UNIVERSITY OF KWAZULU-NATAL

DESIGN, SYNTHESIS AND PHARMACEUTICAL APPLICATION OF NOVEL POLYCYCLIC 'CAGE' DIAMINES

2010

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2010

A thesis submitted to the School of Chemistry, Faculty of Science and Agriculture, University of KwaZulu-Natal, Westville, for the degree of Doctor of Philosophy.

This thesis has been prepared according to **Format 4** as outlined in the guidelines from the Faculty of Science and Agriculture which states:

> This is a thesis in which the chapters are written as a set of discrete research papers, with an Overall Introduction and Final Discussion. Where one (or all) of the chapters has already been published. Typically these chapters will have been published in internationally-recognised, peer-reviewed journals.

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

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ABSTRACT

Despite over 50 centuries of living with the disease, tuberculosis (TB) still remains one of the oldest and deadliest diseases known to man and is gradually becoming a serious threat to the human race. According to the 2009 Global tuberculosis control report of the World Health Organisation (WHO), it is estimated that about 9.4 million incident cases of TB occurred globally. Of these cases an estimated 1.4 million were HIV positive of which 78 % were in the African region while 13 % are located in the South-East Asia Region. An estimate of 1.3 million deaths was reportedly caused by TB among HIV negative people. South Africa has the highest percentage of HIV patients living with tuberculosis. The design, synthesis and evaluation of novel polycyclic 'cage' amines for their pharmaceutical profiles are presented in this thesis. In this project a total of 12 novel intermediates and 31 novel products were synthesised. A thorough NMR elucidation of the various structures was also pursued.

This study was motivated by the reported discovery of SQ109 by Sequella. SQ109 (*N*-Geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine) shares the same 1,2 ethylenediamine pharmacophore with ethambutol (EMB), a commercial TB-drug. SQ109 also possess remarkable activity against MDR-TB which includes the EMB resistant strain suggesting that SQ109 is a new anti-TB drug and not an EMB analogue. SQ109 comprises of a polycyclic adamantane moiety, an isoprenyl moiety and a diamine.

This study had three main aims, namely (a) the design and synthesis of novel polycyclic 'cage' amines derivatives; the polycyclic 'cage' moieties investigated in this study includes adamantane, trishomocubane, oxa-pentacycloundecane, aza-pentacycloundecane, pentacyclodecane and pentacycloundecane, (b) structural elucidation (using 2D NMR techniques) of synthesized novel polycyclic 'cage' amine derivatives (c) the anti-mycobacterial screening of novel polycyclic 'cage' amines derivatives against $H_{37}Rv$, MDR (multi-drug resistant) and XDR (extensively-drug resistant) strains of *Mycobacteria tuberculosis* and (d) the anti-bacterial and anti-fungal screening of selected novel polycyclic 'cage' amine derivatives.

Furthermore, the design, synthesis and NMR elucidation of a family of similar novel PCU diamine ligands are also reported herein. The aim of these ligands is to complex and transport copper ions to the sites of inflammation caused by arthritus. The known pharmaceutical properties of polycyclic 'cage' compounds such as their ability to cross membranes due to improved drug lipophilicity makes them suitable candidates for such a study. This project stems from a logic collaboration between UKZN, UCT (University of Cape Town) and CPUT (Cape

Peninsula University of Technology) to test some of these cage diamines for activity against arthritus. Experimental work in this aspect is performed by the group of Prof. Graham E. Jackson (UCT) and Dr. Sebusi Odisitse (UPUT).

DECLARATIONS

DECLARATION 1 - PLAGIARISM

I, ……., declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1

Onajole, O. K.; Govender, K.; Govender, P.; van Helden, P. D.; Kruger, H. G.; Maguire, G. E. M.; Muthusamy, K.; Pillay, M.; Wiid, I.; Govender, T. **Pentacyclo-undecane derived cyclic tetra-amines: Synthesis and Evaluation as potent anti-tuberculosis agents**; *European Journal of Medicinal Chemistry*, **2009**, 44, 4297-4305.

Contributions: A continuation from my MSc degree. I synthesized three novel final compounds and wrote the paper. All anti-mycobacterial analysis was carried out by Prof. I. Wiid, Prof. van Helden PD and Mrs K. Govender while Ms. K. Muthausamy carried out cytotoxicity analysis. All other authors are supervisors.

Publication 2

Onajole, O. K.; Govender, P.; Govender, T.; Maguire, G. E. M.; and Kruger, H. G. **Synthesis and NMR elucidation of novel pentacyclo-undecane diamine ligands**; *Structural Chemistry* **2009,** 20, 6, 1067.

Contributions: I synthesized all the compounds, carried out NMR elucidations and wrote the paper. All other authors are supervisors.

Publication 3

Onajole, O. K.; Govender, P.; van Helden, P. D.; Kruger, H. G.; Maguire, G. E. M.; Wiid, I.; Govender, T. **Synthesis and evaluation of SQ109 analogues as potential anti-tuberculosis candidates;** *European Journal of Medicinal Chemistry* **2010**, 45, 2075.

Contributions: I synthesized all compounds and wrote the paper. All anti-mycobacterial analysis was carried out by Prof. I. Wiid and Prof. P. D. van Helden. All other authors are supervisors.

Publication 4

Onajole, O. K.; Govender, P.; Kruger, H. G.; Maguire, G. E. M.; Govender, T. **Synthesis and NMR assignment of pentacycloundecane precursors of potential pharmaceutical agents**; *Magnetic Resonance in Chemistry*, **2010,** 48, 245

Contributions: I synthesized all compounds, carried out NMR elucidations with the assistance of Ms. M.M. makatini. I wrote the paper. All other authors are supervisors.

Publication 5

Onajole, O. K.; Govender, P.; Govender, T.; Maguire, G. E. M.; Kruger, H. G. **NMR elucidation of novel SQ109 derivatives**; *Structural Chemistry* **accepted 2010 doi: 10.1007/s11224-010-9661-3**

Contributions: I carried out NMR elucidations and wrote the paper. All other authors are supervisors.

Publication 6

Onajole, O. K.; Coovadia, Y.; Govender, T.; Kruger, H. G.; Maguire, G. E. M.; Naidu, D.; Singh, N.; Govender P. ***In-vitro*** **antifungal and antibacterial activities of pentacycloundecane tetra- amines**; *Chemical Biology and Drug Design* **accepted for publication on 26th October, 2010.**

Contributions: I synthesized the compounds, carried out all anti-microbial analysis under the supervision of Prof. Y. Coovadia, Mrs. D. Naidu and Dr. N. Singh and wrote the paper. All other authors are supervisors.

Publication 7

Onajole, O. K.; Belewa, X. V.; Coovadia, Y.; Govender, T.; Kruger, H. G.; Maguire, G. E. M.; Naidu, D.; Somai, B.; Singh, N.; Govender. P. **SQ109 analogues as potential antimicrobial candidates**; *Medicinal Chemistry Research* **accepted 2010 doi: 10.1007/s00044-010-9490-3**

Contributions: I synthesized all compounds, carried out anti-microbial analysis under the supervision of Prof. Y. Coovadia, Mrs. D. Naidu and Dr. N. Singh and wrote the paper. Dr. B. Somai and Ms X. V. Belewa ran the anti-fungal analysis of all synthesized compounds against a plant fungus. All other authors are supervisors.

Publication 8

Onajole, O. K.; Sosibo, S.; Govender, P.; Govender, T.; van Helden, P. D.; Maguire, G. E. M.; Majerski, M.; Wiid, I.; Kruger, H. G.; **Novel linear diamine disubstituted polycyclic 'cage' derivatives as potential anti-mycobacterial candidates**; manuscript submitted to *Chemical Biology and Drug Design on 3rd November, 2010.*

Contributions: I synthesized all compounds with some assistance from Mr. S. Sosibo. I carried out the NMR elucidation of the compounds and wrote the paper. All anti-TB analysis was carried out by Prof. I. Wiid and Prof P. D. van Helden. All other authors are supervisors.

Publication 9

Onajole, O. K.; Coovadia, Y.; Govender, P.; Kruger, H. G.; Maguire, G. E. M; Pillay, M.; Govender, T.; **Novel polycyclic 'cage'-1,2-diamines as potential anti-tuberculosis agents;** manuscript submission pending, due to possible patent.

Contributions: I synthesized all compounds, carried out NMR elucidations and wrote the paper. All anti-TB analysis was carried out by Prof. Y. Coovadia and M. Pillay. All other authors are supervisors.

Signed:

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To the entire GGKM Research Group, I thank you for the friendly environment provided. You helped to build my passion for chemistry.

To my father, Rev. Aderemi A. Onajole, words can't express my gratitude; thank you for everything. Without your support and endless encourage I would have quited long time ago. I remain eternally grateful to you.

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Much thanks to the University of KwaZulu-Natal, ASPEN pharmaceutical company and NRF (National Researech Foundation) for their generous financial support.

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DEDICATION

This work is dedicated to the memory of my loving mother late Mrs. Olabisi Mercy Olufunmilayo Onajole. “Iya ibeji” you are the best mother and woman I have ever known, a VIRTOUS woman.

I count it a great privilege and honour to be called your son, the fruit of your womb.

Mummy, words can express how much I miss you.

LIST OF PUBLICATIONS

1. Onajole, OK.; Sosibo, S.; Govender, P.; Govender, T.; van Helden, PD.; Maguire, GEM.; Majerski, M.; Wiid, I.; Kruger, HG.; **Novel linear diamine disubstituted polycyclic 'cage' derivatives as potential anti-mycobacterial candidates**; manuscript submitted to *Chemical Biology and Drug Design 2010.*
2. Onajole, OK.; Belewa, XV.; Coovadia, Y.; Govender, T.; Kruger, HG.; Maguire, GEM.; Naidu, D.; Somai, B.; Singh, N.; Govender. P. **SQ109 analogues as potential antimicrobial candidates**; *Medicinal Chemistry Research* **accepted for publication2010.**
3. Onajole, OK.; Coovadia, Y.; Govender, T.; Kruger, HG.; Maguire, GEM.; Naidu, D.; Singh, N.; Govender P. ***In-vitro*** **antifungal and antibacterial activities of pentacycloundecane tetra- amines**; *Chemical Biology and Drug Design* **accepted for publication 2010.**
4. Onajole, OK.; Govender, P.; Govender, T.; Maguire, GEM.; Kruger, HG. **NMR elucidation of novel SQ109 derivatives**; *Structural Chemistry* **accepted 2010 doi: 10.1007/s11224-010-9661-3**
5. Chakka, SK.; Onajole, OK.; Govender, T.; Maguire, GEM.; Su, H.; Kruger, HG. **1,7-Dimethylpentacyclo[5.4.0.02,6.03,10.05,9]undecane-8,11-dione**; *Acta Cryst* **2010**. E66, o1901
6. Altaib, MS.; Arvidsson, PI.; Govender, T.; Maguire, GEM.; Makatini, M.; Onajole, OK.; Kruger, HG.; **Synthesis and NMR elucidation of novel pentacycloundecane based peptides**; *Magnetic Resonance in Chemistry*, **2010,** 48, 6, 435.
7. Onajole, OK.; Govender, P.; Kruger, HG.; Maguire, GEM.; Govender, T. **Synthesis and NMR assignment of pentacycloundecane precursors of potential pharmaceutical agents**; *Magnetic Resonance in Chemistry*, **2010**, 48, 245.
8. Onajole, OK.; Govender, P.; van Helden, PD.; Kruger, HG.; Maguire, GEM.; Wiid, I.; Govender, T. **Synthesis and evaluation of SQ109 analogues as potential anti-tuberculosis candidates;** *European Journal of Medicinal Chemistry* **2010,** 45, 2075.
9. Onajole, OK.; Govender, P.; Govender, T.; Maguire, GEM.; and Kruger, HG. **Synthesis and NMR elucidation of novel pentacyclo-undecane diamine ligands**; *Structural Chemistry* **2009,** 20, 6, 1067.
10. Onajole, OK.; Govender, K.; Govender, P.; van Helden, PD.; Kruger, HG.; Maguire, GEM.; Muthusamy, K.; Pillay, M.; Wiid, I.; Govender, T. **Pentacyclo-undecane derived cyclic tetra-amines: Synthesis and Evaluation as potent anti-tuberculosis agents**; *European Journal of Medicinal Chemistry*, **2009,** 44, 4297-4305.
11. Boyle GA, Govender T, Kruger HG and Onajole OK; **N-(2-Hydroxyethyl)-N-(tricyclo[3.3.1.13,7]dec-2-yl)benzamide**, *Acta Cryst,* **2008**, E**64**, o1029.
12. Boyle GA, Govender T, Kruger HG and Onajole OK, **2-(Tricyclo[3.3.1.13,7]dec-2-ylamino)ethanol hemihydrates**; *Acta Cryst*, **2008**, E64, o1228.
13. Onajole, OK.; Govender, T.; Makatini, M.; Kruger, HG.; **Synthesis and NMR elucidation of pentacyclo-undecane amine derivatives as potential anti-tuberculosis agent**; *Magnetic Resonance in Chemistry*, **2008**, 46, 1007-1014.
14. Govender T, Kruger HG, Makatini M and Onajole OK, **Synthesis and NMR elucidation of pentacyclo-undecane diamine derivatives as potential anti-tuberculosis drugs**; *Structural Chemistry* **2008**, 19, 719-726.

TABLE OF CONTENT

CHAPTER 1
INTRODUCTION

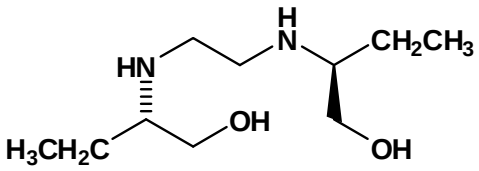
1.1 Background

Tuberculosis (TB) is a highly contagious and infectious disease mainly of the lungs but can easily spread to other organs in the body. Pulmonary tuberculosis is primarily caused by a bacterium called *Mycobacteria tuberculosis* (*M. tuberculosis*). Pulmonary tuberculosis has been in existence for hundreds of centuries with scientific evidence of tuberculosis residues in Egyptian mummies.[1-5] Despite the fact that we have been living for more than 50 centuries with the disease, tuberculosis remains one of the deadliest diseases known to man and is gradually becoming a threat to the human race. The World Health Organisation (WHO) 2010 Global Tuberculosis report, estimated that about 9.4 million cases of TB occurred in 2009 of which 35 % occurred in South-East Asia, 30 % in Africa and 20 % in Western Pacific region. HIV-positive incident cases was estimated at 11-13 %; Africa has the highest percentage (80 %) of HIV-positive people living with tuberculosis.[6]

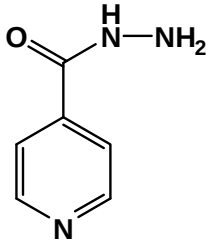
Tuberculosis can be easily managed, prevented and controlled using currently available drugs. The World Health Organisation introduced a drug regimen called DOTS (Directly Observed Treatment Short course) in the mid-90s. This regimen employs a six month treatment with known anti-mycobacterial drugs which includes ethambutol, isoniazid, pyrazinamide, and rifampicin also known as rifampin (Figure 1). These four drugs are prescribed on a daily basis for a two month period. Isonaizid and rifampicin are continued daily for an additional four months; these are referred to as first line drugs.

However, inconsistent treatment, partial intake, in-availability of drugs and negligence during these six month therapy period can easily result in a situation where *M. tuberculosis* becomes resistant to these first line drugs. This appears to be the major cause for the emergence of multi drug-resistant (MDR) strain of tuberculosis. MDR-TB is classified as being resistant to at least isoniazid and rifampicin, the two most powerful existing anti-TB drugs.[7]

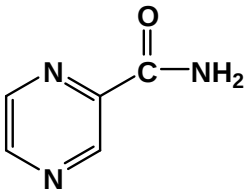
MDR-TB can be cured using second line drugs which include streptomycin, *p*-aminosalicyclic acid, ethionamide cycloserine and fluoroquinolone based drugs such as ciprofloxacin, sparfloxacin, sitafloxacin, moxifloxacin *etc* (Figure 2).



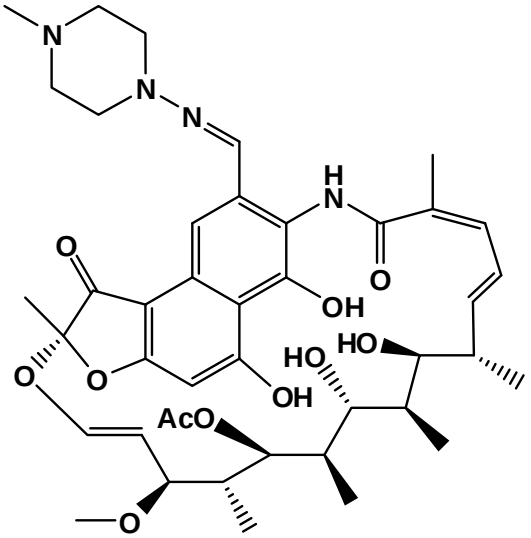
Ethambutol



Isoniazid



Pyrazinamide



Rifampicin

Figure 1: Structures of first-line drugs (1-4)

The second line drugs are very toxic, expensive and possess numerous side effects. Inconsistency in the administration of these drugs renders them less effective against MDR-TB resulting into extensively drug-resistant TB (XDR-TB). At this stage there are little or no therapies that can be offered to XDR-TB patients.[8] The emergence of HIV/AIDS has caused a major setback in the treatment of tuberculosis globally. Tuberculosis infection is very rampant in HIV positive patients due to a drop in their immune system which encourages dormant or growing mycobacteria to grow well and flourish in their host. HIV and TB form a lethal combination and frequently leads to premature death if not attended to on time.[9]

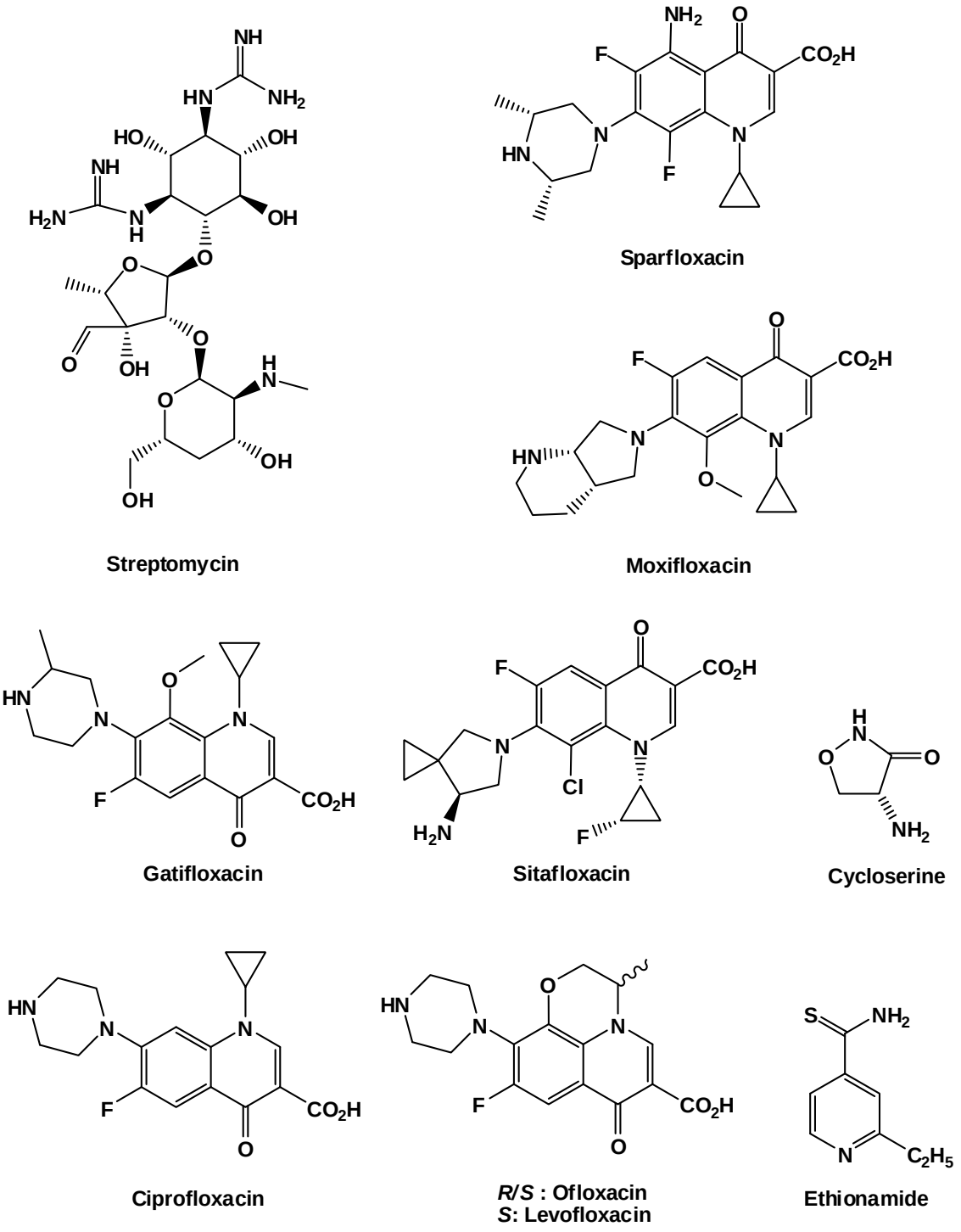


Figure 2: Structure of some known Second line anti-TB drugs[10]

In the context of the current global TB public health disaster, the urgent development of new anti-TB drugs cannot be overemphasized.[7] Researchers have explored the possibility of improving the therapeutic effect of already existing anti-TB drugs such as ethambutol, isonazid *etc*. in order to further enhance/improve the half life and potency of such drugs in the host and

also to lessen any existing side effects. Over the years, several promising drug candidates emerged, each one possessing different site and mode of action on the *mycobacterium*.

1.2 Site and mode of action of current TB drugs

Much research has been undertaken in order to gain more insight to the site and mode of action of current and potential anti-TB drugs. This knowledge has helped a great deal in the design of potent drugs which target specific sites or biosynthesis pathway in the organism or bacillus. The cell wall of *mycobacterium* spp. is essential for its survival and growth in the host.[11] The cell wall comprises mainly of arabinogalactan, peptidoglycan, lipoarabinomannan and mycolic acid.[11] The disruption of the biosynthesis pathway of these molecules most especially peptidoglycan and mycolic acid, should easily damage the assembly of the macromolecules, which leads to the death of the cell.[12-14]

Ethambutol was initially believed to inhibit the biosynthesis of arabinogalactan and lipoarabinomannan of *M. tuberculosis* and other mycobacteria. However, is was subsequently established that ethambutol inhibits the polymerization step of arabinan biosynthesis of arabinogalactan.[12,13] Isoniazid and ethionamide are prodrugs* and are activated within the mycobacterial cell. Isoniazid primarily inhibits the biosynthesis of mycolic acid in the organism.[16,17] Ethionamide is believed to possess a similar mechanism of action as isonaizid.[18,19] Isonaizid and ethionamide are structurally related thus explaining the similarity observed in their mode of action. Streptomycin is a broad-spectrum antibiotic possessing anti-bacterial activity against most Gram-positive, Gram-negative and mycobacterial species.[15,20] Streptomycin inhibits the protein synthesis of the bacilli by interacting with the 30S ribosomal subunit.[20]

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* A prodrug is a pharmacological substance (drug) which is administered in an inactive (less active) form. Once administered, the prodrug is metabolised *in vivo* into an active metabolite.

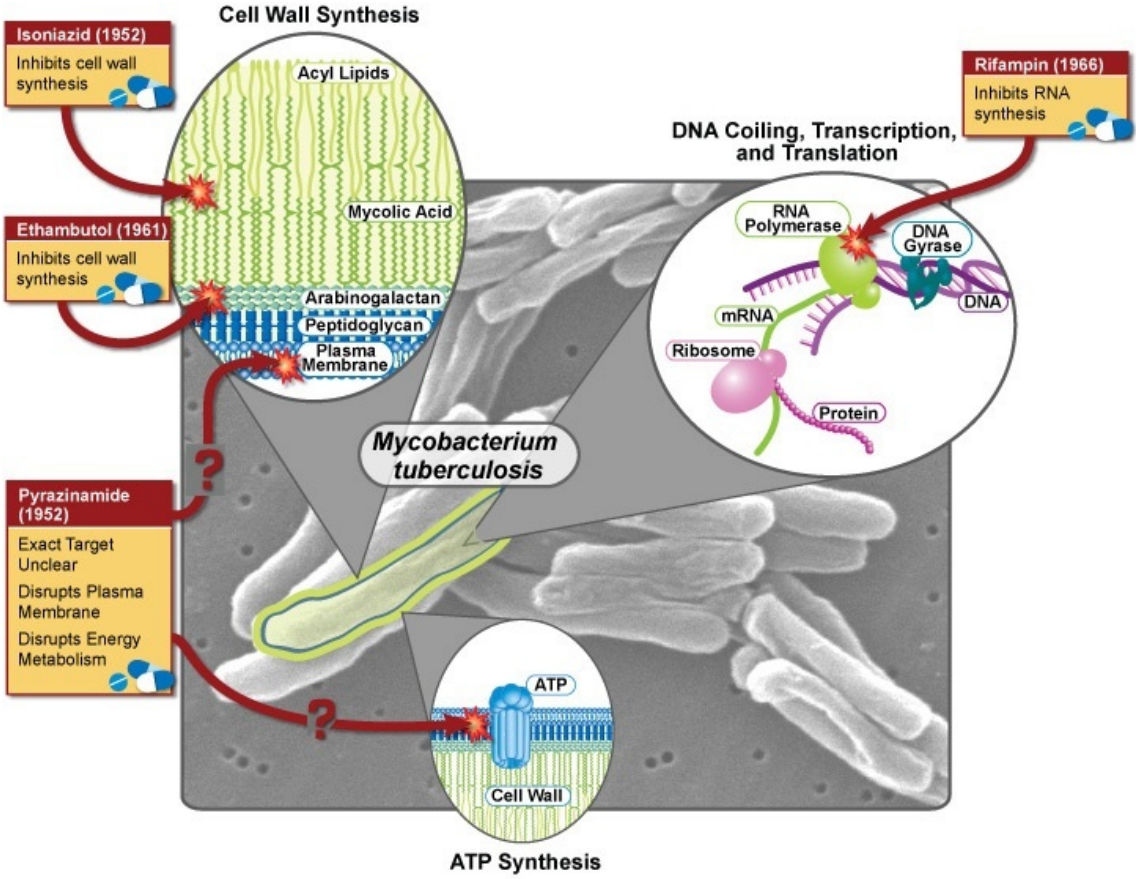

Figure 3: First-Line Treatment of Tuberculosis (TB) for Drug-Sensitive TB[21]

Known anti-TB drugs could either be bacteriostatic or bacteriocidal. Bacteriostatic anti-TB drugs such as ethambutol, ethionamide *etc*. stop the growth or flourishing of the bacilli in its host without necessarily killing it and re-surgence can occur if the treatment is discontinued. Bacterocidal anti-TB drugs such as isonaizid and rifampicin kill the bacilli and in most cases prove to be more effective than static drugs against *M. tuberculosis*. A list of known anti-TB drugs with their mechanisms/site of action, effect on the bacterial cell wall and targets are presented in Table 1.

Table 1: Commonly used TB drugs and their mode of action.[22]

Drug (year of Discovery)	**MIC (g/ml)**	**Effect on bacterial cell**	**Mechanisms of action**	**Targets**	**Genes involved in resistance**
Isoniazid (1952)	0.01-0.2	Bactericidal	Inhibition of cell wall mycolic acid synthesis and other multiple effects on DNA, lipids, carbonhydrates, and NAD metabolism	Multiple targets including acyl carrier protein reductase (InhA)	*katG* *inhA* *ndh*
Rifampicin (1966)	0.05-0.5	Bactericidal	Inhibition of RNA synthesis	RNA polymerase β subunit	*rpoB*
Pyrazinamide (1952)	20-100 pH 5.5 or 6.0	Bacteriostatic/ bactericidal	Disruption of membrane transport and energy depletion	Membrane energy metabolism	*pncA*
Ethambutol (1961)	1-5	Bacteriostatic	Inhibition of cell wall arabinogalactan synthesis	Arabinosyl transferase	*embCAB*
Streptomycin (1944)	2-8	Bactericidal	Inhibition of protein synthesis	Ribosomal S12 protein and 16S rRNA	*rpsL, rrs*
Kanamycin (1957)	1-8	Bactericidal	Inhibition of protein synthesis	16S rRNA	*Rrs*
Quinolones (1963)	0.2-4	Bactericidal	Inhibition of DNA synthesis	DNA gyrase	*gyrA* *gyrB*
Ethionamide (1956)	0.6-2.5	Bacteriostatic	Inhibition of mycolic acid synthesis	Acyl carrier protein reductase (InhA)	*inhA* *etaA/ethA*
p-Aminosalicylic acid PAS (1946)	1-8	Bacteriostatic	Inhibition of folic acid and iron metabolism?	Unknown	Unknown
Cycloserine (1952)	5-20	Bacteriostatic	Inhibition of peptidoglycan synthesis	D-alanine racemase	*alrA, Ddl*

1.3 Development of new generation anti-TB drug candidates

The Stop TB Strategy and Global plan of the World Health Organisation to eliminate TB form part of an on-going campaign to reduce the burden of TB and deaths resulting from TB by the year 2015.[23] The Global Plan to Stop TB '2006-2015' Action for life, was launched in January 2006 at the World Economic Forum in Davos, Switzerland.[24,25] This campaign has led to the fast tracking in the clinical trials of identified anti-TB drug candidates for the past four years. It should be noted that no new anti-TB drug has been approved for use in the past four decades and this shortcoming has resulted in numerous deaths due to the emergence of drug resistant TB.

In order for this set goal to be achieved by the year 2015, there is an urgent need for highly potent chemotherapeutics that are fast acting, have fewer side effects and are also cost effective. Researchers over the last decade have indentified numerous potent anti-tuberculosis candidates some of which are presently undergoing advanced stages of clinical trials; these compounds include derivatives containing diamine (SQ109), diaryl quinoline (TMC207), nitroimidazooxazine (PA-824), pyrrole (LL-3858) and nitrodihydro-imidazooxazole (OPC-67683) functional groups.[26-29] Table 2, shows a list of potential anti-TB candidates at discovery, pre-clinical and clinical stages of development.[47]

TMC207 (a diarylquinoline)

TMC207, a diarylquinoline derivative, (also known as R207910, Figure 5) was first reported in 2005 by Andries *et al*.[30] TMC207 is a pure enantiomer with two chiral centers, its chemical name is 1-(6-bromo-2-methoxyl-quinolin-3-yl)-4-dimethylamino-2-naphthalen-1-yl-1-phenyl-butan-2-ol ($C_{32}H_{31}BrN_2O_2$). The absolute configuration of the two asymmetric centers was identified as *R*,*S i.e.* the carbon bearing the phenyl substituent is of (*R*)-form and the carbon bearing the hydroxyl substituent is of (*S*)-form. Diarylquinolines has a unique structure and mode of action which differs from fluoroquinolones (specific to type II topoisomerases inhibition) and quinolines such as mefloquine. TMC207 possesses anti-TB activity against the $H_{37}Rv$ reference susceptible strain and drug-resistant strains of tuberculosis at a MIC range of 0.030 to 0.120 μg mL^{-1} compared to 0.500 μg/mL for rifampin and 0.120 μg mL^{-1} for isoniazid.[30] TMC207 appears to be mycobacterium specific as significant MIC values were reported for *M. bovis*, *M. kansasii* and *M. ulcerans* including those that are naturally resistant to many known anti-TB agents such as the *M. avium* complex, *M. abscessus*, *M. fortuitum* and *M. marinum*. An increase in MIC values were observed for Gram-positive and Gram-negative bacteria.[30] The target for TMC207 was proposed to be the mycobacterial F1F0 proton ATP synthase. A drug combination of TMC207 with first line anti-TB drugs (rifampin, isonazid and pyrazinamide) carried out in an animal model (mice). When TMC207 is used as a monotherapy, it exhibited similar activity as the combination of rifampin, isoniazid and pyrazinamide. Due to drug resistance, the development of clinical use of TMC207 as monotherapy is not encouraged. TMC207 however displays remarkable bactericidal activities when used in combination

with isonazid and pyrazinamide or rifampin and pyrazinamide proving to be more active than the DOTS regimen. Further study shows that TMC207 is rapidly metabolised by cytochrome P_{450}, which is increased with the use of rifampin thus resulting into an unwanted interaction between TMC207 and rifampin.[32] A recent study also showed that TMC207 posseses excellent activity against *Mycobacterium leprae*, the causal organism of leprosy using a mouse model.[31]

As mentioned earlier the organism *M. tuberculosis* can remain dormant in its host for a long period of time, all known anti-TB drugs including isoniazid and rifampin have no effect on dormant *mycobacteria*. However, treatment of a culture of anaerobic cells (deprived of oxygen) with TMC207 causes a dose-dependent reduction of the ATP synthesis level which results in a reduction in the colony forming units (CFUs) after introducing oxygen.[33] TMC207 also gave impressive results in various mouse models of TB,[32,34-36] whilst initial Phase 1 safety and Phase 2 efficacy studies continue to yield very promising results.[30,37]

LL3858 (Pyrrole derivative)

LL-3858 is a pyrrole derivative (Figure 5) which was developed by Lupin Limited in India. It exhibited potent anti-TB activity against both drug sensitive and resistant strain (MDR) of tuberculosis at a MIC of 0.06-0.5 µg mL^{-1}.[38,39] *In-vitro* anti-TB screening of LL-3858, shows dose dependent mycobactericidal activity (monotherapy) and a synergistic effect when used in combination with rifampin. In murine models, LL-3858 demonstrates superior monotherapeutic activity in comparison to isoniazid. Combination therapy of LL-3858 with other known anti-TB drugs such as isoniazid, rifampin, pyrizinamide and ethambutol showed increased activity.[38] There is little published information available about this compound and its mode or site of action in the mycobacterium is still unknown. LL-3858 is currently undergoing advanced phase 1 clinical trials.[28]

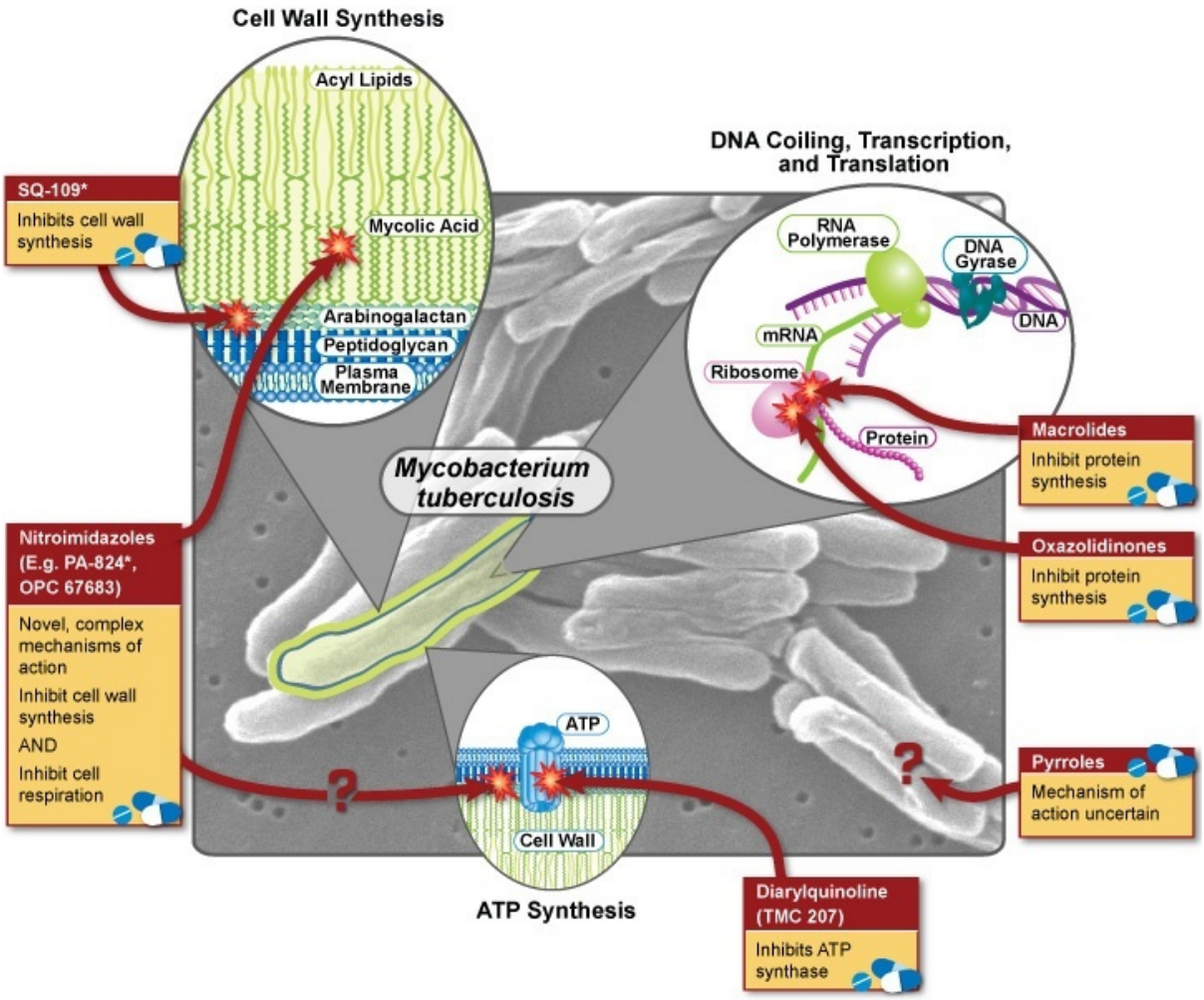

Figure 4: New Tuberculosis (TB) drugs under development[40]

SQ109 (an adamantane diamine derivative)

SQ109 (*N*-geranyl-*N'*-(2-adamantyl)ethane-1,2-diamine is an analogue of ethambutol which was first reported by Lee *et al.* in 2003.[41] They have used a combinatorial approach where a library of 63,238 1,2-diamine analogues of ethambutol were synthesized and screened for their anti-TB activities. SQ109 was indentified from this library as the most active with a 14-35 fold activity over ethambutol as the control drug.[41] Further studies, however showed only a fivefold increase in activity over that of ethambutol.[42] SQ109 inhibits the biosynthesis of the cell wall but its specific target is still unknown. Recent studies showed that SQ109 exhibits activity against numerous multi-drug resistant TB strains which includes an ethambutol resistant strain; thus suggesting that SQ109 is a new anti-TB drug and not an ethambutol analogue.[42] *In vivo* activity of SQ109 in animal models also showed excellent activity against infected mice.[42]

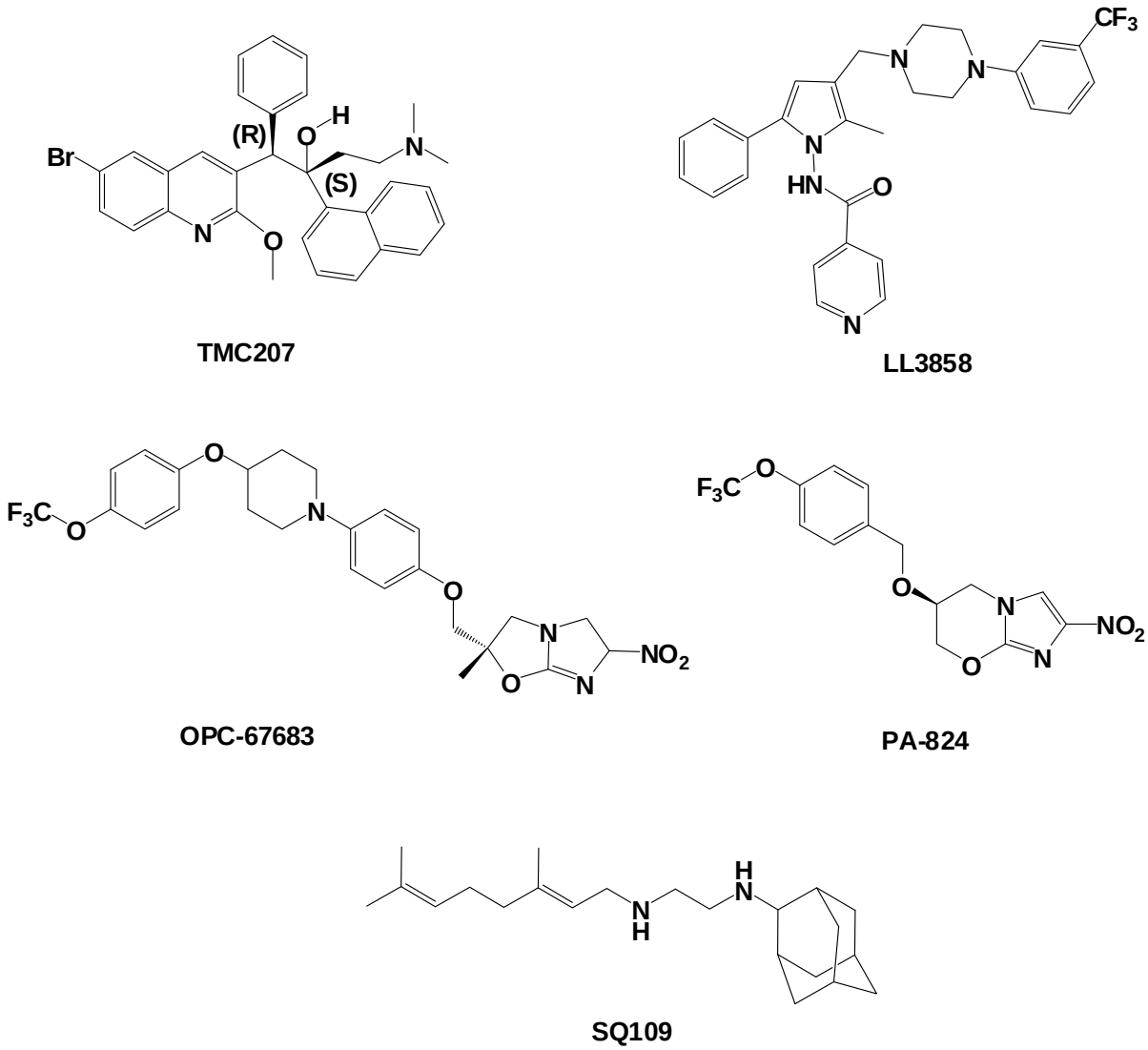

Figure 5: Structures of TMC207, LL3858, OPC-67683, PA-824 and SQ109

Pharmacokinetics and pharmcodynamics of SQ109 in animal models displayed good bioavailability with the highest concentration found in the lungs (>MIC).[43,44] Further *in vitro* drug combination analysis using the BACTEC 460 system shows that SQ109 has a synergistic effect when used in combination with isoniazid and rifampicin, two of the most important first-line anti-TB drugs and an additive effect was observed when used in combination with streptomycin.[45] No synergy or additive effects were observed for the combination of SQ109 with ethambutol or pyrazinamide.[45] SQ109 recently completed phase 1 clinical trials.[46]

Table 2: Compounds targeting tuberculosis in different stages of development[47]

	Source or organization
CLINICAL STUDIES	
Diamine SQ109	Sequella
Diarylquinoline TMC207	Tibotec Inc.
Gatifloxacin	OFLOTUB consortium, others
Moxifloxacin	Bayer, TB Alliance, others
Nitrodihydroimidazo-oxazole OPC-67683	Otsuka Pharmaceutical
Nitroimidazole PA-824	TB Alliance
Pyrrole LL3858	Lupin Pharmaceuticals
Rifapentine	Hoechst
PRECLINICAL TESTING	
Dipiperidine SQ609	Sequella
Nitroimidazole backup compounds	Otsuka Pharmaceutical
Nonfluorinated quinolones	TaiGen
Oxazolidinones	Pfizer
Synthase inhibitor FAS20013	FASgen
Translocase I inhibitors/capuramycins	Sequella, Sankyo
DISCOVERY STAGE	
Cell-wall synthesis inhibitors	Colorado State University, NIAID
Dihydrolipoamide acyltransferase inhibitors	Cornell University, NIAID
Enoyl reductase (InhA) inhibitors	GlaxoSmithKline, TB Alliance
Malate synthase inhibitors	GlaxoSmithKline, others
Multifunctional molecules	TB Alliance, Cumbre Pharmaceuticals
Mycobacterial gyrase inhibitors	TB Alliance, GlaxoSmithKline
Nitrofuranylamides	University of Tennessee, NIAID
Nitroimidazole analogs	TB Alliance, University of Auckland, Uni. of Illinois
Picolinamide imidazoles	NIAID
Pleuromutilins	GlaxoSmithKline, TB Alliance
Promazine analogs	Salisbury University
Protease inhibitors	Medivir
Proteasome inhibitors	Cornell University, NIAID
Quinolones	TB Alliance, KRICT, Yonsei University
Riminophenazines	Institutes of Materia Medica
Thiolactomycin analogs	NIAID, NIH

KRICT = Korea Research Institute of Chemical Technology; NIAID = National Institute of Allergy and Infectious Diseases; NIH = National Institutes of Health; TB Alliance = Global Alliance for TB Drug Development; SOURCES: Stop TB, NIAID, TB Alliance, Doctors Without Borders

Polycyclic 'cage' chemistry

Polycyclic 'cage' chemistry has been of great interest to both medicinal and organic chemists for over four decades.[48-50] These compounds include adamantane, trishomocubane, pentacycloundecane, pentacyclodecane, basketane, cubane *etc*. Of all these compounds, adamantane is the most studied. The chemistry and medicinal value of polycyclic compounds gained popularity with the discovery of amantadine or 1-amino adamantane which exhibited anti-viral activities against several species which includes hepatitis C[51] and the influenza virus.[52,53] The incorporation of 'cage' compounds into pharmaceutical drugs is an interesting application which is fast growing and has not been fully explored yet. Such compounds increases the drug lipophilicity thus serving as a transport aid to carry such drug across cellular membranes including the Blood Brain Barrier (BBB) and the Central Nervous System (CNS).[54,55] They also increase the drug's affinity for lipophilic regions in receptor molecules.[56] It helps to decrease the drug metabolism thus prolonging the pharmaceutical effect of such drug in the body consequently reducing the frequency of dosage intake of such drugs.[57]

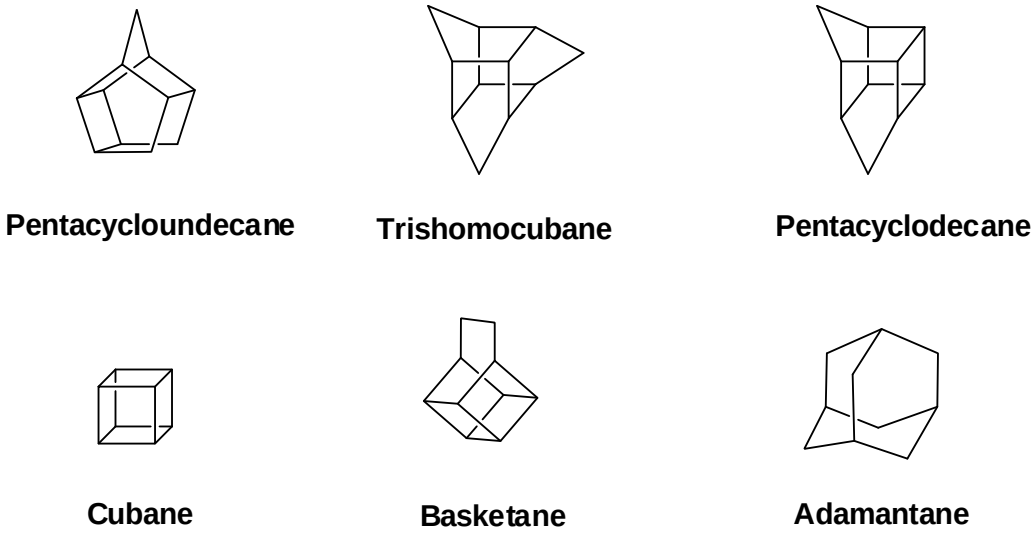

Figure 6: Examples of polycyclic 'cage' compounds[48,49]

This project was motivated by the reported anti-TB activity of SQ109. The structure of SQ109 [*N*-geranyl-*N'*-(2-adamantyl)ethane-1,2-diamine] can be divided into 3 main moieties; which are the adamantyl moiety, ethane-1,2-diamine moiety (EMB pharmacophore) and the isoprenyl (geranyl) moiety. The aim of this project is to evaluate the importance of these moieties and how it contributes to activity. This was achieved by substituting the linear 1,2-diamine with cyclic diamines such as piperazine and homopiperazine. Also the length of the hydrocarbons side chains (saturated or un-saturated) were varied to investigate the effect of these elements on the bioactivity of the compound. Finally, the polycyclic 'cage' moiety was also varied by substituting adamantane with other known polycyclic moieties such as pentacycloundecane, trishomocubane, pentacyclodecane, oxa-pentacycloundecane *etc*.

Research carried out in this thesis

This thesis focuses on the synthesis of novel polycyclic 'cage' amine derivatives, the structural elucidation thereof and their evaluation against *M. tuberculosis*, fungi [yeast and filamentous fungi (moulds)] and bacteria. This work consists of a series of papers (1-9). Based on the format adopted for this thesis, the numbering of structures, figures, schemes and tables in each of the chapters 2-10 will vary. The referencing style will also vary according to the style specified by the journal.

Chapter 2 (paper 1)[58] is a completion of a study carried in my MSc dissertation titled "*Design, synthesis and screening of novel pentacycloundecane tetra-amine derivatives as potential anti-tuberculosis agents*". In this paper, compounds **9a**, **9b** and **9c** were synthesized as part of my PhD studies. The most active compounds (**6a**, **6b**, **8a**, **8b** and **9a**) were re-synthesized and further screened against $H_{37}Rv$ and X194 (an extensively drug-resistant) strains of tuberculosis using the BACTEC 460TB system. In order to complete this study, IC_{50} (inhibition concentration) experiments were also carried out on the most active compounds using the MDBK (Miadin Darby Bovine Kidney epithelium) cell line from the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Westville. The structural activity relationship (SAR) of compounds **8b** and **9a** (paper 1)[58] helped in understanding the different and type of functionalities essential for the activity of these classes of compounds as anti-TB agents. The BACTEC analysis was carried by Prof. I. Wiid at Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa.

Chapter 3 (paper 2)[59] was motivated by reports that SQ109 possesses antifungal activity against *Candida albicans* and fluconazole resistant strains of *Candida albicans*.[60] As a result compounds **8a** (GKM8), **8b** (GKM9) and **9a** (GKM11) were selected for further antimicrobial studies. In this study a total of twenty pathogenic fungi strains and 18 ATCC bacteria (nine Gram-positive and nine Gram-negative) strains were used and the minimum inhibitory concentration (MIC) was determined using the Clinical and Laboratory Standards Institute (CLSI) guidelines. The biological screening was performed by me under the guidance of Dr N. Singh of the School of Biological and Conservation Science (UKZN) and Mrs D. Naidu and Prof. Y. Coovadia of the Microbiology division, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa.

Chapter 4 (paper 3)[61] focuses on the synthesis and evaluation of five novel SQ109 analogues against $H_{37}Rv$ and X174 (an extensively drug-resistant) strains of tuberculosis. Further studies were carried out on these analogues which included the structural elucidation using 2D NMR techniques (Chapter 5, paper 4)[62] and anti-microbial screening of these analogues against fungi and bacteria (Chapter 6, paper 5)[63]. The NMR elucidation was solely done by me. The biological screening was performed by me under the guidance of Dr N. Singh in the School of Biological and Conservation Science (UKZN)

and Mrs D. Naidu and Prof. Y. Coovadia of the Microbiology division, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa.

Chapter 7 (paper 6) evaluates the effect of different polycyclic 'cage' moieties on the anti-TB activity of these class of compounds. Herein, the 2-adamantyl moiety of SQ109 was substituted with pentacycloundecyl, aza-pentcylcoundecyl, oxa-pentacycloundecyl and trishomocubyl moieties and screened for anti-TB activity. The anti-TB analysis was carried out by Mr. M. Pillay and Prof. Y. Coovadia of Microbiology division, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa.

It was postulated that disubstitution on the polycyclic 'cage' moieties compared to the mono-substituted in this case SQ109 (adamantyl moiety), might improve the activity as an anti-TB agent. Three polycyclic 'cage' moieties namely pentacycloundecane, tricyclodecane and pentacyclodecane were employed in this study (Chapter 8, paper 7).[61] The synthesis and structural elucidation of some of the PCU precursors were also reported (Chapter 9, paper 8).[64]

In chapter 10 (paper 9)[65] the synthesis and NMR elucidation of novel PCU diamine ligands were reported. Collaboration established with Prof. Graham Jackson of the School of Chemistry, University of Cape Town and Dr. Sebusi Odistse of the School of Chemistry, Cape Pennisula University of Technology, investigates the application of polycyclic cage compounds as ligands in pharmaceutical applications. Odistse *et al.*[66] recently reported the application of copper (II) diaminediamide derivatives of pentacycloundecane as a potential anti-inflammatory agent. Based on the success of this project, new PCU diamine ligands were designed and proposed. The synthesis and NMR studies are reported herein. Similar anti-inflammatory studies with these compounds are currently being performed at the other institutions.

References

1. Crubezy, E.; Legal, L.; Fabas, G.; Dabernat, H.; Ludes, B. *Infection Genetics and Evolution* **2006**, *6*, 13-21.
2. Zink, A. R.; Molnar, E.; Motamedi, N.; Palfy, G.; Marcsik, A.; Nerlich, A. G. *International Journal of Osteoarchaeology* **2007**, *17*, 380-391.
3. Ziskind, B.; Halioua, B. *Review of Respiratory Diseases* **2007**, *24*, 1277-1283.
4. Retief, F. P.; Cilliers, L. *South Africa Journal of Science and Technology* **2008,** *27*, 229-239.
5. Nerlich, A. G.; Haas, C. J.; Zink, A.; Sziemles, U.; Hagedorn, H. G. *Lancet* **1997,** *350*, 1404-1404.
6. World Health Organisation (W.H.O), http://whqlibdoc.who.int/publications/2010/9789241564069_eng.pdf accessed on 24-07-2010.
7. World Health Organisation (W.H.O), *www.who.int/mediacentre/factsheets/fs104/en/index.html* accessed on 05-08-2009
8. World Health Organisation (W.H.O); *www.who.int/tb/challenges/xdr/en/index.html* accessed on 03-11-2010.
9. National Institute of Allergy and Infectious Diseases (NIAID); webpage: *www.niaid.nih.gov/topics/tuberculosis/Understanding/Pages/tbHIV.aspx* accessed on 03-11-2010.
10. Janin, Y. L. *Bioorganic & Medicinal Chemistry* **2007**, *15*, 2479-2513.
11. Chatterjee, D. *Current Opinion in Chemical Biology* **1997**, *1*, 579-588.
12. Takayama, K.; Kilburn, J. O. *Antimicrobial Agents and Chemotherapy* **1989,** *33*, 1493-1499.
13. Mikusova, K.; Slayden, R. A.; Besra, G. S.; Brennan, P. J. *Antimicrobial Agents and Chemotherapy* **1995**, *39*, 2484-2489.
14. Lee, R. E.; Mikusova, K.; Brennan, P. J.; Besra, G. S. *Journal of the American Chemical Society* **1995**, *117*, 11829-11832.
15. Chopra, I.; Brennan, P. *Tubercle and Lung Disease* **1998**, *78*, 89-98.
16. Winder, F. G.; Collins, P. B. *Journal of General Microbiology* **1970**, *63*, 41-48.
17. Takayama, K.; Schnoes, H. K.; Armstrong, E. L.; Boyle, R. W. *Journal of Lipid Research* **1975**, *16*, 308-317.
18. Winder, F. G.; Collins, P. B.; Whelan, D. *Journal of General Microbiology* **1971,** *66*, 379-&.
19. Wang, F.; Langley, R.; Gulten, G.; Dover, L. G.; Besra, G. S.; Jacobs, W. R.; Sacchettini, J. C. *Journal of Experimental Medicine* **2007**, *204*, 73-78.
20. Zhang, Y.; Young, D. *Journal of Antimicrobial Chemotherapy* **1994**, *34*, 313-319.
21. National Institute of Allergy and Infectious Diseases (NIAID); webpage: *http://www.niaid.nih.gov/topics/tuberculosis/Understanding/WhatIsTB/ScientificIllustrations/Pages/firstLineIllustration.aspx*; NIAD, 25-10-2010.
22. Ying, Z. *Annual Review of Pharmacology and Toxicology* **2005**, *45*, 529-564.
23. World Health Organisation (W.H.O) *www.stoptb.org/global/plan/* accessed on 03-11-2010.
24. World Health Organisation (W.H.O) *www.who.int/tb/features_archive/global_plan_to_stop_tb/en/index.html* accessed on 02-11-2010.
25. World Health Organisation (W.H.O) *www.stoptb.org/assets/documents/global/plan/Davos* speech Marcos.pdf accessed on 03-11-2010.
26. Rivers, E. C.; Mancera, R. L. *Current Medicinal Chemistry* **2008**, *15*, 1956-1967.
27. Rivers, E. C.; Mancera, R. L. *Drug Discovery Today* **2008**, *13*, 1090-1098.
28. Shi, R. R.; Sugawara, I. *Tohoku Journal of Experimental Medicine* **2010**, *221*, 97-106.
29. Barry, C. E.; Blanchard, J. S. *Current Opinion in Chemical Biology***,** *14*, 456-466.
30. Andries, K.; Verhasselt, P.; Guillemont, J.; Gohlmann, H. W. H.; Neefs, J. M.; Winkler, H.; Van Gestel, J.; Timmerman, P.; Zhu, M.; Lee, E.; Williams, P.; de Chaffoy, D.; Huitric, E.; Hoffner, S.; Cambau, E.; Truffot-Pernot, C.; Lounis, N.; Jarlier, V. *Science* **2005**, *307*, 223-227.

31. Gelber, R.; Andries, K.; Paredes, R. M. D.; Andaya, C. E. S.; Burgos, J. *Antimicrobial Agents and Chemotherapy* **2009**, *53*, 3989-3991.
32. Lounis, N.; Gevers, T.; Van Den Berg, J.; Andries, K. *Antimicrobial Agents and Chemotherapy* **2008**, *52*, 3568-3572.
33. Koul, A.; Vranckx, L.; Dendouga, N.; Balemans, W.; Van den Wyngaert, I.; Vergauwen, K.; Goehlmann, H. W. H.; Willebrords, R.; Poncelet, A.; Guillemont, J.; Bald, D.; Andries, K. *Journal of Biological Chemistry* **2008**, *283*, 25273-25280.
34. Ibrahim, M.; Truffot-Pernot, C.; Andries, K.; Jarlier, V.; Veziris, N. *American Journal of Respiratory and Critical Care Medicine* **2009**, *180*, 553-557.
35. Veziris, N.; Ibrahim, M.; Lounis, N.; Chauffour, A.; Truffot-Pernot, C.; Andries, K.; Jarlier, V. *American Journal of Respiratory and Critical Care Medicine* **2009**, *179*, 75-79.
36. Ibrahim, M.; Andries, K.; Lounis, N.; Chauffour, A.; Truffot-Pernot, C.; Jarlier, V.; Veziris, N. *Antimicrobial Agents and Chemotherapy* **2007**, *51*, 1011-1015.
37. Diacon, A. H.; Pym, A.; Grobusch, M.; Patientia, R.; Rustomjee, R.; Page-Shipp, L.; Pistorius, C.; Krause, R.; Bogoshi, M.; Churchyard, G.; Venter, A.; Allen, J.; Palomino, J. C.; De Marez, T.; van Heeswijk, R. P. G.; Lounis, N.; Meyvisch, P.; Verbeeck, J.; Parys, W.; de Beule, K.; Andries, K.; Mc Neeley, D. F. *New England Journal of Medicine* **2009**, *360*, 2397-2405.
38. Abstract no.63 submitted to the American Chemical Society Meeting, Anheim CA, March 28-April 01 2004: *http://wiz2.pharm.wayne.edu/mediabstracts2004.pdf* downloaded on 12-08-2010.
39. Arora, S. K.; Sinha, N.; Jain, S.; Upadhayaya, R. S.; Jain, G.; Ajay, S.; Sinha, R. K. *International patent* **2004**, *WO/2004/026828*.
40. National Institute of Allergy and Infectious Diseases (NIAID); webpage: *http://www.niaid.nih.gov/topics/tuberculosis/Understanding/WhatIsTB/ScientificIllustrations/Pages/newTBdrugs.aspx*, 25-10-2010.
41. Lee, R. E.; Protopopova, M.; Crooks, E.; Slayden, R. A.; Terrot, M.; Barry, C. E. *Journal of Combinatorial Chemistry* **2003**, *5*, 172-187.
42. Protopopova, M.; Hanrahan, C.; Nikonenko, B.; Samala, R.; Chen, P.; Gearhart, J.; Einck, L.; Nacy, C. A. *Journal of Antimicrobial Chemotherapy* **2005**, *56*, 968-974.
43. Jia, J.; Tomaszewski, J. E.; Hanrahan, C.; Coward, L.; Noker, P.; Gorman, G.; Nikonenko, B.; Protopopova, M. *British Journal of Pharmacology* **2005**, *144*, 80-87.
44. Jia, L.; Noker, P. E.; Coward, L.; Gorman, G. S.; Protopopova, M.; Tomaszewski, J. E. *British Journal of Pharmacology* **2006**, *147*, 476-485.
45. Chen, P.; Gearhart, J.; Protopopova, M.; Einck, L.; Nacy, C. A. *Journal of Antimicrobial Chemotherapy* **2006**, *58*, 332-337.
46. National Institute of Allergy and Infectious Diseases (NIAID); webpage: *www.niaid.nih.gov/topics/tuberculosis/Research/treatment/pages/sq109timeline.aspx* accessed 05-08-2010.
47. Lenaerts, A. J.; DeGroote, M. A.; Orme, I. M. *Trends in Microbiology* **2008**, *16*, 48-54.
48. Griffin, G. W.; Marchand, A. P. *Chemical Reviews* **1989**, *89*, 997-1010.
49. Marchand, A. P. *Chemical Reviews* **1989**, *89*, 1011-1033.
50. Marchand, A. P. *Advances in theoretically interesting molecules.*, 1989.
51. Smith, J. P.; Riley, T. R.; Devenyi, A.; Bingaman, S. I.; Kunselman, A. *Journal of General Internal Medicine* **2004**, *19*, 662-668.
52. Davies, W. L.; Hoffmann, C. E.; Paulshock, M.; Wood, T. R.; Haff, R. F.; Grunert, R. R.; Watts, J. C.; Hermann, E. C.; Neumayer, E. M.; McGahen, J. W. *Science* **1964**, *144*, 862-&.
53. Stanicova, J.; Miskovsky, P.; Sutiak, V. *Veterinarni Medicina* **2001**, *46*, 244-256.
54. Nagasawa, H. T.; Elberlin.Ja; Shirota, F. N. *Journal of Medicinal Chemistry* **1973**, *16*, 823-826.
55. Zah, J.; Terre'Blanche, G.; Erasmus, E.; Malan, S. F. *Bioorganic & Medicinal Chemistry* **2003**, *11*, 3569-3578.
56. Geldenhuys, W. J.; Terre'Blanche, G.; Van der Schyf, C. J.; Malan, S. F. *European Journal of Pharmacology* **2003**, *458*, 73-79.

57. Brookes, K. B.; Hickmott, P. W.; Jutle, K. K.; Schreyer, C. A. *South African Journal of Chemistry* **1992**, *45*, 8-11.
58. Onajole, O. K.; Govender, K.; Govender, P.; van Helden, P. D.; Kruger, H. G.; Maguire, G. E. M.; Muthusamy, K.; Pillay, M.; Wiid, I.; Govender, T. *European Journal of Medicinal Chemistry* **2009**, *44*, 4297-4305.
59. Onajole, O. K.; Coovadia, Y.; Govender, T.; Kruger, H. G.; Maguire, G. E. M.; Naidu, D.; Singh, N.; Govender, P. *Chemical Biology and Drug Design* accepted for publication **2010**.
60. *Tuberculosis (Edinb)* **2008**, *88*, 159-61.
61. Onajole, O. K.; Sosibo, S.; Govender, P.; Govender, T.; van Heiden, P. D.; Maguire, G. E. M.; Majerski, K. M.; Wiid, I.; Kruger, H. G. *Chemical Biology and Drug Design* **2010**, *manuscript under review*.
62. Onajole, O. K.; Govender, P.; Govender, T.; Maguire, G. E. M.; Kruger, H. G. *Structural Chemistry* **2010**, article in press.
63. Onajole, O. K.; Belewa, X. V.; Coovadia, Y.; Govender, T.; Kruger, H. G.; Maguire, G. E. M.; Naidu, D.; Somai, B.; Singh, N.; Govender, P. *Medicinal chemistry Research* **2010**, article in press.
64. Onajole, O. K.; Makatini, M. M.; Govender, P.; Govender, T.; Maguire, G. E. M.; Kruger, H. G. *Magnetic Resonance in Chemistry* **2010**, *48*, 249-55.
65. Onajole, O. K.; Govender, P.; Govender, T.; Maguire, G. E. M.; Kruger, H. G. *Structural Chemistry* **2009**, *20*, 1067-1076.
66. Odisitse, S.; Jackson, G. E.; Govender, T.; Kruger, H. G.; Singh, A. *Dalton Transactions* **2007**, 1140-1149.

CHAPTER 2

PENTACYCLOUNDECANE DERIVED CYCLIC TETRA-AMINES: SYNTHESIS AND EVALUATION AS POTENT ANTI-TUBERCULOSIS AGENTS

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Abstract

As part of an ongoing effort to develop highly potent anti-tuberculosis agents, fourteen pentacycloundecane (PCU) tetra-amine compounds were synthesized and screened for their in vitro anti-mycobacterial activity against two TB strains, H_{37}Rv and XDR 194 [an extensively drug-resistant strain of tuberculosis (TB)]. Using the broth macrodilution method, nitrofuranylamide based compounds (**6a** and **6b)** showed almost similar activities against the H_{37}Rv strain of *Mycobacterium tuberculosis* when compared with the control drug, ethambutol. *N*-geranyl piperazine PCU (**8a**) and *trans-trans* farnesyl piperazine PCU (**8b**) were 3.2 and 3.7 times more potent than commercially available ethambutol. Both isoprenyl PCU tetra-amine derivatives and *N*-decyl piperazine PCU (**9a**) were highly active against the XDR 194 strain of tuberculosis with MICs in the range of 0.63 - 3.02 μM. Cytotoxicities (IC_{50}) of isoprenyl based compounds (**8a**, **8b**) and compound **9a** were tested on a mammalian cell line [MDBK (Madin Darby bovine kidney epithelium)] with values of 30, 24 and 25 μM respectively.

Keywords: XDR-tuberculosis (TB), Pentacycloundecane, Isoprenyl, SQ109

1. Introduction

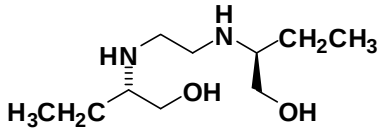
The WHO estimate of 9.2 million new tuberculosis cases with 1.7 million deaths in 2006, indicates that tuberculosis (TB) still accounts for a significantly large proportion of the global disease burden and mortality [**1**]. India, China, Indonesia, South Africa and Nigeria featured as the top five of the highest affected countries. The highest incidence rates were found in Africa, with twelve of the fifteen countries in this continent having the most severe infection rates.

The worldwide increase in prevalence of *Mycobacterium tuberculosis* and the emergence of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB has imposed a serious setback on global TB control [**2**]. There were approximately 0.5 million reported cases of MDR-TB throughout the world in 2006 [**2**]. Aside from protracted treatment periods [**2**] and the use of more toxic and expensive second line drugs [**3**], MDR-TB is associated with low cure rates and high mortality [**4**]. XDR-TB found in most parts of the world [**3**] is almost untreatable and is accompanied by higher mortality rates than MDR-TB [**5**].

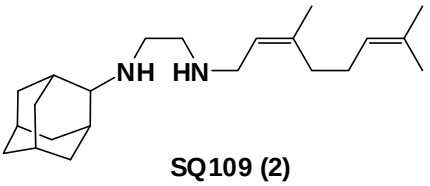
The difficulties associated with the long duration of therapy, such as patient non-compliance following improvement after the intensive phase and ingestion of multiple doses consisting of many tablets have also led to a need to develop simple drug regimen. The ensuing limited treatment options for MDR and XDR-TB have created a renewed interest in the development of novel anti-TB drug candidates. These new drugs must effectively shorten treatment time and act against the subpopulation of slowly metabolising bacilli.

Among the promising potential anti-TB drugs currently undergoing human clinical trials are the third generation fluoroquinolones, gatifloxacin and moxifloxacin, diarylquinolone TMC 207, nitroimidazole PA-824 and nitroimidazo-oxazole OPC-67683 [**6**]. Other drugs that are in the preclinical phase include the diamine SQ109 (**2**), dipiperidines SQ609, nitroimidazo-oxazole back-up, synthase inhibitor FAS20013, translocase I inhibitors and non-fluorinated quinolones [**6**].

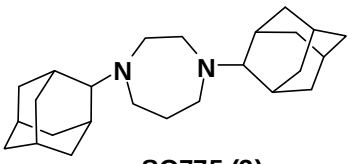
SQ109 (*N*-geranyl-*N′*-(2-adamantyl)ethane-1,2-diamine), developed by Sequella Inc. was first synthesized by Lee et al.[7] using solid phase synthesis of 1,2 diamine analogues of ethambutol (EMB) (1) (Fig. 1). From the several 1,2 diamine derivatives obtained using a combinatorial approach, SQ109 (2) was found to be the most potent new anti-TB lead. SQ109 (2) is an effective compound against EMB resistant strains [8]. Its activity against XDR-TB strains and a 25 % reduction in the time to cure mice by enhancing the activity of isoniazid and rifampicin was also reported. SQ 775 (3) is an example of a cyclic diamine compound with an activity similar to the control drug EMB [9].



Ethambutol (1)



SQ109 (2)



SQ775 (3)

Figure 1: Structures of Ethambutol, SQ109 and SQ775

Non-TB related research showed that polycyclic "cage" compounds (such as adamantane and pentacycloundecane) have been reported to improve drug lipophilicity, thus serving as a transport aid in carrying such drugs across the Blood Brain Barrier (BBB) or Central Nervous System (CNS) [**10,11**]. It can also reduce the bio-degradation of the drug thereby prolonging the pharmaceutical effect of such agents in the body [**11-14**]. SQ109 and SQ775 both have an adamantane moiety in common suggesting that this skeleton might be contributing to its lipophilicity thereby enhancing their anti-TB activities.

In a similar development, Tangallapally et al. [**15, 16**] recently reported novel nitrofuranylamide compounds with promising in vitro anti-tuberculosis activities. However, this activity could not be duplicated in in vivo analysis due to a short serum half life and rapid elimination of the compound. This led to the introduction of a bicyclic (tetrahydroisoquinoline) moiety in order to make it more resistant to proteolysis [**17**]. This particular modification showed an improvement in serum half life while improving its MIC activity for compound **4** [**17**].

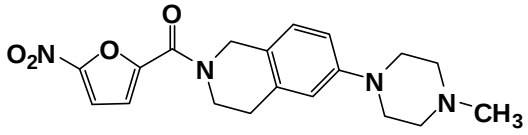


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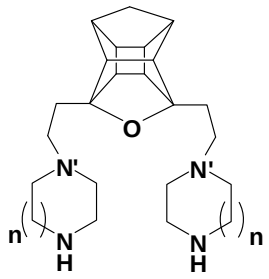
Figure 2: Lead compound **4**.

For our work it was proposed that the replacement of the bicyclic tetrahydroisoquinoline with a more rigid lipophilic compound such as the pentacycloundecane (PCU) moiety, a polycyclic "cage", might further enhance the activity by facilitating the movement of these molecules across the lipid-enriched bacterial cell membrane.

Inspired by the work of Lee et al. [7] and Tangallapally et al. [15-17] a series of novel amine based compounds (5-13) bearing the lipophilic PCU molecule were designed and synthesized (Figure 3).



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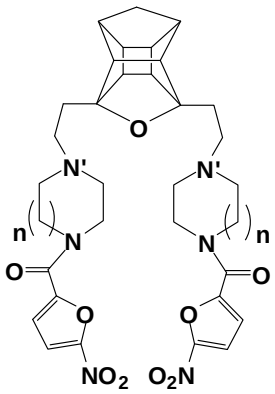
5b: n = 2



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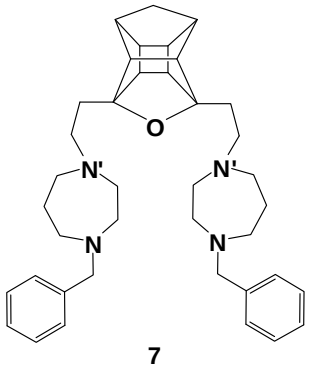

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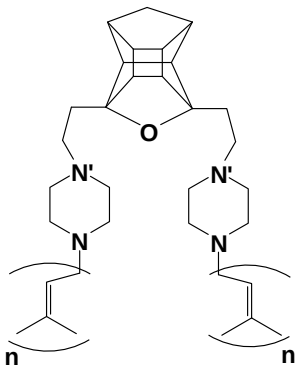
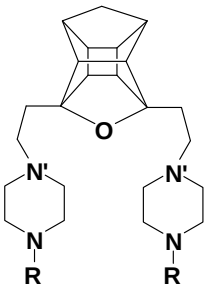
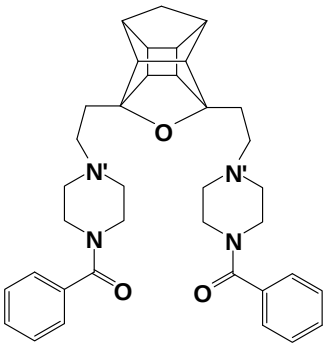


8a: n = 2
8b: n = 3



9a: R = $C_{10}H_{21}$
9b: R = $C_{15}H_{31}$
9c: R = $C_{20}H_{41}$



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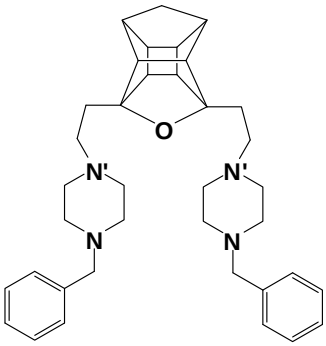

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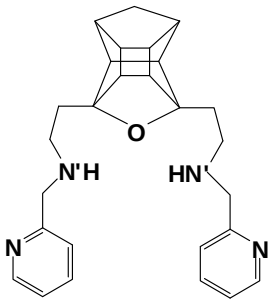

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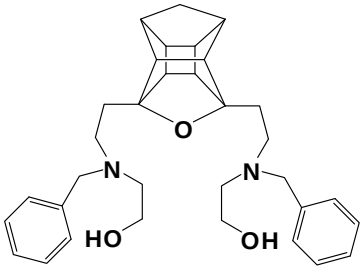

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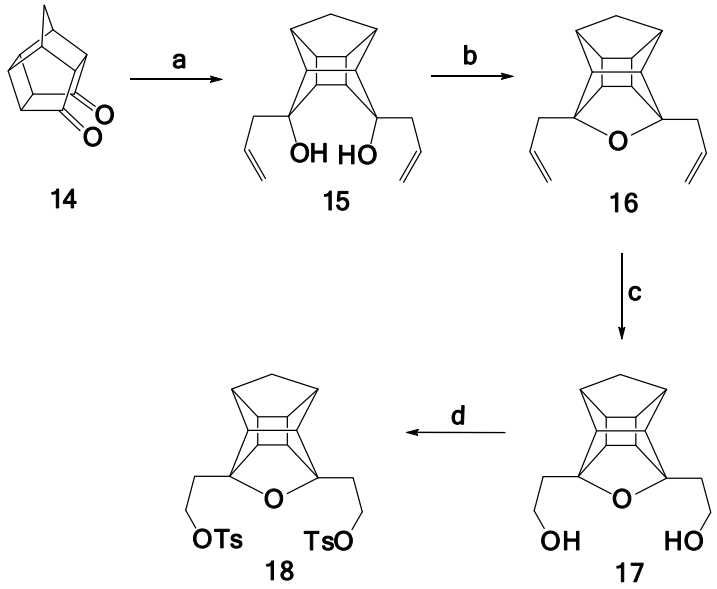
Figure 3: PCU amine derivatives **5-13**.

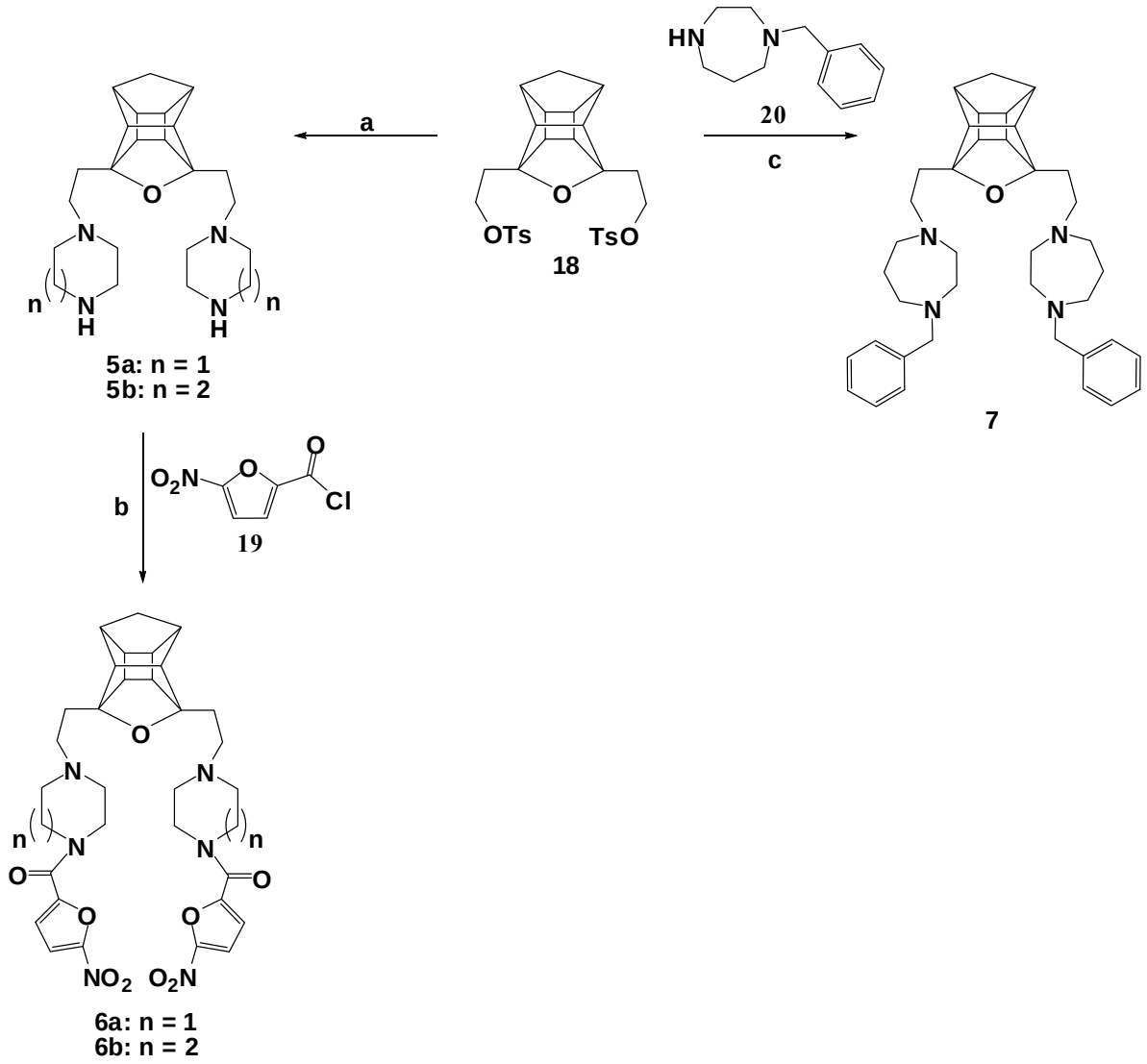
2. Chemistry

The starting material, PCU ditosylate [18] was synthesized from pentacycloundecane-8,11-dione as illustrated in Scheme 1. The starting material, Cookson's dione (14) [19] was reacted with freshly prepared allyl magnesium bromide (Grignard reaction) to afford the endo-8,endo-11 diol (15) which upon dehydration under Dean-Stark conditions, yielded the corresponding 3,5-dially-4-oxahexacyclo [5.4.1.02,6.03,10.05,9.08,11] dodecane (16) [20]. Ozonolysis of the hexacyclic ether (16) followed by reductive work up yielded the PCU diol (17) [18] which upon tosylation gave the PCU ditosylate (18) [18]. The overall yield of (18) from (14) was 60 %.



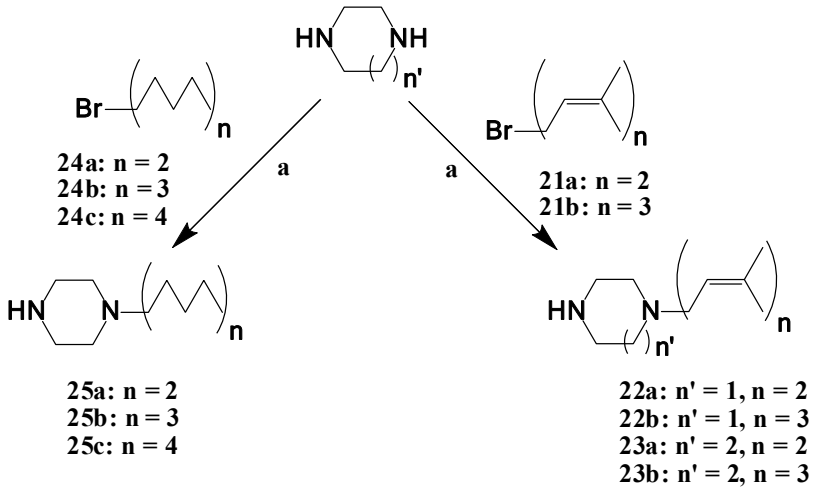
Scheme 1: Reagents and conditions: (a) $H_2C{=}CHCH_2MgBr$, dry THF; (b) Dean-Stark apparatus, H_2SO_4, benzene, reflux; (c) O_3, dry CH_3OH, $NaBH_4$; (d) *p*-TsCl, powdered KOH, THF.

PCU ditosylate (**18**) was reacted with excess piperazine/homopiperazine in dry dichloromethane at -78ºC to afford the tetra-amines **5a** and **5b** respectively (62 %) and these were reacted with 5-nitrofuran-2-carbonyl chloride (**19**) to obtain compounds **6a** and **6b** respectively (65 %). PCU ditosylate (**18**) was reacted with *N*-benzyl homopiperazine (**20)** at a 1:2.2 ratio with triethylamine under reflux conditions to obtain compound **7** (Scheme 2).



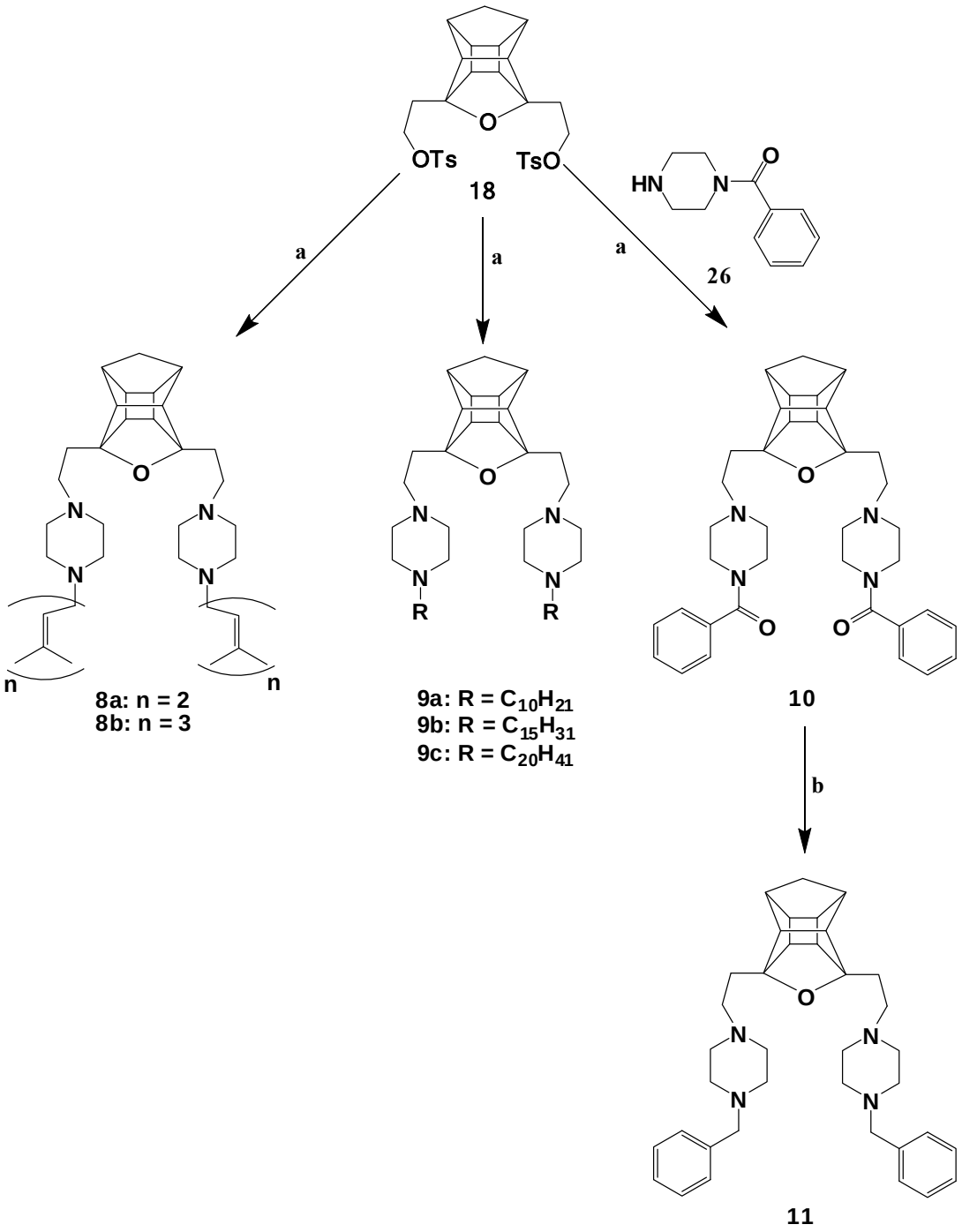
Scheme 2: Reagents and conditions: (a) piperazine/homopiperazine, DCM, -78^{0}C, rt, 24 hrs; (b) DCM, Et_3N, reflux 16 hrs; (c) CH_3CN, Et_3N, reflux, N_2 atm.

Compound **5a** was successfully reacted with geranyl bromide (**21a**) to afford compound **8a** but its purification proved difficult *via* column chromatography. To solve this problem, a new synthetic route was introduced. Applying the methodology employed to synthesize compound **5a**, geranyl bromide (**21a**) was reacted with excess piperazine/homopiperaine to obtain *N*-geranyl piperazine (**22a**) and *N*-geranyl homopiperazine (**23a**) while the reaction of *trans-trans* farnesyl bromide (**21b**) with piperazine/homopiperazine afforded compounds **22b** and **23b**. Piperazine was also reacted with three linear alkane chains (C10, C15 and C20) to obtain compounds **25a**, **25b** and **25c** (Scheme 3).



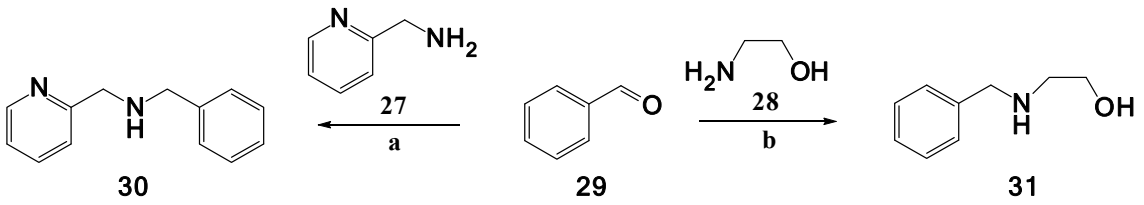
Scheme 3: Reagents and conditions: (a) piperazine/homopiperazine, DCM, -78^{0}C, rt, 24 hrs.

Reaction of PCU ditosylate (**18**) with compound **22a** and **22b** in the presence of K_2CO_3 with reflux resulted in compounds **8a** and **8b** respectively (yield 71 %) which were easily purified *via* column chromatography. Attempts to react PCU ditosylate (**19**) with **23a** and **23b** using a similar methodology employed in the synthesis of compounds **8a** and **8b** were unsuccessful. PCU ditosylate (**18**) was reacted with **25a**, **25b** and **25c** to yield linear alkane derivatives of PCU dipiperazine **9a**, **9b** and **9c** respectively. Reaction of the PCU ditosylate (**18**) with *N*-benzoyl piperazine (**26**) yielded compound **10**, followed by the reduction of the carbonyl group using lithium aluminium hydride to obtain the corresponding benzyl tetra-amine **11** in 55 % yield (Scheme 4).



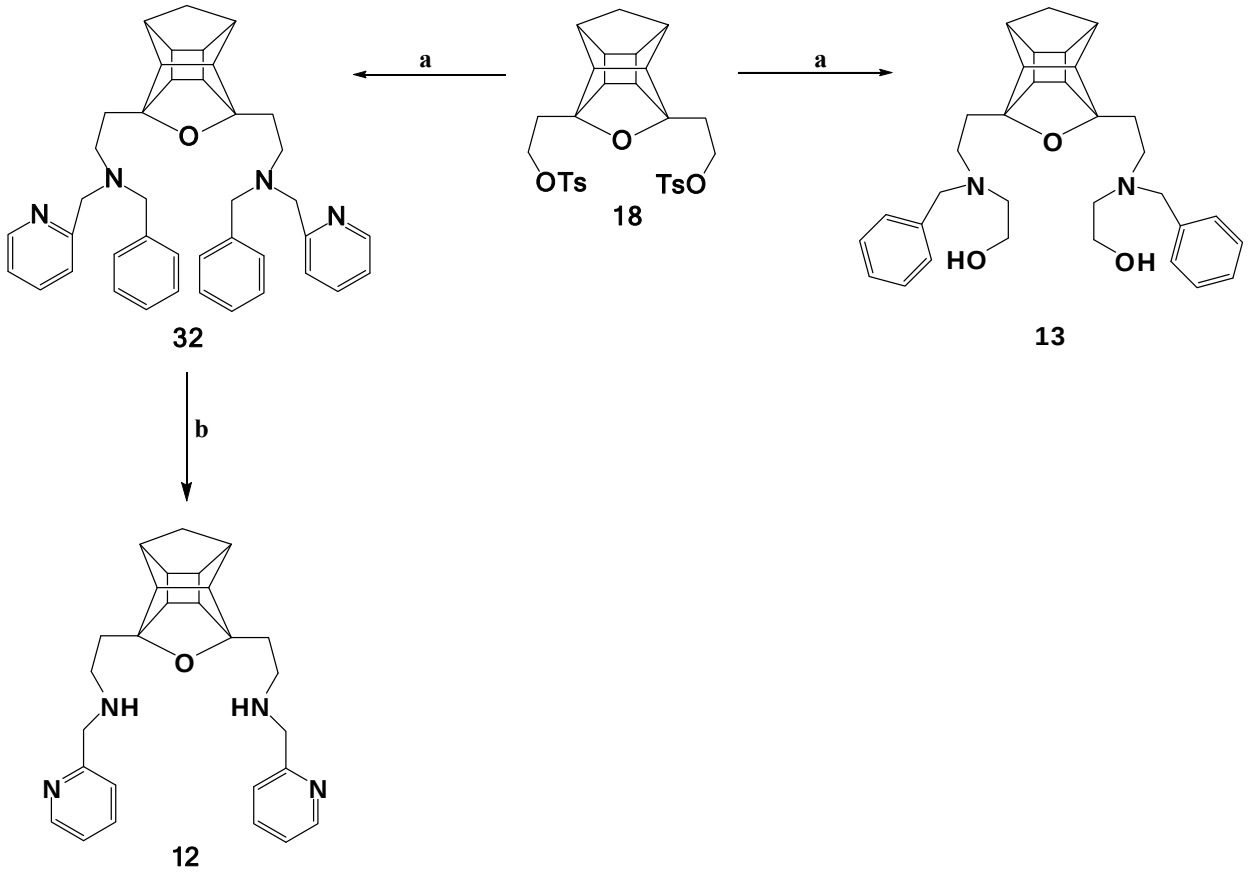
Scheme 4: Reagents and conditions: (a) **22** or **25** or **26**, K_2CO_3, CH_3CN, reflux under N_2 atm; (b) $LiAlH_4$, dry THF and reflux under N_2 atm, 36 hours.

2-(aminomethyl) pyridine **(27)** and ethanolamine **(28)** were reacted with benzaldehyde **(29)** *via* reductive amination to obtain their corresponding secondary amines, *N*-benzyl-*N*-{(pyridin-2-yl)methyl}amine **(30)** [**21,22**] and 2-(Benzylamino)ethanol **(31)** [**23**] (Scheme 5).



Scheme 5: Reagents and conditions: (a) CH_3CH_2OH, rt, l hr, $NaBH_4$, reflux, stir overnight, N_2 atm; (b) CH_3OH, rt, 2 hr, $NaBH_4$, stir overnight, N_2 atm.

N-benzyl-*N*-{(pyridin-2-yl)methyl}amine **(30)** was reacted with PCU ditosylate **(18)** to obtain compound **(32)** after which the benzyl protecting groups were removed by catalytic hydrogenation using ammonium formate with 10 % palladium on carbon to obtain compound **12** (yield 67 %). 2-(benzylamino) ethanol **(31)** was treated with PCU ditosylate **(18)** to obtain compound **13** in an overall yield from **18** of 70 % (Scheme 6).



Scheme 6: Reagents and conditions: (a) 30 or 31, CH_3CN, Et_3N, reflux, N_2 atm; (b) 10 % Pd/C, ammonium formate, dry MeOH, reflux, 16 hrs, N_2 atm.

3. Results and Discussion

All synthesized novel PCU amine derivatives **5-13** excluding **9** were screened in vitro against *M. tuberculosis* $H_{37}Rv$ (ATCC 25618) using a broth macrodilution method (BMM). The ClogP of each novel compound was obtained using the ACDLABS 11.0 program.* The results are depicted in Table 1.

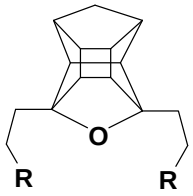
MIC results were obtained after 21 days of incubation. Compounds **5a** and **5b** differ in structure only by the ring sizes of the diamines with **5a** being a six membered and **5b** a seven membered ring. Compound **5b** shows low activity against the $H_{37}Rv$ strain, while **5a** did not show any activity at the highest concentration tested. Modification to these compounds with the introduction of a nitrofuran group to form compounds **6a** and **6b** gave promising activity with MIC values of 24.1 and 23.1 μM respectively which are similar to the control drug, EMB. Compounds **6a** and **6b** did not show any significant difference in calculated ClogP values when compared to **5a** and **5b** respectively.

Compound **7** (the benzyl derivative of **5b**) exhibits similar activity to **5b** (μg/mL) while compound **11** (the benzyl derivative of **5a**) demonstrates an improved activity against $H_{37}Rv$, whilst **5a** did not show comparable activity. The benzoyl derivative **10** (of **5a**) showed a weaker potency compared to **11**. Compound **12** and **13** did not show any significant activity.

Based on results obtained by Lee et al. [**7, 8**] in the development of SQ109 **(2)**, it was decided to synthesize the geranyl and *trans-trans* farnesyl derivative of **5a** and **5b**. As discussed above, the isoprenyl derivatives of **5b** proved to be elusive. Compounds **8a** and **8b** had MIC values of 6.09 and 5.04 μM respectively. The activity of these lipophilic alkene bearing compounds (**8a** and **8b)** were similar (expressed in μg/mL) to the commercially available analogue, EMB, when screened against $H_{37}Rv$.

* downloaded from www.acdlabs.com.

Table 1: ClogP values and in vitro anti-mycobacterial activity on day 21 of novel PCU amine compounds (5-13) against *M. tuberculosis* $H_{37}Rv$.



Compound	R	MW	ClogP[a]	$H_{37}Rv$ (MIC)	
				µg/mL	µM
1	Ethambutol	204	NA	4	19.6
5a	-N'$(CH_2)_4NH$	385	0.41 ± 0.55	>128	ND
5b	-N'$(CH_2)_5NH$	413	1.18 ± 0.54	64	155
6a	-N'$(CH_2)_4NCO(C_4H_2O)NO_2$	663	0.57 ± 0.83	16	24.1
6b	-N'$(CH_2)_5NCO(C_4H_2O)NO_2$	691	1.32 ± 0.81	16	23.2
7	-N'$(CH_2)_5NCH_2C_6H_5$	592	4.11 ± 0.73	64	108.1
8a	-N'$(CH_2)_4NC_{10}H_{17}$	657	7.35 ± 0.73	4	6.09
8b	-N'$(CH_2)_4NC_{15}H_{25}$	793	11.42 ± 0.77	4	5.04
10	-N'$(CH_2)_4NCOC_6H_5$	592	1.47 ± 0.67	128	216.2
11	-N'$(CH_2)_4NCH_2C_6H_5$	565	2.61 ± 0.83	64	113.3
12	2-pyridylmethylamino[c]	429	0.82 ± 0.40	128	298
13	-N[$(CH_2)_2OH$]$CH_2C_6H_5$	515	4.22 ± 0.54	128	248

[a]ClogP was calculated using ACD/labs software v11.0; MIC: Minimal Inhibitory Concentration; ND: Not determined. MW: Molecular weight. NA: Not applicable.

Based on the results for compounds **6a**, **6b**, **8a** and **8b** we decided to test the molecules with a more accurate method (BACTEC 460 TB system) against the $H_{37}Rv$ strain. Compounds **6a** and **6b** (Table 2) showed little difference when compared to the results obtained from the BMM method. Further screening was not carried out, although it is known that MICs of drugs differ slightly depending on the assay method used [**25**]. Due to the activity of **8a** and **8b** we decided to investigate the effect of using long alkane chains in place of the alkene derivatives to determine whether this functionality is essential for efficacy. This led to the synthesis of compounds **9a** (ten carbon alkane), **9b** (fifteen carbon alkane) and **9c** (twenty carbon alkane) (Scheme 4). Anti-mycobacterial screening of compounds **9b** and **9c** was not possible however; due to their insolubility at biological pH presumably due to their highly lipophilic nature (ClogP values are 14.43 ± 0.52 and 19.74 ± 0.52 respectively). Compounds **8a, 8b** and **9a** were tested against an XDR strain of *M. tuberculosis* [strain

X194, resistant to first line drugs (Isoniazid and Rifampicin) and second line drugs (Kanamycin, Ofloxacin and Amikacin)] (BACTEC 460 TB system). These compounds were further analyzed for cytotoxicity on mammalian cells from the MDBK (Madin Darby bovine kidney epithelium) line (Table 2).

Table 2: BACTEC and IC_{50} results using MDBK cell line for selected compounds.

Compound	ClogP	$H_{37}Rv$ (MIC, µM)	$H_{37}Rv$ (MIC, µg/mL)	XDR 194 (MIC, µM)	XDR 194 (MIC, µg/mL)	IC_{50} (µM)
6a	0.57±0.83	MIC>12.07 (SD±7.47)	MIC>8 (SD±4.24)	NT	NT	NT
6b	1.32±0.81	MIC>11.58 (SD±7.16)	MIC>8 (SD±4.24)	NT	NT	NT
8a	7.35 ± 0.73	1.52 > MIC > 0.76 (SD ± 0.54)	1 > MIC > 0.5 (SD ± 0.35)	3.04 > MIC > 1.52 (SD ± 0.54)	2.0 > MIC > 1.0 (SD ± 0.71)	30
8b	11.42 ± 0.77	0.63 > MIC > 0.32 (SD ± 0.22)	0.5 > MIC > 0.25 (SD ± 0.18)	1.26 > MIC > 0.63 (SD ± 0.45)	1 > MIC > 0.5 (SD ± 0.35)	24
9a	9.12 ± 0.52	NT	NT	1.5 > MIC > 0.75 (SD ± 0.53)	1 > MIC > 0.5 (SD ± 0.35)	25
SQ109*	6.04 ± 0.45	0.63	-	-	-	26

*literature value [**8**]; NT: Not Tested.

Compounds **8a** and **8b** show potent activity against the $H_{37}Rv$ strain with **8b** being approximately two-fold more active than **8a** against both $H_{37}Rv$ and XDR 194 strains respectively. The MIC for EMB in $H_{37}Rv$ is 0.94 µg/mL [**25**] the MIC for **8b** against $H_{37}Rv$ was lower (0.5 - 0.25 µg/mL, Table 2). The XDR strain used was resistant to EMB at the breakpoint concentration of 2.5 µg/mL in BACTEC [**24**]. Therefore, the MIC for the XDR strain lies beyond 2.5 µg/mL. This means that the efficacy of compound **8b** is between 2.5 to 5 times more effective in inhibiting the XDR strain than EMB (**8b** killed the strain between 1 µg/mL and 0.5 µg/mL, Table 2). Also, the sensitivity for **8b** between $H_{37}Rv$ and X194 was approximately two fold (Table 2). The results indicate that our compounds properly have a different cellular target to that of EMB.

9a proved to be twice as active as compound **8a** against the XDR strain of *M. tuberculosis*. It was not considered necessary to test **9a** using the BMM method since the BACTEC system is much more accurate. Compound **9a** has a higher ClogP value compared to **8a** (Table 2), suggesting that lipophilicity could play an important role in the activity of this class of compounds. However, this assumption could not be further evaluated due to insolubility problems encountered with compounds **9b** and **9c**. It should be noted that the MICs for **9a** and **8b** fall in the same range (1 > MIC > 0.5), however conversion to µM makes quite a significant difference as compound **8b** is more active than **9a** (**8b** has a larger molecular weight).

Compounds **8a** and **8b** are structurally related to SQ109, they all have polycyclic "cage" moieties, diamines (linear or cyclic) and long alkene chains, which form the basis of this comparison. As illustrated in Table 2, **8a** is only half as active as SQ109 whereas **8b** is twice as active as SQ109 [**8**]. This suggests that the length of the alkene chain (lipophilicity again) may be playing an important role in the activity of these compounds. The cytotoxicities (IC_{50}, Table 2) of these compounds (**8a**, **8b** and **9a**) and that of SQ109 fall in the same range between 20 – 30 μM with compound **8a** being the least toxic.

A potential anti-TB candidate needs to be evaluated as a drug combination, i.e. the possibility of incorporating such an anti-TB candidate with other existing anti-TB drugs. Based on this, further studies were carried out to investigate the interaction of compound **8b** (the most active compound in this series) with two known anti-TB drugs (Isoniazid and Rifampicin) by means of in vitro testing against the H_{37}Rv strain of *M. tuberculosis*. The interaction of compound **8b** with these two anti-TB drugs could be antagonist, additive, synergistic or have no effect at all. Results obtained using once again the BACTEC 460 TB system showed that compound **8b** (0.5 μg/mL) had no antagonist or synergistic effect; it did however have an additive effect.

4. Conclusion

A series of novel PCU tetra-amines (fourteen compounds) were synthesized and screened for their anti-TB activity of which five compounds (**6a**, **6b**, **8a**, **8b** and **9a**) displayed the highest anti-TB activity against *M. tuberculosis* H_{37}Rv. Compounds **8a**, **8b** and **9a** were the most potent with excellent activities giving MICs of 3.04, 1.26 and 1.50 μM respectively against an XDR-TB strain (X194). These results indicate that lipophilicity is an important component of their efficacy (having larger ClogP values than SQ109). This does however limit their potential application due to solubility problems (as seen for molecules **9b** and **9c**). We are examining the design of new derivatives in terms of their structure-activity relationship. It is hoped that new more soluble active species can be obtained. The first generation of these active compounds however, is currently undergoing further in vitro and in vivo analysis.

5. Experimental

The NMR data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments using $CDCl_3$ as a solvent. All chemical shifts (δ) were quoted in parts per million downfield from TMS and coupling constant (*J*) recorded in Hertz. Splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 instrument with an Attenuated Total Reflectance attachment recorded in cm^{-1}. All reactions were monitored using Thin Layer Chromatography (TLC, Merck Kieselgel 60, F254). All purifications were carried by Column Chromatography using Fluka Kieselgel 60 (70 – 230 mesh) and CH_3Cl:CH_3OH:NH_4OH (88:10:2) as the eluent (solvent mixture). Level of purity for all compounds

was judged to be >95% based upon ^{1}H NMR and LC-MS analysis. Mass Spectra were obtained using a Waters LCT Premier Time of Flight mass spectrometer. Tetrahydrofuran was freshly distilled before use from a sodium benzophenone under N_2 atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use. The syntheses of the precursors are described in their corresponding references.

ClogP gives an indication of the lipophilicity of the drug with reference to its pharmacological importance (pharmacokinetics and pharmacodynamics) [**15, 26**].

5.1.1. Synthesis of PCU tetra-amine 5a & 5b

To a vigorously stirred solution of the cyclic diamines (piperazine/homopiperazine) (22 mmol) in DCM (400 mL) at -78 ºC (dry ice, 2-propanol) under N_2 atmosphere was added dropwise PCU ditosylate (**18**, 1.2 g, 2.2 mmol) in DCM (100 mL) over 45 minutes. The reaction mixture was left to attain room temperature with stirring for 24 hours. The solution was washed with water to remove excess diamines, the obtained organic extract was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified *via* column chromatography using CH_3Cl: MeOH: NH_4OH (88:10:2) to obtain a pure product.

5.1.1a. PCU dipiperazine (5a)

A yellow oil (R_f = 0.2, 0.56 g, 68 % yield). IR ν_{max}: broad absorption (N-H) 3367, 2949, 1655, 1463, 1125 and 746 cm^{-1}. HRMS calculated for $C_{23}H_{36}N_4O$ (M + H$^+$) 385.2955, found 385.2967. ^{1}H NMR [$CDCl_3$, 400 MHz]; δ_H 1.52 (*A*B, J_{AB} = 10.1 Hz, 1H), 1.87 (A*B*, J_{AB} = 10.1 Hz, 1H), 1.99 (t, *J* = 7.92 Hz, 2H), 2.38-2.60 (m, 8H), 2.95 (2H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.7 (t), 41.8 (d), 43.4 (t), 44.5 (d), 45.6 (t), 48.0 (d), 53.7 (t), 55.1 (t), 58.8 (d), 94.87 (s).

5.1.1b. PCU dihomopiperazine (5b)

A light yellow oil, (R_f = 0.2, 0.55 g, 62 %). IR ν_{max}: broad absorption (N-H) 3386, 2818, 1465, 1108, 927, 918 and 748 cm^{-1}. HRMS calculated for $C_{25}H_{40}N_4O$ (M + H$^+$) 413.3280 found 413.3287. ^{1}H NMR [$CDCl_3$, 400 MHz] δ_H 1.48 (*A*B, J_{AB} = 10.2 Hz, 1H), 1.84 (A*B*, J_{AB} = 10.2 Hz, 1H), 1.76 (2H), 1.98 (2H), 2.35-2.69 (m, 10H), 2.89-2.94 (m, 4H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 30.0 (t), 30.4 (t), 41.8 (d), 43.4 (t), 44.5 (d), 47.2 (t), 48.0 (d), 48.6 (d), 54.3-55.0 (t), 57.8 (t), 58.8 (d), 94.8 (s).

5.1.2. Synthesis of 5-nitrofuran-2-carbonyl diamine PCU 6a & 6b

To a stirred mixture of PCU tetra-amines (**5a** and **5b**, 1.2 mmol) in DCM (2 mL) and Et_3N (670 µL, 4.8 mmol) under N_2 atmosphere was added freshly prepared 5-nitrofuran-2-carbonyl chloride (**19** [**15**] 3.6 mmol) in DCM (3 mL) and stirred for 18 hours at reflux. The reaction mixture was cooled and diluted with 60 mL of ethyl acetate and washed sequentially with (2 x 50 mL) 10 % $NaHCO_3$, (2 x 50 mL) water and (2 x 50 mL) brine. The organic layer was dried over anhydrous Na_2SO_4 and

concentrated *in vacuo*. The crude residue was purified *via* column chromatography using CH_3Cl: MeOH: NH_4OH (88:10:2).

5.1.2a. 5-nitrofuran-2-carbonyl piperazine PCU (6a)

A brown oil (R_f = 0.7, 0.56 g, 65 %). IR ν_{max}: 2927, 1630, 1352, 1021 and 752 cm^{-1}. HRMS calculated for $C_{33}H_{38}N_6O_9$ (M + H^+) 663.2779, found 663.2758. ^{1}H NMR [$CDCl_3$, 400 MHz]; δ_H 1.52 (J_{AB} = 10.1 Hz, 1H), 1.87 (J_{AB} = 10.1 Hz, 1H), 1.99 (t, J = 7.36 Hz, 2H), 2.38-2.61 (m, 10H), 3.76 (s, 2H), 3.85 (s, 2H), 7.15 (1H), 7.32 (1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.9 (t), 41.8 (d), 43.1 (t), 43.1 (t), 43.5 (t), 44.5 (d), 46.6 (t), 48.1 (d), 53.4 (t), 54.4 (t), 58.8 (d), 94.8 (s), 111.7 (d), 118.1 (d), 148.7 (s), 151.2 (s), 156.6 (s).

5.1.2b. 5-nitrofuran-2-carbonyl homopiperazine PCU (6b)

A brown oil (R_f = 0.51 g, 61 %). IR ν_{max}: 3479, 2951, 2859, 1627, 1530, 1352, 810 and 729 cm^{-1}. HRMS calculated for $C_{35}H_{42}N_6O_9$ (M + H^+) 691.3092, found 691.3105. ^{1}H NMR [$CDCl_3$, 400 MHz]; δ_H 1.52 (J_{AB} = 10.1 Hz, 1H), 1.87 (J_{AB} = 10.1 Hz, 1H), 1.96-2.03 (m, 4H) 2.37-2.88 (m, 10H), 3.72-3.77 (m, 2H), 3.84 (2H), 7.19 (1H), 7.34 (1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 27.0 (t), 28.7 (t), 30.4 (t), 41.8 (d), 43.4 (t), 44.4 (d), 46.2 (t), 47.3 (t), 47.9 (t), 48.0 (d), 48.8 (t), 54.2-54.9 (t), 54.7 (t), 58.8 (d), 94.8 (s), 111.7 (d), 118.0 (d), 149.2 (s), 151.2 (s), 157.9 (s).

5.1.3. Synthesis of N-benzyl homopiperazine PCU (7)

A mixture of *N*-benzyl homopiperazine (**20**, 0.7 g, 3.68 mmol) and PCU ditosylate (**18**, 0.93 g, 1.67 mmol) and triethylamine (350 µL, 2.5 mmol) in CH_3CN (20 mL) was refluxed for four days under N_2 atmosphere. The reaction mixture was cooled, filtered and concentrated *in vacuo*. The crude residue was purified *via* column chromatography on silica gel using CH_3Cl: MeOH: NH_4OH (88:10:2, R_f = 0.65) as eluent to give the product as a yellow oil (0.84 g, 85 %). IR ν_{max}: 2935, 2811, 1452, 1351, 1111, 729 and 695 cm^{-1}. HRMS calculated for $C_{39}H_{52}N_4O$ (M + H^+) 593.4219 found 593.4230. ^{1}H NMR [$CDCl_3$, 400 MHz] δ_H 1.48 (J_{AB} = 10.2 Hz, 1H), 1.84 (J_{AB} = 10.2 Hz, 1H), 1.77 (2H), 1.96 (t, J = 8.0 Hz, 2H), 2.35-2.73 (m, 14H), 3.60 (s, 2H), 7.26-7.31 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 27.5 (t), 30.4 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.4-55.1 (t), 58.8 (d) 62.8 (t), 94.9 (s), 126.8 (d), 128.2 (d), 128.9 (d), 139.5 (s).

5.1.4. Synthesis of N-isoprenyl/linear alkane diamines

To a vigorously stirred solution of diamine (piperazine/homopiperazine) (10 mmol) in DCM (750 mL) at -78 ºC (dry ice, 2-propanol) under N_2 atmosphere was added dropwise a solution of isoprenyl bromide (**21a** or 21b, 2 mmol)/ linear alkane bromide (**24a**, **24b** or **24c**, 2 mmol) in DCM (250 mL) over 45 minutes. The reaction mixture was left to attain room temperature with stirring for 24 hours. The solution was washed with water to remove excess piperazine, the organic extract was dried over

Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified *via* column chromatography using CH_3Cl: MeOH: NH_4OH (88:10:2).

5.1.4a. *N*-geranyl piperazine (22a)

A yellow oil (R_f = 0.5, 0.52 g, 68 %). ^{1}H NMR [$CDCl_3$, 400 MHz]; δ_H 1.56 (s, 3H), 1.60 (s, 3H), 1.64 (s, 3H), 1.98-2.08 (m, 4H), 2.52 (NH proton), 2.98 (d, 2H), 5.04 (t, *J* = 7.12 Hz, 1H), 5.22 (m, 1H). ^{13}C NMR [CDCl3, 100 MHz]: δ_C 16.4 (q), 17.7 (q), 25.7 (q), 26.4 (t), 39.8 (t), 45.1 (t), 52.8 (t), 56.2 (t), 120.1 (d), 124.0 (d), 131.6 (s), 139.7 (s).

5.1.4b. *N-trans-trans* farnesyl piperazine (22b)

A yellow oil (R_f = 0.6, 0.5 g, 65 %). ^{1}H NMR [$CDCl_3$, 400 MHz]; δ_H 1.51 (s, 3H), 1.52 (s, 3H), 1.56 (s, 3H), 1.59 (s, 3H), 1.86-2.01 (m, 8H), 2.40 (NH), 2.90 (m, 2H), 4.98-5.02 (m, 2H), 5.17 (t, 1H, *J* = 6.6 Hz). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.0 (q), 16.4 (q), 17.6 (q), 25.7 (q), 26.3 (t), 26.7 (t), 39.7 (t), 39.7 (t), 45.2 (t), 53.1 (t), 56.0 (t), 56.3 (t), 120.2 (d), 123.8 (d), 124.3 (d), 131.1 (s), 135.0 (s), 139.2 (s).

5.1.4c. *N*-geranyl homopiperazine (23a)

A yellow oil (R_f = 0.5, 0.48 g, 64 %). ^{1}H NMR [$CDCl_3$, 400 MHz]; δ_H 1.47 (s, 3H), 1.50 (s, 3H), 1.55 (s, 3H), 1.69 (*sxt*, 2H), 1.88-1.98 (m, 4H), 2.52-2.55 (m, 4H), 2.82-2.88 (m, 4H), 2.98 (*d*, 2H), 3.51 (NH), 4.93-4.97 (m, 1H), 5.11-5.13 (m, 1H). [$CDCl_3$, 100 MHz]: δ_C 16.1 (q), 17.6 (q), 25.5 (q), 26.1 (t), 29.4 (t), 39.5 (t), 46.5 (t), 48.0 (t), 54.3 (t), 55.9 (t), 56.9 (t), 121.4 (d), 123.9 (d), 131.2 (s), 138.2 (s).

5.1.4d. *N-trans-trans* farnesyl homopiperazine (23b)

A yellow oil (R_f = 0.6, 0.52 g, 66 %). ^{1}H NMR [$CDCl_3$, 400 MHz]; δ_H 1.58 (s, 3H), 1.59 (s, 3H), 1.63 (s, 3H), 1.68 (s, 3H), 1.80 (sxt, 2H), 1.95-1.98 (m, 2H), 2.04-2.12 (m, 6H), 2.59 (NH), 2.63-2.68 (m, 4H), 2.92-2.98 (m, 4H), 3.11 (*d*, 2H), 5.07-5.12 (m, 2H), 5.27 (*t*, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.0 (q), 16.4 (q), 17.6 (q), 25.7 (q), 26.3 (t), 26.3 (t), 26.7 (t), 29.9 (t), 39.7 (t), 39.7 (t), 46.9 (t), 48.3 (t), 54.5 (t), 56.2 (t), 57.7 (t), 121.5 (d), 123.8 (d), 124.3 (d), 131.1 (s), 135.1 (s), 138.5 (s).

5.1.4e. *N*-C10 piperazine (25a)

A white solid (R_f = 0.4, 0.46 g, 58 %). ^{1}H NMR [$CDCl_3$, 600 MHz]; δ_H 0.83 (*t*, 3H), 1.22 (m, 14H), 1.44 (s, 2H), 1.93 (m, 1H), 2.30 (m, 2H), 2.45 (s, 4H), 2.92 (s, 4H). ^{13}C NMR [$CDCl_3$, 150 MHz]: δ_C 14.1 (q), 22.6 (t), 26.5 (t), 27.5 (t), 29.3 (t), 29.5 (t), 31.9 (t), 45.1 (t), 53.2 (t), 59.1 (t).

5.1.4f. *N*-C15 piperazine (25b)

A white solid (R_f = 0.4, 0.45 g, 58 %). ^{1}H NMR [$CDCl_3$, 600 MHz]; δ_H 0.89 (t, 3H), 1.26-1.28 (m, 24H), 1.49 (s, 2H), 1.81 (s, 1H), 2.30 (m, 2H), 2.41 (s, 3H), 2.91 (s, 4H). ^{13}C NMR [$CDCl_3$, 150

MHz]: δ_C 14.1 (q), 22.7 (t), 26.7 (t), 27.6 (t), 29.3 (t), 29.6 (t), 29.6 (t), 29.6 (t), 29.7 (t), 31.9 (t), 46.1 (t), 54.7 (t), 59.5 (t).

5.1.4g. *N*-C20 piperazine (25c)

A white solid (R_f = 0.4, 0.48 g, 53 %). ^{1}H NMR [$CDCl_3$, 600 MHz]; δ_H 0.85 (t, 3H), 1.22-1.24 (m, 30H), 1.45 (m, 2H), 1.99 (s, NH), 2.28 (m, 2H), 2.37 (s, 3H), 2.86 (m, 4H). ^{13}C NMR [$CDCl_3$, 150 MHz]: δ_C 14.0 (t), 22.7 (t), 26.6 (t), 27.6 (t), 29.3 (t), 29.6 (t), 29.6 (t), 29.6 (t), 29.7 (t), 31.9 (t), 46.6 (t), 54.6 (t), 59.5 (t).

5.1.5. Synthesis of PCU piperazine isoprenyl/linear alkane

A mixture of *N*-isoprenyl piperazine/linear alkane piperazine (1.9mmol) was reacted with (0.47 g, 0.84 mmol) PCU ditosylate (18) and K_2CO_3 (0.175 g, 1.27 mmol) in CH_3CN (10 mL) with reflux under nitrogen atmosphere for four days. The reaction was cooled, filtered and concentrated *in vacuo* to obtain a crude product. The residue was purified *via* column chromatography on silica gel using $CHCl_3$/MeOH/NH_4OH: 88:10:2 as eluent to give the product as yellow oil.

5.1.5a. *N*-geranyl piperazine PCU (8a)

A yellow oil (R_f = 0.7, 0.40 g, 71 %). IR ν_{max}: 2929, 1672, 1448, 1375, 1294, 1006 and 821 cm^{-1}. HRMS calculated for C43H68N4O (M + H$^+$) 657.5471, found 657.5446; ^{1}H NMR [$CDCl_3$, 400 MHz] δ_H 1.51 (J_{AB} = 10.2 Hz, 1H), 1.84 (J_{AB} = 10.2 Hz, 1H), 1.95-2.06 (m, 8H), 2.35-2.57 (m, 12H), 2.95 (d, 2H), 5.04 (t, J = 6.72 Hz, 1H), 5.22 (t, J = 6.4 Hz, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (q), 17.7 (q), 25.7 (q), 26.4 (t), 30.1 (t), 39.8 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.0 (t), 53.2 (t), 54.6 (t), 55.8 (t), 56.0 (t), 58.8 (d), 94.9 (s), 120.7 (d), 124.1 (d), 131.5 (s), 138.9 (s).

5.1.5b. *N-trans trans* farnesyl piperazine PCU (8b)

A yellow oil (R_f = 0.8, 0.42 g, 63 %). IR ν_{max}: 2929, 1670, 1448, 1375, 1294, 1153, 1006 and 822 cm^{-1}. HRMS calculated for $C_{53}H_{84}N_4O$ (M + H$^+$) 793.6723, found 793.6714. ^{1}H NMR [$CDCl_3$, 400 MHz] δ_H 1.48 (J_{AB} = 10.2 Hz, 1H), 1.84 (J_{AB} = 10.2 Hz, 1H), 1.56 (s, 3H), 1.57 (s, 3H), 1.60 (s, 3H), 1.65 (s, 3H), 1.91-2.08 (m, 10H), 2.35-2.60 (m, 12H), 2.95 (d, 2H), 5.07 (m, 2H), 5.23 (m, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.0 (q), 16.5 (q), 17.7 (q), 25.7 (q), 26.4 (t), 26.7 (t), 30.1 (t), 39.7 (t), 39.8 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.1 (t), 53.2 (t), 56.0 (t), 58.8 (d), 94.9 (s), 120.6 (d), 124.0 (d), 124.2 (d), 131.3 (s), 135.2 (s), 138.9 (s).

5.1.5c. C10-piperazine PCU (9a)

A dark brown oil (R_f = 0.8, 0.41 g, 73 %). IR ν_{max}: 2852, 2807, 1464, 1.295, 1161, 826 and 721cm^{-1}. HRMS calculated for $C_{43}H_{76}N_4O$ (M + H$^+$) 665.6092, found 665.6114; ^{1}H NMR [$CDCl_3$, 400 MHz] δ_H 0.79 (t, J = 6.44 Hz, 3H), 1.17-1.19 (m, 14H), 1.40 (J_{AB} = 9.2 Hz, 1H), 1.42 (s, 2H), 1.77

(J_{AB} = 10.2 Hz, 1H), 1.91 (m, 2H), 2.28-2.32 (m, 3H), 2.37-2.52 (m, 11H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 14.1 (q), 22.6 (t), 26.4 (t), 27.5 (t), 29.3 (t), 29.5 (t), 29.5 (t), 31.8 (t), 41.7 (d), 43.4 (t), 44.4 (d), 47.9 (d), 52.6 (t), 54.5 (t), 58.6 (t), 58.7 (d), 94.6 (s).

5.1.5d. C15-piperazine PCU (9b)

A light brown oil (R_f = 0.8, 0.48 g, 71 %). IR ν_{max}: 2915, 2850, 2808, 1466, 1375, 1163, 1120, 826 and 720 cm^{-1}. MS (TOF) calculated for $C_{53}H_{96}N_4O$ (M + H$^+$) 805.7657, found 805.7698; ^{1}H NMR [$CDCl_3$, 400 MHz] δ_H 0.77 (m, 3H), 1.15 (s, br, 24H), 1.40 (m, 3H), 1.76 (J_{AB} = 10.0 Hz, 1H), 1.89 (3H), 2.20 (m, 2H), 2.27-2.40 (m, 11H), 2.49 (s, 2H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 14.1 (q), 22.6 (t), 26.9 (t), 27.6 (t), 29.3 (t), 29.5 (t), 29.6 (t), 29.6 (t), 30.1 (t), 31.9 (t), 41.8 (d), 43.4 (t),44.2 (d), 47.9 (d), 53.2 (t), 54.6 (t), 58.8 (d), 58.8 (t), 94.7 (s).

5.1.5e. C20- piperazine PCU (9c)

A yellow solid (R_f = 0.8, 0.58 g, 73 %). IR ν_{max}: 2915, 2849, 2809, 1469, 1375, 1163, 1119, 827 and 718 cm^{-1}. MS (TOF) calculated for $C_{63}H_{116}N_4O$ (M + H$^+$) 945.9222, found 945.9210; ^{1}H NMR [$CDCl_3$, 400 MHz] δ_H 0.86 (m, 3H), 1.22 (s, br, 30H), 1.45 (m, 3H), 1.84 (J_{AB} = 10.2 Hz, 1H), 1.96 (t, 2H), 2.27-2.56 (m, 15H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 14.1 (t), 22.7 (t), 26.8 (t), 27.6 (t), 29.4 (t), 29.4 (t), 29.5 (t), 29.6 (t), 29.7 (t), 30.1 (t), 31.9 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.1 (t), 53.2 (t), 54.6 (t), 58.8 (d), 58.8 (t), 94.8 (s).

5.1.6. *N*-benzoyl piperazine PCU (10)

A mixture of *N*-benzoyl piperazine (**26**, 0.7 g, 3.68 mmol) and PCU ditosylate (**18**, 0.93 g, 1.67 mmol) and K_2CO_3 (0.28 g, 2.0 mmol) in CH_3CN (20 mL) was refluxed for four days under N_2 atmosphere. The reaction mixture was cooled, filtered and concentrated *in vacuo*. The crude residue was purified *via* column chromatography on silica gel using CH_3Cl: MeOH: NH_4OH (88:10:2, R_f = 0.75) as eluent to give the product as a yellow oil (0.84 g, 85 %). IR ν_{max}: 3463, 2952, 1623, 1430, 1292 and 723 cm^{-1}. HRMS calculated for $C_{37}H_{44}N_4O_3$ (M + H$^+$) 593.3492, found 593.3505. ^{1}H NMR [$CDCl_3$, 400 MHz]; δ_H 1.50 (J_{AB} = 10.3 Hz, 1H), 1.85 (J_{AB} = 10.3 Hz, 1H), 2.36-2.59 (m, 10H), 3.42 (s, 2H), 3.78 (s, 2H) and 7.37 (s, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.8 (t), 41.8 (d), 42.0 (t), 43.5 (t), 44.4 (d), 47.6 (t), 48.0 (d), 52.9 (t), 94.7 (s), 127.0 (d), 128.1 (d), 128.5 (d), 135.7 (s), 170.3 (s).

5.1.7. *N*-benzyl piperazine PCU (11)

N-benzoyl piperazine PCU (**10**, 1 g, 1.7 mmol) was added to a stirred suspension of lithium aluminium hydride (0.25 g, 6.8 mmol) in dry THF under nitrogen. The solution was refluxed for 36 hours under N_2. The solution was diluted with diethyl ether and the excess LAH was quenched by the

dropwise addition of saturated aqueous Na_2SO_4. The solution was filtered, dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo* to afford a residue which was purified *via* column chromatography on silica gel using CH_3Cl: MeOH: NH_4OH (88:10:2, R_f = 0.62) as eluent to give the product as a yellow oil (55 %). IR ν_{max}: 2948, 1656, 1149 and 734 cm^{-1}. HRMS calculated for $C_{37}H_{48}N_4O$ (M + H^+) 565.3906, found 565.3911. ^{1}H NMR [$CDCl_3$, 400 MHz]; δ_H 1.48 (J_{AB} = 10.3 Hz, 1H), 1.84 (J_{AB} = 10.3 Hz, 1H), 1.96 (t, J = 7.92 Hz, 2H) 2.34-2.56 (m, 10H), 3.47 (s, 2H) and 7.2-7.28 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.9 (t), 41.8 (d), 43.4 (t), 44.5 (d), 48.0 (d), 53.1 (t), 54.6 (t), 58.8 (d), 63.0 (t), 94.8 (s), 127.0 (d), 128.2 (d), 129.3 (d),138.0 (s).

5.1.8. Synthesis of 2-(aminomethyl) pyridine PCU (12)

A mixture of 2-(aminomethyl) pyridine (**27**, 1 g, 9.2 mmol) and of benzaldehyde (**29**, 0.98 g, 9.2 mmol) in ethanol (15 mL) was stirred for one hour at room temperature under nitrogen atmosphere, the corresponding imine was reduced with solid $NaBH_4$ (0.7 g, 18 mmol) which was added slowly over 30 minutes, the mixture was further stirred for an additional 30 minutes, the mixture was then refluxed overnight. The mixture was allowed to cool to RT, an additional ethanol (15 mL) was added to the reaction vessel after which 10 % HCl was added to quench excess $NaBH_4$. The acidic mixture was basified with 25 % aqueous ammonia solution. The desired product was extracted with dichloromethane (2 x 50 mL) and the solution was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was distilled under vacuum to afford (1.2 g, 65 %) *N*-benzyl-*N*-{(pyridin-2-yl)methyl}amine (**30**) [**21,22**]. 1H NMR [$CDCl_3$, 400 MHz]: δ_H: 2.25 (NH), 3.84 (s, 2H), 3.92 (s, 2H), 7.14 (t, 1H), 7.26 (d, 1H), 7.30-7.37 (m, 5H), 7.62 (t, 1H), 8.56 (d, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 53.4 (t), 54.4 (t), 121.8 (d), 122.2 (d), 126.9 (d), 128.1 (d), 128.3 (d), 136.3 (d), 140.0 (d), 149.2 (s), 156.7 (s).

In the next step, A mixture of *N*-benzyl-*N*-{(pyridin-2-yl)methyl}amine (**30**, 1.5 g, 7.6 mmol), PCU ditosylate (**18**, 1.9 g, 3.4 mmol) and Et_3N (710 µL, 5.1 mmol) in CH_3CN (20 mL) was refluxed under nitrogen for four days. The reaction was monitored on TLC, after completion the reaction was filtered and concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel using CH_3Cl: MeOH: NH_4OH (88:10:2, R_f = 0.8) as eluent to give *N*-benzyl-*N*-(2-methylpyridine) PCU (**32**) (1.55 g, 75 %). ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.39 (J_{AB} = 10.2Hz, 1H), 1.73 (J_{AB} = 10.2Hz, 1H), 2.01 (t, J = 7.8 Hz, 2H), 2.18-2.58 (m, 8H), 3.61 (s, 2H), 3.72 (s, 2H), 7.10 (t, J = 5.2Hz, 1H), 7.19-7.33 (m, 5H), 7.52 (d, J = 7.7Hz, 1H), 7.60 (t, J = 7.5Hz, 1H), 8.47 (d, J = 4.2Hz, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 30.0 (t), 41.6 (d), 43.4 (t), 44.3 (d), 47.8 (d),50.1 (t), 58.4 (t), 58.7 (d), 60.1 (t), 94.8 (s), 121.8 (d), 122.8 (d), 126.9 (d), 128.2 (d), 128.8 (d), 136.4 (d), 139.4 (s), 148.8 (d), 160.4 (s).

A mixture of (1.8 g, 1.7 mmol) *N*-benzyl-*N*-{(pyridin-2-yl)methyl}amine PCU (**32**), ammonium formate (0.54 g, 8.5 mmol) and 150 mg of 10 % Pd/C in methanol was refluxed under nitrogen atmosphere for 15 hours [**27**]. The mixture was cooled to RT, filtered and solution concentrated. The residue obtained was made alkaline with $NaHCO_3$ and extracted with $CHCl_3$. The was mixture dried over Na_2SO_4, concentrated *in vacuo* to afford pure 2 (aminomethyl) pyridine PCU **(12)** ($CHCl_3$: MeOH: NH_4OH, 88:10:2, R_f = 0.65, 0.85 g, 67 %). IR ν_{max}: 3314, 2955, 1665, 1590, 1433 and 753 cm^{-1}. HRMS calculated for $C_{27}H_{32}N_4O$ (M + H^+) 429.2654, found 429.2667. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.48 (J_{AB} = 10.2Hz, 1H), 1.83 (J_{AB} = 10.2Hz, 1H), 2.01 (t, *J* = 7.28 Hz, 2H), 2.3-2.56 (m, 4H), 2.76 (m, 2H), 3.89 (s, 2H), 7.12 (t, 1H), 7.30 (d, 1H), 7.60 (t, 1H), 8.51 (d, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 32.5 (t), 41.5 (d), 43.5 (t), 44.2 (d), 46.1 (t), 47.9 (d), 55.1 (t), 58.5 (d), 95.4 (s), 122.0 (d), 122.2 (d), 136.5 (d), 149.2 (d), 159.3 (s).

5.1.9. Synthesis of *N*-benzyl ethanolamine PCU (13)

A mixture of benzaldehyde (**29**, 2.1 g, 20 mmol) and *N*-ethanolamine (**28**, 1.2 g, 20 mmol) in methanol (20 mL) was stirred at 25 ºC under N_2 atmosphere for two hours. The mixture was cooled to 0 ºC using an external ice-salt bath after which $NaBH_4$ (1.5 g, 40 mmol) was added slowly. The mixture was stirred overnight at RT. Excess $NaBH_4$ was quenched by adding 10 % HCl (20 mL), the mixture was basified with 25 % NH_4OH and the product was extracted from the mixture with dichloromethane (2 x 30 mL). The solvent was dried over Na_2SO_4 and concentrated *in vacuo* to afford pure 2-(Benzylamino) ethanol (**31**) (2.2 g, 72 %). ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 2.71 (t, 2H), 3.04 (NH), 3.60 (t, 2H), 3.75 (s, 2H), 7.23-7.33 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C: 50.6 (t), 53.4 (t), 60.6 (t), 127.0 (d), 128.1 (d), 128.3 (d), 139.6 (s).

A mixture of 2-(Benzylamino) ethanol (**31**, 1.2 g, 7.9 mmol), PCU ditosylate (**18**, 2 g, 3.6 mmol) and K_2CO_3 (0.745 g, 5.4 mmol) in CH_3CN (50 mL) was refluxed under nitrogen for four days. The reaction was monitored using TLC. After completion the reaction was filtered and concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel using CH_3Cl: MeOH: NH_4OH (88:10:2, R_f = 0.7) as eluent to give 2-(Benzylamino)ethanol PCU (**13**, 1.3 g, 70 %). IR ν_{max}: 3371, 2951, 1601, 1452, 1043, 732 and 697 cm^{-1}. HRMS calculated for $C_{33}H_{42}N_2O_3$ (M + H^+) 515.3274, found 515.3288. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.44 (J_{AB} = 10.2Hz, 1H), 1.77 (J_{AB} = 10.2Hz, 1H), 1.96 (t, *J* = 7.36 Hz, 2H), 2.25-2.64 (m, 8H) 3.54 (t, 2H), 3.61 (s, 2H), 7.27-7.28 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 29.3 (t), 41.5 (d), 43.4 (t), 44.1 (d), 47.7 (d), 49.6 (t), 55.0 (t), 58.4 (t), 58.4 (d), 58.7 (t), 95.1 (s), 127.1 (d), 128.3 (s), 129.0 (s).

5.2. Biological testing

5.2.1. Broth Macrodilution Method

All mycobacterial work was carried out in a Level III Biosafety laboratory. All synthesized novel "cage" compounds were evaluated in triplicate against *M. tuberculosis* $H_{37}Rv$. Each PCU amine compound was dissolved in methanol to prepare a final concentration of 12.8 mg/mL and dilution a 100 fold with 7H9 broth medium to give a stock concentration of 128 µg/mL. EMB was dissolved in DMSO and water similarly to serve as a standard. These was diluted two fold in sterile 30 mL universal tubes containing Middlebrook 7H9 broth supplemented with casitone, glycerol and 10 % OADC enrichment to give a concentration range of 128 µg/mL to 0.125 µg/mL. Tubes containing Middlebrook 7H9 broth without compounds served as the compound free controls while Middlebrook 7H9 broth containing solvents alone served as controls to monitor their inhibitory effect.

A standardized inoculum was prepared by vortexing a log phase culture of *M. tuberculosis* $H_{37}Rv$ in a sterile tube containing 4.5 mL phosphate buffer, 0.05 % Tween 80 and 4 to 6 glass beads (5 mm diameter). After allowing the clumps to settle, the supernatant was aspirated and adjusted to a MacFarland number 1 standard, equivalent to $1x10^7$ colony forming units (CFU)/mL. This was diluted in 7H9 broth to obtain a concentration of $1x10^5$ CFU/mL. Of this, 500 µL was added into each tube of broth containing the diluted test compound concentrations, the compound free controls and the solvent controls. Colony counts of the test inoculum were prepared by plating out 20 µL onto Middlebrook 7H11 agar plates. Plates and tubes were incubated aerobically at 37 °C for twenty one days. Macroscopic readings of the tubes were documented every seven days until twenty one days.

5.2.2. BACTEC 460 TB Analysis

Mycobacterium tuberculosis reference strain $H_{37}Rv$ (ATCC 25618) and XDR strain 194 (drug sensitivity: Isoniazid > 0.2 µg/mL; Rifampicin > 1.0 µg/mL; EMB > 2.5 µg/mL; Kanamycin > 5.0 µg/mL; Ofloxacin > 2.0 µg/mL; Amikacin > 5.0 µg/mL) were cultured in Middelbrook 7H9 medium[**28**], enriched with OADC (0.005 %, v/v, oleic acid; 0.5 %, 171 w/v, BSA; 0.2 %, w/v, glucose; 0.02 %, v/v, catalase and 0.085 %, w/v, NaCl)[**24**]. Incubation was with continuous stirring at 37 °C. Reproducible growth (< 1.0 % difference) was recorded under standardized conditions. Cultures with an optical density between 0.4 and 0.6 (at 600 nm) were in exponential growth. At an optical density of approximately 0.16, 0.1 mL of culture was inoculated into a BACTEC vial (Becton Dickinson, Franklin LakesNJ, USA) and incubated at 37 °C to a growth index (GI) of 500 (+/- 50). This culture was used as the primary culture for drug testing. The growth index is a quantitative determination of radioactive CO_2 on a scale from 0 to 999. Drug compounds were diluted with methanol (filter sterilized through Millex LG syringe driven filters, Millipore). Compound

concentrations in BACTEC vials ranged between 8 μg/mL to 0.03125 μg/mL (final concentration). Growth index results were recorded every 24 hours. Growth rates of the mycobacterial strains were calculated as daily growth index difference (ΔGI) where a ΔGI >10 was considered positive growth. Purity of *M. tuberculosis* cultures was monitored by plating onto 7H11 mycobacterium agar plates[**24**].

5.3. Cytotoxicity Analysis

Materials

The RPMI 1640 (with 25 mM HEPES and L-glutamine), penicillin/streptomycin solution, and trypsin-versene mixture were purchased from Lonza and the heat-inactivated foetal bovine serum (FBS) was from Invitrogen. Cytotoxicity was assessed using the Promega CellTiter 96 non-radioactive cell proliferation assay and an EL x 800 automated microplate reader from Bio-Tek Instruments, Inc.

Methods

The toxicities of the compounds were determined on the MDBK (Madin Darby bovine kidney epithelium) cell line (supplied by the Department of Biochemistry, University of Kwa-Zulu Natal, South Africa). The cells were grown in a 37 °C incubator as a monolayer in RPMI 1640 supplemented with 10 % (v/v) heat-inactivated FBS and penicillin/streptomycin at a final concentration of 0.1 mg/mL. Cells were then trypsinised and plated at a density of 5×10^4 cells per well into a 96-well plate and incubated for 6 hours at 37 °C. Media was removed from all wells and replaced with fresh media. The test compounds were dissolved in 1 % methanol with the aid of sonication. Dilutions of the test compounds were then prepared using fresh media before addition to the cells. The control untreated cell culture was incubated in fresh media containing an appropriate volume of the 1 % methanol solution. This was included as a 100 % viability internal control. All test samples were done in quintuplicate and repeated twice on different days. Cells were incubated with the test samples for 42 hours at 37 °C. Cell viability was assessed on the basis of the conversion of the tetrazolium salt {3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide} (MTT) into purple formazan crystals which can be dissolved using acidified isopropanol and then spectrophotometrically analyzed. The Promega CellTiter 96 non-radioactive cell proliferation assay was performed as per manufacturer's instructions. Briefly, MTT was added to each well and the plate incubated at 37 °C for 3 hours before the formazan crystals were dissolved and the absorbance read at 570 nm. Percent cytotoxicity was calculated as follows: percent cytotoxicity = 100 – [(OD of sample/OD of control) × 100 %]. All standard deviations were below 10 %.

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Supplementary data

The ^{1}H, ^{13}C NMR and MS spectra are provided as supplementary information.

References

1. Global tuberculosis control-surveillance, planning, financing, WHO report 2007; http://www.who.int/tb/publications/global_report/2007/pdf/full.pdf (accessed on 26 June 2008)
2. "Tuberculosis" WHO fact sheet No 104, Health Communications, WHO, Geneva, 2006. http://www.who.int/mediacentre/factsheets/fs104/en/index.html (accessed on 14 Nov. 2008).
3. White, V. L. C.; Moore-Gillon, J.; Thorax (**2000**) 55, 962-963.
4. Espinal, M. A.; Kim, S. J.; Suarez, P. G.; Kam, K. M.; Khomenko, A. G.; Migliori, G. B.; Baez, J.; Kochi, A.; Dye, C.; Raviglione, M. C.; J. Am. Med. Assoc. (**2000**) 283, 2537-2545.
5. Gandhi, N. R.; Moll, A.; Sturm, A. W.; Pawinski, R.; Govender, T.; Lalloo, U.; Zeller, K.; Andrews, J.; Friedland, G. Lancet (**2006**) 368, 1575-1580.
6. Medecins Sans Fronteres, "Development of new drugs for TB chemotherapy". http://www.doctorswithoutborders.org/news/tuberculosis/tb_xdr_report_full_10-2006.pdf. (accessed on 26 Sept. 2008)
7. Lee, R. E.; Protopopova, M.; Crooks, E.; Slayden, R. A.; Terrot, M.; Barry, C. E. J. Comb. Chem. (**2003**) 5, 172-187.
8. Protopopova, M.; Hanrahan, C.; Nikonenko, B.; Samala, R.; Chen, P.; Gearhart, J.; Einck, L.; Nacy, C. A. J. Antimicrob. Chemother. (**2005**)56, 968-974.
9. Bogatcheva, E.; Hanrahan, C.; Nikonenko, B.; Samala, R.; Chen, P.; Gearhart, J.; Barbosa, F.; Einck, L.; Nacy, C. A.; Protopopova, M. J. Med. Chem. (**2006**) 49, 3045-3048.
10. Zah, J.; Terre'Blanche, G.; Erasmus, E.; Malan, S. F. Bioorg. Med. Chem. (**2003**) 11, 3569-3578.
11. Nagasawa, H. T.; Elberling, J.A.; Shirota, F. N. J. Med. Chem. (**1973**)16, 823-826.
12. Geldenhuys, W. J.; Malan, S. F.; Bloomquist, J. R.; Marchand, A. P.; Van der Schyf, C. J. Med. Res. Rev. (**2005**)25, 21-48.
13. Nagasawa, H. T.; Elberling, J. A.; Shirota, F. N. J. Med. Chem. (**1975**)18, 826-830.
14. Ito, F. M.; Petroni, J. M.; de Lima, D. P.; Beatriz, A.; Marques, M. R.; de Moraes, M. O.; Costa-Lotufo, L. V.; Montnegro, R. C.; Magalhaes, H. I. F.; Pessoa, C. D. O. Molecules (**2007**) 12, 271-282.
15. Tangallapally, R. P.; Yendapally, R.; Lee, R. E.; Hevener, K.; Jones, V. C.; Lenaerts, A. J. M.; McNeil, M. R.; Wang, Y. H.; Franzblau, S. J. Med. Chem. (**2004**) 47, 5276-5283.
16. Tangallapally, R. P.; Yendapally, R.; Lee, R. E.; Lenaerts, A. J. M. J. Med. Chem. (**2005**) 48, 8261-8269.
17. Tangallapally, R. P.; Lee, R. E. B.; Lenaerts, A. J. M.; Lee, R. E. Bioorg. Med. Chem. Lett. (**2006**) 16, 2584-2589.

18. Boyle, G. A.; Govender, T.; Kruger, H, G.; Maguire G.E.M. Tetrahedron Asym. (**2004**) 15, 2661.
19. Cookson, R. C.; Crundwell, E.; Hill, R. R.; Hudec, J. J. Chem. Soc. (**1964**) 3062-7.
20. Marchand, A. P.; Kumar, K. A.; McKim, A. S.; Mlinaric-Majerski, K.; Kragol, G. Tetrahedron (**1997**) 53, 3467-3474.
21. Spencer, D. J. E.; Johnson, B. J.; Tolman, W. B. Org. Lett. (**2002**) 4, 1391-1393.
22. Mishra, H.; Mukherjee, R. J. Organo. Chem. (**2007**) 692, 3248-3260.
23. Tripathi, R. P.; Saxena, N.; Tiwari, V. K.; Verma, S. S.; Chaturvedi, V.; Manju, Y. K.; Srivastva, A. K.; Gaikwad, A.; Sinha, S. Bioorg. Med. Chem. (**2006**) 14, 8186-8196.
24. Siddiqi, S. H. BACTEC 460 TB system. Product and procedure manual revision; D. Becton Dickinson Microbiology System, Sparks, Md., 1995.
25. Collins, L. A.; Franzblau, S. G. Antimicrob. Agents Chemother. (**1997**) 41, 1004-1009.
26. Navarrete-Vazquez, G.; Molina-Salinas, G. M.; Duarte-Fajardo, Z. V.; Vargas-Villarreal, J.; Estrada-Soto, S.; Gonzalez-Salazar, F.; Hernandez-Nunez, E.; Said-Fernandez, S. Bioorg. Med. Chem. (**2007**) 15, 5502-5508.
27. Scapecchi, S.; Martini, E.; Manetti, D.; Ghelardini, C.; Martelli, C.; Dei, S.; Galeotti, N.; Guandalini, L.; Romanelli, N. M.; Teodori, E. Bio. Med. Chem. (**2004**) 12, 71-85.
28. Middlebrook, G.; Reggiardo, Z.; Tigertt, W. D. Am. Rev. Respir. Dis. (**1977**) 115, 1066-1069.

CHAPTER 3

IN-VITRO ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF PENTACYCLOUNDECANE TETRA-AMINES

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Abstract

The antifungal and antimicrobial activities of three pentacycloundecane (PCU) tetra-amines derivatives are reported herein. The *in vitro* activity of these PCU derivatives against yeasts (*Candida albicans* and non-*albicans* species) and filamentous fungi was evaluated using the Clinical and Laboratory Standards Institute (CLSI) M27-A2 and M38-A2 guidelines and the 2H-terazolium salt, (MTS) colorimetric method. The MIC against most of the tested clinical fungal strains for **GKM8** and **GKM9** derivatives range from 15.6-62.5 μg ml^{-1} while **GKM11** ranged from 3.9-7.8 μg ml^{-1}. The **GKM11** derivative was also active against fluconazole resistant strains of fungi. The **GKM11** derivative also exhibited promising activity against filamentous fungi in that it was 2.5 times more active than amphotericin B against *Sporothrix schenckii*. Antibacterial activity was determined using the broth microdilution method (BMM) and the iodonitrotetrazolium chloride (INT) colorimetric method. The **GKM11** derivative was mainly active against Gram-positive bacteria with MIC ranging from 3.9-7.8 μg ml^{-1}. Activity against Gram-negative bacteria tested was limited to *Escherichia coli* and *Elizabethkingia meningoseptica* (MIC of 31 μg ml^{-1}).

Key words: Polycyclic "cage", pentacycloundecane, antibacterial, antifungal

Introduction

Studies over the past two decades have shown an alarming increase in the number of infections caused by opportunistic fungal pathogens in immuno-compromised/suppressed patients, which are gradually becoming a threat to public health (1-3). These pathogens pose a major setback to effective HIV treatment and management. *Candida albicans* is a recognised opportunistic pathogen, while

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other *Candida* species are increasingly emerging as potential pathogens associated with opportunistic infections (4). Without timely intervention, infections by *Aspergillus* species in immunocompromised/suppressed patients can easily spread from the site of infection to other parts of the body such as the kidneys, liver, spleen, brain and gut (5, 6). A 40 % prevalence of invasive aspergillosis with a mortality rate of 78 to 100 % is reported in high-risk liver transplant recipients (7, 8).

Presently, a limited drug arsenal that primarily includes polyene- and azole-type compounds are available for treatment of fungal infection. Significant drawbacks are associated with the use of some of these compounds, for example, amphotericin B is reported to be highly nephrotoxic and less effective against *Candida* species (9, 10). The emergence of drug-resistant fungal strains coupled with the increasing persistence of mycoses in immunocompromised patients creates an urgent need to design new antifungal drugs. Optimally, these drugs should have a good safety profile and different modes of action that promote a broader antimicrobial spectrum of activity to target multiple infections such as fungi, bacteria and/or tuberculosis, especially in the empirical treatment of hospitalised patients with sepsis of unknown origin.

The incorporation of polycyclic 'cage' compounds into pharmaceutical drugs has enjoyed much attention from researchers for several decades now (11). Polycylic "cage" compounds attached to drug groups improves the drug lipophilicity hence enabling it to cross the blood-brain barrier and to enter the central nervous system (12, 13). It can also reduce metabolic degradation of the drug thereby prolonging its pharmaceutical effect in the body (14, 15). Many researchers have reported the discovery of polycyclic based compounds that possess a broader antimicrobial spectrum against viral, bacterial and fungal infections (16-22). SQ109 (*N*-geranyl-*N´*-(2-adamantyl)ethane-1,2-diamine) an anti-TB agent (23) was recently reported to inhibit *Candida albicans* including fluconazole resistant strain at a MIC of 4-8 μg ml^{-1} (24).

We recently reported the synthesis of fourteen PCU tetra-amine derivatives that were screened against the $H_{37}Rv$ strain of *Mycobacterium tuberculosis* (25). The **GKM8**, **GKM9** and **GKM11** derivatives were screened further against the extensively drug resistance *M. tuberculosis* strain, XDR 194, (drug sensitivity: Isoniazid > 0.2 μg ml^{-1}; Rifampicin > 1.0 μg ml^{-1}; EMB > 2.5 μg ml^{-1}; Kanamycin > 5.0 μg ml^{-1}; Ofloxacin > 2.0 μg ml^{-1}; Amikacin > 5.0 μg ml^{-1}) with MICs of 3.04, 1.26 and 1.50 μM. Motivated by this finding, the three most active pentacycloundecane (PCU) tetra-amines derivatives (Figure 1) were selected for further study. In this paper, we describe the *in vitro* activity of these PCU tetra-amine derivatives against yeast, filamentous fungi and bacteria. This is the first time that PCU-based derivatives are being reported to possess both antifungal and antibacterial activities.

Materials and methods

Antifungal assay

Twenty clinical isolates, ten of yeasts and ten of filamentous fungi (moulds) were used in this study (Table 1) while two ATCC reference strains [(*Candida parapsilosis* (ATCC 22019) and *Candida albicans* (ATCC 90028)] served as controls as recommended by Clinical and Laboratory Standards Institute, CLSI (formerly the National Committee for Clinical Laboratory Standards). The clinical isolates were obtained from the National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Hospital, Durban, South Africa. The isolates were recovered from the respiratory tracts, tissue samples and blood specimens of patients managed by the Hospital. Yeast isolates were identified at the species level using carbohydrate assimilation tests (API 32 ID, bioMérieux, Marcy 1'Etoile, France) while the filamentous fungi were identified by macroscopic and microscopic morphological features.

Antifungal/antibacterial agents: PCU tetra-amine derivatives (**GKM8**, **GKM9** and **GKM11**) were synthesized as described previously (25). The hydrochloride salt of each compound was dissolved in 10 % methanol and diluted with sterile double distilled water. Amphotericin B (Sigma-Aldrich) was used as reference drug. Freshly prepared solutions for all PCU derivatives and reference drugs were used in this study. The final concentrations of the antimicrobial agents ranged from 0.0012 to 80 μg ml^{-1} amphotericin B, 0.0015 to 100 μg ml^{-1} neomycin and 0.0076 to 500 μg ml^{-1} of **GKM 8**, **9** or **11**. All drug dilutions were carried out in a 96-well flat-bottom microtitre plates; each well contained 100 μL of twofold serial dilutions of the drugs tested (2 x final concentrations) and the plates were stored at -70 ºC until the day of testing.

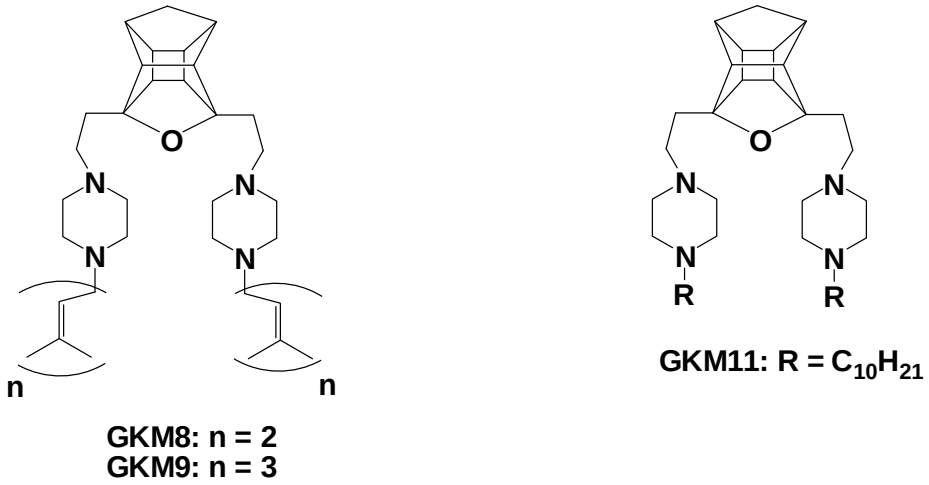

Figure 1: Free salts of PCU tetra-amine derivatives: GKM8, GKM9 and GKM11 (*25*)

Antifungal susceptibility tests: All microbial work was carried out in a Level II Bio-safety cabinet while following Good Laboratory Practice (GLP) procedures. Evaluation of the susceptibility of *Candida albicans*, non-*albicans* species and filamentous fungi was performed using the broth microdilution method according to CLSI M38-A2 (for filamentous fungi) and M27-A2 (for yeast) guidelines (26, 27). Yeast strains were grown aerobically overnight at 35 ºC on Sabouraud dextrose agar (Merck) plates. Yeasts were harvested and suspended in 1 % sterile saline and the turbidity of the supernatants measured spectrophotometrically at 625 nm with an absorbance of 0.08-0.1 equivalent to the No. 0.5 McFarland standard following the NCCLS M27-A2 guideline (26). The working suspension was diluted 1:20 in a mixture containing RPMI 1640 medium (with L-glutamine, without bicarbonate, Cambrex Bio Science Verviers, Belgium) and 0.165 M morpholinepropanesulfonic acid (MOPS, Sigma-Aldrich) buffered to pH 7.0. The working suspension was further diluted with the medium (1:50) to obtain the final test inoculum (1-5 x 10^3 CFU ml^{-1}). The microtitre plates were allowed to thaw and equilibrate to room temperature under aseptic conditions after which 100 µl aliquots of the working inoculum suspension were dispensed into each well and the plates incubated in an aerobic environment at 35 ºC for 24 h. Growth was observed visually with the aid of a concave mirror; MICs were taken on a growth or no-growth (100% visible-growth inhibition) scale.

MTS method: The MICs were also determined by colorimetric method using the dye, 2H-terazolium salt (MTS, Promega Corporation, Madison, USA). After MICs were determined visually on each micotitre plates, 20 µl of 2H-terazolium salt (28) was added directly to each well, incubated at 37 ºC for 4 h and the absorbance recorded at 490 nm on a 96-well plate reader (Biotek, Powerwave XS2). All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

The activity of the PCU tetra-amine derivatives against filamentous fungi was also determined using the broth microdilution method according to the CLSI M38-A2 guideline (27). Cultures were grown on Potato dextrose agar at 35 ºC until sporulation (48 h to 7 days). Spores were harvested and suspended in 1 % sterile saline, allowed to settle and the upper layer aspirated. The turbidity was measured spectrophotometrically at 625 nm and optical density was adjusted to yield a stock suspension of 0.4-5 x 10^6 sporangiospores per millilitre. A working suspension was prepared by diluting 1:50 of the conidia stock suspension in a standard medium (RPMI 1640, MOPS). The fungal inocula (100 µl) were added to each well of 96-well flat-bottom microdilution plates containing 100 µl of drug dilution and incubated for 48 h in an aerobic incubator at 35 ºC. After incubation, potential antimicrobial activity (MICs) was assessed as described previously.

Antibacterial susceptibility assay: All microbial work was carried out in a Level II Bio-safety cabinet while following Good Laboratory Practice (GLP) procedures. Bacterial susceptibility was determined using the broth microdilution method (29, 30). Eighteen ATCC bacterial cultures (identified in Table 2) obtained from the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Westville, South Africa were freshly cultured in Mueller-Hinton broth. The overnight cultures after 16-18 h of incubation at 37 ºC were adjusted to a turbidity of No. 0.5 McFarland standard. At a wavelength of 625 nm, the absorbance was adjusted between 0.08-0.1 to yield a stock suspension of 0.4-5 x 10^8 CFU ml^{-1}, which was diluted one hundred fold to obtain a working suspension of 10^6 CFU ml^{-1}.

The microtitre plates were placed in the laminar flow unit to equilibrate to room temperature under aseptic conditions. Aliquots (100 µl) of bacterial inocula were added to prepared drug samples and incubated aerobically for 16-18 h at 37 ºC. Following incubation, 50 µL of freshly prepared iodonitrotetrazolium chloride (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride, INT, 200 µg ml^{-1}) solution was added to each well and the plate was further incubated for 45 minutes at 37 ºC in the dark. When the colourless INT is reduced to red after incubation, persistent growth of the bacteria is indicated, while no colour change denotes the lack of bacterial growth. Neomycin was used as a control drug in this study. All analyses were performed in triplicates.

Results and discussion

Antifungal activity

Ten clinical strains of yeast and ten clinical strains of filamentous fungi were employed in evaluating the antifungal potency of the novel PCU tetra-amine derivatives. MICs values obtained from the CLSI and dye (terazolium salt) methods were in agreement (31, 32). The control drug, amphotericin B presented a MIC ranging from 0.02-1.25 µg ml^{-1} (Table 1). The **GKM8** and **GKM9** derivatives displayed MIC in the concentration range of 15.6-62.5 µg ml^{-1} against some of the fungal strains while **GKM11** was found to possess a MIC in a concentration range of 1.96-62.5 µg ml^{-1} against most of the tested fungal strains. The **GKM11** derivative showed MIC in a range of 3.9-7.8 µg ml^{-1} against all *Candida* species and *Trischosporon asahii*. Of the three derivatives employed in this study, **GKM11** was the most potent against majority of fungal strains. Against filamentous fungi strains, **GKM11** displays a MIC that is 1.3 and 2.5 times more active than amphotericin B against *Scopularopsis* spp. and *Sporothrix schenckii*. Two *Aspergillus* species namely *A. fumigatus* and *A. flavus* were used in this study. Interestingly, none of the test compounds were active against *A. flavus* (two strains). However, **GKM8** and **GKM9** both recorded a MIC of 31-62.5 µg ml^{-1} while **GKM11** possessed a MIC of 3.9-7.8 µg ml^{-1} against the three *A. fumigatus* strains examined. Cytotoxicity analysis (IC_{50}) of GKM8, GKM9 and GKM11 were carried using mammalian cell line [MDBK

(Madin Darby bovine kidney epithelium)] with IC_{50} values of 30, 24 and 25 µM obtained respectively (25). It is observed that GKM8 and GKM9 only show significant activity as an antifungal agent at MIC: 15.6 µg ml^{-1} (19.4 and 16.6 µM) beyond which it becomes toxic ($1C_{50}$: 30 and 24 µM). GKM11 on the other hand shows promising antifungal activity at MIC of 1.96-7.8 µg ml^{-1} (2.4-9.6 µM) against most fungal strains used. These values fall below the concentration at which it is toxic to healthy cells (IC_{50}; 25 µM).

Table 1: Antifungal activity of PCU tetra-amine derivatives

Strain	**MIC (µg ml^{-1})**			
	GKM8	**GKM9**	**GKM11**	**Ampho. B**
Candida utilis	31	15.6	7.8	0.31
Candida krusei	15.6	15.6	3.9	1.25
Candida tropicalis (2)	15.6-31	15.6-31	3.9-7.8	1.25
Candida parapsilosis (2)	15.6-31	15.6-62.5	3.9	0.04-1.25
Trichosporon asahii	62.5	500	7.8	0.31
Candida albicans	15.6	15.6	3.9	0.62
*Candida krusei**	31	31	7.8	1.25
*Candida parapsilosis**	31	31	7.8	0.02
Aspergillus fumigatus (3)	31-62.5	31-62.5	3.9-7.8	0.63-1.25
Fusarium spp.	125	500	62.5	2.5
Aspergillus flavus (2)	>500	>500	>500	1.25
Sporothrix schenckii	15.6	15.6	1.96	5
Scopularopsis spp.	31	31	7.8	10
Rhizomucor spp.	31	250	62.5	0.32
Pencillium spp.	62.5	31	15.6	1.25

Values in brackets indicate number of isolates that were screened if more than one. * Fluconazole resistant strains; Ampho. B; Amphotericin B. The data are reported as the mean ± standard error of the mean of ≤ 5.

Antibacterial activity

A total of 18 ATCC bacterial strains (nine Gram-positive and nine Gram-negative) were used in this study. Commercially available neomycin was active against all Gram-positive and Gram-negative bacterial strains used in this study. The tested compounds (**GKM8**, **GKM9** and **GKM11**) showed no activity against the following Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 35032 and ATCC 27853), *Proteus mirabilis* (ATCC 29906), *Proteus vulgaris* (ATCC 13315), *Klebsiella pneumoniae* (ATCC 700603), *Klebsiella oxytoca* (ATCC 13812) and *Serratia rubidaea* (ATCC

33670). However, activity was recorded against *Escherichia coli* (ATCC 35218) and *Elizabethkingia meningoseptica* (ATCC 13253) where a MIC ranging between 125-250 μg ml^{-1} was recorded for **GKM8** and **GKM9** while that of **GKM11** was 31 μg ml^{-1} (38.2μM) against both strains (Table 2).

Interestingly, the three PCU tetra-amine derivatives showed promising activity against most of the Gram-positive bacteria except *Staphylococcus saprophyticus* (ATCC 35552), *Enterococcus faecium* (ATCC 19434) and *Streptococcus aerogenes* (ATCC 13048). **GKM8** and **GKM9** displayed a MIC of 15.6-62.5 μg ml^{-1} whilst the MIC activity of **GKM11** ranged from 3.9-7.8 μg ml^{-1} (4.8-9.6 μM) against *Staphylococcus aureus* (ATCC 43300), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus xylosus* (ATCC 35033), *Bacillus subtilis* (ATCC 6051), *Rhodococcus equi* (ATCC 6939) and *Streptococcus agalactiae* (ATCC 13813). Importantly, **GKM9** and **GKM11** were 1.6 and 6.4 times more active than the control drug (neomycin) against *Staphylococcus aureus* (ATCC 43300) only (Table 2).

Table 2: Antibacterial activity of PCU tetra-amine derivatives

Strain	**MIC (μg ml^{-1})**			
	GKM8	**GKM9**	**GKM11**	**Neomycin**
Escherichia coli	250	125	31	3.13
Elizabethkingia meningoseptica	250	125	31	6.25
Staphylococcus aureus	31	15.6	3.9	25
Enterococcus faecalis	62.5	31	7.8	1.56
Staphylococcus xylosus	62.5	31	3.9	0.195
Bacillus subtilis	31	15.6	7.8	0.78
Rhodococcus equi	31	15.6	7.8	1.56
Streptococcus agalactiae	31	15.6	3.9	0.78

In conclusion, the PCU tetra-amine derivatives displayed antimicrobial activities against yeast, filamentous fungi and bacteria. **GKM8** and **GKM9** were least active with an MIC of 15.6-125 μg ml^{-1} against some of the tested fungal strains. **GKM11** [MIC: 1.95-7.8 μg ml^{-1} (2.4-9.6 μM)] proved to be most potent against 17 clinical strains of fungi *i.e.* isolates of *C. albicans, C. krusei*, *C. tropicalis*, *Trichosporon asahii*, *A. fumigatus*, *Sporothrix schenckii, Scopularopsis* spp. with the exception of two *A. flavus* strains and *Rhizomucor* spp.; Antibacterial analysis evaluation shows that **GKM8** and **GKM9** possess a MIC of 15.6-62.5 μg ml^{-1} against most Gram-positive bacterial strains while **GKM11** possess a MIC 3.9-125 μg ml^{-1}. The **GKM8** and **GKM9** (a ten and fifteen carbon alkene moiety) derivatives proved to be less active compared to **GKM11** (a ten carbon alkane moiety) against a variety of yeasts, filamentous fungi and bacteria species, suggesting that the saturated

hydrocarbon alkane moiety plays a key chemical function. It would be logical to screen the fifteen carbon alkane derivative of **GKM11** for its antifungal/bacterial activities however this was not feasible due to its insolubility at biological pH (25). The three derivatives, however, did not show promising activity against Gram-negative bacteria. The **GKM11** derivative thus possesses moderate antifungal activity and antibacterial activity that is seemingly restricted to Gram-positive strains. The low anti-fungal and bacterial activities possessed by **GKM11** against some fungi and bacteria at MIC range of 1.95-7.8μg ml^{-1} (2.4-9.6 μM) which is below its IC_{50} value (25 μM) suggests that **GKM11** could be an potential therapeutic candidate against a broad range of infections.

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References

(1) Singh, N. (2001) Trends in the epidemiology of opportunistic fungal infections: Predisposing factors and the impact of antimicrobial use practices. *Clinical Infectious Diseases 33*, 1692-1696.

(2) Sun, H. Y., and Singh, N. (2008) Emerging importance of infections due to zygomycetes in organ transplant recipients. *International Journal of Antimicrobial Agents 32*, S115-S118.

(3) Netsvyetayeva, I., Swoboda-Kopec, E., Paczek, L., Fiedor, P., Sikora, M., Jaworska-Zaremba, M., Blachnio, S., and Luczak, M. (2009) Trichosporon asahii as a prospective pathogen in solid organ transplant recipients. *Mycoses 52*, 263-265.

(4) Fidel, P. L., Vazquez, J. A., and Sobel, J. D. (1999) *Candida glabrata*: Review of Epidemiology, Pathogenesis, and Clinical Disease with Comparison to *C. albicans*. *Clinical Microbiology Reviews 12*, 80-96.

(5) Walsh, T. J., and Groll, A. H. (1999) Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. *Transplant Infectious Diseases 1*, 247-61.

(6) Eckmanns, T., Ruden, H., and Gastmeier, P. (2006) The influence of high-efficiency particulate air filtration on mortality and fungal infection among highly immunosuppressed patients: A systematic review. *Journal of Infectious Diseases 193*, 1408-1418.

(7) Singh, N., Avery, R. K., Munoz, P., Pruett, T. L., Alexander, B., Jacobs, R., Tollemar, J. G., Dominguez, E. A., Yu, C. M., Paterson, D. L., Husain, S., Kusne, S., and Linden, P. (2003) Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. *Clinical Infectious Diseases 36*, 46-52.

(8) Castroagudin, J. F., Ponton, C., Bustamante, M., Otero, E., Martinez, J., Tome, S., Conde, R., Segade, F. R., Delgado, M., Brage, A., Galban, C., and Varo, E. (2005) Prospective interventional study to evaluate the efficacy and safety of liposomal amphotericin B as prophylaxis of fungal infections in high-risk liver transplant recipients. *Transplantation Proceedings 37*, 3965-3967.

(9) Powderly, W. G., Kobayashi, G. S., Herzig, G. P., and Medoff, G. (1988) Amphotericin B-resistant yeast infection in severely immunocompromised patients. *Americian Journal of Medicine 84*, 826.

(10) Rex, J. H., Cooper, C. R., Merz, W. G., Galgiani, J. N., and Anaissie, E. J. (1995) Detection of amphotericin B-resistant *Candida* isolates in a broth-based system. *Antimicrobial Agents Chemotherapy 39*, 906-909.

(11) Geldenhuys, W. J., Malan, S. F., Bloomquist, J. R., Marchand, A. P., and Van der Schyf, C. J. (2005) Pharmacology and structure-activity relationships of bioactive polycyclic cage compounds: A focus on pentacycloundecane derivatives. *Medicinal Research Reviews 25*, 21-48.

(12) Nagasawa, H. T., Elberlin.Ja, and Shirota, F. N. (1973) 2-Aminoadamantane-2-Carboxylic Acid, a Rigid, Achiral, Tricyclic Alpha-Amino-Acid with Transport Inhibitory Properties. *Journal of Medicinal Chemistry 16*, 823-826.

(13) Zah, J., Terre'Blanche, G., Erasmus, E., and Malan, S. F. (2003) Physicochemical prediction of a brain-blood distribution profile in polycyclic amines. *Bioorganic & Medicinal Chemistry 11*, 3569-3578.

(14) Brookes, K. B., Hickmott, P. W., Jutle, K. K., and Schreyer, C. A. (1992) Introduction of pharmacophoric groups into polycyclic systems .4. aziridine, oxiran, and tertiary beta-

hydroxyethylamine derivatives of adamantane. *South African Journal of Chemistry-Suid-Afrikaanse Tydskrif Vir Chemie 45*, 8-11.

(15) Ito, F. M., Petroni, J. M., de Lima, D. P., Beatriz, A., Marques, M. R., de Moraes, M. O., Costa-Lotufo, L. V., Montnegro, R. C., Magalhaes, H. I. F., and Pessoa, C. D. O. (2007) Synthesis and biological evaluation of rigid polycyclic derivatives of the Diels-Alder adduct tricyclo[6.2.1.0(2,7)]undeca-4-9-dien-3,6-dione. *Molecules 12*, 271-282.

(16) Orzeszko, A., Kaminska, B., and Starosciak, B. J. (2002) Synthesis and antimicrobial activity of new adamantane derivatives III. *Farmaco 57*, 619-624.

(17) Kolocouris, A., Dimas, K., Pannecouque, C., Witvrouw, M., Foscolos, G. B., Stamatiou, G., Fytas, G., Zoidis, G., Kolocouris, N., Andrei, G., Snoeck, R., and De Clercq, E. (2002) New 2-(1-adamantylcarbonyl)pyridine and 1-acetyladamantane thiosemicarbazones-thiocarbonohydrazones: cell growth inhibitory, antiviral and antimicrobial activity evaluation. *Bioorganic & Medicinal Chemistry Letters 12*, 723-727.

(18) Orzeszko, B., Kazimierczuk, Z., Maurin, J. K., Laudy, A. E., Starosciak, B. J., Vilpo, J., Vilpo, L., Balzarini, J., and Orzeszko, A. (2004) Novel adamantylated pyrimidines and their preliminary biological evaluations. *Farmaco (Lausanne) 59*, 929-937.

(19) Papakonstantinou-Garoufalias, S., Pouli, N., Marakos, P., and Chytyroglou-Ladas, A. (2002) Synthesis antimicrobial and antifungal activity of some new 3-substituted derivatives of 4-(2,4-dichlorophenyl)-5-adamantyl-1H-1,2,4-triazole. *Farmaco 57*, 973-977.

(20) Kadi, A. A., El-Brollosy, N. R., Al-Deeb, O. A., Habib, E. E., Ibrahim, T. M., and El-Emam, A. A. (2007) Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles. *European Journal of Medicinal Chemistry 42*, 235-242.

(21) Orzeszko, A., Gralewska, R., Starosciak, B. J., and Kazimierczuk, Z. (2000) Synthesis and antimicrobial activity of new adamantane derivatives I. *Acta Biochimica Polonica 47*, 87-94.

(22) Orzeszko, A., Kaminska, B., Orzeszko, G., and Starosciak, B. J. (2000) Synthesis and antimicrobial activity of new adamantane derivatives II. *Farmaco 55*, 619-623.

(23) Protopopova, M., Hanrahan, C., Nikonenko, B., Samala, R., Chen, P., Gearhart, J., Einck, L., and Nacy, C. A. (2005) Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2-ethylenediamines. *Journal of Antimicrobial Chemotherapy 56*, 968-974.

(24) SQ109. (2008) SQ109. *Tuberculosis (Edinb) 88*, 159-61.

(25) Onajole, O. K., Govender, K., Govender, P., Van Helden, P., Kruger, H. G., Maguire, G. E. M., Muthusamy, K., Pillay, M., Wiid, I., and Govender, T. (2009) Pentacyclo-undecane derived cyclic tetra-amines: Synthesis and evaluation as potent ant-tuberculosis agents. *European Journal of Medicinal Chemistry 44*, 4297 - 4305.

(26) NCCLS. (2002) Clinical and Laboratory Standards Institute. Referencee method for broth dilution antifungal susceptibility testing of yeasts, approved standard M27-A2, vol. 22, no. 15, 2nd ed., CLSI Document. Clinical and Laboratory Standards Institute, Villanova, PA, 2002. *22*.

(27) CLSI. (2008) Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, approved standard M38-A, vol. 22, no. 16, Clinical and Laboratory Standards Institute, Wayne, PA, 2002. *22*.

(28) Promega. (2005) CellTiter 96 AQueous One Solution Cell Proliferation Assay. *Technical Bulletin*.

(29) Schwalbe, R., Steele-Moore, L., and Goodwin, A. C. (2007) *Antimicrobial Susceptibility Testing Protocols*, Vol. 1, CRC Press.

(30) Eloff, J. N. (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica 64*, 711-713.

(31) Hawser, S. P., Norris, H., Jessup, C. J., and Ghannoum, M. A. (1998) Comparison of a 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl-amino)carbonyl]-2H-t etrazolium hydroxide (XTT) colorimetric method with the Standardized National Committee for Clinical Laboratory Standards method of testing clinical yeast isolates for susceptibility to antifungal agents. *Journal of Clinical Microbiology 36*, 1450-1452.

(32) Meletiadis, J., Mouton, J. W., Meis, J. F. G., Bouman, B. A., Donnelly, P. J., Verweij, P. E., and Network, E. (2001) Comparison of spectrophotometric and visual readings of NCCLS method and evaluation of a colorimetric method based on reduction of a soluble tetrazolium salt, 2,3-bis {2-methoxy-4-nitro-5-[(sulfenylamino) carbonyl]-2H-tetrazolium-hydroxide}, for antifungal susceptibility testing of Aspergillus species. *Journal of Clinical Microbiology 39*, 4256-4263.

CHAPTER 4

SYNTHESIS AND EVALUATION OF SQ109 ANALOGUES AS POTENTIAL ANTI-TUBERCULOSIS CANDIDATES

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Abstract

As part of an ongoing project to develop highly potent anti-tuberculosis therapeutics, six **SQ109** derivatives were synthesized and screened *in vitro* for their anti-tuberculosis activity against the ATCC strain $H_{37}Rv$ and the extensively drug-resistant clinical strain XDR 173. Compound **16** with an extended alkene chain was the most active against both strains of *Mycobacterium tuberculosis* within a MIC range of 0.5 to 0.25 µM. Compound **12** and **SQ109** were potent within a MIC range of 1 to 0.5 µM, whilst compound **18** displayed an activity within the MIC range of 0.5 – 2 µM against both *Mycobacterium tuberculosis* strains.

Keywords: anti-tuberculosis (TB), SQ109, 1,2 cage diamine, XDR

1.1 Introduction

Despite thousands of years of living with the disease, tuberculosis (TB) remains one of the most deadly diseases known to man [1,2]. Recently, there has been an alarming increase in the number of TB patients all over the world and an estimated eight million people developed active TB in 2004 with two million dying as a result [1]. The recent emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis resulted in a major setback in the global fight against TB. The prevailing situation is made worse by the continuous increase in the number of immune-

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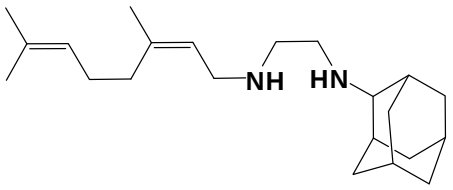
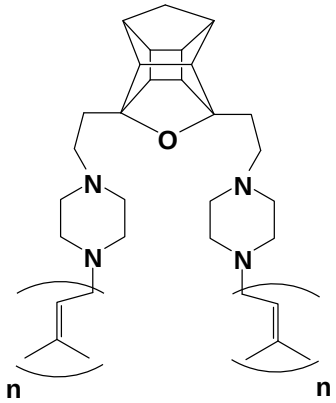
compromised patients living with HIV who are more prone to TB and other bacterial infections [1,3,4].

The urgent need for highly potent and effective drugs to combat the increasing TB pandemic cannot be overemphasized. Presently, **SQ109** (*N*-Geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine) which is a second generation agent developed from the first line drug ethambutol is one of the most promising anti-TB drug candidates at the clinical trials stage [5,6]. **SQ109** displays high potency against the $H_{37}Rv$ reference strain and MDR strains of *Mycobacterium tuberculosis* [7,8]. **SQ109** is relatively potent against *M. tuberculosis, M. bovis, M. marinum*, and less active against *M. avium* and *M. smegmatis* [9]. **SQ109** also shows promising activity against *Candida albicans* (4-8 μg/ml) [9]. **SQ109** in combination with other first line drugs such as isoniazid and rifampicin have been reported to be synergistic and an additive effect was observed when used with streptomycin [10].

We recently reported the anti-TB activities of three PCU tetra-amine derivatives (Figure 1, compounds **1-3**) [11]. Against both the $H_{37}Rv$ and XDR 194 (drug sensitivity: Isoniazid > 0.2 μg/mL; Rifampicin > 1.0 μg/mL; EMB > 2.5 μg/mL; Kanamycin > 5.0 μg/mL; Ofloxacin > 2.0 μg/mL; Amikacin > 5.0 μg/mL) strains of *M. tuberculosis*, it was discovered that the longer alkene side chain (15 carbons) cyclic tetra-amine of compound **2** rendered it two-fold more active than compound **1** (10 carbons in the side chains). Compound **3** has aliphatic side chains (10 carbons) and exhibits similar activity as compound **2** against the XDR 194 *M. tuberculosis* strain [11]. Encouraged by these observations, a series of novel **SQ109** derivatives were synthesized and investigated for their anti-TB activities. In particular we want to examine the importance of chain length and saturation of these **SQ109** derivatives.



SQ109



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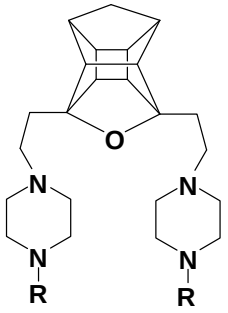
2: n = 3

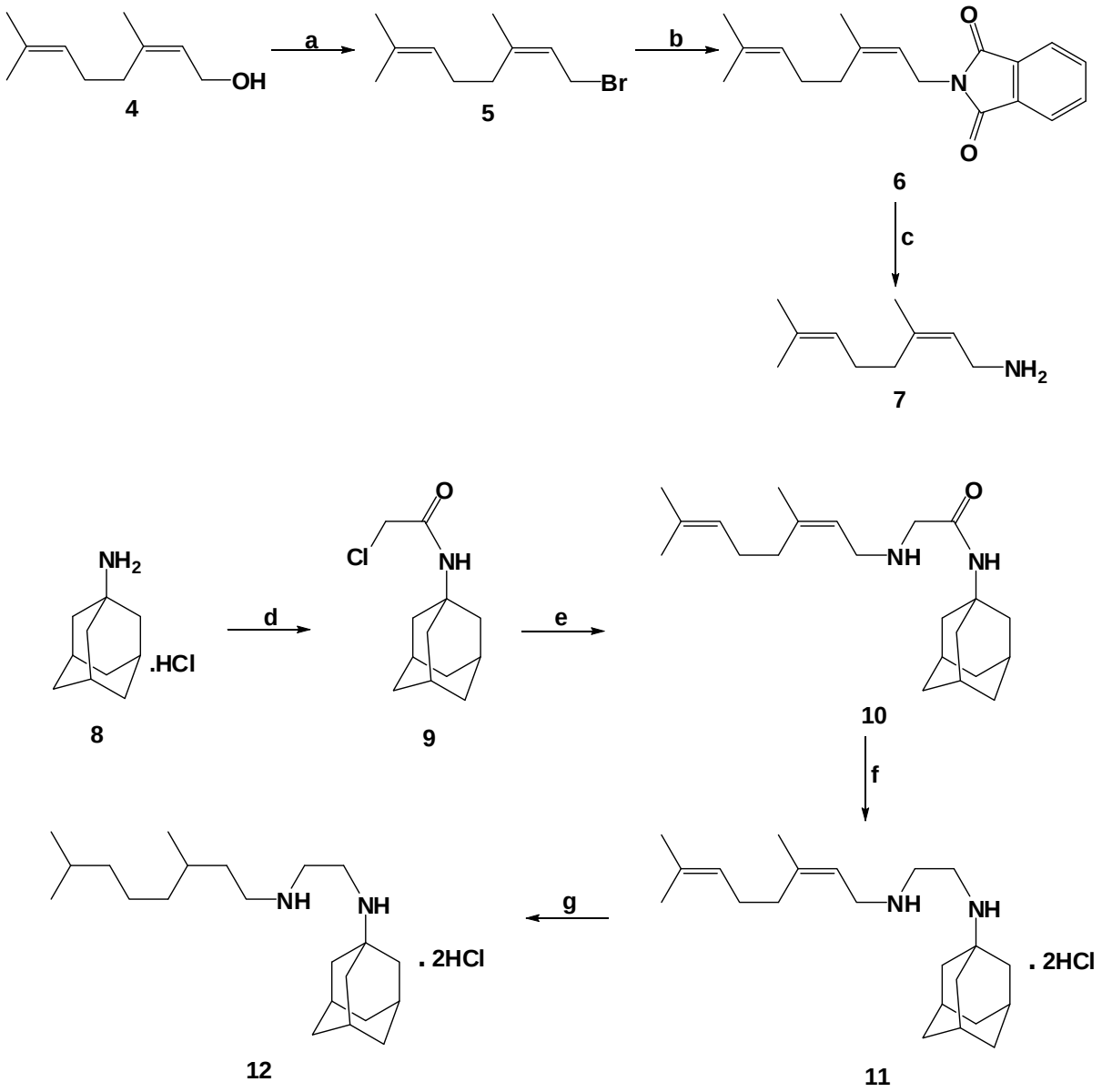


3: R = $C_{10}H_{21}$

Figure 1: Structures of SQ109 [7] and PCU tetra-amine derivatives 1 – 3 [11]

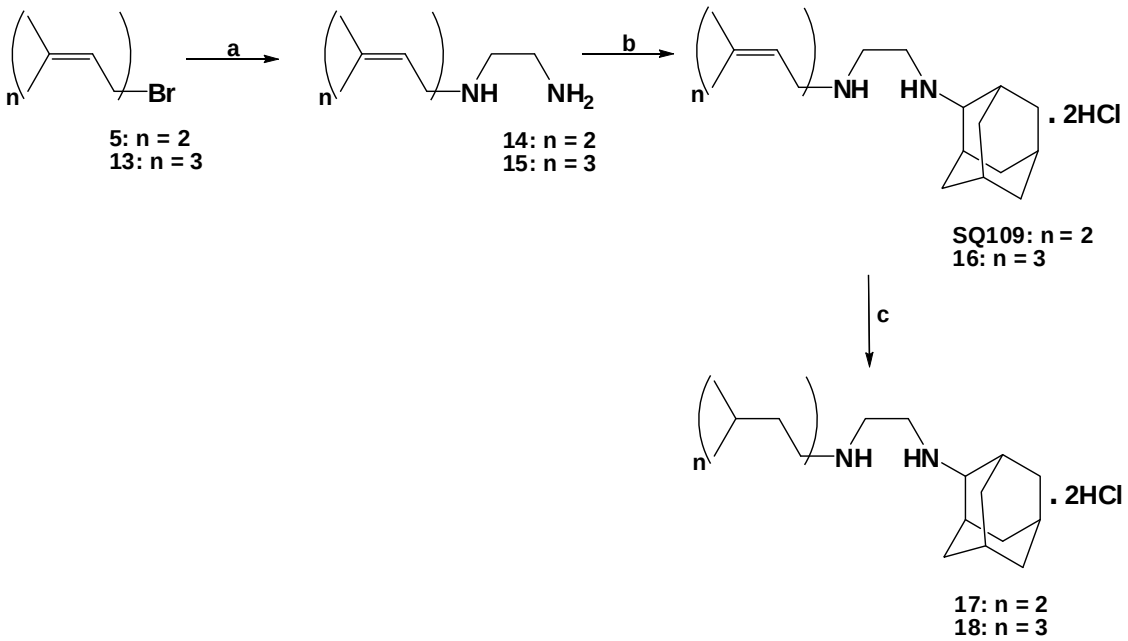
2.1 Chemistry

The starting material geraniol **4** (Scheme 1) was converted to geranyl bromide **5** in 95-96 % yield by treatment with phosphorus tribromide. Compound **5** was reacted with phthalimide in the presence of K_2CO_3 to obtain the corresponding compound **6** (60 % yield), which was converted with hydrazine hydrate to geranylamine **7** with a 34% yield. The free salt of amantadine was reacted with chloroacetyl chloride to obtain compound **9** in 94-96 % yield (Scheme 1). Reaction of **9** with geranylamine **7** resulted in compound **10** in a 40 % yield. The latter compound was subjected to reduction using $LiAlH_4$ to obtain the 1, 2 diamine intermediate, which was converted to the hydrochloride salt **11** in 71 % yield. Compound **11** was subjected to catalytic hydrogenation using 10% Pd/C to obtain derivative **12** in 52 % yield.



Scheme 1: Reagents and conditions: (a) PBr_3, THF, rt, 30 minutes; (b) phthalimide, K_2CO_3, DMF, rt, overnight; (c) MeOH, $H_2NNH_2.H_2O$, rt, overnight; (d) $NH_3/CHCl_3$, chloroacetyl chloride, $CHCl_3$, 2 h; (e) geranylamine (7), Et_3N, DCM, overnight, rt; (f) $LiAlH_4$, THF, N_2, reflux for16 h; then HCl, MeOH; (g) 10 % Pd/C, NH_4HCO_2, MeOH, reflux, 16 h, N_2, then HCl, MeOH.

A different synthetic route from that reported in literature [7] was employed in the synthesis of **SQ109** and its longer branched alkene chain derivative **16** (see Scheme 2). Geranylbromide **5** and *trans-trans* farnesyl bromide **13** were reacted with excess ethylene diamine in dry dichloromethane at – 78 ºC to afford the 1,2 diamines **14** and **15** respectively (86 %) and these were reacted with 2-adamantanone *via* reductive amination to obtain the desired 2-adamantyldiamines; the diamines were treated with hydrochloride to obtain **SQ109** and **16** (57 % and 47 % yield). **SQ109** and **16** were subjected to catalytic hydrogenation using 10 % Pd/C, the obtained products were converted to its HCl salts to obtain compounds **17** and **18** with a 57 % and 54 % yield (Scheme 2).



Scheme 2: Reagents and conditions: (a) ethylene diamine (100:1), -78 ºC, dry DCM; (b) MeOH, 2-adamantanone, N_2, 2 h, $NaBH_4$, overnight; then HCl, MeOH (c) 10 % Pd/C, NH_4HCO_2, MeOH, reflux, 16 h, N_2, then HCl, MeOH.

The successful synthesis of compounds **SQ109**, **11**, **12**, **16**, **17** and **18** and were confirmed using ^{1}H, ^{13}C and HR-MS. All compounds were confirmed to be greater than 95 % pure via LC-MS prior to their use in the evaluation of their biological efficacies. All of these compounds were readily soluble in aqueous media in the HCl salt form at a stock concentration of 100 µM.

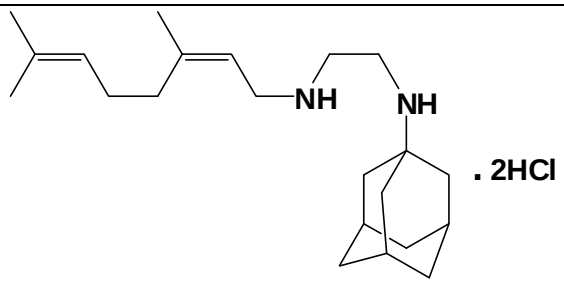
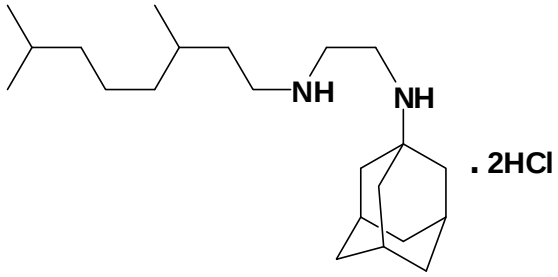
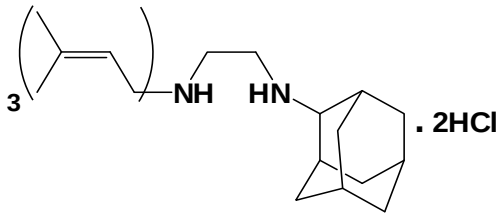
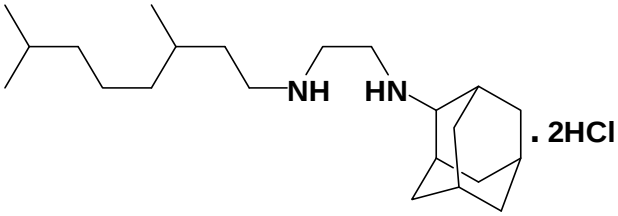
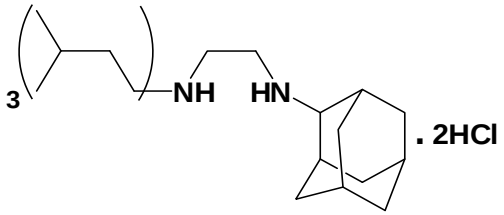
3.1 Results and Discussion

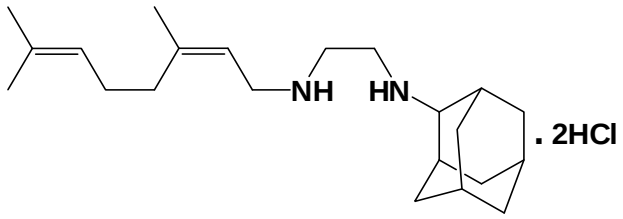
Employing the BACTEC 460 TB system, all compounds underwent an initial *in vitro* screening to determine their activities against the *M. tuberculosis* $H_{37}Rv$ strain [12]. As expected **SQ109** proved to be most potent with a MIC of 0.5-1 µM while it's reduced derivative **17** was not as active with an MIC range of 2 > MIC > 1 µM, suggesting that the alkene moiety is essential for anti-TB activity. The MIC values obtained for compounds **11** and **12** (reduced form of **11**) seemingly contradicts this statement as **12** (MIC: 0.5 - 1 µM) proved to be more active than its precursor **11** (10 > MIC > 1 µM). Compound **11** (1-adamantyl moiety) did not show much improvement over the 2-adamantyl moiety (**SQ109**), whilst compound **12** (reduced derivative of **11**) possess similar activity as **SQ109.**

Compound **16** showed the highest activity with a MIC value of 0.25 µM whereas its reduced analogue **18** showed an activity of 0.5 µM against the $H_{37}Rv$ strain. The most active compounds were selected for further analysis against an extensively drug-resistant (XDR 173 clinical isolate) strain of tuberculosis [resistant to the following drugs at breakpoint concentrations on 7H11 agar plates: first line drugs, Rifampicin (1 µg/mL), Ethambutol (7.5 µg/mL), Isoniazid (0.2 µg/mL). Second line

drugs, Streptomycin (2 µg/mL), Kapriomycin (10 µg/mL), Amikacin (5 µg/mL), Kanamycin (5 µg/mL)]

Table 1: The MICs (µM) of the compounds against bacteria and *M. tuberculosis* (H_{37}Rv and XDR 173 strains)

Compound	Structure	MIC (µM)	
		H_{37}Rv	XDR 173
11		10>MIC>1	Nd
12		1>MIC>0.5	1>MIC>0.5
16		0.5>MIC>0.25	0.5>MIC>0.25
17		2>MIC>1	Nd
18		1>MIC>0.5	2>MIC>1

SQ109		1>MIC>0.5	1>MIC>0.5

Nd; Not determined;

In tests against the XDR 173 strain, compound **16** was the most active (MIC-0.25 μM) and was twice as active as **SQ109.** Compound **12** which displayed similar activities (MIC-0.5 μM) and was four-fold more active than compound **18**.

Our previous studies [11] showed that derivatives of the fifteen carbon alkene chain possessed higher anti-TB activity as compared with the ten carbon alkene analogue. This trend was also observed in this study.

4.1 Conclusion

Novel **SQ109** analogues with potent anti TB activities were identified with compound **16** being the most potent against $H_{37}Rv$ and XDR strains of TB. Further application of compound **18** (ClogP[†]; 9.05 ± 0.39) may be hindered due to a solubility problem experienced in biological conditions however compound **16** (ClogP; 8.08 ± 0.48) did not possess such problem. These compounds are presently undergoing further *in-vitro* and *in-vivo* studies against pathogenic and non-pathogenic strains of bacteria and fungi.

5.1 Experimental

The NMR data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments using $CDCl_3$ as a solvent. All chemical shifts (∂) were quoted in parts per million downfield from TMS and the coupling constants (*J*) recorded in Hertz. Splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. All reactions were monitored using Thin Layer Chromatography (TLC, Merck Kieselgel 60, F254). All purifications were carried by Column Chromatography using Fluka Kieselgel 60 (70–230 mesh) and CH_3Cl:CH_3OH:NH_4OH (88:10:2) as the eluent (solvent mixture). Level of purity for all compounds was judged to be >95% based upon ^1H NMR and LC-MS analysis. Mass Spectra were obtained using a Bruker MicroTOF QII Time of Flight mass spectrometer while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3. Tetrahydrofuran was freshly distilled before use from a sodium benzophenone under N_2 atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use. The syntheses of the precursors are described in their corresponding references. Clog P

[†] ClogP was calculated using ACD/labs software v11.0

gives an indication of the lipophilicity of the drug with reference to its pharmacological importance (pharmacokinetics and pharmacodynamics). The values were calculated using ACD/Labs LogP software v11.0.

Synthesis of compound 11 (*N*-Geranyl-*N*'-(1-adamantyl)ethane-1,2-diamine)

To a stirring solution of **10** (0.82 g, 2.4 mmol) in dry THF (15 mL) was added lithium aluminium hydride LAH (0.27g, 7.2 mmol) and refluxed under nitrogen, reaction was monitored *via* TLC until no starting material is present. The solution was cooled and THF (10 mL) added after which saturated aqueous Na_2SO_4 was added dropwise to quench excess LAH. The solution was filtered, dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo* to afford a residue which was purified *via* column chromatography on silica gel using $CHCl_3$:CH_3OH:NH_4OH (88:10:2) as eluent to give a oil (71%) and converted to its HCl salt. A yellowish solid (HCl salt); 71 % yield; Melting point: 135-138 ºC, HR-MS calculated for $C_{22}H_{39}N_2$ ($[M+H]^+$ of free base) 331.3108, found 331.3104; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 1.51 (s, 5H), 1.55-1.56 (m, 11H), 1.59 (s, 4H), 1.90 – 2.01 (m, 8H), 2.33-2.35 (m, 3H), 2.63 (s, 4H), 3.14 (d, *J* = 6.72 Hz, 2H), 5.01 (m, 1H), 5.17 (t, *J* = 6.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz); δ_C 16.1, 17.5, 25.5, 26.3, 29.4, 36.5, 39.5, 39.6, 42.4, 46.8, 49.4, 122.5, 124.0, 131.3, 137.7

Synthesis of SQ109 (*N*-Geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine) and compound 16 (*N-trans-trans* farnesyl -*N*'-(2-adamantyl)ethane-1,2-diamine)

A mixture of *N* - isoprenyl ethylene diamine (**15** or **16**, 4.0 mmol) and 2-adamantanone (0.72 g, 4.8 mmol) in methanol (15 mL) was stirred for 2 h at room temperature under nitrogen atmosphere. The resulting imine was reduced with solid $NaBH_4$ (0.27 g, 7.2 mmol) which was added slowly over 15 min and the mixture stirred overnight. Additional methanol (15 mL) was added to the reaction vessel after which water (20 mL) was added to quench excess $NaBH_4$. The solution was extracted with ethyl acetate (2 x 50 mL) and the solution dried over Na_2SO_4 and concentration *in vacuo*. The crude product was purified *via* column chromatography on silica gel using $CHCl_3$:CH_3OH:NH_4OH (88:10:2) as eluent to give a yellow oil and converted to its HCl salt.

Data for SQ109 (*N*-Geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine)

A white solid; 57% yield, Melting point: 180-184 ºC, HR-MS calculated for $C_{22}H_{39}N_2$ ($[M+H]^+$ of free base) 331.3108, found 331.3135; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 1.39 – 1.41 (m, 2H), 1.50 (s, 3H), 1.55 (s, 3H), 1.57 – 1.61 (m, 10H), 1.74 -1.78 (m, 5H), 1.84 – 1.93 (m, 5H), 1.96 – 2.00 (m, 2H), 2.63 (s, 1H), 2.68 (s, 4H), 3.20 (2H, d, J = 6.06 Hz), 4.99 (m, 1H), 5.18 (m, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz); δ_C 16.1, 17.5, 25.5, 26.3, 27.4, 27.6, 31.1, 31.8, 37.4, 37.7, 39.5, 45.6, 46.3, 48.3, 61.7, 121.4, 123.9, 131.3, 138.6.

Compound 16 (*N-trans-trans* farnesyl -*N*'-(2-adamantyl)ethane-1,2-diamine)

A white solid (HCl salt); 47 % yield, Melting point: 146–150 ºC, HR-MS calculated for $C_{27}H_{47}N_2$ ($[M+H]^+$ of free base) 399.3734, found 399.3740; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 1.45 – 1.47 (m, 3H), 1.57 (s, 6H), 1.62 (s, 3H), 1.65 (s, 3H), 1.68 (s, 4H), 1.80 – 1.82 (m, 5H), 1.92 – 2.08 (m, 10H), 2.67 (s, 1H0, 2.70 (s, 4H), 3.22 (d, J = 6.72), 5.05 – 5.10 (m, 2H), 5.25 (t, J = 6.5, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz); δ_C 16.0, 16.3, 17.7, 25.7, 26.4, 26.7, 27.6, 27.8, 31.3, 32.2, 37.6, 38.0, 39.6, 39.7, 46.6, 47.1, 49.6, 61.9, 123.0, 124.0, 124.3, 135.1, 137.6.

Synthesis of Compound 12, 17 and 18

A mixture of compound **11**, **SQ109** and **16** (2.4mmol), ammonium formate (0.76 g, 12 mmol) and 350 mg of 10% Pd/C in dry methanol (20 mL) was refluxed under a nitrogen atmosphere for 16 hours. The mixture was cooled to room temperature, filtered through a sintered glass and concentrated *in vacuo*. The residue obtained was made slightly alkaline with $NaHCO_3$ and extracted with chloroform (2 x 50 mL). The solution was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*, purified *via* column chromatography on silica gel using $CHCl_3$:CH_3OH:NH_4OH (88:10:2) as eluent to give the product as a oil and converted to its HCl salt.

Compound 12 (*N*-(3,7-dimethyloctyl)-*N*'-(1-adamantyl)ethane-1,2-diamine)

A white solid (HCl salt); 52 % yield, Melting point: 210 – 214 ºC, HR-MS calculated for $C_{22}H_{43}N_2$ ($[M+H]^+$ of free base) 335.3421, found 335.3418; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 0.74 – 0.77 (m, 9H), 1.03 (m, 2H), 1.11 – 1.21 (m, 4H), 1.38 – 1.41 (m, 2H), 1.48 – 1.57 (m, 14H), 1.96 (s, 3H), 2.50 (s, 2H), 2.63 (s, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz); δ_C 19.7, 22.5, 22.6, 24.6, 27.9, 29.5, 30.9, 36.6, 37.3, 39.2, 39.7, 42.5, 47.7, 50.1, 50.7.

Compound 17 (*N*-(3,7-dimethyloctyl)-*N*'-(2-adamantyl)ethane-1,2-diamine)

A white solid (HCl salt); 57 % yield, Melting point: 182-185 ºC, HR-MS calculated for $C_{22}H_{43}N_2$ ($[M+H]^+$ of free base) 335.3421, found 335.3415; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 0.78 – 0.81 (m, 9H), 1.01 – 1.09 (m, 2H), 1.14 – 1.26 (m, 4H), 1.64 (s, 4H), 1.75 – 1.78 (m, 7H), 1.87 1.90 (m, 2H), 2.52 – 2.54 (m, 2H), 2.63 (s, 1H), 2.66 (s, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz); δ_C 19.6, 22.5, 22.6, 24.6, 27.5, 27.7, 27.9, 30.9, 31.2, 32.1, 37.2, 37.3, 37.5, 37.9, 39.2, 46.3, 47.7, 50.0, 61.8.

Compound 18 (*N*-(3,7,11-trimethyldodecyl)-*N*'-(2-adamantyl)ethane-1,2-diamine)

A white solid (HCl salt); 54 % yield, Melting point: 114-118 ºC, HR-MS calculated for $C_{27}H_{53}N_2$ ($[M+H]^+$ of free base) 405.4203, found 405.4186; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 0.79 – 0.83 (m, 12H), 1.00 – 1.11 (m, 5H), 1.14 – 1.33 (m, 8H), 1.42 1.50 (m, 5H), 1.66 (br s, 6H), 1.78 – 1.80 (m, 5H), 1.90 – 1.93 (m, 2H), 2.54 – 2.60 (m, 2H), 2.66 (s, 1H), 2.68 (s, 4H). ^{13}C NMR ($CDCl_3$, 100

MHz); δ_C 19.8, 22.6, 22.7, 24.4, 24.8, 27.6, 27.8, 27.9, 31.0, 31.3, 32.2, 32.8, 37.3, 37.4, 27.5, 37.6, 38.0, 39.3, 46.5, 47.9, 50.1, 61.9.

Acknowledgements

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References

1. Dye, C. *Lancet* **2006**, *367*, 938-940.
2. "Tuberculosis" WHO fact Sheet No 104, Health Communications, WHO, Geneva, 2006. *http://www.who.int/mediacentre/factsheets/fs104/en/index.html*, (accessed on 14 Nov. 2008).
3. Tuberculosis (TB) WHO Extensively drug-resistant tuberculosis, *http://www.who.int/tb/challenges/xdr/en/index.html*, (accessed on 22 June 2009).
4. McCarthy, M. *Lancet* **1996**, *348*, 393-393.
5. Jia, J.; Tomaszewski, J. E.; Hanrahan, C.; Coward, L.; Noker, P.; Gorman, G.; Nikonenko, B.; Protopopova, M. *Br. J. Pharmacol.* **2005**, *144*, 80-87.
6. Jia, L.; Noker, P. E.; Coward, L.; Gorman, G. S.; Protopopova, M.; Tomaszewski, J. E. *Br. J. Pharmacol.* **2006**, *147*, 476-485.
7. Lee, R. E.; Protopopova, M.; Crooks, E.; Slayden, R. A.; Terrot, M.; Barry, C. E. *J. Comb. Chem.* **2003**, *5*, 172-187.
8. Protopopova, M.; Hanrahan, C.; Nikonenko, B.; Samala, R.; Chen, P.; Gearhart, J.; Einck, L.; Nacy, C. A. *J. Antimicrob. Chemother.* **2005**, *56*, 968-974.
9. *Tuberculosis (Edinb)* **2008,** *88*, 159-61.
10. Chen, P.; Gearhart, J.; Protopopova, M.; Einck, L.; Nacy, C. A. *J. Antimicrob. Chemother.* **2006,** *58*, 332-337.
11. Onajole, O. K.; Govender, K.; Govender, P.; Van Helden, P.; Kruger, H. G.; Maguire, G. E. M.; Muthusamy, K.; Pillay, M.; Wiid, I.; Govender, T. *European J. Med. Chem.* **2009**, *44*, 4297 - 4305.
12. Siddiqi, S. H. *BACTEC 460 TB System. Product and Procedure Manual Revision, D. Becton Dickinson Microbiology System, Sparks, Md,* **1995**.

CHAPTER 5

NMR ELUCIDATION OF NOVEL SQ109 DERIVATIVES

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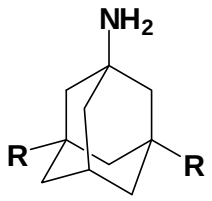
Abstract

The NMR elucidation of five novel SQ109 analogues including SQ109 is reported herein. These compounds were synthesized as potential anti-tuberculosis candidates. One dimensional NMR (^{1}H and ^{13}C) techniques show a series of overlapping signals from the methine and methylene groups of these compounds, thereby making it extremely difficult to assign all NMR signals. Two dimensional (2D) NMR techniques were instrumental in overcoming these difficulties. This paper appears to be a rare report on the complete structure elucidation of mono-substituted adamantane moieties.

Introduction

Polycyclic "cage" chemistry has been attracting the interest of medicinal and organic chemists since the early 1960s. These compounds include pentacycloundecane, trishomocubane, adamantane and basketane. Adamantane is the most studied which has led to the discovery of numerous pharmaceutical candidates [1-6]. The polycyclic "cage" has been reported to improve the pharmacokinetic and pharmacological properties of known drug candidates [6-10]. However, its pharmaceutical importance only drew increased attention after the discovery of 1-amino adamantane or amantadine. This molecule possesses anti-viral activity against several species including hepatitis C[11] and the influenza virus[12,13]. Memantine is another important amino-adamantane used in the clinical treatment of Alzheimer's disease (Figure 1) [14-16]. One of such discoveries is SQ109 (**1**) (*N*-geranyl-*N*'-(2-adamantyl)ethane-1,2 diamine) first reported by Lee *et al.*[17] SQ109 is an ethambutol cage derivative and possesses excellent activity against MDR (multi drug-resistant) strains of tuberculosis [18]. It also exhibits a synergistic effect when used in combination with rifampicin and isonazid while an additive effect is observed when combined with streptomycin [19]. SQ109 is currently in clinical trials.



Amantadine: $R_1 = R_2 = H$
Memantine: $R_1 = R_2 = CH_3$

Figure 1: Structure of amantadine and memantine

The use of 1D NMR techniques and computational studies has been utilized in the elucidation of the adamantane skeleton,[20-24] however 2D NMR studies of mono-substituted adamantane derivatives has not enjoyed much attention. NMR and computational study of "cage" molecules such as pentacycloundecane and trishomocubane based compounds has been a major focus of our group for the past five years [25-36]. We recently reported the NMR elucidation of pentacycloundecane based tetra-amines[37,38] which were screened for their anti-tuberculosis activities [39]. Herein we report the full NMR studies of SQ109 and analogues (**2-6**) (Figure 2) recently synthesized and reported as part of an ongoing project aimed at developing novel molecules with potency against tuberculosis including XDR (extensively drug-resistant) strains of tuberculosis [40].

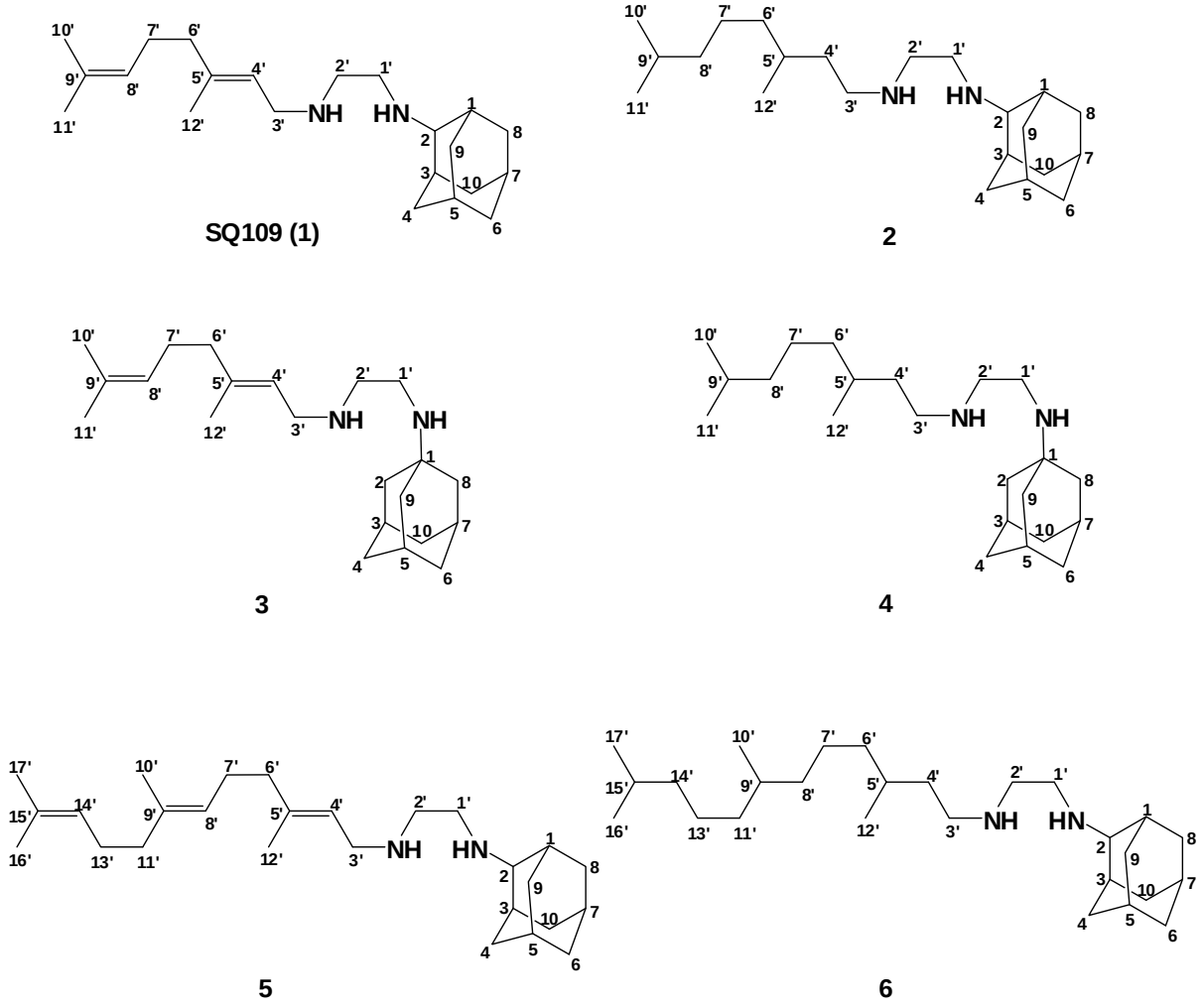


Figure 2: Structure of compounds 1-6

The numbering of the structures was chosen to differ from the IUPAC rules to assist with comparison of NMR data of the same atoms in different compounds. The starting material for SQ109 and compound **5** is 2-adamantanone while amantadine hydrochloride was the starting material for compound **3**. The syntheses of these compounds were reported in literature [40].

Results and discussion

We chose the ten carbon alkene chain as the starting point for the NMR elucidation of SQ109. Two alkene proton signals are observed at δ = 4.99 and 5.18 ppm and represents H-4' or H-8'. H-4' is assigned to the more deshielded peak at 5.18 ppm. A methylene proton (doublet, J = 6.06 Hz) at δ = 3.20 ppm shows a COSY correlation to H-4' (5.18 ppm) and was assigned to H-3'. The remaining alkene proton (H-8' at 4.99 ppm) displays a COSY correlation to H-7' at 2.00 ppm. H-7' exhibits a HMBC correlation to C-8' (δ = 123.9 ppm), two quarternary carbons (δ = 131.3 and 138.6 ppm) which were assigned to C-5' or C-9' and a methylene carbon at δ = 39.5 ppm which was assigned to

C-6'. The corresponding protons of the assigned carbon signals were determined using HSQC spectrum. H-6' (δ = 1.94 ppm) displays a HMBC correlation to a quarternary carbon at δ = 138.6 ppm thus confirming its assignment to C-5'. HMBC correlation of H-6' is also observed with C-4' and C-8' respectively. C-4' and C-5' both show HMBC correlation to a methyl proton (δ = 1.55 ppm) which was assigned to H-12' while C-8' and C-9' also display HMBC correlations to two methyl protons (δ = 1.50 and 1.58 ppm), the former was assigned to H-10' while the latter was assigned to H-11' respectively, based on literature [41]. The H-11' methyl proton displays ROESY interaction with H-8' while H-10' displays ROESY interaction with H-7', confirming this assignment. Also observed on the ROESY spectrum, is the interaction of H-12' with H-3', H-6' and H-7' respectively. Two methylene protons appearing as a singlet at δ = 2.68 ppm were assigned to H-1' and H-2'. These two methylene carbons (δ = 45.6 and 48.3 ppm) were confirmed to correspond to the proton signal at δ = 2.68 ppm using the HSQC spectrum. H-3' (δ = 3.20 ppm) displays HMBC correlation to the methylene carbon at δ = 48.3 ppm which was assigned to C-2' while the other carbon (45.6 ppm) was assigned to C-1'.

The adamantane skeleton was elucidated next. ROESY and HMBC techniques can potentially be used to determine the connection between the side chain and the cage moiety. The adamantyl skeleton has a plane of symmetry (through C-2, C-5, C-6, C-7). This simplifies the NMR spectrum of the cage significantly. Due to the *meso* nature of the 2-adamantyl moiety C-1/3, C-4/9 and C-8/10 appear as single carbon and proton resonances.

A methine carbon (δ = 61.7 ppm) exhibits a HMBC correlation to H-1'/H-2' (δ = 2.68 ppm), this was assigned to C-2. H-2 (δ = 2.64 ppm) shows a COSY correlation to a methine signal at δ = 1.77 ppm (integrating to two protons). The HSQC spectrum shows that this signal corresponds to a methine carbon, thus it was assigned to C-1 and C-3 (δ = 31.8 ppm). The protons attached to these carbon atoms are also identical and register at 1.77 ppm. The remaining two methine protons at δ = 1.68 and 1.74 ppm belongs to H-5 or H-7.

The most convenient handle to identify the NMR signals of the adamantane moiety is by recognizing the through space deshielding experienced by H-4b/H-9b. Since the C-2 to NH bond rotates freely, protons H-1 (δ = 1.77 ppm), H-2 (δ = 2.64 ppm), H-3 (δ = 1.77 ppm) and H-4b/H-9b should experience through space deshielding from the electronegative nitrogen atom.

Two pairs of doublet protons at δ = 1.41 and 1.89 ppm ($^2J_{HH}$ = 12.5 Hz) corresponds to a carbon signal at δ = 31.1 ppm which integrates to two methylene carbons, the former was assigned to H-4a/H-9a while the latter was assigned to H-4b/H-9b. These assignments were confirmed *via* COSY and ROESY spectra. Both pairs of the doublets (H-4/9) displayed COSY correlations with H-1/3 and a methine proton appearing at δ = 1.68 ppm, which was assigned to H-5. H-5 shows a COSY correlation with another signal at δ = 1.62 ppm, this was assigned to H-6. The other methine proton

appearing at δ = 1.74 ppm was assigned to H-7. H-2 exhibits HMBC correlations to C-4/9 and another methylene carbon appearing at δ = 37.4 ppm (integrating to two methylene carbons); this was assigned to C-8/10. The HSQC spectrum confirms that this carbon correlates to two methylene signals at δ = 1.75 and 1.62 ppm respectively (H-8/10). Distinction between H-8a and H-8b (and H-10a/H-10b) is yet to be made.

