SYNTHESIS OF POLYCYCLIC HYDANTOIN DERIVITAVES AND PEPTIDES

BY

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Durban

As the candidate's supervisor, I have approved this dissertation for submission.

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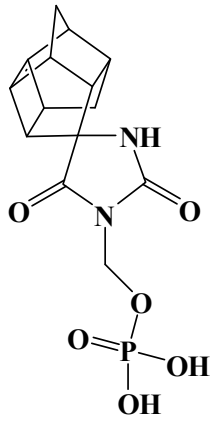
ABSTRACT

Cyclic cage compounds have attracted much attention in pharmaceutical studies. The lipophilic nature of these compounds plays an important role in facilitating the crossing of the cellular membranes, including the blood brain barrier (BBB) and the central nervous system (CNS). Several adamantane and pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane (PCU) derivatives have shown great potential as antiviral, antibacterial and neuroprotective compounds.

The aim of this study includes the synthesis of hydantoin derivatives of adamantane and PCU as anticonvulsant compounds. Fosphentoin sodium (Cerebyx) **48** is a commercial anticonvulsant drug. Structurally, compound **51** and **52** are similar to Cerebyx **48**, where the two phenyl groups have been replaced with PCU or adamantane skeleton respectively. The cage skeleton should increase the lipophilic character of the drug whilst the phosphate group should retain the water solubility of the substrate. The attempted synthesis of these compounds is described in Chapter 2.



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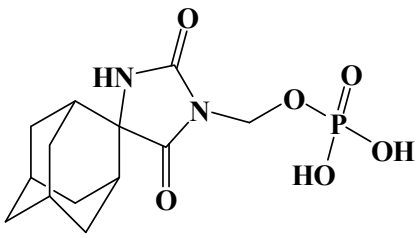

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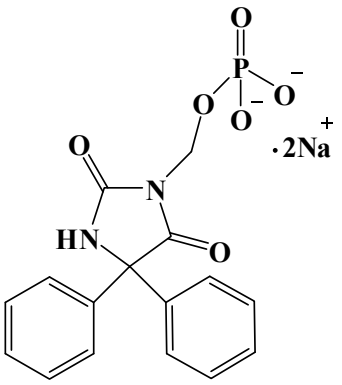

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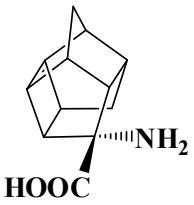
The PCU hydantoin is readily converted to the PCU amino acid. The synthesis of the PCU amino acid **41** and its Fmoc derivative **106** is described in Chapter 3. This compound was incorporated into small peptides, namely Ala-Ala-Ala-PCU-Ala-Ala-Ala-Fmoc and Ala-Val-PCU-Ile for future testing as a potential anti-cancer agent. NMR studies of these peptides are also reported.



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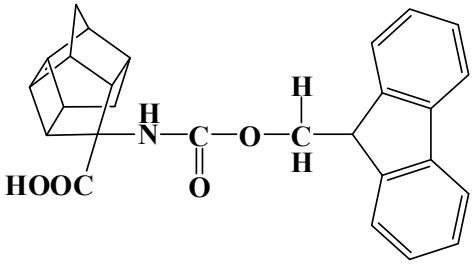

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PREFACE

The experimental work described in this dissertation was carried out in the School of Chemistry, University of KwaZulu-Natal, Durban, from April 2007 to September 2008 under the supervision of Dr. T. Govender and Prof. H.G. Kruger.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

_____

Mohamed Saadaldin Altaib

____ day of _____ 2008

ACKNOWLEDGMENTS

I would like to thank Dr T. Govender, Mr P. Govender Prof H.G. Kruger and Dr G. E. Maguire for their generous guidance and support throughout this project. It was a pleasure to be part of their research group.

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I thank the NRF and University of KwaZulu-Natal for their financial support.

Finally I would like to thank my family for their solid support during my studies in South Africa.

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List of Abbreviations

Ala	Alanine
Asp	Aspartic acid
AVPI	Alanine-Valine-proline-Isoleucine
BBB	Blood Brain Barrier
Boc	tert-Butoxycarbonyl
Bzl	Benzyl
(t-Boc)	di-tert-butyl dicarbonate
$CDCl_3$	Deuterated trichloromethane
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance Spectroscopy
COSY	Correlation Spectroscopy
CNS	Central Nervous System
D	Dextrotatory
DCC	N,N-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIPCDI	N,N-diisopropylcarbodiimide
DMAP	4-dimethyl-aminopyridine
DMF	N,N-Dimethylformamide
$DMSO(d_6)$	Deuterated dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ESR	Electron Spin Resonance
Et_3N	Triethylamine
Fmoc(-Cl)	9-Fluorenylmethyl(chloro)formate
g	Grams
Glu	Glutamic acid
Gly	Glycine
hν	Electromagnetic radiation
1H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
HATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate

HBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium-hexafluorophosphate
HMBC	Heteronuclear multiple bond coherence
HOBt	1-Hydroxybenzotriazole
HOMO	Highest occupied molecular orbital
HPLC	High performance liquid chromatography
HSQC	Heteronuclear Single Quantum Coherence
IAP	Inhibitor of apoptosis protein
Ile	Isoleucine
IR	Infrared spectroscopy
KOH	Potassium hydroxide
L	Levorotatory
LCAO	Linear combination of atomic orbitals
LUMO	Lowest unoccupied molecular orbital
mg	milligrams
MBHA	p-methylbenzhydrylamine
mol	mol
mRNA	messenger RNA
m/z	mass per electron
M	molarity
MHz	Megahertz
Mp	Melting point
NaCN	Sodium cyanide
NaOH	Sodium hydroxide
NOESY	The Nuclear Overhauser Enhancement Spectroscopy
NRF	National Research Foundation
P	Para
p-TS	*p*-Toluenesulphonic acid
PCU	Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane
RNA	Ribonucleic acid
S	*Sinister*
SAR	Structure-Active Relationship
Ser	Serine
SPPS	Solid Phase Peptide Synthesis

S_N2	Nucleophilic substitution
*t*Bu	*tert*-Butyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TNBS	2,4,6-Trinitrobenzenesulfonic acid
Tris	Trishomocubane
UKZN	University of KwaZulu-Natal
Val	Valine
XIAP	X-linked Inhibitor of Apoptosis Protein
Ψ	Wave function
Å	Ångstrom
α	Alpha
ϕ	Atomic orbitals
β	*Beta*
ρ	Density
δ	Delta
^oC	Degrees Celsius
ν_{max}	Frequency of maximum absorption
γ	*Gamma*
ω	*Omega*
%	Percent
ψ	*Psi*
π	*Pi*
ϕ	*Phi*
σ	*Sigma*

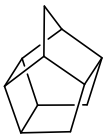
CHAPTER 1

INTRODUCTION

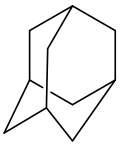
This project involved the synthesis of cage amino acid derivatives having potential pharmaceutical applications. The first part is the synthesis of cage hydantoins (precursor to the amino acid) as potential anti-epileptic drugs. The second part involves the synthesis of a non-natural peptide with the aim of studying the 3D structure induced by the cage amino acid, the so called bio-active conformation. The cage peptide is designed as a potential cancer agent, which will be investigated in subsequent studies. The following is a a discussion of the background of various aspects related to this investigation. First some pharmaceutical aspects of cage compounds will be presented.

Cage compounds

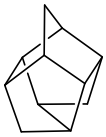
The chemistry of polycyclic "cage" compounds has been of great interest to synthetic chemists for over five decades.[1-4] Pentacycloundecane (PCU) **1** polycyclic "cage" compounds possess unusual carbon-carbon bond lengths as well as C-C-C angles that deviate from 109.5^{o}. This is due to enormous ring strain caused by the cyclobutane ring. With the exception of adamantane **2** and trishomocubane **3**, most of the strained polycyclic "cage" compounds posses unusually high negative heats of decomposition and high positive energy of formation compared to other classes of saturated compounds. This strain energy appears to express itself through unusual chemical reactivity.[4] The following are structural examples of some well known polycyclic "cage" compounds.



1: Pentacycloundecane



2: Adamantane



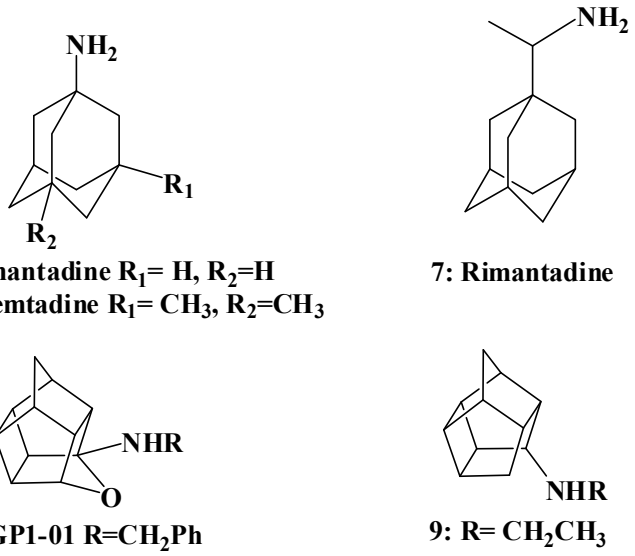
3: Trishomocubane

4: Cubane

The incorporation of cage compounds into medicinal drugs introduces a variety of advantages in terms of drug action and pharmacology.[1] These advantages include:

- The lipophilic nature of cage compounds makes them good candidates for drug delivery. The bulky hydrocarbon framework serves as a transport aid in carrying drugs attached to them across the cellular membrane including the blood brain barrier (BBB) and the central nervous system (CNS).[5-12] The first major breakthrough occurred with the discovery of amantadine **5** which was found to be an effective drug against Parkinson's disease. Amantadine also exhibits antiviral activity against the influenza A_2 Asian virus. Further derivatives of adamantine, **6** and **7**, have been reported to display anti-viral activity with less side effects.[1] This has led to an evolution in drug discovery with the emergence of different classes of polycyclic "cage" based skeletons being synthesized and tested against a series of neurotic diseases. A number of these compounds showed promising activities.[1,7,12,13]

- Cage compounds covalently attached to pharmaceutically active molecules also reduce drug bio-degradation which helps to increase the half life of such compounds in the biological system, thus reducing the dosage frequency.[7]



5: Amantadine R_1= H, R_2=H
6: Memtadine R_1= CH_3, R_2=CH_3

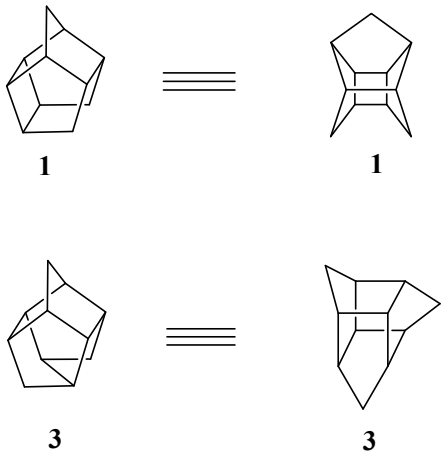
7: Rimantadine

8: NGP1-01 R=CH_2Ph

9: R= CH_2CH_3

Figure 1: Examples of biological active polycyclic compounds

Polycyclic compounds exhibit interesting structural properties e.g. the eleven carbon framework of PCU **1** and trishomocubane **3** have C_2 and D_3 symmetry respectively.[12,14]



1 **1**

3 **3**

Chirality is one of the most important properties for compounds in asymmetric catalysis and pharmaceutical applications. Despite the fact that PCU **1** is a *meso* compound all the methine groups become asymmetric when a chiral arm is attached to the cage skeleton. A number of chiral PCU derivatives have been synthesised for several asymmetric applications.[15-17]

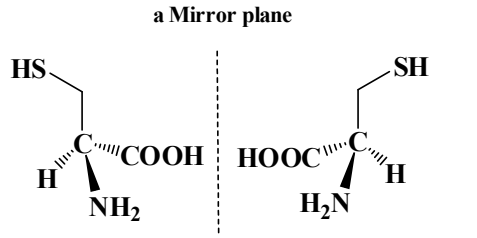
These advantages of cage skeletons make it attractive to incorporate them into non-natural peptides. Other advantages of the cage moiety will be presented in Chapter 3.

Chapter 2 presents the synthesis of cage hydantoin derivatives as potential anti-convulsant derivatives. The back ground of anti-convulsant agents will be presented there. In addition, these hydantoin compounds can be hydrolysed to the corresponding cage amino acids. The

latter will then be incorporated into non-natural peptides. The following is an introduction to peptide chemistry.

Peptide chemistry

The peptide bond or peptide linkage is an amide bond formed by the interaction of an amino group and a carboxyl group of two amino acids. This bond links together amino acids to yield a strand referred to as a peptide or protein.[18,19] Of the 20 known natural amino acids only glycine is achiral.[20]



10a: (*R*)-Cysteine **10b: (*S*)-Cysteine**

With an exception of a few cases (e.g. (*R*)-serine and (*R*)-aspartate in the brain tissue and (*R*)-ornithine in gramicidin S), the 20 natural amino acids predominantly exist as (*S*)-configuration. In contrast to natural amino acids, the carbohydrate units are of opposite configuration.[19]

X-ray studies of peptide bonds shows it contains unusual bond lengths and angles around the amide bond compared to the usual C-N bond.[21] Protein and peptide backbone conformations are usually characterized by four torsion angles φ ψ, χ and ω (see Figure 2). Where ω is normally 180^0 with an exception of proline ($\omega = 0^0$) where the amino acid side chain (R) usually occupies* the *cis*-position with respect to the adjacent amino acids side chain.[21]

* This *cis* or *trans* orientation of proline containing peptides could differ in different solvents.[21]

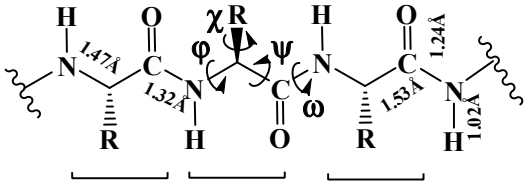


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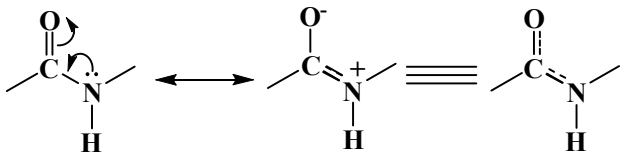
Figure 2: Defining bond lengths and the torsion angles in the peptide backbone[21]

These facts provide clear evidence that amide bonds possess partial double bond character, resulting from the delocalization of the nitrogen lone pair of electrons to the carbonyl group. This partial double bond in **12c** restricts/hinders free rotation around the amide bond (Figure 3).[20,21] The backbone is *trans* across the amide bond (excluding proline) since steric hinderance between the larger groups are minimised.



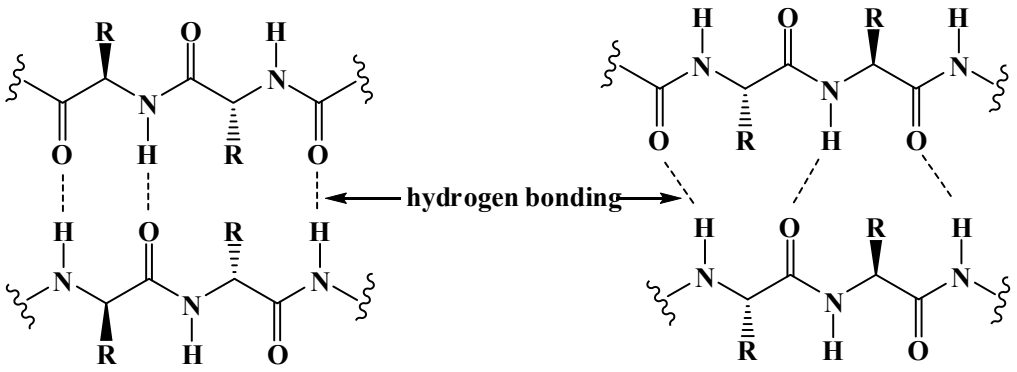
12a **12b** **12c**

Figure 3: Delocalization in the peptide bond[20]

Peptides exist in two major conformational forms, namely helical coils and β-sheets. The nature and classification of helical coils will be discussed below.

β-sheets form due to hydrogen bonding between two neighbouring polypeptides and are classified into two types (Figure 4).

- Parallel β-sheets, the hydrogen bonding occurs between two peptides (C-terminal with C-terminal or N-terminal with N-terminal) align in parallel way.
- Anti-parallel β-sheets, the hydrogen bonds take place between two anti-parallel peptides (one C-terminal and the other N-terminal) to each other.



13: antiparallel plate sheet **14: parallel plate sheet**

Figure 4: Formation of hydrogen bond in parallel and anti-parallel β-sheet plates[21]

Peptides can also exhibit various types of turns or helical structures.

Peptide turns

The formation of a helical structure occurs when successive turns are present in a peptide or protein. In most cases, hydrogen bonding is responsible for stabilizing particular peptide turns. H-bonds occur between the α-amino groups and α-carboxyl groups in a nearby segment or residue.

Depending on the number of the amino acid residues contributing to the specific turn, the type of turn is classified. When three amino acids are involved in the hydrogen bonded turn, then the turn is called a γ-turn. Similarly turns where four, five and six amino acids are involved are respectively called β, α and π-turns.[21] Of these types of turns, β-turns represent by far the most important motives in terms of bio-active peptides.[22] There are different types of β-turns which are classified according to the dihedral angles (ψ, φ) (see Figure 2) of the four amino acids (*i*, *i+1, i*+2*, i*+3).[21] There are three criteria for defining β-turns:[21,22]

- The decisive factor for the classification of β-turns is that the distance between the atoms $C^{\alpha}(i)$ and $C^{\alpha}(i+3)$ should be less than 7Å.
- The torsion angle *t* between C^{α} (*i*), C^{α} (*i*+1), C^{α} (*i*+2) and C^{α} (*i*+3) ranges around $90^0 \leq t \leq 90^0$.
- The distance between the carbonyl group on amino acid (*i*) and the amide hydrogen on the amino acid (*i*+3) is less than 4 Å.

β-turns can also be classified as different types based on the distinctive tetrahedral angles (ψ, φ) of the second and third amino acid residues [(*i*+1) and (*i*+2)].

Some amino acids are known to stabilize β-turns. Proline stabilize β-turns in both *trans* (in position *i*+1 of βI or β II turns) and *cis* (in position *i*+2 of β VIa-turn) configurations. In general, (*R*)-proline and (*R*)-amino acids preferentially form β II (*i*+1) turns.[21,22] The biological and pharmaceutical importance of these turns will be discussed in Chapter 3.

Another important feature in proteins and peptide chemistry is the disulfide bond which is the result of covalent sulfur-sulfur bond formation (Figure 5) between two non-adjacent cysteine residues. This bond plays a major role in determining the overall 3D shape of peptides and proteins.[19,21,23]

History of peptide synthesis

In biological systems peptides and proteins are synthesised through a complicated process. Each natural peptide or protein sequence represents its own genetic information flowing from deoxyribonucleic acid (DNA) to messenger ribonucleic acid (mRNA) then to the target sequence of peptide and protein.[20] The first successful attempt to synthesize a peptide in the laboratory was achieved by Emil Fisher in 1901. Despite the fact that proteins and peptides are responsible for regulating the overall biochemical process in complex organisms, research in this field was neglected until the 1960s when the revolution of increased sensitive analytical techniques were possible. Sanger in 1950 reported the elucidation of the sequence of bovine insulin (Figure 5) which is a polypeptide classified as a hormone secreted from the pancreas to regulate glucose metabolism in the biological system.[19,21]

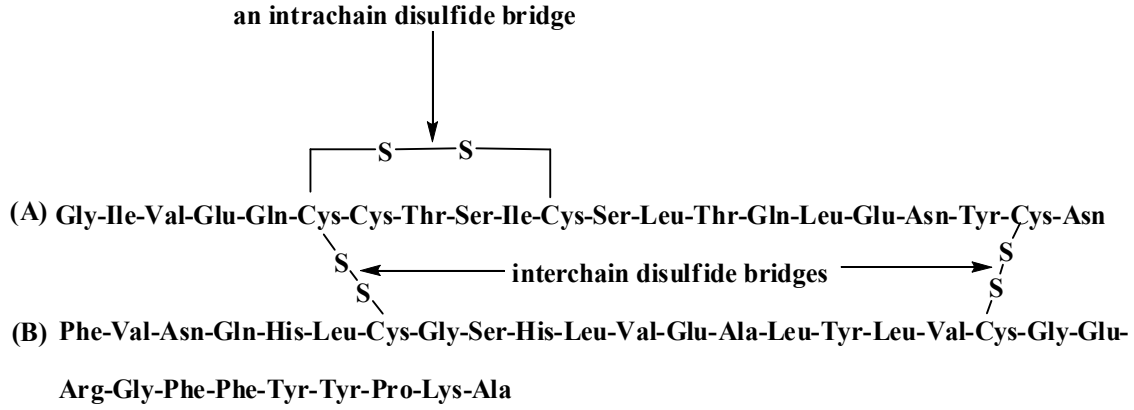
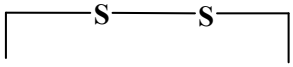


Figure 5: Schematic diagram for insulin[19]

Three years later, Vincent du Vigneaud (winner of the Nobel Prize in chemistry in 1955) synthesised Oxytocin (Figure 6) as the first short peptide to be applied for pharmaceutical

purposes.[19] Oxytocin is a hormone with many physiological functions such as regulating blood pressure and producing breast milk.[19,21]



Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

Figure 6: Oxytocin[19]

Since then the synthesis of biologically active peptides and the development of advanced methods to synthesise more complex peptides, received significant attention.

Methods of peptide synthesis

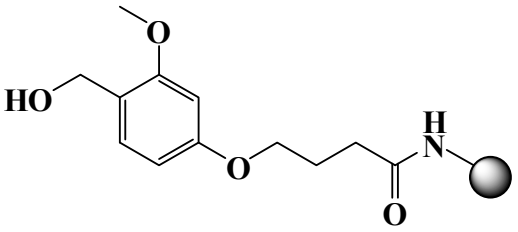
Methods for synthesising peptides have undergone considerable improvements over the years. These methods include classical or solution methods, solid phase peptide synthesis (SPPS) and finally a recombinant DNA method.[21] The recombinant DNA method is a genetic engineering technique which was introduced in the 1980's.[19,21] This method is useful for the synthesis of large amounts of natural peptides and proteins such as insulin.[21] The general idea of this technique is to introduce the coded DNA responsible for the synthesis of the target peptide or protein into a micro-organism cell (bacteria) which will then synthesise the required protein. These cells have the remarkable ability to be cultivated in relatively short time and can synthesise long peptides and proteins without any errors.[19] This method is currently only available for natural proteins of which the specific gene is known.

The synthesis of other peptides and proteins are therefore still largely dependent on chemical synthetic techniques. Initial attempts made use of homogeneous solution phase chemistry.[21] However, due to the problems encountered with classical solution methods an alternative strategy was desperately required. Some of the difficulties inherent to homogeneous synthesis are: purification problems which is required after each coupling step (this is extremely time consuming), the low yields obtained for the total peptide sequence and sometimes the low solubility of the intermediate segments in normal organic solvents.[19,24] Most of these difficulties are brilliantly eliminated with solid phase peptide synthesis (SPPS). It is therefore not surprising that SPPS has become the method of choice for the synthesis of small scale peptides.[24] However, solution synthesis still remains the dominant method for large and commercial scale peptide synthesis.[21]

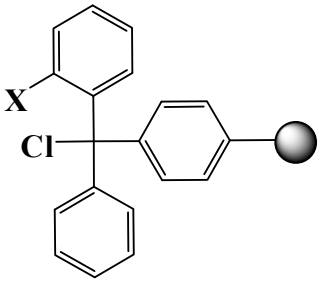
SPPS makes use of C to N coupling (C → N) which has several advantages. C → N coupling in SPPS implies that the N-protected amino acid is coupled with its acid group onto the amino group of an amino acid which is already attached to the resin. SPPS can be summarized into four main steps:[21,24,25]

- Anchoring of the protected amino acid to the solid support resin (see Figure 7 and 8). Depending on the chemicals required (acid or base) for the synthesis and the environment of the desired peptide (*e.g*. peptide amide or peptide acid), the resin should be chosen carefully. The anchor of the protecting amino acids usually results from an esterification reaction (resin/linker with an alcohol terminal group) or through an amide bond (resin/linker with an amino terminal group). The first anchoring method will yield a peptide acid and the second a peptide amide upon stripping of the peptide from the resin.
- Cleavage of the temporary protecting group on the *Nα*-amino acid, should take place before coupling of the next amino acid to the sequence. With the SPPS technique there are two major protecting groups, namely 9-Flourenylmethyl chloroformate (Fmoc) and Di-*tert*-butyl dicarbonate (*t*-Boc). The *t*-Boc group is acid labile [Trifluoroacetic acid (TFA) is in most cases used for cleavage], whilst the Fmoc group is sensitive towards mild basic conditions especially secondary amines. Its stability towards acidic media allows the application of acid-labile semi-permanent protecting groups such as *t*-Boc on the side chain functional groups of the amino acid. The protecting group cleavage is followed by a thorough washing procedure to remove all by-products, unreacted reagents etc. The elegant mechanism of deprotection for the Fmoc group is illustrated in Scheme 1.
- SPPS makes use of *in situ* coupling which is the most efficient and easiest coupling method. Methods were developed in which the carboxylic function of the N-protected amino acid is activated for more facile coupling to the free amino group attached to the solid support. Different *in situ* coupling reagents (Figure 9) are used for this and the main function is to activate the carboxylic group. These two steps as mentioned above (cleavage of the protecting group and coupling of the next amino acid) are repeated a number of times until the target peptide is obtained. As explained, the impurities (by-products and the other reagents) are removed after each step by a thorough washing procedure whilst the pure peptide is attached to the solid phase resin.

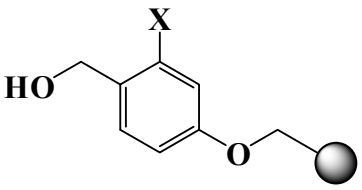
- Cleavage of the desired peptide from the solid support is the final step in the SPPS method in which breaking of the covalent bond between the resin/linker and the peptide take place. Acidolysis† with concentrated TFA is widely used for cleaving the peptide off the resin and simultaneous cleavage of the protection group (e.g. *t*-Boc) off of the peptide. Synthesis of *t*-Boc and *t*-Butyl protected peptides require an acid sensitive linker/resin in order to prevent deprotection of these groups. The Barlos resin **16**, Sasrin resin **19** and Sieber acid resin **20** require between a 1-10% TFA solution to cleave off the peptide.[25]



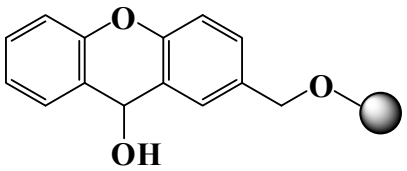
15: HMPB resin



16: Trityl choloride resin (Barlos resin) X = H
17: 2-Chlorotritylchloride resine X = Cl



18: Wang resin X = H,
19: Sasrin resin X = CH_3-O,



20: Sieber acid resin

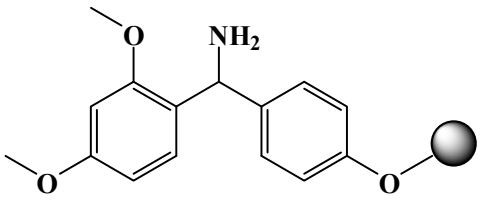
Figure 7: Example of peptide acid resins[24]

All of the resins above will yield peptide acids upon cleavage of the peptide from the resin. It appears that in most cases the resin will form a stabilised carbocation in the process. In many cases Triisopropylsilane (TIS) or 1,2-ethanedithiol (EDT) are widely use as cation scavengers.[25]

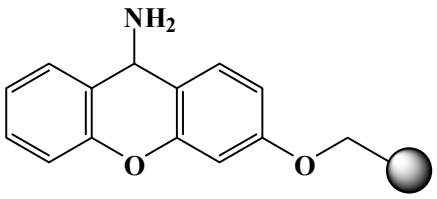
Examples of peptide amide resins are provided in Figure 8. The same principle holds as above. A cation will form upon removal of the peptide amide and the lone pair of electrons on the ether oxygen atoms attached to the aromatic rings are utilized to alleviate the effect of

† Acidolysis is scission of a bond *via* adding $H^+ X^-$, while hydrolysis involves adding of H^+OH^-

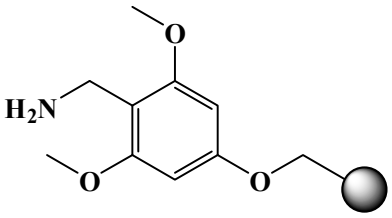
the carbocation. The carbon to amide bond is weakened by the acidic conditions, assisted by delocalization of the resulting positive charge over the complete aromatic system.



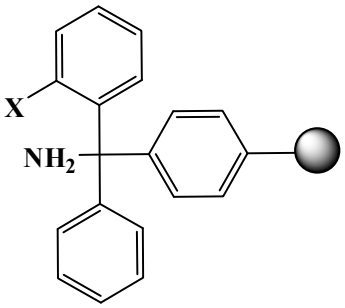
21: Rink amide resin



22: Sieber resin



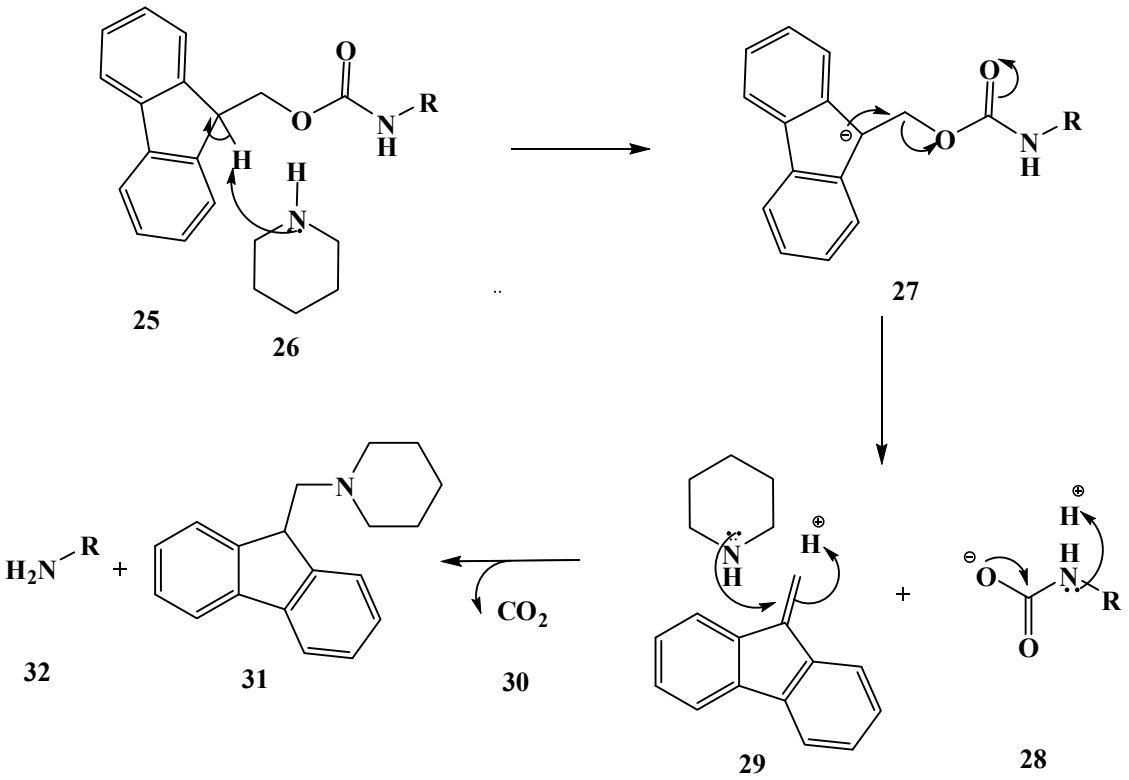
23: Pal resin



24: 2-Chlorotrityl amide resin

Figure 8: Examples of amide peptide resins[24]

A schematic representation of the mechanism of Fmoc deprotection is provided in Scheme 1. The driving force is the fact that the negative charge can be delocalised over three rings (13 carbon atoms).



Scheme 1: Deprotection of Fmoc using piperidine[21]

Coupling agents are required for activating the incoming acid and to remove the equivalent of water that forms during amide bond formation.[25] Initially carbodiimide reagents e.g. diisopropylcarbodiimide (DIC), carbodiimide ethyl(3-diethylaminopropyl)-carbodiimide hydrochloride (EDC) were popular coupling agents. Problems with the use of carbodiimides in the SPPS technique included the formation of insoluble urea by-products and weak coupling efficiency when *N,N*-Dimethylformamide (DMF) is used as solvent.[25] Figure 9 provide an example of more efficient and popular SPPS coupling agents discovered over the years. The effectiveness of these phosphonium and uronium salts stems from the rapid ability to form the corresponding ester, the formation of soluble secondary products and the lack of side products relating to them.[25]

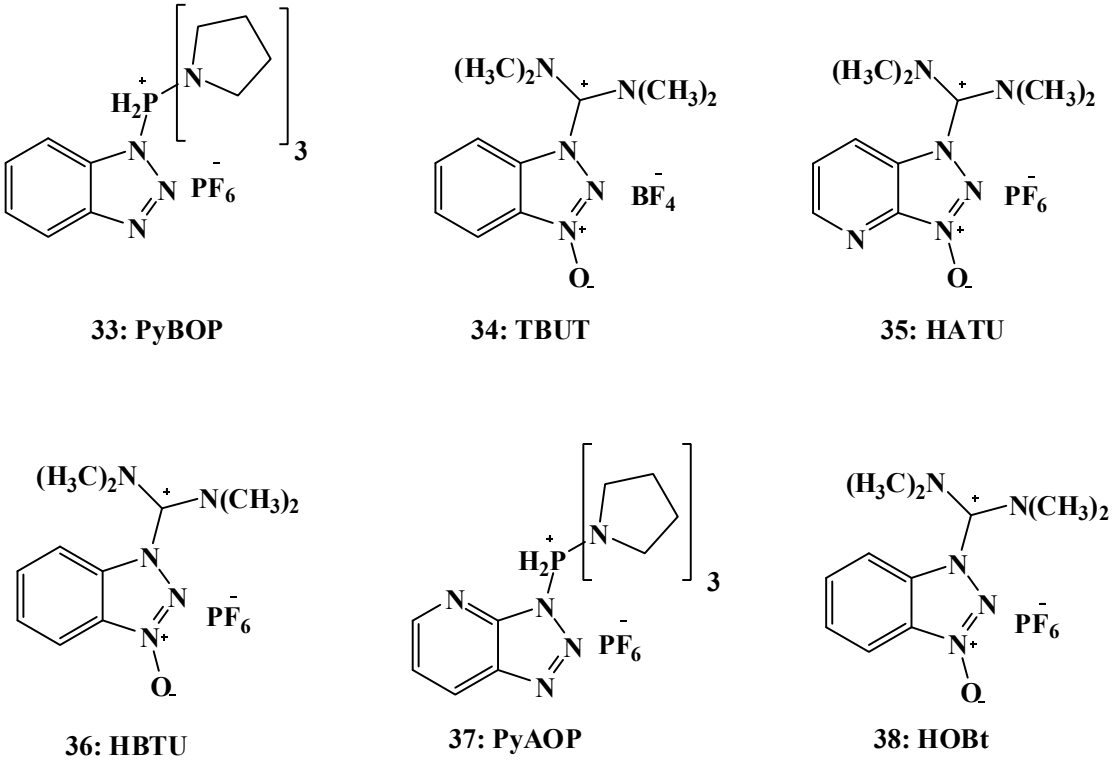


Figure 9: Example of the most common SPPS activation reagents[24,25]

All these SPPS steps can be performed manually where the coupling and deprotection steps are usually monitored by applying the ninhydrin test on a few beads of the resin.[24] In Fmoc chemistry, the absorbance of the reaction solution is another method for monitoring the coupling and the deprotection steps. This is due to the strong absorbance of the dibenzofulvene-piperidine adduct **31**. An absorbance increase after the deprotection and a decrease after the coupling in each step as indicative of the efficiency of the reaction.[21,25]

In 1966 R.B Merrified developed the first automated peptide synthesiser, nowadays many new improved generations of automated peptide synthesis have been developed.[21,24] In automated peptide synthesis methods, the steps can be monitored by either recording the conductivity of the reaction solution or by the absorbance of the reaction when using Fmoc synthesis.[21] The latest peptide synthesisers also make use of microwave synthesis, which allows for faster and more effective coupling.[26] Our group is in the privileged position to have access to an automated microwave assisted SPPS instrument (see details in the experimental section).

Cage amino acid peptides

Incorporation of unnatural polycyclic amino acids into short peptide sequences has been of major interest especially in the pharmaceutical field. This is due to the ability of such amino acids to cross the cellular membrane and to reduce the degradation rate of the peptide in biological systems due to the steric hindrance imposed on the peptide bond.[27] The following are some examples of unnatural lipophilic amino acids that have been synthesised and studied.

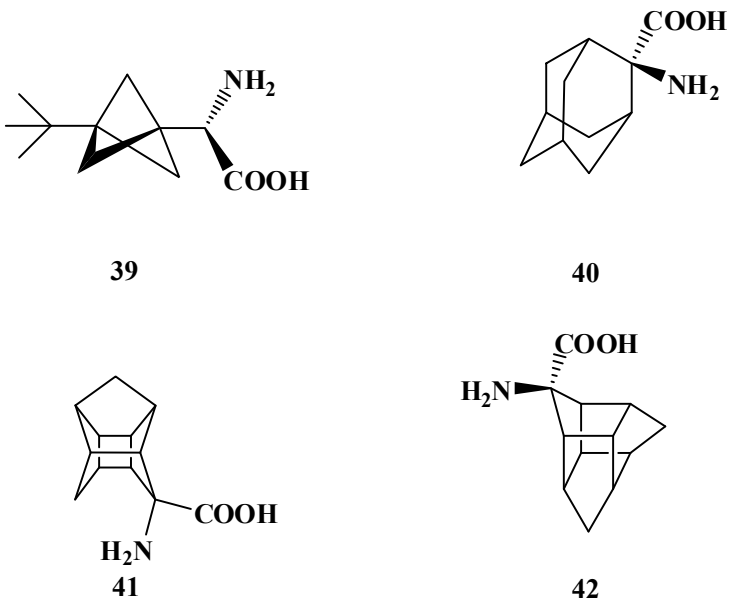
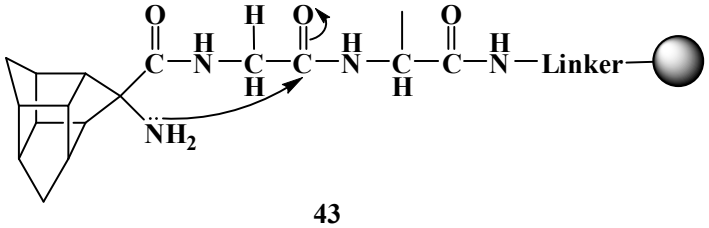


Figure 10: Some examples of polycyclic unnatural amino acids[27-30]

A computational investigation in our group focussing on the incorporation of the PCU amino acid **41** and the trishomocubane amino acid **42** into short peptide sequences have been conducted.[31-33] Previous attempts to incorporate these cage amino acids into peptides were made with limited success.[30,34,35] Initially attempts were conducted with the PCU amino acid **41**[30,34] and it was thereafter decided to use the trishomocubane skeleton **42** since it would impose less steric hindrance.[29,35] Achiral glycine was initially used and was suspected to form a Schiff base (six membered hetero-ring, Figure 11) due to the ability of glycine to fold back.[29,35]



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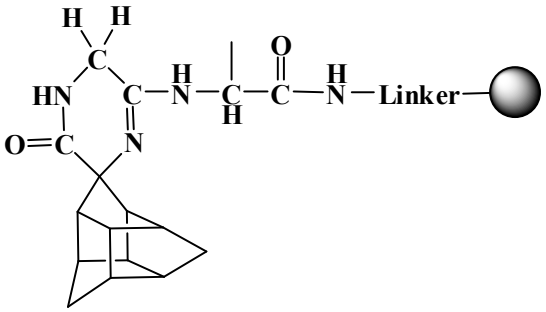

44

Figure 11: A possible Schiff base formation [29]

Alanine was suggested to replace the glycine unit to overcome this problem, the reason being that the methyl group in the alanine side chain could prevent the formation of the Schiff base. Also, the methyl group is expected to be small enough to avoid steric hindrance thus allowing coupling to the cage amino acid. Alanine peptides, according to an electron spin resonance study (ESR), prefer 3_{10}-helix β-turns.[36] Previous attempts in our group to manually synthesize Ala-Ala-Ala-Tris-**42**-Ala-Ala-Ala-Ac (Figure 12) was only partially successful.[29] A correct MS peak was observed, but poor NMR spectra's were achieved. Collaboration with a group at Uppsala University (Sweden) resulted in the successful synthesis of the target sequence followed by a single crystal X-ray study of the peptide.[22]

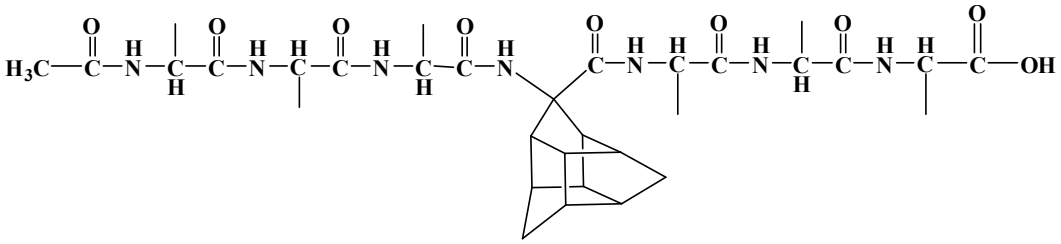

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Figure 12: The structure of Ala-Ala-Ala-Tris-42-Ala-Ala-Ala

The X-ray study of the cage peptide indicated that the trishomocubane amino acid **42** is indeed a very active β-turn inducer and confirmed the computational predictions previously made.[22] As pointed out, a number of efforts to manually synthesise the hepta-peptide Ala-Ala-Ala-PCU-**41**-Ala-Ala-Ala were unsuccessful. Part of this dissertation focuses on the microwave assisted synthesis, purification and NMR (nuclear magnetic resonance) studies of this hepta-peptide.

References

1. Geldenhuys, W. J.; Malan, S. F.; Bloomquist, J. R.; Marchand, A. P.; Van der Schyf, C. J. *Medicinal Research Reviews* **2005**, *25*, 21-48.
2. Kassiou, M.; Nguyen, V. H.; Knott, R.; Christie, M. J.; Hambley, T. W. *Bioorganic & Medicinal Chemistry Letters* **1996**, *6*, 595-600.
3. Marchand, A. P. *In Advances In Theoreticallly Interesting Molecules*, 1989; Vol. 1.
4. Marchand, A. P. *Chemical Reviews* **1989**, *89*, 1011-1033.
5. Zah, J.; Terre'Blanche, G.; Erasmus, E.; Malan, S. F. *Bioorganic & Medicinal Chemistry* **2003**, *11*, 3569-3578.
6. Grobler, E.; Grobler, A.; Van der Schyf, C. J.; Malan, S. F. *Bioorganic & Medicinal Chemistry* **2006**, *14*, 1176-1181.
7. Ito, F. M.; Petroni, J. M.; de Lima, D. P.; Beatriz, A.; Marques, M. R.; de Moraes, M. O.; Costa-Lotufo, L. V.; Montnegro, R. C.; Magalhaes, H. I. F.; Pessoa, C. D. O. *Molecules* **2007**, *12*, 271-282.
8. Geldenhuys, W. J.; Malan, S. F.; Bloomquist, J. R.; Van der Schvf, C. J. *Bioorganic & Medicinal Chemistry* **2007**, *15*, 1525-1532.
9. Geldenhuys, W. J.; Terre'Blanche, G.; Van der Schyf, C. J.; Malan, S. F. *European Journal of Pharmacology* **2003**, *458*, 73-79.
10. Mdzinarishvili, A.; Geldenhuys, W. J.; Abbruscato, T. J.; Bickel, U.; Klein, J.; Van der Schyf, C. J. *Neuroscience Letters* **2005**, *383*, 49-53.
11. Hao, J.; Mdzinarishvili, A.; Abbruscato, T. J.; Klein, J.; Geldenhuys, W. J.; Van der Schyf, C.; Bickel, U. *Brain Research* **2008**, *1196*, 113-120.
12. Oliver, D. W.; Dekker, T. G.; Snyckers, F. O.; Fourie, T. G. *Journal of Medicinal Chemistry* **1991**, *34*, 851-854.
13. Geldenhuys, W. J.; Malan, S. F.; Murugesan, T.; Van der Schyf, C. J.; Bloomquist, J. R. *Bioorganic & Medicinal Chemistry* **2004**, *12*, 1799-1806.
14. Kent, J. K.; Godleski, A. S.; Osawa, E.; Schleyer, R. P. *JOC* **1977,** *42*, 3852-3859.
15. Marchand, A. P.; Kumar, K. A.; McKim, A. S.; MlinaricMajerski, K.; Kragol, G. *Tetrahedron* **1997**, *53*, 3467-3474.
16. Odisitse, S. J., G. E.; Govender, T.; Kruger, H. G.; Singh, A. *The Royal Society of Chemistry* **2007**, 1140–1149.

17. Govender, T.; Hariprakasha, H. K.; Kruger, H. G.; Marchand, A. P. *Tetrahedron Asymmetry* **2003**, 1553–1557.
18. Morrison, R. T.; Boyd, R. N. *Organic Chemistry*, Six Edition ed.; Prentice Hall of India: New Delhi, 2003.
19. Bruice, P. Y. *Organic Chemistry*, Second Edition ed.; Prentice Hall: New Jersey, 1998.
20. Murray, R. K.; Granner, D. K.; Mayes, P. A.; Rodwell, V. W. *Harper's Illustrated Biochemistry*, 26th edition ed.; McGraw-Hill Beirut, 2003.
21. Sewald, N. J. H *Peptide: chemistry and Biology* WILEY-VCH, 2002.
22. Albericio, F.; Arvidson, P.; Bisetty, K.; Giralt, E.; Govender, T.; Jali, S.; Kongsaeree, P.; Kruger, H. G.; Prabpai, S. *Chem Bio Drug Des* **2008**, *71*, 125-130.
23. Powers, S. J.; Hancock, R. E. W. *Peptides* **2003**, *24*, 1681–1691.
24. Howl, J. *Peptide Synthesis and Applications*; Humana Press Inc.: Totowa, New Jersey, 2005.
25. Benoiton, L. N. *Chemistry of Peptide Synthesis*; Taylor & Francis: Boca Raton, 2005.
26. CEM, http://www.cem.com/biosciences/autopep.asp. webpage accessed on 14/10/08.
27. Nagasawa, H. T.; Elberling, J. A.; Shirota, F. N. *Journal of Medicinal Chemistry* **1975**, *18*, 826-830.
28. Pritz, S.; Patzel, M.; Szeimies, G.; Dathe, M.; Bienert, M. *The Royal Society of Chemistry* **2007**, *5*, 1789–1794.
29. Jali, S. *MSc Thesis*, University of KwaZulu-Natal, 2006.
30. Mdluli, P. S. *MSc Thesis*, University of KwaZulu-Natal, 2005.
31. Bisetty, K.; Govender, P.; Kruger, G. H. *Biopolymers* **2006**, *81*, 339-349.
32. Bisetty, K.; Corcho, J. F.; Canto, J.; Kruger, G. H.; Perez, J. J. *Journal of Molecular Structure* **2006**, *759*, 155-157.
33. Bisetty, K.; Kruger, G. H.; Perez. *Molecular stimulation* **2007**, *33*, 1105-1108.
34. Singh, A. *MSc Thesis*, University of Kwazulu-Natal, 2005.
35. Raasch, T. *MSc Thesis*, University of Natal, 2003.
36. Hanson, P.; Martinez, G.; Millhauser, G.; Formaggio, F.; Crisma, M.; Toniolo, C.; Vita, C. *Journal of the American Chemical Society* **1996**, *118*, 271-272.

CHAPTER 2

SYNTHESIS OF HYDANTOIN DERIVATIVES

Introduction

The chemistry of hydantoin derivatives has been of major interest to pharmaceutical/medicinal chemists for many years.[1,2] This is mainly due to the potency and promising activities of hydantoin derivatives in various pharmaceutical applications such as anti-microbial, anti-type-II diabetes (non-insulin dependent), zinc metaloprotease inhibitors and anti-convulsant agents.[3-6] Hydantoins (Figure 13) are heterocyclic five membered ring compounds containing two nitrogen atoms in positions 1 and 3.

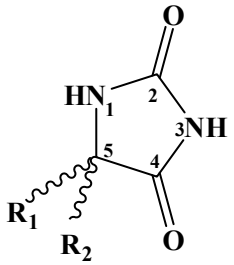

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Figure 13: Hydantoin ring

Phenytoin [(5,5 diphenyl hydantoin)] **47** was the first clinical anti-convulsant drug for electronic shock animal mode of seizure.[2] Phenytoin was introduced over seven decades ago,[2] and according to the American Academy of Neurology (2005), it is still the most prescribed drug for initial therapy.[2,7-9]

Normally, an epileptic seizure causes sudden release of abnormal electrical impulses in the brain resulting in temporary disorder of the normal activity passing through the neuron cells.[10] These seizures are classified as two types:[11]

- General seizure which is the common type, where the seizure starts in a part of the brain and then spreads to other regions.
- Partial seizure which occurs only in a small part of the brain.

The mechanism of action for hydantoin ring /phenytoin is still unclear, but it is believed to act through the blockage of sodium channels and/or Na-K-ATPase (Na-pump) in the brain cells, thereby reducing the high frequency of epileptic seizure.[2,8,12-14]

It was reported that substitution at position one of the hydantoin ring of phenytoin decreases its potency as an anticonvulsant, in contrast to substitution at position three.[7,15,16] Although the nitrogen atom at position three on the hydantoin ring is less nucleophilic than the nitrogen atom at position one, the chances of getting substitution on position three increases with an increase of steric bulk on the carbon atom at the five position.

Due to the low solubility and the bio-availability of phenytoin, considerable efforts were made to improve these properties, by introducing different substituents on the N-3 position or *via* substitution of the phenyl ring.[7,8,17,18] Fosphenytoin **48** is a water soluble prodrug[‡] of phenytoin marketed under the trade name Cerebyx.[19] It metabolises in biological systems to yield phenytoin (the active compound), phosphate and formaldehyde which then converts to formate.[19]

Other active compounds reported involved the replacement of the phenyl groups at the C-5 position.[20] It is obvious that the phenyl ring and aliphatic chains (see Figure 14) are necessary to improve the lipophilicity of the drug.

[‡] A prodrug is an inactive form of drug which needs be metabolically converted to the active form in the biological system. It is in most cases used to carry the drug to the active site where the drug is released.

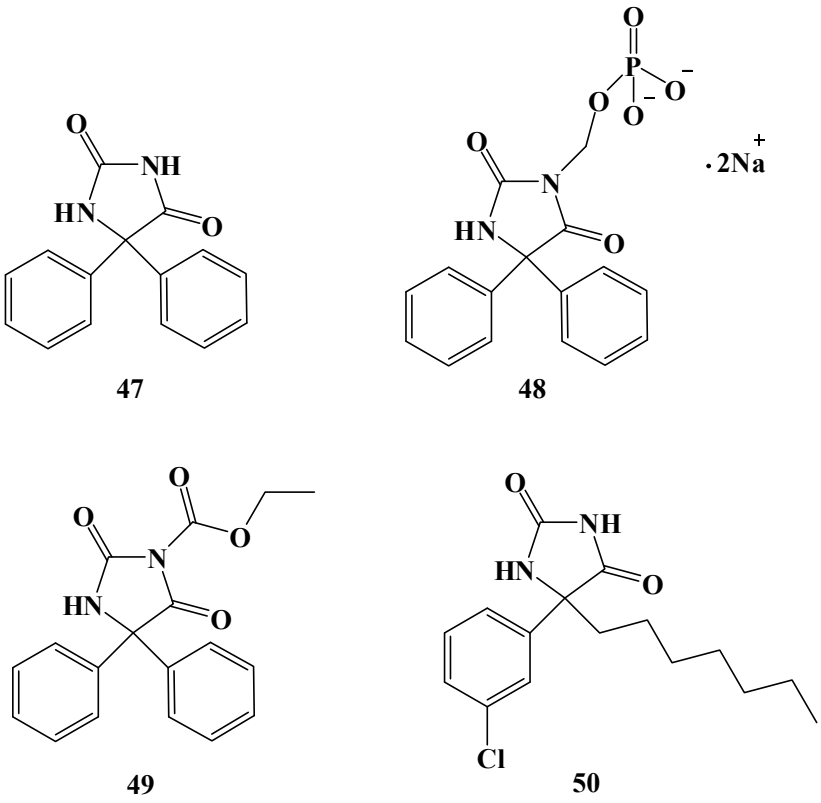
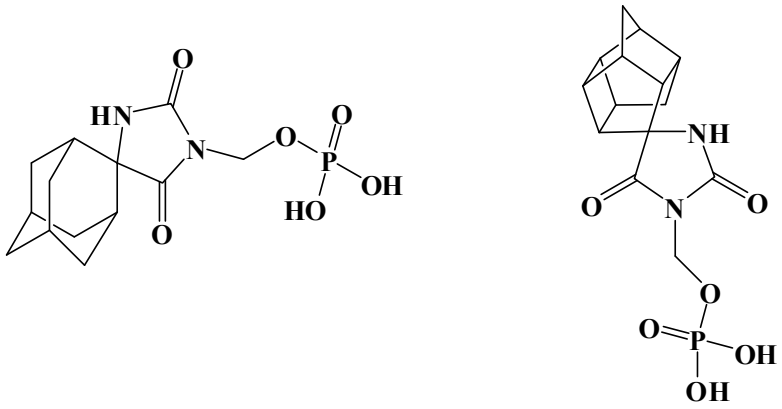


Figure 14: Examples of hydantoin derivatives applied as anticonvulsants[1,7,8]

However a balance between hydrophilic and lipophilic properties (amphilicity) is necessary for designing this type of bioactive compounds, since it allows them to circulate in biological system and achieve their target (cross the BBB).[21] Introducing polar groups (*e.g.* phosphate, carboxylic *ect*) to the parent compound is a popular approach to increase the amphilicity of the drug.[21,22]

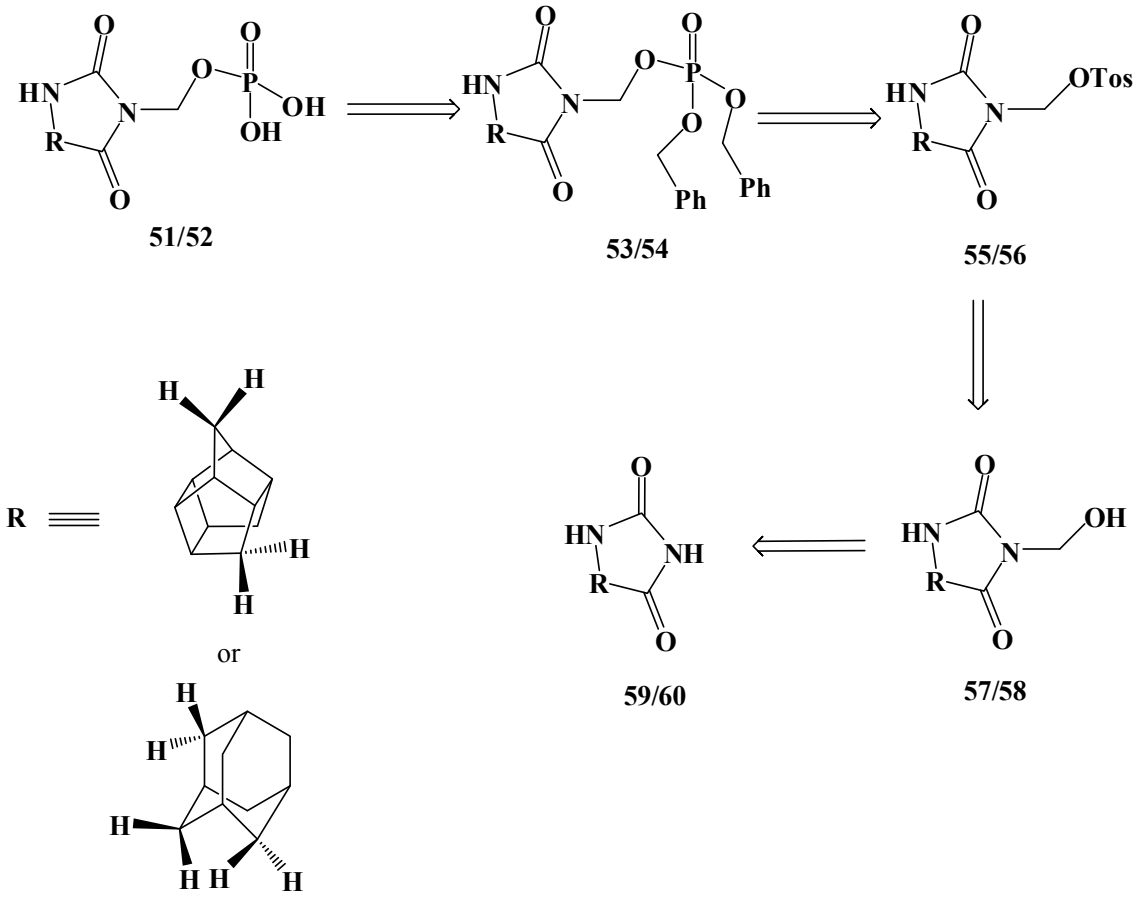
The importance of polycyclic cage compounds in terms of biological applications was discussed in Chapter 1. Two proposed cage analogues of Cerebyx are compounds **51** and **52**. It is envisaged that the cage skeleton which is known to cross the BBB and CNS (as was mentioned in Chapter 1) will be advantageous for potential anti-convulsant drugs.



