SYNTHESIS OF NOVEL CAGE AMINO ACID ANALOGUES

A dissertation submitted to the faculty of Science, University of Natal, Durban, for the degree of Master of Science

by

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DECLARATION

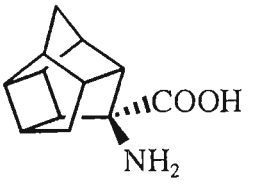
I hereby declare that the work presented in this thesis is my own, unaided work and has never before been submitted for any degree at this or any other university.

T. GOVENDER

--------- day of ---------- 2001

ABSTRACT

Amino acids are important building blocks for the synthesis of a large number of biologically active compounds and drugs. Amino acids with the pentacyclo-undecane (**1**) and trishomocubane (**2**) frameworks fall into the class of conformationally constrained non-natural amino acids. Conformationally constrained amino acids are found in many naturally occurring, biologically active compounds. It was found that incorporating cage structures into drugs induces a range of positive effects: promotes transport across the cell membrane, drugs can be designed to target the central nervous system, increased receptor site specificity, and retards metabolic degradation. In the light of this, it was decided to investigate the incorporation of cage amino acids into peptides. A synthetic route has been established for the efficient synthesis of amino acids **1** and **2**, and for their incorporation into peptides.



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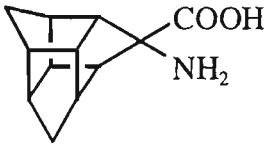

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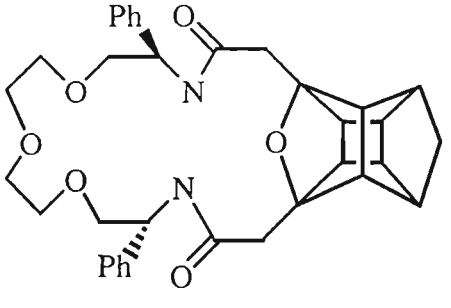
Several chiral macrocyclic crown ethers and related analogues have been shown to be capable of forming complexes enantioselectively with chiral organic ammonium salts. The design and synthesis of host chiral macrocycles which are able to distinguish between the enantiomers of guest organic ammonium salts is of interest in the areas that include synthesis of enzymes, electrodes for specific ions or molecules, drugs targeted for specific sites, and enantiomer separation. A synthetic procedure has been established for the synthesis of cage annulated chiral crown ethers derived from amino acids. The advantage of using cage compounds in crown ethers is due to increased rigidity, increased solubility in non-polar solvents and increased chirality. Various techniques for the determination of enantiomeric recognition have been studied and include NMR spectroscopy, fluorescence emission spectroscopy and computational methods. The cage crown ether **3** represents a typical example of these new cage annulated, chiral crown ethers.



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I dedicate this work to my family, friends and my late beloved father.

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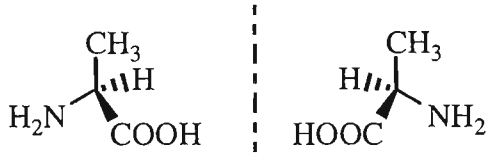
LIST OF ABREVIATIONS

AIB	Aminoisobutyric acid
BOP	benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluoro phosphate
CI	Chemical ionisation
CPU	central processing unit
DCC	Dicyclohexylcarbodiimide
DIEA	N,N-diisopropylethylamine
DIPCDI	Diisopropylcarbodiimide
DMAP	N,N-dimethylaminopyridine
DMF	Dimethylformamide
EtA	Ethylalanine
FAB	fast atom bombardment
Fmoc-Cl	9-Fluorene-9-methyl chloroformate (9H-Fluoren-9-ylmethyl carbonchloridate)
FTICR	fourier transform ion cyclotron resonance
HBTU	(benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate
HF	hydrogen fluoride
THF	Tetrahydrofuran
HOBt	hydroxybenzotriazole
HRMS	High resolution mass spectrometry
MeAIB	N-methylaminoisobutyric acid
PCU	Pentacycloundecane
RAM	Random access memory
RHF	Restricted Hartree-Fock
SPPS	solid-phase peptide synthesis
TFA	Trifluoroacetic acid
TRIS	Trishomocubane
TsOH	p-Toluene sulphonyl chloride

CHAPTER 1

INTRODUCTION

The aim of this study is to synthesise novel chiral crown ethers containing cage compounds and to determine their enantiomeric recognition for chiral guest molecules. The introduction will consist of (a) a general introduction to chirality, (b) a background of chiral crown ethers, (c) motivation for the incorporation of cage compounds into chiral crown ethers, and (d) a short overview of the synthetic approach followed in this study.
Chirality is a fundamental symmetry property of three-dimensional objects. An object is said to be chiral if it cannot be superimposed upon its mirror image. Chiral is a deviation of the Greek word *chiros* (hand). A carbon atom attached to four different groups is chiral e.g. the amino acid alanine (**1**):



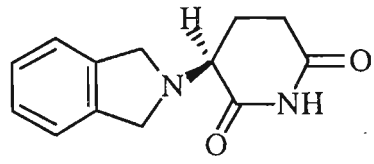
S-(-)-alanine R-(+)-alanine

1

Enantiomers have identical chemical and physical properties in the absence of an external chiral influence. This means that both will have the same melting points, solubility, chromatographic retention time, infrared (IR) and nuclear magnetic resonance (NMR) spectra. Enantiomers rotate plane polarised light in opposite directions. The notation used is (+) for clockwise rotation and (-) for anti clockwise rotation.[1]

If the only difference between enantiomers were their rotation of polarised light, the whole area of chirality would be relegated to little more than a scientific curiosity. That is not so because all forms of life in nature are chiral and most of the important building blocks which make up the biological macromolecules of living systems do so in largely one enantiomeric form only [L-form].[2,3] When, therefore a biologically active chiral compound, such as a drug, interacts with a receptor site which is chiral, it should come as no surprise that the two enantiomers of the drug interact differently and may lead to different biochemical effects.[4]

A good example is the drug thalidomide for which both enantiomers have the same sedative effect (calming or tranquillising) but only the (-)-enantiomer (**2**) causes foetal deformities. Who can forget the tragic consequences brought by this drug in the early 1960s? A high incidence of foetal deaths and malformations occurred due to its use by pregnant women. Unfortunately even if the pure (+)-enantiomer had been used problems would have arisen since the two forms inter convert under physiological conditions.[1]



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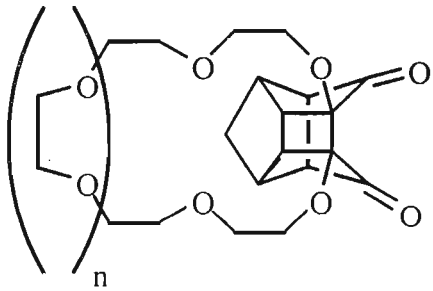
Since then an enormous scientific drive to synthesise chiral molecules in optically active form has been undertaken by many research groups.[1,2,3,4,5] However, a large number of biologically active compounds and pharmaceutical drugs are still synthesised as a racemic mixture either as a result of the lack of a chiral synthetic route or because of the high cost of chiral synthesis.[3,4,6,7] Resolving these mixtures involves enzymatic methods,[8,9] chiral chromatography and in some cases fractional crystallisation with a co-chiral molecule.[4] It is predicted that chiral crown ethers will play a major role in future enantiomeric separations.[10]

Cram synthesised the first chiral crown ether in 1972,[11,12] and since then several other chiral crown ethers have been synthesised.[13,14,15,16,17,18,19,20] Several chiral macrocyclic ligands and analogues have been shown to form enantioselective complexes with chiral organic ammonium salts.[19,21,22,23,24] The design and synthesis of chiral host macrocycles which are able to distinguish between the enantiomers of guest organic ammonium salts is of interest to workers in areas such as catalysis,[25,26] enzyme mimics,[27,28] and enantiomeric separation of racemic mixtures.[29,30]

Hayakawa[31,32,33] was the first to incorporate the pentacyclo-undecane (PCU, **3**) unit into crown ethers but their properties as ligands was not fully explored. The first chiral crown ethers to incorporate the PCU unit was the binaphthol crown (**4**) synthesised in 1999 and it showed promising enantioselectivity towards chiral ammonium salts.[34]



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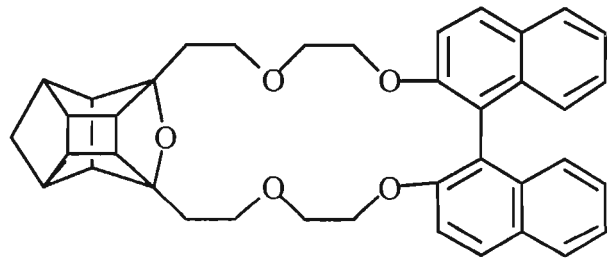

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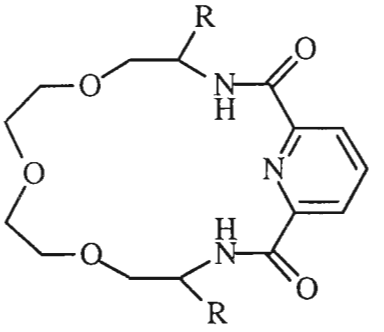
It has been predicted[34] that the incorporation of the PCU framework into chiral crown ethers will enhance their enantioselectivity by providing:

- a high degree of rigidity
- 10 extra chiral centres
- clear differentiation between the 2 faces of the ligand and
- increased solubility in non-polar solvents.

In order to test the validity of these predictions, it was decided to use the pyridine containing macrocycles (**5**) synthesised by Bradshaw and Izatt *et al.*,[35] as a model for the synthesis of new pentacyclo-undecane (PCU) containing crown ethers in this investigation. The synthesis of a new family of chiral PCU crown ethers, containing amino acids as the source of chirality, will be discussed in Chapter 3.



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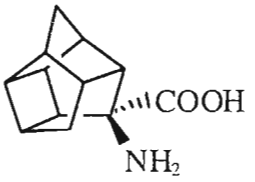
The synthesis of two cage annulated α-amino acids containing the pentacyclo-undecane (**6**) and trishomocubane (**7**) frameworks will be discussed in Chapter 2.



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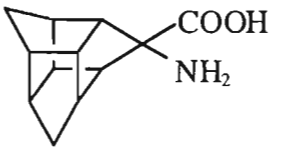

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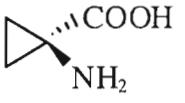
The use of these amino acids in macrocycles is not feasible at this point since they exist as racemates. The enantiomeric resolution of these compounds will be attempted in the future with the use of enzymes.[36,37,38] In the meantime, one of the aims of this investigation is to establish a procedure for incorporating amino acids **6** and **7** into peptides. The difficulty of the esterification of the amino acid **6** has been attributed to steric hindrance[39] and will be discussed in Chapter 2. It was therefore decided to attempt the coupling of glycine onto the amino and carboxylic ends of **6** and **7** since it is the least sterically hindered amino acid and it would also prevent the formation of diastereomers. In the light of the huge steric bulk of cage amino acids, it will be a huge step forward to establish a procedure to incorporate the amino acids **6** and **7** into peptides. The difficulties of incorporating sterically hindered amino acids into peptides will be discussed in Chapter 3. Being successful in this regard will open the doors for future work on the synthesis of macrocyclic peptides containing these cage amino acids.

CHAPTER 2

SYNTHESIS OF CAGE AMINO ACIDS

Amino acids are important building blocks for the synthesis of a large number of biologically active compounds and pharmaceutical drugs.
With the exception of 1-aminocyclopropanecarboxylic acid (**8**), found in cowberry[40] and cider apples,[41] no other cycloaliphatic aminocarboxylic acid occurs naturally.[42] Many homologues of **8** have been synthesised[43,44] and tested for anti-tumor activity[45] but only cycloleucine (**9**) was successful enough to make it to clinical trials.[46] Cycloaliphatic α-aminoacids of 2-amino-2-bornanecarboxylic acid (**10**) was shown to be actively transported by the transport system serving for the natural amino acids with apolar side chains.[47] They competitively inhibit the uptake of valine, leucine and methionine into Ehrlich ascites tumor cells and are therefore potential anti-tumour agents.[48]



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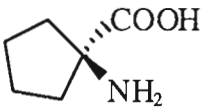

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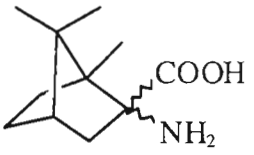

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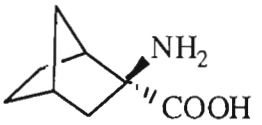

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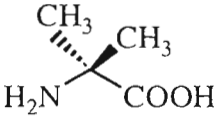
10c

Conformationally constrained amino acids are also found in many naturally occurring biologically active compounds e.g., alamethicin,[49] a peptide antibiotic, is made up of about 50% α-aminoisobutyric acid (AIB, **11**) and α-ethylalanine (EtA, **12**) which are both considered to be sterically hindered amino acids. Other conformationally constrained amino acids, N-methylated α-amino acids (**13**),[50,51] are found in cyclosporines,[52] didemnins[53] and dolastatins[54] and exhibit interesting biological activities.



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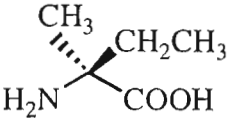

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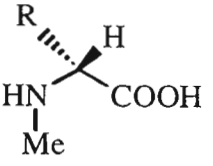

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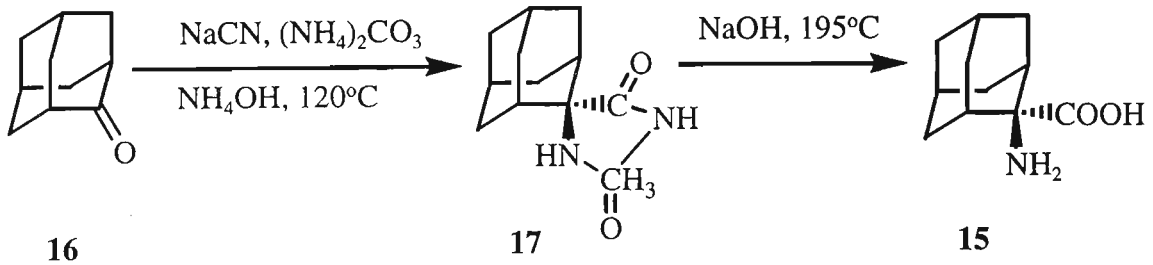
2-Aminoadamantane-2-carboxylic acid (**15**) was the first cage α-amino acid to be reported,[42] and showed excellent anti-tumour activity but its lack of solubility (less than 1.5 x 10^{-3} mol dm^{-3}) at physiological pH has prevented further biological evaluations. The amino acid **15** was synthesised by subjecting adamantan-2-one (**16**) to a Bucherer-Lieb synthesis to form the spirohydantoin **17**, which was then hydrolysed.[42] Amino acid **15** is

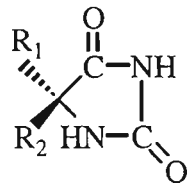
achiral and can therefore be used in peptide synthesis without forming diastereoisomers.



16 **17** **15**

Reaction Scheme I: Synthesis of 2-aminoadamantane-2-carboxylic acid

In this study a hydantoin (**14**) route[55,56,57] was used for the synthesis of the amino acids and it is noteworthy that some hydantoins, are themselves important medicinal and synthetic compounds.

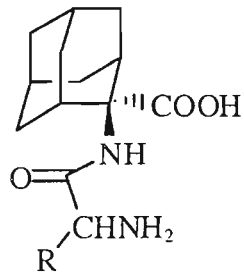


14

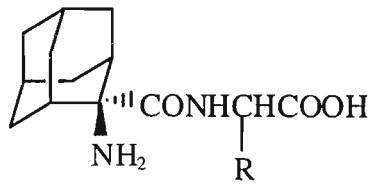
It has been shown[58,59,60] that incorporating cage structures into drugs induces a range of positive effects:

- increased lipophilicity promotes transport across cell membranes, therefore drugs can now be designed to target the central nervous system .
- hydrocarbon moieties increase a drug's affinity for lipophilic regions in receptor molecules.
- bulkiness retards metabolic degradation.

Derivatization of **15** to form dipeptides **18** enhanced their bio-availability by (a) increased water solubility (b) increased transportation across tissue membranes, and (c) retardation of metabolic degradation due to the cage moiety.[61]



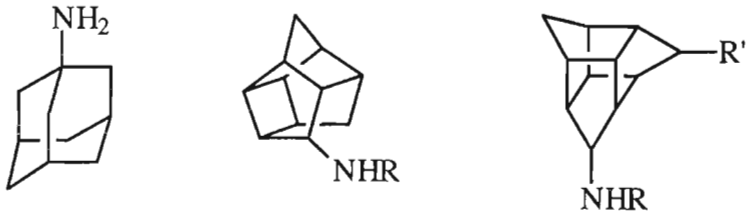
18a R = H, i-Bu



18b R = H, i-Bu, $CH_2C_6H_5$

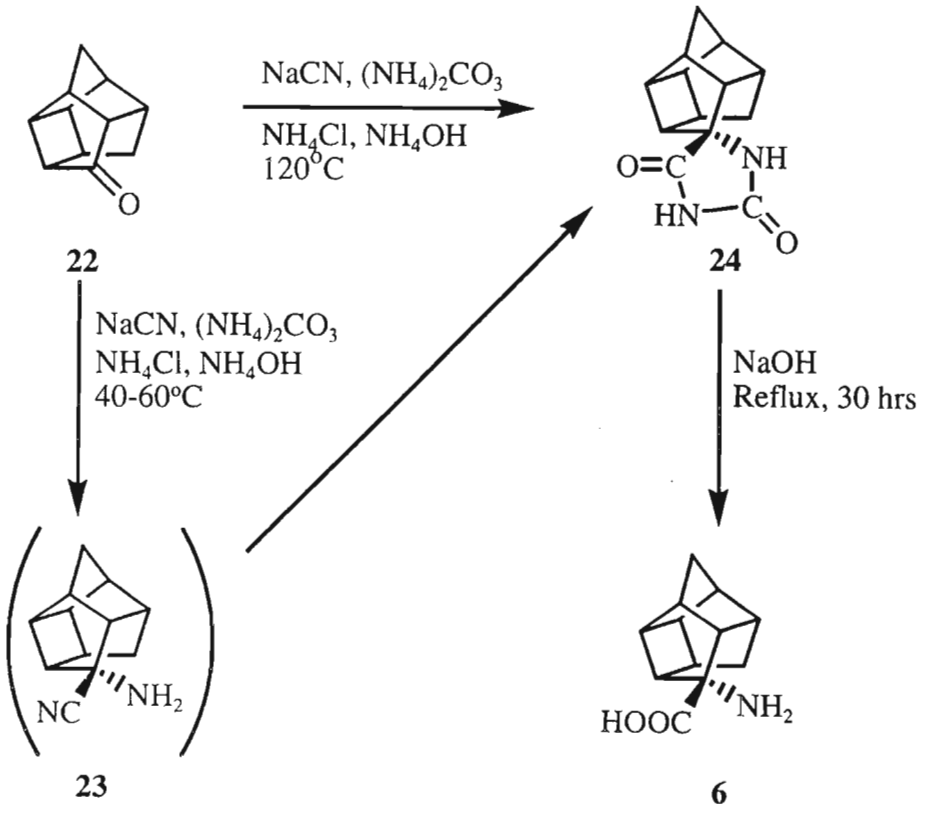
Since the introduction of amantadine (**19**)[62] and 2-aminoadamantane-2-carboxylic acid

(**15**), considerable synthetic effort has taken place in the field of polycyclic compounds.[63,64,65] However, biological activity studies and the synthesis of target molecules with potential biological activities have, to a large extent, been neglected. A number of amino containing cage compounds (such as **20** and **21**) with promising potential as an important new class of medicinal and pharmaceutical agents have been synthesised.[66,67,68,69,70,71,72]



19 **20** **21**

It is clear from the discussion above that the availability of readily accessible amino acids with cage skeletons can open exciting possibilities in a number of research fields.



Reaction Scheme II: Synthesis of the PCU amino acid (**6**)

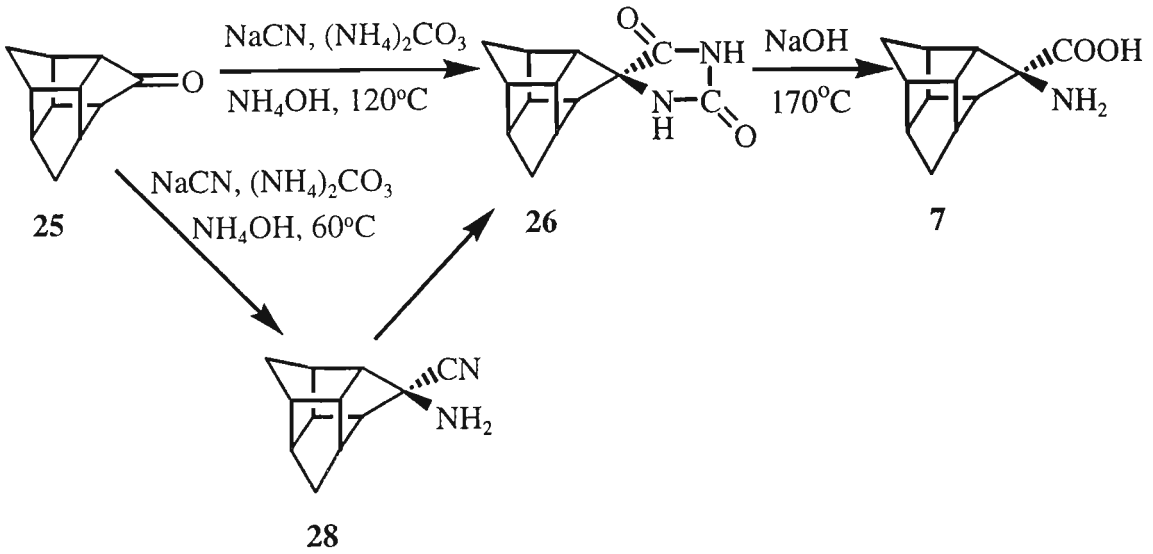
The synthesis of (R)-(+)-8-aminopentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane-8-carboxylic acid (PCU-amino acid, **6**) was recently reported by Martins and co-workers as the first

PCU related α-amino acid.[73] They utilised a similar strategy as was used to obtain the adamantane amino acid (**15**), namely conversion of a ketone to a hydantoin using Strecker or Bucherer-Bergs reagents. Subsequently the hydantoin **24** is hydrolysed to its corresponding amino acid. It was decided to repeat the synthesis of Martins *et al.*,[73] since it would serve as a valuable experience before attempting the synthesis the new TRIS amino acid (**7**) mentioned in the introduction (*vide supra*).

Attempts to repeat synthesis of the hydantoin **24** under the relatively mild conditions reported[73] failed. In this investigation it was found that elevated temperatures and pressures were required to ensure good yield of both hydantoin formation and hydrolysis of the hydantoin to its corresponding amino acid. The reaction temperature was increased gradually, i.e., 60°C for 2 h, 90°C for 2 h and 120°C for 5 h. The synthesis of the PCU amino acid (**6**) is illustrated in Scheme II. Note that the synthesis of the monoketone **22** used for the synthesis of **6** will be discussed at the end of this chapter.

There are so far, no reports on the incorporation of the PCU amino acid (**6**) into peptides. Attempts to esterify **6** via various methods have proven to be unsuccessful, most probably as a result of huge steric hinderance.[39] In the light of the enormous steric bulkiness of the PCU amino acid (**6**), it would be a considerable challenge to incorporate **6** (or **7**) into a peptide.

A literature survey revealed that 4-amino-(D_3)-trishomocubane-4-carboxylic acid (TRIS amino acid, **7**) had not been previously synthesised. Trishomocubanone (**25**) has D_3 symmetry and has an advantage over the PCU monoketone since only two hydantoin isomers can potentially form compared to four possible hydantoin isomers in the case of the PCU monoketone (**22**). It should be noted that both the PCU hydantion (**24**) and the TRIS hydantoin (**26**) do not have α-hydrogens and racemization under basic conditions can not occur, as is the case with many of the naturally occurring amino acids. Apart from the advantage of less isomers, the functional groups on the TRIS amino acid (**7**) are expected to be less sterically hindered, and therefore makes it an excellent candidate for incorporating into peptides. Trishomocubanone (**25**) was converted to the TRIS amino acid (**7**) as discussed for the PCU amino acid (**6**) (scheme III). Synthesis of the TRIS monoketone **25** is discussed at the end of this chapter.

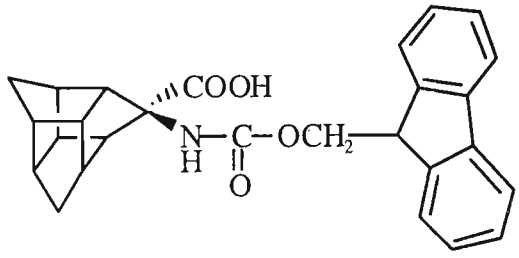


Reaction Scheme III: Synthesis of 4-amino-(D_3)-trishomocubane-4-carboxylic acid (**7**)

Recrystallisation of the hydantoin **26** from tetrahydrofuran rendered a product of which the CI mass spectrum exhibited a molecular ion at m/z 230, confirming the desired hydantoin formation. The infrared spectrum (p60) showed the presence of two carbonyl absorption bands at 1766 and 1720 cm^{-1} and two different N-H stretching vibration absorption at 3314 and 3161 cm^{-1} characteristic of a hydantoin ring. The ^1H NMR (p73) spectrum of the hydantoin **26** recorded in deuterated dimethyl sulphoxide [$(CD_3)_2SO$] exhibited some resonance overlapping. The cage protons resonate between 1.0 – 3.0 ppm, and appeared as a complex signal pattern. The high field signals from 1.15 - 1.47 ppm are assigned to the four methylene protons, and the signals from 1.84 - 2.93 ppm to the eight methine protons. Two deuterium exchangeable protons are registered at 7.89 and 10.55 ppm. The sharp resonance signal at 7.89 ppm can be assigned to the amide proton and the broad signal registered at 10.55 ppm to the imide being shifted to a lower field by the two adjacent carbonyl groups. The ^{13}C NMR (p74) of the hydantoin **26** exhibited two signals of carbonyl carbon atoms characteristic of an amide at 176.7 ppm and an imide at 156.3 ppm.

Hydrolysis of the hydantoin **26** with sodium hydroxide at 150°C produced the TRIS amino acid (**7**) in fair yield (60%). The infrared spectrum (p61) of **7** exhibited a broad complex pattern of peaks between 2345 and 3630 cm^{-1} typical of amino acids. A strong carboxylate ion absorption peak appeared at 1579 cm^{-1}.

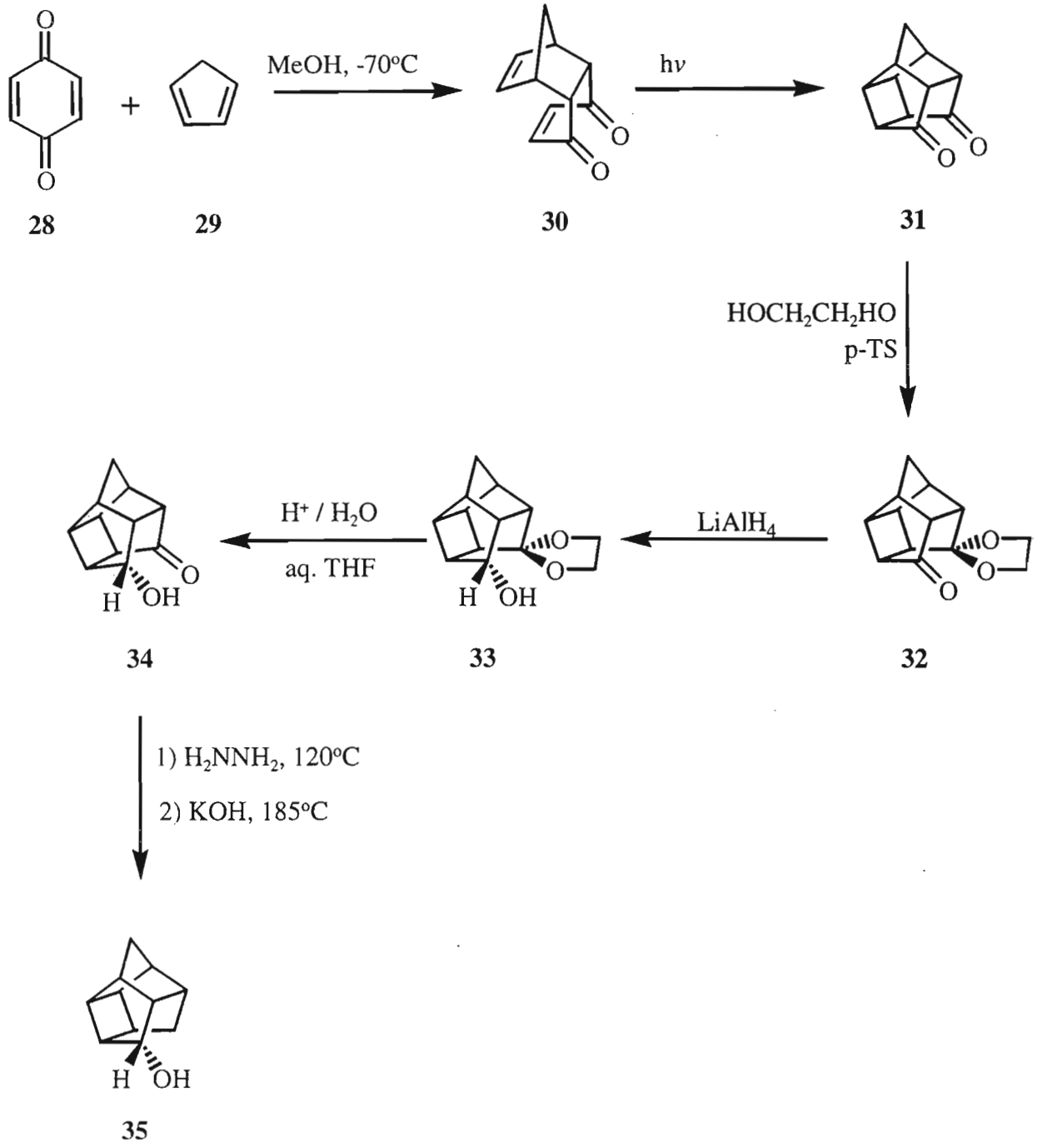
Further characterisation of **7** was done by forming the 9H-fluorene-9-methyl derivative **27** using 9H-fluorene-9-methyl chloroformate (Fmoc-Cl).



27

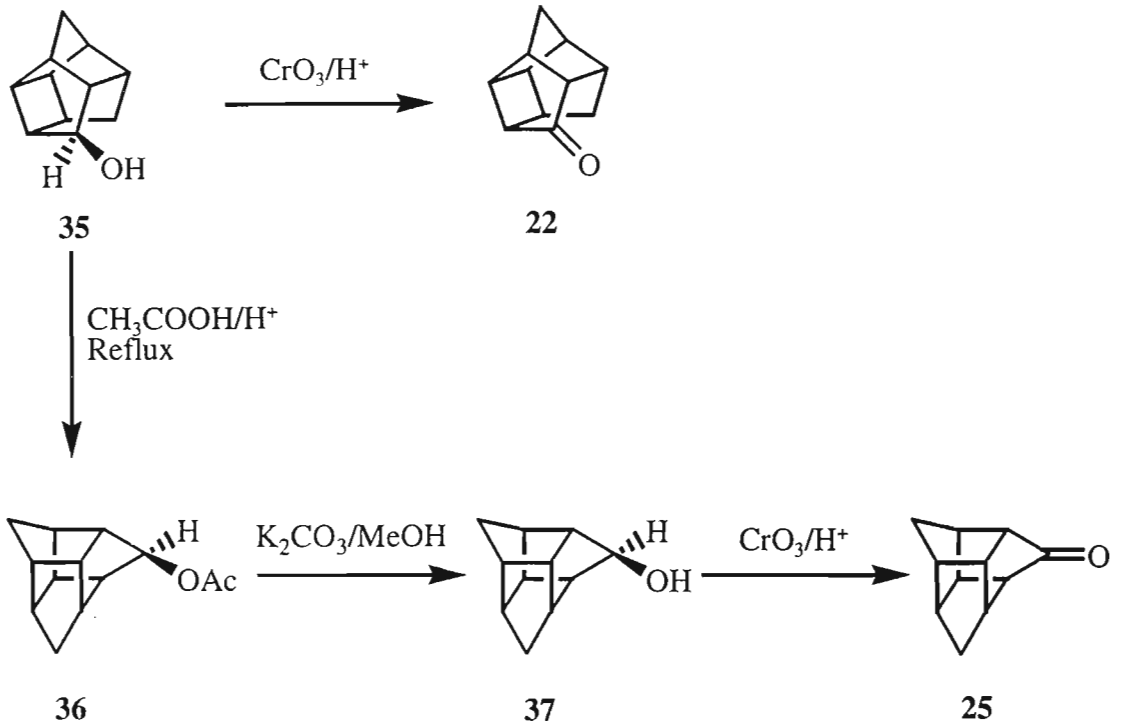
Recrystallisation from dichloromethane and hexane rendered a product for which the CI mass spectrum (p68) exhibited a molecular ion at m/z 430, confirming the desired acylated amino acid. A ninhydrin test (Kaiser Test)[74] was used to confirm the absence of any primary amines. The infrared spectrum (p62) showed absorption at 3358 and 1710 cm^{-1} for the N-H of the secondary amide and the urethane carbonyl respectively. An absorption band at 1510 cm^{-1} for the urethane C-N bond gives further proof that the amine group was acylated. The carbonyl of the carboxylic acid shows a strong absorption peak at 1745 cm^{-1}. The ^1H spectrum (p71) of Fmoc TRIS amino acid (**27**) in $(CD_3)_2SO$ exhibited a complex pattern between 1.0 and 3.0 ppm characteristic of the cage protons. Peaks between 7.2 – 8.2 ppm represent the aromatic protons of the Fmoc group. A low field peak appeared at 12.1 ppm due to the carboxylic acid. The ^{13}C NMR (p78) showed two carbonyl carbon signals at 174.0 ppm for the acid and 155.4 ppm for the amide. Two-methylene carbon resonances were registered at 31.8 ppm and 32.7 ppm for the cage and one signal at 68.7 ppm for the CH_2 of the Fmoc group. The Fmoc group is base labile and a ninhydrin test indicated that some deprotection has occurred after three weeks of storage.

Synthesis of monoketones **22** and **25** was performed according to established procedures (Schemes IV and V). A Diels-Alder Reaction of *p*-benzoquinone (**28**) with cyclopentadiene (**29**) resulted in formation of the adduct **30**, which was photolysed to yield the cage dione **31**.[75,76] Mono-protection of the dione using ethylene glycol in a Dean-Stark apparatus to remove water afforded the monoketal **32**[77] which was subsequently reduced with sodium borohydride to give the hydroxyketal **33**.[77,78] Hydrolysis of the ketal-protecting group gave the keto-alcohol **34**, which was converted via a modified Huang-Minlon reaction to the pentacyclo-undecanol **35**.[77]



Reaction Scheme IV: Synthesis of pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8-ol (**35**)

The alcohol **35** was oxidised with chromium trioxide (Jones Oxidation) under acidic conditions to form the monoketone **22**.[77] The alcohol **35** was then refluxed under strong acidic conditions resulting in rearrangement of the cage skeleton to form the acetate **36**.[77] Hydrolysis of the acetate **36** to the trishomocubanol (**37**) followed by Jones oxidation gave the trishomocubanone (**25**).[77]

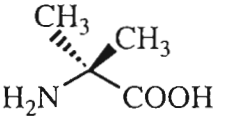


Reaction Scheme V: Synthesis of pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8-one (**22**) and (D_3)-trishomocuban-4-one (**25**)

CHAPTER 3

PEPTIDE CHEMISTRY

Merrifield first reported solid-phase peptide synthesis (SPPS)[79] in 1963, and since then numerous methods of effecting coupling of amino acids have been investigated. The use of acylating agents such as carbodiimides[80,81,82,83] (e.g. dicyclohexylcarbodiimide, DCC), uronium [(benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate, HBTU][84] and phosphonium [benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluoro phosphate, BOP][85] salts, pre-formed anhydrides, active esters, and acid chlorides[86,87] are the most popular for simple amino acids. The efficient coupling of sterically hindered amino acids like α-aminoisobutyric acid (AIB, **11**) and N-methylaminoisobutyric acid (MeAIB, **38**) still proves to be difficult.[88]



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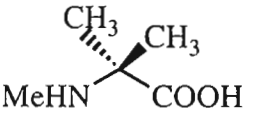
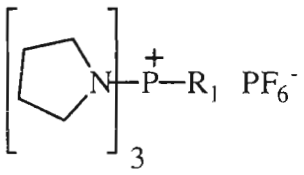
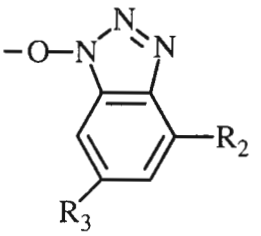


38

Recent reports on the successful coupling of sterically hindered amino acids, such as **11** and **38** in solid phase peptide synthesis include the use of acid fluorides[88,89,90,91,92] and halogenophosphonium derivatives **39**.[50,51,93,94]



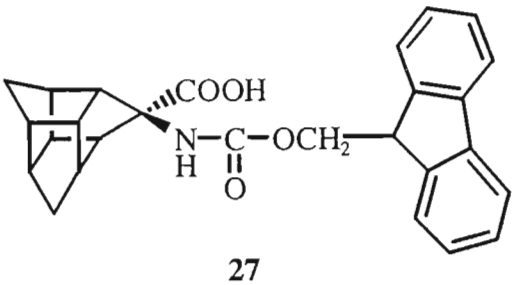
PyBroP: R_1 = Br
PyCloP: R_1 = Cl
PyBOP: R_1 = OBt
CF_3-PyBOP: R_1 = CF_3-OBt
NO_2-PyBOP: R_1 = NO_2-OBt
CF_3-NO_2-PyBOP: R_1 = CF_3-NO_2-OBt



OBt: R_2; R_3 = H
CF_3-OBt: R_2 = H; R_3 = CF_3
NO_2-OBt: R_2 = H; R_3 = NO_2
CF_3-NO_2-OBt: R_2 = NO_2; R_3 = CF_3

39

As mentioned before (see Chapter 1), the TRIS amino acid (**7**) is expected to have less steric hinderance compared to the PCU amino acid (**6**). It was also mentioned that all reasonable attempts[39] to esterify the PCU amino acid (**6**) failed, most possibly as a result of steric hinderance. Incorporating the Fmoc TRIS amino acid (**27**) into short peptides would therefore have a better chance of success. After establishing an effective procedure, coupling of the PCU amino acid (**6**) into peptides might also be investigated.
It was decided to investigate the acid fluoride route for incorporating the Fmoc TRIS amino acid (**27**) into a peptide since the use of BOP compounds **39** would require much more expensive and less stable reagents.[95]



27

= Fmoc TRIS amino acid

Carboxylic acid fluorides have been prepared from potassium fluoride,[95] potassium hydrogen fluoride,[96] potassium fluorosulphinate,[97] sulphur tetrafluoride,[98] diethylene(2-chloro-1,1,2-trifluoroethyl) amine,[99] thionyl fluoride[100] and benzoyl fluoride.[101] The most convenient method however, is the use of cyanuric fluoride in the presence of pyridine.[102]

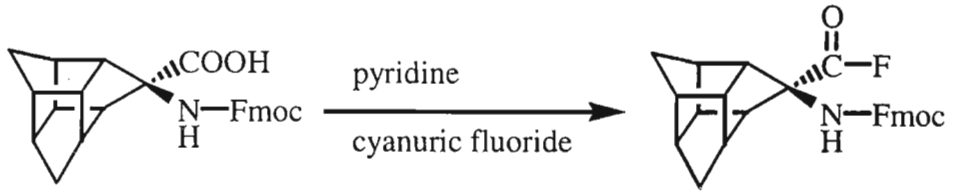
Scheme VI outlines the route followed for the incorporation of the Fmoc TRIS amino acid **27** into a peptide via solid phase synthesis techniques. The acid fluoride **40** was synthesised in 90% yield by reacting **27** with one molar equivalents of pyridine and cyanuric fluoride in dry dichloromethane. The infrared spectrum (p63) of **40** showed strong absorption peaks at 1829 and 1724 cm^{-1} for the carbonyl stretching vibrations of the acyl fluoride and the urethane carbonyl, respectively. ^{13}C NMR (p81) showed that the carbon α (68.6 ppm) to the fluorine being split with a coupling constant of 370 Hz, and the β (174.0 ppm) carbon being split with a coupling constant of 55 Hz. The CI MS was found to be 430.1818, which is consistent with a formula of $C_{27}H_{24}NO_3F$.