Table 1: ^{1}H NMR chemical shifts of compounds 1-6

Atom	1 ($\delta^{1}H^{a,b}$)	2 ($\delta^{1}H^{a,c}$)	3 ($\delta^{1}H^{a,b}$)	4 ($\delta^{1}H^{a,c}$)	5 ($\delta^{1}H^{a,b}$)	6 ($\delta^{1}H^{a,c}$)
1	1.77	1.78	-	-	1.83	1.81
2	2.64	2.63	1.58	1.55	2.67	2.66
3	1.77	1.78	1.99	1.96	1.83	1.81
4a	1.41	1.43	1.56-1.61	1.48-1.57	1.47	1.45
4b	1.89	1.91	-	-	1.93	1.93
5	1.68	1.70	1.99	1.96	1.74	1.72
6	1.62	1.64	1.56-1.61	1.48-1.57	1.68	1.66
7	1.74	1.76	1.99	1.96	1.81	1.78
8a	1.62	1.64	1.58	1.55	1.68	1.66
8b	1.75	1.76	-	-	1.81	1.79
9a	1.41	1.43	1.58	1.55	1.47	1.45
9b	1.89	1.91	-	-	1.93	1.93
10a	1.62	1.64	1.56-1.61	1.48-1.57	1.68	1.66
10b	1.75	1.76	-	-	1.81	1.79
1'	2.68	2.66	2.63	2.63	2.70	2.68
2'	2.68	2.66	2.63	2.63	2.70	2.68
3'	3.20	2.54	3.15	2.50	3.22	2.57
4'	5.18	1.16-1.25	5.20	1.11-1.21	5.25	1.19-1.25
5'	-	1.43	-	1.38	-	1.44
6'	1.94	1.16-1.25	1.94	1.11-1.21	1.99	1.19-1.25
7'	2.00	1.18	2.01	1.16	2.07	1.19-1.25
8'	4.99	1.05	5.03	1.03	5.08	1.07
9'	-	1.44	-	1.41	-	1.46
10'	1.50	0.78/0.79	1.53	0.74-0.77	1.56	0.80-0.84
11'	1.58	0.78/0.79	1.64	0.74-0.77	1.94	1.19-1.25
12'	1.55	0.81	1.57	0.74-0.77	1.62	0.80-0.84
13'					2.03	0.98-1.03
14'					5.06	0.98-1.03
15'					-	1.28
16'					1.57	0.80-0.84
17'					1.65	0.80-0.84

[a] Solvent $CDCl_3$.

[b] 600 MHz for ^{1}H.

[c] 400 MHz for ^{1}H.

H-2 also exhibits a HMBC correlation with C-1' (δ = 45.6 ppm). H-7 displays a COSY correlation with H-6 and H-8/10 respectively. H-2 displays a ROESY interaction with H-1/3 (δ = 1.77 ppm) and H-8a/10a (δ = 1.62 ppm) while H-4a/9a show a ROESY interaction with H-6 (δ = 1.62 ppm), H-4b/9b

(δ = 1.89 ppm) and H-8b/10b (δ = 1.75 ppm). H-1'/2' (δ = 2.68 ppm) also display a ROESY interaction with H-1/3 (δ = 1.77 ppm) and H-4b/9b (δ = 1.89 ppm) further confirming the positioning of these protons in a different environment. C-4/9 displays a HMBC correlation with H-8a/10a, H-6 H-4a/9a. C-8/10 exhibits a HMBC correlation with H-1/3, H-6, H-7, H-4a/9a and H-4b/9b respectively. A HMBC correlation was also observed for C-7 with H-1/3, H-6, H-8/10 (δ = 1.62 and 1.75 ppm). All assignments were further confirmed using the HSQC spectrum and is presented in Tables 1 and 2.

Table 2: ^{13}C NMR chemical shifts of compounds 1-6

Atom	1 ($\delta^{13}C^{a,b}$)	2 ($\delta^{13}C^{a,c}$)	3 ($\delta^{13}C^{a,b}$)	4 ($\delta^{13}C^{a,c}$)	5 ($\delta^{13}C^{a,b}$)	6 ($\delta^{13}C^{a,c}$)
1	31.8 (CH)	32.1 (CH)	50.2 (C)	50.7 (C)	32.3 (CH)	32.1 (CH)
2	61.7 (CH)	61.8 (CH)	42.8 (CH_2)	42.5 (CH_2)	61.9 (CH)	61.8 (CH)
3	31.8 (CH)	32.1 (CH)	29.7 (CH)	29.5 (CH)	32.3 (CH)	32.1 (CH)
4a	31.1 (CH_2)	31.3 (CH_2)	36.8 (CH_2)	36.6 (CH_2)	31.3 (CH_2)	31.3 (CH_2)
4b	31.1 (CH_2)	31.3 (CH_2)	-	-	31.3 (CH_2)	31.3 (CH_2)
5	27.4 (CH)	27.6 (CH)	29.7 (CH)	29.5 (CH)	27.6 (CH)	27.6 (CH)
6	37.7 (CH_2)	37.9 (CH_2)	36.8 (CH_2)	36.6 (CH_2)	38.0 (CH_2)	37.9 (CH_2)
7	27.6 (CH)	27.8 (CH)	29.7 (CH)	29.5 (CH)	27.8 (CH)	27.8 (CH)
8a	37.4 (CH_2)	37.5 (CH_2)	42.8 (CH_2)	42.5 (CH_2)	37.6 (CH_2)	37.5 (CH_2)
8b	37.4 (CH_2)	37.5 (CH_2)	-	-	37.6 (CH_2)	37.5 (CH_2)
9a	31.1 (CH_2)	31.3 (CH_2)	42.8 (CH_2)	42.5 (CH_2)	31.3 (CH_2)	31.3 (CH_2)
9b	31.1 (CH_2)	31.3 (CH_2)	-	-	31.3 (CH_2)	31.3 (CH_2)
10a	37.4 (CH_2)	37.5 (CH_2)	36.8 (CH_2)	36.6 (CH_2)	37.6 (CH_2)	37.5 (CH_2)
10b	37.4 (CH_2)	37.5 (CH_2)	-	-	37.6 (CH_2)	37.5 (CH_2)
1'	45.6 (CH_2)	46.4 (CH_2)	39.9 (CH_2)	39.7 (CH_2)	46.6 (CH_2)	46.5 (CH_2)
2'	48.3 (CH_2)	49.9 (CH_2)	50.0 (CH_2)	50.1 (CH_2)	49.6 (CH_2)	50.1 (CH_2)
3'	46.3 (CH_2)	47.7 (CH_2)	47.1 (CH_2)	47.7 (CH_2)	47.1 (CH_2)	47.8 (CH_2)
4'	121.4 (CH)	37.3 (CH_2)	123.0 (CH)	37.3 (CH_2)	123.0 (CH)	37.2-37.4 (CH_2)
5'	138.6 (C)	30.9 (CH)	137.4 (C)	30.9 (CH)	137.6 (C)	30.9 (CH)
6'	39.5 (CH_2)	37.3 (CH_2)	39.6 (CH_2)	37.2 (CH_2)	39.6 (CH_2)	37.2-37.4 (CH_2)
7'	26.3 (CH_2)	24.6 (CH_2)	26.5 (CH_2)	24.6 (CH_2)	26.4 (CH_2)	24.7 (CH_2)
8'	123.9 (CH)	39.2 (CH_2)	124.1 (CH)	39.2 (CH_2)	124.0 (CH)	39.3 (CH_2)
9'	131.3 (C)	27.9 (CH)	131.4 (C)	27.9 (CH)	135.1 (C)	27.8 (CH)
10'	17.5 (CH_3)	22.5/22.6 (CH_3)	17.6 (CH_3)	22.5/22.6 (CH_3)	15.9 (CH_3)	22.6 (CH_3)
11'	25.5 (CH_3)	22.5/22.6 (CH_3)	25.6 (CH_3)	22.5/22.6 (CH_3)	39.7 (CH_2)	24.4 (CH_2)
12'	16.1 (CH_3)	19.7 (CH_3)	16.3 (CH_3)	19.7 (CH_3)	16.3 (CH_3)	19.6 (CH_3)
13'					26.7 (CH_2)	37.2-37.4 (CH_2)
14'					124.3 (CH)	37.2-37.4(CH_2)
15'					131.3 (C)	32.7 (CH)
16'					17.7 (CH_3)	19.7/22.7 (CH_3)
17'					25.7 (CH_3)	19.7/22.7 (CH_3)

[a] Solvent $CDCl_3$.
[b] 150 MHz for ^{13}C.
[c] 100 MHz for ^{13}C.

The same methodology was utilized for the elucidation of compound **2** (and the remaining molecules); similar details will be omitted for the sake of brevity in the rest of the manuscript. The

only variation to the original structure is the hydrogenation of the alkene bonds to alkanes. The absence of two methylene protons is observed at δ = 4.99 and 5.18 ppm and confirms the successful reduction of compound **1**. The proton signal at δ = 2.54 ppm is assigned to H-3' and it displays a HMBC correlation to a methine carbon (δ = 30.9 ppm) and a methylene carbon (δ = 37.3 ppm), these signals were assigned to C-5' and C-4' respectively. H-3' also shows a HMBC correlation to C-2' (δ = 49.9 ppm) while C-1' was assigned to δ = 46.4 ppm. C-5' displays a HMBC correlation to H-12' (δ = 0.81 ppm). The only remaining methine carbon appears at δ = 27.9 ppm and was assigned to C-9'. H-9' (δ = 1.44 ppm) displays both NOESY and COSY correlations to a methylene proton, H-8' (δ = 1.05 ppm) and methyl protons (δ = 0.78-0.79 ppm) which should be H-10' and/or H-11'. H-8' shows HMBC correlations to C-10' and C-11' (δ = 22.5/22.6 ppm), C-9' (δ = 27.9 ppm) and two other methylene carbons appearing at δ = 24.6 and 37.3 ppm. The confirmation of the H-10'/H-11' assignment is obtained through the HSQC spectrum using the assigned carbon signals. Distinction between the two methyl groups (C-11' and C-10') seems impossible. The methylene carbon (δ = 24.6 ppm) displays a HMBC correlation to H-8' and H-9'; thus it was assigned to C-7' and the remaining carbon signal at δ = 37.3 ppm was assigned to C-6' through elimination. H-8' also displays NOESY and COSY correlations with H-7' and H-9'. The remaining signals were identified using the HSQC spectrum. It is clear from Tables 1 and 2 that there is good correlation of the proton and carbon signals for the 2-adamantyl moiety of SQ109 and compound **2**. This gives some reassurance about the correctness of the assignments. Major differences are observed for the long carbon chain which is as expected.

Unique aspects for the structure elucidation of compound **3** and **4**; will be discussed next. Due to the change in the positioning of the R group from position 2 to 1, there is a change in the plane of symmetry for the adamantyl skeleton. Three planes of symmetry are possible. Due to free rotation of the C-1 to N bond, the following groups are therefore equivalent: C-2/C-8/C-9 (CH_2), C-4/C-6/C-10 (CH_2) and C-3/C-5/C-7 (CH). The ^{13}C APT spectrum displays three methine carbon signals appearing at δ = 29.7, 123.0 and 124.1 ppm. The methine resonances 123.0 and 124.1 ppm were assigned to C-4' and C-8' following the previous elucidation of the alkene chain of SQ109. The remaining methine resonance at δ = 29.7 ppm is quite intense suggesting that there is more than one carbon registered to this signal. The proton signal associated with that (1.99 ppm) integrates to three protons, suggesting three equivalent CH-groups. This signal was assigned to C-3, C-5 and C-7 being the only remaining methine carbons present in the molecule. C-3/5/7 show a HMBC correlation to a signal at δ = 1.56-1.61 ppm, this signal could belong to any of H-2/8/9 or H-4/6/10. The HSQC spectrum shows that two intense methylene carbon signals (δ = 36.8 and 42.8 ppm) integrating to three carbons each correlates to this proton signal (δ = 1.56-1.61 ppm). A quarternary carbon at δ = 50.2 ppm (the only one in the adamantane region) was assigned to C-1. C-1 also shows a HMBC correlation to this signal (δ = 1.56-1.61 ppm). C-1 can only exhibit a HMBC correlation to protons H-

2/8/9 and not to H-4/6/10, hence confirming the location of the remaining adamantyl protons. The position of the carbon resonance for C-2/8/9 at a higher frequency (δ = 42.8 ppm) is in line with the expected negative inductive effect induced by the C-1-NH-R group. The NMR spectra for compound **4** are very similar to that of compound **3** (adamantyl part) and compound **2** (side chain part). These assignments are presented in Tables 1 and 2.

For compound **5** only the discussion on the elucidation of the side chain will be presented. The ^{1}H NMR spectrum of compound **5** shows an intense overlapping of methylene signals at 2.70 ppm, a doublet methylene signal at 3.22 ppm (J = 6.72 Hz) and a methine signal at 5.25 ppm (J = 6.60 Hz) and two overlapping methine signals at 5.06-5.09 ppm. The two overlapping methylene resonances appearing at 2.70 ppm was assigned to H-1'/H-2' as before. The methine proton (5.25 ppm) appearing at a higher frequency was assigned to H-4', this proton displays both COSY and NOESY interactions with the methylene proton appearing at 3.22 ppm (H-3'). The two remaining methine proton signals appearing at 5.06-5.09 ppm should be H-8' or H-14'. These methine protons (5.06-5.09 ppm) display COSY correlations to two methylene signals at δ = 2.03 and 2.07 ppm which should then be H-7' or H-13'. Due to overlap, clarification of these signals follows later.

The ^{13}C spectrum of compound **5** shows three quarternary carbon resonances appearing at 131.3, 135.1 and 137.6 ppm. H-3' displays a HMBC correlation with C-2' (49.6 ppm), C-4' (123.0 ppm) and a quarternary carbon signal appearing at 137.6 ppm; this was assigned to C-5'. C-5' exhibits HMBC correlations to a methylene proton (δ = 1.99 ppm) and methyl proton (δ = 1.62 ppm); these were assigned to H-6' and H-12' respectively. The quarternary carbon signal at 131.3 ppm shows HMBC correlations to a methylene proton at δ = 2.03 ppm and two methyl protons (δ = 1.57 and 1.65 ppm), this quarternary carbon was assigned to C-15', being the only quarternary carbon that can show HMBC correlation with two methyl protons [H-16' (δ = 1.57 ppm) and H-17' (δ = 1.65 ppm) respectively]. The methylene proton at 2.03 ppm was assigned to H-13'. Through elimination the remaining quarternary carbon signal at 135.1 ppm was assigned to C-9'. The signal appearing at 2.07 ppm was confirmed through COSY interaction with H-8' (δ = 5.08 ppm) to be H-7'. C-9' (δ = 135.1 ppm) displays a HMBC correlation with H-7', a methylene proton (δ = 1.94 ppm) and a methyl proton (δ = 1.56 ppm), these were assigned to H-11' and H-10' respectively. H-10' (δ = 1.56 ppm) shows NOESY interaction with H-7' and H-11' while H-12' (δ = 1.62 ppm) shows NOESY interaction with H-3', H-6' and H-7' respectively. H-17' (δ = 1.65 ppm) also displays NOESY interactions with H-14' (δ = 5.06 ppm) while H-16' (δ = 1.57 ppm) shows NOESY interaction with H-13'. The methine proton, H-2 (δ = 2.67 ppm) displays NOESY interactions with H-1/3 (δ = 1.83 ppm) and H-8a/10a (δ = 1.68 ppm). H-4b/9b (δ = 1.93 ppm) show NOESY interactions with H-1/3 (δ = 1.83 ppm), H-5 (δ = 1.74 ppm) and H-1'/2' (δ = 2.70 ppm) while H-4a/9a display NOESY interactions with H-1/3, H-5, H-6 (δ = 1.68 ppm) and H-8b/10b (δ = 1.81 ppm). The NMR data of compound **5** is presented in

Tables 1 and 2. Compound **6** was elucidated following similar methodology as carried out for compound **2**; the NMR data for compound **6** is also presented in Tables 1 and 2.

Conclusion

The full NMR elucidation of SQ109 and its derivatives was successfully carried out. Although considerable overlapping of proton and carbon signals was observed, 2D NMR techniques proved to be a useful tool in their elucidation. The adamantyl moiety is *meso* depending on the positioning of the R group, hence the similarities in the cage atom signals for all carbon and protons signals (Table 1 and 2) which are almost identical for compounds 1, 2, 5 and 6 (R-group on position 2) and compounds 3 and 4 (R-group on position 1). These studies supplement a small number of reports in literature on the complete elucidation of the mono-substituted adamantane skeleton.

Experimental

The NMR spectra were recorded on a Bruker AVANCE III 400 and 600 MHz NMR spectrometers using Topspin 2.1 (Bruker, Karlsruhe, Germany). The chemical shifts were referenced to the solvent peak δ = 7.24 ppm (^{1}H) and 77.0 ppm (^{13}C) for $CDCl_3$ at ambient temperature. The ^{1}H NMR spectra were recorded at a transmitter frequency of 600.103 MHz (spectral width 11.38 ppm; acquisition time of 2.398 s; the 30° pulse width equals 10 μs and 16 scans were recorded with a relaxation delay of 1s) while the ^{1}H NMR spectra were recorded at a transmitter frequency of 400.222 MHz (spectral width, 20.547 ppm; acquisition time, 1.99 s; pulse width, 10μs; scans, 16; relaxation delay, 1.0 s) for the AVANCE III 400 MHz spectrometer. The ^{13}C NMR spectra were recorded at 150.910 MHz (spectral width 238.9 ppm; acquisition time 0.908 s; 30° pulse width, 9.00 μs; 4800 scans; relaxation delay 2.00 s) for the AVANCE III 600 MHz spectrometer while the ^{13}C NMR spectra were recorded at 100.645 MHz (spectral width, 238.843 ppm, acquisition time 1.363 s, 30° pulse width, 8.40 μs; 1024 scans, relaxation delay 2.00 s) for the AVANCE III 400 MHz spectrometer.

The 2D experimental data parameters obtained on the Bruker AVANCE III 400 MHz were as follows: 90^{o} pulse width, 10 μs for all spectra; spectral width for ^{1} H, 3084.42, 3289.47 and 1851.85 Hz for **2**, **4** and **6** respectively (NOESY, COSY, HSQC and HMBC); spectral width for ^{13}C, 16670.416, and 22352.855 Hz (HSQC and HMBC) for **2**, **4** and **6**; number of data points per spectrum in F2 were 2048 (COSY), 2048 (NOESY), 4096 (HMBC), 1024 (HSQC) for compounds **2**, **4** and **6**; number of time-increments in F1 were 128 (COSY), 256 (NOESY), 128 (HMBC), 256 (HSQC) for compounds **2**, **4** and **6**; relaxation delay for compounds **2**, **4** and **6** were 1.35, 1.37 and 1.13 s (COSY experiments), 1.92, 1.94 and 1.70 s (NOESY experiments), 1.22, 1.26 and 0.78 s (HMBC experiments) 1.43, 1.44 and 1.32 s (HSQC experiments).

All 2D experimental data obtained on the Bruker AVANCE III 600 MHz for **SQ109**, **3** and **5** are as follows; 90^{o} pulse width, 15.1 μs for all spectra; spectral width for ^{1} H are 6830.60, 4261.36 and 2495.01 Hz for **SQ109**, **3** and **5** (NOESY, COSY, HSQC and HMBC) and 7211.54 Hz for **SQ109** (ROESY); spectral width for ^{13}C, 25000 and 36057. 691 Hz (HSQC and HMBC) for **SQ109**, **3** and **5**, number of data points per spectrum in F2 were 2048 (COSY), 2048 (NOESY), 4096 (HMBC), 1024 (HSQC) while number of time – increments in F1 were 128 (COSY), 256 (NOESY), 128 (HMBC), 256 (HSQC) for **SQ109**, **3** and **5**. The relaxation delay for compound **SQ109**, **3** and **5** are 1.0 s for COSY, NOESY, HSQC and HMBC respectively and a relaxation delay of 1.50 s was recorded for **SQ109** (ROESY); spectra acquired in phase-sensitive mode for all compounds are NOESY and HSQC; spectra for all compounds were acquired in absolute value mode. Gradients were used for the COSY, HSQC and HMBC spectra of **SQ109** and **2-6**. All NMR spectra are available as supplementary material.

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References

1. Dabur, R.; Chhillar, A. K.; Yadav, V.; Kamal, P. K.; Gupta, J.; Sharma, G. L. (2005) J Med Microbio 54: 549-552.
2. Orzeszko, A.; Gralewska, R.; Starosciak, B. J.; Kazimierczuk, Z. (2000) Acta Biochimica Polonica, 47: 87-94.
3. Orzeszko, A.; Kaminska, B.; Orzeszko, G.; Starosciak, B. J. (2000) Farmaco, 55: 619-623.
4. Orzeszko, A.; Kaminska, B.; Starosciak, B. J. (2002) Farmaco, 57: 619-624.
5. Kolocouris, A.; Dimas, K.; Pannecouque, C.; Witvrouw, M.; Foscolos, G. B.; Stamatiou, G.; Fytas, G.; Zoidis, G.; Kolocouris, N.; Andrei, G.; Snoeck, R.; De Clercq, E. (2002) Bioorg Med Chem Lett 12: 723-727.
6. Geldenhuys, W. J.; Malan, S. F.; Bloomquist, J. R.; Marchand, A. P.; Van der Schyf, C. J. (2005) Med Res Rev 25: 21-48.
7. Nagasawa, H. T.; Elberlin.Ja; Shirota, F. N. (1973) J Med Chem 16: 823-826.
8. Nagasawa, H. T.; Elberling, J. A.; Shirota, F. N. (1975) J Med Chem 18: 826-830.
9. Zah, J.; Terre'Blanche, G.; Erasmus, E.; Malan, S. F. (2003) Bioorg Med Chem 11: 3569-3578.
10. Jia, L.; Tomaszewski, J. E.; Hanrahan, C.; Coward, L.; Noker, P.; Gorman, G.; Nikonenko, B.; Protopopova, M. (2005) British J Pharmaco 144: 80-87.
11. Smith, J. P.; Riley, T. R.; Devenyi, A.; Bingaman, S. I.; Kunselman, A. (2004) J General Internal Med 19: 662-668.
12. Davies, W. L.; Hoffmann, C. E.; Paulshock, M.; Wood, T. R.; Haff, R. F.; Grunert, R. R.; Watts, J. C.; Hermann, E. C.; Neumayer, E. M.; McGahen, J. W. (1964) Science, 144: 862.
13. Stanicova, J.; Miskovsky, P.; Sutiak, V. (2001) Veterinarni Medicina, 46: 244-256.
14. Toggas, S. M.; Masliah, E.; Mucke, L. (1996) Brain Res 706: 303-307.
15. Parsons, C. G.; Danysz, W.; Quack, G. (1999) Neuropharmacology, 38: 735-767.
16. McKeage, K. (2009) Cns Drugs, 23: 881-897.
17. Lee, R. E.; Protopopova, M.; Crooks, E.; Slayden, R. A.; Terrot, M.; Barry, C. E. (2003) J Comb Chem 5: 172-187.
18. Protopopova, M.; Hanrahan, C.; Nikonenko, B.; Samala, R.; Chen, P.; Gearhart, J.; Einck, L.; Nacy, C. A. (2005) J Antimicrob Chemother 56: 968-974.
19. Chen, P.; Gearhart, J.; Protopopova, M.; Einck, L.; Nacy, C. A. (2006) J Antimicrob Chemother 58: 332-337.
20. Anderson, J. E.; de Meijere, A.; Kozhushkov, S. I.; Lunazzi, L.; Mazzanti, A. (2003) J Org Chem 68: 8494-8499.
21. Ganguly, B.; Singh, A.; Basaric, N.; Matkovic, M.; Mlinaric-Majerski, K. (2008) J Molecular Struct 888: 238-243.
22. Mikhova, B.; Stamboliyska, B.; Koch, A.; Duddeck, H.; Kleinpeter, E. (2008) Magn Reson Chem 46: 1153-1157.
23. Vikic-Topic, D.; Pejov, L. (2000) Croatica Chemica Acta, 73: 1057-1075.
24. Vikic-Topic, D.; Pejov, L. (2001) J Chem Information and Computer Sci, 41: 1478-1487.

25. Govender, T.; Kruger, H. G.; Raasch, T. (2005) Struct Chem 16: 129-134.

26. Kruger, H. G.; Mdluli, P. S. (2006) Struct Chem 17: 121-125.

27. Kruger, H. G.; Ramdhani, R. (2006) Magn Reson Chem, 44: 1058-1062.

28. Kruger, H. G.; Ramdhani, R. (2006) S Afr J Chem, 59, 71-U28.

29. Boyle, G. A.; Kruger, H. G.; Maguire, G. E. M.; Singh, A. (2007) Struct Chem 18: 633-639.

30. Boyle, G. A.; Govender, T.; Kruger, H. G.; Maguire, G. E. M.; Naicker, T. (2008) Magn Reson Chem 46: 1089-1095.

31. Boyle, G. A.; Govender, T.; Kruger, H. G.; Maguire, G. E. M.; Naicker, T. (2008) Struct Chem, 19: 429-434.

32. Onajole, O. K.; Govender, P.; Govender, T.; Maguire, G. E. M.; Kruger, H. G. (2009) Struct Chem 20: 1067-1076.

33. Onajole, O. K.; Makatini, M. M.; Govender, P.; Govender, T.; Maguire, G. E. M.; Kruger, H. G. (2010) Magn Reson Chem 48: 249-255.

34. Fourie, L.; Govender, T.; Hariprakasha, H. K.; Kruger, H. G.; Raasch, T. (2004) Magn Reson Chem 42: 617-623.

35. Kruger, H. G.; Mdluli, P.; Power, T. D.; Raasch, T.; Singh, A. (2006) J Molecular Structure-Theochem 771: 165-170.

36. Altaib, M. S.; Arvidson, P. I.; Govender, T.; Maguire, G. E. M.; Makatini, M.; Onajole, O. K.; Kruger, H. G. (2010) Magn Reson Chem 48: 435-442.

37. Govender, T.; Kruger, H. G.; Makatini, M.; Onajole, O. K. (2008) Struct Chem 19: 719-726.

38. Onajole, O. K.; Govender, T.; Makatini, M.; Kruger, H. G. (2008) Magn Reson Chem 46: 1007-1014.

39. Onajole, O. K.; Govender, K.; Govender, P.; Van Helden, P.; Kruger, H. G.; Maguire, G. E. M.; Muthusamy, K.; Pillay, M.; Wiid, I.; Govender, T. (2009) Euro J Med Chem 44: 4297 - 4305.

40. Onajole, O. K.; Govender, P.; Van Helden, P.; Kruger, H. G.; Maguire, G. E. M.; Wiid, I.; Govender, T. (2010) Euro J Med Chem 45: 2075-2079.

41. Bohlmann, F.; Zeisberg, R.; Klein, E. (1975) Org Magn Reson 7: 426-432.

CHAPTER 6

SQ109 ANALOGUES AS POTENTIAL ANTIMICROBIAL CANDIDATES

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Abstract

The antimicrobial activity of five novel **SQ109** derivatives including **SQ109** against bacteria, yeast and filamentous fungi is reported herein. Using broth microdilution techniques, compounds **2** and **3** were found to be active against most tested fungi and bacteria, with minimum inhibitory concentrations (MICs) ranging from 0.98-31 µg mL^{-1}, except for *Klebsiella pneumonia* where the MIC was 250 µg mL^{-1}. **SQ109** and derivative **4** did not show any significant activity against most of the organisms used. However, their reduced derivatives **1** and **5** showed promising activity with MICs between 0.49 and 62.5 µg mL^{-1} against most of the microorganisms used.

Keywords*:* Antimicrobial activity; structural activity relationship; SQ109; 1,2 diamine

Introduction

Fungal infection in humans is commonly caused by Candida species mainly *Candida albicans*. Opportunistic fungal pathogens are increasingly becoming a global epidemic coupled with the emergence of drug resistant fungi. Fungal infection caused by non-*Candida* species is also becoming rampant especially in immuno-compromised/suppressed patients (Castroagudin *et al.*, 2005; Netsvyetayeva *et al.*, 2009). Fungal and bacterial infections are most prominent in transplant patients

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resulting in high mortality rates (Shi *et al.*, 2009; Singh *et al.*, 2003; Zeglen *et al.*, 2009). The emergence of multidrug resistant fungal and bacterial strains has narrowed down possible treatment options; hence the need to design new antimicrobial therapeutics cannot be over emphasized. Preferably these new classes of drugs should have a broader antimicrobial spectrum of activity with little or no side effects.

SQ109 (*N*-Geranyl-*N′*-(2-adamantyl)ethane-1,2-diamine), a second generation agent developed from the first line drug ethambutol (Lee *et al.*, 2003) is a promising anti-tuberculosis (TB) candidate. **SQ109** is specific for mycobacteria showing high activity against *Mycobacterium tuberculosis, M. bovis* and *M. marinum*, but shows reduced activity against *M. avium* and *M. smegmatis* (SQ109, 2008).

We recently reported the anti-mcyobacterial activities of **SQ109** and its analogues (1-5). It was found that the longer 15 carbon alkene side chain diamine [compound **2]** was twofold more active than **SQ109** (which has a ten carbon side chain) against both H_{37}Rv and XDR 173 (an extensively resistant) strains of *M. tuberculosis* (Onajole *et al.*, 2010). The anti-mycobacterial activity of these compounds was lost when their alkene chain was reduced to a branched alkane chain. However, compound **5** demonstrated better activity than compound **4** against both H_{37}Rv and X173 strains of *M. tuberculosis* (Onajole *et al.*, 2010). **SQ109** was also reported to possess promising activity against susceptible and fluconazole resistant strains of *Candida albicans* at a MIC of 4-8 μg mL^{-1} (SQ109, 2008). Inspired by this report, all **SQ109** analogues (**1-5**) were screened in this study for other potential antimicrobial (fungal and bacterial) activities.

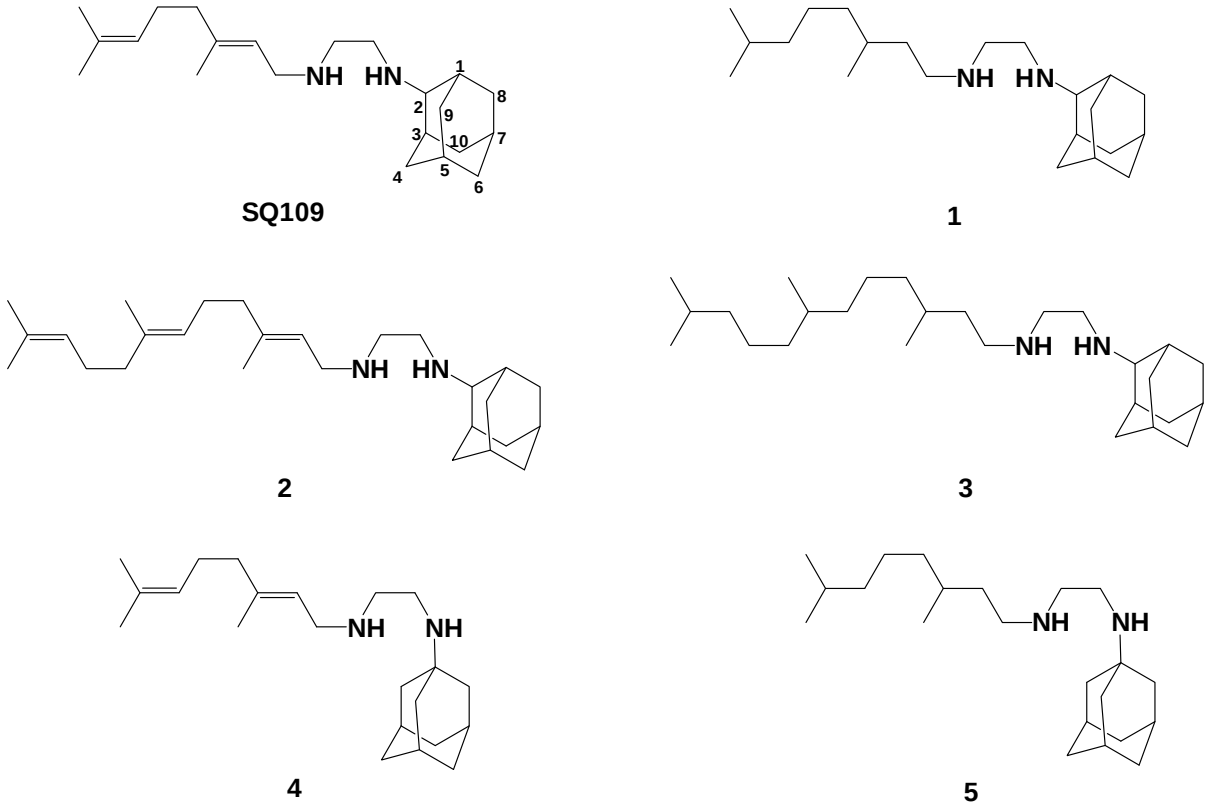
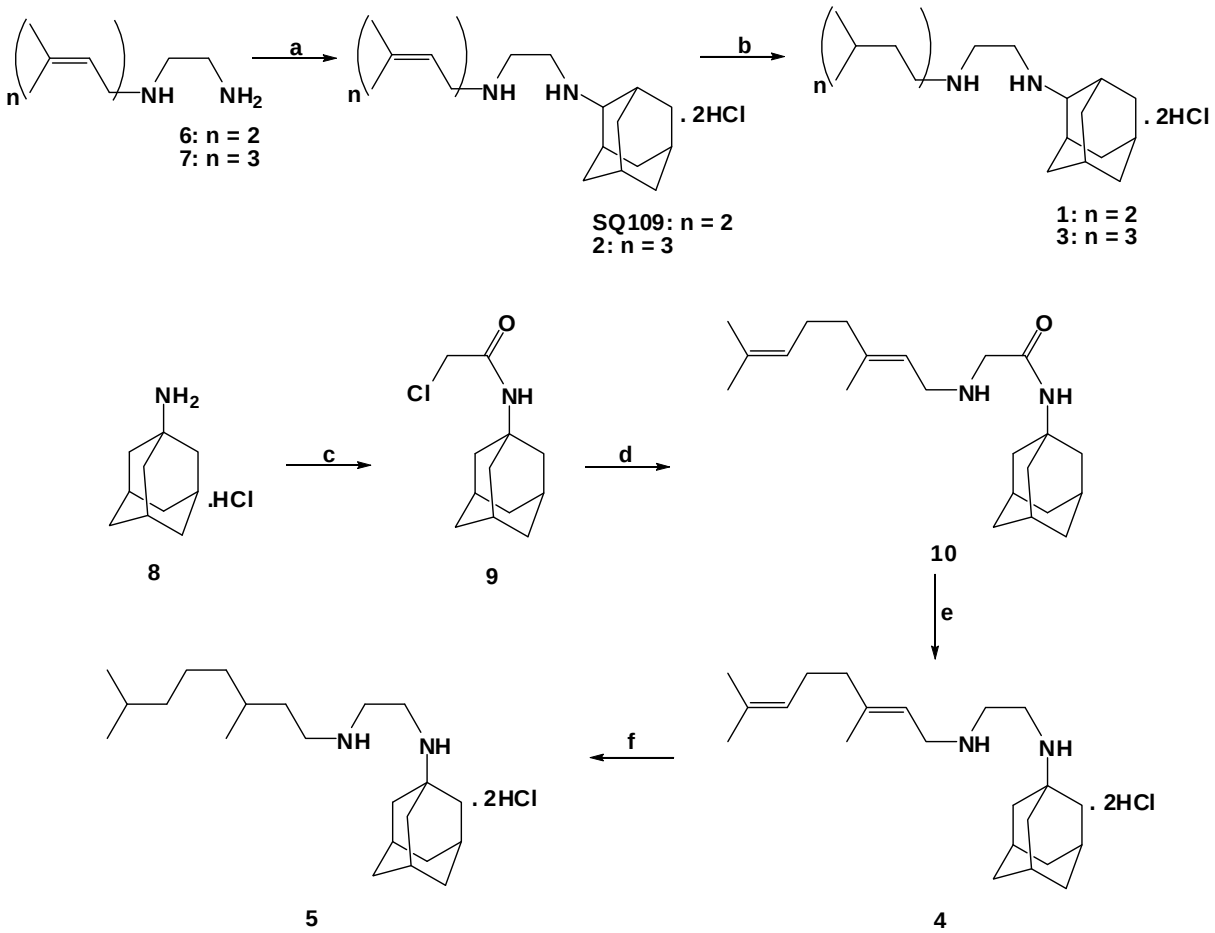


Figure 1: Free salts of SQ109 and its derivatives 1-5

Results and discussion

Geranyl bromide and *trans*, *trans* farnesyl bromide were reacted with excess ethylene diamine in dry dichloromethane at –78 ºC to afford (*E*)-*N′*-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (**6**) and *N′*-[(2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl]ethane-1,2-diamine (**7**) in a 86 % yield. Isoprenyl diamines **6** and **7** were reacted with 2-adamantanone (1.2:1) *via* reductive amination to obtain the desired substituted diamines; the diamines were treated with hydrochloride to obtain **SQ109** and **2** (57 % and 47 % yield). **SQ109** and **2** were subjected to catalytic hydrogenation using 10 % Pd/C, the obtained products were converted to their HCl salts to yield compounds **1** and **3** with a 57 % and 54 % yield (Scheme 1). The free salt of amantadine (**8**) was reacted with chloroacetyl chloride to obtain compound **9** in 96 % yield. Reaction of **9** with geranylamine resulted in compound **10** (40 % yield). Compound **10** was subjected to reduction using $LiAlH_4$ to obtain the 1,2 diamine intermediate, which was converted to its hydrochloride salt **4** in 71 % yield. Compound **4** was subjected to catalytic hydrogenation using 10% Pd/C to obtain derivative **5** (52 % yield).



Scheme 1: Reagents and conditions: (a) CH_3OH, 2-adamantanone, N_2 atmosphere, 2 h, $NaBH_4$, overnight; then HCl, MeOH. (b) 10 % Pd/C, NH_4HCO_2, MeOH, reflux, 16 h, N_2 atmosphere, then HCl, MeOH. (c) $NH_3/CHCl_3$, chloroacetyl chloride, $CHCl_3$, 2 h; (d) Geranylamine, Et_3N, DCM, overnight, rt; (e) $LiAlH_4$, THF, N_2 atmosphere, reflux for16 h; then HCl, MeOH; (f) 10 % Pd/C, NH_4HCO_2, MeOH, reflux, 16 h, N_2 atmosphere, then HCl, MeOH.

Twenty pathogenic isolates, nine yeast, ten filamentous fungi (moulds) and one plant filamentous fungus were employed in this study (Table 1). For amphotericin B, sixteen of the nineteen (84%) tested clinical fungal strains exhibited an MIC ranging between 0.02 and 1.25 μg mL^{-1} (Table 1). **SQ109** and compound **4** show slight activity between 62.5 and 125 μg mL^{-1} against most of the fungal strains used with the exception of *Sporothrix schenckii* where a MIC of 1.95 and 3.9 μg mL^{-1} were obtained. Interestingly, compounds **1** and **5** (reduced derivatives of **SQ109** and **4**) displayed higher activities (MIC ranging between 0.49-31 μg mL^{-1}) against all fungal strains when compared to **SQ109** and **4**. A MIC value of 25 μg mL^{-1} was also obtained for compounds **1**, **2** and **5** against *Aspergillus flavus* (a plant filamentous fungus) while **SQ109**, **3** and **4** did not show any activity against this organism even at the highest concentration tested (100 μg mL^{-1}). Compound **2** (a fifteen carbon alkene chain derivative of **SQ109**) and **3** (reduced derivative of **2**) possessed MIC values ranging between 0.98-31 μg mL^{-1}. Compound **2** (fifteen carbon alkene chain derivative) exhibited greater activity than **SQ109** (a ten carbon alkene chain), suggesting that the longer alkene chain contributes to

higher antifungal activity. Although compound **3** were expected to be more active than compound **2**, this however was not the case especially with the plant filamentous fungus. It is worth noting that all tested compounds (**SQ109**, analogues **1-5**) exhibited greater antifungal activity than amphotericin B (control drug) for *Sporothrix schenckii*. Compounds **1** and **5** were five times more active while compounds **2** and **3** were approximately three times more active than amphotericin B against Scopularopsis spp. (Table 1). Interestingly, all tested compounds showed good activity against fluconazole-sensitive strain of *Candida krusei*, more importantly their MIC improved against the fluconazole-resistant strain of *Candida krusei*. Compounds **2** and **3** displayed slightly lower activity than amphotericin B while compounds **1** and **5** possess similar MIC values as the control drug against fluconazole-resistant strain of *Candida krusei*.

Table 1: Antifungal activity of **SQ109** and its derivatives

Strain	**MIC (μg mL^{-1})**						
	SQ109	**1**	**2**	**3**	**4**	**5**	**Amph. B**
Candida utilis	15.6	1.95	1.95	3.13	31.25	1.95	0.31
Candida krusei	250	7.8	3.9	6.25	125	15.6	1.25
Candida tropicalis (2)	62.5-125	7.8	3.9-7.8	6.25	62.5	3.9-7.8	1.25
Candida parapsilosis (2)	250-500	7.8-15.6	7.8	6.25	250-500	15.6	0.04-1.25
Trichosporon asahii	125	7.8	3.9	6.25	125	7.8	0.31
Candida albicans	125	3.9	7.8	6.25	31.25	3.9	0.62
*Candida krusei**	15.6	0.98	1.95	3.13	15.6	0.98	1.25
*Candida parapsilosis**	250	7.8	3.9	6.25	125	7.8	0.02
Aspergillus fumigatus (2)	250	7.8	7.8	12.5	125-250	15.6	1.25
Fusarium spp.	125	7.8	7.8	12.5	250	7.8	2.5
Aspergillus flavus (2)	500	31.25	15.6-31	25	>500	31.25	1.25
Sporothrix schenckii	1.95	0.49	0.98	1.56	3.9	0.49	5
Scopularopsis spp.	62.5	1.95	3.9	3.13	125	1.95	10
Rhizomucor spp.	125	7.8	7.8	12.5	125	7.8	0.31
Pencillium spp.	125	3.9	3.9	6.25	62.5	7.8	1.25
Aspergillus flavus **	>100	25	25	>100	>100	25	Nd

* Fluconazole-resistant strains; ** Plant fungus (MRC2527); Values in brackets indicate number of isolates that were screened if more than one. Amph. B = Amphotericin B; Nd = Not done. The data are reported as the mean ± standard error of the mean of ≤ 5.

Four Gram-positive and four Gram-negative ATCC bacterial strains were employed in this study. Commercially available neomycin (Sigma) was active against bacterial strains used in this study (Table 2). **SQ109** and derivative **4** did not show any significant activity against any of the bacterial strains tested. Interestingly compound **1** and **5** showed activity against all the strains which seems to indicate that activity is enhanced by the reduction of the branched alkene chain to a branched alkane chain. Compound **2** and **3** proved to be approximately two- and three-fold more active than neomycin respectively against *Staphylococcus aureus* (ATCC 43300). Generally compound **3** exhibited two-fold greater activity than compound **2** except for *E. Coli* (same MIC) and *Klebsiella* spp. This trend is reversed for *Klebsiella* spp. It can be tentatively suggested that compound **2** may penetrate the capsule layer surrounding *Klebsiella* spp. with greater ease than compound **3**.

Table 2: Antibacterial activity of SQ109 and its derivatives

Strains (ATCC number)		**MIC (μg mL^{-1})**						
		SQ109	**1**	**2**	**3**	**4**	**5**	**Neomycin**
Gram-positive	*Bacillus subtilis* (6051)	500	15.6	7.8	3.9	125	15.6	0.78
	Rhodococcus equi (6939)	125	7.8	7.8	3.9	125	15.6	1.56
	Staphylococcus xylosus (35033)	500	15.6	7.8	3.9	250	15.6	0.20
	Staphylococcus aureus (43300)	1000	31.25	15.6	7.8	500	62.5	25
Gram-negative	*Escherichia coli* (35218)	500	31.25	7.8	7.8	500	31.25	3.13
	Klebsiella oxytoca (13182)	500	62.5	15.6	31.25	500	62.5	6.25
	Klebsiella pneumoniae (700603)	1000	125	125	250	1000	62.5	6.25
	Elizabethkingia meningoseptica (13253)	500	31.25	15.6	7.8	500	31.25	12.5

Conclusion

The susceptibility data obtained in this study strongly suggests that compound **2** could serve as an effective therapeutic agent against a broad range of infections. Compound **2** was reported (Onajole *et al.*, 2010) to be highly potent against an extensively resistant strain of mycobacterium (XDR-TB), and the same compound has further shown promising antifungal and antibacterial activity in this study. In our previous study (Onajole *et al.*, 2010), it was observed that **SQ109** and compound **2** lost activity against *Mycobacterium* spp. when reduced to compounds **1** and **3**, but compound **4** gained activity when reduced to **5**. This is believed to be due to the positioning of the long carbon chains i.e. position 1 (compound **5**) on the adamantyl moiety compared to position 2 (compound **1**). However in the present study, the positioning of the long alkene/alkane chain either on position 1 or 2 on the

adamantyl moiety had little effect on either the antibacterial or antifungal activities, as the alkane chain derivatives (**1** and **5**) were more favoured as antifungal and antibacterial agents compared to their alkene chain derivatives (**SQ109** and **4**).

Experimental

Chemistry

All necessary chemicals were purchased from Sigma-aldrich, Merck and Fluka. Reactions were monitored using thin layer Chromatography (TLC, Merck Kieselgel 60, F254). All purifications were carried by Column Chromatography using Fluka Kieselgel 60 (70–230 mesh) and CH_3Cl:CH_3OH:NH_4OH (88:10:2) as the eluent (solvent mixture). The NMR data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments in $CDCl_3$ using trimethylsilane (TMS) as internal standard: Mass spectra were obtained using a Bruker MicroTOF QII Time of Flight mass spectrometer while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3, melting point results were un-corrected. Level of purity for all compounds was judged to be ≥ 95% based upon ^{1}H NMR prior to their use in the evaluation for biological efficacies.

(E)-N′-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (6)

A light yellow oil (86 %, R_f = 0.45). ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.36 (s, NH), 1.56 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.97 (2H, m, CH_2), 2.05 (2H, m, CH_2), 2.63 (2H, t, *J* = 6.1, 5.6 Hz, CH_2), 2.78 (2H, t, *J* = 5.7, 6.1 Hz, CH_2), 3.21 (2H, d, *J* = 6.8 Hz, CH_2), 5.06 (1H, t, *J* = 6.8 Hz, CH), 5.23 (1H, t, *J* = 6.8 Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.3 (CH_3), 17.6 (CH_3), 25.7 (CH_3), 26.5 (CH_2), 39.6 (CH_2), 41.8 (CH_2), 47.1 (CH_2), 52.1 (CH_2), 122.8 (CH), 124.1 (CH), 131.5 (C), 137.7 (C).

N′-[(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trienyl]ethane-1,2-diamine (7)

A light yellow oil (86 %, R_f = 0.50). ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.32 (s, NH), 1.60 (6H, s, 2xCH_3), 1.65 (3H, s, CH_3), 1.68 (3H, s, CH_3), 1.95-2.11 (8H, m, 4xCH_2), 2.67 (2H, t, *J* = 6.0, 5.7 Hz, CH_2), 2.81 (2H, t, *J* = 5.7 Hz, 6.0 Hz, CH_2), 3.24 (2H, d, *J* = 6.7 Hz, CH_2), 5.09 (2H, m, 2xCH), 5.27 (1H, m, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.0 (CH_3), 16.3 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.4 (CH_2), 26.7 (CH_2), 39.6 (CH_2), 39.7 (CH_2), 41.9 (CH_2), 47.1 (CH_2), 52.2 (CH_2), 122.9 (CH), 124.0 (CH), 124.3 (CH), 131.3 (C), 135.1 (C), 137.7 (C).

N-Geranyl-N′-(2-adamantyl)ethane-1,2-diamine (SQ109)

A white solid; 57% yield, Melting point: 180-184 ºC, HR-MS calculated for $C_{22}H_{39}N_2$ ($[M+H]^+$ of free base) 331.3108, found 331.3135; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 1.41 (2H, d, *J* = 12.5 Hz, H-

4a/9a), 1.50 (3H, s, CH_3), 1.55 (3H, s, CH_3), 1.58 (3H, s, CH_3), 1.59-1.61 (4H, m, H-6, H-8a/10a), 1.68 (1H, s, H-5), 1.74-1.78 (5H, m, H-1, H-3, H-7, H-8b/10b), 1.89 (2H, d, *J* = 12.5 Hz, H-4b/9b), 1.93 (2H, m, CH_2), 2.00 (2H, m, CH_2), 2.64 (1H, s, H-2), 2.68 (4H, s, 2xCH_2), 3.20 (2H, d, *J* = 6.06 Hz, CH_2), 4.99 (1H, m, CH), 5.18 (1H, m, CH). ^{13}C NMR ($CDCl_3$, 150 MHz); δ_C 16.1 (CH_3), 17.5 (CH_3), 25.5 (CH_3), 26.3 (CH_2), 27.4 (C-5), 27.6 (C-7), 31.1 (C-4, 9), 31.8 (C-1, 3), 37.4 (C-8, 10), 37.7 (C-6), 39.5 (CH_2), 45.6 (CH_2), 46.3 (CH_2), 48.3 (CH_2), 61.7 (C-2), 121.4 (CH), 123.9 (CH), 131.3 (C), 138.6 (C).

N-(3,7-dimethyloctyl)-N´-(2-adamantyl)ethane-1,2-diamine (1)

A white solid (HCl salt); 57 % yield, Melting point: 182-185 ºC, HR-MS calculated for $C_{22}H_{43}N_2$ ($[M+H]^+$ of free base) 335.3421, found 335.3415; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 0.78–0.81 (9H, m, 3xCH_3), 1.01–1.09 (m, 3H, (2xCH_2)), 1.14–1.26 (4H, m, 2xCH_2), 1.40-1.48 (m, 4H (H-4a/9a, 2xCH), 1H (CH_2)), 1.64 (4H, s, H-6, H-8a/10a), 1.70 (1H, s, H-5), 1.75–1.78 (5H, m, H-1, H-3, H-7, H-8b/10b), 1.91 (2H, d, *J* = 12.2 Hz, H-4b/9b), 2.52–2.54 (2H, m, CH_2), 2.63 (1H, s, H-2), 2.66 (4H, s, 2xCH_2). ^{13}C NMR ($CDCl_3$, 100 MHz); δ_C 19.6 (CH_3), 22.5 (CH_3), 22.6 (CH_3), 24.6 (CH_2), 27.5 (C-5), 27.7 (C-7), 27.9 (CH), 30.9 (CH), 31.2 (C-4, 9), 32.1 (C-1, 3), 37.2 (CH_2), 37.3 (CH_2), 37.5 (C-8, 10), 37.9 (C-6), 39.2 (CH_2), 46.3 (CH_2), 47.7 (CH_2), 50.0 (CH_2), 61.8 (C-2).

N-trans-trans farnesyl -N´-(2-adamantyl)ethane-1,2-diamine (2)

A white solid (HCl salt); 47 % yield, Melting point: 146–150 ºC, HR-MS calculated for $C_{27}H_{47}N_2$ ($[M+H]^+$ of free base) 399.3734, found 399.3740; ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 1.47 (2H, d, *J* = 12.2 Hz, H-4a/9a), 1.57 (6H, s, 2xCH_3), 1.62 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.68 (4H, s, H-6, H-8a/10a), 1.74 (1H, s, H-5), 1.80–1.82 (5H, m, H-1, H-3, H-7, H-8b/10b), 1.93-1.96 (4H, m, H-4b/9b, CH_2),1.98-2.08 (6H, m, 3xCH_2), 2.67 (1H, s, H-2), 2.70 (4H, s, 2xCH_2), 3.22 (2H, d, *J* = 6.72 Hz, CH_2), 5.05–5.10 (2H, m, 2xCH), 5.25 (1H, t, *J* = 6.5 Hz, CH); ^{13}C NMR ($CDCl_3$, 150 MHz); δ_C 16.0 (CH_3), 16.3 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.4 (CH_2), 26.7 (CH_2), 27.6 (C-5), 27.8 (C-7), 31.3 (C-4, 9), 32.2 (C-1, 3), 37.6 (C-8, 10), 38.0 (C-6), 39.6 (CH_2), 39.7 (CH_2), 46.6 (CH_2), 47.1 (CH_2), 49.6 (CH_2), 61.9 (C-2), 123.0 (CH), 124.0 (CH), 124.3 (CH), 131.3 (C), 135.1 (C), 137.6 (C).

N-(3,7,11-trimethyldodecyl)-N´-(2-adamantyl)ethane-1,2-diamine (3)

A white solid (HCl salt); 54 % yield, Melting point: 114-118 ºC, HR-MS calculated for $C_{27}H_{53}N_2$ ($[M+H]^+$ of free base) 405.4203, found 405.4186; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 0.79–0.83 (12H, m, 4xCH_3), 0.98-1.11 (m, 4H, (2xCH_2), 1H (CH_2)), 1.13-1.33 (9H, m, CH, 4xCH_2), 1.42-1.50 (m, 4H, (H-4a/9a, 2xCH), 1H (CH_2)), 1.66 (4H, br s, H-6, H-8a/10a), 1.72 (1H, s, H-5), 1.78–1.81 (5H, m, H-1, H-3, H-7, H-8b/10b), 1.93 (2H, d, *J* = 12.2 Hz, H-4b/9b), 2.54–2.60 (2H, m, CH_2), 2.66 (1H, s, H-2), 2.68 (4H, s, 2xCH_2). ^{13}C NMR ($CDCl_3$, 100 MHz); δ_C 19.6 (CH_3), 19.7 (CH_3), 22.6 (CH_3), 22.7 (CH_3), 24.4 (CH_2), 24.8 (CH_2), 27.6 (C-5), 27.8 (C-7), 27.9 (CH), 31.0 (CH), 31.3 (C-4, 9), 32.2 (C-1,

3), 32.8 (CH), 37.3 (CH_2), 37.4 (CH_2), 37.5 (C-8, 10), 37.6 (CH_2), 38.0 (C-6), 39.3 (CH_2), 46.5 (CH_2), 47.9 (CH_2), 50.1 (CH_2), 61.9 (C-2).

N-Geranyl-N´-(1-adamantyl)acetamide (10)

A light yellow oil, 40 % yield; ^{1}H NMR ($CDCl_3$, 600 MHz): 1.57 (3H, s, CH_3), 1.60 (CH_3), 1.65 (9H, s, H-4, H-6, H-10, CH_3), 1.98 (8H, s, H-2, H-8, H-9, CH_2), 2.04-2.06 (5H, m, H-3, H-5, H-7, CH_2), 3.08 (2H, s, CH_2), 3.15 (2H, d, J = 6.9 Hz, CH_2), 5.05 (1H, t, J = 7.0 Hz, CH), 5.16 (1H, t, J = 6.6 Hz, CH), 6.94 (CONH). ^{13}C NMR ($CDCl_3$, 150 MHz); δ_C 16.3 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.4 (CH_2), 29.4 (C-3, 5, 7), 36.3 (C-4, 6, 10), 39.6 (CH_2), 41.6 (C-2, 8, 9), 47.0 (CH_2), 51.0 (C-1), 52.6 (CH_2), 121.9 (CH), 123.9 (CH), 131.6 (C), 138.8 (C), 170.6 (C=O).

N-Geranyl-N´-(1-adamantyl)ethane-1,2-diamine (4)

A yellowish solid (HCl salt); 71 % yield; Melting point: 135-138 ºC, HR-MS calculated for $C_{22}H_{39}N_2$ ($[M+H]^+$ of free base) 331.3108, found 331.3104; ^{1}H NMR ($CDCl_3$, 600 MHz): 1.53 (3H, s, CH_3), 1.57 (CH_3), 1.58 (6H, s, H-2, H-8, H-9), 1.56-1.61 (6H, m, H-4, H-6, H-10), 1.64 (3H, s, CH_3), 1.94 (2H, m, CH_2), 1.99 (3H, s, H-3, H-5, H-7), 2.01 (2H, m, CH_2), 2.63 (4H, s, 2xCH_2), 3.14 (2H, d, J = 6.72 Hz, CH_2), 5.01 (1H, m, CH), 5.17 (1H, t, J = 6.6 Hz, CH).. ^{13}C NMR ($CDCl_3$, 150 MHz); δ_C 16.3 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.3 (CH_2), 29.7 (C-3, 5, 7), 36.8 (C-4, 6, 10), 39.6 (CH_2), 39.9 (CH_2), 42.8 (C-2, 8, 9), 47.1 (CH_2), 50.0 (CH_2), 50.2 (C-1), 123.0 (CH), 124.1 (CH), 131.4 (C), 137.4 (C).

N-(3,7-dimethyloctyl)-N´-(1-adamantyl)ethane-1,2-diamine (5)

A white solid (HCl salt); 52 % yield, Melting point: 210 – 214 ºC, HR-MS calculated for $C_{22}H_{43}N_2$ ($[M+H]^+$ of free base) 335.3421, found 335.3418; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 0.74–0.77 (9H, m, 3xCH_3), 0.98-1.05 (m, 3H, (2xCH_2)), 1.11–1.21 (4H, m, 2xCH_2), 1.35–1.44 (m, 2H (2xCH), 1H (CH_2)), 1.48–1.57 (12H, m, H-2, H-4, H-6, H-8, H-9, H-10), 1.96 (3H, s, H-3, H-5, H-7), 2.50 (2H, s, CH_2), 2.63 (4H, s, 2xCH_2). ^{13}C NMR ($CDCl_3$, 100 MHz); δ_C 19.7 (CH_3), 22.5 (CH_3), 22.6 (CH_3), 24.6 (CH_2), 27.9 (CH), 29.5 (C-3, 5, 7), 30.9 (CH), 36.6 (C-4, 6, 10), 37.2 (CH_2), 37.3 (CH_2), 39.2 (CH_2), 39.7 (CH_2), 42.5 (C-2, 8, 9), 47.7 (CH_2), 50.1 (CH_2), 50.7 (C-1).

Antimicrobial evaluation

Antifungal assay

Twenty pathogenic isolates, nine human yeast, ten human filamentous fungi and one plant filamentous fungus were used in this study while two reference strains [*Candida parapsilosis* (ATCC 22019) and *Candida albicans* (ATCC 90028)] served as controls as recommended by Clinical and Laboratory Standards Institute, (CLSI, formerly the National Committee for Clinical Laboratory Standards). The clinical isolates were obtained from the National Health Laboratory Services

(NHLS), Inkosi Albert Luthuli Hospital, Durban, South Africa. The fungal isolates were recovered from the respiratory tracts, tissue samples and blood specimens of patients managed by the Hospital. Yeast isolates were identified at the species level using carbohydrate assimilation tests (API 32 ID, bioMérieux, Marcy 1'Etoile, France) while the filamentous fungi were identified by macroscopic and microscopic morphological features.

Antifungal/antibacterial agents

The hydrochloride salt of each compound [**SQ109** and its derivatives (**1-5**)] was dissolved in 10 % methanol and diluted with sterile double distilled water. Amphotericin B (Sigma-Aldrich) and neomycin (Sigma) were used as reference drugs. Freshly prepared solutions of **SQ109**, its derivatives and the reference drugs were used in this study. The final concentrations of the antimicrobial agents ranged from 0.0012 to 80 μg mL^{-1} amphotericin B, 0.0015 to 100 μg mL^{-1} neomycin and derivative **3**, 0.0076 to 1000 μg mL^{-1} for **SQ109** and derivatives **1**, **2**, **4** and **5**. All drug dilutions were performed in 96-well flat-bottom microtitre plates. Each well contained 100 μL of twofold serial dilutions of the tested drugs (2 x final concentrations) and were stored at -70 ºC until the day of testing.

Antifungal susceptibility assay

Evaluation of the susceptibility of *Candida albicans*, non-*Candida albicans* species and filamentous fungi (moulds) was performed using the broth microdilution method according to CLSI (formerly NCCLS) M27-A2 (for yeast) and M38-A2 (for filamentous fungi) guidelines (CLSI, 2008; NCCLS, 2002). Yeast strains were grown aerobically overnight at 35 ºC on Sabouraud dextrose agar (Merck) plates. Each yeast strain was harvested and suspended in 1 % sterile saline and the turbidity of the supernatants measured spectrophotometrically at 625 nm to achieve an absorbance of 0.08-0.10 units equivalent to the No. 0.5 McFarland standard of the NCCLS M27-A2 guidelines (NCCLS, 2002). The working suspension was diluted 1:20 in a mixture containing RPMI 1640 medium (with L-glutamine, without bicarbonate, Cambrex Bio Science Verviers, Belgium) and 0.165 M morpholinepropanesulfonic acid (MOPS, Sigma-Aldrich) buffered to pH 7.0. The working suspension was further diluted (1:50) with the medium to obtain the final test inoculum concentration of 1-5 x 10^3 CFU mL^{-1}. Microtitre plates containing frozen serial dilutions of the antibiotics and compounds 1-5 were allowed to thaw and equilibrate to room temperature under aseptic conditions after which 100 μL aliquots of the working inoculum suspension were dispensed into each well and the cultures were aerobically incubated at 35ºC for 24 h. Growth was observed visually with the aid of a concave mirror; MICs were taken on a growth or no-growth (100% visible-growth inhibition) scale.

MTS reduction Analysis

The MICs were also determined by colorimetric method using the dye, 2H-terazolium salt (MTS, Promega Corporation, Madison, USA). After MICs were determined visually on each micotitre plates, 20 µL of 2H-terazolium salt (Promega, 2005) was added directly to each well, incubated at 37 ºC for 4 h and the absorbance was spectrophotometrically determined at 490 nm using a microplate reader (*PowerWave* XS2, BioTek). All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

The anti-fungal activities of **SQ109** and its derivatives were also determined against human pathogenic filamentous fungi using the broth microdilution method according to the CLSI M38-A2 guidelines (CLSI, 2008). Cultures were grown on potato dextrose agar at 35ºC until sporulation (48 h to 7 days). Spores were harvested and suspended in 1 % sterile saline, allowed to settle and the upper layer aspirated. The turbidity was measured spectrophotometrically and the optical density was adjusted to yield a stock suspension of 0.4-5.0 x 10^6 sporangiospores per millilitre. A working suspension was prepared by making a 1:50 dilution of the conidia stock suspension in a standard medium (RPMI 1640, MOPS). Each fungal inoculum (100 µL) was added to individual wells of 96-well flat-bottom microdilution plates containing 100 µL of the appropriate dilution of the drug and aerobically incubated for 48 h at 35ºC. After incubation, potential antimicrobial activity (MICs) was assessed as described in the yeast section. All analyses were performed in triplicate and data are reported as the mean ± standard error of the mean of ≤ 5.

XTT reduction assay

Plant pathogenic *Aspergillus flavus* (MRC2527) was routinely grown on glucose minimal agar (pH 6.5) (50 mL/L of 20x nitrate salts, 1 mL/L trace elements, 10 g/L glucose) plates in an incubator at 28ºC for 4 to 5 days prior to use. When required, spores were harvested by flooding the surface of the plates with sterile water containing 0.1% Tween 20 and gently scraping off the spores with a sterile glass rod. Spore counts were done using a haemocytometer. Therefore the metabolic activity of *A. flavus* against **SQ109** and its derivatives was investigated using a modified procedure of Antachopoulos *et al*.(Antachopoulos *et al.*, 2006; Antachopoulos *et al.*, 2007). A concentration of 1.0 x 10^5 *A. flavus* spores was first inoculated into RPMI 1640 medium (with L-glutamine; without bicarbonate) (Gibco BRL, Life Technologies) in flat-bottomed 96-well microtiter plates. For each compound, the required concentrations were prepared by diluting each to attain a final volume of 200 µL per well. The concentrations of each tested compound ranged between 25 µg mL^{-1} to 100 µg mL^{-1}. A positive control, containing only fungal spores and no antibiotic was prepared for examination of spore viability. Negative controls, containing each compound with no spores were also prepared. These negative control wells served as a measure of the background absorbance (blank wells) contributed by the reagents used in the study. The plate was incubated at 28°C for 24 h. After the

incubation period, 50 µL of 1.25 mM XTT/menadione solution was added to each well, resulting in a final concentration of 100 µg mL^{-1} XTT and 25 µM menadione. The reactions were incubated for a further 2 h at 37 ^{o}C to allow for the conversion of the XTT into its formazan derivative and subsequently shaken for two minutes to mix the reagents. Thereafter, the absorbance was determined at 450 nm (*PowerWave* XS2, BioTek). The specific amount of XTT conversion was determined by subtracting the background absorbance (blank) from each experimental well as described by Antachopoulos *et al*. (Antachopoulos *et al.*, 2007). Each experiment was done in triplicate and repeated at least once.

Antibacterial susceptibility assay

Bacterial susceptibility was determined using the broth microdilution method (Eloff, 1998; Schwalbe *et al.*, 2007). Eight ATCC bacterial cultures obtained from the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Westville, South Africa were freshly cultured in Mueller-Hinton broth. The turbidity of overnight cultures that were grown for 16-18 h at 37ºC was adjusted to that of No. 0.5 McFarland standard. At a wavelength of 625 nm, the absorbance was adjusted between 0.08-0.10 units to yield a stock suspension of 0.4-5.0 x 10^{8} CFU mL^{-1}, which was diluted one hundred fold to obtain a working suspension of 10^{6} CFU mL^{-1}.

The microtitre plates containing frozen dilutions of compounds were allowed to equilibrate to room temperature under aseptic conditions. Aliquots (100 µL) of bacterial inocula were added to the prepared drug samples and incubated aerobically for 16-18 h at 37ºC. Following incubation, 50 µL of freshly prepared iodonitrotetrazolium chloride (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride), (INT) solution (200 µg mL^{-1}) was added to each well and the plate was further incubated for 45 minutes at 37ºC in the dark. When the colourless INT is reduced to red after incubation, persistent growth of the bacteria is indicated, while no colour change denotes a lack of bacterial growth. Neomycin was used as a control drug in this study and all analyses were performed in triplicate.

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References

Antachopoulos, C., Meletiadis, J., Roilides, E., Sein, T. & Walsh, T. J. (2006) Rapid susceptibility testing of medically important zygomycetes by XTT assay. J. Clin Microbiol 44, 553-560.

Antachopoulos, C., Meletiadis, J., Sein, T., Roilides, E. & Walsh, T. J. (2007) Use of high inoculum for early metabolic signalling and rapid susceptibility testing of Aspergillus species. J Antimicrob Chemother 59, 230-237.

Castroagudin, J. F., Ponton, C., Bustamante, M., Otero, E., Martinez, J., Tome, S., Conde, R., Segade, F. R., Delgado, M., Brage, A., Galban, C. & Varo, E. (2005) Prospective interventional study to evaluate the efficacy and safety of liposomal amphotericin B as prophylaxis of fungal infections in high-risk liver transplant recipients. Transplant Proc 37, 3965-3967.

CLSI (2008) Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, approved standard M38-A, vol. 22, no. 16, Clinical and Laboratory Standards Institute, Wayne, PA, 2002.

Eloff, J. N. (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 64, 711-713.

Lee, R. E., Protopopova, M., Crooks, E., Slayden, R. A., Terrot, M. & Barry, C. E. (2003) Combinatorial lead optimization of [1,2]-diamines based on ethambutol as potential antituberculosis preclinical candidates. J Comb Chem 5, 172-187.

NCCLS (2002) Clinical and Laboratory Standards Institute. Referencee method for broth dilution antifungal susceptibility testing of yeasts, approved standard M27-A2, vol. 22, no. 15, 2nd ed., CLSI Document. Clinical and Laboratory Standards Institute, Villanova, PA, 2002.

Netsvyetayeva, I., Swoboda-Kopec, E., Paczek, L., Fiedor, P., Sikora, M., Jaworska-Zaremba, M., Blachnio, S. & Luczak, M. (2009) Trichosporon asahii as a prospective pathogen in solid organ transplant recipients. Mycoses 52, 263-265.

Onajole, O. K., Govender, P., Van Helden, P., Kruger, H. G., Maguire, G. E. M., Wiid, I. & Govender, T. (2010) Synthesis and evaluation of SQ109 analogues as potential anti-tuberculosis candidates. Euro J Med Chem 45, 2075-2079.

Promega (2005) CellTiter 96 AQueous One Solution Cell Proliferation Assay. Technical Bulletin.

Schwalbe, R., Steele-Moore, L. & Goodwin, A. C. (2007) Antimicrobial Susceptibility Testing Protocols: CRC Press.

Shi, S. H., Kong, H. S., Xu, J., Zhang, W. J., Jia, C. K., Wang, W. L., Shen, Y., Zhang, M. & Zheng, S. S. (2009) Multidrug resistant gram-negative bacilli as predominant bacteremic pathogens in liver transplant recipients. Transplant Infect Dis 11, 405-412.

Singh, N., Avery, R. K., Munoz, P., Pruett, T. L., Alexander, B., Jacobs, R., Tollemar, J. G., Dominguez, E. A., Yu, C. M., Paterson, D. L., Husain, S., Kusne, S. & Linden, P. (2003) Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. Clin Infect Dis 36, 46-52.

SQ109 (2008) SQ109. Tuberculosis (Edinb) 88, 159-161.

Zeglen, S., Wojarski, J., Wozniak-Grygiel, E., Siola, M., Jastrzebski, D., Kucewicz-Czech, E. & Zembala, M. (2009) Frequency of Pseudomonas aeruginosa Colonizations/Infections in Lung Transplant Recipients. Transplant Proc 41, 3222-3224.

CHAPTER 7

NOVEL POLYCYCLIC 'CAGE'-1,2-DIAMINES AS POTENTIAL ANTI-TUBERCULOSIS AGENTS

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ABSTRACT

SQ109 (*N*-geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine) is a novel anti-tuberculosis candidate presently in clinical trials. In this report, a series of polycyclic 'cage' derivatives of *N*-geranyl-1,2 diamines were synthesized and screened for their anti-mycobacterial activity against $H_{37}Rv$, multidrug resistant (MDR) and extensively drug-resistant (XDR) strains of tuberculosis. By substituting the adamantyl skeleton of SQ109 with trishomocubanyl (**9**), oxa-pentacycloundecyl (**14**, **16**), pentacycloundecyl, PCU, (**10**, **15**) and aza-pentacycloundecyl (**22**, **23**), the effect of other polycyclic "cage" skeletons could be investigated. Using the BACTEC MGIT 960 system, compound **9** (trishomocubanyl moiety) proved to be the most active (MICs: 0.5 -2 µg/mL) while PCU hydroxyl derivatives (**15** and **23**), oxa-pentacycoundecyl and aza-pentacycloundecyl derivatives displayed similar activity to SQ109 (MICs: 0.5-4 µg/mL) against all three strains of TB used in this study. Compounds **10**, **14** and **21** displayed similar activities (MICs: 1-8 µg/mL) against all three strains of tuberculosis.

Keywords: SQ109, polycyclic "cage", tuberculosis, anti-tuberculosis

INTRODUCTION

Tuberculosis (TB) is a highly contagious and insidious disease with a high infection rate that has been present in humans since antiquity. TB is predominantly caused by *Mycobacterium tuberculosis* which is a slow growing bacterium. The causal organism can remain dormant in the host (human) for a very long time and may only become active when the person falls sick or has a back drop in his/her

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immune system. This event in particular has become most prominent in immuno-compromised patients such as those living with HIV (Human Immunodeficiency Virus). The 2009 global tuberculosis control report of the World Health Organisation (WHO), estimated that about 9.4 million incident cases of TB occurred globally. An estimated 1.4 million were HIV positive, of which, 78 % were in Africa while 13 % are located in South-East Asia [1].

There is an urgent need for highly potent, more effective drugs with fewer or no side effects and shorter treatment periods to combat the increasing TB pandemic. Present studies, identify molecules such as diarylquinolone (TMC207), nitroimidazole (OPC67683 and PA824), pyrrole (LL3858), diamine (SQ109) are in different stages of clinical trials [2-5].

SQ109 (**2**) first reported by Lee *et al.* [6] shares the same 1,2 ethylenediamine pharmacophore with ethambutol (**1**). SQ109 proved to be 14-35 fold more active than ethambutol against the $H_{37}Rv$ strain of tuberculosis [6]. SQ109 also possesses remarkable activity against MDR-TB which includes the EMB resistant strain suggesting that SQ109 is a new anti-TB drug with new mechanism and not an EMB analogue [7]. SQ109 induces a synergistic effect when used in combination with other first line drugs such as isoniazid and rifampicin, however an additive effect is observed when used with streptomycin [8].

The incorporation of polycyclic "cage" compounds (such as adamantane and pentacyclo-undecane) in potential pharmaceutical application has enjoyed much attention from researchers for four decades starting with the discovery of amantadine an anti-viral drug [9-11]. Polycyclic "cage" compounds possess the ability to improve drug lipophilicity, thus serving as a transport aid in carrying such drugs across cell membranes. It's been reported that 'cage' moieties such as adamantane and PCU are able to cross the Blood Brain Barrier (BBB) and the Central Nervous System (CNS) [9,12,13]. It also helps to reduce the bio-degradation of drugs in biological systems thus prolonging their pharmaceutical effect in the body [9,14,15]. SQ109 (**2**) and SQ117 (**3**) both have a lipophilic moiety, namely the 2-adamantly moiety in SQ109 (log P; 5.26) and a diphenyl moiety in SQ117 (log P; 5.50). However, different MIC values were reported for each [7]. SQ109 was five-fold more active than SQ117 suggesting that the nature of the lipophilic group does play a role in their activities even though SQ117 possesses a higher log P value [7].

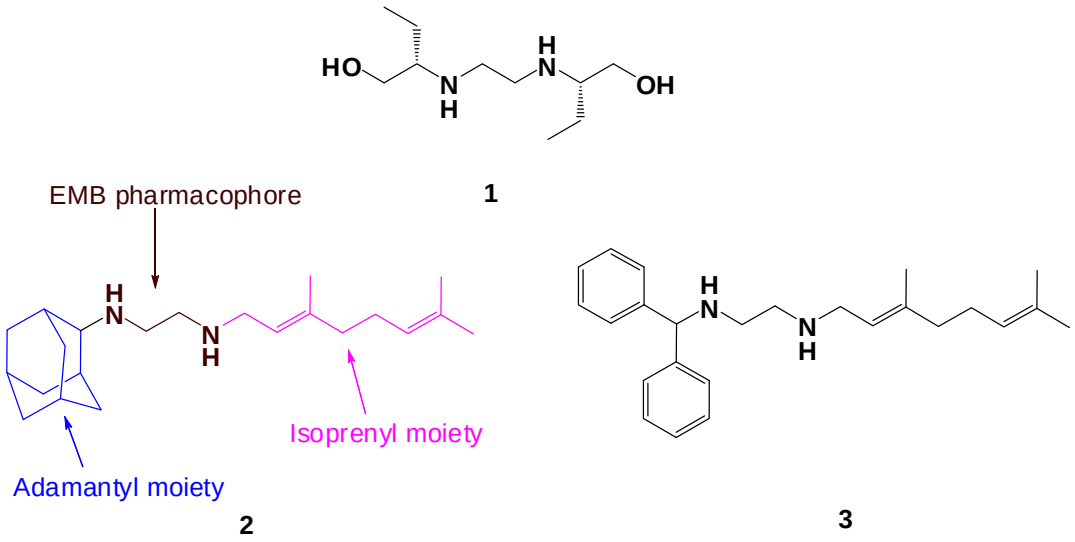
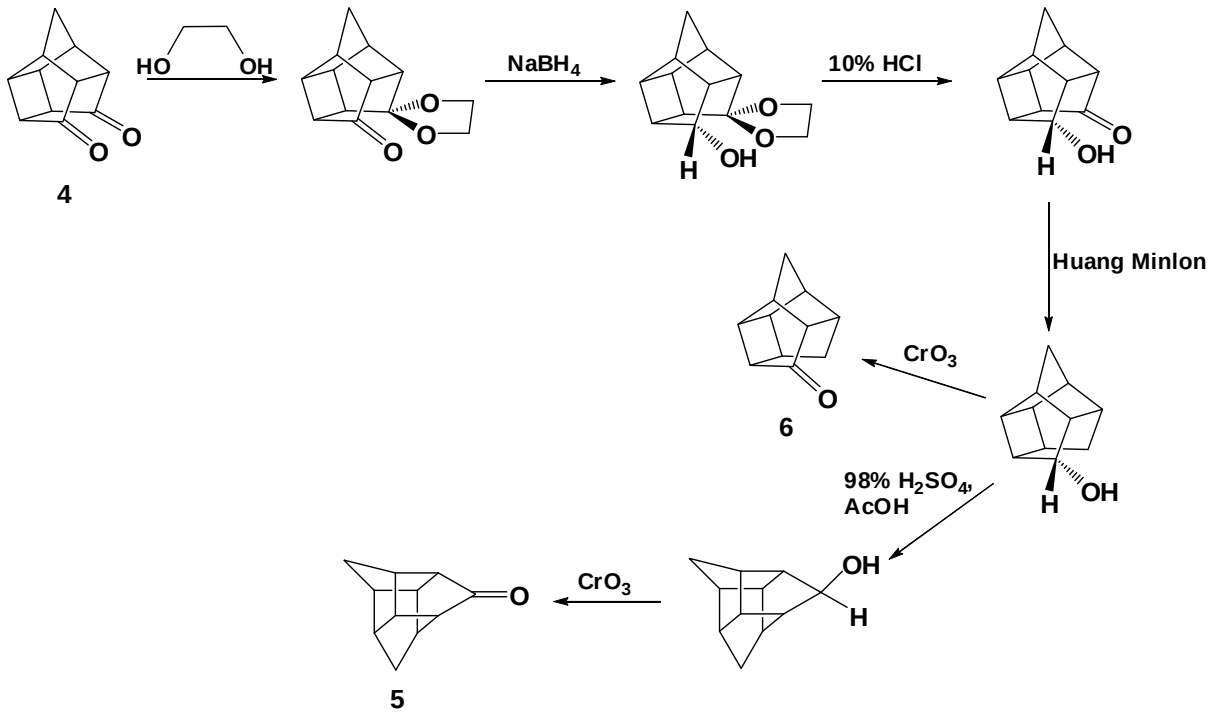


Figure 1: Structure of ethambutol (1), SQ109 (2) and SQ117 (3)

In the course of our research for novel anti-tubercular compounds,[16] we recently demonstrated the importance of chain length and saturation of some SQ109 derivatives [17]. This present study, aims at investigating the possibility of further enhancing/improving the anti-TB activity of the diamine *via* substitution of the adamantyl moiety with other polycyclic compounds such as trishomocubane and pentacycloundecane. Based on this; seven novel diamine based compounds bearing the lipophilic pentacycloundecane and trishomocubane cages were synthesized and screened for activity against drug sensitive ($H_{37}Rv$) and drug resistant strains of tuberculosis.

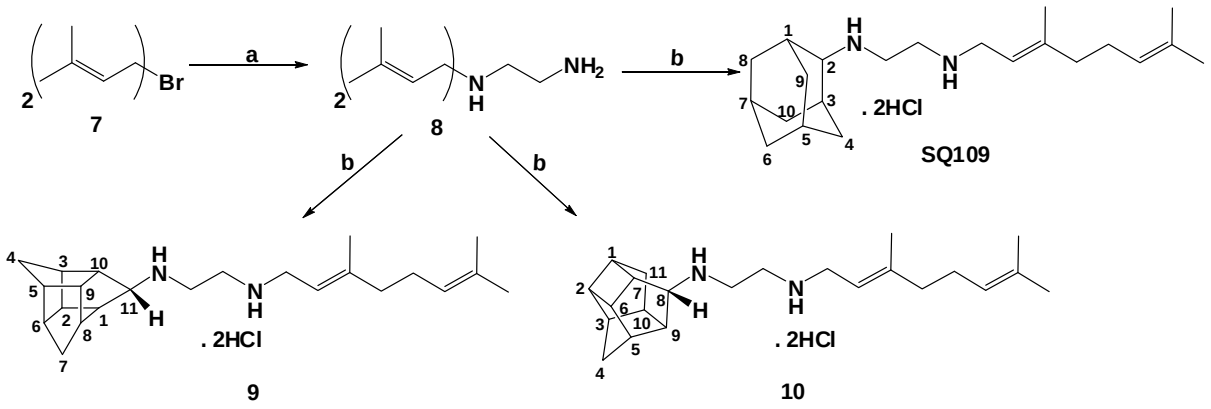


Scheme 1: Synthetic route for the synthesis of PCU monoketone and trishomocubanone

Results and discussion

Chemistry

Cookson's dione **4** [18,19] was the starting material in the synthesis of trishomocubanone (**5**) and the PCU-monoketone (**6**). Trishomocubanone was synthesized *via* a six step reaction pathway as reported in literature [20,21] and PCU monoketone was synthesized *via* a five step reaction pathway as reported in literature [20-22] (Scheme 1). Geranyl bromide **7** was reacted with excess ethane-1,2-diamine in dry dichloromethane at – 78 ºC to afford (*E*)-*N′*-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine **8** (86 % yield). Isoprenyl ethane-1,2-diamine **8** was reacted with trishomocubanone, PCU monoketone and 2-adamantanone *via* reductive amination; the resulting imines were reduced with $NaBH_4$ to obtain polycyclic-diamines; the diamines were converted to their HCl salts to obtain compounds **9** [*N*-geranyl-*N′*-(11-trishomocubanyl)ethane-1,2-diamine hydrochloride], **10 [***N*-geranyl-*N′*-(8-pentacycloundecyl)ethane-1,2-diamine hydrochloride] and SQ109 **[***N*-geranyl-*N′*-(2-adamantyl)ethane-1,2-diamine hydrochloride] (55-57 % yield). The expected orientation of the nitrogen atom on the PCU skeleton upon reductive amination with $NaBH_4$ is the *endo*-form [23,24]. This orientation was confirmed by a NOESY experiment as H-8 (2.67 ppm) displayed through space interaction with H-5 and H-9 (2.16-2.18 ppm).

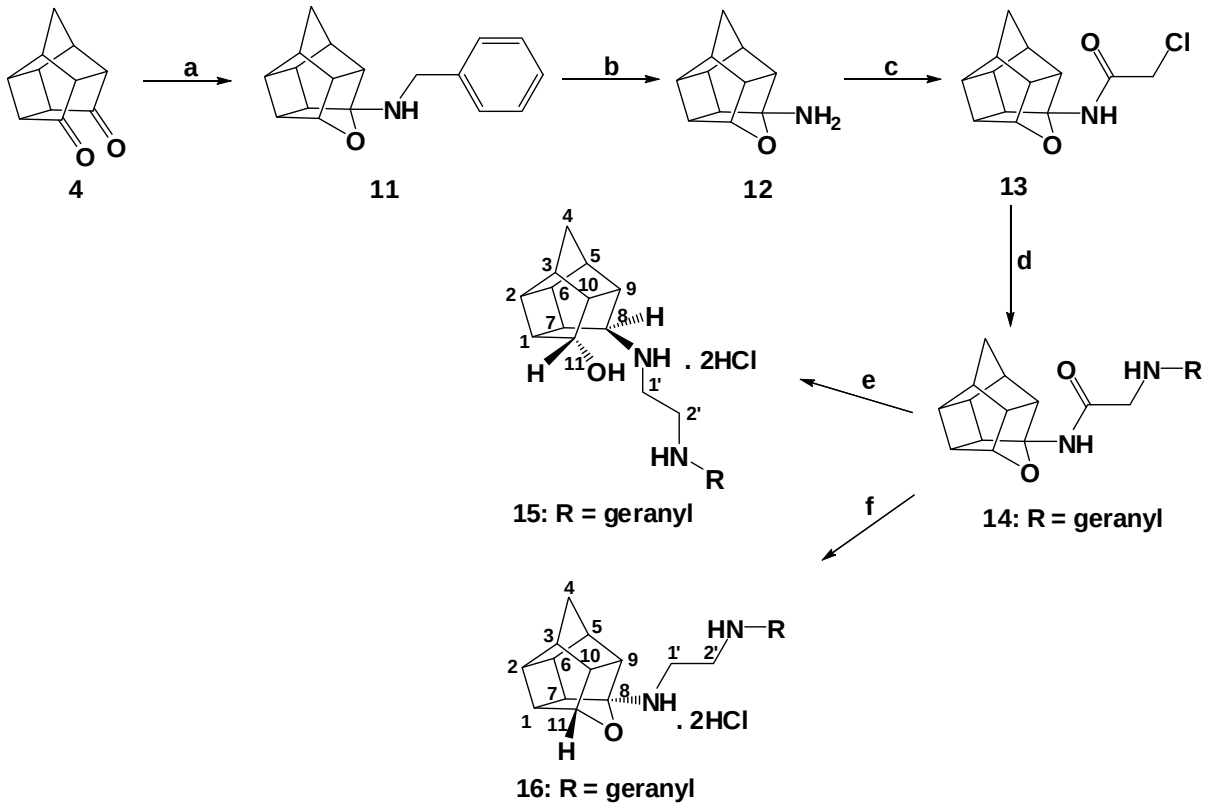


7 8 9 10

Scheme 2: Reagents and conditions: (a) ethylene diamine (100:1), -78 ºC, dry DCM; (b) MeOH, polycyclic 'cage' monoketone (1.2:1), N_2 atmosphere, 2 h, $NaBH_4$, overnight; then HCl, MeOH

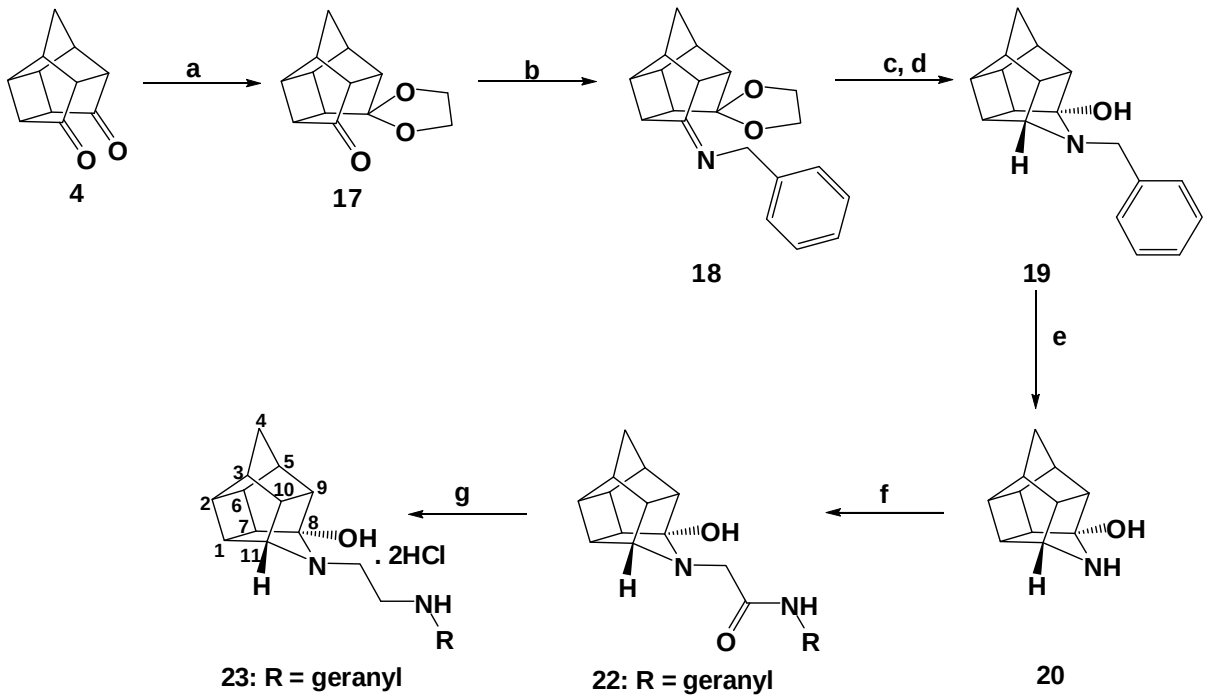
It was anticipated that the presence of a hydroxyl group (*endo*/*exo* positioning) on the cage moiety might contribute significantly to its activity; this led to the design and successful synthesis of compound **15** and **23**. 8-Benzylamino-8, 11-oxapentacyclo-[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**11)** was synthesized as reported in literature [13,25]. Compound **11** was debenzylated using 10% Pd/C under hydrogen gas at atmospheric pressure to obtain **12** in 60.8 % yield [26]. Compound **12** was reacted with chloroacetyl chloride to obtain compound **13** (62 % yield), which was reacted with geranylamine[17] to obtain compound **14** (58.5 % yield). Compound **14** was reduced using a strong reducing agent (LAH) at a 1:4 ratio in dry THF with refluxing to obtain a compound with m/z of 357.2897 (M+H$^+$, 33.7 % yield). Compound **15** was proposed and this structure was confirmed using 2D NMR experiments. A through space interaction of H-11 (3.86 ppm) with H-1 (2.68 ppm), H-3 (2.30 ppm) and H-10 (2.36 ppm) was observed thus proving that the hydroxyl group was at an *endo* position. H-8 also displayed a NOESY interaction with H-5 (2.34 ppm), H-7 (2.80 ppm), H-9 (2.51 ppm) and H-1'/2' (ethylene protons at 3.18 ppm) while the methylene protons, H-1'/2', displayed NOESY interactions with methyl protons (1.61 ppm), H-7, H-8 and H-9 respectively. These assignments confirm the *endo* positioning of the isoprenyl diamine moiety on the PCU cage as a result of the breakage of the ether bridge.

Attempts to synthesize the H-8 *endo*-orientated compound **10** prove unsuccessful; however an *exo* positioning of the R-group would be obtained if an oxa-pentacycloundecyl moiety is used, this led to the design and successful synthesis of compound **16**. This was achieved by using a milder reducing reagent (65 % Red-Al in toluene) on compound **14** to obtain **16** (45.2 % yield). NMR spectroscropy of compound **14** showed the successful synthesis as no extra methine carbon was observed at position 57.4 ppm and a quarternary carbon (C-8) at position 110.1 ppm was observed. C-8 displays a HMBC correlation with methylene protons (H-1') at 3.02 ppm while H-11 (4.58 ppm) also displayed NOESY interactions with H-1, H-3 and H-10 respectively, thus confirming the successful synthesis of compound **16**.



Scheme 3: Reagents and conditions: (a) benzylamine, THF, 0 ºC-5 ºC, 20 minutes; azeotropic distillation, benzene, 1 h, $NaBH_4$, 24 h; (b) 10 % Pd/C (1:1) mass ratio, H_2 gas, atmospheric pressure; (c) chloroacetyl chloride, dry DCM, K_2CO_3, reflux for 12 h; (d) geranylamine (1:2) mole ratio, K_2CO_3, dry THF, reflux; (e) LAH (1:5) mole ratio, dry THF, reflux, N_2 atmosphere, 12 h; then HCl, MeOH. (f) Red-Al (1:5) mole ratio, dry THF, reflux, N_2 atmosphere; then HCl, MeOH

Replacement of the oxo-bridge of **16** with an aza-bridge to give the isomeric hemiaminal **23** was also achieved (Scheme 4) and this compound was screened for anti-TB activity. Mono-protection of Cookson's diketone **4** was carried out to obtain the ethylene ketal **17** in 74 % yield and condensation with benzylamine gave the imine **18**. Reduction with $NaBH_4$, followed by hydrolysis resulted in the formation of the racemic hexacyclic cage amine **19**. Benzyl deprotection of **19** led to compound **20** (44 % yield). Geranylamine was reacted with chloroacetyl chloride to afford (*E*)-2-chloro-N-(3,7-dimethylocta-2,6-dienyl)acetamide (**21**, 81 % yield) which was reacted with 4-azahexacyclo [5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$] dodecan-3-ol (**20**) to obtain **22** (81 % yield). Reduction of **22** to yield the desired compound **23** (72 % yield) was achieved using 65 % Red-Al in toluene.



Scheme 4: Reagent and conditions: (a) ethylene glycol, *p*-toluenesulfonic acid (cat.), toluene, reflux, Dean-Stark Conditions; (b) Benzylamine, EtOH, 100 ºC, 18 h; (c) $NaBH_4$, EtOH, rt, 8 h; (d) Acetone, 4M HCl, 12 h, basified with 1M NaOH; (e) MeOH, 10% Pd/C, H_2 atm; (f) 21, K_2CO_3, THF, reflux, H_2 atm; (g) Red-Al (1:5) mole ratio, dry THF, reflux, N_2 atmosphere; then HCl, MeOH

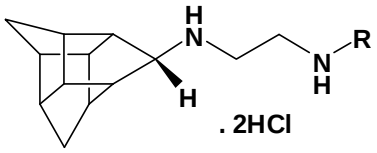
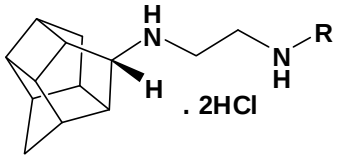
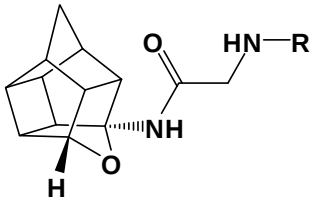
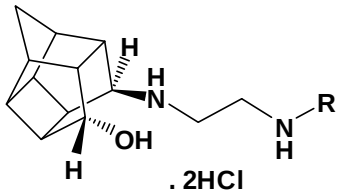
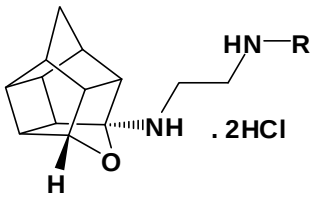
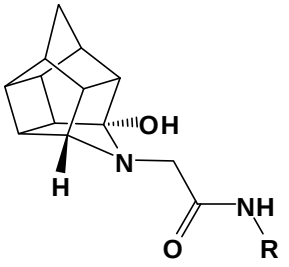
The successful syntheses of all novel compounds were confirmed using ^{1}H, ^{13}C, 2D NMR experiments (COSY, HSQC, HMBC, ROESY and NOESY), IR and HR-MS. All compounds were tested as racemate and confirmed to be greater than 95 % pure *via* NMR spectroscopy prior to their evaluation for biological activities.

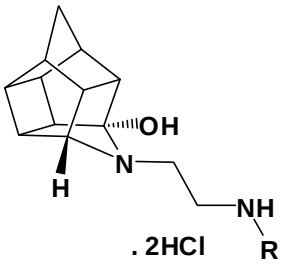
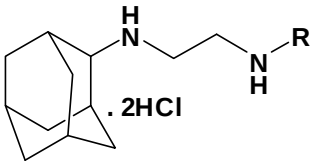
Anti-tubercular activity

The *in vitro* anti-mycobacterial activity of all the compounds against *M. tuberculosis* strain was carried out using the Mycobacteria Growth Indicator Tube system (MGIT). The minimum inhibitory concentration (MIC, µg/mL) was detected by BACTEC 960TB (Becton Dickinson). All compounds were screened against $H_{37}Rv$ (ATCC No: 25177), MDR and XDR strains of tuberculosis and the results are summarized in Table 1. Compounds **14** and **22** did not show any promising anti-TB activity implied that the carbonyl group contributes negatively to their inhibitory effect. Compounds **15** (*endo*-positioned hydroxyl group) and **23** (*exo*-positioned hydroxyl group) displayed similar activity as SQ109 against both MDR and XDR strains of TB at a MIC of 2 and 4µg/mL respectively. On the other hand, compound **10** (similar to **15**) without the hydroxyl group displayed reduced activity against all tested TB stains; this appears that the hydroxyl group not withstanding its positioning is essential for anti-tubercular activity. The oxa-pentacycloundecyl derivative **16** proves to be more active (two-fold) than **10** suggesting that the *exo* positioning of the isoprenyl diamine moiety on the pencycloundecanyl moiety is also essential for the anti-TB activity of this class of

compounds. Compound **9** proves to be the most active in this series; with a twofold increase in activity against MDR and XDR-TB when compared to **10**, **15**, **16**, **23** and **SQ109**. The D_3-trishomocubyl derivative **9** displayed significantly higher activity than its pentacycloundecyl and adamantyl counterparts.

Table 1: The MICs of the target compounds against M. tuberculosis ($H_{37}Rv$, MDR and XDR) strains

Compound	Structure	MIC (µg/mL)		
		$H_{37}Rv$	MDR	XDR
9		0.5	1	2
10		1	4	8
14		1	4	8
15		0.5	2	4
16		1	2	4
22		1	4	8

23		1	2	4
SQ109		0.5	2	4

R = geranyl moiety

This result suggests that the nature or type of polycyclic "cage" compounds is important for activity. As observed by many researchers in the field [9,11] the substitution of the polycyclic "cage" moiety (adamantyl) with similar moieties such as trishomocubane, pentacycloundecane, oxa-pentacycloundecane *etc.* in most cases maintains the activity of such compounds. Of all reported polycyclic 'cage' compounds only trishomocubane possess a unique D3 stereochemistry which could have contributed to its activity in this series.

Conclusion

We have synthesized a series of novel polycyclic 'cage' diamine derivatives with potent anti-TB activity. As observed, the positioning of the isoprenyl diamine on the PCU moiety either *endo* or *exo* does influence its anti-TB activity while the hydroxyl group is also essential for activity. Compound **9** (trishomocubyl moiety) was identified as the most potent against MDR and XDR strains of TB used with a two-fold increase in activity than **10**, **15** (pentacycloundecyl), **16** (oxapentacycloundecyl), **23** (azapentacycloundecyl), and SQ109 (2-adamantyl). This suggests that the type of polycyclic 'cage' moiety used has an influence on the activity of such compound. Further studies are ongoing in our laboratory to derivatise the lead compound with the possibility of enhancing its anti-TB activity.

Experimental

The NMR spectroscopic data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments using $CDCl_3$ as a solvent. All chemical shifts (δ) were quoted in parts per million downfield from TMS and the coupling constants (J) recorded in Hertz. Splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Reactions were

monitored using thin layer chromatography (TLC, Merck Kieselgel 60, F254). All purifications were carried by column chromatography using Fluka Kieselgel 60 (70–230 mesh) and CH_3Cl:CH_3OH:NH_4OH (88:10:2) as the eluent (solvent mixture). Level of purity for all compounds was judged to be >95% based upon ^1H NMR analysis. Purification of compound **15** were done *via* semi-preparative HPLC on a Shimadzu, LC-6AD instrument using water (solvent A) and acetronitrile (solvent B) while methanol (as Solvent B) was used for compound **23**. An ACE 5C18 150 x 21.2 mm column was used. A gradient elution system of 95 % solution A and 5 % solution B which changes linearly over 25 min to 20 % solution A and 80 % solution B at 15 mL/min, detected on a UV-VIS detector at 215 and 254 nm. Mass Spectra were obtained using a Bruker MicroTOF QII Time of Flight mass spectrometer while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3. Melting points results were uncorrected. Tetrahydrofuran was freshly distilled before use from a flask containing sodium benzophenone under N_2 atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use.

Synthesis of (*E*)-*N'*-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (8)[17]

To a vigorously stirred solution of ethane-1,2-diamine (55.4 g, 0.92 mol) in dichloromethane (400 mL) at -78 ºC under N_2 atmosphere was dropwise added geranyl bromide **7** (2 g, 9.2 mmol) in 1200 mL of dichloromethane over 4 hours. The reaction mixture was left to attain room temperature with stirring over night. The solution mixture was reduced *in vacuo* to about 500 mL and washed with water to remove excess ethane-1,2 diamine, the organic extract was dried over Mg_2SO_4 and concentrated *in vacuo*. The crude residue was purified using column chromatography [eluent; $CHCl_3$:CH_3OH:NH_4OH (88:10:2)] to give a light yellow oil (1.56 g, 86 %, R_f = 0.45). ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.36 (NH), 1.56 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.97 (2H, m, CH_2), 2.05 (2H, m, CH_2), 2.63 (2H, *t*, *J* = 5.6 Hz, CH_2), 2.78 (2H, t, J = 5.7, 6.1 Hz, CH_2), 3.21 (2H, *d*, *J* = 6.8 Hz, CH_2), 5.06 (1H, *J* = 6.9 Hz, CH), 5.23 (1H, *J* = 6.8 Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.3 (CH_3), 17.6 (CH_3), 25.7 (CH_3), 26.5 (CH_2), 39.6 (CH_2), 41.8 (CH_2), 47.1 (CH_2), 52.1 (CH_2), 122.8 (CH), 124.1 (CH), 131.5 (C), 137.7 (C).

Synthesis of *N*-Geranyl-N'-(trishomocubanyl)ethane-1,2-diamine (9), *N*-Geranyl-*N*'-(pentacycloundecyl)ethane-1,2-diamine (10), *N*-Geranyl-N'-(2-adamantyl)ethane-1,2-diamine (SQ109)

A mixture of isoprenyl diamine **8** (1.2 mol) and polycyclic 'cage' mono ketone (1 mol) in methanol (15 mL) was stirred for 2 h at room temperature under a nitrogen atmosphere. The resulting imine was reduced with solid $NaBH_4$ (1.2 mol) which was added slowly over 15 min and the mixture stirred overnight. Additional methanol (15 mL) was added to the reaction vessel after which water (20 mL) was added to quench excess $NaBH_4$. The solution was extracted with ethyl acetate (2 x 50 mL) and

the solution dried over Na_2SO_4 and concentration *in vacuo*. The crude product was purified *via* column chromatography on silica gel using $CHCl_3$:CH_3OH:NH_4OH (88:10:2) as eluent to give a yellow oil and converted to its HCl salt.

Data for *N*-Geranyl-*N′*-(11-trishomocubyl)ethane-1,2-diamine dihydrochloride (9)

A white solid (Mp; 174-178 ºC, 0.65 g, yield 57%, R_f = 0.64). IR ν_{max}: 3388, 2949, 2871, 2736, 1585, 1443, 1034, 773 and 555 cm^{-1}. MS (TOF) calculated for $C_{23}H_{37}N_2$ (M + H^+ of free base) 341.2951, found 341.2940. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.21-1.28 (3H, m, H-4a, H-7a, H-7s), 1.37 (1H, AB, d, *J* = 10.2 Hz, H-4s), 1.53 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.85-1.88 (2H, m, H-1, 2), 1.91-1.96 (4H, m, H-8, 10, CH_2), 1.97-2.03 (5H, m, H-5, 6, 9, CH_2), 2.37 (1H, s, H-3), 2.63-2.69 (4H, m, 2xCH_2), 2.91 (1H, s, H-11), 3.17 (2H, d, *J* = 6.8 Hz, CH_2), 5.03 (1H, m, CH), 5.19 (1H, m, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.2 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.4 (CH_2), 32.8 (C-4), 33.4 (C-7), 39.5 (CH_2), 40.6 (C-8), 41.4 (C-2), 44.2 (C-3), 44.7 (C-9), 46.9 (CH_2), 47.0 (C-5), 47.4 (C-6), 48.0 (CH_2), 49.2 (CH_2), 50.4 (C-10), 51.6 (C-1), 64.2 (C-11), 122.8 (CH), 124.0 (CH), 131.3 (C), 137.5 (C).

Data for *N*-Geranyl-*N′*-(8-pentacycloundecyl)ethane-1,2-diamine dihydrochloride (10)

A white solid (Mp; 158-160 ºC, 0.59 g, yield 55 %, R_f = 0.62). IR ν_{max}: 3140, 3048, 2948, 2694, 1444, 1405, 1034, 793 and 557 cm^{-1}. MS (TOF) calculated for $C_{23}H_{37}N_2$ (M + H^+ of free base) 341.2951, found 341.2928. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 0.94 (1H, AB, *J* = 11.9 Hz, H-11a), 1.13 (1H, AB, *J* = 10.3 Hz, H-4a), 1.55 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.62 (3H, s, CH_3), 1.64 (1H, s, H-4s), 1.83 (2H, br s, NH), 1.91-1.98 (2H, m, CH_2), 2.02-2.07 (2H, m, CH_2), 2.16-2.18 (3H, m, H-3, 5, 9), 2.26 (1H, s, H-10), 2.31 (1H, AB, *J* = 11.7 Hz, H-11s), 2.43 (1H, m, H-6), 2.49-2.54 (2H, m, H-2, 7), 2.60-2.62 (1H, m, H-1), 2.63-2.70 (5H, m, H-8, 2xCH_2), 3.19 (2H, *d*, *J* = 6.8 Hz, CH_2), 5.05 (1H, t, *J* = 5.6 Hz, CH), 5.21 (1H, t, *J* = 5.9 Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.2 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.4 (CH_2), 28.7 (C-11), 34.6 (C-4), 36.2 (C-1), 37.7 (C-7), 39.6 (CH_2), 40.8 (C-6), 41.8 (C-10), 41.9 (C-2), 44.2 (C-3/5), 44.6 (C-3/5), 46.9 (CH_2), 47.2 (C-9), 48.6 (CH_2), 49.3 (CH_2), 61.9 (C-8), 122.6 (CH), 124.1 (CH), 131.4 (C), 137.7 (C).

Data for SQ109 (*N*-Geranyl-*N′*-(2-adamantyl)ethane-1,2-diamine) dihydrochloride

A white solid (Melting point; 180-184 ºC, 0.35 g, 60 % yield, R_f = 0.62). IR ν_{max}: 3142, 3050, 2910, 2850, 1588, 1459, 1408, 1102, 778 and 553 cm^{-1}. HR-MS calculated for $C_{22}H_{39}N_2$ $(M+H)^+$ 331.3108, found 331.3135; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.47 (2H, d, *J* = 12.5 Hz, H-4a/9a), 1.57 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.66-1.68 (4H, m, H-6, 8a/10a), 1.74 (1H, s, H-5), 1.80-1.82 (5H, m, H-1, 3, 7, 8b/10b), 1.95 (2H, d, *J* = 12.6 Hz, H-4b/9b), 1.98-2.00 (2H, m, CH_2), 2.03-2.09 (2H, m, CH_2), 2.67 (1H, s, H-2), 2.70 (4H, s, 2xCH_2), 3.22 (2H, d, *J* = 6.8 Hz, CH_2), 5.06 (1H, m, CH), 5.24 (1H, t, *J* = 6.7 Hz, CH). ^{13}C NMR ($CDCl_3$, 100 MHz); δ_C 16.3 (CH_3), 17.6 (CH_3), 25.7

(CH_3), 26.5 (CH_2), 27.6 (C-5), 27.8 (C-7), 31.3 (C-4/9), 32.1 (C-1/3), 37.6 (C-8/10), 37.9 (C-6), 39.6 (CH_2), 46.5 (CH_2), 47.1 (CH_2), 49.5 (CH_2), 61.9 (C-2), 122.9 (CH), 124.1 (CH), 131.5 (C), 137.6 (C).

8-Benzylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (11)[13]

PCU-8,11-dione (Cookson's dione) was synthesized *via* photocyclization of Diels-Alder adducts obtained from reacting freshly cracked cyclopentadiene with *p*-benzoquionone [18,19]. PCU-dione (5g, 28.7 mmol) was dissolved in anhydrous tetrahydrofuran (THF, 50 mL) and cooled with stirring to 5ºC with an external ice bath. Benzylamine (3.39 g, 31.6 mmol) was added slowly with continuous stirring while maintaining the temperature. The reaction mixture was stirred over 30 minutes and the resulting hydroxylamine (a white precipitate) was filtered and washed with cold THF. Dehydration of the hydroxylamine in dry benzene was achieved under Dean-Stark condition for 1 h or until no more water collected in the trap. The resulting solution was concentrated *in vacuo* to obtain the Schiff base (a yellow oil) which was reduced with $NaBH_4$ (1.63 g, 43.05 mmol) in dry methanol (30 mL) and dry THF (150 mL) with stirring for 24 hr at room temperature. The solution was concentration *in vacuo* and water (2 x 100 mL) was added and the resulting mixture extracted with CH_2Cl_2 (4 x 50 mL) and the combined organic solution dried over Mg_2SO_4 and concentrated *in vacuo* to yield a yellow oil. Purification was carried using column chromatography with solvent system; Hexane:CH_2Cl_2 (1:1) to obtain **11** as a colourless solid. Melting point: 79-80 ºC, 2.73 g, 36 % yield, R_f = 0.20. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.55 (AB, *J* = 10.44 Hz, 1H), 1.91 (AB, *J* = 10.48 Hz, 1H), 2.14 (br s, 1H, NH), 2.42 (t, *J* = 4.9 Hz, 1H), 2.51-2.62 (m, 4H), 2.71-2.84 (m, 3H), 3.98 (AB, *J* = 13.36 Hz, 1H), 4.03 (AB, *J* = 13.36 Hz, 1H), 4.66 (t, *J* = 5.3 Hz, 1H), 7.21-7.37 (m, 5H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 41.5 (CH), 42.0 (CH), 43.1 (CH), 43.2 (CH_2), 44.5 (CH), 44.8 (2xCH), 47.8 (CH_2), 54.7 (CH), 55.2 (CH), 82.5 (CH), 109.5 (C), 126.8 (CH), 127.8 (CH), 128.3 (CH), 140.8 (C).

Synthesis of 8-amino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (12)

To a solution of compound **11** (2.73 g, 10.3 mmol) dissolved in dry methanol (100 mL) was added 10% Pd/C (1.37 g) and stirred under hydrogen gas at atmospheric pressure for 16 h or until no starting material was observed on TLC. The spent Pd/C was filtered using celite and a sintered funnel, the solution was concentrated *in vacuo* and the crude product purified on silica using CH_2Cl_2:CH_3OH (95:5) to obtain **12** in pure form. A white solid (Melting point: 143-145 ºC, 1.66 g, yield 60.8 % yield, R_f = 0.52). ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.50 (AB, *J* = 10.44 Hz, 1H), 1.86 (AB, *J* = 10.48 Hz, 1H), 2.09 (NH_2), 2.30-2.37 (m, 3H), 2.51-2.56 (m, 2H), 2.69-2.80 (m, 3H), 4.56 (t, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 41.4 (CH), 42.4 (CH), 43.3 (CH), 43.4 (CH_2), 44.8 (CH), 47.0 (CH), 55.1 (CH), 57.4 (CH), 83.0 (CH), 106.3 (C).

Synthesis of compound 8-chloroacetylamine-8,11-oxapentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]-undecane (13)

A mixture of compound **12** (1.62 g, 9.2 mmol), chloroacetyl chloride (0.807 mL, 10.1 mmol) and K_2CO_3 (2.54 g, 18.4 mmol) in dry DCM (20 mL) was stirred and heated gently at 40 ºC for 1 h and allowed to stir overnight without heat. Purification was carried on silica, CH_2Cl_2:CH_3OH (95:5) to obtain pale yellow oil which solidified on standing at room temperature to afford a yellowish solid. Melting point; 152-154 ºC, 1.44 g, 62 %, R_f = 0.67. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.53 (AB, *J* = 10.6 Hz, 1H), 1.91 (AB, 10.6 Hz, 1H), 2.42 (s, 1H), 2.59-2.62 (m, 2H), 2.74-2.79 (m, 1H), 2.89-1.98 (m, 4H), 4.02 (s, 2H), 4.73 (t, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 41.4 (CH), 41.9 (CH), 42.6 (CH_2), 43.2 (CH_2), 43.6 (CH), 44.6 (CH), 44.8 (CH), 46.1 (CH), 55.0 (CH), 56.7 (CH), 83.7 (CH), 102.9 (C), 166.2 (C).

Synthesis of 8-[(*E*)-*N*-3,7-dimethylocta-2,6-dienylamino]acetamide-8,11-oxapentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]-undecane (14)

To a solution of geranylamine (0.33 g, 3.57 mmol) in dry THF (15 mL) was added K_2CO_3 (0.37 g) and compound **13** (0.45 g, 1.8 mmol), the mixture was stirred with reflux, and the reaction was monitored by TLC until no starting material was observed. The reaction mixture was cooled, filtered and concentrated *in vacuo*, the crude product was purified on silica gel; $CHCl_3$:CH_3OH (95:5) to obtain **14**. A yellow oil (380 mg, 57.5 % yield, R_f = 0.34,). IR ν_{max}: 3308, 2966, 1672, 1514, 1002 and 747 cm^{-1}. MS (TOF) calculated for $C_{23}H_{33}N_2O_2$ (M + H$^+$) 369.2537, found 369.2537. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.54 (1H, AB, *J* = 10.5 Hz, H-4a), 1.57 (3H, s, CH_3), 1.58 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.90 (1H, AB, *J* = 10.5 Hz, H-4s), 1.95-2.06 (4H, m, 2xCH_2), 2.41 (1H, t, *J* = 4.76 Hz, H-3), 2.53 (1H, m, H-5), 2.57-2.60 (1H, m, H-2), 2.69-2.73 (1H, m, H-6), 2.85-3.00 (4H, m, H-1, 7, 9, 10), 3.18 (2H, d, *J* = 6.88 Hz, CH_2), 3.19 (2H, s, CH_2), 4.69 (1H, t, J = 5.16 Hz, H-11), 5.02-5.06 (1H, m, CH), 5.13-5.17 (1H, m, CH), 7.94 (CONH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.2 (CH_3), 17.7 (CH_3), 25.6 (CH_3), 26.4 (CH_2), 39.5 (CH_2), 41.4 (C-2), 41.8 (C-6), 43.4 (C-4), 43.5 (C-5), 44.6 (C-1), 44.8 (C-3), 45.9 (C-7), 47.1 (CH_2), 52.2 (CH_2), 54.8 (C-10), 56.4 (C-9), 83.5 (C-11), 102.2 (C-8), 121.7 (CH), 123.9 (CH), 131.6 (C), 139.0 (C), 172.1 (C=O).

Synthesis of 11-hydroxylpentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]-undecane-8-aminoethyl[(*E*)-*N*-3,7-dimethylocta-2,6-dien-amine] dihydrochloride (15)

Compound **14** (460 mg, 1.25 mmol) dissolved in dry THF (15 mL) was added LAH (0.24 g, 6.25 mmol) gently; the mixture was refluxed overnight under nitrogen atmosphere. The reaction vessel was cooled and the mixture quenched with aqueous Na_2SO_4, the obtained precipitate was filtered off and the filtrate concentrated to obtain the crude product. The crude product was purified *via* preparative HPLC (as specified above, retention time: 9.1 min) sample was lyophilized to obtain a **15**

(150 mg, 33.7 % yield) and converted to its HCl salt to obtain a yellowish slurry. IR ν_{max}: 3149, 3048, 2960, 2809, 1452, 1071 and 568 cm^{-1} MS (TOF) calculated for $C_{23}H_{37}N_2O$ ($M + H^+$ of free base) 357.2900, found 357.2897. ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.08 (1H, AB, *J* = 10.8 Hz, H-4a), 1.53 (3H, s, CH_3), 1.61-1.63 (7H, m, H-4s, 2xCH_3), 1.98-2.01 (4H, m, 2xCH_2), 2.30-2.38 (3H, m, H-3, 5, 10), 2.50-2.55 (2H, m, H-2, 9), 2.64-2.69 (2H, m, H-1, 6), 2.80 (1H, s, H-7), 2.94 (s, 1H, H-8), 3.18 (4H, s, 2xCH_2), 3.49 (2H, d, *J* = 7.2 Hz, CH_2), 3.86 (1H, t, *J* = 3.2 Hz, H-11), 4.98 (1H, t, *J* = 5.2 Hz, CH), 5.22 (1H, t, *J* = 6.7 Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.1 (CH_2), 34.0 (C-4), 34.9 (C-7), 37.8 (C-1), 39.5 (CH_2), 39.7 (C-2), 40.2 (C-6), 42.1 (C-9), 43.0 (C-3), 43.1 (CH_2), 43.2 (CH_2), 44.7 (C-5/10), 44.8 (C-5/10), 45.3 (CH_2), 57.4 (C-8), 70.4 (C-11), 114.2 (CH), 123.3 (CH), 131.9 (C), 145.2 (C).

Synthesis of 8,11-oxapentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}]undecane-8-aminoethyl[(*E*)-*N*-3,7-dimethylocta-2,6-dien-amine] dihydrochloride (16)

To a solution of compound **14** (0.38 g, 1.03 mmol) in dry THF (10 mL) at 0 ºC was added slowly 65 % Red Al in toluene (1.02 mL, 5.16 mmol) and kept at this temperature for 10 minutes. The reaction was kept at 35 ºC for an hour and refluxed. The reaction was monitored until no starting material was observed on TLC. THF (20 mL) was added to the reaction mixture and quenched with 5N NaOH (10 mL), the organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified on silica gel; the column was first flushed with 100 mL of $CHCl_3$:CH_3OH (95:5) after which a $CHCl_3$:CH_3OH:NH_4OH (88:10:2) as eluent mixture was introduced to obtain **16** (165 mg, 45.2 %, R_f = 0.57) in pure form and converted to its HCl salt to obtain a yellow slurry. IR ν_{max}: 3359, 2960, 1449, 1370, 1009 and 556 cm^{-1}. MS (TOF) calculated for $C_{23}H_{35}N_2O$ ($M + H^+$ of free base) 355.2744, found 355.2744. ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.52 (1H, AB, *J* = 10.5 Hz, H-4a), 1.57 (3H, s, CH_3), 1.62 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.88 (1H, AB, *J* = 10.5 Hz, H-4s), 1.97-2.07 (4H, m, 2xCH_2), 2.39 (1H, t, H-3), 2.46 (1H, t, J = 4.7 Hz, H-5), 2.50-2.59 (3H, m, H-2, 7, 9), 2.66-2.69 (1H, m, H-6), 2.73-2.81 (4H, m, H-1, 10, CH_2), 3.00 (2H, t, *J* = 5.4, 5.9 Hz, CH_2), 3.29 (2H, d, *J* = 6.96 Hz, CH_2), 4.58 (1H, t, *J* = 5.2 Hz, H-11), 5.03-5.07 (1H, m, CH), 5.22-5.26 (1H, m, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.5 (CH_2), 39.6 (CH_2), 41.5 (C-2), 41.9 (C-6), 42.3 (CH_2), 43.1 (C-5), 43.2 (C-4), 44.5 (C-1, 7), 44.8 (C-3), 46.3 (CH_2), 49.3 (CH_2), 54.7 (C-10), 55.1 (C-9), 82.5 (C-11), 110.1 (C-8), 120.2 (CH), 124.0 (CH), 131.8 (C), 140.4 (C).

Synthesis of pentacyclo[5.4.0.0.^{2,6}.0^{3,10}.0^{5,9}] undecane-8,11-dione ethylene acetal (17)[27-29]

A mixture of PCU dione **4** (10g, 57 mmol), ethylene glycol (4.5 mL, 80 mmol) and *p*-toluenesulfonic acid (0.33g, 1.9 mmol) was dissolved in toluene (45 mL) and refluxed using a Dean-Stark apparatus for three days. The reaction mixture was allowed to cool down followed by addition of cold aqueous solution of 10% (m/v) Na_2CO_3 (60 mL) and extracted with dichloromethane (3 x 25 mL). The organic layer was dried using anhydrous Na_2SO_4. The mixture was filtered and the solvent evaporated *in vacuo*. The crude product was purified via silica gel column chromatography (40:60; EtOAc: Hexane) and obtained as a white solid (Melting point = 71 ºC; 10.3 g, 80 %, R_f = 0.55). ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.55 (AB, *J* = 11.0 Hz, 1H), 1.84 (AB, *J* = 11.0 Hz, 1H), 2.37-2.38 (m, 1H), 2.44-2.47 (m, 1H), 2.51-2.63 (m, 3H), 2.74-2.79 (m, 2H), 2.89-2.94 (m, 1H), 3.80-3.91 (m, 4H). ^{13}C NMR [$CDCl_3$, 100 MHz] δ_C 36.3 (CH), 38.7 (CH_2), 41.3 (CH), 41.4 (CH), 42.2 (CH), 42.8 (CH), 45.8 (CH), 50.7 (CH), 52.9 (CH), 64.4 (CH_2), 65.6 (CH_2), 113.8 (C), 215.2 (C).

Synthesis of 4-benzyl-4-azahexacyclo[5.4.1.0.0.^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (19)

A mixture of compound **17** (1g, 4.59 mmol) and benzylamine (0.59g, 5.5 mmol) in EtOH (10 mL) was heated at 100 ^{0}C using a sealed high pressure glass tube for 16 h. The solution was cooled and $NaBH_4$ (0.35g, 9.17 mmol) was added gradually and the mixture was stirred at room temperature for 8 h. The solution was concentrated *in vacuo*, water (20 mL) was added and the intermediate was extracted twice with a 3x15 mL portion of CH_2Cl_2. The combined organic solution was washed with brine (15 mL) dried ($MgSO_4$) and concentrated *in vacuo*. To the crude product was added acetone (30 mL) and aqueous 4M HCl (20 mL) with stirring at room temperature for 12 hours. Water was added (250 mL) and the solution was basified to pH 14 with aqueous 1M NaOH and extracted with CH_2Cl_2 (3x25 mL). The combined organic extract was dried over $MgSO_4$ and concentrated to obtain crude product, which was recrystallised from isopropanol to yield the desired product **19** (0.35 g, 29 % yield). Melting point; 158-159 ºC, ^{1}H NMR [DMSO-d_6, 400 MHz]: δ_H 1.43 (AB, *J* = 10.2 Hz, 1H), 1.76 (AB, *J* = 10.2 Hz, 1H), 2.27 (t, *J* = 4.7 Hz, 1H), 2.46-2.51 (m, 4H), 2.60-2.69 (m, 2H), 2.77-2.80 (m, 1H), 3.17 (t, *J* = 5.0 Hz, 1H), 3.23 (br, 1H), 3.61 (d, *J* = 13.3 Hz, 1H), 5.76 (s, OH), 7.17-7.31 (m, 5H). ^{13}C NMR [DMSO-d_6, 100 MHz] δ_C 41.2 (CH), 41.5 (CH), 41.6 (CH), 41.8 (CH_2), 42.5 (CH), 44.7 (CH), 45.1 (CH), 50.4 (CH), 50.8 (CH_2), 55.3 (CH), 65.2 (CH), 105.9 (C), 126.2 (CH), 127.9 (CH), 128.4 (CH), 140.6 (C).

Synthesis of 4-azahexacyclo[5.4.1.0.0.^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (20)

To a solution of compound **18** (2.2 g, 8.3 mmol) in of dry CH_3OH (30 mL) was added gently 10 % Pd/C (1.1 g) and stirred under H_2 atmospheric pressure for 16 h or until no starting material was

observed on TLC. The spent Pd/C was filtered using celite and a sintered funnel, the solution was concentrated *in vacuo* and the crude product purified on silica using $CHCl_3$:CH_3OH:NH_4OH (88:10:2) to obtain **19** in pure form as a white solid (M.p: 81-83 ºC, 0.64 g, yield 44.1 %, R_f = 0.30).^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.48 (AB, *J* = 10.5 Hz, 1H), 1.83 (AB, *J* = 10.4 Hz, 1H), 2.37-2.46 (m, 3H), 2.57-2.62 (m, 2H), 2.67-2.82 (m, 3H), 3.53 (t, *J* = 5.0 Hz, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 41.6 (CH), 41.8 (CH_2), 42.6 (CH), 43.4 (CH), 44.9 (CH), 45.8 (CH), 46.1 (CH), 54.5 (CH), 55.2 (CH), 60.5 (CH) 105.9 (C).

Synthesis of (*E*)-2-chloro-N-(3,7-dimethylocta-2,6-dienyl)acetamide (21)

A mixture of chloroacetyl chloride (0.88 g, 7.8 mmol), geranylamine (1 g, 6.5 mmol) and K_2CO_3 (1.08 g, 7.8 mmol,) in dry THF (30 mL) was refluxed for 16 h. The solution was filtered and concentrated *in vacuo* and purified on silica using $CHCl_3$:EtOAc (70:30) to afford (*E*)-2-chloro-N-(3,7-dimethylocta-2,6-dienyl)acetamide [**20**, 1.20 g, 81 % yield, R_f = 0.80]. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.56 (s, 3H) 1.64 (s, 6H), 1.97-2.08 (m, 4H), 3.86 (t, *J* = 6.24 Hz, 2H), 4.00 (s, 2H), 5.03 (t, *J* = 6.72 Hz, 1H), 5.16 (t, *J* = 6.96 Hz, 1H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.2 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.2 (CH_2), 37.7 (CH_2), 39.3 (CH_2), 42.5 (CH_2), 118.9 (CH), 123.6 (CH), 131.8 (C), 140.7 (C), 165.5 (C=O).

Synthesis of 4-[(*E*)-*N*-3,7-dimethylocta-2,6-dienyl]acetamide-4-azahexacyclo[5.4.1.0.0.^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol (22)

To a stirred solution of compound **20** (0.3 g, 1.7 mmol) in dry THF (15 mL) was added (*E*)-2-chloro-N-(3,7-dimethylocta-2,6-dienyl)acetamide (**21**, 0.39 g, 1.7 mmol) and K_2CO_3 (0.35 g, 2.55 mmol). The mixture was then refluxed overnight under N_2 atmosphere. The reaction was allowed to cool to room temperature, filtered and concentrated *in vacuo*. The crude product was purified on silica using $CHCl_3$:CH_3OH (90:10,) to afford the product **22** as light yellow oil. (445 mg, 72 % yield, R_f = 0.53) IR ν_{max}: 3230, 2966, 2868, 1661, 1562, 1348 and 728 cm^{-1}. MS (TOF) calculated for $C_{23}H_{33}N_2O_2$ (M + H$^+$) 369.2537, found 369.2539. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.49 (3H, s, CH_3), 1.52 (1H, m, H-4a), 1.56 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.84 (1H, m, H-4s), 1.87-1.98 (4H, m, 2xCH_2), 2.56 (1H, s, H-3), 2.66-2.70 (2H, m, H-2, 5), 2.78-2.84 (3H, m, H-6, 7, 9), 2.95 (1H, s, H-10), 3.06 (1H, s, H-1), 3.39 (br s, 1H), 3.71 (d, *J* = 15.4 Hz, 1H), 3.75 (2H, m, CH_2), 4.05 (1H, t, *J* = 4.8 Hz, H-11), 4.97 (1H, t, *J* = 5.6 Hz, CH), 5.08 (1H, t, *J* = 6.3 Hz, CH), 7.93 (s, CONH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.1 (CH_3), 17.5 (CH_3), 25.4 (CH_3), 26.2 (CH_2), 37.5 (CH_2), 39.3 (CH_2) 40.4 (C-1), 41.1 (C-4), 41.4 (C-2, 6), 43.2 (C-7), 43.8 (C-5), 46.2 (C-3), 47.5 (CH_2), 49.6 (C-10), 52.8 (C-9), 65.9 (C-11), 111.8 (C-8), 119.1 (CH), 123.6 (CH), 131.4 (C), 139.5 (C), 164.5 (C=O).

Synthesis of 4-[(*E*)-*N*-ethyl-3,7-dimethylocta-2,6-dienyl]amine-4-azahexacyclo[5.4.1.0.0^{2,6}.0^{3,10}.0^{5,9}.0^{8,11}]dodecan-3-ol dihydrochloride (23)

To a solution of compound **22** (0.36 g, 0.97 mmol) in dry THF (10 mL) at 0 ºC was added slowly 65 % Red Al in toluene (0.58 mL, 2.9 mmol) and the mixture was kept at this temperature for 10 minutes. The reaction was kept at 35 ºC for an hour and refluxed; the reaction was monitored until no starting material was observed on LC-MS. 20 mL of THF was added to the reaction mixture and quenched with 5N NaOH (10 mL), the organic layer was separated, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was dissolved in methanol (5 mL) and purified using preparative HPLC (as specified above, retention time: 13.6 mins). The sample was lyophilized to obtain **23** (200 mg, 55 % yield) and converted to its HCl salt to obtain a light yellow slurry. IR ν_{max}: 2967, 2866, 1668, 1198, 1178, 1129, 831 and 719 cm^{-1}. MS (TOF) calculated for $C_{23}H_{35}N_2O$ ($M + H^+$ of free base) 355.2744, found 355.2748. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.57 (3H, s, CH_3), 1.60 (1H, H-4a), 1.65 (6H, s, 2xCH_3), 1.93 (1H, d, *J* = 10.92 Hz, H-4s), 2.00-2.07 (4H, m, 2xCH_2), 2.60 (1H, m, H-3), 2.73 (1H, m, H-2), 2.78 (1H, m, H-5), 2.83-2.87 (3H, m, H-6, 7, 9), 2.96-2.98 (1H, m, H-10), 3.01-3.24 (5H, m, H-1, 2xCH_2), 3.41 (2H, d, *J* = 7.16 Hz, CH_2), 3.90 (1H, t, *J* = 5.1 Hz, H-11), 5.03 (1H, m, CH), 5.21 (1H, t, *J* = 6.5 Hz, CH), 5.61 (OH, NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.3 (CH_2), 39.6 (CH_2), 41.1 (C-1), 41.2 (C-4), 41.6 (C-2), 41.7 (C-6), 43.5 (C-7), 44.1 (C-5), 44.3 (CH_2), 44.5 (CH_2), 45.9 (CH_2), 46.3 (C-3), 50.1 (C-10), 53.5 (C-9), 67.3 (C-11), 113.5 (C-8), 117.0 (CH), 123.7 (CH), 132.1 (C), 143.4 (C).

Biological testing

Compounds **9**, **10**, **14**, **15**, **16**, **22**, **23** and SQ109 were first dissolved in 100 % Methanol, sonicated and filter sterilized using 0.22 µm polycarbonate sterile filters to obtain a stock concentration of 10 mg/mL. This was diluted in sterile water and twofold serial dilutions were made to give working concentration ranges of 8 µg/mL to 0.125 µg/m L. 1mL volume of each concentration was aliquoted in cryovials and stored at -70 ^{0}C.

Bactec MGIT 960 analysis

Suspectibility testing with the BACTEC MGIT 960 system (Becton Dickinson) was performed according to the manufacturer's recommendations. Mycobacterial work was carried out in a level III biosafety laboratory. *M.tuberculosis* reference strain H_{37}Rv (ATCC No. 25177), MDR (drug sensitivity: isonaizid > 0.2 µg/mL, rifampicin > 1.0 µg/mL and EMB > 5 µg/mL) and XDR strains (drug sensitivity: isonaizid > 0.2 µg/mL, rifampicin > 1.0 µg/mL, EMB > 5 µg/mL, streptomycin > 2.0 µg/mL, ofloxacin > 2.0 µg/mL and kanamycin > 5.0 µg/mL) were cultured in Middlebrook 7H9 medium [30], enriched with OADC (0.00 5%, v/v, oleic acid; 0.5 %, 171 w/v, BSA; 0.2 %, w/v,

glucose; 0.02 %, v/v, catalase and 0.085 %, w/v, NaCl) and incubated at 37 ºC. Freshly grown cultures were used to prepare a standardised inoculum in a sterile tube containing 4.5 mL phosphate buffer, 0.05 % tween 80 with glass beads (5mm diameter) by vortexing. Once the clumps were allowed to settle for 45 min, the supernatant was aspirated and adjusted to a McFarland No. 1 standard, equivalent to a 10^7 colony forming units CFU/mL. 0.5 mL of the standardised inoculum was diluted tenfold to obtain a final concentration of 10^5 CFU/mL, after which 0.5 mL of the standardised inoculum was added to each of the MGIT containing the compounds. A 1:100 inoculum dilution was used to inoculate the drug free control tubes. This represents 1 % of the bacterial population. The MGITs were loaded in the BACTEC 960 drawers and the MIC was determined to be the lowest dilutions that were negative by the automated system in the compound containing tubes when the control tube showed positive. Antimycobacterial analysis of all compounds was done in triplicate.

All compounds containing tubes belonging to the positive drug free control were unloaded from the BACTEC 960 system and a Ziehl Neelson stain was performed to confirm the presence of *M. tuberculosis*[31]. Colony counts of the test inoculum were also prepared by plating out 20 µL onto Middelbrook 7H11 agar plates. Plates were incubated aerobically at 37 ºC for 21 days.

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References

1. World Health Organisation (WHO); webpage: http://www.who.int/tb/publications/global_report/2009/update/en/index.html, accessed on 23-07-2009.
2. Rivers, E. C.; Mancera, R. L. *Current Medicinal Chemistry* **2008,** *15*, 1956-1967.
3. Rivers, E. C.; Mancera, R. L. *Drug Discovery Today* **2008,** *13*, 1090-1098.
4. Barry, C. E.; Blanchard, J. S. *Current Opinion in Chemical Biology* **2010,** *14*, 456-466.
5. Shi, R. R.; Sugawara, I. *Tohoku Journal of Experimental Medicine* **2010,** *221*, 97-106.
6. Lee, R. E.; Protopopova, M.; Crooks, E.; Slayden, R. A.; Terrot, M.; Barry, C. E. *Journal of Combinatorial Chemistry* **2003,** *5*, 172-187.
7. Protopopova, M.; Hanrahan, C.; Nikonenko, B.; Samala, R.; Chen, P.; Gearhart, J.; Einck, L.; Nacy, C. A. *Journal of Antimicrobial Chemotherapy* **2005,** *56*, 968-974.
8. Chen, P.; Gearhart, J.; Protopopova, M.; Einck, L.; Nacy, C. A. *Journal of Antimicrobial Chemotherapy* **2006,** *58*, 332-337.
9. Geldenhuys, W. J.; Malan, S. F.; Bloomquist, J. R.; Marchand, A. P.; Van der Schyf, C. J. *Medicinal Research Reviews* **2005,** *25*, 21-48.
10. Van der Schyf, C. J.; Geldenhuys, W. J. *Neurotherapeutics* **2009,** *6*, 175-186.
11. Oliver, D. W.; Malan, S. F. *Medicinal Chemistry Research* **2008,** *17*, 137-151.
12. Nagasawa, H. T.; Elberlin.Ja; Shirota, F. N. *Journal of Medicinal Chemistry* **1973,** *16*, 823-826.

13. Zah, J.; Terre'Blanche, G.; Erasmus, E.; Malan, S. F. *Bioorganic & Medicinal Chemistry* **2003,** *11*, 3569-3578.
14. Nagasawa, H. T.; Elberling, J. A.; Shirota, F. N. *Journal of Medicinal Chemistry* **1975,** *18*, 826-830.
15. Ito, F. M.; Petroni, J. M.; de Lima, D. P.; Beatriz, A.; Marques, M. R.; de Moraes, M. O.; Costa-Lotufo, L. V.; Montnegro, R. C.; Magalhaes, H. I. F.; Pessoa, C. D. O. *Molecules* **2007,** *12*, 271-282.
16. Onajole, O. K.; Govender, K.; Govender, P.; Van Helden, P.; Kruger, H. G.; Maguire, G. E. M.; Muthusamy, K.; Pillay, M.; Wiid, I.; Govender, T. *European Journal of Medicinal Chemistry* **2009,** *44*, 4297 - 4305.
17. Onajole, O. K.; Govender, P.; Van Helden, P.; Kruger, H. G.; Maguire, G. E. M.; Wiid, I.; Govender, T. *European Journal of Medicinal Chemistry* **2010,** *45*, 2075-2079.
18. Cookson, R. C.; Crundwell, E.; Hill, R. R.; Hudec, J. *Journal of the Chemical Society* **1964**, 3062-7.
19. Marchand, A. P.; Allen, R. W. *Journal of Organic Chemistry* **1974,** *39*, 1596-1596.
20. Dekker, T. G.; Oliver, D. W. *South African Journal of Chemistry* **1979,** *32*, 45-48.
21. Dekker, T. G.; Oliver, D. W.; Pachler, K. G. R.; Wessels, P. L.; Woudenberg, M. *Organic Magnetic Resonance* **1981,** *15*, 188-192.
22. Eaton, P. E.; Hudson, R. A.; Giordano, C. *Journal of the Chemical Society-Chemical Communications* **1974**, 978-978.
23. Marchand, A. P.; Laroe, W. D.; Sharma, G. V. M.; Suri, S. C.; Reddy, D. S. *Journal of Organic Chemistry* **1986,** *51*, 1622-1625.
24. Marchand, A. P.; Dave, P. R.; Satyanarayana, N.; Arney, B. E. *Journal of Organic Chemistry* **1988,** *53*, 1088-1092.
25. Grobler, E.; Grobler, A.; Van der Schyf, C. J.; Malan, S. F. *Bioorganic & Medicinal Chemistry* **2006,** *14*, 1176-1181.
26. Marchand, A. P.; Arney, B. E.; Dave, P. R.; Satyanarayana, N.; Watson, W. H.; Nagl, A. *Journal of Organic Chemistry* **1988,** *53*, 2644-2647.
27. Eaton, P. E.; Cassar, L.; Hudson, R. A.; Dengrueyhwang. *Journal of Organic Chemistry* **1976,** *41*, 1445-1448.
28. Boeyens, J. C. A.; Burger, J.; Dekker, J. J.; Fourie, L. *South African Journal of Chemistry* **1978,** *31*, 101-106.
29. Kruger, H. G.; Ramdhani, R. *Magnetic Resonance in Chemistry* **2006,** *44*, 1058-1062.
30. Middlebrook, G.; Reggiardo, Z.; Tigertt, W. D. *American Review of Respiratory Disease* **1977,** *115*, 1066-1069.
31. Laifangbam, S.; Singh, H. L.; Singh, N. B.; Devi, K. M.; Singh, N. T. *Kathmandu Univ Med J (KUMJ)* **2009,** *7*, 226-30.

CHAPTER 8

NOVEL LINEAR DIAMINE DISUBSTITUTED POLYCYCLIC 'CAGE' DERIVATIVES AS POTENTIAL ANTI-MYCOBACTERIAL CANDIDATES

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Abstract

As part of an ongoing project to develop highly potent anti-tuberculosis therapeutics, a series novel polycyclic 'cage' tetra-amines were synthesized and screened for *in-vitro* anti-tuberculosis activities against the $H_{37}Rv$ strain of tuberculosis. Three disubstituted polycyclic moieties, namely pentacyclodecane, pentacycloundecane (PCU), and tricyclodecane were used in this study. Compounds **5** and **7** showed similar activity to **SQ109** at a MIC of 1 μM while compounds **4**, **6** and **8** displayed MIC activity at 1<MIC<10 μM. This study demonstrates the first reported analysis of pentacyclo[5.3.0.$0^{2,5}$.$0^{3,9}$.$0^{4,8}$]decane as a potential therapeutic agent.

Keywords: Anti-tuberculosis activity, **SQ109**, pentacyclodecane, pentacycloundecane and tricyclodecane, isoprenyl diamine

Introduction

Tuberculosis is a curable deadly contagious disease and has been in existence for over 5 centuries. According to the 2009 Global tuberculosis control report of the World Health Organisation, it is estimated that about 9.4 million incident cases of TB occurred globally. Of these cases an estimated 1.4 million were HIV positive of which 78 % were in Africa while 13 % are located in South-East

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Asia. An estimate of 1.3 million deaths was reportedly caused by TB among HIV negative people. South Africa has the highest percentage of HIV patients living with tuberculosis (1).

The need for the development of new, faster-acting therapeutics with reduced side effects and which are active against emerging drug resistant TB strains cannot be overemphasized. Researchers over the last decade have indentified numerous potent anti-tuberculosis candidates some of which are presently undergoing advanced stages of clinical trials; these compounds include derivatives containing diamine (SQ109), diaryl quinoline (TMC 207), nitroimidazooxazine (PA-824), pyrrole (LL-3858) and nitrodihydro-imidazooxazole (OPC-67683) functional groups (2-4).

SQ109 (*N*-Geranyl-*N′*-(2-adamantyl)ethane-1,2-diamine) first reported in 2003 by Lee *et al*(5) is an analogue of ethambutol possessing remarkable activity against MDR (multi-drug resistant)(6) and XDR (extensively-drug resistant) TB (7). **SQ109** also has excellent distribution into various tissues, with the highest concentration in the lung followed by the spleen and kidney tissues (8). **SQ109** recently completed phase one human clinical trials (9).

As part of our ongoing studies aimed at developing new polycylic 'cage' amines with anti-TB properties,(7, 10) we recently reported the activity of novel **SQ109** derivatives. Compound **1** (*N-trans-trans* farnesyl -*N′*-(2-adamantyl)ethane-1,2-diamine) was discovered to be more active than **SQ109** suggesting that the length of the isoprenyl group plays a significant role in its activity (7). We also reported novel pentacycloundecane (PCU) cyclic tetra-amine derivatives (Figure 1, compounds **2** and **3**) with excellent activity against susceptible and resistant (XDR) strains of tuberculosis (10).

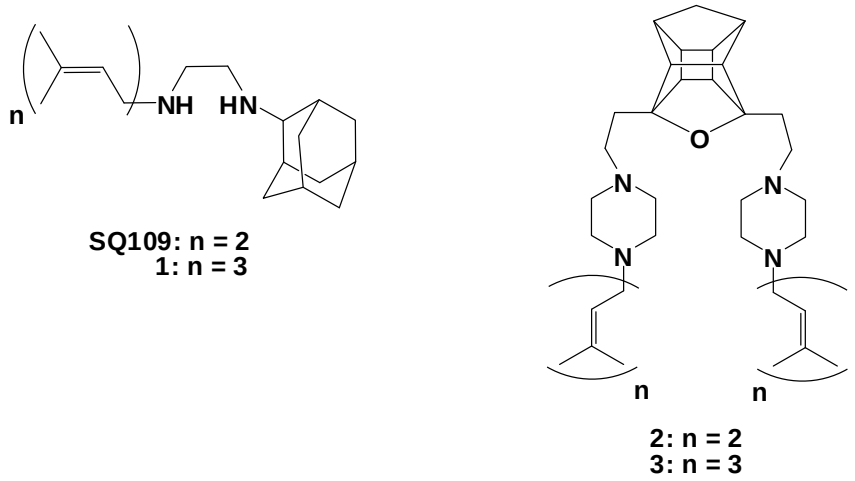


Figure 1: Structure of SQ109 and compounds 1-3

Inspired by this, we synthesized a series of novel disubstituted polycyclic 'cage' bearing linear diamine isoprenyl moieties and screened against *M. tuberculosis*. It was postulated that disubstitution

on the polycyclic 'cage' moieties compared to the mono-substituted **SQ109** (adamantyl moiety), might improve the activity as an anti-TB agent. Three polycyclic 'cage' moieties pentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane, tricyclo[3.3.1.1^{3,7}]decane and pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane were employed in this study. Over a century ago, Thiele(11) reported the carbonation of cyclopentadienylpotassium to obtain a Diels-Alder dimer of cyclopentadienecarboxylic acid ("Thiele's acid"). Pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dicarboxylic acid which was used in this study was synthesized from Thiele's acid (12). Although this 1,3-bishomocubane (pentacyclodecane) has been extensively studied with regards to its geometry (C_2 symmetry) and chirality (12-17); its use in medicinal chemistry has not yet been investigated. This is the first time that a pentacyclodecane based derivative has been evaluated for potential pharmaceutical properties.

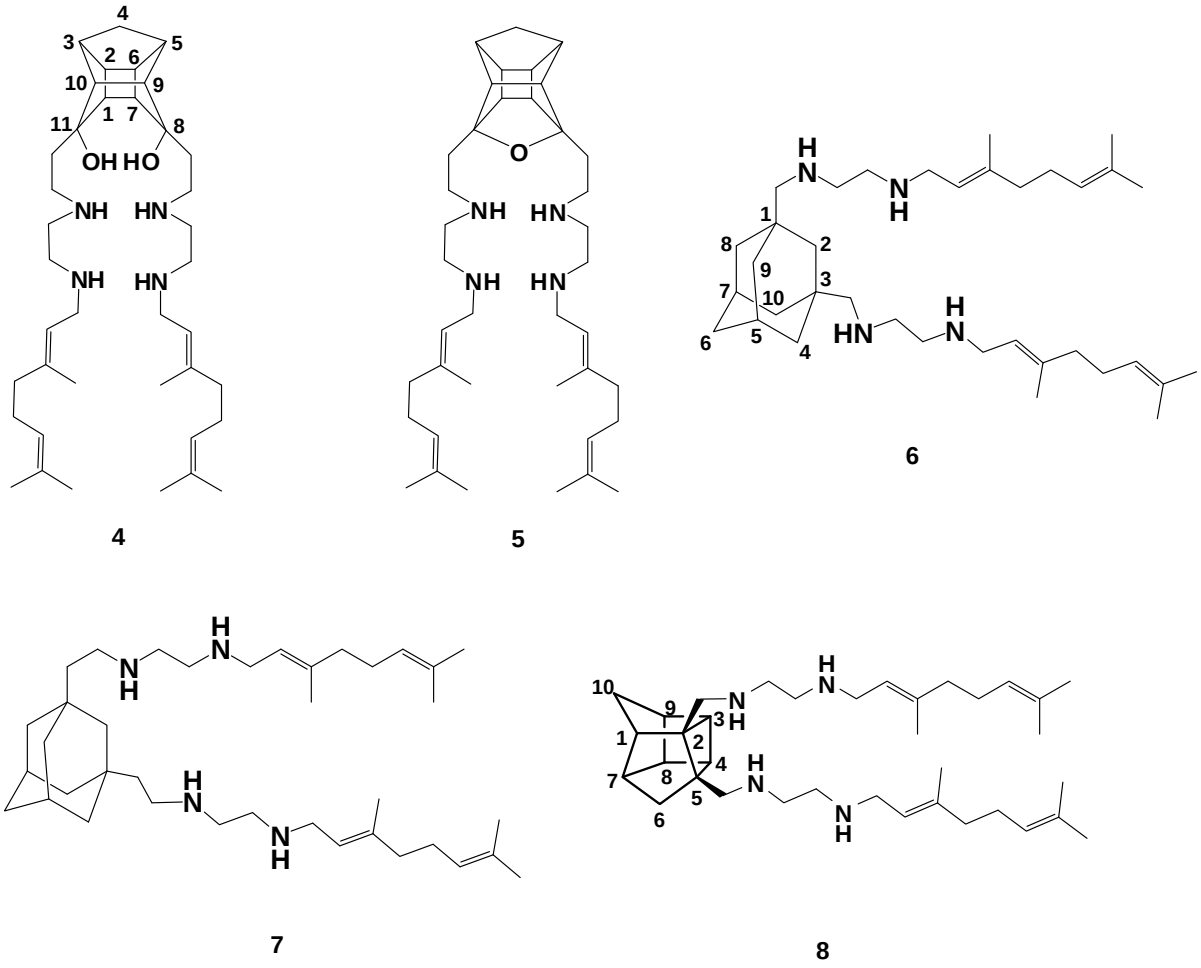
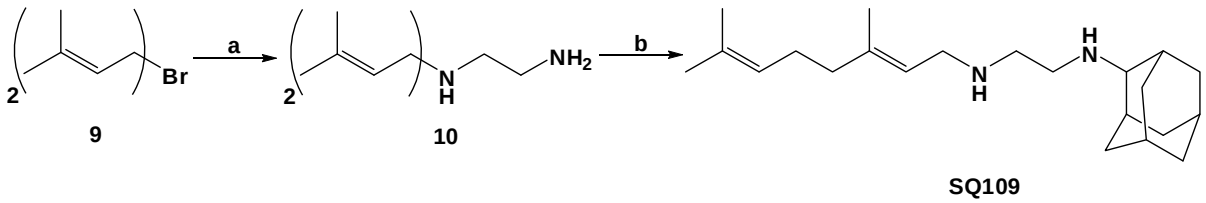


Figure 2: Structure of polycyclic 'cage' tetra-amine derivatives (4-8)

Results and discussion

Chemistry

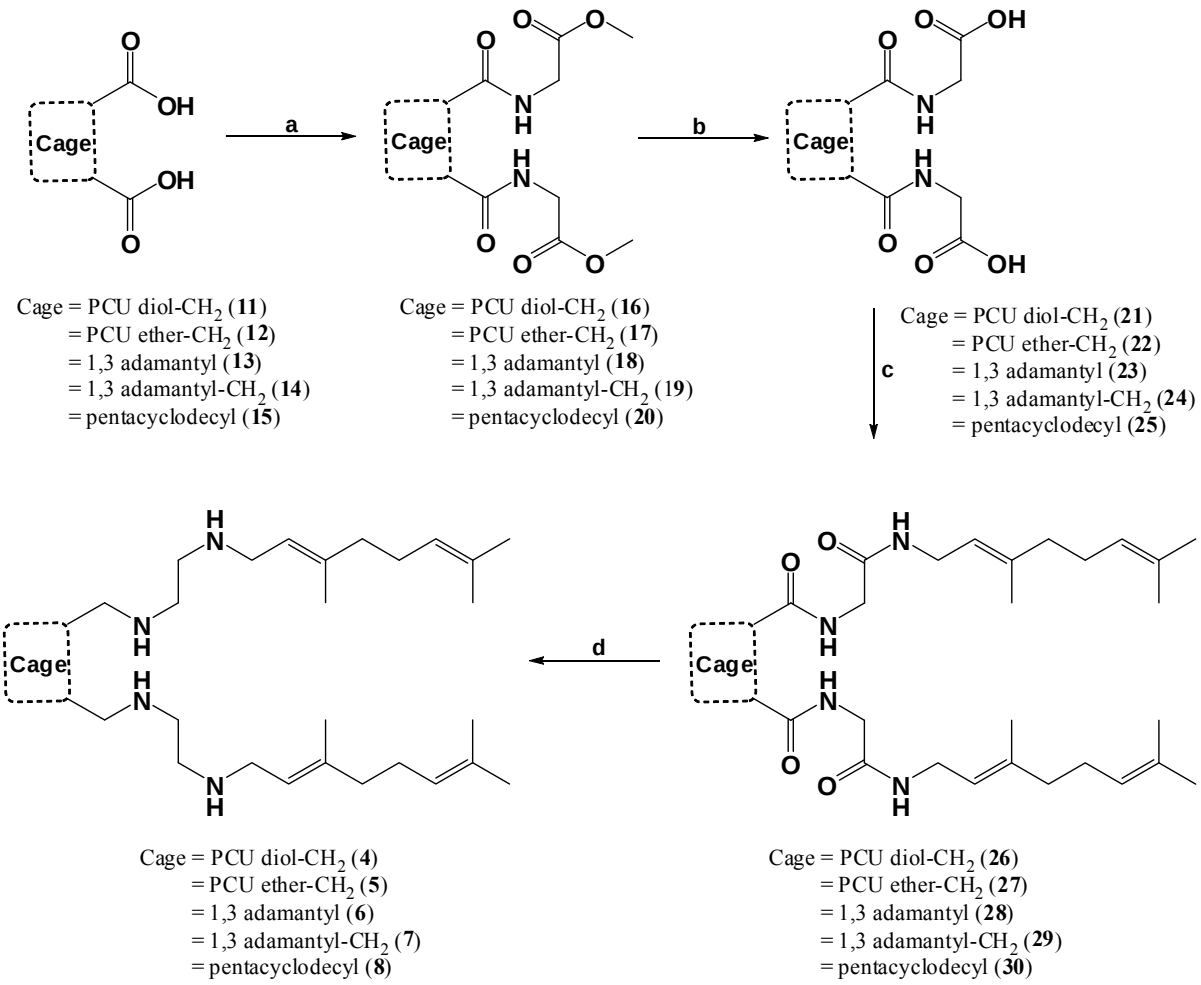
Following similar methodology as reported in literature,(7) (*E*)-*N'*-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (**10**) was reacted with 2-adamantanone (1.2:1) (reductive amination) to obtain **SQ109** (57%). The polycyclic diacid compounds were synthesized as reported in literature. PCU diol diacid **11**,(18) PCU ether diacid **12**,(19, 20) 1,3-adamantanecarboxylic acid (**13**),(21) 1,3-adamantanediacetic acid (**14**)(22) and pentacyclodecane-2,5-diacid (**15**)(12, 15, 16) were reacted with glycine methyl ester using coupling reagents hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-methylmorphine (NMM) to obtain compounds **16** (51.5 %),**17** (68.7 %), **18**, **19** (quantitative), and **20** (42 %) respectively.



Scheme 1: Reagents and conditions: (a) ethylene diamine (100:1), -78 ºC, dry DCM; (b) MeOH, 2-adamantanone (1.2:1), N_2 atmosphere, 2 h, $NaBH_4$, overnight.

PCU dimethoxyl diamide **16** and **17**, adamantyl dimethoxyl diamide **18** and **19**, and pentacyclodecyl dimethoxyl diamide **20** were hydrolyzed using lithium hydroxide in THF to obtain the corresponding polycyclic glycine derivatives **21**, **22**, **23**, **24** and **25** in quantitative yields. Polycyclic 'cage' glycine derivatives **21**-**25** were coupled with geranylamine(7) using HOBt, EDC and NMM as coupling reagents to obtain compounds **26** (28 %), **27** (59 %), **28** (69 %), **29** (72 %) and **30** (67 %). Compounds **27** and **30** were purified on preparative HPLC while compounds **26**, **28** and **29** were re-crystallized from acetronitrile to obtain white crystalline solids. Reduction of the tetra-carbonyl groups of PCU derivatives **26** and **27** were achieved using $LiAlH_4$ at a 1:20 ratio to yield compounds **4** and **5** (30 and 34 %). Interestingly, the reduction of **26** yielded both **4** and **5** as products, suggesting that LAH dehydrated the diol to form an ether bridge. Separation was achieved using preparative HPLC. Following similar reduction conditions, compounds **28**, **29** and **30** were reduced to their corresponding tetra-amines **6** (41.8 %), **7** (33.0 %) and **8** (28.1 %) respectively (Scheme 2). Purification of compounds **4**, **5**, **6**, **7** and **8** was achieved *via* preparative HPLC using acetronitrile and water as the eluent; it should be noted that compound **8** was obtained as a racemate. All compounds were characterized by ^{1}H, ^{13}C (APT), COSY, NOESY/ROESY, HSQC, HMBC, IR

and HR-MS experiments. Compounds **4**, **5**, **6** and **7** with the exception of **8** are *meso* compounds thus simplifying their elucidation using 2D NMR techniques.



Scheme 2: (a) DMF, glycine methyl ester, EDC, HOBt and NMM, stirring, 16 hr; (b) THF, 1M LiOH solution, stirring overnight; (c) DMF, geranylamine, EDC, HOBt and NMM, stirring, 16 hr; (d) dry THF, $LiAlH_4$ (1:20), reflux 5 days, N_2 atm.

Anti-tubercular activity

The anti-TB activity of the novel polycyclic compounds **26-30** were assayed against *M. tuberculosis* ($H_{37}Rv$). The inhibitory activity of all these compounds is summarized in Table 1, together with the results obtained for **SQ109**. No anti-TB activity was observed for precursors **26-30** even at the highest tested concentration (50 μM), suggesting that the carbonyl group contributes negatively to the activity of these compounds. Compound **8** was less active than **SQ109**; this compound has a carbon atom spacer between the pentacyclodecyl moiety and the bis-isoprenyl diamine moiety suggesting that spacer length maybe critical to activity.

Compound **6** having one carbon atom spacer between the 'cage' and bis-isoprenyl diamine was not as active as its two atom spacer analogue **7**, once again suggesting that spacer length contributes to the

efficacy of these molecules. Compounds **4**, **5** (pentacycloundecyl) and **7** (adamantyl) are quite similar as they have a two carbon atom spacers. Diol **4**, however was not as active, whereas compound **5** with an ether bridge was as effective as **7** both being in the same range as **SQ109**. We have previously reported a study of PCU ether cyclic tetra-amines (**1-3**) (two "armed" ether compounds similar to **6**) that displayed excellent activity against both susceptible and resistant strains of tuberculosis (10).

Table 1: *In-vitro* activity of compounds 4-8, 27-30 against *M. tuberculosis* $H_{37}Rv$ strain

Compound	Mol. formula	MW ($gmol^{-1}$)	MIC (μM, $H_{37}Rv$)
4	$C_{39}H_{66}N_4O_2$	622.5	1<MIC<10
5	$C_{39}H_{64}N_4O$	604.5	1
6	$C_{36}H_{64}N_4$	552.5	1<MIC<10
7	$C_{38}H_{68}N_4$	580.5	1
8	$C_{36}H_{60}N_4$	548.5	1<MIC<10
26	$C_{39}H_{58}N_4O_6$	678.4	>50
27	$C_{39}H_{56}N_4O_5$	660.4	>50
28	$C_{36}H_{56}N_4O_4$	608.4	>50
29	$C_{38}H_{60}N_4O_4$	636.5	>50
30	$C_{36}H_{52}N_4O_4$	604.4	>50
SQ109	$C_{22}H_{38}N_2$	330.3	1

The MICs are reported as the Standard derivation of ≤ 6.36 with the exception of compounds **26-30**.

Conclusion

We have synthesized and evaluated a series of novel polycyclic linear tetra-amines as inhibitors of *M. tuberculosis* $H_{37}Rv$. This study has investigated the use of a number of disubstituted polycyclic 'cages' with isoprenyl diamines side arms. Compounds **6** and **8** (one carbon atom spacers) gave encouraging inhibitory results. This is the first example of the pentacyclodecane being employed in medicinal chemistry. Compounds **5** and **7** (two carbon atom spacer) were the most efficacious. As in our previous studies an ether functional group tends to improve overall activity.

Experimental Section

General

The NMR data were recorded on Bruker AVANCE III 400 MHz and 600 MHz instruments NMR spectrometer using Topspin 2.1 (Bruker, Karlsruhe, Germany). The chemical shifts were referenced to the solvent peak for $CDCl_3$ (^1H: 7.24 ppm, ^{13}C: 77.0 ppm), D_20 (^1H: 4.79 ppm), CD_3OD (^1H: 3.32 ppm, ^{13}C: 49.3 ppm) at 25 ºC. All chemical shifts (δ) were quoted in parts per million downfield from TMS and the coupling constants (*J*) recorded in Hertz. Splitting pattern abbreviations are as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. All reactions were monitored using thin layer chromatography (TLC, Merck Kieselgel 60, F254). Purifications were carried by column chromatography using Fluka Kieselgel 60 (70–230 mesh). Purification of final compounds was done via semi-preparative HPLC on a Shimadzu, LC-6AD instrument using water (solvent A) and Acetronitrile (solvent B). An ACE 5C18 150 x 21.2 mm column was used. A gradient elution system of 95 % solvent A and 5 % solvent B which changes linearly over 25 min to 20 % solvent A and 80 % solvent B and stays at 95 % solvent B (5 % solvent A) for 5 extra minutes at 15 ml/min was used and detected on a UV-VIS detector at 215 and 254 nm. High Resolution Mass Spectroscopic analysis was performed on a Bruker MicroTOF QII mass spectrometer in positive mode with an internal calibration while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3 and melting points are uncorrected. Tetrahydrofuran was freshly distilled from a flask containing sodium benzophenone under N_2 atmosphere while dichloromethane was dried using phosphorus pentoxide prior to use.

Synthesis of (*E*)-*N'*-(3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine (10)

To a vigorously stirred solution of ethylene diamine (55.4 g, 0.92 mol) in dichloromethane (400 mL) at -78 ºC under N_2 atmosphere was dropwise added geranyl bromide **9** (2 g, 9.2 mmol) in 1200 mL of dichloromethane over 4 hours. The reaction mixture was left to attain room with stirring over night. The solution mixture was reduced *in vacuo* to about 500 mL and washed with water to remove excess ethane-1,2 diamine, the organic extract was dried over Mg_2SO_4 and concentrated *in vacuo*. The crude residue was purified using column chromatography [eluent; $CHCl_3$:CH_3OH:NH_4OH (88:10:2)] to give a light yellow oil (1.56 g, 86 %, R_f = 0.45). ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.36 (NH), 1.56 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.97 (2H, m, CH_2), 2.05 (2H, m, CH_2), 2.63 (2H, *t*, *J* = 5.6 Hz, CH_2), 2.78 (2H, t, J = 5.7, 6.1 Hz, CH_2), 3.21 (2H, *d*, *J* = 6.8 Hz, CH_2), 5.06 (1H, *J* = 6.9 Hz, CH), 5.23 (1H, *J* = 6.8 Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.3 (CH_3), 17.6 (CH_3), 25.7 (CH_3), 26.5 (CH_2), 39.6 (CH_2), 41.8 (CH_2), 47.1 (CH_2), 52.1 (CH_2), 122.8 (CH), 124.1 (CH), 131.5 (C), 137.7 (C).

Data for SQ109 [*N*-Geranyl-*N′*-(2-adamantyl)]ethane-1,2-diamine

A mixture of isoprenyl diamine **10** (0.35 g, 1.78 mmol) and 2-adamantane (0.29 g, 1.96 mmol) in methanol (10 mL) was stirred for 2 h at room temperature under a nitrogen atmosphere. The resulting imine was reduced with solid $NaBH_4$ (0.13 g, 3.56 mmol) which was added slowly over 15 min and the mixture stirred overnight. Additional methanol (15 mL) was added to the reaction vessel after which water (20 mL) was added to quench the excess $NaBH_4$. The solution was extracted with ethyl acetate (2 x 50 mL), the combined organic extracts dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel using $CHCl_3$:CH_3OH:NH_4OH (88:10:2, R_f = 0.6) as eluent to obtain a colourless oil (57 % yield). HR-MS calculated for $C_{22}H_{39}N_2$ $(M+H)^+$ 331.3108, found 331.3135; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 1.47 (2H, d, *J* = 12.5 Hz, H-4a/9a), 1.57 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.66-1.68 (4H, m, H-6, 8a/10a), 1.74 (1H, s, H-5), 1.80-1.82 (5H, m, H-1, 3, 7, 8b/10b), 1.95 (2H, d, *J* = 12.6 Hz, H-4b/9b), 1.98-2.00 (2H, m, CH_2), 2.03-2.09 (2H, m, CH_2), 2.67 (1H, s, H-2), 2.70 (4H, s, $2CH_2$), 3.22 (2H, d, *J* = 6.8 Hz, CH_2), 5.06 (1H, m, CH), 5.24 (1H, t, *J* = 6.7 Hz, CH). ^{13}C NMR ($CDCl_3$, 100 MHz); δ_C 16.3 (CH_3), 17.6 (CH_3), 25.7 (CH_3), 26.5 (CH_2), 27.6 (C-5), 27.8 (C-7), 31.3 (C-4/9), 32.1 (C-1/3), 37.6 (C-8/10), 37.9 (C-6), 39.6 (CH_2), 46.5 (CH_2), 47.1 (CH_2), 49.5 (CH_2), 61.9 (C-2), 122.9 (CH), 124.1 (CH), 131.5 (C), 137.6 (C).

General synthesis of polycyclic dimethoxyl diamide

A mixture of the polycyclic diacid (**11-15**) (1 mol), glycine methyl ester (2.6 mol), HOBt (2.2 mol), EDC (2.2 mol), NMM (2.6 mol) in DMF (50 mL) was stirred for 24 hours. The reaction mixture was concentrated *in vacuo*. The crude product was dissolved in 1M HCl (100 mL) and extracted with DCM (3 x 50 mL). The combined organic solution was washed successively with 10 % $NaHCO_3$ (2 x 100 mL) and brine (2 x 100 mL). The resulting organic solution was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified *via* column chromatography using silica to obtain the desired products **16-20** respectively.

Data for *N′*,*N′*-bis(2-oxo-2-methoxyethyl)-8,11-dihydroxy-pentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diacetylcarboxamide (16)

A yellow slurry ($CHCl_3$: CH_3OH: NH_4OH (88:10:2), R_f = 0.6, yield 51.5 %). IR ν_{max}: 3266, 2953, 1744, 1639, 1533, 1435 and 1204 cm^{-1}. MS (TOF) calculated for $C_{21}H_{29}N_2O_8$ $(M + H^+)$ 437.1918, found 437.1893. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.06 (1H, d, *J* = 10.8 Hz, H-4a), 1.52 (1H, d, *J* = 10.8 Hz, H-4s), 2.25 (1H, d, *J* = 15.0 Hz, CH_2), 2.31 (1H, s, H-9/10), 2.38 (1H, s, H-3/5), 2.40 (1H, d, *J* = 15.0 Hz, CH_2), 2.46 (1H, s, H-1/7), 2.49 (1H, s, H-2/6), 3.67 (3H, s, CH_3), 3.94 (2H, m, CH_2),

7.61 (*t*, 15.5 Hz, NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C.34.0 (C-4), 39.3 (C-2/6), 40.9 (CH_2), 42.9 (C-1/7), 44.3 (C-3/5), 44.3 (CH_2), 49.5 (C-9/10), 52.3 (CH_3), 77.3 (C-8/11), 170.2 (C=O), 172.9 (C=O).

Data for *N′,N′*-bis(2-oxo-2-methoxyethyl)-8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diacetylcarboxamide (17)

A yellow slurry [$CHCl_3$: CH_3OH: NH_4OH (88:10:2), R_f = 0.8, yield 68.7 %]. IR ν_{max}: 3320, 2954, 1741, 1649, 1534 and 1201 cm^{-1}. MS (TOF) calculated for $C_{21}H_{27}N_2O_7$ (M + H$^+$) 419.1813, found 419.1803. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.49 (1H, *d*, *J* = 10.5 Hz, H-4a), 1.85 (1H, *d*, *J* = 10.5 Hz, H-4s), 2.43 (1H, s, H-3/5), 2.62 (2H, m, H-2/6, 9/10), 2.65 (1H, s, H-1/7), 2.68-2.80 (2H, *t*, *J* = 15.7 Hz, CH_2), 3.65 (3H, s, CH_3), 3.98 (2H, *t*, *J* = 5.5 Hz, CH_2), 7.07 (*t*, *J* = 5.2 Hz, NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C.39.4 (CH_2), 41.0 (CH_2), 41.4 (C-2/6), 43.4 (C-4), 44.0 (C-3/5), 48.1 (C-1/7), 52.1 (CH_3), 58.6 (C-9/10), 94.0 (C-8/11), 170.3 (C=O), 170.9 (C=O).

Data for *N′,N′*-bis(2-oxo-2-methoxyethyl)tricyclo[3.3.1.1^{3,7}]decane-1,3-dicarboxamide (18)

A yellow slurry [$CHCl_3$: CH_3OH; (9:1), R_f = 0.5, quantitative yield]. IR ν_{max}: 3350, 2907, 1741, 1639, 1517, 1201 and 1180 cm^{-1}. MS (TOF) calculated for $C_{18}H_{27}N_2O_6$ (M + H$^+$) 367.1864, found 367.1864. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.65 (1H, s, H-6), 1.78-1.86 (4H, m, H-4/9, 8/10), 1.97 (1H, s, H-2), 2.17 (1H, s, H-5/7), 3.70 (3H, s, CH_3), 3.96 (2H, m, CH_2), 6.29 (br s, NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 28.0 (C-5/7), 35.3 (C-6), 38.1 (C-4/9, 8/10), 40.2 (C-2) 41.0 (C-1/3), 41.1 (CH_2), 52.2 (CH_3), 170.5 (C=O), 177.1 (C=O).

Data for *N′,N′*-bis(2-oxo-2-methoxyethyl)tricyclo[3.3.1.1^{3,7}]decane-1,3-diacetylcarboxamide (19)

A yellow slurry [$CHCl_3$: CH_3OH; (9:1), R_f = 0.4 quantitative yield]. IR ν_{max}: 3300, 2901, 1743, 1644, 1535, 1203 and 1181 cm^{-1}. MS (TOF) calculated for $C_{20}H_{31}N_2O_6$ (M + H$^+$) 395.2177, found 395.2178. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.45-1.51 (6H, complex m, H-2, 4/9, 6, 8/10), 1.97-1.98 (3H, m, H-5/7, CH_2), 3.68 (3H, s, CH_3), 3.97 (2H, d, *J* = 5.5 Hz, CH_2), 6.34 (t, *J* = 5.4 Hz, NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 28.8 (C-5/7), 33.4 (C-1/3), 35.7 (C-6), 40.8 (CH_2), 41.6 (C-4/9, 8/10), 46.9 (C-2), 50.7 (CH_2), 52.1 (CH_3), 170.7 (C=O), 171.1 (C=O).

Data for *N′,N′*-bis(2-oxo-2-methoxyethyl)pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dicarboxamide (20)

A whitish yellow solid [$CHCl_3$:CH_3OH:NH_4OH; (88:10:2), R_f = 0.5 42% yield]. Melting point: 156-158 ^{0}C. IR ν_{max}: 3313, 2984, 1755, 1652, 1638, 1521, 1197, 1173 and 1156 cm^{-1}. MS (TOF) calculated for $C_{18}H_{23}N_2O_6$ (M + H$^+$) 363.1551, found 363.1589. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.48

(1H, AB, *J* = 11.4 Hz, H-10a), 1.64 (1H, AB, *J* = 11.4 Hz, H-10b), 1.70 (1H, AB, *J* = 11 Hz, H-6a), 2.18 (1H, AB, *J* = 10.8 Hz, H-6b), 2.56 (1H, m, H-7), 2.75-2.83 (2H, m, H-4, 8), 2.87-2.90 (2 H, m, H-1, 9), 2.99 (1H, q, *J* = 9.0 Hz, H-3), 3.67 (6H, s, $2CH_3$), 3.86-4.02 (4H, m, $2CH_2$), 6.55 (s, NH), 6.77 (s, NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 37.7 (C-10), 39.5 (C-4), 40.8 (C-9), 41.0 (CH_2), 41.1 (C-6), 41.2 (CH_2), 43.4 (C-8), 43.8 (C-3), 47.2 (C-7), 52.0 (CH_3), 52.1 (CH_3), 54.9 (C-1), 61.6 (C-2), 62.3 (C-5), 170.2 (C=O), 170.3 (C=O), 172.9 (C=O), 173.4 (C=O).

General synthesis of polycyclic diamide diacid 21-25

Polycyclic 'cage' dimethoxyl diamide (**16-20**) dissolved in THF (20 mL) was added 1 M LiOH solution (12.4 mL) with stirring overnight. The resulting solution was acidified to pH 7. The desired product was extracted with ethyl acetate (3 x 100 mL) and the combined organic layer was dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. Quantitative yields were obtained for all steps.

Data for 8,11-dihydroxypentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diacetylcarboxamido diacetic acid (21)

A white solid. IR ν_{max}: 3249, 2934, 1599, 1390, 1290 and 1101 cm^{-1}. MS (TOF) calculated for $C_{19}H_{25}N_2O_8$ (M + H$^+$) 409.1605, found 409.1589. ^{1}H NMR [CD_3OD, 400 MHz]: δ_H 1.20 (1H, d, *J* = 10.7 Hz, H-4a), 1.65 (1H, d, *J* = 10.7 Hz, H-4s), 2.36 (1H, d, *J* = 14.6 Hz, CH_2), 2.37 (1H, s, H-9/10), 2.48 (1H, d, *J* = 14.6 Hz, CH_2), 2.56 (1H, s, H-3/5), 2.60 (1H, s, H-1/7), 2.64 (1H, s, H-2/6), 3.80 (2H, t, *J* = 5.3 Hz, CH_2). ^{13}C NMR [CD_3OD, 100 MHz]: δ_C.34.8 (C-4), 40.7 (C-2/6), 44.4 (C-1/7), 44.6 (CH_2), 45.7 (CH_2), 45.8 (C-3/5), 51.0 (C-9/10), 78.6 (C-8/11), 174.0 (C=O), 176.4 (C=O).

Data for 8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diacetylcarboxamido diacetic acid (22)

A cream white solid obtained. IR ν_{max}: 3284, 2960, 1593, 1397 and 1297 cm^{-1}. MS (TOF) calculated for $C_{19}H_{23}N_2O_7$ (M + H$^+$) 397.1605, found 397.1631. ^{1}H NMR [CD_3OD, 400 MHz]: δ_H 1.59 (1H, d, *J* = 10.4 Hz, H-4a), 1.97 (1H, d, *J* = 10.4 Hz, H-4s), 2.50 (1H, s, H-3/5), 2.73-2.75 (2H, s, H-2/6, 9/10), 2.77 (1H, s, H-1/7), 2.81 (2H, s, CH_2), 3.78 (2H, s, CH_2). ^{13}C NMR [CD_3OD, 100 MHz]: δ_C.40.1 (CH_2), 42.8 (C-2/6), 44.3 (C-4), 44.6 (CH_2), 45.6 (C-3/5), 49.5 (C-1/7), 59.9 (C-9/10), 95.2 (C-8/11), 172.4 (C=O), 176.2 (C=O).

Data for tricyclo[3.3.1.1^{3,7}]decane-1,3-dicarboxamidodiacetic acid (23)

A white solid obtained. IR ν_{max}: 3341, 2909, 1590, 1533, and 1397 cm^{-1}. MS (TOF) calculated for $C_{16}H_{23}N_2O_6$ (M + H$^+$) 339.1551, found 339.1551. ^{1}H NMR [CD_3OD, 400 MHz]: δ_H 1.74 (1H, s, H-6), 1.85-1.93 (4H, m, H-4/9, 8/10), 1.99 (1H, s, H-2), 2.19 (1H, s, H-5/7), 3.72 (2H, s, CH_2), 8.51 (s, NH). ^{13}C NMR [CD_3OD, 100 MHz]: δ_C 30.0 (C-5/7), 36.8 (C-6), 39.5 (C-4/9, 8/10), 41.7 (C-2), 42.5 (C-1/3), 44.8 (CH_2), 176.6 (C=O), 179.7 (C=O).

Data for tricyclo[3.3.1.1^{3,7}]decane-1,3-diacetylcarboxamidodiacetic acid (24)

A white solid obtained. IR ν_{max}: 3291, 2901, 1589, 1396 and 1314 cm^{-1}. MS (TOF) calculated for $C_{18}H_{27}N_2O_6$ (M + H$^+$) 367.1864, found 367.1855. ^{1}H NMR [CD_3OD, 400 MHz]: δ_H 1.54-1.64 (6H, complex m, H-2, 4/9, 6, 8/10), 2.01-2.04 (3H, m, H-5/7, CH_2), 3.74 (2H, s, CH_2), 8.54 (s, NH). ^{13}C NMR [CD_3OD, 100 MHz]: δ_C 30.9 (C-5/7), 34.9 (C-1/3), 37.3 (C-6), 43.1 (C-4/9, 8/10), 44.8 (CH_2), 48.9 (C-2), 52.0 (CH_2), 173.7 (C=O), 176.8 (C=O).

Data for pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dicarboxamido diacetic acid (25)

A whitish yellow slurry obtained. IR ν_{max}: 3289, 2968, 1593, 1533, 1428, 1396 and 1310 cm^{-1}. MS (TOF) calculated for $C_{16}H_{19}N_2O_6$ (M + H$^+$) 335.1238, found 335.1237. ^{1}H NMR [D_2O, 400 MHz]: δ_H 1.50 (1H, AB, *J* = 11.5 Hz, H-10a), 1.72 (1H, AB, *J* = 11.5 Hz, H-10b), 1.79 (1H, AB, *J* = 10.6 Hz, H-6a), 2.20 (1H, AB, *J* = 10.7 Hz, H-6b), 2.67 (1H, m, H-7), 2.78-2.81 (1H, m, H-4), 2.84-2.86 (2H, m, H-1, 8), 2.97 (1H, m, H-9), 3.12 (1H, q, *J* = 9.1 Hz, H-3), 3.68-3.86 (4H, m, 2CH_2). ^{13}C NMR [D_2O, 100 MHz]: δ_C 37.0 (C-10), 38.3 (C-6), 39.9 (C-4), 40.4 (C-9), 43.1 (CH_2), 43.2 (CH_2), 43.2 (C-8), 43.7 (C-3), 47.1 (C-7), 54.6 (C-1), 61.0 (C-2), 62.8 (C-5), 175.4 (C=O), 175.7 (C=O), 176.4 (C=O), 176.6 (C=O).

General synthesis of compounds 26-30

A mixture of the polycyclic diamide diacid (**21**-**25**) (1 mol), geranylamine(7) (2.6 mol), HOBt (2.2 mol), EDC (2.2 mol), NMM (2.6 mol) in DMF (50 mL) was stirred for 24 hours. The reaction mixture was concentrated *in vacuo*. The crude product was dissolved in 1M HCl (100 mL) and extracted with DCM (3 x 50 mL). The combined organic solution was washed successively with 10 % $NaHCO_3$ (2 x 100 mL) and brine (2 x 100 mL). The resulting organic solution was dried over Na_2SO_4 and concentrated *in vacuo*.

Data for *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-dihydroxypentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-dimethylenecarboxamide (26)

The crude product was recrystallized from acetonitrile to obtain **26** as a white solid (0.57 g, 38 %). Melting point: 149-152 ºC. IR ν_{max}: 3267, 3143, 2967, 2922, 1636, 1522, 1222 and 769 cm^{-1}. MS (TOF) calculated for $C_{39}H_{59}N_4O_6$ (M + H$^+$) 679.4429, found 679.4428. ^{1}H NMR [$CDCl_3$, 600 MHz]: δ_H 1.11 (1H, AB, *J* = 10.7 Hz, H-4a), 1.53 (1H, H-4s), 1.56 (3H, s, CH_3), 1.62 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.97 (2H, m, CH_2), 2.04 (2H, m, CH_2), 2.24 (1H, s, H-9/10), 2.29 (1H, AB, *J* = 14.6 Hz, CH_2), 2.39 (1H, AB, *J* = 14.6 Hz, CH_2), 2.43 (1H, s, H-3/5), 2.52-2.55 (2H, m, H-1/7, 2/6), 3.81 (2H, t, *J* = 6.08 Hz, CH_2), 3.89 (2H, t, *J* = 5.46 Hz, CH_2), 5.04 (1H, t, *J* = 6.54 Hz, CH), 5.13 (1H, t, *J* = 6.78 Hz, CH), 6.51 (NH), 7.29 (NH). ^{13}C NMR [$CDCl_3$, 150 MHz]: δ_C 16.3 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.4 (CH_2), 34.0 (C-4), 37.5 (CH_2), 39.4 (C-2/6), 39.5 (CH_2), 42.7 (C-1/7), 43.0 (CH_2), 44.4

(C-3/5), 44.5 (CH_2), 50.1 (C-9/10), 77.4 (C-8/11), 119.3 (CH), 123.8 (CH), 131.7 (C), 140.1 (C), 168.6 (C=O), 172.6 (C=O).

Data for *N′,N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-dimethylenecarboxamide (27)

The crude product was purified using preparative HPLC to obtain yellow oil, retention time: 19.3 min (0.83 g, 59 %). IR ν_{max}: 3288, 2965, 2927, 2862, 1642, 1529, 1440 and 1234 cm^{-1}. MS (TOF) calculated for $C_{39}H_{57}N_4O_5$ (M + H$^+$) 661.4323, found 661.4307. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.51 (1H, H-4a), 1.55 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.88 (1H, d, *J* = 10.64 Hz, H-4s), 1.94 (2H, m, CH_2), 2.02 (2H, m, CH_2), 2.45 (1H, s, H-3/5), 2.60 (1H, s, H-9/10), 2.63 (2H, s, H-1/7, H-2/6), 2.66-2.78 (2H, m, CH_2), 3.79 (2H, t, *J* = 6.08 Hz, CH_2), 3.87 (2H, d, *J* = 5.4 Hz, CH_2), 5.02 (1H, m, CH), 5.09 (1H, m, CH), 6.33 (NH), 7.54 (NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.3 (CH_3), 17.6 (CH_3), 25.6 (CH_3), 26.4 (CH_2), 37.5 (CH_2), 39.4 (CH_2), 39.5 (CH_2), 41.5 (C-2/6), 43.2 (CH_2), 43.4 (C-4), 44.0 (C-3/5), 48.2 (C-1/7), 58.7 (C-9/10), 93.9 (C-8/11), 119.5 (CH), 123.7 (CH), 131.8 (C), 140.0 (C), 168.9 (C=O), 170.3 (C=O).

Data for *N′,N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dicarboxamide (28)

A white crystalline solid, re-crystallized from acetronitrile was obtained (69 % yield). Melting point: 106-108 ºC,. IR ν_{max}: 3288, 3075, 2908, 2856, 1668, 1643, 1529, 1448, and 1236 cm^{-1}. MS (TOF) calculated for $C_{36}H_{57}N_4O_4$ (M + H$^+$) 609.4374, found 609.4364. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.56 (3H, s, CH_3), 1.63 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.66 (1H, s, H-6), 1.80-1.87 (4H, m, H-4/9, 8/10), 1.96-2.06 (5H, m, H-2, 2CH_2), 2.18 (1H, s, H-5/7), 3.81 (2H, t, *J* = 6.0 Hz, CH_2), 3.86 (2H, d, *J* = 4.96 Hz, CH_2), 5.04 (1H, t, *J* = 6.5 Hz, CH), 5.14 (1H, t, *J* = 6.8 Hz, CH), 6.58 (br s, NH), 6.96 (br s, NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.3 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.4 (CH_2), 28.1 (C-5/7), 35.3 (C-6), 37.5 (CH_2), 38.1 (C-4/9, 8/10), 39.5 (CH_2), 40.4 (C-2), 41.0 (C-1/3), 43.4 (CH_2), 119.5 (CH), 123.8 (CH), 131.7 (C), 139.9 (C), 168.9 (C=O), 177.7 (C=O).

Data for *N′,N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dimethylenecarboxamide (29)

A white crystalline solid, re-crystallized from acetronitrile was obtained [72 % yield]. Melting point: 115-117 ºC. IR ν_{max}: 3290, 2905, 2851, 1628, 1534, 1445 and 1267 cm^{-1}. MS (TOF) calculated for $C_{38}H_{61}N_4O_4$ (M + H$^+$) 637.4687, found 637.4686. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.46-1.53 (6H, m, H-2, 6, 4/9, 8/10), 1.56 (3H, s, CH_3), 1.63 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.94-2.06 (7H, m, H-5/7, 3CH_2), 3.81 (2H, t, *J* = 6.0 Hz, CH_2), 3.85 (2H, d, *J* = 5.4 Hz, CH_2), 5.03 (1H, t, *J* = 6.6 Hz, CH), 5.14 (1H, t, *J* = 6.0 Hz, CH), 6.53 (t, *J* = 4.8 Hz, NH), 6.94 (t, *J* = 5.2 Hz, NH). ^{13}C NMR [$CDCl_3$, 100

MHz]: δ_C 16.3 (CH_3), 17.7 (CH_3), 25.6 (CH_3), 26.4 (CH_2), 28.9 (C-5/7), 33.5 (C-1/3), 35.8 (C-6), 37.5 (CH_2), 39.5 (CH_2), 41.7 (C-4/9, 8/10), 43.3 (CH_2), 47.4 (C-2), 50.7 (CH_2), 119.5 (CH), 123.7 (CH), 131.7 (C), 139.9 (C), 169.1 (C=O), 171.7 (C=O).

Data for *N′,N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dicarboxamide (30)

The crude product was purified using preparative HPLC to obtain yellowish oil, retention time: 22.5 min (67 % yield). IR ν_{max}: 3314, 2968, 2927, 2875, 1636, 1521, 1266 and 729 cm^{-1}. MS (TOF) calculated for $C_{36}H_{53}N_4O_4$ (M + H$^+$) 605.4061, found 605.4077. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.42 (1H, AB, *J* = 11.4 Hz, H-10a), 1.56 (6H, s, 2CH_3), 1.61-1.67 (14H, m, H-6a, 10b, 4CH_3), 1.94-1.97 (4H, m, 2CH_2), 2.01-2.04 (4H, m, 2CH_2), 2.20 (1H, AB, *J* = 10.4 Hz, H-6b), 2.60 (1H, s, H-7), 2.78-2.84 (2H, m, H-4, 8), 2.88 (1H, s, H-1), 2.93 (1H, m, H-9), 3.08 (1H, q, *J* = 8.9 Hz, H-3), 3.60-3.66 (2H, m, CH_2), 3.69-3.74 (2H, m, CH_2), 3.82-3.89 (2H, m, CH_2), 4.03-4.13 (2H, m, CH_2), 5.05 (2H, t, *J* = 6.6 Hz, 2CH), 5.18 (2H, t, *J* = 6.6 Hz, 2CH), 6.44 (br s, NH), 6.59 (br s, NH), 6.88 (br s, NH), 6.93 (br s, NH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.2 (CH_3), 17.7 (CH_3), 25.6 (CH_3), 26.5 (CH_2), 37.6 (C-10), 37.6 (CH_2), 38.8 (C-6), 39.5 (CH_2), 39.6 (C-4), 40.7 (C-9), 43.1 (CH_2), 43.2 (CH_2), 43.5 (C-8), 44.0 (C-3), 47.4 (C-7), 53.6 (C-1), 61.9 (C-2), 62.9 (C-5), 120.0 (CH), 120.1 (CH), 124.0 (CH),131.5 (C), 138.8 (C), 138.9 (C), 168.4 (C=O), 173.7 (C=O), 173.8 (C=O).

General synthesis of Compounds 4-8

To a stirring solution of **26-30** (1 mol) in dry THF as added $LiAlH_4$ (20 mol) and reflux under N_2 atm., reaction was monitored *via* LC-MS. After completion, excess $LiAlH_4$ was quenched by dropwise addition of aqueous Na_2SO_4, the resulting white precipitate was filtered and the filtrate concentrated *in vacuo*. The crude products were dissolved in acetonitrile (5-8 mL) and purified using preparative HPLC.

Data for [*N′,N′*-(8,11-dihydroxypentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diethylene)]-bis[(*N′′*-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (4)

A yellow oil, retention time: 14.7 min (50 mg, 30 %). IR ν_{max}: 2962, 2859, 2775, 2685, 1575, 1449, 1342 and 758 cm^{-1}. MS (TOF) calculated for $C_{39}H_{67}N_4O_2$ (M + H$^+$) 623.5259, found 623.5235. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.09 (1H, d, *J* = 10.0 Hz, H-4a), 1.48 (1H, H-4s), 1.54 (3H, s, CH_3), 1.62 (3H, s, CH_3), 1.65 (3H, s, CH_3), 1.73 (2H, s, CH_2), 2.02 (4H, s, 2CH_2), 2.19 (1H, s, H-9/10), 2.28 (1H, s, H-3/5), 2.44 (1H, s, H-1/7), 2.48 (1H, s, H-2/6), 3.04-3.08 (2H, m, CH_2), 3.25 (2H, m, CH_2), 3.32 (2H, m, CH_2), 3.52 (2H, m, CH_2), 4.99 (1H, m, CH), 5.23 (1H, m, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.5 (CH_3), 17.7 (CH_3), 25.6 (CH_3), 26.2 (CH_2), 34.0 (C-4), 34.6 (CH_2), 39.5 (C-2/6), 39.6

(CH_2), 42.7 (C-1/7), 42.7 (CH_2), 43.8 (C-3/5), 44.2 (CH_2), 44.3 (CH_2), 45.0 (CH_2), 49.4 (C-9/10), 77.1 (C-8/11), 114.1 (CH), 123.3 (CH), 132.0 (C), 145.6 (C).

Data for [*N′*,*N′*-(8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diethylene)]-bis[(*N′′*-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (5)

A yellow slurry oil, retention time: 16.3 min (0.12 g, 34 %). IR ν_{max}: 2965, 2858, 2765, 2683, 1560, 1453, 1342 and 763 cm^{-1}. MS (TOF) calculated for $C_{39}H_{65}N_4O$ (M + H$^+$) 605.5153, found 605.5137. ^{1}H NMR [$CDCl_3$, 600 MHz]: δ_H 1.49 (1H, d, *J* = 10.4 Hz, H-4a), 1.53 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.63 (3H, s, CH_3), 1.85 (1H, d, *J* = 10.4 Hz, H-4s), 2.01-2.03 (4H, m, 2CH_2), 2.10 (2H, m, CH_2), 2.34 (1H, s, H-3/5), 2.51 (2H, s, H-1/7, 9/10), 2.56 (1H, s, H-2/6), 3.02 (2H, m, CH_2), 3.13 (2H, m, CH_2), 3.21 (2H, m, CH_2), 3.43-3.46 (2H, m, CH_2), 4.99 (1H, m, CH), 5.21 (1H, m, CH). ^{13}C NMR [CDCl3, 150 MHz]: δ_C 16.5 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.3 (CH_2), 28.7 (CH_2), 39.7 (CH_2), 41.2 (C-2/6), 43.1 (CH_2), 43.6 (C-4), 43.8 (C-3/5), 45.0 (CH_2), 45.1 (CH_2), 45.2 (CH_2), 47.5 (C-1/7), 58.0 (C-9/10), 95.5 (C-8/11), 115.9 (CH), 123.5 (CH), 132.0 (C), 143.9 (C).

Data for [*N′*,*N′*-(tricyclo[3.3.1.1^{3,7}]decane-1,3-dimethylene)]-bis[(*N′′*-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (6)

A yellow slurry oil, retention time: 11.1 min (0.56 g, 41.8 %). IR ν_{max}: 2907, 2849, 2763, 2678, 1563, 1449, 1343 and 763 cm^{-1}. MS (TOF) calculated for $C_{36}H_{65}N_4$ (M + H$^+$) 553.5204, found 553.5207. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.35 (2H, d, *J* = 11.5 Hz, H-4/9 or H-8/10), 1.55 (3H, s, CH_3), 1.52-1.56 (2H, m, H-4/9 or H-8/10), 1.58 (2H, s, H-2, 6), 1.63 (3H, s, CH_3), 1.64 (3H, s, CH_3), 2.01-2.06 (5H, br s, H-5/7, 2CH_2), 2.49 (2H, s, CH_2), 3.12 (2H, m, CH_2), 3.18 (2H, m, CH_2), 3.51 (2H, d, *J* = 7.3 Hz, CH_2), 5.00 (1H, m, CH), 5.25 (1H, t, *J* = 6.8 Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.4 (CH_3), 17.7 (CH_3), 25.6 (CH_3), 26.2 (CH_2), 28.0 (C-5/7), 33.6 (C-1/3), 36.1 (C-6), 39.5 (C-4/9, 8/10), 39.6 (CH_2), 40.4 (C-2), 44.0 (CH_2), 44.8 (CH_2), 46.5 (CH_2), 60.4 (CH_2), 114.6 (CH), 123.3 (CH), 132.1 (C), 145.1 (C).

Data for [*N′*,*N′*-(tricyclo[3.3.1.1^{3,7}]decane-1,3-diethylene)]-bis[(*N′′*-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (7)

A yellow slurry oil, retention time: 11.2 min (0.29 g, 33 %). IR ν_{max}: 2911, 2847, 1765, 2682, 1567, 1449, 1340 and 763 cm^{-1}. MS (TOF) calculated for $C_{38}H_{69}N_4$ (M + H$^+$) 581.5517, found 581.5506. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.25 (1H, s, H-2), 1.35-1.43 (4H, m, H-4/9, 8/10), 1.47-1.50 (2H, m, CH_2), 1.54 (1H, s, H-6), 1.56 (3H, s, CH_3), 1.64 (3H, s, CH_3), 1.67 (3H, s, CH_3), 1.99 (1H, s, H-5/7), 2.02-2.05 (4H, m, 2CH_2), 2.89 (2H, s, CH_2), 3.22 (2H, m, CH_2), 3.26 (2H, m, CH_2), 3.52 (2H, d, *J* = 7.2 Hz, CH_2), 5.01 (1H, m, CH), 5.26 (1H, t, *J* = 6.7 Hz, CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.5 (CH_3), 17.7 (CH_3), 25.7 (CH_3), 26.2 (CH_2), 28.5 (C-5/7), 32.4 (C-1/3), 36.0 (C-6), 39.5 (CH_2), 39.6

(CH_2), 41.4 (C-4/9, 8/10), 42.6 (CH_2), 43.7 (CH_2), 44.3 (CH_2), 45.2 (CH_2), 45.8 (C-2), 114.3 (CH), 123.3 (CH), 132.1 (C), 145.5 (C).

Data for [*N´*,*N´*-(pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dimethylene)]-bis[(*N´´*-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (8)

A yellow slurry oil, retention time: 11.8 min (0.52 g, 28.1 %). IR ν_{max}: 2966, 2925, 2855, 2774, 1578, 1446, 1342 and 756 cm^{-1}. MS (TOF) calculated for $C_{36}H_{61}N_4$ (M + H$^+$) 549.4891, found 549.4929. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.24 (1H, AB, *J* = 12.4 Hz, H-6a), 1.27 (1H, AB, *J* = 12.9 Hz, H-10a), 1.55 (6H, s, 2CH_3), 1.59 (1H, H-10b), 1.63 (6H, s, 2CH_3), 1.65 (6H, s, 2CH_3), 1.86 (1H, AB, *J* = 10.7 Hz, H-6b), 2.01-2.07 (8H, m, 4CH_2), 2.35 (2H, m, H-3, 4), 2.49 (1H, s, H-7), 2.59 (1H, s, H-1), 2.64 (1H, AB, *J* = 13.1 Hz, CH_2), 2.66 (1H, m, H-8), 2.76 (1H, m, H-9), 2.82 (1H, AB, *J* = 12.8 Hz, CH_2), 2.96 (1H, AB, *J* = 13.1 Hz, CH_2), 3.07-3.29 (8H, m, 4CH_2), 3.25 (1H, AB, *J* = 13.1 Hz, CH_2), 3.49 (4H, d, *J* = 7.1 Hz, 2CH_2), 5.00 (2H, m, 2CH), 5.26 (2H, t, *J* = 6.9 Hz, 2CH). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 16.5 (CH_3), 17.7 (CH_3), 25.6 (CH_3), 26.2 (CH_2), 37.6 (C-10), 39.6 (CH_2), 40.2 (C-4), 40.3 (C-6), 40.5 (C-9), 42.3 (C-3), 43.2 (C-8), 43.9 (CH_2), 44.3 (CH_2), 45.3 (CH_2), 46.1 (CH_2), 46.2 (CH_2), 46.9 (C-7), 48.9 (CH_2), 50.4 (CH_2), 50.8 (C-1), 53.0 (C-2), 54.2 (C-5), 114.6 (CH), 114.7 (CH), 123.4 (CH), 132.0 (C), 144.9 (C), 145.0 (C).

Anti-mycobacterial assay

The BACTEC system has been devised to monitor mycobacterial growth of the slow growing species. *M. tuberculosis* H_{37}Rv reference strain (ATCC 25618) was used to evaluate the compounds for their anti-tuberculosis activity. The strain is sensitive to Isoniazid with an MIC for INH at 0.03 µg/mL in BACTEC 12B medium. All work was carried out in the BSL3 laboratory of the Division of Molecular Biology of the University of Stellenbosch. Bacterial colonies were cultured and selected from Loewenstein-Jensen slant cultures. A colony of *M.tuberculosis* reference strain H_{37}Rv was cultured in 7H9 mycobacterial medium (Difco) enriched with ADC (Biolab art. C70) with continuous stirring at 37 °C. When cultures reached a density of approximately 0.16 at A600 nm (one McFarland), 0.1 mL was inoculated into a Bactec vial (23). These primary cultures were incubated at 37 °C until a growth index of 500 (+/-50) was reached. These primary cultures were used for drug testing of anti-tuberculosis compounds. Cultures were regularly stained by acid-fast staining (ZN staining) to control for contamination (24).

The compounds **4-8**, **26-30** and **SQ109** were dissolved in methanol to make a stock solution of 10 mM concentration. 0.1 mL of primary culture and 0.1 mL drug compound were added to a BACTEC vial, the vials incubated at 37 ºC, and the growth monitored every 24 hours. The final concentrations in BACTEC 12B medium were 50 µM, 10 µM, and 1 µM and the final methanol concentration in the

12B medium was 2.5%. Read-out values are expressed as growth index (GI). Controls included cultures with and without solvent (2.5 % Methanol). GI readings were continued until the controls reached the maximum GI value approximately500 or more. Control GI values between 50 and 800 are normally used to evaluate the efficacy of compounds with possible anti-tuberculosis activity (23).

Acknowledgements

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Supplementary data

Supplementary data (^{1}H, ^{13}C NMR and HRMS spectra) associated with this article can be found in the online version at XXXXXX

References

(1) World Health Organisation (WHO); Global tuberculosis control: a short update to the 2009 report; http://www.who.int/tb/publications/global_report/2009/update/en/index.html, accessed on 05-02-10.

(2) Rivers, E. C., and Mancera, R. L. (2008) New anti-tuberculosis drugs with novel mechanisms of action. Curr Med Chem; 15: 1956-1967.

(3) Rivers, E. C., and Mancera, R. L. (2008) New anti-tuberculosis drugs in clinical trials with novel mechanisms of action. Drug Discovery Today; 13: 1090-1098.

(4) Shi, R. R., and Sugawara, I. (2010) Development of New Anti-tuberculosis Drug Candidates. Tohoku J Exp Med; 221: 97-106.

(5) Lee, R. E., Protopopova, M., Crooks, E., Slayden, R. A., Terrot, M., and Barry, C. E. (2003) Combinatorial lead optimization of [1,2]-diamines based on ethambutol as potential antituberculosis preclinical candidates. J Comb Chem; 5: 172-187.

(6) Protopopova, M., Hanrahan, C., Nikonenko, B., Samala, R., Chen, P., Gearhart, J., Einck, L., and Nacy, C. A. (2005) Identification of a new antitubercular drug candidate, SQ109, from a combinatorial library of 1,2-ethylenediamines. J Antimicrob Chemother; 56: 968-974.

(7) Onajole, O. K., Govender, P., Van Helden, P., Kruger, H. G., Maguire, G. E. M., Wiid, I., and Govender, T. (2010) Synthesis and evaluation of SQ109 analogues as potential anti-tuberculosis candidates. Euro J Med Chem; 45: 2075-2079.

(8) Jia, J., Tomaszewski, J. E., Hanrahan, C., Coward, L., Noker, P., Gorman, G., Nikonenko, B., and Protopopova, M. (2005) Pharmacodynamics and pharmacokinetics of SQ109, a new diamine-based antitubercular drug. Brit J Pharmacol; 144: 80-87.

(9) National Institute of Allergy and Infectious Diseases: Timeline of Events in Development of SQ109. www.niaid.nih.gov/topics/tuberculosis/Research/treatment/pages/sq109timeline.aspx; accessed on 05-08-10.

(10) Onajole, O. K., Govender, K., Govender, P., Van Helden, P., Kruger, H. G., Maguire, G. E. M., Muthusamy, K., Pillay, M., Wiid, I., and Govender, T. (2009) Pentacyclo-undecane derived cyclic tetra-amines: Synthesis and evaluation as potent ant-tuberculosis agents. Euro J Med Chem; 44: 4297 - 4305.

(11) Thiele, J. (1901) About derivatives of cyclopentadienyl. Chem Ber; 34: 68.

(12) Marchand, A. P., Namboothiri, I. N. N., Lewis, S. B., Watson, W. H., and Krawiec, M. (1998) Thiele's acid revisited: Isolation and characterization of two minor products formed by carbonation of cyclopentadienide anion. Tetrahedron; 54: 12691-12698.

(13) Dunn, G. L., and Donohue, J. K. (1968) The structure of thiele's ester, a dimethyl dicyclopentadienedicarboxylate. Tetrahedron lett; 9: 3485-3487.

(14) Marchand, A. P. (1989) Synthesis and Chemistry of Homocubanes, Bishomocubanes, and Trishomocubanes. Chem Rev; 89: 1011-1033.

(15) Marchand, A. P., Hariprakasha, H. K., and Namboothiri, I. N. N. (2001) Synthesis of 2,5-dimethyl-pentacyclo-[5.4.0.0^{2,5}.0^{3,9}0^{4,8}]decane. Synth Comm; 31: 1863-1869.

(16) Minter, D. E., Smith, W. B., Marchand, A. P., Etukala, J. R., and Sivappa, R. (2004) Regio- and stereochemistry of Michael addition of methanol to Thiele's ester. J Phy Org Chem; 17: 174-179.

(17) Watson, W. H., Marchand, A. P., and Sivappa, R. (2004) Structure of a minor reaction product formed via base promoted hydrolysis of Thiele's ester. Arkivoc; 66-73.

(18) Onajole, O. K., Makatini, M. M., Govender, P., Govender, T., Maguire, G. E. M., and Kruger, H. G. (2010) Synthesis and NMR assignment of pentacycloundecane precursors of potential pharmaceutical agents. Magn Reson Chem; 48: 249-255.

(19) Govender, T., Hariprakasha, H. K., Kruger, H. G., and Marchand, A. P. (2003) Synthesis and transport studies of a new class of cage-annulated chiral macrocycles. Tetrahedron-Asym; 14: 1553-1557.

(20) Boyle, G. A., Kruger, H. G., Maguire, G. E. M., and Singh, A. (2007) NMR elucidation of some pentacycloundecane derived ligands. Struct Chem; 18: 633-639.

(21) Stetter, H., and Wulff, C. (1960) Through links with urotropine structure XVIII. On the bromination of adamantane. Chem Ber; 93: 1366-1371.

(22) Bott, K., and Hellmann, H. (1966) Syntheses of carboxylic acids from 1.1-dichloroethylene. Angew Chem; 5: 870.

(23) Siddiqi, S. H. (2006) BACTEC 460 TB system. Product and procedure manual, revision D. Becton Dickinson Microbiology System, Sparks, Md.

(24) Laifangbam, S., Singh, H. L., Singh, N. B., Devi, K. M., and Singh, N. T. (2009) A comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. Kathmandu Univ Med J (KUMJ); 7: 226-30.

CHAPTER 9
SYNTHESIS AND NMR ASSIGNMENT OF PENTACYCLOUNDECANE PRECURSORS OF POTENTIAL PHARMACEUTICAL AGENTS

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Abtract

The synthesis and complete NMR elucidation of eight novel pentacycloundecane (PCU) derivatives are reported. These compounds are precursors in the synthesis of PCU based anti - tuberculosis (TB) agents and potential HIV protease inhibitors. Two dimensional NMR techniques were used to assign the NMR spectra for these compounds. Substitution of the cage molecule at (C-8/11) further complicates the assignment, since some of the substituted alkyl chain groups overlap with the cage proton signals. The side chain heteroatoms also introduce rare through-space deshielding effect to some of the carbon atoms of the cage skeleton. Ring strain in the rigid cage skeleton appears to induce drastic electronic changes some parts of the cage framework. This observation is more dramatic for the C-4 methylene group of the cage diols and the cage ethers.

Introduction

Polycyclic “cage” molecules have been of interest to scientists for many years.[1-5] The chemistry and applications of “cage” compounds (such as pentacycloundecane and trishomocubane) were the focus of a number of South African scientists over the years.[6-13] The value of 2D NMR techniques to determine the structure of cage compounds has been reported by several authors.[1,14-16] Our group has successfully utilized these techniques in the elucidation of numerous PCU derivatives synthesized in our laboratory.[17-24] As part of our ongoing programme in this field, the NMR assignment of eight novel PCU derivatives (**1-8**) is reported (Figure 1).

Onajole *et al.* [25] recently reported novel PCU tetra amine compounds as potential anti-TB agents. The eight compounds communicated herein are all precursors in the synthesis of these series of potential anti-TB and HIV protease agents presently being studied in our laboratory.

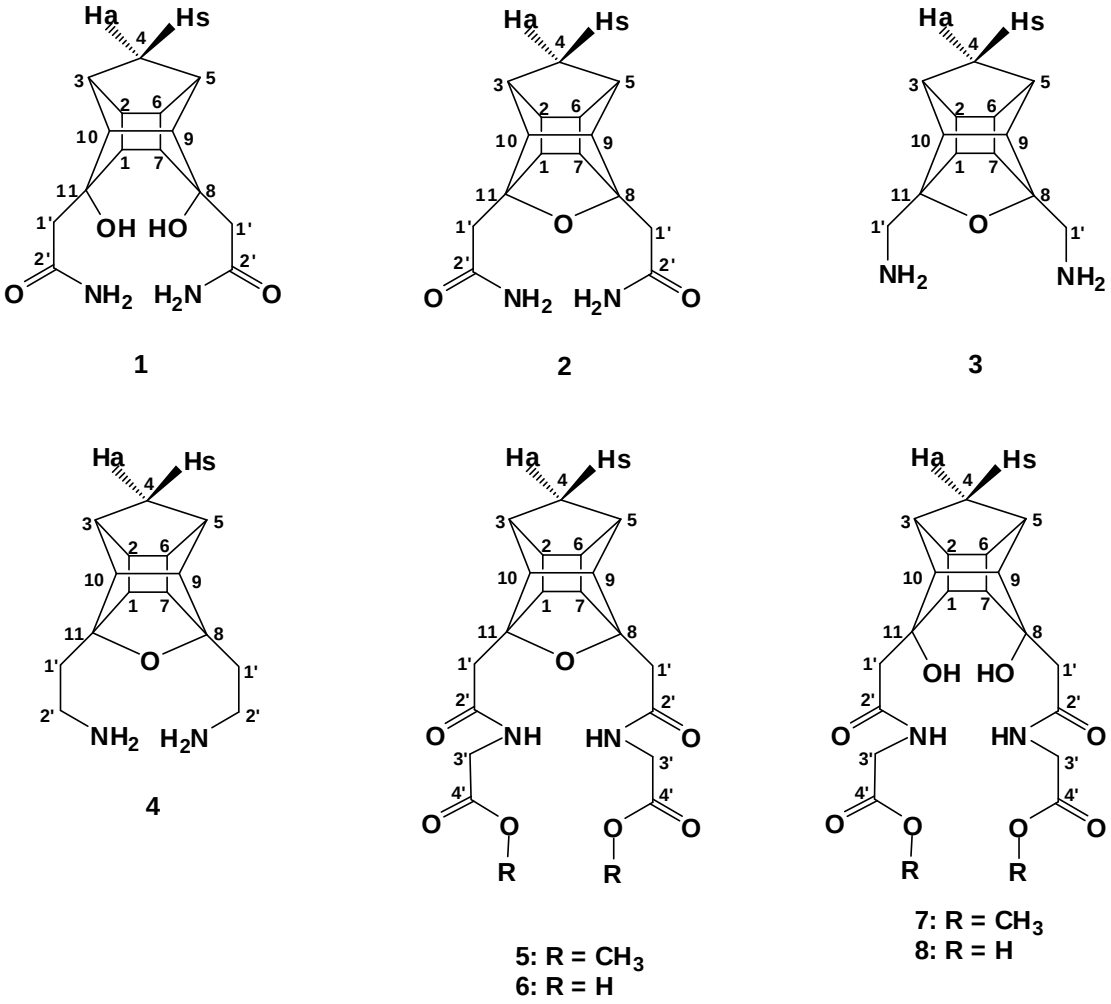
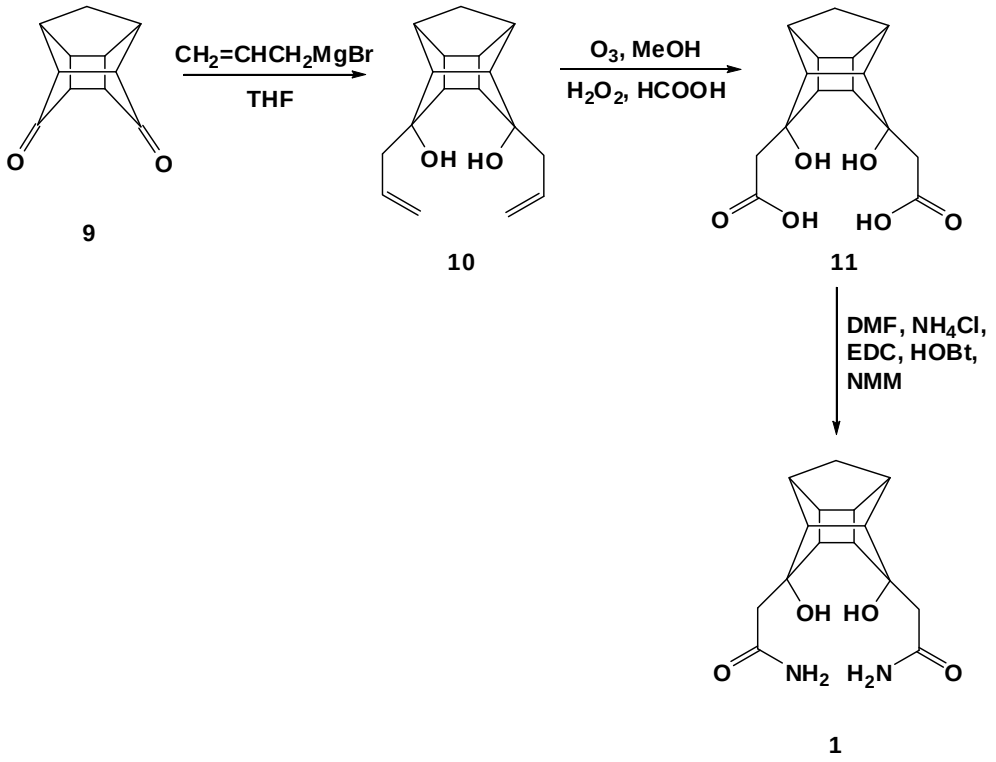


Figure 1: PCU derivatives **(**1-8**)**

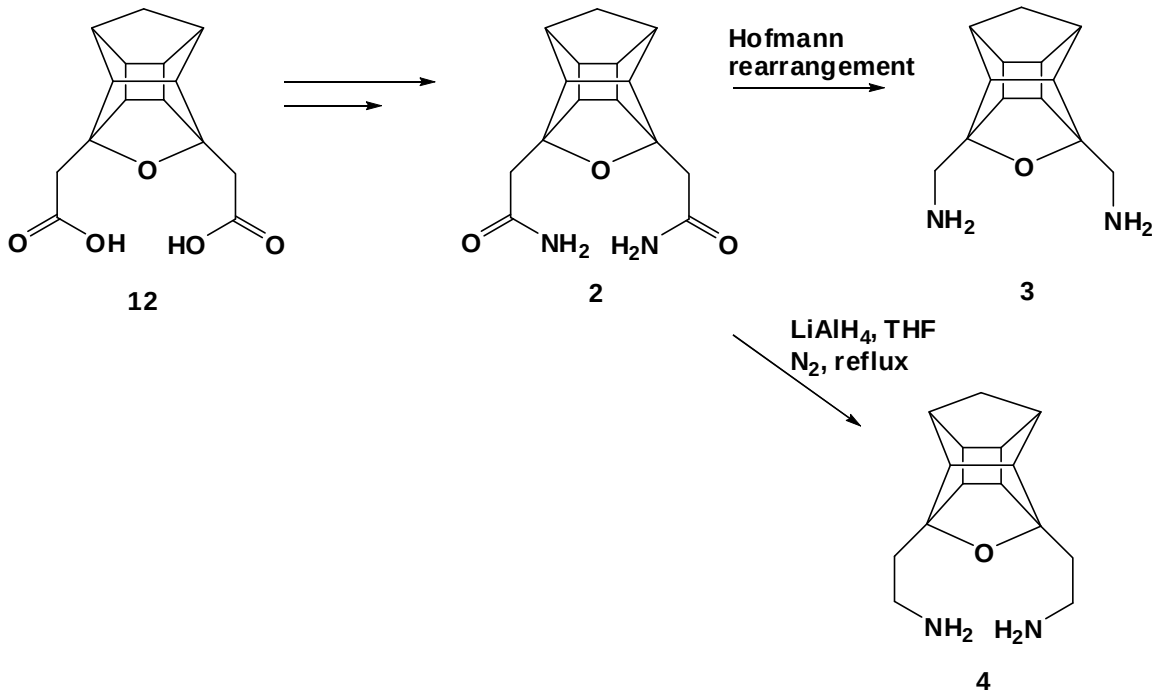
The starting material for these molecules is Cookson's dione **9**.[26] Cookson's dione is reacted with freshly prepared allyl magnesium bromide (Grignard reaction) to obtain *endo*-8,*endo*-11 diol **10**.[10] Ozonolysis of the diol **10** followed by an oxidative workup yielded the PCU diol dicarboxylic acid **11**. Compound **11** is reacted with ammonium chloride using coupling reagents hydroxybenzotriazole (HOBt), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-methylmorpholine (NMM) to obtain compound **1** (Scheme 1).



Scheme 1: Synthesis of PCU derivative 1*

The starting material in the synthesis of compounds **2-5** is the PCU ether dicarboxylic acid **12**.[10,21] Compound **12** was converted to its corresponding diacid chloride[10] and reacted with ammonia-saturated chloroform at 0 ºC to obtain **2** in good yield. Compound **2** was subjected to a Hofmann rearrangement reaction using sodium hypochlorite and sodium hydroxide to obtain compound **3**. Reduction of compound **2** using lithium aluminium hydride yielded the corresponding derivative **4** (scheme 2).

* The synthesis of compound **11** was first achieved by Mr. R. Karpoormath in our group (unpublished results).



Scheme 2: Synthesis of PCU derivatives 2-4

Compounds **12** and **11** were converted to **5** and **7** respectively using glycine methyl, HOBt, EDC and NMM. Compounds **5** and **7** were hydrolyzed using lithium hydroxide to obtain their corresponding acid **6** and **8**.

Results and discussions

All PCU compounds (**1-8**) was analogous to those described before[19,21] and the results are presented in Tables 1 and 2.

The C-N bond in compound **1** and **2** experiences partial double bond character which hinders "free" rotation of the sp^2 amide nitrogen. Thus for compound **1**, the *cis*-amide proton (δ=7.33 ppm) to the carboxamide oxygen appears at a higher frequency compared to the *trans*-amide proton (δ=7.08 ppm) due to a through-space deshielding effect of the *cis*-proton.[24,27] A similar observation was noted in the elucidation of compound **2**.

An interesting observation in Table 1 begs an explanation. Why would H-1/7 and H-9/10, H-1' and C-8/11, in compound **2** be more deshielded than the corresponding signals in compound **1**? Protons on the cage are normally deshielded if a hetero atom of a side chain is positioned such that the electron rich hetero atom is in close proximity of the cage proton(s).[4,19,28,29] It is possible that the OH and NH_2 groups in **1** cause intramolecular hydrogen bond interactions. This will largely "tie" the two groups together at room temperature. However, for compound **2** much less intramolecular hydrogen bond interactions are possible. The result is that the side arms are "free" to rotate around C-8/11 and

C-1' at room temperature, spending on average more time in close proximity to H-1/7 and H-9/10. This will cause the through-space deshielding effect observed for protons H-1/7 and H-9/10.

The tertiary carbon signals of a tertiary alcohol and that of tertiary ether normally appears quite close together between 60 - 70 ppm,[†] however the corresponding values for **1** and **2** are quite far apart. The tertiary alcohol carbon of compound **1** (C-8/11) appears at 76.6 ppm while the C-8/11 for compound **2** appears at δ=93.6 ppm. This observation suggests that the intramolecular hydrogen bond interaction experienced in compound **1** contributes to the shielding effect observed for C-8/11. The hydrogen bond is absent in **2** and it appears that a through-space deshielding effect from the side chain heteroatoms on the *exo*-side of C-8/11 causes that this carbon atom is registered at a much higher frequency. This through-space deshielding of carbon atoms has been observed before for two similar compounds in our group,[19] as well as by other researchers for different molecules.[19,30,31]

Another large chemical shift to a higher frequency is observed for C-4 when the cage diol (such as **1**) is converted to the cage ether (such as **2**). The side arms are too short to induce a through-space deshielding effect to C-4. To obtain some insight in this interesting phenomenon, it was decided to optimize the two structures with a Density Functional Theory calculation [B3LYP[32-34] with the 6-31+G(d) basis set]. The PCU skeleton resembles a rigid cyclohexane boat structure (C-1, C-7, C-8, C-9, C-10 and C-11). It was shown in previous studies[35] that a reduction in distance (of ~0.1 Å) between C-8 and C-11 is responsible for large ring strain in the molecule. The calculated distance between C-8 and C-11 in the cage diol **1** is 2.94 Å and the cage ether **2** is 2.21 Å; a difference of about ~0.7 Å. A deviation in the angles between C-11, C-10 and C-3 for these two compounds was also observed (101.4º for compound **1** and 109.0º for compound **2**). These deviations appear to somehow influence the electron densities in the two compounds for C-4.

The ^{13}C NMR spectrum of the two molecules were calculated (GIAO)[36-40] and it was found that C-4 for compound **1** appears at ~ 45 ppm and for compound **2** at ~ 54 ppm. This theoretical result coincides with the experimental observation in which C-4 for compound **2** registers at higher frequency of about 9 ppm more than for compound **1**. Since this is also observed in the theoretical case, it is expected that C-4 for compound **2** experience larger electron density than the corresponding carbon for **1**. The molecular orbitals for the two molecules were calculated and the 10 highest occupied molecular orbitals (MOs) were compared. For compound **1**, only the fifth highest MO showed electron density from C-3 through C-4 to C-5. All the other nine MOs showed either no electron density in that part of the molecule or showed a node (non bonding interaction) at C-4. For compound **2**, both the fifth and tenth highest MOs showed electron density involving C-3 through C4

[†] Results generated using Chemdraw Ultra 10.

to C-5. The increased electron density of C-4 for compound **2** should explain the difference in carbon shifts for C-4 in these two molecules.

The signal for C-2' (δ=173.0 ppm) is absent in the ^{13}C and HMBC spectra of **3**, thus confirming the success of the Hofmann rearrangement reaction. The ^{13}C spectrum shows two methylene carbons appearing at δ=43.4 and 43.7 ppm. The assignment of the former carbon was made to C-4 and the latter to C-1' since the positions of H-4a and H-4s are known. The H-1' protons display NOESY interactions with the NH_2 protons (δ=1.39 ppm), H-1/7 (δ=2.58 ppm) and H-9/10 (δ=2.57 ppm) respectively. Carbons C-9/10 (δ=56.4 ppm) show HMBC correlations with H-1', H-1/7, H-4a and H-4s respectively. All assignments were further confirmed using the HSQC spectrum; these assignments are presented in Table 1 and 2.