51 52

Figure 15: Structures of the proposed hydantoin derivatives

The proposed retro synthetic pathway for the synthesis of **51** and **52** is presented below. The pathway was adapted from Fosphenytoin literature.[23,24]



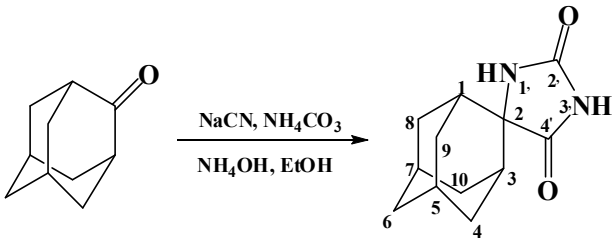
Scheme 2: The proposed retro synthetic scheme for compounds 51 and 52[23]

The next sections describe the attempted synthesis of compounds **51** and **52**

Synthesis of adamantane hydantoin analogues

Synthesis of the adamantane hydantoin **59**[25,26] was achieved *via* the Bucherer-Lieb[27] method. adamantanone **61** is reacted with sodium cyanide, ammonium carbonate and ammonium hydroxide in ethanol producing **59** in 89%.yield.

Note that Adamantanone is commercially available and can be used without further purification.

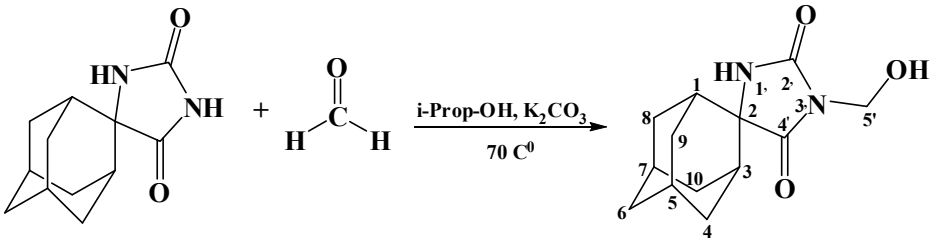


61 **59**

Scheme 3: Synthesis of adamantane hydantoin 59[25]

Compound **59** doesn't have a chiral centre due to the plane of symmetry which crosses each of the following atoms: C-6, C-5, C-7, C-2, N-1', C-2', N-3' and C-4'.

The adamantane hydantoin was further reacted with formaldehyde **62** in the presence of a base in isopropanol at 70^0C to obtain the novel compound **57** in 91% yield.



59 **62** **57**

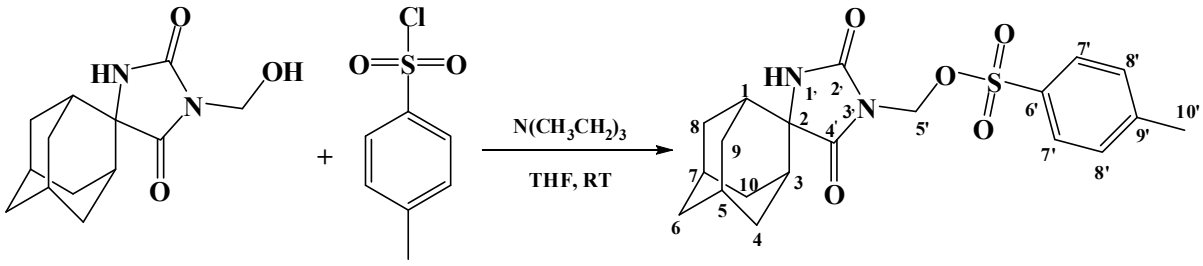
Scheme 4: Synthesis of novel compound 57

The ^{1}H NMR spectrum of compound **57** showed the absence of the singlet at 10.48 ppm assigned to the N-3' position and the presence of a singlet integrating to two protons at 4.67 ppm which was assigned to the 5' protons. The N-1' proton shifted from 8.38 ppm to 8.71 ppm.

The ^{13}C NMR spectrum of compound **57** displays a signal at 60.2 ppm representing the C-5' position while the carbon signals at 154.7 and 174.9 ppm show HMBC correlation to H-5' representing the C-2' and C-4' respectively on the hydantoin ring. These assignments

correlate to those by Martins *et al.* for the PCU hydantoin **60**.[28] The C-2' and C-4' shifts for this study were assigned to each of the compounds **55-58** based on the results by Martins *et al***.**

Reacting a suspension of compound **57**, *p*-toluenesulfonyl chloride (tosyl chloride) **63** and triethylamine in THF at room temperature for 12 hours gave **55** in 73% yield. Attempts to remove triethylamine *via* aqueous extraction and silica gel chromatography resulted in decomposition of **55** to **59**. The same problem was encountered for compound **56**.



Scheme 5: Synthesis of novel compound 55

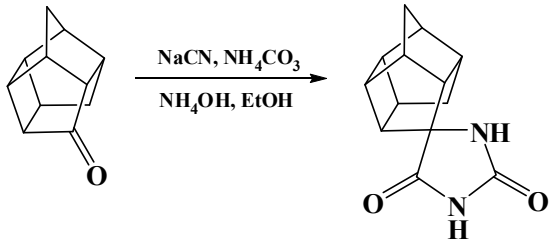
The ^{1}H NMR spectrum of compound **55** shows a singlet at 4.71 ppm for the two methyl protons at the H-5'position. The N-1' proton of **55** has shifted to 9.22 ppm from 8.71 ppm for the starting material **57**. This is due to the introduction of the electron withdrawing tosyl group present in compound **55**. Electron withdrawing groups cause (through delocalization in the ring) the N-1' proton to be more de-shielded, hence it will register at a higher frequency. The ^{13}C NMR spectrum shows a peak at 20.7 ppm for the C-10' carbon on the tosyl group; the peaks at 125.4, 128.0, 145.6, 153.9 ppm belong to the aromatic ring carbons. The two carbonyl carbons C-2'and C-4' in the hydantoin ring are observed at 154.0 and 176.0 ppm respectively.

Synthesis of PCU hydantoin analogous

The starting material for the synthesis of PCU hydantoin **60** is pentacyclo-[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]undecane-8-one (PCU monoketone **64**) which was synthesized from the PCU-8,11-dione **73**. Note that the synthesis of **64** will be discussed at the latter part of this chapter in order not to disrupt the flow of the current theme. It is important also to note that the monoketone **64** starting material is normally obtained as a racemate.[28] However, it is

possible to obtain it in optically pure form by using a horse liver alcohol dehydrogenase enzyme.[29]

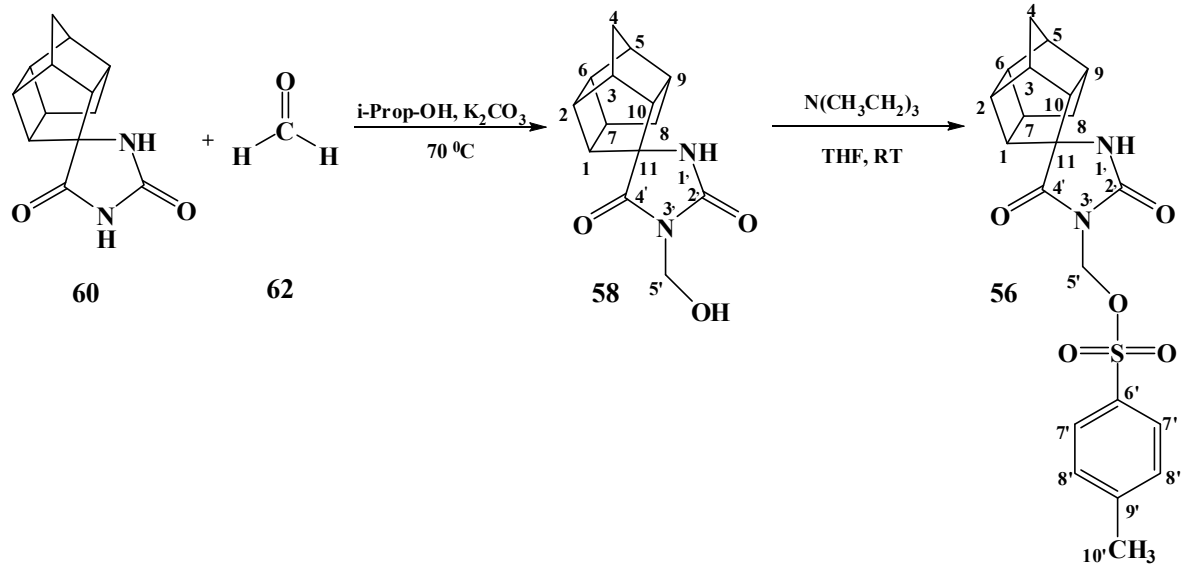
The PCU hydantoin **60** was synthesized *via* a similar synthetic pathway as described for the synthesis of the adamantine based hydantoin **59** (Scheme 6).



64 **60**

Scheme 6: Synthesis of PCU hydantoin 60[15,28]

Compound **60** above was further derivatized using similar conditions as reported for the adamantine based hydantoin to obtain the novel compounds **58** and **56** (Scheme 7).



60 **62** **58** **56**

Scheme 7: Synthesis of novel compounds 58 and 56

All attempts to purify compound **58** through silica gel chromatography were unsuccessful due to degradation to the starting material **60**. Recrystallization of **58** from ethyl acetate resulted in the pure form.

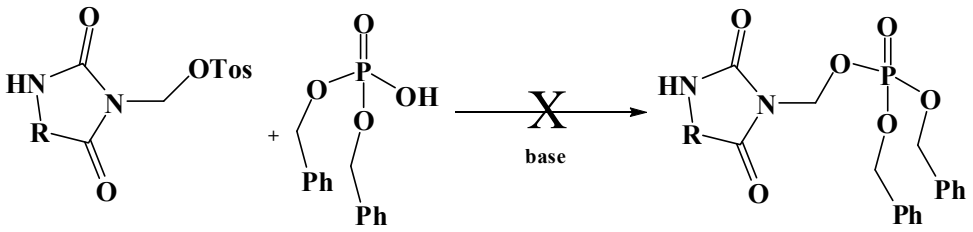
The N-3'peak in the ^{1}H NMR spectrum of compound **58** is absent while the corresponding signal for compound **60** is observed at 10.49 ppm. The signal registering at 4.68 ppm is

assigned to the methylene protons of H-5' while the triplet peak at 6.20 ppm was assigned to the -OH group; these assignments were based on (a) the proton integration values, (b) the fact that the peak at 6.20 ppm exchanges with D_2O and (c) the observed COSY correlation between H-5' and O-H. The signal at 7.91 ppm was assigned to the N-1' proton on the hydantoin ring. The ^{13}C NMR spectrum of compound **58** shows a peak at 60.4 ppm which was assigned to C-5' while the carbon atoms associated with 156.2 and 176.5 ppm which show HMBC correlation to H-5'. These carbon atoms were assigned to the carbonyl carbons C-2' and C-4' respectively. The assignment for the C-5' was confirmed by the correlation in the HSQC spectrum with the corresponding hydrogens (H-5', 4.68 ppm).

The ^{1}H NMR spectrum of compound **56** shows a singlet peak for N-1' at 8.44 ppm. This corresponding peak had shifted from 7.91 ppm for compound **58** and is similar to what was observed for the corresponding adamantane analogue **55** as discussed above. The absence of the –OH peak observed in compound **58** at 6.20 ppm also confirms the successful synthesis of compound **56**. Doublet signals were observed at 7.12 and 7.48 ppm, which were assigned to the aromatic protons of the tosyl group while the singlet at 2.27 ppm was assigned to C-10' of the tosyl group. The ^{13}C NMR spectrum shows a peak at 20.7 ppm for C-10'; the peaks at 125.4, 128.1, 138.0, and 145.2 ppm were assigned to the aromatic ring.
The next step in the project was to attempt the attachment of a phosphate groups to the side chains.

Attempted synthesis of dibenzyl phosphate derivatives of PCU and adamantane

According to the synthetic route for Fosphenytoin,[23] compounds **55/56** should be treated with dibenzyl phosphate **64** in the presence of a base in an organic polar solvent to give the phosphate derivatives **53/54**.

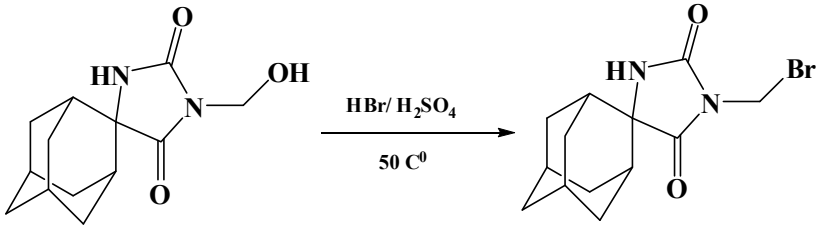


55/56 **64** **53/54**

Scheme 8: Attempted synthesis of compounds 53 and 54

The procedure from literature for the synthesis of Cerebyx[19] was followed but this synthesis of **53/54** was unsuccessful. Different solvents such as tetrahydrofuran, dichloromethane and acetonitrile were used under various conditions (room temperature and refluxing) with different bases (such as triethylamine, sodium hydride and sodium hydroxide). The reactions were monitored up to 24 hours and showed no sign of product formation using TLC analysis and NMR spectroscopy, only starting materials were observed.

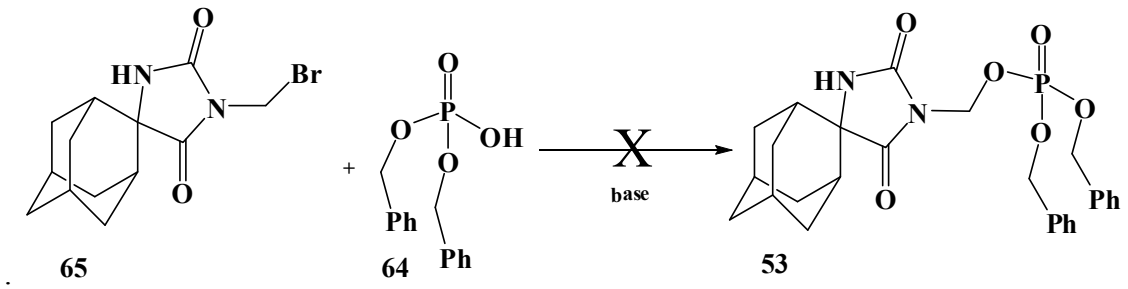
It was then decided to use bromine as a leaving group instead of the tosyl group. First, the adamantane derivative **65** was synthesised as presented in Scheme 9.



57 **65**

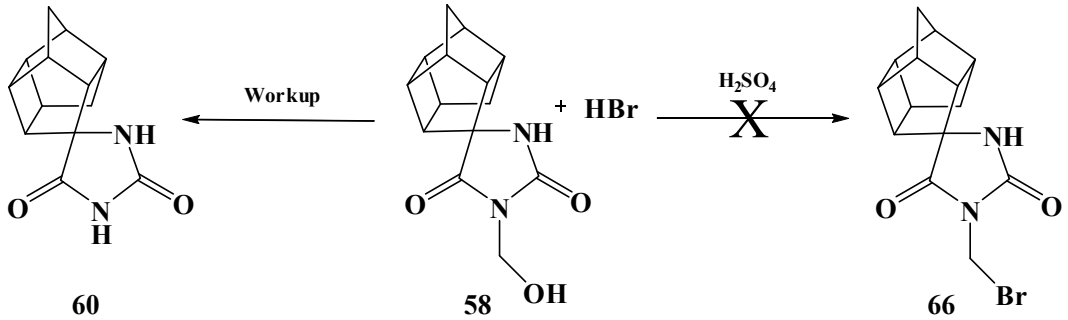
Scheme 9: Synthesis of novel compound 65

Compound **65** was refluxed with dibenzylphosphate in DCM in the presence of KOH as base for 12 hrs; again no product was obtained according to TLC and NMR analysis (Scheme 10).



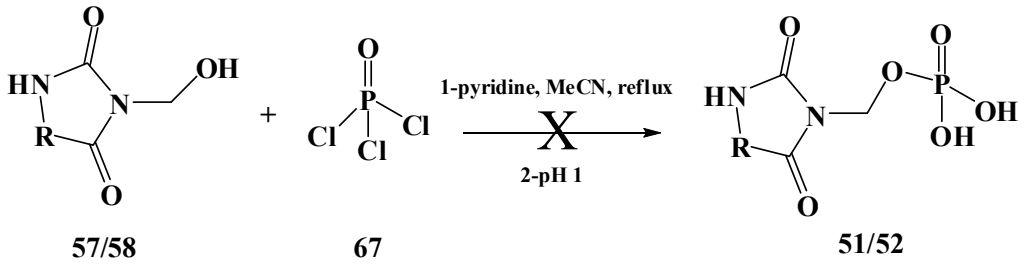
Scheme 10: Attempted synthesis of compound 53

Applying the same technique for the synthesis of the PCU hydantoin bromide **65** proved to be futile as the reaction workup resulted in the isolation of the PCU hydantoin **60** as was confirmed by NMR.



Scheme 11: Attempted synthesis of compound 66

Based on the previous unsuccessful attempts, a new synthetic pathway was designed (Scheme 12).[30] This reaction was also unsuccessful as the only products identified *via* TLC and NMR were compounds **59** and **60**.



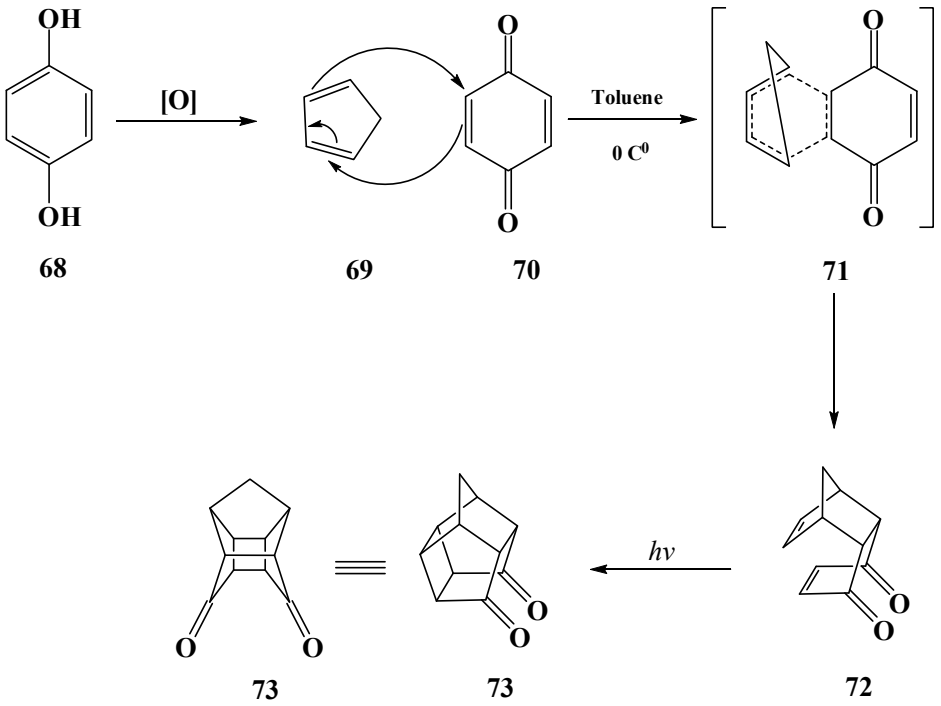
57/58 **67** **51/52**

Scheme 12: Attempted synthesis of compounds 51 and 52

It was decided that the proposed synthesis of **51/52** was sufficiently exhausted and that this part of the project would not be pursued further. The following section describes the synthesis of the PCU mono ketone **64** as starting material for the PCU hydantoin **60**.

Synthesis of PCU mono ketone 64

The starting material for the PCU mono ketone **64** is the dione **73**. The dione was synthesized[31] by reacting cyclopentadiene **69** with *p*-benzoquinone **70**. This reaction takes place *via* a Diels–Alder mechanism ([4+2] cycloaddition reaction) to form 5,8-methano-4a,5,8,8a-tetrahydro-1,4-naphthoquinone **72** which upon irradiation with UV light resulted in the PCU dione **73** (Scheme 13).



Scheme 13: Synthesis of the PCU dione 73[31]

The mechanism of converting the adduct **72** to PCU dione **73** was initially considered to be a concerted photochemical [2+2] cycloaddition reaction.[31] The concerted cycloaddition reaction for 4n π-electron type of molecules are thermally not allowed but photochemical possible. This is attributed to the symmetry forbidden overlapping between the two double bonds while they are in ground state. Excitation with UV light excites one of the double bonds and cause promotion to one of its electrons from π to π^* as indicated below ("π" indicates the ground state filled π-orbital and "π^*" indicates the excited state (empty or filled) π-orbital).

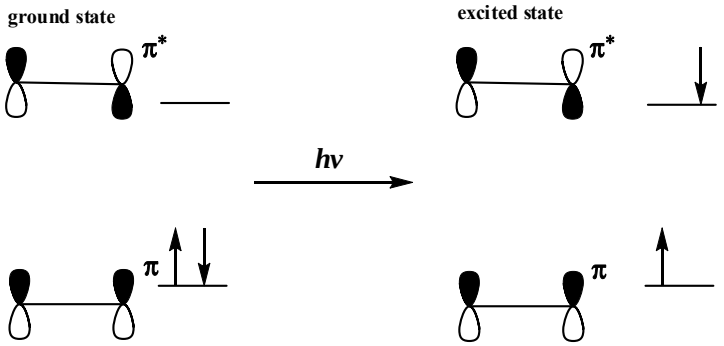


Figure 16: Photochemical excitation of the alkene bond[32]

According to the Hofmann Rules, the following electronic changes occur during the reaction.[32] Combination of the alkene exited state with the ground state one, results in two different energy molecular orbitals for each of π- and π*-electronic states (Figure 17).[32] In the case of the π-orbitals, two electrons are in the lower energy orbital and one is present in the higher molecular π-orbital. The only π*-excited electron collapses to a lower π*-orbital. The result is that three electrons are located in a low energy molecular orbital and one electron is excited allowing for the cyclization to take place.[32] The two single electrons have opposite spins, which simplifies the process of bond formation.

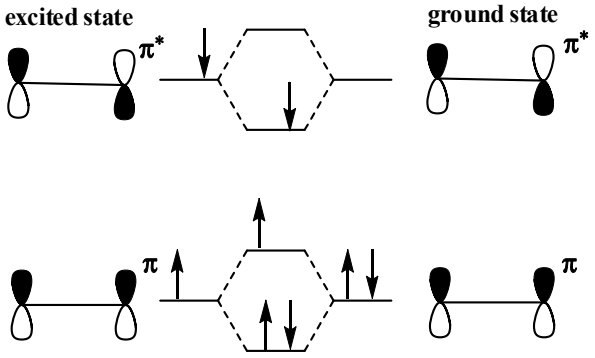
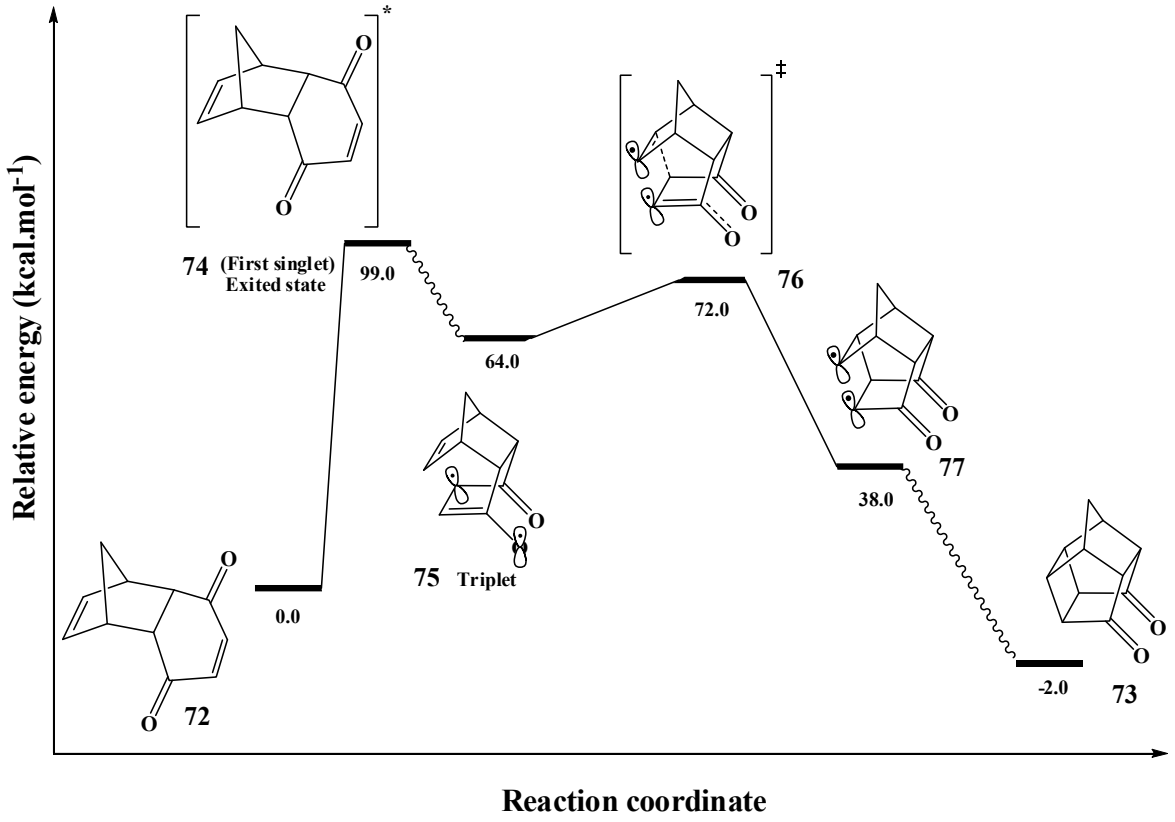


Figure 17: Electrons distribution in π and π* orbitals after combining the alkene excited state with the ground state[32]

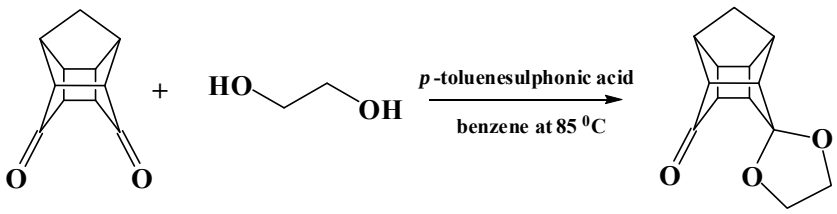
A subsequent computational study of this mechanism using *ab initio* software revealed a di-radical step wise mechanism is possible which takes place through the triplet exited state rather than the classical concerted [2+2] mechanism.[33]



Scheme 14: *Ab initio* calculated mechanism for adduct cyclisation[33]

Formation of the **75** (triplet) enedione through the first singlet excited state, **74**, requires relatively high energy (99 kcal/mol) in order to promote one of the nonbonding electrons on the oxygen atom from the n- to π^*-orbital.[33] The one unpaired electron of **75** (from the π-orbital in **74**) interacts to form the first new carbon-carbon cyclization bond in the transition state **76**.[33] Homolytic cleavage of the π-orbital of the other alkene will yield the diradical **76** (72 kcal/mol) in which both radicals have the same spin direction. This step (formation of **77**) was suggested to act as the rate determining step for this reaction. Finally **73** is obtained through the exothermic reaction after the one radical electron undergoing a change in spin to allow for cyclization of the precursor **77**.[33]

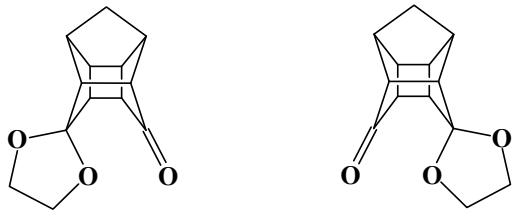
Synthesis of the PCU mono-ketone **64** was achieved following a series of well established reactions. The reaction started with protection of one of the carbonyl groups of the dione as a ketal group using ethylene glycol **78** with a catalytic amount of acid in a Dean-Stark apparatus.[34,35]



73 78 79

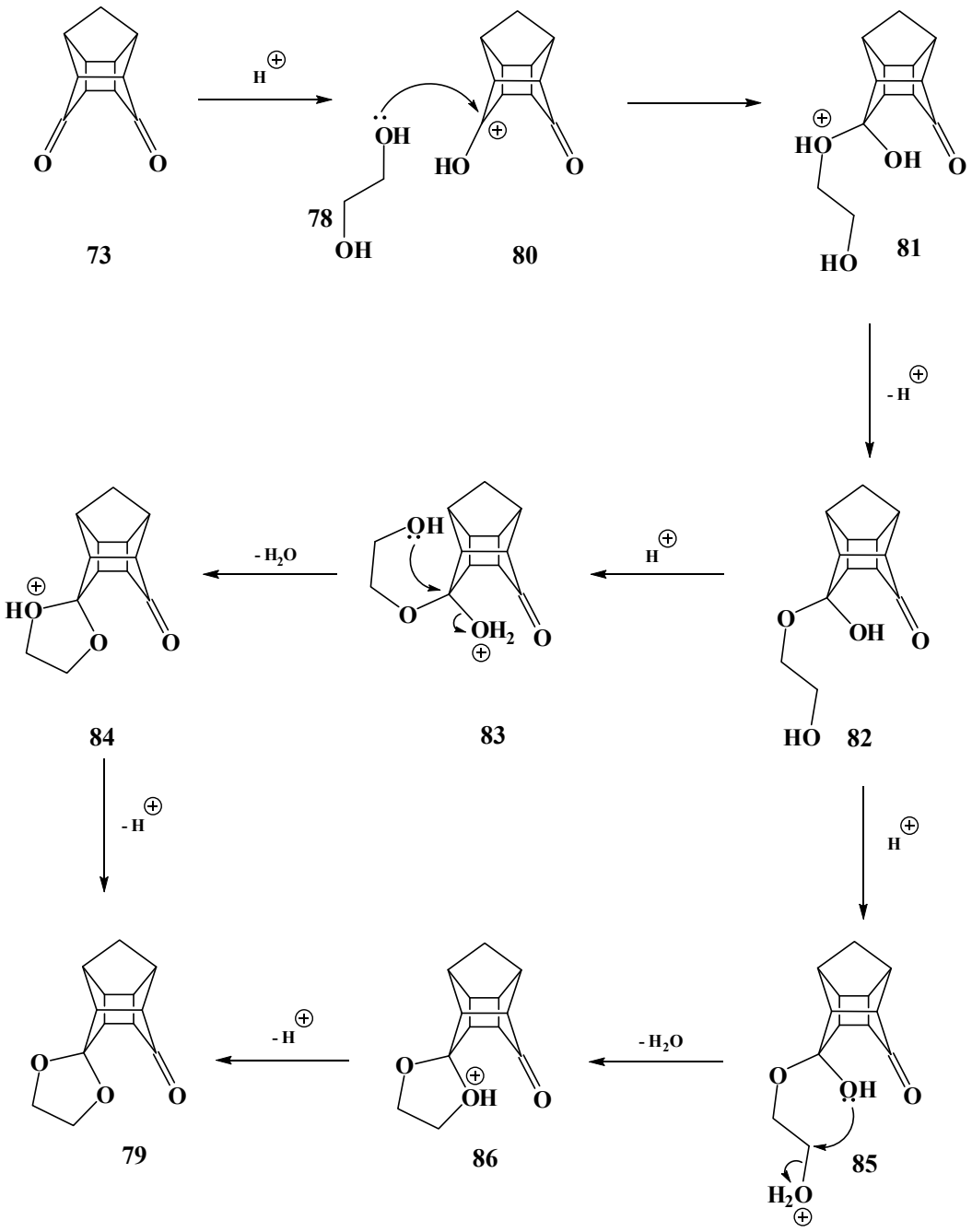
Scheme 15: Synthesis of the PCU keto-ketal 79[34]

Due to steric effects, the monoethylene ketal (keto-ketal) **79** is obtained as the only product, even with modest excess ethylene glycol. Since the reaction is performed under non-chiral conditions, both enantiomers **79a** and **79b** are obtained in 88% overall yield.



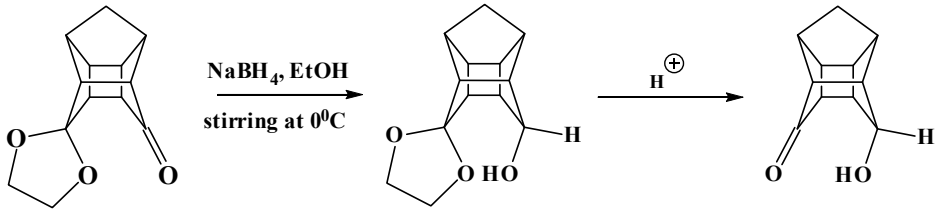
79a **79b**

The mechanism of this reaction is described below. It is known that nucleophilic attack on the dione **73** almost exclusively occurs from the *exo*-face of the cage.[35,36] In order to simplify the reaction mechanism, only one enantiomer will be used for all subsequent presentations.



Scheme 16: Proposed mechanism for keto-ketal 79 synthesis[37]

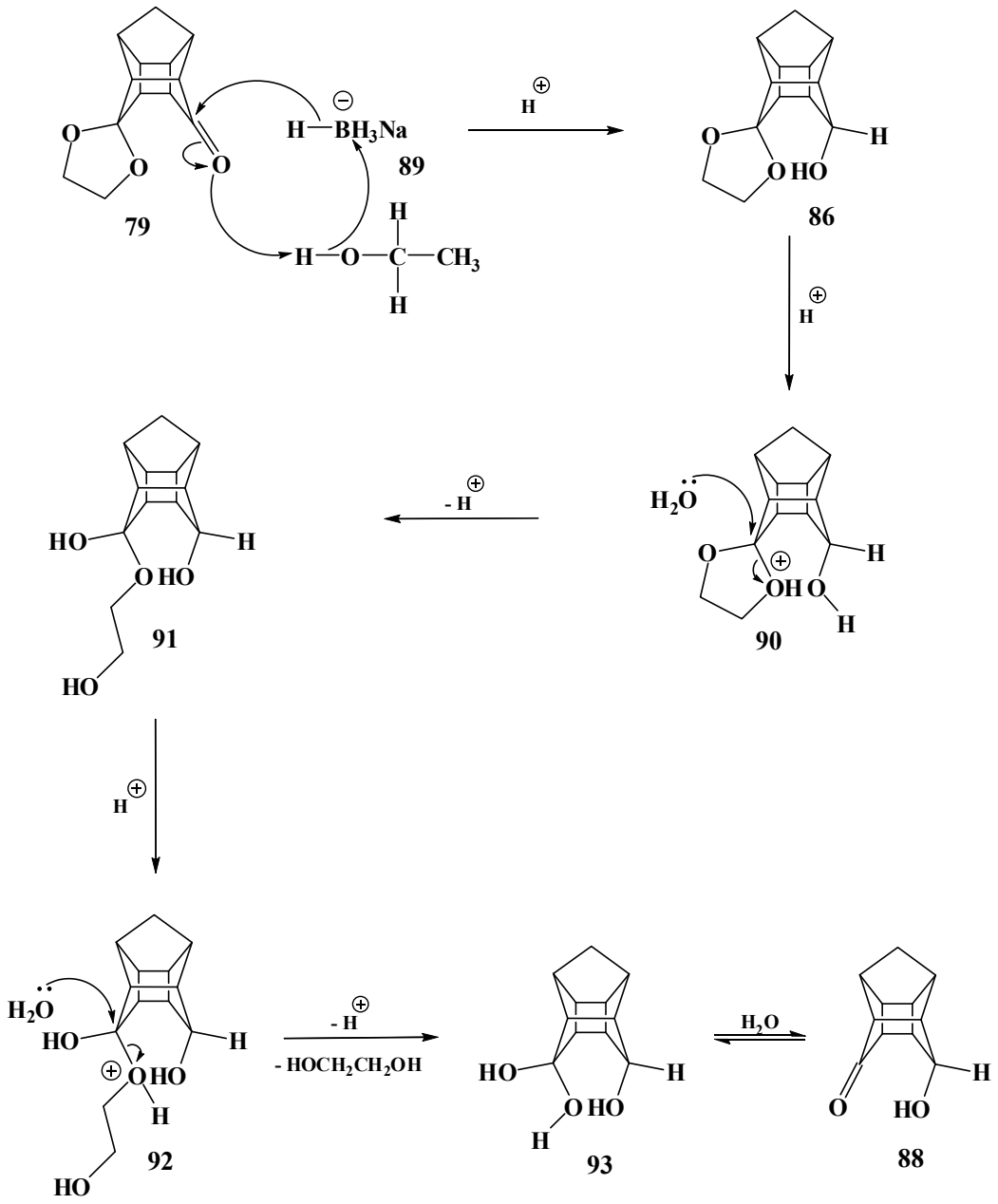
Sodium borohydride was employed to reduce the carbonyl group on the ketal **79** to the alcohol **87**.[34,35] The reduction product, 11-hydroxy-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8-one-ethylene ketal (hydroxy-ketal) **87**, was deprotected using 10% HCl ($^{v}/_{v}$) to obtain 11-hydroxy-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8-one (hydroxy ketone) **88**.[35]



79 **87** **88**

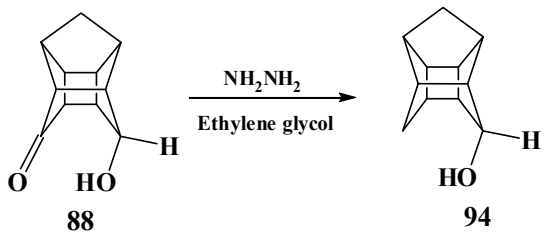
Scheme 17: Synthesis of hydroxy-ketal 87 and hydroxy-ketone 88[34,35]

Exo nucleophilic attack of the hydride ion on the carbonyl group gives hydroxyl ketal **87**. Deprotection of the ketal group proceeds *via* protonation of the oxygen atom followed by S_N2 nucleophilic attack of water. This step is repeated for the other oxygen atom on the ketal group resulting in compound **88** (Scheme 18).



Scheme 18: Proposed mechanism for synthesis of the hydroxy-ketone 88[37]

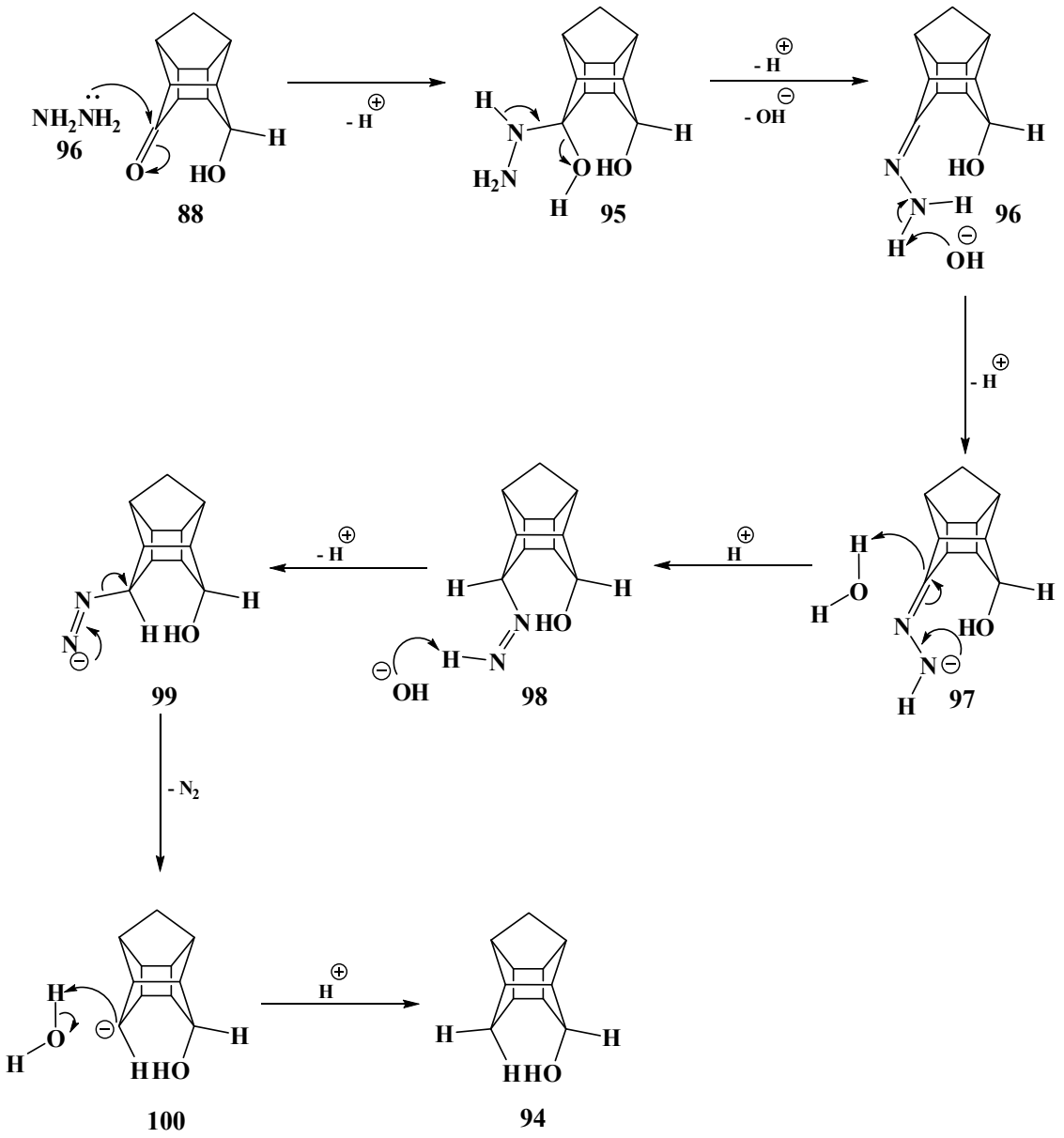
Reduction of the carbonyl group to methylene was carried out by refluxing the hydroxy-ketone **88** with hydrazine hydrate followed by addition of excess of KOH. The volatile pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8-ol (*endo*-PCU alcohol) product **94** was purified through steam distillation 83% yield.



88 94

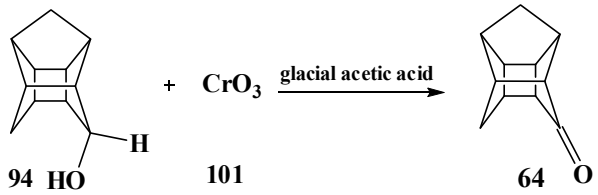
Scheme 19: Synthesis of the PCU alcohol 94[35]

The proposed mechanism for this reactions is presented in Scheme 20. Hydrazine attacks the carbonyl carbon forming the hydrazone **96** after elimination of the hydroxyl group.[21] The hydrazone undergoes further decomposition in presence of base forming the alkyldiimide **99**. The latter loses nitrogen, followed by protonation to obtain the methylene group in **94**.[21]



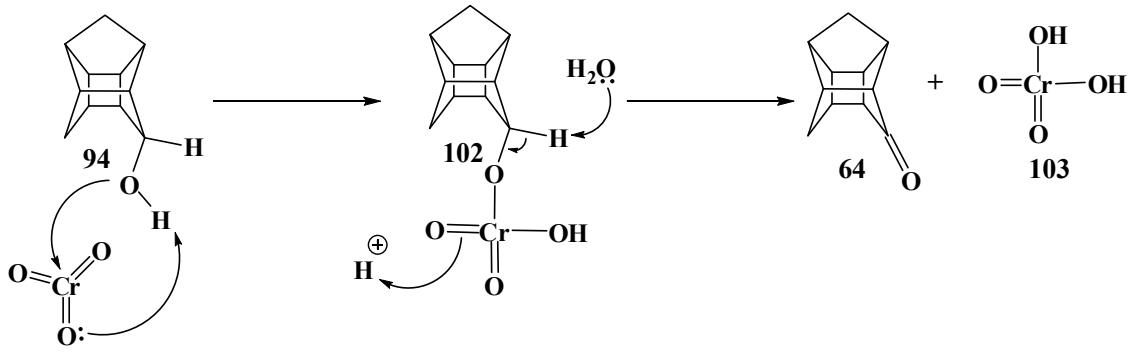
Scheme 20: Proposed mechanism for PCU alcohol 94 synthesis[37]

Oxidation of the PCU alcohol **94** was achieved utilizing Jone's reagent, chromium tri-oxide **101**, in glacial acetic acid to yield the PCU monoketone **64** in approximately 77 %.



Scheme 21: Synthesis of PCU mono ketone 64[35]

The mechanism of the reaction is presented in Scheme 22.



Scheme 22: Proposed mechanism for PCU mono ketone 64 synthesis[37]

References

1. Merritt, H. H.; Putnam, T. J. *Arch. Neurol. Psychiatry* **1938**, *39*, 1003-1015.
2. Tunnicliff, G. *General Pharmacology-the Vascular System* **1996**, *27*, 1091-1097.
3. Sheppeck, J. E.; Gilmore, J. L.; Tebben, A.; Xue, C. B.; Liu, R. Q.; Decieco, C. P.; Duan, J. J. W. *Bioorganic & Medicinal Chemistry Letters* **2007**, *17*, 2769-2774.
4. Tan, K. T.; Obendorf, S. K. *Journal of Membrane Science* **2007**, *289*, 199-209.
5. Barnes, K.; Liang, J.; Wu, R.; Worley, S. D.; Lee, J.; Broughton, R. M.; Huang, T. S. *Biomaterials* **2006**, *27*, 4825-4830.
6. Sergent, D.; Wang, Q.; Sasaki, N. A.; Ouazzani, J. *Bioorganic & Medicinal Chemistry Letters* **2008**, *18*, 4332-4335.
7. Tanino, T.; Ogiso, T.; Iwaki, M.; Tanabe, G.; Muraoka, O. *International Journal of Pharmaceutics* **1998**, *163*, 91-102.
8. Lenkowski, P. W.; Batts, T. W.; Smith, M. D.; Ko, S. H.; Jones, P. J.; Taylor, C. H.; McCusker, A. K.; Davis, G. C.; Hartmann, H. A.; White, H. S.; Brown, M. L.; Patel, M. K. *Neuropharmacology* **2007**, *52*, 1044-1054.
9. Smith, A. *Current Therapeutic Research* **2005**, *66*, 255-265.
10. Epilepsy action, http://www.epilepsy.org.uk/, webpage accessed on 06/09/08.
11. University of Washington, http://faculty.washington.edu/chudler/epi.html, webpage accessed on 01/09/08.
12. Stefan, H.; Feuerstein, T. J. *Pharmacology & Therapeutics* **2007,** *113*, 165-183.
13. White, H. S.; Smith, M. D.; Wilcox, K. S. *Neurobiology of Epilepsy and Aging* **2007,** *81*, 85-110.
14. Czapinski, P.; Blaszczyk, B.; Czuczwar, J. S. *Current Topics in Medicinal Chemistry* **2005**, *5*, 3-14.
15. Kruger, H. G.; Mdluli, S. P. *Journal of Structural Chemistry* **2006**.
16. Nakamura, K.; O'Hashi, K.; Nakatsuji, K.; Hiroka, T.; Fujimoto, K.; Ose, S. *Arch. Int. Pharmacodyn* **1965**, *156 (2)*, 261–270.
17. Dylag, T.; Zygmunt, M.; Maciag, D.; Handzlik, J.; Bednarski, M.; Filipek, B.; Kiec-Kononowicz, K. *European Journal of Medicinal Chemistry* **2004**, *39*, 1013-1027.
18. Mehta, N. B.; Diuguid, C. A. R.; Soroko, F. E. *Journal of Medicinal Chemistry* **1981**, *24*, 465-468.

19. CERYBYX, http://www.pfizer.com/files/products/cerebyxpi.pdf, webpage accessed on 25/12/2007.
20. Zhu, O.; Pan, Y.; Xu, Z.; Li, R.; Qiu, G.; Xu, W.; Ke, X.; Wu, L.; Hu, X. *European Journal of Medicinal Chemistry* **2008**, 1-7.
21. Carey, A. F.; Sundberg, J. R.; *Advanced Organic Chemistry*; Springer, 2001.
22. Gad, C. S. *Drug Discovery Handbook*; Wiley-IEEE, 2005.
23. Kirsch; Volke. office, U. P. T. Ed.: United States, 2005.
24. Stella, J. V. *Advanced Drug Delivery Reviews* **1996**, *19*, 31 I-330.
25. Nagasawa, H. T.; Elberlin, J.; Shirota, F. N. *Journal of Medicinal Chemistry* **1973**, *16*, 823-826.
26. Edward, J. T.; Jitrangsri, C. *Canadian Journal of Chemistry-Revue Canadienne De Chimie* **1975**, *53*, 3339-3350.
27. Bucherer, H. T.; Steiner, W. J. *J. Prakt. Chem* **1934**, 291-314.
28. Martins, F. J. C.; Viljoen, A. M.; Kruger, H. G.; Fourie, L.; Roscher, L.; Joubert, A. J.; Wessels, P. L. *Tetrahedron* **2001**, *57*, 1601-1607.
29. Naemura, K.; Fujii, T.; Chikamatsu, H. *Chemistry Letters* **1986**, 923-926.
30. Bello, M. A.; Poduch, E.; Liu, Y.; Wei, L.; Crandall, I.; Wang, X.; Dyanand, C.; Kain, C. K.; Pai, F. E.; Kotra, L. P. *Journal of Medicinal Chemistry* **2008**, *51*, 439–448.
31. Marchand, P. A.; Allen, W. R. *Journal of Organic Chemistry* **1974**, *39*, 1596.
32. Clayden; Greeves; Warren; Wothers *Organic chemistry*; Oxford University Press: New York, 2001.
33. Marchand, P. A.; Power, D. T.; Kruger, H. G. *Croatica Chemica Acta* **2001**, *74*, 265-270.
34. Eaton, E. P.; Cassar, L.; Hudson, A. R.; Hwang, R. D. *Journal of Organic Chemistry* **1976**, *41*, 1445-1449.
35. Dekker, T. G.; Oliver, D. W. *South African Journal of Chemistry* **1979**, 45-48
36. Marchand, P. A.; LaRoe, D. W.; Sharma, M. G. V.; Suri, C. H.; Reddy, S. D. *Journal of Organic Chemistry* **1986**, *51*, 1622-1625.
37. Raasch, T. *MSc thesis*, University of Natal, 2003.

CHAPTER 3

SYNTHESIS AND INCORPERATION OF THE PCU AMINO ACID INTO SMALL PEPTIDES

Introduction

In biological systems specific protein-protein interactions are responsible for regulating many biological processes.[1] Some of these processes are naturally initiating in the biological system *e.g.* immune responses, while other processes are inhibiting e.g. apoptosis.[1,2] Peptidomimetics are small/shorter modified peptides synthesised in the laboratory to mimic natural proteins/peptides in terms of the 3D space required to interact with the biological host system.[2] When the peptidomimetics bind to the host, it prevents the natural protein-protein interaction and subsequently inhibits the process that initiates from the normal protein-protein interaction.[3]

However, peptides with low molecular (less than 500) weight have a high degradation rate in the biological system (short half life time) coupled with rapid excretion by the kidney and the liver.[4,5]

The design of peptidomimetics depend on the structure–activity relationship (SAR) of the guest (peptidomimetics) with the host (*e.g.* enzyme, DNA) in order to identify the active side and its three dimensional arrangement.[6] Peptidomimetics are basically classified into three types as[6]

- Type I: The design of the peptide mimic (sometimes atom for atom) the parent peptide backbone.
- Type II: Also known as functional mimetic: These are non-peptide molecules which bind to the active site of the peptide.
- Type III: The design of the mimic peptide is not related to the parent peptide/protein, but still contains the required scaffold and groups to bind with the active site of the host system.

