Use of conformational control in the design of novel abiotic oligomers, building blocks and biomedically potential combretastatin analogs

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(IN CHEMISTRY)

By

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CERTIFICATE

Certified that the work incorporated in the thesis entitled **"Use of conformational control in the design of novel abiotic oligomers, building blocks and biomedically potential combretastatin analogs"**, submitted by **Mr. Arup Roy** for the degree of **Doctor of Philosophy** was carried out by the candidate under my supervision in Organic Chemistry Division, National Chemical Laboratory, Pune, India. Materials obtained from other sources have been duly acknowledged in the thesis.

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DECLARATION

I hereby declare that the thesis entitled **"Use of conformational control in the design of novel abiotic oligomers, building blocks and biomedically potential combretastatin analogs"**, submitted for the Degree of Doctor of Philosophy in Chemistry to the University of Pune, has not been submitted by me to any other university or institution. This work has been carried out at Division of Organic Chemistry, National Chemical Laboratory, Pune under the supervision of Dr. G. J. Sanjayan (Research guide).

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Dedicated to my family and friends

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Abbreviations

A

Å	Ångström
AA	Amino acid
Ac	Acyl
AcOH	Acetic acid
AcOEt	Ethyl acetate
Ala	Alanine
Aib	Amino isobutyric acid
Ant	Anthranilic acid

B

Boc	tert.- Butyloxycarbonyl
Bn	Benzyl

C

$CDCl_3$	Chloroform-d
COSY	Correlated spectroscopy

D

d	doublet (NMR)
δ	Chemical shift (NMR)
DCC	*N, N'*-dicyclohexyl-carbodimide
DCM	Dichloromethane
DMF	Dimethyl formamide
DIPEA	Diisopropyl ethylamine
DMSO	Dimethylsulfoxide
DMAP	4-dimethyl aminopyridine

E

ESI	Electron spray ionization
Et	Ethyl
EDC	1-Ethyl-3-(3-dimethyl aminopropyl)carbodiimide

H

H-bond	Hydrogen bond
HMBC	Hetero Multiple Bond Correlation
HBTU	O-benzotriazol-1-yl-N,N, N',N'-tetramethyluronium hexafluorophosphate
HOBt	1-hydroxybenzotriazole
HSQC	Hetero Nuclear Single Quantum Coherence
Hz	Hertz

L

LCMS	Liquid chromatography–mass spectrometry
Leu	Leucine

M

m	Multiplet (NMR)
MS	Mass Spectrometry
Me	Methyl

N

NOESY	Nuclear Overhauser and Exchange Spectroscopy

P

Ph	Phenyl
Pro	Proline
Pd-C	palladium 10 % on activated carbon

S

s	Singlet (NMR)
^{S}Ant	2-aminobenzenesulfonic acid
$SnCl_2$	Stannous chloride

T

TFA	Trifluroacetic acid
THF	Tetrahydrofuran
t	Triplet (NMR)

V

Val	Valine

ABSTRACT

The prime intention of this work was to design and synthesize a range of molecules containing biomedically potential combretastatin analogs as the core moiety, as well as an array of carbohydrate based hydroxyethylamine isosteres. Efforts were also expended for the development of foldamers and building blocks which are structurally different from the classically observed ones, as detailed below under appropriate headings.

Chapter 1: This chapter describes an array compounds having combretastatin as the core moiety (Figure-1). Combretastatins are very effective as anti-mitotic agents due to their efficiency of binding to tubulin at the colchicine site. The specificity of the drug can further be improved by incorporating the therapeutics to ligands that can recognize tumour-associated antigens. A class of small and potent molecules containing glutamate urea, called ARM-Ps (antibody-recruiting molecules targeting prostate cancer) capable of enhancing antibody-mediated immune recognition of prostate cancer cells and a xanthine-based adenosine receptor antagonist were chosen to function as the ligand.

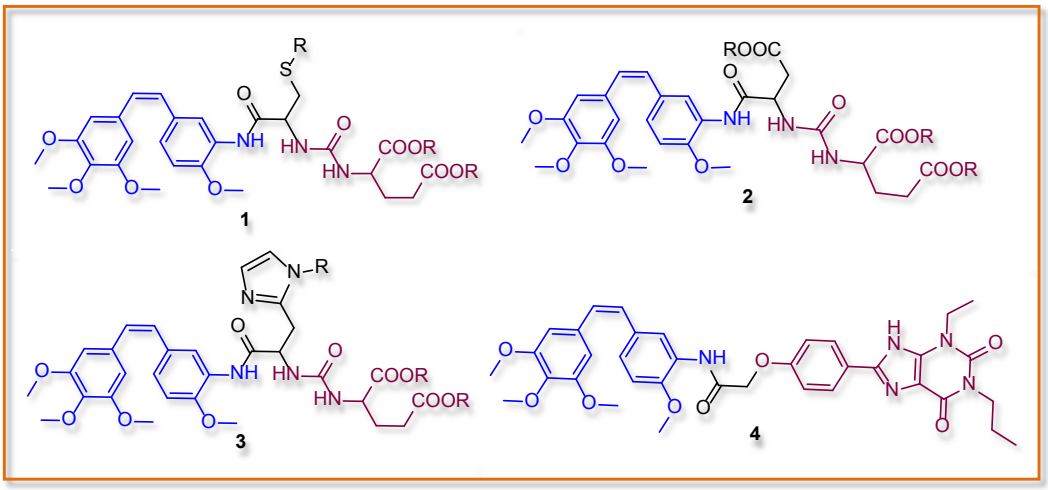


Figure-1 Hybrid molecules having combretastatin as the core moiety.

This section also describes a general synthetic route, starting from the readily available D-glucose, to access carbohydrate-based conformationally constrained hydroxyethyl amine (HEA) isosteres featuring amino acid side chains (Figure-2).

A carbohydrate-based starting material was chosen owing to the enormous scope of functionalization and ready availability, as well as the ease of tuning of

the ring size of these constrained HEA isosteres. This work provides an elegant synthetic route to access amino-acid-tethered carbohydrate conjugates, which may eventually find potential applications in the development of conformationally restricted HEA isosteres.

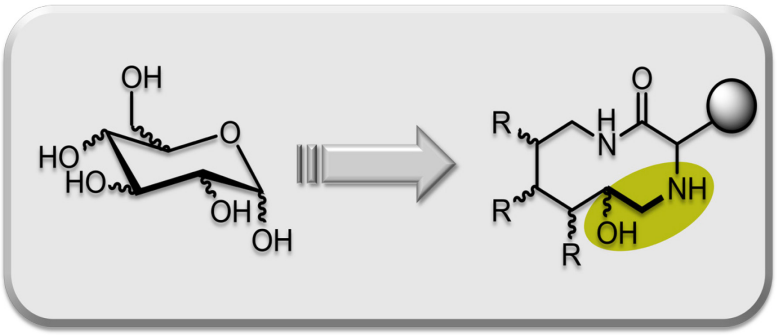


Figure-2 Design strategy to form conformationally constrained carbohydrate-derived macrocyclic HEA isosteres featuring amino acid side chain.

Chapter 2: This chapter deals with the development of conformationally constrained foldamer building blocks, constructed on rigid backbone wherein the chain propagating groups project in two dimensions (planes) on the aromatic framework. Such a feature offers the possibility of design and development of conformationally ordered synthetic oligomers with intriguing structural architectures distinct from those classically observed (Figure-3)

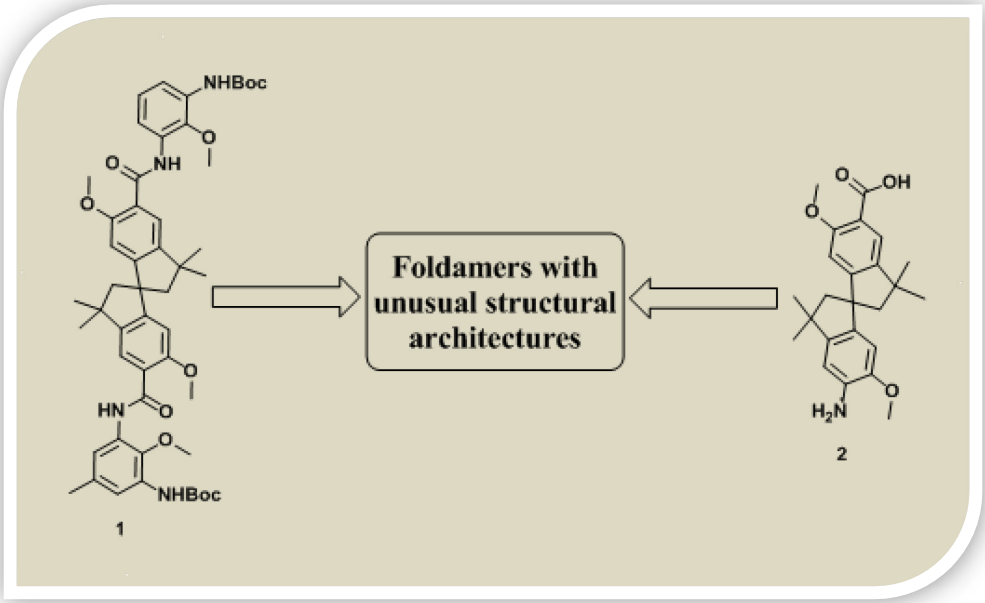


Figure-3 Spirobiindane-based two-dimensional foldamer building blocks, useful for the development of foldamers with distinctly different architecture.

These foldamer building blocks will have the potential to extend the conformational space available for foldamer design with diverse backbone conformation and structural architectures.

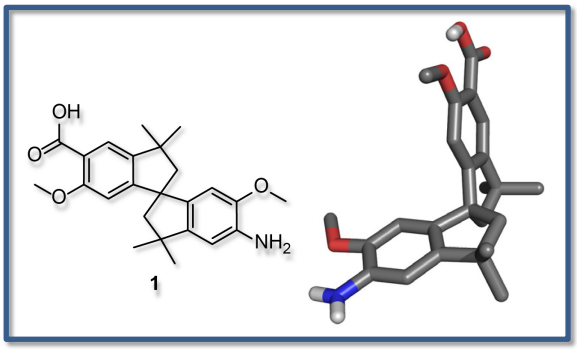


Figure-4. Molecular and single crystal X-ray structure of **2** showing *anti-periplanar* disposition of the chain propagating groups.

Single crystal X-ray diffraction studies of spirobiindane-based amino acid **2** (Figure-4) reveals that the aryl rings projecting the amino and carboxy groups assume anti-periplanar conformation, as anticipated.

Chapter 3: This chapter describes the consequence of replacing carboxamide by sulphonamide on the foldamer backbone. Owing to the geometric disparity of sulphonamide and carboxamide (sulphonamide bond has a dihedral angle $\omega \sim 180^{o}$ and in carboxamides $\omega \sim 90^{o}$), the conformation of peptides featuring sulphonamide will be markedly different from those observed in typical oligopeptides. Moreover sulfonamides also form a very important class of biologically important compounds called sulpha drugs. We, therefore, designed a collection of oligopeptides with sulfonamide as a connective-entity.

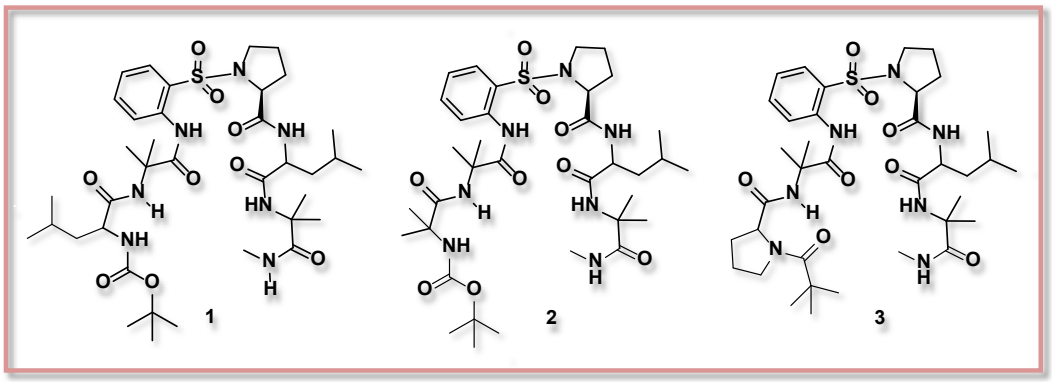


Figure-5. Peptide sequences having sulphonamide linkage in central dipeptide featuring 2-amino sulphonic acid and proline.

The peptides **1-3** which differ in the N-terminal amino acids feature a common central dipeptide motif made of 2-amino sulfonic acid (^{S}Ant) and proline (Pro). Biophysical investigation of these peptides was anticipated to throw light on the consequences of introducing the aforesaid dipeptide motif (^{S}Ant-Pro) into peptide backbones.After relentless efforts, compound **1** could be crystallized. Closer investigation of its crystal structure reveals that there are three distinctly different intramolecular hydrogen bonding interactions in the molecule, which stabilize its folded conformation (figure-6).

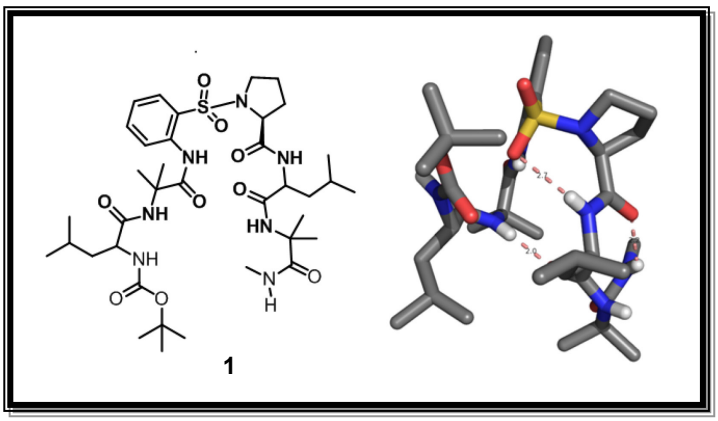
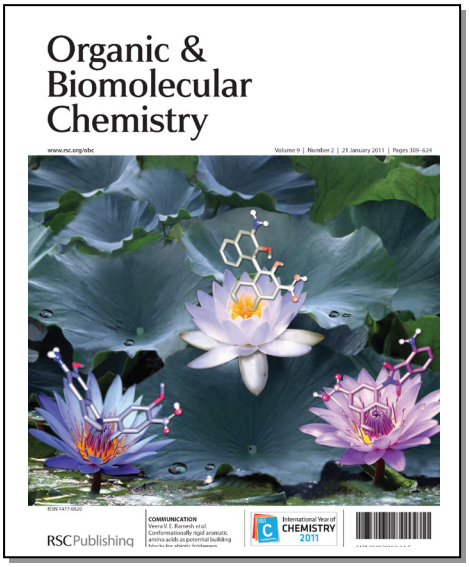


Figure-6. Molecular and single crystal X-ray structure of **1** showing a folded conformation.

General Remarks

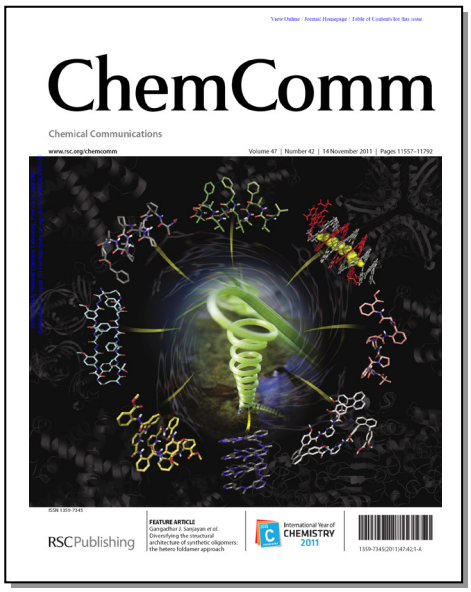
- Unless otherwise stated, all the chemicals and reagents were obtained commercially.
- Required dry solvents and reagents were prepared using the standard procedures.
- All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel plates (kieselgel $60F_{254}$, Merck) with UV, I_2 or ninhydrin solution as the developing reagents in the concerned cases.
- Column chromatographic purifications were done with 100-200 Mesh Silica gel or with Flash silica gel (230-400) mesh in special cases.
- Melting points were determined on a Buchi Melting Point B-540 and are uncorrected.
- IR spectra were recorded in nujol or $CHCl_3$ using Shimadzu FTIR-8400 spectrophotometer.
- NMR spectra were recorded on Ac 200 MHz, AV 400 MHz or DRX-500 MHz Bruker NMR spectrometers. All chemical shifts are reported in δ ppm downfield to TMS and peak multiplicities as singlet (s), doublet (d), quartet (q), broad (br), broad singlet (bs) and multiplet (m).
- Elemental analyses were performed on a Elmentar-Vario- EL.
- Electron Scattered Ionization (ESI) Mass Spectrometric measurements were done with API QSTAR Pulsar mass Spectrometer and MALDI-TOF mass spectrometric measurements were done on Voyager-DE STR mass spectrometer.
- Solution state structure determination was done using the using Maestro v9.3.518 from Schrödinger.
- Single crystal X-ray data were collected on a *Bruker SMART APEX* CCD Area diffractometer.

List of Publications



(1) **Conformationally rigid aromatic amino acids as building blocks for abiotic foldamers**

V. V. E. Ramesh, **A. Roy**, K. N. Vijayadas, A. M. Kendhale, P. Prabhakaran, R. G. Gonnade, V. G. Puranik and G. J. Sanjayan, *Org. Biomol. Chem.,* 2011, **9**, 367. ***(Front Cover page)***



(2) **Diversifying the structural architecture of synthetic oligomers: the hetero foldamer approach**

A. Roy, P. Prabhakaran, P. K. Baruah and G. J. Sanjayan, *Chem. Commun.,* 2011, **47**, 11593. ***(Front Cover page)***

(3) **Sugar–amino acid cyclic conjugates as novel conformationally constrained hydroxyethylamine transition-state isosteres**

A. Roy and G. J. Sanjayan, *Tetrahedron Lett.,* 2012, **53,** 3361.

4) **Influence of 2-amino sulphonic acid (^{S}Ant) in the structural modulation of peptide backbones**

A. Roy, A. S. Kotmale, R. L. Gawade, V. G. Puranik, P. R. Rajamohanan and G. J. Sanjayan. (Manuscript under preparation)

5) **Cancer cell binding terminus (CBT)-Combretastatin hybrid analogs as potential anticancer agents**

A. Roy and G. J. Sanjayan. (Manuscript under preparation)

CHAPTER 1

PART A

Sugar-Amino Acid Cyclic Conjugates as Novel Conformationally Constrained Hydroxyethyl amine Transition-State Isostere

PART B

Combretastatin Based Analogs as Potential Anticancer Agents

Sugar–Amino Acid Cyclic Conjugates as Novel Conformationally Constrained Hydroxyethylamine Transition-State Isostere

1.1 Proteases

Proteolytic enzymes, known as proteases are essential enzymes, accounting for about 2% of the genes in humans, contagious organisms, and other forms of life.[1] Consequently, proteases regulate most physiological processes by controlling the activation, synthesis and turnover of all proteins. Hence, proteases play prominent roles in a wide array of physiological processes such as regulation of conception, birth, growth, maturation, ageing, diseases and death. Many of these processes are proteolytic cascades, which, once set in action, lead very rapidly and irreversibly to a specific cellular response.

Genetic and environmental factors can disturb the balance of protease-catalyzed human physiology leading to abnormal development, poor health, disease and death. Proteases also contribute to the replication and transmission of microorganisms like viruses, parasites and bacterias that cause diseases and hence its inhibition is a promising approach to treat conditions like hepatitis,[2] herpes,[3] various forms of cancer,[4] malaria,[5] Alzheimer's disease,[6] AIDS[7] etc. Most of the proteases are sequence-specific and hydrolyze a particular amide bond after a certain amino acid sequence.[8] Schechter and Berger first proposed a standard nomenclature for the residues in a protease/substrate complex which is now widely used to illustrate the sites in such a complex (Fig. 1.1).[8] The residues in the N-terminal side of the scissile amide bond nd are known as the nonprime side and is being designated as P_n (n = 1, 2, 3 etc), wherein the C-terminal direction is

referred to as the prime side and labeled as P_n', and the corresponding sub-sites in the enzyme are called S_n and S_n' for the N- and C-terminal direction respectively.

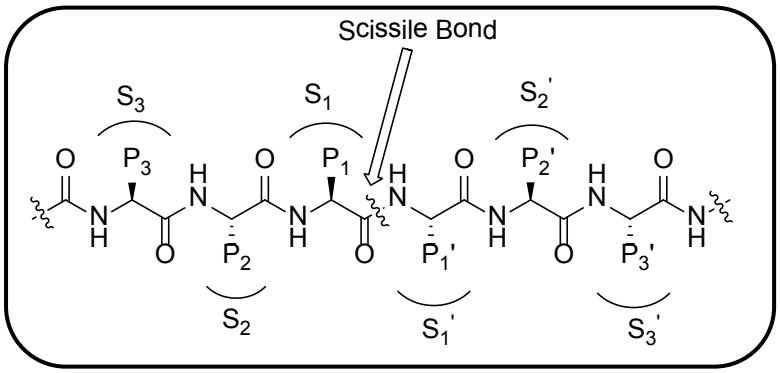


Fig. 1.1 Standard nomenclature P_1……P_n, P_1'….. P_n' is used to designate amino acid residues of peptide substrates. The corresponding binding sites on the protease are referred to as S_1……S_n, S_1'….. S_n' subsites.[8]

1.2 Classification of Proteases

Proteases can broadly be subdivided into two major classes, *viz.* exopeptidases and endopeptidases, depending on their site of action. Exopeptidases cleave the peptide bond close to the amino or carboxy termini of the substrate, whereas endopeptidases cleave peptide bonds distant from the termini of the substrate. Based on the functional groups present at the active site, proteases are further classified into five prominent groups, i.e. serine proteases, aspartic proteases, cysteine proteases, threonine protease and metallo proteases.[10] Each class of protease has a specific set of reactive amino acid residues organized in a specific configuration which constitutes the active site. Serine protease, the most studied class possesses a serine residue at the active site, in addition to having histidine and aspartic acid. The cysteine proteases are identified by the cysteine residue at the catalylic centre. Similarly aspartic proteases are distinguished by the presence of an aspartic acid counterpart at the active site and shows optimum activity at acidic pH . Threonine proteases belong to the family of

proteolytic enzymes harbouring a threonine (Thr) residue within the active site. The prototype members of this class of enzymes are the catalytic subunits of the proteasome. Metallo proteases contain a glutamic acid residue at the catalytic centre and require a metal cation (e.g. Zn, Ca, Mg etc.) to bring about the peptide hydrolysis. Given below (Table 1.1) are examples of enzymes which belong to the selected classes of protease.

Table 1.1 Examples of enzymes and their origin which belong to the selected classes of protease.

Class	Enzyme	Origin
Serine Protease	Trypsin	Pancreas
	Chymotrypsin	Pancreas
	Subtilisin	*Bacillus subtilis*
Cysteine Protease	Papain	Papaya latex
	Chymopapain	Papaya latex
	Ficin	Ficus latex
Aspartic Protease	Pepsin	Gastric juice
	Chymosin	Gastric juice (Young animals)
	Cathepsin D	Liver, Spleen
Metallo Protease	Thremolysin	*Bacillus thermoproteolyticus*
	Carboxypeptidase A	Bovine pancreas
Threonine Protease	Archaean proteasome, β-component	*Thermoplasma acidophilum*

1.3 Protease inhibitors

Biological machineries can be inactivated by proteolytic degradation or by binding with inhibitor molecules. Protease inhibitors often mimic the tetrahedral intermediate that is formed in the enzyme-catalyzed reaction in proteases (Fig. 1.2) and can bind at the active site of proteases,[11] thereby inhibiting the enzyme completely. Naturally occurring protease inhibitors control the proteolysis in an organism.

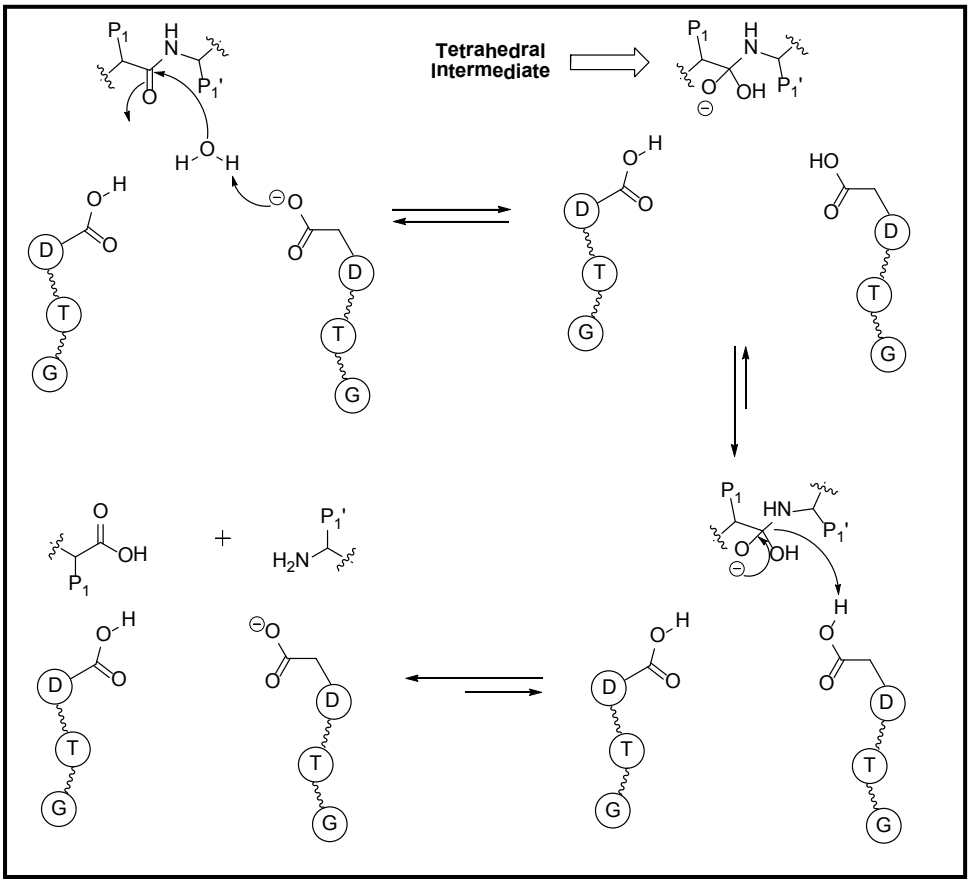


Fig. 1.2 Proposed catalytic mechanism for aspartic proteases involving tetrahedral intermediate.

Protease inhibitors can broadly be classified into two groups depending on their structural disparity: low molecular weight peptidomimetic inhibitors and protein protease inhibitors that are composed of one or more peptide chains. Protease inhibitors can further be classified into five groups (serine, cysteine,

aspartyl, threonine and metallo protease inhibitors) according to their working mechanism at the active site of proteases they inhibit. Some of the protease inhibitors can also act against more than one type of protease. For instance, the serine protease inhibitors (serpins) which are generally active against serine proteases, contain several important inhibitors of cysteine proteases as well.

Proteolytic inhibition by protease inhibitors can occur via two different mechanistic pathways: reversible tight-binding reactions and irreversible trapping reactions.[12] In reversible tight-binding reactions, the protease inhibitor binds to the active site of the protease directly; these reactions are reversible and the inhibitor can dissociate itself from the enzyme in either the initial stage or after modification by the protease. However, in an irreversible trapping reaction the inhibitor binds through a trapping mechanism and changes its conformation after cleaving a peptide bond and traps the enzyme molecule covalently.

Protease inhibitors have been studied for the last two decades primarily because of their effectiveness as therapeutic agents, and have been used successfully for the treatment of malaria, AIDS, cancer, Alzheimer's disease, nosocomial infections etc. Introduction of new protease inhibitors have been credited for the significant increase in life expectancy in patients suffering from acquired immune deficiency syndrome (AIDS).[13] At present, there are ten FDA approved protease inhibitors for HIV, which include Saquinavir, Indinavir, Amprenavir, Atazanavir, Fosamprenavir, Tipranavir, Darunavir etc. (Fig. 1.3).

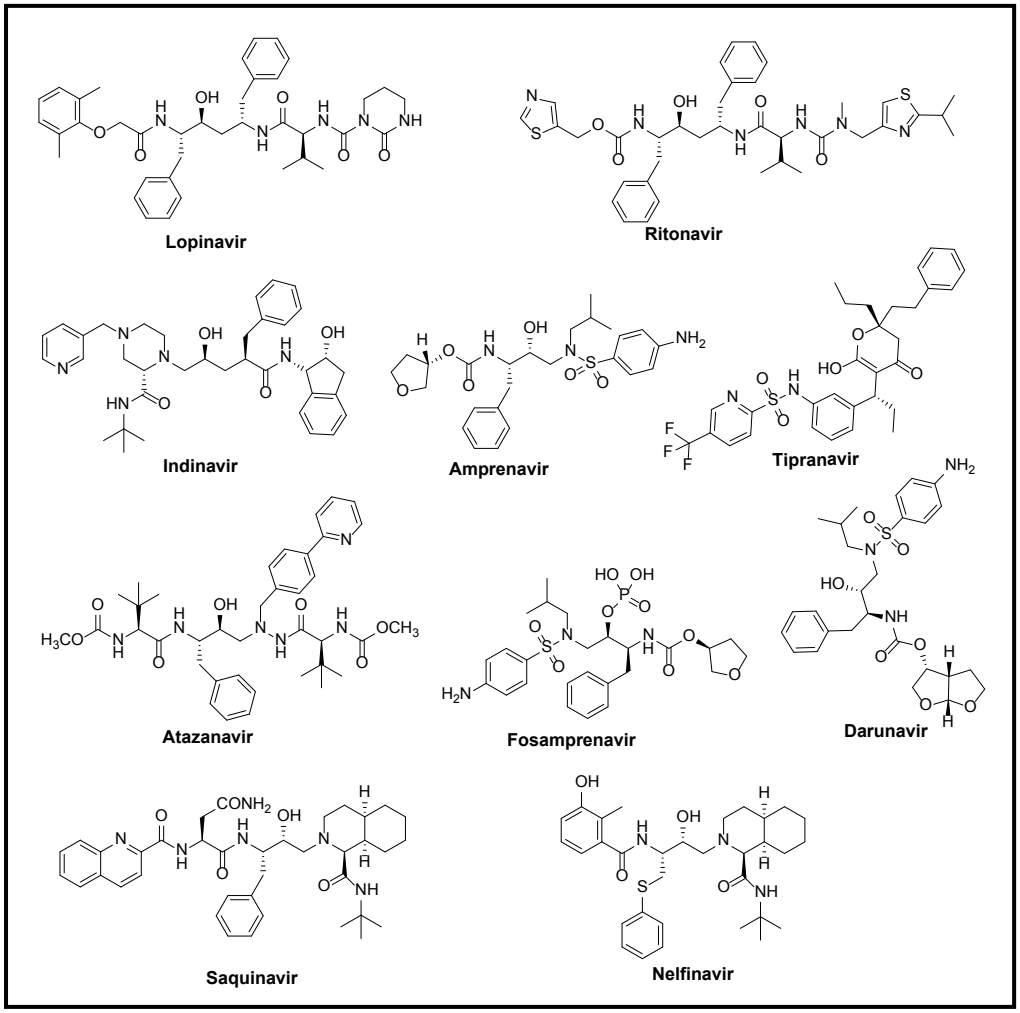


Fig. 1.3 FDA approved HIV-protease inhibitors.

Angiotensin converting enzyme (ACE) inhibitors are excellent examples of protease inhibitor which act as therapeutic agents. Hydrolysis of angiotensin I to angiotensin II, a potent vasoconstrictor is catalyzed by ACE,[14] thus reducing the blood pressure by reducing peripheral vascular resistance. As a result, ACE inhibitors are used for treatment of various ailments like hypertension, myocardial infarction etc. Success of ACE and HIV protease inhibitors led to the design and development of many more classes of protease inhibitors to treat different conditions (Table 1.2).

Table 1.2 Selected examples of protease inhibitors approved for clinical use.

Conditions	Compound	Company	Target	Protease Class
Thrombosis	Argatroban	Mitsubishi pharma	Thrombin	Serine
	Lepirudin	Aventis (Hoechst marion Roussell)		
	Desirudin	Novartis		
AIDS	Ritonavir	Abbott	HIV Protease	Aspartic
	Fosamprenavir	Glaxo Smithkline		
	Saquinavir	Hoffmann-La-Roche		
Hypertension, Myocardial infarction	Captopril	Bristol-Myers Squibb	ACE	Metallo
	Enalapril	Merck		
	Lisinopril	Astra Zeneca		
	Trandolapril	Abbott		
	Ramipril	Aventis		
	Moexipril	Boehringer mannheim		
	Qinapril	Pfizer		
Cancer	Bortezomib	Millennium	Proteasome	Threonine

1.4 Transition state isosteres

Protease inhibitors can be obtained by the isosteric replacement of the scissile peptide bond.[15] Thus, transition-state isosteres of the hydrolyzable amide bond is very important in the design of protease inhibitors[16] for developing therapeutic agents and catalytic antibodies.[17] Many of the commercially available drugs and inhibitors that are currently in clinical phases contains noncleavable transition state isosteres such as hydroxyethylamine,[18-20] hydroxyethylene,[21] statin,[22] norstatin,[23] phosphinate,[24] reduced amide, dihydroxyethylene,[25] α-keto amide[26] and silicon-based inhibitors[27] etc.(Fig. 1.4).

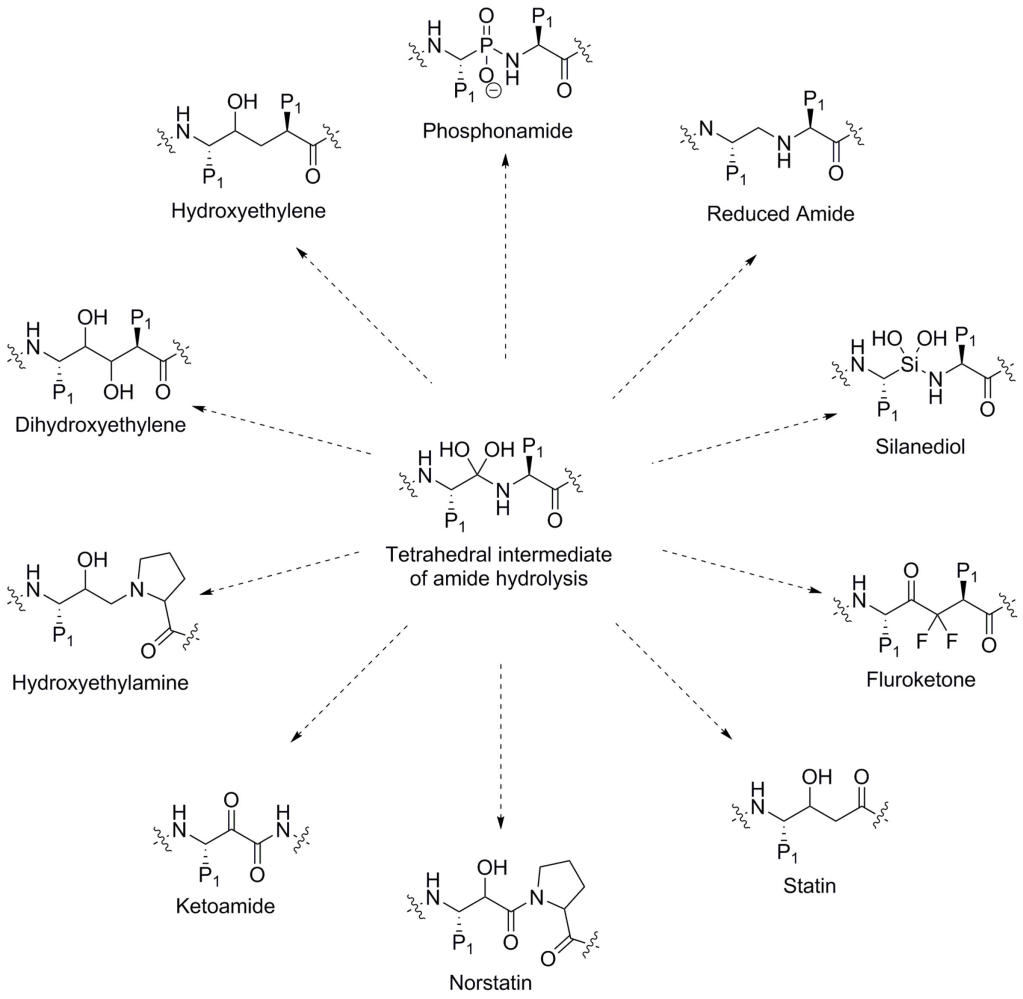


Fig. 1.4 Selected examples of transition-state isosteres developed for the synthesis of protease inhibitors.

Activity of these transition-state isosteres can be further enhanced by enforcing conformational restriction,[28] because a stretched out or randomly orientated protein is generally devoid of biological activity. Moreover, owing to poor receptor selectivity and metabolic instability, it may lead to undesired biological effect. Hence, it is very important to achieve a desired bioactive conformation which can be obtained by incorporating additional structural elements into peptidomimetic design to force rigidity. A rigid molecule ensures the correct positioning of specific functionalities in order to optimize factors like hydrogen bonding, electrostatic and hydrophobic interactions between the peptidomimetic ligand and the receptor. Entropy of the rigid peptidomimetic analogs are also lower when binding to the target receptor, and therefore conformationally rigid peptidomimetics should bind more effectively when compared to their flexible analogs.

1.5 Objective of the present work and design strategy

One of the most important transition-state isosteres developed so far is the hydroxyethylamine (HEA) motif. Ever since the successful development of the first protease inhibitor based on transition-state simulation principle, the amount of attention paid to this class of compounds have increased manifolds. Hydroxyethylamine isosteres already have been proven to be promising candidates for various curative programs (*vide supra*). Success of using this moiety as a peptide bond surrogate can readily be understood by the number of HEA-based drugs which have been available in the market that includes saquinavir, indinavir, nelfinavir and amprenavir which are the FDA approved

protease inhibitors (PIs), and several other therapeutically significant HEA isosters are known.[18-20]

The approach of incorporation of a transition-state analog into a peptide/peptidomimetic structure, carrying an amino acid side chain has time and again been proved very successful. As per the literature precedents, the design improvements can be achieved through amino acid variation, different side chain lengths, conformational constraints, and substitutions, which in turn can be the fruitful ways to enhance the binding affinity.[28-29]

In an effort to augment the structural diversity further, we made an attempt to design and synthesize a series of carbohydrate based conformationally restricted macrocyclic hydroxyethylamine (HEA) transition-state isosteres featuring various amino acid side chains (Fig 1.5). A carbohydrate-based starting material was chosen herein owing to the enormous scope of its functionalization and ready availbaility,[30] as well as the ease of tuning the ring size of these constrained HEA isosteres.

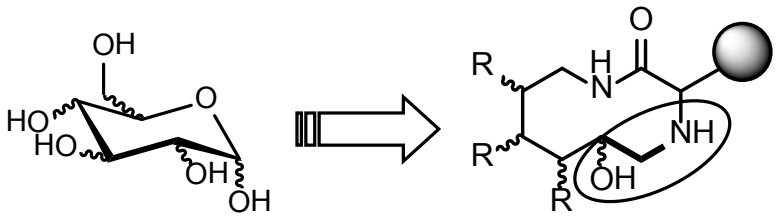


Fig. 1.5 Design strategy to develop conformationally constrained carbohydrate-derived macrocyclic HEA isosteres featuring amino acid side chain.

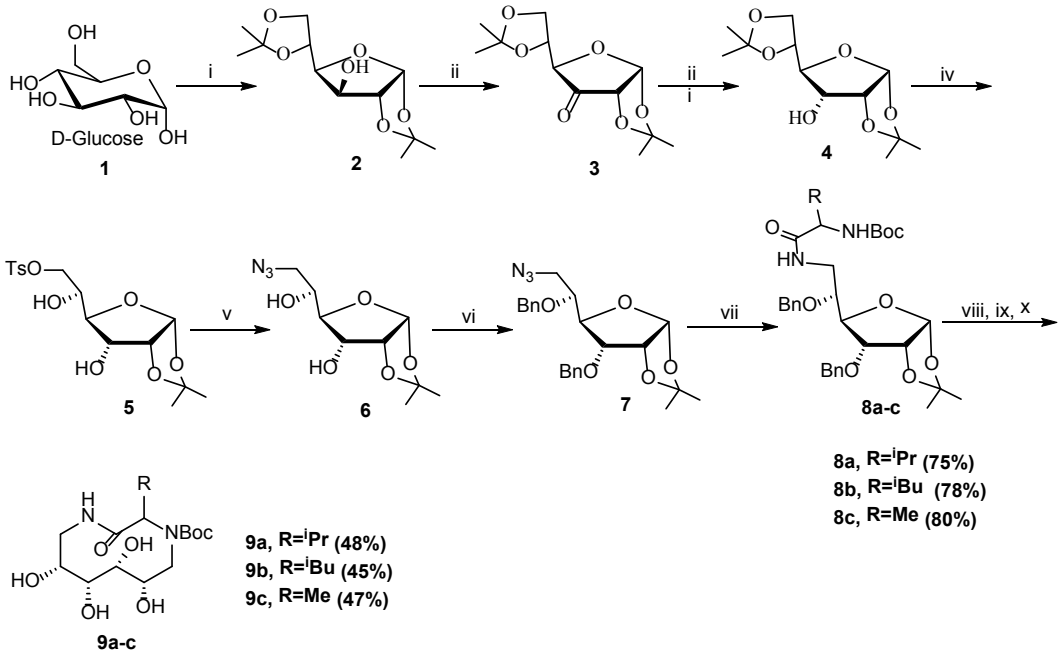
The design strategy illustrated herein is a straightforward one which makes use of established synthetic protocols. The main challenge of our synthetic strategy was the formation of the 10-membered macrocycle which was finally achieved through a two-step 1,2 acetonide deprotection *cum* reductive amination

process. The constrained HEA isosteres were synthesized in thirteen steps starting from D-glucose.

1.6 Synthesis of carbohydrate-based conformationally constrained HEA isosteres

To synthesize the carbohydrate-based conformationally constrained HEA isosteres **9a-c**, we began with D-glucose which was transformed into the tosyl analog **5** in four steps, following the known protocols reported in the literature.[31]

Scheme 1.1 Synthesis of amino-acid-tethered carbohydrate conjugates **9a-c**



Reagents and conditions: (i) H_3PO_4, $ZnCl_2$, acetone, reflux, 36 h, 43%; (ii) PDC, DCM, Ac_2O, 2 h, 95%; (iii) $NaBH_4$, EtOH, 2 h, 70%; (iv) a. 80% aq. AcOH, 8 h, 92%; b. Bu_2SnO, $CHCl_3$, TsCl, 5 h, 86%; (v) NaN_3, DMF, 50 °C, 10 h, 94%; (vi) NaH, BnBr, TBAI, THF, 2.5 h, 68% ; (vii) a. TPP, THF-H_2O, rt, 3 h; b. HBTU, DIPEA, BOC-amino acids, CH_3CN, 10 h; (viii) a. 80% aq. TFA, 30 h; b. $NaBH_3CN$, AcOH, 40 h; (ix) BOC_2O, H_2O, 12 h; (x) H_2, $Pd(OH)_2$, MeOH, 150 psi, 16 h.

The tosyl protected furanose sugar **5** furnished the azide displaced product **6** in excellent yield upon heating it at 50 °C in DMF containing NaN_3.[32] Benzyl protection of the two secondary hydroxyl groups were carried out by reacting with

benzyl bromide in THF, having NaH as the base and TBAI as a phase transfer catalyst affording the di-benzyl ether **7**. The furanose **7** was then subjected to selective azide reduction using triphenylphosphine/water followed by coupling with different BOC-protected amino acids to furnish **8a-c** in very good yields. The 1,2 acetonide protected furanose sugar bearing amino acid side chains were then subjected to a two-step TFA-assisted acetonide cleavage followed by reductive amination in presence of $NaBH_3CN$. The crude secondary amine was then protected using BOC anhydride in water as a solvent. Finally the benzyl groups were removed using 20% $Pd(OH)_2$ in methanol at 150 psi under hydrogen atmosphere for 16 h to furnish the macrocyclic HEA isosteres **9a-c**.

It is noteworthy that at some stage during the formation of the 10-membered macrocyclic ring from the amino acid coupled furanose precursor, a water molecule got associated with the molecule, as clearly evident from their spectral data (*vide infra*), which is a common feature in carbohydrates and their analogs bearing multiple hydroxyl groups.[33]

1.7 Conclusion

In summary, this work has provided an elegant synthetic route to access amino-acid-tethered carbohydrate conjugates, which may eventually find potential applications in the development of conformationally restricted HEA isosteres. The synthetic strategy reported herein is a convenient and straightforward one starting from the readily available D-glucose. Also, the generality of this synthetic strategy has been demonstrated by the introduction of three different amino acid residues into the carbohydrate cyclic frame work.

Combretastatin-based analogs as potential anticancer agents

1.8 Introduction

Microtubules are one of the important targets for developing anticancer agents. Certain compounds attack microtubules through tubulin and disrupt its cellular structure causing mitotic arrest. Bio-active compounds like paclitaxel, vincristine etc. can inhibit microtubule polymerization and are therefore suitable candidates for anticancer drugs. However, most of these drugs possess undesirable side effects in spite of being potent antitumor agents. Consequently, new antitumor agents with increased specificity and least side effect need to be developed in order to minimize the damage to the healthy cells.

1.8.1 Microtubules: a key target for anticancer drugs

Microtubules are hollow cylindrical aggregates that consist of repeating α/β-tubulin heterodimers and are present in all eukaryotes.[34] Microtubules are vital for determining various cellular functions like maintaining cell shape, polarity, intracellular transport, secretion, and neurotransmissions.[35] On binding with guanosine triphosphate (GTP), tubulin self-assembles to form microtubules which are helical array of alternating α and β subunits with a diameter of approximately 24 nm (Fig. 1.6).[36] Microtubles also play very crucial role in mitosis, a process in which a cell duplicates the chromosome in the nucleus and generates two identical daughter cells. Advance research in this field made it possible to study the microtubule dynamics in living cells and has established that microtubules in mitotic spindles have uniquely rapid dynamics that are crucial for a successful mitosis.[37] Compounds that interact with α/β-tubulin or microtubules and suppress the microtubule dynamics, subsequently blocking cell cycle progression can therefore

be used for different purposes, including pesticides, antiparasitics and most importantly as anticancer agents.[38]

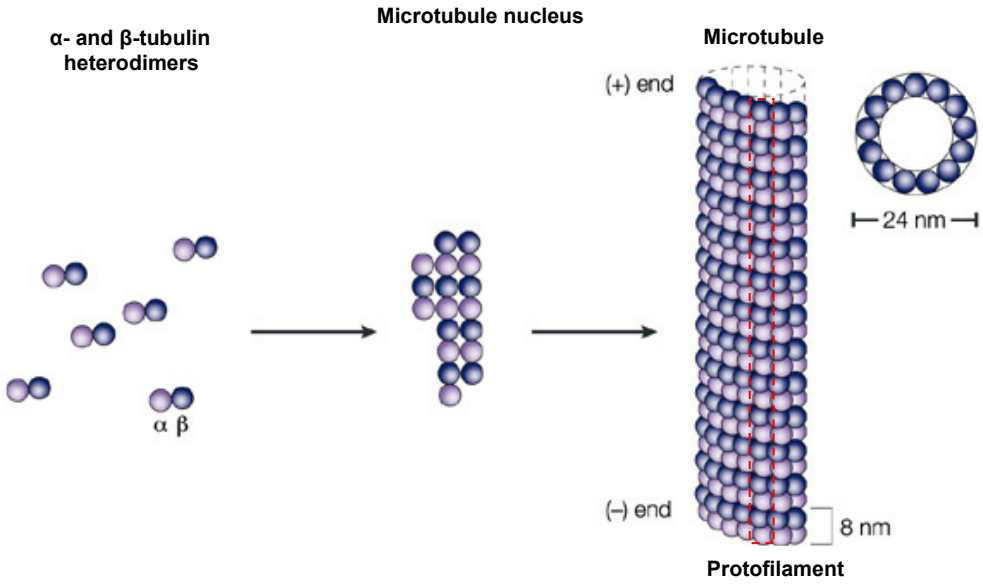


Fig. 1.6 Tubulin dimers, having an α-tubulin and a β-tubulin peptide, polymerize to form a microtubule nucleus. Additional dimers are added head-to-tail and the resulting microtubules are extremely dynamic structures containing a (+) end, characterized by an exposed β-tubulin peptide and a (–) end, characterized by an exposed α-tubulin peptide.[38]

1.8.2 Microtubule interacting agents

There are various sites in a microtubule for interaction of tubulin and/or microtubule-interacting agents. Currently, there are three well established sites on β-tubulin *viz.* vinca domain,[39] taxane domain[40] and colchicine site[41] for microtubule binding (Fig. 1.7). The vinca domain is situated at its plus end surface, to which many agents like vinblastine, cevipabulin, eribulin etc. can bind with high affinity, thereby reducing both treadmilling and dynamic instability of microtubules. The taxane domain lies within a hydrophobic cleft near the surface of β-tubulin and allows compounds like paclitaxel to interact with proteins through hydrogen bonding and hydrophobic interactions.[42] Colchicine was originally isolated from the meadow saffron *Colchicum autumnale*,[43] but could

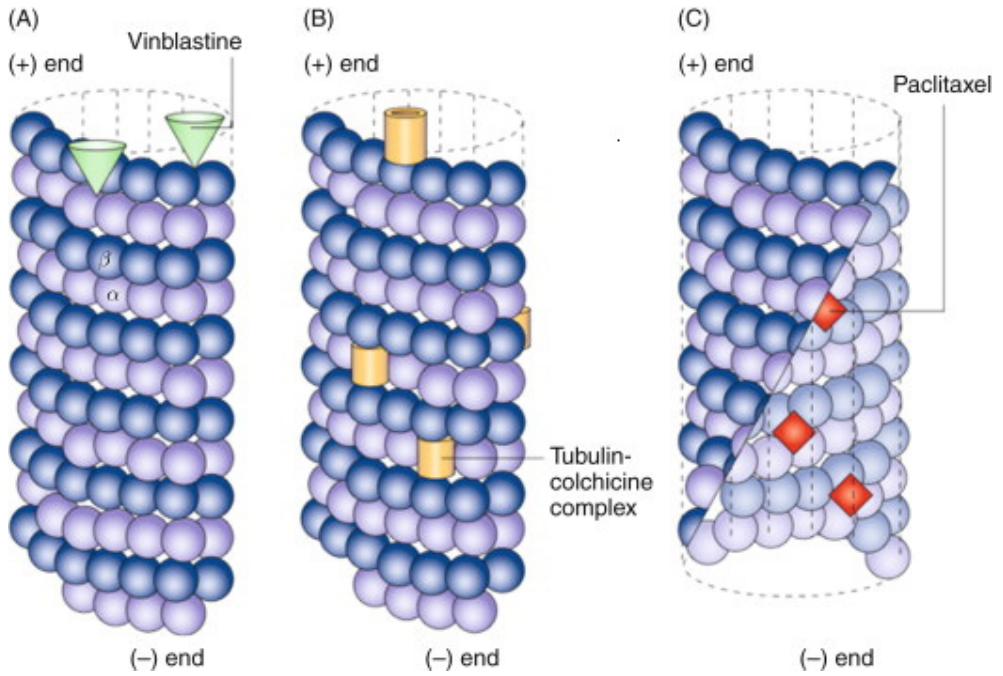
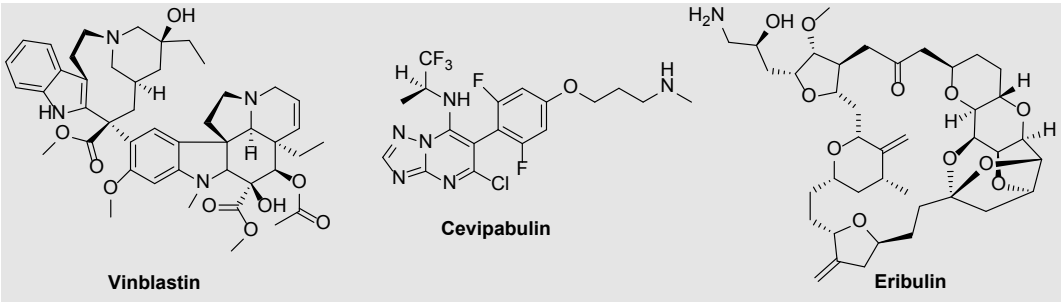


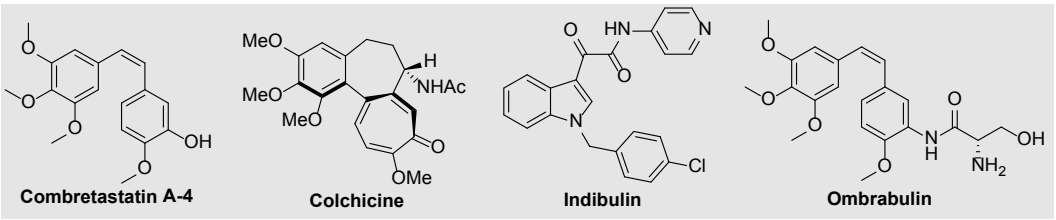
Fig. 1.7 Binding sites of microtubule interacting agents are shown (in green funnels, yellow cylinders and red diamonds). Although vinca alkaloids, such as vinblastine, interact in the microtubule ends, colchicine binds to soluble dimmers that can be incorporated in the microtubules. Taxanes, such as paclitaxel, bind to the interior surface of the microtubules.[38]

not be used for the treatment of cancer due of its increased cytotoxicity to normal cells. However, colchicine played a major role in explaining the function and properties of microtubules.[44] The colchicine site is located between the β-tubulin and α-tubulin interface.[43] Other agents which interact with colchicine site include combretastatins, ombrabulin, indibulin etc. In addition to the three binding sites, there are many compounds like laulimalide, noscapine which bind to some other sites that are yet to be revealed (Fig. 1.8).

