy)-6-oxo-2-(9-phenyl-9H-fluoren-9-ylamino)-Oct-4-enoic acid *tert*-butyl ester 3.50:



To a stirred solution of β-keto phosphonate **3.39** (655 mg, 1.19 mmol, 100 mol%) in CH_3CN (5 mL), Cs_2CO_3 (388.3 mg, 1.19mmol, 100 mol%) was added and the suspension was stirred for 30 min at room temperature, cooled to 0°C and treated with a solution of aldehyde **3.42** (491.4 mg, 1.19 mmol, 100 mol%, prepared according to reference 28) in CH_3CN (5 mL). The mixture was stirred at room temperature for 24 h, diluted with EtOAc (60 mL), and quenched with NaH_2PO_4 (7.0 mL, 1M). The layers were separated, and the aqueous layer was extracted with EtOAc (2 x 20mL). The combined organic layers were washed with brine, dried, and evaporated to give yellowish oil that was chromatographed using a gradient of 3-6% EtOAc in hexanes to afford **3.50** as clear oil (710 mg and 72% yield). $[\alpha]^{20}_D$ –30.85° (*c* 0.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.03 (s, 9H); 1.21 (s, 9 H); 1.48 (s, 9H); 2.12-2.31 (m, 2H); 2.68 (t, J = 5.7 Hz, 1H); 3.31 (s, 1H); 3.95-4.01 (m, 2H); 4.58-4.69 (dd, J = 7.0, 3.3 Hz 1H); 5.62-5.72 (t, J = 7.7 Hz, 1H); 6.2 (d, J = 15.8 Hz, 1H), 6.79-7.04 (m,1H); 7.20-7.72 (m, 23H). ^{13}C NMR δ 19.6; 27.1; 28.2; 28.7; 39.3; 55.9; 59.8; 64.6; 73.3; 79.9; 81.6; (120.1-149.4); 155.6; 174.3; 196.0. HRMS calcd. For $C_{52}H_{61}N_2O_6Si$ $(M+H)^+$ 837.42934, found 837.43226.

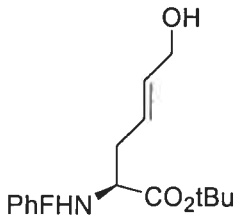
(2*S*,7*R*)-7-tert-Butoxycarbonylamino-8-(*tert*-butyl-diphenyl-silanyloxy)-6-hydroxy-2-(9-phenyl-9H-fluoren-9-ylamino)-Oct-4-enoic Acid *tert* -Butyl Ester 3.51:



α,β-Unsaturated ketone **3.50** (335 mg, 0.4 mmol, 100 mol%) was dissolved in 20 mL of a solution of 0.4 M $CeCl_3.7H_2O$ in methanol, cooled to -15 °C, treated slowly with $NaBH_4$ (22.7 mg, 0.6 mmol, 150 mol%), stirred for 2h at -15 °C, and quenched with H_2O (1 mL). The mixture was extracted with Et_2O (2 x 10 mL) and EtOAc (2 x 10 mL). The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated to give a residue that was purified by column chromatography using an eluent of 10-15% EtOAc in hexanes to furnish alcohol **3.51** (263 mg, 78% yield) as a pure diastereomer (*E*-olefin isomer) (confirmed by LC/MS and ^1H NMR) as a white foam; $[\alpha]^{20}_D$ –65.8° (*c* 0.01, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.11 (s, 9H); 1.22 (s, 9 H); 1.44(s, 9H); 2.12 (dd, *J* = 13.6, 6.5, Hz, 2H); 2.64 (dd, *J* = 11.6, 5.8 Hz , 1H); 3.2 (bs, 1H); 3.71-3.77 (m, 2H); 3.83 (d, *J* = 4.1 Hz , 1H); 3.92 (dd, *J* = 10.3, 3.6 Hz , 1H); 4.30 (d, *J* = 4.8 Hz , 1H); 5.2 (d, *J* = 8.1 Hz, 1H); 5.5 (dd, *J* = 15.4, 5.8 Hz, 1H), 5.77-5.87 (m, 1H); 7.20-7.71 (m, 23H). ^{13}C NMR δ 14.1; 19.1; 26.7; 27.8; 28.2; 55.0; 55.9; 63.8; 72.9; 73.6; 79.2; 80.5; (119.5-149.3); 157.2; 174.4. HRMS calcd. For $C_{52}H_{63}N_2O_6Si$ $(M+H)^+$ 839.44499, found 839.44499.

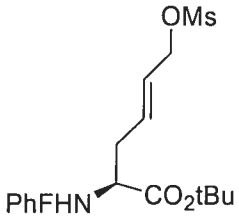
3.4.3.3 Attempted synthesis of 4-vinyl Aze 3.59 from allylic alcohol 3.55

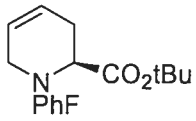
(2S)-6-Hydroxy-[*N*-(PhF)amino]-hex-4-enoic Acid *tert*-Butyl Ester 3.55:



To a stirred solution of diester **3.54** (1g, 2.01 mmol, 100 mol%, prepared according to reference 16) in THF (40.5 mL) at -40 °C, DIBAL-H (1 M solution in toluene, 8.06 mmol, 400 mol%) was added. The mixture was stirred for 1.5 h, quenched with acetone (1.5 mL) diluted with MeOH (8 mL), and allowed to warm to room temperature and evaporated. The residue was dissolved in ether (50 mL) and extracted with 1 M NaOH (3 x 30 mL), washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography using 20% EtOAc in hexanes as eluent to yield 893 mg of **3.55** as oil (95% yield). $[\alpha]^{20}_D$ –248.51° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28 (s, 9H); 2.26 (t, J = 6.02 Hz, 2H); 2.42 (t, J = 7.21 Hz, 1H); 2.71-2.74(m, 1H); 4.12 (d, J = 4.24 Hz, 2H); 5.68-5.72 (m, 2H); 7.26-7.75 (m, 13H). ^{13}C NMR δ 28.0; 38.0; 76.1; 56.2; 63.4; 73.2; 80.8; (119.8-149.6); 174. HRMS calcd 442.2378 For $C_{28}H_{31}NO_3$ $(M+H)^+$ 442.2376.

6-Methansulfonyloxy-*N*-PhF-hex-4-enoic Acid *tert*-Butyl Ester 3.57 and *N*(PhF)-1,2,3,4-tetrahydro-Pyridine-2-Carboxylic Acid *tert*-Butyl Ester 3.58:





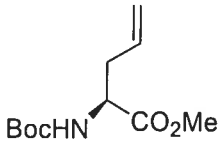
To an ice-cooled solution of alcohol **3.55** (589 mg, 1.33 mmol, 100 mol%), triethylamine (405.2 mg, 4.00 mmol, 300 mol%), DMAP (16.2 mg, 0.133 mmol, 10 mol%) in dichloromethane, methanesulfonyl chloride (304.5 mg, 2.66 mmol, 200 mol%) was added. The bath was removed and the mixture was allowed to warm to r.t. After 24h, the reaction mixture was diluted with water (15 mL) and extracted twice with EtOAc. The organic layers were washed with brine, dried and evaporated to a residue, that was chromatographed with 3-10% EtOAc in hexanes as eluent to give 297 mg of mesylate **3.57** (43% yield) and 214 mg of pipecolate **3.58** (38% yield).

Mesylate **3.57**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.10 (s, 9H); 2.08 (t, J = 5.7 Hz, 2H); 2.51 (t, J = 8.08 Hz, 1H); 2.88 (s, 3H); 3.08-3.18 (bs, 1H); 4.57 (d, J = 8.8 Hz, 2H); 5.45-5.51 (m, 1H); 5.70-5.85 (m, 1H); 7.11-7.58 (m, 13H). ^{13}C NMR δ 28.0; 38.0; 76.1; 56.2; 63.4; 73.2; 80.8; (119.8-149.6); 174.8; ESMS m/z for $C_{30}H_{33}NO_5S$ (M+) 519.9.

Pipecolate **3.58**: $[\alpha]^{20}_{D}$ –109.51° (*c* 0.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.11 (s, 9H); 2.07 (dd, J = 12.5, 6.2 Hz, 2H); 2.51 (t, J = 6.09 Hz, 1H); 3.92 (d, J = 6.8 Hz, 2H); 5.46-5.53 (m, 1H); 5.59-5.65 (m, 1H); 7.10-7.58 (m, 13H). ^{13}C NMR δ 28.1; 38.7;45.4; 56.1; 73.2; 81.1; (120.0-149.6); 174.5. HRMS calcd. For $C_{29}H_{30}NO_2$ $(M+H)^+$ 424.2271, found 424.22537.

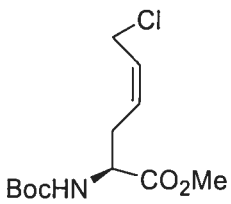
3.4.3.4 Synthesis of 4-vinyl Aze 3.65 via S_N2' reaction of alylic chloride 3.64

(2*S*)2-*tert*-Butoxycarbonylamino-pent-4-enoic Acid Methyl Ester 3.63:



To a suspension of *N*-(Boc)allylglycine **3.62** (340mg, 1.58 mmol,100mol% prepared according to reference 28, 20) and sodium hydrogen carbonate (265.5 mg, 3.16 mmol, 200mol%) in dimethylformamide (5 mL), methyl iodide (1.11 g, 7.9 mmol, 500 mol% in 5 ml DMF) was added at room temperature. The mixture was stirred for 24 h, treated with water (20 mL) and extracted with ethyl acetate (4 x 10 mL). The organic layer was washed with water, dried with Na_2SO_4, evaporated to a small volume that was purified by column chromatography using 10% ethyl acetate in hexanes as eluent. Evaporation of the collected fractions provided ester **3.63** as an oil (315 mg, 87 % yield). $[\alpha]^{20}_D$ +15.75° (*c* 0.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.33 (s, 9H); 2.39 (m, 2H); 3.61 (s, 3H); 4.27 (dd, J = 13.7, 6.2 Hz, 1H); 5.04 (m, 3H); 5.57 (m, 1H). ^{13}CNMR δ 28.4; 36.7; 53.0 ; 54.5; 80.5; 119.5; 132.4; 155.8; 176.4. ESMS For $C_{11}H_{20}NO_4$ $(M+H)^+$ 230.1 . HRMS calcd. For $C_{11}H_{19}NO_4Na$ $(M+Na)^+$ 252.1206, found 252.1205.

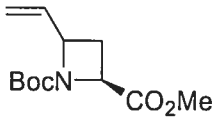
(2*S*)-2-tert-Butoxycarbonylamino-6-chloro-hex-4-enoic Methyl Ester 3.64:



To a solution of *N*-(Boc)allylglycine methyl ester **3.63** (230 mg, 1.003 mmol, 100 mol%) and allyl chloride (307.2 mg, 4.015 mmol, 400 mol%) in CH_2Cl_2 (17 mL), Grubbs second

generation catalyst benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium (87.6 mg, 0.1 mmol, 10 mol%) was added. The mixture was heated at reflux for 17 h. The solvents were evaporated under reduced pressure. The residue was chromatographed on silica gel using of 3-10% EtOAc in hexanes as eluent afforded **3.65** (172 mg, 62%) as a clear oil. $[\alpha]^{20}_{D}$ +33.73 (*c* 0.01, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 9 H); 2.40-2.47 (m, 1H); 2.52-2.58 (m, 1H); 3.72 (s, 3H); 3.98 (dd, J = 19.2, 8.2 Hz, 2H); 4.35 (dd, J = 13.2, 6.1 Hz, 1H); 5.07 (J = 7.7 Hz, 1H); 5.60-5.72 (m, 2H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 27.5; 34.5; 43.7; 51.6; 52.1; 79.3; 128.3; 129.5; 154.3; 171.5. HRMS calcd. For $C_{12}H_{20}ClNO_4Na$ $(M+Na)^+$ 300.0973, found 300.0985.

_N_-(Boc)-4-Vinyl Azetidine 2-Methyl Ester 3.65:

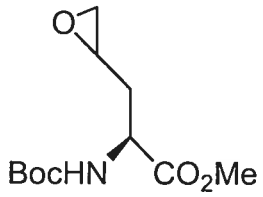


Under argon, allyl chloride methyl ester **3.64** (50 mg, 0.18 mmol, 100 mol %) was dissolved in THF (0.7 mL), cooled to -78 °C, treated dropwise with a solution of *n*-BuLi (0.07 mL, in cyclohexane, 2.5 M), stirred at -78 °C for 15 min, treated with silver triflate (74.2 mg, 0.28 mmol, 160 mol%) and the resulting light brown solution was allowed to warm to r.t. over 1.5h and stirred for an additional 1h. The resulting heterogeneous reaction mixture was diluted with ether, washed successively with 2N HCl, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated under reduced pressure to a residue that was chromatographed using 10-25% EtOAc in hexanes as eluant to provide vinyl azetidine **3.65** (12 mg, 25% yield) as a colorless oil; The ^{1}H NMR and ^{13}C NMR

were complicated by carbamate *cis* and *trans*-isomers and diastereomers of *N*-Boc 4-vinyl azetidine 2-methyl ester **3.65** and showed multiple signal for some carbons: ^{1}H NMR ($CDCl_3$, 500 MHz, at 25 °C) in the vinylic region exhibited two *trans* and two *cis* in a 5.7:1 ratio as assessed by measurement of the *cis* doublet signals at δ 5.38 and 5.18 ppm. δ 1.50 (s, 9 H); 1.61-1.70 (m, 1H); 1.87-2.05 (m, 1H); 3.75 (s, 3H); 4.39-4.58 (m, 1H); 5.10 (bs, 1H); 5.20 and 5.35 (2d, J = 5.8, 8.2 Hz, total 1H); 5.33 and 5.48 (2d, J = 14.2 , 18.3 Hz, total 1H); 5.78-5.91 (m, 1H). ^{1}H NMR ($CDCl_3$, 500 MHz at -55 °C) δ in the vinylic region exhibited two *trans* and two *cis* in a 2.7:1 ratio as assessed by measurement of the *cis* doublet signals at the same chemical shift as described above. δ 1.49 (s, 9 H); 1.60-1.71 (m, 1H); 1.90-2.15 (m, 1H); 3.81 (s, 3H); 4.52-4.61 (m, 0.5H); 4.83-4.92 (m, 0.5H); 5.18 (d, J = 14.3 Hz, 1H); 5.20 (2d, J = 6.2, 6.5 Hz, total 1H); 5.35, 5.50 (2d, J = 15.2, 17.8 Hz, total 1H); 5.83-5.92 (m, 1H). ^{13}C NMR ($CDCl_3$, 500 MHz at -55 °C) δ 28.3; 36.9; 41.3; 50.2; 51.2; 52.2; 53.5; 68.0; 78.5; 78.5; 81.0; 81.3; 115.2; 120.7; 134.0; 134.2; 138.7; 155.4; 157.0; 173.5; 175.6. ^{13}C NMR ($CDCl_3$, 500 MHz at 25 °C) 28.5; 35.0; 37.4; 41.4; 51.0; 51.7; 52.9; 68.6; 78.4; 80.9; 114.9; 119.4; 134.7; 139.8; 156.9; 173.4. HRMS calcd. For $C_{12}H_{20}NO_4$ $(M+H)^+$ 242.10038, found 242.10159.

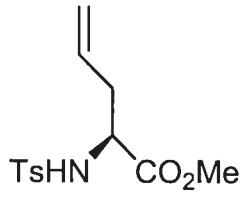
3.4.4 Attempted synthesis of 4-substituted Aze via intramolecular nucleophilic ring opening of an epoxide:

(2S)-2-*tert*-Butoxycarbonylamino-3-oxiranyl-Propionic Acid Methyl Ester 3.70:



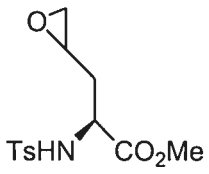
To a stirred solution of *N*-Boc allylglycine **3.63** (100mg, 0.436 mmol, 100 mol%, prepared according to reference 19) in CH_2Cl_2 was added *m*-chloroperbenzoic acid (*m*-CPBA, 370 mg, 2.13 mmol, 490 mol%). After 5 h, the mixture was filtered through a glass filter, the solids were washed with CH_2Cl_2, and the combined organic filtrate and washings were cooled to 0 °C and washed with Na_2SO_3 (2 mL). The mixture was filtered and organic phase was washed with 10% Na_2SO_3, 10% $NaHCO_3$ and water. After drying ($MgSO_4$), filtration and evaporation of the volatiles, the residue was chromatographed on silica gel using a gradient of 20:80 EtOAc in hexanes to afford a mixture of two diastereomers of epoxide **3.70** in a 4:1 ratio (according to LC/MS) as clear oil (70 mg, 70% yields). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.29 (s, 9 H); 1.60-1.75 (m, 1H); 1.88-2.11 (m, 1H); 2.33 (dd, J = 4.8, 2.6 Hz, 1H); 2.62 (dd, J = 7.2, 3.9 Hz, 1H); 2.84-2.87 (m, 1H); 3.60 (s, 3H); 4.29 (t, J = 6.9 Hz, 1H); 5.17 (s, 1H); ^{13}C NMR δ 28.6; 35.7; (35.9); 46.8; (47.0); 49.4; 52.1; 52.8; 76.9; 155.5; (155.9); 172.8. HRMS calcd. For $C_{11}H_{20}NO_5$ $(M+H)^+$ 246.1342, found 246.1342.

(2*S*)-2-(Toluene-4-sulfonylamino)-pent-4-enoic Acid Methyl Ester 3.73:



To a solution of allylglycine methyl ester hydrochloride **3.72** (20 mg, 0.119 mmol, 100 mol %, prepared according to reference 6) in CH_2Cl_2 (1.4 mL), pyridine (18.8 mg, 0.238 mmol, 200 mol%) followed by *p*-toluene sulfonyl chloride (23 mg, 0.119 mmol, 100 mol%) were added. The mixture was stirred for 36 h at room temperature, and then washed with brine and water. After drying ($MgSO_4$) and concentration of the volatiles, the residue was chromatographed on silica gel using a gradient of 15-20% EtOAc in hexanes to give sulfonamide **3.73** as an oil (27 mg, 80% yield) $[\alpha]^{20}_D$ +11.12 (*c* 0.04, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.41 (s, 3H); 2.45 (t, J = 6.6 Hz, 2H); 3.51 (s, 3H); 4.01-4.04 (m, 1H); 5.05-5.10 (m, 2H); 5.22 (d, J = 8.9 Hz, 1H); 5.58-5.62 (m,1H); 7.26-7.72 (m, 4H). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 21.8; 37.8; 52.7; 55.5;120.0; 127.5; 129.9; 131.5; 137.0; 144.0; 171.6. ESMS m/z 284.0 For $C_{13}H_{17}NO_4S$ $(M+H)^+$. HRMS calcd. For $C_{13}H_{17}NO_4S$ $(M+H)^+$ 284.09511, found 284.09570.

(2*S*)-3-Oxiranyl-2-(toluene-4-sulfonylamino)-Propionic Acid Methyl Ester 3.74:



Epoxide **3.74** was synthesized according to the procedure described for the synthesis of

epoxide **3.70** above using *N*-toluenesulfonyl allyglycine methyl ester **3.73** (50 mg, 0.176 mmol, 100 mol %) in CH_2Cl_2 (2 mL) and *m*-chloroperbenzoic acid (*m*-CPBA, 370 mg, 2.13 mmol, 500 mol%). The residue was purified on silica gel using a gradient of 20-35% EtOAc in hexanes afforded a mixture 1:1 of two diastereomers in a ratio 1:1 as assessed by measurement of the diastereomeric methyl ester singlet at δ 3.52 and 3.56 ppm in the ^{1}H NMR spectrum **3.74** as white solid (38 mg, 72% yields). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.81-2.10 (m, 2H); 2.41 (s, 3H); 2.49 (t, J = 5.3 Hz, 1H); 2.73-2.77 (m, 1H); 2.92-3.02 (m, 1H); 3.52 (s, 3H); 4.12 (t, J = 6.2 Hz, 1H); 5.52 (d, J = 7.8 Hz, 1H); 7.26-7.74 (m, 4H). ^{13}C NMR δ 21.8; 36.4; 36.8; 47.1; 47.3; 48.6; 49.1; 49.0; 53.0; 53.1; 53.8; 54.1; 127.5; 129.9; 130.0; 136.6; 136.8; 144.1; 171.6; 171.8. HRMS calcd. for $C_{13}H_{18}NO_5S$ $(M+H)^+$ 300.0900, found 300.0908.

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CHAPTER 4

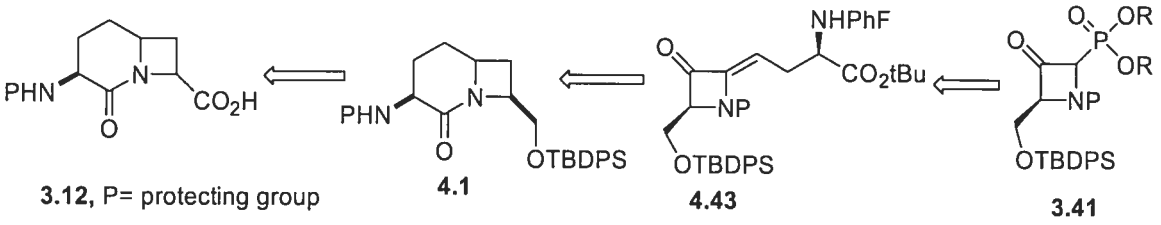
Conclusions and Perspectives

4.1 Conclusion, future work and perspectives:

Incorporation of conformational constraints, such as a proline or proline homologue, into a biologically active peptide can enhance receptor selectivity and modulate ligand efficacy. With this aim we have developed methodologies for the synthesis of 3- and 4-substituted azetidine-2-carboxylic acids to serve as tools for studying the conformational requirements of biologically active peptides.

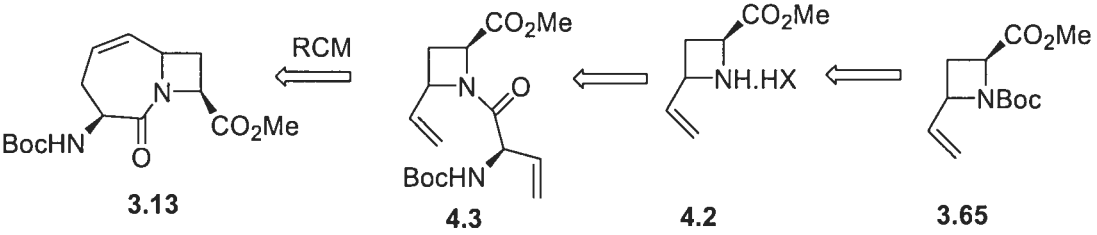
In Chapter 2, enantiopure 3-substituted azetidine-2-carboxylic acids containing different heteroatomic side chains were synthesized from L-Asp as chiral educt. 3-Substituted Aze analogues were prepared to serve as azetidine amino acid chimeras of Lys, nor-leucine and aminoadipate. In light of the activity of 3-carboxymethyl Aze (*t*-CAA) **1.36**, a Glu-Aze chimera, to inhibit excitatory amino acid transporters, the potential for aminoadipate-Aze **2.4** to exhibit activity merits investigation and may now be explored, because of our efficient synthesis.

In Chapter 3, preliminary experiments were made to prepare 2,4-disubstituted azetidines. For example, 4-substituted azetidine 2-phosphonate **3.41** was synthesized starting from L-serine **3.38** by a route featuring an intramolecular diazo insertion reaction of α-diazo β-ketophosphonate **3.40**. This Aze analogue may be considered as a mimic of the corresponding α-aminocarboxylic acid and was used in Wittig chemistry to provide 2,4-disubstituted azetidine-3-one **3.43**. A potential application of 2,4-disubstituted azetidine-3-one **3.43** as an intermediate for the synthesis of target azabicyclo[4.2.0]alkane amino acid **3.12** is illustrated in Scheme 4.1.



Scheme 4.1: Retro synthesis of target azabicyclo[4.2.0]alkane amino acid **3.12** from azetidine phosphonate **3.41**.

Another investigation to synthesize 4-substituted Aze analogues via S_N2' reaction, has produced 4-propenyl Aze analogue **3.48** as a diastereomeric mixture. Moreover, a novel pipecolate **3.58** was prepared as an interesting alternative product to 4-vinyl *N*-(PhF)Aze **3.59** when DMAP was used in the S_N2' reaction. 4-vinyl Aze **3.65** was synthesized using Ag(I) to effect the intramolecular S_N2' *N*-alkylation of **3.64**. 4-Vinyl Aze **3.65** may serve as precursor for the preparation of fused azabicyclo[5.2.0]alkane amino acid **3.13**, a novel β-turn mimic (Scheme 4.2).



Scheme 4.2: Retro synthesis of target azabicyclo[5,2,0]alkane **3.13** from vinyl azetidine **3.65**.

In this route, the key step would be *N*-acylation of 4-vinyl Aze **3.65** to provide dipeptide **4.3** for ring-closing metathesis and construction of β-lactam **3.13**.

Although an investigation failed to produce 4-substituted Aze by intramolecular ring opening of an epoxide by nucleophilic attack of a β-amine, new amino epoxides **3.70**, **3.74** were synthesized for the first time from L-Asp. These novel amino epoxides could be considered as intermediates for synthesis of functionalized compounds such as amino alcohols.[5]

This research has thus touched on the field of peptide mimicry by providing new methodology and design for the synthesis of conformationally constrained compounds that may induce turn motifs when introduced into peptides and proteins. The important roles turns play in peptide recognition and biological activity suggest that the 3- and 4-substituted Aze analogues have interesting potential for studying conformation-activity relationships effecting potency, metabolic stability, and activity of biologically active peptides in medicinal chemistry and peptide science. Approaches for synthesizing 4-substituted azetidinone **3.43** and 4-substituted Aze analogues **3.48** and **3.65** offer potential for the construction of azabicyclo[X.2.0]alkane amino acids such as **3.12** and **3.13**. Future work on the synthesis of fused ring systems such as **3.12** and **3.13** will require improved methods for 4-substituted Aze synthesis. In this light, the described methods for making 3- and 4-substituted Aze analogues provides a solid foundation for the research activity of future colleagues.

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Appendix 1

^{1}H NMR and ^{13}C NMR of compounds related to chapter 2

Sajjadi, Z; Lubell, W. D., "Amino Acid-Azetidine Chimeras: Enantiopure 3-Substituted Azetidine-2-Carboxylic Acids", *J. Peptide. Res.* **2005,** *65*, 298-310.

Appendix 2

^{1}H NMR and ^{13}C NMR and HRMS of compounds related to chapter 3

Appendix 1

^{1}H NMR and ^{13}C NMR of compounds related to chapter 2

Sajjadi, Z; Lubell, W. D., "Amino Acid-Azetidine Chimeras: Enantiopure 3-Substituted Azetidine-2-Carboxylic Acids", *J. Peptide. Res.* **2005,** *65*, 298-310.

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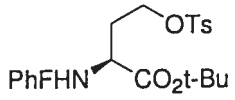

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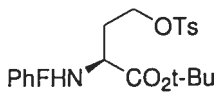

2.15

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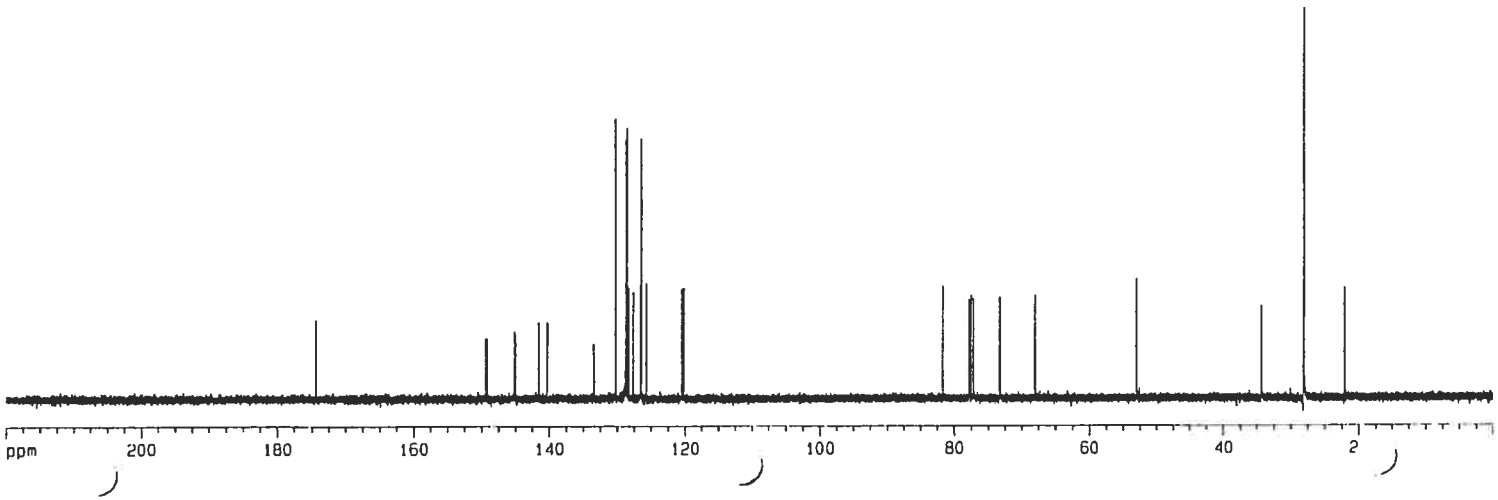

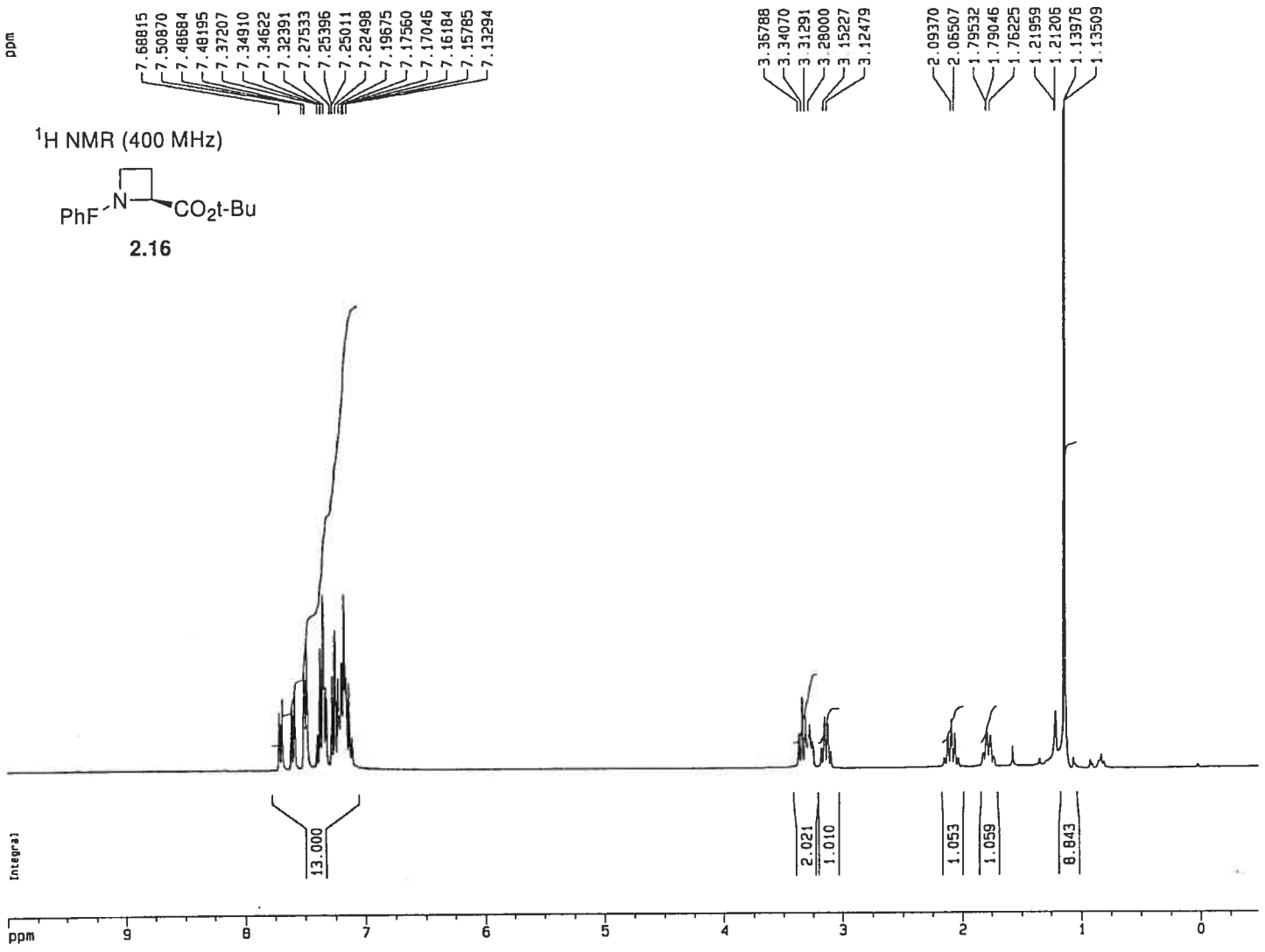
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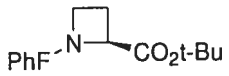

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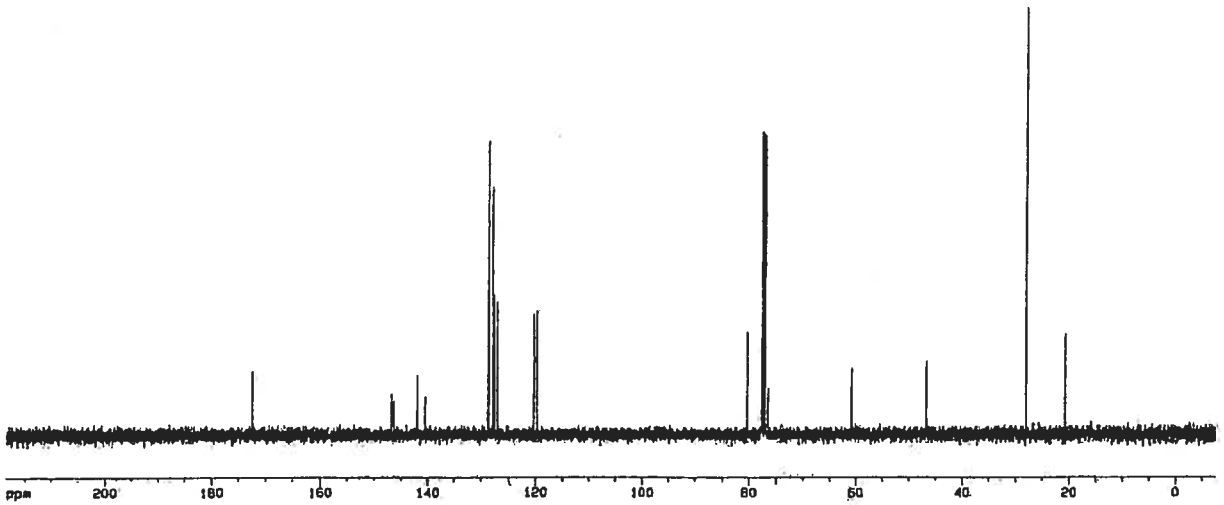
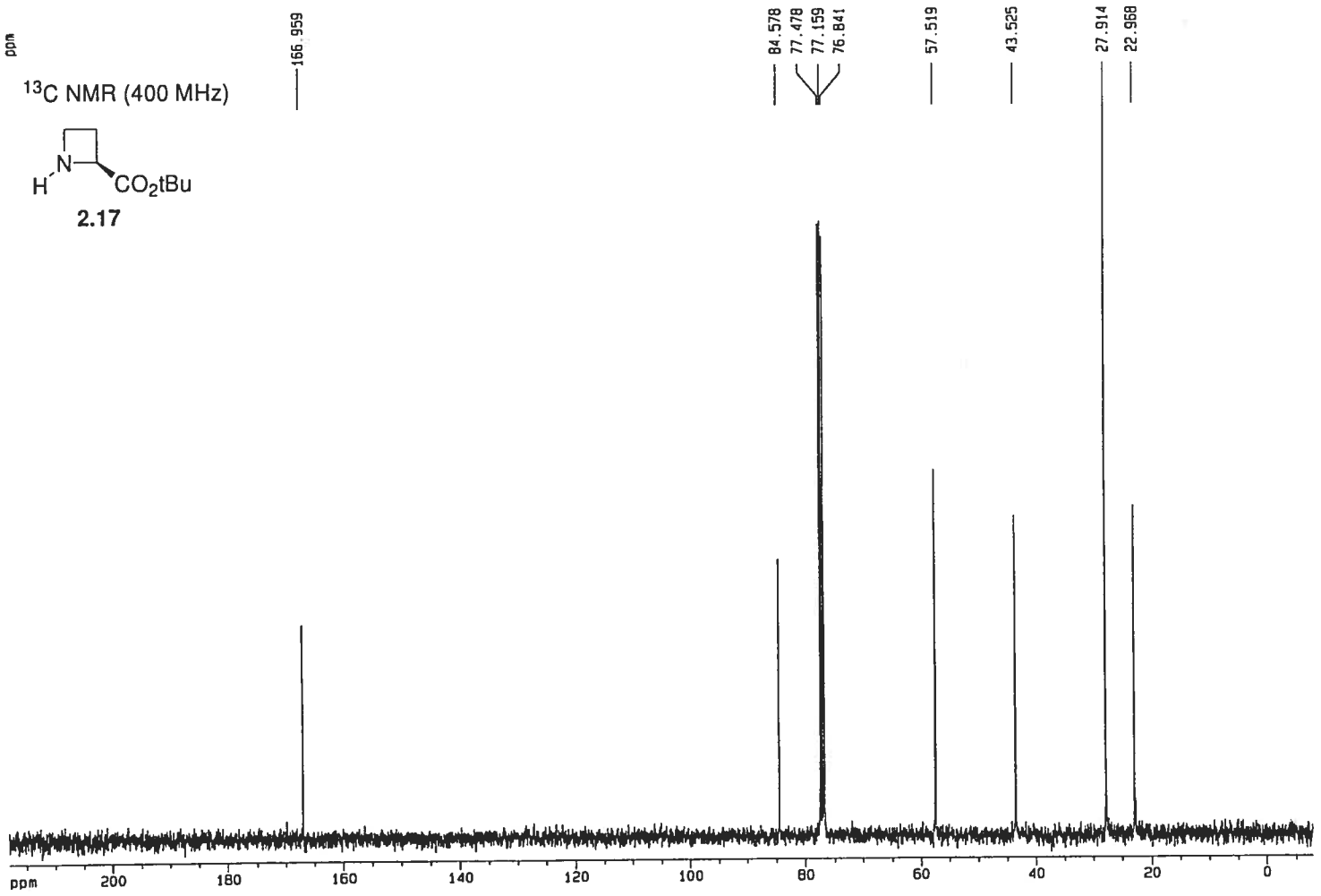


MHz)

H NMR (400 MHz)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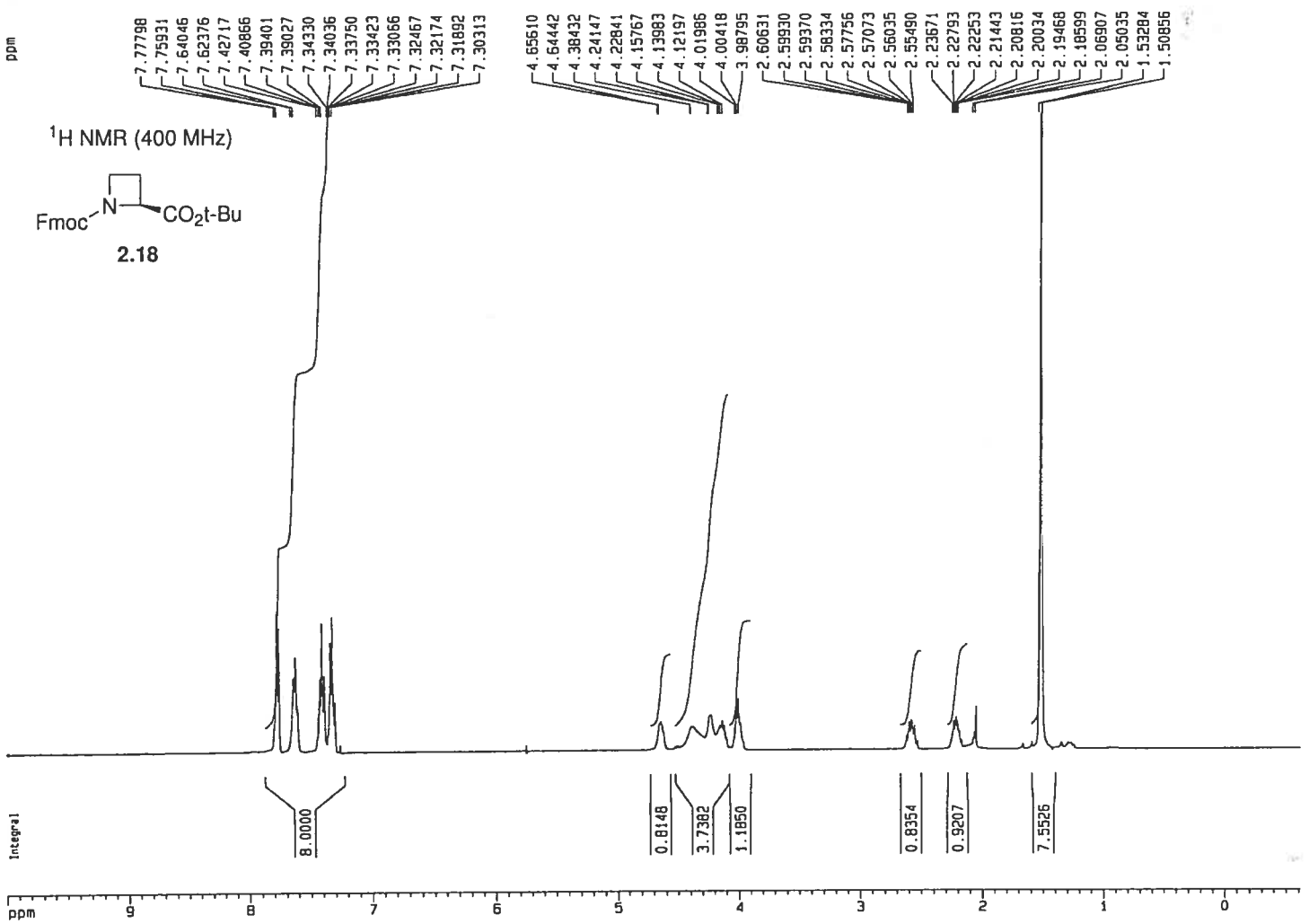
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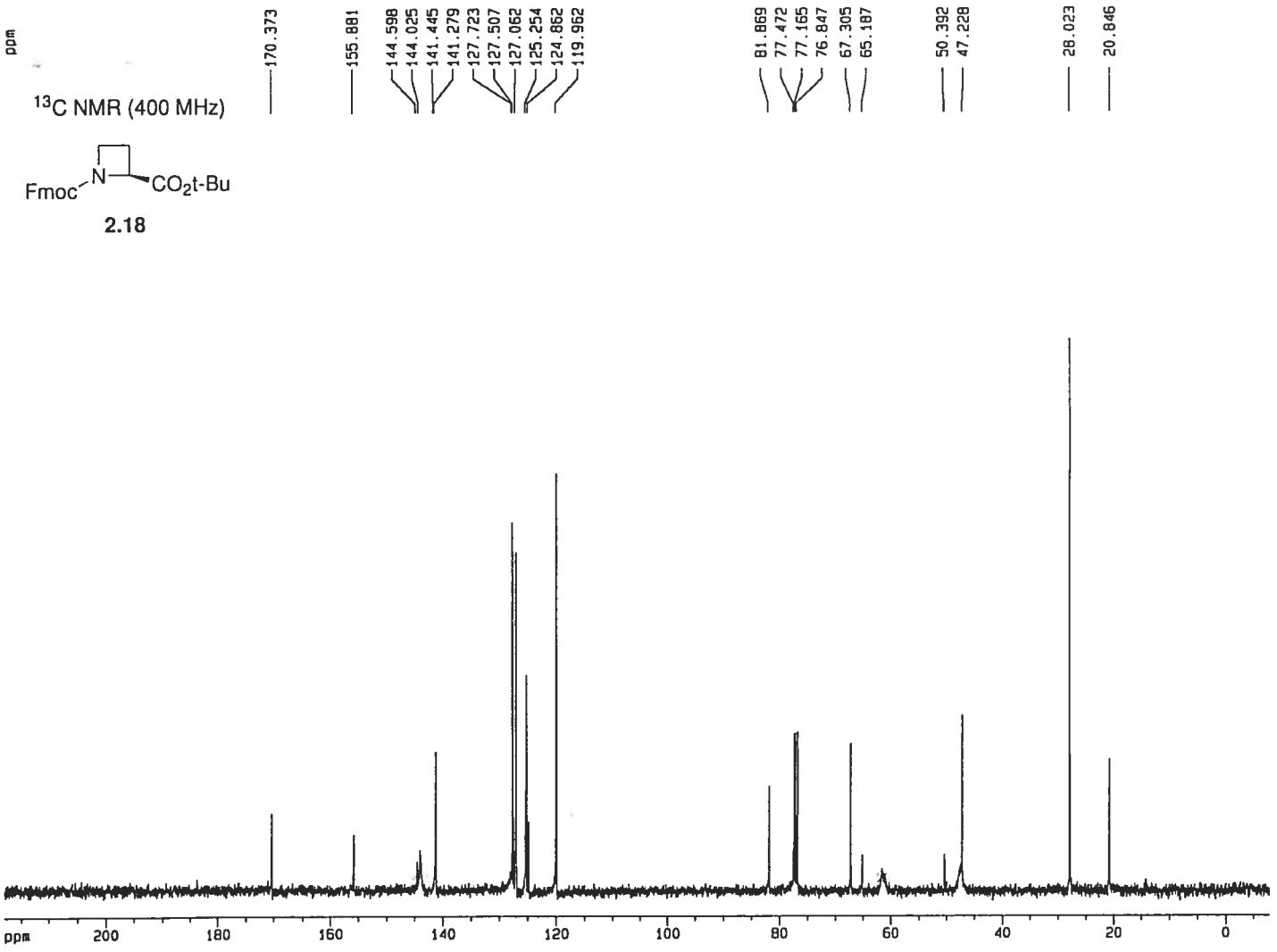
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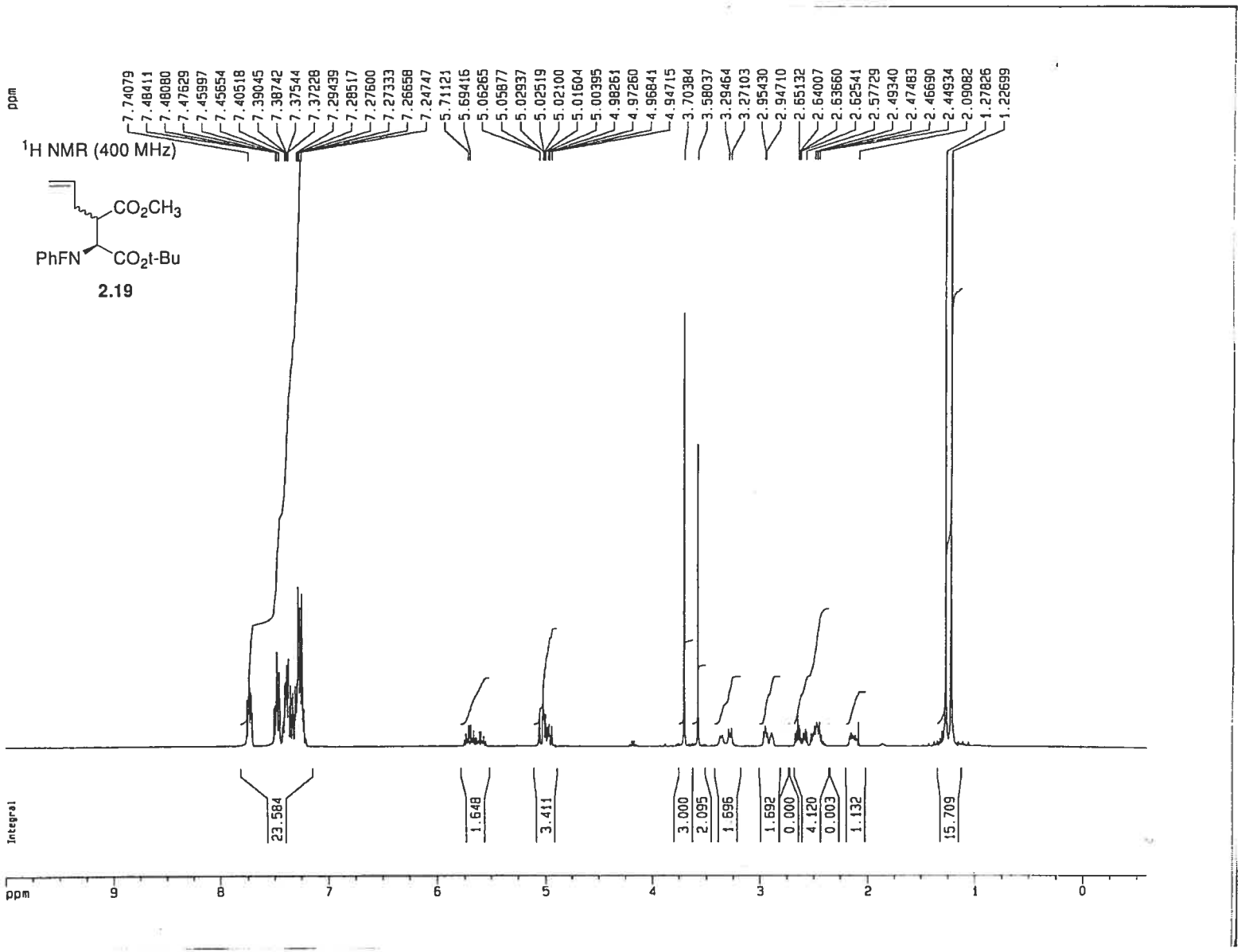
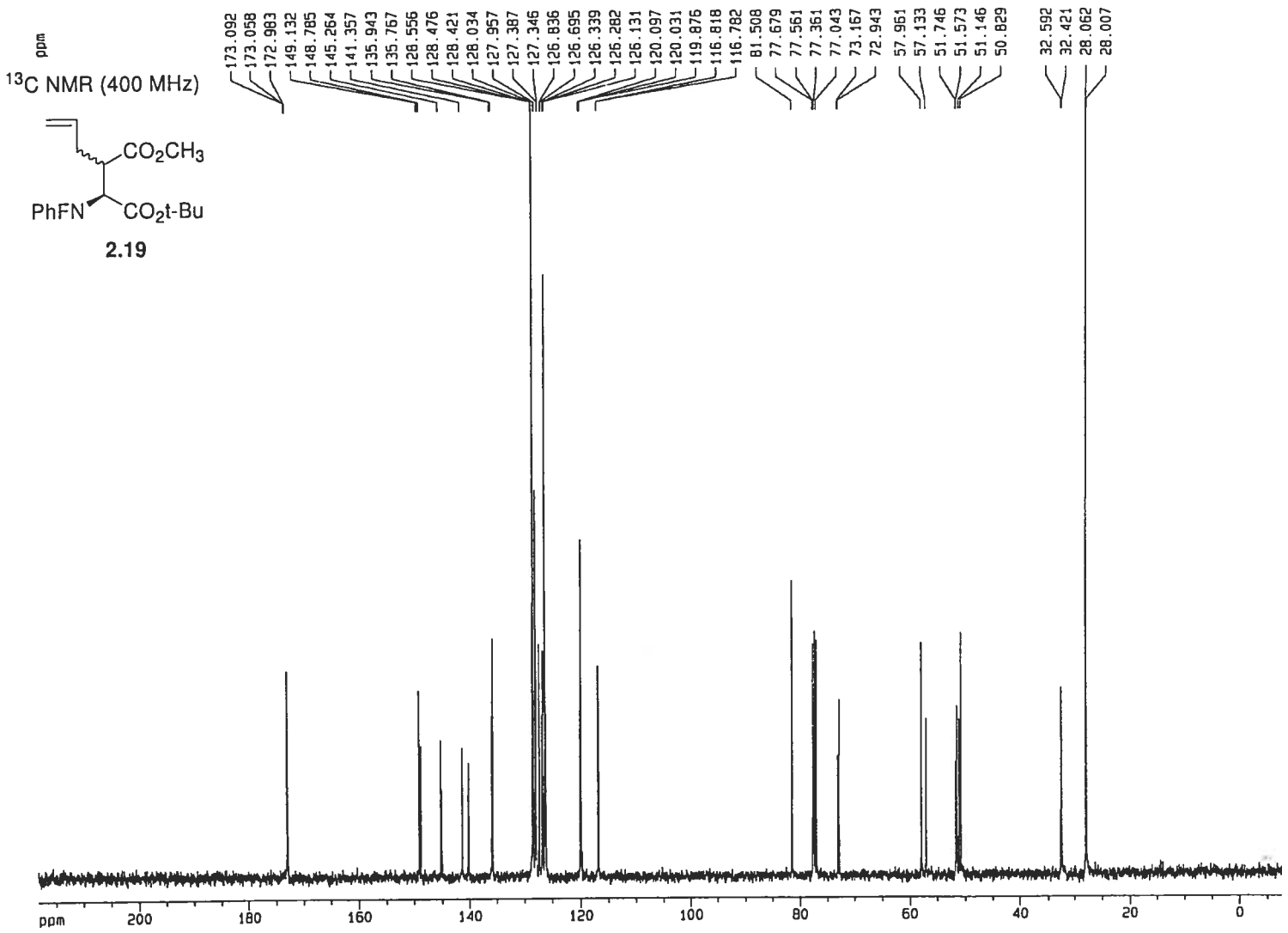
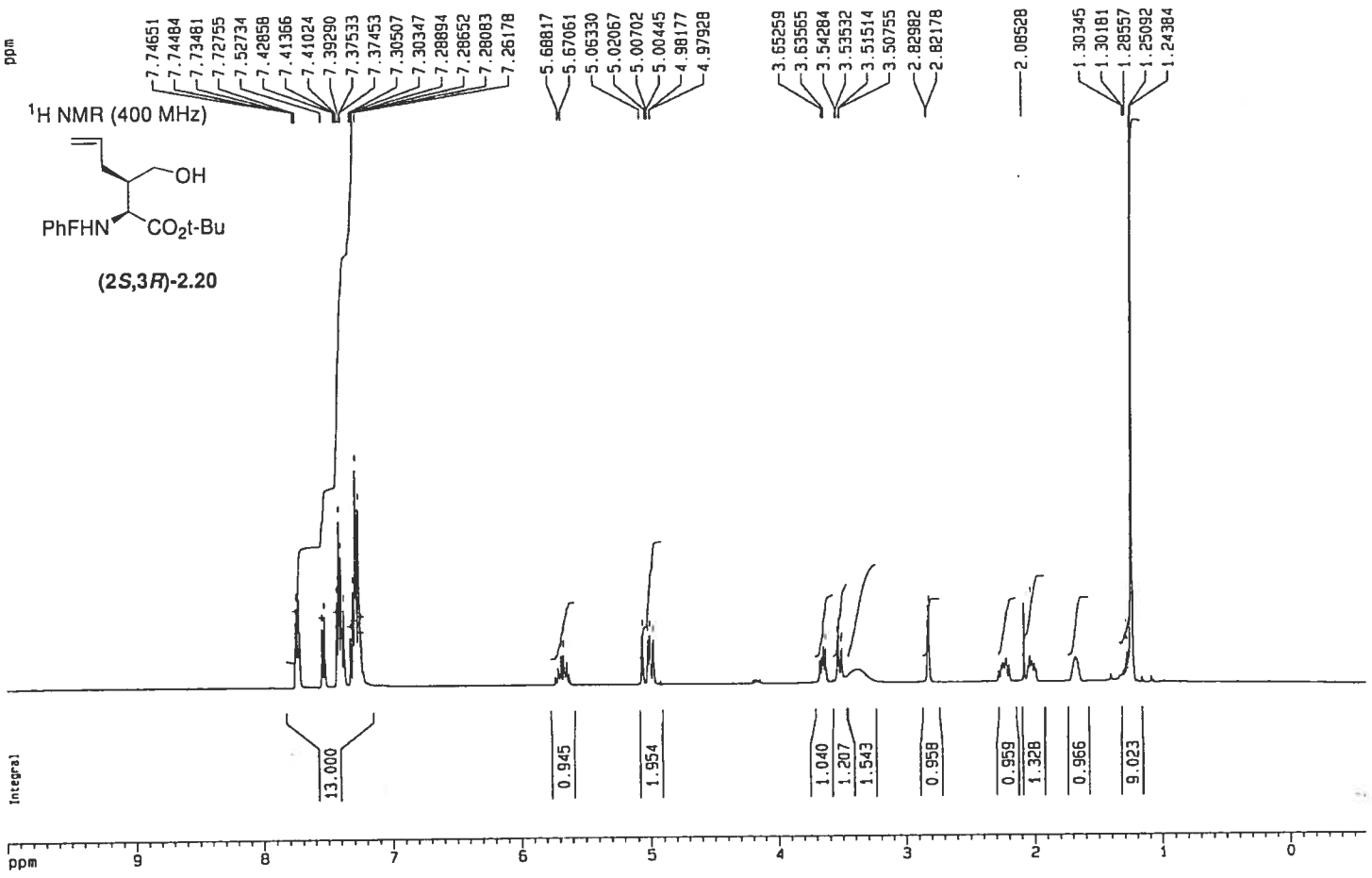
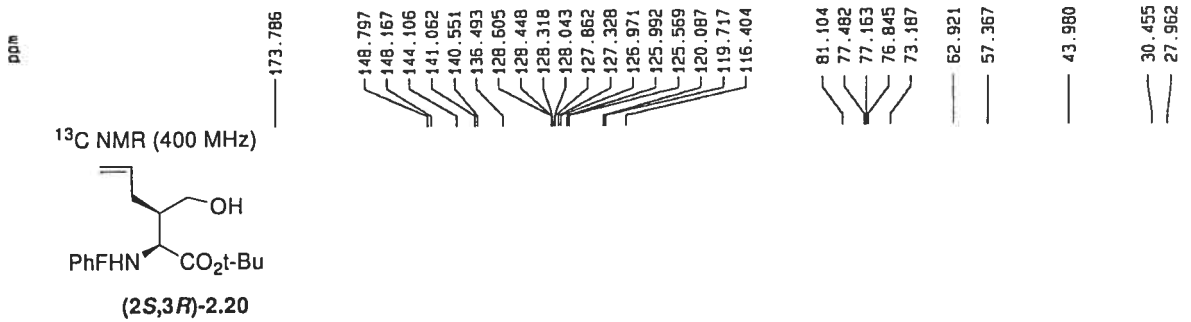
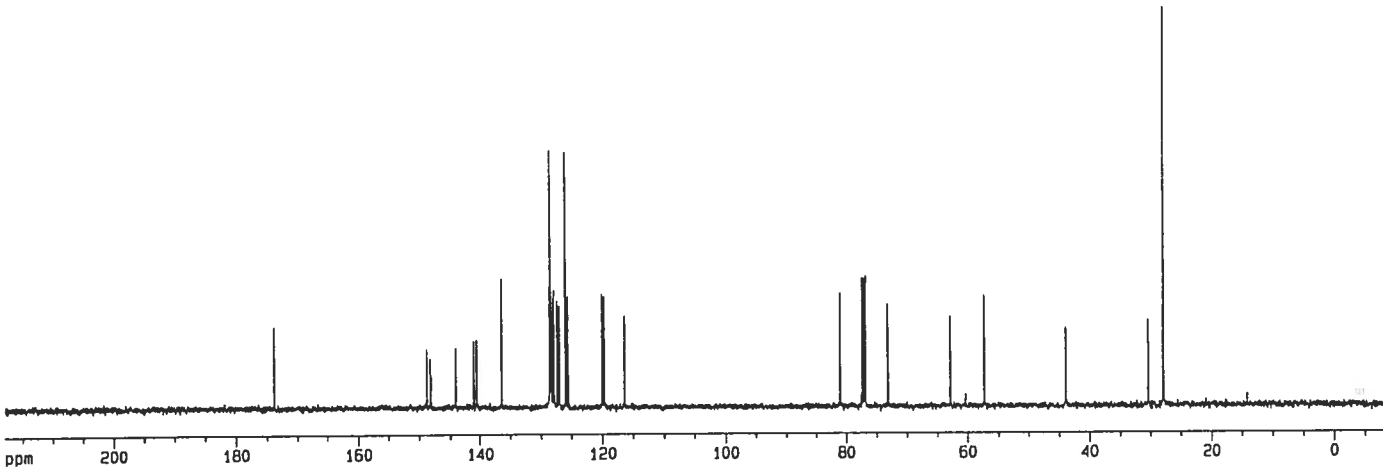
141.279
127.723
127.507
127.062
125.254
124.862
119.962
81.869
77.472
77.165
76.847
67.305
65.187
50.392
47.228
28.023
20.846
^{13}C NMR (400 MHz)
Fmoc
N
CO_2t-Bu
2.18
ppm
200
180
160
140
120
100
80
60
40
20
0