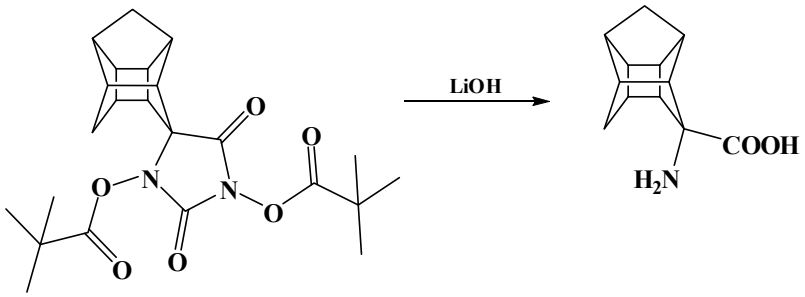
In many cases α-helices and β-turns are responsible for protein host systems (enzymes, hormones) to identify their partner/guest molecule. Note that DNA sequences can also

operate as a host system which can interact with a guest molecule to induce a certain biological reaction.[7,8] This resulted in an increased of research into the design of peptidomimetic molecules. Incorporation of unnatural amino acids into the peptidomimetic sequences became a widely used method for stabilised conformations especially for adopting the different possible β-turns.[7,9]

Part of the research interests in the GGKM group[§] is to study the influence of the incorporation of polycyclic cage amino acids into short peptides.[10-12] These studies revealed that cage moieties serve as very active β-turn inducers. As indicated in Chapter 1, the synthesis of PCU amino acid **41** containing short peptides had not yet been achieved. The challenge therefore remained to successfully synthesise such a peptide and to determine (if possible) its X-ray structure. The next section describes the synthesis of PCU amino acid peptides.

Synthesis of Fmoc PCU amino acid

The established method for the synthesis of polycyclic amino acids proceeds *via* hydrolysis of the corresponding hydantoin derivatives.[13-16] In our laboratory alternative methods were explored that involve the hydrolysis of *bis*-*t*-boc protected PCU hydantoin **104** with aqueous LiOH at room temperature (Scheme 23).

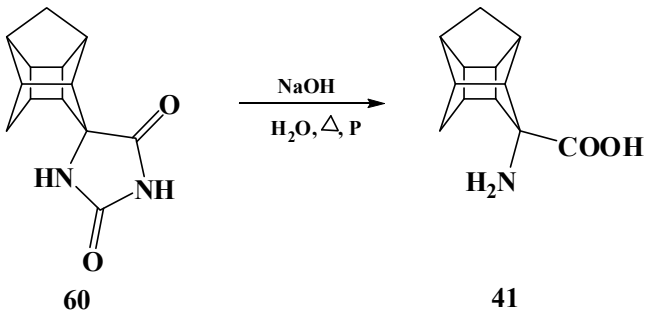


104 **41**

Scheme 23: Hydrolysis of bis-*t*-boc protected PCU hydantoin

The method which was applied in this dissertation involves direct hydrolysis of the PCU hydantoin in a medium pressure reaction vessel using sodium hydroxide (Scheme 24).

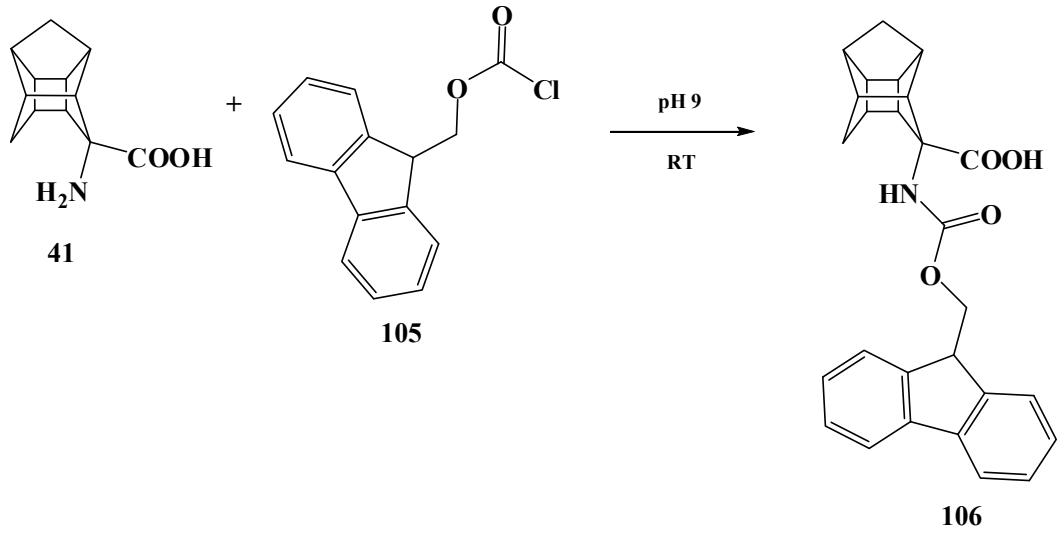
[§] www.ukzn.ac.za/ggkm/ggkm.htm, webpage accessed on 2008



60 41

Scheme 24: Hydrolysis PCU hydantoin

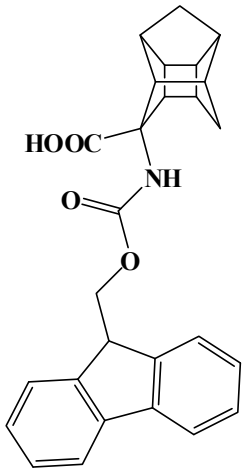
Due to the solubility problem of PCU amino acid **41** in water and organic solvents, characterization (NMR analysis) was performed on the Fmoc derivative **106**. An aqueous solution of **41** was adjusted to pH 9 and Fmoc-Cl **105** in dioxane was added with stirring for 24 hrs at room temperature, providing **106** in 73 % overall yield. While the alternative procedure affords **106** in 75% overall yield.[17]



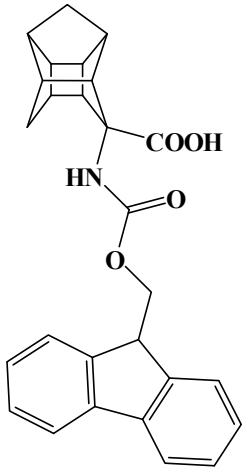
41 105 106

Scheme 25: Synthesis of Fmoc PCU amino acid 106

As discussed in Chapter 2, two enantiomers of the PCU hydantoin were obtained, leading to the two enantiomeric Fmoc PCU amino acids presented below.



106a



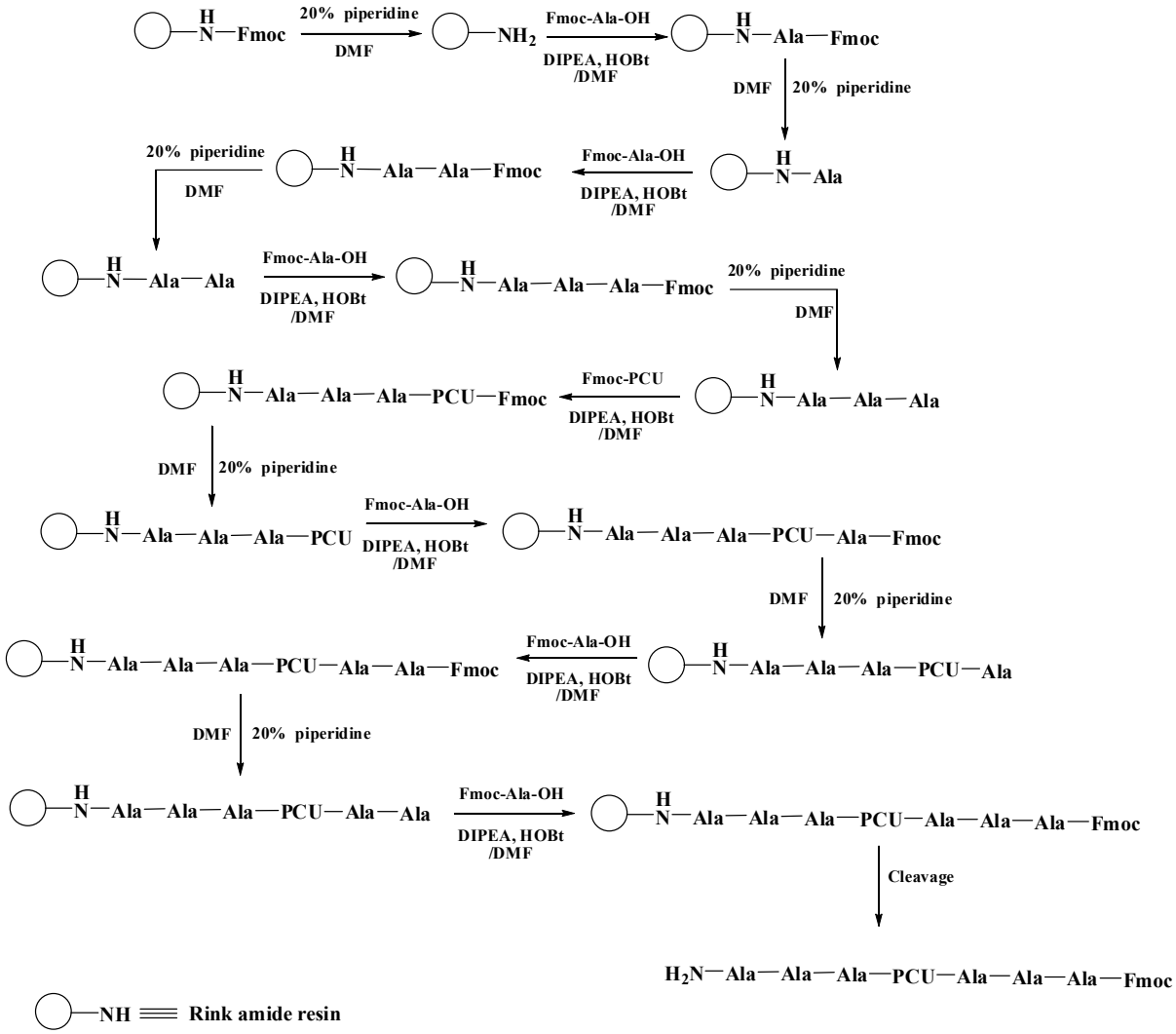
106b

These Fmoc protected amino acids were used as a racemate in the peptide synthesis described next.

Synthesis of $(Ala)_3$-PCU-41-$(Ala)_3$-Fmoc

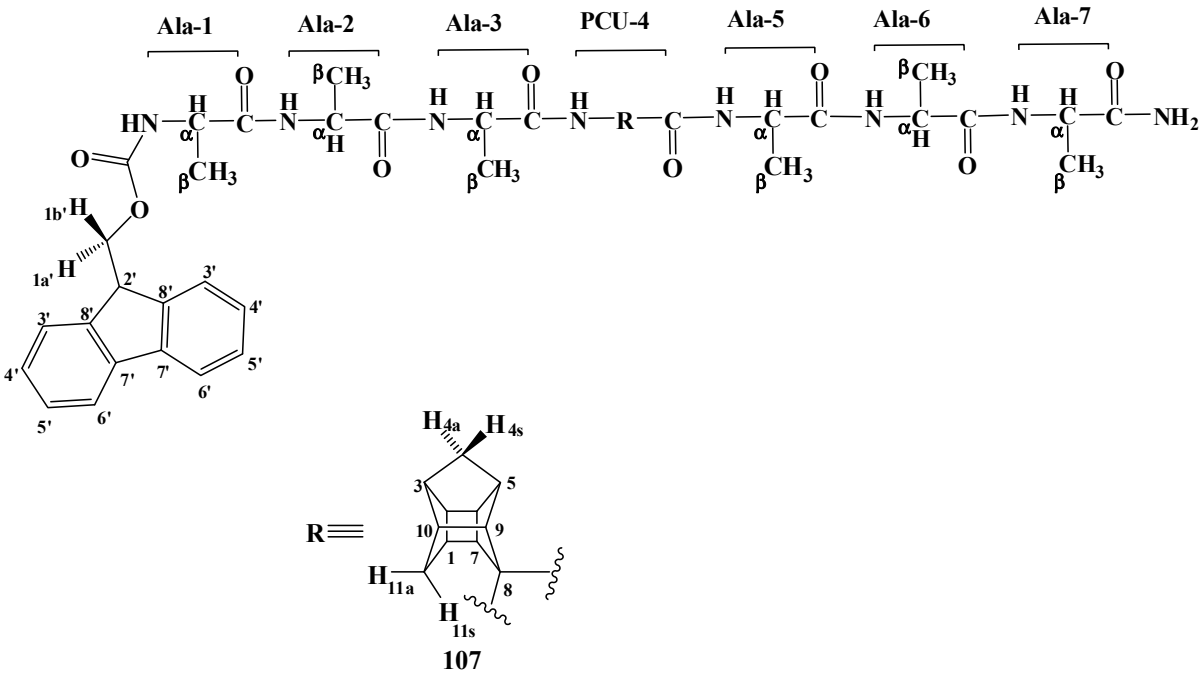
SPPS techniques were used for the synthesis of the target peptide on Rink amide resin. Monitoring the coupling of the first alanine into the Resin-$(Ala)_3$-PCU-NH_2 sequence was problematic as a positive result with the ninhydrin test could not be observed, even upon extended reaction times. This was attributed to the steric hindrance exerted by H-11s on the amino group at C-8 (see Figure 18).[18]

The target peptides were successfully synthesised using the microwave automated peptide synthesiser. The representation of the sequence of reactions that was followed is shown in Scheme 26. The complete experimental details are provided in Chapter 5. As with the case with the trishomocubane amino acid **42**,[18] a diastereomeric mixture of peptides was obtained. Two diastereomeric peptides were obtained since (*S*)-Ala was used with (*R*)- and (*S*)-**106a** and **106b**.



Scheme 26: Synthesis of $(Ala)_3$-PCU-$(Ala)_3$-Fmoc using automated microwave synthesis

Unlike the trishomocubane peptide sequence **45**, it was decided to leave the Fmoc protecting group on the peptide in order to assist in separating the diastereomers after unsuccessful attempts to separate them when unprotected. Two dimension NMR spectra were obtained on the separated diastereomeric sample at retention time 11.45 minutes (see experimental details in Chapter 5). The other diastereomer at 12.28 minutes was obtained in too low a yield for 2D NMR analysis.



107

Figure 18: $(Ala)_3$-PCU-$(Ala)_3$-Fmoc peptide

NMR elucidation of the peptide sequence $(Ala)_3$-PCU-$(Ala)_3$-Fmoc 107.

NMR and the electro spray ionization (ESI) mass spectroscopy were used to confirm the successful synthesis of the desired product. Since the peptide was dissolved in deuterated methanol (CD_3OD), it was expected that deuterium exchange would take place with the amide hydrogens. ESI mass spectra showed a molecular ion peak at 880 [M + 23(Na) + 4 ($4D^+$)]. In order to confirm the structure of the peptide product from the NMR spectra, firstly the presence and structure of the PCU amino acid **41** (segment PCU-4, Figure 18) was established, thereafter that the rest of the peptide structure was confirmed. Possible NOESY interactions between the different amino acid segments was searched for in an effort to establish information about the 3D structure of the peptide.

When the NMR of the cage amino acid is considered, one should realize that all the methine carbons are chiral. Nevertheless, the skeleton exhibits a reasonable symmetric character, especially for groups further removed from α-carbon (C-8). Previous NMR studies on smaller cage structures managed to assign each of the proton and carbon signals individually.[19,20] A very handy approach to elucidate the NMR spectra of the cage amino acid **41** is the fact that the coupling constants for the two CH_2-groups are known.[16] It is important to note that the protons on each methylene group are in a different environments

due to the asymmetric nature of the cage moiety, also since there is no free rotation possible in the rigid cage skeleton.

The ^{1}H NMR spectrum of the peptide **107** shows a pair of doublet peaks for H-4a and H-4s at 1.23 and 1.71 ppm. These protons exhibit a COSY and NOESY correlation to each other with a coupling constant of about 10.35 Hz. The other pair of doublets were assigned to H-11a (0.96 ppm) and H-11s (2.06 ppm) and also shows similar COSY and NOESY correlation to each other with a coupling constant of about 13.0 Hz. These coupling constants are similar to that of the PCU hydantoin **60**.[16]

The signal at 2.2 ppm shows COSY and NOESY correlation to H-4a and H4s and could be assigned to H-3 or H-5. H-11a exhibits a COSY and NOESY correlation with a signal at 2.46 ppm, whilst the latter shows a COSY and NOESY correlation to the signal at 2.2 ppm. This cross correlations enables one to assign the signal at 2.26 and 2.46 ppm to H-3 and H-10 respectively. The resonance at 2.58 ppm shows a COSY correlation with H-4a and a NOESY correlation with both H-4a and H-4s; through elimination this signal was assigned to H-5. Both H-10 (2.4 ppm) and H-5 (2.5 ppm) exhibit COSY and NOESY correlations with a peak at 2.8 ppm, which was assigned to H-9. The signal at 2.73 ppm shows a COSY correlation to H-11a and a NOESY correlation to both H-11a and H-11s; through elimination this resonance was assigned to H-1. A COSY correlation was observed between a signal registering at 2.73 ppm and with the two methine protons H-1 and H-3. It also exhibits a NOESY correlation with the resonance at 2.73 ppm and H-3 only. The only remaining proton that satisfies these correlations is H-2 (2.73 ppm). The resonance at 3.28 ppm was assigned to H-7 because of an observed NOESY and COSY correlation with H-1. The COSY and NOESY correlations of H-7 and H-2 with a signal overlapping with H-5 at 2.58 ppm was assigned to H-6 (also at 2.58 ppm). The HSQC spectrum was used to identify the carbon signals of all assigned protons, however the identification of C-5 and C-6 seems complicated as their protons overlap with each other while C-1 overlaps with an impurity in the sample.

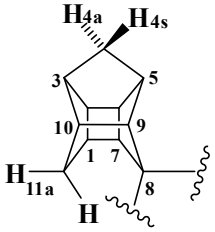


Table 1: ^{1}H and ^{13}C NMR data[a] for PCU-4 skeleton

PCU-4			
Atom	$\delta^{1}H^{b}$	*J*(Hz)	$\delta^{13}C^{b}$
1	2.73	-	33.6/36.2
2	2.62	-	41.25
3	2.26	-	46.5
4a	1.23	10.35	33.6
4s	1.71	10.35	33.6
5	2.57	-	40.5/44.1
6	2.57	-	40.5/44.1
7	3.28	-	40.5
8	-	-	65.4
9	2.85	-	46.5
10	2.46	-	42.4
11a	0.96	13.0	28.4
11s	2.06	13.0	28.4

[a] 600MHz for ^{1}H and 150MHz for ^{13}C.
[b] Solvent CD_3OD.

The next section describes as far as possible the observed NMR signals for the rest of the peptide sequence.

Using the APT ^{13}C NMR spectrum, the six alanine methyl carbons registering at 15.53-16.42 ppm were identified. These carbon signals show HSQC correlation to multiplets registering in the region 1.25-1.56 ppm, which were assigned to all eighteen protons belonging to Ala-H^{β} (CH_3) in the sequence. This region (1.25-1.56 ppm) shows COSY and NOESY correlations to three multiplet signals (4.02 (2H), 4.15 (3H) and 4.24 (2H) ppm). Those peaks were assigned to the all six Ala-H^{α} protons in the sequence. As mentioned earlier, all eighteen

Ala-H^{β} (CH_3) appears in regions 1.25-1.56 ppm and due to these intense overlapping each Ala-H^{β} cannot be individually assigned. However, from the HSQC spectrum it is clear that the carbon signals of all eighteen Ala-H^{β} (CH_3) appears at 15.5, 15.8, 16.1, 16.44 ppm, where the first peak seems to be overlapping with the other two Ala-H^{β} (CH_3) groups. The Ala-H^{β} protons show HMBC correlation with the carbonyl carbons attached to Ala-C^{α} registered at 173.8, 174.3, 175.1, 175.6 and 175.7 ppm. Also, observed in the HMBC spectrum is the correlation of the Ala-H^{β} with the Ala-C^{α} regions, further confirming the assignment of the methyl groups.

The next section will focus on the elucidation of the Fmoc part of the peptide sequence. Two multiplet resonances at 4.41 and 4.52 ppm show COSY and NOESY correlation with each other and both correlate to a carbon signal registering at 66.9 ppm as illustrated in the HSQC spectrum. These resonances were assigned to H-1a' and H-1b' respectively since both H-1a' and H-1b' shows a COSY correlation to a peak at 4.25 ppm (overlapping with one Ala-H^{α}) which was assigned to H-2'. The HMBC spectrum shows a correlation of H-1a' and H-1b' with a quaternary carbon at 143.9 ppm, this was assigned to C-8'. Also evident on the HMBC spectrum, are the correlations of C-8' with H-2' and two unknown signals at 7.35 and 7.85 ppm which are probably H-4' and H-6'. However, only the peak at 7.35 ppm shows NOESY and COSY correlation to two signals at 7.43 and 7.69 ppm; based on the Fmoc structure the signal at 7.35 ppm was assigned to H-4' while that at 7.85 ppm was assigned to H-6'. H-6' (7.85 ppm) exhibits a COSY correlation with 7.43 ppm which was assigned to H-5'; it follows by way of elimination that H-3' registers at 7.69 ppm. The resonances of H-3' and H-5' show HMBC correlations to a quaternary carbon at 141.3 ppm, this was assigned to C-7'. The above assignments were further verified using the HSQC spectrum with all proton and carbon signals reported in Table 2.

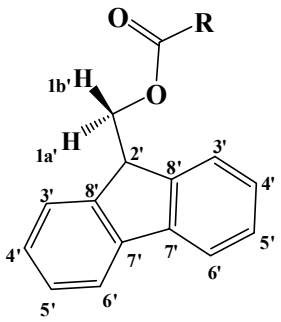


Table 2: 1H and 13C NMR data[a] of Fmoc group on the peptide sequence

Fmoc			
Atom	$\delta^{1}H^{b}$	*J*(Hz)	$\delta^{13}C^{b}$
1’	4.41	m	66.9
1’	4.52	m	66.9
2’	4.29	-	49.3/46.5
3’	7.69	-	124.71
4’	7.35	-	126.82
5’	7.43	-	127.62
6’	7.85	-	119
7’	-	-	141.3
8’	-	-	143.9

[a] 600MHz for ^{1}H and 150MHz for ^{13}C.
[b] Solvent CD_3OD.

The attempts to confirm the existence of a β turn in peptide **107** was attempted by obtaining a low temperature (-50°C) NOESY spectrum. It was hoped that more pronounced NOESY interactions between NH and other protons could be observed at lower temperatures. However, the low temperature NOESY did not reveal much more. This was attributed to the deuterium exchange between the amide hydrogen and CD_3OD.

After the successful synthesis of the former peptide, it was decided to incorporate the PCU amino acid **41** into a short peptide template of the AVPI peptide. AVPI exhibits anti-cancer activity. A brief introduction on the AVPI peptide template, its activity and the synthetic steps of the modified AVPI peptide are provided below.

Synthesis of Ala-Val-PCU-Ile 108

Apoptosis or programmed cell death is an important process for growing and homeostasis[**] in metazoan[††]. Although the normal cells are programmed for apoptosis when they are no longer in use, cancer cells have the ability to prevent the process using inhibitors binding to a number of apoptosis's enzyme of the general family caspases[‡‡].

X-linked inhibitor of apoptosis protein (XIAP) is one of those inhibitors. Its potency is believed to be the result of selective binding to the two effectors caspases-3 and-7 *via* the Baculoviral IAP Repeats2 (BIR2) domain[§§]. It also binds to the peptide linker at caspases-9 through the BIR3 domain.[21,22] XIAP became a research target for designing the peptidomimetic sequence aiming to bind with BIR3 and BIR2 to prevent its protein-protein interaction with the apoptosis enzyme. An important discovery was made when it was realised that the peptide sequence Ala-Val-Pro-Ile (AVPI, **Error! Reference source not found.**) shows the ability to bind with both the BIR3 and BIR2 domains with K_d values of 0.4-0.7 μM and 6-9 μM respectively. Unfortunately AVPI failed in cell permeability tests[22] and the obvious reason for that is the lack of lipophilic groups in the peptide.[22,23]

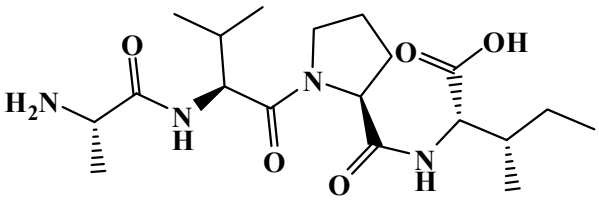


Figure 19: The structure of AVPI

Computational results in our laboratory[10,12,24] demonstrated that the PCU amino acid can promote similar β-turns to/like proline, which is used in AVPI. The high lipophilic character of this cage moiety was discussed in Chapter 1; it was therefore decided to synthesise the peptide sequence Ala-Val-PCU-Ile as a potential anti-cancer agent. Note that the actual

[**] Homeostasis is the ability of the body to maintain its physiological system at constant condition, despite the variation of the environment around it.

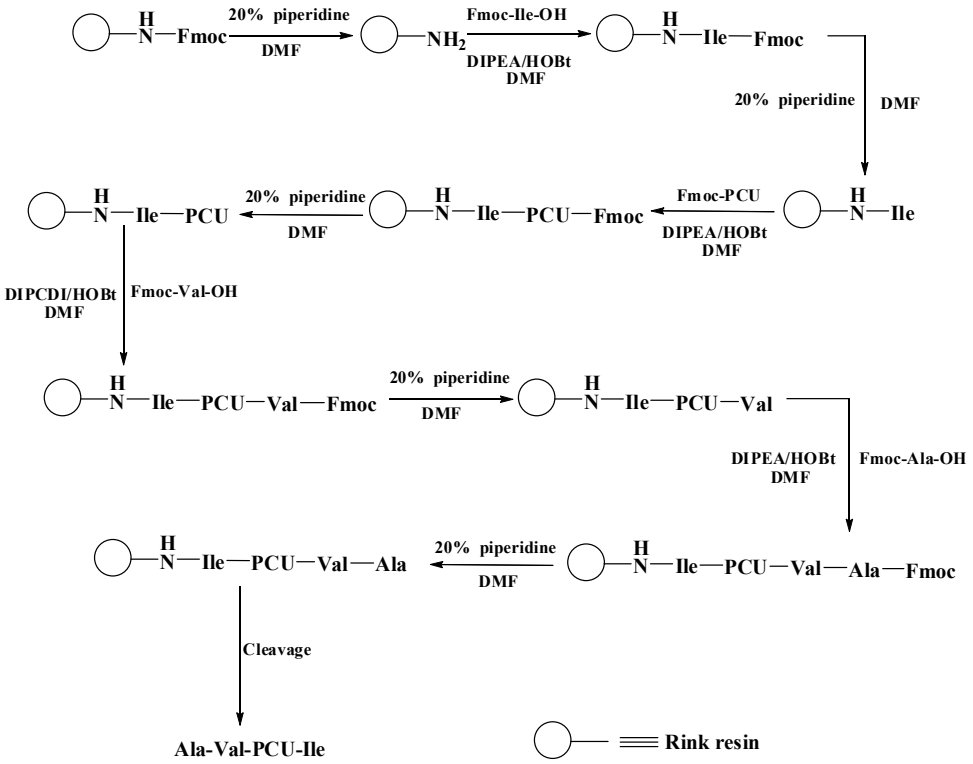
[††] Metazoan or kingdom of animalia are multicellular organisms that depend on the other organisms (directly or indirectly) for their nutrients.

[‡‡] Caspases part of the cystiene proteases family, play an important role in apoptosis.

[§§] BIR zinc finger domains form part of the IAP protein sequence and play an important role in programmed cell death. They consist of four α-helics, three anti-parallel β-sheets and coordinate with Zn ion through three Cys and one His as a ligand.

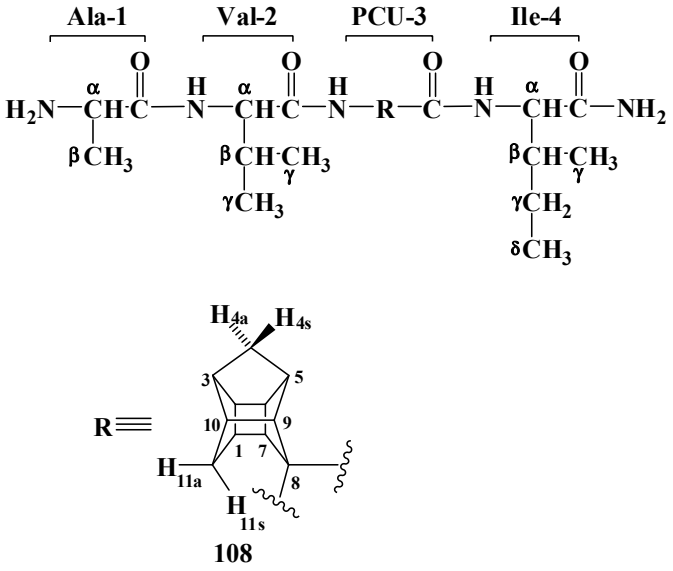
biochemical/microbiology anti-cancer tests of the product falls outside the scope of this dissertation.

Once again the same automated peptide synthesiser and the same approach were used for the synthesis of the A-V-PCU-I sequence.



Scheme 27: Synthesis of Ala-Val-PCU-Ile

The peptide was synthesised and purified using HPLC (See the details in the experimental section – Chapter 5) and the NMR analysis was done on a separated diastereomeric sample collected at 6.24 minutes retention time.



108

Figure 20 Ala-Val-PCU-Ile

The successful synthesis of the peptide was confirmed using (a) ESI mass spectrum shows molecular ion peak at 488.4 [M+1] and (b) the NMR spectra which is discussed below.

NMR elucidation of the peptide sequence Ala-Val-PCU-Ile 108

The same methodology as for the previous peptide **107** was followed. The ^{1}H NMR spectrum shows the geminal PCU bridge methylene protons resonances at 1.26 and 1.72 ppm (*d*, *J* = 10.5 *Hz*) which were assigned to H-4a and H-4s respectively. From literature,[16] it is known that this pair of doublets are normally observed in this region with a similar coupling constant. The COSY and NOESY spectra show correlations of H-4a with H-4s as expected. H-11a and H-11s (1.04 and 2.10 ppm, *d, J* = 12.8 *Hz*) were assigned as above due to the relative positions and the size of the coupling constant.

H-4a and H-4s show both COSY and NOESY correlations with two resonances at 2.15 and 2.30 ppm, which could be attributed to H-3 or H-5. The pair of doublets H-11a and H-11s also shows both COSY and NOESY correlations with the resonances at 2.47 and 2.75 ppm which can be assigned to either H-1 or H-10. The COSY and NOESY spectra show distinct correlation of the protons at 2.30 ppm with a signal at 2.75 ppm; thus confirming that the resonance registered at 2.30 and 2.75 ppm are H-3 and H-1 respectively. The signals at 2.15 and 2.47 ppm were assigned to H-5 and H-10. The methine protons (H-5 and H-10) show COSY and NOESY correlations to a proton peak at 2.84 ppm which was assigned to H-9. H-3 (2.30 ppm) shows both COSY and NOESY correlations with a signal at 2.62 ppm which was assigned to H-2. Also, observed on both COSY and NOESY spectra are the correlation

of H-5 (2.15 ppm) with a signal at 2.64 ppm which was assigned to H-6. A resonance at 3.33 ppm shows both COSY and NOESY correlations with H-1 (2.75 ppm) and H-6 (2.64 ppm), thus this resonance was assigned to H-7. It is evident from the NOESY spectrum that H-4a shows correlations with H-2 and H-6 while H-4s shows correlations with H-9 and H-10. H-11s and H-11a show NOESY correlations with H-1 (2.75 ppm) and H-10 (2.47 ppm) while H-11a shows an extra NOESY correlation with H-3 (2.30 ppm). The HSQC spectrum was used to further confirm the assignments of the proton and carbon signals of the "cage" moiety. These assignments are presented in Table 3 below.

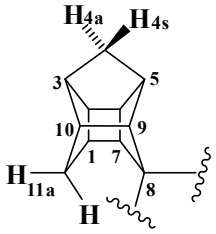


Table 3: ^{1}H and ^{13}C NMR data[a] of PCU-3 side chain

PCU-3			
Atom	$\delta^{1}H^{b}$	*J*(Hz)	$\delta^{13}C^{b}$
1	2.75		35.5
2	2.62		41.4
3	2.30		46.7
4a	1.26	10.5	33.7
4s	1.72	10.5	33.7
5	2.15		43.0
6	2.64		41.4
7	3.33		38.5
8	-		65.8
9	2.84		47.5
10	2.47		42.5
11a	1.04	12.8	28.1
11s	2.10	12.8	28.1

[a] 600MHz for ^{1}H and 150MHz for ^{13}C.
[b] Solvent CD_3OD.

Using the APT ^{13}C NMR, a methylene signal registering at 24.2 ppm was observed. This carbon shows a HSQC interaction with two proton resonances at 1.36 and 1.19 ppm were assigned to Ile-4 H^{γ} (CH_2); the peptide has only the one methylene group apart from the two PCY methylene groups. The proton-split experienced by Ile-4 H^{γ} (CH_2) is the result of the interaction with the chiral carbon C^{β} to which the Ile-4 C^{γ} (CH_2) is attached. This effect is due to the diastereotopic nature of the two H^{γ} protons. Ile-4 H^{γ} (CH_2) shows a COSY correlation to the methyl protons at 0.92 ppm and a signal at 1.84 ppm which were assigned to Ile-4 $3H^{\delta}$ and Ile-4 H^{β} respectively. Ile-4 H^{β} shows a COSY correlation to a methyl signal registering at 0.95 ppm, which was assigned to Ile-4 H^{γ} (CH_3). The signal at 4.19 ppm shows COSY correlation to Ile-4 H^{β} and was assigned to Ile-4 H^{α}. This assignment was further confirmed through HMBC correlation between Ile-4 C^{α} (59.1ppm) and Ile-4 H^{γ} (CH_3) at 0.95ppm.

Using the APT ^{13}C NMR and HSQC spectra two methyl proton signals at 0.96 and 0.98 ppm show was observed exhibiting a HMBC correlation to the carbon atoms of each other. The only groups that can exhibit such a HMBC correlations are the 2 x Val-2 CH_3^{γ} groups (the $3H^{\gamma}$ at 0.98 ppm correlates with C^{γ} at16.89 ppm while the other $3H^{\gamma}$ at 0.96ppm correlates with the C^{γ} at 18.7ppm). These two methyl groups show a COSY correlation to a signal at 2.13 ppm which was attributed to Val-2 H^{β}. The former protons show a COSY correlation to a peak at 4.13 ppm overlapping with Ile-4 H^{α}; these protons were assigned to Val-2 H^{α}. The HSQC spectrum was used to assign the remaining proton and carbon signals Val-2 and Ile-4.

A methyl signal registering at 16.7 ppm was observed in the APT ^{13}C NMR spectrum. This methyl groups shows a HSQC correlation to a doublet peak at 1.47 ppm which was assigned to Ala-1 H^{β}. Ala-1 H^{β} shows a COSY correlation with a resonance registering at 3.94 ppm which was assigned to Ala-1 H^{α}. HSQC was used to assign the remaining proton and carbon signals for Ala-1.

Table 4: ^{1}H and ^{13}C NMR data[a] of Ala-1, Val-2 and Ile-4

Amino acid	$\delta^{1}H^{\alpha b}$	$\delta^{13}C^{\alpha b}$	$\delta^{1}H^{\beta b}$	$\delta^{13}C^{\beta b}$	$\delta^{1}H^{\gamma b}$	$\delta^{13}C^{\gamma b}$	$\delta^{1}H^{\delta b}$	$\delta^{13}C^{\delta b}$
Ala-1	3.94	49.2	1.47	16.7	-	-	-	-
Val-2	4.13	57.2	2.13	30.14	0.96 0.98	16.89 18.7	-	-
Ile-4	4.19	59.1	1.84	37.1	$CH_{2(}$1.36, 1.19) CH_3(0.95)	CH_2(24.2) CH_3(14.66)	0.92	10.25

[a] 600MHz for ^{1}H and 150MHz for ^{13}C
[b] Solvent CD_3OD

References

1. Peczuh, W. M.; Hamilton, D. A. *Chem. Rev.* **2000**, *100*, 2479-2494.
2. Vagner, J.; Qu, H. C.; Hruby, V. J. *Current Opinion in Chemical Biology* **2008**, *12*, 292-296.
3. Veerapandian, P. *Structure-based Drug Design*; Informa Health Care, 1997.
4. Hanessian, S.; Auzzas, L. *Account of Chemical Research* **2008**.
5. Cochran, A. G. *Chemistry & Biology* **2000**, *7*, 85-94.
6. Ripka, A. S.; Rich, D. H. *Current Opinion in Chemical Biology* **1998**, *2*, 441-452.
7. Cochran, A. G. *Current Opinion in Chemical Biology* **2001**, *5*, 654-659.
8. Garner, J.; Harding, M. M. *Organic and Biomolecular Chemistry* **2007**, *5*, 3577–3585.
9. Kotha, S. *Account of Chemical Research* **2003**, *36*, 342-351.
10. Bisetty, K.; Kruger, G. H.; Perez. *Molecular stimulation* **2007**, *33*, 1105-1108.
11. Bisetty, K.; Govender, P.; Kruger, G. H. *Biopolymers* **2006**, *81*, 339-349.
12. Bisetty, K.; Corcho, J. F.; Canto, J.; Kruger, G. H.; Perez, J. J. *Journal of Molecular Structure* **2006**, *759*, 155-157.
13. Gaudry, R. *Canadian Journal of Research* **1948**, *26*, 773.
14. Govender, T.; Hariprakasha, K. H.; Kruger, G. H.; Raasch, T. *South African Journal of. Chemistry* **2005,** *58*, 37-40.
15. Nagasawa, H. T.; Elberlin.Ja; Shirota, F. N. *Journal of Medicinal Chemistry* **1973**, *16*, 823-826.
16. Martins, F. J. C.; Viljoen, A. M.; Kruger, H. G.; Fourie, L.; Roscher, L.; Joubert, A. J.; Wessels, P. L. *Tetrahedron* **2001**, *57*, 1601-1607.
17. Mdluli, P. S., University of KwaZulu-Natal, 2005.
18. Albericio, F.; Arvidson, I. P.; Bisetty, K.; Girlalt, E.; Govender, T.; Jali, S.; Kongsaeree, P.; Kruger, G. H.; Prabpai, S. *Chemical Biology Drug Design* **2008**, *71*, 125-130.
19. Boyle, A. G.; Govender, T.; Kruger, G. H.; Maguire, M. G.; Nicker, T. *Structural. Chemistry* **2008**, *19*.
20. Gonder, T.; Kruger, G. H.; Makatini, M.; Onajole, K. O. *Structural Chemistry* **2008**, *19*, 719-726.

21. Park, C.; Sun, C.; Olejniczak, E. T.; Wilson, E. A.; Meadows, P. R.; Betz, F. S.; Elmore, W. S.; Fesik, W. S. *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, 771-775.

22. Sun, H.; Nikolovska-Coleska, Z.; Lu, J.; Meagher, J. L.; Yang, C.; Qiu, S.; Tomita, Y.; Ueda, Y.; Jiang, S.; Krajewski, K.; Roller, P. P.; Stuckey, A. P.; Wang, S. *Journal of American Chemical Society* **2007**, *129*, 15279-15294.

23. Sun, H.; Nikolovska-Coleska, Z.; Lu, J.; Chen, J.; Yang, C.; Tomita, Y.; Pan, H.; Yoshioka, Y.; Krajewski, K.; Roller, P. P.; Wang, S. *Bioorganic & Medicinal Chemistry Letters* **2005**, *15*, 793–797.

24. Bisetty, K.; Corcho, J. F.; Canto, J.; Kruger, G. H.; Perez, J. J. *Journal of Peptide Science* **2006**, *12*, 92-105.

CHAPTER 4

CONCLUSION

Attempts to synthesis the polycyclic cage hydantoin derivatives **51** and **52** as anticonvulsant agents were unsuccessful. The poor stability of the novel PCU hydantoin derivatives **56** and **58** and the adamantane hydantoin derivatives **55** and **57** were the reason for these failed attempts.

PCU hydantoin was successfully hydrolysed to the PCU amino acid **41** and was successfully incorporated into the hepta-peptide **108** using a SPPS microwave peptide synthesiser. The peptide was obtained in relatively good yield (53%) and suitably pure for two dimensional NMR analysis. The NMR elucidation was only partially possible for the alanine segments due to massive overlap of proton and carbon signals. The PCU segment was successfully identified from the 2D NMR spectra. Attempt to determine the 3D structure of **108** were done through NOESY spectra at -50^0C. Unfortunately there were no guide correlations that could confirm the β-turn in **108**. Several attempts to obtain a suitable crystal for X-ray analysis also met with no success.

Incorporation of the PCU amino acid **41** into the semantic peptide backbone AVPI as a potential anticancer agent was successful. The target peptide **109** was purified and analysed with NMR and MS. This peptide is currently undergoing biological evaluation at the School of Biochemistry, University of KwaZulu-Natal for anti-cancer activity.

CHAPTER 5

EXPERIMENTAL

Melting points were recorded using a Gallenkams melting point instrument. All melting points are uncorrected. NMR data were recorded on a Bruker ANANCE III 400 MHz spectrophotometer while the two dimensional NMR spectra were recorded on a Bruker ANANCE III 600 MHz spectrophotometer. The peptides sequences were synthesised *via* CEM Liberty automated peptide synthesizer instrument and the mass spectra were carried out on a Bruker miro TOF-Q II instrument. Purification was done *via* semi-prep HPLC on a Young Lin Acme 9000 instrument. The column and solvent conditions are provided below where applicable. Infrared spectra were obtained on a Perkin Elmer spectrum 100 instrument with an attenuated total reflection attachment.

Synthesis of pentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]undecane-8-11-dione 73.[1]

Freshly cracked cyclopentadiene **69** (76 g 1.12 mol) was added to a stirred solution of *p*-benzoquinone **70** (110 g 1.02 mol) in toluene (2000 ml) at 0^0C and left overnight. The solution was transferred to an evaporating dish and placed in a dark fume hood, the crude adduct was thereafter recrystallised from petroleum ether (40-60 ^{0}C) resulting in greenish yellow crystals. A solution of adduct **72** in ethyl acetate (5000 ml) was placed directly under sun light until the yellow solution turned clear, which is an indication of the completion of the reaction. The solvent was evaporated under reduced pressure and the crude product was purified *via* silica gel column chromatography (ethyl acetate: hexane 40:60, R_f = 20) to afford the product **73** as a white powder (110 g, 88%, mp = 240^0C).

The NMR spectra were identical to an authentic sample.

Synthesis of pentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]undecane-8-11-dione-mono-ethylene ketal 79.[2,3]

A mixture of PCU dione **73** (10 g, 0.057 mol), ethylene glycol **78** (4.5 ml, 0.080 mol) and *p*-toluenesulfonic acid (0.33 g, 0.19 x 10^{-2} mol) was dissolved in toluene (45 ml) and refluxed using a Dean-Stark apparatus for three days. The reaction mixture was allowed to cool down followed by addition of a cold aqueous solution of 10% Na_2CO_3 (60 ml) and extracted with DCM (3x25 ml); the organic layer was dried using anhydrous Na_2SO_4. Filtration of Na_2SO_4

was carried out and the solvent was evaporated *in vacuo.* The crude product was purified via silica gel column chromatography (40:60 ethyl acetate : hexane, R_f = 0.55) and obtained as a white solid (10.3 g, 80%, mp = 71^0C).
The NMR spectra were identical to an authentic sample.

Synthesis of 11-hydroxy-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane-8-one-ethylene ketal 87.[2,3]

An ethanolic solution of sodium borohydride **89** (1.75 g in 50 ml) was added over five minutes to a stirred solution of PCU monoketal **79** (5 g 2.3 x 10^{-2}mol) in ethanol (50 ml). The reaction vessel was placed in an ice bath. The reaction was stirred for two hours in the ice bath and for another two hours at room temperature. Completion of reduction was monitored via TLC technique (ethyl acetate:hexane 40:60, R_f = 0.53). The mixture was diluted with deionised water (50 ml) and extracted with DCM (3 x 40 ml); the organic layer was evaporated *in vacuo* the product was obtained as a white solid (4.6 g, 90%, mp = 245^0C).
The NMR spectra were identical to an authentic sample.

Synthesis of 11-hydroxy-pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane-8-one 88.[3]

The hydroxy ketal **87** (22.5 g 0.10 mol) was kept in an ice bath; 10 (v/v) of HCl (100 ml) was added cautiously and the reaction mixture was allowed to return to room temperature and stirred for 18 hours. The aqueous solution was extracted with DCM (2 x 40 ml) and the organic layer was evaporated *in vacuo* leaving a brown powder. Purification was achieved through silica gel column chromatography (ethyl acetate:hexane 40:60, R_f = 0.37). (16.1 g, 89.8%, mp = 255^0C).
The NMR spectra were identical to an authentic sample

Synthesis of Pentacyclo [5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane-8-ol 94.[3,4]

A mixture of hydroxy ketone **88** (9 g 0.05 mol) and hydrazine hydrate **96** (21 ml 0.67 mol) in ethylene glycol (130 ml) was refluxed at 120^0C for six hours. The reaction mixture was allowed to cool to 80^0C. At this stage excess KOH (15 g) was added cautiously. The reaction mixture was distilled until the temperature reached 185^0C in order to remove the excess of hydrazine hydrate and water. The reaction mixture was refluxed for another three hours at 185^0C. The mixture was cooled, followed by dilution with deionised water (150 ml) and extracted with DCM (3x100 ml). The organic layer was removed *in vacuo* and the crude product was purified *via* steam distillation giving compound **94** as a white power with a distinct camphor odour (ethyl acetate:hexane 40:60, R_f = 0.74) (6.8 g 83%, mp = 228^0C).

The NMR spectra were identical to an authentic sample.

Synthesis of Pentacyclo [5.4.0.0^{2,6}.0^{3,10}.0^{5,9}] undecane-8-one 64.[3,4]

A solution of PCU alcohol **94** (5 g 0.03 mol) in ethyl acetate (35 ml) was added drop wise to a stirred mixture of chromium trioxide **101** (6.7 g 6.7x10^{-2} mol), water (11 ml) and acetic acid (110 ml) and the mixture was maintained at 90^{0}C overnight. The reaction mixture turned gradually from the dark brown to a green colour which was an evident of the reduction of Cr^{+6} to Cr^{+3} (Jone's oxidation). The reaction mixture was cooled and diluted with deionised water (350 ml) followed by extraction with DCM (3 x 100 ml). The organic layer was collected and washed with a saturated solution of $NaHCO_3$ (3 x 100 ml), water (150 ml) and dried using anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the product **64** was isolated as a white powder (ethyl acetate:hexane 40:60, R_f = 0.78) (3.8 g, 77%, mp = 165^{0}C). The NMR spectra were identical to an authentic sample

Synthesis of PCU hydantoin 60.[5,6]

A mixture of monoketone **64** (1g, 0.62x10^{-2} mol), sodium cyanide (1g 2.04x10^{-2} mol), ammonium carbonate (2g 2.08x10^{-2} mol), ethanol (10 ml) and ammonia solution (15 ml) was sealed in glass pressure vessel and placed in an oil bath at 60^{0}C for two hours, 100^{0}C for another two hours and at 120^{0}C overnight. The reaction mixture was allowed to cool and added to deionised water (100 ml) followed by extraction with ethyl acetate (150 ml). The organic layer was washed extensively with water and evaporated *in vacuo* to obtain the product as white crystals (ethyl acetate:hexane 50:50, R_f = 0.29) (0.9 g 63%, mp = 227^{0}C). The NMR spectra were identical to an authentic sample.

Synthesis of compound 58 (novel compound).

A mixture of PCU hydantoin **60** (0.5g, 0.21 x 10^{-2} mol), 34% formaldehyde **62** (2 ml) and K_2CO_3 (0.05 g 3.6x10^{-4} mol) was dissolved in isopropanol (8 ml) and stirred for five hours at 60^{0}C. Deionized water (20 ml) was added to the mixture and extracted with DCM (2 x 25 ml). The organic layer was evaporated *in vacuo* and the crude product was recrystallized from ethyl acetate yielding **58** as a white powder (ethyl acetate:hexane 50:50, R_f = 0.20) (0.4 g 70%, mp = 197-199^{0}C). IR ν_{max} 3375.74 cm^{-1}, 2936 cm^{-1}, 1768.77 cm^{-1}, 1714 cm^{-1}. ^{1}H NMR [$(CD_3)_2SO_2$, 400MHz]: δ_H 1.05 (AB, J_{AB} 13.5Hz, 1H), δ_H 1.16 (AB, J_{AB} 10.3Hz, 1H), δ_H 1.58 (AB, J_{AB} 10.3Hz, 1H), δ_H 1.85 (AB, J_{AB} 13.5Hz, 1H), δ_H 2.22 (m, 2H), δ_H 2.43 (m, 2H), δ_H 2.63 (m, 2H), δ_H 2.74 (s, 1H), δ_H 2.91 (s, 1H), δ_H 4.68 (d, 2H), δ_H 7.91 (s, 1H deuterium exchangeable). ^{13}C NMR [$(CD_3)_2SO_2$, 100MHz]: δ_C 28.3 (t), δ_C 33.8 (t), δ_C 35.3

(d), δ_C 41.1(d), δ_C 41.4 (d), δ_C 42.0(d), δ_C 42.5(d), δ_C 42.5 (d), δ_C 48.0 (d), δ_C 60.3 (d), δ_C 67.3 (s), δ_C 67.3 (t), δ_C 156.2 (s), δ_C 176.5 (s).

Synthesis of compound 56 (novel compound).

A solution of compound **58** (0.5g, 0.19 x 10^{-2} mol), triethylamine (3 ml, 2.1 x 10^{-2} mol) and *p*-toluensulfonyl chloride **63** (0.6 g, 0.31 x 10^{-2} mol) in THF (15 ml) was stirred overnight at room temperature. The reaction mixture was refrigerated for four hours. A white precipitate formed which was filtered and recrystallised from THF providing compound **56** as a white powder (0.5 g, 63%, mp = 86^0C) (chloroform:methanol 95:5, R_f=0.28,). IR ν_{max} 2951.13 cm^{-1}, 1781.33 cm^{-1}, 1723.90 cm^{-1}, 680.39 cm^{-1}, 564.21. cm^{-1}. ^{1}H NMR [$(CD_3)_2SO_2$, 400MHz] δ_H ^{1}H NMR [$(CD_3)_2SO_2$, 400MHz]: δ_H 1.1 (AB, J_{AB} 12Hz, 1H), δ_H 1.6 (d, 1H), δ_H 1.90 (AB, J_{AB} 12Hz, 1H), δ_H 2.28 (s, 3H), δ_H 2.45 (m, 2H), δ_H 2.60 (m, 2H), δ_H 2.74 (m, 1H), δ_H 2.77 (m, 1H), δ_H 4.66 (s, 2H), δ_H 7.13 (d, 2H), δ_H 7.49 (d, 2H), δ_H 8.44 (s, 1H deuterium exchangeable). ^{13}C NMR [$(CD_3)_2SO_2$, 100MHz] δ_C 20.7 (q), δ_C 28.1 (t), δ_C 33.8 (t), δ_C 38.8 (d), δ_C 47.9 (d), δ_C 41.2 (d), δ_C 41.8 (d), δ_C 46.0 (d), δ_C 41.8 (d), δ_C 47.9(d), δ_C 57.9 (t), δ_C 79.2 (s), δ_C 125.4 (d), δ_C 128.0 (d), δ_C 137.6 (s), δ_C 145 2(s), δ_C 155.4 (s), δ_C 177.7 (s).

Synthesis of adamantane hydantoin 59.

A mixture of 2-adamantanone **61** (1 g 0.66 x 10^{-2} mol), sodium cyanide (1 g 2.04x10^{-2} mol), ammonium carbonate (2 g 2.08x10^{-2} mol), ethanol (10 ml) and ammonia solution (15 ml) was sealed in a glass pressure vessel and placed in an oil bath at 60^0C for two hours, 100^0C for two hours and 120^0C overnight. The mixture was allowed to cool and the crude product was obtained as a brown precipitate which was filtered and recrystallised from isopropanol yielding compound **59** as white crystals (1.3 g, 89%, mp = 301^0C). ^{1}H NMR [$(CD_3)_2SO_2$, 400MHz] : δ_H 1.54 (m, 4H), δ_H 1.67 (s, 2H), δ_H 1.76(m, 2H), δ_H 2.0 (d, 2H), δ_H 8.38 (s, 1H deuterium exchangeable), δ_H 10.48 (s, 1H deuterium exchangeable). ^{13}C NMR [$(CD_3)_2SO_2$, 100MHz]: δ_C 25.6 (d), 26.2 (d), 31.5 (t), 32.8 (t), 34.6 (d), 37.3 (t), 65.5 (s), 155.8 (s), 177.2(s).

Synthesis of compound 57 (novel compound).

A suspension of adamantane hydantoin **59** (1 g 0.45 x1 0^{-2} mol), 37% formaldehyde **62** (4 ml) and K_2CO_3 (0.1 g 7.2 x 10^{-4} mol) was stirred overnight in isopropanol (15 ml) at 60 ^{0}C. The product was obtained as white precipitate which was filtered and washed extensively with water and dried (chloroform:methanol 90:10, R_f = 0.41) (1.1 g 91%, mp = 118-120^0C). IR ν_{max} 3371.6 cm^{-1}, 2894.56 cm^{-1}, 1763.5 cm^{-1}, 1707.8 cm^{-1}. ^{1}H NMR [$(CD_3)_2SO_2$, 400MHz]:

δ_H 1.56 (m, 4H), δ_H 1.69 (s, 2H), δ_H 1.73 (m, 2H), δ_H 1.77 (m, 1H), δ_H 1.82 (m, 1H), δ_H 2.04 (d, 2H), δ_H 2.55(d, 2H), δ_H 4.70 (s, 2H), δ_H 8.71 (s, 1H deuterium exchangeable). ^{13}C NMR [$(CD_3)_2SO_2$, 100MHz]: δ_C 25.5(d), δ_C 26.2(d), δ_C 31.5(t), δ_C 32.7 (t), δ_C 34.4 (d), δ_C 37.2 (t), δ_C 60.2 (t), δ_C 64.4 (s), δ_C 154.7 (s), δ_C 174.9 (s).

Synthesis of compound 55 (novel compound).

A suspension of compound **57** (0.8 g 0.32 x 10^{-2} mol), triethylamine (5 ml 3.6x10^{-2} mol) and *p*-toluenesulfonyl chloride **63** (0.9 g 0.47x10^{-2} mol) was stirred overnight in THF (30 ml) at room temperature. The precipitate was filtered and washed extensively with THF and the white product was dried *in vacuo* (chloroform:methanol 95:5, R_f = 0.26) (0.9 g 73%, mp = 110^{0}C). IR ν_{max} 2901.6 cm^{-1}, 1772.9 cm^{-1}, 1719.1 cm^{-1}, 679.5 cm^{-1}564.3 cm^{-1}. ^{1}H NMR [$(CD_3)_2SO_2$, 400MHz]: δ_H 1.62 (m, 4H), δ_H 1.70 (s, 2H), δ_H 1.79-1.82 (m, 2H), δ_H 2.00-2.08 (m, 4H), δ_H 2.28 (s, 3H), δ_H 2.42-2.44 (d, 2H), δ_H 4.70 (s, 2H), δ_H 7.12 (d, 2H), δ_H 7.48 (d, 2H), δ_H 9.22 (s, 1H deuterium exchangeable). ^{13}C NMR [$(CD_3)_2SO_2$, 100MHz]: δ_C 20.7 (q), δ_C 25.4 (d), δ_C 26.0 (d), δ_C 31.6 (t), δ_C 32.4 (t), δ_C 33.9 (d), δ_C 37.1 (t), δ_C 58.1 (t), δ_C 65.2 (s), δ_C 125.4 (d), δ_C 128.0 (d), δ_C 137.6 (s), δ_C 145.6 (s), δ_C 153.9 (s), δ_C 176.0 (s).