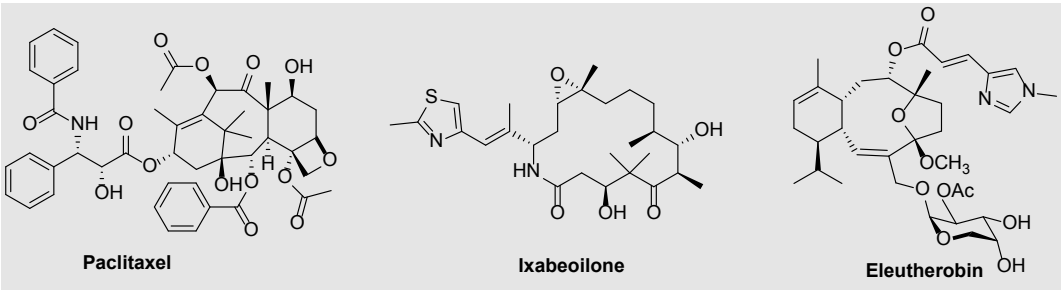
(A)Vinca-domain binders



(B) Colchicine-domain binders



(C) Taxol-domain binders



(D) Others

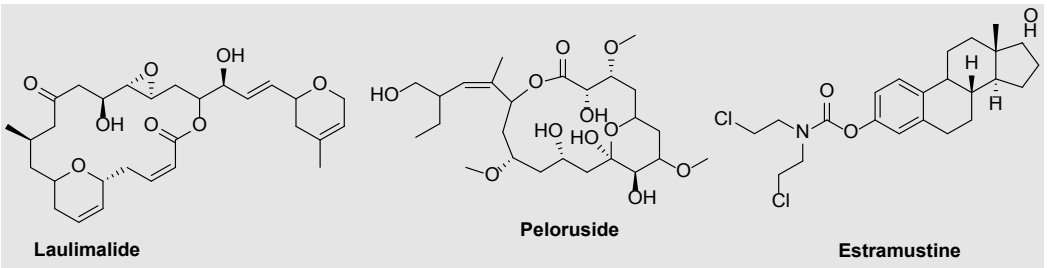


Fig. 1.8 Selected examples of microtubule-binding agents arranged according to their binding domains.[38]

1.8.3 Combretastatins: The vascular-targeting agents

Combretastatins represent a group of small and structurally uncomplicated antimitotic agents found to be potent inhibitors of tubulin assembly.[45] Pettit and co-workers first isolated combretastatin A-4 (CA-4) in 1982 from the root bark of the South-African bush willow tree *Combretum caffrum*.[46] CA-4[47] was found to inhibit a broad class of cancer cells, including the multi-drug resistant (MDR) cancer cell lines, following which there was a major impetus in research in this field.[48] Owing to its high affinity of binding to the colchicines site,[49] combretastatins act as highly potent cancer cell inhibitors. It selectively acts on the vascular system of tumors, restricting the blood flow to the affected cells while retaining blood supply to the healthy cells.[50] Structure activity relation (SAR) studies of CA-4 analogs reveal that the *cis*-disposition of the stilbene moiety and 3,4,5-trimethoxyphenyl as the A-ring substituents are essential for optimal biological activity.[51] (Fig. 1.9). It was also found that B-ring modification is tolerable especially at 3'postion.[51e]

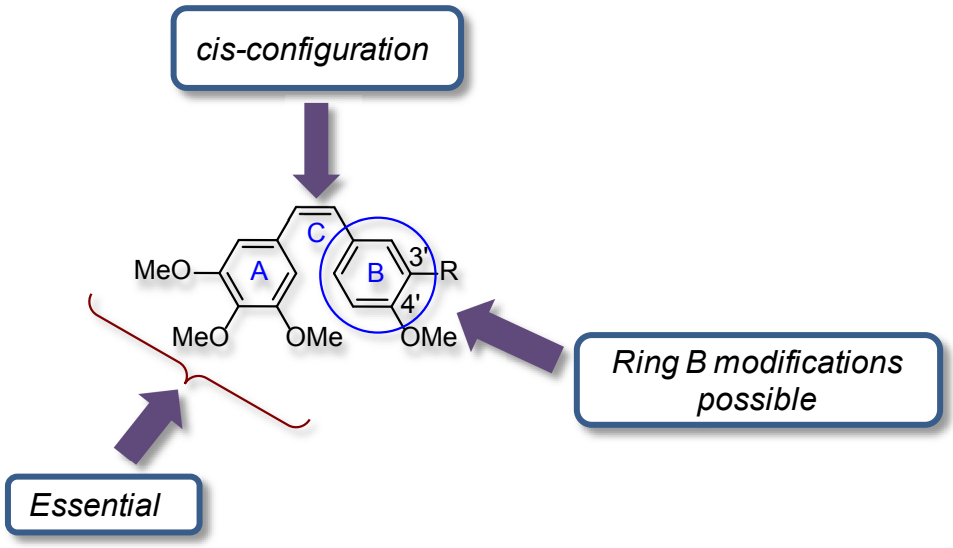


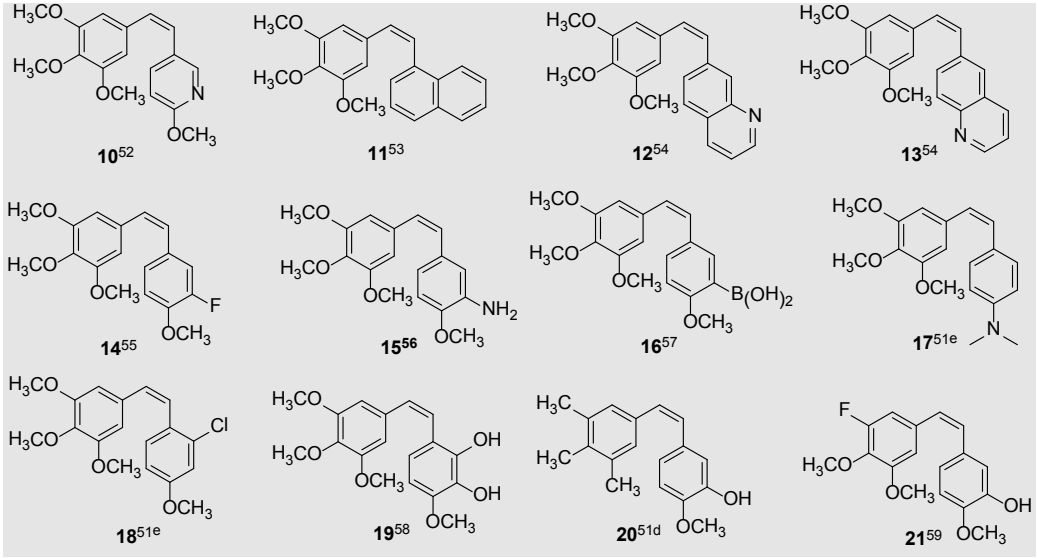
Fig. 1.9 SAR study of CA-4 showing the possibilities for modification.

Although SAR studies reveal the likely alteration possibilities, modification of combretastatins analogs is practically possible in three different places: ring-A, ring- B, and the double bond (Fig. 1.10).[51-67] As established, it was historically believed that ring B modifications were only bearable yielding potent compounds, and consequently, this ring has received greater attention from synthetic chemists. Modifications on this ring has been done by replacement with various phenyl substituted rings, heterocyclic rings, non-substituted aromatic rings etc.[51-59] It was also long been assumed that presence of the trimethoxyphenyl moiety was vital for the antitubulin responses, mainly because of repetition of this chemical motif in other antitubulin drugs.[51b] Also, loss of potency was observed in CA-3 wherein the *meta*-methoxy group of ring-A was replaced with a hydroxyl group.[51a] Loss of potency was also noticed when a simple aromatic ring is placed or deletions of the *meta* or *para* position methoxy groups were performed.[51c] Similar was the result when the methoxy groups were substituted with ethoxy groups.[51d] It is noteworthy that a sizable amount of modification has also been done on this ring (Fig. 1.10A). Single crystal X-ray structure investigation of CA-4 and analogs revealed that the two rings are at an angle of ~50 ° maintaining a *quasi-cisoid* conformation.[58] Thus, modification about the double bond that allows to retain the conformation required for biological activity was possible and hence been done (Fig. 1.10B).[60-63] Additionally, there are analogs where the olefinic group has been replaced by a ring (Fig. 1.10C). [64-67]

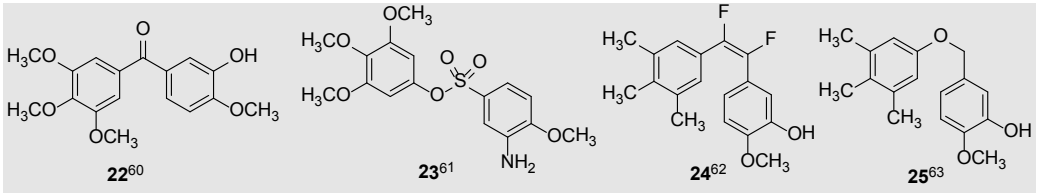
Even though the expedition for developing newer analogs continue, it is important to understand that to get optimum result out of a given bioactive compound, and to improve the selectivity and efficacy further, two things can be attempted: (i) incorporation of the therapeutics to ligands that can recognize

tumour-associated antigens; (ii) following the hybrid strategy (wherein two bioactive compounds that can act as different pharmacophores are attached). Rest of this section will deal with the potential modifications of combretastatins based on the above mentioned strategies.

(A) Selected examples of A and B ring modifications in CA-4



(B) Selected analogs of CA-4 with modifications on the double bond



(C) Selected examples of *cis*-restricted CA-4

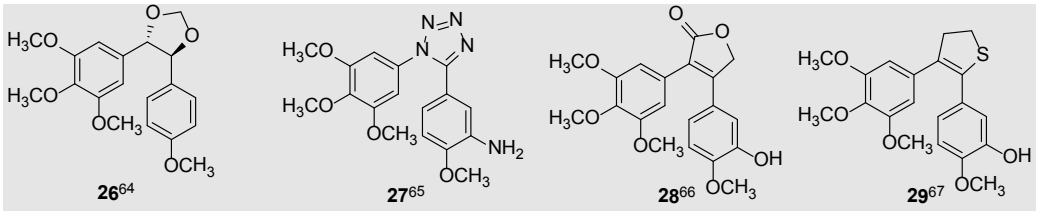


Fig. 1.10 Selected examples of modified CA-4 analogs.

1.9 Objective of the present work and design strategy

Most of the chemotherapies involve treatment with cytotoxic drugs which act randomly on the malignant as well as sensitive normal cells. Thus, the conventional treatment may lead to side-effects like nausea/vomiting, fatigue, myelosuppression, mucositis, alopecia, etc.[69] Therefore, there are various targeted therapies which are currently being developed to minimize the cytoxicity to the healthy cells.[70] Our objective here was to develop novel combretastatin analogs with a ligand capable of identifying tumour-associated antigens hooked to it. It has recently been reported that CA-4 analogues modified with nitrogen at the 3' position were strong inhibitors of tubulin polymerization, having an IC50 value for amine **15** almost equal to that of CA-4 (IC50 value, 1.2 mM).[71] Also these analogs showed activity against different colon cell line in virto and *in vivo*. Moreover the aniline analog **15** was found to be potent against pancreas, neuroblast, thyroid, lung-NSC, pharynx and prostate cancer cell lines,[71] and is currently in clinical trials. Consequently B-ring modified potent combretastatin analog **15** (Fig. 1.10) was chosen as our core moiety. Literature precedents ascertain that certain glutamate ureas are potent inhibitors of glutamate carboxypeptidase II (GCP II),[72] also known as PSMA, a type II integral membrane glycoprotein which is overexpressed in prostate tissues. So if a potent anticancer agent like **15** is coupled with such a ligand which can deliver it to the receptor site, the specificity of the said drug may increase significantly.

Our endeavour in this direction was therefore to attach N-analog of CA-4 (**15**) with a glutamate urea moiety, which is known to bind with PSMA and inhibit its enzymatic activity. These ligands have already been analysed for its targeted

drug delivery action.[73] The glutamate urea ligand (**L**) (Fig. 1.11) has also been shown to tolerate various R groups at C2 including alkyl heterocycles.[74] The synthetic strategy herein was to couple N-analog of combretastatin **15** with different amino acids followed by attaching with pre-formed urea to get an array of potential bioactive compounds having combretastatin core. Furthermore, we aimed to develop hybrid combretastatin analogs by attaching **15** with a potent xanthine derivative that has been proven to act as adenosine receptor (AR) antagonist (Fig. 1.11).[75]

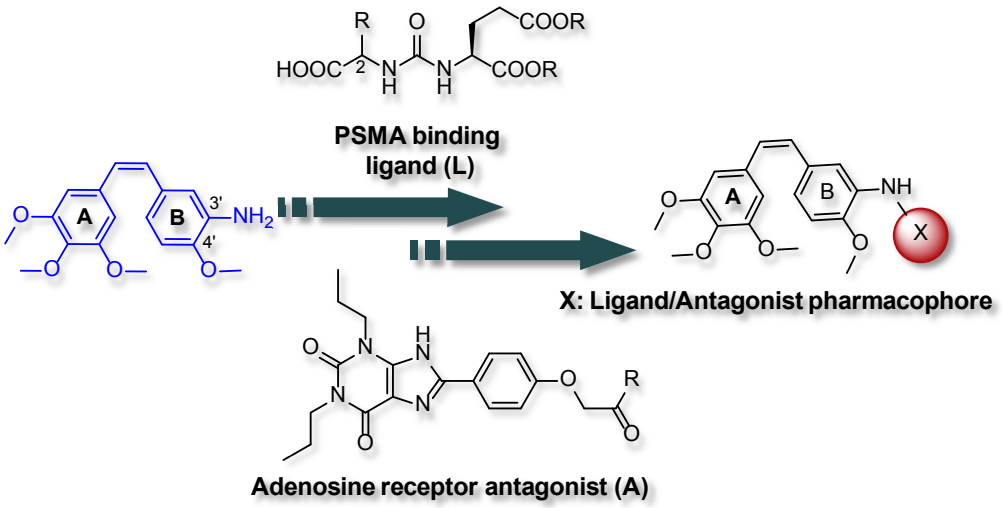


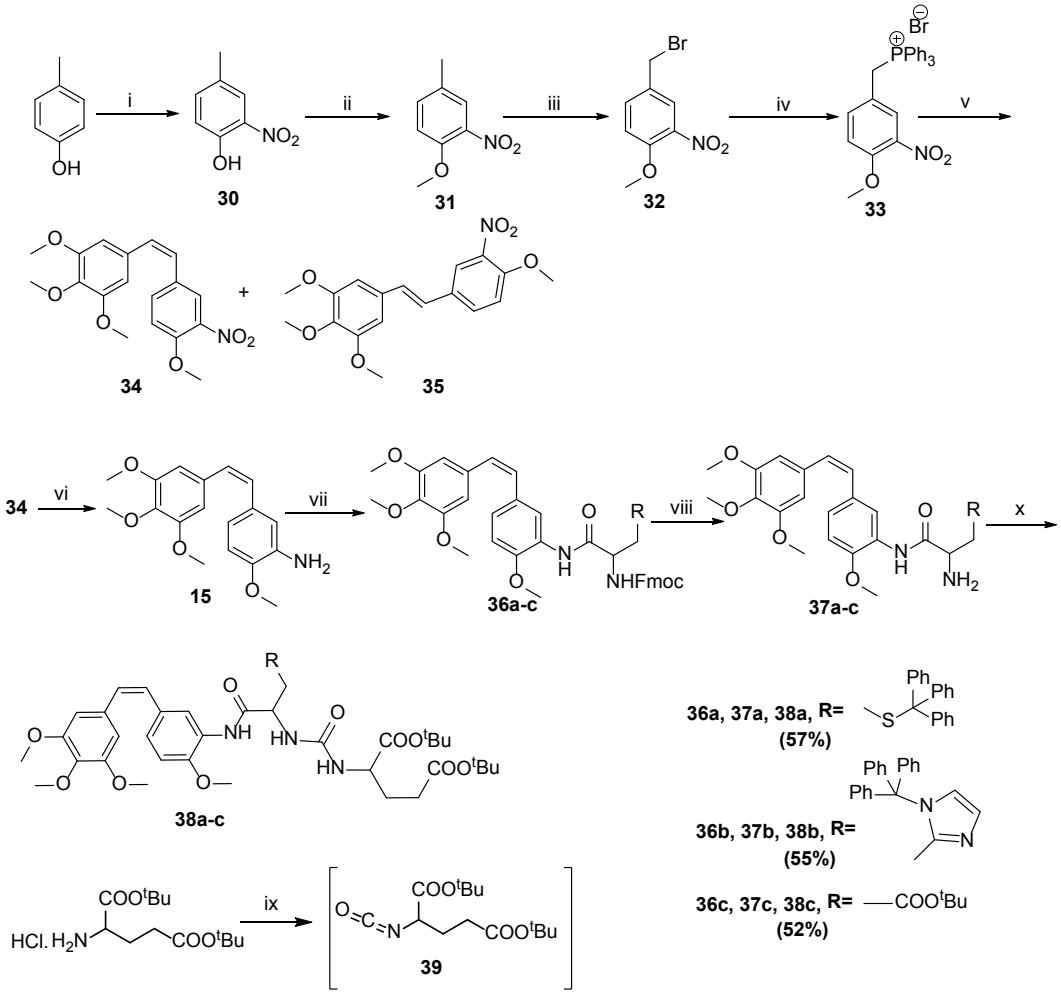
Fig. 1.11 Design strategy to develop combretastatin based analogs.

1.10 Synthesis of glutamate urea tethered combretastatin analogs

Synthesis of the hybrid combretastatin analog started with commercially availbale *p*-cresol, which gave the N-analog of CA-4 (**15**) in six steps on following the literature protocol[71] (Scheme 1.2). Amine **15** was then coupled with various Fmoc protected amino acids to furnish **36a-c**, which were carried forward without further characterization. Deprotection of Fmoc protecting group was carried out using diethylamine as base in DCM to obtain free amines **37a-c**. On the other hand, the isocyanate of glutamate ester **39** was generated *in situ* when

hydrochloride salt of L-glutamic acid di-tert-butyl ester was treated with 0.33 equivalent of triphosgene in presence of DIPEA in DCM as a solvent. The crude free amine was then added to the *in situ* genetared isocyanate to obtain the ligand associated combretastatin analogs **38a-c** in very good to excellent yields.

Scheme 1.2 Synthesis of combretastatin analogs **38a-c**

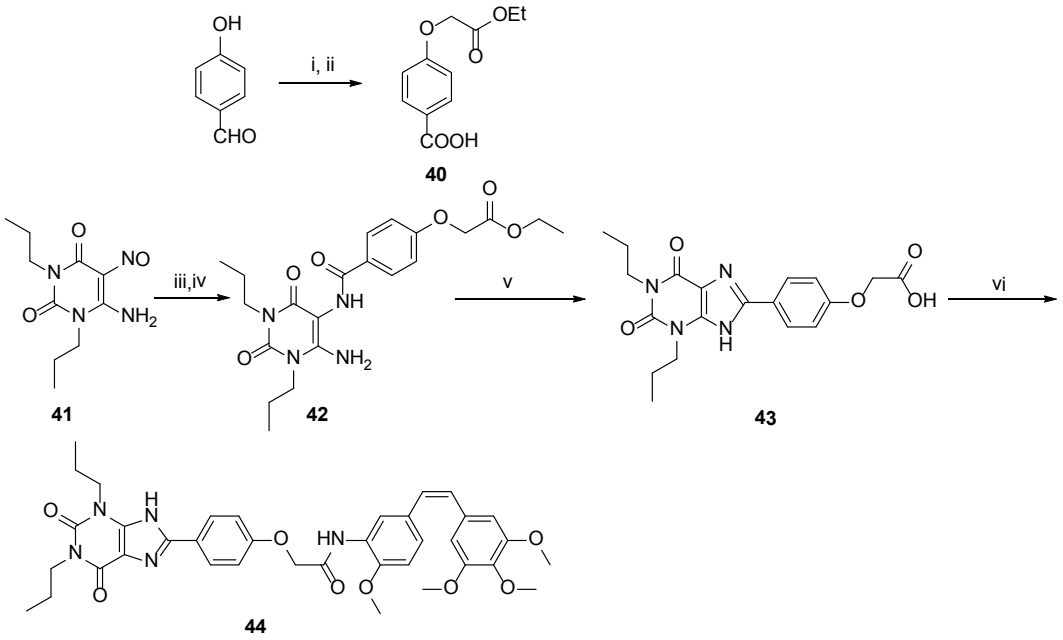


Reagents and conditions: (i) KNO_3, H_2SO_4, 2 h, -10 °C, 50%; (ii) NaOH, DMS, 7 h, 90%; (iii) AIBN, NBS, CCl_4, reflux, 5 h; (iv) PPh_3, DCM, reflux, 2 h, 78%; (v) 3,4,5-trimethoxybenzaldehyde, NaH, DCM, 15h, 75% [**34**:**35**→3:1 ratio]; (vi) Zn, AcOH, 2 h, rt, 50%; (vii) Fmoc-AA, EDC.HCl, HOBt, DMF,12h; (viii) Et_2NH, DCM, 4 h, quantitative; (ix) Triphosgene, DIPEA, DCM, -78 °C, rt, 0.5 h; (x)11, DIPEA, DCM, 16 h.

1.11 Synthesis of xanthine-coupled hybrid combretastatin analog

Hybrid combretastatin analog **44** was synthesized by coupling modified CA-4 **15** with the xanthine counterpart **43.**[76] Synthesis started with 4-hydroxy benzaldehyde which was subjected to *O*-alkylation followed by oxidation of aldehyde functionality to get the acid **40** in 80% over two steps following a reported procedure[76] (Scheme 1.3).

Scheme 1.3 Synthesis of hybrid analog **44**



Reagents and conditions: (i) Ethyl bromo acetate, K_2CO_3, NaI, CH_3CN, reflux 18 h; (ii) $KMnO_4$, Acetone, rt, 3 h, 80%; (iii) H_2, Pd/C, 60 psi, MeOH, 8 h; (iv) **14**, EDC.HCl, DMAP, MeOH, rt, 12 h; (v) 10% NaOH, MeOH, reflux, 4 h, 64%; (vi) EDC.HCl, HOBt, DMF, rt, 12 h, 90%.

Nitroso pyrimidanone **41** on the other hand was subjected to hydrogenation at 60 psi followed by coupling with the benzoic acid analog **40** to obtain the coupled product **42** which was carried forward without further purification. Compound **42** when refluxed in methanol in the presence of 10% NaOH in 4:1 ratio for 5 hours gave us the ester deprotected purine analog **43** in 64% yield, in four steps. The free acid **43** was then coupled with the CA-4 analog

15 in presence of EDC.HCl as coupling agent to attain the hybrid molecule **44** in 90% yield.

1.12 Conclusion

In summary, we have been able to synthesize an array of PSMA inhibitor ligand bound CA-4 analogs in very good yields. Also, we were able to develop a hybrid analog comprising of combretastatin and xanthine based adenosine receptor in excellent yield. These compounds may have the potential to become key members of the ever-increasing assembly of anticancer agents.

1.13 Experimental Section (Part A)

1, 2- O-isopropylidene-6-Azido-α-D-allofuranose 6:[32]

Dry DMF (20 ml) was added to 1,2-O-isopropylidene-6-O-*p*-toluenesulphonyl-α-D-allofuranose **5** (2.8 g, 7.48 mmol), followed by NaN_3 (2.43 g, 37.4 mmol, 5 equiv) and the reaction mixture was heated at 50 °C and stirred for 10 h. DMF was then completely evaporated under reduced pressure and the residue was extracted with ethyl acetate, dried (Na_2SO_4), concentrated and was subjected to purification by column chromatography to afford a white solid. Yield: 1.75 g (94%); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.81-5.79 (d, *J*= 3.92 Hz, 1H), 4.63-4.59 (m, 1H), 4.14-4.02 (m, 2H), 3.81-3.74 (m, 1H), 3.50-3.47 (d, *J*= 5.56 Hz, 2H), 2.72 (bs, 1H), 2.61 (bs, 1H), 1.59 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 113.0, 103.9, 79.7, 78.9, 71.6, 70.6, 52.8, 26.5, 26.4.

1,2- O-isopropylidene-3,5-Di-O-benzyl-6-azido-α-D-allofuranose 7:

A solution of 1,2- O-isopropylidene-6-Azido-α-D-allofuranose **6** (1.73 g, 7.09 mmol, 1 equiv) in THF (10 ml) was added to NaH (0.766 g, 31.9 mmol) at 0 °C and stirred for 30 min, and to this reaction mixture, benzyl bromide (2.65 g, 15.5 mmol) in THF (10 ml) and TBAI (0.13 g, 0.3 mmol) were added. After stirring for 2.5 h, the reaction mixture was poured into 10 mL of ice cold water. Aqueous layer was then extracted with chloroform, dried over anhy. Na_2SO_4, concentrated and was purified by column chromatography to afford a colorless viscous liquid. Yield: 2.049 g (68%); $[\alpha]^{30}_D$: 82.27° (*c* =1.58, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3471, 2928, 2102, 1724, 1603, 1454, 1383, 1270, 1026, 757, 699; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36-7.24 (m, 10H), 5.68-5.66 (d, *J*= 3.66 Hz, 1H), 4.77-4.70 (m, 3H), 4.56-4.50 (m, 2H), 4.17-4.12 (m, 1H), 4.02-3.88 (m, 2H), 3.50-3.39 (m, 1H), 3.22-

3.13 (m, 1H), 1.58 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.2, 137.2, 128.4, 128.3, 128.1, 128.0, 127.8, 127.6, 113.1, 104.0, 79.4, 77.7, 77.4, 74.1, 51.6, 26.8, 26.5; GC MS: 448.2766 $(M+Na)^+$, 464.2981 $(M+K)^+$; Anal. Calcd for $C_{23}H_{27}N_3O_5$: C,64.93; H, 6.40; N,9.88; Found: C,64.70; H,6.58; N,9.93.

General method for preparation of compounds 8a and 8b, 8c:

***tert*-butyl-1-(2-(benzyloxy)-2-((*3aR,5R,6aR*)-6-(benzyloxy)-2,2-dimethyltetrahydro-furo[2,3-*d*][1,3]dioxol-5-yl)ethylamino)-3-methyl-1-oxobutan-2-ylcarbamate 8a:**

Representative procedure: PPh_3 (1.11 g, 4.23 mmol) was added to a solution of 1,2- O-isopropylidene-3,5-Di-O-benzyl-6-azido-α-D-allofuranose **7** (1.2 g, 2.82 mmol) in 4:1 (v/v) THF:H_2O mixture (15 ml) and was stirred for 3 h. THF was then completely evaporated and the residue was extracted with ethyl acetate, dried over anhy. Na_2SO_4 and was concentrated under reduced pressure. The residue containing the crude amine (1.22 g, 3.05 mmol) was dissolved in acetonitrile containing Boc ^{L}Valine (0.729 g, 3.36 mmol). To the resulting mixture at 0 °C, HBTU (1.5 g, 3.97 mmol) was added followed by the addition of DIPEA (1.04 mL, 6.11 mmol) and the mixture was allowed to stir at room temperature for 10 h. The reaction mixture was then taken into ethyl acetate, sequentially washed with aq. $NaHCO_3$ and $KHSO_4$, dried over anhy. Na_2SO_4, concentrated under reduced pressure and was finally purified by column chromatography to furnish a white solid. Yield: 1.35 g (75%); mp: 73-75 °C; $[\alpha]^{28}_D$: 333.3° (*c* = 0.6, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3436, 3018, 2299, 1666, 1369, 1215, 756; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.35-7.28 (m, 10H), 6.24 (bs, 1H), 5.71-5.69 (d, *J*= 3.66 Hz, 1H), 4.96-4.92 (d, *J*= 8.59 Hz, 1H), 4.81-4.54 (m, 5H), 4.24-4.19 (m, 1H), 3.99-3.92 (m, 1H), 3.82-3.75 (m, 2H), 3.68-3.55 (m, 1H), 3.29-3.22 (m, 1H), 2.07-2.00 (m,1H), 1.80 (s, 1H), 1.58 (s, 3H), 1.44 (s, 9H), 1.36 (s, 3H), 0.89-0.85 (d, *J*= 6.82

Hz, 3H), 0.79-0.76 (d, *J*= 6.82 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.3, 155.5, 138.2, 137.1, 128.4, 128.3, 128.0, 127.8, 127.6, 112.9, 103.9, 79.7, 77.2, 77.1, 75.9, 72.9, 71.9, 59.7, 39.2, 30.7, 28.2, 26.7, 26.5, 19.0, 17.3; ESI MS: 599.2980 $(M+H)^+$, 621.2883 $(M+Na)^+$, 637.2506 $(M+K)^+$; Anal. Calcd for $C_{33}H_{46}N_2O_8$: C,66.20; H,7.74; N, 4.68; Found: C, 66.32; H,.82;N, 4.48.

tert-butyl-1-(2-(benzyloxy)-2-((*3aR,5R,6aR*)-6-(benzyloxy)-2,2-dimethyl tetrahydrofuro[2,3-*d*][1,3]dioxol-5-yl)ethylamino)-4-methyl-1-oxopentan-2-ylcarbamate 8b:

The product **8b** was obtained as a colourless viscous liquid. Yield: 78%; $[\alpha]^{28}_D$: 44° (*c* = 1.5, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3436, 3018, 1672, 1496, 1369, 1215, 1026, 757, 699; ^{1}H NMR (200 MHz, $CDCl_3$): δ= 7.34-7.31 (m, 10H), 6.36 (bs, 1H), 5.71-5.69 (d, *J*= 3.66 Hz, 1H), 4.80-4.54 (m, 6H), 4.24-4.19 (m, 1H), 3.99-3.93 (m, 2H), 3.65-3.51 (m, 1H), 3.29-3.16 (m, 1H), 2.00 (bs, 1H), 1.58 (m, 5H), 1.43 (s, 9H), 1.35 (s, 4H), 0.90-0.87 (d, *J*= 5.94 Hz, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.4, 155.3, 138.4, 137.2, 128.4, 128.3, 128.0, 127.9, 127.6, 113.0, 104.0, 79.7, 77.3, 77.2, 76.1, 73.1, 72.0, 53.0, 41.5, 39.3, 28.2, 26.8, 26.5, 24.6, 22.9, 21.7; ESI MS: 613.1911 $(M+H)^+$, 635.1877 $(M+Na)^+$, 651.1275 $(M+K)^+$; Anal. Calcd for $C_{34}H_{48}N_2O_8$: C, 66.64; H, 7.90,; N, 4.57; Found: C,66.52; H,7.81; N,4.78.

tert-butyl-1-(2-(benzyloxy)-2-((*3aR,5R,6aR*)-6-(benzyloxy)-2,2-dimethyltetra hydrofuro[2,3-*d*][1,3]dioxol-5-yl)ethylamino)-1-oxopropan-2-ylcarbamate 8c:

The product **8c** was obtained as a colourless viscous liquid. Yield 80%; $[\alpha]^{28}_D$: 106.97° (*c* = 0.86, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3433, 2981, 2250, 1672, 1496, 1375, 1166, 1026, 908, 732; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36-7.31 (m, 10H), 6.36 (bs, 1H), 5.71-5.69 (d, *J*= 3.66 Hz, 1H), 4.94 (bs, 1H), 4.80-4.53 (m, 5H), 4.24-4.19 (dd, J_1= 2.02 Hz, J_2= 8.97 Hz, 1H), 3.99-3.93 (m, 2H), 3.85-3.79 (m,

1H), 3.65-3.52 (m, 1H), 3.28-3.15 (m, 1H), 1.58 (s, 3H), 1.43 (s, 9 H), 1.35 (s, 3H), 1.23-1.20 (d, *J*= 7.08 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.4, 155.0, 138.3, 137.1, 128.4, 128.3, 128.0, 127.9, 127.6, 112.9, 103.9, 79.7, 77.3, 77.2, 75.9, 50.0, 39.3, 28.2, 296.7, 26.5, 18.7; ESI MS: 571.0034 $(M+H)^+$, 592.9886 $(M+Na)^+$, 608.9503 $(M+K)^+$; Anal. Calcd for for $C_{31}H_{42}N_2O_8$: C,65.24; H,7.42; N,4.91; Found: C,65.00; H,7.51; N,5.06.

General method for preparation of compounds 9a, 9b and 9c:

(*6R,7R,8R,9S*)-*tert*-butyl-6,7,8,9-tetrahydroxy-2-isopropyl-3-oxo-1,4-diazecane-1-carboxylate monohydrate 9a:

Representative procedure: Valine coupled furanose sugar **8a** (0.34 g, 0.56 mmol, 1 equiv) was taken in a 25 mL two-neck round bottom flask containing 6 mL aq. trifluoro acetic acid (80%) and the reaction mixture was stirred for 30 h at room temperature. The residue (0.4 g, 0.73 mmol) obtained after evaporation of TFA at reduced pressure was then taken into methanol and $NaBH_3CN$ (0.13 g, 2.1 mmol) was added followed by the addition of AcOH (0.04 mL, 0.73 mmol) at 0 °C. The reaction mixture was then allowed to come to rt and was further stirred for 40 h. Methanol was then evaporated under reduced pressure and the aq. layer was washed with ethyl acetate, followed by neutralization with saturated $NaHCO_3$. The neutralized aq. layer thus obtained was subsequently extracted with dichloromethane. The organic layer was concentrated and the crude product obtained was carried forward for the next reaction without further purification. To the crude free amine (0.13 g, 0.30 mmol, 1 equiv), BOC anhydride (0.33 g, 1.5 mmol, 5 equiv) was added followed by water (3 mL) and the resulting mixture was stirred for 12h, when a sticky material settled down, which was taken into DCM, washed sequentially with aq. $KHSO_4$ and $NaHCO_3$, dried over Na_2SO_4, and

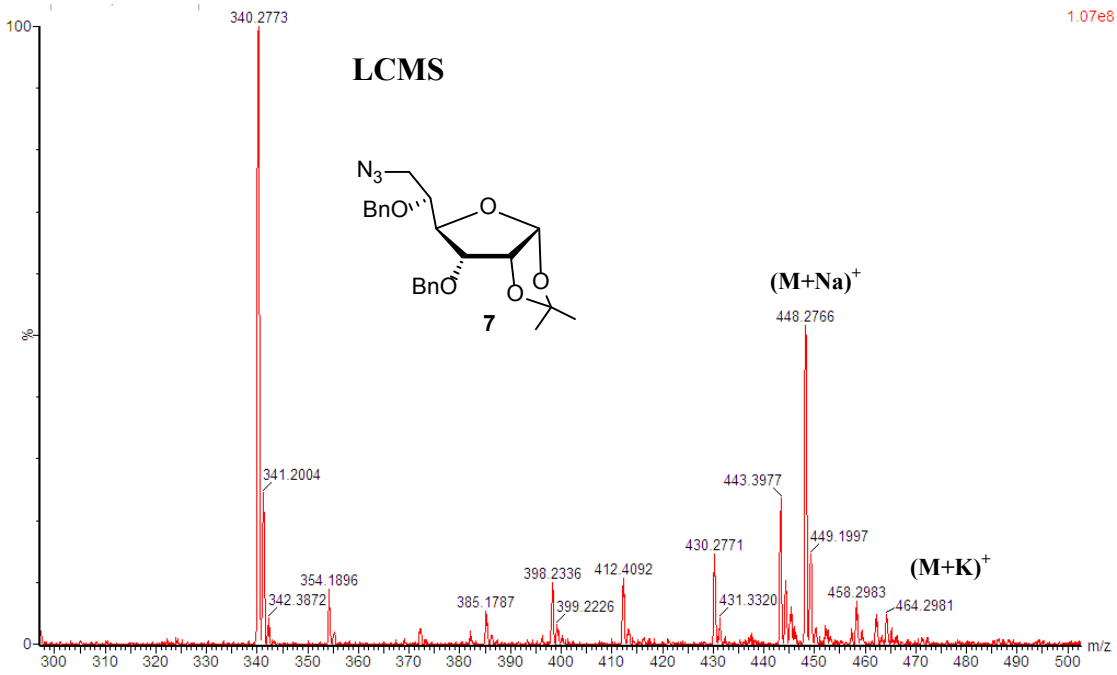
concentrated under reduced pressure to obtain a residue whose benzyl groups were subjected to hydrogenolysis using 20% $Pd(OH)_2$ in methanol at 150 psi under hydrogen atmosphere for 16 h to furnish **9a**, which was then purified using preparative TLC to furnish the pure product as an off white fluffy solid. Yield over three steps (48%); mp: 82-85 °C; $[\alpha]^{28}_D$: -7.07° (*c* = 5.09, $CHCl_3$); IR (Nujol) ν (cm^{-1}): 3323, 2922, 2359, 1633, 1454, 1163, 727; ^{1}H NMR (500 MHz, CD_3OD): δ 3.94-3.93 (m, 2H), 3.89 (bs, 1H), 3.85-3.79 (m, 2H), 3.72-3.70 (m, 2H), 3.61-3.58 (m, 1H), 3.45-3.42 (m, 1H), 3.37 (bs, 1H), 2.14-2.10 (m, 1H), 1.51 (s, 9H), 1.02-1.01 (d, *J*= 6.60 Hz, 3H), 0.99-0.98 (d, *J*= 6.88 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 175.2, 158.1, 80.7, 74.8, 74.4, 74.3, 72.6, 64.3, 61.8, 43.2, 31.9, 28.8, 19.8, 18.4; ESI MS: 381.2047 $(M+H)^+$, 403.1407 $(M+Na)^+$; Anal. Calcd for $C_{16}H_{32}N_2O_8$: C,50.51; H, 8.48; N, 7.36; Found: C, 50.35,; H,8.40; N, 7.60.

(*6R,7R,8R,9S*)-*tert*-butyl-6,7,8,9-tetrahydroxy-2-isobutyl-3-oxo-1,4-diazecane-1-carboxylate monohydrate 9b:

The product **9b** was obtained as a white fluffy solid. Yield over three steps (45%); mp: 160-165 °C; $[\alpha]^{28}_D$: -10.2° (*c* = 4.5, $CHCl_3$); IR (Nujol) ν (cm^{-1}): 3450, 2924, 2359, 1634, 1454, 1377, 727; ^{1}H NMR (500 MHz, CD_3OD): δ 8.60 (s, 1H), 4.15-4.12 (m, 1H), 3.93-3.88 (m, 2H), 3.85-3.78 (m, 2H), 3.74-3.69 (m, 2H), 3.58-3.55 (m, 1H), 3.46-3.43 (m, 1H), 3.37 (bs, 1H), 1.76-1.74 (m, 1H), 1.62-1.57 (m, 2H), 1.51 (s, 9H), 1.02-0.98 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.5, 158.0, 80.8, 74.9, 74.5, 74.3, 72.8, 64.3, 55.0, 43.2, 42.3, 28.8, 26.0, 24.3, 23.5, 22.0; ESI-MS: 395.2959 $(M+H)^+$, 417.3022 $(M+Na)^+$; Anal. Calcd for $C_{17}H_{34}N_2O_8$: C, 51.76; H, 8.69; N, 7.10; Found: C, 51.51; H, 8.78; N= 7.26.

(*6R,7R,8R,9S*)-*tert*-butyl-6,7,8,9-tetrahydroxy-2-methyl-3-oxo-1,4-diazecane-1-carboxylate monohydrate 9c:

The product **9c** was obtained as a colourless viscous liquid. Yield over three steps (47%); $[\alpha]^{28}_{D}$: -14.9° (*c* = 0.67, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3344, 3018, 2360, 1658, 1215, 769; ^{1}H NMR (500 MHz, CD_3OD): δ= 4.15-4.14 (m, 1H), 3.95 (bs, 1H), 3.90 (bs, 1H), 3.87-3.84 (m, 1H), 3.81 (bs, 1H), 3.73-3.71 (m, 2H), 3.61-3.58 (m, 1H), 3.47-3.45 (m, 1H), 3.39 (bs, 1H), 1.52 (s, 9H), 1.40-1.39 (d, *J*= 7.16 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.6, 157.7, 80.8, 74.7, 74.3, 72.6, 64.3, 57.8, 43.2, 28.7, 18.4; ESI MS: 353.2823 $(M+H)^+$, 375.3724 $(M+Na)^+$; Anal. Calcd for $C_{14}H_{28}N_2O_8$: C, 47.72; H, 8.01; N, 7.95; Found: C, 47.40; H, 8.25; N, 8.03.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%
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412.4092
430.2771
431.3320
443.3977
448.2766
449.1997
458.2983
464.2981
(M+Na)+
(M+K)+
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BnO
BnO
O
O
O
7
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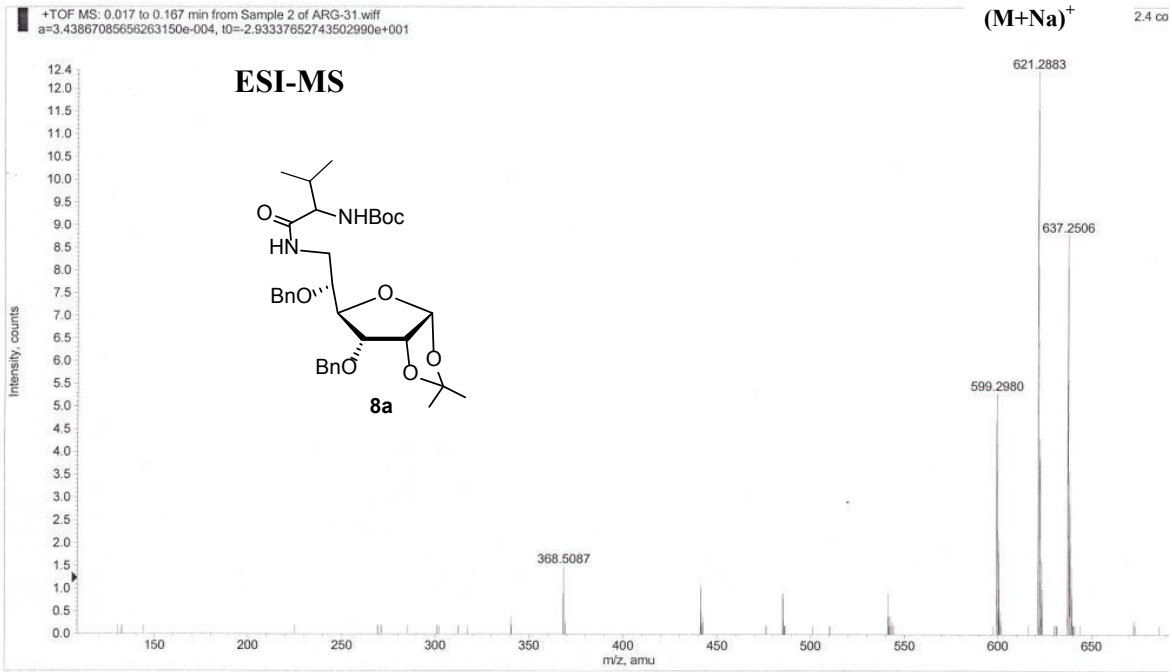
m/z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ESI-MS
(M+Na)+
2.4 co
Intensity, counts
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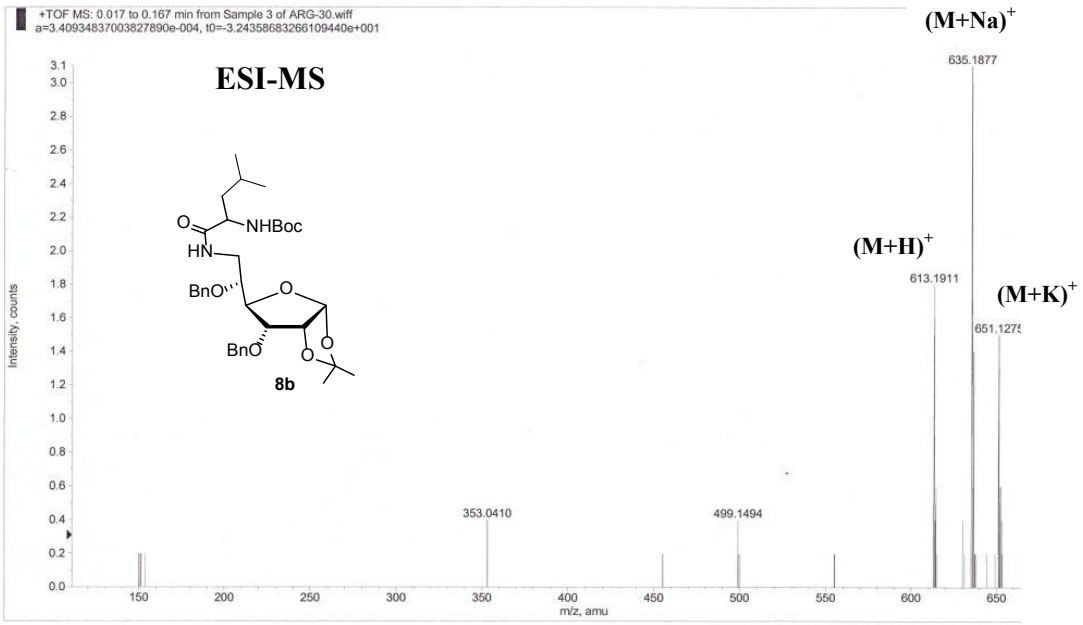
637.2506
599.2980
368.5087
O
NHBoc
HN
BnO
BnO
O
O
O
8a
150 200 250 300 350 400 450 500 550 600 650
m/z, amu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ESI-MS
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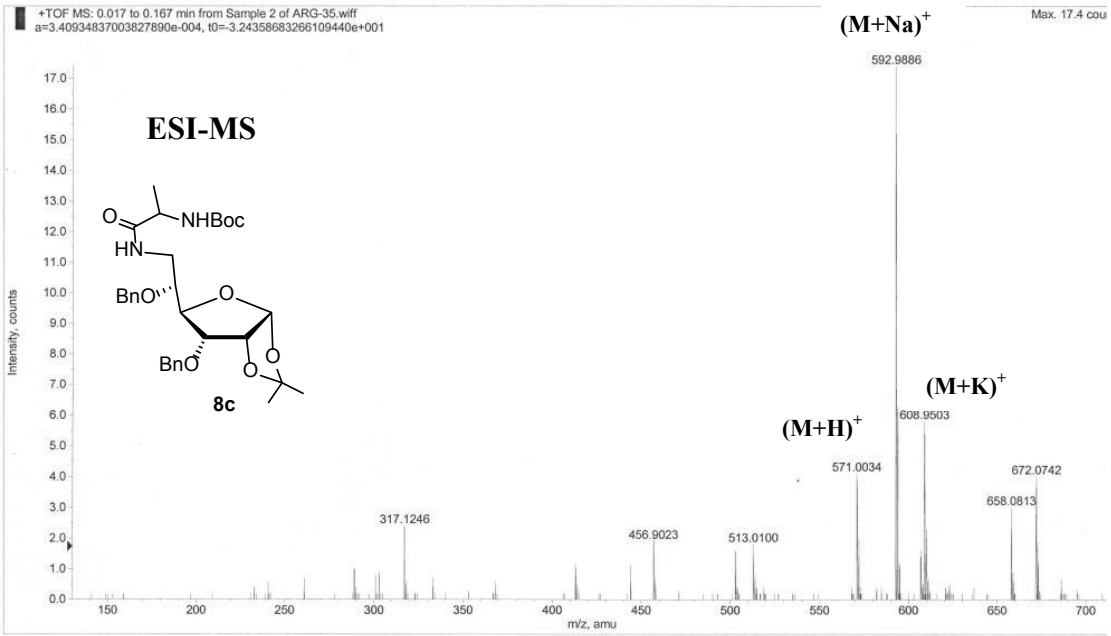
(M+H)+
613.1911
(M+K)+
651.127
353.0410
499.1494
NHBoc
HN
BnO
BnO
8b
Intensity, counts
m/z, amu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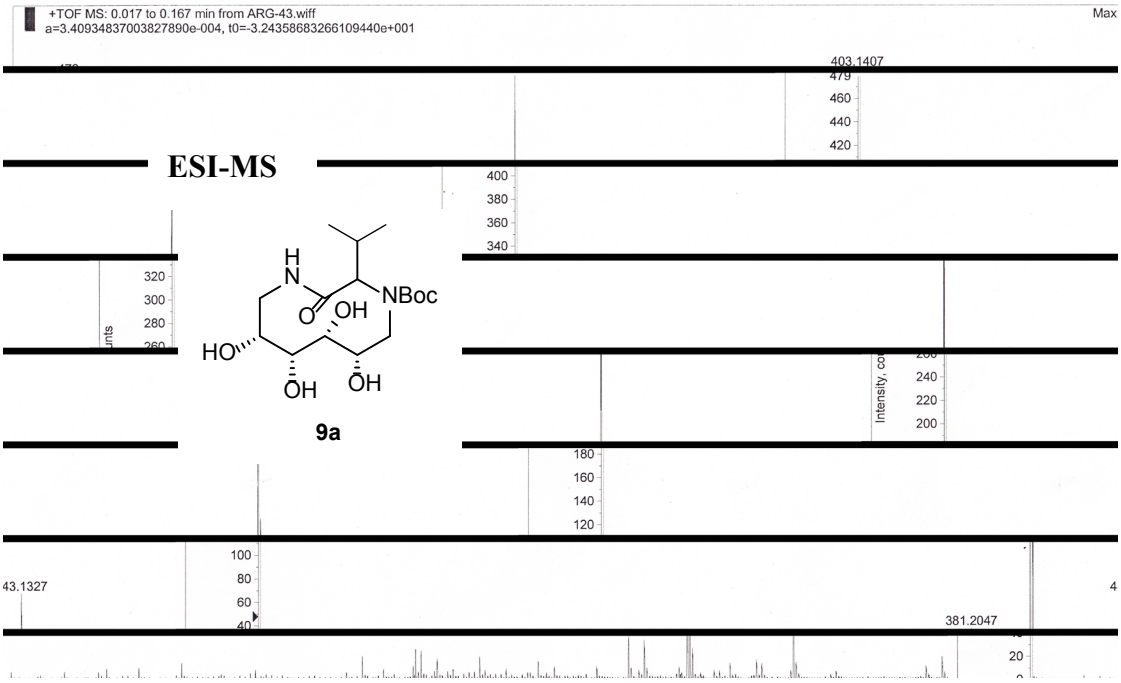
Max. 17.4 cou
ESI-MS
(M+Na)+
592.9886
(M+K)+
608.9503
(M+H)+
571.0034
672.0742
658.0813
317.1246
456.9023
513.0100
NHBoc
HN
BnO
BnO
8c
Intensity, counts
m/z, amu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ESI-MS
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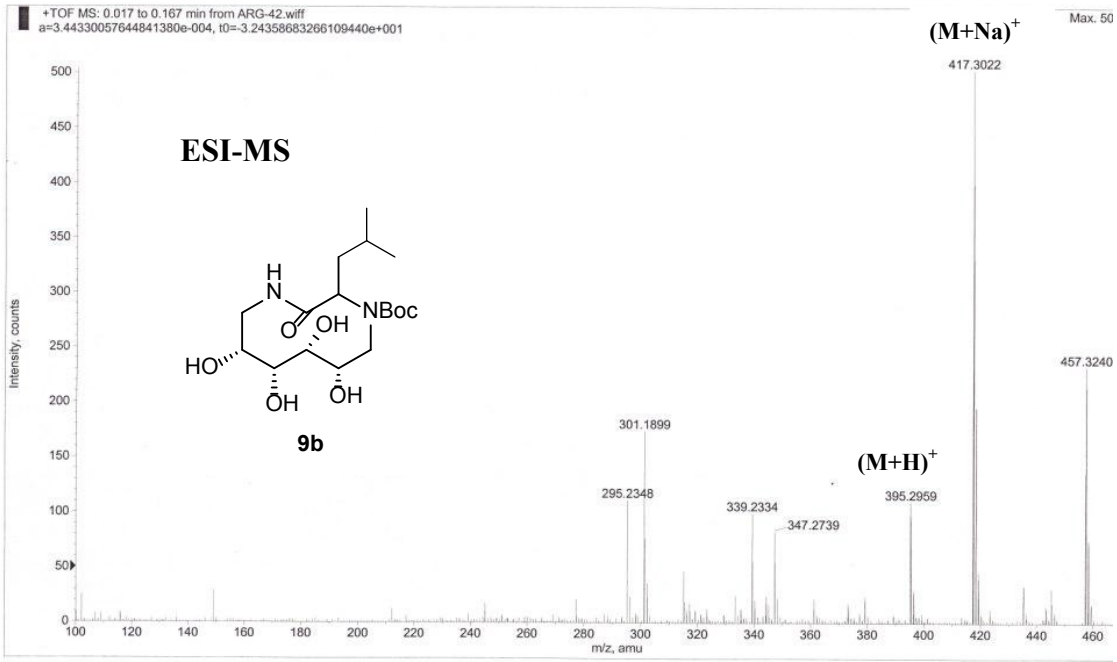
381.2047
43.1327
NBoc
OH
HO
OH
OH
9a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Max. 50
ESI-MS
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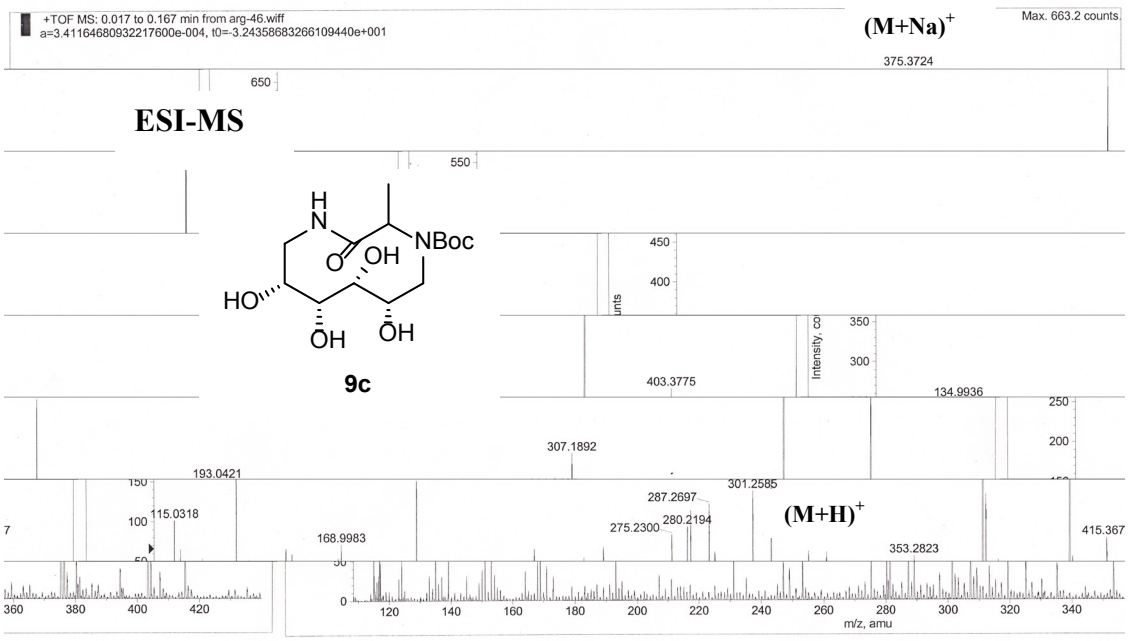
417.3022
457.3240
(M+H)+
395.2959
339.2334
347.2739
301.1899
295.2348
Intensity, counts
m/z, amu
NBoc
OH
HO
OH
OH
9b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Max. 663.2 counts
(M+Na)+
375.3724
ESI-MS
9c
403.3775
134.9936
307.1892
193.0421
115.0318
301.2585
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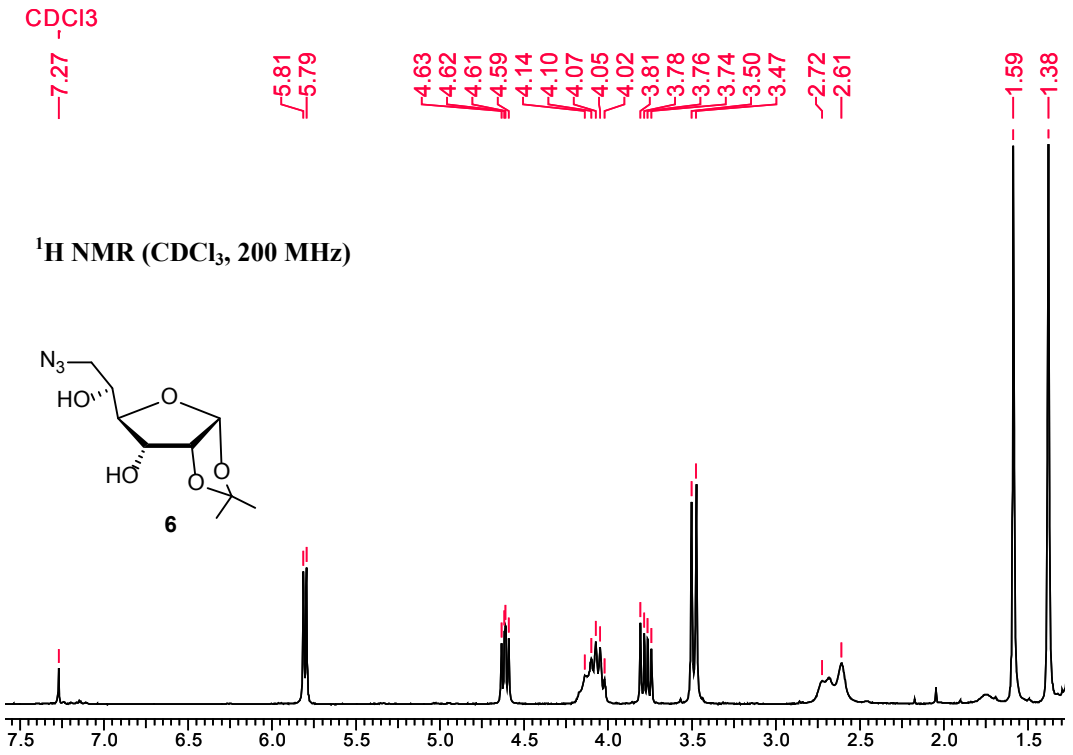
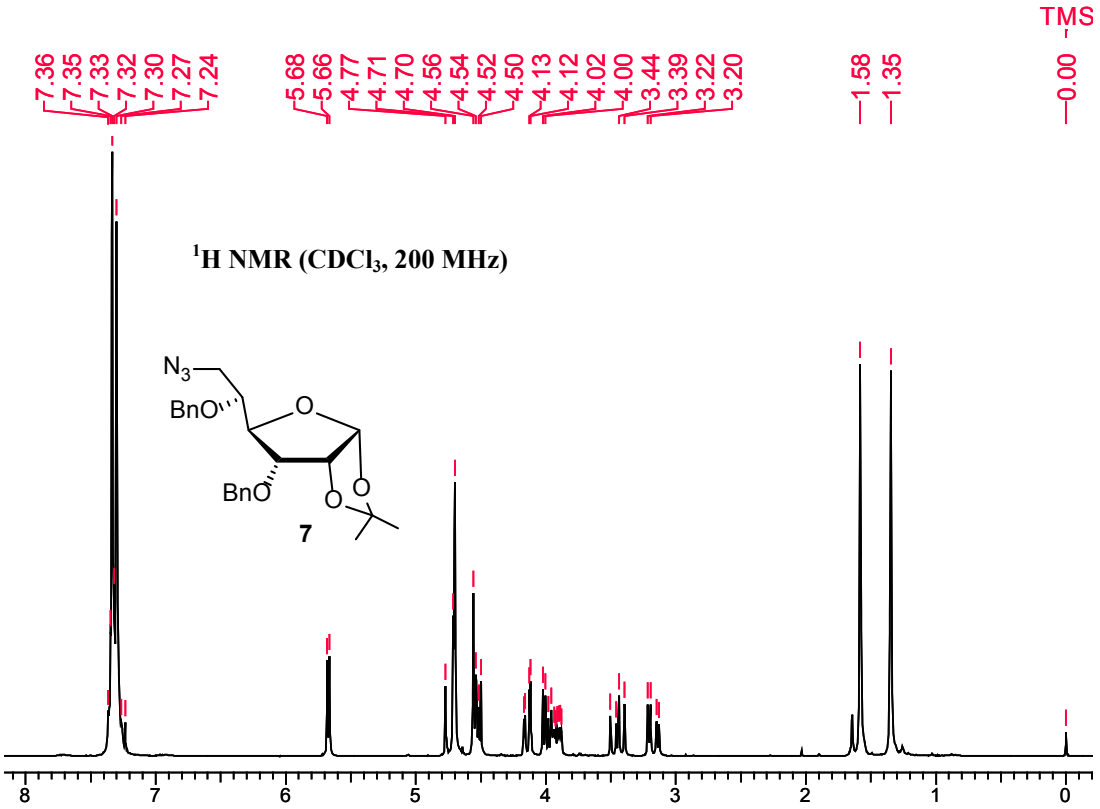
280.2194
275.2300
(M+H)+
168.9983
353.2823
415.367
Intensity, co
m/z, amu