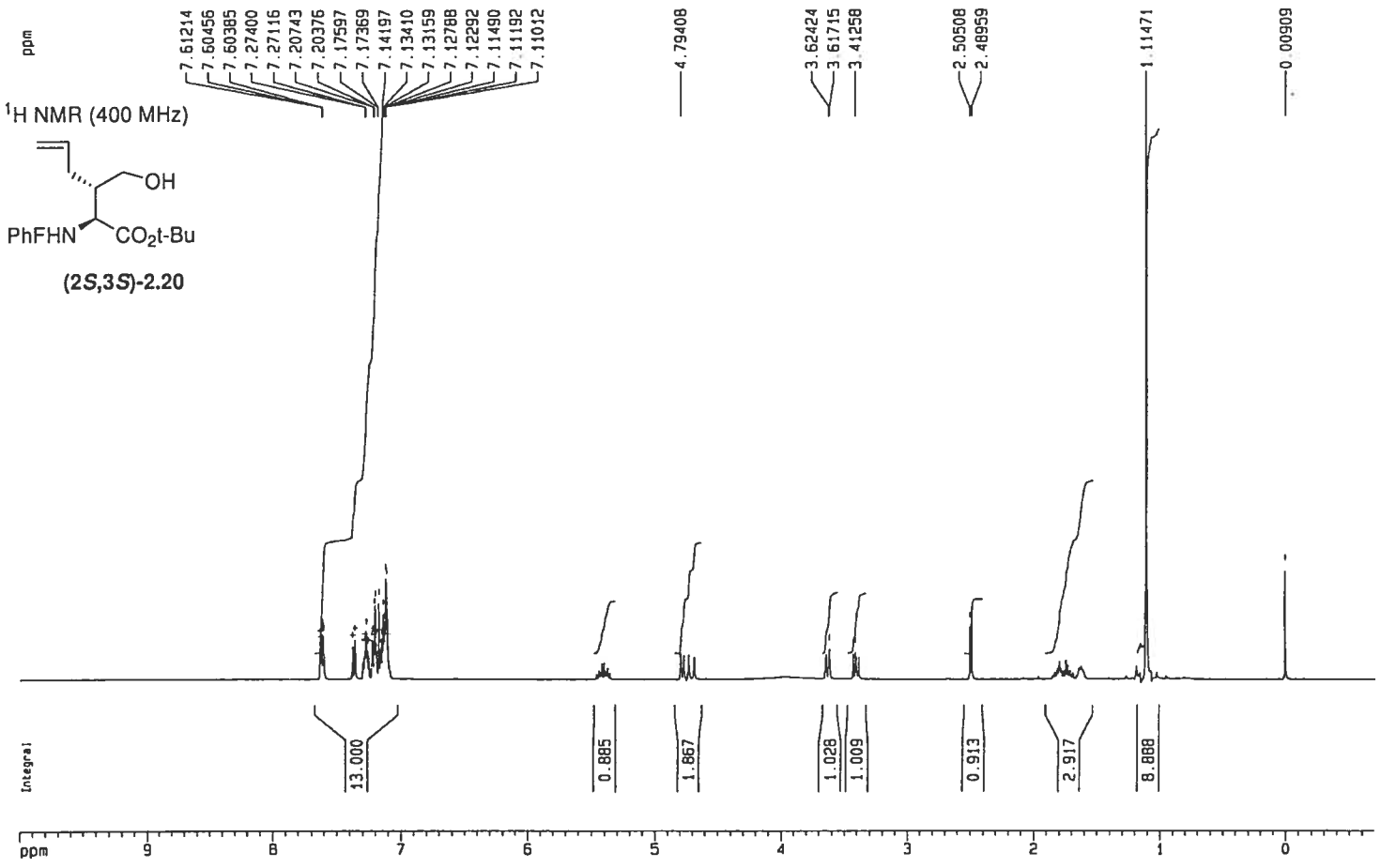

ppm
7.74079
7.48411
7.48080
7.47629
7.45997
7.45654
7.40518
7.39045
7.38742
7.37544
7.37228
7.29439
7.28517
7.27600
7.27333
7.26658
7.24747
5.71121
5.69416
5.06265
5.05877
5.02937
5.02519
5.02100
5.01604
5.00395
4.98261
4.97260
4.96841
4.94715
3.70384
3.58037
3.29464
3.27103
2.95430
2.94710
2.65132
2.64007
2.63660
2.62541
2.57729
2.49340
2.47483
2.46690
2.44934
2.09082
1.27826
1.22699
^{1}H NMR (400 MHz)
CO_2CH_3
PhFN
CO_2t-Bu
2.19
Integral
23.584
1.648
3.411
3.000
2.095
1.696
1.692
0.000
4.120
0.003
1.132
15.709
ppm
9
8
7
6
5
4
3
2
1
0


ppm
^{13}C NMR (400 MHz)
CO_2CH_3
PhFN
CO_2t-Bu
2.19
173.092
173.058
172.983
149.132
148.785
145.264
141.357
135.943
135.767
128.556
128.476
128.421
128.034
127.957
127.387
127.346
126.836
126.695
126.339
126.282
126.131
120.097
120.031
119.876
116.818
116.782
81.508
77.679
77.561
77.361
77.043
73.167
72.943
57.961
57.133
51.746
51.573
51.146
50.829
32.592
32.421
28.062
28.007
ppm
200
180
160
140
120
100
80
60
40
20
0

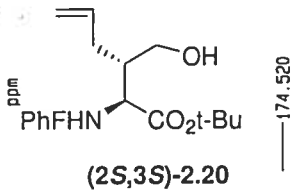

ppm
7.74651
7.74484
7.73481
7.72755
7.52734
7.42858
7.41366
7.41024
7.39290
7.37533
7.37453
7.30507
7.30347
7.28894
7.28652
7.28083
7.26178
5.68817
5.67061
5.06330
5.02067
5.00702
5.00445
4.98177
4.97928
3.65259
3.63565
3.54284
3.53532
3.51514
3.50755
2.82982
2.82178
2.08528
1.30345
1.30181
1.28557
1.25092
1.24384
^{1}H NMR (400 MHz)
OH
PhFHN
CO_2t-Bu
(2S,3R)-2.20
Integral
13.000
0.945
1.954
1.040
1.207
1.543
0.958
0.959
1.328
0.966
9.023
ppm
9
8
7
6
5
4
3
2
1
0


ppm
173.786
148.797
148.167
144.106
141.062
140.551
136.493
128.605
128.448
128.318
128.043
127.862
127.328
126.971
125.992
125.569
120.087
119.717
116.404
81.104
77.482
77.163
76.845
73.187
62.921
57.367
43.980
30.455
27.962
13C NMR (400 MHz)
OH
PhFHN
CO2t-Bu
(2S,3R)-2.20


ppm
200
180
160
140
120
100
80
60
40
20
0

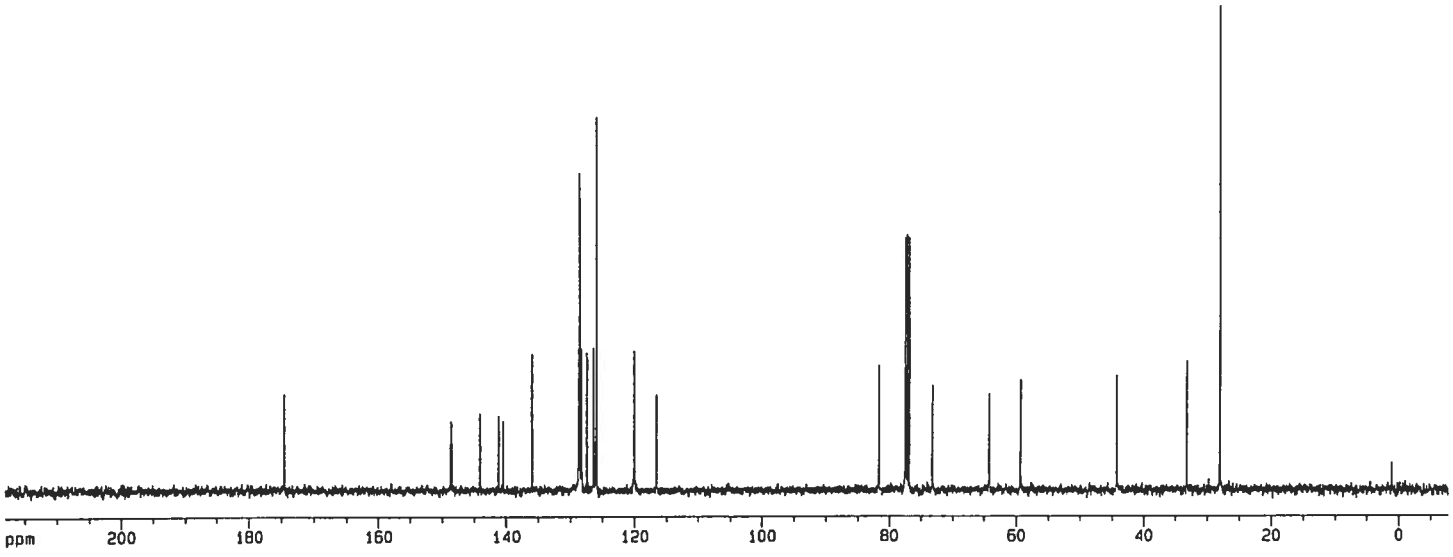

ppm
7.61214
7.60456
7.60385
7.27400
7.27116
7.20743
7.20376
7.17597
7.17369
7.14197
7.13410
7.13159
7.12788
7.12292
7.11490
7.11192
7.11012
4.79408
3.62424
3.61715
3.41258
2.50508
2.48959
1.11471
0.00909
^{1}H NMR (400 MHz)
OH
PhFHN
CO_2t-Bu
(2S,3S)-2.20
Integral
13.000
0.885
1.867
1.028
1.009
0.913
2.917
8.888
ppm
9
8
7
6
5
4
3
2
1
0

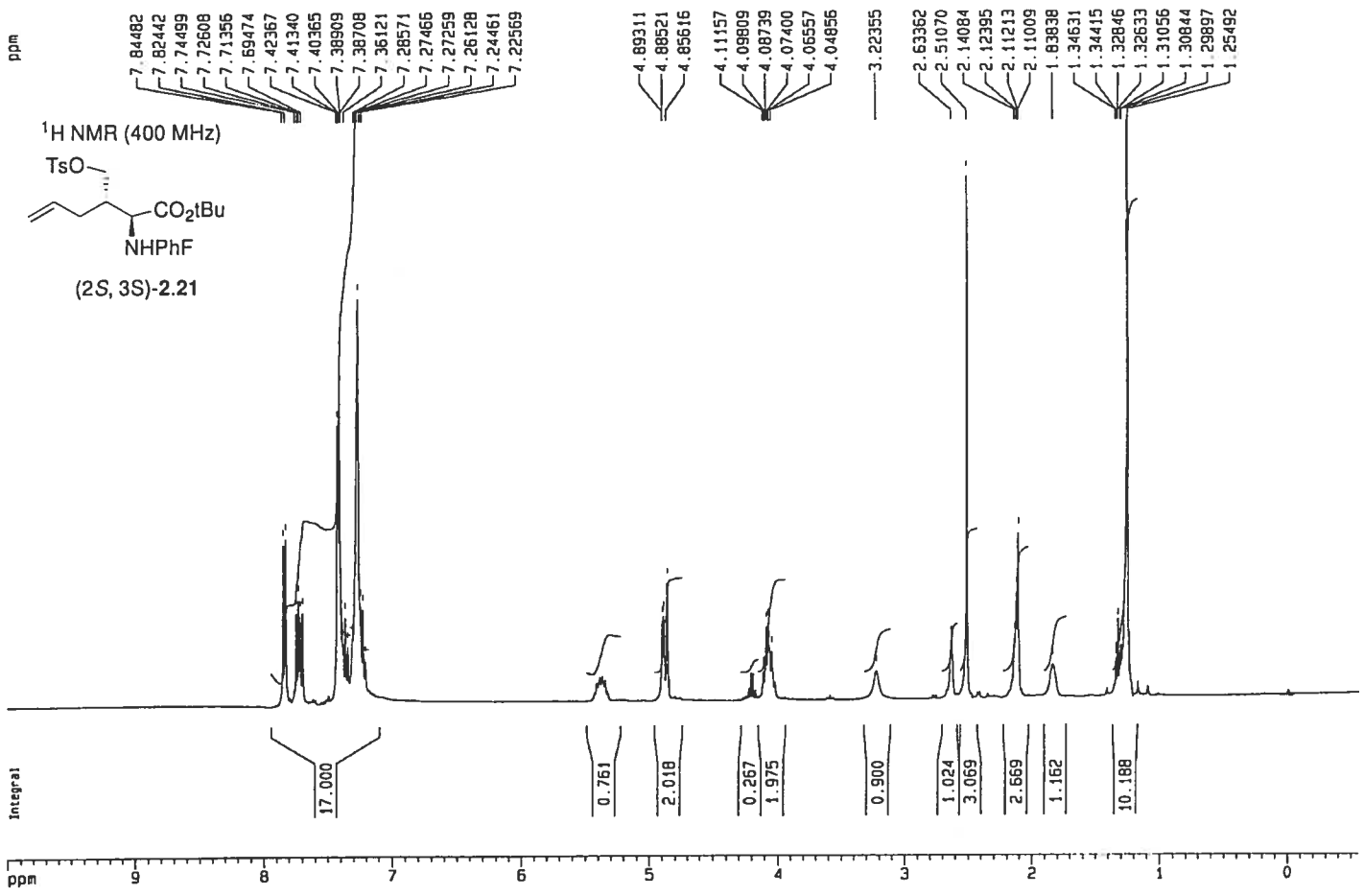
^{13}C NMR (400 MHz)

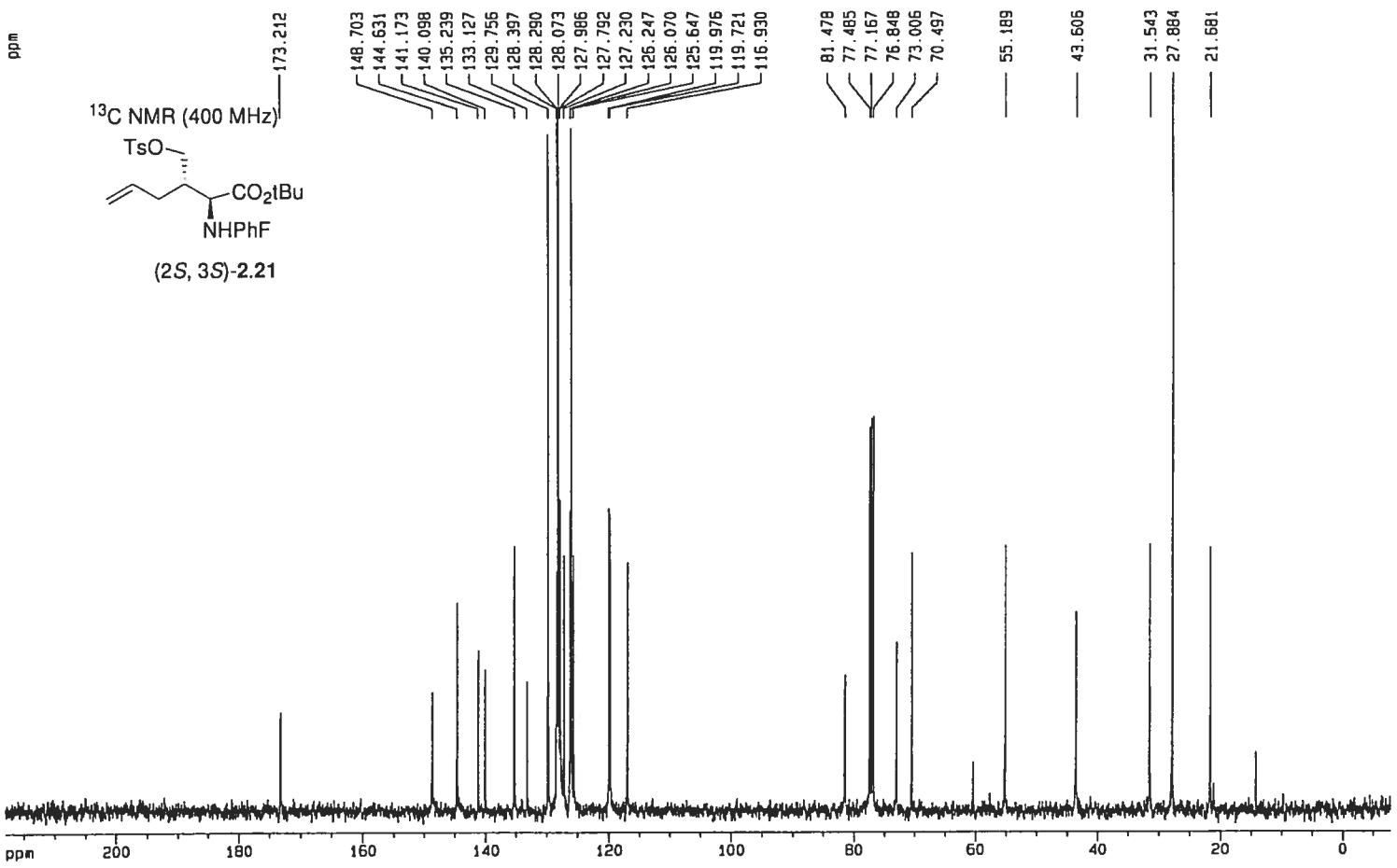


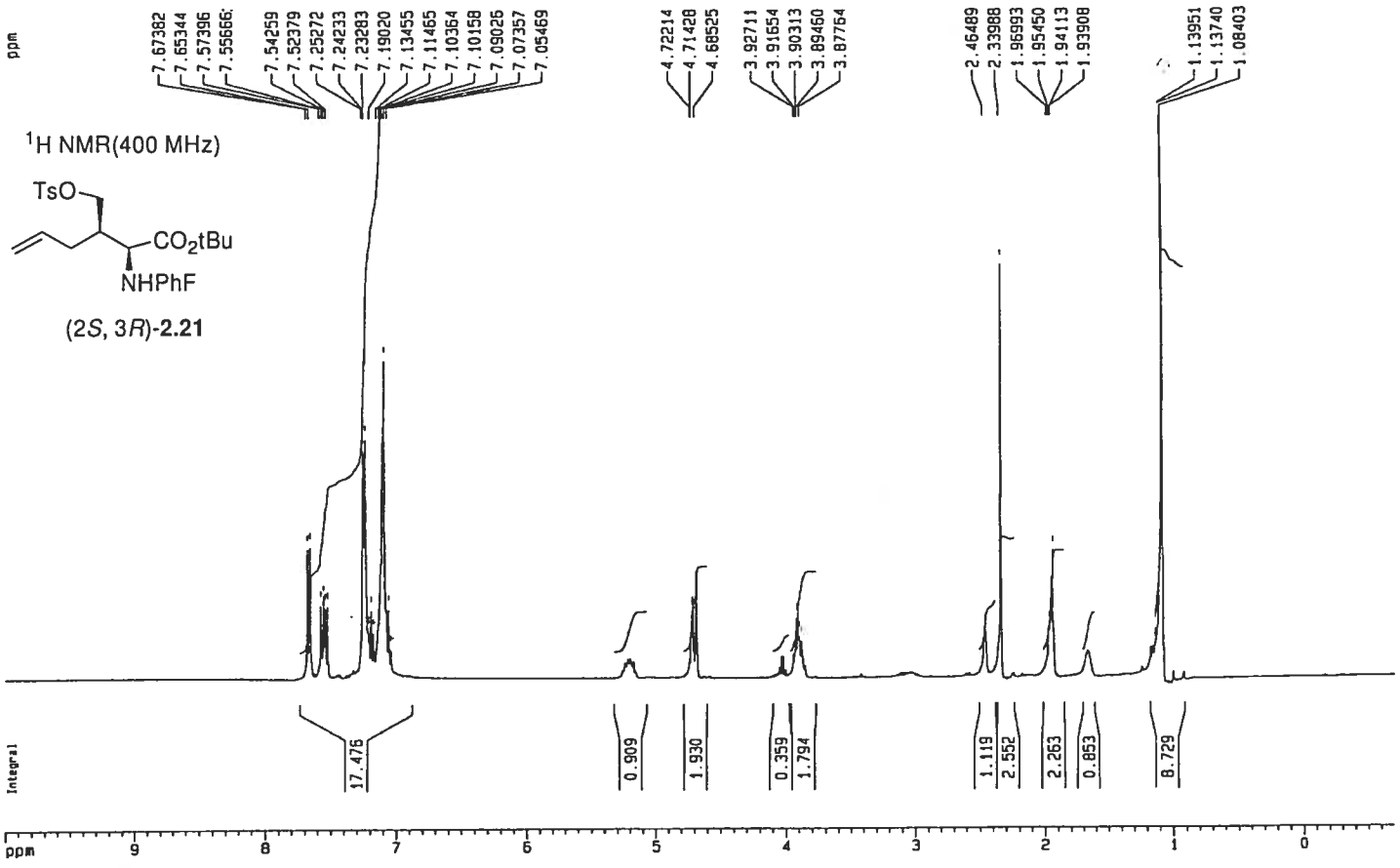
(2*S*,3*S*)-2.20

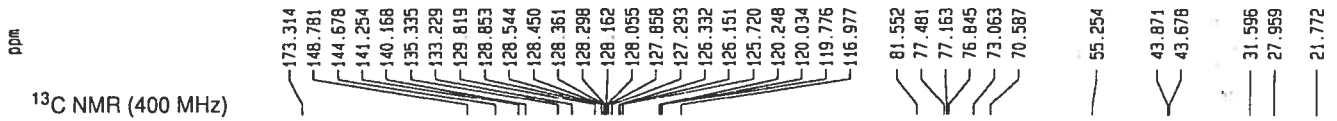
174.520
148.641
148.468
144.116
141.201
140.465
135.981
128.714
128.572
128.488
128.285
128.197
127.388
126.398
125.948
120.070
119.949
116.564
81.647
77.481
77.367
77.163
76.845
73.145
64.182
59.315
44.266
33.178
28.034



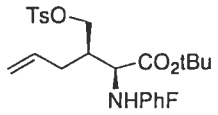

ppm
7.84482
7.82442
7.74499
7.72608
7.71356
7.69474
7.42367
7.41340
7.40365
7.38909
7.38708
7.36121
7.28571
7.27466
7.27259
7.26128
7.24461
7.22569
4.89311
4.88521
4.85616
4.11157
4.09809
4.08739
4.07400
4.06557
4.04856
3.22355
2.63362
2.51070
2.14084
2.12395
2.11213
2.11009
1.83838
1.34631
1.34415
1.32846
1.32633
1.31056
1.30844
1.29897
1.25492
^{1}H NMR (400 MHz)
TsO
CO_2tBu
NHPhF
(2*S*, 3S)-**2.21**
Integral
17.000
0.761
2.018
0.267
1.975
0.900
1.024
3.069
2.669
1.162
10.188
ppm
9
8
7
6
5
4
3
2
1
0


ppm
173.212
148.703
144.631
141.173
140.098
135.239
133.127
129.756
128.397
128.290
128.073
127.986
127.792
127.230
126.247
126.070
125.647
119.976
119.721
116.930
81.478
77.485
77.167
76.848
73.006
70.497
55.189
43.606
31.543
27.884
21.681
^{13}C NMR (400 MHz)
TsO
CO_2tBu
NHPhF
(2S, 3S)-2.21
ppm
200
180
160
140
120
100
80
60
40
20
0

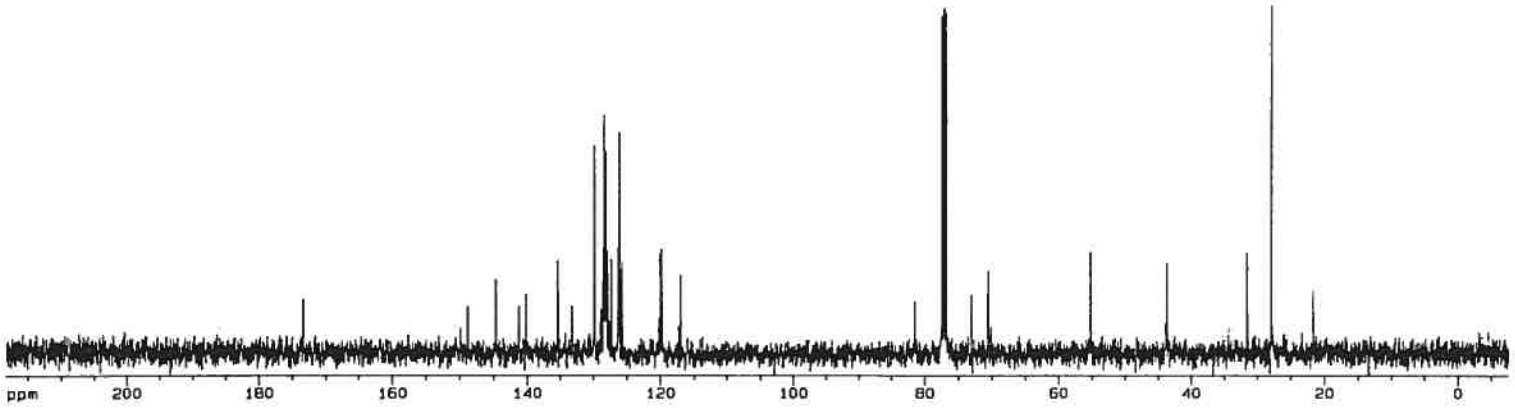

ppm
7.67382
7.65344
7.57396
7.55666
7.54259
7.52379
7.25272
7.24233
7.23283
7.19020
7.13455
7.11465
7.10364
7.10158
7.09026
7.07357
7.05469
4.72214
4.71428
4.68525
3.92711
3.91654
3.90313
3.89460
3.87764
2.46489
2.33988
1.96993
1.95450
1.94113
1.93908
1.13951
1.13740
1.08403
^{1}H NMR(400 MHz)
TsO
CO_2tBu
NHPhF
(2S, 3R)-2.21
Integral
17.476
0.909
1.930
0.359
1.794
1.119
2.552
2.263
0.853
8.729
ppm
9
8
7
6
5
4
3
2
1
0

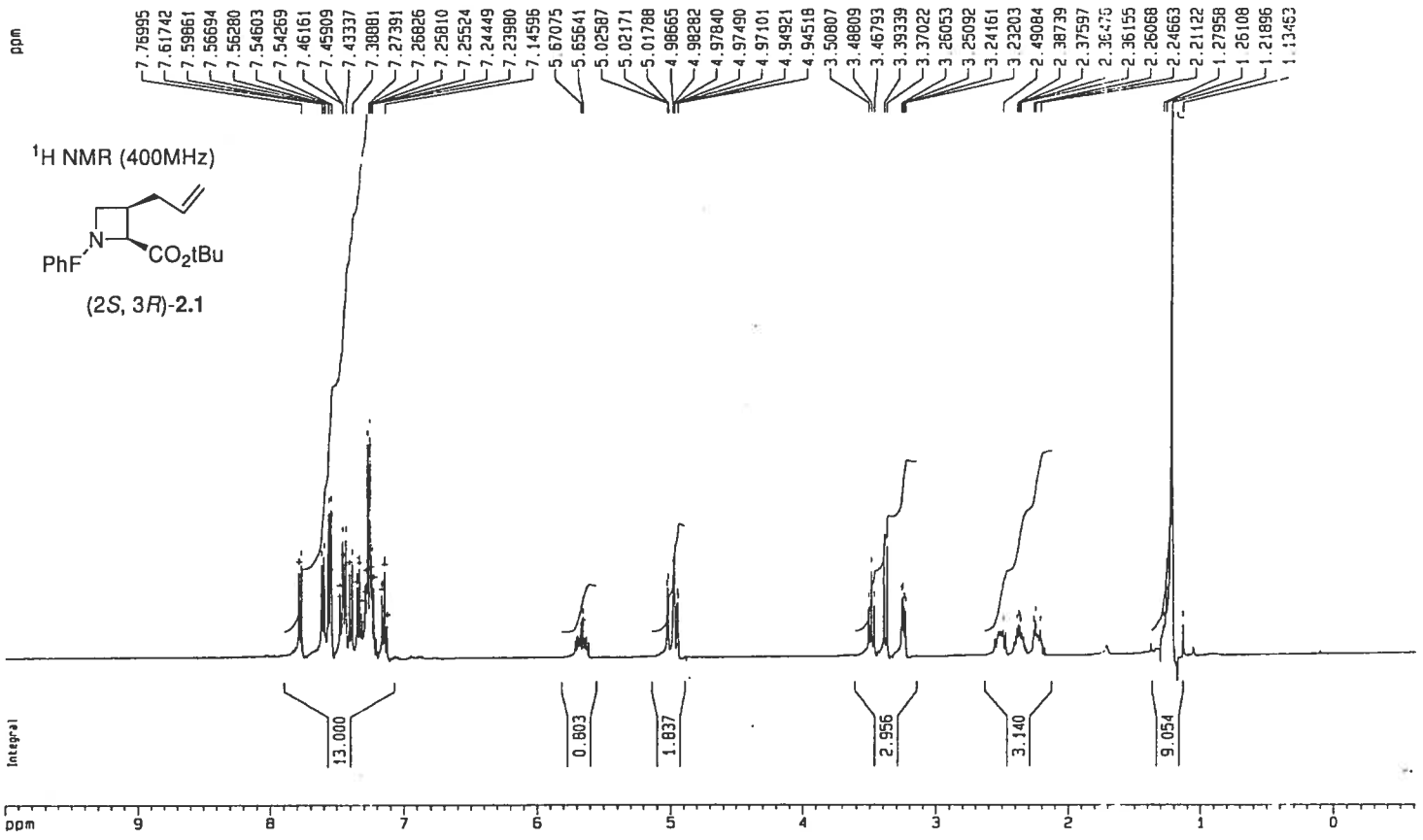


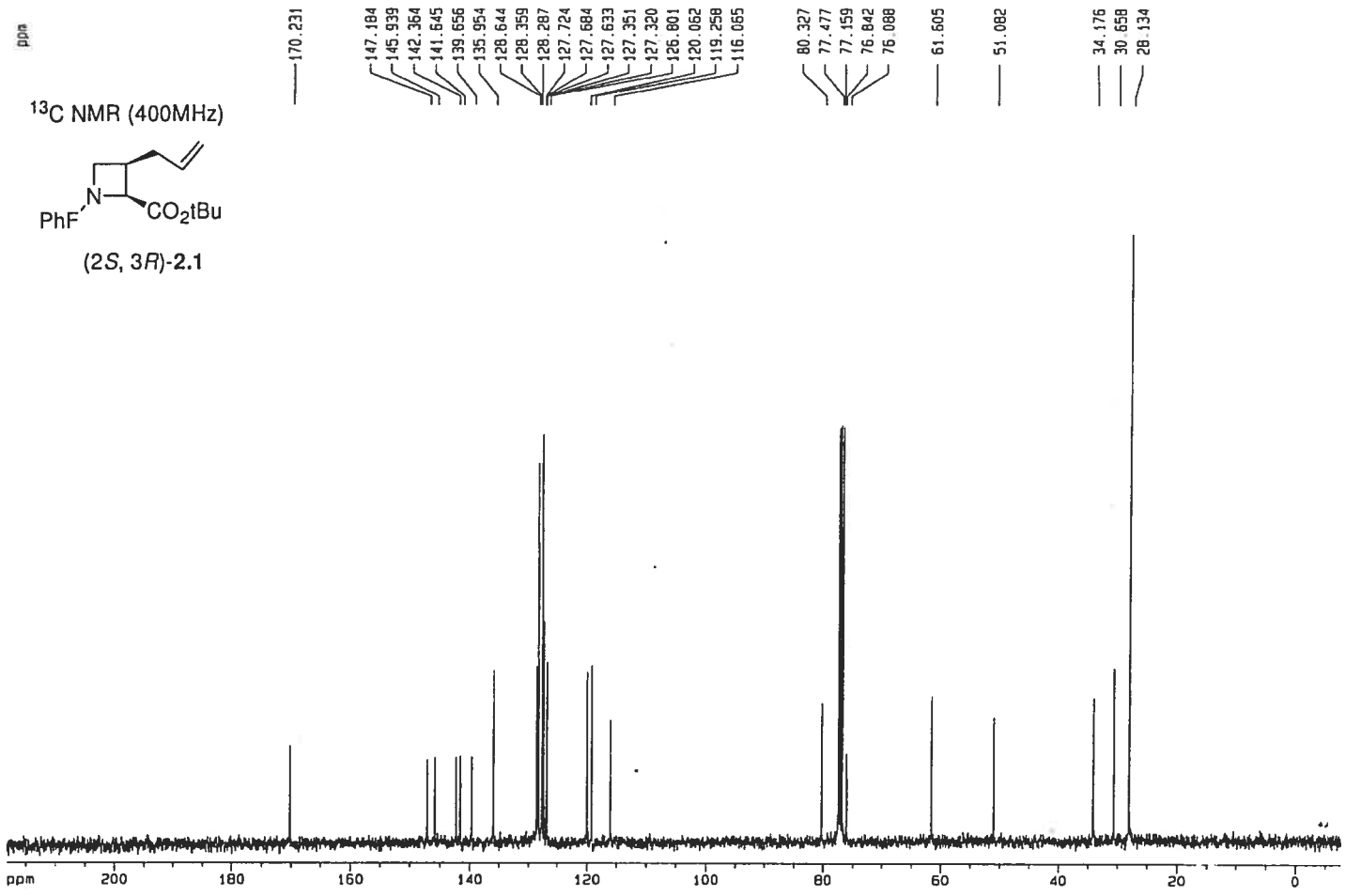
^{13}C NMR (400 MHz)



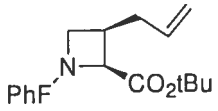
(2*S*, 3*R*)-**2.21**



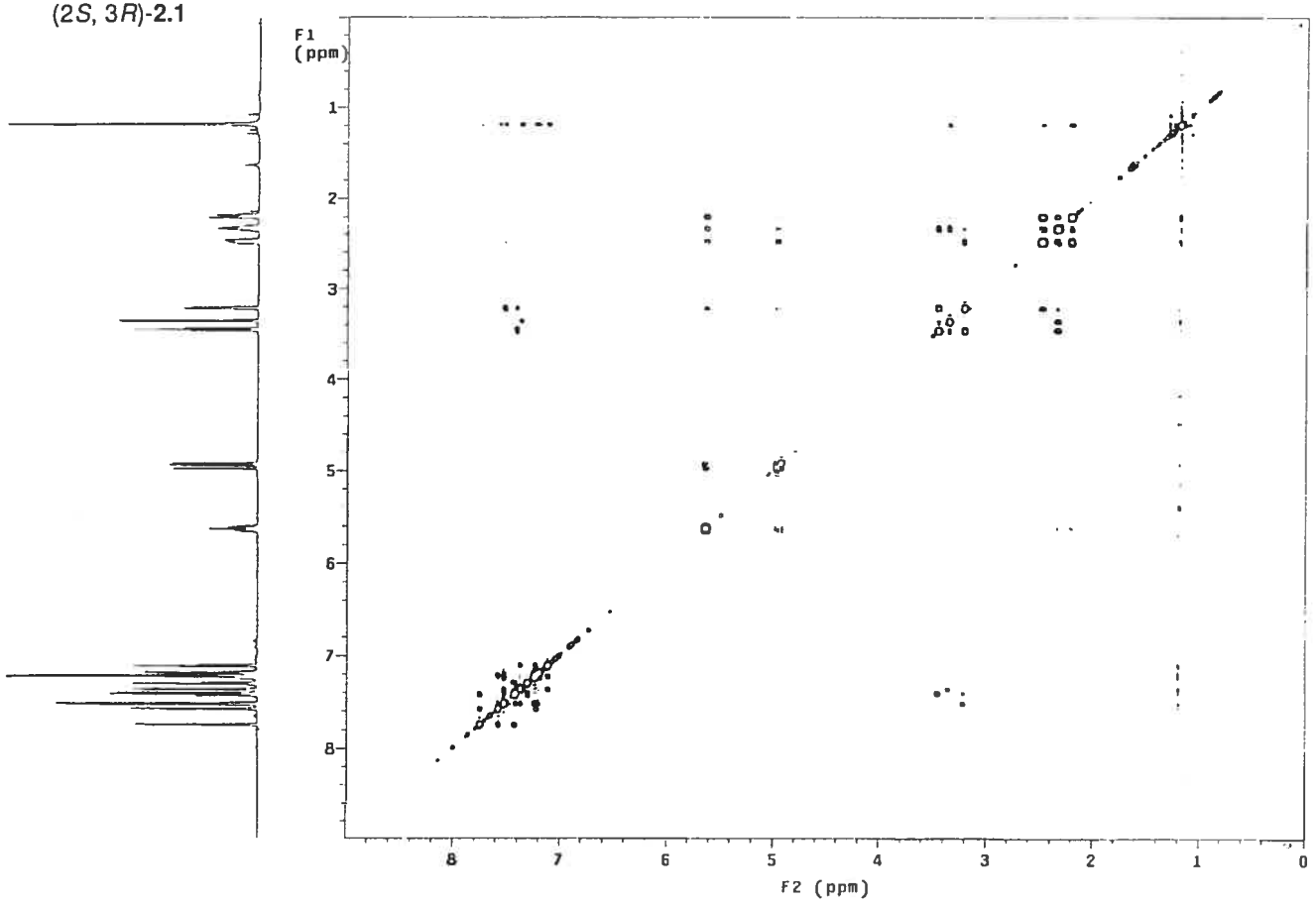

ppm
7.76995
7.61742
7.59861
7.56694
7.56280
7.54603
7.54269
7.46161
7.45909
7.43337
7.38881
7.27391
7.26826
7.25810
7.25524
7.24449
7.23980
7.14596
5.67075
5.65641
5.02587
5.02171
5.01788
4.98665
4.98282
4.97840
4.97490
4.97101
4.94921
4.94518
3.50807
3.48809
3.46793
3.39339
3.37022
3.26053
3.25092
3.24161
3.23203
2.49084
2.38739
2.37597
2.36155
2.26068
2.24663
2.21122
1.27958
1.26108
1.21896
1.13453
^{1}H NMR (400MHz)
N
PhF
CO$_2$tBu
(2*S*, 3*R*)-**2.1**
Integral
13.000
0.803
1.837
2.956
3.140
9.054
ppm
9
8
7
6
5
4
3
2
1
0


ppm
170.231
147.184
145.939
142.364
141.645
139.656
135.954
128.644
128.359
128.287
127.724
127.684
127.633
127.351
127.320
126.801
120.062
119.258
116.065
80.327
77.477
77.159
76.842
76.088
61.605
51.082
34.176
30.658
28.134
^{13}C NMR (400MHz)
PhF
N
CO_2tBu
(2S, 3R)-2.1
ppm
200
180
160
140
120
100
80
60
40
20
0