Synthesis of compound 66 (novel compound).

To an ice bath cooled mixture of 48% HBr (1.5 ml) and compound **57** (0.5 g 0.20x10^{-2} mol) was added concentrated H_2SO_4 (0.5 ml) cautiously. After that the mixture was stirred at 60^{0}C for five hrs. The precipitate was filtered followed by thorough washing with water (50 ml) and 10 % ammonia solution (20 ml). The crude product was recrystallised from THF and gave compound **66** as a white powder (DCM:methanol 90:10, R_f = 0.33) (0.78 g, 62%, mp = 263-265^{0}C). IR ν_{max} 3379.8 cm^{-1}, 2895 cm^{-1}.01, 1779.83 cm^{-1}, 1714.09 cm^{-1}, 1433.3 cm^{-1}, 609.5 cm^{-1}. δ_H 1.56 (m, 4H), δ_H 1.69 (s, 2H), δ_H 1.73 (m, 2H), δ_H 1.77 (m, 1H), δ_H 1.82 (m, 1H), δ_H 2.04 (d, 2H), δ_H 2.55(d, 2H), δ_H 4.70 (s, 2H), δ_H 8.72 (s, 1H deuterium exchangeable). ^{13}C NMR [$(CD_3)_2SO_2$, 100MHz]: δ_C 25.5(d), δ_C 26.2(d), δ_C 31.5(t), δ_C 32.7 (t), δ_C 34.4 (d), δ_C 37.2 (t), δ_C 60.2 (t), δ_C 64.4 (s), δ_C 154.7 (s), δ_C 174.9 (s).

Attempted synthesis of compounds 53 and 54.

Attempt A: A solution of compound **64** (0.44 g 0.16 x 10^{-2} mol) in DCM (3 ml) was added drop wise to a stirred solution of (a) compound **55** (0.25 g 0.06 x 10^{-2} mol) (b) compound **56** (0.25 g 0.06 x 10^{-2} mol) and triethylamine (0.4 ml 0.3 x 10^{-2} mol) in DCM (10 ml) and the mixture was stirred overnight at room temperature. There was no evidence of product

formation when the reaction was monitored using NMR and LCMS. This was also the result after the reaction was refluxed for 24 hours.

Attempt B: A solution of (a) compound **55** (0.1 g 0.02 x 10^{-2} mol) (b) compound **56** (0.1 g 0.02x10^{-2} mol) in acetonitrile (2 ml) was added drop wise to a stirred solution of compound **64** (0.15 g 0.05 x 10^{-2} mol) and triethylamine (0.35 ml 0.25 x 10^{-2} mol) in acetonitrile (8 ml). The solution was then stirred overnight at ambient temperature. There was no evidence of product formation when the reaction was monitored with NMR and LCMS. This was also the result after the reaction was refluxed for 24 hours.

Attempt C: A suspension of (a) compound **55** (0.1 g 0.02 x 10^{-2} mol) (b) compound **56** (0.1 g 0.02 x 10^{-2} mol) in THF (3.5 ml) was added drop wise to a stirred solution of compound **64** (0.15 g 0.05 x 10^{-2} mol) and sodium hydride (0.1 g 0.25 x 10^{-2}mol) in THF (8 ml) under inert atmosphere. The mixture was stirred over night at ambient temperature. The precipitate was filtered and the filtrate was dried in *vacuo*. There was no evidence of product formation when the filtrate and precipitate were analysed with NMR and LCMS. This was also the result after the reaction was refluxed for 24 hours.

Attempt D A solution of (a) compound **55** (0.1 g 0.02 x 10^{-2} mol) (b) compound **56** (0.1 g 0.02 x 10^{-2}mol) in THF (3.5 ml) was added dropwise to a stirred solution of compound **64** (0.15 g 0.05 x 10^{-2} mol) and sodium hydroxide (0.1 g 0.25 x 10^{-2} mol) in THF (13 ml). The reaction mixture was stirred over night at ambient temperature. There was no evidence of product formation when the filtrate and precipitate were analysed with NMR and LCMS. This was also the result after the reaction was refluxed for 24 hours.

Attempt E A solution of compound **64** (0.3 g 0.1 x 10^{-2} mol) in DCM (3 ml) was added drop wise to a stirred solution of compound **65 (**0.12 g 0.04 x 10^{-2} mol) and sodium hydroxide (0.15 g 0.37 x 10^{-2} mol) in DCM (6 ml), the reaction was stirred for 12 hrs at 50^0C. There was no evidence of product formation when the filtrate and precipitate were analysed with NMR and LCMS. This was also the result after the reaction was refluxed for 12 hours.

Attempted synthesis of compound 66.

Concentrated H_2SO_4 (0.5 ml) was added cautiously to an ice bath cooled solution of compound **58** (0.5 g 0.20 x 10^{-2} mol) in 48% HBr (1.5 ml) and the mixture was stirred at 60^0C for 5 hrs. The precipitate was filtered followed by thorough washing with water (3 x 50 ml)

and 10 % ammonia solution (3x20 ml). The only observable product was PCU hydantoin **60** (using NMR and TLC analysis).

Attempted synthesis of compounds 51 and 52.

A solution of (a) compound **57** (0.4 g 0.16 x 10^{-2} mol) (b) compound **58** (0.4 g 0.16x10^{-2} mol) in acetonitrile (5 ml) was added drop wise to a solution of phosphorus chloride oxide **67** (0.25 g 0.16x10^{-2} mol) and pyridine (1 ml 1x10^{-2} mol) in acetonitrile (10 ml). The reaction mixture was stirred for 5 hrs at 0^0C and thereafter adjusted to pH 1 *via* addition of 10 % aqueous HCl and stirred at room temperature for another 1 hour. Deionised water (20 ml) was added to the mixture and the product was extracted with ethyl acetate (3 x 25 ml). The organic layer was dried and there was no evidence of product formation.

Synthesis of Fmoc PCU amino acid 106.[6]

A suspension of PCU hydantoin **60** (1.0 g, 4.3 x 10^{-3} mol) in NaOH (1.25 M, 50 ml) was sealed in a pressure reactor vessel and heated in an oil bath at 170^0C for 12 hours. The reaction mixture was cooled and adjusted to pH 9 by addition of concentrated HCl. The mixture was added slowly to a stirred solution of 9-flourenylmethyl chloroformate **105** (1.7 g, 0.6x10^{-2} mol) in dioxan (20 ml) and stirred at ambient temperature overnight. The reaction mixture was acidified to pH 2 *via* addition of HCl (37%) and extracted with ethyl acetate (2x100 ml). The organic layer was evaporated and the crude product was purified via silica gel column chromatography (choloroform:methanol:acetic acid 95:4:1, R_f = 0.42) giving the product **106** as a white powder (1.3 g, 73%, mp = 216 ^{0}C).

The NMR spectra were identical to an authentic sample.

General procedure for the synthesis of peptides using microwave power.

Solutions of required amino acids (0.2 x 10^{-3} mol), N,N-diisopropylethylamine (DIPEA) (1x10^{-3} mol) and hydroxybenzotriazole (HOBt) (2 x 10^{-3} mol) in DMF were made up and fitted in the appropriate reaction vessels on the automatic peptide synthesizer. All peptides were synthesized on a 0.1 x 10^{-3} mol scale using microwave power. The details of the microwave conditions are given in table 5.

Table 5: details of the microwave conditions for synthesis of peptide 106 and 107

	Microwave power/W	Time/s	Temperature/°C
Arg Coupling	0	1500	156.1
	25	300	
Normal Coupling	25	300	71
Deprotection	60	180	71

An arginine coupling method was used for coupling the PCU amino acid (*i*) to each of (*i*-1) and (*i*+1)[***] The normal coupling method was used for subsequent couplings and for Fmoc deprotection, the deprotection method was used as indicated in table.

Cleavage of peptides from rink amide resin.

The rink amide resin bound peptide was washed with DCM (3 x 10 ml) in a manual peptide synthesis reaction vessel. The resin was dried and the cleavage mixture (10 ml) of 2.5% (v/v) (TIS), 2.5% (v/v) water and 95% (v/v) TFA was added to the resin and stirred for 1 hour. The resin was filtered and washed with methanol (30 ml). TFA was removed *via* bubbling air through the mixture and the crude peptide was precipitated with cold ether.

General Procedure for preparative HPLC purification of novel compounds.

A solvent system containing acetonitrile (solvent B) and water (Solvent A) was used for HPLC analysis of compounds. A ACE 5 C18 150x21.2 mm column was used. Gradient elution system of 5% (Solution B) and 95% (Solution A) was used initially and this was changed linearly over 60 minutes to 95% (Solution B) and 5% (Solution A) at 17.00 ml per minute.

Data for Novel peptides.

H_2N-$(Ala)_3$-PCU-$(Ala)_2$-Ala-Fmoc 106a and 106b.

Yield: 35% and 18%. LC-MS Retention time: 11.4 and 12.2 min. ESI-MS 880 [M + 23(Na) + 4 ($4D^+$)] m/z. NMR data provided in Tables **1** and **2**.

[***] (*i*-1) and (*i*+1) are Ala and Ala in case of Ala3-PCU-Ala-2-Ala-Fmoc sequence and Val and Ile respectively in case of Ala-Val-PCU-Ile

Ala-Val-PCU-Ile 107a and 107b.

Yield: 27% and 23%. LC-MS Retention time: 6.2 and 7.3 min. ESI-MS m/z 488.4 [M + 1] m/z. NMR data provided in Tables **3** and **4**.

References

1. Marchand, P. A.; Allen, W. R. *Journal of Organic Chemistry* **1974,** *39*, 1596.

2. Eaton, E. P.; Cassar, L.; Hudson, A. R.; Hwang, R. D. *Journal of Organic Chemistry* **1976,** *41*, 1445-1449.

3. Dekker, T. G.; Oliver, D. W. *South African Journal of Chemistry* **1979**, 45-48

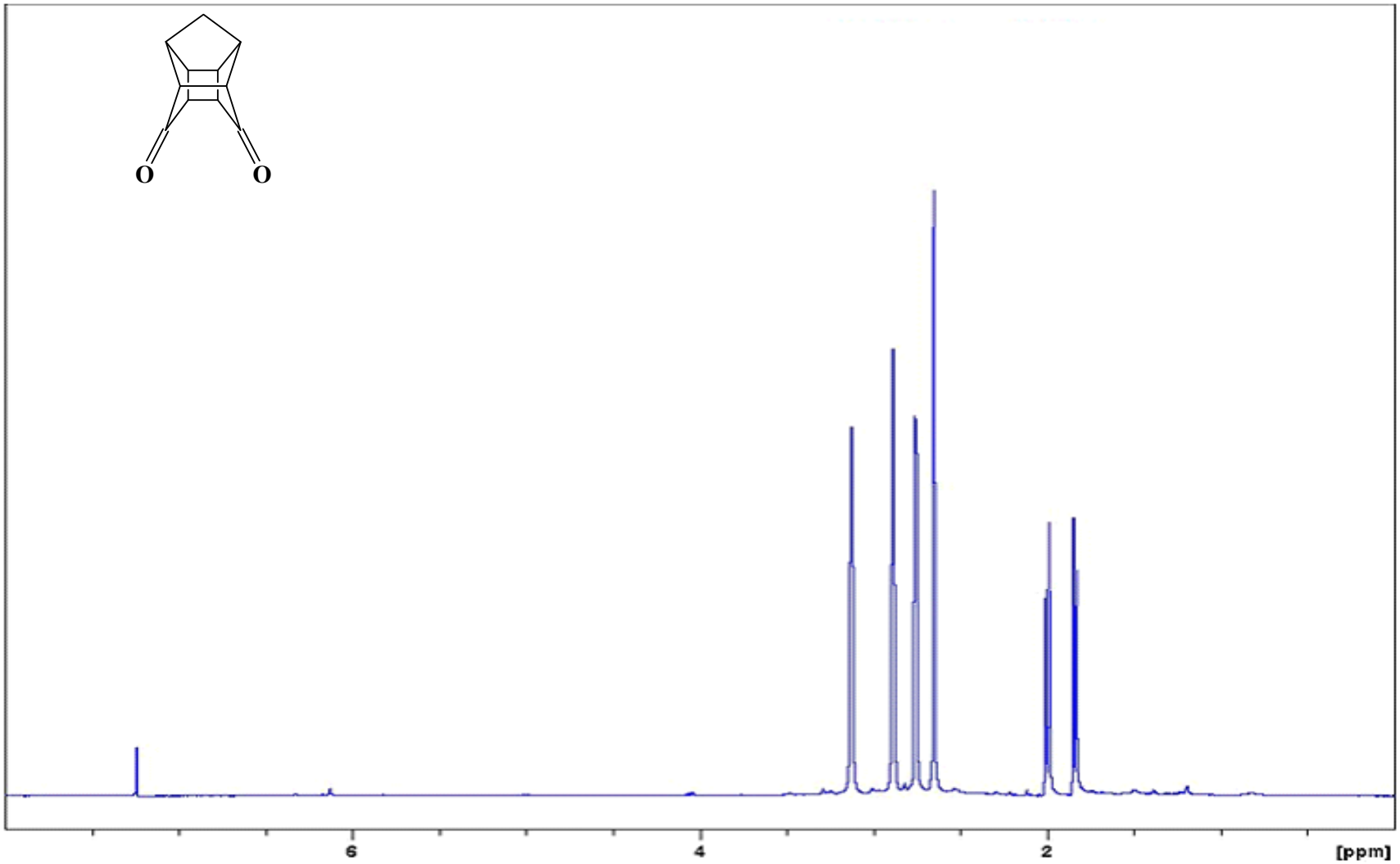
4. Dekker, T. G.; Oliver, D. W.; Pachler, K. G. R.; Wessels, P. L.; Woudenberg, M. *Journal of Organic Magnetic Resonance* **1981,** *15*, 188-192.

5. Martins, F. J. C.; Viljoen, A. M.; Kruger, H. G.; Fourie, L.; Roscher, L.; Joubert, A. J.; Wessels, P. L. *Tetrahedron* **2001,** *57*, 1601-1607.

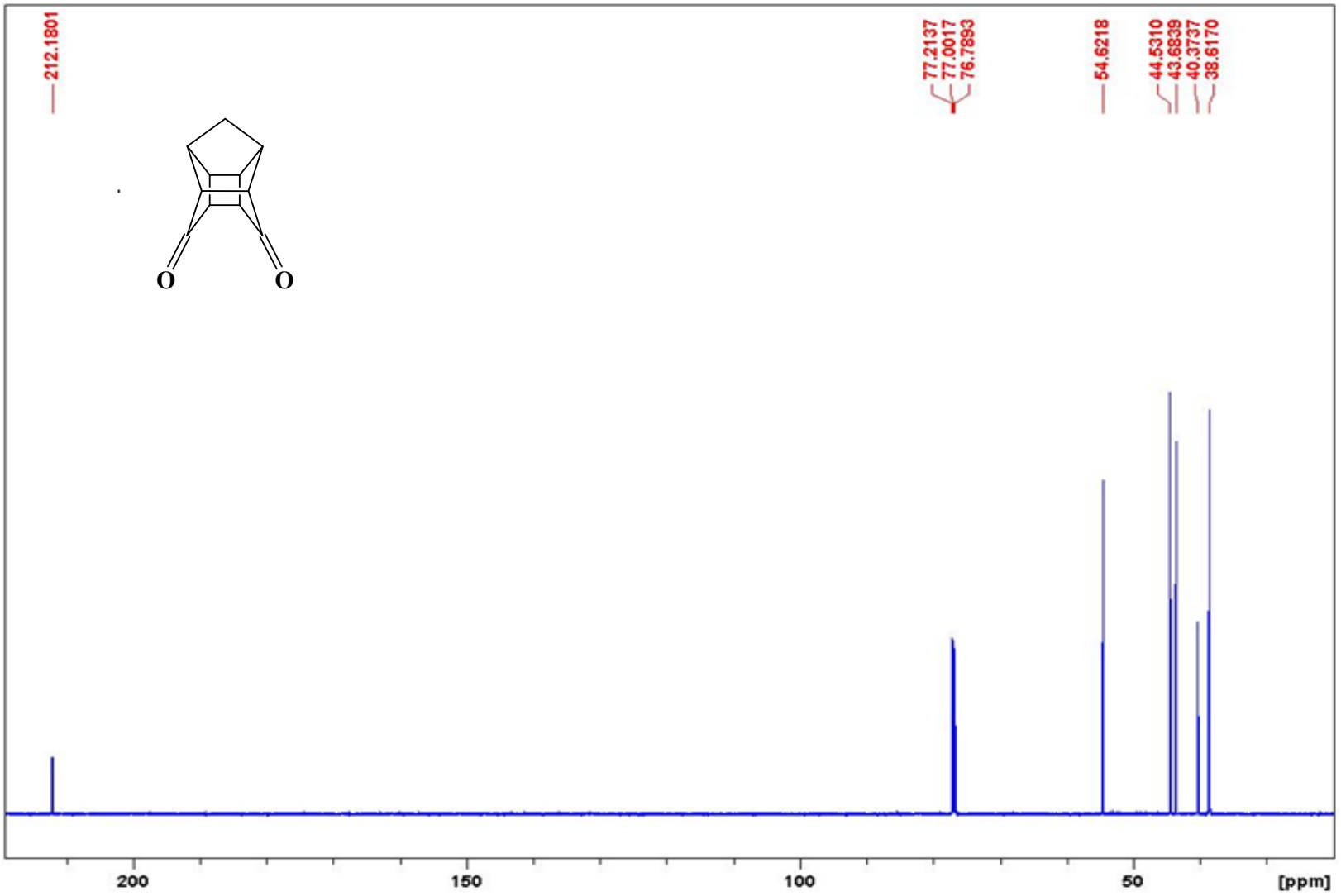
6. Singh, A. *MSc thesis*, University of Kwazulu-Natal, 2005.

APPENDIX

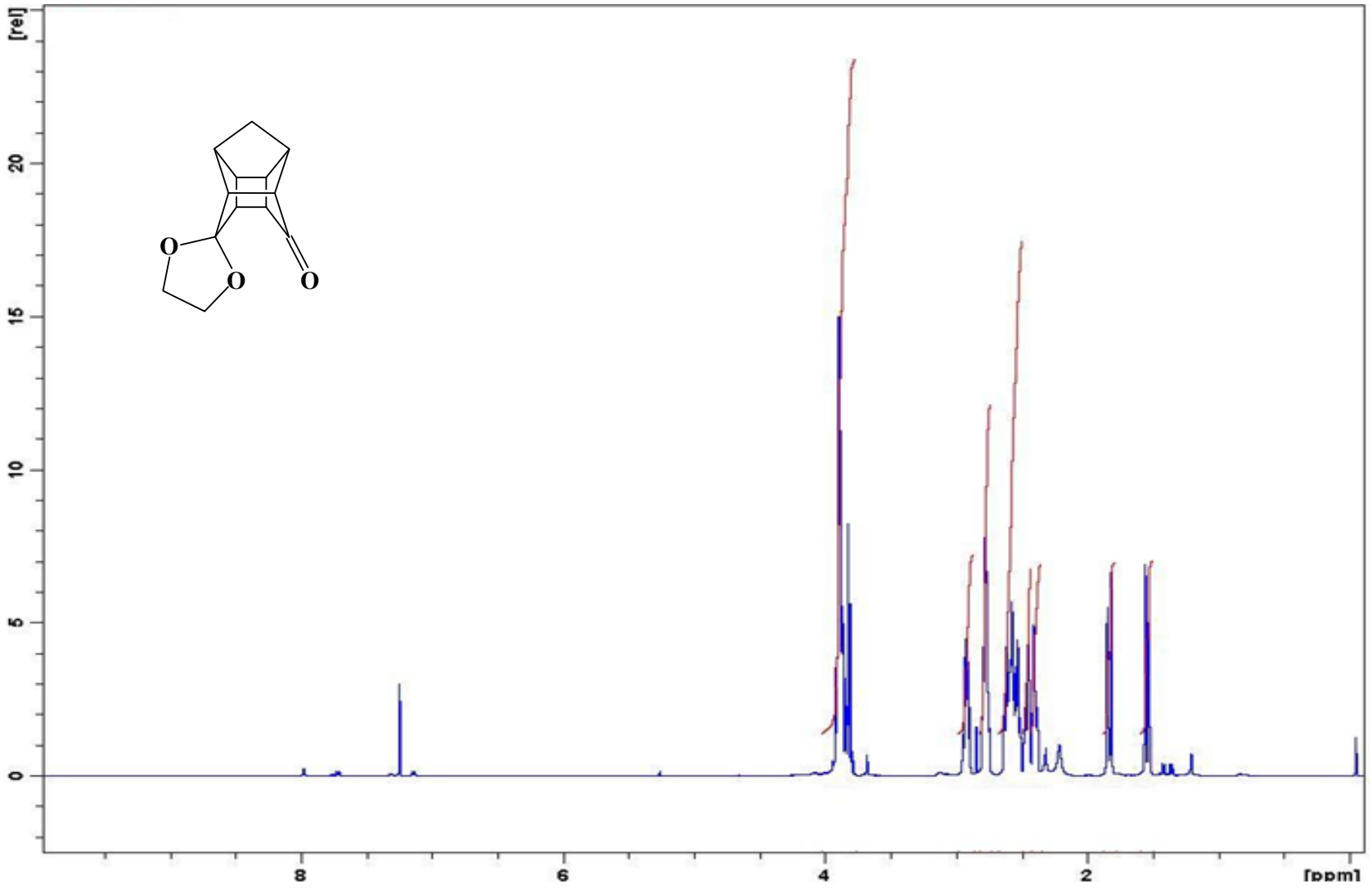
SPECTRA



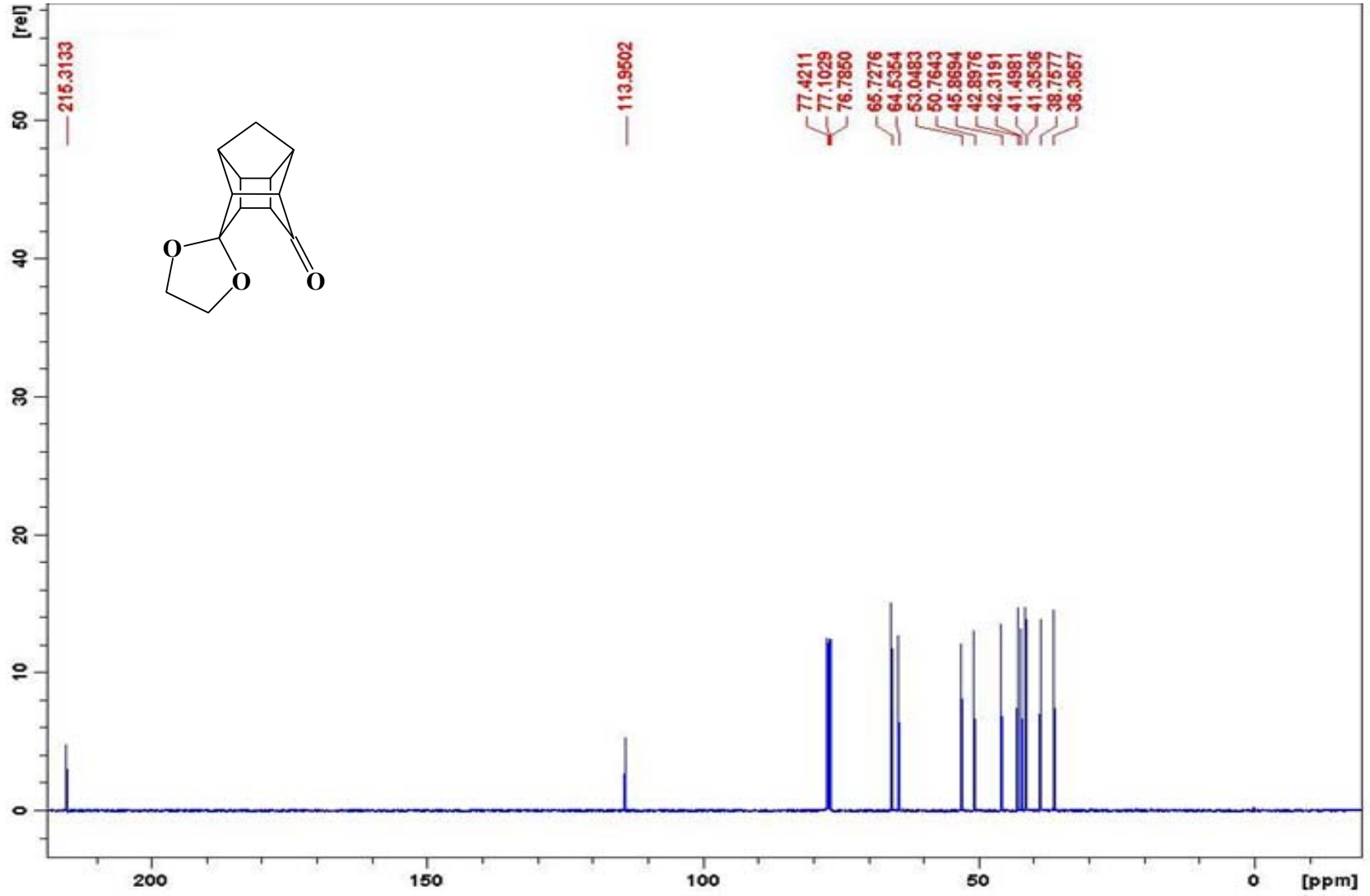
Spectrum 1: ^{1}H NMR spectrum of PCU dione 73 in $CDCl_3$



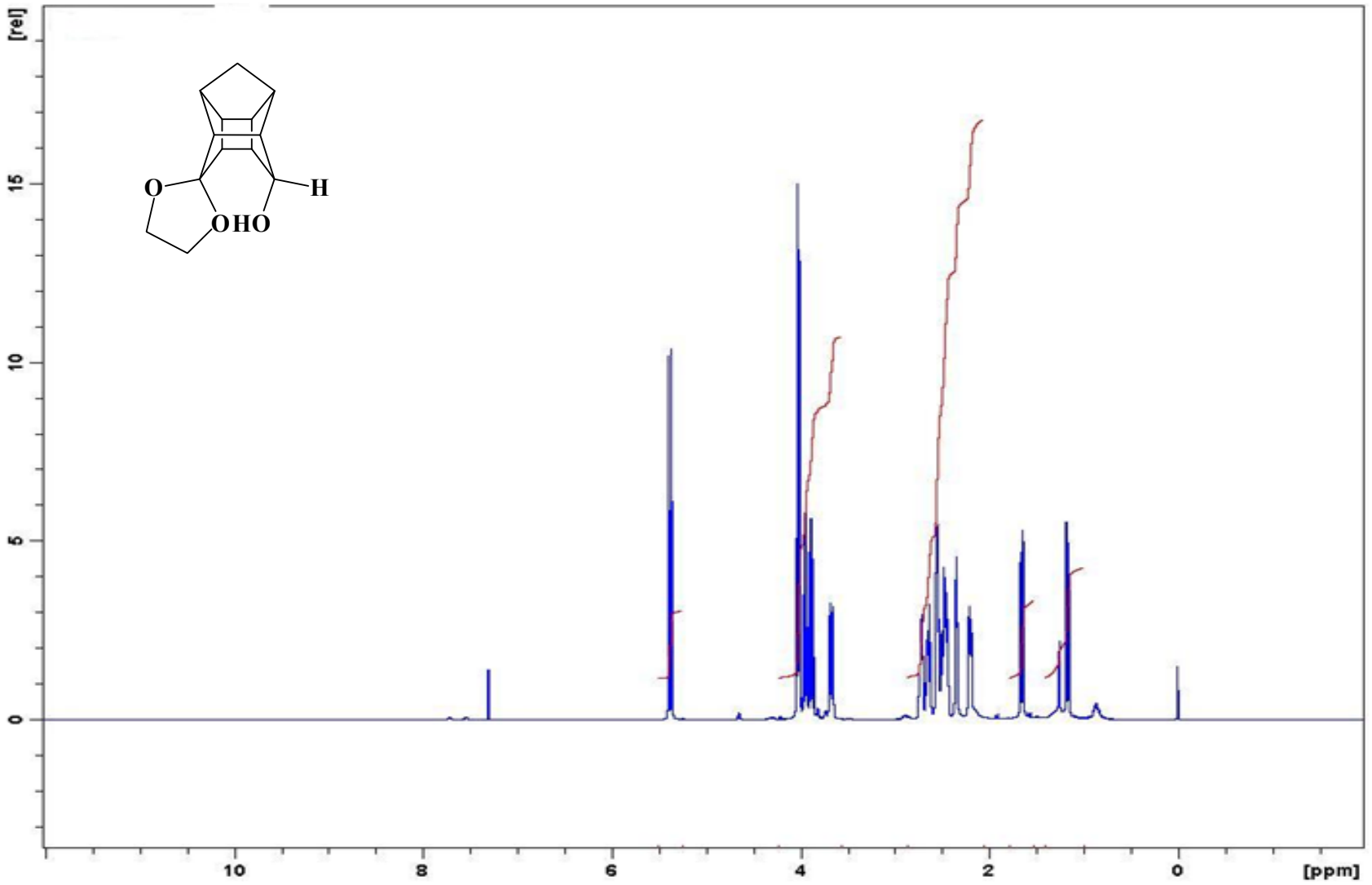
Spectrum 2: ^{13}C NMR spectrum of PCU 73 dione in $CDCl_3$



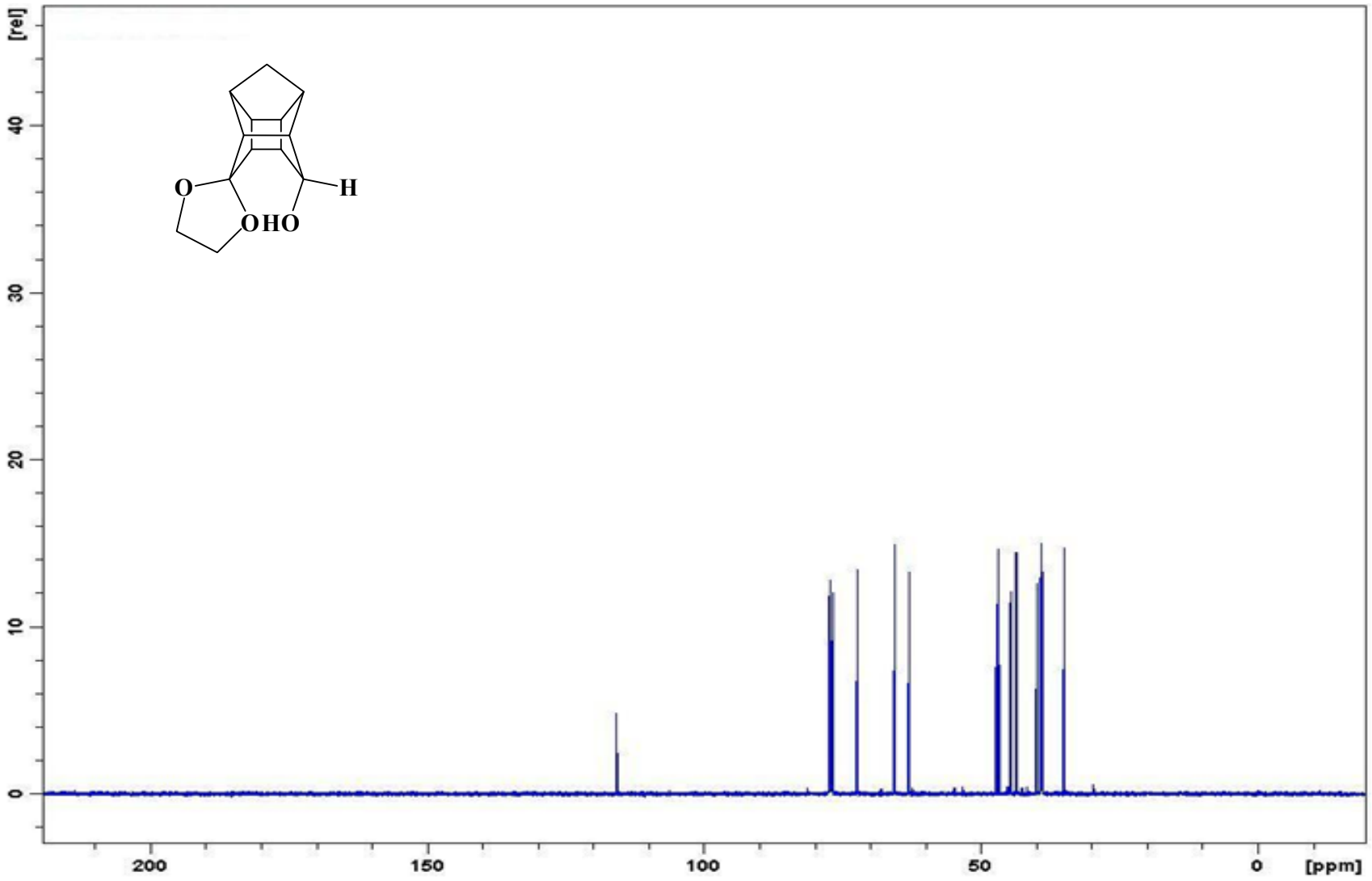
Spectrum 3: ^{1}H NMR spectrum of PCU ketal 79 in $CDCl_3$



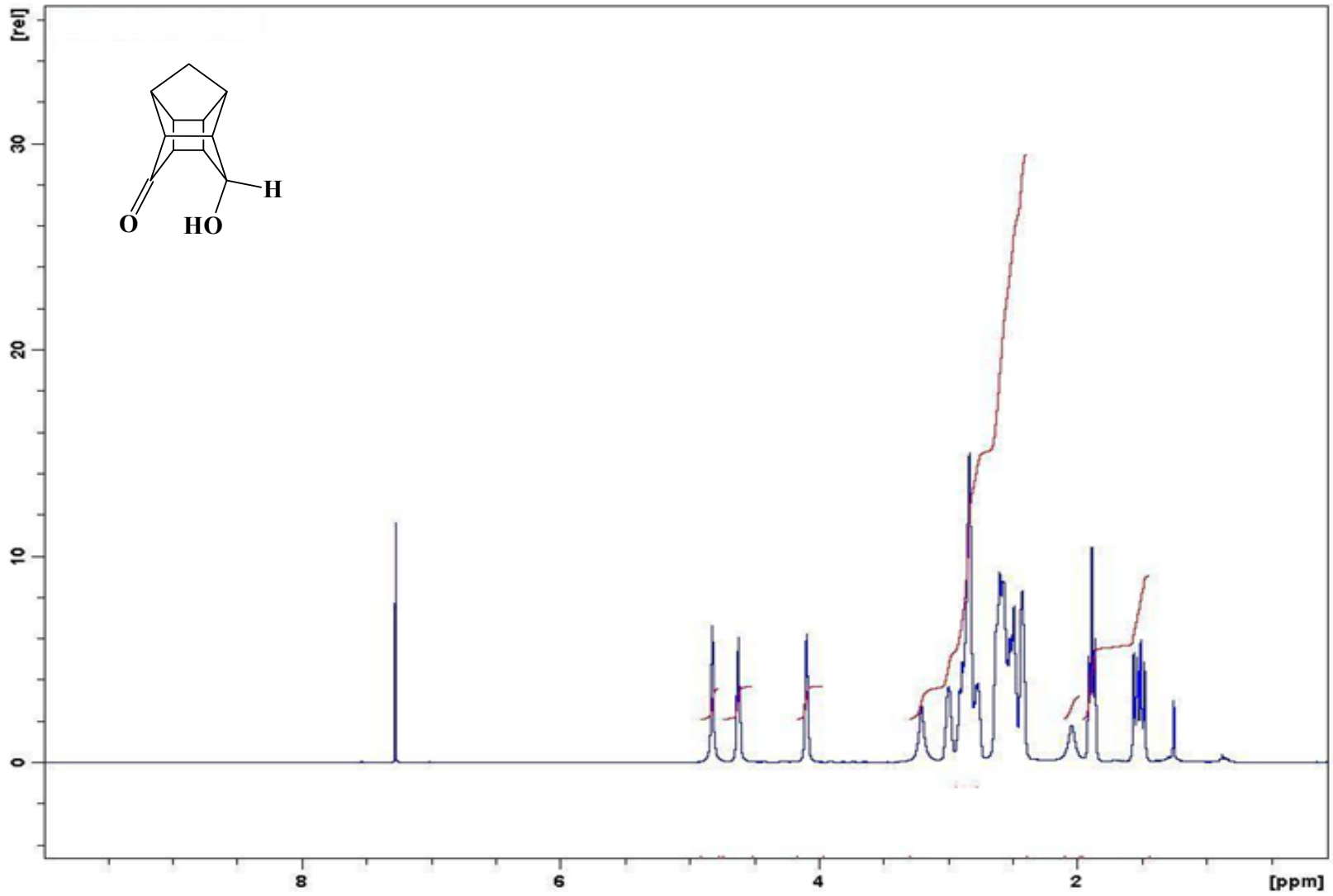
Spectrum 4: ^{13}C NMR spectrum of PCU ketal 79 in $CDCl_3$



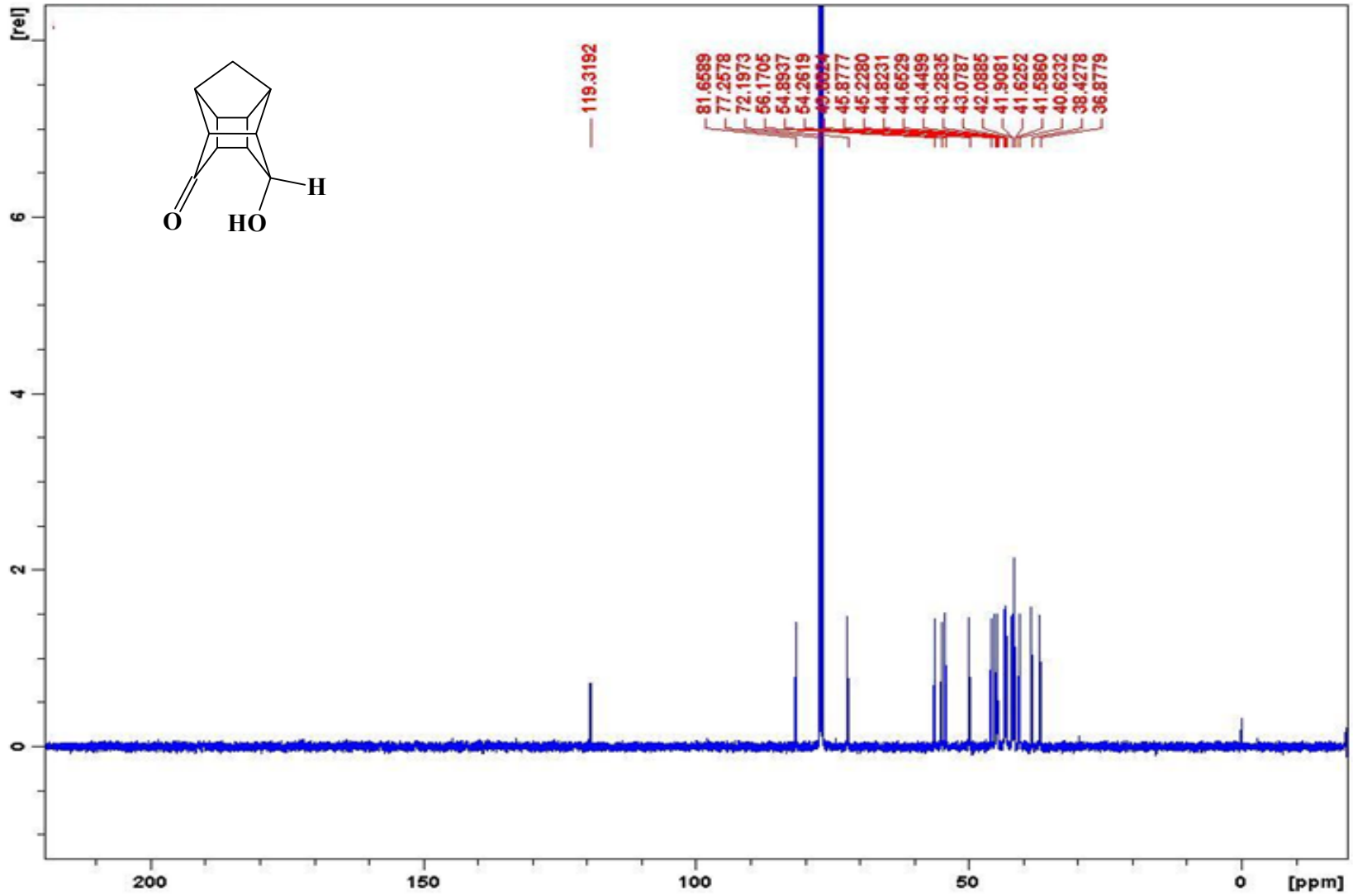
Spectrum 5: ^{1}H NMR spectrum of PCU hydroxy-ketal 87 in $CDCl_3$



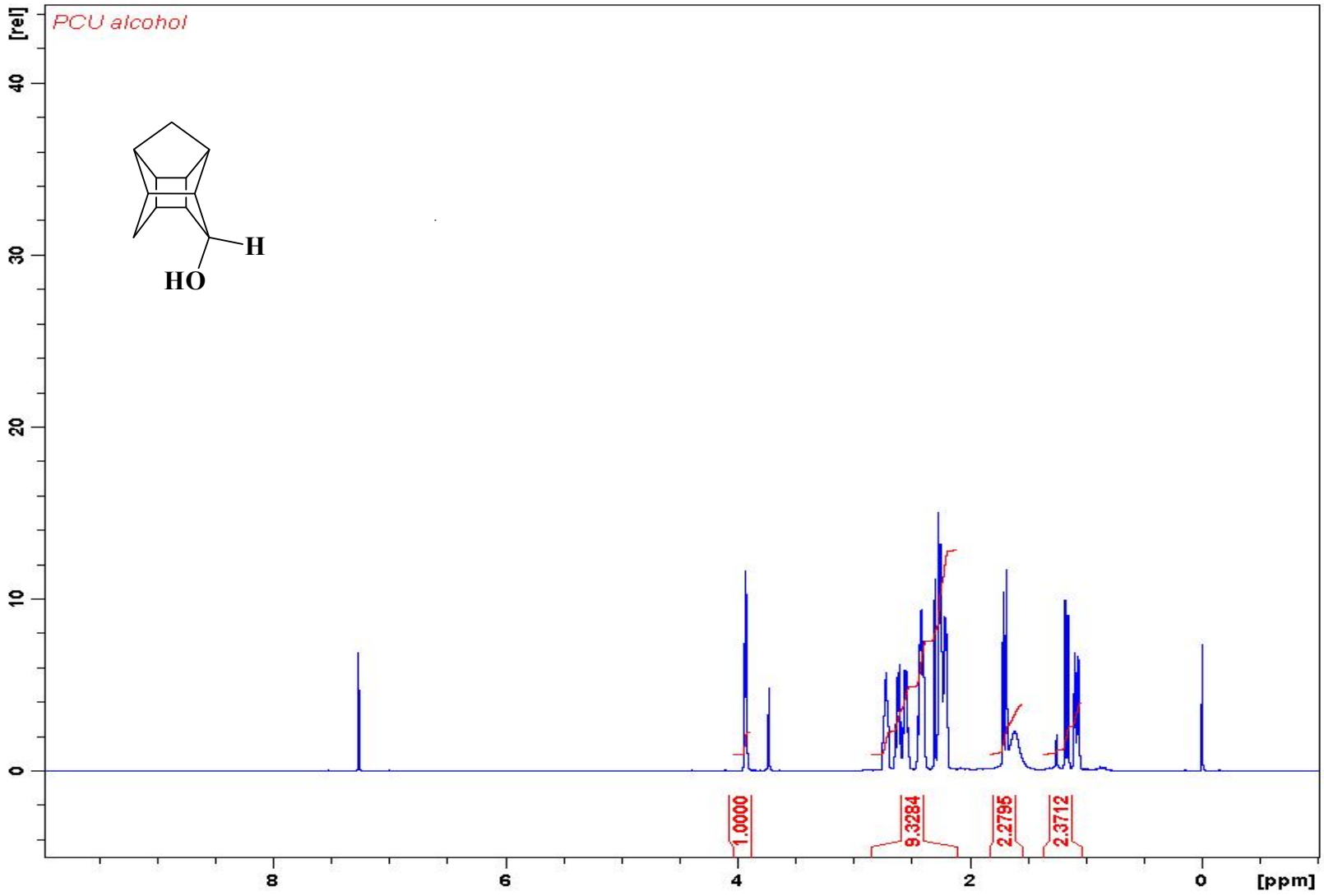
Spectrum 6: ^{13}C NMR spectrum of PCU hydroxy-ketal 87 in $CDCl_3$



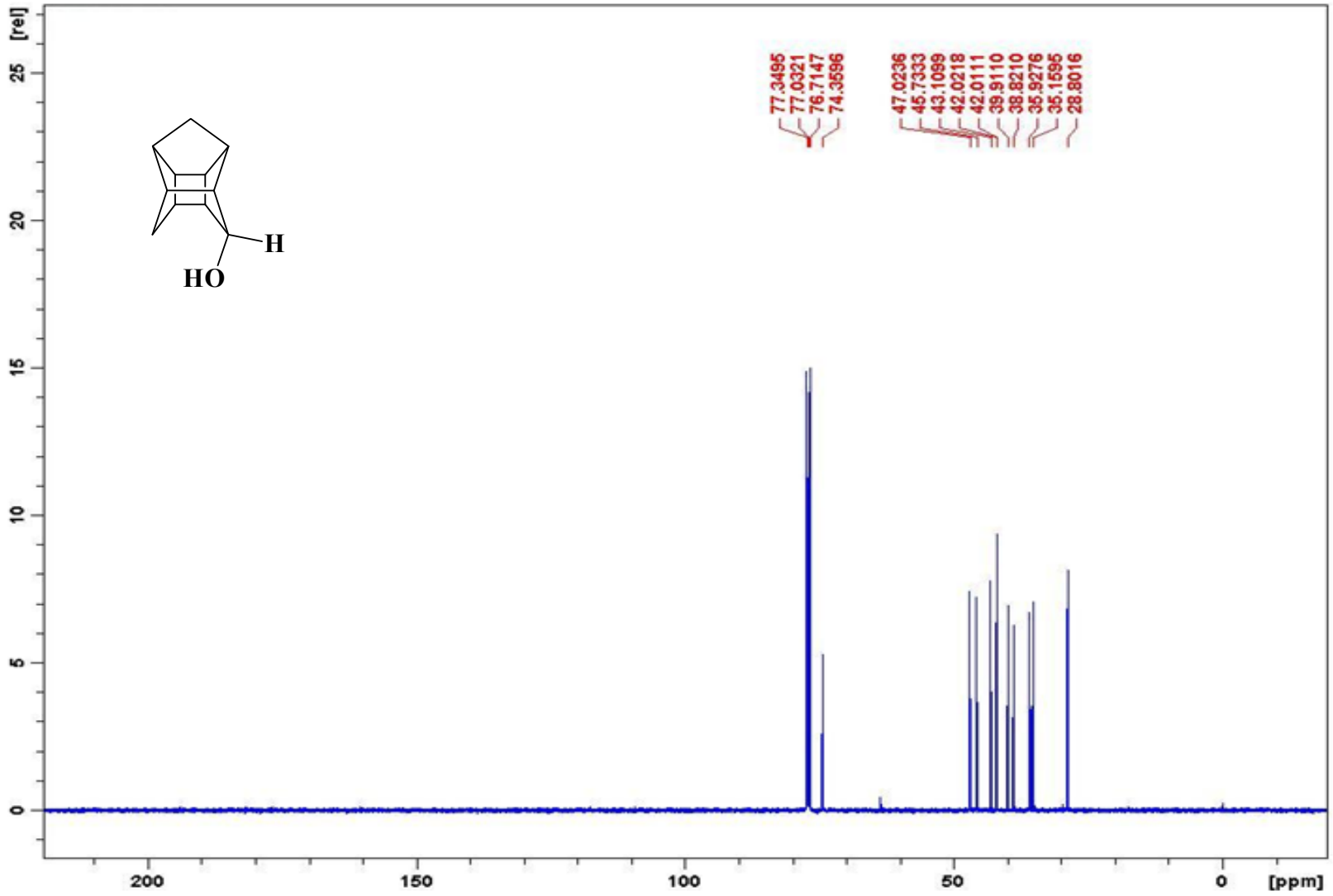
Spectrum 7: ^{1}H NMR spectrum of PCU hydroxy-ketone 88 in $CDCl_3$



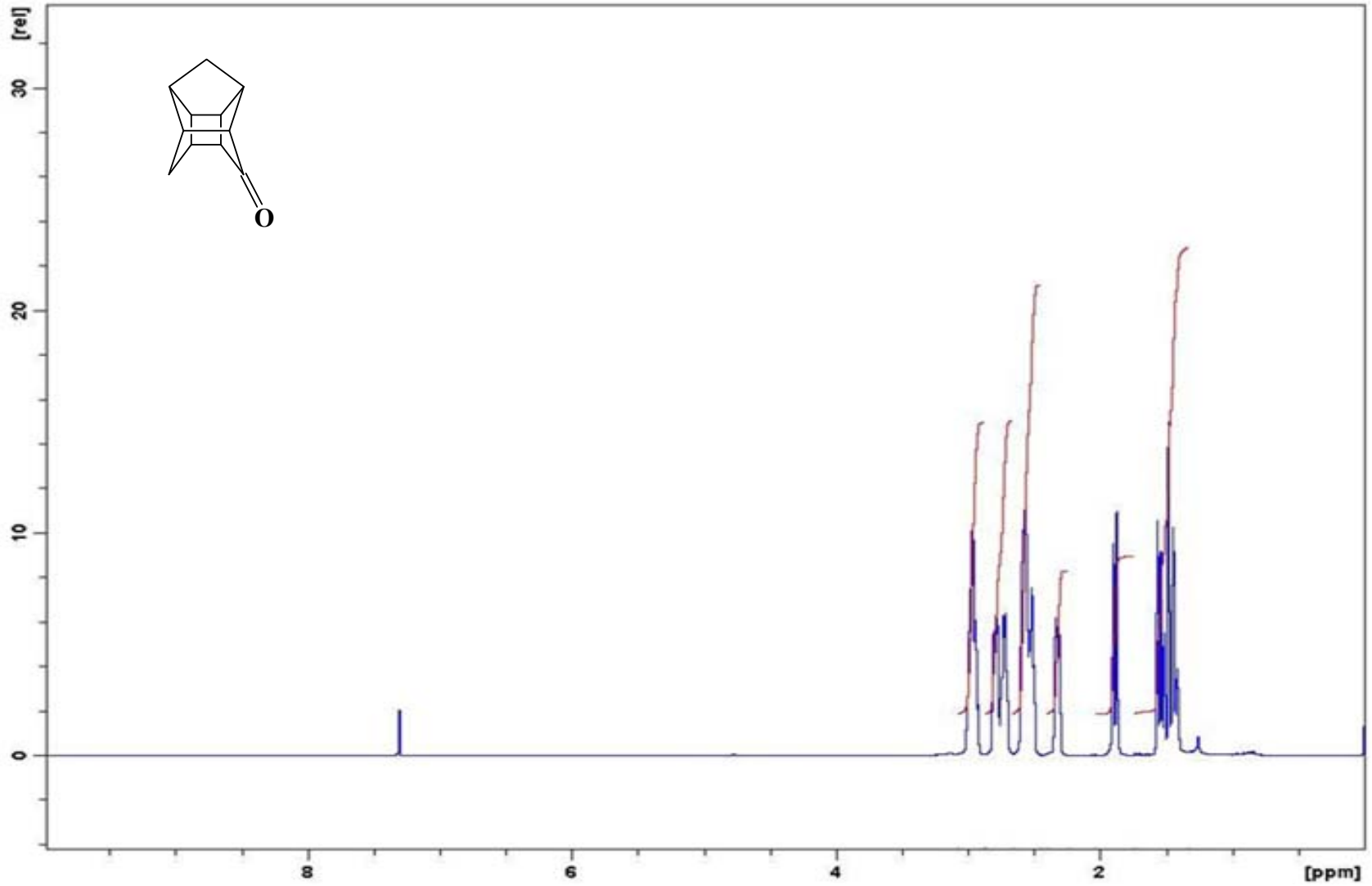
Spectrum 8: ^{13}C NMR spectrum of PCU hydroxy-ketone 88 in $CDCl_3$



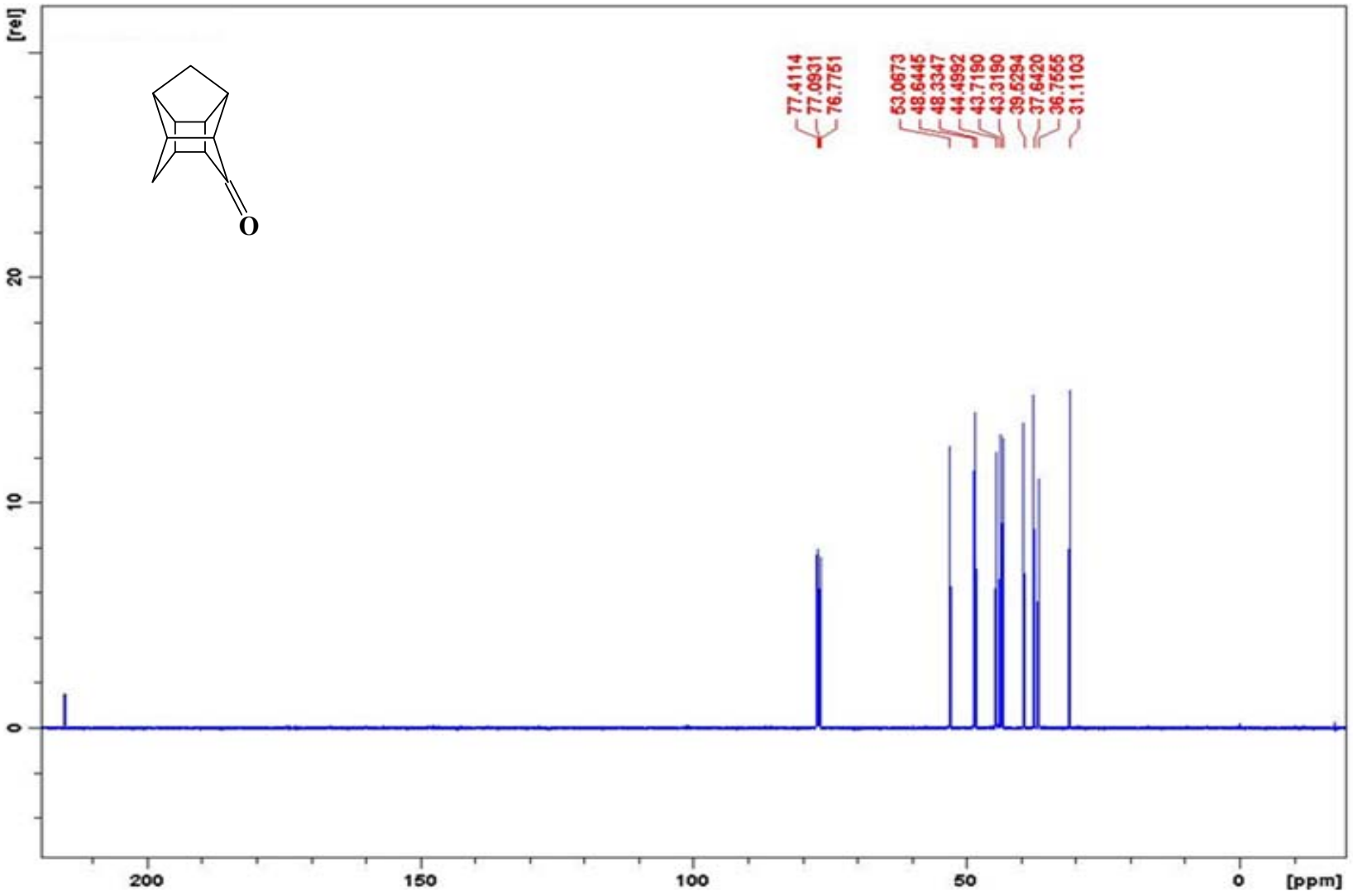
Spectrum 9: ^{1}H NMR spectrum of PCU alcohol 94 in $CDCl_3$