The TRIS amino acid (**7**) was obtained as a racemate (see chapter 2) and in order to avoid the formation of diastereomeric mixtures it was decided to attach glycine (gly) onto both the functional groups of the cage amino acid in proof that its sterical hindrance could be overcome in SPPS. Since glycine is the least sterically hindered amino acid, it would also be advantageous in coupling of the highly hindered Fmoc TRIS amino acid fluoride (**40**). Glycine is also an achiral compound and will not form diastereomeric products when coupled to a racemic amino acid. Fmoc glycine (**41**) was coupled to the p-benzyloxybenzyl alcohol resin (Wang Resin)[103,104] via a standard esterification method using diisopropylcarbodiimide (DIPCDI) and hydroxybenzotriazole (HOBt).[80,81,82,83] The Wang Resin was chosen because it is the most economical and therefore most widely used of all the resins available for Fmoc SPPS. The only disadvantage of using the Wang resin is the lack of an amino group. Since it is functionalized with a hydroxyl group, it is not possible to monitor the loading of the first amino acid with the use of the ninhydrin test (Kaiser Test).[84]

The base labile Fmoc protecting group was removed by treating the resin with 20% Piperidine in dimethylformamide (DMF) for thirty minutes. The coupling of Fmoc amino acid fluorides is normally carried out in the presence of base to trap the hydrogen fluoride (HF) released,[92,105,106] but can also be carried out in the absence of base with less efficiency.[88,107] N,N-diisopropylethylamine (DIEA) is the base of choice[107] for coupling of Fmoc amino acid fluorides but there is a danger of premature deblocking,[108] especially in the case of slow coupling reactions.[92] Coupling of the Fmoc TRIS amino acid fluoride

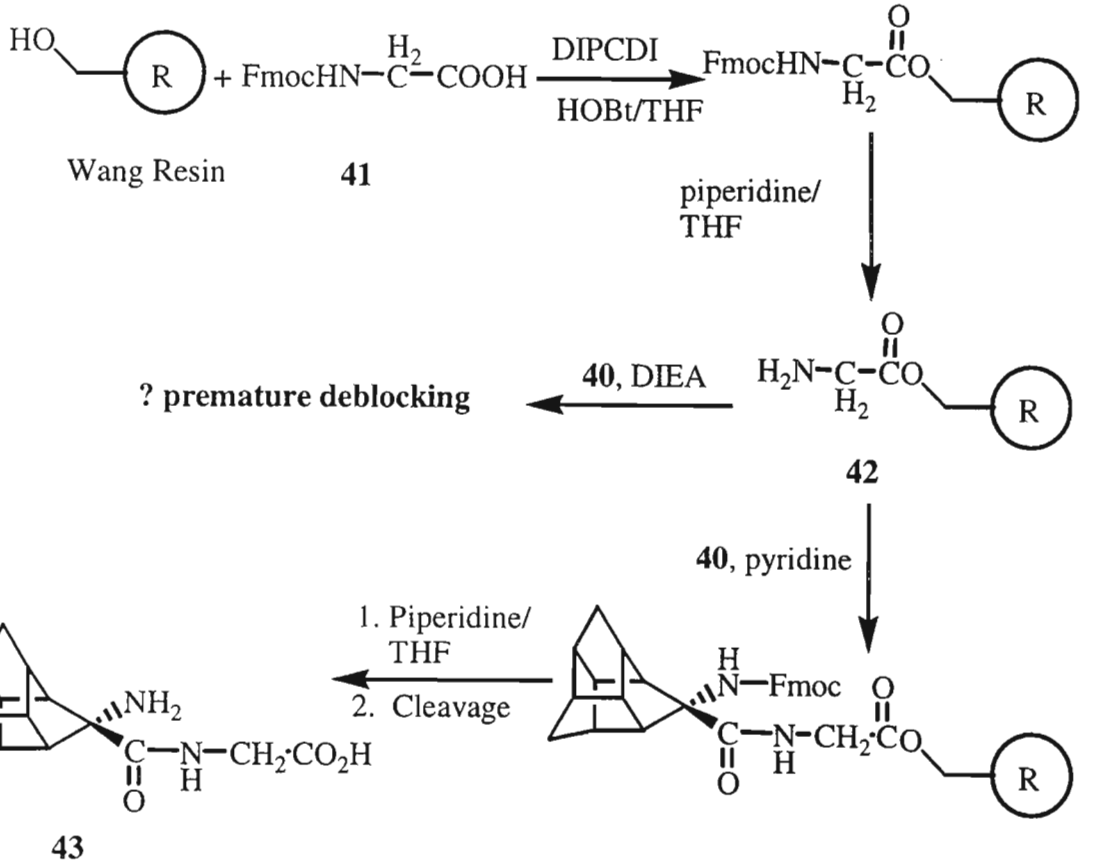
(**40**) to the glycine containing resin **42**, in the presence of one equivalent of DIEA, showed a positive ninhydrin test (presence of primary amines) after six hours of reaction time indicating that either premature deblocking had occurred or the reaction was incomplete. It was decided to investigate the use of pyridine as the HF scavenger since it is used in the synthesis of the acid fluoride without any sign of deblocking. After six hours of reaction time, the Kaiser Test was negative, indicating the completion of the coupling reaction and no sign of premature deblocking. After twenty hours of reaction time, there was still no sign of premature deblocking suggesting that pyridine is a better base for this purpose. Some of the resin was treated with trifluoroacetic acid (TFA), after deprotection, to cleave the dipeptide **43** for analysis. Peptides are normally purified using preparative HPLC and the purity is determined with regular HPLC. This equipment was unfortunately not available during this study. Attempts to purify the peptide on a gravitational silica gel column proved to be difficult.

A:



27 **40**

B:



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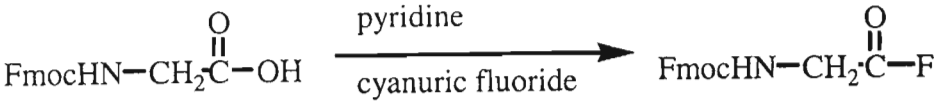
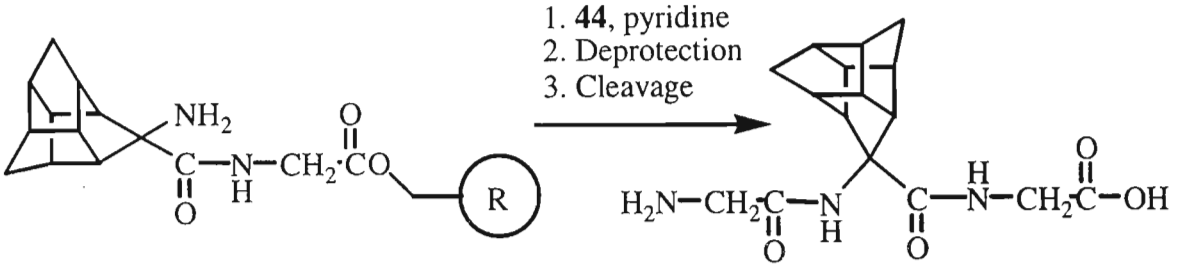


41 **44**

D:



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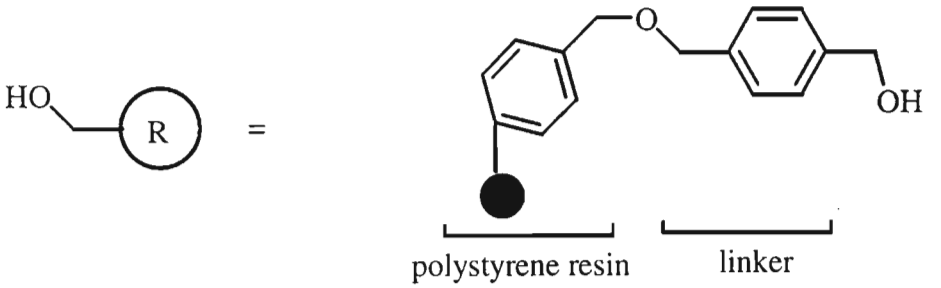


46

Reaction Scheme VI: Synthesis of the peptides

Standard cleavage procedures were used to confirm formation of the dipeptide **43** (see experimental section). ^{13}C NMR (p84) in methanol-d_4 showed the presence of the cage with two methylenes at 33.6 and 34.0 ppm, and eight methines from 42.9 to 55.3 ppm. A methylene at 44.2 ppm indicates the presence of the glycine, and two quaternary carbons at 171.5 and 176.3 ppm for the amide and carboxylic carbonyls respectively. HRMS shows a signal at m/z 263 ($M + H^+$) confirming the desired dipeptide **43**.

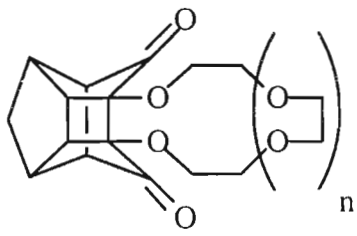
The protecting group of the rest of the resin bound peptide was removed and the second glycine was coupled to the cage via its Fmoc acid fluoride (**44**) in the presence of pyridine. The tripeptide **45** was deprotected and cleaved from the resin, and proved to be more difficult to purify than the dipeptide **43**. HRMS (p69) of the crude product shows a signal at m/z 320 ($M + H^+$) confirming the presence of the desired tripeptide.

Even though a procedure has been established for incorporating cage amino acids into peptide, it was not feasible to attempt the same reactions with the PCU amino acid without the use of HPLC. Future studies will focus on the incorporation of the PCU amino acid into a similar tripeptide. After successful coupling with glycine the optically pure PCU amino acid synthesised by Martins and co-workers[73] can be used to establish procedures to incorporate the PCU amino acid into enantiomeric pure peptides containing other naturally occurring amino acids via SPPS.

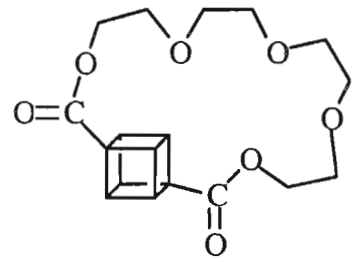
CHAPTER 4

SYNTHESIS OF CAGE ANNULATED CHIRAL CROWN ETHERS

Relatively few crown ethers containing a "cage" moiety as part of its "backbone" have been synthesised.[109] Compounds **47**[110] and **48**[111] are examples of cage containing crown ethers but the cage in either case simply functions as a lipophilic "spacer".[1]

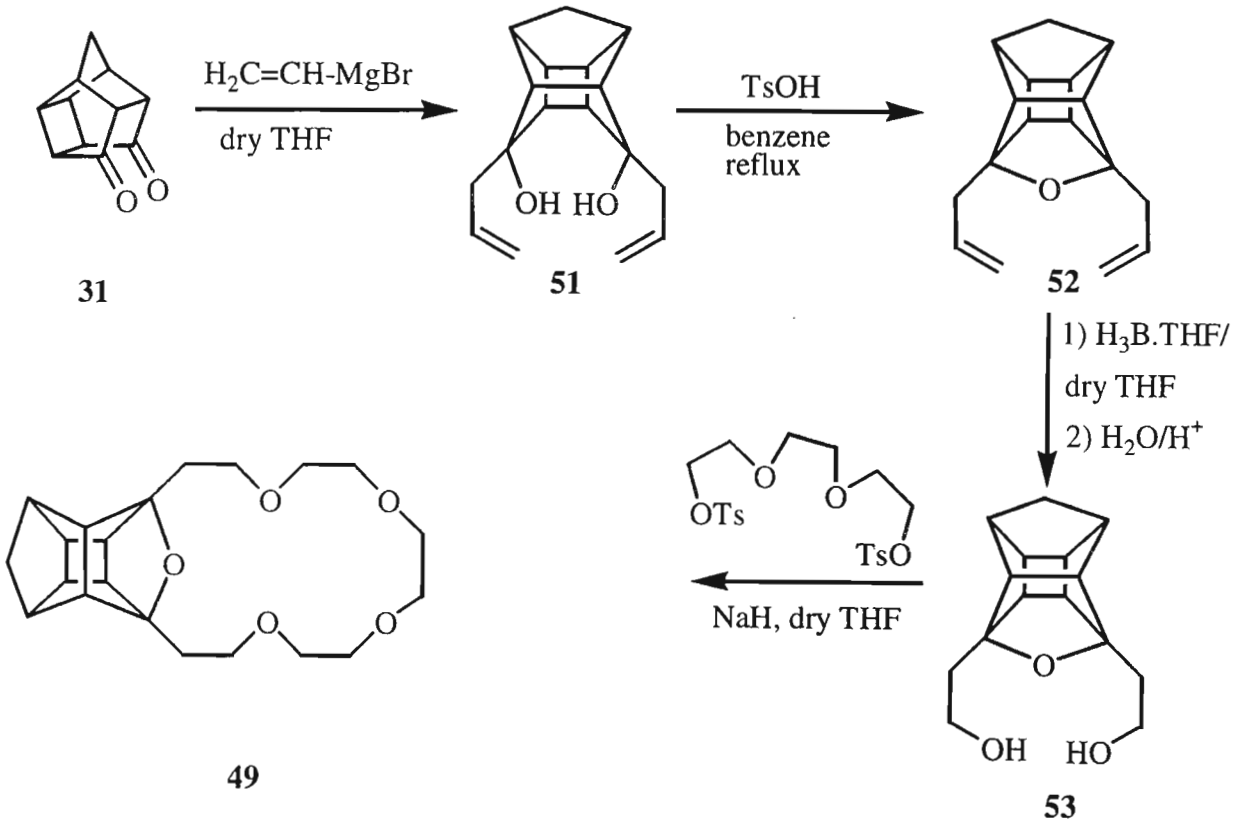


47



48

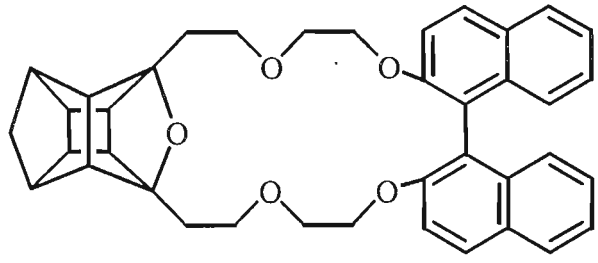
An improved system of incorporating cage moieties into crown ethers using the PCU cage diol **53** was developed by Marchand *et al.*[109] The synthesis of **49**[109] is illustrated in Scheme VII. The cage is now part of the ligand's backbone and not only serves as a rigidifying moiety but enables the bridgehead oxygen atom to participate, with the other donating atoms, in complexation of a guest.



31 **51** **52** **53** **49**

Reaction Scheme VII: Improved PCU cage annulated crown ether

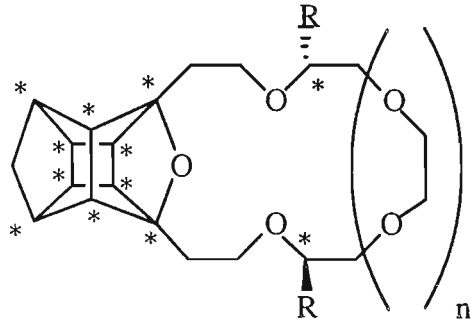
In addition, the pentacyclo-undecane (PCU) cage renders the "faces" of the crown ether inherently diastereotopically non-equivalent. Alkali metal picrate extraction experiments on this type of crown ethers have shown[109] that they are better hosts than their corresponding non-cage annulated systems. Recently, Marchand *et al.*[34] reported the synthesis of the first PCU-cage annulated chiral crown ether **4**[34] using a binaphthol group as the source of chirality and was proven to exhibit promising enantioselectivity.



4

Many chiral agents are used in the synthesis of chiral macrocyclic ligands such as carbohydrates,[112,113] D-α-ephedrine,[114] tartaric acid,[115] cyclohexane-1,2-diol,[116] binaphthol,[117] spirobifluorene,[118] and amino acid derivatives.[119]

In this study it was decided to use amino acids because of their natural availability and cost effectiveness. Note that the PCU unit is a meso compound but becomes chiral when incorporated into a chiral crown. One of the main aims when designing the synthesis was to get the chiral centres closer to the cage to take maximum advantage of the ten inducible chiral centres (see structure **54**)



54

From the past investigations, five rules have emerged which are regarded as being essential in estimating chiral recognition ability of a macrocyclic receptor and therefore used as the basis of designing such systems.[23]

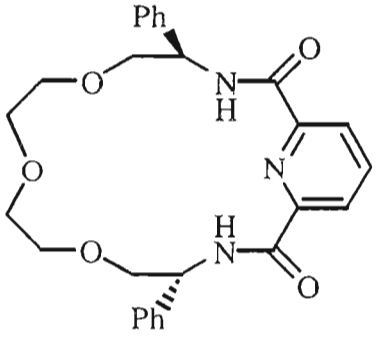
- in order to obtain good enantiomeric selectivity, the host and the guest need to form stable diastereomeric complexes and the enantiomeric discrimination will depend on the steric repulsion between the substituents at the chiral portions of the host macrocycle and the guest molecules.[120,121]

- larger chiral barrier(s) in a macrocycle usually results in an increase in the degree of enantiomeric recognition, but it should not be too large as to prevent complexation.[122,123,124,125]
- Still and co-workers[126] have shown that rigid diastereomeric complex between the host and guest is essential for good enantiomeric recognition. If flexible complexes form, the enantiomers can orientate themselves as to avoid the steric hindrance created by the chiral centers.[119,121,127]
- The chiral macrocyclic compound should be stereochemically complimentary with the guest enantiomers, interaction of the chiral centres should not be prevented by steric hindrance.[128,129]
- higher enantioselectivity is shown by macrocyclic ligands possessing C_2, C_3 and D_2 symmetry over those with C_1 or D_3 symmetry.[127,128,130]

Incorporation of the pentacyclo-undecane (PCU) cage into a chiral macrocyclic ligands would satisfy four out of the five requirements (*vide supra*). Chiral macrocycles containing the PCU cage will have a C_1 symmetry, which may not be regarded as the most favourable symmetry.[127,128,130] Though chiral crown ethers of type **54** possesses a C_1 symmetry, we anticipate that the cage would offer:

- 10 extra chiral centers when associated with a source of chirality.
- high degree of rigidity.
- clear differentiation between the 2 faces of the ligand and,
- increased solubility in non-polar solvents, which might enable such a macrocycle to show increased enantiomeric recognition.

The pyridine containing macrocycles[131,132,133,134,135,136,137] **55** and **56** were chosen as the model systems for this study in order to obtain a good comparison between the cage macrocycles and other relatively rigid systems. Systems **55** and **56** have been shown to form strong complexes with ammonium salts, via NMR and fluorescence studies respectively,[136,138] and in some cases display enantiomeric recognition.[135,136]



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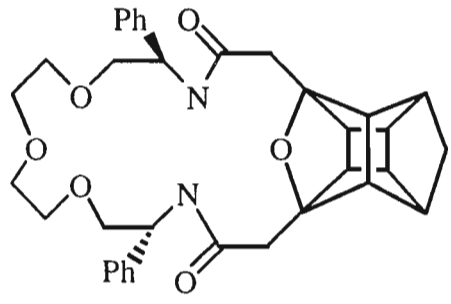

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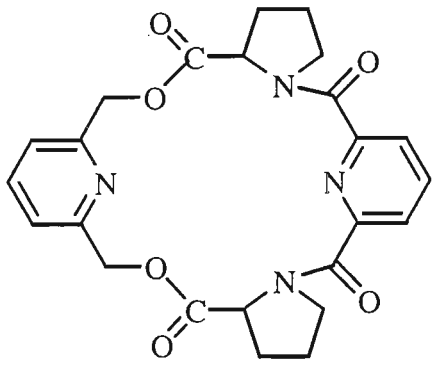

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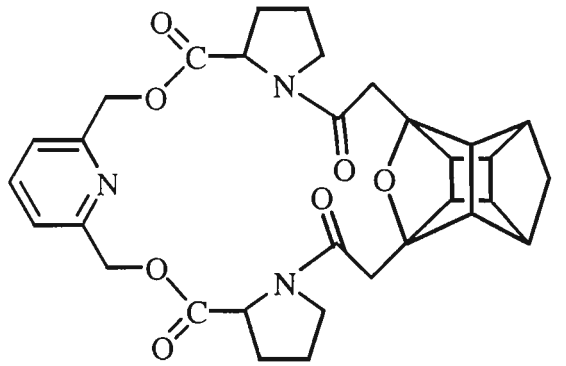
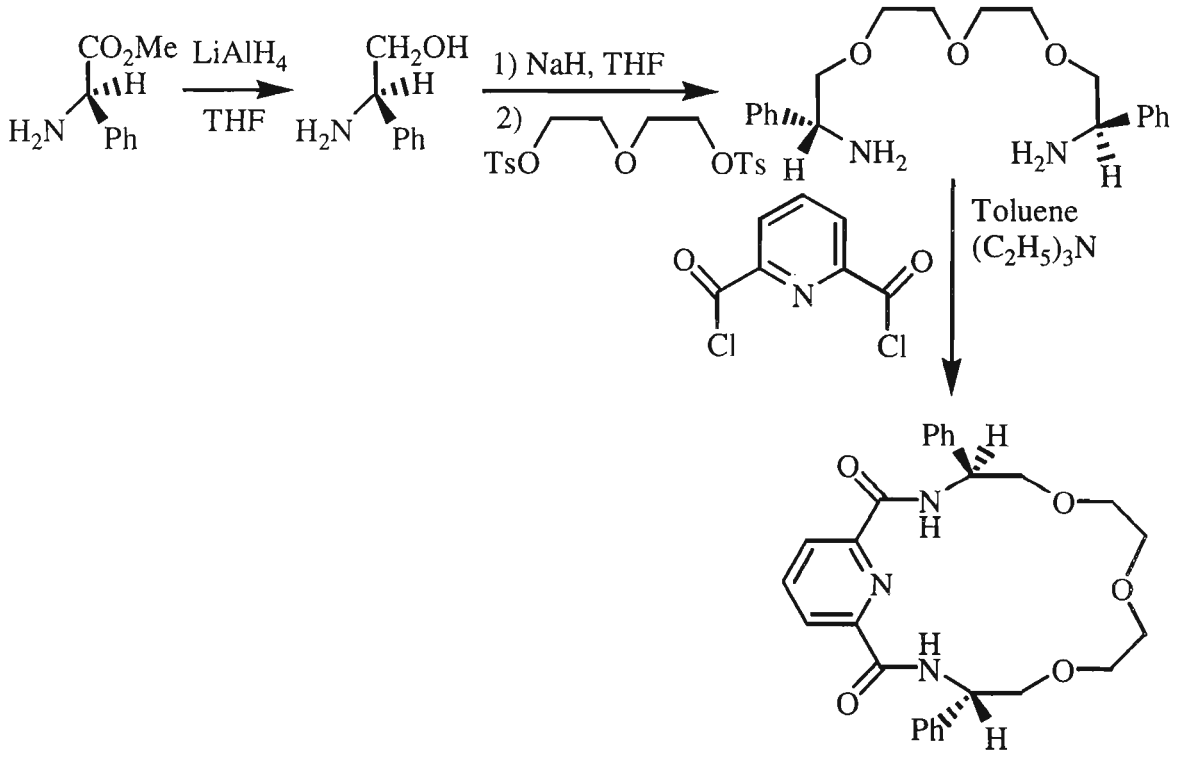


58

The novel cage macrocyclic analogues **57** and **58** were intended to be synthesised in this study. The use of phenylglycine derivatives as a source of chirality in these systems has shown less promising complexation with NMR studies. It was however decided to use phenylglycine in this study of new chiral PCU macrocycles in order to be able to apply and learn the technique of complexation studies using Fluorescence Spectroscopy which was recently reported[138] to be an easy tool for determining enantiomeric recognition of macrocycles. Three model systems were chosen that would allow for Fluorescence Spectroscopy studies[138] on the corresponding PCU cage analogues. The discussion on Fluorescence Spectroscopy and the results obtained are detailed in chapter 4.



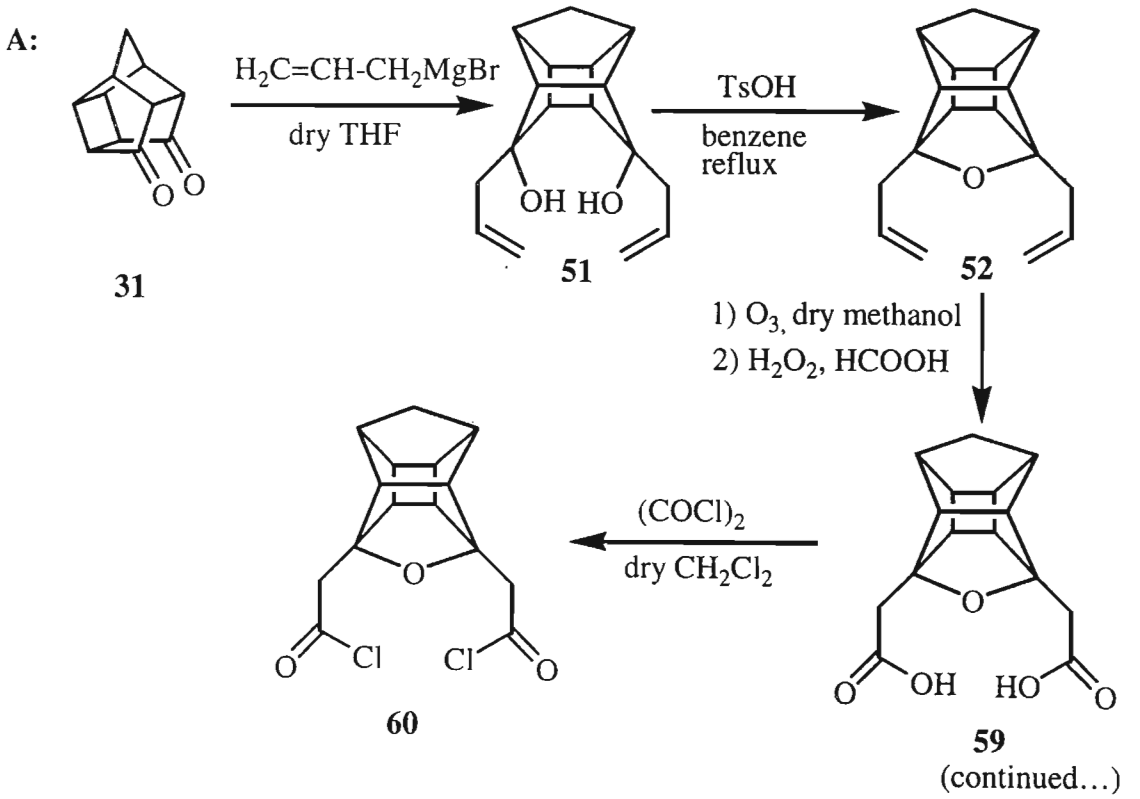
55

Reaction Scheme VIII: Synthesis of chiral pyridine macrocycle **55**[132]

The PCU cage is substituted for the rigid pyridine unit in the model compounds **55** and **56**. Synthesis of model crown, (4S,14S)-(-)-4,14-diphenyl-6,9,12-trioxa-3,15,21-triaza-bicyclo[15.3.1]heneicosa-1(21),17,19-triene-2,16-dione (**55**), is shown in Scheme VIII.[132] Ring closure with diacid dichlorides and diamines is usually an effective method to form cyclic macrocyclic diamides[139,140,141] but the yield reported is relatively low (16%).

It was decided to follow a similar route for the synthesis of the corresponding PCU macrocyle **57**, but with slight modification of the reaction conditions in hope of achieving a better yield. Scheme IX outlines the route followed for the synthesis of the PCU macrocycle **57** as corresponding PCU cage analogue.

The reaction of the PCU-8,11-dione (**31**) with excess allylmagnesium bromide (obtained from reacting allylbromide with magnesium in ether) afforded the corresponding *endo*-8,*endo*-11 diol **51** (91%).[142,143] Subsequent dehydration of the diol **51** produced the corresponding hexacyclic ether, **52**.[144] Ozonolysis of the diene followed by an oxidative work-up gave the PCU-diacid **59** (76%). The ^{1}H NMR showed an AB pattern at 1.45 and 1.83 ppm with a coupling constant of 10 Hz indicating the presence of the PCU cage. The ^{13}C NMR (p90) showed a triplet at 42.8 ppm which is characteristic of the methylene on the cage. A triplet at 37.9 ppm represents the methylene joining the carboxylic acid to the cage and a singlet at 171.4 ppm for the carbon of the carboxylic acid.



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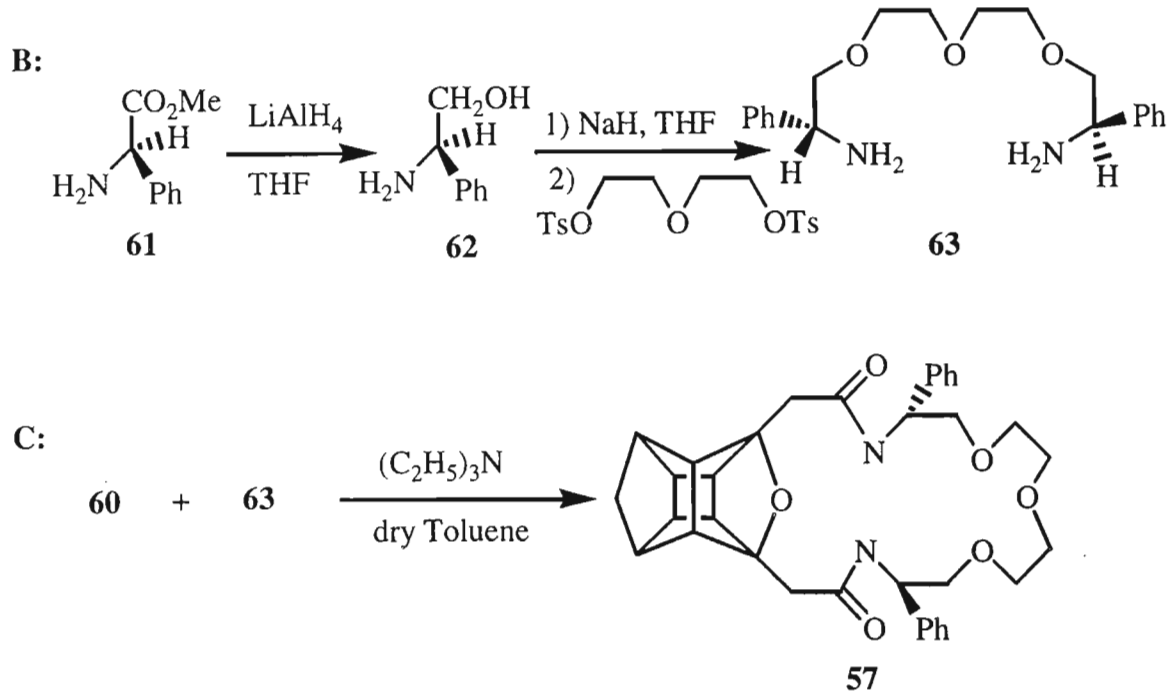


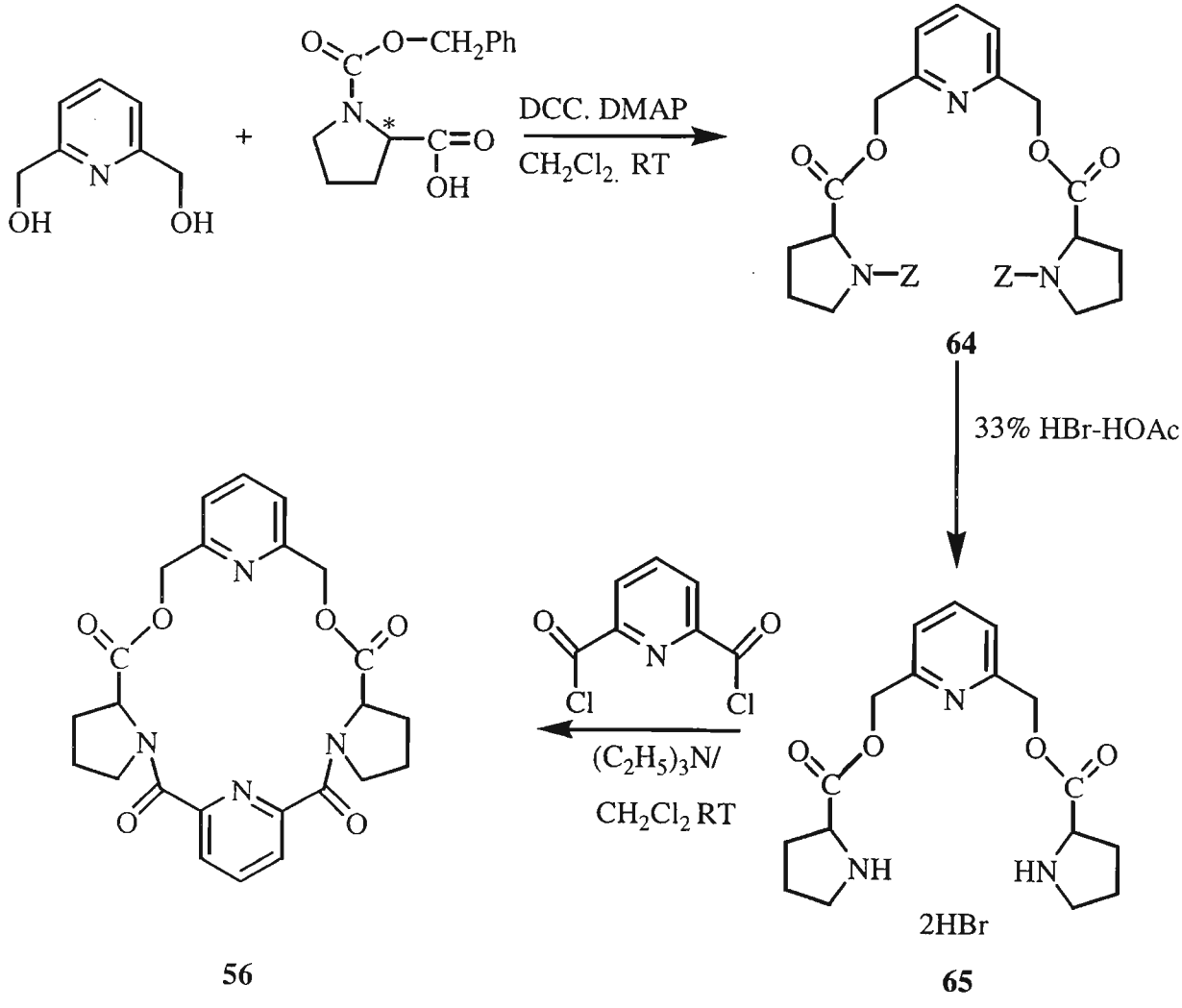
Reaction Scheme IX: Synthesis of macrocycle (R)-**57**

The diacid **59** was subsequently reacted with oxalylchloride to form the diacyl chloride **60** and used directly in the next step. (R)-(+)-2-phenylglycine methyl ester (**61**) was reduced using $LiAlH_4$ in THF to (R)-2-phenylglycinol (**62**) in 67% yield after recrystallisation from toluene.[145] The diamine **63** was prepared in 65% yield by treating two equivalents of (R)-2-phenylglycinol (**62**) with one equivalent of diethylene glycol ditosylate (obtained by reacting diethylene glycol and tosyl chloride in aqueous potassium hydroxide) in the presence of sodium hydride in THF.[146] The alkoxide of the aminoalcohol formed under these conditions, acts as the nucleophile. It was difficult to obtain pure diamine **63** and hence it was used as obtained in the next step.

The coupling of the PCU diacyl chloride **60** and the diamine **63** was performed in pure and dry toluene, with triethylamine as the base, to give the macrocycle (R)-**57** (37.5%). The increase in yield as compared to the model compound **55** is perhaps a combination of two factors; (i) the reaction was carried out at a much higher dilution (2 mmol dm^{-3}) and (ii) because the cage diacyl chloride **60** is much more rigid and helps in tying up the loose ends of the diamine **63**. The crude product was purified by chromatography and recrystallised from toluene. The infrared spectrum (p64) of **57** showed multiple peaks from 3307 - 3343 cm^{-1} representative of secondary amide NH stretching and a peak at 1517 cm^{-1} representing secondary amide NH bending. A characteristic CO stretching vibration peak at 1674 cm^{-1} confirmed the amide group. ^1H NMR (p91) shows an AB pattern at 1.49 and 1.82 ppm with a coupling constant of 10 Hz and a complex multiplet from 2.15 to 2.80 ppm representing the PCU cage. Another complex multiplet from 3.45 to 3.95

ppm indicates the presence of the OCH_2 protons. The aromatic protons exhibited a multiplet from 7.12 to 7.53 ppm. ^{13}C NMR (p92) of **57** showed eleven signals for the cage carbons (unlike in the regular cage annulated crowns and chiral crown **50** reported by Marchand *et al.*,[34] which showed only six signals for the cage carbons). The lack of symmetry on the cage part of **57** indicates that the positioning of the chiral agents (phenylglycinol (**62**)) successfully induced chirality on all ten of the inducible carbons of the cage. The CI MS (p70) confirms the [1:1] cyclization product with a m/z peak at 585.2973 and supports a formula of $C_{35}H_{41}N_2O_6$.

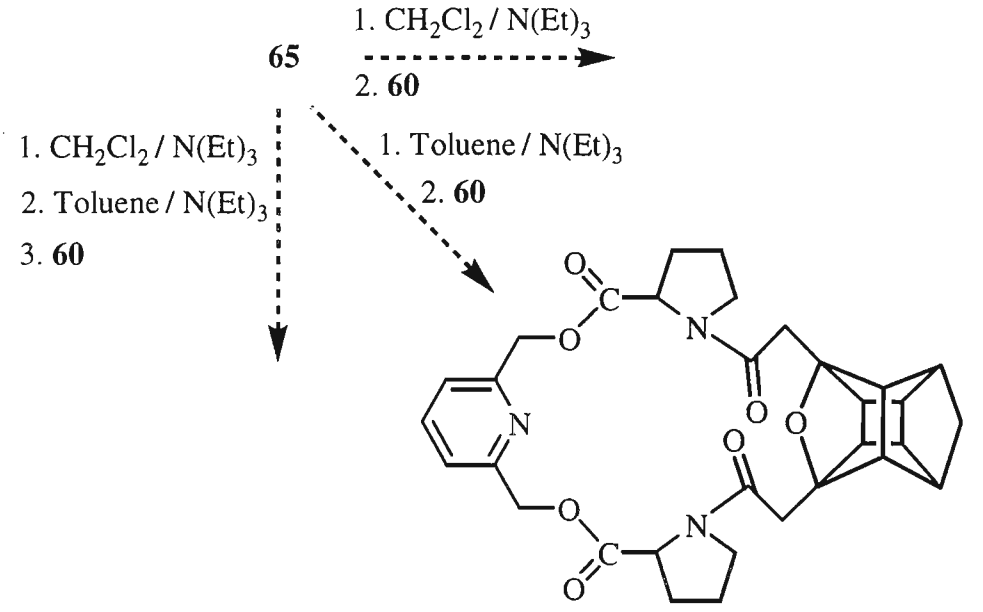
The synthesis of the dipyridine crown **56**, is outlined in Scheme X and has been shown, via Fluorescence Spectroscopy studies, to exhibit good enantiomeric discrimination for D- and L-amino acid methyl ester hydrochloride.[138]



Reaction Scheme X: Synthesis of dipyridine macrocycle **56**[138]

The method used in literature[138] to prepare **56** was employed for synthesising the novel macrocycle **58** as the corresponding cage analogue. The key intermediate **65** was prepared from condensation of benzyloxycarbonyl-L-proline and 2,6-bishydroxymethyl

pyridine (scheme X) in the presence of dicyclohexylcarbodiimide (DCC) and N,N-dimethylaminopyridine (DMAP) followed by deprotection. Acylation of the chiral diamine dihydrobromide **65** with PCU diacyl chloride (**60**), under various conditions did not result in the desired [1:1] cyclization product **58**, shown in Scheme XI. This may be attributed to steric effects from the relatively rigid proline system. The diamine dihydrobromide **65** was dissolved in a mixture of dichloromethane and triethylamine and then reacted with **60**. In the second method, toluene was used as the solvent in an attempt to decrease the polarity of the medium to slow the reaction down. In the third method, the free diamine (obtained by treating a solution of **65** in dichloromethane with triethylamine and then washed with water) was reacted with **60** using toluene as the medium. All three methods proved unsuccessful in synthesising the macrocycle **58** possibly as result of aforementioned steric reasons.



58

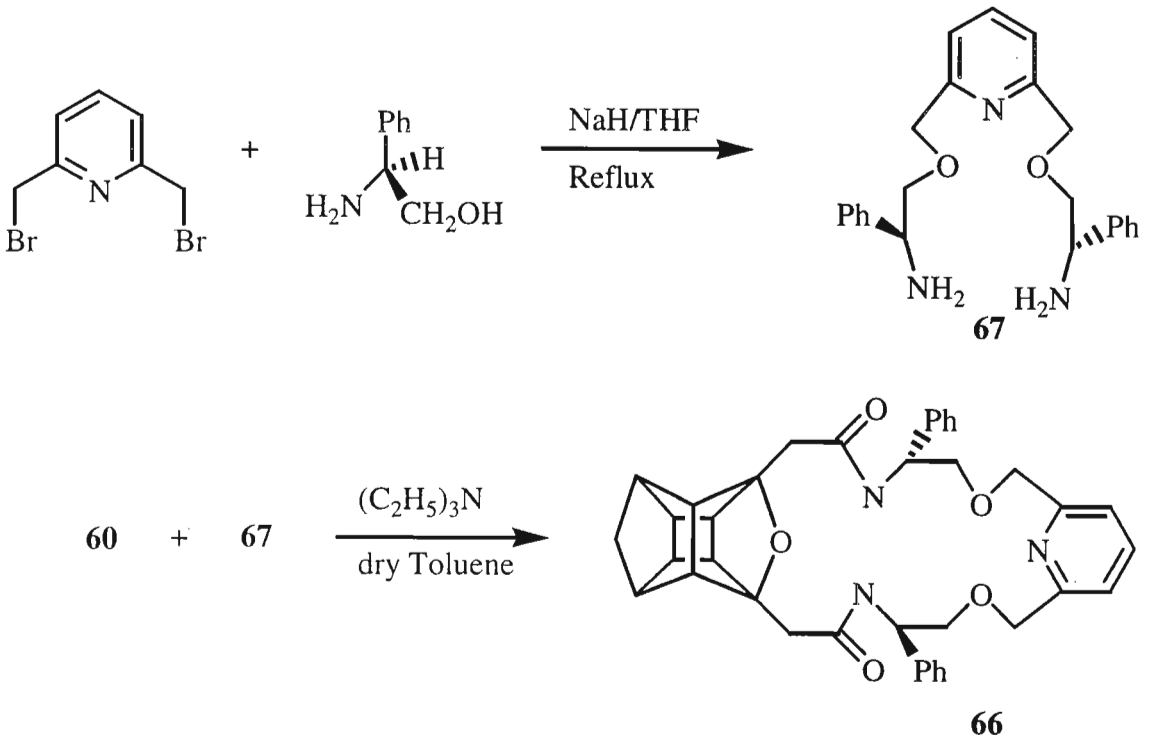
Reaction Scheme XI: Attempted synthesis of the chiral macrocycle **58**

It was decided to synthesise the PCU containing macrocycle **66** instead, since phenylglycine will be used as the source of chirality rather than the more sterically hindered proline. It would also enable us to compare **66** and **57** since they both have just two amide groups as compared to the two additional ester groups in **58**.