NOESY

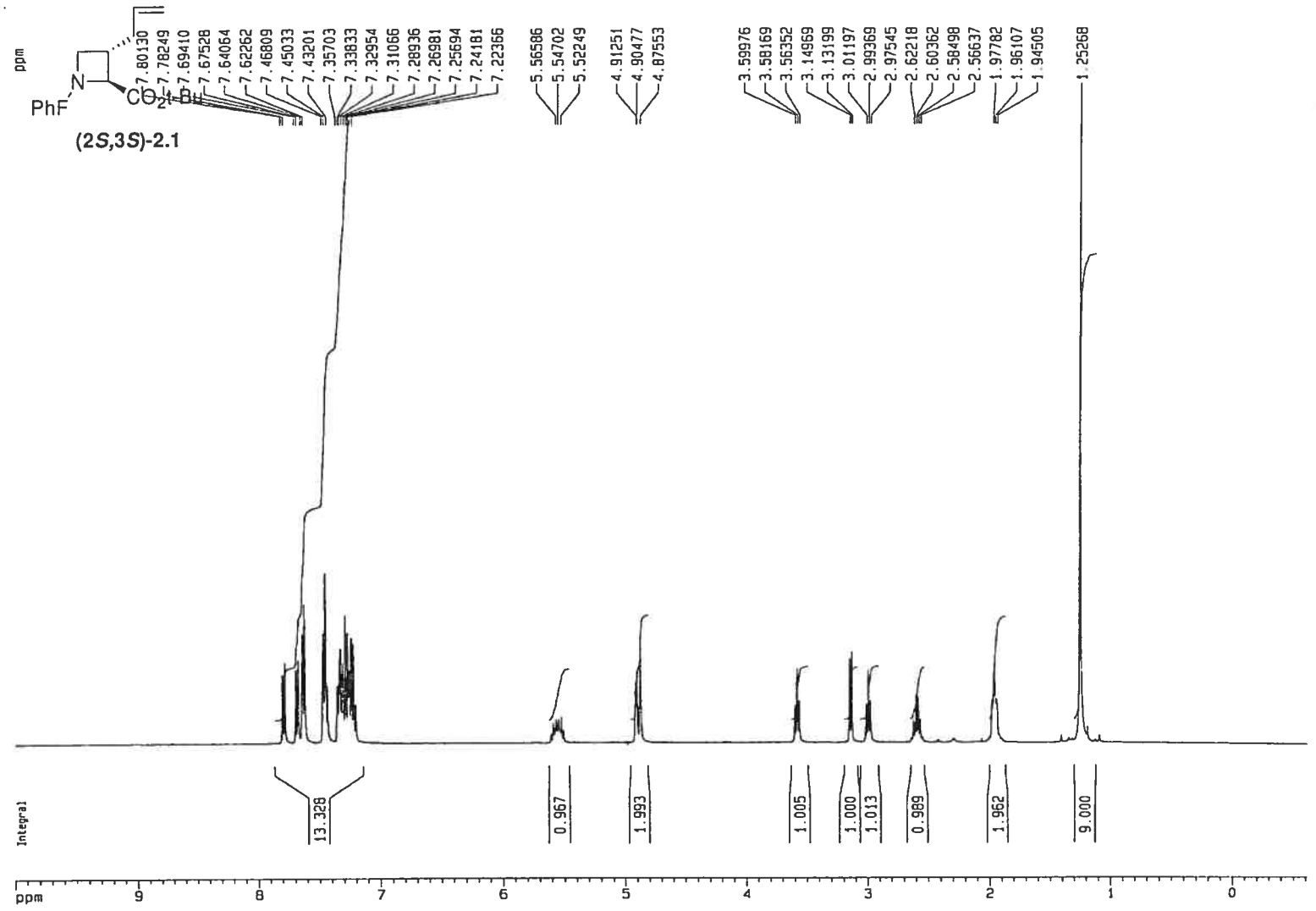


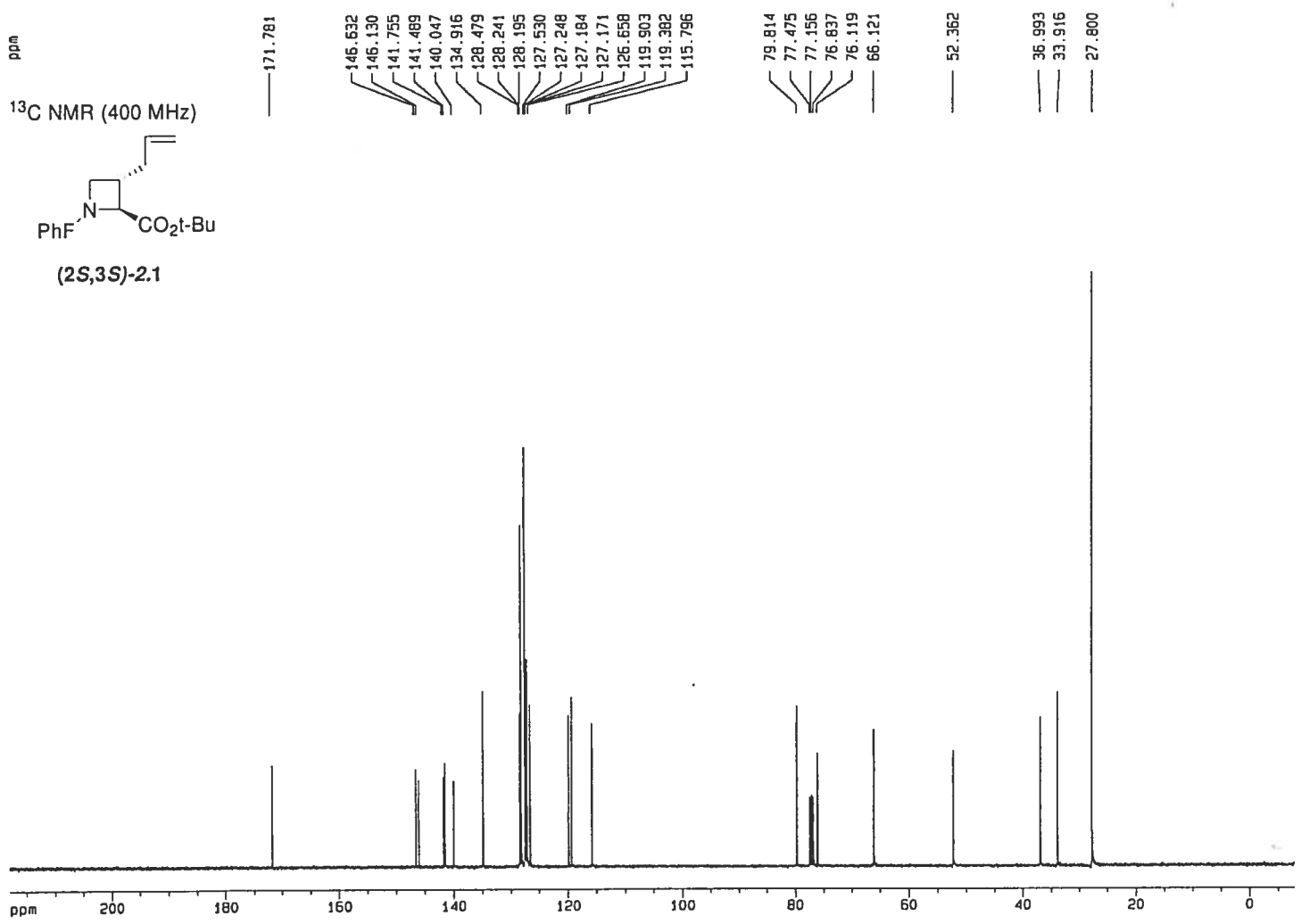
(2*S*, 3*R*)-**2.1**

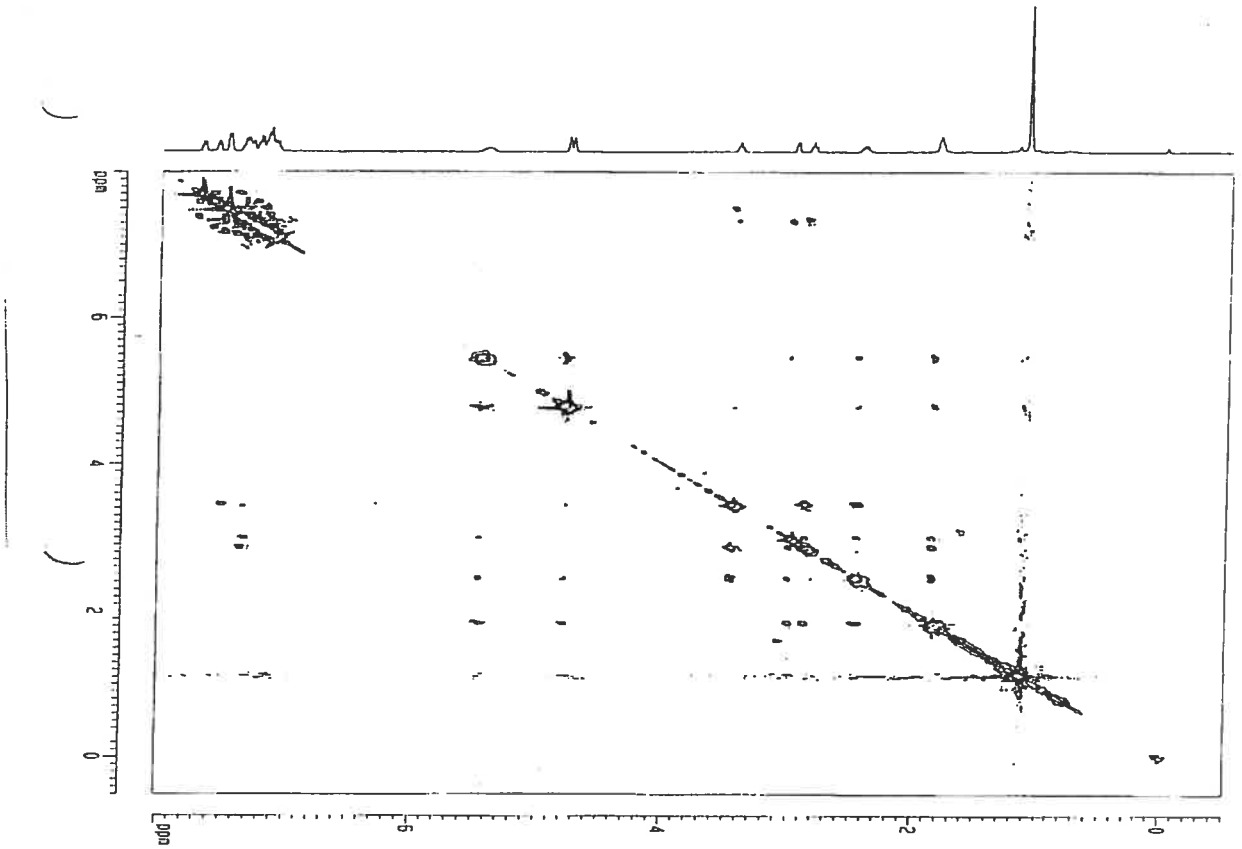


NOESY Idea.

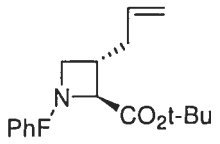
^{1}H NMR (400 MHz)



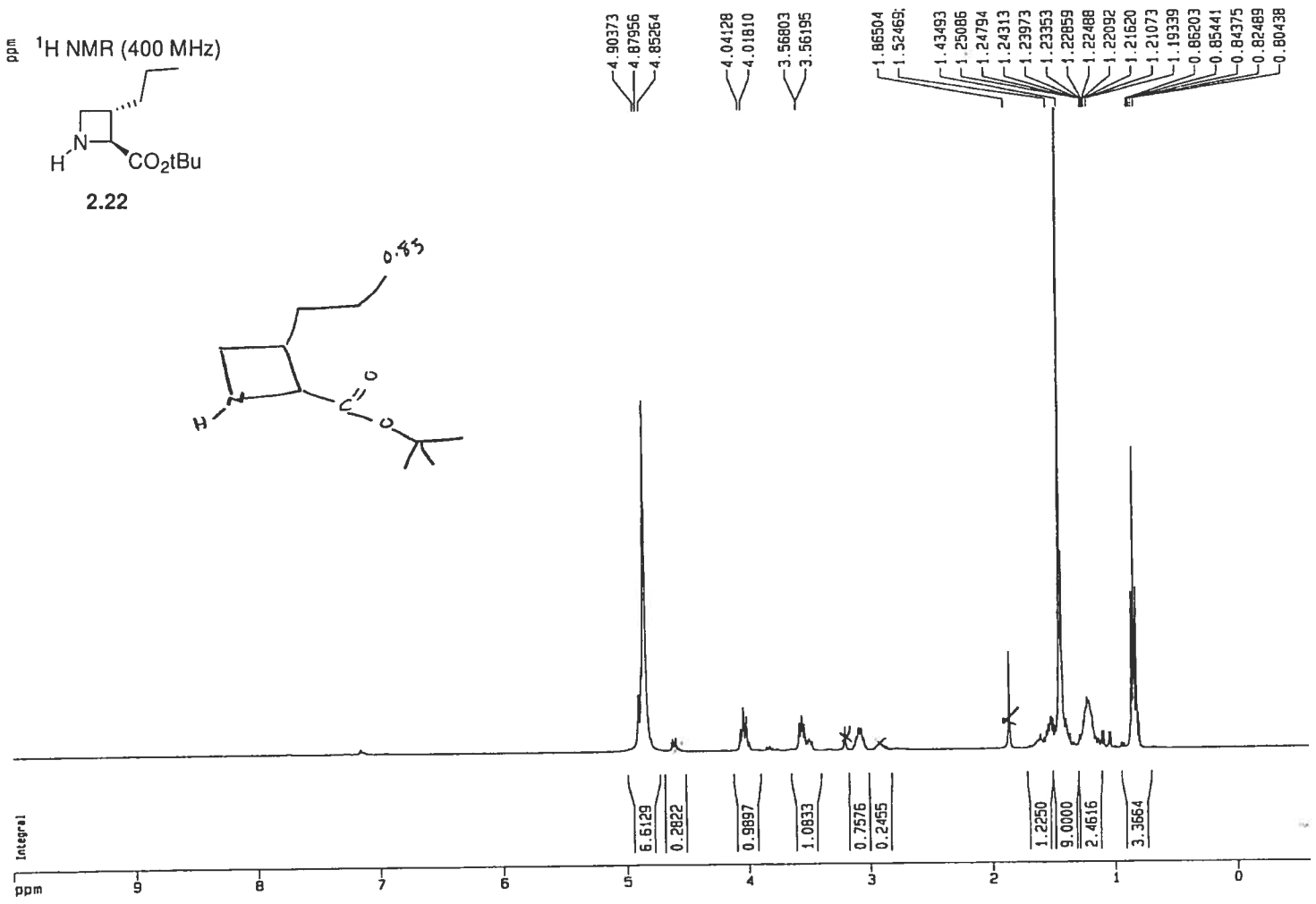

ppm
171.781
146.632
146.130
141.755
141.489
140.047
134.916
128.479
128.241
128.195
127.530
127.248
127.184
127.171
126.658
119.903
119.382
115.796
79.814
77.475
77.156
76.837
76.119
66.121
52.362
36.993
33.916
27.800
13C NMR (400 MHz)
PhF
N
CO2t-Bu
(2S,3S)-2.1
ppm
200
180
160
140
120
100
80
60
40
20
0



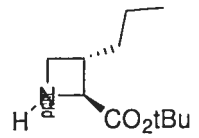
NOSEY



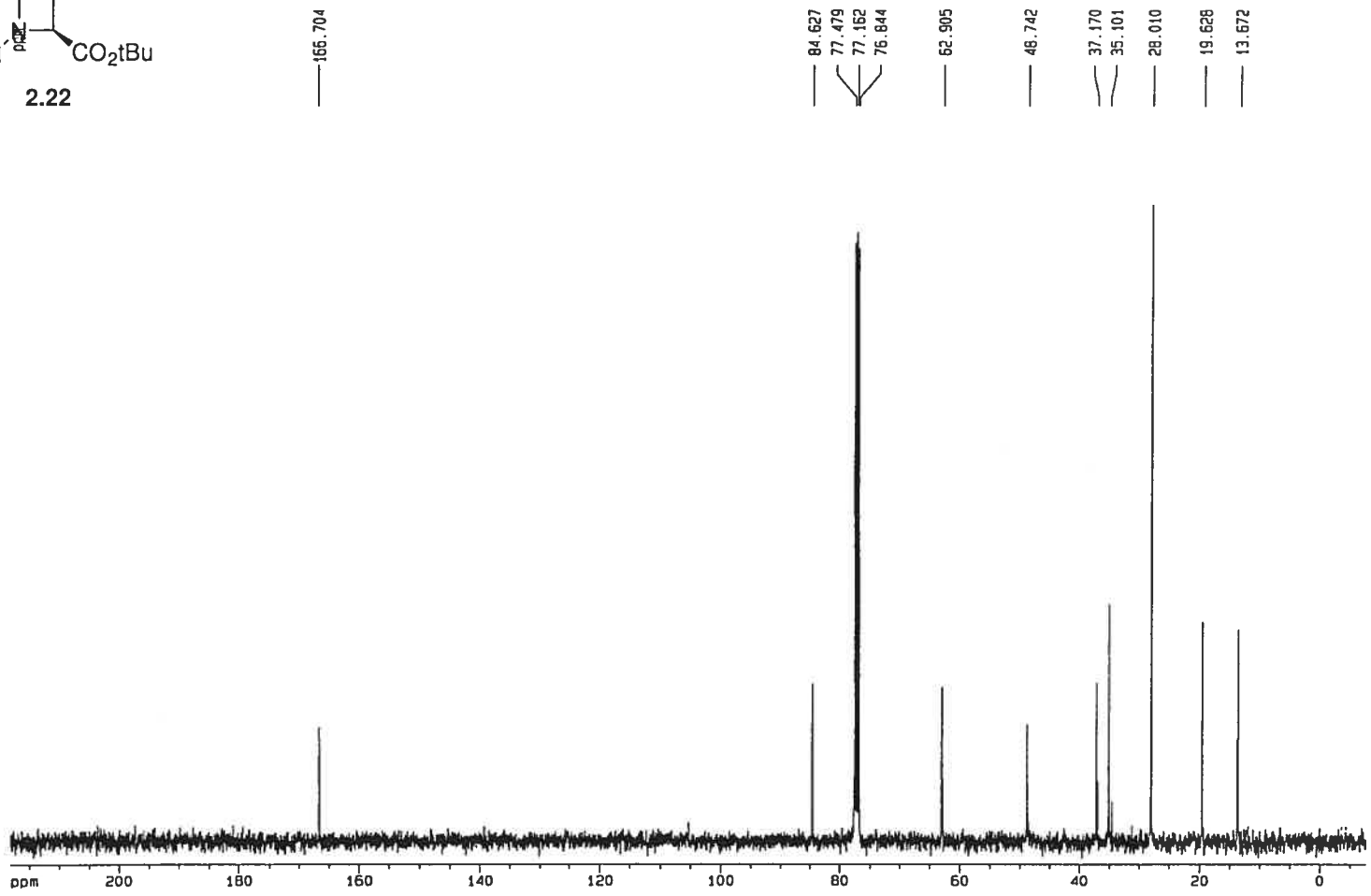
(2*S*,3*S*)-2.1

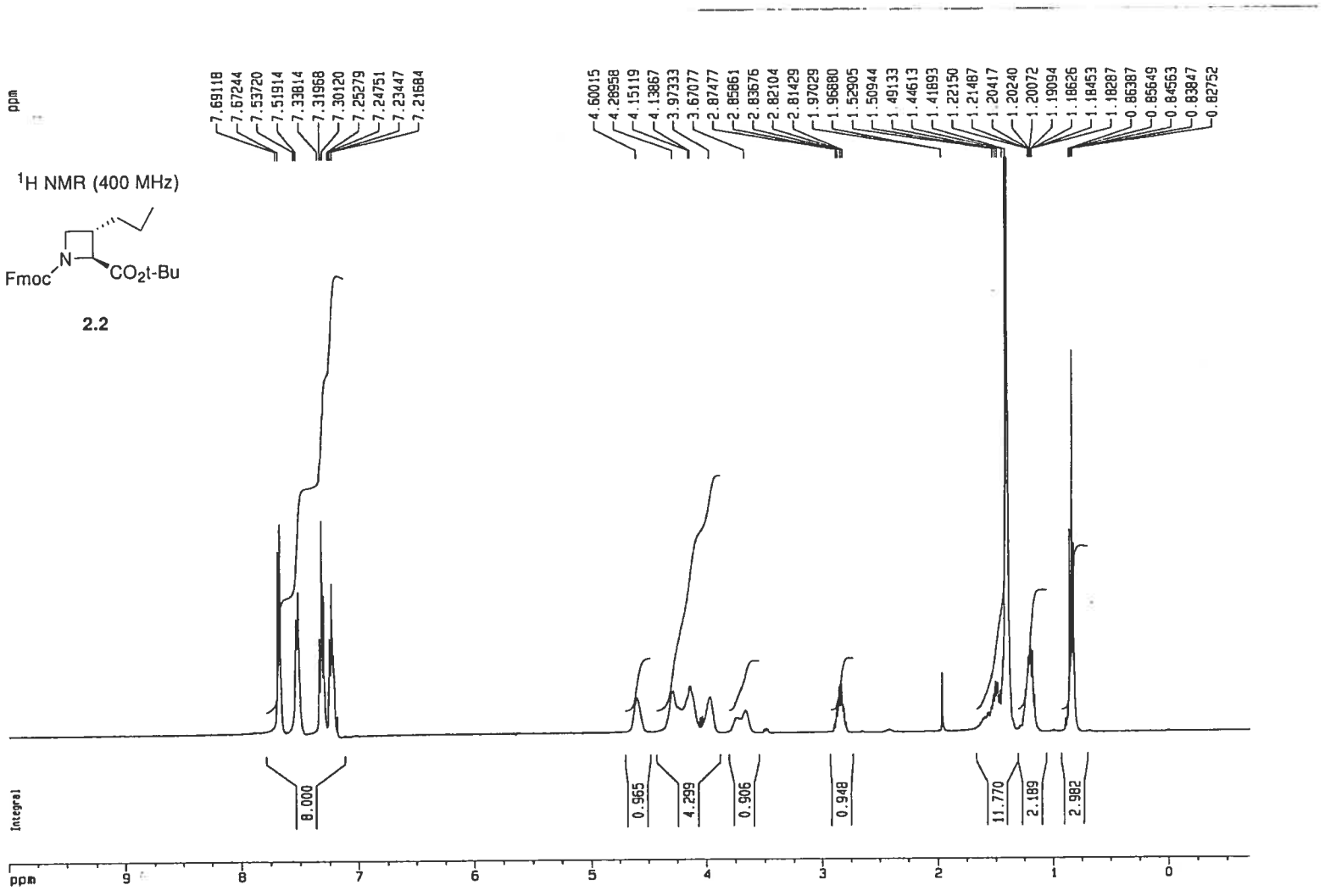

ppm
¹H NMR (400 MHz)
N
H
CO2tBu
2.22
0.83
4.90373
4.87956
4.85264
4.04128
4.01810
3.56803
3.56195
1.86504
1.52469
1.43493
1.25086
1.24794
1.24313
1.23973
1.23353
1.22859
1.22488
1.22092
1.21620
1.21073
1.19339
0.86203
0.85441
0.84375
0.82489
0.80438
Integral
6.6129
0.2822
0.9897
1.0833
0.7576
0.2465
1.2250
9.0000
2.4616
3.3664
ppm
9
8
7
6
5
4
3
2
1
0

^{13}C NMR (400 MHz)

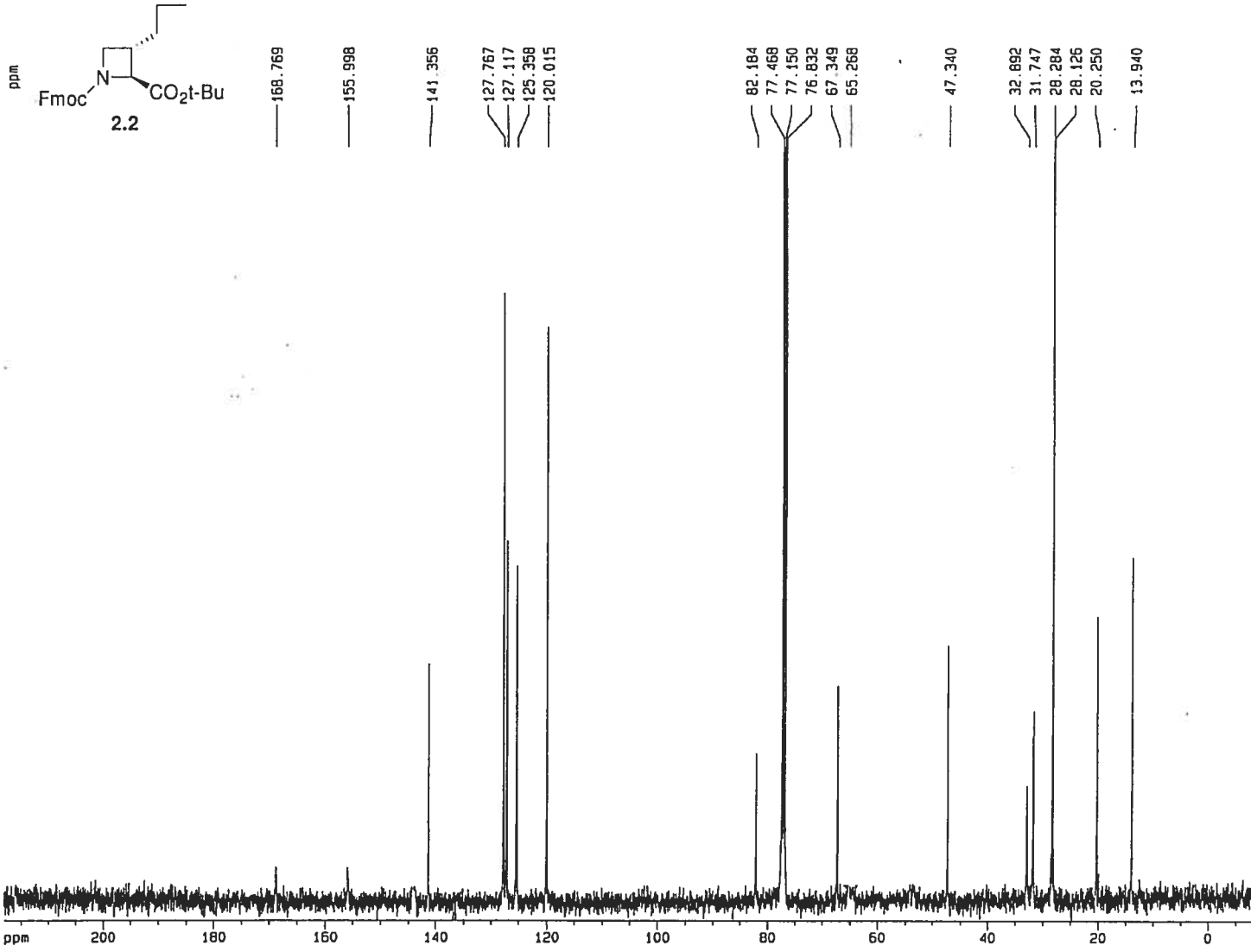


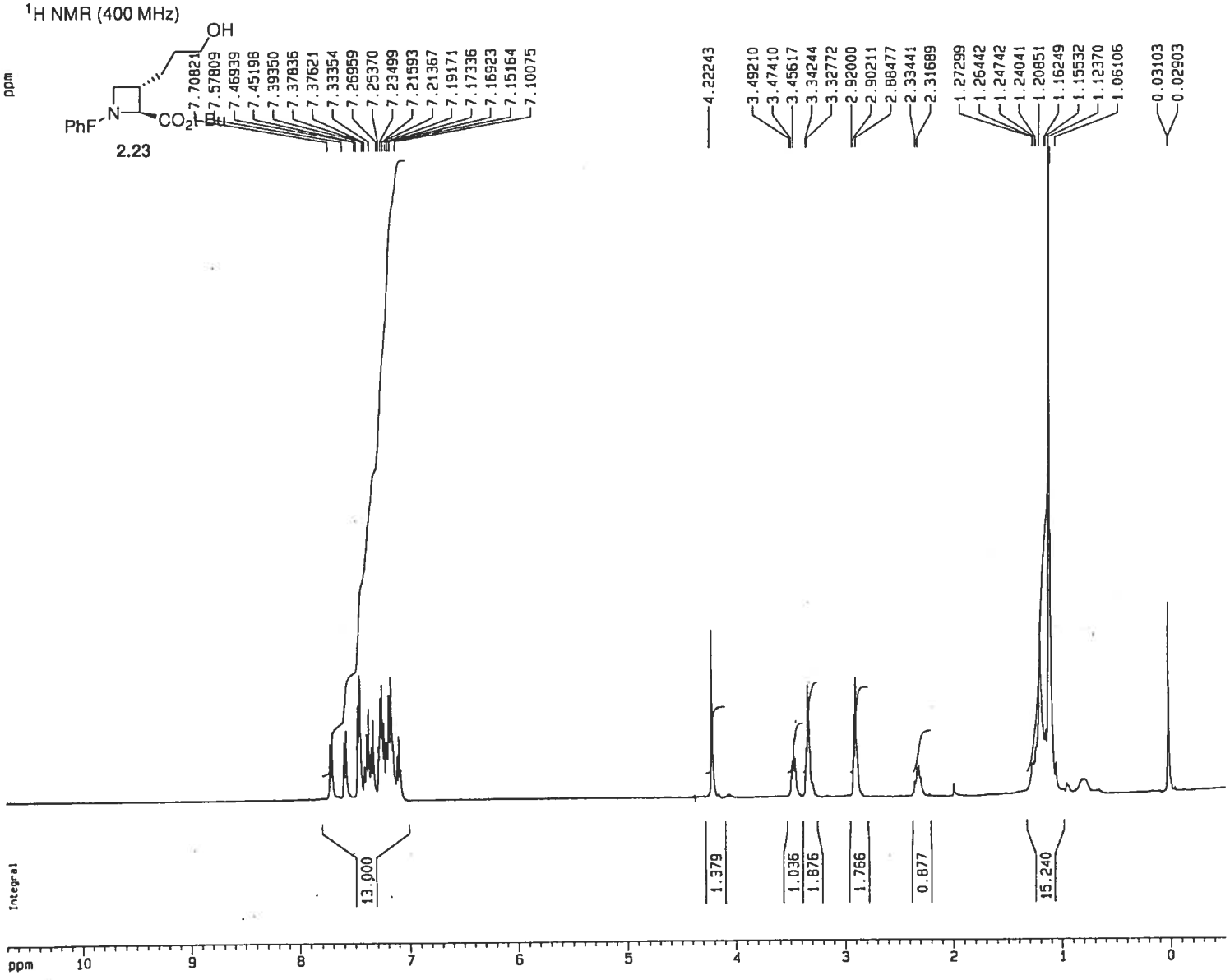
2.22

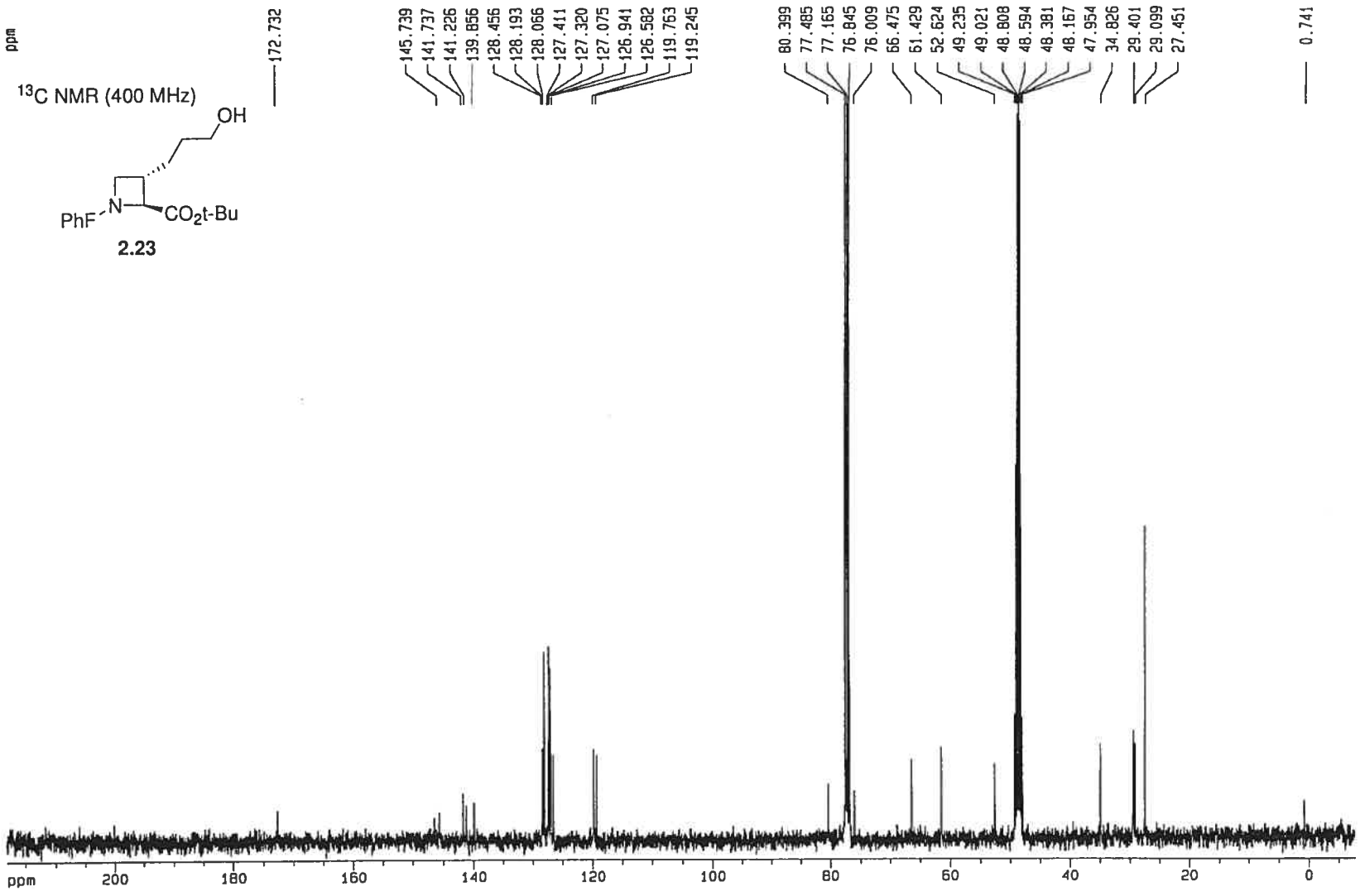



ppm
7.69118
7.67244
7.53720
7.51914
7.33814
7.31968
7.30120
7.25279
7.24751
7.23447
7.21684
4.60015
4.28958
4.15119
4.13867
3.97333
3.67077
2.87477
2.85861
2.83676
2.82104
2.81429
1.97029
1.96880
1.52905
1.50944
1.49133
1.44613
1.41893
1.22150
1.21487
1.20417
1.20240
1.20072
1.19094
1.18626
1.18453
1.18287
0.86387
0.85649
0.84563
0.83847
0.82752
^{1}H NMR (400 MHz)
Fmoc
N
CO_2t-Bu
2.2
Integral
8.000
0.965
4.299
0.906
0.948
11.770
2.189
2.982
ppm
9
8
7
6
5
4
3
2
1
0

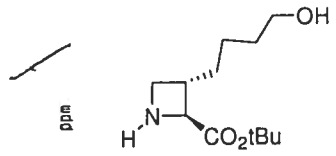
^{13}C NMR (400 MHz)



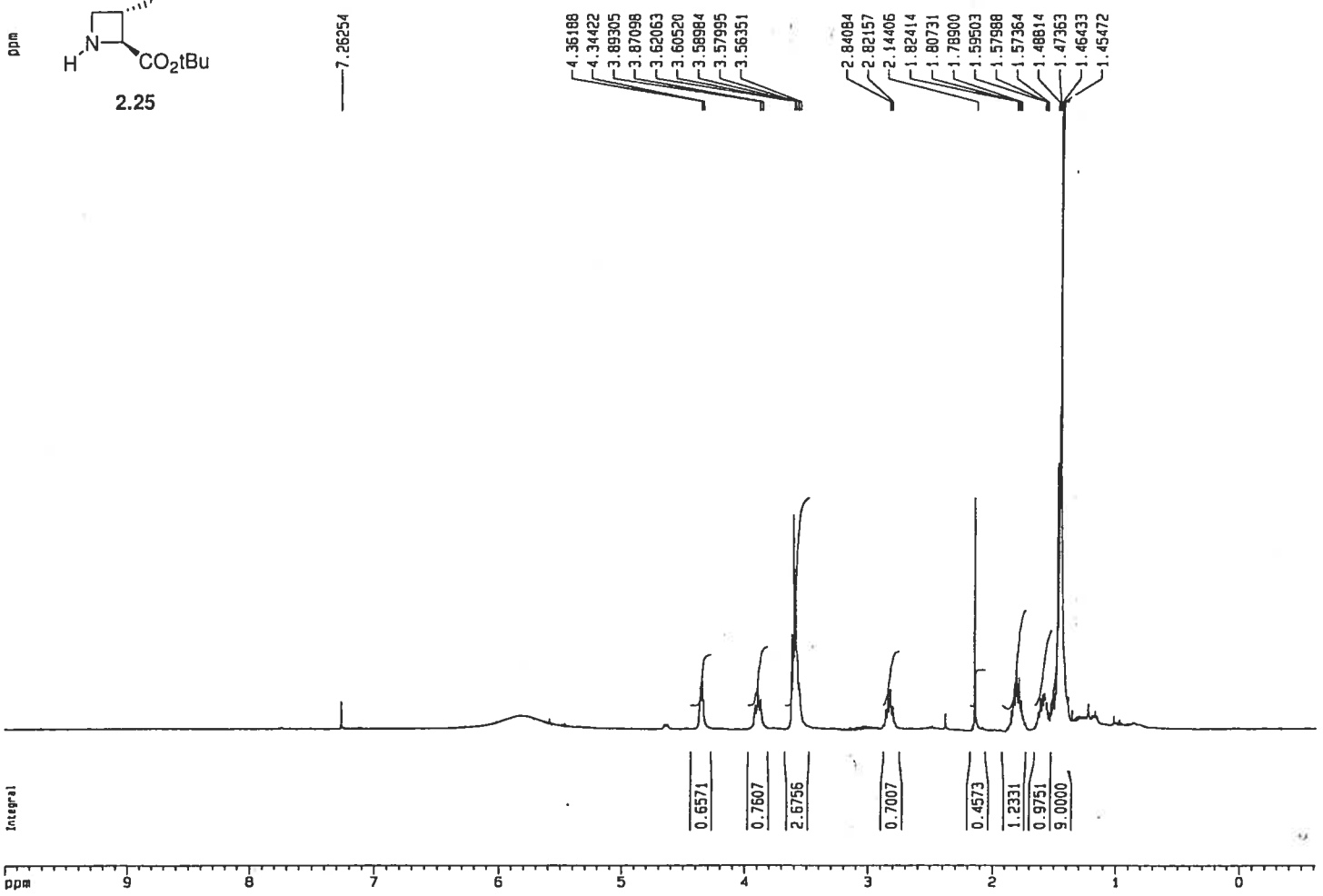

^{1}H NMR (400 MHz)
OH
PhF
N
CO_2t-Bu
2.23
ppm
7.70821
7.57809
7.46939
7.45198
7.39350
7.37836
7.37621
7.33354
7.26959
7.25370
7.23499
7.21593
7.21367
7.19171
7.17336
7.16923
7.15164
7.10075
4.22243
3.49210
3.47410
3.45617
3.34244
3.32772
2.92000
2.90211
2.88477
2.33441
2.31689
1.27299
1.26442
1.24742
1.24041
1.20851
1.16249
1.15532
1.12370
1.06106
0.03103
0.02903
Integral
13.000
1.379
1.036
1.876
1.766
0.877
15.240
ppm
10
9
8
7
6
5
4
3
2
1
0


ppm
172.732
145.739
141.737
141.226
139.856
128.456
128.193
128.066
127.411
127.320
127.075
126.941
126.582
119.763
119.245
80.399
77.485
77.165
76.845
76.009
66.475
61.429
52.624
49.235
49.021
48.808
48.594
48.381
48.167
47.954
34.826
29.401
29.099
27.451
0.741
^{13}C NMR (400 MHz)
OH
PhF
N
CO$_2$t-Bu
2.23
ppm
200
180
160
140
120
100
80
60
40
20
0

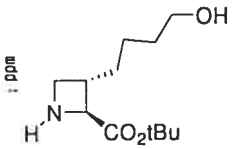
^{1}H NMR (400 MHz)



2.25



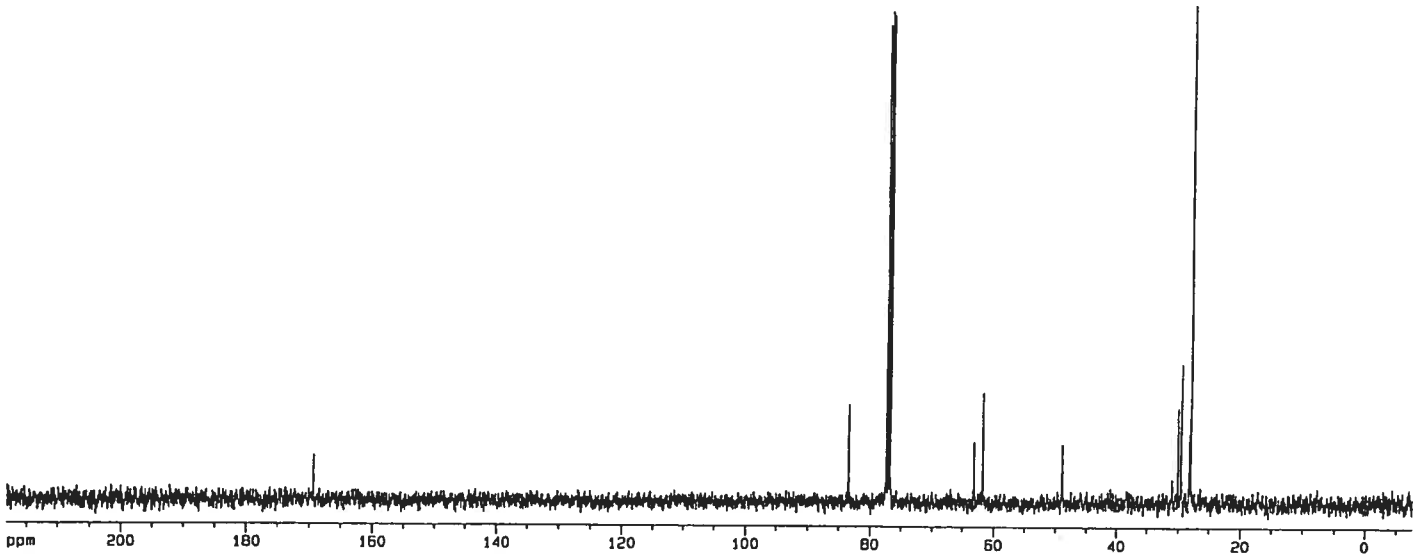
^{13}C NMR (400 MHz)

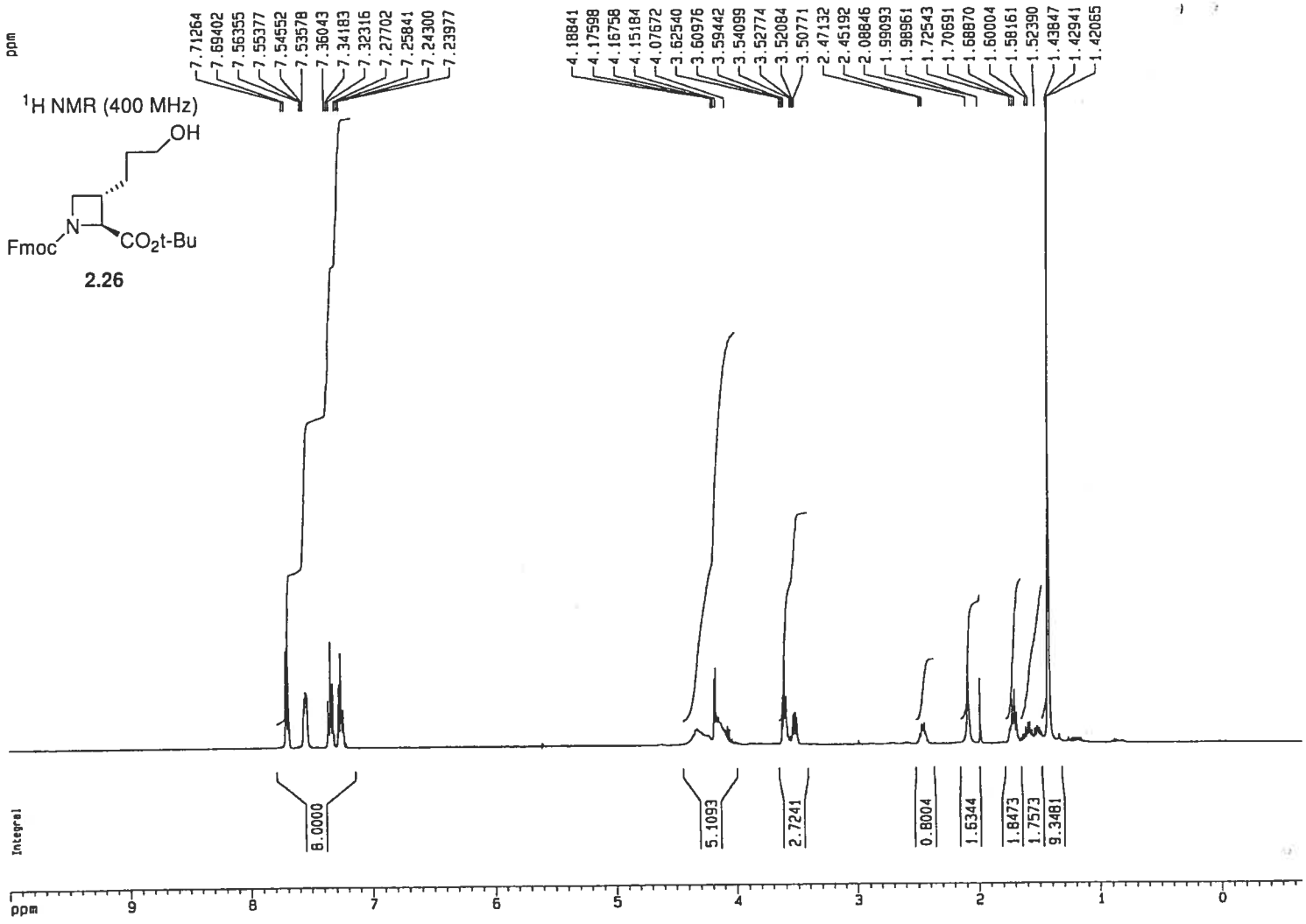


2.25

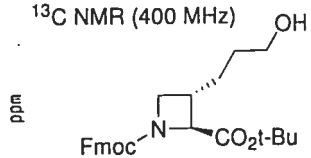
169.350

83.466
77.472
77.154
75.837
63.249
61.829
48.900
30.999
30.058
29.571
28.202
28.010




ppm
7.71264
7.69402
7.56355
7.55377
7.54552
7.53578
7.36043
7.34183
7.32316
7.27702
7.25841
7.24300
7.23977
4.18841
4.17598
4.16758
4.15184
4.07672
3.62540
3.60976
3.59442
3.54099
3.52774
3.52084
3.50771
2.47132
2.45192
2.08846
1.99093
1.98961
1.72543
1.70691
1.68870
1.60004
1.58161
1.52390
1.43847
1.42941
1.42065
^{1}H NMR (400 MHz)
OH
N
Fmoc
CO_2t-Bu
2.26
Integral
8.0000
5.1093
2.7241
0.8004
1.6344
1.8473
1.7573
9.3481
ppm
9
8
7
6
5
4
3
2
1
0

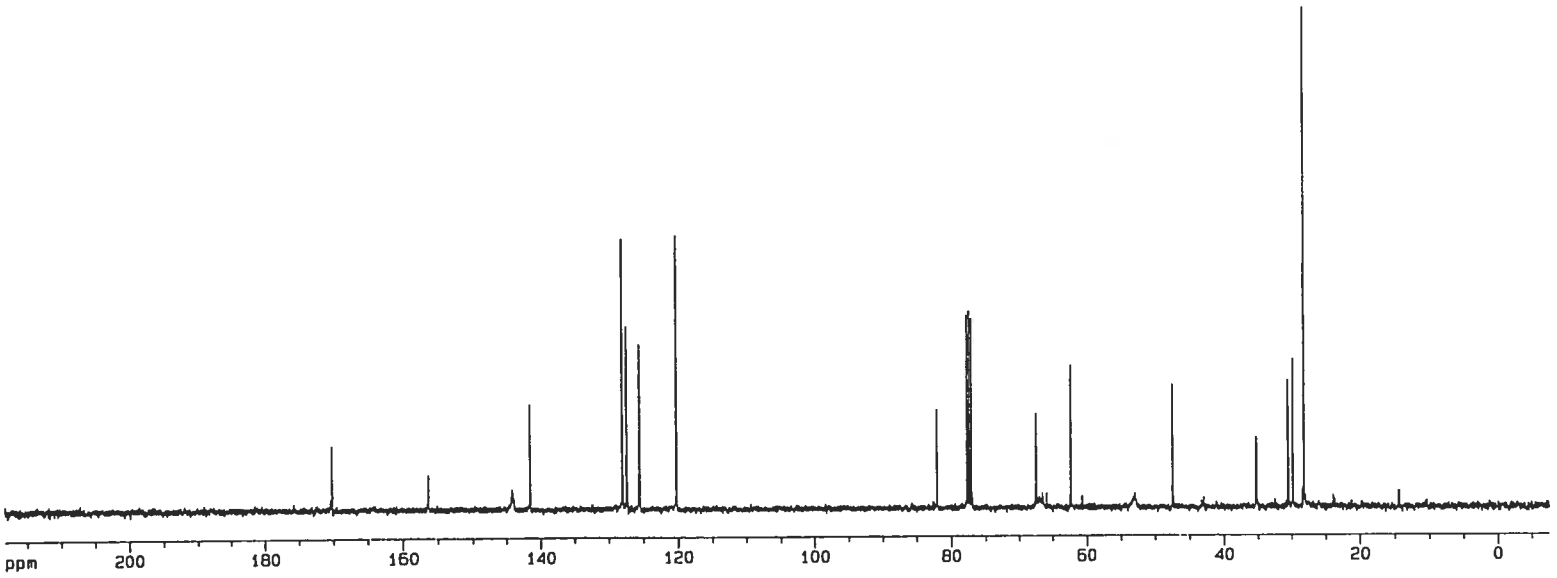
^{13}C NMR (400 MHz)

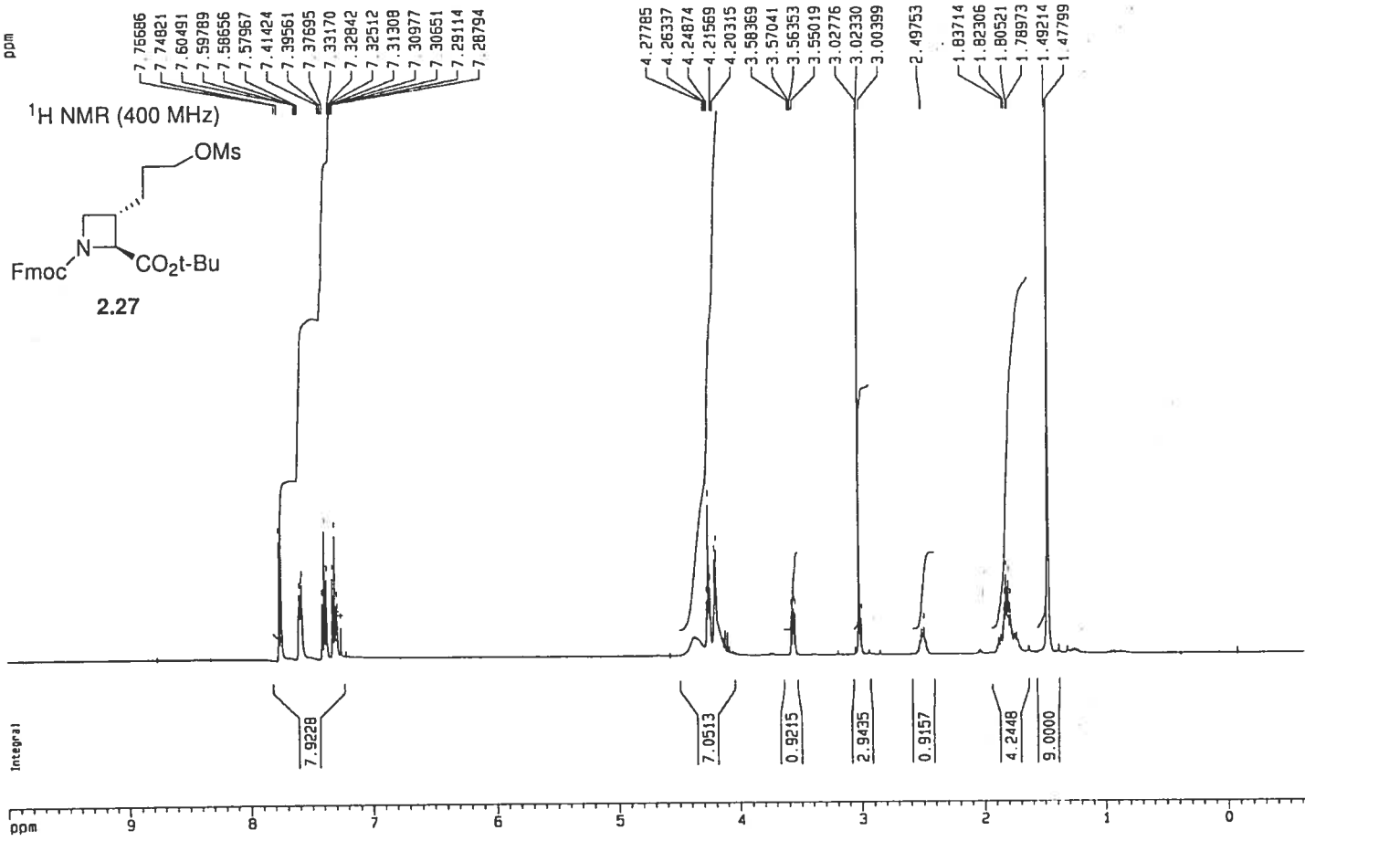


2.26

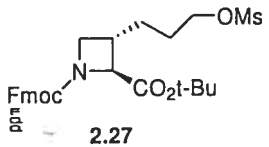
ppm

170.214 156.364 141.517 127.955 127.325 127.295 125.513 125.449 120.175 82.117 77.685 77.366 77.048 67.528 62.276 47.486 35.296 30.562 29.843 28.261