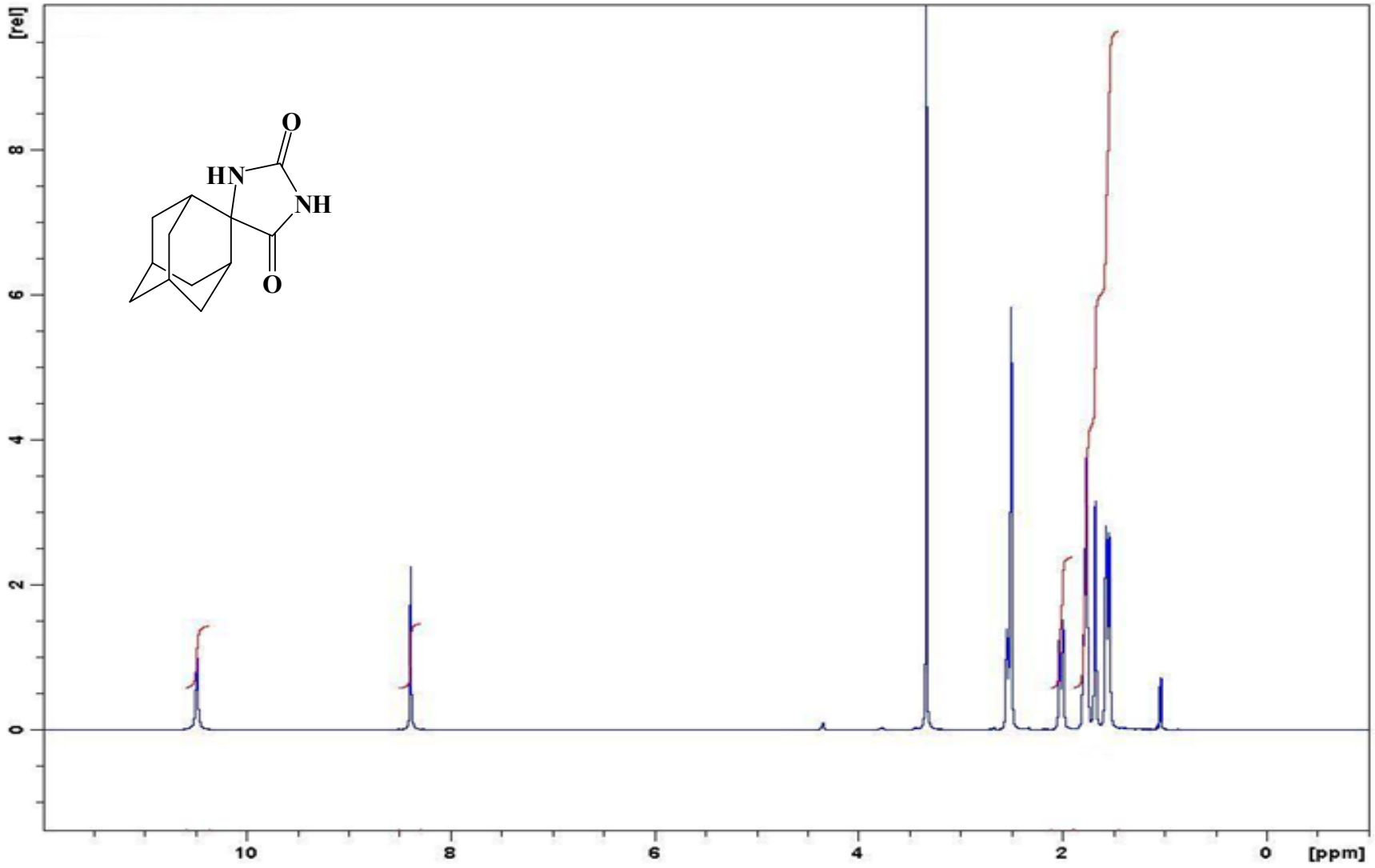
Spectrum 10: ^{13}C NMR spectrum of PCU alcohol 94 in $CDCl_3$



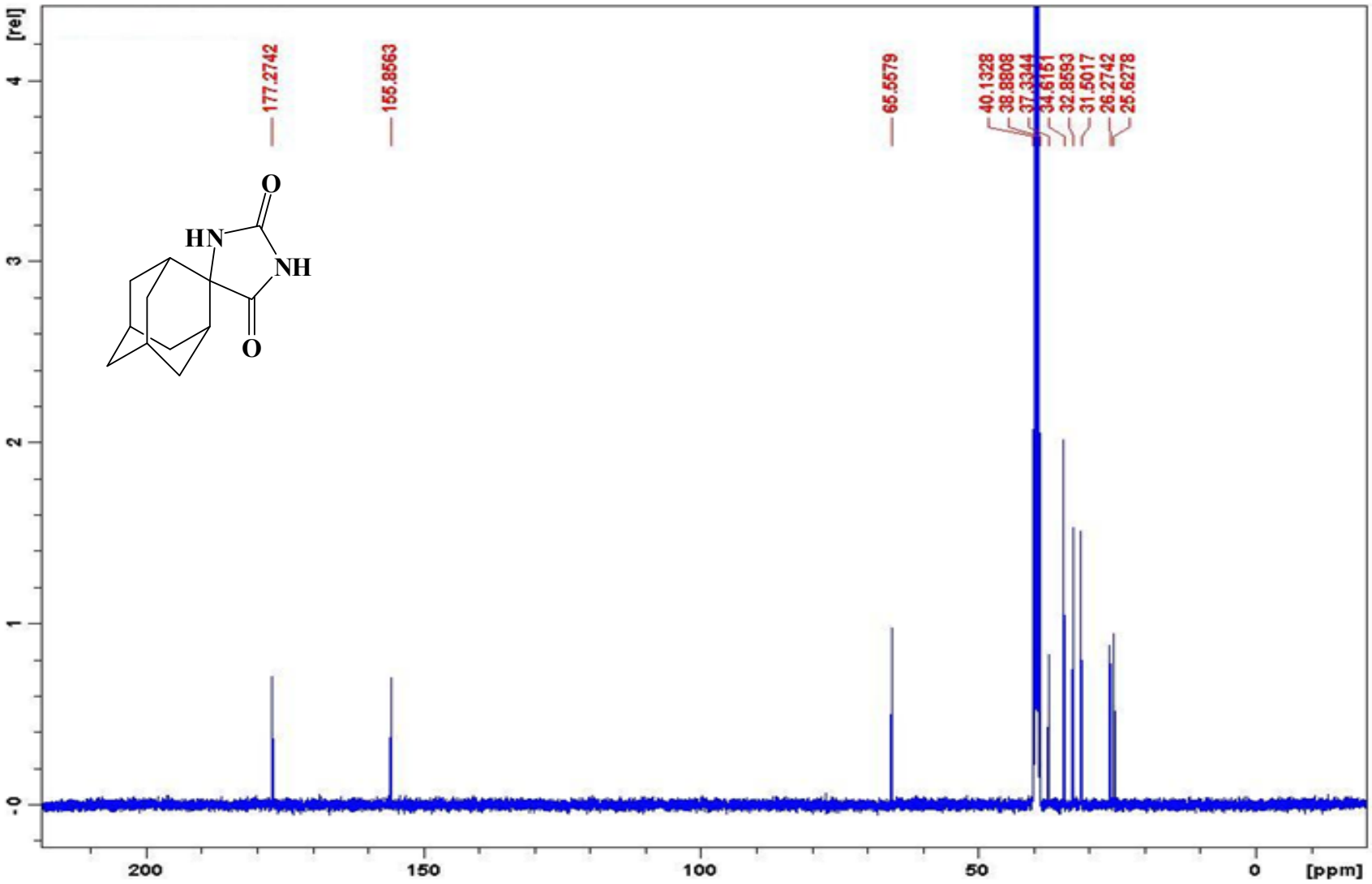
Spectrum 11: ^{1}H NMR spectrum of PCU mono-ketone 64 in $CDCl_3$



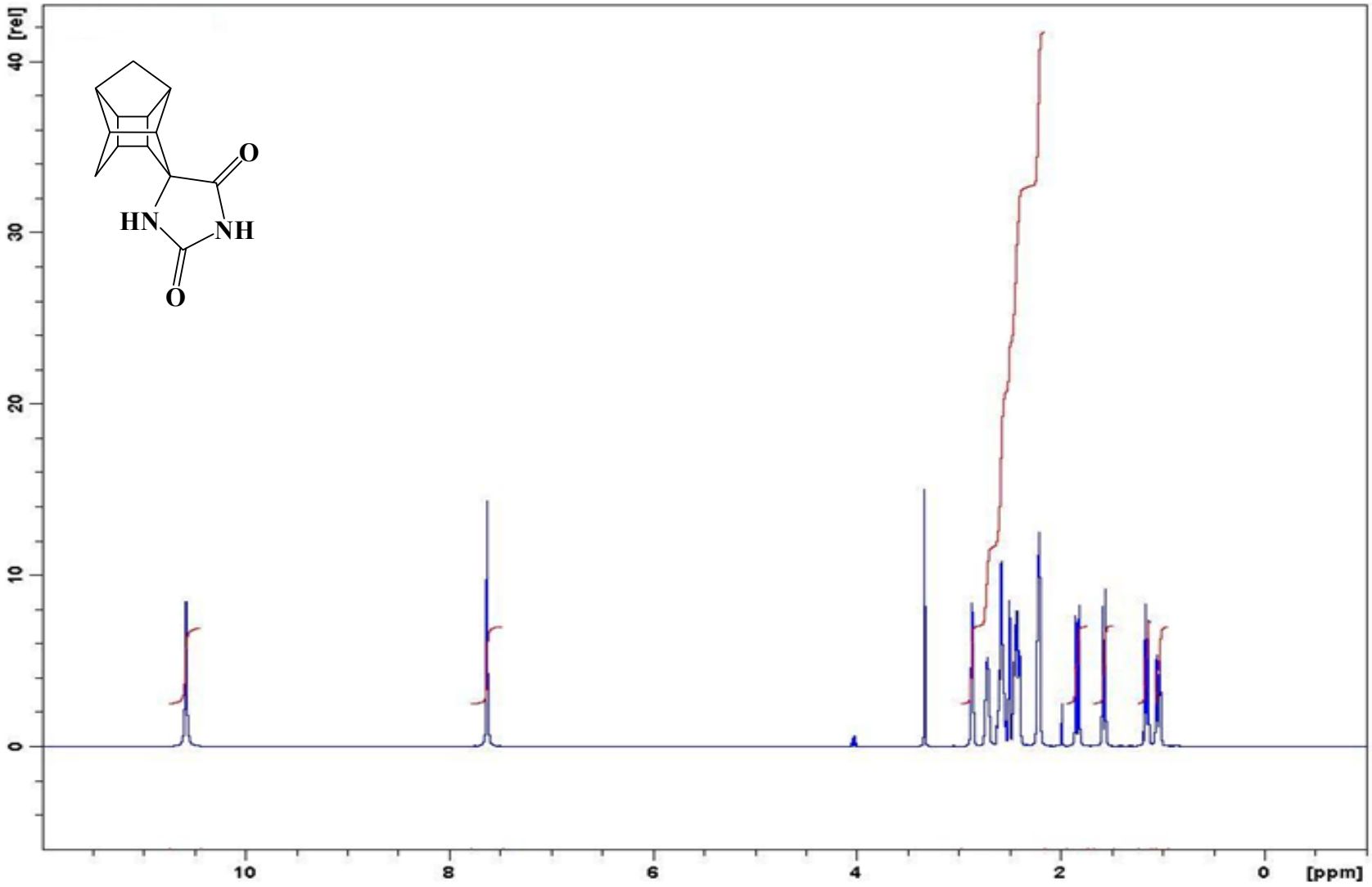
Spectrum 12: ^{1}C NMR spectrum of PCU mono ketone 64 in $CDCl_3$



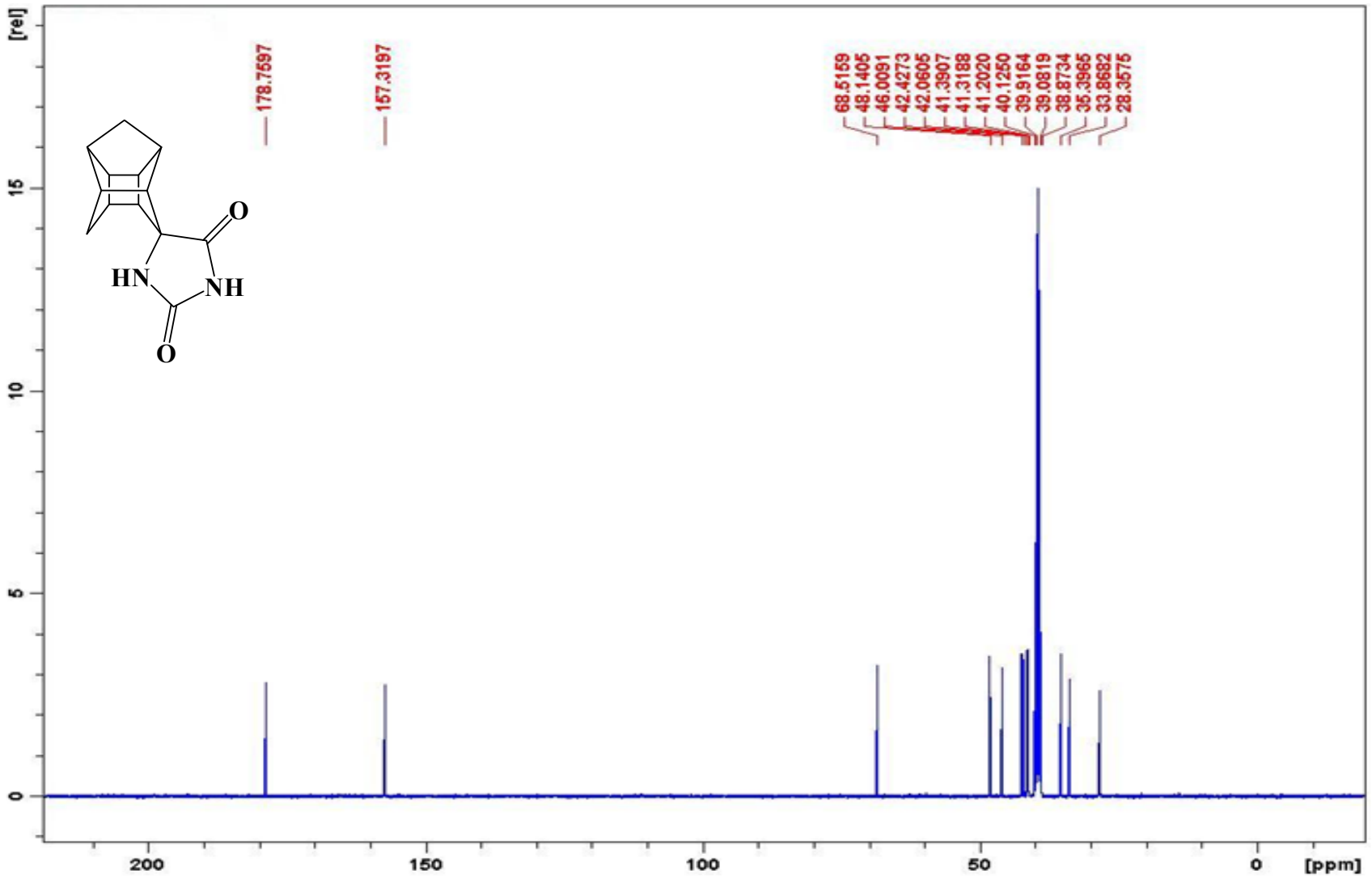
Spectrum 13: ^{1}H NMR spectrum of adamantane hydantoin 59 in $(CD_3)_2SO_2$



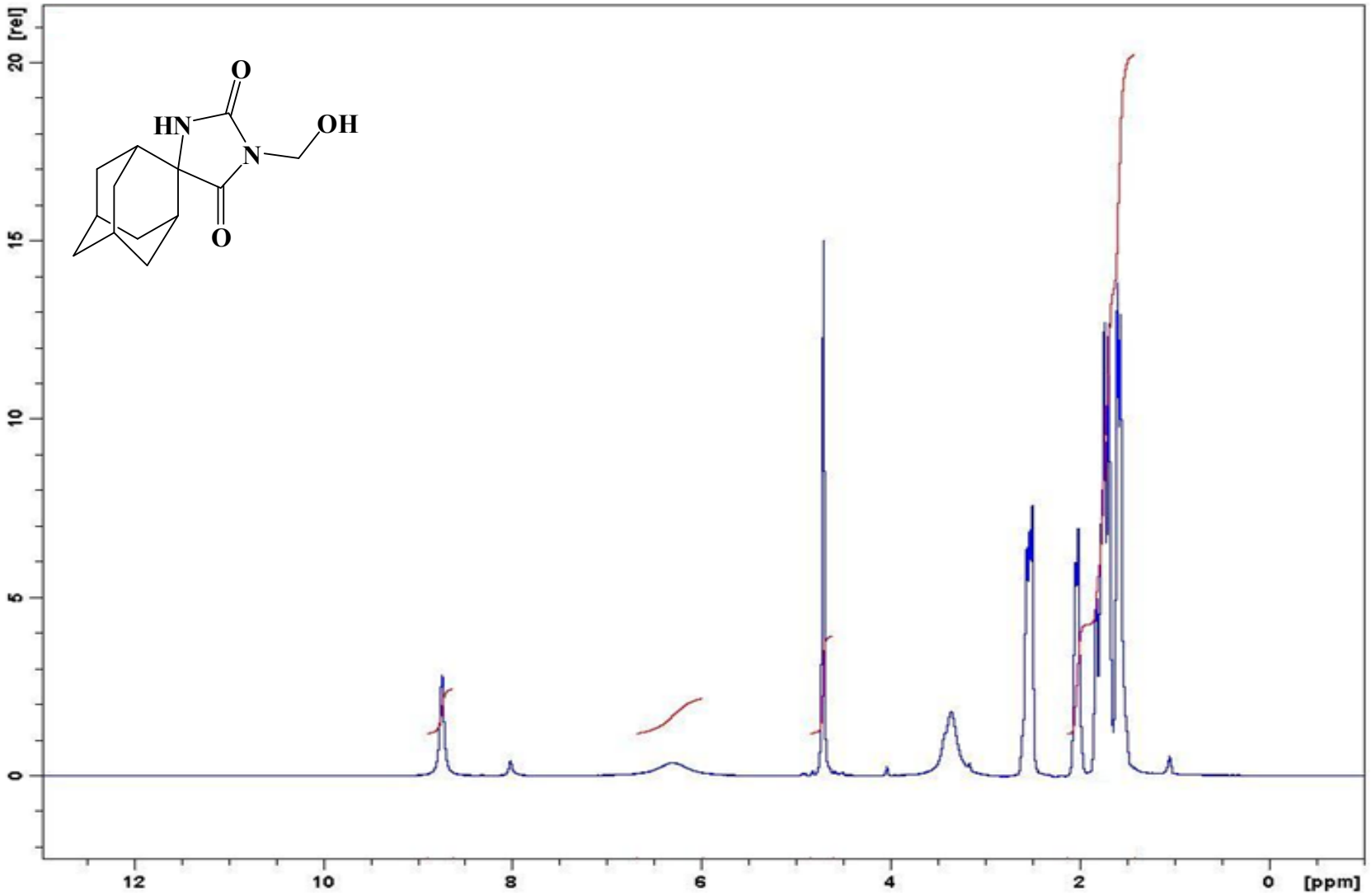
Spectrum 14: ^{13}C NMR spectrum of adamantane hydantoin 59 in $(CD_3)_2SO_2$



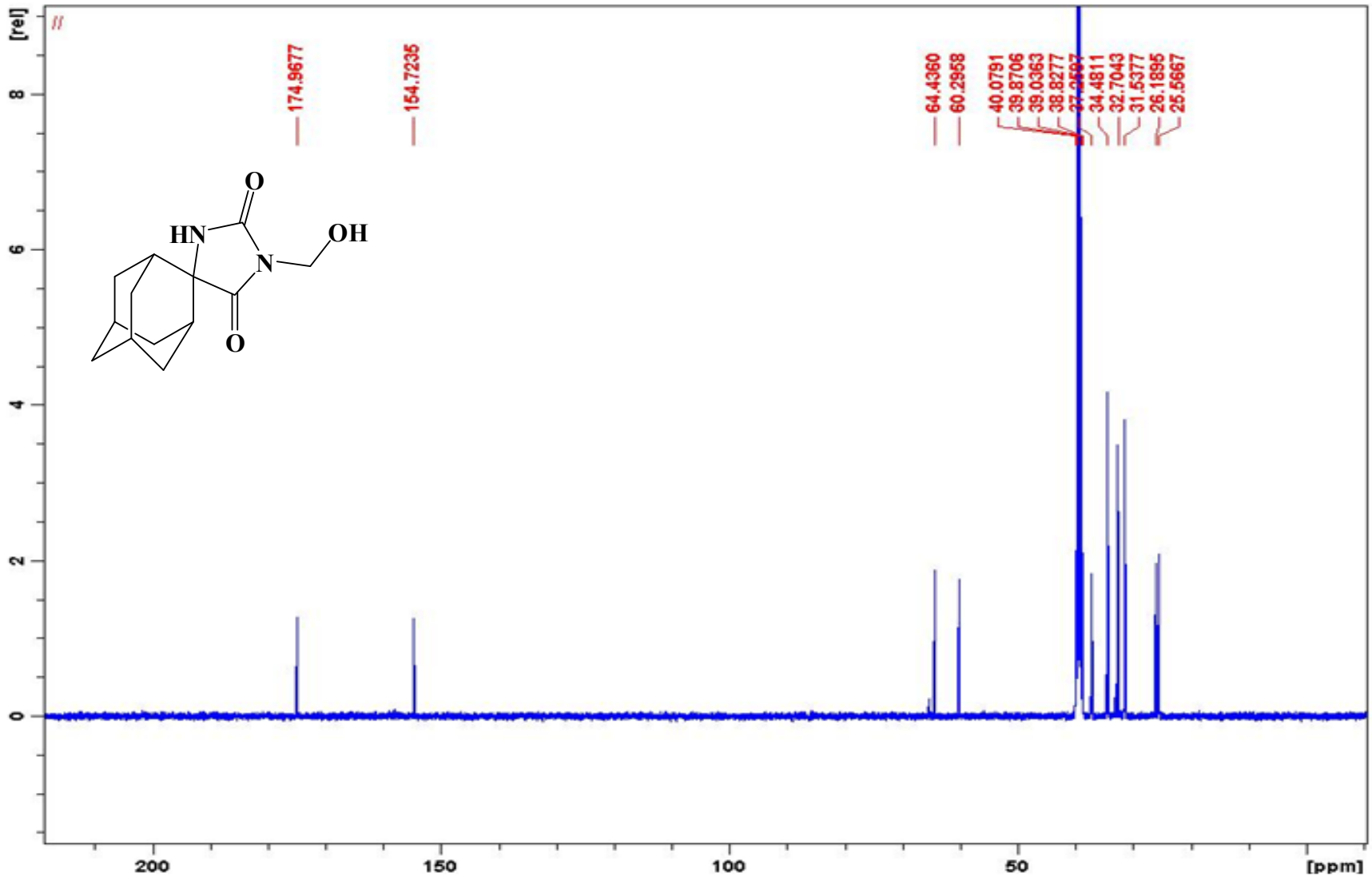
Spectrum 15: ^{1}H NMR spectrum of PCU hydantoin 60 in $(CD_3)_2SO_2$



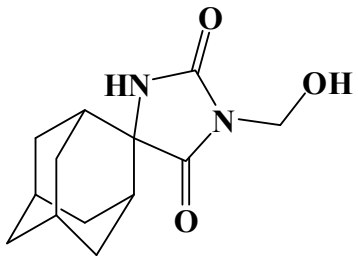
Spectrum 16: ^{13}C NMR spectrum of PCU hydantoin 60 in $(CD_3)_2SO_2$

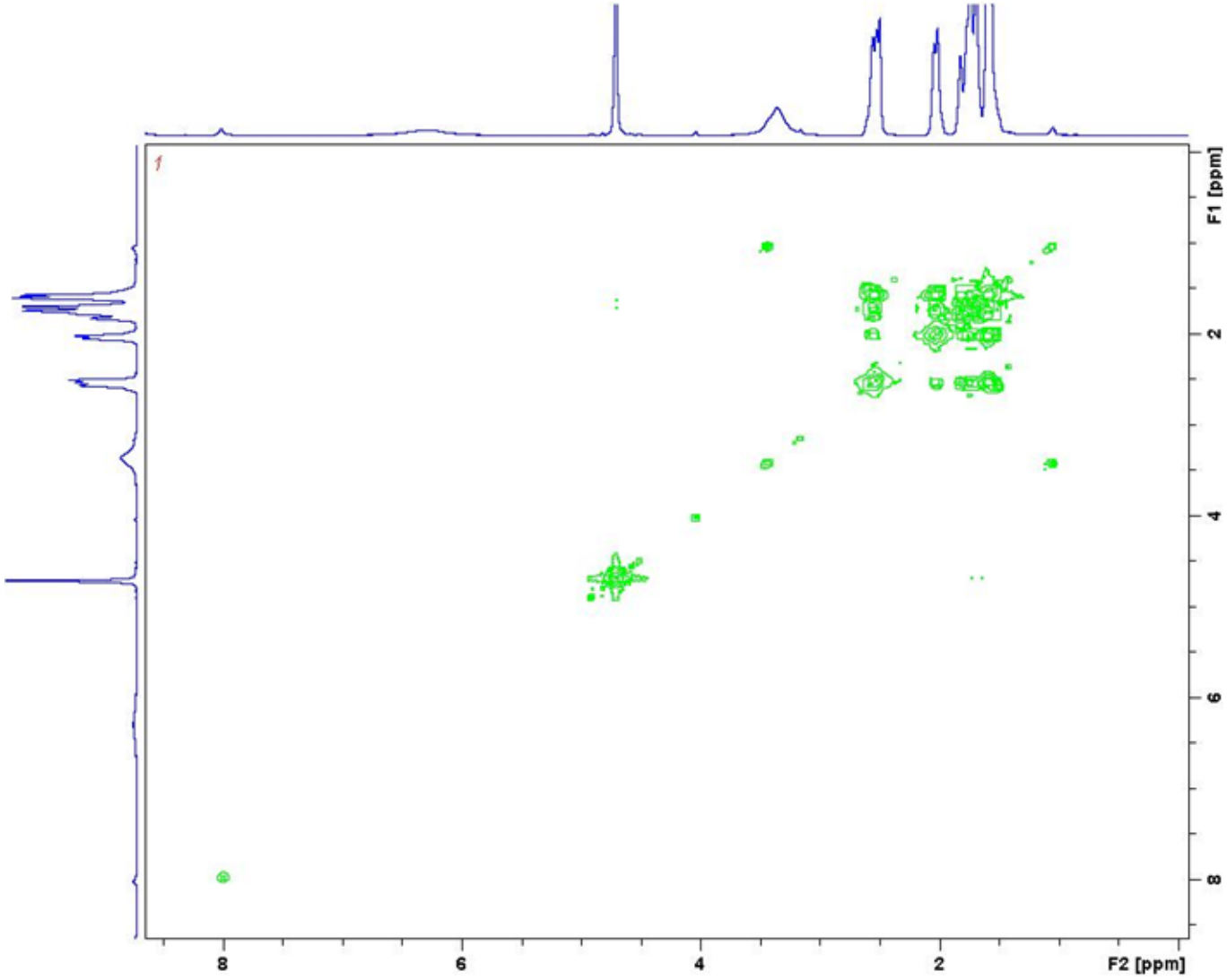


Spectrum 17: ^{1}H NMR spectrum of compound 57 in $(CD_3)_2SO_2$

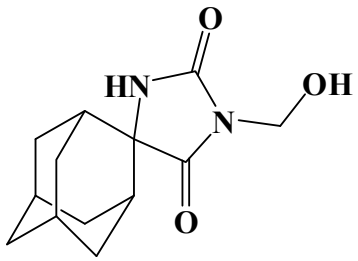


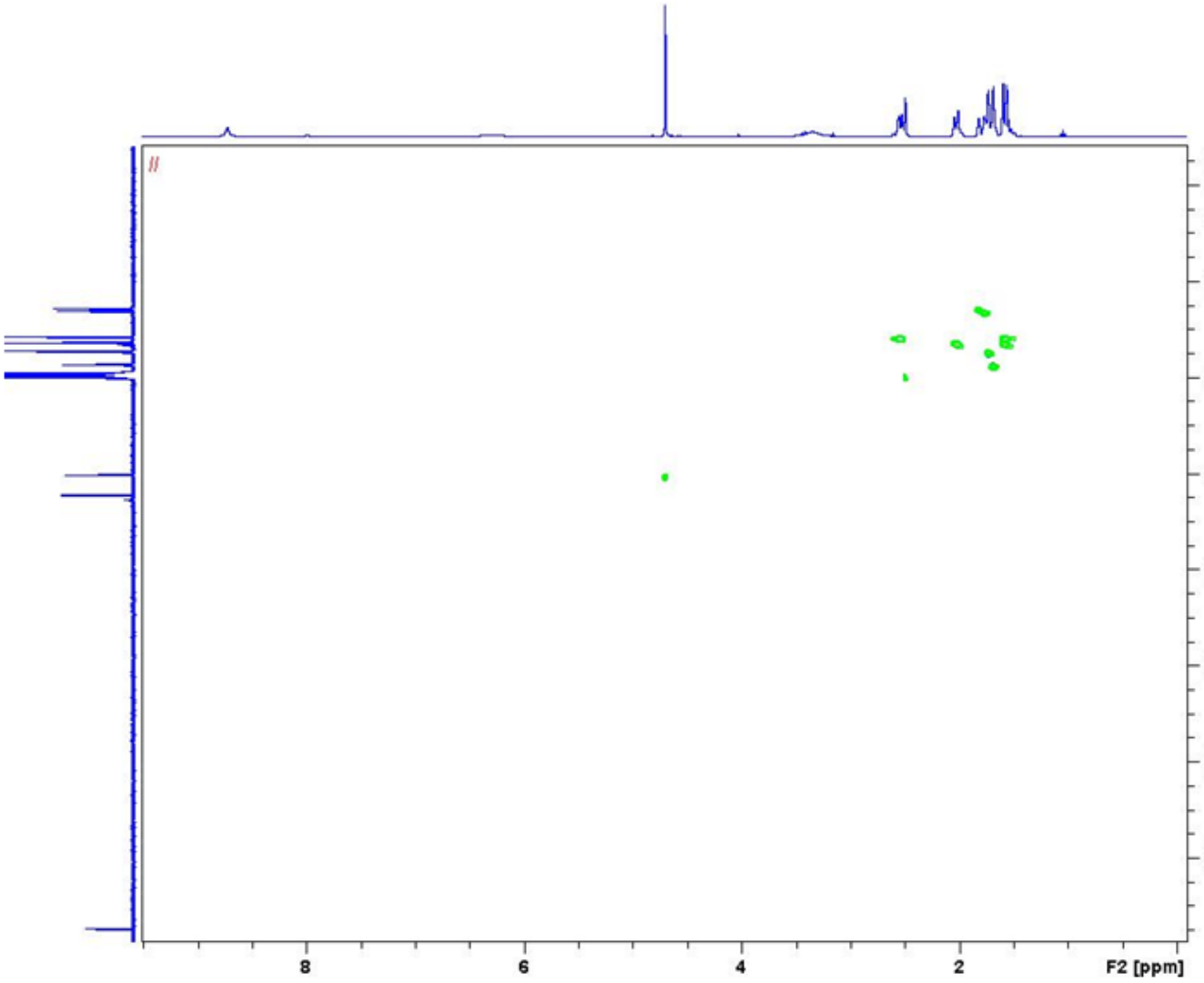
Spectrum 18: ^{13}C NMR spectrum of compound 57 in $(CD_3)_2SO_2$



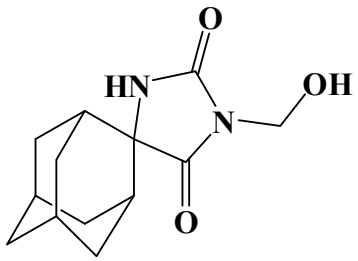


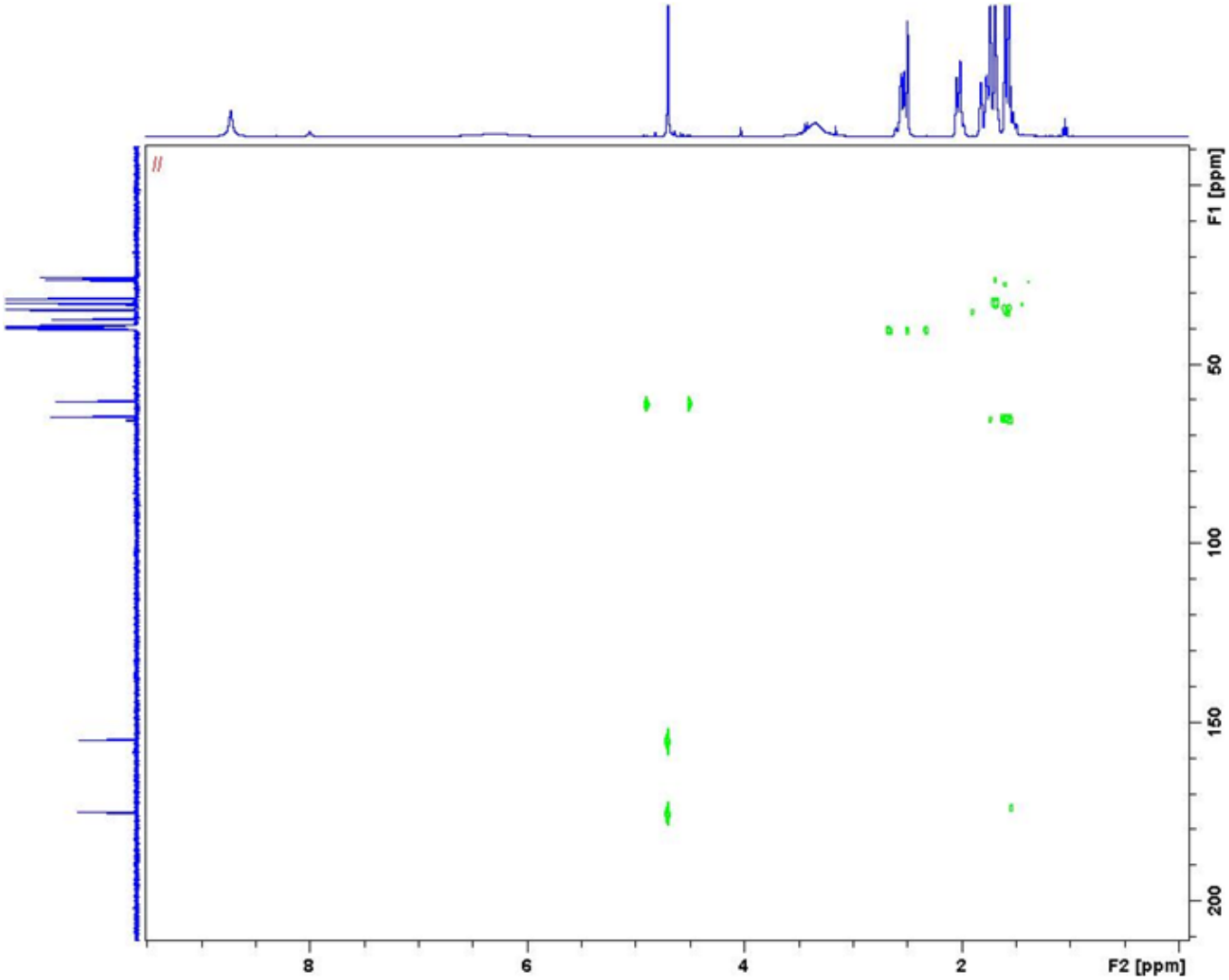
Spectrum 19: COSY spectrum of compound 57 in $(CD_3)_2SO_2$



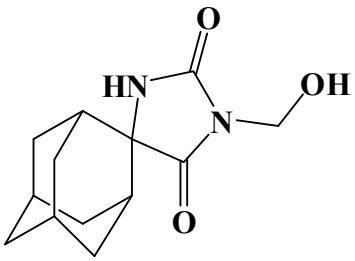


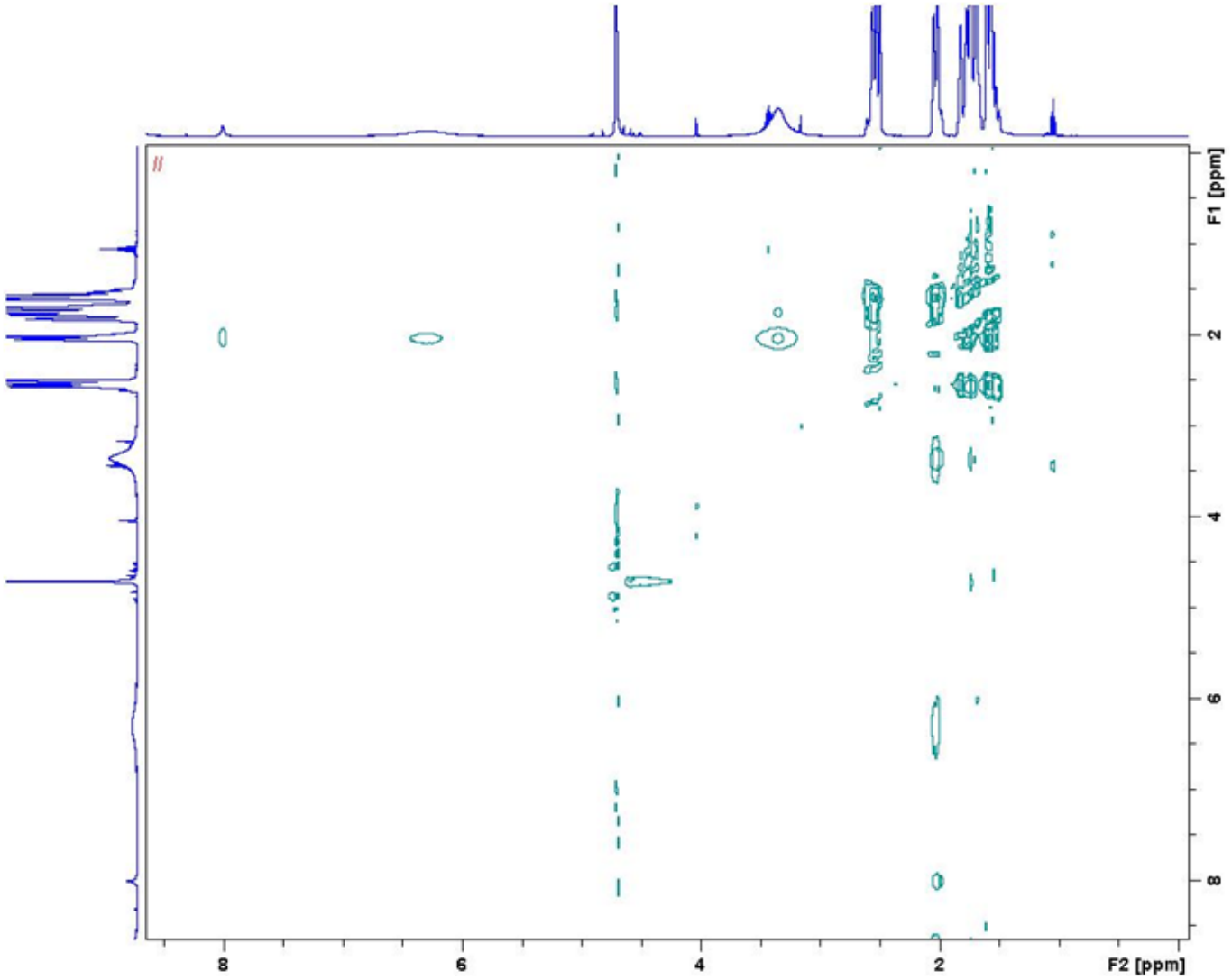
Spectrum 20: HSQC spectrum of compound 57 in $(CD_3)_2SO_2$



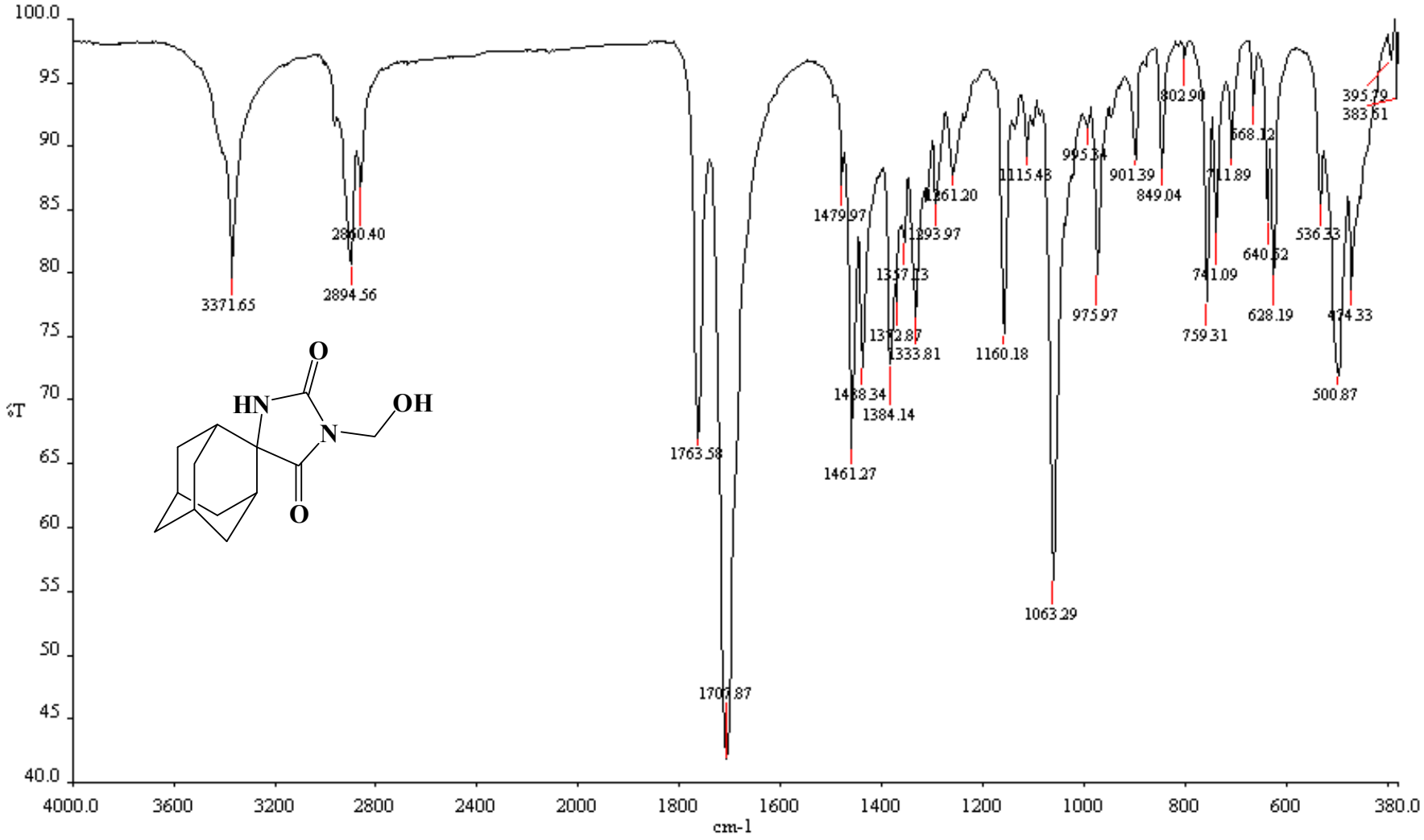


Spectrum 21: HMBC spectrum of compound 57 in $(CD_3)_2SO_2$

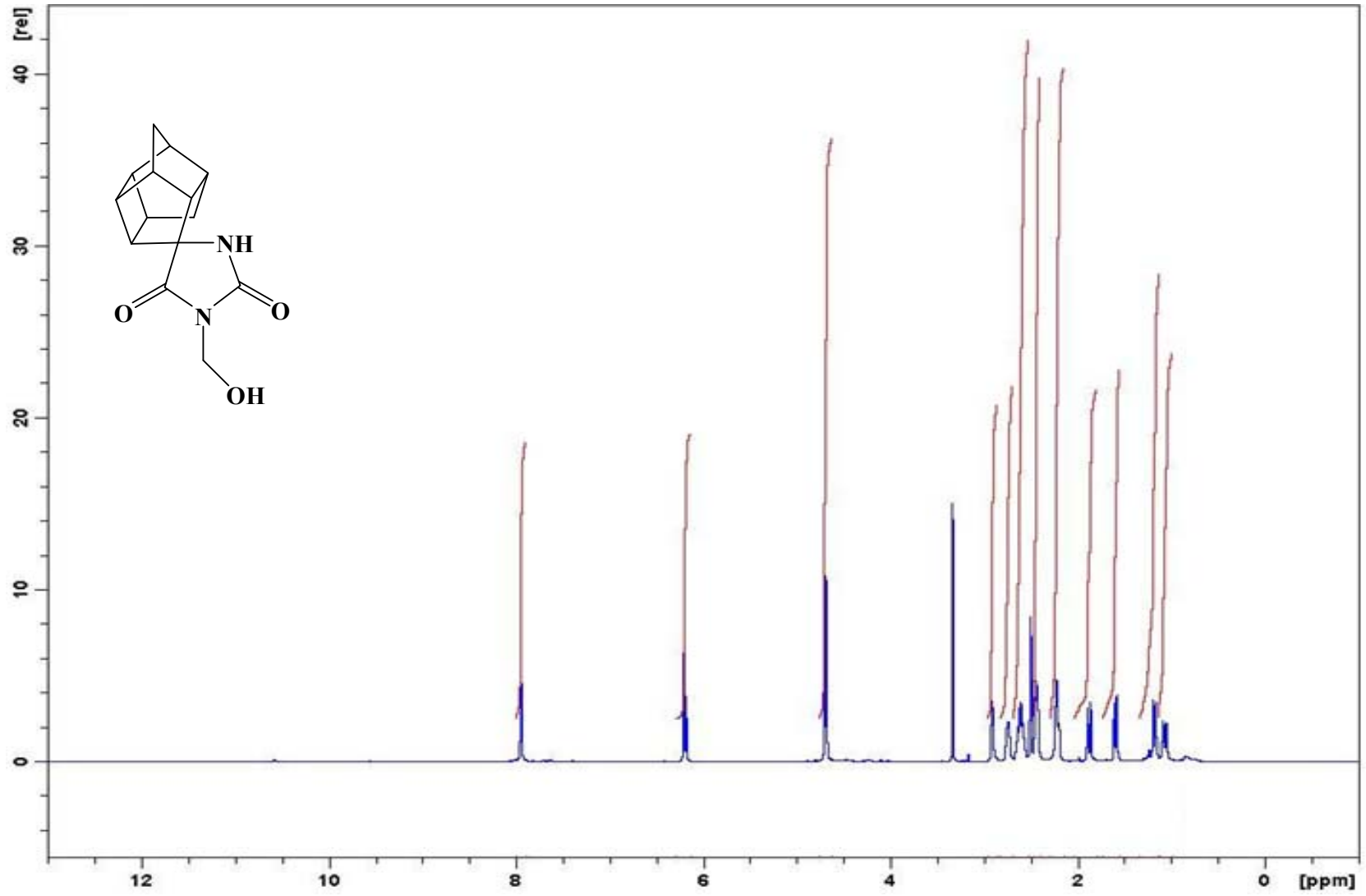




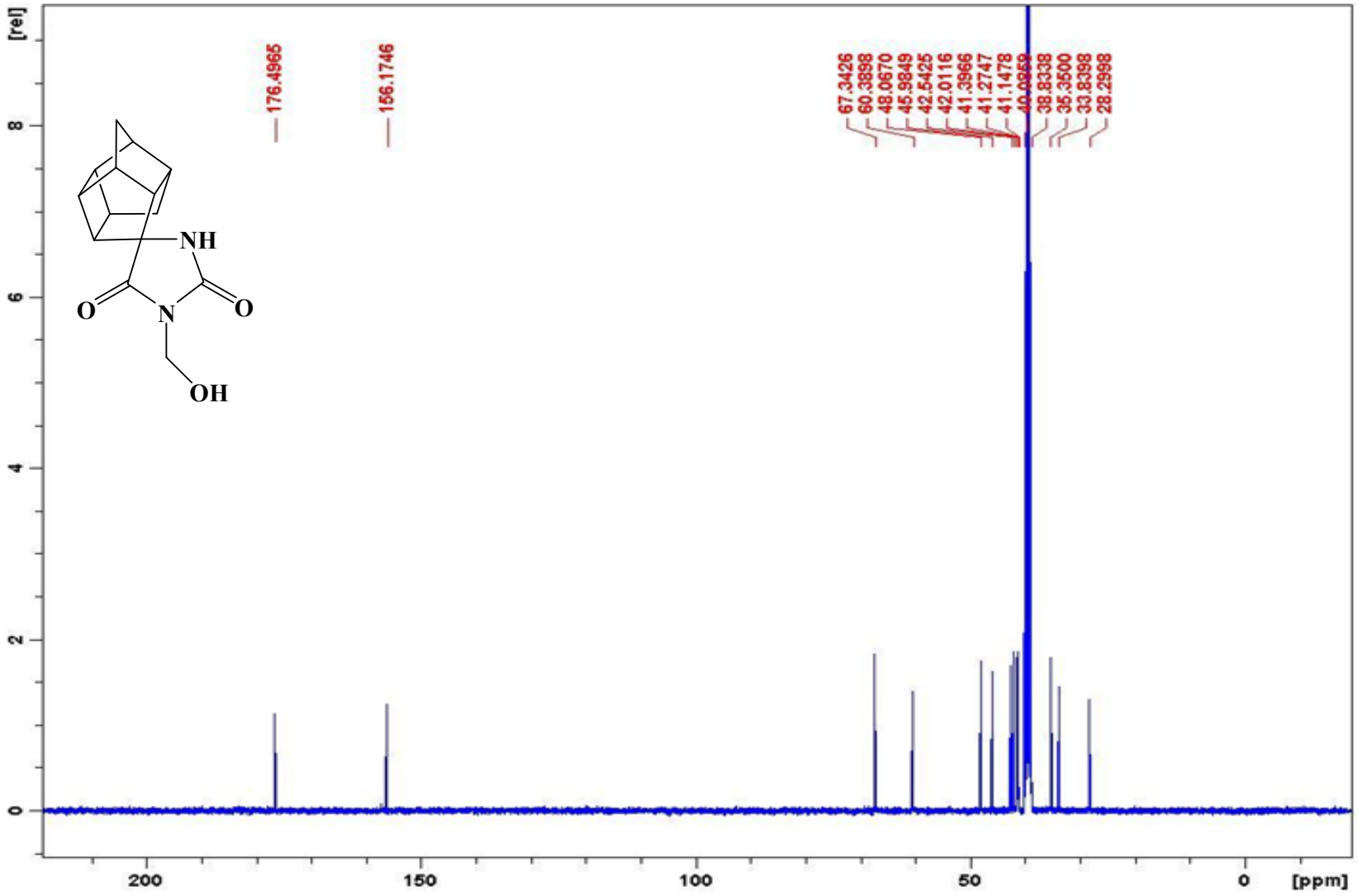
Spectrum 22: NOESY spectrum of compound 57 in $(CD_3)_2SO_2$