The synthesis of macrocycle **66** is outlined in Scheme XII. Macrocycle **66** is similar to **57** (Scheme IX), only differing by the presence of a pyridine moiety as part of the ring. The diamine **67** was prepared in 72% yield by treating two equivalents of (S)-2-phenylglycinol with one equivalent of pyridine dibromide in the presence of sodium hydride in THF.[137] The diamine **67** was difficult to purify and used as obtained in the next step. The infrared spectrum (p65) of **67** showed peaks between 3025 and 3370 cm^{-1} for the primary amines, and peaks at 1104 cm^{-1} for the ether groups. The ^1H NMR (p95) showed a singlet at 1.91 ppm for four D_2O exchangeable protons indicating the presence of the

diamine. A multiplet at 3.59 ppm represented the four OCH_2 protons and a pair of doublets at 4.25 and 4.29 ppm for the two-methine protons at the chiral centre. A singlet at 4.65 ppm is for the four protons of the methylene group attached to the pyridine. The ^{13}C NMR (p96) showed peaks for a methine at 55.4 ppm and two methylene peaks at 73.9 and 77.1 ppm. CI MS (p72) confirmed the compound with a m/z peak at 378.2175.

Coupling of the PCU diacyl chloride (**60**) and the diamine **67** was performed in pure and dry toluene, with triethylamine as the base, to give the novel crown **66** (24%). The infrared spectrum of **66** showed broad peaks from 3294 to 3425 cm^{-1} representative of secondary amide NH stretching and a peak at 1523 cm^{-1} representing secondary amide NH bending. A characteristic CO stretching vibration peak at 1656 cm^{-1} confirms the presence of an amide bond. A broad peak at 1116 cm^{-1} is indicative of the CO stretching vibrations of the ether linkages. ^1H NMR showed an AB pattern at 1.40 and 1.75 ppm with a coupling constant of 10.6 Hz and a complex multiplet from 2.22 to 2.74 ppm representing the PCU cage. Another complex multiplet from 3.80 to 4.00 ppm indicates the presence of the OCH_2 protons. A triplet at 4.66 ppm with a coupling constant of 14.3 Hz is registered for the methylene protons bonded to pyridine. The aromatic protons are found as a multiplet from 7.12 to 7.44 ppm. One of the two NH protons registered as a doublet at 7.79 ppm with a coupling constant of 7.7 Hz and the other proton was masked with one of the pyridine protons between 7.62 and 7.74 ppm. The CI MS confirms the [1:1] cyclization product with a m/z peak at 618.2959, which supports a formula of $C_{38}H_{39}N_3O_5$.



Reaction Scheme XII: Synthesis of macrocycle **66**

CHAPTER 5

ENANTIOMERIC RECOGNITION STUDIES OF CHIRAL MACROCYCLES

Many methods exist for enantiomeric recognition studies and include fast atom bombardment mass spectrometry (FAB/MS),[147] fourier transform ion cyclotron resonance mass spectrometry (FTICR/MS),[148] low temperature ^{1}H NMR,[149] and fluorescence spectroscopy.[138] The extent of enantiomeric recognition displayed in these types of studies was found to parallel separation factors demonstrated by chromatographic methods.[23,150,151,152,153,154,155,156,157] Computational chemistry is a theoretical method which has been applied to a limited extend[34,133,136] in order to calculated the stability of host-guest complexes. It is important to note that a literature survey showed application of mostly one, if any, of the analytical techniques combined with extraction or transport studies. Extraction or transport studies make use of U or W-tube experiments.[117] A single study of MS, NMR, fluorescence, computational techniques in relation to extraction studies have not yet been reported. Most of the chiral recognition studies focussed only on one of the techniques mentioned above and a lack of calibration methods between these techniques is apparent. It should also be pointed out that good chiral discrimination alone may not be sufficient proof of successful chiral separation by extraction or transport techniques, since complexation might still be too weak for phase transfer or too strong for releasing of the guest. The aim of this chapter is to provide a short overview on the theory and procedures of some of the methods used to determine enantiomeric recognition.

5.1. Extraction Studies with U and W tube

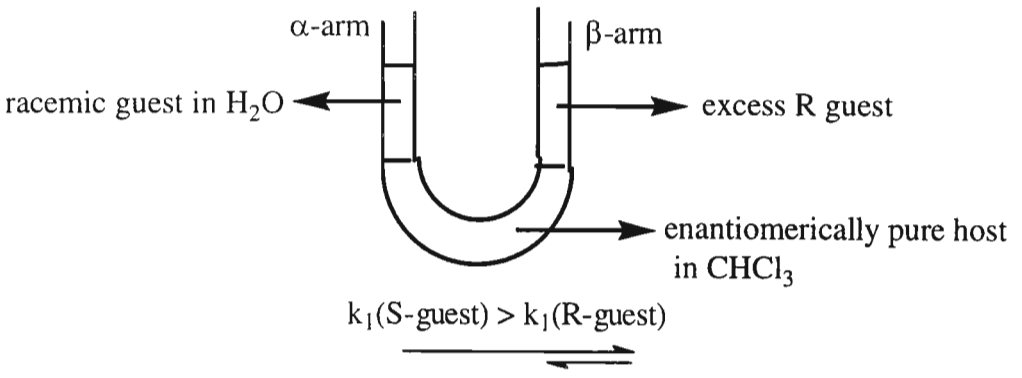


Figure 1: U-tube

A racemic solution of guests in an aqueous phase (α-arm) form a complex with the host in an organic phase ($CHCl_3$) at the solvent interface and is transferred into the organic phase. Subsequent transport to the other side of the U or W-tube enables releasing of the guest to the second water phase (β-arm). If the rate (k_1) of transport of R-guest is larger that of S-guest, then after some time, the optical activity of the second aqueous phase will indicate enantiomeric excess for R-guest. In a U-tube the enantiomeric excess will decrease with time since equilibrium will ultimately be obtained. In a W-tube with R-host in one side of the W-tube and S-guest on the other side, a racemic mixture of guest in the centre, complete resolution of the host can be achieved after infinite time.[117]

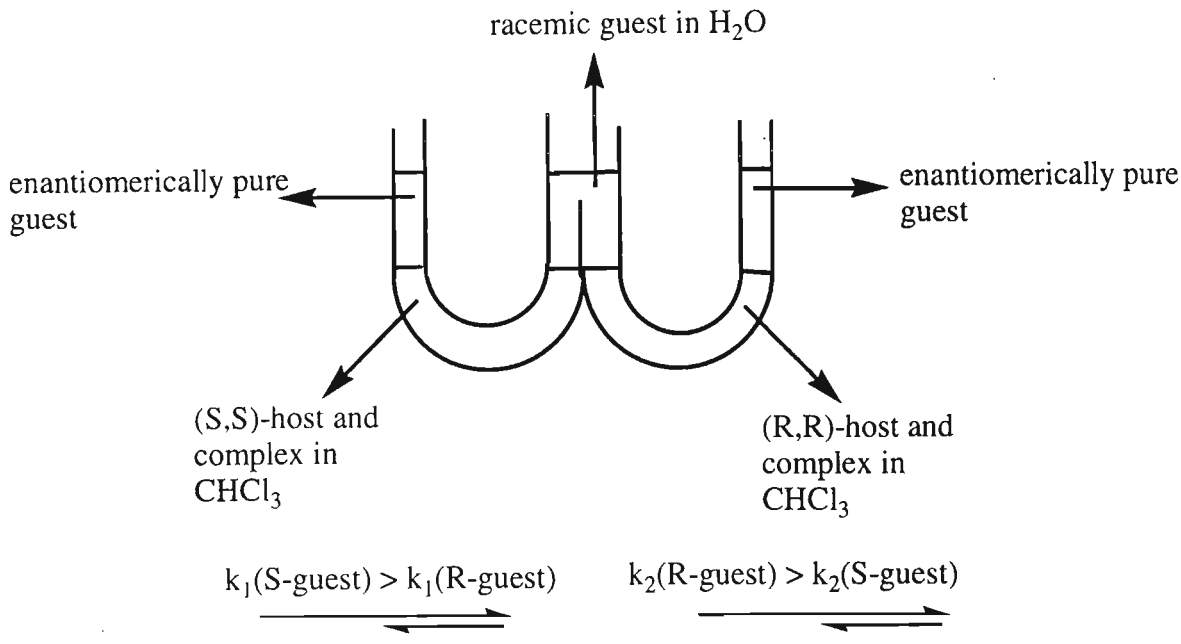


Figure 2: W-tube[117]

At the solvent interface of the reservoir, an equilibrium is built up for the complexation between host (H) and guest (G) and is expressed as ('*' indicates organic phase):

$$G^+ + X^- + H^* \leftrightarrow GX^*.H^* \qquad (1)$$

The complex reaches equilibrium in the organic phase and is expressed as:

$$GX^*.H^* \leftrightarrow G^* + X^* + H^* \qquad (2)$$

Transportation is said to have occurred when the guest (G) and counter ion (X) is released into the collecting arm. An equilibrium is established at the delivery arm for the complex and is expressed as:

$$GX^*.H^* \leftrightarrow G^+ + X^- + H^* \qquad (3)$$

In the left side of the W-tube, S-guest is transported faster than R guest to the left arm. Some R guest will be transported as well. After some time, the left arm will become enriched with the R guest as the S guest is transported back to the racemic reservoir (centre of the tube). With increased time, the enantiomeric excess in each arm will increase.[117]

5.2. Low Temperature ^{1}H NMR Studies

NMR line-shape methods are a valuable tool for studying a variety of processes that become slow on the NMR time scale at temperatures between room temperature and -90^oC.[149,158] Reinhoudt and de Jong have discussed[159] the specific application of NMR line-shape methods to the determination of rate constants and activation energies for the dissociation of crown ether complexes. The concept can be explained using Figure 3. In

the absence of complex formation, protons H_a and H_b are indistinguishable on a NMR spectrum. When complex formation occurs, the proton on the same side of the crown as the alkyl group of the alkyl-ammonium salt experiences a different magnetic environment than the proton on the opposite side. At ambient temperatures, the rate of complexation and dissociation is so rapid on the NMR time scale that only an average chemical shift of the protons is observed. Lowering the temperature of the NMR probe, "freezes" complexes that have high activation energies for dissociation (i.e. more stable complexes). This slows down the exchange rate on the NMR time scale resulting in the observation of different signals for H_a and H_b.

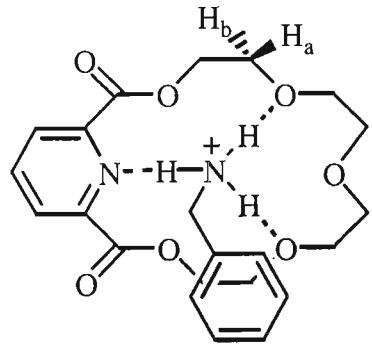


Figure 3: Ammonium ion complexation in a macrocycle

The rate of the dissociation process is thought to be the rate-determining step and can be expressed as:

$$k_c = \pi \Delta v / 2^{1/2} \qquad (1)$$

Where k_c is the rate of the exchange process at the coalescence temperature (temperature at which the signals just merge) and Δv is the chemical shift separation (Hz) between the resolved signals.[160] The free energy of activation at the coalescence temperature ($\Delta G^{\neq}_c$) can be calculated from the rate constant (k_c), the molar gas constant (R), the coalescence temperature (T_c), Planck's constant (h), and the Boltzmann constant (K) using equation 2.[160]

$$\Delta G^{\neq}_c = -RT_c \ln k_c h / KT_c \qquad (2)$$

Greater enantiomeric resolution is observed when the difference in the free energies between the two isomers, Δ (ΔG), is maximised and the ΔG is minimised.[149] Stronger complexation between host and guest will require a lower coalescence temperature (k_c) and therefore result in a lower free energy (ΔG).

This procedure of calculating free energy values for different complexes requires a vast amount of time on a NMR instrument mainly due to continuously changing the temperature of the probe.

A qualitative measure of the enantiomeric recognition can be obtained from the ^1H spectra of the diastereomeric complexes with the use of equations 3 and 4.[161]

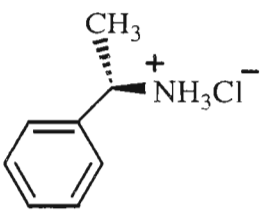
$$[(R)G,(SS)H]/[(S)G,(SS)H] = (\delta_S{}^H - \delta_{2RS}{}^H)/(\delta_{2RS}{}^H - \delta_R{}^H) \quad (3)$$

$$[(R)G,(SS)H]/[(S)G,(SS)H] = (\delta_{2RS}{}^{SG} - \delta_F{}^G)(\delta_S{}^{SG} - \delta_F{}^G)/(\delta_R{}^{RG} - \delta_R{}^{RG})(\delta_{2RS}{}^{RG} - \delta_F{}^G) \quad (4)$$

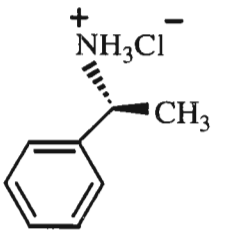
The relative concentrations of the diastereomeric complexes formed by the (R) and (S) guests [(R)G and (S)G] with the (S,S)-host [(SS)H] are obtained from the chemical shifts ($\delta_S{}^H$, $\delta_R{}^H$ and $\delta_{2RS}{}^H$) of a selected host signal in the presence of one equivalent of (S)-guest, one equivalent of (R)-guest, and two equivalents of (R,S)-guest respectively. $\delta_S{}^{SG}$ and $\delta_{2RS}{}^{SG}$ are the chemical shifts of a selected (S)-guest signal in solutions containing the host and one equivalent of (S)-guest and two equivalents of (R,S)-guest respectively, $\delta_R{}^{SG}$ and $\delta_{2RS}{}^{RG}$ have the same significance for the (R)-guest signals, and $\delta_F{}^G$ is the corresponding chemical shift for the uncomplexed guest.[161]
This type of experiment is performed at one set temperature and therefore requires less time on a NMR spectrometer and can be used as a preliminary test for complexation.

^1H NMR (300 MHz) spectra of one equivalents of (R)-α-methylbenzylammonium hydrochloride (**68**) and (S)-α-methylbenzylammonium hydrochloride (**69**) in solution ($CDCl_3$) with macrocycles **57** and **66** were obtained at 20, 0 and -20°C. It was not feasible to go to any lower temperatures since it would require longer instrument time and a more expensive NMR solvent (CD_2Cl_2).

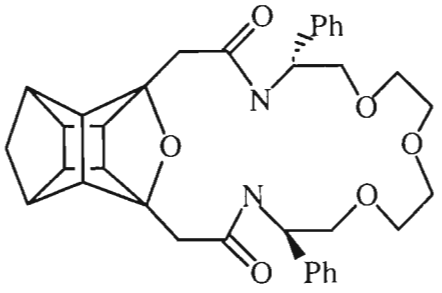
None of the spectra (p102-105) showed any significant change in chemical shifts between the free hosts (**57** and **66**), and the mixtures of the hosts and guests (**57** + **68**, **57** + **69**, **66** + **68** and **66** + **69**). If any complexation were to occur then protons at the chiral centre would be expected to split the most. It is obvious that there is no sign of complexation, let alone any enantiomeric discrimination. It can be concluded from this observation that either the macrocycles **57** and **66** are poor hosts or, that –20°C is not a low enough temperature to prevent the activation energy for the dissociation of the complexes to be reached.



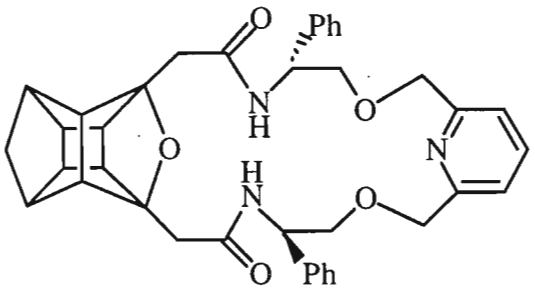
(R)-α-methylbenzylamine (**68**)



(S)-α-methylbenzylamine (**69**)

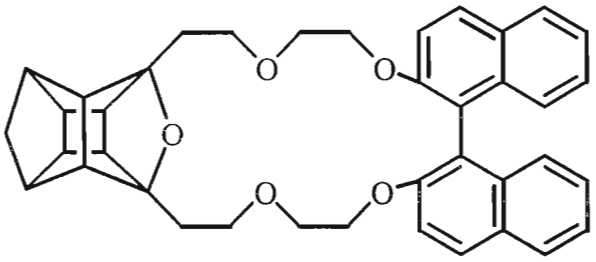


57



66

Macrocycle **4** has been shown[34] via transport experiments to be selective for (S)-α-methylbenzylamine (**69**). ^{1}H NMR spectra (p100-101) of **4** with **68** and **69** at –20^{0}C shows a change in the chemical shifts of some protons of the binaphthyl group (2.4 ppm and 7.9-8.0 ppm), indicating the formation of a complex. There was no notable differentiation between the spectra of the two diastereomeric complexes (**4** + **68** and **50** + **69**). Since it has been proven in literature[34] that the host **4** transported (S)-α-methylbenzylamine in enantiomeric excess, it is clear that -20°C is also not low enough to show enantiomeric discrimination. It was reported that only 12% of guest (3 times excess with respect to host) were transported (4 hours) which might indicate that the activation energy for the dissociation of the complex might be high.



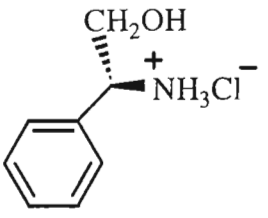
4

It is clear that in order to practice this exercise, it is important to use CD_2Cl_2 (m.p. -90°C) rather than $CDCl_3$ (m.p. -50°C) as solvent in order to experiment at lower temperatures, and to have access to a NMR instrument for long periods at a time.

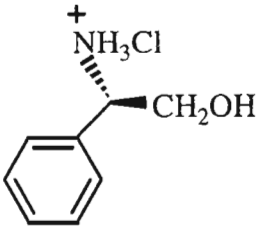
5.3. Fluorescence Spectroscopy Studies

Recently, Hua, 2000, reported the use of fluorescence spectroscopy in determining enantiomeric recognition.[138] With this technique,[138] fluorescence emission was measured for hosts and for mixtures of hosts and guests, and the difference in wavelength at maximum intensity ($\Delta\lambda$) between the two diastereoisomers was taken as a measure of enantiomeric discrimination. Stronger complexation between host and guest would result in greater significant change in configuration of the molecules and therefore a larger change in the wavelength (50 nm) at maximum intensity (λ_{max}).

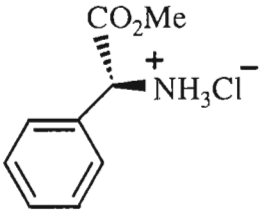
Solutions, 1 x 10^{-3} molar, of **57** with **68**, **69**, **70**, **71**, **72**, **73** in 1:3 methanol:dichloromethane were excited at 257 nm and the fluorescence emission recorded. The excitation wavelength was determined by finding the maximum wavelength the solution absorbed when subjected to ultra violet radiation. There was no change in λ_{max} between the pure macrocycle **57** and mixtures of the macrocycle with the various guests. Similar experiments were done using macrocycles **66** (excitation wavelength = 278nm) and **4**[160] (excitation wavelength = 307 nm) and even these showed no sign of conformational change. It can be concluded from these results that either no complexation had occurred or that there is no significant conformational change in these systems upon complex formation. These measurements were performed at room temperature and it would be interesting to investigate the temperature dependence of this method.



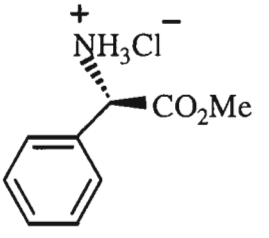
(S)-phenylglycinol (**70**)



(R)-phenylglycinol (**71**)



(S)-phenylglycine methyl ester(**72**)



(R)-phenylglycine methyl ester (**73**)

5.3. Computational Calculations

Computational chemistry due to spectacular advances in both hard and software[162] has become a valuable tool to enhance our understanding about many complex chemical systems. There are three areas of computational tools available. Molecular mechanics or force field derived calculations, semi-empirical methods and *ab initio* calculations. Molecular mechanics are typically used for very large biochemical systems such as proteins and although it is the cheapest method in regard to computer time, good force fields for macrocyclic hosts with ammonium ion guest interactions have not yet been developed. In general molecular mechanics also give the least accurate results.[163] Molecular mechanics results of such systems gave meaningless geometry.[164]

The cheapest *ab initio* calculation (in respect of computer time) that would give a reasonable answer is a Restricted Hartree-Fock (RHF) calculation with the 3-21+G basis set. Diffuse functions (+) are used to give a better description of systems containing pi elec-

trons and lone pairs. One of these calculations takes between 3 and 5 days on a supercomputer (SGI Origin 2000 with 64 CPU's, 20GB of RAM and huge disc space – note that due to limitations of the Gaussian 98 code, little gain in performance is obtained using more than 2 CPU's in parallel calculations). In the light of the 40 calculations per R and S host with R and S guest, a study using *ab initio* calculations would have taken several years to complete.

The only alternative option is to use semi empirical calculations, provided that the answers obtained are reliable enough. This was verified in our laboratory[164] by comparing PM3 semi empirical results of such a system with the RHF/3-21+G *ab initio* results of the same system. The route of mean square (RMS) overlay error of the PM3 result with the *ab initio* result is about 0.01Å, which is very acceptable. The average time for PM3 calculations of these systems takes between 2 and 9 hours.

Geometry optimisations of the ligands **57** and **66** with guests **68**, **69**, **70**, and **71** were done using Gaussian 98 at a PM3 semi-emperical level of theory. Starting geometries of the complexes were drawn in Chem 3D and converted to Gaussian input files.

Host molecules containing the PCU cage present two potential complexation "faces" to an approaching guest molecule. As the host-guest complex formation progresses, the guest can pursue either a "topside" approach i.e., along a trajectory proximal to the methylene carbon of the cage moiety, or a "bottomside" approach in which the approaching guest follows a trajectory that lies distal to the methylene carbon atom in this moeity.[34]

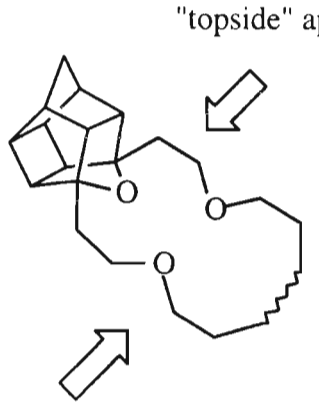


Figure 4: Designation of topside and bottomside in PCU crown ethers

The guest molecule was placed at a distance of about 3-4 Å away from the host molecule and rotated at about 36° to produce ten different starting geometries. A full geometry optimisation for each of the ten topside complexes were performed and the same was done for the bottom side approach. The optimised complexes were then recorded according to their relative energies.[164]

Since extraction study data of macrocycle **4** and racemic α-methylbenzylamine as guest has been reported,[34] it was decided to perform PM3 calculations on this system and use it

as a "benchmark" for the calculations of the other systems.
The calculation results (see table 1) for **4** imply that the S-α-methylbenzylamine is preferred over R-α-methylbenzylamine by 2.4 kcal mol^{-1}. The S-α-methylbenzylamine is predicted to bind preferentially to the topside by ca. 1.3 kcal. mol^{-1}. This observation is consistent with the extraction study data that found macrocycle (R)-**4** to selectively transport S-α-methylbenzylamine in 78.7 % enantiomeric excess.

Table 1: Relative energies (kcal. mol^{-1}) for PM3 calculations of macrocycle (R)-**4** with guests (R) / (S)-α-methylbenzylamine

R-α-methylbenzylamine		S-α-methylbenzylamine	
Topside	**Bottomside**	**Topside**	**Bottomside**
2.4	4.1	0.0	2.8
5.0	4.1	3.0	4.0
5.1	4.1	4.1	4.8
5.5	5.8	4.1	5.2
5.6	6.9	5.1	5.2
6.4	7.2	5.5	6.0
6.6	7.6	6.7	6.3
6.6	7.8	7.9	6.3
6.6	8.1	8.1	6.5
7.1	8.5	9.3	7.0

Table 2: Relative energies (kcal. mol^{-1}) for PM3 calculations of macrocycle (R)-**57** with guests (R) / (S) -α-methylbenzylamine

R-α-methylbenzylamine		S-α-methylbenzylamine	
Topside	**Bottomside**	**Topside**	**Bottomside**
1.2	7.0	0.0	0.7
1.5	7.1	1.1	3.8
2.0	7.5	1.7	5.3
5.2	7.5	2.0	5.9
3.0	8.5	2.6	7.9
3.0	11.0	2.8	10.8
4.4	12.3	2.9	11.6
4.8	12.5	3.3	14.1
6.6	10.3	6.0	14.4
7.8	16.6	6.5	17.7

Table 2 represents the relative energies of complexes formed between macrocycle **57** and (R) or (S)-α-methylbenzylamine in kcal mol^{-1}. (S)-α-methylbenzylamine is preferred over (R)-α-methylbenzylamine by 1.2 kcal mol^{-1}, which indicates moderate enantioselectivity when compared to the host **4**. There is moderate preference for the topside approach of the guest (0.7 kcal mol^{-1}). The structural conformation for the most stable

complex formed between macrocycle (R)-**57** with guest (S)-α-methylbenzylamine is shown in figure 5. The only sign of hydrogen bonding is between a carbonyl oxygen atom of the crown and a quaternary ammonium hydrogen atom of the guest.

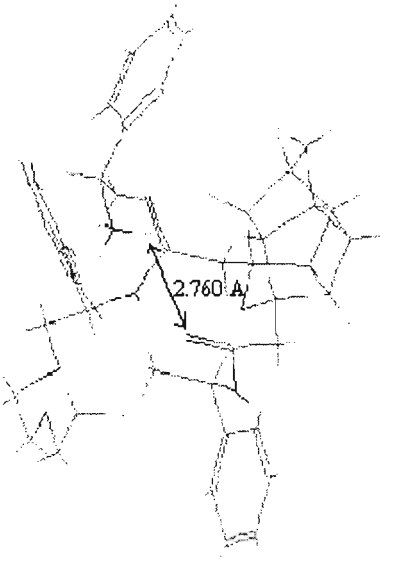


Figure 5: Structural conformation of the most stable complex formed between macrocycle (R)-**57** and guest (S)-α-methylbenzylamine. *

Table 3: Relative energies (kcal. mol^{-1}) for PM3 calculations of macrocycle (R)-**57** with guests (R) / (S)–phenylglycinol with hydroxyl group facing away from the crown

(R)-phenylglycinol		(S)-phenylglycinol	
Topside	**Bottomside**	**Topside**	**Bottomside**
3.7	8.4	2.7	0.0
3.8	8.5	3.5	4.7
3.8	8.8	5.1	7.6
3.9	9.0	6.8	8.5
4.1	9.1	7.0	8.8
5.6	10.3	7.0	9.4
6.5	10.8	7.1	15.2
6.9	15.3	8.2	15.2
10.4	15.9	8.4	16.7
11.5	19.1	9.3	23.5

* All structures available on CD in pdb format

Table 4: Relative energies (kcal. mol^{-1}) for PM3 calculations of macrocycle (R)-**57** with guests (R) / (S)–phenylglycinol with hydroxyl group facing towards the crown

(R)-phenylglycinol		(S)-phenylglycinol	
Topside	**Bottomside**	**Topside**	**Bottomside**
0.6	4.8	0.0	4.2
2.1	8.2	0.3	6.1
2.4	8.4	0.4	7.7
2.4	9.1	0.5	10.5
3.1	9.1	1.9	11.0
3.8	10.1	3.0	12.4
4.9	10.6	4.0	15.6
10.4	15.3	5.2	16.5
10.6	15.9	6.4	17.8
11.3	19.2	10.5	19.3

Tables 3 and 4 gives the relative energies of complexes formed between macrocycle **57** and (R) or (S)-phenylglycinol. Table 3 represents complexes in which the OH group of the phenylglycinol is pointing away from the macrocycle and the NH_3 group towards the macrocycle. Table 4 represents the complexes in which the OH and NH_3 groups of the glycinol are pointing towards the macrocycle. Tables 3 and 4 indicate that the (S)-phenylglycinol is favoured over (R)-phenylglycinol by 3.7 and 0.6 kcal mol^{-1} respectively. These calculations imply that there will be greater enantiomeric recognition with racemic phenylglycinol (3.7 kcal mol^{-1}) as a guest, compared to racemic α-methylbenzyl amine (1.1 kcal mol^{-1}). Figures 6 and 7 are the structural conformations for the most stable complexes formed between (R)-**57** with guest (S)–phenylglycinol (OH group facing away and towards the crown respectively). Figure 6 implies that there is significant hydrogen bonding between the quaternary ammonium hydrogen atoms of the guest and a carbonyl oxygen atom, an ether oxygen atom and an amide hydrogen atom of the host. In this conformation, the OH group of the phenylglycinol does not participate in hydrogen bonding with any part of the guest. In figure 7, the hydroxyl hydrogen atom of the phenylglycinol forms a hydrogen bond with a carbonyl oxygen atom of the crown. Two of the quaternary ammonium hydrogen atoms of the guest form hydrogen bonds with two of the carbonyl oxygen atoms of the crown.

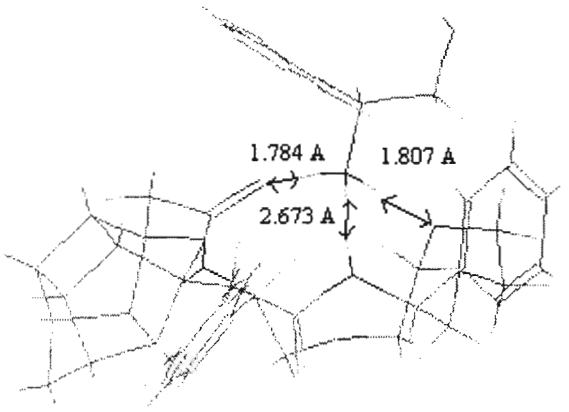


Figure 6: Structural conformation of the most stable complex formed between macrocycle (R)-**57** and guest (S)-phenylglycinol with the OH group facing away from the crown.*

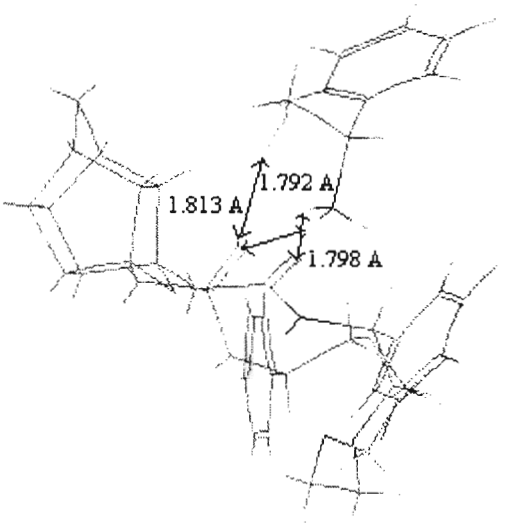


Figure 7: Structural conformation of the most stable complex formed between macrocycle (R)-**57** and guest (S)-phenylglycinol with the OH group facing towards from the crown.*

* All structures available on CD in pdb format

Table 5: Relative energies (kcal. mol^{-1}) for PM3 calculations of macrocycle (S)-**66** with guests (R) / (S) -α-methylbenzylamine

R-α-methylbenzylamine		S-α-methylbenzylamine	
Topside	**Bottomside**	**Topside**	**Bottomside**
13.6	1.2	0.0	17.9
14.1	1.8	1.5	17.9
14.7	1.9	5.8	19.6
14.9	1.9	6.0	21.4
15.3	2.2	6.4	21.6
15.6	5.6	6.7	22.9
16.8	9.8	7.9	23.7
17.5	10.2	9.5	24.8
17.9	15.6	10.6	26.4
17.9	18.6	11.9	30.4

Table 5 gives the relative energies of complexes formed between macrocycle **66** and (R) or (S)-α-methylbenzylamine. (S)-α-methylbenzylamine is preferred over (R)-α-methylbenzylamine by 1.2 kcal mol^{-1}, which indicates great enantioselectivity. The structural conformation for the most stable complex formed between macrocycle (R)-**66** with guest (S)-α-methylbenzylamine is shown in figure 8. It implies that two of the quaternary ammonium hydrogen atoms of the guest form hydrogen bonds with two carbonyl oxygen atoms of the crown.

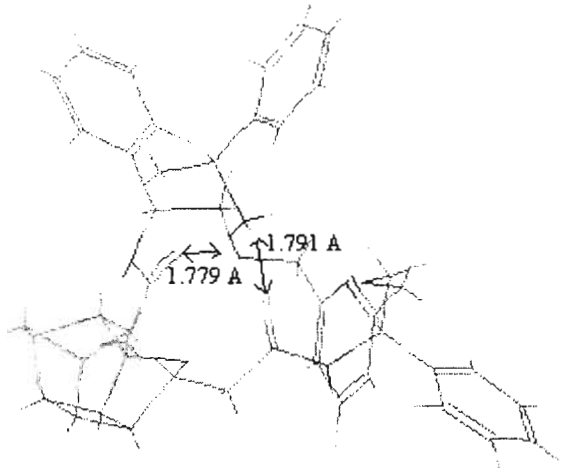


Figure 8: Structural conformation of the most stable complex formed between macrocycle (S)-**66** and guest (S)-α-methylbenzylamine. *

* All structures available on CD in pdb format

Table 6: Relative energies (kcal. mol^{-1}) for PM3 calculations of macrocycle (S)-**66** with guests (R) / (S)–phenylglycinol with hydroxyl group facing away from the crown

(R)-phenylglycinol		(S)-phenylglycinol	
Topside	**Bottomside**	**Topside**	**Bottomside**
1.1	0.7	0.0	4.8
1.5	5.8	0.8	6.5
1.8	8.7	1.3	9.3
2.6	9.4	1.5	9.7
3.0	9.7	1.9	10.2
3.2	10.6	2.1	10.4
3.3	11.4	2.2	10.5
6.7	11.7	4.4	10.8
6.8	11.9	4.6	11.0
7.0	12.5	5.0	11.5

Table 7: Relative energies (kcal. mol^{-1}) for PM3 calculations of macrocycle (S)-**66** with guests (R) / (S)–phenylglycinol with hydroxyl group facing towards the crown

(R)-phenylglycinol		(S)-phenylglycinol	
Topside	**Bottomside**	**Topside**	**Bottomside**
1.1	6.0	0.0	9.2
2.3	9.4	1.0	9.7
3.1	10.0	1.9	9.9
3.5	10.1	2.0	10.2
3.9	10.5	2.2	10.3
4.7	11.1	2.6	11.2
4.8	11.6	2.8	11.6
6.3	11.6	2.9	11.8
6.5	12.7	3.1	12.0
8.4	12.9	5.0	12.6

Tables 6 and 7 gives the relative energies of complexes formed between macrocycle **66** and (R) or (S)-phenylglycinol. Table 6 represents complexes in which the OH group is pointing away from the crown and table 7 represents the complexes in which the OH group is pointing towards the crown. Tables 6 and 7 indicate that the (S)-phenylglycinol is favoured over (R)-phenylglycinol by 0.7 and 2.1 kcal mol^{-1} respectively and the complex with the OH facing towards the crown is favoured by 0.8 kcal mol^{-1} over the complex where the OH is facing away from the crown. These calculations imply that there will be greater enantiomeric recognition with racemic phenylglycinol (2.1 kcal mol^{-1}) as guest, compared to racemic α-methylbenzylamine (1.2 kcal mol^{-1}). Figures 9 and 10 are the structural conformations for the most stable complexes formed between (S)-**66** with guest (S)–phenylglycinol (OH group facing away and towards the crown respectively). Figure 9 implies that there is significant hydrogen bonding between the quaternary am-

monium hydrogen atoms of the guest and two carbonyl oxygen atoms and an ether oxygen atom the host. In this conformation, the OH group of the phenylglycinol does not participate in hydrogen bonding with any part of the guest. In figure 10, the hydroxyl hydrogen atom of the phenylglycinol forms a hydrogen bond with a carbonyl oxygen atom of the crown. The quaternary ammonium hydrogen atoms of the guest form hydrogen bonds with two of the carbonyl oxygen atoms and one of the ether oxygen atoms of the crown.

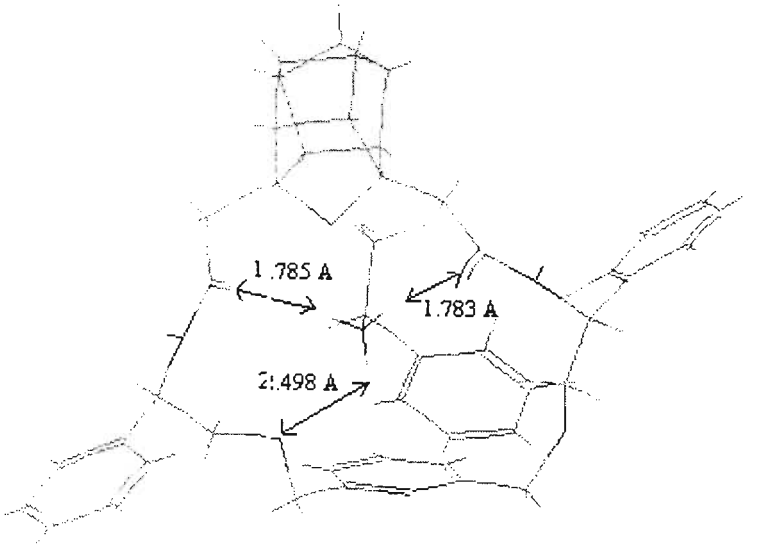


Figure 9: Structural conformation of the most stable complex formed between macrocycle (S)-**66** and guest (S)-phenylglycinol with the OH group facing away from the crown. *

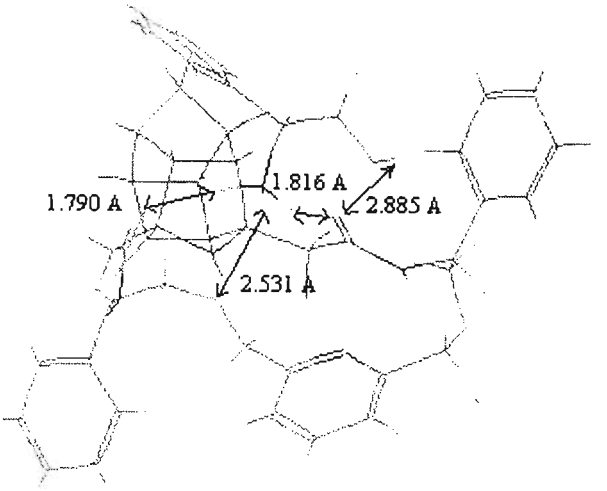


Figure 10: Structural conformation of the most stable complex formed between macrocycle (S)-**66** and guest (S)-phenylglycinol with the OH group facing towards from the crown.*

* All structures available on CD in pdb format

In the systems studied, α-methylbenzylamine formed only one hydrogen bond with the host macrocycle as compared to phenylglycinol, which formed at least three hydrogen bonds with the host macrocycle. From the above study it can be concluded that greater enantiomeric discrimination is observed in systems that form a larger number of hydrogen bonds between the host and guest.

Using macrocycle **4** as a benchmark (since extraction study results are published) we found that a 78% enantiomeric recognition for α-methylbenzylamine corresponds to a calculated relative complexation energy of 2.4 kcal mol^{-1}. Although novel macrocycles **57** and **66** exhibit weaker enantiomeric recognition with α-methylbenzylamine, it can be concluded that they would exhibit promising enantiomeric discrimination with phenylglycinol (3.7 kcal mol^{-1} and 2.1 kcal mol^{-1} respectively).

CHAPTER 6

CONCLUSION

Establishing a method for the efficient incorporation of sterically hindered amino acids into peptides via solid phase peptide synthesis (SPPS) is an important topic in peptide chemistry. The incorporation of the TRIS amino acid into a peptide via Fmoc SPPS has been accomplished using an acid fluoride method. This method normally makes use of diisopropylethylamine (DIEA) as HF scavenger when incorporating sterically hindered, non-cage amino acids, like α-aminoisobutyric acid, into peptides. The use of DIEA resulted in premature deblocking of the Fmoc protecting group (base labile) in the case of the TRIS amino acid. Pyridine was proven to be a better scavenger for this purpose since it was not basic enough to cause deprotection of the Fmoc group. This discovery could prove to be very important with respect to solid phase peptide synthesis of sterically hindered amino acids since there have been numerous reports of premature deblocking when DIEA was used as the scavenger. Qualitative proof for the success of this method comes in the form of ninhydrin Tests and crude mass spectra data. Future work will involve determining the efficiency of this method with the use of HPLC and NMR instruments.

An efficient procedure has been established for the synthesis of PCU cage annulated, chiral crown ethers containing amino acids as the source of chirality. Similar PCU crown ethers have never been synthesised via ring closure with diacid dichlorides and diamines and non-PCU crown ethers gave relatively poor yields (< 20%). In this study the PCU diacid was synthesised in excellent yields (> 80%) and converted to the corresponding diacid dichloride in equally good yields (> 80%). The use of dry, non-polar solvents (toluene or benzene) and high dilution conditions (1 mmol. dm^{-3}), have proven to be some of the determining factors in obtaining relatively good yields (> 30%). The rigidity of the cage may also be assisting in tying up the loose ends of the crown ethers. Positioning of the chiral centres closer to the cage successfully induced chirality into the ten inducible centres of the PCU cage, resulting in an increase in the overall chirality of the macrocycle.

Enantiomeric recognition of the chiral PCU cage crown ethers was studied using NMR, fluorescence and computational methods. The aim of this study was to get familiar with different techniques available for enantiomeric recognition. Efficiency of the enantiomeric recognition capabilities of the novel crown ethers was compared with a PCU cage annulated crown ether for which extraction study data already existed. The known and novel crown ethers showed no significant complexation activity in the NMR and Fluorescence studies. The computational studies indicate that greater enantiomeric recognition occurs between the novel crown ethers synthesised in this study and various guests, as compared to the known crown ether with the same guests. Future work will involve calibrating extraction studies with computational studies.

Synthesis of sterically hindered amino acids, like cage amino acids, requires elevated temperatures and pressures with respect to non-sterically hindered amino acids. An efficient method has been established for the synthesis of racemic pentacyclo-undecane

(PCU) and trishomocubane (TRIS) cage amino acids via a hydantoin route. Future work will involve synthesis of the enantiomerically pure cage amino acids with the use of enzymes (hydantoinases). Hydantoinases enantiomerically catalyse the hydrolysis of hydantoins to the corresponding amino acids.

CHAPTER 7

EXPERIMENTAL

Melting points (uncorrected) were determined using a Mel-Temp apparatus in sealed capillary tubes with the head space above the sample filled with a glass rod to minimise sublimation. High-resolution mass spectral data reported herein were obtained by Professor Jennifer S. Brodbelt at the Mass Spectrometry Facility at the Department of Chemistry and Biochemistry, University of Texas at Austin by using a ZAB-E double sector high-resolution mass spectrometer (Micromass,.Manchester, England) that was operated in the chemical ionisation mode. NMR spectra were recorder on a Varian Gemini-200 and a Varian Gemini-300 spectrophotometer.