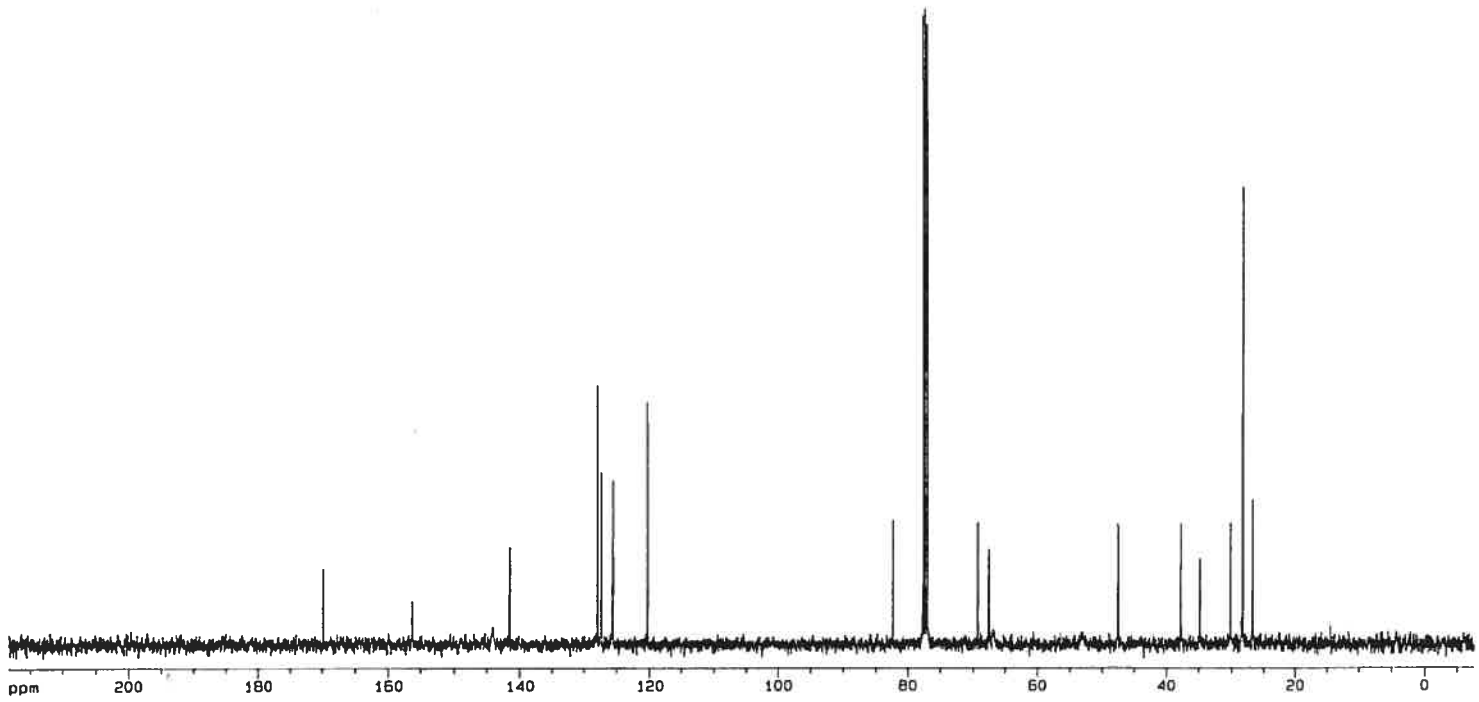

ppm
7.76686
7.74821
7.60491
7.59789
7.58656
7.57967
7.41424
7.39561
7.37695
7.33170
7.32842
7.32512
7.31308
7.30977
7.30651
7.29114
7.28794
4.27785
4.26337
4.24874
4.21569
4.20315
3.58369
3.57041
3.56353
3.55019
3.02776
3.02330
3.00399
2.49753
1.83714
1.82306
1.80521
1.78973
1.49214
1.47799
^{1}H NMR (400 MHz)
OMs
N
Fmoc
CO$_2$t-Bu
2.27
Integral
7.9228
7.0513
0.9215
2.9435
0.9157
4.2448
9.0000
ppm
9
8
7
6
5
4
3
2
1
0

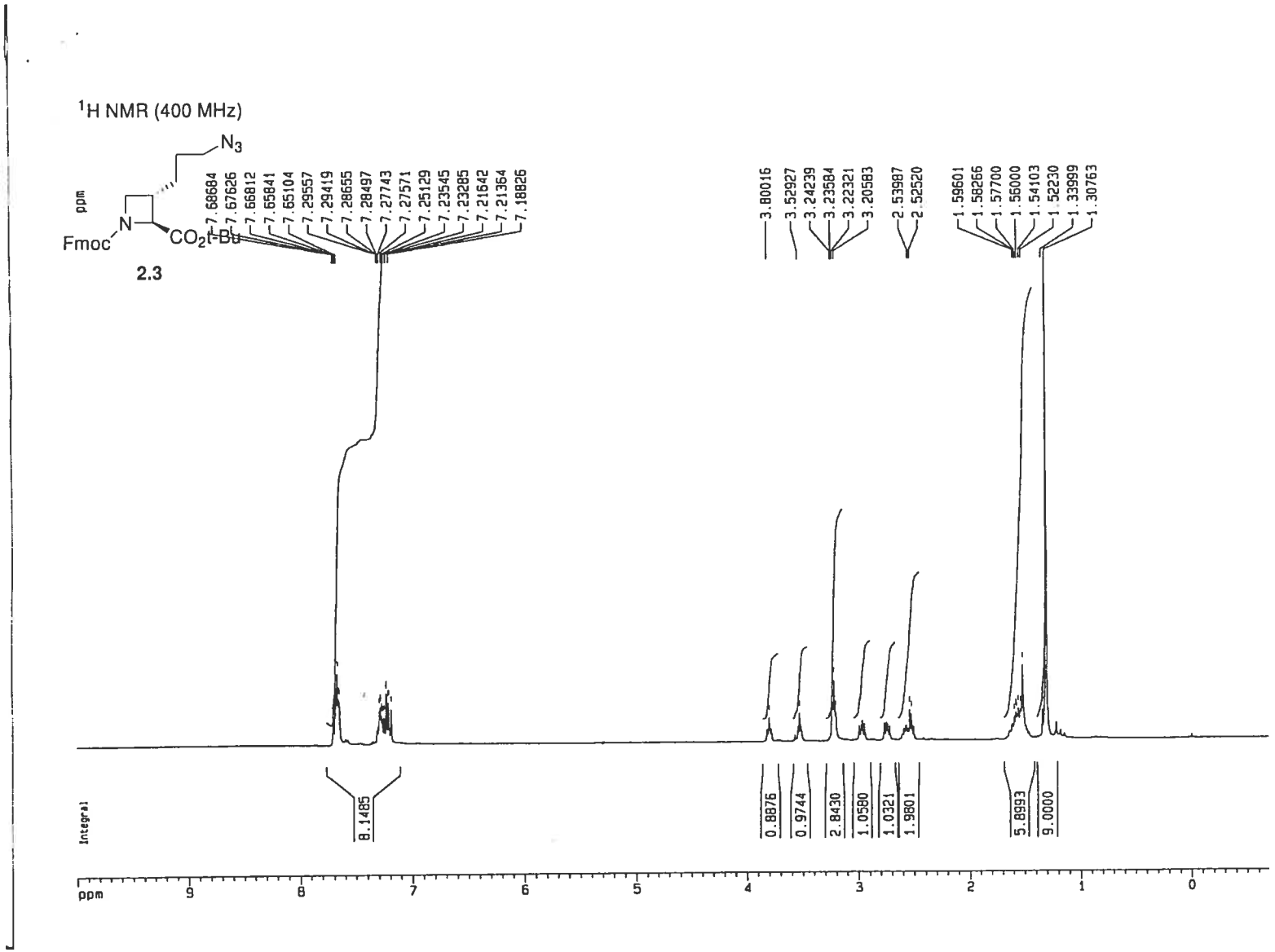
^{13}C NMR (400 MHz)

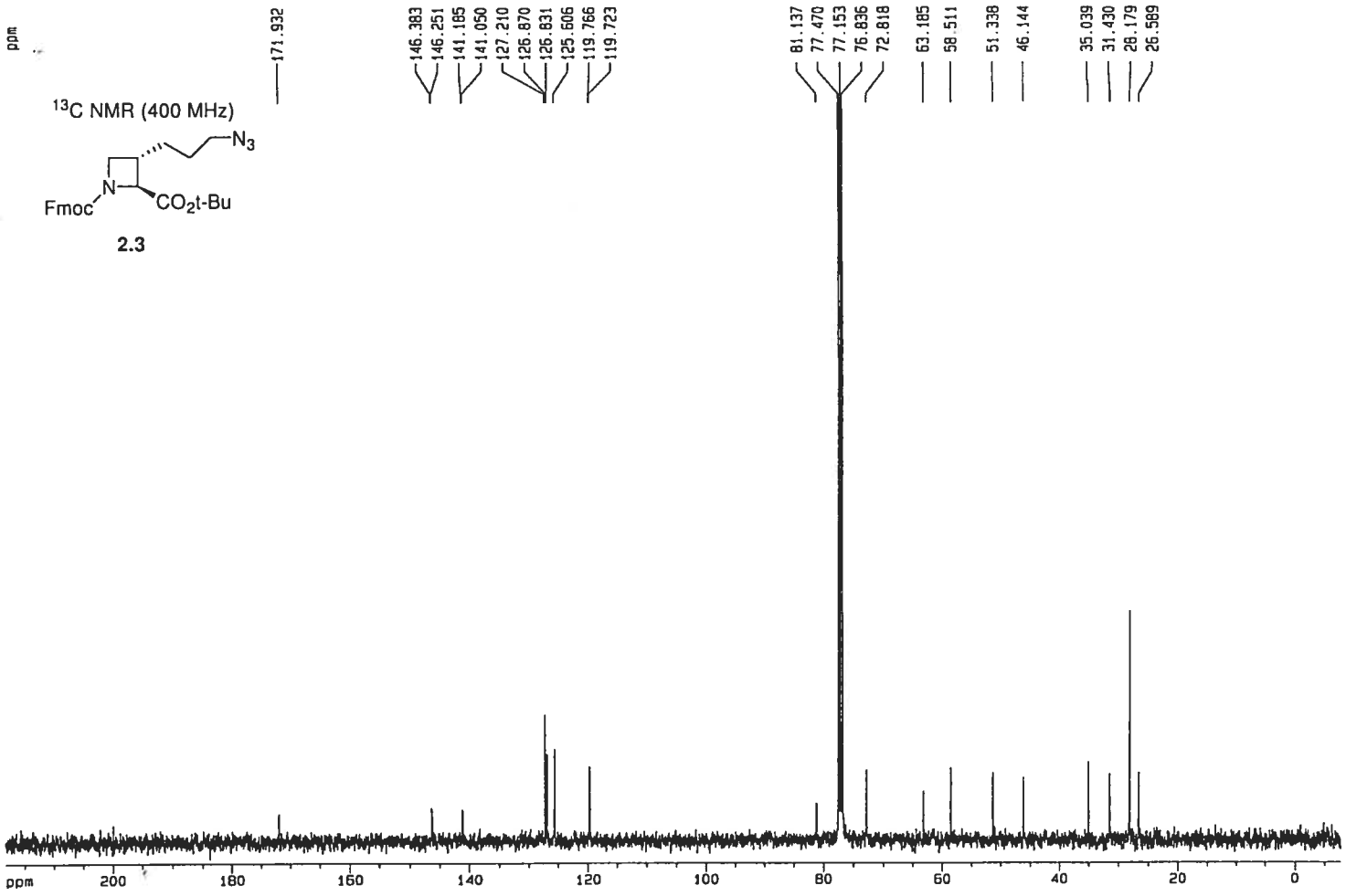


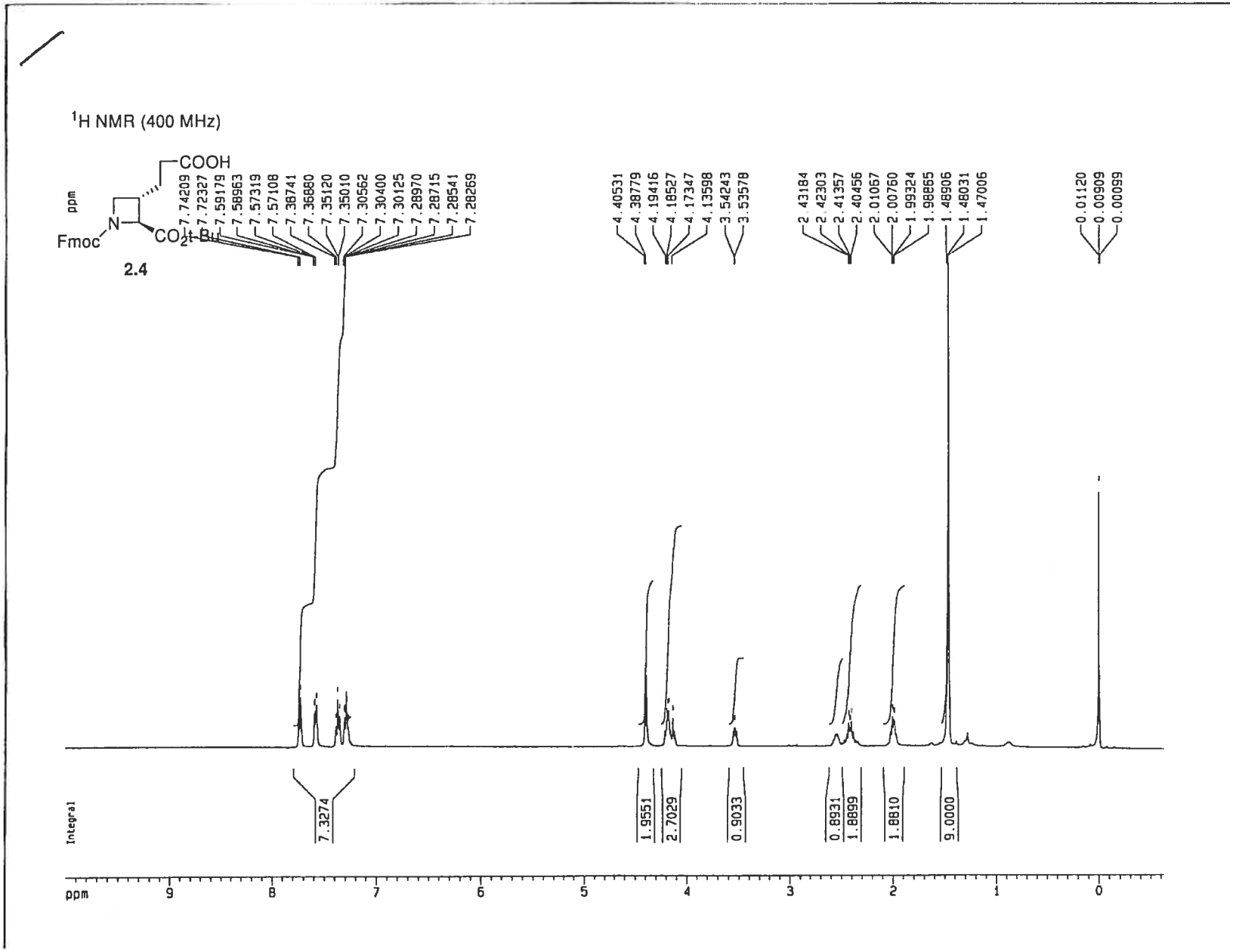
2.27

169.890
156.316
141.580
128.011
127.378
127.350
125.538
125.490
120.229
82.344
77.677
77.359
77.041
69.261
67.540
47.544
37.758
34.852
30.172
28.459
28.315
26.728

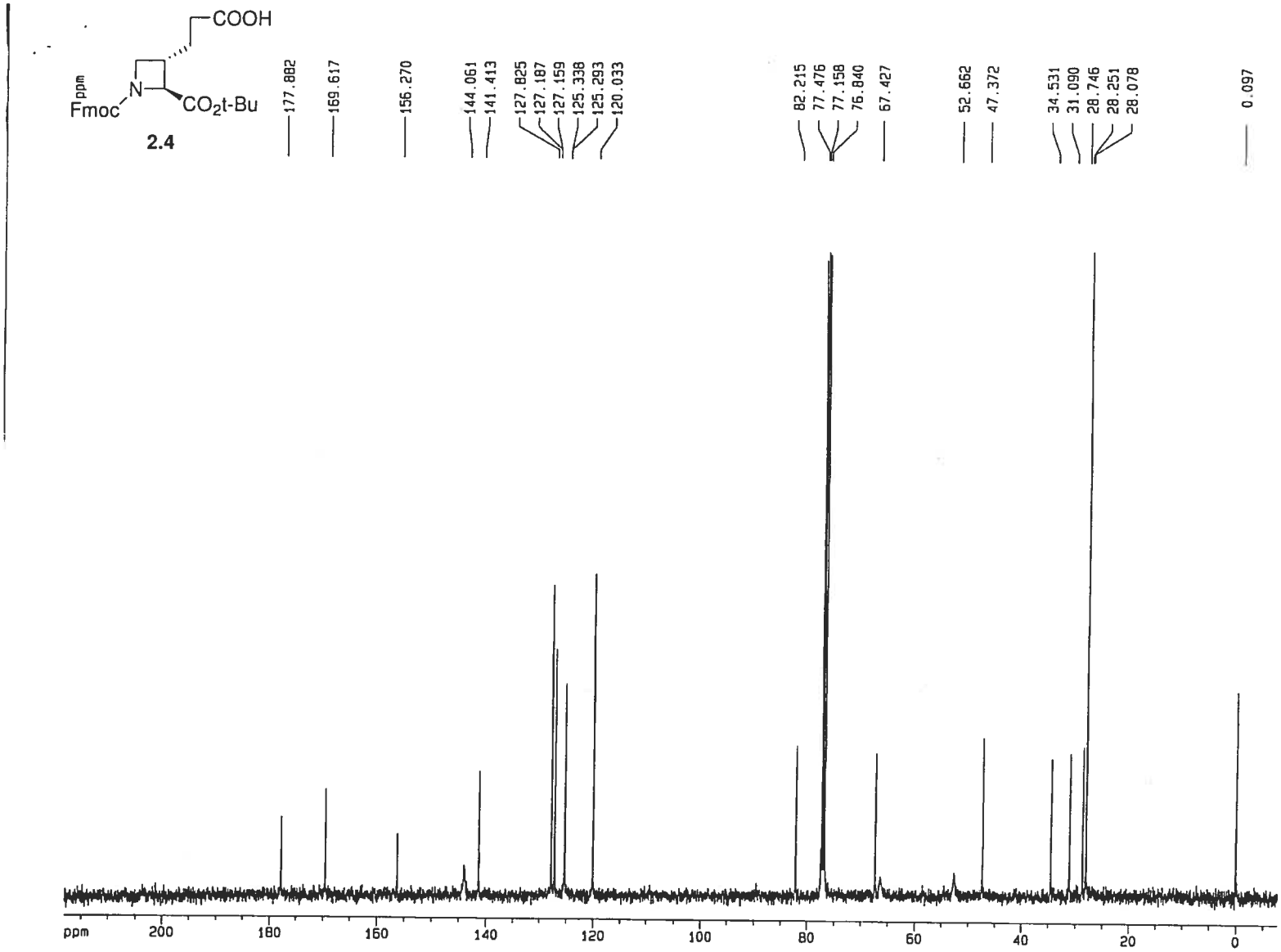



^{1}H NMR (400 MHz)
N_3
Fmoc
N
CO_2t-Bu
2.3
ppm
7.68684
7.67626
7.66812
7.65841
7.65104
7.29557
7.29419
7.28655
7.28497
7.27743
7.27571
7.25129
7.23545
7.23285
7.21642
7.21364
7.18826
3.80016
3.52927
3.24239
3.23584
3.22321
3.20583
2.53987
2.52520
1.59601
1.58266
1.57700
1.56000
1.54103
1.52230
1.33999
1.30763
Integral
8.1485
0.8876
0.9744
2.8430
1.0580
1.0321
1.9801
5.8993
9.0000
ppm
9
8
7
6
5
4
3
2
1
0


ppm
171.932
146.383
146.251
141.185
141.050
127.210
126.870
126.831
125.606
119.766
119.723
81.137
77.470
77.153
76.836
72.818
63.185
58.511
51.338
46.144
35.039
31.430
28.179
26.589
13C NMR (400 MHz)
N3
N
Fmoc
CO2t-Bu
2.3
ppm
200
180
160
140
120
100
80
60
40
20
0

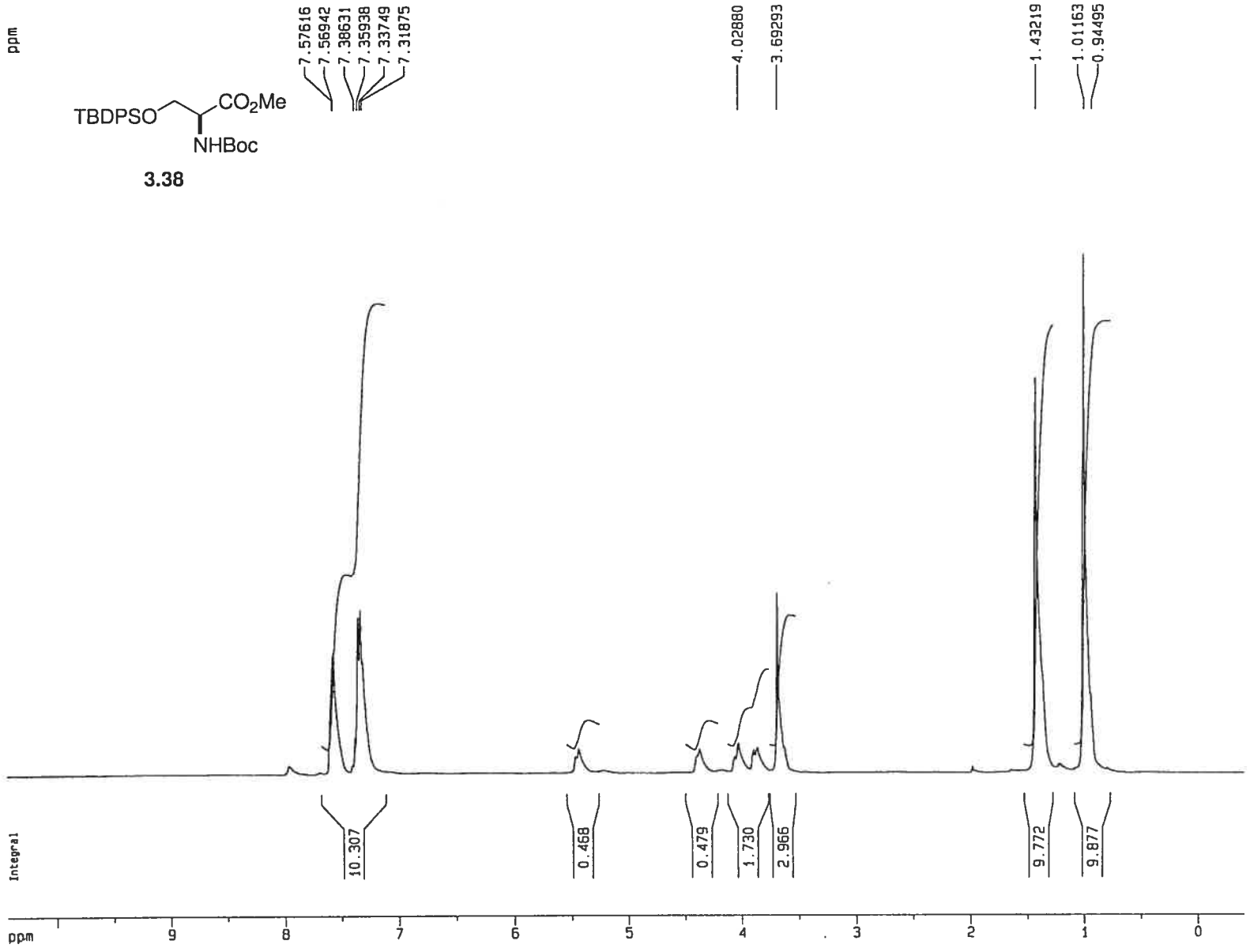

^{1}H NMR (400 MHz)
COOH
Fmoc
N
CO2t-Bu
2.4
ppm
7.74209
7.72327
7.59179
7.58963
7.57319
7.57108
7.38741
7.36880
7.35120
7.35010
7.30562
7.30400
7.30125
7.28970
7.28715
7.28541
7.28269
4.40531
4.38779
4.19416
4.18527
4.17347
4.13598
3.54243
3.53578
2.43184
2.42303
2.41357
2.40456
2.01067
2.00760
1.99324
1.98865
1.48906
1.48031
1.47006
0.01120
0.00909
0.00099
Integral
7.3274
1.9551
2.7029
0.9033
0.8931
1.8899
1.8810
9.0000
ppm
9
8
7
6
5
4
3
2
1
0

^{13}C NMR (400 MHz)



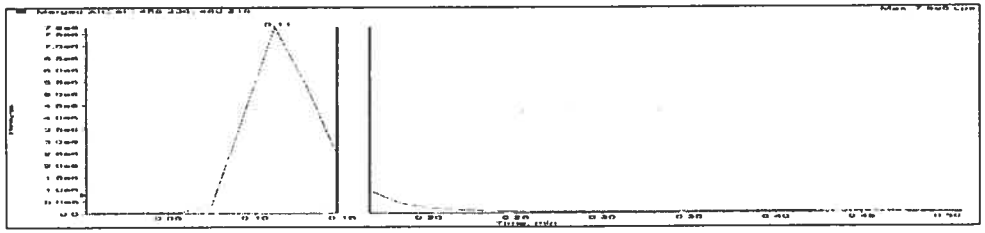
Appendix 2

^{1}H NMR and ^{13}C NMR and HRMS of compounds related to chapter 3

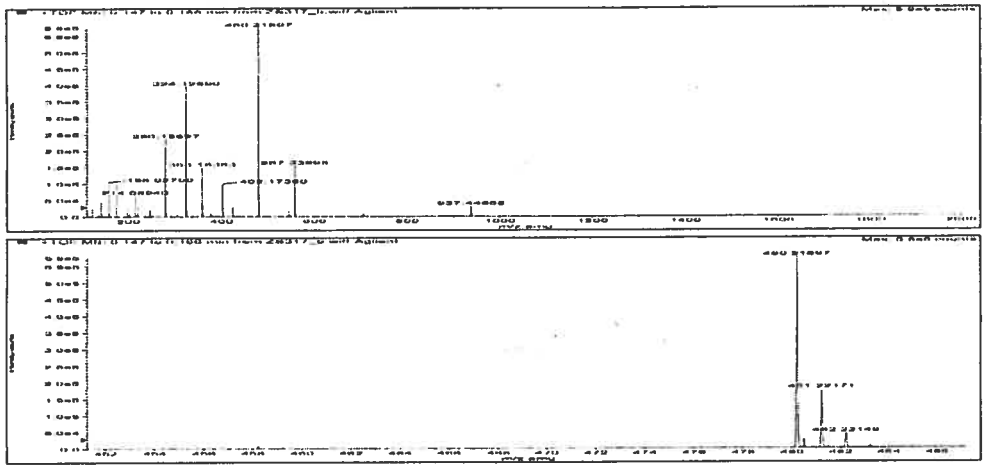

ppm
7.57616
7.56942
7.38631
7.35938
7.33749
7.31875
4.02880
3.69293
1.43219
1.01163
0.94495
TBDPSO
CO2Me
NHBoc
3.38
Integral
10.307
0.468
0.479
1.730
2.966
9.772
9.877
ppm
9
8
7
6
5
4
3
2
1
0

Empirical Formula Confirmation Report

Sample Name: ZS317 Sample Location: P1-C5 Sample Id: Operator:
Data File Name: d:\PE Sciex Data\Projects\02mar_2006\Data\ZS317_b.wiff Acq Time: March 02,2006, 02:47:18 PM
Method: C:\Program Files\Agilent\TOF Software\damethods\test.anm\efc.xml

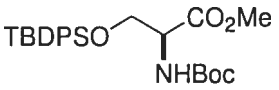


Merged XIC, Period# : 1 Experiment# : 1

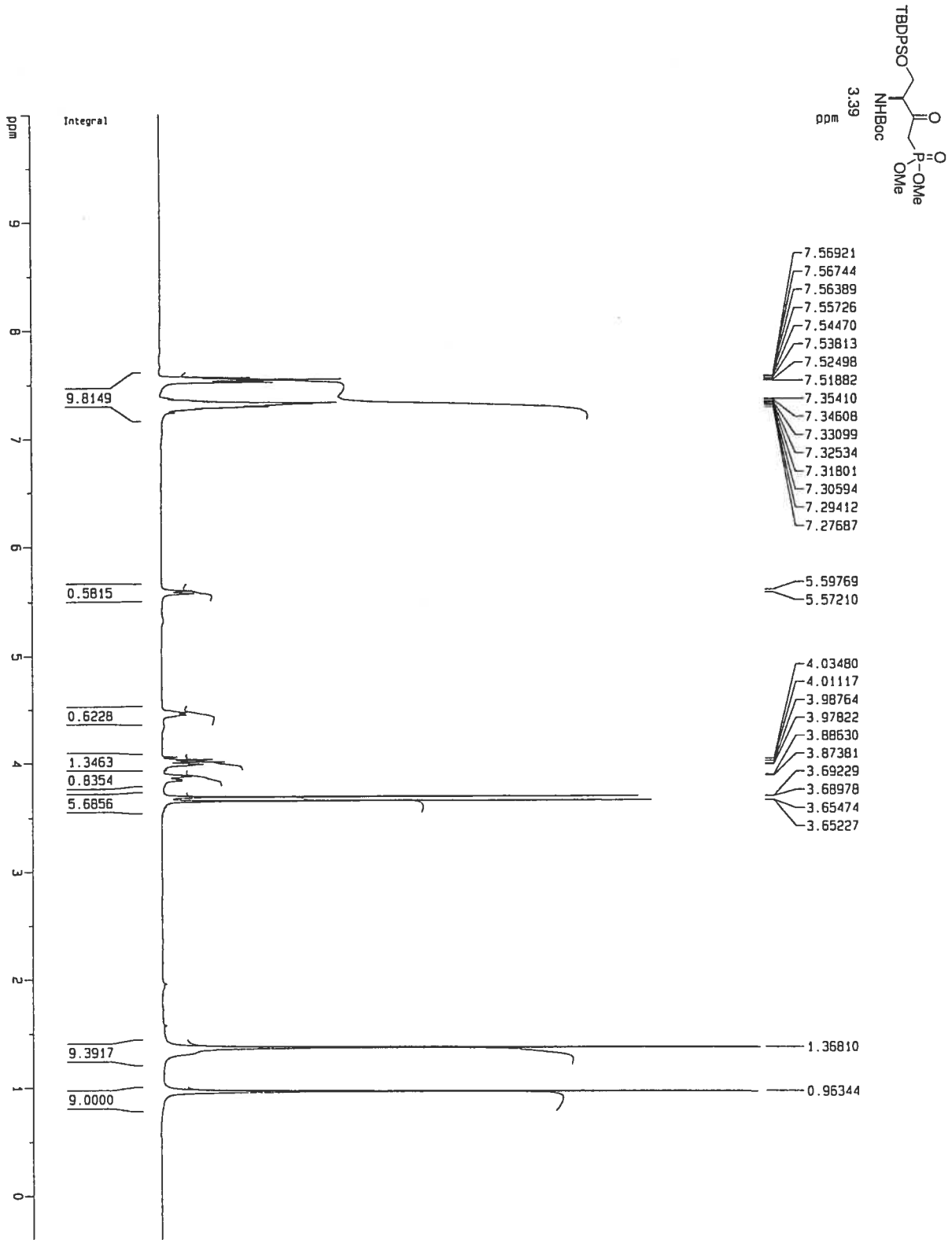


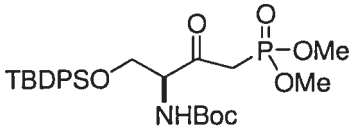
Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C25H35NO5Si	--	457.22845	0.11	2.27611 E7	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	6453.64	458.23573	458.23586	0.00014	0.30	--
[M+Na]+	587722.20	480.21767	480.21897	0.00130	2.72	--

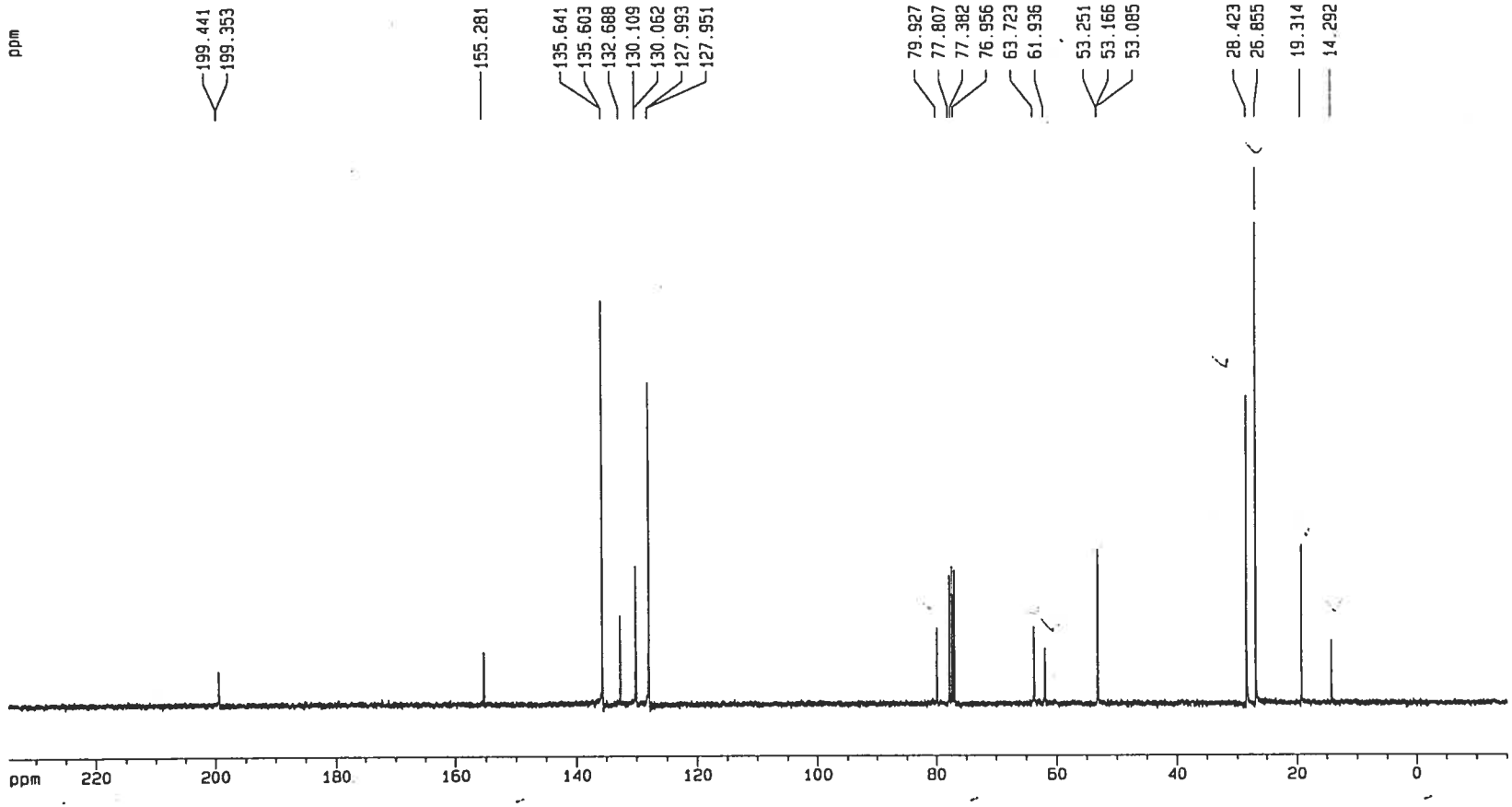


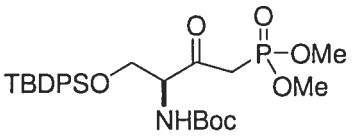
3.38


TBDPSO
NHBoc
O
O
P
OMe
OMe
3.39
ppm
Integral
ppm
9.8149
0.5815
0.6228
1.3463
0.8354
5.6856
9.3917
9.0000
7.56921
7.56744
7.56389
7.55726
7.54470
7.53813
7.52498
7.51882
7.35410
7.34608
7.33099
7.32534
7.31801
7.30594
7.29412
7.27687
5.59769
5.57210
4.03480
4.01117
3.98764
3.97822
3.88630
3.87381
3.69229
3.68978
3.65474
3.65227
1.36810
0.96344


TBDPSO
O
O
P–OMe
OMe
NHBoc

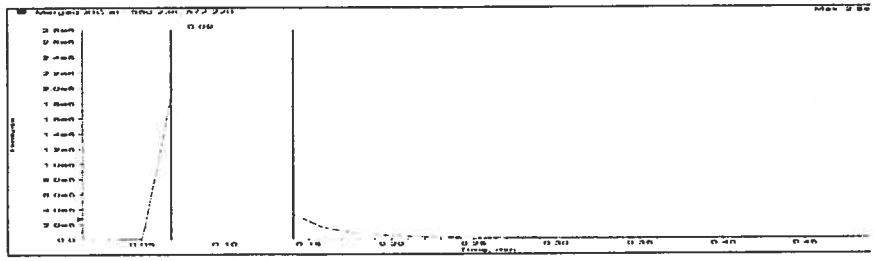
3.39


ppm
199.441
199.353
155.281
135.641
135.603
132.688
130.109
130.062
127.993
127.951
79.927
77.807
77.382
76.956
63.723
61.936
53.251
53.166
53.085
28.423
26.855
19.314
14.292
ppm
220
200
180
160
140
120
100
80
60
40
20
0

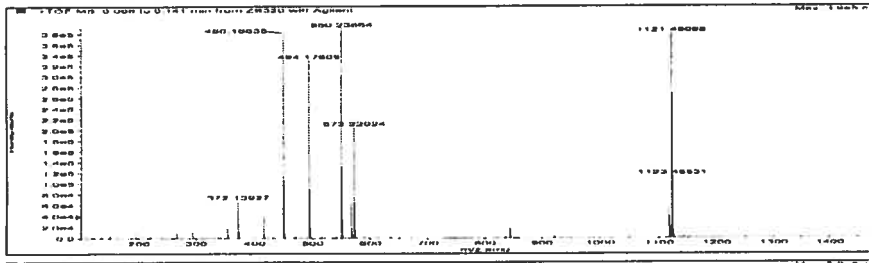


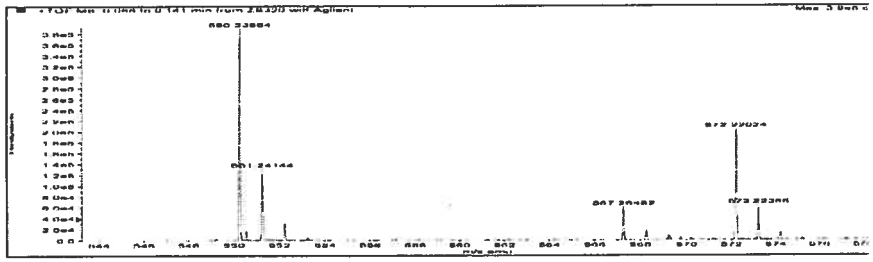
3.39

Sample Name: ZS320 Sample Location: P1-A1 Sample Id: Operator: Alex
Data File Name: d:\PE Sciex Data\Projects\31oct_2005\Data\ZS320.wiff Acq Time: October 31 2005, 03:19:23 PM
Method: C:\Program Files\Agilent\TOF Software\damethods\test.anm\ofc.xml



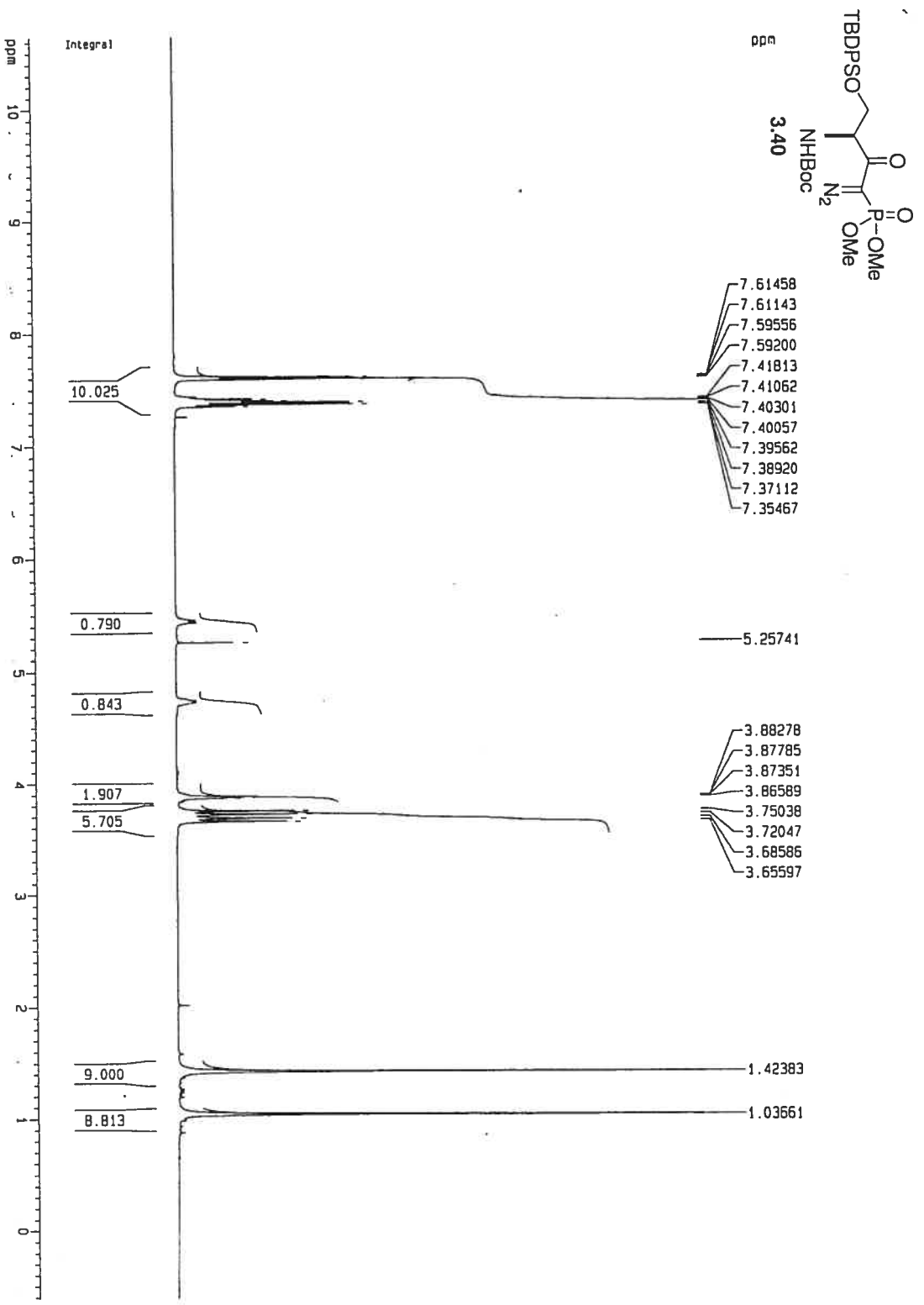
Merged XIC, Period# : 1 Experiment# : 1



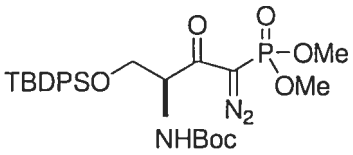


Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C27H40NO7PSi	–	549.23117	0.09	8.57980 E6	–

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	406873.32	550.23844	550.23864	0.00019	0.35	–
[M+Na]+	216469.34	572.22039	572.22024	-0.00015	-0.26	–

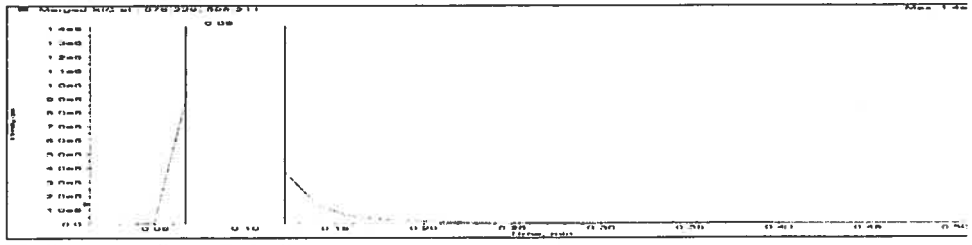

TBDPSO
NHBoc
O
N2
O
P
OMe
OMe
3.40
ppm
Integral
7.61458
7.61143
7.59556
7.59200
7.41813
7.41062
7.40301
7.40057
7.39562
7.38920
7.37112
7.35467
5.25741
3.88278
3.87785
3.87351
3.86589
3.75038
3.72047
3.68586
3.65597
1.42383
1.03661
10.025
0.790
0.843
1.907
5.705
9.000
8.813

ppm
TBDPSO
NHBoc
N2
O
P=O
OMe
OMe
3.40
155.353
135.776
135.711
132.824
132.769
130.219
130.171
128.048
128.009
80.225
77.679
77.361
77.043
64.033
57.664
54.059
54.004
53.923
53.868
28.503
26.927
19.407
ppm
200
180
160
140
120
100
80
60
40
20
0

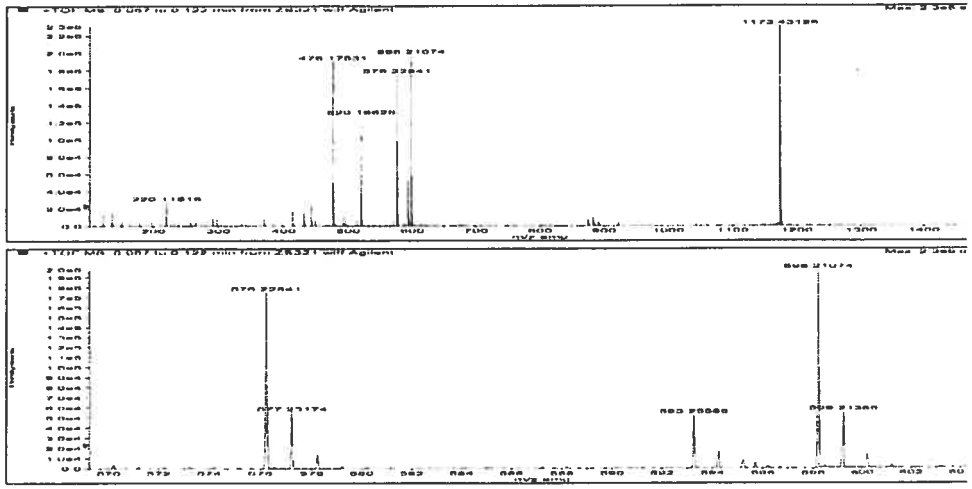


3.40

Sample Name: ZS321 Sample Location: P1-A2 Sample Id: Operator: Alex
Data File Name: d:\PE Sciex Data\Projects\31oct_2005\Data\ZS321.wiff Acq Time: October 31 2005, 03:20:41 PM
Method: C:\Program Files\Agilent\TOF Software\damethods\testanm\efc.xml