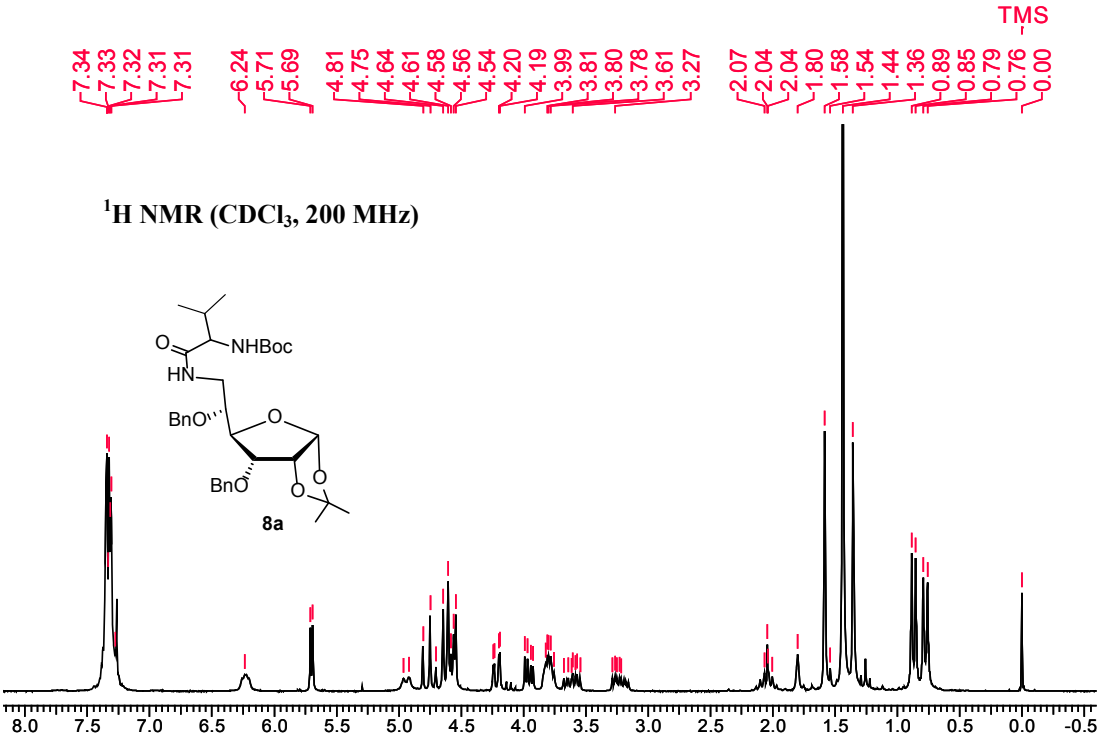
^{1}H NMR ($CDCl_3$, 200 MHz)

6



^{1}H NMR ($CDCl_3$, 200 MHz)

7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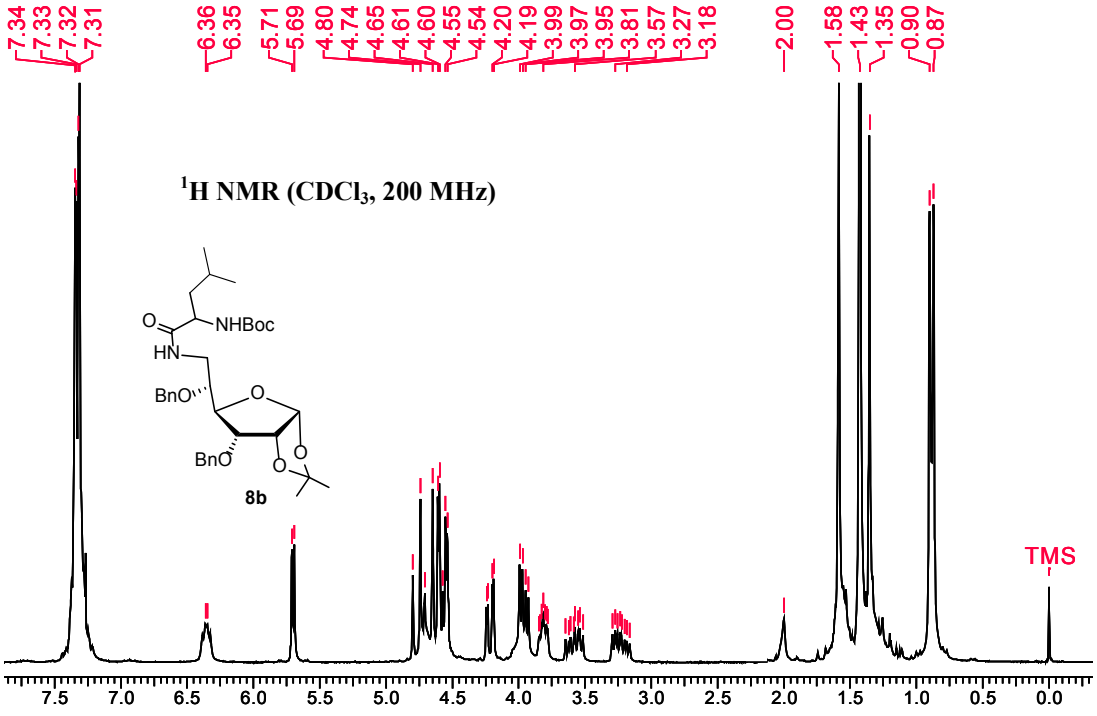
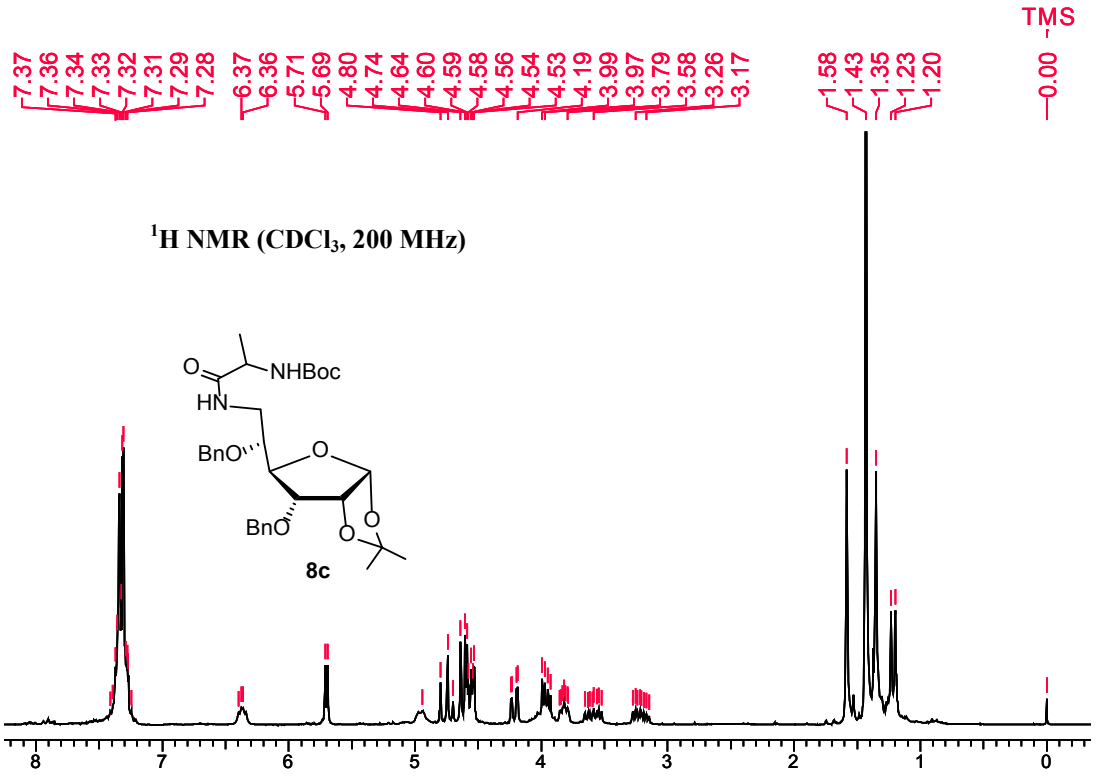
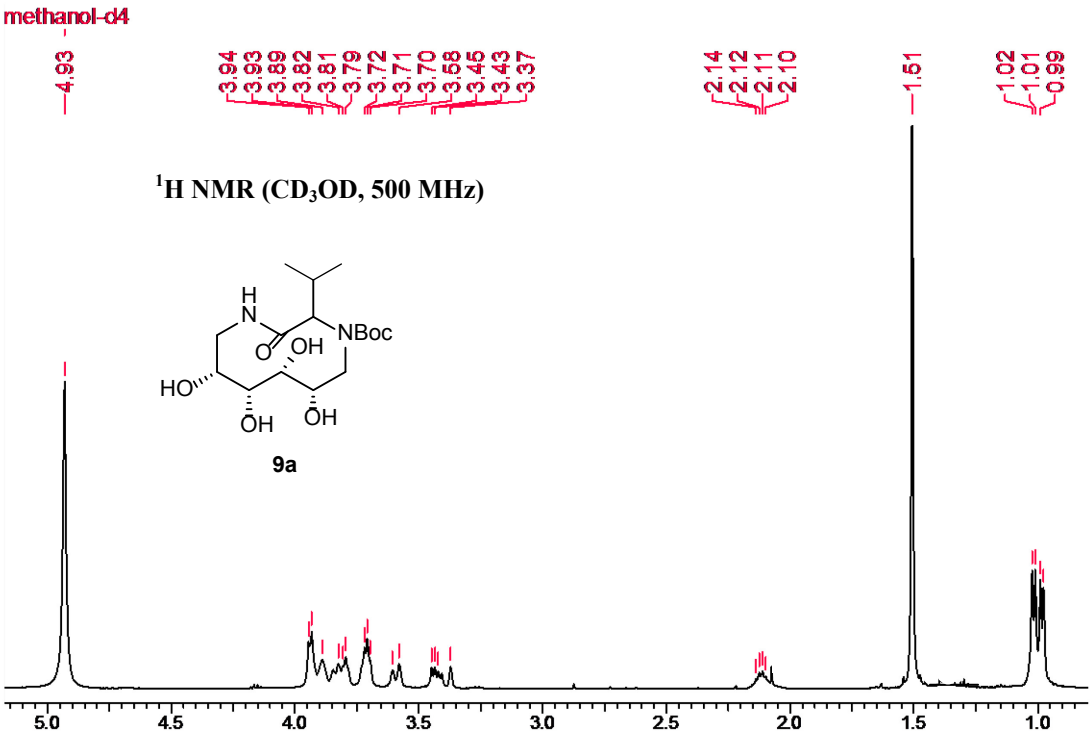
^{1}H NMR ($CDCl_3$, 200 MHz)
NHBoc
HN
BnO
BnO
O
O
O
8a
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5


7.34 7.33 7.32 7.31 6.36 6.35 5.71 5.69 4.80 4.74 4.65 4.61 4.60 4.55 4.54 4.20 4.19 3.99 3.97 3.95 3.81 3.57 3.27 3.18 2.00 1.58 1.43 1.35 0.90 0.87
^{1}H NMR ($CDCl_3$, 200 MHz)
NHBoc
HN
BnO
BnO
O
O
O
8b
TMS
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0



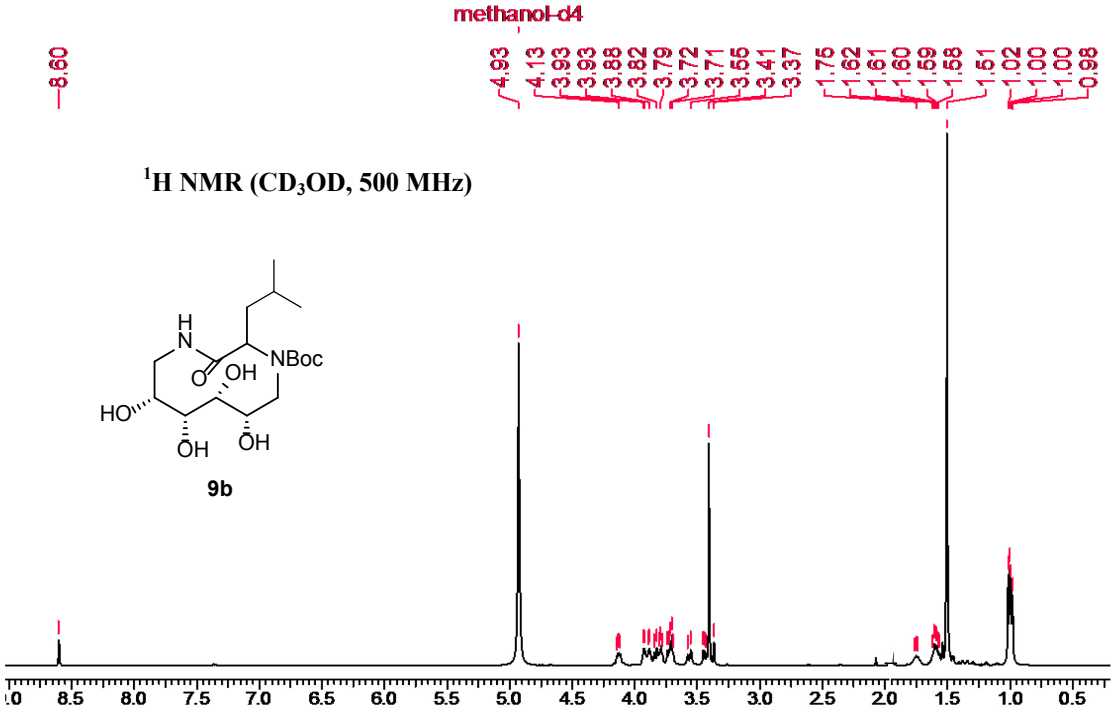
^{1}H NMR ($CDCl_3$, 200 MHz)

8c



^{1}H NMR (CD_3OD, 500 MHz)

9a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8.60
4.93
4.13
3.93
3.93
3.88
3.82
3.79
3.72
3.71
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3.41
3.37
1.75
1.62
1.61
1.60
1.59
1.58
1.51
1.02
1.00
1.00
0.98
1H NMR (CD3OD, 500 MHz)
H
N
O
OH
NBoc
HO
OH
OH
9b
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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2.5
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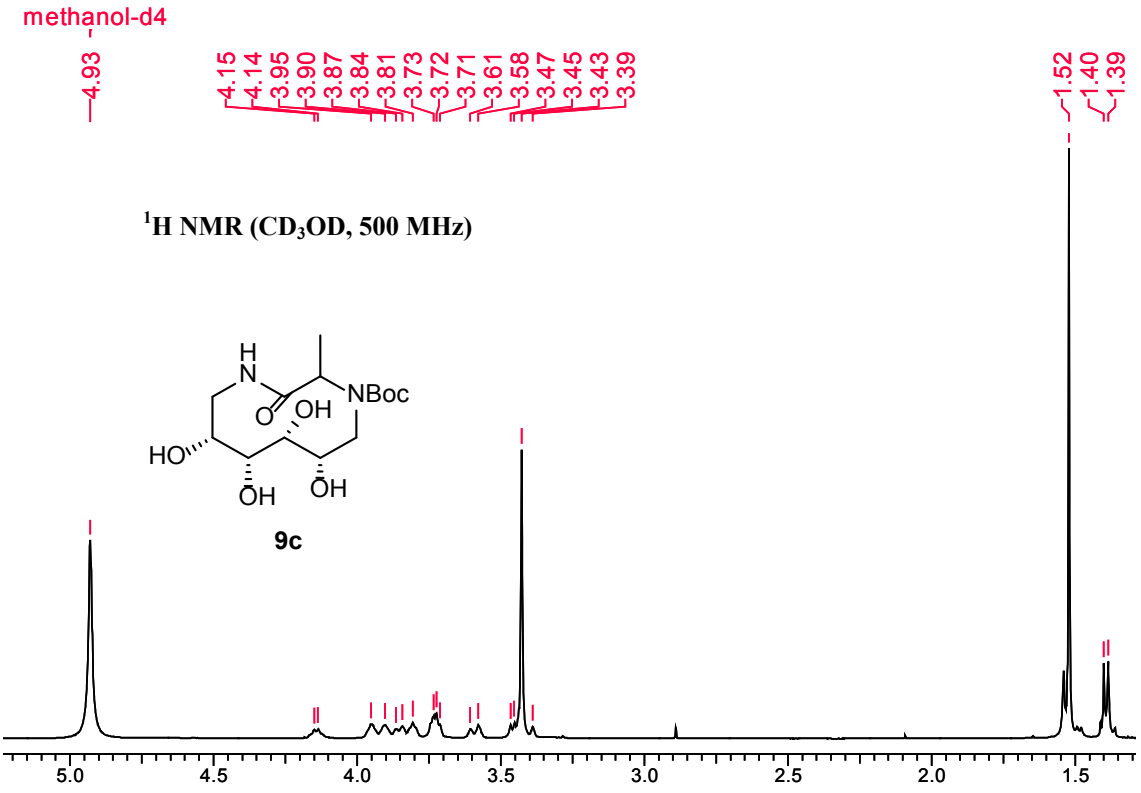
0.5


methanol-d4
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4.15
4.14
3.95
3.90
3.87
3.84
3.81
3.73
3.72
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3.58
3.47
3.45
3.43
3.39
1.52
1.40
1.39
1H NMR (CD3OD, 500 MHz)
H
N
O
OH
NBoc
HO
OH
OH
9c
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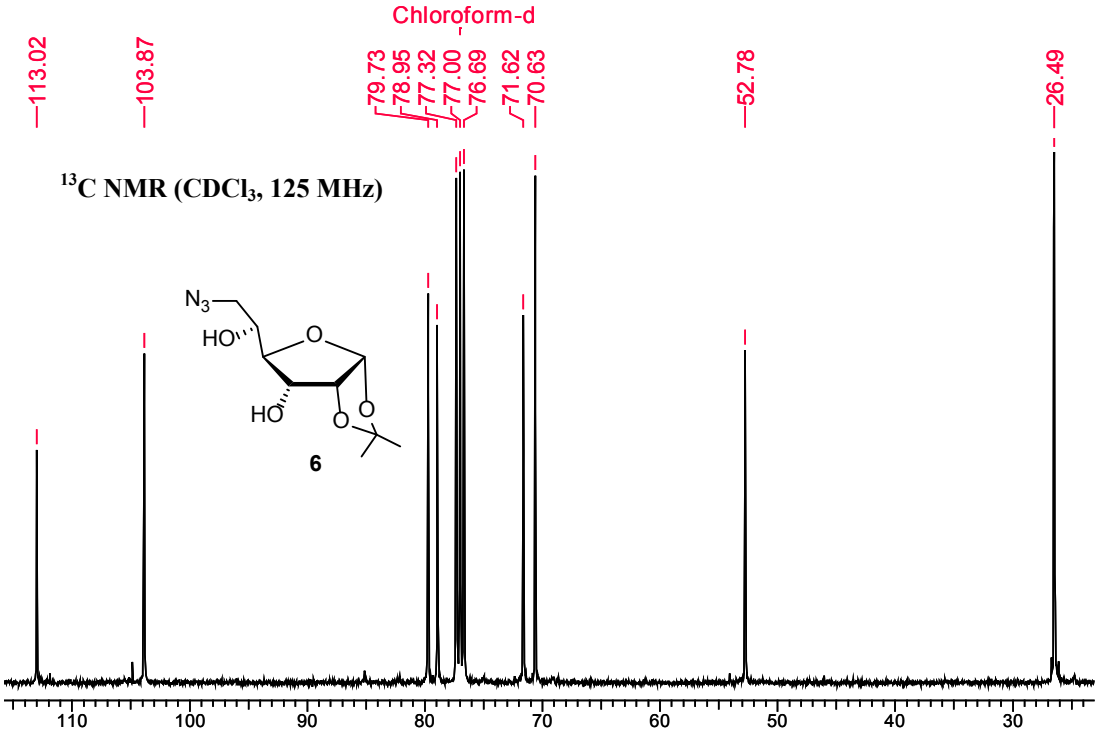
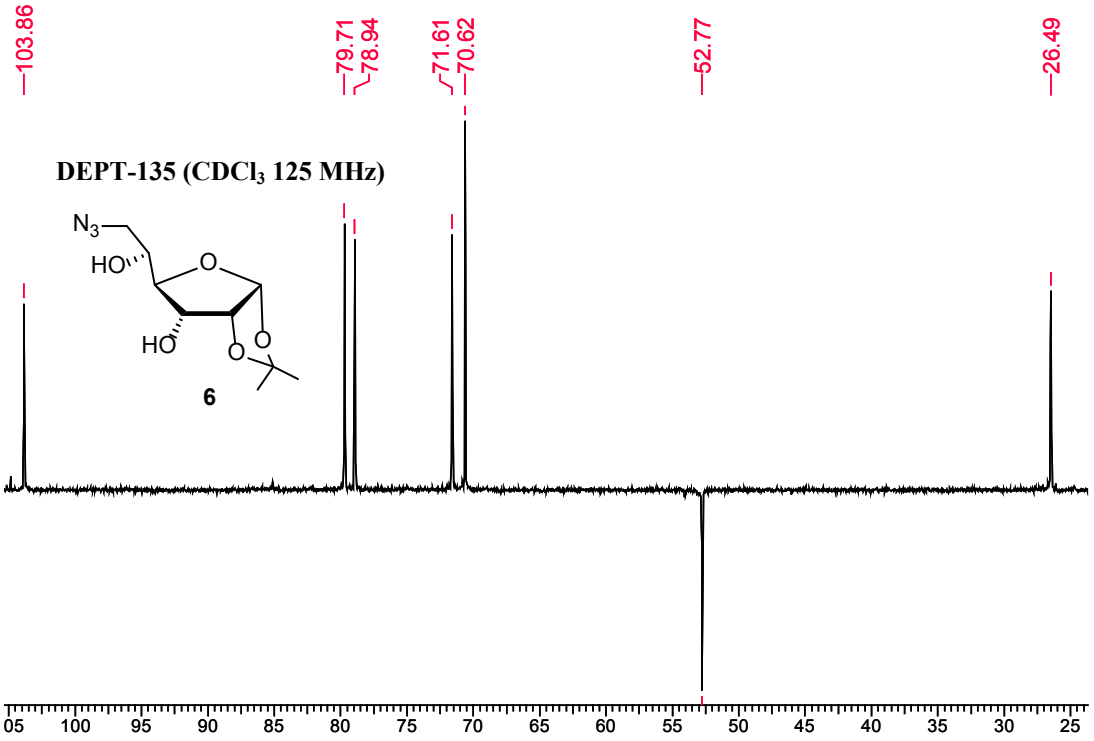
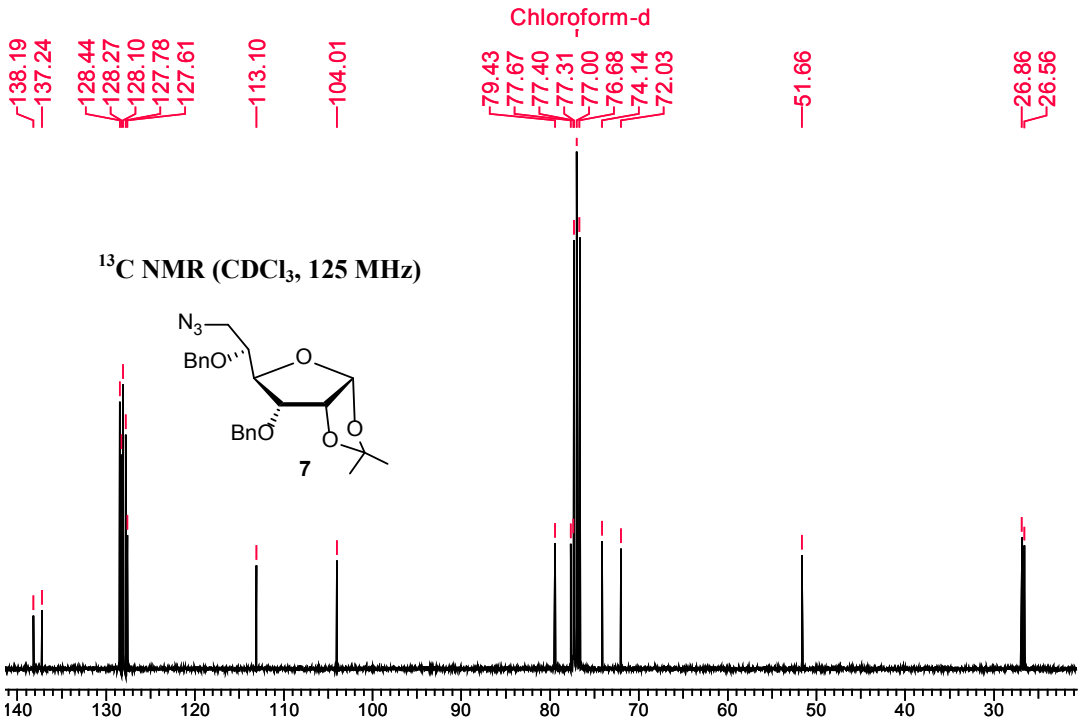
4.5
4.0
3.5
3.0
2.5
2.0
1.5

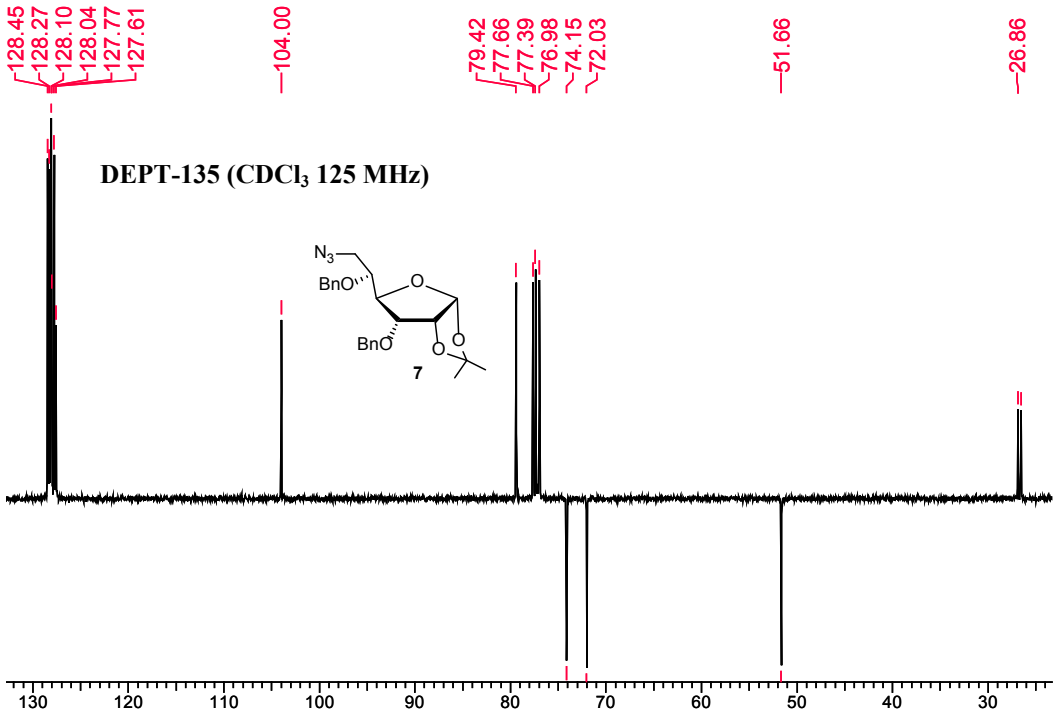

Chloroform-d
113.02
103.87
79.73
78.95
77.32
77.00
76.69
71.62
70.63
52.78
26.49
13C NMR (CDCl3, 125 MHz)
N3
HO
O
HO
O
O
6
110
100
90
80
70
60
50
40
30


103.86
79.71
78.94
71.61
70.62
52.77
26.49
DEPT-135 (CDCl3 125 MHz)
N3
HO
O
HO
O
O
6
05
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25



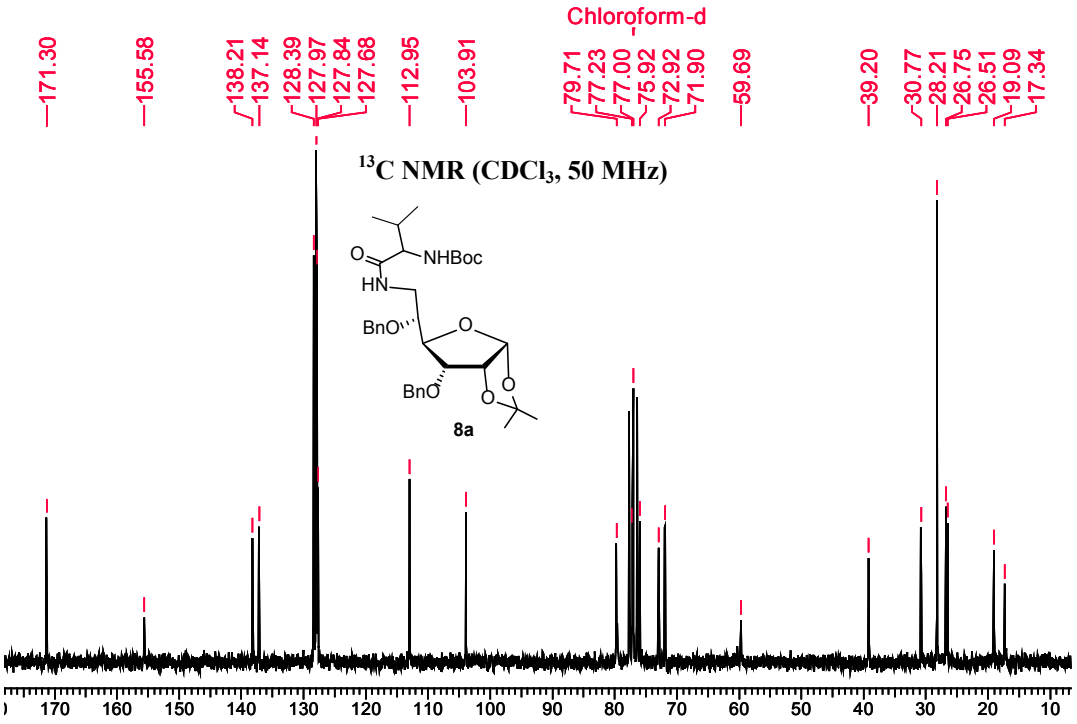
^{13}C NMR ($CDCl_3$, 125 MHz)

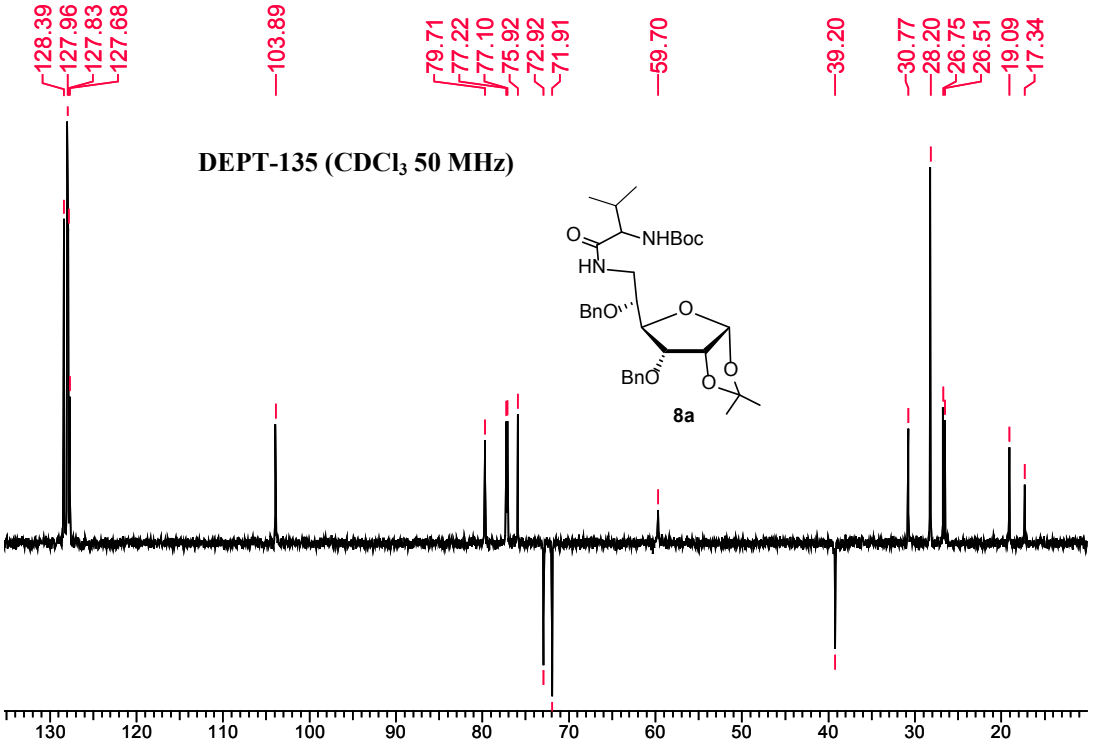
7

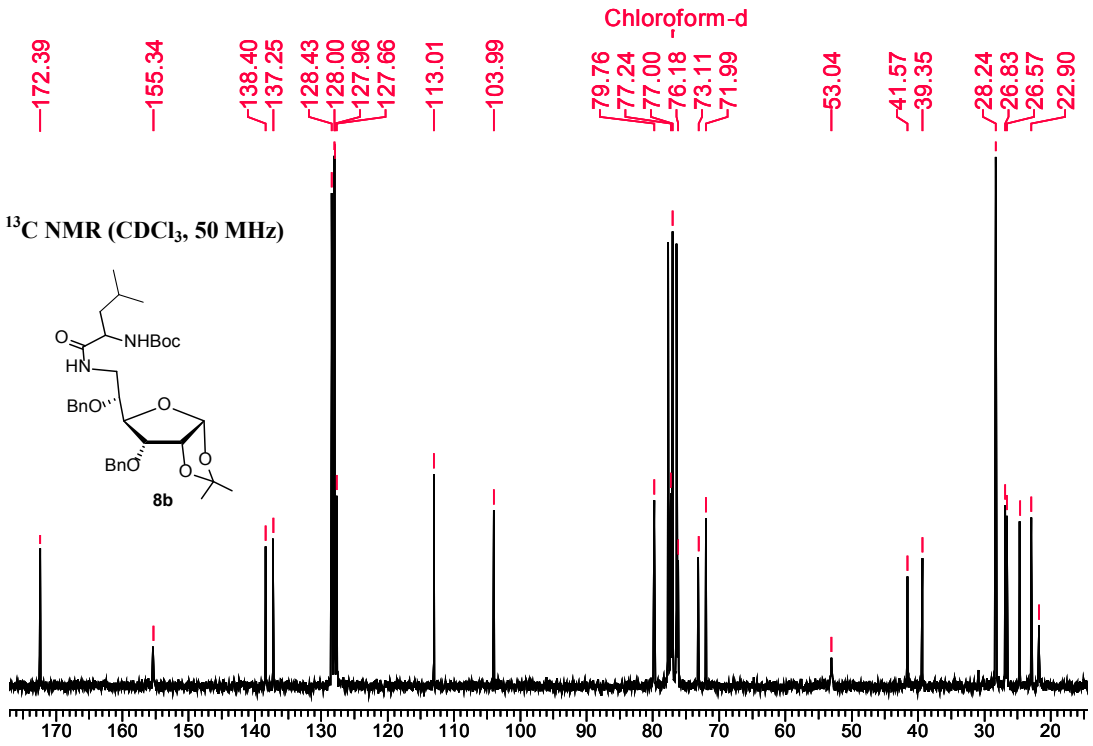


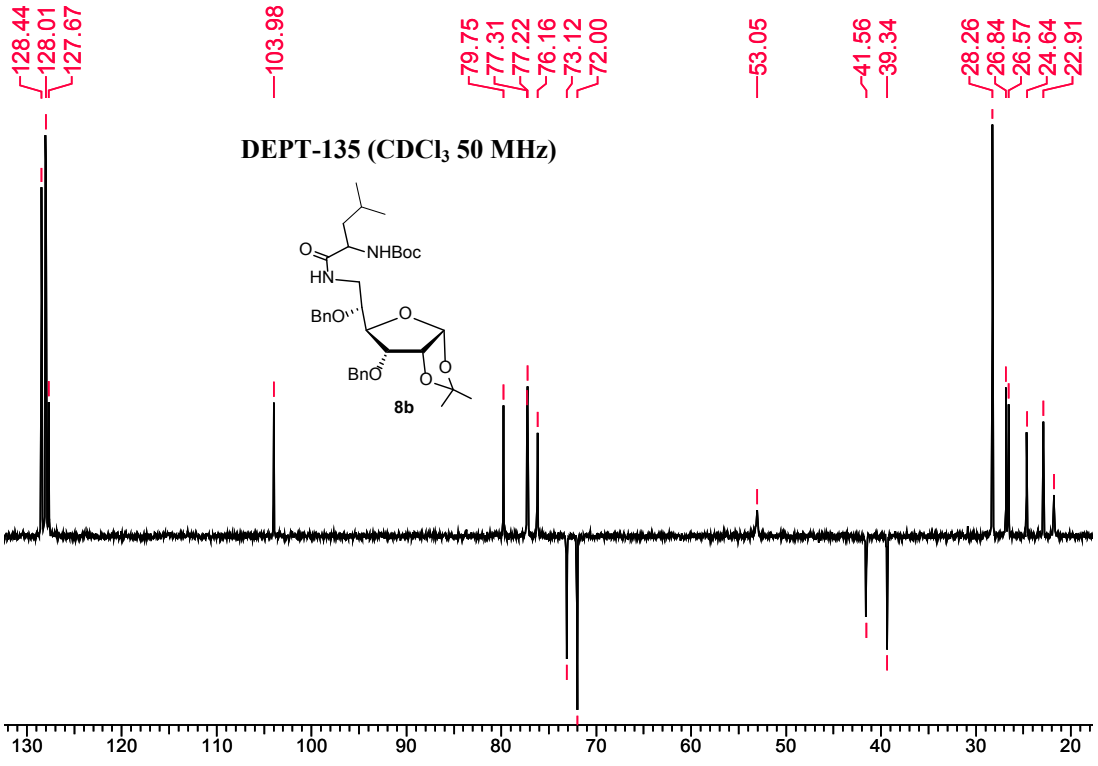
DEPT-135 ($CDCl_3$ 125 MHz)

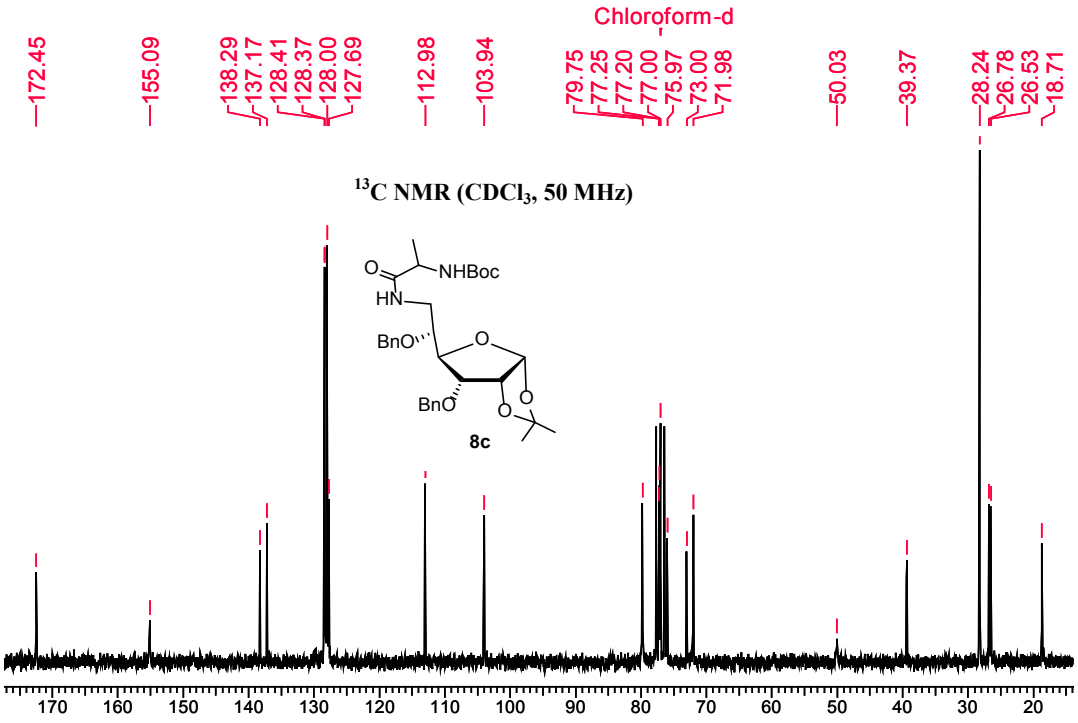
7

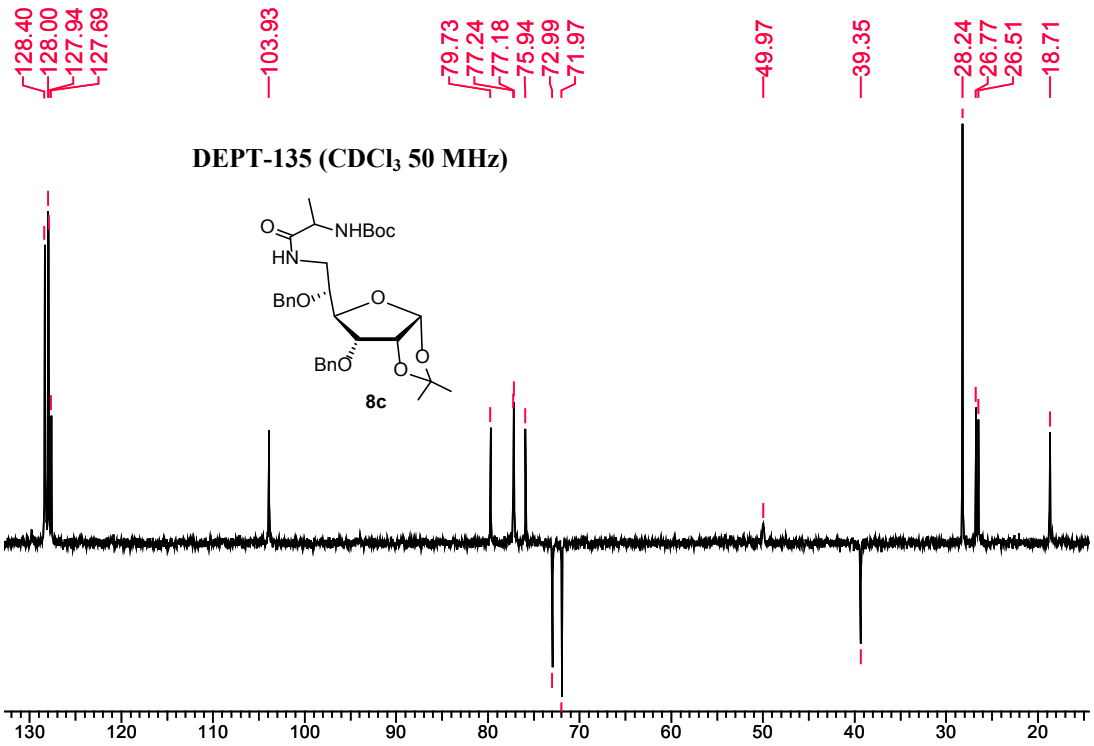

Chloroform-d
171.30
155.58
138.21
137.14
128.39
127.97
127.84
127.68
112.95
103.91
79.71
77.23
77.00
75.92
72.92
71.90
59.69
39.20
30.77
28.21
26.75
26.51
19.09
17.34
13C NMR (CDCl3, 50 MHz)
NHBoc
HN
BnO
BnO
O
O
O
8a
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

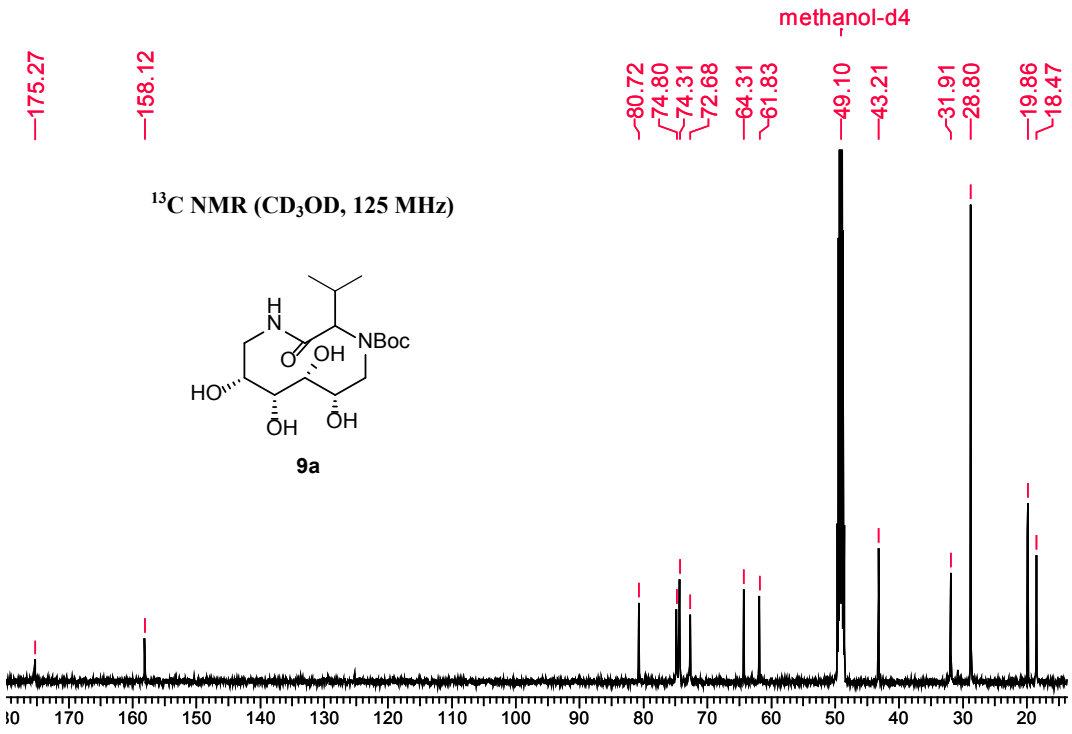

128.39
127.96
127.83
127.68
103.89
79.71
77.22
77.10
75.92
72.92
71.91
59.70
39.20
30.77
28.20
26.75
26.51
19.09
17.34
DEPT-135 (CDCl3 50 MHz)
NHBoc
HN
BnO
BnO
O
O
O
8a
130
120
110
100
90
80
70
60
50
40
30
20


Chloroform-d
172.39
155.34
138.40
137.25
128.43
128.00
127.96
127.66
113.01
103.99
79.76
77.24
77.00
76.18
73.11
71.99
53.04
41.57
39.35
28.24
26.83
26.57
22.90
13C NMR (CDCl3, 50 MHz)
NHBoc
HN
BnO
BnO
O
8b
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20


128.44
128.01
127.67
103.98
79.75
77.31
77.22
76.16
73.12
72.00
53.05
41.56
39.34
28.26
26.84
26.57
24.64
22.91
DEPT-135 (CDCl3 50 MHz)
NHBoc
HN
BnO
BnO
O
8b
130 120 110 100 90 80 70 60 50 40 30 20

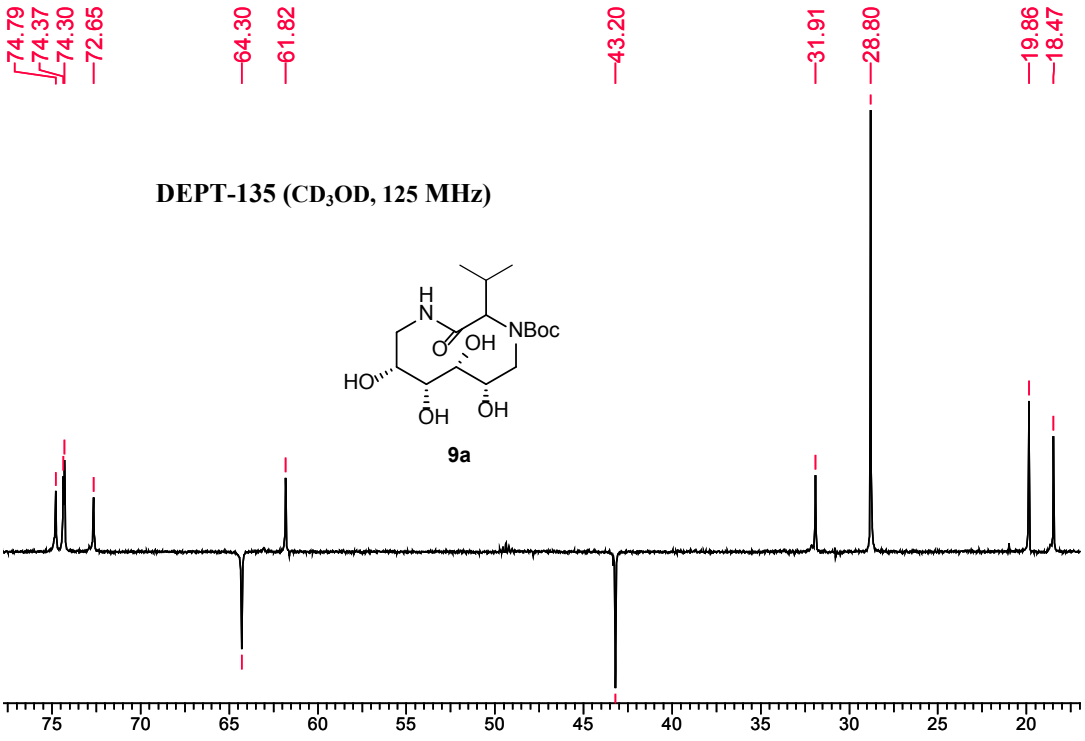

Chloroform-d
172.45
155.09
138.29
137.17
128.41
128.37
128.00
127.69
112.98
103.94
79.75
77.25
77.20
77.00
75.97
73.00
71.98
50.03
39.37
28.24
26.78
26.53
18.71
13C NMR (CDCl3, 50 MHz)
NHBoc
HN
BnO
BnO
O
O
O
8c
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20


128.40
128.00
127.94
127.69
103.93
79.73
77.24
77.18
75.94
72.99
71.97
49.97
39.35
28.24
26.77
26.51
18.71
DEPT-135 (CDCl3 50 MHz)
NHBoc
HN
BnO
BnO
O
O
O
8c
130 120 110 100 90 80 70 60 50 40 30 20



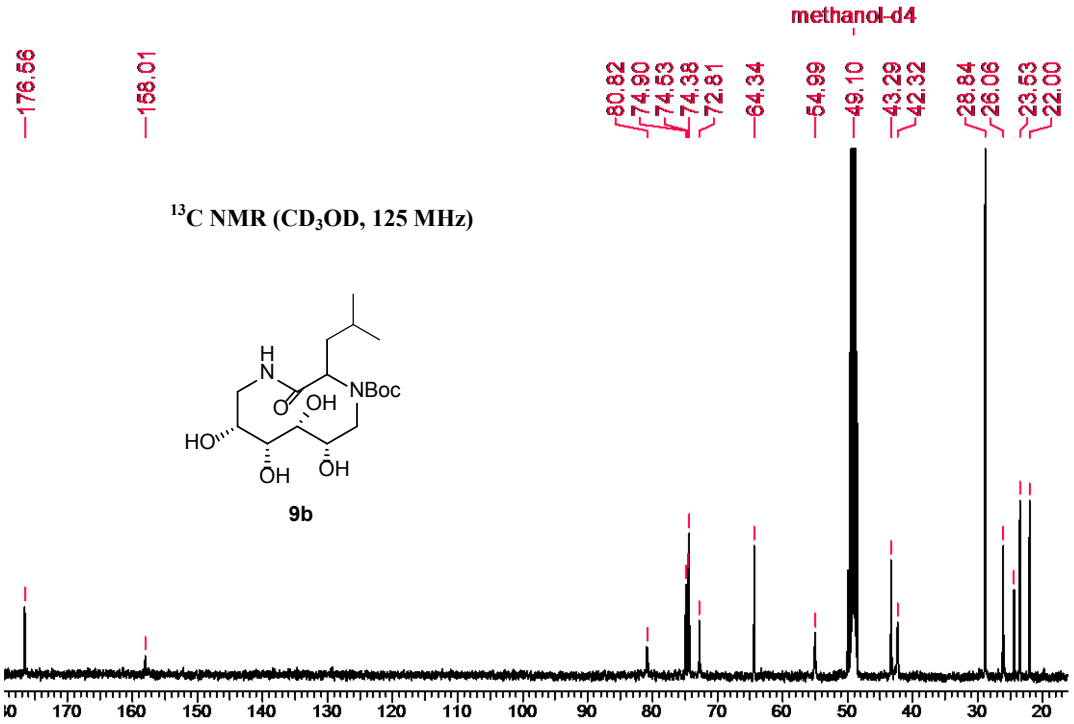
^{13}C NMR (CD_3OD, 125 MHz)

9a



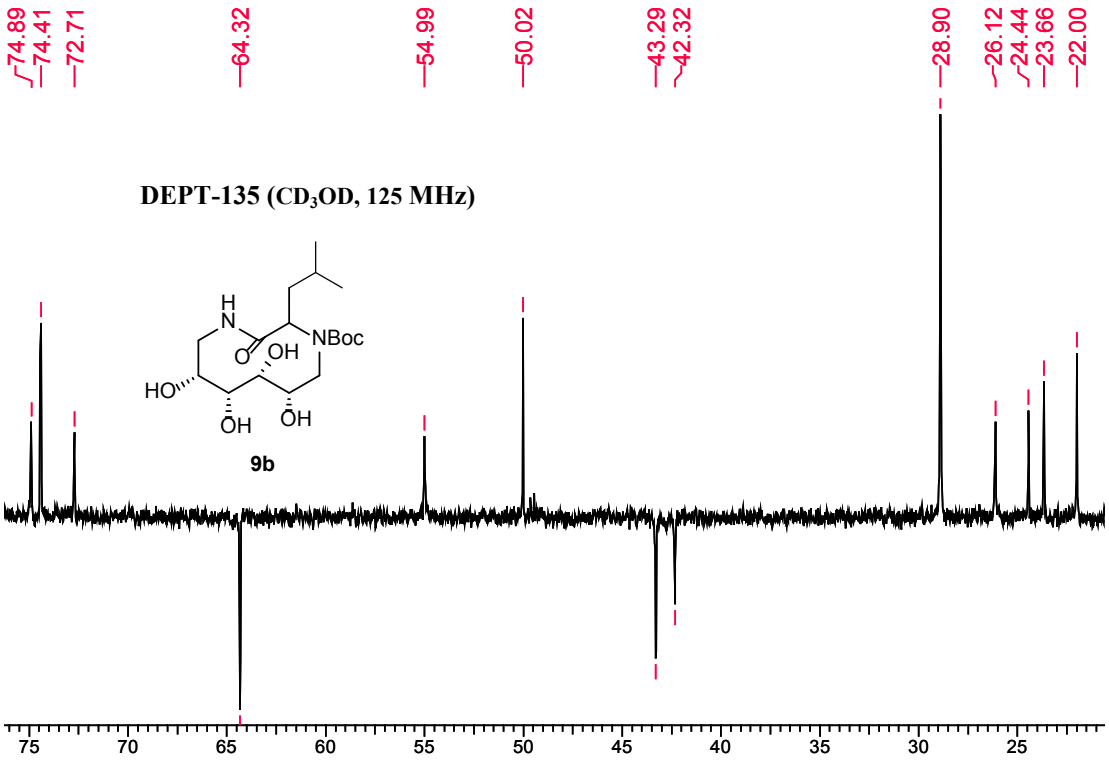
DEPT-135 (CD_3OD, 125 MHz)