Synthesis of Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-8,11-dione (31)[76,77]

p-Benzoquinone (240g, 2.22mol) in methanol (1L) was cooled to -70°C via application of an external dry ice-acetone cold bath. To this was added, over 8 hours, with stirring a solution of freshly cracked cyclopentadiene (171.6g, 2.6 mol) in cold methanol (200ml). The reaction mixture was allowed to warm gradually to room temperature with stirring. The product was filtered and recrystallized from *n*-hexane to yield the adduct as yellowish-green crystals **30** (280g, 68%).

The above adduct (**30**, 60g) was dissolved in 600ml acetone and irradiated with a 500W mercury lamp until the TLC (4:1 hexane:ethyl acetate) indicated the absence of starting material (6 – 8 hours). The solvent was evaporated to give the product as a colourless microcrystalline solid **31** (52g, 86%), m.p 233°C.

The IR and NMR data were identical to an authentic sample.

Synthesis of the Ketal (31)[77]

A mixture of the dione (99.2g, 0.5731mol), ethylene glycol (44ml, 0.78mol) and *p*-toluenesulfonic acid (3.31g, catalytic amount) in benzene (600ml) was refluxed (Dean Stark trap) with stirring for 5 hours. The reaction mixture was cooled and slowly poured into 500ml of ice cold 10% aqueous Na_2CO_3, extracted with dichloromethane (500ml x 2) and the combined extracts were dried over anhydrous $MgSO_4$. Evaporation of solvent followed by recrystallisation of the crude residue yielded the mono-ketal **31** (93.2g, 74.62%), as colourless crystals.

The IR and NMR data were identical to an authentic sample.

Synthesis of 11-Hydroxypentacyclo [5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecan-8-one (34)[77,78]

To a suspension of $LiAlH_4$ (12.08g, 0.3183 mol) and dry ether (2 L) was added, under argon very slowly, the ketal **32** (92.5g, 0.4243 mol). The mixture was then refluxed for 12 hours. The reaction was quenched by drop-wise addition of saturated Na_2SO_4 solution with vigorous stirring. The white slurry thus formed was filtered and washed with ether. The filtrate was concentrated and dried to give the hydroxy-ketal **33** (89.5g, 96%).

A mixture of the hydroxy-ketal **33** (89.5g, 0.41 mol), THF (200ml), water (100ml) and concentrated HCl (15ml) was stirred at room temperature for 18 hours. The mixture was diluted with water (500ml) and extracted with dichloromethane (4 x 300ml). The combined organic phase was washed with water (2x), then with saturated aqueous $NaHCO_3$ solution (2x), dried over anhydrous Na_2SO_4 and concentrated to give the product as white solid **34** (62.7g, 76.5%), m.p. 255°C.

The IR and NMR data were identical to an authentic sample.

Synthesis of Pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecan-8-ol (35)[77]

A mixture of the hydroxy-ketone **34** (13g, 0.074 mol) and hydrazine hydrate (20ml, 98%, 0.56 mol) in diethylene glycol (200ml) was maintained at 120°C for 2hrs. The mixture was allowed to cool down to 80°C after which KOH (10g) was added and the excess hydrazine hydrate and water was distilled off until the temperature reached 185°C. The reaction mixture was refluxed for 3hrs at 185°C, cooled, diluted with water (300ml), extracted with dichloromethane. The combined organic phase was concentrated and dried. The residue was purified by steam distillation to yield the alcohol **35** (11.2g, 56.3%), m.p. 231-232°C.

The IR and NMR data were identical to an authentic sample.

Synthesis of Pentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecan-8-one (22)[77]

To a mixture of CrO_3 (14g, 0.14 mol) in water (15ml) and acetic acid (150ml, 98%), was added the alcohol **35** (10g, 0.0625 mol) in acetic acid (50ml) drop-wise over a period of 10 minutes with stirring. The reaction mixture was stirred at 90°C for 5 h, cooled, diluted with water (600ml) and extracted with CH_2Cl_2 (3 x 300ml). The organic extract was washed successively with water (2 x 500ml), saturated aqueous $NaHCO_3$ (2 x 500ml), water (2 x 500ml) and dried over anhydrous $MgSO_4$. Removal of the solvent gave the crude mono-ketone **22** which was purified by steam distillation to give the product (7,5g, 76%), m.p. 194-195°C.

The IR and NMR data were identical to an authentic sample.

Synthesis of the PCU-hydantoin (24)[73]

A glass pressure reaction vessel was charged with a mixture of the mono-ketone **22** (1g, 6.2 mmol), NaCN (1g, 20 mmol), $(NH_4)_2CO_3$ (2g, 20 mmol), EtOH (10ml) and concentrated NH_4OH (15ml). After heating at 60°C for 2 hours, 100°C for 2 hours and 120°C for 3 hours, the reaction mixture was cooled, diluted with water (100ml) and the solids were collected by suction filtration. The solids were washed with H_2O, acetone, and Et_2O and air-dried to yield dense white crystals **24** (1.4g, 98%), m.p. 230°C.

The IR and NMR data were identical to an authentic sample.

Synthesis of the PCU-Amino Acid (6)
A suspension of the hydantoin **24** (1g, 4.3 mmol) in 1.25N NaOH solution (50ml) was heated in a pressure vessel for 12 hours with stirring at 170°C. The cooled mixture was diluted with water (200ml) and the solution acidified with concentrated HCl. The solution was filtered, concentrated (~100ml) under reduced pressure and neutralised with NaOH solution, whereupon fine white crystals were precipitated. The product was collected under suction, recrystallised from water, washed with acetone and diethyl ether, and air-dried to give the product **6** (0.5g, 56%).

The IR and NMR data were identical to an authentic sample.

Synthesis of Pentacyclo[6.3.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecan-4-ol (37)[77]
The alcohol **35** (31.2g, 0.195 mol) in glacial acetic acid (360ml) containing conc. H_2SO_4 (9g) was refluxed for 6hrs. (The reaction mixture turned dark in colour after a few minutes of refluxing.) The reaction mixture was cooled, diluted with water (1500ml) and extracted with CH_2Cl_2. The dichloromethane extract was washed successively with water and sat.aq. $NaHCO_3$ and decolourised with activated charcoal. Removal of the solvent gave the crude acetate **36** as dark yellow oil that was stirred overnight in a mixture of methanol (330ml) and K_2CO_3 (18g). The reaction mixture was concentrated, the residue obtained was diluted with water and the solid that separated was filtered and dried to afford pure alcohol **37** (20.6g, 66%) as a white solid, m.p. 167-168°C.

The IR and NMR data were identical to an authentic sample.

Synthesis of Pentacyclo[6.3.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecan-4-one (25)[77]
To a mixture of CrO_3 (32g, 0.32 mol) in water (27ml) and acetic acid (320ml), was added the alcohol (20.6g, 0.13 mol) in acetic acid (100ml) drop-wise over a period of 10 minutes with stirring. The reaction was then stirred at 90°C for 6 h, cooled, diluted with water (1.2 L) and extracted with dichloromethane. The organic extract was washed successively with water (3 x 500ml), saturated sodium bicarbonate solution (3 x 500ml), and water (3 x 500ml). Removal of the solvent gave the product **25** (16.2g, 79.6%) as colourless microcrystalline solid.

The IR and NMR data were identical to an authentic sample.

Synthesis of the Trishomocubane hydantoin (26)
A mixture of the monoketone **25** (1g, 6 mmol), NaCN (1g, 20 mmol), $(NH4)_2CO_3$ (2g, 20 mmol), EtOH (10ml) and concentrated NH_4OH (15ml) was taken in a glass pressure reaction vessel. After heating at 60°C for 2 hours, 100°C for 2 hours and 120°C for 3 hours, the reaction mixture was cooled, diluted with water (100ml) and the solids were collected under suction. The solids were washed with H_2O, acetone, and Et_2O and air-dried to yield dense white crystals **26** (1.4g, 98%). The analytical sample was recrystallised from

tetrahydrofuran: m.p. 324.5-325°C; IR (KBr): V_{max} 3314, 3161, 1766, 1720, 1403, 1286 cm^{-1}; ^{1}H-NMR [DMSO, 200 MHz]: δ_H 1.15-1.46 (m, 4H), 1.86-2.05 (m, 2H), 2.07-2.26 (m, 4H), 2.46 (br s, 1H), 2.85 (br s, 1H), 7.89 (s, 1H, D_2O exchangeable), 10.55 (s, 1H, D_2O exchangeable); ^{13}C-NMR [DMSO, 50 MHz]: δ_C 32.25 (t), 32.78 (t), 41.51 (d), 42.06 (d), 42.99 (d), 44.96 (d), 45.97 (d), 46.41 (d), 54.14 (d), 54.98 (d), 72.78 (s), 156.33 (s), 176.74 (s); CI MS: Calc. for $C_{13}H_{15}N_2O_2$ [M+H$^+$] m/z 231.1133, Found [M+H$^+$] 231.1133.

Synthesis of the Trishomocubane-Amino Acid (7)[77]

A suspension of the hydantoin **26** (1g, 4.3 mmol) in 1.25N NaOH solution (50ml) in a pressure vessel was heated for 12 hours with stirring at 170°C. The cooled reaction mixture was diluted with water (200ml) and acidified with concentrated HCl. The solution was filtered, concentrated (~100ml) and neutralised with NaOH solution, whereupon fine white crystals precipitated. The product was collected, recrystallised from water, washed with acetone and diethyl ether, and air-dried to give the product **7** (0.5g, 56%). IR (KBr): V_{max} 3250, 3204, 2955, 2854, 2735, 1579, 1497, 1386 cm^{-1}.

Fmoc TRIS Amino Acid (27)

To a solution of the amino acid **7** (0.1g, 4.88 x 10^{-4} moles) in dioxane (7.5ml) and 10% Na_2CO_3 (20ml) was added slowly with stirring and ice-bath cooling a solution of 9-fluorenylmethyl chloroformate (FMOC) (0.151g, 5.8 x 10^{-4}moles) in dioxane (5ml) for 4 hours and at room temperature for 8 hours, diluted with water (50ml) and extracted with diethyl ether (2 x 50ml). The aqueous layer was cooled in an ice-bath and acidified to pH 2 with concentrated HCl. Extraction with ethyl acetate (3 x 50ml) gave the product **27** as a white solid (0.152g, 72%), m.p.213-214°C; IR (KBr): V_{max} 3360, 2952, 1748, 1702, 1294, 1231, 1041 cm^{-1}; ^{1}H NMR [DMSO, 200MHz]: δ_H 1.15-1.50 (m, 4H), 1.90-2.30 (m, 7H), 2.47 (m, 1H), 3.35 (s, 1H, D_2O exchangeable), 4.10-4.43 (m, 3H), 7.22-8.08 (m, 8H), 12.17 (s, 1H, D_2O exchangeable); ^{13}C NMR [DMSO, 50 MHz]: δ_C 31.7 (t), 32.7 (t), 42.5 (d), 42.6 (d), 42.8 (d), 43.8 (d), 46.1 (d), 46.4 (d), 46.7 (d), 52.8 (d), 53.3 (d), 65.1 (s), 68.6 (t), 120.0 (d), 125.1 (d), 125.2 (d), 126.9 (d), 127.9 (d), 140.6 (s), 140.7 (s), 143.7 (s), 143.8 (s), 155.3 (s), 174.0 (s); CI MS: Calc. for $C_{27}H_{26}NO_4$ [M+H$^+$] m/z 428.1861, Found 428.1860

Fmoc TRIS Amino Acid Fluoride (40)

Cyanuric fluoride (40.5μl, 4.68 x 10^{-4}moles) was added to a suspension of Fmoc TRIS Amino Acid **27** (0.2g, 4.68 x 10^{-4}moles) in dry CH_2Cl_2 (50ml) and dry pyridine (37.8μl, 4.68 x 10^{-4}moles), and stirred under argon for 5 hours. The mixture, from which a water-soluble white precipitate had settled, was extracted with ice water (2 x 20ml). The organic phase was dried over $MgSO_4$ and concentrated to give a white solid that was recrystallised from CH_2Cl_2/hexane to give the pure acid fluoride (0.188g, 90%), m.p. 84-85°C; IR (KBr): V_{max} 3346, 2966, 1829, 1724, 1504, 1457, 1277 cm^{-1}; ^{1}H NMR [$CDCl_3$, 200 MHz]: δ_H 1.18-1.16 (m, 4H), 1.96-2.46 (m, 7H), 2.73 (br s, 1H), 4.22 (t, J = 6.6 Hz, 1H), 4.75 (d, J = 6.2 Hz, 2H), 5.38 (s, 1H, D_2O exchangeable), 7.25-7.48 (m, 4H), 7.59 (d, J = 7Hz, 2H), 7.77 (d, J = 7Hz, 2H); ^{13}C NMR [$CDCl_3$, 50 MHz]: δ_C 32.0 (t), 33.0 (t), 42.7 (d), 42.9 (d), 43.2 (d), 44.2 (d), 46.9 (d), 47.0 (d), 47.2 (d), 53.7 (d), 54.1 (d), 66.7 (t), 68.1 (s), 69.2 (s), 119.3 (d), 124.8 (d), 127.0 (d), 127.6 (d), 141.3 (s), 143.5 (s), 155.2

(s), 158.6 (s), 166.0 (s); ^{19}F NMR [$CDCl_3$, 282 MHz]: δ_F –180.3; CI MS: Calc. for $C_{27}H_{25}NO_3F$ [M+H$^+$] m/z 430.1818, Found 430.1810

Coupling of Glycine onto Wang-Resin[80,81,82,83]

The Fmoc Glycine **41** (0.6g, 2 x 10^{-3}moles), DIPCDI (0.254g, 2 x 10^{-3}moles) and 1-hydroxybenzotriazole (0.270g, 2 x 10^{-3}moles) was added at room temperature to the resin (0.5g, 6.25 x 10^{-4}moles) in dimethylformamide (DMF). The mixture was shaken occasionally for 12 hours and then washed.

Deprotection of Fmoc group

The Fmoc group was cleaved by shaking the resin with 20% piperidine in DMF for 30 minutes. Kaiser Test should show positive.

Washing procedure for Solid Phase Peptide Synthesis

The resin was washed using DMF (5 x 10ml, 30 seconds), isopropanol (3 x 10ml, 30 seconds) and DMF (3 x 10ml, 30 seconds).

Coupling of Fmoc TRIS Acid Fluoride (40) using Diisopropylethylamine (DIEA) as HF scavenger

The amino acid fluoride **40** (0.670g, 1.56 x 10^{-3}moles) and DIEA (271 *u*l, 1.56 x 10^{-3}moles) was added to the resin **42** in DMF and shaken every 30 minutes for 6 hours. Resin was washed and showed a positive Kaiser Test.

Coupling of Fmoc TRIS Acid Fluoride using pyridine as HF scavenger (Dipeptide 43)

The amino acid fluoride **40** (0.670g, 1.56 x 10^{-3} moles) and pyridine (151 *u*l, 1.87 x 10^{-3}moles) was added to the resin **42** in DMF and shaken every 1 hour for 24 hours. Resin was washed and showed a negative Kaiser Test. The Fmoc group was cleaved by shaking the resin with 20% piperidine in DMF for 30 minutes. Kaiser Test showed positive. The dipeptide **43** was cleaved as described in the general procedure below.

FAB MS Calc. for $C_{14}H_{18}N_2O_3$ [M+H$^+$] m/z 263, Found 263

Coupling of Fmoc Glycine Acid Fluoride to the TRIS Amino Acid containing resin (Tripeptide 45)

The Fmoc Glycine Acid Fluoride **44** (0.600g, 2.00 x 10^{-3}moles) and pyridine (162 *u*l, 2.00 x 10^{-3}moles) was added to the resin containing the dipeptide **43** in DMF and shaken occasionally for 24 hours. Resin was washed and showed a negative Kaiser Test. The Fmoc group was cleaved by shaking the resin with 20% piperidine in DMF for 30 minutes. Kaiser Test showed positive. The tripeptide **45** was cleaved as described in the general procedure below.

FAB MS Calc. for $C_{16}H_{21}N_3O_4$ [M+H$^+$] m/z 320, Found 320

Cleavage of the peptide from the resin (Novabiochem General Procedure)

Resin was washed several times with CH_2Cl_2, methanol and Et_2O, and dried overnight on a vacuum pump. The dry resin was placed in a flask and the cleavage mixture (95% Trifluoroacetic acid (TFA), 2.5% H_2O, 2.5% triisopropylsilane) added. The flask was left at

room temperature for 5 hours with occasional stirring. The resin was removed by filtration and washed twice with TFA. The filtrate was concentrated by blowing air over it while continuously adding Et_2O.

(R)-(+)-2-Phenylglycinol (62)[145]

To an ice cooled suspension of $LiAlH_4$ (2g) in dry THF (150ml) was added drop-wise the (R)-(+)-2-phenylglycine methyl ester **61** (3g, 18.18mmol) in dry THF (100ml). The mixture was refluxed under argon for 2 hours and quenched with saturated Na_2SO_4 solution (10ml). The white precipitate was filtered and washed successively with THF, the filtrate was dried over Na_2SO_4 and concentrated.
The product was recrystallised from toluene to yield the (R)-(+)-2-phenylglycinol **62** (1.6g, 65%): m.p. 69-71°C (lit. 72-73°C)[145]

The IR and NMR data were identical to an authentic sample.

***exo*-8-*exo*-11-Diallylpentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane-*endo*-8-*endo*-11-diol (51)**[109]

A solution of dione **31** (58.47 g, 336 mmol) in dry THF (500 mL) was added dropwise over 2 hours to a mechanically stirred suspension of freshly prepared_allylmagnesium bromide (2.2 L of 0.392 M in dry THF; excess) under argon at 0°C. After the addition had been completed, the external ice-water bath was removed, and the reaction mixture was allowed to warm gradually to ambient temperature while stirring under argon during 24 h. The reaction was quenched via addition of saturated aqueous NH_4Cl (until pH is 6~7), the layers were separated, and the aqueous layer was extracted with EtOAc (2 x 500 mL). The combined organic extracts were dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was recrystallized from hexane, thereby affording pure **51** (78.89 g, 91%) as a colourless microcrystalline solid: m.p. 82-83 °C; IR (KBr): V_{max} 3169, 2976, 1639 cm^{-1} ; ^{1}H-NMR [$CDCl_3$, 200 MHz]: δ_H 1.05 (*AB*, J_{AB} = 10.8 Hz, 1 H), 1.49 (A*B*, J_{AB} = 10.8 Hz, 1 H), 1.97-2.24 (m, 6 H), 2.30-2.61 (m, 6 H), 5.01 (dd, J = 8.0 & 2.6 Hz, 2 H), 5.04 (dd, J = 16.85 & 2.6 Hz, 2 H), 5.90 (m, 2 H), 6.52 (br s, 2 H); ^{13}C-NMR [$CDCl_3$, 50 MHz]: δ_C 33.9 (t), 40.0 (d), 42.8 (d), 44.0 (d), 44.1 (t), 49.1 (d), 77.2 (s), 117.5 (t), 133.8 (d). CI MS: Calc for $C_{17}H_{22}O_2$: [M + H]+ *m/z* 259.16981. Found: [M + H]+ *m/z* 259.16994. Anal. Calcd for $C_{17}H_{22}O_2$: C, 79.03; H, 8.58. Found: C, 79.14; H, 8.42.

3,5-Diallyl-4-oxahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}] dodecane (52)[109]

A solution of **51** (61 g, 0.236 mol) and TsOH (1.5 g, 0.79 mmol, catalytic amount) in benzene (1.2 L) was refluxed in a Dean-Stark apparatus and the resulting water was removed azeotropically. After every 12 hours, additional TsOH (500 mg) was added. When TLC indicated the absence of **51** (72 hours), the reaction mixture was allowed to cool gradually to ambient temperature and washed sequentially 10% aqueous $NaHCO_3$ (100 mL), water (100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified via column chromatography on silica gel by eluting with 5% EtOAc-hexane. Pure **52** (46.53 g, 82%) was thereby obtained as a colourless oil; IR (neat): V_{max} 3075, 2965, 1640, 997, 910 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ_H 1.46 (*AB*, J_{AB} = 10.2 Hz, 1 H), 1.82 (A*B*, J_{AB} = 10.2 Hz, 1 H),

2.35 (br s, 2 H), 2.45-2.65 (m, 10 H), 5.01 (dd, J = 9.8 & 2.2 Hz, 2 H), 5.07 (dd, J = 15.4 & 1.4 Hz, 2 H), 5.78 (m, 2 H); ^{13}C NMR ($CDCl_3$): δ_C37.5 (t), 41.7 (d), 43.3 (t), 44.5 (d), 47.8 (d), 58.6 (d), 95.1 (s), 116.8 (t), 134.4 (d). CI MS: Calc. for $C_{17}H_{20}O$: [M + H]+ m/z 241.1592. Found: [M + H]+ m/z 241.1601.

3,5-dicarboxymethyl-4-oxahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]-dodecane (59)

A solution of the diene **52** (3.3g) in dry methanol (100ml) was purged with argon for 20 minutes while cooling in a dry ice-acetone bath. Ozone was bubbled into the mixture until the blue-purple colour persisted indicating the presence of excess ozone and completion of reaction. The excess ozone was flushed out with argon and the solvent was removed under reduced pressure. Hydrogen peroxide (30ml, 30%) was added drop-wise to a stirred, ice bath cooled mixture of the ozonide and formic acid (21ml). The reaction was stirred at ambient temperature for 1 hour, refluxed gently for 12 hours and concentrated to yield the diacid **59** (2.895g, 76%) as a white solid, m.p. 175-175.5°C; IR (KBr): V_{max} 3180, 2980, 1720 cm^{-1}; 1H NMR [DMSO, 200 MHz]: δ_H 1.45 (AB, J_{AB}=10 Hz, 1H), 1.83 (AB, J_{AB}=10 Hz, 1H), 2.36-2.80 (m, 12H), 12.15 (br s, 2H, D_2O exchangeable); 13C NMR [DMSO, 50 MHz]: δ_C 37.94 (t), 41.27 (d), 42.81 (t), 44.04 (d), 47.91 (d), 58.55 (d), 92.56 (s) and 171.40 (s); Anal. Calc. for $C_{15}H_{16}O_4$ C,65.21; H,5.84: Found C,65.39; H,5.71

(R,R)-1,11-Diamino-1,11-diphenyl-3,6,9-trioxaundecane (63)[146]

The (R)-amino alcohol **62** (1.476g, 10.78mmol) in dry THF (50ml) was added drop-wise at 0°C to a stirred suspension of NaH (55-60% in oil, 0.8g) in dry THF (80ml) under argon. The mixture was stirred at room temperature for 2 hours, treated with the diethylene glycol bis-toluene-p-sulphonate (2.23g, 5.395mmol) in dry THF (50ml) and stirred at room temperature for 72 hours. The reaction was quenched with water (60ml) and the solution concentrated to about 40ml. The concentrate was acidified with HCl (15%, 50ml), washed with ethyl acetate (3 x 50ml), made basic (Na_2CO_3), and extracted with ethyl acetate (3 x 50ml). This extract was dried and evaporated to give the crude diamine **63** as an oil which was suitable for use in the next step, ^{1}H-NMR [$CDCl_3$, 200 MHz]: δ_H 7.45-7.19 (m, 2 x C_6H_5), 4.20 (m, 2 x NCH), 3.65-3.40 (m, 6 x OCH_2) and 2.68 (br s, 2 x NH_2).

Synthesis of macrocycle (R,R)-57

To a suspension of the diacid **59** (0.552g, 2mmol) in dry CH_2Cl_2 (10ml) was added oxalylchloride (2ml, 23mmol) drop-wise with stirring under argon. Stirred for a further 5 hours after the reaction became homogeneous and concentrated (using a vacuum pump) to yield the diacyl choride **60** as a clear oil. Mixtures of the diamine **63** (0.688g, 2mmol) and $N(Et)_3$ (0.56ml) in toluene (250ml) and the diacyl choride **60** (2mmol) in toluene (250ml) were added simultaneously over 10 hours to toluene (250ml) with stirring at 0°C under argon. The mixture was then stirred at ambient temperature for 1 day and concentrated. The product **57** was obtained by chromatography (alumina, 1:5 ethyl acetate: dichoromethane) and recrystallised from toluene to give clear crystals (0.43g, 37%), m.p. 94-97°C; $[\alpha]^{24}_D$ + 6.56° (c=1, CH_2Cl_2); IR (KBr): V_{max} 3343, 3307, 2958, 2870, 1674, 1517, and 1109 cm^{-1}; ^{1}H NMR [$CDCl_3$, 200 MHz]: δ_H 1.49 (AB, J_{AB}=10.5 Hz, 1H), 1.82 (AB, J_{AB}=10.6 Hz, 1H), 2.15-2.80 (m, 10H), 2.89 (AB, J_{AB}=15.5 Hz, 1H), 2.98 (AB,

J_{AB}=14.8 Hz, 1H), 3.45-3.95 (m, 12H), 5.09-5.30 (m, 2H), 7.12-7.53 (m, 10H), 7.65 (d, J = 8.1 Hz, 1H), 7.74 (d, J = 8 Hz, 1H); ^{13}C NMR [$CDCl_3$, 50 Hz]: δ_C 39.55 (t), 39.58 (t), 41.00 (d), 41.35 (d), 43.15 (t), 43.68 (d), 43.98 (d), 46.22 (d), 49.76 (d), 52.80 (d), 52.85 (d), 56.36 (d), 60.06 (d), 70.22 (t), 70.89 (t), 70.94 (t), 73.91 (t), 73.94 (t), 93.98 (s), 94.05 (s), 126.62 (d), 127.01 (d), 128.04 (d), 140.25 (s), 140.32 (s), 169.14 (s); CI MS: Calc. for $C_{35}H_{41}N_2O_6$: [M + H^+] 585.2964, Found: [M + H^+] 585.2973.

N-Benzyloxycarbonyl-L-proline diester (64)[138]
In dry CH_2Cl_2 (200ml) were dissolved 2,6-pyridinedimethanol (1g, 7.2 x 10^{-3}moles), benzyloxycarbonyl-L-proline (3.66g, 17 x 10^{-3}moles), 4-(dimethylamino)pyridine (0.4g, 20 x 10^{-3}moles), and DIPCDI (2.5g, 20 x 10^{-3}moles). After stirring overnight at room temperature, the reaction was concentrated and the residue was chromatographed on silica gel to give a colourless oil **64** (2.7g, 72%).
^{1}H NMR: δ_H 1.95 (m, 4H), 2.11 (brs, 2H), 2.26 (d, 2 H), 3.64 (m, 4H), 4.51 (m, 2H), 5.11 (m, 6H), 5.27 (d, 2H), 7.26 - 7.56 (m, 13H)

L-Proline diester diamine dihydrobromide[138]
N-benzyloxycarbonyl-L-proline diester **64** (1g, 1.87 x 10^{-3}moles) was dissolved in 33% HBr-HOAc (25ml). The mixture was stirred at room temperature overnight and the precipitate was filtered and washed with diethyl ether. The product was recovered as a light yellow powder **65** (0.833g, 90%).

The IR and NMR data were identical to the authentic sample.

S-PhenylGlycine Pyridine Diamine (67)
The (S)-amino alcohol (0.7g, 5.1 x 10^{-3}moles) in dry THF (50ml) was added drop-wise at 0°C to a stirred suspension of NaH (55 - 60% in oil, 0.45g, 15 x 10^{-3}moles) in dry THF (80ml) under argon. The mixture was stirred at room temperature for 2 hours, treated with the 2,6-pyridine dimethylbromide (0.675g, 2.5 x 10^{-3}moles) in dry THF (50ml) and stirred at room temperature for 72 hours. The reaction was quenched with water (60ml) and the solution concentrated to about 40ml. The concentrate was acidified with HCl (15%, 50ml), washed with ethyl acetate (3 x 50ml), made basic (Na_2CO_3), and extracted with ethyl acetate (3 x 50ml). This extract was dried and evaporated to give the crude diamine as a light yellow oil **67** (0.672g, 71%) which was suitable for use in the next step. A small amount was chromatographed on alumina for characterisation. $[\alpha]^{24}_D$ + 40.00°; IR (neat): V_{max} 3370, 3061, 3025, 2898, 1585, 1449, 1357, 1104 cm^{-1}; ^{1}H-NMR [$CDCl_3$, 200 MHz]: δ_H 1.91 (s, 4H, D_2O exchangeable), 3.46-3.72 (m, 4H), 4.25 (d, J = 3.7 Hz, 1H), 4.29 (d, J = 4Hz, 1H), 4.65 (s, 4H), 7.18-7.45 (m, 12H), 7.67 (t, J = 7.7 Hz, 1H); ^{13}C NMR [$CDCl_3$, 50 MHz]: δ_C 55.4 (d), 73.9 (t), 77.1 (t), 119.9 (d), 126.7 (d), 127.3 (d), 128.3 (d), 137.2 (d), 142.2 (s), 157.7 (s); CI MS: Calc. for $C_{23}H_{27}N_3O_2$: [M + H^+] 378.2181, Found: [M + H^+] 378.2175

Synthesis of macrocycle (S,S)-66
To a suspension of the diacid **59** (0.552g, 2mmol) in dry CH_2Cl_2 (10ml) was added oxalyl chloride (2ml, 23mmol) drop-wise with stirring under argon. The reaction mixture was then stirred for a further 5 hours, and concentrated using a vacuum pump to yield the

diacyl choride **60** as a colourless oil. Mixtures of the diamine **67** (0.755g, 2mmol) and $N(Et)_3$ (0.56ml) in toluene (250ml) and the diacyl choride **60** (2mmol) in toluene (250ml) were added simultaneously over 10 hours to toluene (250ml) with stirring at $0^{O}C$ under argon. The mixture was then stirred at ambient temperature for 1 day and concentrated. The product was obtained by chromatography (alumina, 1:5 ethyl acetate:dichloromethane) as white solid **66** (0.3g, 24%), m.p. 89-91°C; $[\alpha]^{24}_{D}$ + 36.06° IR (KBr): V_{max} 3425, 3327, 2957, 1656, 1523, 1488, 1116 cm^{-1}; ^{1}H NMR [$CDCl_3$, 200 MHz]: δ_H 1.40 (AB, J_{AB} = 10.6 Hz, 1H), 1.75 (AB, J_{AB} = 10.6 Hz, 1H), 2.22-2.74 (m, 12H), 3.80-4.00 (m, 4H), 4.66 (t, J = 14.3 Hz, 4H), 5.09-5.23 (m, 2H), 7.12-7.44 (m, 12H), 7.62-7.74 (m, 2H), 7.79 (d, J = 7.7 Hz, 1H); ^{13}C NMR [$CDCl_3$, 50 MHz]: δ_C 39.8 (t), 39.9 (t), 41.3 (d), 41.4 (d), 43.1 (t), 44.1 (d), 44.2 (d), 47.6 (d), 48.9 (d), 53.1 (d), 53.2 (d), 58.0 (d), 59.2 (d), 74.1 (t), 74.2 (t), 74.3 (t), 74.4 (t), 93.7 (s), 93.8 (s), 121.7 (d), 121.8 (d), 126.8 (d), 127.2 (d), 128.2 (d), 137.5 (d), 140.1 (s), 157.4 (s), 157.7 (s), 169.2 (s), 169.3 (s); CI MS: Calc. for $C_{38}H_{39}N_3O_5$: $[M + H^+]$ 618.2967, Found: $[M + H^+]$ 618.2959

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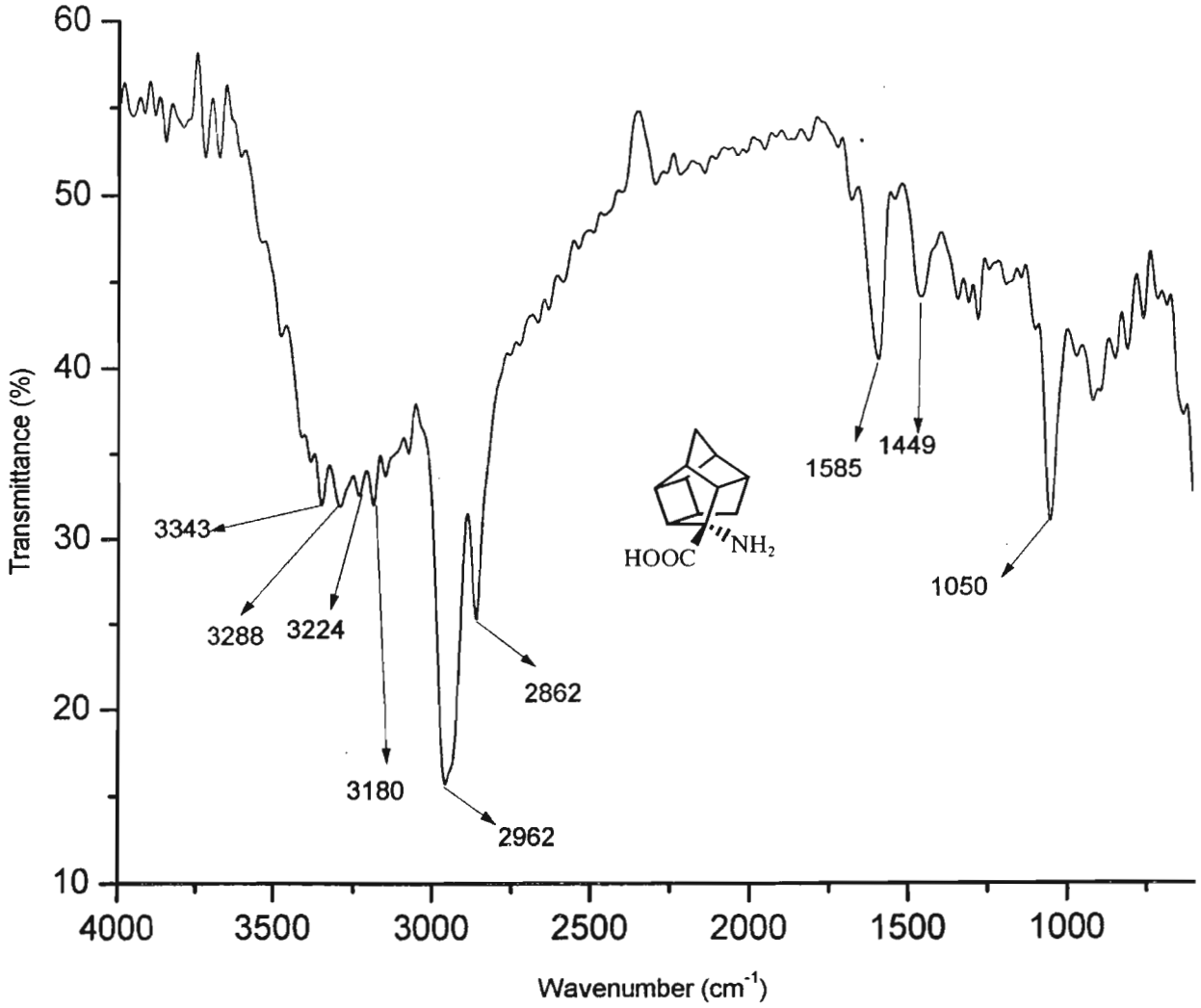
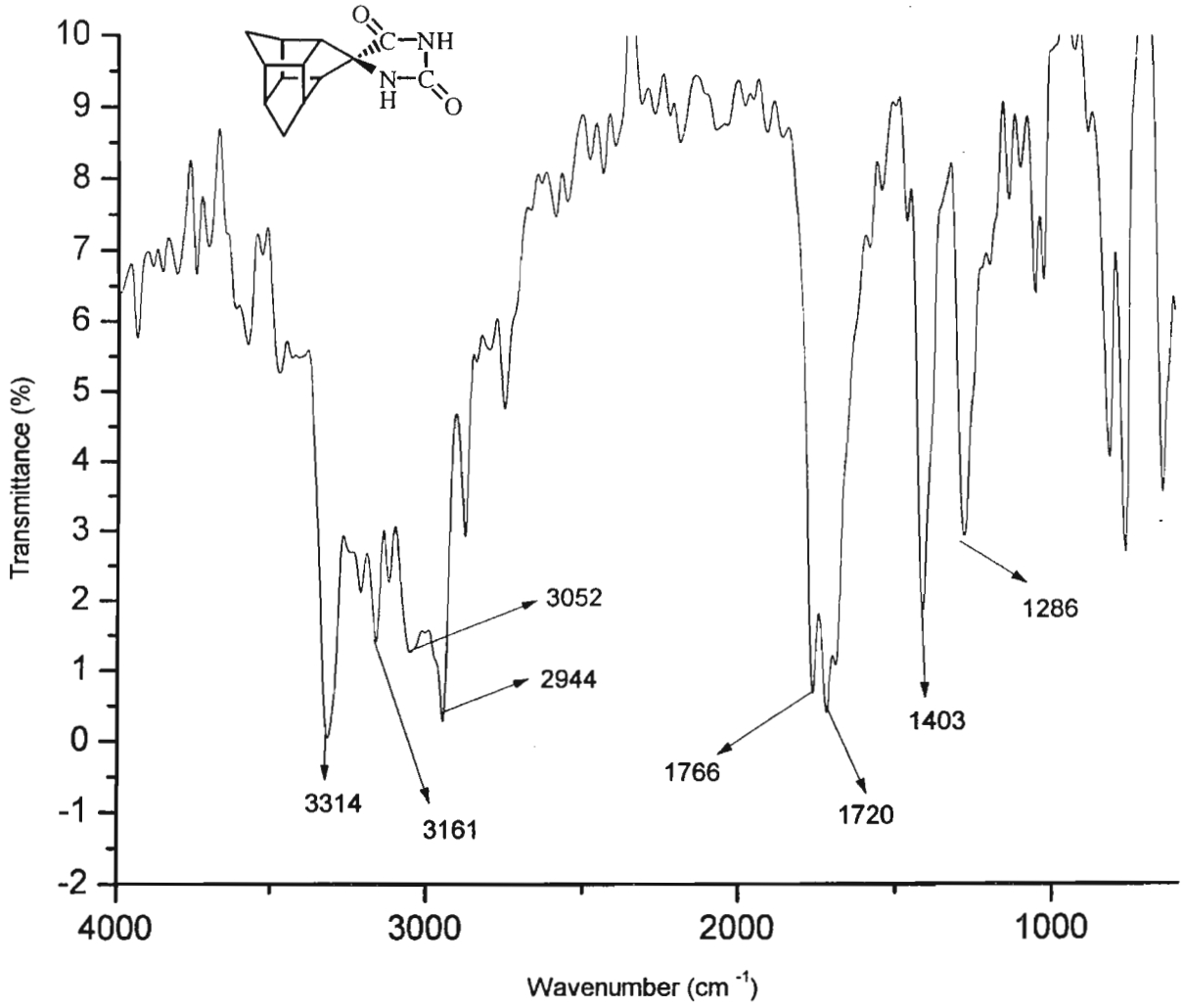
164. Private communication with Dr. T. D. Power, University of North Texas, who is studying similar systems.