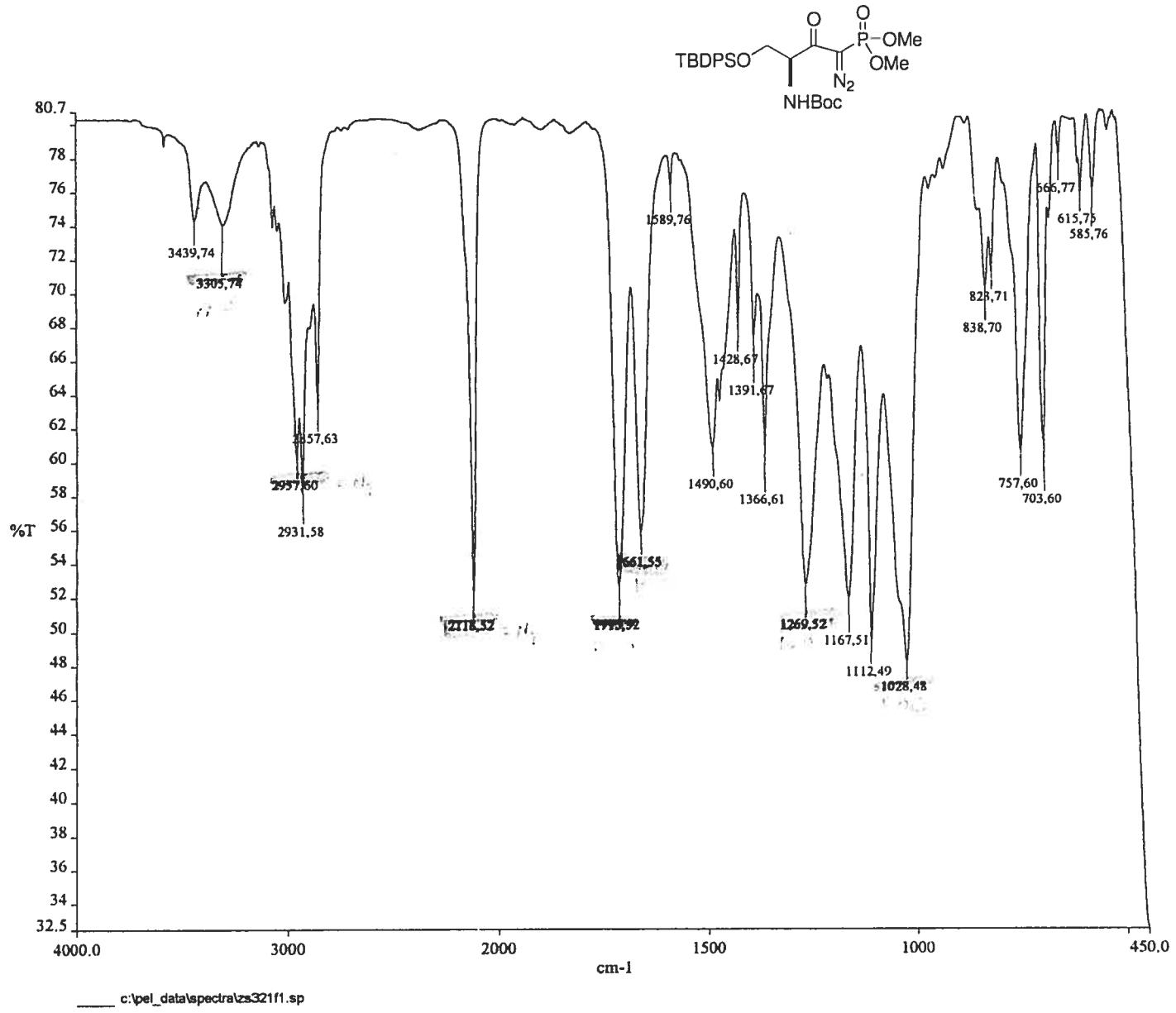


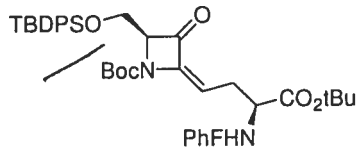
Merged XIC, Period# : 1 Experiment# : 1



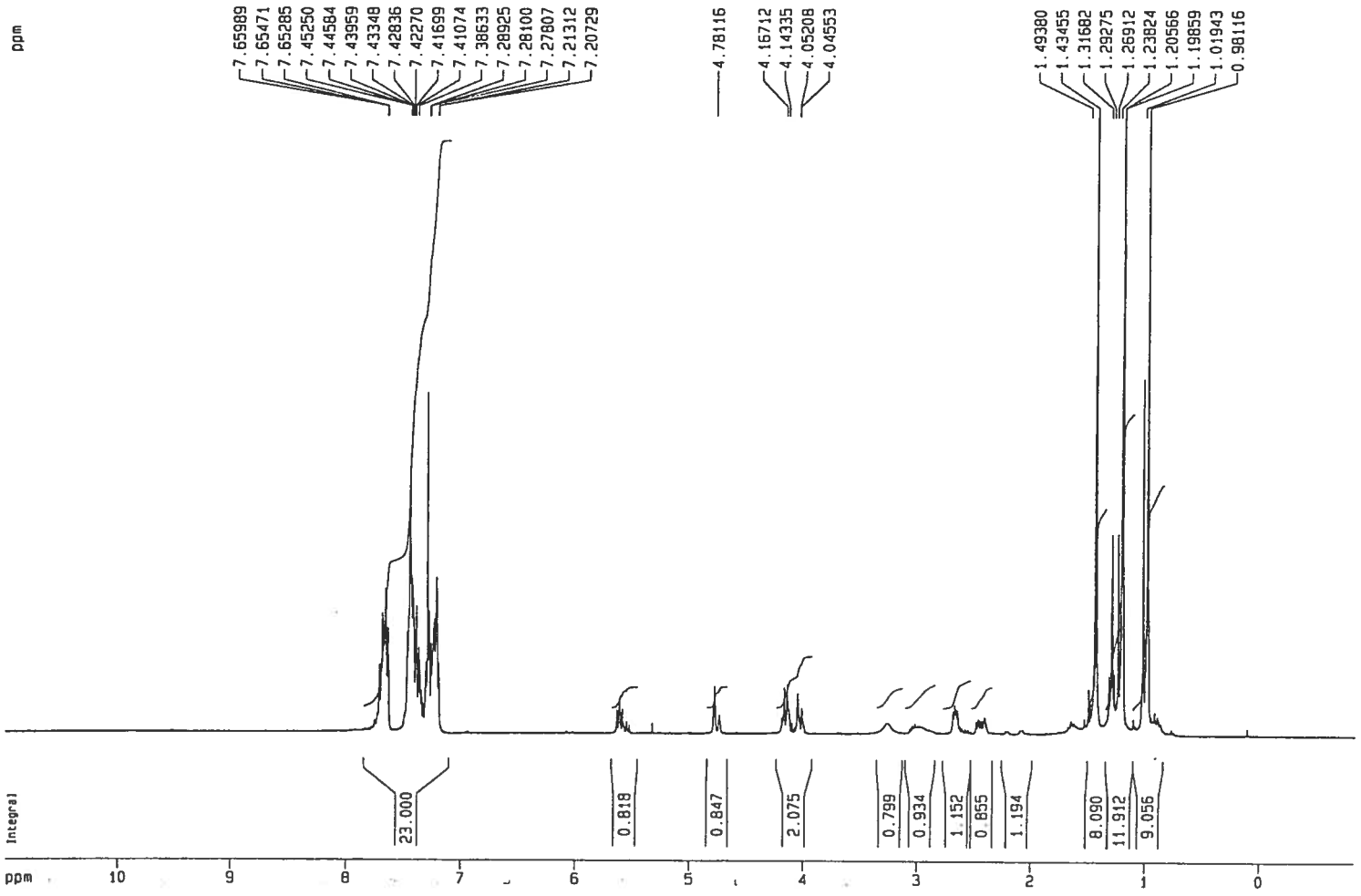
Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C27H38N3O7PSi	–	575.22188	0.09	4.13594 E6	–

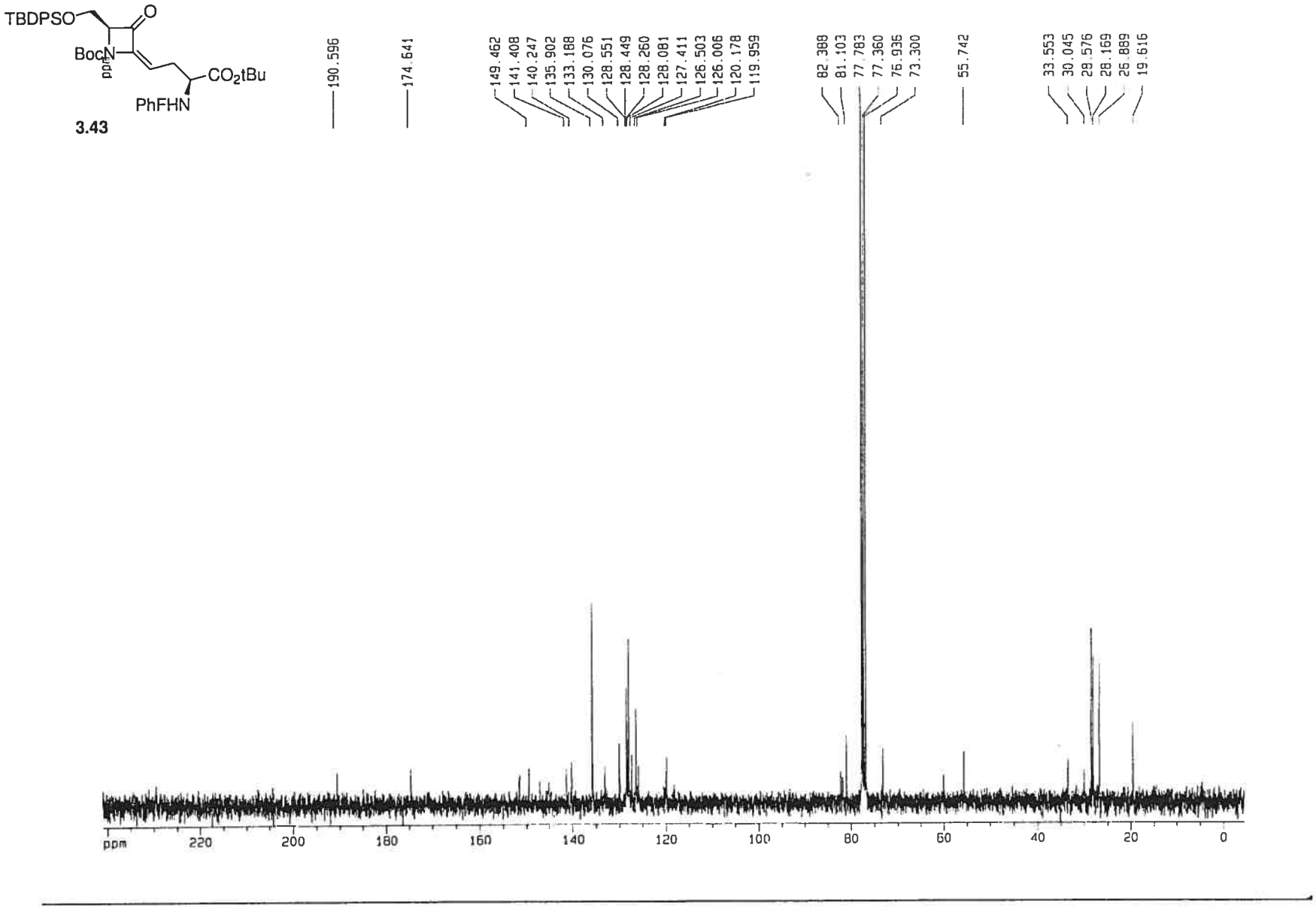
Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	183988.60	576.22894	576.22841	-0.00053	-0.91	–
[M+Na]+	198119.50	598.21088	598.21074	-0.00014	-0.24	–

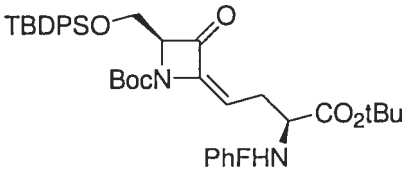

TBDPSO
O
O
P–OMe
OMe
N2
NHBoc
%T
cm-1
3439,74
3305,74
2957,60
2931,58
2857,63
2118,52
1661,55
1589,76
1490,60
1428,67
1391,67
1366,61
1269,52
1167,51
1112,49
1028,48
838,70
828,71
757,60
703,60
666,77
615,75
585,76
c:\pel_data\spectra\zs321f1.sp


TBDPSO
O
BocN
CO2tBu
PhFHN

3.43

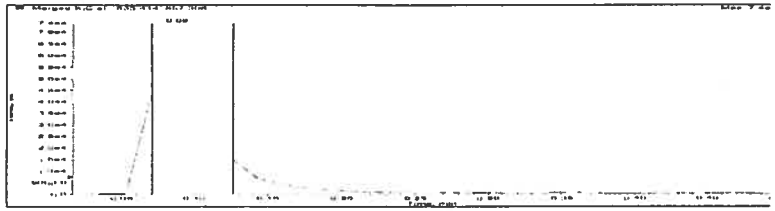

ppm
7.65989
7.65471
7.65285
7.45250
7.44584
7.43959
7.43348
7.42836
7.42270
7.41699
7.41074
7.38633
7.28925
7.28100
7.27807
7.21312
7.20729
4.78116
4.16712
4.14335
4.05208
4.04553
1.49380
1.43455
1.31682
1.29275
1.26912
1.23824
1.20566
1.19859
1.01943
0.98116
Integral
23.000
0.818
0.847
2.075
0.799
0.934
1.152
0.855
1.194
8.090
11.912
9.056
ppm
10
9
8
7
6
5
4
3
2
1
0


TBDPSO
O
BocN
ppm
CO2tBu
PhFHN
3.43
190.596
174.641
149.462
141.408
140.247
135.902
133.188
130.076
128.551
128.449
128.260
128.081
127.411
126.503
126.006
120.178
119.959
82.388
81.103
77.783
77.360
76.936
73.300
55.742
33.553
30.045
28.576
28.169
26.889
19.616
ppm
220
200
180
160
140
120
100
80
60
40
20
0

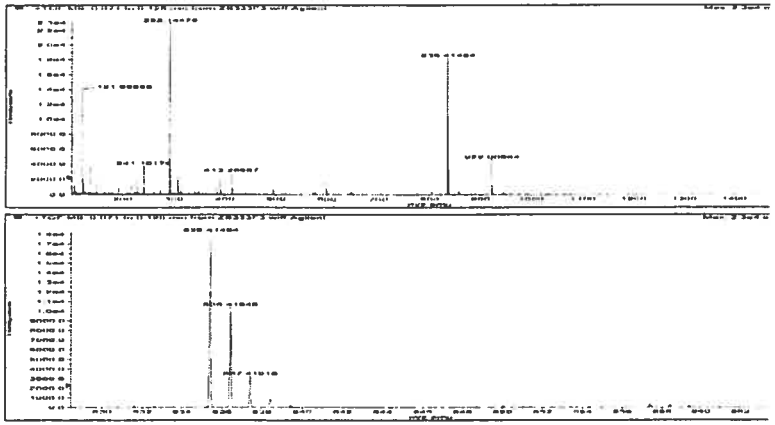


3.43

Sample Name: ZS333F3 Sample Location: P1-A3 Sample Id: Operator: Alex
Data File Name: d:\PE Sciex Data\Projects\31oct_2005\Data\ZS333F3.wiff Acq Time: October 31 2005, 03:21:59 PM
Method: C:\Program Files\Agilent\TOF Software\damethods\test.anmiefc.xml

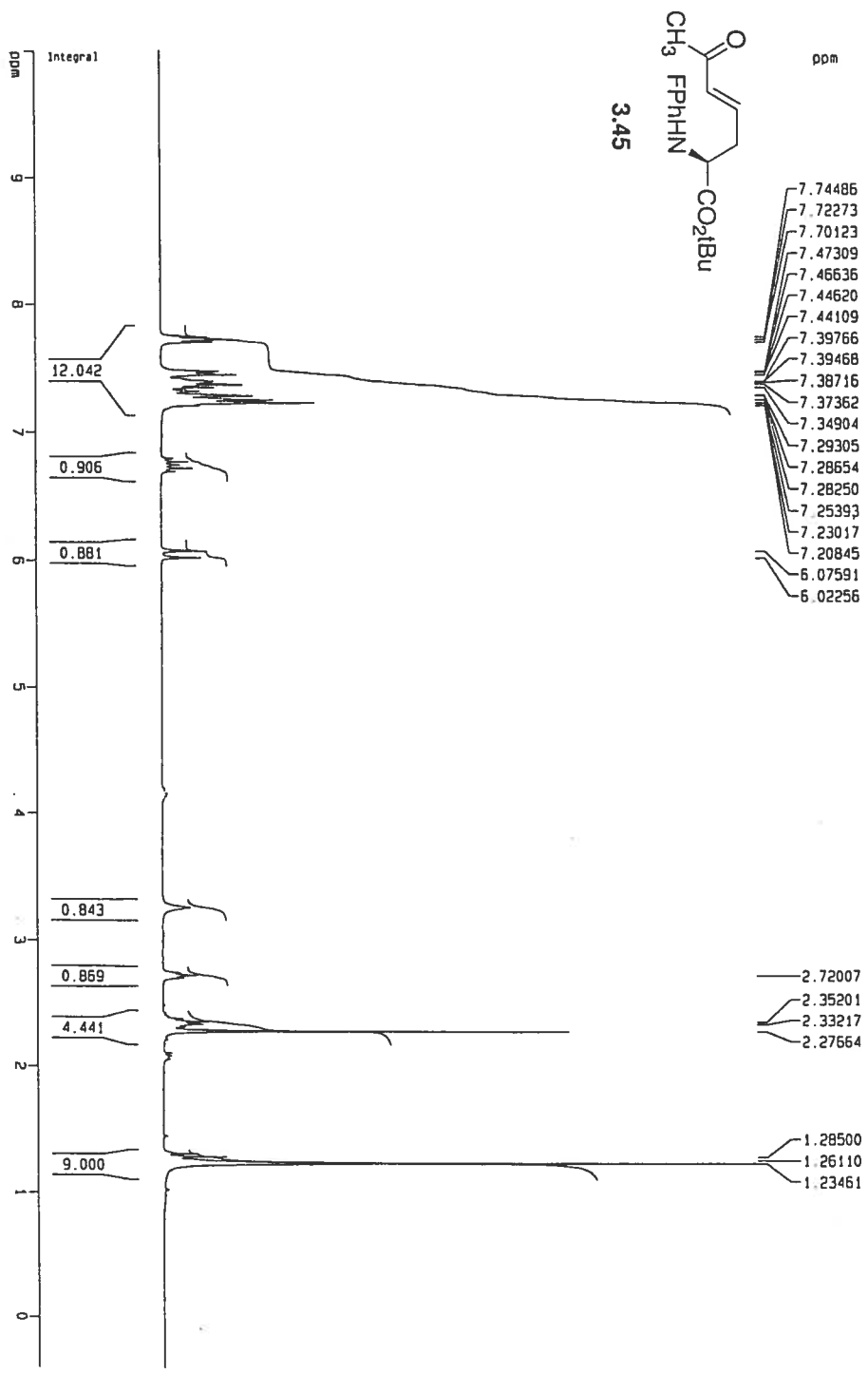


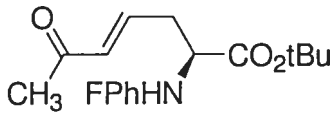
Merged XIC, Period# : 1 Experiment# : 1



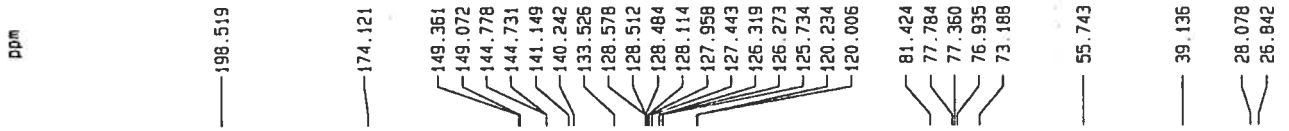
Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C52H58N2O6Si	--	834.40641	0.09	1.98310 E5	--

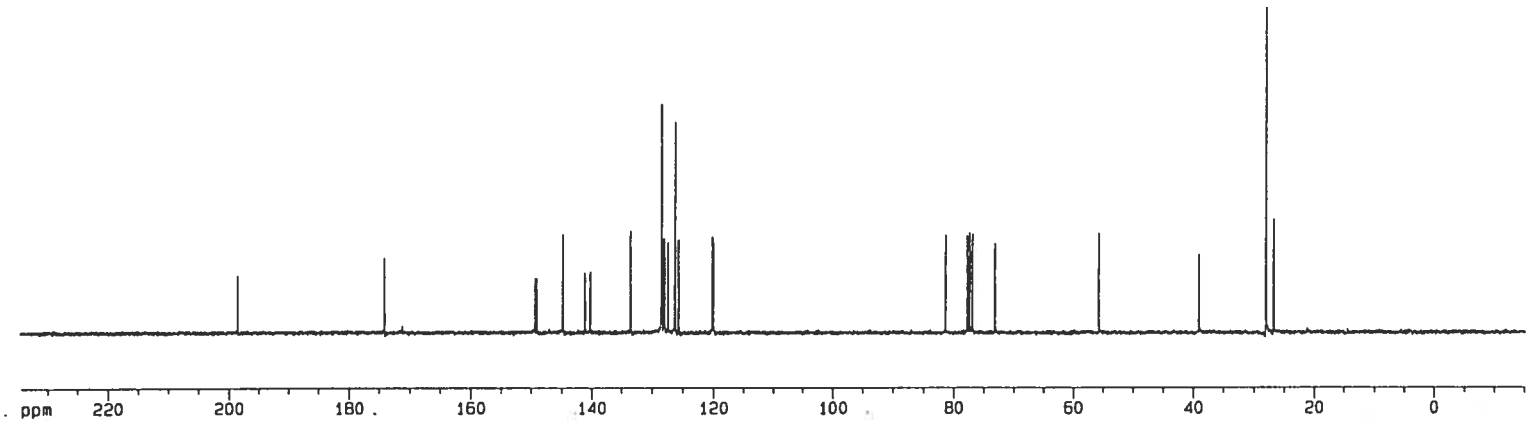
Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	18229.90	835.41369	835.41484	0.00115	1.37	--

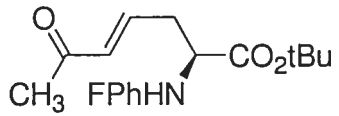

3.45
CH3
O
FPhHN
CO2tBu
ppm
Integral
12.042
0.906
0.881
0.843
0.869
4.441
9.000
7.74486
7.72273
7.70123
7.47309
7.46636
7.44620
7.44109
7.39766
7.39468
7.38716
7.37362
7.34904
7.29305
7.28654
7.28250
7.25393
7.23017
7.20845
6.07591
6.02256
2.72007
2.35201
2.33217
2.27664
1.28500
1.26110
1.23461


O
CO2tBu
CH3
FPhHN

3.45


ppm
198.519
174.121
149.361
149.072
144.778
144.731
141.149
140.242
133.526
128.578
128.512
128.484
128.114
127.958
127.443
126.319
126.273
125.734
120.234
120.006
81.424
77.784
77.360
76.935
73.188
55.743
39.136
28.078
26.842


ppm
220
200
180
160
140
120
100
80
60
40
20
0

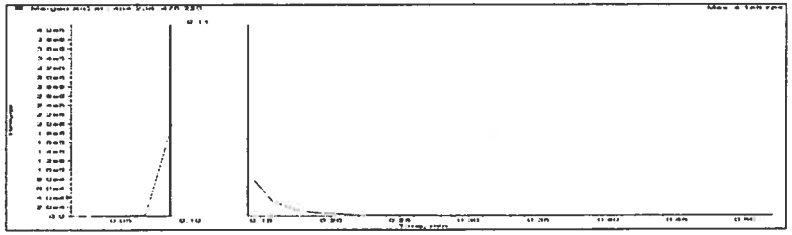


3.45

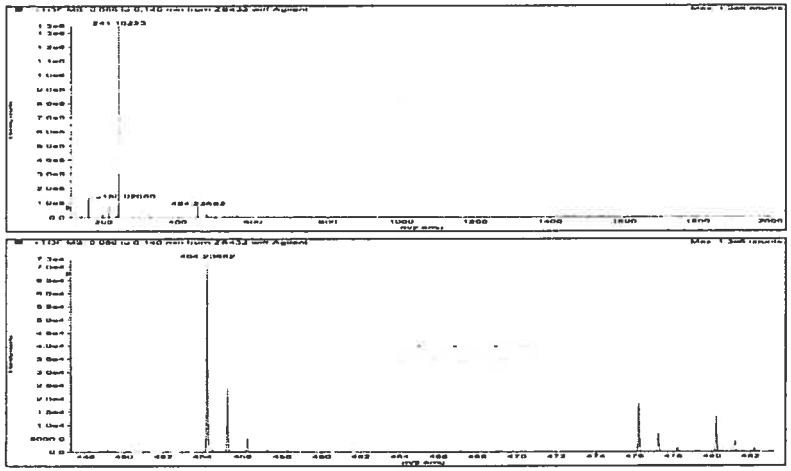
Empirical Formula Confirmation Report

Page 1 of 1

Sample Name: ZS432 Sample Location: P1-C2 Sample Id: Operator:
Data File Name: d:\PE Sciex Data\Projects\02mar_2006\Data\ZS432.wiff Acq Time: March 02 2006, 02:15:49 PM
Method: C:\Program Files\Agilent\TOF Software\damethods\testLanmeic.xml



Merged XIC, Period# : 1 Experiment# : 1



Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C30H31NO3	--	453.23039	0.11	1.08714 E6	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	73342.98	454.23767	454.23682	-0.00086	-1.88	--
[M+Na]+	19011.07	476.21961	476.21898	-0.00063	-1.33	--

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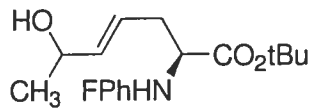
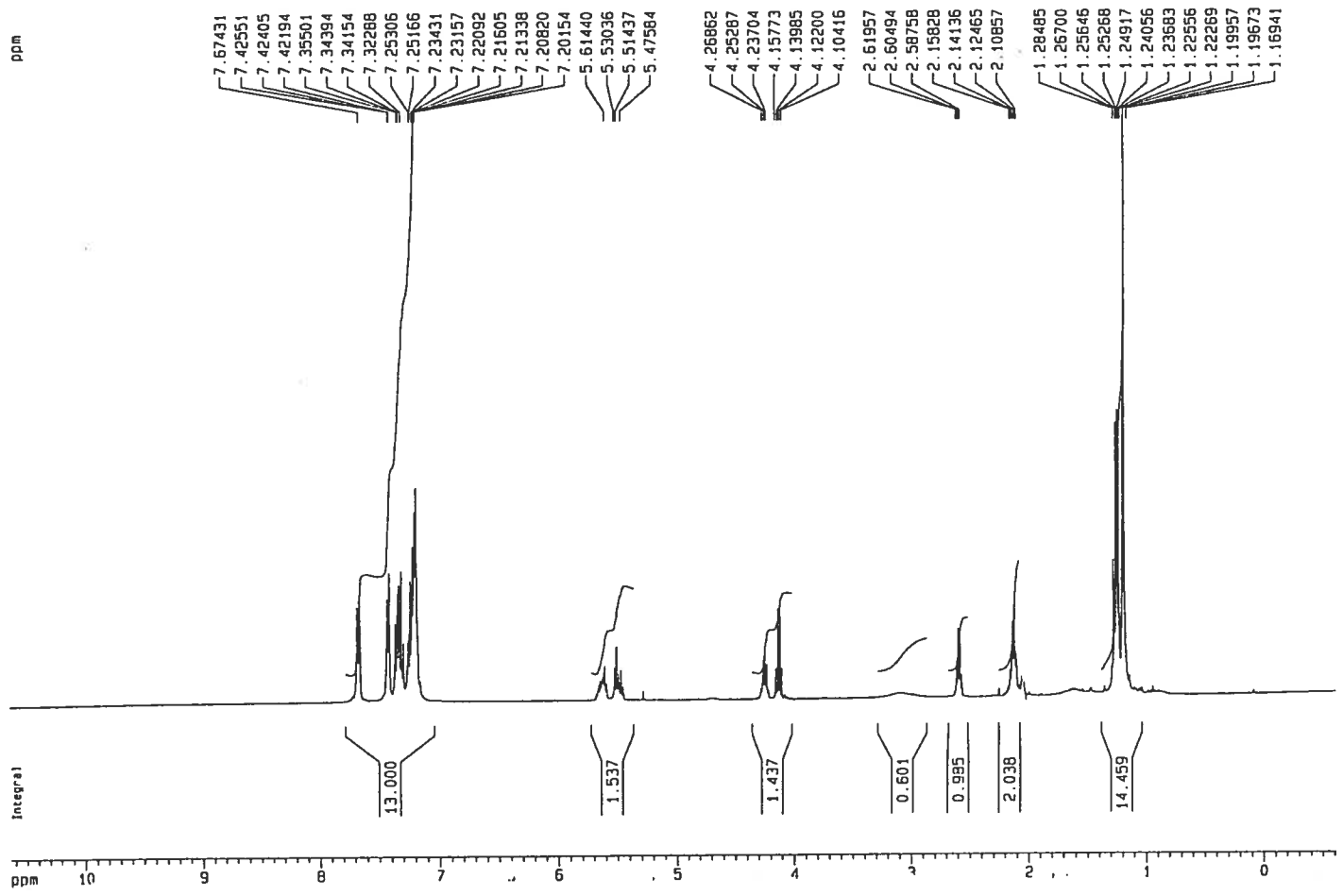
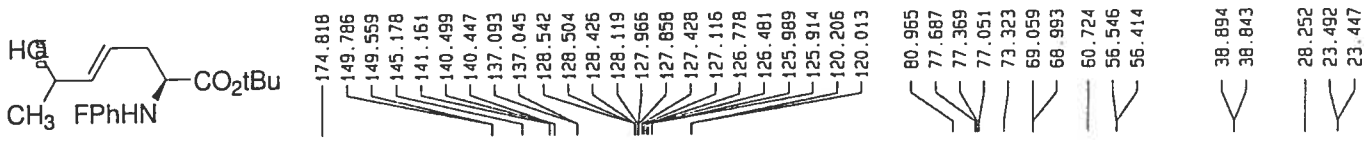
Thursday, March 02, 2006

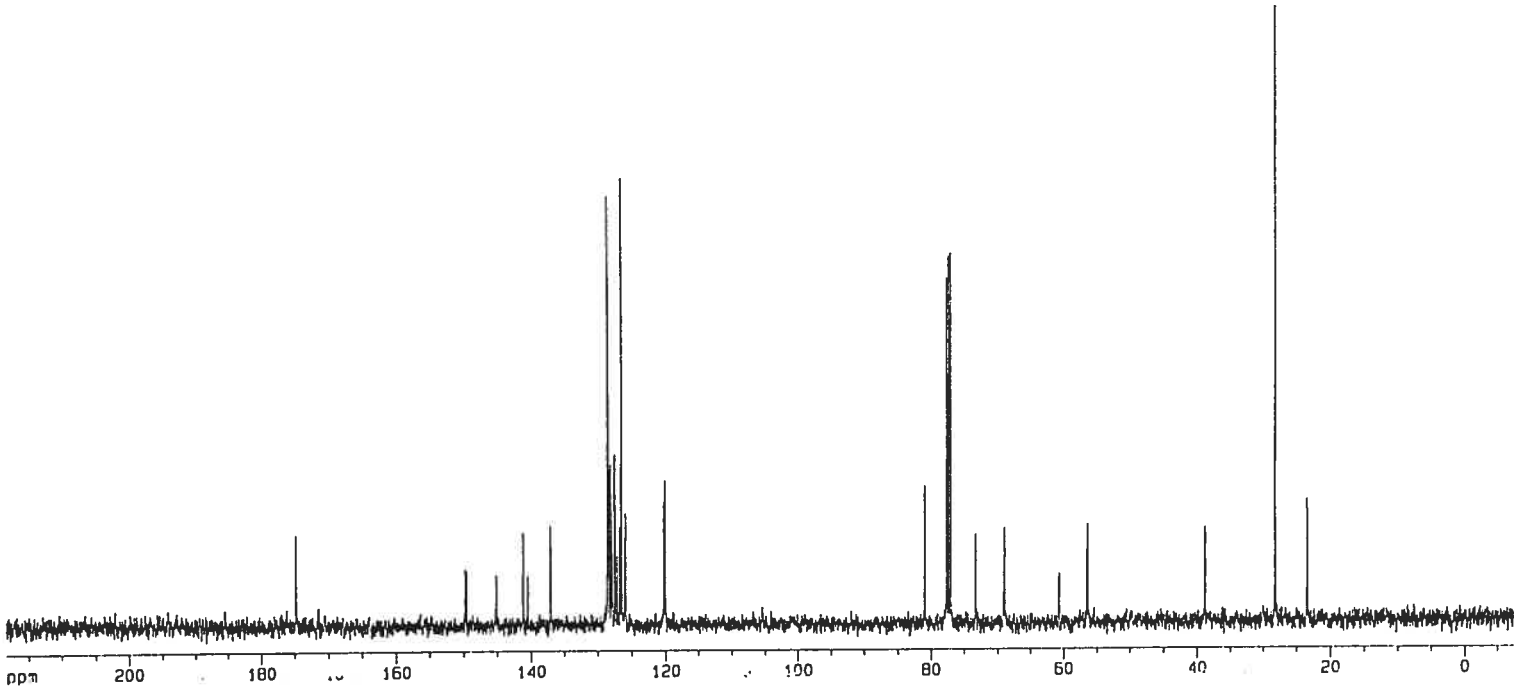

HO
CO2tBu
CH3
FPhHN

3.46

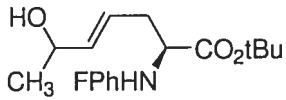

ppm
7.67431
7.42551
7.42405
7.42194
7.35501
7.34394
7.34154
7.32288
7.25306
7.25166
7.23431
7.23157
7.22092
7.21605
7.21338
7.20820
7.20154
5.61440
5.53036
5.51437
5.47584
4.26862
4.25287
4.23704
4.15773
4.13985
4.12200
4.10416
2.61957
2.60494
2.58758
2.15828
2.14136
2.12465
2.10857
1.28485
1.26700
1.25646
1.25268
1.24917
1.24056
1.23683
1.22556
1.22269
1.19957
1.19673
1.16941
Integral
13.000
1.537
1.437
0.601
0.995
2.038
14.459
ppm
10
9
8
7
6
5
4
3
2
1
0


HO
CH3
FPhHN
CO2tBu
174.818
149.786
149.559
145.178
141.161
140.499
140.447
137.093
137.045
128.542
128.504
128.426
128.119
127.966
127.858
127.428
127.116
126.778
126.481
125.989
125.914
120.206
120.013
80.965
77.687
77.369
77.051
73.323
69.059
68.993
60.724
56.546
56.414
38.894
38.843
28.252
23.492
23.447

3.46

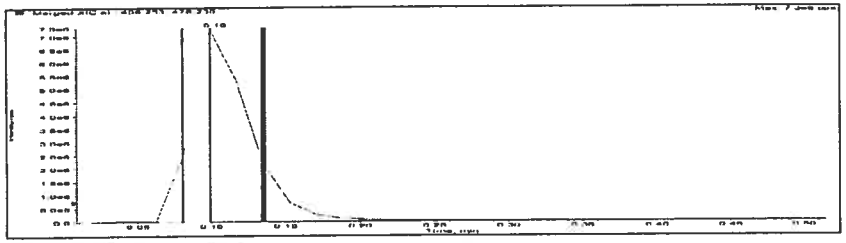

ppm
200
180
160
140
120
100
80
60
40
20
0

Empirical Formula Confirmation Report

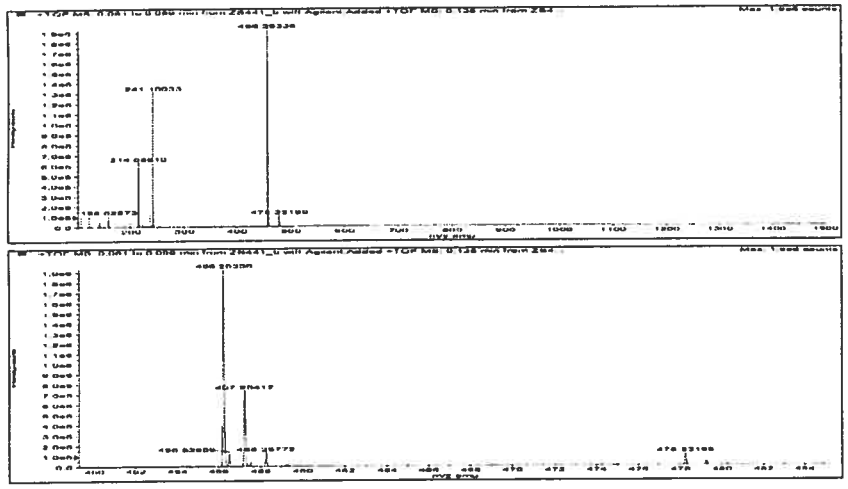


3.46

Sample Name: ZS441 Sample Location: P1-E3 Sample Id: Operator:
Data File Name: d:\PE Sciex Data\Projects\01fev_2006\Data\ZS441_b.wiff Acq Time: February 01,2006, 10:14:53 AM
Method: C:\Program Files\Agilent\TOF Software\damethods\test.anm\efc.xml

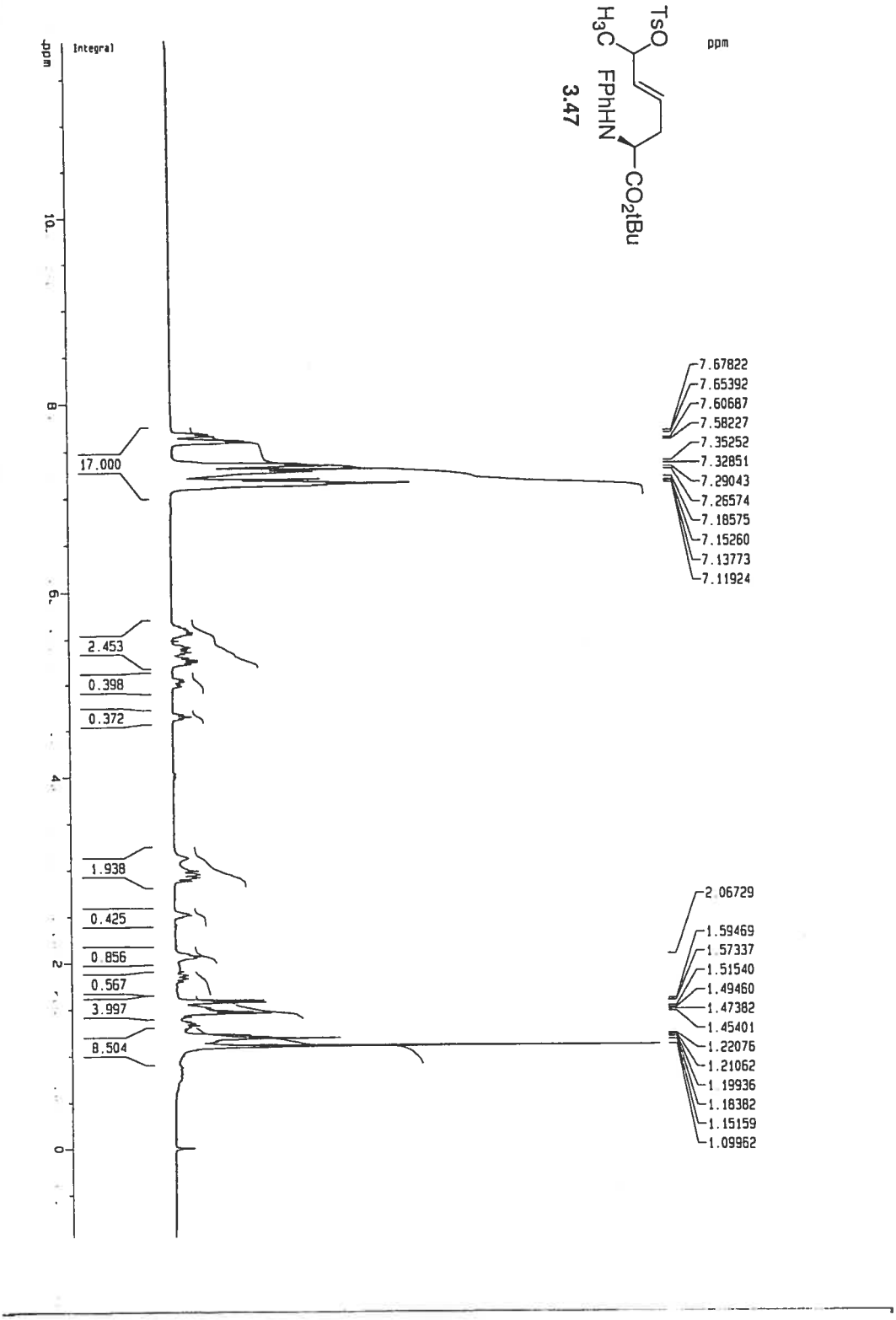


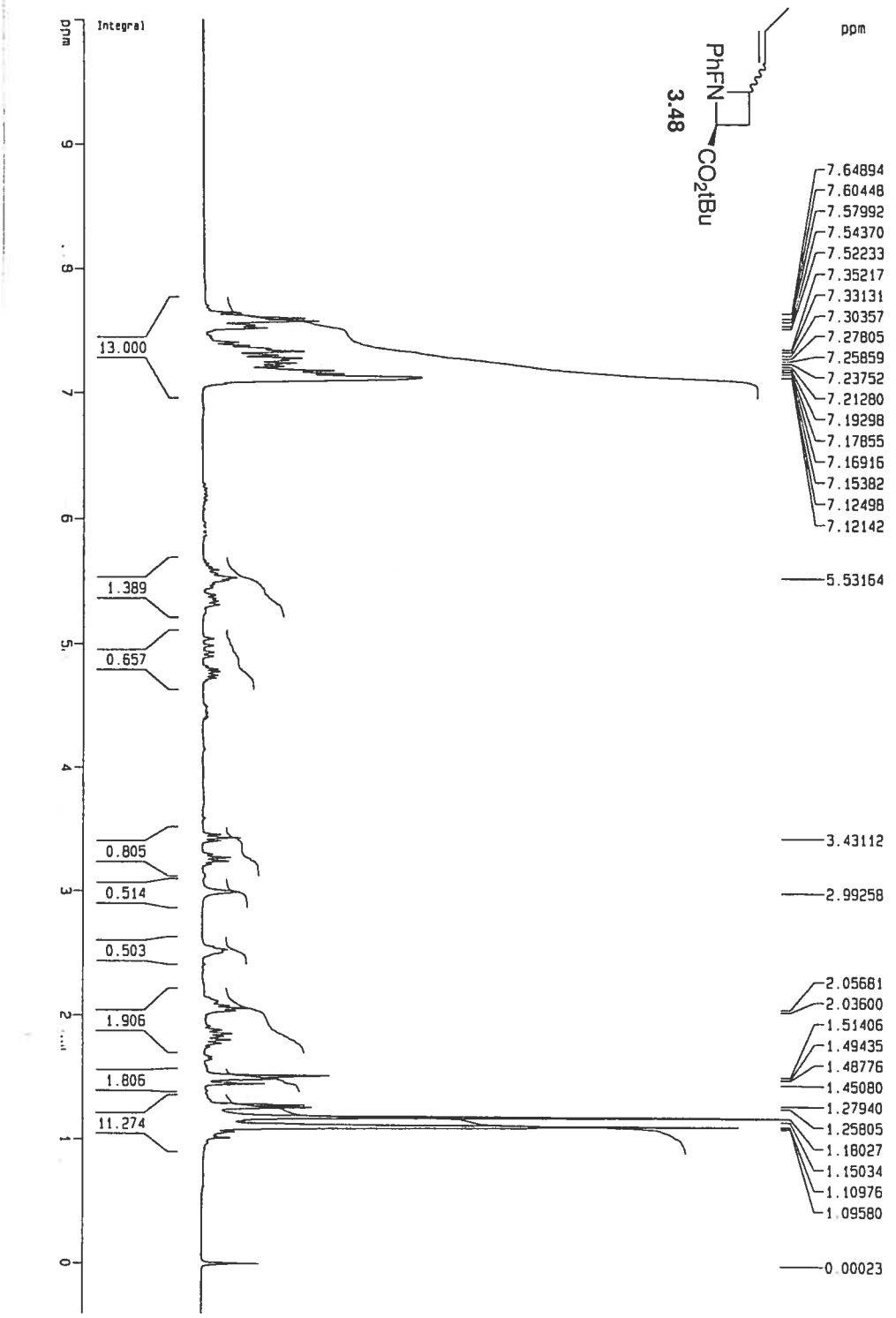
Merged XIC, Period# : 1 Experiment# : 1

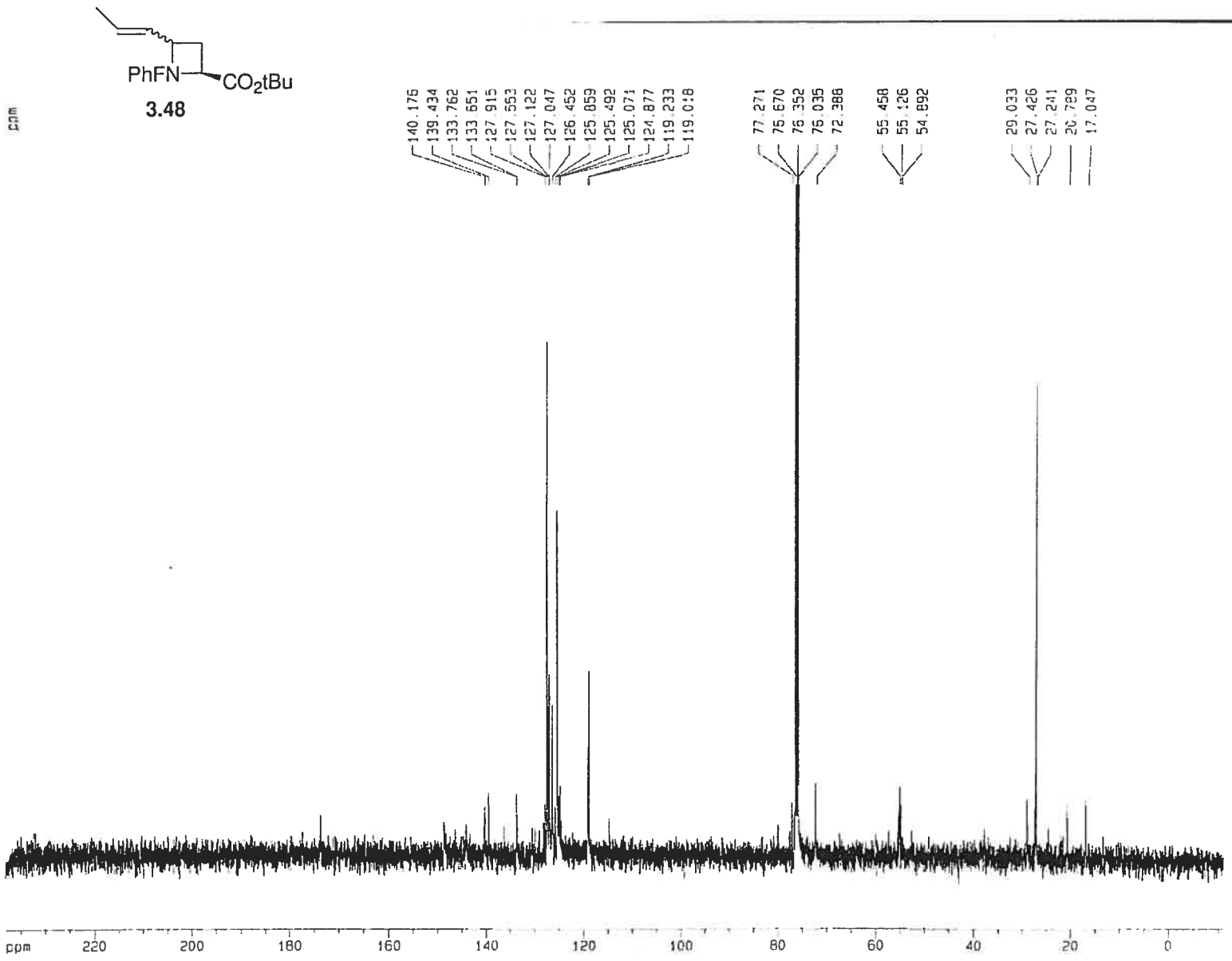


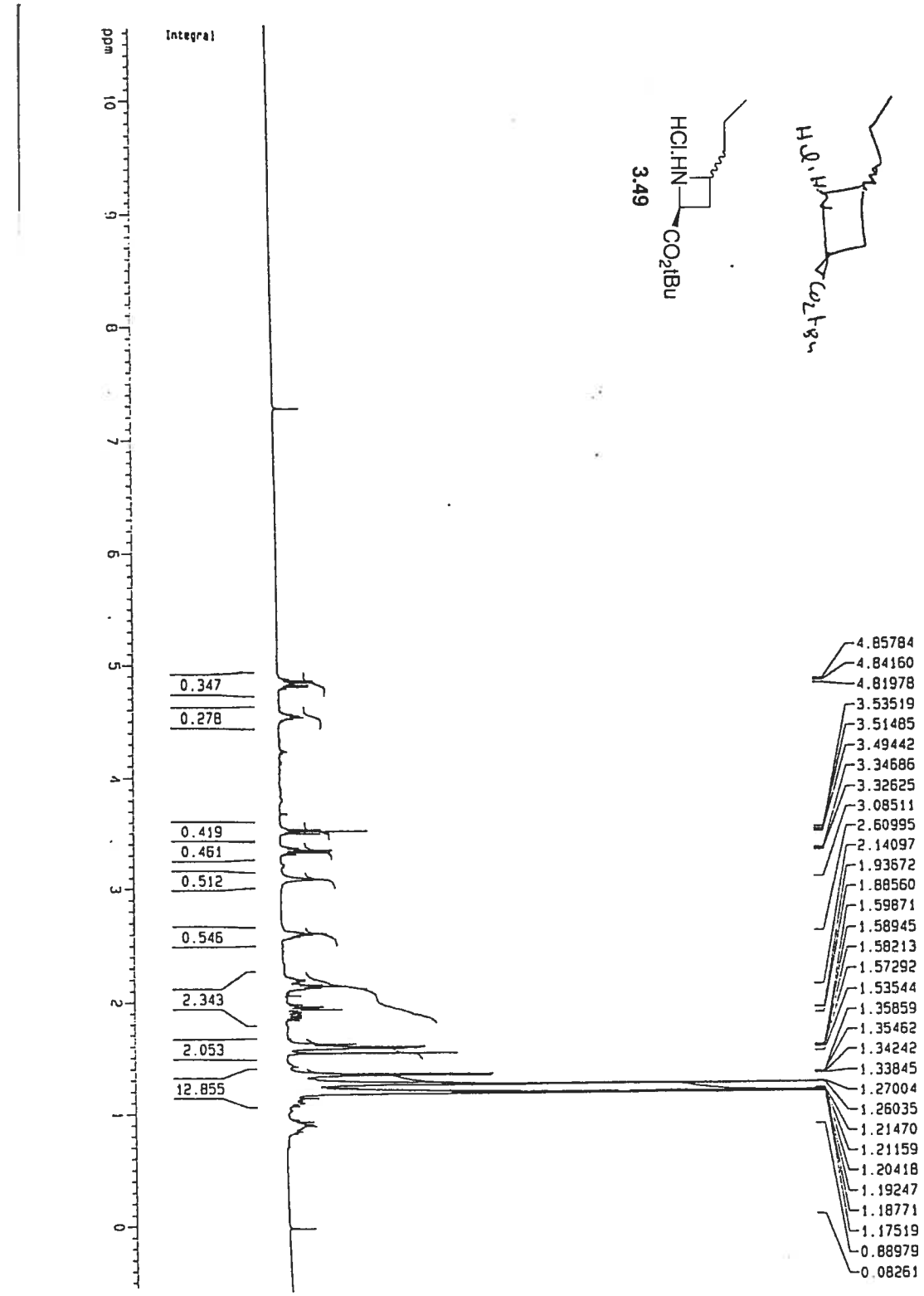
Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C30H33NO3	–	455.24604	0.10	1.98378 E7	–

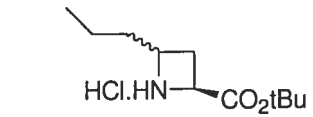
Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
(M+H)+	1988180.69	456.25332	456.25336	0.00004	0.09	–
(M+Na)+	109889.26	478.23527	478.23199	-0.00327	-6.84	–