9a



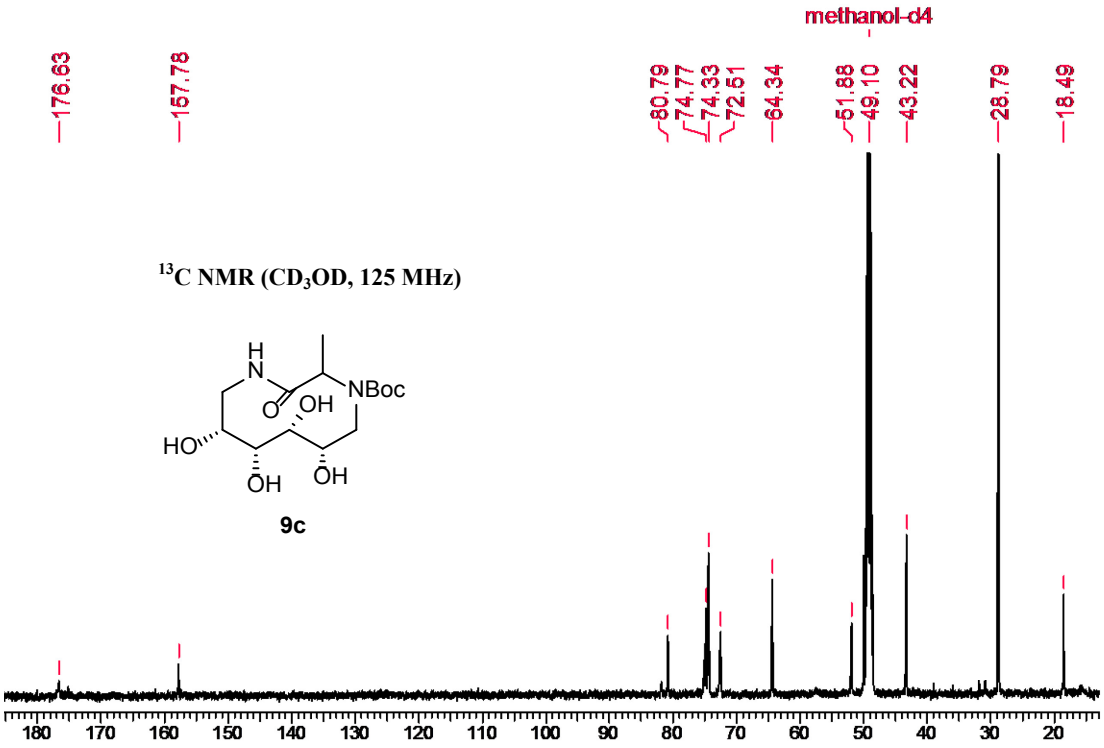
^{13}C NMR (CD_3OD, 125 MHz)

9b



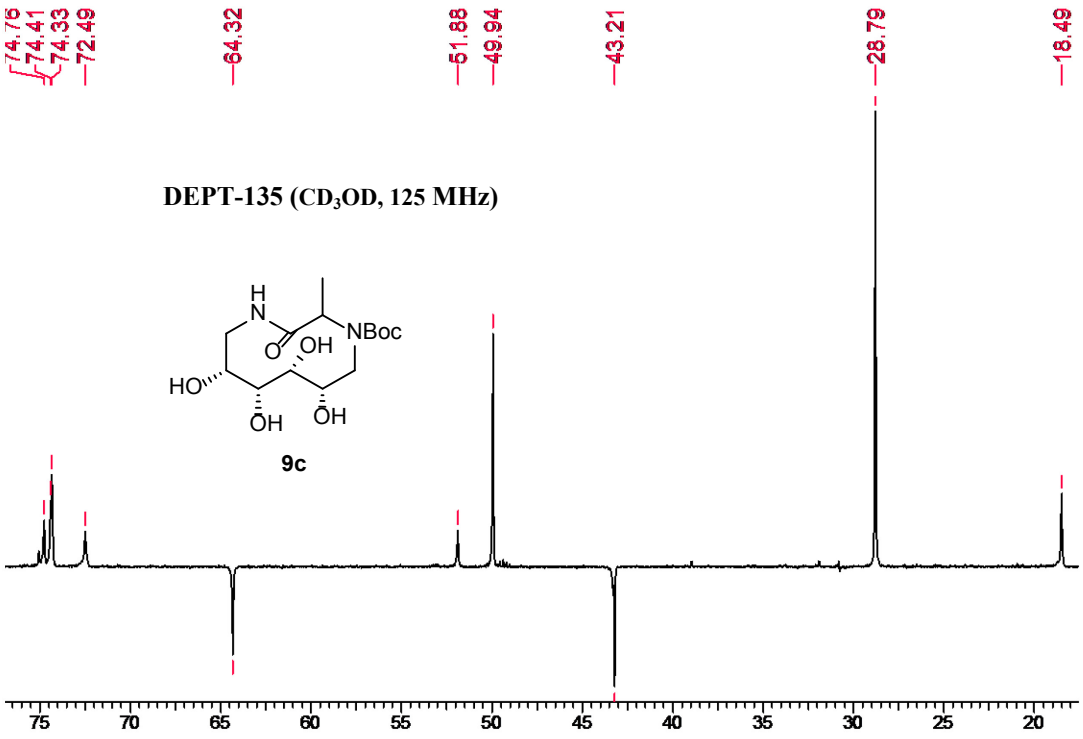
DEPT-135 (CD_3OD, 125 MHz)

9b



^{13}C NMR (CD_3OD, 125 MHz)

9c



DEPT-135 (CD_3OD, 125 MHz)

9c

1.14 Experimental Section (Part B)

di-*tert*-butyl (Z)-((1-((2-methoxy-5-(3,4,5-trimethoxystyryl)phenyl)amino)-1-oxo-3-(tritylthio)propan-2-yl)carbamoyl)glutamate 38a:

A solution of combretastatin analog **15** (0.33 g, 1.04 mmol) and Fmoc-Cys(Trt)-OH (0.68 g, 1.15 mmol) in DMF (8 mL) was cooled to 0 °C and EDC.HCl (0.26 g, 1.25 mmol) was added it followed by HOBt (0.21 g, 1.57 mmol). The resulting mixture was allowed to stir at the same temperature for 10 min. followed by 8 h at room temperature. The reaction mixture was then taken into ethyl acetate and repeatedly washed with sat. brine solution to remove DMF, followed by aq. $NaHCO_3$ and $KHSO_4$, dried over anhy. Na_2SO_4, concentrated under reduced pressure and was purified by column chromatography. The coupled product was carried forward without further characterization. Fmoc protecting group was removed when diethylamine (1.5 mL) was added to a solution of **36a** (0.5 g, 0.56 mmol) in DCM (8 mL) and the resulting mixture was stirred for 4 h at room temperature, followed by evaporation of the volatiles under reduced pressure to get the crude free amine. A solution of free amine **37a** (0.3 g, 0.45 mmol) in DCM was then added to the preformed isocyanate **39** (0.15 g, 0.54 mmol) (*vide infra*) at 0 °C, followed by DIPEA (0.31 mL, 1.8 mmol) and was allowed to stir at room temperature for 12 h. The reaction mixture was then taken into DCM, sequentially washed with aq. $NaHCO_3$ and $KHSO_4$, dried over anhy. Na_2SO_4, concentrated under reduced pressure and was finally purified by column chromatography to furnish a colourless viscous liquid. Yield (over three steps) (57%); $[\alpha]^{25}_D$: -16° (*c* = 0.5, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3019, 2400, 1720, 1523, 1427, 1215, 1045, 877, 759, 669; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (S, 1H), 8.32 (bs, 1H), 7.48-7.46 (m, 6H), 7.33-7.30 (m, 6H), 7.27 (S, 1H), 7.25-7.22 (m, 3H), 6.99-6.98 (m,

1H), 6.67-6.66 (m, 1H), 6.51 (s, 2H), 6.50-6.47 (d, 1H, *J*= 12.21 Hz), 6.43-6.41 (d, 1H, *J*= 12.21 Hz), 4.97-4.95 (m, 1H), 4.62-4.61 (m, 1H), 4.32-4.28 (m, 1H), 3.83 (s, 3H), 3.79 (S, 3H), 3.68 (S, 3H), 2.96-2.92 (m, 1H), 2.62-2.59 (m, 1H), 2.33-2.18 (m, 2H), 2.09-2.03 (m, 1H), 1.92-1.84 (m, 1H), 1.49 (S, 6H), 1.40 (S, 6H), 1.26 (S, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 171.6, 168.8, 156.4, 152.8, 147.2, 144.5, 137.0, 132.8, 129.9, 129.6, 128.0, 127.1, 126.9, 124.4, 120.8, 109.5, 105.9, 82.2, 80.6, 60.8, 55.9, 53.4., 52.9, 33.6, 31.3, 29.6, 28.4, 28.0; LC-MS: 968.56 $(M+Na)^+$; Anal. Calcd. for $C_{54}H_{63}N_3O_{10}S$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.70; H, 6.98; N, 4.20.

di-*tert*-butyl (Z)-((1-((2-methoxy-5-(3,4,5-trimethoxystyryl)phenyl)amino)-1-oxo-3-(1-trityl-1H-imidazol-2-yl)propan-2-yl)carbamoyl)glutamate 38b:

The product 3**8b** was obtained as a colourless viscous liquid. Yield (over three steps) (55%); $[\alpha]^{25}_D$: -11.36° (*c* = 0.88, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3020, 2400, 1715, 1524, 1215, 1130, 928, 754, 669; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.52 (S, 1H), 8.34 (bs, 1H), 7.40 (S, 1H), 7.28-7.26 (m, 3H), 7.24-7.21 (m, 3H), 7.03-7.02 (m, 6H), 6.50-6.48 (m, 6H), 6.44-6.42 (d, 1H, *J*= 12.21 Hz), 5.78 (bs, 1H), 4.65-4.60 (m, 1H), 4.41-4.40 (m, 1H), 3.83 (S, 3H), 3.77 (S, 3H), 3.66 (S, 3H), 3.17-3.14 (m, 1H), 3.03-3.00 (m, 1H), 2.36-2.31 (m, 2H), 2.17-2.10 (m, 1H), 1.93-1.88 (m, 1H), 1.43 (S, 6H), 1.41 (S, 6H), 1.39 (S, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 171.6, 170.0, 157.5, 152.8, 147.2, 141.9, 138.1, 137.0, 132.9, 129.8, 129.6, 128.7, 128.0, 127.3, 124.1, 120.6, 120.3, 109.4, 105.9, 92.0, 81.7, 80.4, 60.8, 55.9, 52.8, 36.6, 31.6, 28.4, 28.0, 27.9; LC-MS: 980.63 $(M+H)^+$, 968.56 $(M+Na)^+$; Anal. Calcd. For $C_{57}H_{65}N_5O_{10}$: C, 69.85; H, 6.68; N, 7.15. Found: C, 69.52; H, 6.95; N, 7.22.

di-*tert*-butyl-(Z)-((4-(tert-butoxy)-1-((2-methoxy-5-(3,4,5-trimethoxystyryl) phenyl)amino)-1,4-dioxobutan-2-yl)carbamoyl)glutamate 38c:

Compound 3**8c** was obtained as a colourless viscous liquid Yield (over three steps) 1.35 g (52%); $[\alpha]^{25}_{D}$: -4.68° (*c* = 1.28, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3019, 2403, 1722, 1583, 1523, 1427, 1215, 1045, 928, 1217, 758, 699; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90 (S, 1H), 8.27 (S, 1H), 7.27 (S, 1H), 6.97-6.95 (d, 1H, *J*= 8.56 Hz), 6.66-6.64 (d, 1H, *J*= 8.56 Hz), 6.48 (S, 2H), 6.38-6.35 (d, 1H, *J*= 12.22 Hz), 6.10-6.08 (d, 1H, *J*= 8.31 Hz), 5.58-5.56 (d, 1H, *J*= 8.07 Hz), 4.80-4.77 (m, 1H), 4.40-4.39 (m, 1H), 3.80 (m, S, 6H), 3.65 (m, S, 6H), 2.90-2.85 (m, 1H), 2.67-2.61 (m, 1H), 2.34-2.26 (m, 2H), 2.12-2.05 (m, 1H), 1.91-1.84 (m, 1H), 1.44 (S, 9H), 1.38 (S, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.1, 171.9, 171.3, 169.3, 156.7, 152.6, 147.2, 136.7, 132.7, 129.6, 128.7, 127.2, 124.1, 120.6, 109.3, 105.8, 82.1, 81.4, 80.4, 77.2, 60.7, 55.7, 52.8, 51.0, 39.9, 36.5, 31.3, 28.2, 27.8; LC-MS: 794.47 $(M+Na)^+$; Anal. Calcd. For $C_{40}H_{57}N_3O_{12}$: C, 62.24; H, 7.44; N, 5.44. Found: C, 62.03; H, 7.63; N, 5.21.

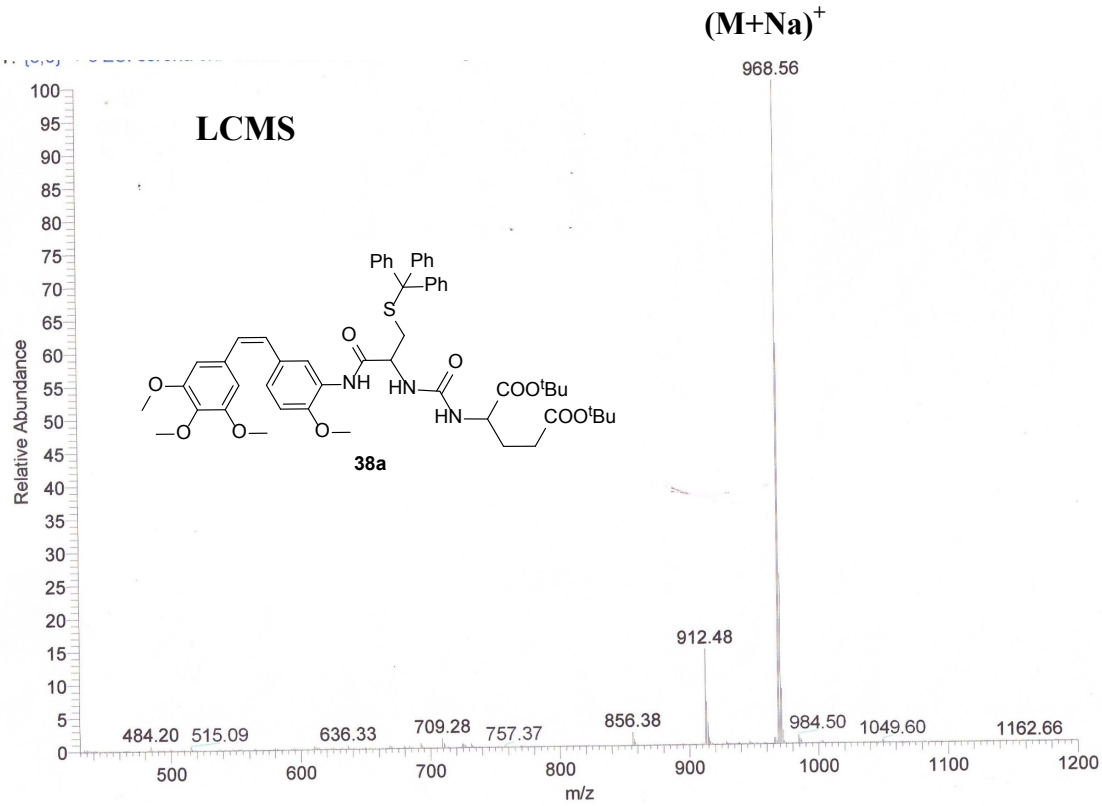
di-*tert*-butyl 2-isocyanatopentanedioate 39:

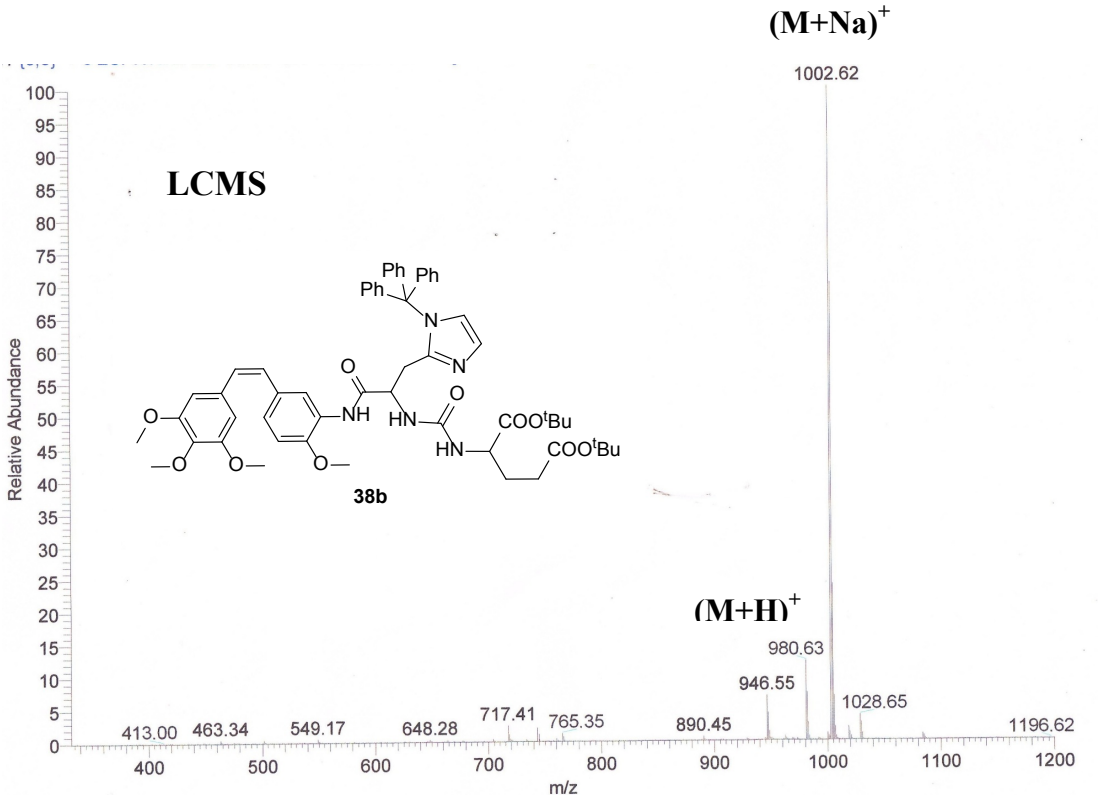
A mixture of hydrochloride salt of di-*tert*-butyl glutamate (0.21 g, 0.71 mmol) and DIPEA (0.48 mL, 2.8 mmol) in DCM at was added drop-wise to a solution triphosgene (0.07 g, 0.24 mmol) in DCM at -78 °C. It was allowed to come to room temperature and was stirred for 1 h. The volatiles were stripped off under inert atmosphere and the crude isocyanate formed was used for further reactions.

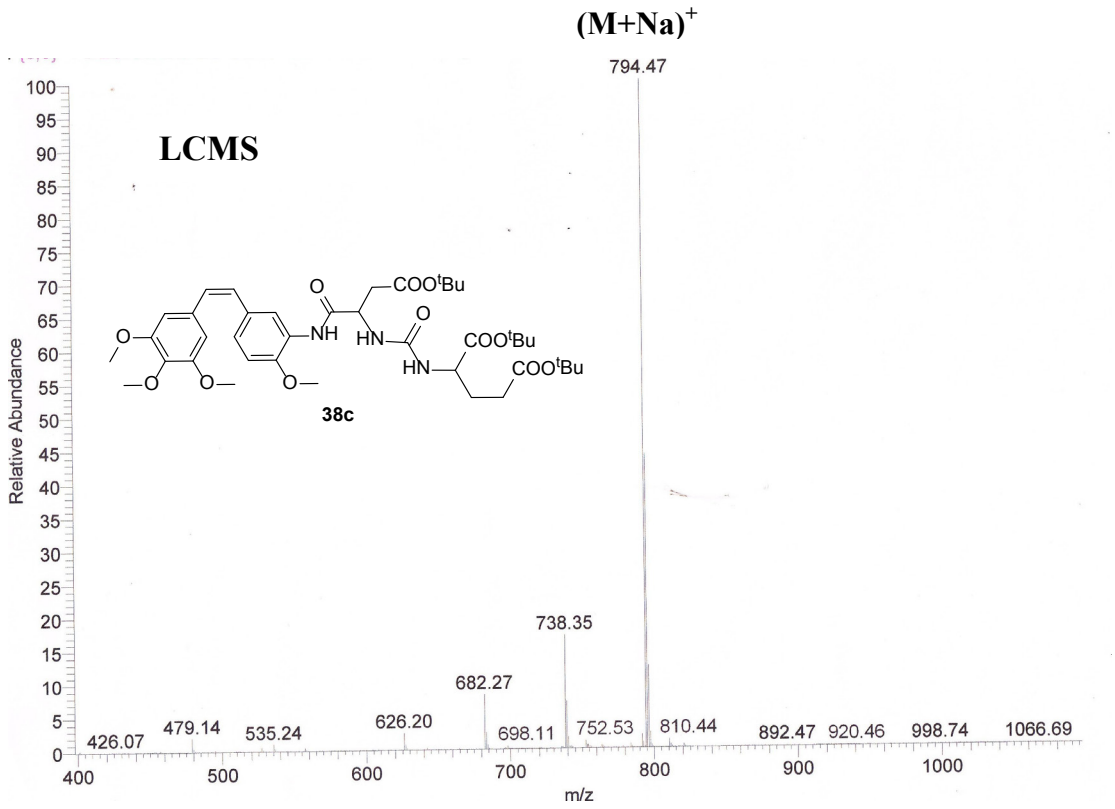
(*Z*)-2-(4-(2,6-dioxo-1,3-dipropyl-2,3,6,9-tetrahydro-1*H*-purin-8-yl)phenoxy)-*N*-(2-methoxy-5-(3,4,5-trimethoxystyryl)phenyl)acetamide 44:

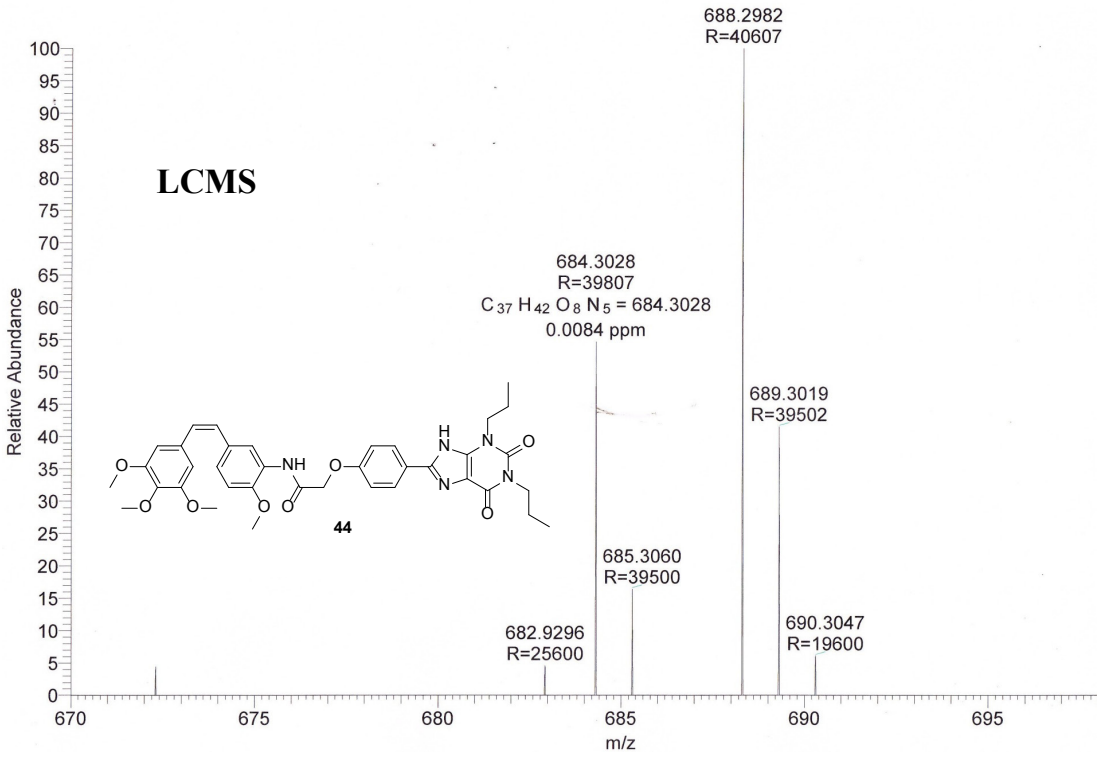
A solution of acid **43** (0.15 g, 0.36 mmol) and combretastatin analog **15** (0.13 g, 0.4 mmol) in DMF (5 mL) was cooled to 0 °C and EDC.HCl (0.097 g, 0.47 mmol) was added to it followed by HOBt (0.07 g, 0.54 mmol). The resulting mixture was

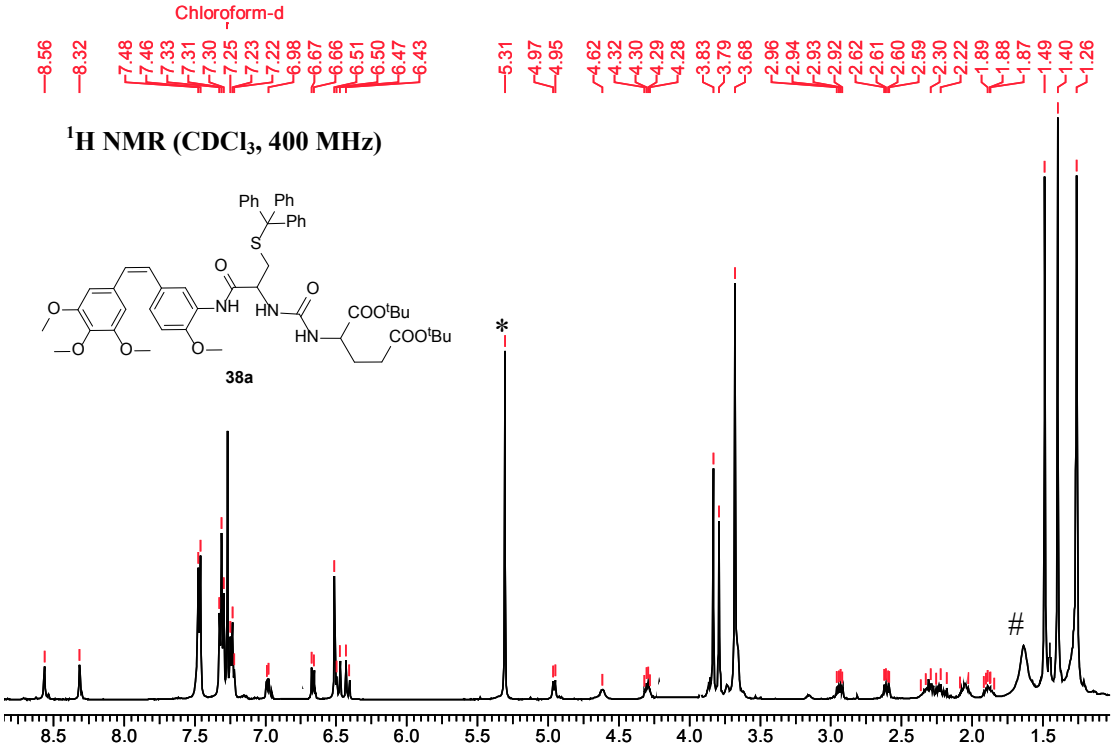
allowed to stir at the same temperature for 10 min. followed by 8 h at room temperature. The reaction mixture when taken into ethyl acetate formed a white precipitate which was repeatedly washed with sat. brine solution followed by aq. $NaHCO_3$ and $KHSO_4$ and filtered under suction. The residue thus collected was again repeatedly washed with ethyl acetate to obtain the pure product as an off-white solid. Yield: 0.21 g (80%), mp: 215-217 °C, IR (Nujol) ν (cm^{-1}): 3367, 3169, 2919, 2725, 1709, 1456, 1377, 1169, 972, 846, 722; ^1H NMR (400 MHz, $CDCl_3$): δ 9.26 (S, 1H), 8.81 (S, 1H), 8.07 (S, 1H), 7.99-7.97 (m, 2H), 7.09-7.05 (m, 3H), 6.71 (S, 2H), 6.58 (S, 2H), 6.54-6.45 (m, 1H), 4.63 (S, 2H), 3.66 (bs, 5H), 3.72 (S, 3H), 3.64 (S, 3H), 3.60 (S, 5H), 1.56-1.52 (m, 4H), 0.90-0.84 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1, 159.9, 159.3, 152.7, 151.9, 150.9, 148.6, 136.9, 132.2, 130.0, 129.3, 129.2, 127.9, 126.4, 125.4, 121.5, 114.1, 111.1, 106.1, 87.6, 67.2, 60.2, 56.2, 55.7, 43.9, 42.0, 21.0, 11.4, 10.9; HRMS: $C_{37}H_{41}N_5O_8$ Calcd. 683.2955, Found: 684.3028 $(M+H)^+$.

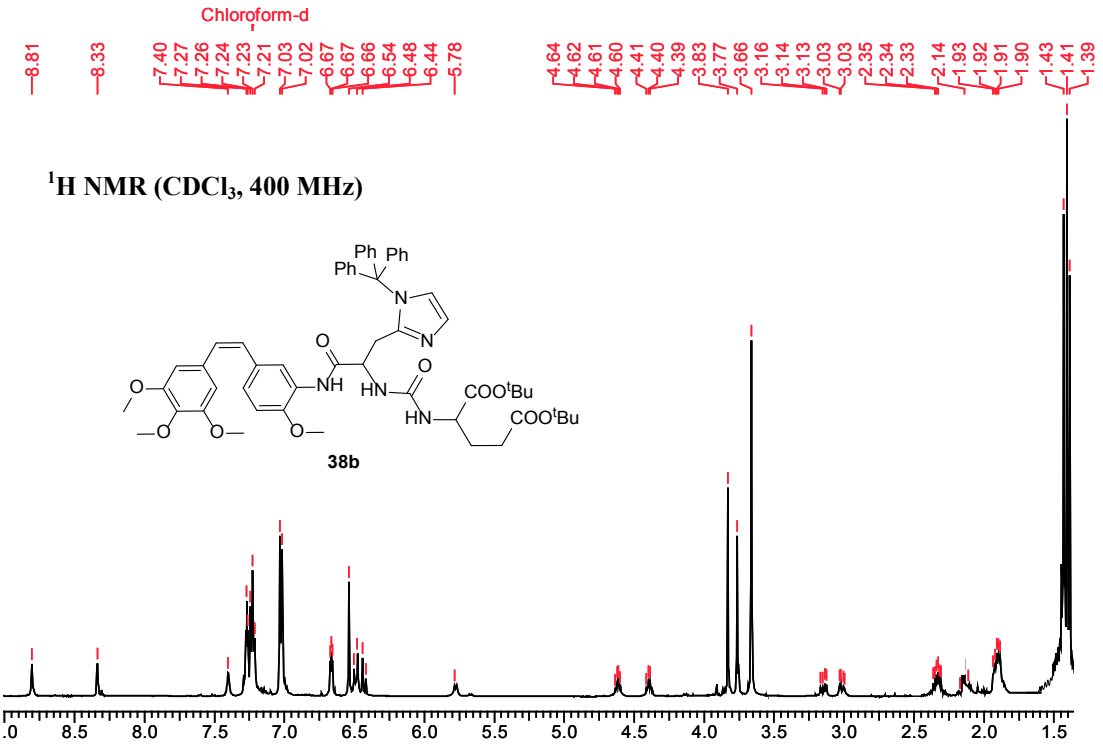

(M+Na)+
LCMS
968.56
912.48
484.20
515.09
636.33
709.28
757.37
856.38
984.50
1049.60
1162.66
Relative Abundance
m/z
38a


(M+Na)+
1002.62
LCMS
(M+H)+
980.63
946.55
1028.65
413.00
463.34
549.17
648.28
717.41
765.35
890.45
1196.62
Relative Abundance
m/z
38b

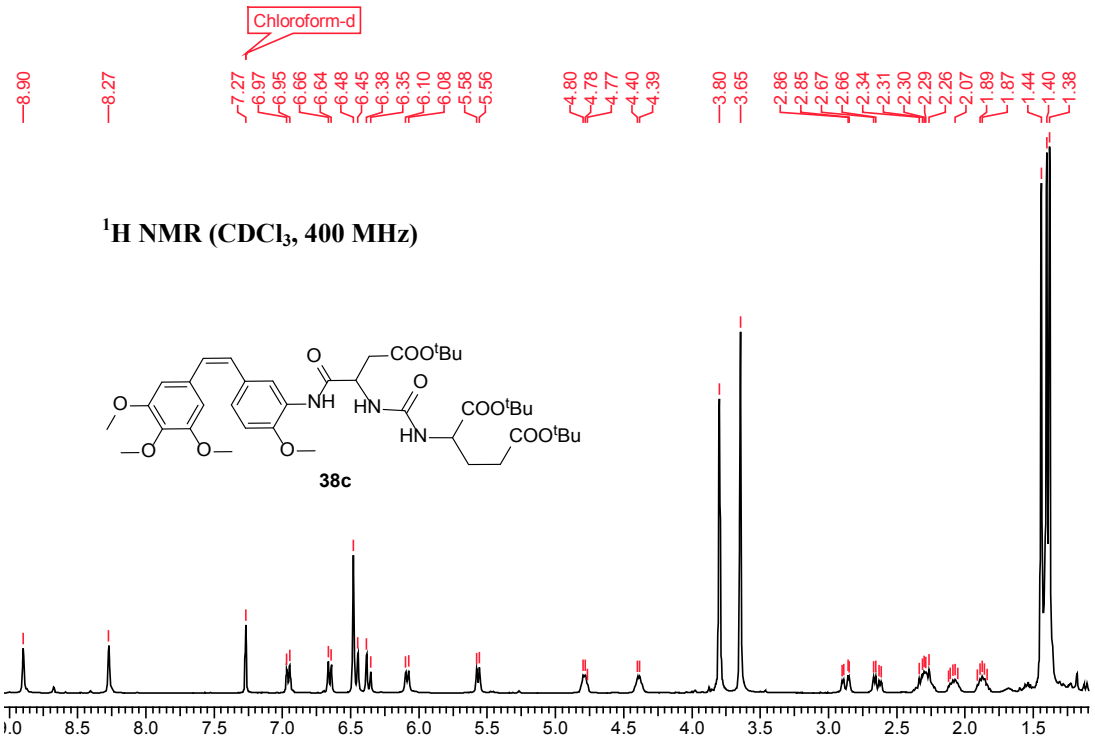

(M+Na)+
LCMS
794.47
738.35
682.27
426.07
479.14
535.24
626.20
698.11
752.53
810.44
892.47
920.46
998.74
1066.69
Relative Abundance
m/z
38c

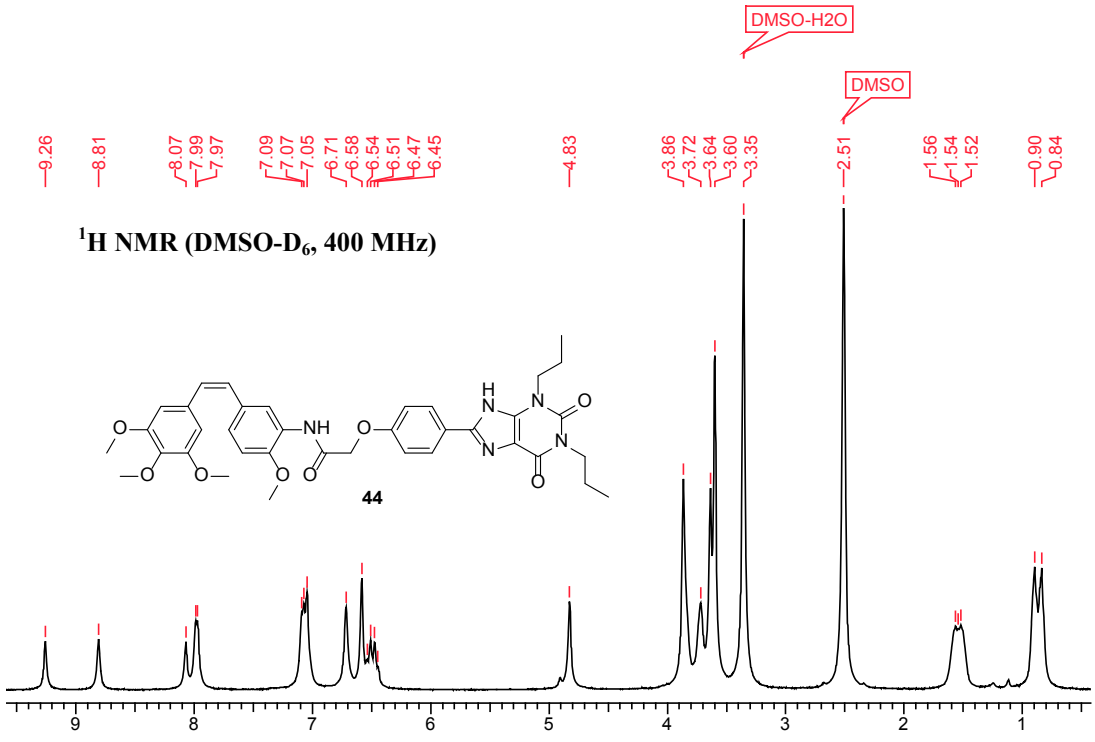

LCMS
688.2982
R=40607
684.3028
R=39807
C37 H42 O8 N5 = 684.3028
0.0084 ppm
689.3019
R=39502
685.3060
R=39500
682.9296
R=25600
690.3047
R=19600
Relative Abundance
m/z
44

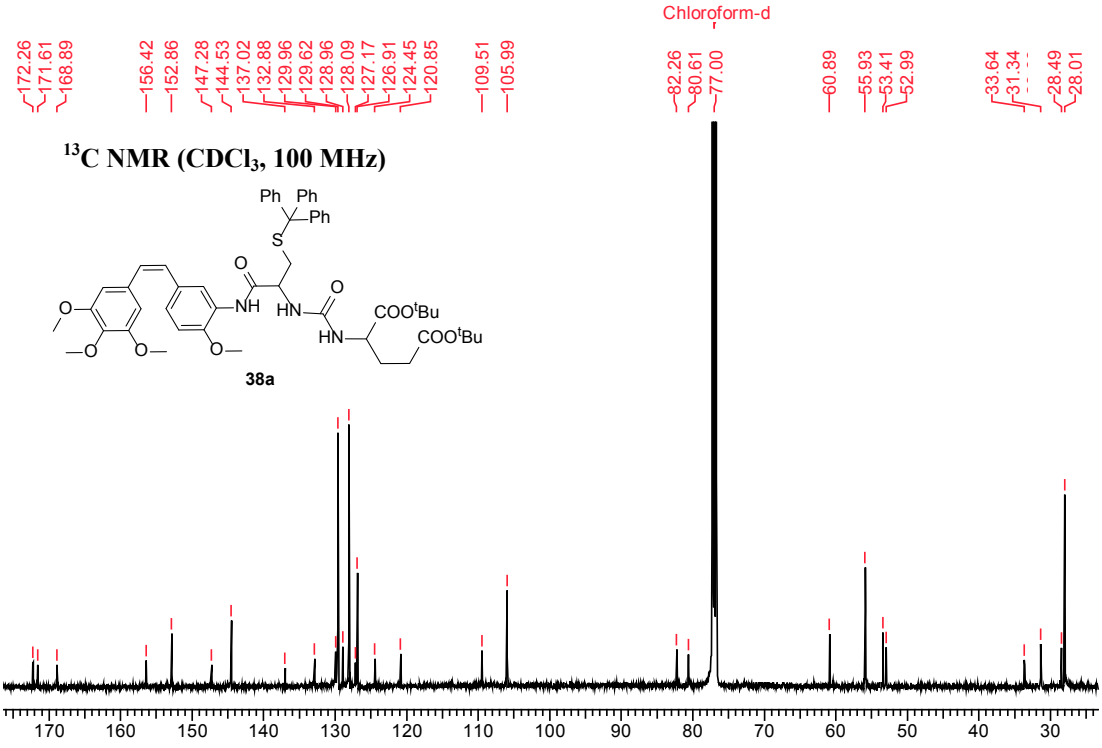


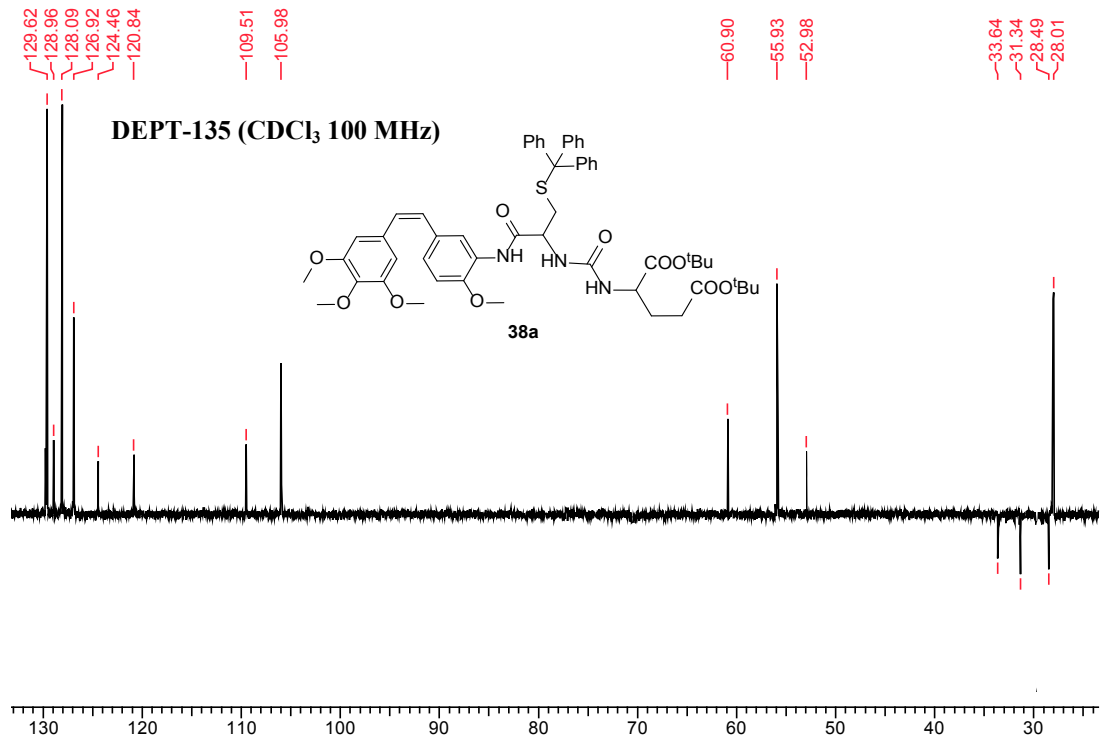


* DCM, # $CDCl_3$-H_2O


Chloroform-d
8.90
8.27
7.27
6.97
6.95
6.66
6.64
6.48
6.45
6.38
6.35
6.10
6.08
5.58
5.56
4.80
4.78
4.77
4.40
4.39
3.80
3.65
2.86
2.85
2.67
2.66
2.34
2.31
2.30
2.29
2.26
2.07
1.89
1.87
1.44
1.40
1.38
^{1}H NMR (CDCl$_3$, 400 MHz)
38c
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5


DMSO-H2O
DMSO
9.26
8.81
8.07
7.99
7.97
7.09
7.07
7.05
6.71
6.58
6.54
6.51
6.47
6.45
4.83
3.86
3.72
3.64
3.60
3.35
2.51
1.56
1.54
1.52
0.90
0.84
^{1}H NMR (DMSO-D$_6$, 400 MHz)
44
9
8
7
6
5
4
3
2
1


Chloroform-d
172.26
171.61
168.89
156.42
152.86
147.28
144.53
137.02
132.88
129.96
129.62
128.96
128.09
127.17
126.91
124.45
120.85
109.51
105.99
82.26
80.61
77.00
60.89
55.93
53.41
52.99
33.64
31.34
28.49
28.01
13C NMR (CDCl3, 100 MHz)
38a
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30


129.62
128.96
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120.84
109.51
105.98
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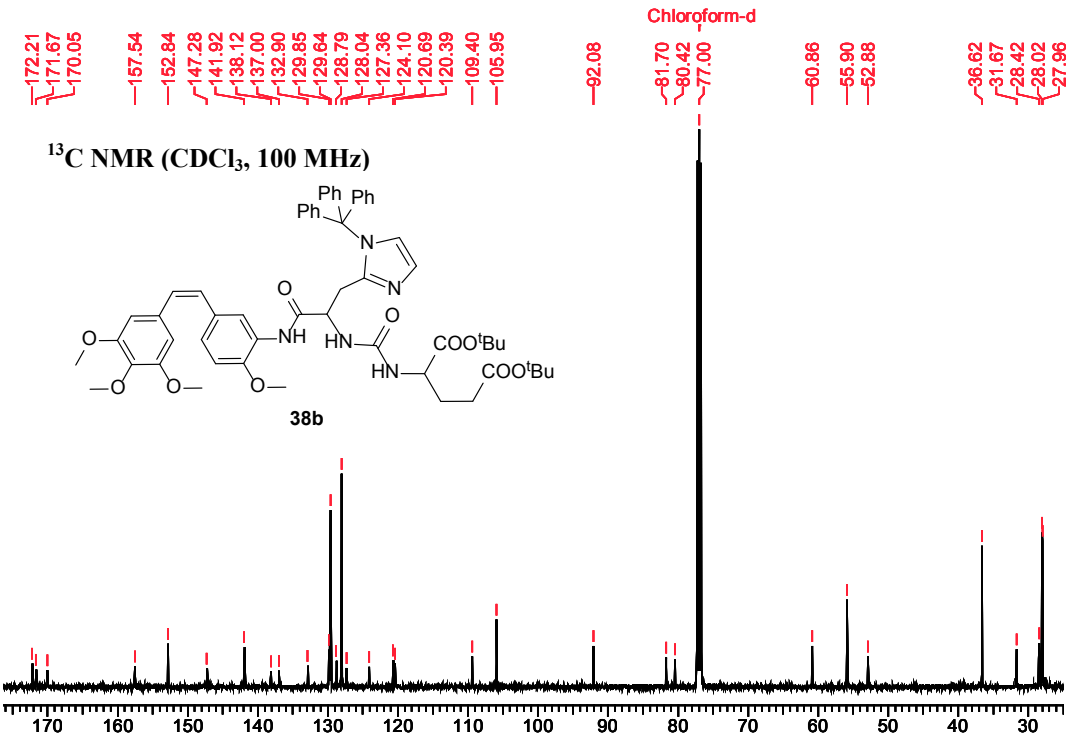
52.98
33.64
31.34
28.49
28.01
DEPT-135 (CDCl3 100 MHz)
38a
130 120 110 100 90 80 70 60 50 40 30


Chloroform-d
172.21
171.67
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157.54
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147.28
141.92
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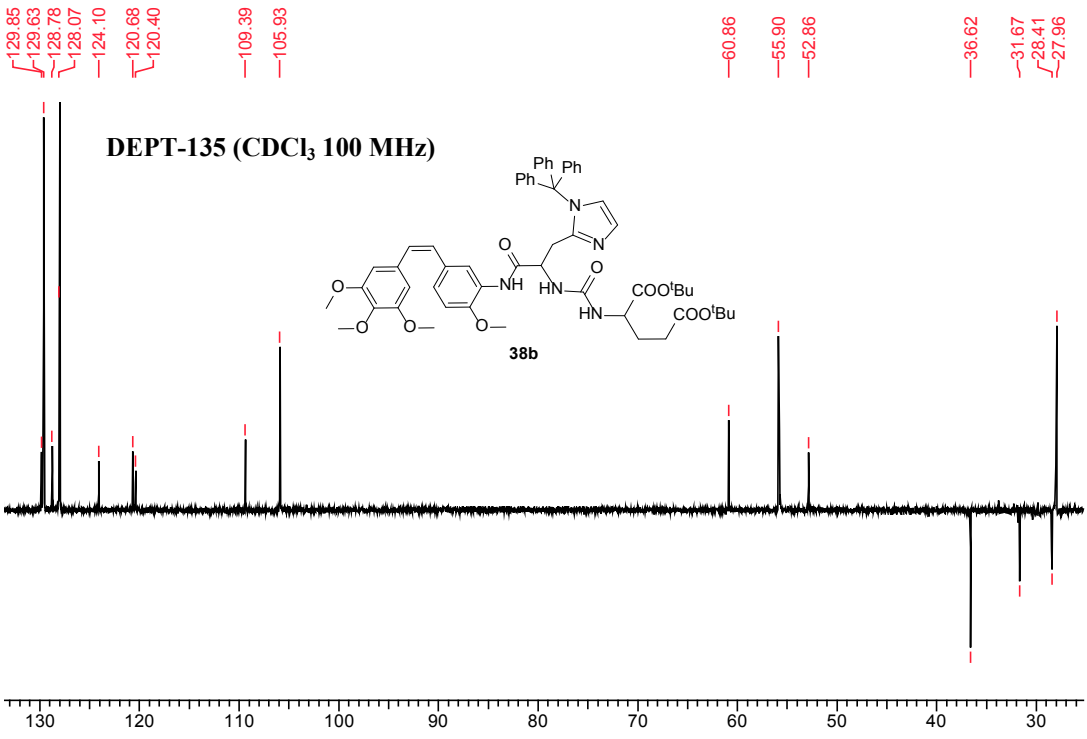
137.00
132.90
129.85
129.64
128.79
128.04
127.36
124.10
120.69
120.39
109.40
105.95
92.08
81.70
80.42
77.00
60.86
55.90
52.88
36.62
31.67
28.42
28.02
27.96
13C NMR (CDCl3, 100 MHz)
38b
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30


129.85
129.63
128.78
128.07
124.10
120.68
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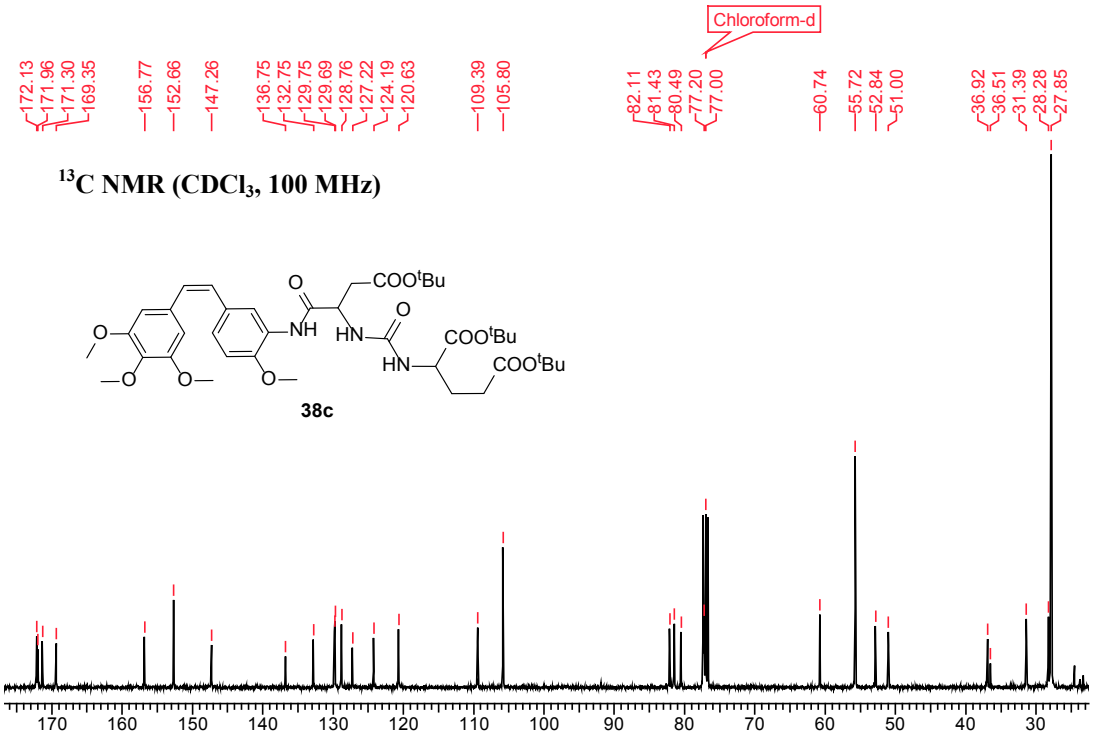
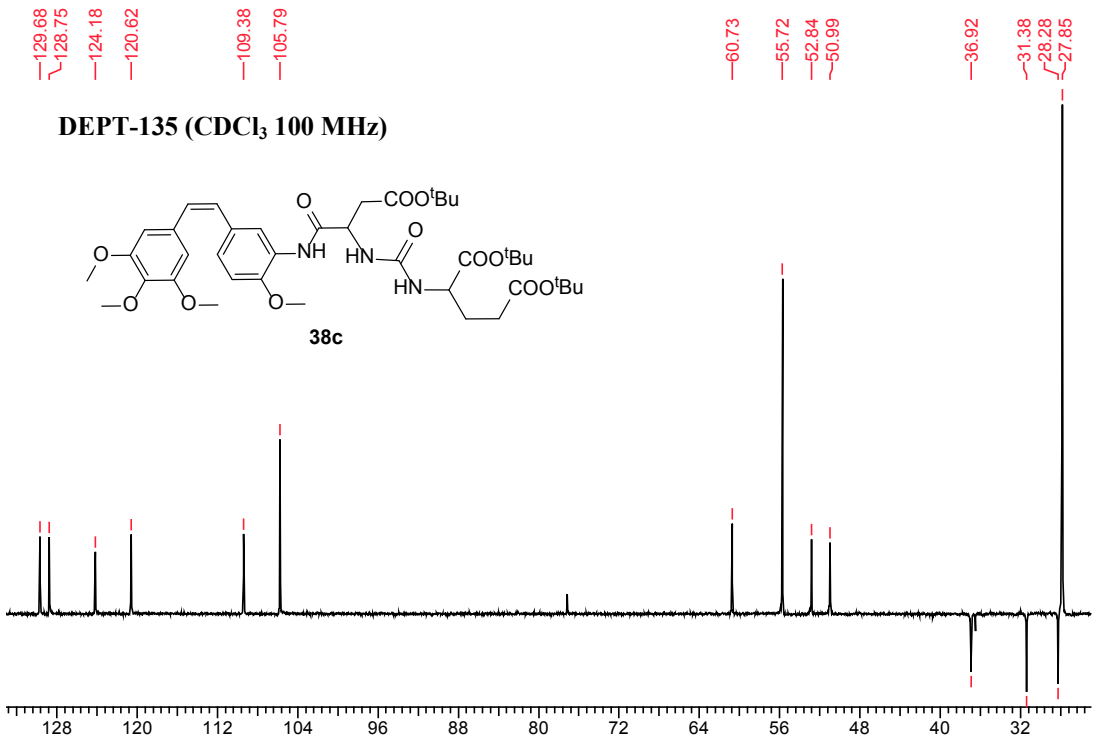
105.93
60.86
55.90
52.86
36.62
31.67
28.41
27.96
DEPT-135 (CDCl3 100 MHz)
38b
130
120
110
100
90
80
70
60
50
40
30



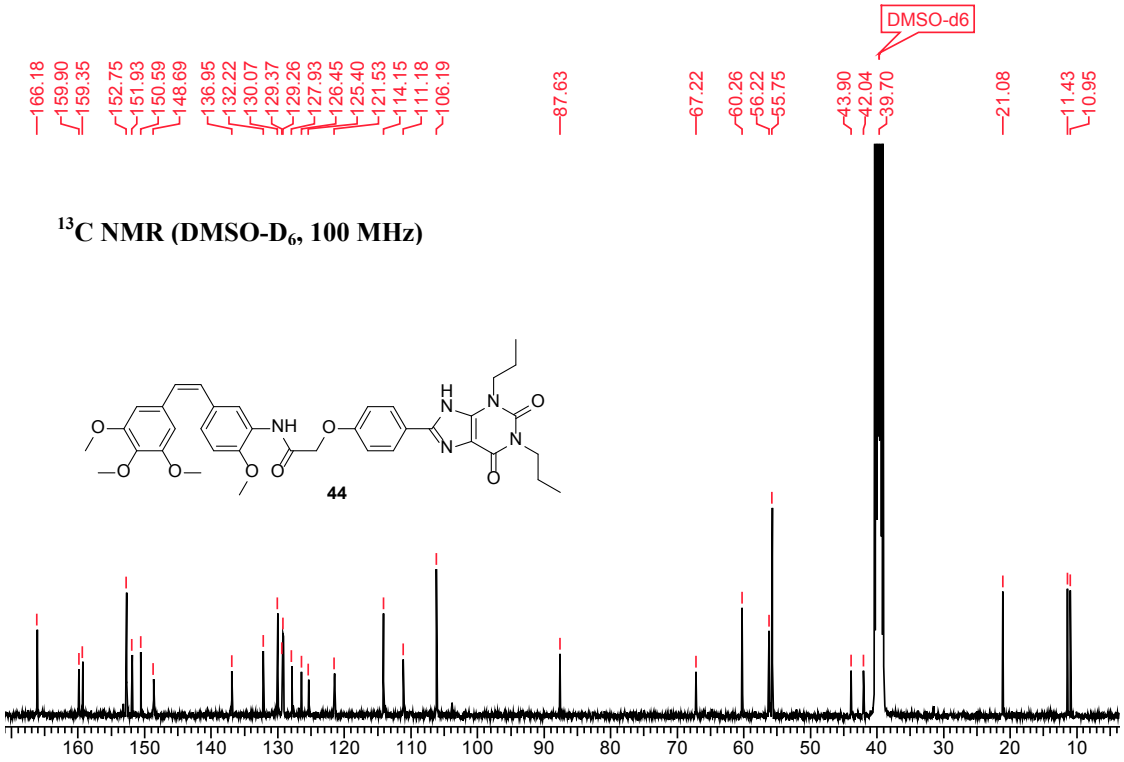
^{13}C NMR ($CDCl_3$, 100 MHz)

38c



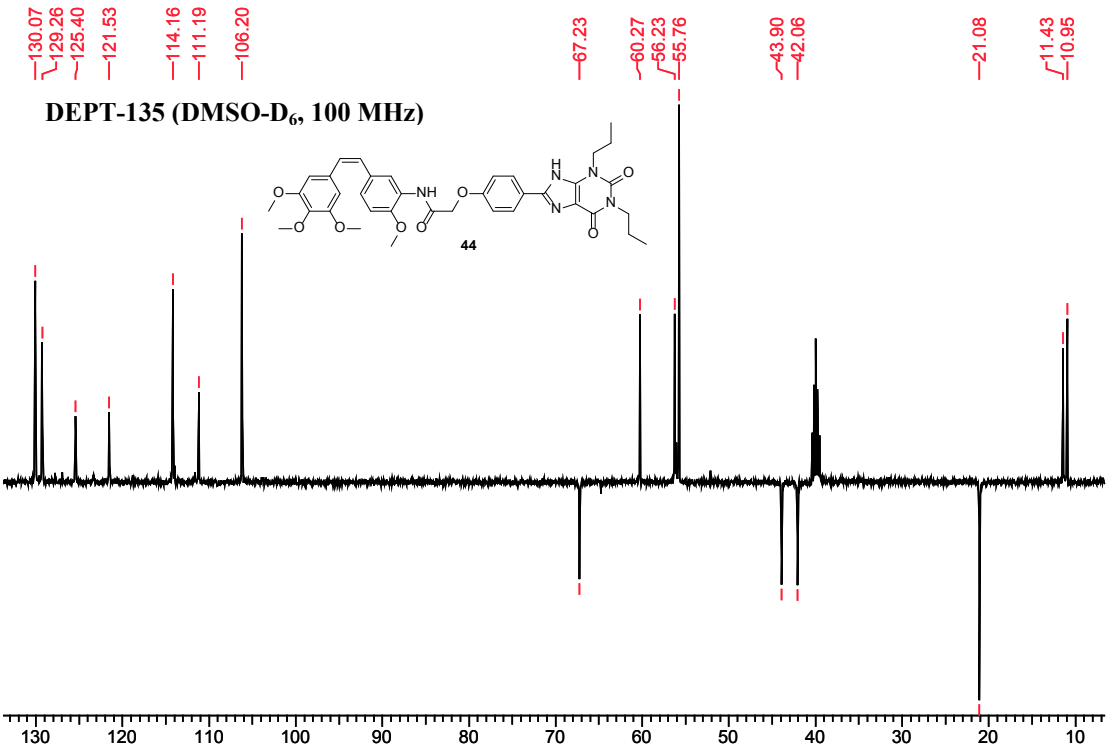
DEPT-135 ($CDCl_3$ 100 MHz)

38c



^{13}C NMR (DMSO-D$_6$, 100 MHz)

44



DEPT-135 (DMSO-D$_6$, 100 MHz)

44

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CHAPTER 2

Conformationally rigid two-dimensional abiotic foldamer building blocks

2.1 Foldamer-An overview

Folding and assembly of biomolecules are two of the most significant characteristics observed in the area of biomolecular science. The DNA-duplex formation till date symbolizes one of the most elegant and superlative examples of both self-assembly and folding in biomacromolecules.[1] Nature employs the twenty amino acid-set to generate a collection of biomolecules with diverse structures and functions. Structural and conformational analyses of biopolymers reveal that most of the biological events result from their stable compact conformation, stabilized by a set of non-covalent interactions.[2] As proposed by Linderstrøm-Lang,[3] proteins have structures at several different levels (Fig. 2.1). The primary structure of a protein consists of the order in which amino acids are linked to one another by peptide bonds. The secondary structure involves the way by which chain of amino acids twists or folds to form either α-helical, β-sheet or a variety of other possible arrangements.[4] An assembly of secondary structure is referred to as the tertiary structure, and is responsible for the bioactivity of proteins. If a protein consists of more than one chain, the shape in which those separate chains bind together by non-covalent interaction is referred to as the quaternary structure.[5] The combined shape of the secondary, tertiary and the quaternary structure, if there is one, makes up the conformation of a protein.[6]

The three-dimensional conformations of biopolymers may have information-rich surfaces, which in fact are responsible for different biological processes.[7] Biological machines play a multitude of roles in the living organisms such as molecular recognition, information storage, biocatalysis (enzymes), transmission of signals (hormones) etc. These mysterious events have always been

a fascination to chemists and biochemists, and in an attempt to mimic these bio-machineries, both structurally and functionally with man-made constructs, small molecules were designed and developed.