The loss of the carbonyl signal at 173.0 ppm (^{13}C NMR spectrum of **4**) and the presence of methylene protons at 2.62 – 2.74 ppm (^{1}H NMR spectrum) confirm the successful reduction of compound **1** to **4**. The APT ^{13}C spectrum shows three methylene carbons at 36.2, 38.8 and 43.4 ppm, the signal at δ=43.4 ppm was assigned to C-4 (due to HSQC correlation with the H-4a and H-4s protons) while the remaining two signals should belong to C-1' and C-2'. COSY and NOESY spectra show correlations between the two methylene protons appearing at δ=1.81 and 2.62 – 2.74 ppm which belongs to H-1' and H-2' respectively. H-1' (δ=1.81 ppm) displays HMBC spectrum correlations with C-1/7 (δ=47.7 ppm), C-9/10 (δ=58.3 ppm), C-8/11 (δ=95.3 ppm) and C-2' (δ=38.8 ppm) respectively. The H-2' protons show HMBC correlations with C-1' (δ=36.2 ppm) and C-8/11. H-1' exhibit a NOESY interactions with H-1/7 and H-9/10 respectively. These assignments are presented in Table 1 and 2.

The APT ^{13}C spectrum of compound **5** shows a quaternary carbon at δ=94.0 ppm which was assigned to C-8/11. C-8/11 displays a HMBC correlation with a methylene proton at δ=2.68 – 2.80 ppm this was assigned to H-1'. The ^{13}C spectrum shows two carbonyl carbons at δ=170.3 and 170.9 ppm. HMBC correlation was observed between H-1' and a carbonyl carbon (170.3 ppm) which was then assigned to C-2', thus the second carbonyl carbon (δ=170.9 ppm) was assigned to C-4'. C-4' display a HMBC correlation to H-3' (δ=3.98 ppm) and H-5' (δ=3.65 ppm). The NH protons at δ=7.07 ppm show a HMBC correlation to C-2' (δ=170.3 ppm), C-3' (δ=41.0 ppm) and C-4' (δ=170.9 ppm). The NH proton also displays a NOESY interaction with H-1'and H-3' respectively and a COSY correlation with H-3' only. All assignments were further confirmed using the HSQC spectrum; these assignments are presented in Table 1 and 2.

Table 1: ^{1}H NMR chemical shifts of compounds 1 - 8

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Atom	**$\delta^{1}H^{a,b}$**	**$\delta^{1}H^{c,b}$**	**$\delta^{1}H^{c,d}$**	**$\delta^{1}H^{b,c}$**	**$\delta^{1}H^{b,c}$**	**$\delta^{1}H^{b,e}$**	**$\delta^{1}H^{b,c}$**	**$\delta^{1}H^{b,e}$**
1/7	2.41	2.59	2.58	2.41	2.65	2.77	2.46	2.60
2/6	2.48	2.57	2.60	2.50	2.62	2.74	2.49	2.64
3/5	2.40	2.39	2.37	2.27	2.43	2.50	2.38	2.56
4a	1.05 d, *J* = 10.5	1.47, d, *J* = 10.5 Hz	1.52, d, *J* = 10.4	1.42, d, *J* = 10.4	1.49, d, *J* = 10.5	1.59, d, *J* = 10.4	1.06 d, *J* = 10.8	1.20 d, *J* = 10.7
4s	1.50, d, *J* = 10.5	1.82, d, *J* = 10.5 Hz	1.88, d, *J* = 10.4	1.78, d, *J* = 10.4	1.85, d, *J* = 10.5	1.97, d, *J* = 10.4	1.52 d, *J* = 10.8	1.65 d, *J* = 10.7
8/11	-	-	-	-	-	-	-	-
9/10	2.19	2.58	2.57	2.39	2.62	2.73	2.31	2.37
1'a	2.09, d, *J* = 14.4	2.56-2.68	2.98, t, *J* = 13.5	1.81, t, *J* = 7.2	2.68 – 2.80, t, *J* = 15.7	2.81	2.40 d, *J* = 15.0	2.36 d, *J* = 14.6
1'b	2.21, d, *J* = 14.4	-	-	-	-	-	2.25 d, *J* = 15.0	2.48 d, *J* = 14.6
2'	-	-	-	2.62-2.74	-	-	-	-
3'	-	-	-	-	3.98, t, *J* = 5.5	3.78	3.94	3.80 t, *J* = 5.3
4'	-	-	-	-	-	-	-	-
5'	-	-	-	-	3.65		3.67	
NH	7.08	6.45	1.39	1.85	7.07, t, *J* = 5.2		7.61 t, *J* = 15.5	
NH	7.33	6.67	1.39	1.85				
OH	7.66							

[a] Solvent $(CD_3)_2SO$; [b] 400 MHz for ^{1}H; [c] Solvent $CDCl_3$, [d] 600 MHz for ^{1}H; [e] Solvent CD_3OD

Table 2: ^{13}C NMR chemical shifts of compounds 1 - 8

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Atom	**$\delta^{13}C^{a,b}$**	**$\delta^{13}C^{c,b}$**	**$\delta^{13}C^{c,d}$**	**$\delta^{13}C^{b,c}$**	**$\delta^{13}C^{b,c}$**	**$\delta\ ^{13}C^{b,e}$**	**$\delta^{13}C^{b,c}$**	**$\delta^{13}C^{b,e}$**
1/7	42.5 (CH)	48.0 (CH)	45.9 (CH)	47.7 (CH)	48.1 (CH)	49.5 (CH)	42.9 (CH)	44.4 (CH)
2/6	38.9 (CH)	41.3 (CH)	41.5 (CH)	41.3 (CH)	41.4 (CH)	42.8 (CH)	39.3 (CH)	40.7 (CH)
3/5	43.8 (CH)	43.9 (CH)	44.0 (CH)	44.0 (CH)	44.0 (CH)	45.6 (CH)	44.3 (CH)	45.8 (CH)
4a	33.5 (CH_2)	43.3 (CH_2)	43.4 (CH_2)	43.4 (CH_2)	43.4 (CH_2)	44.3 (CH_2)	34.0 (CH_2)	34.8 (CH_2)
4s	33.5 (CH_2)	43.3 (CH_2)	43.4 (CH_2)	43.4 (CH_2)	43.4 (CH_2)	44.3 (CH_2)	34.0 (CH_2)	34.8 (CH_2)
8/11	76.6 (C)	93.6 (C)	97.4 (C)	95.3 (C)	94.0 (C)	95.2 (C)	77.3 (C)	78.6 (C)
9/10	49.3 (C)	58.4 (CH)	56.4 (CH)	58.3 (CH)	58.6 (CH)	59.9 (CH)	49.5 (CH)	51.0 (CH)
1'a	43.9 (CH_2)	39.0 (CH_2)	43.7 (CH_2)	36.2 (CH_2)	39.4 (CH_2)	40.1 (CH_2)	44.3 (CH_2)	45.7 (CH_2)
1'b	43.9 (CH_2)	-	-	-	-	-	44.3 (CH_2)	45.7 (CH_2)
2'	173.8 (C)	173.0 (C)	-	38.8 (CH_2)	170.3 (C)	172.4 (C)	172.9 (C)	174.0 (C)
3'	-	-	-	-	41.0 (CH_2)	44.6 (CH_2)	40.9 (CH_2)	44.6 (CH_2)
4'	-	-	-	-	170.9 (C)	176.2 (C)	170.2 (C)	176.4 (C)
5'	-	-	-	-	52.1 (CH_3)	-	52.3 (CH_3)	-

[a] Solvent $(CD_3)_2SO$; [b] 100 MHz for ^{13}C; [c] Solvent $CDCl_3$, [d] 150 MHz for ^{13}C; [e] Solvent CD_3OD

The ^{13}C spectrum of **6** shows two carboxylic carbon signals appearing at δ=172.4 and 176.2 ppm. The carbon at 172.4 ppm exhibits HMBC correlations with two proton signals at δ=2.81 and 3.78 ppm which should be protons H-1' and H-3'. This carbon (δ=172.4 ppm) was therefore assigned to C-2' while δ=176.2 ppm was assigned to C-4'. The carboxylic carbon (C-4') shows HMBC correlation to protons at δ=3.78 ppm which was assigned to H-3' while δ=2.81 ppm was assigned to H-1'. H-1' displays HMBC correlations with C-1/7 (δ=49.5 ppm), C-9/10 (δ=59.9 ppm), C-8/11 (δ=95.2 ppm) and C-2' (δ=172.4 ppm) respectively while H-3' shows HMBC correlations to C-2' (δ=172.4 ppm) and C-4' (δ=176.2 ppm) respectively. As was the case with the previous NMR signals, a similar trend is observed between the two related structures. The assigned signals are presented in Table 1 and 2.

A similar route, employed in the synthesis of **5** and **6** was used to obtain compounds **7** and **8**. The ^{13}C spectrum shows two carbonyl carbons at δ=170.2 and 172.9 ppm. The signal at δ=170.2 ppm shows an HMBC correlation with the methyl (δ=3.67 ppm) and methylene (δ=3.94 ppm) protons. This carbonyl was therefore assigned to C-4' and the methylene and methyl groups to H-3' and H-5' respectively. The remaining carbonyl carbon was assigned to C-2' (δ=172.9 ppm). The C-2' carbon resonance displays a HMBC correlation to a set of doublets at [δ=2.25 and 2.40 ppm (J = 15 Hz)] and to the H-3' protons. The set of doublets were assigned to H-1'. The signal at 7.61 ppm was assigned to the NH proton, this proton displayed COSY and NOESY interactions with H-3' and H-1', thus confirming the assignment of the nitrogen proton. The assignment of compound **7** is presented in Table 1 and 2.

The absence of a methyl signal both in the ^{1}H and ^{13}C spectrum of compound **8** suggests the successful hydrolysis of compound **7** to its corresponding diacid **8**. A methylene proton appearing at 2.36 and 2.48 ppm (J = 14.6 Hz) shows a HMBC correlation to the quaternary carbon C-8/11 (δ=78.6 ppm) and was thus assigned to H-1'. The H-1' signal displays a HMBC correlation with C-1/7 (δ=44.4 ppm), C-9/10 (δ=51.0 ppm) and a carbonyl signal at δ=174.0 ppm which was assigned to C-2'. The remaining carbonyl was assigned to C-4'. C-4' shows a HMBC correlation with a methylene proton (δ=3.80 ppm) which was assigned to H-3'. The assignments are presented in Table 1 and 2.

Conclusion

The synthesis and complete elucidation of eight novel PCU derivatives was successfully carried out. The synthesis was achieved utilizing known protocols from literature. Compounds **1** and **2** exhibit interesting conformation effects of the 'cage' side chains due to the presence of intramolecular hydrogen bonding in compound **1**. Similar through-space deshielding of the cage protons when in proximity to a side chain hetero atom was reported previously by our research group. A rare through-space deshielding effect on some of the cage carbons is observed. It also appears that ring strain

experienced by different PCU skeletons is responsible for large electronic differences of certain groups in the cage framework.

Experimental

Infrared spectra were obtained from a Perkin Elmer Spectrum 100 instrument with an Attenuated Total Reflectance attachment. Accurate mass spectra were measured with a Bruker Micro TOF-QII instrument while melting point analysis (uncorrected) was performed on a Stuart Scientific digital melting point apparatus SMP3. Tetrahydrofuran was freshly distilled using sodium wire/benzophenone under a N_2 atmosphere. Dichloromethane was dried using phosphorus pentoxide. The NMR data were recorded on a Bruker AVANCE III 400 and 600 instruments; the chemical shifts were referenced to the solvent peak, namely 7.24 ppm for $CDCl_3$, 2.50 ppm for $(CD_3)_2SO$ and 3.34 ppm for CD_3OD at ambient temperature. The ^1H NMR spectra were recorded at a transmitter frequency of 600.103 MHz (spectral width, 12335.526 Hz; acquisition time, 1.328 s; 90^o pulse width, 15μs; scans, 16; relaxation delay, 1.0 s) for the Bruker AVANCE III 600 instrument while the ^1H NMR spectra were recorded at a transmitter frequency of 400.222 MHz (spectral width, 8223.685 Hz; acquisition time, 3.98 s; 90^o pulse width, 10μs; scans, 16; relaxation delay, 1.0 s) for the Bruker AVANCE III 400 instrument. The ^{13}C NMR spectra were recorded at 150.910 MHz (spectral width, 36057.69 Hz; acquisition time, 0.908 s; 90^o pulse width, 9.00 μs; scans, 4800; relaxation delay, 2.00 s) for the Bruker AVANCE III 600 instrument while the ^{13}C NMR spectra were recorded at 100.645 MHz (spectral width, 24038.461 Hz; acquisition time, 1.363 s; 90^o pulse width, 8.40 μs; scans, 3200; relaxation delay, 2.00 s) for the Bruker AVANCE III 400 instrument.

The 2D experimental data parameters obtained on the Bruker AVANCE III 400 were as follows: 90^o pulse width, 10 μs for all spectra; spectral width for ^1H, 3355.705, 2403.846, 2577.320, 3144.654, 3875.969 and 3623.188 Hz for **1**, **2**, **4**, **5**, **6**, **7** and **8** respectively (NOESY, COSY, HSQC and HMBC); spectral width for ^{13}C, 1666.666 Hz (HSQC and HMBC) for **1**, 16670.416, 22352.855 Hz (HSQC and HMBC) for **2**, **5**, **7** and **8**, 16670.416, 27932.961 Hz (HSQC and HMBC) for **4**; number of data points per spectrum, 2048 (COSY), 2048 (NOESY), 4096 (HMBC), 1024 (HSQC) for compounds **1** - **8**; number of time-incremented spectra, 128 (COSY), 256 (NOESY), 128 (HMBC), 256 (HSQC) for compounds **1** - **8**; relaxation delay for compounds **1**, **2**, **5** -**8** was 1.4 s and **4** was 1.3 s for COSY experiments, the relaxation delay for NOESY experiments are 2.0 s for compounds **1**, **6**, **7** and **8**, 1.8 s for **2** and 1.9 s for **4** and **5** respectively. The relaxation delay for HMBC experiments are 1.3 s for **1**, **6** and **8**, 1.0 s for **2**, 1.1 s for **4** and 1.2 s for **5** while HSQC experiments had 1.4 s for **1**, **2**, **4** and **5** and 1.5 s for **6** – **8** respectively. All 2D experimental data obtained on the Bruker AVANCE III 600 for compound **3** are as follows; 90^o pulse width, 15.1 μs for all spectra; spectral width for ^1H is 6009.615 Hz (NOESY, COSY, HSQC and HMBC); spectral width for ^{13}C, 25000 and 36057.691 Hz (HSQC and HMBC) for **3**, number of data points *per* spectrum, 2048 (COSY), 2048 (NOESY), 4096

(HMBC), 1024 (HSQC) while number of time – incremented spectra, 128 (COSY), 256 (NOESY), 128 (HMBC), 256 (HSQC). The relaxation delay for compound **3** is 1.0 s for COSY, NOESY, HSQC and HMBC respectively; spectra acquired in phase-sensitive mode, **1 - 8** (NOESY and HSQC); spectra acquired in absolute value mode, **1 – 8** (COSY and HMBC); gradients used for **1 - 8** (COSY, HSQC and HMBC). All NMR spectra are available as supplementary material.

Synthesis of PCU diol diacid 11

A solution of the diene **10**[10] (15.0 g, 58.0 mmol) in dry methanol (100 mL) was purged with nitrogen for six hours while cooling in a dry ice-isopropanol bath (-78 ºC). Ozone was bubbled into the reaction mixture until a blue-purple colour persisted indicating the presence of excess ozone in the system and hence the completion of the reaction. The excess ozone gas was purge with nitrogen and the solvent (MeOH) removed *in vacuo*. Formic acid (100 mL) was added to the ozonide and the mixture was cooled in an ice bath with stirring. Hydrogen peroxide (150 mL, 30%) was then added drop-wise to the stirring cooled reaction mixture. The reaction was left to attain ambient temperature for one hour and refluxed gently for twelve hours, the resulting mixture was concentrated *in vacuo* to yield the diol diacid 11 (15.6 g, 92 % yield) as a white solid. M.p: 153 – 157 ºC, ^{1}H NMR [$(CD_3)_2SO$, 400 MHz]: δ_H 1.03 (*A*B, J_{AB} = 10.4 Hz, 1H), 1.51 (A*B*, J_{AB} = 10.4 Hz, 1H), 2.23 (s, 3H), 2.26 (s, 2H), 2.37 (s, 2H), 2.48 – 2.50 (m, 3H), 2.57 – 2.59 (m, 2H). ^{13}C NMR [$(CD_3)_2SO$, 100 MHz]: δ_C 33.5 (t), 38.8 (d), 42.2 (d), 43.9 (d), 44.1 (t), 49.9 (d), 76.5 (s), 172.4 (s).

Synthesis of PCU diol diamide 1

A mixture of the PCU diol diacid **11** (5.0 g, 17.0 mmol), NH_4Cl (2.4 g, 44.2 mmol), HOBt (5.7 g, 37.4 mmol), EDC (7.1 g, 37.4 mmol) and NMM (4.9 mL, 44.2 mmol) was dissolved in DMF (30 mL) and stirred at room temperature for 36 hours. The reaction mixture was concentrated *in vacuo*. The crude product was dissolved in 1M HCl (50 mL) and extracted with DCM (2 x 50 mL). The combined organic solution was washed successively with 10% $NaHCO_3$ (2 x 50 mL) and brine (2 x 50 mL). The resulting organic solution was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel using $CHCl_3$: MeOH: 25 % NH_4OH (88:10:2, R_f = 0.3, 72 % yield) to obtain a white solid. M.p.; 207 – 209 ºC, IR ν_{max}: 3172, 2961, 1656, 1408, 1287, 728, 634, and 436 cm^{-1}. MS (TOF) calculated for $C_{15}H_{21}N_2O_4$ (M + H$^+$) 293.1496, found 293.1484. The NMR data for **1** are presented in Table 1 and 2.

Synthesis of PCU ether diamide 2

To a solution of the PCU ether diacid **12**[10] (1.5 g, 5.4 mmol) in DCM (10 mL)was added oxalyl chloride in DCM (10 mL) dropwise over ten minutes. Two drops of DMF was added and stirred for two hours under nitrogen. The resulting mixture was concentrated *in vacuo* to obtain **13**.[10] Compound **13** was re-dissolved in dry DCM (20 mL) and added drop wise to a stirring solution of

$NH_3/CHCl_3$ at 0 ºC over 20 minutes. The reaction was stirred for two hours, filtered and the filtrate concentrated *in vacuo*. Purification was carried out on silica gel using $CHCl_3$: MeOH: 25 % NH_4OH (88:10:2, R_f = 0.5) to obtain a yellow slurry (1.1 g, 74 % yield). IR ν_{max}: 3343, 3193, 2961, 1653, 1397, 643 and 587 cm^{-1}. MS (TOF) calculated for $C_{15}H_{19}N_2O_3$ ($M + H^+$) 275.1396, found 275.1397. The NMR data for **2** are listed in Table 1 and 2.

Synthesis of PCU ether derivative 3

A mixture of crude PCU ether diamide **2** (3 g, 10.9 mmol), sodium hypochlorite (45 mL) and 4M sodium hydroxide (45 mL) in a 250 round bottom flask was heated to 80 ºC overnight. The mixture was concentrated *in vacuo* and purified *via* column chromatography on silica gel using $CHCl_3$: MeOH: 25 % NH_4OH (88:10:2, R_f = 0.6) to obtain a compound **3** as a yellow slurry (1.2 g, 50 %). IR ν_{max}: 3355, 2949, 2925, 2857, 1563, 1468, 1381, 1304 and 523 cm^{-1}. MS (TOF) calculated for $C_{13}H_{19}N_2O$ ($M + H^+$) 219.1492, found 219.1487. The NMR data for **3** are presented in Table 1 and 2.

Synthesis of PCU ether derivative 4

To a solution of the PCU ether diamide **2** (7 g, 25.0 mmol) in THF (40 mL) in an external ice cold bath was added slowly ten equivalence of LAH (9.6 g, 25.3 mmol), the resulting mixture was refluxed for 24 hours under nitrogen. The reaction mixture was allowed to cool to room temperature after which aqueous Na_2SO_4 was added drop wise over one hour. The resulting precipitate was filter off and the filtrate concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel using $CHCl_3$: MeOH: 25 % NH_4OH (88:10:2, R_f = 0.5) to obtain compound **4** as a yellow slurry (2.48 g, 39.5 %). IR ν_{max}: 3358, 2949, 2859, 1562, 1467, 1330 and 527 cm^{-1}. MS (TOF) calculated for $C_{15}H_{19}N_2O$ ($M + H^+$) 247.1805, found 247.1827. The NMR data for **4** are listed in Table 1 and 2.

Synthesis of PCU dimethoxyl diamide **5** and **7**

A mixture of the PCU diacid (**11**/**12**) (1 mol), glycine methyl ester (2.6 mol), HOBt (2.2 mol), EDC (2.2 mol), NMM (2.6 mol) in DMF (50 mL) was stirred for 24 hours. The reaction mixture was concentrated *in vacuo*. The crude product was dissolved in 1M HCl (100 mL) and extracted with DCM (3 x 50 mL). The combined organic solution was washed successively with 10 % $NaHCO_3$ (2 x 100 mL) and brine (2 x 100 mL). The resulting organic solution was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel using $CHCl_3$: MeOH: 25 % NH_4OH (88:10:2) to obtain the desired products **5** and **7** respectively.

Data for PCU ether dimethoxyl diamide 5

A yellow oil (2.59 g, R_f = 0.8, yield 68.7 %). IR ν_{max}: 3320, 2954, 1741, 1649, 1534 and 1201 cm^{-1}. MS (TOF) calculated for $C_{21}H_{27}N_2O_7$ ($M + H^+$) 419.1813, found 419.1803. The NMR data for **5** are presented in Table 1 and 2.

Data for PCU diol dimethoxyl diamide 7

A yellow oil (2.3 g, R_f = 0.6, yield 51.5 %). IR ν_{max}: 3266, 2953, 1744, 1639, 1533, 1435 and 1204 cm^{-1}. MS (TOF) calculated for $C_{21}H_{29}N_2O_8$ ($M + H^+$) 437.1918, found 437.1893. The NMR data for **7** are listed in Table 1 and 2.

Synthesis of PCU diamide diacid 6 and 8

PCU diacid dimethoxyl (**5** and **7**) (2.59 g) dissolved in THF (20 mL) was added 1 M LiOH solution (12.4 mL) with stirring overnight. THF was removed *in vacuo* and the aqueous solution was neutralized using 12M HCl to pH 7. The desired product was extracted with ethyl acetate (3 x 100 mL) and the combined organic layer was dried with anhydrous Na_2SO_4 and concentrated *in vacuo*.

Data for PCU ether diamide diacid 6

A yellow white solid (2.32 g). IR ν_{max}: 3284, 2960, 1593, 1397 and 1297 cm^{-1}. MS (TOF) calculated for $C_{19}H_{23}N_2O_7$ ($M + H^+$) 397.1605, found 397.1631. The NMR data for **6** are presented in Table 1 and 2.

Data for PCU diol diamide diacid 8

A yellow white solid (2.0 g). IR ν_{max}: 3249, 2934, 1599, 1390, 1290 and 1101 cm^{-1}. MS (TOF) calculated for $C_{19}H_{25}N_2O_8$ ($M + H^+$) 409.1605, found 409.1589. The NMR data for **8** are listed in Table 1 and 2.

Computational details

DFT gas phase calculations were executed by using GAUSSIAN 09.[41] The density functional hybrid method B3LYP[32-34] was employed in combination with the 6-31+G(d) basis set. In the present case, diffuse functions on heavy atoms only is justified because the negative charge is mainly located on oxygen and nitrogen atoms. Polarization functions remove some limitations of the basis set by expansion of the virtual space. Solvation and catalytic effects were not considered in order to simplify the model. The side chains were manually rotated to find the lowest energy structure for each compound (**1** and **2**). NMR shifts for the optimized structures were calculated at the same level using the GIAO[36-40] method. The molecular orbitals of the optimized structures were calculated with

the “pop=full” command. The Cartesian coordinates of these two low energy structures are available as supplementary material.

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Reference

1. A. P. Marchand, *Chem. Rev.* **1989**, *89*, 1011-1033.
2. G. W. Griffin, A. P. Marchand, *Chem. Rev.* **1989**, *89*, 997-1010.
3. A. P. Marchand, *Advances in Theorectically Interesting Molecules*; JAI Press, Greenwich CT, 1989; Vol. 1. p 357.
4. T. G. Dekker, D. W. Oliver, *S. Afr. J. Chem.* **1979**, *32*, 45-48.
5. W. J. Geldenhuys, S. F. Malan, J. R. Bloomquist, A. P. Marchand, C. J. Van der Schyf, *Med. Res. Rev.* **2005**, *25*, 21-48.
6. Brookes, K. B.; Hickmott, P. W.; Jutle, K. K.; Schreyer, C. A. *S. Afr. J. Chem.* **1992**, *45*, 8-11.
7. F. J. C. Martins, A. M. Viljoen, H. G. Kruger, J. A. Joubert, *Tetrahedron* **1993**, *49*, 9573-9580.
8. F. J. C. Martins, A. M. Viljoen, H. G. Kruger, P. L. Wessels, *Tetrahedron* **1993**, *49*, 6527-6532.
9. F. J. C. Martins, A. M. Viljoen, H. G. Kruger, J. A. Joubert, P. L. Wessels, *Tetrahedron* **1994**, *50*, 10783-10790.
10. T. Govender, H. K. Hariprakasha, H. G. Kruger, A. P. Marchand, *Tetrahedron-Asym.* **2003**, *14*, 1553-1557.
11. G. A. Boyle, T. Govender, H. G. Kruger, G. E. M. Maguire, *Tetrahedron-Asym.* **2004**, *15*, 2661-2666.
12. T. Govender, H. K. Hariprakasha, H. G. Kruger, T. Raasch, *S. Afr. J. Chem.* **2005**, *58*, 37-40.
13. S. Odisitse, G. E. Jackson, T. Govender, H. G. Kruger, A. Singh, *Dalton Trans.* **2007**, 1140-1149.
14. F. J. C. Martins, G. H. Coetzee, L. Fourie, H. J. Venter, A. M. Viljoen, P. L. Wessels, *Magn. Reson. Chem.* **1993**, *31*, 578-584.
15. G. A. Craze, I. Watt, *J. Chem. Soc.-Perkin Trans. 2* **1981**, 175-184.
16. D. H. Cadd, W. J. Feast, A. M. Kenwright, J. M. Say, *Magn. Reson. Chem.* **1993**, *31*, 801-807.
17. H. G. Kruger, P. S. Mdluli, *Struct. Chem.* **2006**, *17*, 121-125.
18. H. G. Kruger, R. Ramdhani, *Magn. Reson. Chem.* **2006**, *44*, 1058-1062.
19. H. G. Kruger, R. Ramdhani, *S. Afr. J. Chem.* **2006**, *59*, 71-U28.
20. G. A. Boyle, T. Govender, H. G. Kruger, G. E. M. Maguire, T. Naicker, *Struct. Chem.* **2008**, *19*, 429-434.
21. G. A. Boyle, H. G. Kruger, G. E. M. Maguire, A. Singh, *Struct. Chem.* **2007**, *18*, 633-639.
22. G. A. Boyle, T. Govender, H. G. Kruger, G. E. M. Maguire, T. Naicker, *Magn. Reson. Chem.* **2008**, *46*, 1089-1095.
23. T. Govender, H. G. Kruger, M. Makatini, O. K. Onajole, *Struct. Chem.* **2008**, *19*, 719-726.
24. O. K. Onajole, T. Govender, M. Makatini, H. G. Kruger, *Magn. Reson. Chem.* **2008**, *46*, 1007-1014.

25. O. K. Onajole, K. Govender, P. Govender, P. Van Helden, H. G. Kruger, G. E. M. Maguire, K. Muthusamy, M. Pillay, I. Wiid, T. Govender, *European J. Med. Chem.* **2009**, *44*, 4297 - 4305.

26. R. C. Cookson, E. Crundwell, R. R. Hill, J. Hudec, *J. Chem. Soc.* **1964**, 3062 - 3067

27. E. Breitmaier, *Structure elucidation by NMR in Organic Chemistry*; Wiley and Sons Ltd: England, 1995.

28. L. Fourie, T. Govender, H. K. Hariprakasha, H. G. Kruger, T. Raasch, *Magn. Reson. Chem.* **2004**, *42*, 617-623.

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

30. E. Kleinpeter, P. R. Seidl, *J. Phys. Org. Chem.***2004**, *17*, 680-685.

31. H. B. Lee, H. Y. Park, B. S. Lee, Y. G. Kim, *Magn. Reson. Chem.* **2000**, *38*, 468-471.

32. D. J. Becke, *J. Chem. Phys.* **1993**, *98*, 5648.

33. W. Lee, R. G. Yang, Parr. *Phys. Rev. B* **1988**, *37*, 785.

34. A. Mielich, H. S. Savin, H. Peus, *Chem. Phys. lett.* **1989**, *157*, 200.

35. K. Bisetty, F. J. Corcho, J. Canto, H. G. Kruger, J. J. Perez, *J. Mol. Struct. Theochem* **2006,** *770*, 221-228.

36. F. London, *J. Phys. Radium* **1937**, *8*, 397 - 409.

37. R. McWeeny, *Phys. Rev.* **1962**, *126*, 1028.

38. R. Ditchfield, *Mol. Phys.* **1974**, *27*, 789 - 807.

39. K. Wolinski, J. F. Hilton, P. Pulay, *J. Am. Chem. Soc.* **1990**, 8251 - 60.

40. J. R. Cheeseman, G. W. Trucks, T. A. Keith, M. J. Frisch, *J. Chem. Phys.* **1996**, 5497 - 509.

41. Gaussian 09, Revision ***A.1***, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

CHAPTER 10
SYNTHESIS AND NMR ELUCIDATION OF NOVEL PENTACYCLO-UNDECANE DIAMINE LIGANDS

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Abstract

The synthesis and NMR elucidation of eight novel pentacyclo-undecane (PCU) diamine compounds are reported. These ligands are potential anti-inflammatory agents to be used against rheumatoid arthritus (RA). One dimensional NMR techniques (^{1}H and ^{13}C spectra) show major overlapping of methine resonances of the "cage" (PCU) thereby making it extremely difficult to assign all NMR signals. This overlapping occurs as a result of the substitutions made at the quaternary carbons (C-8/C-11) of the cage. Two dimensional NMR techniques proved to be a useful tool in overcoming this problem.

Keywords: 2D NMR, Pentacyclo-undecane diamine, Ligands.

Introduction

The chemistry of polycyclic "cage" compounds have been of major interest to organic chemists for decades.[**1-4**] As part of an ongoing programme to utilize NMR spectroscopy for the structure elucidation of PCU derivatives, the NMR assignments of eight PCU diamine derivatives (**1-8**) was attempted (Figure 1).

Many authors over the years have attributed the difficulties encountered in the elucidation of cage based compounds.[**1,5-11**] Geminal/vicinal proton-proton coupling and long-range proton-proton interactions result in broad unresolved resonances. However, the emergence of a range of standard 2D NMR techniques has helped a great deal in overcoming these problems. Our group has successfully used 2D NMR techniques to elucidate numerous PCU derivatives.[**12-21**] Odisitse *et al.*[**22**] recently reported potent anti-inflammatory activity for the PCU diamine **9**; this discovery led

to the design and synthesis of compounds **3**-**8** which are currently being investigated as potential inflammatory agents while compounds **1** and **2** are precursors to **3** and **4**.

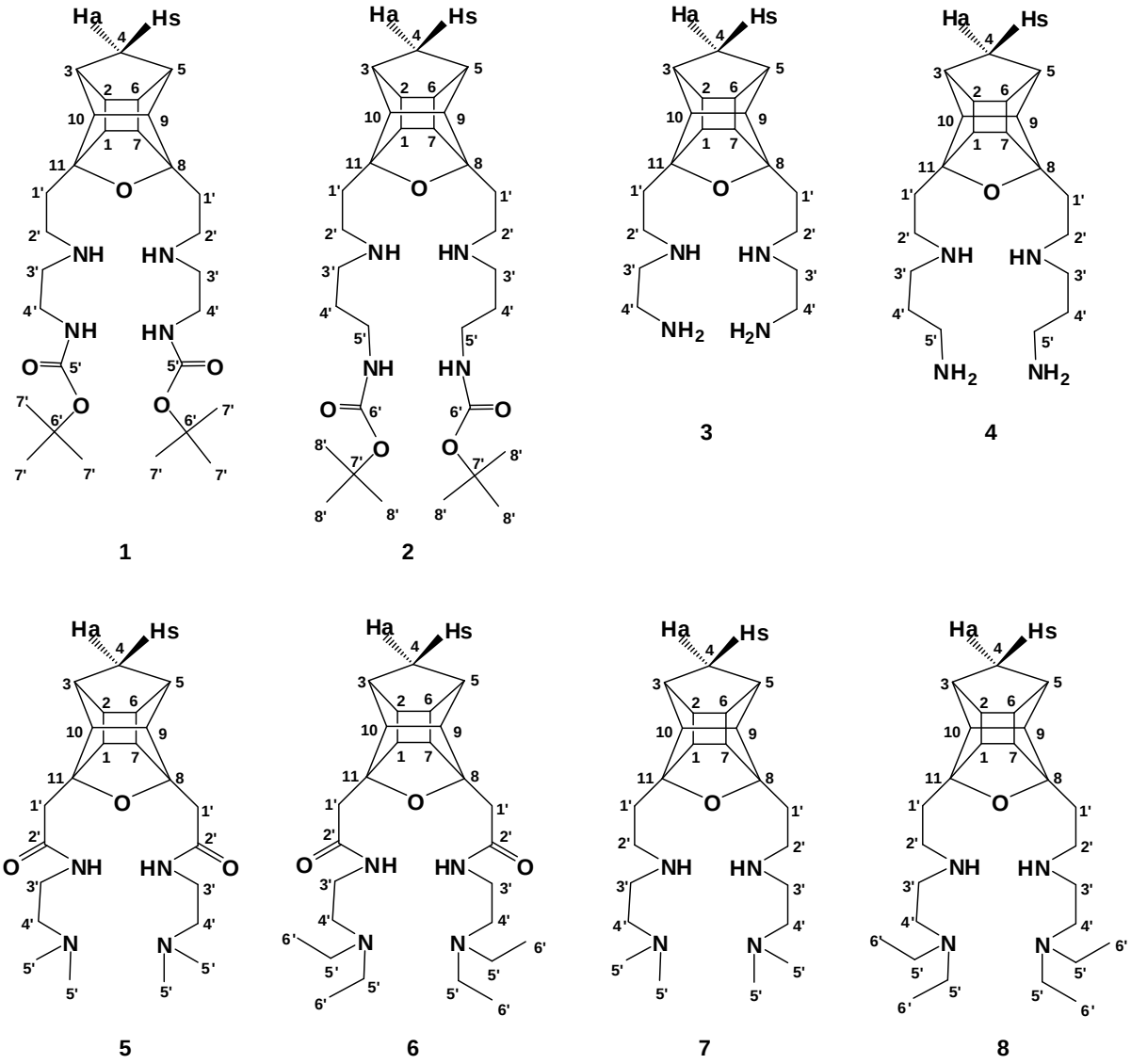
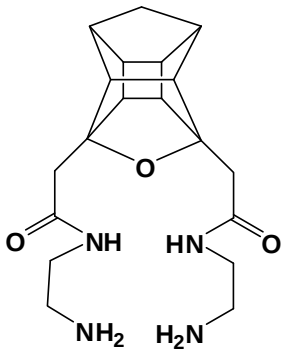


Figure 1: PCU diamine compounds 1-8



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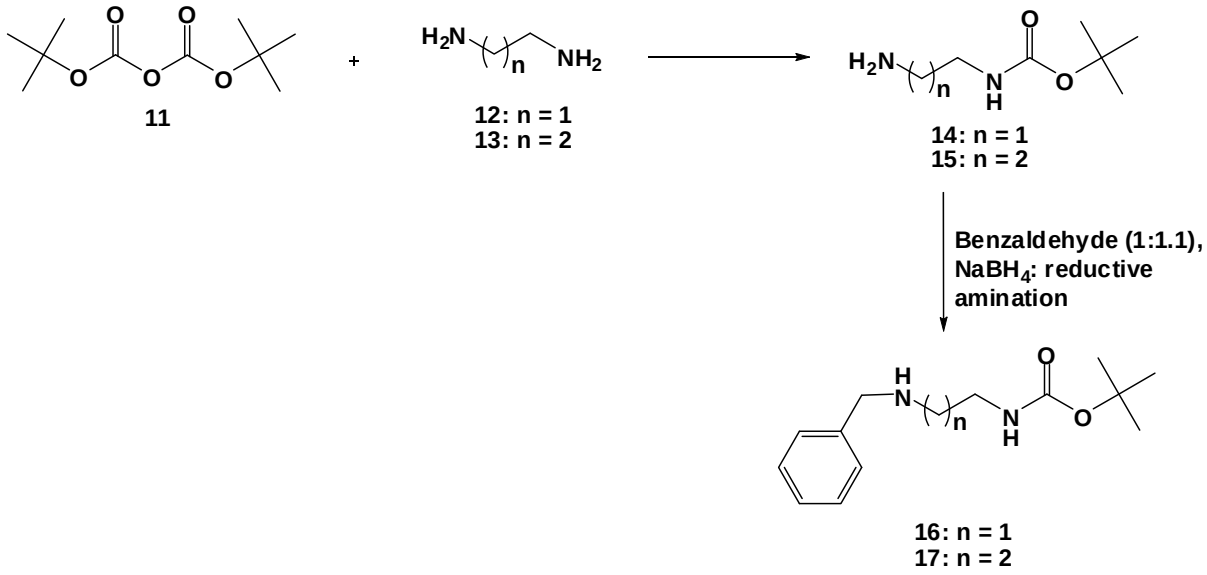
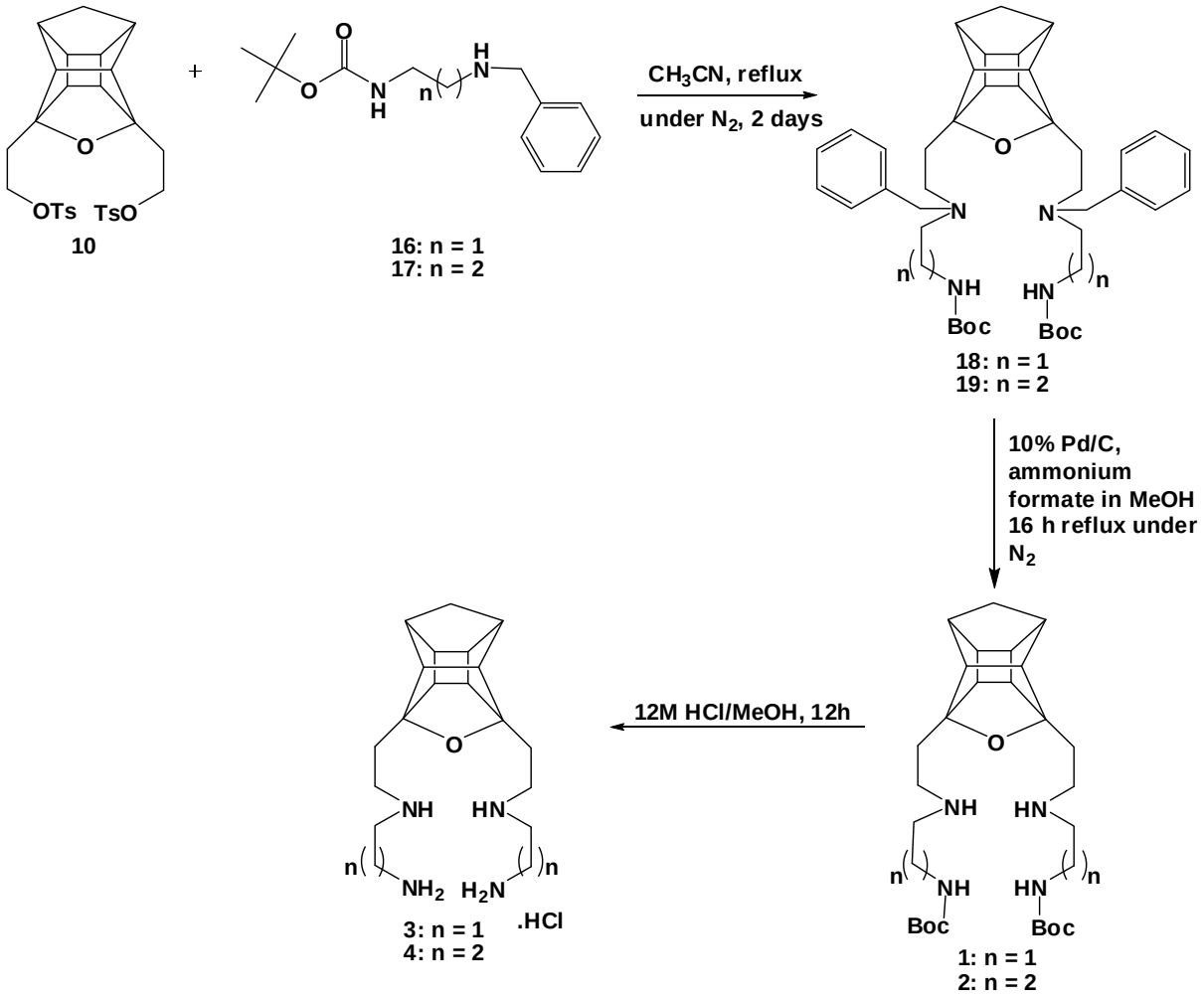
The starting material for the synthesis of PCU diamine derivatives **1-4** is the PCU ditosylate **10**.[**23**] Di-*tert*-butyl dicarbonate **11** is reacted with excess diamine **12**/**13** (1:10 ratio) to yield *N*-*tert*-butoxycarbonyl diamines **14** and **15** in quantitative yields.[**24**] To a solution of *N*-*tert*-butoxycarbonyl diamine in methanol was added 1.1 mole equivalent of benzaldehyde to yield compounds **16** and **17**.[**24**]



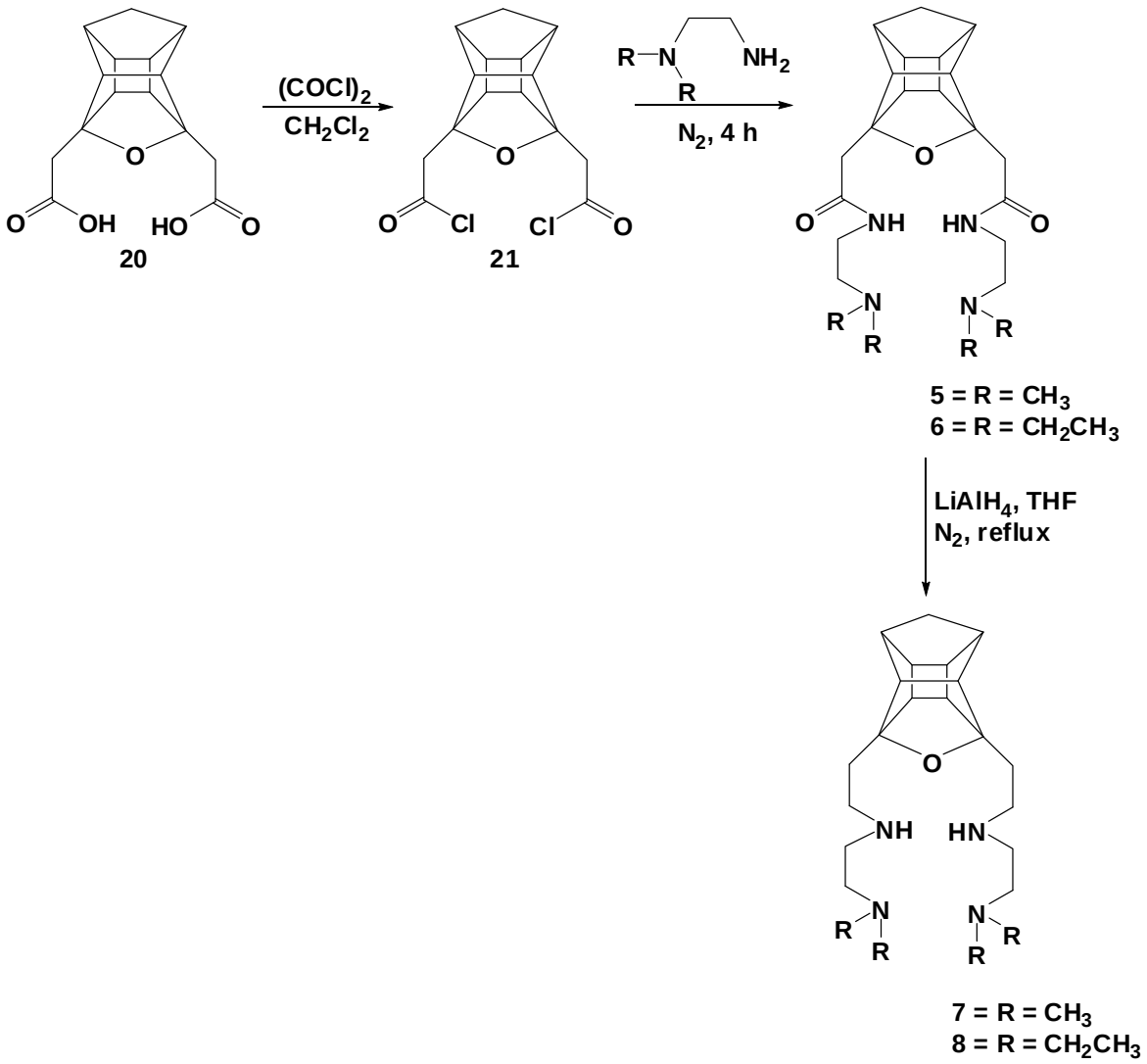
Scheme 1: Synthesis of *N*′-benzyl-*N*″-*tert*-butoxycarbonyl diamine (16 and 17)

A mixture of PCU ditosylate **10** and *N*′-benzyl-*N*″-*tert*-butoxycarbonyl diamine (**16** and **17**) in acetronitrile and triethylamine was refluxed under nitrogen for 36 hours to obtain the novel compounds **18** and **19**. The benzyl groups of compounds **18** and **19** were deprotected using 10% Pd/C and ammonium formate to yield compounds **1** and **2** respectively. Compounds **3** and **4** were obtained after removal of the *Boc* protecting groups.



Schemee 2: Synthesis of novel PCU diamine compounds (1-4)

The starting material for the synthesis of PCU carbonyl diamine derivatives (**5-8**) is the PCU ether diacid **20[25**]. PCU ether diacid **20** was converted to its corresponding acid chloride **21** as described in literature.[**25**] Compound **21** was reacted with *N*-substituted diamines **22** and **23** under a nitrogen atmosphere to obtain compounds **5** and **6** (Scheme **3**). Compounds **5** and **6** were subjected to reduction using lithium aluminium hydride to obtain the corresponding diamine compounds **7** and **8**.



Scheme 3: Synthesis of PCU diamine compounds 5 - 8

Results and discussion

All pentacyclo-undecane diamine compounds (**1-8**) reported are meso compounds thereby simplifying the NMR elucidations, since all groups on the cage, except the methylene group at C-4, exit as pairs. From literature,[**16,17**] it is known that the H-4 protons appear as a pair of doublets (geminal protons) one for each of H-4a and H-4s with AB spin resonances of approximately 1.50 and 1.80 ppm ($J \sim 10$Hz) respectively.

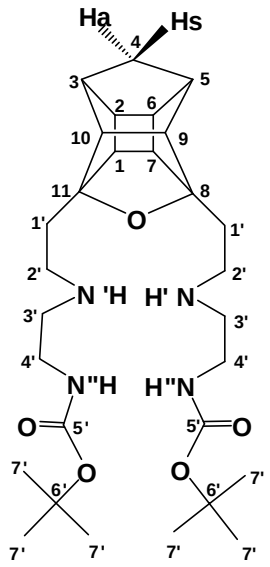
In the ^{1}H NMR spectrum of compound **1** the geminal protons H-4a and H-4s appear at 1.49 and 1.85 ppm respectively with a coupling constant of 10.4 Hz. This pair of doublets shows both COSY and NOESY correlations with resonances registering at 2.34 ppm which was assigned to H-3/H-5. H-3/H-5 exhibits COSY correlations to two signals at 2.46 and 2.57 ppm which should be H-2/H-6 and H-9/H-10. Since only H-2/H-6 should show COSY interaction with one more set of protons (H-1/H-7) the signal at 2.57 ppm was assigned to H-2/H-6; these protons exhibit a COSY correlation with an additional signal at 2.47 ppm, which was assigned to H-1/H-7. On the other hand, H-9/H-10 is only

supposed to show one COSY correlation, namely with H-3/H-5. By way of elimination the signal at 2.46 ppm was assigned to H-9/H-10. H-4a exhibits a NOESY interaction with H-2/H-6 (2.57 ppm) and H-3/H-5 (2.34 ppm) while H-4s shows a NOESY interaction with H-3/H-5 (2.34 ppm) and H-9/H-10 (2.46 ppm) respectively. This reinforced the assignments made before.

The methylene protons, H-1' (1.93 ppm) exhibit a NOESY interaction with H-9/H-10 (2.46 ppm) and a signal at 2.68 ppm (2H) which was assigned to H-2'. The ^{1}H spectrum of compound **1** shows the absence of methylene benzyl protons at 3.55 ppm and its ring protons (7.17 – 7.26 ppm) indicating the successful deprotection of the precursor **18**. A NOESY interaction between the methylene protons of H-1' (1.93 ppm) and that of H-3' (2.68 ppm) was observed, the same unique through space effect was reported for similar cage compounds [**17**]. Distinction between H-2' and H-3' is possible by looking at their COSY correlations. H-1' shows a COSY correlation with H-2' (2.68 ppm) while H-3' exhibits COSY correlations to H-4' (3.18 ppm). The two NH protons register at 2.13 and 5.34 ppm show NOESY interactions with H-3' and H-4' respectively. All corresponding carbons of the assigned protons were identified using the HSQC spectrum. H-4' exhibits a HMBC correlation with the *Boc* carbonyl carbon (C-5') registering at 156.1 ppm and also with C-3' (49.0 ppm). The methyl protons (H-7') exhibit a HMBC interaction with the quaternary carbon, C-6' (79.0 ppm). Also evident on the HMBC spectrum, is the correlation of C-8/11 (95.6 ppm) with H-1' (1.93 ppm) and H-2' (2.68 ppm) while C-1' (32.1 ppm) shows an interaction with H-2' (2.68 ppm) and H-1/H-7 (2.47 ppm) respectively. C-9/C-10 (58.4 ppm) exhibit a HMBC correlation with H-2/H-6, H-1/H-7, H-3/H-5, H-1' and H-4a while H-4' (3.18 ppm) interacts with C-3' (49.0 ppm). Further confirmation of these assignments was performed using the HSQC spectrum. The NMR assignments of compound **1** are presented in Table **1**.

A similar methodology as applied in the elucidation of compound **1** was used to elucidate the cage protons and carbons of the remaining compounds. Details of the subsequent NMR assignments of the PCU skeleton will be omitted in further discussions.

The ^{1}H spectrum of compound **2,** confirms the successful removal of the benzyl protecting group when compared to that of compound **19**. H-4' (1.47 ppm) exhibits NOESY and COSY correlations with H-3' (2.47 ppm) and H-5' (2.99 ppm) while NOESY interactions between H-5' (2.99 ppm) and the H-8' methyl protons (1.25 ppm) were also observed. H-5' shows HMBC correlations with C-4' (29.8 ppm), C-3' (47.4 ppm) and the C-6' carbonyl carbon registering at 156.1 ppm. A HMBC correlation was also observed between the C-7' quaternary carbon (78.6 ppm) and H-8' (1.25 ppm). C-8/11 shows HMBC correlations with H-2' (2.51 ppm) and H-1' (1.77 ppm) while C-9/10 interacts with H-2/6 (2.43 ppm), H-1/H-7 (2.33 ppm), H-3/H-5 (2.20 ppm), H-1' (1.77 ppm), H-4s (1.70 ppm) and H-4a (1.35 ppm) respectively. These assignments are presented in Table **1**.



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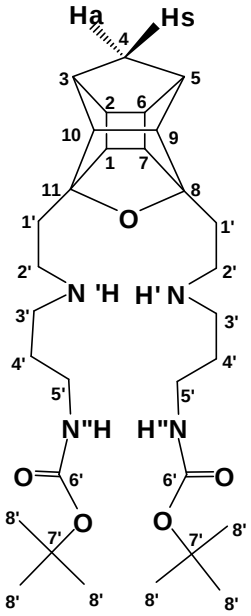

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Table 1: NMR data for PCU derivatives 1 and 2

Compound **1**				Compound **2**			
Atom	$\delta^{1}H^{a,b}$	***J*(Hz)**	$\delta^{13}C^{a,b}$	Atom	$\delta^{1}H^{a,b}$	***J*(Hz)**	$\delta^{13}C^{a,b}$
1/7	2.47		47.8	**1/7**	2.33		47.8
2/6	2.57		41.5	**2/6**	2.43		41.5
3/5	2.34		44.2	**3/5**	2.20		44.1
4a	1.49	10.44	43.5	**4a**	1.35	10.26	43.4
4s	1.85	10.32	43.5	**4s**	1.70	10.26	43.4
8	-		95.6	**8**	-		95.2
9/10	2.46		58.4	**9/10**	2.31		58.5
11	-		95.6	**11**	-		95.2
1'	1.93	6.96	32.1	**1'**	1.77	7.26, 7.20	32.5
2'	2.68		46.0	**2'**	2.51		46.2
3'	2.68		49.0	**3'**	2.47		47.4
4'	3.18		40.1	**4'**	1.47	6.36, 6.42	29.8
5'	-		156.1	**5'**	2.99		38.9
6'	-		79.0	**6'**	-		156.1
7'	1.40		28.4	**7'**	-		78.6
N'H	2.13			**8'**	1.25		28.3
N"H	5.34			**N"H**	5.30		-

[a] Solvent $CDCl_3$

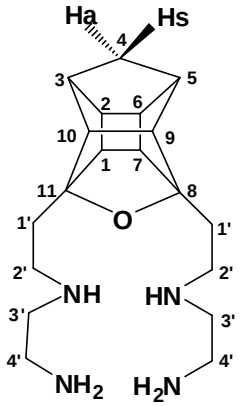
[b] 600MHz for ^{1}H and 150 MHz for ^{13}C

It is clear from Table **1** that the proton and carbon signals for compounds **1** and **2** correlate. This gives some reassurance about the correctness of the assignments.

The PCU diethylene diamine was obtained as its HCl salt and basified to obtain compound **3** which was dissolved in $CDCl_3$. The ^{1}H spectrum confirms the absence of the *Boc* methyl protons positioned at 1.40 ppm in the precursor, compound **1**. It also appears that H-4' in compound **3** registers at a

lower frequency compared to that for compound **1**. COSY interaction between H-1' (1.93 ppm) and H-2' (2.67 ppm) was observed while H-3' shows both COSY and NOESY correlations with H-4' (2.75 ppm). The NOESY spectrum displays an interaction of H-1' (1.93 ppm) with H-9/H-10 (2.45 ppm), H-1/H-7 (2.47 ppm), H-2' (2.67 ppm) and also like the precursor (**1**), with H-3' (2.63 ppm). H-3' shows a HMBC correlation with C-4' (41.6 ppm) and C-2' (46.3 ppm) while C-8/11 correlates with H-2' and H-1'respectively. C-9/10 (58.6 ppm) show HMBC correlations with H-1' and H-4a (1.48 ppm) while C-1/C-7 (47.9 ppm) correlate with H-1' only. Comparison of the ^{13}C data for compound **3** with that of the precursor **1** again display remarkable agreement. All assignments were further confirmed using the HSQC spectrum; these assignments are presented in Table **2**.

A similar rational was carried out for compound **4** but unlike **3**, the NMR spectra of **4** were unresolved suggesting that the compound is unstable, hence the HCl salt of compound **4** was used, requiring D_2O as the NMR solvent. As expected, no NH protons were observed for the ^1H NMR spectrum (as observed in compound **3**) due to deuterium exchange of the NH protons. A methylene proton registering at 2.13 ppm was assigned to H-4'. H-4' shows both NOESY and COSY correlations to two methylene protons registered at 3.14 and 3.20 ppm (H-5' and/or H-3') further confirmation for the assignment of H-4'. H-1' shows a COSY interaction to a signal registering at 3.16 – 3.24 ppm; this was assigned to H-2', H-1' also exhibits a NOESY correlation to H-9/H-10 (2.67 ppm), H-1/H-7 (2.69 ppm) and a overlapping signal at 3.16 – 3.24 ppm; this signal was assigned to H-2 and H-3. Carbon signals were assigned using the HSQC spectrum. C-5' (36.6 ppm) shows a HMBC interaction with H-3' (3.20 ppm) and H-4' (2.13 ppm) while C-3' (44.5 ppm) correlates with H-2' (2.16 - 2.24 ppm) and H-4' (2.13 ppm) respectively. C-8/C-11 show a HMBC correlation with H-2' and H-1'. These assignments are presented in Table **2**.



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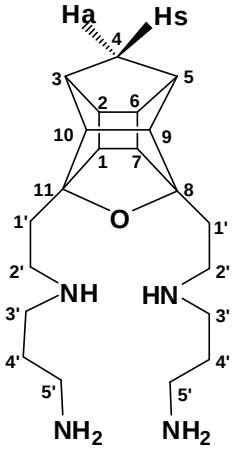

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Table 2: NMR data for PCU derivatives 3 and 4

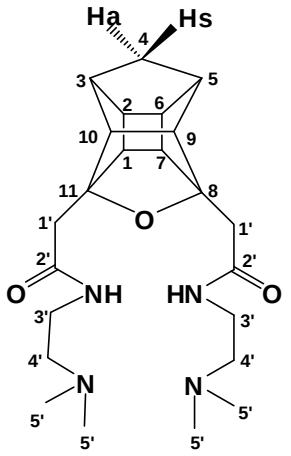
Compound **3**				Compound **4**			
Atom	δ ^{1}H[a,b]	***J*(Hz)**	δ ^{13}C[a,b]	**Atom**	δ ^{1}H[c,d]	***J*(Hz)**	δ ^{13}C[c,d]
1/7	2.47		47.9	1/7	2.69		47.3
2/6	2.57		41.6	2/6	2.73		41.2
3/5	2.34		44.3	3/5	2.52		44.1
4a	1.48	10.44	43.5	4a	1.60	10.56	43.0
4s	1.84	10.32	43.5	4s	1.97	10.56	43.0
8	-		95.4	8	-		94.9
9/10	2.45		58.6	9/10	2.67		57.9
11	-		95.4	11	-		94.9
1'	1.93	7.02, 7.14	32.7	1'	2.34		28.2
2'	2.67		46.3	2'	3.16-3.24		44.5
3'	2.63		52.5	3'	3.20		44.5
4'	2.75		41.6	4'	2.13		23.8
NH/NH_2	1.86		-	5'	3.14		36.6

[a] Solvent $CDCl_3$, [b] 600MHz for ^{1}H and 150 MHz for ^{13}C

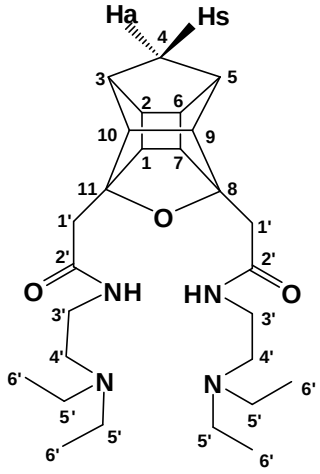
[c] Solvent D_2O, [d] 400MHz for ^{1}H and 100 MHz for ^{13}C

Compound **5** shows a NOESY interaction between H-9/H-10 (2.54 ppm) and H-1' (2.65 ppm) while H-3' (3.25 ppm) interacts with H-4' (2.33 ppm) and H-5' (2.15 ppm) respectively. The ^{1}H NMR spectrum displays an amide proton registering at 7.08 ppm; the amide signal exhibits a NOESY interaction with H-1', H-3', H-4' and H-5' respectively. Each corresponding carbon signal was confirmed using the HSQC spectrum. H-3' exhibits HMBC correlations with C-4' (57.9 ppm) and the carbonyl carbon (C-2') registering at 170 ppm. H-1' also shows HMBC correlations with C-1/7 (48.2 ppm), C-9/10 (58.6 ppm), C-8/11 (93.8 ppm) and C-2' (170.0 ppm) respectively. The assigned signals are presented in Table **3**.

The ^{1}H NMR spectrum of compound **6** displays a similar pattern when compared to that of **5**. Major differences were observed for the region from 2.0 – 2.40 ppm. This is due to the methyl protons H-6' (0.95 ppm) in compound **6** which are further removed from the nitrogen atom. The methylene protons (H-5') are overlapping with the methylene protons of H-4' registering at 2.47 – 2.49 ppm. H-3' and the amide N-H' proton shows NOESY interactions with H-4', H-5' (2.47 – 2.49 ppm) and H-6' (0.95 ppm) respectively. Due to the overlapping of the proton peaks for H-4' and H-5', the HSQC spectrum was as useful as before. The HMBC spectrum was thus vital in assigning the carbon signals of H-4' and H-5'. H-3' exhibits a HMBC correlation to a carbon signal registering at 51.5 ppm; this was assigned to C-4' while H-6' shows correlation to a carbon signal registering at 11.7 ppm; this was assigned to C-5'. The carbonyl carbon (C-2') registering at 169.8 ppm shows a HMBC correlation with H-3' and H-1' respectively. Further assignments and confirmation of all other proton signals were done using the HSQC spectrum. The assignments are presented in Table **3**.



5



6

Table 3: NMR data for PCU derivatives 5 and 6

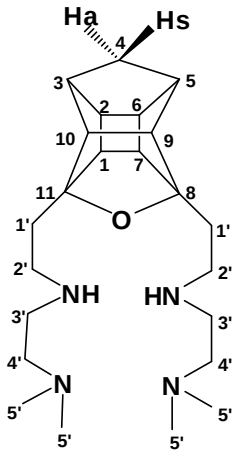
Compound **5**				Compound **6**			
Atom	δ $^{1}H^{a,b}$	***J*(Hz)**	$\delta^{13}C^{a,b}$	**Atom**	$\delta^{1}H^{a,b}$	***J*(Hz)**	$\delta^{13}C^{a,b}$
1/7	2.57		48.3	1/7	2.59		48.2
2/6	2.59		41.5	2/6	2.61		41.4
3/5	2.40		44.1	3/5	2.42		44.1
4a	1.49	10.56	43.4	4a	1.50	10.56	43.4
4s	1.83	10.56	43.4	4s	1.85	10.56	43.4
8	-		93.8	8	-		93.9
9/10	2.54		58.6	9/10	2.56		58.5
11	-		93.8	11	-		93.9
1'	2.65		39.7	1'	2.70		39.7
2'	-		170.0	2'	-		169.8
3'	3.25		36.8	3'	3.26		36.9
4'	2.33	5.64, 6.42	57.9	4'	2.47-2.49		51.5
5'	2.15		45.1	5'	2.47-2.49		46.5
NH	7.08			6'	0.95	7.32, 6.96	11.7
				NH	7.08		

[a] Solvent $CDCl_3$, [b] 600MHz for ^{1}H and 150 MHz for ^{13}C

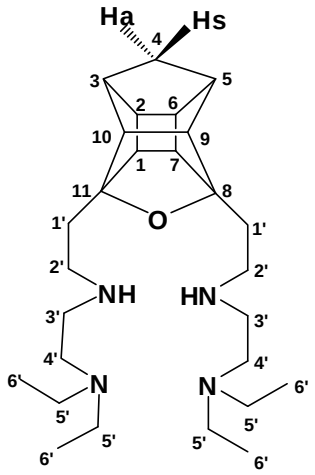
For compound **7**, the absence of a carbonyl signal registering at 170 ppm (^{13}C spectrum) and the presence of a methylene proton at 3.23 ppm (^{1}H NMR spectrum) confirms the successful reduction of compound **5**. H-1' (2.22 ppm) shows NOESY and COSY interactions with H-2' (δ 3.23). Two methylene protons registering at 3.50 – 3.59 ppm were assigned to H-3' and H-4' while the methyl protons registering at δ 2.97 was assigned to H-5'. Each corresponding carbon signal was confirmed

using the HSQC spectrum. The HMBC spectrum was vital in assigning C-3' and C-4'. The HMBC spectrum exhibits a correlation of a carbon signal registering at 52.4 ppm with H-3' and H-5'; this carbon was assigned to C-4' while C-3' was assigned to a signal registering at 41.8 ppm. All assignments were further confirmed using the HSQC spectrum and are presented in Table **4**.

For compound **8**, once again the absence of a carbonyl signal at 169.8 ppm (^{13}C NMR spectrum) and the presence of a methylene proton at 3.22 ppm (^{1}H NMR spectrum) confirmed the successful synthesis of **8** from **6**. H-1' (2.22 ppm) shows a COSY correlation with H-2' (3.22 ppm) and a NOESY interaction with H-1/H-7 (2.65 ppm) and H-9/H-10 (2.63 ppm) respectively. The methyl proton registering at 1.30 ppm was assigned to H-6', H-6' exhibits a COSY and NOESY interaction with a signal at 3.28 ppm which was assigned to H-5'. H-6' also shows a NOESY interaction with a signal registering at 3.52 ppm (4H) which was assigned to H-3' and H-4'. All carbon signals were assigned to their corresponding protons using the HSQC spectrum. C-6' exhibits a HMBC correlation to H-5' while a carbon signal registering at 46.7 ppm shows a HMBC interaction to H-5' while another carbon signal registering at 41.4 ppm shows a correlation with H-2'; the latter was assigned to C-4' and the latter to C-3'. Further assignments were confirmed using the HSQC spectrum and are presented in Table **4**.



7



8

Table 4: NMR data for PCU derivatives 7 and 8

Compound **7**				Compound **8**			
Atom	δ ^{1}H^{a,b}	***J*(Hz)**	δ^{13}C^{a,b}	**Atom**	δ^{1}H^{a,b}	***J*(Hz)**	δ^{13}C^{a,b}
1/7	2.65		47.2	1/7	2.65		47.2
2/6	2.69		41.1	2/6	2.68		41.1
3/5	2.48		44.0	3/5	2.47		44.1
4a	1.55	10.48	43.0	4a	1.55	7.04	43.0
4s	1.93	10.48	43.0	4s	1.93	7.04	43.0
8	-		94.9	8	-		94.8
9/10	2.63		57.9	9/10	2.63		57.9
11	-		94.9	11	-		94.8
1'	2.22	8.24, 7.72	28.1	1'	2.22	8.04, 7.92	28.1
2'	3.23		45.2	2'	3.22		45.1
3'	3.50-3.59		41.8	3'	3.52		41.4
4'	2.50-3.59		52.4	4'	3.52		46.7
5'	2.97		43.4	5'	3.28		48.1
				6'	1.30	7.28	8.2

[a] Solvent D_2O, [b] 400MHz for ^{1}H and 100 MHz for ^{13}C

Conclusion

The full NMR elucidation of eight novel PCU diamine analogues was successfully carried out. Although considerable overlap of proton and carbon signals occurs, 2D NMR techniques again proved to be a useful and convenient tool in the elucidation of these PCU cage ligands. These compounds are currently being tested as potential anti-inflammatory agents.

Supporting information

All NMR spectra mentioned in the text are available as supportive information.

Experimental

The NMR data were recorded on Bruker AVANCE III 400 and 600 MHz instruments; the chemical shifts were referenced to the solvent peak 4.79 ppm for D_2O and 7.24 ppm for $CDCl_3$ at ambient temperature. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 instrument with an Attenuated Total Reflectance attachment. Accurate mass spectra were carried out on a Bruker Micro TOF-QII instrument while melting point analysis was performed on a Stuart Scientific digital melting point apparatus SMP3. Tetrahydrofuran was freshly distilled from a sodium benzophenone ketyl solution under N_2 atmosphere while dichloromethane was dried using phosphorus pentoxide as the drying agent.

General synthesis of *N*-tert-butoxycarbonyl diamine[24] (14 and 15)

Di-*tert*-butyl dicarbonate (50.0 mmol, 1 eq) was dissolved in CH_2Cl_2 (450 mL) and added dropwise to a solution of diamine (0.50 mol, 10 eq) in CH_2Cl_2 (450 mL) over a period of 3 h, whilst the reaction flask was kept in an ice bath. The reaction mixture was stirred overnight at room temperature after which it was washed with water (4 x 250 mL). The CH_2Cl_2 solution was separated then dried over $MgSO_4$, removed *in vacuo* to give the product.

Data for *N*-tert-butoxycarbonylethylene diamine (14)

A colorless oil. [4.8 g, yield 60 %, R_f = 0.30-0.40 (solvent system; CH_3Cl: MeOH: NH_4OH: 88:10:2)]. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.37 (s, 9H), 2.04 (s, 2H, NH_2), 2.68 (t, 2H), 3.07 (q, 2H), 5.22 (s, 1H, NH), ^{13}C NMR [$CDCl_3$, 100 MHz]: δ 28.2 (q), 41.5 (t), 43.0 (t), 79.0 (s), 156.2 (s).

Data for *N*-tert-butoxycarbonylpropane 1, 3- diamine (15)

A colourless oil [Yield 70 %, 5.7 g, R_f = 0.30-0.40 (solvent system; $CHCl_3$: MeOH: NH_4OH: 88:10:2)]. ^{1}H NMR [$CDCl_3$, 600 MHz]: δ_H 1.15 (s, 9H), 1.33 (m, 2H), 2.47 (t, 2H), 2.91 (2H), 5.41 (s, 1H, NH). ^{13}C NMR [$CDCl_3$, 150 MHz]: δ 27.9 (q), 32.9 (t), 37.6 (t), 39.0 (t), 78.1 (s), 155.8 (s).

General synthesis of *N′*-benzyl-*N″*-*tert*-butoxycarbonyl diamine[24] (16 and 17)

A mixture of benzaldehyde (39.2 mmol, 1.1 eq) and *N*-tert-Butoxycarbonyl diamine (35.6 mmol, 1 eq) with 1g of freshly dried 3Å molecular sieve in methanol (60 mL) was stirred at 25 ºC under N_2 atmosphere for 4 hours. The mixture was cooled to 0ºC using an external ice-salt bath after which solid $NaBH_4$ (4 eq.) was added slowly over 30 min and stirred overnight at RT. The reaction mixture was concentrated *in vacuo* and the residue partitioned between ethyl acetate and water (100 mL). The organic solution was extracted with a solution of 0.5 N HCl (3 x 100 mL). The aqueous solution was cooled to 0°C and basified with ammonia solution. The result solution was extracted with

dichloromethane (2 x 200 mL), separated then dried over $MgSO_4$ and concentrated *in vacuo* to afford the product.

Data for *N′*-benzyl-*N″*-*tert*-butoxycarbonylethylene diamine (16)

A colourless oil. [5.0 g, yield 56%, R_f = 0.60 (solvent system; $CHCl_3$: MeOH: NH_4OH: 88:10:2)]. ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 7.21-7.26 (m, 5H), 5.07 (s, 1H, NH), 3.77 (s, 2H), 3.23 (q, 2H), 2.73 (t, 2H), 1.43 (s, 9H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 28.3 (q), 40.1 (t), 48.4 (t), 53.4 (t), 79.1 (s), 127.0 (d), 128.0 (d), 128.4 (d), 140.0 (s), 156.1 (s).

Data for *N′*-benzyl-*N″*-*tert*-butoxycarbonylpropane 1, 3- diamine (17)

A colourless oil. [5.0 g, yield 67 %, R_f = 0.62 (solvent system; $CHCl_3$: MeOH: NH_4OH: 88:10:2)]. ^1H NMR [$CDCl_3$, 600 MHz]: δ_H 1.43 (s, 9H), 1.64 (m, 2H), 2.67 (t, 2H), 3.19 (2H), 3.74 (s, 2H), 5.47 (s, 1H, NH), 7.23-7.34 (m, 5H). ^{13}C NMR [$CDCl_3$, 150 MHz]: δ_C 28.3 (q), 29.5 (t), 39.3 (t), 47.0 (t), 53.8 (t), 78.6 (s), 126.7 (d), 127.9 (d), 128.2 (d), 140.0 (s), 156.0 (s).

General synthesis of *N′*-benzyl-*N″*-*tert*-butoxycarbonyl diamine PCU (18 and 19)

A mixture of *N′*-benzyl-*N″*-*tert*-butoxycarbonyl diamine (9.90 mmol) and PCU ditosylate[**23**] (4.50 mmol) and triethylamine (9.9 mmol) in CH_3CN (30 mL) was refluxed for two days under N_2 atmosphere. The reaction mixture was cooled and concentrated *in vacuo*. The crude residue was dissolved in CH_2Cl_2 (150 mL) and washed successively with water (3 x 50 mL) after which the CH_2Cl_2 solution was dried over Na_2SO_4 and concentrated *in vacuo.* The crude product was purified *via* column chromatography on silica gel using $CHCl_3$: MeOH: NH_4OH (88:10:2).

Data for *N′*-benzyl-*N″*-*tert*-butoxycarbonylethylene diamine PCU (18)

A yellow oil (2.61 g, 82 %, R_f = 0.68). IR ν_{max}: 3136 cm^{-1}, 2964 cm^{-1}, 1702 cm^{-1}, 1364 cm^{-1}, 1163 cm^{-1}, 735 cm^{-1}, 698 cm^{-1}. MS (TOF) calculated for $C_{43}H_{60}N_4O_5$ ($M + H^+$) 713.4590, found 713.4714. ^1H NMR [$CDCl_3$, 400 MHz]; δ_H 1.41 (s, 9H), 1.45 (AB, J_{AB} = 10.28 Hz, 1H), 1.80 (A*B*, J_{AB} = 10.28 Hz, 1H), 1.95 (t, 2H), 2.27 (s, 1H), 2.38-2.40 (m, 2H), 2.47-2.58 (m, 5H), 3.14 (m, 2H), 3.55 (s, 2H), 5.03 (br, NH), 7.17-7.27 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 28.2 (q), 29.4 (t), 37.9 (t), 41.3 (d), 43.2 (t), 44.0 (d), 47.6 (d), 49.5 (t), 52.6 (t), 58.1 (t), 58.4 (d), 78.5 (s), 94.6 (s), 126.7 (d), 128.0 (d), 128.5 (d), 139.0 (s), 155.7 (s).

Data for *N′*-benzyl-*N″*-*tert*-butoxycarbonylpropane 1, 3-diamine PCU (19)

A yellow oil (2.63 g, 79 %, R_f = 0.60). IR ν_{max}: 3345 cm^{-1}, 2962 cm^{-1}, 2863 cm^{-1}, 1694 cm^{-1}, 1508 cm^{-1}, 1165 cm^{-1}, 732 cm^{-1}, 698 cm^{-1}. MS (TOF) calculated for $C_{45}H_{64}N_4O_5$ ($M + H^+$) 741.4949, found 741.4899. ^1H NMR [$CDCl_3$, 600 MHz]; δ_H 1.43 (s, 9H), 1.46 (1H), 1.61 (s, 2H), 1.81 (AB, J_{AB} = 10.14 Hz, 1H), 1.97 (t, 2H), 2.28 (s, 1H), 2.37-2.39 (m, 2H), 2.42-2.55 (m, 5H), 3.13 (2H), 3.53 (s,

2H), 5.53 (br, NH), 7.21-7.30 (m, 5H). ^{13}C NMR [$CDCl_3$, 150 MHz]; δ_C 26.5 (t), 28.5 (q), 29.4 (t), 39.7 (t), 41.6 (d), 43.4 (t), 44.3 (d), 47.8 (d), 49.7 (t), 52.0 (t), 58.7 (t), 58.7 (d), 78.5 (s), 94.9 (s), 126.9 (d), 128.4 (d), 128.9 (d), 139.4 (s), 156.0 (s).

General synthesis of *N*-tert-butoxycarbonyldiamine PCU (1 and 2)

A mixture of (4.9 mmol) *N′*-benzyl-*N″*-*tert*-butoxycarbonyl diamine PCU, ammonium formate (24.5 mmol) and 350 mg of 10% Pd/C in methanol (30 mL) was refluxed under a nitrogen atmosphere for 15 hours. The mixture was cooled to room temperature, filtered and concentrated. The residue obtained was made slightly alkaline with $NaHCO_3$ and extracted with $CHCl_3$ (2 x 100mL) The mixture was dried over anhydrous Na_2SO_4, concentrated *in vacuo* to afford pure *N*-tert-butoxycarbonyl diamine PCU.

Data for *N*-tert-butoxycarbonylethylene diamine PCU (1)

A light yellow oil ($CHCl_3$: MeOH: NH_4OH - 88:10:2, R_f = 0.42; 1.9 g, 73 %). IR ν_{max}: 2965 cm^{-1}, 1692 cm^{-1}, 1513 cm^{-1}, 1248 cm^{-1}, 1163 cm^{-1},753 cm^{-1}. MS (TOF) calculated for $C_{29}H_{48}N_4O_5$ (M + H$^+$) 533.3651, found 533.3654. ^{1}H NMR [$CDCl_3$, 600 MHz]: δ_H 1.40 (br, 9H), 1.49 (AB, J_{AB} = 10.4Hz, 1H), 1.84 (AB, J_{AB} = 10.3Hz, 1H), 1.93 (t, 2H), 2.34 (s, 1H), 2.46-2.48 (m, 2H), 2.57 (s, 1H), 2.62-2.71 (m, 4H), 3.18 (t, 2H), 5.34 (br, NH). ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 28.4 (q), 32.1 (t), 40.1 (t), 41.5 (d), 43.5 (t), 44.2 (d), 46.0 (t), 47.8 (d), 49.0 (t), 58.4 (d), 79.0 (s), 95.6 (s), 156.1 (s).

Data for *N*-tert-butoxycarbonylpropane 1, 3-diamine PCU (2)

A light yellow oil ($CHCl_3$: MeOH: NH_4OH - 88:10:2, R_f = 0.41; 1.7 g, 68 %). IR ν_{max}: 3320 cm^{-1}, 2962 cm^{-1}, 1690 cm^{-1}, 1517 cm^{-1}, 1166 cm^{-1},751 cm^{-1}. MS (TOF) calculated for $C_{31}H_{52}N_4O_5$ (M + H$^+$) 561.3963, found 561.3971. ^{1}H NMR [$CDCl_3$, 600 MHz]: δ_H 1.47 (br, 9H), 1.35 (AB, J_{AB}=10.26 Hz, 1H), 1.47 (t, 2H), 1.70 (AB, J_{AB} = 10.3 Hz, 1H), 1.77 (t, 2H), 2.20 (s, 1H), 2.31-2.33 (m, 2H), 2.43 (s, 1H), 2.46-2.52 (m, 4H), 2.99 (t, 2H), 5.30 (br, NH). ^{13}C NMR [$CDCl_3$, 150 MHz]; δ_C 28.3 (q), 29.8 (t), 32.5 (t), 38.9 (t), 41.5 (d), 43.4 (t), 44.1 (d), 46.2 (t), 47.4 (t), 47.8 (d), 58.5 (d), 78.6 (s), 95.2 (s), 156.1 (s).

General synthesis of PCU diamine.HCl (3 and 4)

***N*-*tert*-butoxycarbonyl diamine PCU (1** or **2**, 3.9 mmol) was dissolved in MeOH (30 mL) and a concentrated HCl solution (12 M, 5 mL) was added. The reaction was stirred for 16 h after which the solvent was evaporated under reduced pressure; the obtained residue was filtered and washed with petroleum ether and diethyl ether to obtain PCU diamine hydrochloric salt.

Data for PCU ethylene diamine.HCl (3)

A white solid (1.12 g). Melting point: 192-196 ºC. IR ν_{max}: 3394 cm^{-1}, 2955 cm^{-1}, 2791 cm^{-1}, 2677 cm^{-1}, 2438 cm^{-1}, 1637 cm^{-1}, 1159 cm^{-1}, 1025 cm^{-1}, 915 cm^{-1}. MS (TOF) calculated for $C_{19}H_{32}N_4O$ (M + H$^+$) 333.2649, found 333.2649. ^{1}H NMR [$CDCl_3$, 600 MHz]: δ_H 1.48 (AB, J_{AB} = 10.3 Hz, 1H), 1.84

(AB, J_{AB} = 10.4 Hz, 1H), 1.93 (t, 2H), 2.34 (s, 1H), 2.46 (s, 1H), 2.47 (s, 1H), 2.57 (s, 1H), 2.63 (m, 2H), 2.68 (m, 2H), 2.75 (t, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 32.6 (t), 41.5 (d), 41.6 (t), 43.4 (t), 44.2 (d), 46.2 (t), 47.8 (d), 52.4 (t), 58.5 (d), 95.4 (s).

Data for PCU propane 1, 3-diamine.HCl (4)

A white solid (1.0 g). Melting point: 276-284ºC. IR ν_{max}: 3389 cm^{-1},2954 cm^{-1}, 2837 cm^{-1}, 2696 cm^{-1}, 1520 cm^{-1}, 1467 cm^{-1}, 1179 cm^{-1}. MS (TOF) calculated for $C_{21}H_{36}N_4O$ (M + H$^+$) 361.2961, found 361.2940. ^{1}H NMR [$CDCl_3$, 400 MHz]: δ_H 1.60 (AB, J_{AB} = 10.48 Hz, 1H), 1.97 (AB, J_{AB} = 10.48 Hz, 1H), 2.13 (m, 2H), 2.34 (m, 2H), 2.52 (s, 1H), 2.67 (s, 1H), 2.69 (s, 1H), 2.73 (s, 1H), 3.14 (t, 2H), 3.12-3.24 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 23.8 (t), 28.2 (t), 36.6 (t), 41.2 (d), 43.0 (t), 44.1 (d),44.5 (t), 44.5 (t), 47.3 (d), 57.9 (d), 94.9 (s).

General synthesis of *N'*-substituted diamine carbonyl PCU (5 and 6)

To a vigorously stirring solution of *N*'-substituted diamine (4 mole equivalence) in CH_2Cl_2 is added dropwise a solution of freshly prepared PCU ether acid chloride **21[25**] in CH_2Cl_2 for 30 minutes under inert atmosphere. The reaction was allowed to stir for 4 hours at room temperature and dried *in vacuo*. The resulting brown oil was dissolved in water and the product extracted with CH_2Cl_2, the organic solution was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified *via* column chromatography on silica gel using $CHCl_3$: MeOH: NH_4OH (88:10:2).

Data for *N', N'* dimethylamino ethylamine carbonyl PCU (5)

A yellow slurry ($CHCl_3$: MeOH: NH_4OH - 88:10:2, R_f = 0.52; 73 %). IR ν_{max}: 3288 cm^{-1}, 2951 cm^{-1}, 2863 cm^{-1}, 2828 cm^{-1}, 2783 cm^{-1}, 1642 cm^{-1}, 1544 cm^{-1}, 1460 cm^{-1}, 1036 cm^{-1}, 847 cm^{-1}. MS (TOF) calculated for $C_{23}H_{36}N_4O_3$ (M + H$^+$) 417.2860, found 417.2848. ^{1}H NMR [$CDCl_3$, 600 MHz]: δ_H 1.49 (AB, J_{AB} = 10.56 Hz, 1H), 1.83 (AB, J_{AB} = 10.56 Hz, 1H), 2.15 (s, 6H), 2.33 (t, 2H), 2.40 (s, 1H), 2.54 (s, 1H), 2.57 (s, 1H), 2.59 (s, 1H), 2.65 (t, 2H), 3.25 (m, 2H),. ^{13}C NMR [$CDCl_3$, 150 MHz]: δ_C 36.8 (t), 39.7 (t), 41.5 (d), 43.4 (t), 44.1 (d), 45.1 (q), 48.3 (d), 57.9 (t), 58.6 (d), 93.8 (s), 170.0 (s).

Data for *N', N'* diethylamino ethylamine carbonyl PCU (6)

A yellow slurry ($CHCl_3$: MeOH: NH_4OH - 88:10:2, R_f = 0.57; 60 %). IR ν_{max}: 3300 cm^{-1}, 2967 cm^{-1}, 2864 cm^{-1}, 2816 cm^{-1}, 1642 cm^{-1}, 1542 cm^{-1}, 1448 cm^{-1}, 1179 cm^{-1}, 1067 cm^{-1}, 730 cm^{-1}. MS (TOF) calculated for $C_{27}H_{44}N_4O_3$ (M + H$^+$) 473.3486, found 473.3468. ^{1}H NMR [$CDCl_3$, 600 MHz]: δ_H 0.95 (t, 6H), 1.50 (AB, J_{AB} = 10.56Hz, 1 H), 1.85 (AB, J_{AB} = 10.56Hz, 1H), 2.42 (s, 1H), 2.47 – 2.49 (m, 6H), 2.56 (s, 1H), 2.59 (s, 1H), 2.61 (s, 1H), 2.70 (t, 2H), 3.26 (m, 2H),. ^{13}C NMR [$CDCl_3$, 150 MHz]: δ_C 11.7 (q), 36.9 (t), 39.7 (t), 41.4 (d), 43.4 (t), 44.1 (d), 46.5 (q), 48.2 (d), 51.5 (t), 58.5 (d), 93.9 (s), 169.8 (s).

General synthesis of *N'*-substituted diamine PCU (7 and 8)

To a stirring solution of *N'*-substituted diamine carbonyl PCU (**5** or **6**, 1 mole) in dry THF as added $LiAlH_4$ (5 mol) and refluxed under N_2, reaction was monitored *via* TLC. After completion, excess

$LiAlH_4$ was quenched by drop-wise addition of aqueous Na_2SO_4, the resulting precipitate was filtered and the filtrate concentrated *in vacuo*. The crude product was purified *via* column chromatography on alumina using $CHCl_3$:MeOH (90:10). The resulting pure product was converted to its corresponding hydrochloric salt.

Data for PCU *N'*, *N'* dimethylamino ethylamine HCl (7)

A grey solid ($CHCl_3$: MeOH - 90:10, R_f = 0.56; 24.7 %), melting point: 269-272ºC. IR ν_{max}: 3312 cm^{-1}, 2959 cm^{-1}, 2743 cm^{-1}, 2433 cm^{-1}, 1586 cm^{-1}, 1472 cm^{-1}, 1294 cm^{-1}, 1134 cm^{-1}, 994 cm^{-1}, 529 cm^{-1}. MS (TOF) calculated for $C_{23}H_{40}N_4O$ ($M + H^+$) 389.3275, found 389.3269. ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.55 (AB, J_{AB} = 10.48 Hz, 1H), 1.93 (AB, J_{AB} = 10.48 Hz, 1H), 2.22 (t, 2H), 2.48 (s, 1H), 2.63 (s, 1H), 2.65 (s, 1H), 2.69 (s, 1H), 2.97 (s, 6H), 3.23 (m, 2H), 3.50 – 3.59 (m, 4H),. ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 28.1 (t), 41.1 (d), 41.8 (t), 43.0 (t), 43.4 (q), 44.0 (d), 45.2 (t), 47.2 (d), 52.4 (t), 57.9 (d), 94.9 (s).

Data for PCU *N'*, *N'* diethylamino ethylamine HCl (8)

A viscous brown oil ($CHCl_3$:MeOH - 90:10, R_f = 0.59; 25.8 %). IR ν_{max}: 3488 cm^{-1}, 3417 cm^{-1}, 2976 cm^{-1}, 2585 cm^{-1}, 2426 cm^{-1}, 1461 cm^{-1}, 1388 cm^{-1}, 1388 cm^{-1}, 1009 cm^{-1}, 531 cm^{-1}. MS (TOF) calculated for $C_{27}H_{48}N_4O$ ($M + H^+$) 445.3900, found 445.3907. ^1H NMR [$CDCl_3$, 400 MHz]: δ_H 1.30 (t, 6H), 1.55 (AB, J_{AB} = 7.04 Hz, 1H), 1.93 (AB, J_{AB} = 7.04 Hz, 1H), 2.22 (t, 2H), 2.47 (s, 1H), 2.63 (s, 1H), 2.65 (s, 1H), 2.68 (s, 1H), 3.22 (m, 2H), 3.28 (q, 4H), 3.52 (s, 4H). ^{13}C NMR [$CDCl_3$, 100 MHz]: δ_C 8.2 (q), 28.1 (t), 41.4 (t), 43.0 (t), 44.1 (d), 45.1 (t), 46.7 (t), 47.2 (d), 48.1 (t), 57.9 (d), 94.8 (s).

Acknowledgements

This work was supported by grants from the National Research Foundation, Gun 2073251, Aspen Pharmacare and the University of KwaZulu-Natal.

References

1. Marchand, A. P. Chemical Reviews **1989**, 89, 1011-1033.
2. Marchand, A. P. Advances in Theorectically Interesting Molecules; JAI Press, Greenwich CT, 1989; Vol. 1.p 357.
3. Griffin, G. W.; Marchand, A. P. Chem. Rev. **1989**, 89, 997-1010.
4. Geldenhuys, W. J.; Malan, S. F.; Bloomquist, J. R.; Marchand, A. P.; Van der Schyf, C. J. Med. Res. Rev. **2005**, 25, 21-48.
5. Martins, F. J. C.; Coetzee, G. H.; Fourie, L.; Venter, H. J.; Viljoen, A. M.; Wessels, P. L. Magn. Reson. Chem. **1993**, 31, 578-584.
6. Martins, F. J. C.; Viljoen, A. M.; Kruger, H. G.; Joubert, J. A. Tetrahedron **1993,** 49, 9573-9580.
7. Martins, F. J. C.; Viljoen, A. M.; Kruger, H. G.; Joubert, J. A.; Wessels, P. L. Tetrahedron **1994**, 50, 10783-10790.
8. Martins, F. J. C.; Viljoen, A. M.; Kruger, H. G.; Wessels, P. L. Tetrahedron **1993**, 49, 6527-6532.
9. Martins, F. J. C.; Viljoen, A. M.; Kruger, H. G.; Wessels, P. L. Magn. Reson. Chem. **2004**, 42, 402-408.
10. Cadd, D. H.; Feast, W. J.; Kenwright, A. M.; Say, J. M. Magn. Reson. Chem. **1993**, 31, 801-807.
11. Craze, G. A.; Watt, I. J. Chem. Soc. Perkin Trans. 2 **1981**, 175-184.
12. Fourie, L.; Govender, T.; Hariprakasha, H. K.; Kruger, H. G.; Raasch, T. Magn. Reson. Chem. **2004**, 42, 617-623.
13. Govender, T.; Hariprakasha, H. K.; Kruger, H. G.; Raasch, T. S. A. J. Chem. **2005**, 58, 37-40.
14. Kruger, H. G.; Mdluli, P. S. Struct. Chem. **2006**, 17, 121-125.
15. Kruger, H. G.; Ramdhani, R. Magn. Reson. Chem. **2006**, 44, 1058-1062.
16. Kruger, H. G.; Ramdhani, R. S. A. J. Chem. **2006**, 59, 71-U28.
17. Boyle, G. A.; Kruger, H. G.; Maguire, G. E. M.; Singh, A. Struct. Chem. **2007**, 18, 633-639.
18. Boyle, G. A.; Govender, T.; Kruger, H. G.; Maguire, G. E. M.; Naicker, T. Struct. Chem. **2008**, 19, 429-434.
19. Boyle, G. A.; Govender, T.; Kruger, H. G.; Maguire, G. E. M.; Naicker, T. Magn. Reson. Chem. **2008**, 46, 1089-1095.
20. Govender, T.; Kruger, H. G.; Makatini, M.; Onajole, O. K. Struct. Chem. **2008**, 19, 719-726.
21. Onajole, O. K.; Govender, T.; Makatini, M.; Kruger, H. G. Magn. Reson. Chem. **2008**, 46, 1007-1014.
22. Odisitse, S.; Jackson, G. E.; Govender, T.; Kruger, H. G.; Singh, A. Dalton Trans. **2007**, 1140-1149.
23. Boyle, G. A.; Govender, T.; Kruger, H. G.; Maguire, G. E. M. Tetrahedron-Asym. **2004**, 15, 2661-2666.
24. Millet, R.; Urig, S.; Jacob, J.; Amtmann, E.; Moulinoux, J. P.; Gromer, S.; Becker, K.; Davioud-Charvet, E. J. Med. Chem. **2005**, 48, 7024-7039.

25. Govender, T.; Hariprakasha, H. K.; Kruger, H. G.; Marchand, A. P. Tetrahedron-Asym. **2003**, 14, 1553-1557.

CHAPTER 11
SUMMARY AND CONCLUSIONS

SUMMARY

The chemistry of polycyclic 'cage' compounds remains an interesting and attractive field to many organic and medicinal chemists. The unique chemical and biochemical characteristics of these polycyclic moieties, since the discovery of adamantine, have led to the exploration of their medicinal and pharmaceutical properties.

This project focused mainly on the design, synthesis and screening of polycyclic 'cage' analogues as potential anti-tuberculosis and anti-microbial (anti-fungal and bacterial) agents. A range of polycyclic 'cage' moieties such as adamantane, pentacycloundecane, pentacyclodecane, trishomocubane, oxa-pentacycloundecane and aza-pentacycloundecane were utilised in this study. In this project a total of 12 novel intermediates and 31 novel products were synthesised. A thorough NMR elucidation of the various structures was also pursued.

Compounds **GKM8**, **GKM9** and **GKM11** (Figure 1) showed similar anti-TB activities as **SQ109**. These three PCU cyclic tetra-amine derivatives were screened against selected clinical pathogenic strains of fungi (yeasts and moulds) and ATCC strains of bacteria (Gram-positive and Gram-negative strains). **GKM11** (a 10 carbon linear alkane chain derivative) however showed promising anti-microbial activities against most of the strains used while **GKM8** (10 carbon branched alkene chain) and **GKM9** (15 carbon branched alkene chain) showed reasonable activity against similar strains of microorganisms.

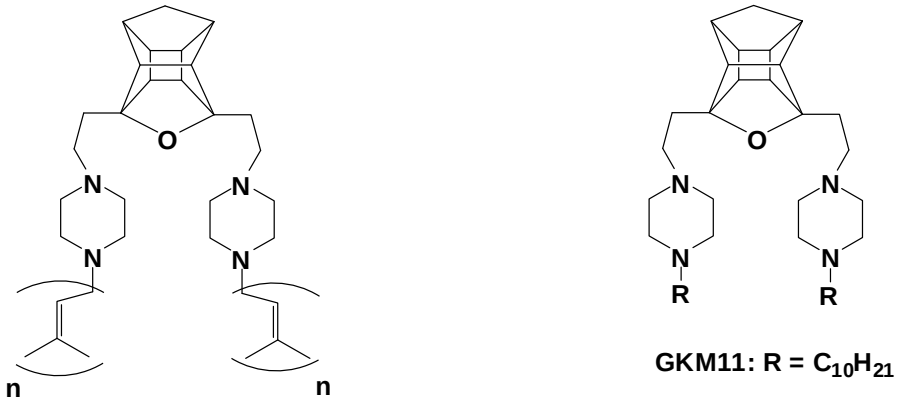


Figure 1: Free salts of PCU tetra-amine derivatives: GKM8, GKM9 and GKM11

This discovery led to the synthesis of several **SQ109** analogues where the type of chain and the positioning of the chain on the adamantyl moiety were varied. Compound **3** (a 15 carbon branched

alkene chain) proved to be most active against TB with a twofold increase activity over **SQ109** (a 10 carbon alkene chain). This finding supports an earlier report where it was shown that **GKM9** was twice as active than **GKM8** against tuberculosis. However, the reduction of the alkene chain to branched alkane chains resulted in a loss of anti-TB activity with the exception of compound **2** where anti-TB activity was gained over its alkene version **1**. This anomaly is believed to be due the positioning of the hydrocarbon chain diamine on the adamantyl moiety. Synthesis of the 1-adamantyl version of compounds **3** and **5** would proof this conclusion. Attempts to achieved that failed due inability to synthesis the *trans-trans* farnesyl amine required.

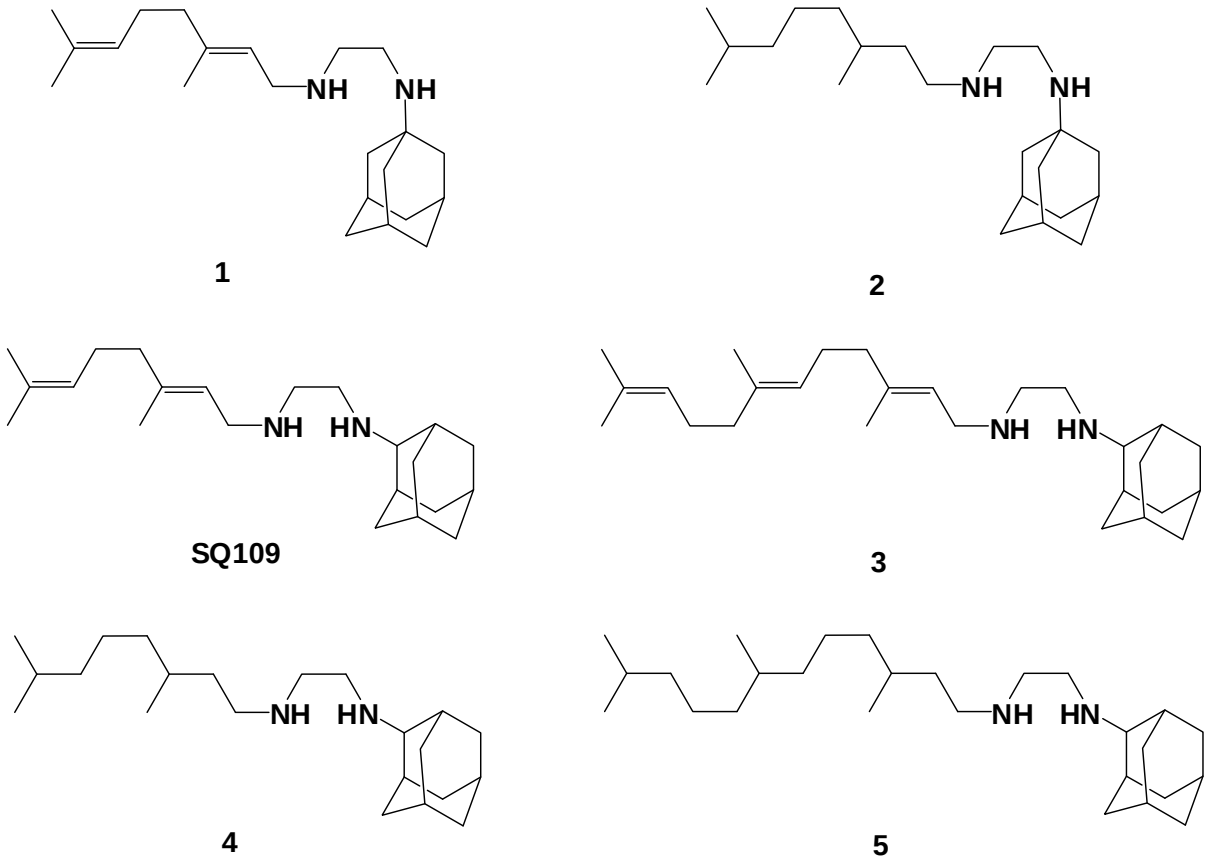
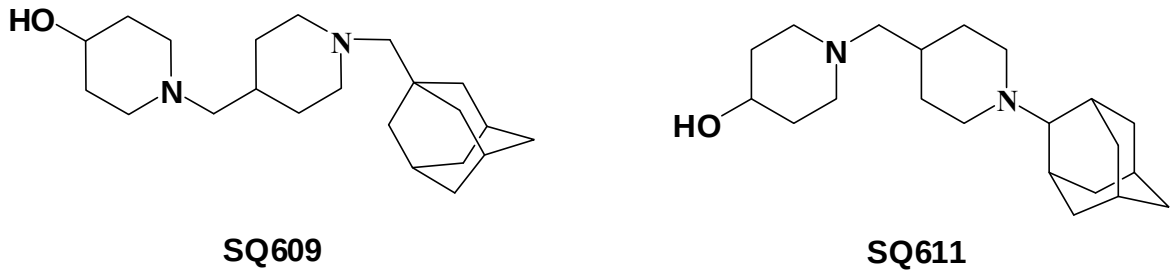


Figure 2: Structure of SQ109 and its analogues

The next step was to investigate the anti-microbial activity of these compounds (Figure 2). This result showed that the branched alkane derivatives **2**, **4** and **5** possessed promising anti-fungal and anti-bacterial (mainly against Gram-positive strains). The branched alkene derivatives (**SQ109** and **1**) did not show any promising anti-microbial activities with the exception of **3** which displayed significant anti-microbial activity against most of the strains used. It should be noted that compound **GKM11** (linear alkane chain derivative) possess higher antimicrobial activities than **GKM8** and **GKM9** (alkene chain derivatives); compounds **2**, **4** and **5** (branched alkane chain derivatives) also possess

higher antimicrobial activities than its branched alkene chain counterparts **1**, **3** and **SQ109**. This observation shows that antimicrobial activity is enhanced with the alkane chain.

In a related study, Bogatcheva *et al.*[1] of Sequella Inc reported the discovery of dipiperidines as new antitubercular agents. Using combinatory chemistry a library of 10,358 compounds were synthesized and screened for activity against *Mycobacterium tuberculosis*. This led to the discovery of **SQ609** with a MIC of 6.25 μM while its counterpart **SQ611** posses a MIC of 31.25 μM.[1] These two compounds have in common the adamantyl and dipiperidine moiety however the dipiperidine moiety is on position 1 of the adamantane for **SQ609** and on position 2 of the adamantane for **SQ611** thus supporting the hypothesis that different positioning of the R-group could influence the activities of such compounds (Figure 3).



SQ609 **SQ611**

Figure 3: Structure of SQ609 and SQ611

Figure 4 shows monosubstituted polycyclic 'cage' compounds with the isoprenyl diamine moiety maintained while varying the polycyclic 'cage' group (such as trishomocubane, pentacycloundecane, aza-pentacycloundecane and oxa-pentacycloundecane). In this series, trishomocubanyl diamine derivative **6** exhibited a twofold activity over that of compounds **7**, **8**, **9**, **10**, **11**, **12** and **SQ109** against MDR and XDR strains of tuberculosis.

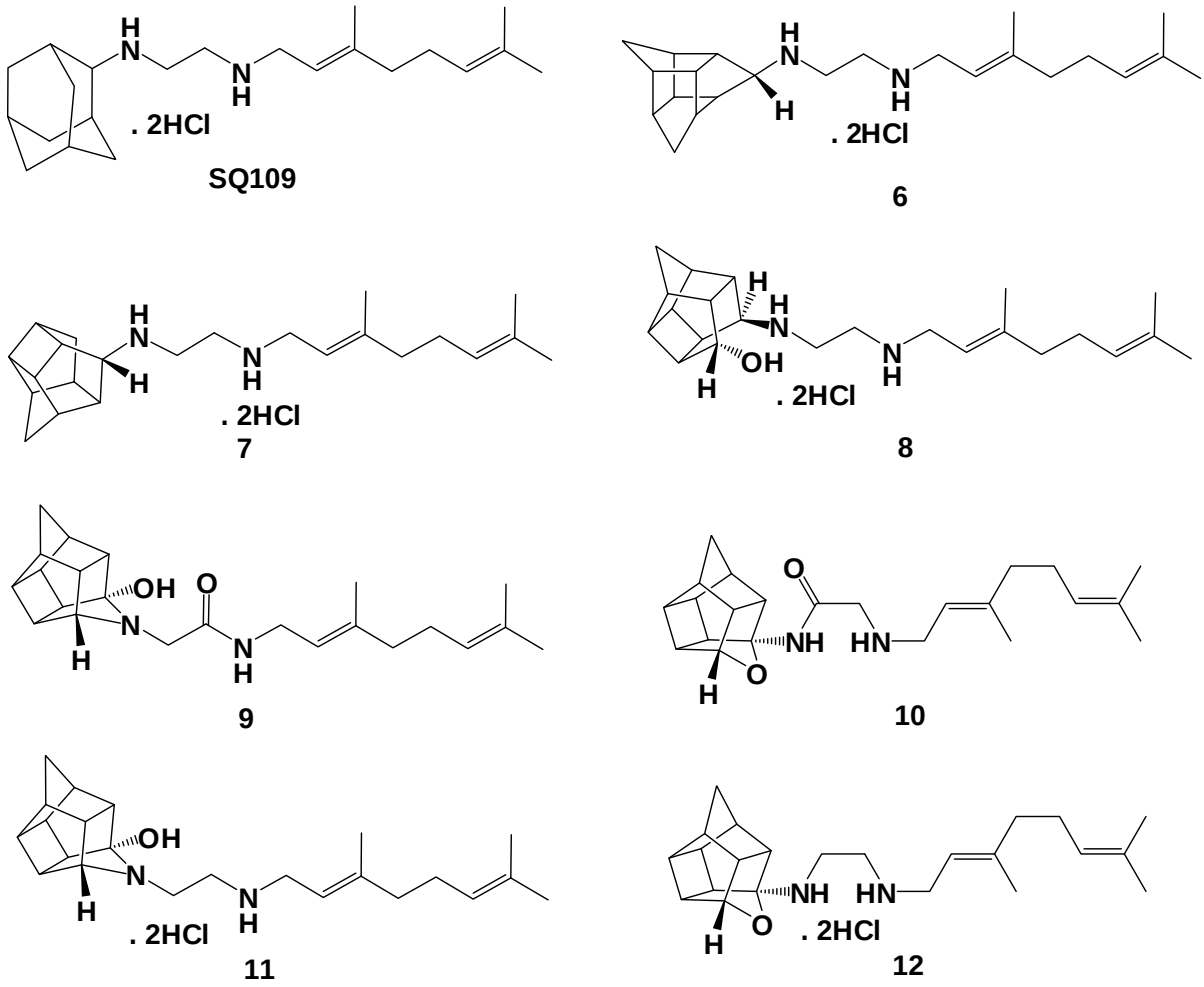


Figure 4: Structure of novel polycyclic 'cage' diamine derivatives

It was postulated that disubstitution on the polycyclic 'cage' moieties compared to the mono-substituted **SQ109** (adamantyl moiety), might improve the activity as an anti-TB agent. This was not observed as the disubstituted polycyclic 'cage' derivatives **14** and **16** showed similar activity than **SQ109** while **13**, **15** and **17** showed reduced activities (Figure 5). This study produced the first report of the pentacyclodecane 'cage' moiety **17** for medicinal use.

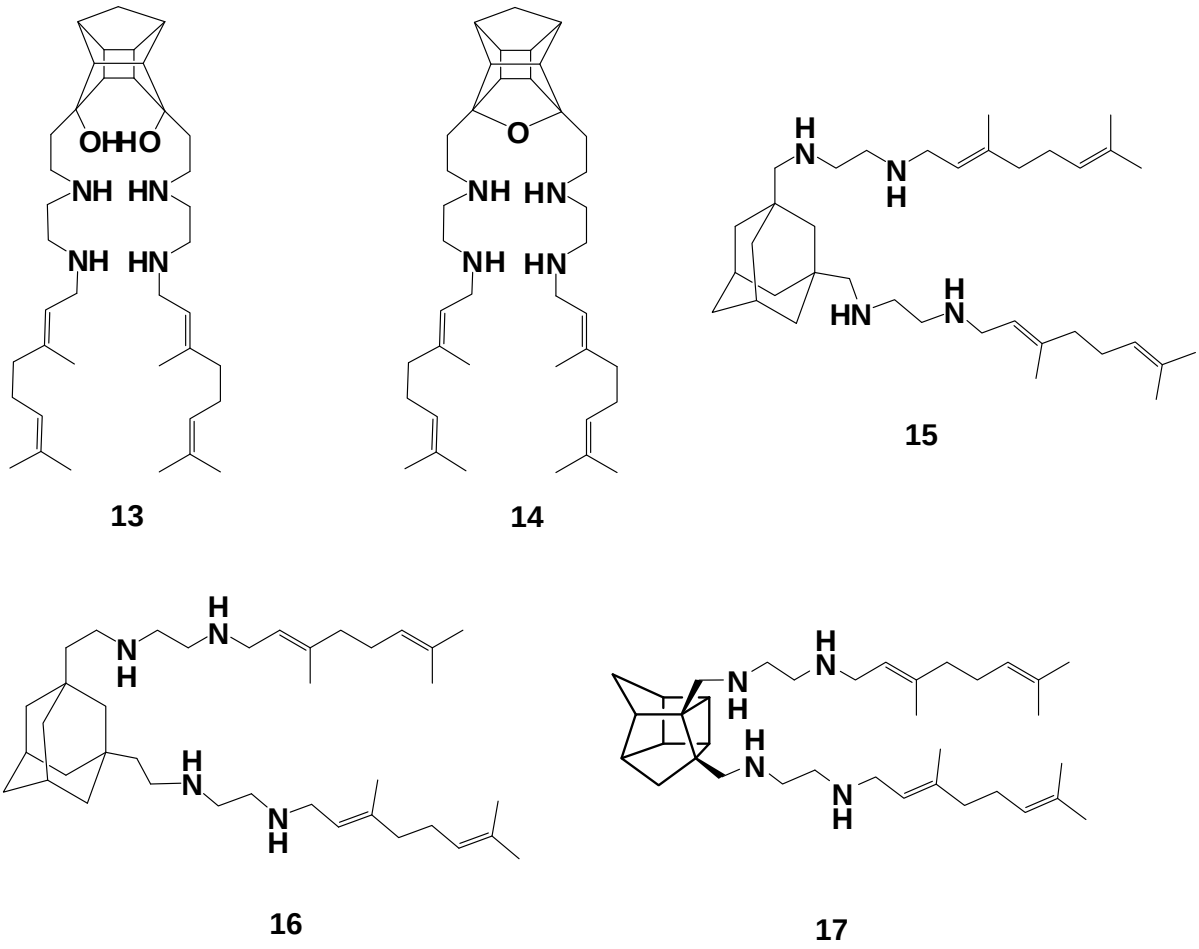


Figure 5: Structure of disubstituted polycyclic 'cage' diamine derivatives

An interesting observation in the structural elucidation of compounds **18** and **19** using NMR techniques showed and un-usual deshielding of H-1/7, H-9/10, H-1' and C-8/11 in compound **18** than the corresponding signals in compound **19** (Figure 6). This is due to the ability of the side arms to freely rotate around C-8/11 and C-1' at room temperature, spending on average more time in close proximity to H-1/7 and H-9/10 while factors such as intramolecular hydrogen bonding and thorough space deshielding effects contributed to the C-8/11 of compound **19** to appear at a higher frequency compared to **18**. Computational chemistry was also instrumental in explaining the large chemical shift to a higher frequency observed for the C-4 of cage diol **18** and cage ether **19**. A similar observation was noted for compounds **22**/**23** and **24**/**25**.

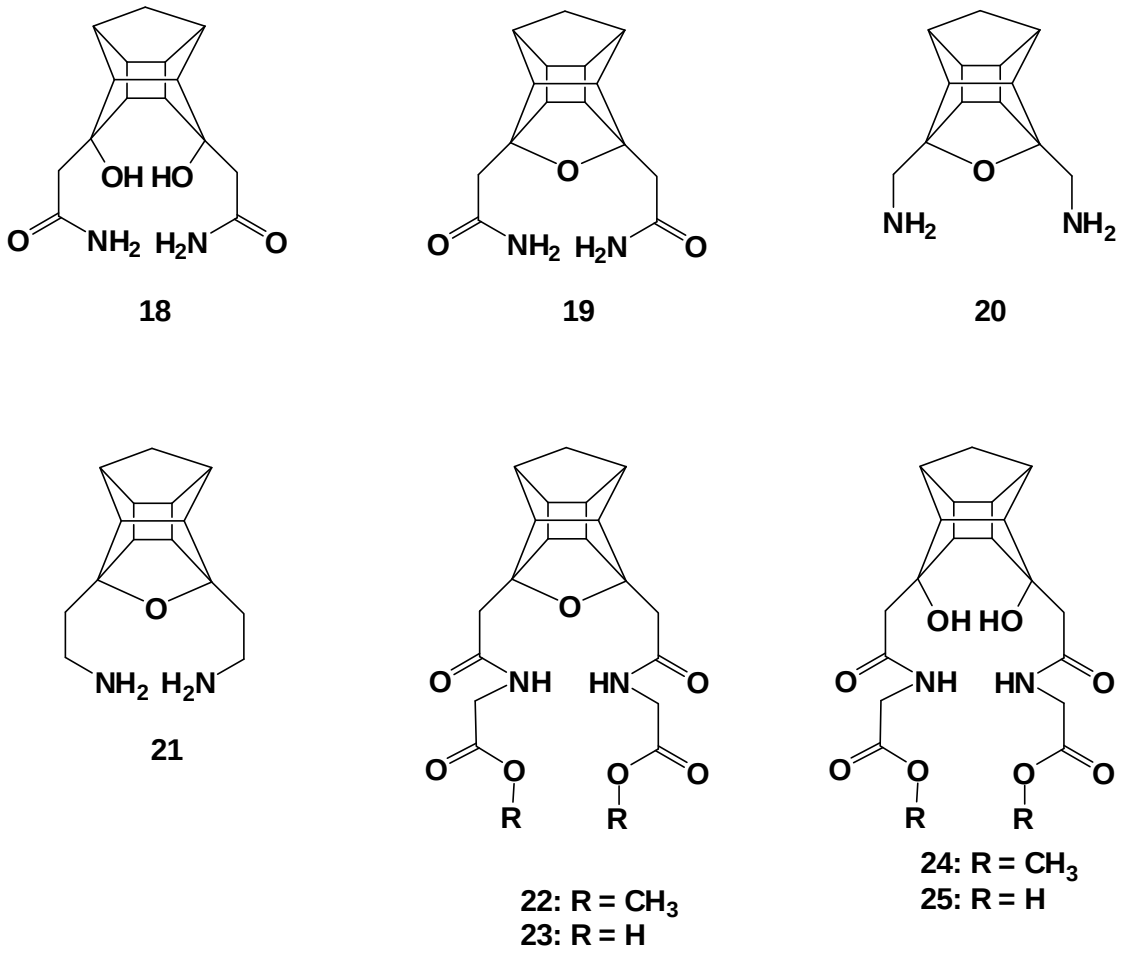


Figure 6: Structure of PCU diamine precursors

A series of PCU based diamine ligands were also synthesized and elucidated. The full structural elucidation of these compounds adds to an impressive library utilising 2D NMR experiments in overcoming severe overlapping proton and carbon NMR signals. A family of the PCU-diamine compounds (**28-33**, Figure 7) are currently being investigated by collaborators at UCT and CPUT for activity against arthritus.

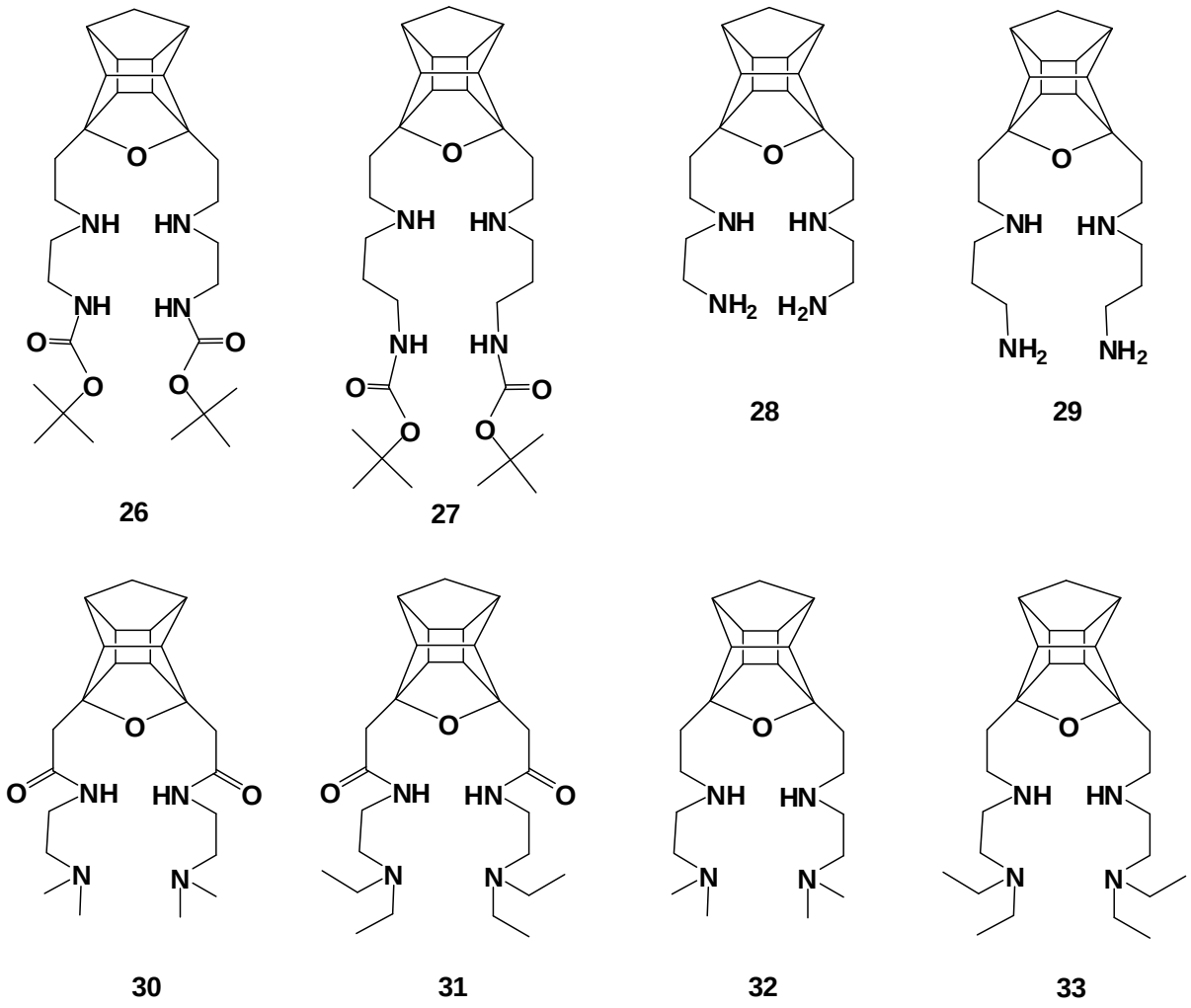


Figure 7: Structure of PCU diamines (26-33)

CONCLUSION

This study has led to the identification of a series of novel polycyclic based diamines with potent activities against tuberculosis, bacteria and fungi. A total of 22 novel polycyclic amine derivatives were screened against tuberculosis while nine novel PCU diamine ligands are being investigated for complexation and transport of copper ions for potential activity against arthritus.

Reference

1. Bogatcheva, E.; Hanrahan, C.; Chen, P.; Gearhart, J.; Sacksteder, K.; Einck, L.; Nacy, C.; Protopopova, M. *Bioorganic & Medicinal Chemistry Letters*, *20*, 201-205.

SUPPORTING INFORMATION

Supporting information includes 1D NMR, 2D NMR, IR and HR-MS experiments.

CHAPTER 3

PENTACYCLOUNDECANE DERIVED CYCLIC TETRA-AMINES: SYNTHESIS AND EVALUATION AS POTENT ANTI-TUBERCULOSIS AGENTS

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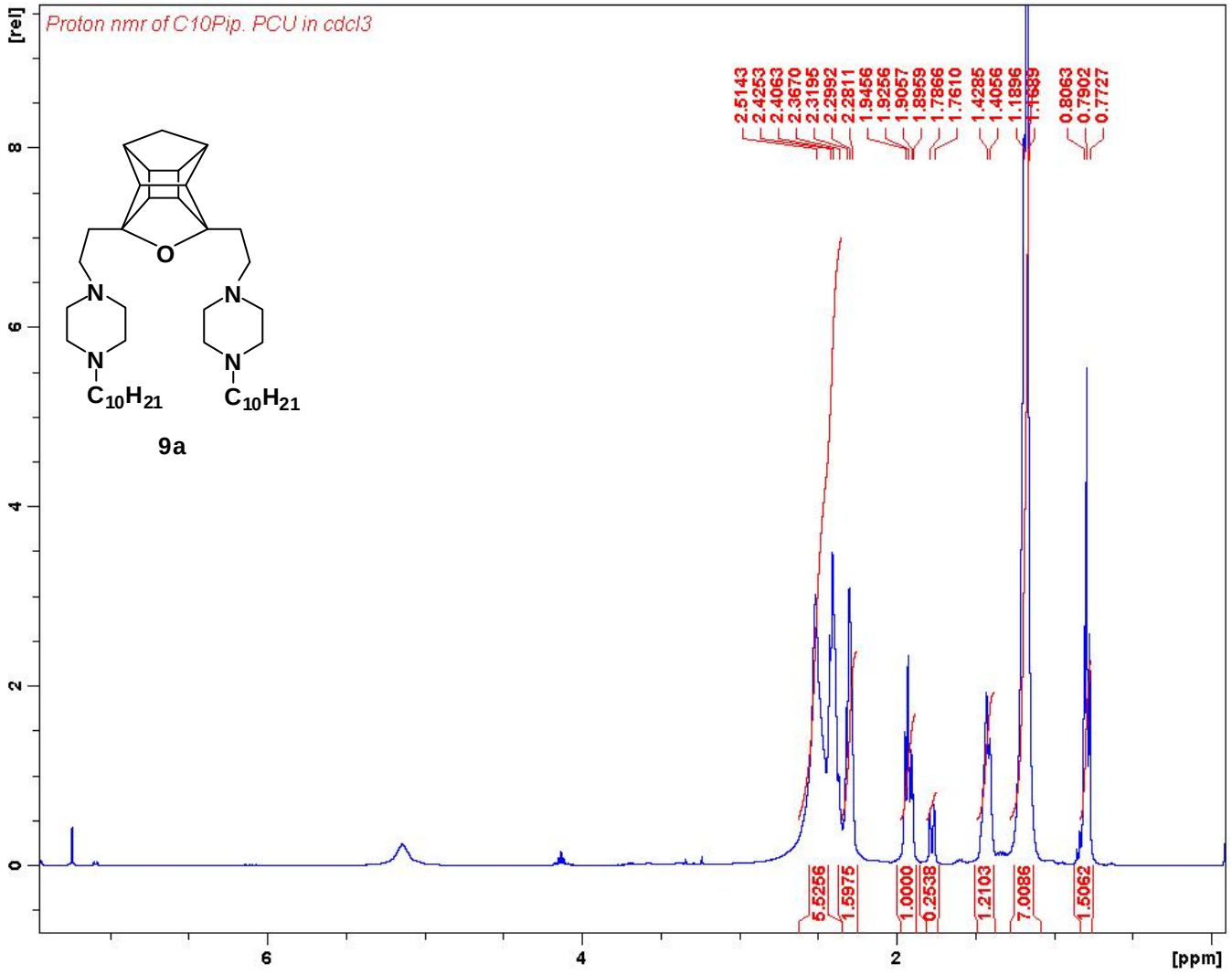
[b] Department of Medical Microbiology Nelson R Mandela School of Medicine, Durban, University of KwaZulu-Natal, South Africa.

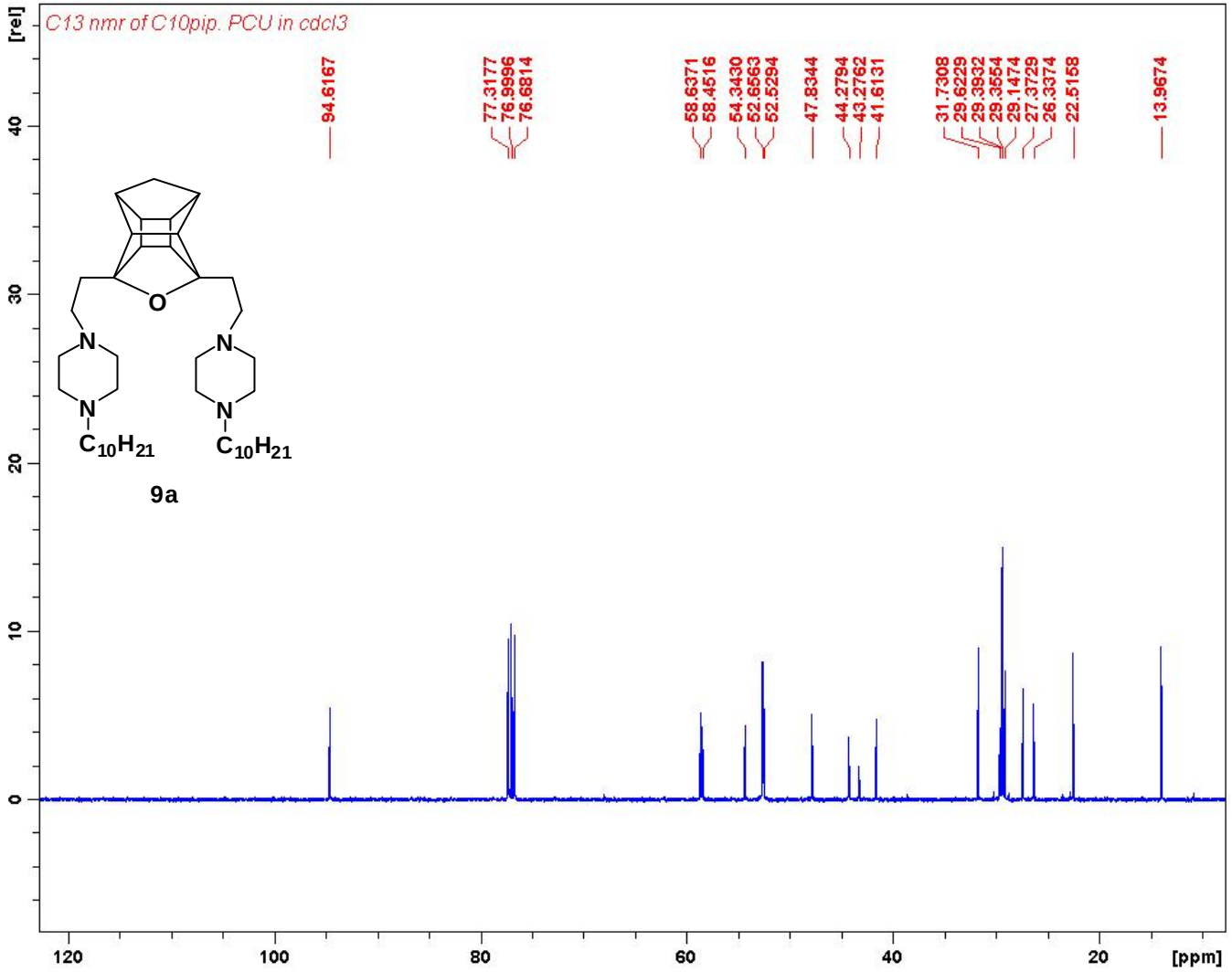
[c] School of Biochemistry, University of KwaZulu-Natal, Durban, South Africa.

[d] Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa.

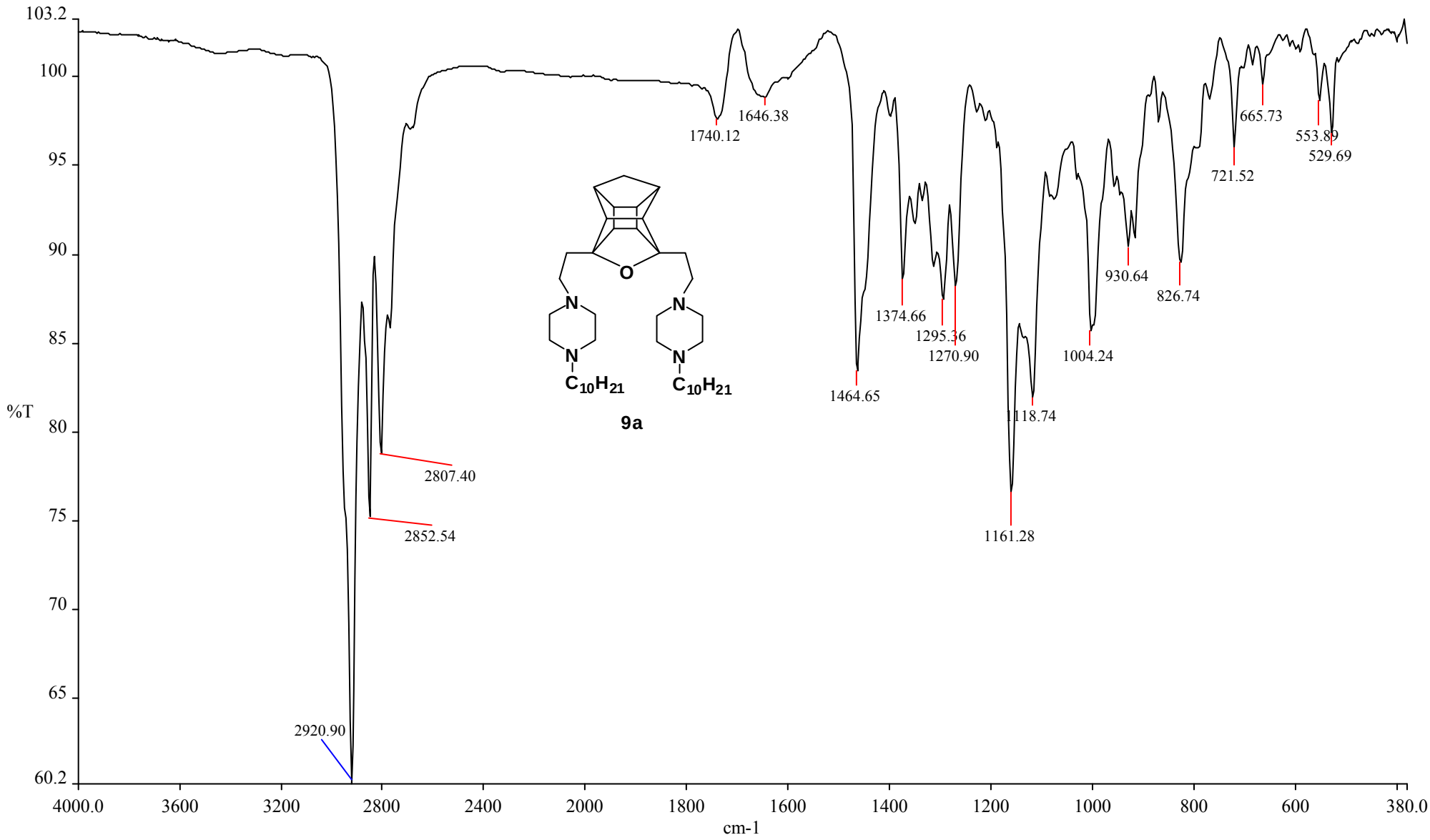
[e] School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa.



^{1}H NMR spectrum of C10 piperazine PCU **(9a)**



^{13}C NMR spectrum of C10 piperazine PCU **(9a)**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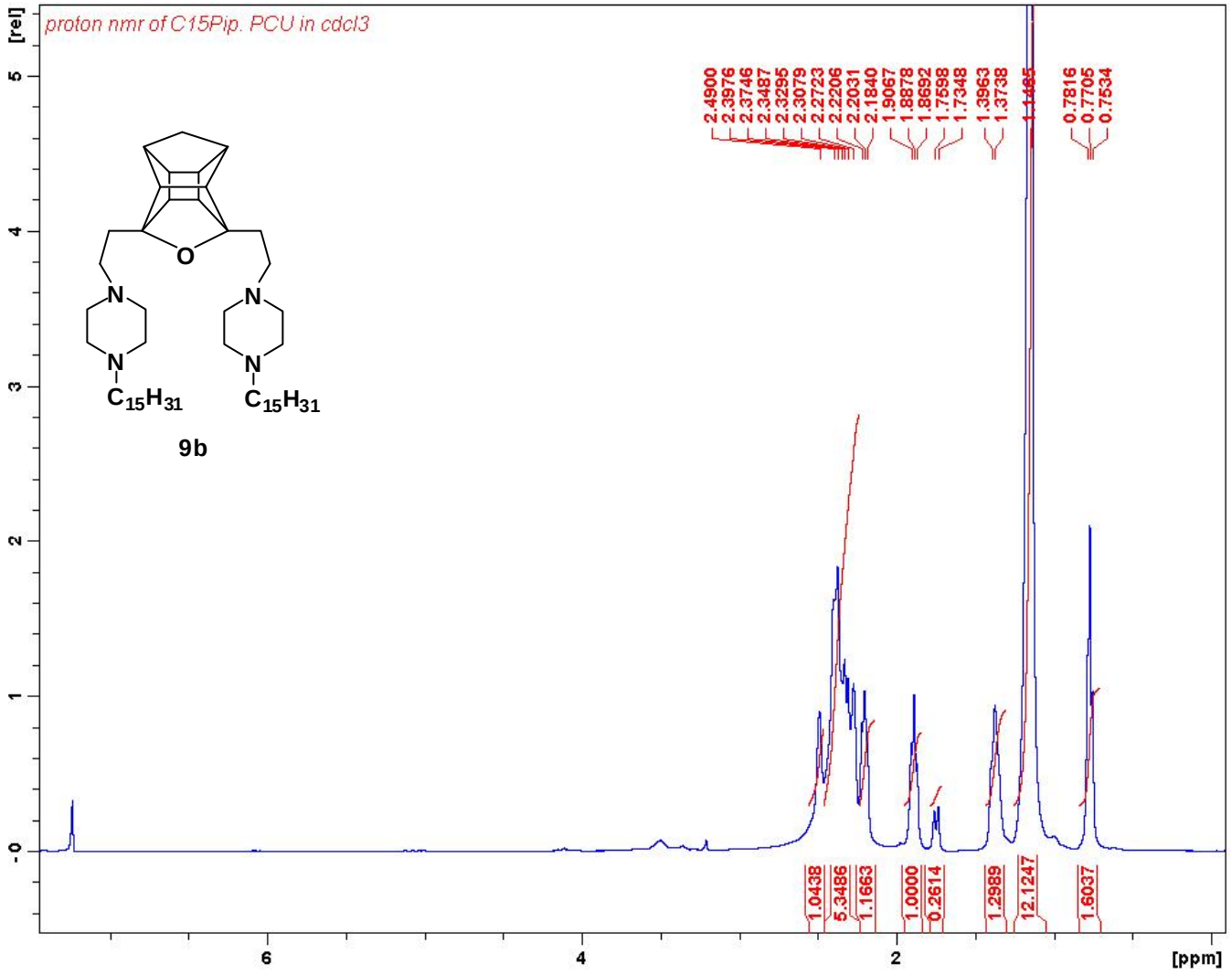
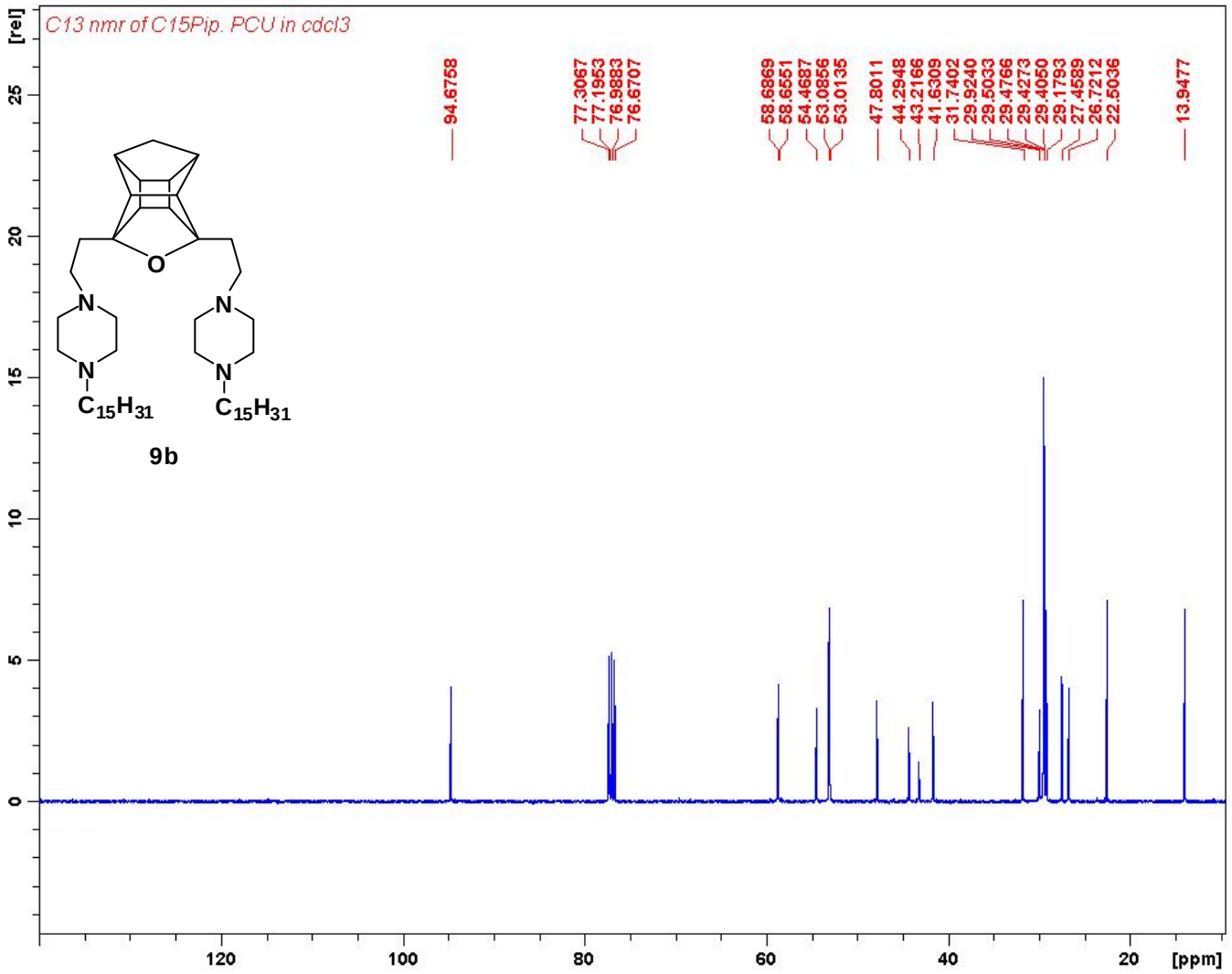
C10H21
9a

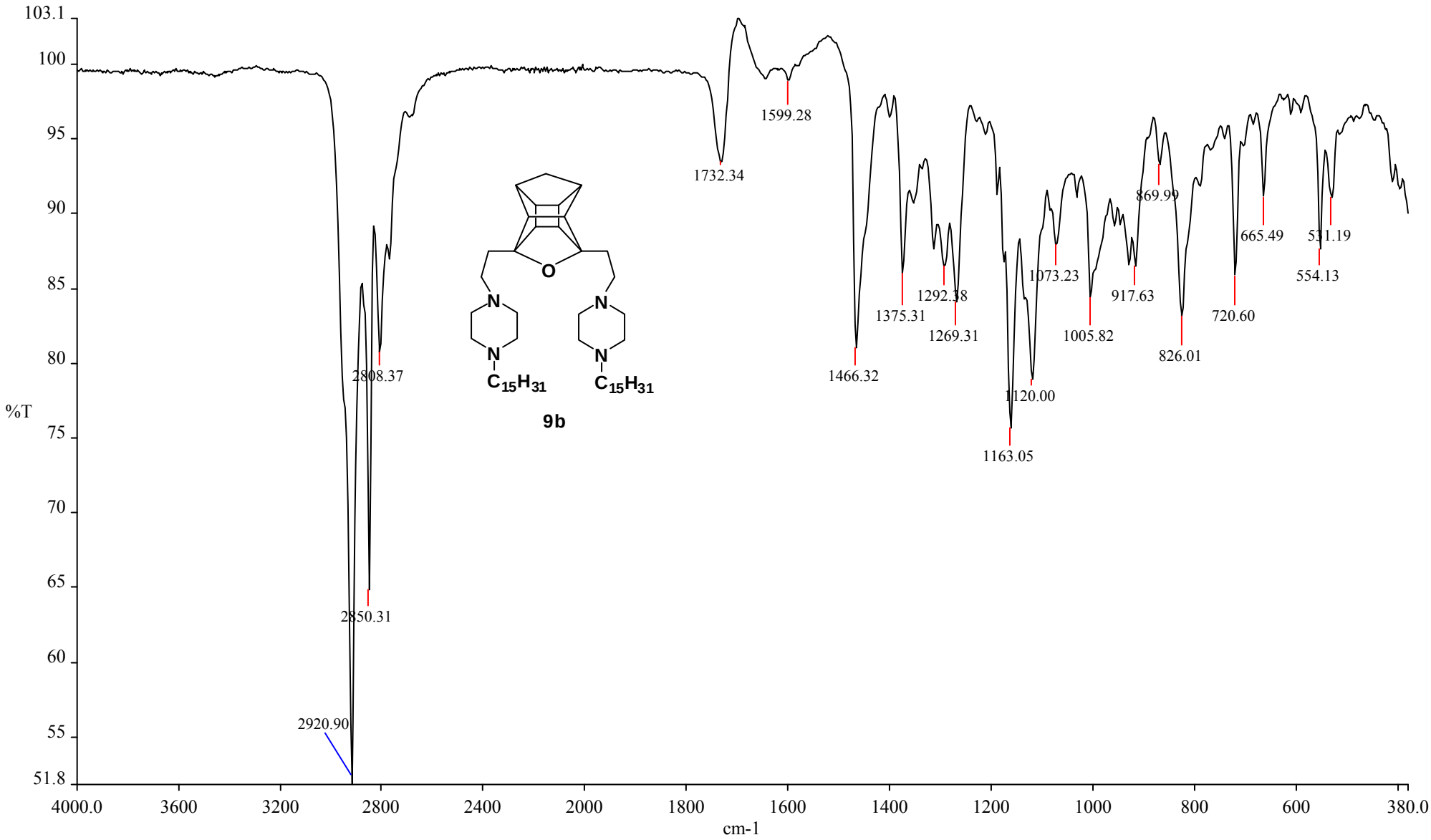
IR spectrum of C10 piperazine PCU **(9a)**



^{1}H NMR spectrum of C15 piperazine PCU **(9b)**



^{13}C NMR spectrum of C15 piperazine PCU **(9b)**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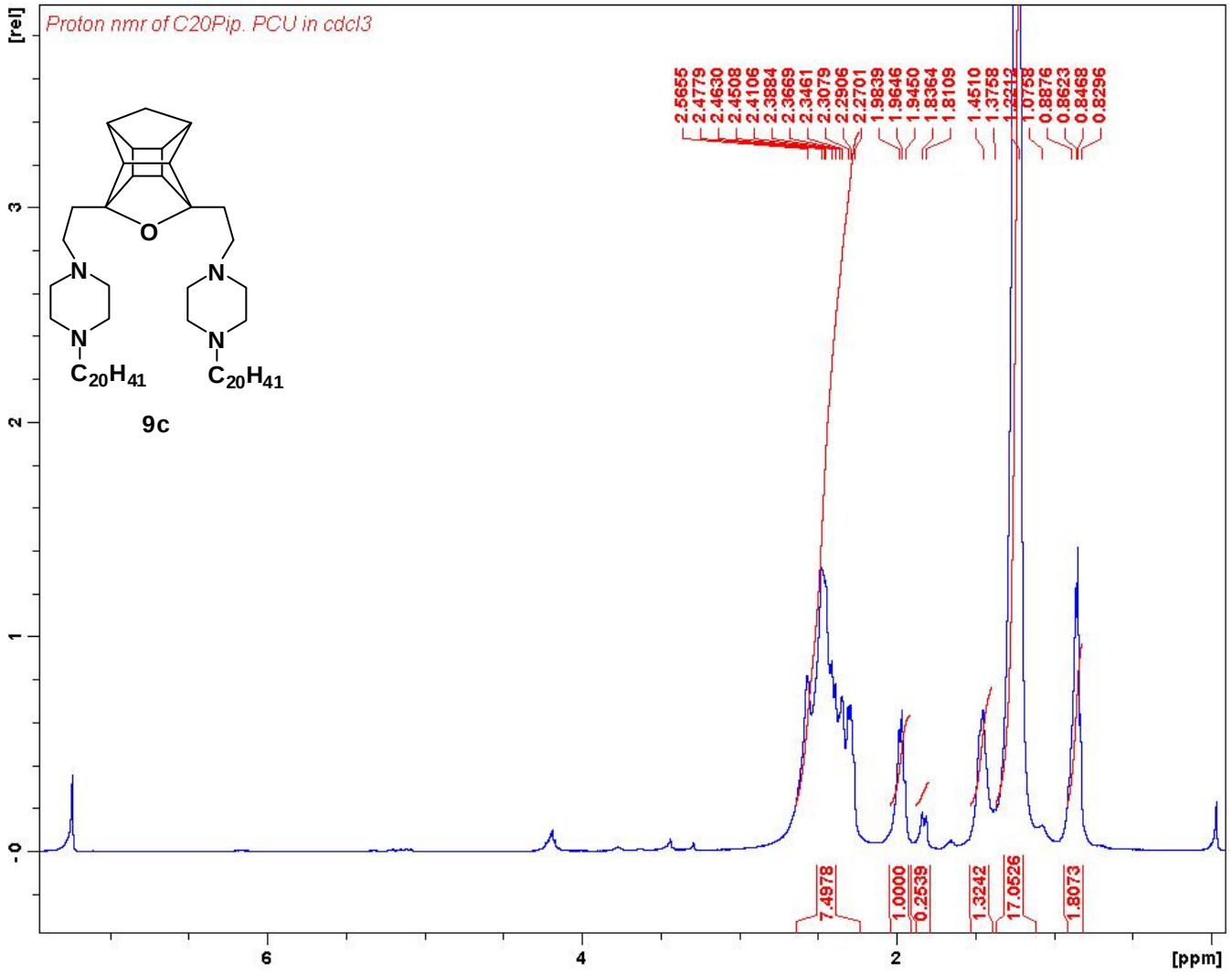
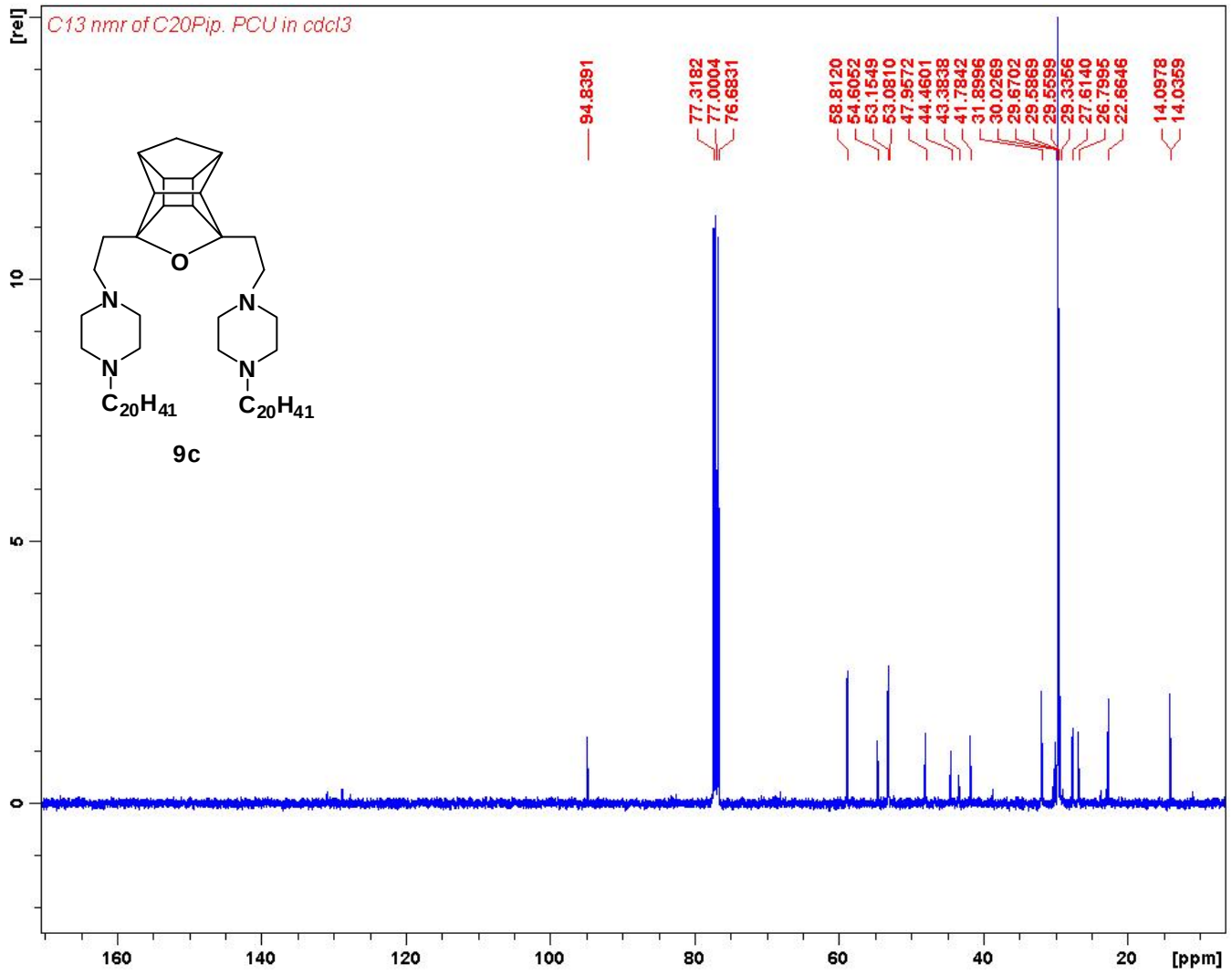
9b

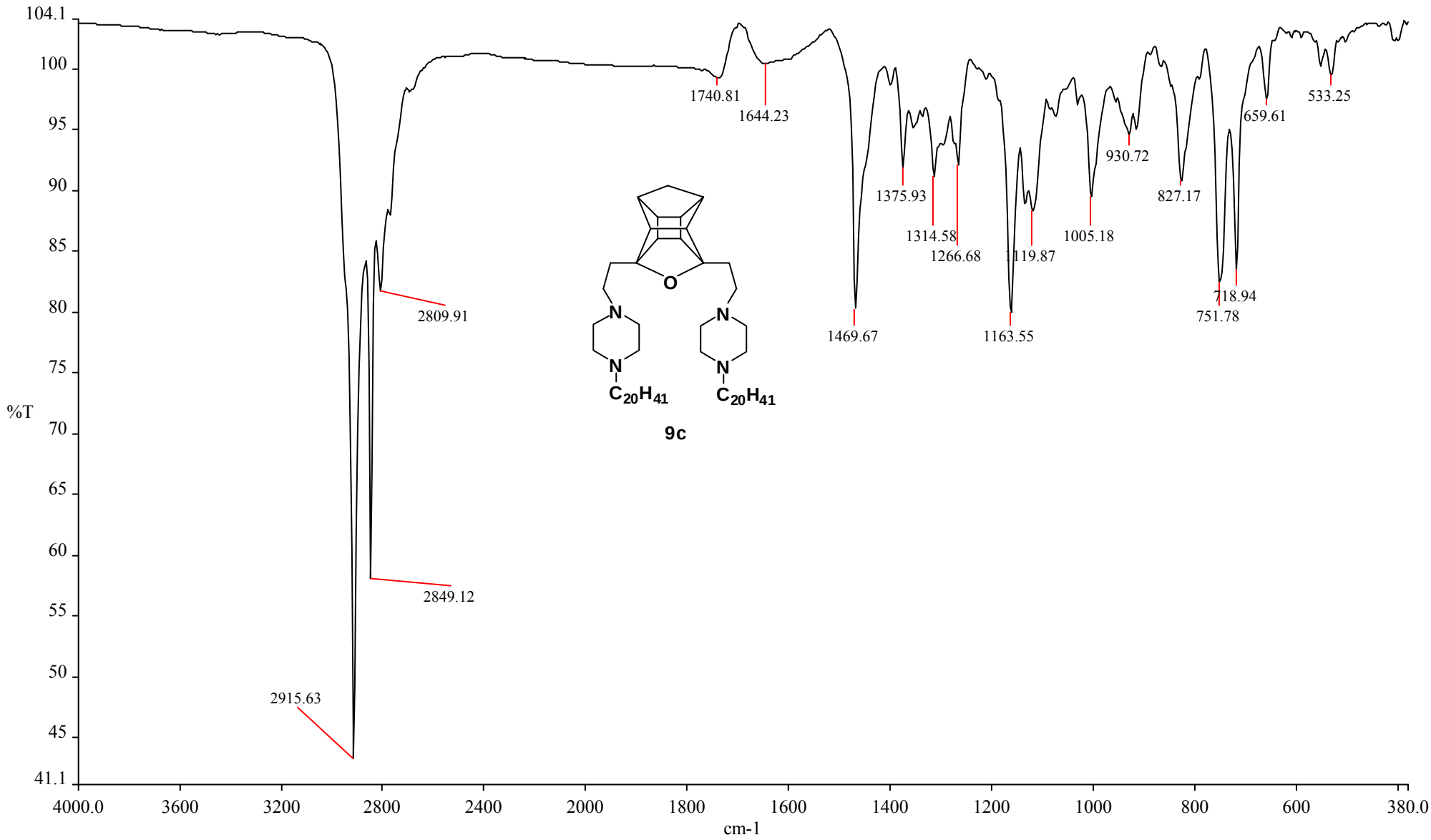
IR spectrum of C15 piperazine PCU **(9b)**



^{1}H NMR spectrum of C20 piperazine PCU **(9c)**



^{13}C NMR spectrum of C20 piperazine PCU **(9c)**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C20H41

9c

IR spectrum of C20 piperazine PCU **(9c)**

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Comment			

Acquisition Parameter

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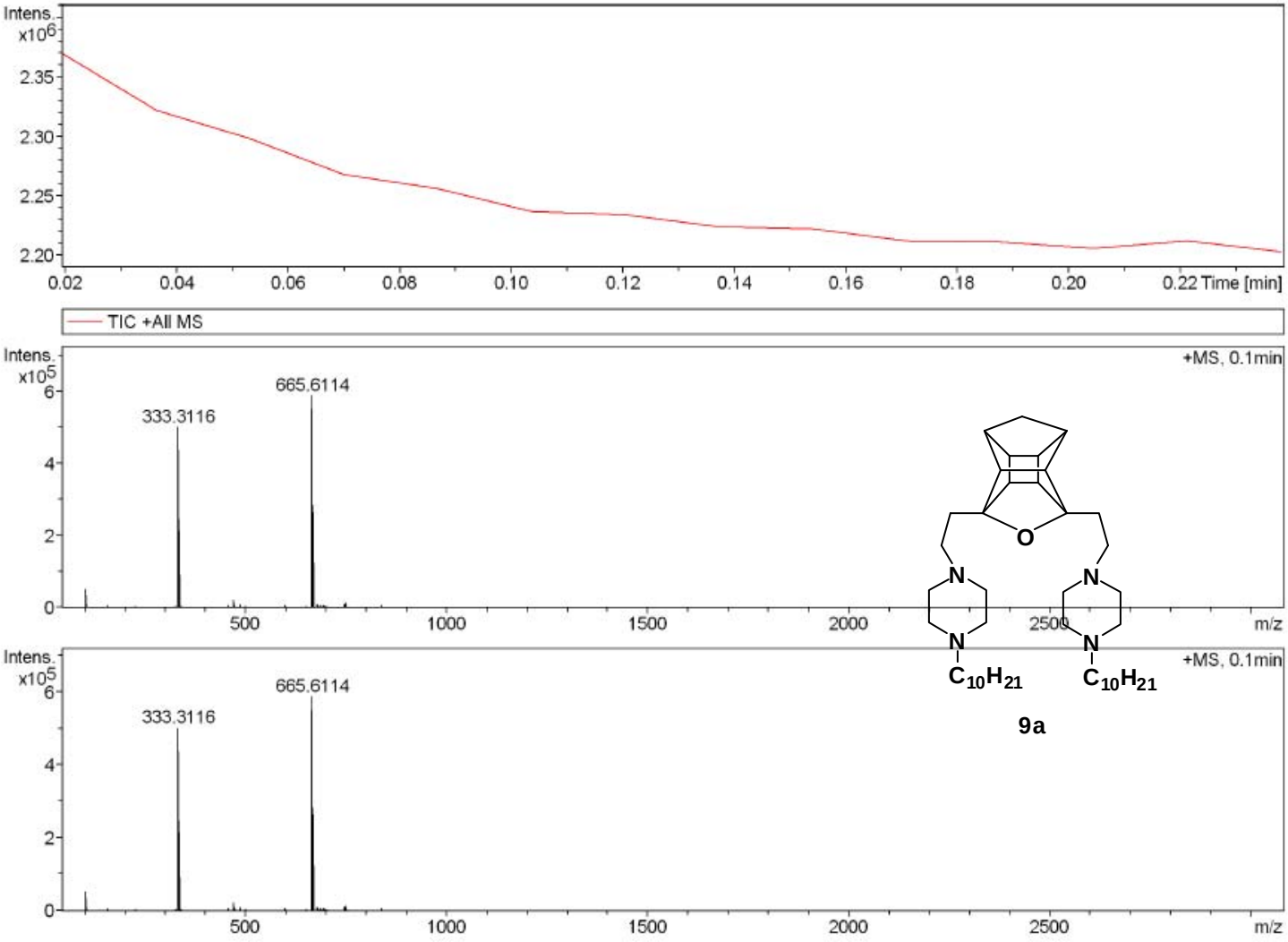

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	2	C 43 H 77 N 4 O	665.6092	-3.3	-2.5	7.5	ok	even	4.27	0.0058	0.0020	0.0020	0.0021	0.8427
	3	C 42 H 81 O 5	665.6079	-5.3	-4.5	2.5	ok	even	9.59	0.0110	0.0032	0.0040	0.0021	0.9409
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Mass spectrum of C10 piperazine PCU **(9a)**

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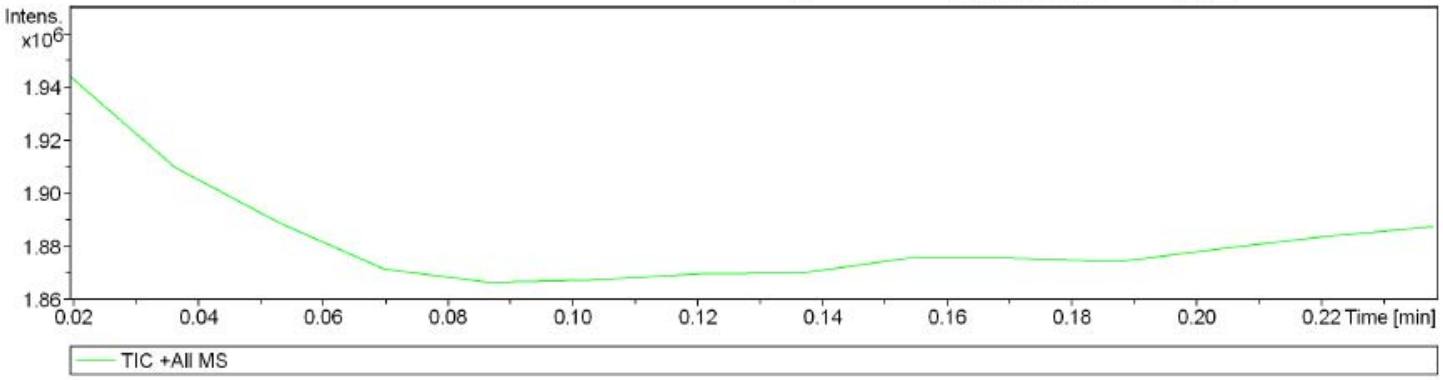
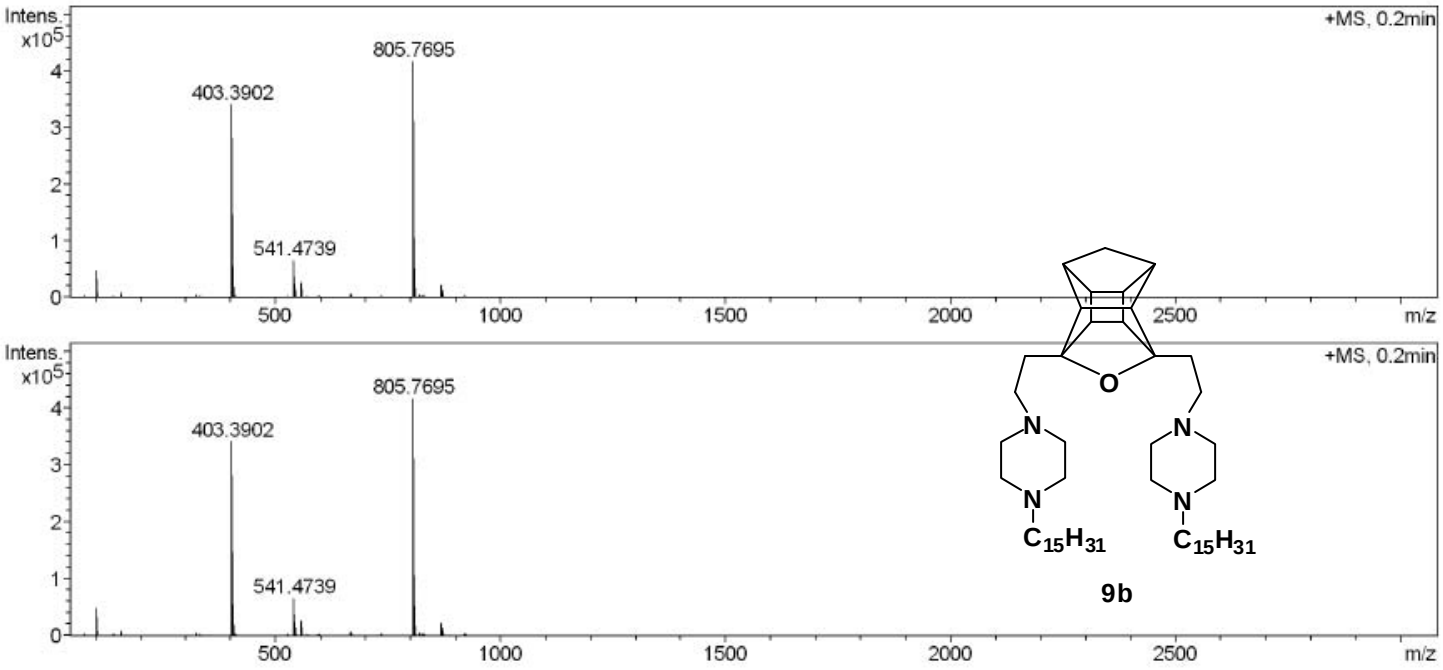
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



TIC +All MS



9b

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
805.7695														
	1	C 53 H 97 N 4 O	805.7657	-4.7	-4.4	7.5	ok	even	4.94	0.0075	0.0036	0.0025	0.0009	0.8322
	2	C 55 H 99 N O 2	805.7670	-3.1	-2.8	7.0	ok	odd	11.36	0.0164	0.0023	0.0052	0.0009	0.8424
	3	C 47 H 97 N 8 O 2	805.7729	4.2	4.5	3.5	ok	even	25.62	0.0268	0.0036	0.0088	0.0005	0.9303

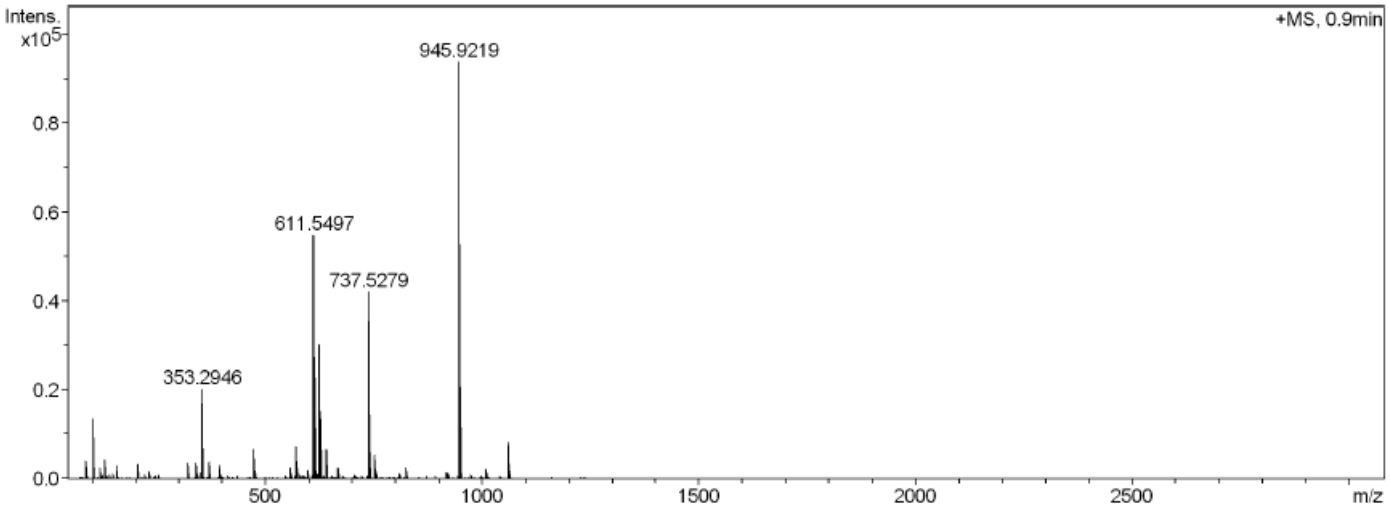
Mass spectrum of C15 piperazine PCU **(9b)**

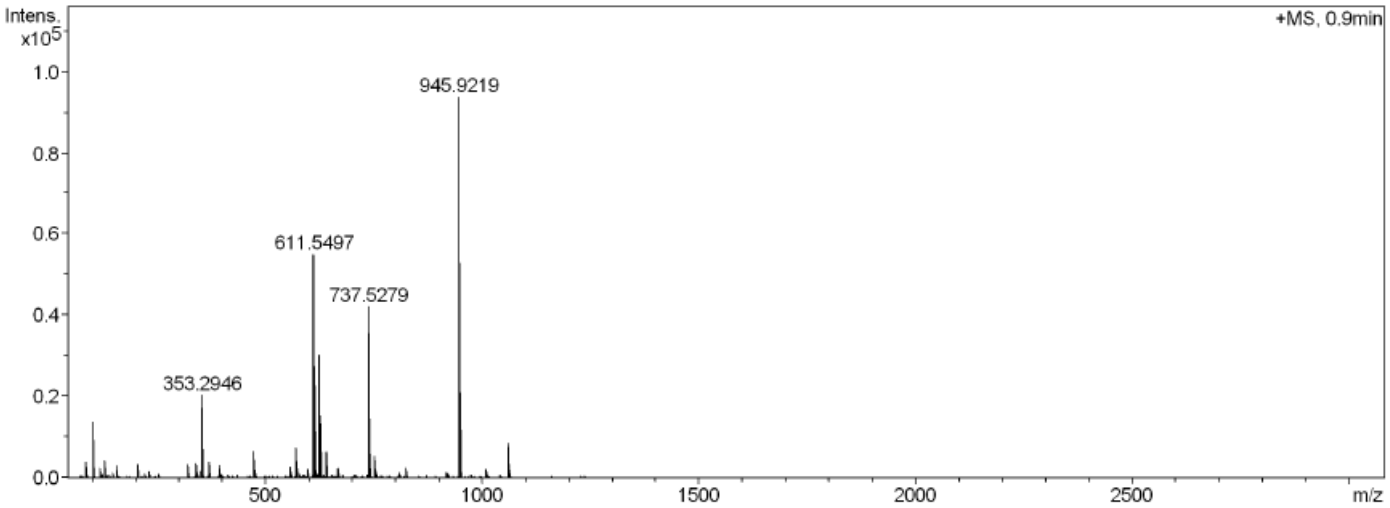
Analysis Info

Analysis Name	D:\Data\kenny\C20ppcu000001.d	Acquisition Date	10/3/2008 8:07:26 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	C20 PIP PCU	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source





Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
945.9219														
	1	C 58 H 117 N 6 O 3	945.9182	-3.9	-3.7	3.5	ok	even	3.19	0.0032	0.0036	0.0010	0.0017	0.8865
	2	C 60 H 119 N 3 O 4	945.9195	-2.5	-2.3	3.0	ok	odd	5.58	0.0075	0.0023	0.0025	0.0017	0.8713
	3	C 62 H 121 O 5	945.9209	-1.1	-0.9	2.5	ok	even	11.83	0.0146	0.0011	0.0046	0.0017	0.8401

4	C 61 H 115 N 7	945.9208	-1.1	-0.9	8.0	ok	odd	15.76	0.0164	0.0012	0.0046	0.0017	0.8427
5	C 63 H 117 N 4 O	945.9222	0.3	0.5	7.5	ok	even	21.80	0.0226	0.0009	0.0064	0.0017	0.8585
6	C 65 H 119 N O 2	945.9235	1.8	2.0	7.0	ok	odd	29.18	0.0308	0.0020	0.0090	0.0017	0.9450

Mass spectrum of C20 piperazine PCU **(9c)**

CHAPTER 4

SYNTHESIS AND EVALUATION OF SQ109 ANALOGUES AS POTENTIAL ANTI-TUBERCULOSIS CANDIDATES

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[c] Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa.

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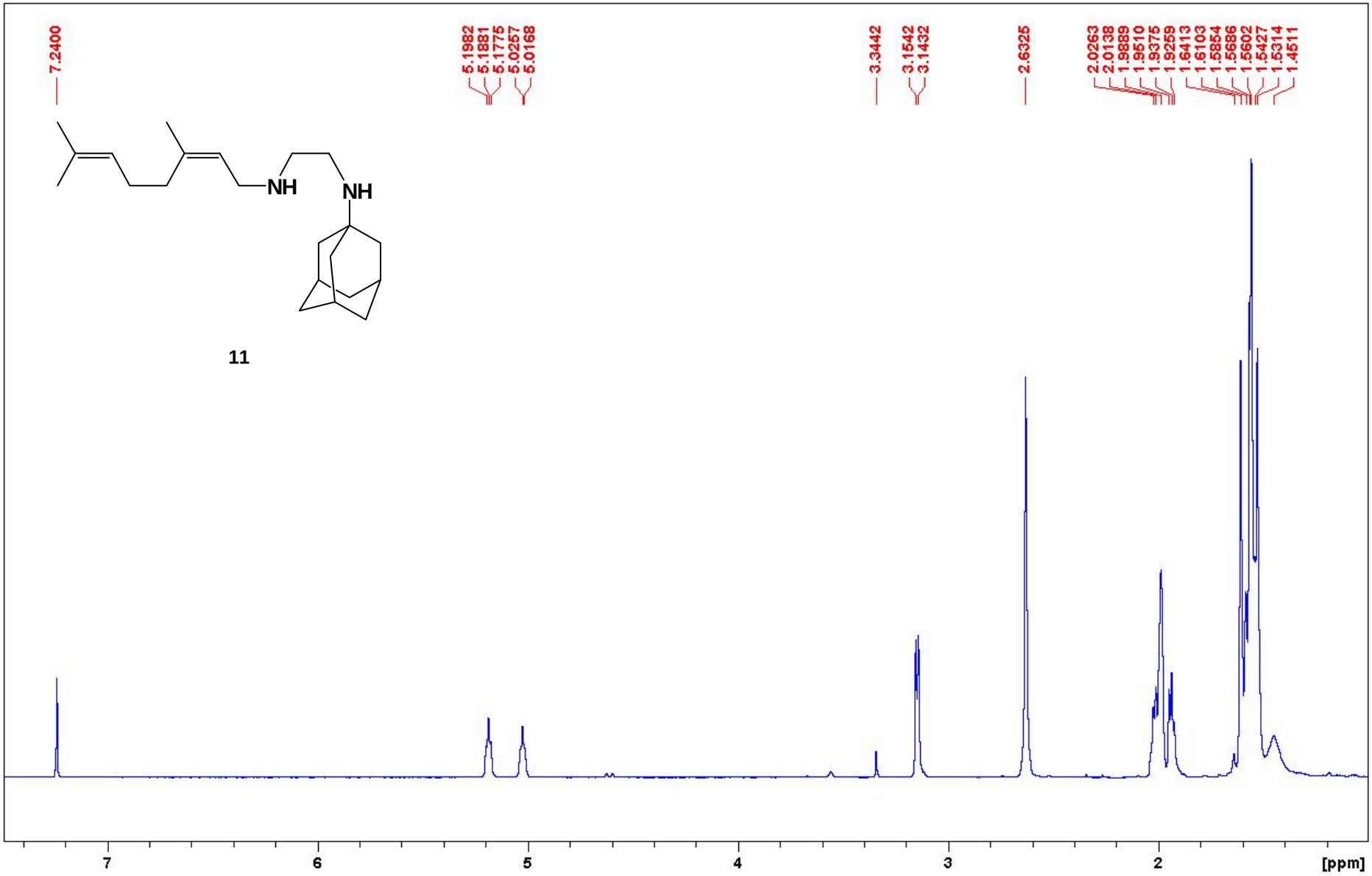
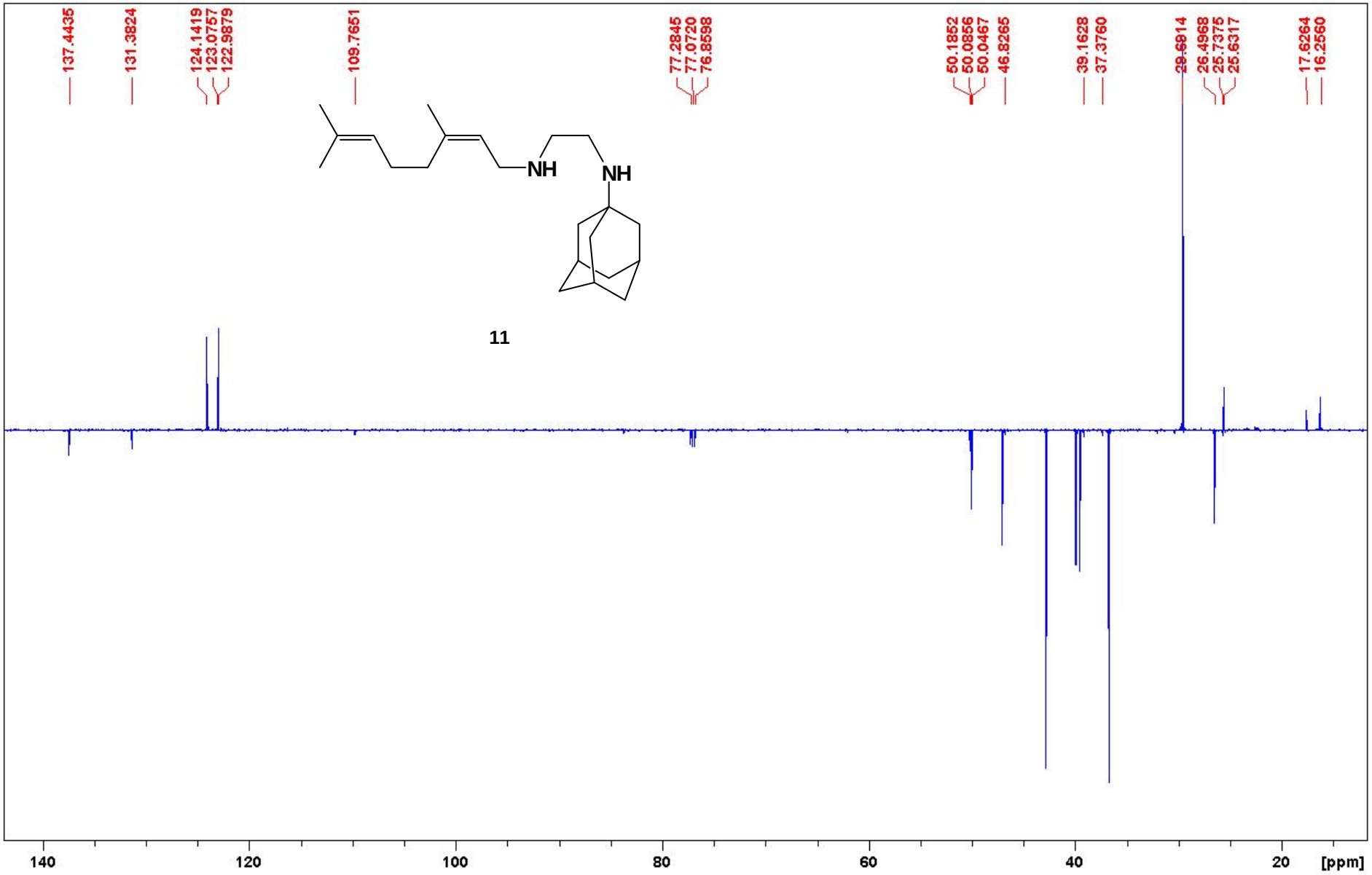
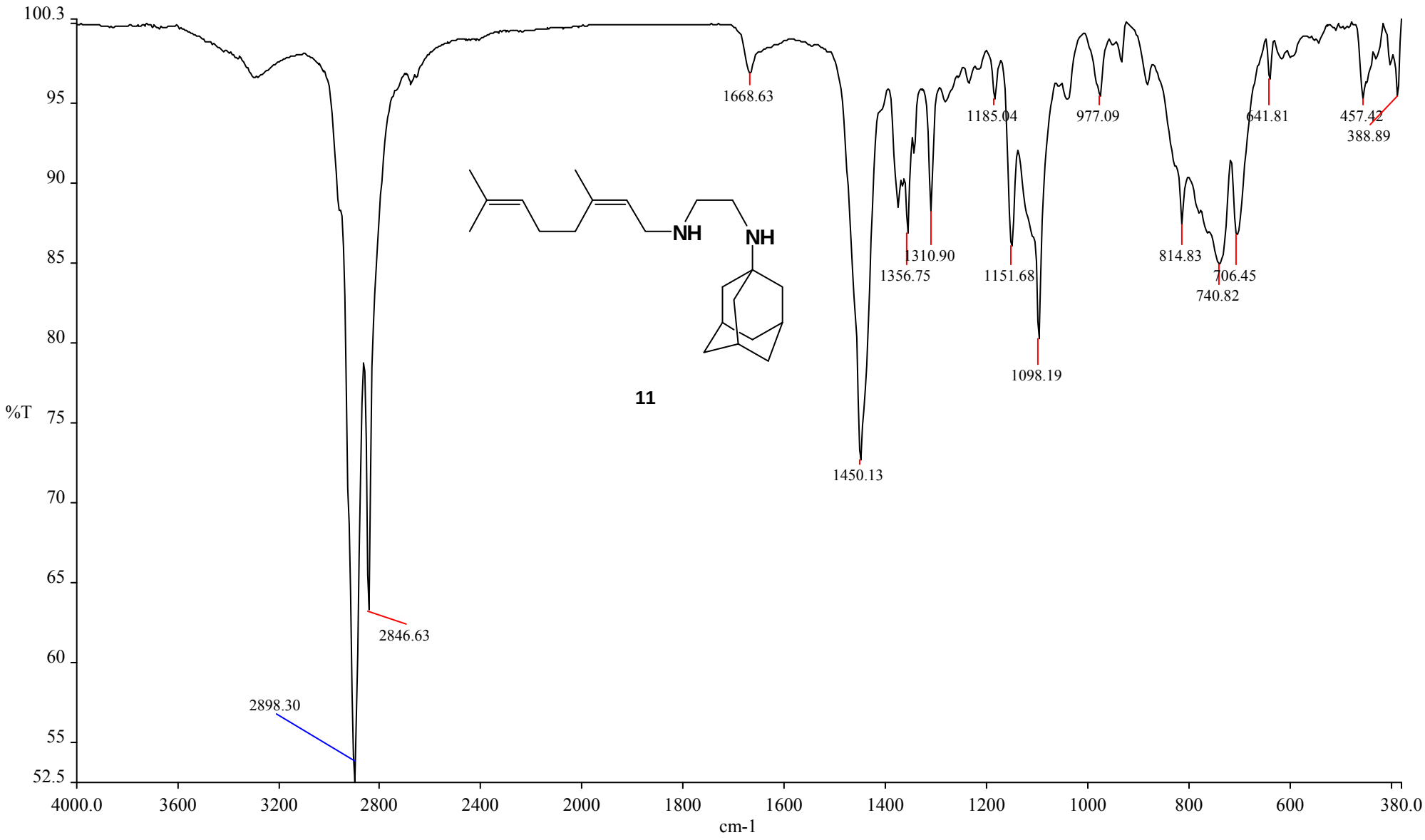

7.2400
5.1982
5.1881
5.1775
5.0257
5.0168
3.3442
3.1542
3.1432
2.6325
2.0263
2.0138
1.9889
1.9510
1.9375
1.9259
1.6413
1.6103
1.5854
1.5686
1.5602
1.5427
1.5314
1.4511
NH
NH
7
6
5
4
3
2
[ppm]

11

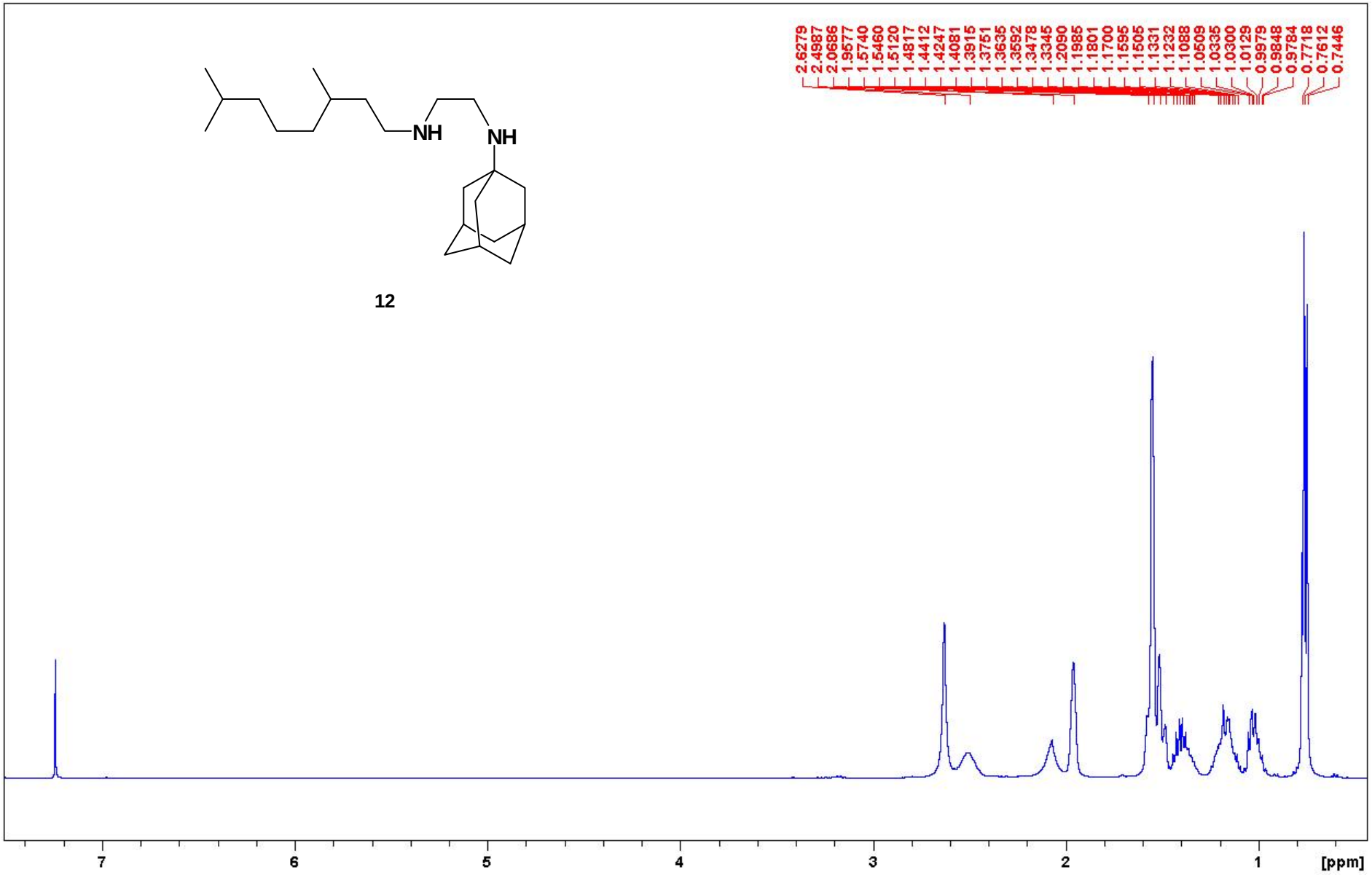
^{1}H NMR spectrum of compound 11 in $CDCl_3$


137.4435
131.3824
124.1419
123.0757
122.9879
109.7651
77.2845
77.0720
76.8598
50.1852
50.0856
50.0467
46.8265
39.1628
37.3760
29.6814
26.4968
25.7375
25.6317
17.6264
16.2560
NH
NH
11
140
120
100
80
60
40
20
[ppm]

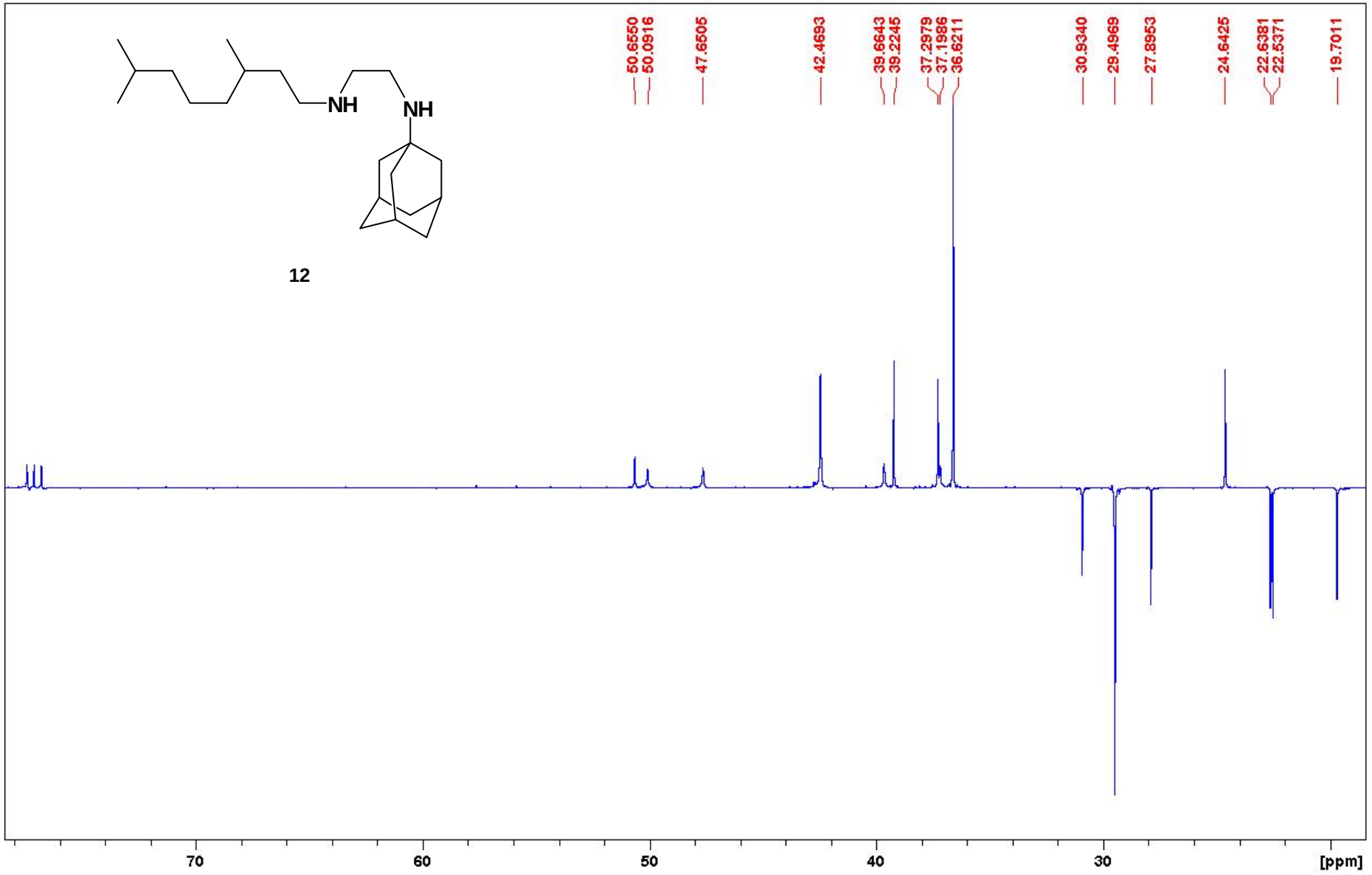
^{13}C NMR spectrum of compound 11 in $CDCl_3$


100.3
95
90
85
80
75
70
65
60
55
52.5
%T
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380.0
cm-1
NH
NH
11
1668.63
1450.13
1356.75
1310.90
1185.04
1151.68
1098.19
977.09
814.83
740.82
706.45
641.81
457.42
388.89
2898.30
2846.63

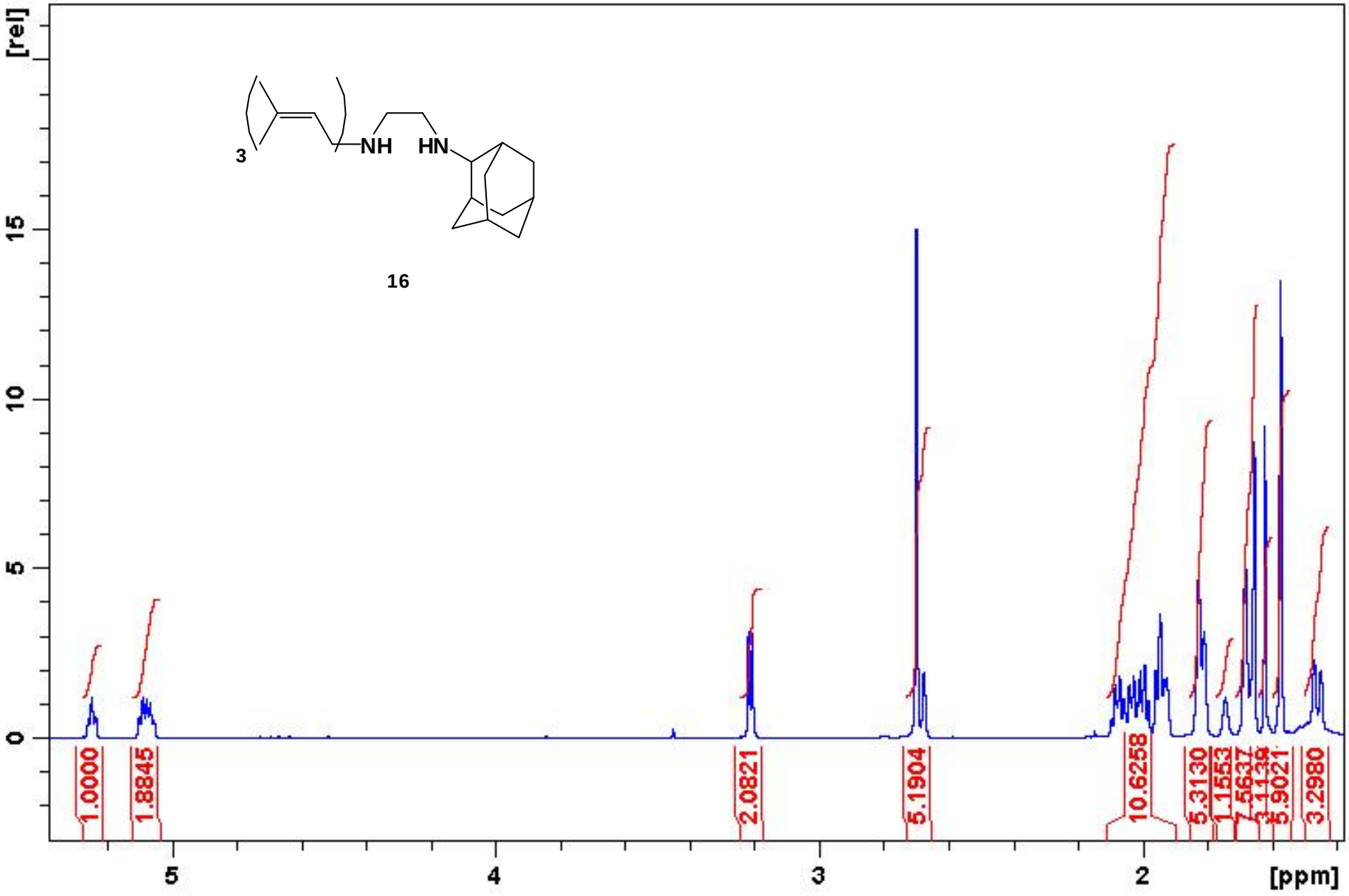
IR spectrum of compound 11


NH
NH
12
2.6279
2.4987
2.0686
1.9577
1.5740
1.5460
1.5120
1.4817
1.4412
1.4247
1.4081
1.3915
1.3751
1.3635
1.3592
1.3478
1.3345
1.2090
1.1985
1.1801
1.1700
1.1595
1.1505
1.1331
1.1232
1.1088
1.0509
1.0335
1.0300
1.0129
0.9979
0.9848
0.9784
0.7718
0.7612
0.7446
7
6
5
4
3
2
1
[ppm]

^{1}H NMR of Compound 12 in $CDCl_3$


NH
NH
12
50.6550
50.0916
47.6505
42.4693
39.6643
39.2245
37.2979
37.1986
36.6211
30.9340
29.4969
27.8953
24.6425
22.6381
22.5371
19.7011
70
60
50
40
30
[ppm]

^{13}C NMR of Compound 12 in $CDCl_3$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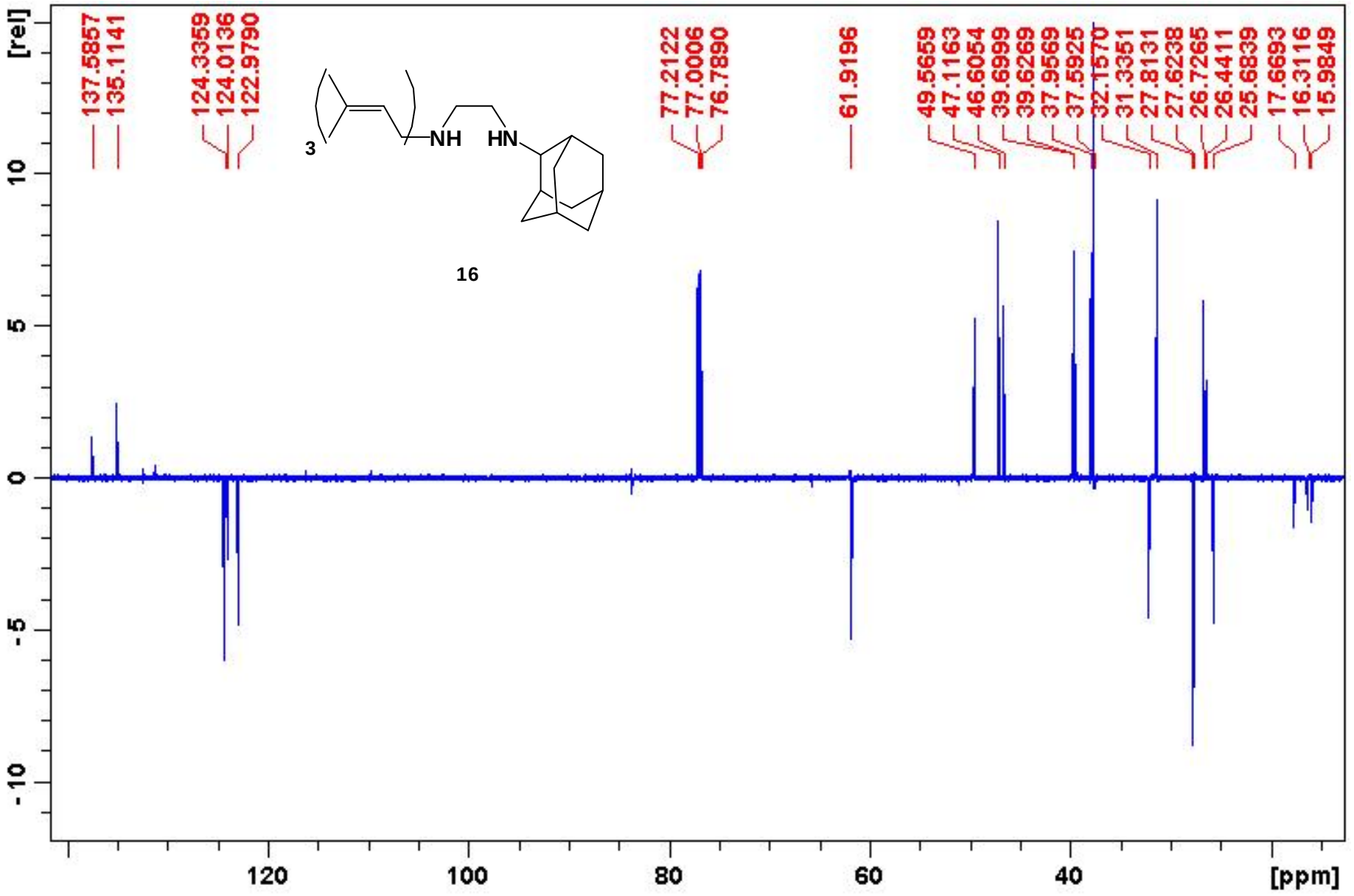
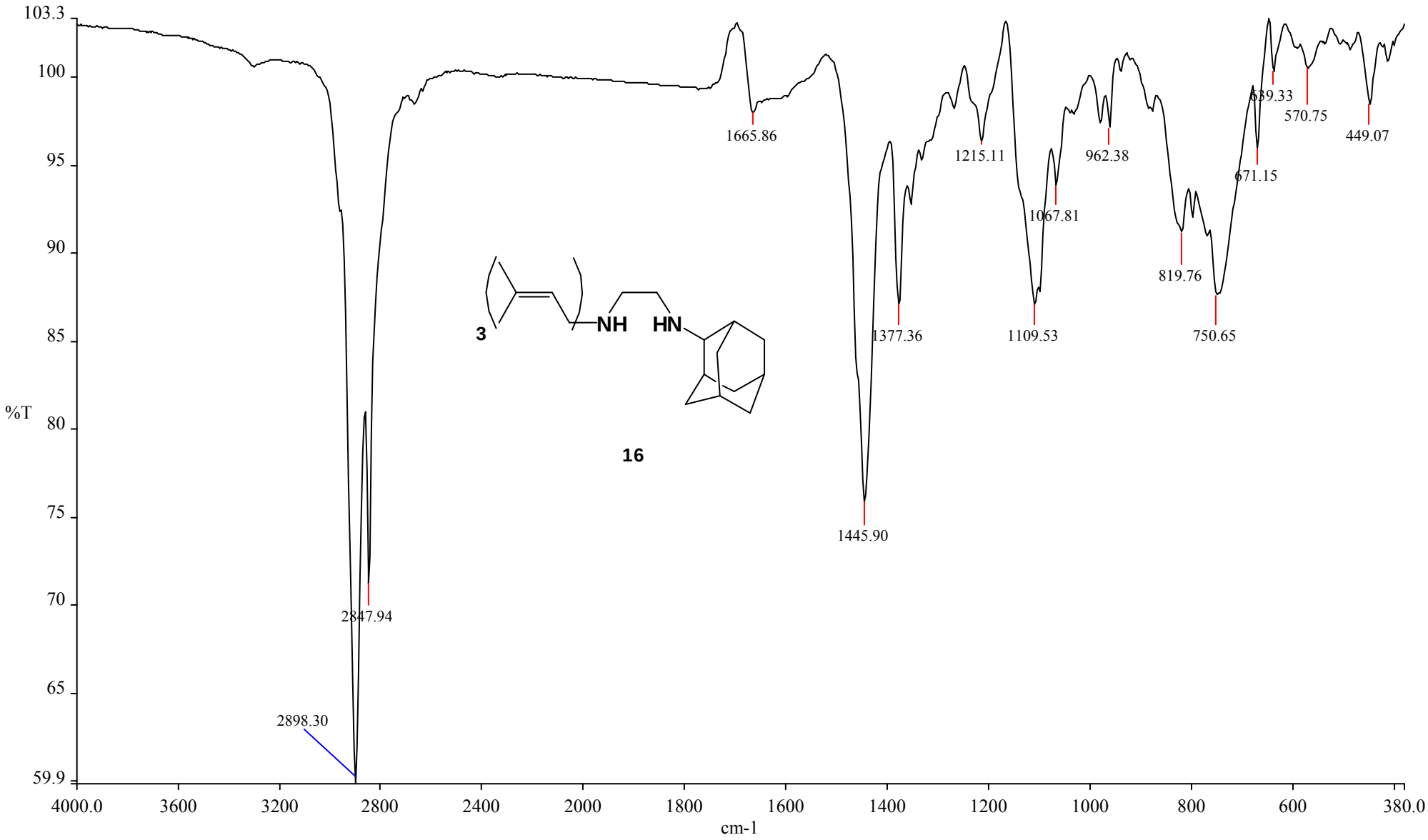
15
10
5
0
3
NH
HN
16
1.0000
1.8845
2.0821
5.1904
10.6258
5.3130
1.1553
7.5637
3.1139
5.9021
3.2980
5
4
3
2
[ppm]

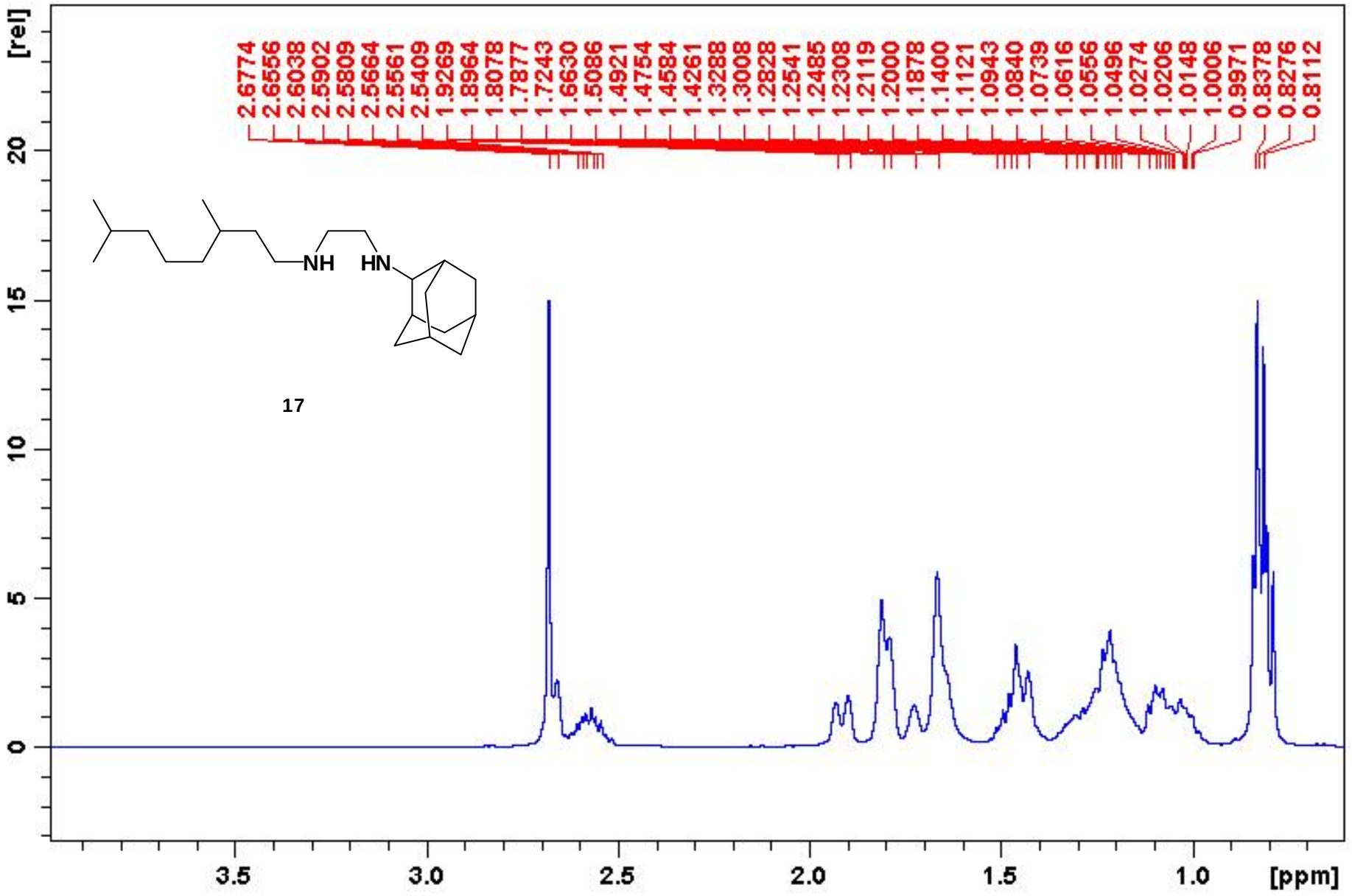
^{1}H NMR spectrum of compound 16 in $CDCl_3$


137.5857
135.1141
124.3359
124.0136
122.9790
77.2122
77.0006
76.7890
61.9196
49.5659
47.1163
46.6054
39.6999
39.6269
37.9569
37.5925
32.1570
31.3351
27.8131
27.6238
26.7265
26.4411
25.6839
17.6693
16.3116
15.9849
NH
HN
3
16
[rel]
10
5
0
-5
-10
120
100
80
60
40
[ppm]

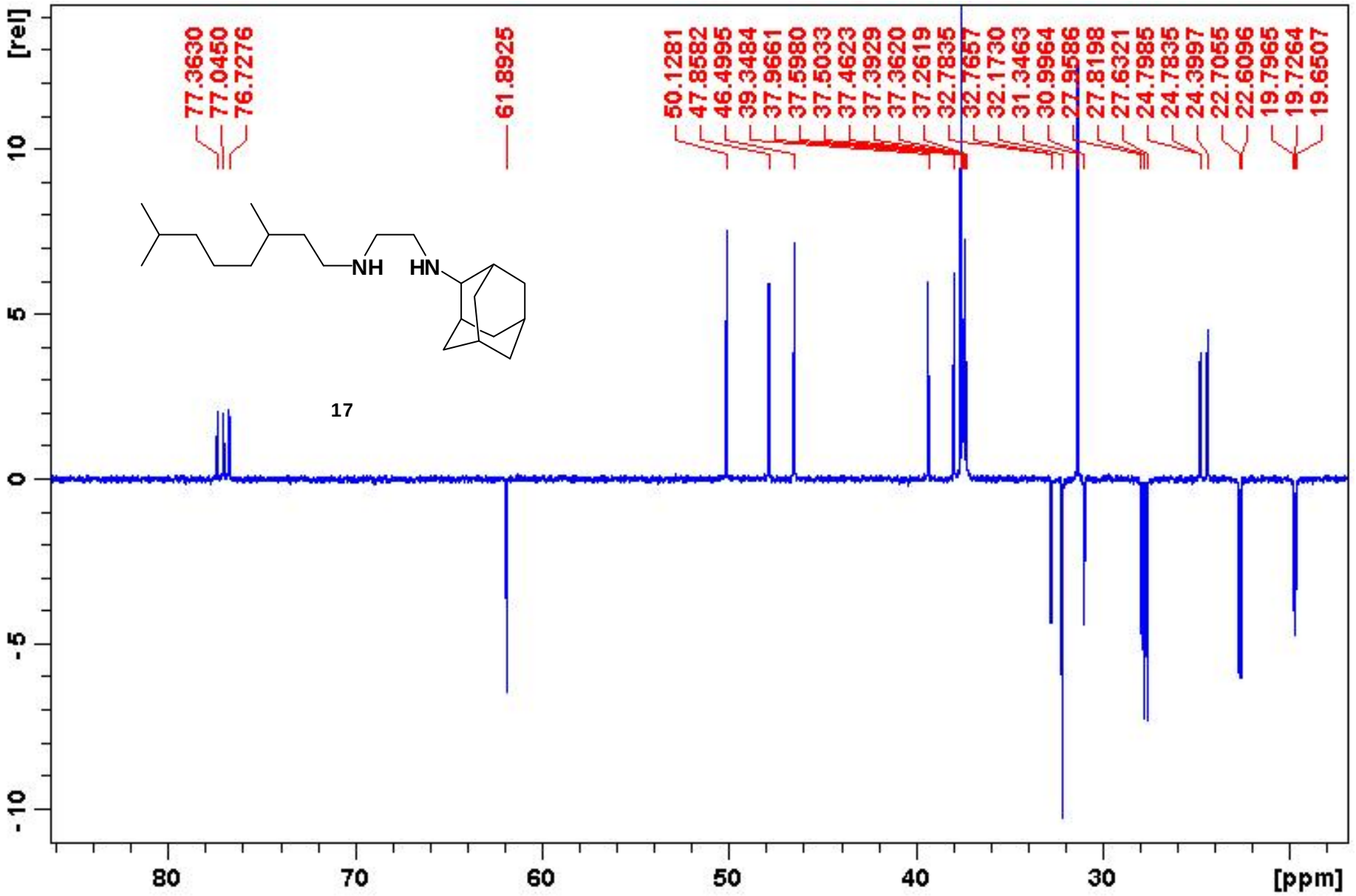
^{13}C NMR spectrum of compound 16 in $CDCl_3$


103.3
100
95
90
85
80
75
70
65
59.9
%T
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380.0
cm-1
2898.30
2847.94
1665.86
1445.90
1377.36
1215.11
1109.53
1067.81
962.38
819.76
750.65
671.15
639.33
570.75
449.07
3
NH
HN
16

IR spectrum of compound 16


2.6774
2.6556
2.6038
2.5902
2.5809
2.5664
2.5561
2.5409
1.9269
1.8964
1.8078
1.7877
1.7243
1.6630
1.5086
1.4921
1.4754
1.4584
1.4261
1.3288
1.3008
1.2828
1.2541
1.2485
1.2308
1.2119
1.2000
1.1878
1.1400
1.1121
1.0943
1.0840
1.0739
1.0616
1.0556
1.0496
1.0274
1.0206
1.0148
1.0006
0.9971
0.8378
0.8276
0.8112
NH
HN
17
[rel]
20
15
10
5
0
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]

^{1}H NMR spectrum of compound 17 in $CDCl_3$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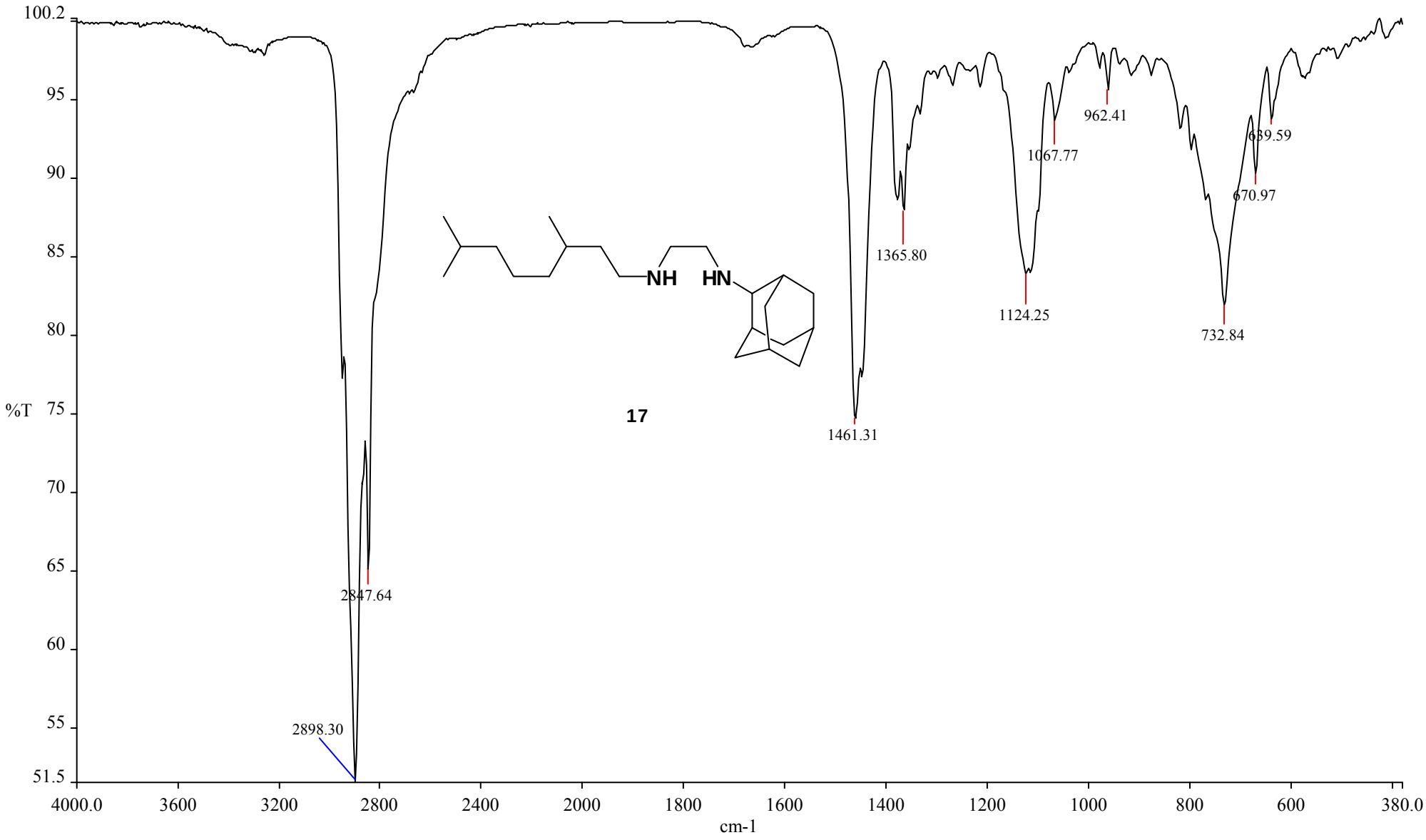
77.0450
76.7276
61.8925
50.1281
47.8582
46.4995
39.3484
37.9661
37.5980
37.5033
37.4623
37.3929
37.3620
37.2619
32.7835
32.7657
32.1730
31.3463
30.9964
27.9586
27.8198
27.6321
24.7985
24.7835
24.3997
22.7055
22.6096
19.7965
19.7264
19.6507
NH
HN
17
[rel]
10
5
0
-5
-10
80
70
60
50
40
30
[ppm]