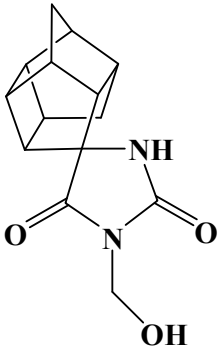
Spectrum 23: IR spectrum of compound 57

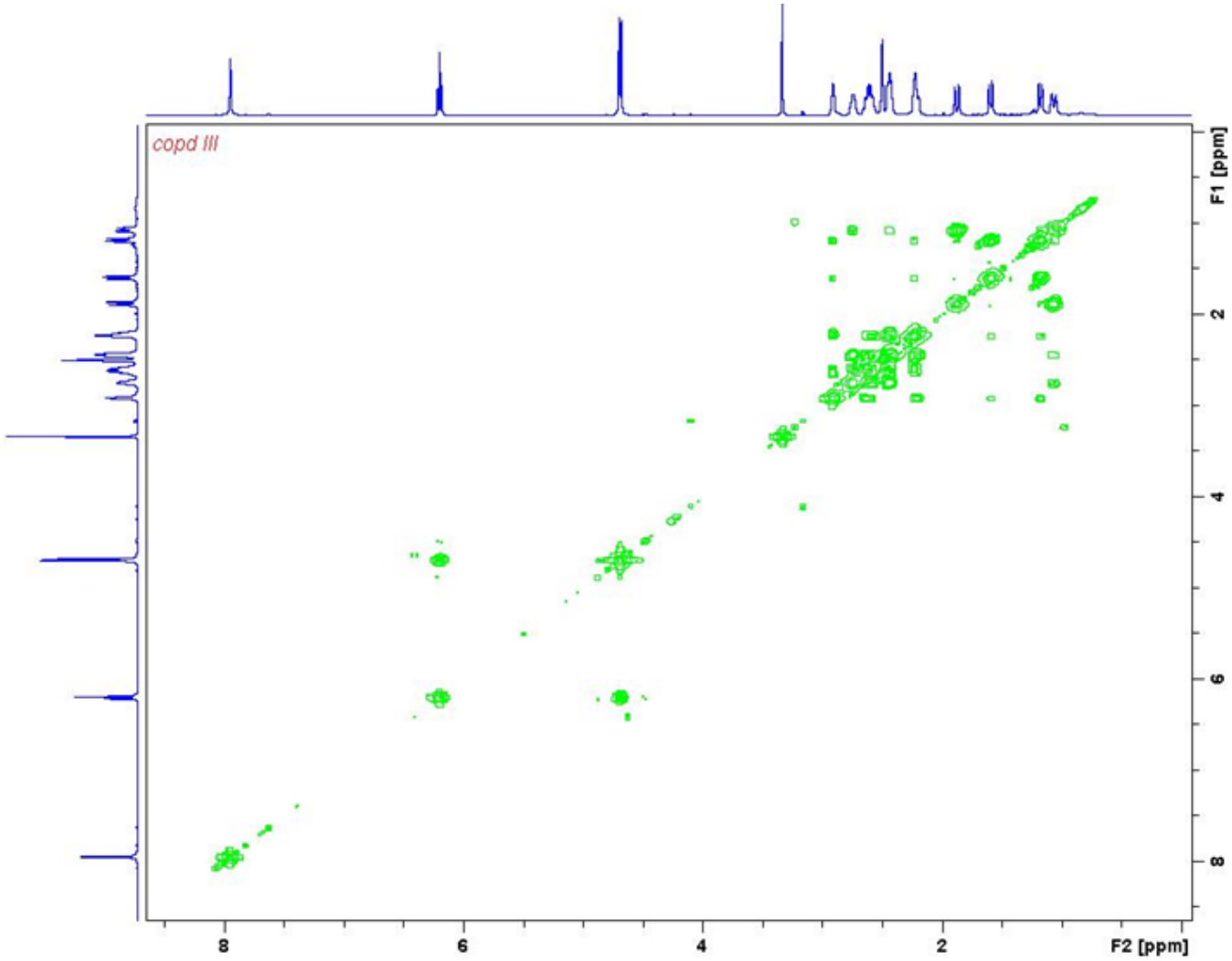


Spectrum 24: ^{1}H NMR spectrum of compound 58 in $(CD_3)_2SO_2$

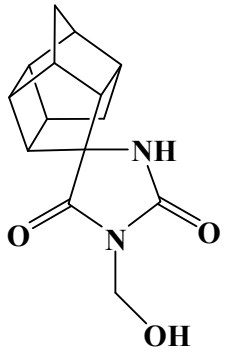


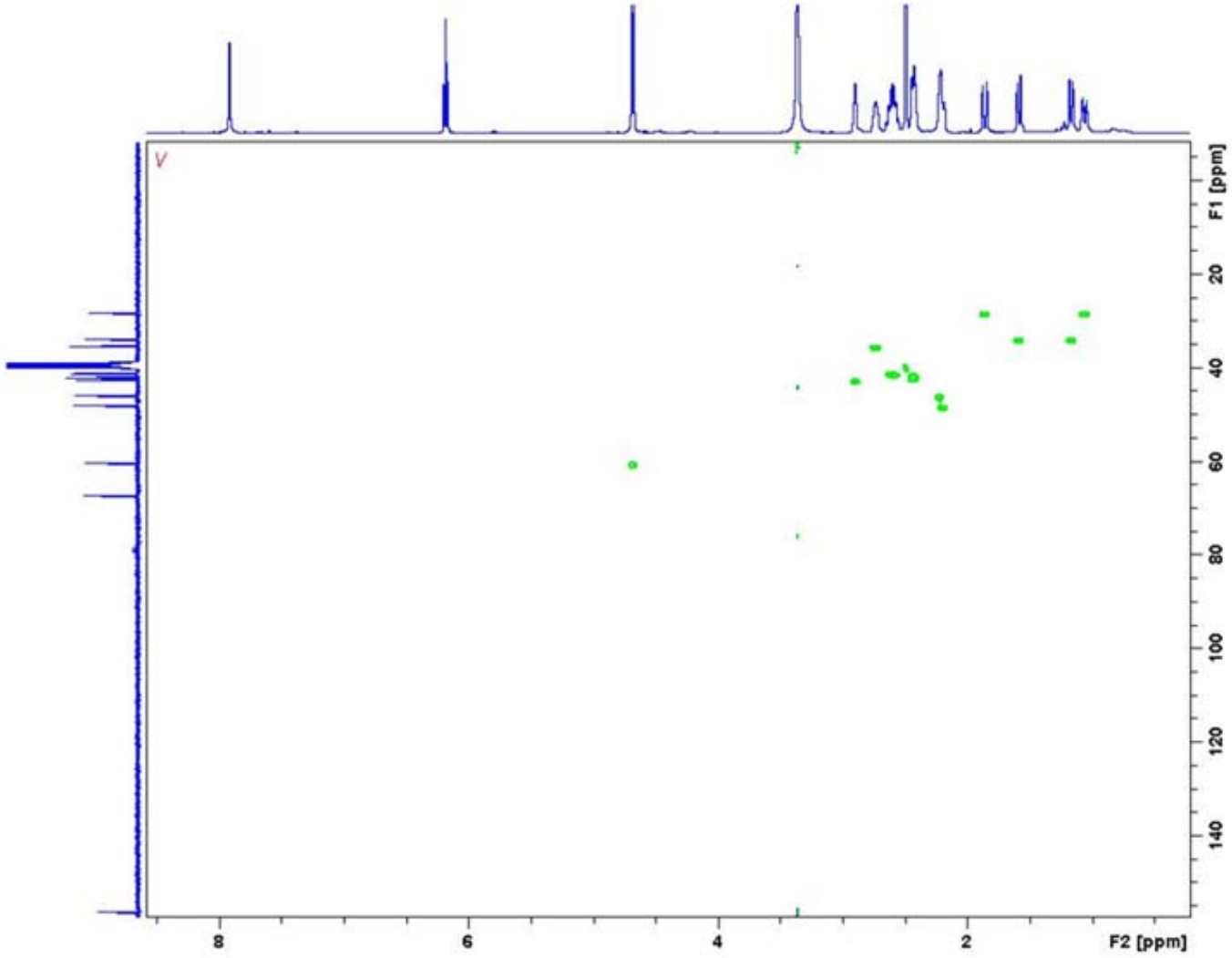
Spectrum 25: ^{13}C NMR spectrum of compound 58 in $(CD_3)_2SO_2$



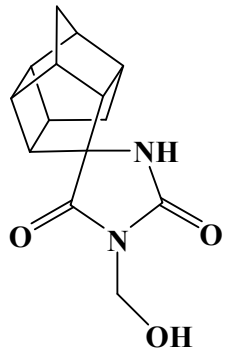


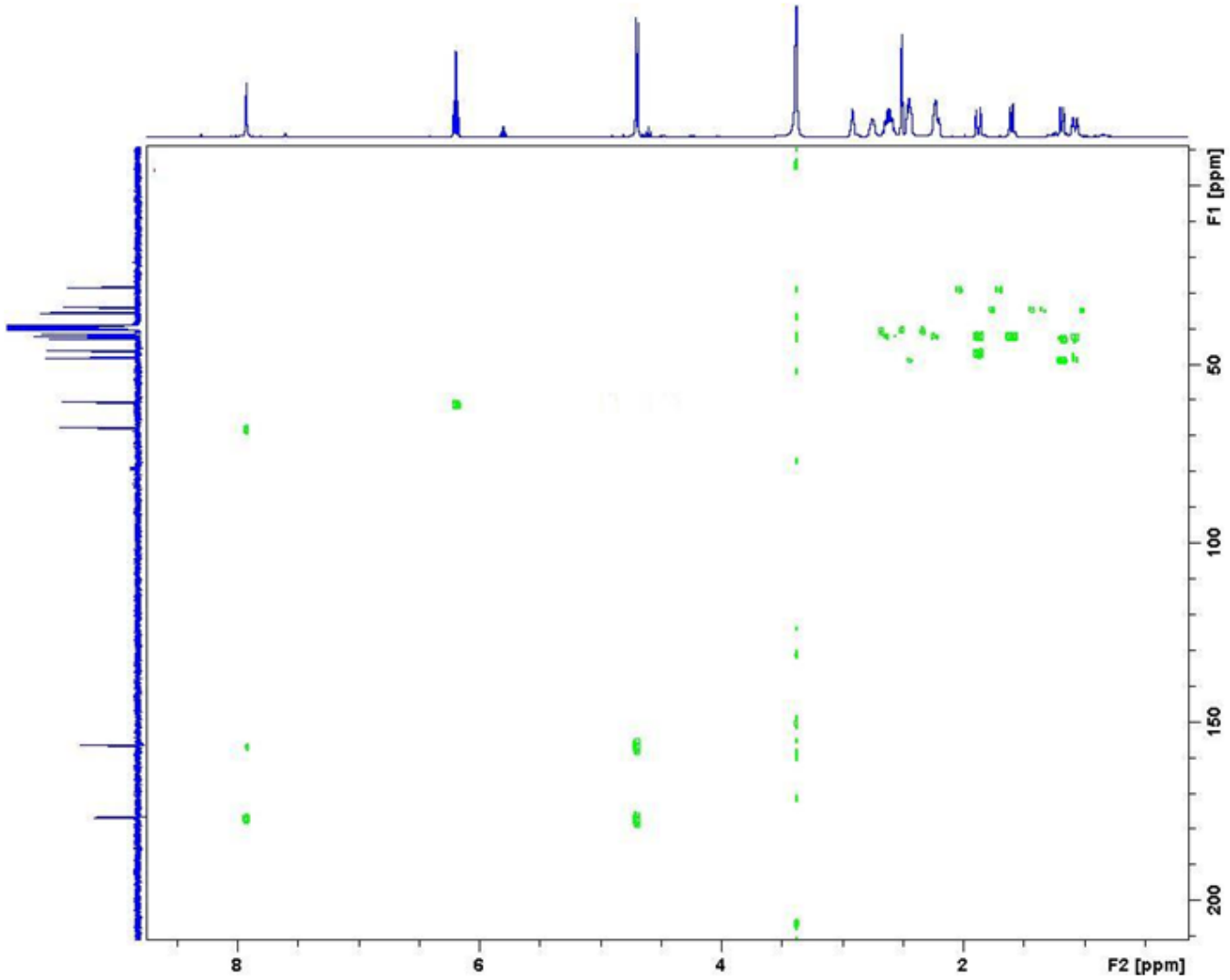
Spectrum 26: COSY spectrum of compound 58 in $(CD_3)_2SO_2$



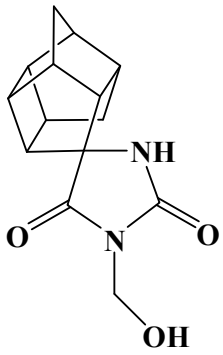


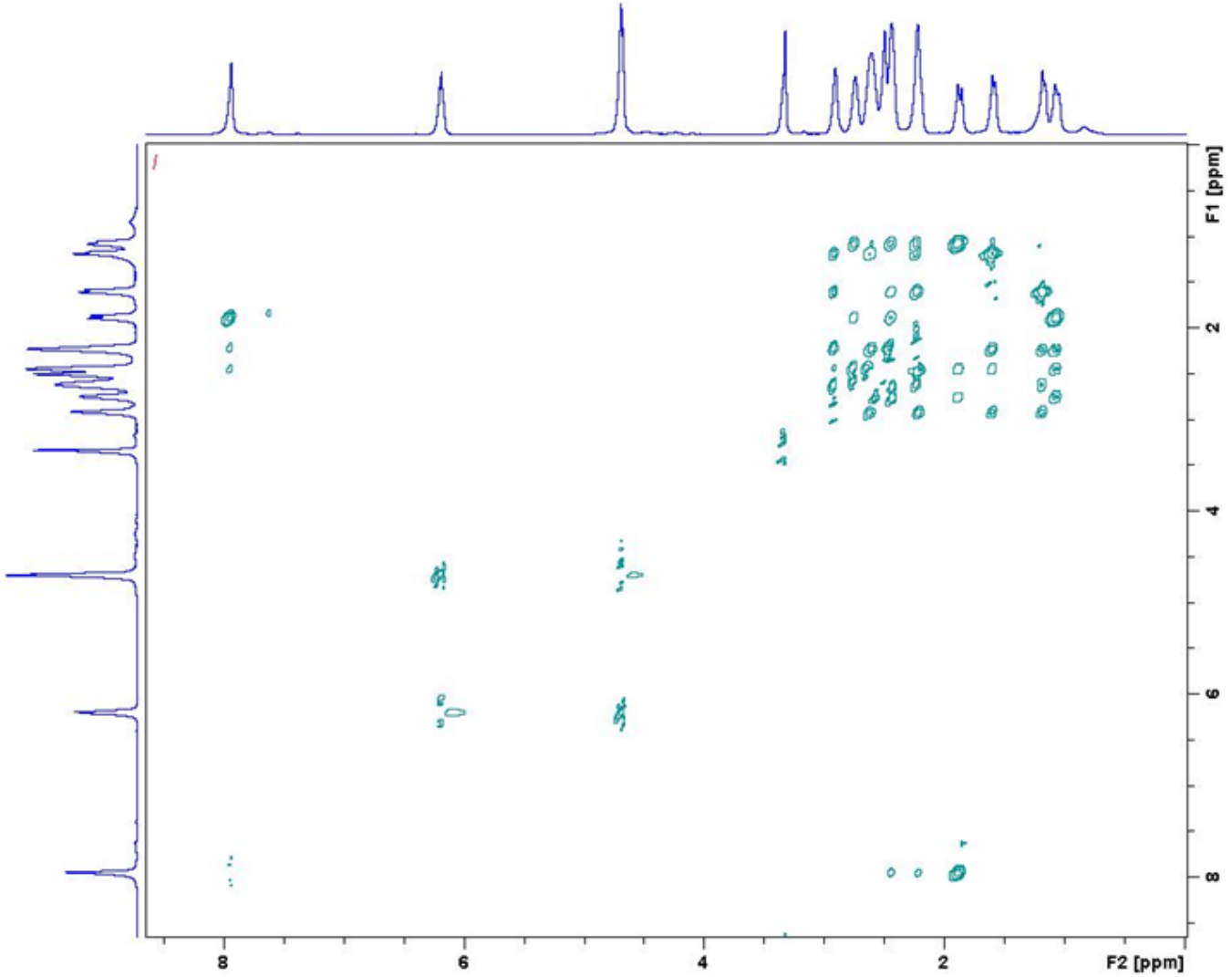
Spectrum 27: HSQC spectrum of compound 58 in $(CD_3)_2SO_2$



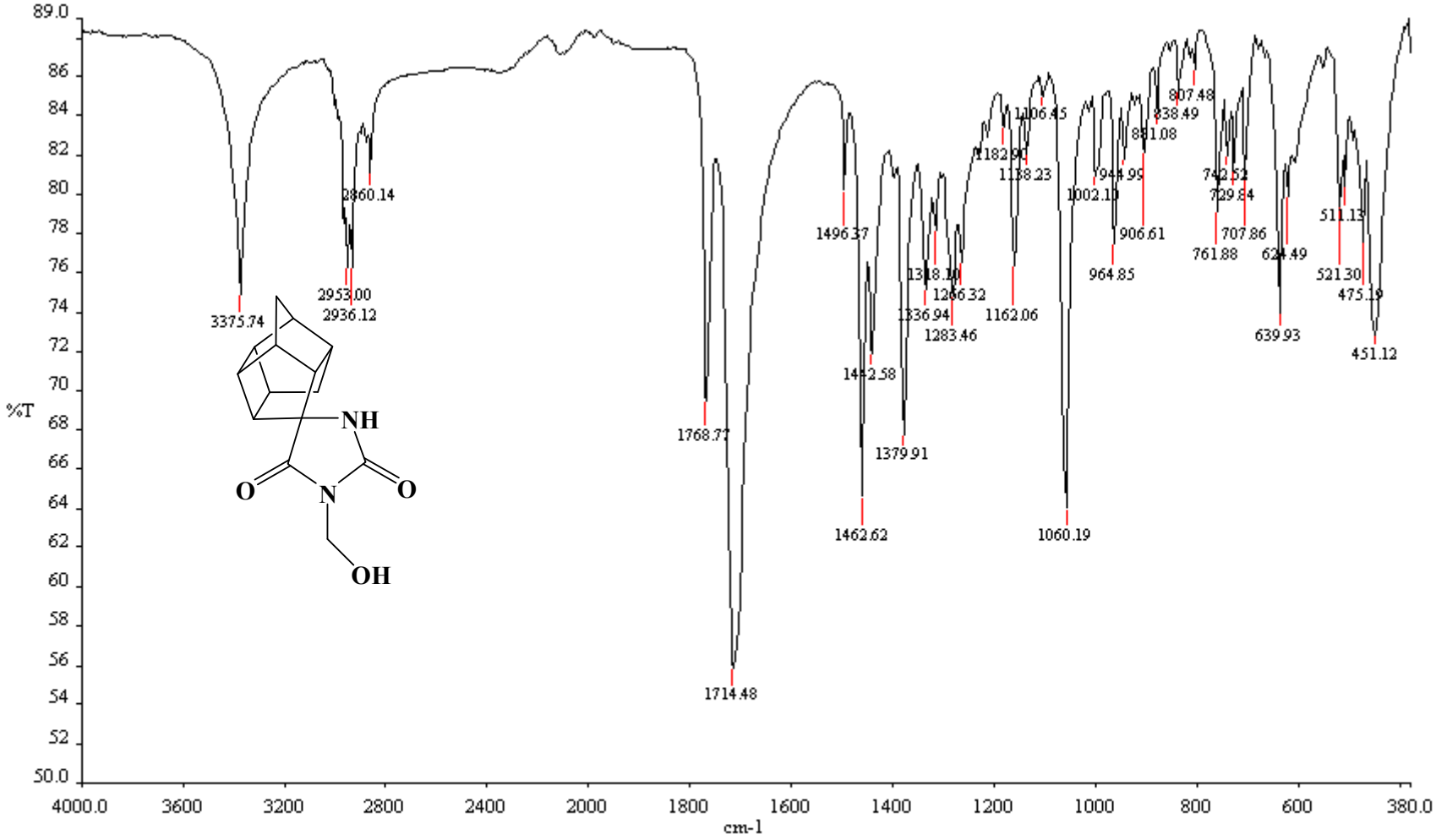


Spectrum 28: HMBC spectrum of compound 58 in $(CD_3)_2SO_2$

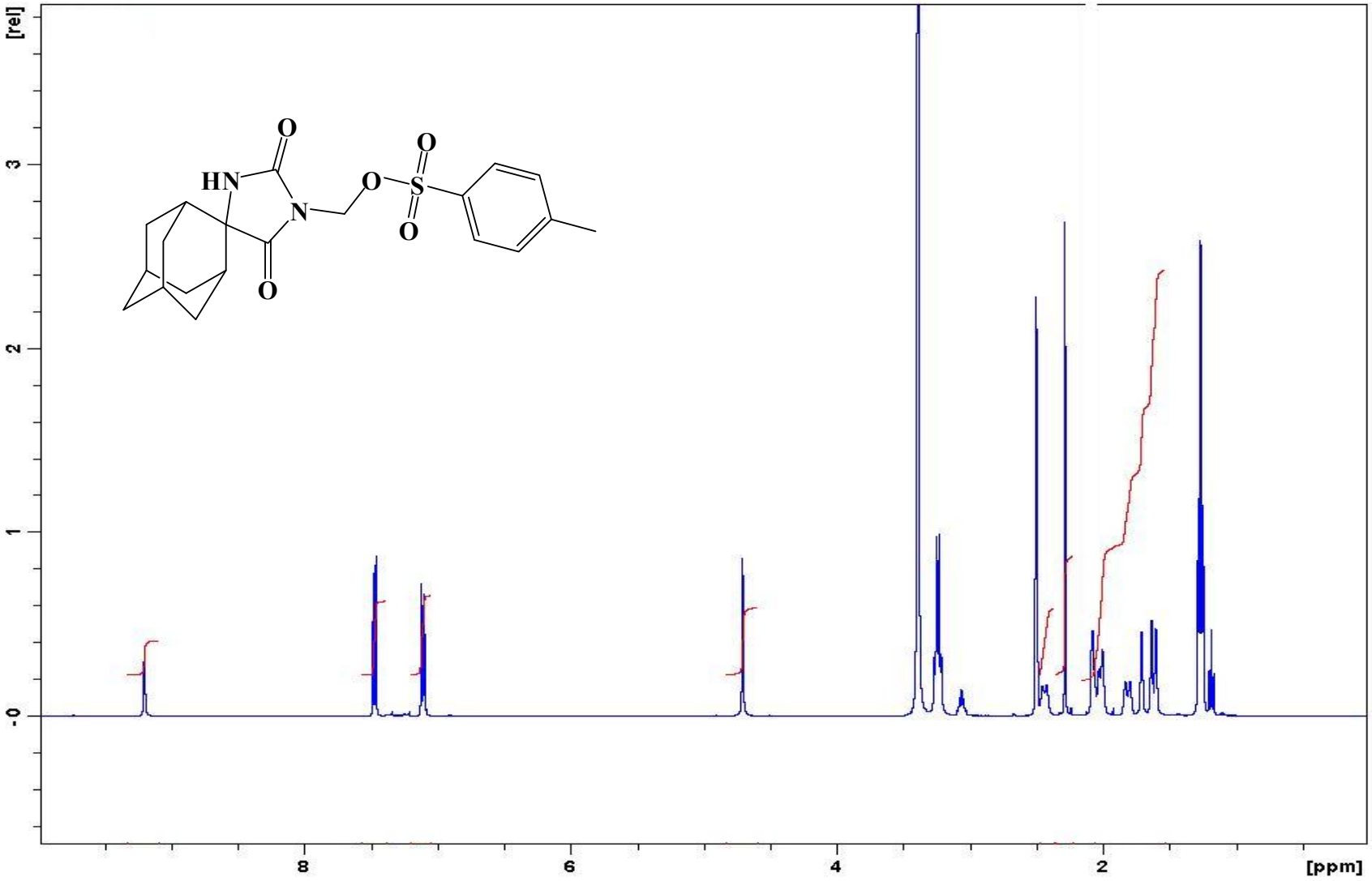




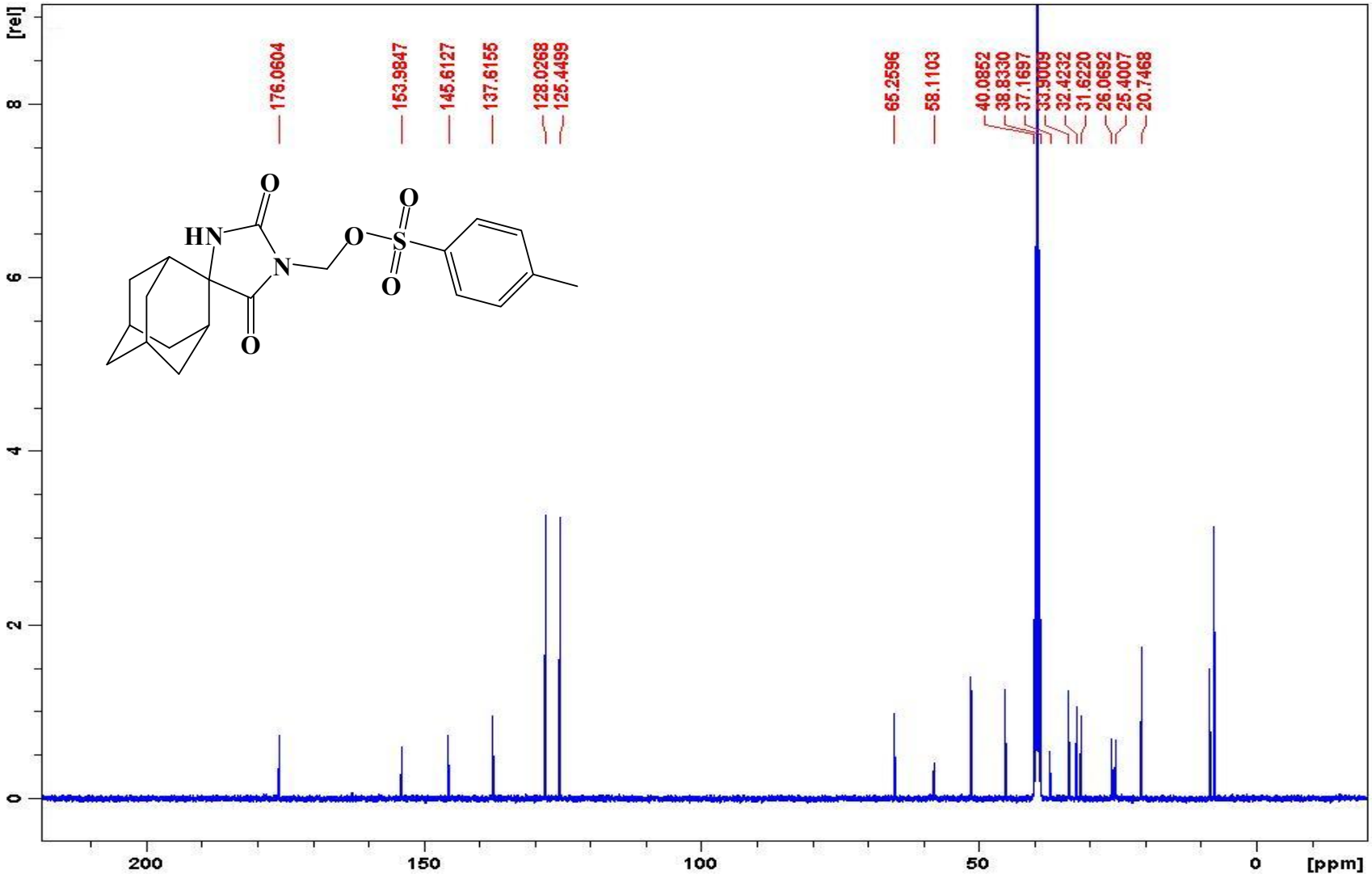
Spectrum 29: NOESY spectrum of compund 58 in $(CD_3)_2SO_2$



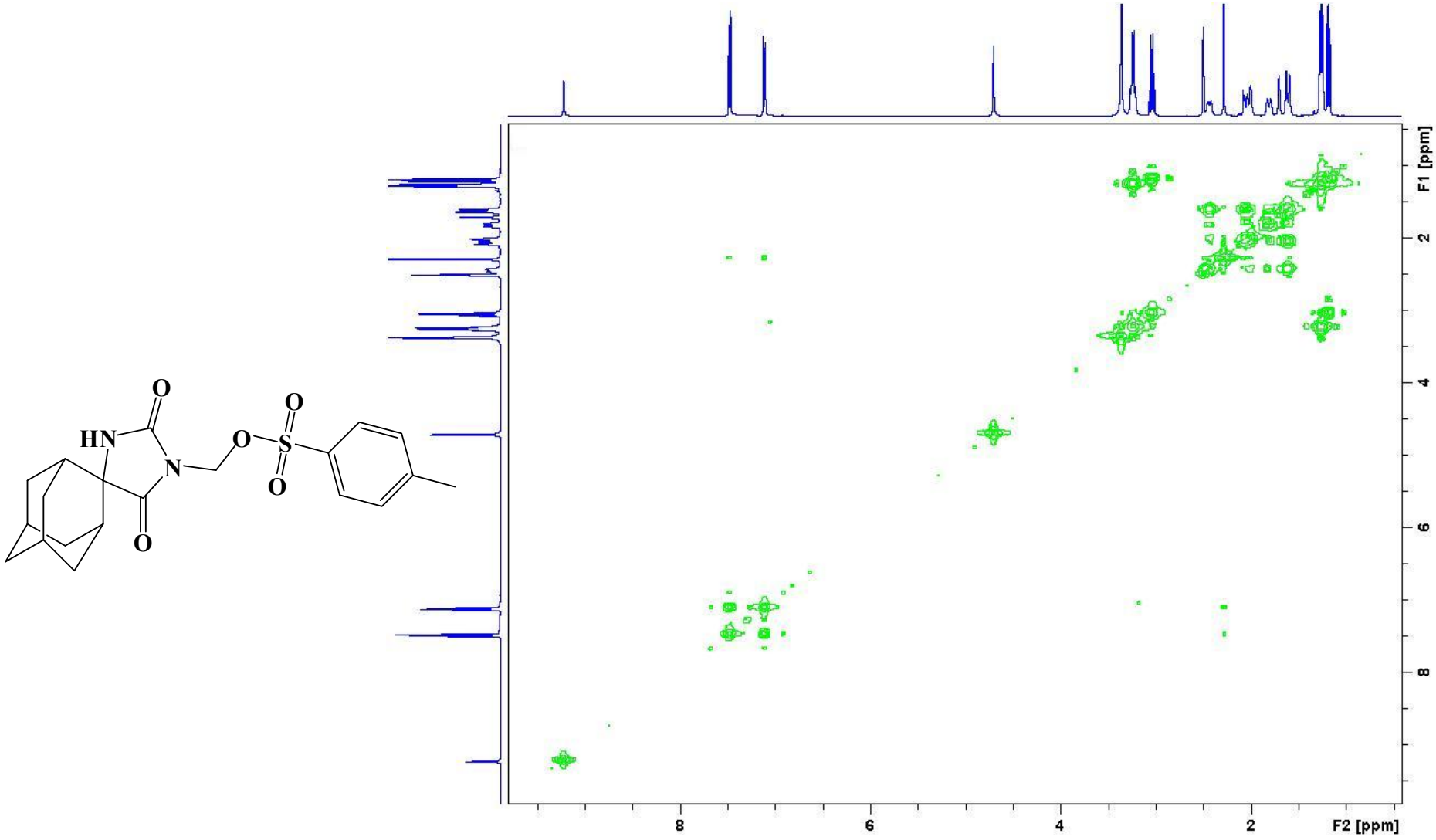
Spectrum 30: IR spectrum of compound 58



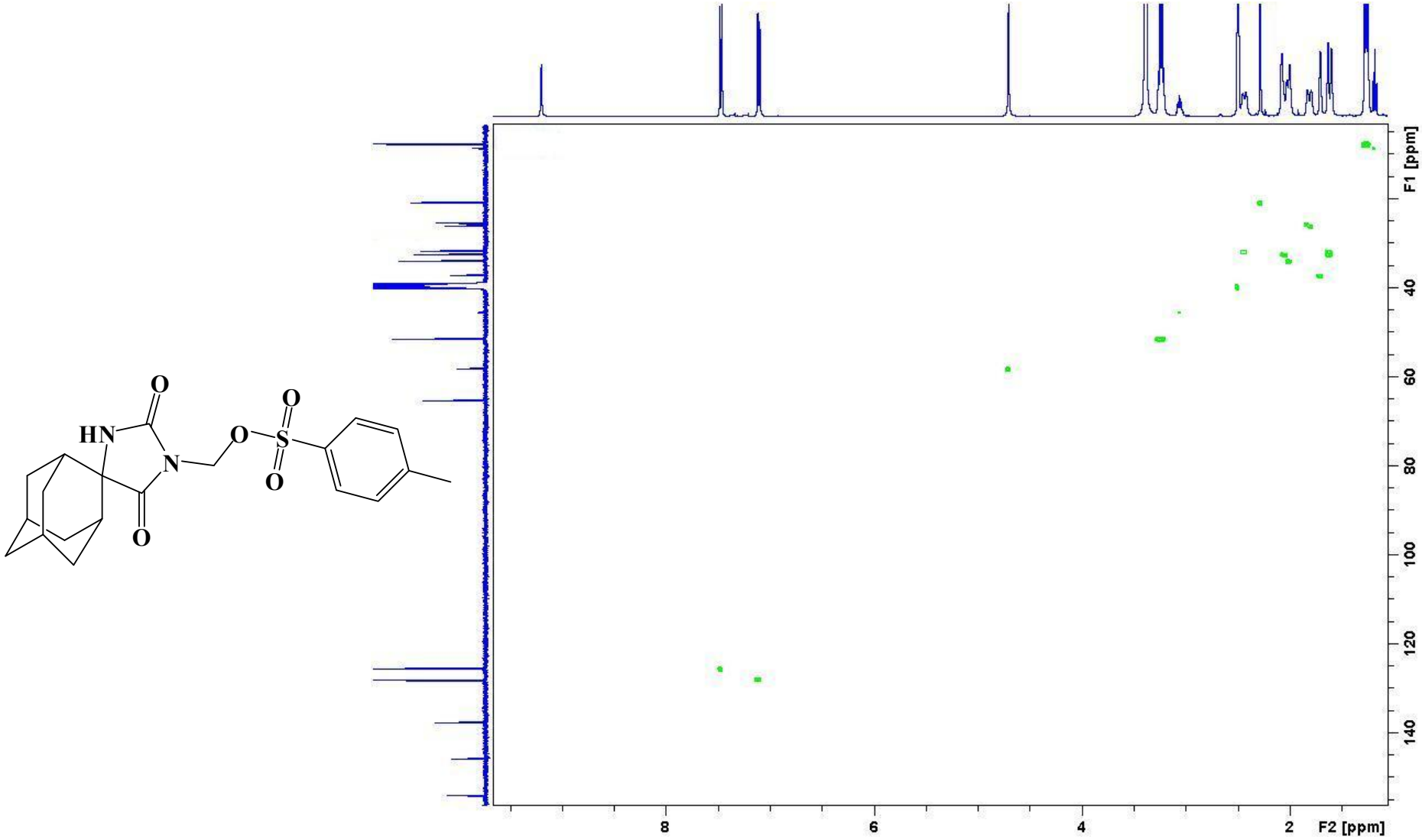
Spectrum 31: ^{1}H NMR spectrum of compound 55 in $(CD_3)_2SO_2$



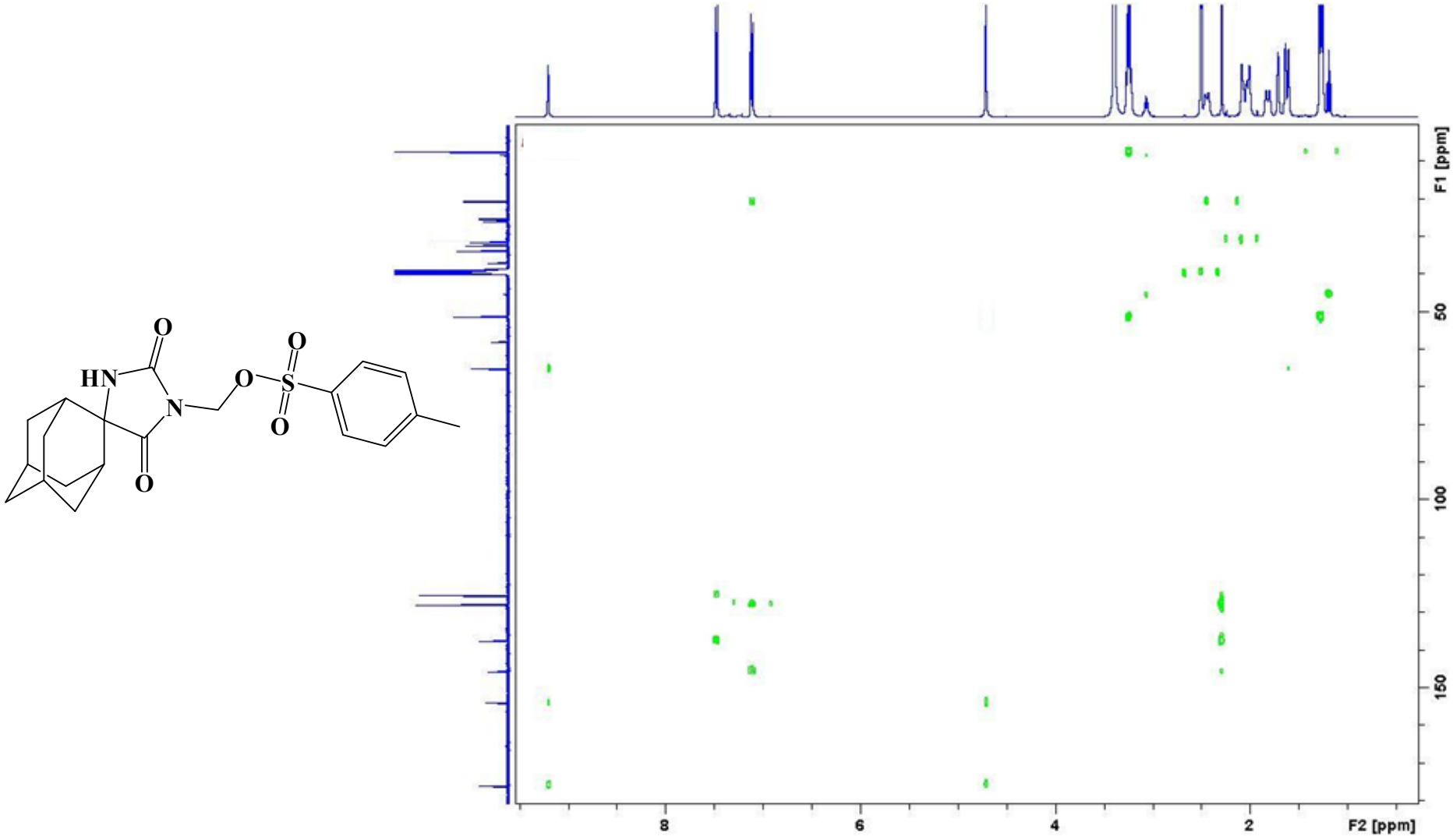
Spectrum 32: ^{13}C NMR spectrum of compound 55 in $(CD_3)_2SO_2$



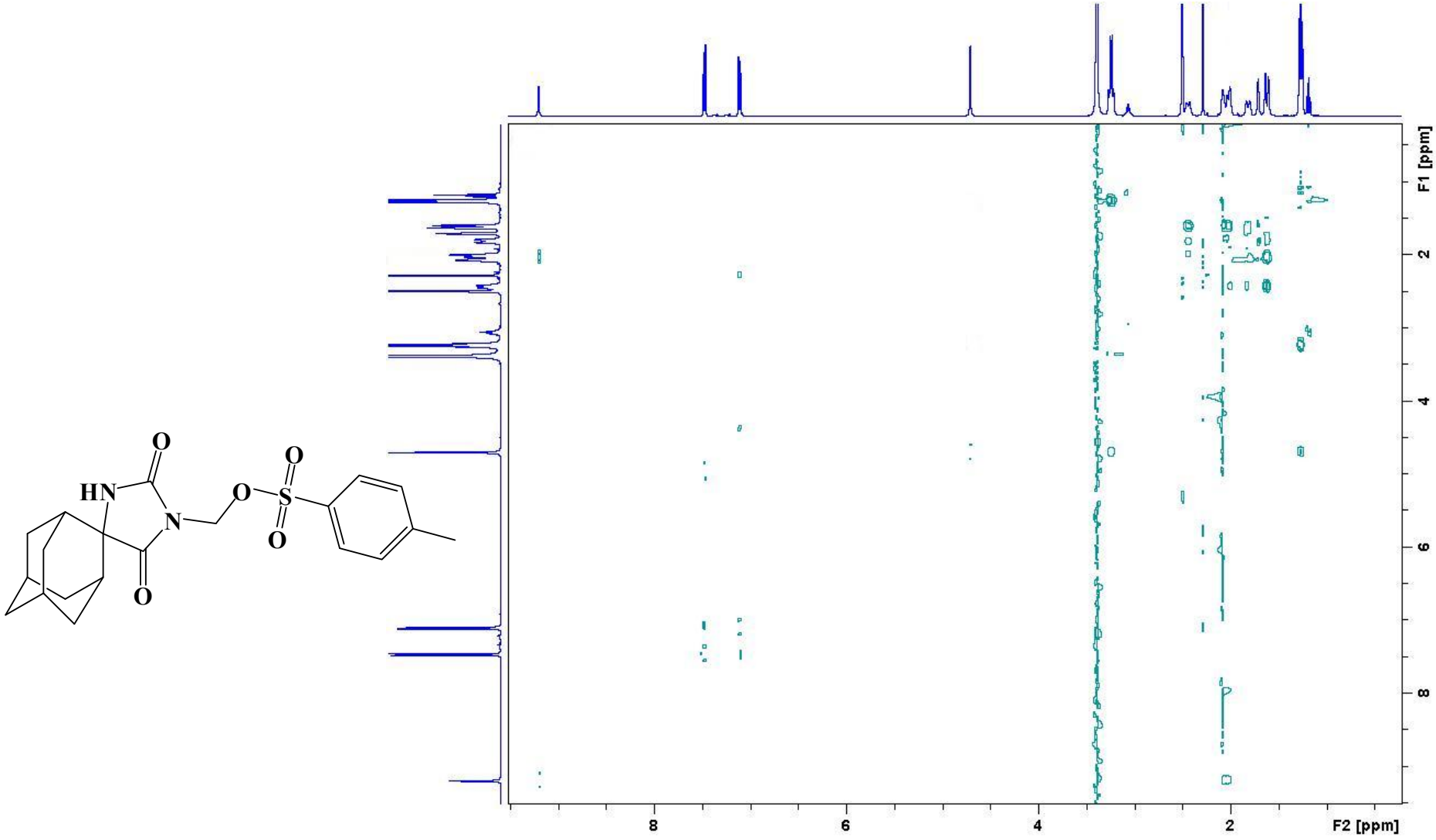
Spectrum 33: COSY spectrum of compound 55 in $(CD_3)_2SO_2$



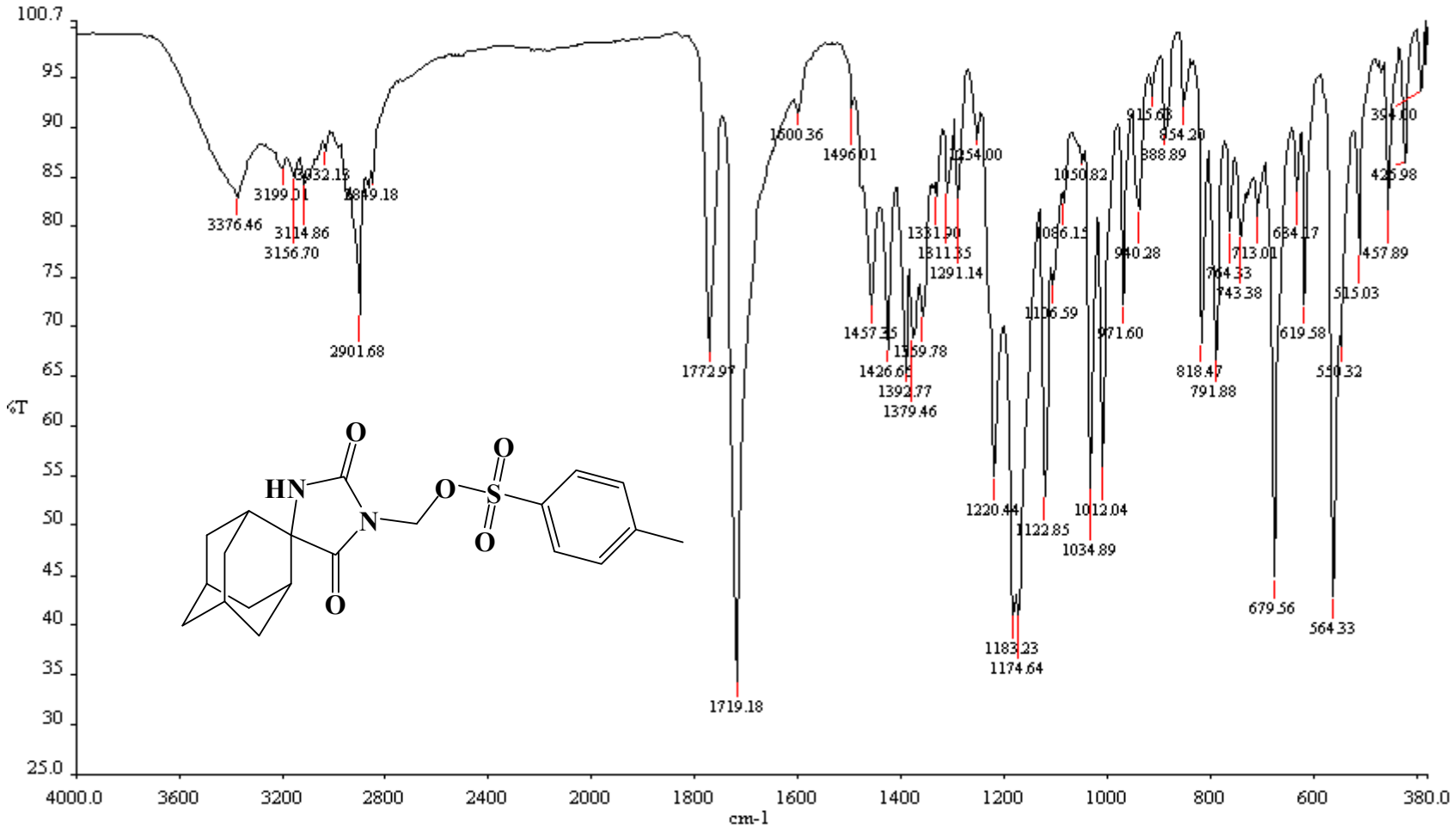
Spectrum 34: HSQC spectrum of compound 55 in $(CD_3)_2SO_2$



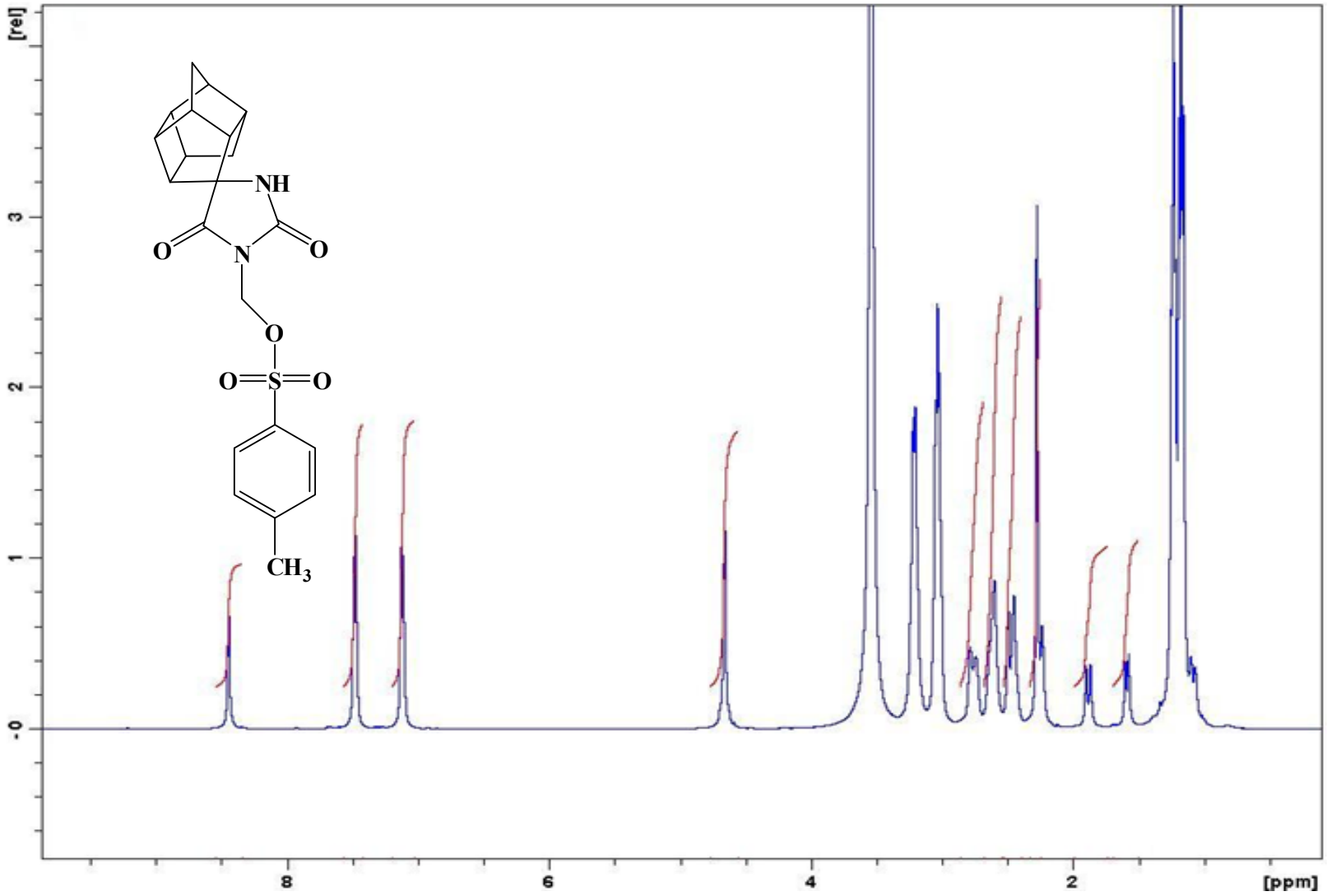
Spectrum 35: HMBCspectrum of compound 55 in $(CD_3)_2SO_2$



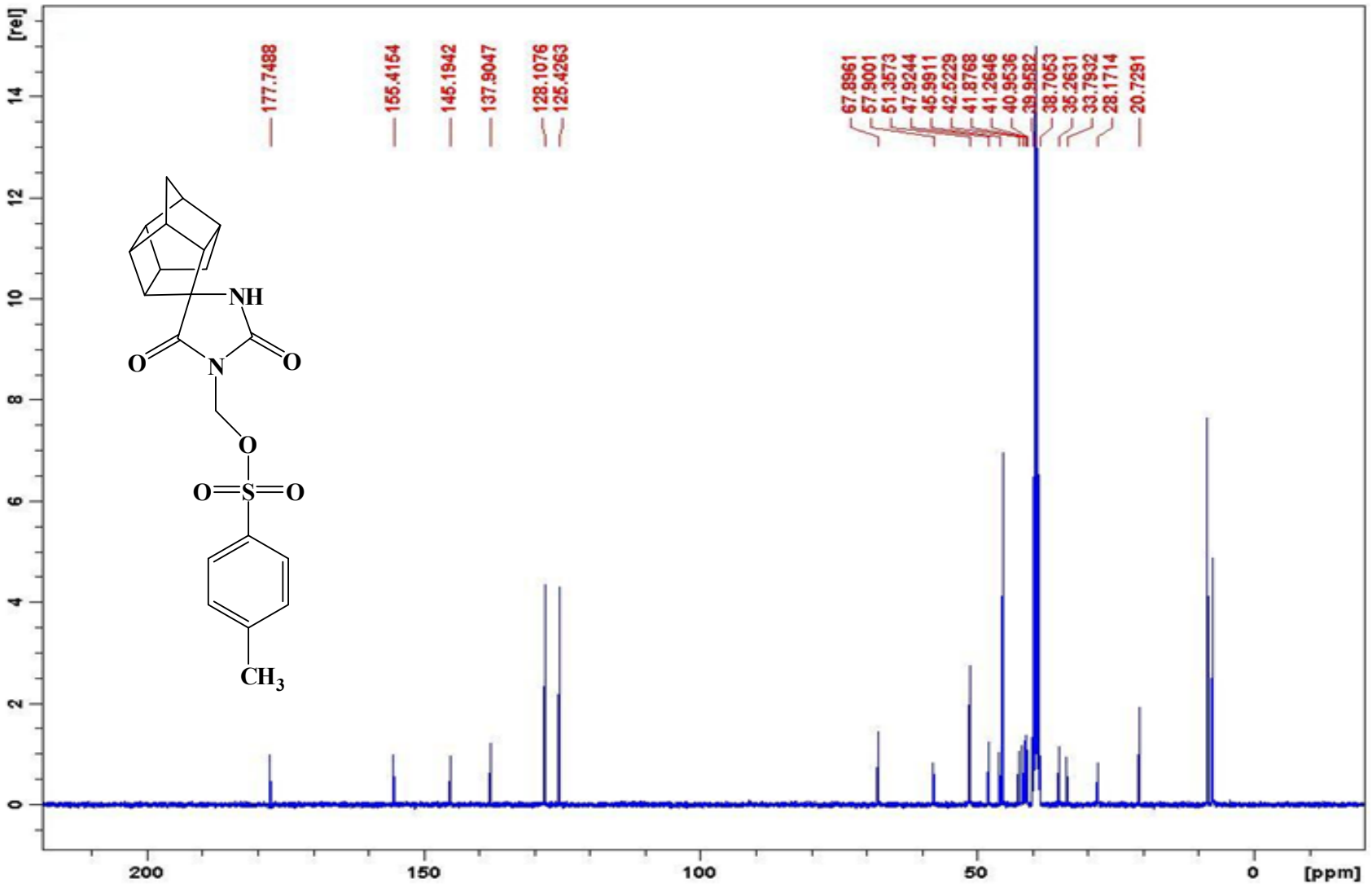
Spectrum 36: NOESY spectrum of compound 55 in $(CD_3)_2SO_2$