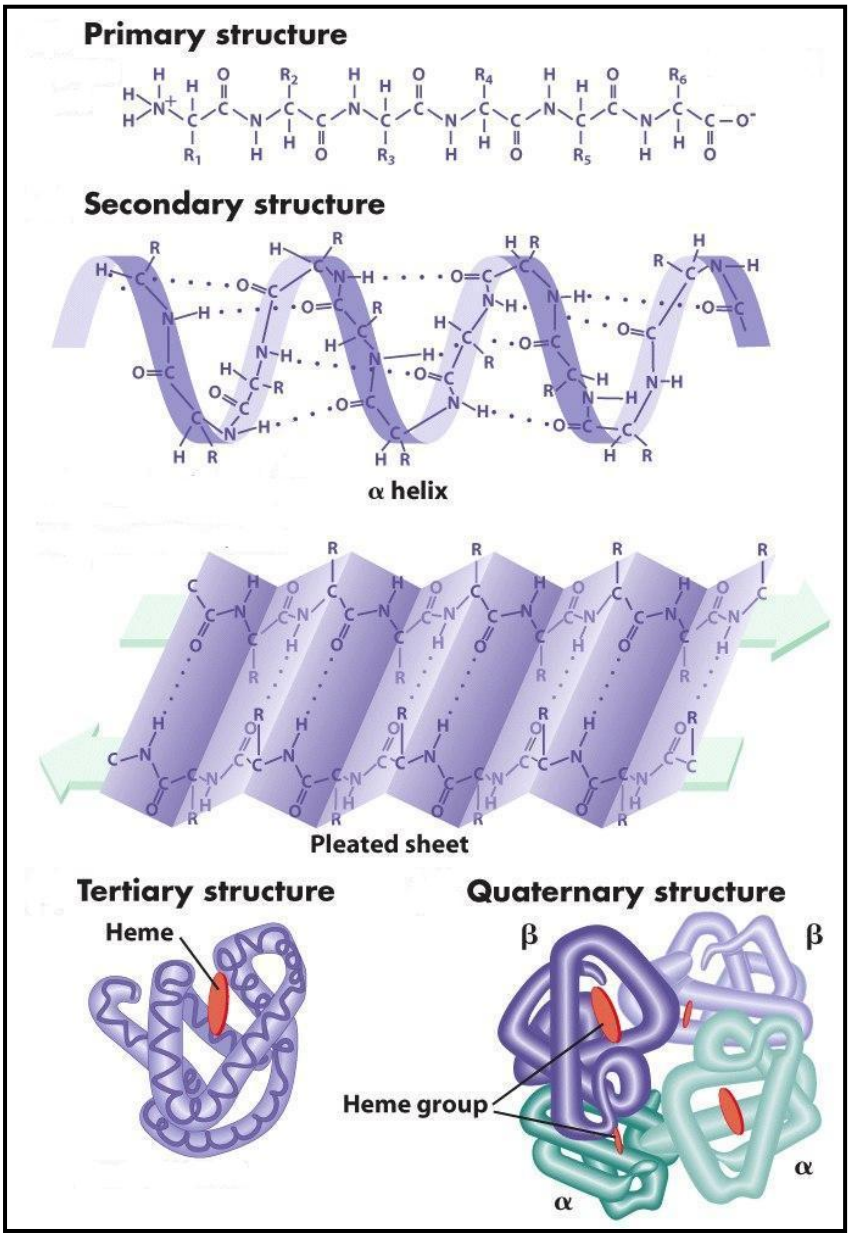


Fig. 2.1 The linear sequence of amino acids (primary structure) folds into helices or sheets (secondary structure) which pack into a globular or fibrous domain (tertiary structure). Some individual proteins self-associate into complexes (quaternary structure).

In this context, an area of research that has attained considerable attention in recent years is foldamers which are synthetic oligomers that mimic the

conformational features of biopolymer.[8] The term "foldamer" coined by Prof. Gellman describes *any oligomer with a strong tendency to adopt a specific compact conformation.* The term *compact* is often used to describe the tertiary structure of proteins. Because there are very few synthetic polymers that display a specific tertiary structure, the primary step in designing a foldamer is therefore to identify new backbones with well-defined secondary structural preferences. By utilizing diverse synthetic tools, this 'bottom up' approach may help to engineer new frameworks that may be molded to mimic the structure and functions of the biopolymers.[9] The scope and feasibility of this concept is reflected in the exponential growth from its foundation in the early 20th century to the present stage, as would be evident from the recent literature.[10] Although foldamer chemistry started with the modification of natural α-peptides with their β- and γ-counterparts,[11,12] wide range of backbones have been developed and reported later.[13-16]

2.2 Classification of foldamers

Based on the nature of the backbone, foldamers can be classified into two broad categories: biotic (or bio-inspired foldamers) and abiotic (or synthetic foldamers). Wherein most biotic foldamers chemically resemble proteins (Fig. 2.2),[17-22] majority of the abiotic foldamers include aromatic rings like Amb (2-aminomethyl benzoic acid), Adb (3-amino-4,6 dimethoxy benzoic acid), anthranilic acid (2-amino benzoic acid) etc. in the backbone (Fig. 2.3).[23-29] Recent advancements relating to bio-inspired foldamers include elucidating the sequence specificity and stability of various secondary structures in solution and generating tertiary structures like helix bundles. Abiotic foldamer research has mainly been focused on designing the basic building block to modulate the structure of

oligomers. Conformationally constrained building blocks which have obvious edge over the flexible counterparts have been utilized in biotic as well as abiotic foldamers.

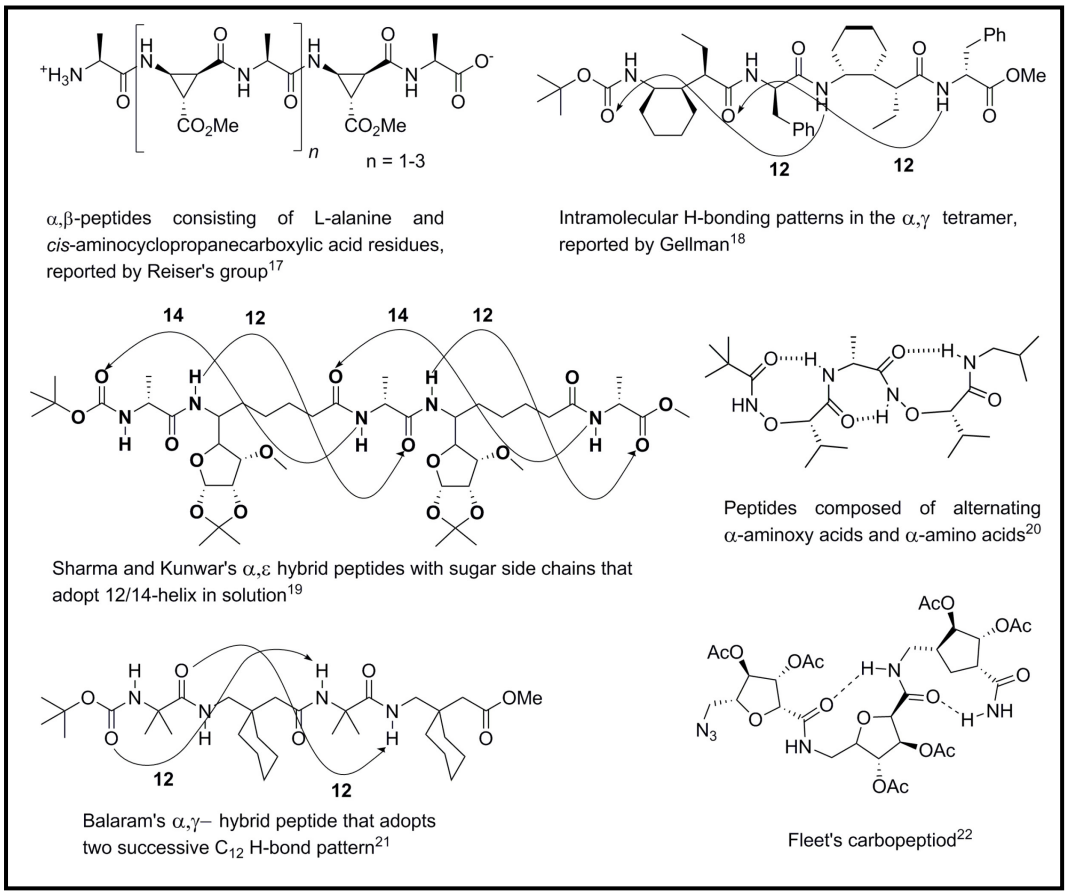


Fig. 2.2 Selected examples of biotic (bio-inspired) foldamers.

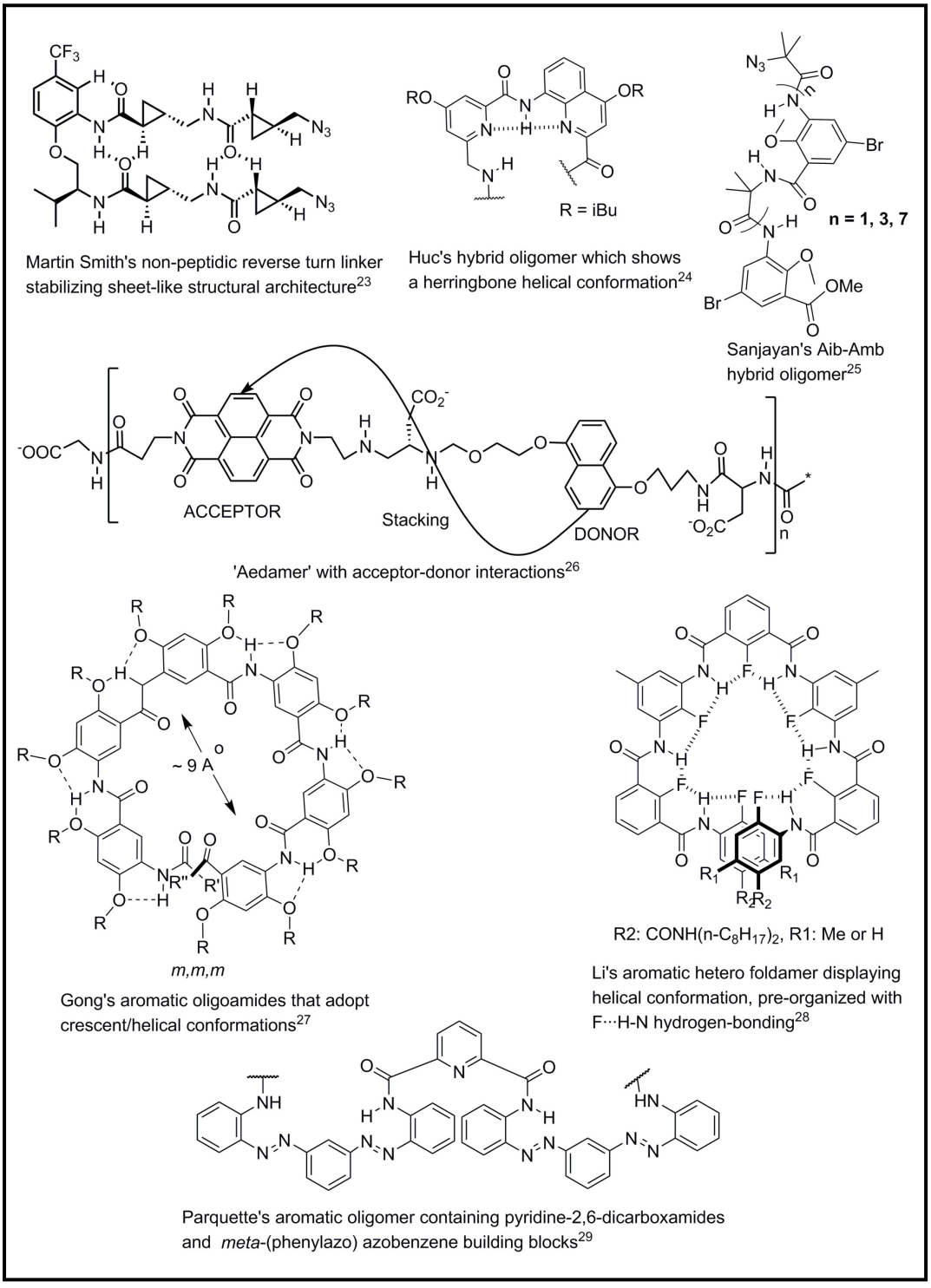


Fig. 2.3 Selected examples of abiotic foldamers.

2.3 Diversity at the building block level

Intensive research in the field of peptidomimetics during the last decade has been proven fruitful with researchers in quest of alternative building blocks that can mimic the structure and function of peptides.[30] It has been well established that a slight change in the backbone or the side chain of these residues generate a myriad of structures which is still counting. With ever-increasing number of building blocks available in the armory of foldamer design, many unseen structures and functions are waiting to be discovered. Given below, under appropriate sub-headings, are some of the selected building blocks used to generate diverse class of foldamers.

2.3.1 β-Peptides

β-amino acid is one of the most utilized building blocks for peptidomimetic foldamers. It has an additional methylene group in between the amine and acid counterparts. Although minute, it opens up a new dimension in the structural architecture of the designed molecule. The chemical similarity with α-peptides and higher proteolytic stability *in vitro* and *in vivo* has inspired many researchers to design well-defined secondary and quternary structures using β-peptide as a scaffold.[31-35] The field of β-peptide foldamers has been established by the research groups of Seebach[30] and Gellman.[31] Some of the frequently used β-amino acid building blocks include 2-aminocyclopentanecarboxylic acid (ACPC),[32] 2-aminocyclohexanecarboxylic acid (ACHC)[33] and 2-amino benzoic acid (anthranilic acid)[34] (Fig. 2.4 A). A foldamer reported by our group[35] that displays an unusual periodic *pseudo* β-turn network of 9-membered ring

hydrogen-bonded network formed in the forward direction of the sequence by 1→2 amino acid interactions both in solid-state as well as in solution is depicted in Fig. 2.4B.

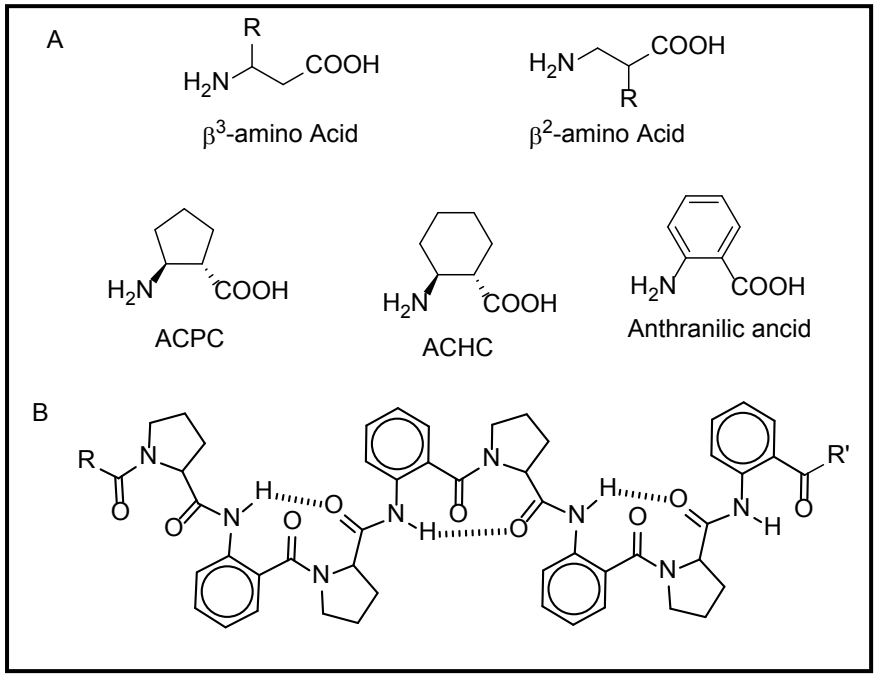


Fig. 2.4 Selected examples of β-peptide building blocks (A) and an oligomer featuring anthranilic acid as one of the building blocks (B).

2.3.2 γ-Peptides

γ-Peptides are the higher homologues of β-Peptides. In spite of receiving less attention, oligomers of γ-peptides often display versatility as compared to that of β-counterparts. The conventional β-turn secondary structure (C_{10} turn) with an αα segment can be expanded to C_{12} in a αγ counterpart.[36] For instance, 1-(aminomethyl)-cyclohexaneacetic acid, "gabapentin" (Gpn) (Fig 2.5A) is a conformationally constrained γ-amino acid,[37] extensively investigated by Balaram's group in recent years for designing peptides with diverse secondary structures. Gellman's group developed a cyclically constrained chiral γ-amino acid building block, featuring side chains, which was used for developing αγ

heterogeneous peptides,[18] which showed clear preference for C_{12} H-bond pattern in solid as well as in the solution state (Fig 2.5B).

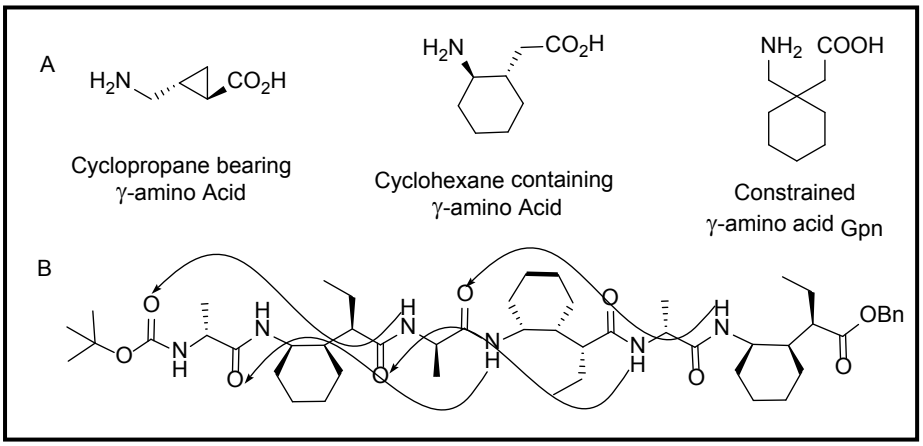


Fig. 2.5 Selected examples of γ-peptide building blocks (A) and an αγ oligomer developed from alanine and cyclically constrained chiral γ-amino acid building blocks (B).

2.3.3 δ-Peptides

δ-Peptides are the isosteric replacements of dipeptide units. β-turn is a common structural feature of proteins associated with the dipeptide fragment. Obviously, most of the work in the δ-peptide family has been intended to create a β-turn mimic. Most of the research in this area involves carbopeptoid backbones and the idea of using carbohydrate (sugar) amino acids for peptidomimetics[38] has gained immense popularity in the recent past. The conformational preference of sugar residues incorporated into peptide chains were initially exploited for the rational design of a β-turn mimetic. Eventually, it was understood that appropriately linked sugar amino acids can serve as a dipeptide replacement. The research groups of Fleet,[39] Chakraborty,[40] Fuchs[41] and many others[13a] have put an extensive effort in generating diverse class of sugar amino acids (SAAs) with varying preferences for secondary structure formation. Selected examples of such

building blocks and a peptidomimetic containing 3,4-dideoxy furanoid sugar amino acid[40] are shown in Fig. 2.6.

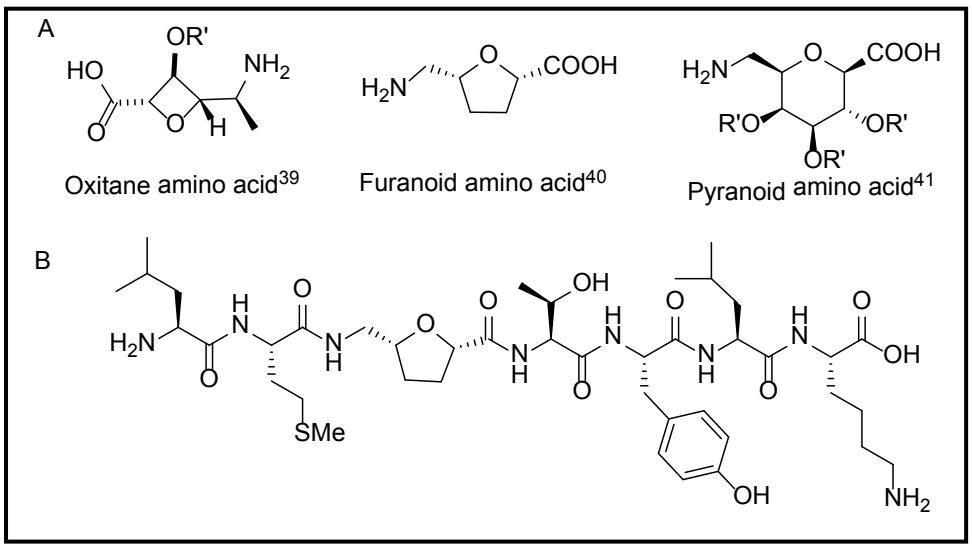


Fig. 2.6 Selected examples δ-peptide building blocks (A) and a peptide featuring furanoid amino-acid (B).

2.3.4 Aminoxy acids

In contrast to the amino acids devoid of backbone oxygen, the N–O bond in aminoxy acids demonstrates unusual torsional characteristics because of the repulsion between the lone pairs of electrons on the CO and O atoms. As a result, the backbone of aminoxy peptides is endowed with extra rigidity. Oligomers comprising α-, β- and γ-aminoxy acids have been shown to form several well-defined secondary structures.[42] Introduction of α-aminoxy acid in a peptide chain strongly favors secondary structures stabilized by 8-membered H-bond featuring i→i+3 C=O···NH (N-O turn, Fig. 2.7) – which is not very common.[43] As compared to α-aminoxy peptides, the β-counterpart have an extra carbon atom in the backbone, which offers a greater variation in the substitution pattern.[44] Interestingly, a peptide containing alternating L-aminoxy valine and D-alanine has

been shown to adopt a secondary structure wherein γ-turn can be initiated by a succeeding α-N-O turn (Fig. 2.7).[45]

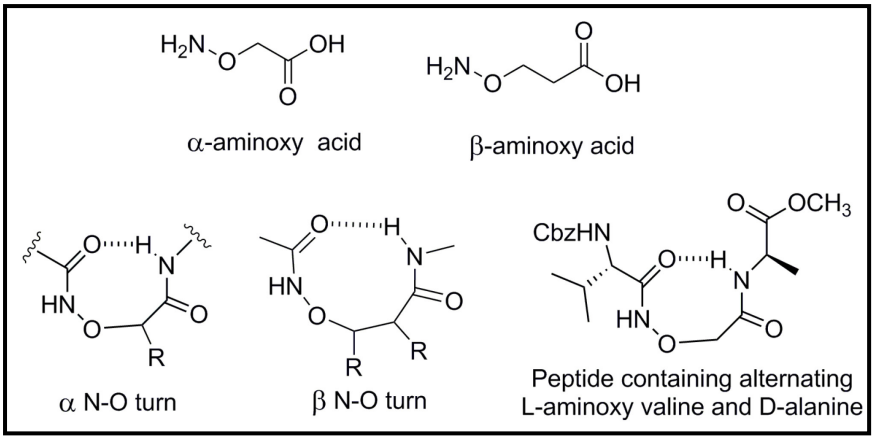


Fig. 2.7 α- and β-aminoxy acids and the characteristic N–O turns,[42-44] and a peptide containg L-aminoxy valine.[45]

2.3.5 Azapeptides

When the β^3-carbon of a β-peptide is replaced by a trivalent nitrogen atom, we obtain an azapeptide. This little change brings about a marked difference in the H-bonding preferences of the backbone. Similarly, as for the aminoxy peptides, the structure is organized by short range turn-like H-bonded rings. Azapeptide homo-oligomers tend to achieve strand structures that rely on a framework of hydrazino turns (Fig. 2.8A).[46] The β^3-peptide foldamer shown in Fig. 2.8B prove that the backbone organization consistently depends on a framework of consecutive eight-membered hydrogen-bonded pseudocycles. [47] These findings were also supported by solid and solution state studies.

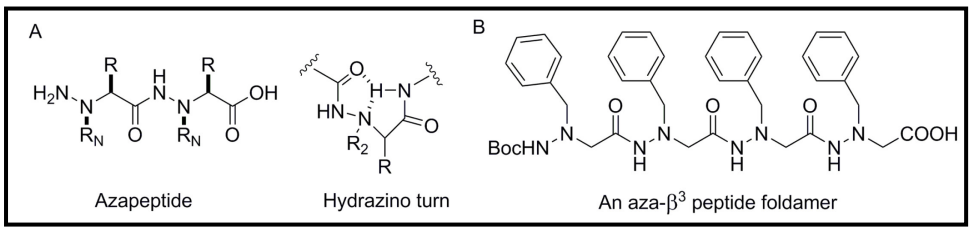


Fig. 2.8 An azapeptide moiety and hydrazino turn (A)[46] and an aza- β^3-peptides foldamer (B).[47]

2.3.6 Oligoureas

Oligoureas are obtained when the γ^3-carbon in the γ-peptide family is replaced with nitrogen. The urea functionality in itself is known to be one of the strongest H-bond mainstay, and this is augmented by the likelihood of bifurcated H-bonding and consequently restricted flexibility of the backbone. Recent studies reveal that oligourea sequences can adopt a 14-helix (Fig. 2.9B) suggestive of the γ-H14 helix.[48] The folding of these structures is robust enough to allow further applications.[48c] Also, given below is an example of an oligourea (Fig. 2.9C), wherein the hydrogen-bonding between the heteroatom on the aromatic ring and the backbone NH has been utilized to a great extent in the design of foldamers with pre-disposed conformation.[49]

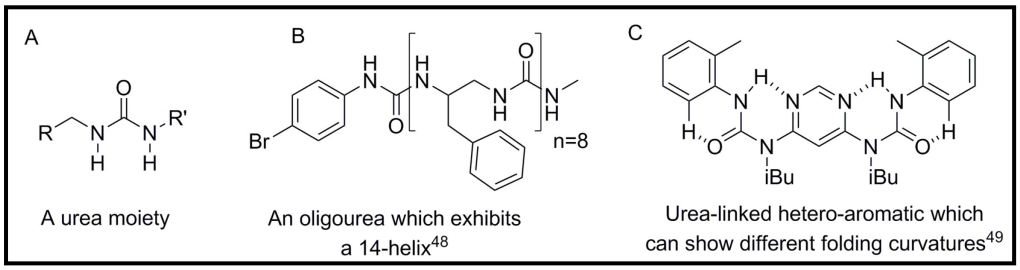


Fig. 2.9 A urea moiety (A) and oilgoureas (B, C).

2.3.7 Peptoids

Peptoids or N-substituted glycines are a class of peptidomimetic compounds wherein the nitrogen atom of the peptide bond is protected. Application of peptoids in biology is immense.[50] The group of Taillefumier and Edwards first reported the synthesis and structural investigations of a linear and cyclic α/β-alternating peptoids.[51] Given is an example of peptoid foldamer reported by Kirshenbaum's group (Fig 2.10B)[52] to explore the possibilities to augment conformational ordering of N-substituted glycine oligomers which

describes the use of N-aryl side chains as an instrument to enforce the presence of *trans*-amide bonds, thereby ensuring structural order.

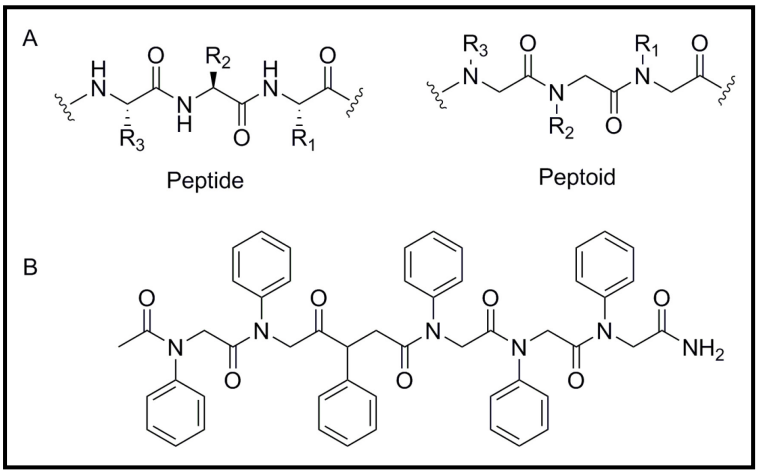


Fig. 2.10 Comparison of peptide and peptoids structures (A), and a peptoid foldamer (B) reported by Kirshenbaum's group.[52]

2.3.8 Foldamers based on heterocyclic skeleton

Foldamers based on heterocyclic skeleton have regularly been used to generate well defined secondary structures.[53] Our efforts in this direction culminated in the development of a foldamer having a structural feature that adopts co-facial architecture (Fig. 2.11A), using diamino-pyridine as one of the building blocks.[53d] Stable helical conformations, even in a polar solvent like methanol were feasible with aromatic oligoamides derived from 1,10-phenanthroline diacid and *O*-phenylenediamine as reported by Chen and co-workers (Fig. 2.11B).[54] Based on extensive spectroscopic studies, these conformations have been shown to be stabilized through a combination of intramolecular hydrogen-bondings and aromatic π–π stacking interactions. Also given is an example of aromatic oligomer reported by Hamilton and co-workers that is composed of anthranilic acid and 2,6-pyridine dicarboxylic acid[55] (Fig. 2.11C). A drastic change in conformation from linear to helical was observed upon incorporation of pyridine-2, 6-dicarboxylic acid unit.

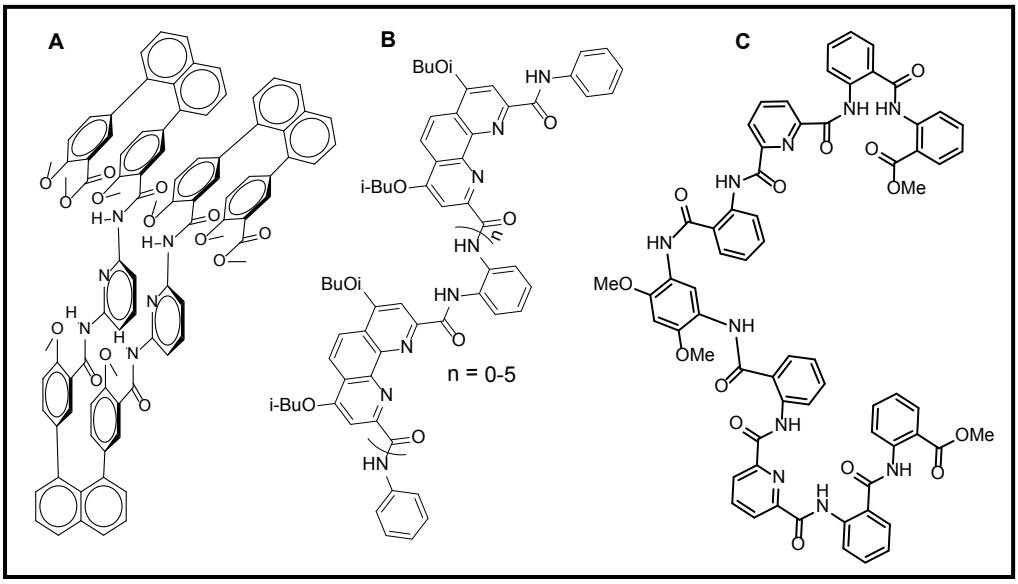


Fig. 2.11 Selected examples of foldamers which use heterocycles as building blocks.

2.4 Applications of foldamer

Due to their structural tunability and stability, foldamers can be potential candidates for diverse applications.[56] Helical foldamers with hollow cavity can find applications in host-guest chemistry, drug delivery, catalysis, chemical transformation etc. β-sheet foldamers can provide insights into various β-sheet-mediated diseases. Due to its dynamic nature, foldamers are suited for the design of stimuli-responsive materials that can respond to temperature, chemicals, pH, light, etc. For instance, the photoswitchable *trans*-azobenzene-incorporated amphiphilic oligo-(*m*-phenylenes) shows considerable promise for developing photo-responsive materials.[57] Some of the promising foldamers that act as molecular receptors, sensors, biomimetics, catalysts etc. are given in Fig. 2.12.[58-61]

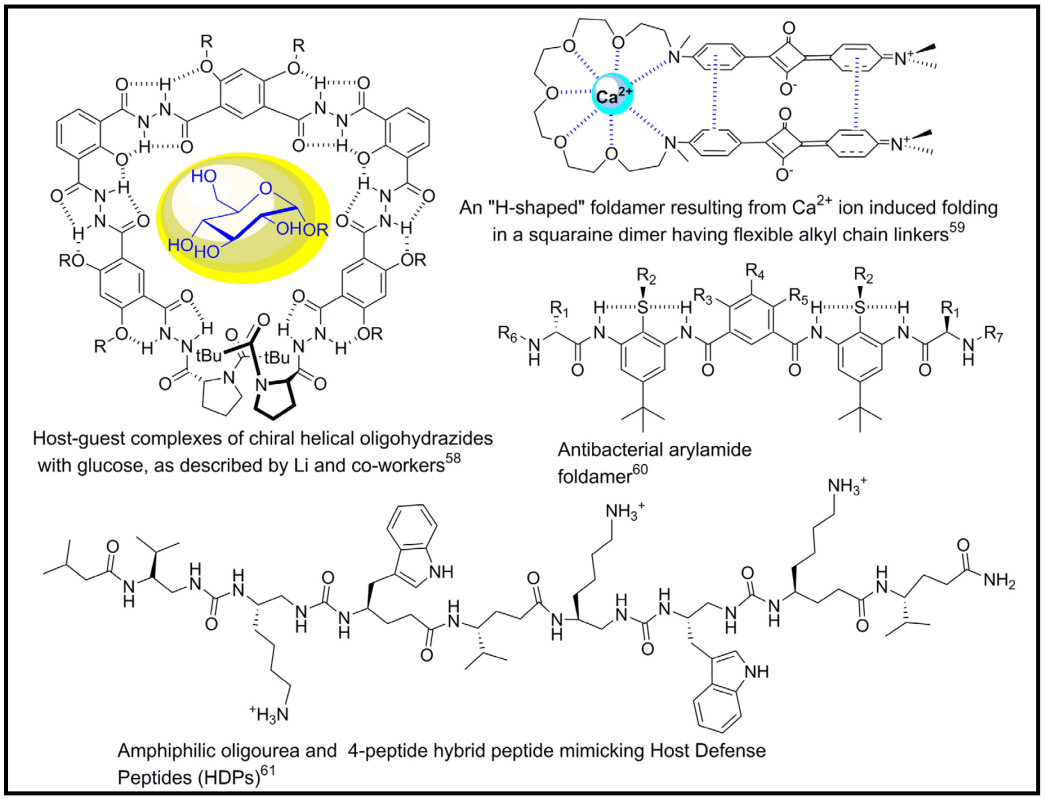


Fig. 2.12 Some of the foldamers that find practical applications.[58-61]

2.5 Objective of the present work and design strategy

Alteration of the amino acid order and torsional parameters of the individual units have been shown to have a marked influence on the overall conformation and structural architecture (shape) of biopolymers. In the last decade, a sizable amount of work has been done in this area of foldamers to obtain novel molecular architectures with wide range of applications (*vide supra*). In this regard, the synthetic oligomers may provide an excellent starting point for the elaboration of peptide mimics that could be developed only with difficulties on the basis of small-molecule scaffolds.[62] Herein, our endeavor was to develop building blocks which may lead to foldamers that would be structurally different and unique in their conformation. Although there are innumerable unnatural amino

acids and other building blocks reported in the literature, aromatic building blocks with two-dimensional orientations of the chain propagating groups appended on conformationally rigid framework suitable for foldamer generation have not yet been explored. Positioning of the chain propagating groups on a rigid aromatic framework can be expected to show a marked influence on the overall shape of the oligomers containing such building blocks. Furthermore, such a strategy could furnish synthetic oligomers with dazzling structural architectures.[63]

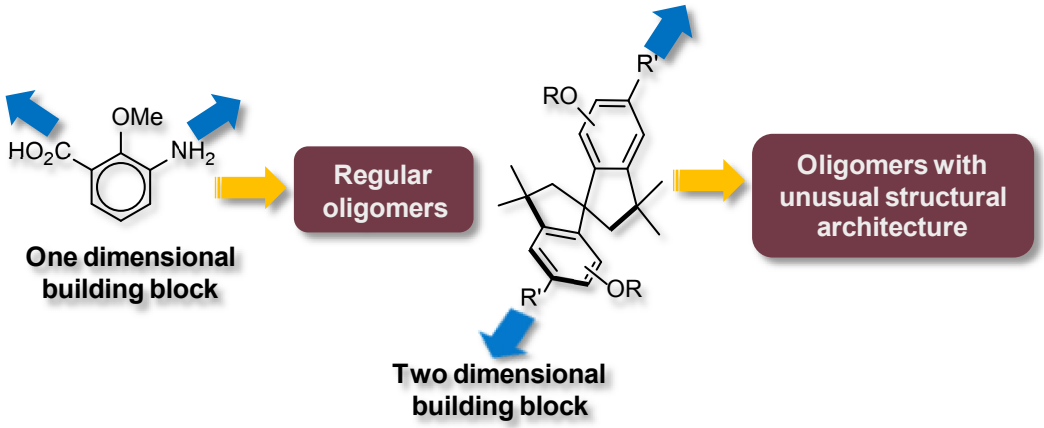


Fig. 2.13 Design strategy to synthesize conformationally rigid two-dimensional foldamer building blocks.

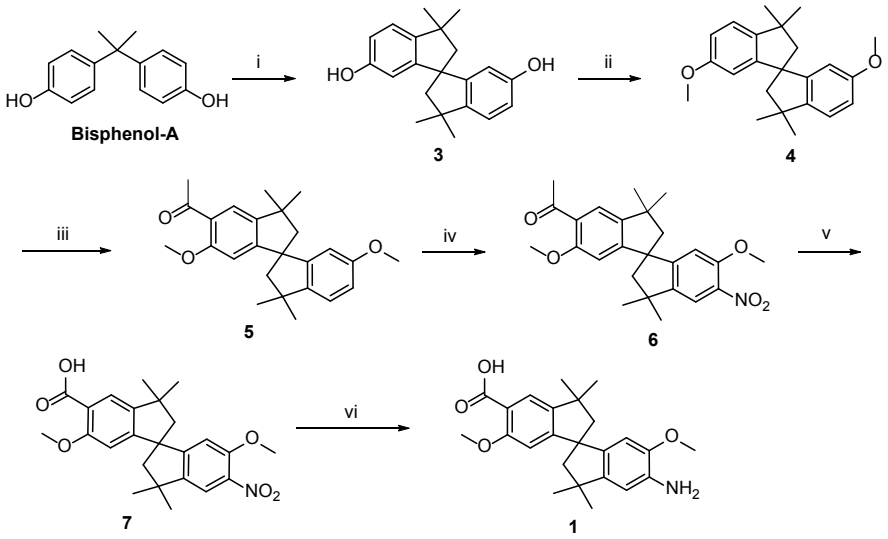
Considering the high conformational rigidity (because of the presence of spiro-annulated rings that restricts the rotation of aryl rings) and the ease of synthesis, we anticipated that 1,1'- spirobiindane building block (Fig. 2.13) would be a useful basic unit in the design and development of synthetic oligomers with unique structural architectures. Moreover, the introduction of H-bond directing alkoxy groups on the aromatic rings would exert directional effect for H-bonding interactions and hence would control the conformational feature of the oligomers. Furthermore, introducing the chain propagating groups, such as amino (NH_2) and carboxylic acid (CO_2H), *ortho* to the alkoxy groups followed by coupling would result in the formation of oligomers.

2.6 Synthesis of two-dimensional building blocks

2.6.1 Conformationally rigid two-dimensional amino acid

The spirobiindane based amino acid building block **1** was synthesized starting from[63b] spirobiindane bis-ether **4** (Scheme 1), obtained in quantitative yield by the exhaustive methylation of the known spirobiindanol **3**, was subjected to Friedel–Crafts acylation-haloform reaction sequence to introduce the carboxyl group on the aromatic framework. This procedure for the installation of the carboxyl group on the aryl rings *ortho* to alkoxy groups was preferred over a possible metal directed (*O*-lithiation)-one-step carboxylation procedure, due to the anticipated difficulties associated with the latter procedure, particularly when working on a larger scale. The nitro derivative **6**, obtained by the careful nitration of **5**, was subjected to haloform reaction to afford the nitro acid **7**, which after catalytic nitro reduction readily furnished the novel amino acid **1** in an overall yield of 16% starting from **3**.

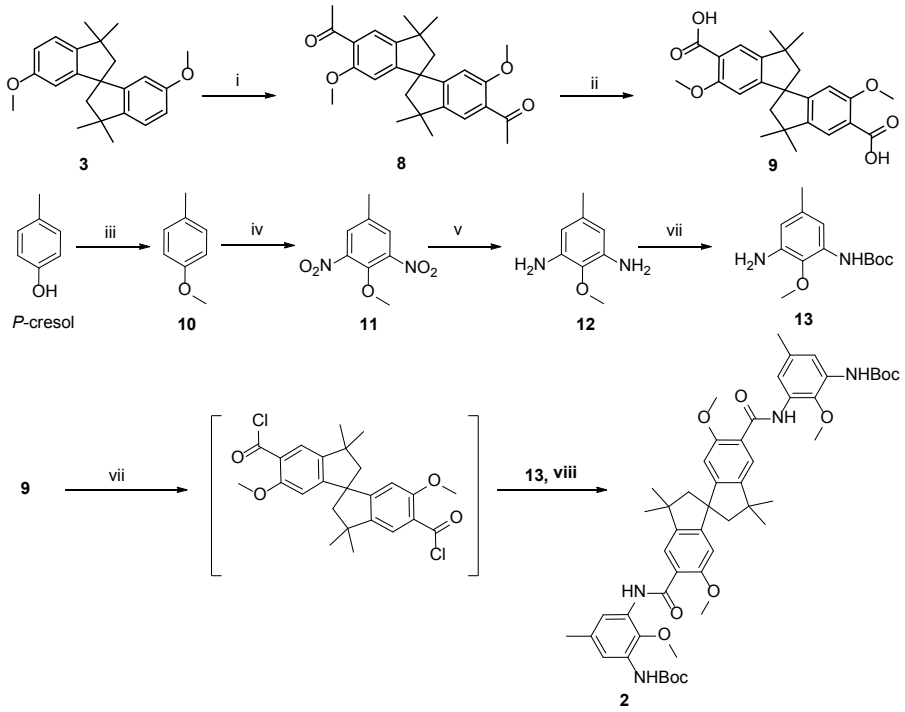
Scheme 2.1 Synthesis of building block **1**



Reagents and conditions: (i) CH_3SO_3H, rt, 96 h, 67%; (ii) Me_2SO_4, K_2CO_3, acetone, reflux, 8 h, 96%; (iii) CH_3COCl, $SnCl_4$, DCM, -10 °C, 1.5 h, 70%; (iv) HNO_3, H_2SO_4, CH_3COOH, 2 min, 60%; (v) NaOCl (4%), NaOH (50%), dioxane, 12 h, 69%; (vi) $HCOONH_4$, Pd-C, MeOH, reflux, 6 h, 62%.

2.6.2 Conformationally rigid two-dimensional abiotic foldamer building block

Scheme 2.2 Synthesis of building block **2**



Reagents and conditions: (i) CH_3COCl, $SnCl_4$, DCM, rt, 24h, 94%; (ii) NaOCl (4%), NaOH, dioxane, 24h, 76%; (iii) K_2CO_3, acetone, reflux, 8h, 90%; (iv) HNO_3, Ac_2O, 0 °C, 1.5 h, 50%; (v) H_2, Pd-C, 60 psi, 8h; (vi) LiHMDS, Boc_2O, THF, rt, 3h, 45%; (vii) $(COCl)_2$, DCM, DMF(cat), 3h; (viii) Et_3N, THF, reflux, 5h, 90%.

The foldamer building block **2** was synthesized starting from bisphenol A by one-step acid-mediated rearrangement followed by exhaustive methylation of the spirobiindanol to obtain **3** (Scheme 2.2).[64] To get the bis-acid **9**, the bis-alkoxy derivative **3** was first subjected to Friedel-Craft's acylation to deliver bis-acylated building block **8**, followed by the haloform reaction. The amine counterpart **13** was prepared starting from commercially available *p*-cresol which on *O*-methylation and nitration afforded the di-nitro analog **11** in 50% yield. On catalytic hydrogenation, **11** afforded reduced product **12** in quantitative yield, which was subjected to mono Boc protection of one of the amine functionalities in presence of one equivalent of Boc-anhydride and LiHMDS to furnish the amine

counterpart **13** in 45% yield. Bis-acid chloride derivative was obtained by reacting the bis-acid **9** with oxalyl chloride, which was then reacted with two equivalents of mono-amine **13** to obtain the foladmer building block **2** in 90% yield.

2.7 Single crystal X-ray diffraction studies of building block 1

Extensive efforts to grow crystals of the conformationally restricted aromatic amino acid building block culminated in the formation of crystals of **1** (Fig. 2.14A). Investigations reveal that in the spirobiindane based unnatural aromatic amino acid **1**, the amine and acid groups are oriented in such a way that they lie in two different planes, reversing the growth of the backbone by 83.5° (Fig. 2.14B). Furthermore, intra-residual hydrogen bonding between the chain propagating amino and carboxylic acid functionalities and the adjacent methoxy groups help the backbone to attain additional rigidity, as anticipated.

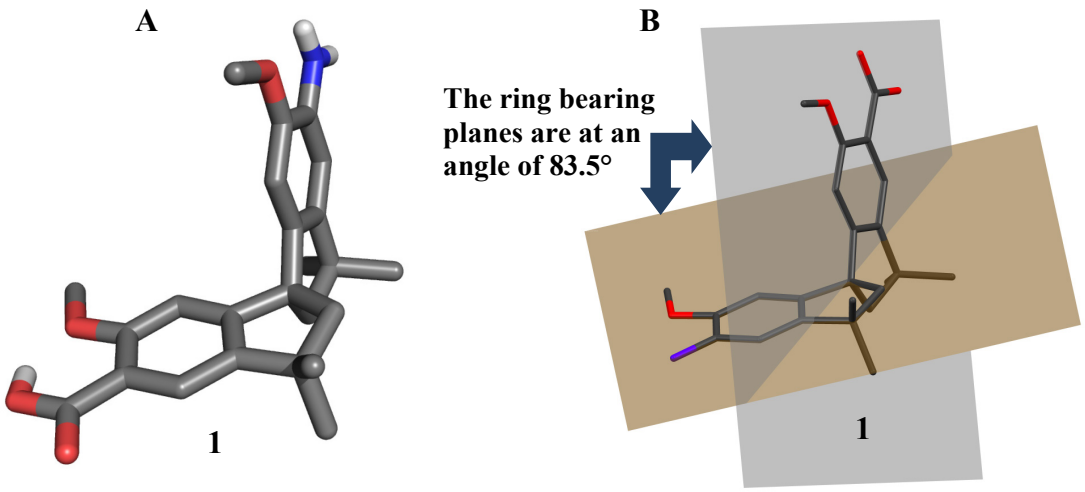


Fig. 2.14 Crystal structure of **1** exhibiting the two-dimensional orientation of the chain propagating groups (A), and the ring bearing planes at an angle of 83.5° (B).

2.8 Conclusion

Summarizing our results, we have developed two novel conformationally rigid foldamer building blocks, wherein the chain propagating groups, embedded

on the aryl rings, are projected in an anti-periplanar arrangement (two-dimensional arrangement). Oligomers containing such building blocks are expected to have overwhelming 'conformational ordering', facilitating ease of characterization.[65] Structural investigations of **1** by single-crystal X-ray studies provided clear evidence for the two-dimensional orientation of the chain propagating groups. The strategy disclosed herein for the construction of conformationally restricted building blocks would be useful for the construction of oligomers displaying novel molecular architectures with conformations distinctly different from those classically observed.

2.9 Experimental Section

Crystal data for **1:** $C_{24}H_{29}NO_4$, M = 395.48, Pale yellow colored crystals, approximate size 0.50 x 0.48 x 0.07 mm, Multiscan data acquisition. Total scans = 3, total frames = 1818, Oscillation / frame -0.3º, exposure / frame = 15.0 sec / frame, θ range = 2.24 to 25.00º, completeness to θ of 25.00 º is 99.8 %. Crystals belong to Monoclinic, space group P 2_1/n, a = 10.881 (7), b = 17.932 (11), c = 11.015 (7) Å, β = 104.754 (10)º , V = 2078 (2) $Å^3$, Z = 4, D_c = 1.264 mg m^{-3}, μ (MoK_α) = 0.085 mm^{-1}, T = 297(2) K, 14637 reflections measured, 3648 [R(int) = 0.0488] unique [I>2σ(I)], R value 0.0912, wR2 = 0.2283.

5-Acyl -6, 6'-dimethoxy-3, 3, 3', 3'-tetramethyl-1, 1'-spirobiindane 5:

To a stirred solution containing 6,6'-Dimethoxy 3,3,3',3'-tetramethyl-1,1' spirobiindane **4**[63b] (5 g, 14.8 mmol, 1 equiv.) in dichloromethane (50 mL) at -10 °C, acetyl chloride (1.27 mL, 17.8 mmol, 1.2 equiv.) was added drop wise for 10 minutes, followed by $SnCl_4$ (3.48 mL, 29.7 mmol, 2 equiv.). The reaction mixture was allowed to stir at -10 °C for one and half hour, diluted with dichloromethane (50 mL), acidified with dil. HCl, and the product was extracted into the organic layer. Drying and purification by column chromatography furnished **5**. Yield: 3.72 g (66%); mp: 116-118 °C; IR ($CHCl_3$) ν (cm^{-1}): 3299, 3236, 3020, 2958, 1668, 1602, 1485, 1465, 1404, 1217, 1155 ; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.58 (s, 1H), 7.09 (d, J = 8.33 Hz ,1H), 6.84-6.79 (dd, J_1 = 2.40 Hz, J_2 = 5.94 Hz, 1H), 6.37 (s, 1H), 6.30(d, J = 4.27 Hz, 1H), 3.74 (s, 3H), 3.70 (s, 3H), 2.60 (s, 3H), 2.40-2.21 (q, 4H), 1.39(d, J = 1.39 Hz, 6H), 1.33(d, J = 1.64 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 200.06, 159.45, 159.31, 156.75, 151.19, 144.62, 144.61, 127.84, 123.79, 122.59,113.57, 108.97, 107.12, 59.49, 59.39, 58.14, 55.70, 55.45,

42.97, 42.89, 31.80, 31.70, 31.62, 30.52, 30.34; LC-MS: 379.24 $(M+H)^+$; Anal. Calcd. for $C_{30}H_{36}N_4O_7$: C, 61.88.; H, 7.99.; N, 11.10. Found: C, 61.50.; H, 8.14.; N, 11.23.

5-Acyl-5'-nitro-6,6'-dimethoxy-3,3,3',3'-tetramethyl -1,1'-spirobiindane 6:

To a solution of 5-Acyl -6, 6'-dimethoxy-3, 3, 3', 3'-tetramethyl-1, 1'-spirobiindane **5** (3.5 g, 9.25 mmol, 1 equiv.) in AcOH (50 mL), H_2SO_4 (5 mL) was added drop wise followed by the drop wise addition of HNO_3 (14 mL) and stirred for two minutes. The solid residue was then filtered, washed with water and was purified by column chromatography furnishing **6** as yellow solid. Yield: 2.35 g (60%), m.p: 215-217 °C; IR ($CHCl_3$) ν (cm^{-1}): 3326, 3245, 3217, 3018, 2958, 2866, 1670, 1604, 1521, 1521, 1217, 1126, 1010; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.64 (s, 1H), 7.57 (s, 1H), 6.42 (s, 1H), 6.30 (s, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 2.60 (s, 3H), 2.44-2.17 (m, 4H), 1.42-1.40 (d, J = 2.53 Hz, 6H), 1.35-1.33 (d, J = 4.67 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ & DMSO-D_6 (5%)) δ: 199.30, 158.86, 156.12, 154.55, 152.59, 144.02, 143.96, 139.06, 127.82, 123.40, 118.88, 108.47, 106.30, 58.65, 58.28, 58.03, 56.29, 55.30, 42.72, 42.64, 31.27, 31.06, 30.95, 29.79, 29.63; LC-MS: 446.22 $(M+Na)^+$; Anal. Calcd. for $C_{25}H_{29}NO_5$: C, 70.90; H, 6.90; N, 3.31; Found: C, 71.02; H, 6.77; N, 3.54.