^{13}C NMR spectrum of compound 17 in $CDCl_3$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90
85
80
75
70
65
60
55
51.5
%T
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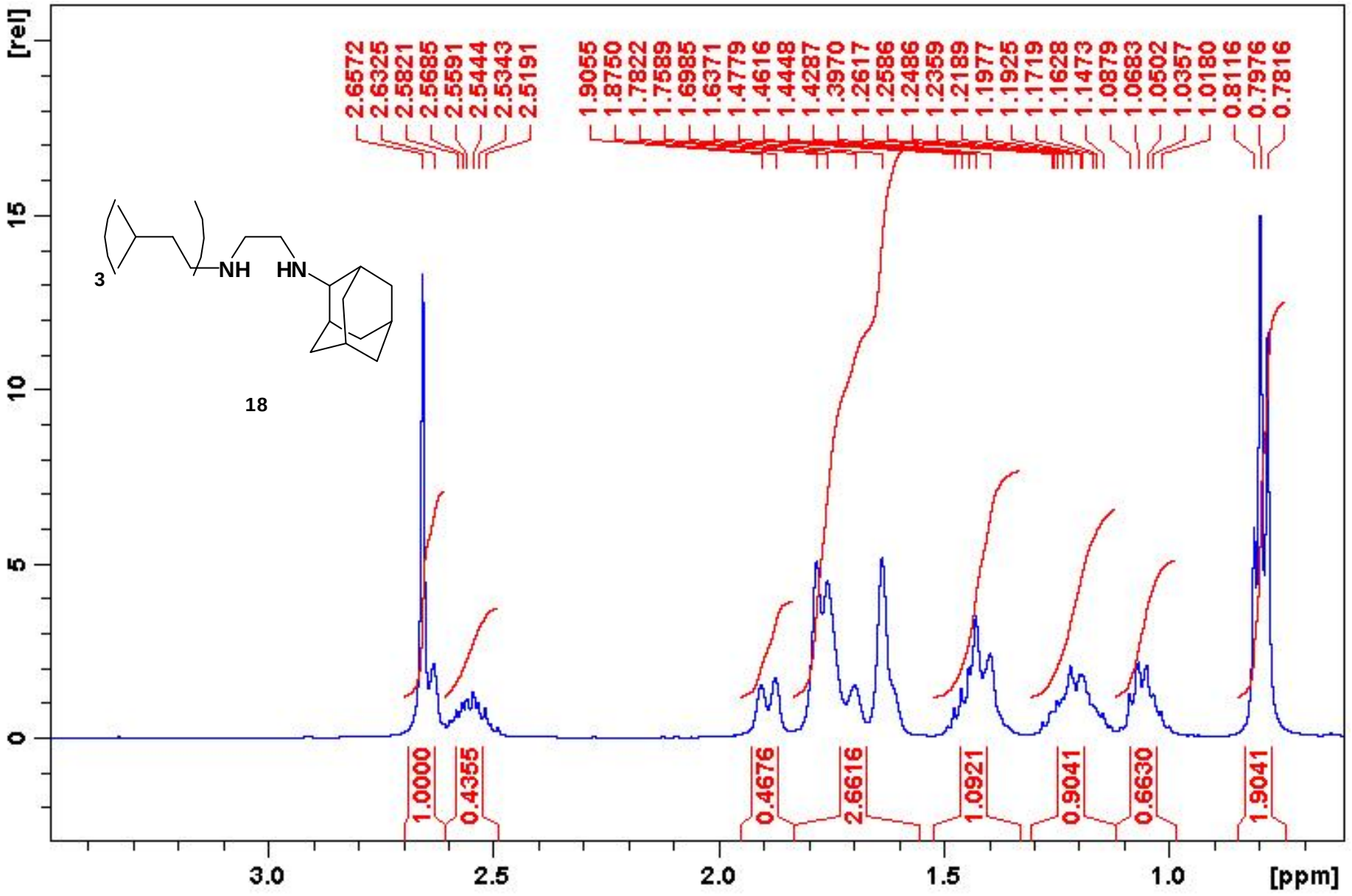
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380.0
cm-1
NH
HN
17
2898.30
2847.64
1461.31
1365.80
1124.25
1067.77
962.41
732.84
670.97
639.59

IR spectrum of compound 17


[rel]
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10
5
0
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2.6325
2.5821
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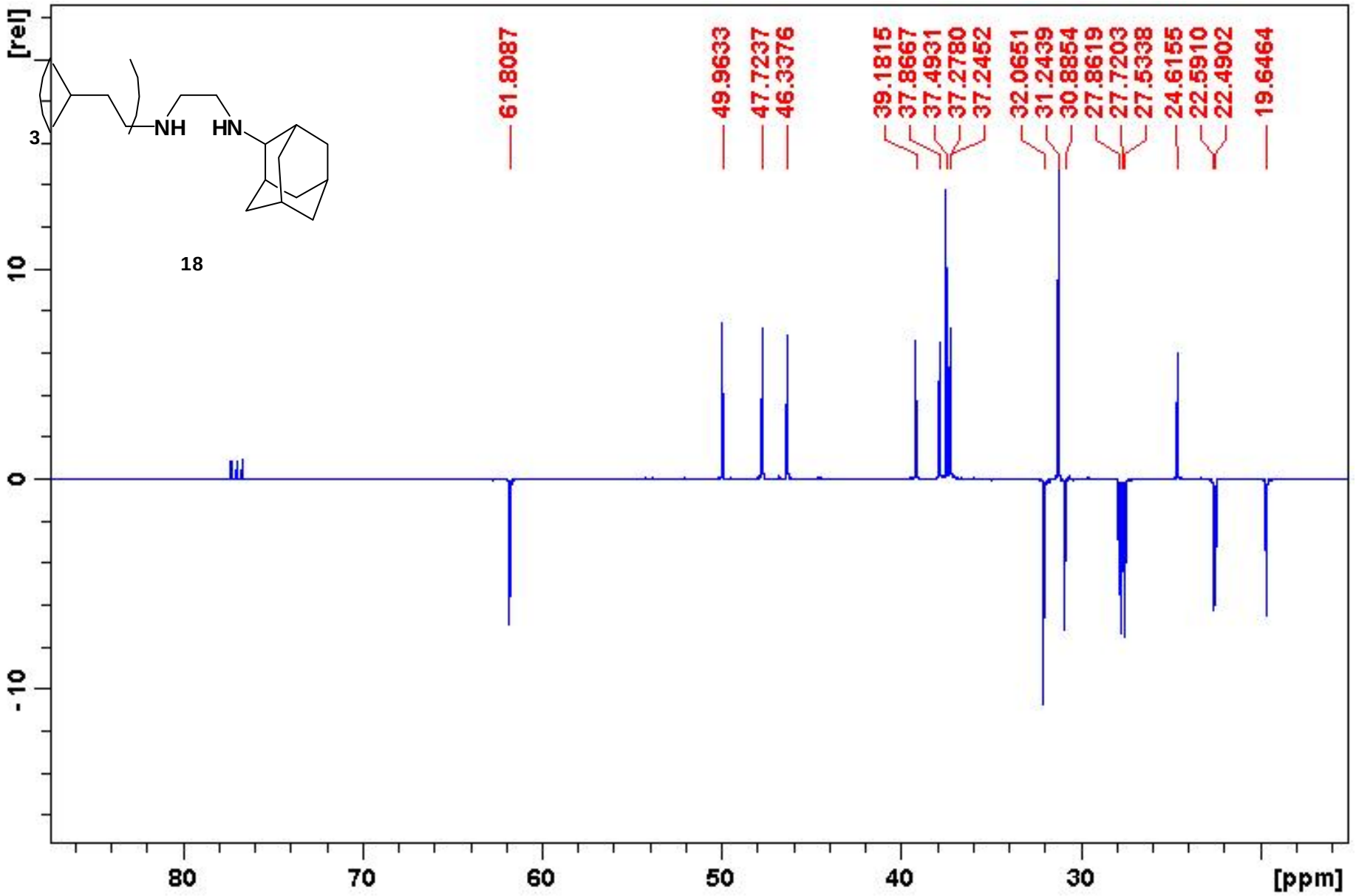
2.5591
2.5444
2.5343
2.5191
1.9055
1.8750
1.7822
1.7589
1.6985
1.6371
1.4779
1.4616
1.4448
1.4287
1.3970
1.2617
1.2586
1.2486
1.2359
1.2189
1.1977
1.1925
1.1719
1.1628
1.1473
1.0879
1.0683
1.0502
1.0357
1.0180
0.8116
0.7976
0.7816
3
NH
HN
18
1.0000
0.4355
0.4676
2.6616
1.0921
0.9041
0.6630
1.9041
3.0
2.5
2.0
1.5
1.0
[ppm]

^{1}H NMR spectrum of compound 18 in $CDCl_3$


[rel]
10
0
- 10
80
70
60
50
40
30
[ppm]
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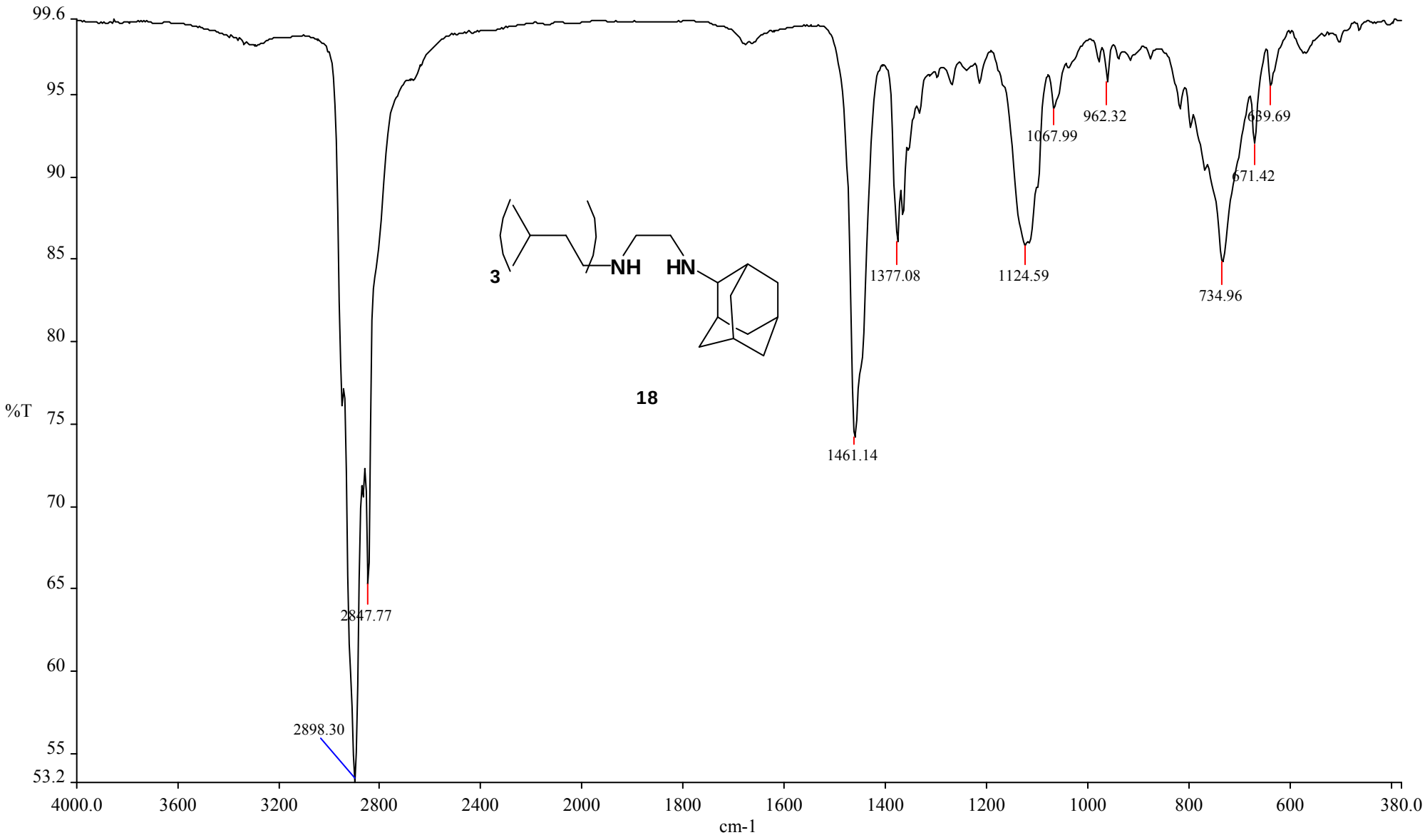
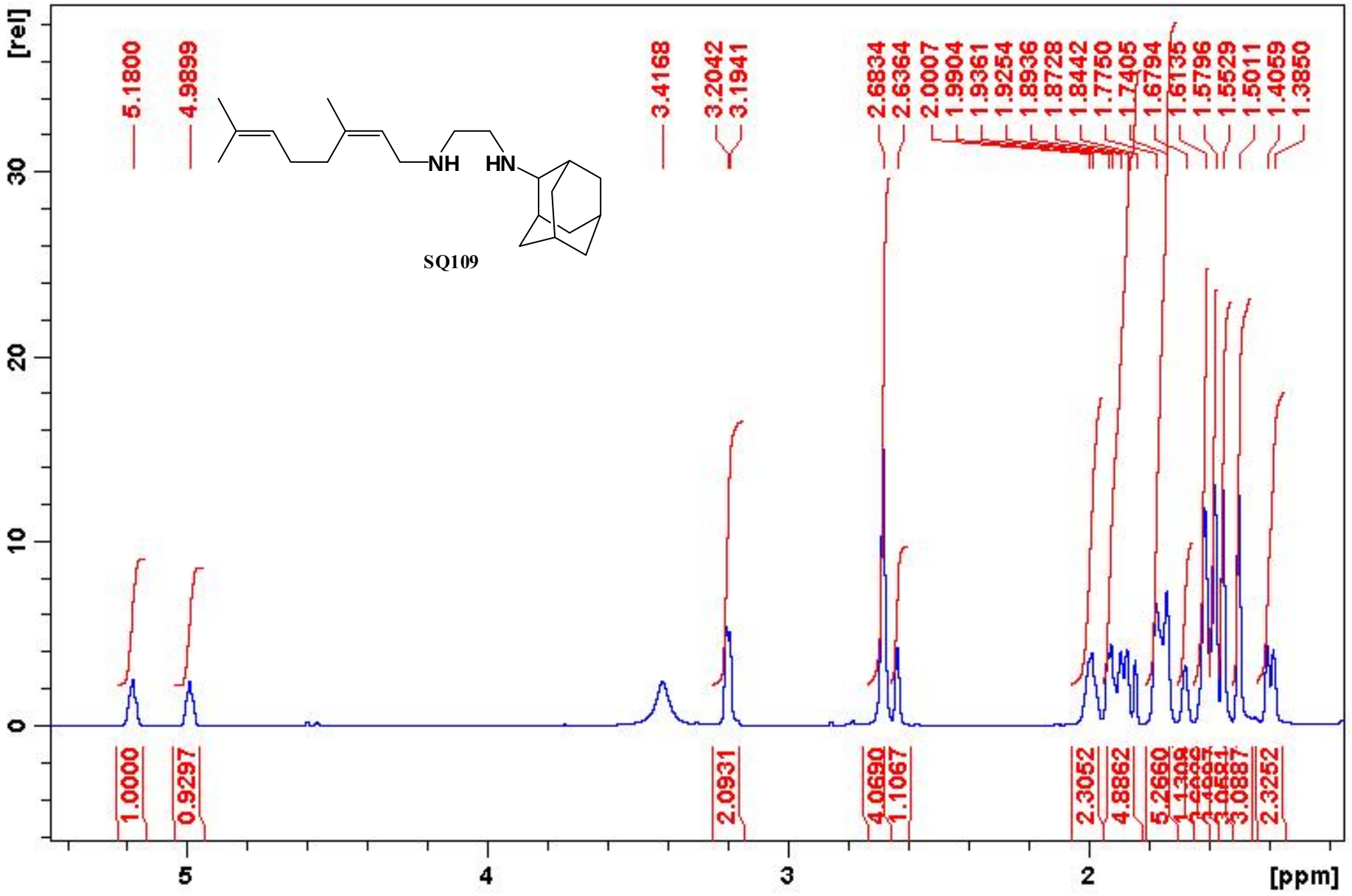
47.7237
46.3376
39.1815
37.8667
37.4931
37.2780
37.2452
32.0651
31.2439
30.8854
27.8619
27.7203
27.5338
24.6155
22.5910
22.4902
19.6464
NH
HN
3

18

^{13}C NMR spectrum of compound 18 in $CDCl_3$


99.6
95
90
85
80
75
70
65
60
55
53.2
%T
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380.0
cm-1
2898.30
2847.77
1461.14
1377.08
1124.59
1067.99
962.32
734.96
671.42
639.69
3
NH
HN
18

IR spectrum of compound 18


[rel]
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20
10
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3.2042
3.1941
2.6834
2.6364
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1.9254
1.8936
1.8728
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1.7750
1.7405
1.6794
1.6135
1.5796
1.5529
1.5011
1.4059
1.3850
NH
HN
SQ109
1.0000
0.9297
2.0931
4.0690
1.1067
2.3052
4.8862
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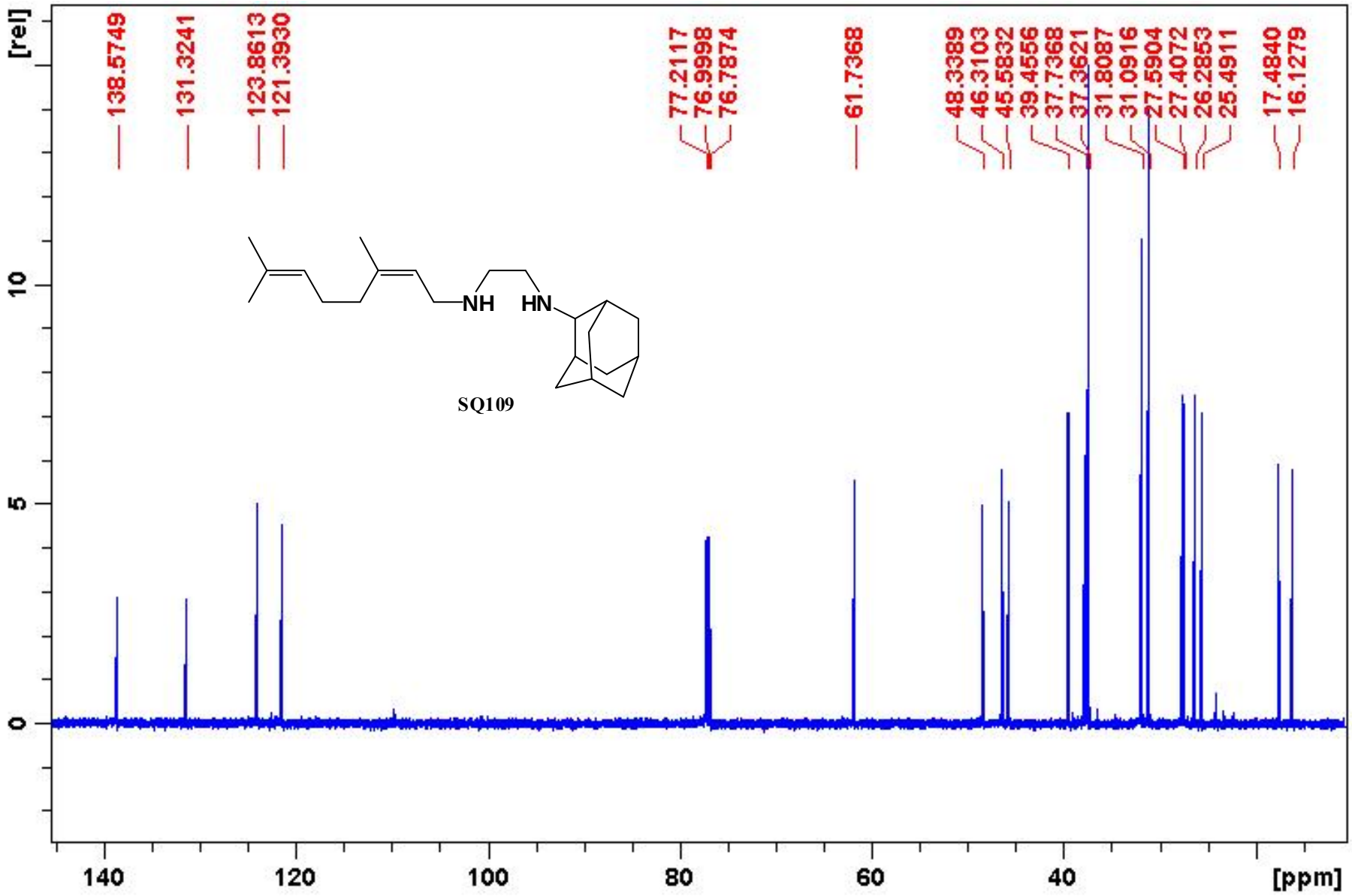
3.0887
2.3252
5
4
3
2
[ppm]

^{1}H NMR spectrum of SQ109 in $CDCl_3$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123.8613
121.3930
77.2117
76.9998
76.7874
61.7368
48.3389
46.3103
45.5832
39.4556
37.7368
37.3621
31.8087
31.0916
27.5904
27.4072
26.2853
25.4911
17.4840
16.1279
NH
HN
SQ109
10
5
0
140
120
100
80
60
40
[ppm]

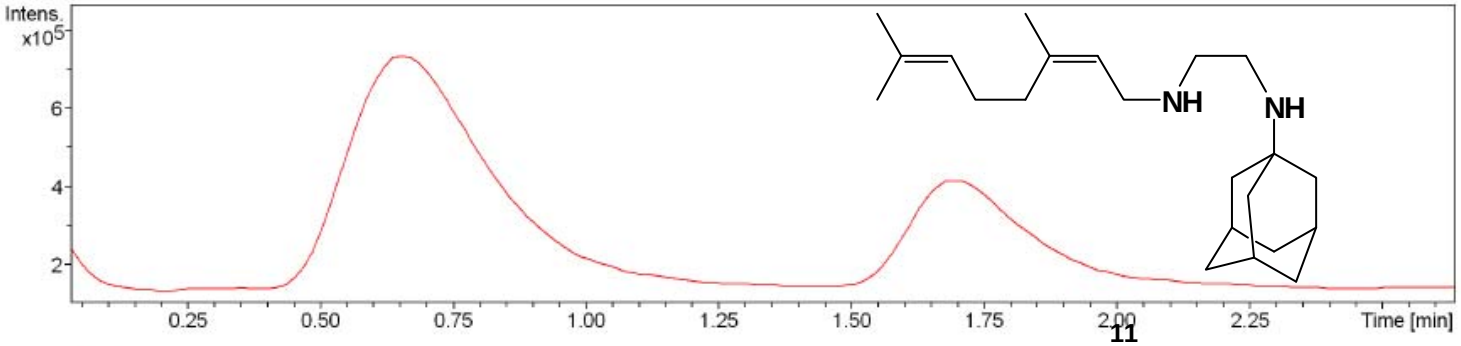
^{13}C NMR spectrum of SQ109 in $CDCl_3$

Analysis Info

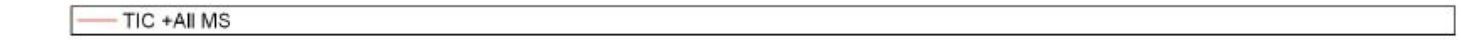
Analysis Name	D:\Data\kenny\GKM4A000001.d	Acquisition Date	9/10/2009 7:21:01 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM4A	Instrument / Ser#	micrOTOF-Q 10139
Comment	geranyl ethylene diamine 1-adamantane		

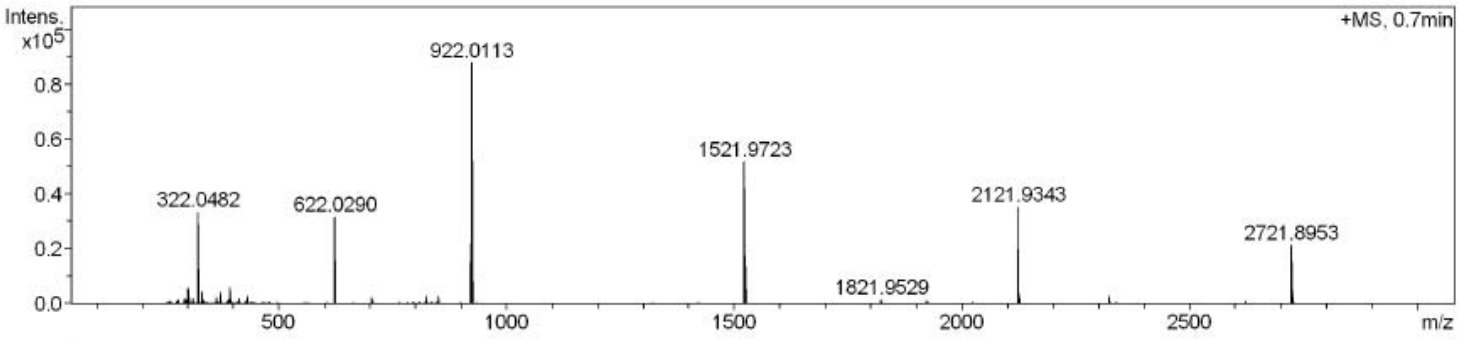
Acquisition Parameter

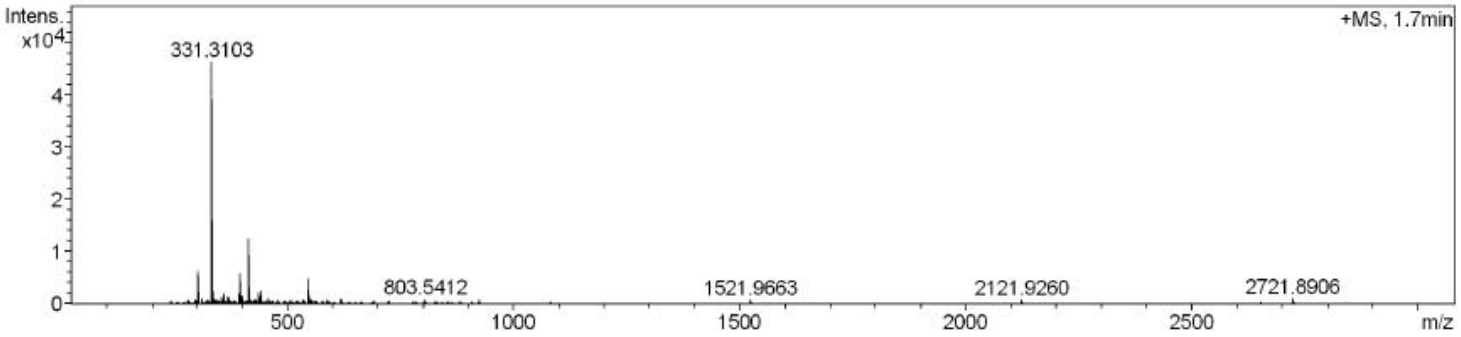
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



11







Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
331.3103	1	C 22 H 39 N 2	331.3108	1.5	0.9	4.5	ok	even	18.43	0.0295	0.0008	0.0203	0.0003	0.6976

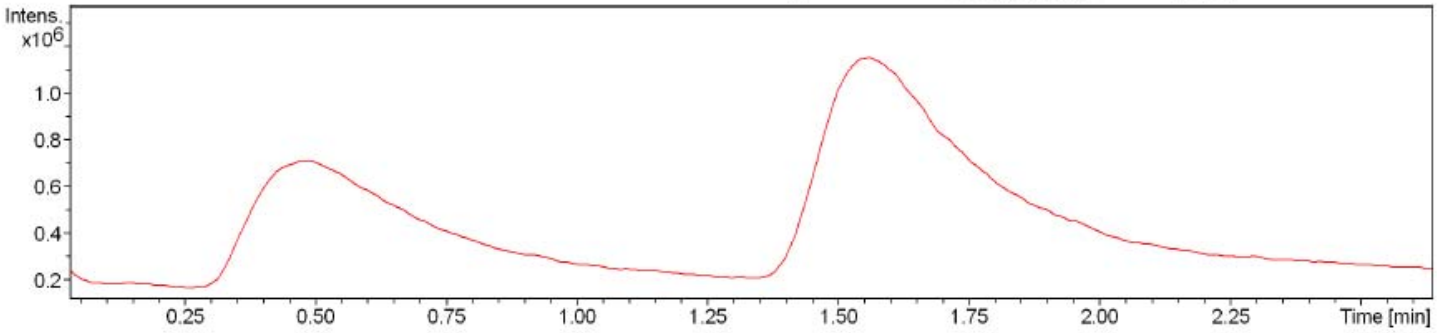
HRMS spectrum of compound 11

Analysis Info

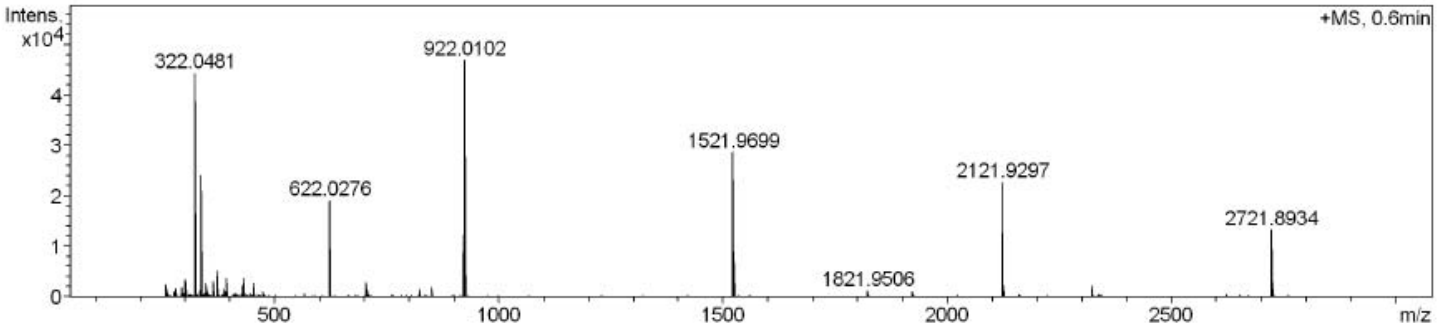
Analysis Name	D:\Data\kenny\GKM4ARR000004.d	Acquisition Date	10/7/2009 12:20:34 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	BRANCHED ALKANE 1-ADAMANTYL	Instrument / Ser#	micrOTOF-Q 10139
Comment			

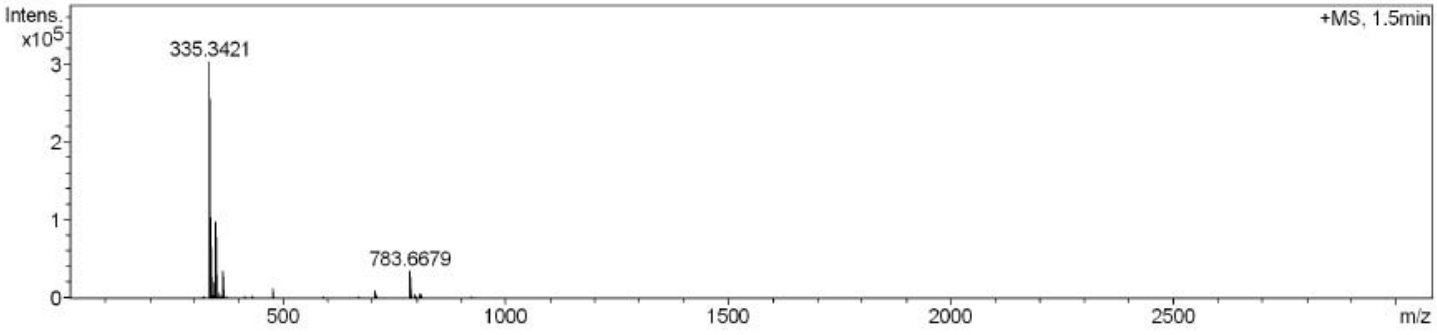
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



TIC +All MS





Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
335.3421	1	C 22 H 43 N 2	335.3421	-0.0	0.4	2.5	ok	even	1.41	0.0020	0.0003	0.0014	0.0006	0.8427

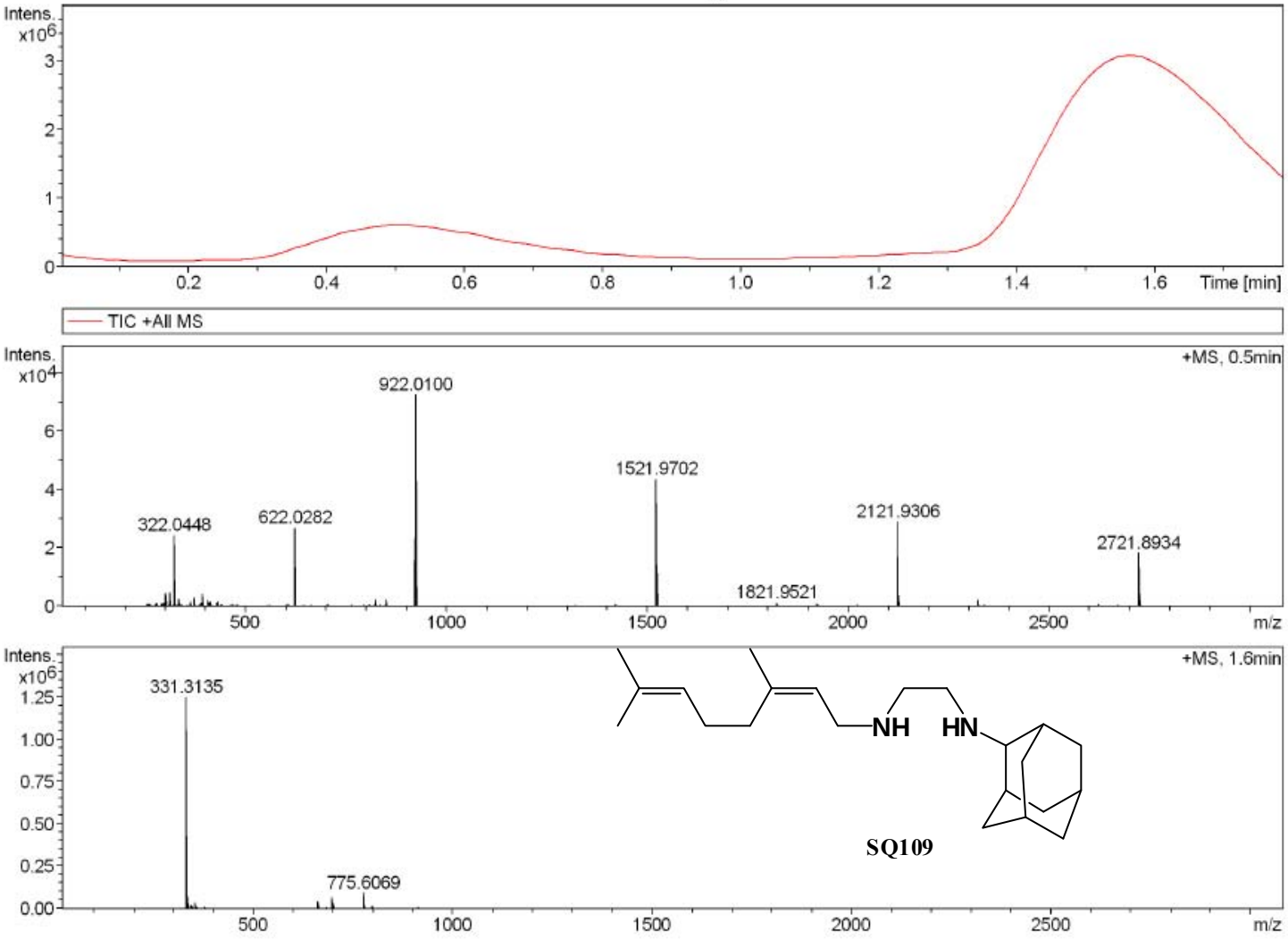
HRMS spectrum of compound 12

Analysis Info

Analysis Name	D:\Data\kenny\SQ109000001.d	Acquisition Date	9/10/2009 6:43:51 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	SQ109	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
331.3135														
	1	C 22 H 39 N 2	331.3108	-8.1	-3.3	4.5	ok	even	175.20	0.2195	0.0023	0.0898	0.0041	0.8427
	2	C 14 H 37 N 9	331.3166	9.6	14.2	1.0	ok	odd	211.08	0.2817	0.0051	0.1111	0.0039	0.9347

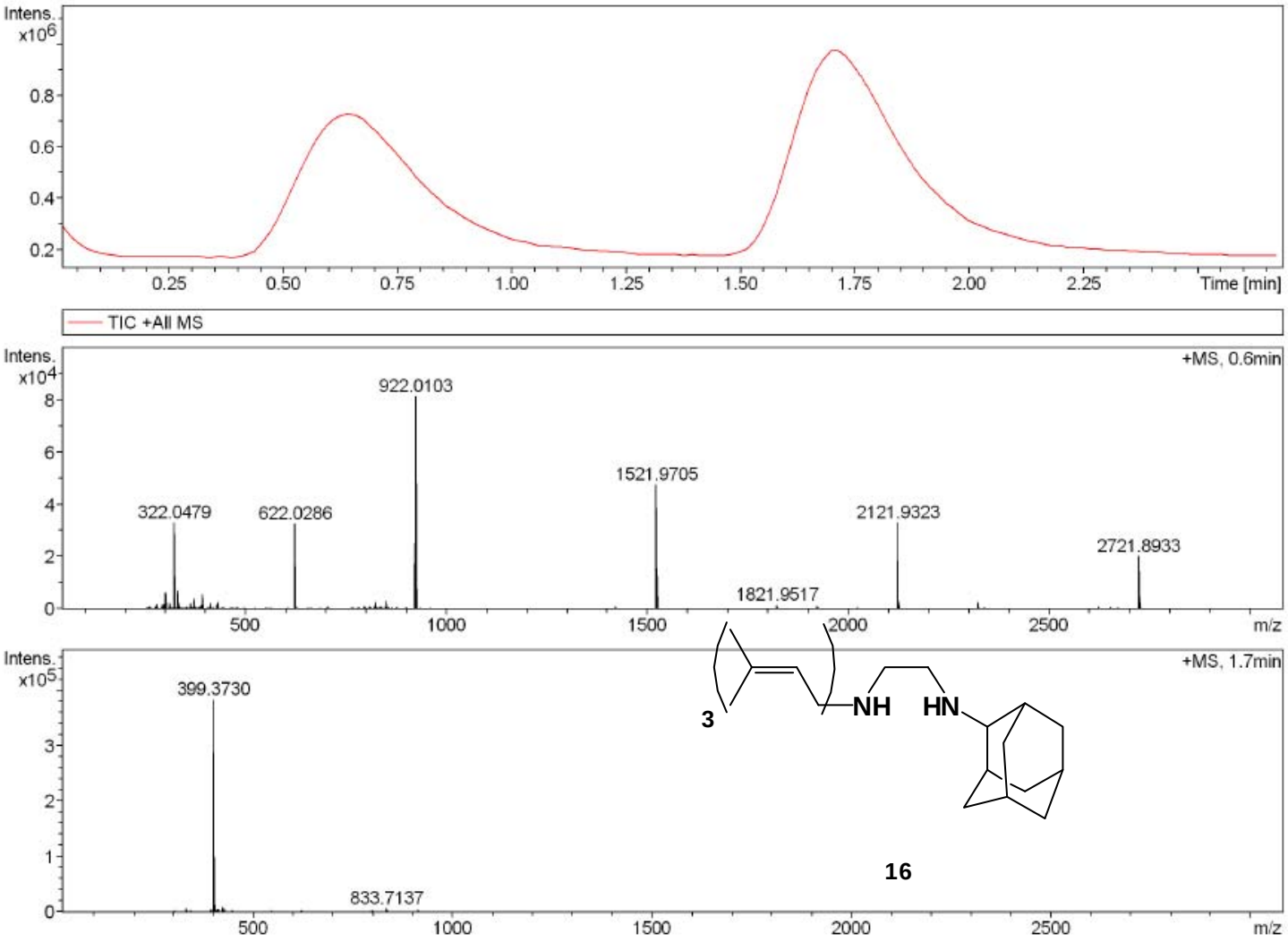
HRMS spectrum of SQ109

Analysis Info

Analysis Name	D:\Data\kenny\GKM1A000001.d	Acquisition Date	9/10/2009 6:51:51 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM1A	Instrument / Ser#	micrOTOF-Q 10139
Comment	trans- trans farnesyl ethylene diamine 2-adamantane		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



16

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
399.3730														
	1	C 27 H 47 N 2	399.3734	1.0	2.0	5.5	ok	even	1.62	0.0023	0.0010	0.0009	0.0014	0.5212
	2	C 24 H 49 N O 3	399.3707	-5.7	-4.7	1.0	ok	odd	19.21	0.0285	0.0020	0.0122	0.0015	0.8427

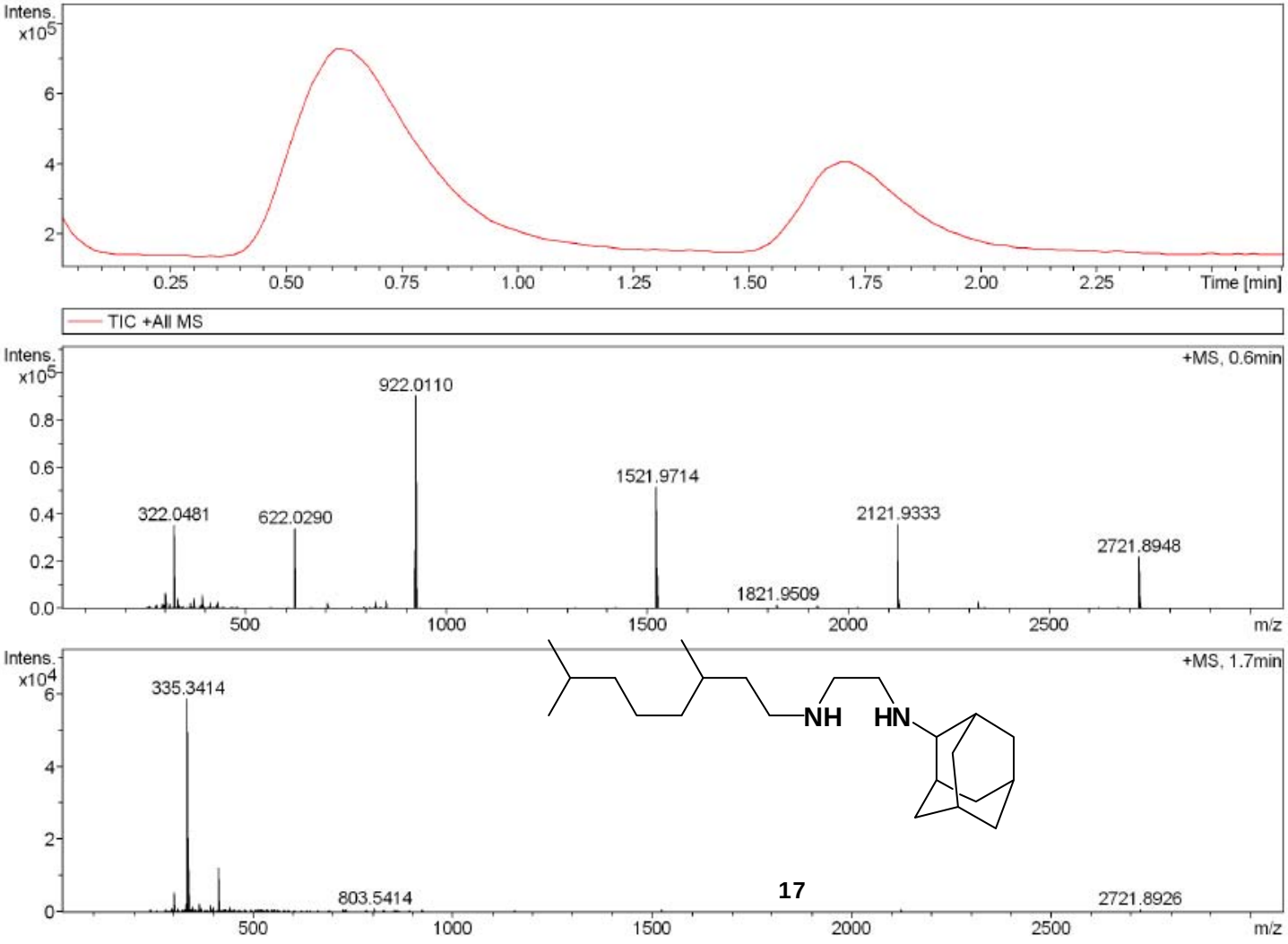
HRMS spectrum of compound 16

Analysis Info

Analysis Name	D:\Data\kenny\GKM3A000001.d	Acquisition Date	9/10/2009 7:11:22 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM3A	Instrument / Ser#	micrOTOF-Q 10139
Comment	reduced geranyl ethylene diamine 2-adamantane (reduced SQ109)		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
335.3414	1	C 22 H 43 N 2	335.3421	2.0	2.5	2.5	ok	even	7.52	0.0110	0.0009	0.0048	0.0007	0.8427

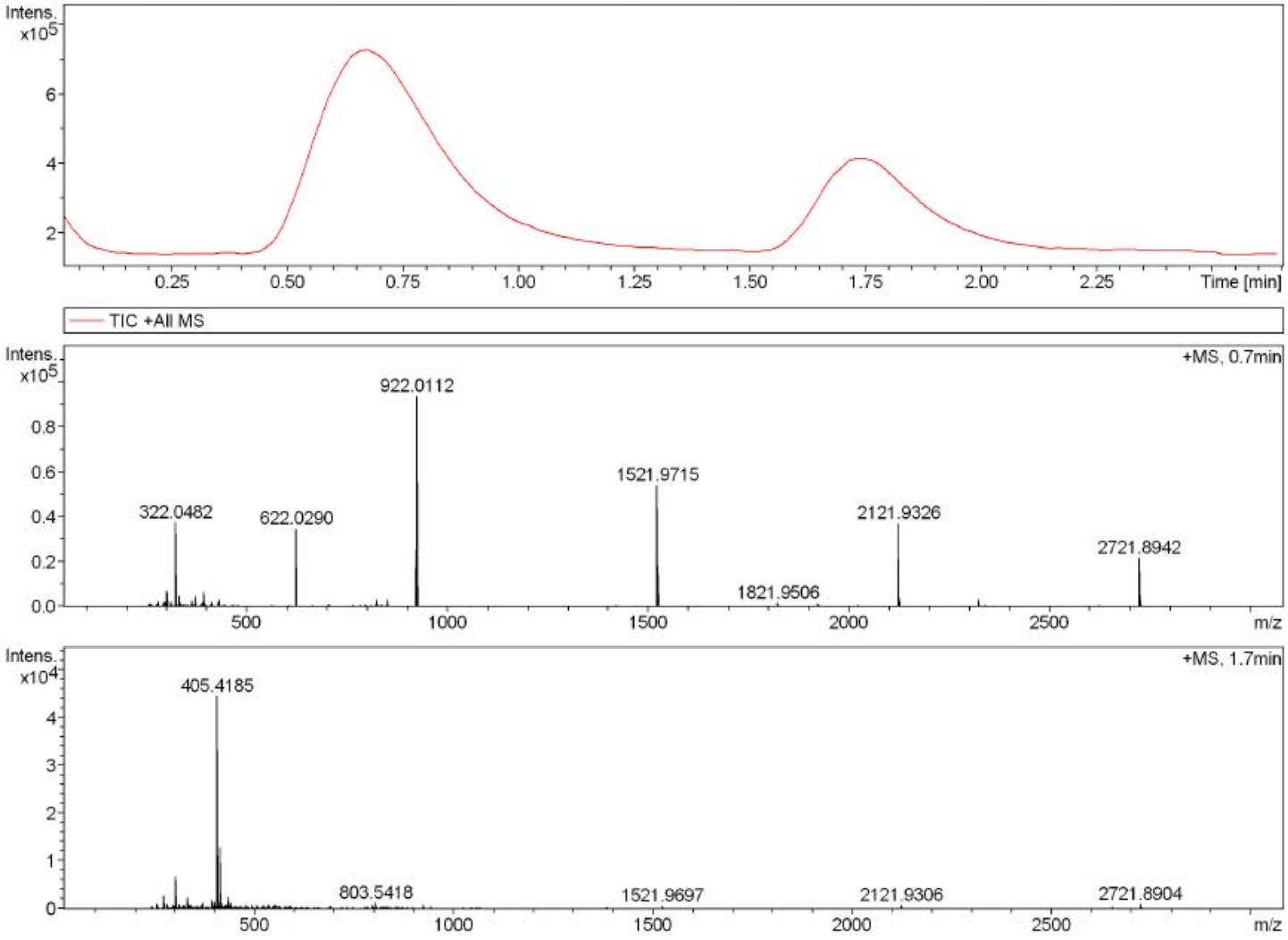
HRMS spectrum of compound 17

Analysis Info

Analysis Name	D:\Data\kenny\GKM2A000001.d	Acquisition Date	9/10/2009 7:07:00 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM2A	Instrument / Ser#	micrOTOF-Q 10139
Comment	reduced trans- trans farnesyl ethylene diamine 2-adamantane		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
405.4185	1	C 27 H 53 N 2	405.4203	4.4	4.8	2.5	ok	even	13.83	0.0203	0.0021	0.0075	0.0015	0.8427

HRMS spectrum of compound 18

CHAPTER 5

NMR ELUCIDATION OF NOVEL SQ109 DERIVATIVES

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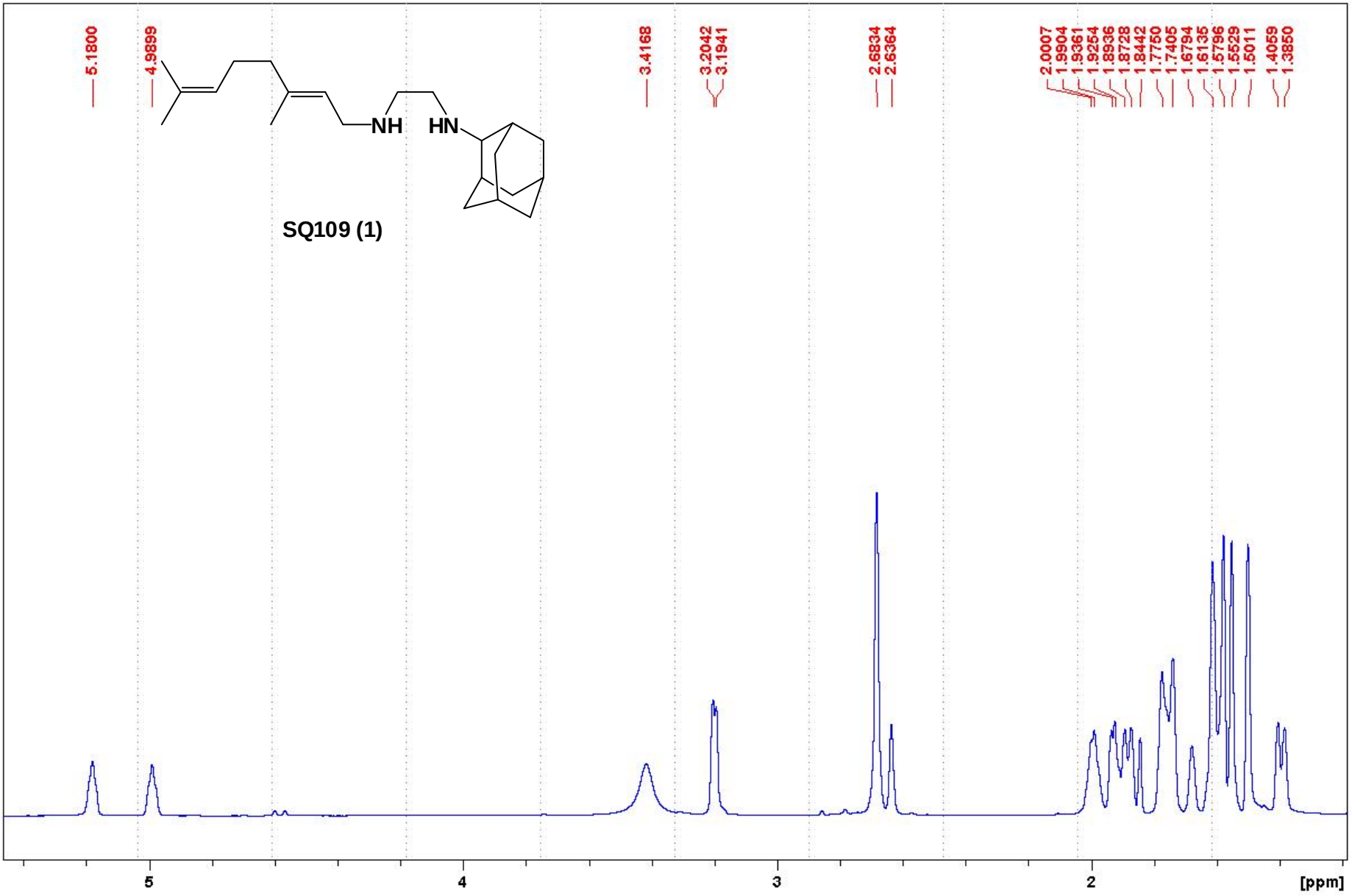
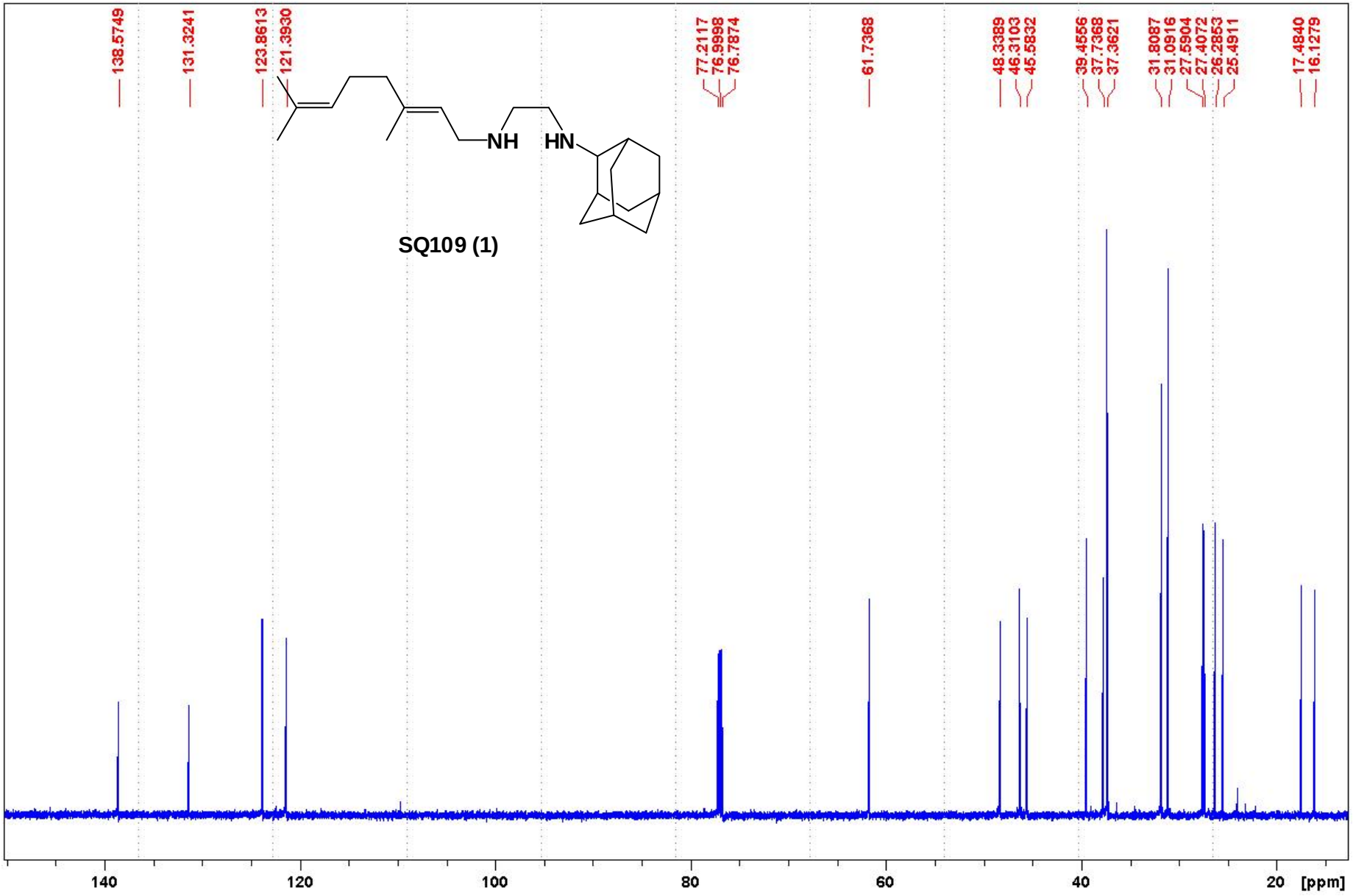
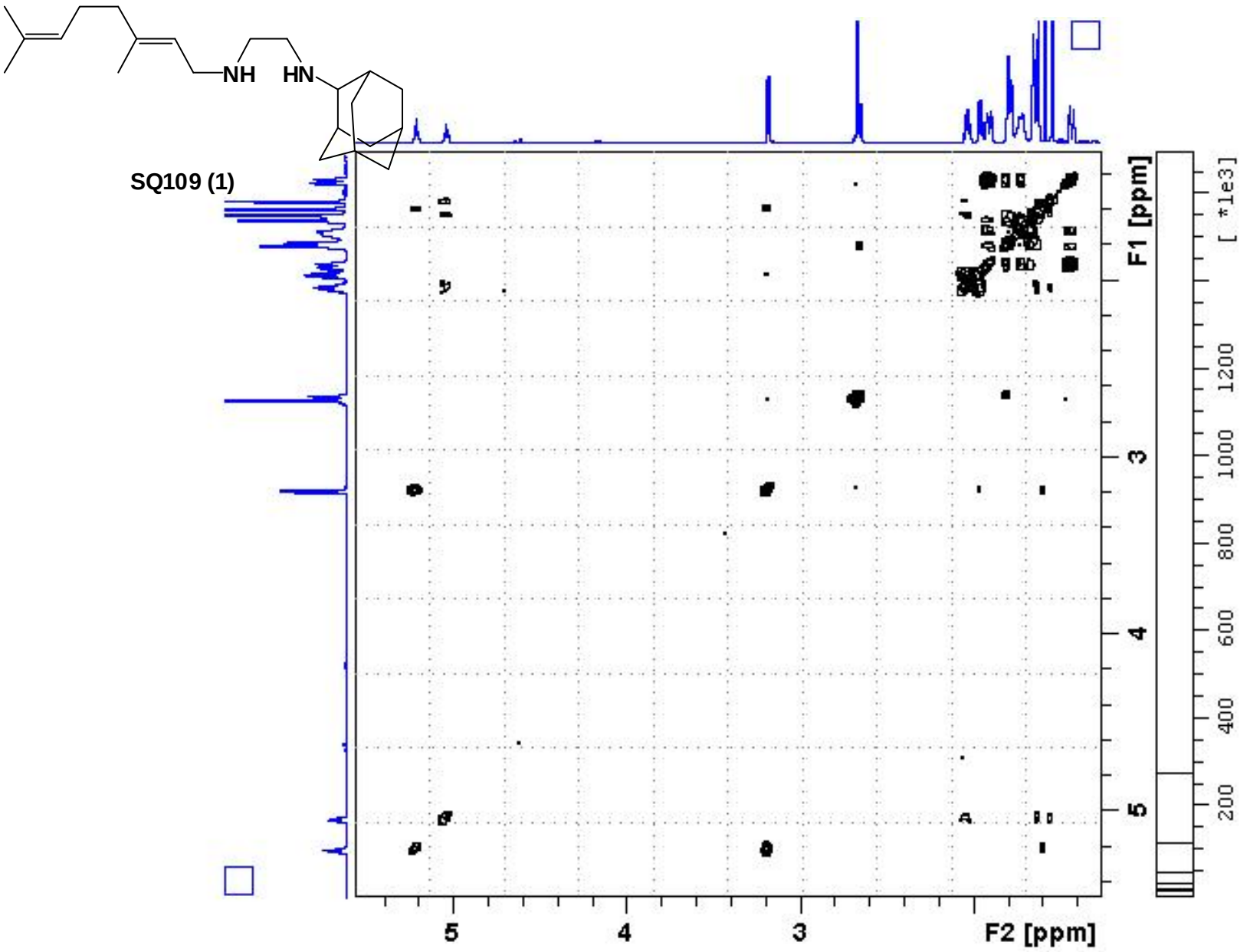
[c] School of Pharmacy and Pharmacology, University of KwaZulu-Natal, Durban, South Africa


5.1800
4.9899
3.4168
3.2042
3.1941
2.6834
2.6364
2.0007
1.9904
1.9361
1.9254
1.8936
1.8728
1.8442
1.7750
1.7405
1.6794
1.6135
1.5796
1.5529
1.5011
1.4059
1.3850
NH
HN
SQ109 (1)
5
4
3
2
[ppm]

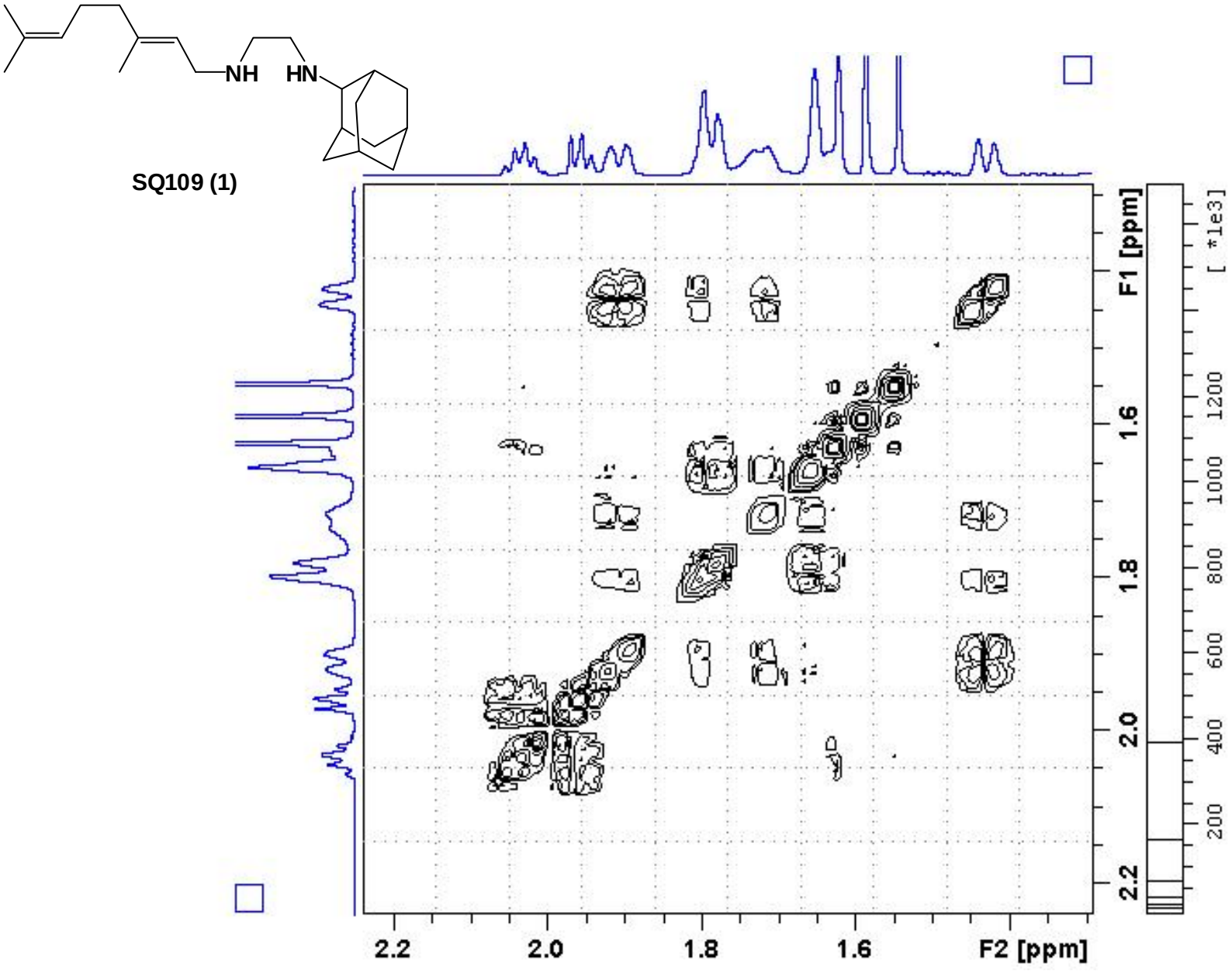
^{1}H spectrum of SQ109


138.5749
131.3241
123.8613
121.3930
77.2117
76.9998
76.7874
61.7368
48.3389
46.3103
45.5832
39.4556
37.7368
37.3621
31.8087
31.0916
27.5904
27.4072
26.2853
25.4911
17.4840
16.1279
NH
HN
SQ109 (1)
140
120
100
80
60
40
20
[ppm]

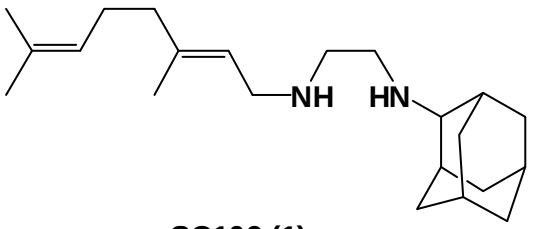
^{13}C APT spectrum of SQ109


NH
HN
SQ109 (1)
F1 [ppm]
F2 [ppm]
[*1e3]
200
400
600
800
1000
1200
3
4
5

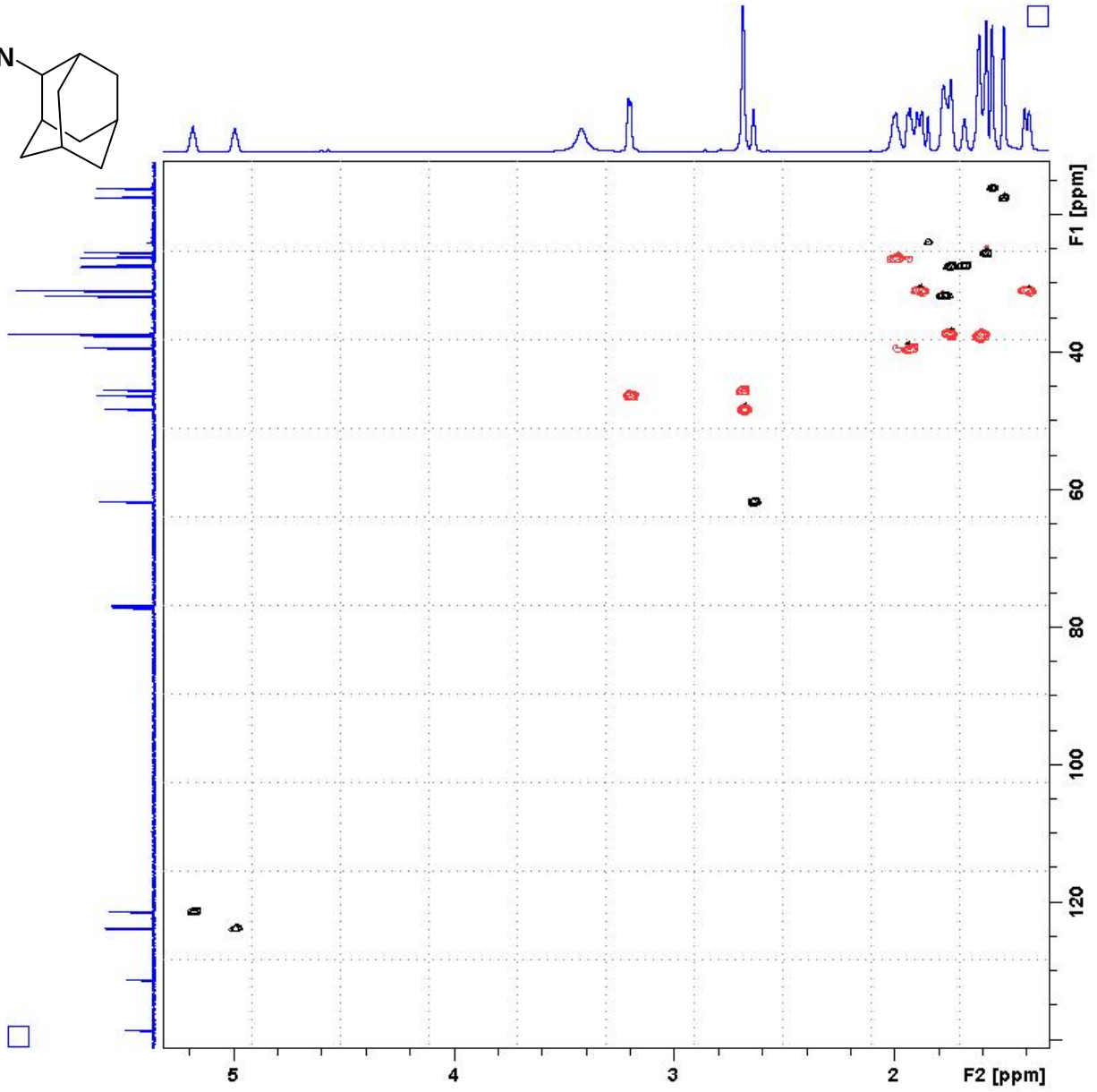
COSY spectrum of SQ109


NH
HN
SQ109 (1)
F1 [ppm]
F2 [ppm]
2.2
2.0
1.8
1.6
[*1e3]
200
400
600
800
1000
1200

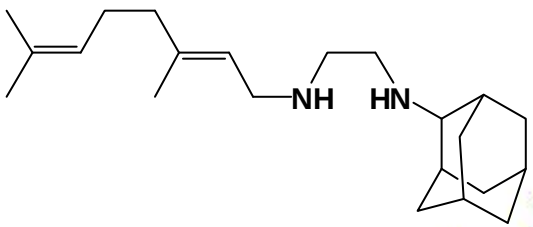
Expanded COSY spectrum of SQ109



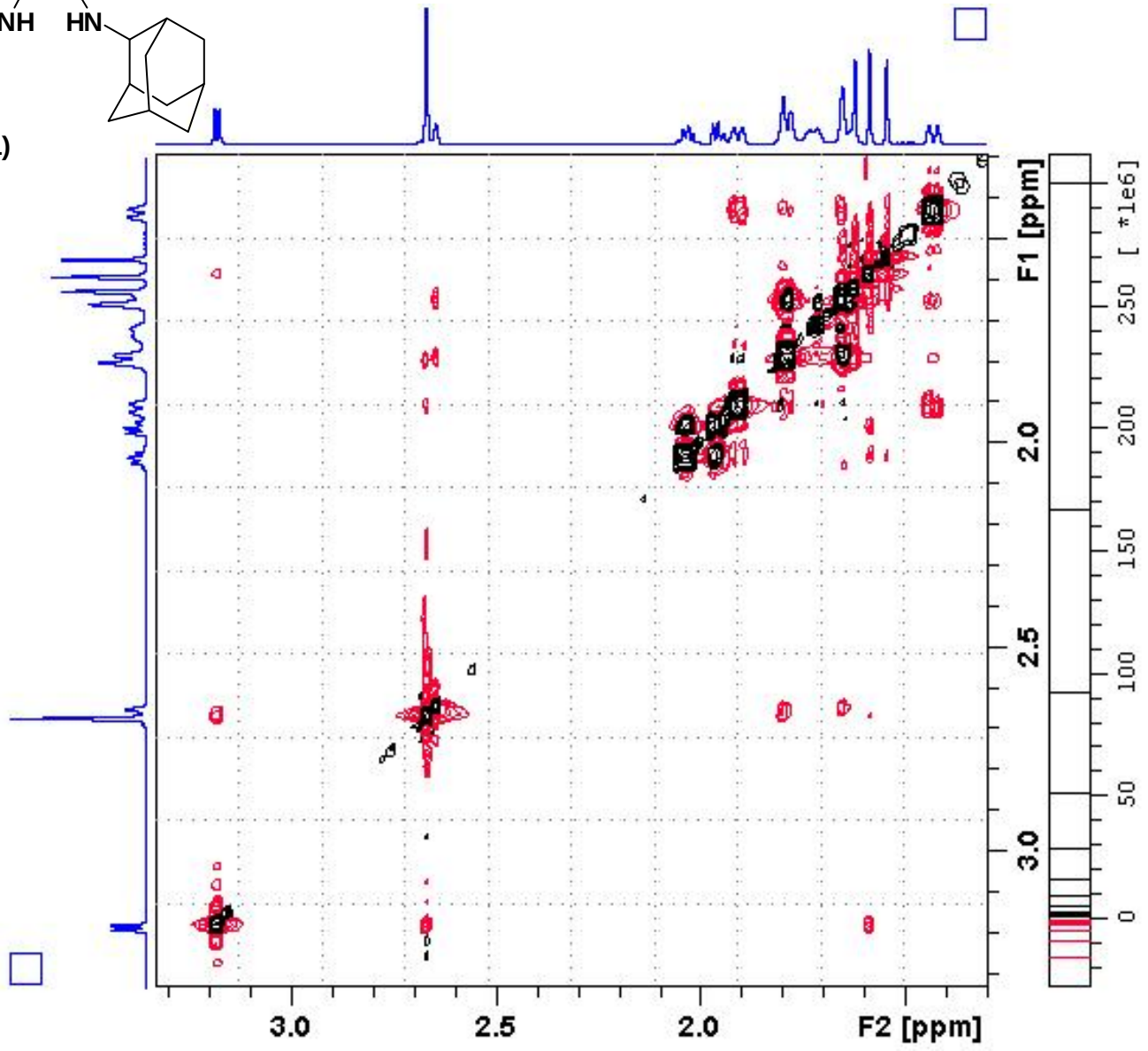
SQ109 (1)



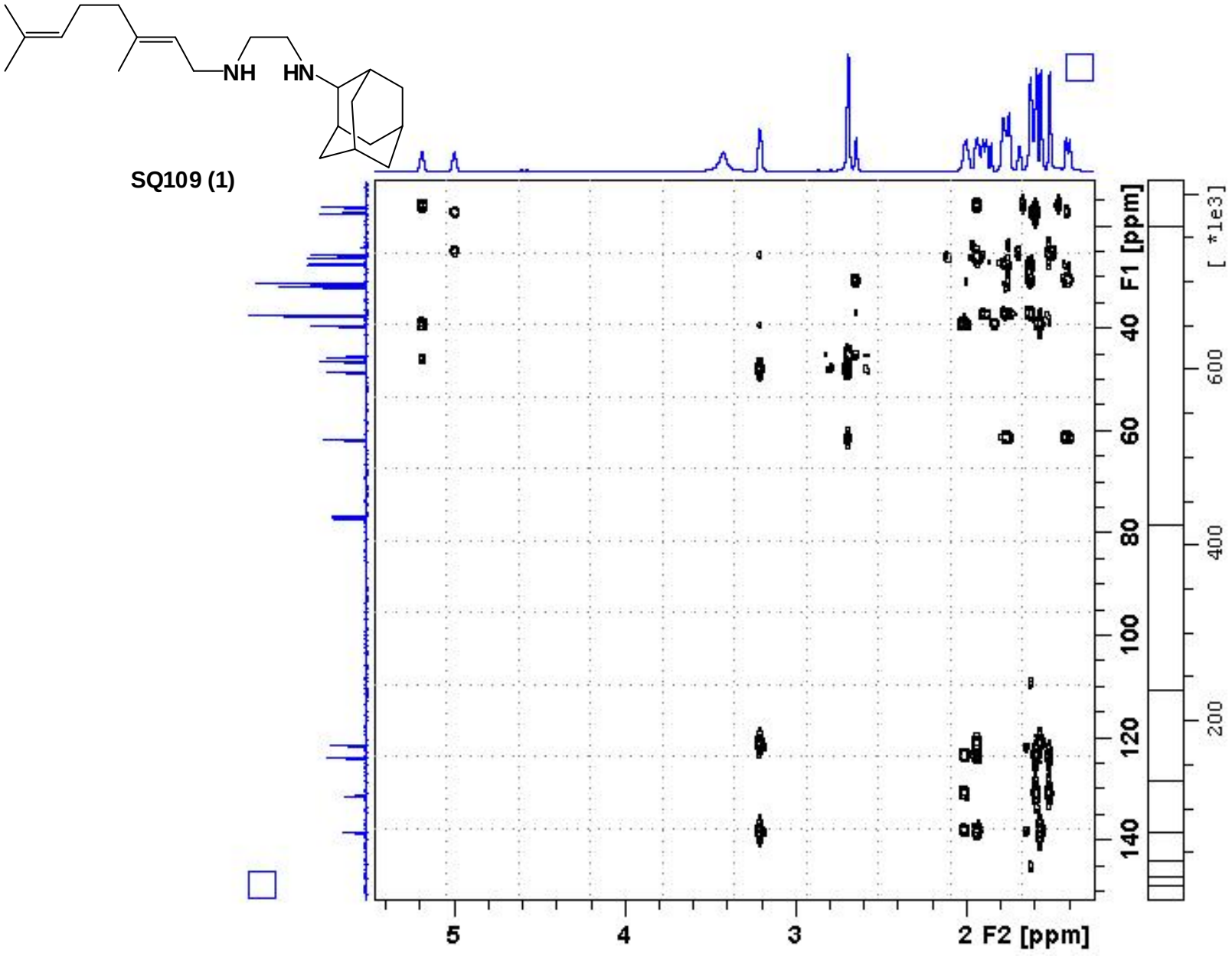
HSQC spectrum of SQ109



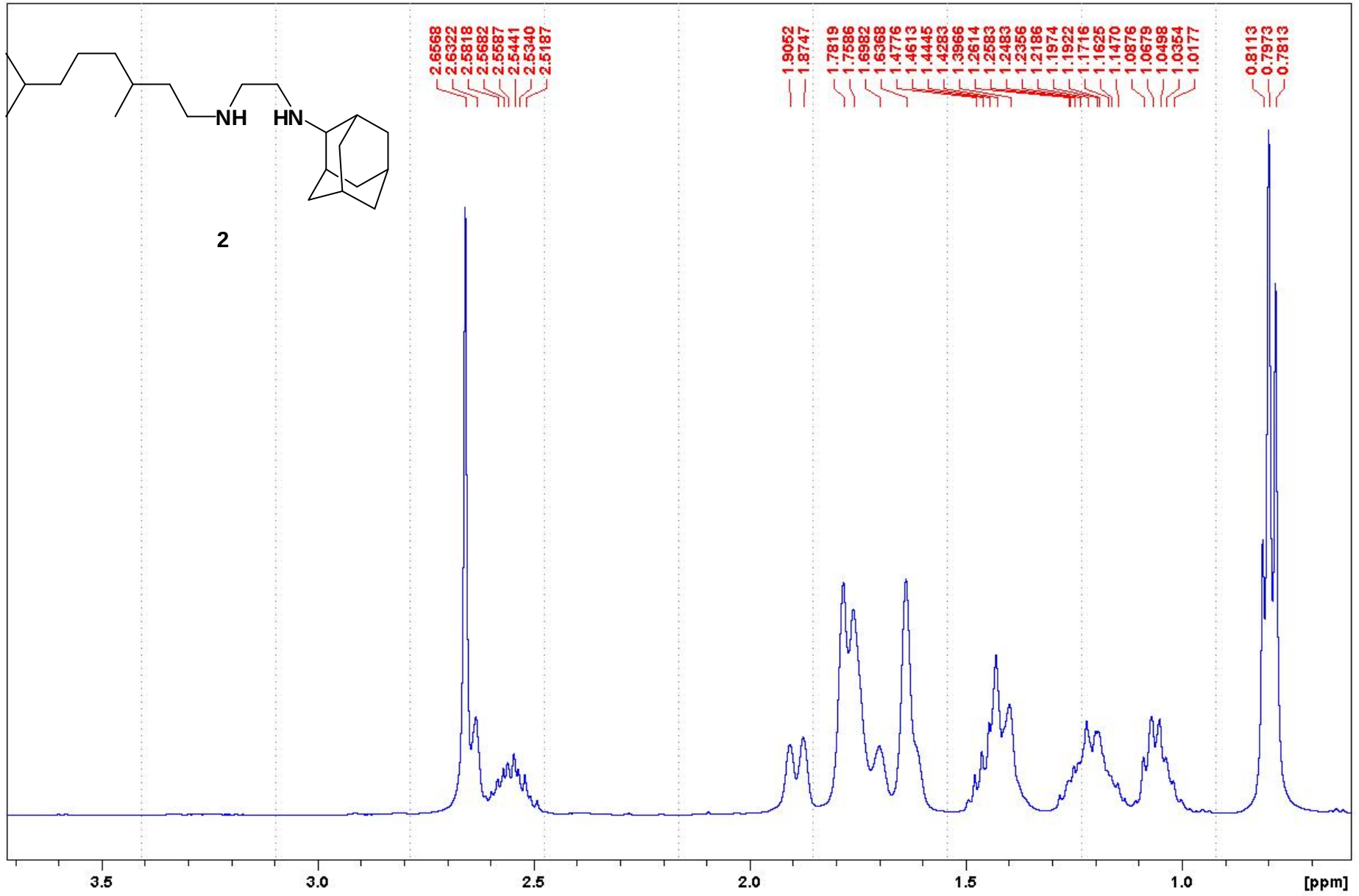
SQ109 (1)



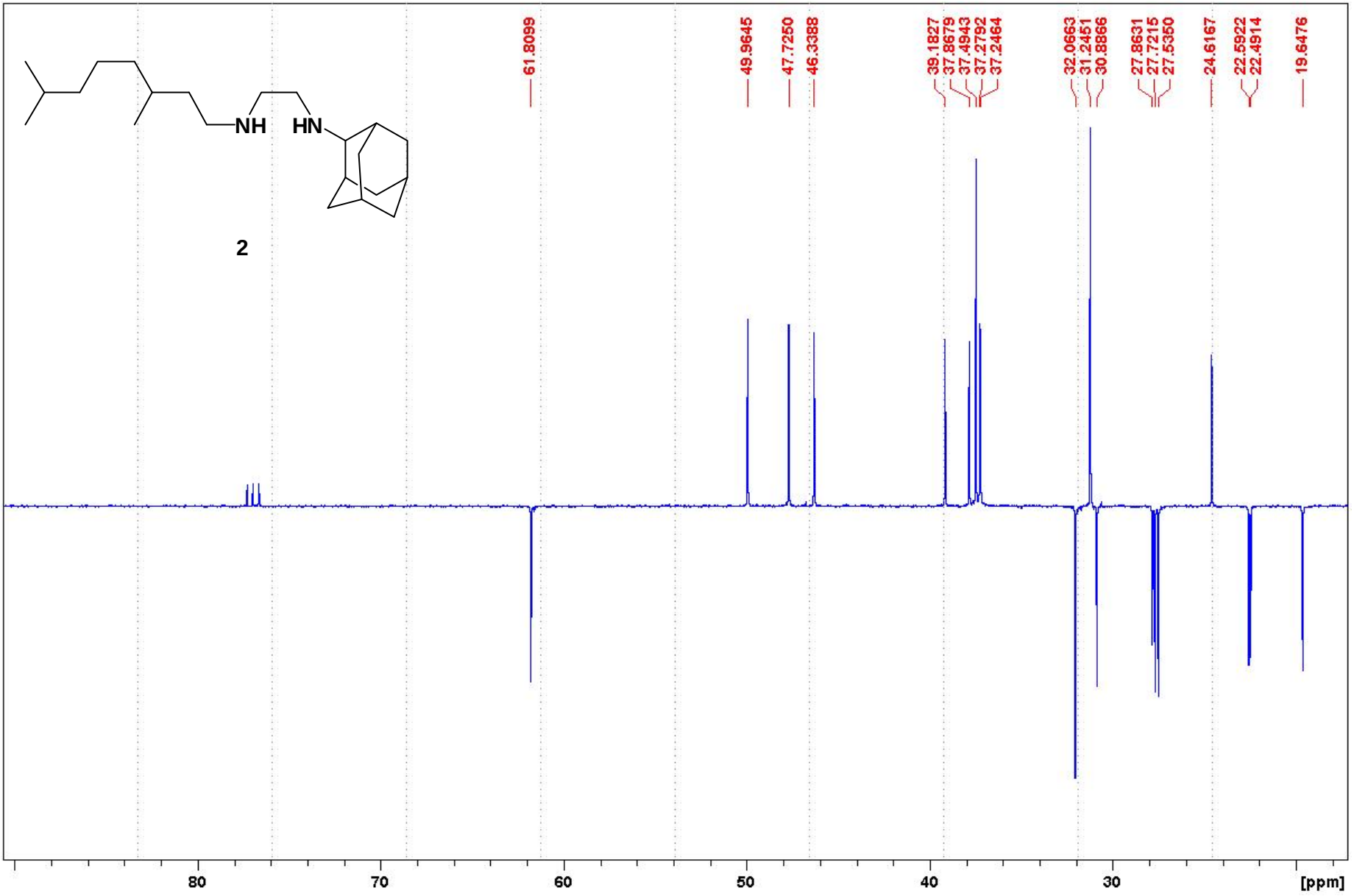
Expanded ROESY spectrum of SQ109


NH
HN
SQ109 (1)
F1 [ppm]
40
60
80
100
120
140
[*1e3]
600
400
200
5
4
3
2 F2 [ppm]

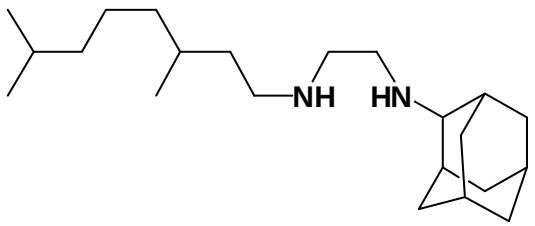
HMBC spectrum of SQ109


NH
HN
2
2.6568
2.6322
2.5818
2.5682
2.5587
2.5441
2.5340
2.5187
1.9052
1.8747
1.7819
1.7586
1.6982
1.6368
1.4776
1.4613
1.4445
1.4283
1.3966
1.2614
1.2583
1.2483
1.2356
1.2186
1.1974
1.1922
1.1716
1.1625
1.1470
1.0876
1.0679
1.0498
1.0354
1.0177
0.8113
0.7973
0.7813
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]

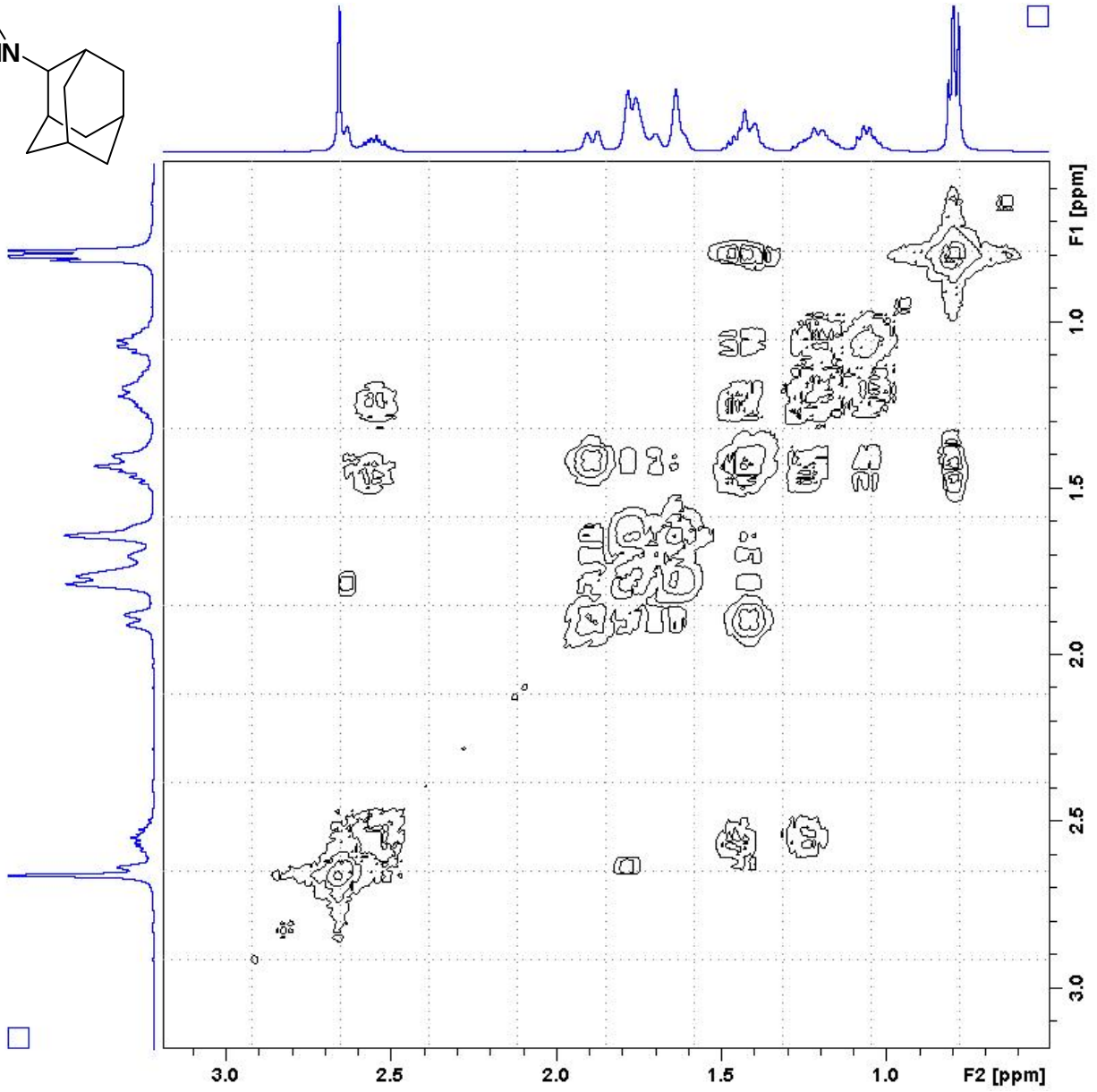
^{1}H spectrum of Compound 2


NH
HN
2
61.8099
49.9645
47.7250
46.3388
39.1827
37.8679
37.4943
37.2792
37.2464
32.0663
31.2451
30.8866
27.8631
27.7215
27.5350
24.6167
22.5922
22.4914
19.6476
80
70
60
50
40
30
[ppm]

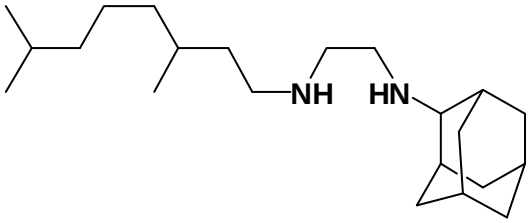
^{13}C APT spectrum of Compound 2



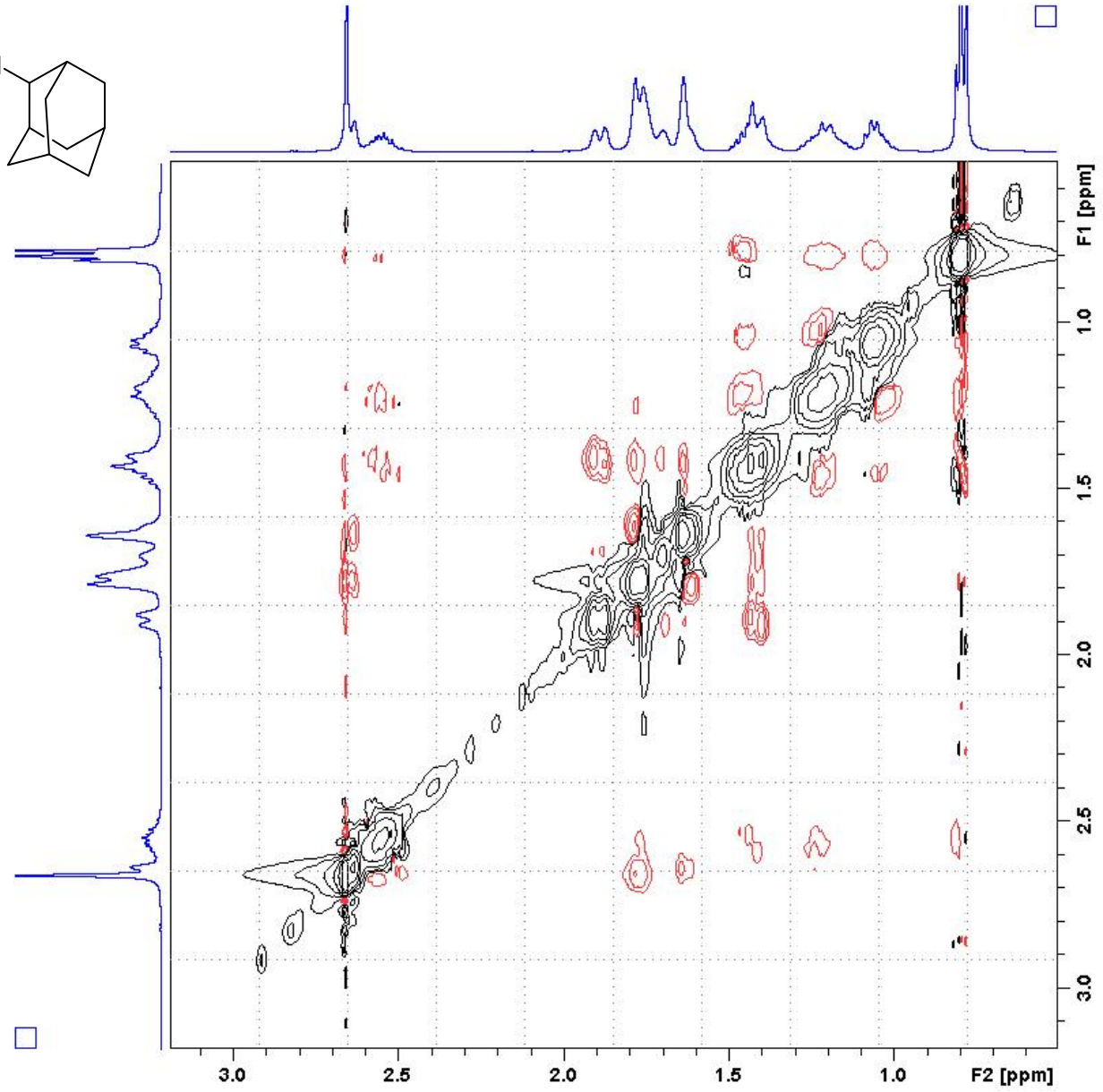
2



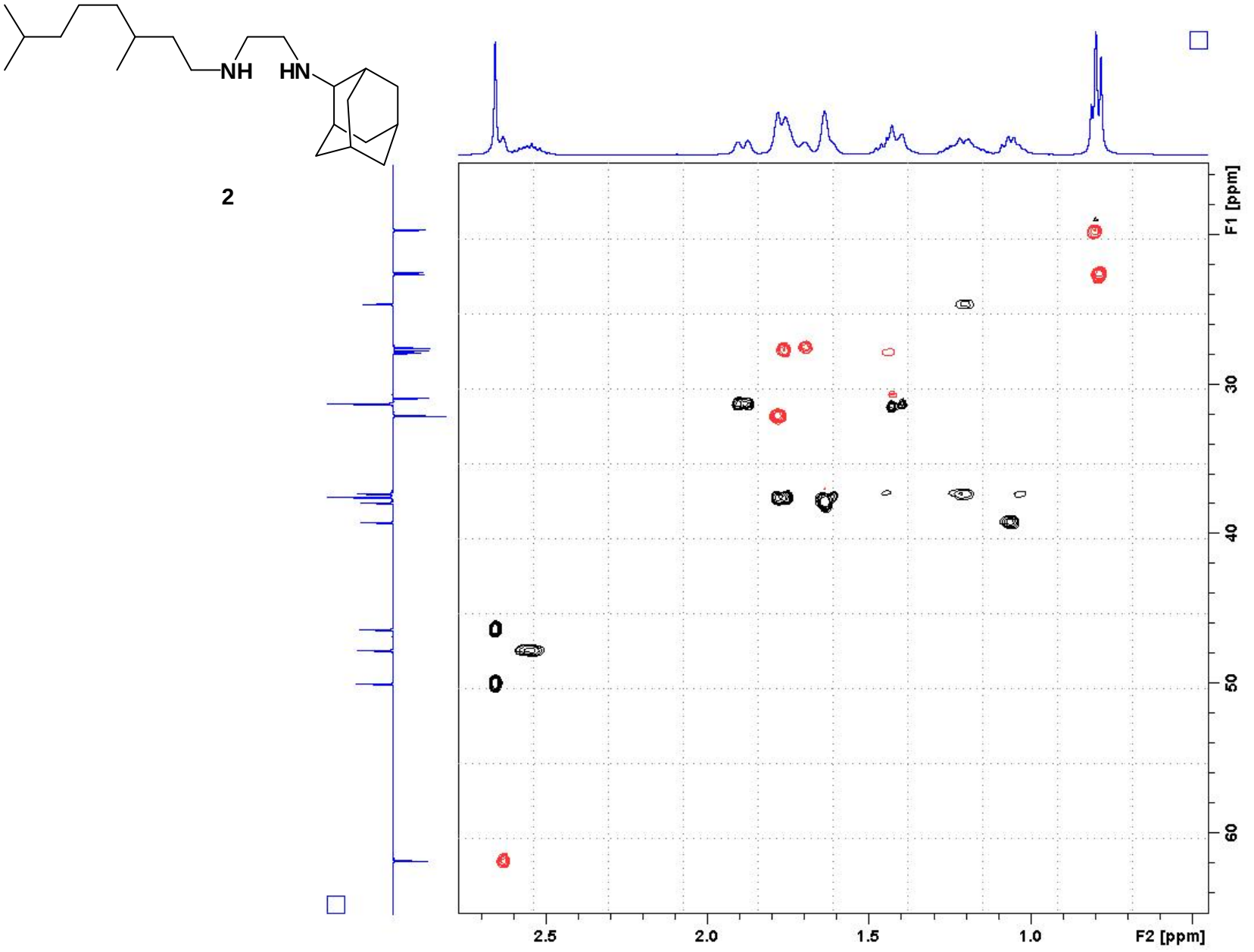
COSY spectrum of Compound 2



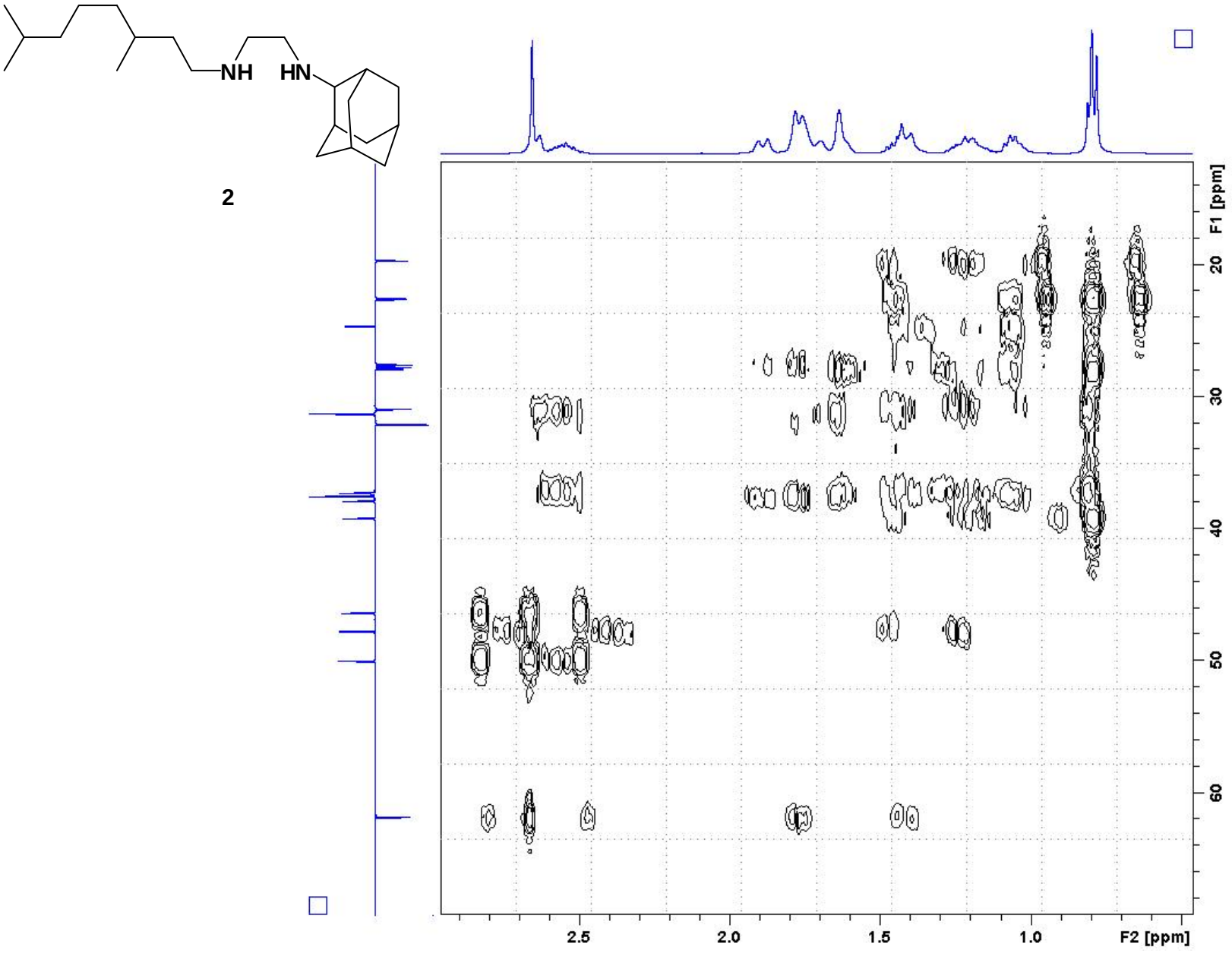
2



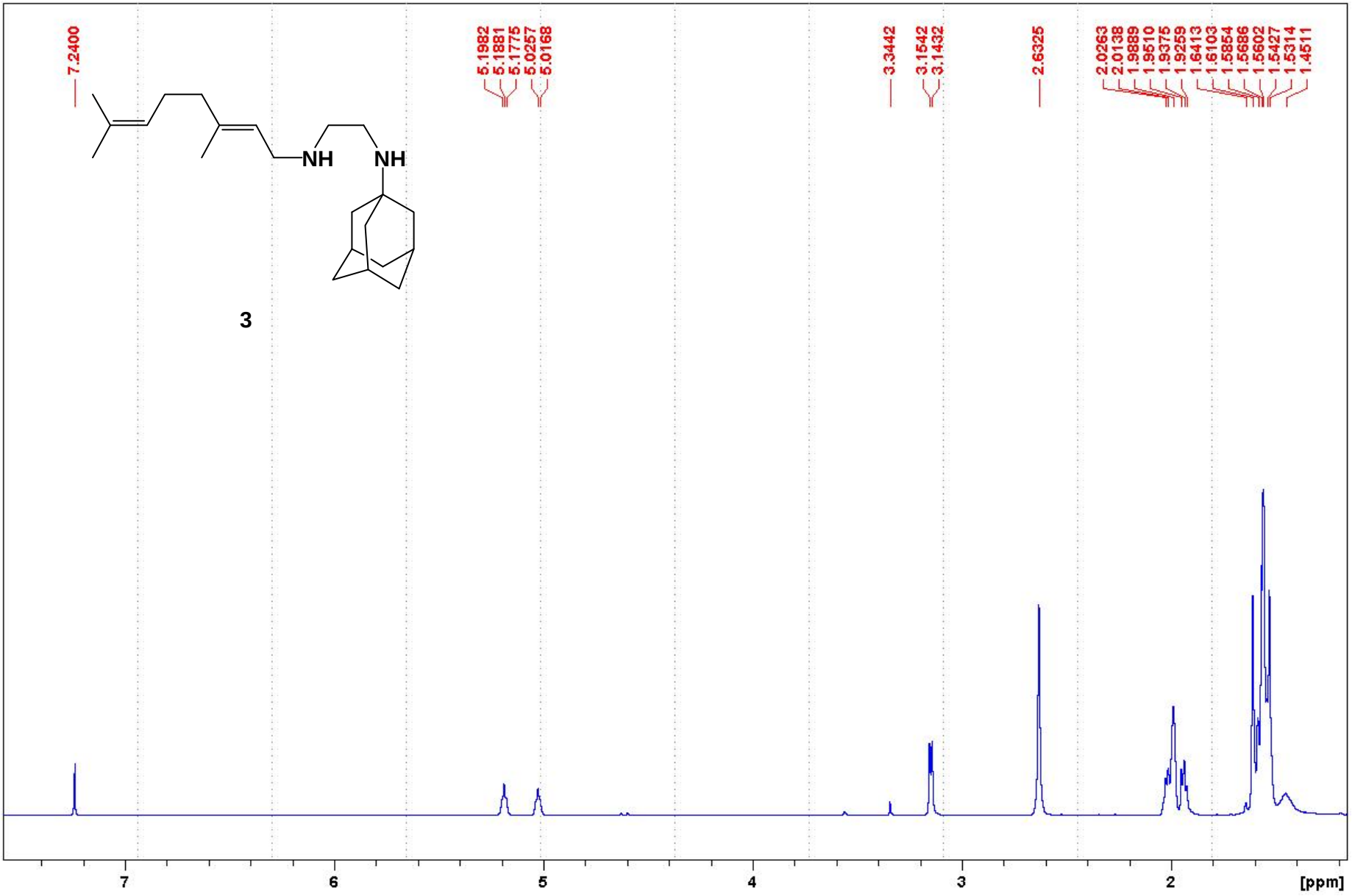
NOESY spectrum of Compound 2


NH
HN
2
F1 [ppm]
30
40
50
60
2.5
2.0
1.5
1.0
F2 [ppm]

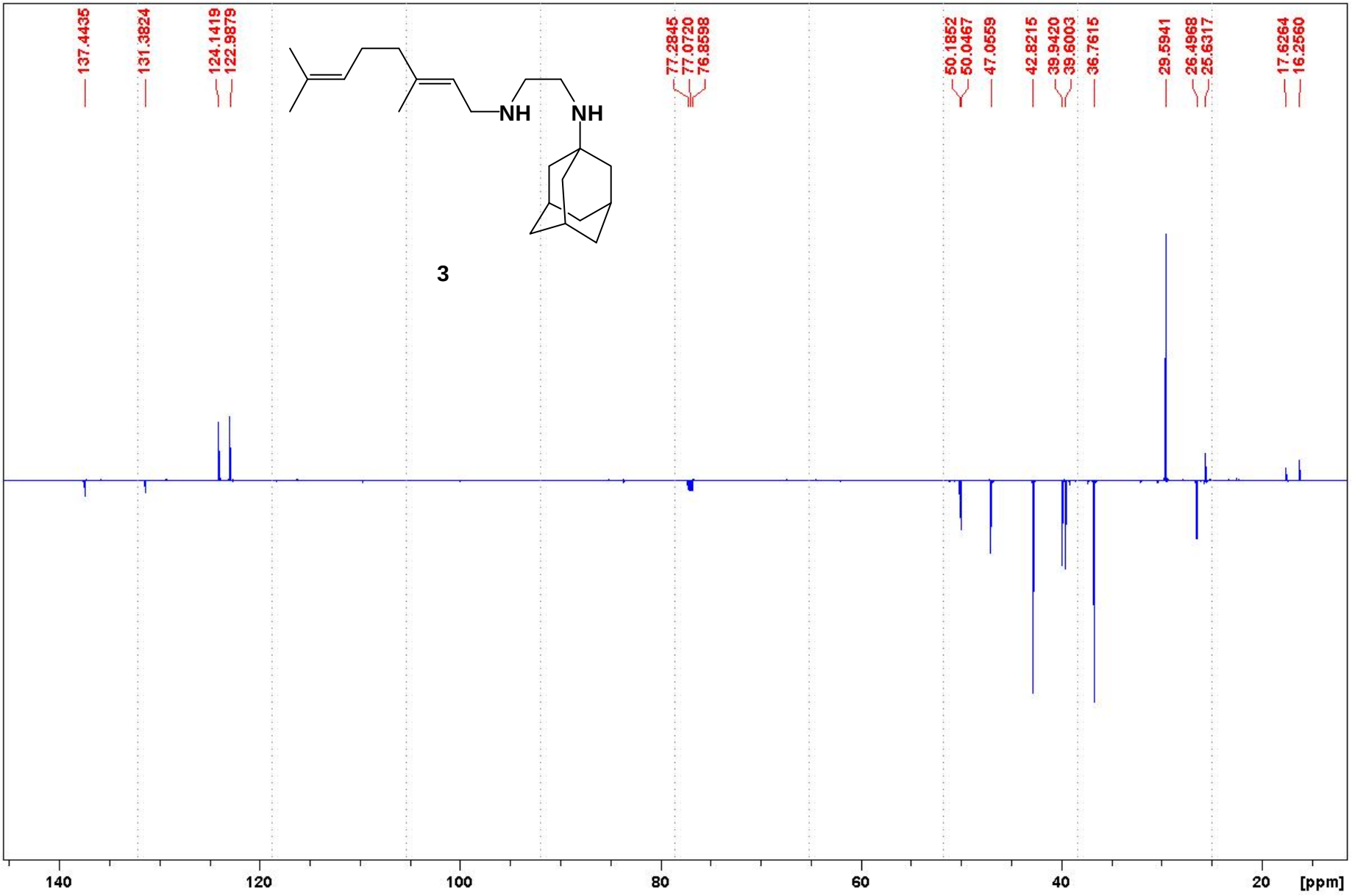
HSQC spectrum of Compound 2


NH
HN
2
F1 [ppm]
20
30
40
50
60
2.5
2.0
1.5
1.0
F2 [ppm]

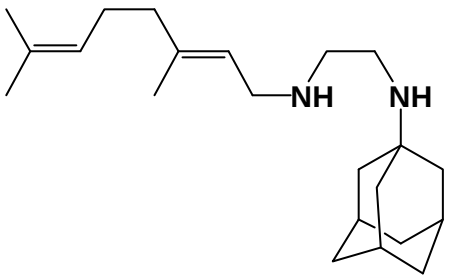
HMBC spectrum of Compound 2


7.2400
5.1982
5.1881
5.1775
5.0257
5.0168
3.3442
3.1542
3.1432
2.6325
2.0263
2.0138
1.9889
1.9510
1.9375
1.9259
1.6413
1.6103
1.5854
1.5686
1.5602
1.5427
1.5314
1.4511
NH
NH
3
7
6
5
4
3
2
[ppm]

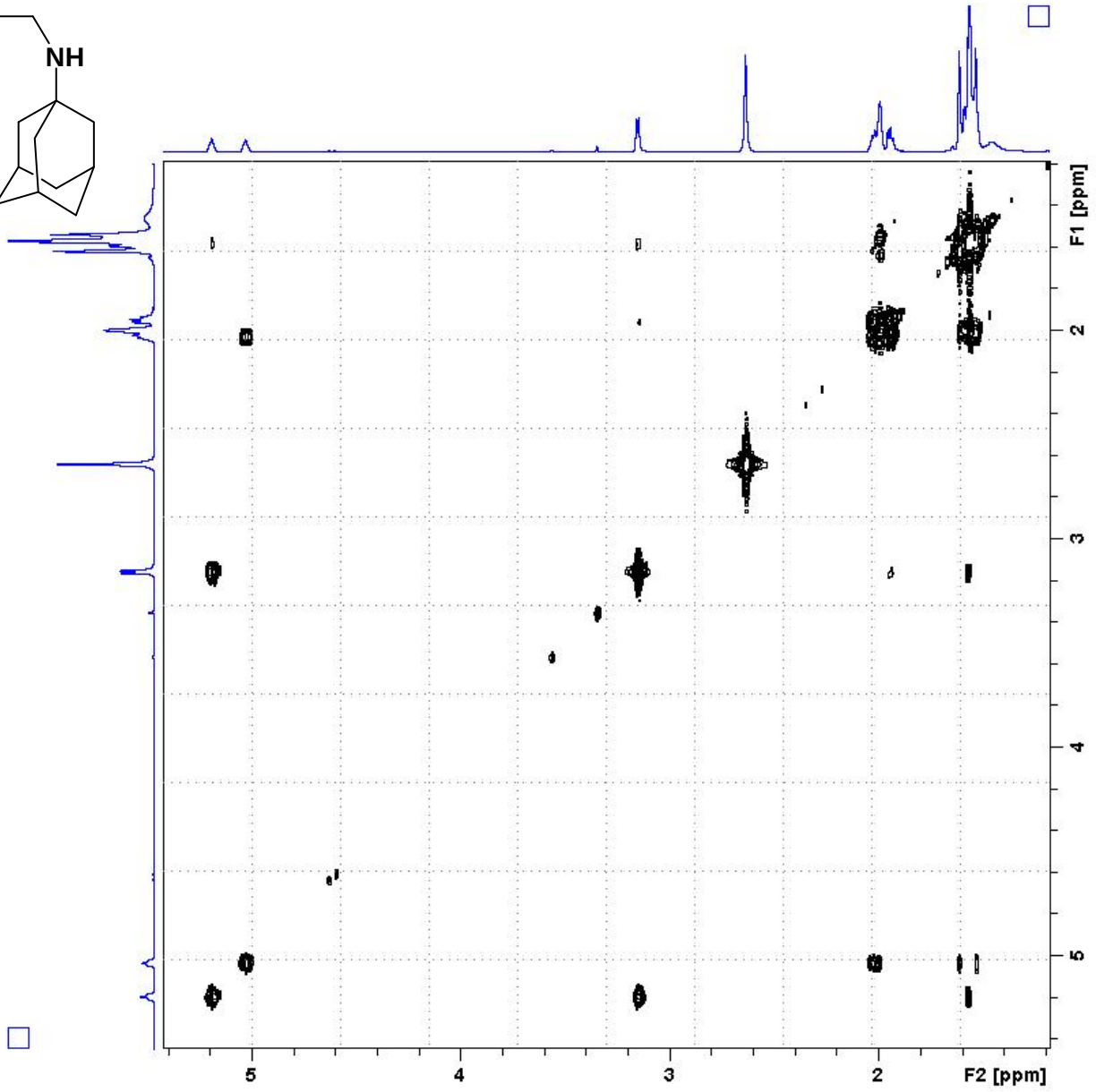
^{1}H spectrum of Compound 3


137.4435
131.3824
124.1419
122.9879
77.2845
77.0720
76.8598
50.1852
50.0467
47.0559
42.8215
39.9420
39.6003
36.7615
29.5941
26.4968
25.6317
17.6264
16.2560
NH
NH
3
140
120
100
80
60
40
20
[ppm]

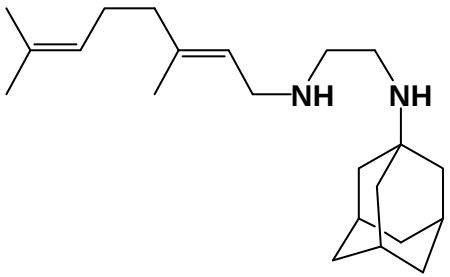
^{13}C APT spectrum of Compound 3



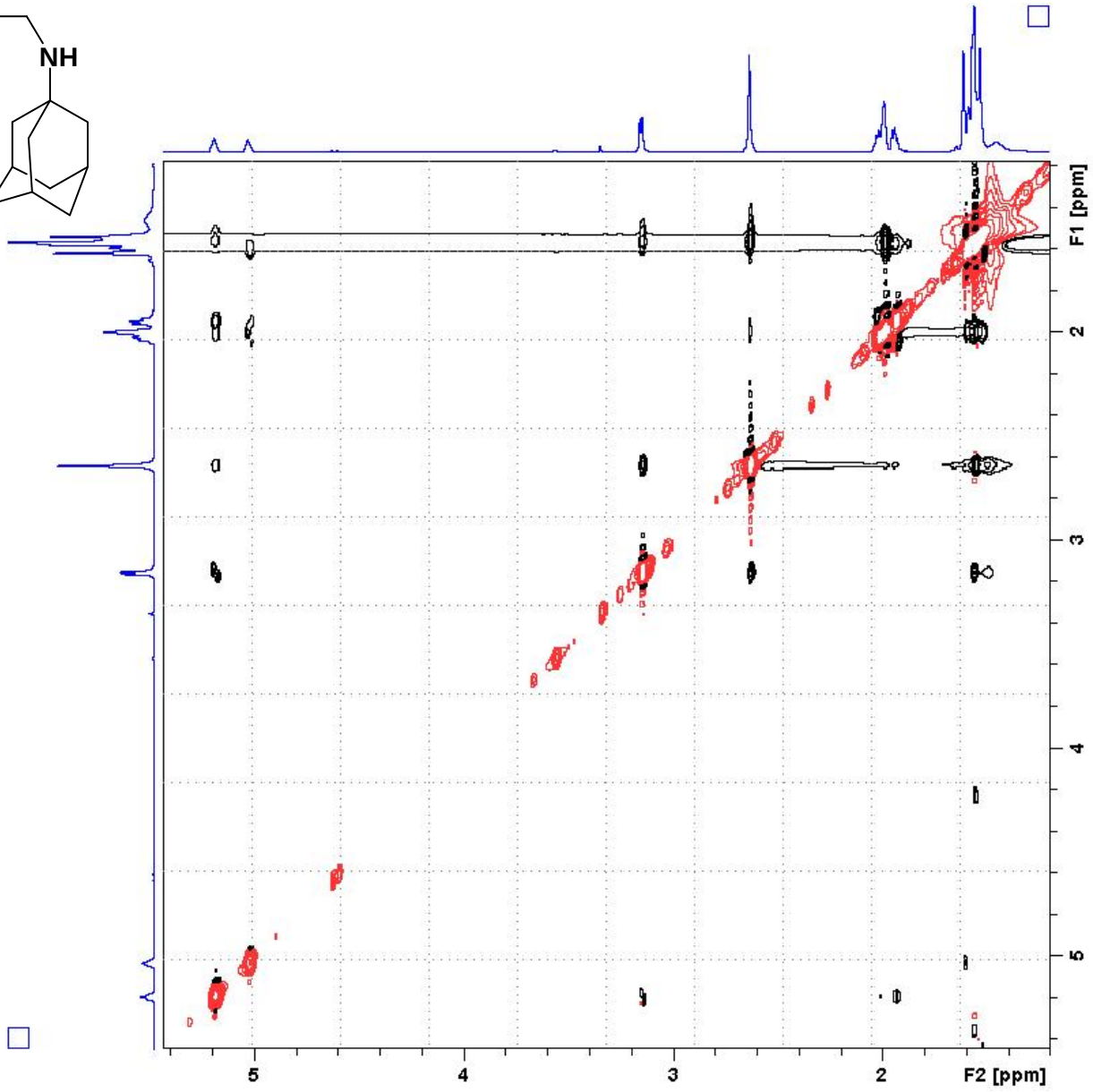
3



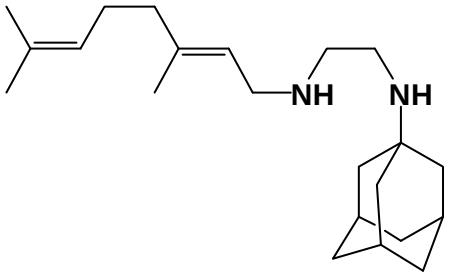
COSY spectrum of Compound 3



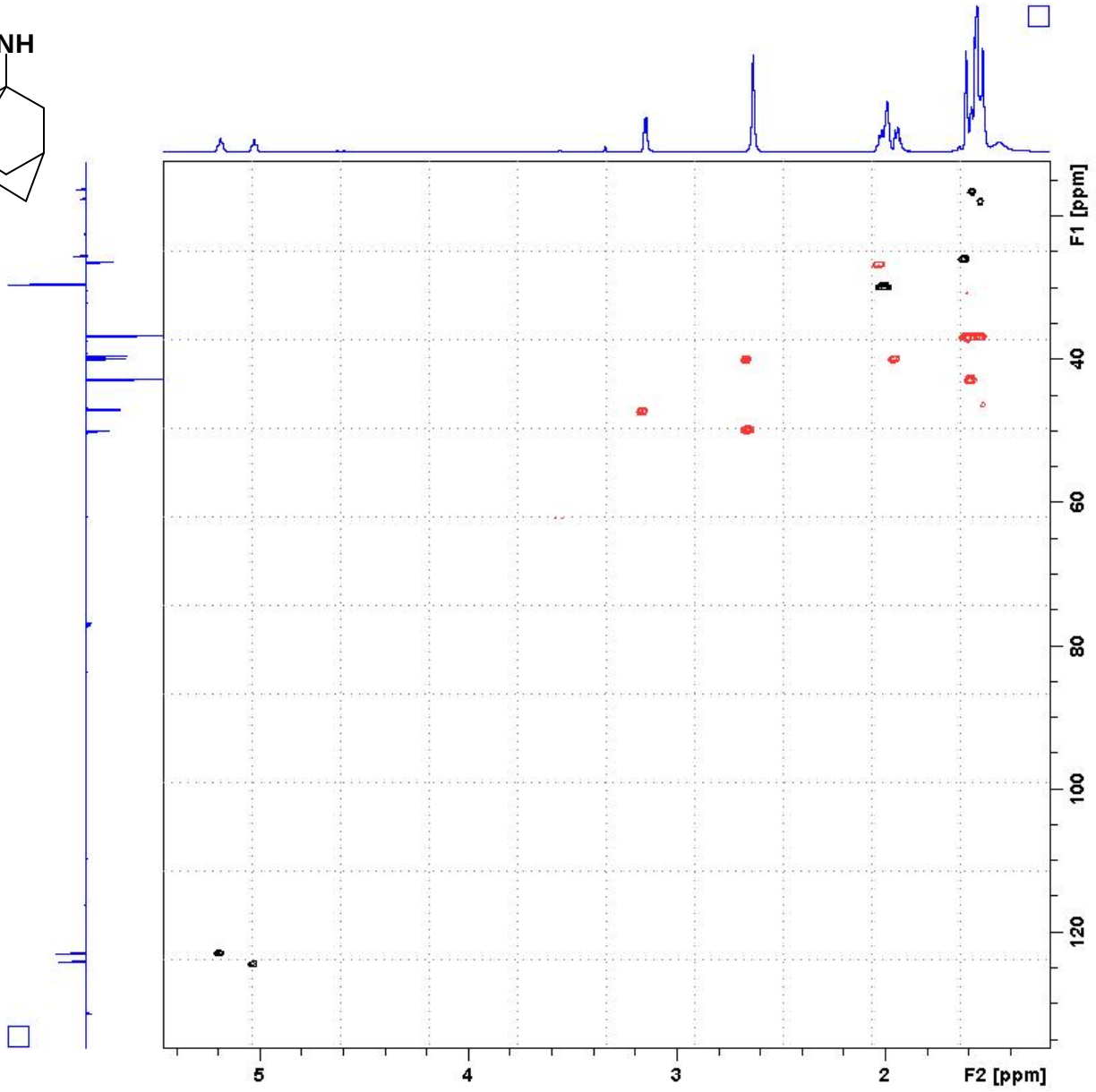
3



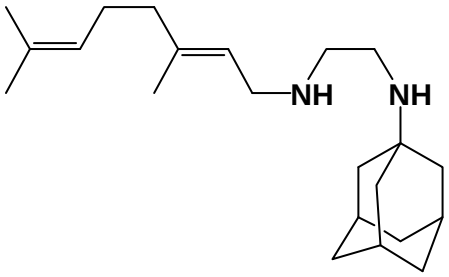
NOESY spectrum of Compound 3



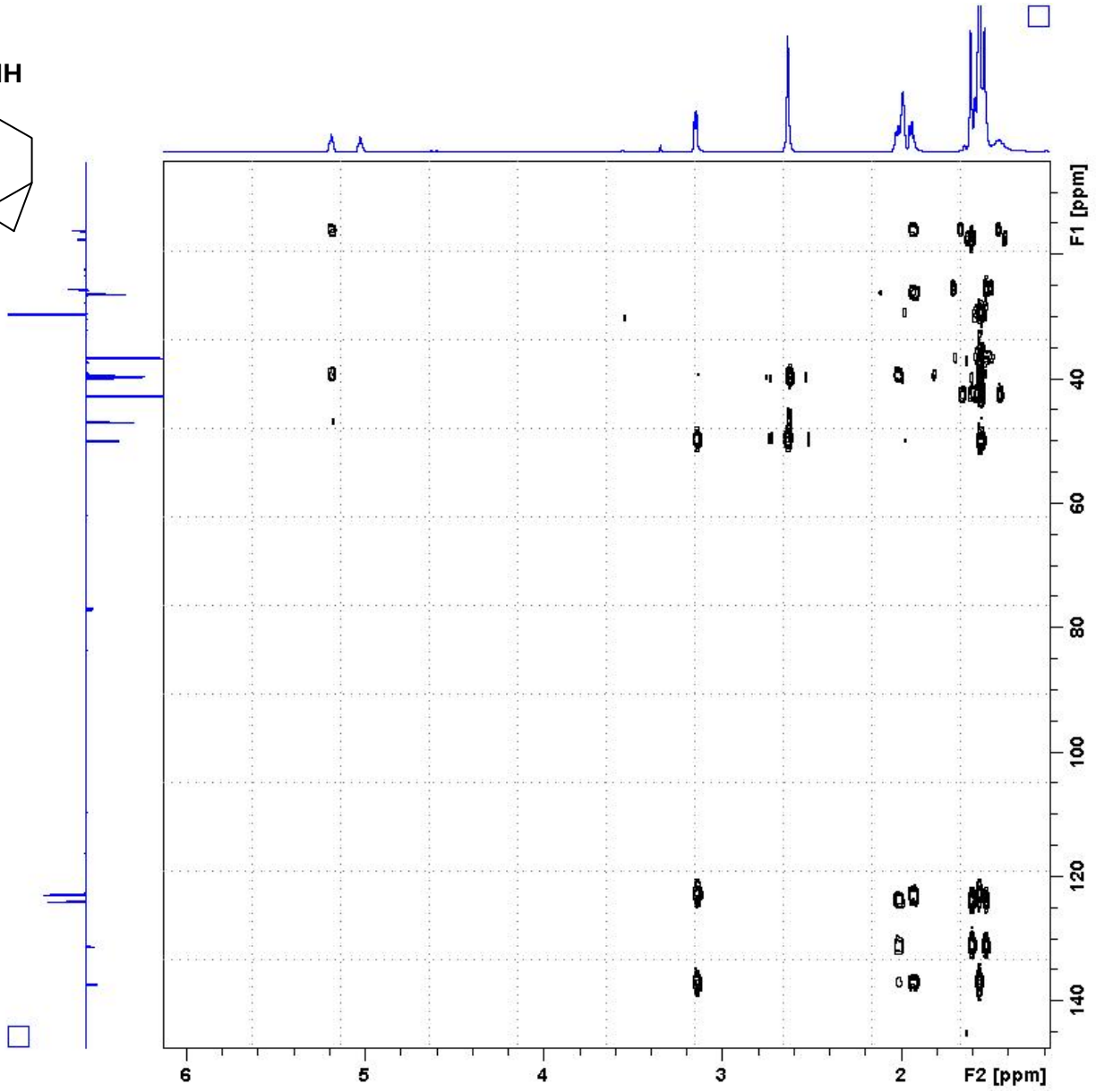
3



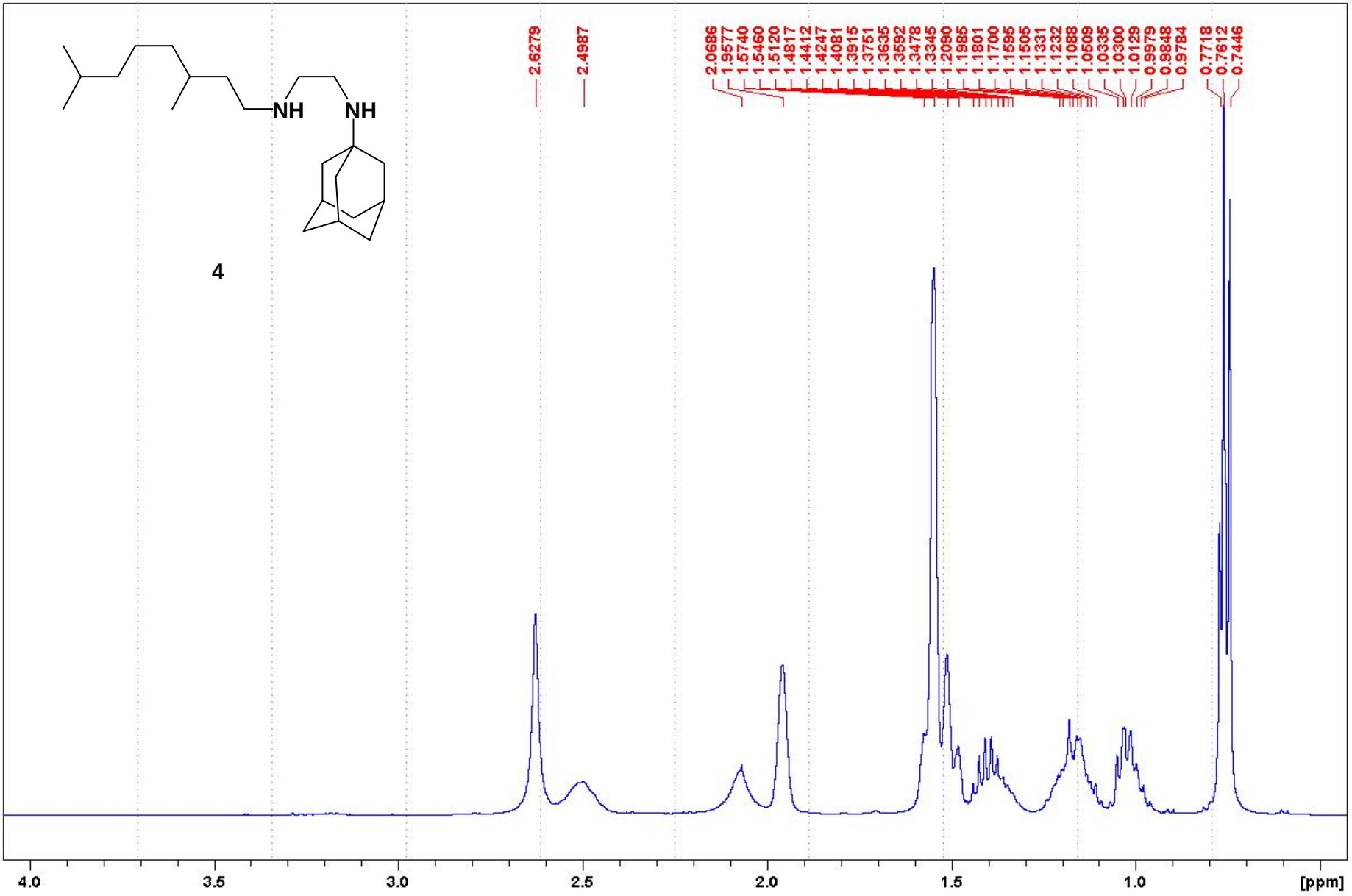
HSQC spectrum of Compound 3



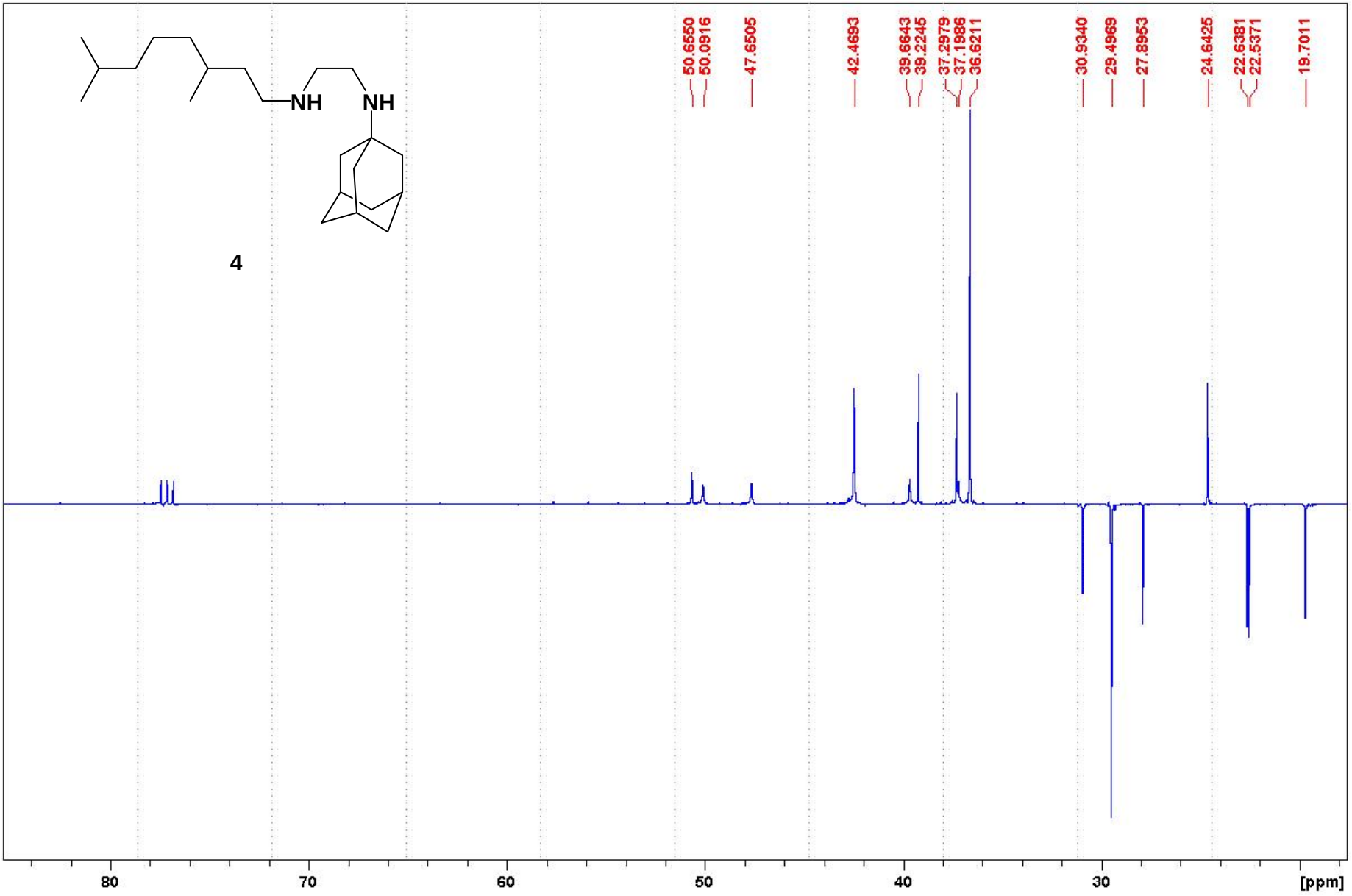
3



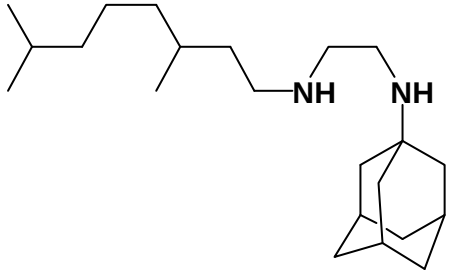
HMBC spectrum of Compound 3


NH
NH
4
2.6279
2.4987
2.0686
1.9577
1.5740
1.5460
1.5120
1.4817
1.4412
1.4247
1.4081
1.3915
1.3751
1.3635
1.3592
1.3478
1.3345
1.2090
1.1985
1.1801
1.1700
1.1595
1.1505
1.1331
1.1232
1.1088
1.0509
1.0335
1.0300
1.0129
0.9979
0.9848
0.9784
0.7718
0.7612
0.7446
4.0
3.5
3.0
2.5
2.0
1.5
1.0
[ppm]

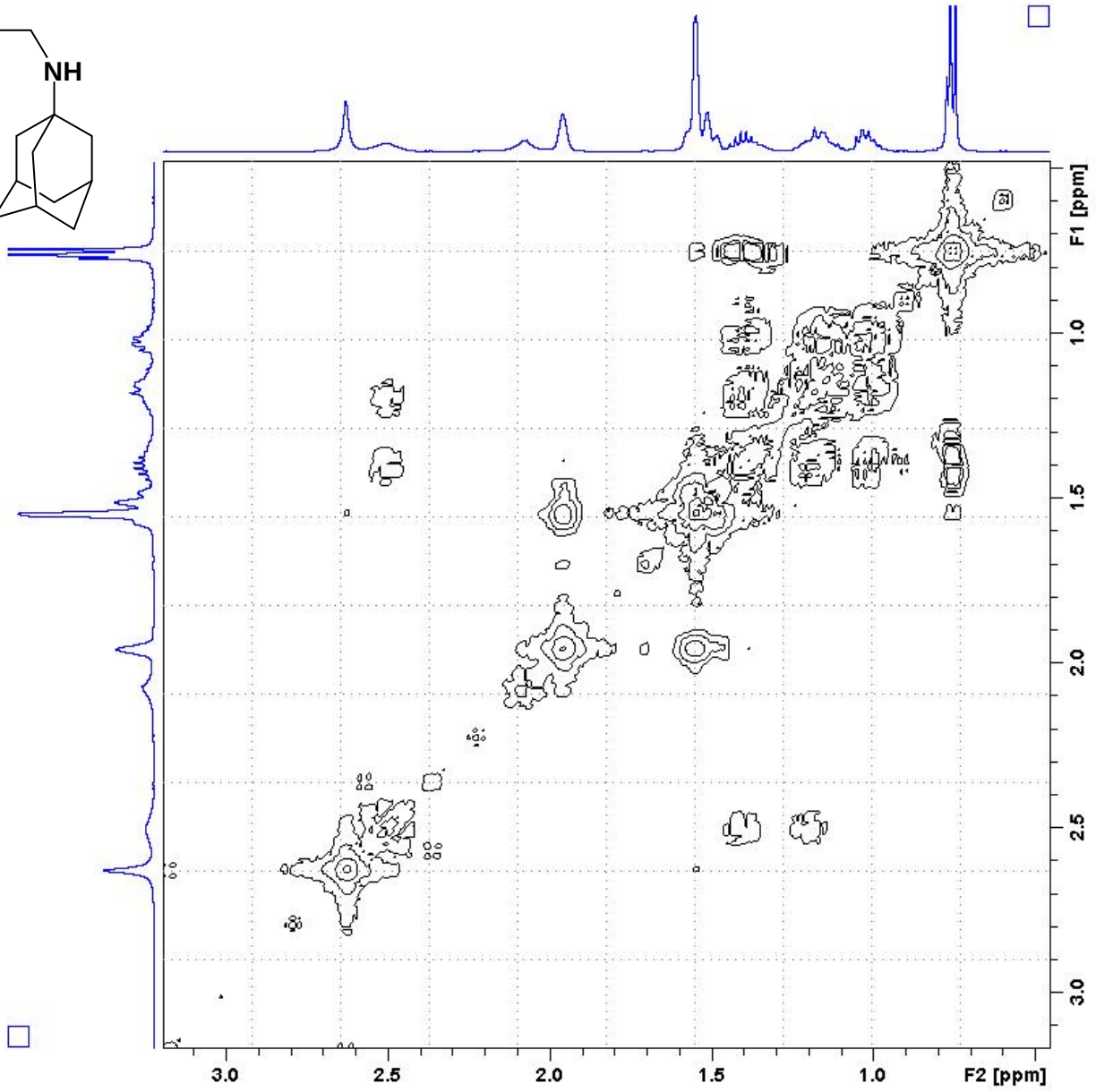
^{1}H spectrum of Compound 4


NH
NH
4
50.6550
50.0916
47.6505
42.4693
39.6643
39.2245
37.2979
37.1986
36.6211
30.9340
29.4969
27.8953
24.6425
22.6381
22.5371
19.7011
80
70
60
50
40
30
[ppm]

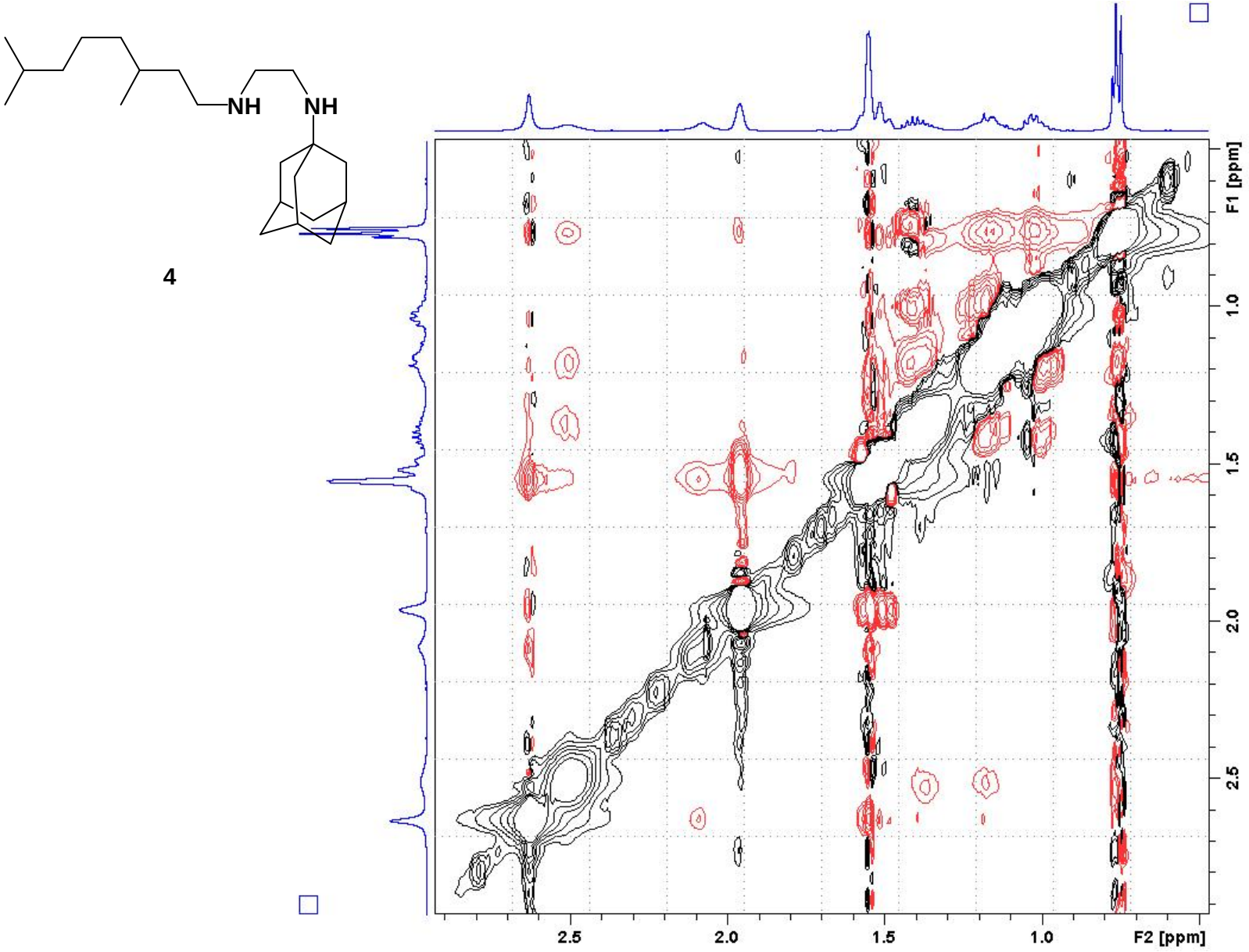
^{13}C APT spectrum of Compound 4



4

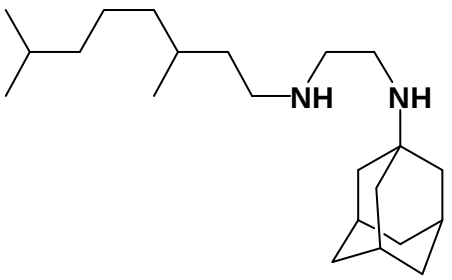


COSY spectrum of Compound 4

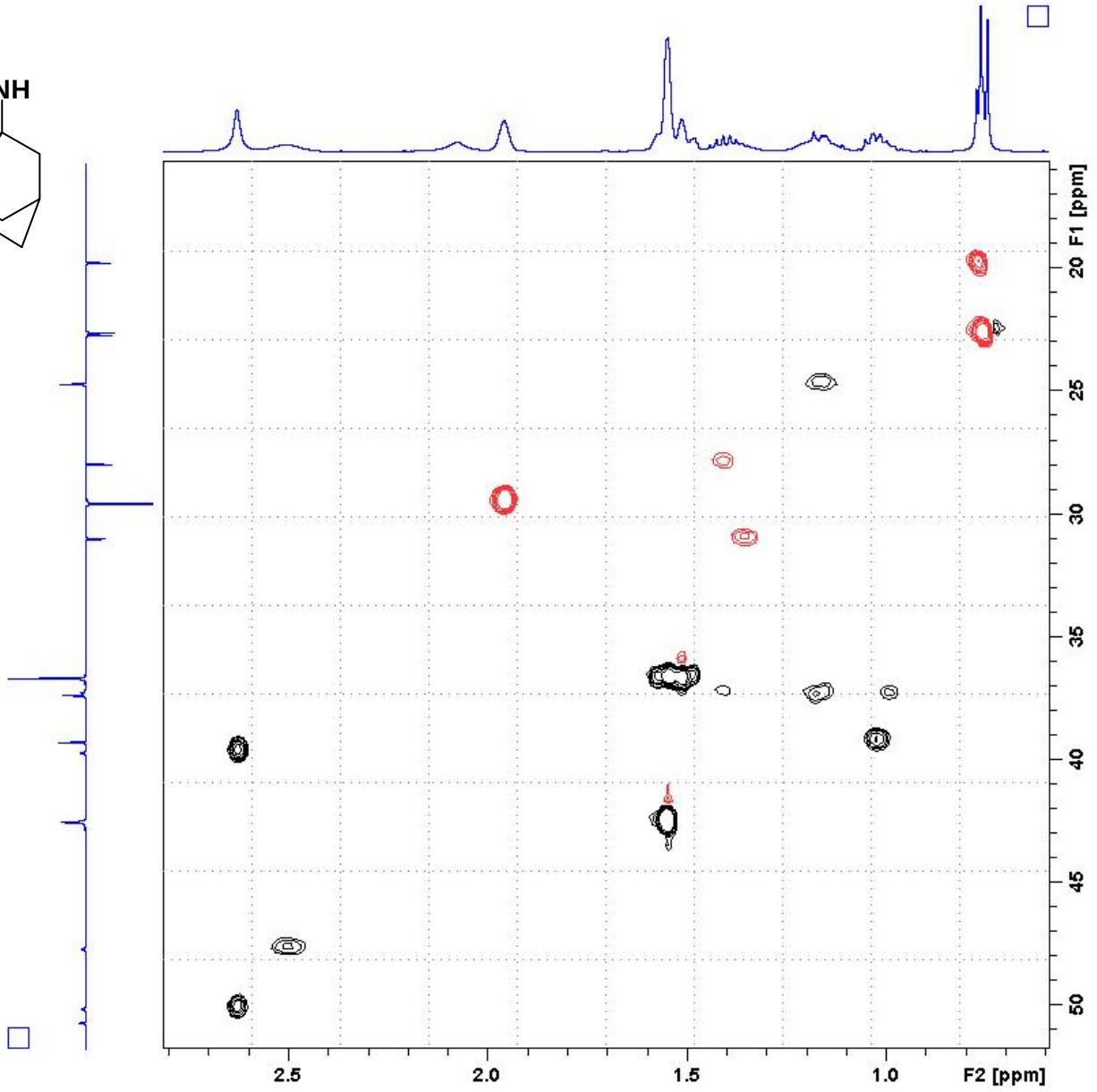


4

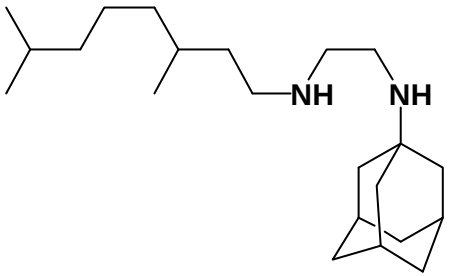
NOESY spectrum of Compound 4



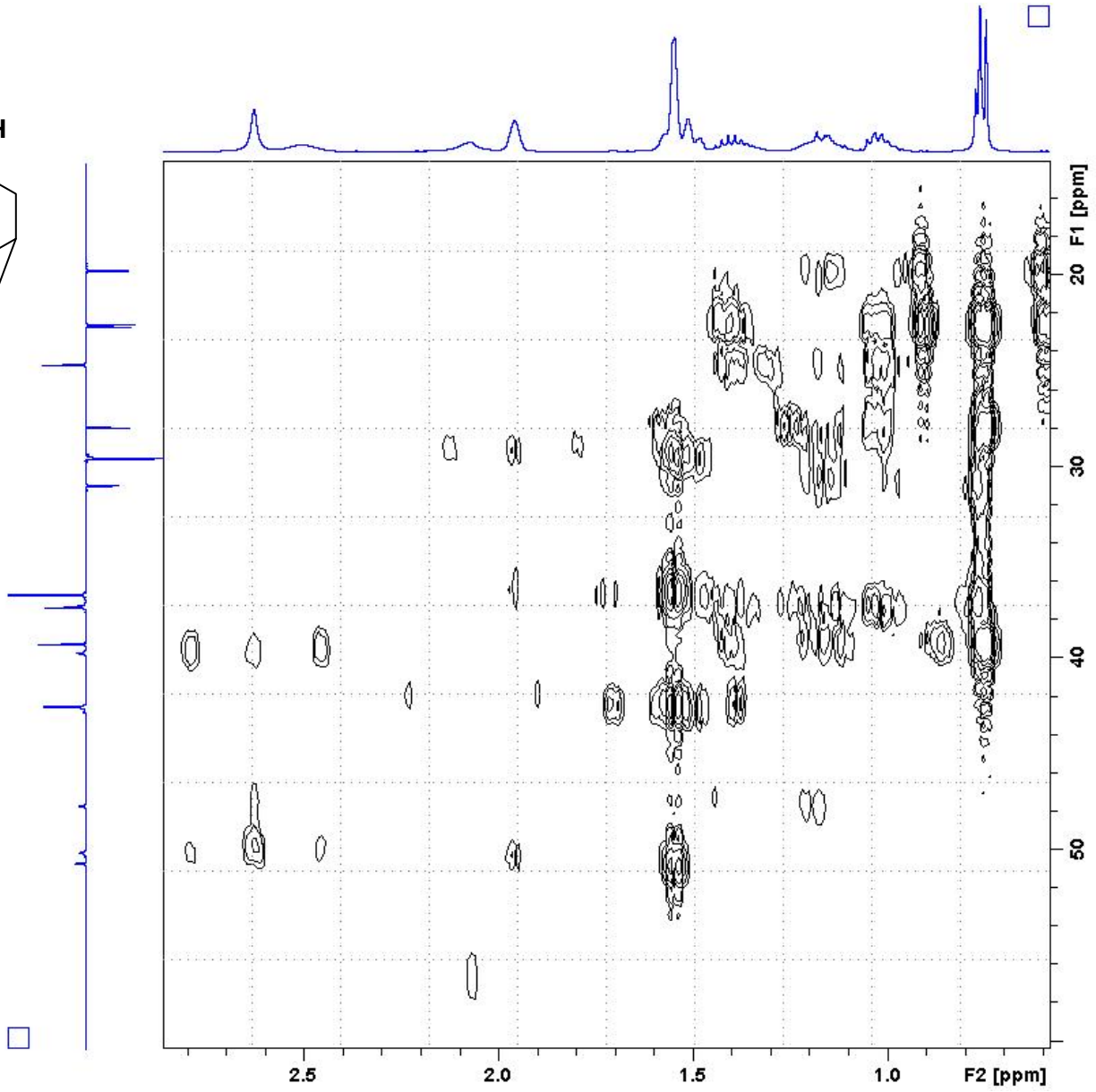
4



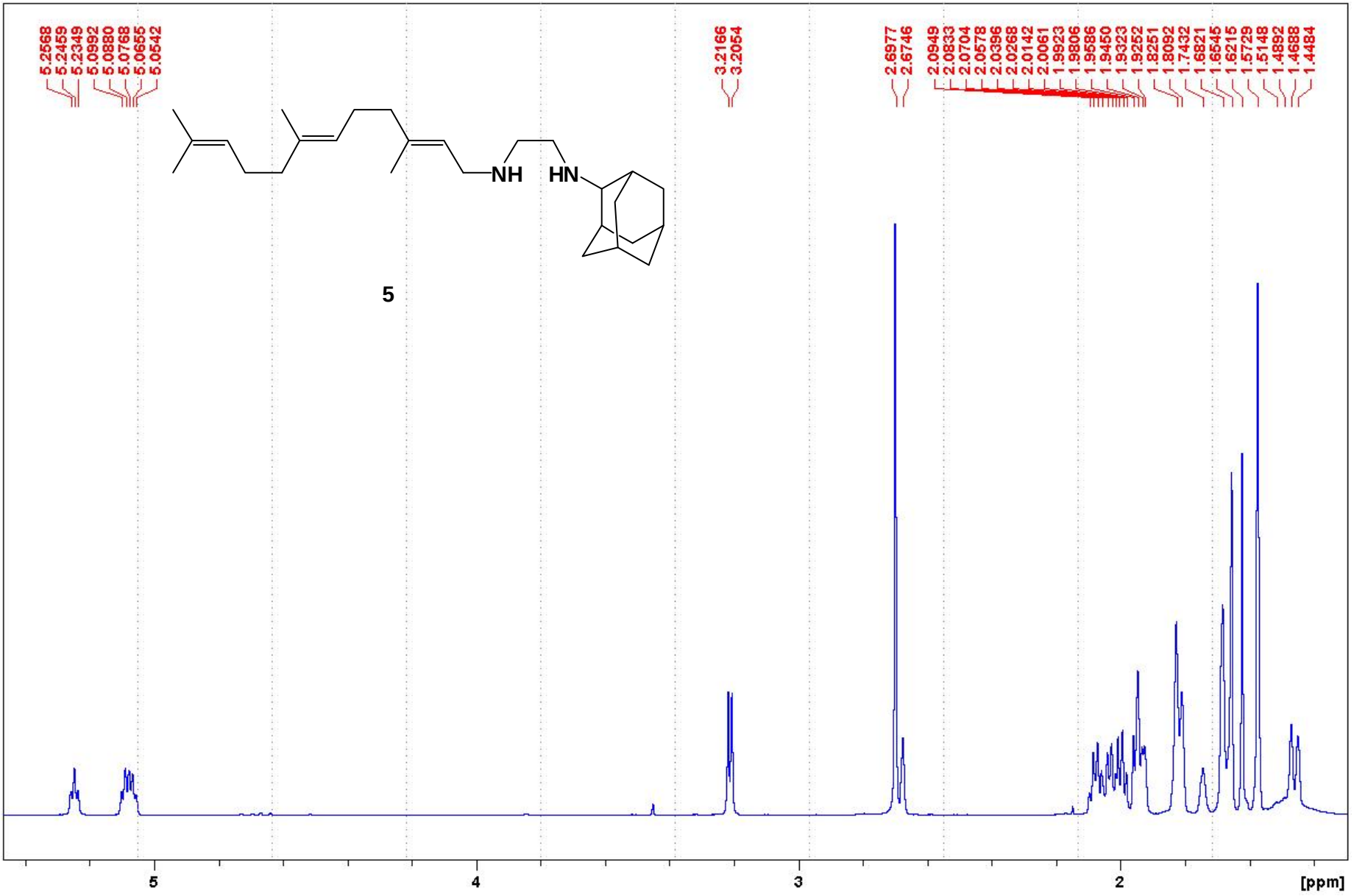
HSQC spectrum of Compound 4



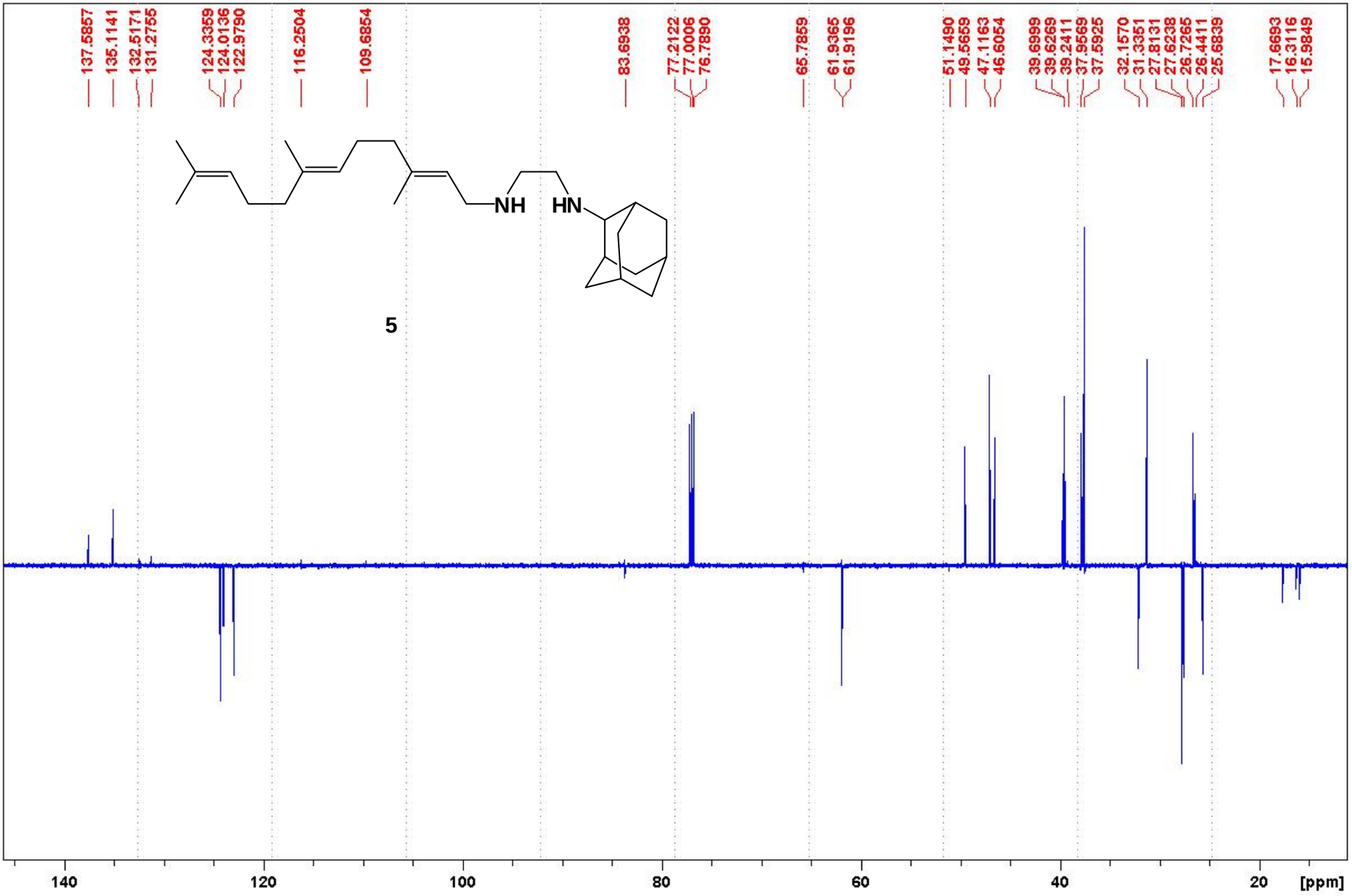
4



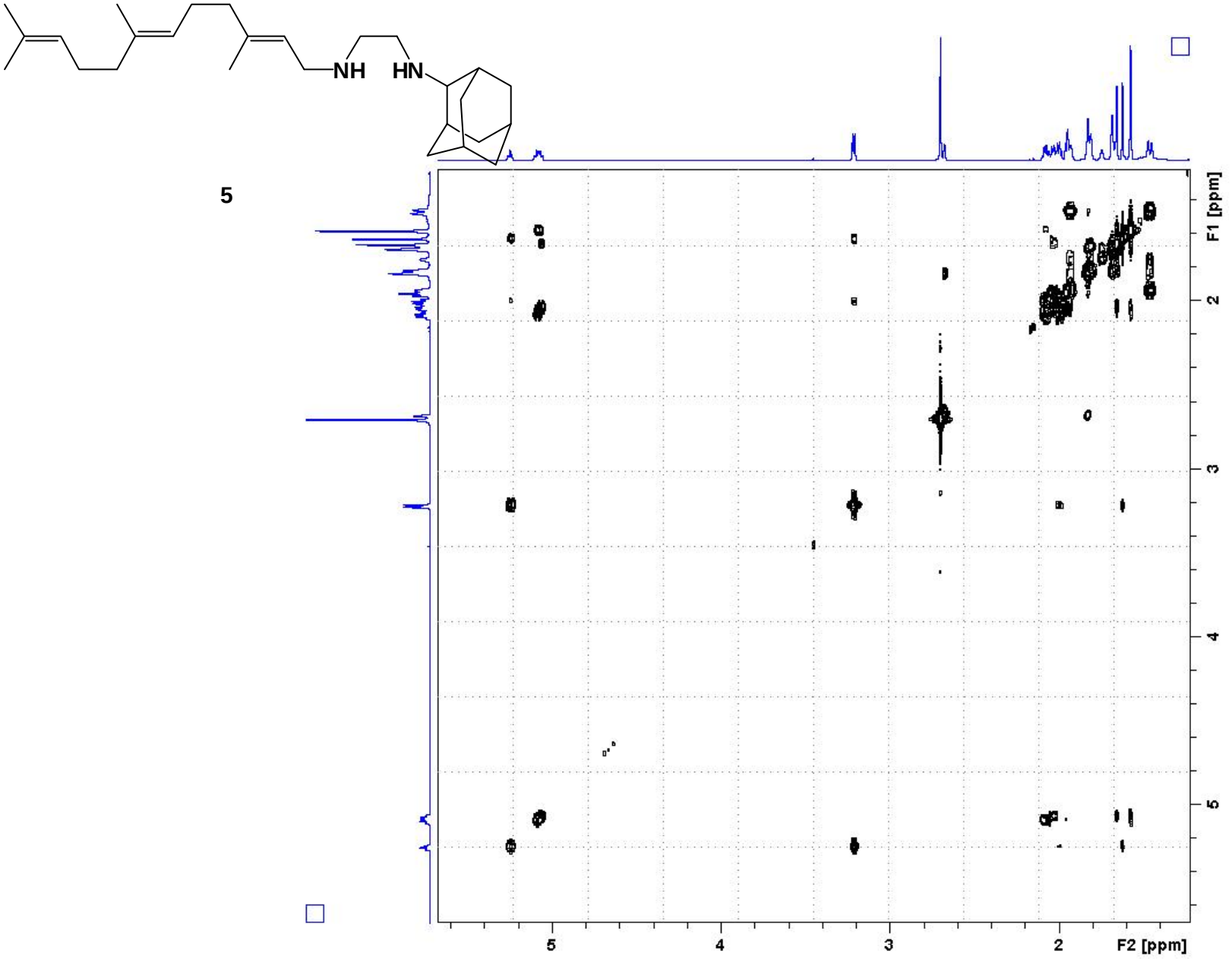
HMBC spectrum of Compound 4


5.2568
5.2459
5.2349
5.0992
5.0880
5.0768
5.0655
5.0542
3.2166
3.2054
2.6977
2.6746
2.0949
2.0833
2.0704
2.0578
2.0396
2.0268
2.0142
2.0061
1.9923
1.9806
1.9586
1.9450
1.9323
1.9252
1.8251
1.8092
1.7432
1.6821
1.6545
1.6215
1.5729
1.5148
1.4892
1.4688
1.4484
NH
HN
5
5
4
3
2
[ppm]

^{1}H spectrum of Compound 5

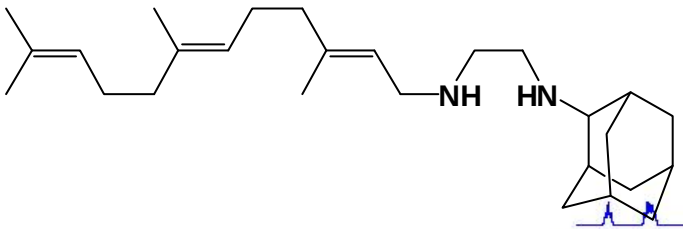

137.5857
135.1141
132.5171
131.2755
124.3359
124.0136
122.9790
116.2504
109.6854
83.6938
77.2122
77.0006
76.7890
65.7859
61.9365
61.9196
51.1490
49.5659
47.1163
46.6054
39.6999
39.6269
39.2411
37.9569
37.5925
32.1570
31.3351
27.8131
27.6238
26.7265
26.4411
25.6839
17.6693
16.3116
15.9849
NH
HN
5
140
120
100
80
60
40
20
[ppm]

^{13}C APT spectrum of Compound 5

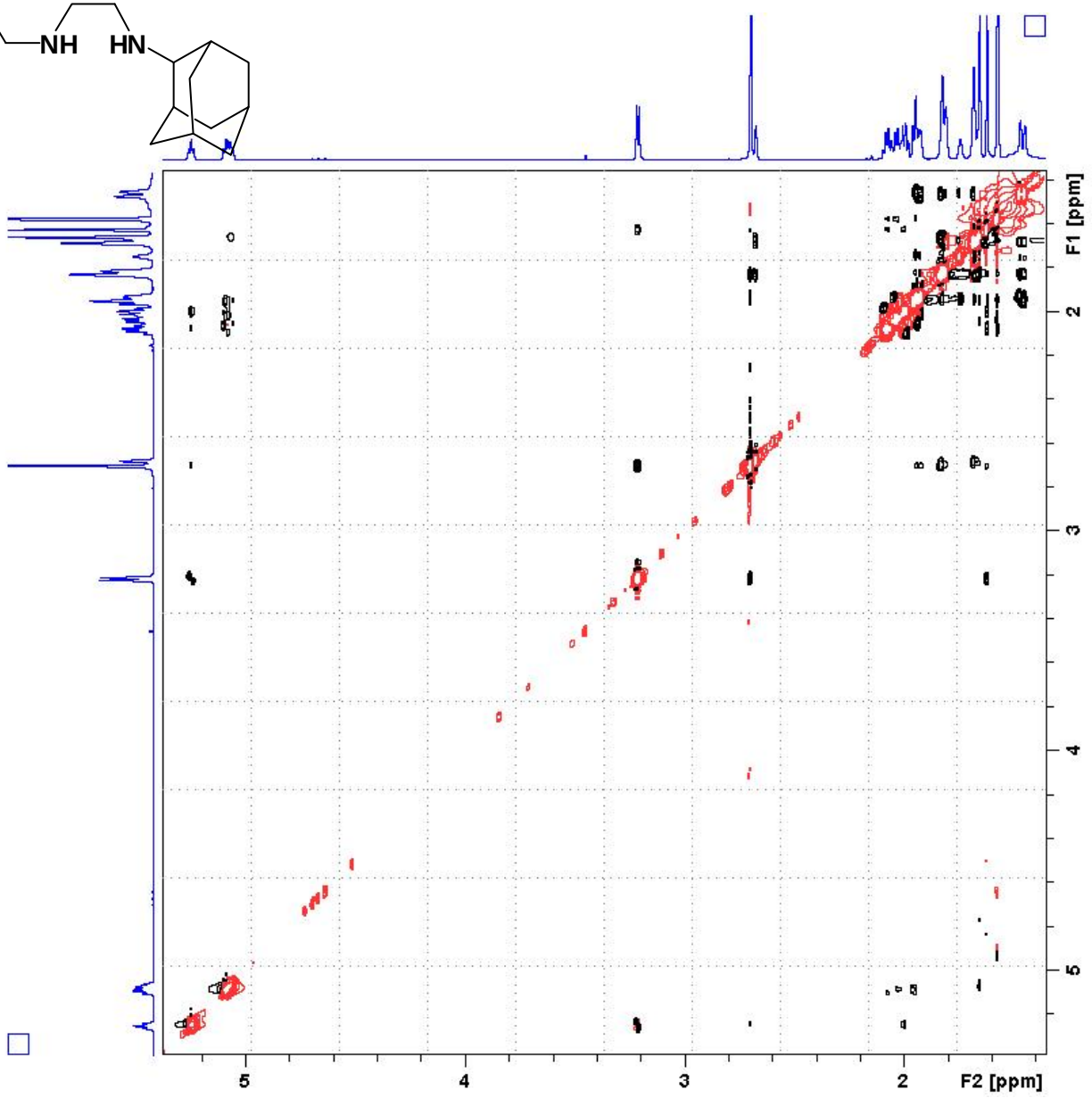


5

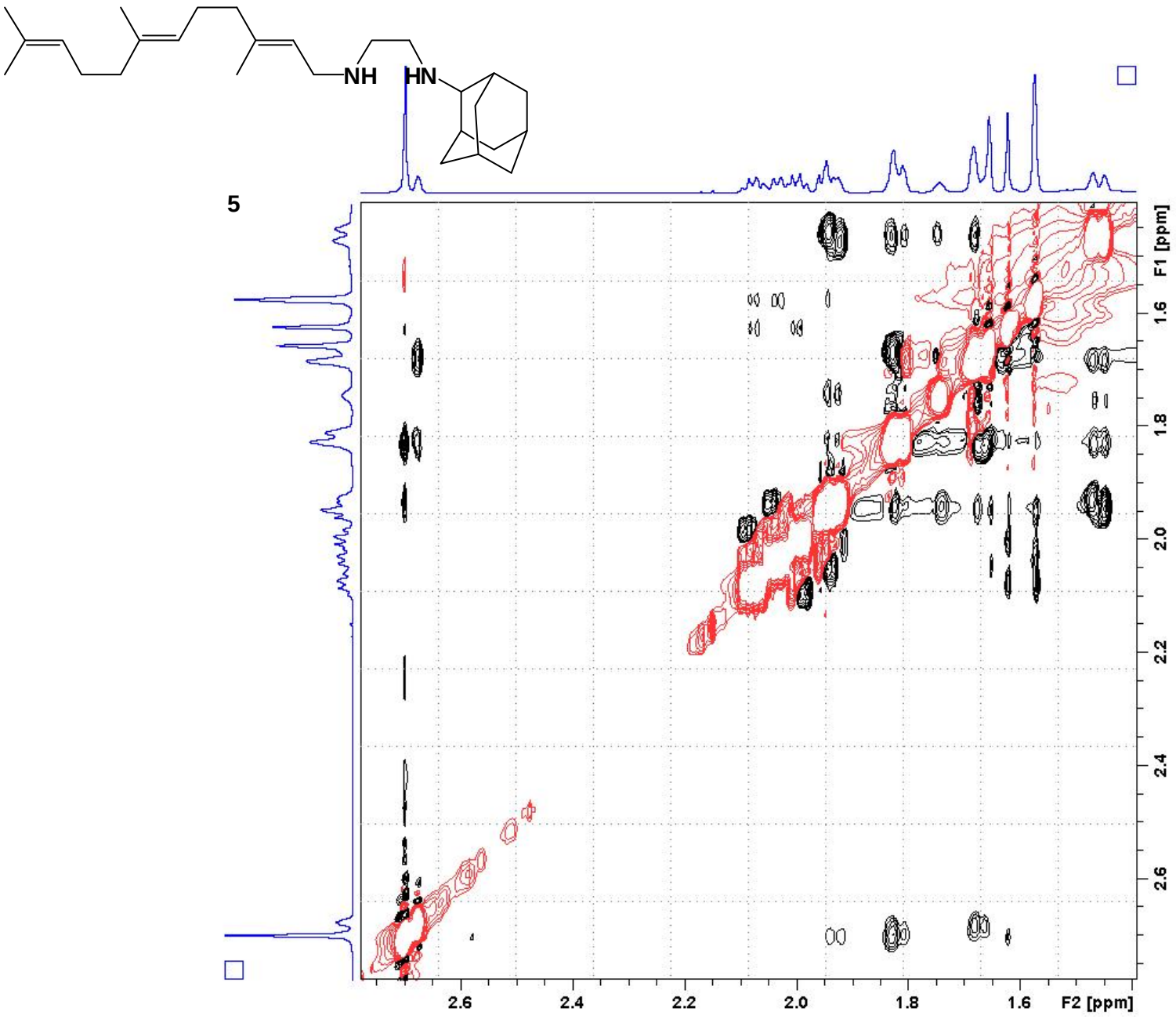
COSY spectrum of Compound 5



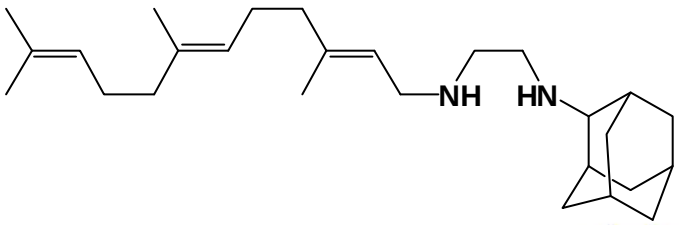
5



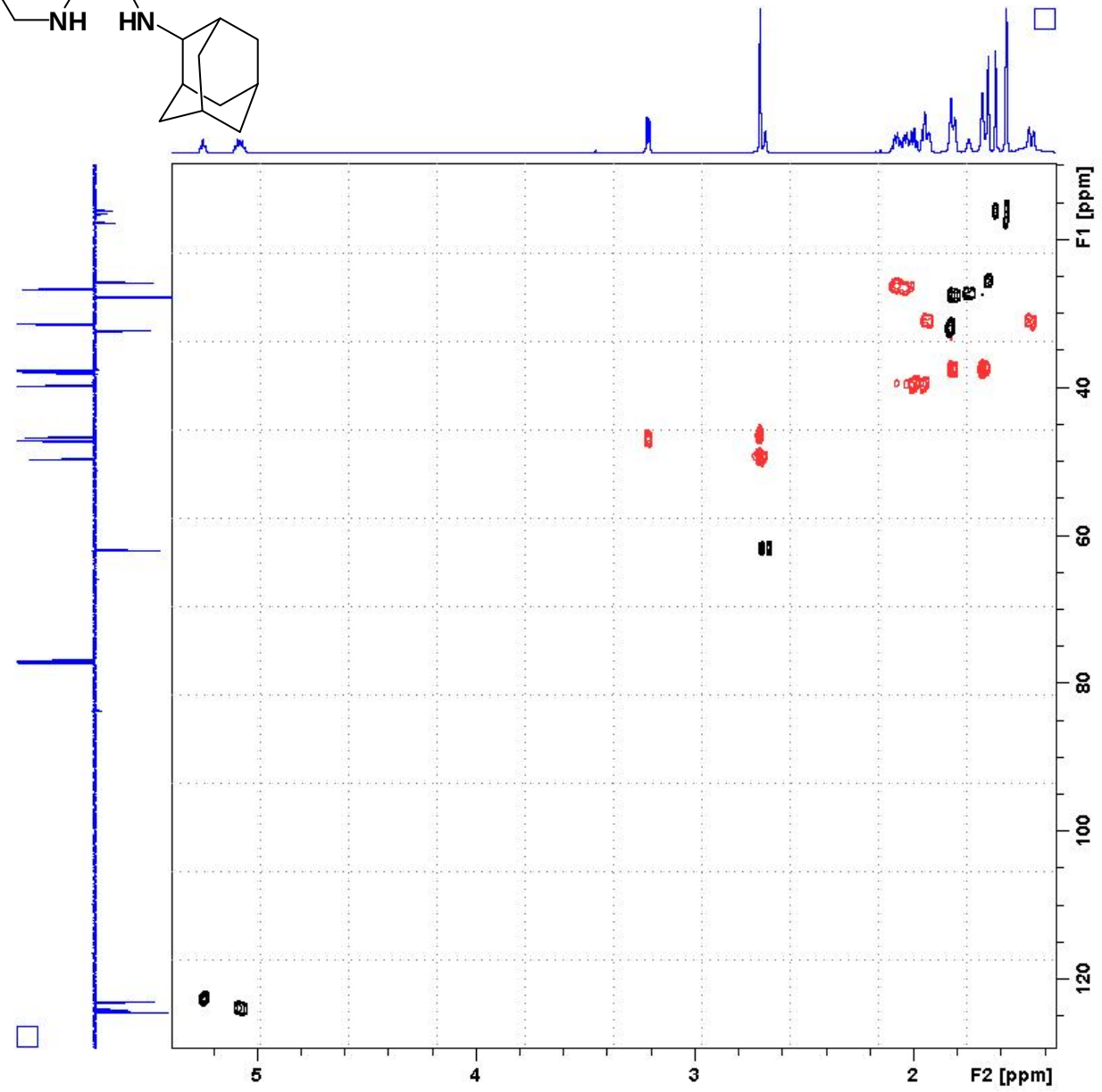
NOESY spectrum of Compound 5


NH
HN
5
F1 [ppm]
1.6
1.8
2.0
2.2
2.4
2.6
F2 [ppm]
2.6
2.4
2.2
2.0
1.8
1.6

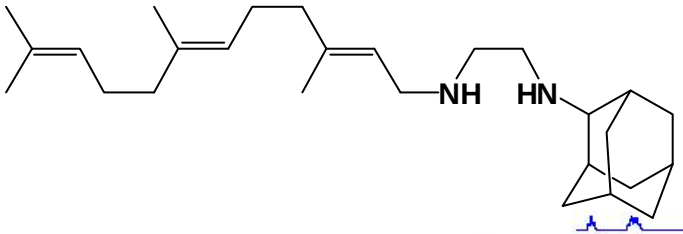
Expanded NOESY spectrum of Compound 5



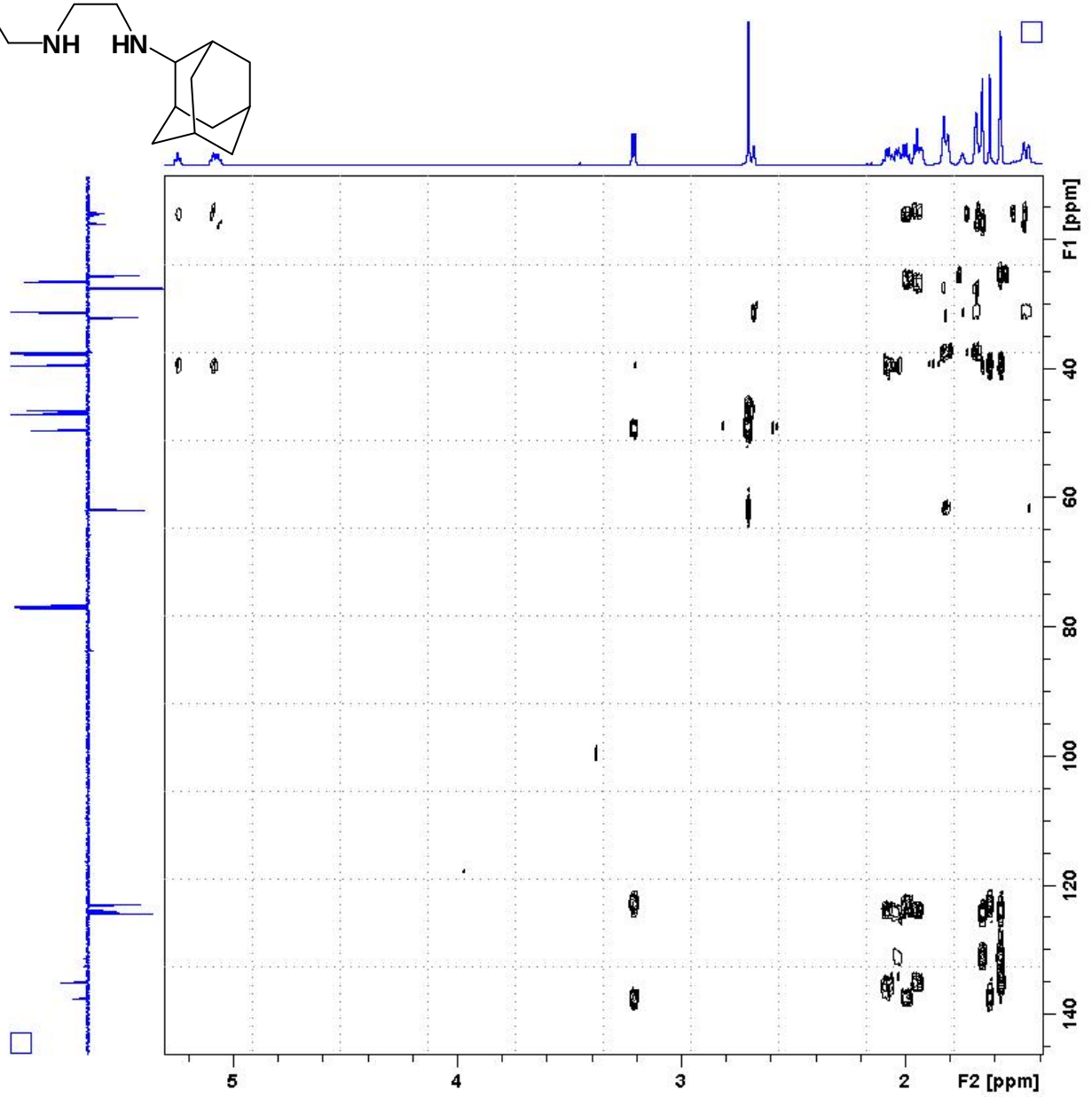
5



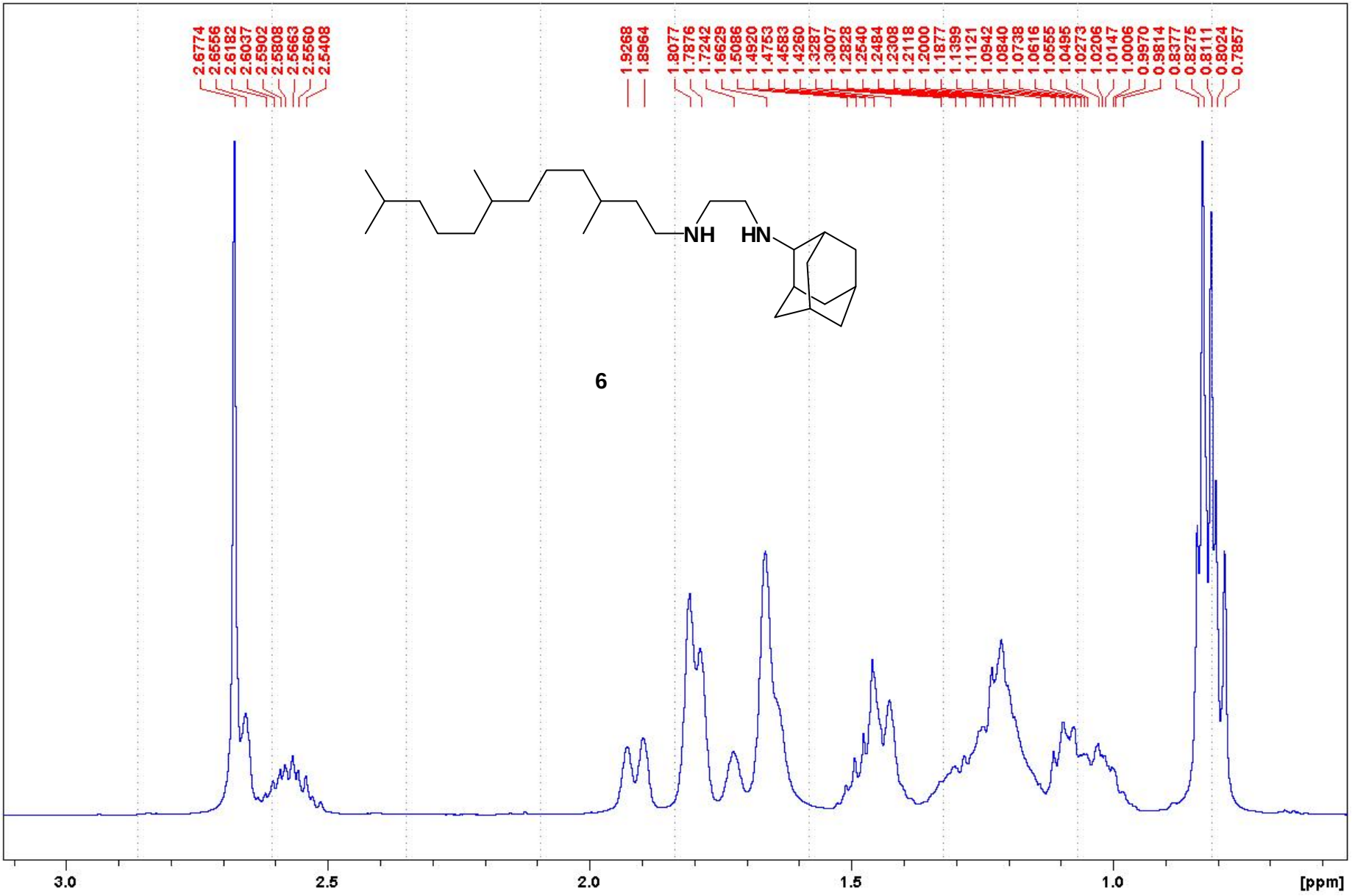
HSQC spectrum of Compound 5



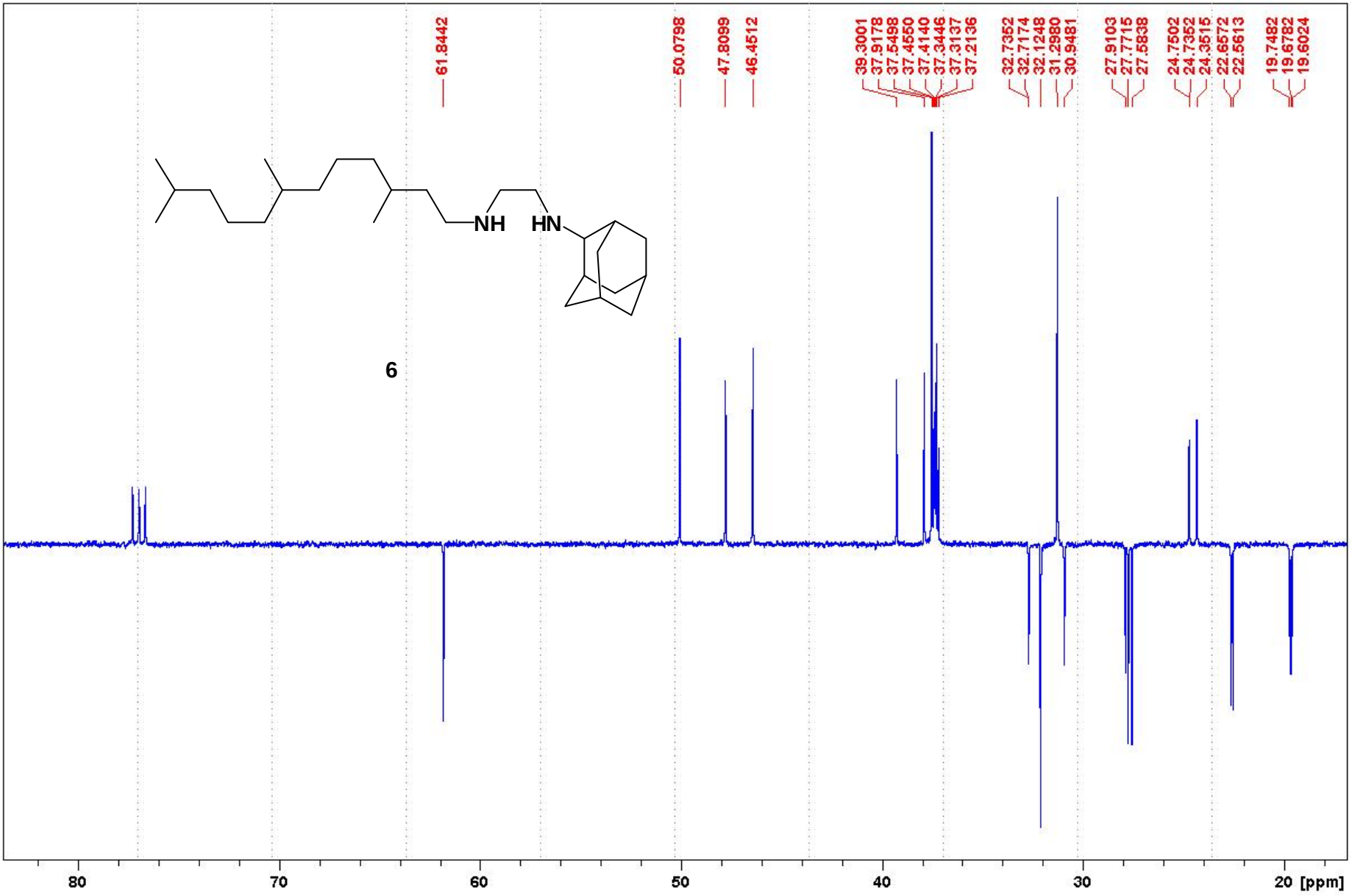
5



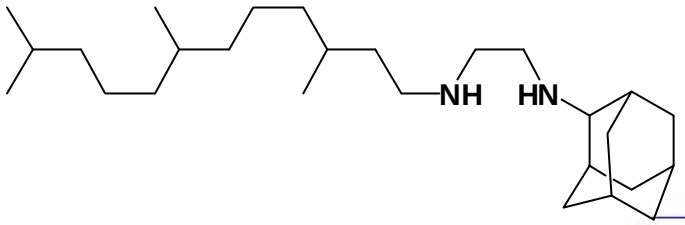
HMBC spectrum of Compound 5


2.6774
2.6556
2.6182
2.6037
2.5902
2.5808
2.5663
2.5560
2.5408
1.9268
1.8964
1.8077
1.7876
1.7242
1.6629
1.5086
1.4920
1.4753
1.4583
1.4260
1.3287
1.3007
1.2828
1.2540
1.2484
1.2308
1.2118
1.2000
1.1877
1.1399
1.1121
1.0942
1.0840
1.0738
1.0616
1.0555
1.0495
1.0273
1.0206
1.0147
1.0006
0.9970
0.9814
0.8377
0.8275
0.8111
0.8024
0.7857
NH
HN
6
3.0
2.5
2.0
1.5
1.0
[ppm]

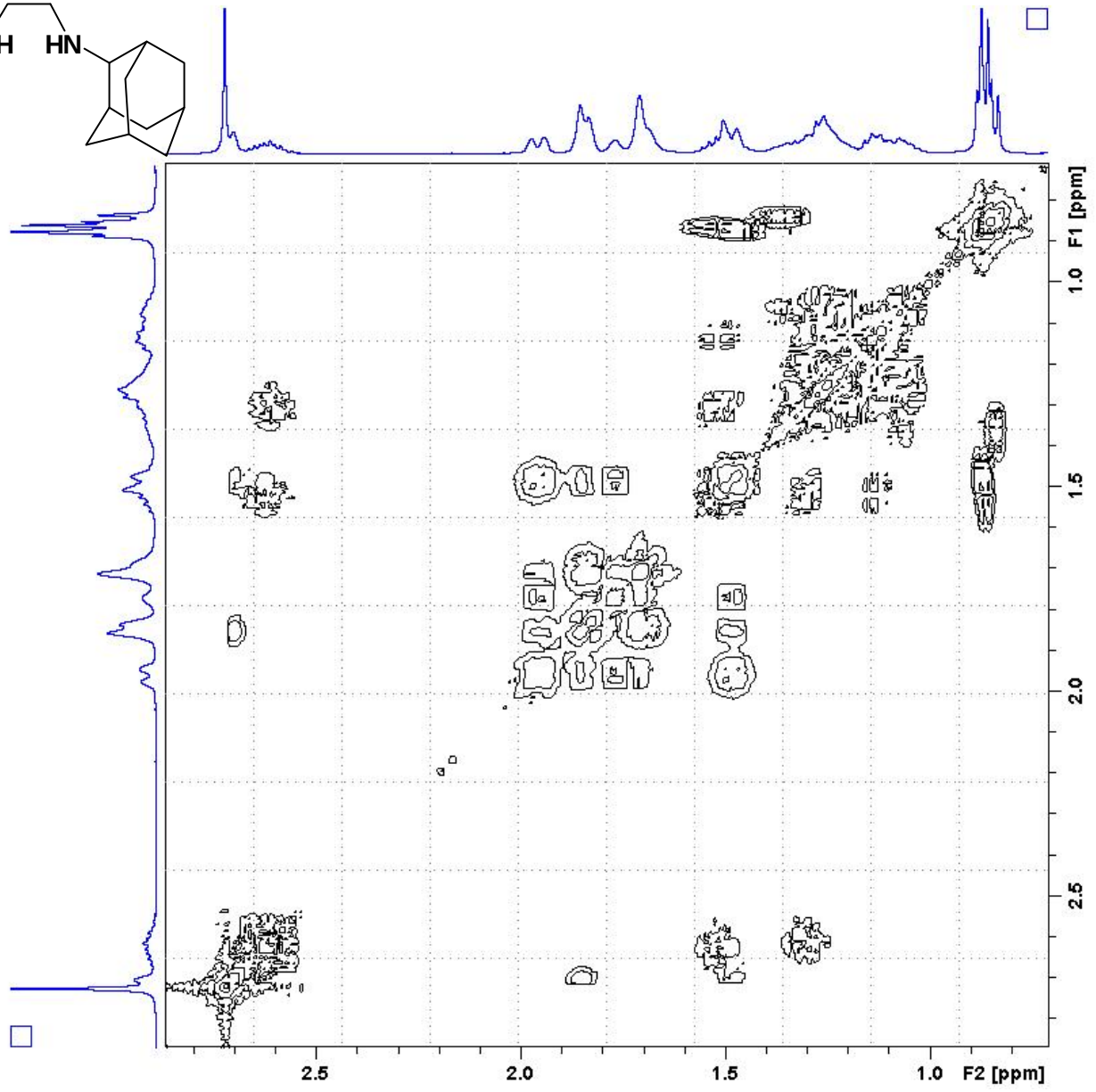
^{1}H spectrum of Compound 6


61.8442
50.0798
47.8099
46.4512
39.3001
37.9178
37.5498
37.4550
37.4140
37.3446
37.3137
37.2136
32.7352
32.7174
32.1248
31.2980
30.9481
27.9103
27.7715
27.5838
24.7502
24.7352
24.3515
22.6572
22.5613
19.7482
19.6782
19.6024
NH
HN
6
80
70
60
50
40
30
20 [ppm]

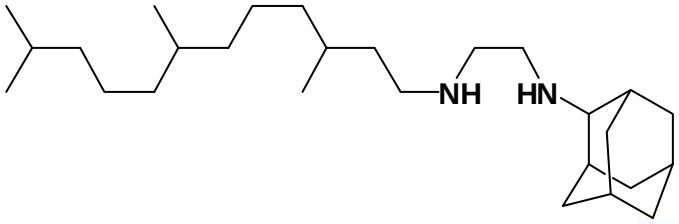
^{13}C APT spectrum of Compound 6



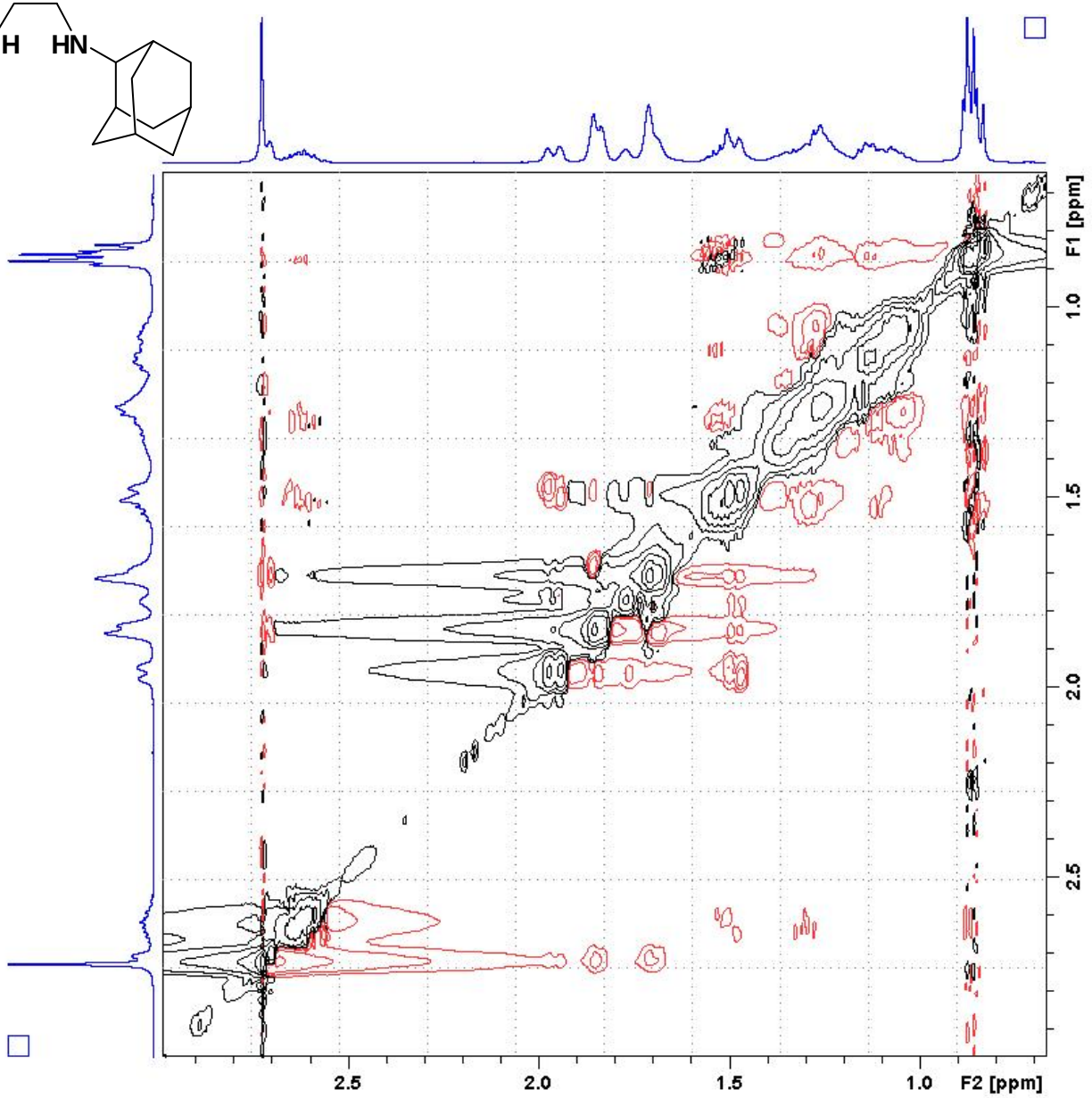
6



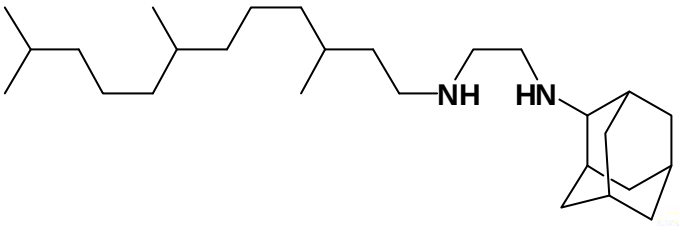
COSY spectrum of Compound 6



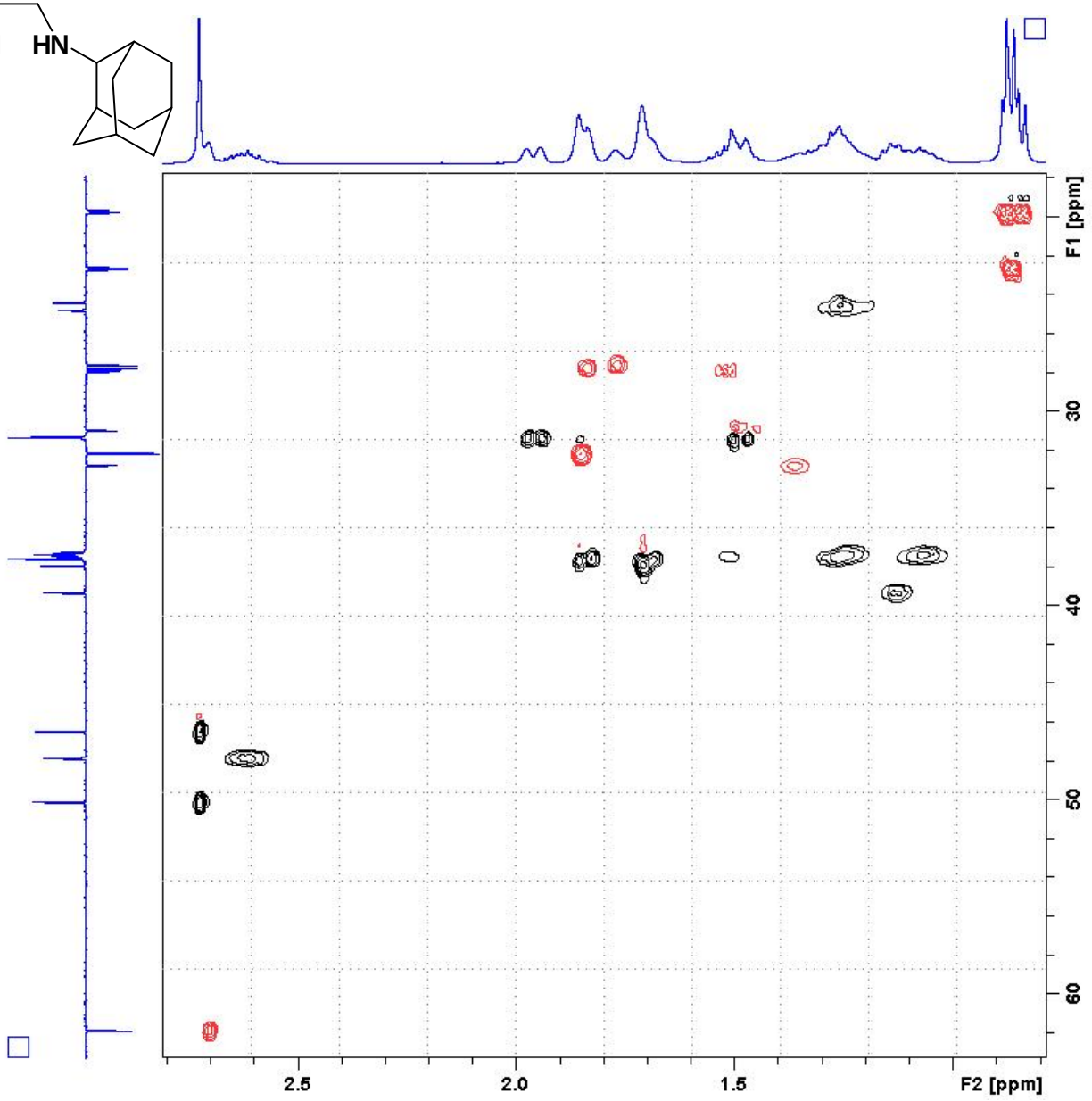
6



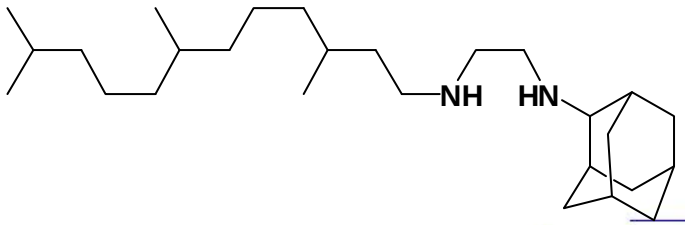
NOESY spectrum of Compound 6



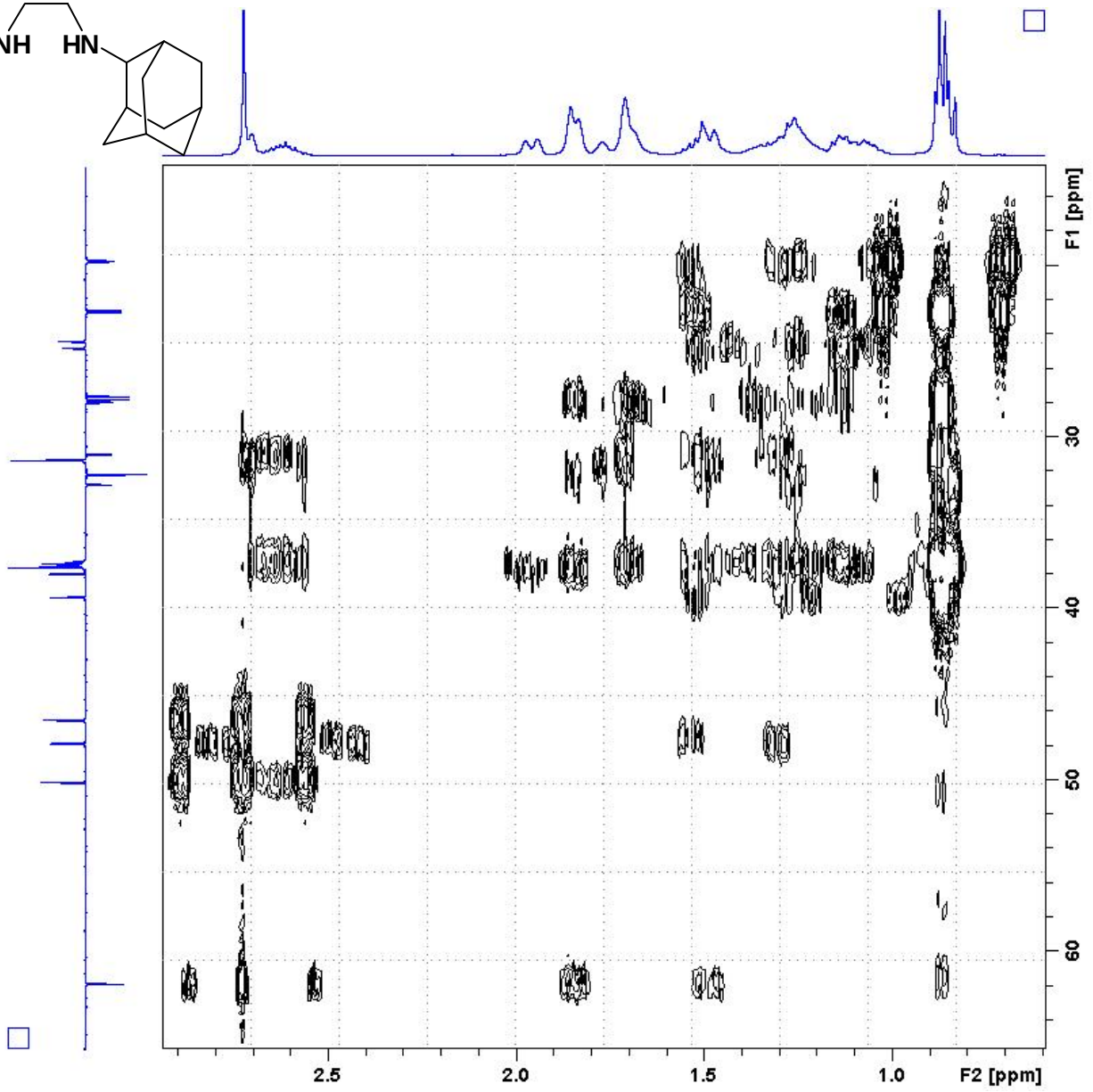
6



HSQC spectrum of Compound 6



6



HMBC spectrum of Compound 6

CHAPTER 7

NOVEL POLYCYCLIC 'CAGE'-1,2-DIAMINES AS POTENTIAL ANTI-TUBERCULOSIS AGENTS

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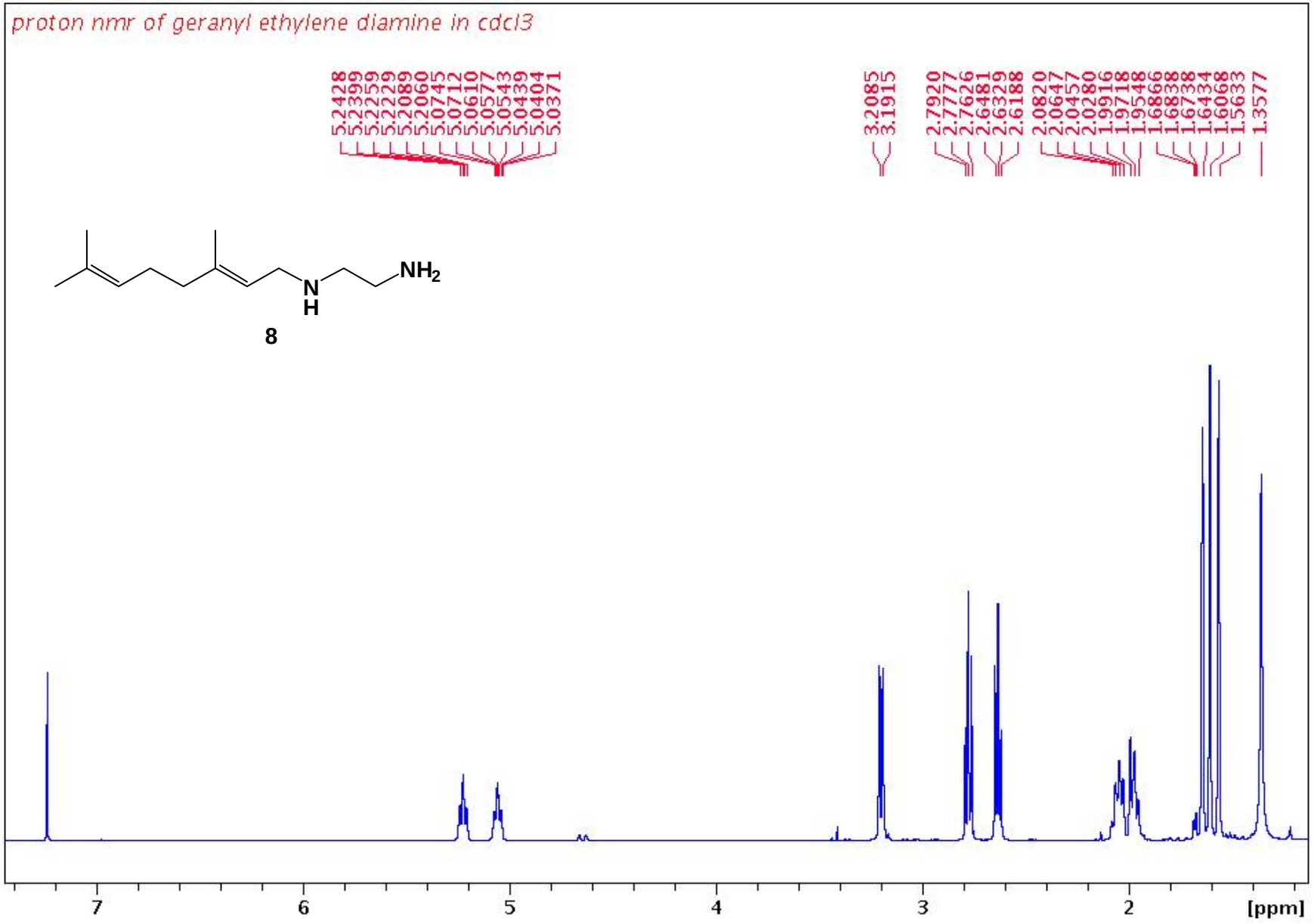
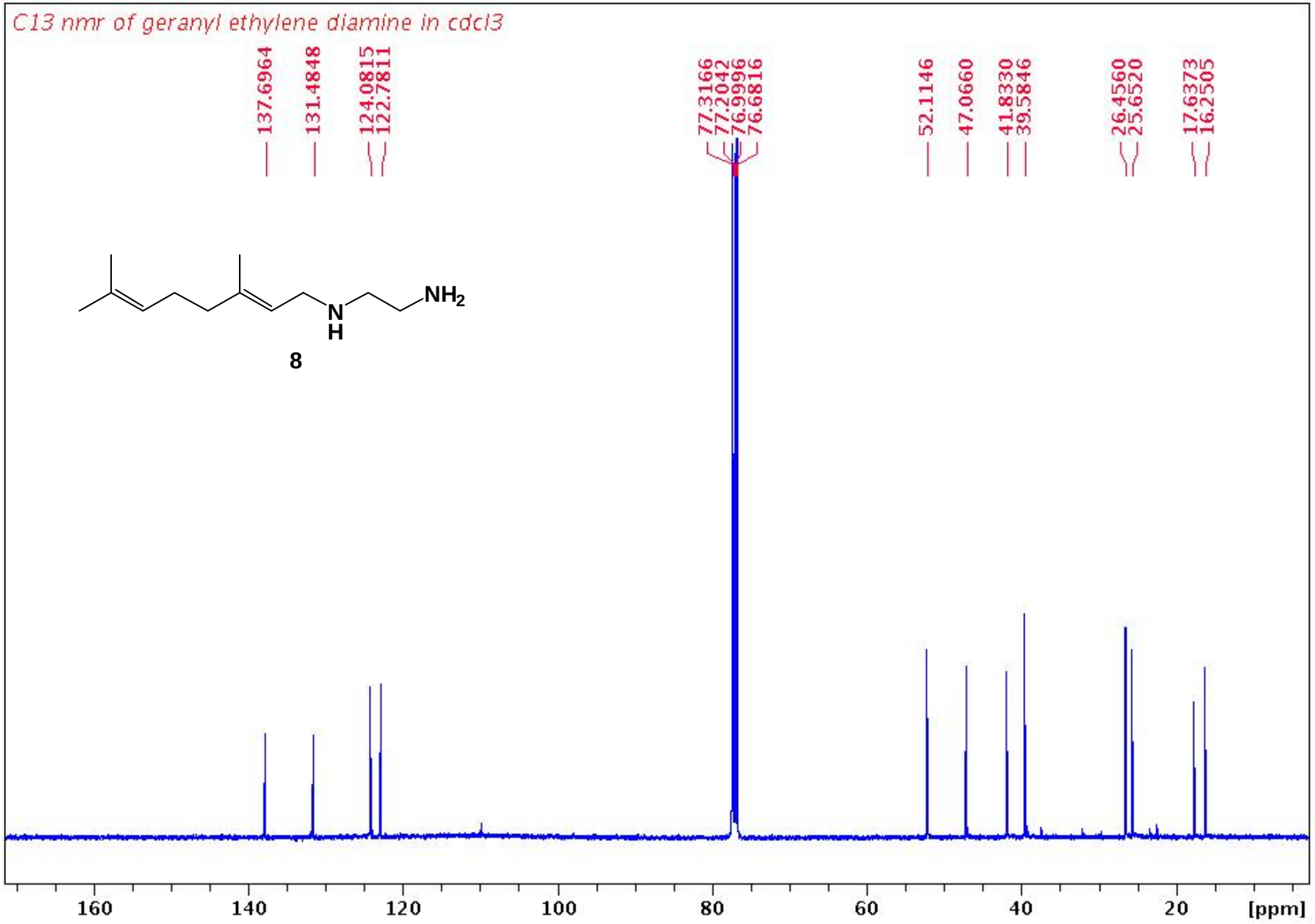
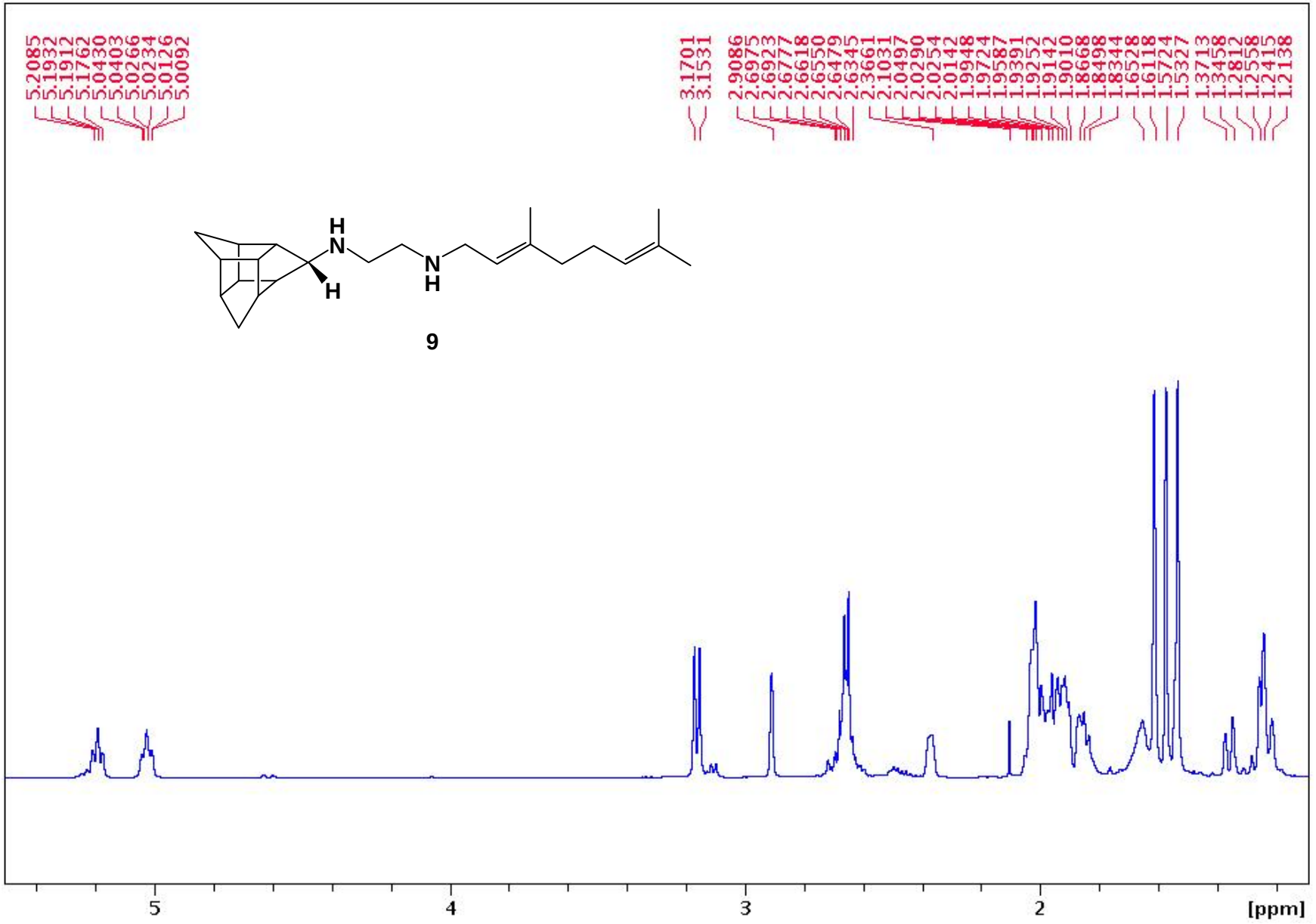

proton nmr of geranyl ethylene diamine in cdcl3
5.2428
5.2399
5.2259
5.2229
5.2089
5.2060
5.0745
5.0712
5.0610
5.0577
5.0543
5.0439
5.0404
5.0371
3.2085
3.1915
2.7920
2.7777
2.7626
2.6481
2.6329
2.6188
2.0820
2.0647
2.0457
2.0280
1.9916
1.9718
1.9548
1.6866
1.6838
1.6738
1.6434
1.6068
1.5633
1.3577
NH2
N
H
8
7
6
5
4
3
2
[ppm]