TsO
H3C
FPhHN
CO2tBu
3.47
ppm
Integral
17.000
2.453
0.398
0.372
1.938
0.425
0.856
0.567
3.997
8.504
7.67822
7.65392
7.60687
7.58227
7.35252
7.32851
7.29043
7.26574
7.18575
7.15260
7.13773
7.11924
2.06729
1.59469
1.57337
1.51540
1.49460
1.47382
1.45401
1.22076
1.21062
1.19936
1.18382
1.15159
1.09962

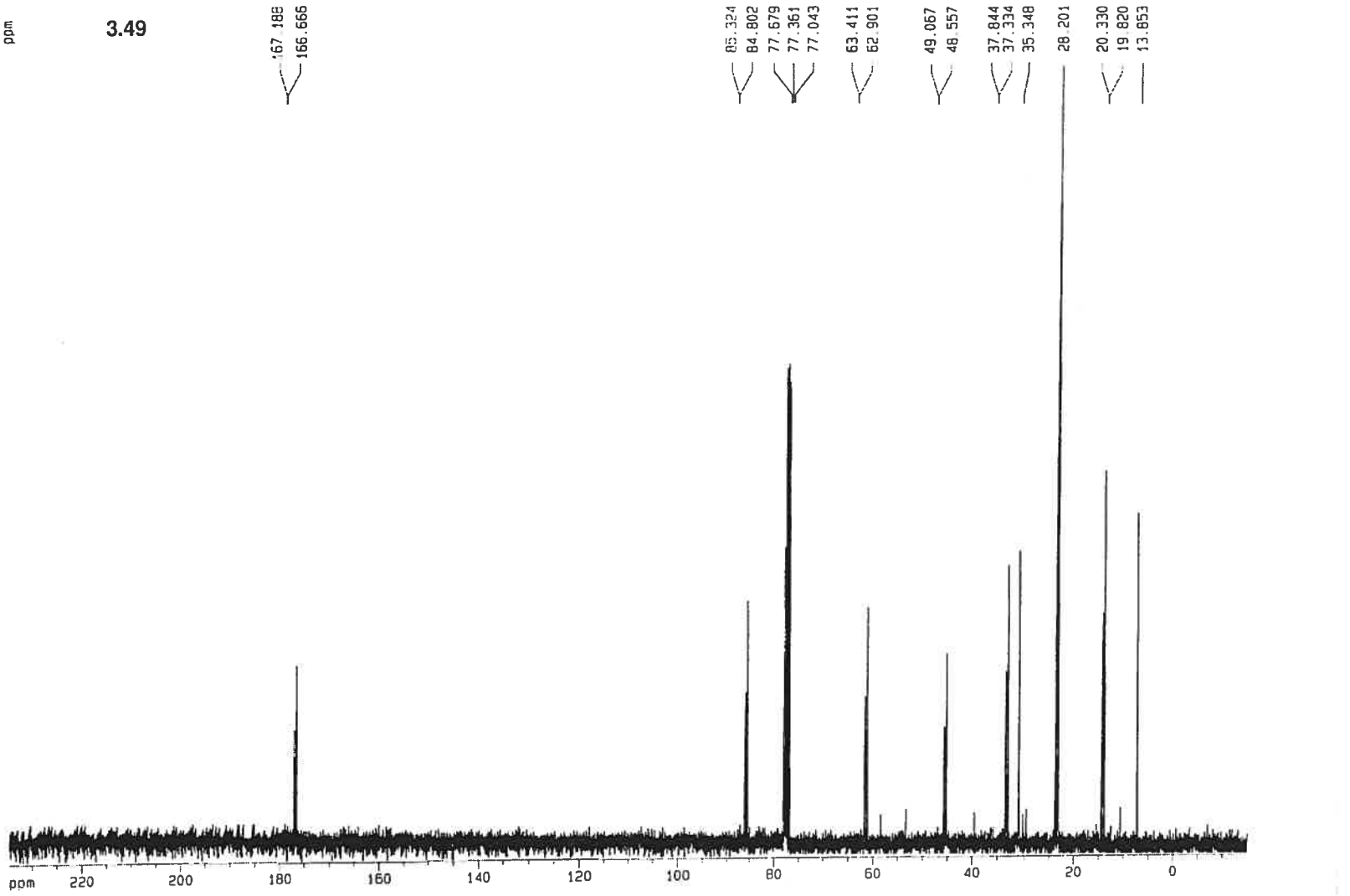

PhFN
CO2tBu
3.48
ppm
Integral
7.64894
7.60448
7.57992
7.54370
7.52233
7.35217
7.33131
7.30357
7.27805
7.25859
7.23752
7.21280
7.19298
7.17855
7.16916
7.15382
7.12498
7.12142
5.53164
3.43112
2.99258
2.05681
2.03600
1.51406
1.49435
1.48776
1.45080
1.27940
1.25805
1.18027
1.15034
1.10976
1.09580
0.00023
13.000
1.389
0.657
0.805
0.514
0.503
1.906
1.806
11.274


PhFN
CO2tBu
3.48
ppm
140.176
139.434
133.762
133.651
127.915
127.553
127.122
127.047
126.452
125.859
125.492
125.071
124.877
119.233
119.018
77.271
76.670
76.352
76.035
72.386
55.458
55.126
54.892
29.033
27.426
27.241
20.729
17.047
220
200
180
160
140
120
100
80
60
40
20
0


HCl.HN
CO2tBu
3.49
Integral
ppm
0.347
0.278
0.419
0.461
0.512
0.546
2.343
2.053
12.855
4.85784
4.84160
4.81978
3.53519
3.51485
3.49442
3.34686
3.32625
3.08511
2.60995
2.14097
1.93672
1.88560
1.59871
1.58945
1.58213
1.57292
1.53544
1.35859
1.35462
1.34242
1.33845
1.27004
1.26035
1.21470
1.21159
1.20418
1.19247
1.18771
1.17519
0.88979
0.08261


HCl.HN
CO2tBu

3.49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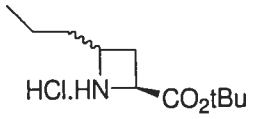
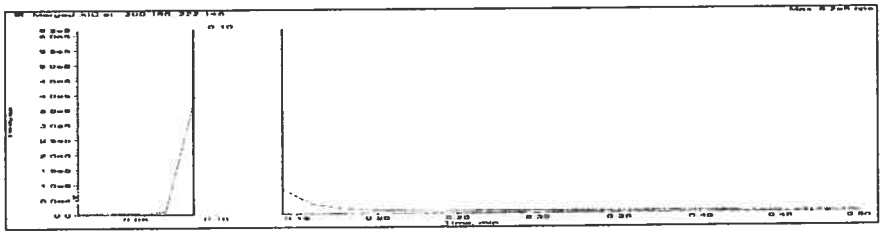
167.188
166.666
85.324
84.802
77.679
77.361
77.043
63.411
62.901
49.067
48.557
37.844
37.334
35.348
28.201
20.330
19.820
13.853
ppm
220
200
180
160
140
120
100
80
60
40
20
0

Empirical Formula Confirmation Report

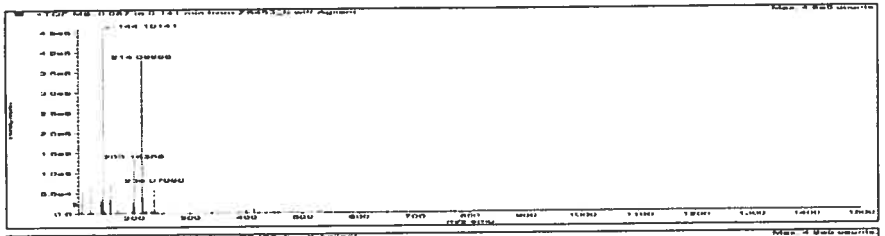


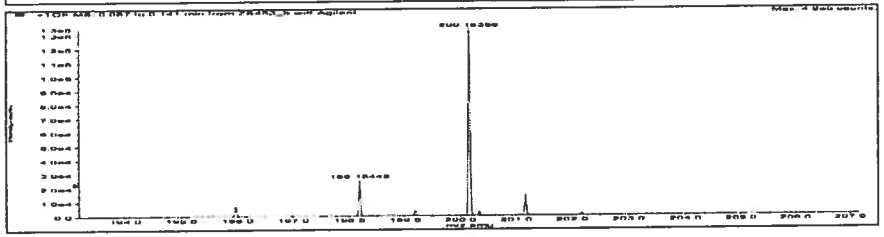
3.49

Sample Name: ZS453 Sample Location: P1-E4 Sample Id: Operator:
Data File Name: d:\PE Sciex Data\Projects\01fev_2006\Data\ZS453_b.wiff Acq Time: February 01 2006, 10:19:38 AM
Method: C:\Program Files\Agilent\TOF Software\damethods\test.anm\efc.xml



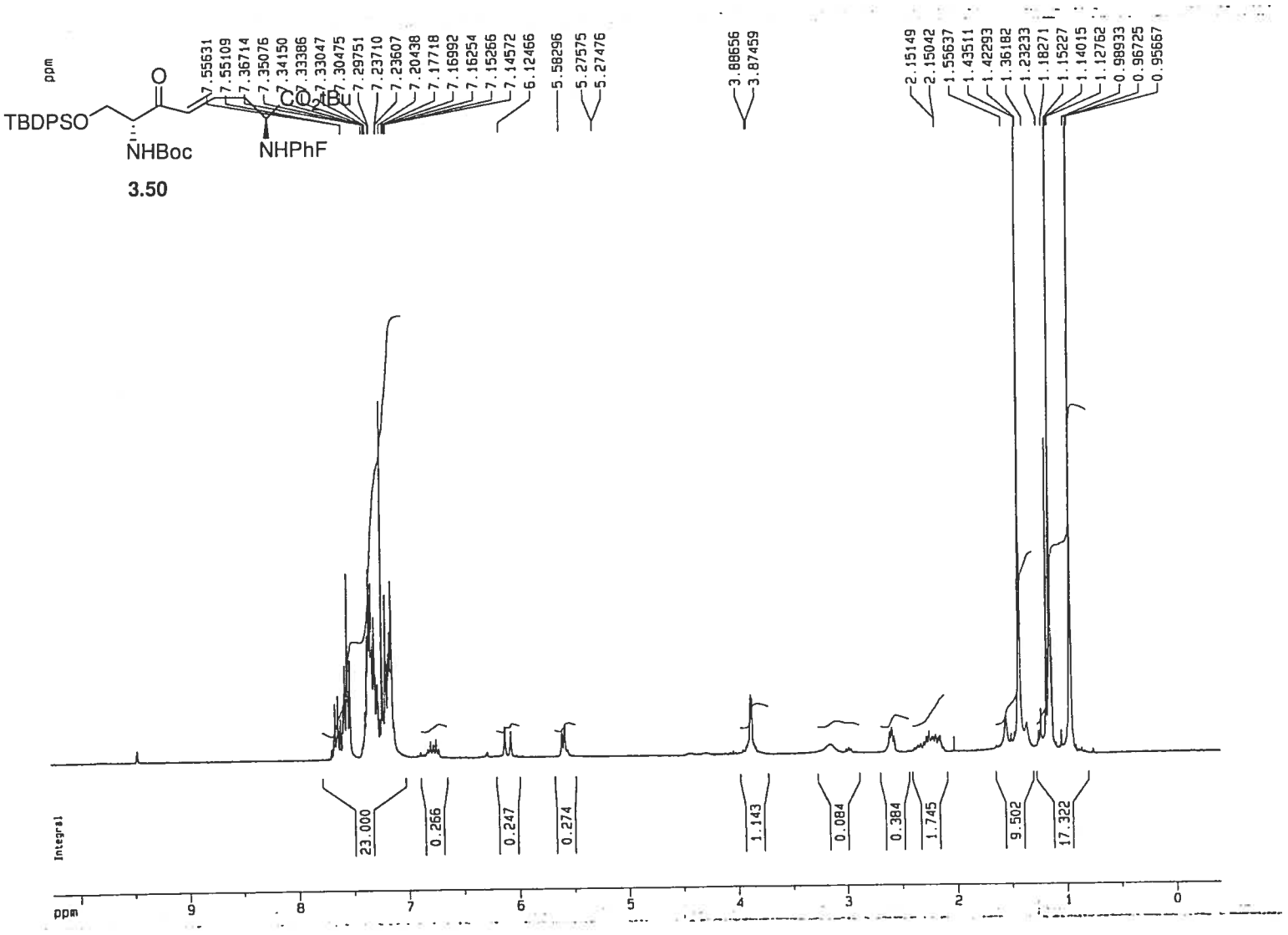
Merged XIC, Period# : 1 Experiment# : 1

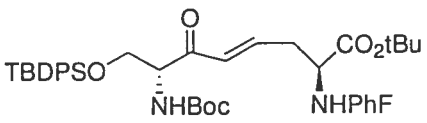




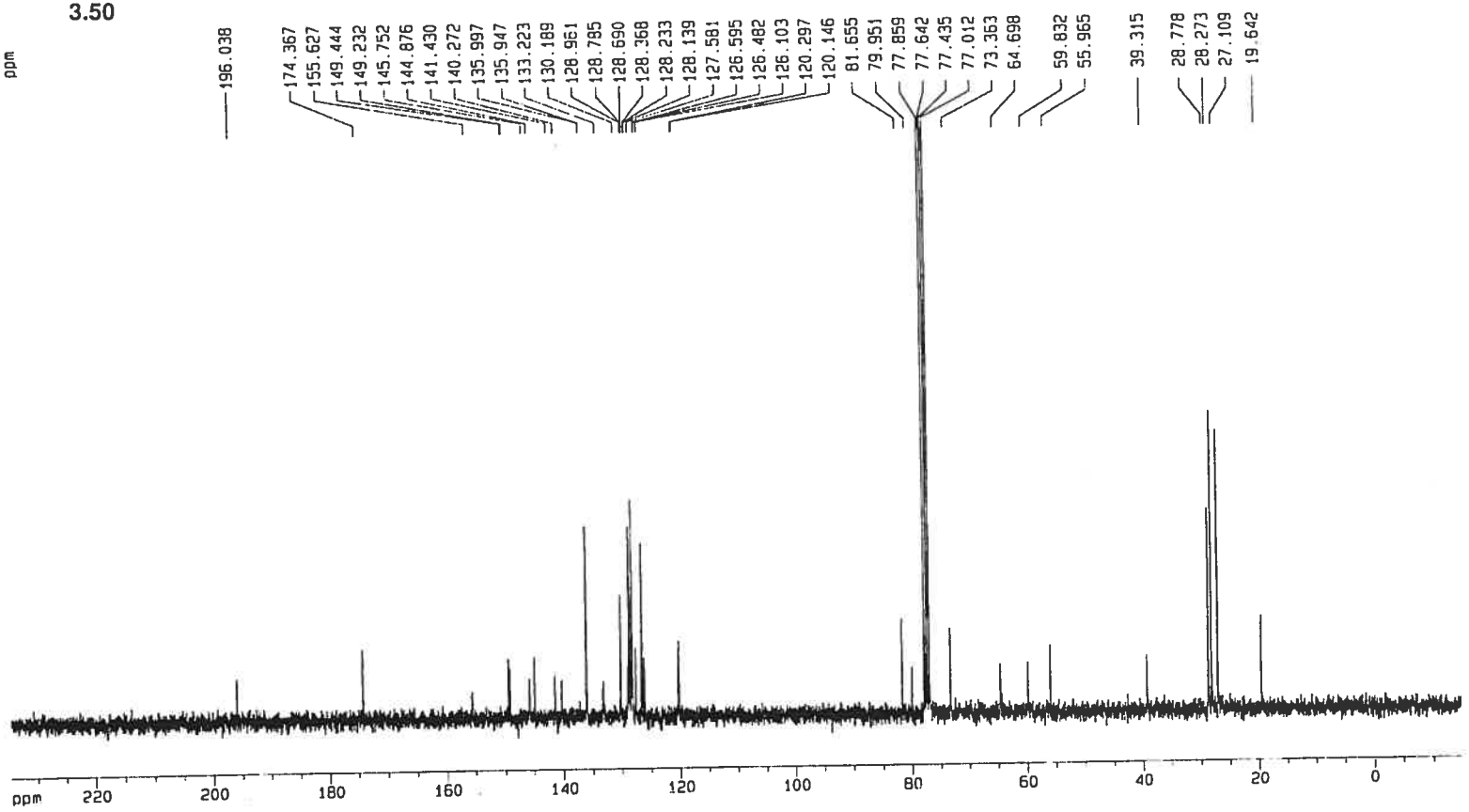
Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C11H21NO2	--	199.15723	0.10	1.49262 E6	--

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	139101.04	200.16451	200.16358	-0.00092	-4.60	--

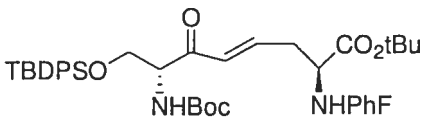

TBDPSO
O
NHBoc
CO2tBu
NHPhF
3.50
ppm
7.55631
7.55109
7.36714
7.35076
7.34150
7.33386
7.33047
7.30475
7.29751
7.23710
7.23607
7.20438
7.17718
7.16992
7.16254
7.15266
7.14572
6.12466
5.58296
5.27575
5.27476
3.88656
3.87459
2.15149
2.15042
1.55637
1.43511
1.42293
1.36182
1.23233
1.18271
1.15227
1.14015
1.12762
0.98933
0.96725
0.95667
Integral
23.000
0.266
0.247
0.274
1.143
0.084
0.384
1.745
9.502
17.322
ppm
9
8
7
6
5
4
3
2
1
0


O
TBDPSO
CO2tBu
NHBoc
NHPhF

3.50

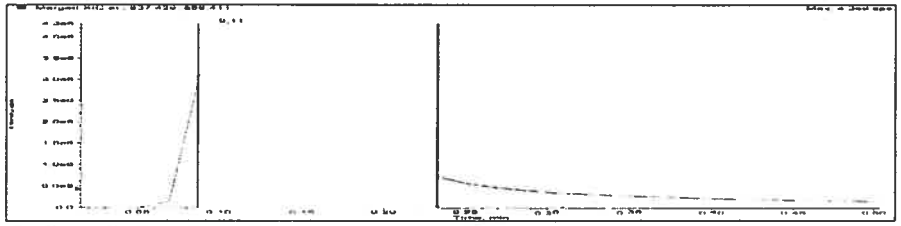

ppm
196.038
174.367
155.627
149.444
149.232
145.752
144.876
141.430
140.272
135.997
135.947
133.223
130.189
128.961
128.785
128.690
128.368
128.233
128.139
127.581
126.595
126.482
126.103
120.297
120.146
81.655
79.951
77.859
77.642
77.435
77.012
73.363
64.698
59.832
55.965
39.315
28.778
28.273
27.109
19.642
ppm 220 200 180 160 140 120 100 80 60 40 20 0

Empirical Formula Confirmation Report

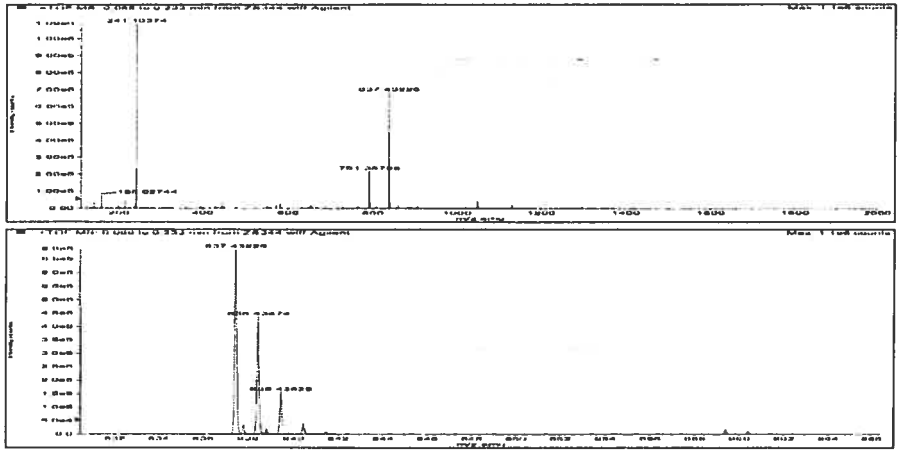


3.50

Sample Name: ZS344 Sample Location: P1-C3 Sample Id: Operator:
Data File Name: d:\PE Sciex Data\Projects\02mar_2006\Data\ZS344.wiff Acq Time: March 02 2006, 02:17:24 PM
Method: C:\Program Files\Agilent\TOF Software\damethods\test.anm\efc.xml

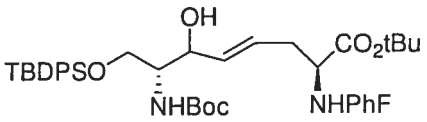


Merged XIC, Period# : 1 Experiment# : 1

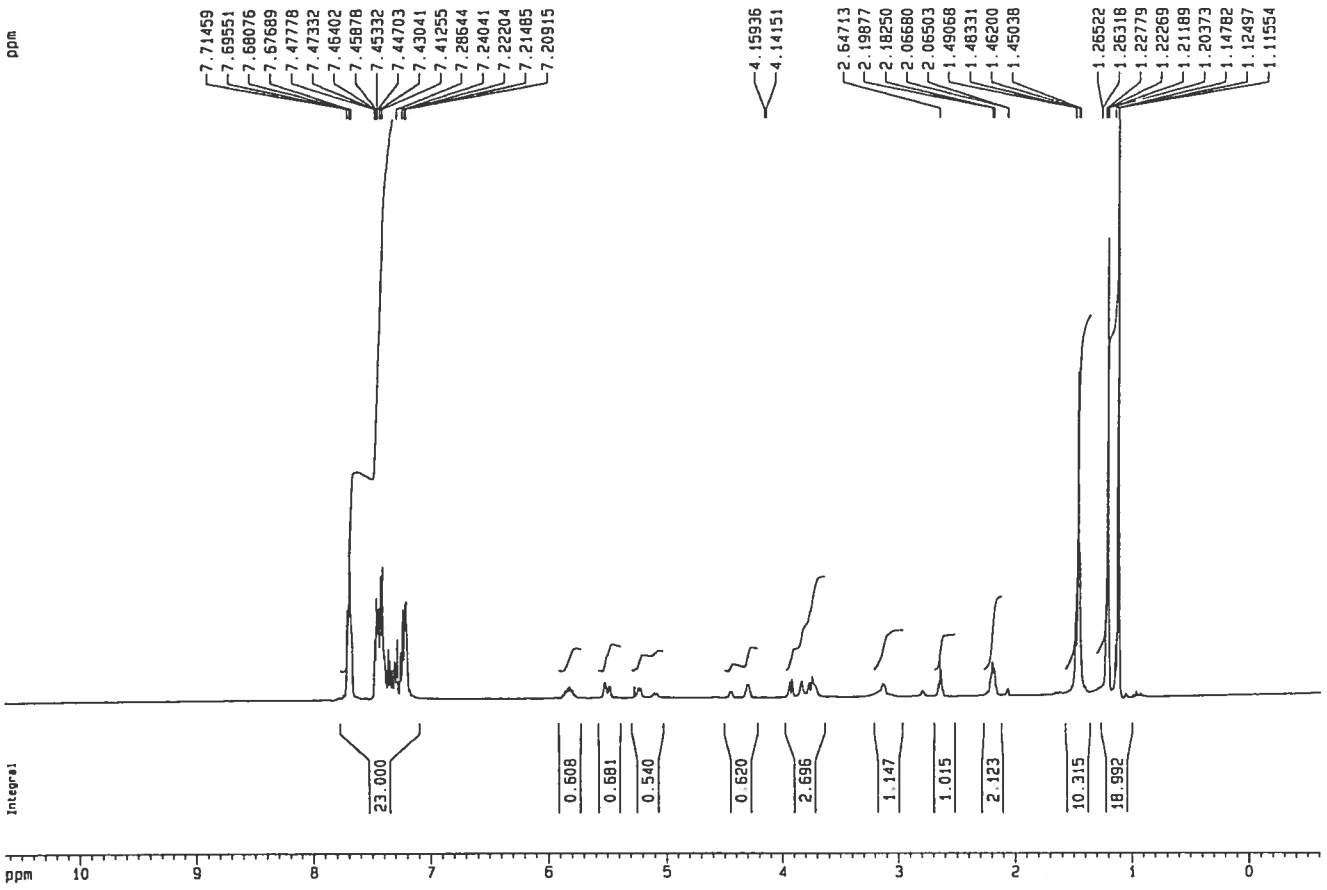


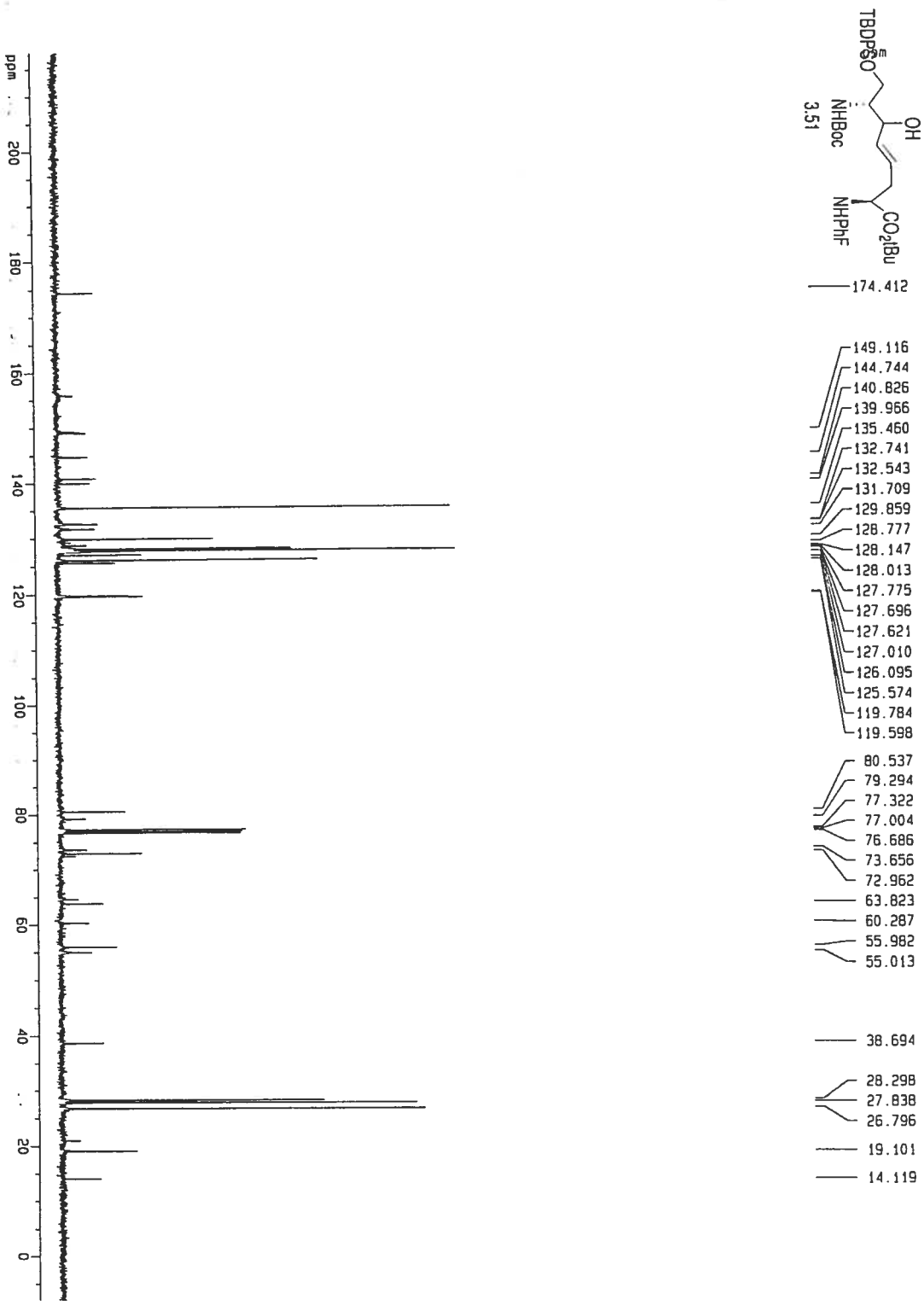
Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C52H60N2O6Si	--	836.42206	0.11	2.31078 E7	--

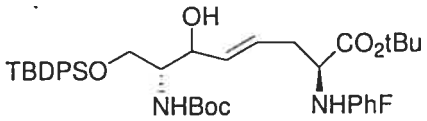
Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	717994.15	837.42934	837.43226	0.00292	3.48	--
[M+Na]+	16975.86	859.41129	859.41308	0.00179	2.09	--


OH
CO2tBu
TBDPSO
NHBoc
NHPhF

3.51


ppm
7.71459
7.69551
7.68076
7.67689
7.47778
7.47332
7.46402
7.45878
7.45332
7.44703
7.43041
7.41255
7.28644
7.24041
7.22204
7.21485
7.20915
4.15936
4.14151
2.64713
2.19877
2.18250
2.06680
2.06503
1.49068
1.48331
1.46200
1.45038
1.26522
1.26318
1.22779
1.22269
1.21189
1.20373
1.14782
1.12497
1.11554
Integral
23.000
0.608
0.681
0.540
0.620
2.696
1.147
1.015
2.123
10.315
18.992
ppm
10
9
8
7
6
5
4
3
2
1
0

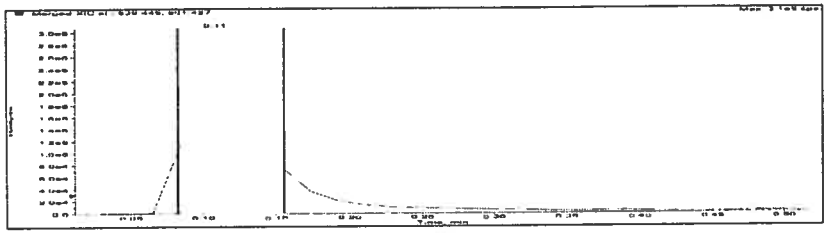

TBDPSO
OH
NHBoc
3.51
NHPhF
CO2tBu
174.412
149.116
144.744
140.826
139.966
135.460
132.741
132.543
131.709
129.859
128.777
128.147
128.013
127.775
127.696
127.621
127.010
126.095
125.574
119.784
119.598
80.537
79.294
77.322
77.004
76.686
73.656
72.962
63.823
60.287
55.982
55.013
38.694
28.298
27.838
26.796
19.101
14.119
ppm
200
180
160
140
120
100
80
60
40
20
0



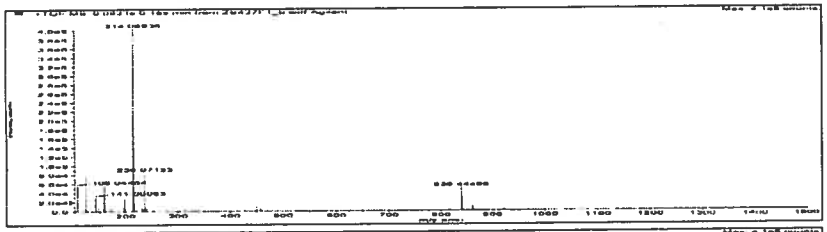
3.51

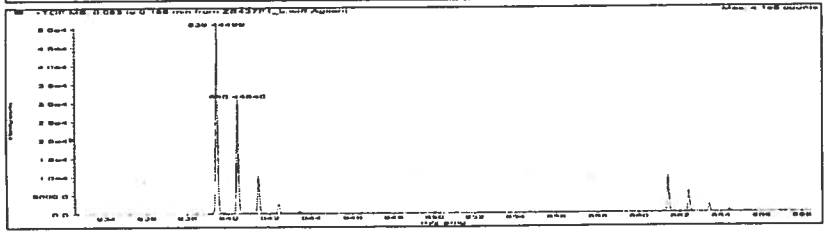
Empirical Formula Confirmation Report

Sample Name: ZS437F1 Sample Location: P1-E5 Sample Id: Operator:
Data File Name: d:\PE Sciex Data\Projects\01(ey_2006)\Data\ZS437F1_b.wiff Acq Time: February 01 2006, 10:17:36 AM
Method: C:\Program Files\Agilent\TOF Software\damethods\test.anmeth.xml



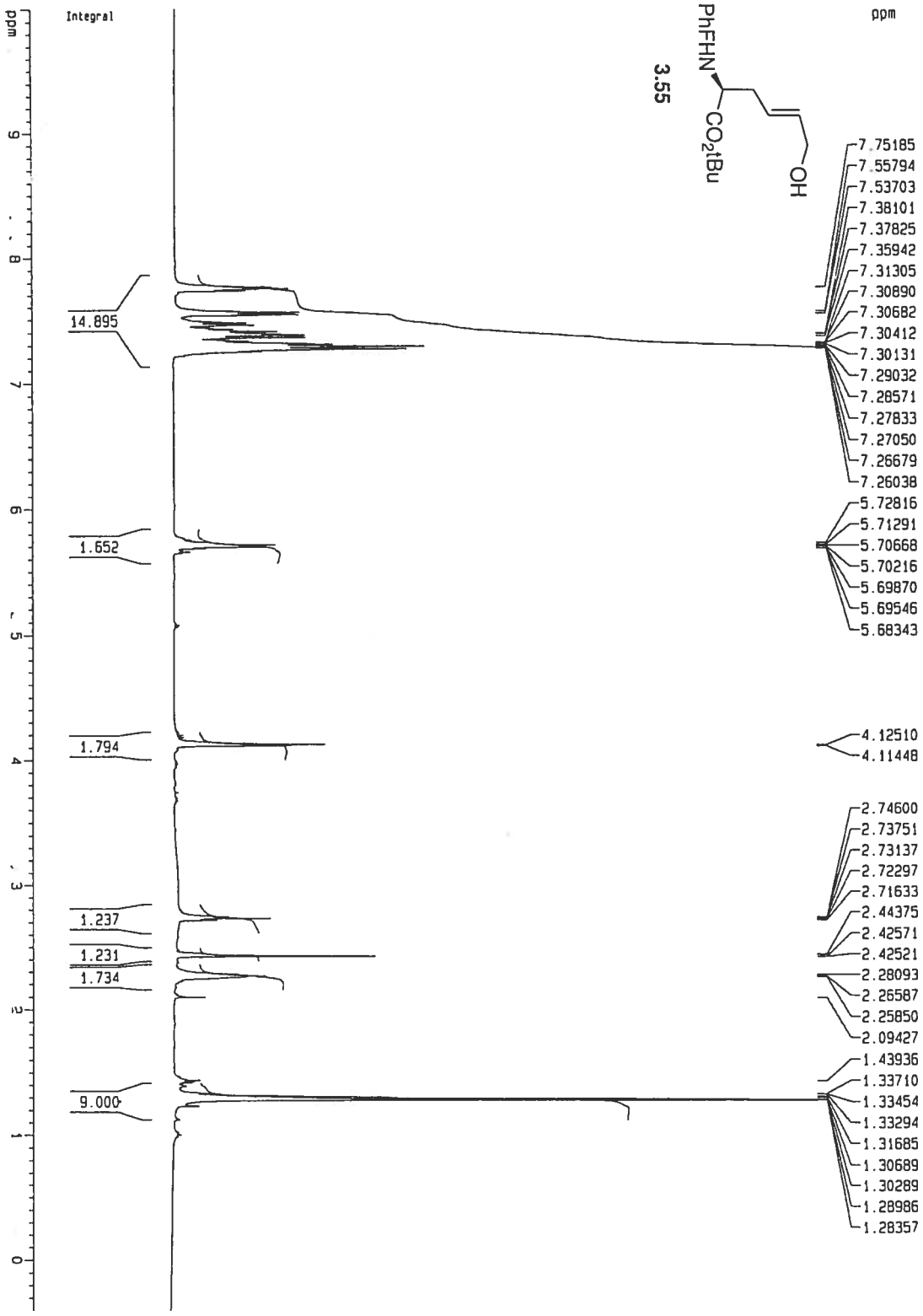
Merged XIC, Period# : 1 Experiment# : 1

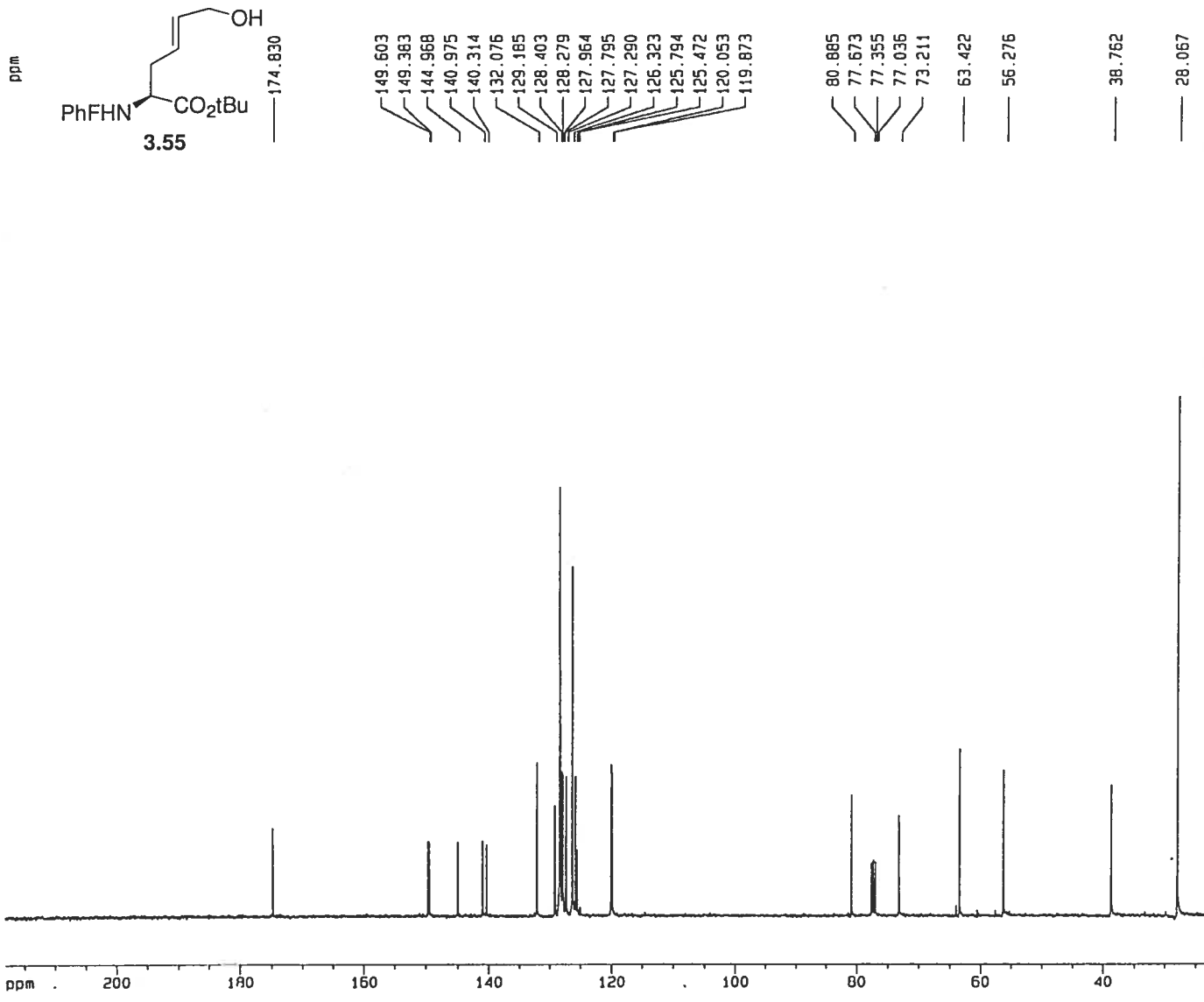


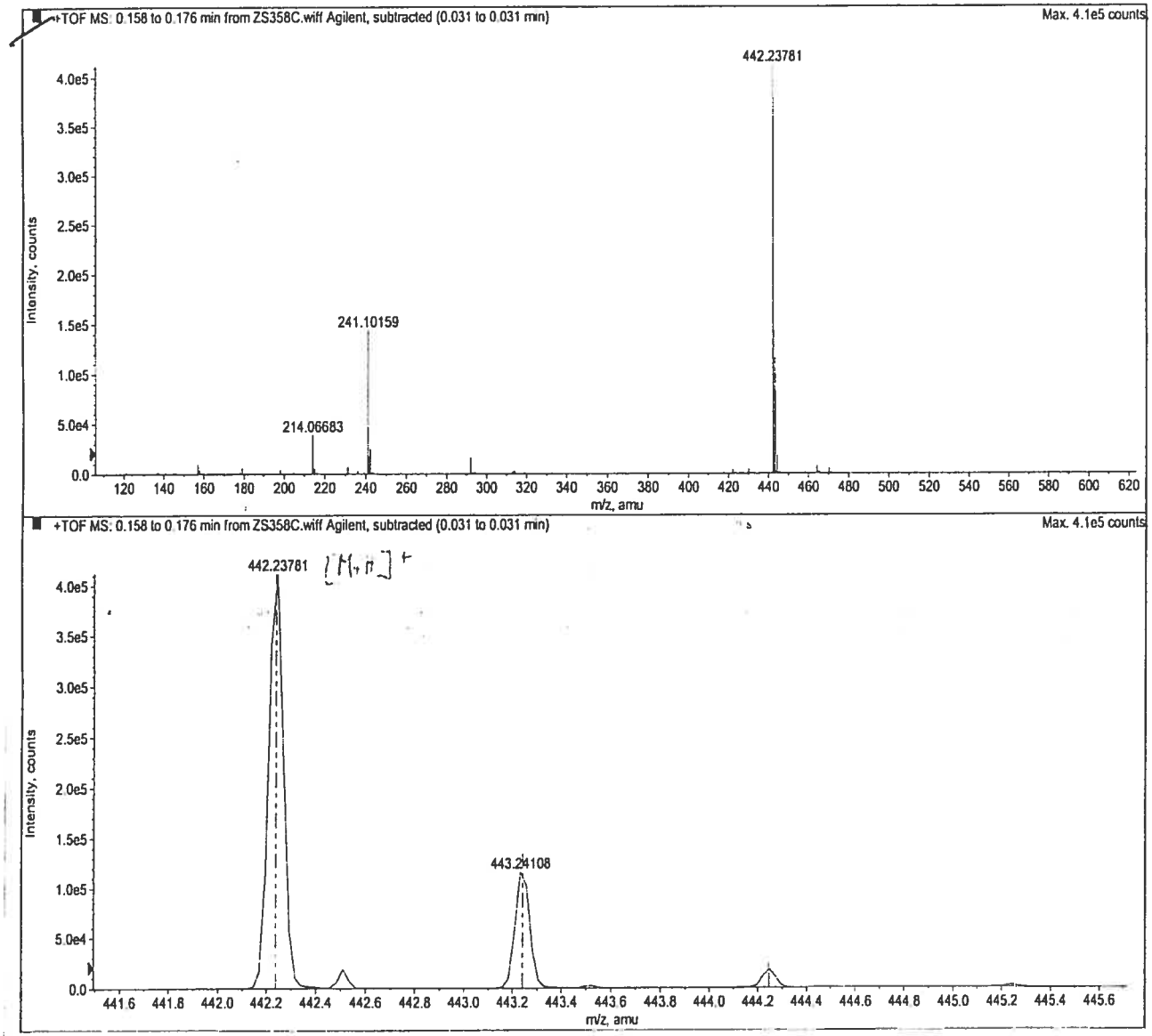


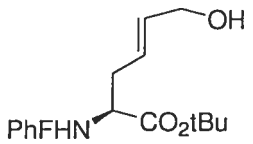
Formula	Compound name	Mass	Peak RT (min)	Peak area	Description
C52H62N2O6Si	–	838.43771	0.11	1.05672 E6	–

Species	Abundance (counts)	Ion Mass	Measured Mass	Error (mDa)	Error (ppm)	Ret. Time Error (min)
[M+H]+	50608.18	839.44499	839.44499	0.00000	0.00	–
[M+Na]+	9942.93	861.42694	861.42762	0.00068	0.79	–

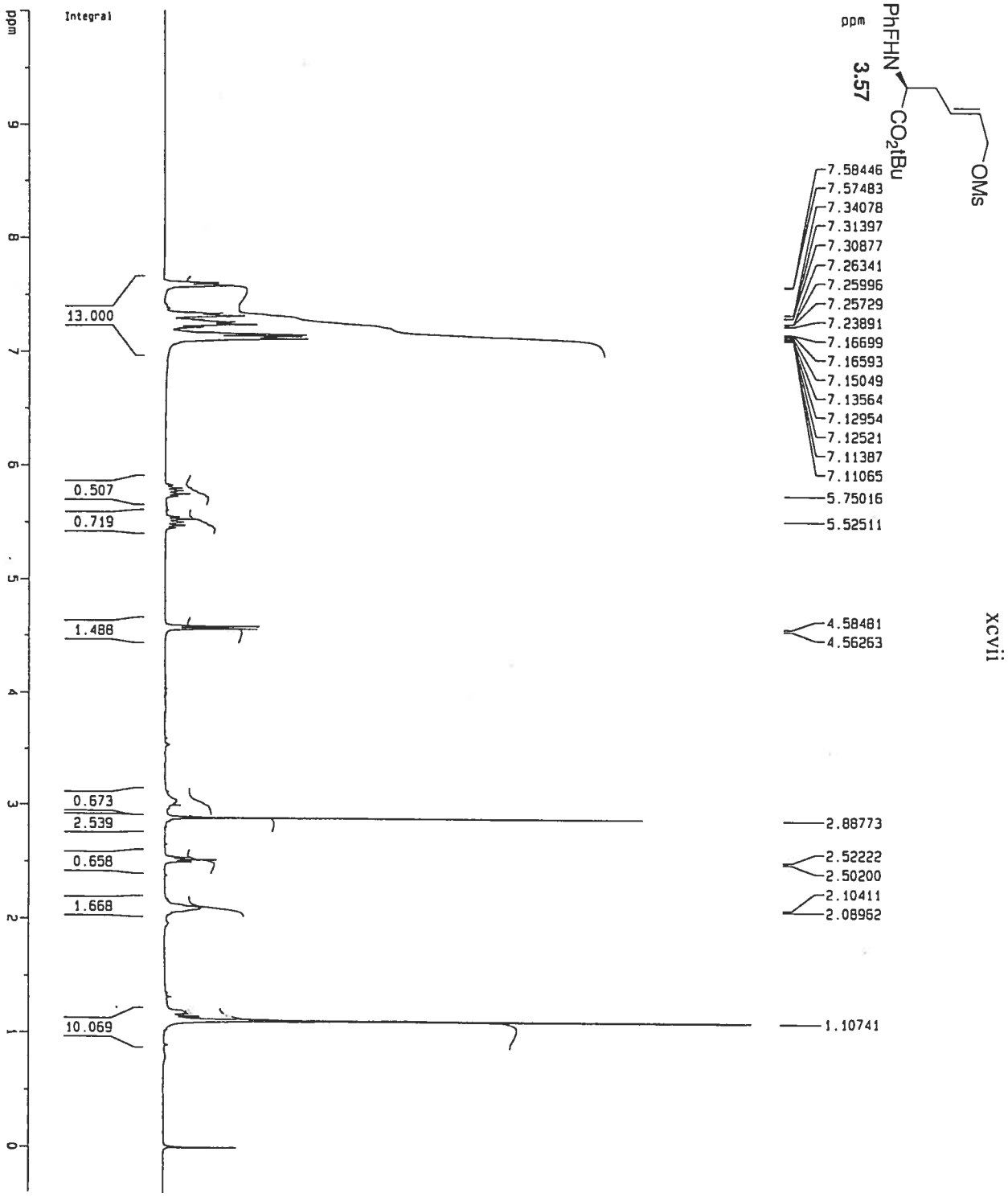

Integral
ppm
PhFHN
CO2tBu
OH
3.55
14.895
1.652
1.794
1.237
1.231
1.734
9.000
7.75185
7.55794
7.53703
7.38101
7.37825
7.35942
7.31305
7.30890
7.30682
7.30412
7.30131
7.29032
7.28571
7.27833
7.27050
7.26679
7.26038
5.72816
5.71291
5.70668
5.70216
5.69870
5.69546
5.68343
4.12510
4.11448
2.74600
2.73751
2.73137
2.72297
2.71633
2.44375
2.42571
2.42521
2.28093
2.26587
2.25850
2.09427
1.43936
1.33710
1.33454
1.33294
1.31685
1.30689
1.30289
1.28986
1.28357


OH
PhFHN
CO2tBu
3.55
ppm
174.830
149.603
149.383
144.968
140.975
140.314
132.076
129.185
128.403
128.279
127.964
127.795
127.290
126.323
125.794
125.472
120.053
119.873
80.885
77.673
77.355
77.036
73.211
63.422
56.276
38.762
28.067
ppm
200
180
160
140
120
100
80
60
40
20
0


+TOF MS: 0.158 to 0.176 min from ZS358C.wiff Agilent, subtracted (0.031 to 0.031 min)
Max. 4.1e5 counts.
442.23781
241.10159
214.06683
Intensity, counts
m/z, amu
+TOF MS: 0.158 to 0.176 min from ZS358C.wiff Agilent, subtracted (0.031 to 0.031 min)
Max. 4.1e5 counts.
442.23781
[M+H]+
443.24108
Intensity, counts
m/z, amu