5-Carboxy-5'-nitro-6,6'-dimethoxy-3,3,3',3'-tetramethyl -1,1'-spirobiindane 7:

To 5-Acyl-5'-nitro-6,6'-dimethoxy-3,3,3',3'-tetramethyl -1,1'-spirobiindane **6** (1.7 g, 4 mmol, 1 equiv.), NaOCl (4% W/V) (80 ml) was added drop wise over a period of 5 minutes followed by the addition NaOH (50% W/V, 40 ml). The reaction mixture was then heated to 70 °C and stirred for 12 h, allowed to come down to room temperature and 5% HCl was added drop wise until pH = 1, extracted with ethyl acetate, dried over anhydrous.Na_2SO_4 and was purified by

column chromatography. Yield: 1.17 g, (69 %); m.p >300°C ; IR (Nujol) ν (cm^{-1}): 3296, 3242, 2923, 1733, 1616, 1458, 1377, 1220, 1130, 1049; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.05 (s, 1H), 7.64 (s, 1H), 6.40 (bs, 2H), 3.94 (s, 3H), 3.79 (s, 3H), 2.46-2.21 (m, 4H), 1.43-1.42 (d, *J* = 1.26 Hz, 6H), 1.36-1.35 (d, *J* = 1.52 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.1, 156.8, 156.5, 152.3, 149.0, 144.2, 143.3, 139.3, 122.3, 118.8, 109.0, 106.7, 58.99, 58.57, 58.13, 56.83, 55.87, 42.84, 31.59, 30.35; LC-MS: 426.08 $(M+H)^+$; Anal. Calcd. for $C_{24}H_{27}NO_6$: C, 67.75; H, 6.40; N, 3.29; Found: C, 67.62; H, 6.15; N, 3.60.

5-Carboxy-5'-amino-6,6'-dimethoxy 3,3,3',3'-tetramethyl -1,1'-spirobiindane 1:

5-Carboxy-5'-nitro-6,6'-dimethoxy-3,3,3',3'-tetramethyl -1,1'-spirobiindane **8** (1g, 2.35 mmol, 1equiv) in methanol (30 ml) was subjected to the reduction of nitro group by transfer hydrogenation using ammonium formate (0.74 g, 11.7 mmol, 5 equiv) and Pd-C (10%, 0.10 g) After reflux for 6 hours, the reaction mixture was filtered over celite pad, washed with methanol (3 x 10 ml) and on purification by column chromatography gave amine **1.** Yield: 0.57 g (61%); mp: 289-292 °C; IR (Nujol) ν (cm^{-1}): 3444, 3330, 3120, 2954, 2925, 2854, 2725, 1718, 1610, 1461, 1377, 1149; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.01 (s, 1H), 6.77 (s, 1H), 6.45 (s, 1H), 6.20 (s, 1H), 3.92 (s, 3H), 3.73 (s, 3H), 2.39-2.12 (m, 4H), 1.41-1.31(m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.85, 158.25, 156.37, 146.78, 144.30, 143.61, 137.41, 136.49, 124.22, 120.71, 107.52, 107.04, 105.64, 59.37, 59.10, 57.64, 56.13, 55.69, 42.93, 42.28, 31.77, 31.45, 30.69, 30.28; LC MS mass: 396.12 $(M+H)^+$; Anal. Calcd. for $C_{24}H_{29}NO_4$: C, 72.89; H, 7.39, N, 3.54; Found: C, 72.55; H, 7.21; N, 3.78.

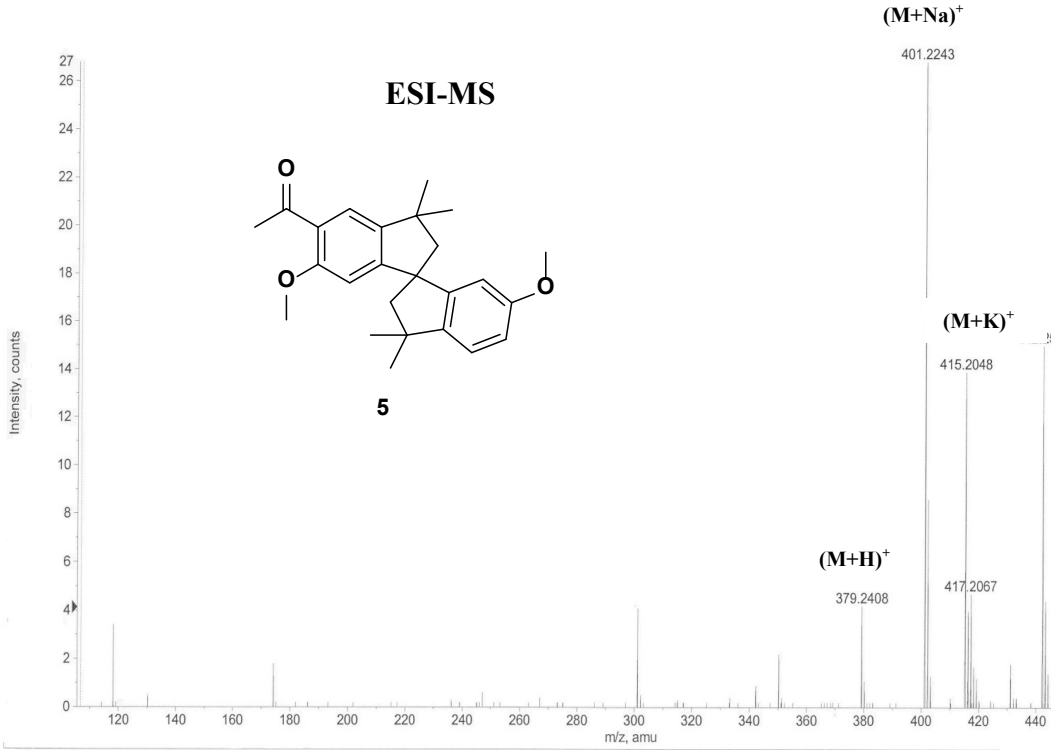
***tert*-butyl (3-amino-2-methoxy-5-methylphenyl)carbamate 13:**

1 molar LiHMDS solution in THF (2.62 ml, 2.62 mmol) was added drop-wise to a solution of compound **12**[66] (0.20 g, 1.31 mmol) in 5 mL THF at room temperature and was stirred for 10 min. To the resulting mixture a solution of Boc-anhydride (0.28 g, 1.31 mmol) in THF (5 mL) was added slowly for 5 min. Reaction mixture was then allowed to stir at room temperature for 3 h, and volatiles were evaporated. The residue was purified by column chromatography. Yield: 0.15 g (45%), mp: 117-119 °C, IR ($CHCl_3$) ν (cm^{-1}): 3428, 3019, 2400, 1725, 1619, 1535, 1463, 1368, 1215, 1158, 993, 758,669; ^{1}H NMR (400 MHz,$CDCl_3$): δ 7.33 (S, 1H), 6.94 (S, 1H), 6.26 (S, 1H), 3.73 (S, 3H), 3.69 (bs, 1H), 2.22 (S, 3H), 1.53 (S, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.7, 138.7, 134.7, 133.4, 131.6, 110.9, 109.3, 80.3, 59.3, 28.3, 21.4. HRMS: $C_{13}H_{20}N_2O_3$ Calcd. 252.1474, Found: 253.1574 $(M+H)^+$, 275.1366 $(M+Na)^+$.

di-tert-butyl (((6,6'-dimethoxy-3,3,3',3'-tetramethyl-2,2',3,3'-tetrahydro-1,1'-spirobi[indene]-5,5'-dicarbonyl)bis(azanediyl))bis(2-methoxy-5-methyl-3,1-phenylene))dicarbamate 2:

To a solution of spirobiindane based bis-acid **9** (0.9 g, 2.12 mmol) in DCM (15 ml) containing dry DMF (cat.), oxalyl chloride (1.06 g, 0.73 ml, 8.45 mmol) was added drop-wise at 0 °C, and the reaction mixture was allowed to stir at room temperature for 3 h. The volatiles were stripped off and to a solution of mono-amine **13** (1.16 g, 4.6 mmol) in anhy. DCM (25 ml) and Et_3N (1.06 g, 1.46 ml, 10.5 mmol) was added drop-wise at 0 °C. To the resulting mixture crude bis-acid chloride dissolved in anhy. DCM was added drop-wise maintaining 0 °C, which was then stirred at room temperature for 12 h. Purification of the crude material by column chromatography furnished **2** as a white solid. Yield: 1.7 g (90%), mp: 117-119 °C, IR ($CHCl_3$) ν (cm^{-1}): 3422, 3354, 3019, 2400, 1731, 1604, 1504,

1215, 1156, 988, 756, 699; ^{1}H NMR (400 MHz,$CDCl_3$): δ 10.44 (S, 2H), 8.20 (S, 2H), 8.10 (bs, 2H), 7.66 (bs, 2H), 6.45 (S, 2H), 3.94 (S, 6H), 3.79 (S, 6H), 2.46-2.42 (d, 2H, *J*= 13.3 Hz), 1.54 (S, 18 H), 1.48 (S. 6H), 1.39 (S, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.3, 157.4, 155.5, 152.6, 145.6, 135.6, 135.1, 131.4, 130.8, 126.2, 121.4, 115.7, 114.5, 106.5, 106.8, 80.6, 60.4, 59.2, 58.4, 56.2, 43.2, 31.5, 30.3, 28.2, 21.8; LC MS mass: 915.61 $(M+Na)^+$; Anal. Calcd. for $C_{51}H_{64}N_4O_{10}$: C, 68.59; H, 7.22; N, 6.27. Found: C, 68.70; H, 6.98; N, 6.51.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(M+H)+
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417.2067
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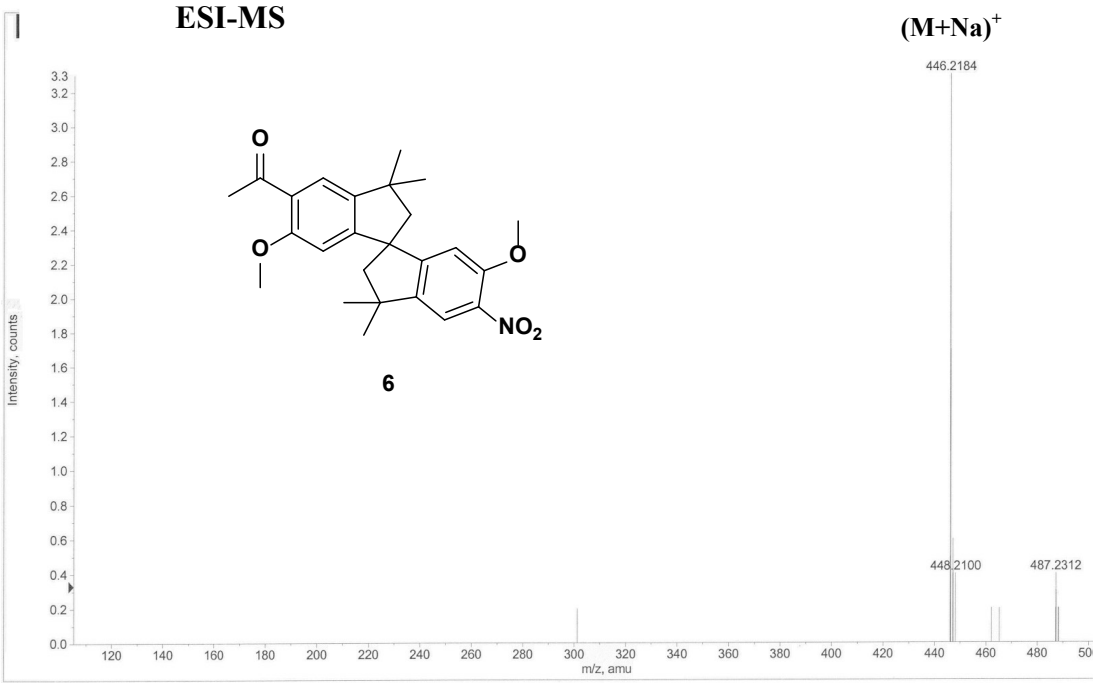
O
O
5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m/z, amu
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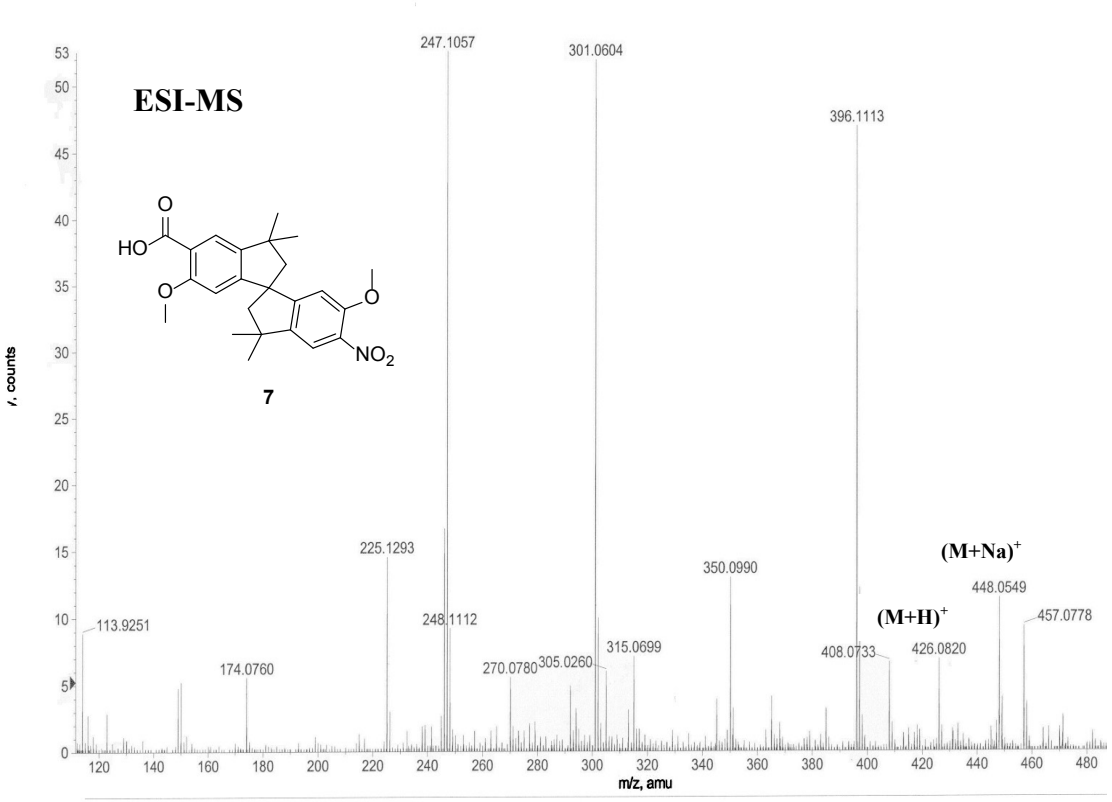
O
O
NO2
6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m/z, amu
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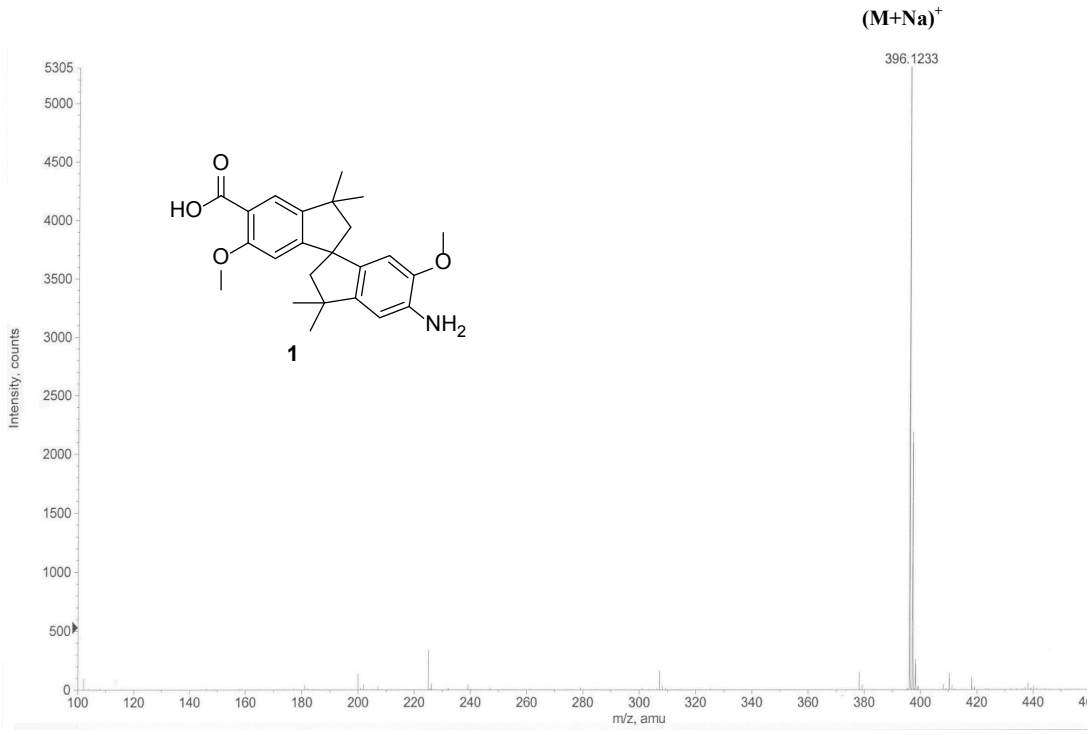
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m/z, amu
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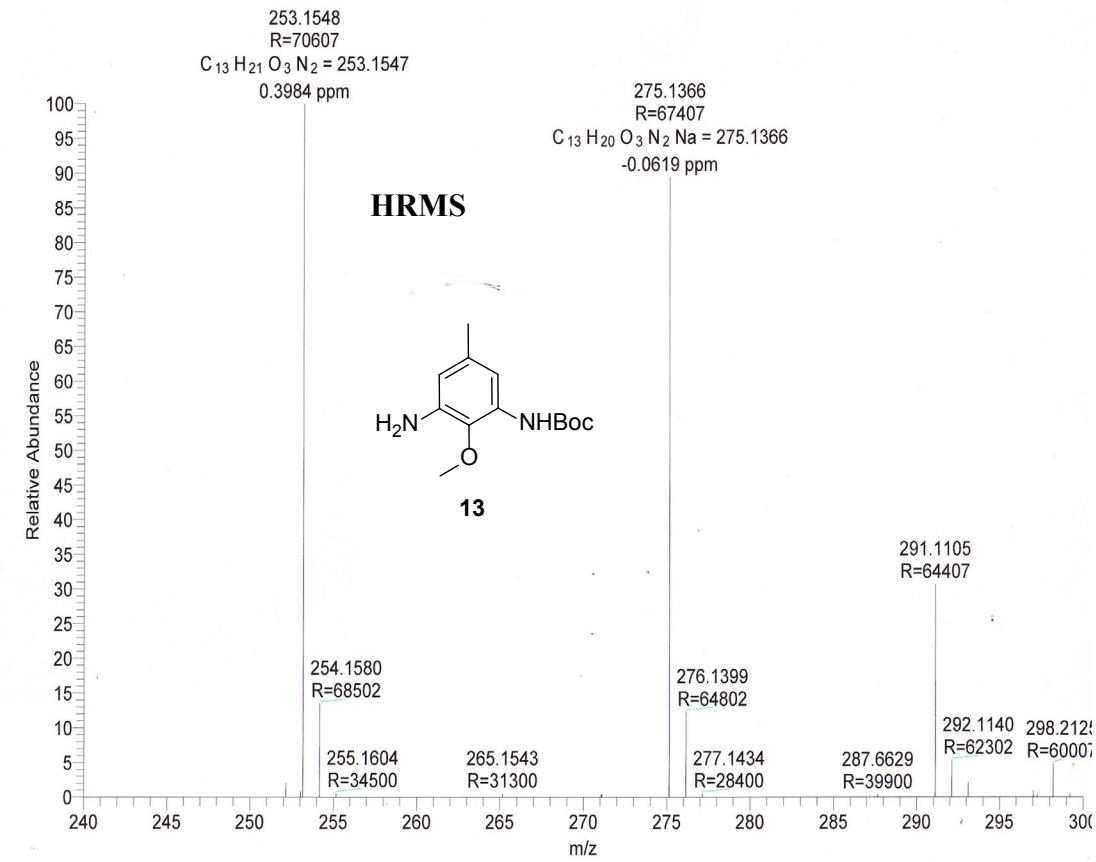
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O
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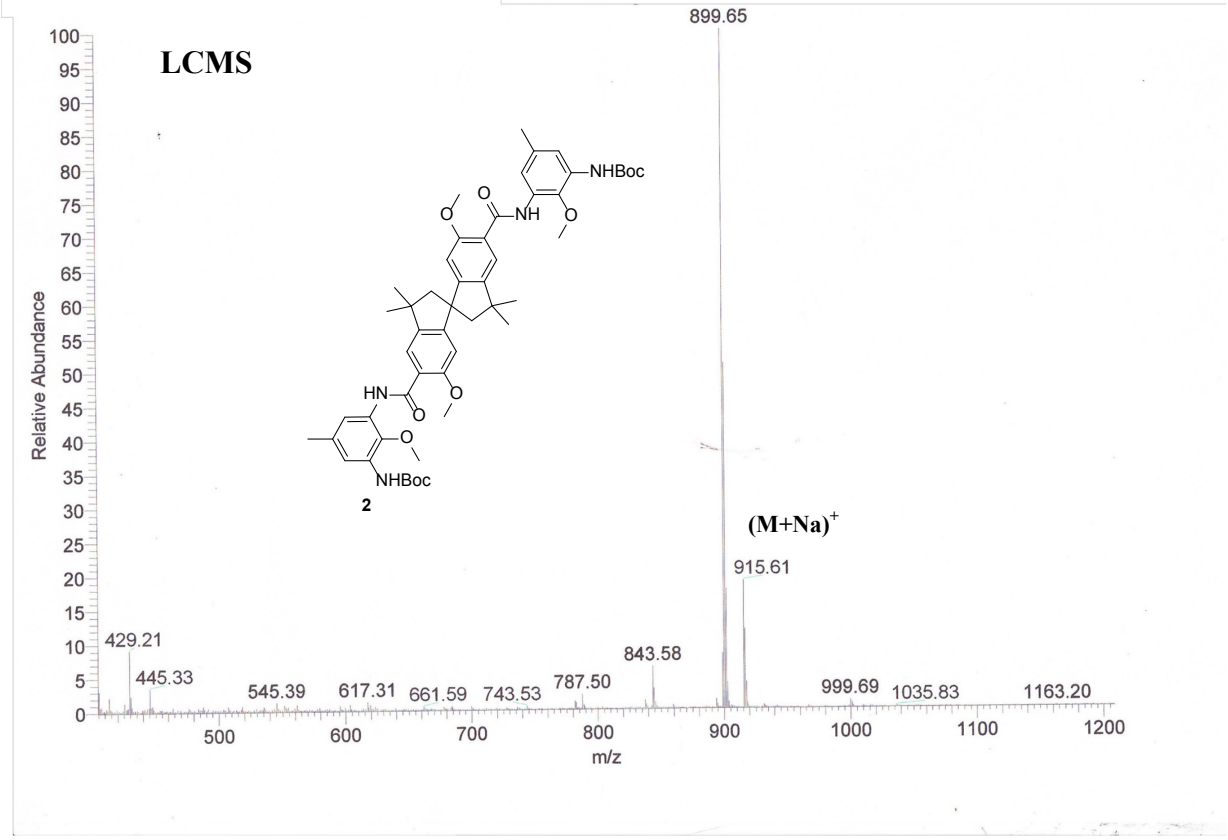
Relative Abundance
m/z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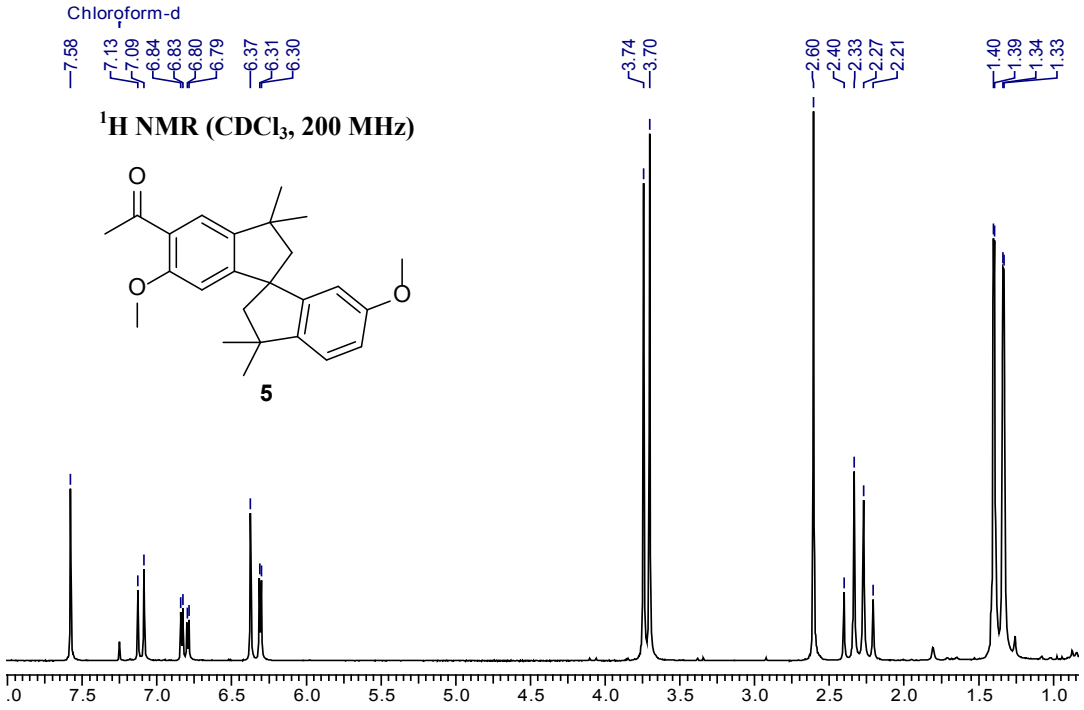
m/z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2.60
2.40
2.33
2.27
2.21
1.40
1.39
1.34
1.33
1H NMR (CDCl3, 200 MHz)
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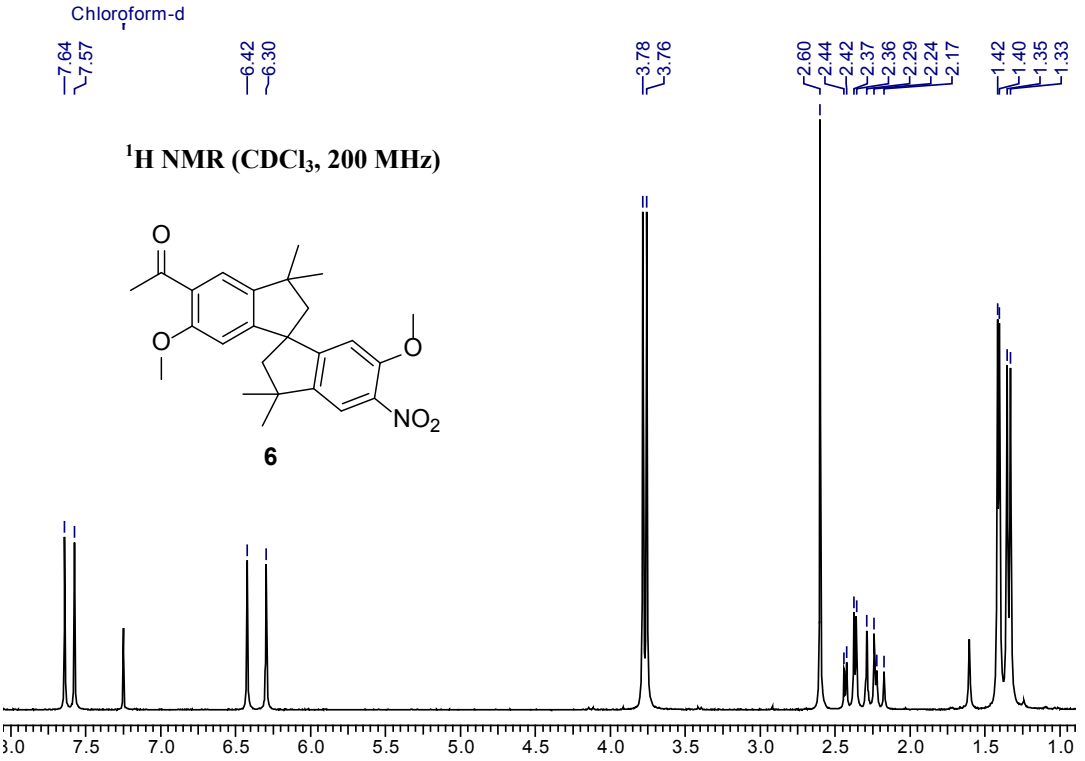
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1.42
1.40
1.35
1.33
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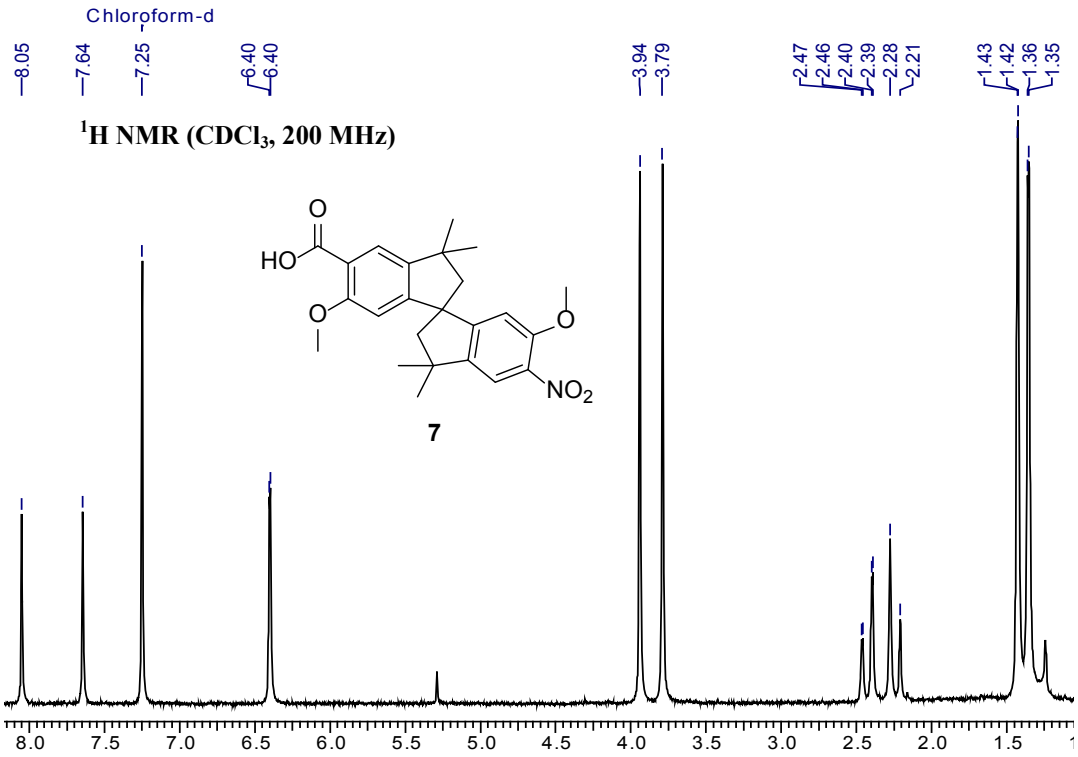
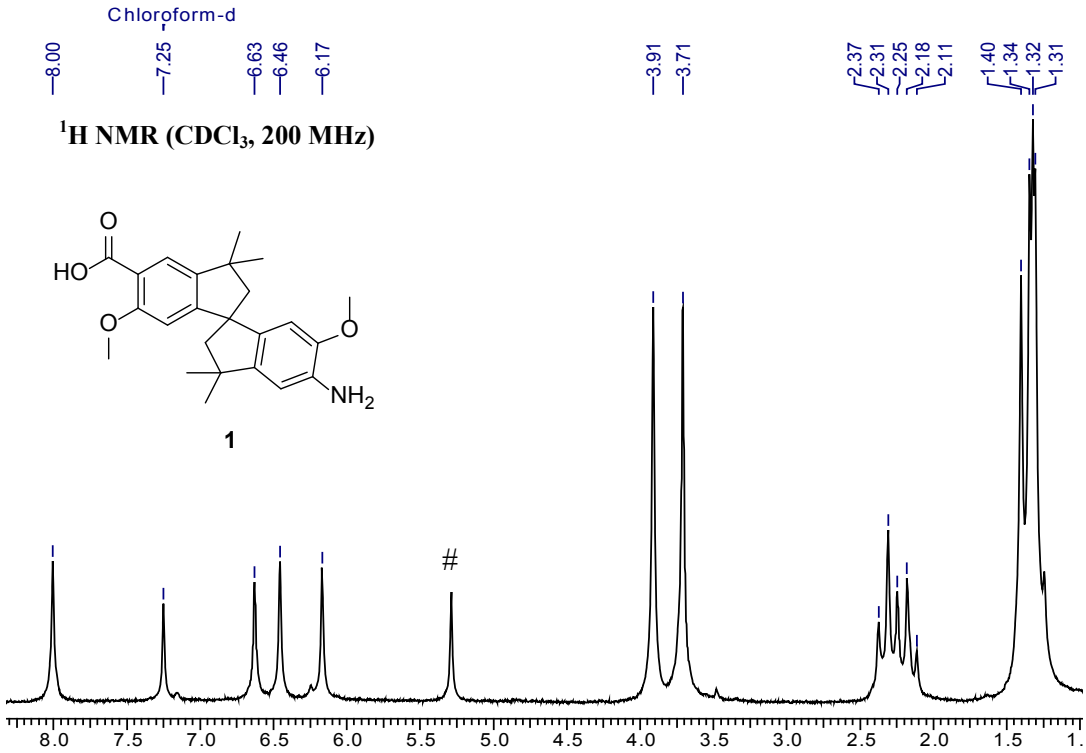
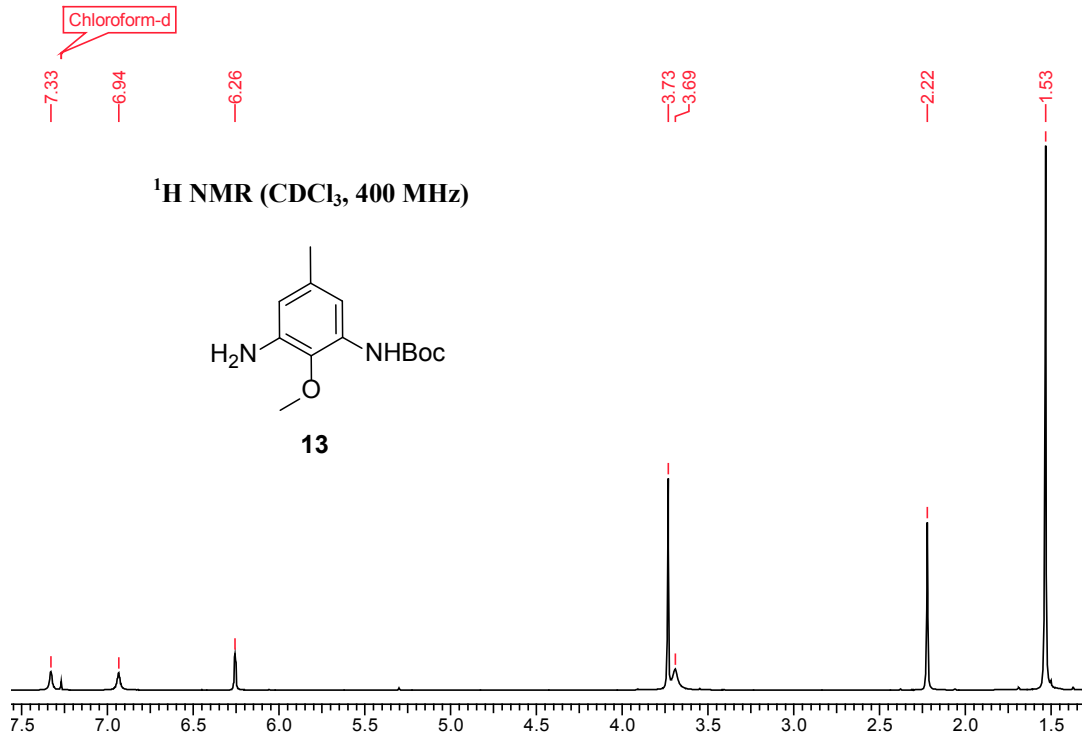




Dichloromethane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O
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7.5
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3.5
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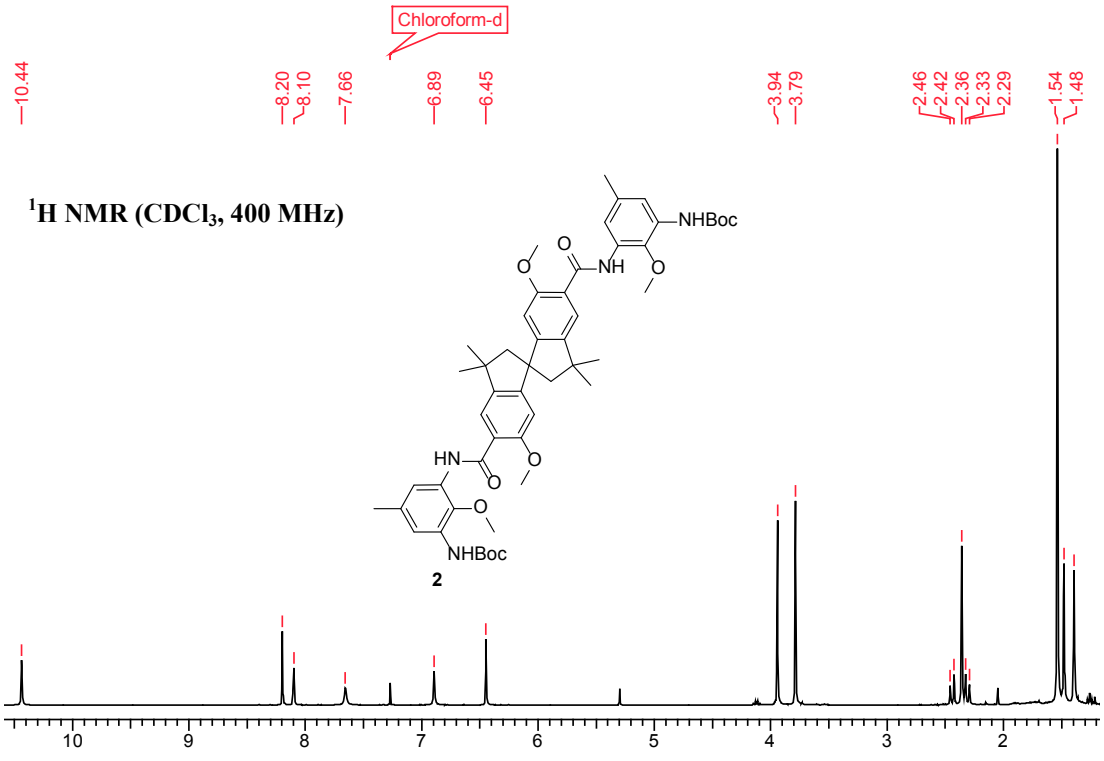
2.5
2.0
1.5


Chloroform-d
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8.10
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2.29
1.54
1.48
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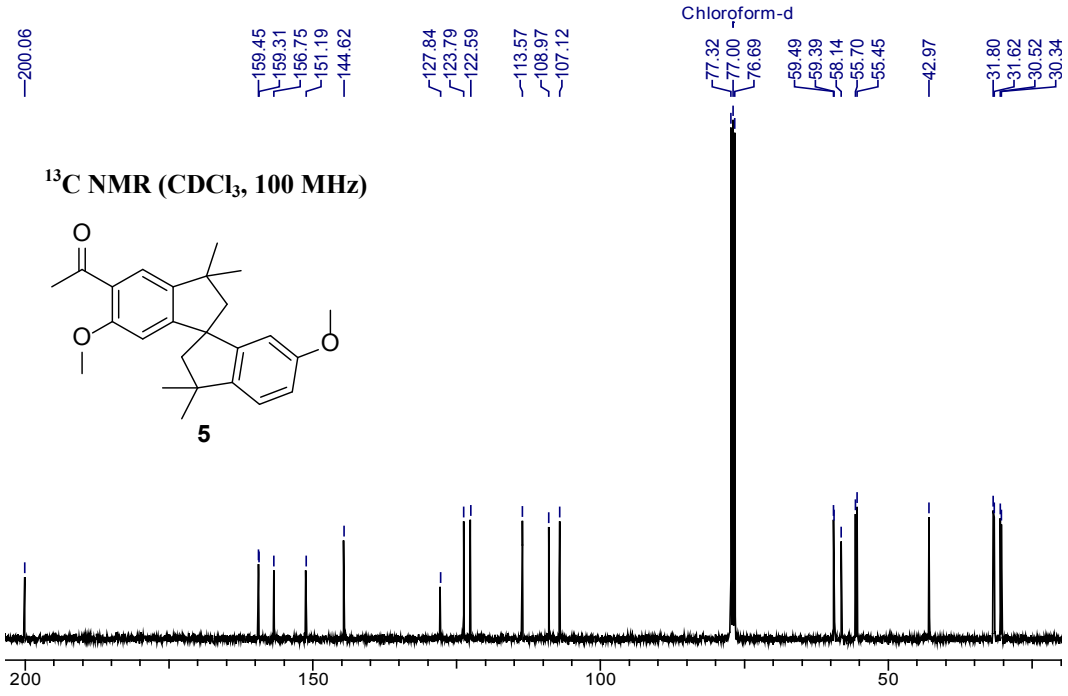
O
NH
O
O
HN
O
O
O
NHBoc
2
10
9
8
7
6
5
4
3
2



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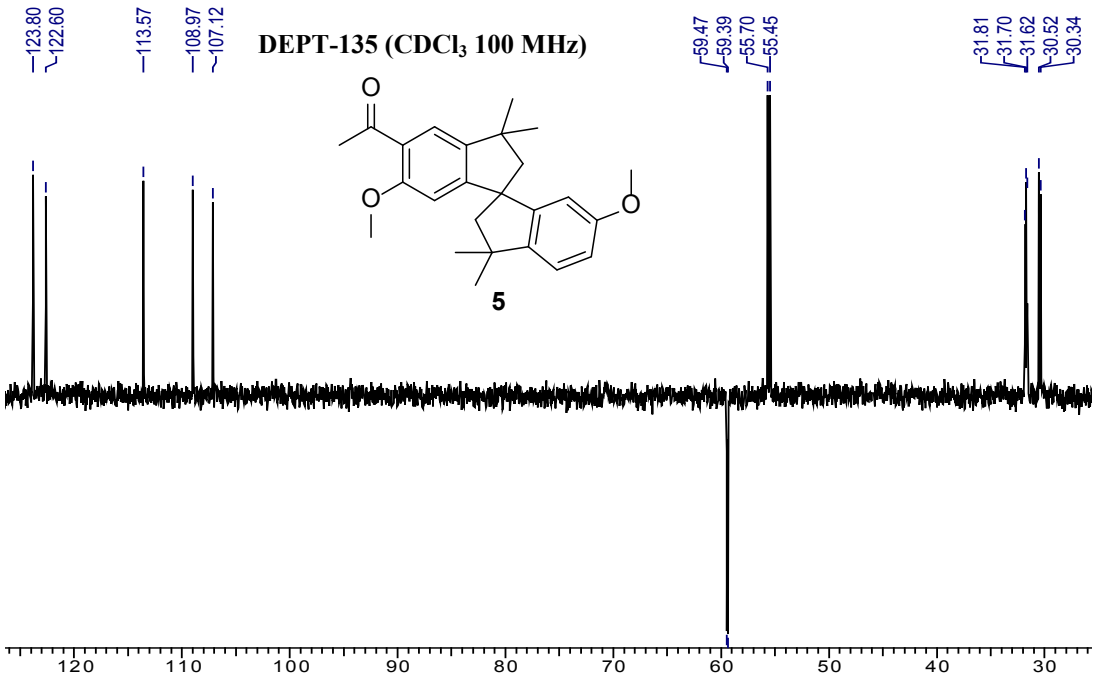

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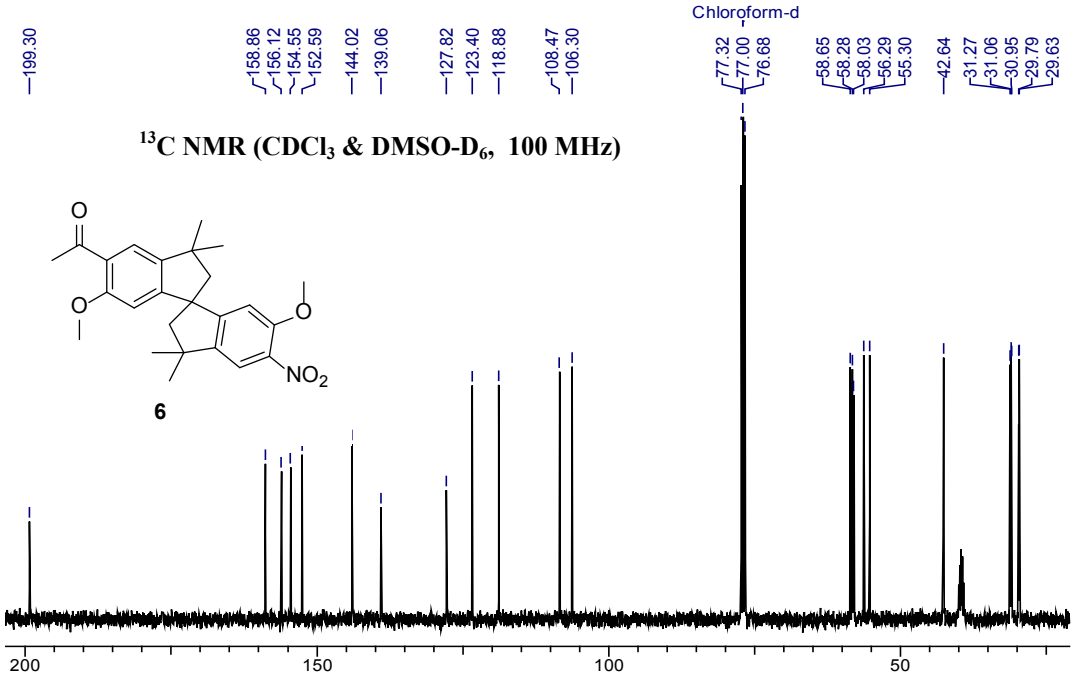

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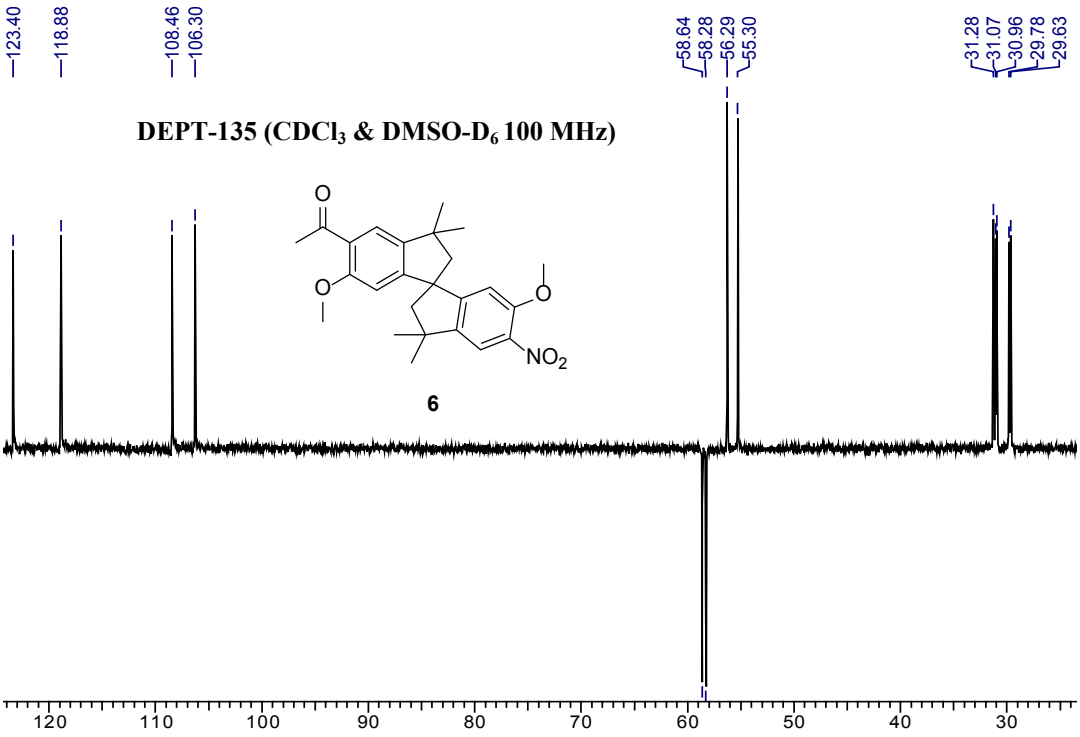
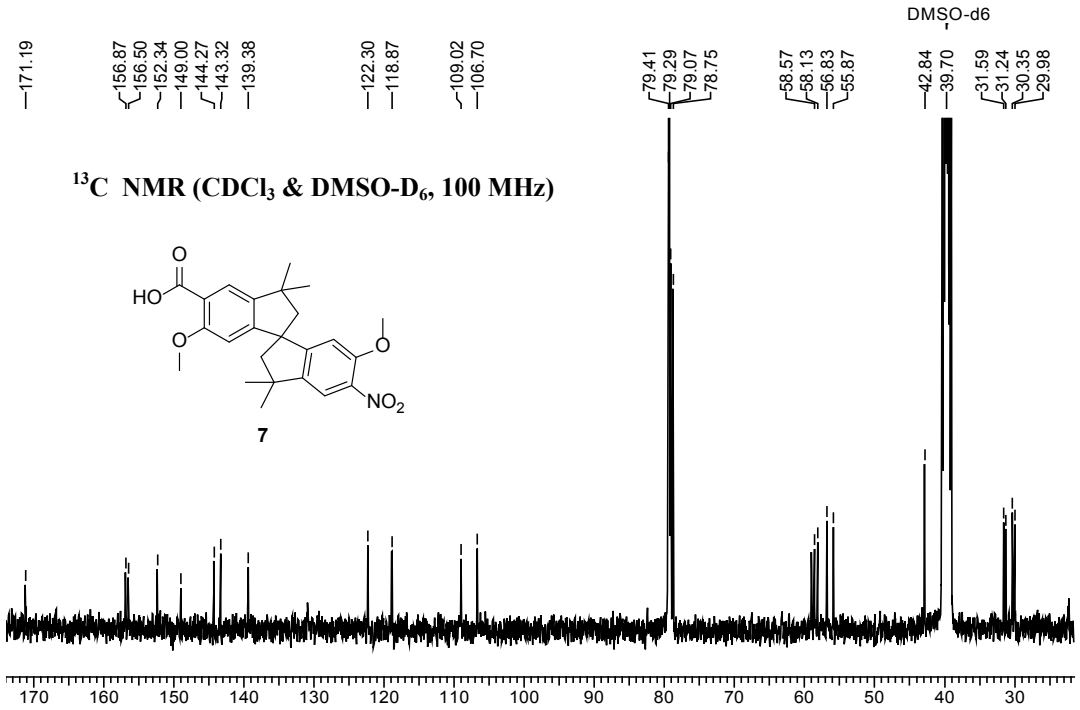
6



DEPT-135 ($CDCl_3$ & DMSO-D_6 100 MHz)

6



^{13}C NMR ($CDCl_3$ & DMSO-D_6, 100 MHz)

7

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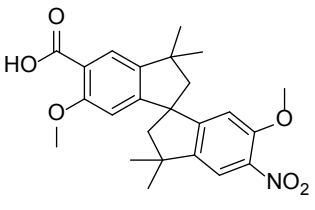

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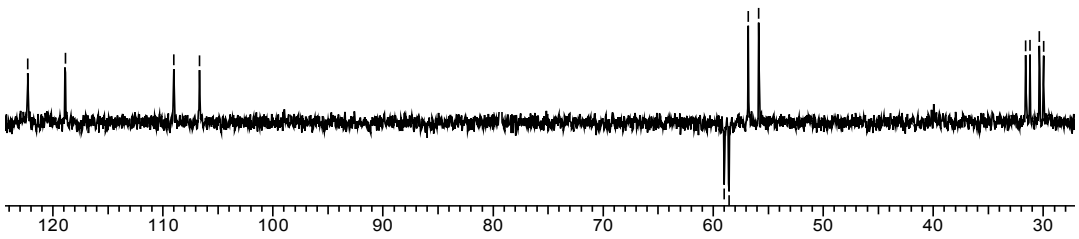

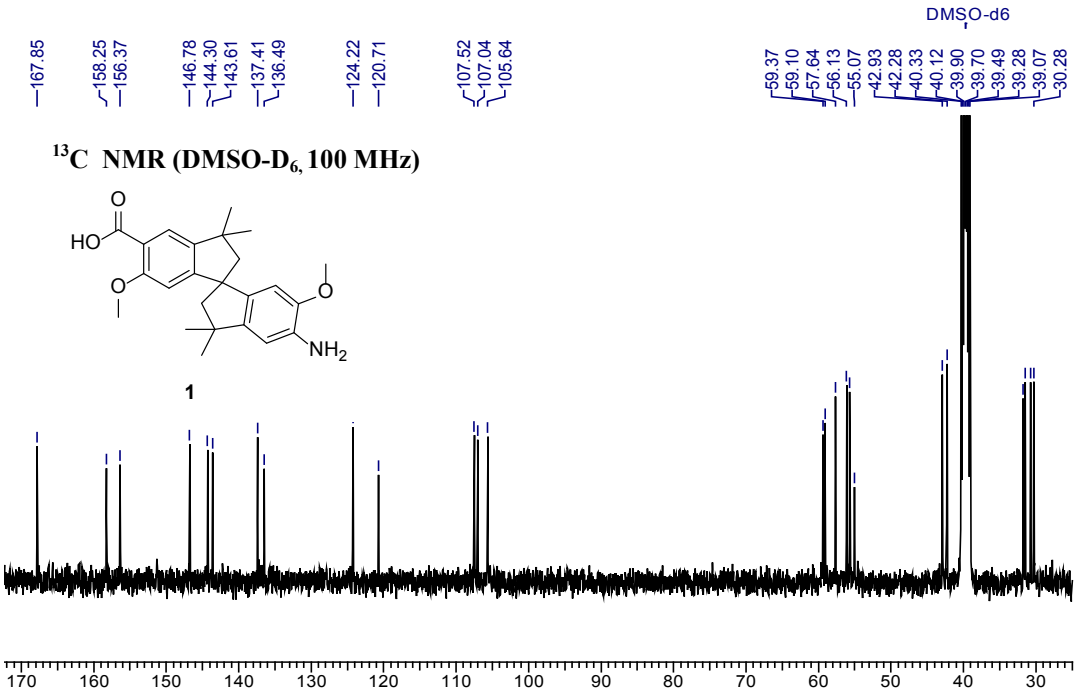

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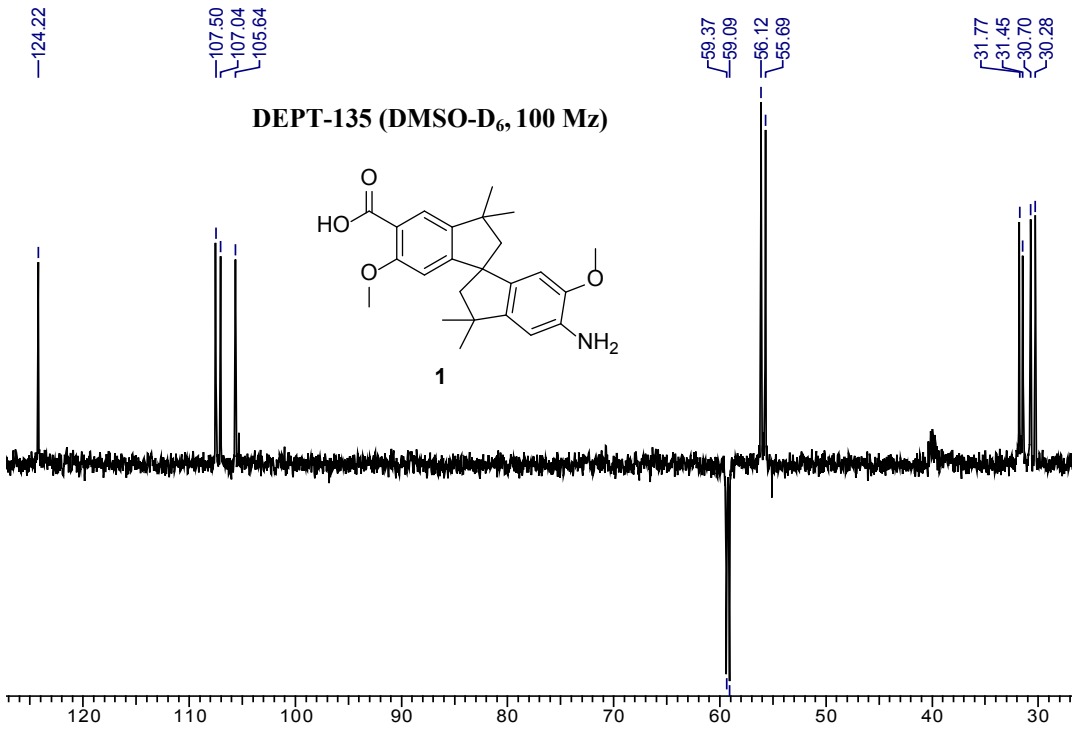