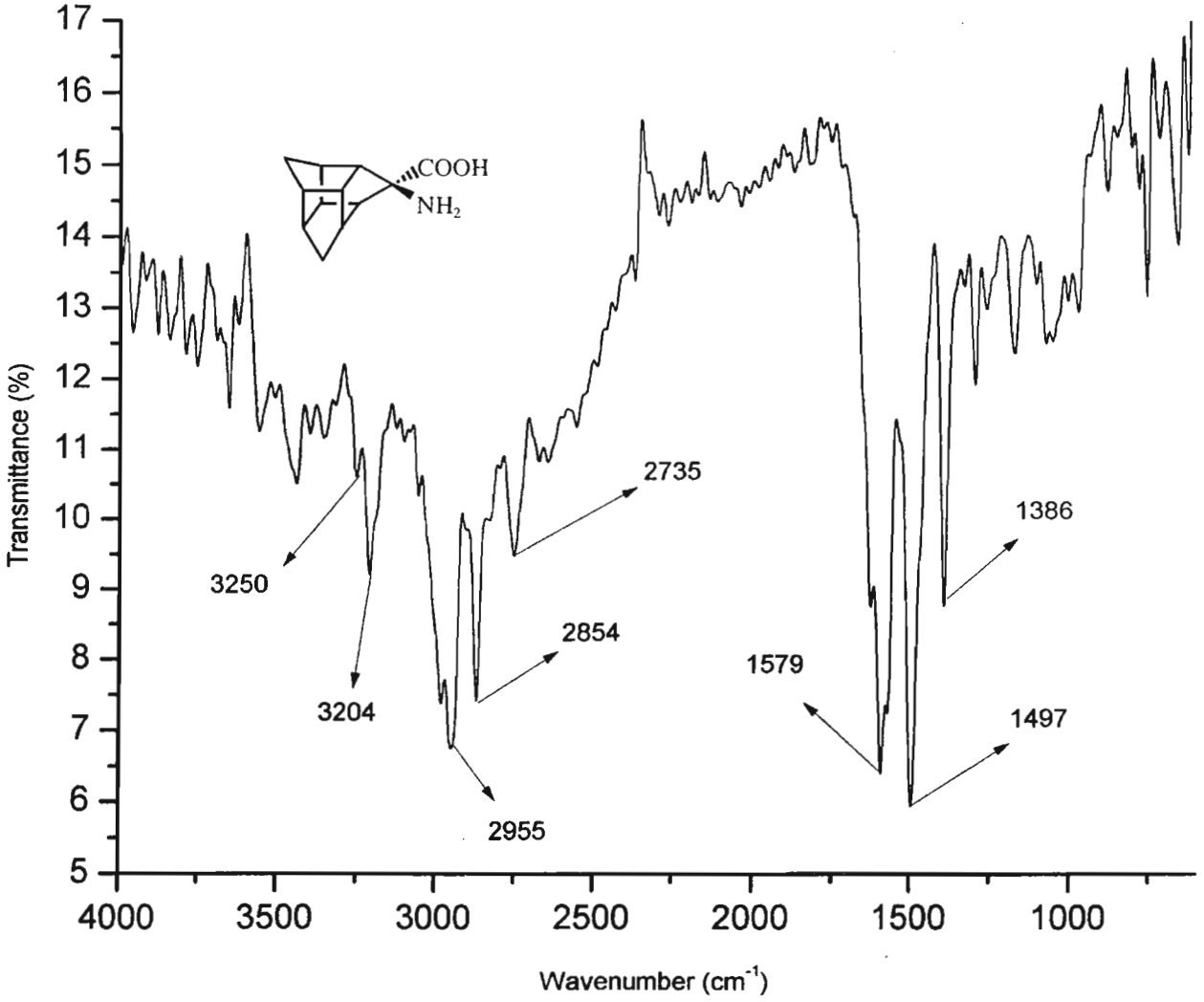
APPENDIX



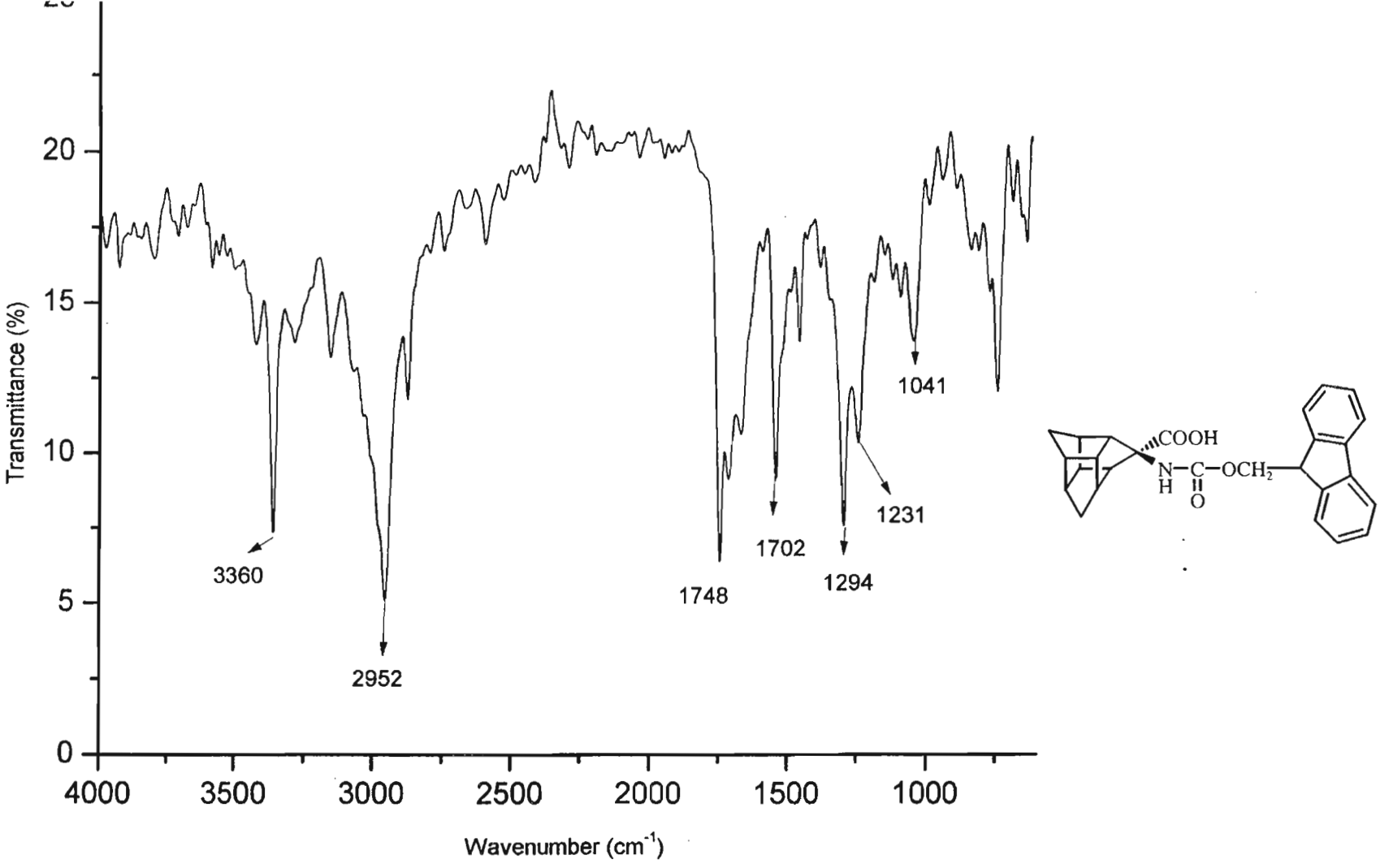
IR spectrum of PCU amino acid (6)



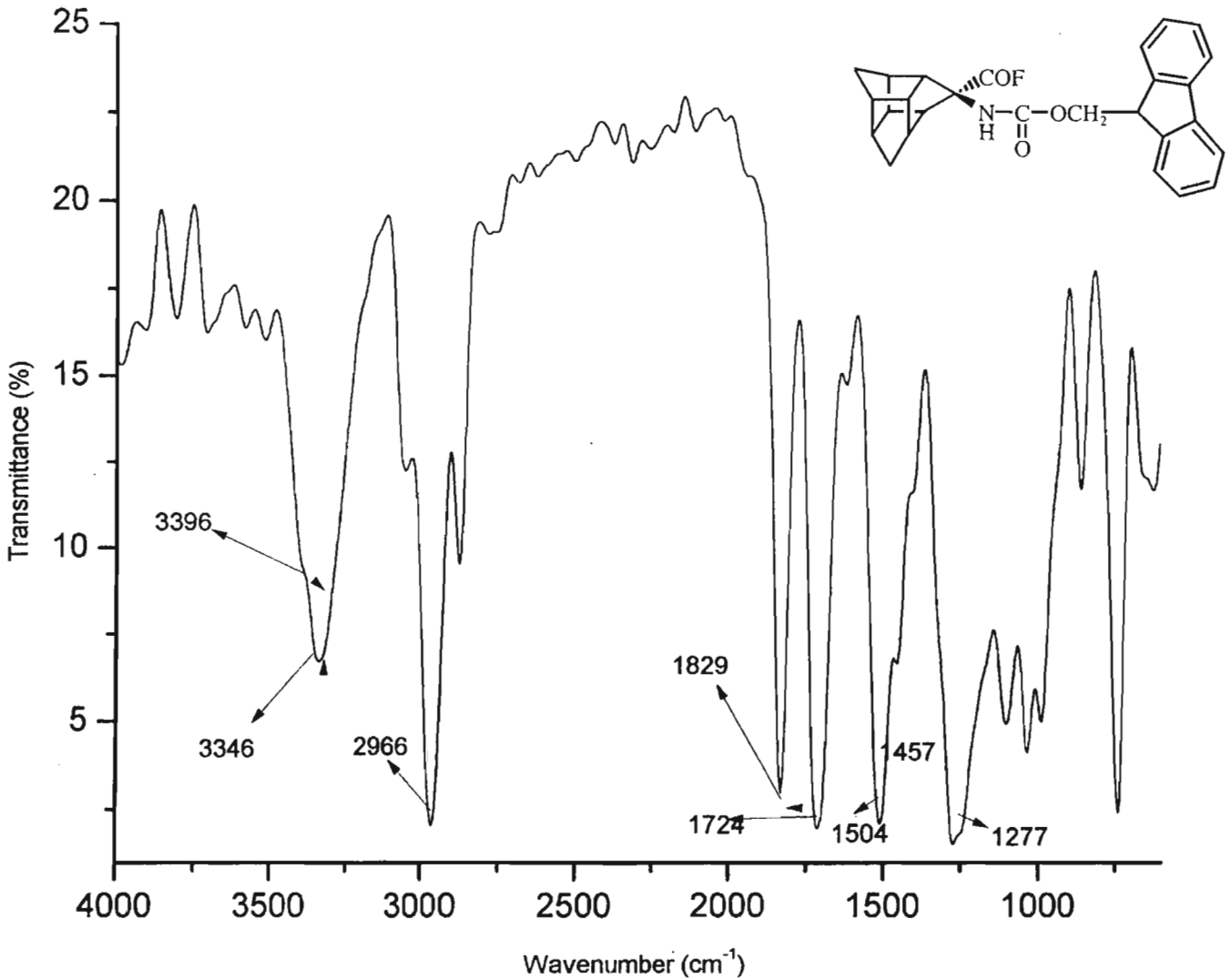
IR spectrum of TRIS hydantoin (26)



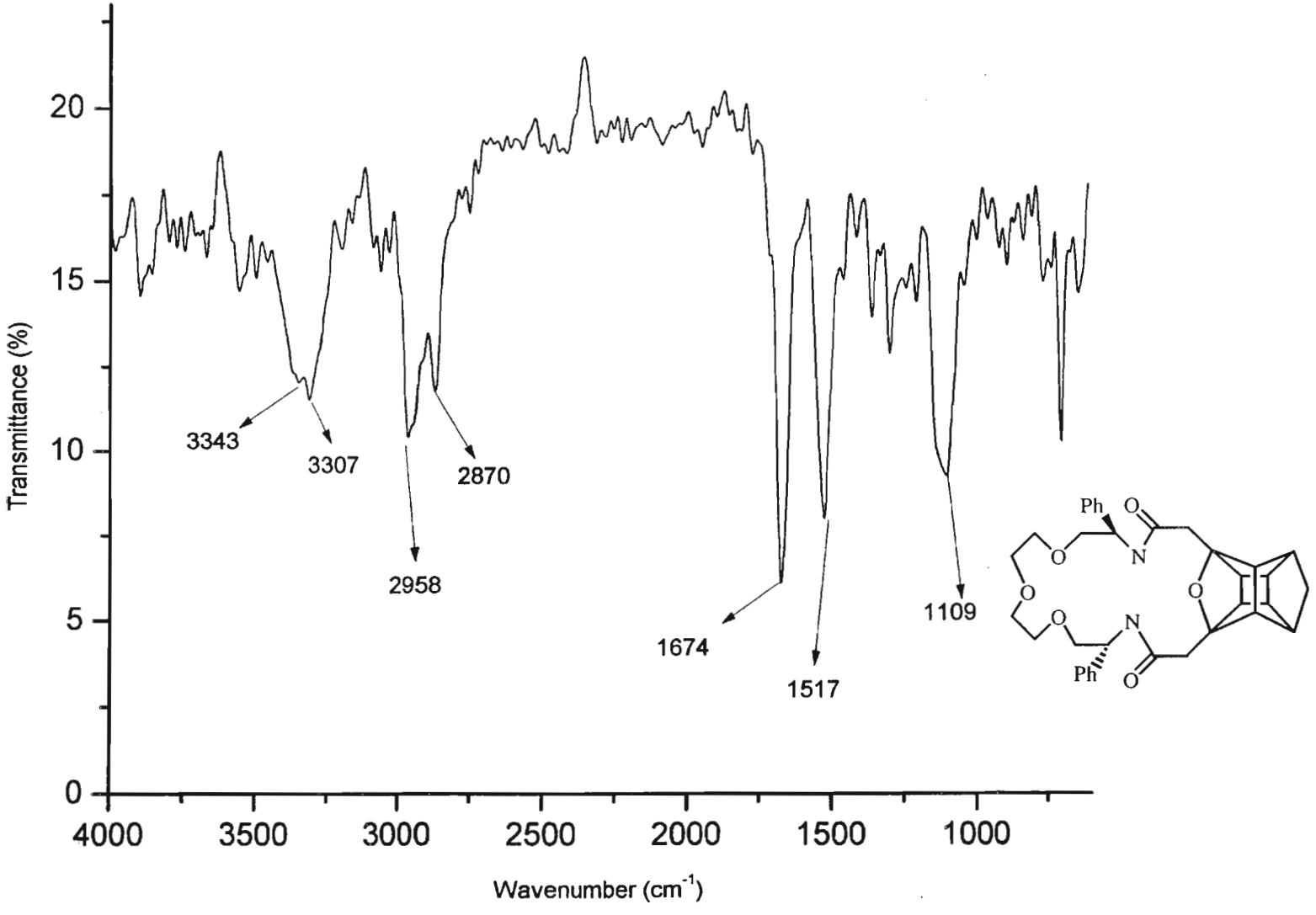
IR spectrum of TRIS amino acid (7)



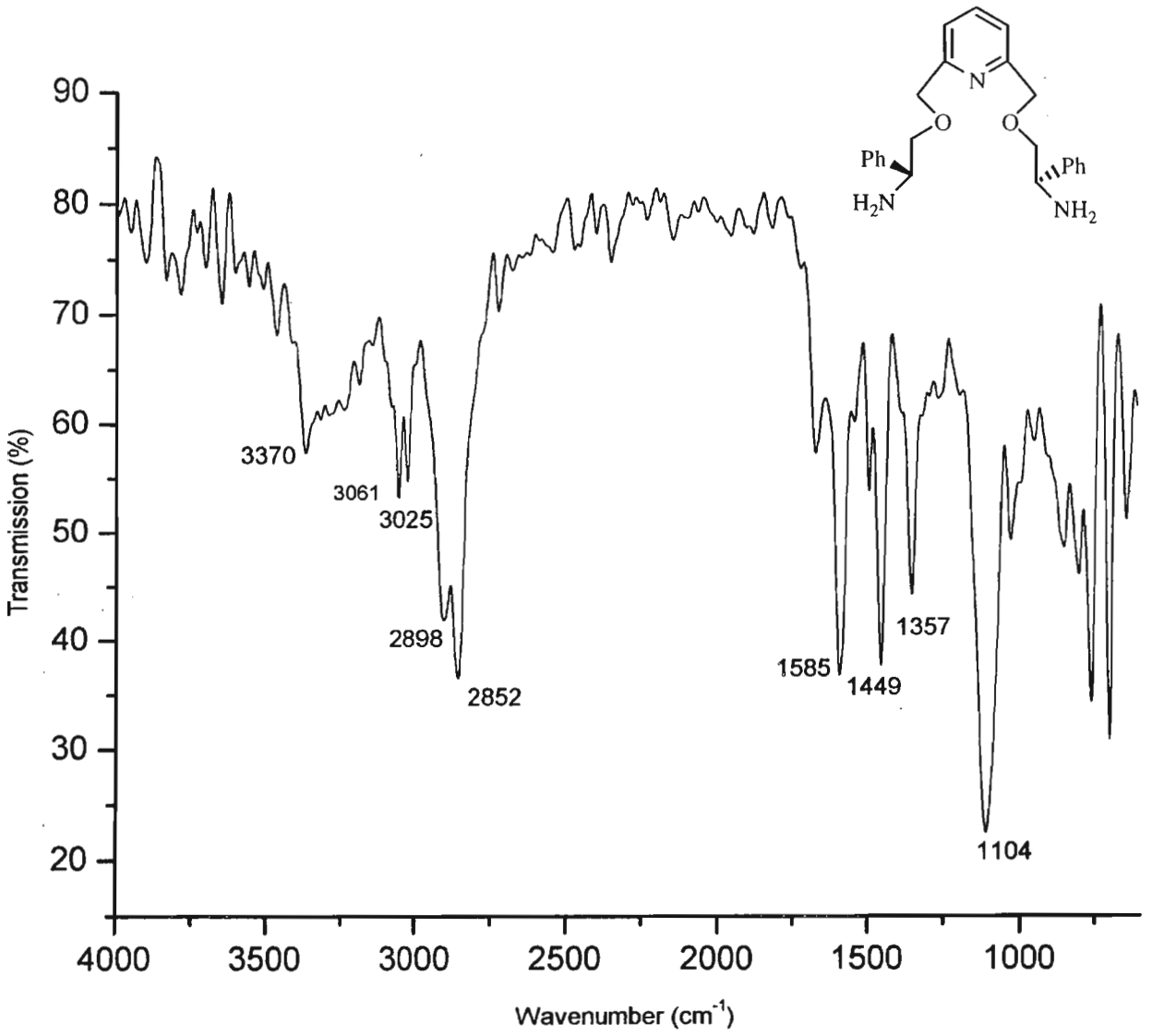
IR spectrum of Fmoc TRIS amino acid (27)



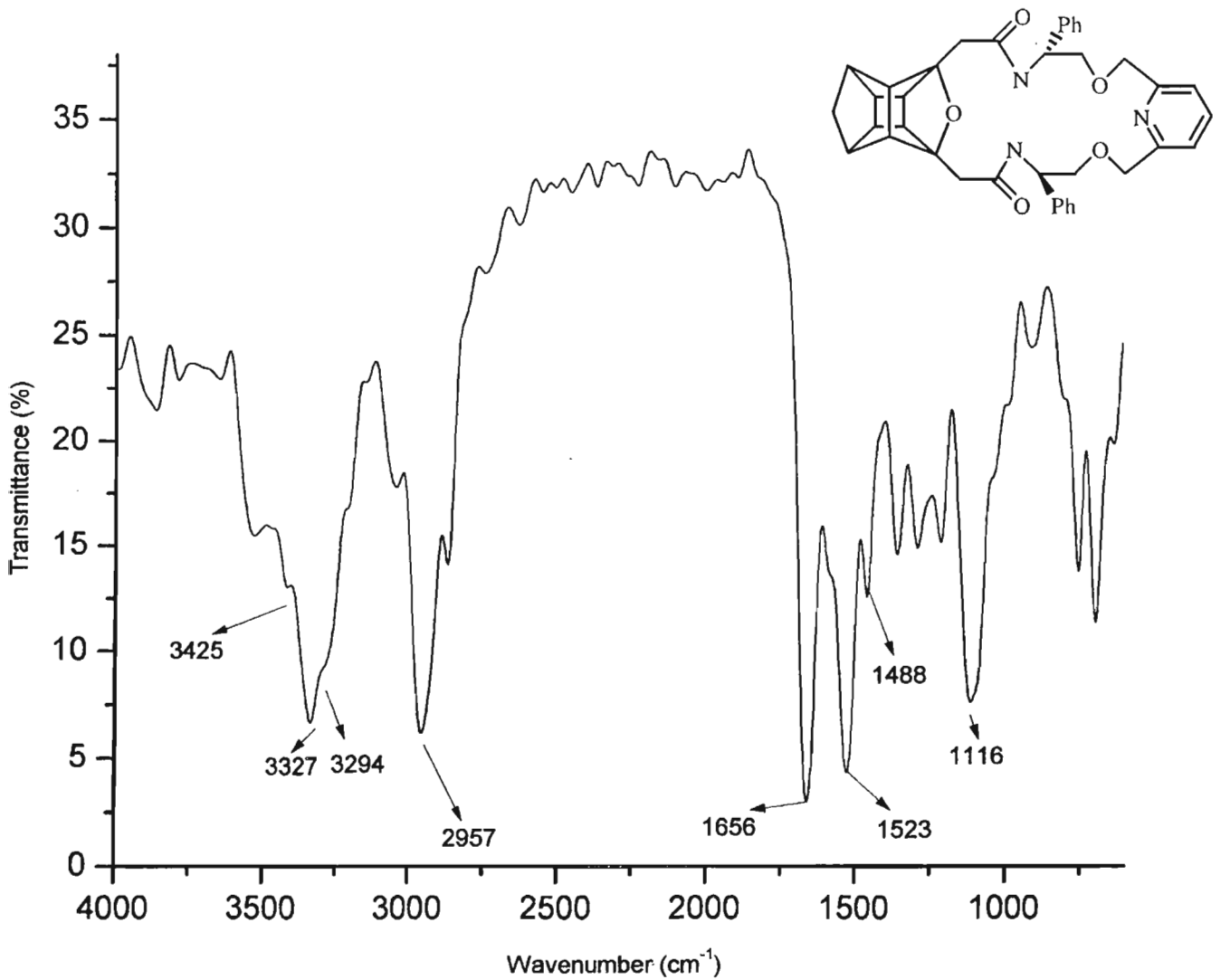
IR spectrum of Fmoc TRIS acid fluoride (40)



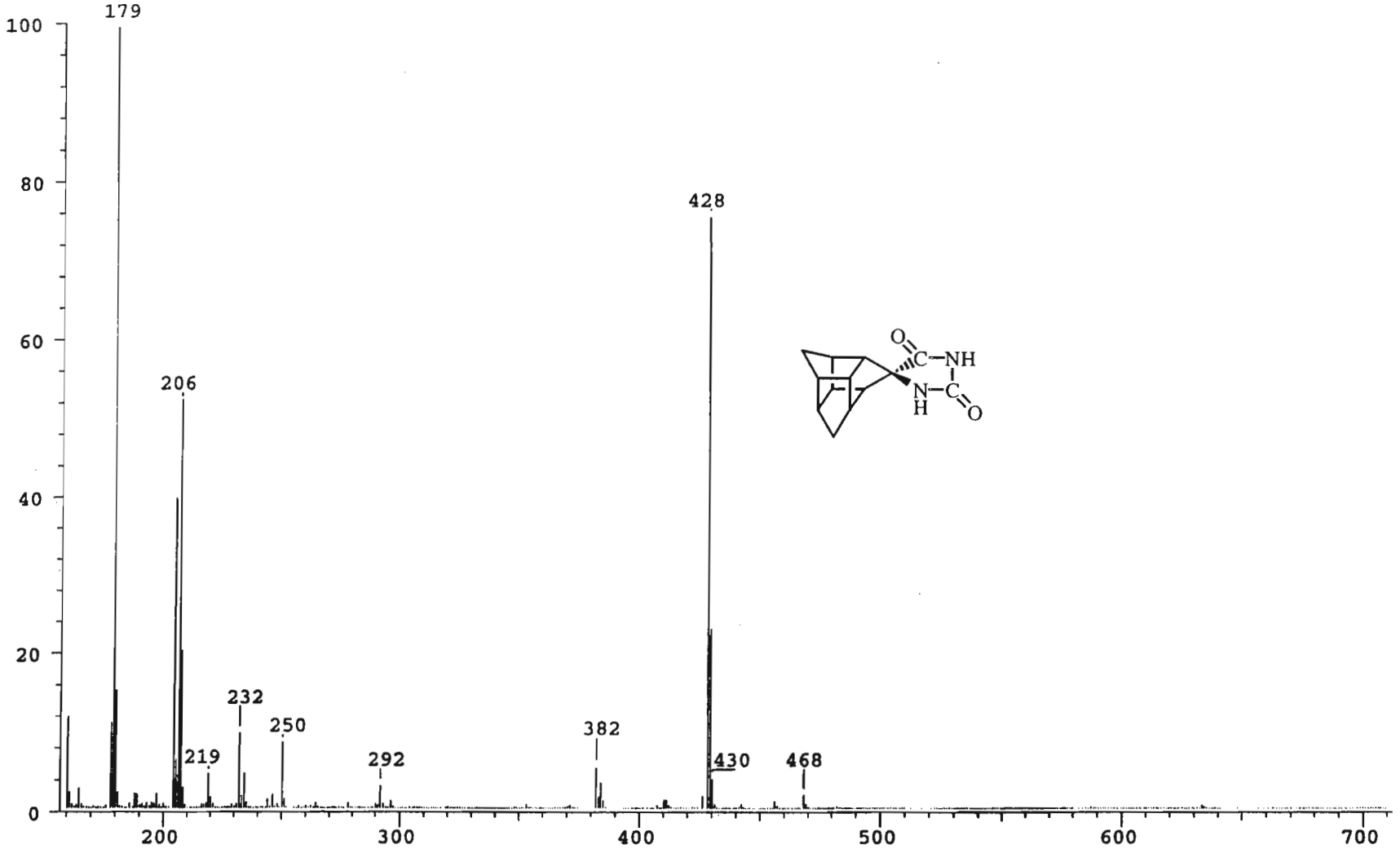
IR spectrum of macrocycle 57



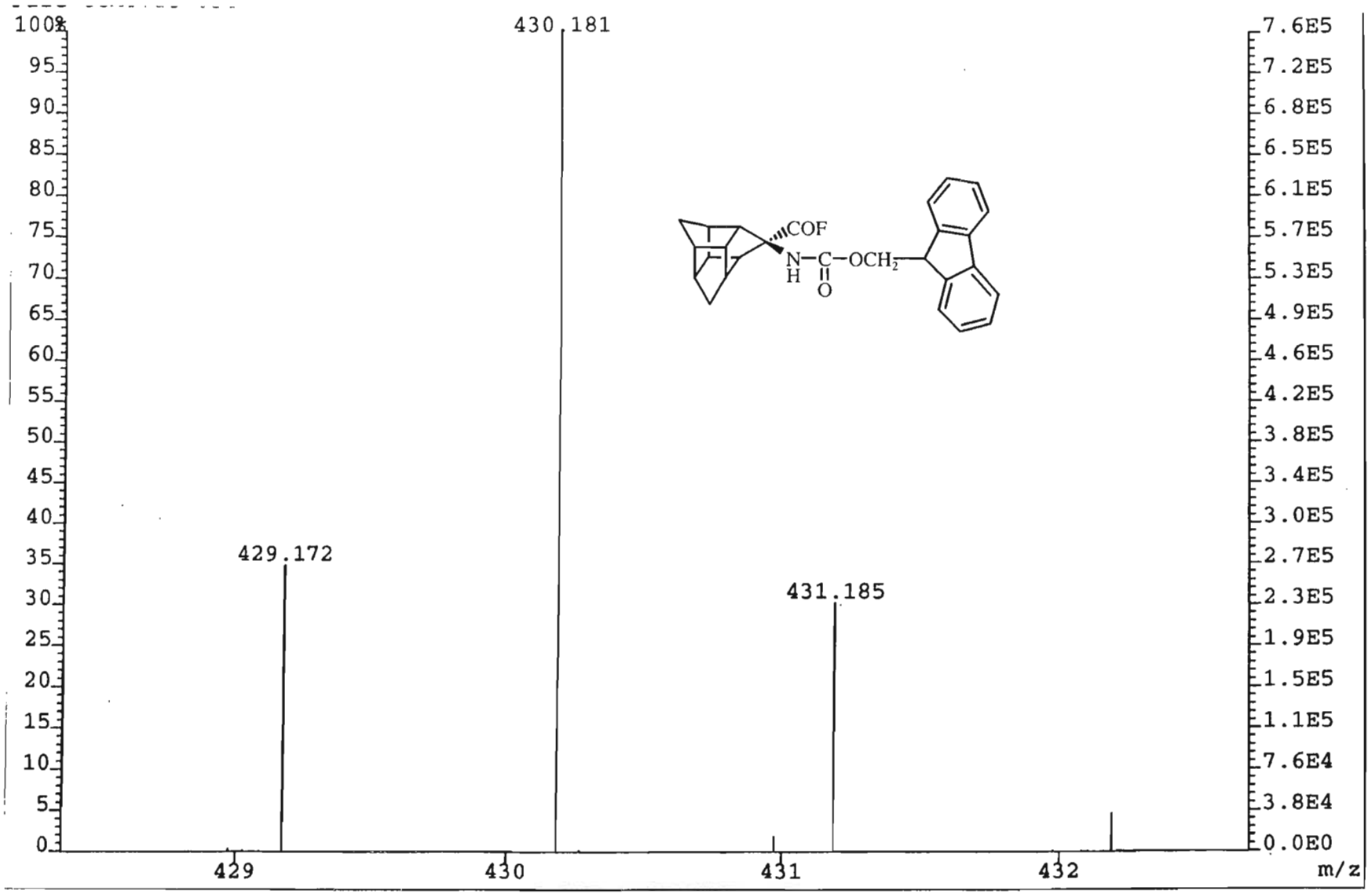
IR spectrum of S-PhenylGlycine Pyridine Diamine (67)



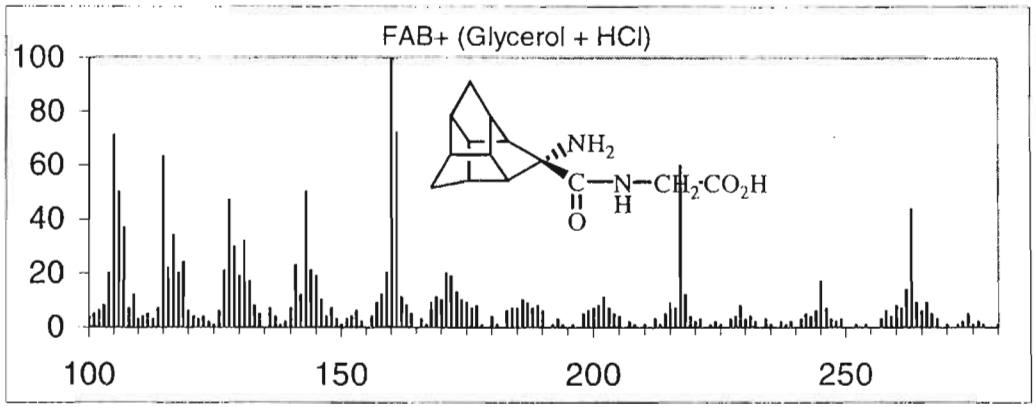
IR spectrum of macrocycle 66



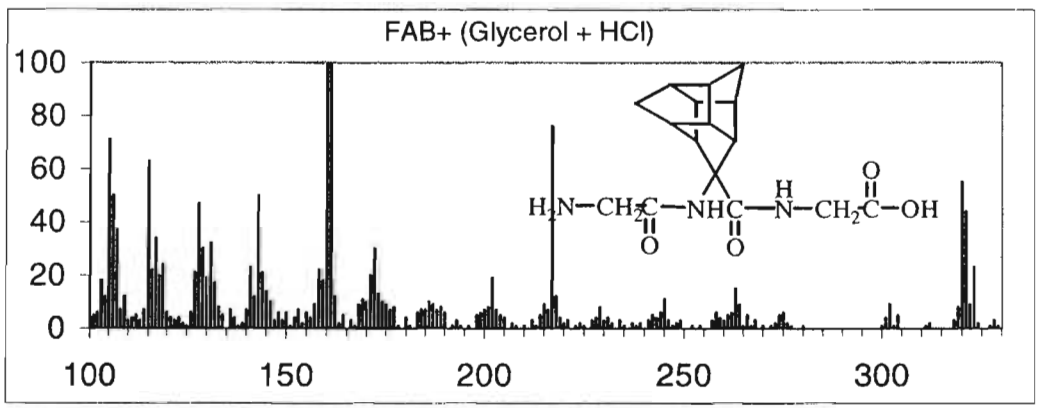
CI MS of TRIS hydantoin (26)



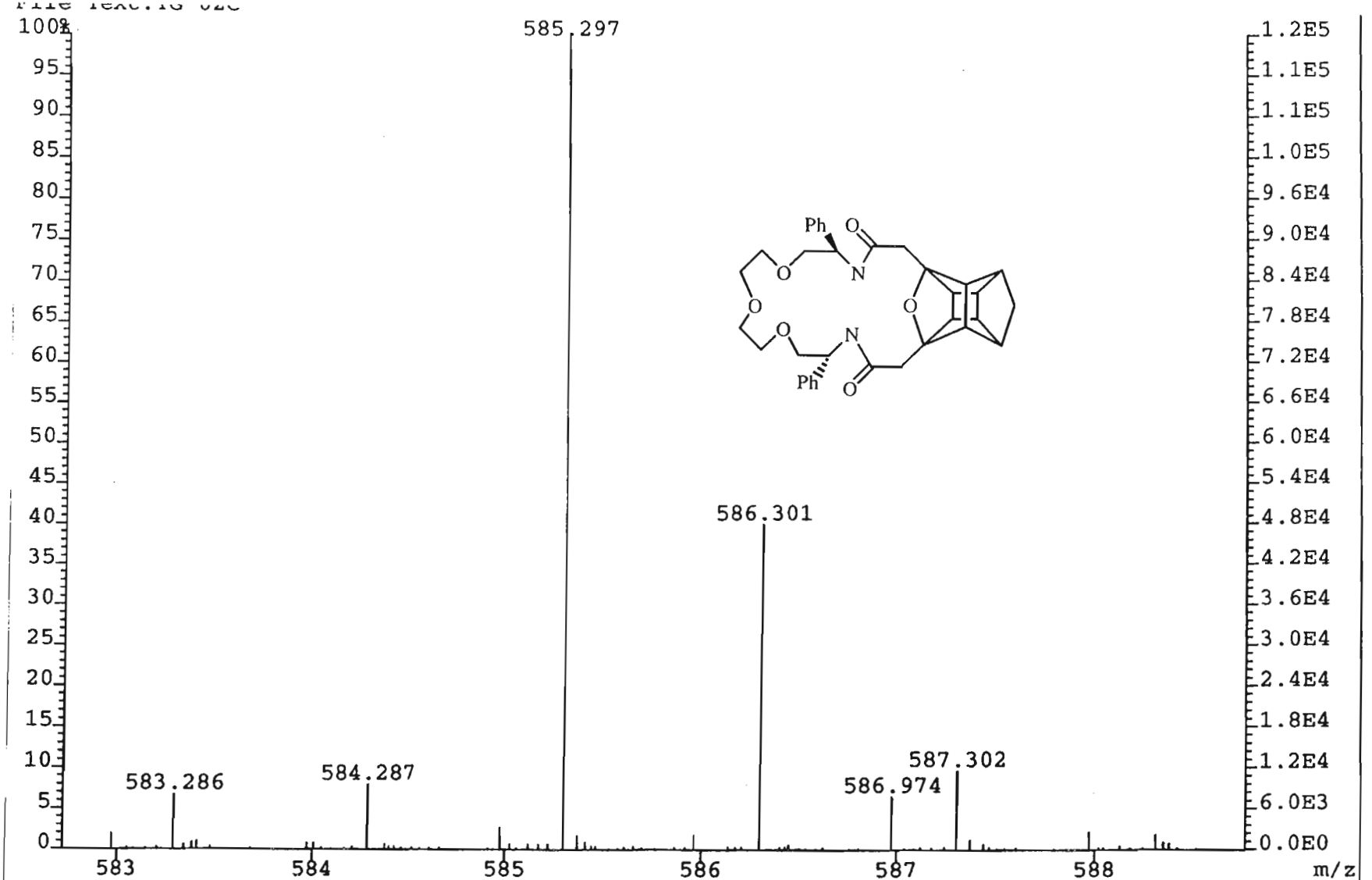
CI MS of Fmoc TRIS acid fluoride (40)



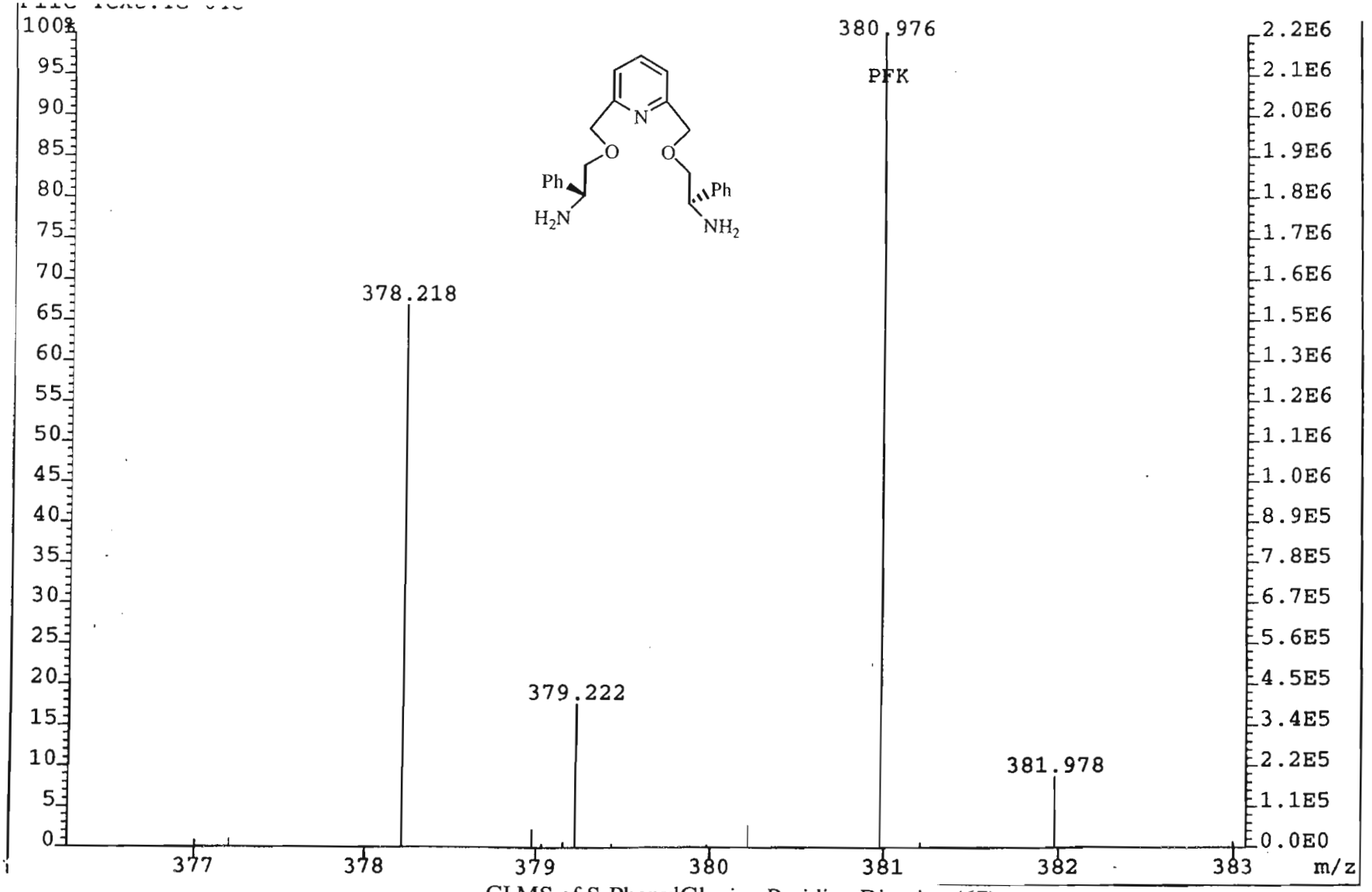
FAB MS of TRIS-Gly dipeptide (43)



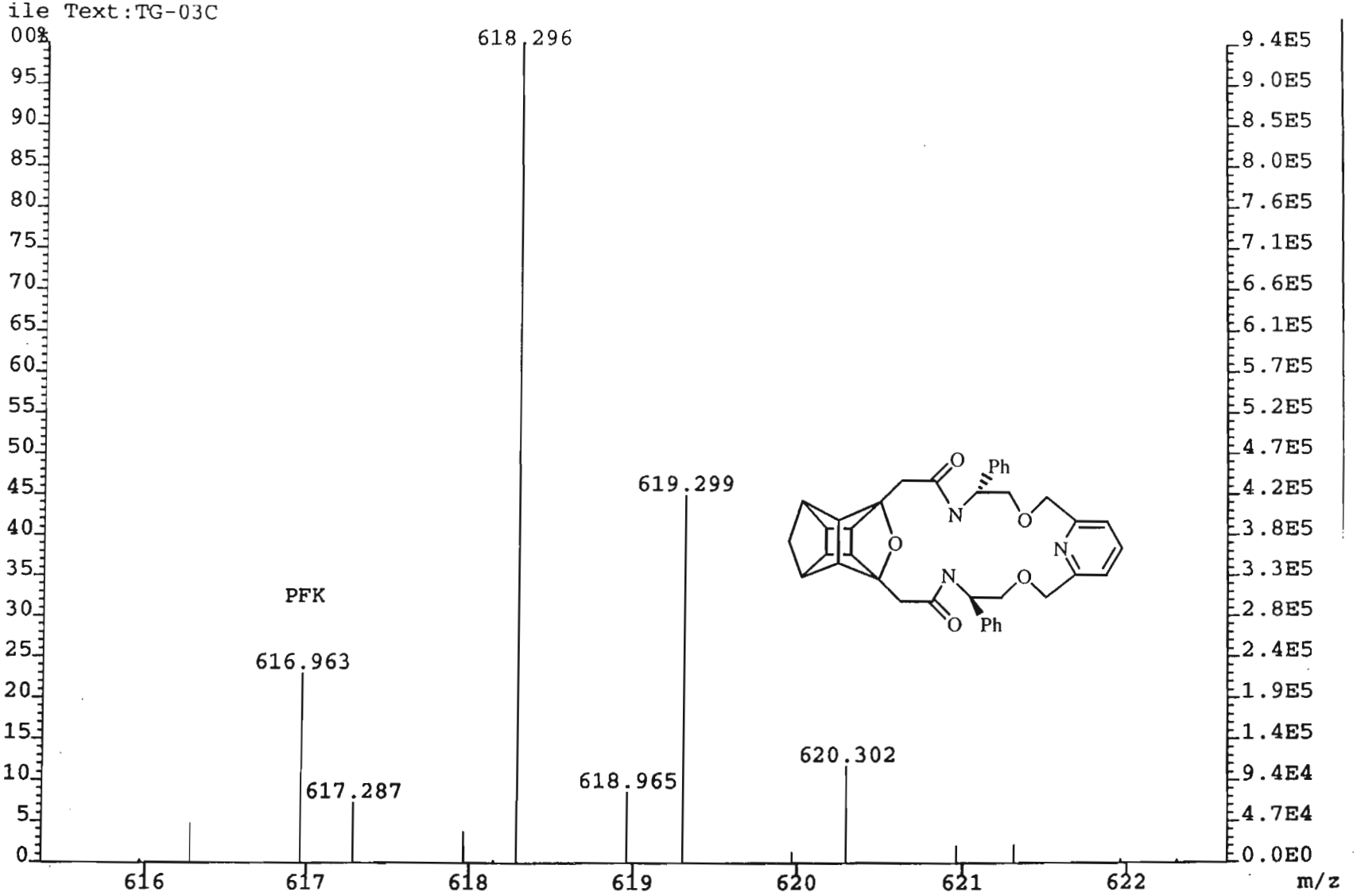
FAB MS of Gly-TRIS-Gly tripeptide (45)