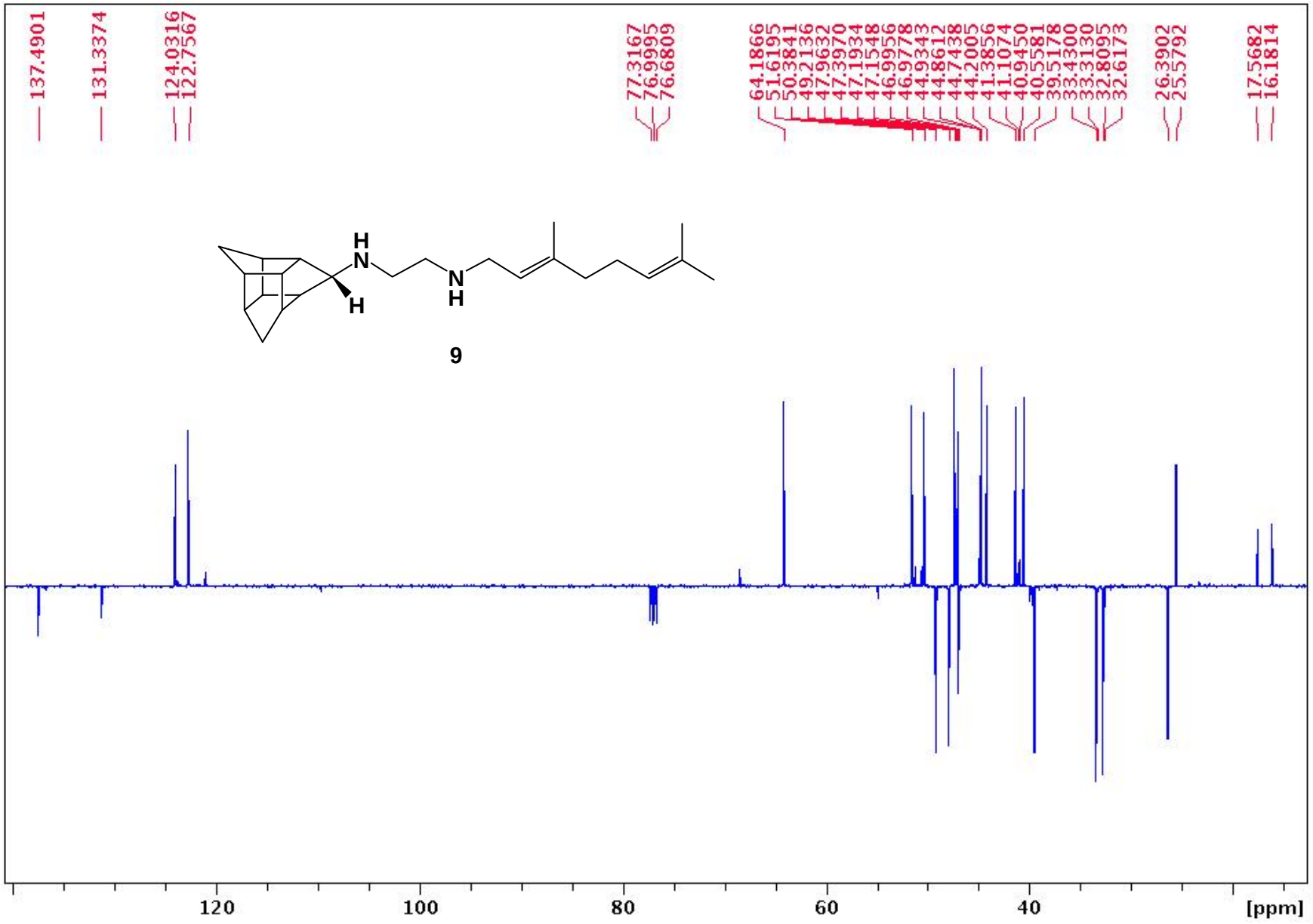
^{1}H NMR of *N*-geranyl ethane-1,2-diamine (8) in $CDCl_3$


C13 nmr of geranyl ethylene diamine in cdcl3
137.6964
131.4848
124.0815
122.7811
77.3166
77.2042
76.9996
76.6816
52.1146
47.0660
41.8330
39.5846
26.4560
25.6520
17.6373
16.2505
NH2
N
H
8
160
140
120
100
80
60
40
20
[ppm]

^{13}C (APT) NMR of *N*-geranyl ethane-1,2-diamine (8) in $CDCl_3$

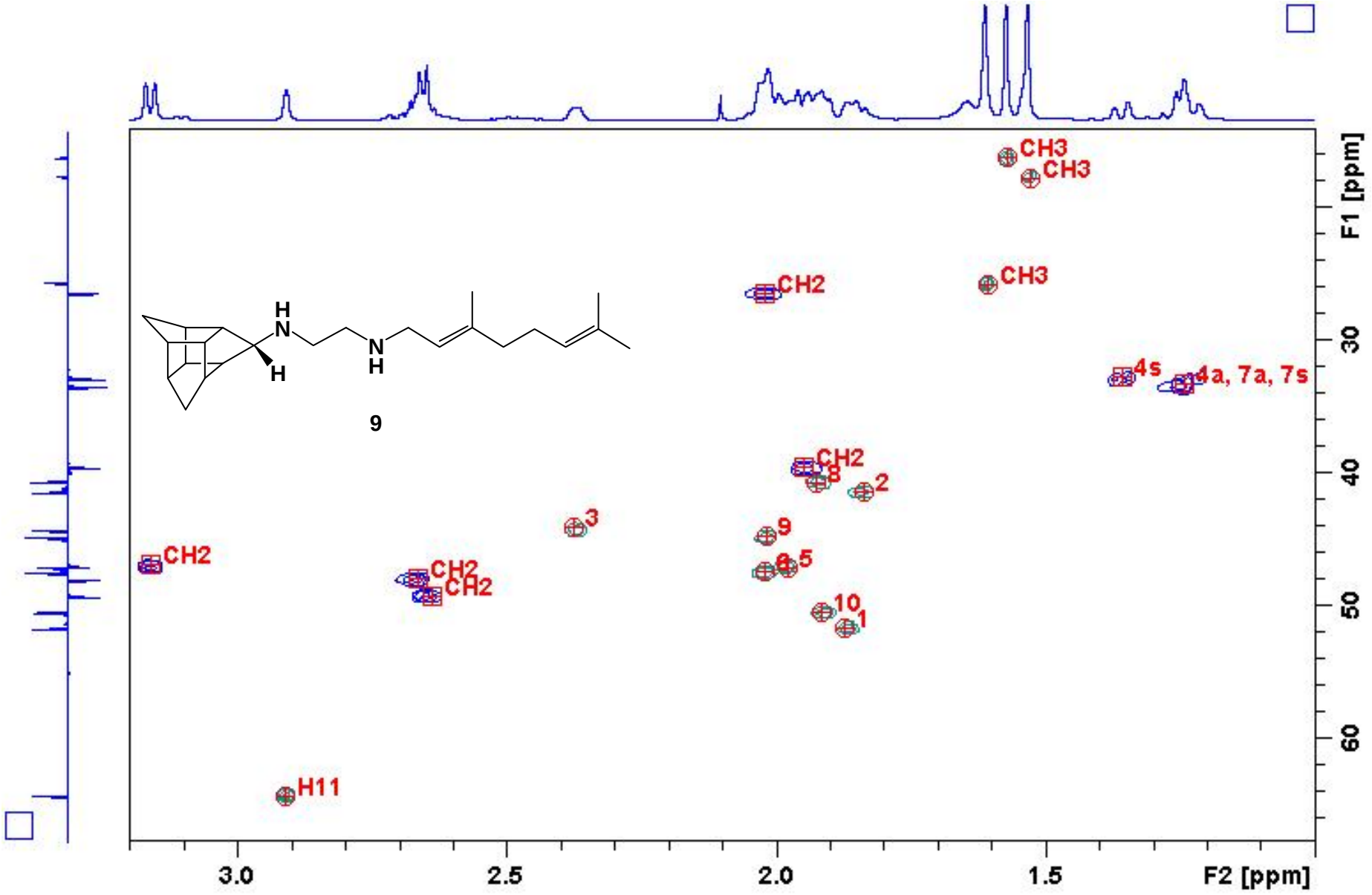

5.2085
5.1932
5.1912
5.1762
5.0430
5.0403
5.0266
5.0234
5.0126
5.0092
3.1701
3.1531
2.9086
2.6975
2.6923
2.6777
2.6618
2.6550
2.6479
2.6345
2.3661
2.1031
2.0497
2.0290
2.0254
2.0142
1.9948
1.9724
1.9587
1.9391
1.9252
1.9142
1.9010
1.8668
1.8498
1.8344
1.6528
1.6118
1.5724
1.5327
1.3713
1.3458
1.2812
1.2558
1.2415
1.2138
H
N
H
N
H
9
5
4
3
2
[ppm]

^{1}H NMR of *N*-Geranyl-*N'*-(trishomocubanyl)ethane-1,2-diamine (9) in $CDCl_3$


137.4901
131.3374
124.0316
122.7567
77.3167
76.9995
76.6809
64.1866
51.6195
50.3841
49.2136
47.9632
47.3970
47.1934
47.1548
46.9956
46.9778
44.9343
44.8612
44.7438
44.2005
41.3856
41.1074
40.9450
40.5581
39.5178
33.4300
33.3130
32.8095
32.6173
26.3902
25.5792
17.5682
16.1814
H
N
H
N
H
120
100
80
60
40
[ppm]

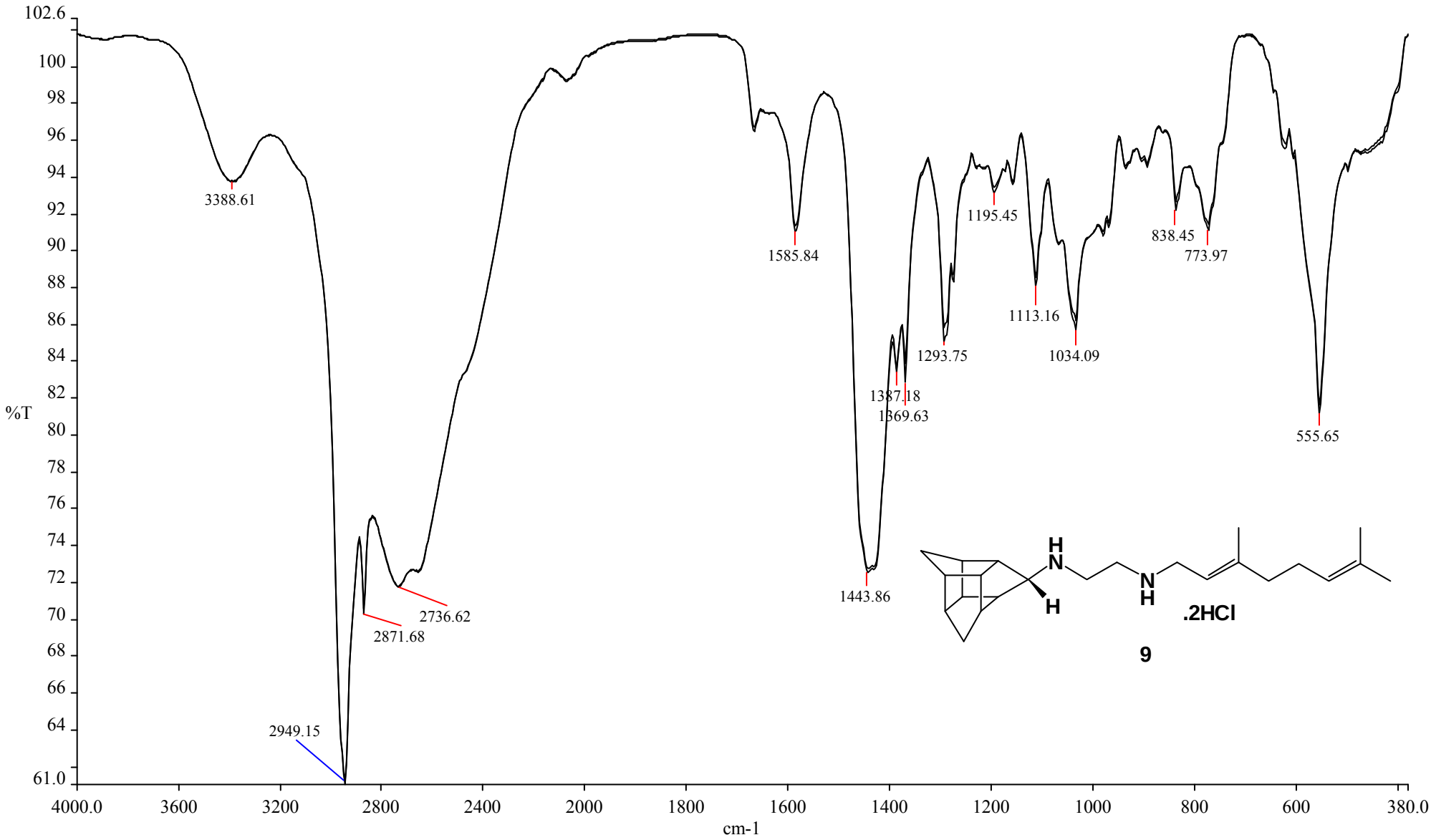
9

^{13}C (APT) NMR of *N*-Geranyl-*N'*-(trishomocubanyl)ethane-1,2-diamine (9) in $CDCl_3$

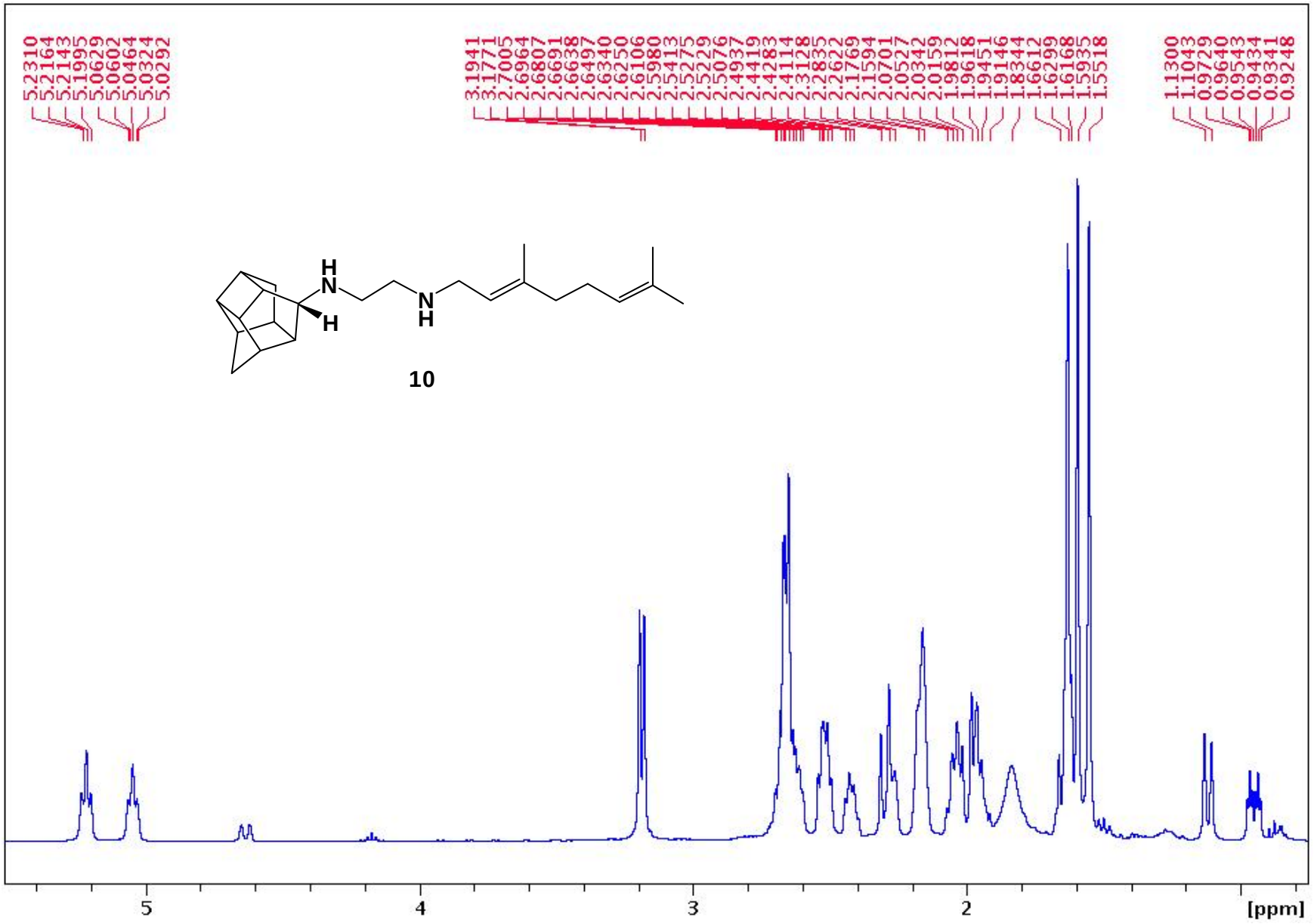

CH3
CH3
CH2
CH3
4s
4a, 7a, 7s
CH2
8
2
3
9
CH2
CH2
CH2
5
10
1
H11
F1 [ppm]
30
40
50
60
3.0
2.5
2.0
1.5
F2 [ppm]

9

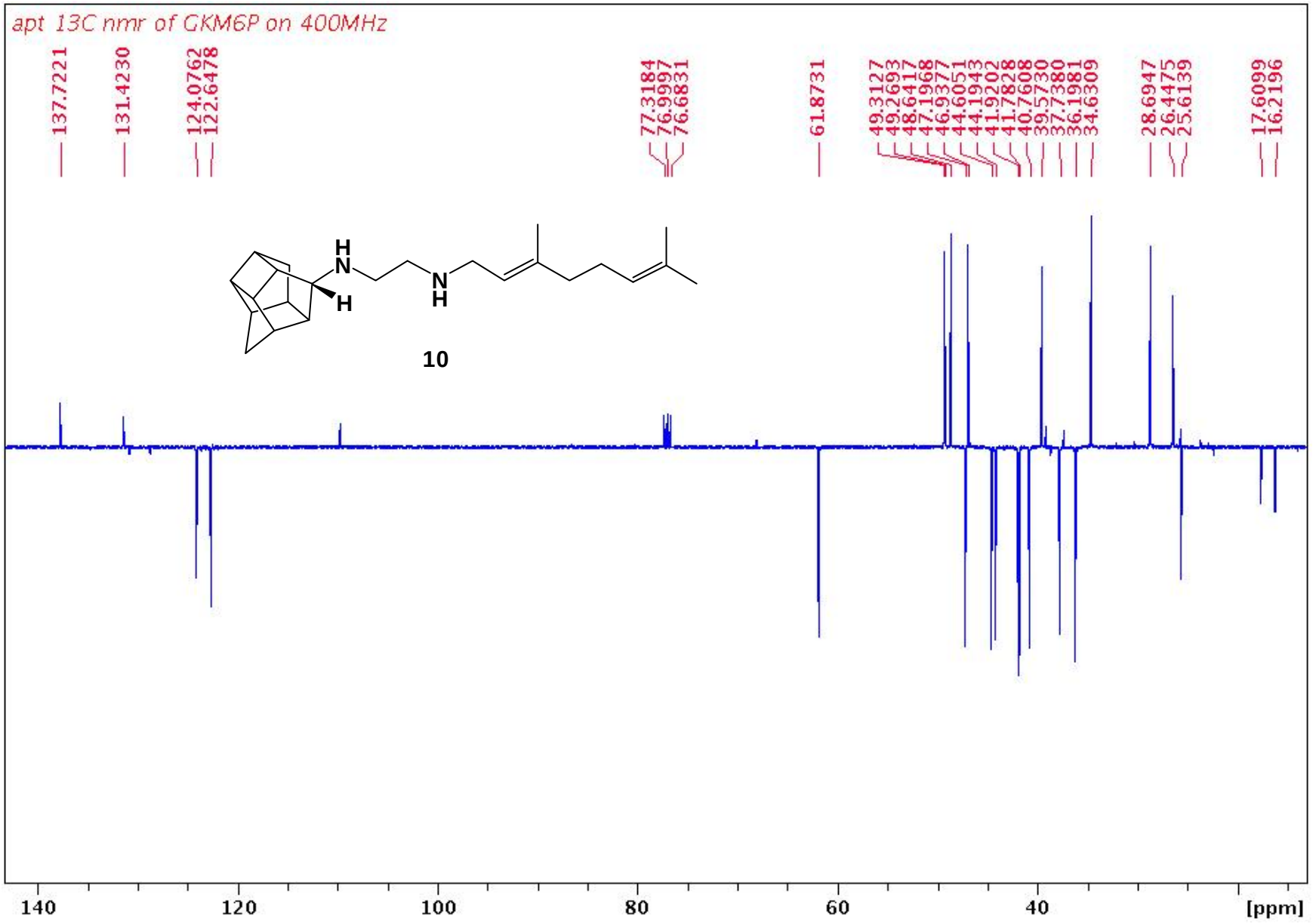
HSQC spectrum of *N*-Geranyl-*N'*-(trishomocubanyl)ethane-1,2-diamine (9) in $CDCl_3$


102.6
100
98
96
94
92
90
88
86
84
82
80
78
76
74
72
70
68
66
64
61.0
%T
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380.0
cm-1
3388.61
2949.15
2871.68
2736.62
1585.84
1443.86
1387.18
1369.63
1293.75
1195.45
1113.16
1034.09
838.45
773.97
555.65
H
N
H
N
H
.2HCl
9

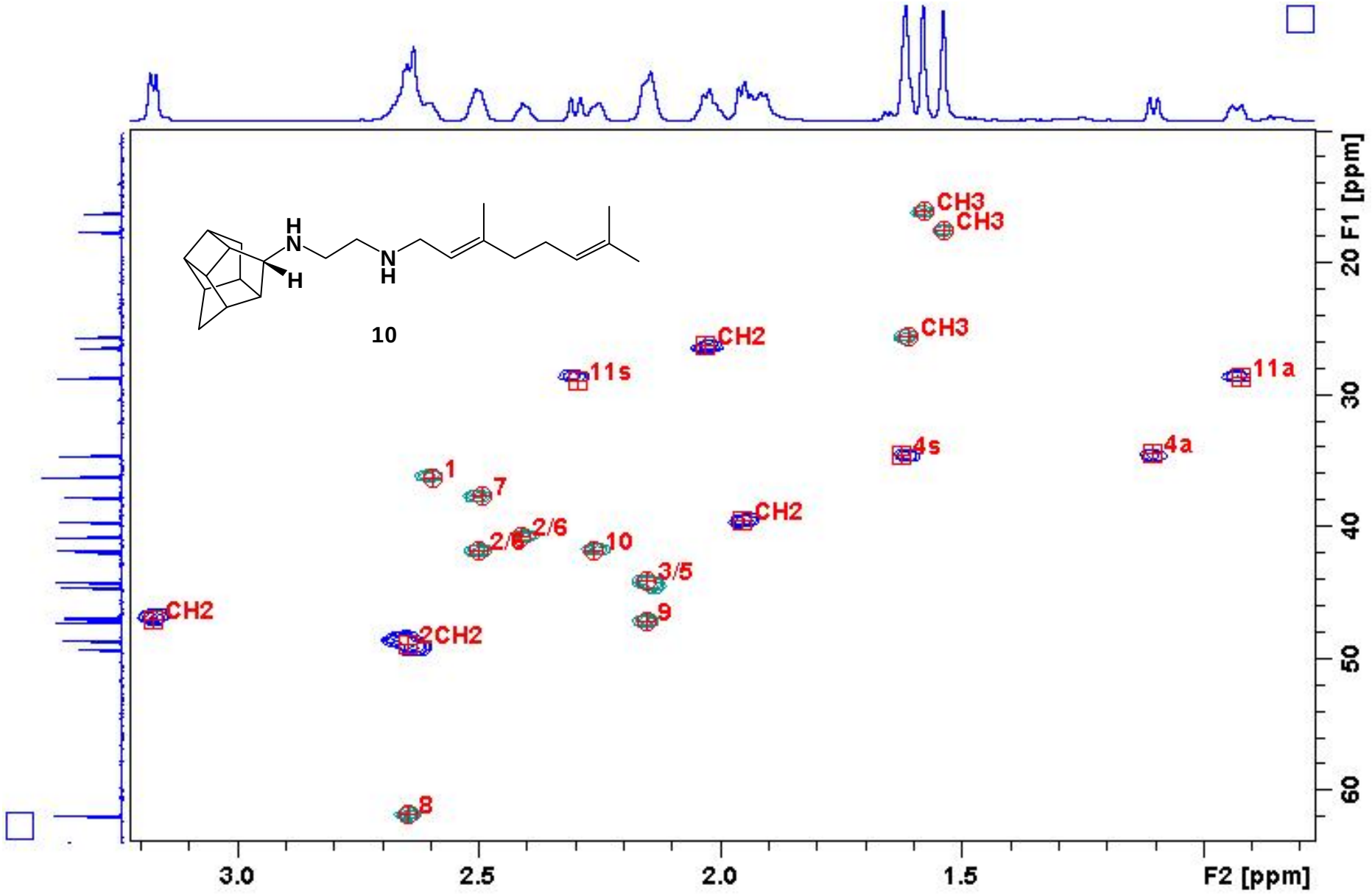
IR spectrum of *N*-Geranyl-*N'*-(trishomocubanyl)ethane-1,2-diamine (9)


5.2310
5.2164
5.2143
5.1995
5.0629
5.0602
5.0464
5.0324
5.0292
3.1941
3.1771
2.7005
2.6964
2.6807
2.6691
2.6638
2.6497
2.6340
2.6250
2.6106
2.5980
2.5413
2.5275
2.5229
2.5076
2.4937
2.4419
2.4283
2.4114
2.3128
2.2835
2.2622
2.1769
2.1594
2.0701
2.0527
2.0342
2.0159
1.9812
1.9618
1.9451
1.9146
1.8344
1.6612
1.6299
1.6168
1.5935
1.5518
1.1300
1.1043
0.9729
0.9640
0.9543
0.9434
0.9341
0.9248
H
N
H
N
H
10
5
4
3
2
[ppm]

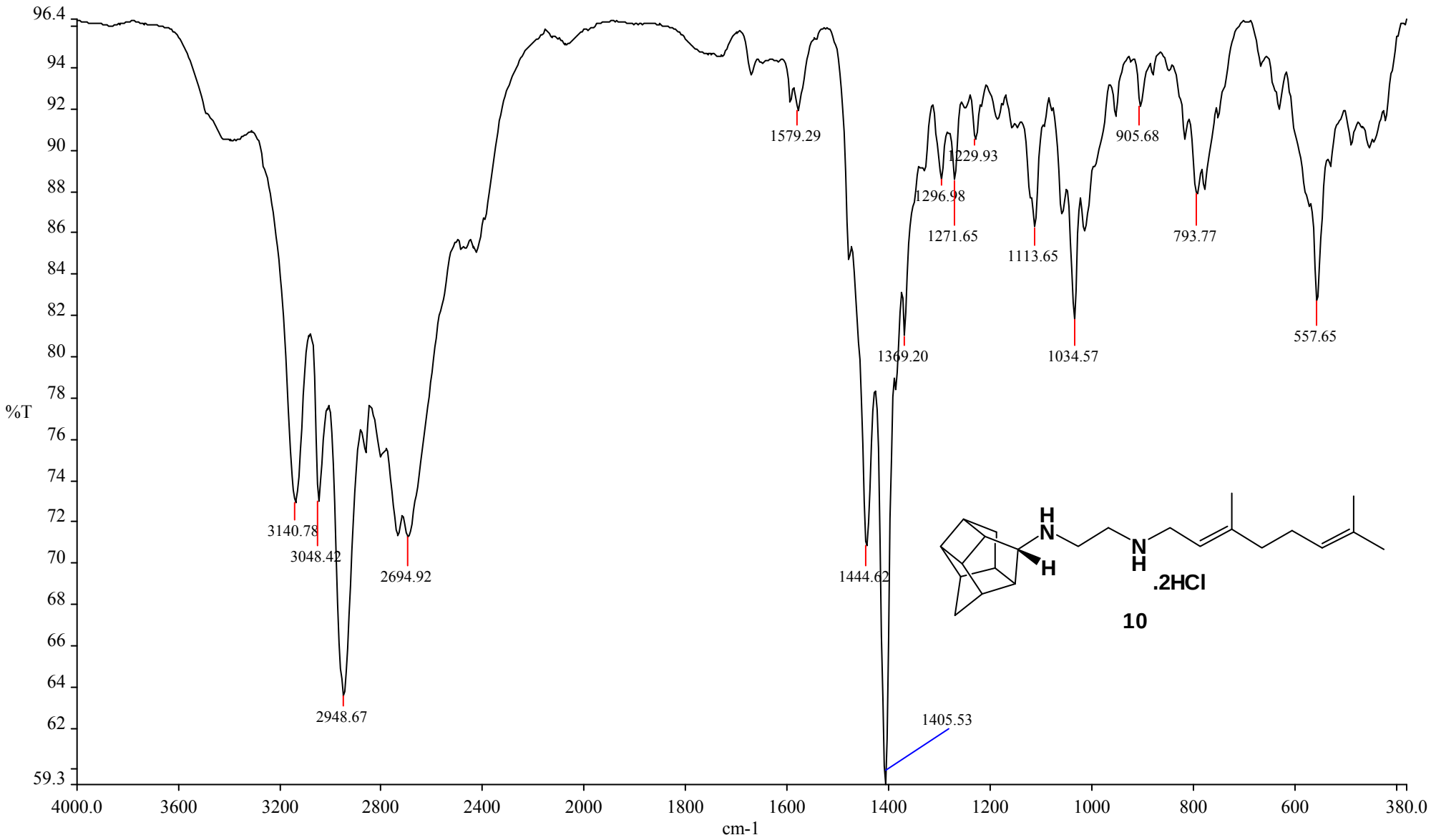
^{1}H NMR of *N*-Geranyl-*N'*-(pentacycloundecyl)ethane-1,2-diamine (10) in $CDCl_3$


apt 13C nmr of GKM6P on 400MHz
137.7221
131.4230
124.0762
122.6478
77.3184
76.9997
76.6831
61.8731
49.3127
49.2693
48.6417
47.1968
46.9377
46.6051
44.1943
41.9202
41.7828
40.7608
39.5730
37.7380
36.1981
34.6309
28.6947
26.4475
25.6139
17.6099
16.2196
H
N
H
N
H
10
140
120
100
80
60
40
[ppm]

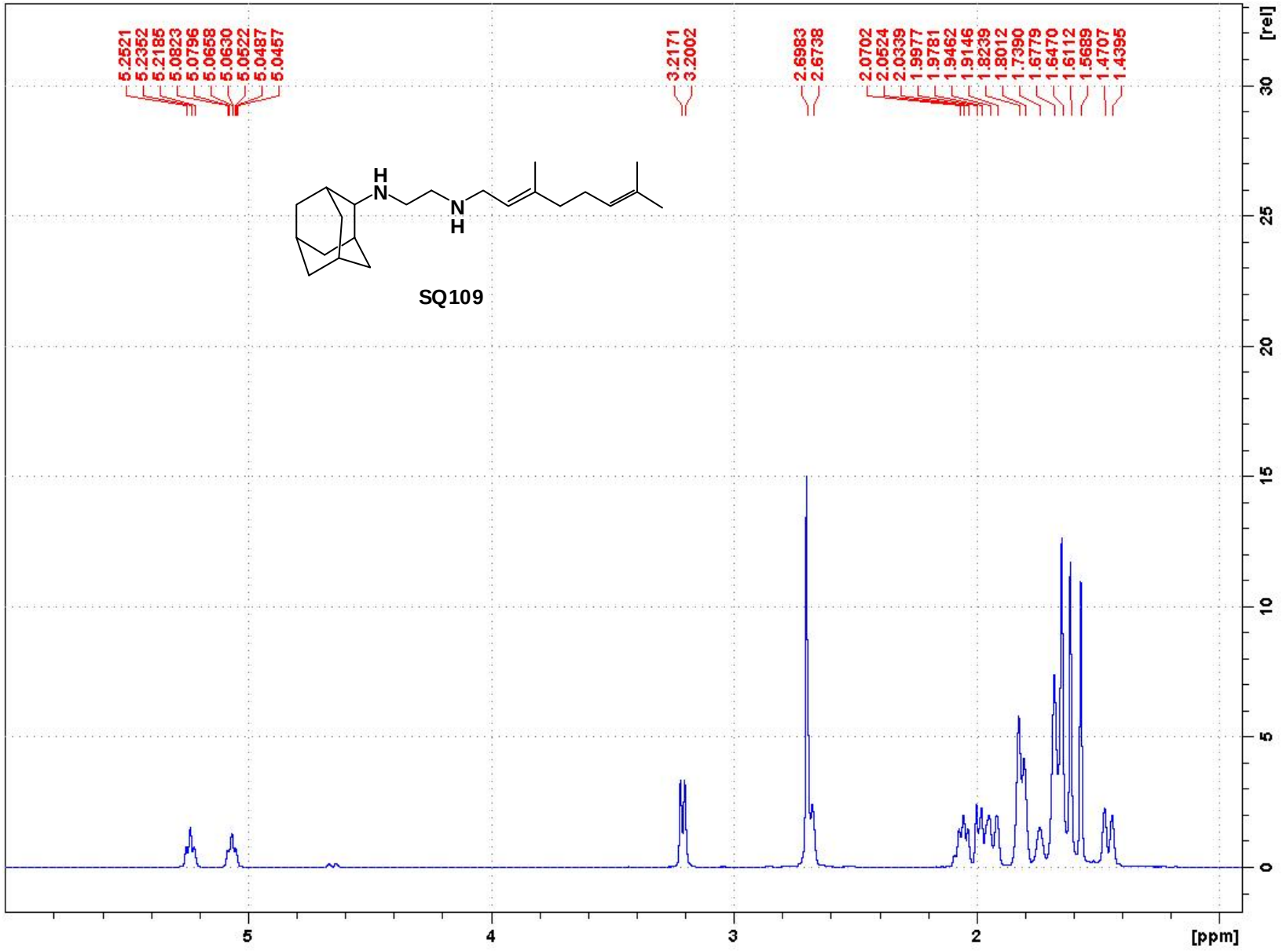
^{13}C (APT) NMR of *N*-Geranyl-*N'*-(pentacycloundecyl)ethane-1,2-diamine (10) in $CDCl_3$


H
N
H
N
H
10
CH3
CH3
CH3
CH2
11s
11a
4s
4a
1
7
2/6
2/6
10
CH2
3/5
9
CH2
2CH2
8
20 F1 [ppm]
30
40
50
60
3.0
2.5
2.0
1.5
F2 [ppm]

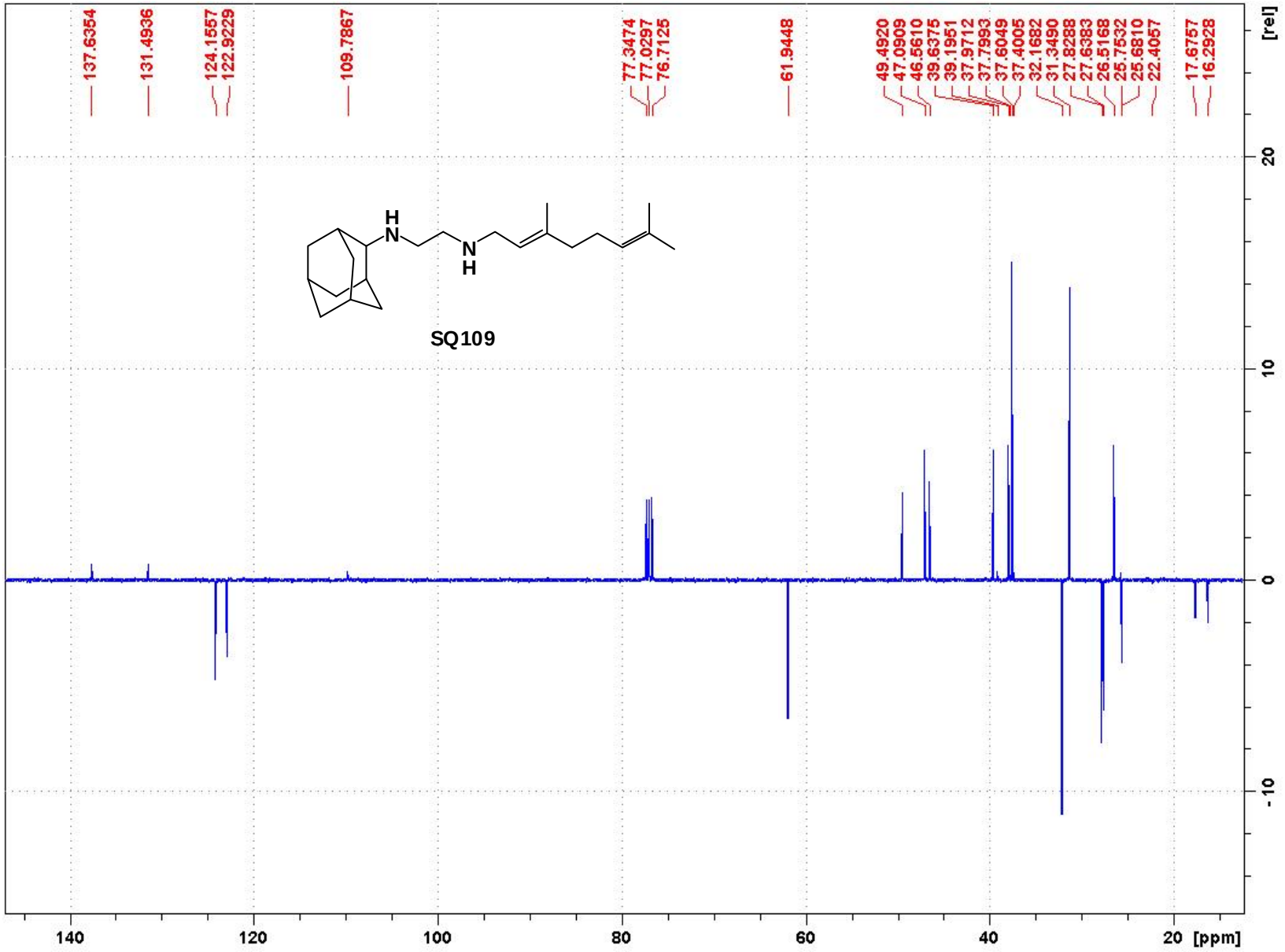
HSQC spectrum of *N*-Geranyl-*N'*-(pentacycloundecyl)ethane-1,2-diamine (10) in $CDCl_3$



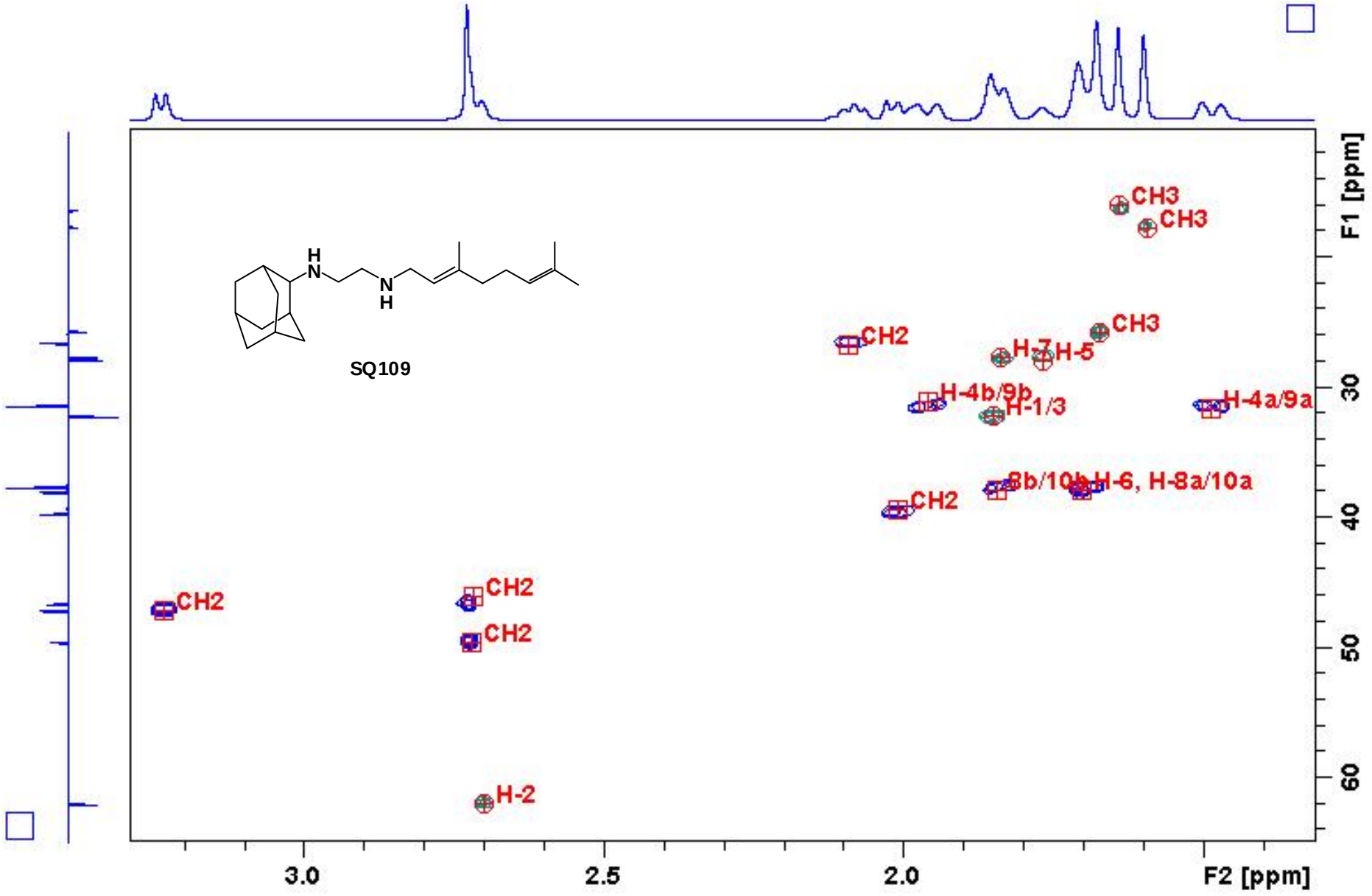
IR spectrum of *N*-Geranyl-*N'*-(pentacycloundecyl)ethane-1,2-diamine (10)


5.2521
5.2352
5.2185
5.0823
5.0796
5.0658
5.0630
5.0522
5.0487
5.0457
3.2171
3.2002
2.6983
2.6738
2.0702
2.0524
2.0339
1.9977
1.9781
1.9462
1.9146
1.8239
1.8012
1.7390
1.6779
1.6470
1.6112
1.5689
1.4707
1.4395
H
N
N
H
SQ109
[rel]
30
25
20
15
10
5
0
5
4
3
2
[ppm]

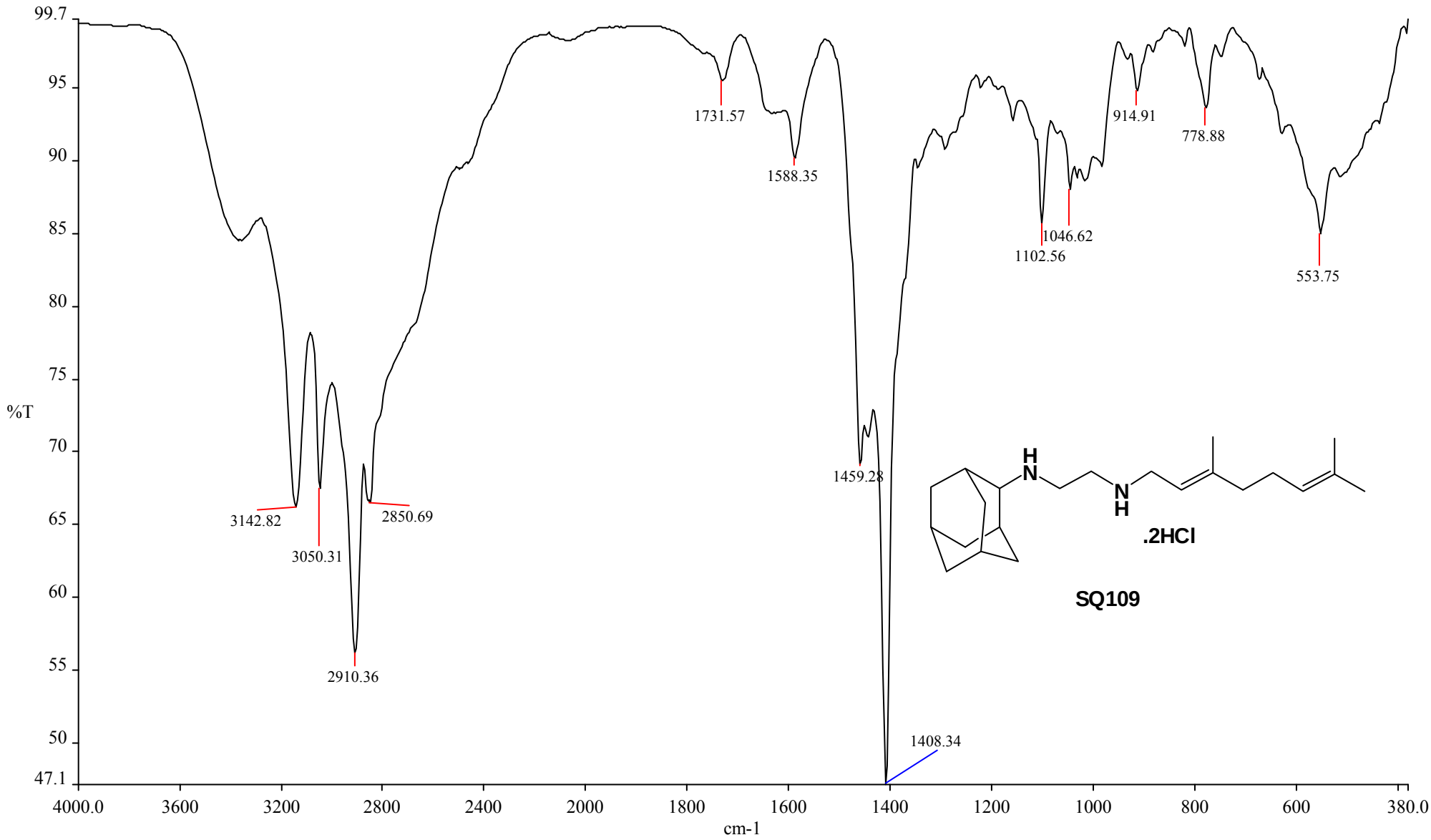
^{1}H NMR of *N*-Geranyl-*N'*-(2-adamantyl)ethane-1,2-diamine (SQ109) in $CDCl_3$


137.6354
131.4936
124.1557
122.9229
109.7867
77.3474
77.0297
76.7125
61.9448
49.4920
47.0909
46.5610
39.6375
39.1951
37.9712
37.7993
37.6049
37.4005
32.1682
31.3490
27.8288
27.6383
26.5168
25.7532
25.6810
22.4057
17.6757
16.2928
[rel]
20
10
0
-10
140
120
100
80
60
40
20 [ppm]
H
N
N
H
SQ109

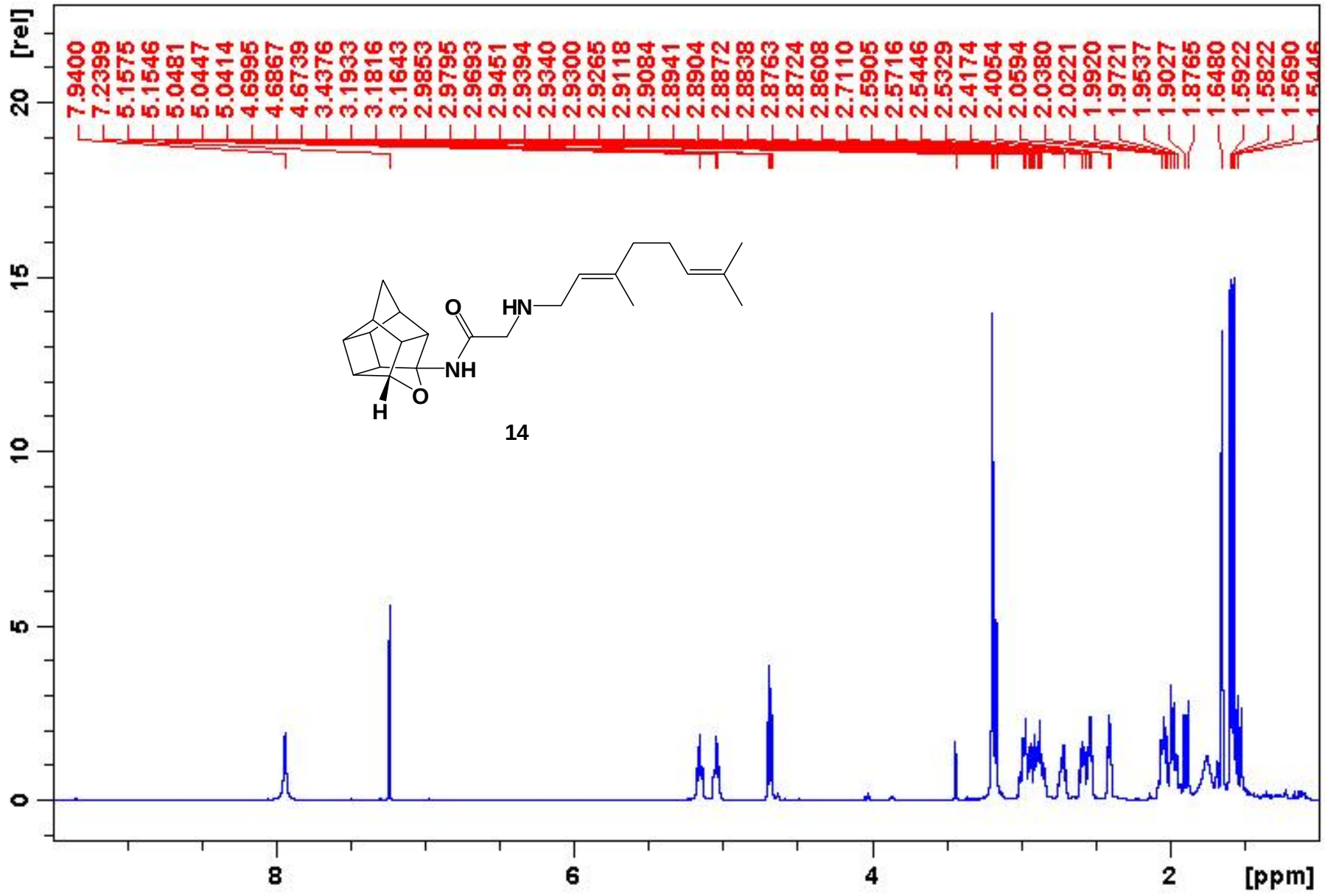
^{13}C (APT) NMR of *N*-Geranyl-*N'*-(2-adamantyl)ethane-1,2-diamine (SQ109) in $CDCl_3$


SQ109
CH3
CH3
CH2
CH3
H-7
H-5
H-4b/9b
H-1/3
H-4a/9a
8b/10b
H-6, H-8a/10a
CH2
CH2
CH2
CH2
H-2
F1 [ppm]
F2 [ppm]
3.0
2.5
2.0
30
40
50
60

HSQC spectrum of *N*-Geranyl-*N'*-(2-adamantyl)ethane-1,2-diamine (SQ109) in $CDCl_3$

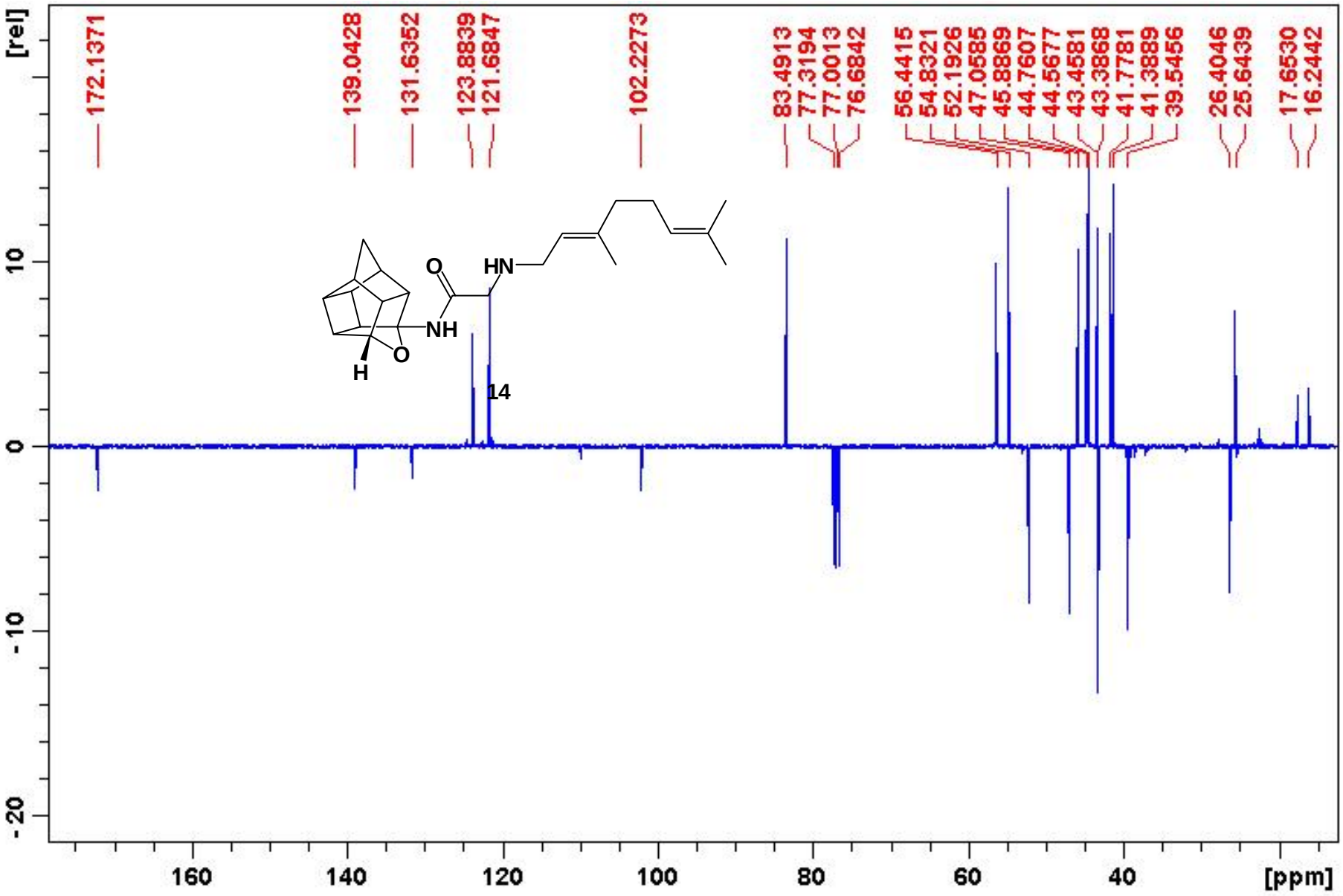

99.7
95
90
85
80
75
%T
70
65
60
55
50
47.1
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380.0
cm-1
3142.82
3050.31
2910.36
2850.69
1731.57
1588.35
1459.28
1408.34
1102.56
1046.62
914.91
778.88
553.75
H
N
N
H
.2HCl
SQ109

IR spectrum of *N*-Geranyl-*N'*-(2-adamantyl)ethane-1,2-diamine (SQ109)

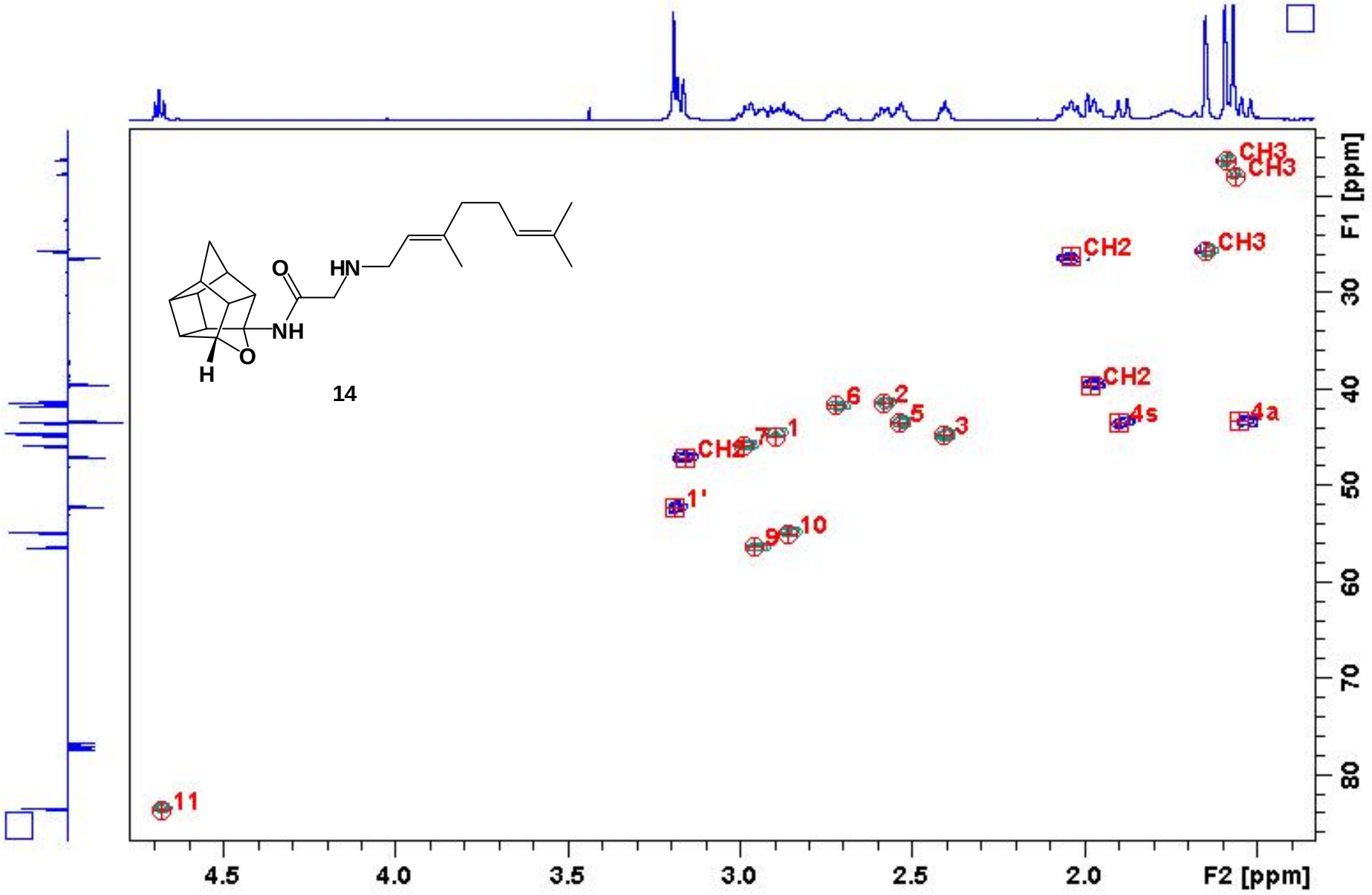

7.9400
7.2399
5.1575
5.1546
5.0481
5.0447
5.0414
4.6995
4.6867
4.6739
3.4376
3.1933
3.1816
3.1643
2.9853
2.9795
2.9693
2.9451
2.9394
2.9340
2.9300
2.9265
2.9118
2.9084
2.8941
2.8904
2.8872
2.8838
2.8763
2.8724
2.8608
2.7110
2.5905
2.5716
2.5446
2.5329
2.4174
2.4054
2.0594
2.0380
2.0221
1.9920
1.9721
1.9537
1.9027
1.8765
1.6480
1.5922
1.5822
1.5690
1.5446
20
15
10
5
0
[rel]
8
6
4
2
[ppm]
O
HN
NH
O
H

14

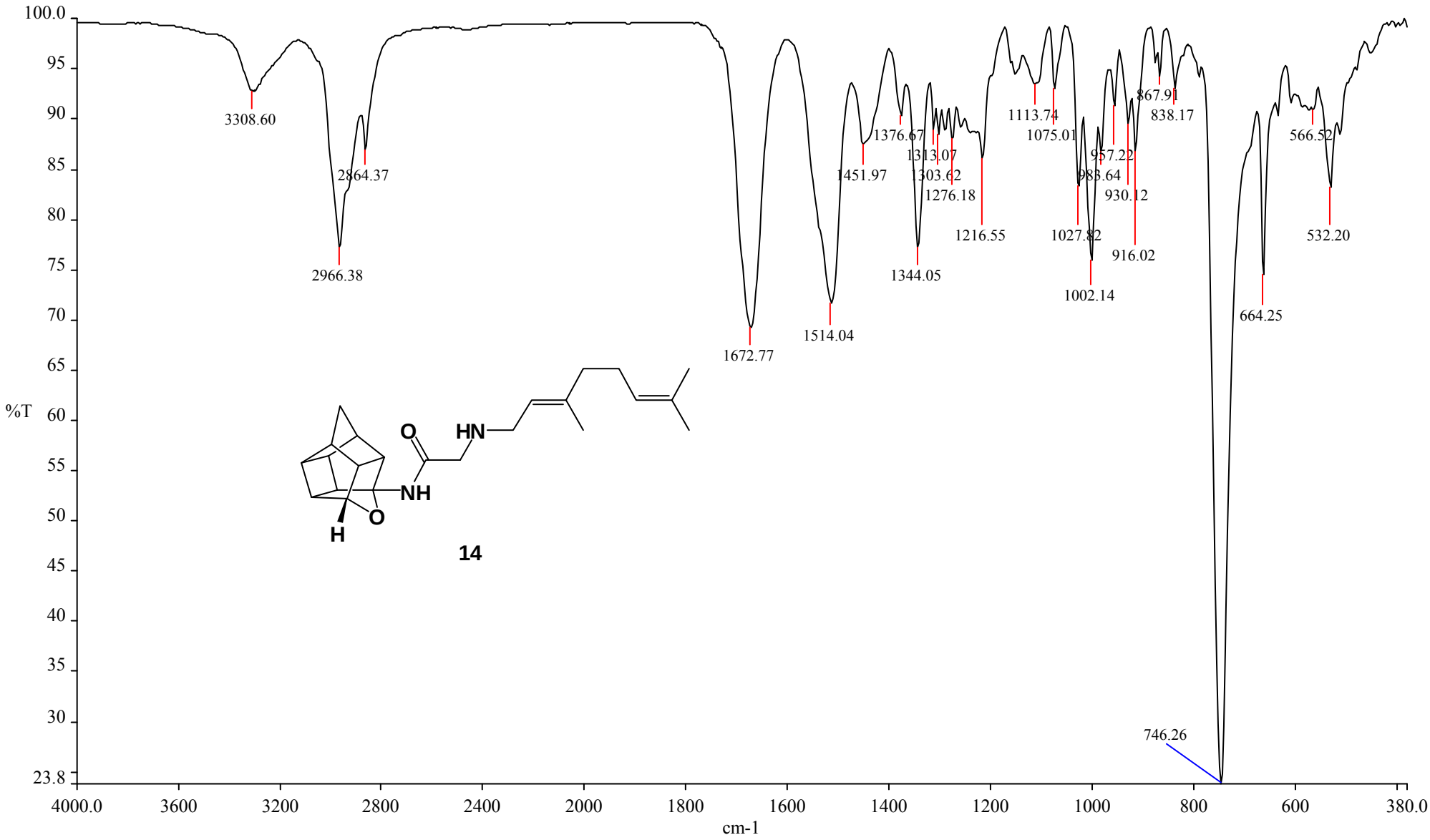
^{1}H NMR spectrum of compound 14 in $CDCl_3$


[rel]
172.1371
139.0428
131.6352
123.8839
121.6847
102.2273
83.4913
77.3194
77.0013
76.6842
56.4415
54.8321
52.1926
47.0585
45.8869
44.7607
44.5677
43.4581
43.3868
41.7781
41.3889
39.5456
26.4046
25.6439
17.6530
16.2442
O
HN
NH
O
H
14
10
0
-10
-20
160
140
120
100
80
60
40
[ppm]

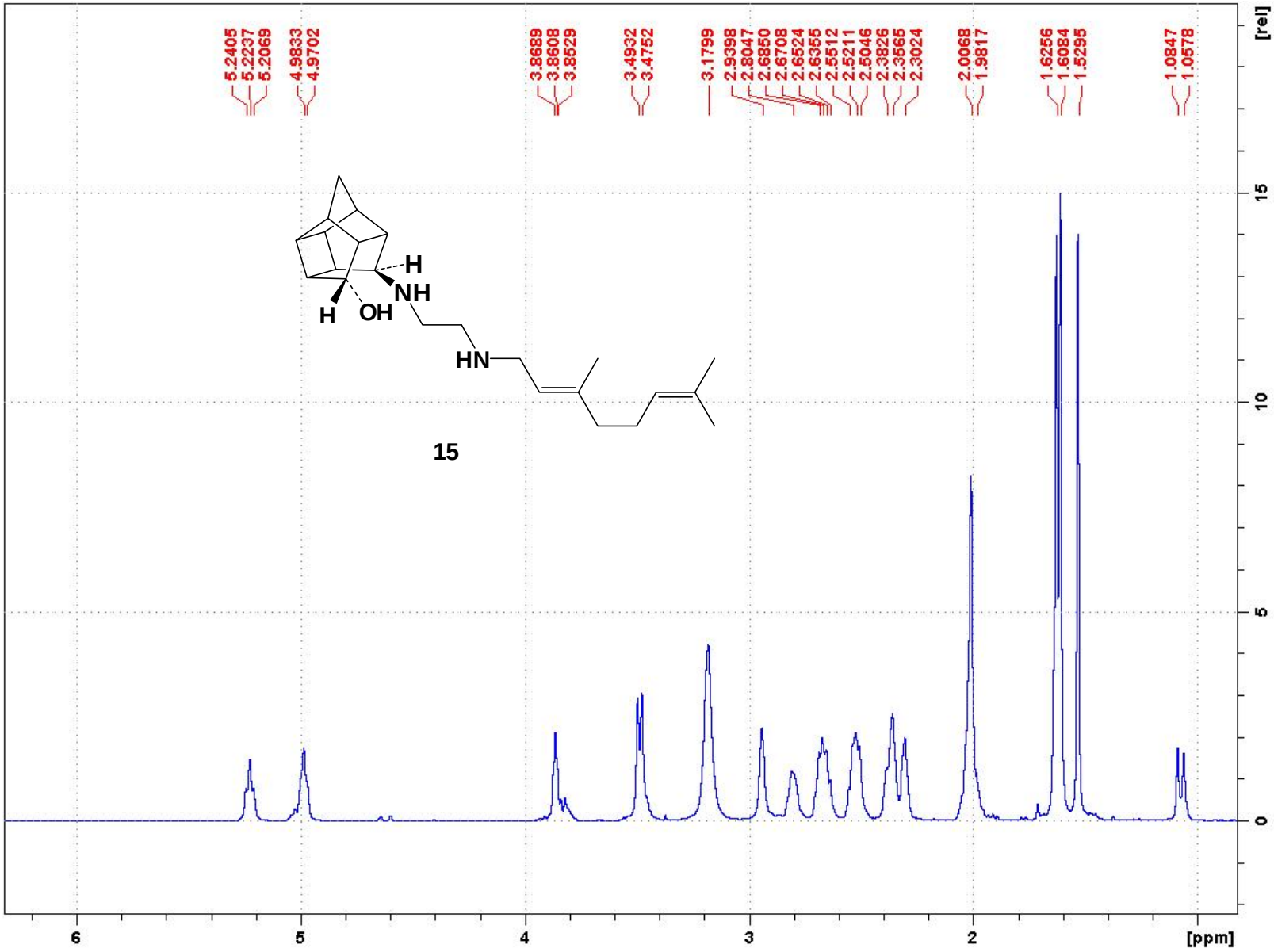
^{13}C APT NMR spectrum of compound 14 in $CDCl_3$


O
HN
NH
O
H
14
CH3
CH3
CH2
CH3
CH2
4s
4a
6
2
5
3
7
1
CH2
1'
9
10
11
F1 [ppm]
30
40
50
60
70
80
4.5
4.0
3.5
3.0
2.5
2.0
F2 [ppm]

HSQC spectrum of compound 14 in $CDCl_3$

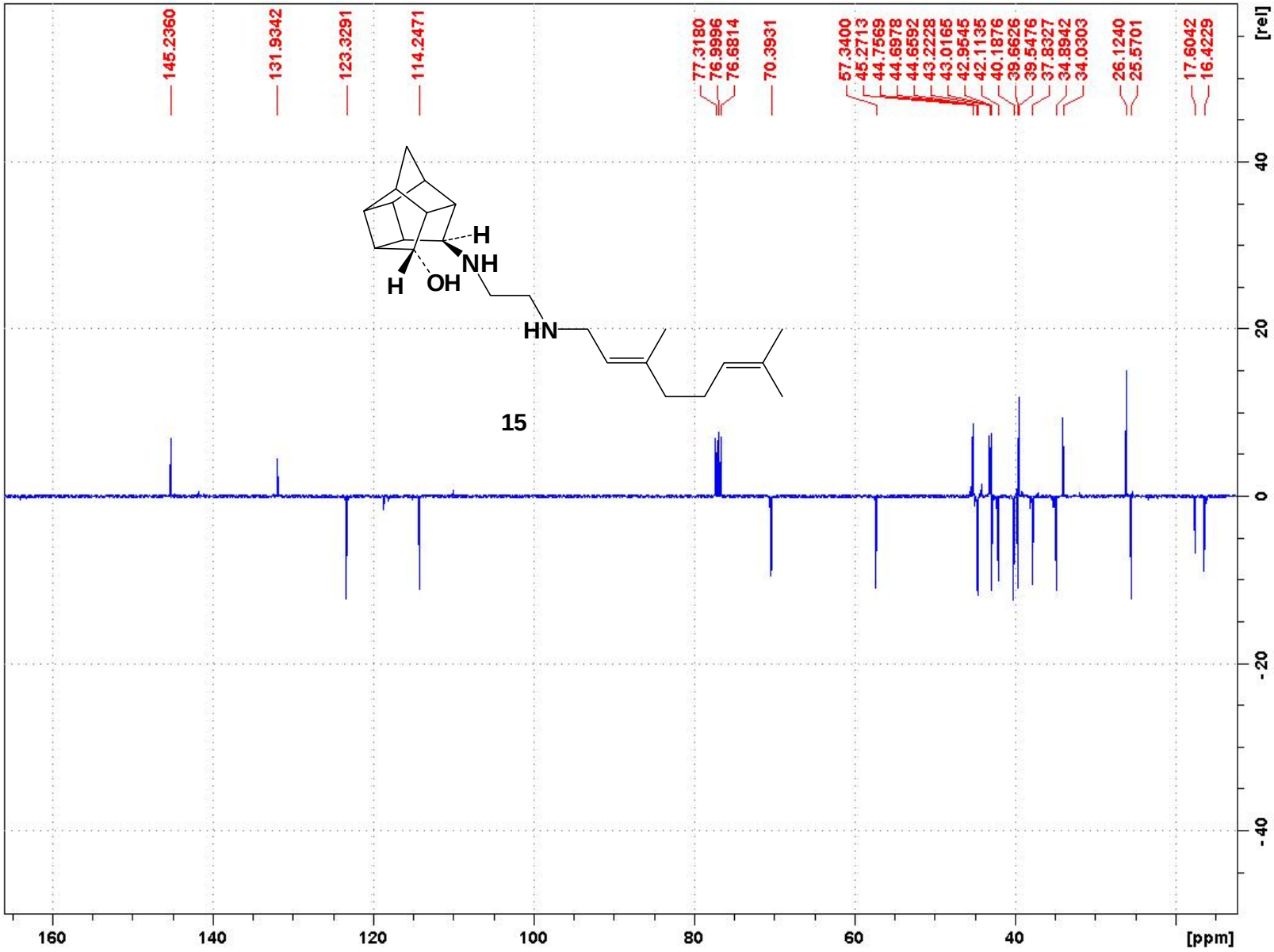


IR spectrum of compound 14

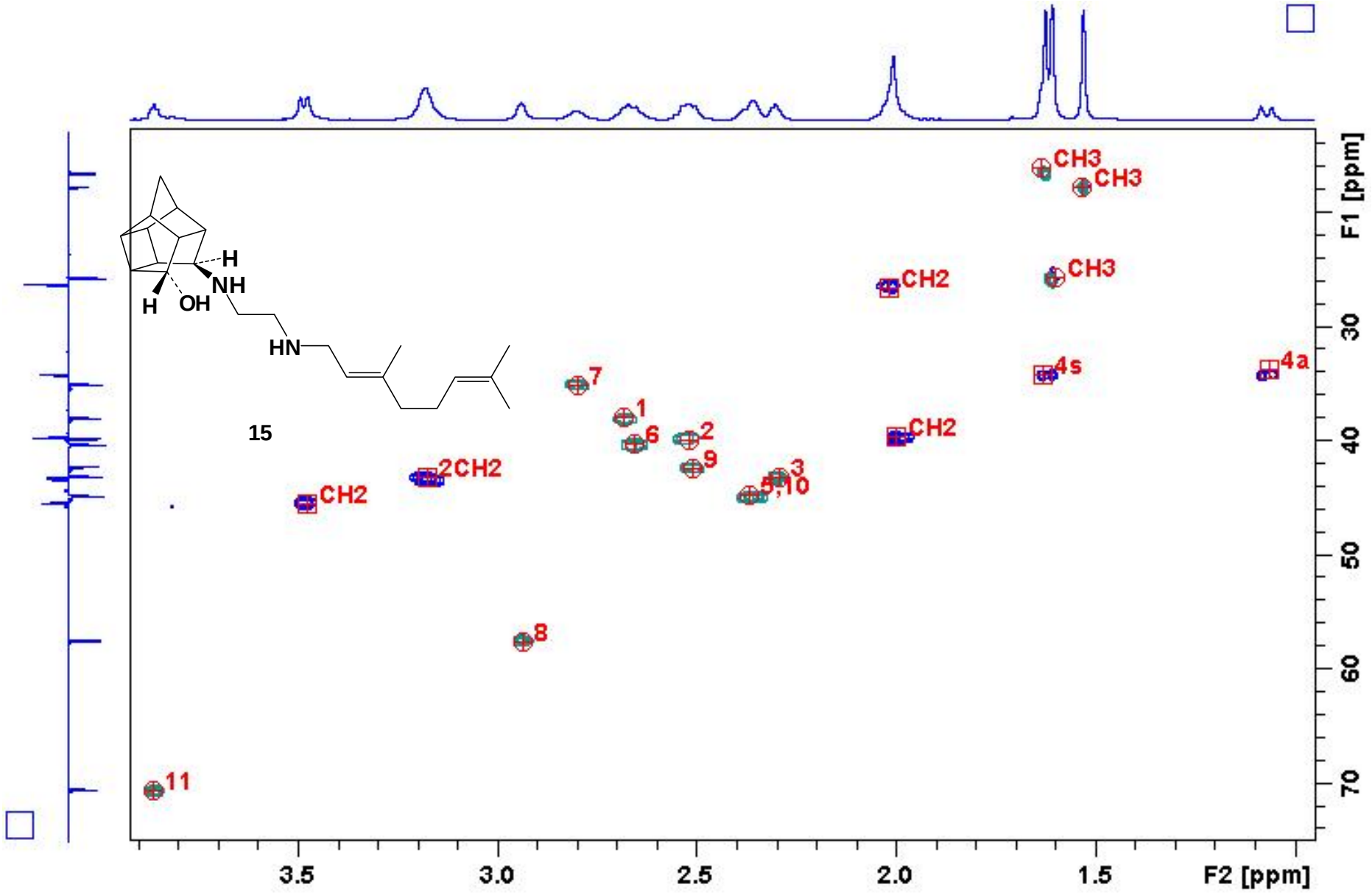

5.2405
5.2237
5.2069
4.9833
4.9702
3.8689
3.8608
3.8529
3.4932
3.4752
3.1799
2.9398
2.8047
2.6850
2.6708
2.6524
2.6355
2.5512
2.5211
2.5046
2.3826
2.3565
2.3024
2.0068
1.9817
1.6256
1.6084
1.5295
1.0847
1.0578
[rel]
15
10
5
0
H
NH
H
OH
HN
6
5
4
3
2
[ppm]

15

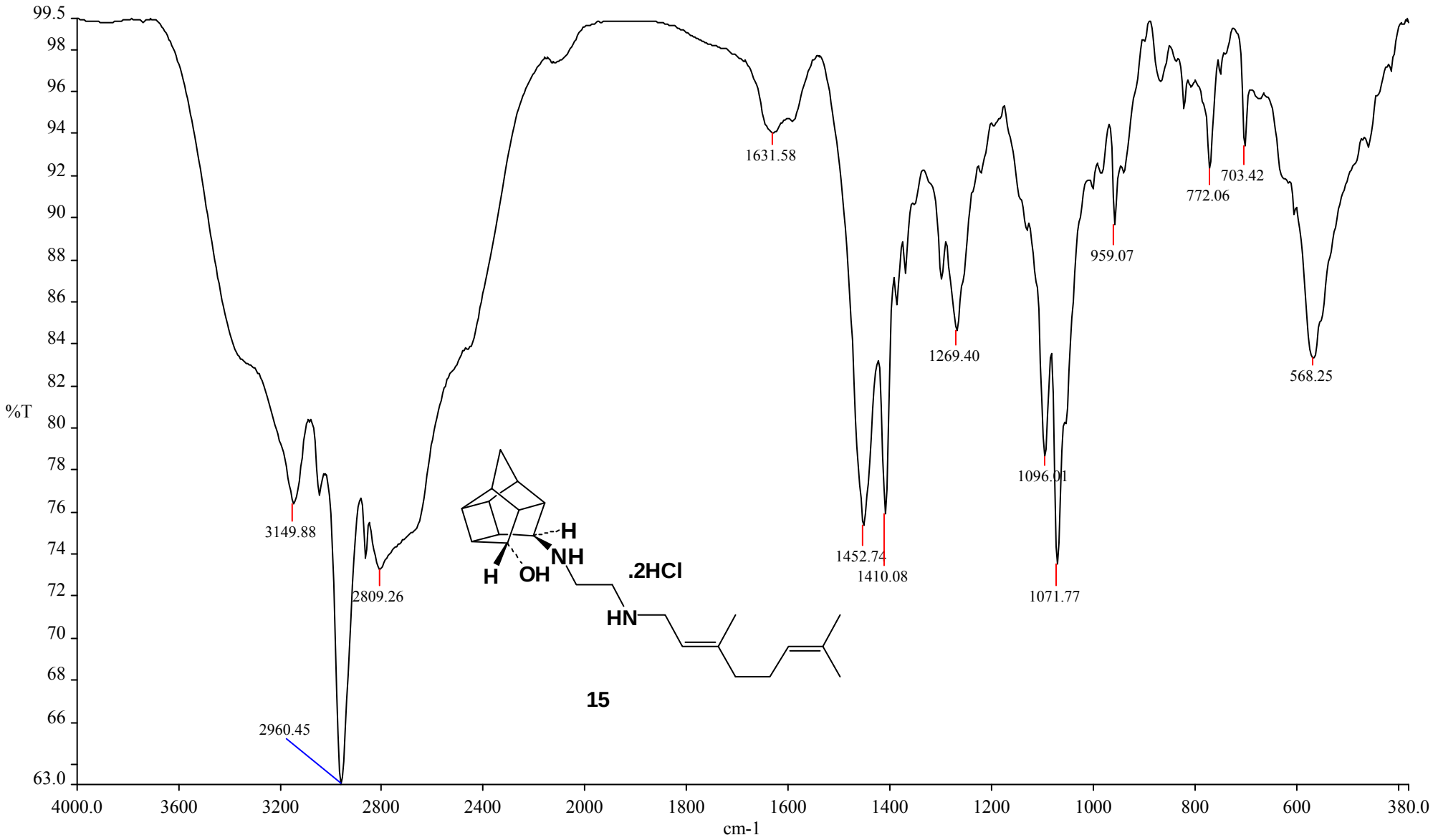
^{1}H NMR spectrum of compound 15 in $CDCl_3$


145.2360
131.9342
123.3291
114.2471
77.3180
76.9996
76.6814
70.3931
57.3400
45.2713
44.7569
44.6978
44.6592
43.2228
43.0165
42.9545
42.1135
40.1876
39.6626
39.5476
37.8327
34.8942
34.0303
26.1240
25.5701
17.6042
16.4229
[rel]
40
20
0
-20
-40
160
140
120
100
80
60
40
[ppm]
H
NH
H
OH
HN
15

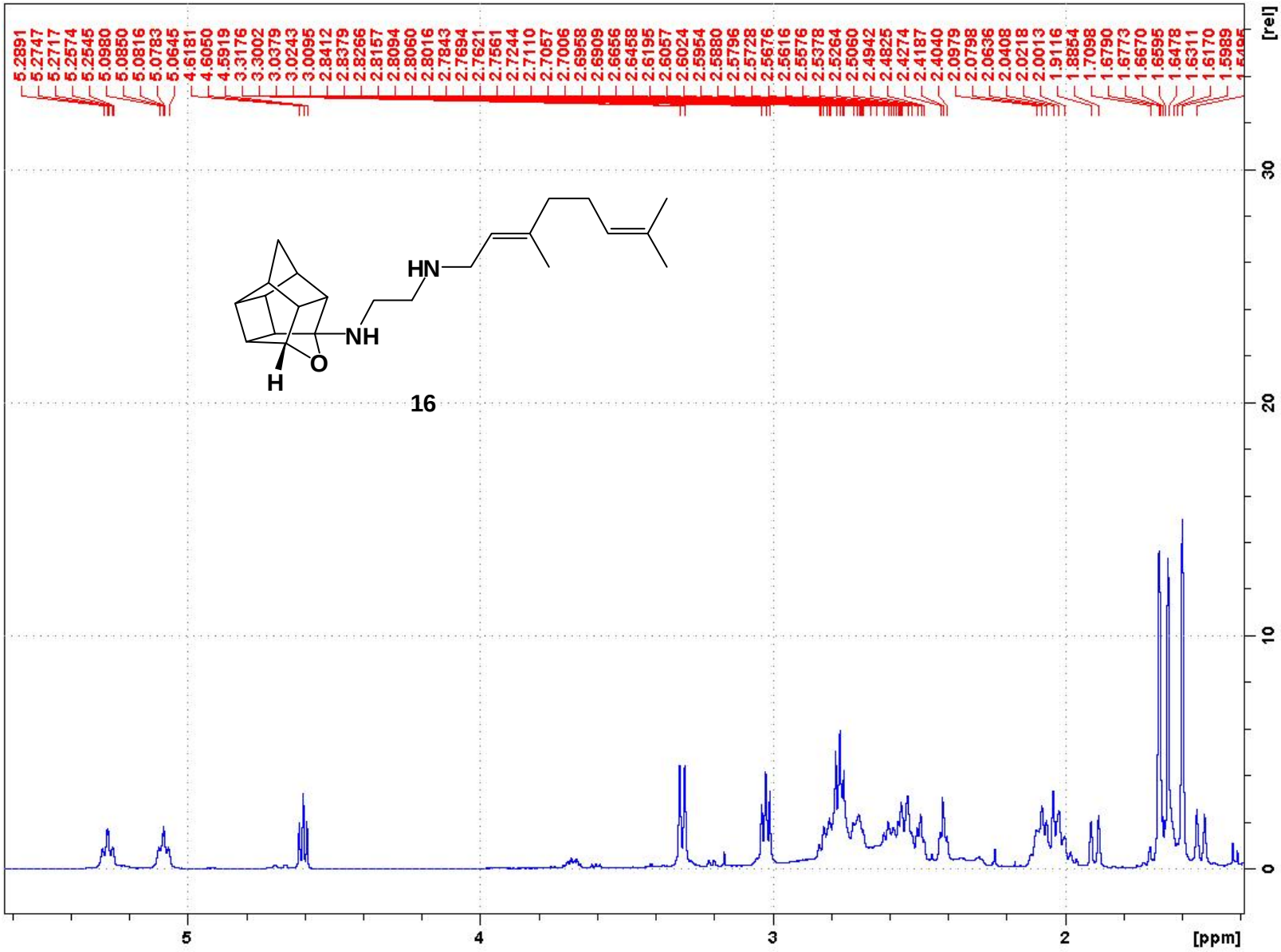
^{13}C (APT) NMR spectrum of compound 15 in $CDCl_3$


H
NH
H
OH
HN
15
CH3
CH3
CH3
CH2
CH2
4s
4a
7
1
6
2
9
3
5,10
2CH2
CH2
8
11
F1 [ppm]
30
40
50
60
70
3.5
3.0
2.5
2.0
1.5
F2 [ppm]

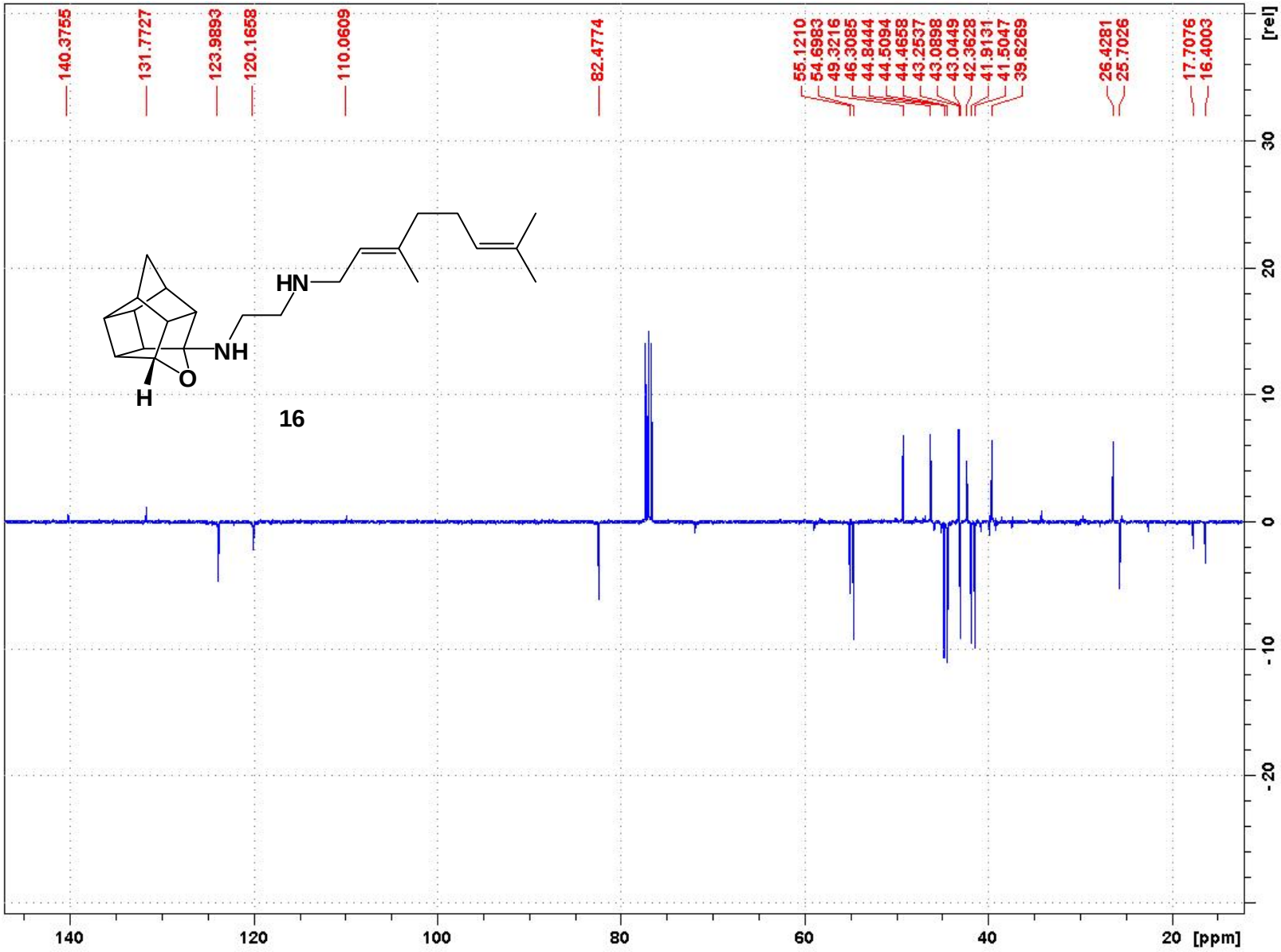
HSQC spectrum of compound 15 in $CDCl_3$



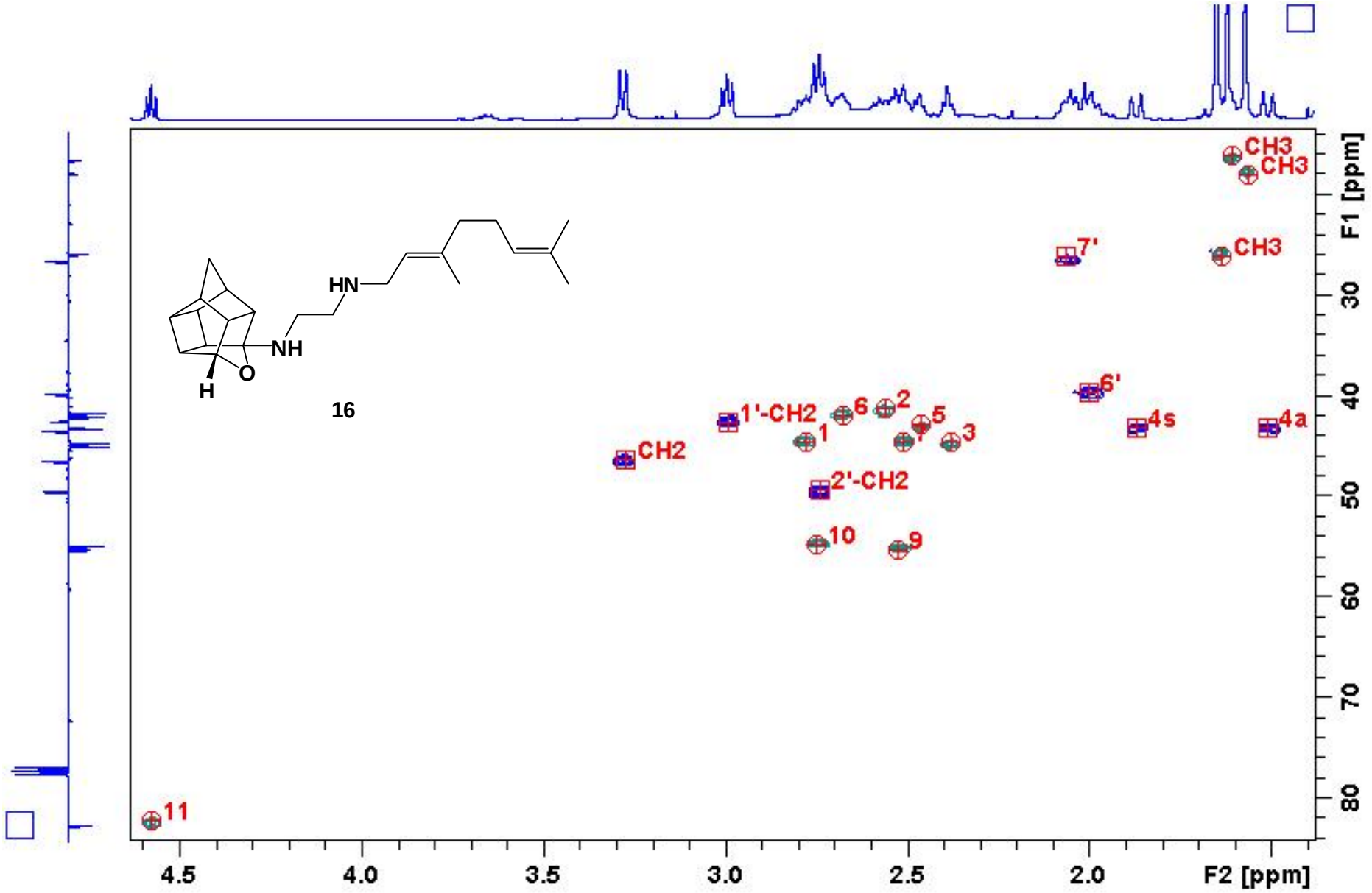
IR spectrum of compound 15


5.2891
5.2747
5.2717
5.2574
5.2545
5.0980
5.0850
5.0816
5.0783
5.0645
4.6181
4.6050
4.5919
3.3176
3.3002
3.0379
3.0243
3.0095
2.8412
2.8379
2.8266
2.8157
2.8094
2.8060
2.8016
2.7843
2.7694
2.7621
2.7561
2.7244
2.7110
2.7057
2.7006
2.6958
2.6909
2.6656
2.6458
2.6195
2.6057
2.6024
2.5954
2.5880
2.5796
2.5728
2.5676
2.5616
2.5576
2.5378
2.5264
2.5060
2.4942
2.4825
2.4274
2.4187
2.4040
2.0979
2.0798
2.0636
2.0408
2.0218
2.0013
1.9116
1.8854
1.7098
1.6790
1.6773
1.6670
1.6595
1.6478
1.6311
1.6170
1.5989
[rel]
30
20
10
0
HN
NH
O
H
16
5
4
3
2
[ppm]

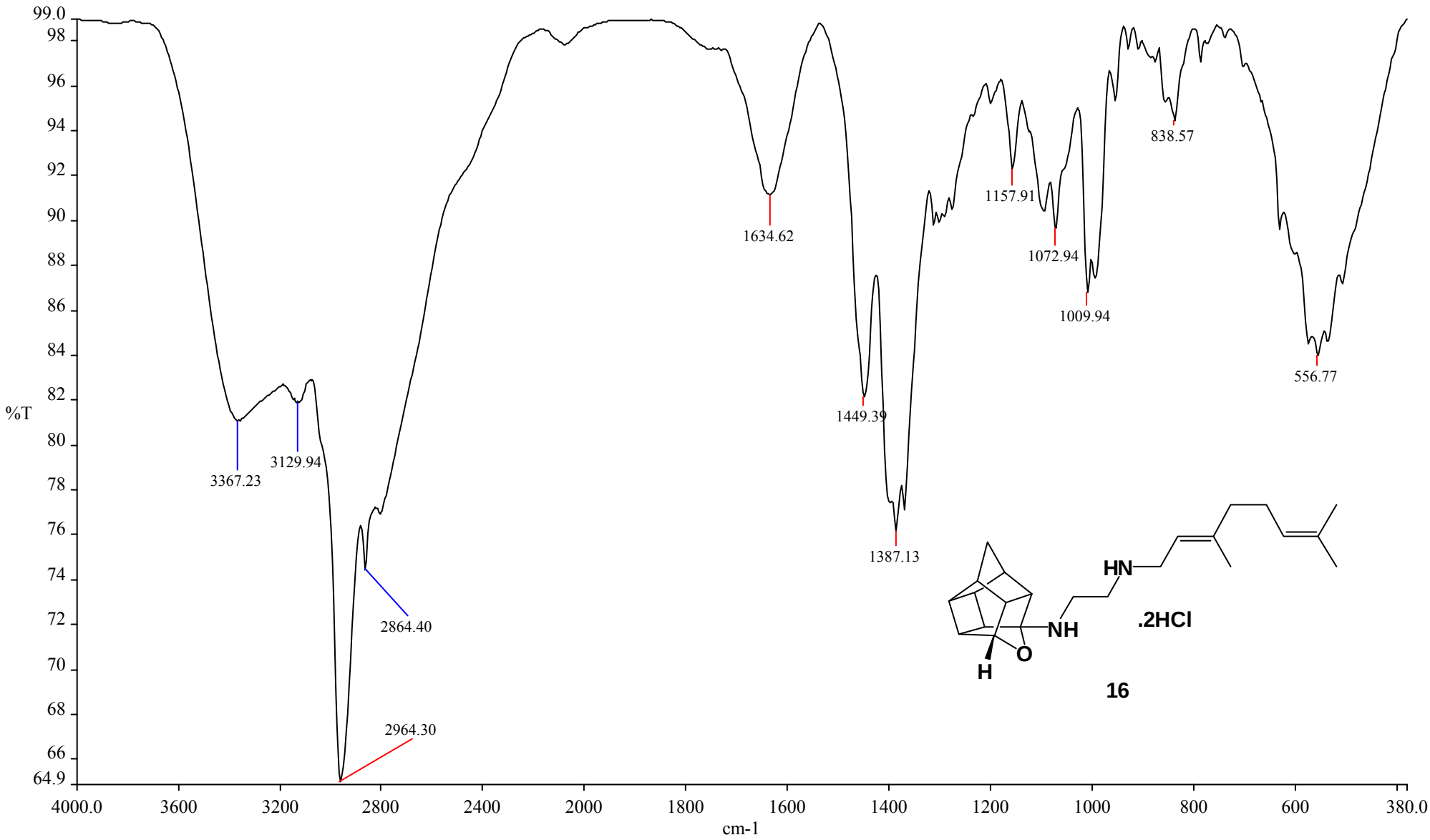
^{1}H NMR spectrum of compound 16 in $CDCl_3$


140.3755
131.7727
123.9893
120.1658
110.0609
82.4774
55.1210
54.6983
49.3216
46.3085
44.8444
44.5094
44.4658
43.2537
43.0898
43.0449
42.3628
41.9131
41.5047
39.6269
26.4281
25.7026
17.7076
16.4003
[rel]
30
20
10
0
-10
-20
HN
NH
O
H
16
140
120
100
80
60
40
20
[ppm]

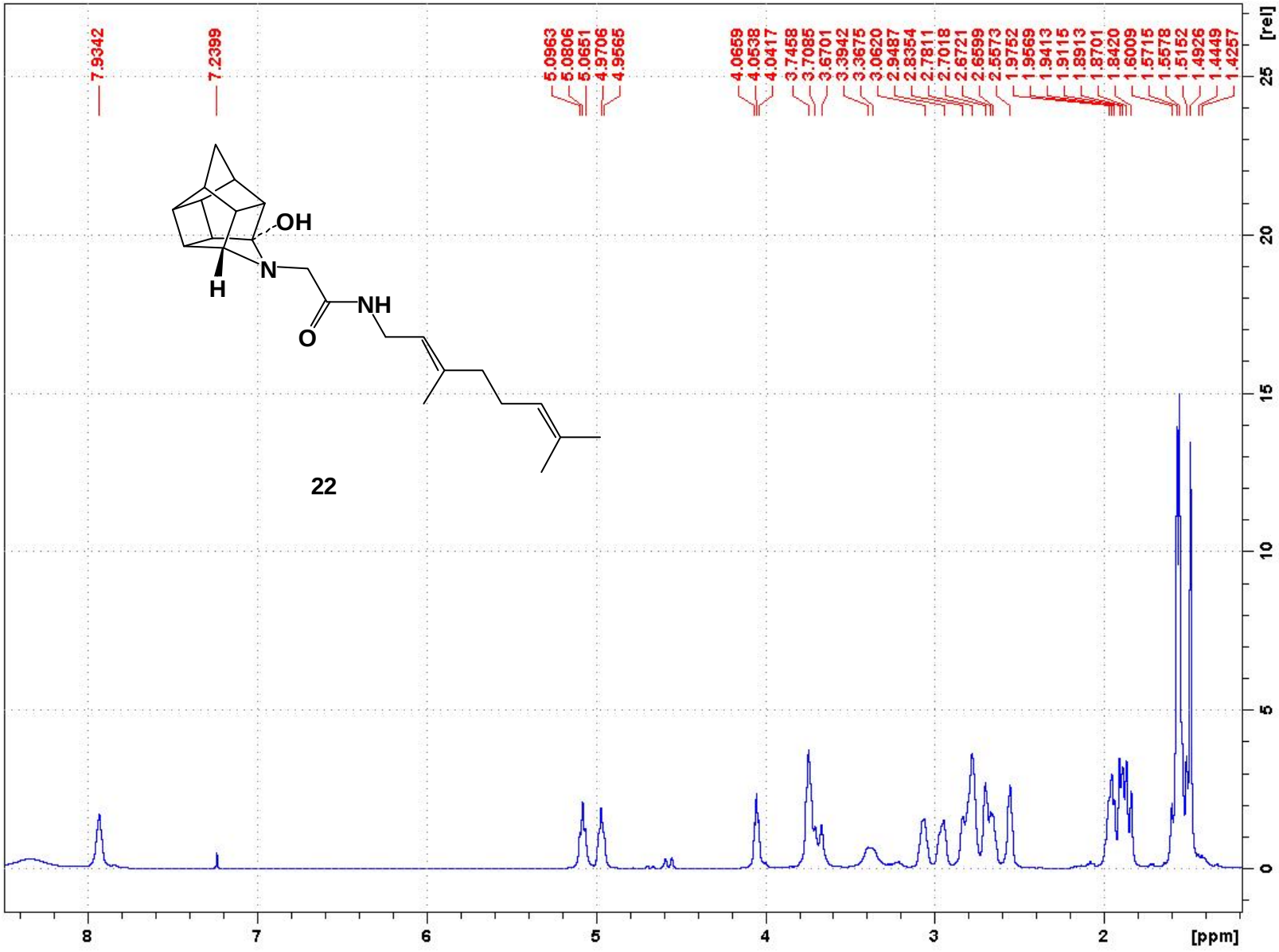
^{13}C (APT) NMR spectrum of compound 16 in $CDCl_3$


HN
NH
O
H
16
CH3
CH3
CH3
7'
6'
4s
4a
1'-CH2
CH2
2'-CH2
1
2
3
5
6
10
9
11
F1 [ppm]
30
40
50
60
70
80
4.5
4.0
3.5
3.0
2.5
2.0
F2 [ppm]

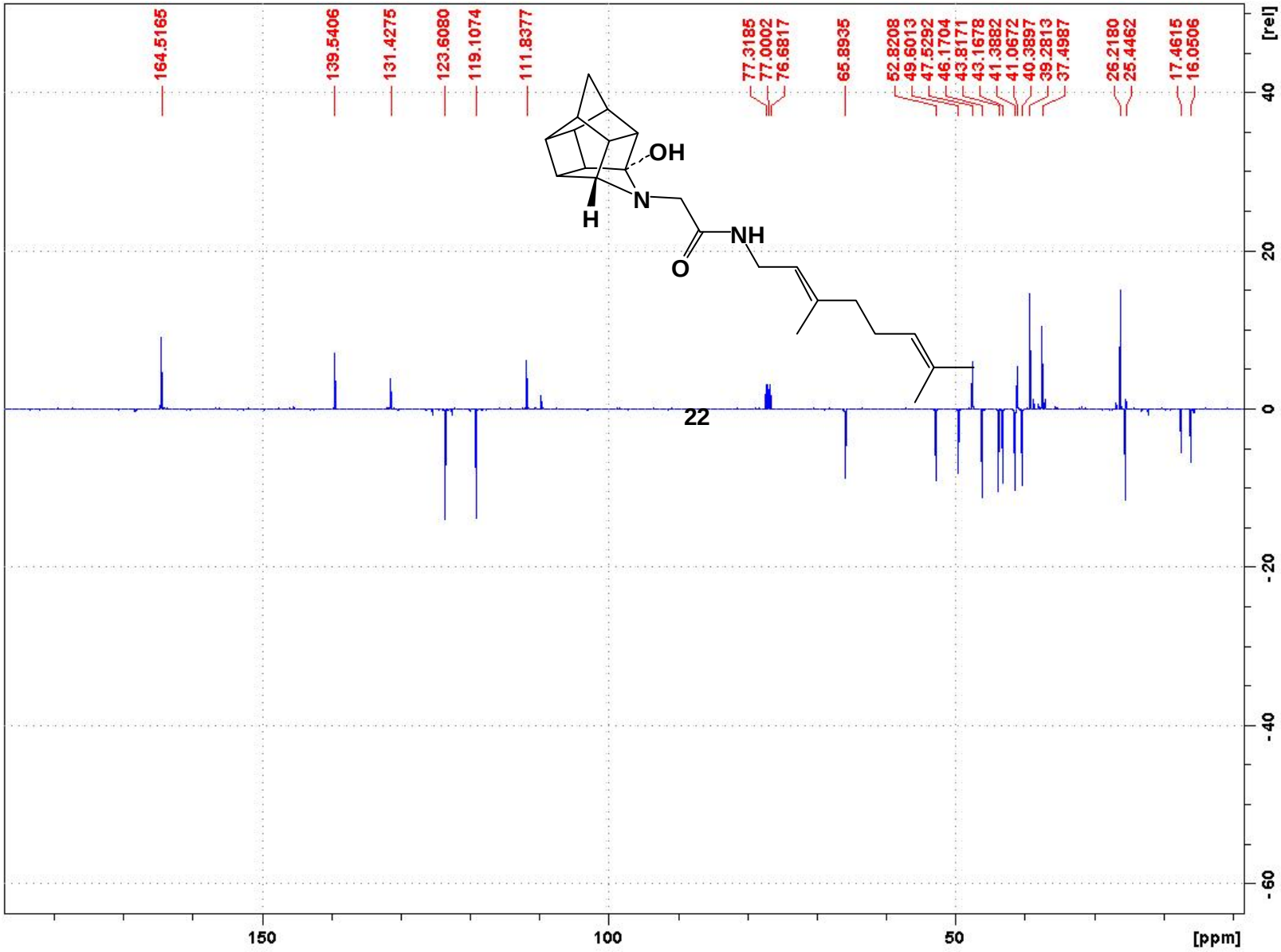
HSQC spectrum of compound 16 in $CDCl_3$


99.0
98
96
94
92
90
88
86
84
82
80
78
76
74
72
70
68
66
64.9
%T
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380.0
cm-1
3367.23
3129.94
2864.40
2964.30
1634.62
1449.39
1387.13
1157.91
1072.94
1009.94
838.57
556.77
HN
NH
.2HCl
O
H
16

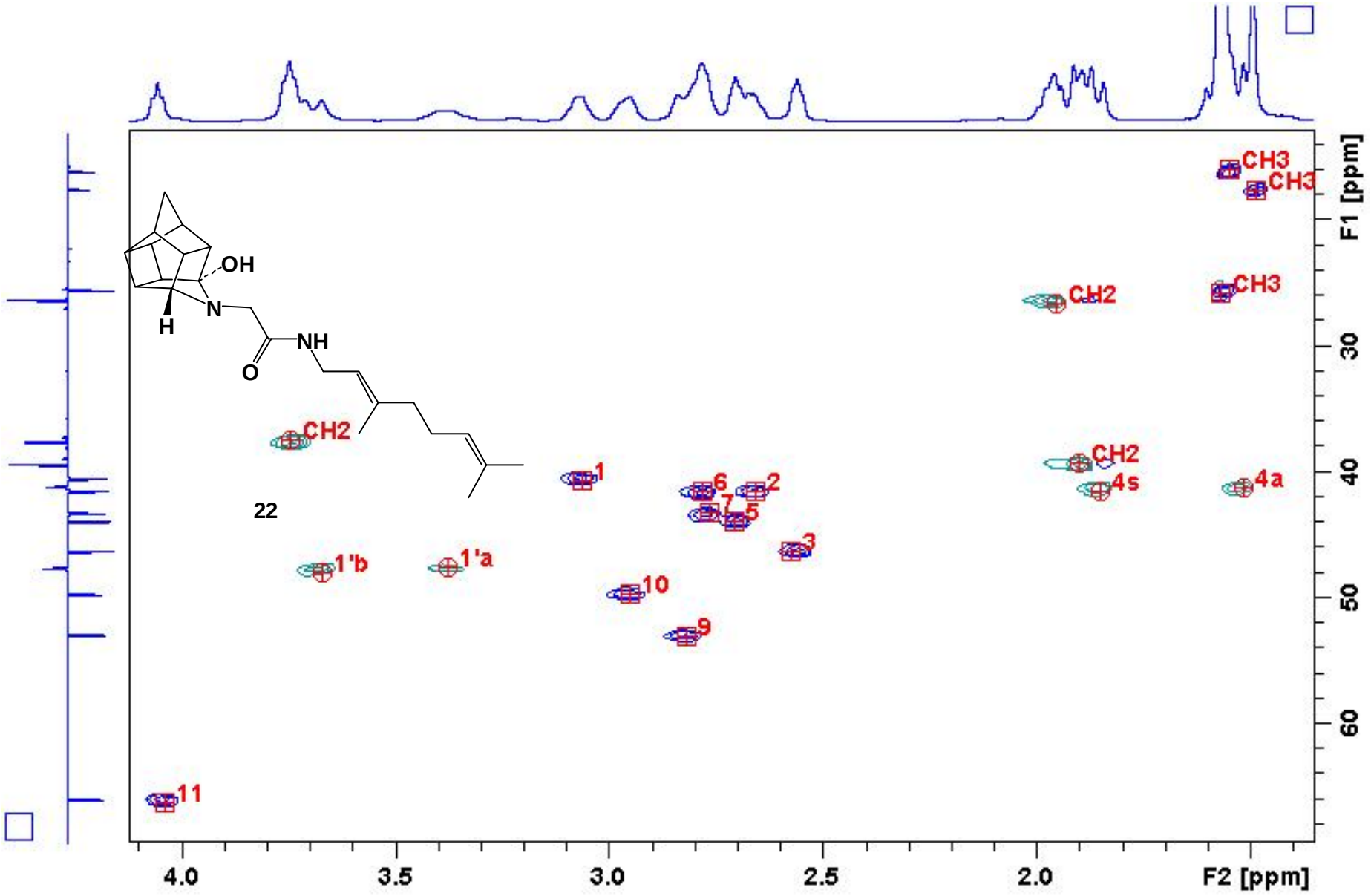
IR spectrum of compound 16


7.9342
7.2399
5.0963
5.0806
5.0651
4.9706
4.9565
4.0659
4.0538
4.0417
3.7458
3.7085
3.6701
3.3942
3.3675
3.0620
2.9487
2.8354
2.7811
2.7018
2.6721
2.6599
2.5573
1.9752
1.9569
1.9413
1.9115
1.8913
1.8701
1.8420
1.6009
1.5715
1.5578
1.5152
1.4926
1.4449
1.4257
[rel]
25
20
15
10
5
0
OH
N
H
NH
O
22
8
7
6
5
4
3
2
[ppm]

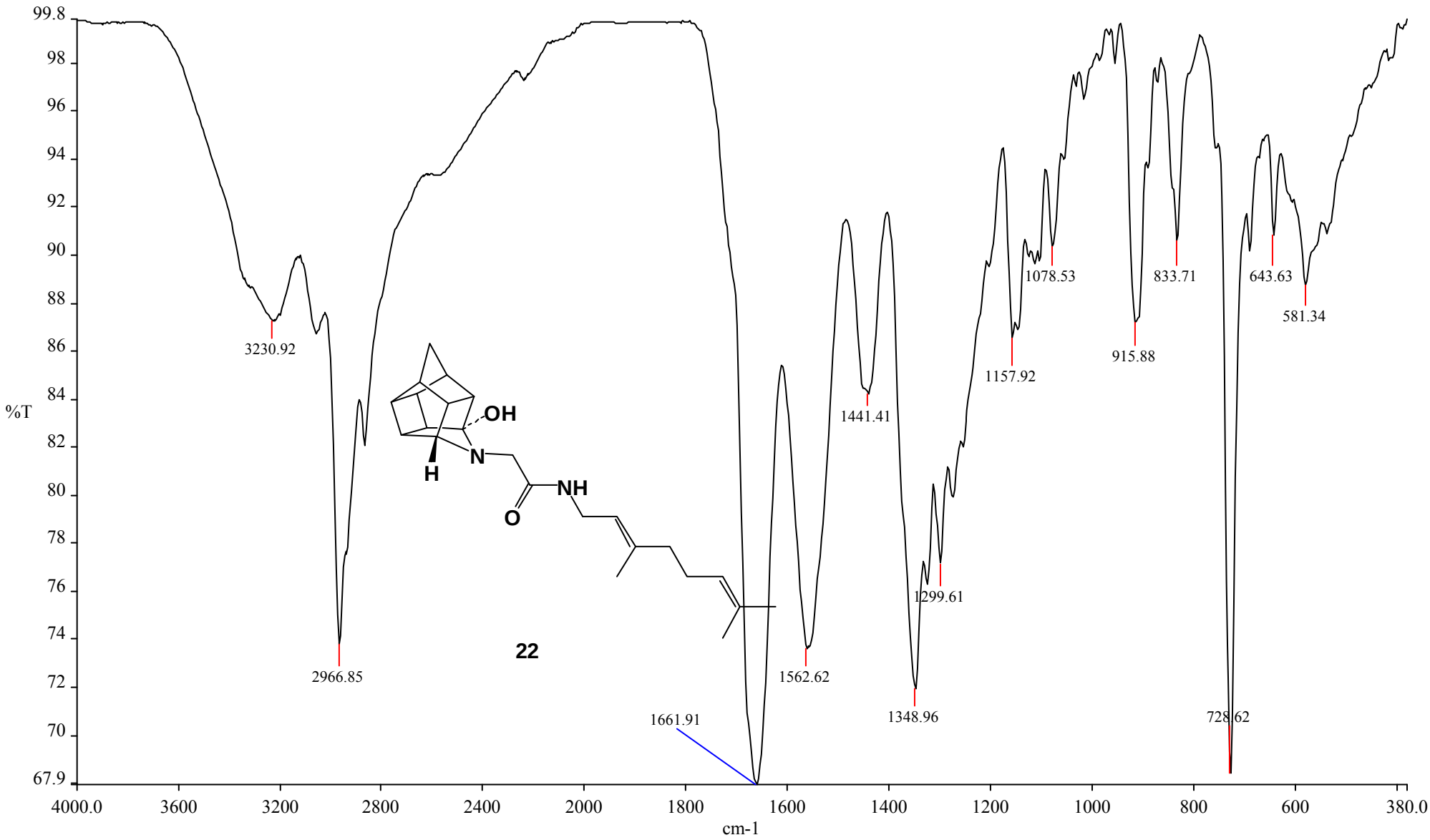
^{1}H NMR spectrum of compound 22 in $CDCl_3$


164.5165
139.5406
131.4275
123.6080
119.1074
111.8377
77.3185
77.0002
76.6817
65.8935
52.8208
49.6013
47.5292
46.1704
43.8171
43.1678
41.3882
41.0672
40.3897
39.2813
37.4987
26.2180
25.4462
17.4615
16.0506
OH
N
H
NH
O
22
[rel]
40
20
0
-20
-40
-60
150
100
50
[ppm]

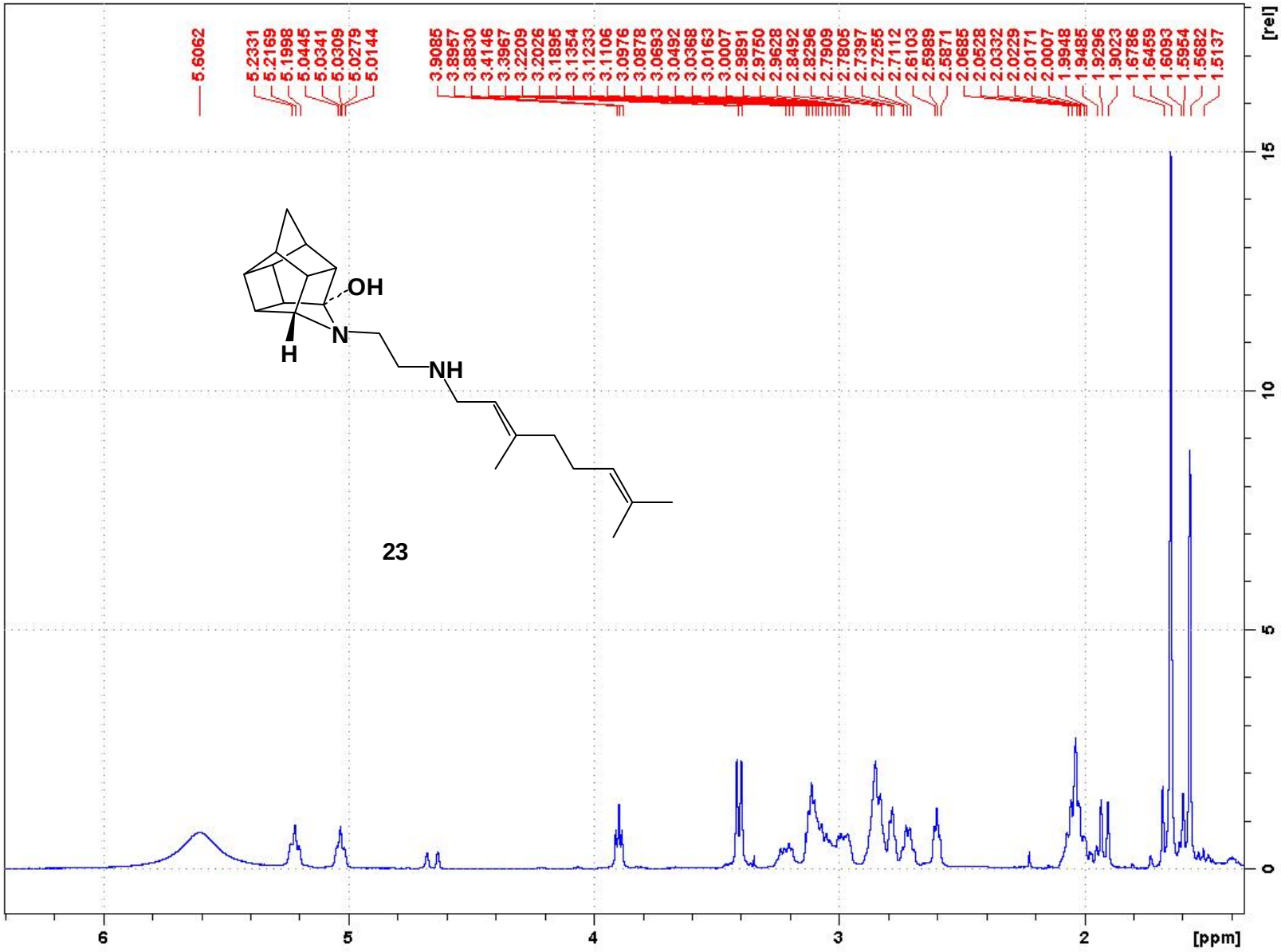
^{13}C (APT) NMR spectrum of compound 22 in $CDCl_3$


OH
N
H
NH
O
CH2
22
1
6
2
7
5
3
1'b
1'a
10
9
11
CH3
CH3
CH2
CH3
CH2
4s
4a
F1 [ppm]
30
40
50
60
4.0
3.5
3.0
2.5
2.0
F2 [ppm]

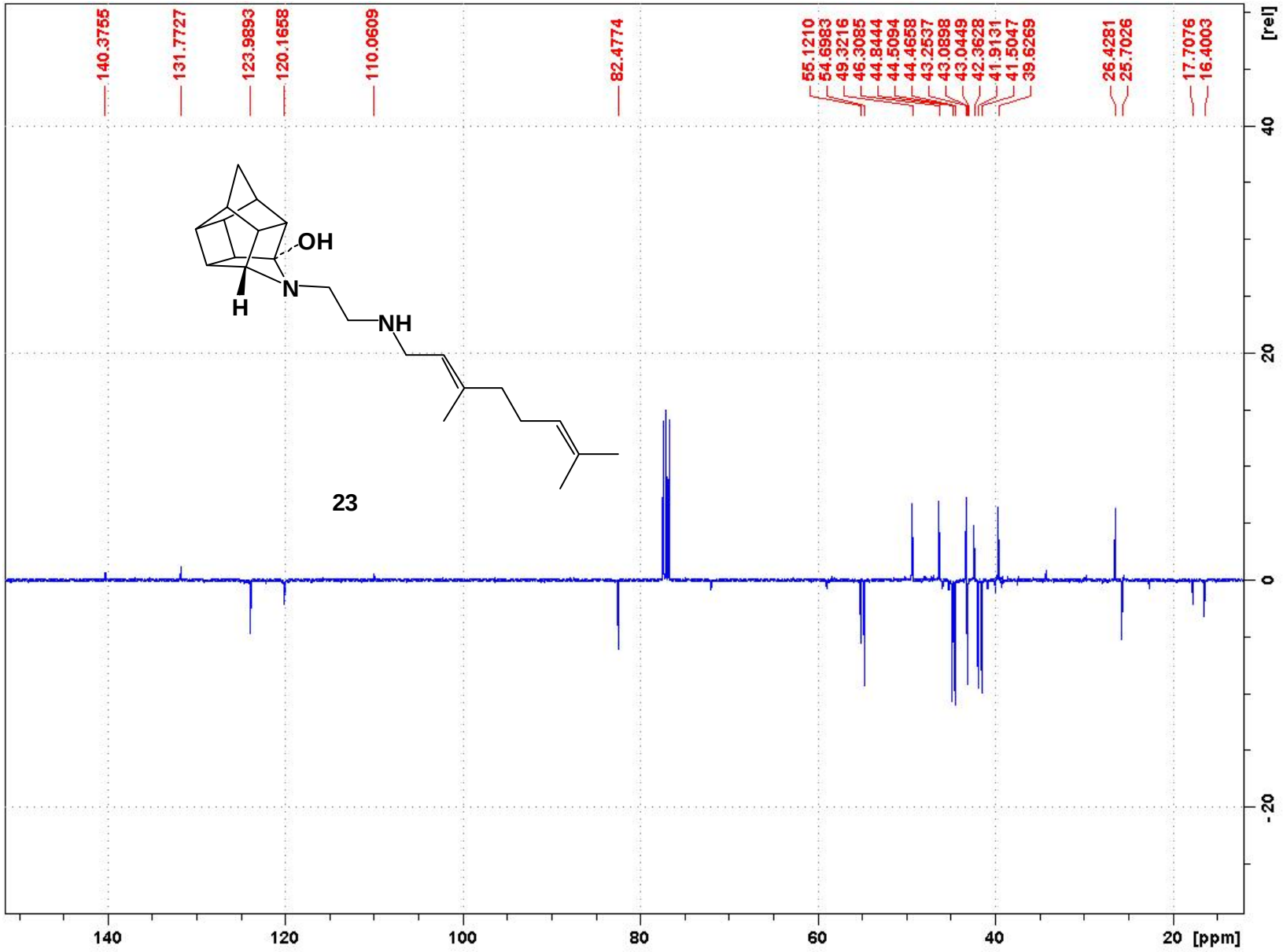
HSQC spectrum of compound 22 in $CDCl_3$


99.8
98
96
94
92
90
88
86
84
82
80
78
76
74
72
70
67.9
%T
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
380.0
cm-1
3230.92
2966.85
1661.91
1562.62
1441.41
1348.96
1299.61
1157.92
1078.53
915.88
833.71
728.62
643.63
581.34
OH
N
H
NH
O
22

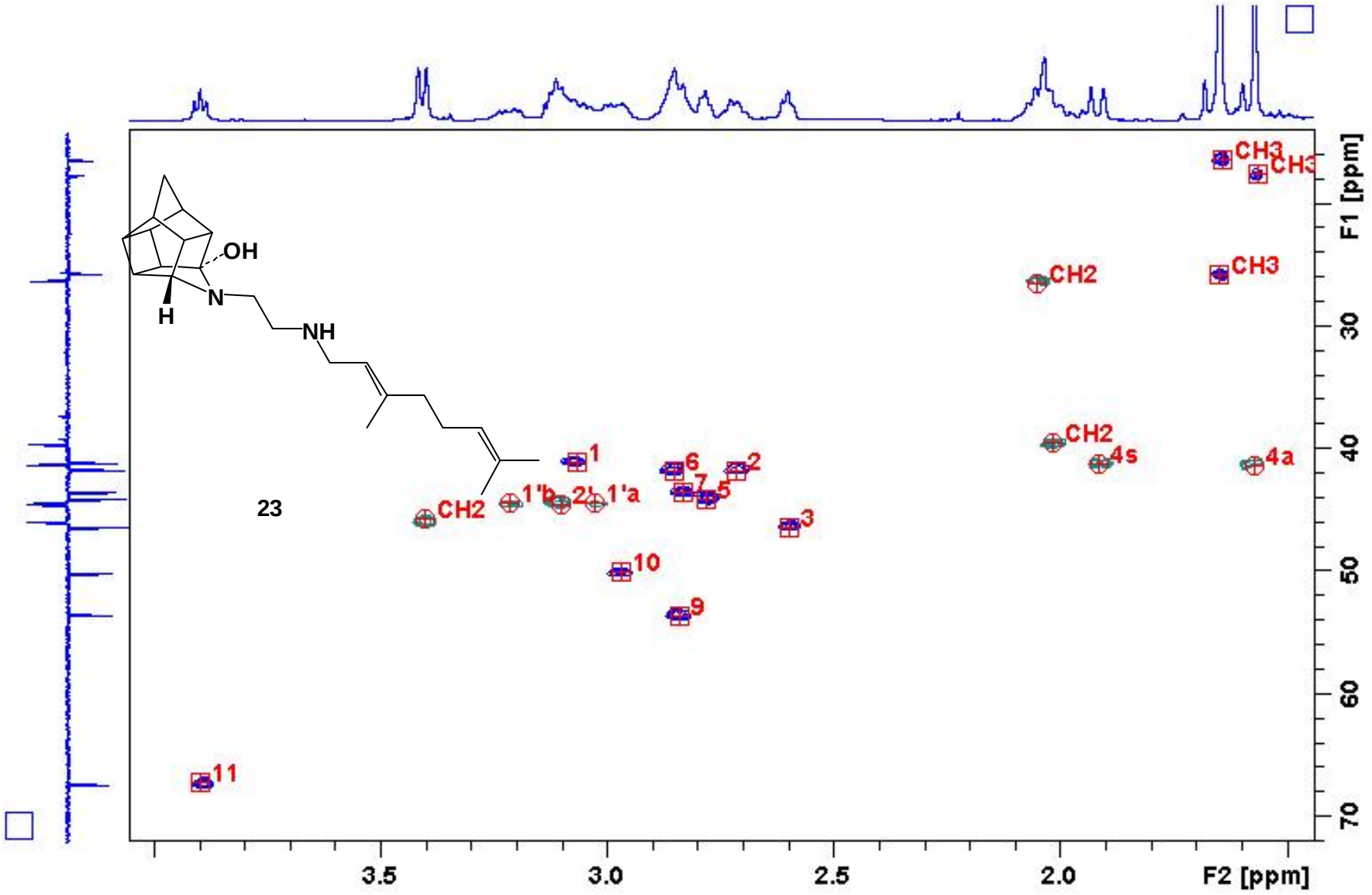
IR spectrum of compound 22


5.6062
5.2331
5.2169
5.1998
5.0445
5.0341
5.0309
5.0279
5.0144
3.9085
3.8957
3.8830
3.4146
3.3967
3.2209
3.2026
3.1895
3.1354
3.1233
3.1106
3.0976
3.0878
3.0693
3.0492
3.0368
3.0163
3.0007
2.9891
2.9750
2.9628
2.8492
2.8296
2.7909
2.7805
2.7397
2.7255
2.7112
2.6103
2.5989
2.5871
2.0685
2.0528
2.0332
2.0229
2.0171
2.0007
1.9948
1.9485
1.9296
1.9023
1.6786
1.6459
1.6093
1.5954
1.5682
1.5137
[rel]
15
10
5
0
OH
N
H
NH
23
6
5
4
3
2
[ppm]

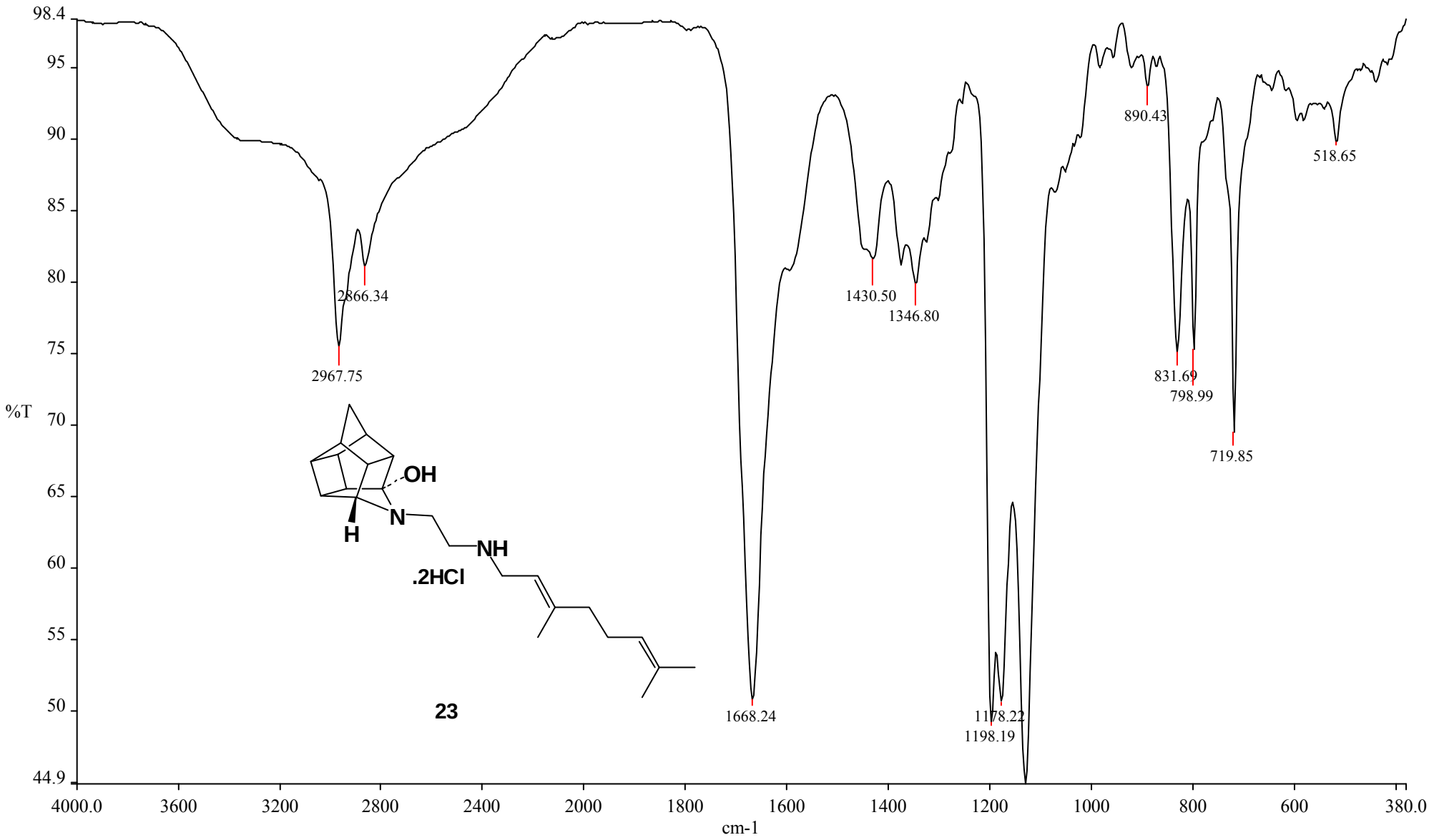
^{1}H NMR spectrum of compound 23 in $CDCl_3$


140.3755
131.7727
123.9893
120.1658
110.0609
82.4774
55.1210
54.6983
49.3216
46.3085
44.8444
44.5094
44.4658
43.2537
43.0898
43.0449
42.3628
41.9131
41.5047
39.6269
26.4281
25.7026
17.7076
16.4003
[rel]
40
20
0
-20
OH
N
H
NH
23
140
120
100
80
60
40
20 [ppm]

^{13}C (APT) NMR spectrum of compound 23 in $CDCl_3$



HSQC spectrum of compound 23 in $CDCl_3$



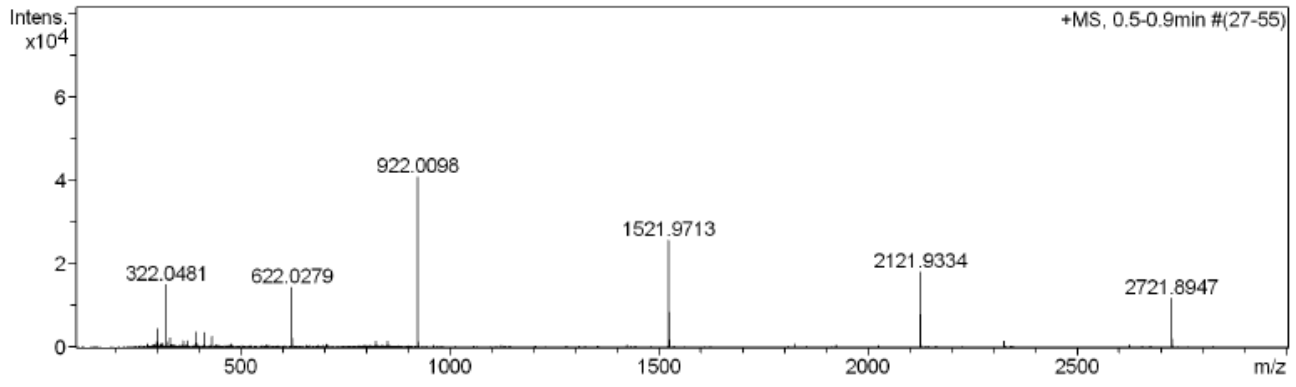
IR spectrum of compound 23

Analysis Info

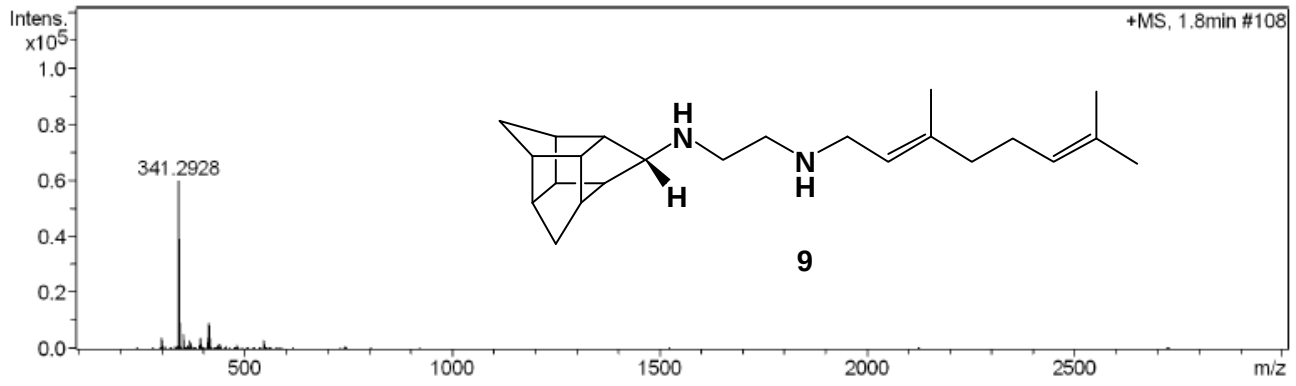
Analysis Name	D:\Data\kenny\GKM6P000001.d	Acquisition Date	9/10/2009 7:39:13 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM6P	Instrument / Ser#	micrOTOF-Q 10139
Comment	geranyl ethylene diamine mono PCU (endo position)		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev



9

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
341.2928														
	1	C 23 H 37 N 2	341.2951	6.9	6.8	6.5	ok	even	1.40	0.0025	0.0023	0.0015	0.0002	0.8727
	2	C 18 H 37 N 4 O 2	341.2911	-4.9	-5.0	2.5	ok	even	26.69	0.0429	0.0017	0.0174	0.0003	0.9979

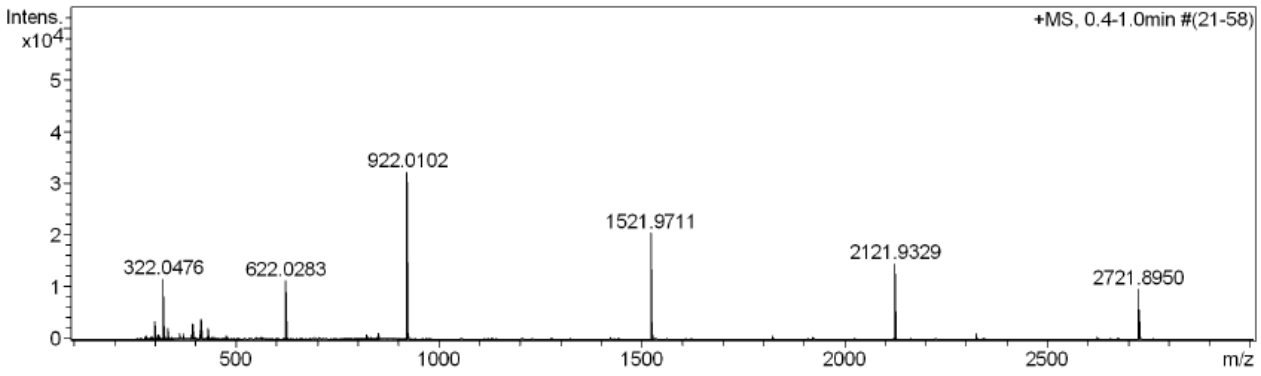
HRMS spectrum of Compound 9

Analysis Info

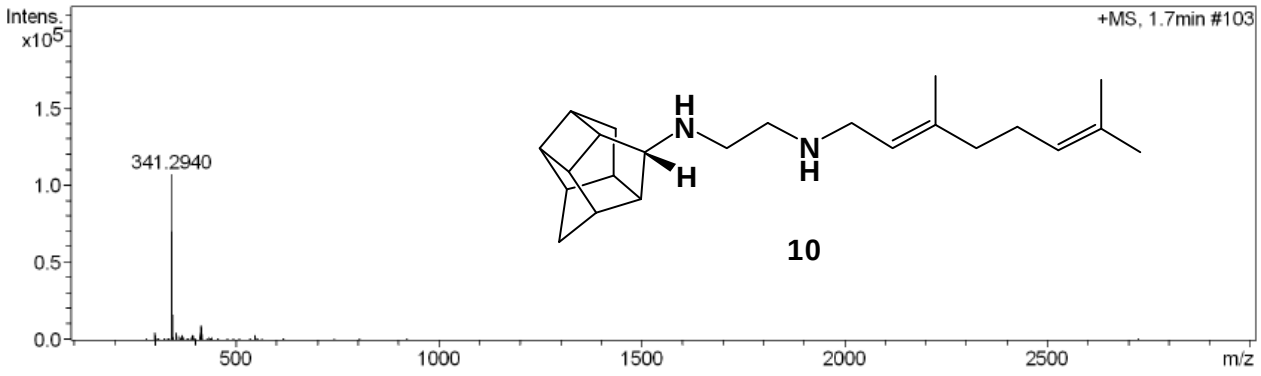
Analysis Name	D:\Data\kenny\GKM5T000001.d	Acquisition Date	9/10/2009 7:26:00 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM5T	Instrument / Ser#	micrOTOF-Q 10139
Comment	geranyl ethylene diamine trishomocubanyl		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev



10

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
341.2940														
	1	C 23 H 37 N 2	341.2951	3.4	3.2	6.5	ok	even	7.13	0.0114	0.0011	0.0047	0.0003	0.8427
	2	C 18 H 37 N 4 O 2	341.2911	-8.4	-8.6	2.5	ok	even	20.11	0.0330	0.0029	0.0134	0.0004	0.9811

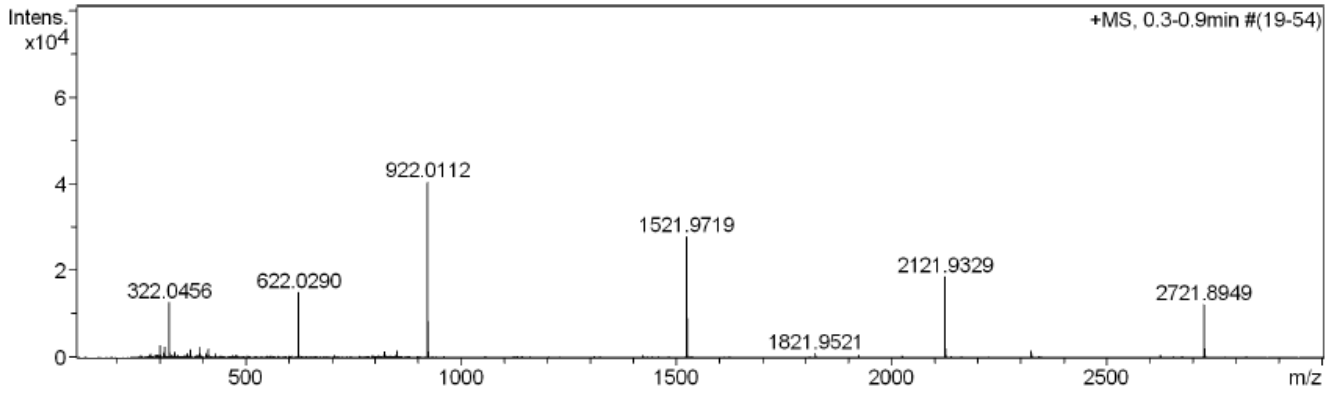
HRMS spectrum of Compound 10

Analysis Info

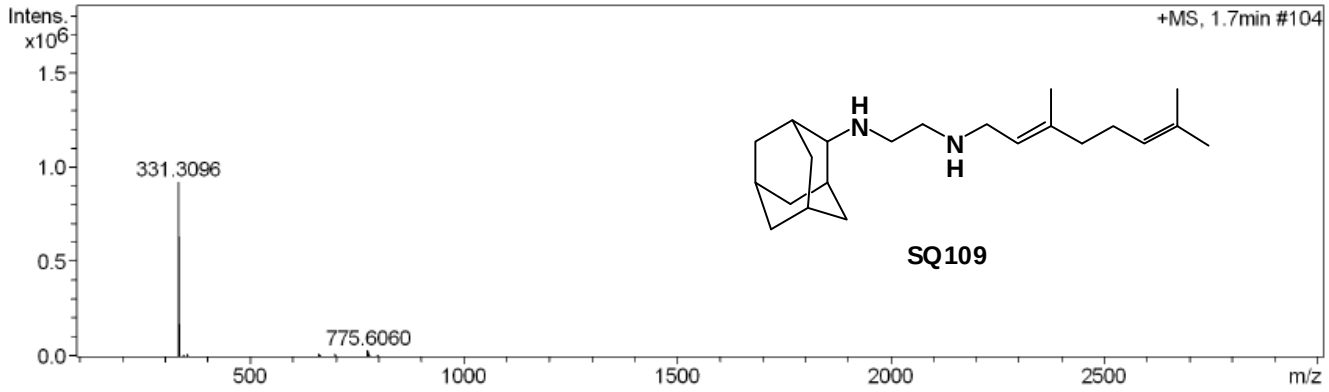
Analysis Name	D:\Data\kenny\SQ109000001.d	Acquisition Date	9/10/2009 6:43:51 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	SQ109	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev



SQ109

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
331.3096														
	1	C 22 H 39 N 2	331.3108	3.5	4.2	4.5	ok	even	40.38	0.0604	0.0015	0.0247	0.0010	0.8427
	2	C 17 H 39 N 4 O 2	331.3068	-8.7	-7.9	0.5	ok	even	67.58	0.1055	0.0027	0.0424	0.0009	0.9392

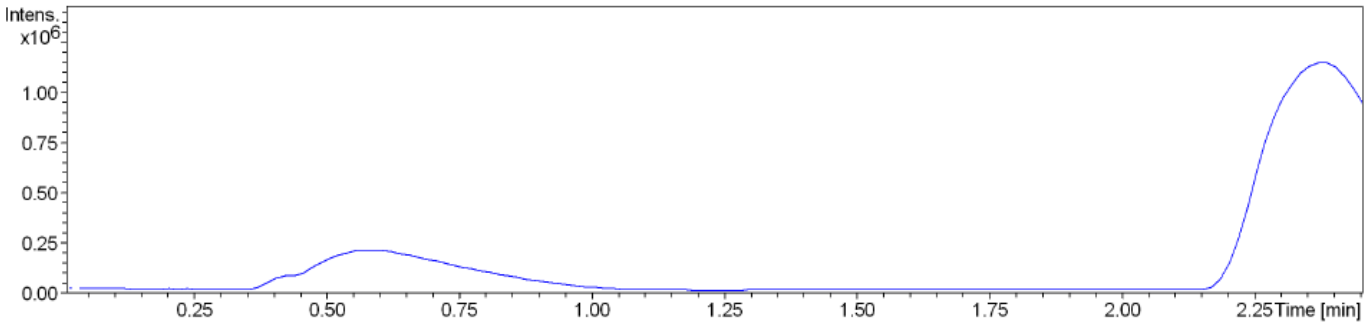
HRMS spectrum of SQ109

Analysis Info

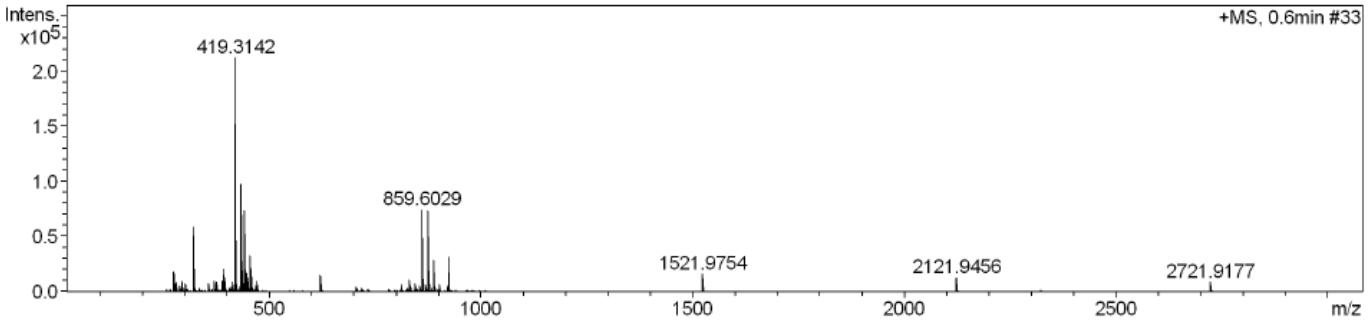
Analysis Name	D:\Data\kenny\GKM10P carbonyl000001.d	Acquisition Date	5/8/2010 5:19:17 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	PCU ether exo NH carbonyl CH2NH2 geranyl	Instrument / Ser#	micrOTOF-Q 10139
Comment			

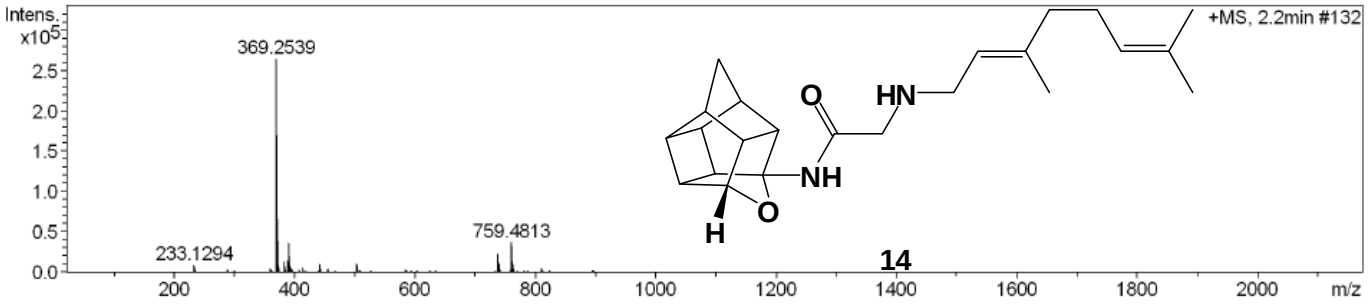
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



BPC 99.0000-3001.0000 +All MS





14

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
369.2539														
	1	C 28 H 33	369.2577	10.3	8.5	12.5	ok	even	67.62	0.1091	0.0038	0.0527	0.0046	0.8066
	2	C 23 H 33 N 2 O 2	369.2537	-0.6	-2.0	8.5	ok	even	74.02	0.1115	0.0021	0.0748	0.0008	0.7823
	3	C 19 H 29 N 8	369.2510	-7.9	-9.4	9.5	ok	even	80.26	0.1287	0.0040	0.0832	0.0007	0.8583

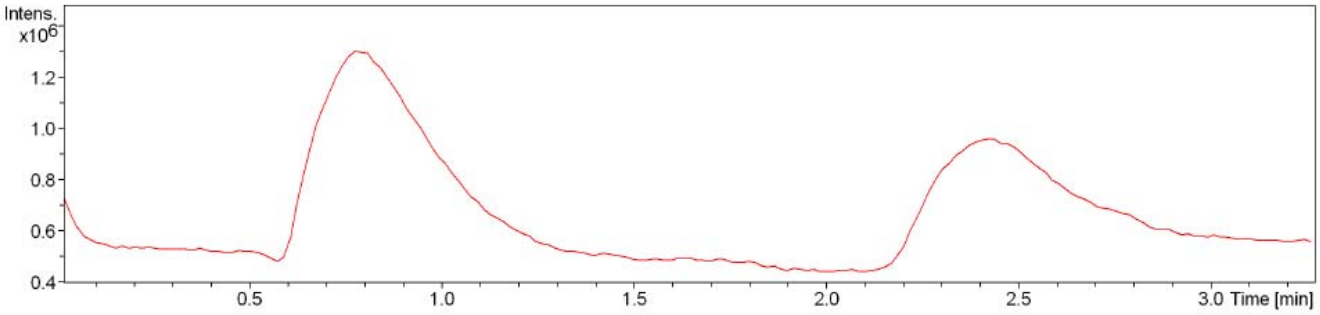
HRMS spectrum of Compound 14

Analysis Info

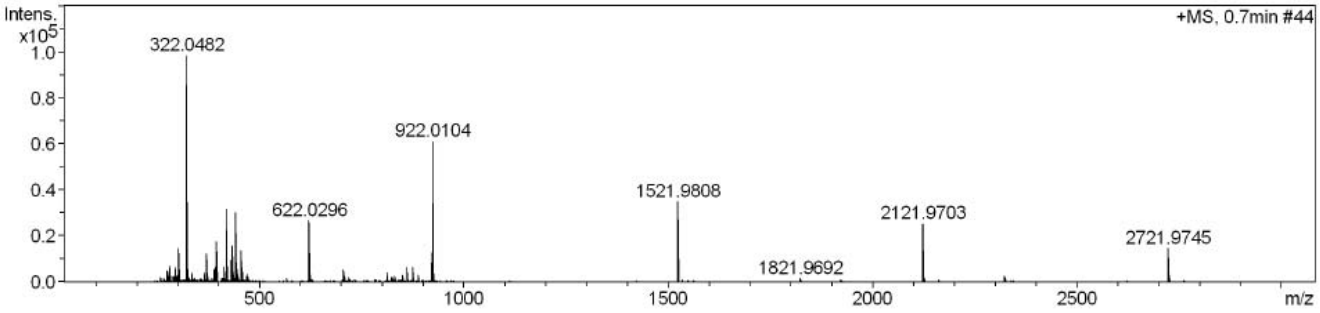
Analysis Name	D:\Data\kenny\GKM10P000001.d	Acquisition Date	5/8/2010 5:07:27 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	PCU OH GERANYL DIAMINE	Instrument / Ser#	micrOTOF-Q 10139
Comment			

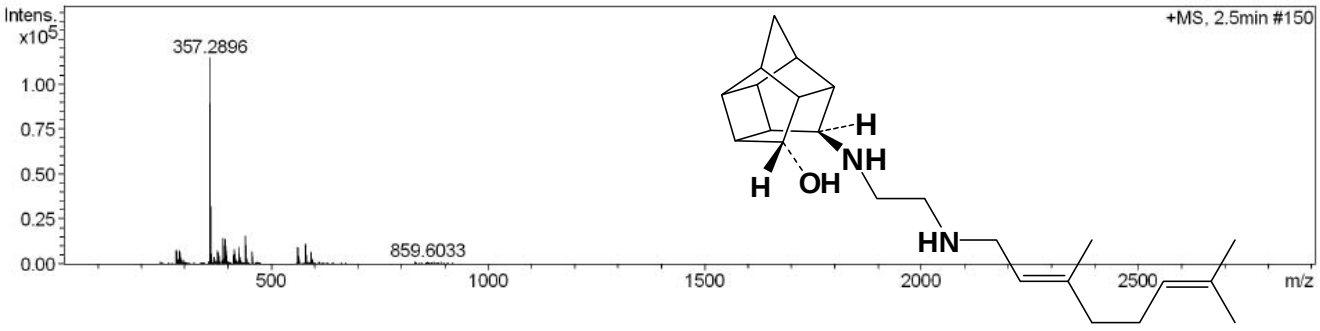
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



TIC +All MS





15

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
357.2896														
	1	C 23 H 37 N 2 O	357.2900	1.3	0.8	6.5	ok	even	22.81	0.0326	0.0010	0.0229	0.0002	0.8427
	2	C 18 H 37 N 4 O 3	357.2860	-10.0	-10.5	2.5	ok	even	27.95	0.0522	0.0039	0.0279	0.0002	0.9689

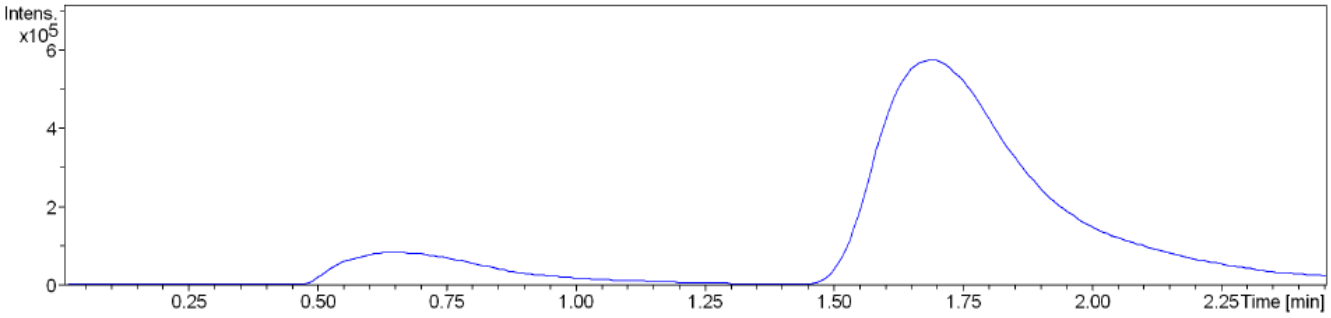
HRMS spectrum of Compound 15

Analysis Info

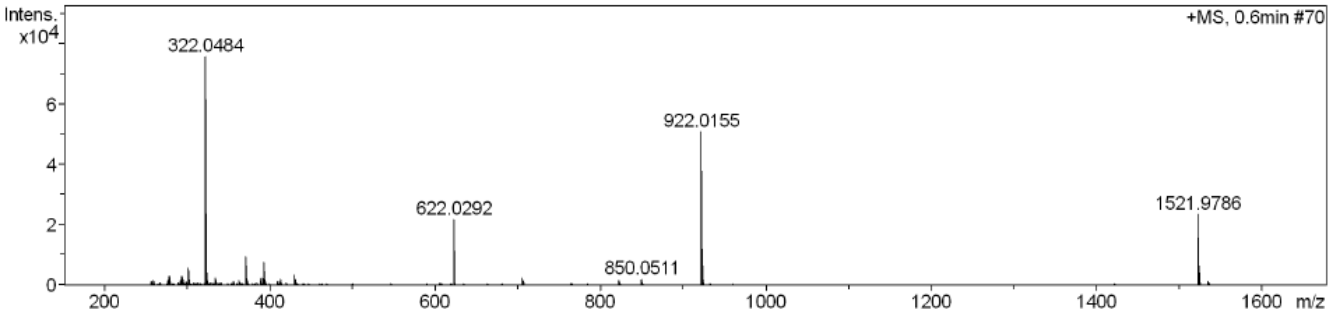
Analysis Name	D:\Data\kenny\GKM11P.d	Acquisition Date	5/24/2010 8:20:45 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM11P, PCU ether exo NHCH2CH2NH geranyl	Instrument / Ser#	micrOTOF-Q 10139
Comment			

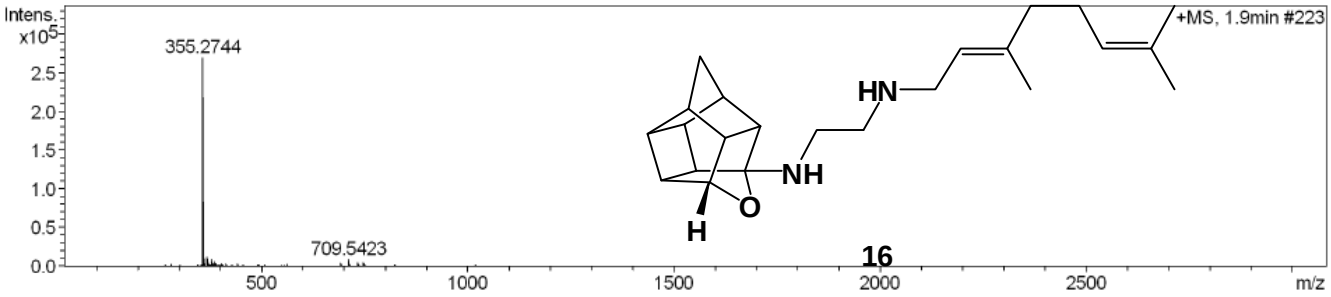
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



BPC 99.0000-3001.0000 +All MS





Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
355.2744	1	C 23 H 35 N 2 O	355.2744	-0.0	-2.3	7.5	ok	even	112.70	0.1644	0.0026	0.1102	0.0010	0.8427

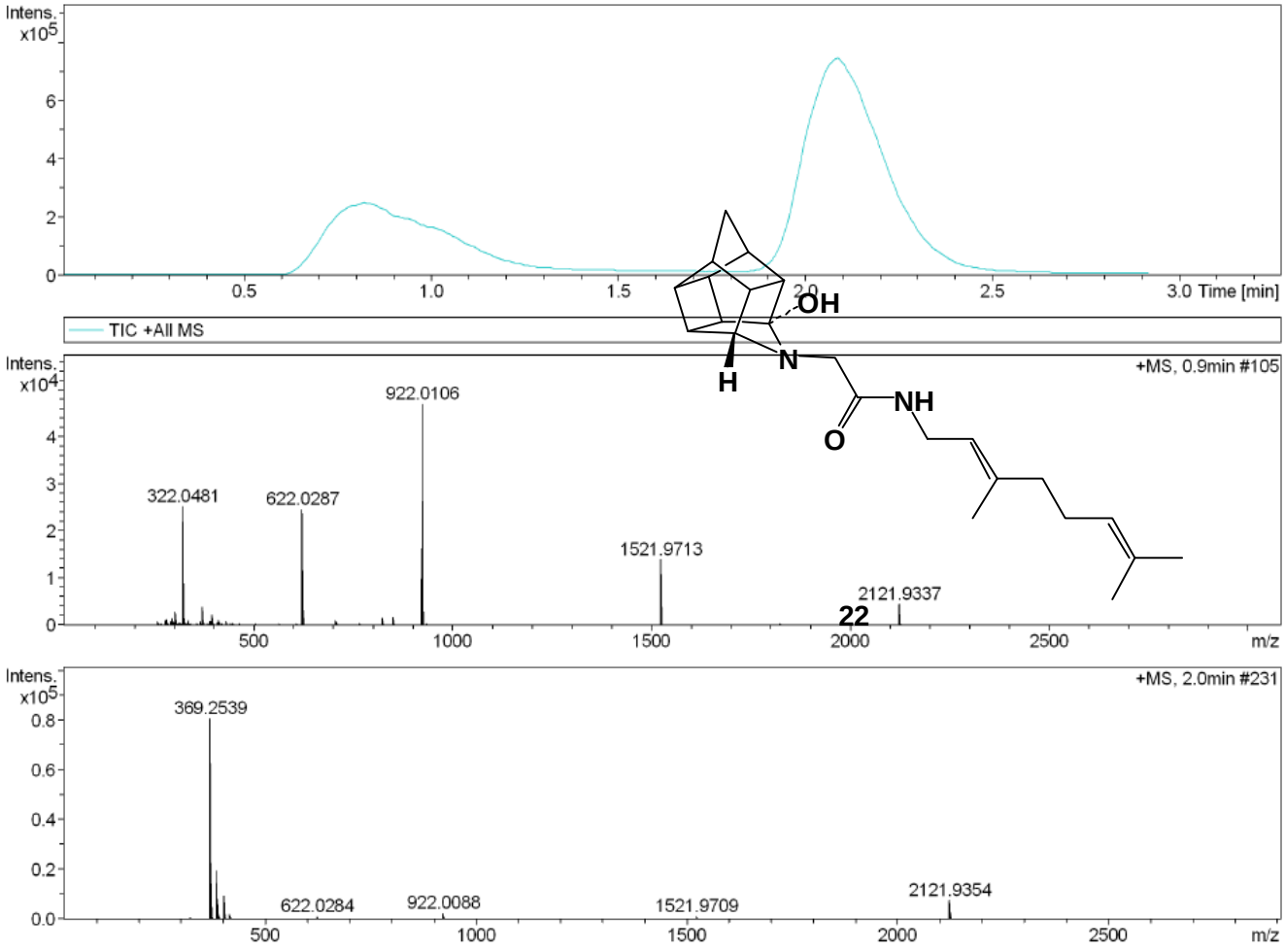
HRMS spectrum of Compound 16

Analysis Info

Analysis Name	D:\Data\kenny\GKM12PAC000001.d	Acquisition Date	7/30/2010 10:33:53 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM12PAC	Instrument / Ser#	micrOTOF-Q 10139
Comment	MASS = 369 PCU AZA CH2CONH2 GERANYL		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
369.2539														
	1	C 19 H 29 N 8	369.2510	-8.0	-8.2	9.5	ok	even	13.52	0.0215	0.0031	0.0150	0.0003	0.8427
	2	C 23 H 33 N 2 O 2	369.2537	-0.7	-0.8	8.5	ok	even	19.22	0.0261	0.0007	0.0137	0.0004	0.6889
	3	C 28 H 33	369.2577	10.2	10.0	12.5	ok	even	38.96	0.0626	0.0038	0.0203	0.0021	0.8908

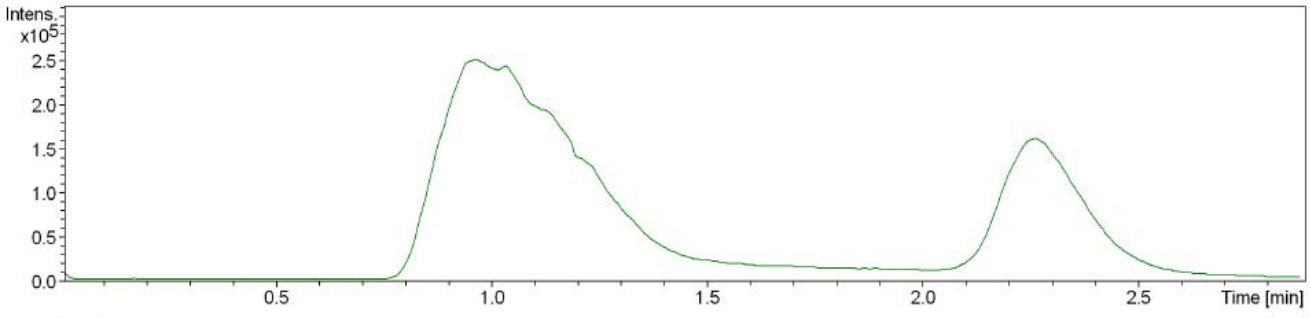
HRMS spectrum of Compound 22

Analysis Info

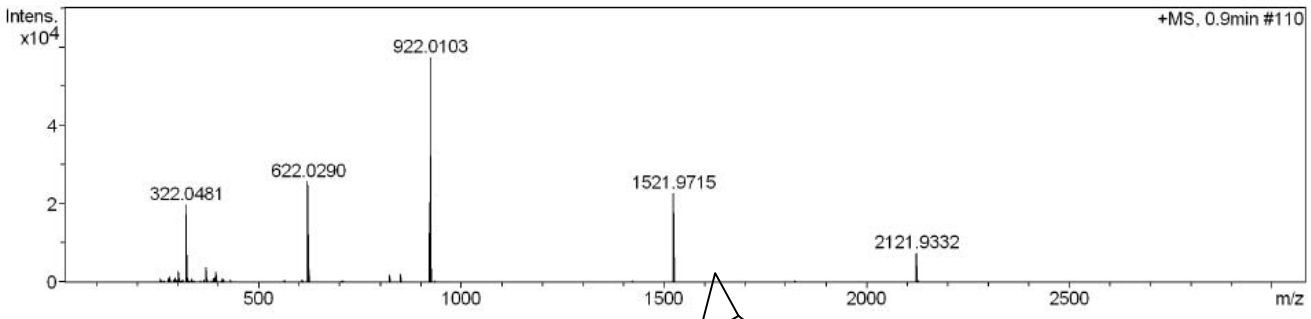
Analysis Name	D:\Data\kenny\GKM13000001.d	Acquisition Date	7/30/2010 10:29:54 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM13	Instrument / Ser#	micrOTOF-Q 10139
Comment	MASS = 354 PCU AZA CH2CH2NH2 GERANYL		

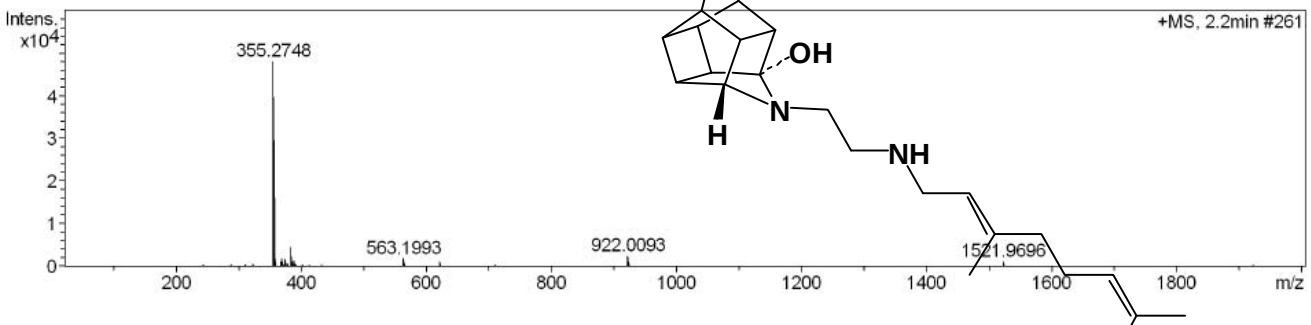
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



TIC +All MS





23

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
355.2748	1	C 23 H 35 N 2 O	355.2744	-1.3	-0.8	7.5	ok	even	10.13	0.0134	0.0005	0.0077	0.0009	0.8427

HRMS spectrum of Compound 23

CHAPTER 8

NOVEL LINEAR DIAMINE DISUBSTITUTED POLYCYCLIC 'CAGE' DERIVATIVES AS POTENTIAL ANTI-MYCOBACTERIAL CANDIDATES

**Oluseye K. Onajole,[a] Sphelele Sosibo,[a] Patrick Govender,[b] Thavendran Govender,[c] Paul D. van Helden,[d] Glenn E. M. Maguire,[a] Kata Mlinarić-Majerski,[e] Ian Wiid,[c] and Hendrik G. Kruger,[a]*

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[d] Department of Biomedical Sciences, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, South Africa

[e] Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia

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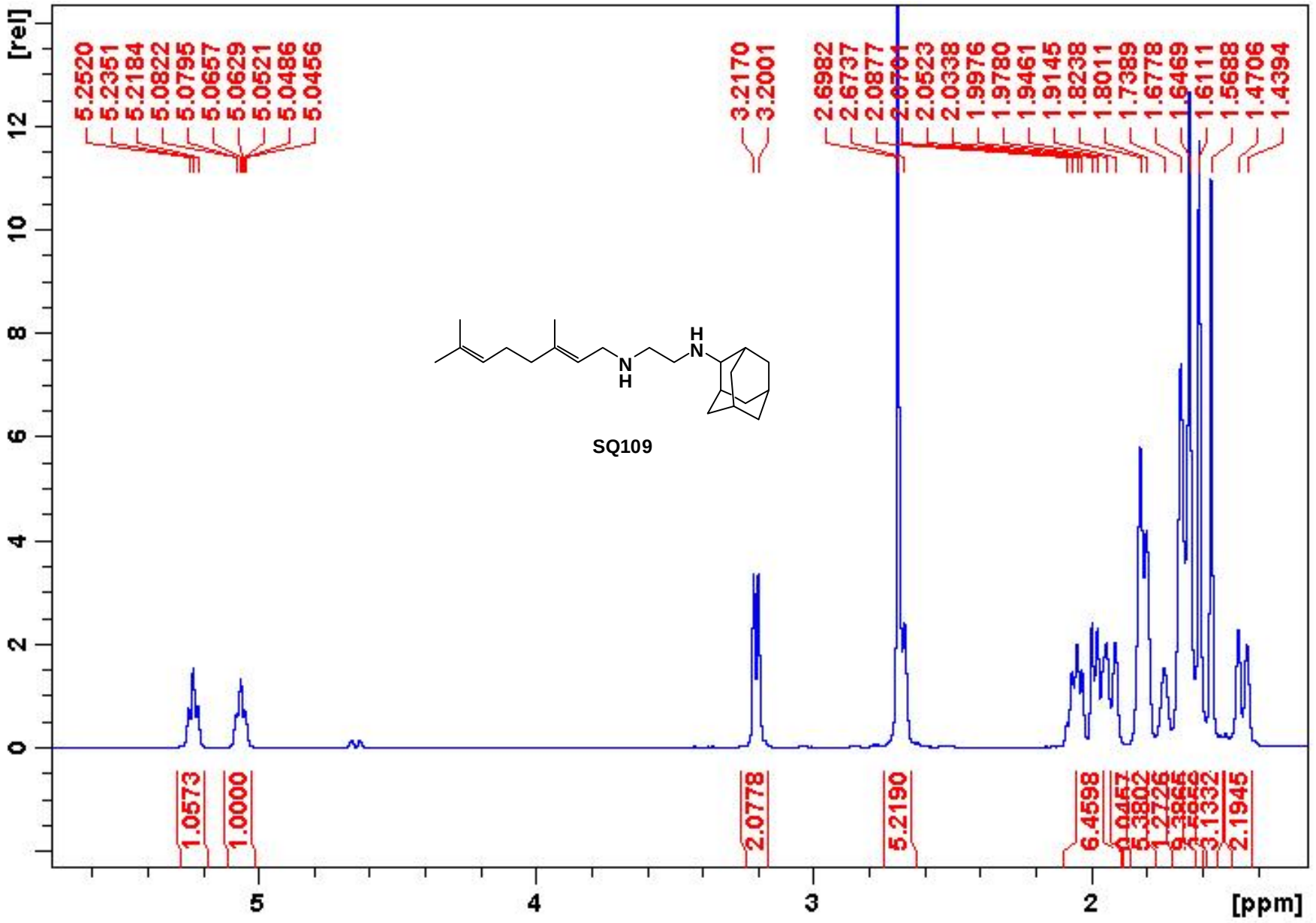
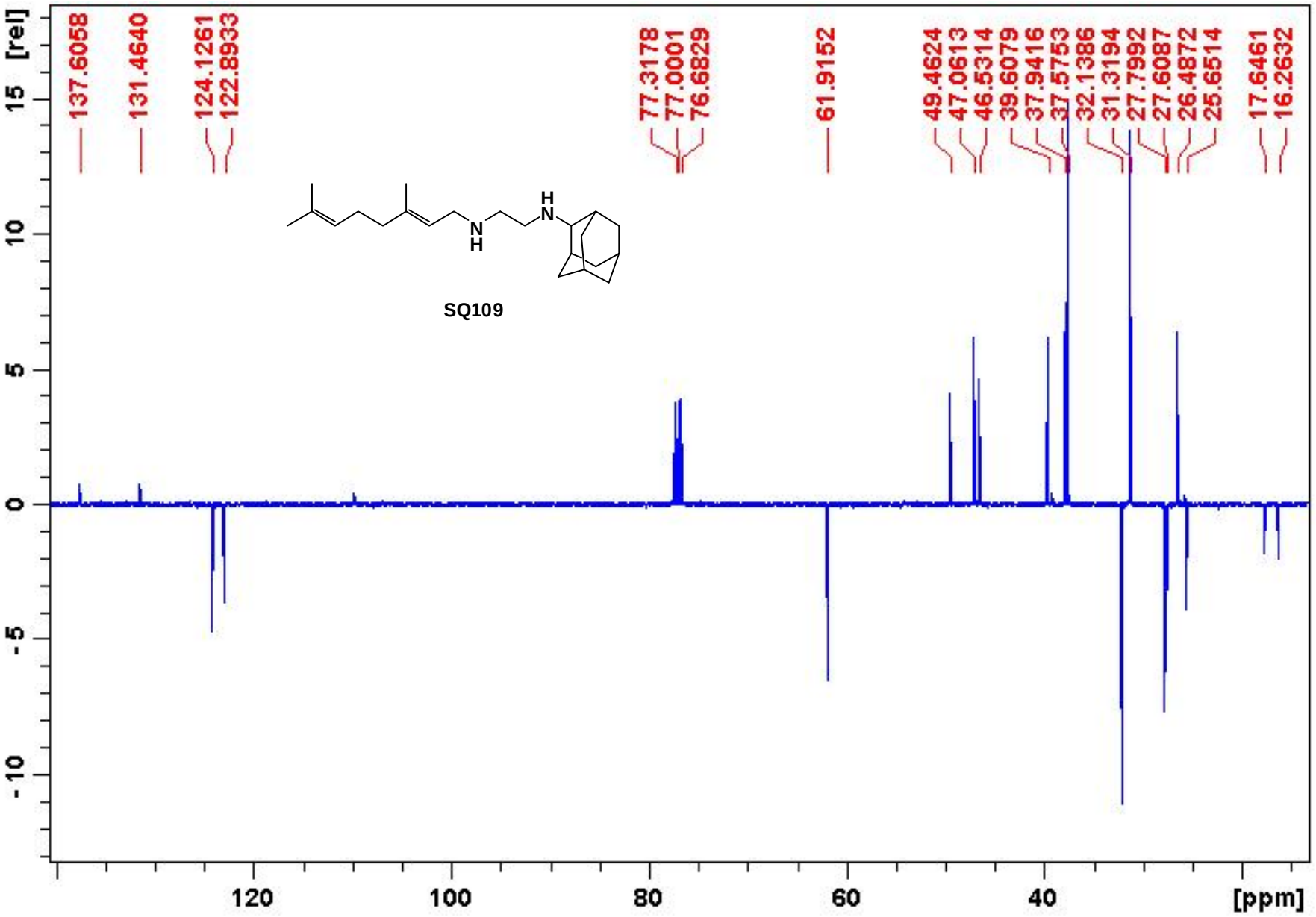
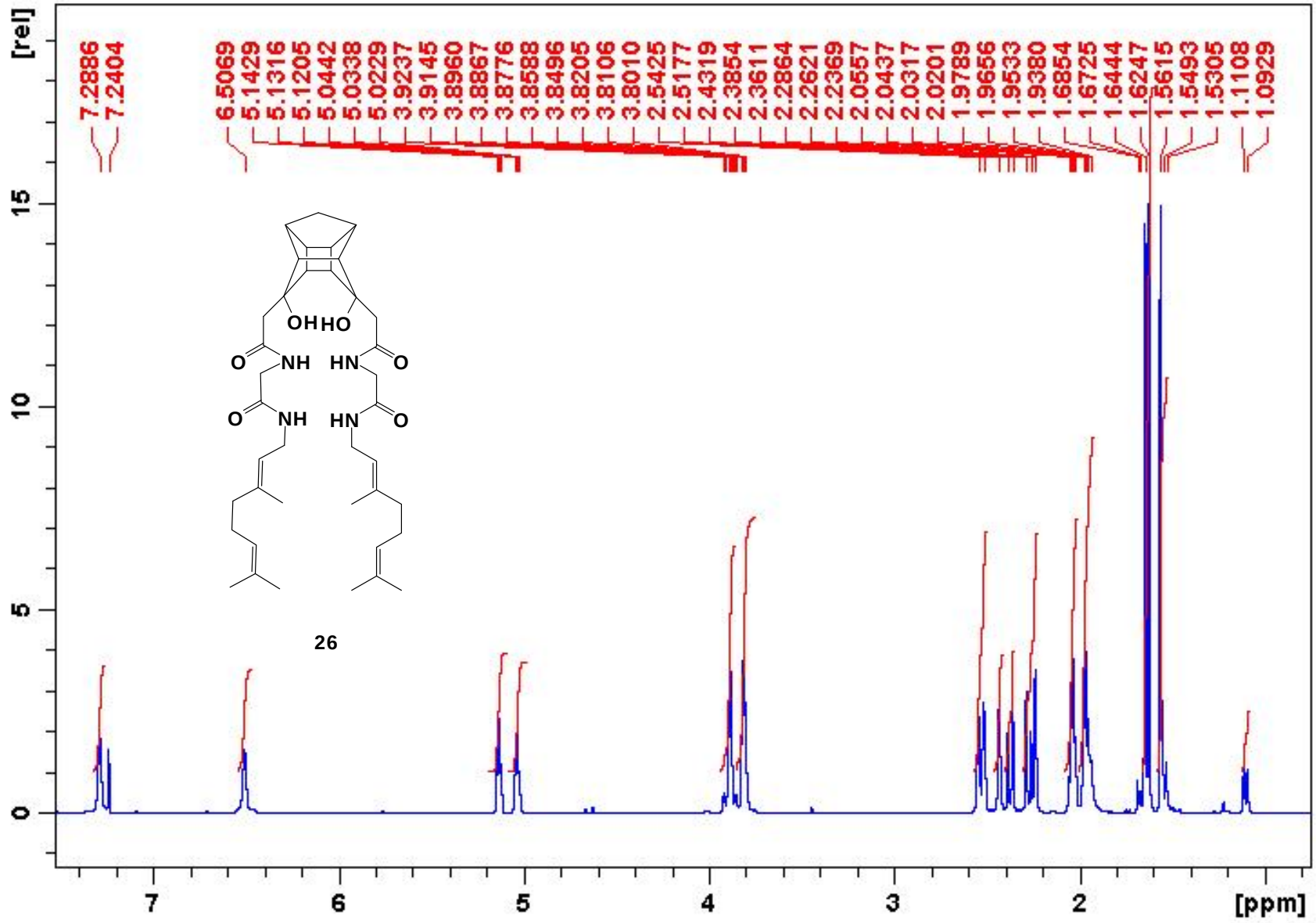

[rel]
12
10
8
6
4
2
0
5.2520
5.2351
5.2184
5.0822
5.0795
5.0657
5.0629
5.0521
5.0486
5.0456
3.2170
3.2001
2.6982
2.6737
2.0877
2.0701
2.0523
2.0338
1.9976
1.9780
1.9461
1.9145
1.8238
1.8011
1.7389
1.6778
1.6469
1.6111
1.5688
1.4706
1.4394
H
N
N
H
SQ109
1.0573
1.0000
2.0778
5.2190
6.4598
5.3802
1.2726
3.1332
2.1945
5
4
3
2
[ppm]

^{1}H NMR spectrum of (*N*-Geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine) SQ109

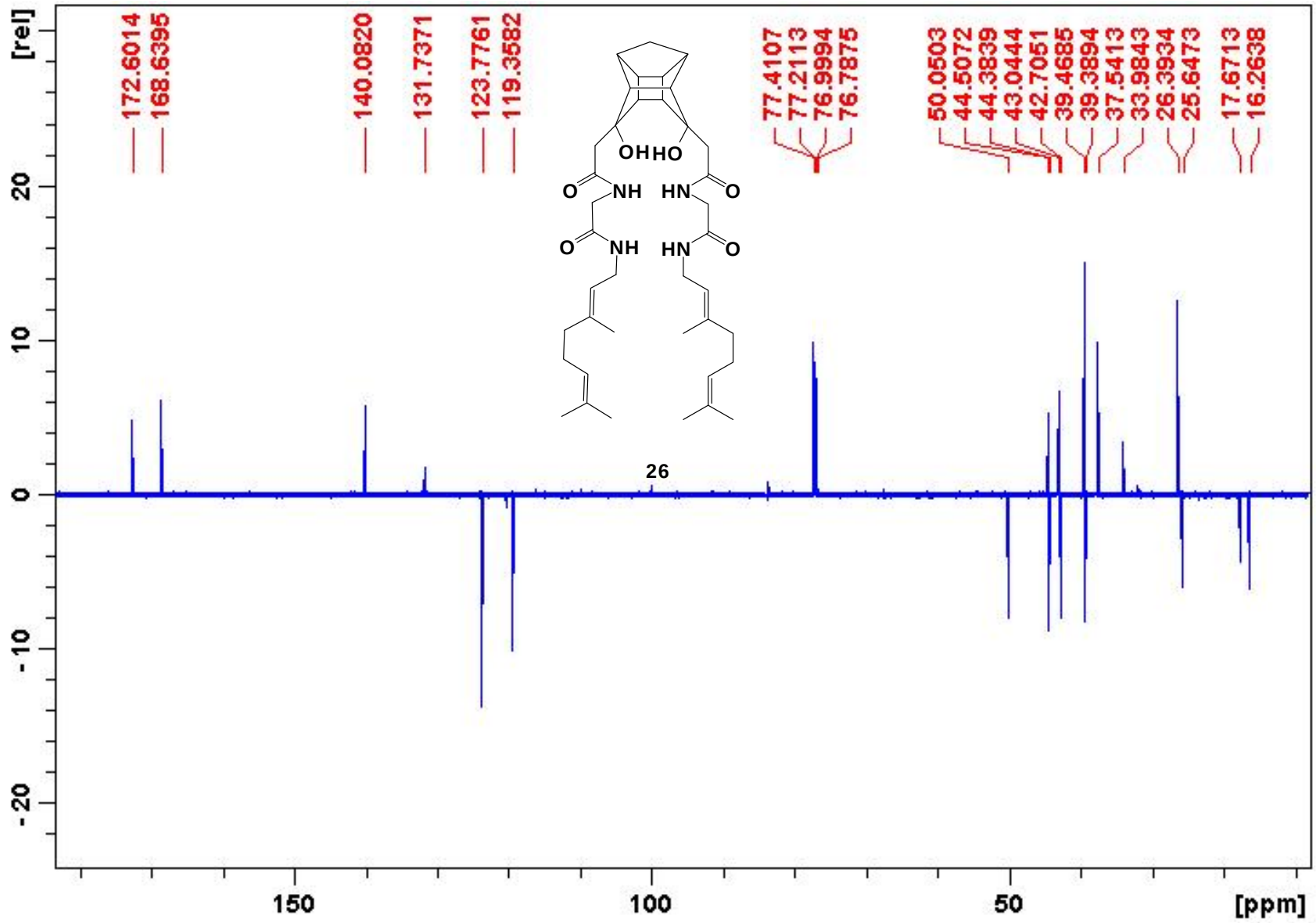

137.6058
131.4640
124.1261
122.8933
77.3178
77.0001
76.6829
61.9152
49.4624
47.0613
46.5314
39.6079
37.9416
37.5753
32.1386
31.3194
27.7992
27.6087
26.4872
25.6514
17.6461
16.2632
15 [rel]
10
5
0
-5
-10
120
100
80
60
40
[ppm]
SQ109

^{13}C NMR spectrum of (*N*-Geranyl-*N*'-(2-adamantyl)ethane-1,2-diamine) SQ109

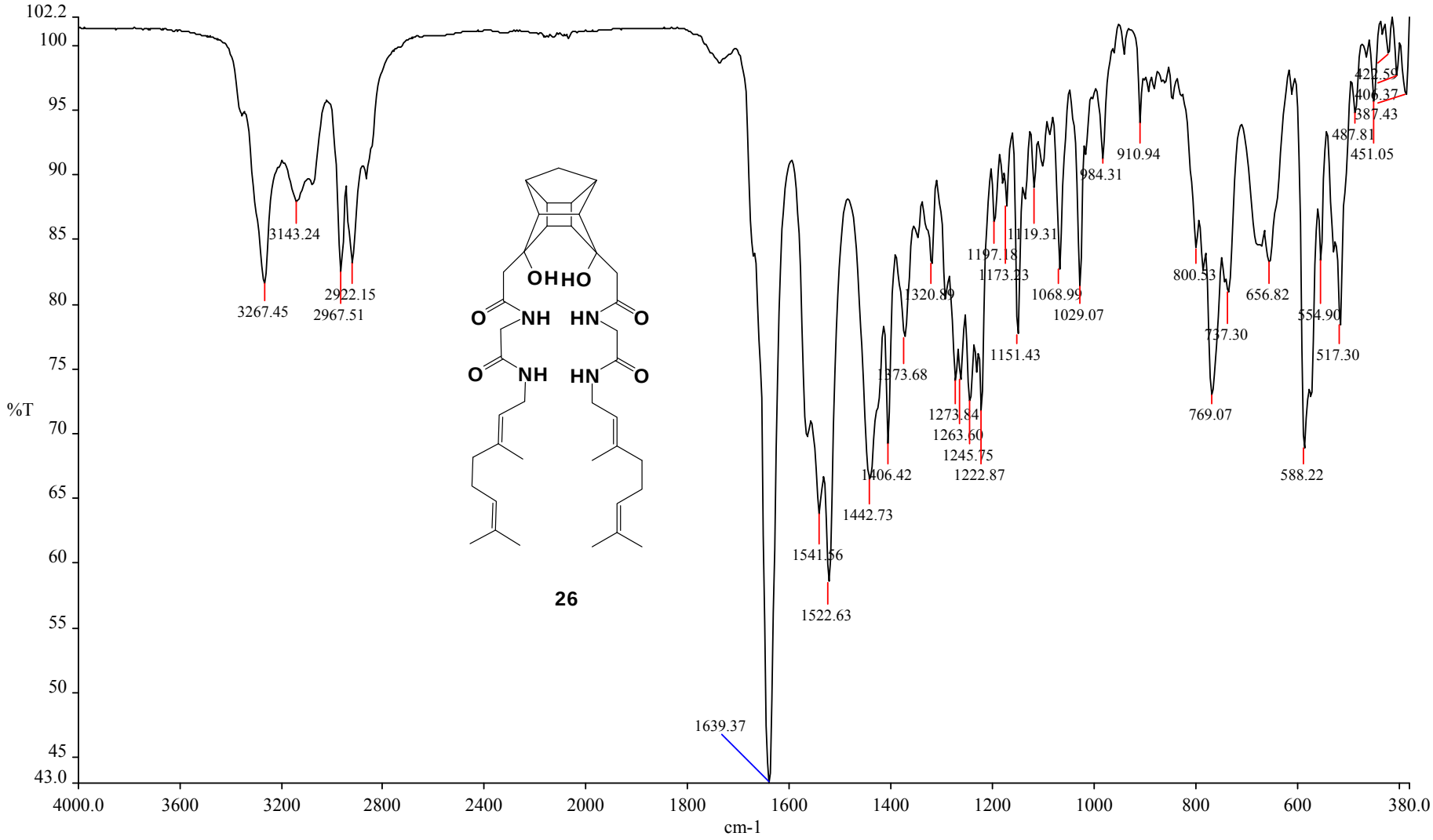


26

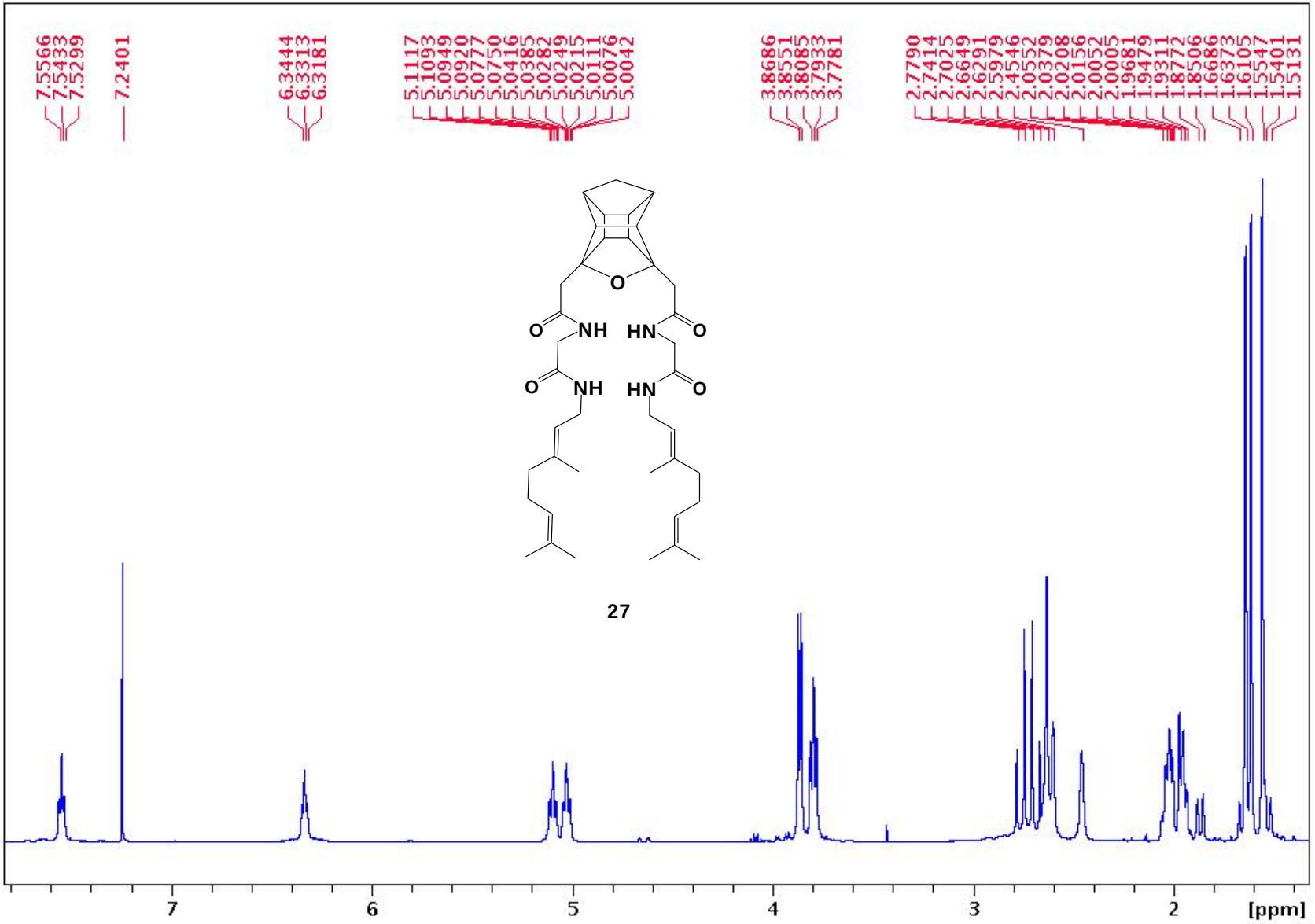
^{1}H NMR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-dihydroxypentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-dimethylenecarboxamide (26)


[rel]
172.6014
168.6395
140.0820
131.7371
123.7761
119.3582
77.4107
77.2113
76.9994
76.7875
50.0503
44.5072
44.3839
43.0444
42.7051
39.4685
39.3894
37.5413
33.9843
26.3934
25.6473
17.6713
16.2638
OH HO
O NH HN O
O NH HN O
26
20
10
0
-10
-20
150
100
50
[ppm]

^{13}C NMR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-dihydroxypentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-dimethylenecarboxamide (26)



IR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-dihydroxypentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-dimethylenecarboxamide (26)


7.5566
7.5433
7.5299
7.2401
6.3444
6.3313
6.3181
5.1117
5.1093
5.0949
5.0920
5.0777
5.0750
5.0416
5.0385
5.0282
5.0249
5.0215
5.0111
5.0076
5.0042
3.8686
3.8551
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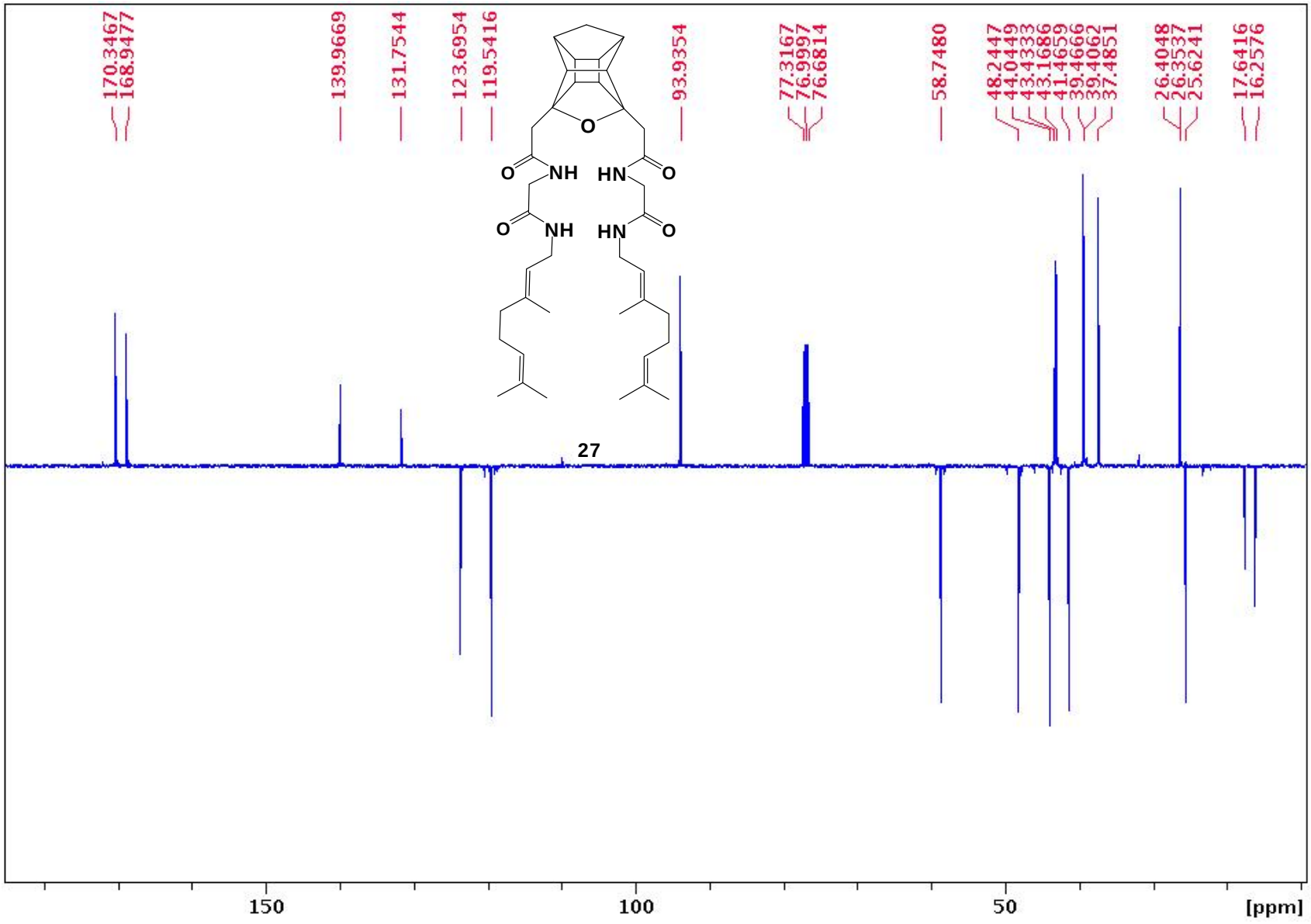
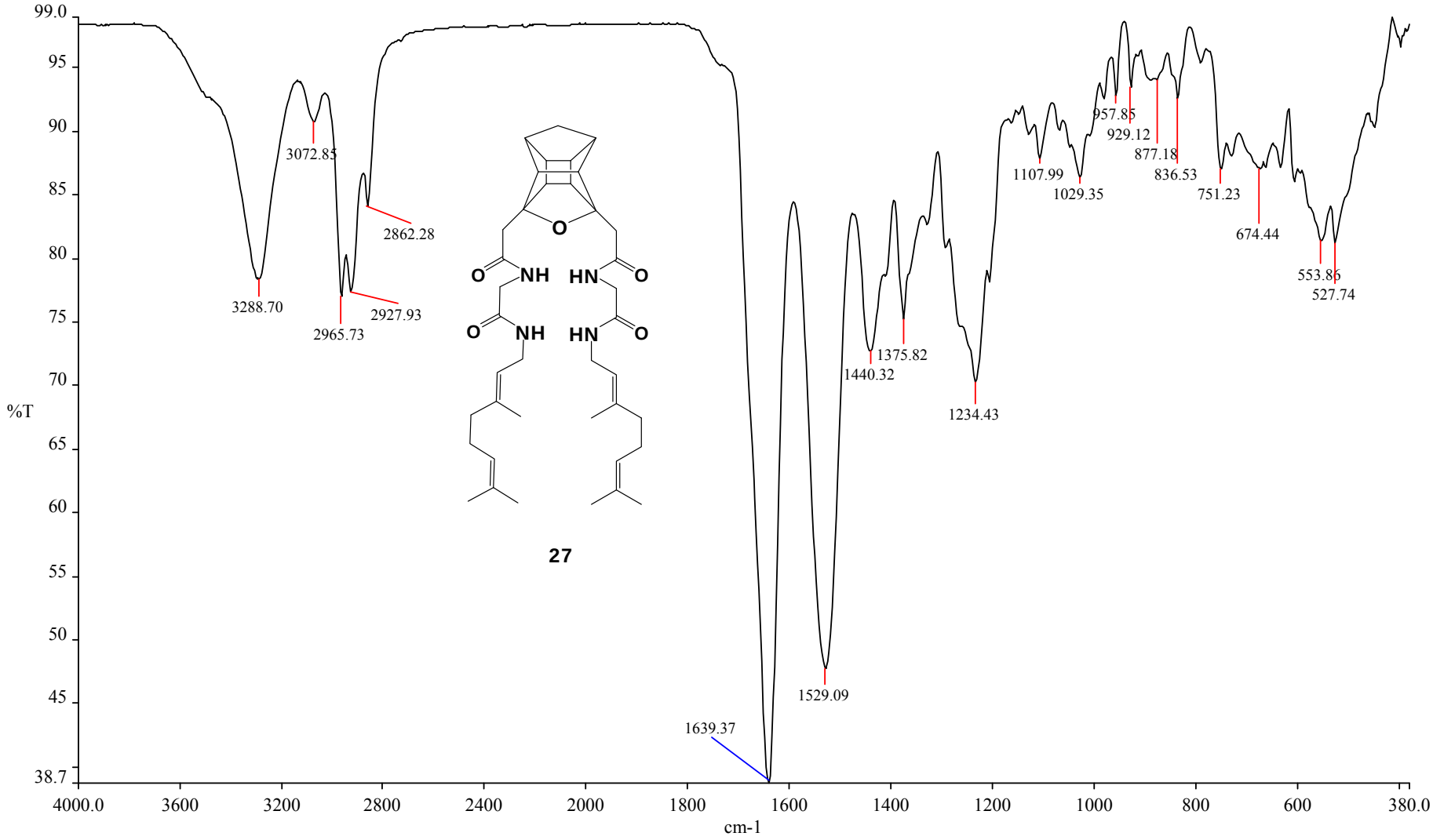
3.7781
2.7790
2.7414
2.7025
2.6649
2.6291
2.5979
2.4546
2.0552
2.0379
2.0208
2.0156
2.0052
2.0005
1.9681
1.9479
1.9311
1.8772
1.8506
1.6686
1.6373
1.6105
1.5547
1.5401
1.5131
O
O
NH
HN
O
O
NH
HN
O
7
6
5
4
3
2
[ppm]

27

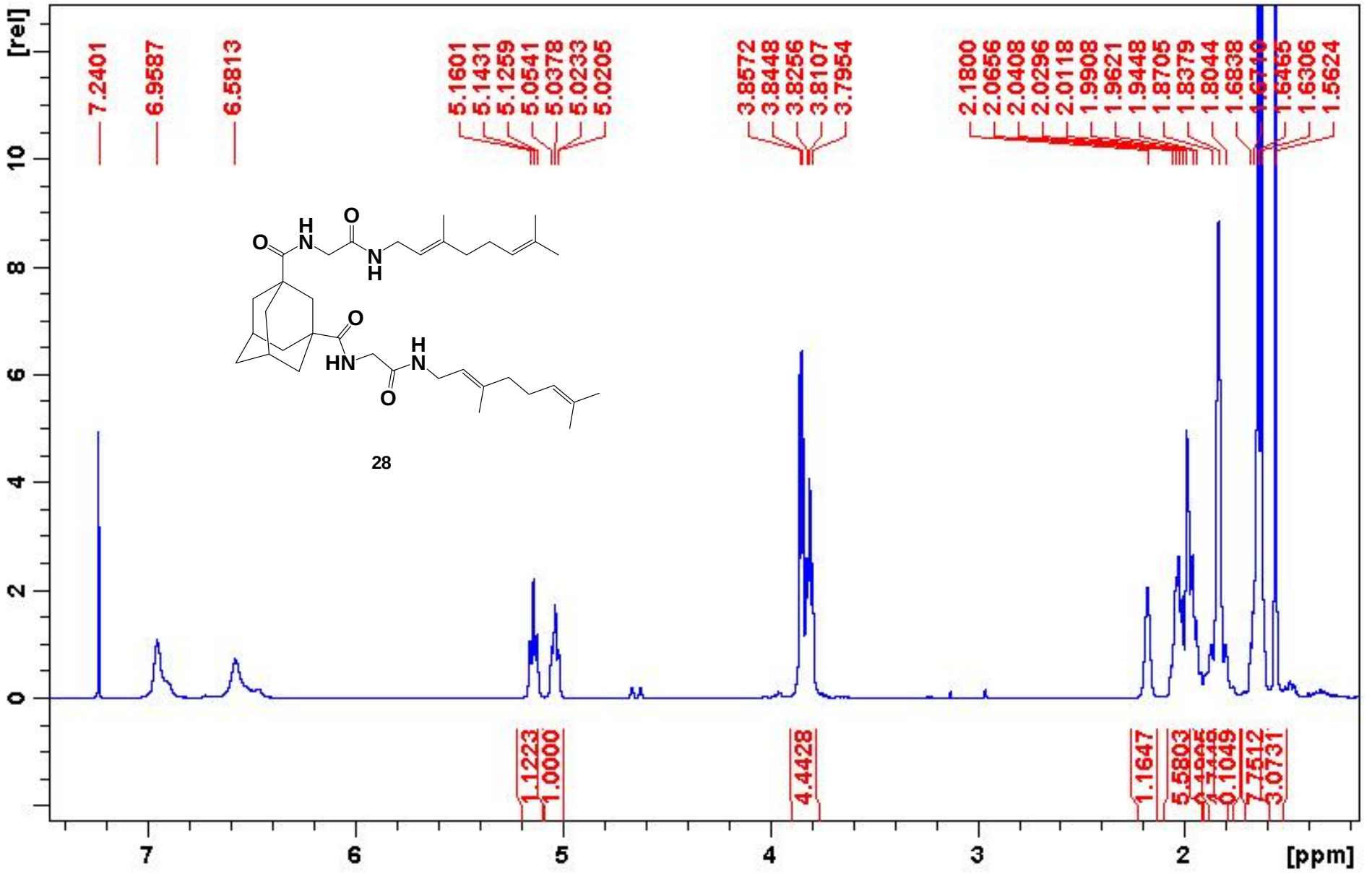
^{1}H NMR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-dimethylenecarboxamide (27)


170.3467
168.9477
139.9669
131.7544
123.6954
119.5416
93.9354
77.3167
76.9997
76.6814
58.7480
48.2447
44.0449
43.4333
43.1686
41.4659
39.4666
39.4062
37.4851
26.4048
26.3537
25.6241
17.6416
16.2576
O
O
NH
HN
O
O
NH
HN
O
27
150
100
50
[ppm]

^{13}C NMR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-dimethylenecarboxamide (27)

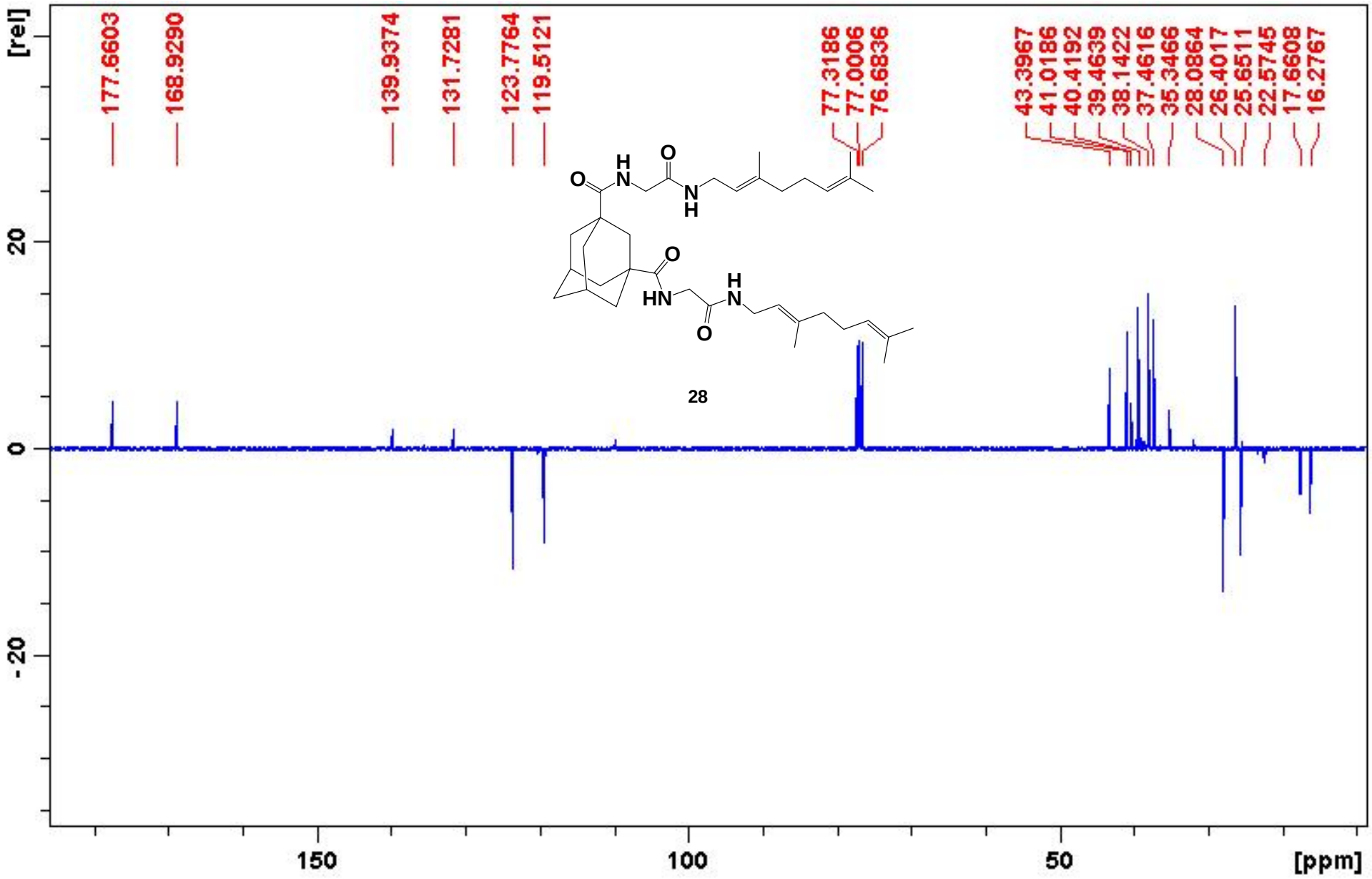


IR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-dimethylenecarboxamide (27)


[rel]
7.2401
6.9587
6.5813
5.1601
5.1431
5.1259
5.0541
5.0378
5.0233
5.0205
3.8572
3.8448
3.8256
3.8107
3.7954
2.1800
2.0656
2.0408
2.0296
2.0118
1.9908
1.9621
1.9448
1.8705
1.8379
1.8044
1.6838
1.6740
1.6465
1.6306
1.5624
1.1223
1.0000
4.4428
1.1647
5.5803
0.1049
7.7512
3.0731
[ppm]

28

^{1}H NMR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dicarboxamide (28)


[rel]
177.6603
168.9290
139.9374
131.7281
123.7764
119.5121
77.3186
77.0006
76.6836
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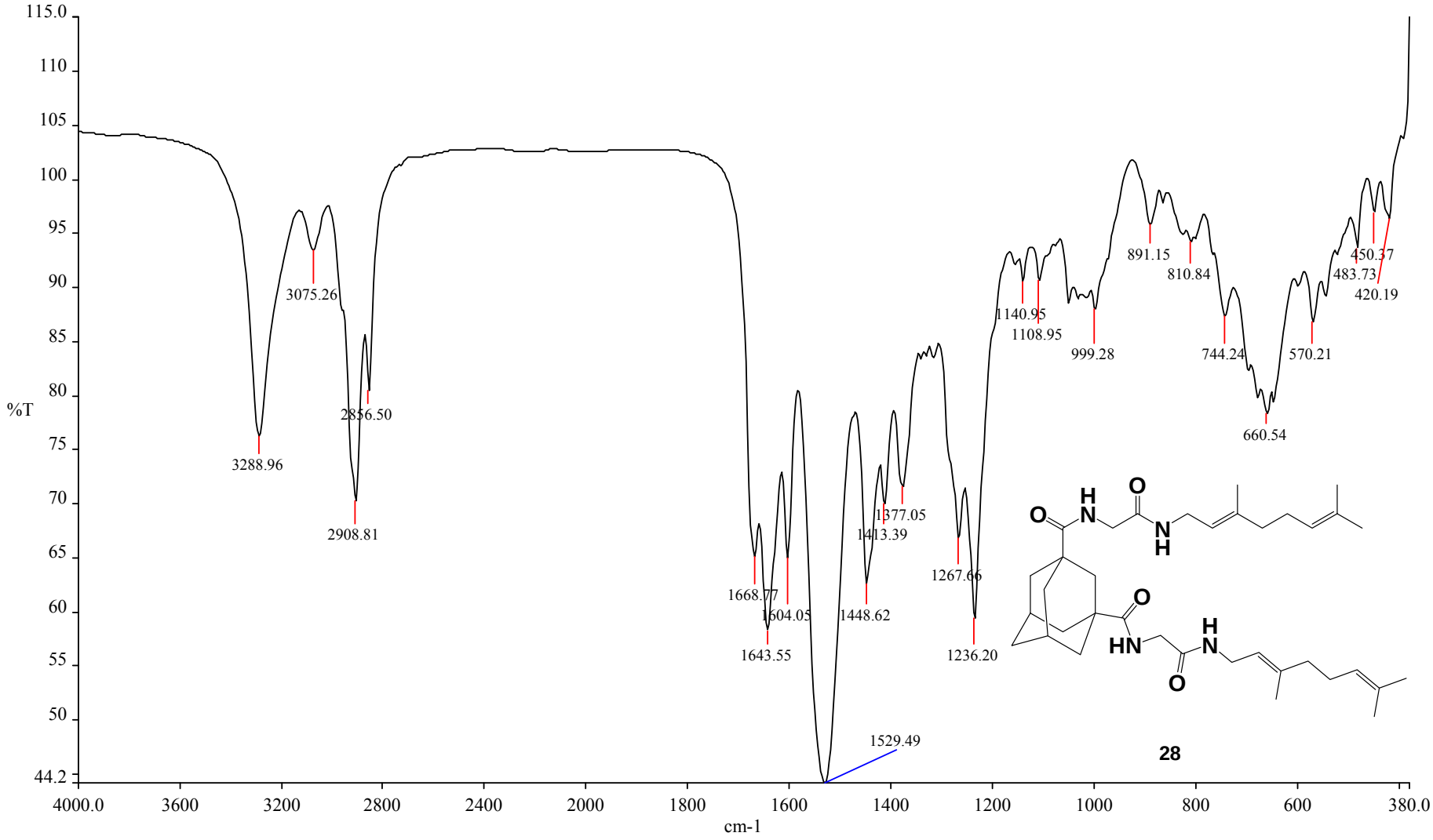
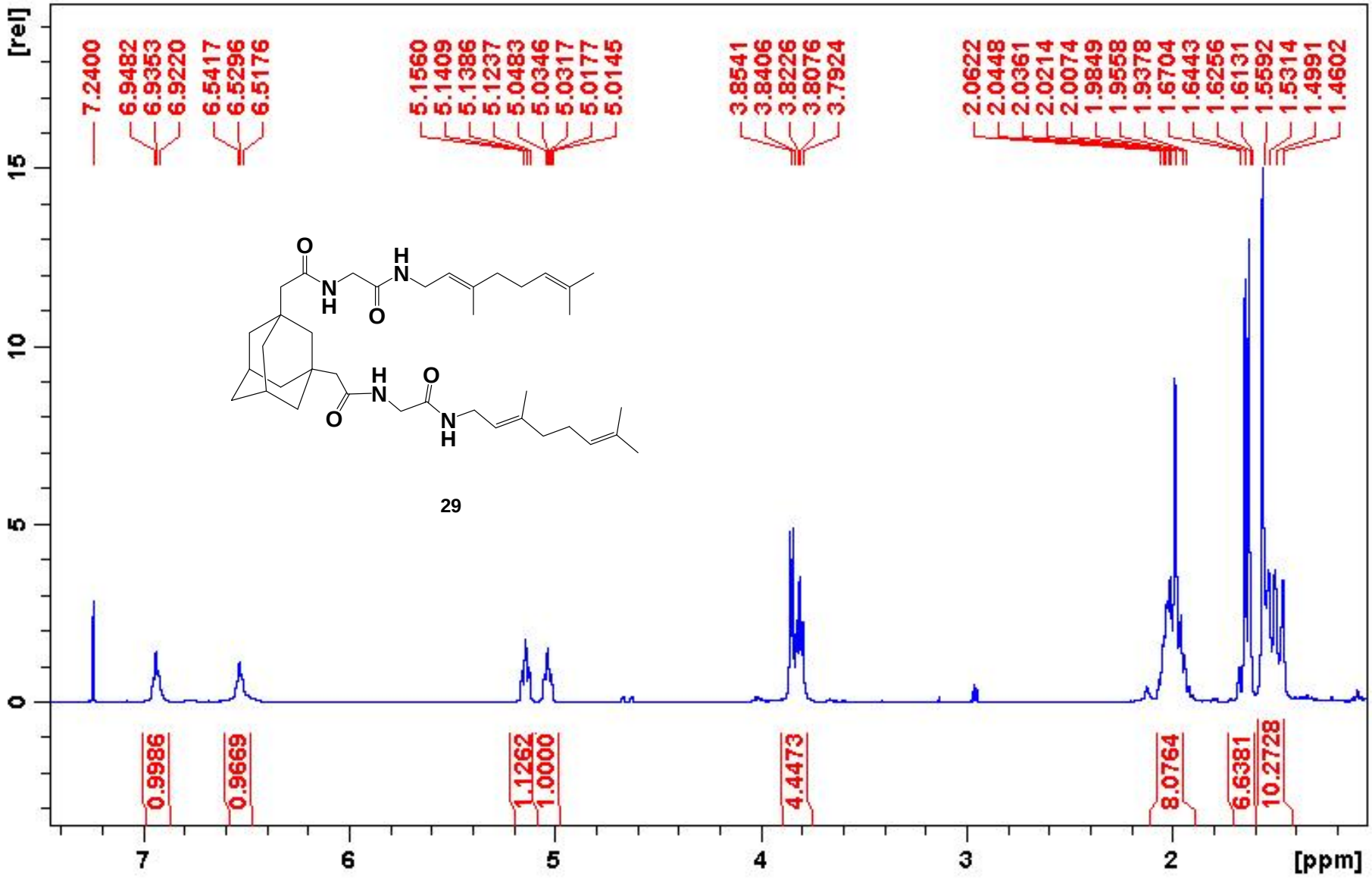
41.0186
40.4192
39.4639
38.1422
37.4616
35.3466
28.0864
26.4017
25.6511
22.5745
17.6608
16.2767
20
0
-20
150
100
50
[ppm]