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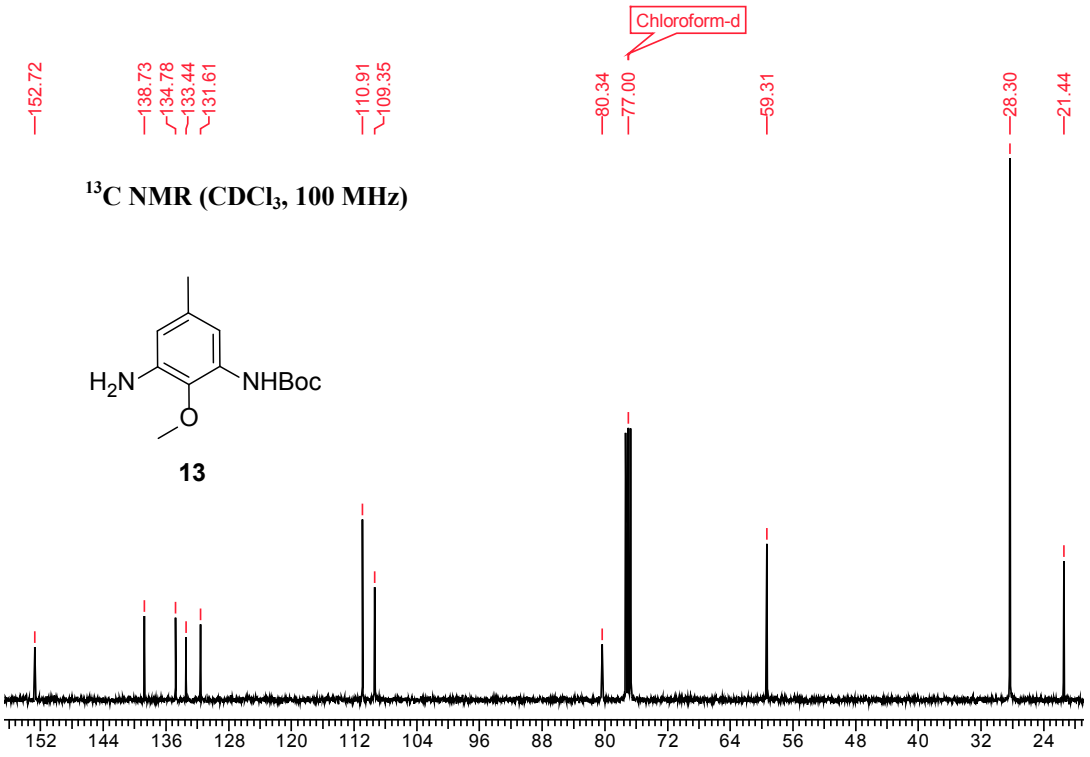

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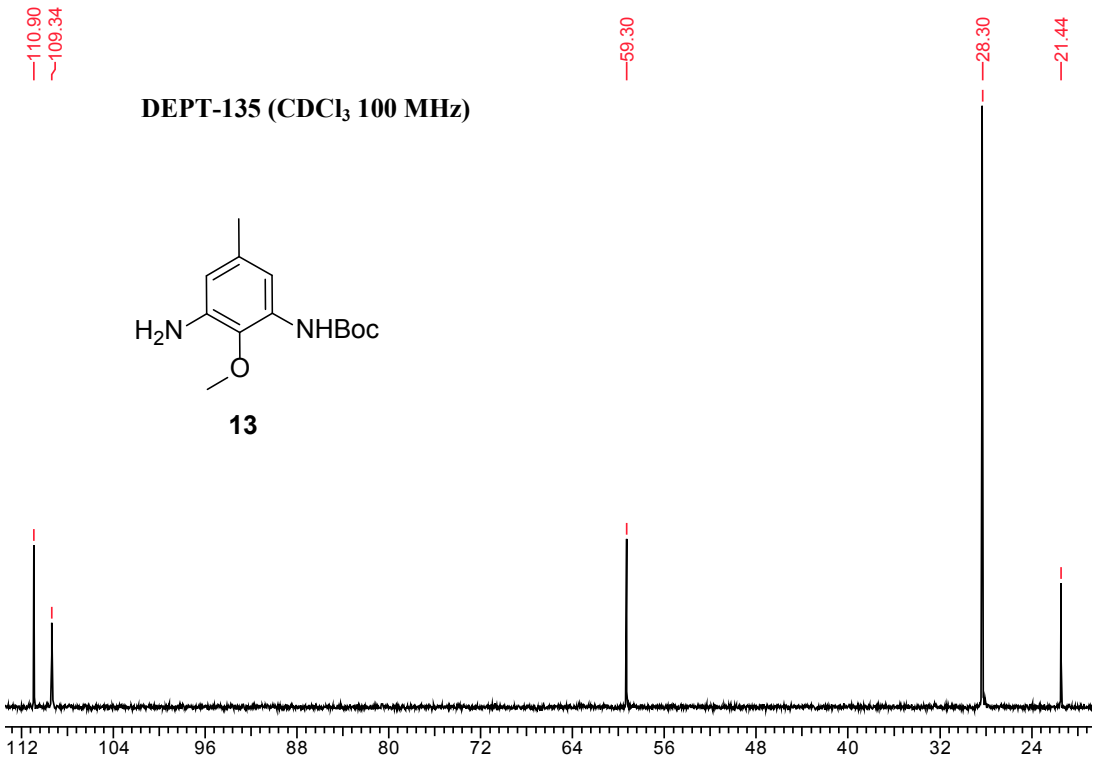

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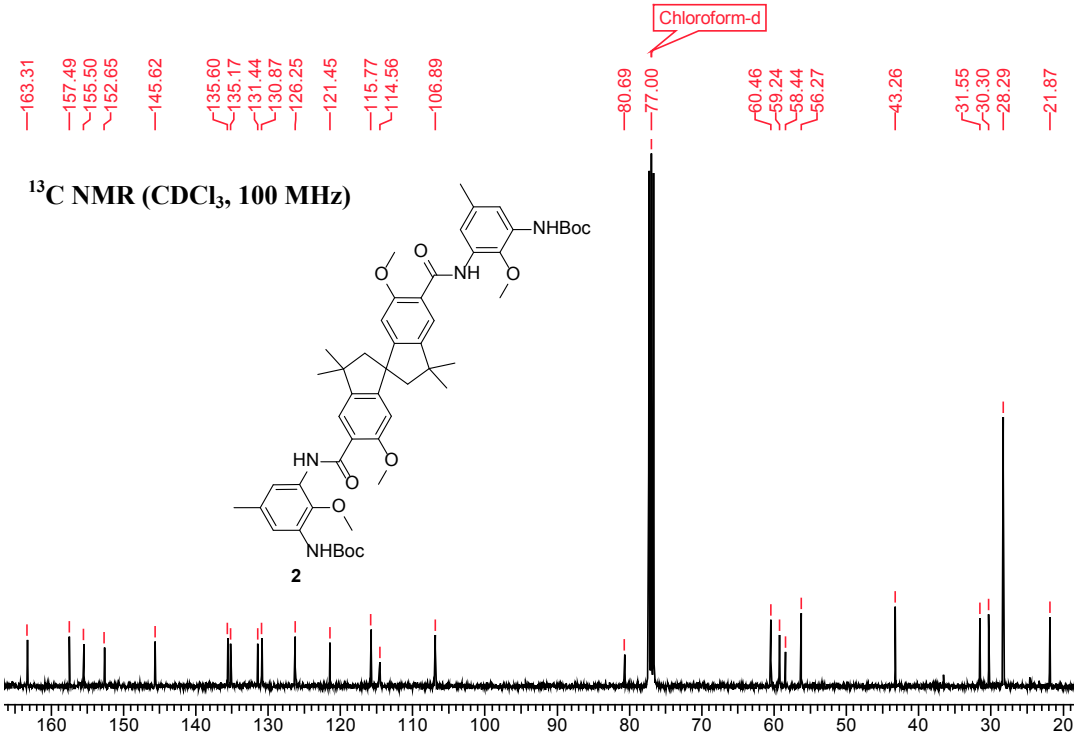
13



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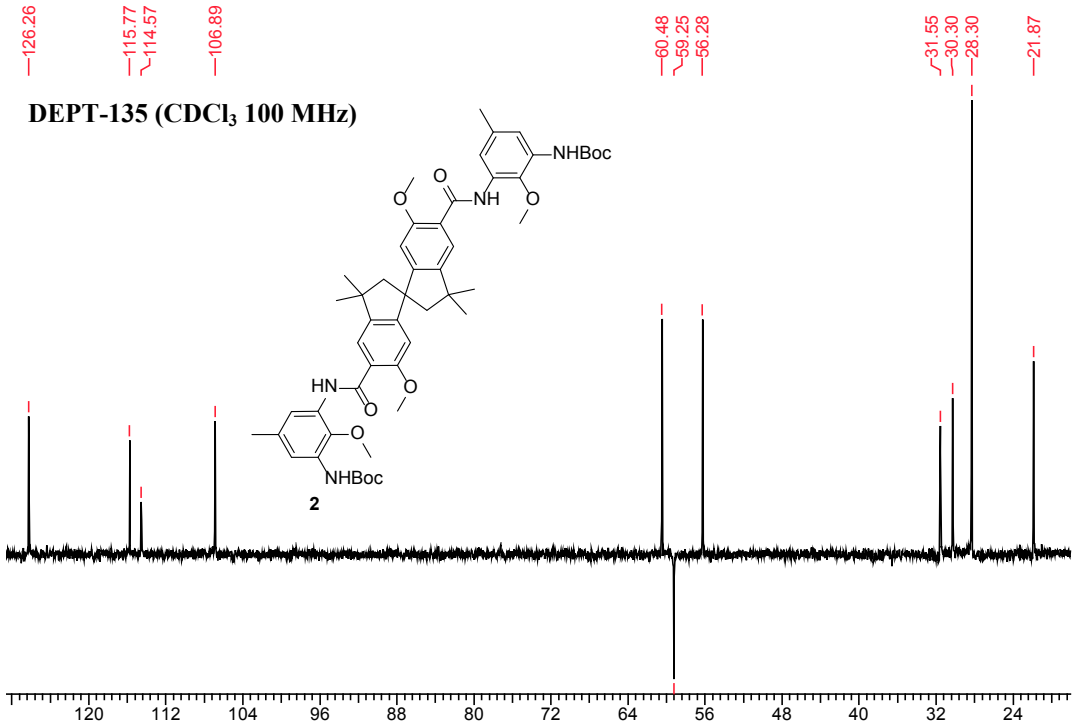
2



DEPT-135 ($CDCl_3$ 100 MHz)

2

2.10 References and notes

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CHAPTER 3

Probing the Folding Induction Ability of Orthanilic Acid in peptides: Some Observations

3.1 Introduction

In the past three decades, an extensive amount of attention has been given towards finding the best possible replacement for the peptide bond. This resulted in the development of a large array of peptide surrogates/isosteres.[1] In the previous two chapters, we already have discussed many such peptide bond surrogates in detail. Among the most promising candidates that can be used in place of peptide connectivity, a special place should be given to sulphonamides. A sulphonamide connecting-entity possesses chemical and physiochemical properties, some of which are extremely useful in overcoming the well-known shortcomings of the scissile peptide bond. In addition, sulphonamides possess higher proteolytic stability, polar character, and tetrahedral sulphur atom which mimics the intermediate formed during proteolysis.[2] These qualities make sulphonamides an ideal candidate to be used for developing therapeutic agents. Sulphonamides consequently forms an important class of drug, with many pharmacological agents showing antibacterial, antitumor, anticarbonic anhydrase, diuretic, hypoglycemic, antithyroid, or protease inhibitory activity among others (Fig. 3.1).[3,4]

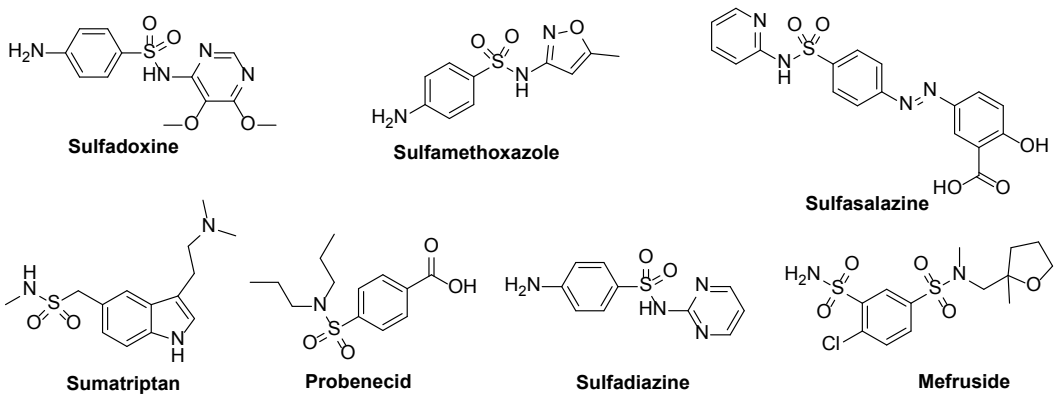


Fig. 3.1 Selected examples of sulphonamide-based drugs.

3.1.1 Sulphonamide as a connecting entity

Sulphonamide as a connecting entity is of great interest because it matches both the shape and electronic environment of the tetradedral transition-state of an amide bond. Sulphonamides have long been used as an amide bond replacement to generate various synthetic peptides.[5,6] Although similar, sulphonamide and a carboxamide have substantial conformational disparity due to the following factors: (i) sulphonamide NH is more acidic than carboxamide NH (RSO_2-NHR', pK_a~ l0-11), which makes it a stronger hydrogen bonding donor; (ii) presence of an additional H-bond acceptor oxygen atom provides sulphonamides an edge over carboxamides in the H-bonding predilection; (iii) rotation barrier about the S-N bond is much lower as compared to C-N bond, and consequently a sulphonamide bond is more flexible than the amide bond helping it to attain a twisted conformation that may induce folding,[7] and most importantly; (iv) the dihedral angle 'ω' in sulphonamides is found to be ~90°, whereas 'ω' is 180° in the case of carboxamides. Also, one of the H-N-S=O torsion angles is about 0°, whereas the H-N-C=O torsion angle is approximately around 180° (Fig. 3.2).

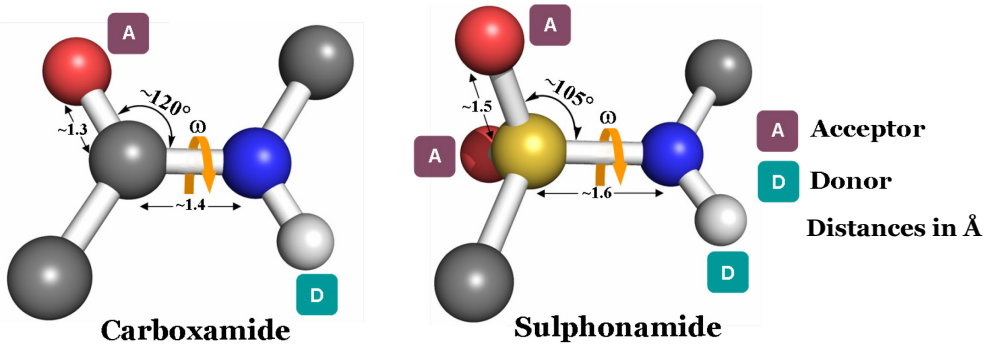


Fig 3.2 Comparison of the geometry of a carboxamide and sulphonamide bond.

These properties mentioned above makes sulphonamide a moiety of choice to be utilized as a connecting entity for developing oligopeptides. Moreover, the

conformational disparities may also lead to a difference in hydrogen bonding behavior thereby displaying interesting arrangements.

3.2 Objective of the present work and design strategy

Unnatural amino-acids are crucial for understanding and mimicking the bio-constructs. In the recent past, chemists have taken advantage of using unnatural amino-acids to develop peptidomimetic-based leads for drug discovery.[8] This is primarily because of the proteolytic stability of the unnatural amino-acids as compared to their natural counterparts which make them unsuitable to be used in therapeutics. Moreover, incorporation of unnatural amino-acids in a peptide sequence offers possibilities of generating diverse secondary structures. Over the years, various research groups have developed and utilized unnatural amino-acids to generate numerous peptidomimetic and foldamer molecules with interesting secondary structural preferences (Fig. 3.3).[9,10]

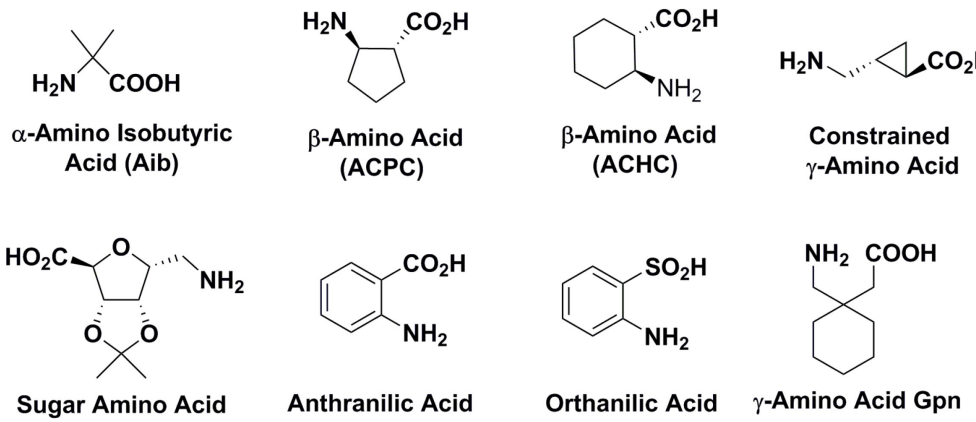


Fig. 3.3 Some of the important unnatural amino acids which have frequently been used for developing various peptidomimetic molecules.

We have already demonstrated the geometrical and H-bonding similarity between sulphonamide and carboxamide by putting orthanilic acid (2-amino benzenesulphonic acid, ^{S}Ant) in place of anthranilic acid (Ant) in the Ant-Pro (anthranilic acid-proline) turn segment which showed the existence of a strong 9-

membered-ring H-bonding.[11] The results obtained were quite fascinating given the fact that these groups have relatively different H-bonding and geometrical preferences.[12] But the literature precedents and the geometric disparity of sulphonamides as compared to carboxamides are too significant to be satisfied with a single result. Consequently, to explore further in the direction of developing novel peptidomimetics, we thought of designing a range of short chain oligopeptides featuring ^{S}Ant-Pro as the potential turn inducer (Fig. 3.4).

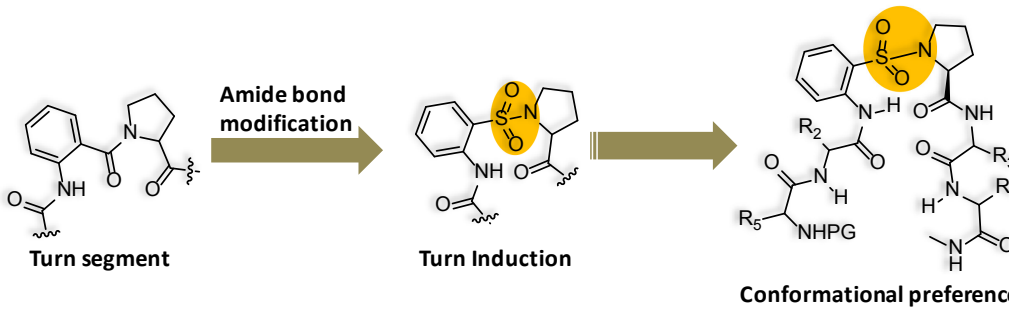


Fig. 3.4 Design strategy to develop oligomer sequences containing orthanilic acid as a connecting entity

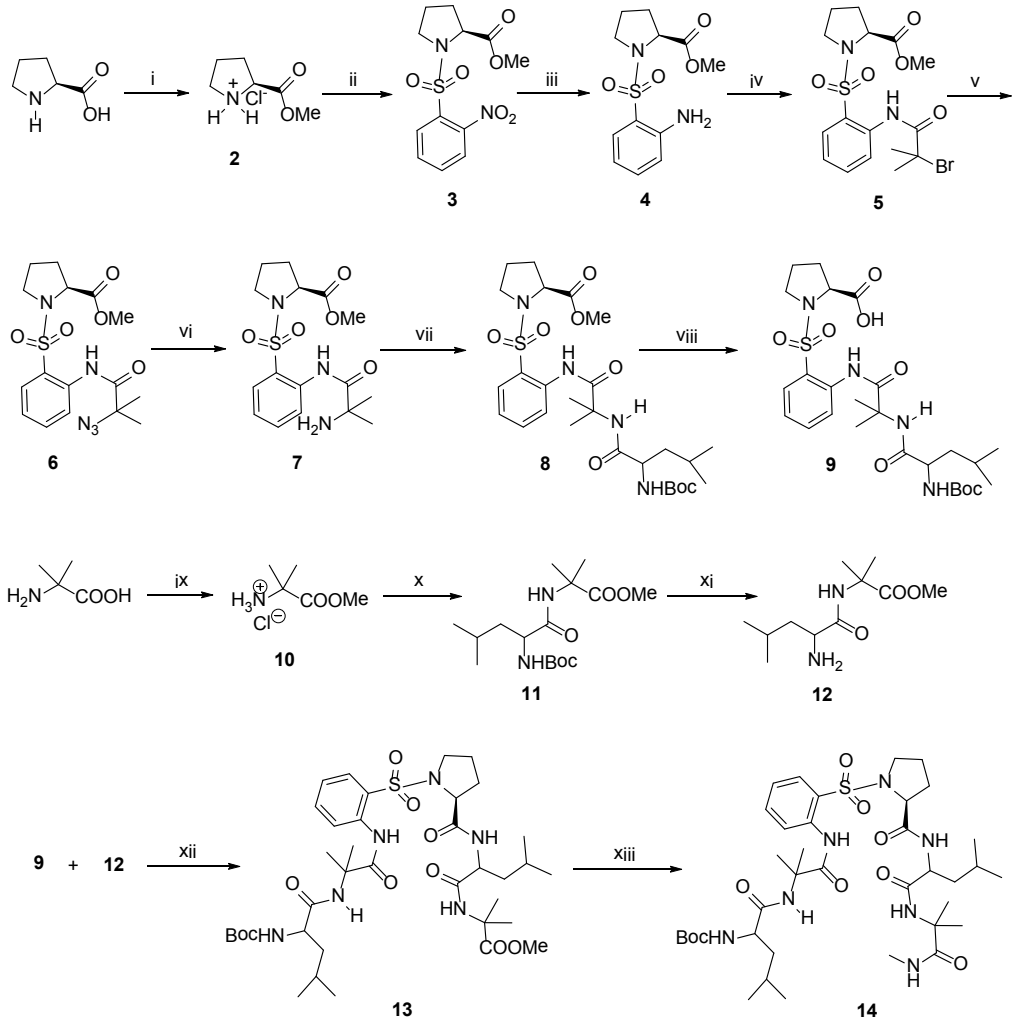
3.3 Results and discussion

3.3.1 Synthesis

Synthesis of the foldamer sequence bearing sulphonamide connection started with L-proline**,** which was subjected to esterification followed by coupling with 2-nitro sulphonyl chloride to obtain the coupled product **3** in 57% yield (Scheme 3.1). Dimer **3** on catalytic reduction furnished amine **4** in quantitative yield. Coupling of amine **4** with 2-bromo-2-methylpropanoyl bromide gave **5** in 90% yield, which was subjected to nucleophilic substitution to furnish **6** in very good yield. The trimer azide **6** was then reduced in presence of H_2, Pd-C and was coupled with Boc-Leu-OH in presence of EDC.HCl as coupling agent to obtain tetramer **8** in 85% yield. Ester hydrolysis of **8** was followed by coupling with free amine of dipeptide **12** (which was synthesized by coupling Boc-Leu-OH with H-

Aib-OMe and subsequent Boc deprotection in presence of TFA),[13] furnished hexamer **13** in 75% yield.

Scheme 3.1 Synthesis of oligomer **14**

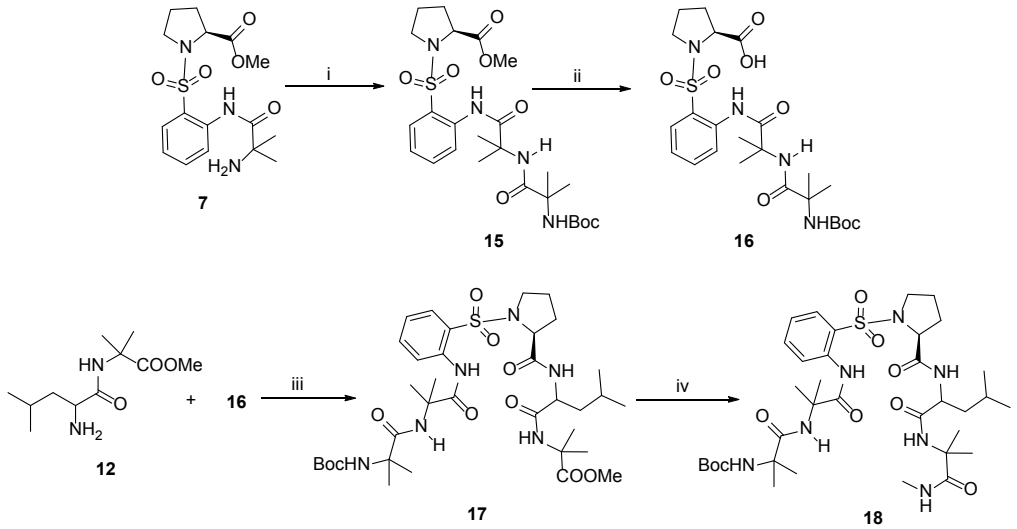


Reagents and conditions: (i) $SOCl_2$, MeOH, reflux, 8 h; (ii) 2-nitrobenzene-1-sulphonyl chloride, Et_3N, DCM, 8 h, 57%; (iii) H_2, Pd-C, MeOH, 12 h; (iv) 2-bromo-2-methylpropanoyl bromide, Et_3N, DCM, 12 h, 90% (v) NaN_3, DMF, 75 °C, 12 h, 81%; (vi) H_2, Pd-C, 60 psi, 10 h; (vii) Boc-Leu-OH, EDC.HCl, HOBt, DCM, 10h, 85%; (viii) LiOH, MeOH, H_2O, 4 h; (ix) $SOCl_2$, MeOH, reflux, 8 h; (x) (a) DIPEA, DCM; (b) Boc-Leu-OH, EDC.HCl, HOBt, 10h, 90%; (xi) TFA, DCM, 2h; (xii) EDC.HCl, HOBt, DCM, 10 h, 75%; (xiii) sat. methanolic $MeNH_2$, 8 h, 80%.

Hexamer **13** on treatment with sat. methanolic $MeNH_2$ produced the methyl amide analog **14** in 80% yield. Results obtained during the conformational analysis (*vide infra*) of oligomer **14** prompted us to replace the Leu moiety at the

N-terminal by Aib and Pro for developing two more sequences.

Scheme 3.2 Synthesis of oligomer **18**

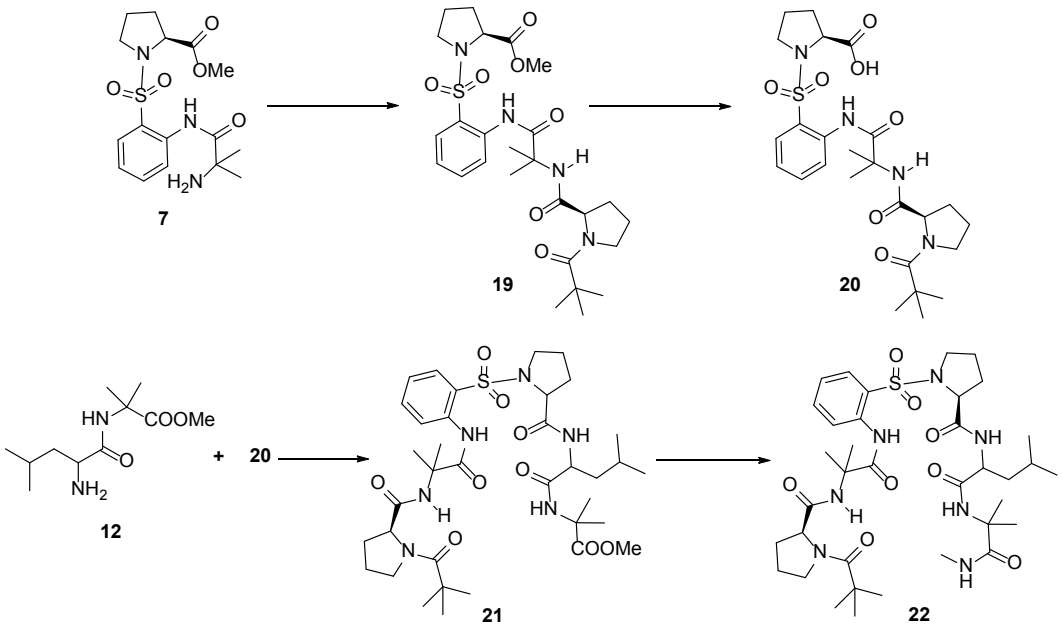


Reagents and conditions: (i) Boc-Aib-OH, EDC.HCl, HOBt, DCM, 10 h, 90%; (ii) LiOH, MeOH, H_2O, 4 h; (iii) EDC.HCl, HOBt, DCM, 10 h, 82%; (iv) sat. methanolic $MeNH_2$, 8 h, 82%.

To prepare the sequence with α-amino isobutyric acid (Aib) at the N-terminus, we started with the trimer amine **7** (Scheme 3.2) which was coupled with Boc-Aib-OH, in presence of EDC.HCl as coupling agent to obtain the tetramer **15** in 85% yield. Ester hydrolysis of the tetramer **15** was followed by coupling it with the free amine of dipeptide **12** produced hexamer **17** in 82% yield. Hexapeptide **17** containing constrained amino acid Aib at the N-terminus was then treated with sat. methanolic $MeNH_2$ furnishing the methyl amide analog **18** in 82% yield.

The third sequence having proline at the N-terminus was synthesized starting from the same free amine **7** (Scheme 3.3) which when coupled with Piv-Pro-OH, furnished the tetramer **19** in 86% yield. Free acid **20** obtained after basic hydrolysis was coupled with the free amine of dipeptide **12** to attain the hexamer **21** in 73% yield, which on stirring with sat. methanolic $MeNH_2$ furnished the methyl amide analog **22** in 86% yield.

Scheme 3.3 Synthesis of oligomer **22**



Reagents and conditions: (i) Piv-Pro-OH, EDC.HCl, HOBt, DCM, 10 h, 88%; (ii) LiOH, MeOH, H_2O, 4 h; (iii) EDC.HCl, HOBt, DCM, 10 h, 73%; (iv) sat. methanolic $MeNH_2$, 8 h, 86%.

3.3.2 Solid-state conformational analysis

Intensive effort to crystallize the oligomers resulted in the formation of crystals of oligomer **14** (Fig. 3.5). Careful analysis of the crystal data revealed the presence of three inter-residual H-bonding, and a 6-membered intra-residual H-bonded ring between NH and S=O of the orthanilic acid moiety [d(C=O…H-N) = 1.98 Å, bond angle (N-H…O) = 141°]. Of the three different types of inter-residual H-bonding patterns, (i) one was a 10-membered ring (C10 H-bonding) between the C-terminus amide NH and the carbonyl of proline moiety [d(C=O…H-N) 2.24 Å, bond angle (N-H…O) = 130°], (ii) a 7-membered ring H-bonding between S=O of ^{S}Ant and N-H of Leu_2 [d(C=O…H-N) 2.71 Å, bond angle (N-H…O) = 123°], and (iii) an unusual, long range (15-membered) H-bonding between N-H of Aib_1 and C=O Leu_2 [d(C=O…H-N) 2.02 Å, bond angle (N-H…O) = 167°].

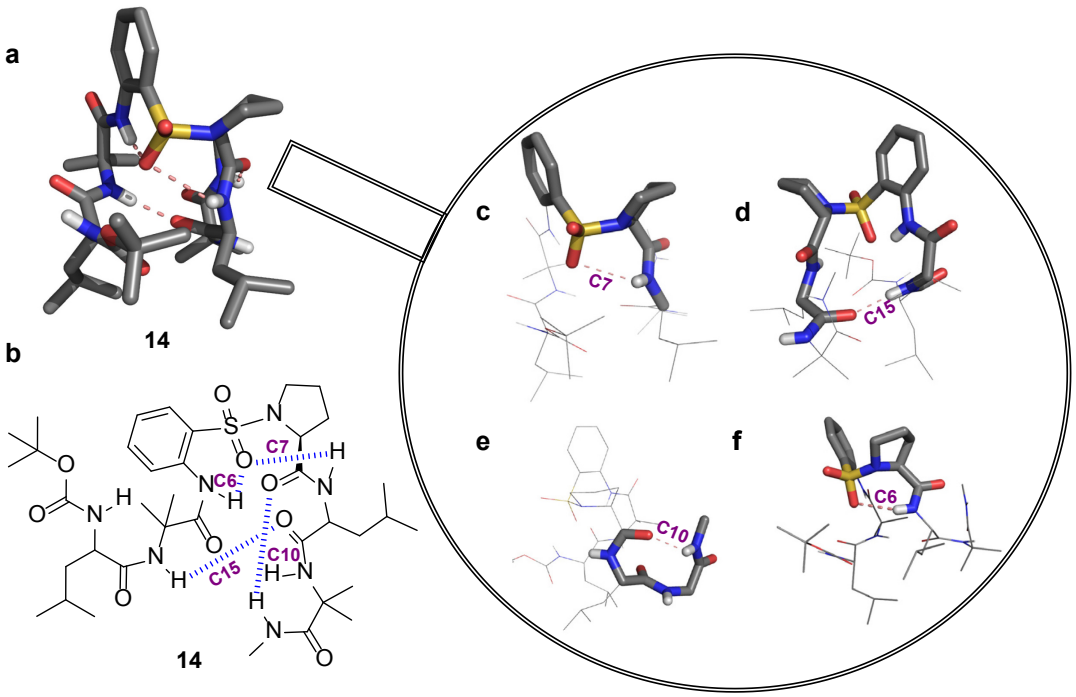


Fig. 3.5 Crystal (a) and molecular structure (b) of oligomer **14**, and different types of H-bondings observed (c-f).

Dihedral angle values shed more light on the observed conformation of the oligomer **14** containing sulphonamide connecting entity. As evident from the crystal structure, torsion angles of ^{S}Ant were: ϕ= 174.68°, θ= 5.01°, ψ= 92.55° and ω= –55.31°. In the case of proline, ϕ and ψ was found to be -107.85° and 19° respectively. Thus, the solid-state conformational analysis clearly indicates that the torsional constraint of the orthanilic acid (^{S}Ant) residue is a key factor for the folded conformation seen in oligomer **14**. Crystal structure investigation also suggested that the Leu_1 residue at the C-terminus has reversed. This can happen by virtue of the flexible nature of leucine, owing to which rotation about the single bond was possible, or probably because that was the most favourable conformation the molecule could adopt in the solid state.

3.3.3 Conformational Investigation in Solution-State

We undertook extensive NMR studies to provide insights into the solution-state conformation of the oligomers. Details of the peak assignments with spectra are provided in the ESI. All the oligomers were readily soluble in nonpolar organic solvents (>>100 mM in $CDCl_3$) at ambient temperature. Signal assignments were made unambiguously using a combination of two-dimensional COSY, TOCSY, HSQC, HMBC and NOESY experiments. Oligomers **14** and **18** exhibited sharp signals for Boc-CH_3 in ^{1}H NMR, whereas in case of the oligomer **22** having proline at the N-terminus showed multiple signals for the pivoloyl group implying the existence of *cis-trans* isomerization.[14] This is presumably due to the slow rotation of the bond connecting the N-terminus proline nitrogen and the carbonyl of pivoloyl protecting group. As the crystal structure of **14** revealed the presence of an unusual 15-membered H-bonded ring, most characteristic nOes should be a long range inter-residual dipolar coupling of CH_3 of Aib_1 with NH5 and C33H in order to be in agreement with the solid state conformation. Furthermore, the formation of the C15 H-bonding requires the oligomer backbone to fold back considerably resulting in a short distance between the residues (Fig. 3.6). The characteristic nOes supporting the folded conformation in **14** were the inter-residual coupling between Aib_1-CH_3 (C10H) and NH5 along with Aib_1-CH_3 (C10H) and C33H. Other important nOes were found between: Pro-αH (C18H) and Aib_1-CH_3 (C10H), Pro-αH (C18H) and ^{S}Ant (C14H). Dipolar coupling also was seen between Pro-αH (C18H) and NH3. However, there were no nOes observed between Boc-CH_3 and the C-terminus methyl (C33H) as anticipated from the single crystal X-ray diffration analysis.

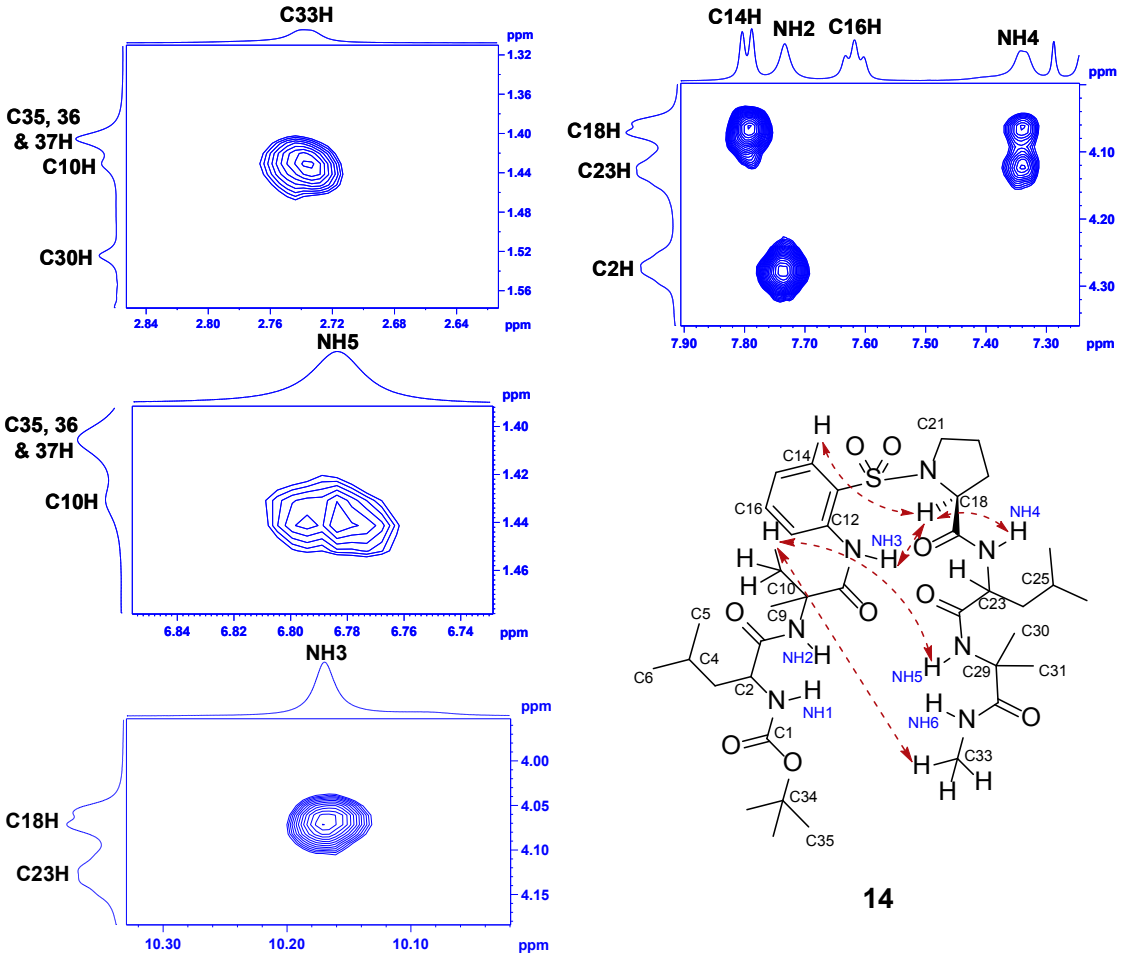


Fig. 3.6 Selected nOe excerpts from the 2D NOESY data of oligomer **14** in $CDCl_3$.

For the oligomer **18,** nOes that supported the folded conformation were between Pro-αH (C16H) and NH3, NH3 and NH4, C33H and Aib_2-CH3 (C8H). Inter-residual nOes were also observed between NH3 with NH2 and NH5 (Fig. 3.7a)

In the case of oligomer **22**, inter-residual dipolar couplings were observed between Pro_2-αH (C17H) and NH2, Pro_2-αH (C17H) and Aib_1-CH_3 (C9H), C-terminus methyl (C33H) and Aib_1-CH_3 (C9H). Also, observed were nOes of Pro_1-αH (C2H) with NH2 and Aib_2-CH_3 (C30H) (Fig. 3.7b). Similar nOe patterns of the identical central residues in all the oligomers suggest the prevalence of folded conformation, as anticipated.

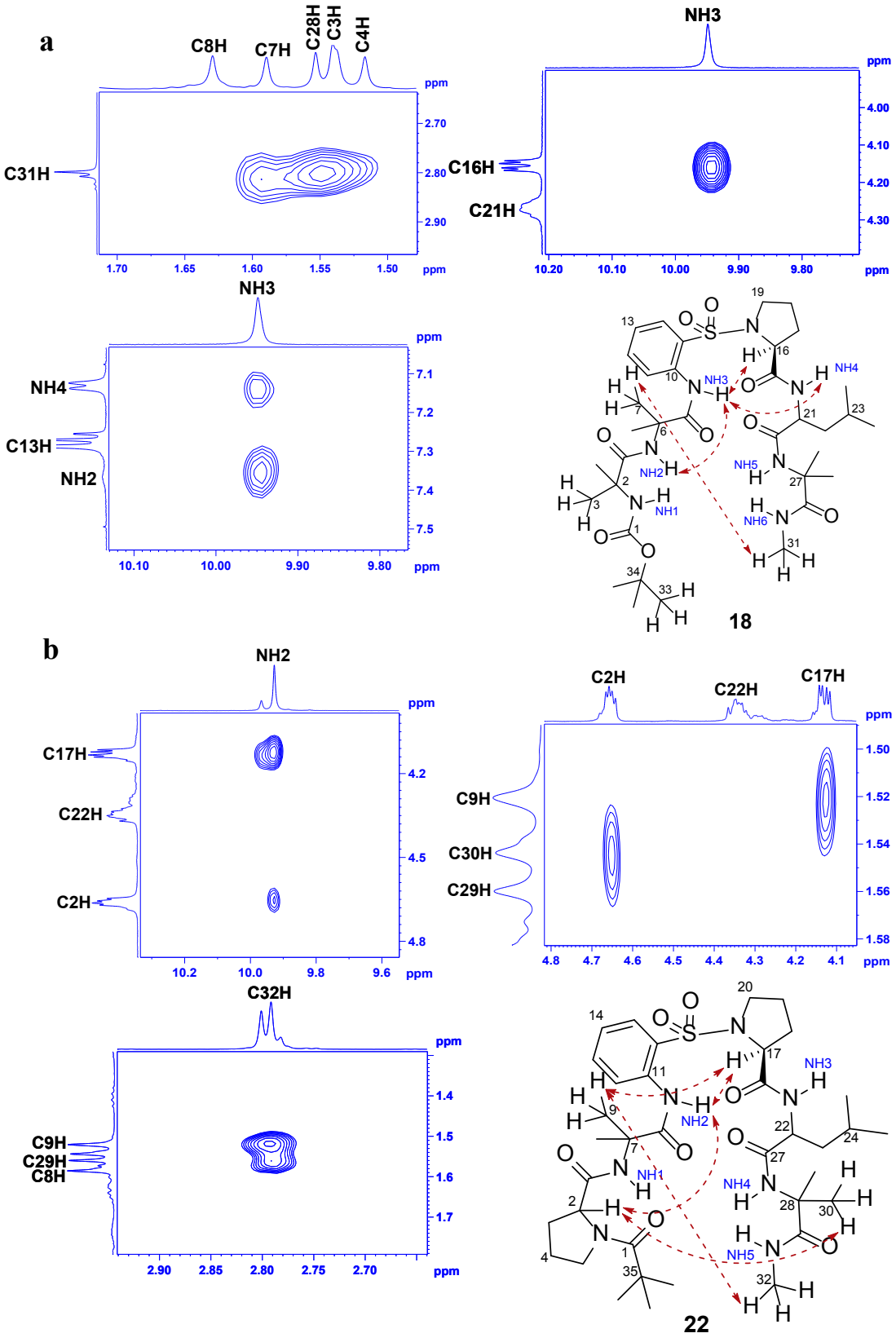


Fig. 3.7 Selected NOE excerpts from the 2D NOESY data of oligomer **18** (a) and **22** (b) in $CDCl_3$.

To investigate the existence of intramolecular hydrogen-bonding in the oligomers, we also undertook DMSO-d_6 titration and variable temparature experiments (Fig. 3.8). All the NHs of **14** involved in intramolecular hydrogen bonding showed very little shift in the titraton studies [Δδ (NH) < 0.25 ppm]. Only NH5 underwent relatively larger chemical shift change [Δδ (NH5) < 0.7 ppm] on incremental addition of DMSO-d_6, suggesting its non-participation in H-bonding. Similar was the case for oligomer **18** where a considerable change in chemical shift was observed for NH5 and NH1 [Δδ (NH) < 0.52 ppm], while rest of the NHs showed negligible shift [Δδ (NH) < 0.18 ppm]. Oligomer **15** on the other hand showed very little change in chemical shift values for all the NHs [Δδ (NH) < 0.25 ppm], except for NH3 [Δδ (NH) < 0.49 ppm], implying the weakness of H-bonding.

Variable temperature experiment of **14, 18** and **22** were mostly in agreement with the observations from the other studies, showing temperature coefficient (Δδ/ΔT) >-3.8 ppb/K for all NHs, except for NH2 of compound **14** and NH4 of **18** [(Δδ/ΔT) for NH2 of **9** = -6.5 ppb/K and (Δδ/ΔT) for NH4 of **18** = -5.6 ppb/K] (Fig. 3.8d-f). This suggests that the 15-membered H-bonding in compound **9** is relatively weak, specially at higher temperature. Same is applicable for NH4 of compound **18.**

All the interesting results found during the solid and solution state studies amplified our desire to study the conformational features of the oligomer **18** and **22** which could not be crystallized, in spite of best efforts put in. This impelled us to undertake nOe based molecular modeling studies.

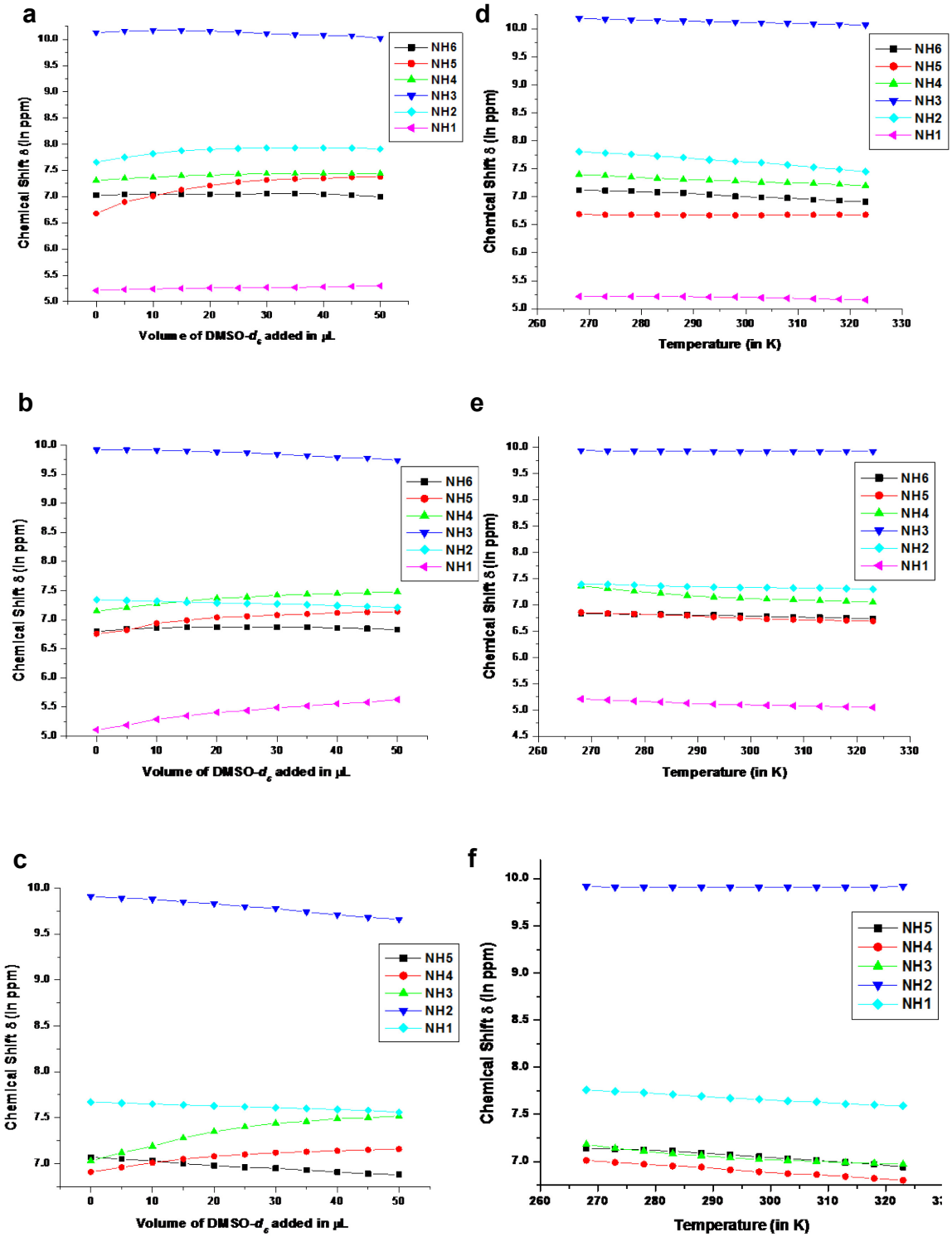


Fig. 3.8 DMSO-d_6 NMR titration plots of **14** (a), **18** (b) and **22** (c) in $CDCl_3$ (10 mmol) and variable temparture plots of **14** (d), **18** (e) and **22** (f) in $CDCl_3$ (10 mmol).

3.3.4 Solution-state structural investigations

NMR-based structures of compound **18** and **22** were derived from nOe

cross peaks by using Maestro v9.3.518 from Schrödinger (for complete table please refer to experimental section) (Fig. 3.9). The twenty lowest-energy superimposed structures of compound **18** and **22** showed a root mean square deviation (RMSD) of 0.26 ± 0.06 Å and 0.15 ± 0.06 Å respectively. The dynamic ensemble structures of compound **18** reveals that the N-terminus did not reverse as expected when Aib was put in place of Leu. The H-bonding pattern although remained the same as observed for oligomer **14**. However, the solution state structural analysis of compound **18** showed the presence of a second 10-membered ring (C10 H-bonding) between the NH of ^{S}Ant and the carbonyl of pivoloyl moiety. Also, the 7-membered ring H-bonding between S=O of ^{S}Ant and N-H of Leu_2 seen in the previous cases was no more intact, which was obvious from the DMSO titration studies. Rest of the H-bonding patterns remains the same. Thus, the nOe-derived structural investigations concludes that the oligomers exist in a folded conformation.

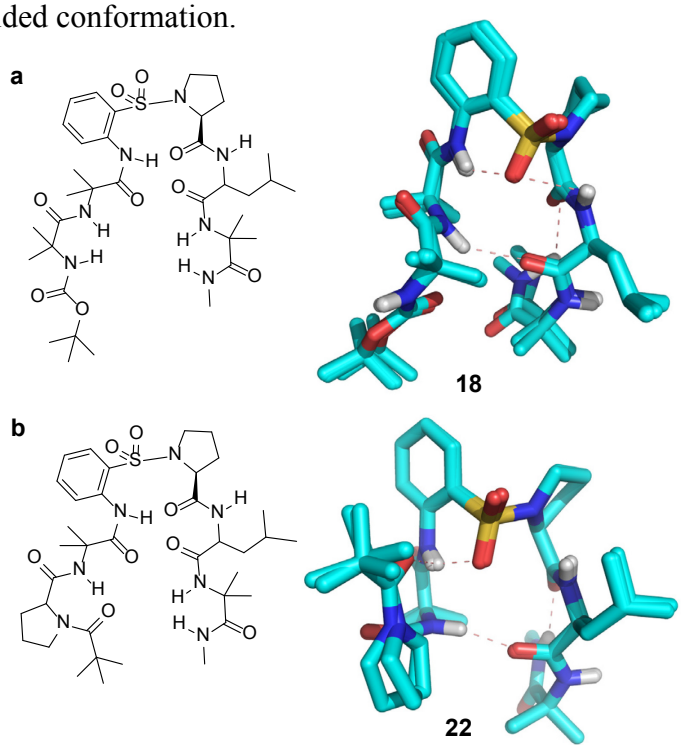


Fig. 3.9 Molecular and nOe-derived structures of compounds **18** (a) and **22** (b) showing folded conformation

3.4 Conclusion

In summary, all the oligomers carrying ^{S}Ant as the turn inducer showed compact structures with folded conformation, which supports the abilty of sulphonamides to promote folding. Moreover, all the oligomers containing ^{S}Ant connect showed unusual and long range 15-membered ring H-bonding involving four amino-acid residues. These results further validate our observations that orthanilic acid (2-aminobenzenesulfonic acid, ^{S}Ant) is a strong reverse-turn inducer when incorporated into peptide sequences.[15]

3.5 Experimental Section

Crystal data for **14:** $C_{37}H_{61}N_7O_9S$, M = 802.41, Colourless crystals, approximate size 0.50 x 0.48 x 0.07 mm, Total frames = 1651, Crystals belong to orthorhombic, space group P 212121, a = 10.4234(3), b = 10.5165(2), c = 39.4004(9) Å, β = 104.754 (10)º , V = 4318.98(18) Å^3, Z = 6, D_c = 1.291 mg m^{-3}, μ (MoK$_\alpha$) = 0.175 mm^{-1}, T = 296(2) K, 34067 reflections measured, 10683 [R(int) = 0.0347] unique [I>2σ(I)], R1 = 0.0744, wR2 = 0.2194.

***(S)*-methyl 1-(2-nitrophenylsulphonyl)pyrrolidine-2-carboxylate 3:**

L-proline methyl ester hydrochloride (0.82 g, 4.96 mmol) was added to a solution of 2-nitrobenzenesulphonylchloride (1.0 g, 4.51 mmol) in anhy. DCM (10 mL) at 0 °C followed by the addition of Et_3N (1.45 mL, 10.38 mmol). The resulting mixture was then allowed to attain room temperature and was stirred for 12 h. It then was sequentially washed with sat. $NaHCO_3$, water, dil. HCl and brine. Organic layer was then dried over anhy. Na_2SO_4 and evaporated under reduced pressure to get the crude product which on purification by column chromatography furnished **3** as an off-white solid. Yield: 0.81 g (57%); mp: 85-86 °C; $[\alpha]^{26}_D$: -100.0° (*c* 1.6, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3621, 3418, 3020, 1746, 1640, 1546, 1371, 1216, 1163, 770; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.08 (s, 1H), 7.71-7.69 (m, 2H), 7.63-7.62 (s, 1H), 4.58-4.57 (d, J = 8 Hz, 1H), 3.66 (s, 3H), 3.62-3.60 (m, 1H), 3.55-3.52 (m, 1H), 2.29-2.22 (m, 1H), 2.10-1.94 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 172.2, 148.0, 133.6, 133.5, 132.7, 132.6, 131.6, 130.9, 124.0, 60.8, 52.3, 48.4, 30.8, 24.4; ESI MS: 337.04 $(M+Na)^+$; Anal. Calcd for $C_{12}H_{14}N_2O_6S$: C, 45.85; H, 4.49; N, 8.91; Found: C, 45.32; H, 4.73; N, 9.28.

***(S)*-methyl 1-(2-aminophenylsulphonyl)pyrrolidine-2-carboxylate 4:**

10% Pd/C (0.015 g) was added to a solution of **3** (0.15 g, 0.47 mmol) in methanol (6 mL). The reaction mixture was then stirred at 60 psi under hydrogen atmosphere for 8 h, followed by filtration of the catalyst through celite and the filtrate was evaporated to get product **4** which was carried forward without further purification.