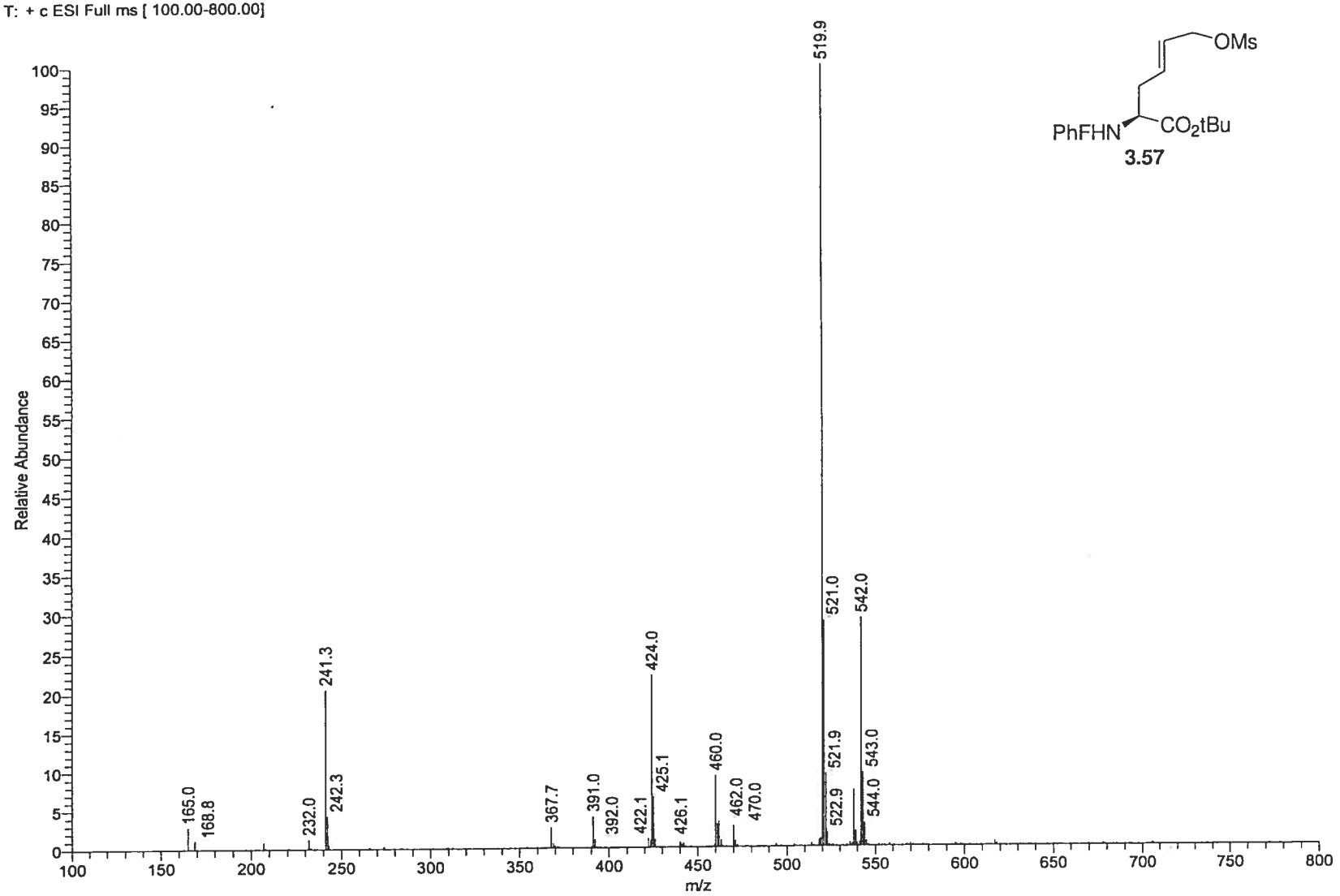

OH
PhFHN
CO2tBu

3.55

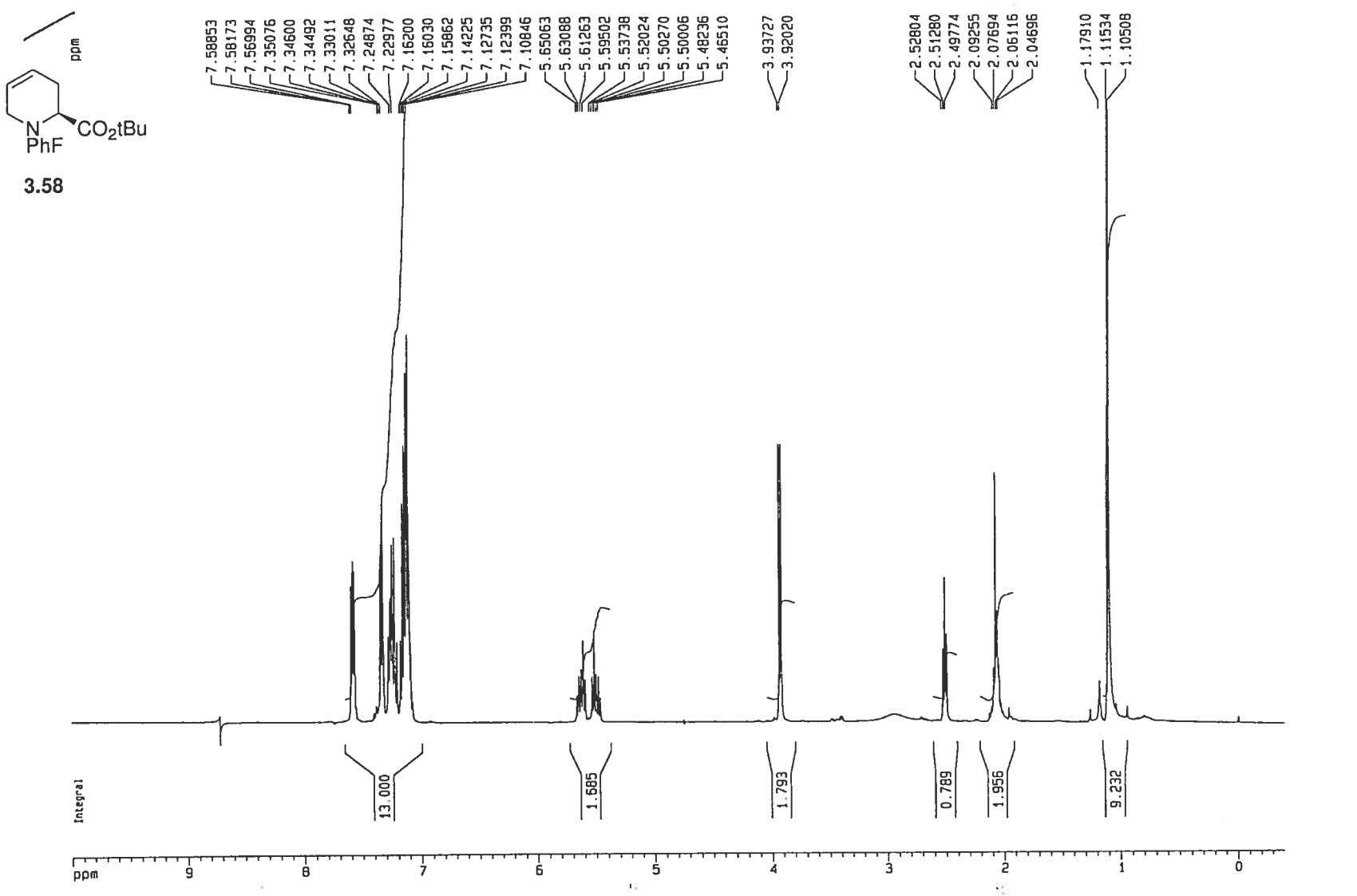

Integral
ppm
13.000
0.507
0.719
1.488
0.673
2.539
0.658
1.668
10.069
PhFHN
CO2tBu
OMs
ppm
3.57
7.58446
7.57483
7.34078
7.31397
7.30877
7.26341
7.25996
7.25729
7.23891
7.16699
7.16593
7.15049
7.13564
7.12954
7.12521
7.11387
7.11065
5.75016
5.52511
4.58481
4.56263
2.88773
2.52222
2.50200
2.10411
2.08962
1.10741

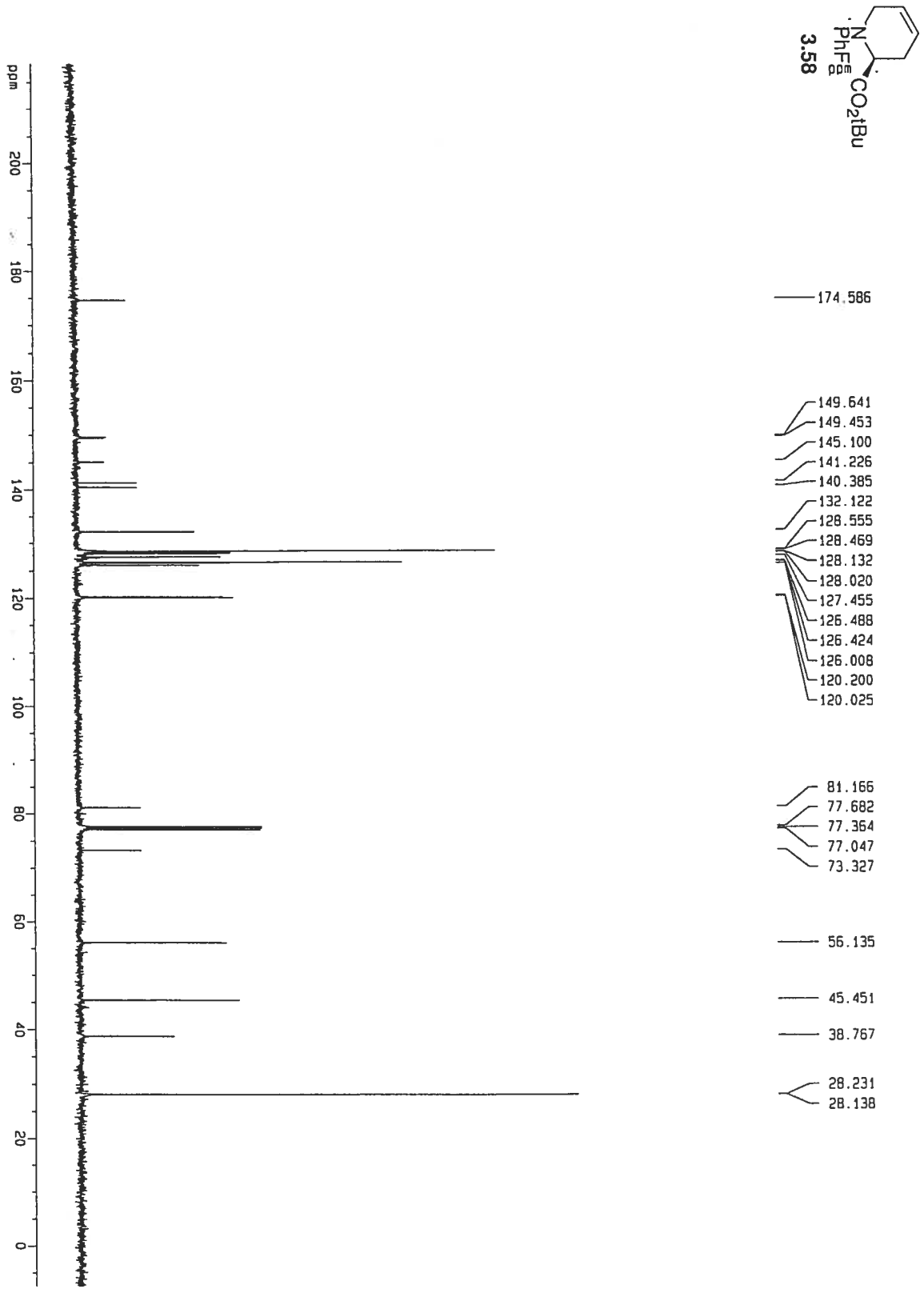
C:\Ana Direct Injection\Data\ZS-360 05/12/2005 01:09:18 PM

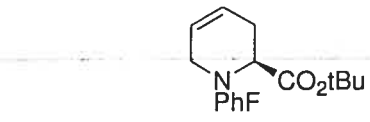
ZS-360 #93-110 RT: 1.23-1.46 AV: 18 SB: 37 0.04-0.28, 2.64-2.91 NL: 1.12E7
T: + c ESI Full ms [100.00-800.00]



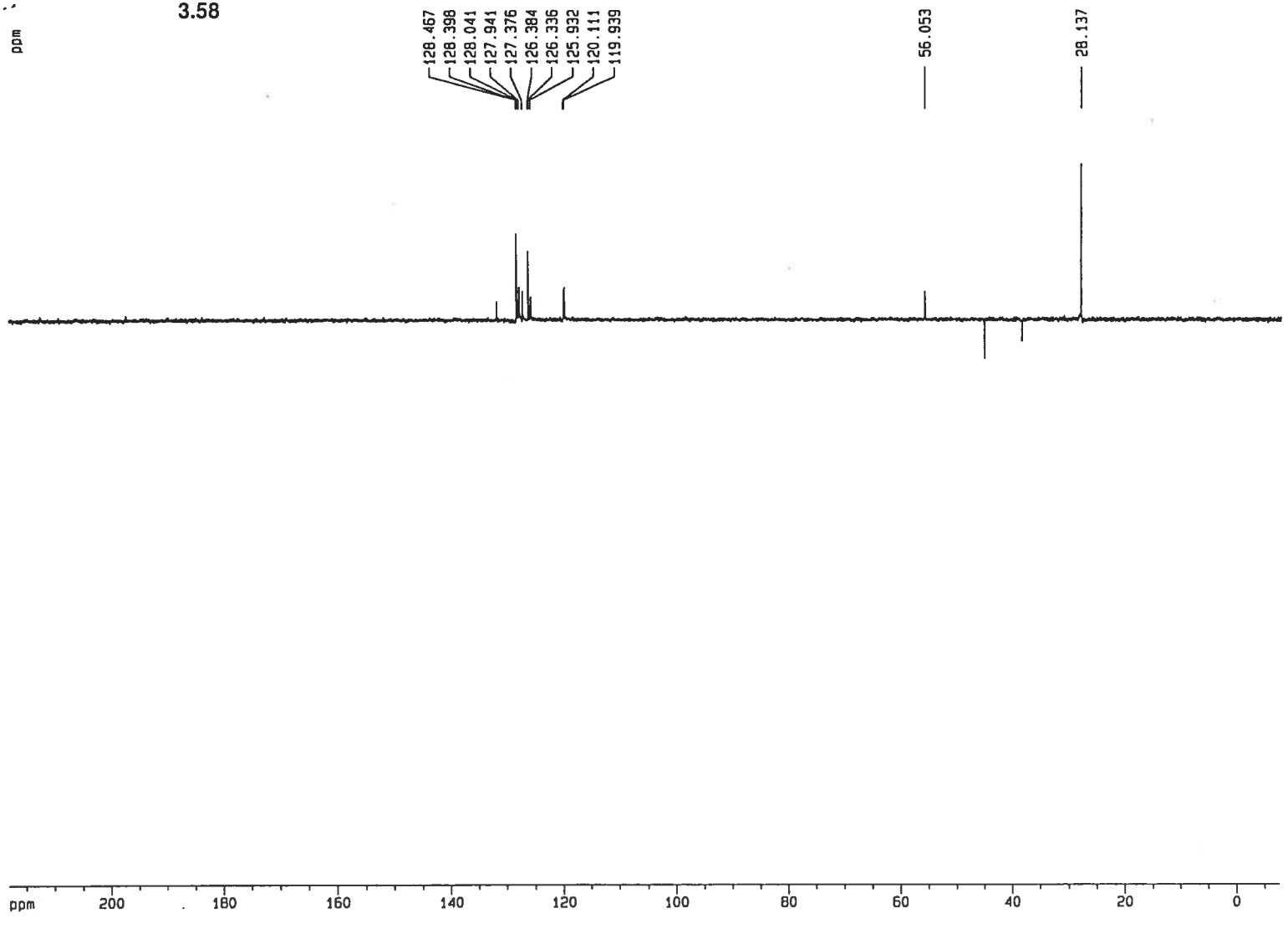
3.57

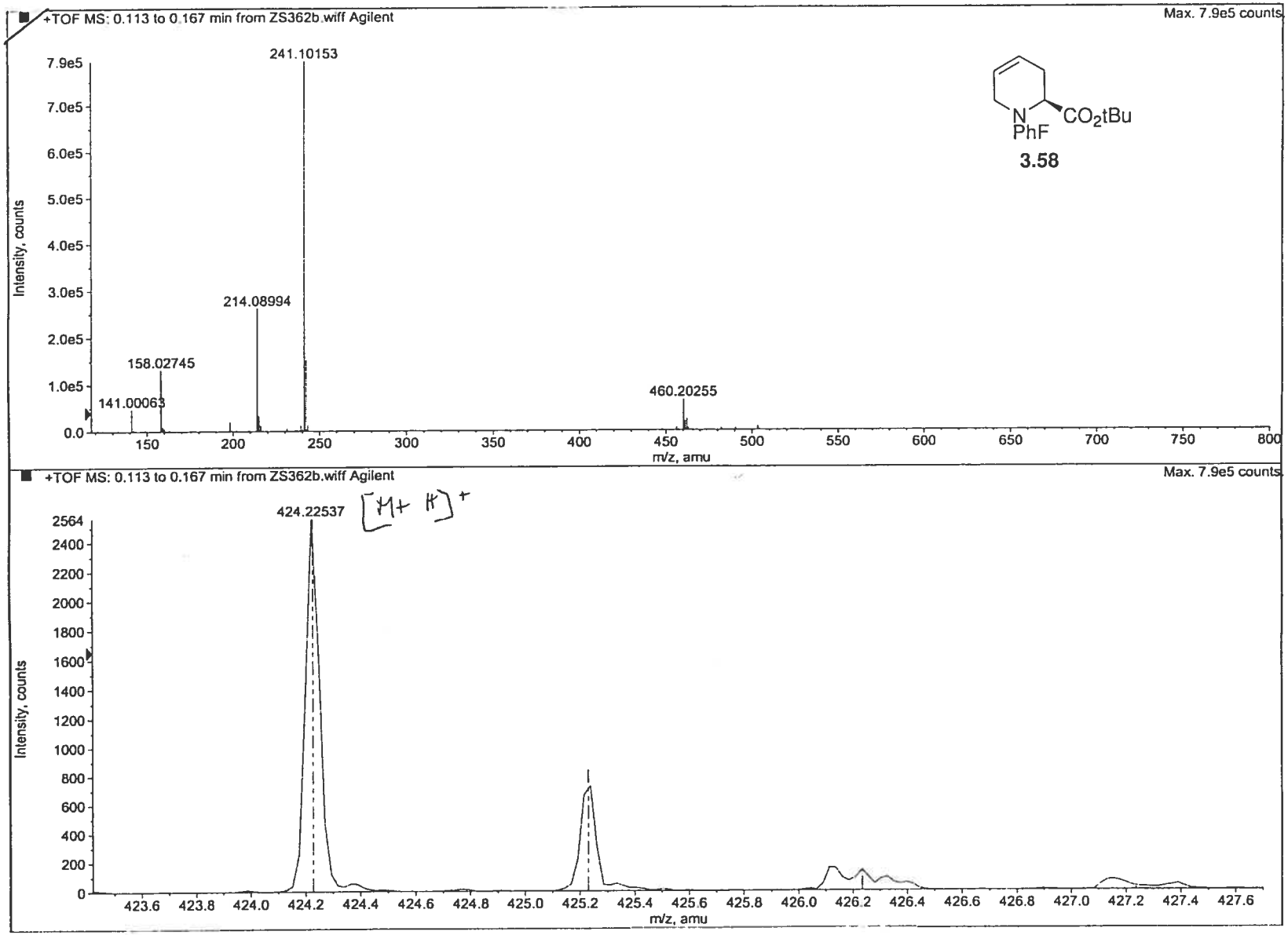

ppm
N
PhF
CO2tBu
3.58
7.58853
7.58173
7.56994
7.35076
7.34600
7.34492
7.33011
7.32648
7.24874
7.22977
7.16200
7.16030
7.15862
7.14225
7.12735
7.12399
7.10846
5.65063
5.63088
5.61263
5.59502
5.53738
5.52024
5.50270
5.50006
5.48236
5.46510
3.93727
3.92020
2.52804
2.51280
2.49774
2.09255
2.07694
2.06116
2.04696
1.17910
1.11534
1.10508
Integral
13.000
1.685
1.793
0.789
1.956
9.232
ppm
9
8
7
6
5
4
3
2
1
0

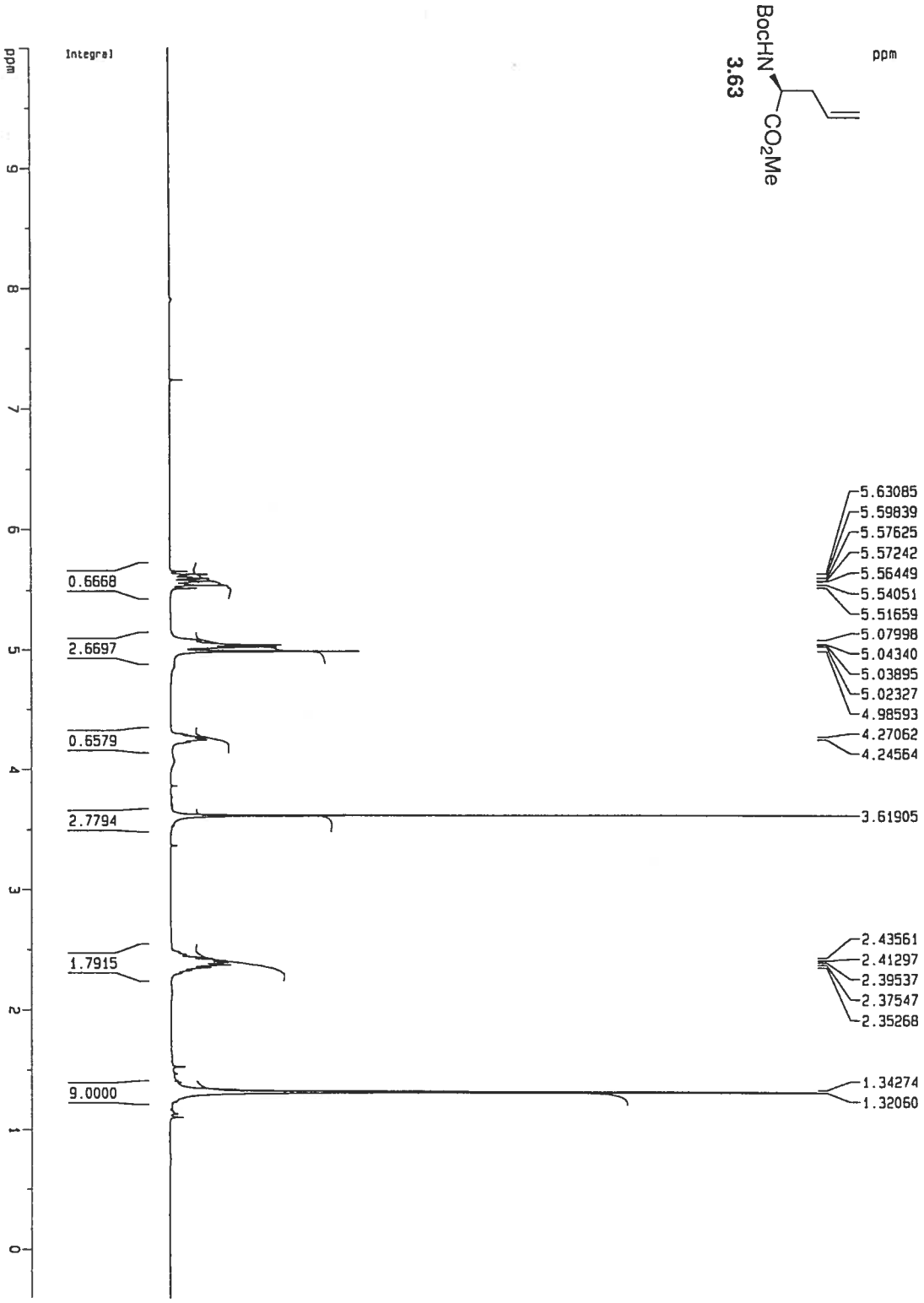

3.58
N
PhF
pp
CO2tBu
ppm
200
180
160
140
120
100
80
60
40
20
0
174.586
149.641
149.453
145.100
141.226
140.385
132.122
128.555
128.469
128.132
128.020
127.455
126.488
126.424
126.008
120.200
120.025
81.166
77.682
77.364
77.047
73.327
56.135
45.451
38.767
28.231
28.138

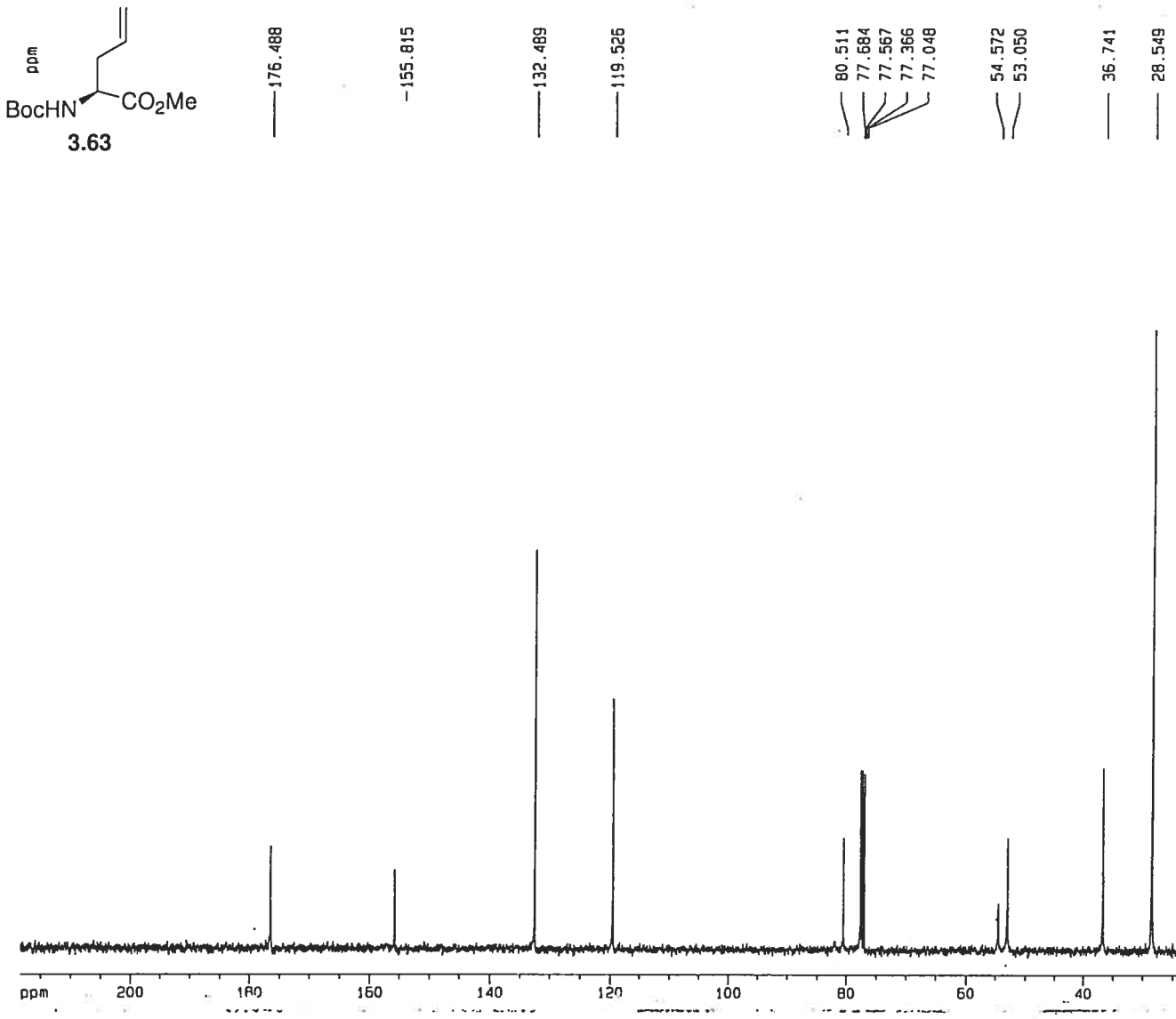

N
PhF
CO2tBu

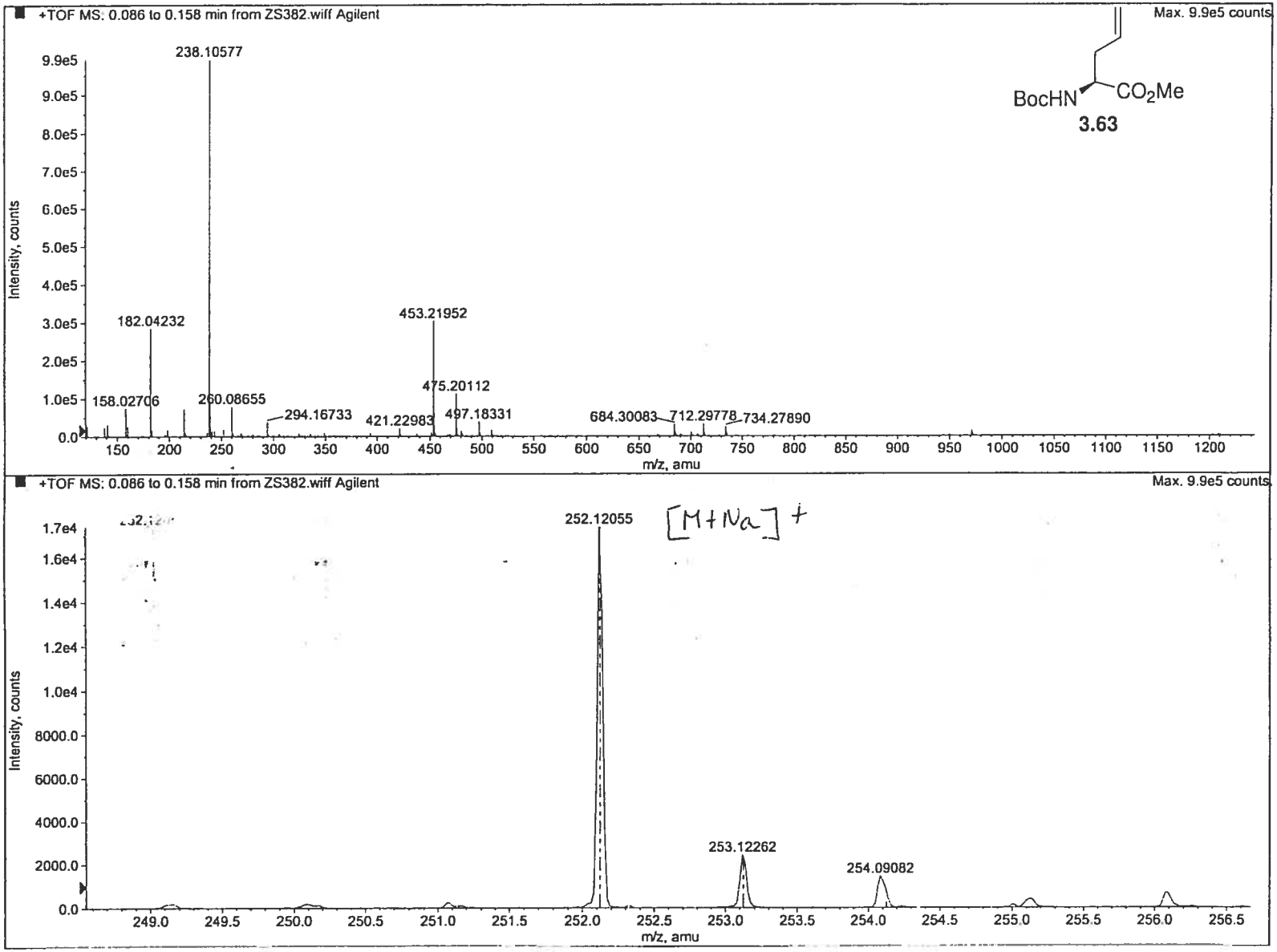
3.58

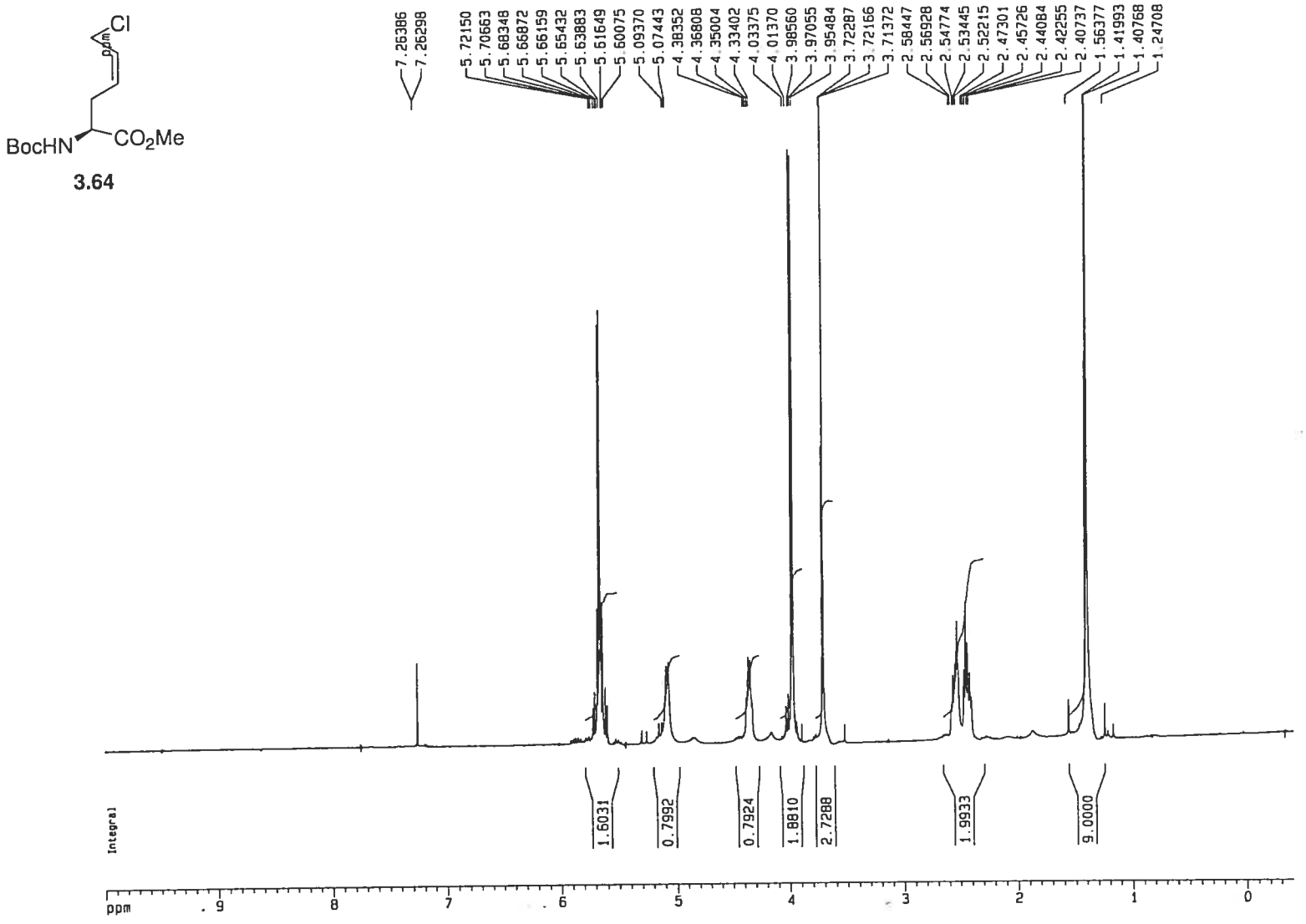

ppm
128.467
128.398
128.041
127.941
127.376
126.384
126.336
125.932
120.111
119.939
56.053
28.137
ppm
200
180
160
140
120
100
80
60
40
20
0

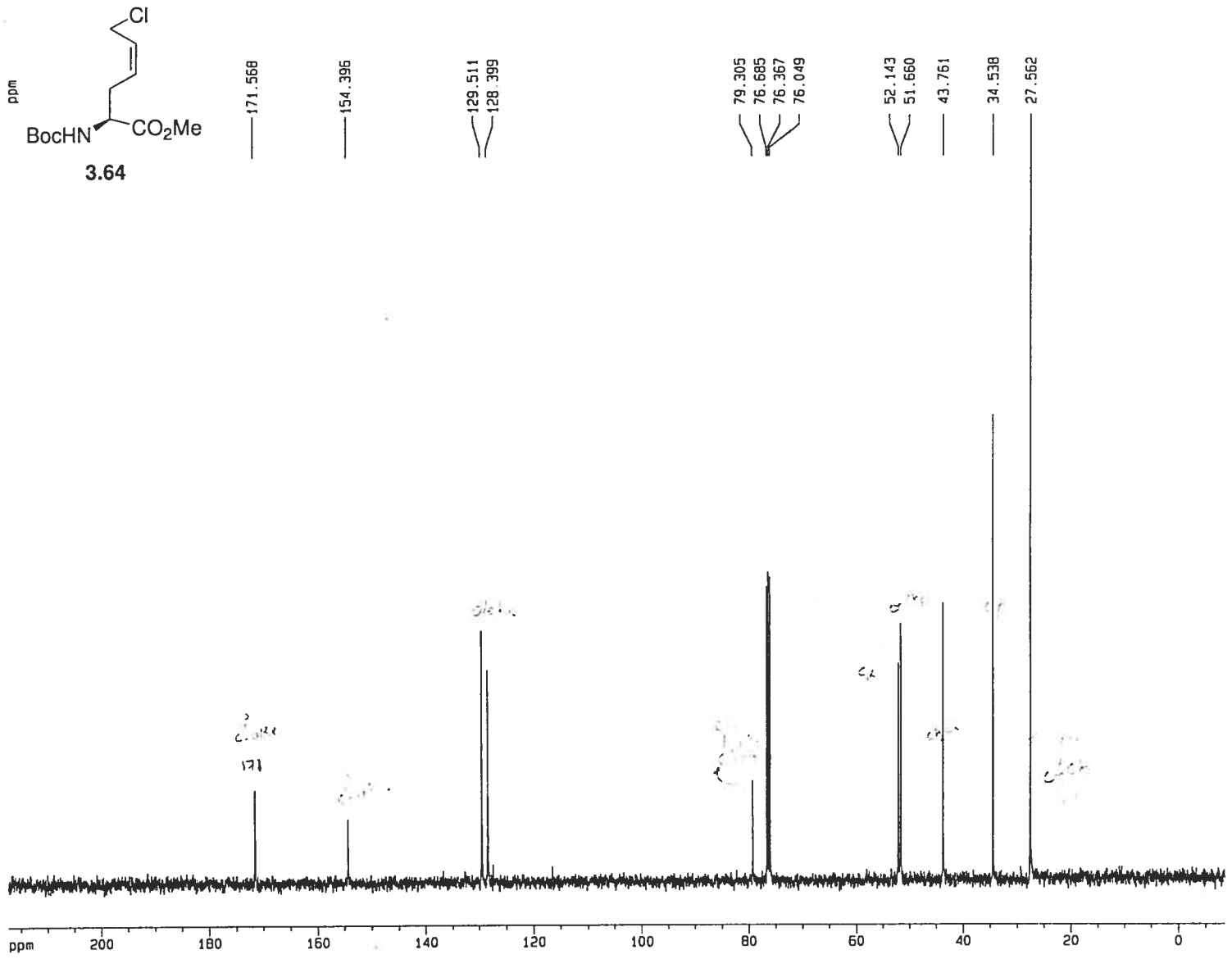

+TOF MS: 0.113 to 0.167 min from ZS362b.wiff Agilent
Max. 7.9e5 counts
Intensity, counts
m/z, amu
241.10153
214.08994
158.02745
141.00063
460.20255
N
PhF
CO2tBu
3.58
+TOF MS: 0.113 to 0.167 min from ZS362b.wiff Agilent
Max. 7.9e5 counts
424.22537
[M+H]+

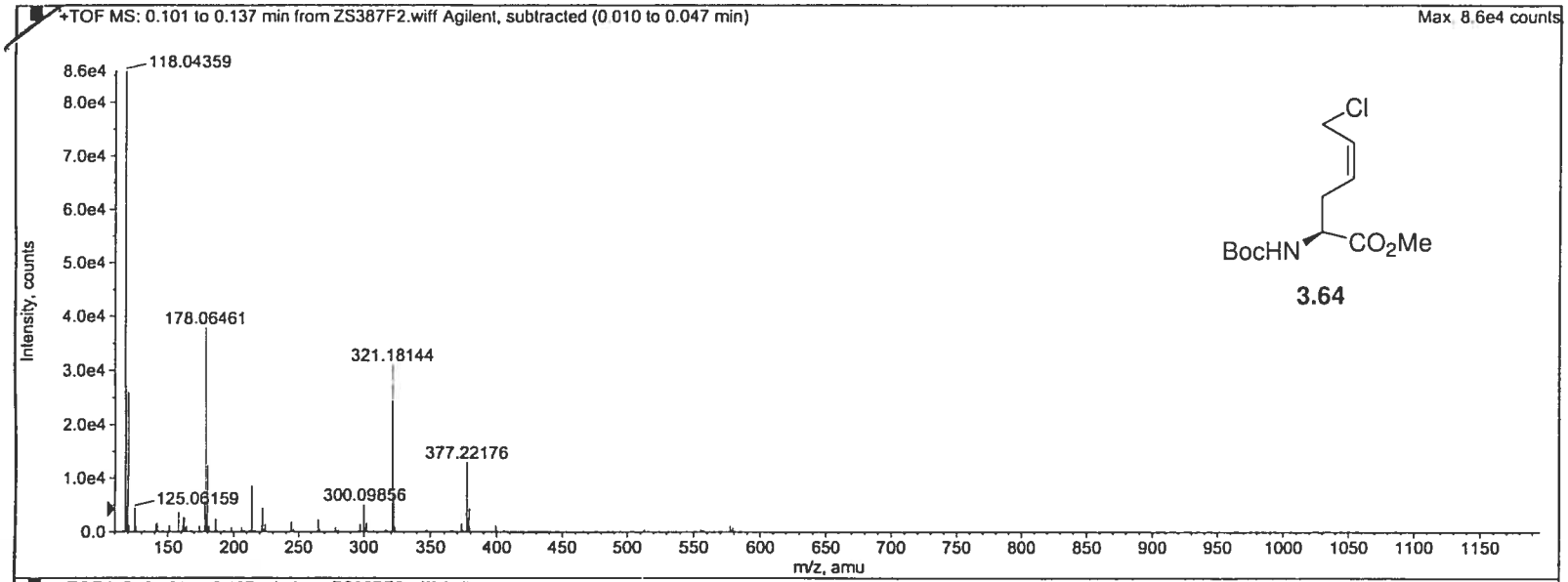

BocHN
CO2Me
3.63
ppm
Integral
0.6668
2.6697
0.6579
2.7794
1.7915
9.0000
5.63085
5.59839
5.57625
5.57242
5.56449
5.54051
5.51659
5.07998
5.04340
5.03895
5.02327
4.98593
4.27062
4.24564
3.61905
2.43561
2.41297
2.39537
2.37547
2.35268
1.34274
1.32060

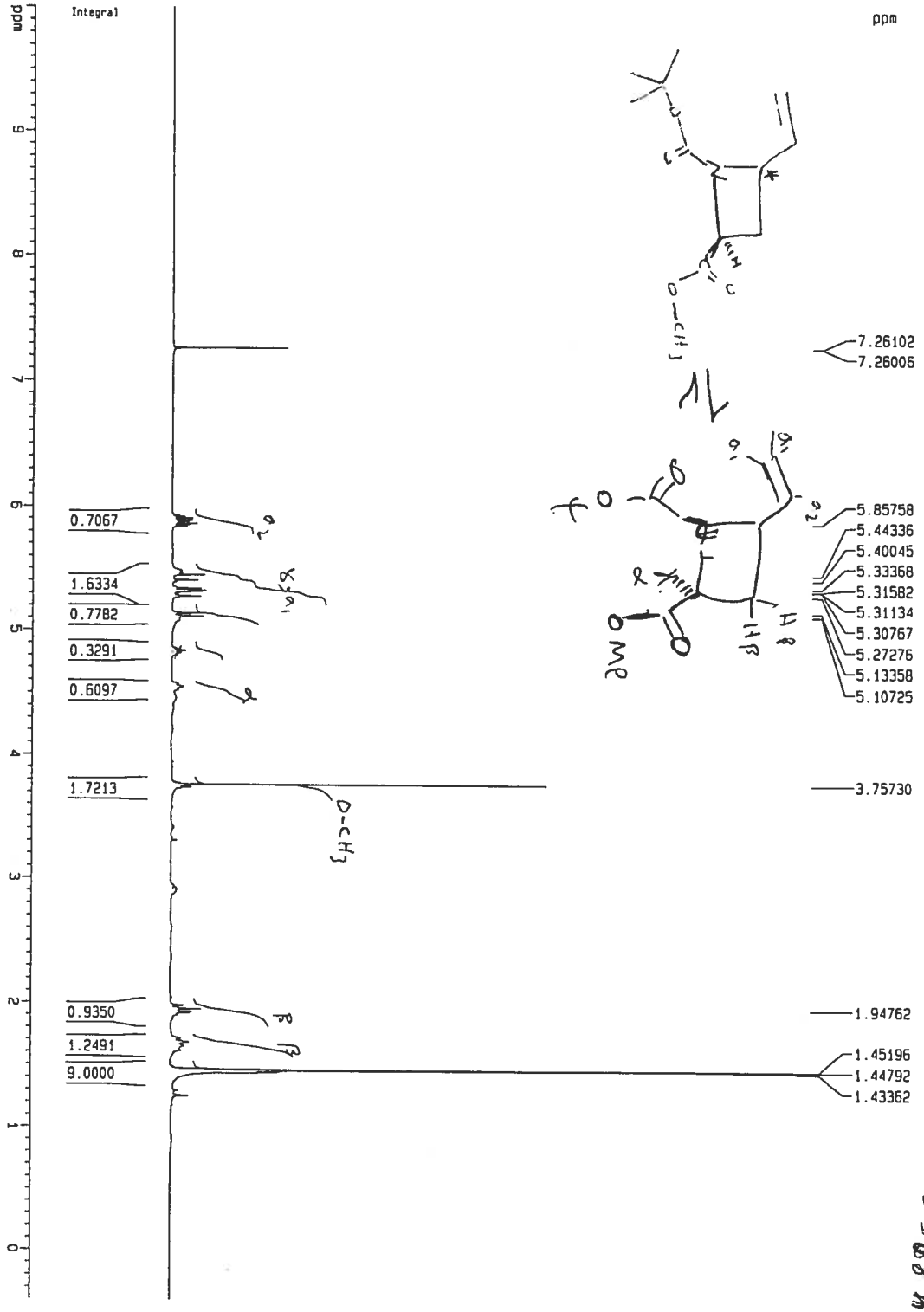

ppm
BocHN
CO2Me
3.63
176.488
155.815
132.489
119.525
80.511
77.684
77.567
77.366
77.048
54.572
53.050
36.741
28.549
ppm
200
180
160
140
120
100
80
60
40
20
0


+TOF MS: 0.086 to 0.158 min from ZS382.wiff Agilent
Max. 9.9e5 counts
238.10577
182.04232
453.21952
475.20112
158.02706
260.08655
294.16733
421.22983
497.18331
684.30083
712.29778
734.27890
Intensity, counts
m/z, amu
BocHN
CO2Me
3.63
+TOF MS: 0.086 to 0.158 min from ZS382.wiff Agilent
Max. 9.9e5 counts
252.12055
[M+Na]+
253.12262
254.09082
Intensity, counts
m/z, amu

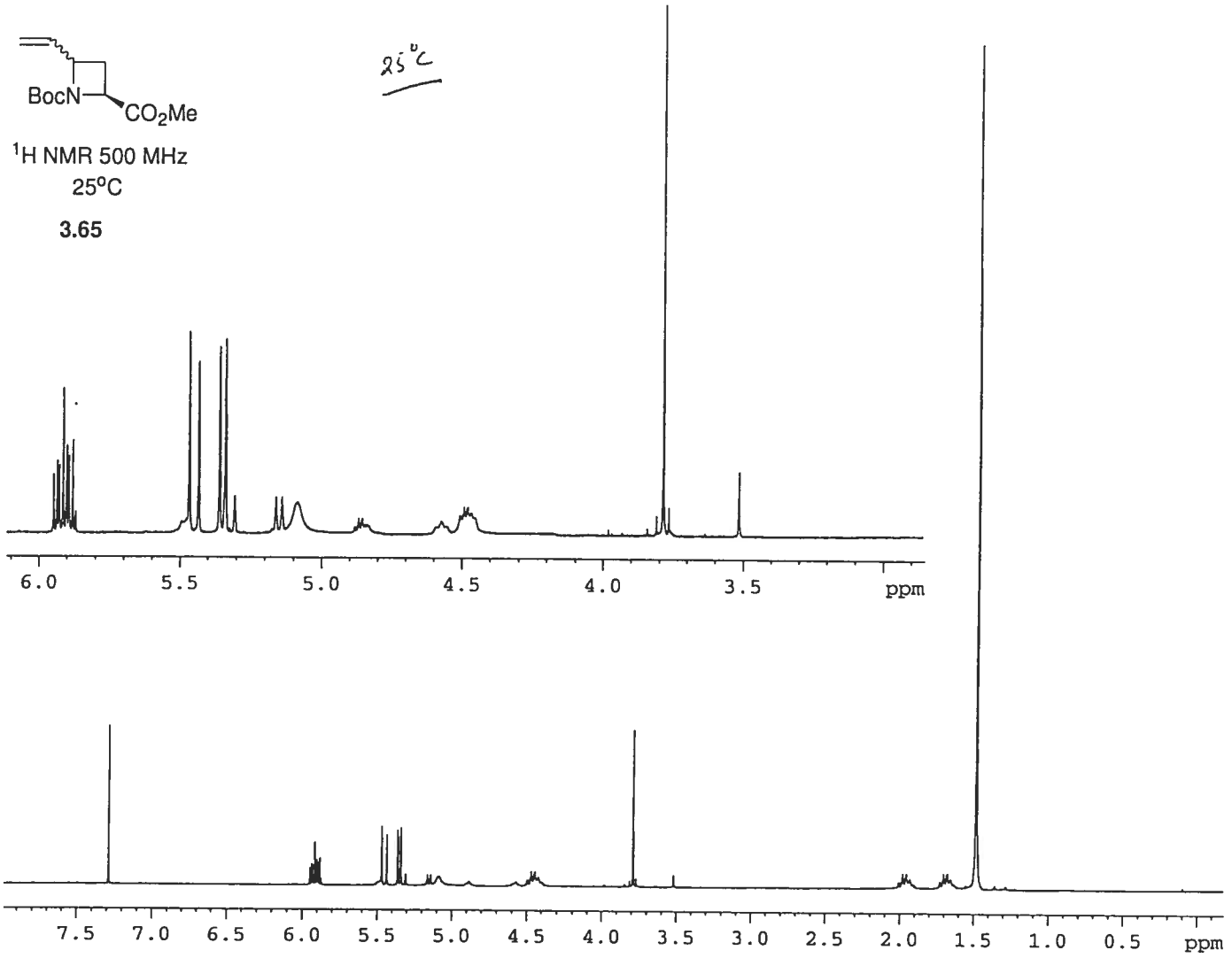

Cl
BocHN
CO2Me
3.64
7.26386
7.26298
5.72150
5.70663
5.68348
5.66872
5.66159
5.65432
5.63883
5.61649
5.60075
5.09370
5.07443
4.38352
4.36808
4.35004
4.33402
4.03375
4.01370
3.98560
3.97055
3.95484
3.72287
3.72166
3.71372
2.58447
2.56928
2.54774
2.53445
2.52215
2.47301
2.45726
2.44084
2.42255
2.40737
1.56377
1.41993
1.40768
1.24708
1.6031
0.7992
0.7924
1.8810
2.7288
1.9933
9.0000
Integral
ppm
9
8
7
6
5
4
3
2
1
0