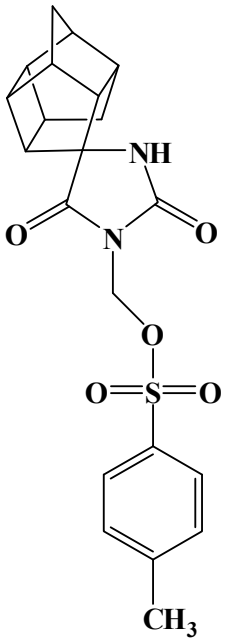
Spectrum 37: IR spectrum of compound 55

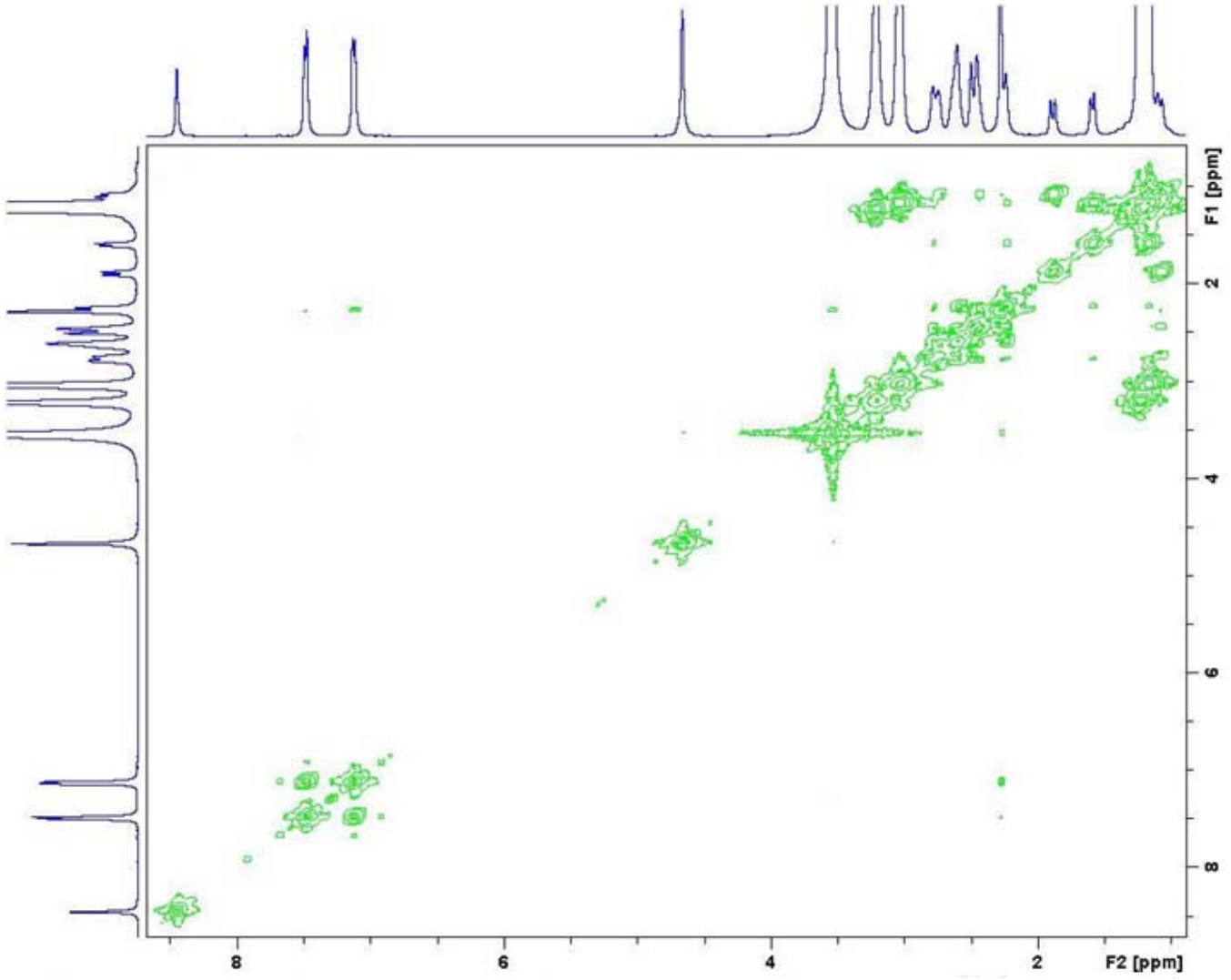


Spectrum 38: ^{1}H NMR spectrum of compound 56 in $(CD_3)_2SO_2$

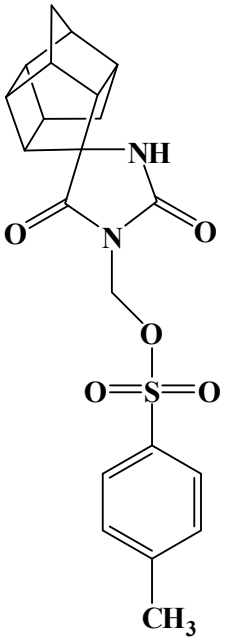


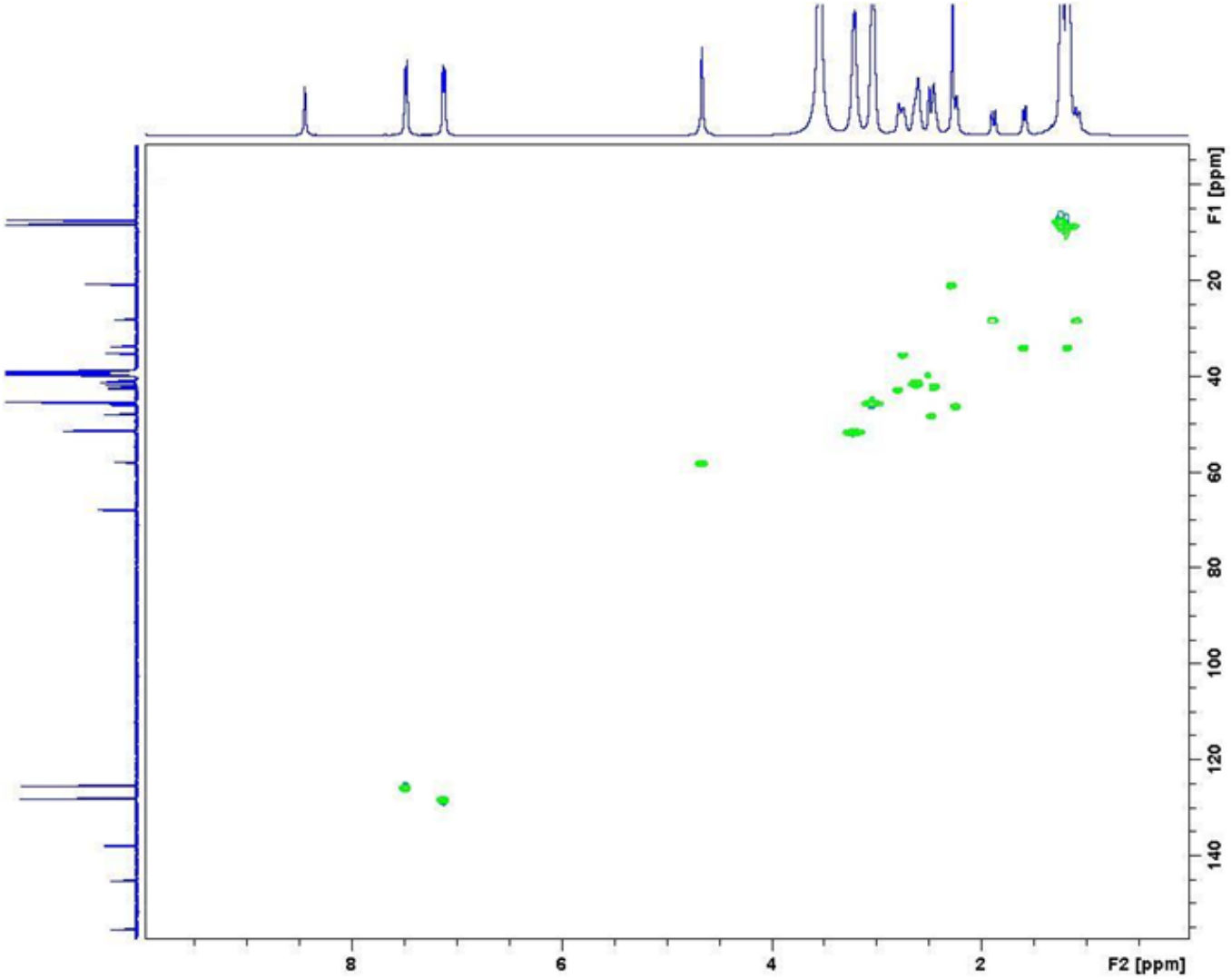
Spectrum 39: ^{13}C NMR spectrum of compound 56 in $(CD_3)_2SO_2$



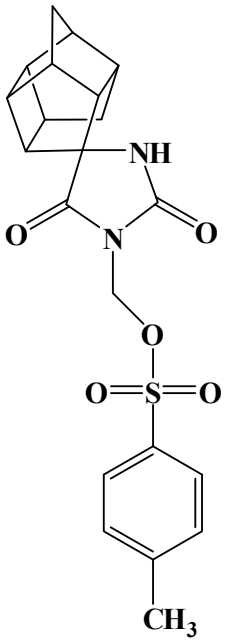


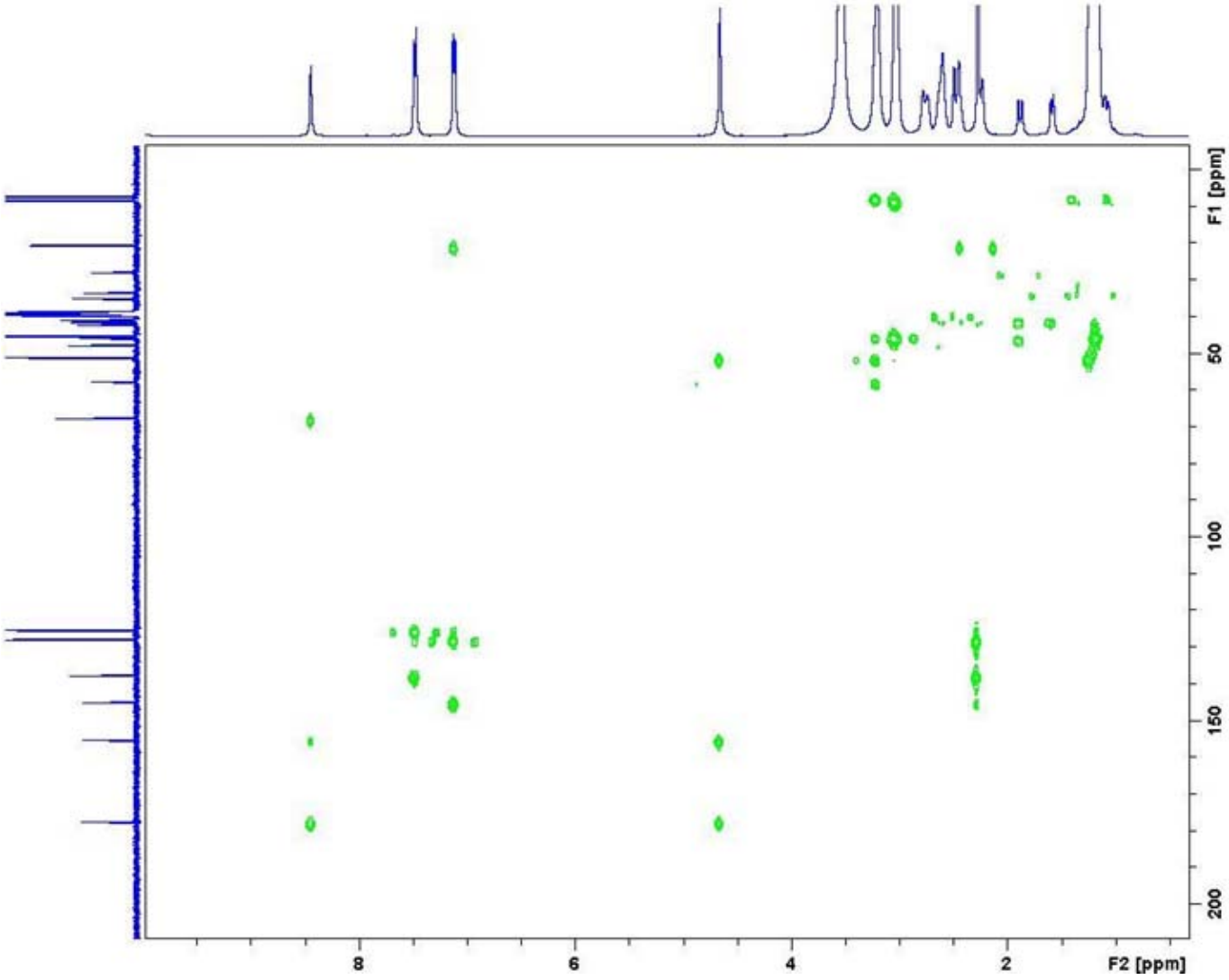
Spectrum 40: COSY spectrum of compound 56 in $(CD_3)_2SO_2$



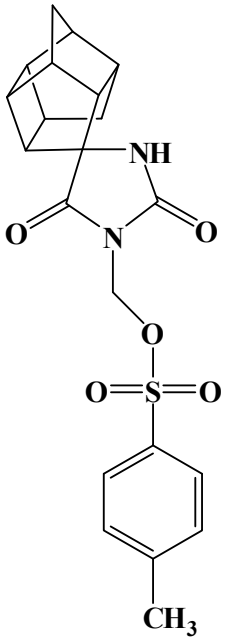


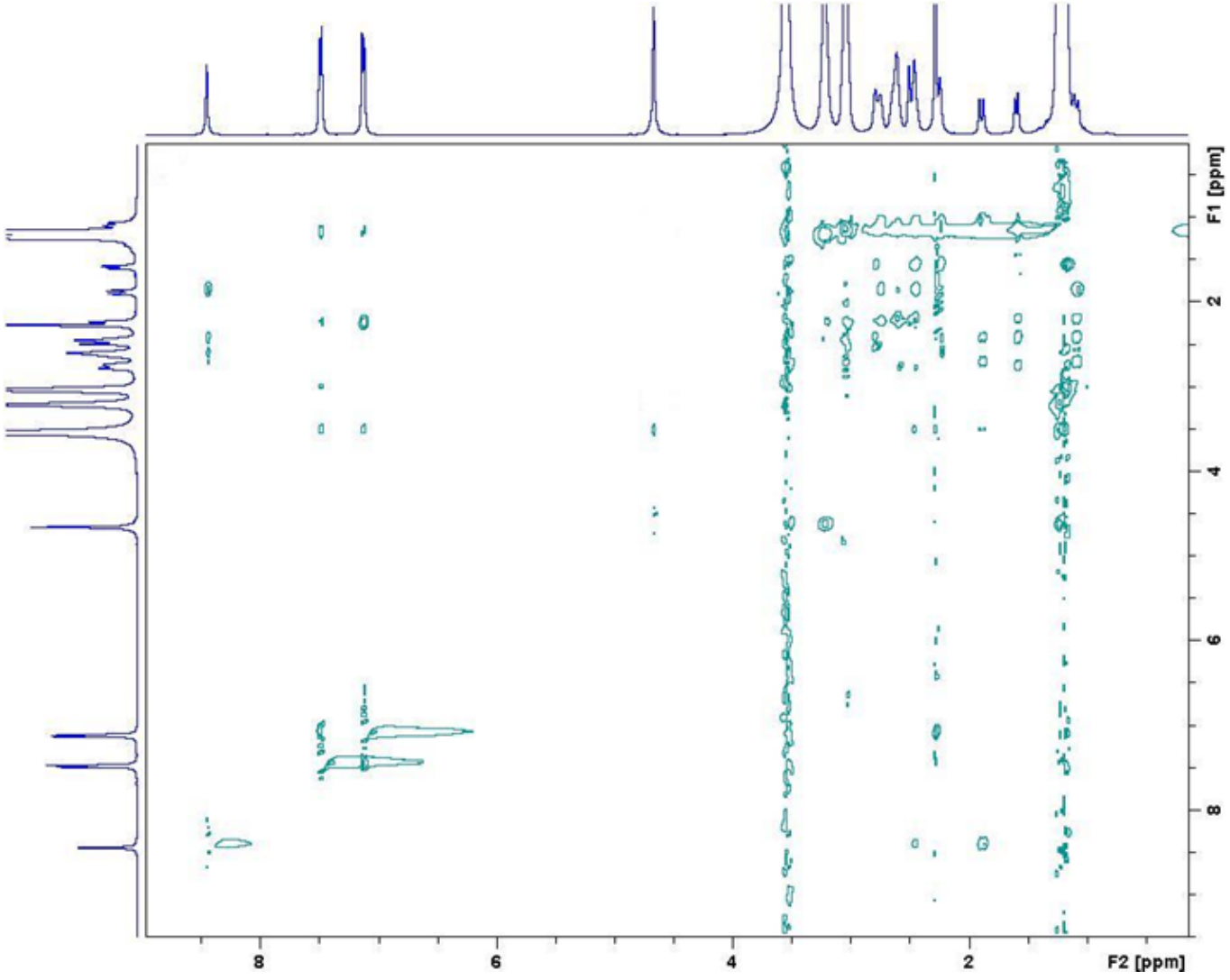
Spectrum 41: HSQC spectrum of compound 56 in $(CD_3)_2SO_2$



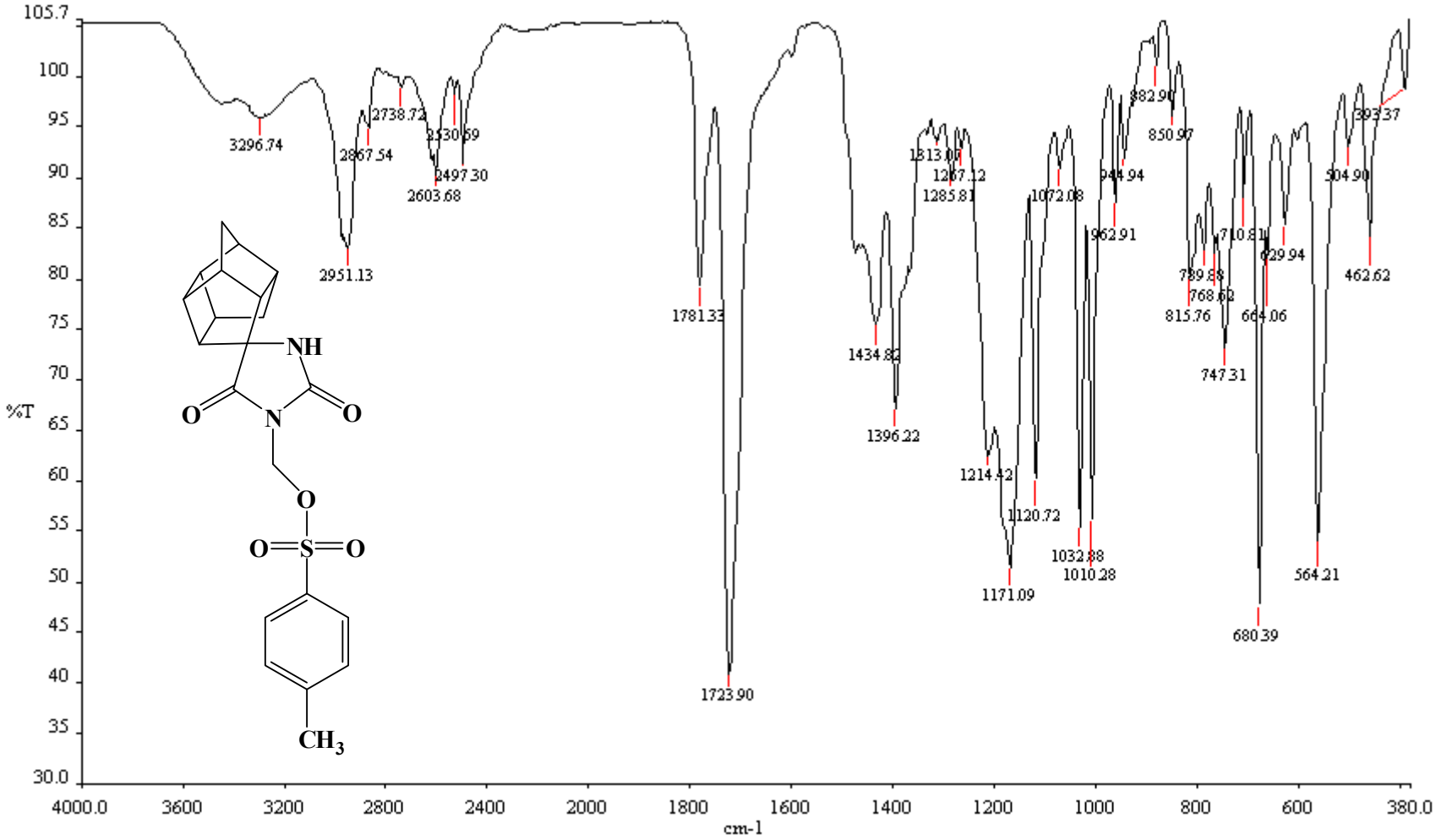


Spectrum 42: HMBC spectrum of compound 56 in $(CD_3)_2SO_2$

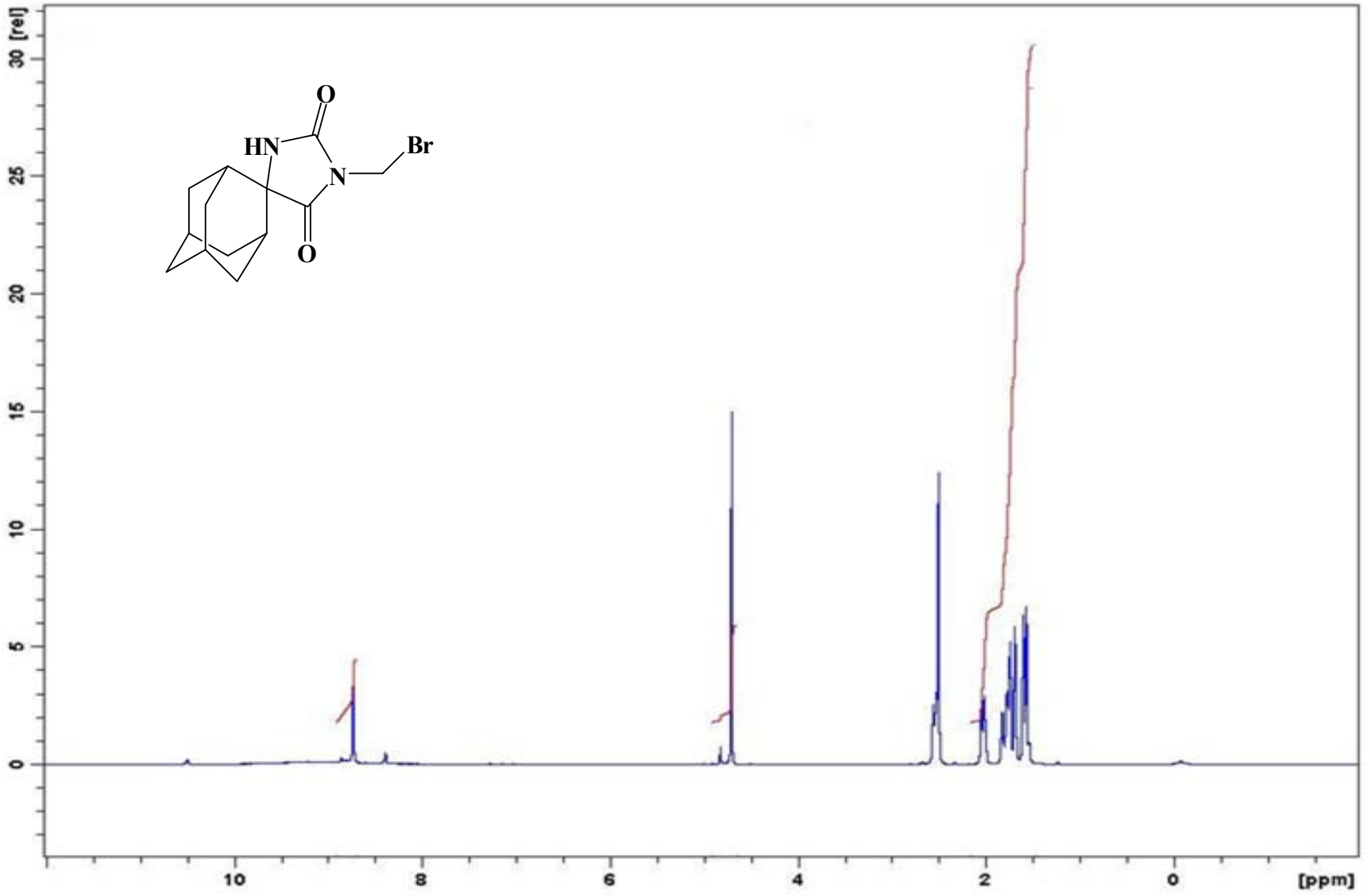




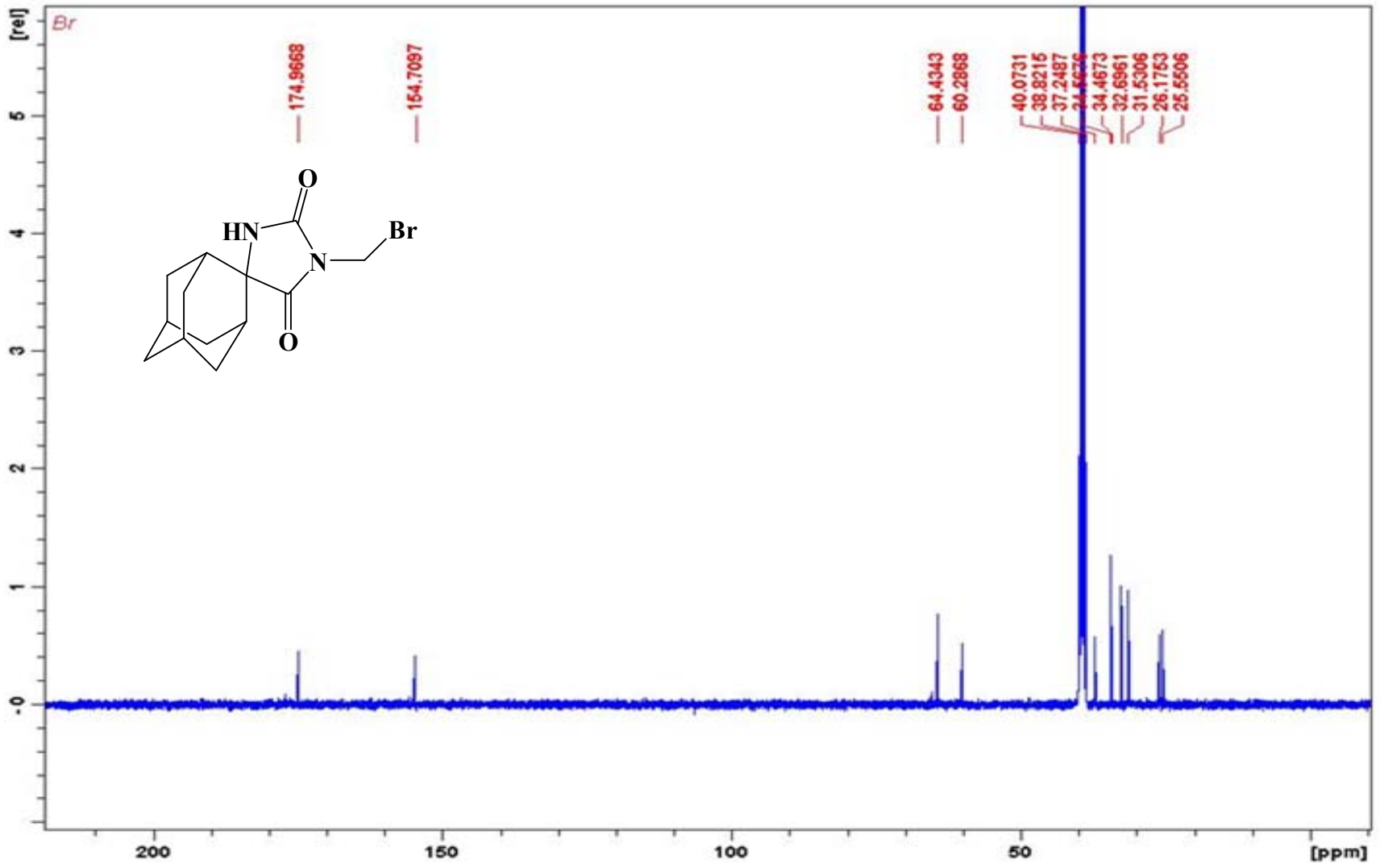
Spectrum 43: NOESY spectrum of compound 56 in $(CD_3)_2SO_2$



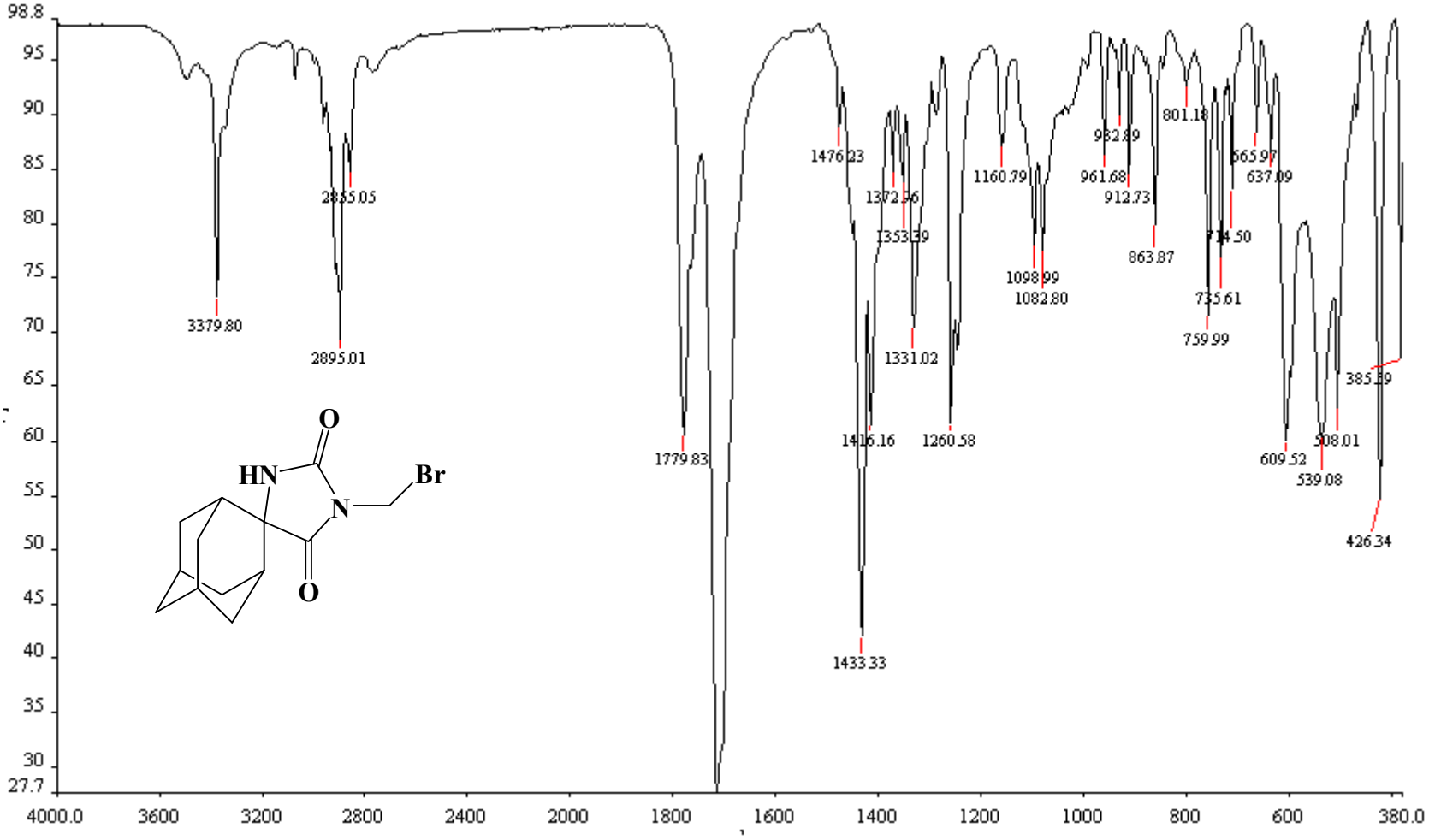
Spectrum 44: IR spectrum of compound 56



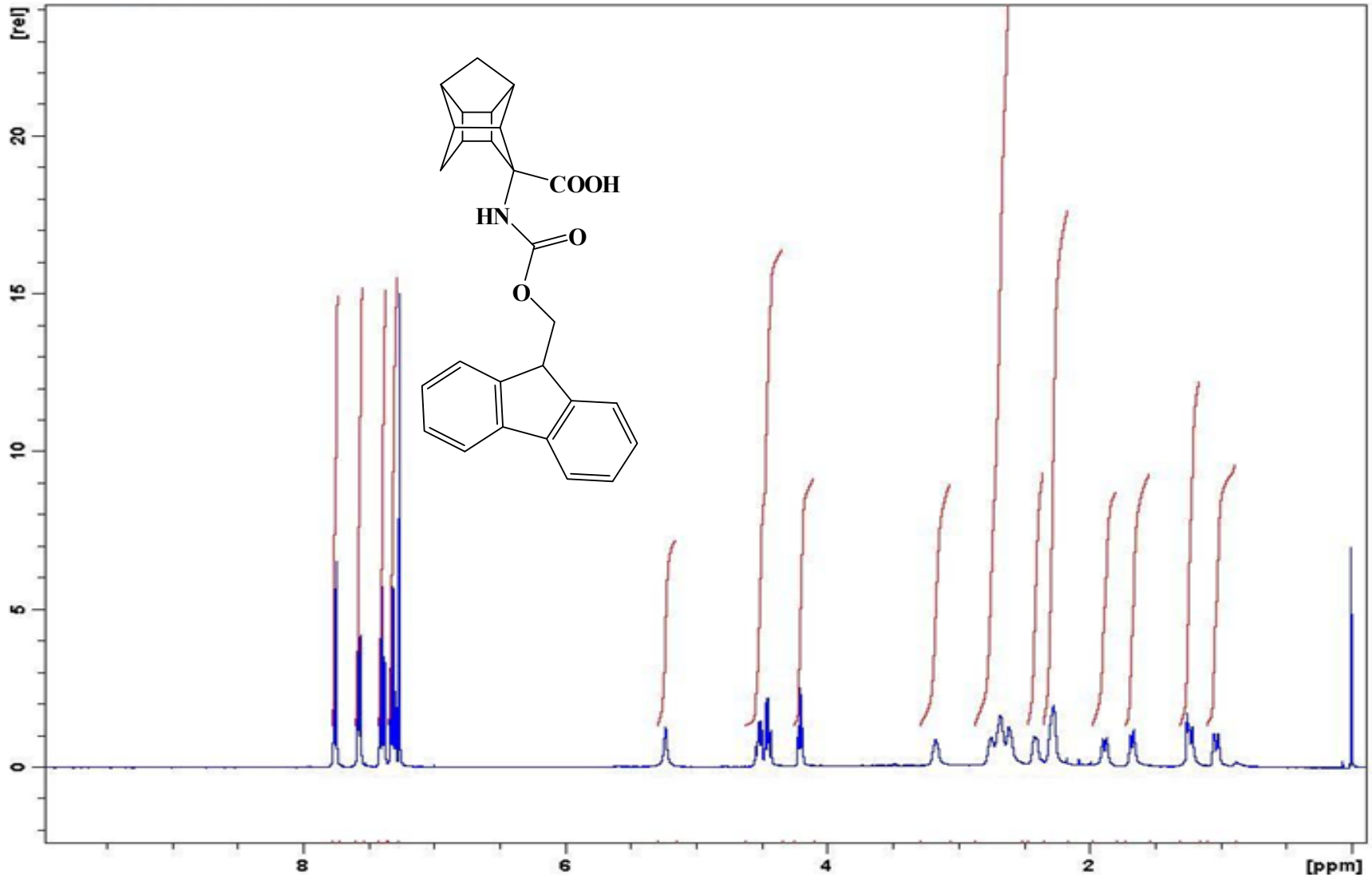
Spectrum 45: ^{1}H NMR spectrum of compound 65 in $(CD_3)_2SO_2$



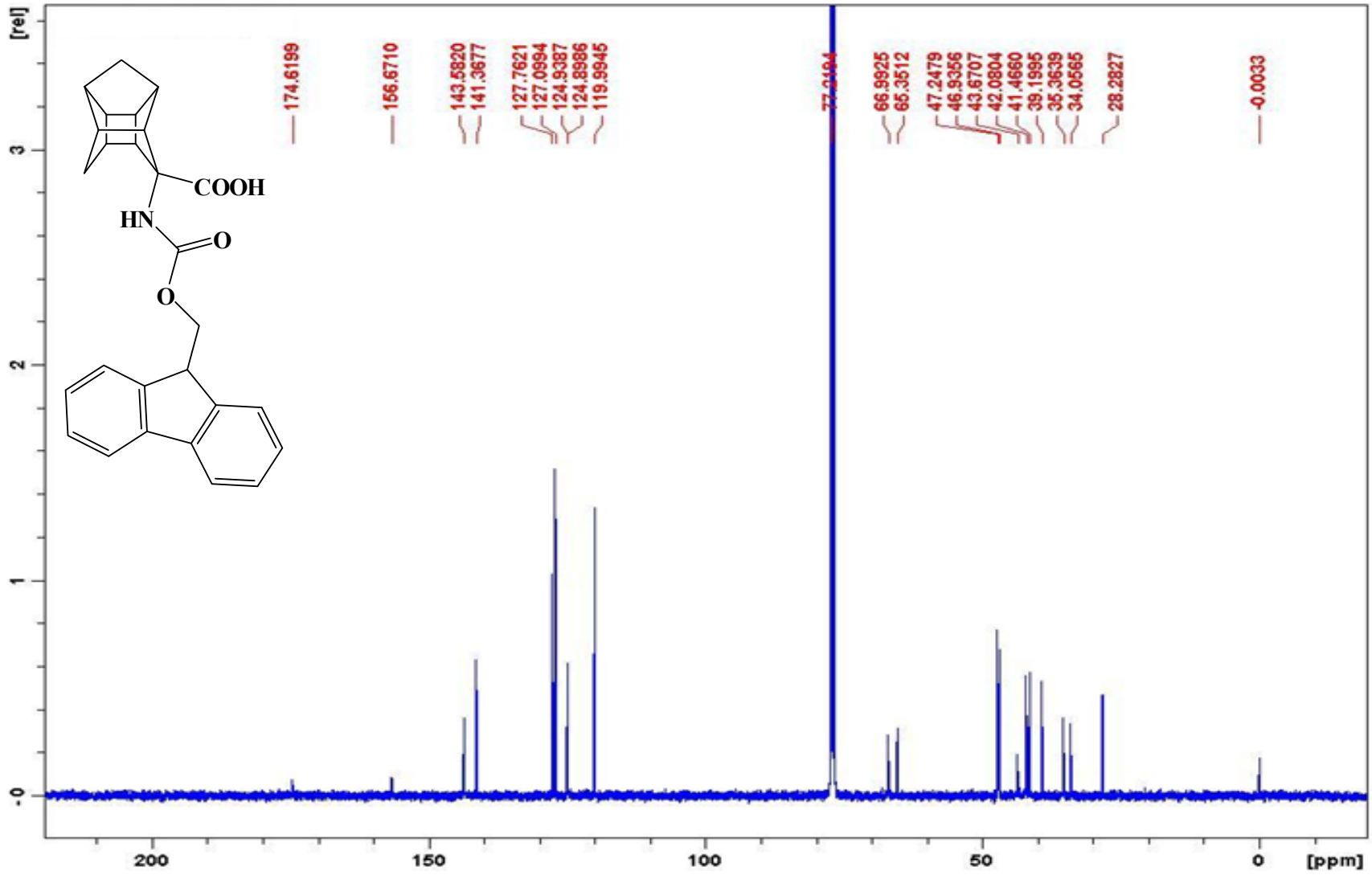
Spectrum 46: ^{13}C NMR spectrum of compound 65 in $(CD_3)_2SO_2$



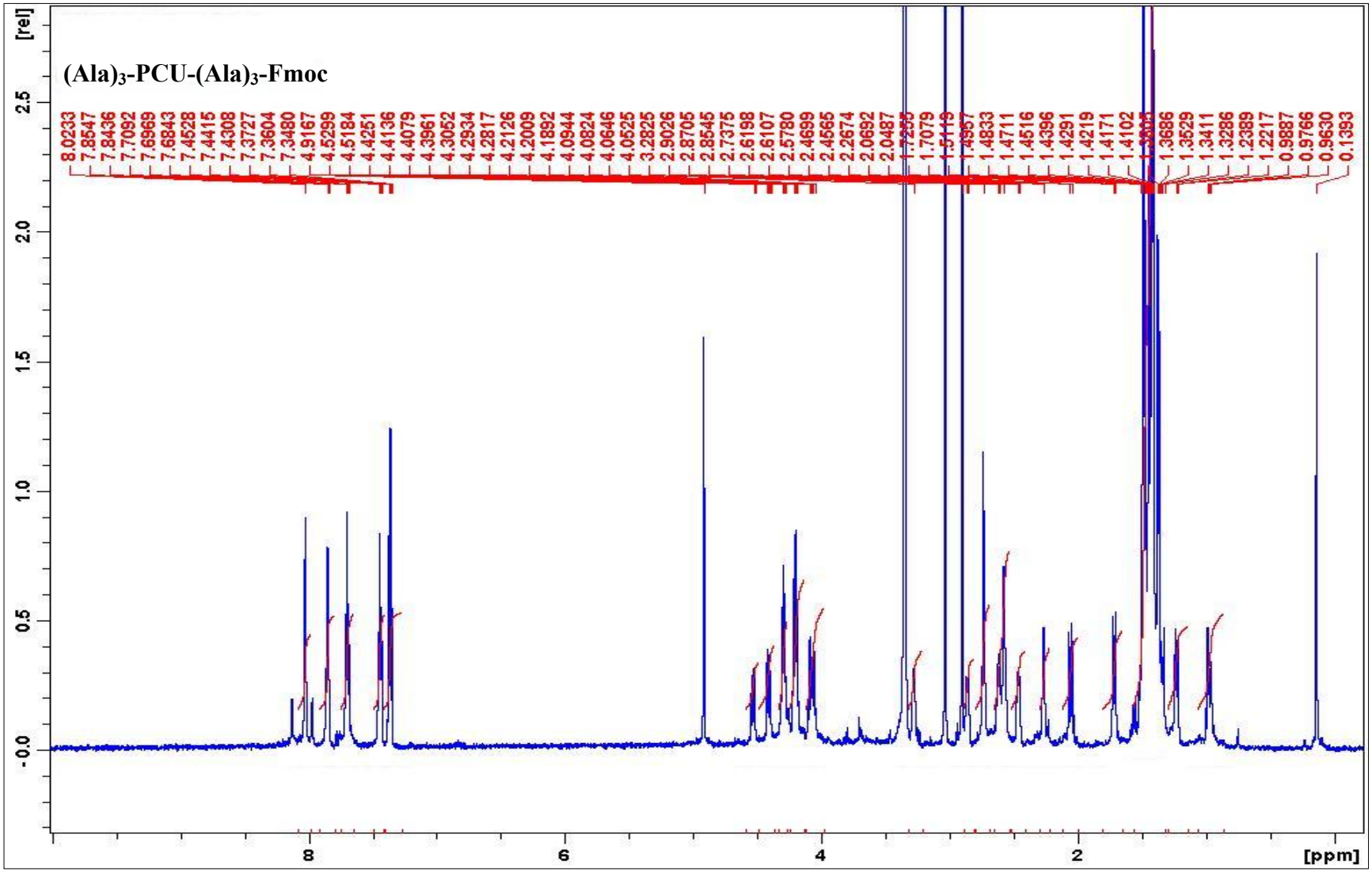
Spectrum 47: IR spectrum of compound 65



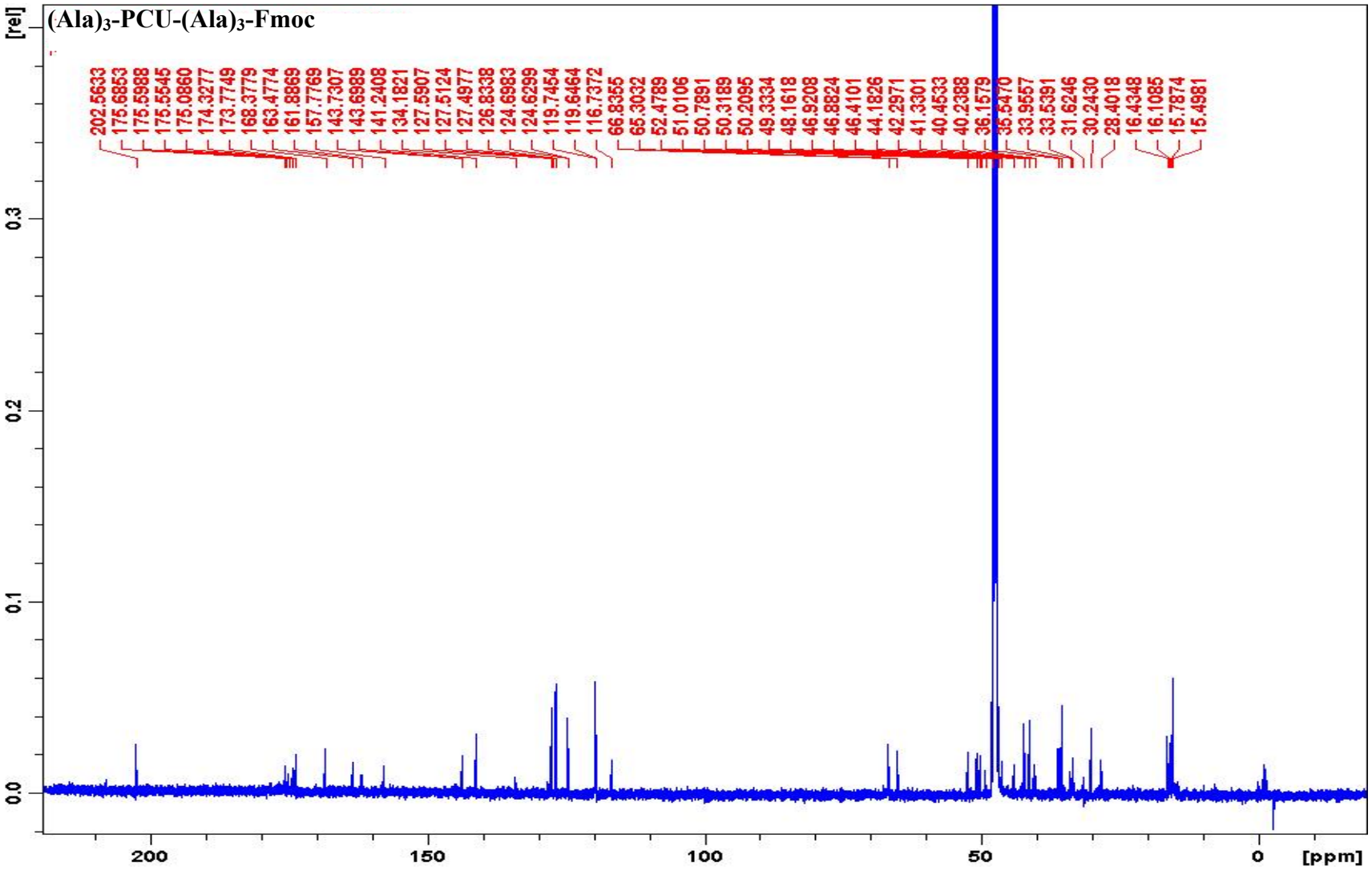
Spectrum 48: ^{1}H NMR spectrum of Fmoc PCU 106 amino acid in CD_3Cl



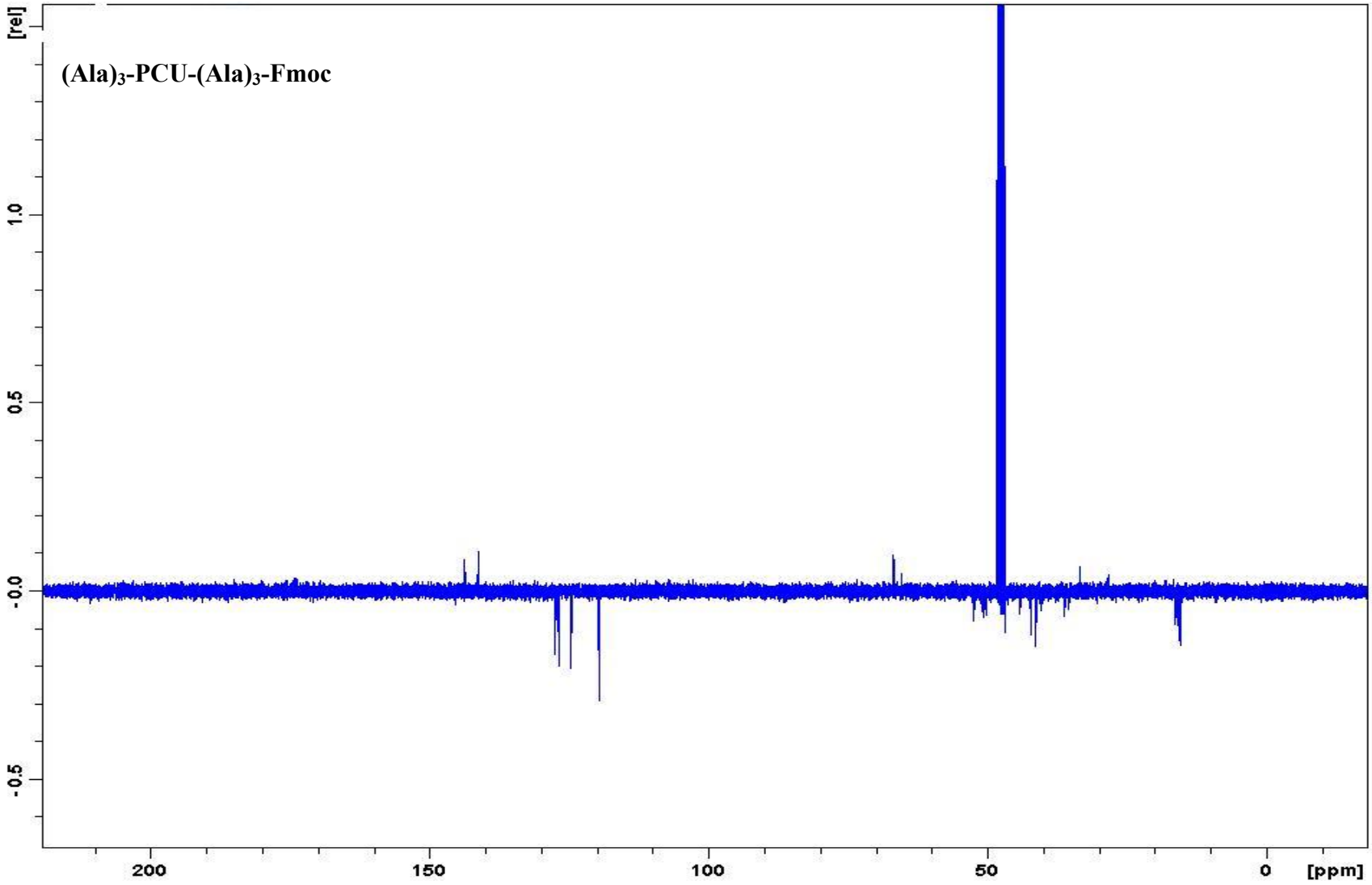
Spectrum 49: ^{13}C NMR spectrum of Fmoc PCU amino 106 acid in CD_3Cl



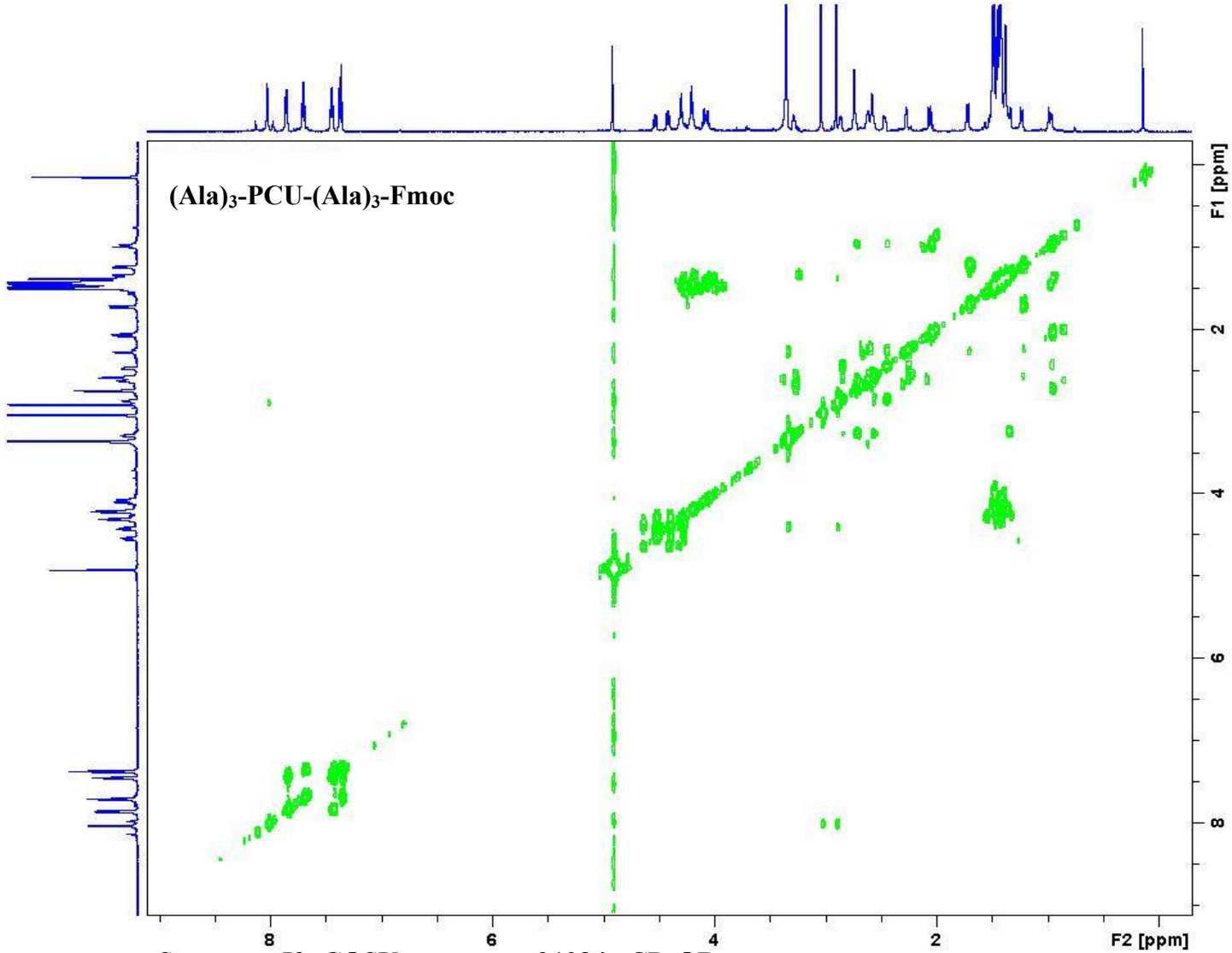
Spectrum 50: ^{1}H NMR spectrum of peptide 108 in CD_3OD



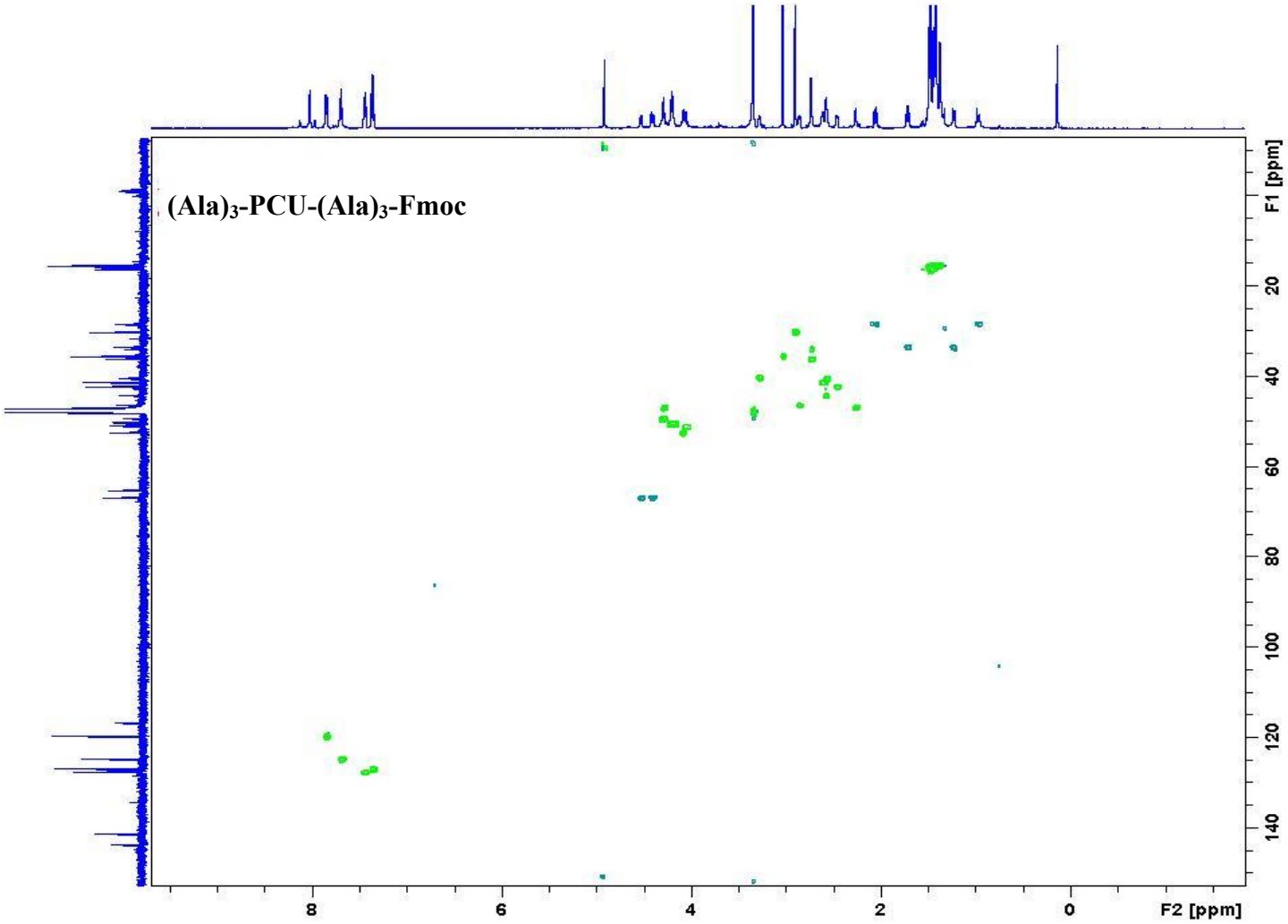
Spectrum 51: ^{13}CNMR spectrum of peptide 108 in CD_3OD