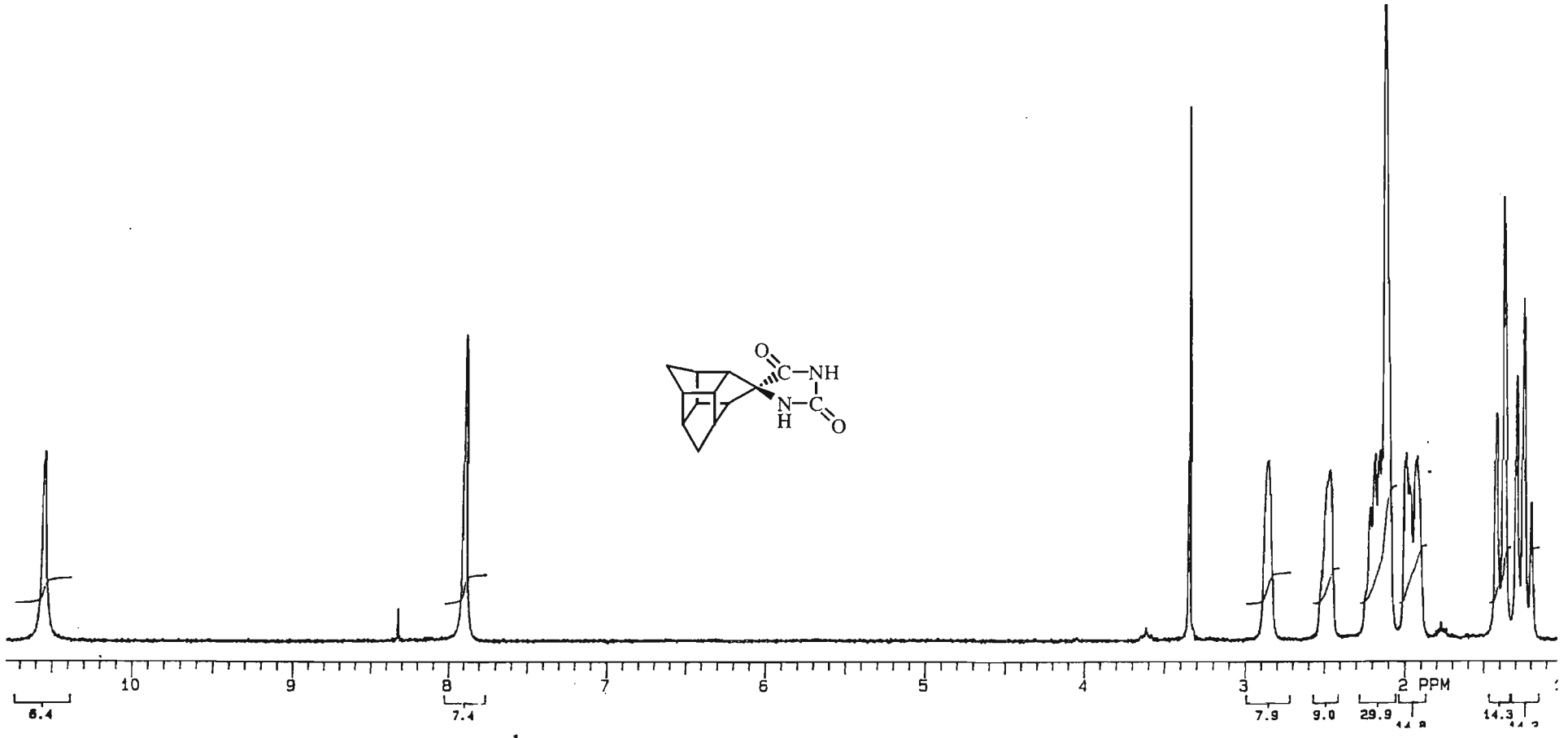
CI MS of macrocycle 57



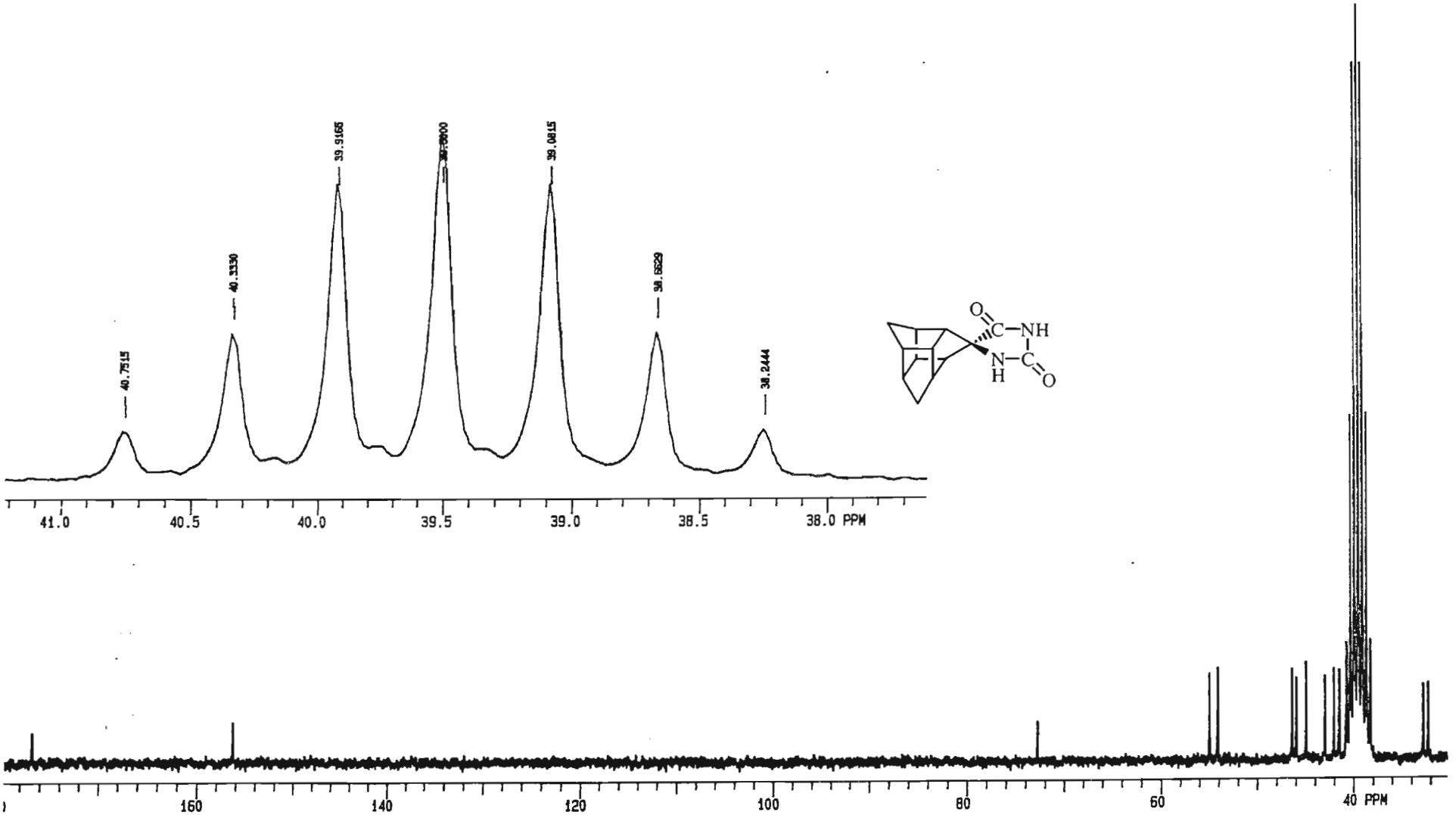
CI MS of S-PhenylGlycine Pyridine Diamine (67)



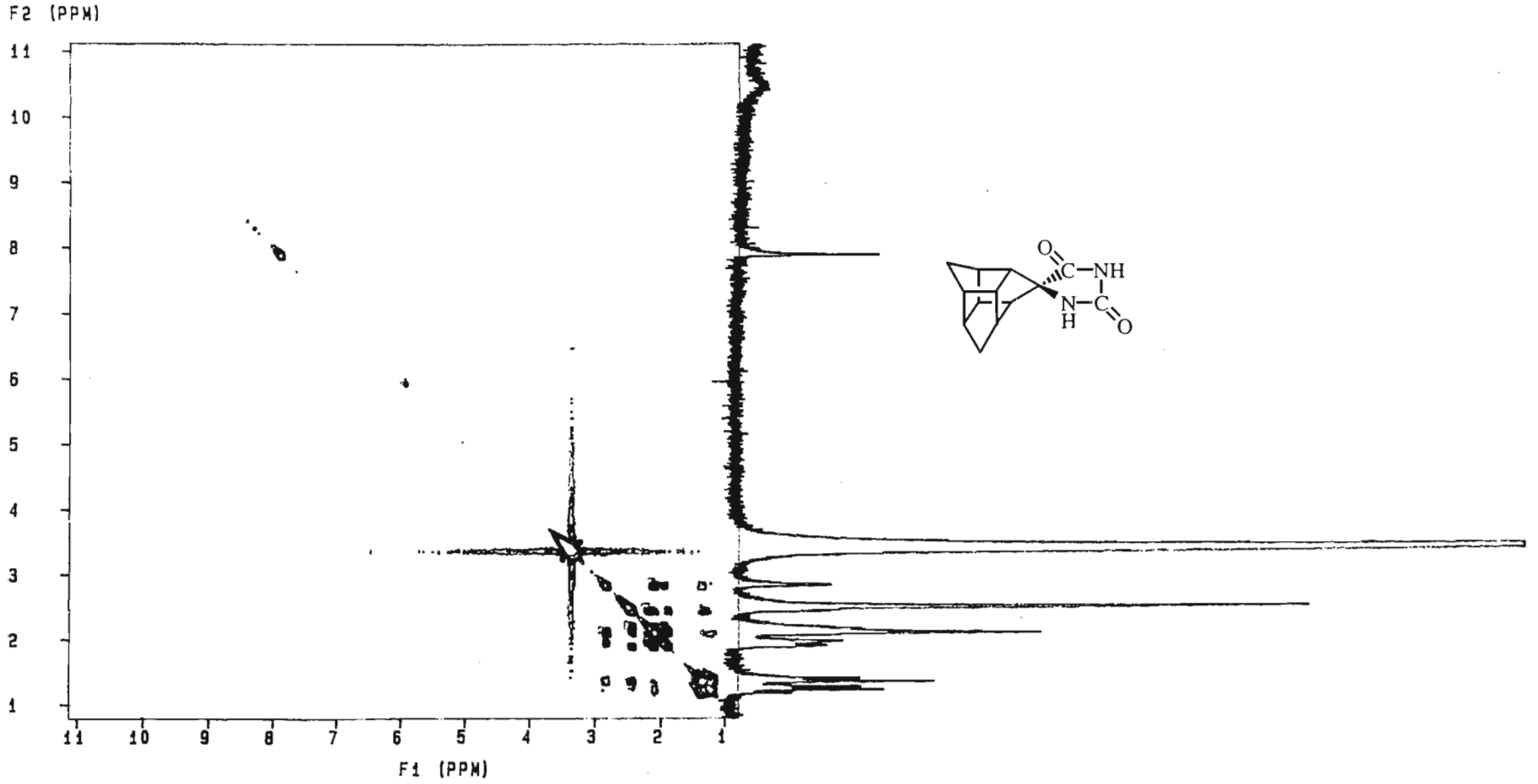
CI MS of macrocycle 66



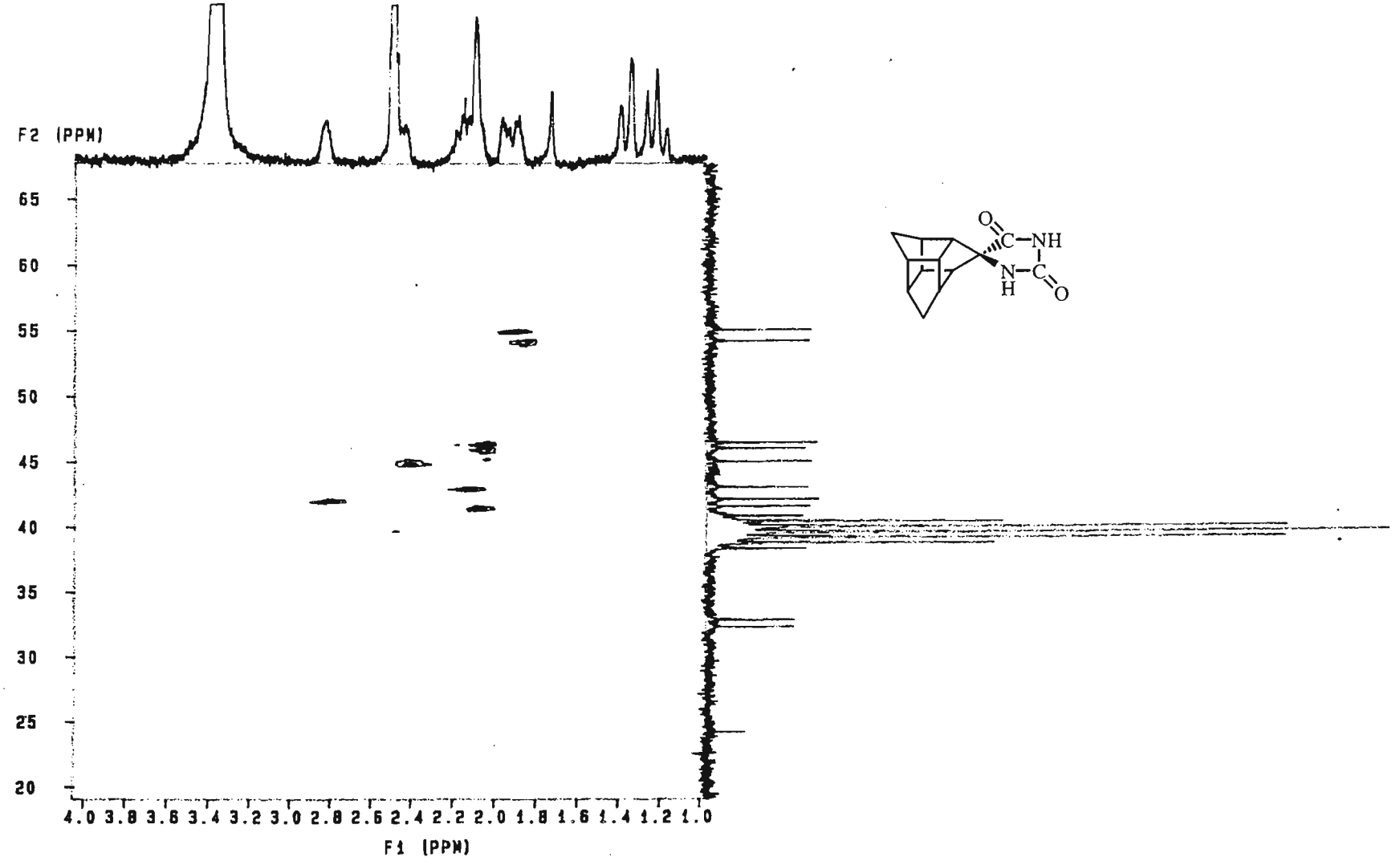
^{1}H nmr [$(CD_3)_2SO$] 200 MHz of TRIS hydantoin (26)



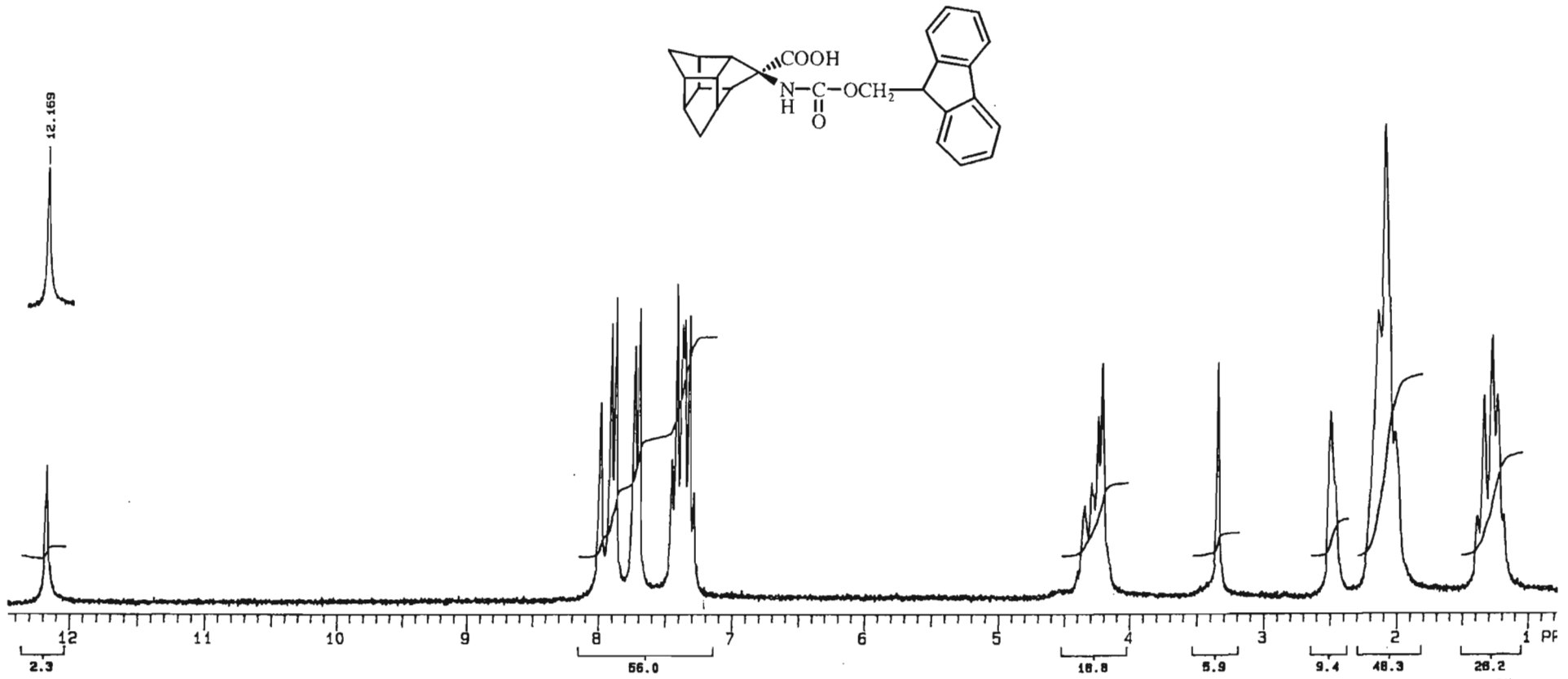
^{13}C nmr [$(CD_3)_2SO$] 50 MHz of TRIS hydantoin (26)



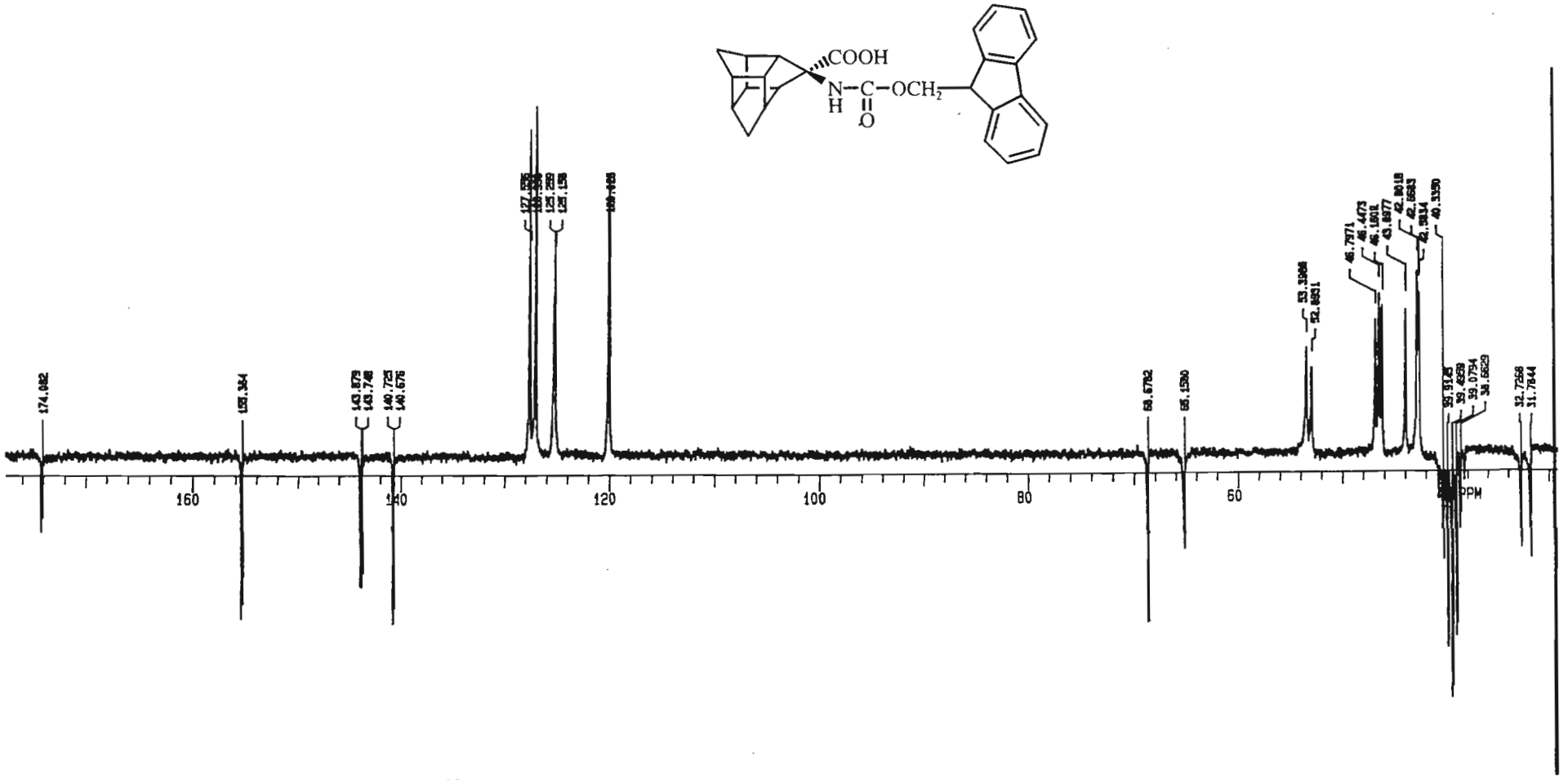
COSY [$(CD_3)_2SO$] of TRIS hydantoin (26)



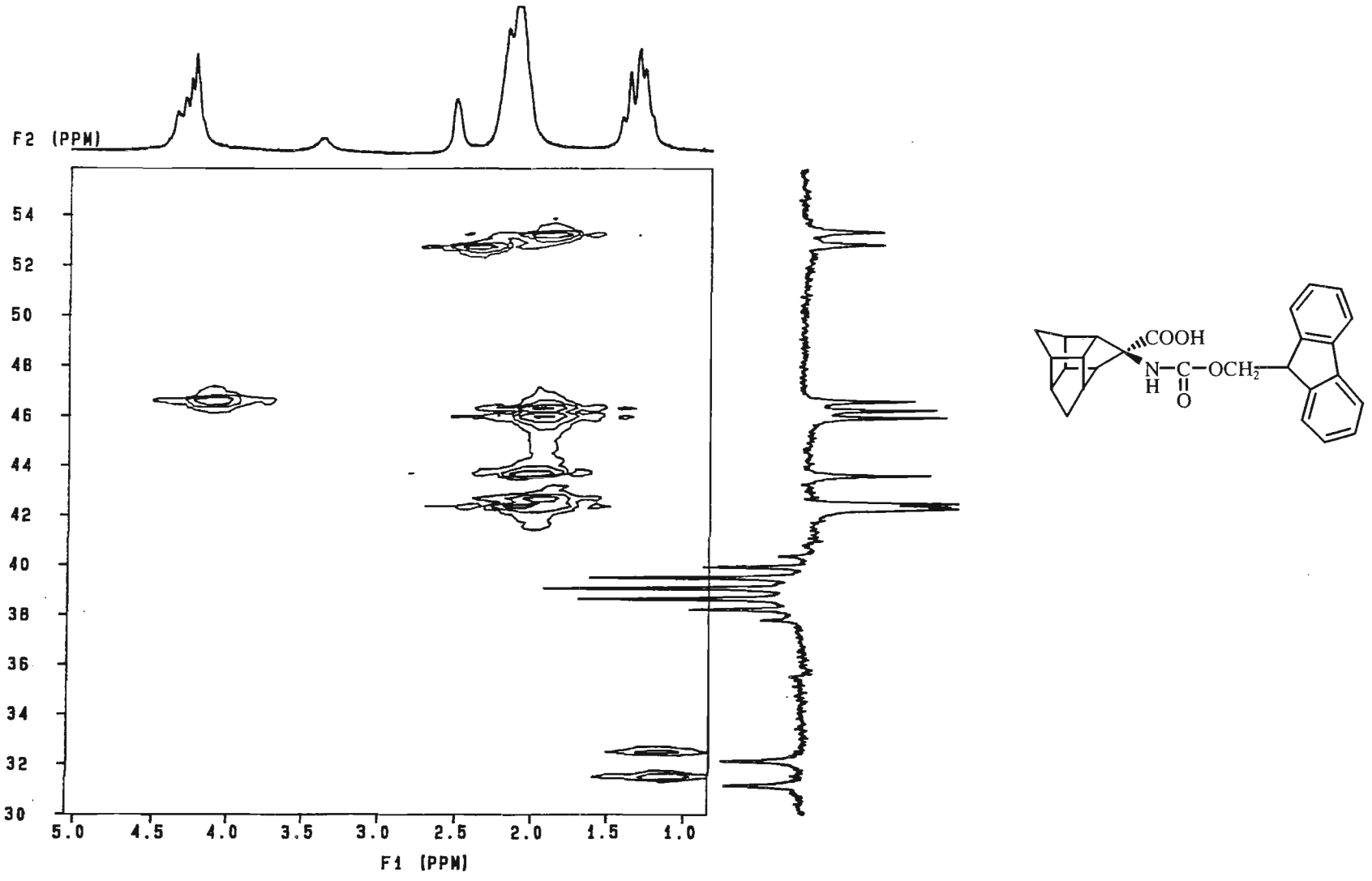
HETCOR [$(CD_3)_2SO$] of TRIS hydantoin (26)



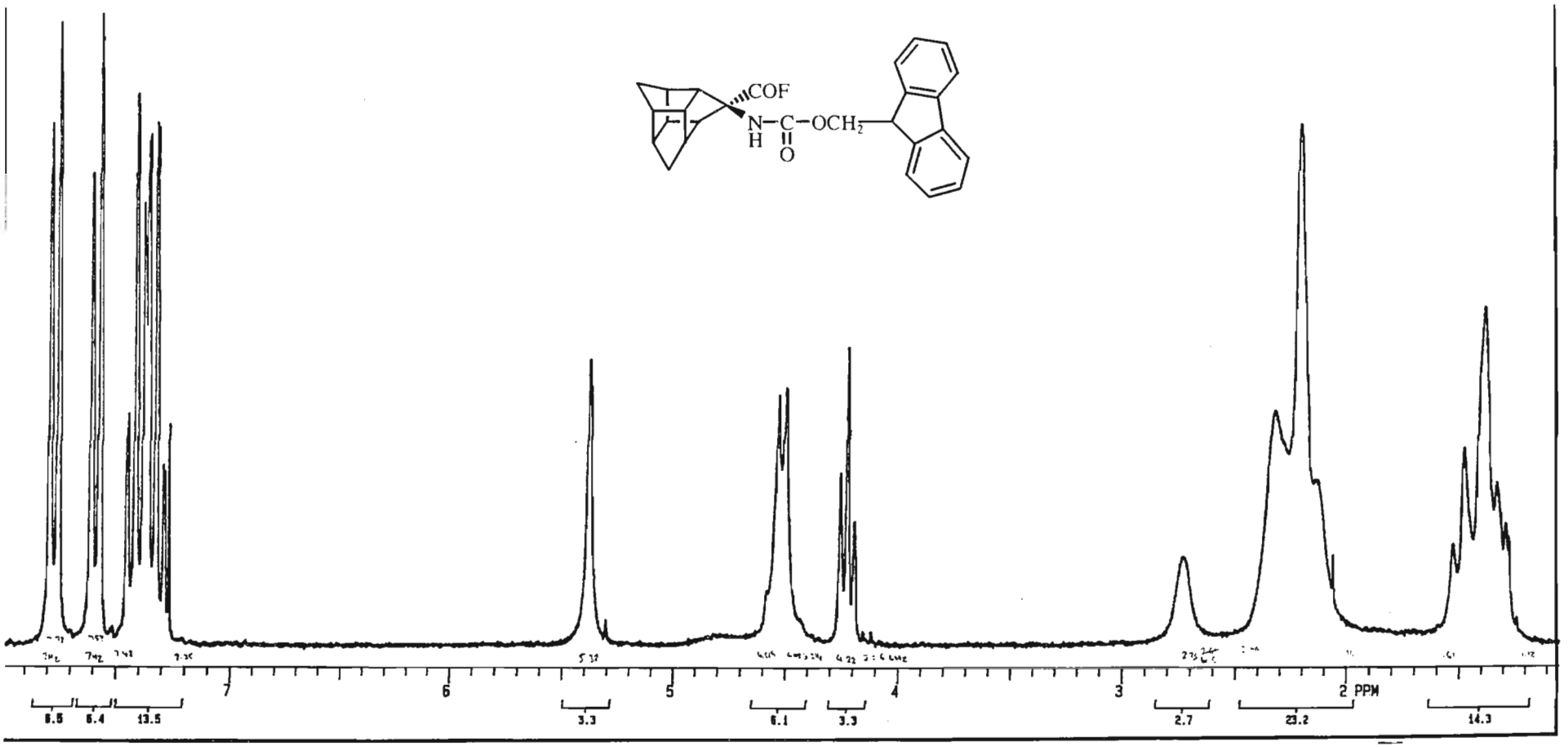
^{1}H nmr [$(CD_3)_2SO$] 200 MHz of Fmoc TRIS amino acid (27)



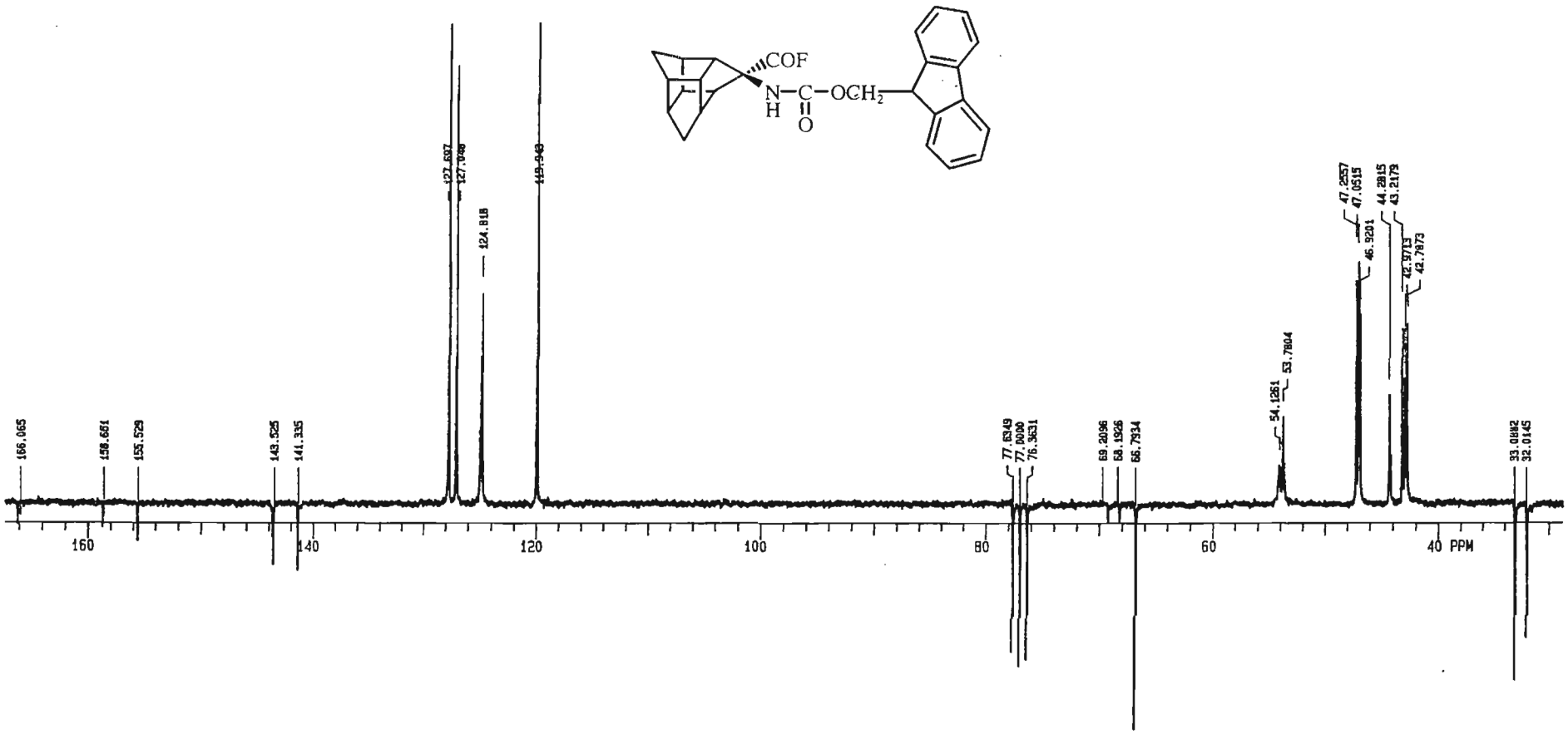
^{13}C nmr [$(CD_3)_2SO$] 50 MHz of Fmoc TRIS amino acid (27)



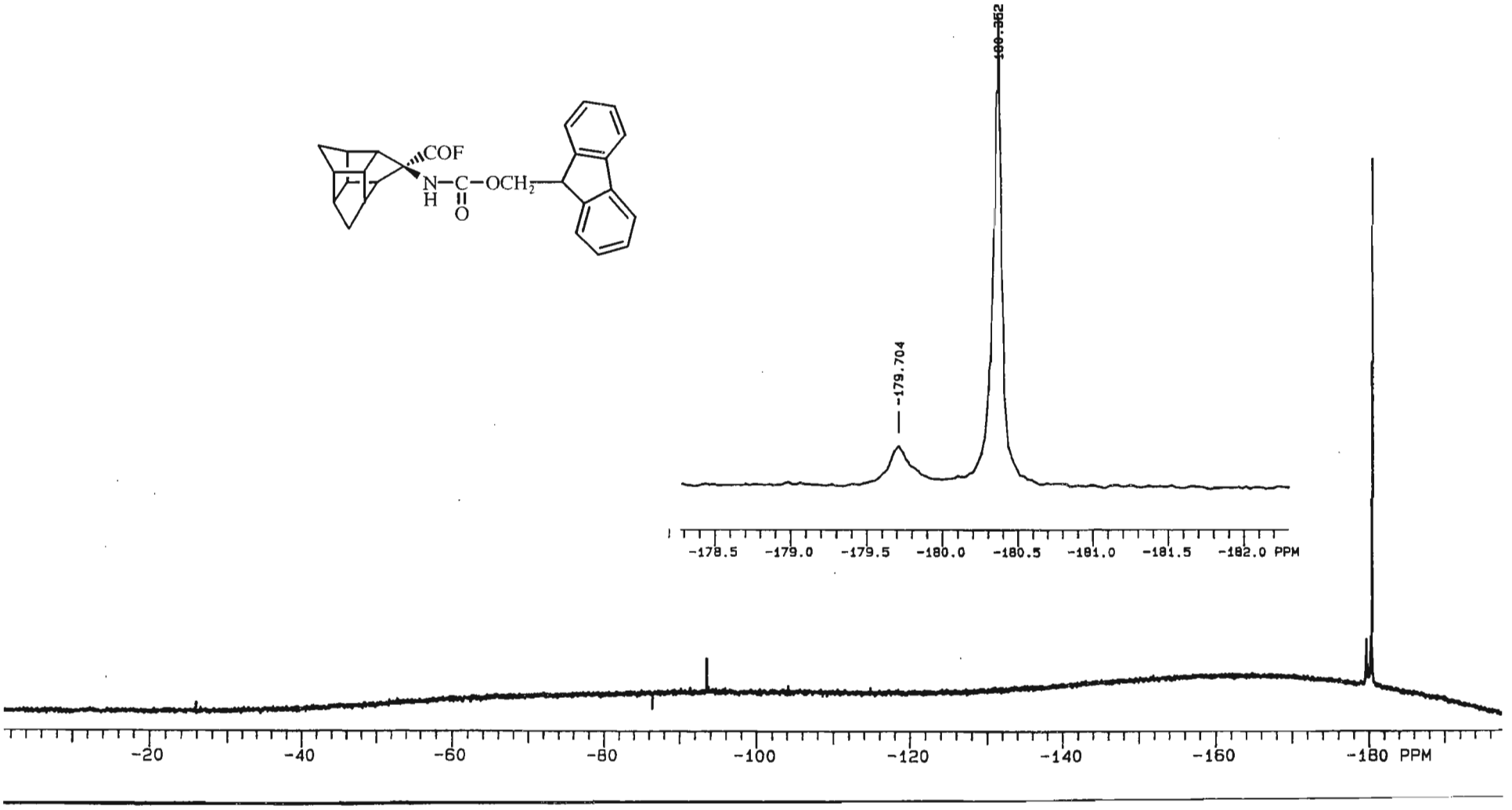
HETCOR [$(CD_3)_2SO$] of Fmoc TRIS amino acid (27)



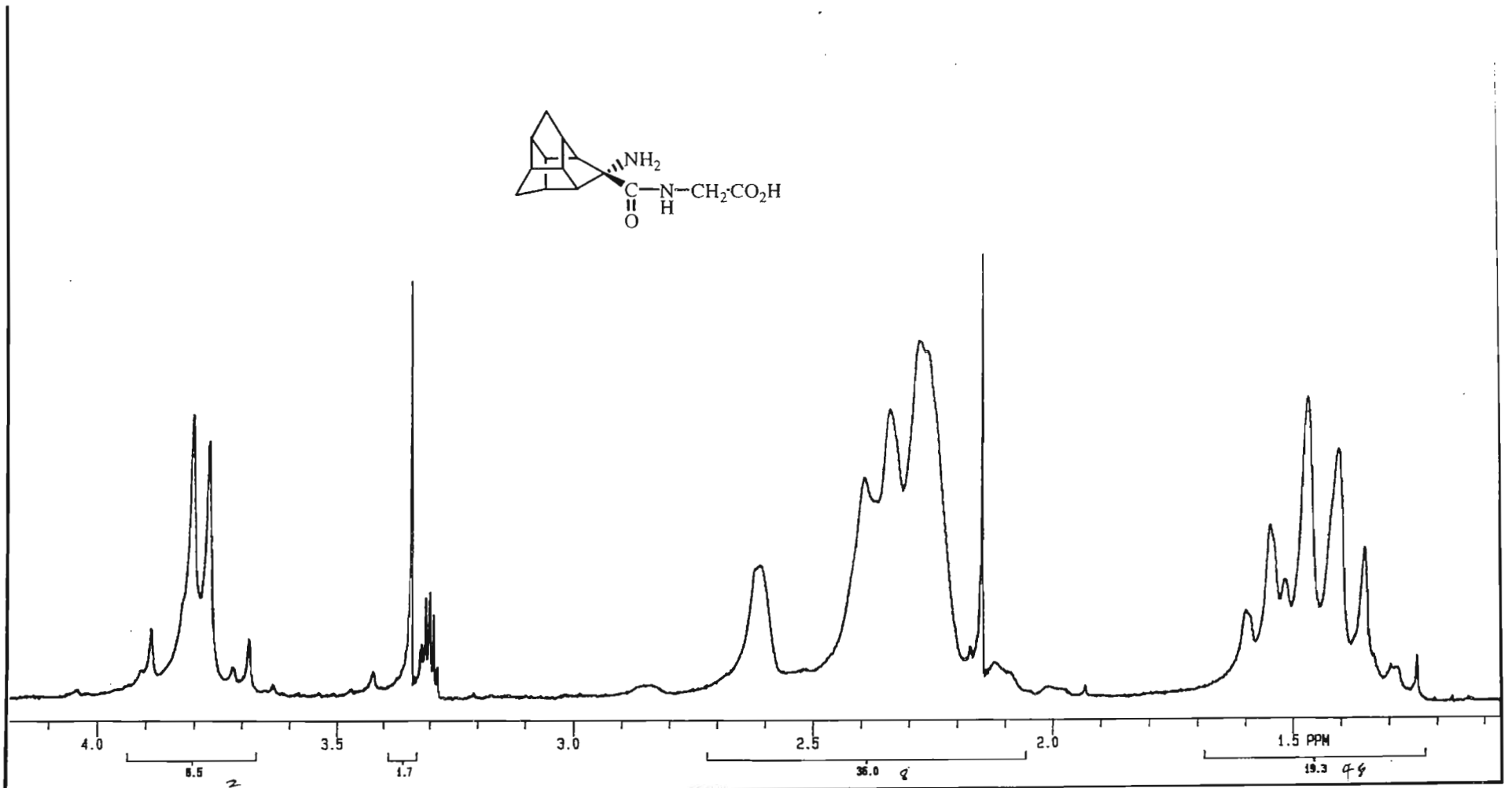
^{1}H nmr $CDCl_3$ 200 MHz of Fmoc TRIS amino acid fluoride (40)



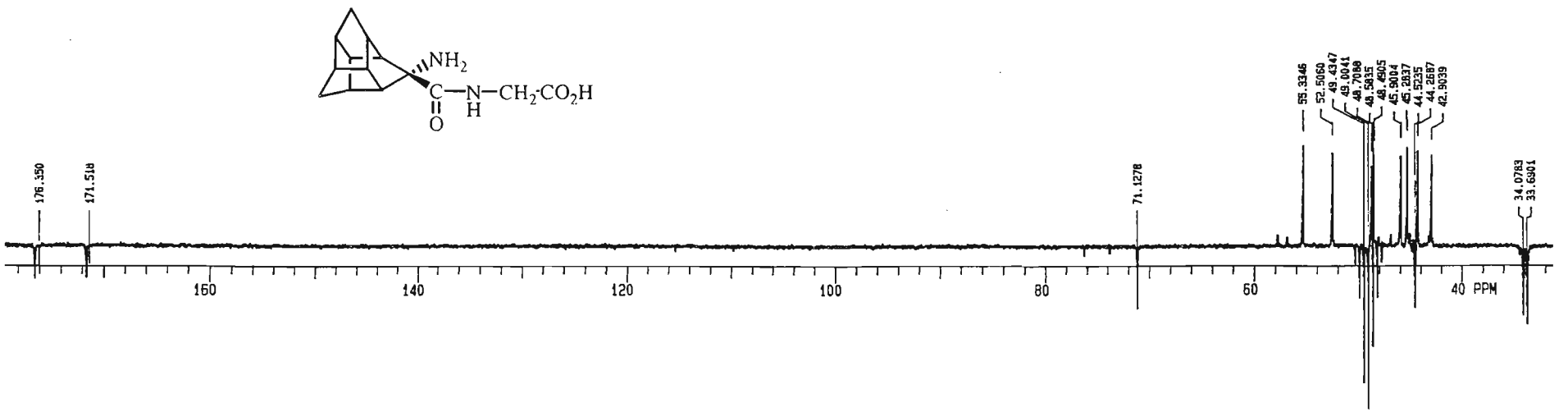
^{13}C nmr $CDCl_3$ 50 MHz of Fmoc TRIS amino acid fluoride (40)