28

^{13}C NMR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dicarboxamide (28)

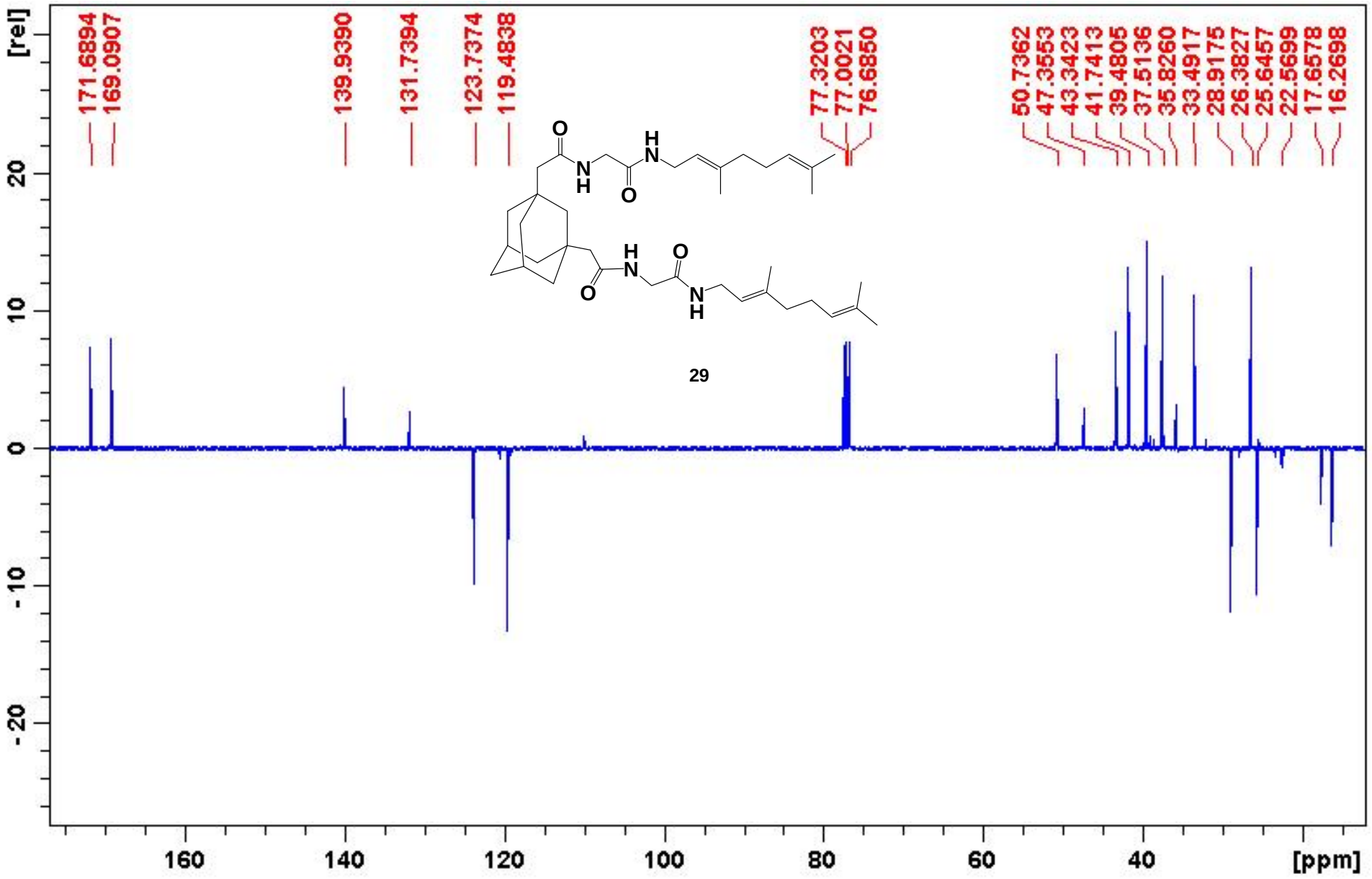


IR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dicarboxamide (28)


[rel]
7.2400
6.9482
6.9353
6.9220
6.5417
6.5296
6.5176
5.1560
5.1409
5.1386
5.1237
5.0483
5.0346
5.0317
5.0177
5.0145
3.8541
3.8406
3.8226
3.8076
3.7924
2.0622
2.0448
2.0361
2.0214
2.0074
1.9849
1.9558
1.9378
1.6704
1.6443
1.6256
1.6131
1.5592
1.5314
1.4991
1.4602
0.9986
0.9669
1.1262
1.0000
4.4473
8.0764
6.6381
10.2728
[ppm]

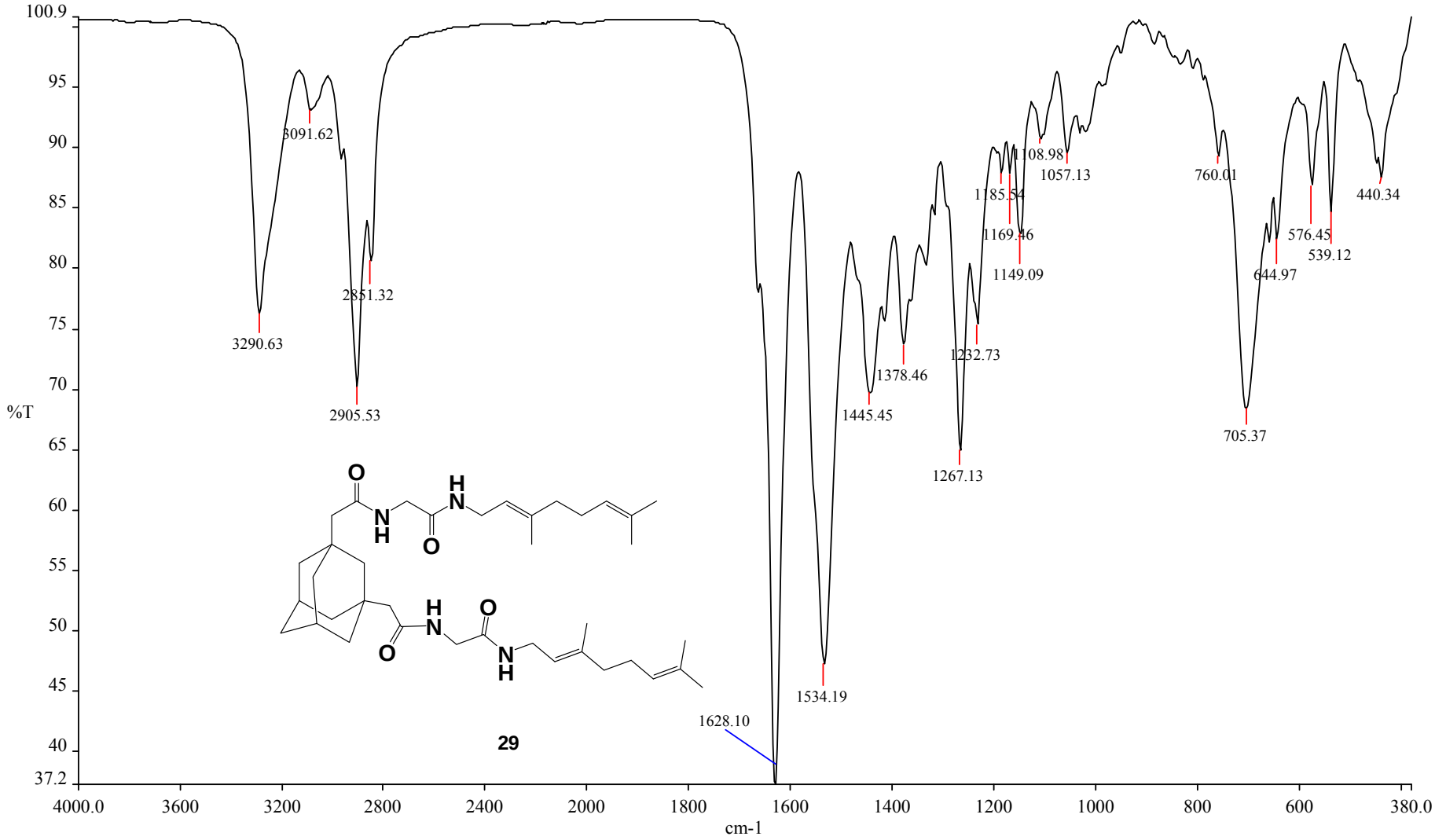
29

^{1}H NMR spectrum *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dimethylenecarboxamide (29)

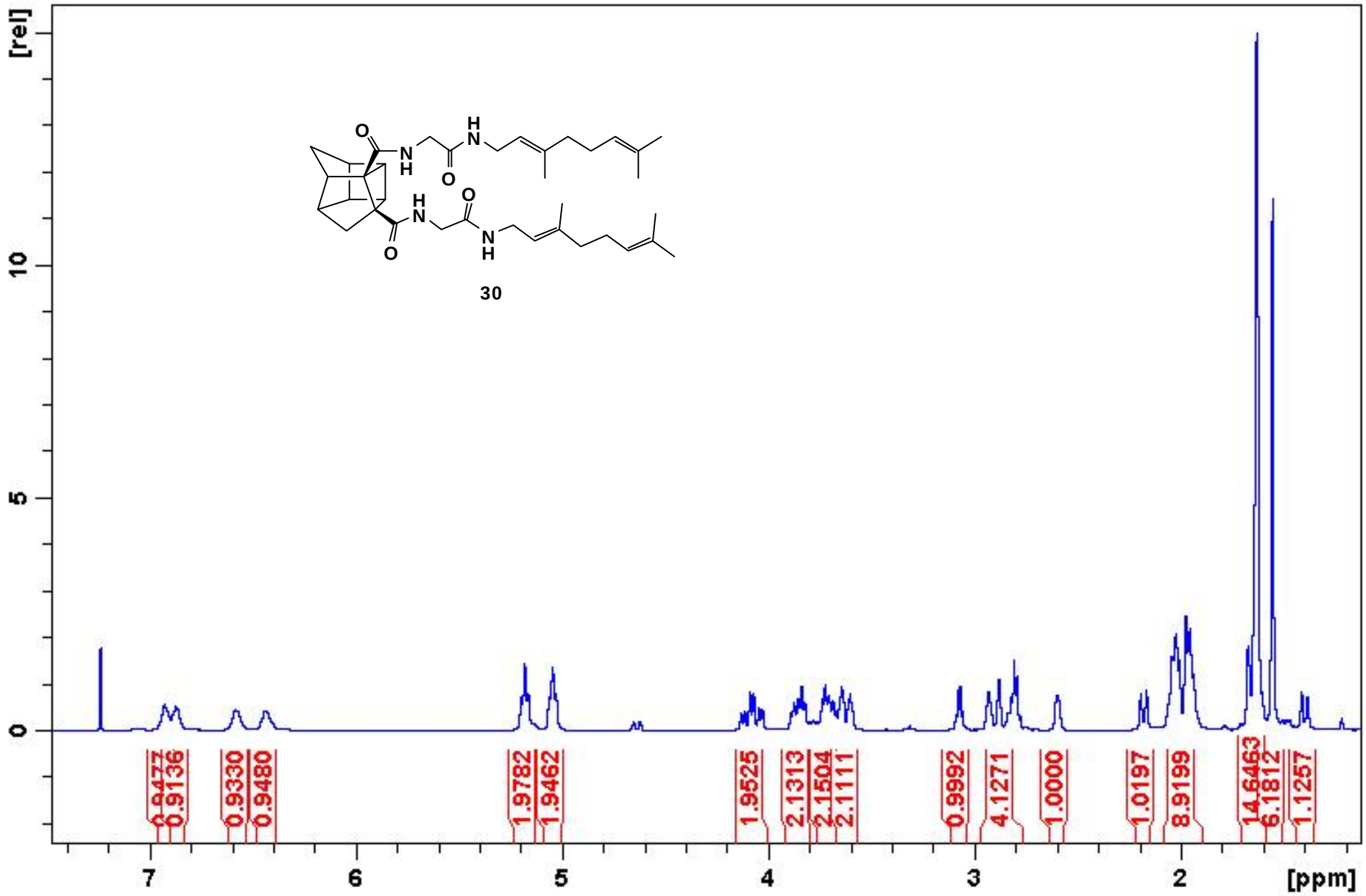

171.6894
169.0907
139.9390
131.7394
123.7374
119.4838
77.3203
77.0021
76.6850
50.7362
47.3553
43.3423
41.7413
39.4805
37.5136
35.8260
33.4917
28.9175
26.3827
25.6457
22.5699
17.6578
16.2698
[rel]
20
10
0
-10
-20
160
140
120
100
80
60
40
[ppm]
O
H
N
N
H
O
O
H
N
O
O
N
H

29

^{13}C NMR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dimethylenecarboxamide (29)

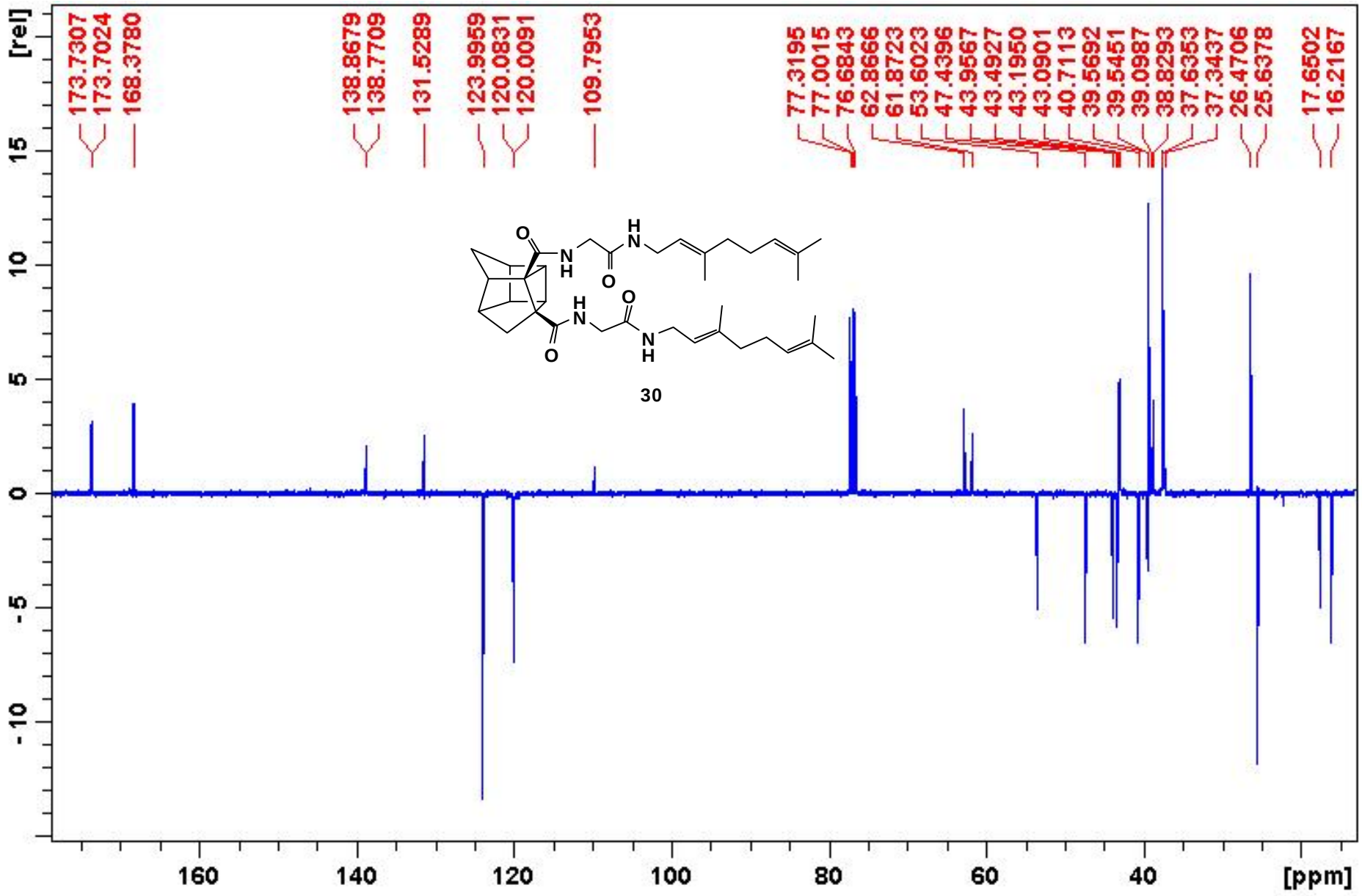


IR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-tricyclo[3.3.1.1^{3,7}]decane-1,3-dimethylenecarboxamide (29)


[rel]
10
5
0
7
6
5
4
3
2
[ppm]
0.9477
0.9136
0.9330
0.9480
1.9782
1.9462
1.9525
2.1313
2.1504
2.1111
0.9992
4.1271
1.0000
1.0197
8.9199
14.6463
6.1812
1.1257

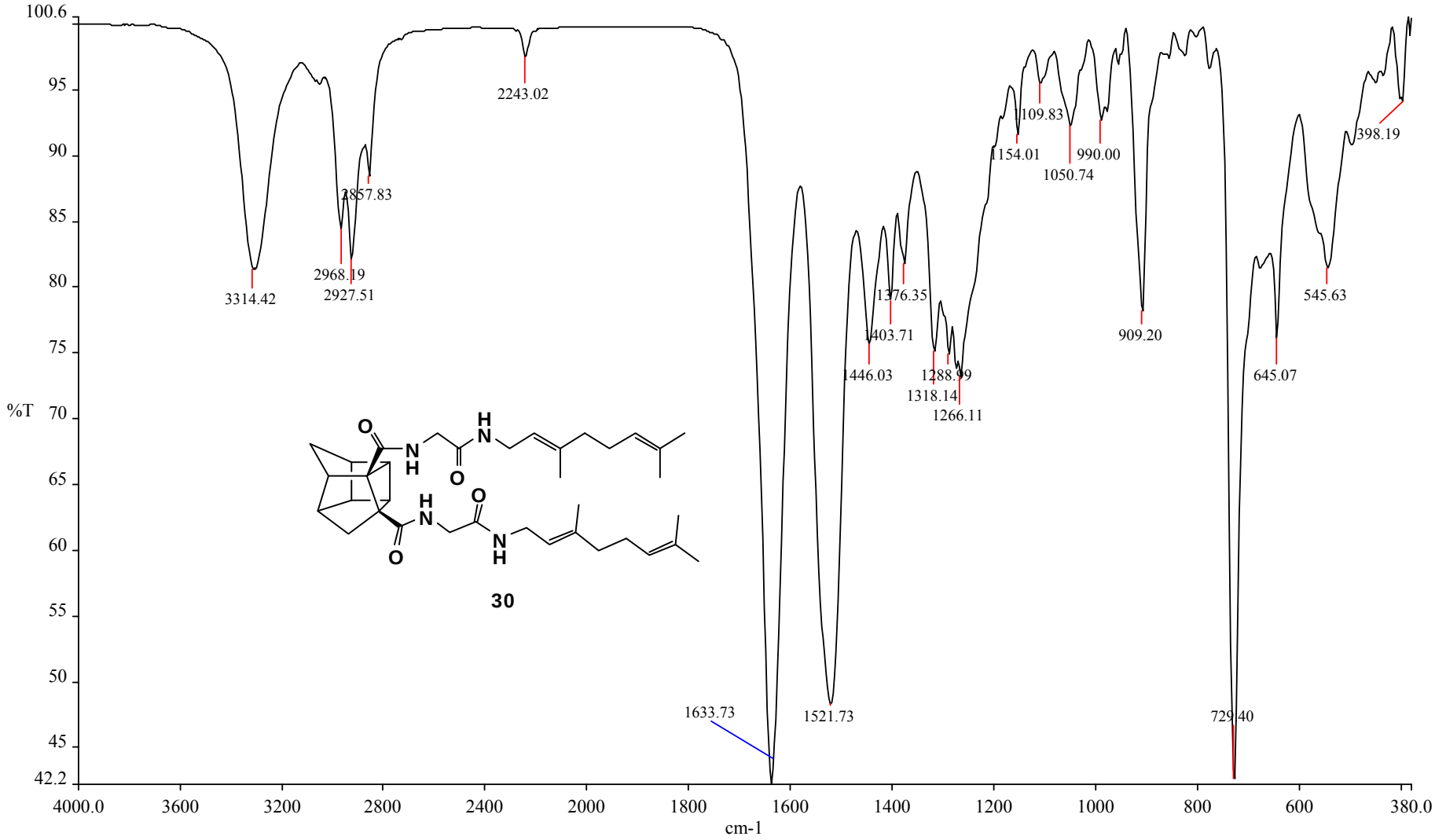
30

^{1}H NMR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dicarboxamide (30)

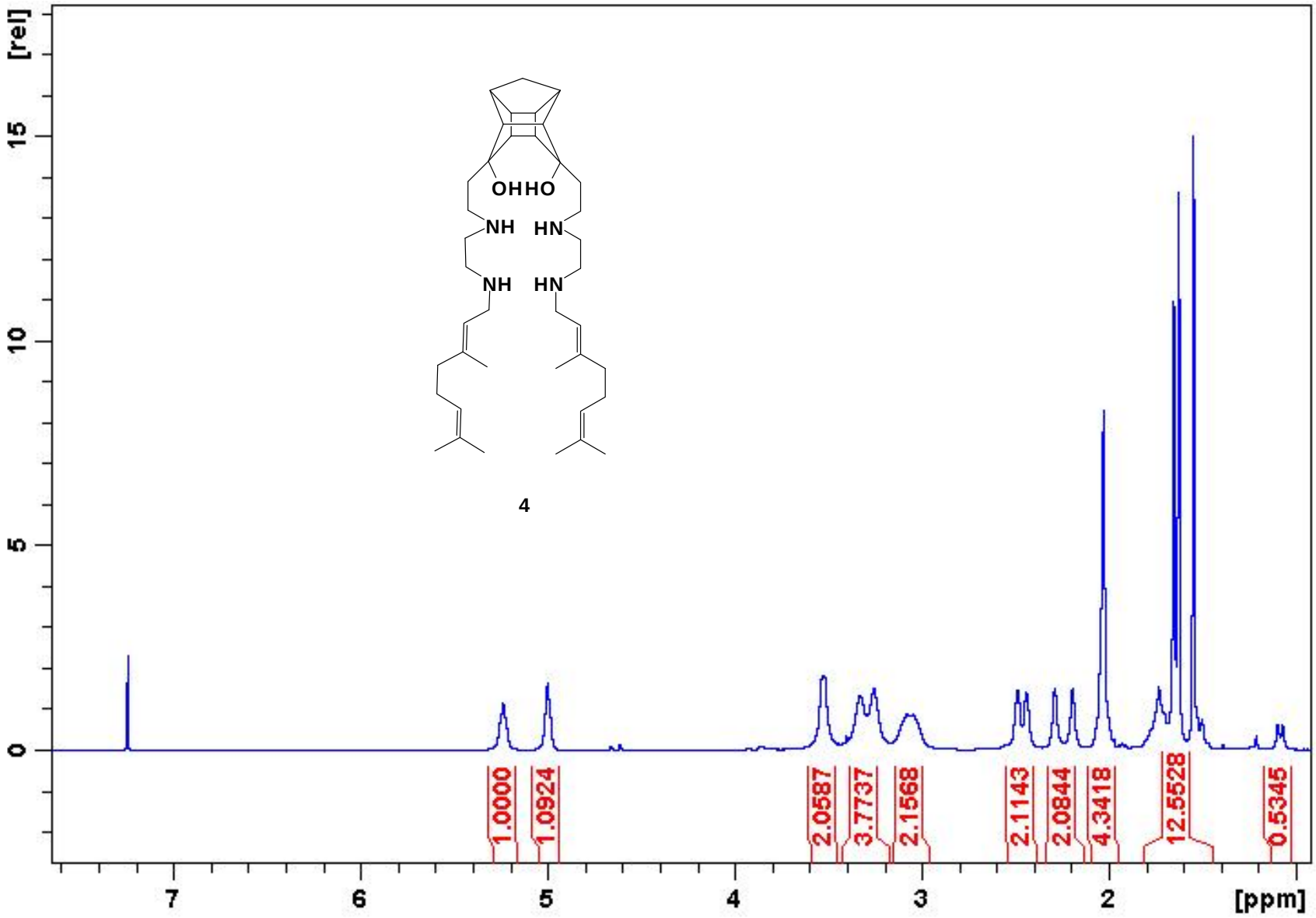

[rel]
15
10
5
0
-5
-10
173.7307
173.7024
168.3780
138.8679
138.7709
131.5289
123.9959
120.0831
120.0091
109.7953
77.3195
77.0015
76.6843
62.8666
61.8723
53.6023
47.4396
43.9567
43.4927
43.1950
43.0901
40.7113
39.5692
39.5451
39.0987
38.8293
37.6353
37.3437
26.4706
25.6378
17.6502
16.2167
160
140
120
100
80
60
40
[ppm]

30

^{13}C NMR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dicarboxamide (30)

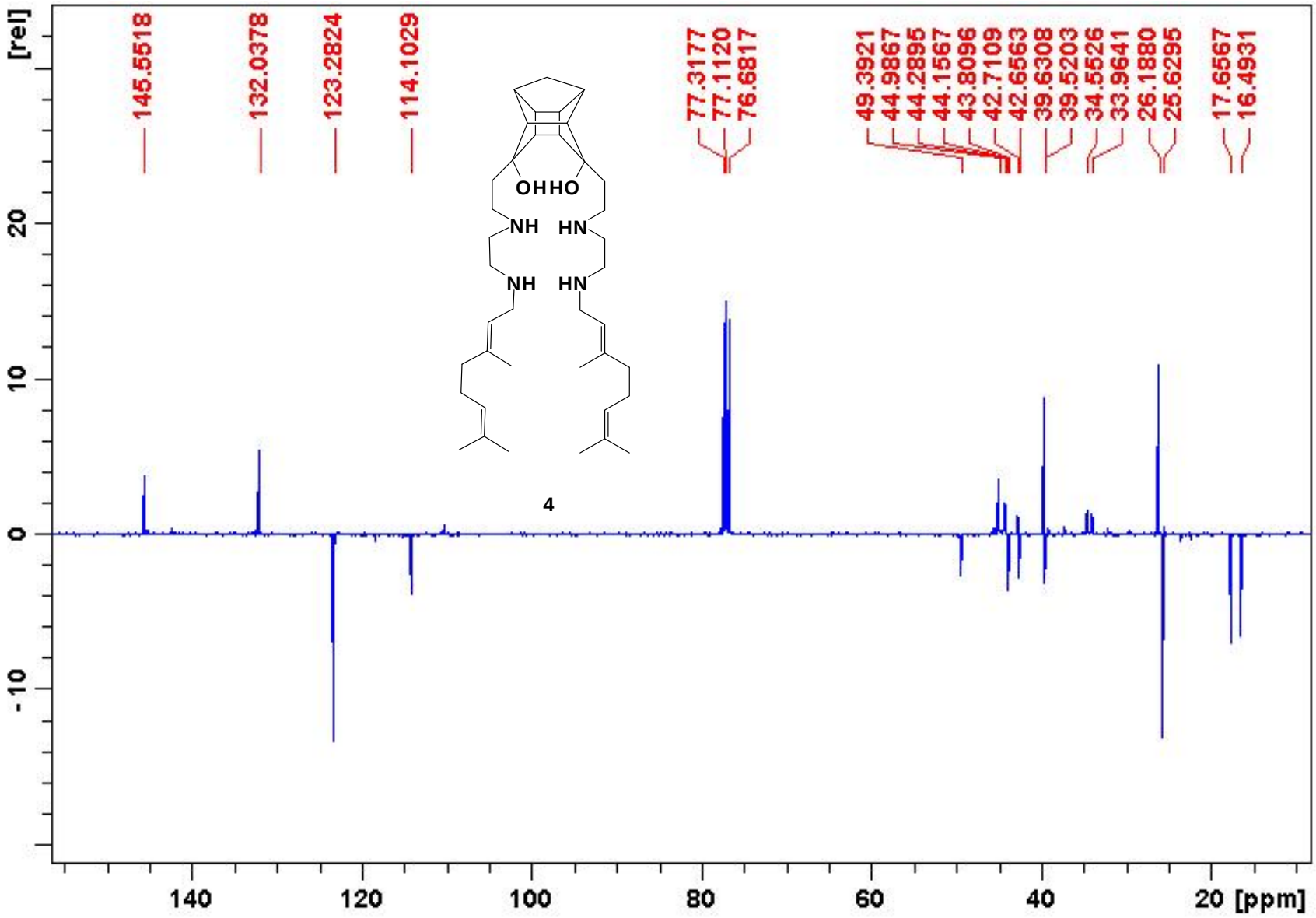


IR spectrum of *N′*,*N′*-bis[2-((*E*)-3,7-dimethylocta-2,6-dienylamino)-2-oxoethyl]-pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dicarboxamide (30)


[rel]
15
10
5
0
OH HO
NH HN
NH HN
7
6
5
4
3
2
[ppm]
1.0000
1.0924
2.0587
3.7737
2.1568
2.1143
2.0844
4.3418
12.5528
0.5345

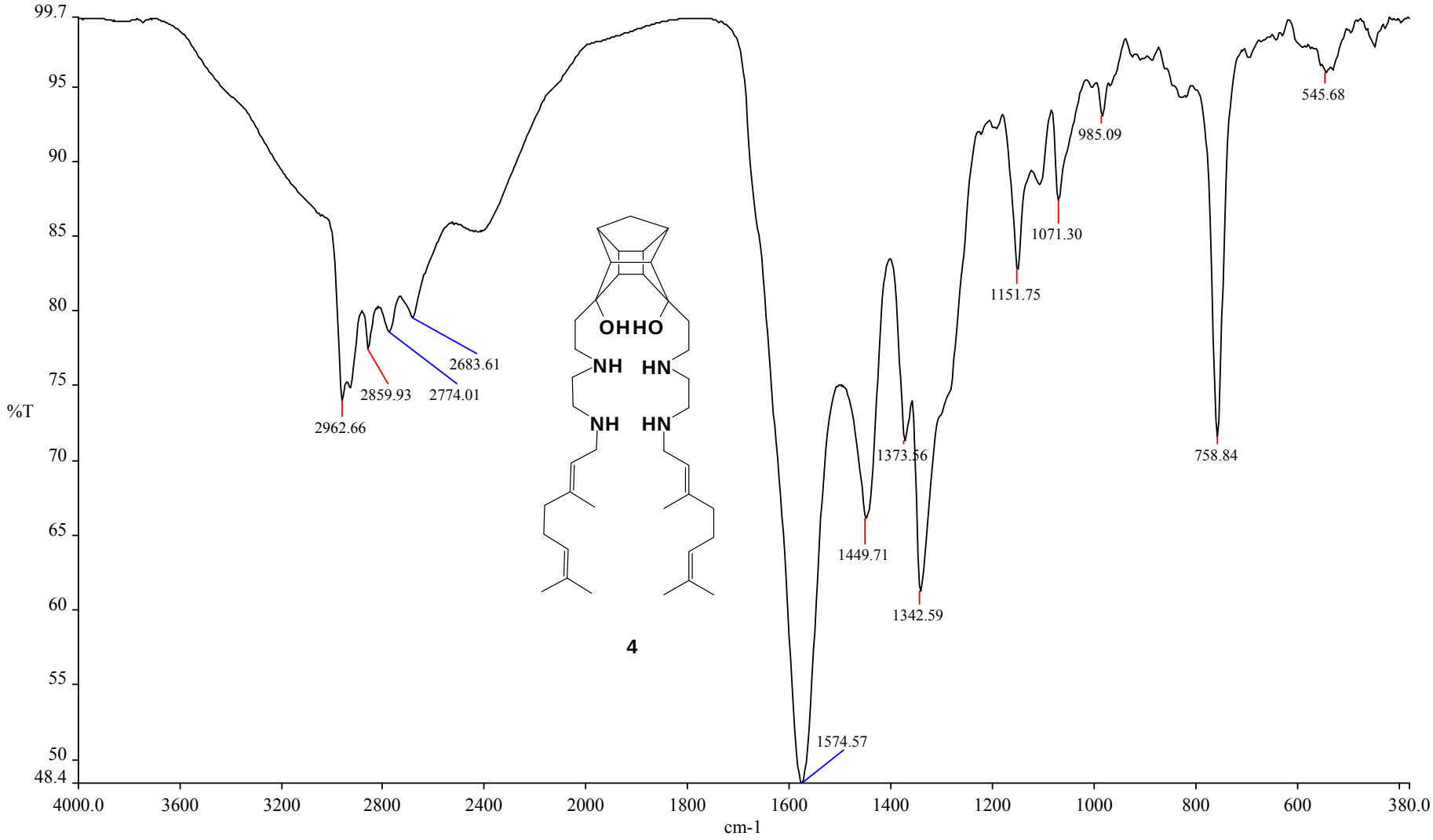
4

^{1}H NMR spectrum of [*N′*,*N′*-(8,11-dihydroxypentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diethylene)]-bis[(*N′′*-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (4)

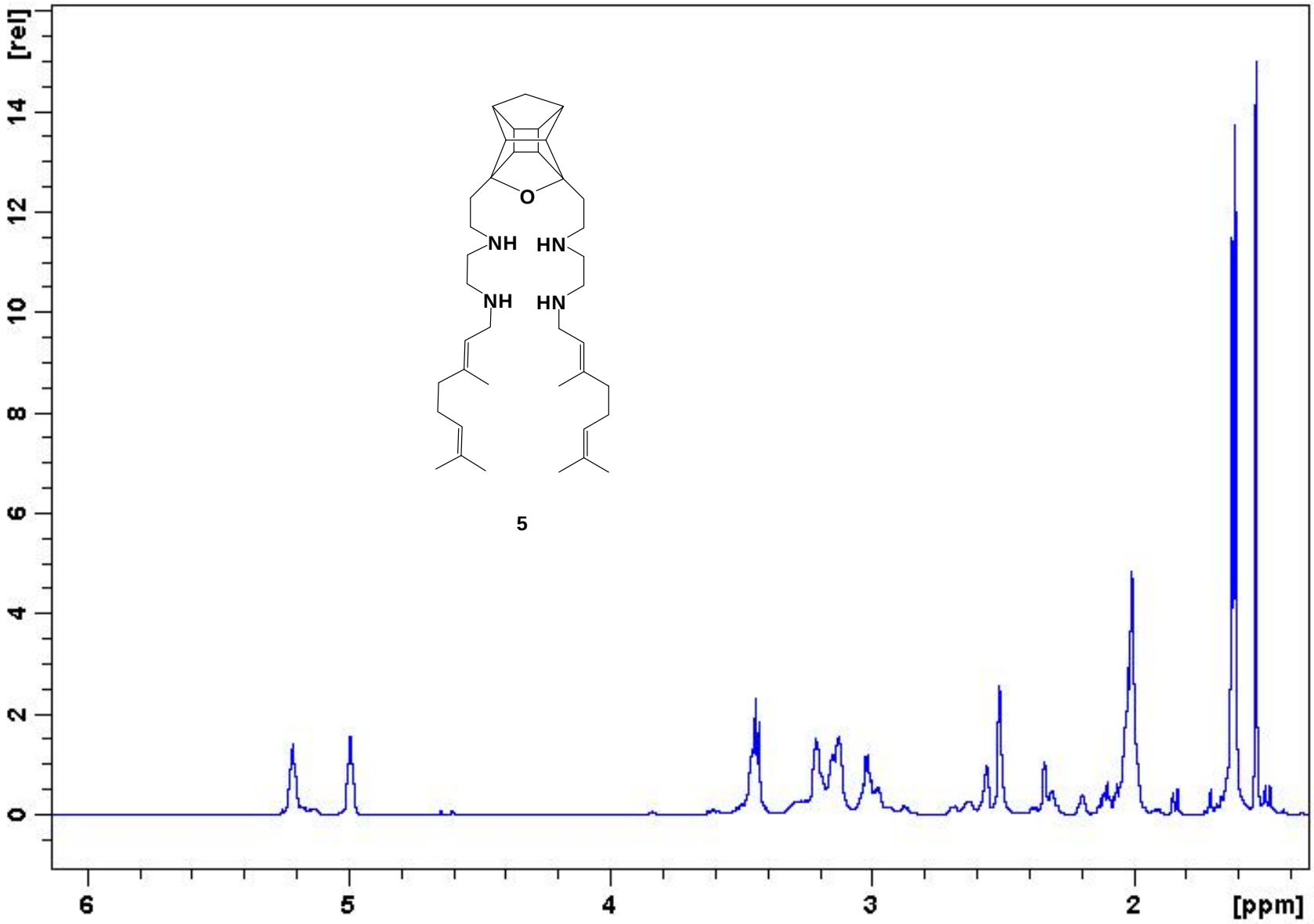

[rel]
145.5518
132.0378
123.2824
114.1029
77.3177
77.1120
76.6817
49.3921
44.9867
44.2895
44.1567
43.8096
42.7109
42.6563
39.6308
39.5203
34.5526
33.9641
26.1880
25.6295
17.6567
16.4931
OH HO
NH HN
NH HN
20
10
0
-10
140
120
100
80
60
40
20 [ppm]

4

^{13}C NMR spectrum of spectrum of [*N*′,*N*′-(8,11-dihydroxypentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (4)

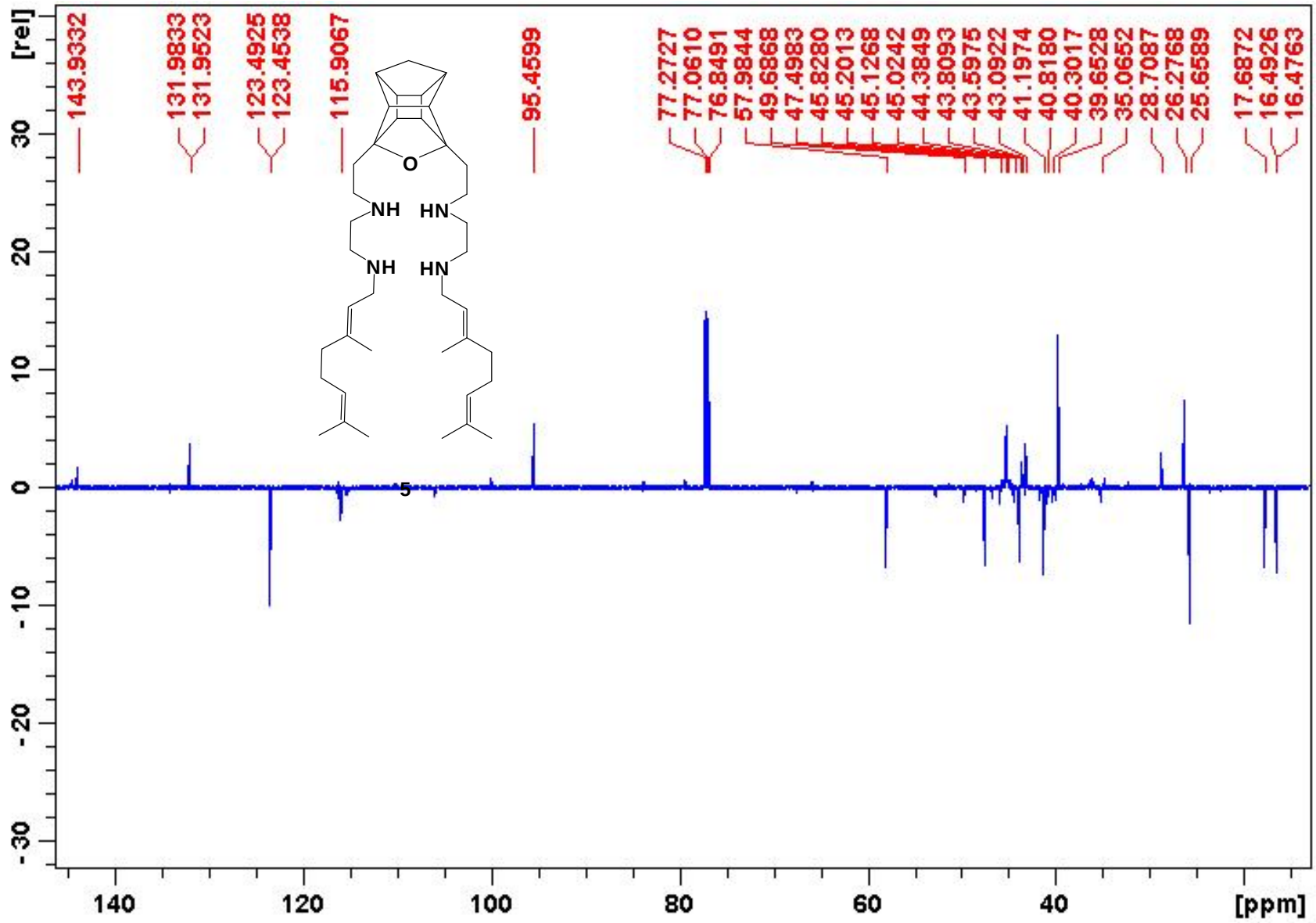


IR spectrum of spectrum of [*N*′,*N*′-(8,11-dihydroxypentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (4)

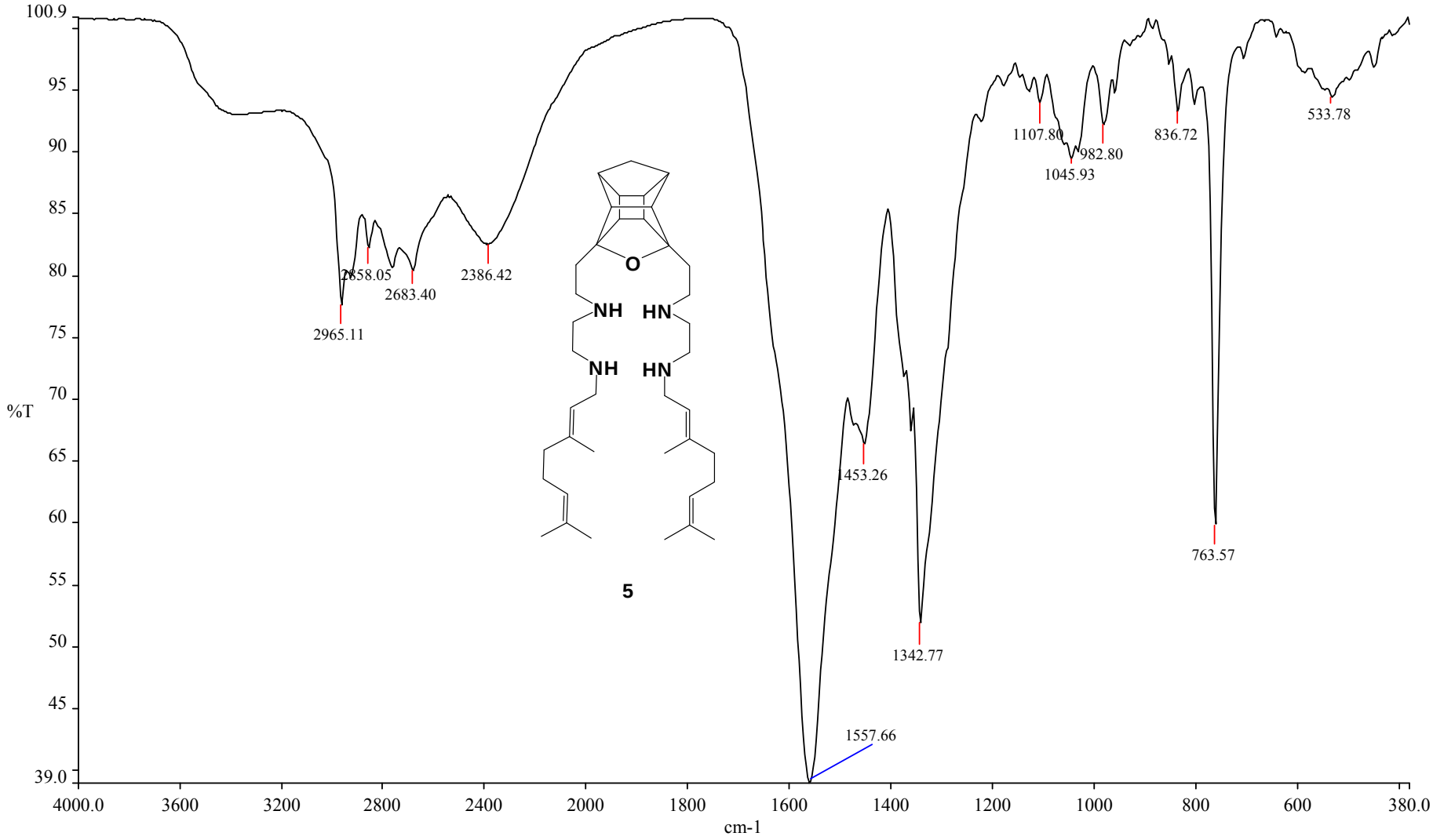


5

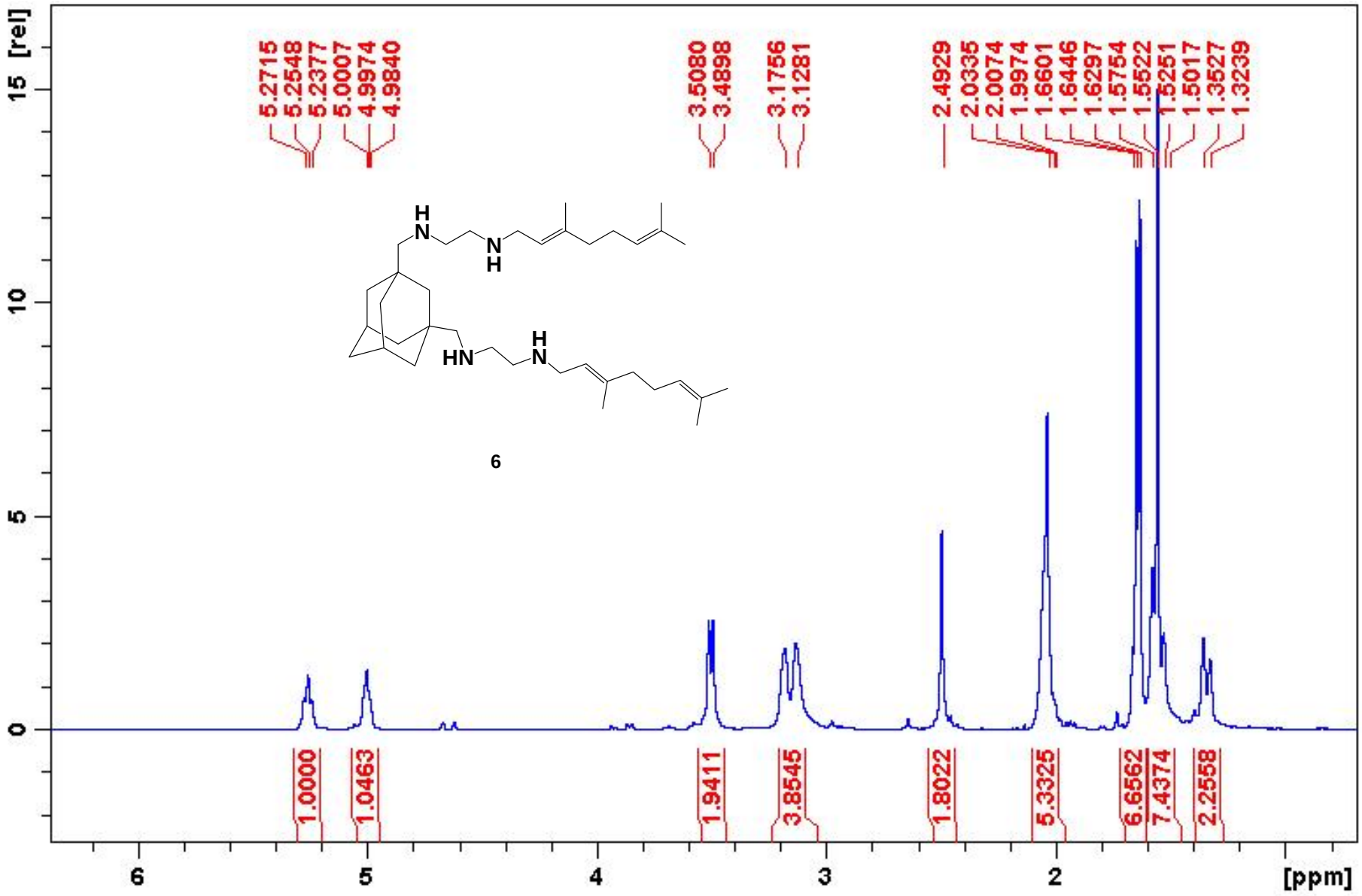
^{1}H NMR spectrum of [*N*′,*N*′-(8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (5)


143.9332
131.9833
131.9523
123.4925
123.4538
115.9067
95.4599
77.2727
77.0610
76.8491
57.9844
49.6868
47.4983
45.8280
45.2013
45.1268
45.0242
44.3849
43.8093
43.5975
43.0922
41.1974
40.8180
40.3017
39.6528
35.0652
28.7087
26.2768
25.6589
17.6872
16.4926
16.4763
[rel]
30
20
10
0
-10
-20
-30
O
NH
HN
NH
HN
5
140
120
100
80
60
40
[ppm]

^{13}C NMR spectrum of [*N*′,*N*′-(8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (5)

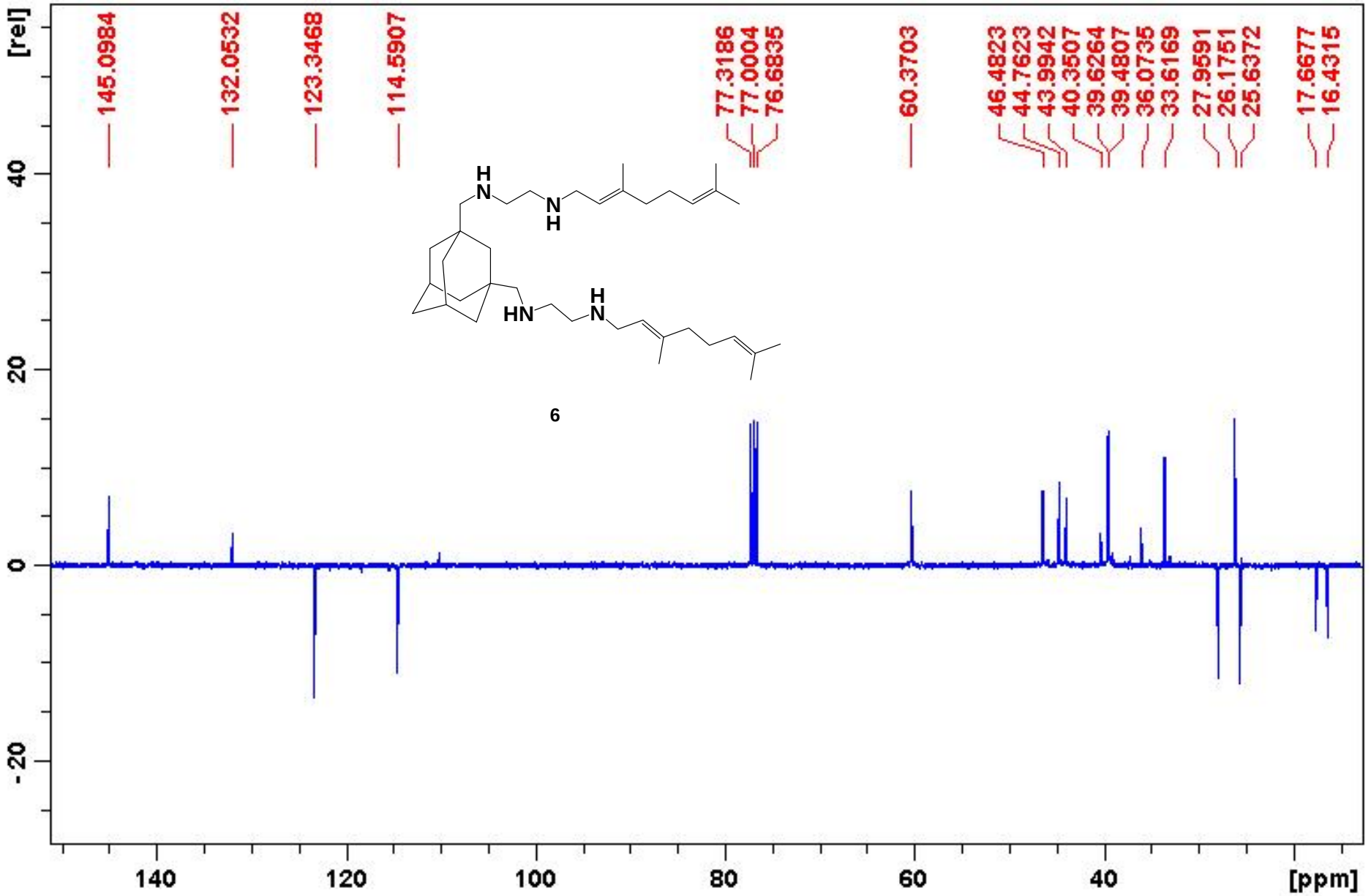


IR spectrum of [*N*′,*N*′-(8,11-oxapentacyclo[5.4.0.0^{2,6}.^{3,10}.0^{5,9}]undecane-8,11-diethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (5)


15 [rel]
10
5
0
5.2715
5.2548
5.2377
5.0007
4.9974
4.9840
3.5080
3.4898
3.1756
3.1281
2.4929
2.0335
2.0074
1.9974
1.6601
1.6446
1.6297
1.5754
1.5522
1.5251
1.5017
1.3527
1.3239
H
N
N
H
HN
H
N
1.0000
1.0463
1.9411
3.8545
1.8022
5.3325
6.6562
7.4374
2.2558
6
5
4
3
2
[ppm]

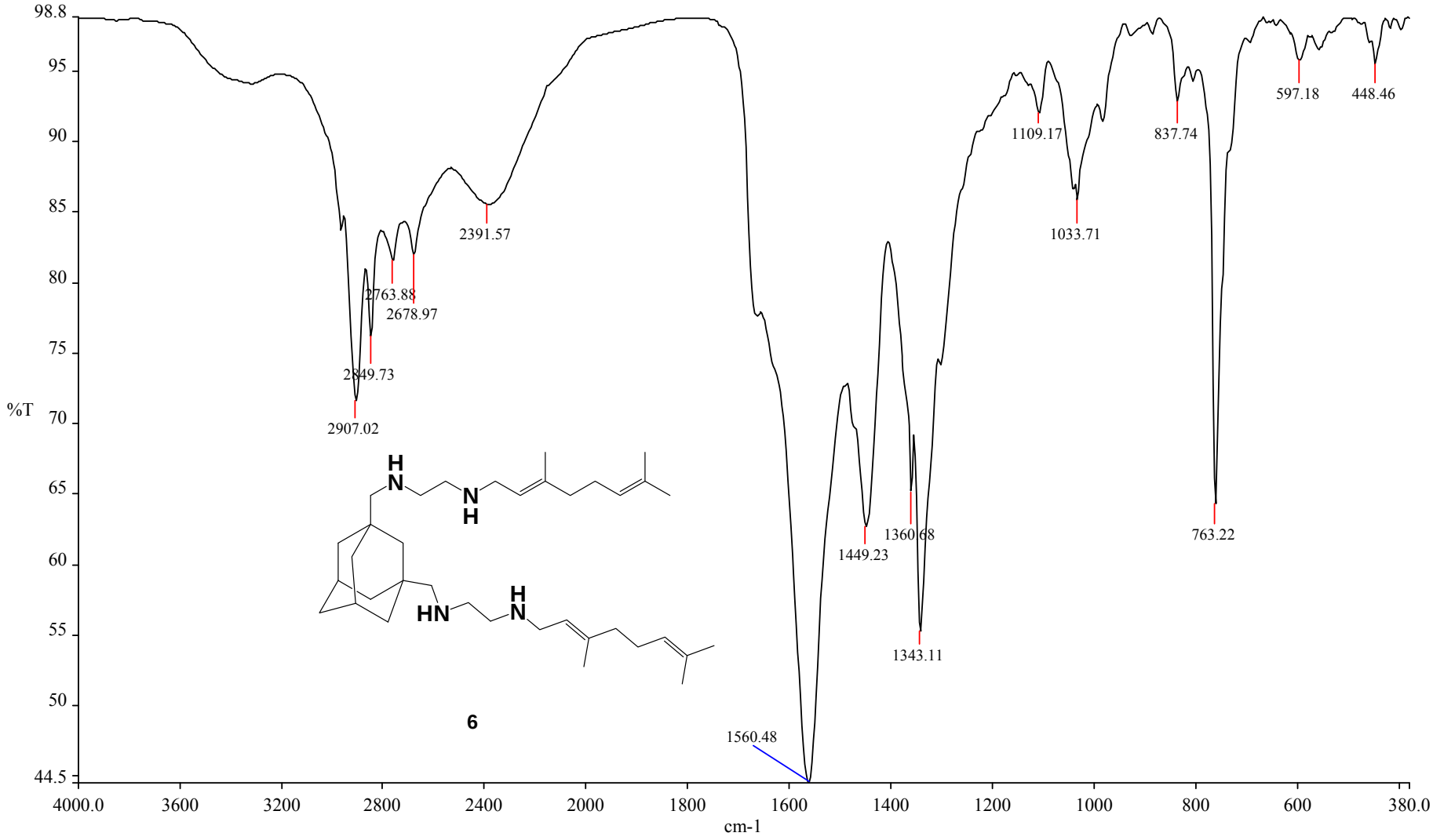
6

^{1}H NMR spectrum of [*N*′,*N*′-(tricyclo[3.3.1.1^{3,7}]decane-1,3-dimethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (6)

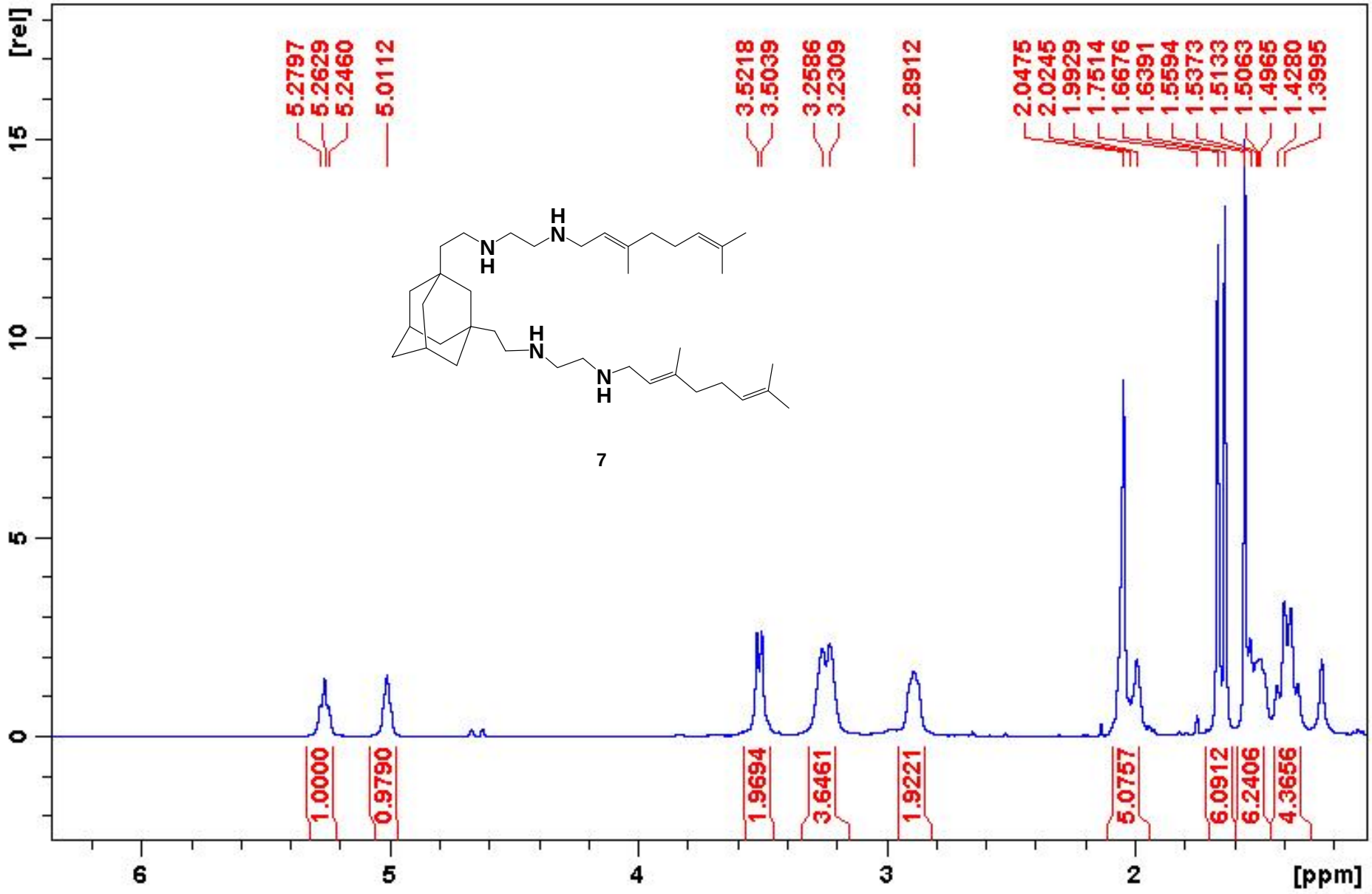

145.0984
132.0532
123.3468
114.5907
77.3186
77.0004
76.6835
60.3703
46.4823
44.7623
43.9942
40.3507
39.6264
39.4807
36.0735
33.6169
27.9591
26.1751
25.6372
17.6677
16.4315
[rel]
40
20
0
-20
140
120
100
80
60
40
[ppm]
H
N
N
H
HN
H
N

6

^{13}C NMR spectrum of [*N*′,*N*′-(tricyclo[3.3.1.1^{3,7}]decane-1,3-dimethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (6)

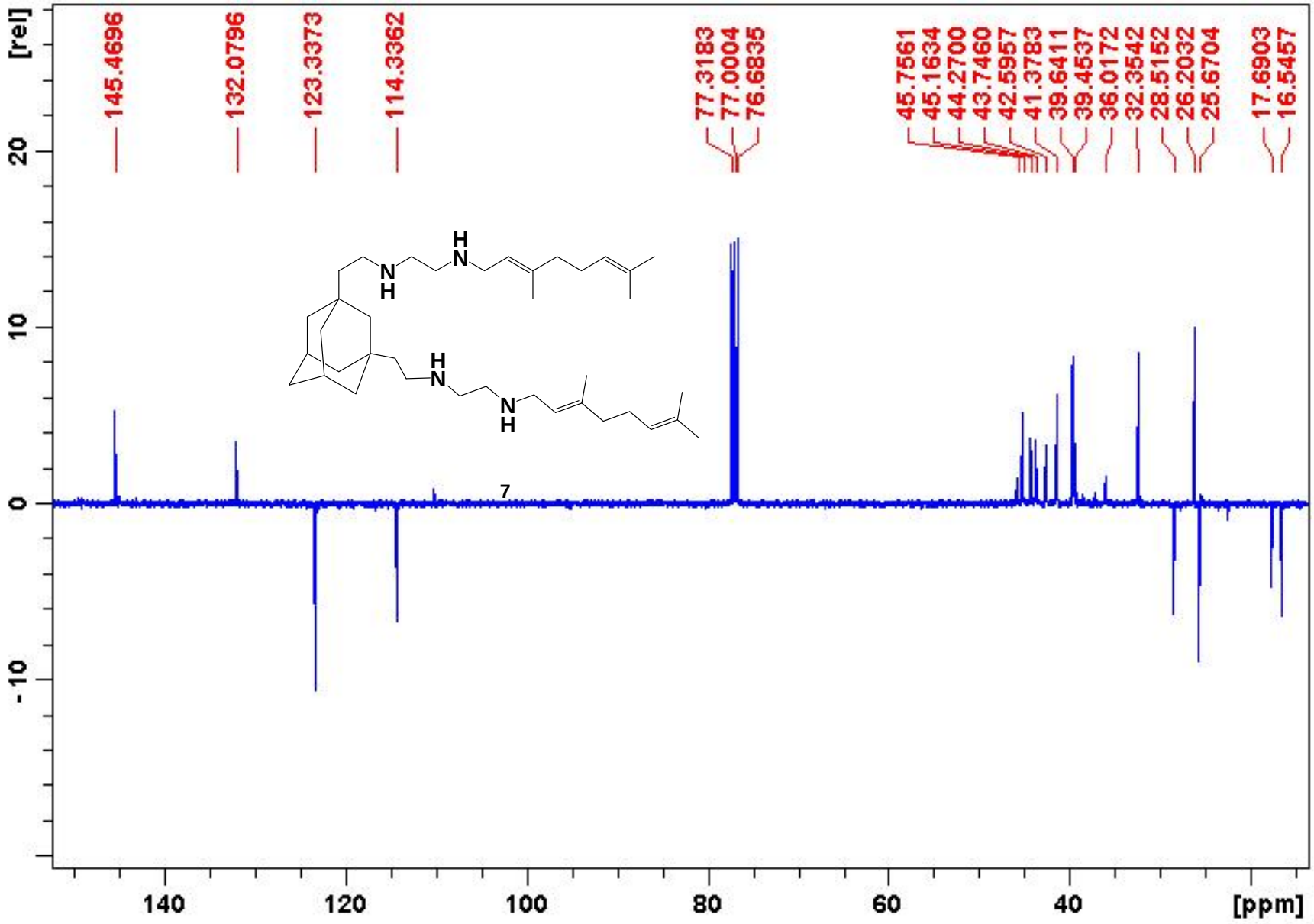


IR spectrum of [*N*′,*N*′-(tricyclo[3.3.1.1^{3,7}]decane-1,3-dimethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (6)

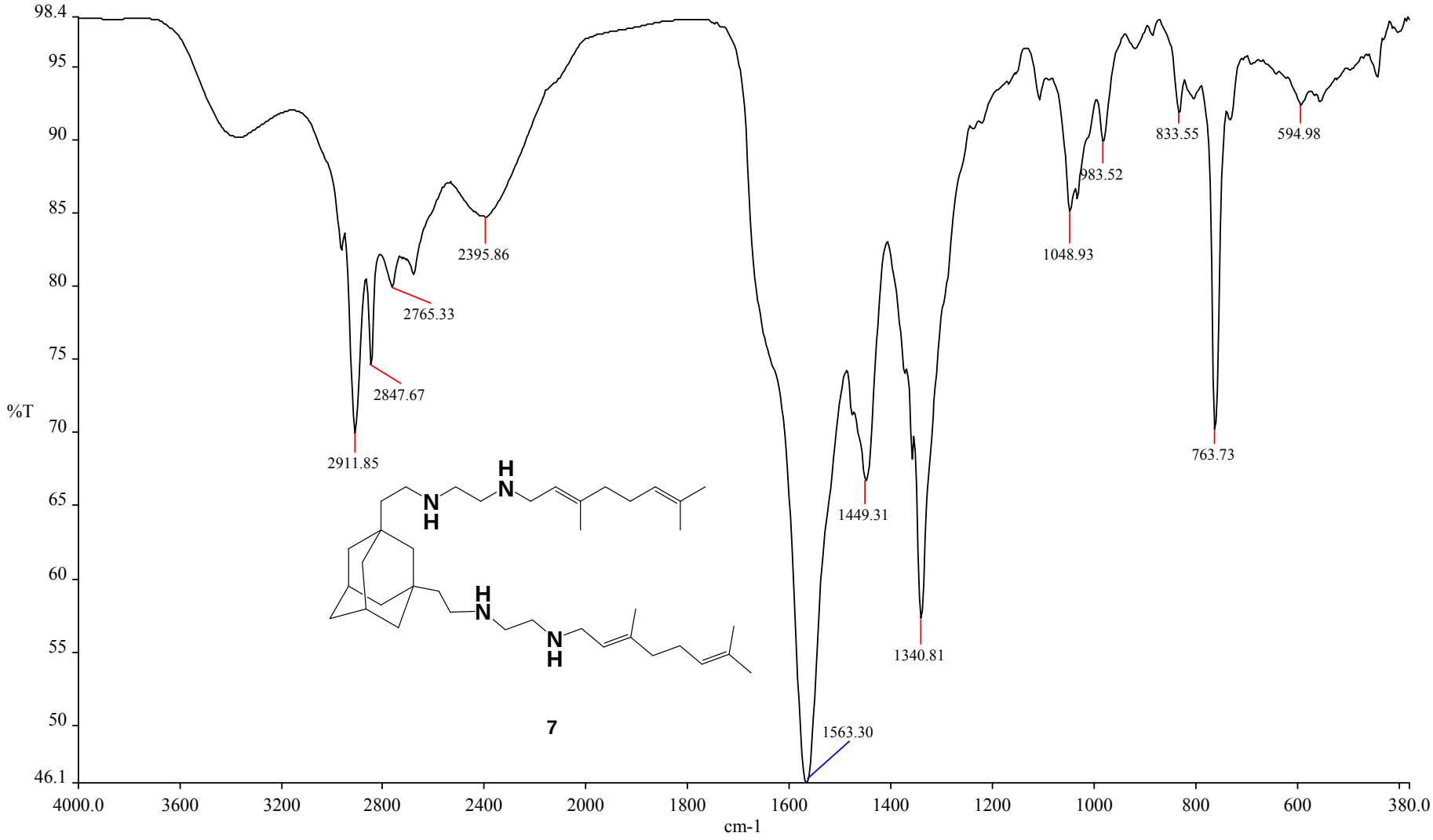

[rel]
15
10
5
0
5.2797
5.2629
5.2460
5.0112
3.5218
3.5039
3.2586
3.2309
2.8912
2.0475
2.0245
1.9929
1.7514
1.6676
1.6391
1.5594
1.5373
1.5133
1.5063
1.4965
1.4280
1.3995
1.0000
0.9790
1.9694
3.6461
1.9221
5.0757
6.0912
6.2406
4.3656
6
5
4
3
2
[ppm]

7

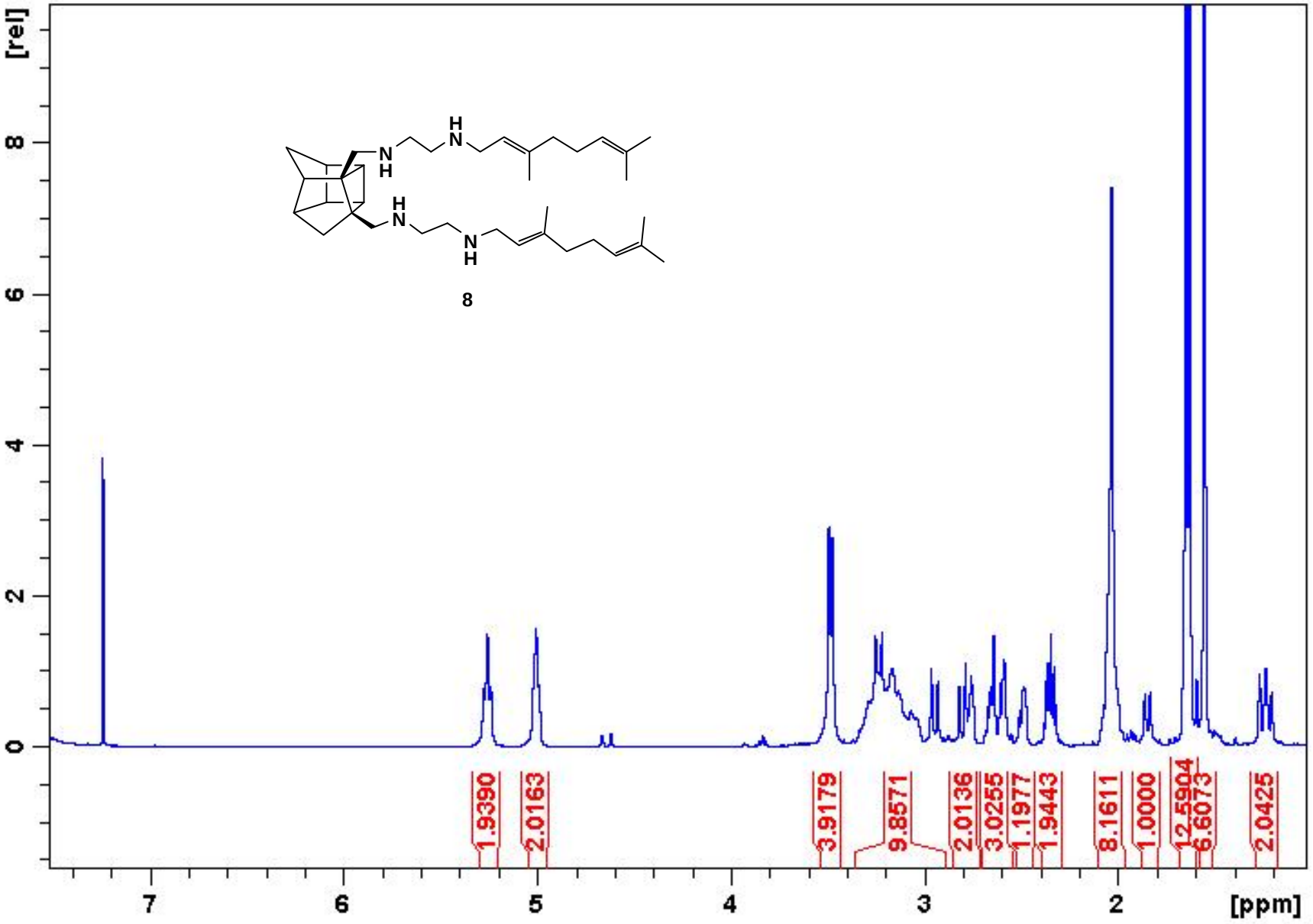
^{1}H NMR spectrum of [*N*′,*N*′-(tricyclo[3.3.1.1^{3,7}]decane-1,3-diethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (7)


145.4696
132.0796
123.3373
114.3362
77.3183
77.0004
76.6835
45.7561
45.1634
44.2700
43.7460
42.5957
41.3783
39.6411
39.4537
36.0172
32.3542
28.5152
26.2032
25.6704
17.6903
16.5457
[rel]
20
10
0
-10
140
120
100
80
60
40
[ppm]
H
N
N
H
H
N
N
H
7

^{13}C NMR spectrum of [*N*′,*N*′-(tricyclo[3.3.1.1^{3,7}]decane-1,3-diethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (7)

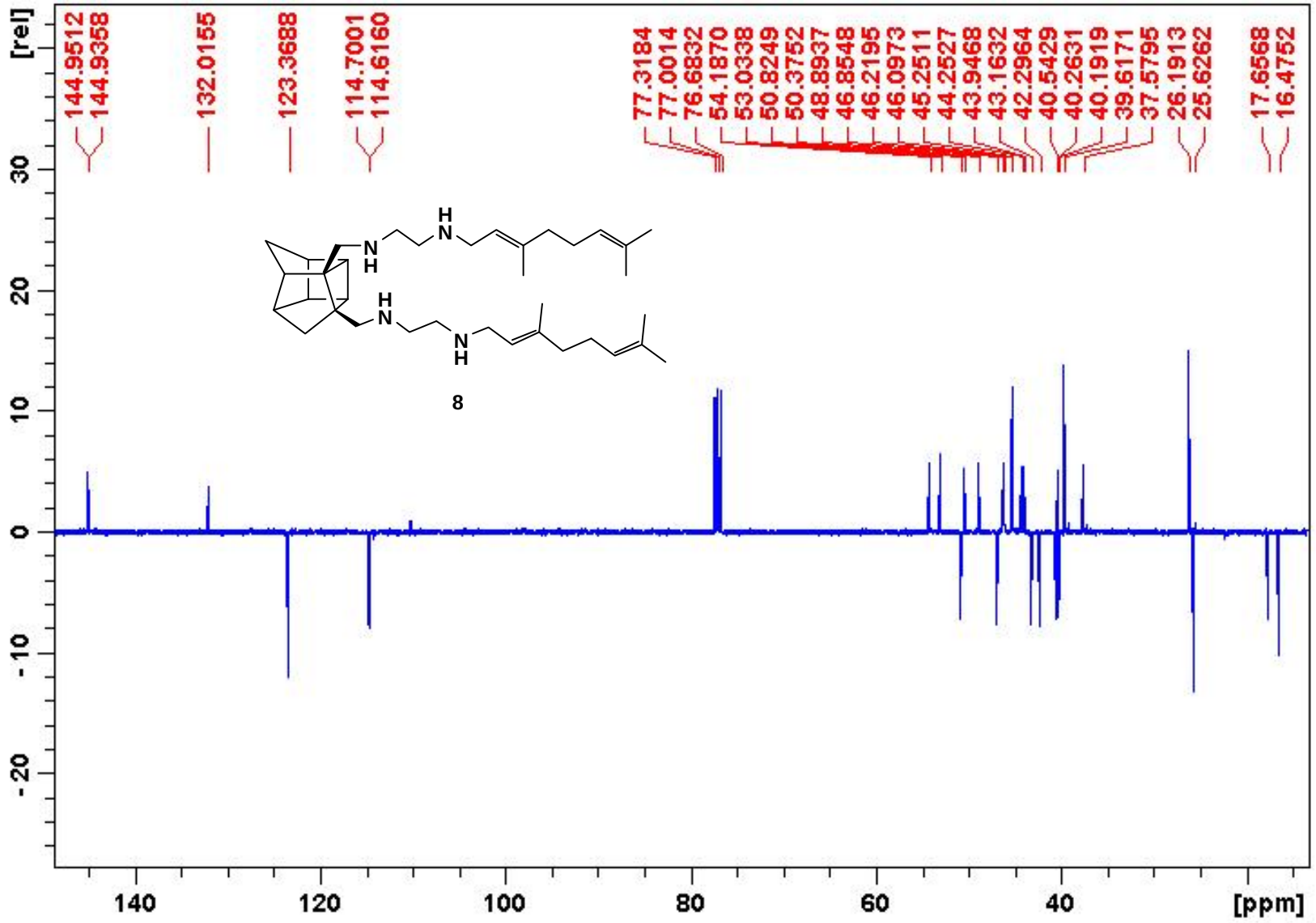


IR spectrum of [*N*′,*N*′-(tricyclo[3.3.1.1^{3,7}]decane-1,3-diethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (7)


[rel]
8
6
4
2
0
7
6
5
4
3
2
[ppm]
1.9390
2.0163
3.9179
9.8571
2.0136
3.0255
1.1977
1.9443
8.1611
1.0000
12.5904
6.6073
2.0425

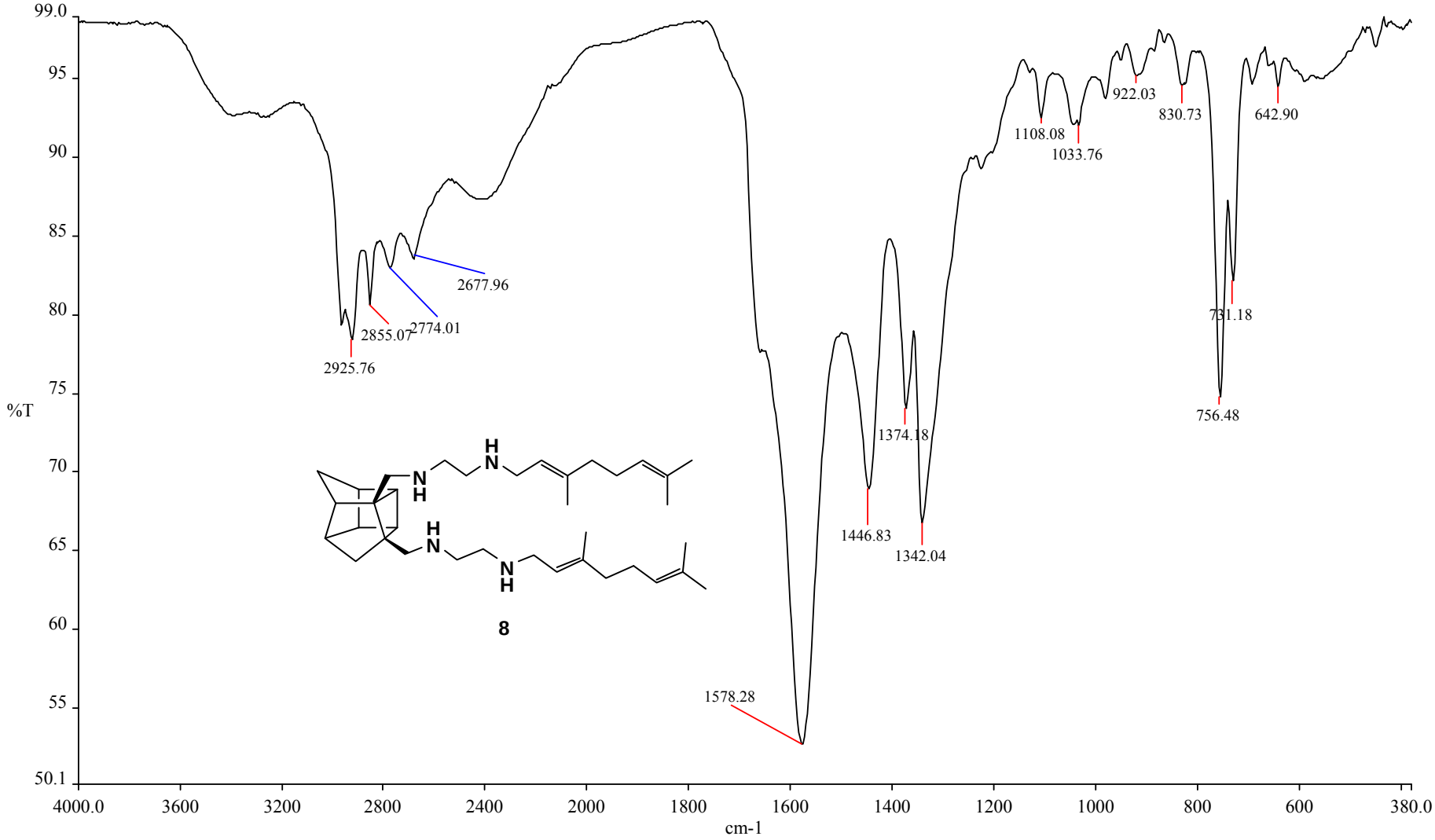
8

^{1}H NMR spectrum of [*N*′,*N*′-(pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dimethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (8)



8

^{13}C NMR spectrum of [*N*′,*N*′-(pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dimethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (8)



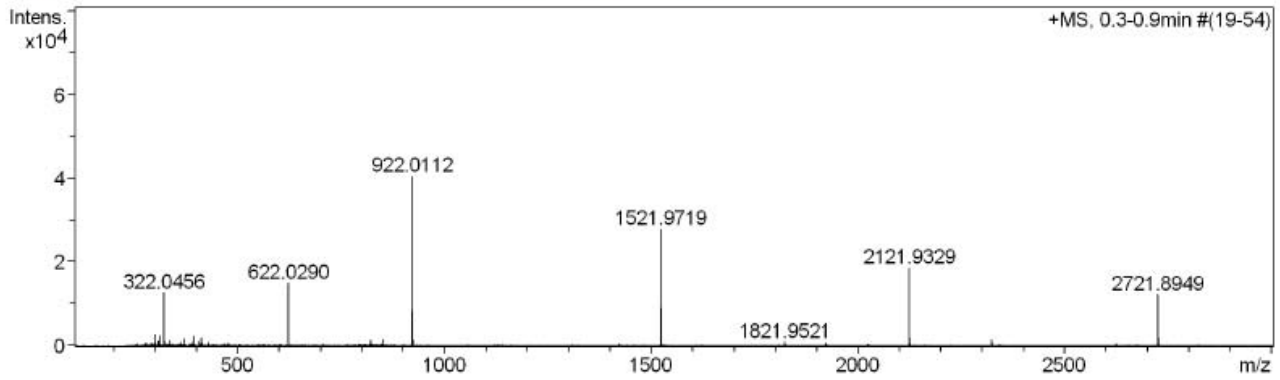
IR spectrum of [*N*′,*N*′-(pentacyclo[5.3.0.0^{2,5}.0^{3,9}.0^{4,8}]decane-2,5-dimethylene)]-bis[(*N*′′-(*E*)-3,7-dimethylocta-2,6-dienyl)ethane-1,2-diamine] (8)

Analysis Info

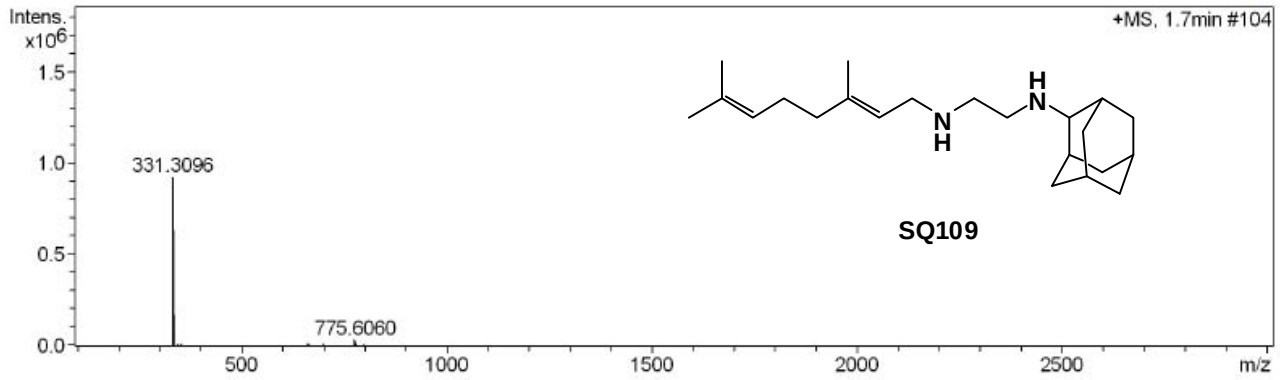
Analysis Name	D:\Data\kenny\SQ109000001.d	Acquisition Date	9/10/2009 6:43:51 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	SQ109	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev



SQ109

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
331.3096														
	1	C 22 H 39 N 2	331.3108	3.5	4.2	4.5	ok	even	40.38	0.0604	0.0015	0.0247	0.0010	0.8427
	2	C 17 H 39 N 4 O 2	331.3068	-8.7	-7.9	0.5	ok	even	67.58	0.1055	0.0027	0.0424	0.0009	0.9392

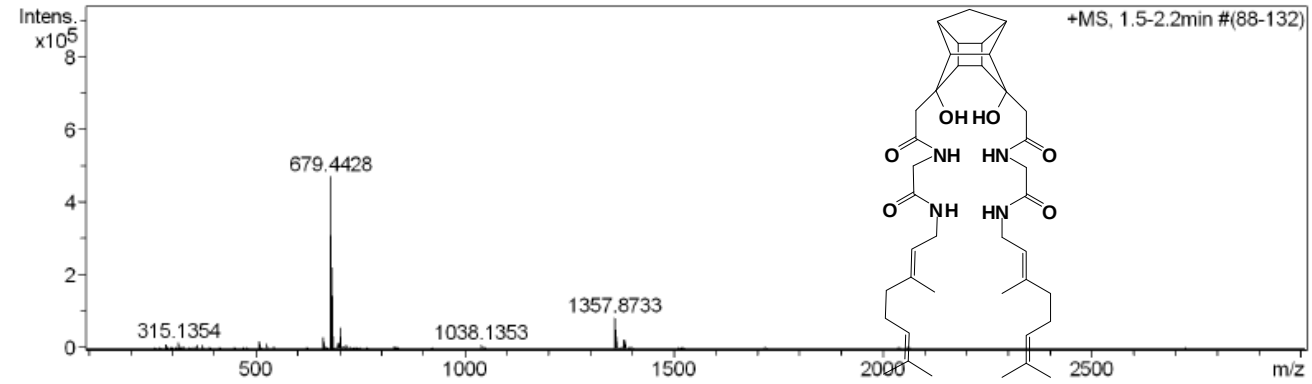
HRMS spectrum of SQ109

Analysis Info

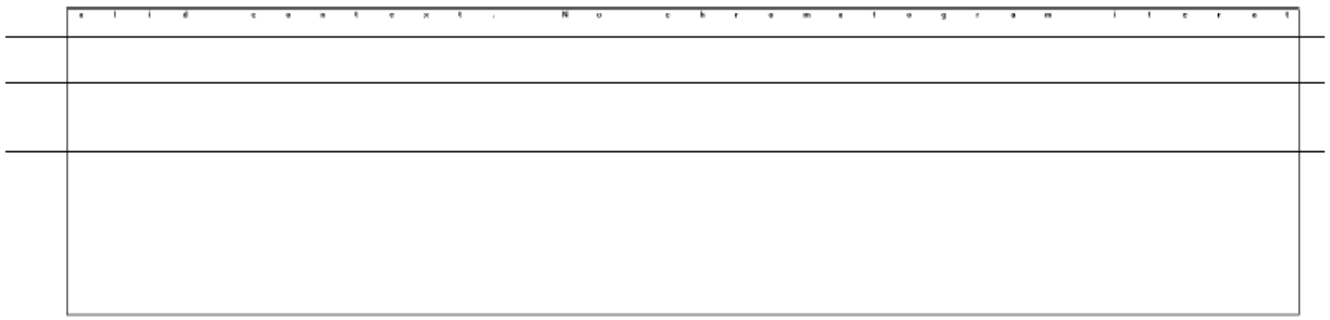
Analysis Name	D:\Data\kenny\GGKM8PD-R000001.d	Acquisition Date	6/13/2009 7:32:34 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GGKM8PD-R	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
679.4428														
	1	C 40 H 55 N 8 O 2	679.4442	2.1	3.1	17.5	ok	even	3.16	0.0050	0.0023	0.0021	0.0018	0.8115
	2	C 39 H 59 N 4 O 6	679.4429	0.2	1.2	12.5	ok	even	13.56	0.0175	0.0013	0.0055	0.0018	0.8001
	3	C 36 H 51 N 14	679.4416	-1.8	-0.9	18.5	ok	even	15.43	0.0233	0.0011	0.0084	0.0018	0.8273
	4	C 38 H 63 O 10	679.4416	-1.8	-0.7	7.5	ok	even	23.97	0.0302	0.0011	0.0096	0.0019	0.8432
	5	C 35 H 55 N 10 O 4	679.4402	-3.8	-2.8	13.5	ok	even	26.28	0.0364	0.0021	0.0118	0.0018	0.9207



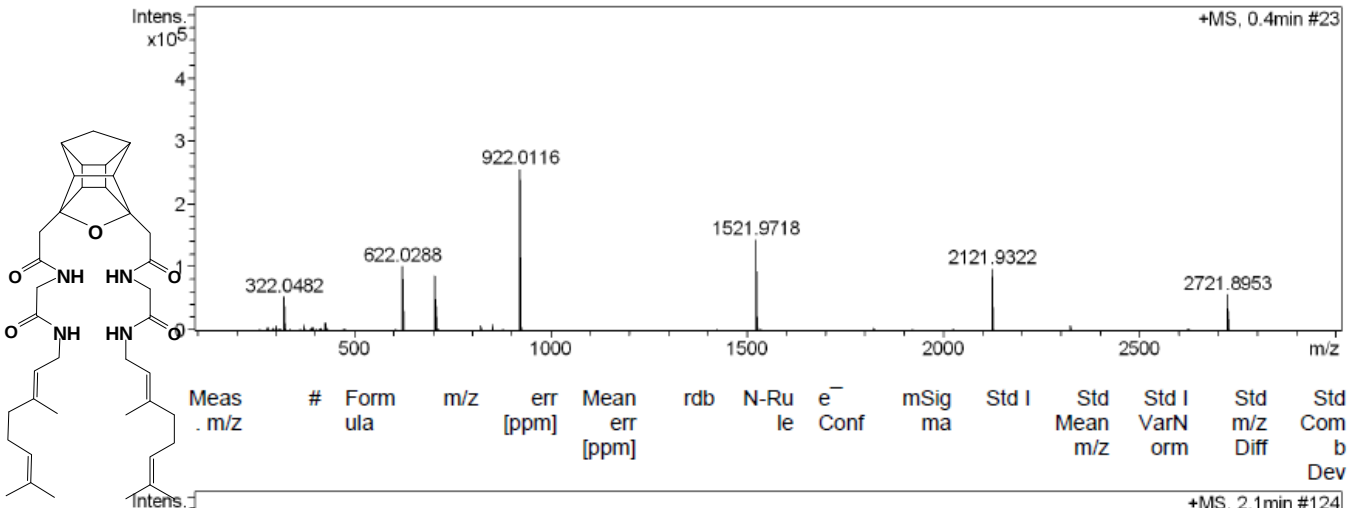
HRMS spectrum compound 26

Analysis Info

Analysis Name	D:\Data\kenny\GGKM8PE-R000001.d	Acquisition Date	6/13/2009 7:24:17 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GGKM8PE-R	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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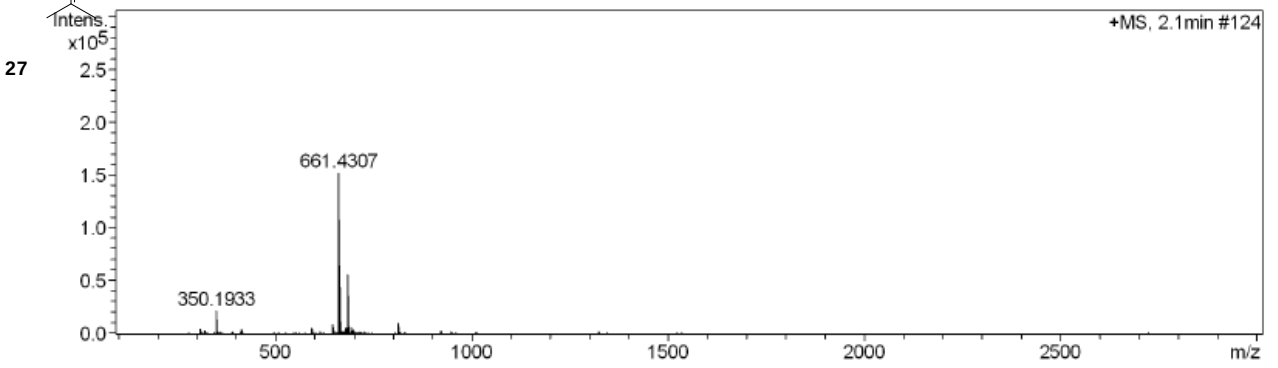
Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.4307														
	1	C 38 H 61 O 9	661.4310	0.4	0.9	8.5	ok	even	5.25	0.0065	0.0009	0.0021	0.0014	0.5846
	2	C 35 H 53 N 10 O 3	661.4297	-1.6	-1.2	14.5	ok	even	6.79	0.0116	0.0010	0.0046	0.0014	0.6934
	3	C 39 H 57 N 4 O 5	661.4323	2.5	2.9	13.5	ok	even	7.49	0.0096	0.0020	0.0030	0.0014	0.7340
	4	C 34 H 57 N 6 O 7	661.4283	-3.6	-3.2	9.5	ok	even	17.24	0.0249	0.0022	0.0079	0.0014	0.8439
	5	C 31 H 49 N 16 O	661.4270	-5.7	-5.3	15.5	ok	even	20.02	0.0330	0.0036	0.0120	0.0014	0.9235

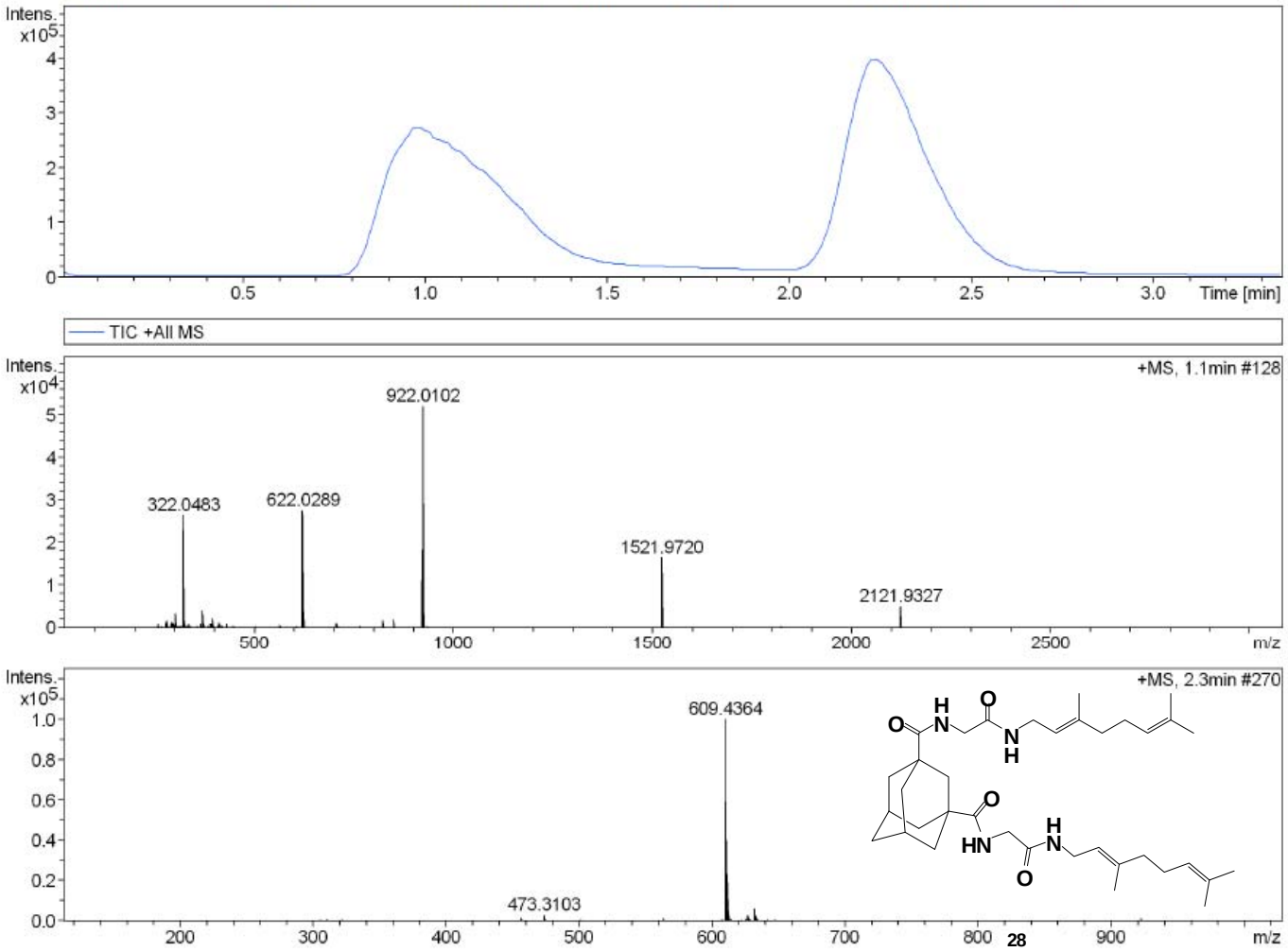
HRMS spectrum compound 27

Analysis Info

Analysis Name	D:\Data\kenny\1,3 ADAMANTYL CO TETRACARBONYL GERANYL000001.d	Acquisition Date	7/30/2010 10:25:30 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	1,3 ADAMANTYL CO TETRACARBONYL GERANYL	Instrument / Ser#	micrOTOF-Q 10139
Comment	MASS = 609		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
609.4364														
	1	C 36 H 57 N 4 O 4	609.4374	1.7	0.9	10.5	ok	even	54.65	0.0851	0.0017	0.0376	0.0030	0.8041
	2	C 35 H 61 O 8	609.4361	-0.5	-1.3	5.5	ok	even	55.44	0.0894	0.0018	0.0378	0.0030	0.8111
	3	C 37 H 53 N 8	609.4388	3.9	3.0	15.5	ok	even	56.58	0.0838	0.0025	0.0379	0.0030	0.8427

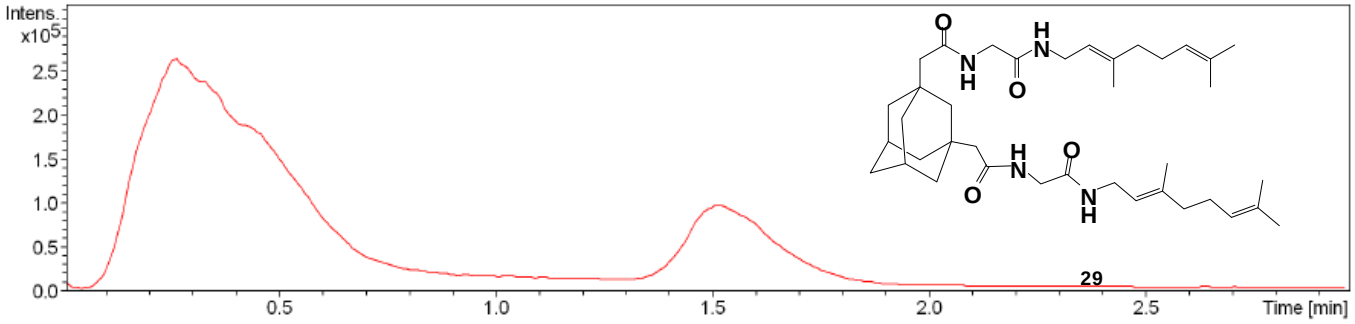
HRMS spectrum compound 28

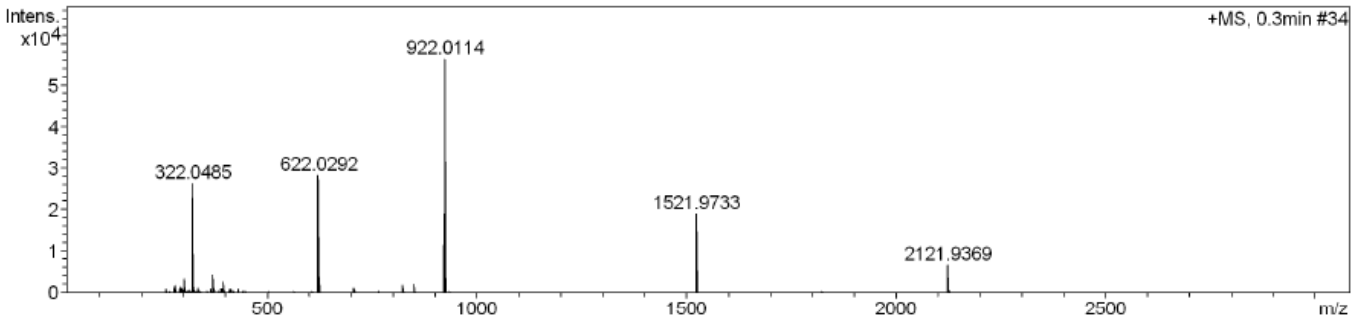
Analysis Info

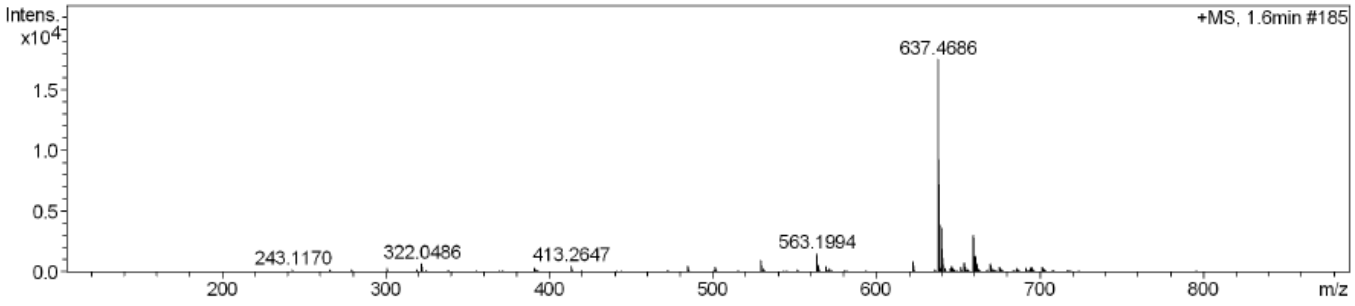
Analysis Name	D:\Data\kenny\1,3 ADAMANTYL CH2 TETRACARBONYL GERANYL000001.d	Acquisition Date	7/30/2010 10:49:04 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	1,3 ADAMANTYL CH2 TETRACARBONYL GERANYL	Instrument / Ser#	micrOTOF-Q 10139
Comment	MASS = 638		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source







Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
637.4686														
	1	C 38 H 61 N 4 O 4	637.4687	0.2	-0.5	10.5	ok	even	59.51	0.0899	0.0016	0.0403	0.0030	0.8071
	2	C 37 H 65 O 8	637.4674	-1.9	-2.5	5.5	ok	even	59.69	0.0933	0.0023	0.0404	0.0030	0.8419
	3	C 39 H 57 N 8	637.4701	2.3	1.6	15.5	ok	even	61.83	0.0890	0.0019	0.0407	0.0030	0.8234

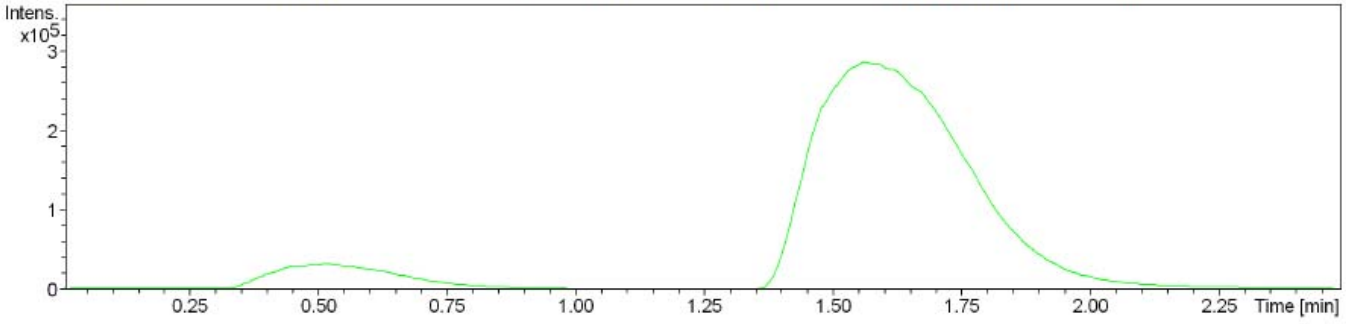
HRMS spectrum compound 29

Analysis Info

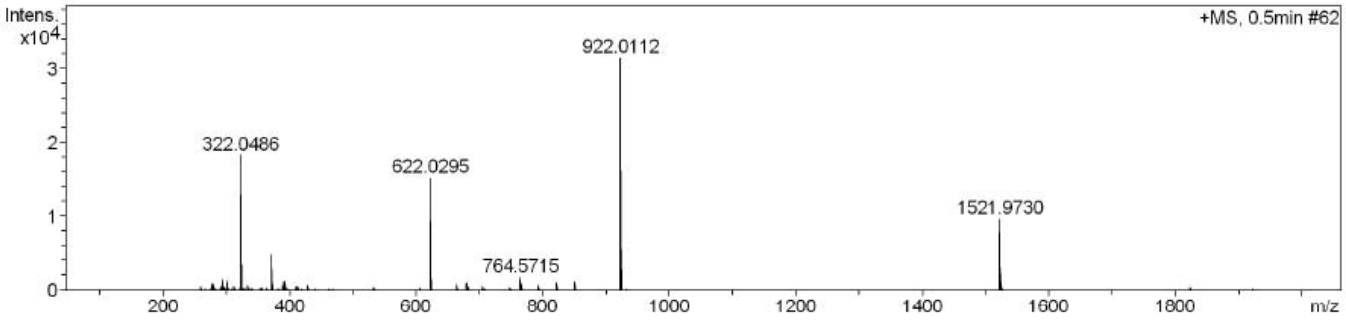
Analysis Name	D:\Data\kenny\GKM-K1000001.d	Acquisition Date	8/10/2010 7:23:43 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	THIELE TETRACARBONYL EDA GERANYL	Instrument / Ser#	micrOTOF-Q 10139
Comment	MASS = 603		

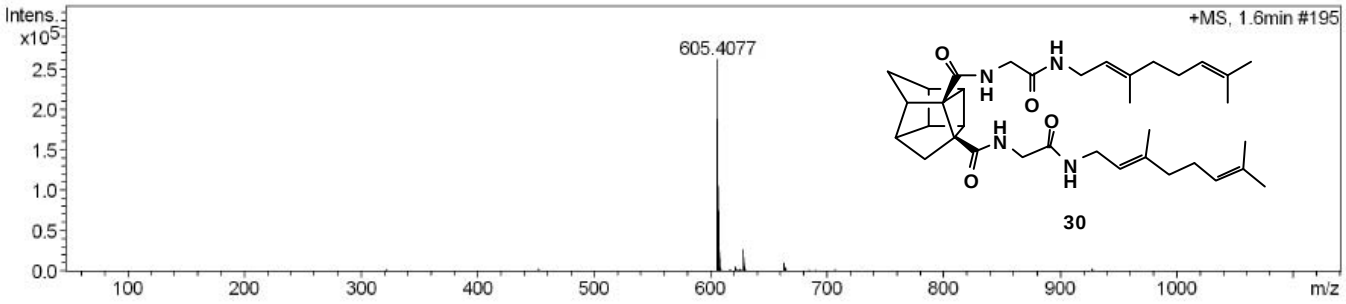
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



BPC 98.9949-2001.0439 +All MS





30

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
605.4077														
	1	C 36 H 53 N 4 O 4	605.4061	-2.6	-2.0	12.5	ok	even	6.65	0.0086	0.0013	0.0037	0.0011	0.8312
	2	C 35 H 57 O 8	605.4048	-4.8	-4.1	7.5	ok	even	7.88	0.0124	0.0026	0.0041	0.0012	0.9103
	3	C 37 H 49 N 8	605.4075	-0.4	0.2	17.5	ok	even	18.54	0.0230	0.0006	0.0080	0.0011	0.8519

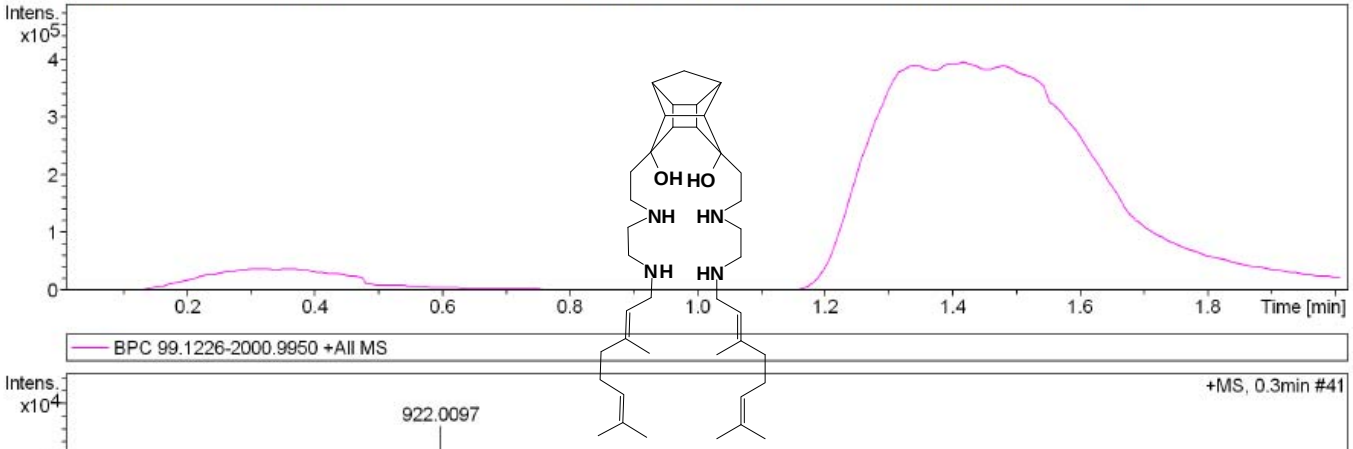
HRMS spectrum compound 30

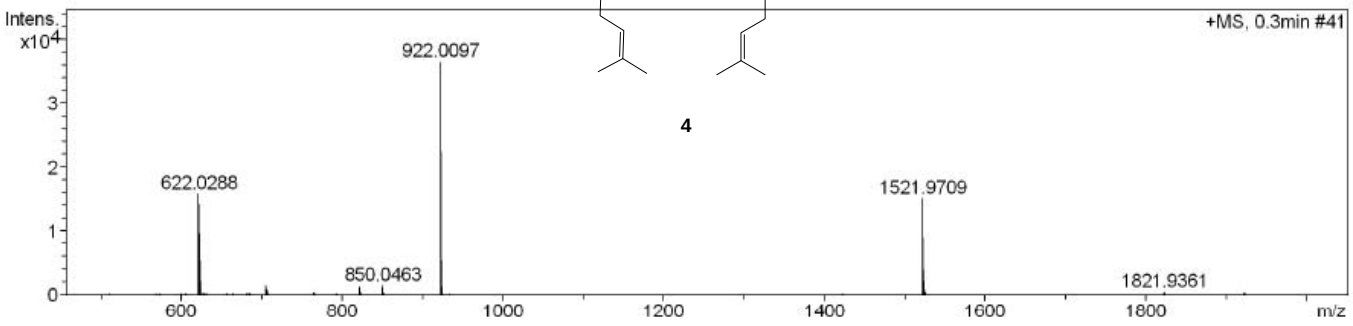
Analysis Info

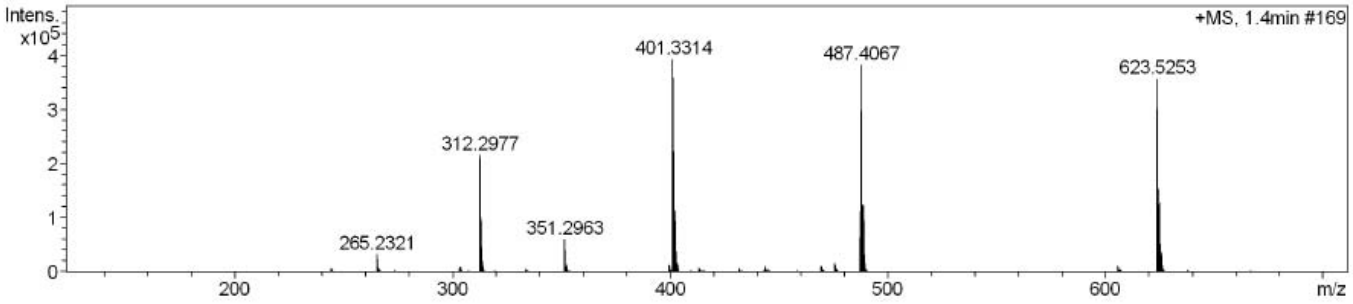
Analysis Name	D:\Data\kenny\623-GKM9PD.d	Acquisition Date	8/12/2010 1:57:03 AM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	623-GKM9PD	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	500 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	1000.0 Vpp	Set Divert Valve	Source







4

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
623.5253														
	1	C 38 H 71 O 6	623.5245	-1.2	-0.2	3.5	ok	even	5.24	0.0065	0.0009	0.0024	0.0020	0.7648
	2	C 35 H 63 N 10	623.5232	-3.4	-2.5	9.5	ok	even	7.56	0.0125	0.0018	0.0050	0.0020	0.9055
	3	C 39 H 67 N 4 O 2	623.5259	0.9	1.9	8.5	ok	even	7.62	0.0098	0.0015	0.0031	0.0020	0.8427

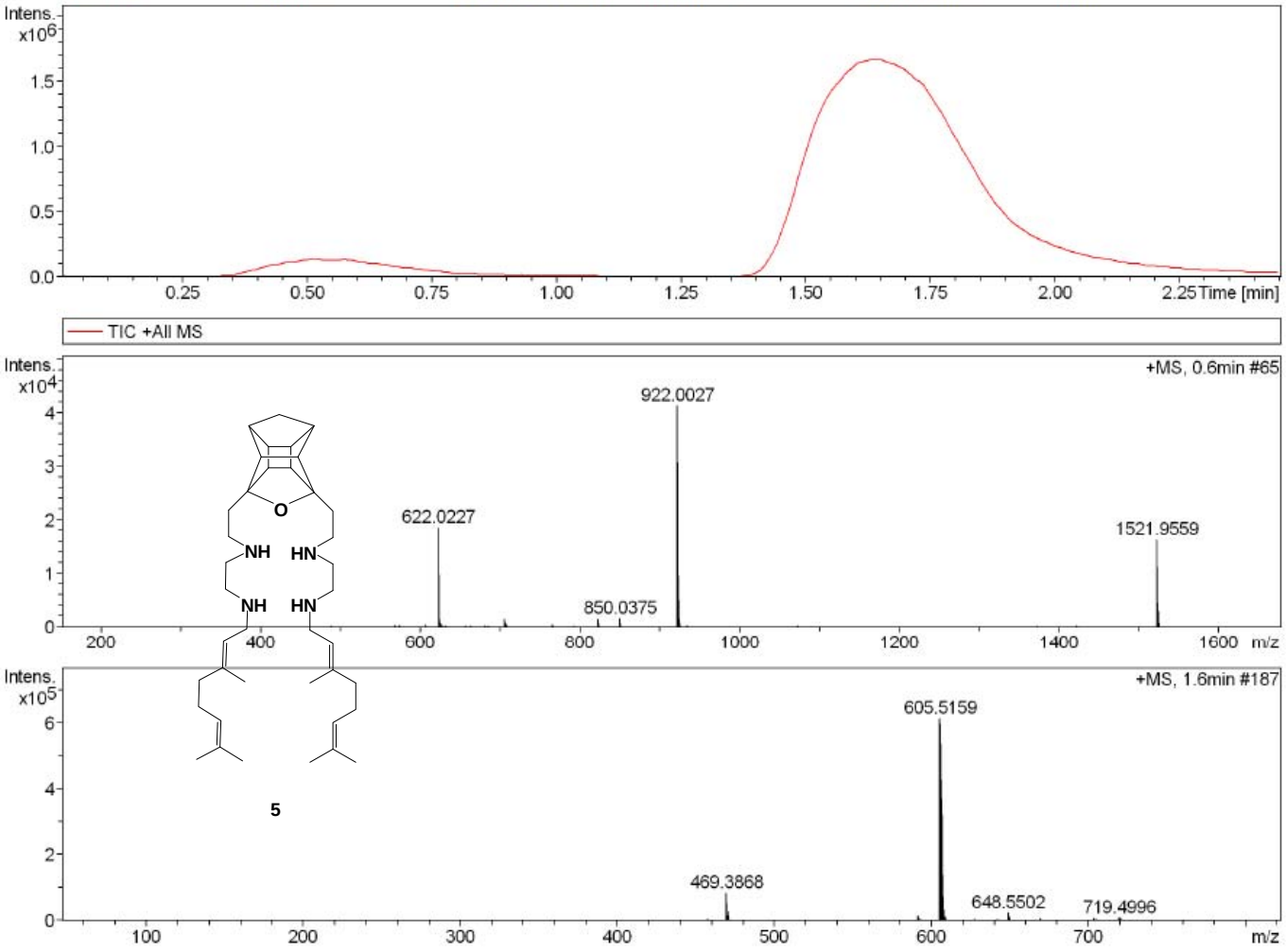
HRMS spectrum compound 4

Analysis Info

Analysis Name	D:\Data\kenny\605-GKM9PE.d	Acquisition Date	8/12/2010 2:00:07 AM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	605-GKM9PE	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set Collision Cell RF	1000.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
605.5159														
	1	C 39 H 65 N 4 O	605.5153	-1.0	1.9	9.5	ok	even	80.98	0.0938	0.0025	0.0293	0.0042	0.7935
	2	C 38 H 69 O 5	605.5140	-3.2	-0.3	4.5	ok	even	93.27	0.1089	0.0022	0.0341	0.0042	0.7958
	3	C 29 H 61 N 14	605.5198	6.4	9.3	6.5	ok	even	119.55	0.1512	0.0060	0.0473	0.0041	0.9301

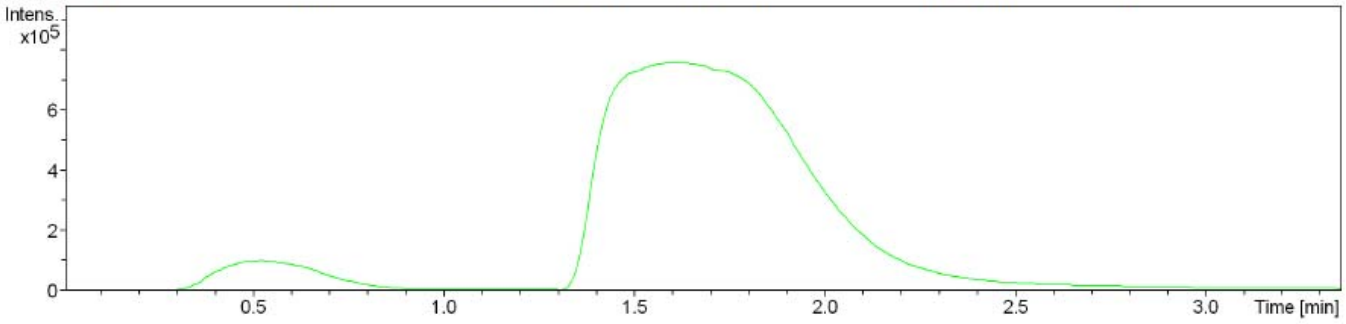
HRMS spectrum compound 5

Analysis Info

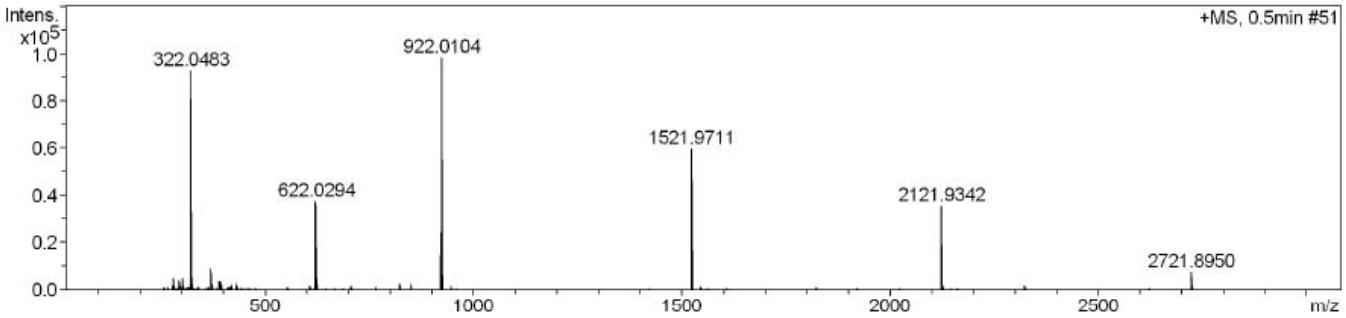
Analysis Name	D:\Data\kenny\GKM-KA-553000001.d	Acquisition Date	8/20/2010 10:55:03 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM-KA-553	Instrument / Ser#	micrOTOF-Q 10139
Comment	1,3 ADAMATYL EDA GERANYL		

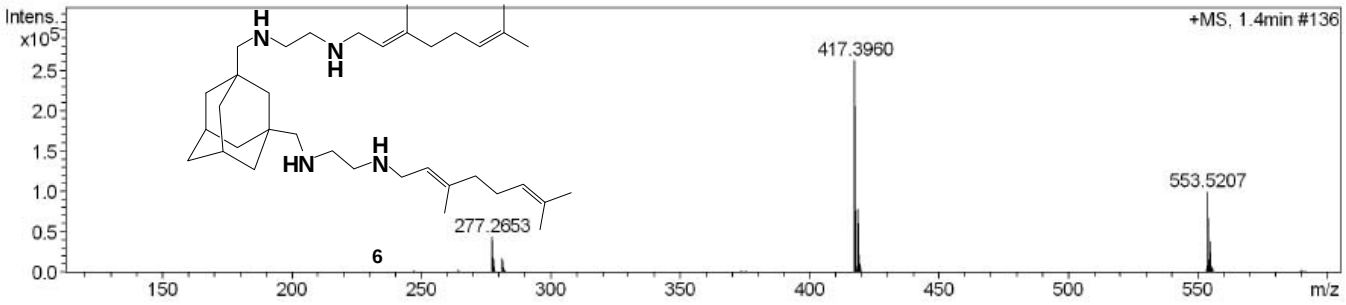
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



BPC 98.9942-3001.0640 +All MS





Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
553.5207														
	1	C 35 H 69 O 4	553.5190	-2.9	-2.5	1.5	ok	even	6.97	0.0117	0.0014	0.0053	0.0009	0.9563
	2	C 36 H 65 N 4	553.5204	-0.5	-0.1	6.5	ok	even	12.98	0.0209	0.0004	0.0071	0.0009	0.8731

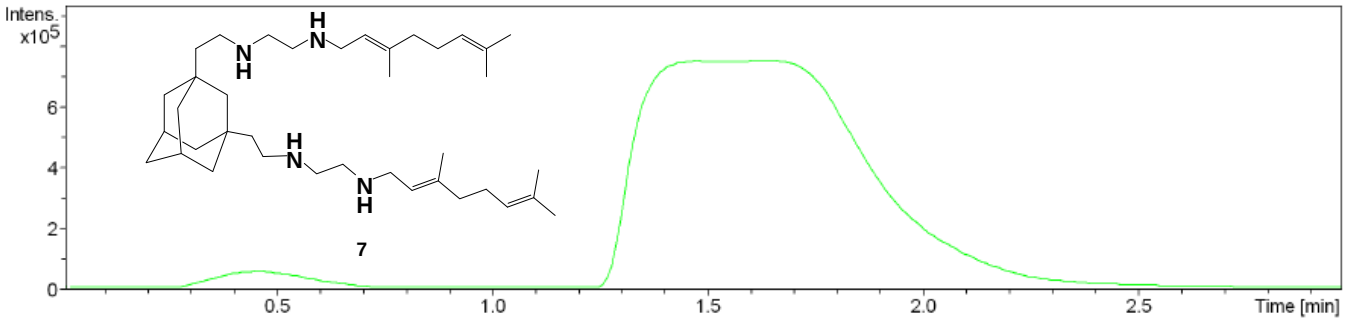
HRMS spectrum compound 6

Analysis Info

Analysis Name	D:\Data\kenny\GKM-KB (581).d	Acquisition Date	8/23/2010 4:05:59 AM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM-KB	Instrument / Ser#	micrOTOF-Q 10139
Comment	MASS = 581, 1,3 ADAMANTYL (CH2)2 DIEDA GERANYL		

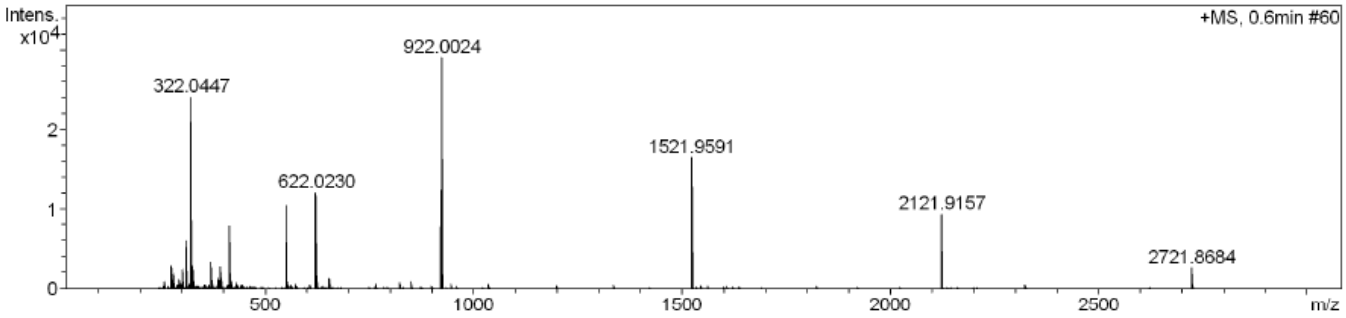
Acquisition Parameter

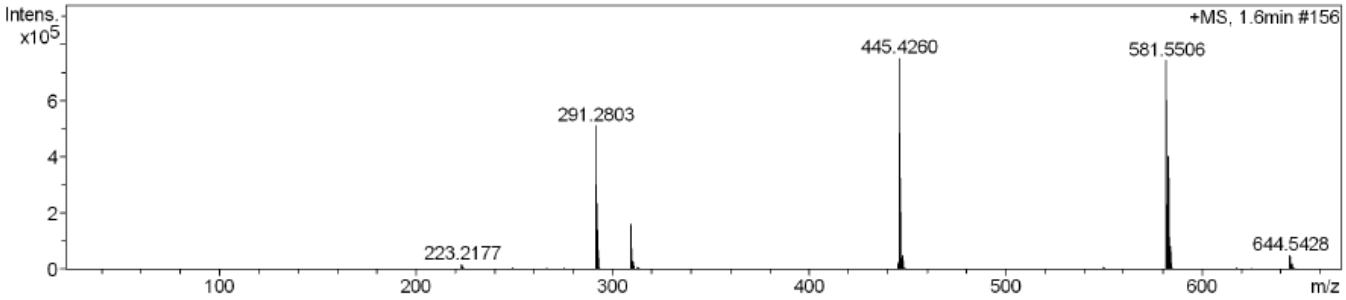
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



7

BPC 99.0000-3001.0000 +All MS





Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
581.5506														
	1	C 38 H 69 N 4	581.5517	1.9	3.4	6.5	ok	even	56.12	0.0673	0.0023	0.0210	0.0022	0.8196
	2	C 37 H 73 O 4	581.5503	-0.4	1.1	1.5	ok	even	68.71	0.0835	0.0013	0.0262	0.0022	0.7863
	3	C 33 H 69 N 6 O 2	581.5477	-5.0	-3.5	2.5	ok	even	80.41	0.1019	0.0023	0.0317	0.0022	0.8654

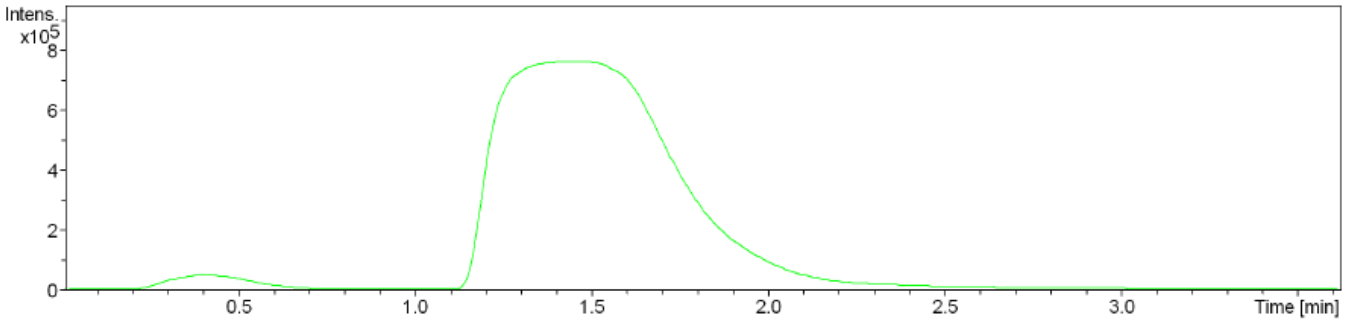
HRMS spectrum compound 7

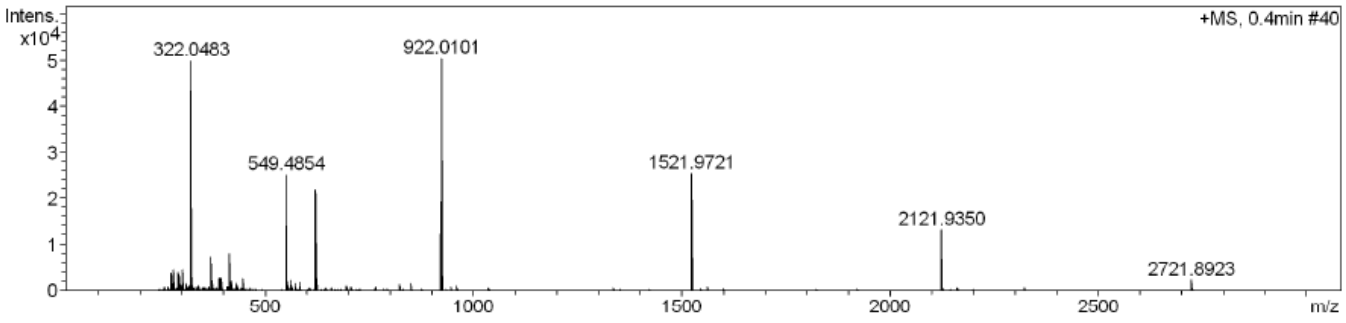
Analysis Info

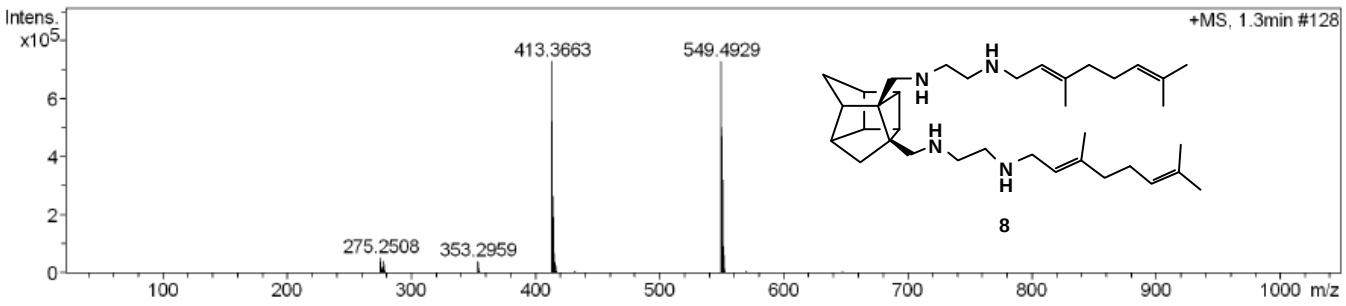
Analysis Name	D:\Data\kenny\GKM-KT (547).d	Acquisition Date	8/23/2010 4:11:20 AM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	GKM-KT	Instrument / Ser#	micrOTOF-Q 10139
Comment	MASS = 587, THIELE CH2 DIEDA GERANYL		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source







8

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
549.4929														
	1	C 36 H 61 N 4	549.4891	-7.0	-4.3	8.5	ok	even	118.51	0.1367	0.0029	0.0425	0.0031	0.8328
	2	C 30 H 61 N 8 O	549.4963	6.2	8.8	4.5	ok	even	144.98	0.1774	0.0051	0.0550	0.0031	0.9136
	3	C 25 H 61 N 10 O 3	549.4923	-1.2	1.4	0.5	ok	even	194.90	0.2157	0.0018	0.0948	0.0030	0.8723

HRMS spectrum compound 8

CHAPTER 9

SYNTHESIS AND NMR ASSIGNMENT OF PENTACYCLOUNDECANE PRECURSORS OF POTENTIAL PHARMACEUTICAL AGENTS

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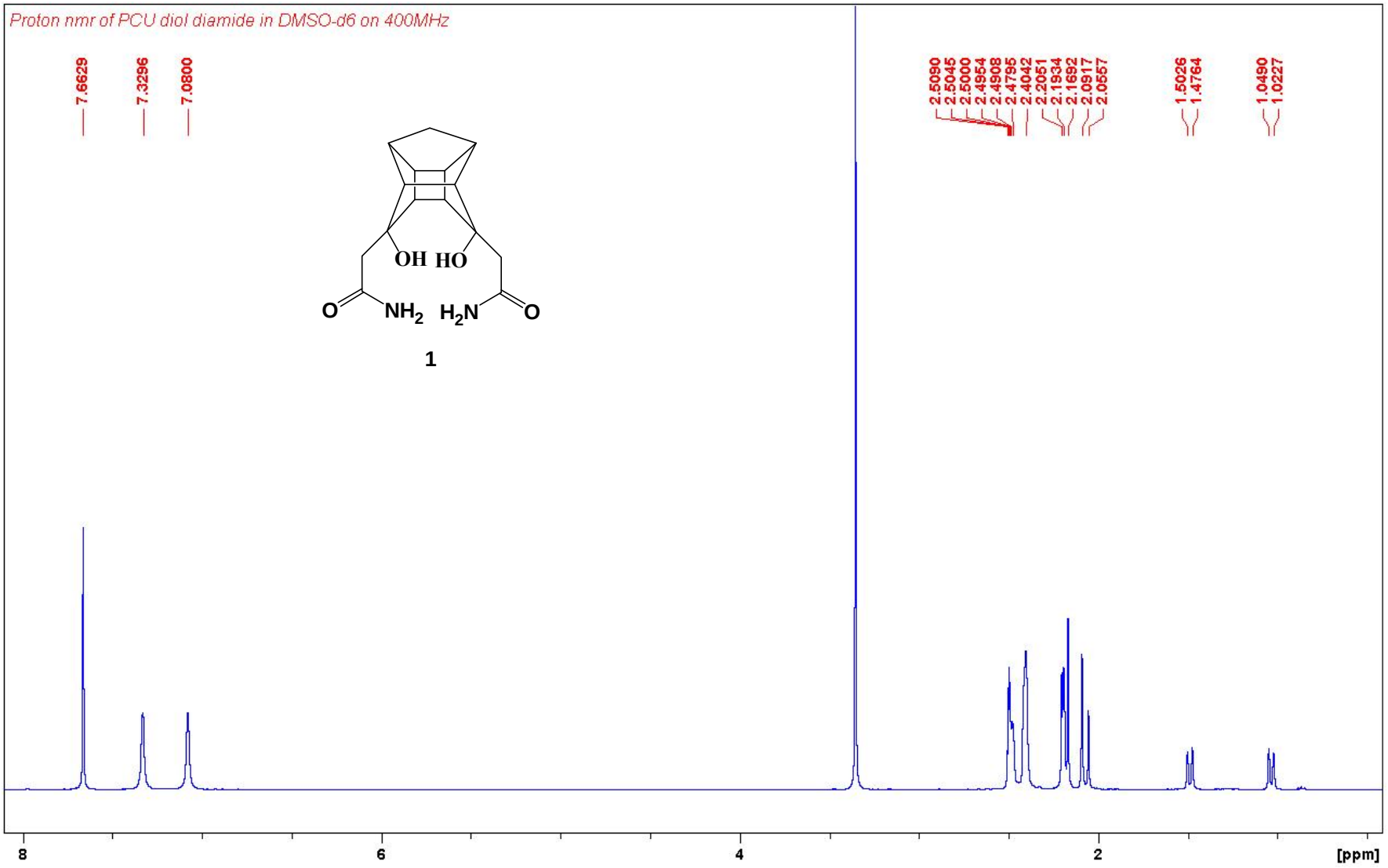
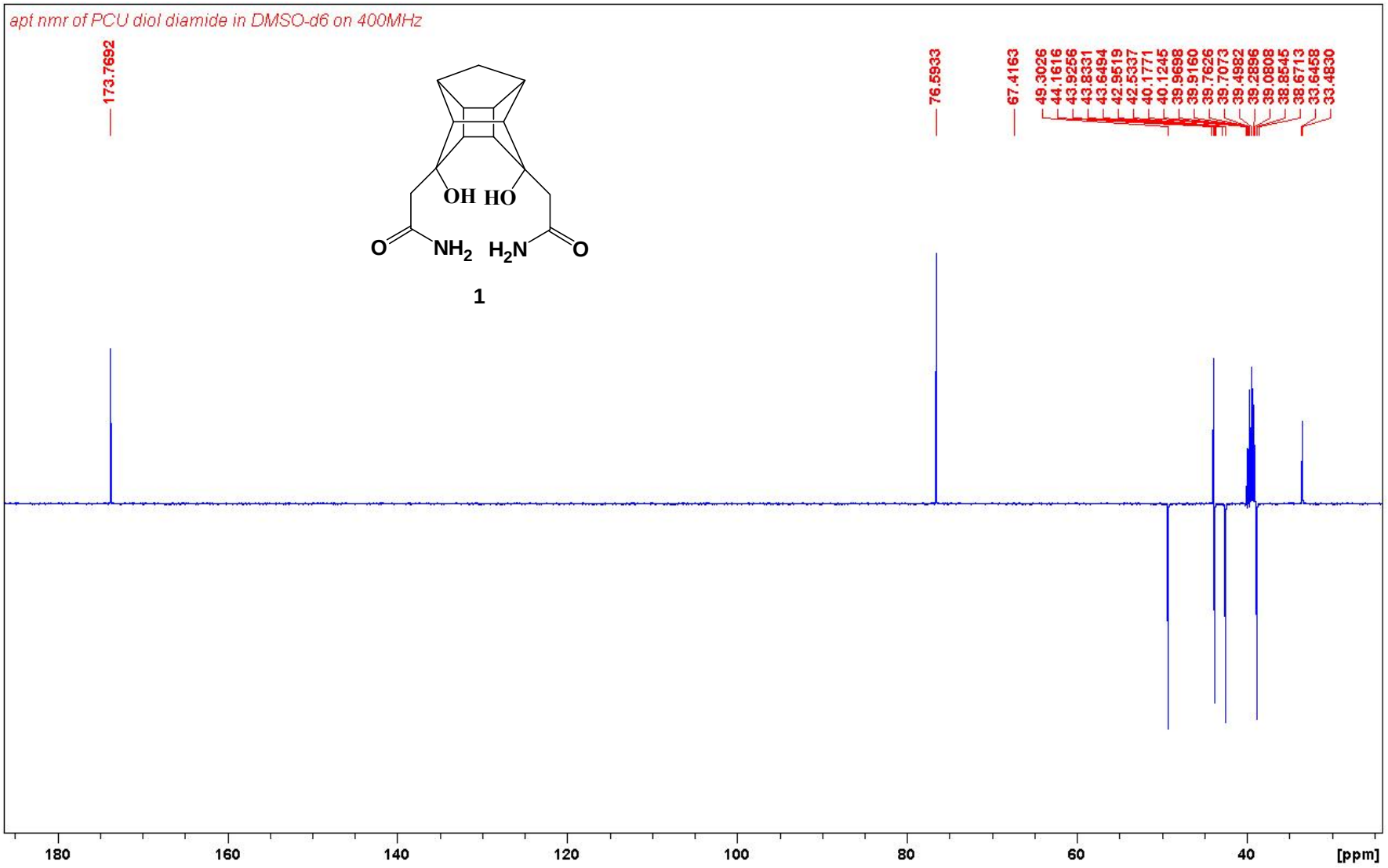
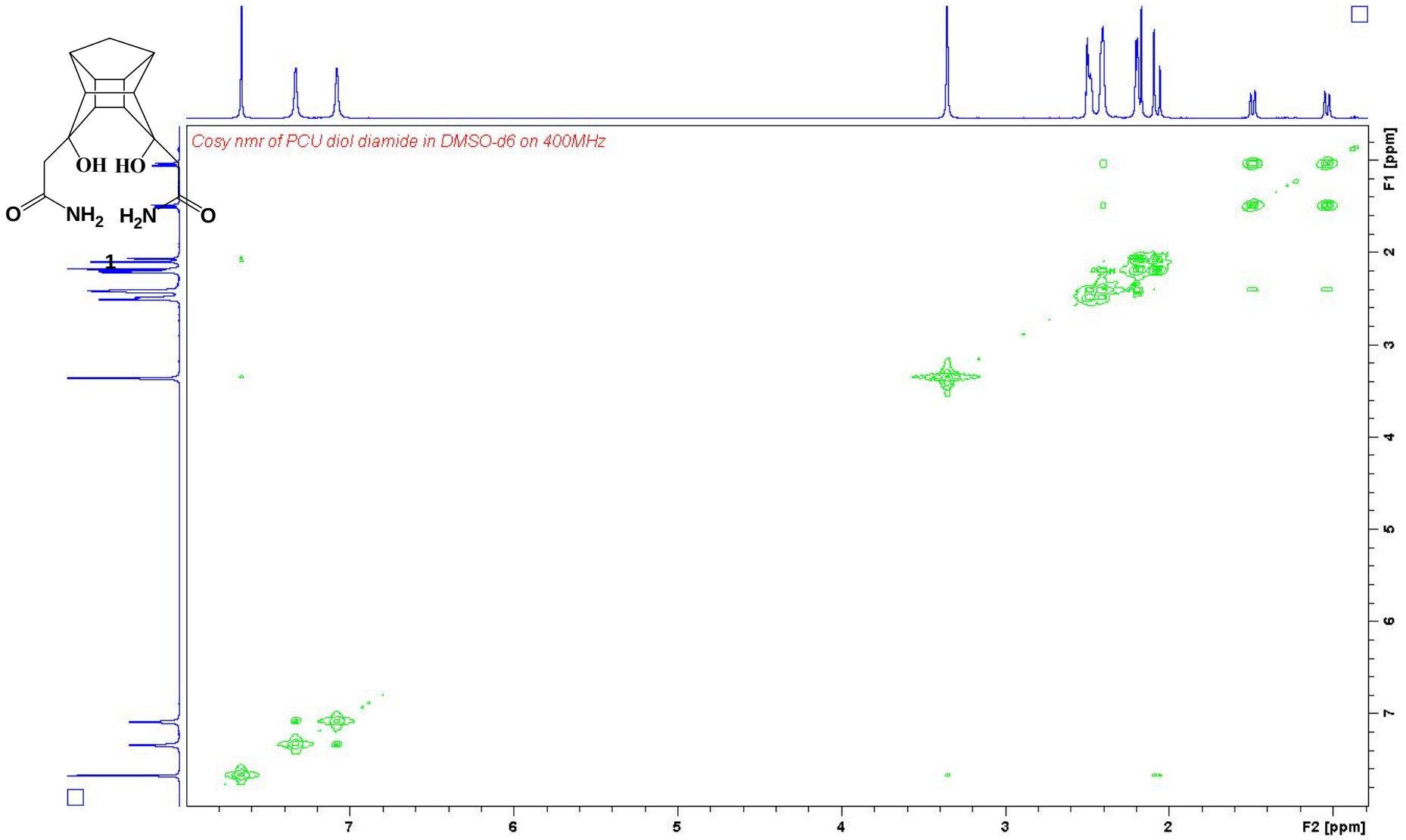
Corresponding author: *Email: kruger@ukzn.ac.za, Fax: +27-31-2603091



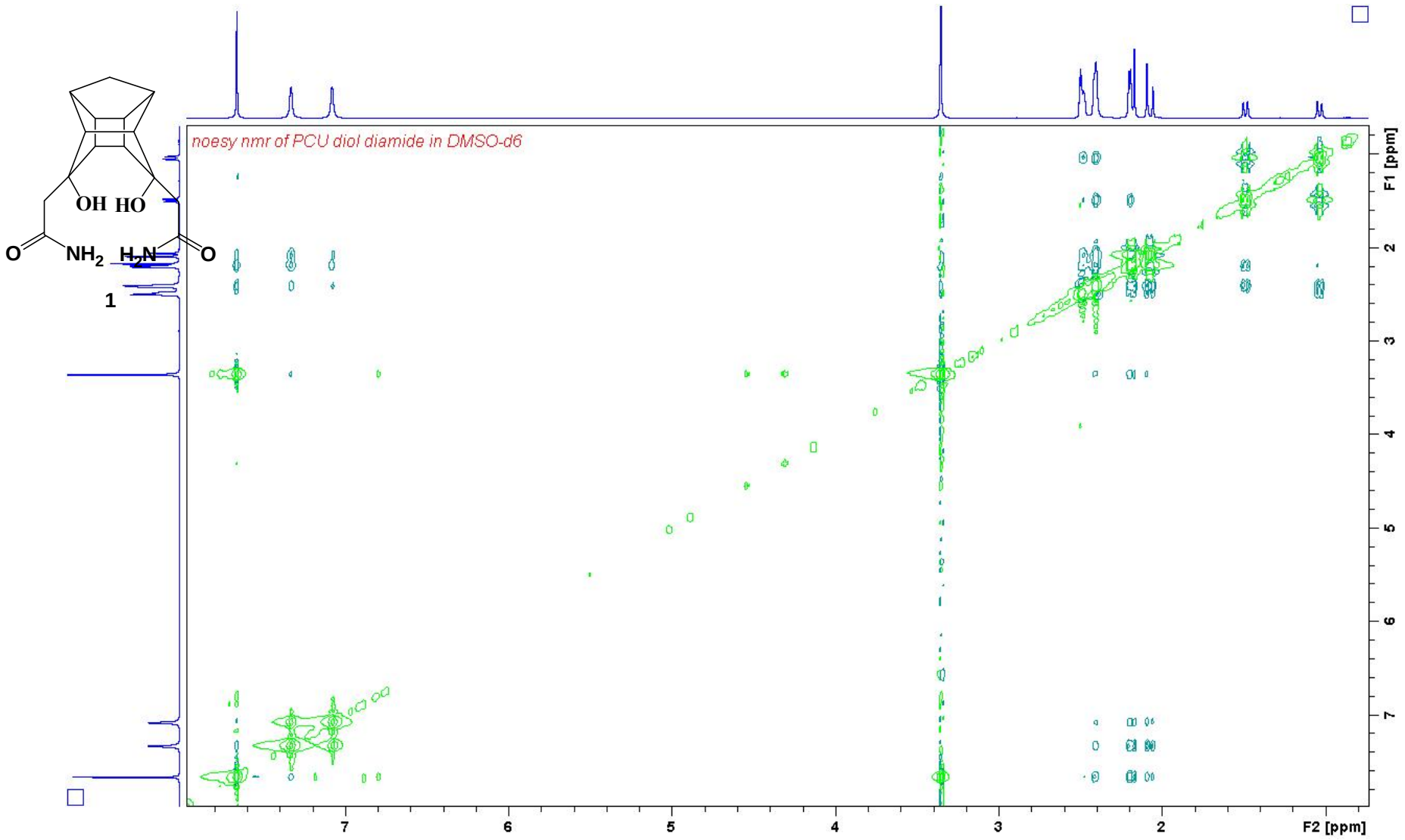
^{1}H spectrum of compound 1 in $(CD_3)_2SO$



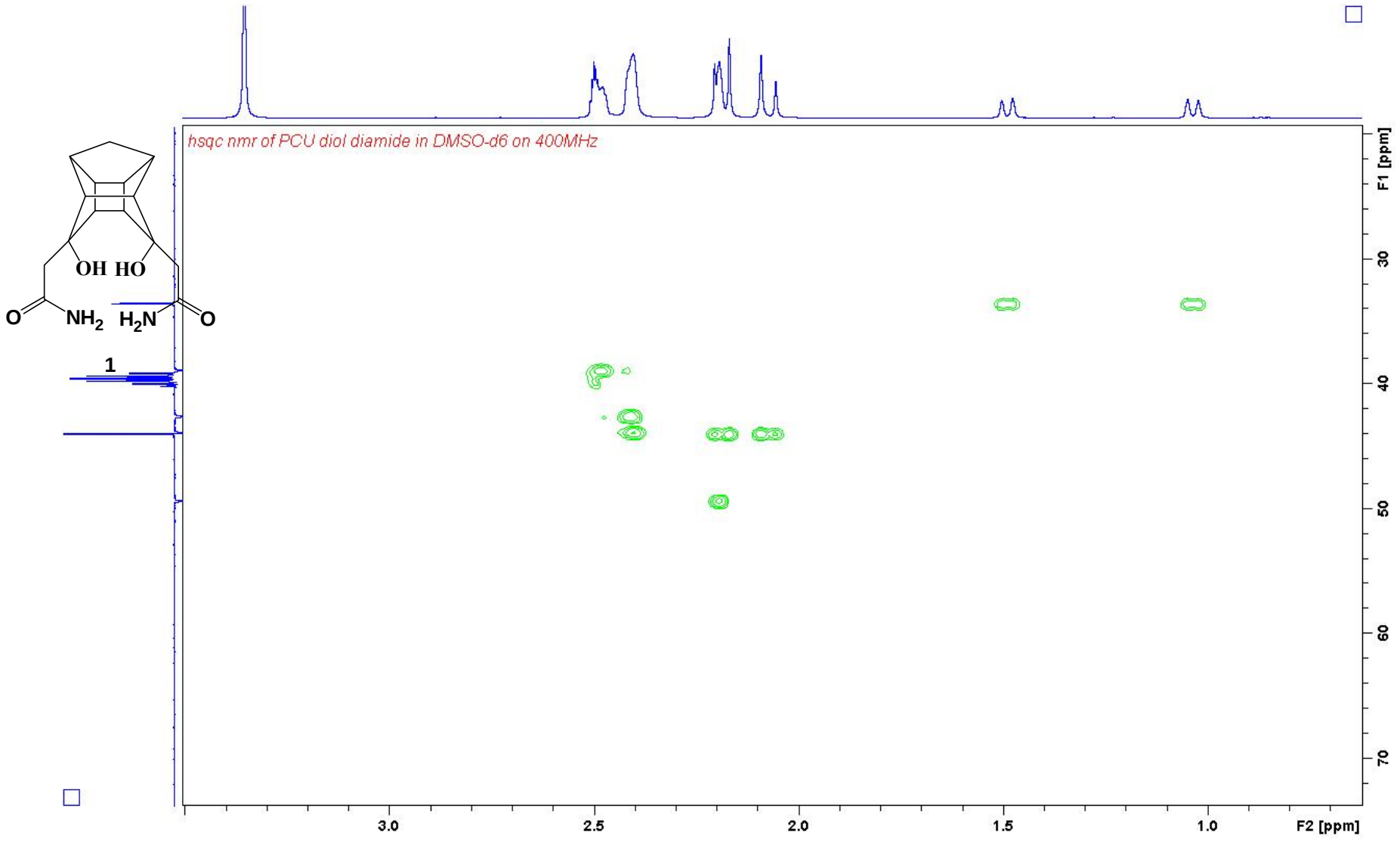
^{13}C spectrum of compound 1 in $(CD_3)_2SO$



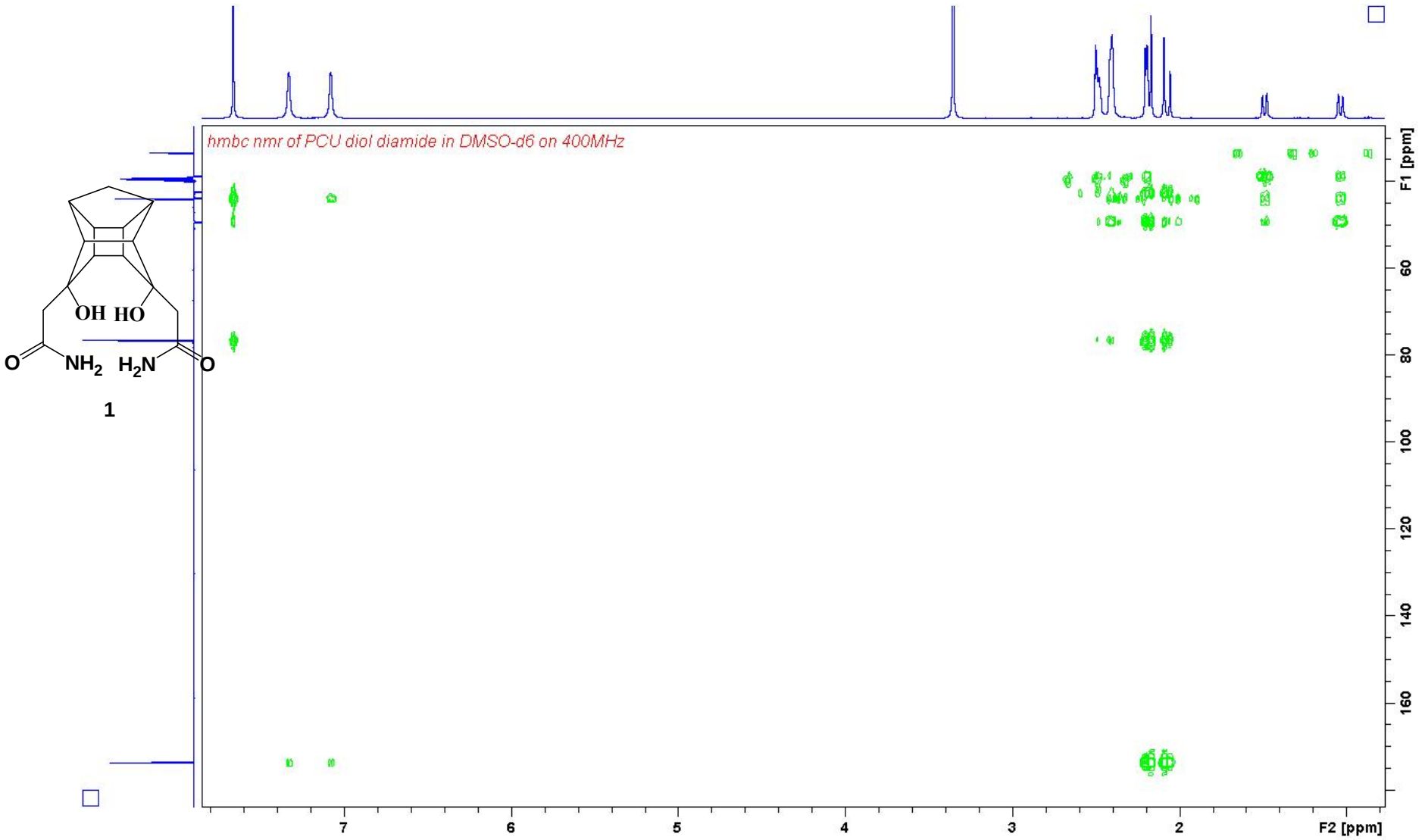
COSY spectrum of compound 1 in $(CD_3)_2SO$



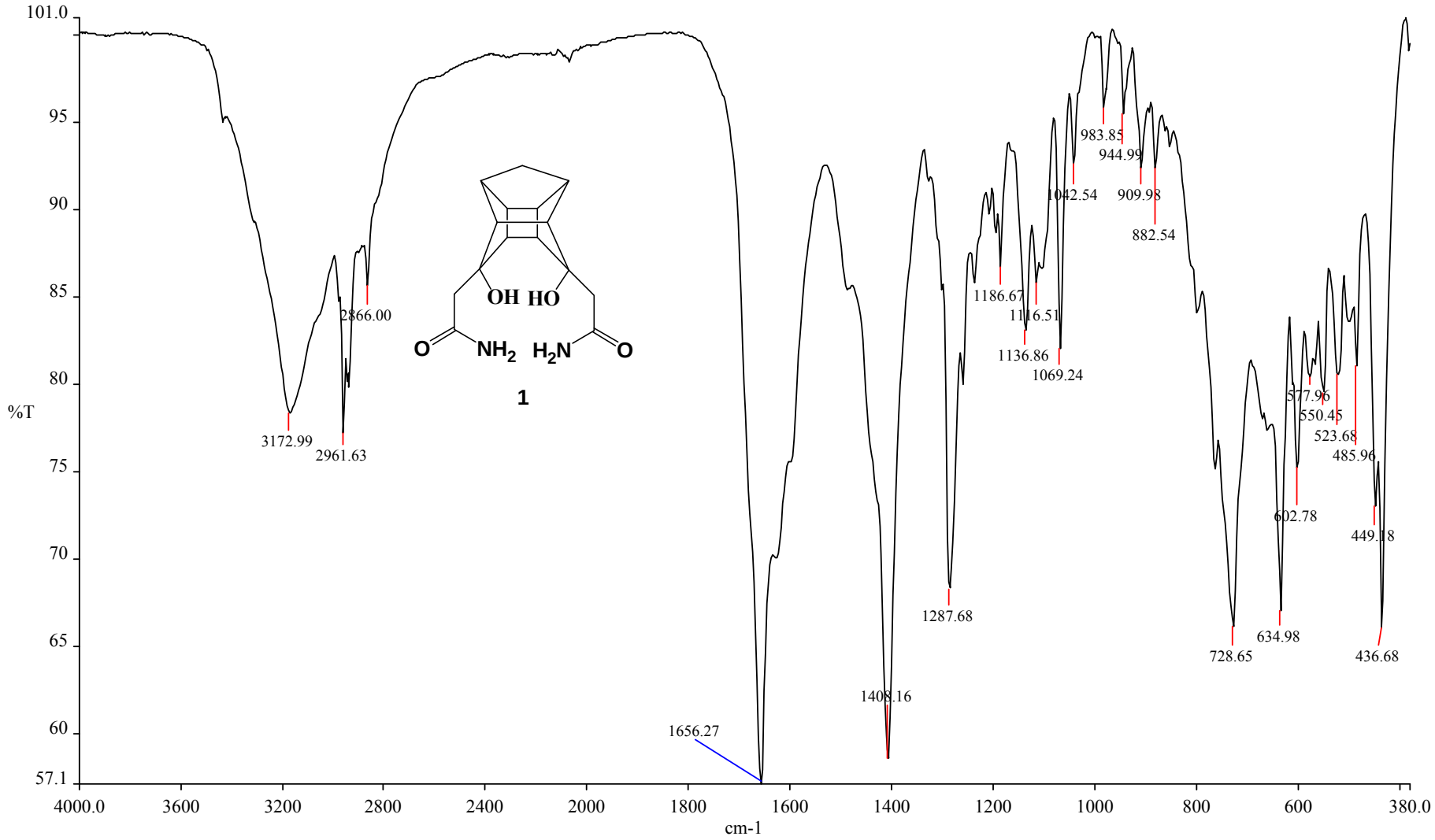
NOESY spectrum of compound 1 in $(CD_3)_2SO$



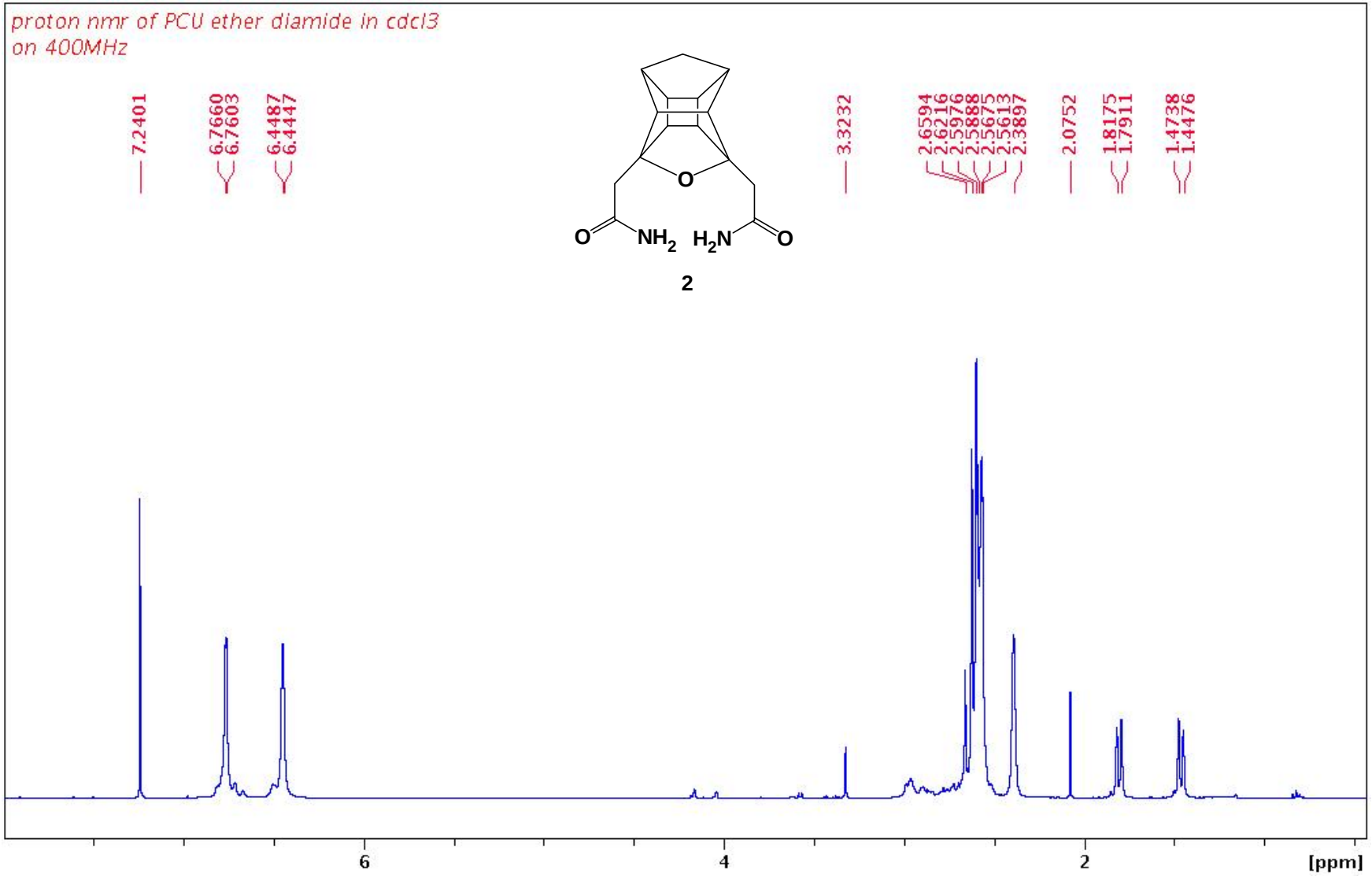
HSQC spectrum of compound 1 in $(CD_3)_2SO$



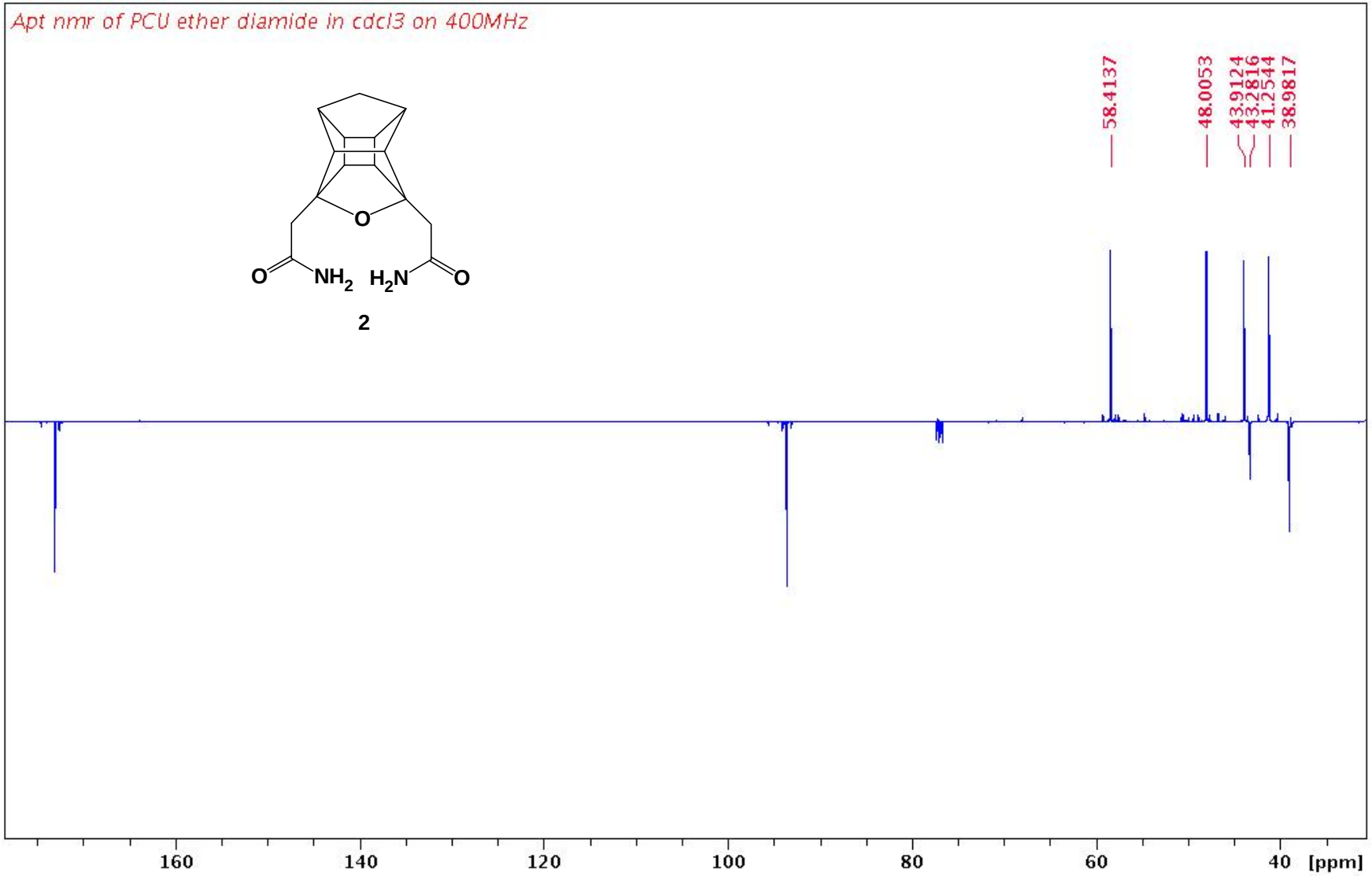
HMBC spectrum of compound 1 in $(CD_3)_2SO$



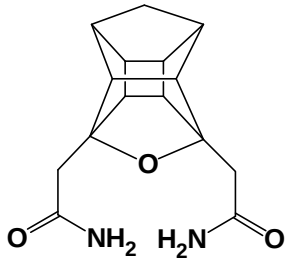
IR spectrum of compound 1



^{1}H spectrum of compound 2 in $CDCl_3$



^{13}C spectrum of compound 2 in $CDCl_3$



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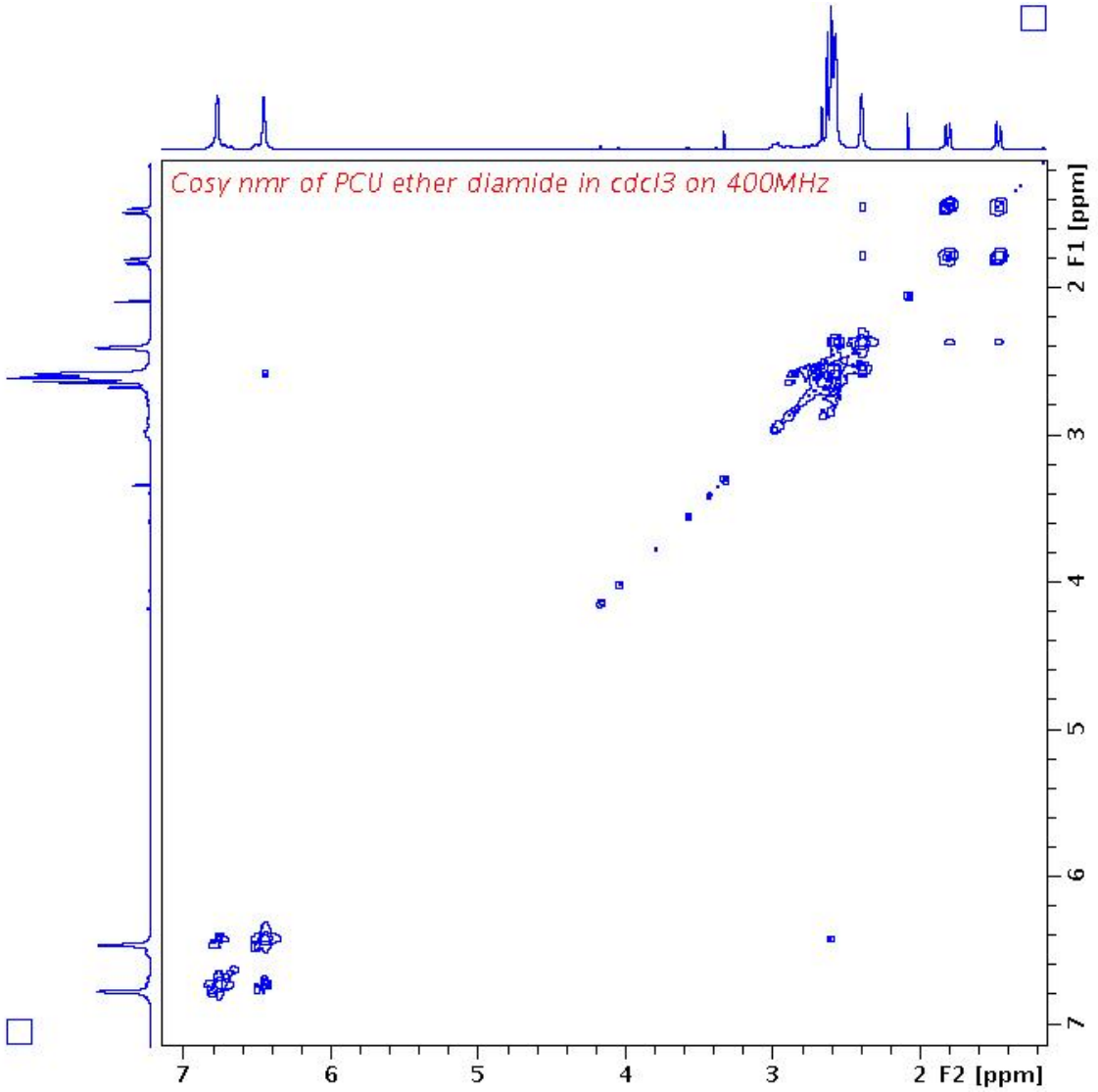
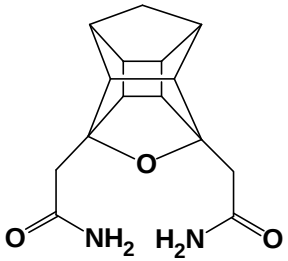


COSY spectrum of compound 2 in $CDCl_3$



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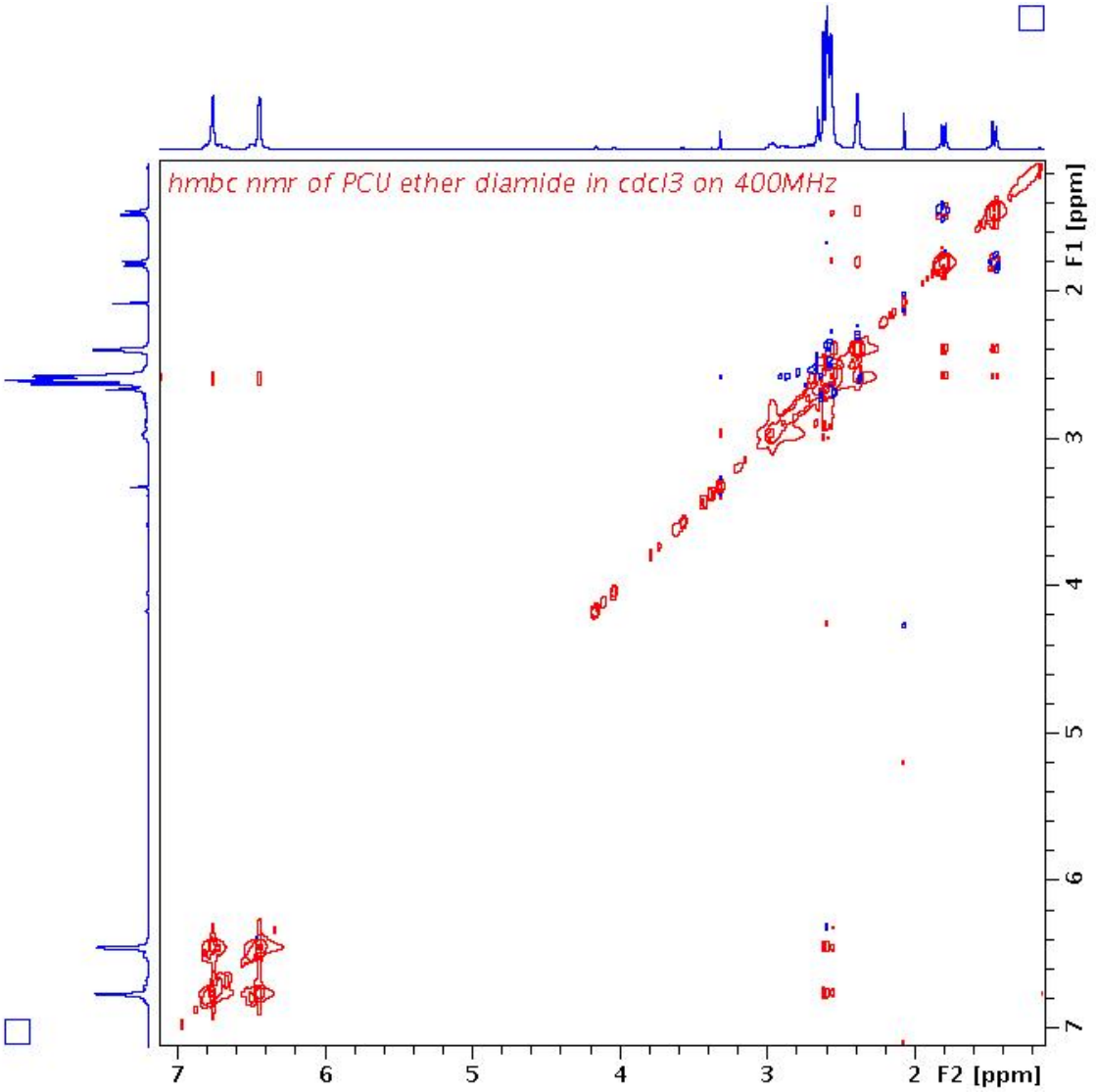
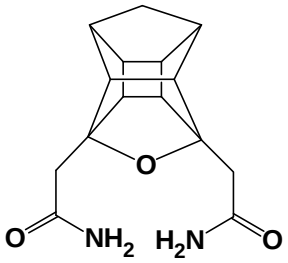


NOESY spectrum of compound 2 in $CDCl_3$



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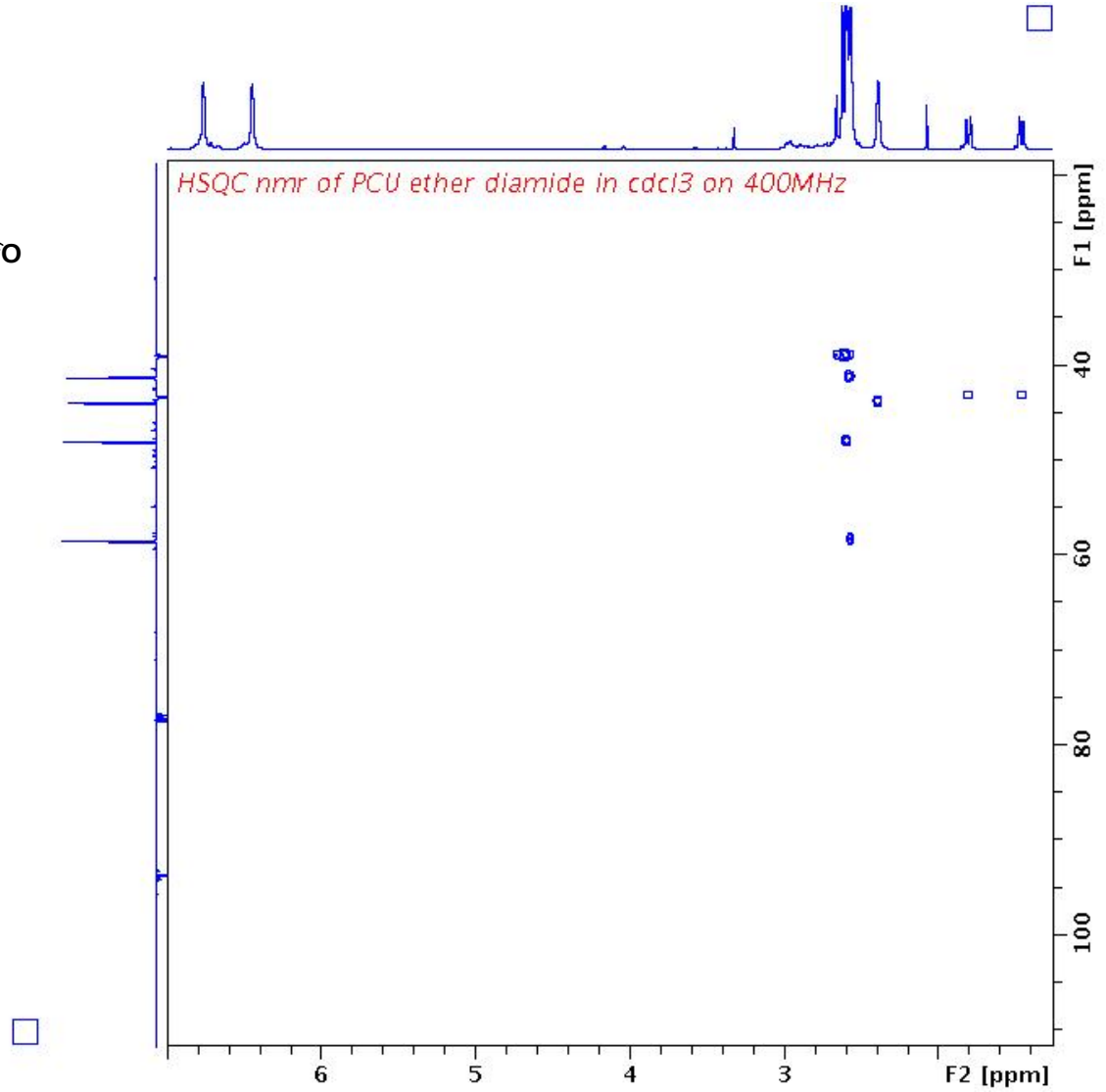
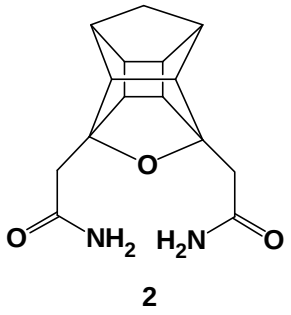


HSQC spectrum of compound 2 in $CDCl_3$



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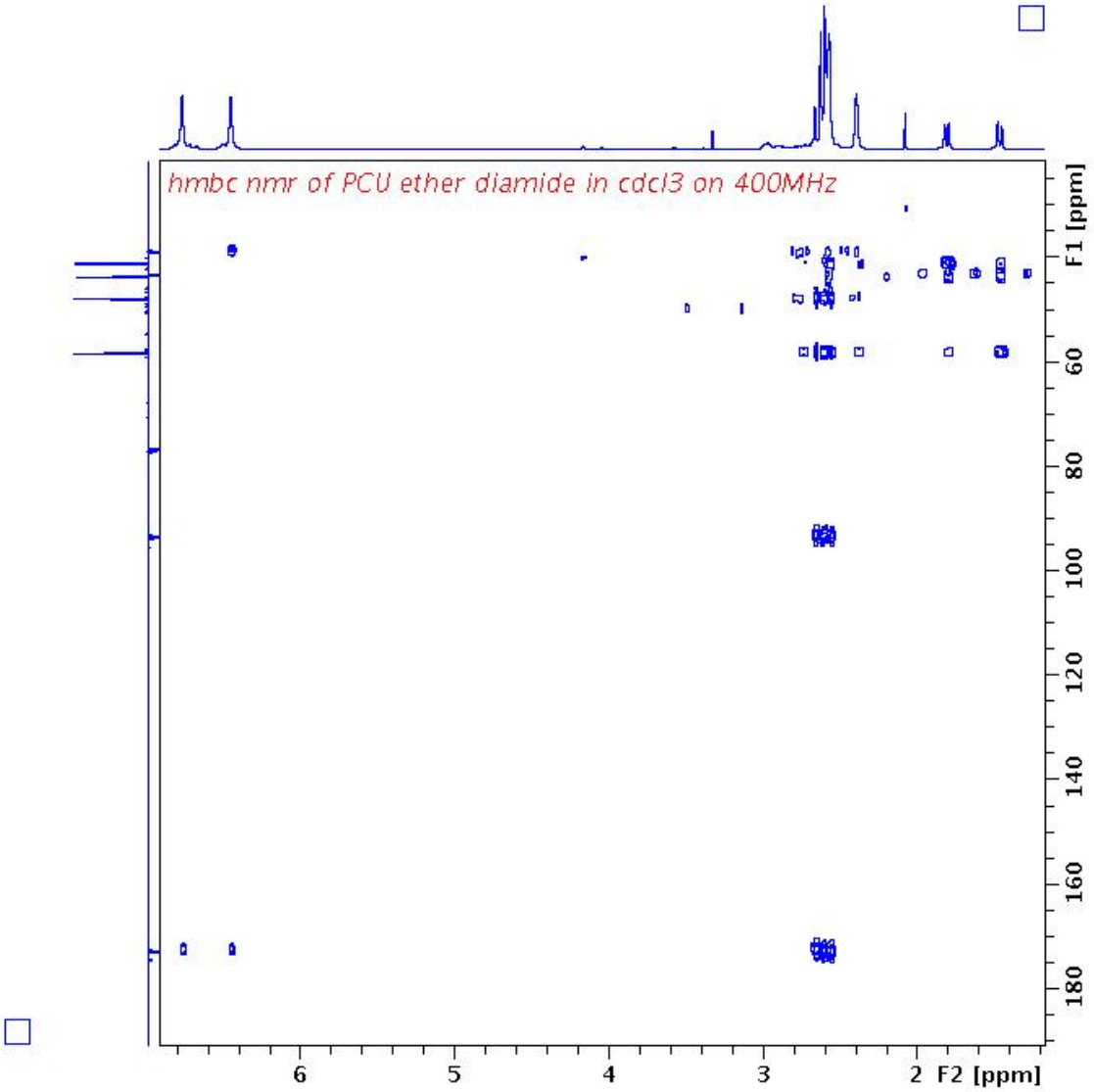
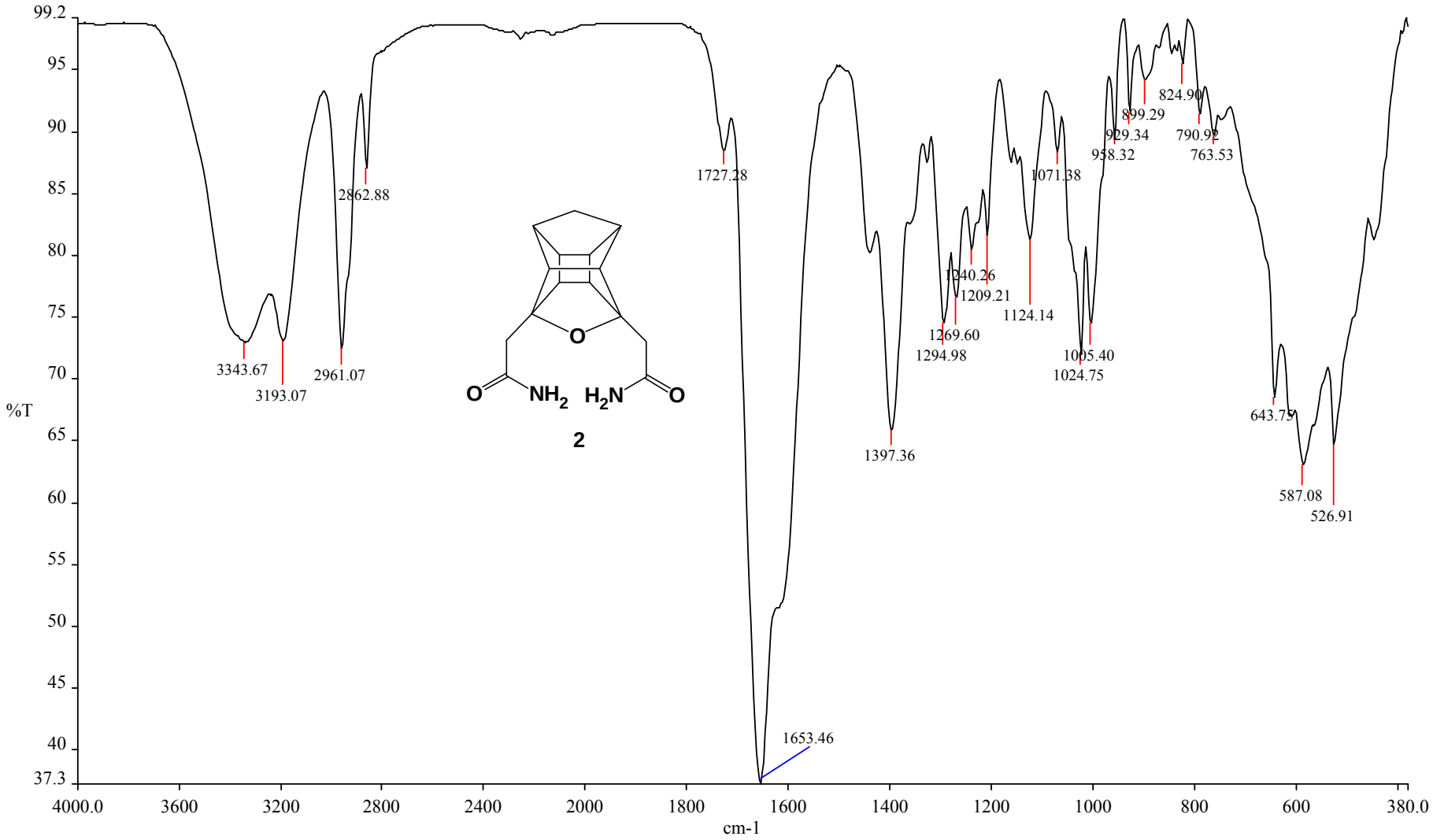
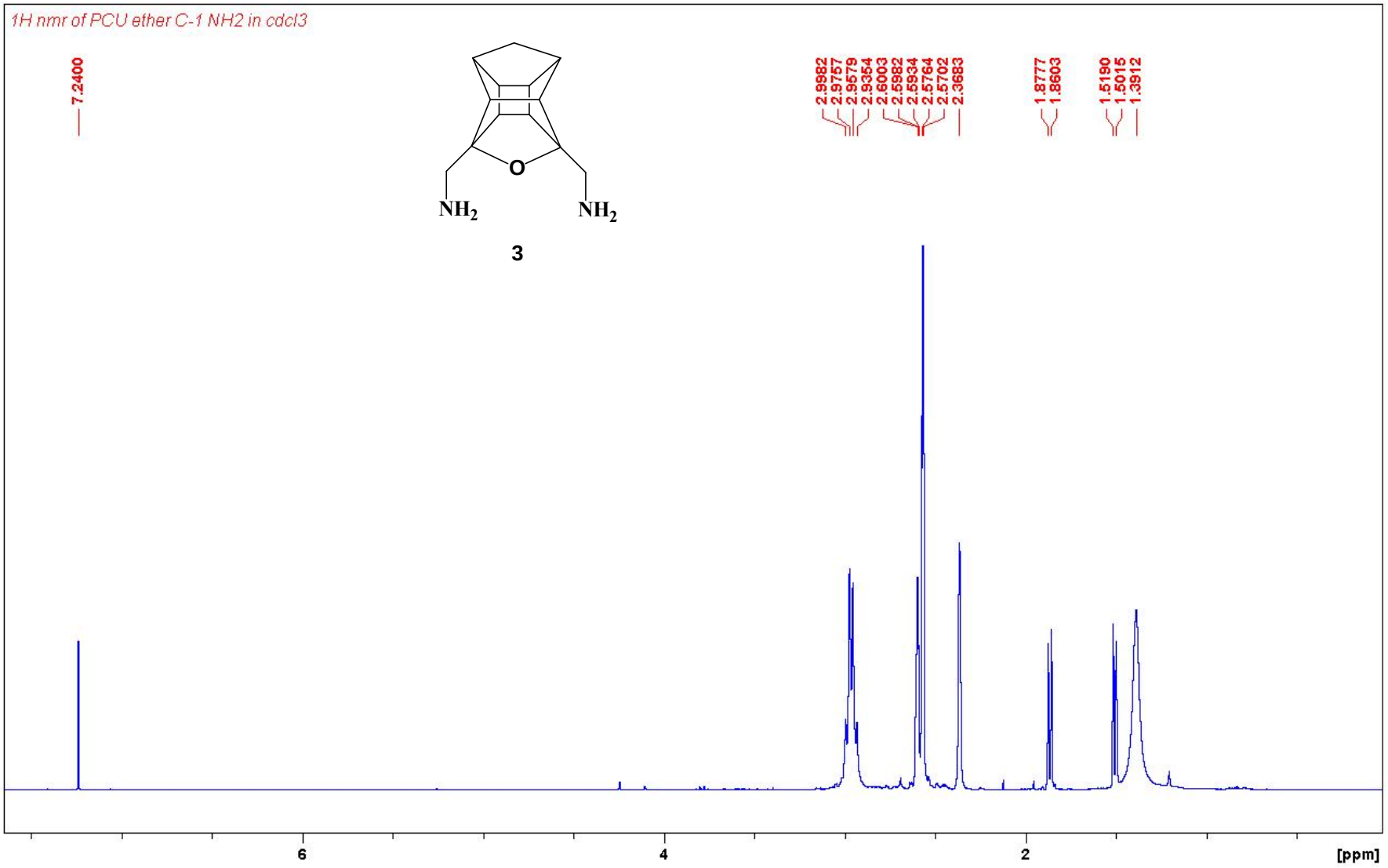


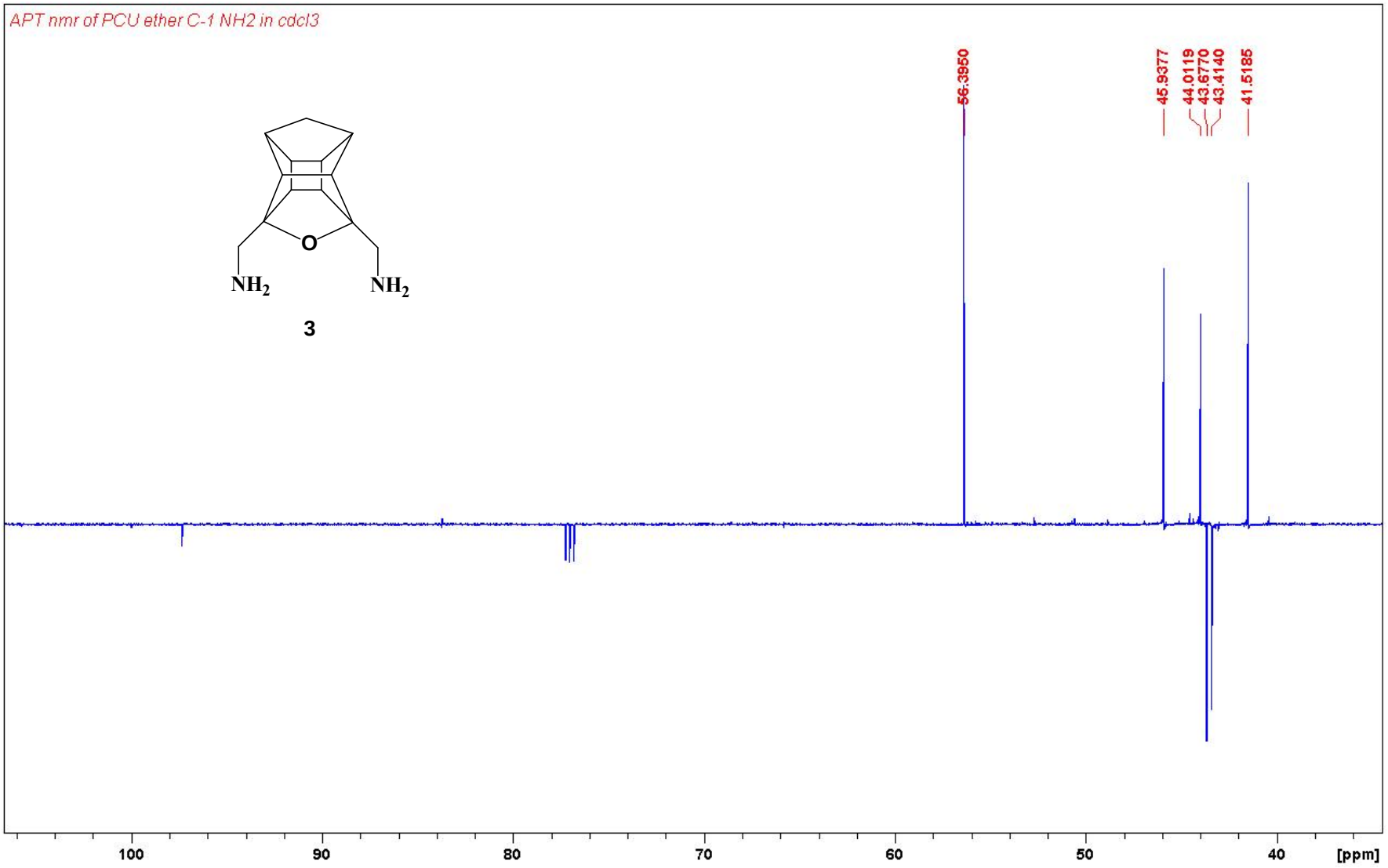
HMBC spectrum of compound 2 in $CDCl_3$



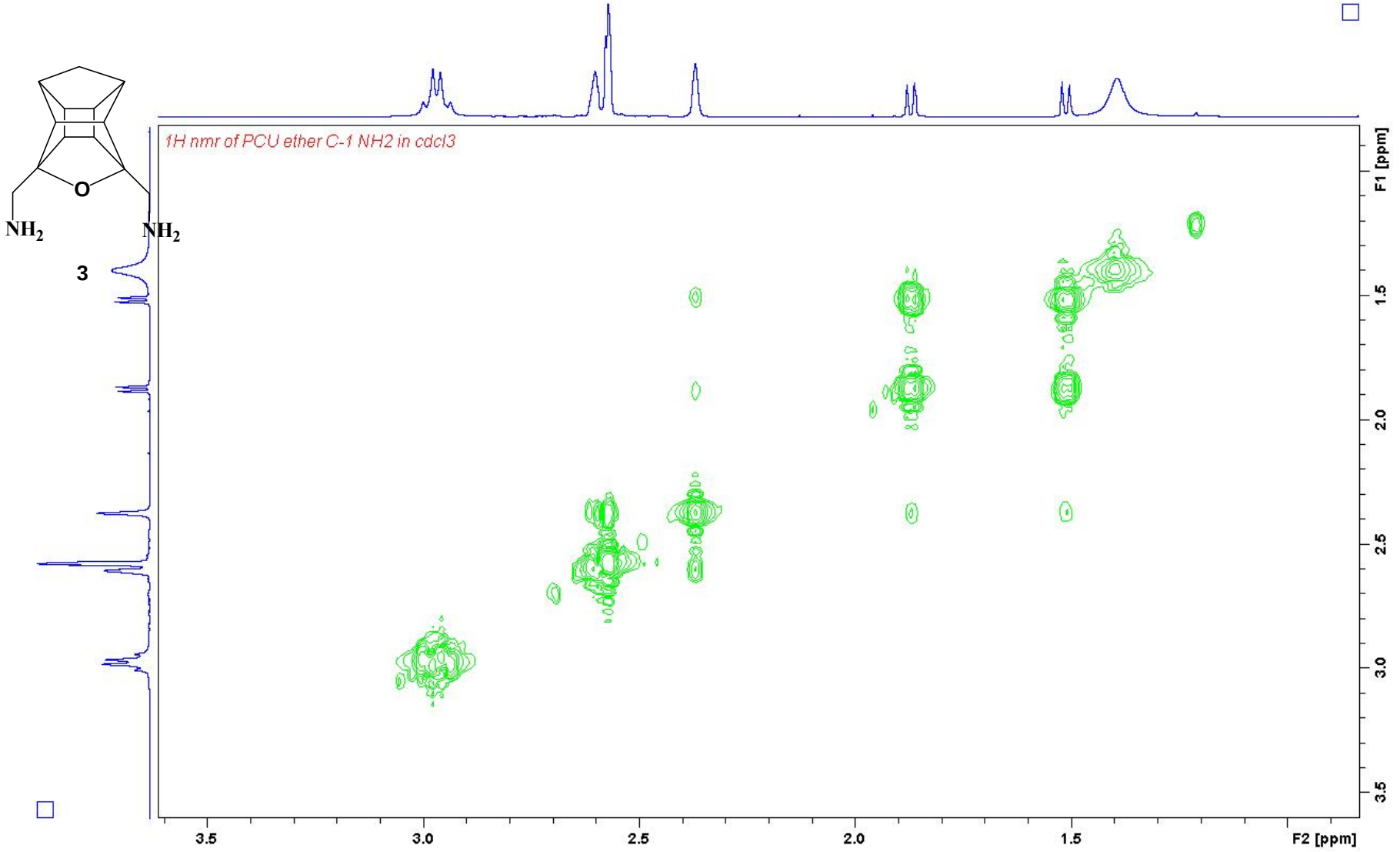
IR spectrum of compound 2



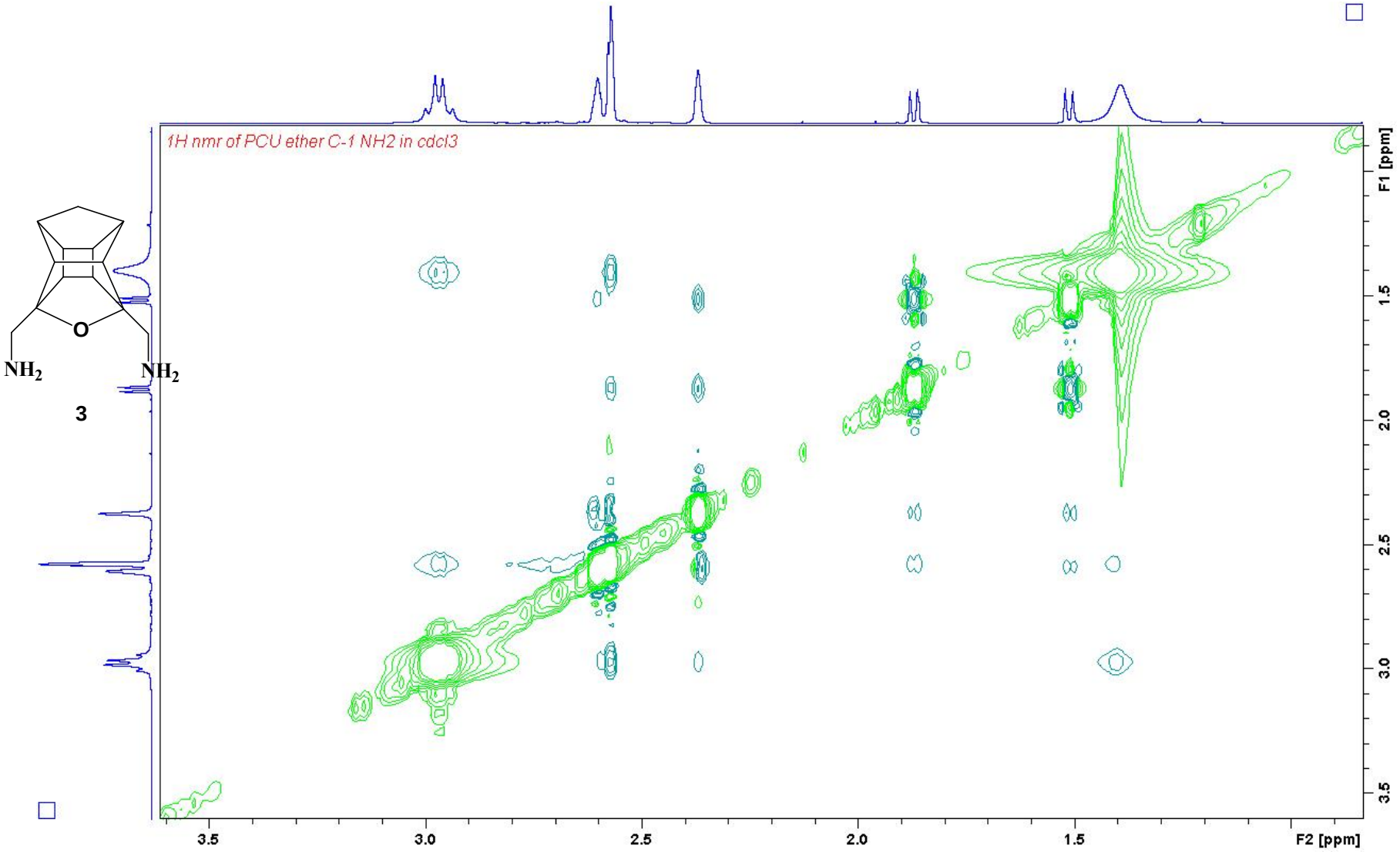
^{1}H spectrum of compound 3 in $CDCl_3$



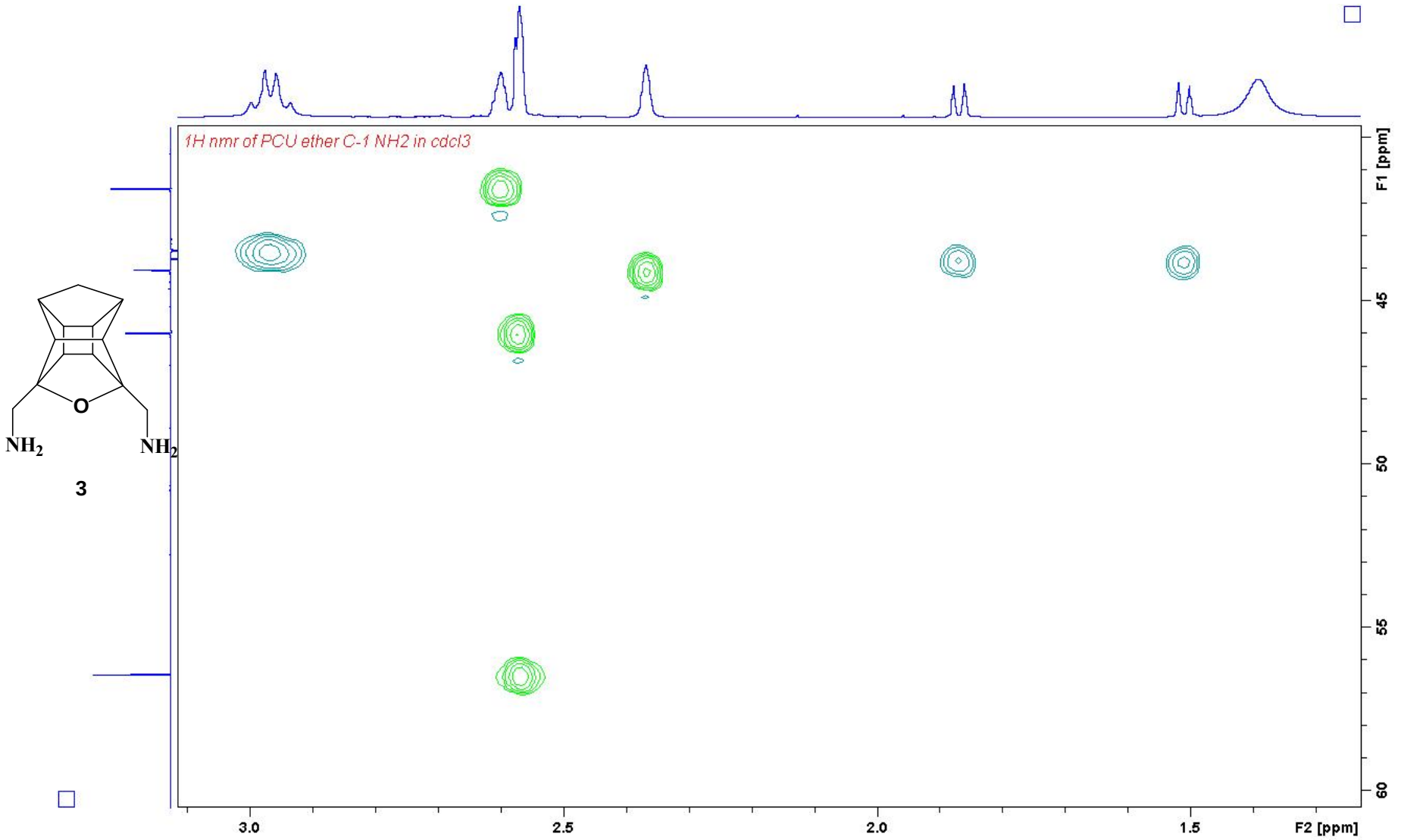
^{13}C spectrum of compound 3 in $CDCl_3$



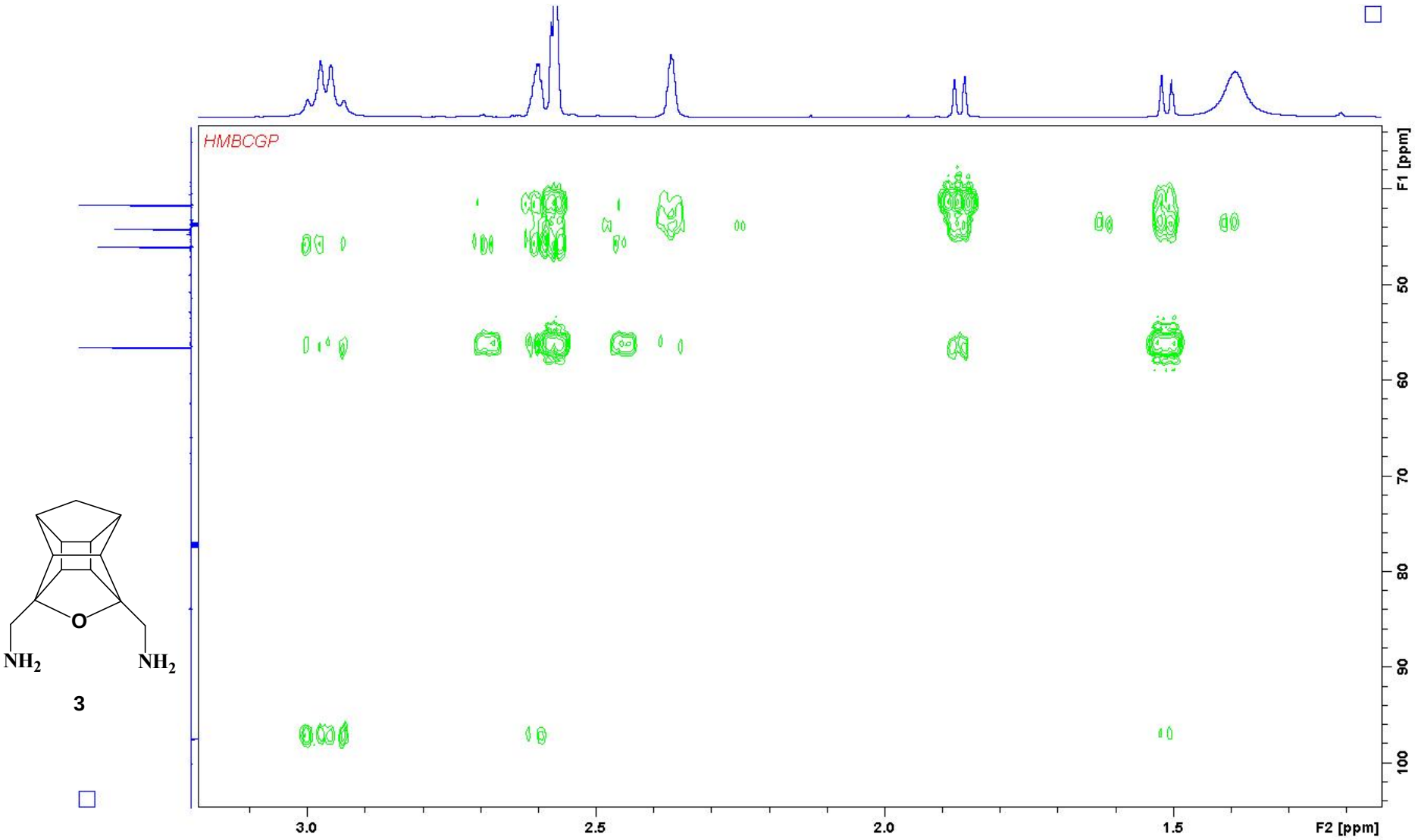
COSY spectrum of compound 3 in $CDCl_3$



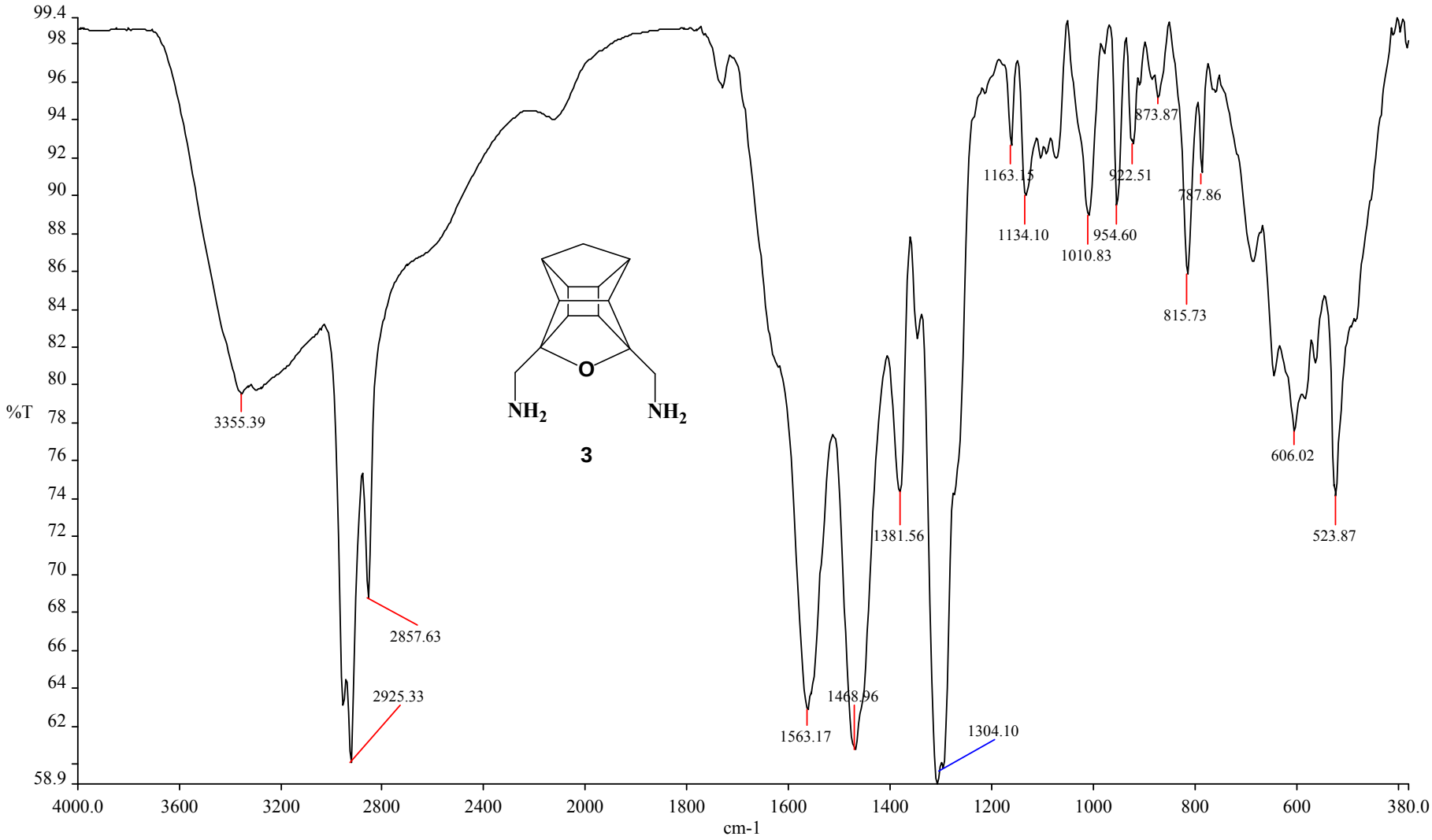
NOESY spectrum of compound 3 in $CDCl_3$