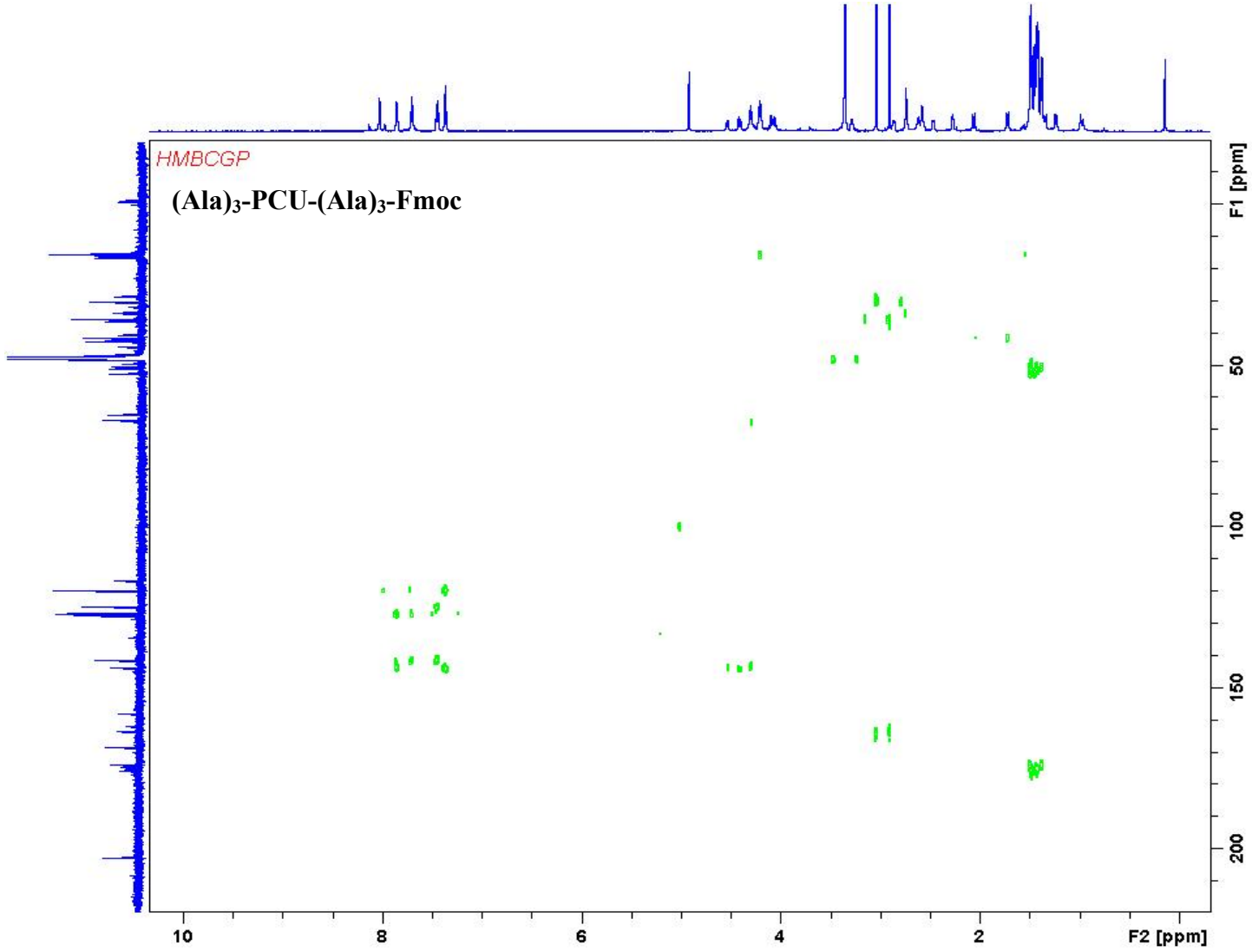
Spectrum 52: ^{13}C APT spectrum of 108 in CD_3OD



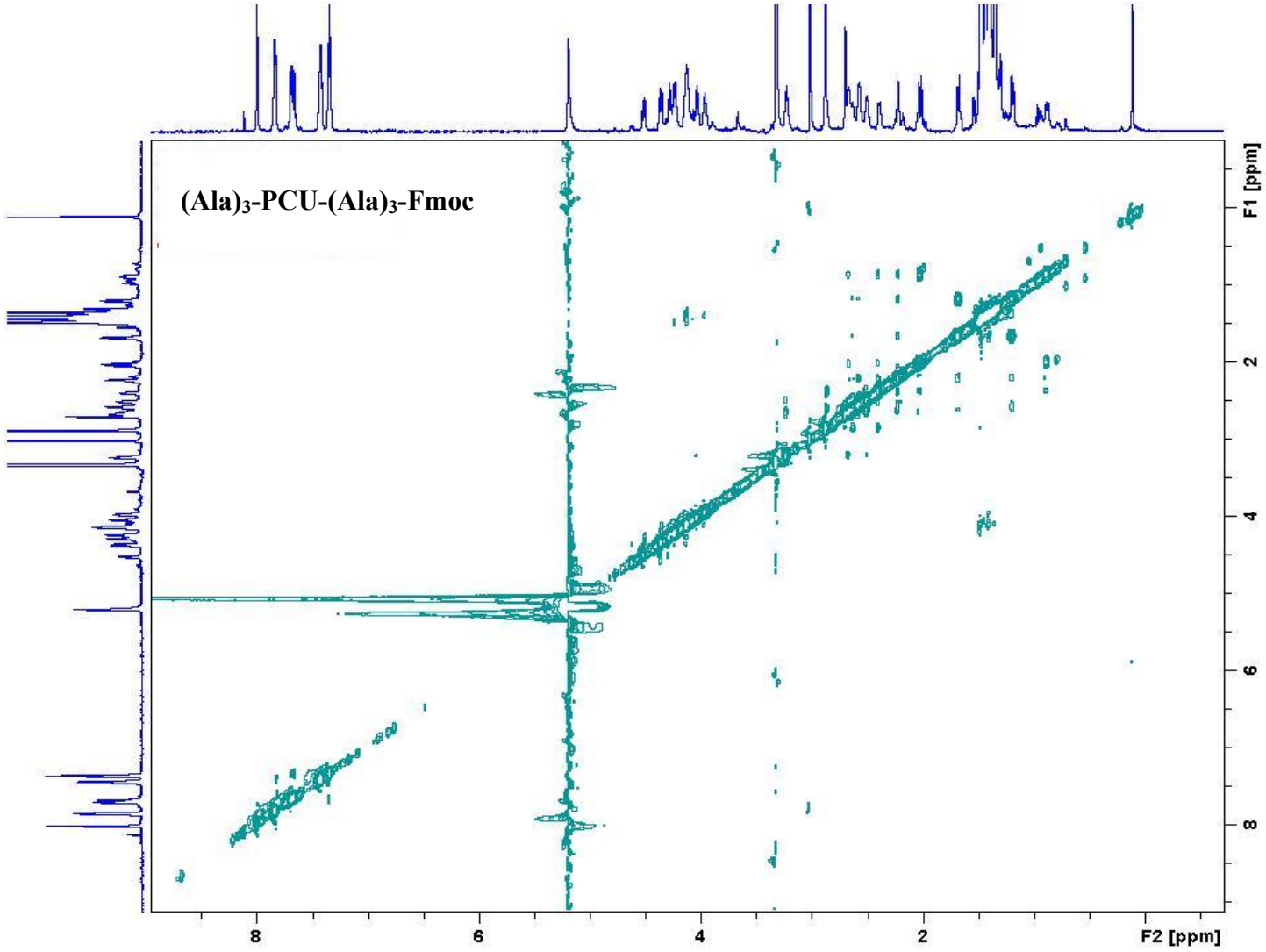
Spectrum 53: COSY spectrum of 108 in CD_3OD



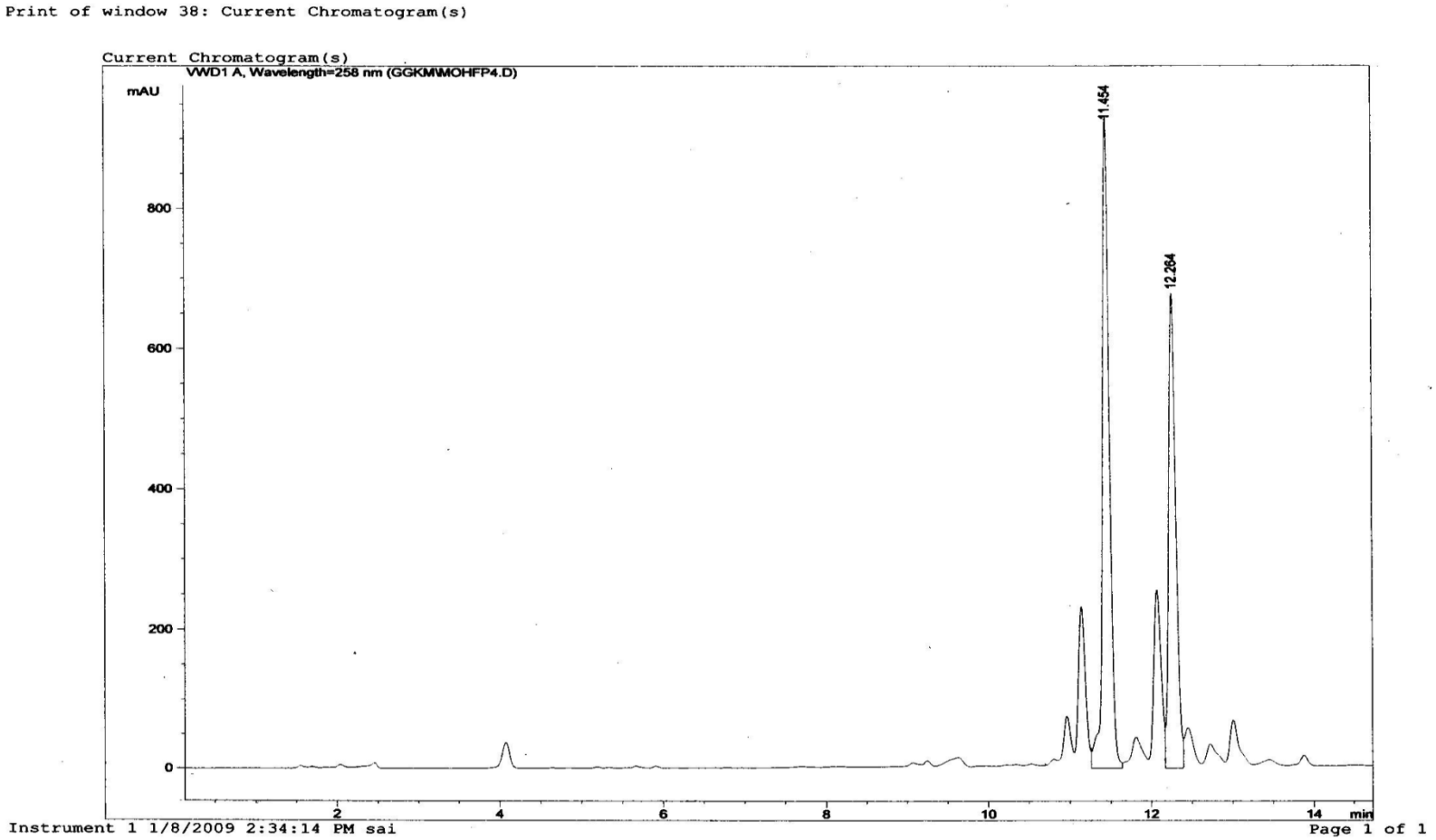
Spectrum 54: HSQC spectrum of peptide 108 in CD_3OD



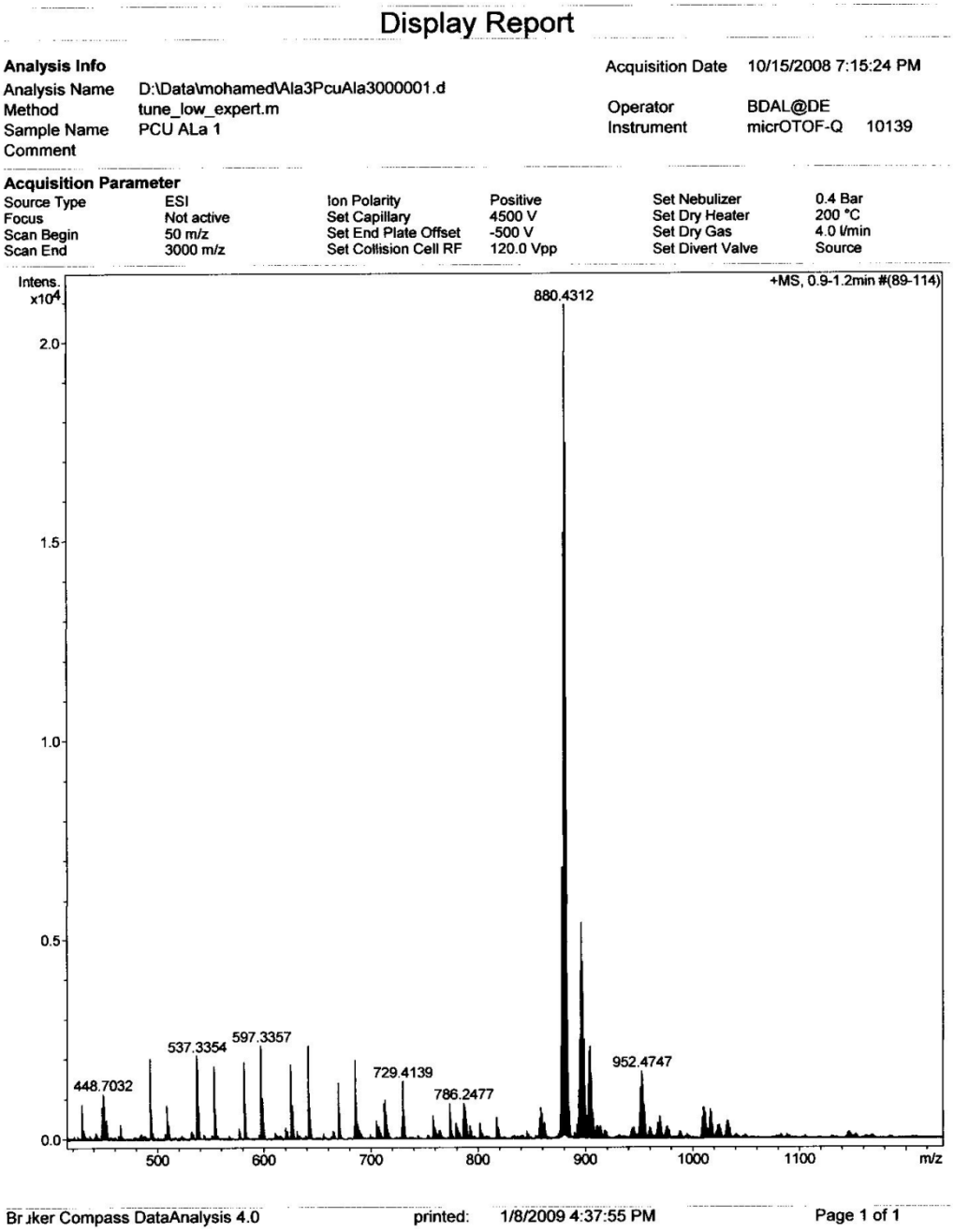
Spectrum 55: HMBC spectrum of peptide 108 in CD_3OD



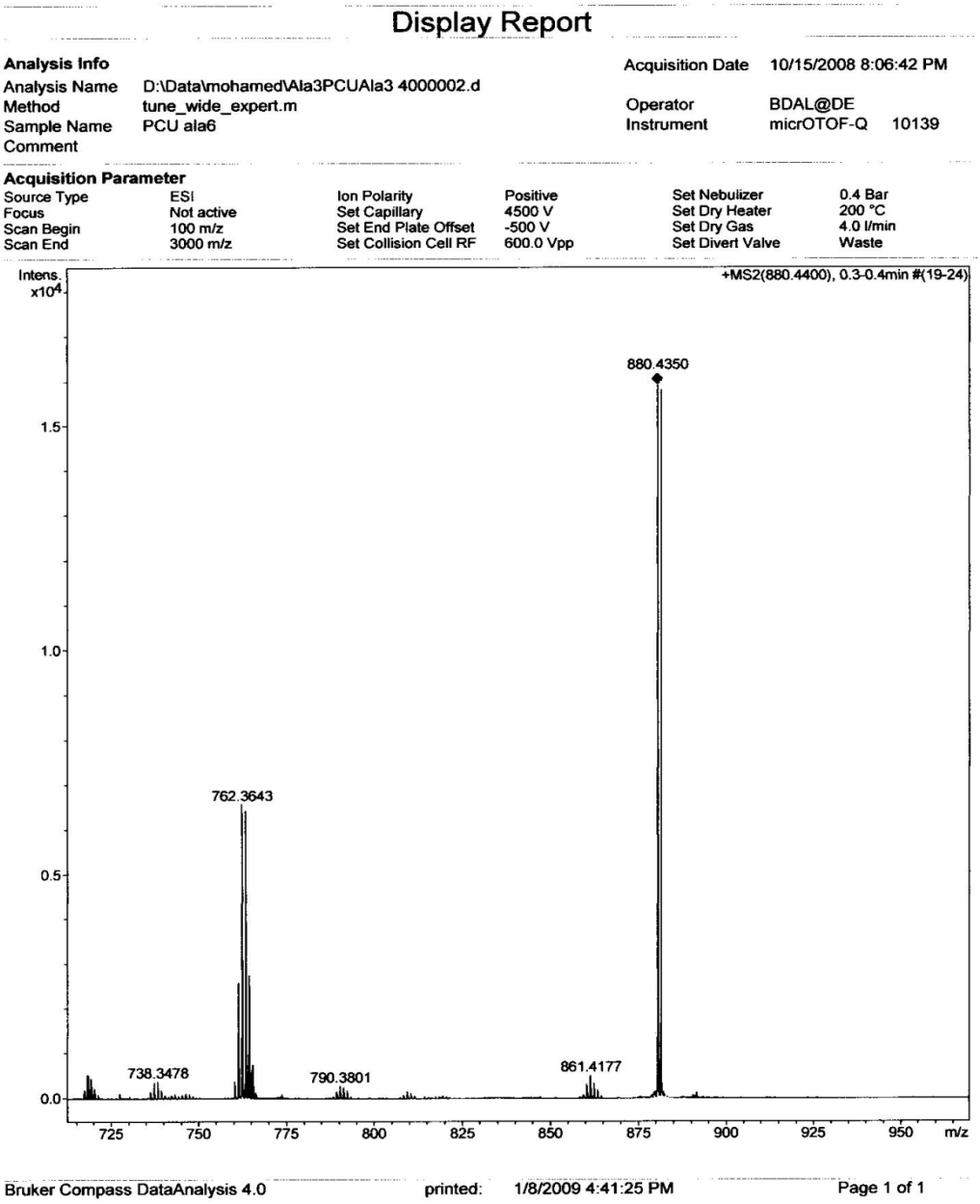
Spectrum 55: NOESY spectrum of peptide 108 at -50^{0}C in CD_3OD



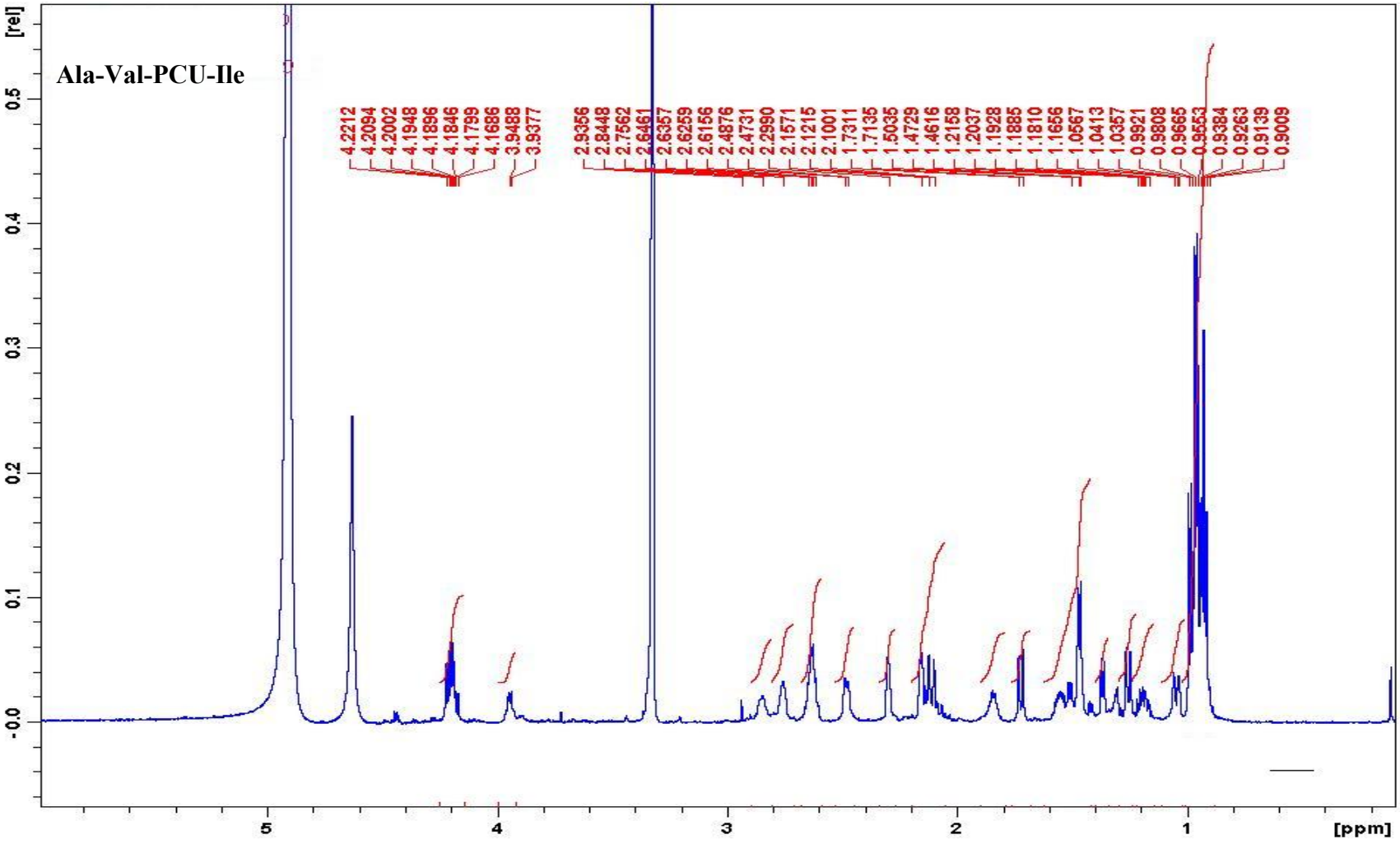
Spectrum 56: HPLC chromatogram of peptide 108a and 108b



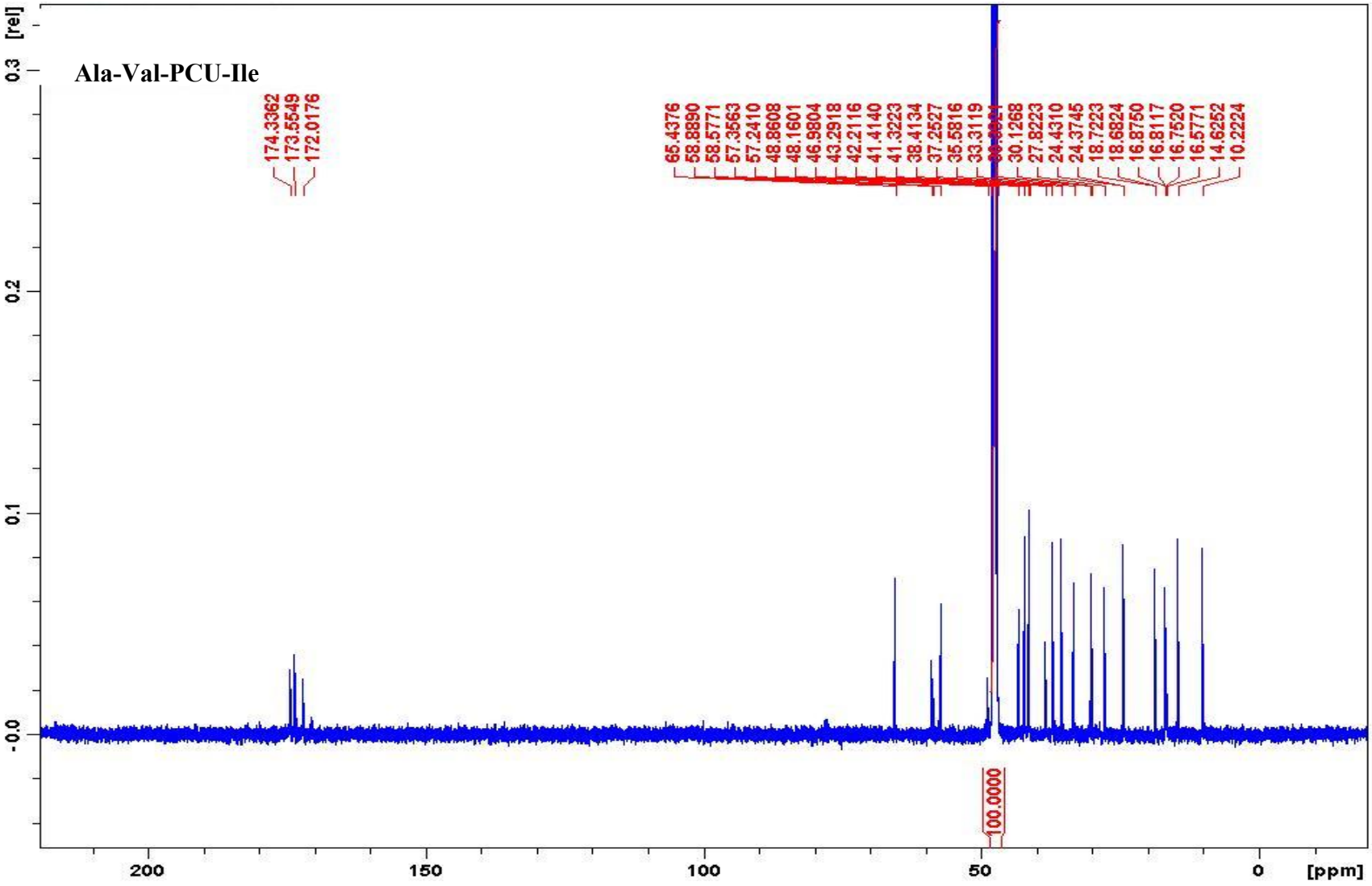
Spectrum 57: ESI Mass spectrum of peptide 108a



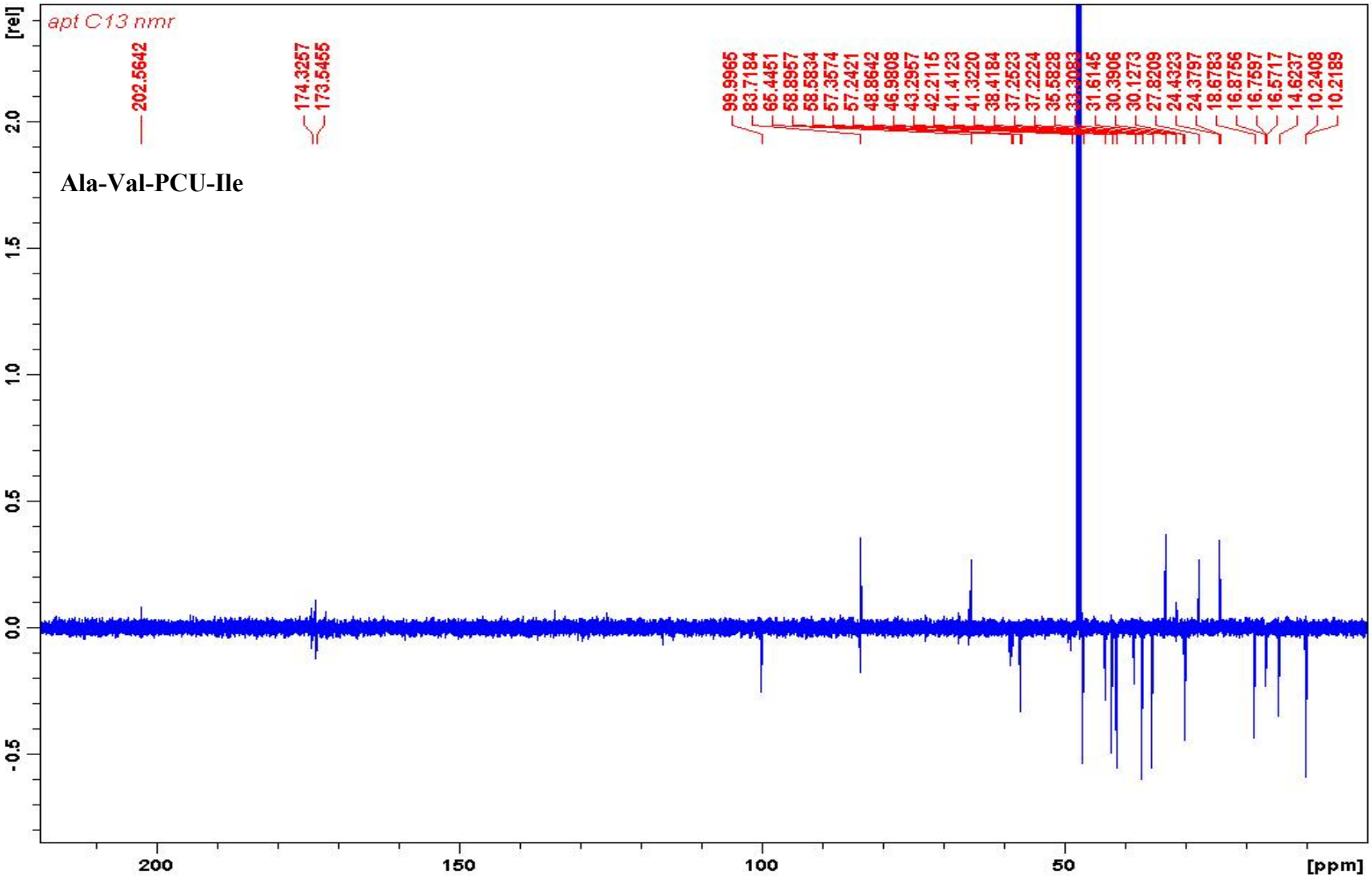
Spectrum 58: ESI Mass spectrum of peptide 108b



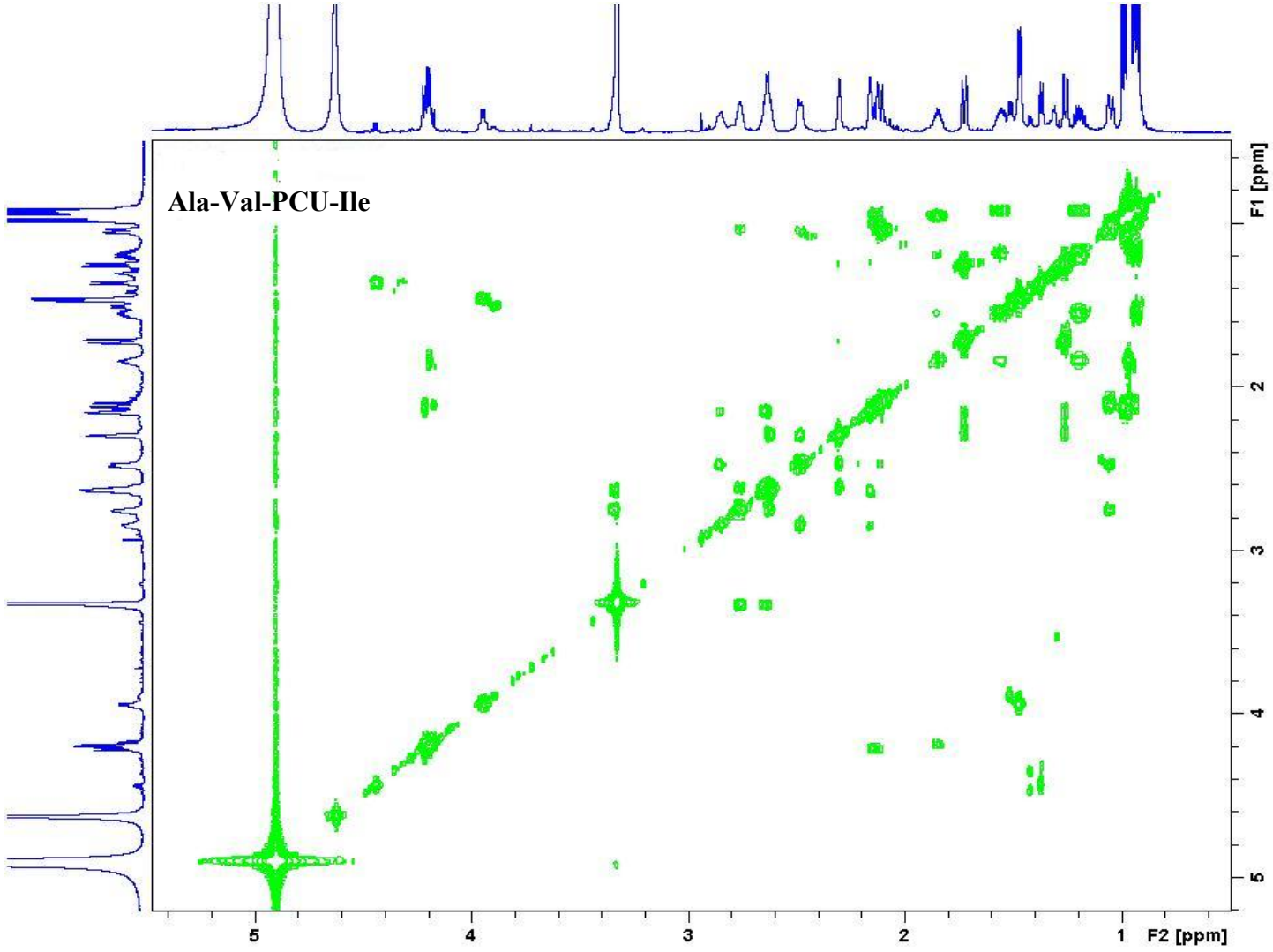
Spectrum 59: ^{1}H NMR spectrum of peptide 109 in CD_3OD



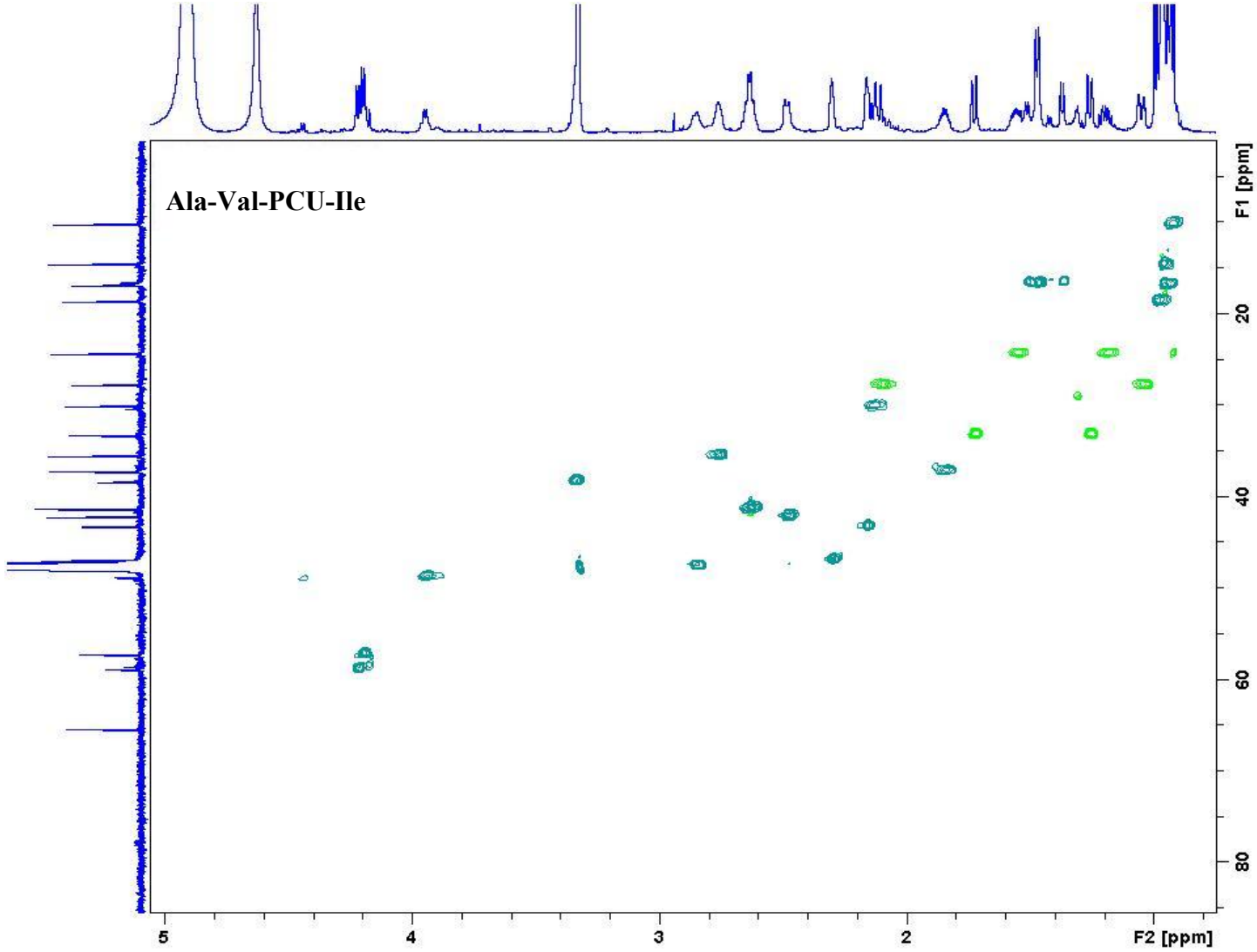
Spectrum 60: ^{13}C NMR spectrum of peptide 109 in CD_3OD



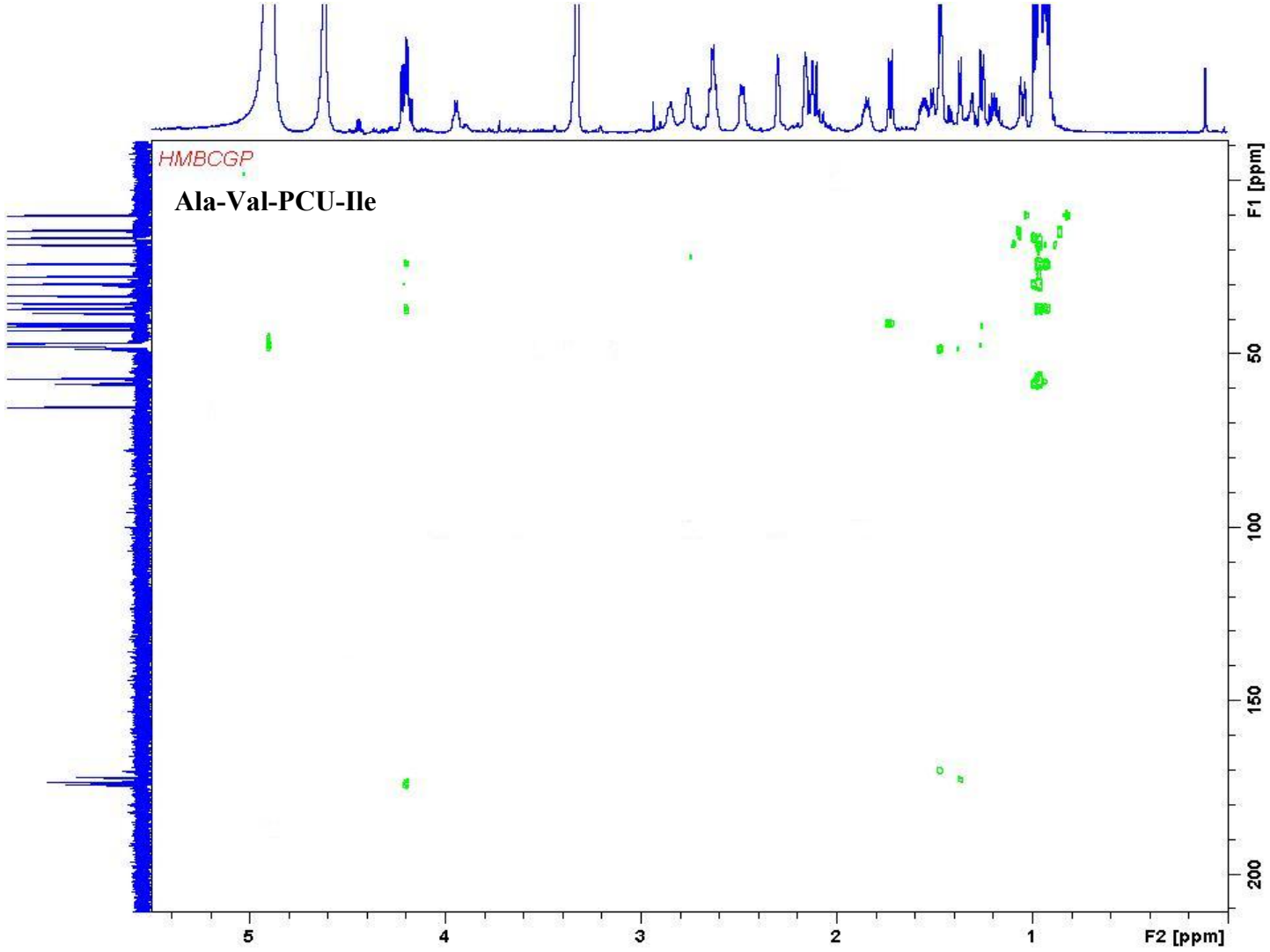
Spectrum 61: ^{13}C APT spectrum of peptide 109 in CD_3OD



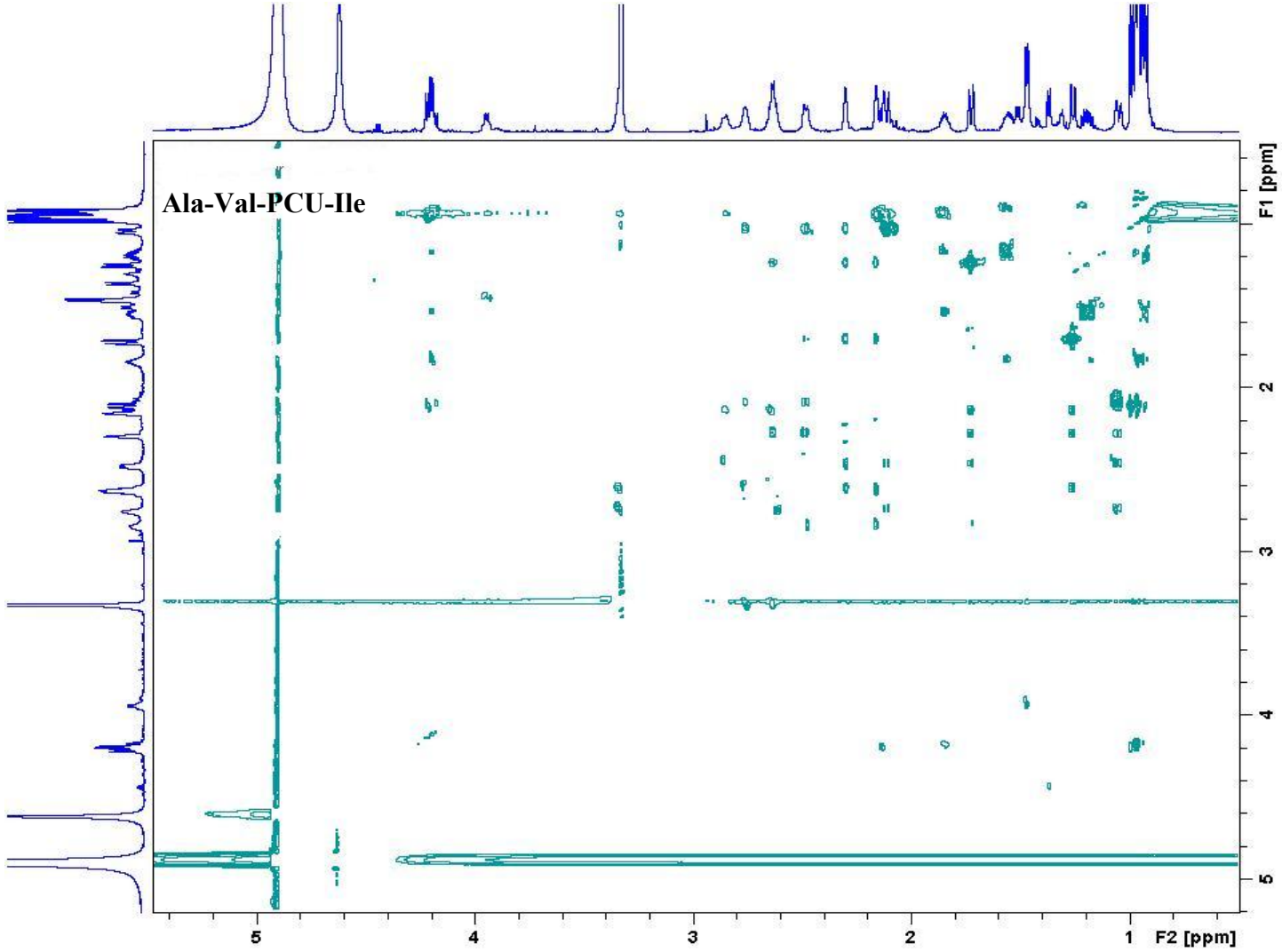
Spectrum 62: COSY spectrum of peptide 109 in CD_3OD



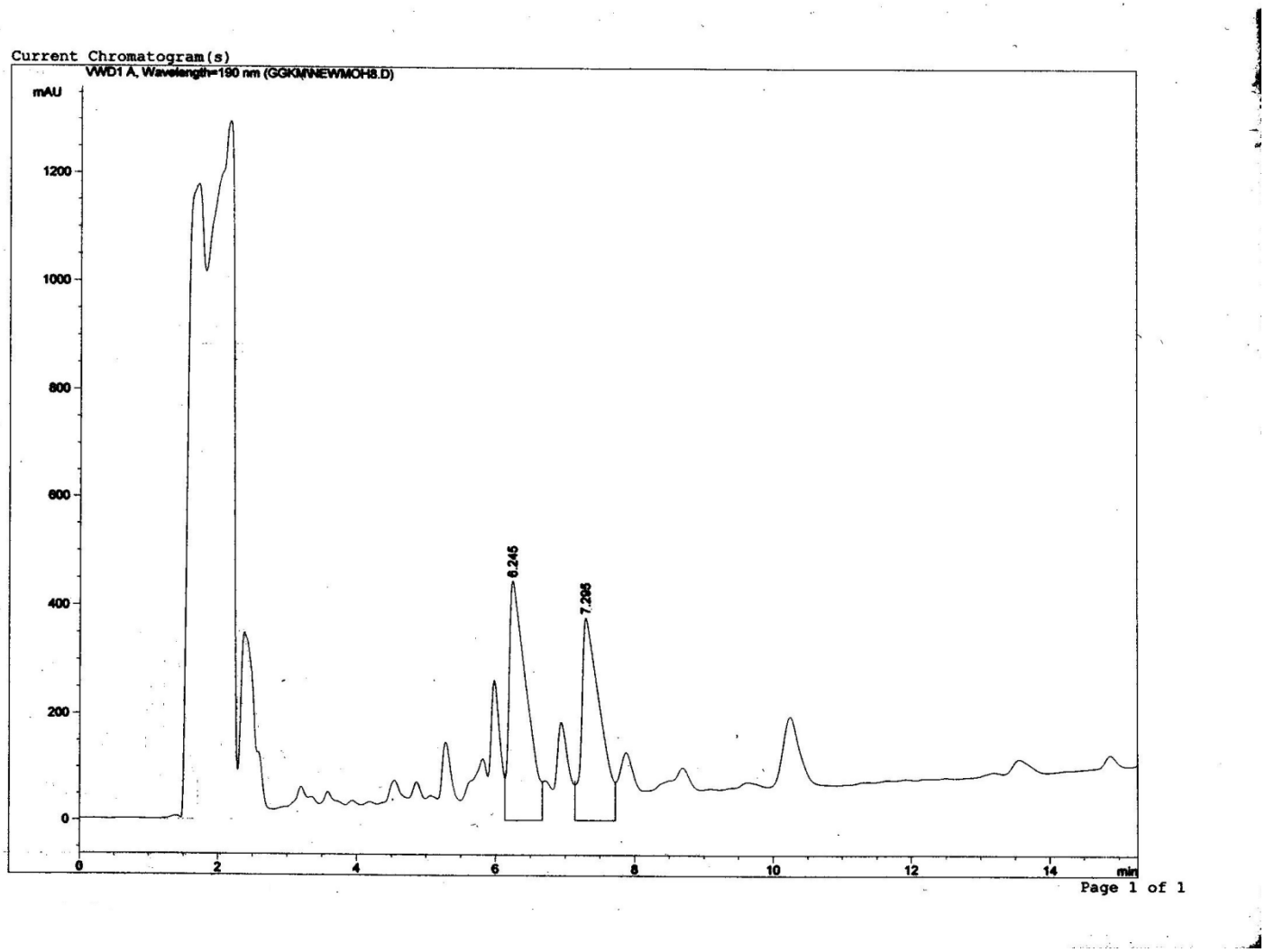
Spectrum 63: HSQC spectrum of peptide 109 in CD_3OD



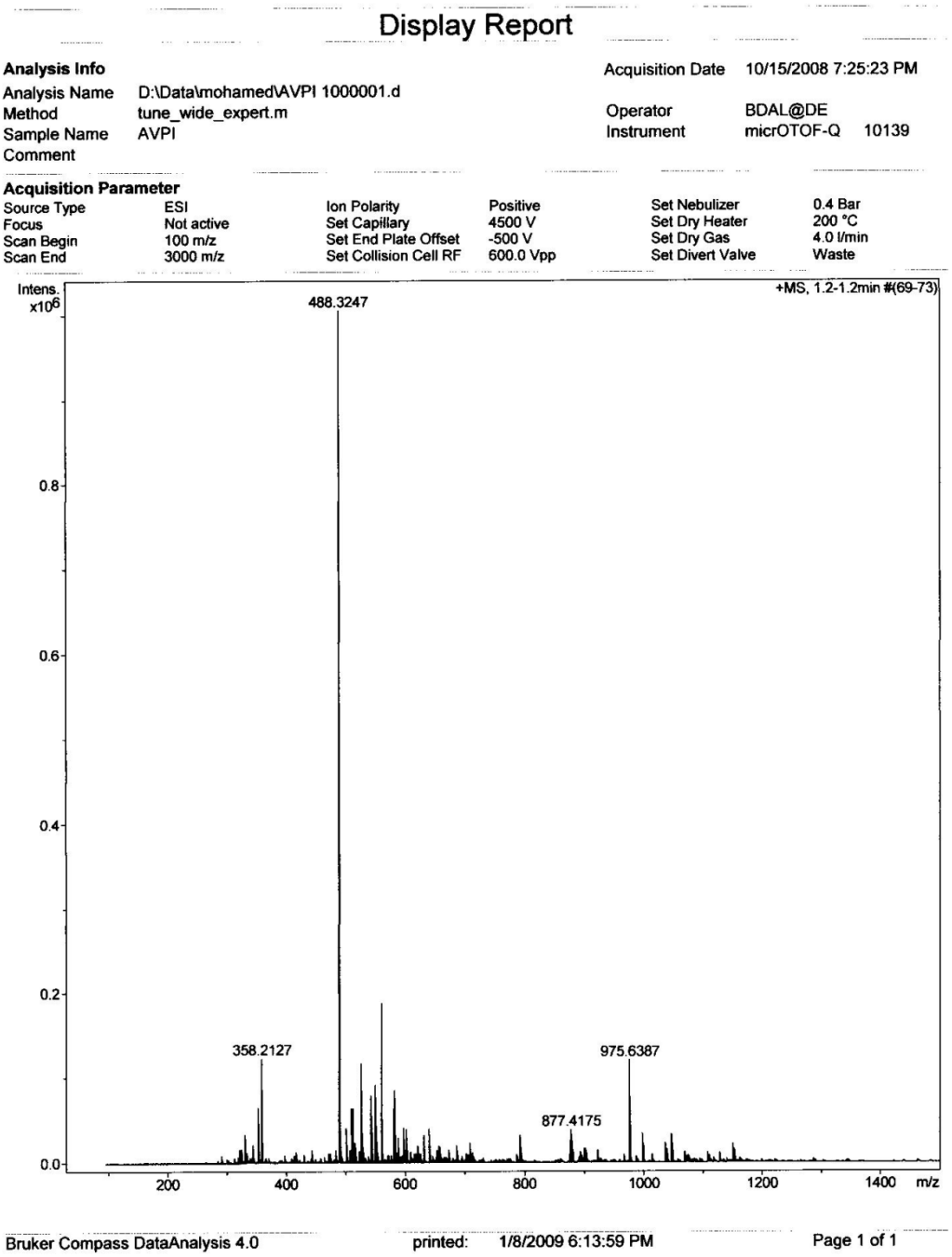
Spectrum 64: HMBC spectrum of peptide 109 in CD_3OD



Spectrum 65: NOESY spectrum of peptide 109 in CD_3OD



Spectrum 66: HPLC chromatogram of peptide 109a and 109b



Spectrum 58: ESI Mass spectrum of peptide 109