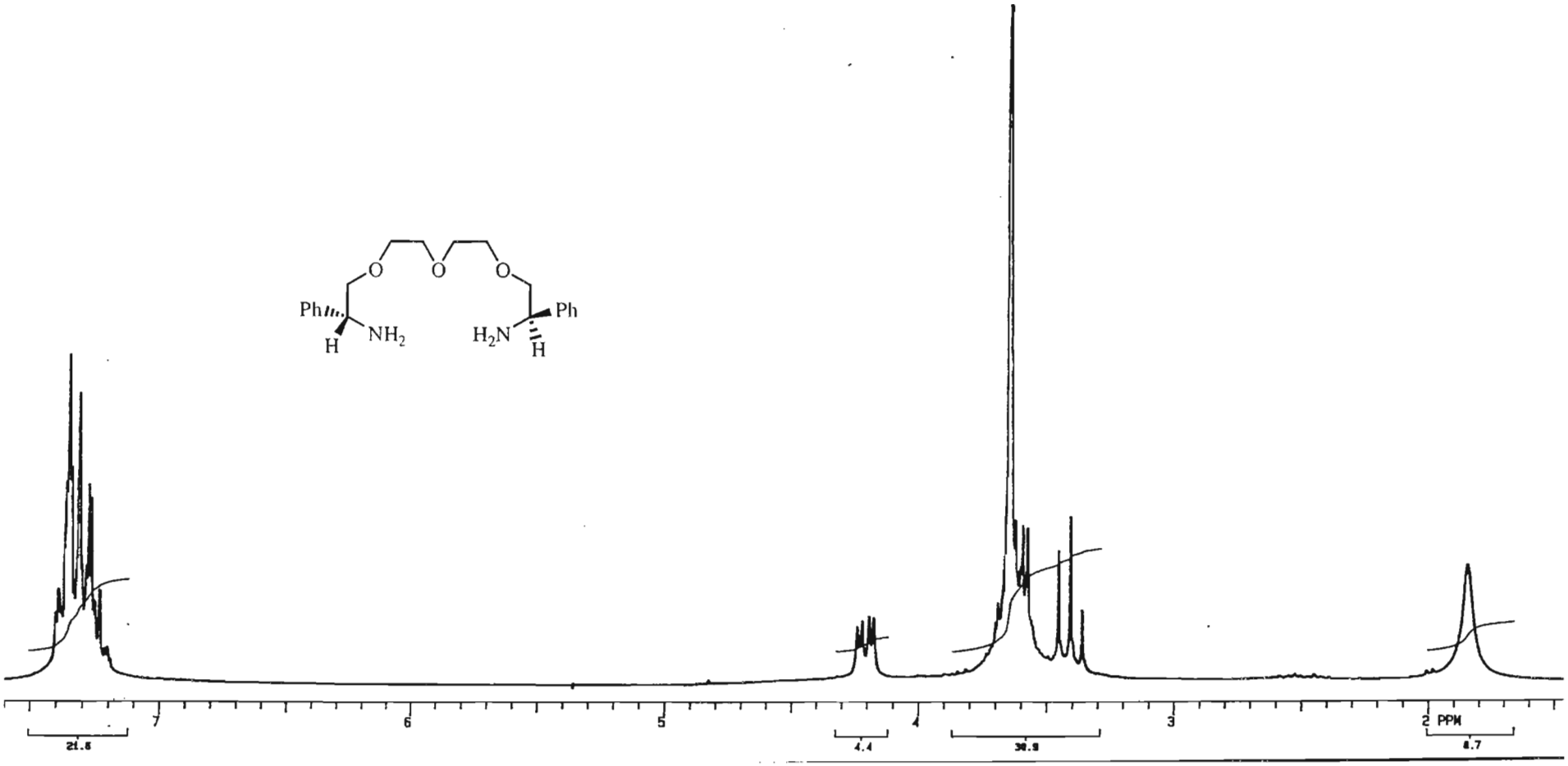
^{19}F nmr $CDCl_3$ 280 MHz of Fmoc TRIS amino acid fluoride (40)



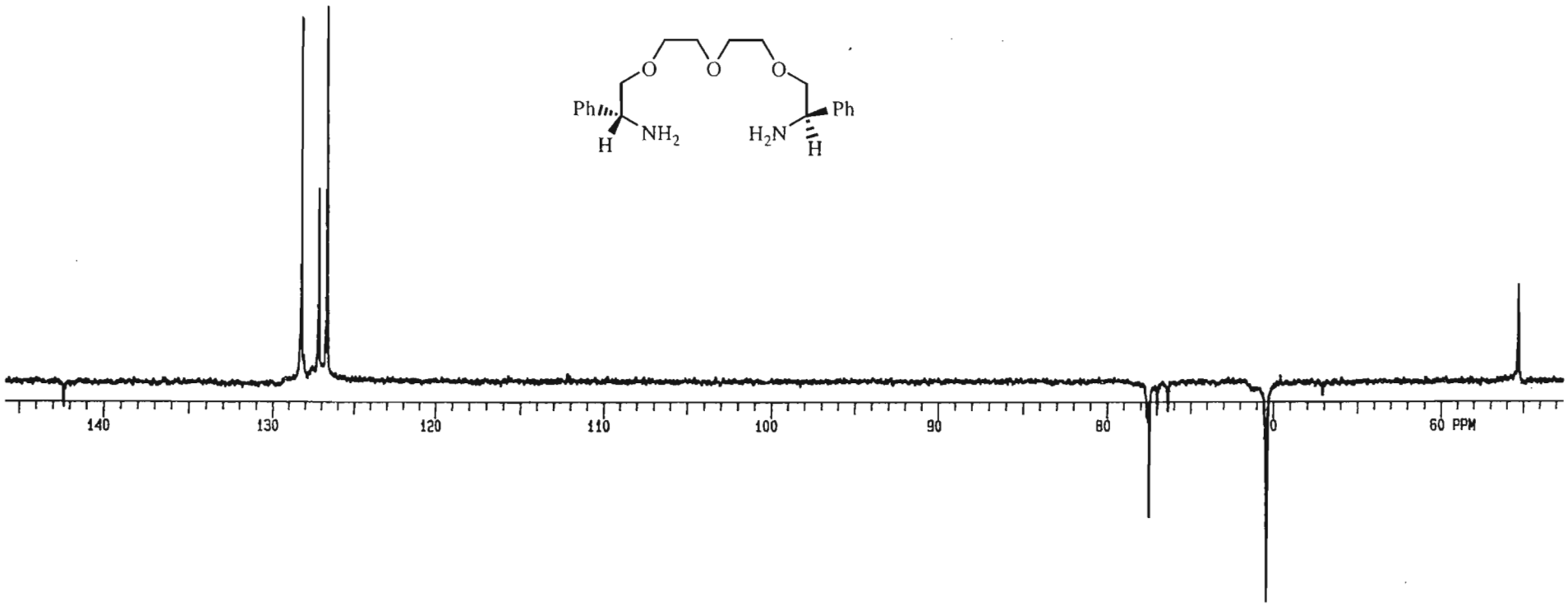
^{1}H nmr [$(CD_3)_2SO$] 200 Mhz of TRIS-Gly dipeptide (43)



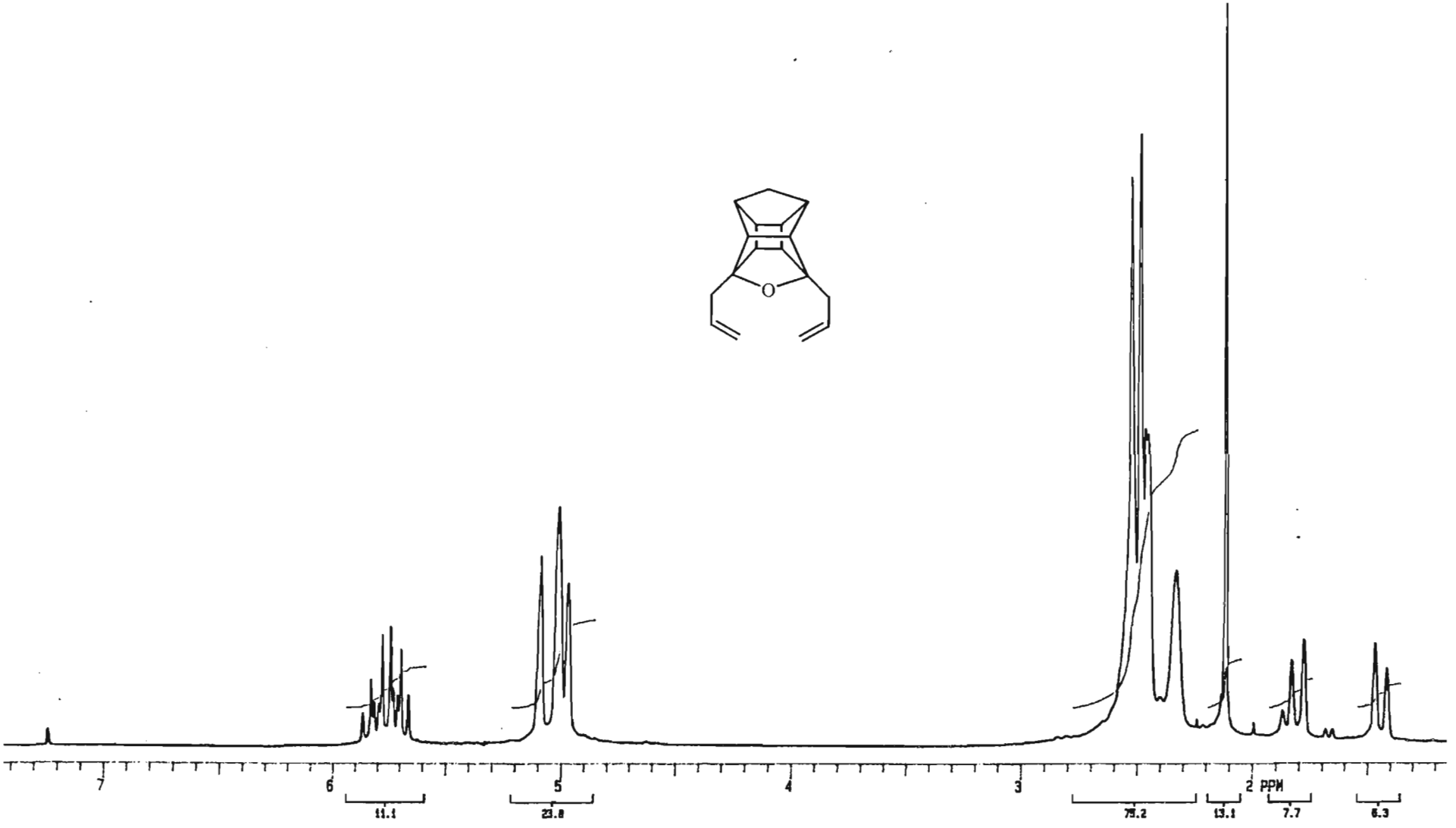
^{13}C nmr [$(CD_3)_2SO$] 50 MHz of TRIS-Gly dipeptide (43)



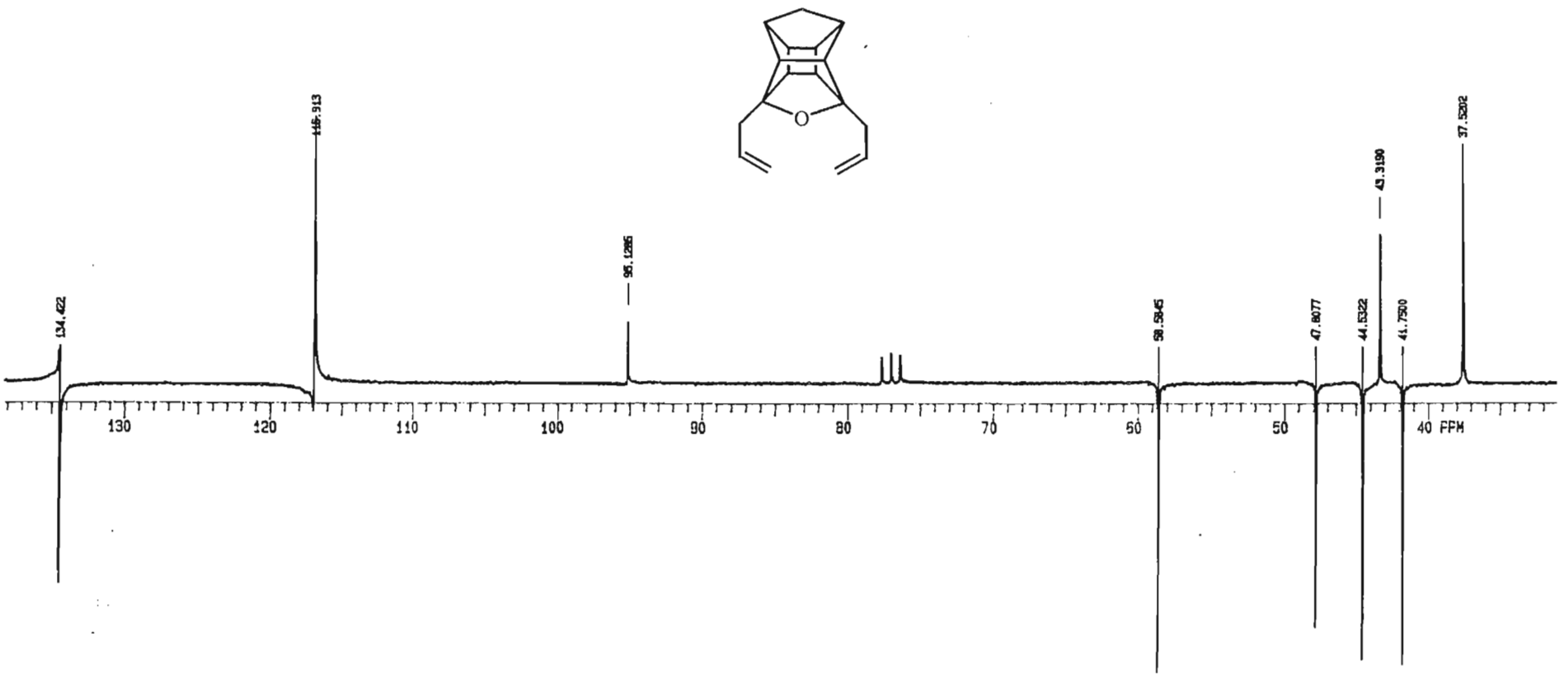
^{1}H nmr $CDCl_3$ 200 MHz of (R,R)-1,11-Diamino-1,11-diphenyl-3,6,9-trioxaundecane (63)



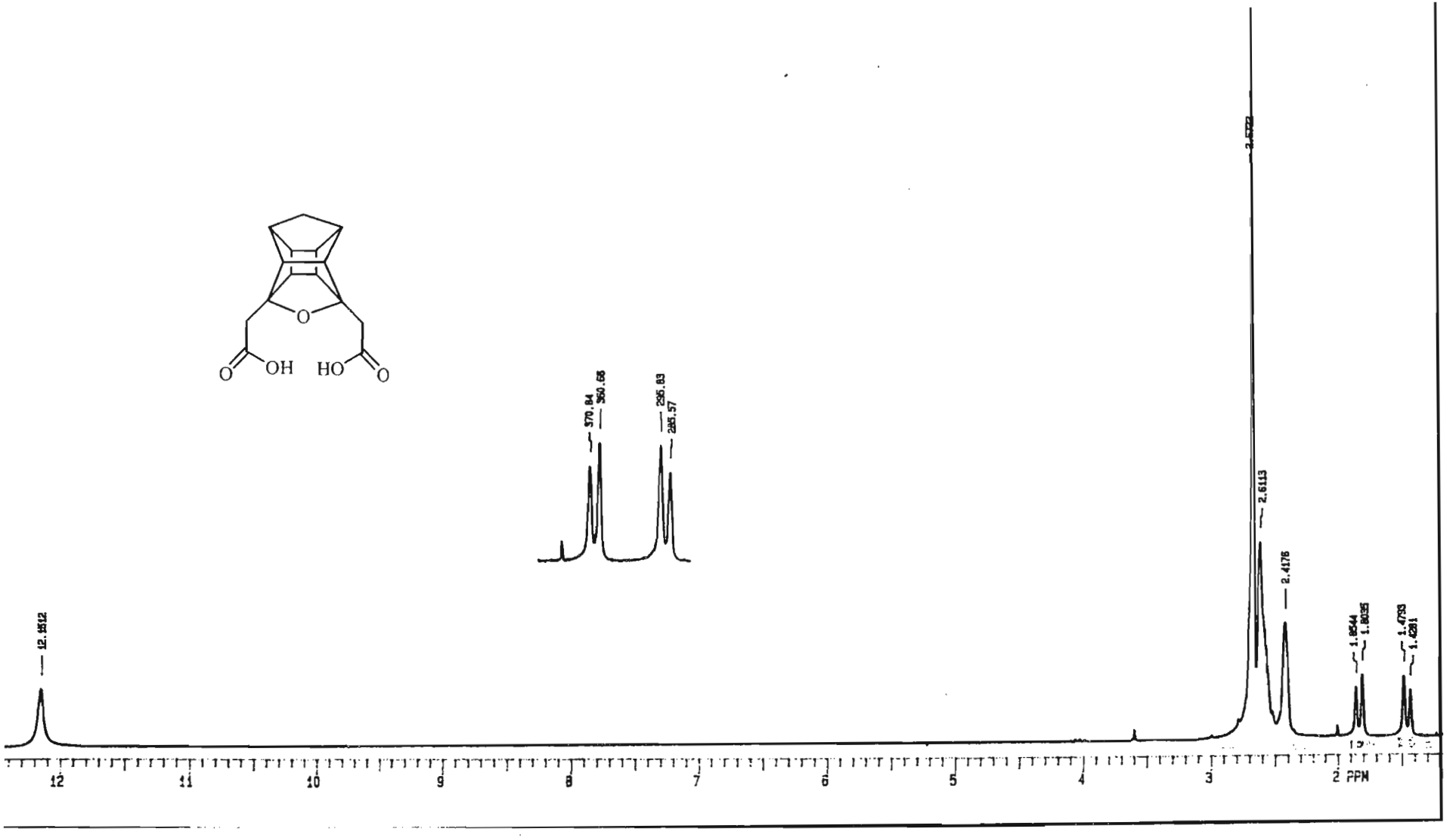
^{13}C nmr $CDCl_3$ 50 MHz of (R,R)-1,11-Diamino-1,11-diphenyl-3,6,9-trioxaundecane (63)



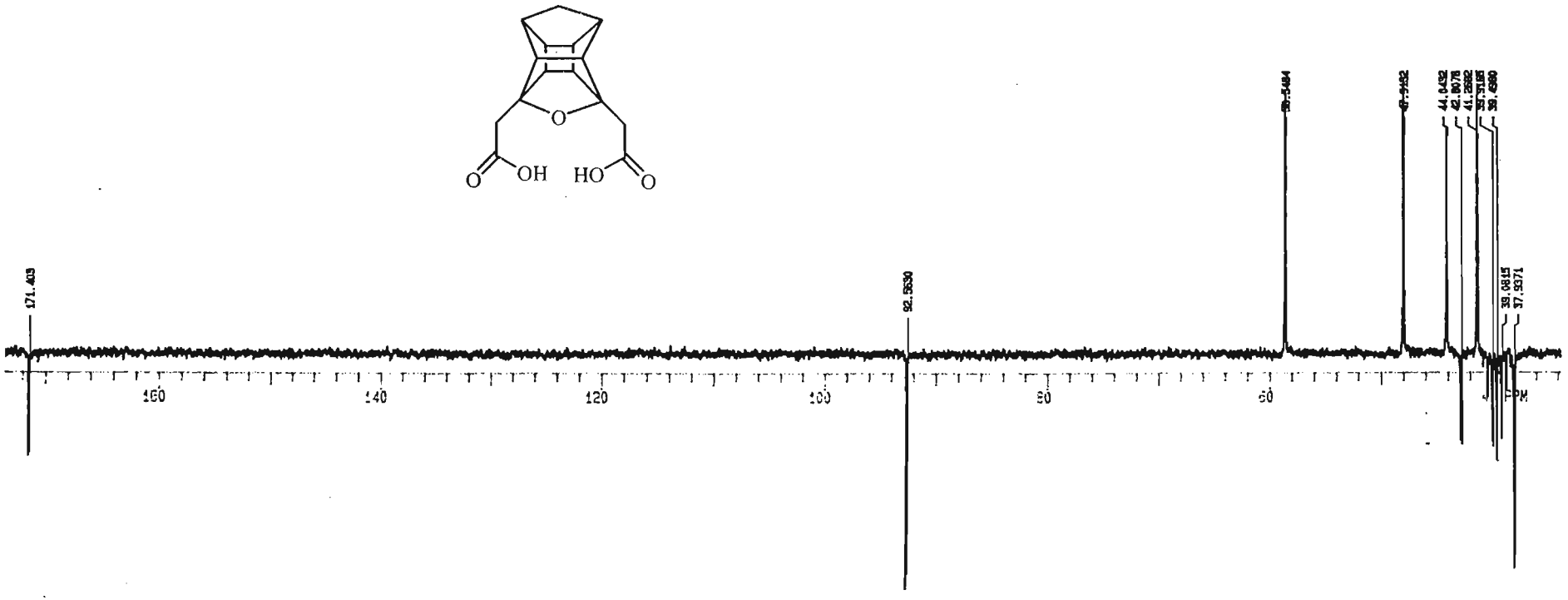
^{1}H nmr $CDCl_3$ 200 MHz of 3,5-Diallyl-4-oxahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$] dodecane (52)



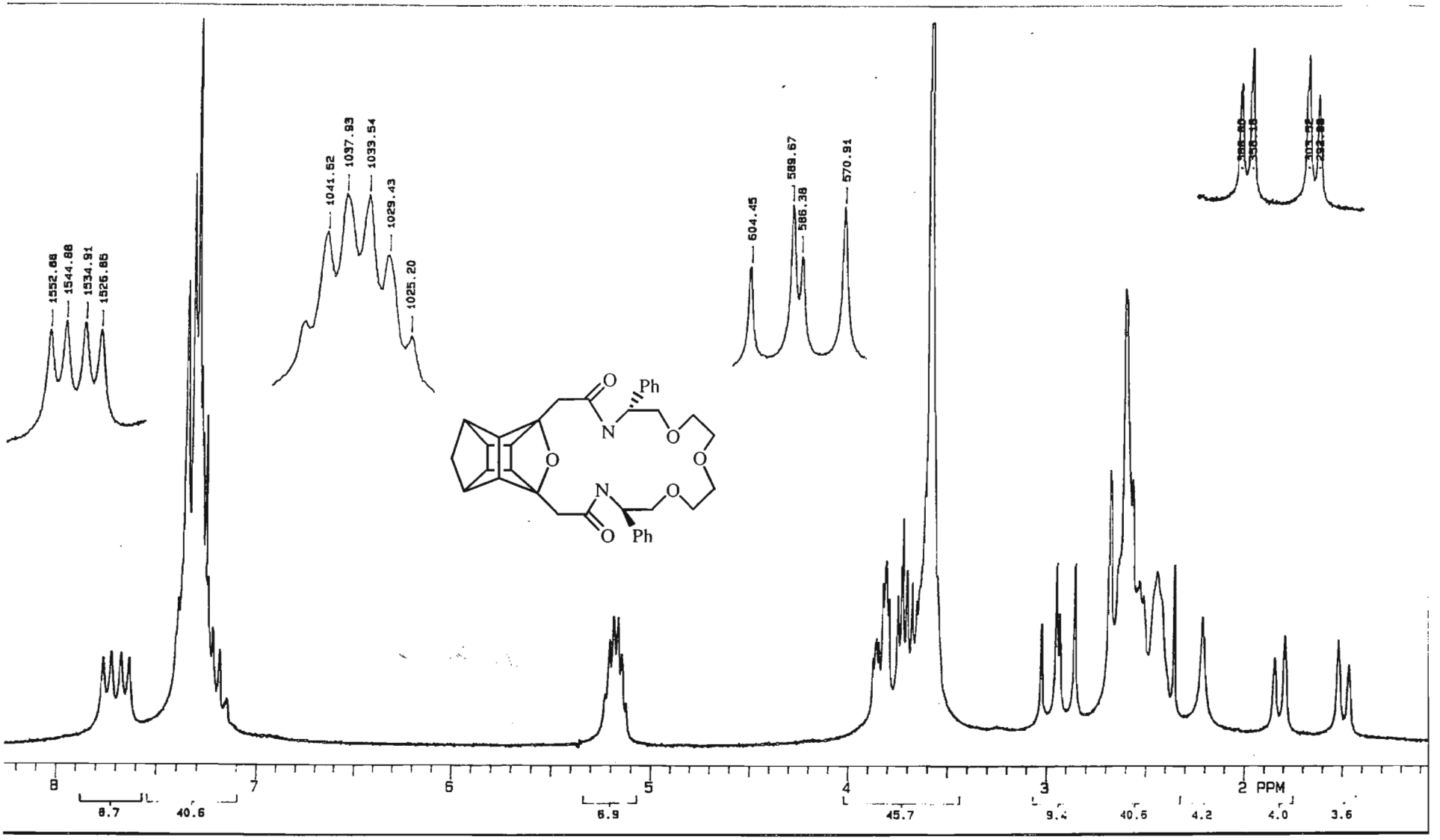
^{13}C nmr $CDCl_3$ 50 MHz of 3,5-Diallyl-4-oxahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$] dodecane (52)



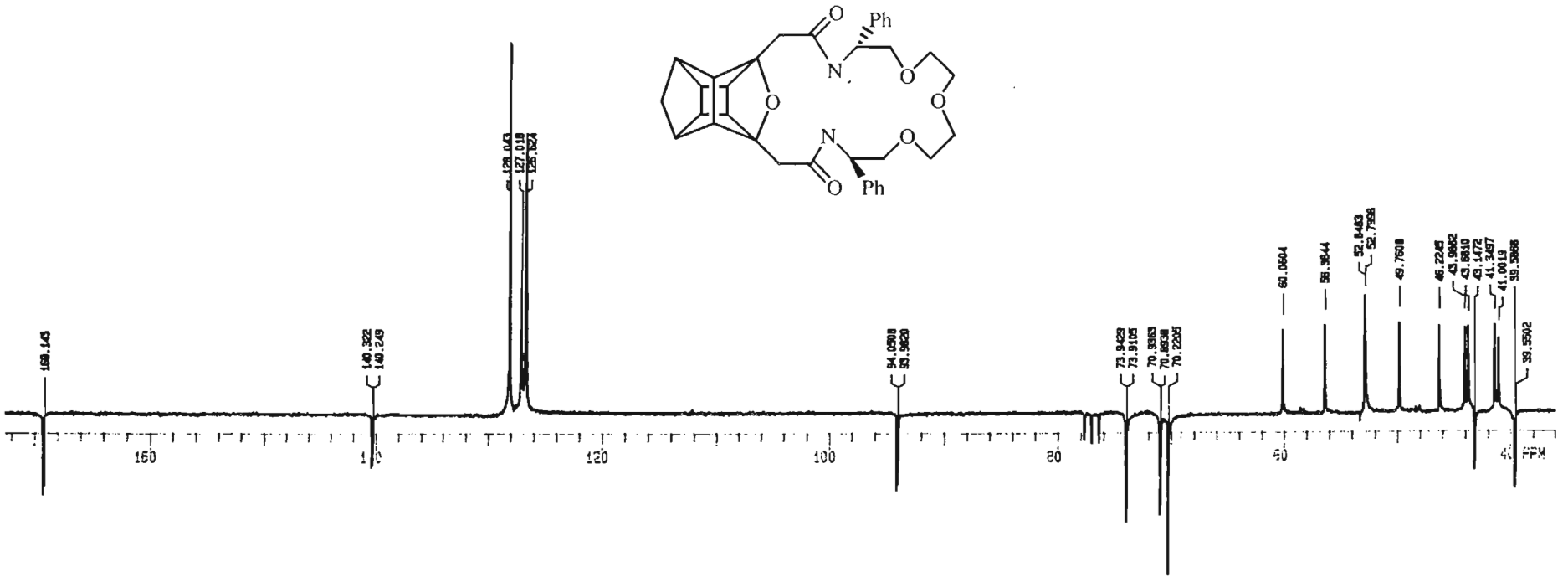
^{1}H nmr [$(CD_3)_2SO$] 200 MHz of 3,5-dicarboxymethyl-4-oxahexacyclo[5.4.1.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]-dodecane (59)



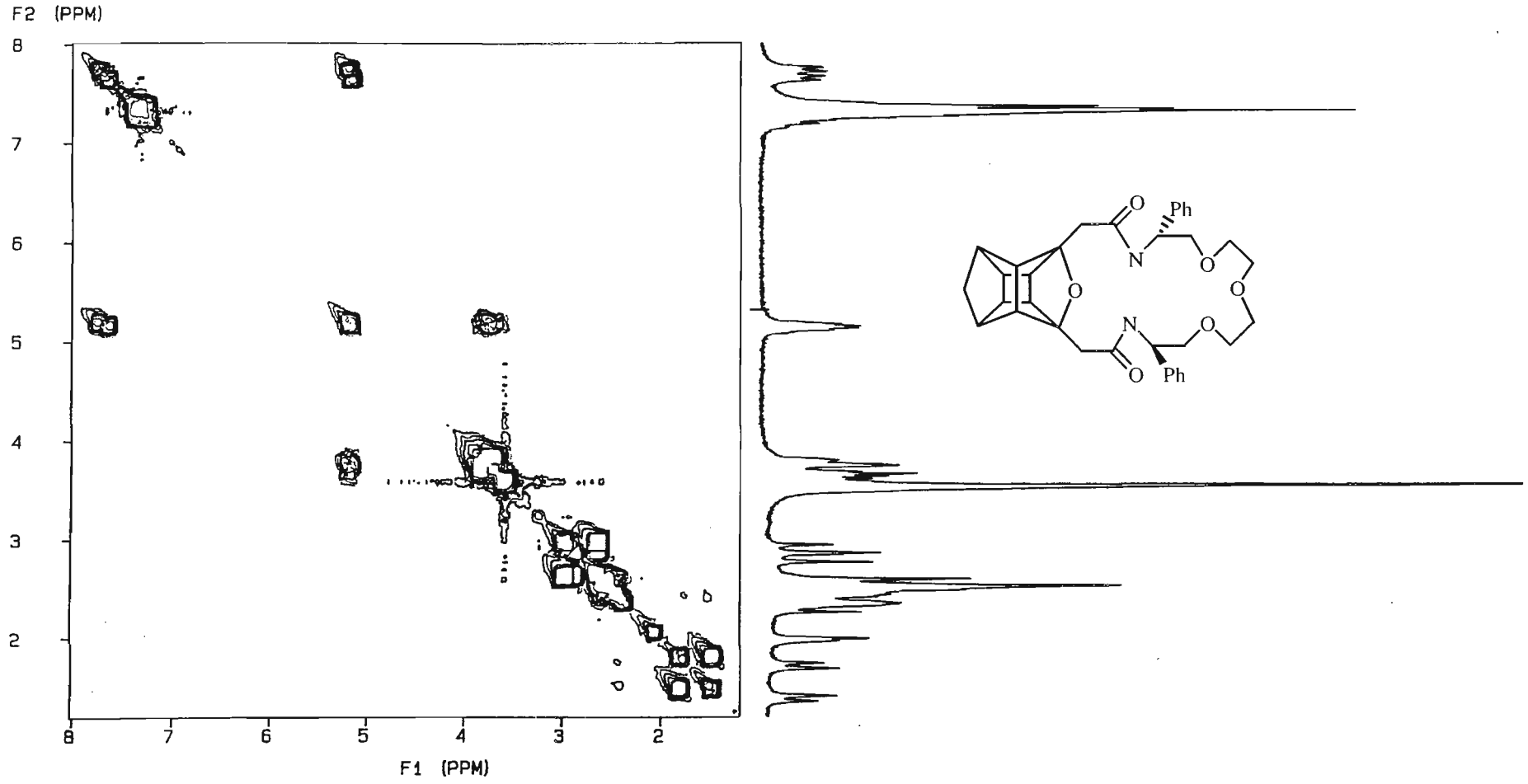
^{13}C nmr [$(CD_3)_2SO$] 50 MHz of 3,5-dicarboxymethyl-4-oxahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]-dodecane (59)



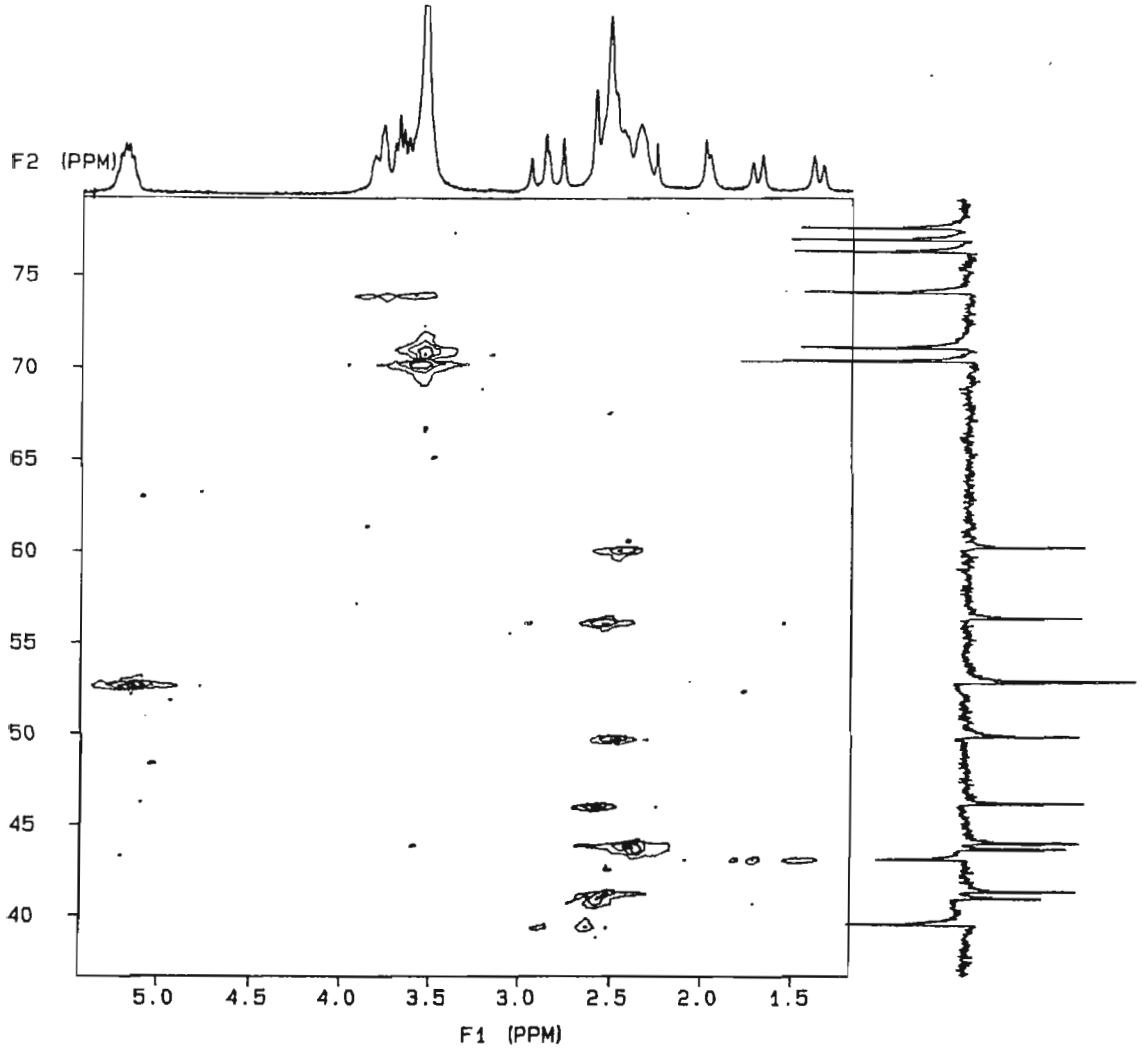
^{1}H nmr $CDCl_3$ 200 MHz of macrocycle 57