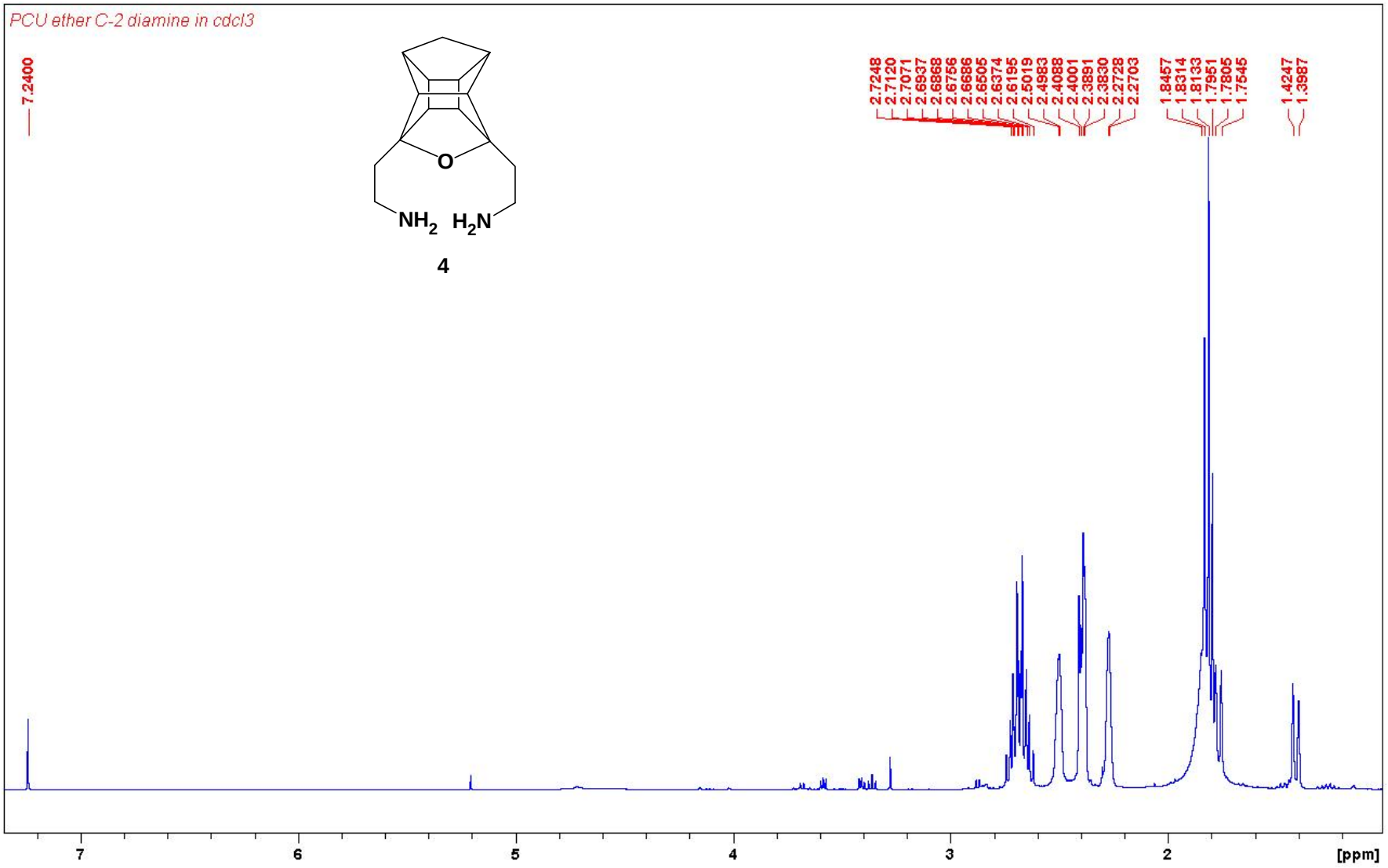
HSQC spectrum of compound 3 in $CDCl_3$



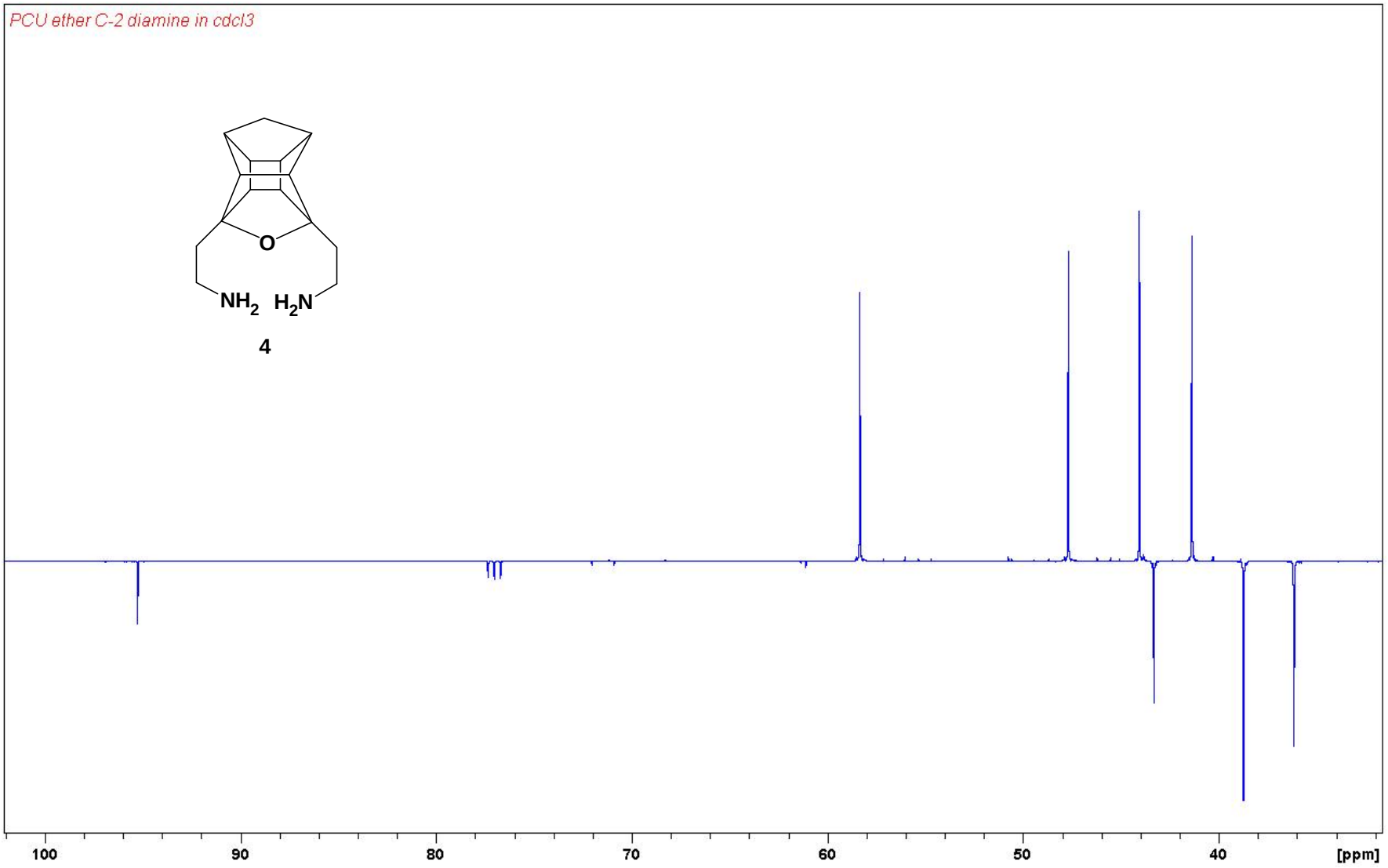
HMBC spectrum of compound 3 in $CDCl_3$



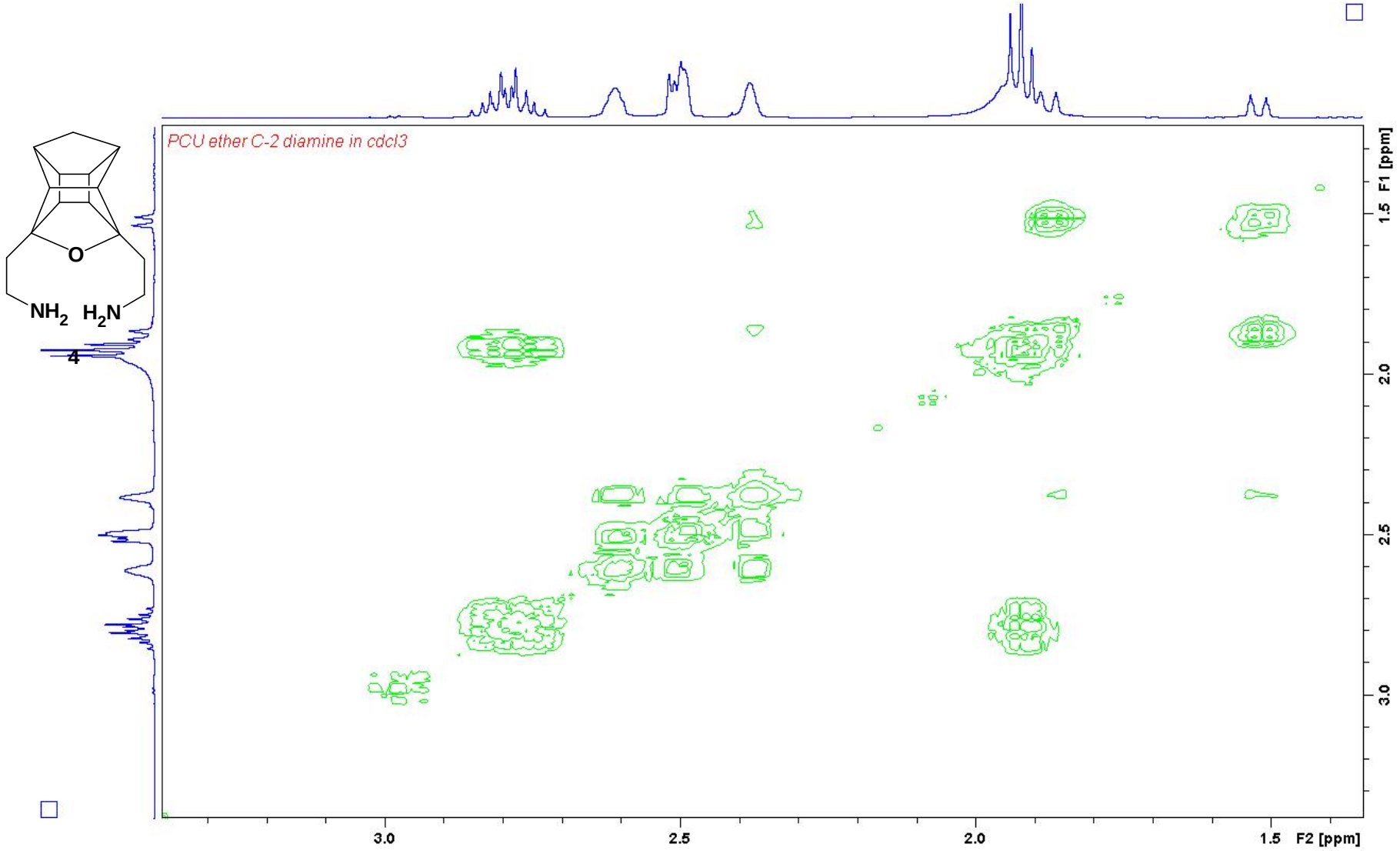
IR spectrum of compound 3



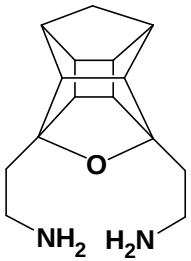
^{1}H spectrum of compound 4 in $CDCl_3$



^{13}C spectrum of compound 4 in $CDCl_3$



COSY spectrum of compound 4 in $CDCl_3$



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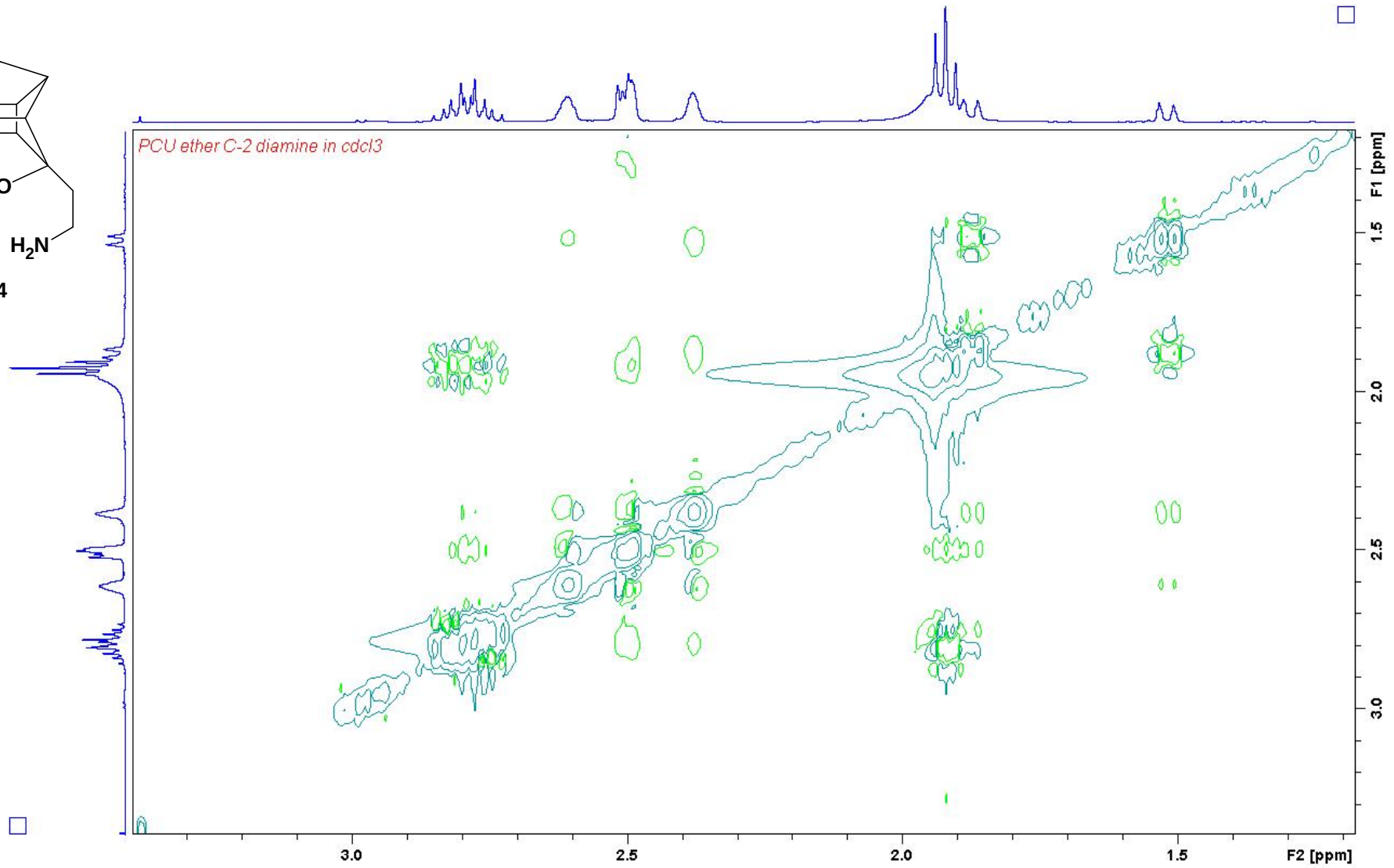
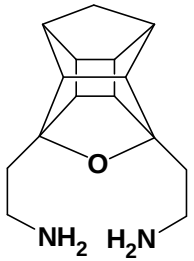
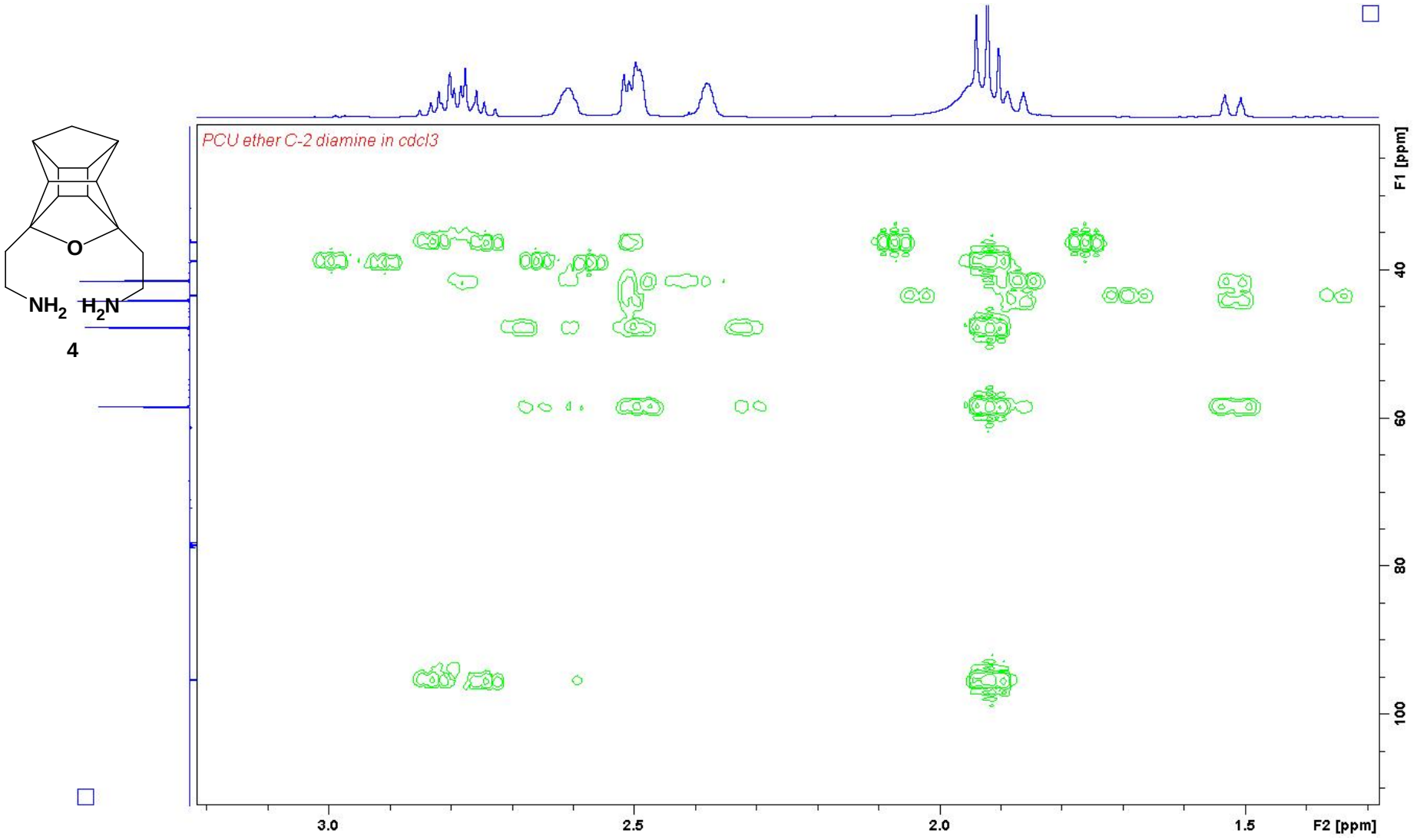


NOESY spectrum of compound 4 in $CDCl_3$

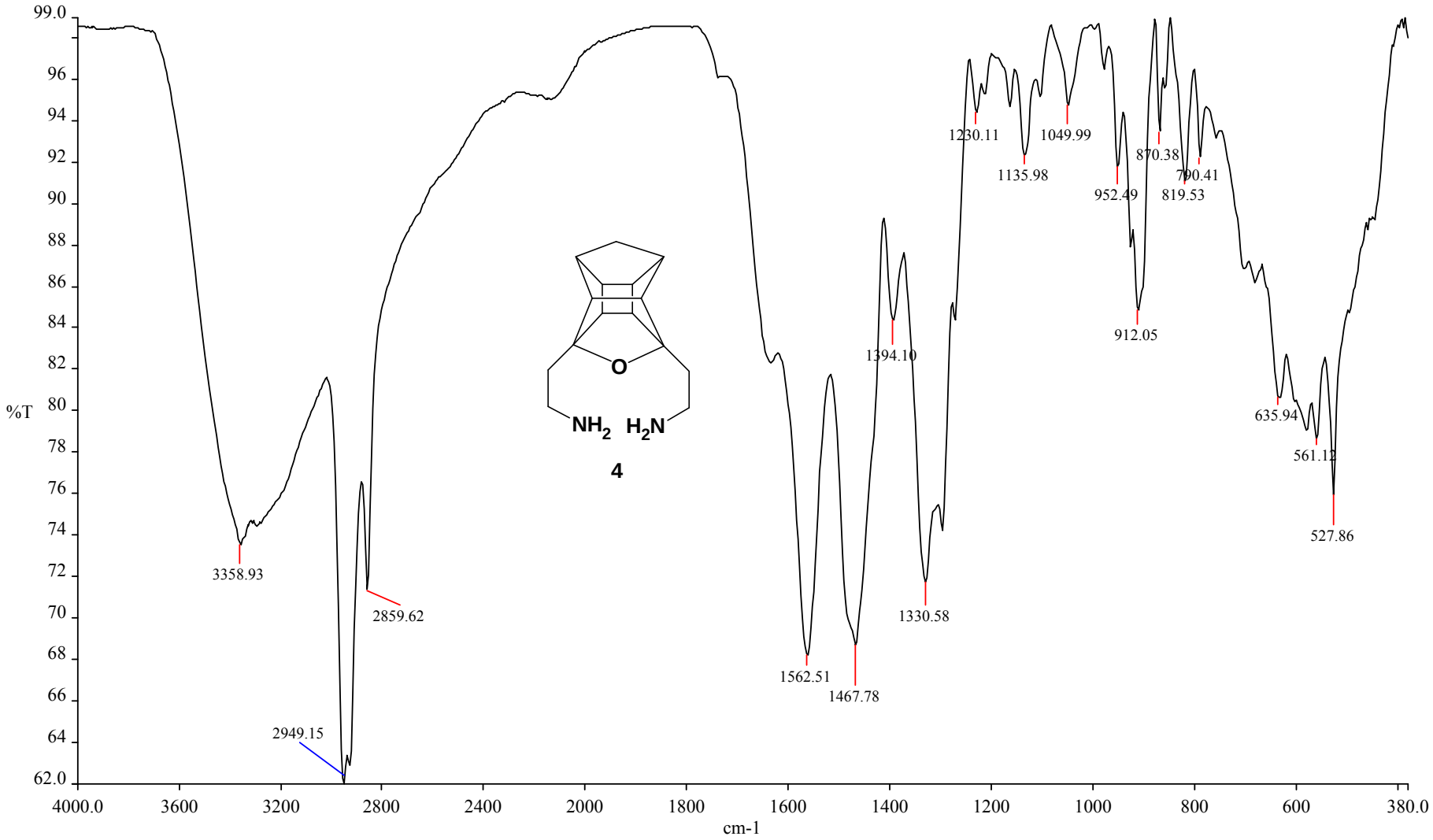


4

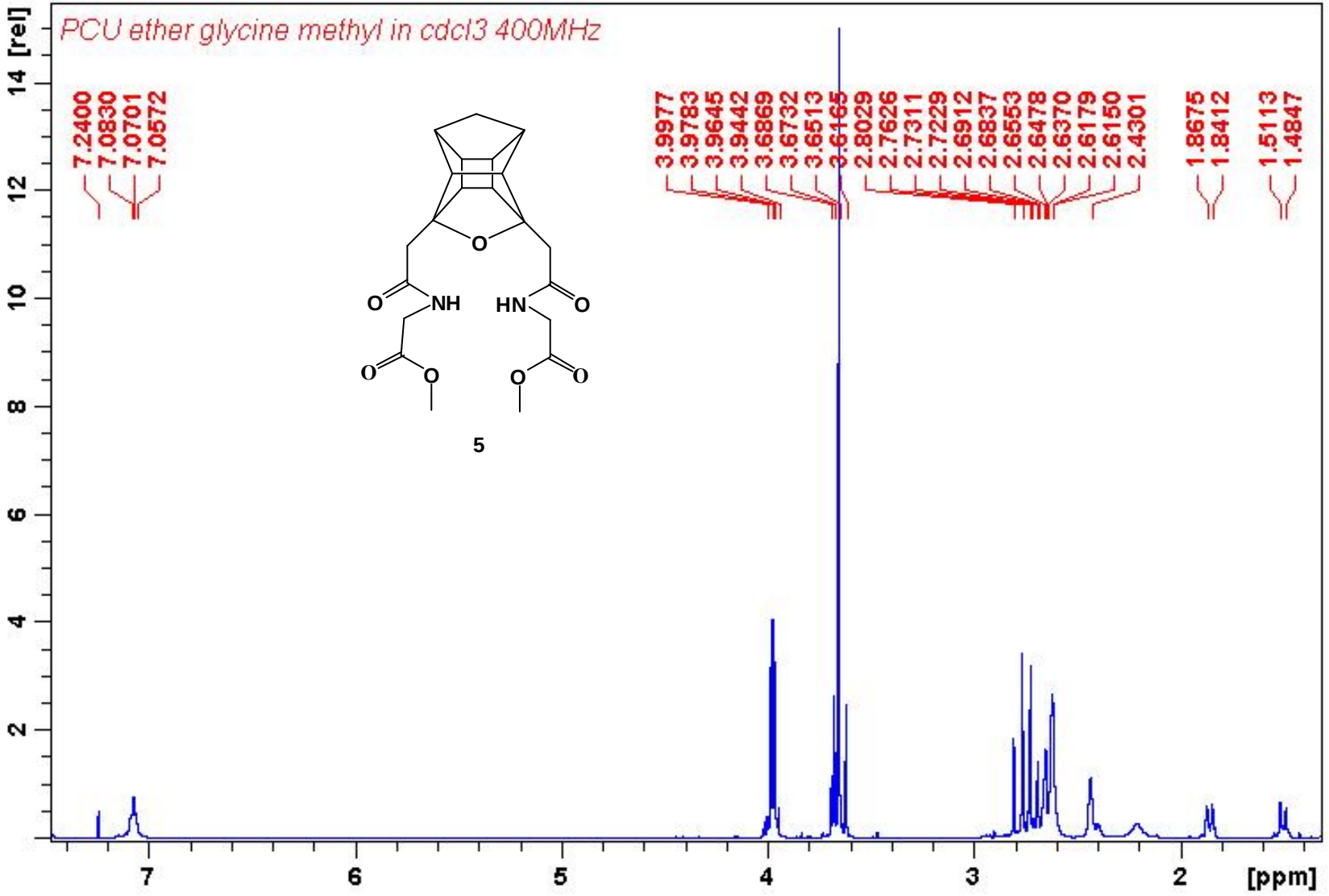
HSQC spectrum of compound 4 in $CDCl_3$



HMBC spectrum of compound 4 in $CDCl_3$

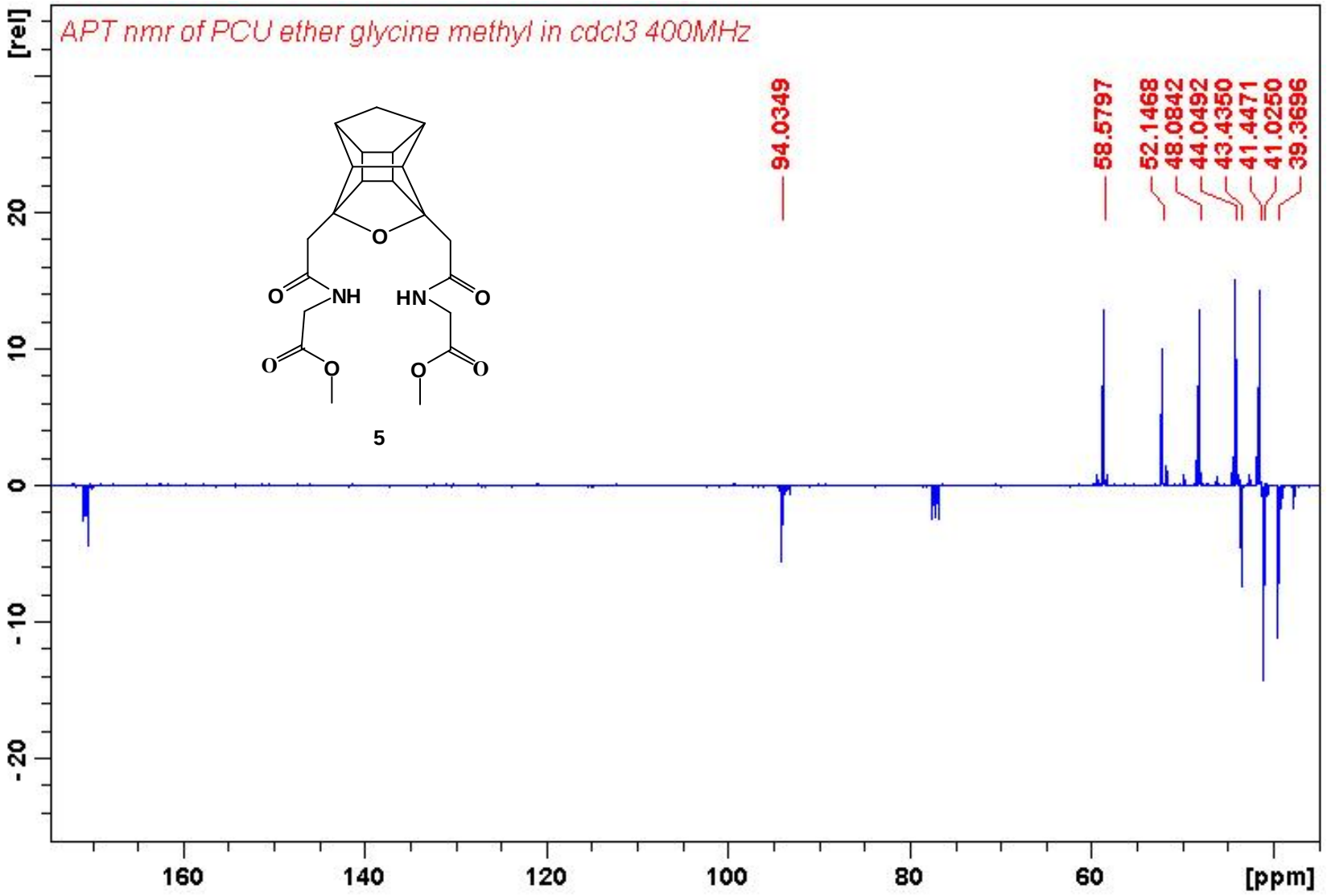


IR spectrum of compound 4



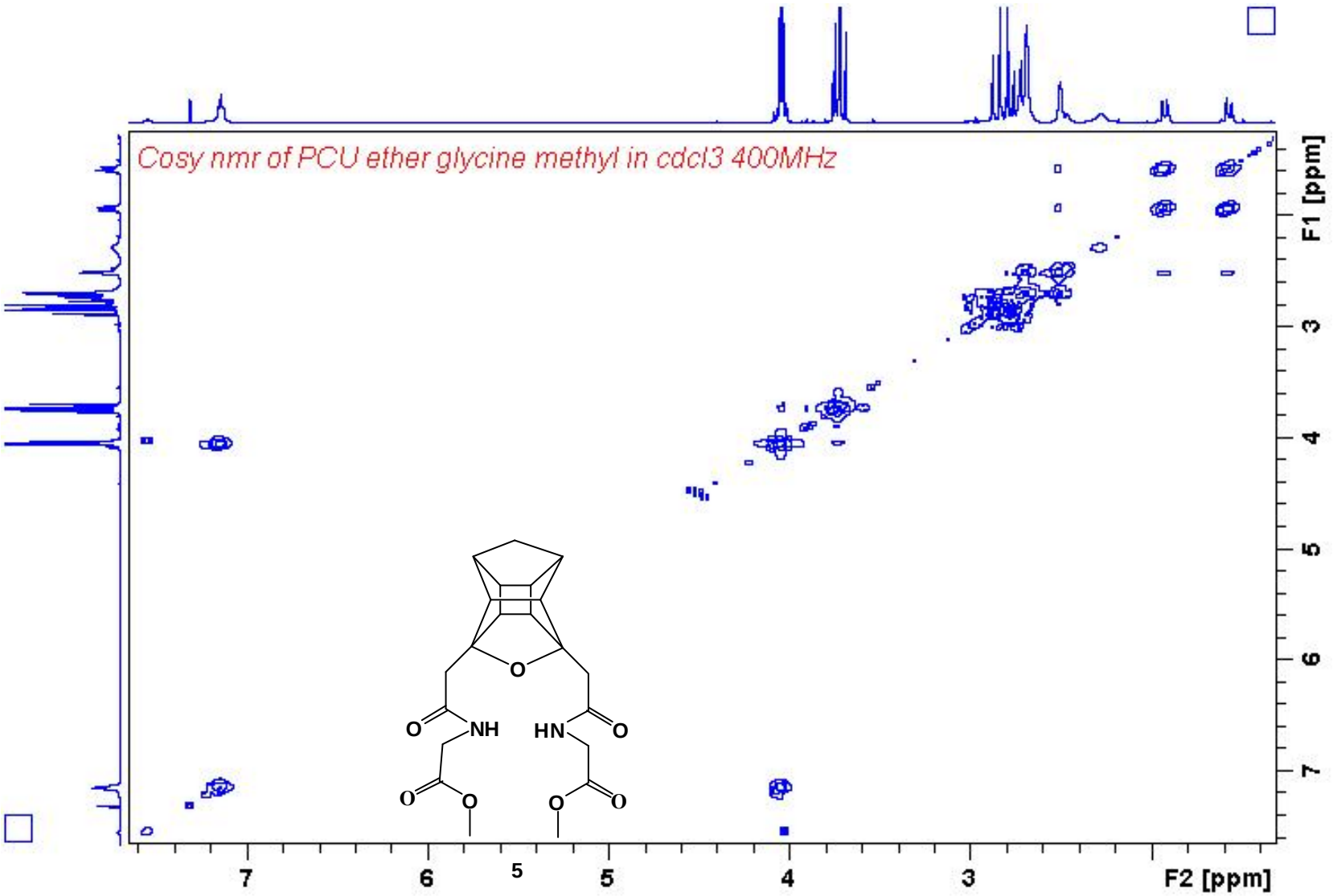
5

^{1}H spectrum of compound 5 in $CDCl_3$

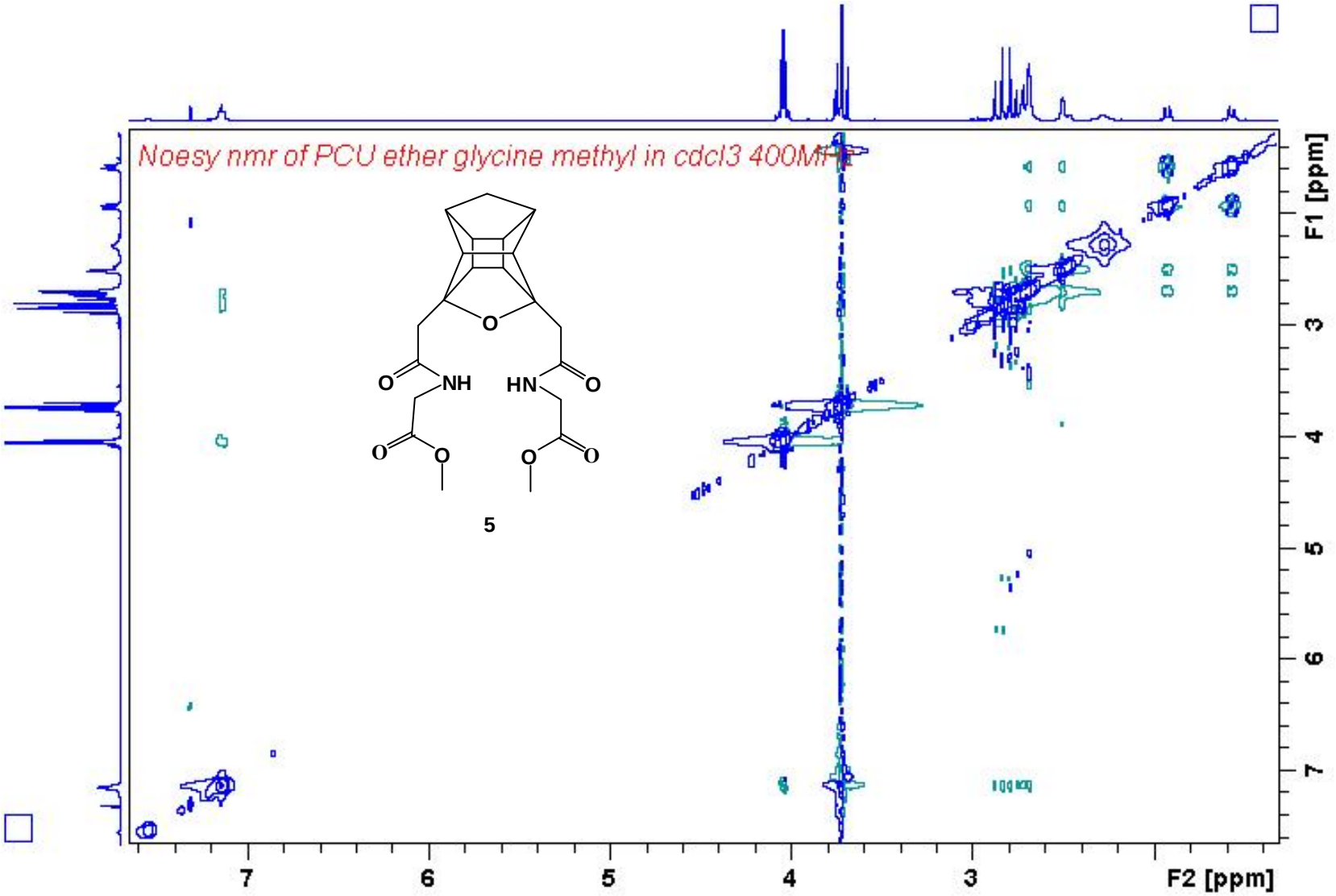


5

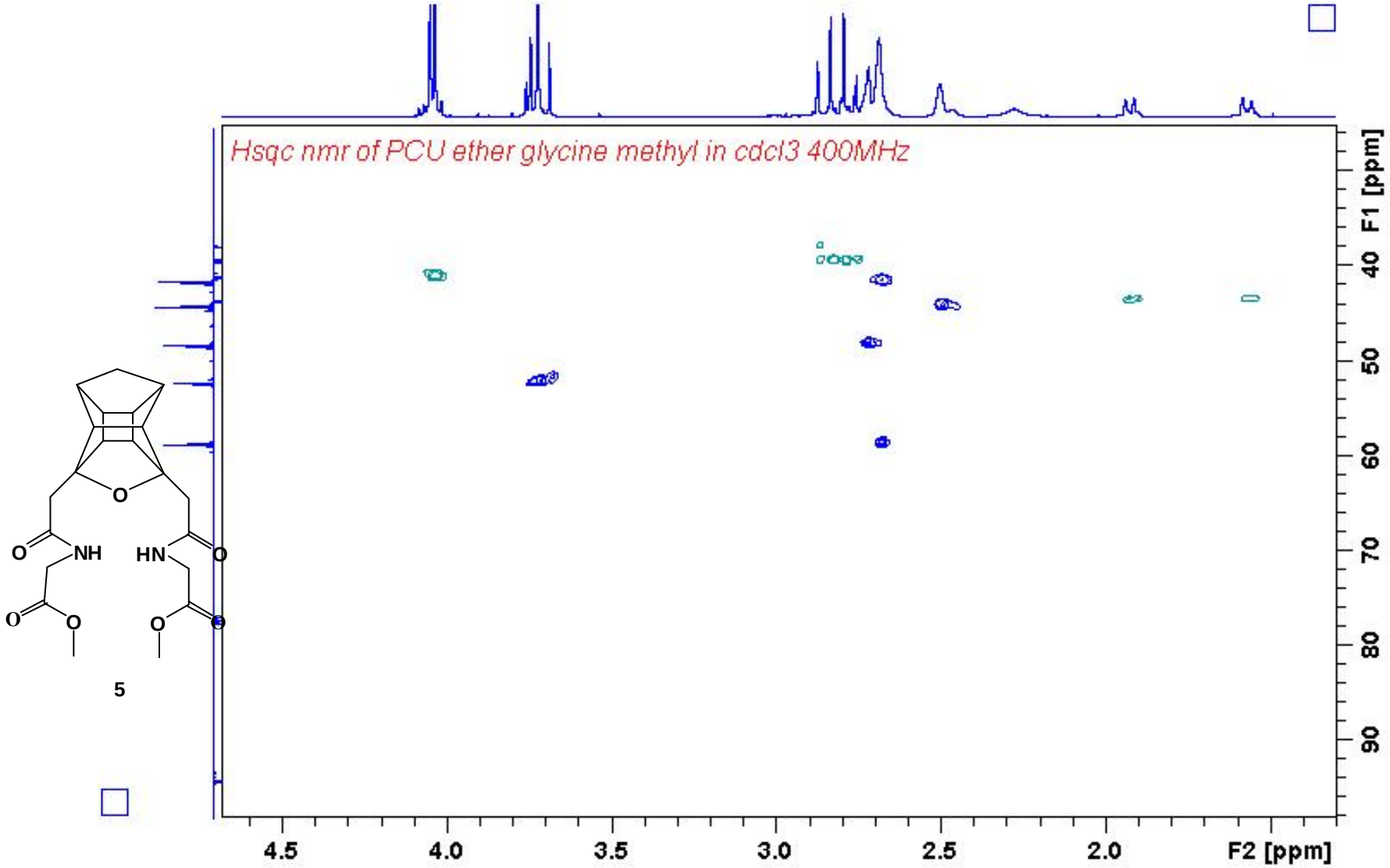
^{13}C spectrum of compound 5 in $CDCl_3$



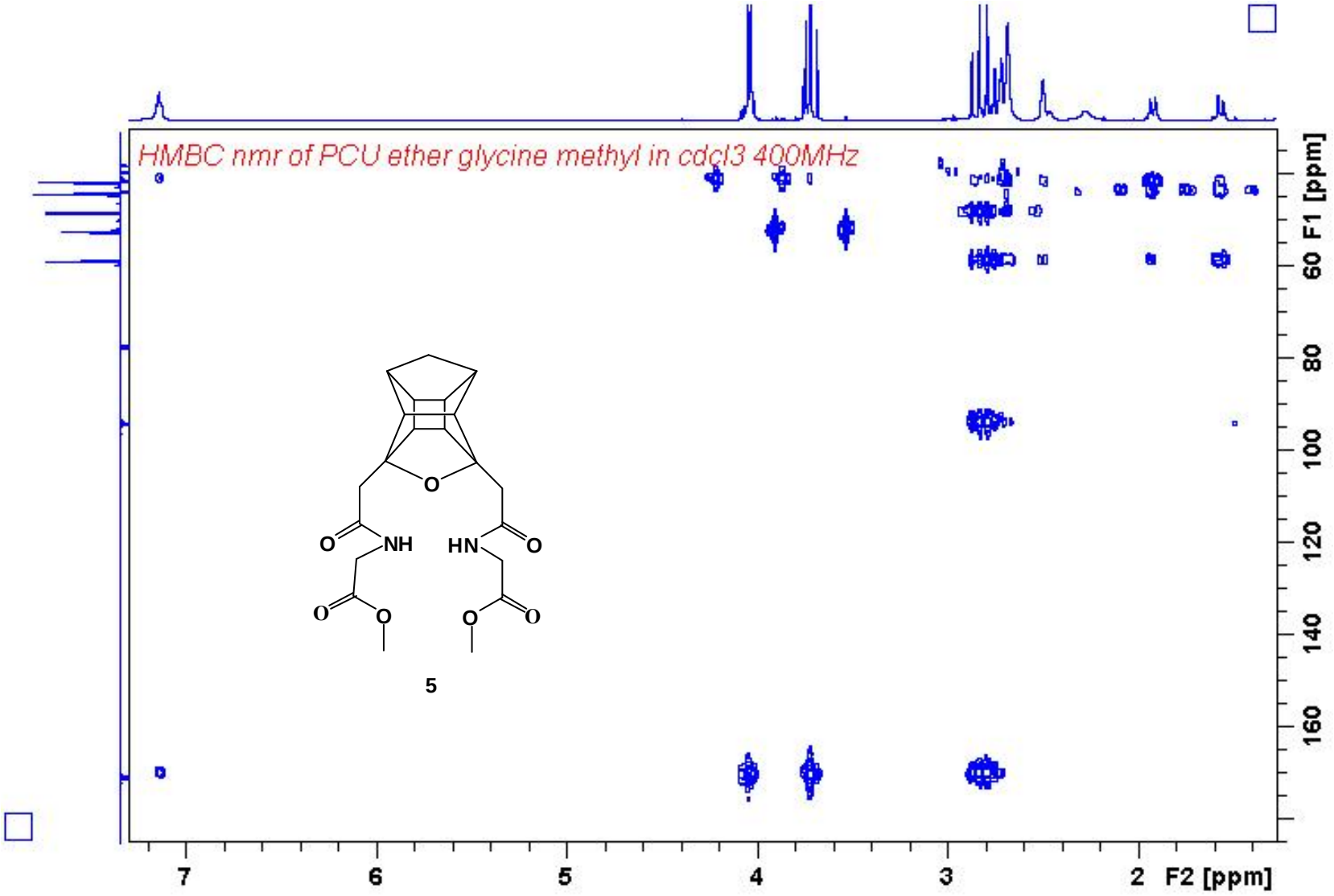
COSY spectrum of compound 5 in $CDCl_3$



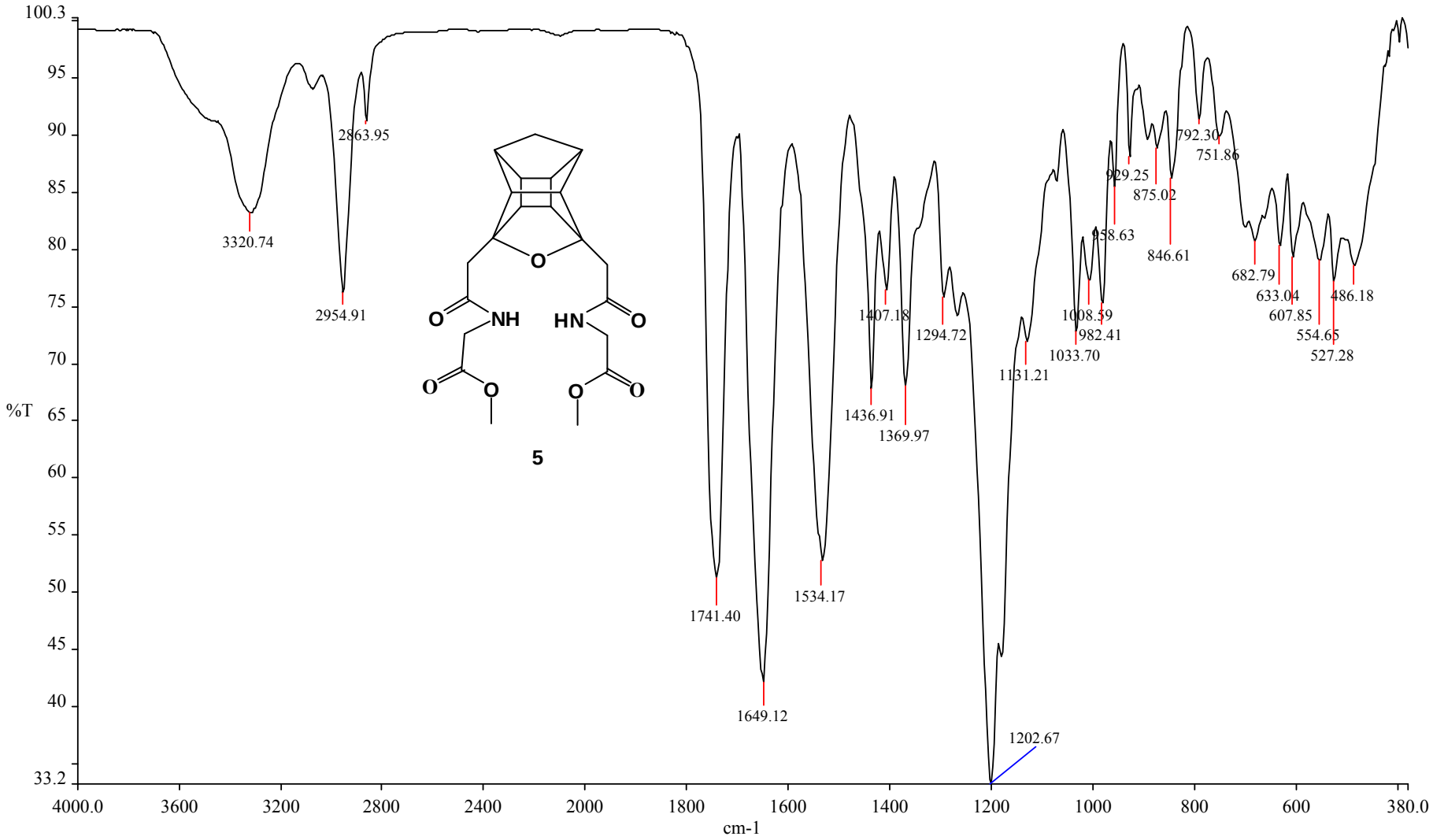
NOESY spectrum of compound 5 in $CDCl_3$



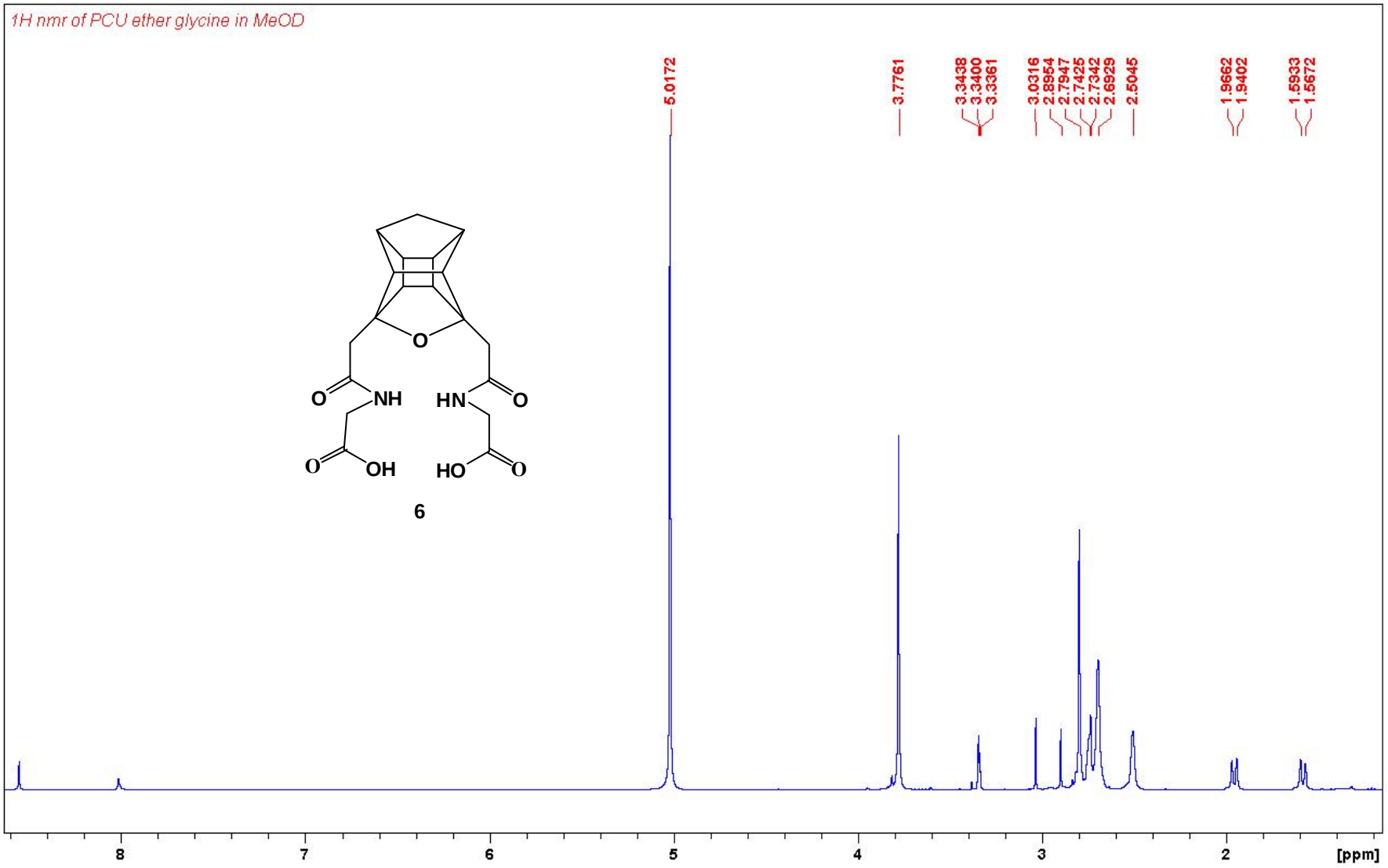
HSQC spectrum of compound 5 in $CDCl_3$



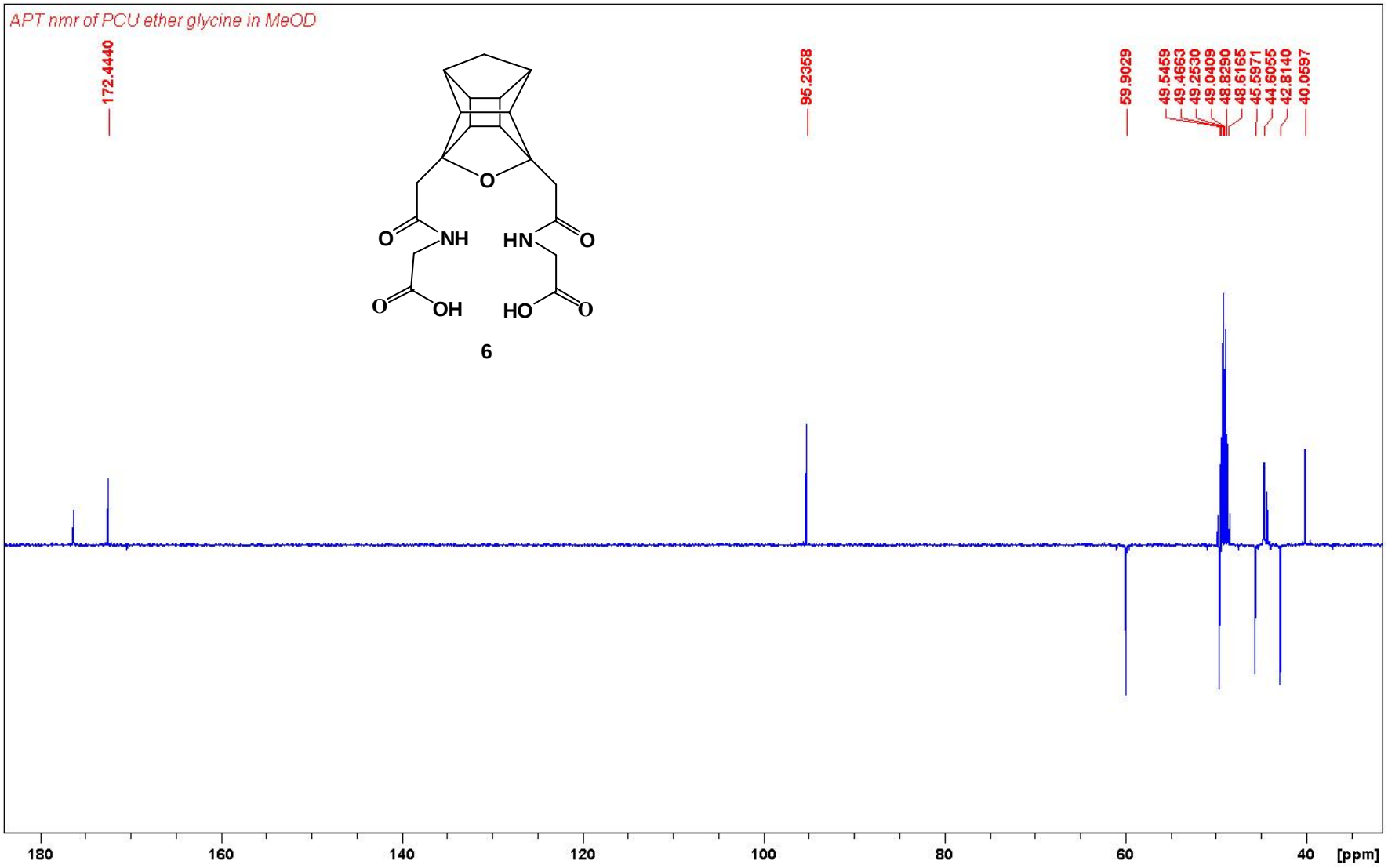
HMBC spectrum of compound 5 in $CDCl_3$



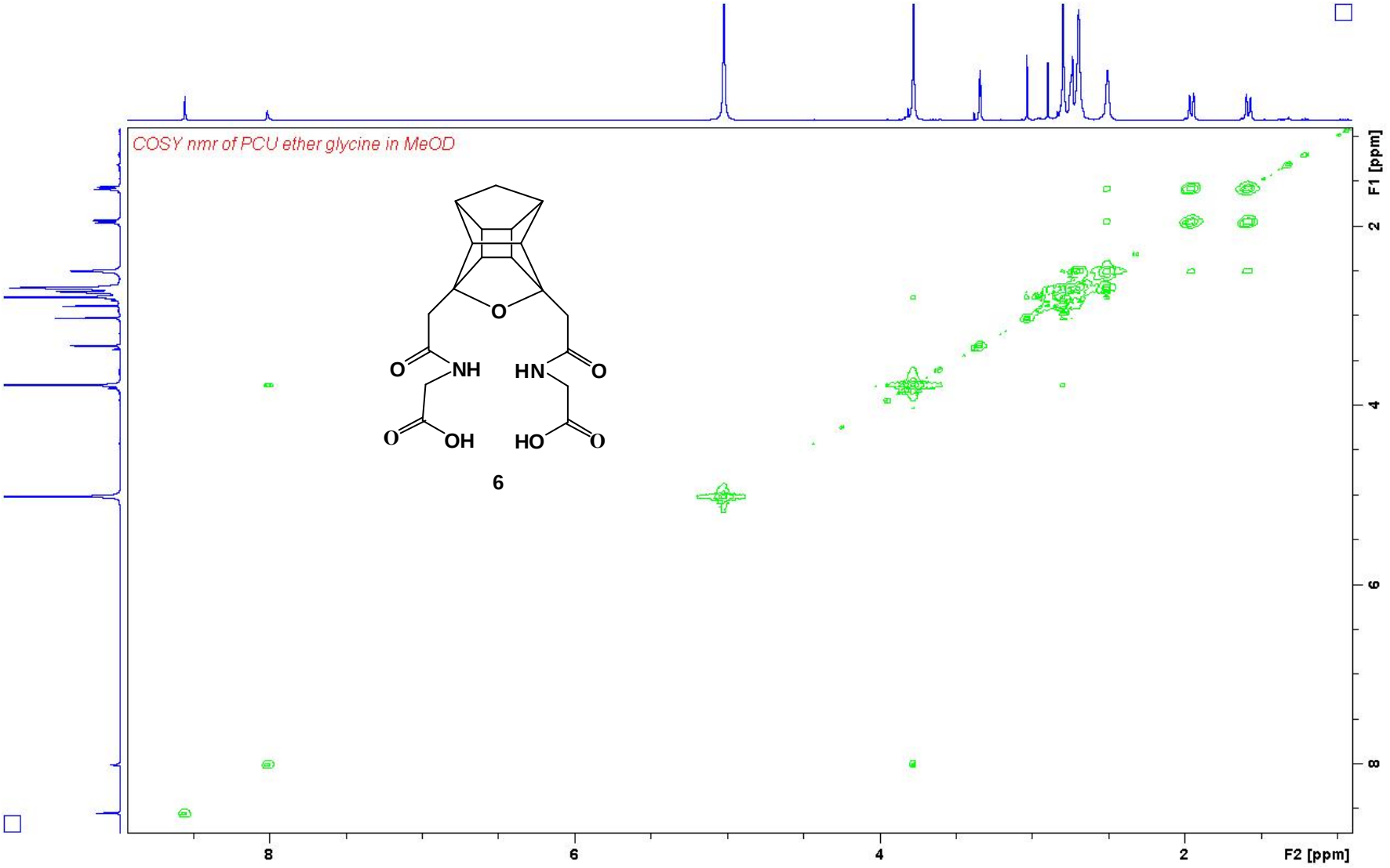
IR spectrum of compound 5



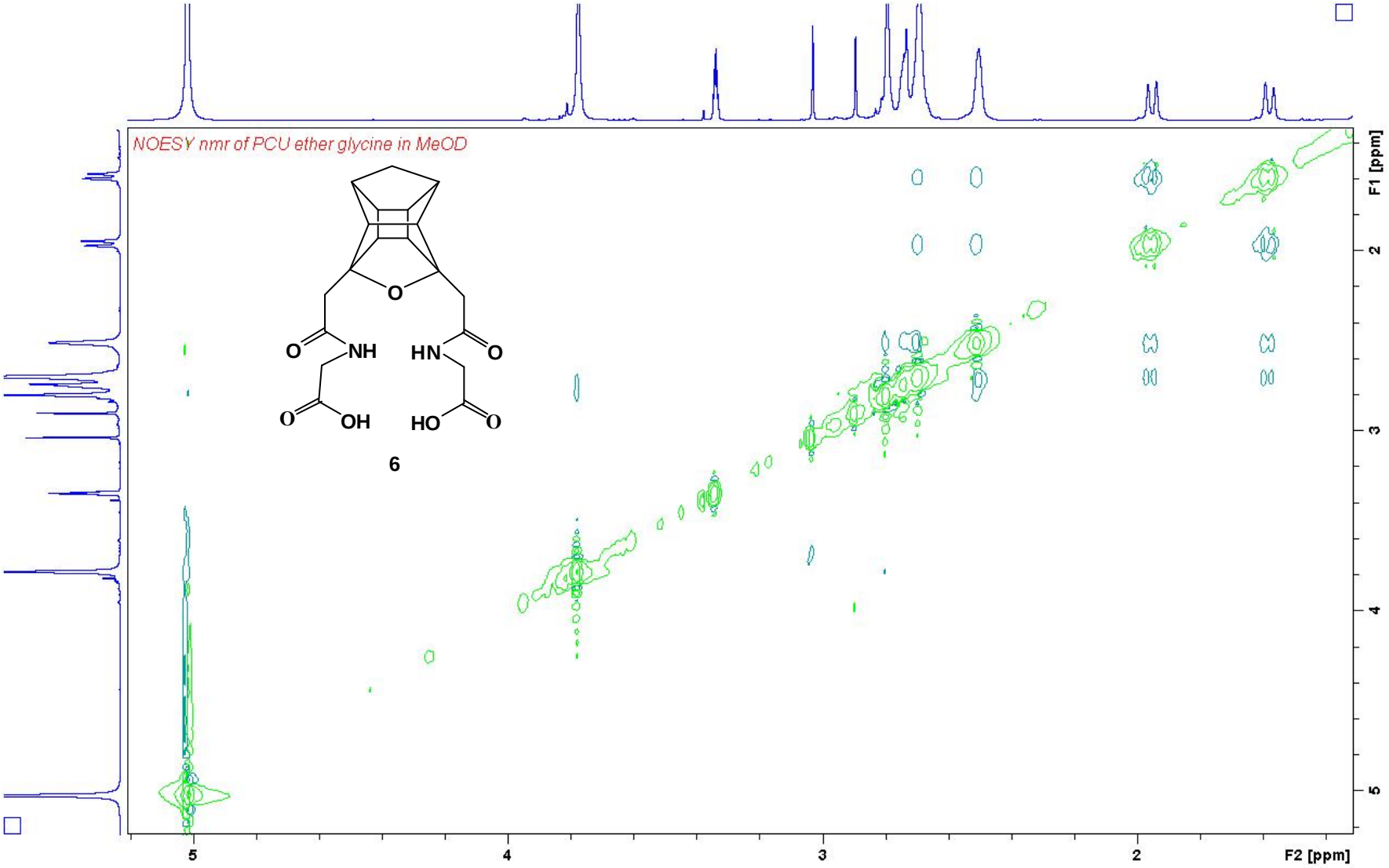
^{1}H spectrum of compound 6 in CD_3OD



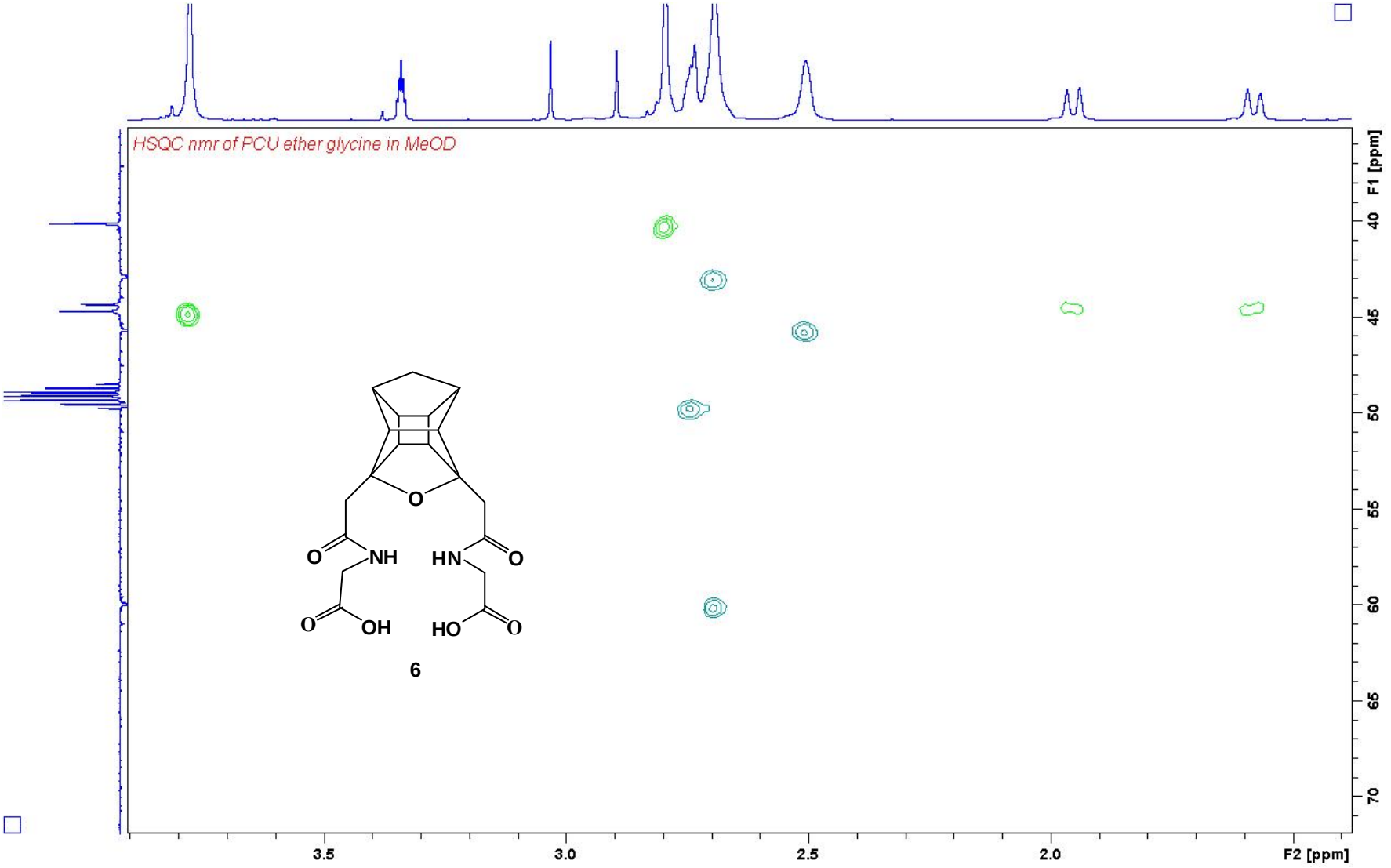
^{13}C spectrum of compound 6 in CD_3OD



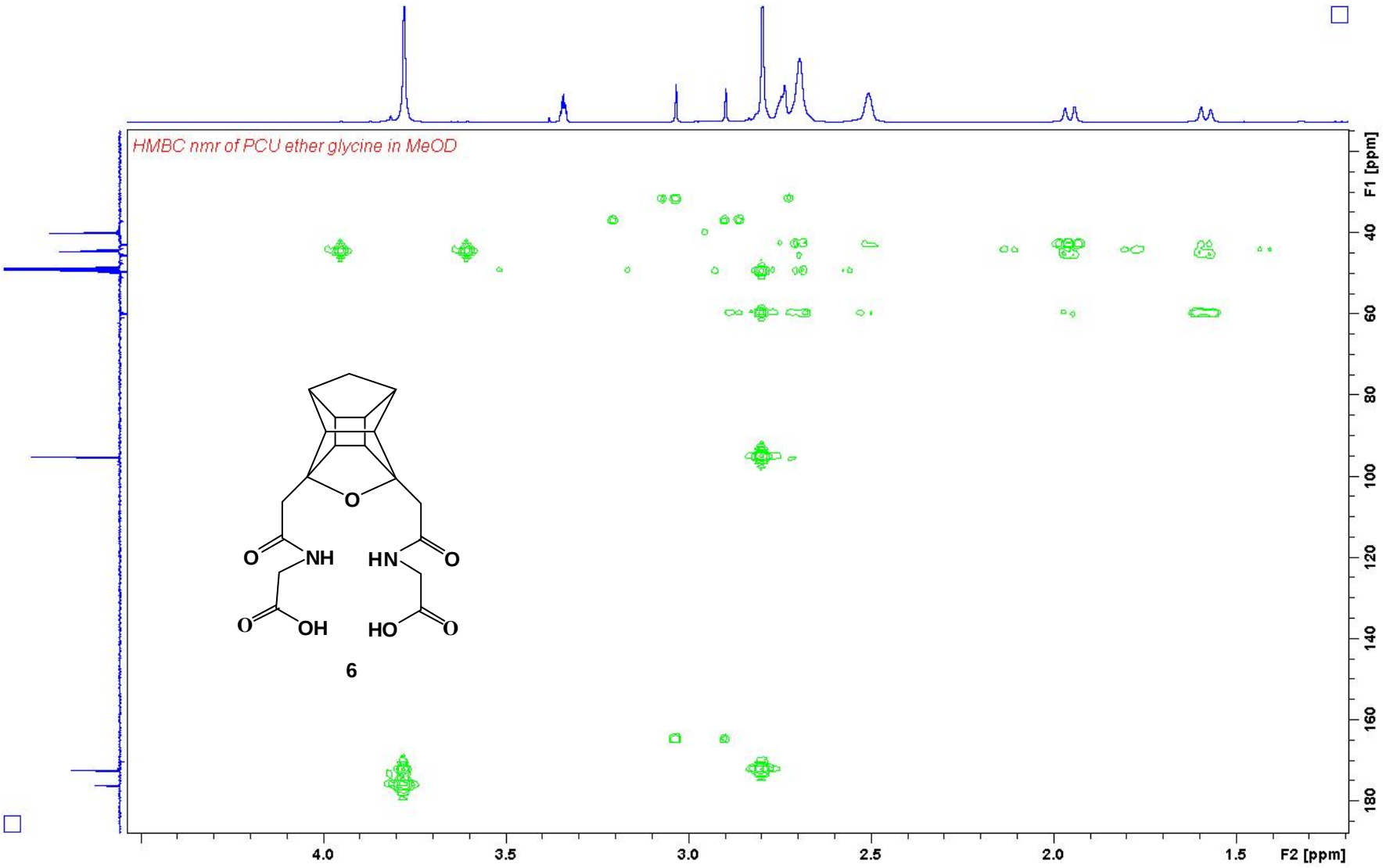
COSY spectrum of compound 6 in CD_3OD



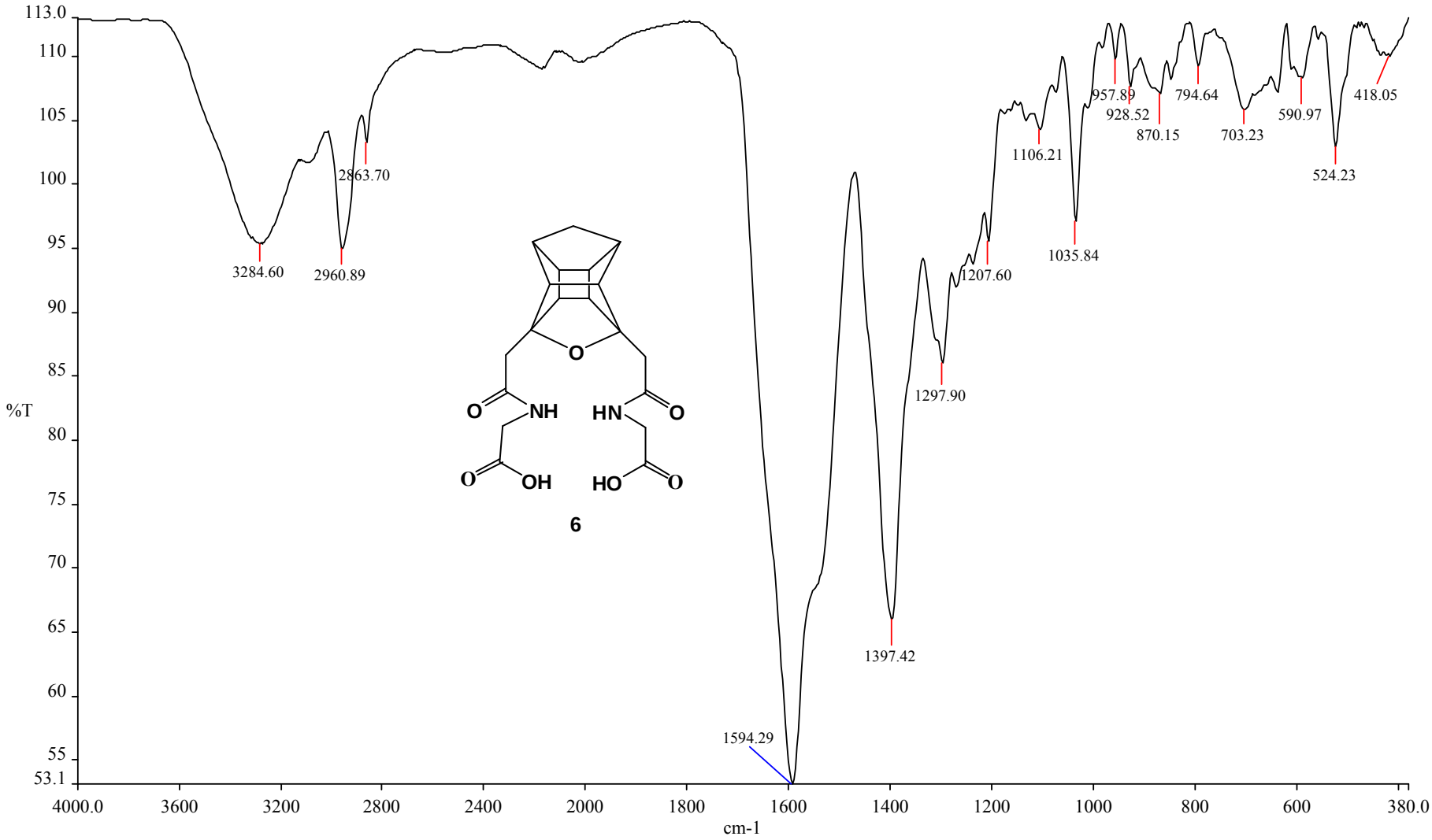
NOESY spectrum of compound 6 in CD_3OD



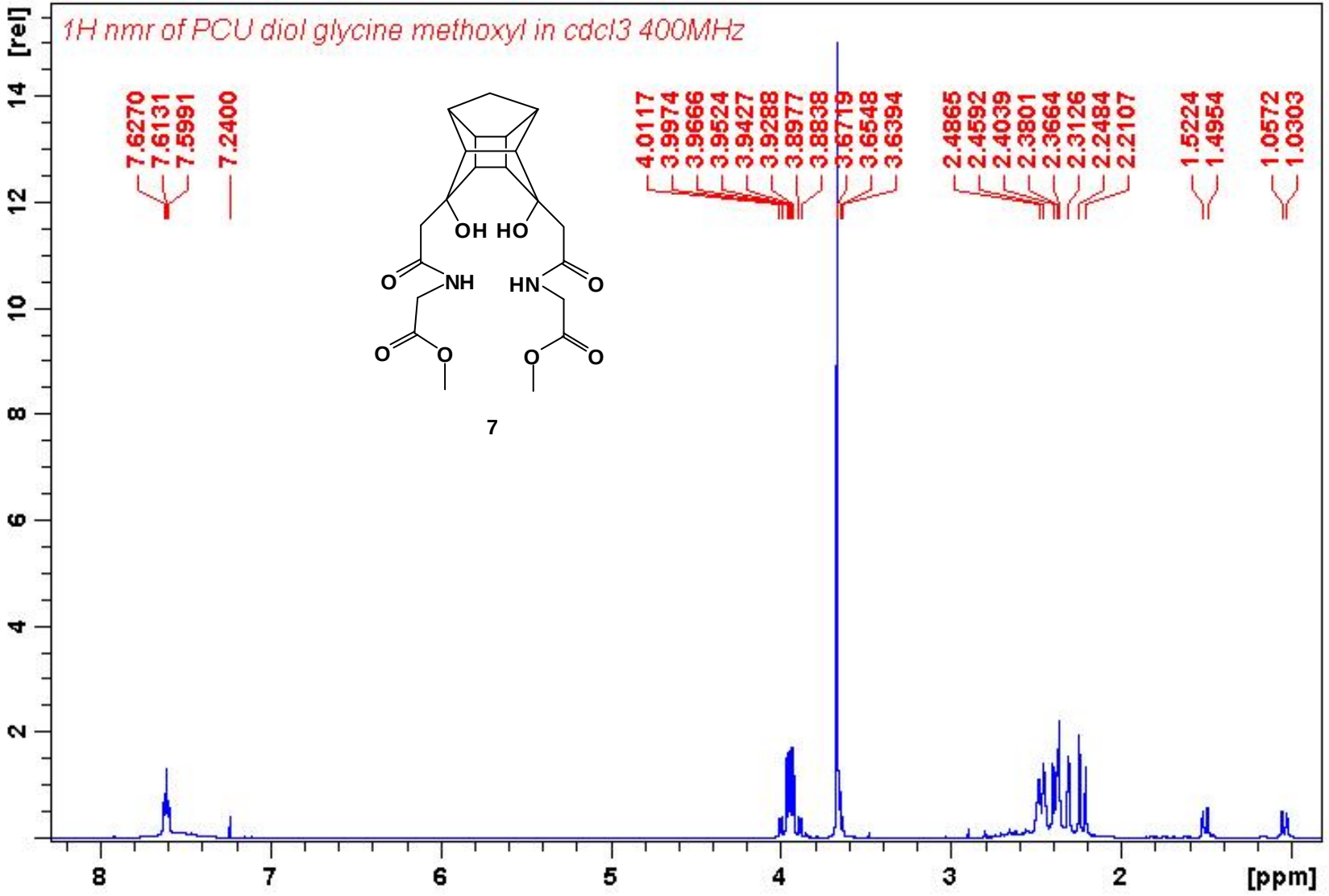
HSQC spectrum of compound 6 in CD_3OD



HMBC spectrum of compound 6 in CD_3OD

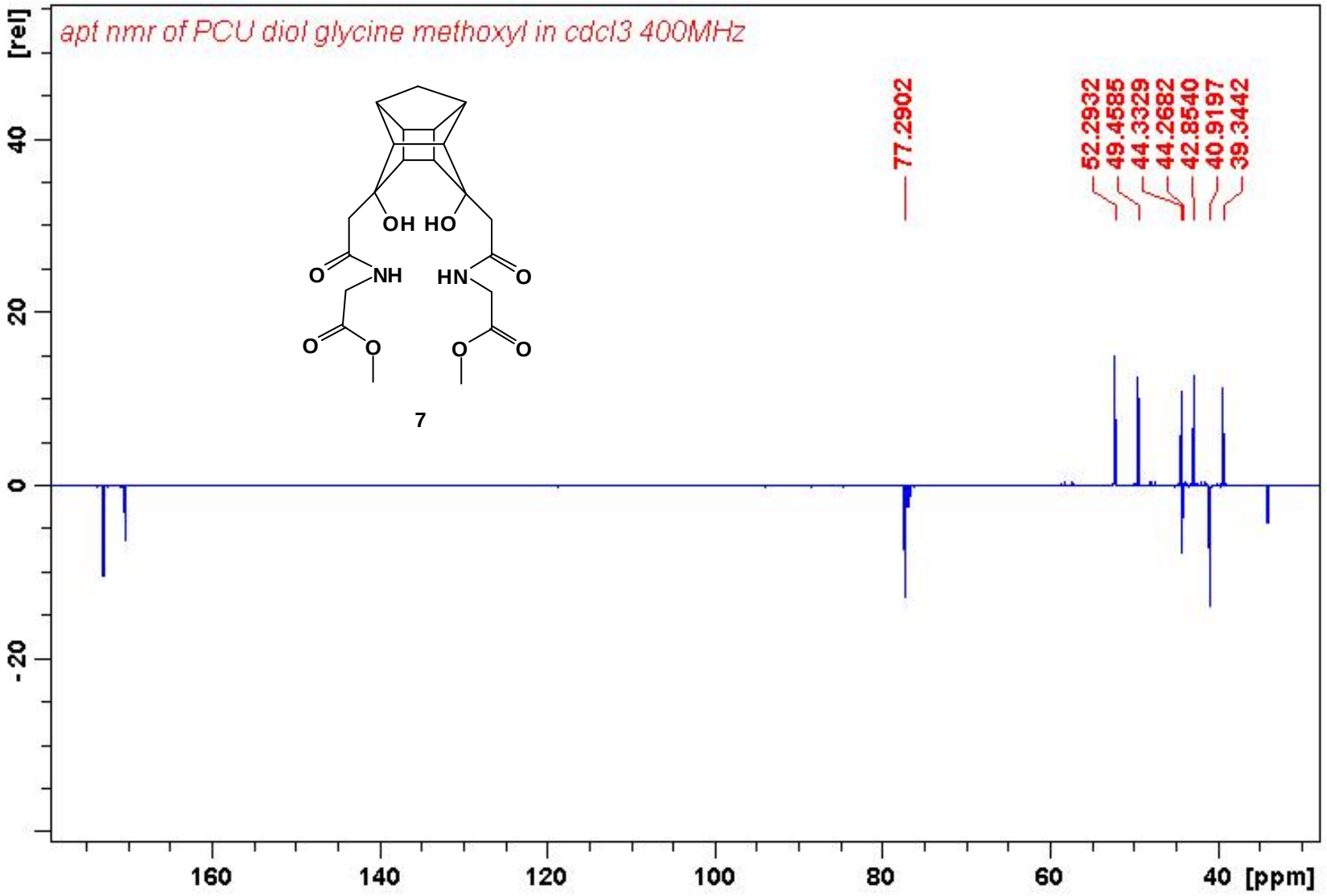


IR spectrum of compound 6

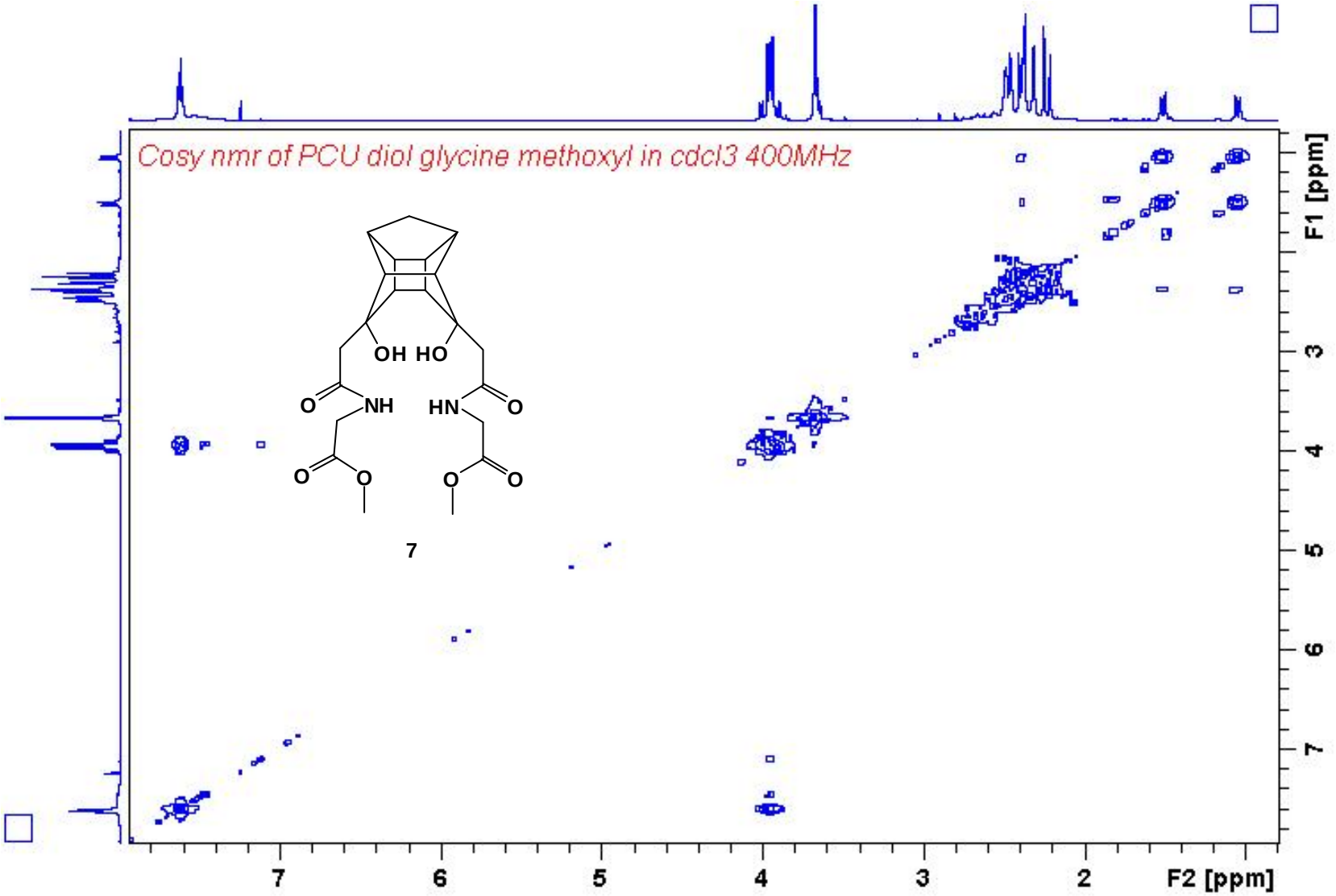


7

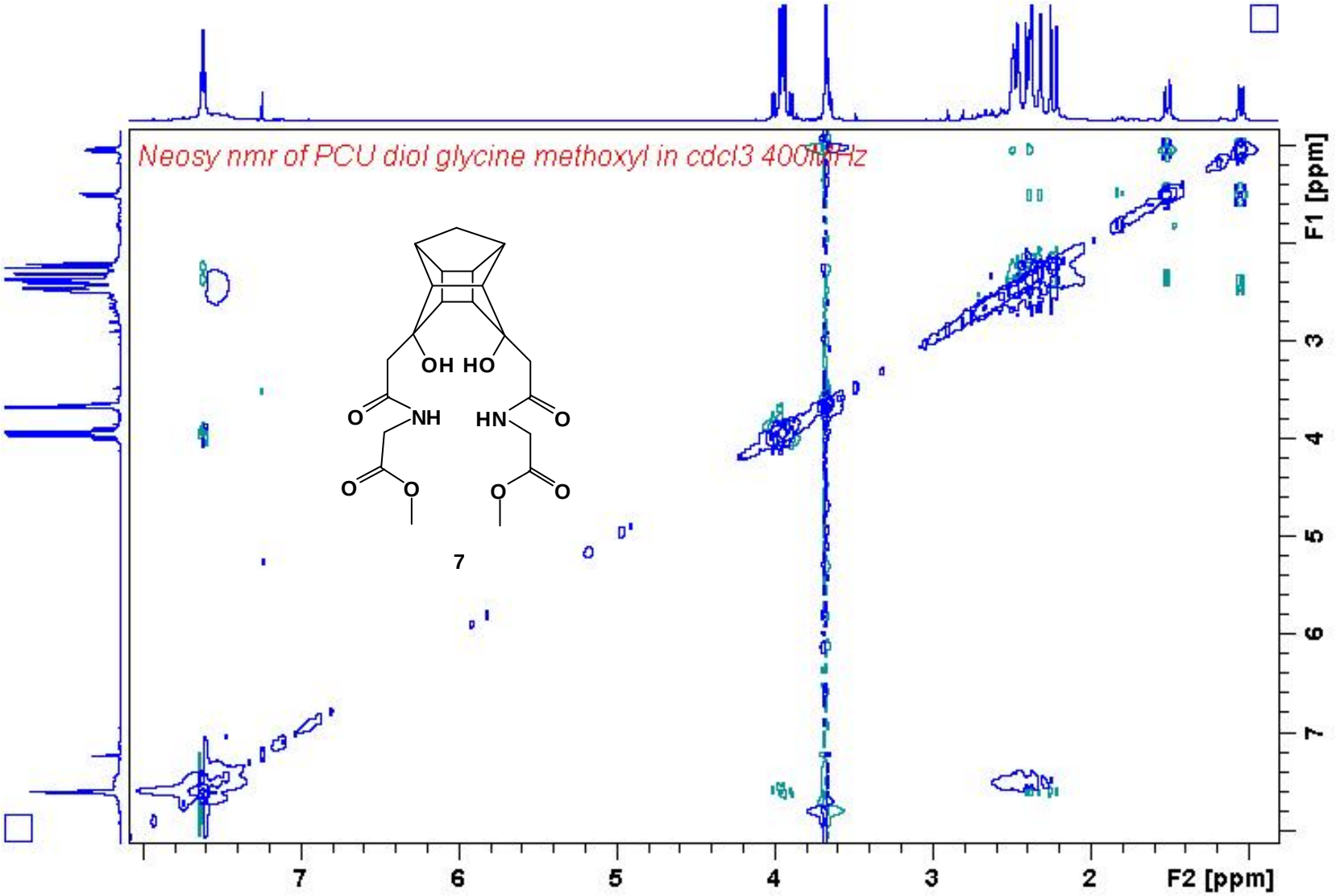
^{1}H NMR spectrum of compound 7 in $CDCl_3$



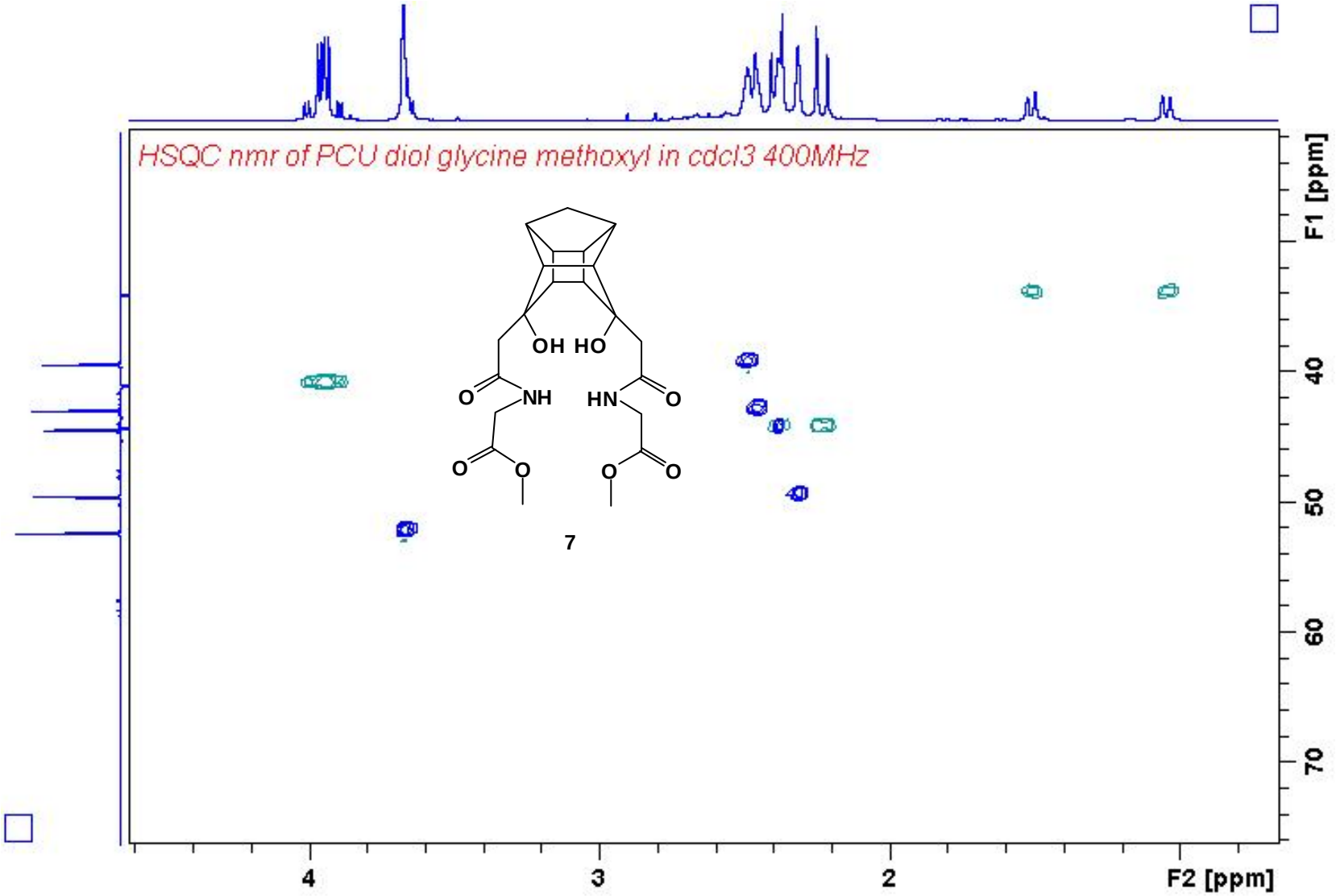
^{13}C NMR spectrum of compound 7 in $CDCl_3$



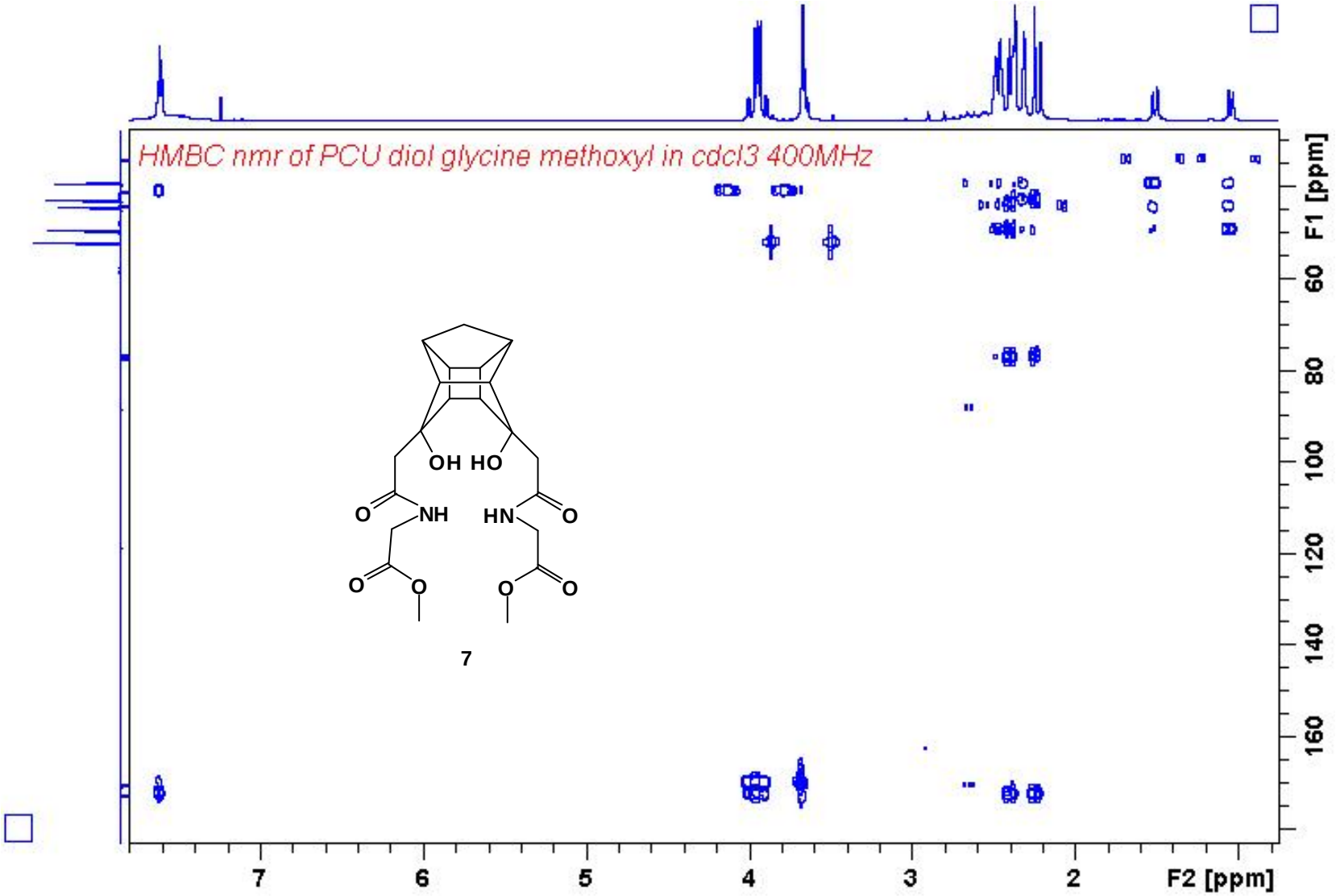
COSY spectrum of compound 7 in $CDCl_3$



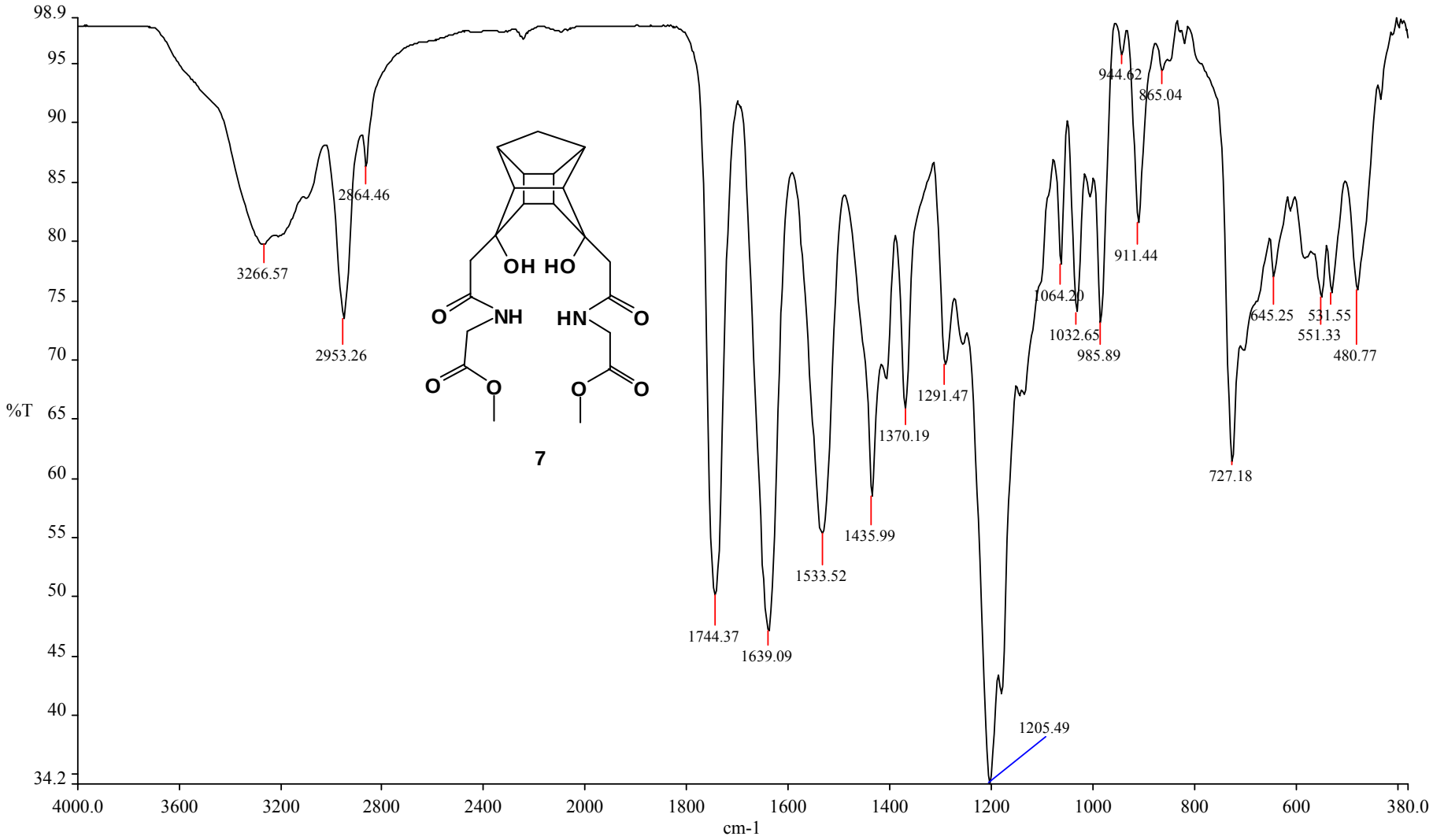
NOESY spectrum of compound 7 in $CDCl_3$



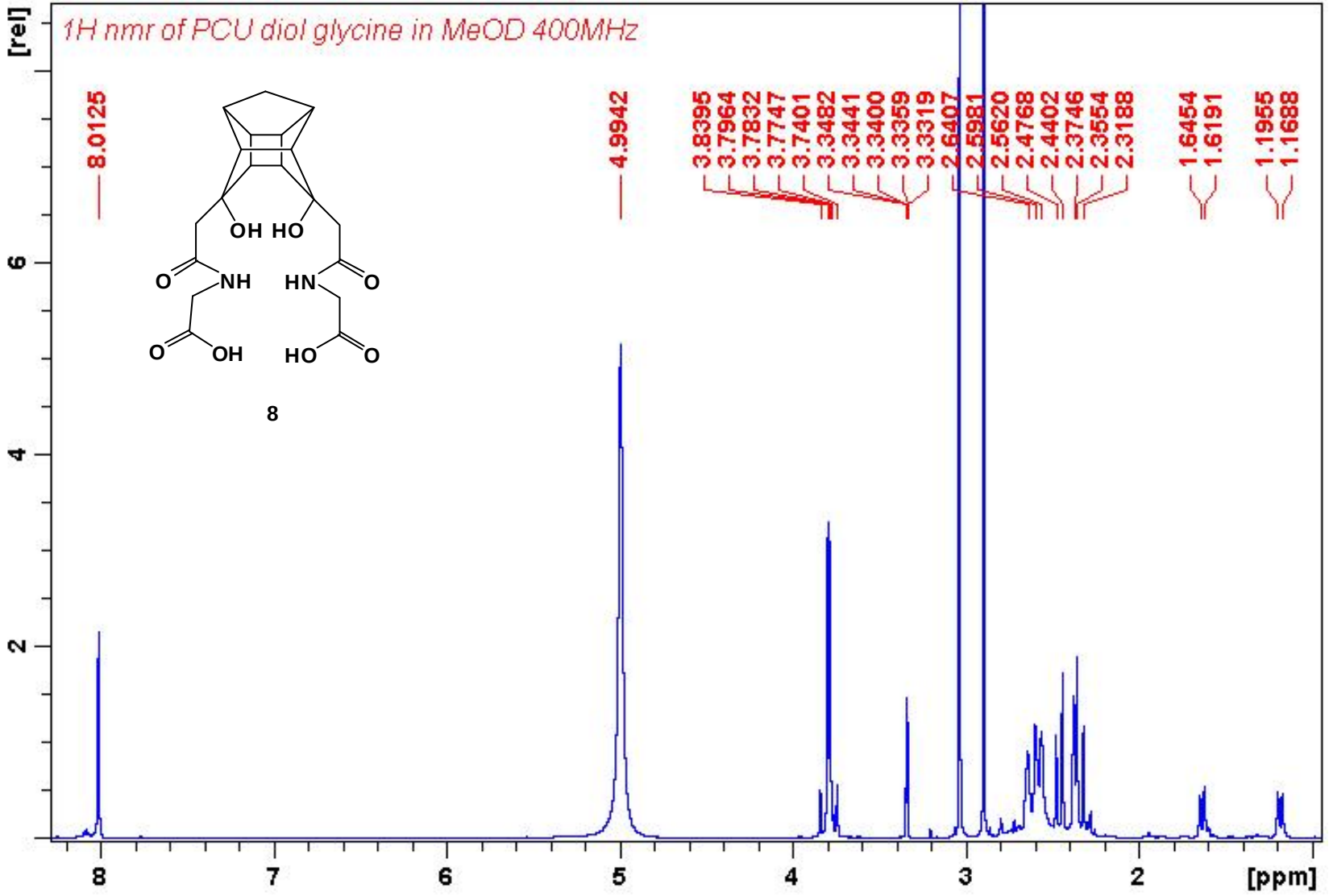
HSQC spectrum of compound 7 in $CDCl_3$



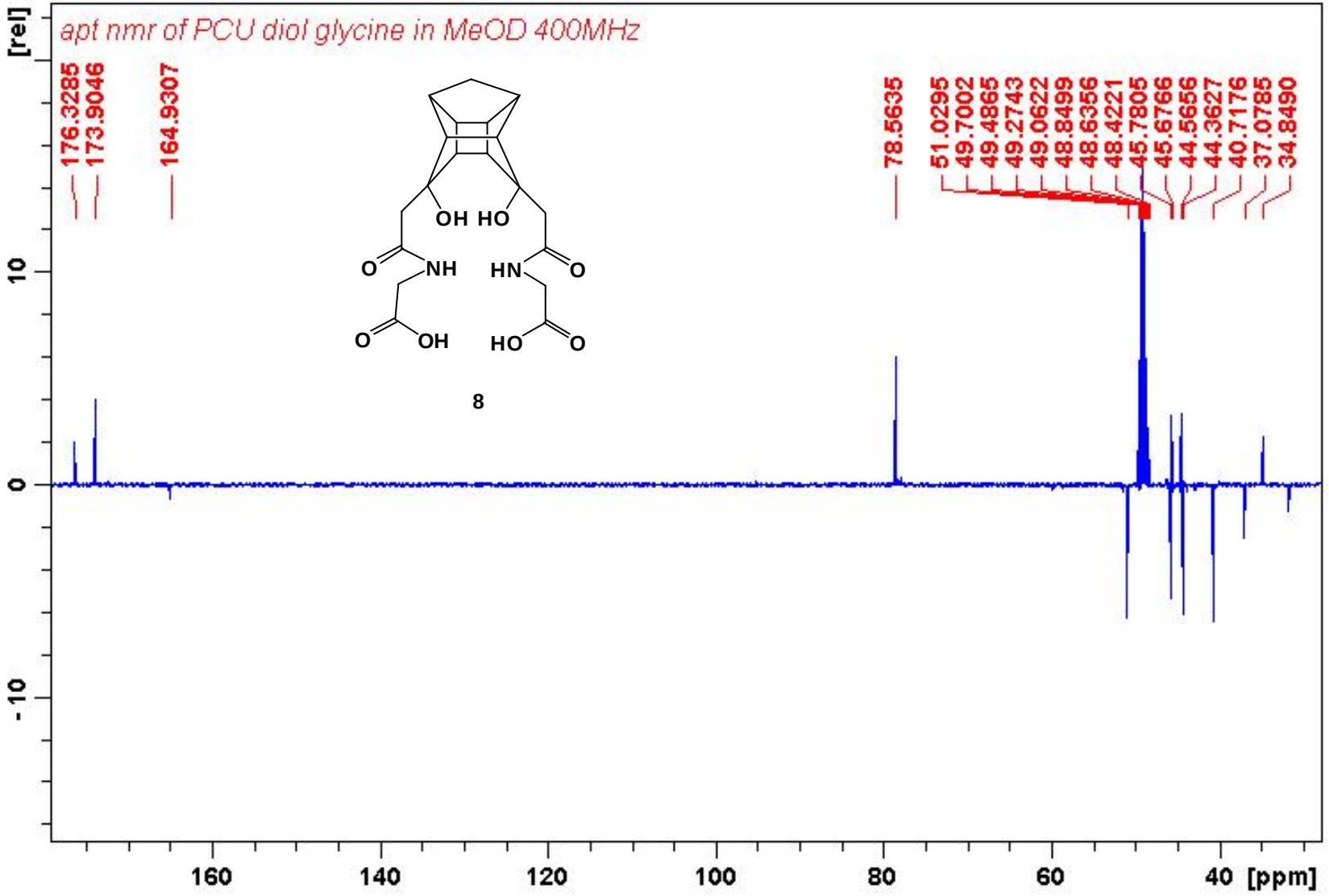
HMBC spectrum of compound 7 in $CDCl_3$



IR spectrum of compound 7

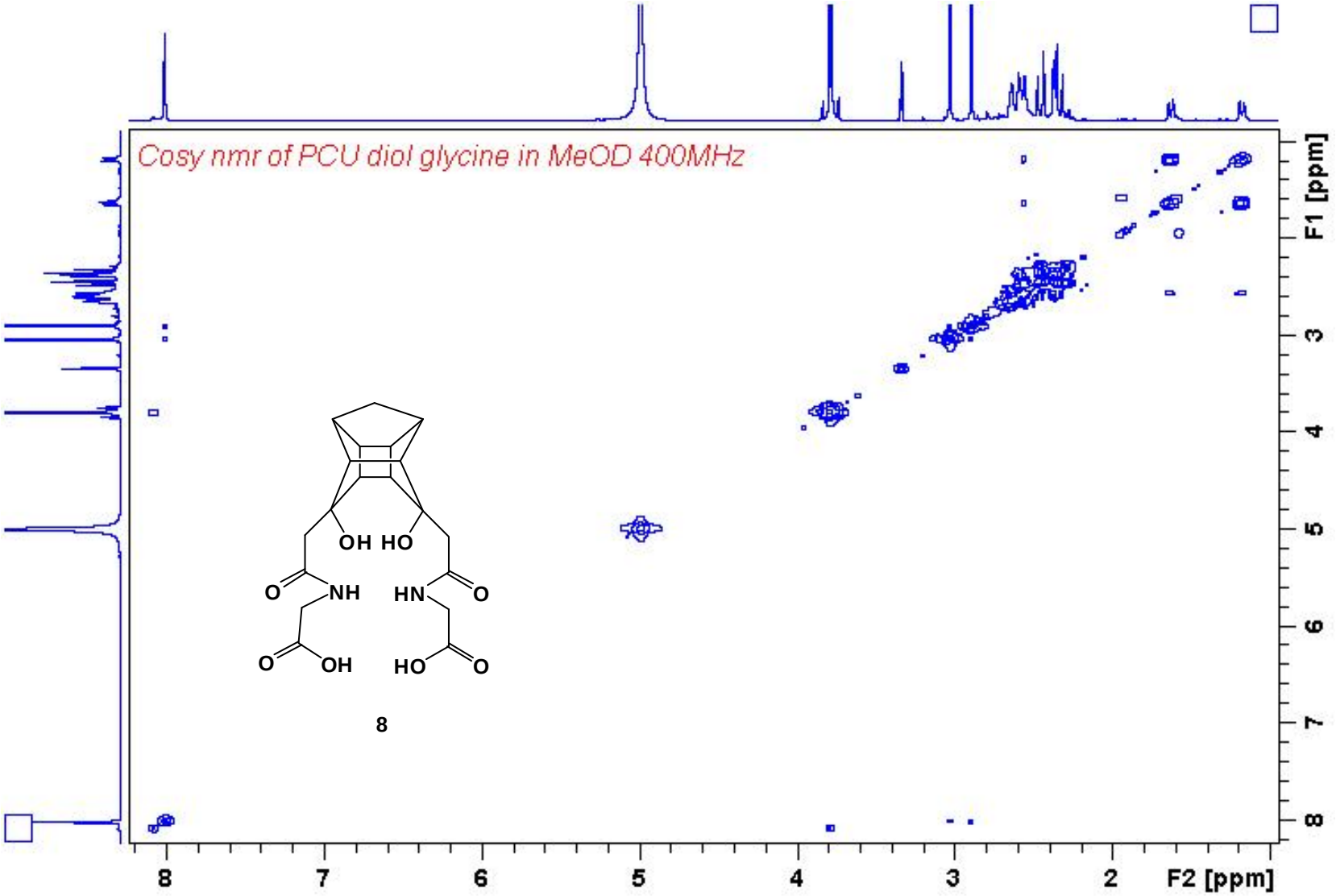


^{1}H NMR spectrum of compound 8 in CD_3OD



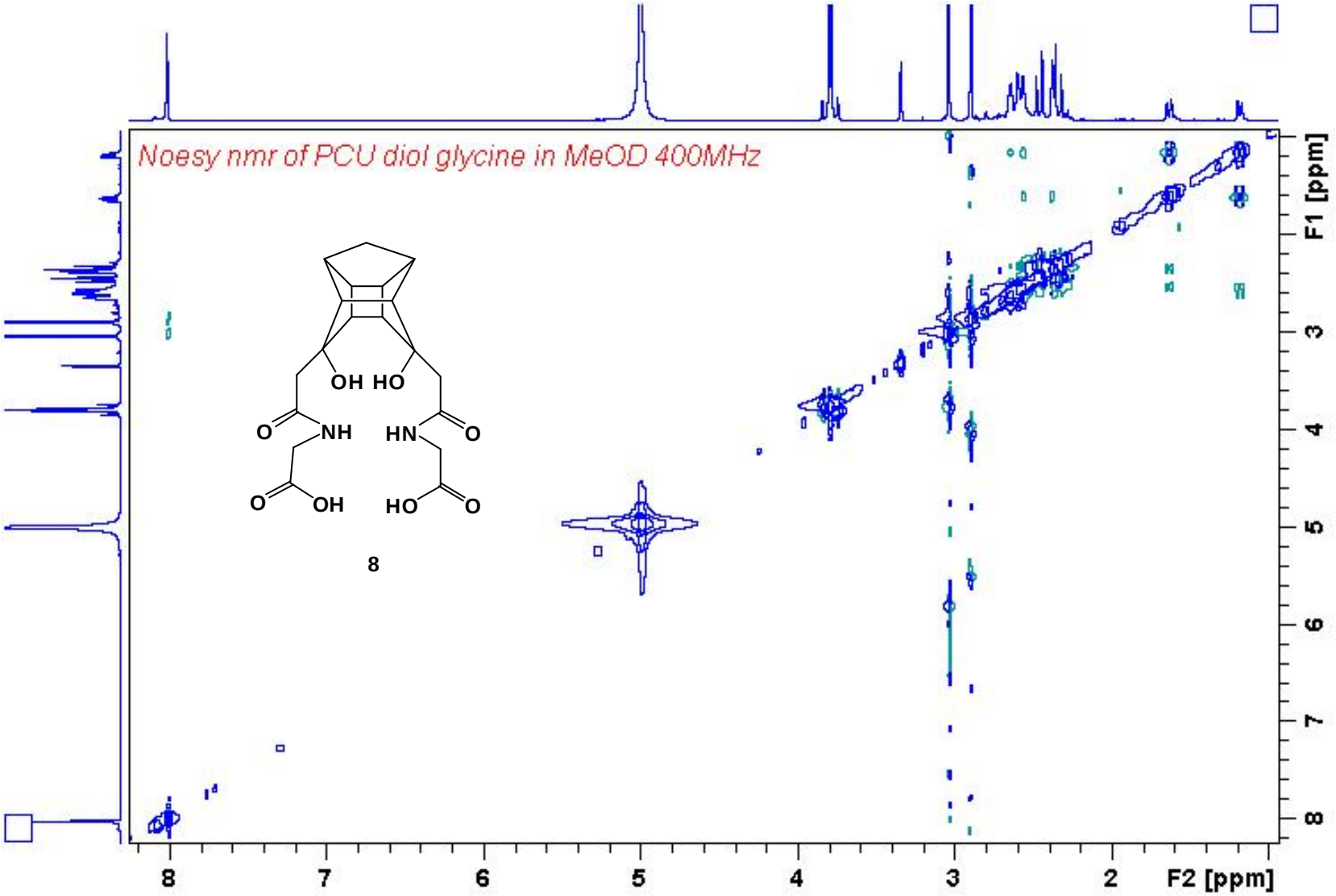
8

^{13}C NMR spectrum of compound 8 in CD_3OD

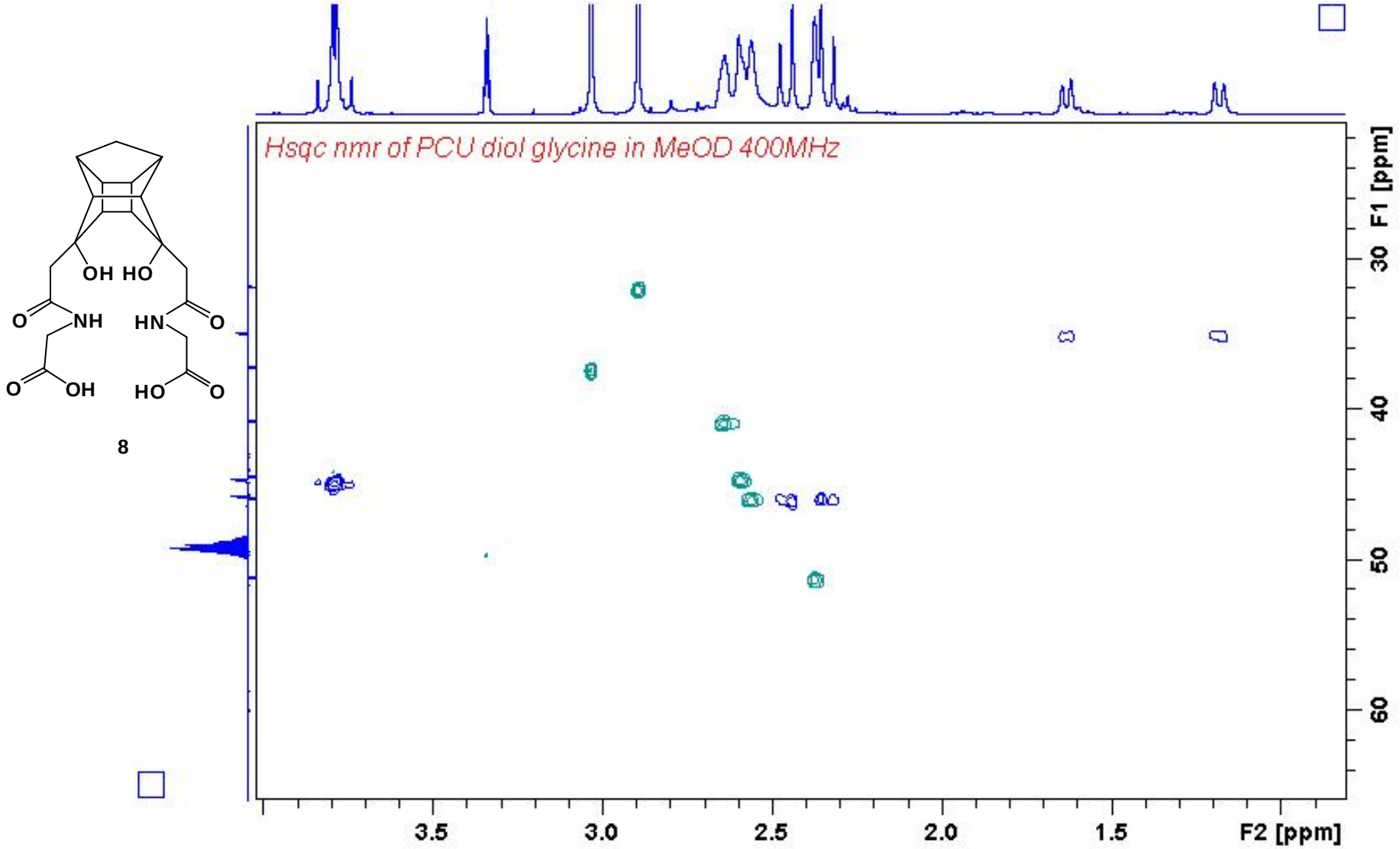


8

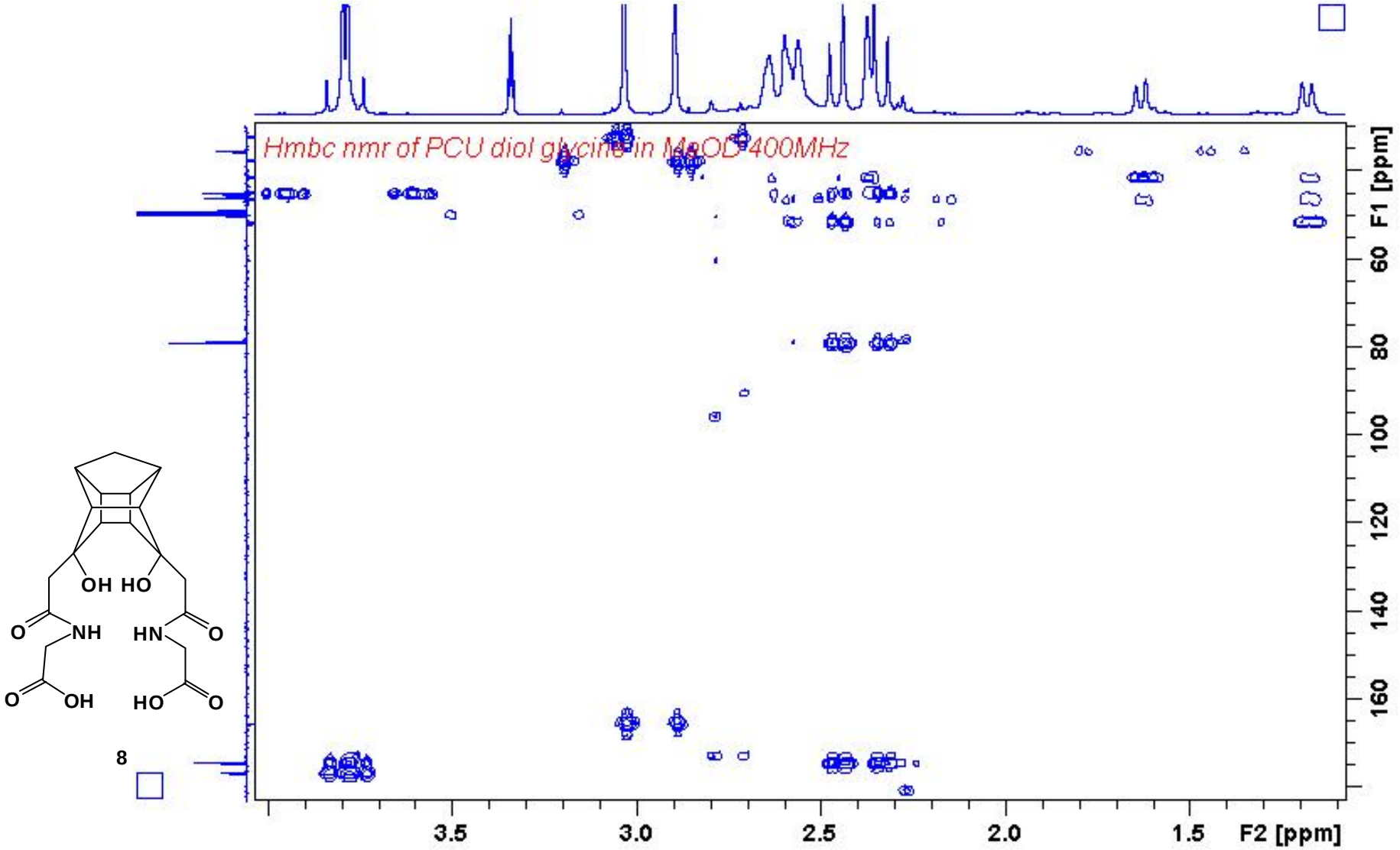
COSY spectrum of compound 8 in CD_3OD



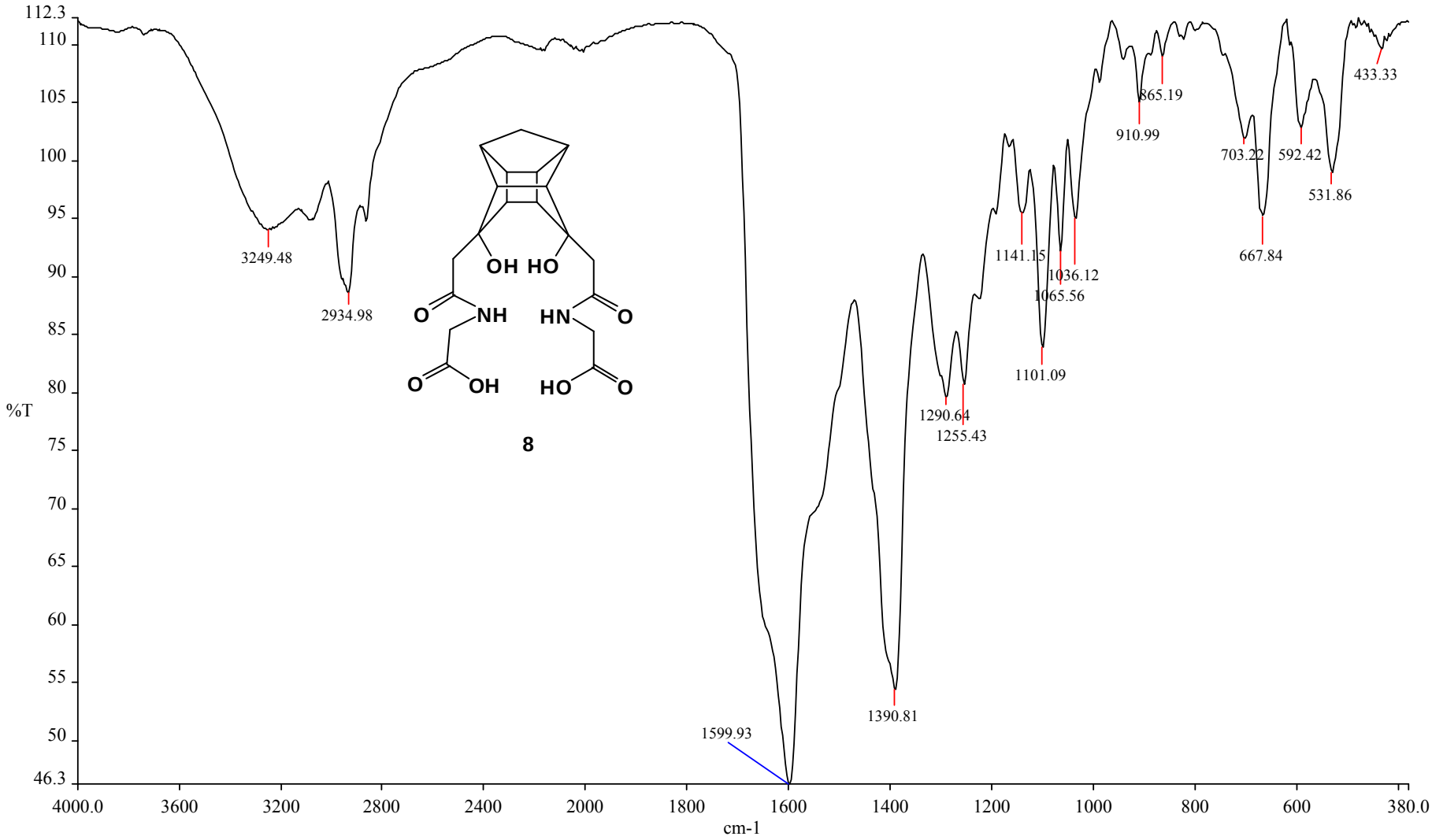
NOESY spectrum of compound 8 in CD_3OD



HSQC spectrum of compound 8 in CD_3OD



HMBC spectrum of compound 8 in CD_3OD



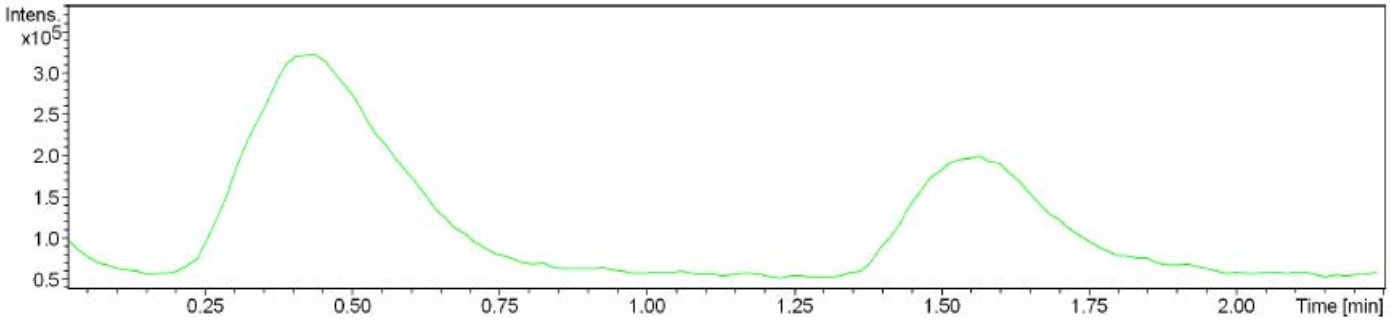
IR spectrum of compound 8

Analysis Info

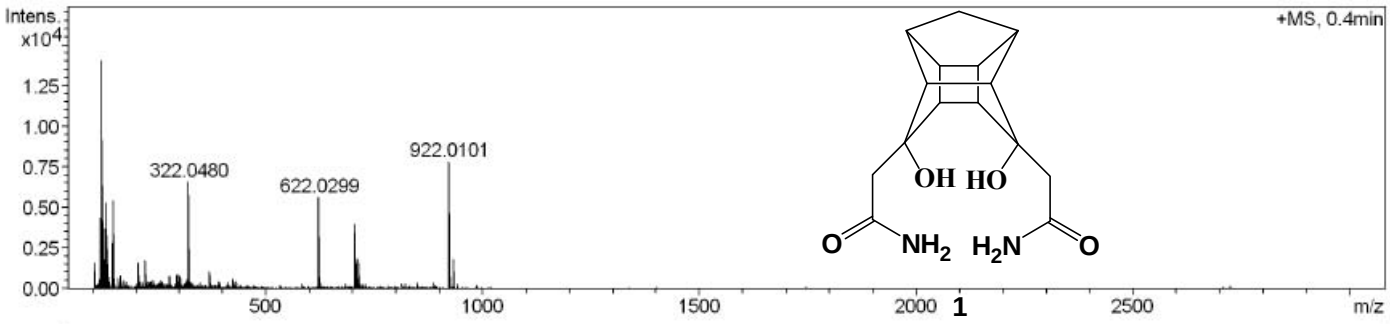
Analysis Name	D:\Data\kenny\PCU diol diamide000001.d	Acquisition Date	6/13/2009 5:34:55 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU diol diamide	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

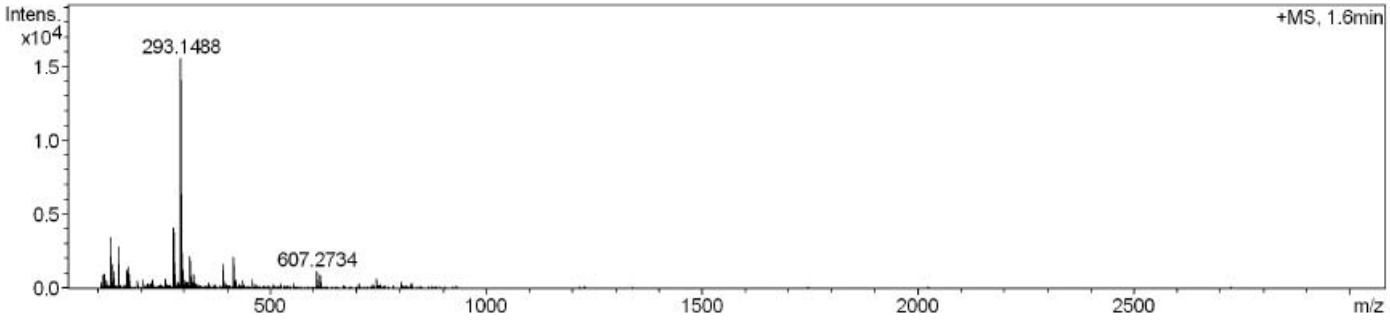
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



TIC +All MS



1



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
293.1488														
	1	C 15 H 21 N 2 O 4	293.1496	2.5	2.1	6.5	ok	even	2.35	0.0049	0.0007	0.0023	0.0010	0.5205
	2	C 13 H 19 N 5 O 3	293.1482	-2.0	-2.5	7.0	ok	odd	8.75	0.0177	0.0008	0.0075	0.0011	0.6744
	3	C 16 H 17 N 6	293.1509	7.1	6.6	11.5	ok	even	13.39	0.0206	0.0020	0.0096	0.0011	0.8036
	4	C 11 H 17 N 8 O 2	293.1469	-6.6	-7.1	7.5	ok	even	15.22	0.0309	0.0021	0.0130	0.0011	0.8436

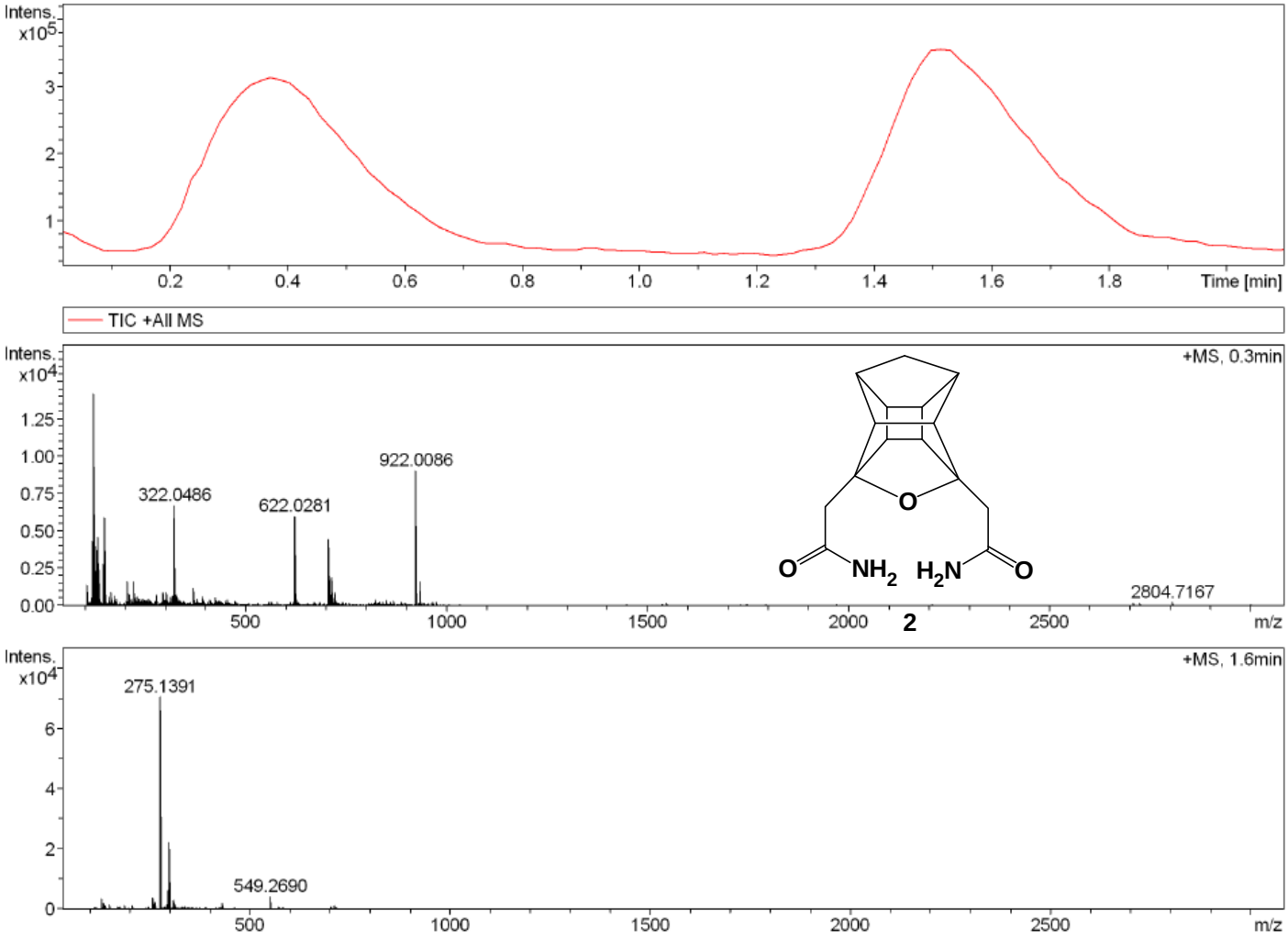
HRMS spectrum of compound 1

Analysis Info

Analysis Name	D:\Data\kenny\PCU ether diamide000001.d	Acquisition Date	6/13/2009 5:16:40 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU ether diamide	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
275.1391														
	1	C 15 H 19 N 2 O 3	275.1390	-0.1	-0.0	7.5	ok	even	0.19	0.0003	0.0001	0.0002	0.0001	0.1786
	2	C 13 H 17 N 5 O 2	275.1377	-5.0	-4.9	8.0	ok	odd	6.47	0.0128	0.0014	0.0053	0.0001	0.6729
	3	C 11 H 15 N 8 O	275.1363	-9.9	-9.9	8.5	ok	even	13.39	0.0276	0.0027	0.0117	0.0000	0.8445
	4	C 18 H 17 N 3	275.1417	9.6	9.7	12.0	ok	odd	20.81	0.0356	0.0027	0.0141	0.0001	0.8618

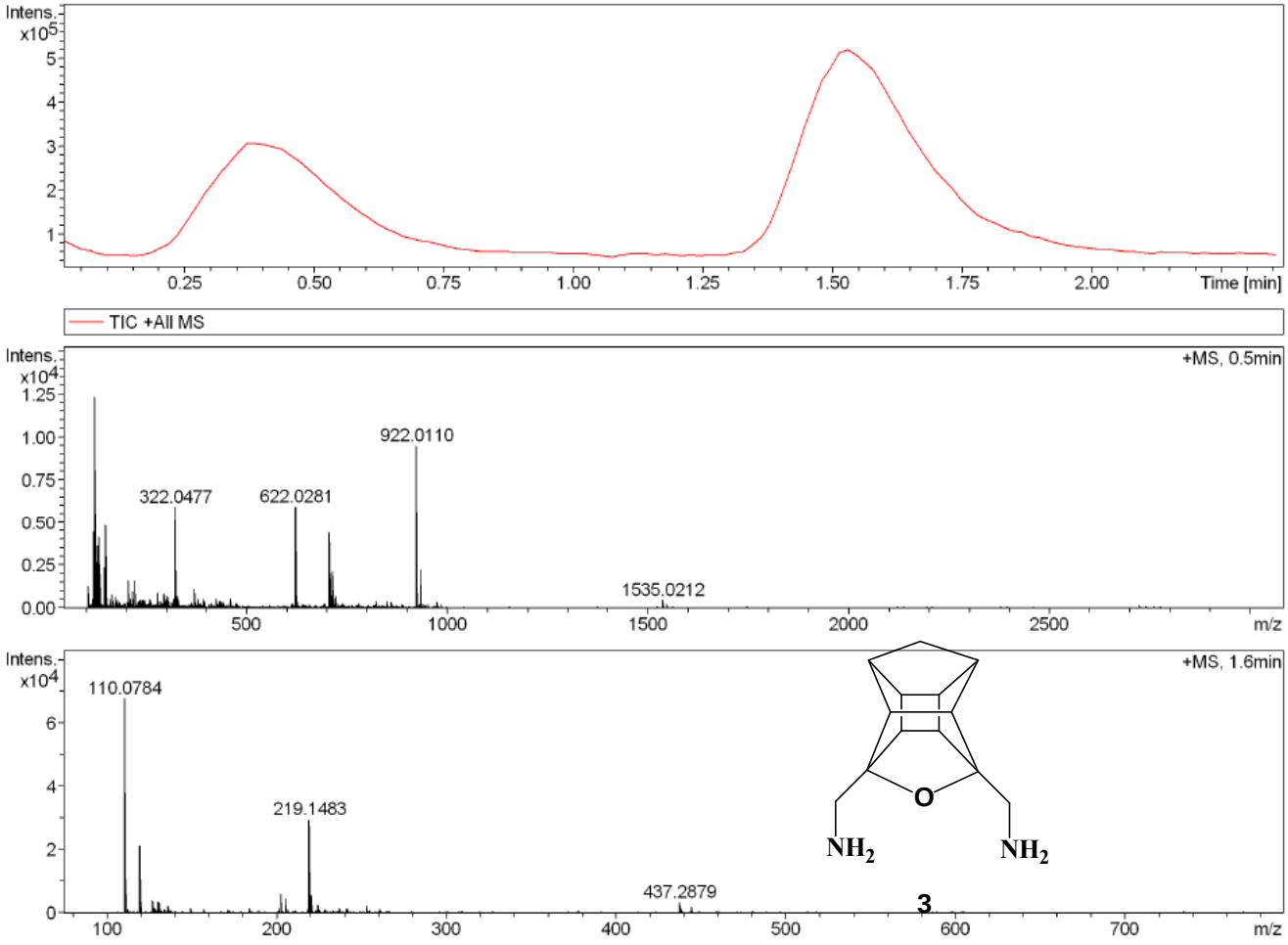
HRMS spectrum of compound 2

Analysis Info

Analysis Name	D:\Data\kenny\PCU ether C1-NH2 (Hofmann rearrangement)000001.d	Acquisition Date	6/13/2009 5:11:42 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU ether C1-NH2 (Hofmann rearrangement)	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
219.1483														
	1	C 13 H 19 N 2 O	219.1492	3.8	10.0	5.5	ok	even	23.03	0.0450	0.0037	0.0184	0.0074	0.8613
	2	C 11 H 17 N 5	219.1478	-2.3	3.9	6.0	ok	odd	29.14	0.0571	0.0031	0.0234	0.0074	0.8672

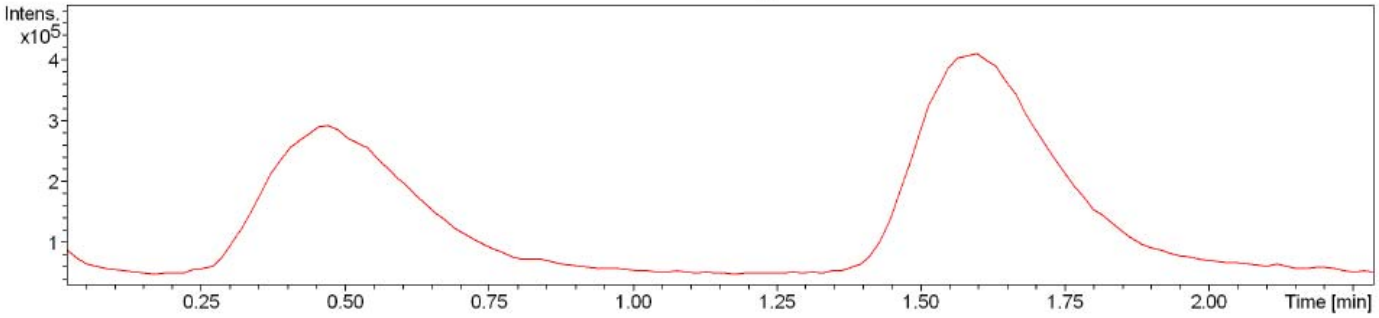
HRMS spectrum of compound 3

Analysis Info

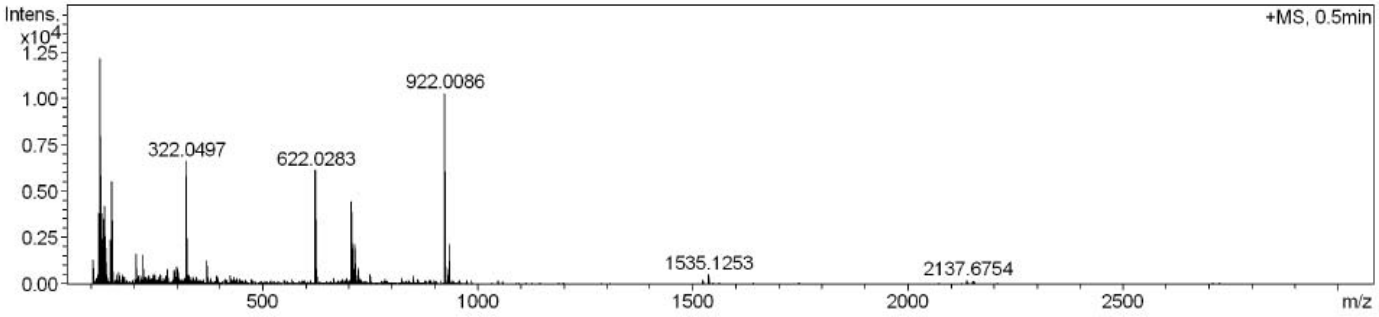
Analysis Name	D:\Data\kenny\PCU ether C2-NH2 (LAH reduction)000001.d	Acquisition Date	6/13/2009 5:05:20 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU ether C2-NH2 (LAH reduction)	Instrument / Ser#	micrOTOF-Q 10139
Comment			

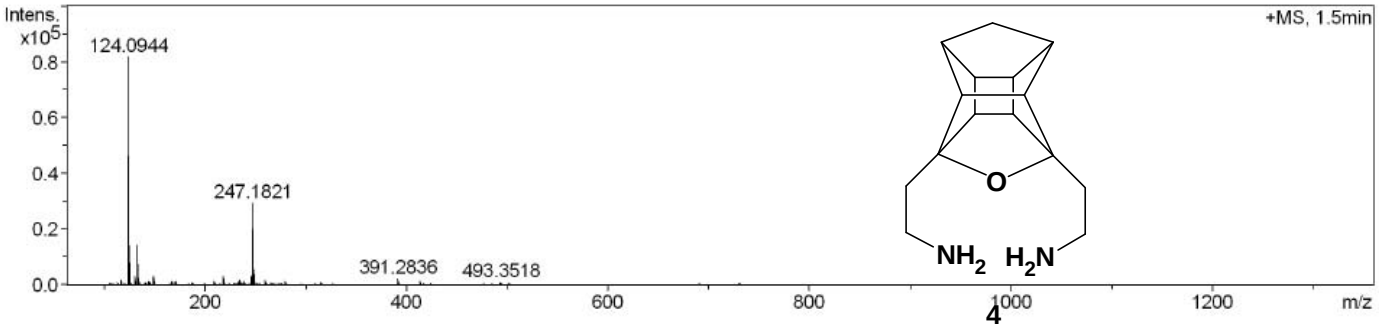
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



TIC +All MS





4

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
247.1821														
	1	C 15 H 23 N 2 O	247.1805	-6.6	-5.5	5.5	ok	even	6.87	0.0135	0.0016	0.0056	0.0012	0.8427
	2	C 13 H 21 N 5	247.1791	-12.1	-11.0	6.0	ok	odd	12.99	0.0251	0.0028	0.0102	0.0012	0.9421

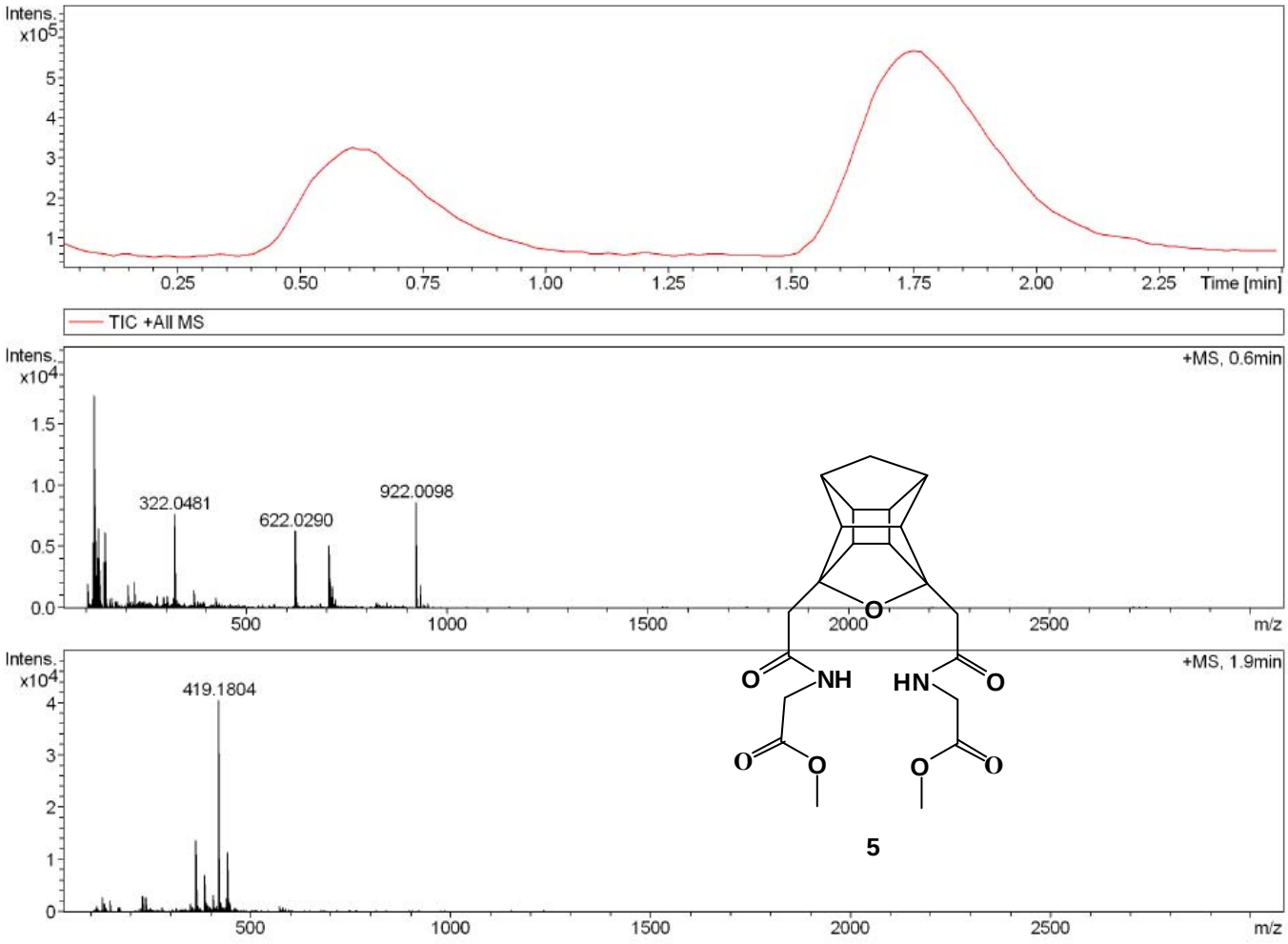
HRMS spectrum of compound 4

Analysis Info

Analysis Name	D:\Data\kenny\PCU ether glycine methoxyl000001.d	Acquisition Date	6/13/2009 5:38:38 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU ether glycine methoxyl	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



5

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
419.1804														
	1	C 21 H 27 N 2 O 7	419.1813	2.0	2.3	9.5	ok	even	4.23	0.0078	0.0010	0.0040	0.0004	0.6529
	2	C 20 H 21 N 9 O 2	419.1813	2.0	2.3	15.0	ok	odd	9.37	0.0129	0.0010	0.0088	0.0003	0.7381
	3	C 19 H 25 N 5 O 6	419.1799	-1.2	-0.9	10.0	ok	odd	10.60	0.0192	0.0005	0.0090	0.0004	0.6602

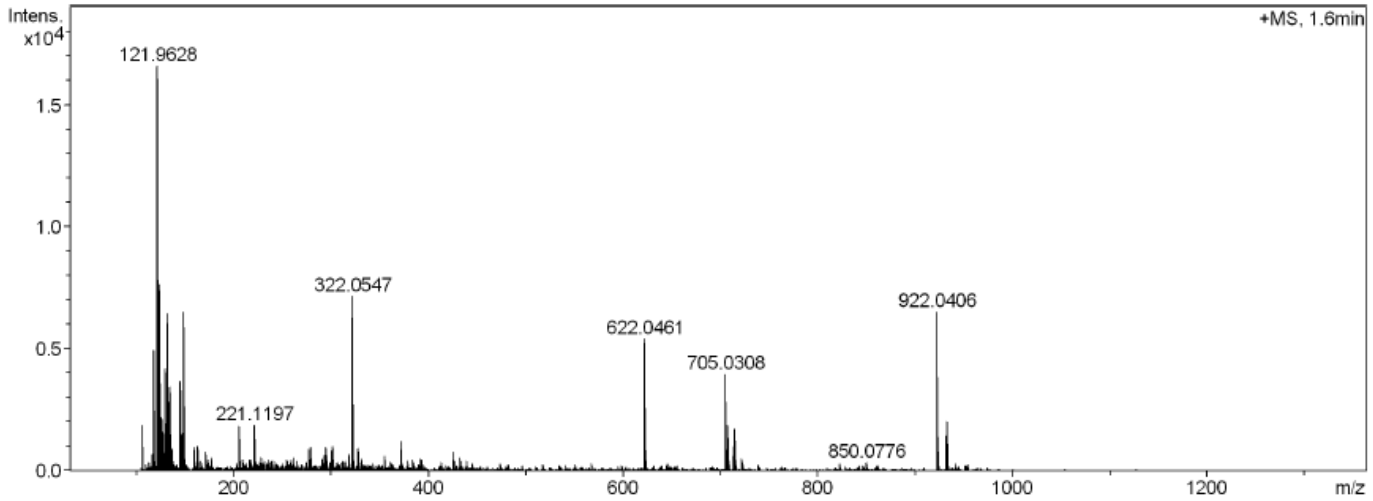
HRMS spectrum of compound 5

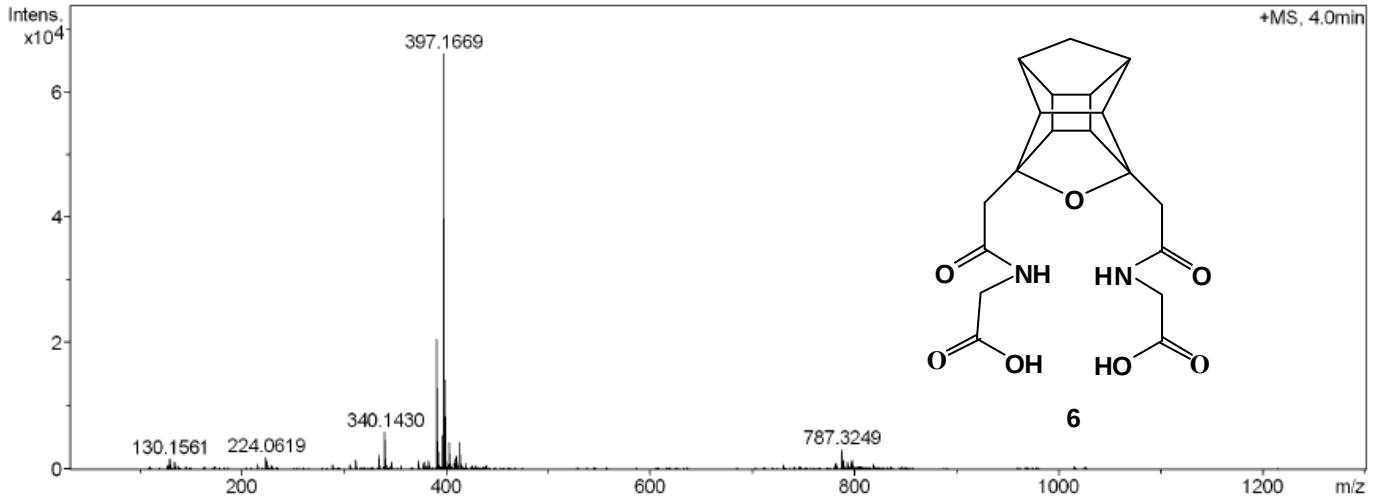
Analysis Info

Analysis Name	D:\Data\kenny\PCU ether glycine 000001.d	Acquisition Date	6/13/2009 6:07:00 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU ether glycine	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source





6

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev

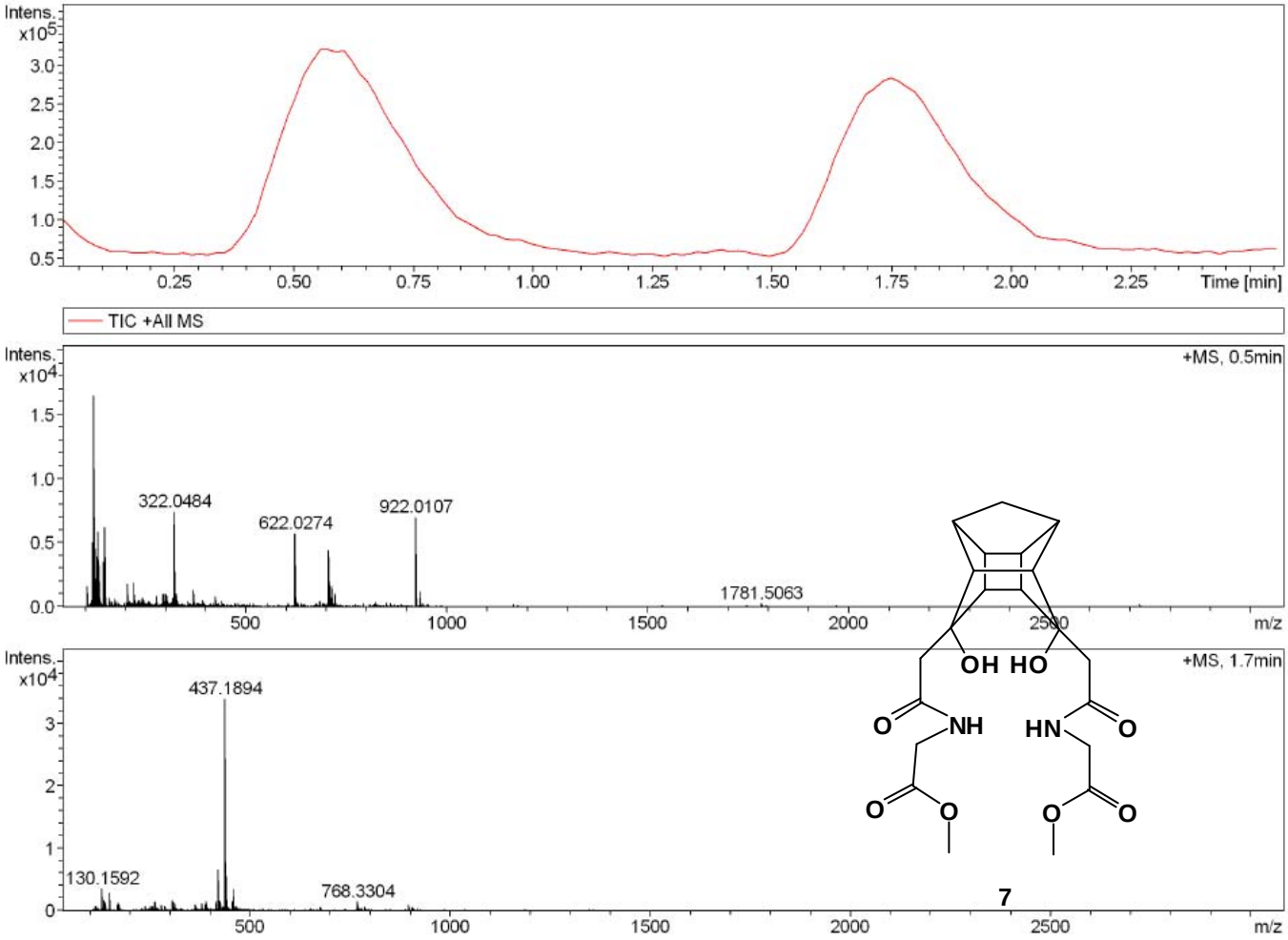
HRMS spectrum of compound 6

Analysis Info

Analysis Name	D:\Data\kenny\PCU diol glycine methoxyl000001.d	Acquisition Date	6/13/2009 5:42:02 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU diol glycine methoxyl	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
437.1894														
	1	C 19 H 27 N 5 O 7	437.1905	2.4	2.7	9.0	ok	odd	3.06	0.0058	0.0013	0.0029	0.0002	0.6713
	2	C 21 H 29 N 2 O 8	437.1918	5.5	5.8	8.5	ok	even	3.56	0.0060	0.0026	0.0021	0.0016	0.7669
	3	C 18 H 21 N 12 O 2	437.1905	2.4	2.6	14.5	ok	even	9.33	0.0127	0.0012	0.0085	0.0001	0.7740

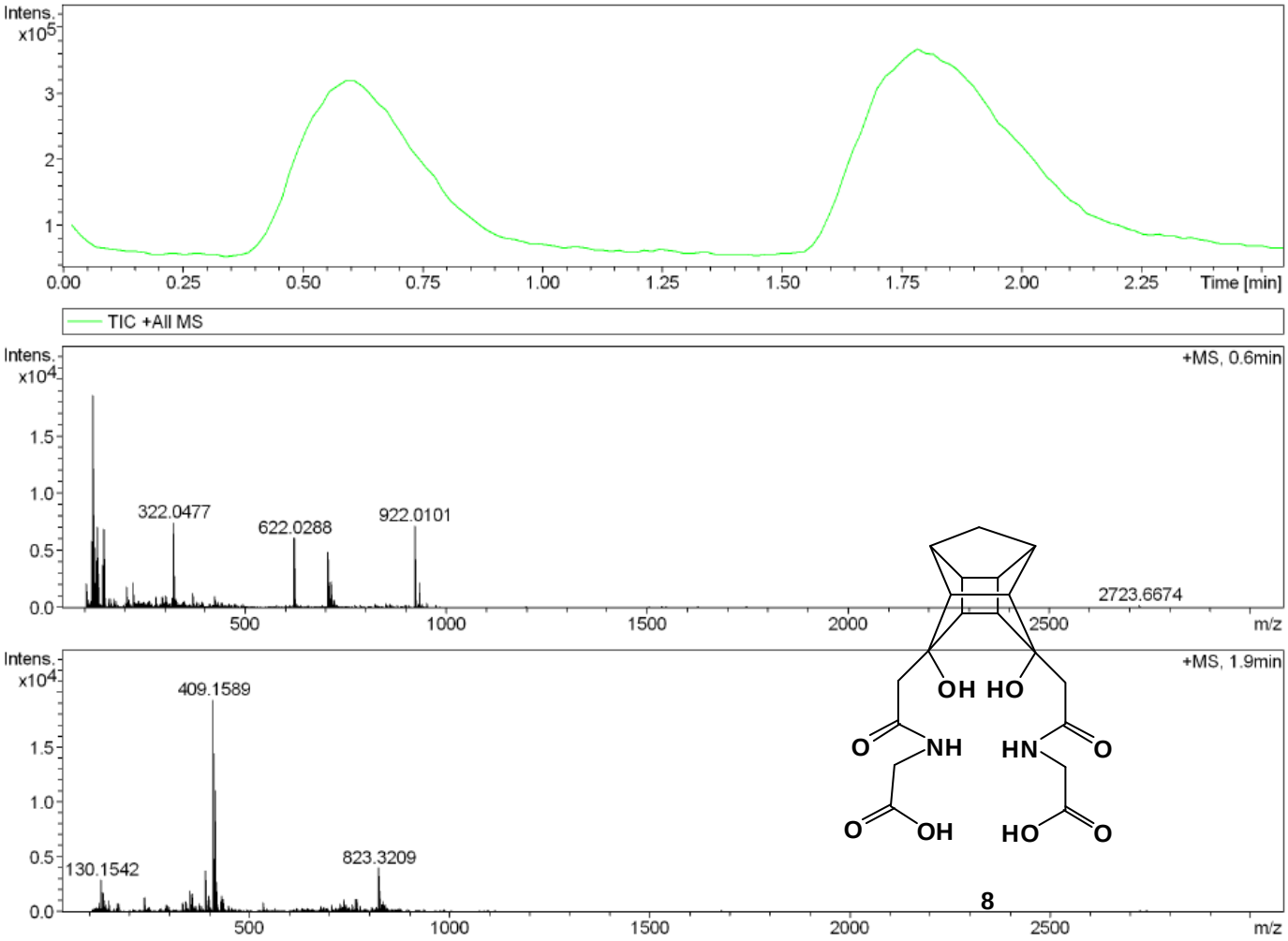
HRMS spectrum of compound 7

Analysis Info

Analysis Name	D:\Data\kenny\PCU diol glycine 000001.d	Acquisition Date	6/13/2009 5:45:24 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU diol glycine	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
409.1589														
	1	C 19 H 25 N 2 O 8	409.1605	4.1	4.1	8.5	ok	even	2.71	0.0048	0.0017	0.0030	0.0001	0.7828
	2	C 17 H 23 N 5 O 7	409.1592	0.8	0.8	9.0	ok	odd	8.16	0.0157	0.0004	0.0075	0.0001	0.6816
	3	C 16 H 17 N 12 O 2	409.1592	0.8	0.8	14.5	ok	even	9.99	0.0163	0.0004	0.0113	0.0003	0.7380

HRMS spectrum of compound 8

Cartesian coordinates of optimised structures for compound 1 and 2

[B3LYP/6-31+G(d)] opt freq

PCU diol **1** (HF=-994.0593253)

0 1

C	-0.49313600	0.13320300	1.53298500
C	-0.79317200	1.64595100	1.25644500
C	-1.50590500	1.73411000	-0.13457700
C	-1.08951500	0.35851700	-0.73532000
C	-1.33878100	-0.60047900	0.46816800
C	1.48295900	-0.13189900	-0.21978000
C	0.45266000	0.60855200	-1.10647200
C	0.66388100	2.08722000	-0.65696500
C	0.70722400	1.89026000	0.89379200
C	1.02274300	0.37784200	1.16616100
C	-0.68196100	2.77036700	-0.89894200
O	-1.04729200	-1.97955700	0.25750000
O	1.42082700	-1.55216300	-0.45100300
C	-2.82204200	-0.61161200	0.92562600
C	2.93130300	0.33359500	-0.57094200
C	-3.84066600	-0.90440800	-0.17735400
C	4.06085700	-0.63643600	-0.22835000
O	-4.76163200	-0.13055200	-0.43454500
N	-3.67167300	-2.09448800	-0.82053400
O	4.96273800	-0.88833600	-1.01010200
N	3.98114000	-1.27229900	1.00512400
H	-0.65286300	-0.23906100	2.54889000
H	-1.23699800	2.24852700	2.05281300
H	-2.58337600	1.90207200	-0.10609400
H	-1.65773600	0.07594500	-1.62560600
H	0.63490400	0.43546500	-2.17136600
H	1.53683900	2.57748000	-1.09469500
H	1.26080200	2.65020600	1.45181600
H	1.70889300	0.16629600	1.99365600

H	-0.94955200	2.83985600	-1.96036700
H	-0.73663600	3.77438100	-0.45851100
H	-0.12960800	-2.04700800	-0.08551500
H	2.08381300	-1.99212600	0.11291900
H	-2.89815600	-1.39094600	1.69421200
H	-3.11921500	0.33332100	1.37975100
H	3.01404500	0.49904800	-1.64876600
H	3.15718100	1.28855300	-0.08067800
H	-2.79804400	-2.59724900	-0.69998700
H	4.81657800	-1.78479000	1.27018100
H	-4.26383400	-2.29457000	-1.61595600
H	3.51087100	-0.80173300	1.76951800

PCU ether **2** (HF=-917.649667)

0 1

C	-0.26294300	0.63829200	1.57612100
C	-0.59851300	2.12817100	1.26670800
C	-1.52655100	2.17121500	0.02234400
C	-1.23584600	0.79399200	-0.62272500
C	-0.96181600	-0.24182800	0.50299600
C	1.05398500	0.02293500	-0.35865300
C	0.20519300	0.98306400	-1.23803700
C	0.55611400	2.44515800	-0.86994000
C	0.83599200	2.31685000	0.65190000
C	1.16183500	0.82639100	0.96779000
C	-0.80842100	3.16568100	-0.91447900
O	0.12634600	-1.05030600	-0.02830900
C	-2.11615000	-1.10887800	0.98317000
C	2.33027400	-0.51739200	-0.98589200
C	-2.87680800	-1.91098200	-0.07928500
C	3.23261800	-1.38626600	-0.10198100
O	-4.10261900	-1.97116100	-0.08084100
N	-2.10390000	-2.58871900	-0.97549900
O	4.45293400	-1.25569400	-0.11003000
N	2.59720600	-2.33696700	0.64057500
H	-0.34551700	0.26900200	2.60138100
H	-0.88826900	2.77871100	2.09536000
H	-2.58197400	2.37046500	0.23014100
H	-1.98017600	0.46540300	-1.35254300

H	0.25538900	0.75234700	-2.30633000
H	1.36363000	2.89143700	-1.45803900
H	1.47799000	3.08989600	1.08054000
H	2.02721800	0.58932100	1.59010700
H	-1.24544200	3.20658800	-1.91981000
H	-0.76890000	4.18552500	-0.51044800
H	-1.74242500	-1.81392000	1.73905800
H	-2.85825200	-0.47501300	1.47717800
H	2.07039200	-1.09964500	-1.88127000
H	2.94788500	0.32154100	-1.31967000
H	-1.10576100	-2.42232500	-1.02649100
H	3.15424700	-2.91621800	1.25487000
H	1.58660000	-2.34659000	0.71360800
H	-2.56683700	-3.10784800	-1.70996200

CHAPTER 10

SYNTHESIS AND NMR ELUCIDATION OF NOVEL PENTACYCLO-UNDECANE DIAMINE LIGANDS

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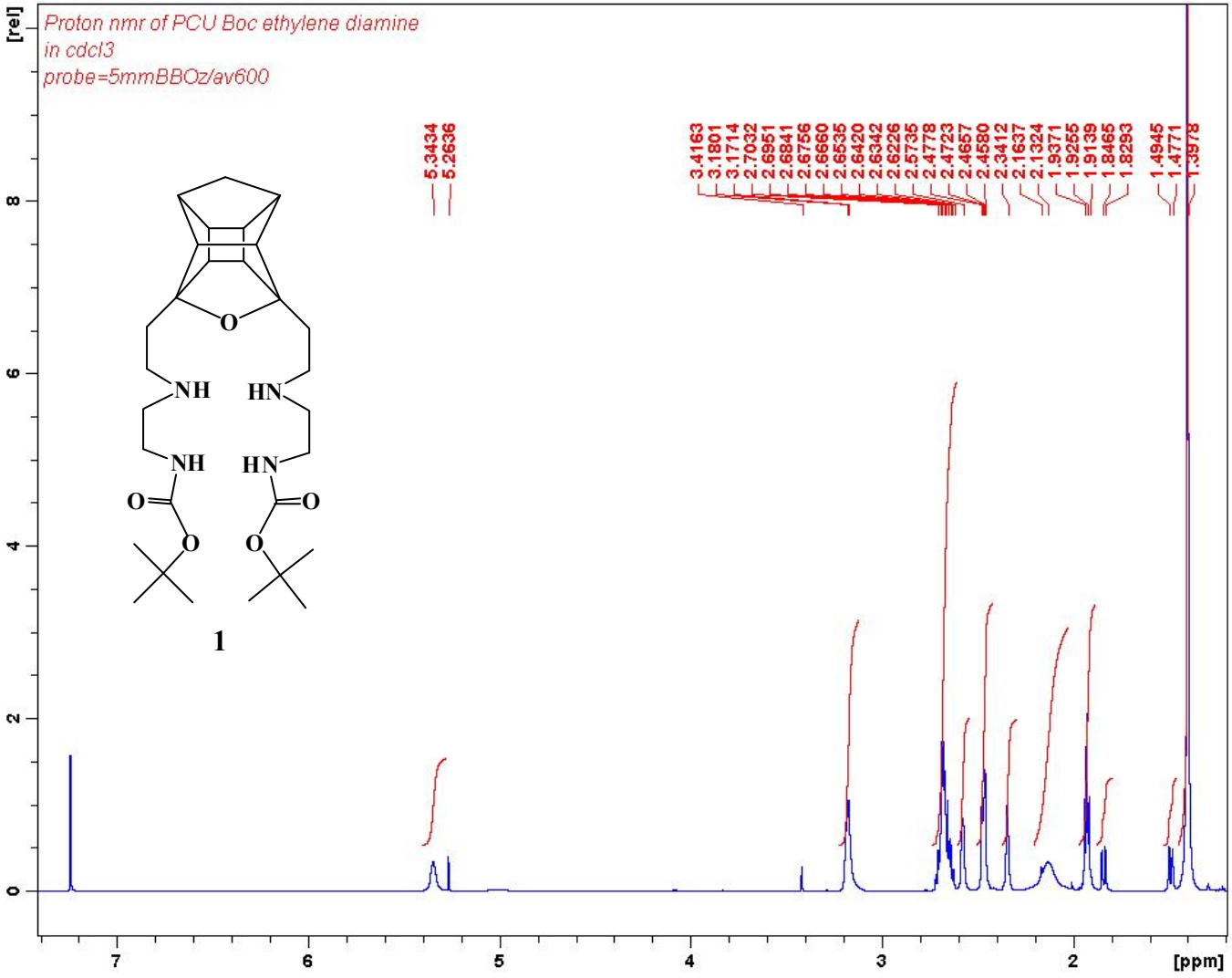

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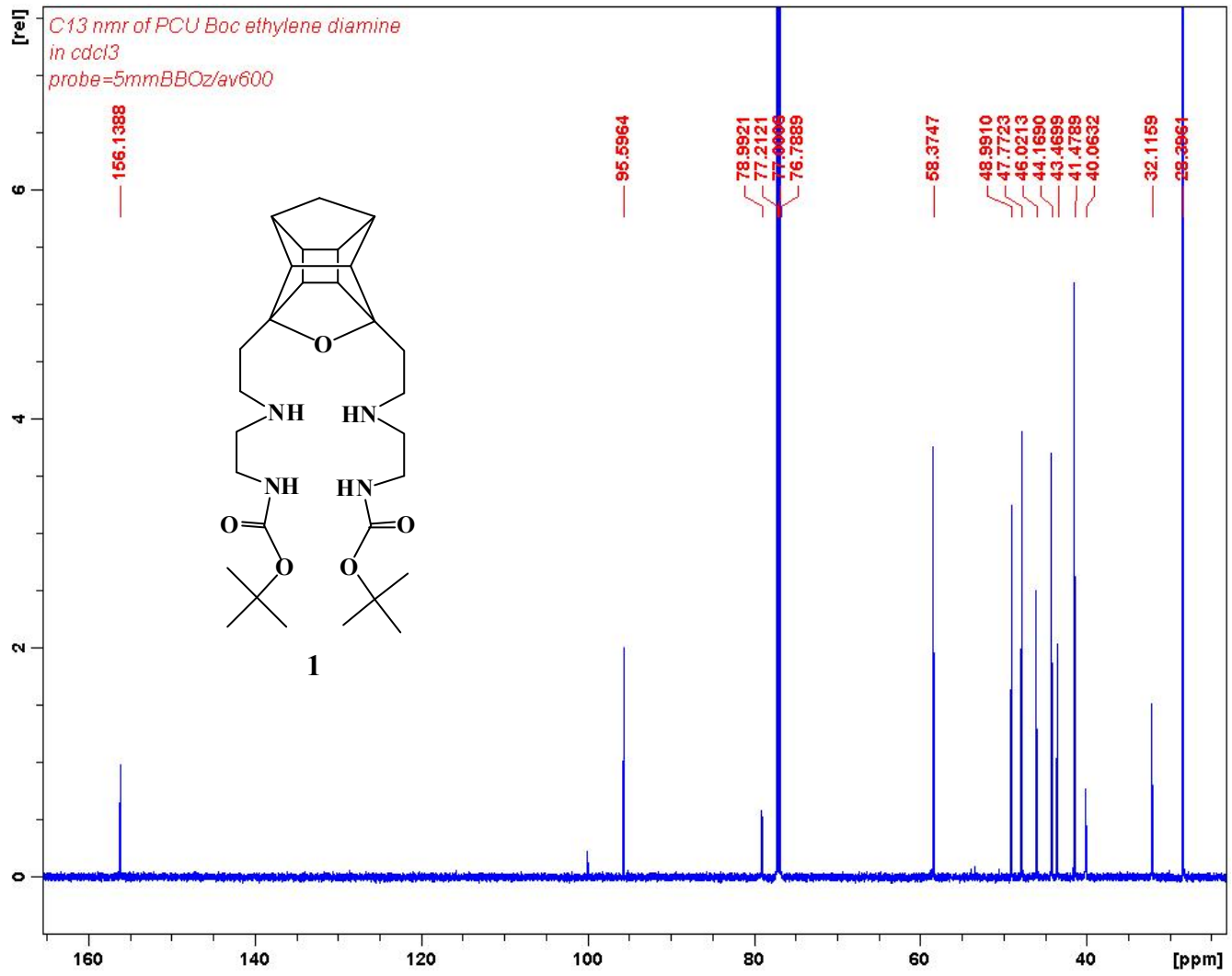
^{1}H NMR spectrum of *N*-tert-butoxycarbonylethylene diamine PCU (1)



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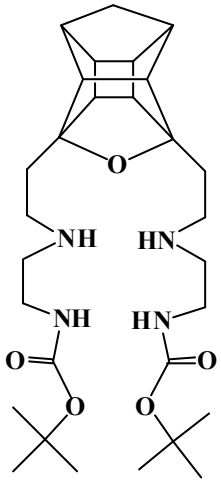

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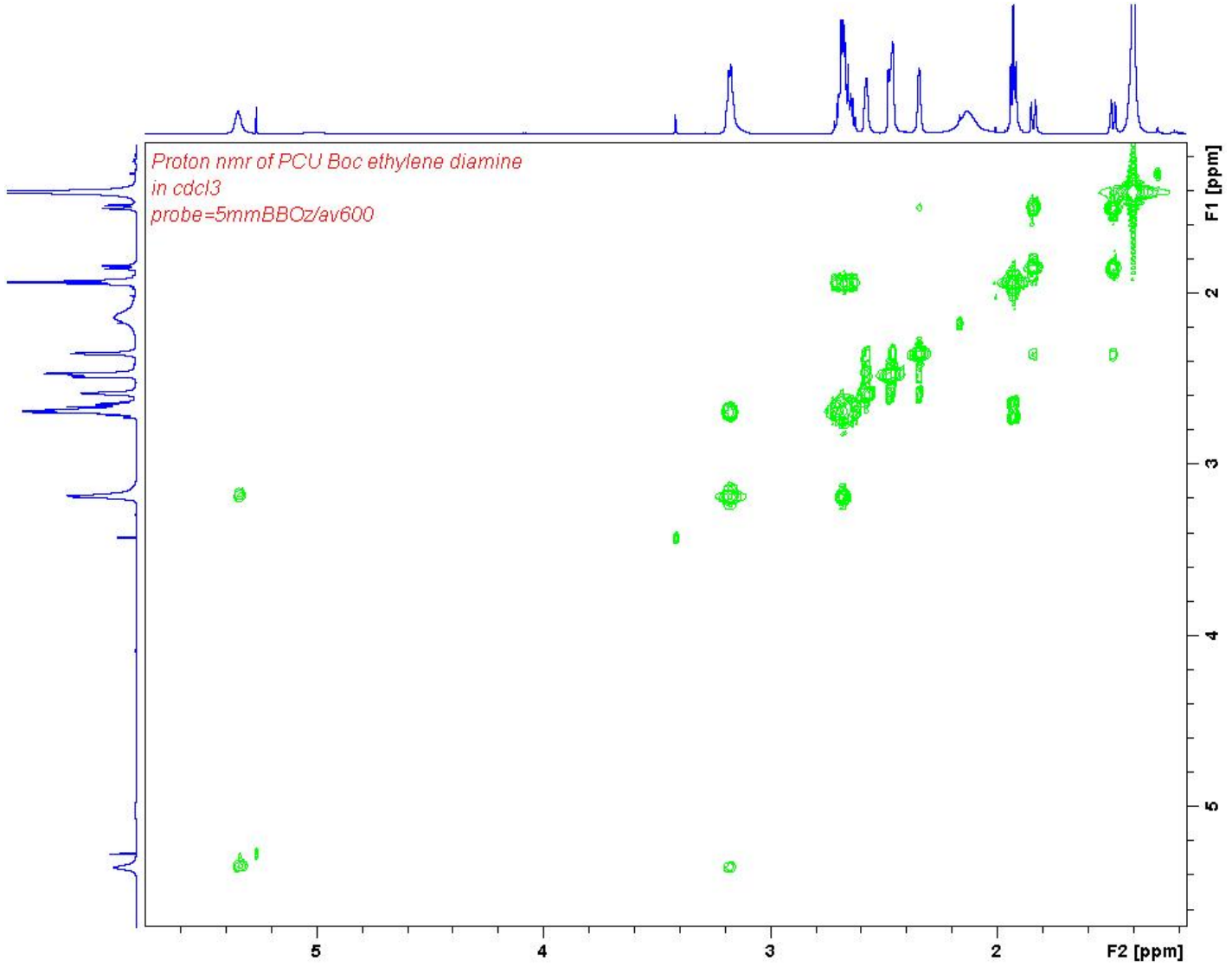
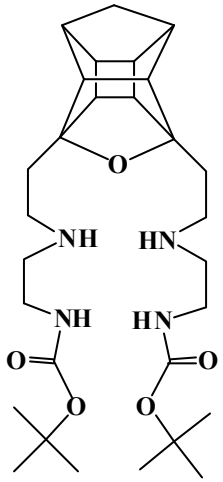


COSY spectrum of *N*-tert-butoxycarbonylethylene diamine PCU (1)



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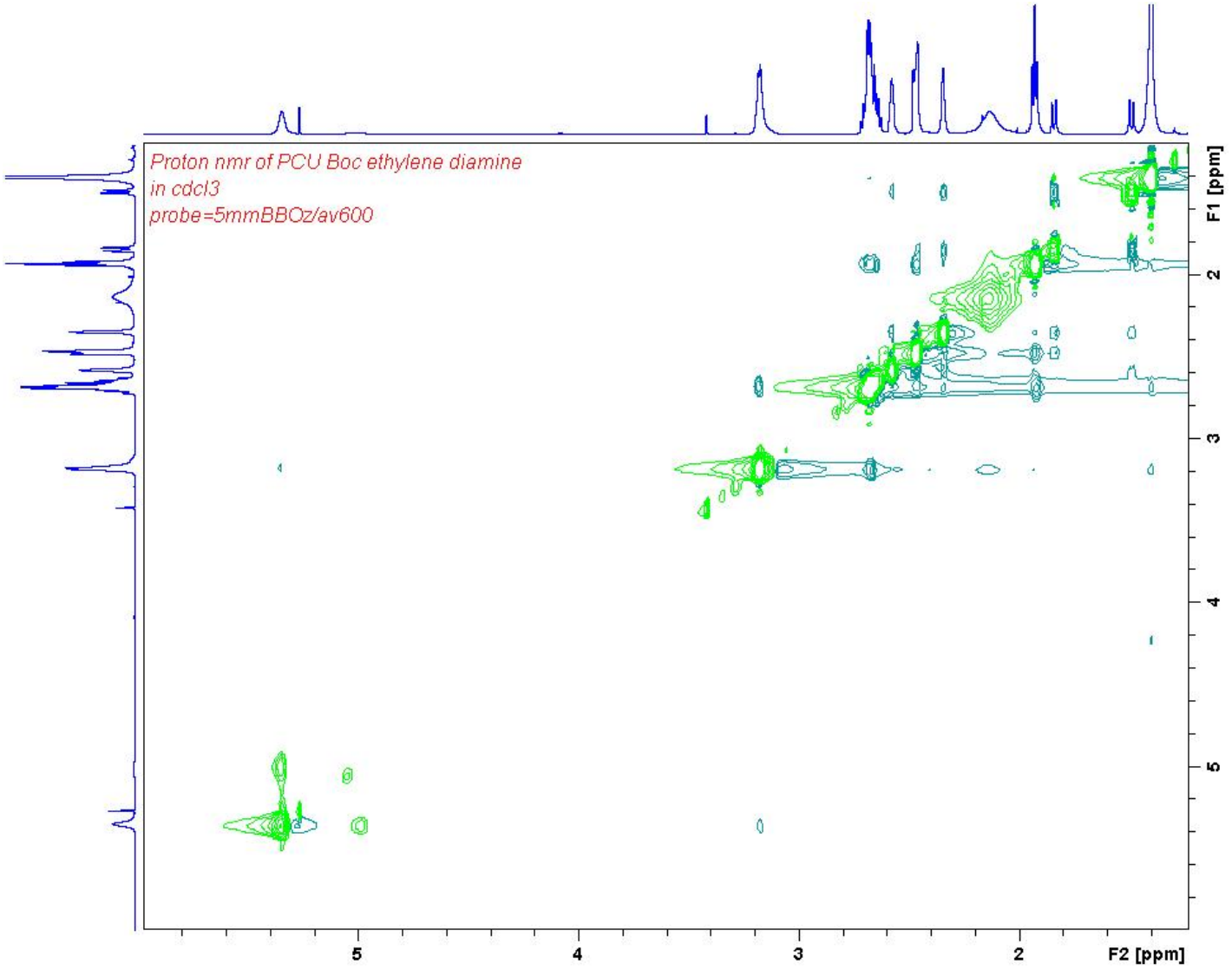
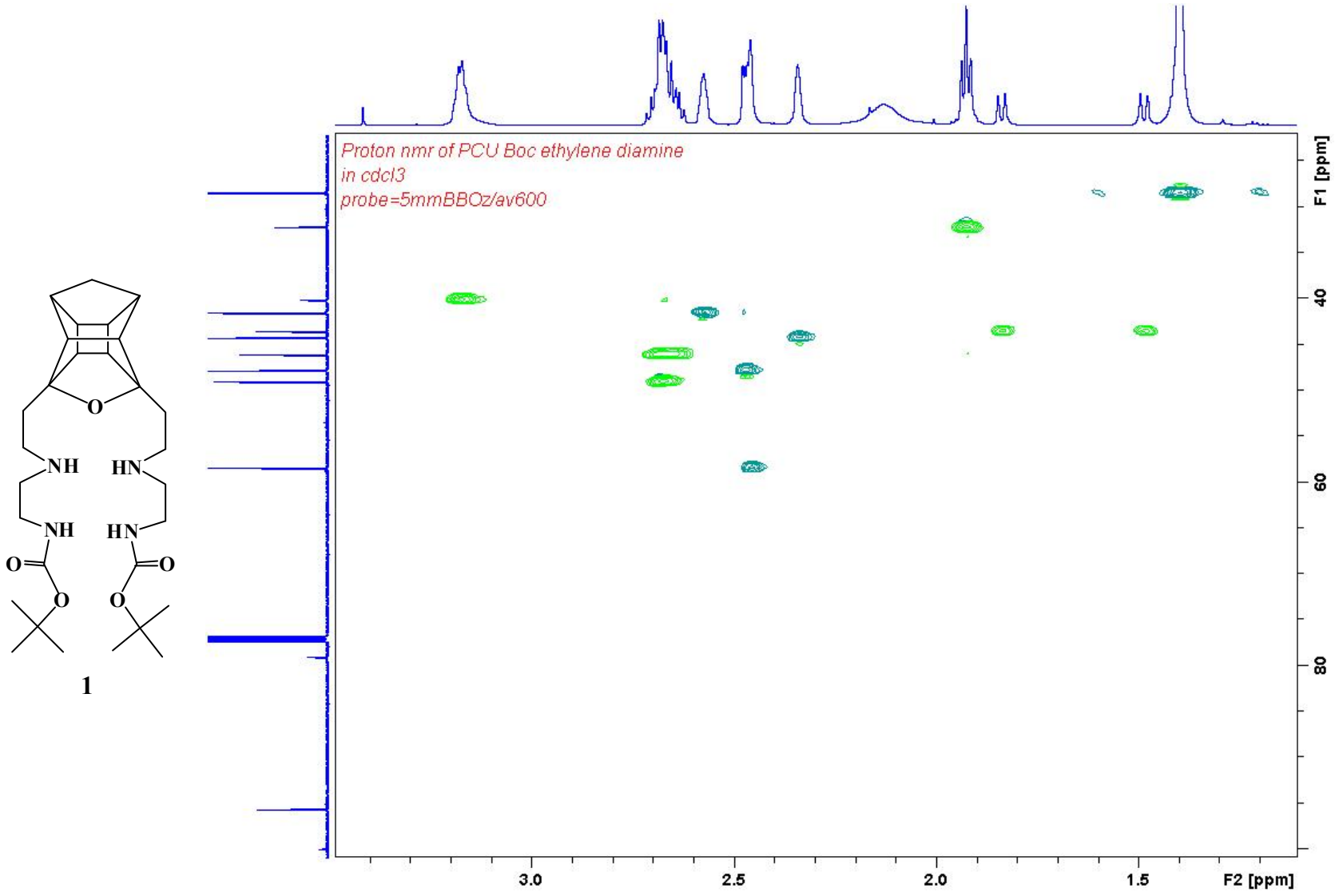


NOESY spectrum of *N*-tert-butoxycarbonylethylene diamine PCU (1)



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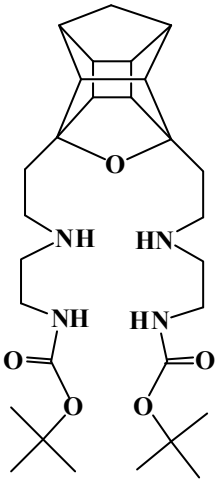
HSQC spectrum of *N*-tert-butoxycarbonylethylene diamine PCU (1)



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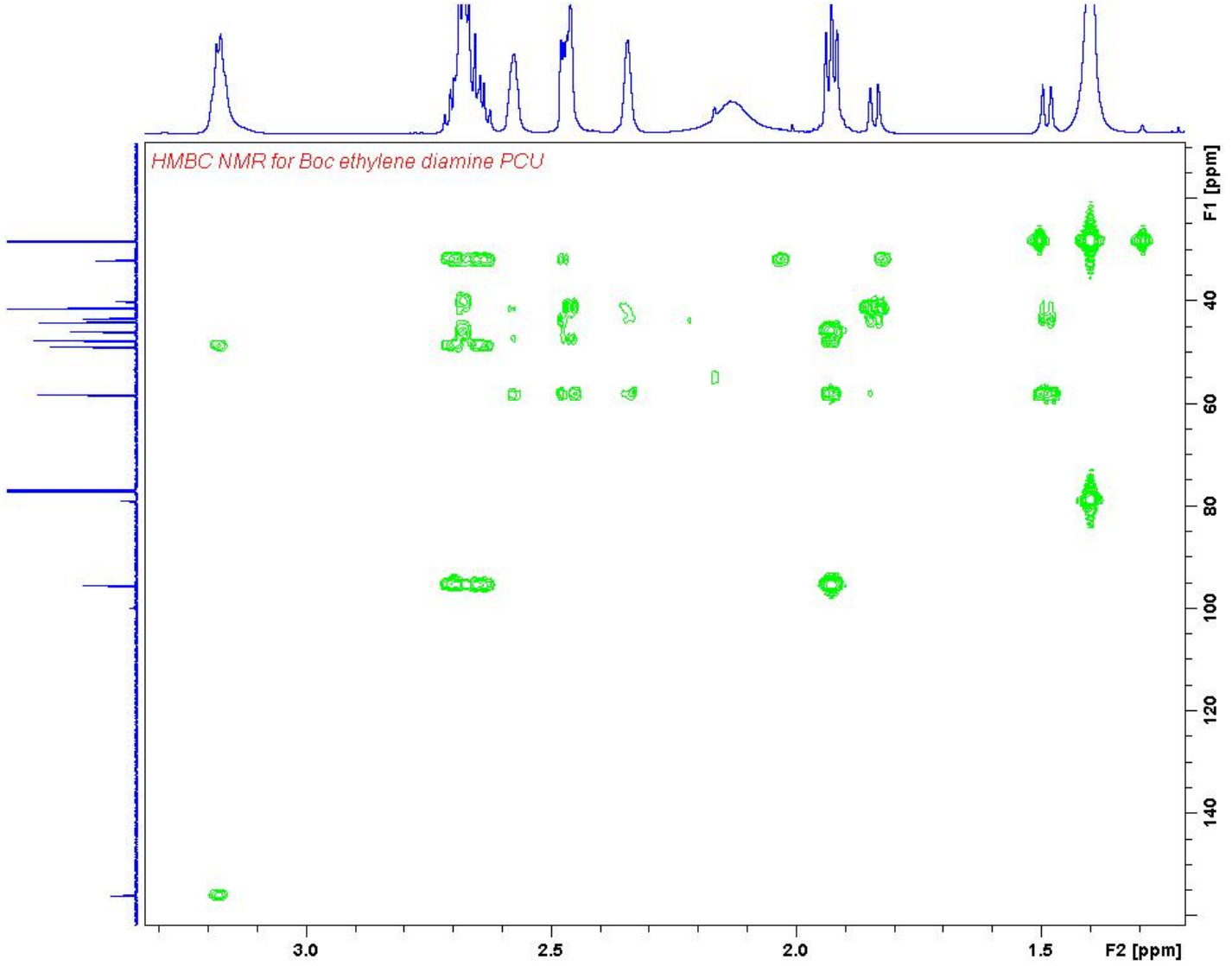
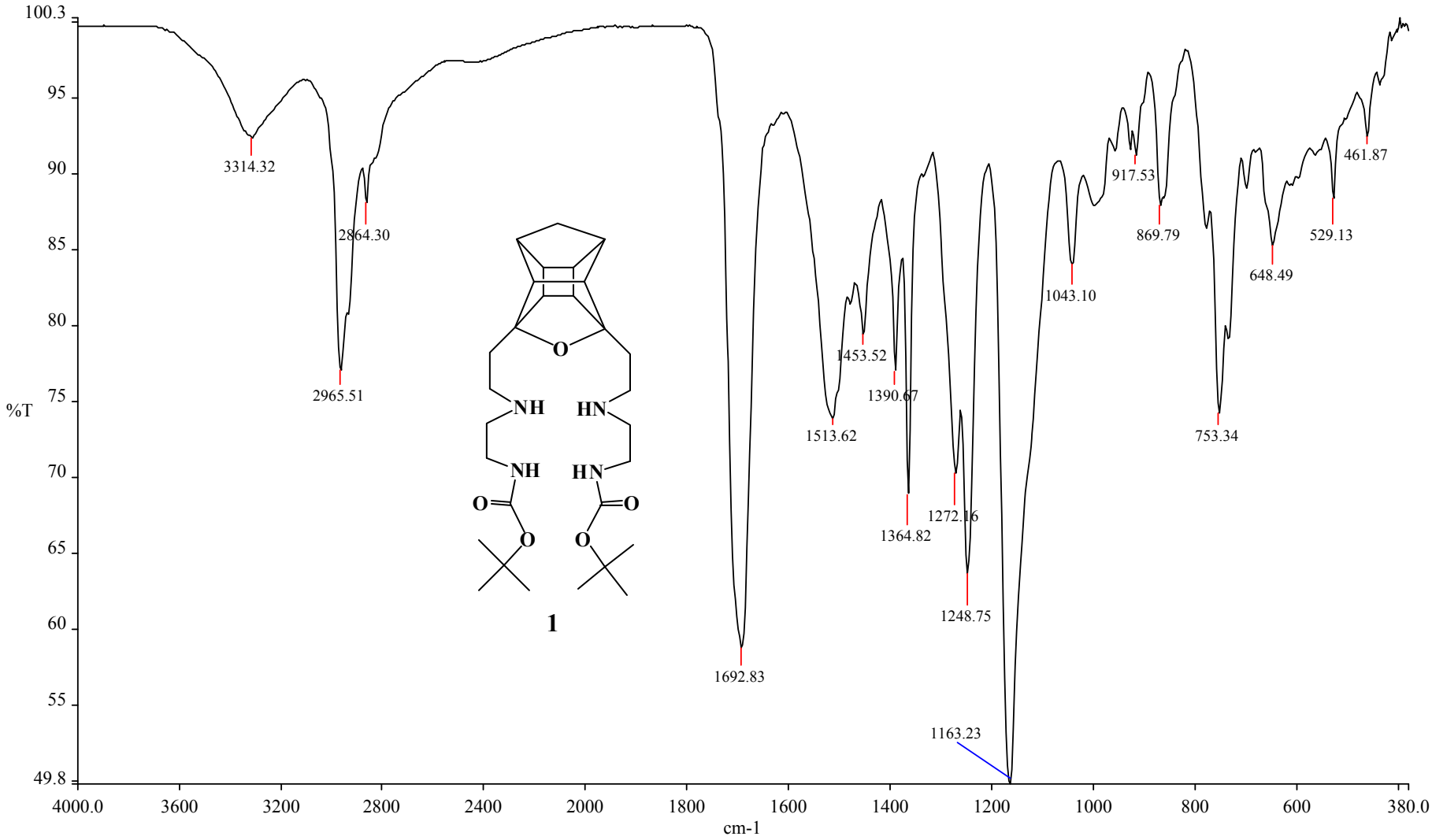
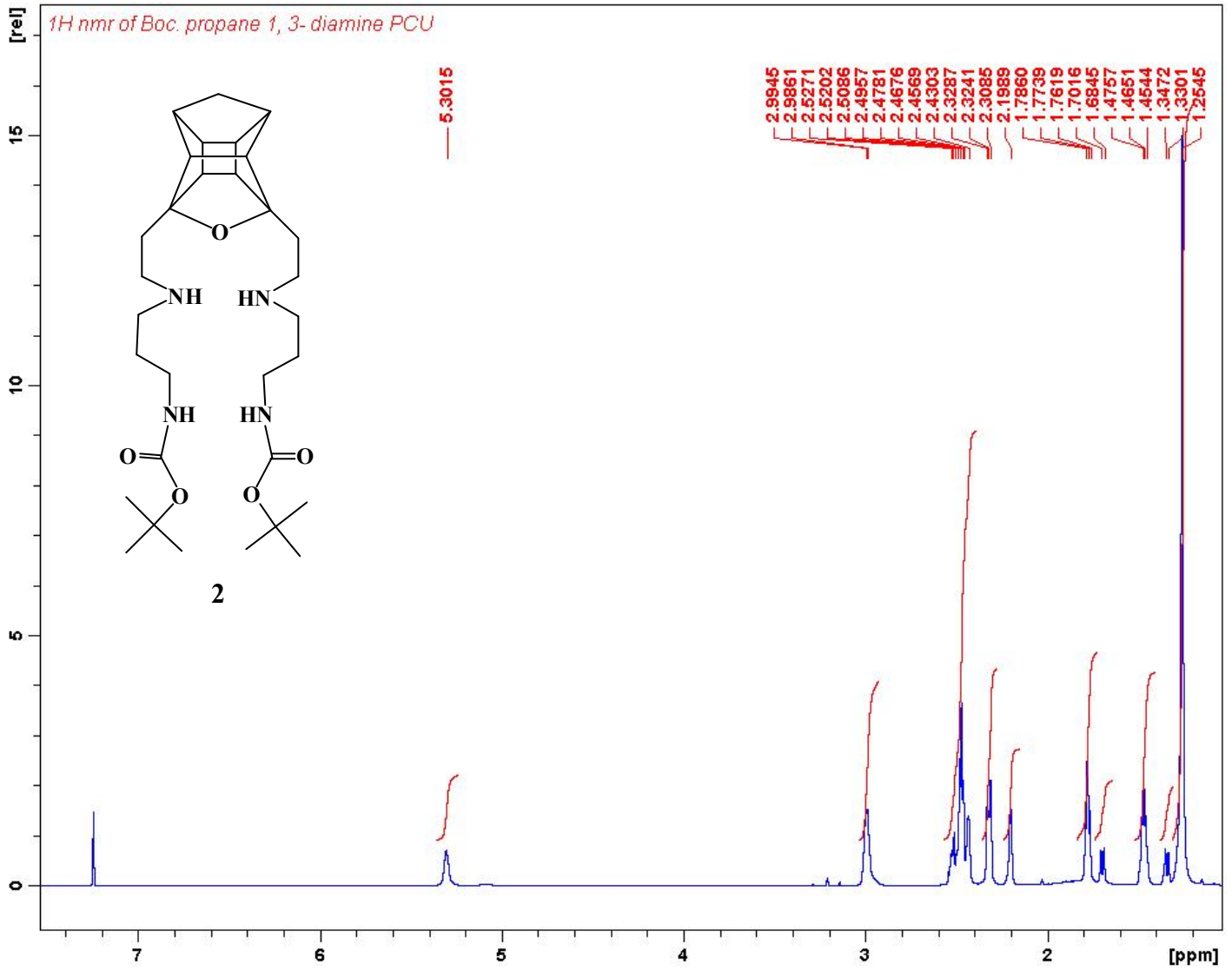


HMBC spectrum of *N*-tert-butoxycarbonylethylene diamine PCU (1)



IR spectrum of *N*-tert-butoxycarbonylethylene diamine PCU (1)



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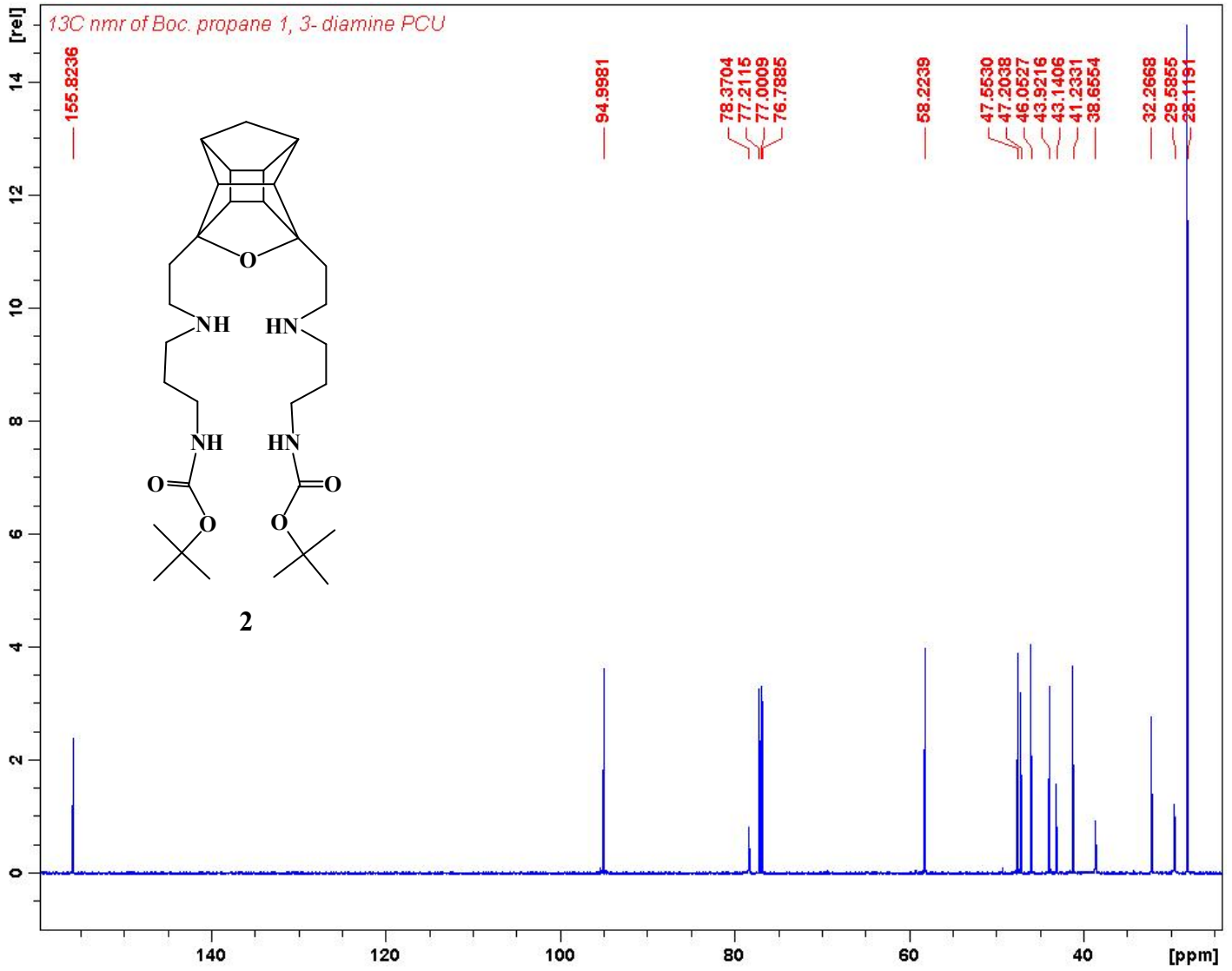
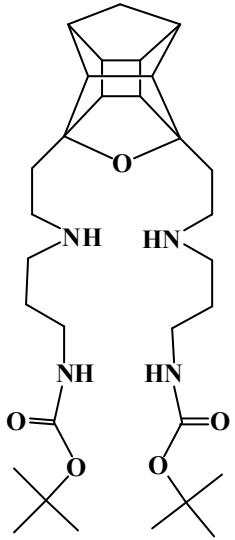
^{1}H NMR spectrum of *N*-tert-butoxycarbonylpropane diamine PCU (2)



^{13}C NMR spectrum of *N*-tert-butoxycarbonylpropane diamine PCU (2)



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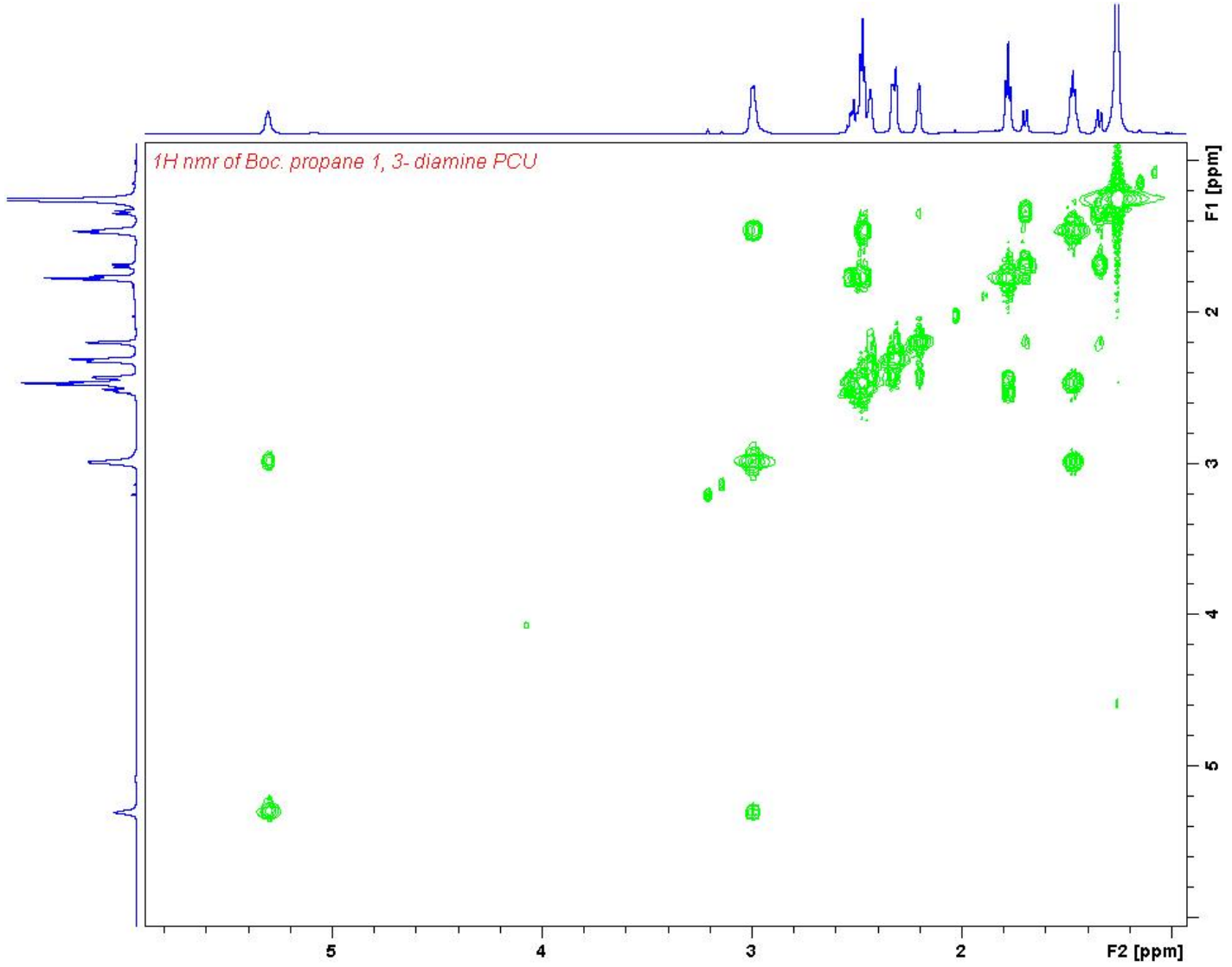
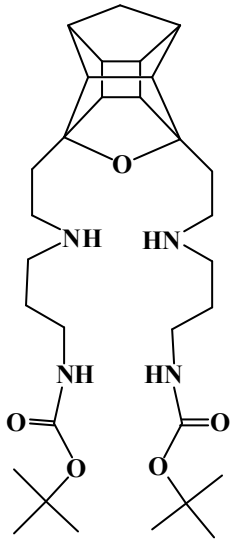


COSY spectrum of *N*-tert-butoxycarbonylpropane diamine PCU (2)