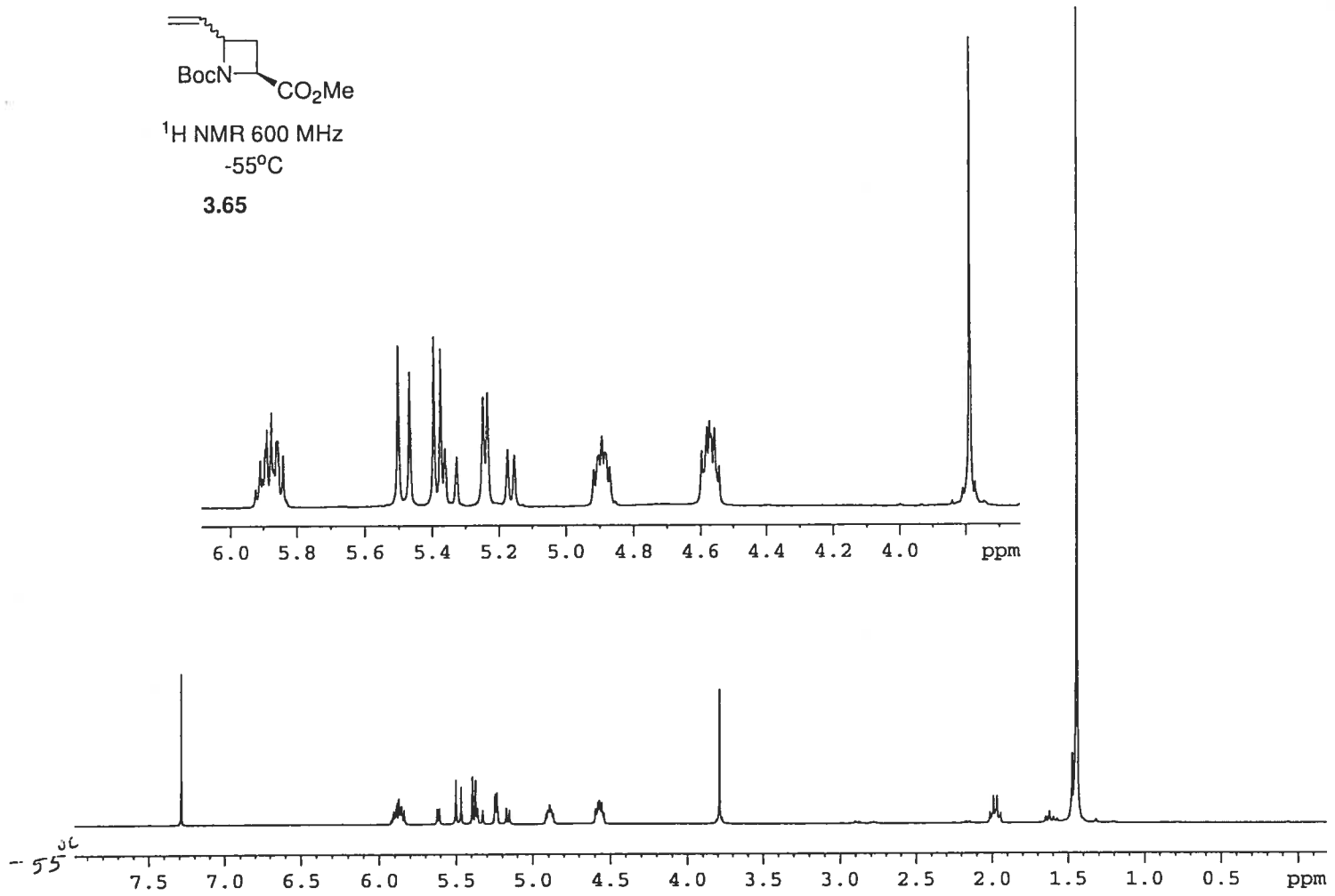

Cl
BocHN
CO2Me
3.64
ppm
171.568
154.396
129.511
128.399
79.305
76.685
76.367
76.049
52.143
51.660
43.761
34.538
27.562
ppm
200
180
160
140
120
100
80
60
40
20
0

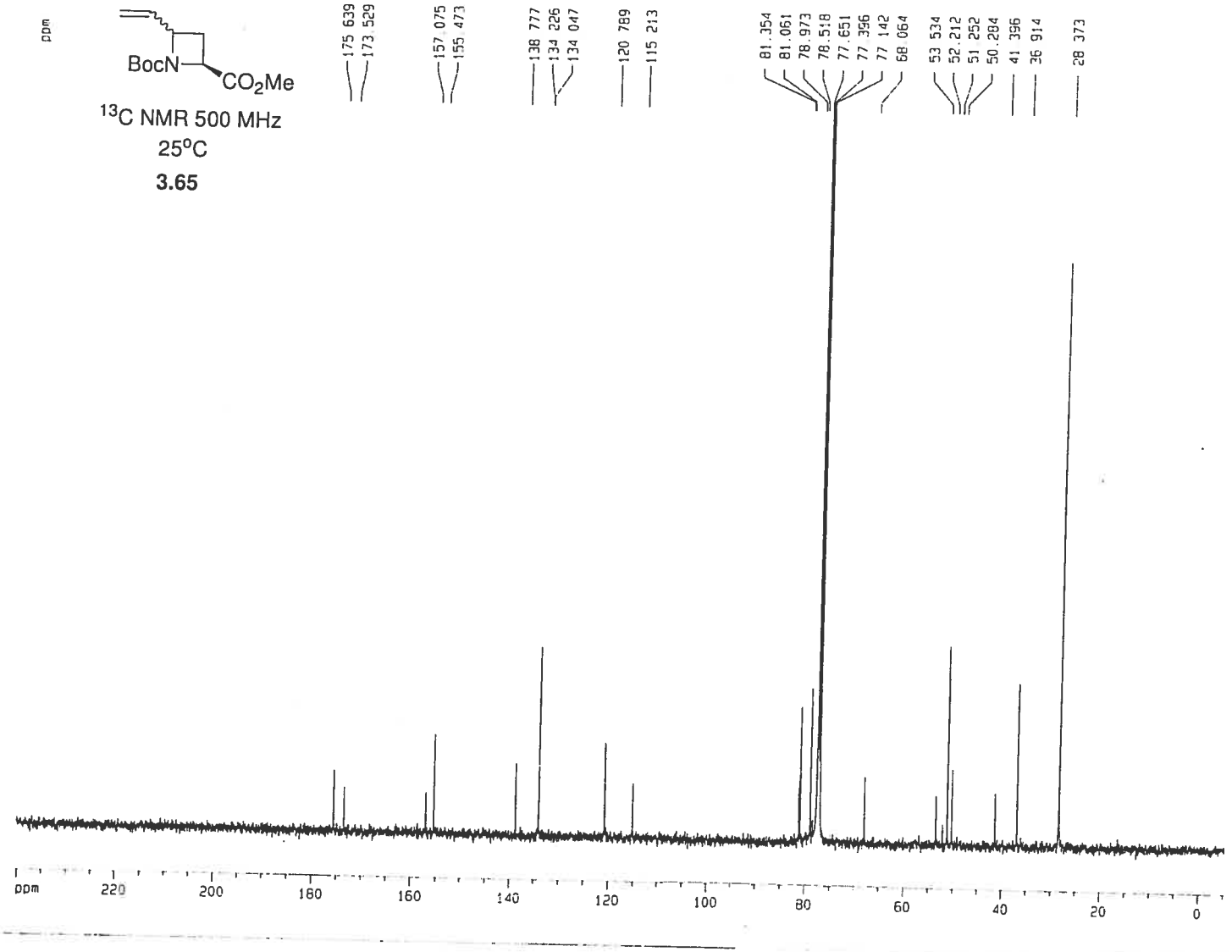

+TOF MS: 0.101 to 0.137 min from ZS387F2.wiff Agilent, subtracted (0.010 to 0.047 min)
Max. 8.6e4 counts.
Intensity, counts
m/z, amu
118.04359
178.06461
321.18144
377.22176
125.06159
300.09856
Cl
BocHN
CO2Me
3.64
+TOF MS: 0.101 to 0.137 min from ZS387F2.wiff Agilent
Max. 2.5e5 counts.
300.09851
[M+Na]+

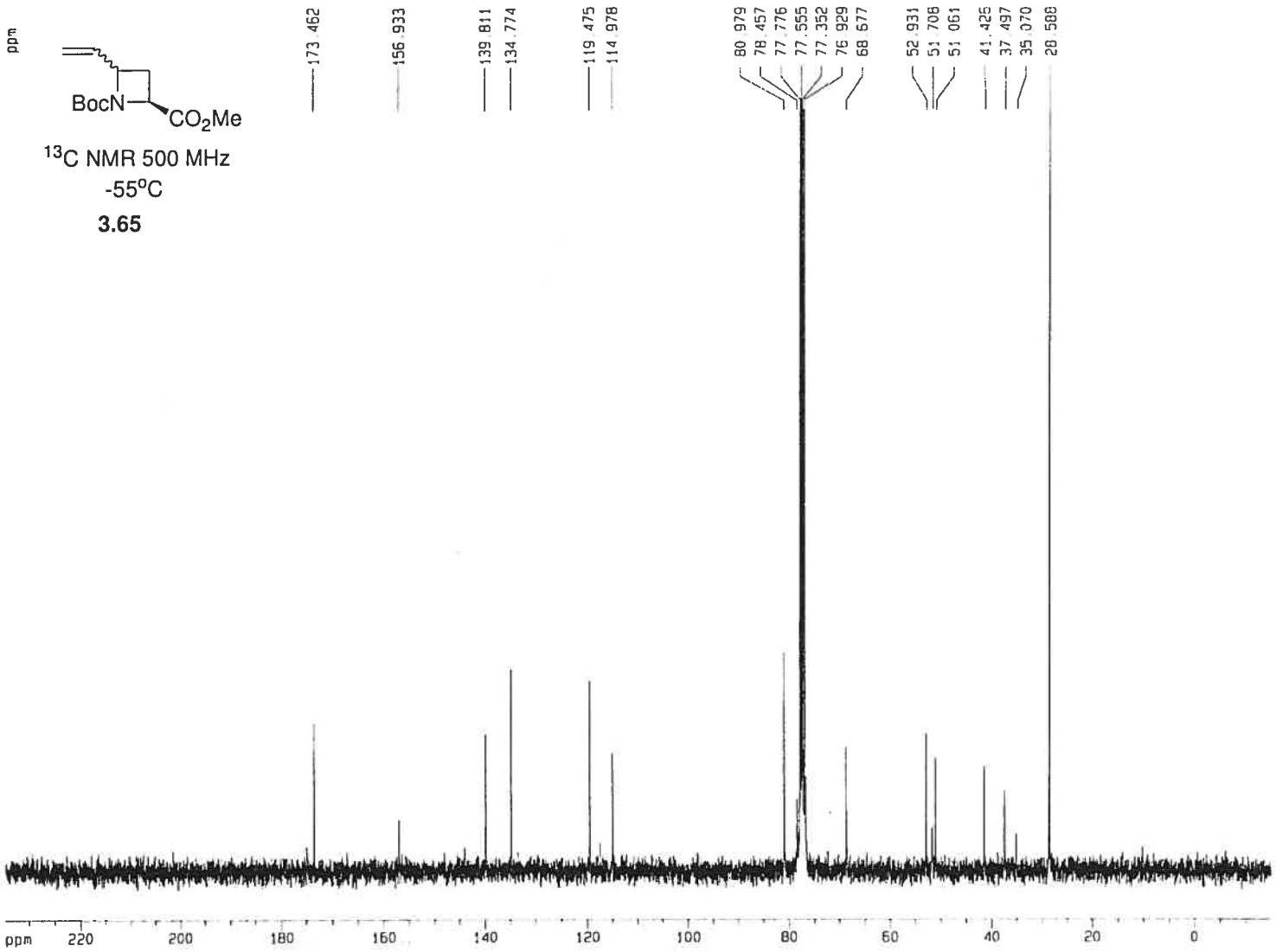

Integral
ppm
0.7067
1.6334
0.7782
0.3291
0.6097
1.7213
0.9350
1.2491
9.0000
7.26102
7.26006
5.85758
5.44336
5.40045
5.33368
5.31582
5.31134
5.30767
5.27276
5.13358
5.10725
3.75730
1.94762
1.45196
1.44792
1.43362
O-CH3
OMe
Hβ
Hα

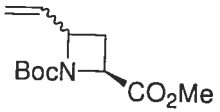
ZS3688


BocN
CO2Me
1H NMR 500 MHz
25°C
3.65
25°C
6.0
5.5
5.0
4.5
4.0
3.5
ppm
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

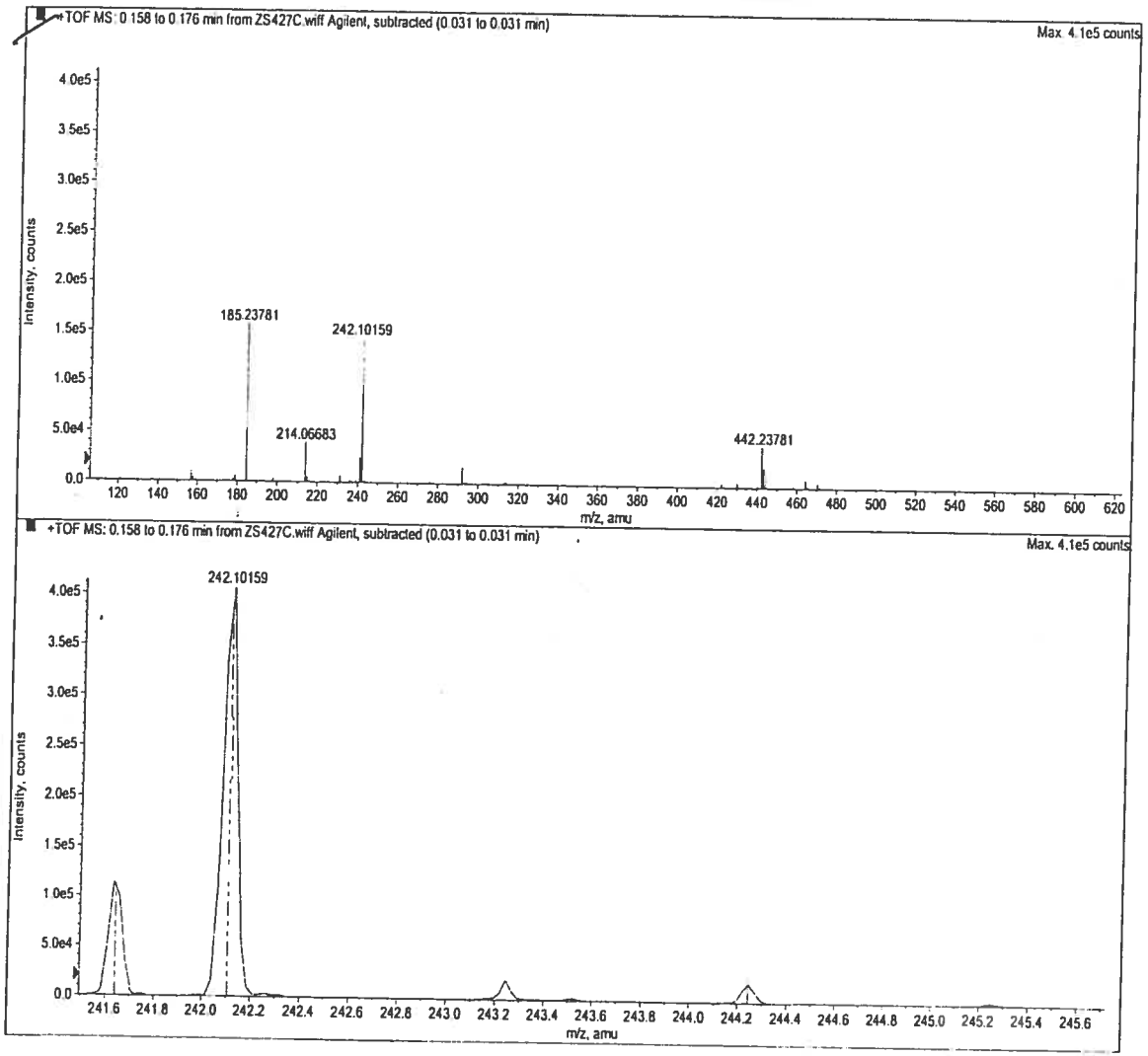

BocN
CO2Me
1H NMR 600 MHz
-55°C
3.65
6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 ppm
-55°C
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

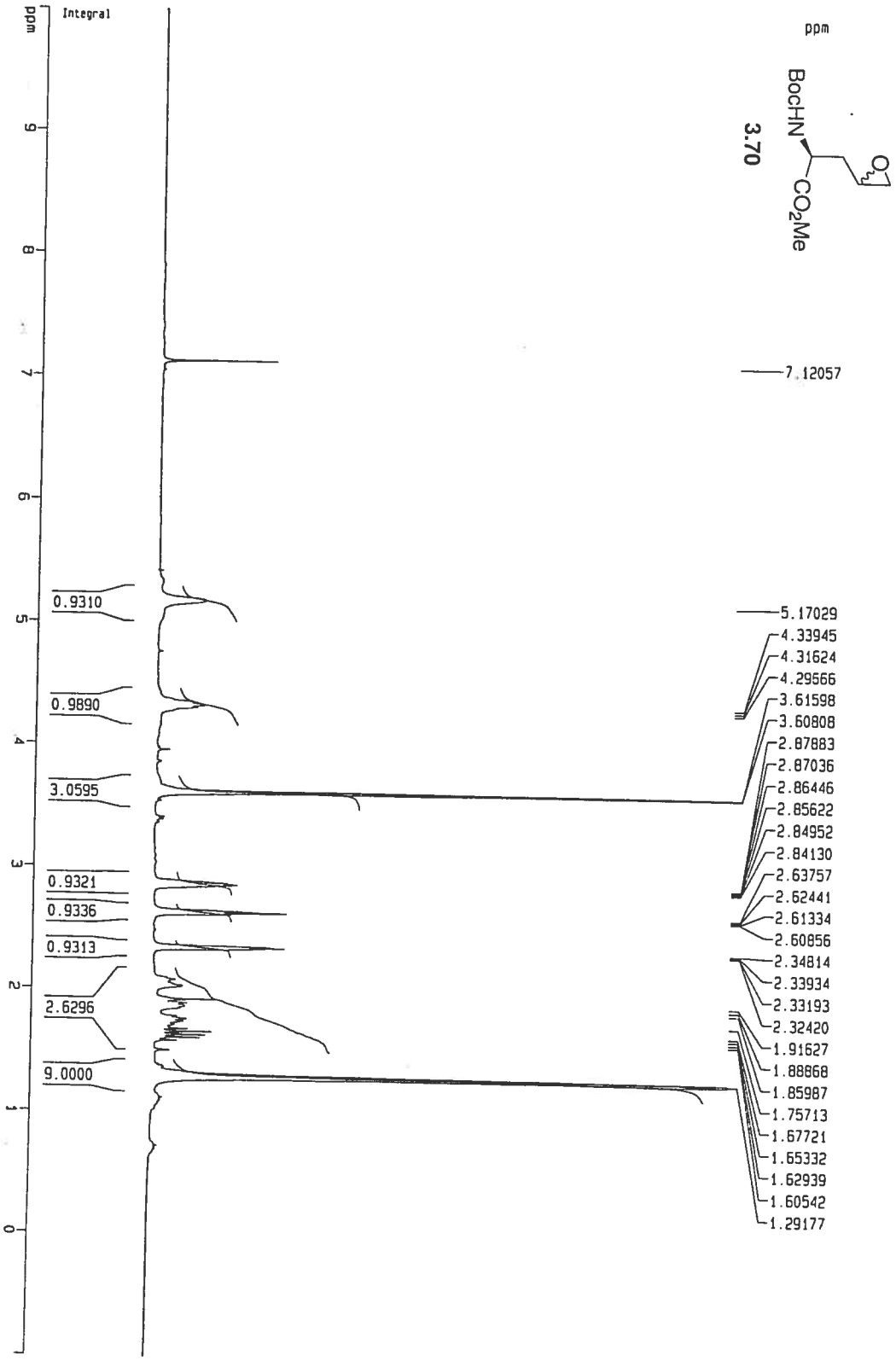

ppm
BocN
CO2Me
13C NMR 500 MHz
25°C
3.65
175.639
173.529
157.075
155.473
138.777
134.226
134.047
120.789
115.213
81.354
81.061
78.973
78.518
77.651
77.356
77.142
68.064
53.534
52.212
51.252
50.284
41.396
36.914
28.373
ppm
220
200
180
160
140
120
100
80
60
40
20
0

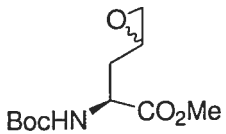

ppm
BocN
CO2Me
13C NMR 500 MHz
-55°C
3.65
173.462
156.933
139.811
134.774
119.475
114.978
80.979
78.457
77.776
77.555
77.352
76.929
68.677
52.931
51.708
51.061
41.426
37.497
35.070
28.588
ppm
220
200
180
160
140
120
100
80
60
40
20
0


BocN
CO2Me

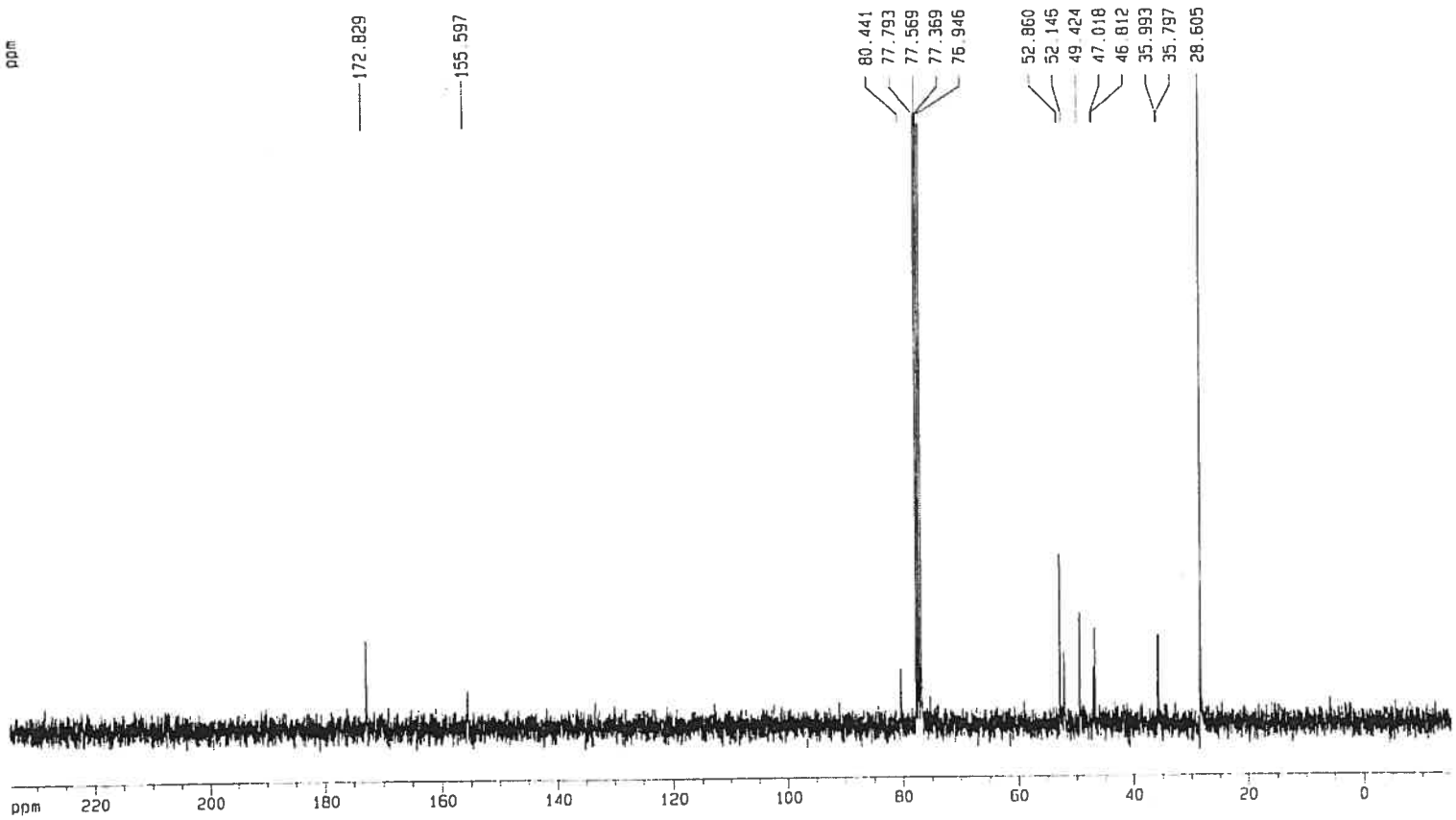
3.65

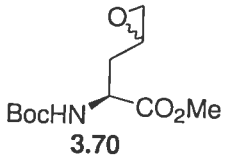

+TOF MS: 0.158 to 0.176 min from ZS427C.wiff Agilent, subtracted (0.031 to 0.031 min)
Max. 4.1e5 counts
Intensity, counts
185.23781
242.10159
214.06683
442.23781
m/z, amu
+TOF MS: 0.158 to 0.176 min from ZS427C.wiff Agilent, subtracted (0.031 to 0.031 min)
Max. 4.1e5 counts
242.10159
Intensity, counts
m/z, amu


ppm
Integral
9
8
7
6
5
4
3
2
1
0
0.9310
0.9890
3.0595
0.9321
0.9336
0.9313
2.6296
9.0000
ppm
BocHN
CO2Me
3.70
7.12057
5.17029
4.33945
4.31624
4.29566
3.61598
3.60808
2.87883
2.87036
2.86446
2.85622
2.84952
2.84130
2.63757
2.62441
2.61334
2.60856
2.34814
2.33934
2.33193
2.32420
1.91627
1.88868
1.85987
1.75713
1.67721
1.65332
1.62939
1.60542
1.29177

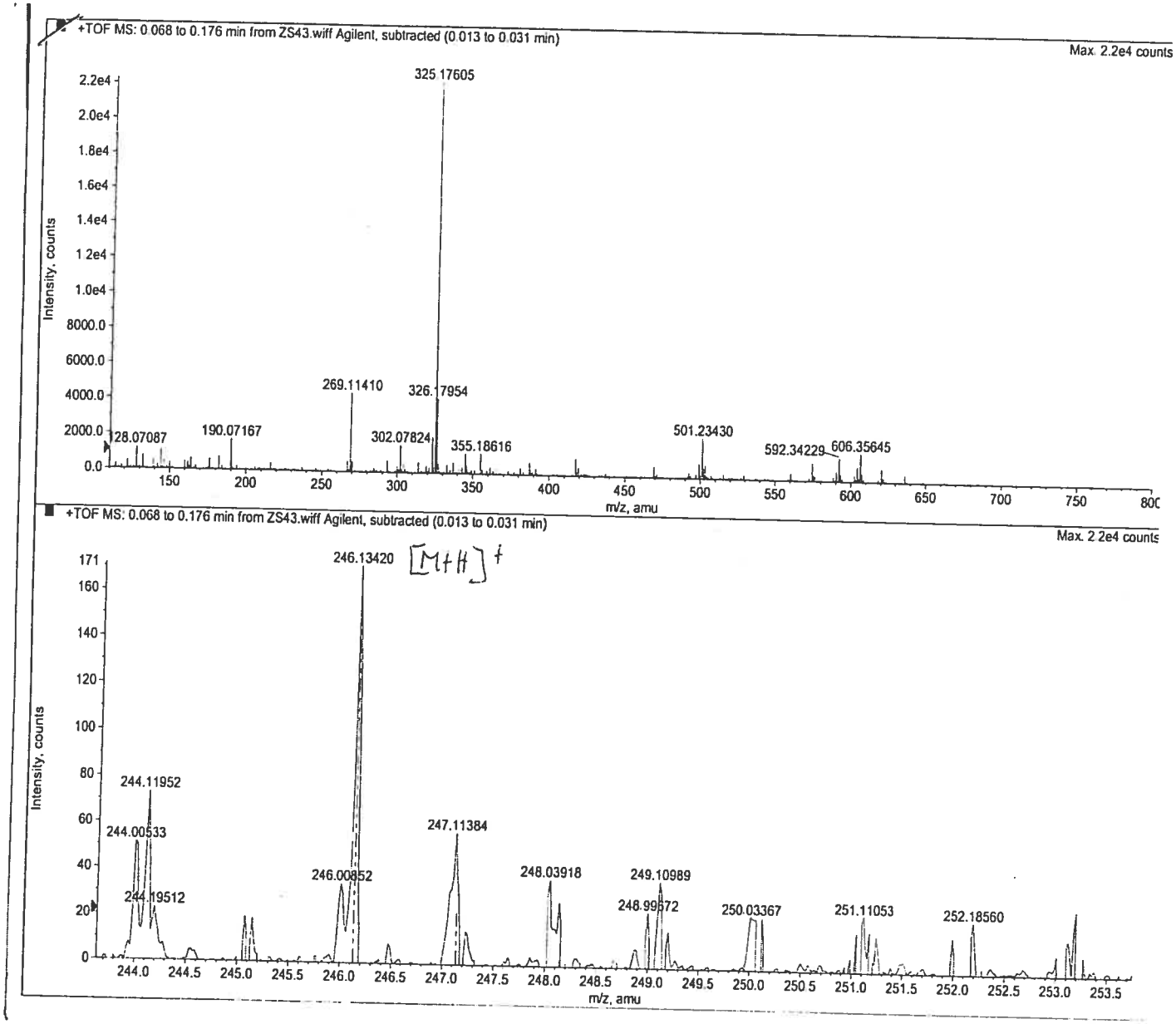

O
BocHN
CO2Me

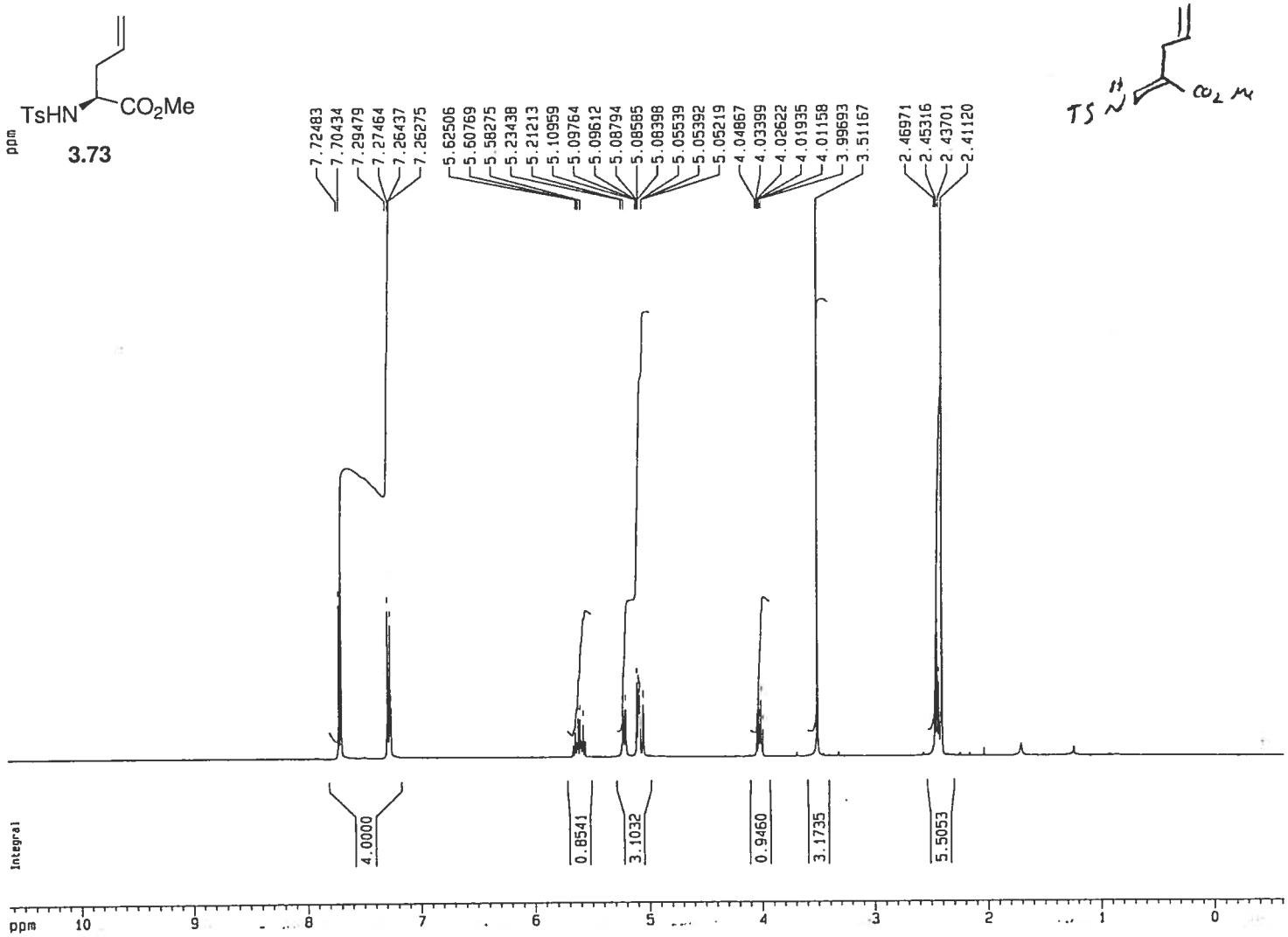
3.70

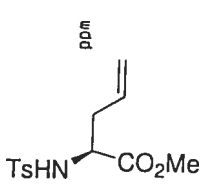

ppm
172.829
155.597
80.441
77.793
77.569
77.369
76.946
52.860
52.145
49.424
47.018
46.812
35.993
35.797
28.605
ppm
220
200
180
160
140
120
100
80
60
40
20
0


O
BocHN
CO2Me

3.70

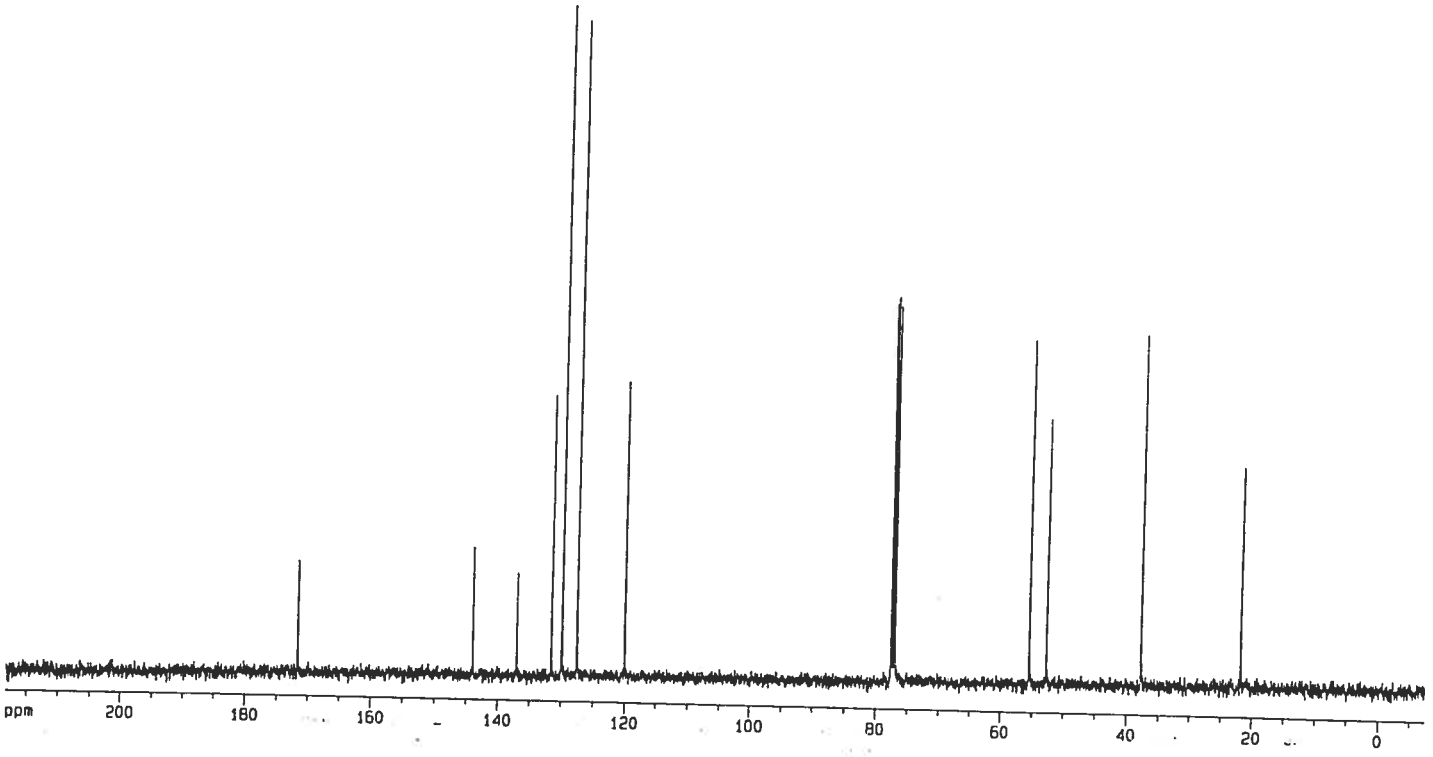

+TOF MS: 0.068 to 0.176 min from ZS43.wiff Agilent, subtracted (0.013 to 0.031 min)
Max. 2.2e4 counts
Intensity, counts
m/z, amu
325.17605
269.11410
326.17954
128.07087
190.07167
302.07824
355.18616
501.23430
592.34229
606.35645
+TOF MS: 0.068 to 0.176 min from ZS43.wiff Agilent, subtracted (0.013 to 0.031 min)
Max. 2.2e4 counts
246.13420 [M+H]+
244.11952
244.00533
244.19512
246.00852
247.11384
248.03918
249.10989
248.99572
250.03367
251.11053
252.18560

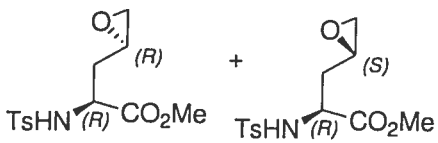

TsHN
CO2Me
3.73
ppm
7.72483
7.70434
7.29479
7.27464
7.26437
7.26275
5.62506
5.60769
5.58275
5.23438
5.21213
5.10959
5.09764
5.09612
5.08794
5.08585
5.08398
5.05539
5.05392
5.05219
4.04867
4.03399
4.02622
4.01935
4.01158
3.99693
3.51167
2.46971
2.45316
2.43701
2.41120
Integral
4.0000
0.8541
3.1032
0.9460
3.1735
5.5053
ppm 10 9 8 7 6 5 4 3 2 1 0



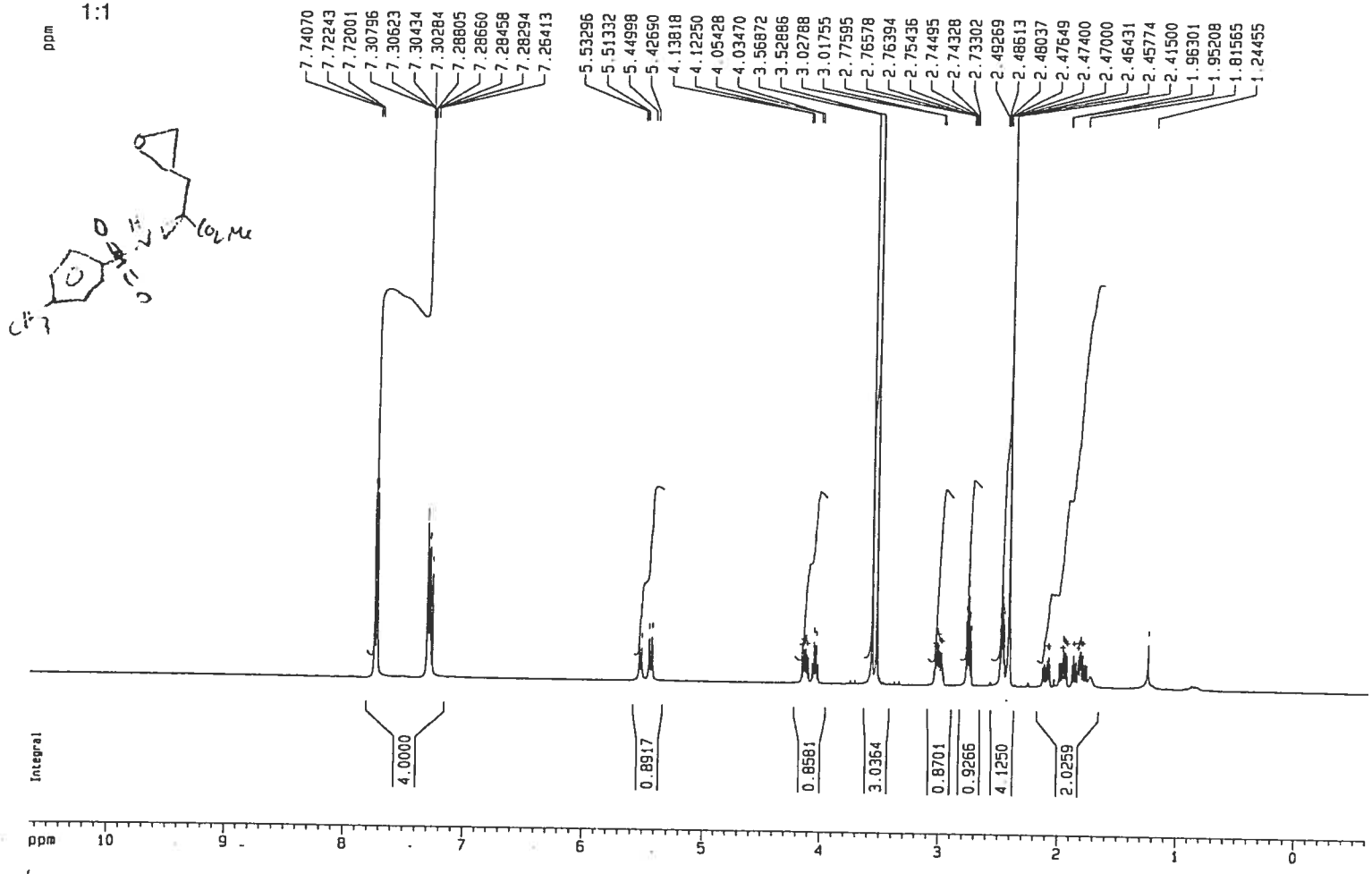
3.73

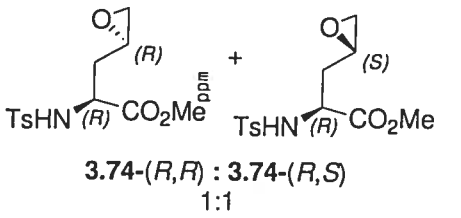
171.654
144.005
137.066
131.588
129.937
127.548
120.082
77.675
77.358
77.040
55.521
52.737
37.848
21.842





3.74-(*R*,*R*) : 3.74-(*R*,*S*)
1:1





3.74-(*R*,*R*) : 3.74-(*R*,*S*)
1:1

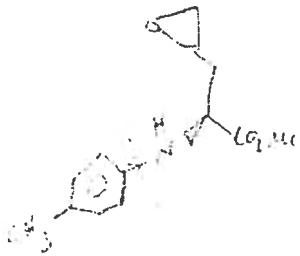
171.835
171.686

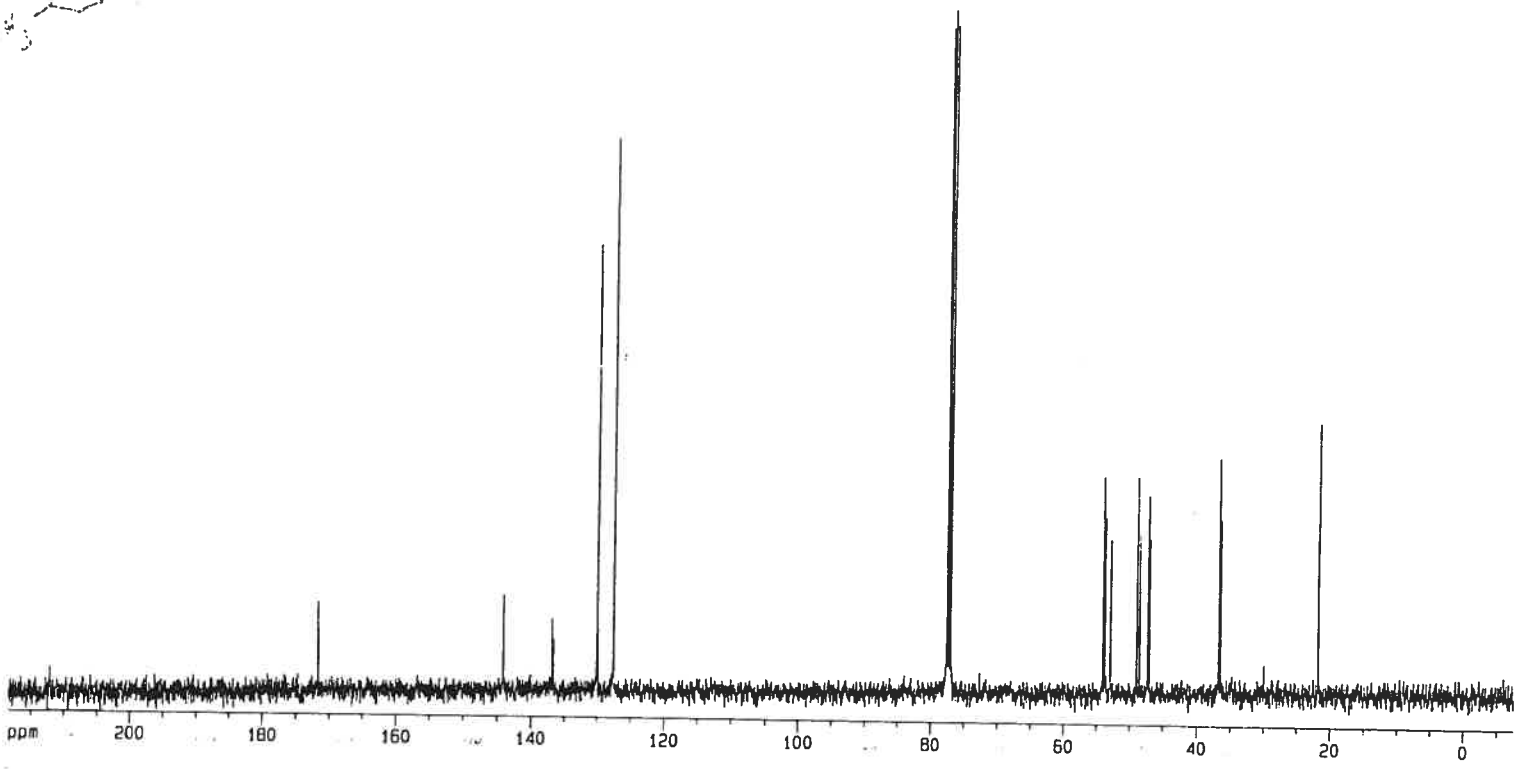
144.186
144.116
136.863
136.640
130.054
129.980
127.576

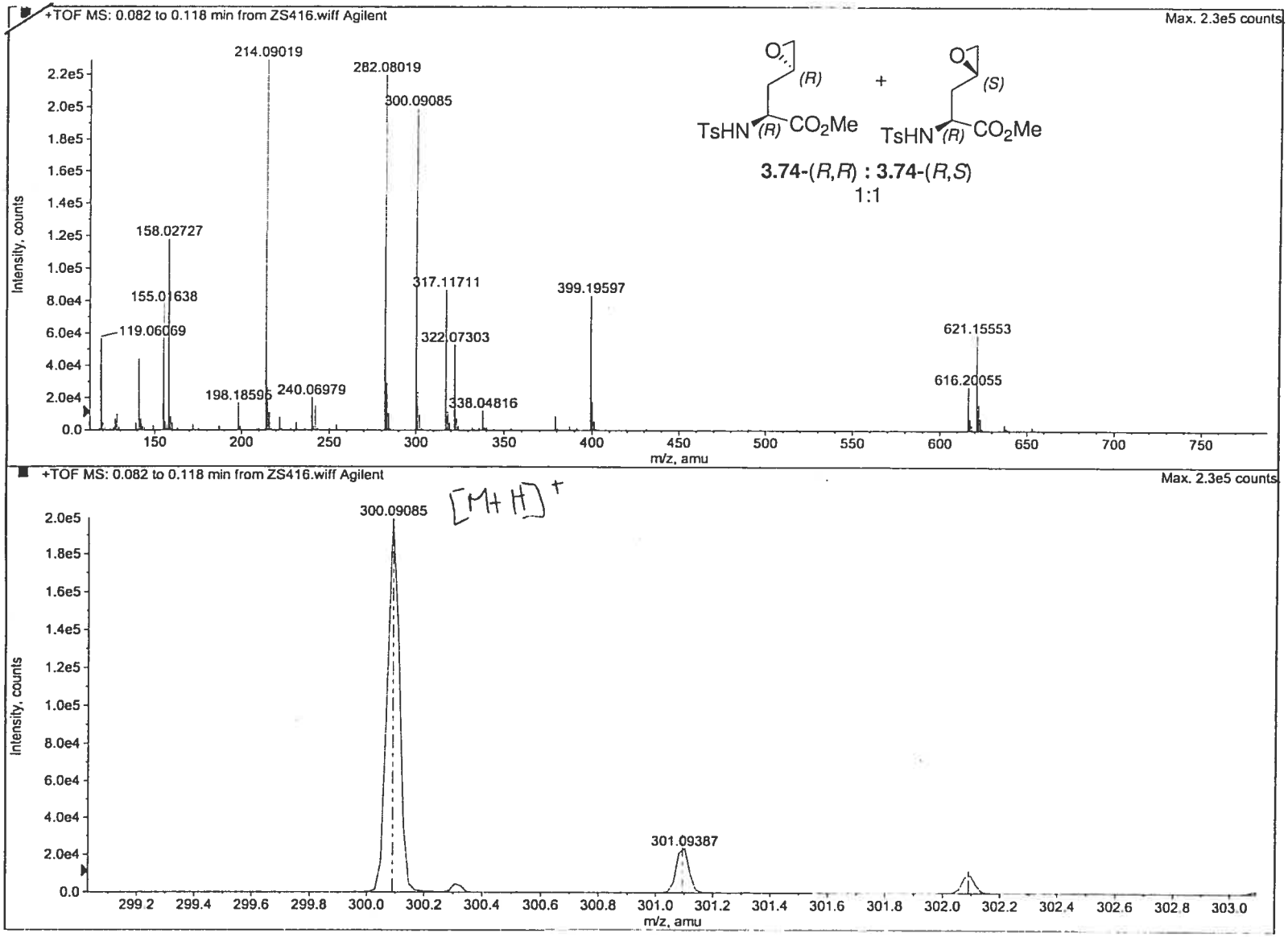
77.671
77.353
77.036

54.195
53.882
53.119
53.030
49.060
48.647
47.372
47.101
36.805
36.499
29.996

21.859






+TOF MS: 0.082 to 0.118 min from ZS416.wiff Agilent
Max. 2.3e5 counts.
214.09019
282.08019
300.09085
158.02727
155.01638
119.06069
317.11711
399.19597
322.07303
621.15553
616.20055
198.18595
240.06979
338.04816
Intensity, counts
m/z, amu
(R)
(S)
TsHN (R) CO2Me
3.74-(R,R) : 3.74-(R,S)
1:1
+TOF MS: 0.082 to 0.118 min from ZS416.wiff Agilent
Max. 2.3e5 counts.
300.09085
[M+H]+
301.09387
Intensity, counts
m/z, amu