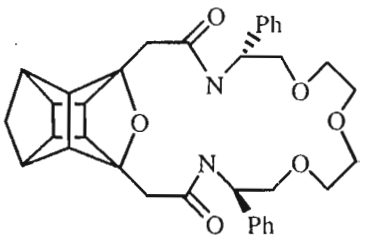


^{13}C nmr $CDCl_3$ 50 MHz of macrocycle 57

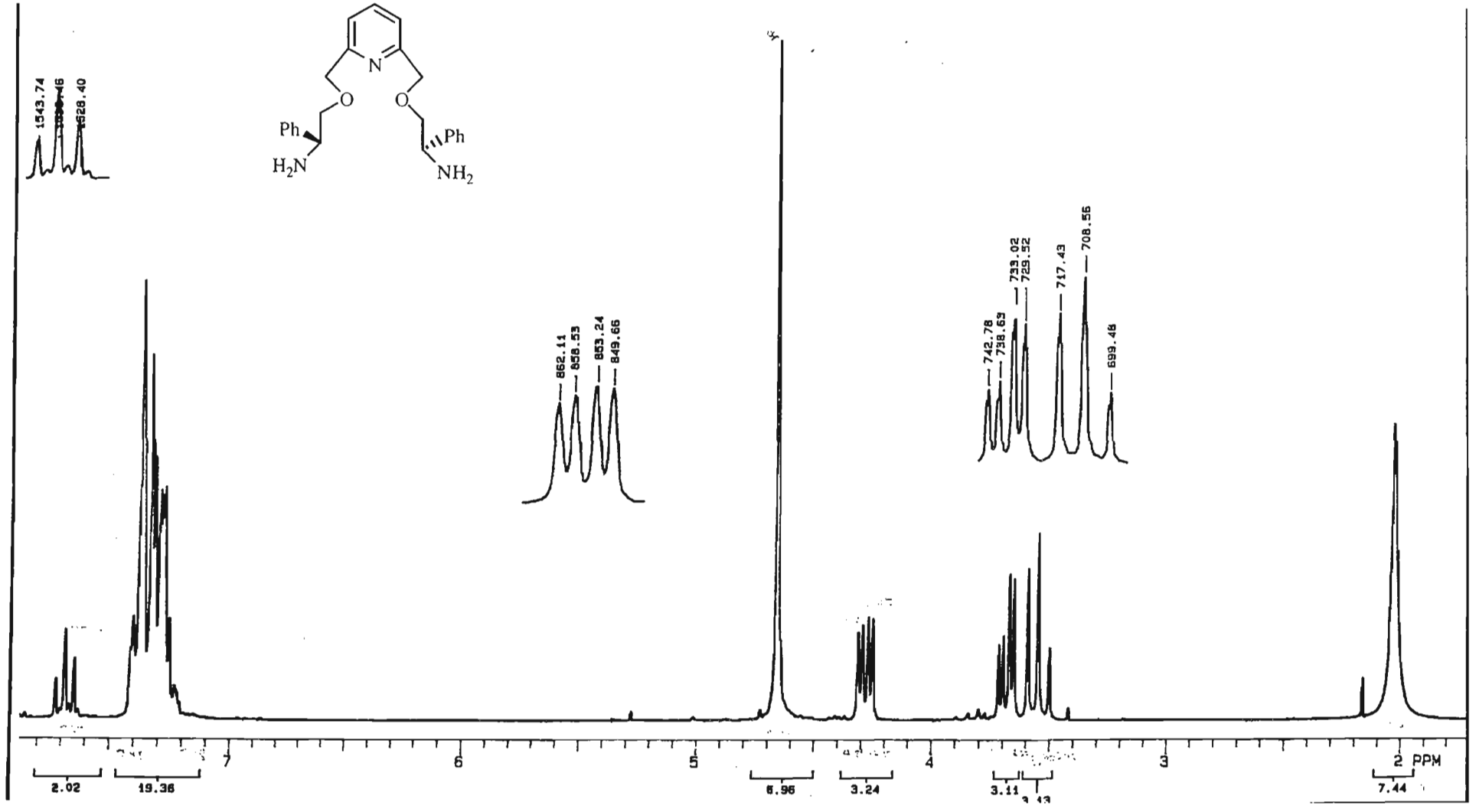


COSY $CDCl_3$ of macrocycle 57

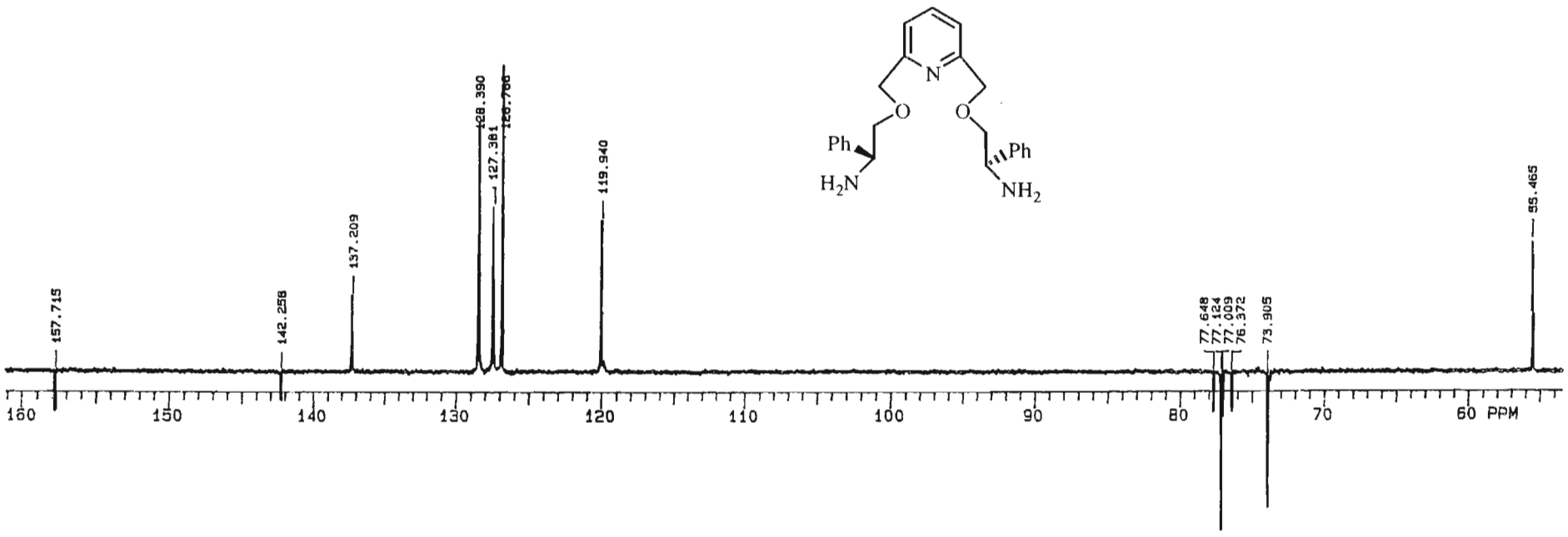




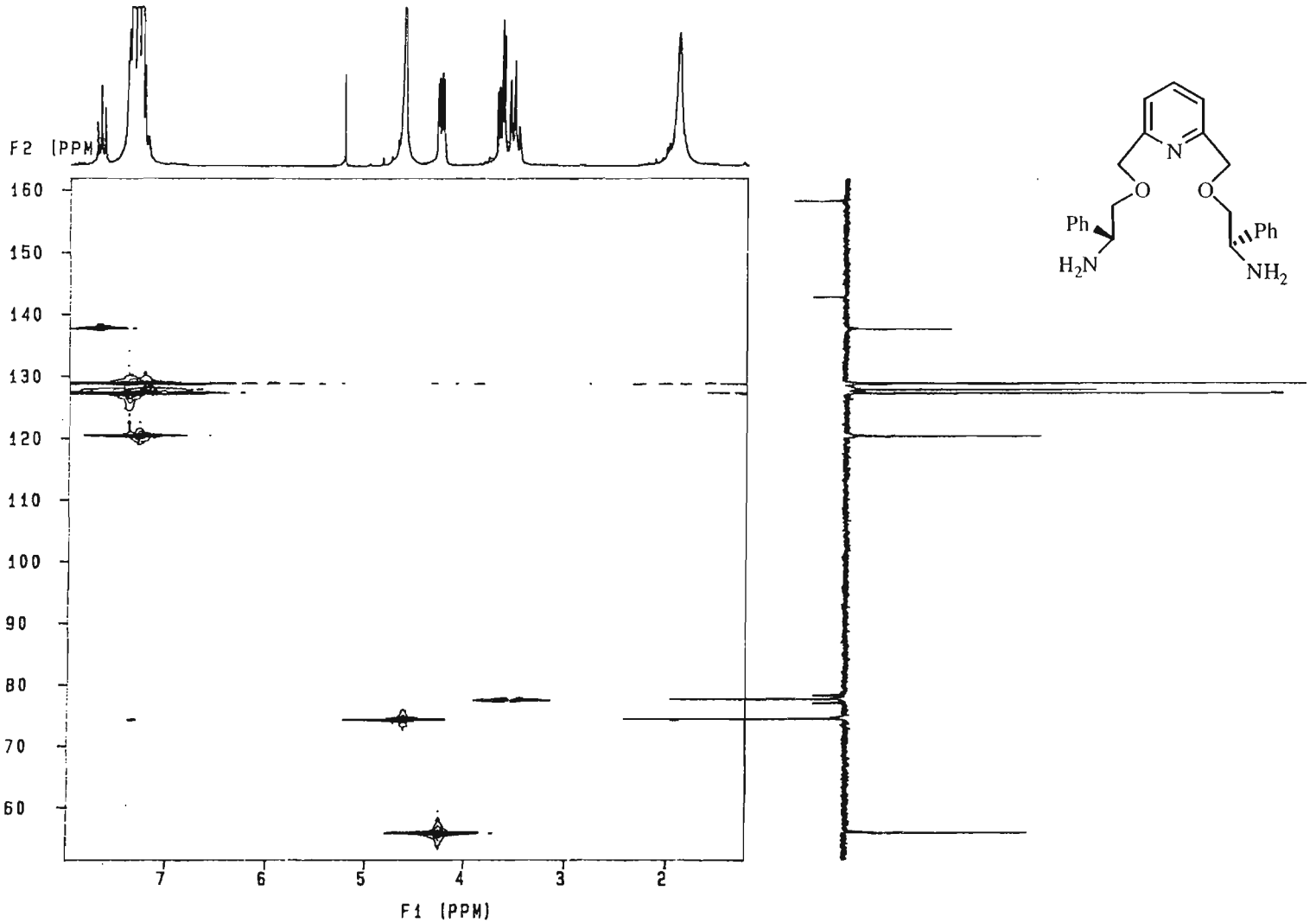
HETCOR $CDCl_3$ of macrocycle 57



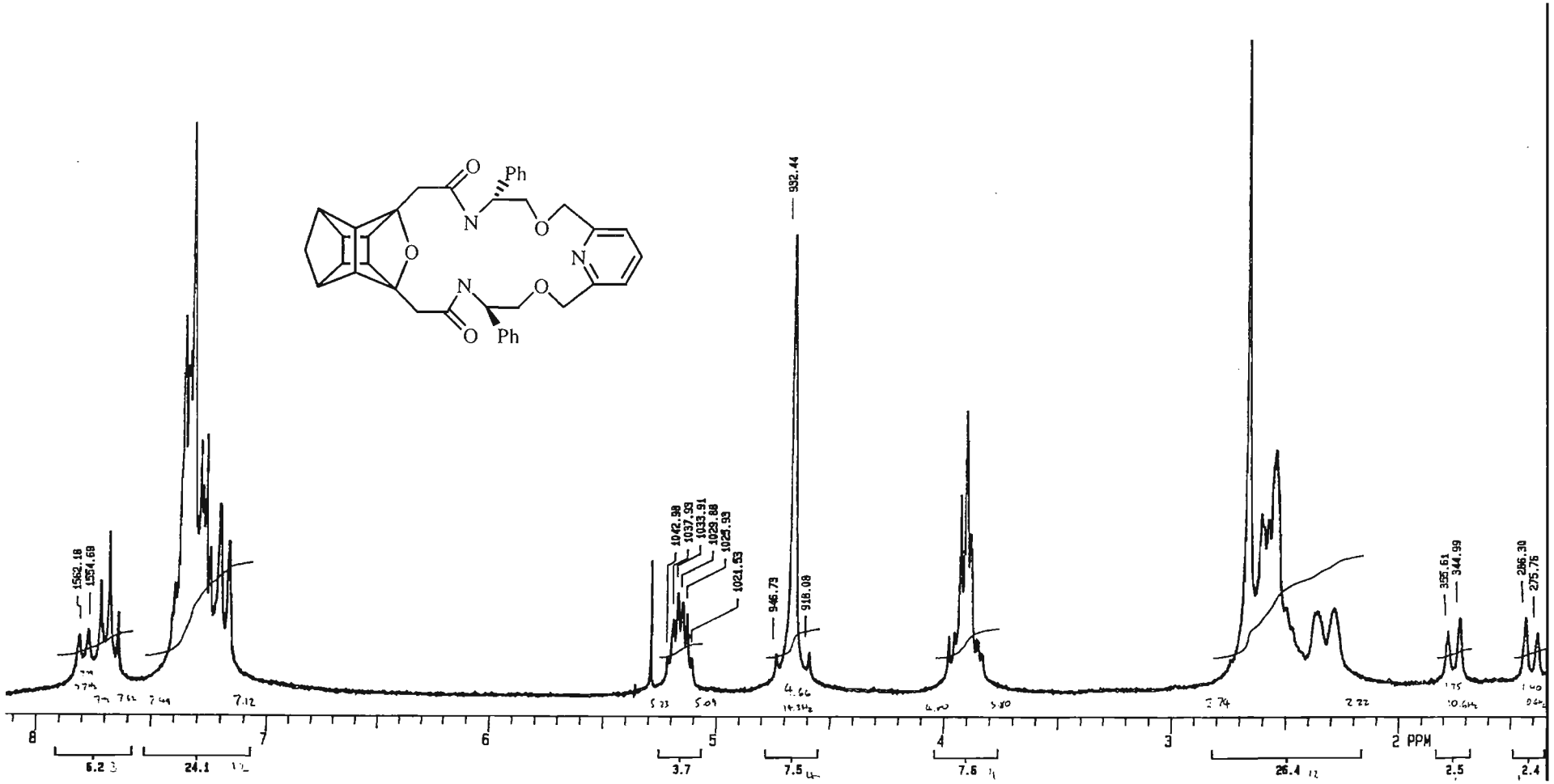
^{1}H nmr $CDCl_3$ 200 MHz of (S)-phenylglycine pyridine diamine (67)



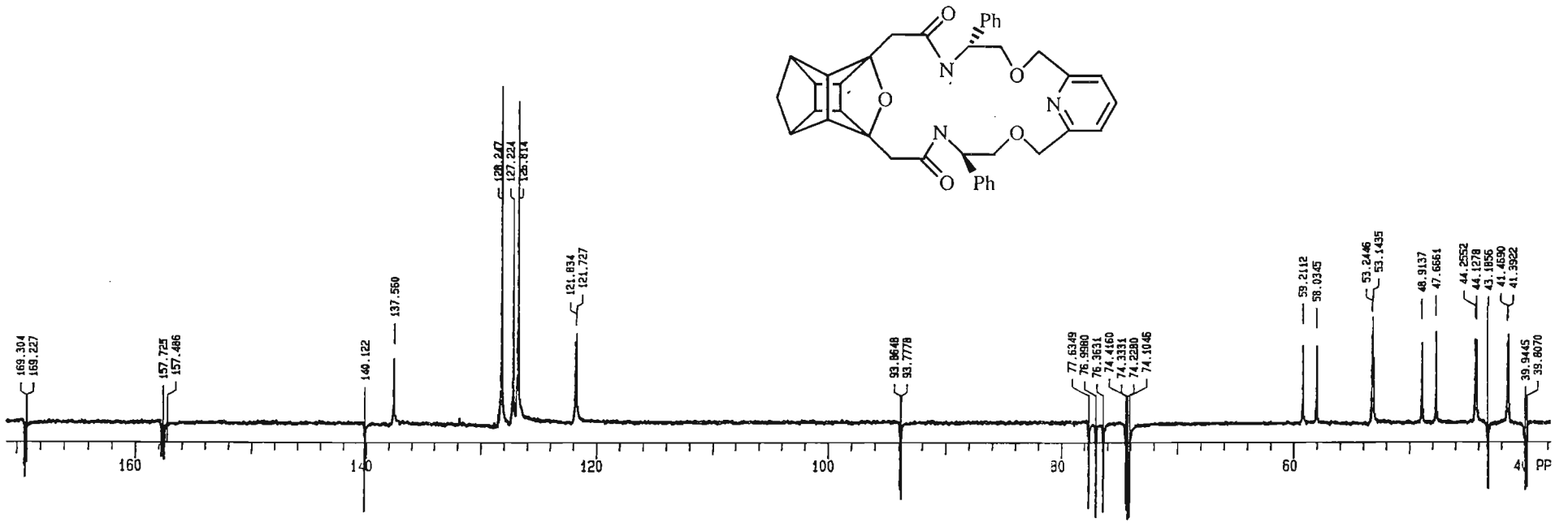
^{13}C nmr $CDCl_3$ 50 MHz of (S)-phenylglycine pyridine diamine (67)



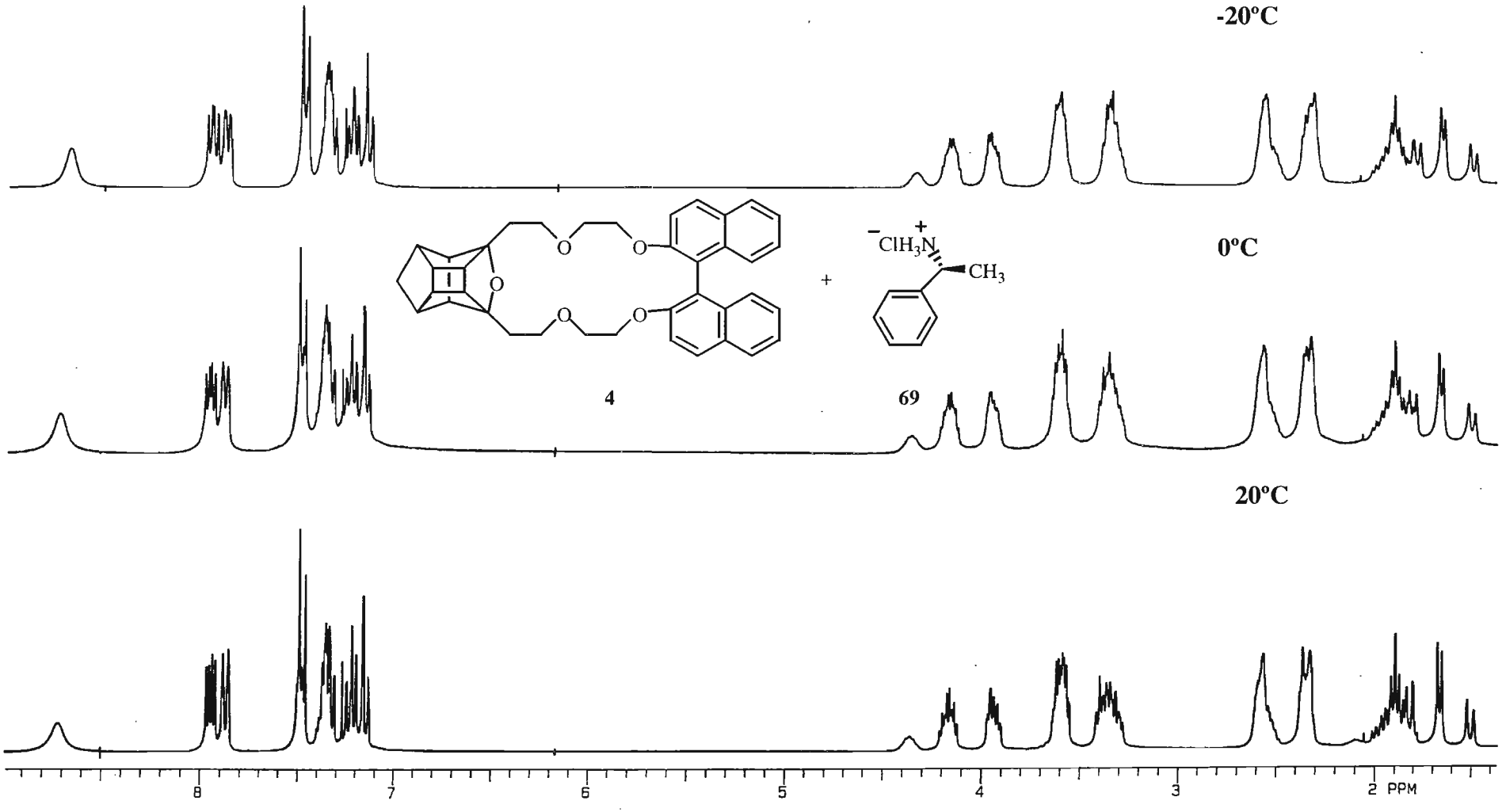
HETCOR $CDCl_3$ of (S)-phenylglycine pyridine diamine (67)



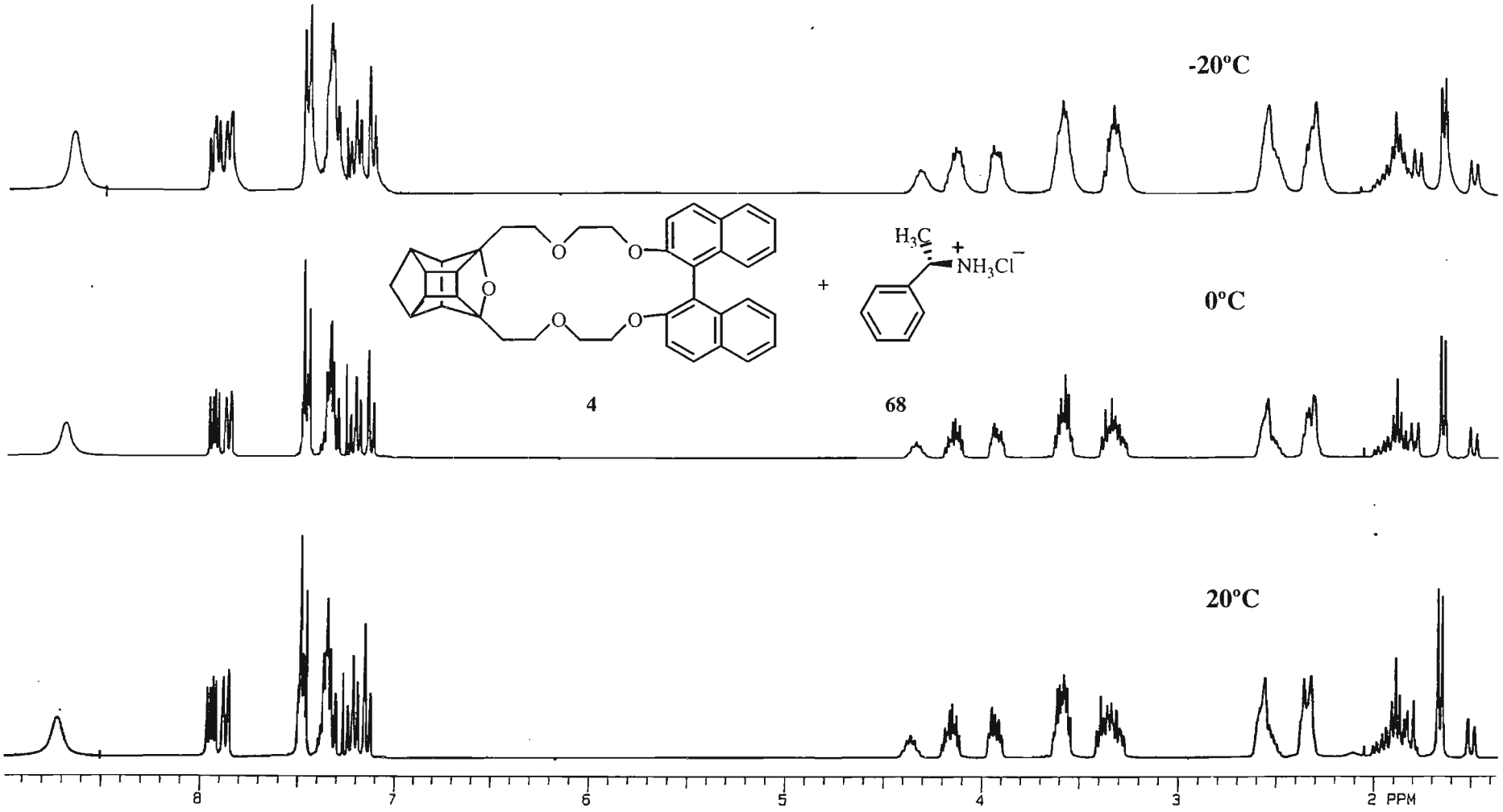
^{1}H nmr $CDCl_3$ 200 MHz of macrocycle 66



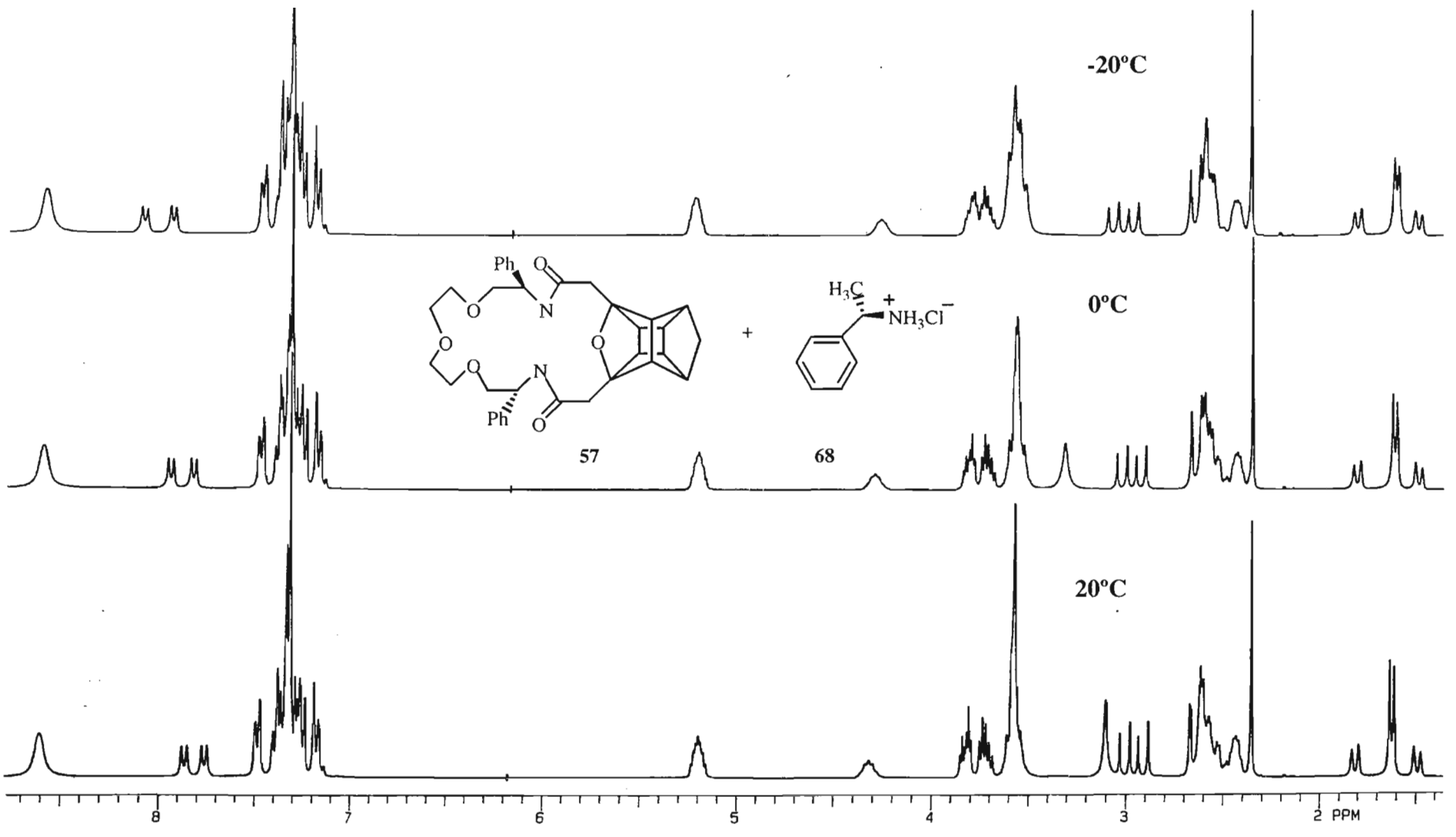
^{13}C nmr $CDCl_3$ 50 MHz of macrocycle 66



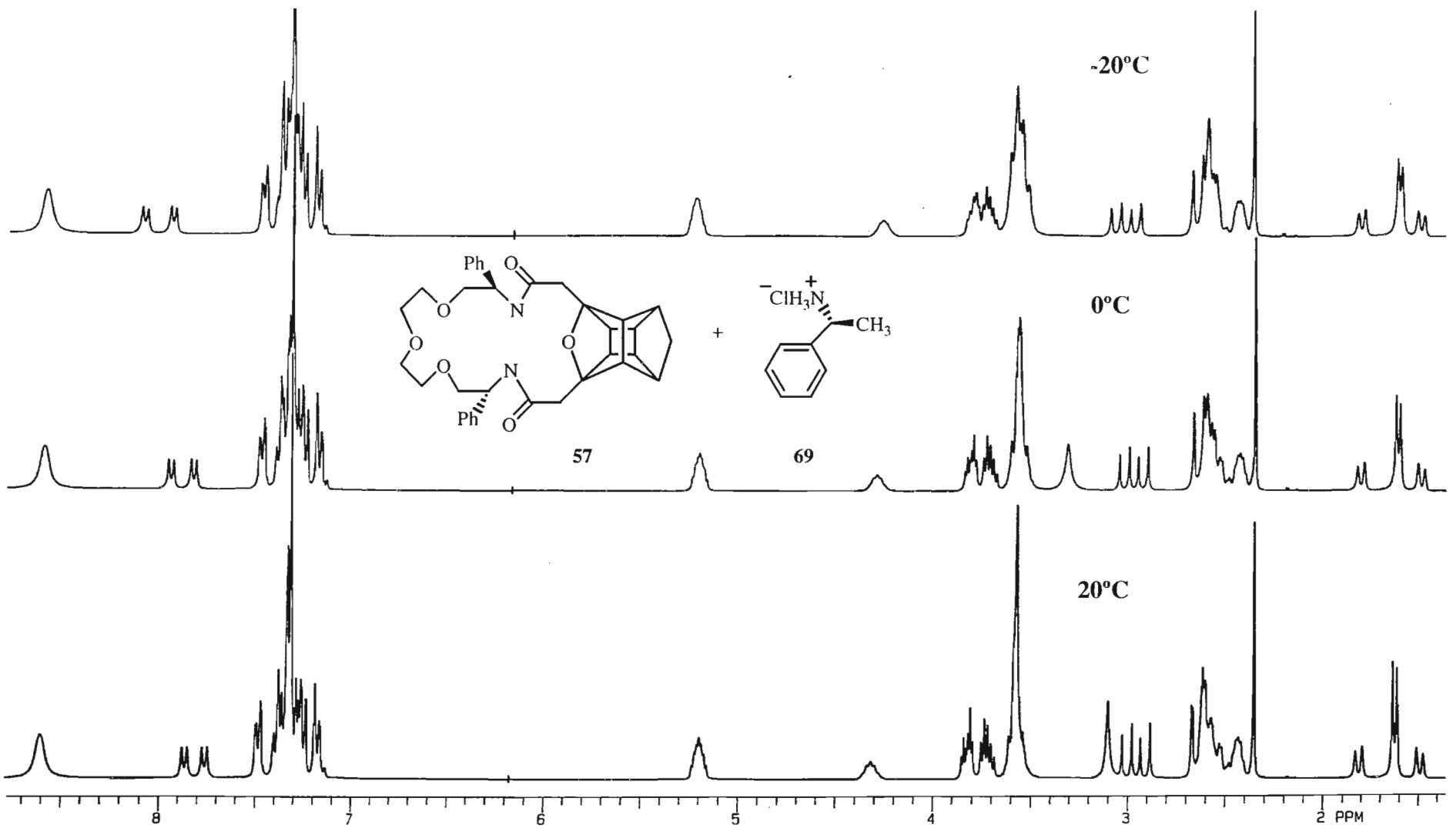
^{1}H nmr $CDCl_3$ 200 MHz



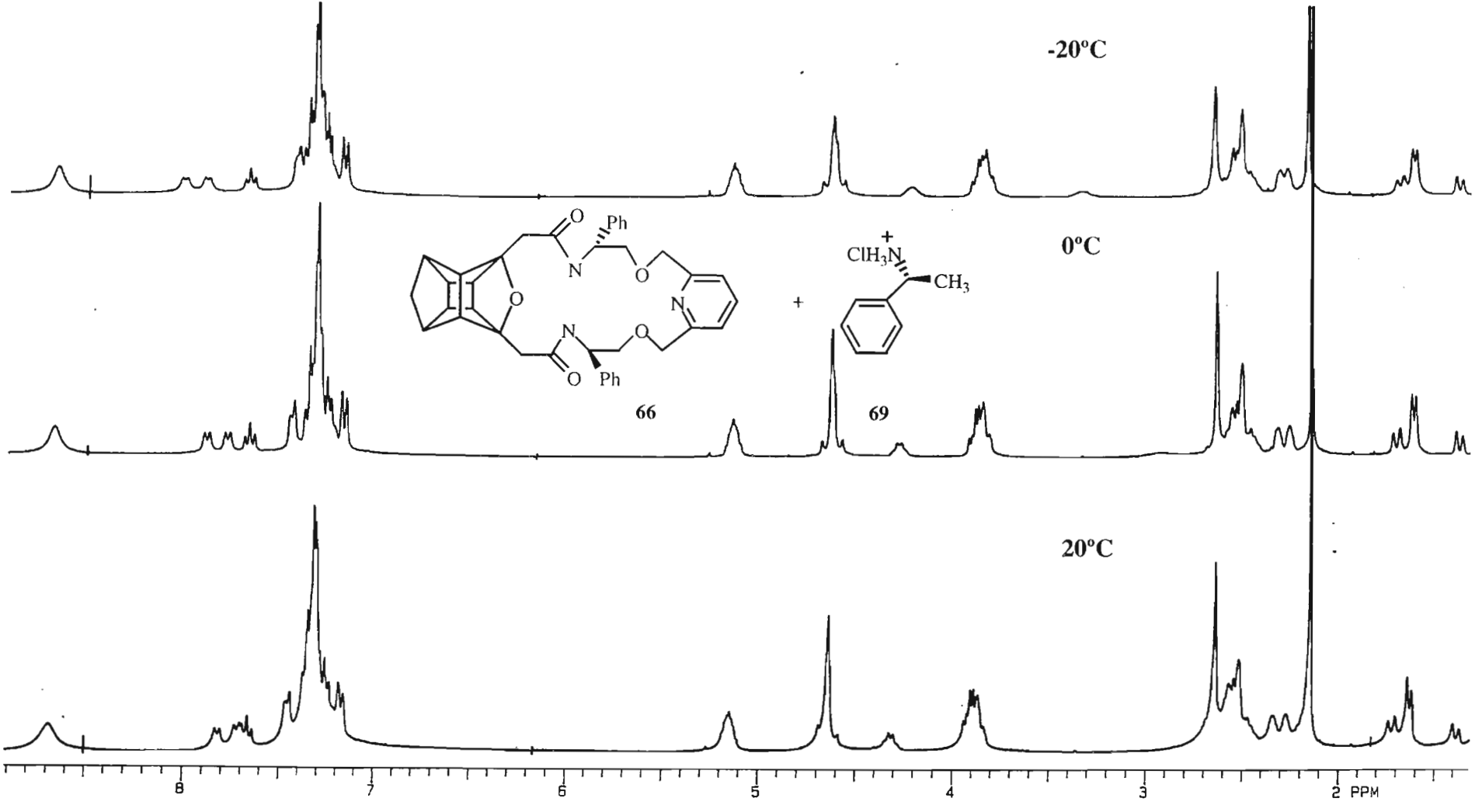
^{1}H nmr $CDCl_3$ 300 MHz



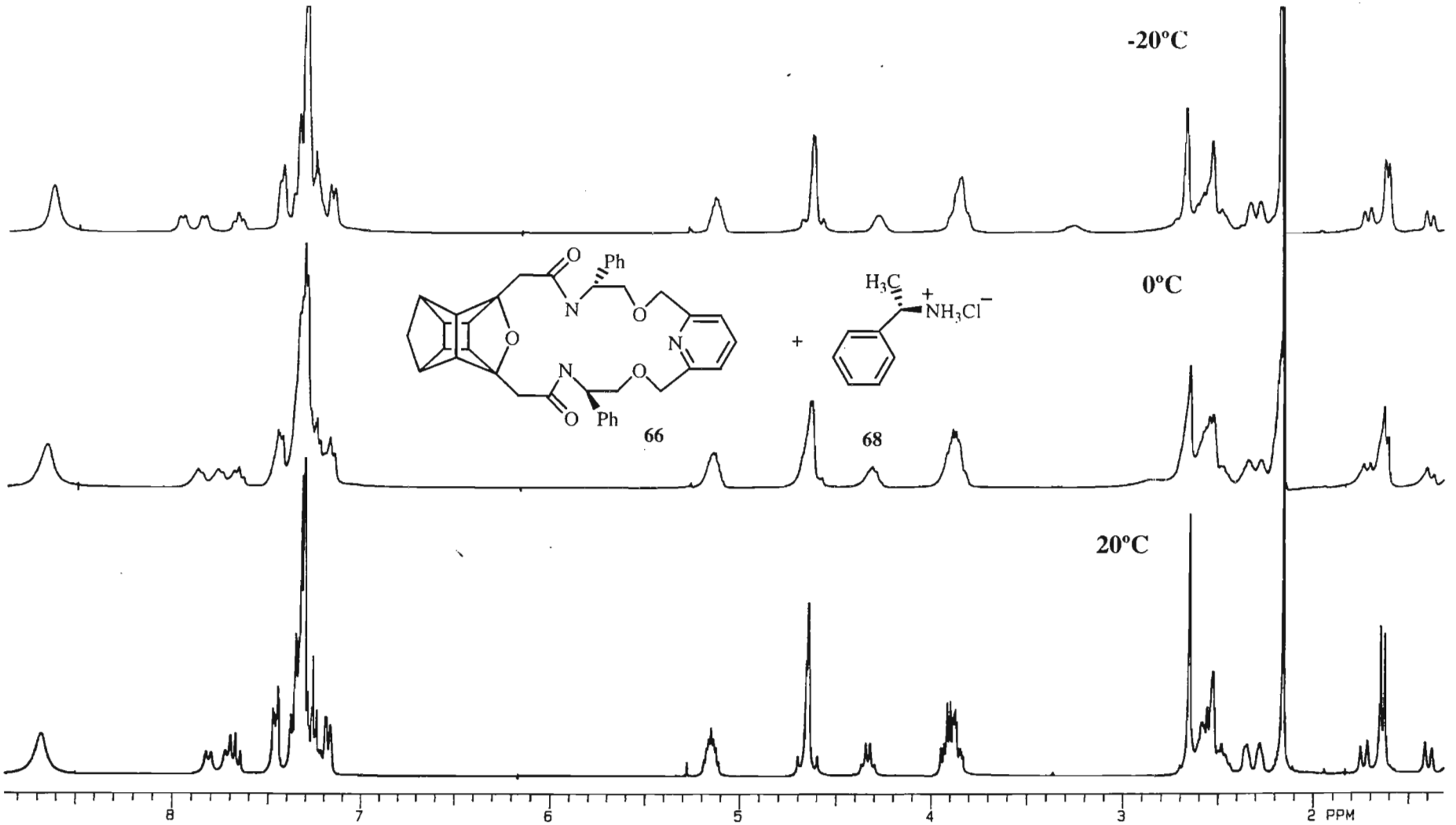
^{1}H nmr $CDCl_3$ 300 MHz



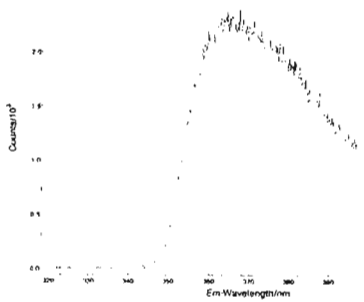
^{1}H nmr $CDCl_3$ 300 MHz



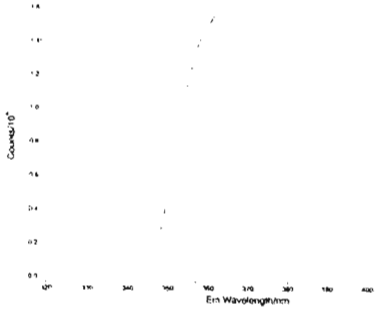
^{1}H nmr $CDCl_3$ 300 MHz



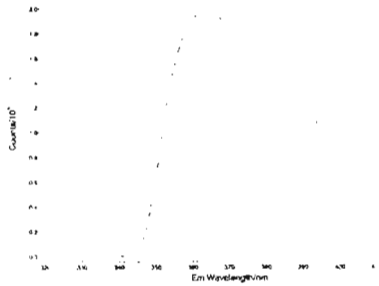
^{1}H nmr $CDCl_3$ 300MHz



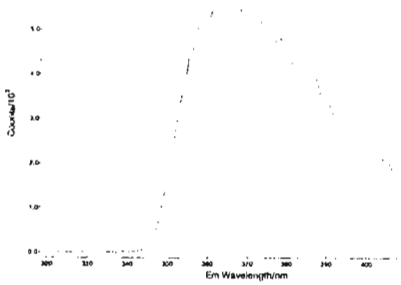
50



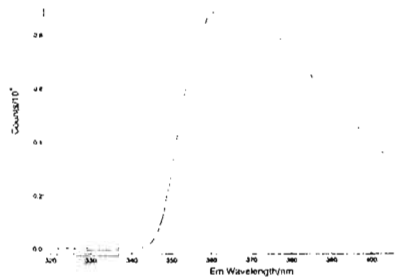
50 + (R)-α-methylbenzylamine



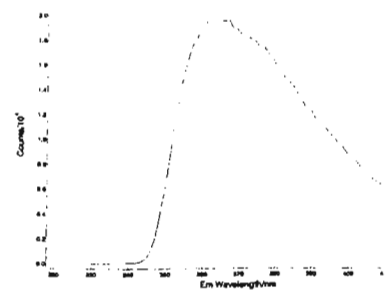
50 + (S)-α-methylbenzylamine



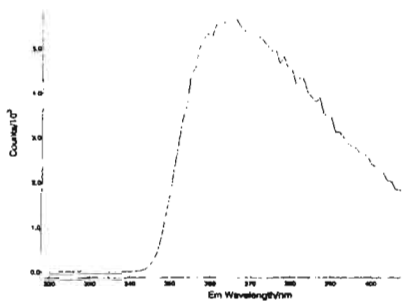
50 + (R)-phenylglycinol



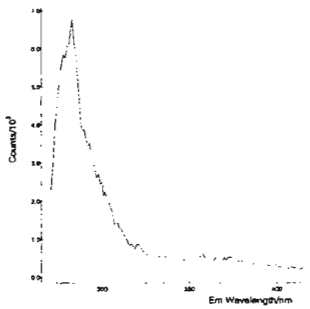
50 + (S)-phenylglycinol



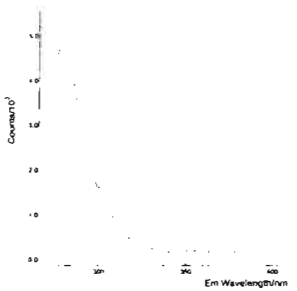
50 + (R)-phenylglycine methyl ester



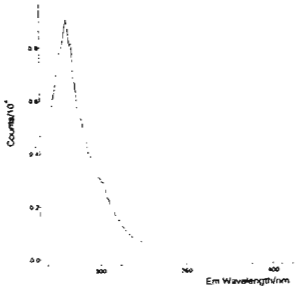
50 + (S)-phenylglycine methyl ester



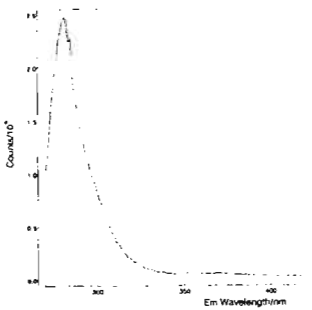
57



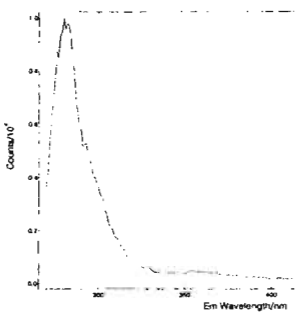
57 + (R)-α-methylbenzylamine



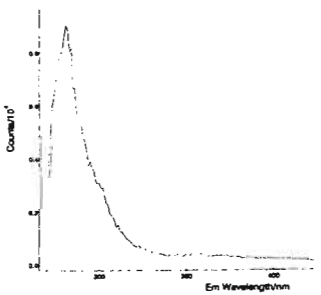
57 + (S)-α-methylbenzylamine



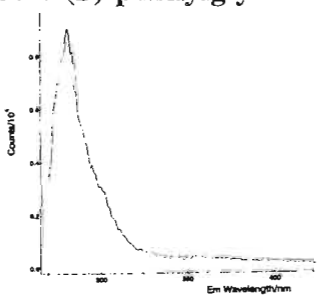
57 + (R)-phenylglycinol



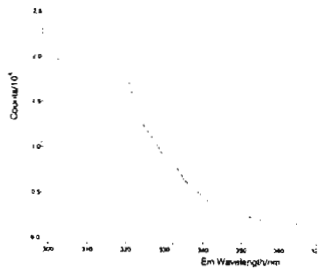
57 + (S)-phenylglycinol



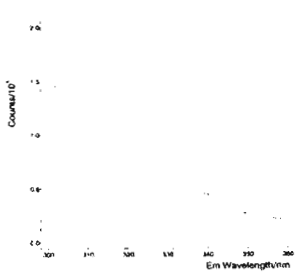
57 + (R)-phenylglycine methyl ester



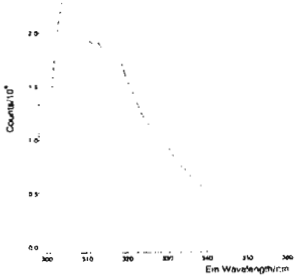
57 + (S)-phenylglycine methyl ester



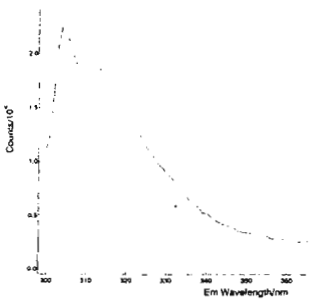
66



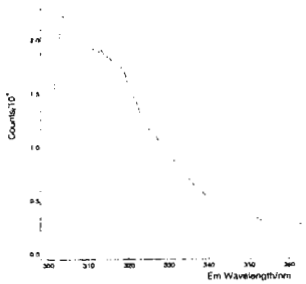
66 + (R)-α-methylbenzylamine



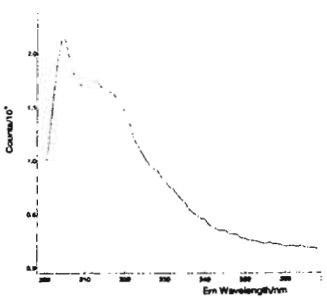
66 + (S)-α-methylbenzylamine



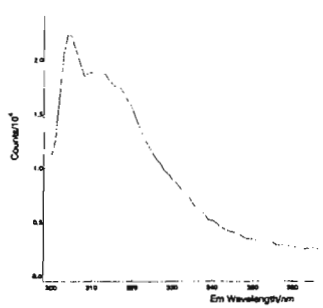
66 + (R)-phenylglycinol



66 + (S)-phenylglycinol



66 + (R)-phenylglycine methyl ester



66 + (S)-phenylglycine methyl ester