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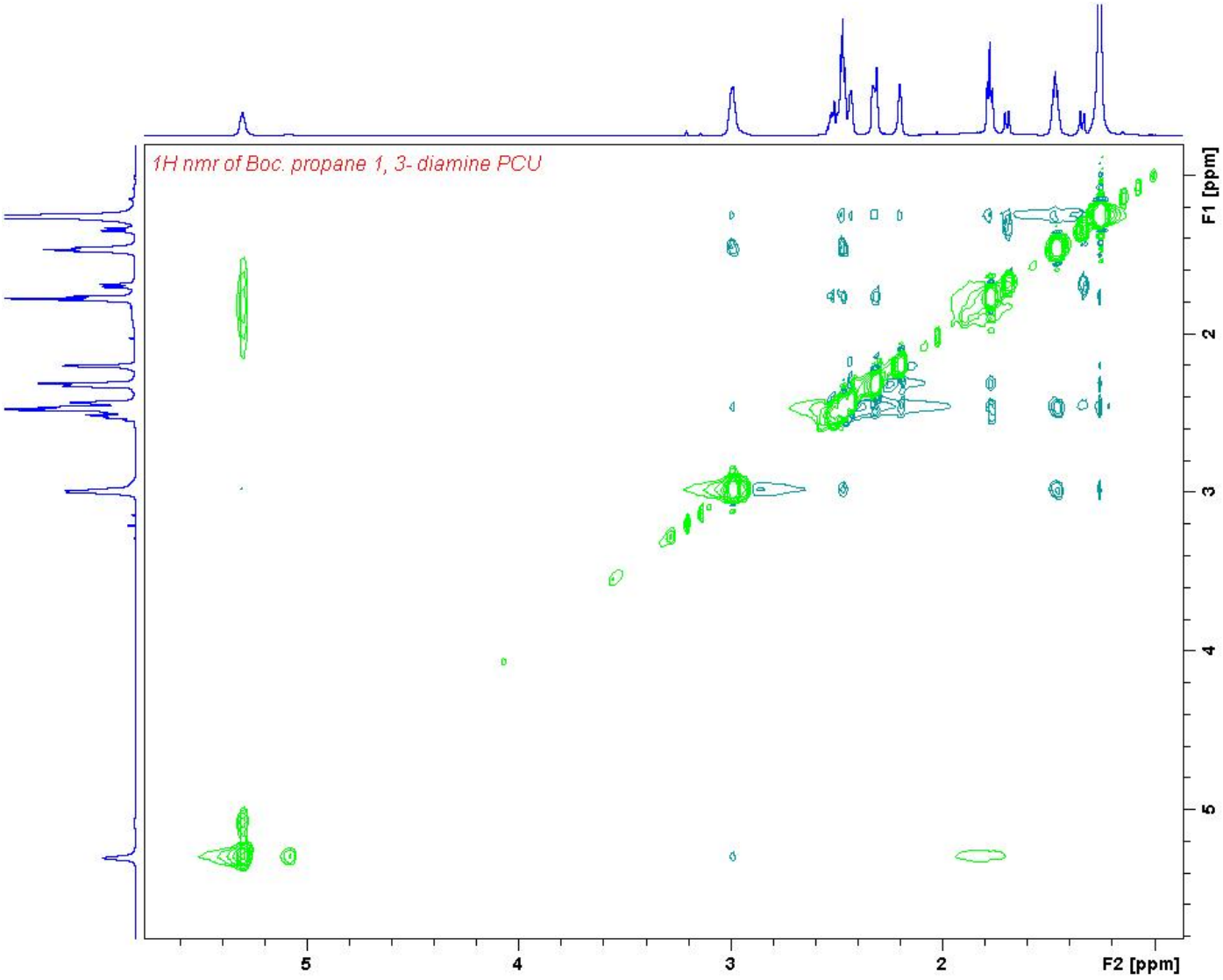
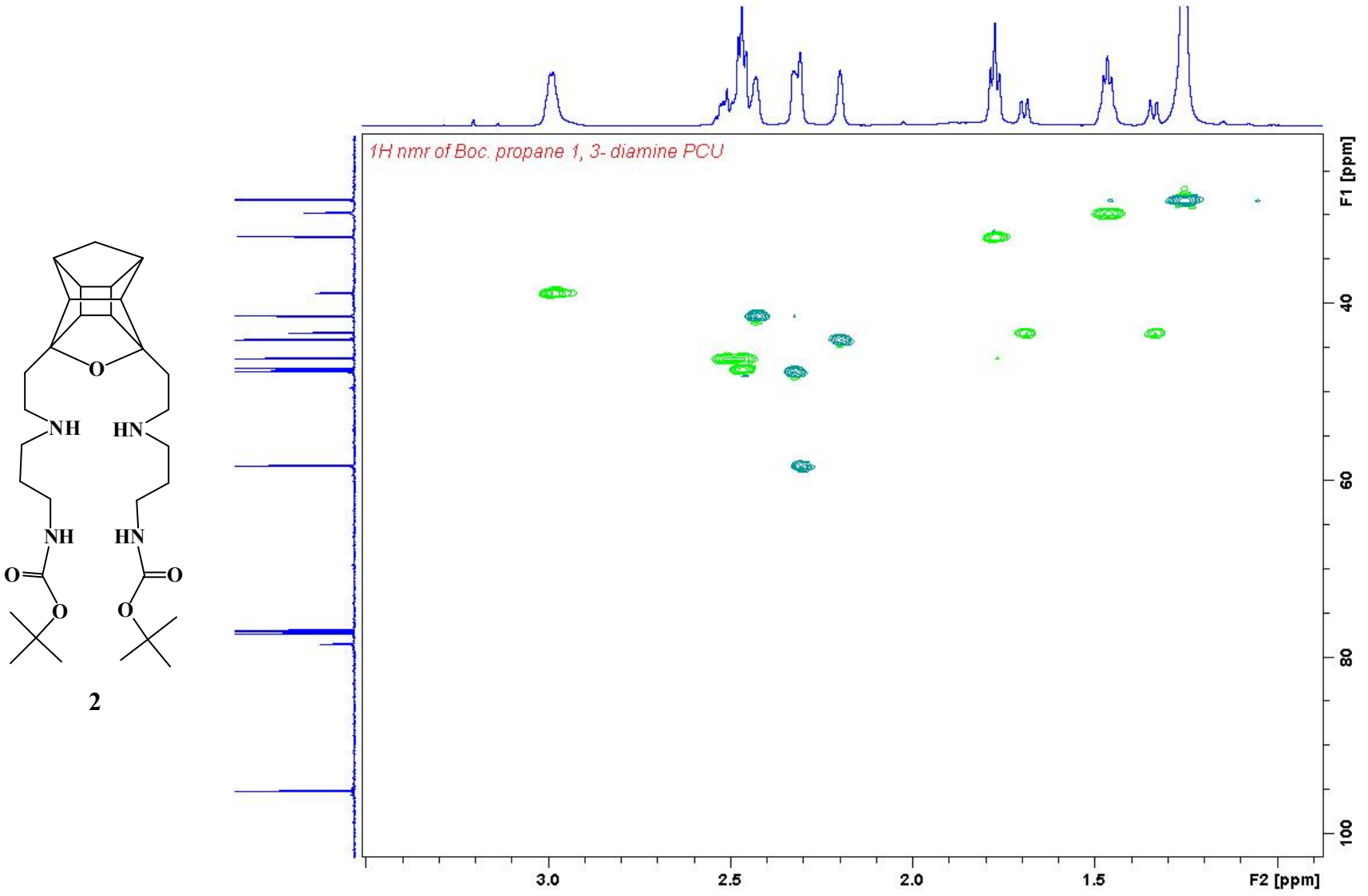


NOESY spectrum of *N*-tert-butoxycarbonylpropane diamine PCU (2)



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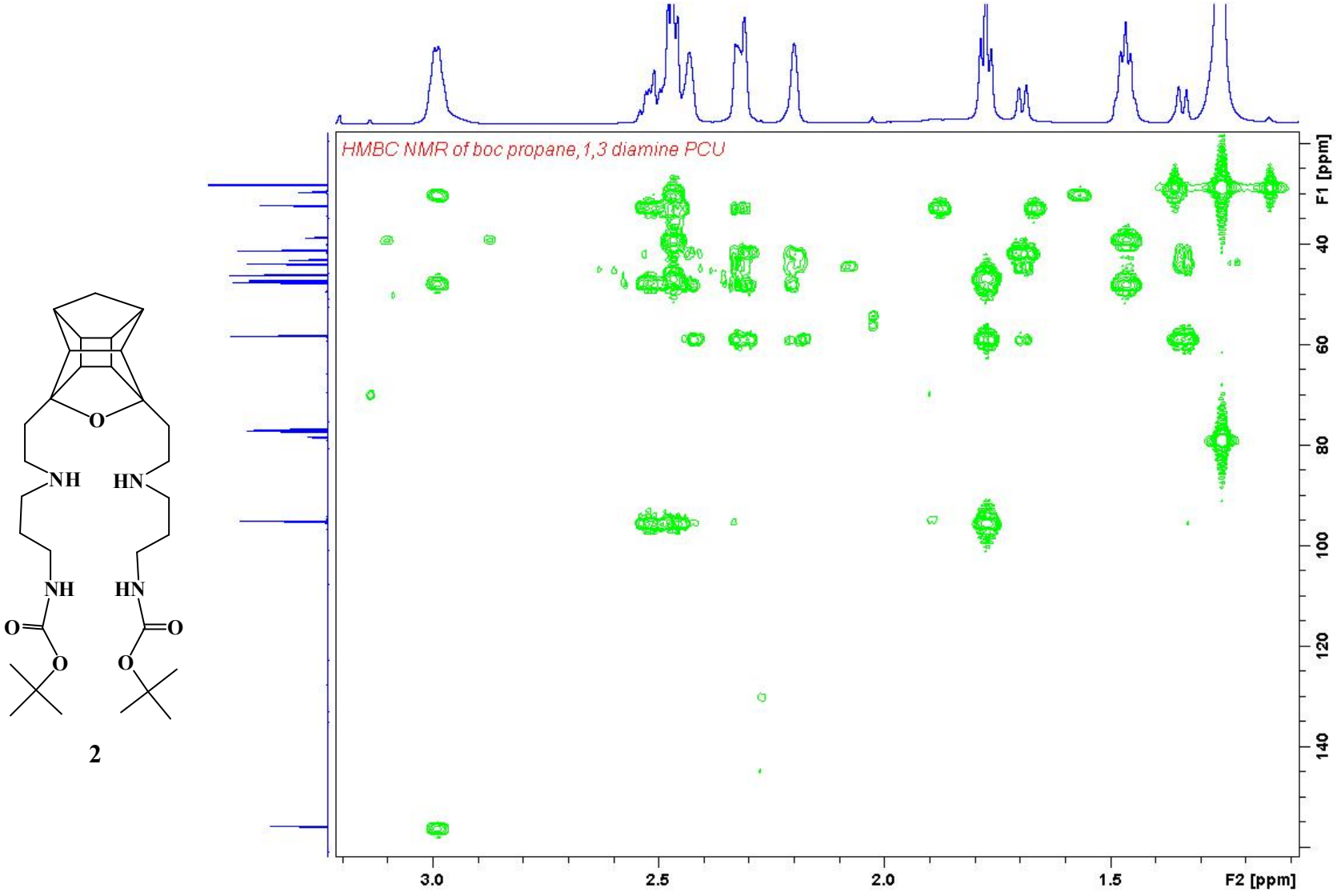
HSQC spectrum of *N*-tert-butoxycarbonylpropane diamine PCU (2)



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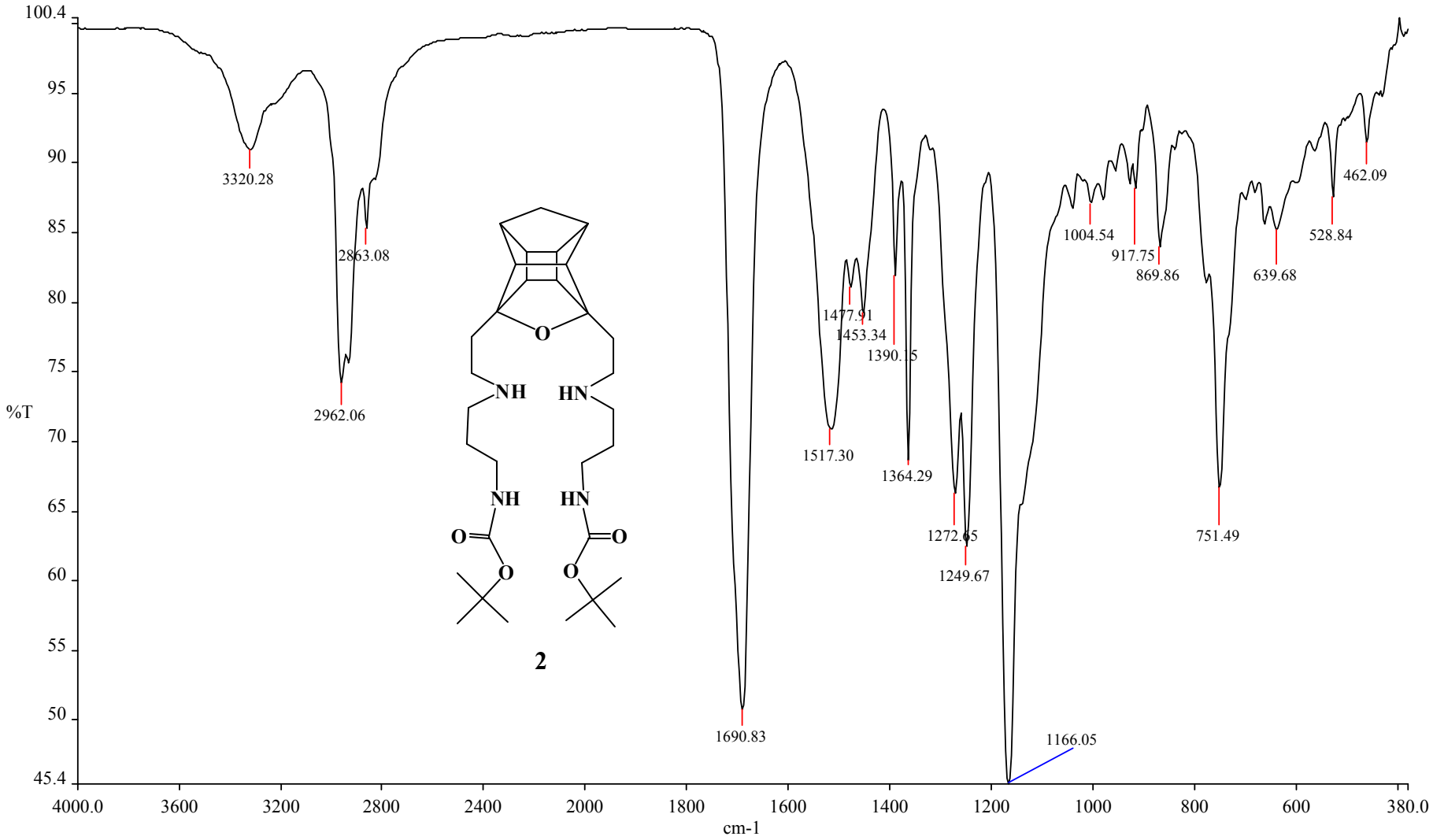
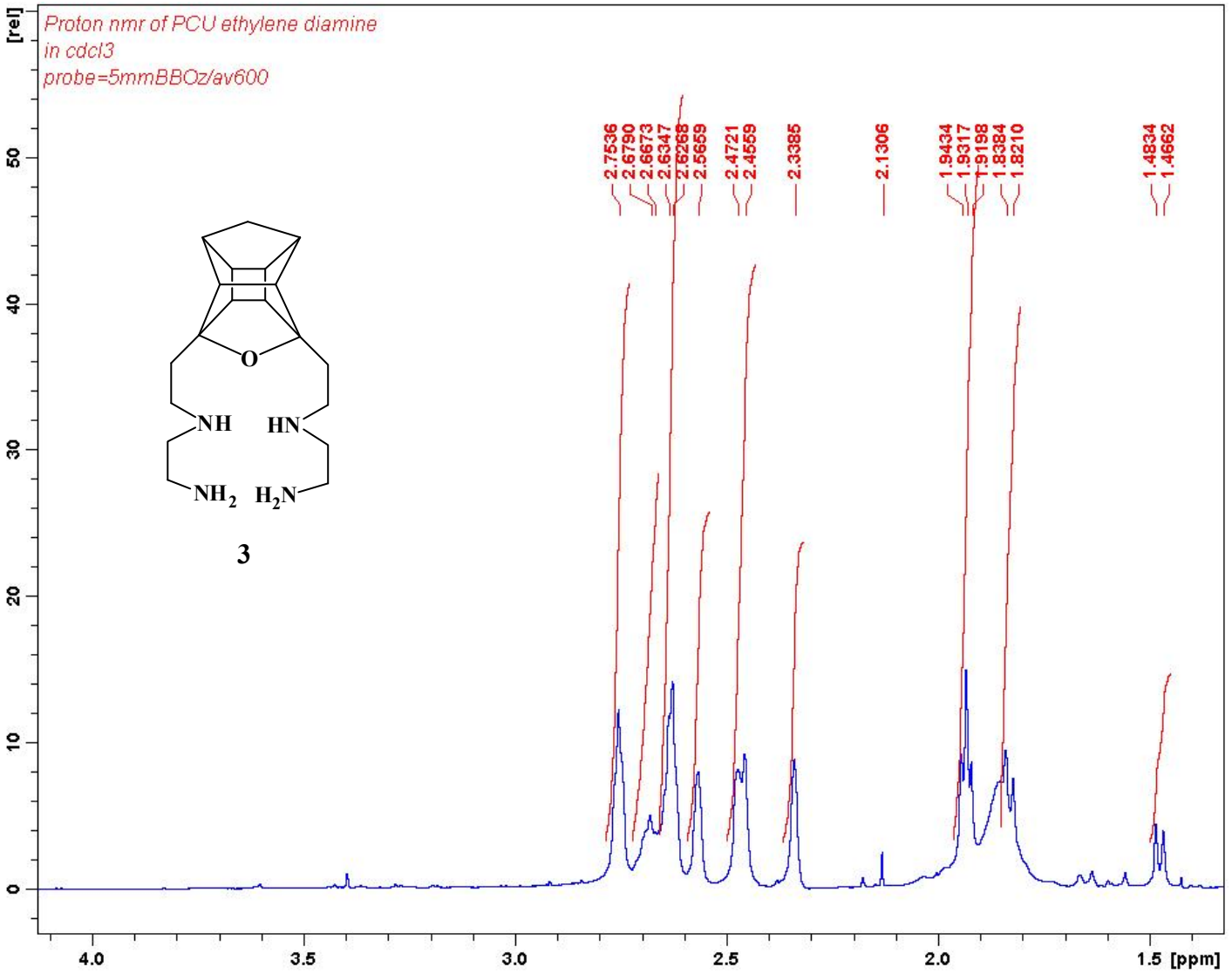
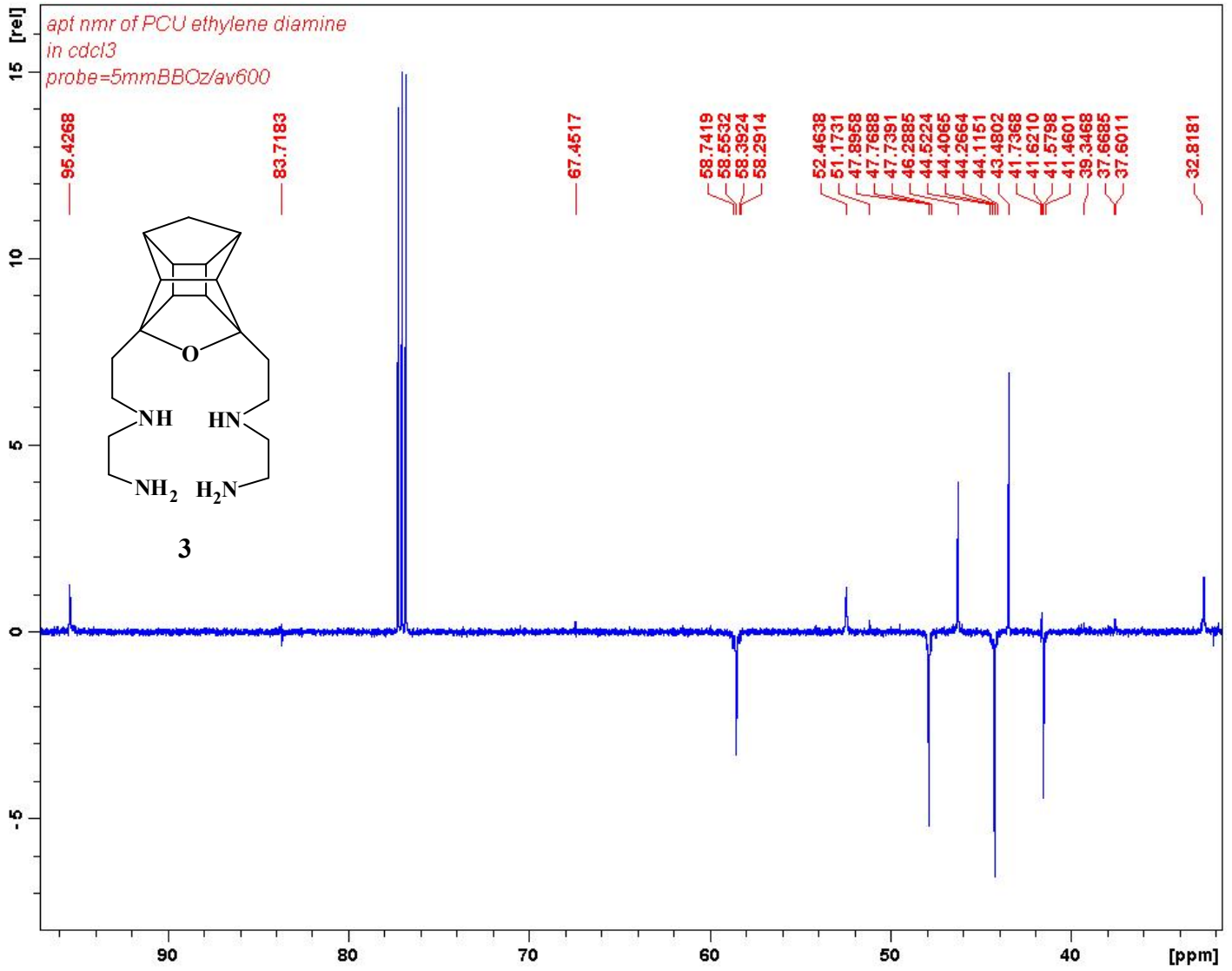
HMBC spectrum of *N*-tert-butoxycarbonylpropane diamine PCU (2)



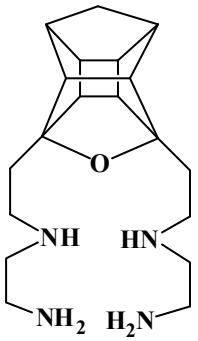
IR spectrum of *N*-tert-butoxycarbonylpropane diamine PCU (2)



^{1}H NMR spectrum of PCU ethylene diamine (3)



^{13}C (APT) NMR spectrum of PCU ethylene diamine (3)



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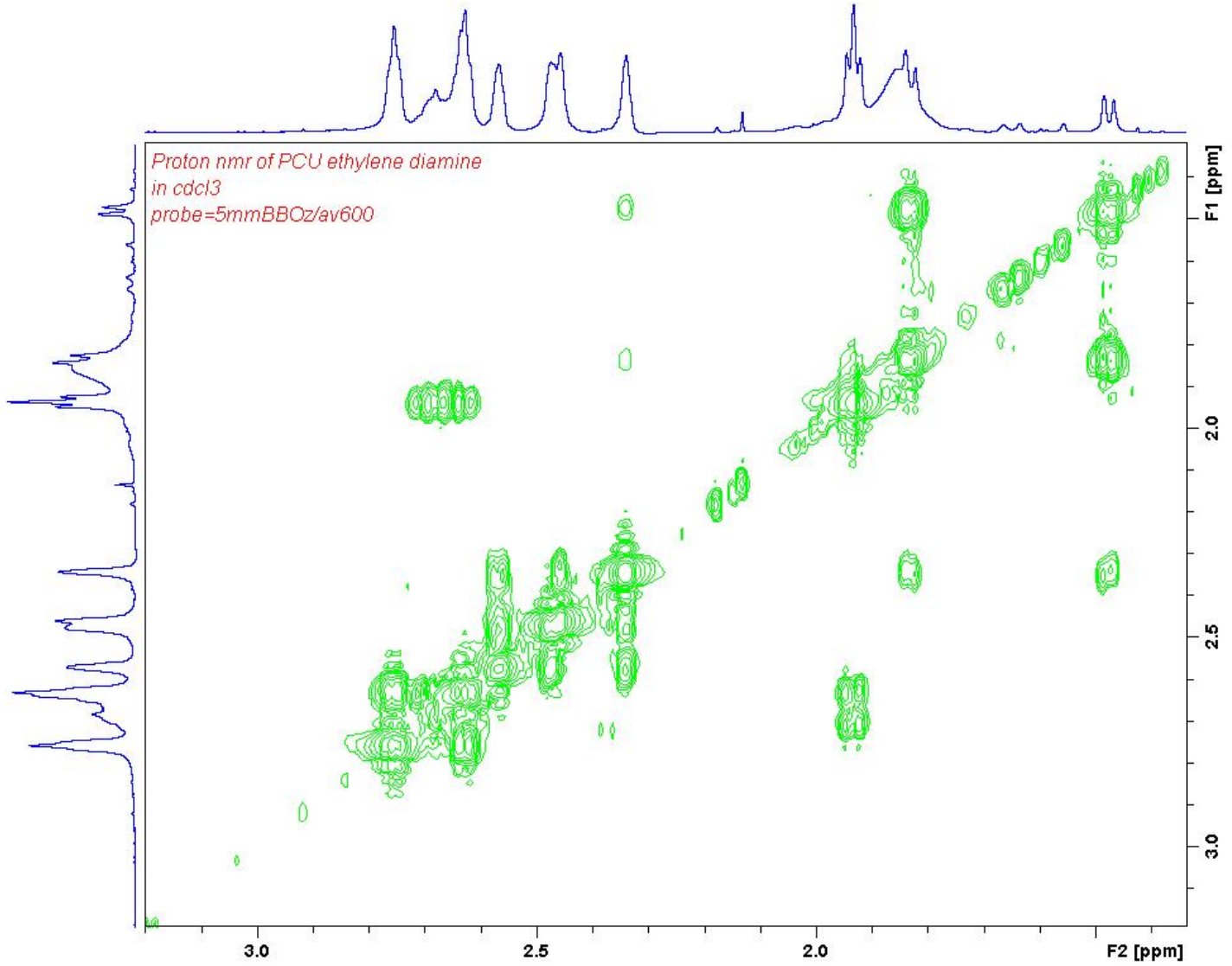
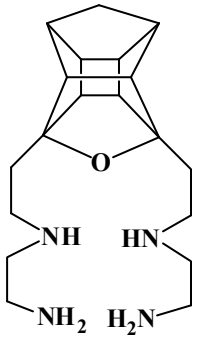


COSY spectrum of PCU ethylene diamine (3)



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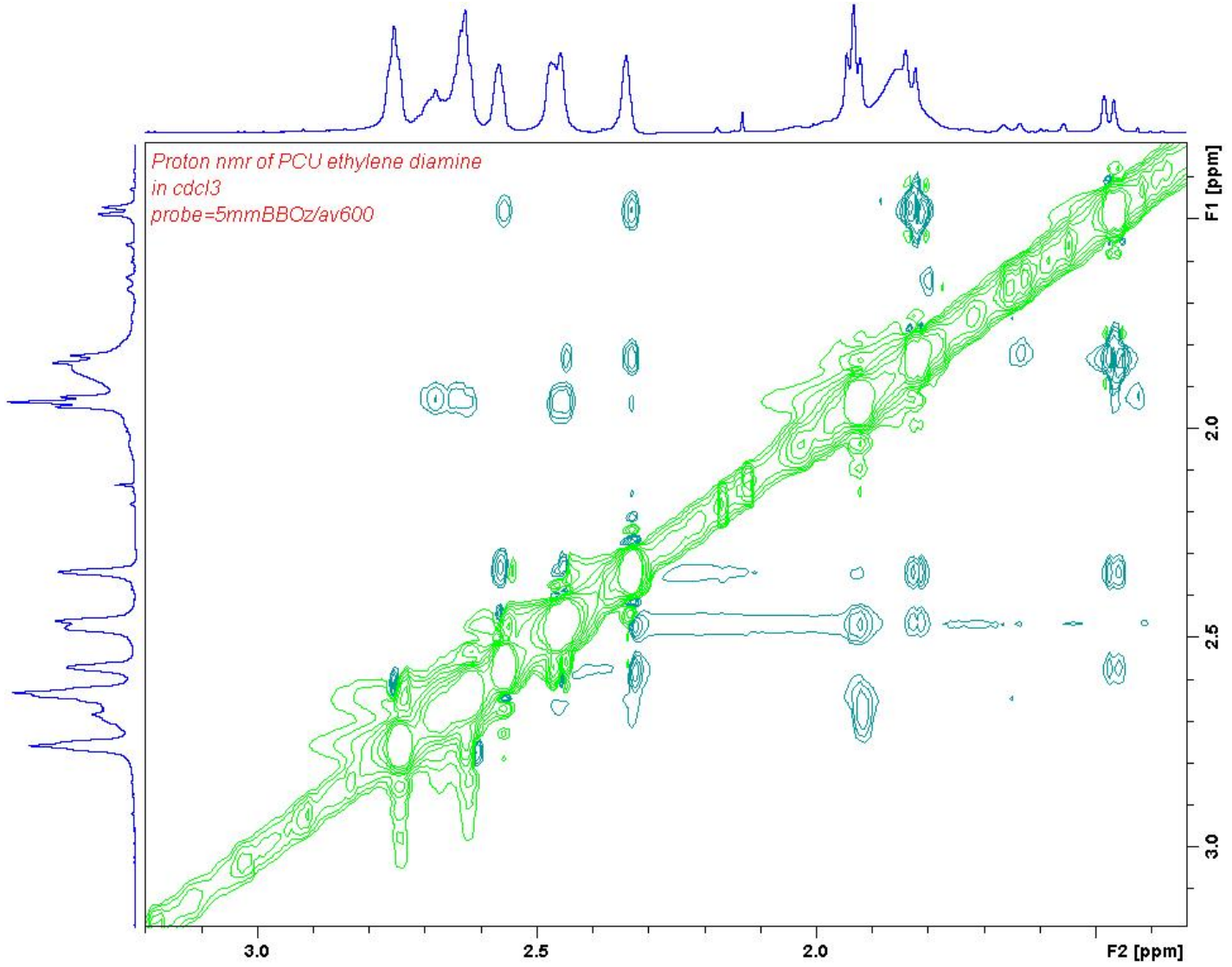
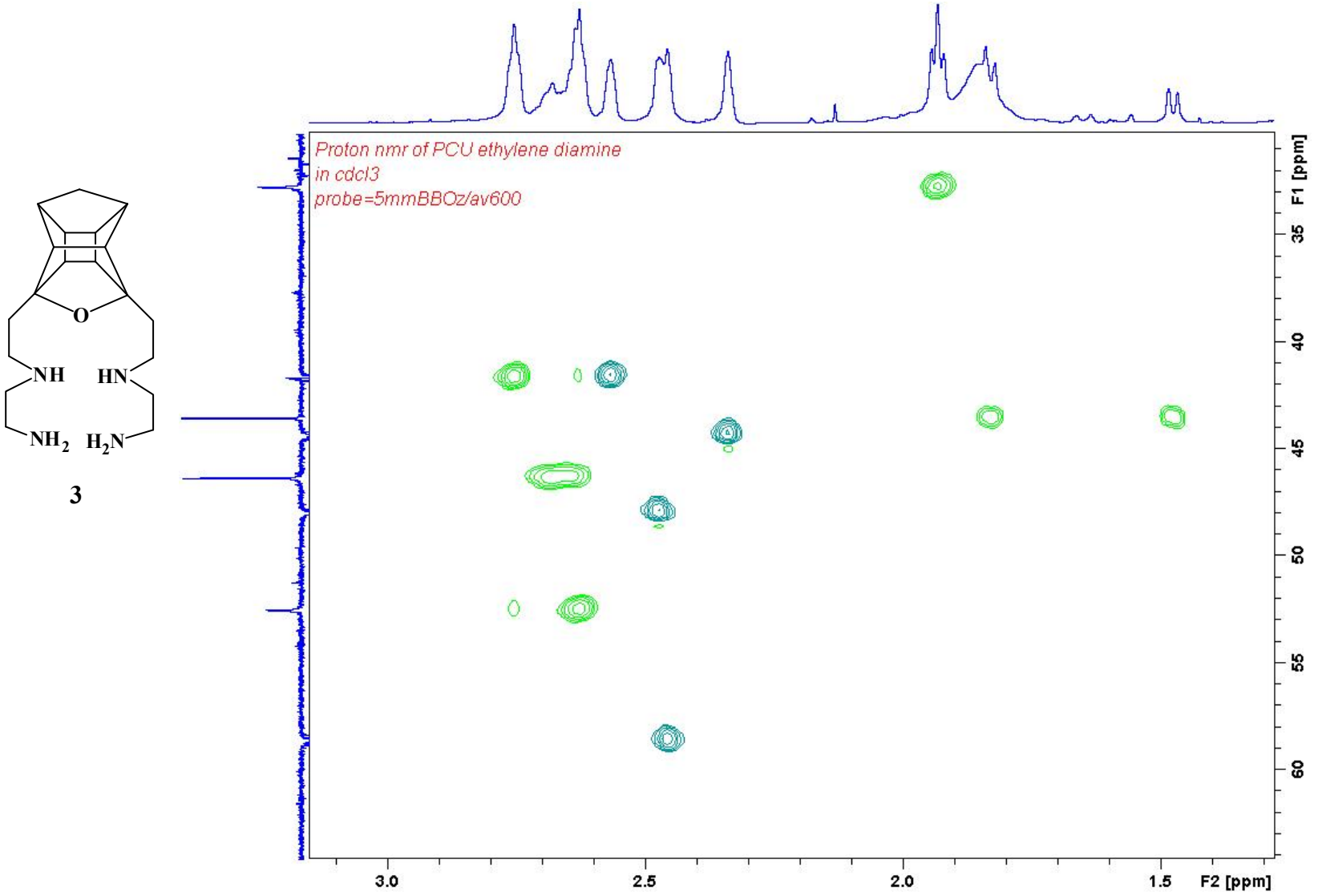
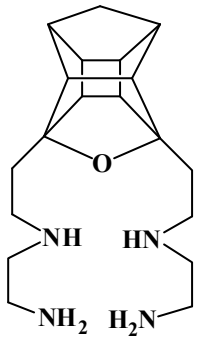


NOESY spectrum of PCU ethylene diamine (3)



HSQC spectrum of PCU ethylene diamine (3)



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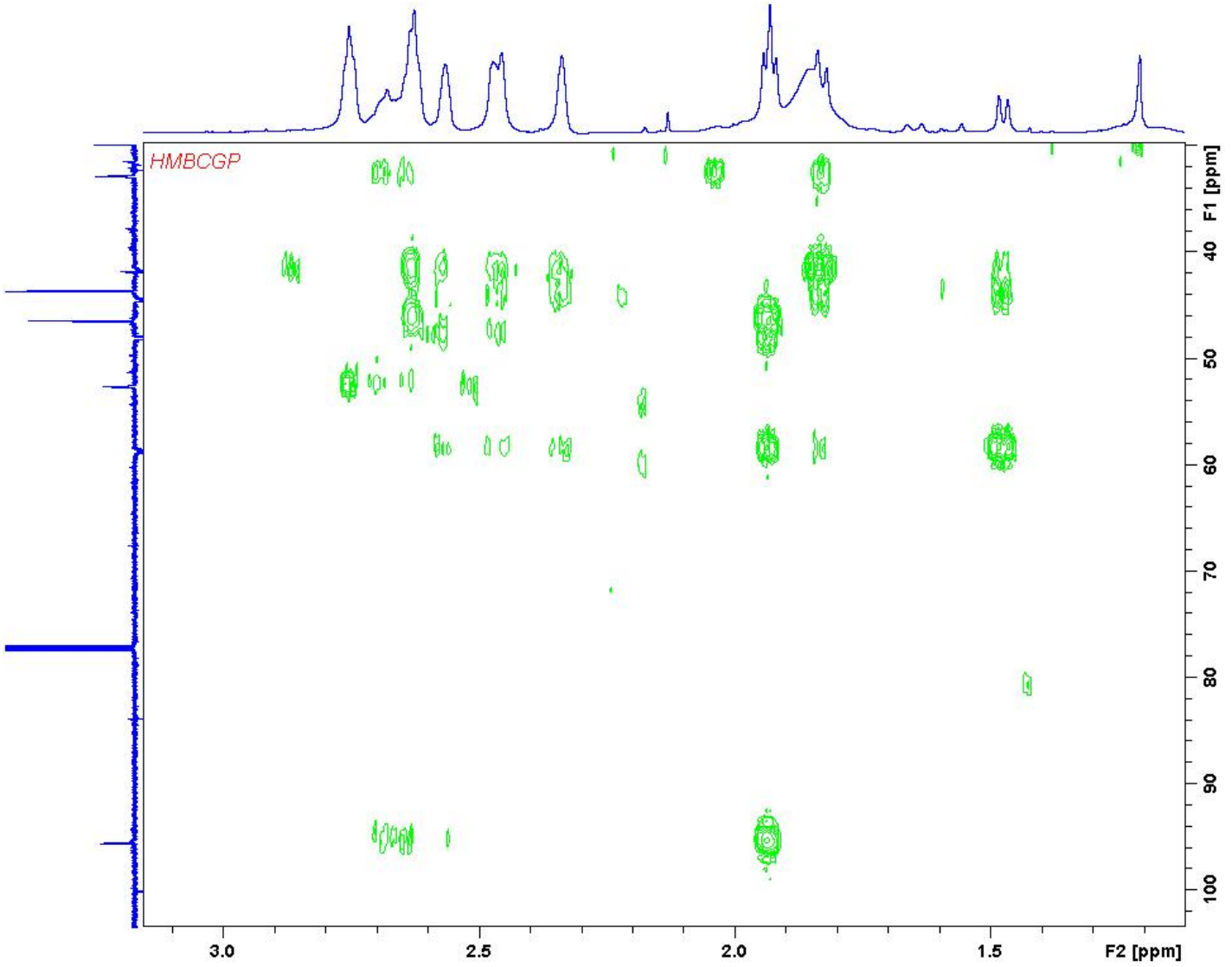
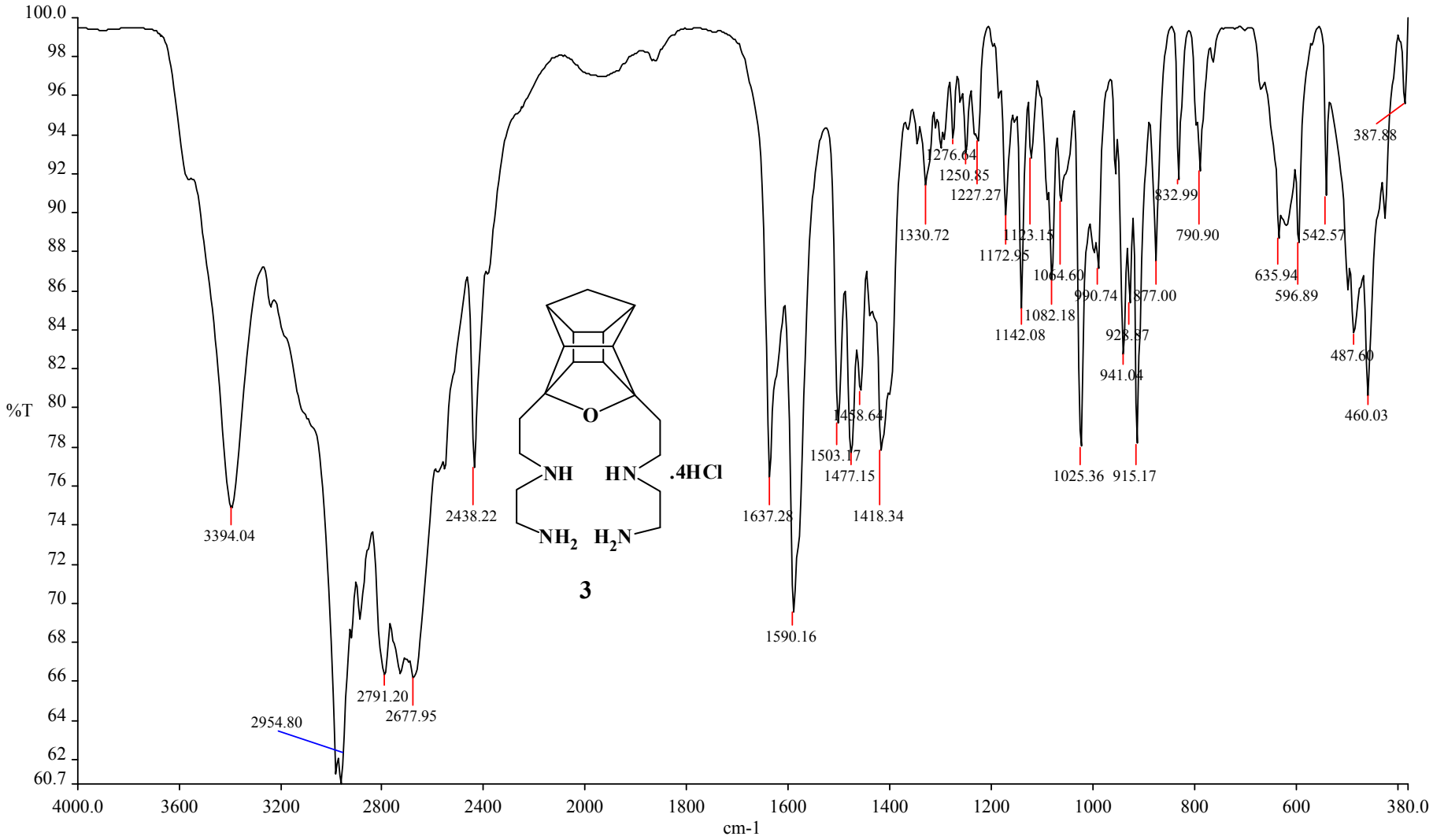
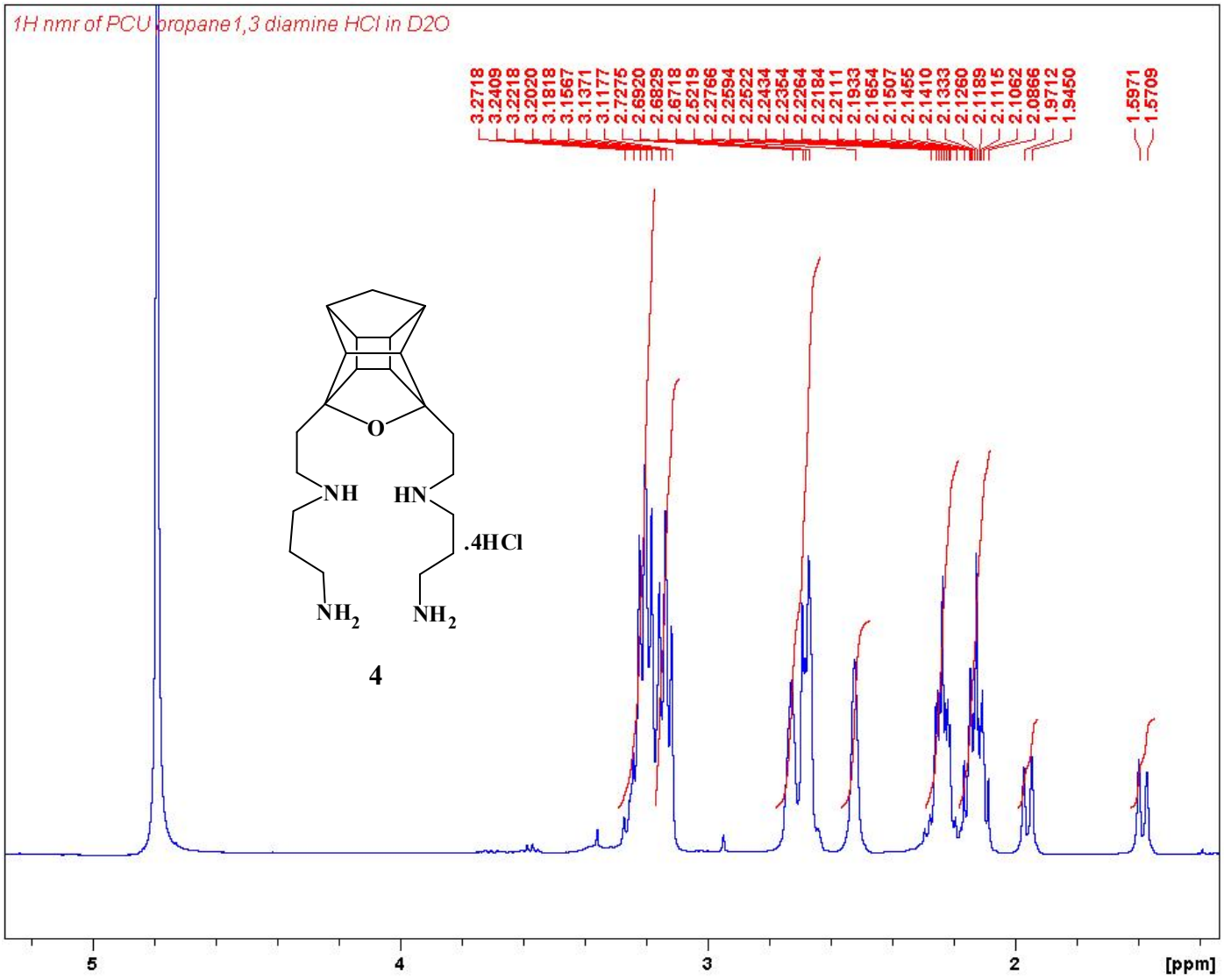


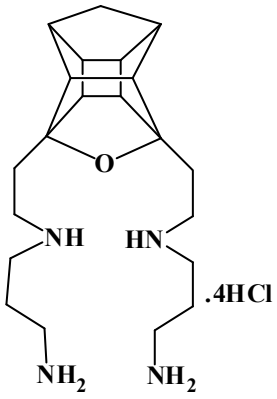
HMBC spectrum of PCU ethylene diamine (3)



spectrum of PCU ethylene diamine (3)



[1]H NMR spectrum of PCU propane 1,3- diamine HCl (4)



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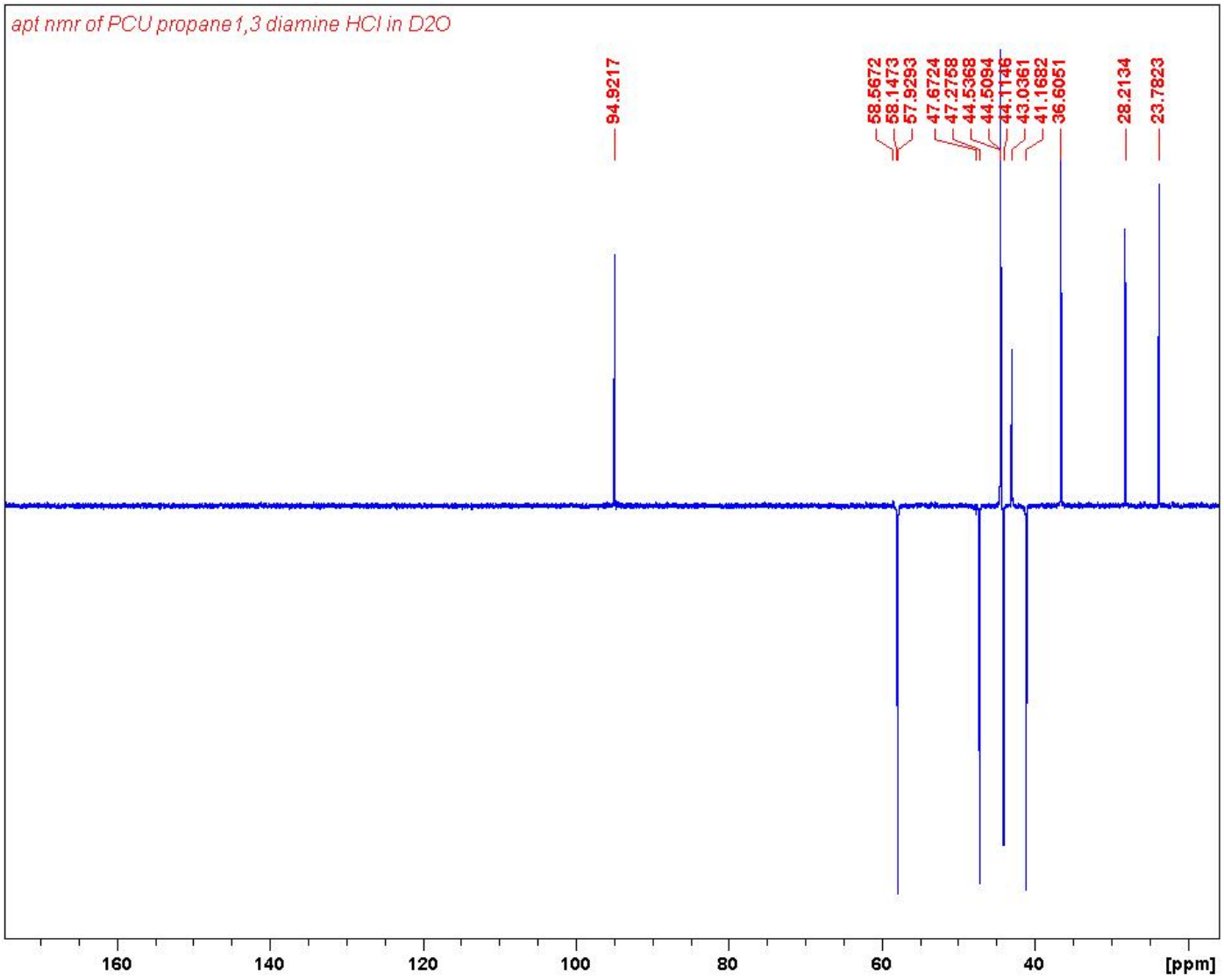
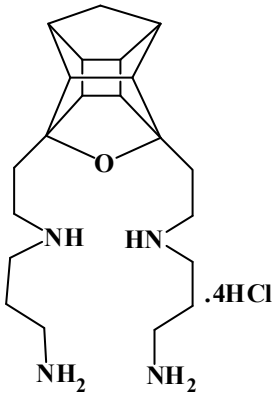


^{13}C NMR spectrum of PCU propane 1,3- diamine HCl (4)



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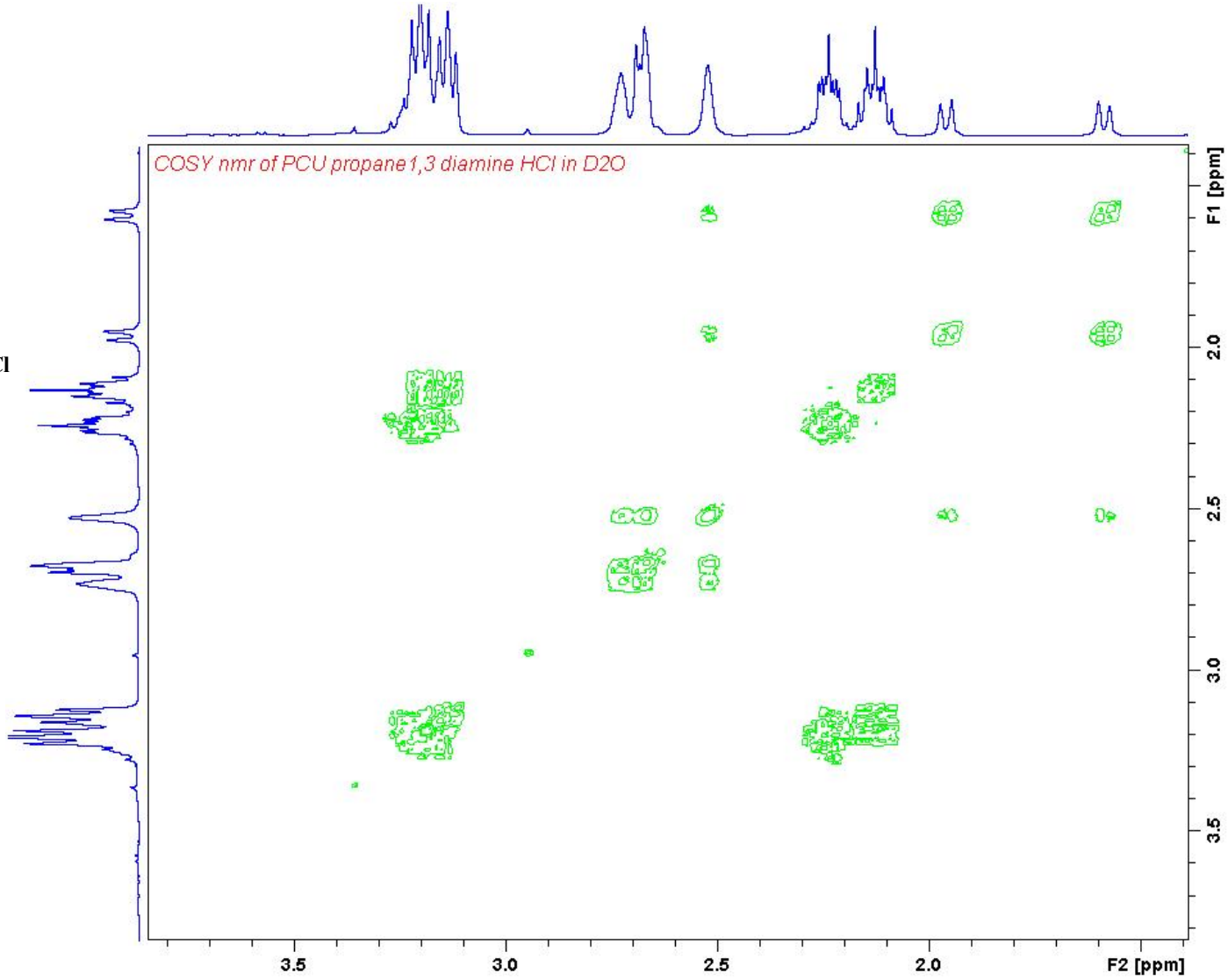
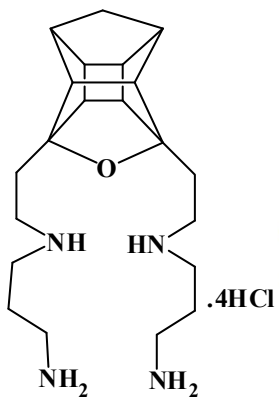


COSY NMR spectrum of PCU propane 1,3- diamine HCl (4)



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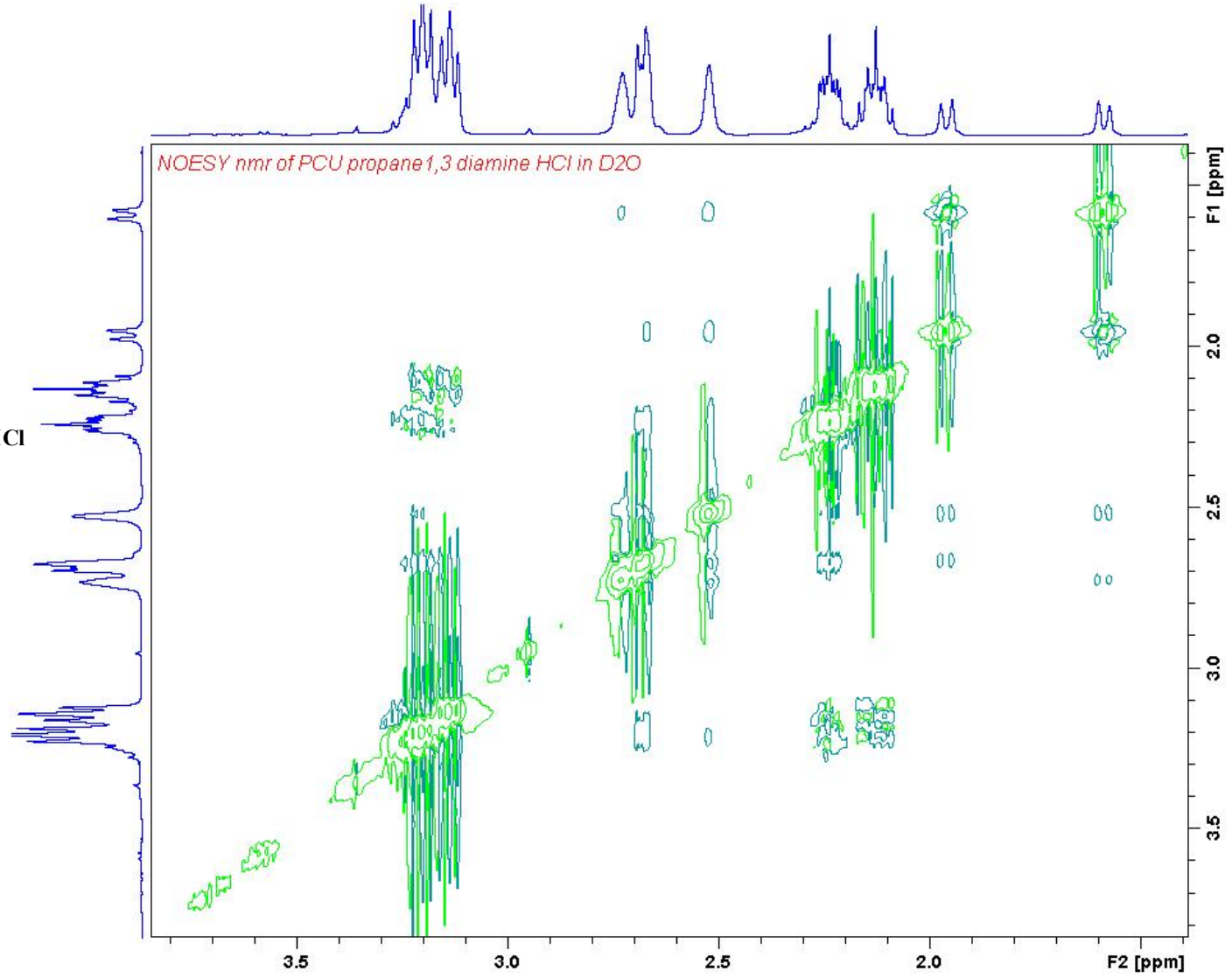
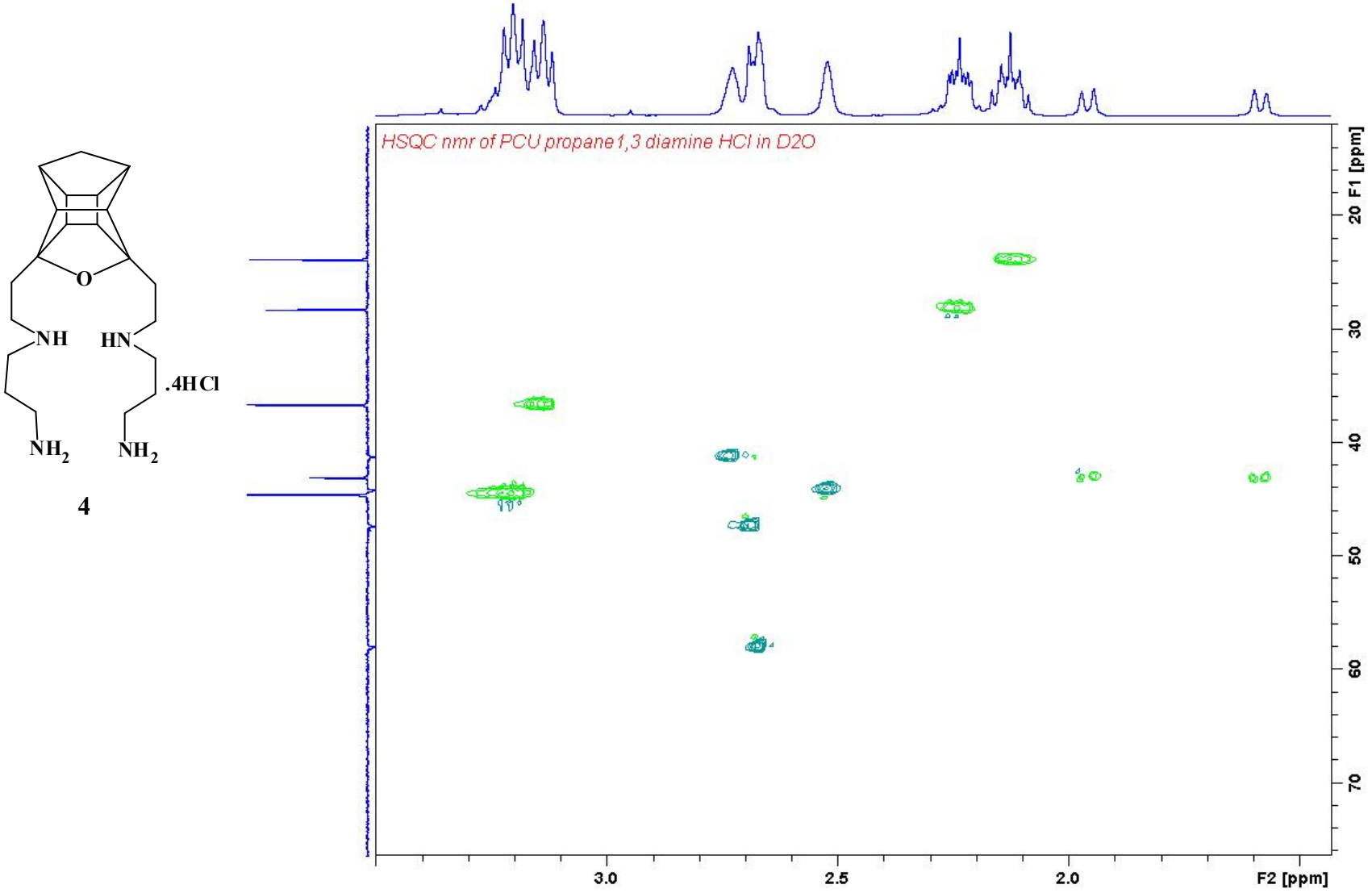


NOESY NMR spectrum of PCU propane 1,3- diamine HCl (4)



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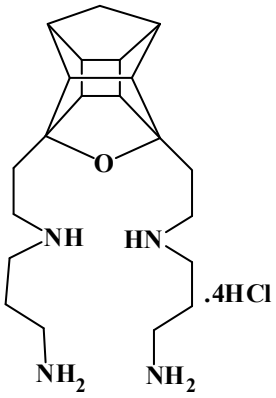
HSQC NMR spectrum of PCU propane 1,3- diamine HCl (4)



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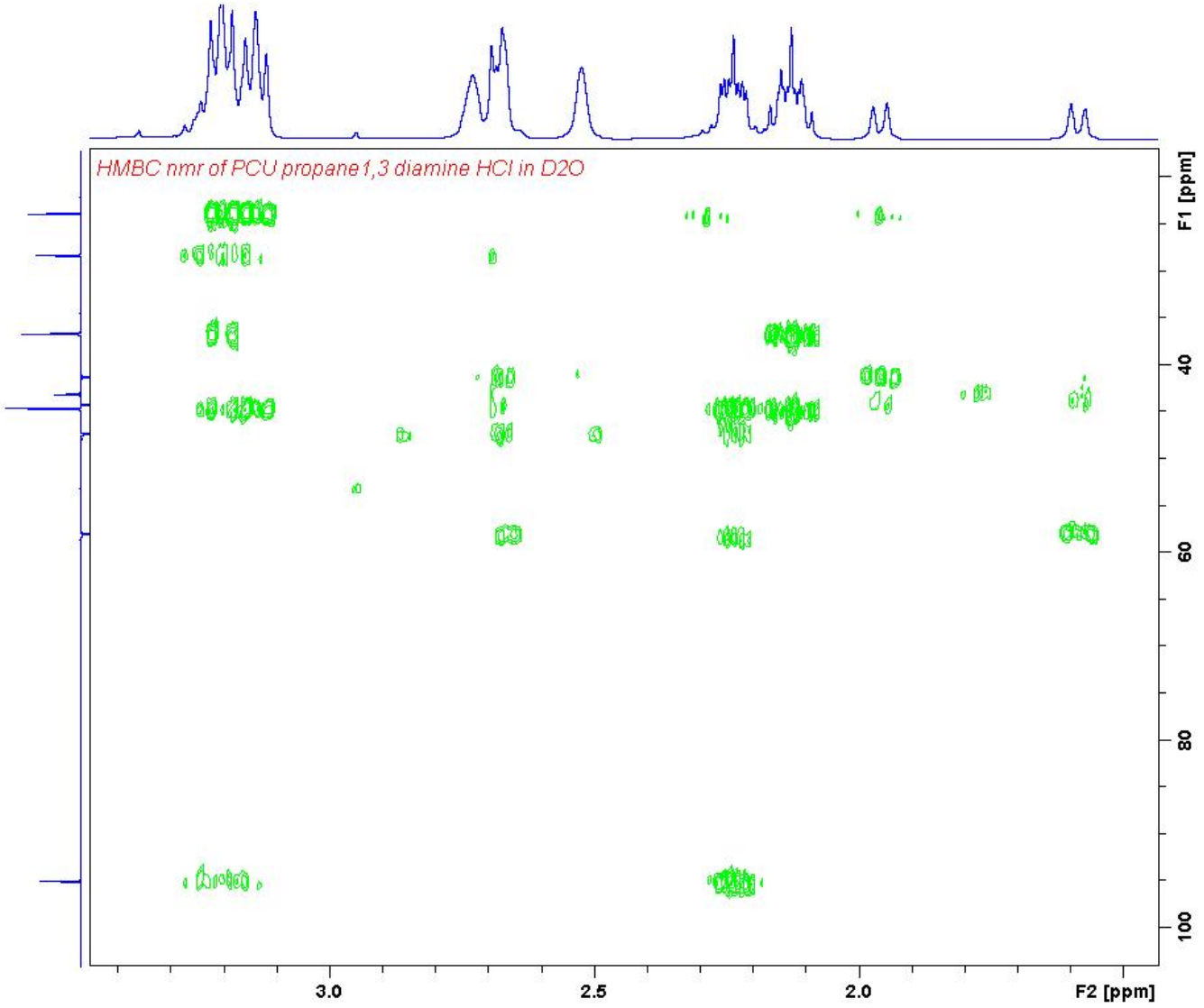
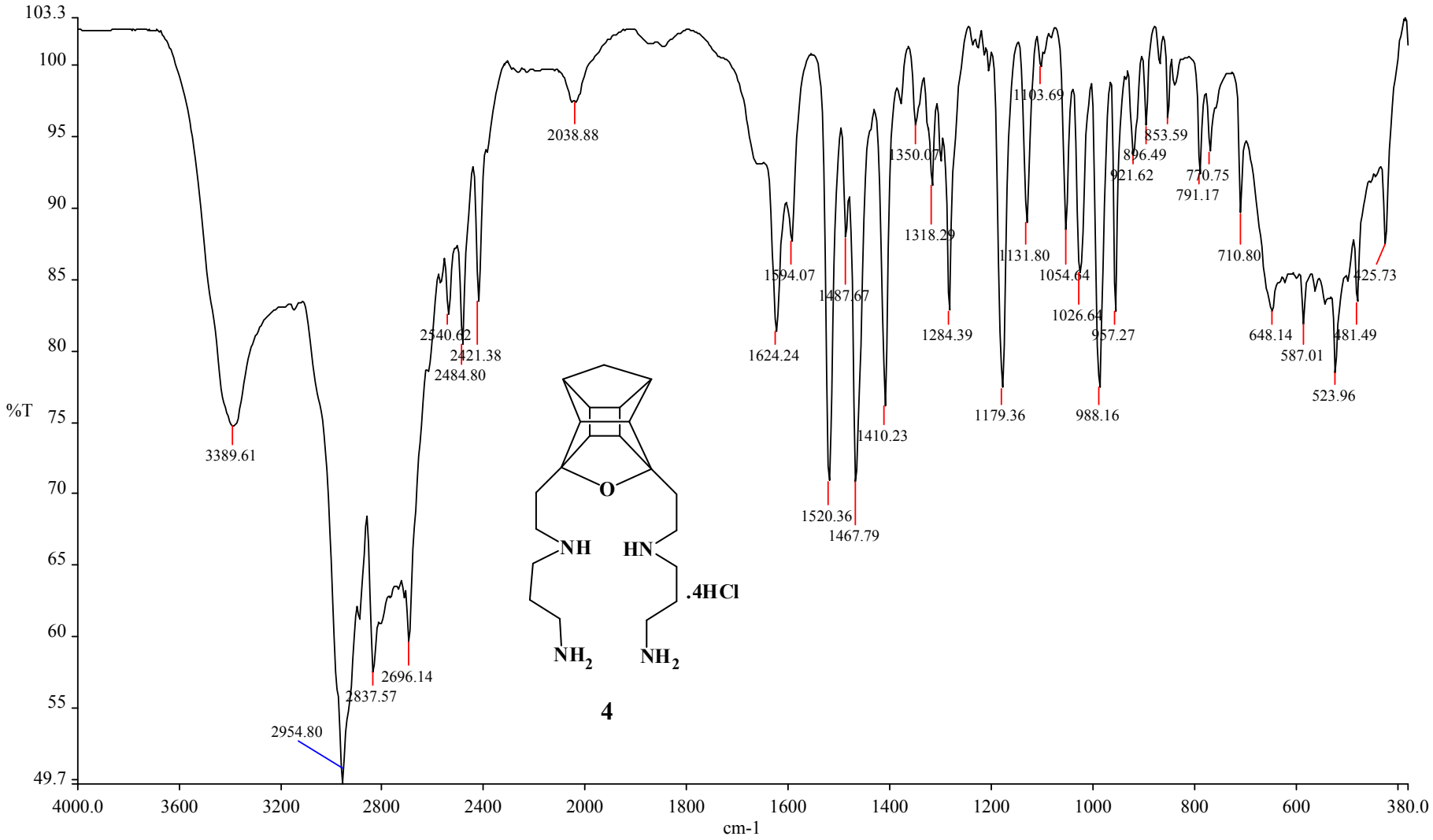
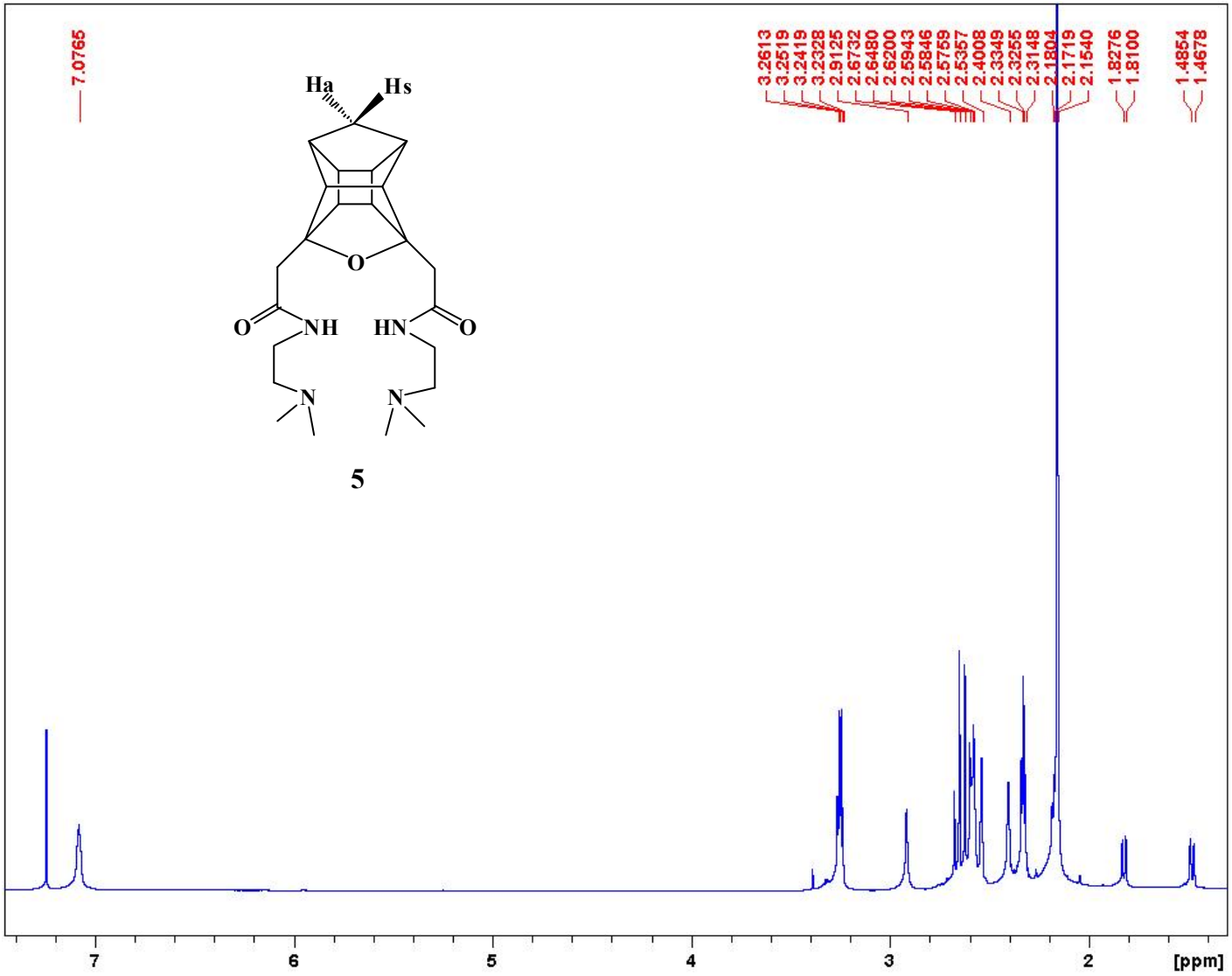


HMBC spectrum of PCU propane 1,3- diamine HCl (4)

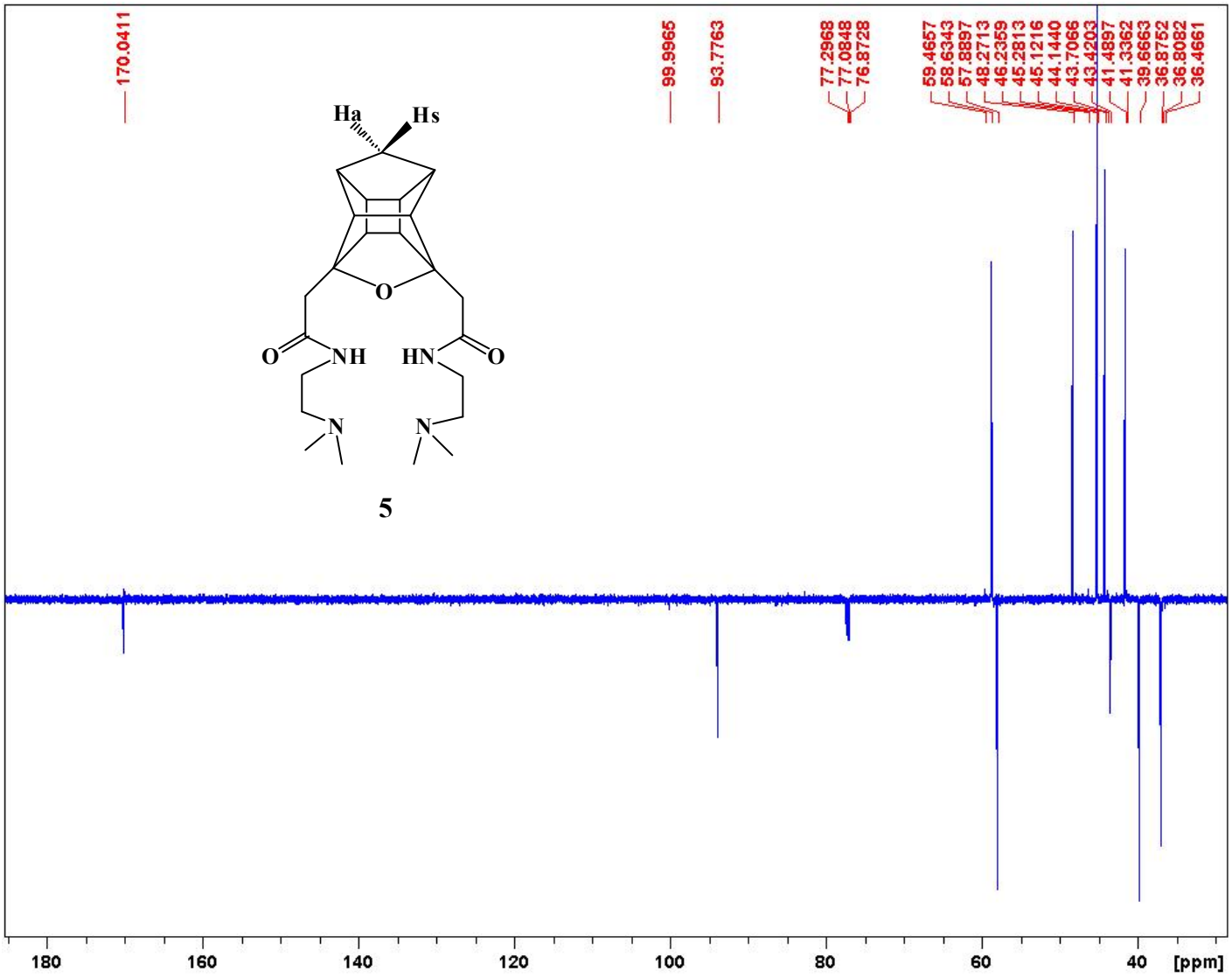


IR spectrum of PCU propane 1,3- diamine HCl (4)

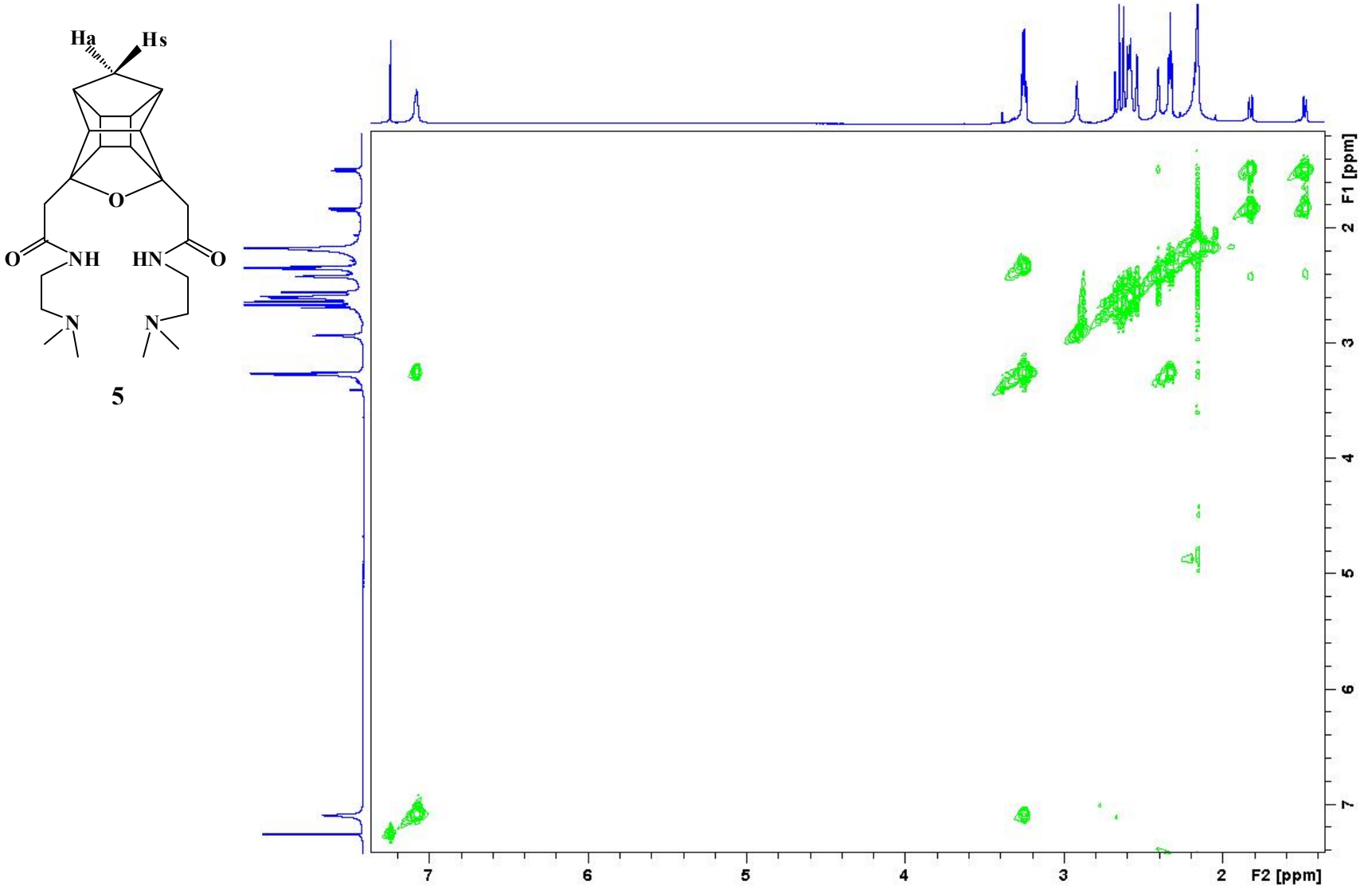


5

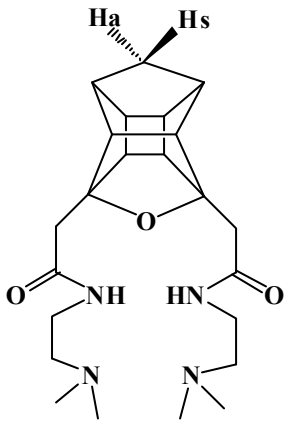
^{1}H NMR spectrum of PCU carbonyl-*N*,*N* dimethyl amino ethylamine (5)



^{13}C NMR spectrum of PCU carbonyl-*N*,*N* dimethyl amino ethylamine (5)



COSY spectrum of PCU carbonyl-*N*,*N* dimethyl amino ethylamine (5)



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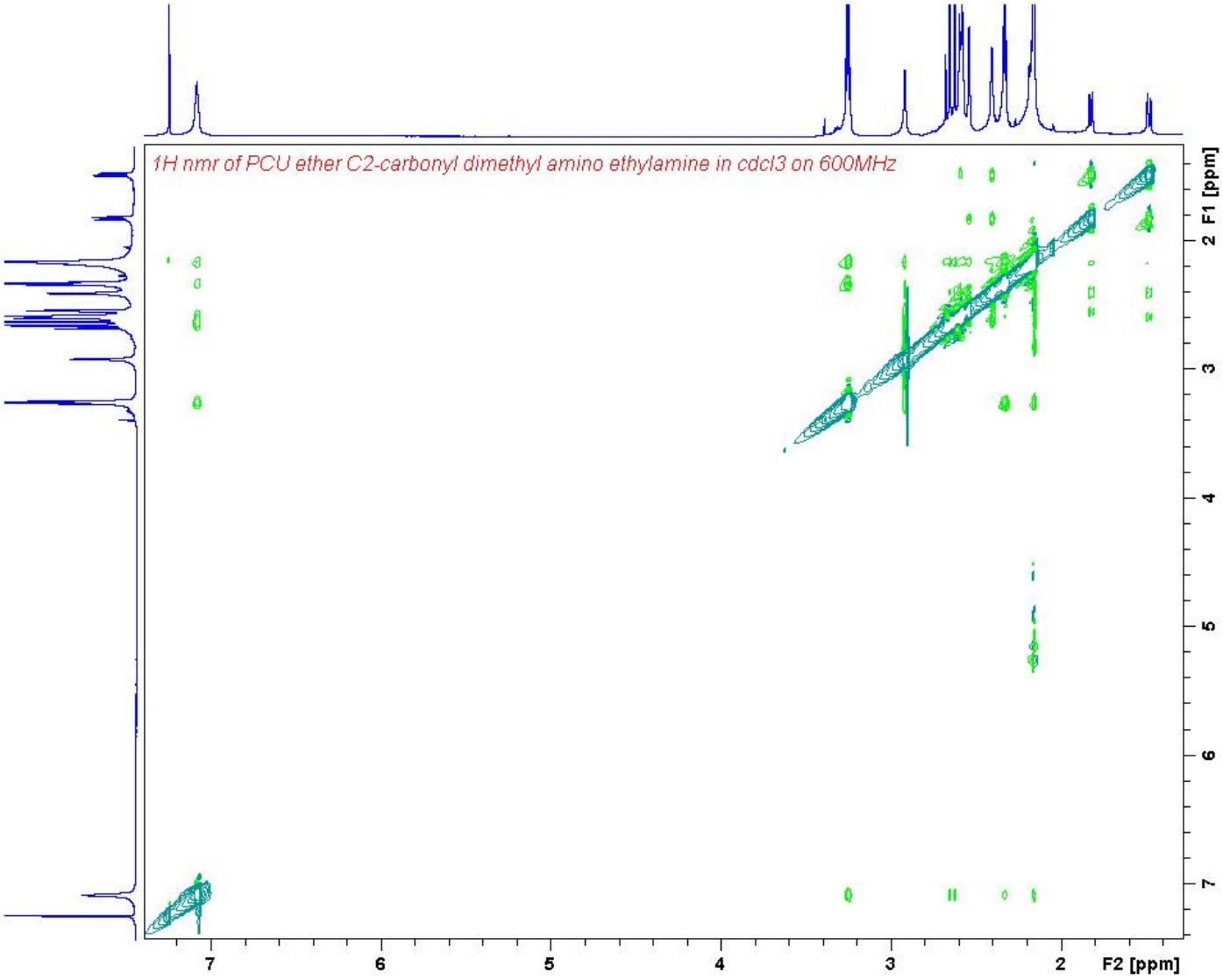
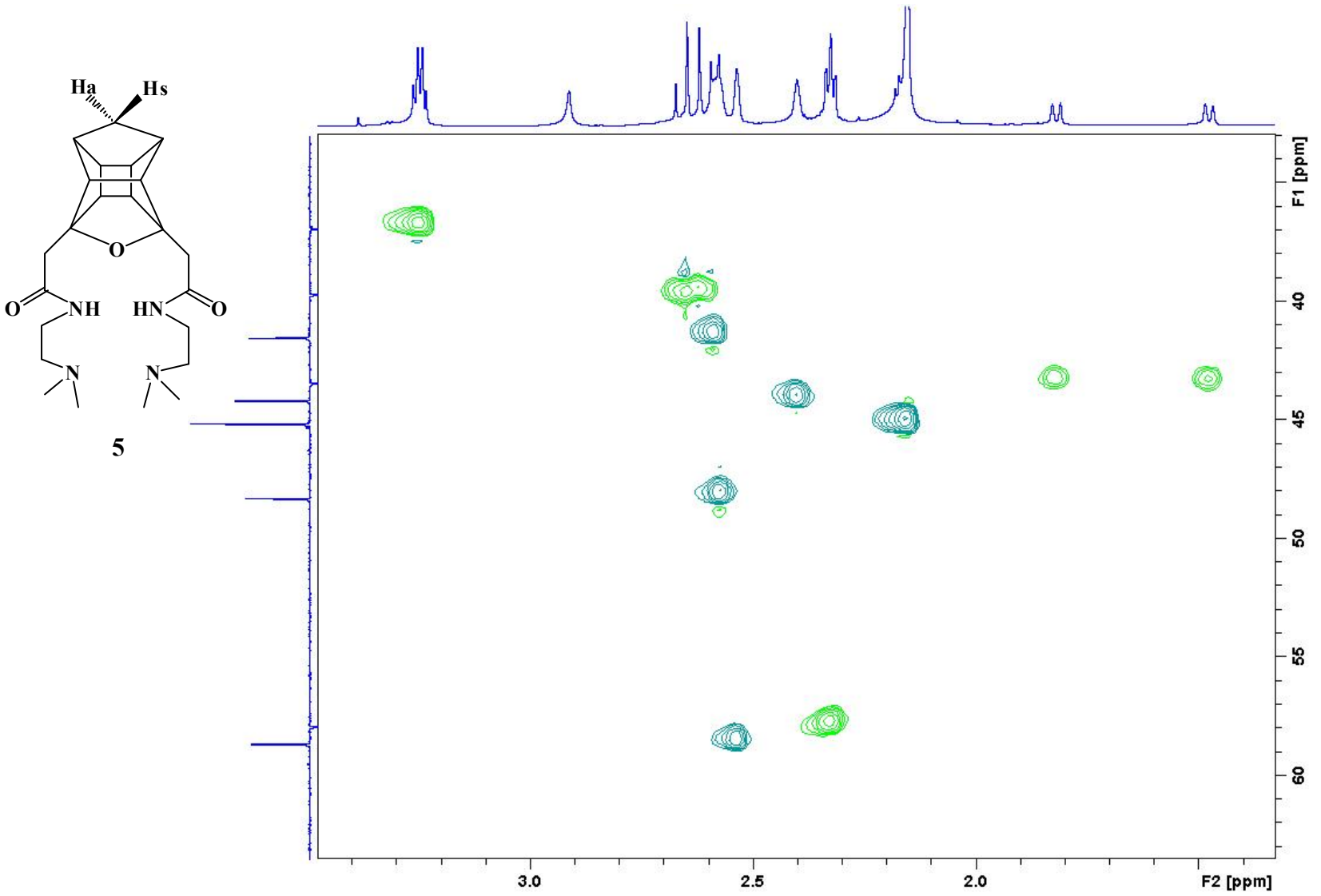
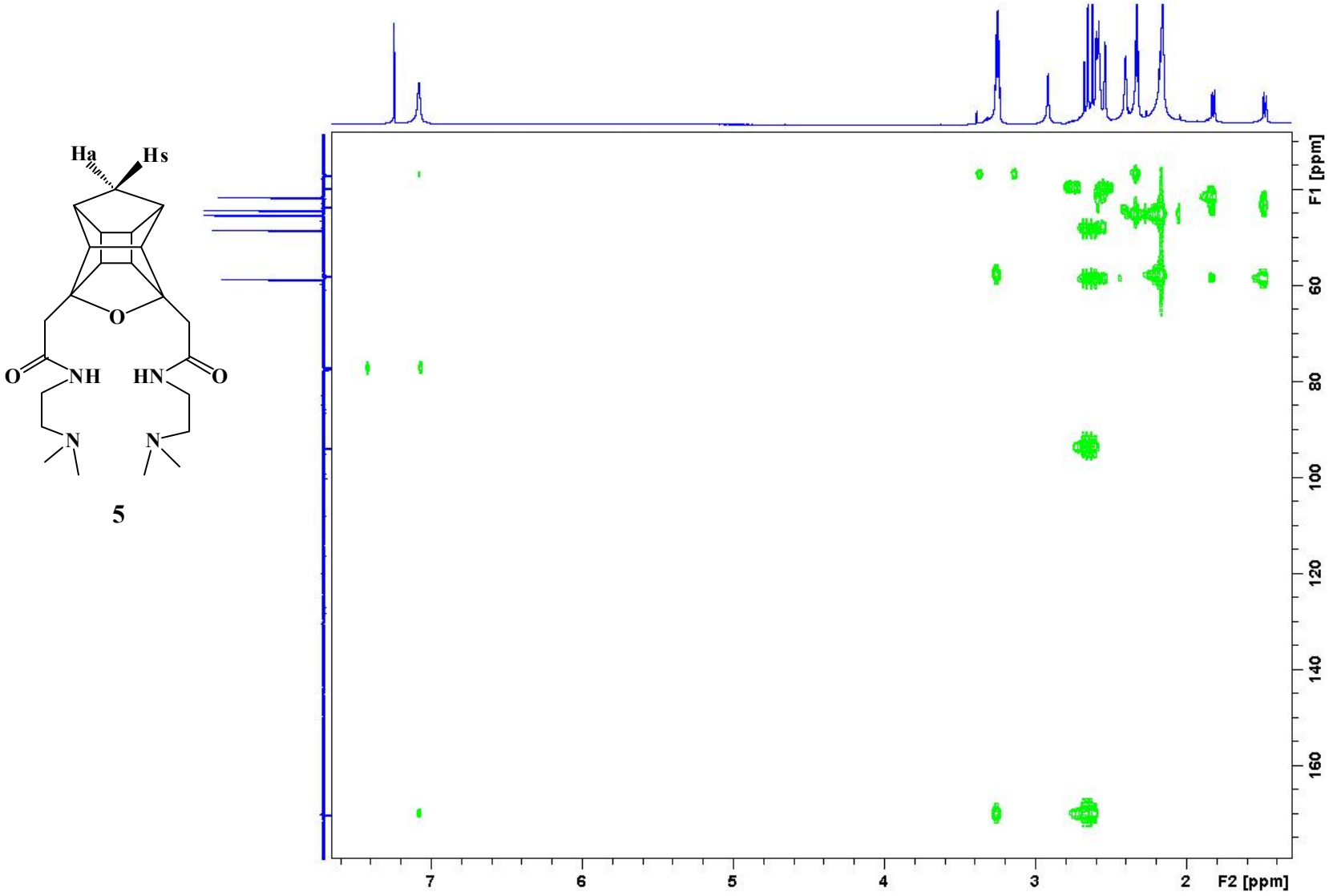


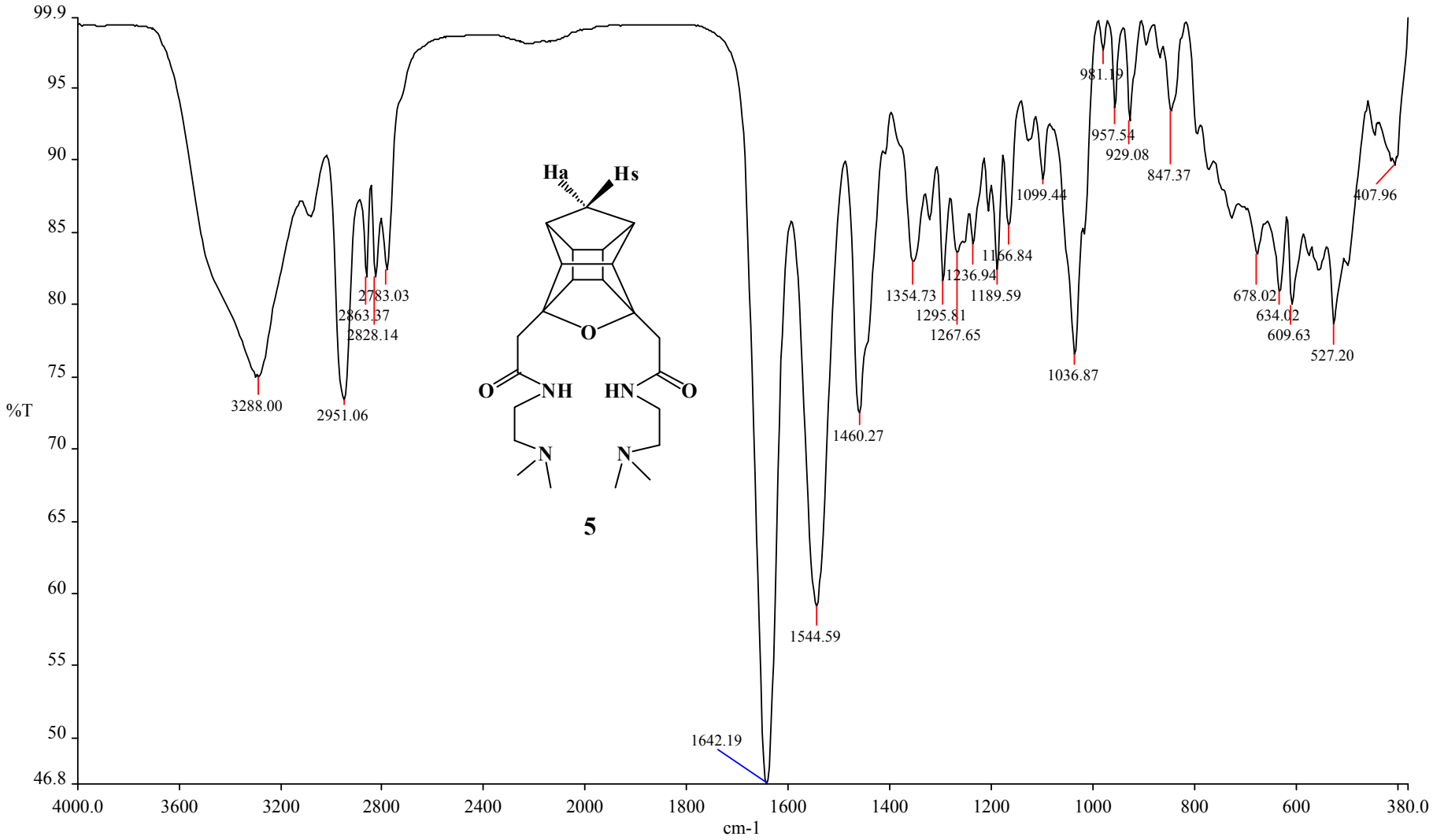
NOESY spectrum of PCU carbonyl- *N,N* dimethyl amino ethylamine (5)



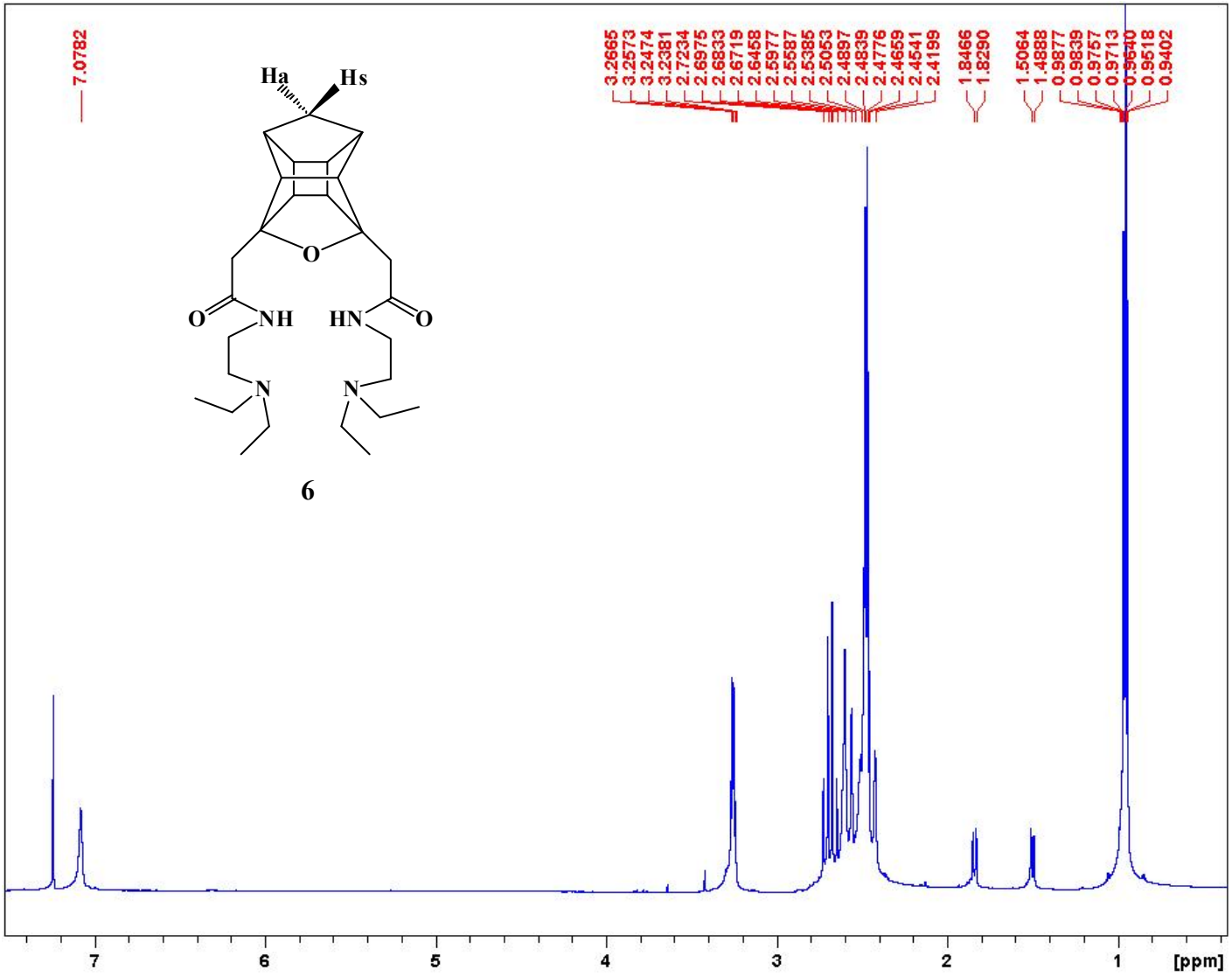
HSQC spectrum of PCU carbonyl- *N,N* dimethyl amino ethylamine (5)



HMBC spectrum of PCU carbonyl- *N*,*N* dimethyl amino ethylamine (5)

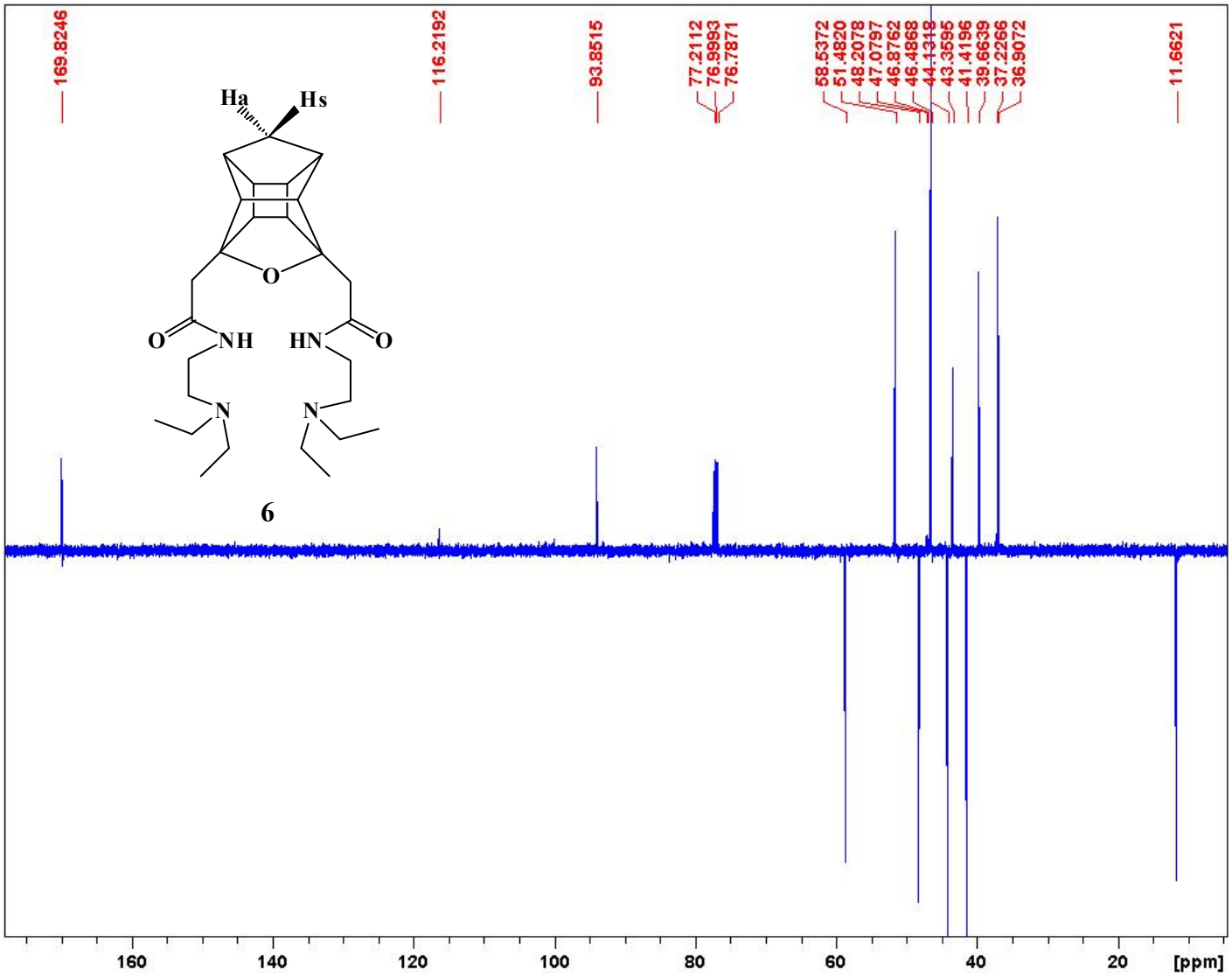


IR spectrum of PCU carbonyl- *N,N* dimethyl amino ethylamine (5)

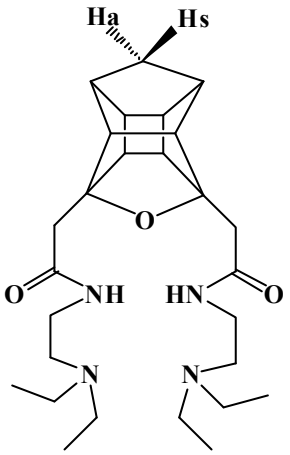


6

^{1}H NMR spectrum of PCU carbonyl- *N,N* diethyl amino ethylamine (6)



^{13}C NMR spectrum of PCU carbonyl-*N*,*N* diethyl amino ethylamine (6)



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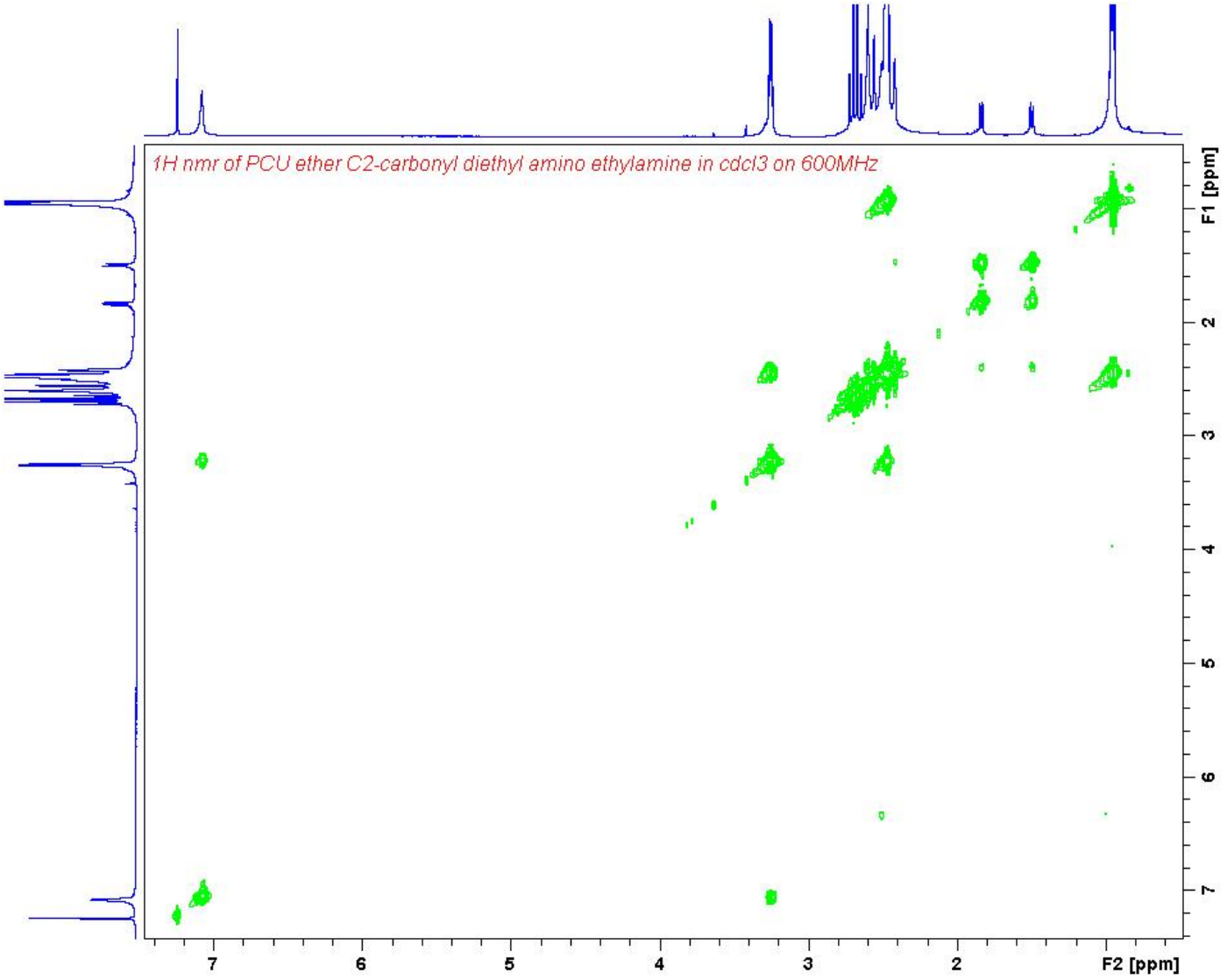
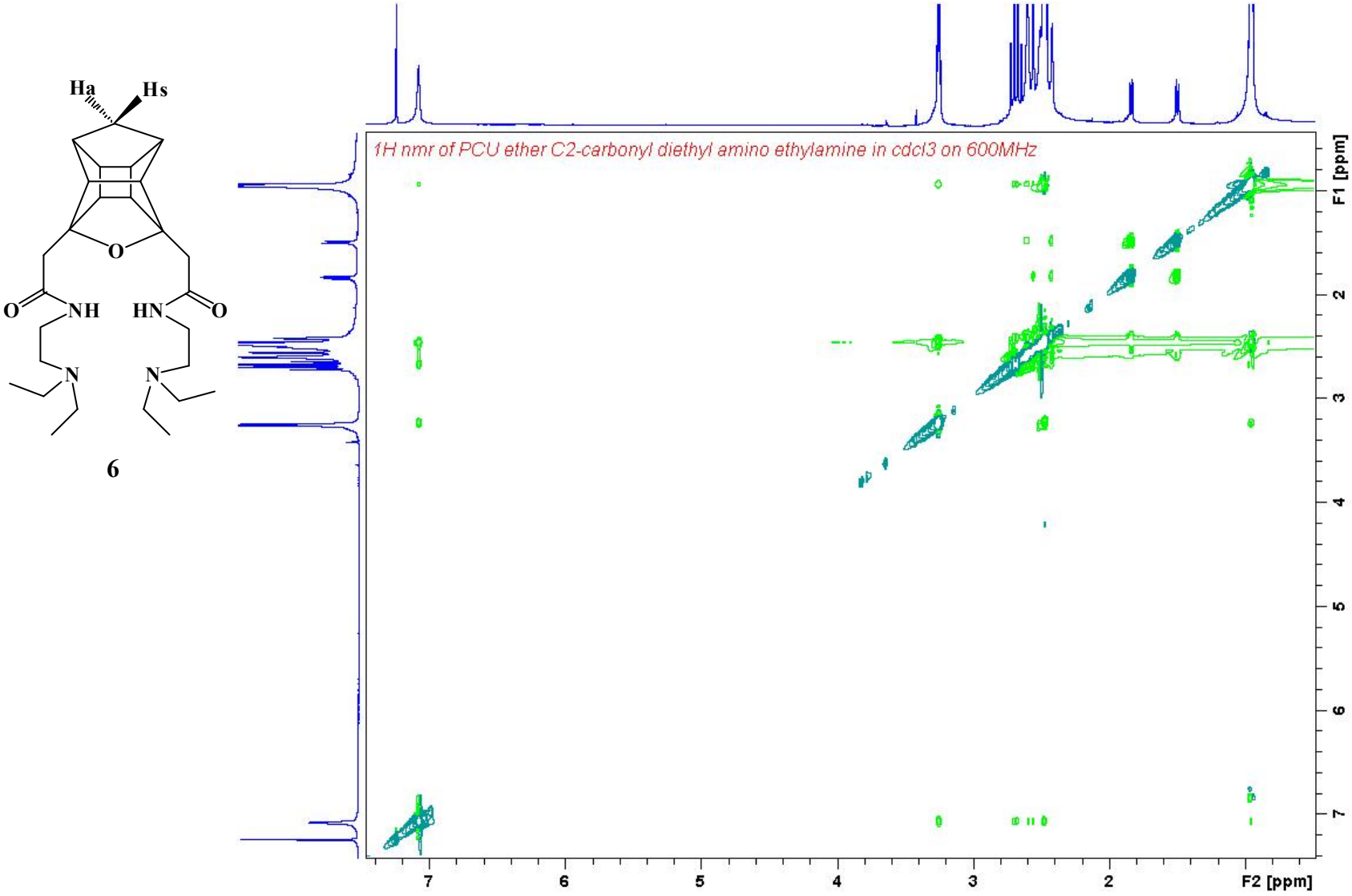
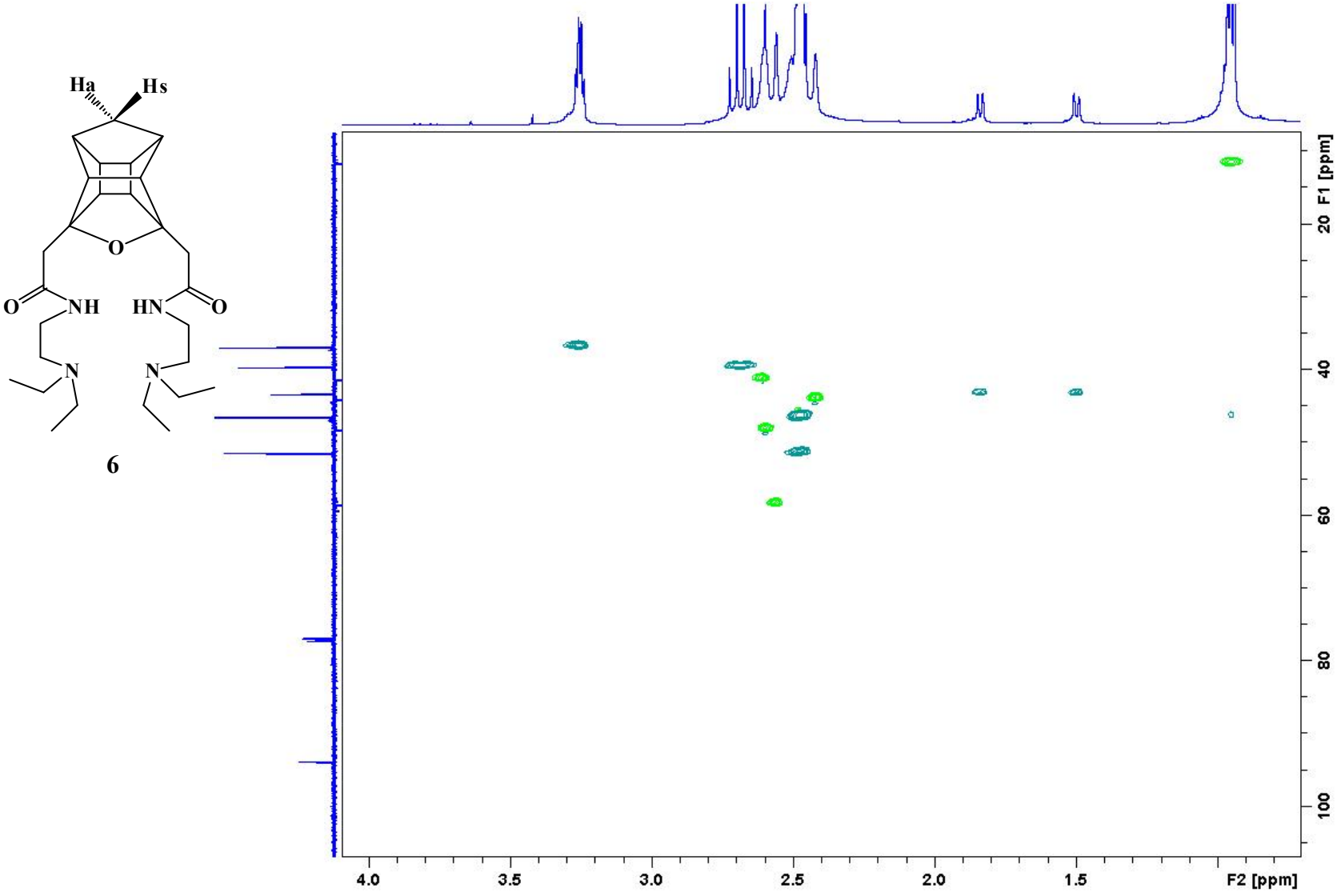


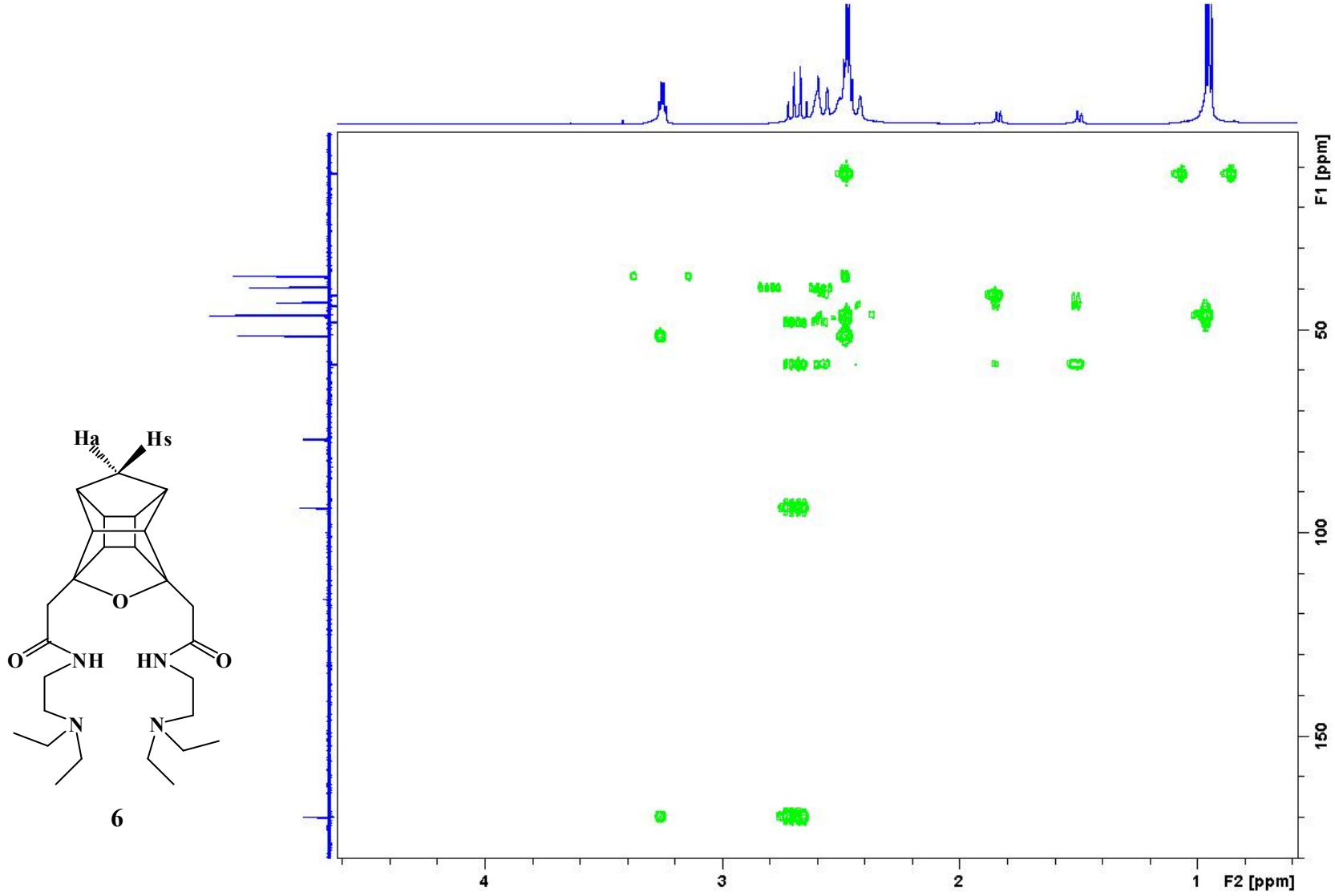
COSY spectrum of PCU carbonyl- *N,N* diethyl amino ethylamine (6)



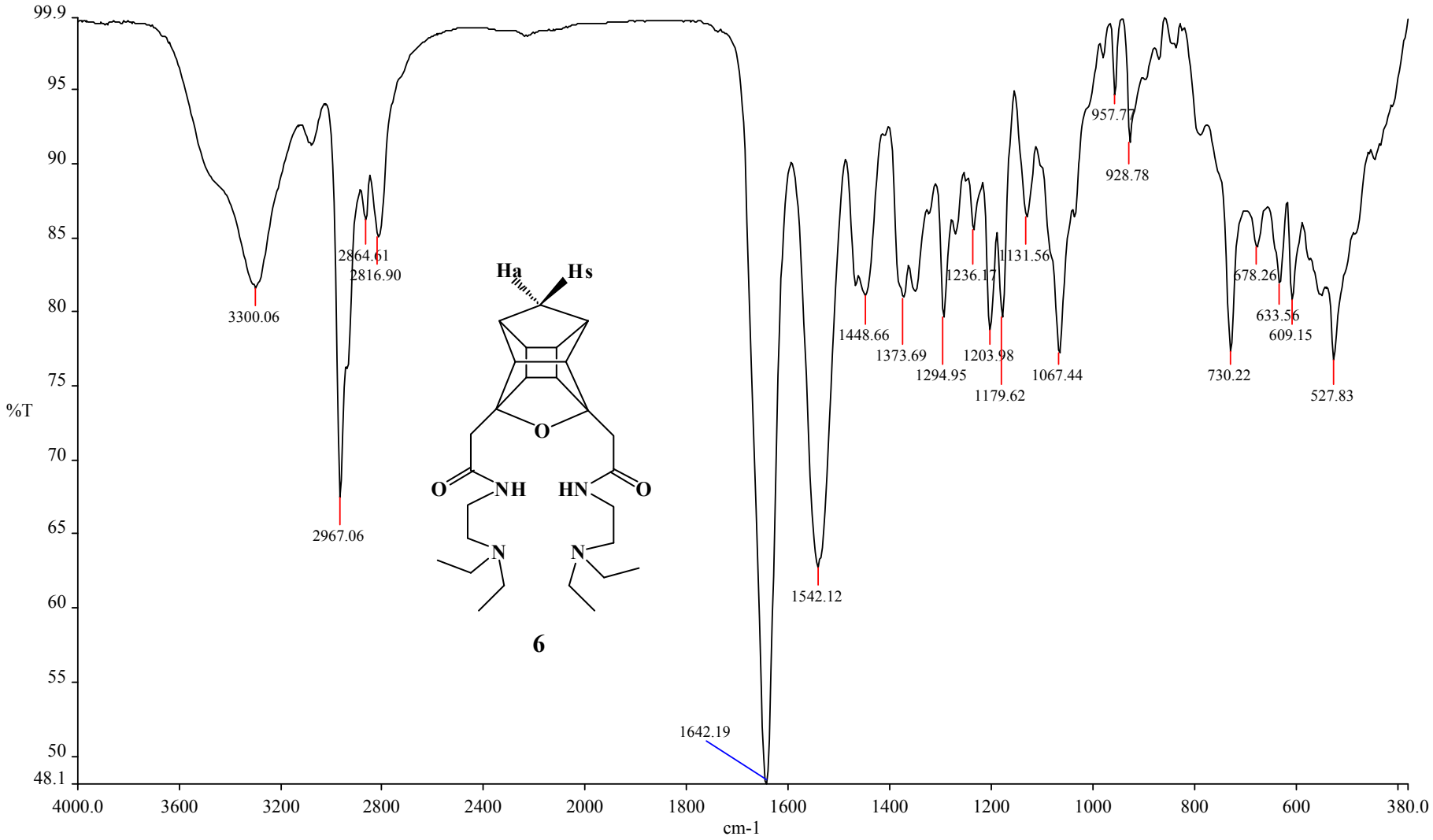
NOESY spectrum of PCU carbonyl- *N,N* diethyl amino ethylamine (6)



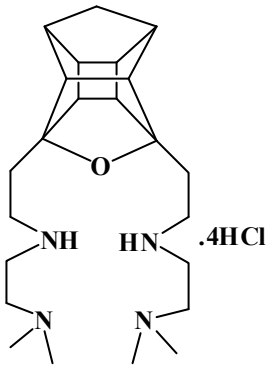
HSQC spectrum of PCU carbonyl- *N,N* diethyl amino ethylamine (6)



HMBC spectrum of PCU carbonyl- *N,N* diethyl amino ethylamine (6)



IR spectrum of PCU carbonyl- *N,N* diethyl amino ethylamine (6)



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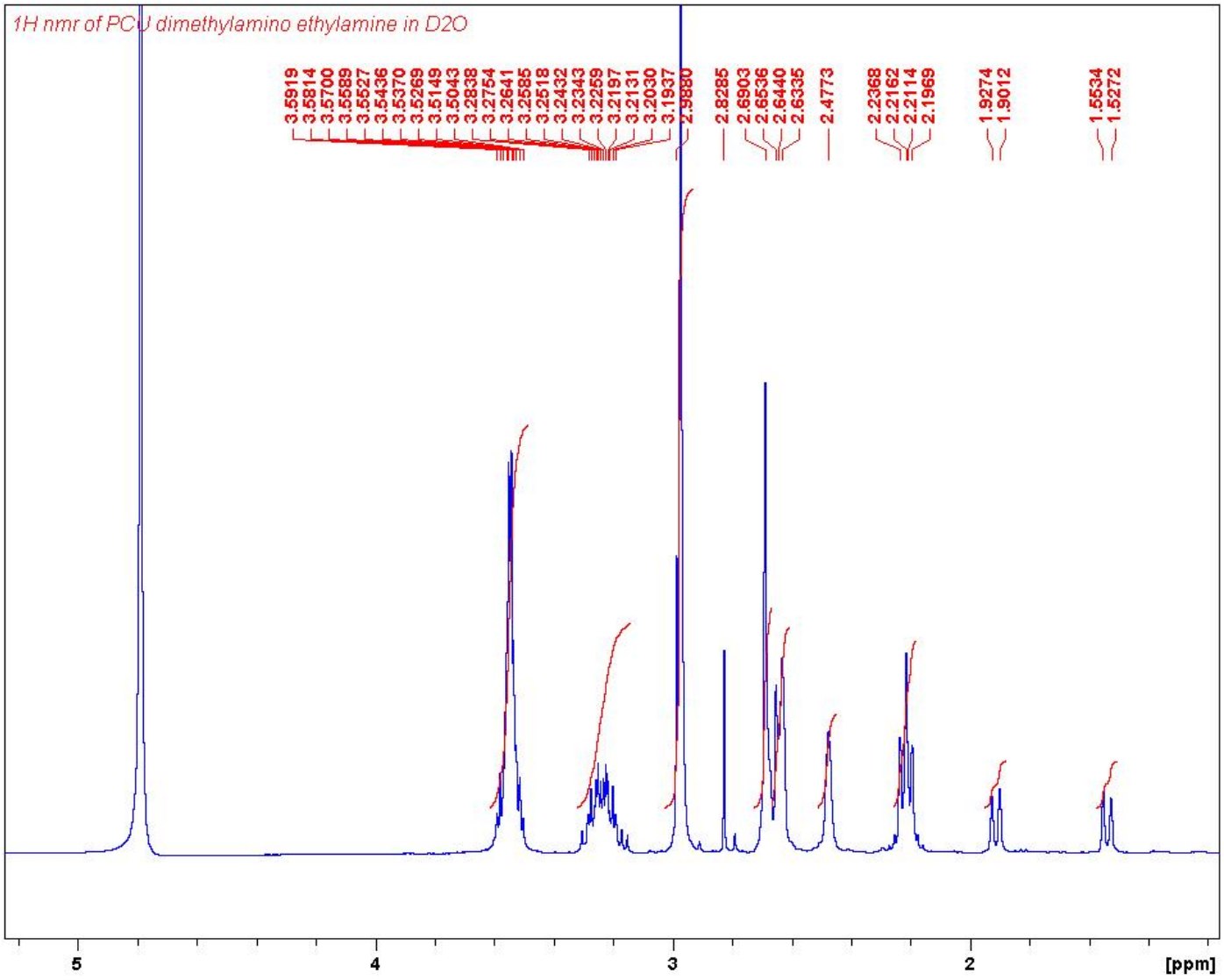
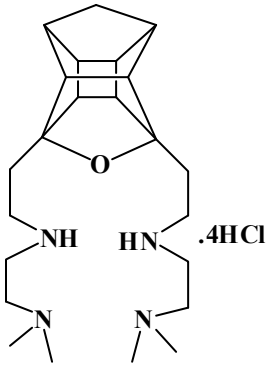


^{1}H NMR spectrum of PCU - *N,N* dimethyl amino ethylamine HCl (7)



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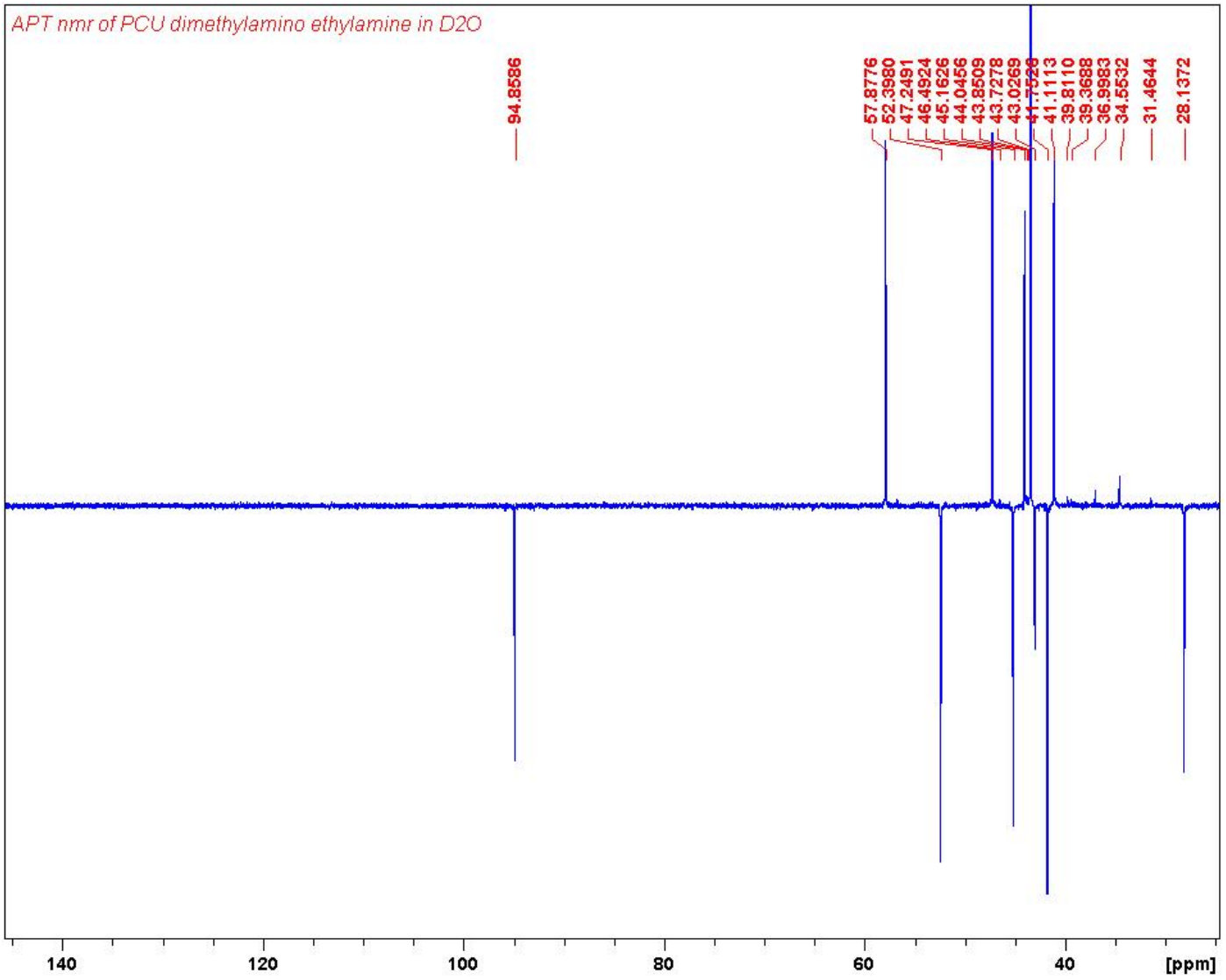
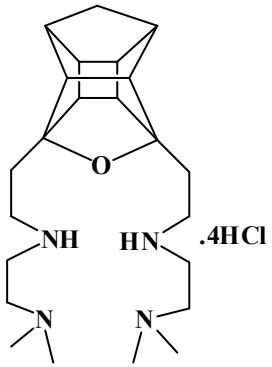


^{13}C NMR spectrum of PCU - *N,N* dimethyl amino ethylamine HCl (7)



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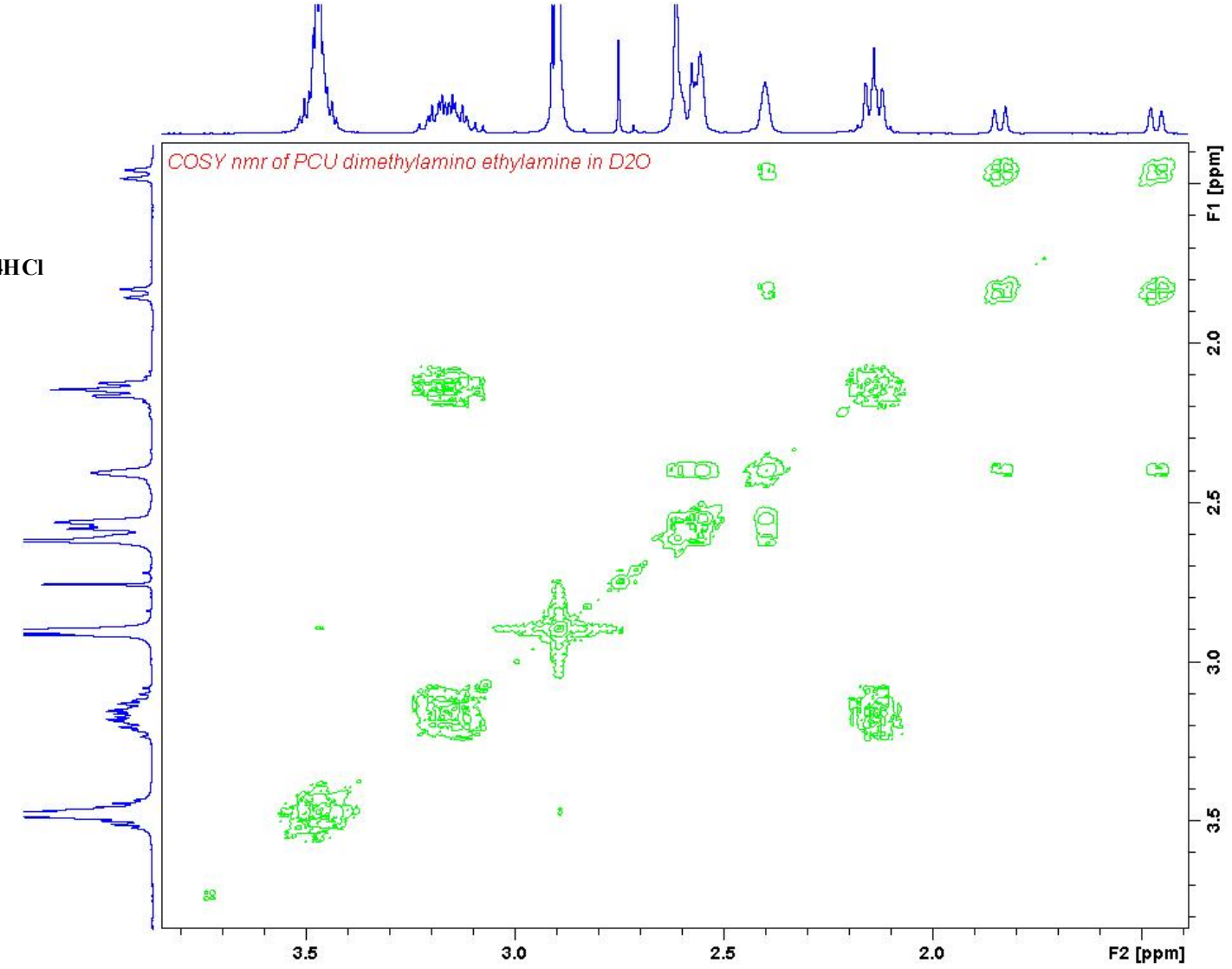
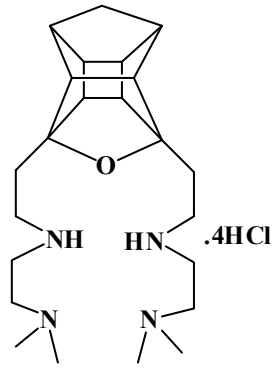


COSY spectrum of PCU - *N,N* dimethyl amino ethylamine HCl (7)



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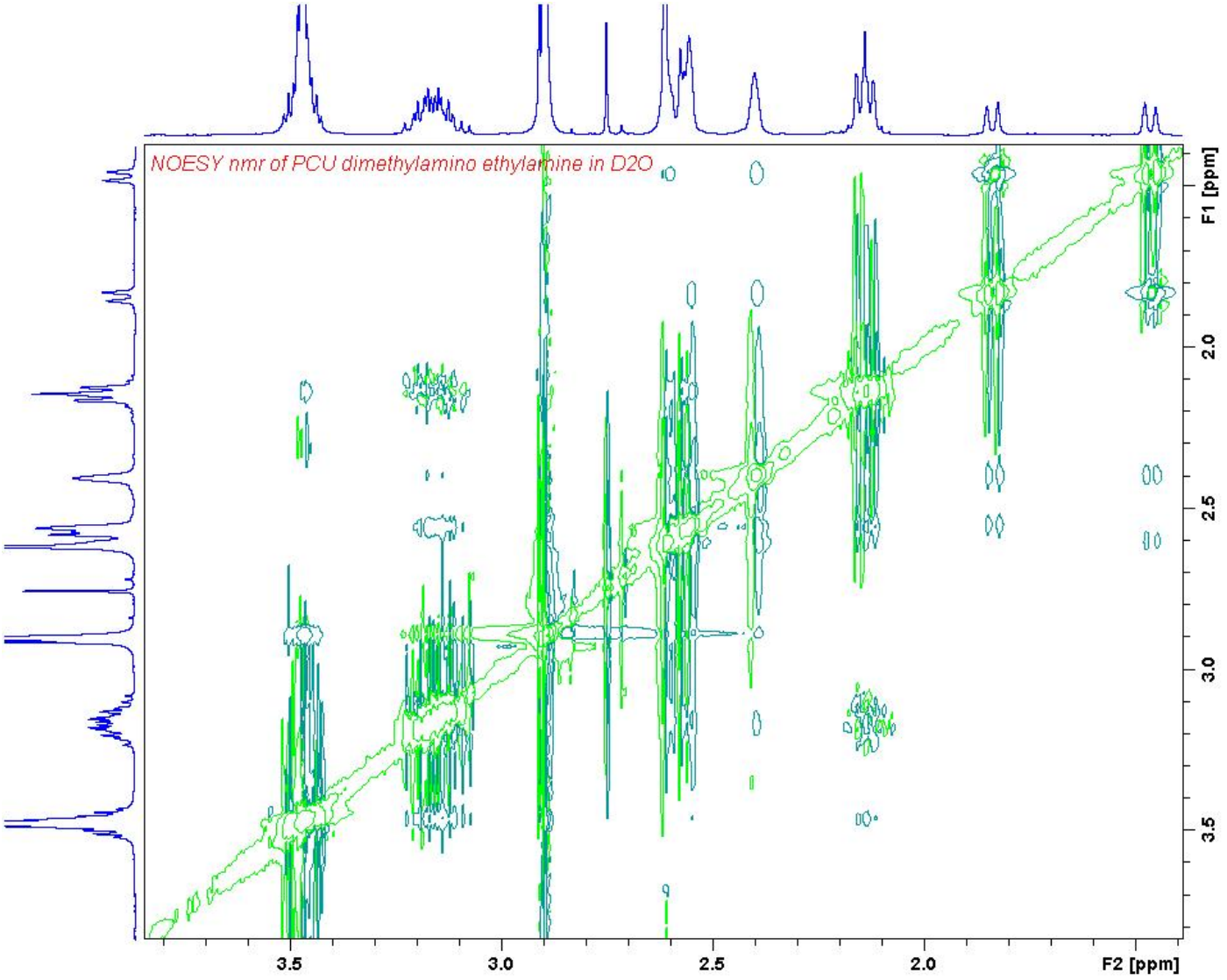
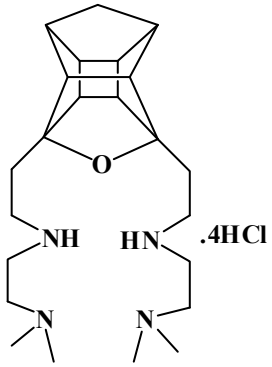


NOESY spectrum of PCU - *N,N* dimethyl amino ethylamine HCl (7)



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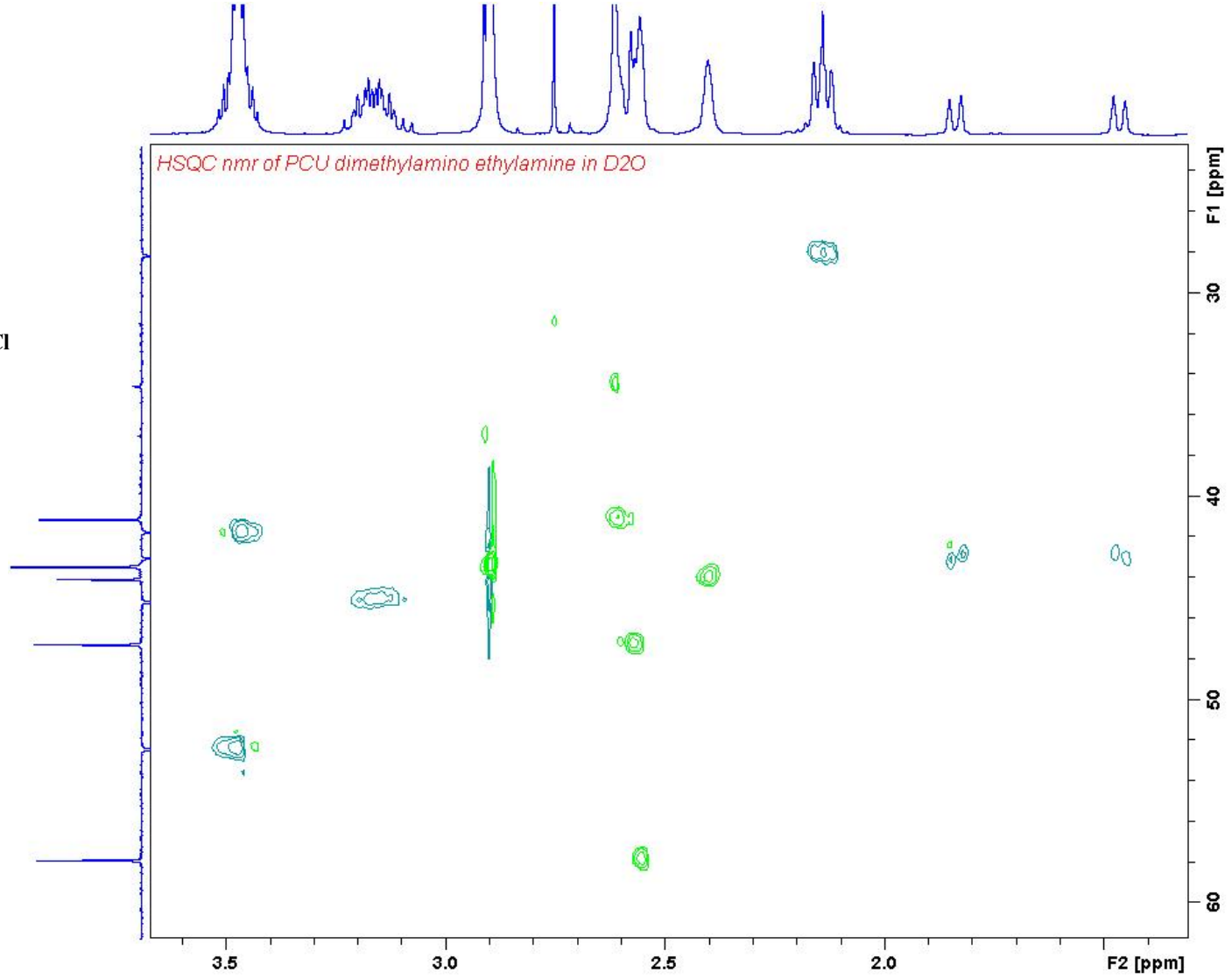
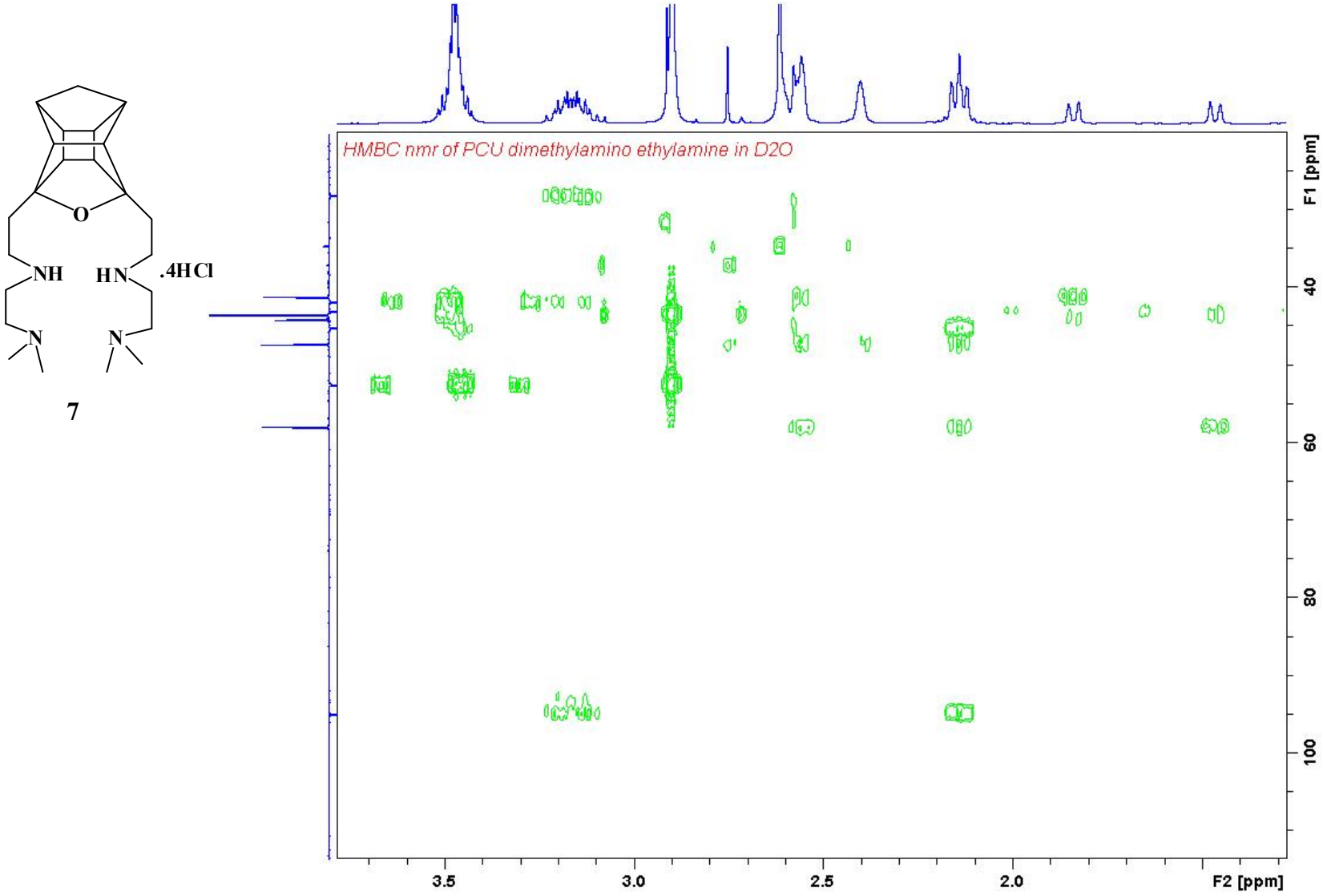
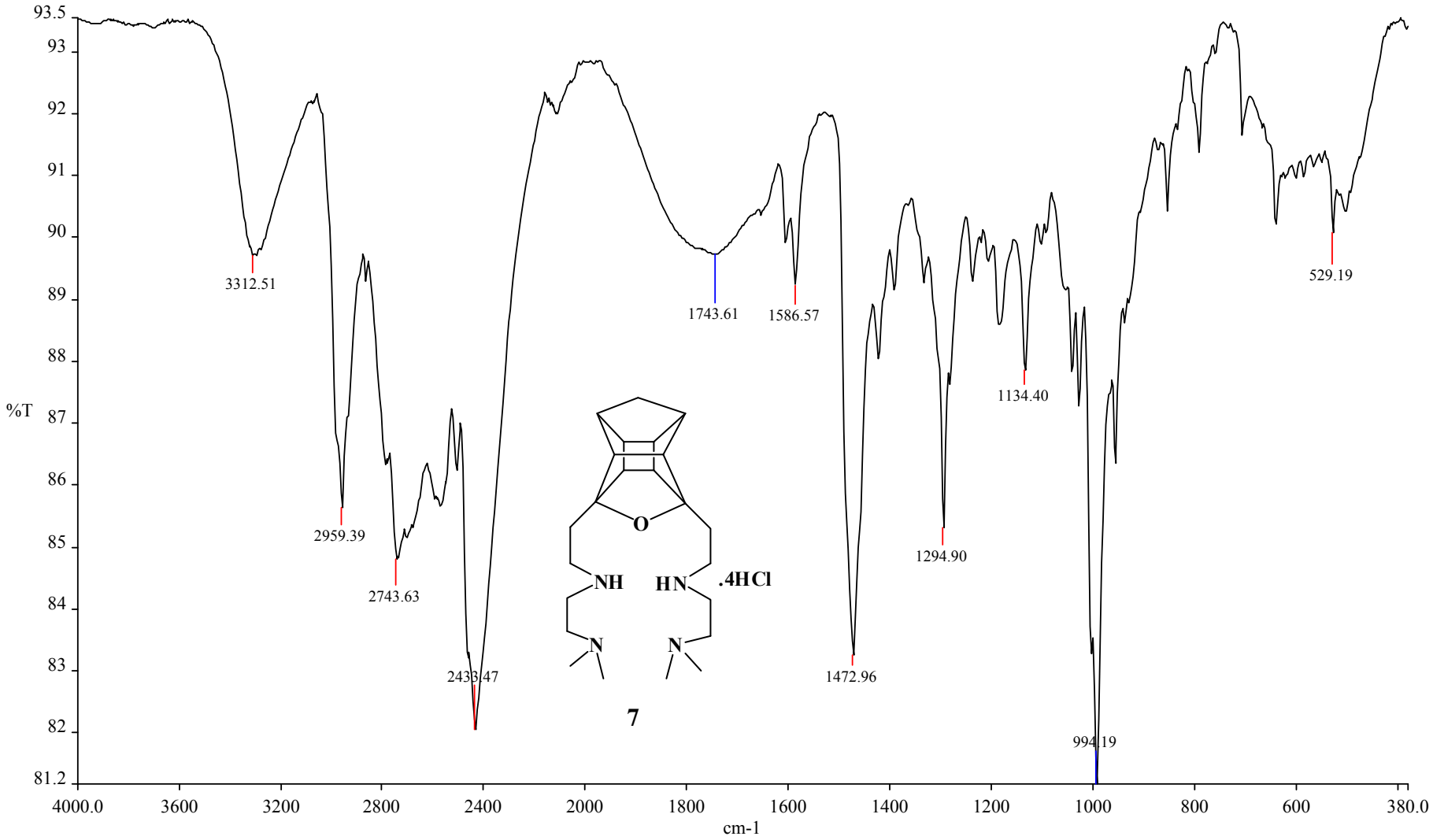


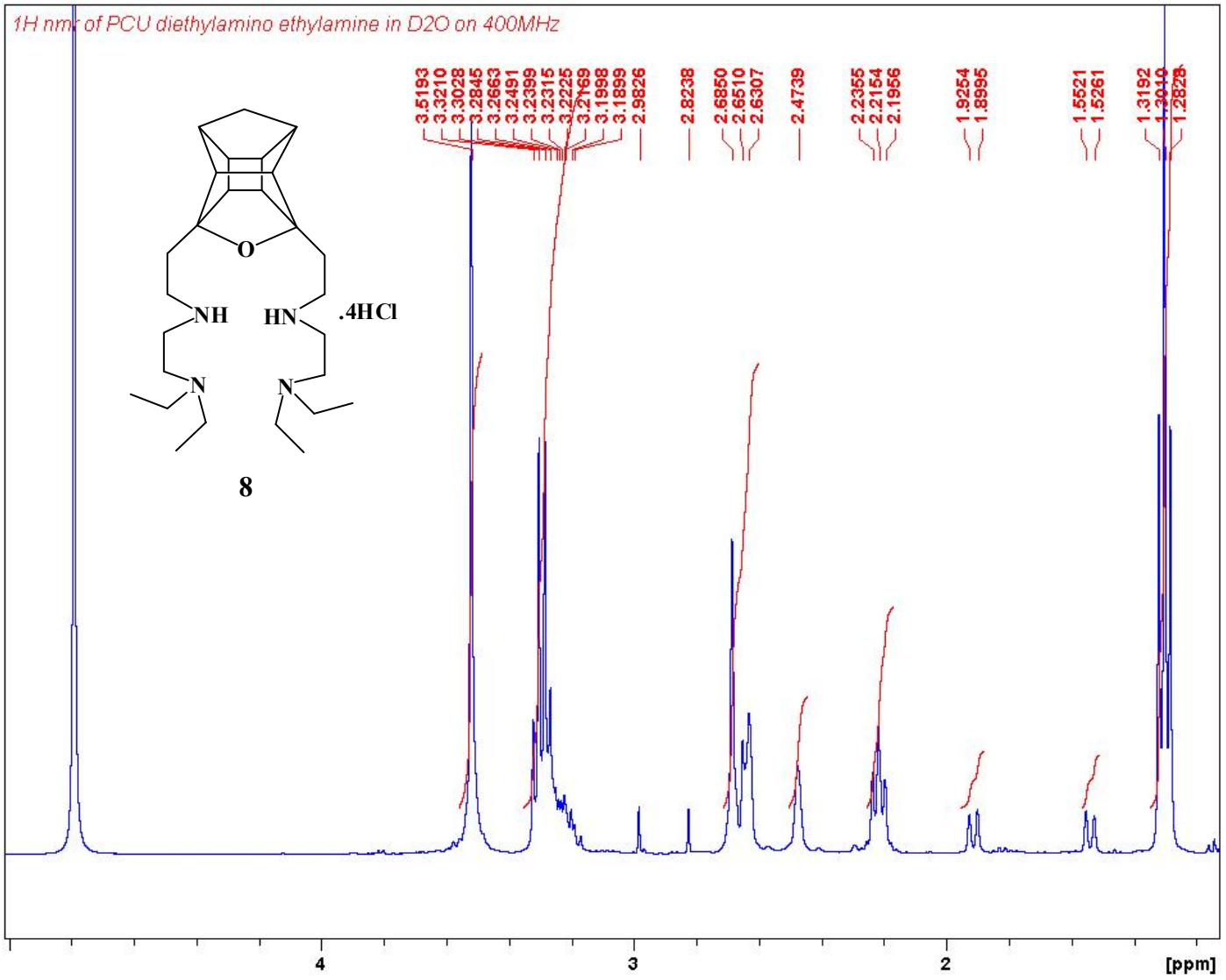
HSQC spectrum of PCU - *N,N* dimethyl amino ethylamine HCl (7)



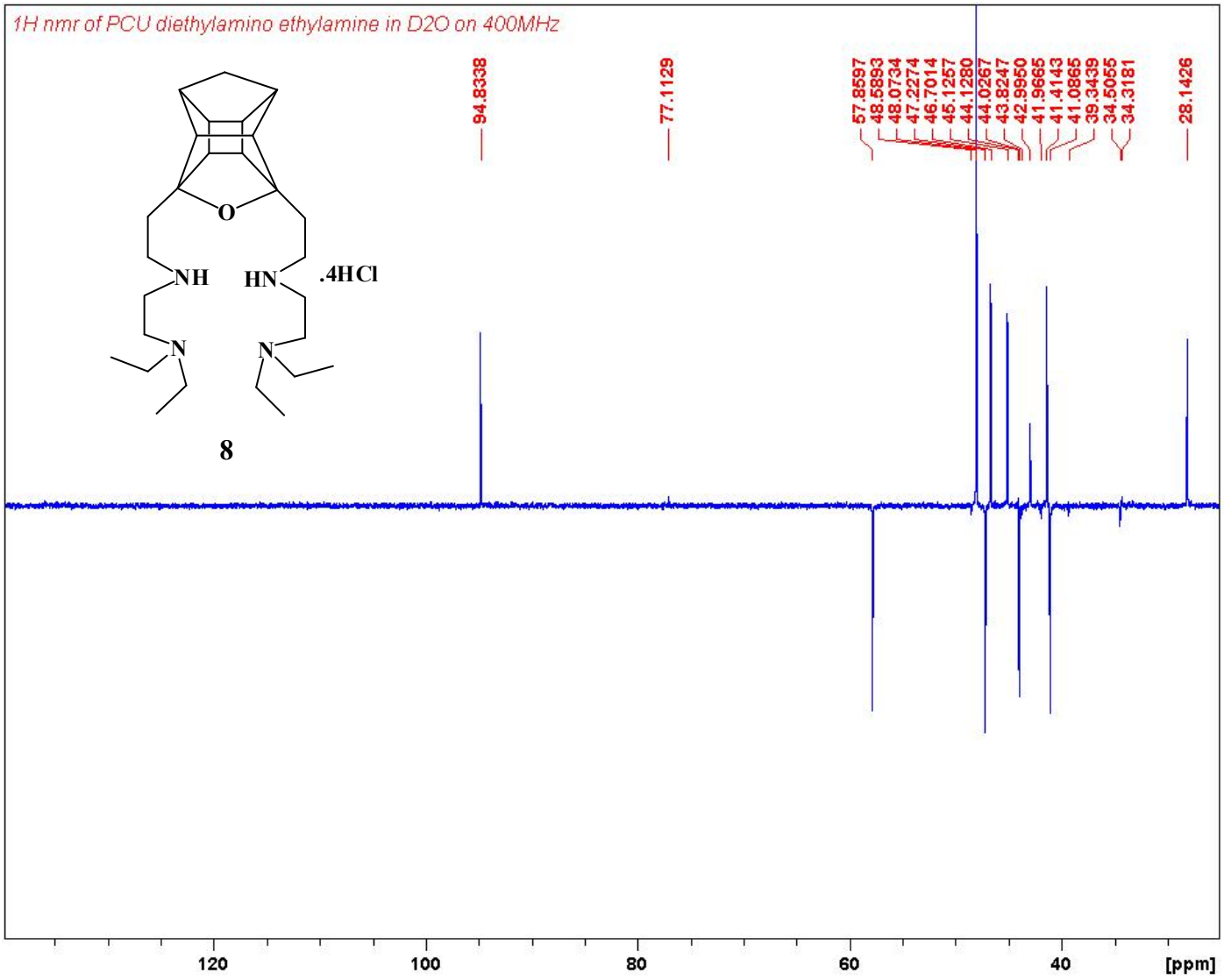
HMBC spectrum of PCU - *N,N* dimethyl amino ethylamine HCl (7)



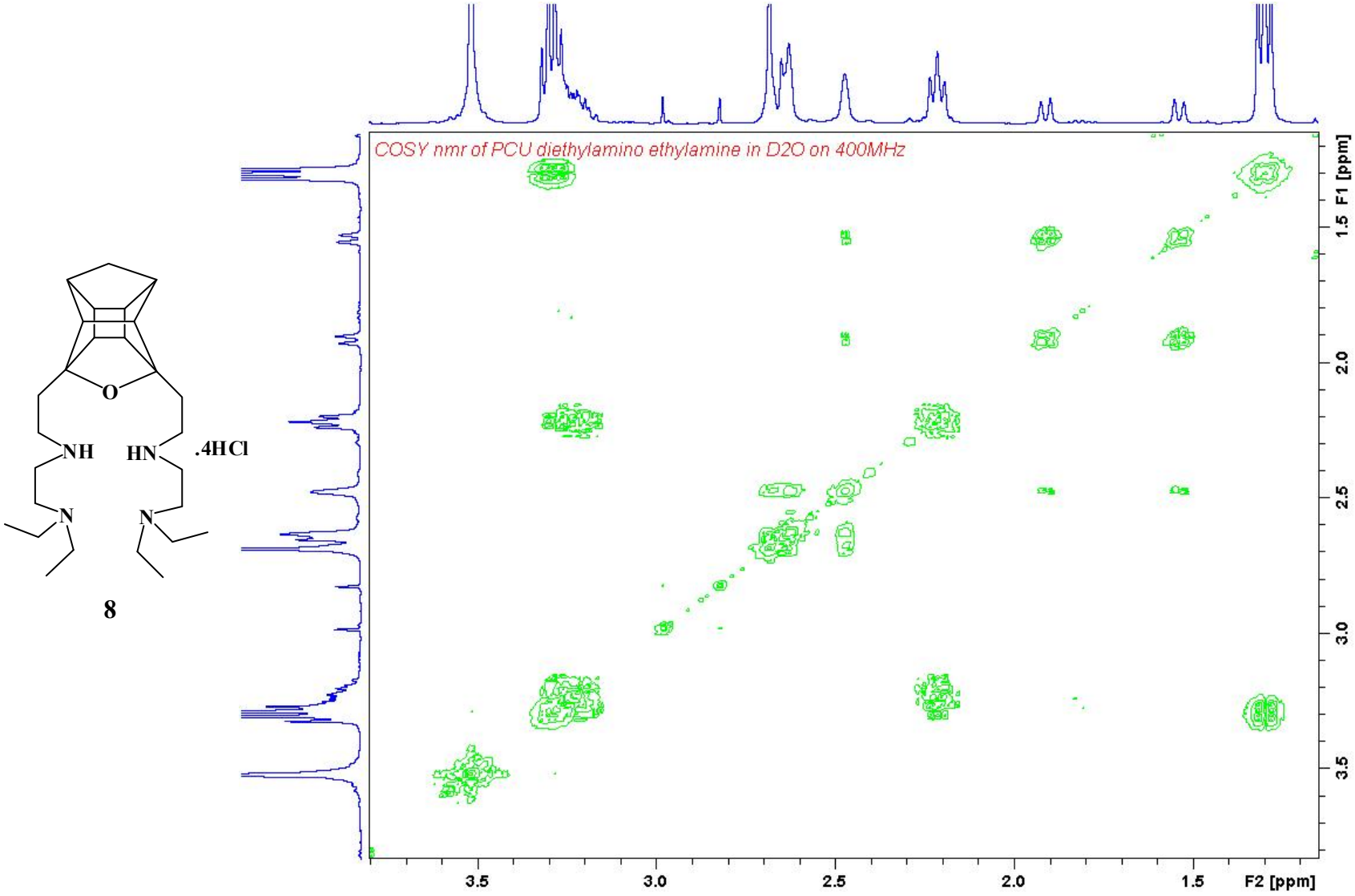
IR spectrum of PCU - *N,N* dimethyl amino ethylamine HCl (7)



^{1}H NMR spectrum of PCU - *N*,*N* diethyl amino ethylamine HCl (8)



^{13}C NMR spectrum of PCU - *N,N* diethyl amino ethylamine HCl (8)



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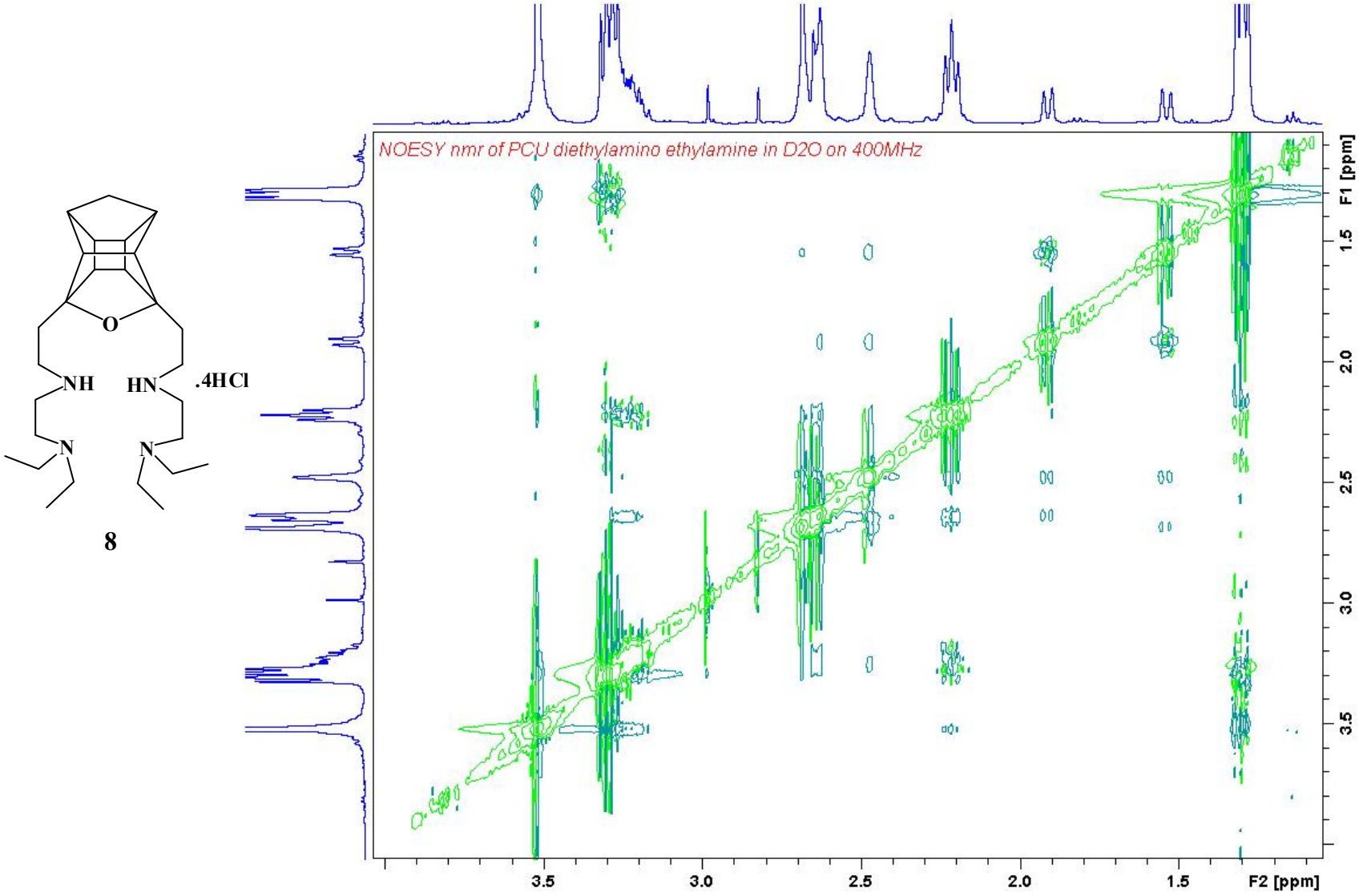
COSY spectrum of PCU - *N,N* diethyl amino ethylamine HCl (8)



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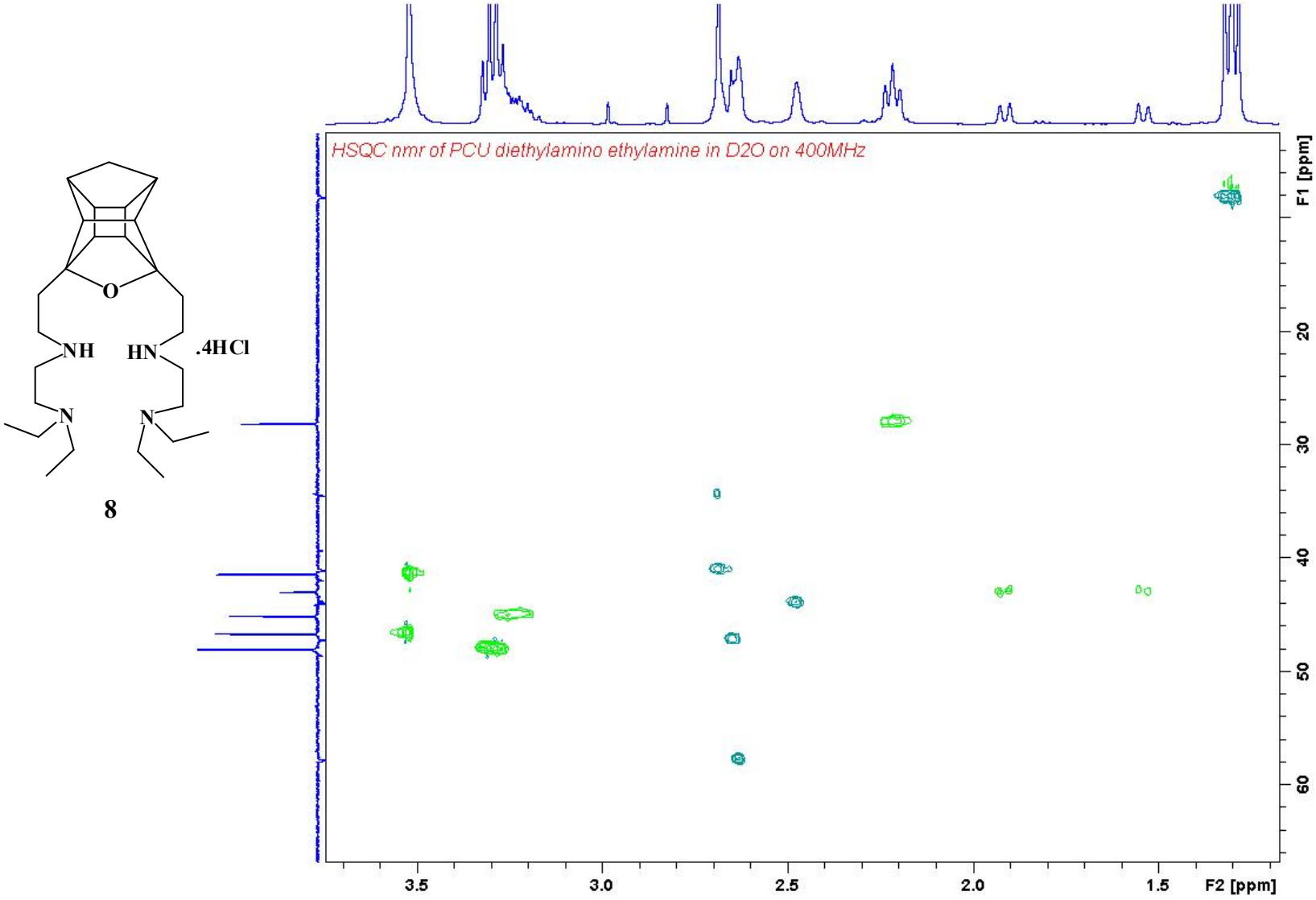
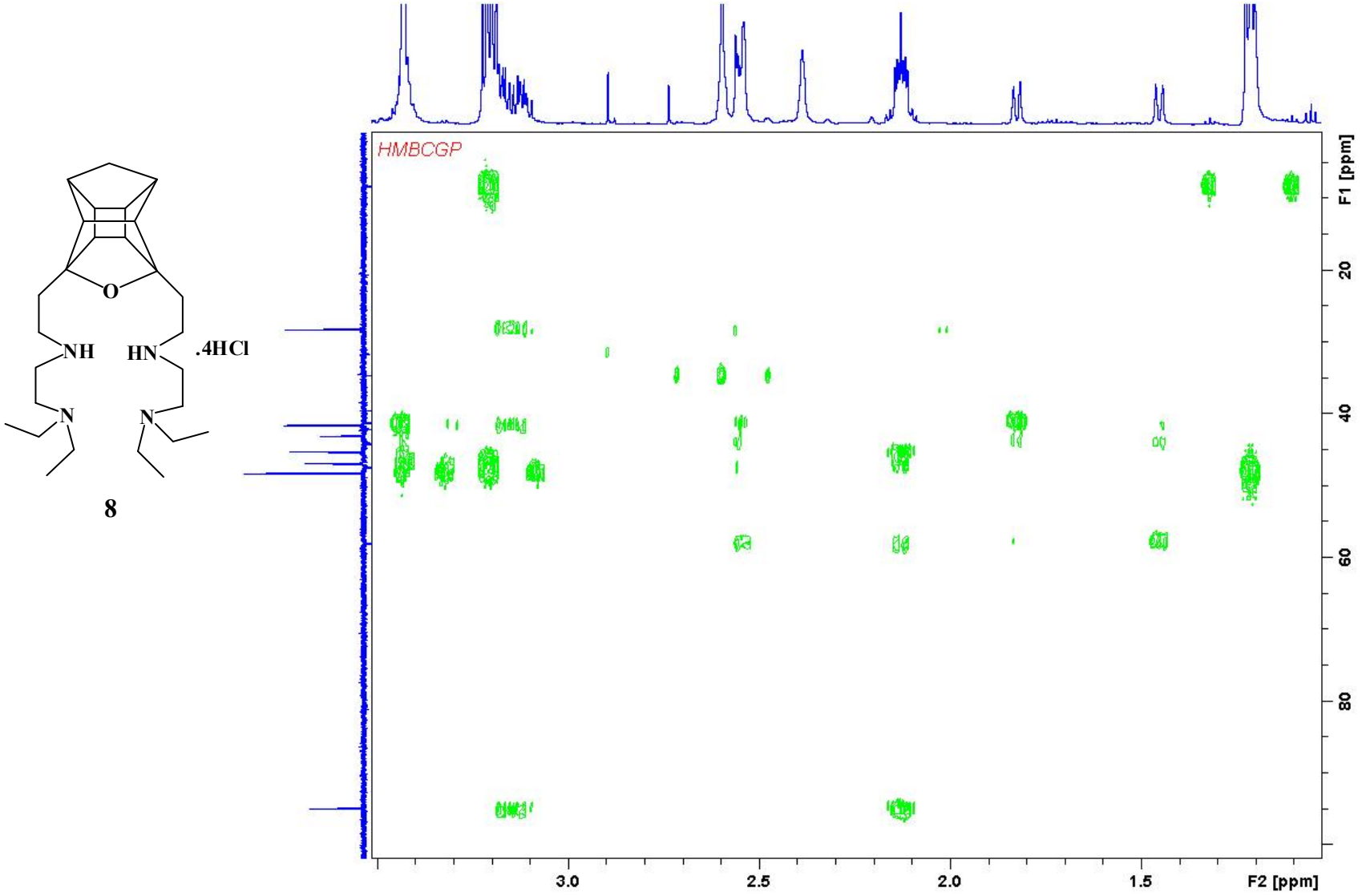
NOESY spectrum of PCU - *N*,*N* diethyl amino ethylamine HCl (8)

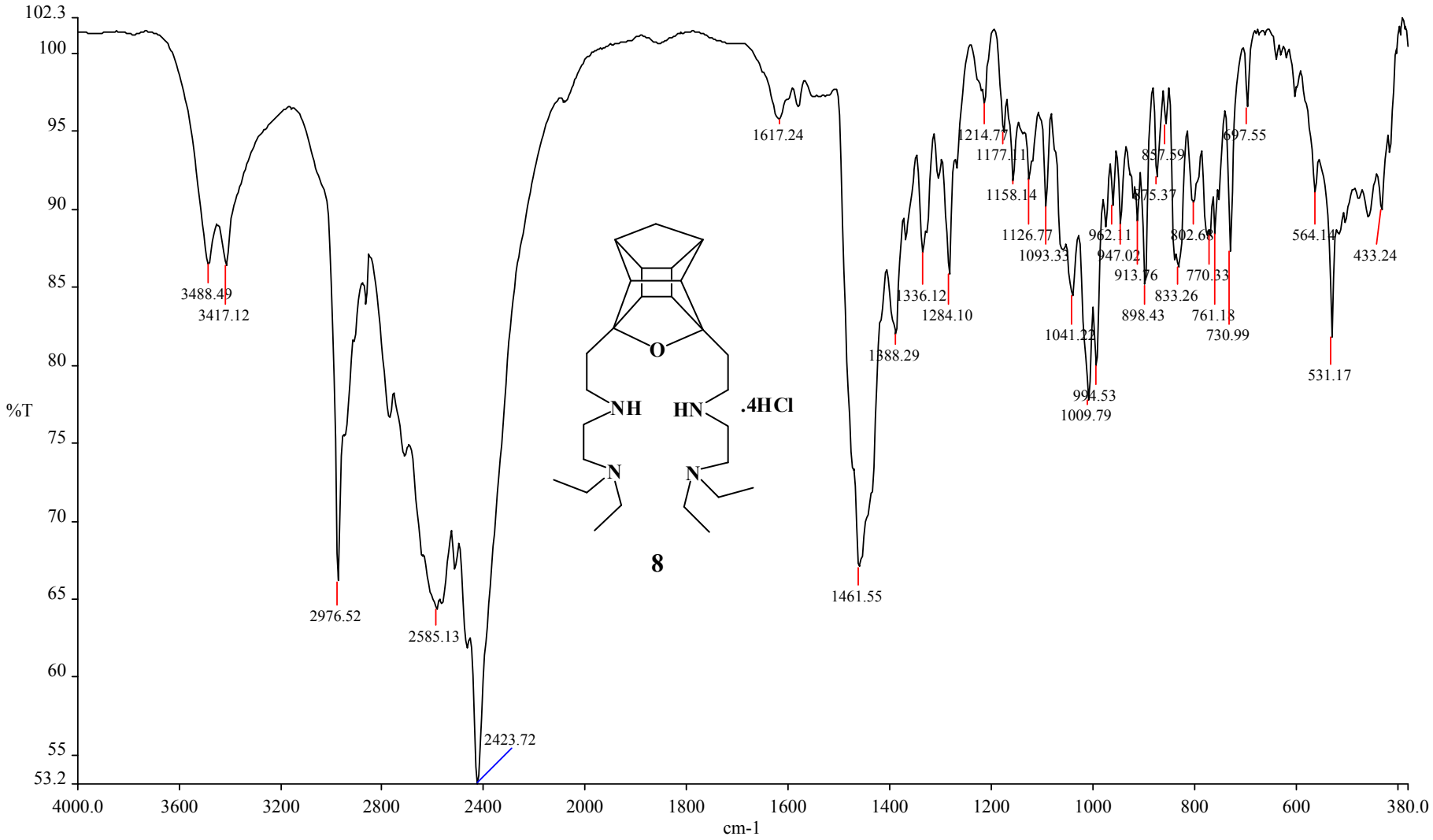


8

HSQC spectrum of PCU - *N,N* diethyl amino ethylamine HCl (8)



HMBC spectrum of PCU - *N,N* diethyl amino ethylamine HCl (8)



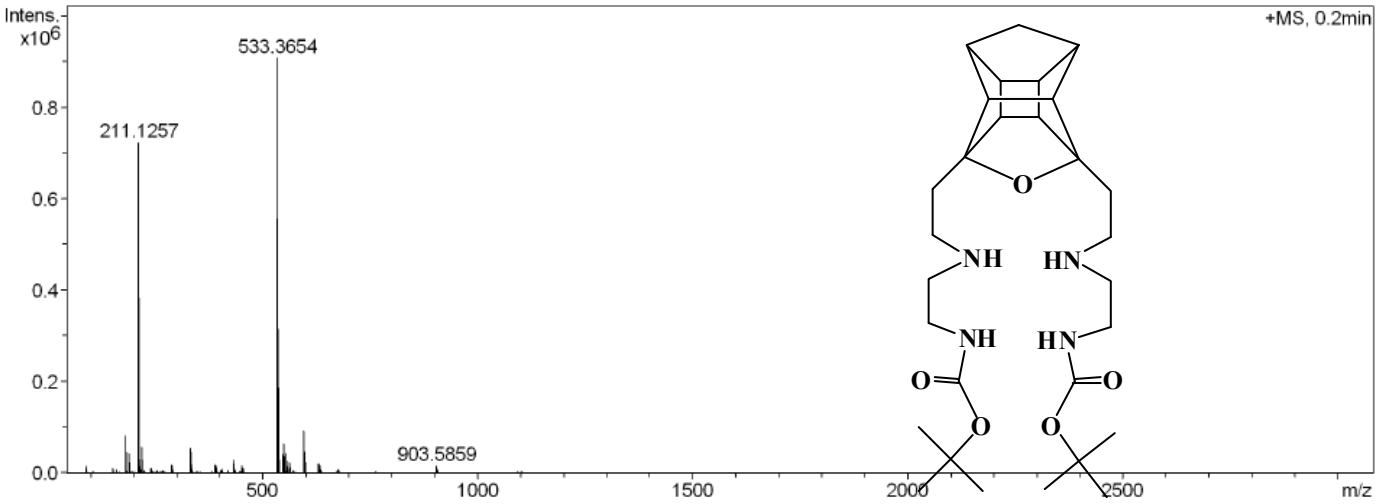
IR spectrum of PCU - *N,N* diethyl amino ethylamine (8)

Analysis Info

Analysis Name	D:\Data\kenny\boc eda PCU000002.d	Acquisition Date	10/15/2008 7:55:25 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	boc eda PCU	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



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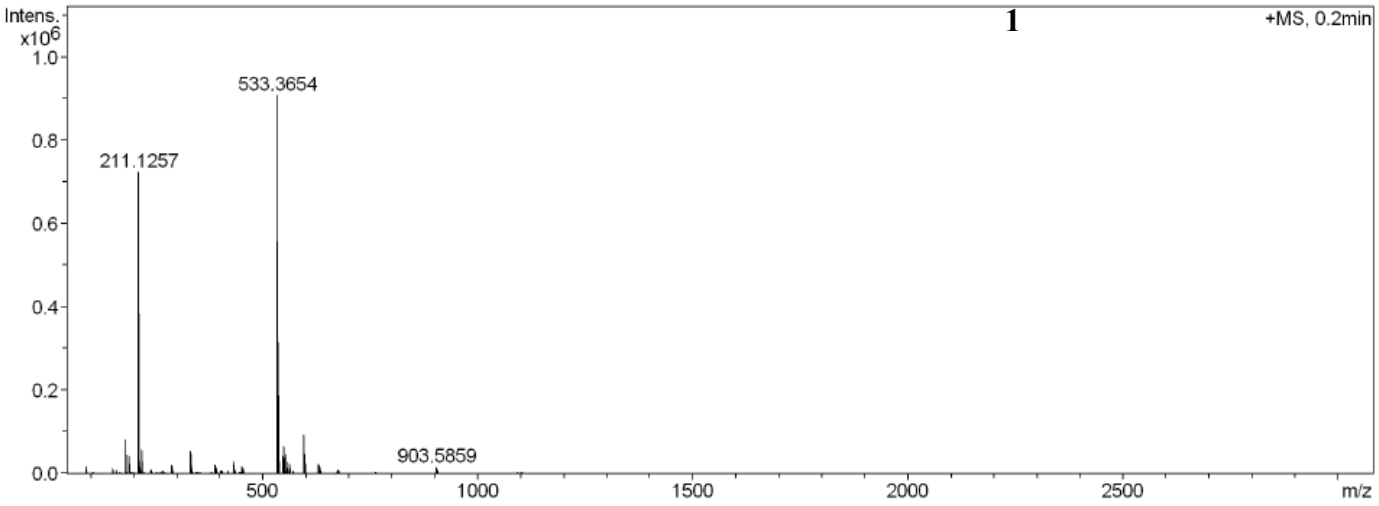


Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev

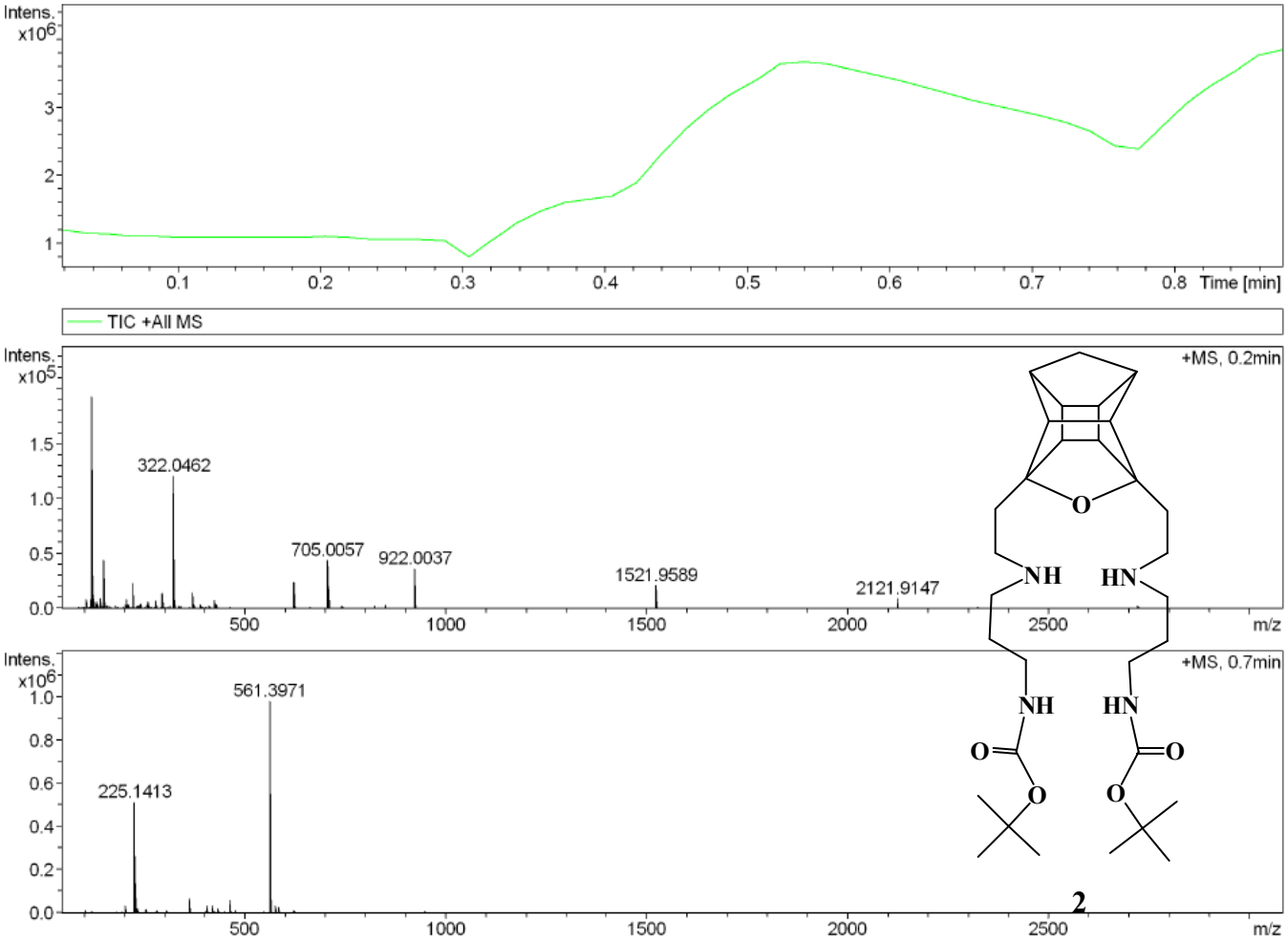
HRMS spectrum of compound 1

Analysis Info

Analysis Name	D:\Data\kenny\boc PPD PCU000001.d	Acquisition Date	10/15/2008 8:25:44 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	boc PPD PCU	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
561.3971														
	1	C 30 H 47 N 11	561.4010	6.9	7.8	13.0	ok	odd	6.12	0.0107	0.0045	0.0037	0.0018	0.8002
	2	C 31 H 53 N 4 O 5	561.4010	7.0	7.9	7.5	ok	even	12.06	0.0171	0.0045	0.0054	0.0018	0.8286
	3	C 37 H 53 O 4	561.3938	-5.9	-4.9	11.5	ok	even	15.29	0.0243	0.0029	0.0081	0.0019	0.8082

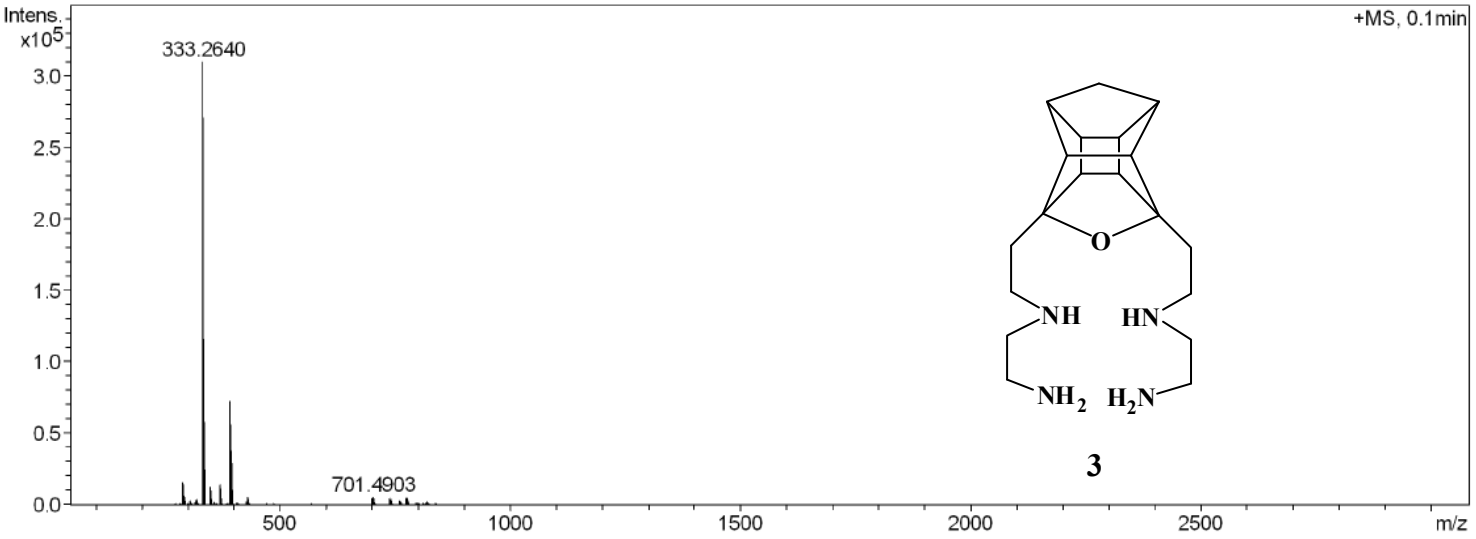
HRMS spectrum of compound 2

Analysis Info

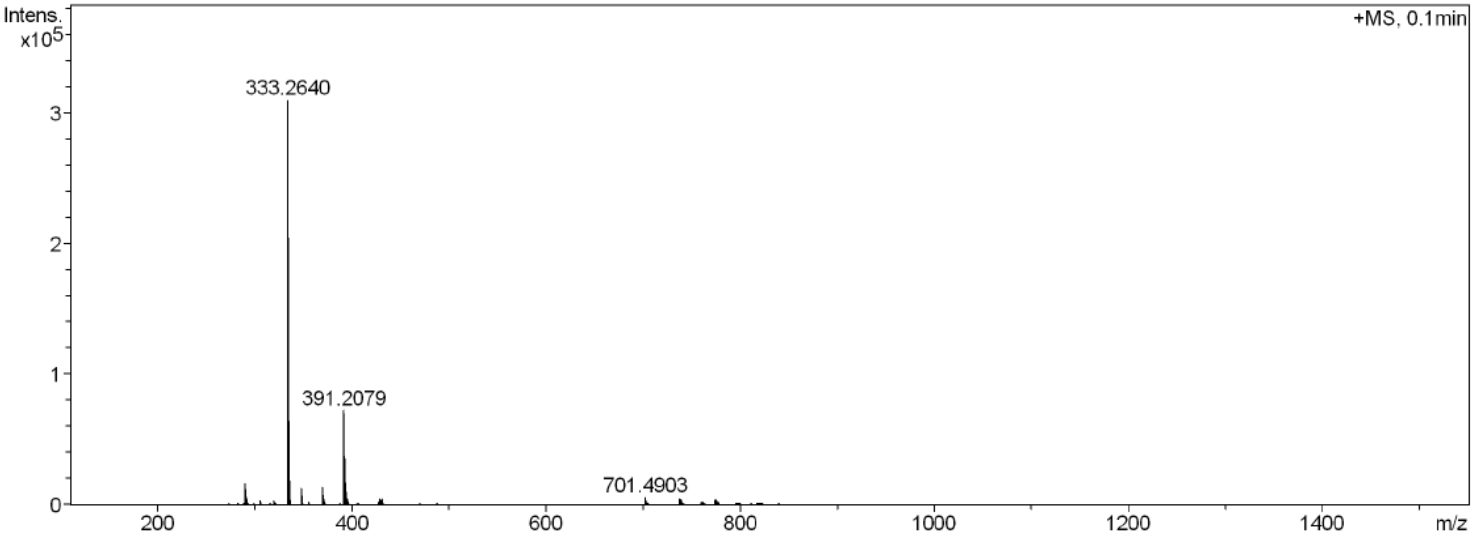
Analysis Name	D:\Data\test\kenny000010.d	Acquisition Date	7/19/2008 6:48:06 PM
Method	test.m	Operator	BDAL@DE
Sample Name	PCU ethylene diamine	Instrument / Ser#	micrOTOF-Q 10139
Comment	PCU ethylene diamine		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	180 °C
Scan Begin	150 m/z	Set End Plate Offset	-500 V	Set Dry Gas	5.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Waste



3



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
333.2640														
	1	C 17 H 31 N 7	333.2635	-1.3	-0.1	6.0	ok	odd	0.82	0.0016	0.0009	0.0008	0.0017	0.5483
	2	C 19 H 33 N 4 O	333.2649	2.8	3.9	5.5	ok	even	5.66	0.0096	0.0016	0.0038	0.0017	0.7759
	3	C 18 H 37 O 5	333.2636	-1.2	-0.0	0.5	ok	even	9.72	0.0139	0.0009	0.0074	0.0018	0.7862

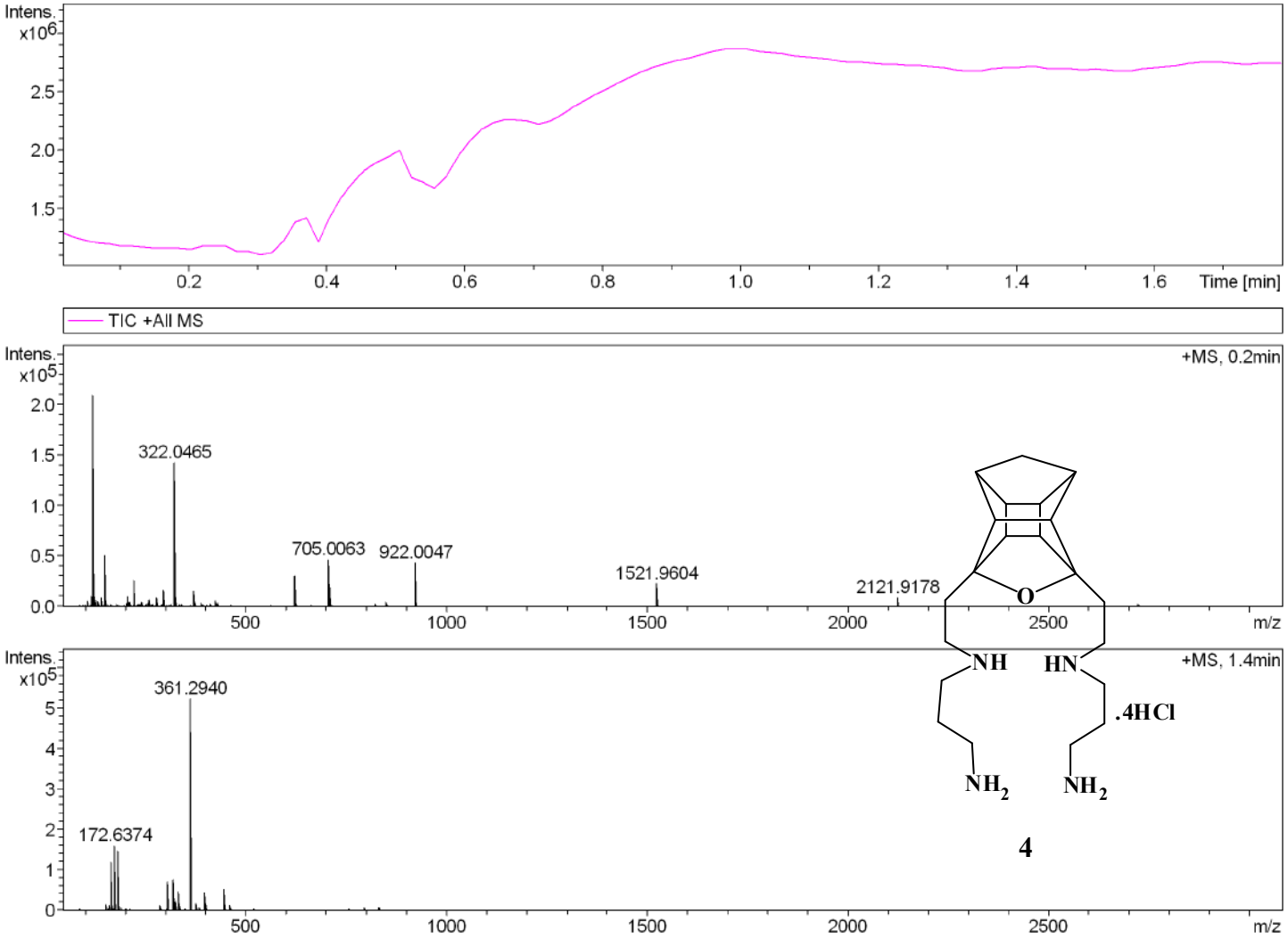
HRMS spectrum of compound 3

Analysis Info

Analysis Name	D:\Data\kenny\PPD PCU000002.d	Acquisition Date	10/15/2008 8:33:06 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PPD PCU	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
361.2940														
	1	C 19 H 35 N 7	361.2948	2.3	2.9	6.0	ok	odd	3.45	0.0065	0.0012	0.0032	0.0008	0.7547
	2	C 21 H 37 N 4 O	361.2962	6.0	6.6	5.5	ok	even	3.81	0.0054	0.0025	0.0025	0.0009	0.8431
	3	C 23 H 39 N O 2	361.2975	9.7	10.4	5.0	ok	odd	10.42	0.0175	0.0038	0.0075	0.0010	0.9498
	4	C 20 H 41 O 5	361.2949	2.3	3.0	0.5	ok	even	10.94	0.0161	0.0012	0.0074	0.0010	0.8427

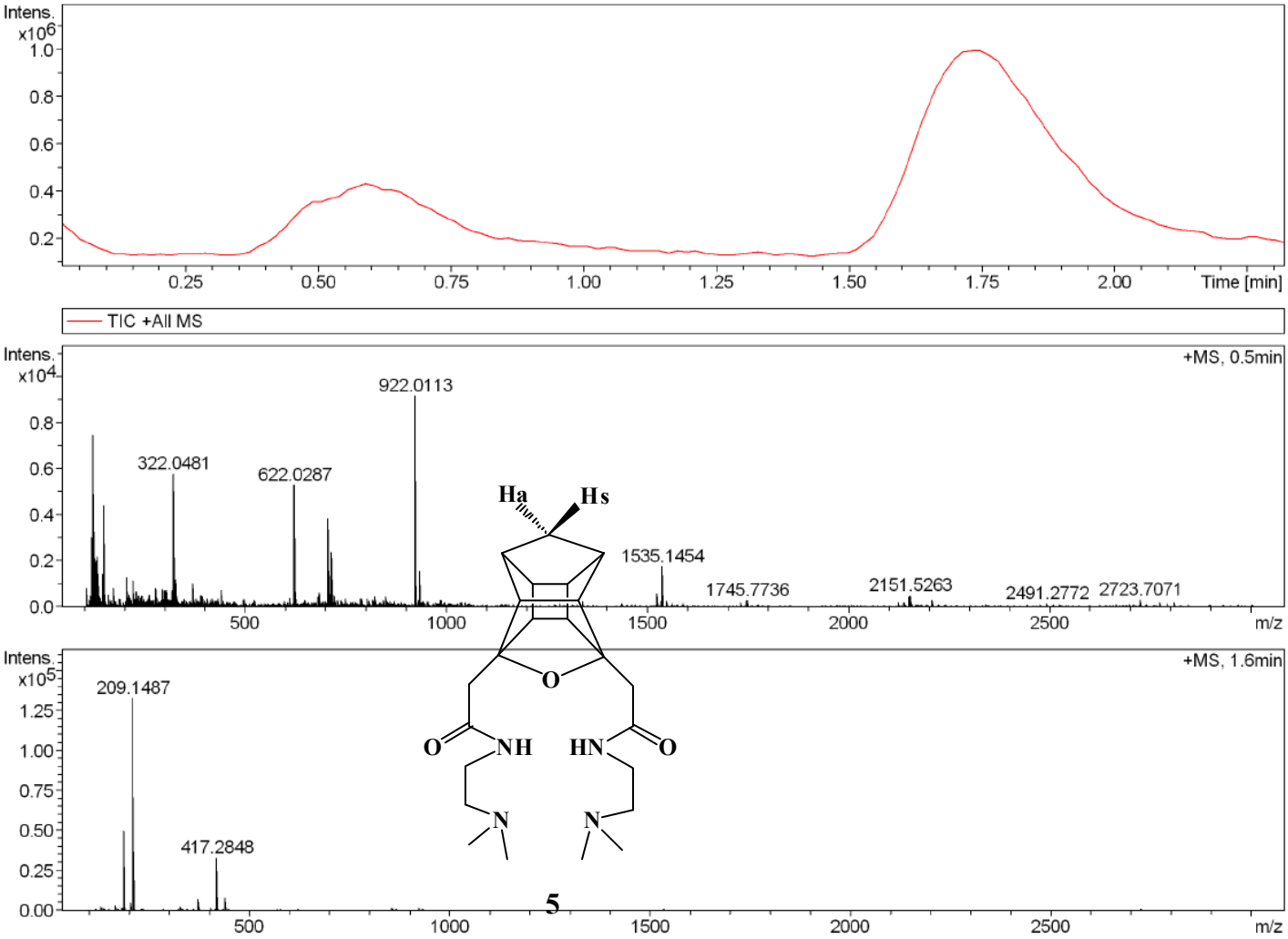
HRMS spectrum of compound 4

Analysis Info

Analysis Name	D:\Data\kenny\PCU dimethylamino ethylamine000006.d	Acquisition Date	6/13/2009 4:27:23 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU dimethylamino ethylamine	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



5

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
417.2848														
	1	C 25 H 39 N O 4	417.2874	6.2	6.5	7.0	ok	odd	2.18	0.0028	0.0027	0.0017	0.0004	0.8014
	2	C 23 H 37 N 4 O 3	417.2860	2.9	3.2	7.5	ok	even	6.10	0.0106	0.0014	0.0054	0.0003	0.7761
	3	C 21 H 35 N 7 O 2	417.2847	-0.3	-0.0	8.0	ok	odd	12.80	0.0220	0.0002	0.0103	0.0003	0.6849

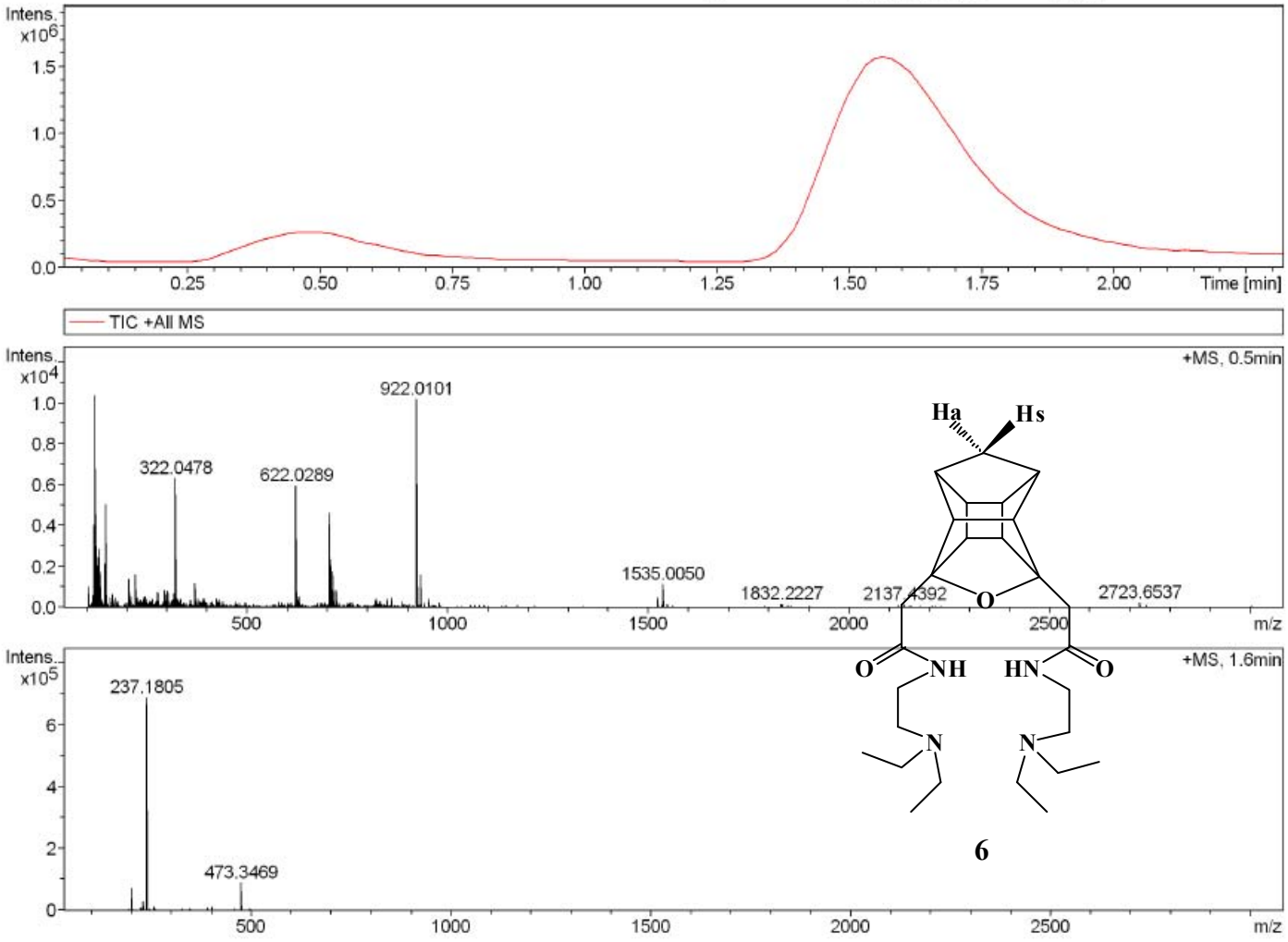
HRMS spectrum of compound 5

Analysis Info

Analysis Name	D:\Data\kenny\PCU diethylamino ethylamine000001.d	Acquisition Date	6/13/2009 4:40:40 PM
Method	tune_low_expert.m	Operator	BDAL@DE
Sample Name	PCU diethylamino ethylamine	Instrument / Ser#	micrOTOF-Q 10139
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	110 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	120.0 Vpp	Set Divert Valve	Source



6

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
473.3469														
	1	C 27 H 45 N 4 O 3	473.3486	3.5	4.1	7.5	ok	even	3.70	0.0056	0.0020	0.0035	0.0012	0.8787
	2	C 29 H 47 N O 4	473.3500	6.4	7.0	7.0	ok	odd	5.82	0.0096	0.0034	0.0048	0.0013	0.9479
	3	C 25 H 43 N 7 O 2	473.3473	0.7	1.3	8.0	ok	odd	8.98	0.0137	0.0008	0.0062	0.0009	0.8075

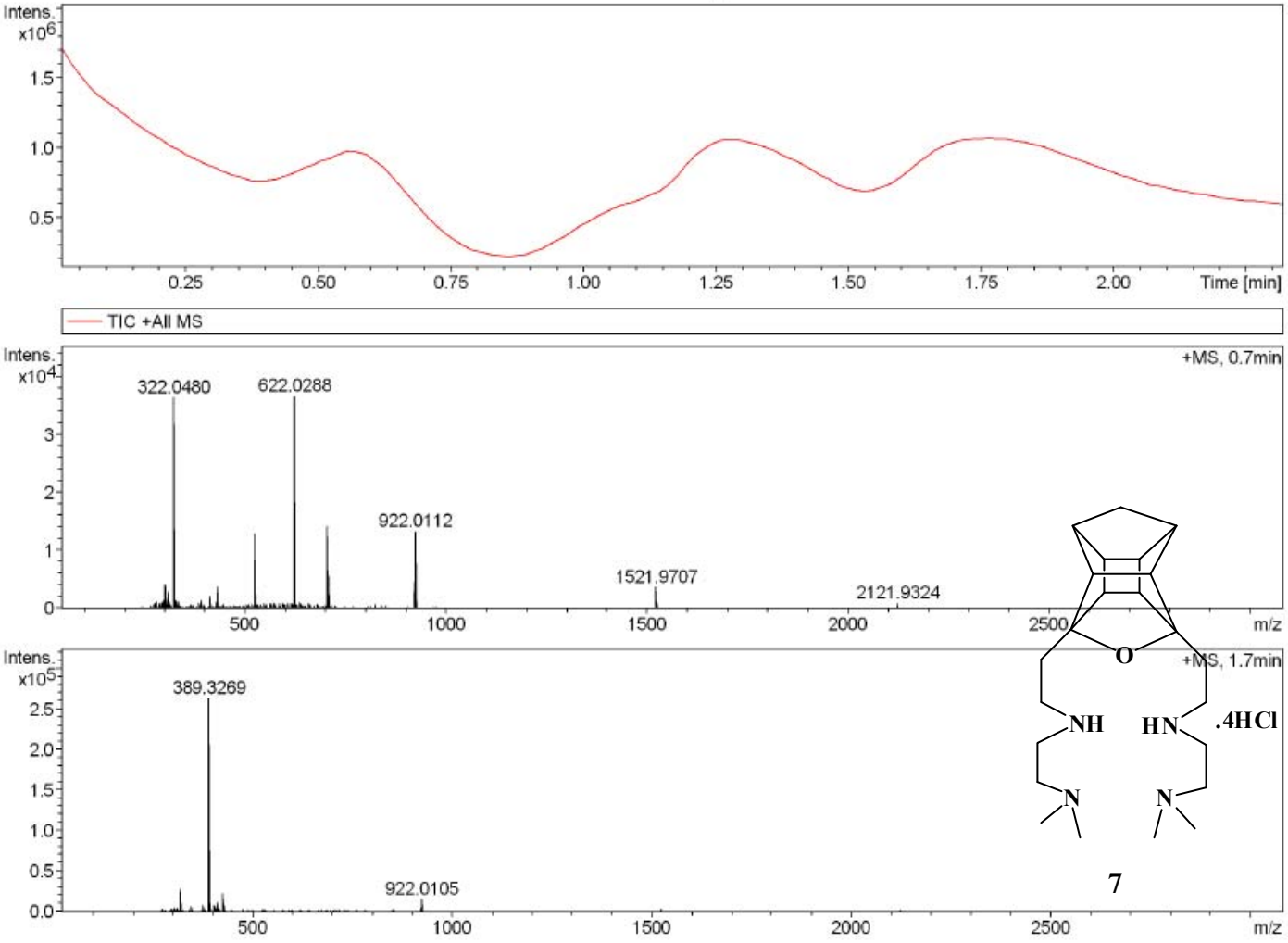
HRMS spectrum of compound 6

Analysis Info

Analysis Name	D:\Data\kenny\PCU dimethylamino ethylamine (reduced)000001.d	Acquisition Date	6/17/2009 7:55:13 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	PCU dimethylamino ethylamine (reduced)	Instrument / Ser#	micrOTOF-Q 10139
Comment	amine amide peptoid		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



7

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
389.3269														
	1	C 23 H 41 N 4 O	389.3275	1.6	2.4	5.5	ok	even	10.05	0.0149	0.0011	0.0104	0.0013	0.8415
	2	C 25 H 43 N O 2	389.3288	5.0	5.8	5.0	ok	odd	11.24	0.0146	0.0023	0.0083	0.0013	0.9064

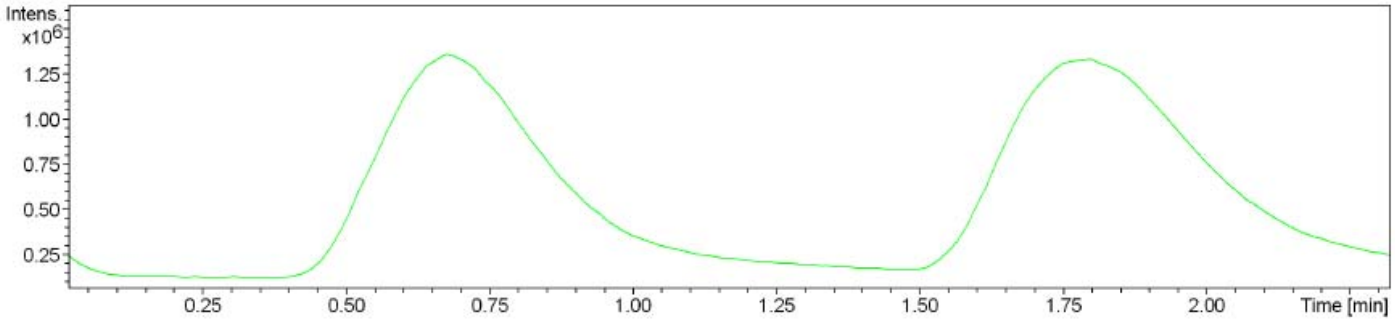
HRMS spectrum of compound 7

Analysis Info

Analysis Name	D:\Data\kenny\PCU diethylamino ethylamine (reduced)000001.d	Acquisition Date	6/17/2009 8:04:06 PM
Method	tune_wide_expert.m	Operator	BDAL@DE
Sample Name	PCU diethylamino ethylamine (reduced)	Instrument / Ser#	micrOTOF-Q 10139
Comment	amine amide peptoid		

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	200 °C
Scan Begin	100 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set Collision Cell RF	500.0 Vpp	Set Divert Valve	Source



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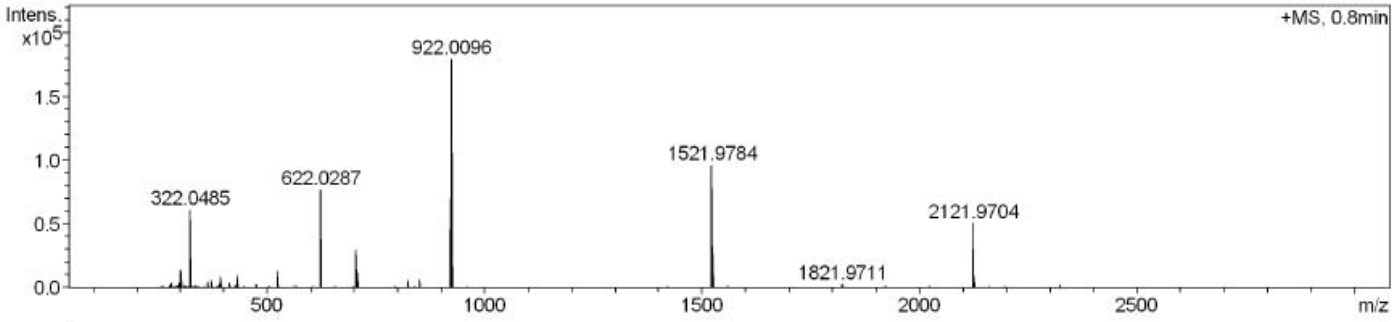

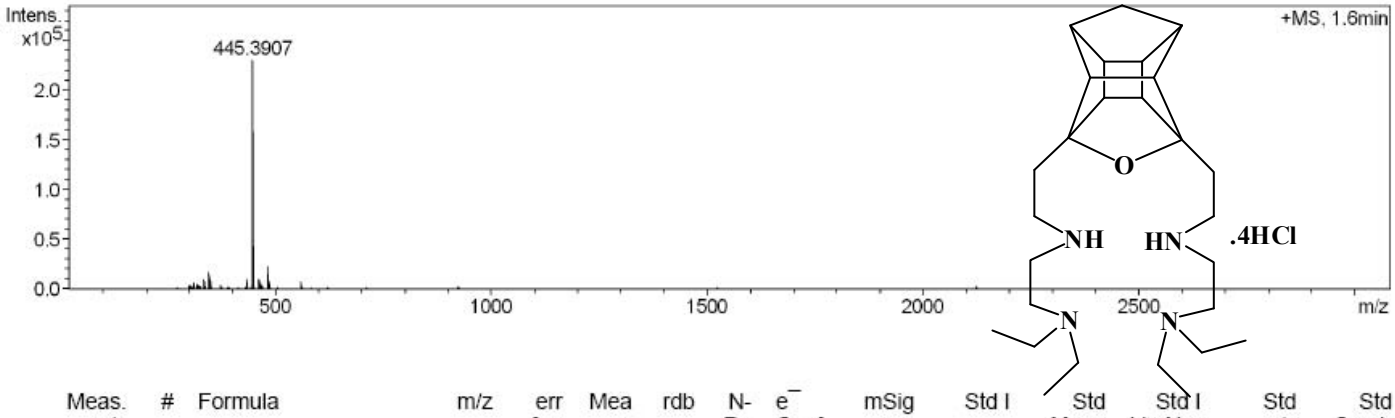

8

Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
445.3907														
	1	C 27 H 49 N 4 O	445.3901	-1.3	-0.9	5.5	ok	even	8.45	0.0140	0.0005	0.0068	0.0006	0.6845
	2	C 25 H 47 N 7	445.3887	-4.3	-3.9	6.0	ok	odd	9.86	0.0159	0.0018	0.0102	0.0007	0.8611

HRMS spectrum of compound 8