***(S)*-methyl-1-(2-(2-bromo-2-methylpropanamido)phenylsulphonyl) pyrrolidine-2-carboxylate 5:**

To a solution of **4** (4.0 g, 14.1 mmol) in dry DCM (25 mL), anhy. Et_3N (2.55 mL, 18.3 mmol) was added at 0 °C followed by slow addition of 2-bromo-2-methylpropanoyl bromide (1.92 mL, 15.5 mmol). The resulting mixture was then allowed to come to room temperature and was stirred for 12 h, following which it was sequentially washed with sat. $NaHCO_3$, water and brine. Organic layer was then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which on purification by column chromatography furnished **5** as a viscous liquid. Yield: 5.5 g (90%); $[\alpha]^{26}_D$: -63.08° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3349, 3020, 1740, 1688, 1588, 1338, 1215, 1154, 759; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.41 (s, 1H), 8.55-8.53 (d, *J* = 8 Hz, 1H), 7.91-7.89 (dd, *J* = 8.0, 1.6 Hz, 1H) 7.61-7.57 (t, *J* = 7 Hz, 1H), 7.26-7.22 (t, *J* = 7 Hz, 1H), 4.39-4.36 (dd, *J* = 8.0, 2.5 Hz, 1H), 3.64-3.59 (m, 4H), 3.49-3.43 (m, 1H), 2.13-2.09 (m, 1H), 2.06 (s, 6H), 2.04-1.98 (m, 2H), 1.93-1.84 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.9, 170.4, 136.5, 134.2, 129.6, 125.8, 124.0, 122.1, 60.4, 59.9, 52.5, 48.4, 31.7, 31.0, 24.5; ESI MS: 455.01 $(M+Na)^+$; Anal. Calcd. for $C_{16}H_{21}BrN_2O_5S$: C, 44.35; H, 4.88; N, 6.46; Found: C, 44.62; H, 5.26; N, 6.08.

Methyl ((2-(2-azido-2-methylpropanamido)phenyl)sulphonyl)-*L*-prolinate 6:

Sodium azide (0.68 g, 10.5 mmol) was added to a solution of **5** (1.2 g, 3.5 mmol) in anhy. DMF (10 mL) and the reaction mixture was maintained 70 °C for 12 h. It was then cooled to room temperature, following which EtOAc (40 mL) was added and the organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to get the crude product which on purification by column chromatography gave **6** as a viscous liquid. Yield: 0.88 g (81%); $[\alpha]^{26}_D$: -50.53° (*c* 0.95, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3436, 3020, 2120, 1739, 1685, 1585, 1340, 1217, 1140, 770; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.39 (s, 1H), 8.52-8.50 (d, *J* = 8 Hz, 1H), 7.89-7.87 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.58-7.54 (t, *J* = 7 Hz, 1H), 7.24-7.20 (t, *J* = 7 Hz, 1H), 4.39-4.36 (dd, *J* = 8.0, 2.8 Hz, 1H), 3.60-3.54 (m, 4H), 3.46-3.40 (m, 1H), 2.15-2.07 (m, 1H), 2.05-1.95 (m, 1H), 1.93-1.84 (m, 1H), 1.62 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.9, 171.1, 136.1, 134.1, 129.7, 126.2, 124.0, 122.2, 64.6, 60.2, 52.4, 48.3, 31.0, 24.5; ESI-MS: 418.07 (M+Na; Anal. Calcd for $C_{16}H_{21}N_5O_5S$: C, 48.60; H, 5.35; N, 17.71; Found: C, 48.29.; H, 5.11.; N, 17.09.

Methyl ((2-(2-amino-2-methylpropanamido)phenyl)sulphonyl)-*L*-prolinate 7:

The product **7** was obtained from **6**, following the procedure mentioned for **4**.

Methyl-((2-(2-(2-((tert-butoxycarbonyl)amino)-4-methylpentanamido)-2-methylpropanamido)phenyl)sulphonyl)-*L*-prolinate 8:

Representative procedure: EDC.HCl (0.142 g, 0.69 mmol) was added to a solution of **7** (0.17 g, 0.46 mmol) and Boc-Leu-OH (0.12 g, 0.50 mmol) in anhy. DCM at 0 °C followed by HOBt (0.062 g, 0.46 mmol). The resulting mixture was then stirred at 0 °C for 10 min and at room temperature for 12 h. To the reaction

mixture 30 mL DCM was added and the organic layer was washed sequentially with sat. $NaHCO_3$, water, sat. $KHSO_4$ and brine. It was concentrated under reduced pressure and finally purified by column chromatography to furnish a white solid. Yield: 0.24 g (85%); mp: 90-92 °C; $[\alpha]^{25}_D$: -82° (*c*=1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3337, 3020, 2400, 1700, 1503, 1337, 1215, 1155, 1022, 759, 699; ^1H NMR (400 MHz, $CDCl_3$) δ:10.12 (s, 1H), 8.63-8.61 (d, 1H, *J*= 8.53 Hz), 7.82-7.80 (d, 1H, *J*= 8.03 Hz), 7.58-7.54 (m, 1H), 7.20-7.16 (m, 1H), 6.93 (bs, 1H), 5.01 (bs, 1H), 4.34-4.31 (m, 1H), 4.19 (bs, 1H), 3.68 (s, 3H), 3.52-3.50 (m, 1H), 3.32-3.26 (m, 1H), 2.08-1.98 (m, 3H), 1.82-1.66 (m, 3H), 1.66 (s, 3H), 1.59 (s, 3H), 1.54-1.48 (m, 1H), 1.45 (s, 9H), 0.94-0.91 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ:174.6, 172.9, 171.5, 137.3, 134.8, 129.8, 123.4, 123.1, 122.7, 79.4, 61.8, 57.4, 56.1, 53.4, 52.4, 49.7, 40.0, 30.9, 28.2, 26.0, 25.0, 24.9, 24.7, 24.6, 24.3, 24.1, 22.9, 22.7, 22.0; LC-MS: 605.25 $(M+Na)^+$; Anal. Calcd. for $C_{27}H_{42}N_4O_8S$: C, 55.65; H, 7.27; N, 9.62; Found: C, 55.81; H, 7.05.; N, 9.75.

Methyl((2-(2-(2-((tert-butoxycarbonyl)amino)-2-methylpropanamido)-2-methylpropanamido)phenyl)sulphonyl)-*L*-prolinate 15:

Tetramer **15** was obtained as a white fluffy solid. Yield: 90%; mp: 67-69 °C; $[\alpha]^{25}_D$: -12° (*c*=1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3337, 3019, 2400, 1735, 1699, 1523, 1338, 1215, 1045, 928, 758, 669; ^1H NMR (400 MHz, $CDCl_3$) δ: 10.04 (s, 1H), 8.58-8.56 (d, 1H, *J*= 8.54 Hz), 7.82-7.79 (d, 1H, *J*= 8.03 Hz), 7.56-7.52 (m, 1H), 7.27 (bs, 1H), 7.19-7.15 (m, 1H), 5.03 (s, 1H), 4.33-4.30 (m, 1H), 3.64 (s, 3H), 3.53-3.48 (m, 1H), 3.35-3.29 (m, 1H), 2.11-1.92 (m, 3H), 1.86-1.77 (m, 1H), 1.61 (s, 6H), 1.49 (s, 6H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.5, 173.2, 172.0, 155.0, 137.1, 134.2, 129.2, 124.8, 123.4, 122.3, 60.3, 57.4, 56.9, 52.4, 48.3, 30.8, 28.2, 25.3, 25.2, 24.9, 24.4; LC MS: 477.15 $(M+Na)^+$, 493.14

$(M+K)^+$; Anal. Calcd. for $C_{25}H_{38}N_4O_8S$: C, 54.14; H, 6.91; N, 10.10; Found: C, 54. 43; H, 6.68; N, 10.28.

Methyl((2-(2-methyl-2-((R)-1-pivaloylpyrrolidine-2-carboxamido)propan amido) phenyl)sulphonyl)-L-prolinate 19:

Compound **19** was obtained as a white fliffy solid. Yield: 88%; mp: 60-62 ^oC; $[\alpha]^{25}_D$: -88° (*c*=1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3336, 3019, 2400, 1739, 1699, 1585, 1522, 1338, 1215, 1152, 762, 669; ^1H NMR (400 MHz, $CDCl_3$) δ: $10.08_{rotamer}$ (0.2H), $10.04_{rotamer}$ (0.8H), $8.57_{rotamer}$ (0.2H), 8.56-$8.54_{rotamer}$ (0.8H), 7.78-7.77 (m, 1H), $7.55_{rotamer}$ (0.2H), $7.52_{rotamer}$ (0.8H), 7.50-7.49 (m, 1H), 7.15-7.12 (m, 1H), $4.66_{rotamer}$ (0.2H), 4.64-$4.62_{rotamer}$ (0.8H), 4.30-4.28 $_{rotamer}$ (0.8H), 4.26-4.25 $_{rotamer}$ (0.2H), 3.67-3.65 (m, 2H), $3.61_{rotamer}$ (2H), $3.61_{rotamer}$ (1H), 3.50-3.46 (m, 1H), 3.32-3.27 (m, 1H), 2.25-2.22 (m, 1H), 2.04-2.00 (m, 2H), 1.98-1.91 (m, 2H), 1.87-1.77 (m, 3H), 1.55-1.54 (m, 6H), $1.23_{rotamer}$ (7H), $1.23_{rotamer}$ (2H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.0, 173.1, 173.0, 171.9, 171.7, 171.6, 137.2, 134.1, 129.2, 129.1, 124, 9, 124.8, 123.3, 123.2, 122.2, 122.0, 61.8, 61.6, 60.3, 60.1, 57.4, 52.3, 48.2, 39.1, 30.7, 27.4, 25.8, 25.6, 24.5. 24.4, 23.9; LC MS: 573.23 $(M+Na)^+$, 589.20 $(M+K)^+$; Anal. Calcd. for $C_{25}H_{36}N_4O_7S$: C, 55.95; H, 6.76; N, 10.44; Found: C, 55.52; H, 6.99; N, 10.50.

((2-(2-(2-((tert-butoxycarbonyl)amino)-4-methylpentanamido)-2-methylpropanamido)phenyl)sulphonyl)-*L*-proline 9:

Representative procedure: LiOH ·H_2O (0.06 g, 1.3 mmol) was added to a solution of **8** (0.2 g, 0.34 mmol) in methanol (5 mL), followed by water (1 mL) at 0 °C. After complete consumption of the starting material (4 h), solvent was evaporated under reduced pressure, and the free acid was generated by treating with sat. $NaHSO_4$ solution followed by extraction with DCM (2 × 10 mL). Residue of **9**

obtained after evaporation of the solvent under vacuum was carried forward without further purification.

Methyl-2-(2-((2S)-1-((2-(2-(2-((tert-butoxycarbonyl)amino)-4-methylpentan-amido)-2-methylpropanamido)phenyl)sulphonyl)pyrrolidine-2-carboxamido)-4-methylpentanamido)-2-methylpropanoate 13:

Representative procedure: A solution of free acid **9** (0.2 g 0.35 mmol) and dimer amine **12** (0.09 g, 0.38 mmol) in anhy. DCM was cooled to 0 °C. To this mixture EDC.HCl (0.11 g, 0.52 mmol) was added followed by HOBt (0.05 g, 0.35 mmol), and was stirred at 0 °C for 10 min followed by 12 h at room temperature. DCM (30 mL) was then added to the reaction mixture and the organic layer washed sequentially with sat. $NaHCO_3$, water, sat. $KHSO_4$ and brine, and concentrated under reduced pressure and was finally purified by column chromatography to furnish a white solid. Yield: 0.2 g (75%); mp: 115-117 °C; $[\alpha]^{25}_{D}$: -101.69° (*c*=1.18, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3394, 3019, 2400, 1674, 1523, 1338, 1215, 1046, 928, 755, 669; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.17 (s, 1H), 8.52-8.51 (d, 1H, *J*= 7.93 Hz), 7.83-7.82 (d, 1H, *J*= 7.93 Hz), 7.61-7.58 (m, 1H), 7.43 (bs, 1H), 7.32 (bs, 1H), 7.23-7.20 (m, 1H), 5.64 (s, 1H), 4.17-4.14 (m, 2H), 3.70 (s, 3H), 3.67 (bs, 1H), 3.26-3.15 (m, 2H), 2.08-2.06 (m, 1H), 1.90 (bs, 1H), 1.84-1.82 (m, 2H), 1.75-1.72 (m, 1H), 1.64 (s, 3H), 1.61 (bs, 1H), 1.59-1.57 (m, 2H), 1.53 (s, 3H), 1.50 (s, 3H), 1.47 (s, 3H), 1.43 (s, 9H), 0.96-0.95 (d, 3H, *J*= 6.71 Hz), 0.91-0.89 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.7, 173.0, 171.5, 156.3, 137.3, 134.9, 129.8, 123.9, 123.2, 123.0, 79.9, 61.9, 57.5, 56.2, 53.4, 52.4, 49.8, 40.0, 31.0, 28.2, 26.1, 25.1, 24.9, 24.8, 24.7, 24.4, 24.1, 23.0, 22.8, 22.06; LC MS: 803.40 $(M+Na)^{+}$; Anal. Calcd. for $C_{37}H_{60}N_6O_{10}S$: C, 56.90; H, 7.74; N, 10.76; Found: C, 56.63.; H, 7.92.; N, 10.68.

Methyl-2-(2-((S)-1-((2-(2-(2-((tert-butoxycarbonyl)amino)-2-methylpropan amido)-2-methylpropanamido)phenyl)sulphonyl)pyrrolidine-2-carboxamido)-4-methylpentanamido)-2-methylpropanoate 17:

Hexapeptide **17** was obatined as a white fluffy soild. Yield: 82%; mp: 93-95 °C; $[\alpha]^{25}_{D}$: -101° (*c*=1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3393, 3019, 2981, 2401, 1725, 1675, 1523, 1294, 1216, 1155, 1073, 926, 759, 668; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.97 (s, 1H), 8.51-8.49 (d, 1H, *J*= 8.24 Hz), 7.81-7.79 (d, 1H, *J*= 7.33 Hz), 7.61-7.58 (m, 1H), 7.36 (bs, 1H), 7.24-7.21 (m, 1H), 7.08-7.06 (d, 1H, *J*= 8.55 Hz), 5.20 (s, 1H), 4.46-4.41 (m, 1H), 4.19-4.17 (m, 1H), 3.69 (s, 3H), 3.60 (bs, 1H), 3.18-3.13 (m, 1H), 2.22 (bs, 1H), 2.07-2.04 (m, 1H), 1.91-1.86 (m, 1H), 1.82-1.79 (m, 2H), 1.72-1.71 (m, 1H), 1.60 (s, 3H), 1.58 (s, 3H), 1.54-1.52 (m, 1H), 1.50-1.49 (m, 6H), 1.47 (bs, 6H), 1.42 (s, 9H), 0.94-0.93 (d, 3H, *J*= 6.41 Hz), 0.91-0.89 (d, 3H, *J*= 6.41 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.8, 174.6, 173.1, 171.1, 170.9, 155.1, 137.3, 134.9, 129.7, 123.9, 123.5, 123.0, 80.2, 77.2, 62.1, 57.3, 56.7, 56.1, 52.3, 51.8, 49.6, 40.3, 30.7, 28.2, 25.2, 25.1, 25.0, 24.6, 24.5, 22.9, 21.6; LC MS: 775.39 $(M+Na)^+$, 791.30 $(M+K)^+$; Anal. Calcd. for $C_{35}H_{56}N_6O_{10}S$: C, 55.83; H, 7.50; N, 11.16; Found: C, 56.03.; H, 7.29; N, 11.30.

methyl 2-methyl-2-(4-methyl-2-(1-((2-(2-methyl-2-((S)-1-pivaloylpyrrolidine-2-carboxamido)propanamido)phenyl)sulphonyl)pyrrolidine-2-carboxamido) pentanamido)propanoate 21:

Hexapeptide **17** was obatined as a white fluffy soild. Yield: 73%; mp: 98-100 ^{o}C; $[\alpha]^{26}_{D}$: -129.52° (*c*=1.05, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3333, 3019, 2973, 2400, 1738, 1681, 1584, 1524, 1337, 1215, 1153, 926, 758, 668; ^{1}H NMR (400 MHz, $CDCl_3$) δ: $10.03_{rotamer}$ (0.8H), $9.96_{rotamer}$ (0.2H), 8.52-$8.50_{rotamer}$ (0.8 H), 8.43-$8.41_{rotamer}$ (0.2H), 7.79-7.77 (d, 1H, *J*= 7.94 Hz), 7.60-7.56 (m, 2H), 7.22-7.19 (m, 1H), 7.06 (bs, 1H), 6.98-$6.96_{rotamer}$ (0.8H), $6.94_{rotamer}$ (0.2H), 4.66-4.65 (m, 1H), 4.49-4.46 (m, 1H), 4.18-$4.17_{rotamer}$ (0.2H), 4.15-$4.12_{rotamer}$ (0.8H), 3.68 (s, 3H),

3.65-3.61 (m, 2H), 3.15-3.10 (m, 1H), 2.20-2.18 (m, 1H), 2.07-2.01 (m, 2H), 1.91-1.76 (m, 5H), 1.70-1.68 (m, 1H), 1.55 (s, 3H), 1.54 (s, 3H), 1.53-1.50 (m, 1H), 1.48 (s, 3H), 1.45 (s, 3H), $1.22_{rotamer}$ (7H), $1.19_{rotamer}$ (2H), 0.93-0.92 (m, 3H), 0.89-0.88 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.1, 174.5, 173.3, 173.2, 172.0, 171.9, 171.1, 171.0, 170.8, 137.5, 134.8, 134.7, 129.6, 124.0, 123.9, 123.8, 123.2, 122.8, 62.3, 62.2, 61.7, 61.6, 57.4, 56.1, 52.3, 52.2, 51.6, 49.4, 48.4, 48.3, 40.3, 40.2, 39.0, 30.8, 30.7, 27.4, 27.3, 25.9, 25.8, 25.6, 25.1, 25.0, 24.9, 24.6, 24.5, 24.0, 21.6, 21.5; LC MS: 771.41 $(M+Na)^+$, 783.38 $(M+K)^+$; Anal. Calcd. for $C_{36}H_{56}N_6O_9S$: C, 57.73; H, 7.54; N, 11.22; Found: C, 57.51; H, 7.38; N, 11.60.

tert-butyl-(4-methyl-1-((2-methyl-1-((2-(((*2S*)-2-((4-methyl-1-((2-methyl-1-(methylamino)-1-oxopropan-2-yl)amino)-1-oxopentan-2-yl)carbamoyl) pyrrolidin-1-yl)sulphonyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopentan-2-yl)carbamate 14:

Representative procedure: To the ester **13** (0.15 g, 0.02 mmol), a saturated solution of methylamine in methanol was added at 0 °C and stirred for 12 h. Solvent was then stripped off to get the methyl amide **14** as a white solid. Yield: 0.13 g (90%); mp: 203-205 °C; $[\alpha]^{25}_D$: -55.55° (*c* 1.56, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3615, 3393, 3019, 2400, 1674, 1523, 1421, 1338, 1215, 1046, 759, 669; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.15 (s, 1H), 8.63-8.62 (d, 1H, *J*= 7.93 Hz), 7.79-7.77 (d, 1H, *J*= 7.93 Hz), 7.72 (s, 1H), 7.61-7.58 (m, 1H), 7.32 (bs, 1H), 7.23-7.20 (m, 1H), 7.04 (s, 1H), 6.77 (s, 1h), 5.24 (bs, 1H), 4.25 (bs, 1H), 4.11 (bs, 1H), 4.05-4.04 (m, 1H), 3.64 (bs, 1H), 3.22-3.20 (m, 1H), 2.72 (s, 3H), 2.09 (bs, 2H), 1.78-1.72 (m, 3H), 1.71-1.64 (m, 3H), 1.61 (s, 3H), 1.57 (s, 3H), 1.51 (s, 3H), 1.46 (bs, 2H), 1.41 (s, 3H), 1.39 (s, 9H), 1.02-1.01 (m, 3H), 0.94-0.90 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.6, 173.4, 173.1, 172.1, 171.9, 155.7, 137.6, 135.0, 129.6, 123.7, 122.9, 122.5, 79.5, 61.4, 57.6, 54.0, 52.6, 50.2, 41.5, 39.5,

30.6, 28.2, 26.5, 25.2, 24.9, 24.5, 24.3, 23.6, 23.0, 22.7, 22.0, 21.7; LC MS: 802.41 $(M+Na)^+$, 818.37 $(M+K)^+$; Anal. Calcd. for $C_{37}H_{61}N_7O_9S$: C, 56.98; H, 7.88; N, 12.57; Found: C, 56.50; H, 8.02; N, 12.89.

***tert*-butyl(2-methyl-1-((2-methyl-1-((2-(((*2S*)-2-((4-methyl-1-((2-methyl-1-(methylamino)-1-oxopropan-2-yl)amino)-1-oxopentan-2-yl)carbamoyl) pyrrolidin-1-yl)sulphonyl)phenyl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate 18:**

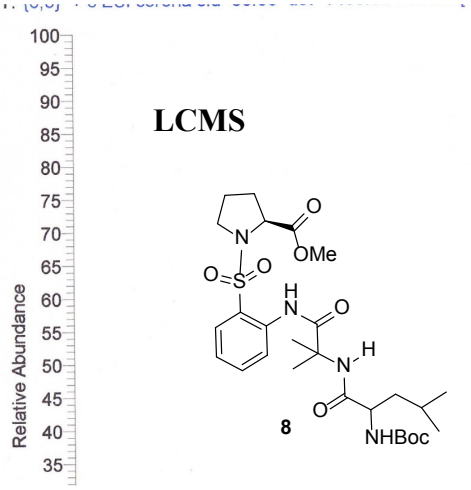
Compound **18** was obtained as a white solid. Yield: 82%, mp: 136-138 ^oC; $[\alpha]^{25}_D$: -63.52° (*c*= 0.85, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3627, 3393, 3019, 2400, 1674, 1522, 1422, 1338, 1215, 1046, 928, 757, 669; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.93 (s, 1H), 8.50-8.49 (d, 1H, *J*= 8.24 Hz), 7.78-7.77 (m, 1H), 7.63-7.60 (m, 1H), 7.35 (bs, 1H), 7.25-7.20 (m, 2H), 6.82-6.81 (m, 1H), 5.17 (s, 1H), 4.28-4.26 (m, 1H), 4.15-4.13 (m, 1H), 3.66-3.62 (m, 1H), 3.18-3.13 (m, 1H), 2.79-2.76 (m, 3H), 2.06-2.02 (m, 2H), 1.94-1.88 (m, 1H), 1.82-1.79 (m, 2H), 1.75-1.69 (m, 1H), 1.63-1.62 (m, 1H), 1.62 (s, 3H), 1.60 (s, 3H), 1.51 (s, 6H), 1.50 (s, 3H), 1.49 (s, 3H), 0.98-0.97 (d, 3H, *J*= 6.41 Hz), 0.93-0.92 (d, 3H, *J*= 6.41 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.9, 174.7, 173.2, 172.1, 171.3, 155.0, 137.3, 134.9, 129.6, 124.0, 123.7, 122.9, 80.2, 77.2, 62.1, 57.5, 57.4, 56.7, 53.1, 49.6, 39.3, 30.7, 28.2, 26.5, 25.7, 25.5, 25.4, 25.3, 25.1, 24.6, 24.5, 22.9, 21.6; LC MS: 774.45 $(M+Na)^+$, 790.43 $(M+K)^+$; Anal. Calcd. for $C_{35}H_{57}N_7O_9S$: C, C, 55.91; H, 7.64; N, 13.04; Found: C, 56.13; H, 7.45; N, 13.28.

(*2S*)-N-(2-methyl-1-((2-(((*2S*)-2-((4-methyl-1-((2-methyl-1-(methylamino)-1-oxopropan-2-yl)amino)-1-oxopentan-2-yl)carbamoyl)pyrrolidin-1-yl)sulphonyl) phenyl)amino)-1-oxopropan-2-yl)-1-pivaloylpyrrolidine-2-carboxamide 22:

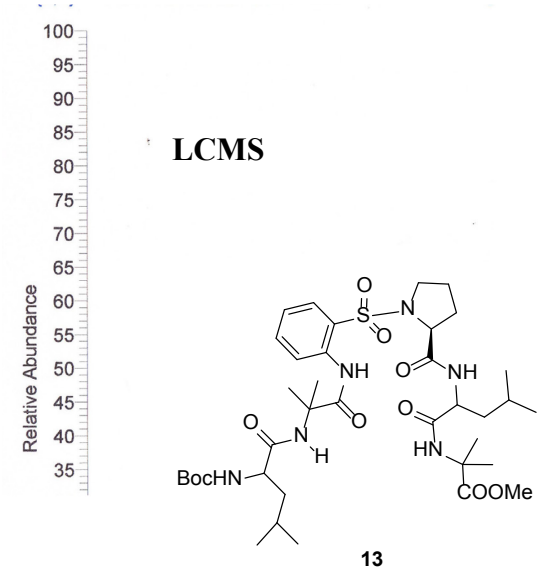
Compound **22** was obtained as a white solid. Yield: 86%; mp: 162-164 ^oC; $[\alpha]^{25}_D$: -104° (*c*=1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}) 3615, 3393, 3019, 2400, 1674, 1523, 1421, 1338, 1215, 1046, 928, 767, 669; ^1H NMR (400 MHz, $CDCl_3$) δ: $9.94_{rotamer}$

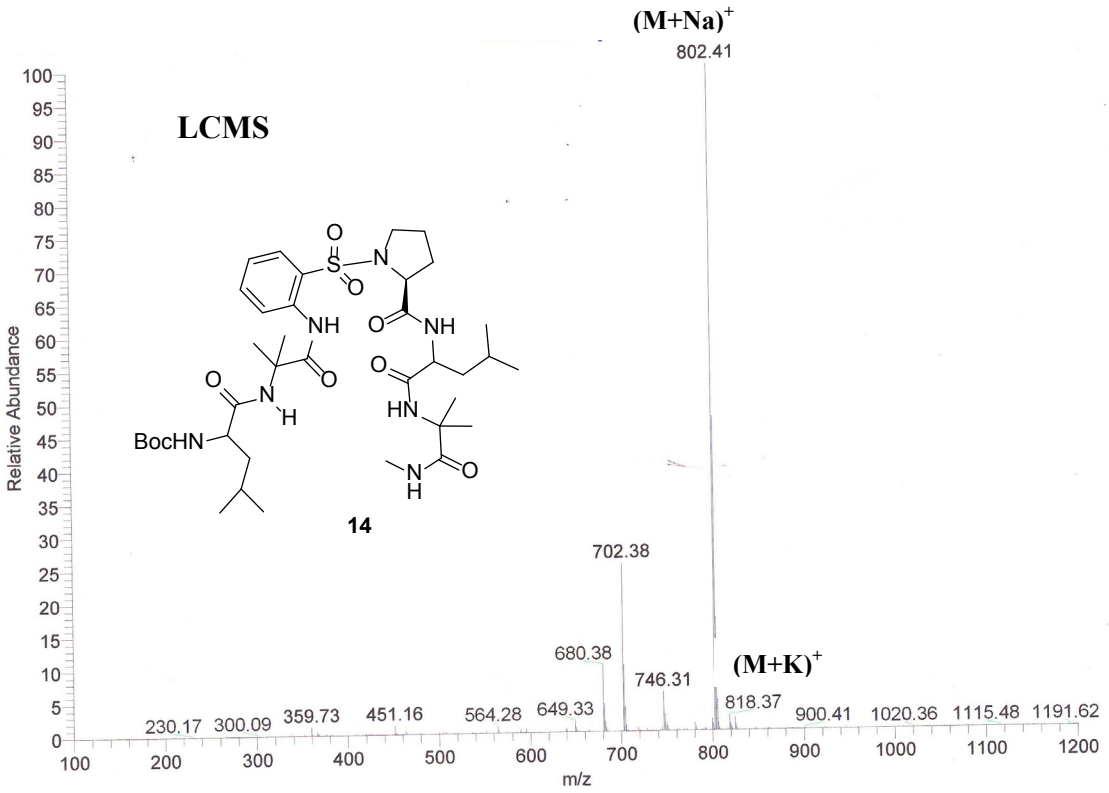
(0.2 H), 9.91$_{rotamer}$ (0.8 H), 8.43-8.42 (d, 1H, *J*= 8.31 Hz), 7.79-7.77$_{rotamer}$ (0.9H), 7.76$_{rotamer}$ (0.7 H), 7.67$_{rotamer}$ (0.7 H), 7.64$_{rotamer}$ (0.3H), 7.61-7.58 (m, 1H), 7.25-7.22 (m, 1H), 7.07-7.04 (m, 2H), 6.92 (bs, 1H), 4.65-4.62 (m, 1H), 4.35-4.30$_{rotamer}$ (0.8 H), 4.28-4.26$_{rotamer}$ (0.2 H), 4.14-4.13$_{rotamer}$ (0.1 H), 4.12-4.10$_{rotamer}$ (0.9 H), 3.72-3.59(m, 3H), 3.16-3.11 (m, 1H), 2.78-2.77$_{rotamer}$ (2.5H), 2.76$_{rotamer}$ (0.5 H), 2.19-2.14 (m, 1H), 2.12-2.08 (m,1 H), 2.04-1.96 (m, 3H), 1.93-1.87 (m, 2H), 1.82-1.77 (m, 2H), 1.73-1.69 (m, 1H), 1.63-1.58 (m, 1H), 1.55 (s, 3H), 1.54 (s, 3H), 1.53 (s, 3H), 1.50 (s, 3H), 1.20$_{rotamer}$ (2H), 1.16$_{rotamer}$ (7H), 0.99-0.97 (m, 3H), 0.93-0.91 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.1, 177.8, 174.9, 174.7, 172.3, 172.2, 172.2, 172.0, 171.7, 137.7, 137.6, 134.9, 129.7, 129.5, 124.3, 124.2, 124.1, 123.4, 61.9, 61.7, 57.6, 57.5, 57.3, 53.1, 52.9, 49.6, 48.3, 40.0, 39.1, 39.0, 30.9, 27.3, 26.5, 25.8, 25.7, 25.6, 25.5, 24.3, 25.1, 25.0, 24.6, 24.5, 23.0, 22.9, 21.6, 21.5; LC MS: 770.47 $(M+Na)^+$, 786.41 $(M+K)^+$; Anal. Calcd. for $C_{36}H_{57}N_7O_8S$: C, 57.81; H, 7.68; N, 13.11; Found: C, 57.55; H, 7.89; N, 13.35.

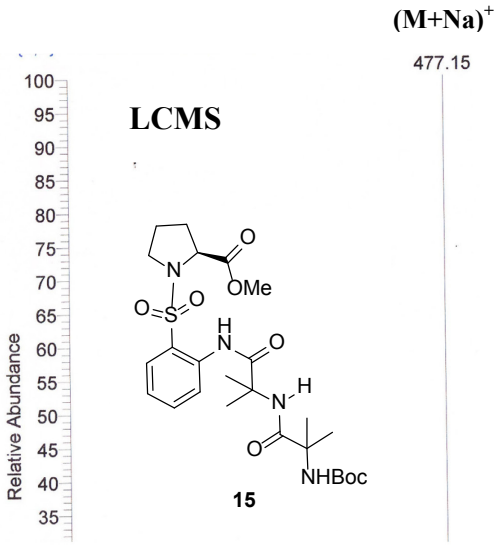
$(M+Na)^+$


100
95
90
85
80
75
70
65
60
55
50
45
40
35
Relative Abundance
LCMS
O
N
OMe
O=S=O
H
N
O
N
H
O
NHBoc
8

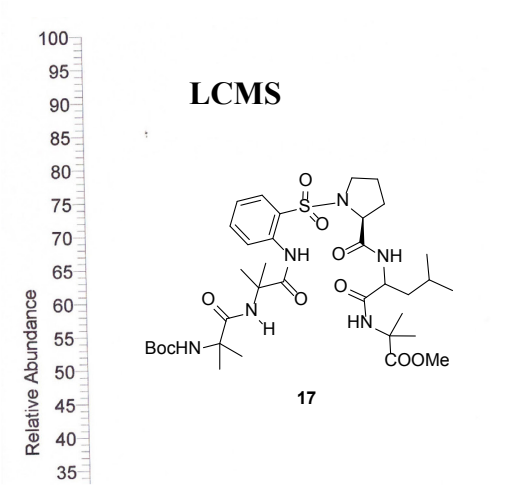
$(M+Na)^+$


100
95
90
85
80
75
70
65
60
55
50
45
40
35
Relative Abundance
LCMS
O
S
N
O
NH
O
NH
O
N
H
O
BocHN
HN
COOMe
13


(M+Na)+
802.41
LCMS
Relative Abundance
m/z
14
702.38
680.38
746.31
(M+K)+
818.37
230.17
300.09
359.73
451.16
564.28
649.33
900.41
1020.36
1115.48
1191.62

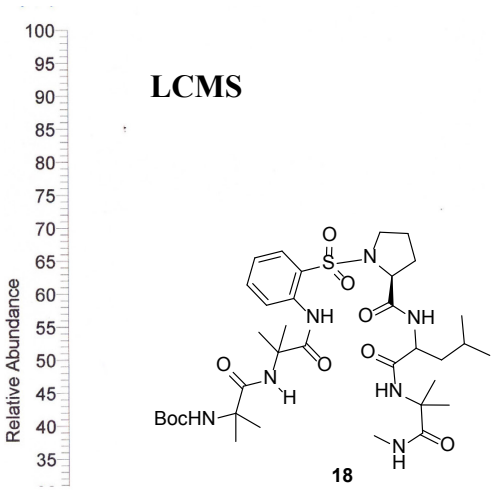

(M+Na)+
477.15
LCMS
Relative Abundance
15
(M+K)+

$(M+Na)^+$

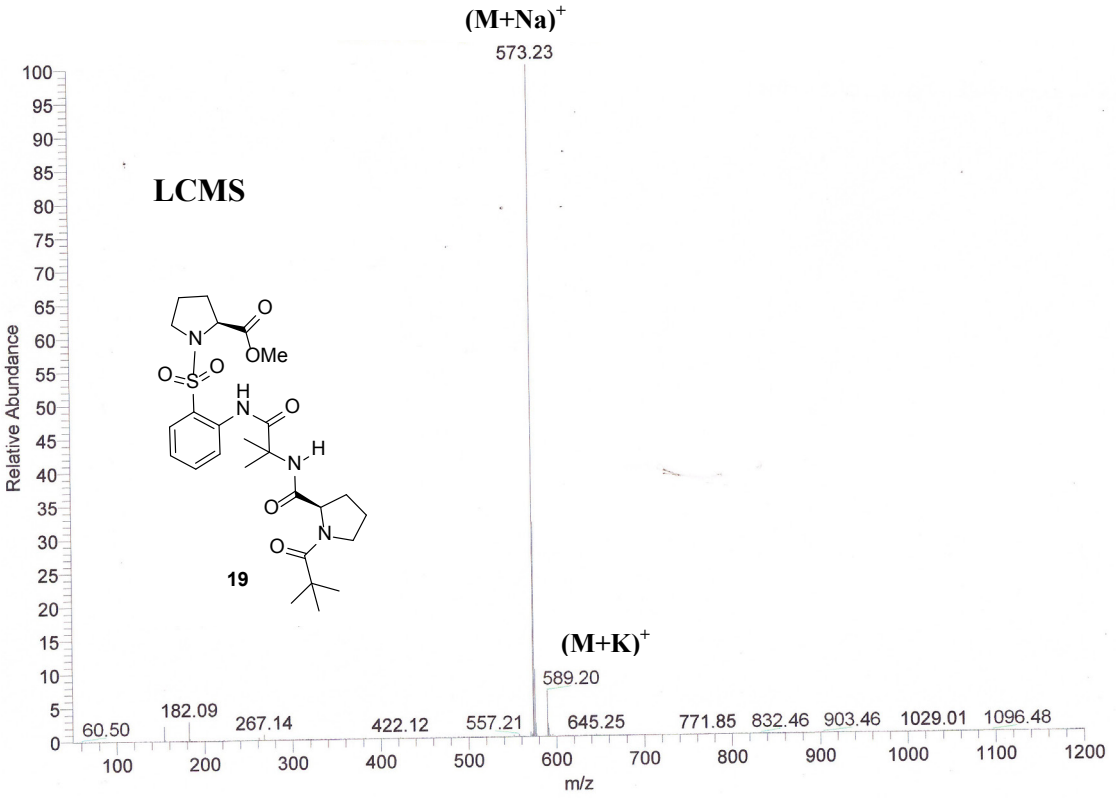

LCMS
Relative Abundance
100
95
90
85
80
75
70
65
60
55
50
45
40
35
BocHN
COOMe
17

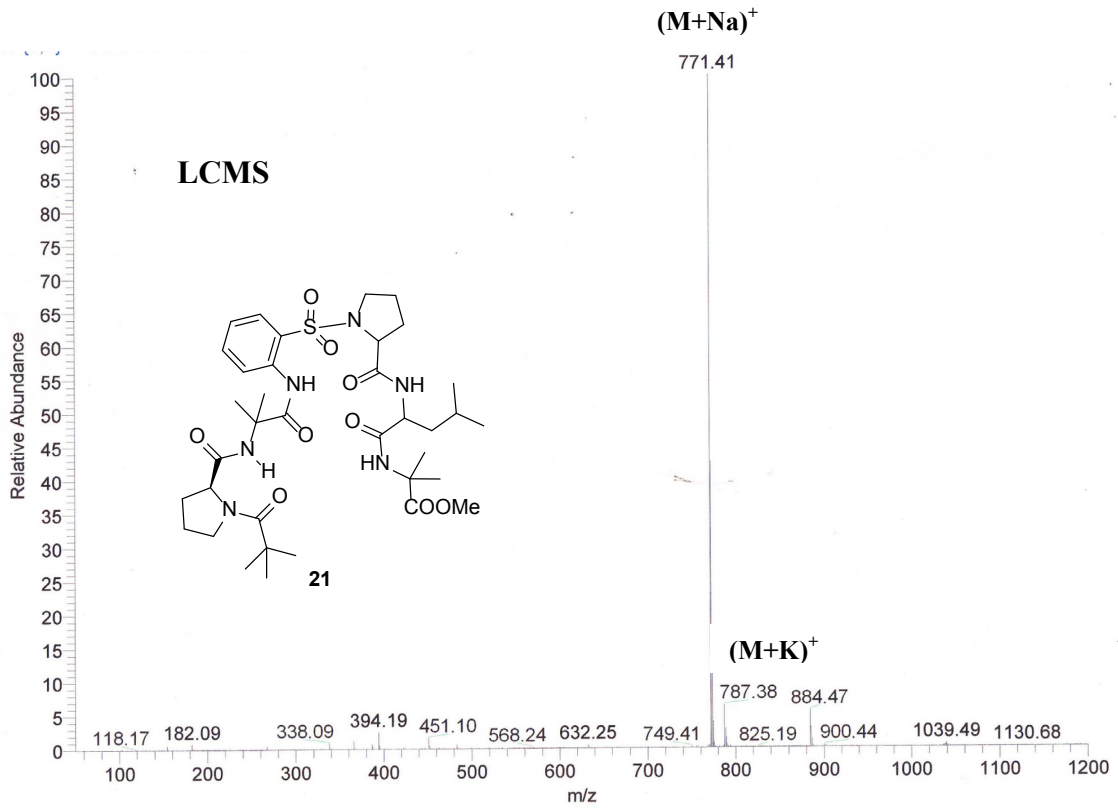
$(M+K)^+$

$(M+Na)^+$

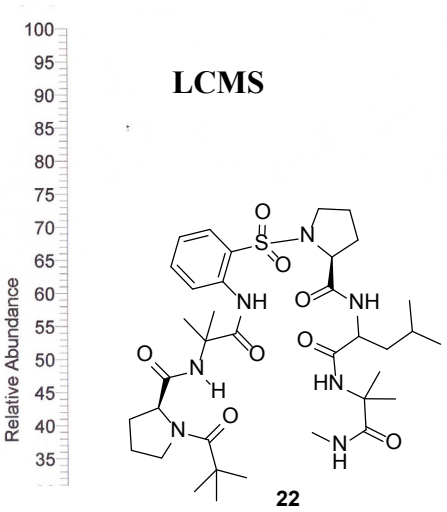

LCMS
Relative Abundance
100
95
90
85
80
75
70
65
60
55
50
45
40
35
BocHN
18

$(M+K)^+$

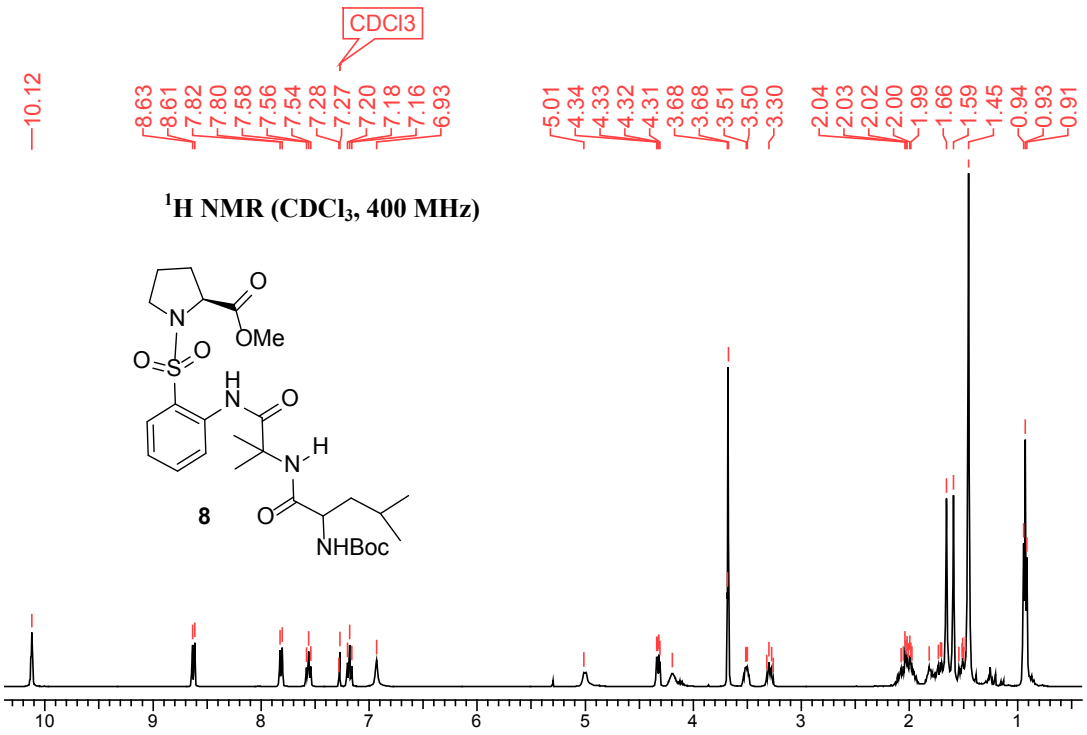

(M+Na)+
573.23
LCMS
19
(M+K)+
589.20
Relative Abundance
m/z
60.50
182.09
267.14
422.12
557.21
645.25
771.85
832.46
903.46
1029.01
1096.48


(M+Na)+
771.41
LCMS
21
(M+K)+
787.38
884.47
Relative Abundance
m/z
118.17
182.09
338.09
394.19
451.10
568.24
632.25
749.41
825.19
900.44
1039.49
1130.68

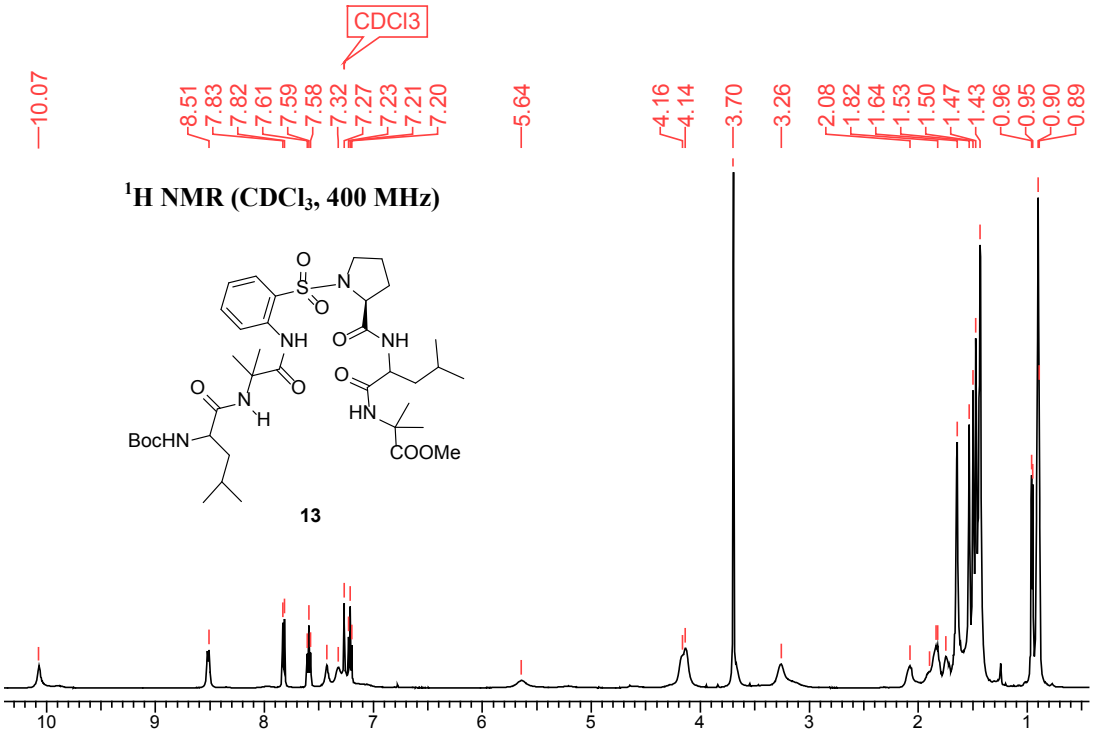
(M+Na)$^+$

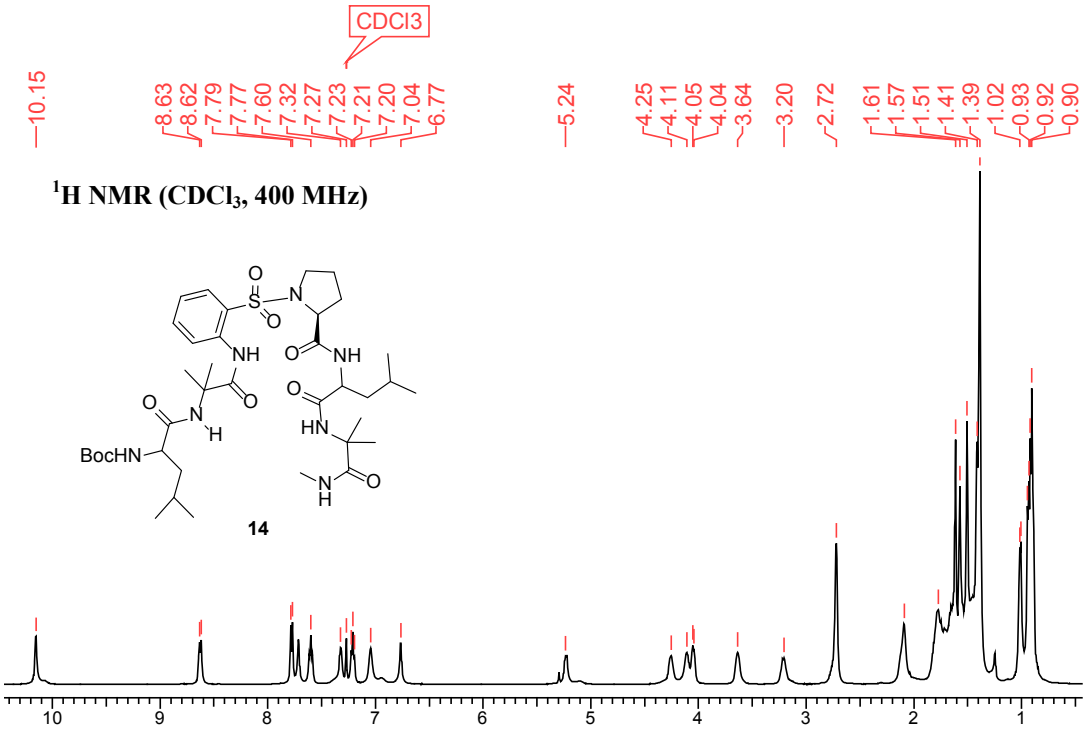


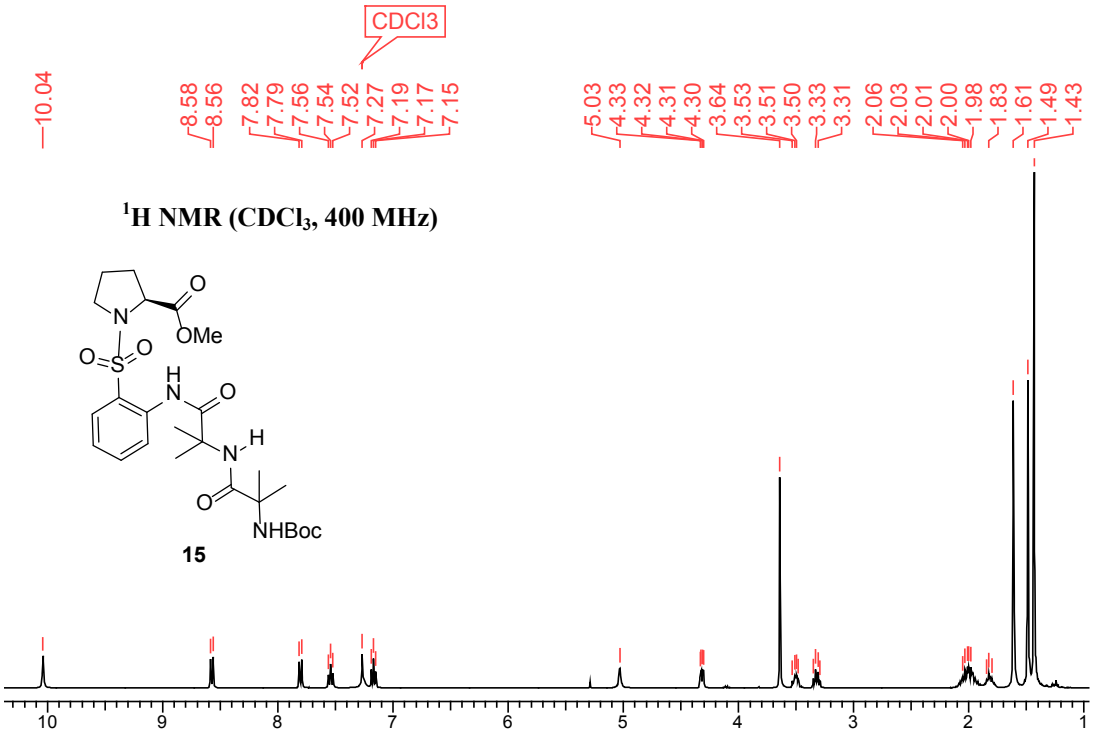
(M+K)$^+$



^{1}H NMR ($CDCl_3$, 400 MHz)

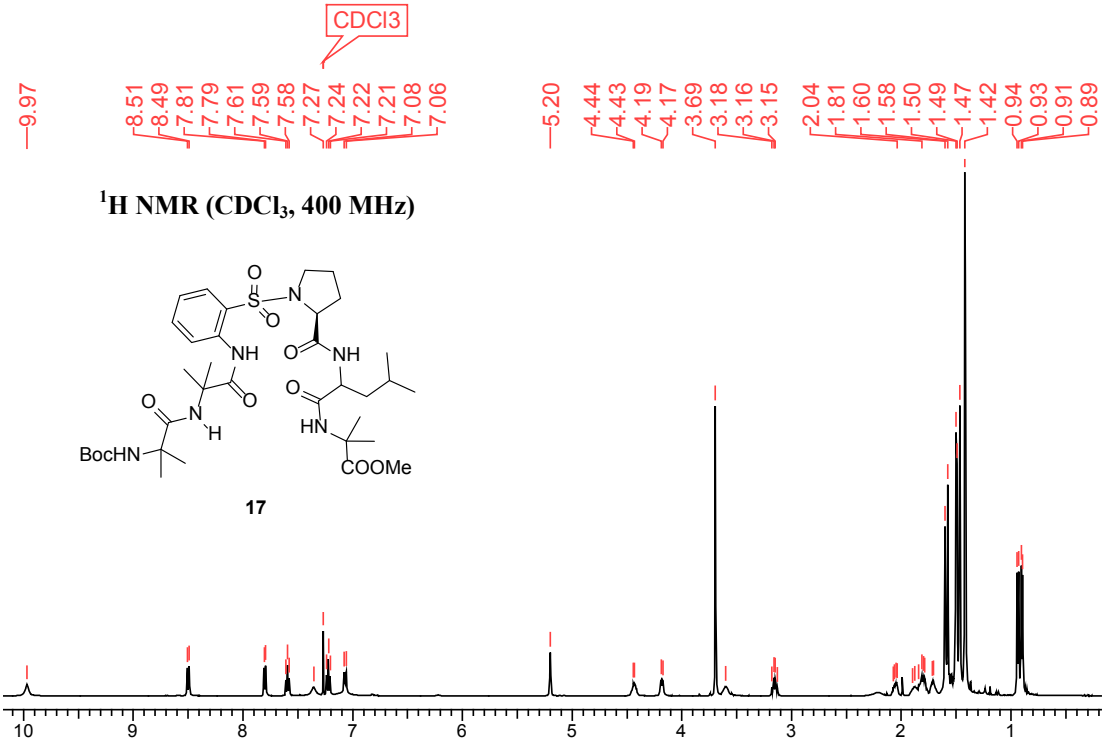

CDCl3
10.07
8.51
7.83
7.82
7.61
7.59
7.58
7.32
7.27
7.23
7.21
7.20
5.64
4.16
4.14
3.70
3.26
2.08
1.82
1.64
1.53
1.50
1.47
1.43
0.96
0.95
0.90
0.89
1H NMR (CDCl3, 400 MHz)
BocHN
COOMe
13


CDCl3
10.15
8.63
8.62
7.79
7.77
7.60
7.32
7.27
7.23
7.21
7.20
7.04
6.77
5.24
4.25
4.11
4.05
4.04
3.64
3.20
2.72
1.61
1.57
1.51
1.41
1.39
1.02
0.93
0.92
0.90
1H NMR (CDCl3, 400 MHz)
BocHN
14



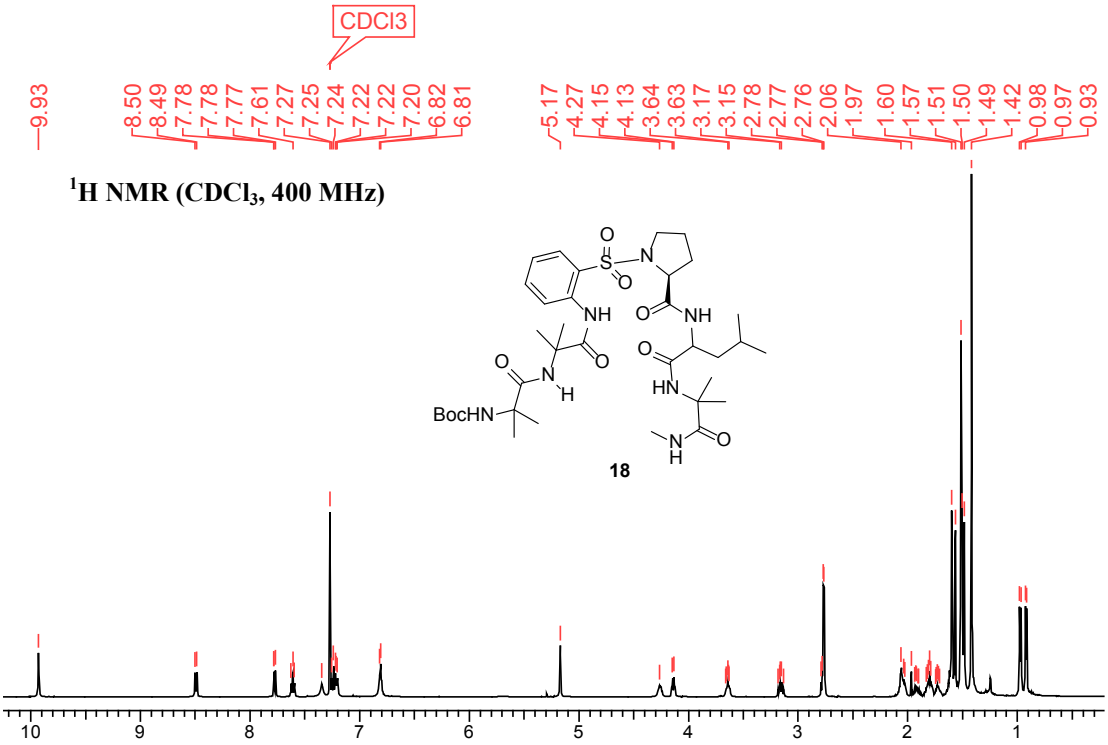
^{1}H NMR ($CDCl_3$, 400 MHz)

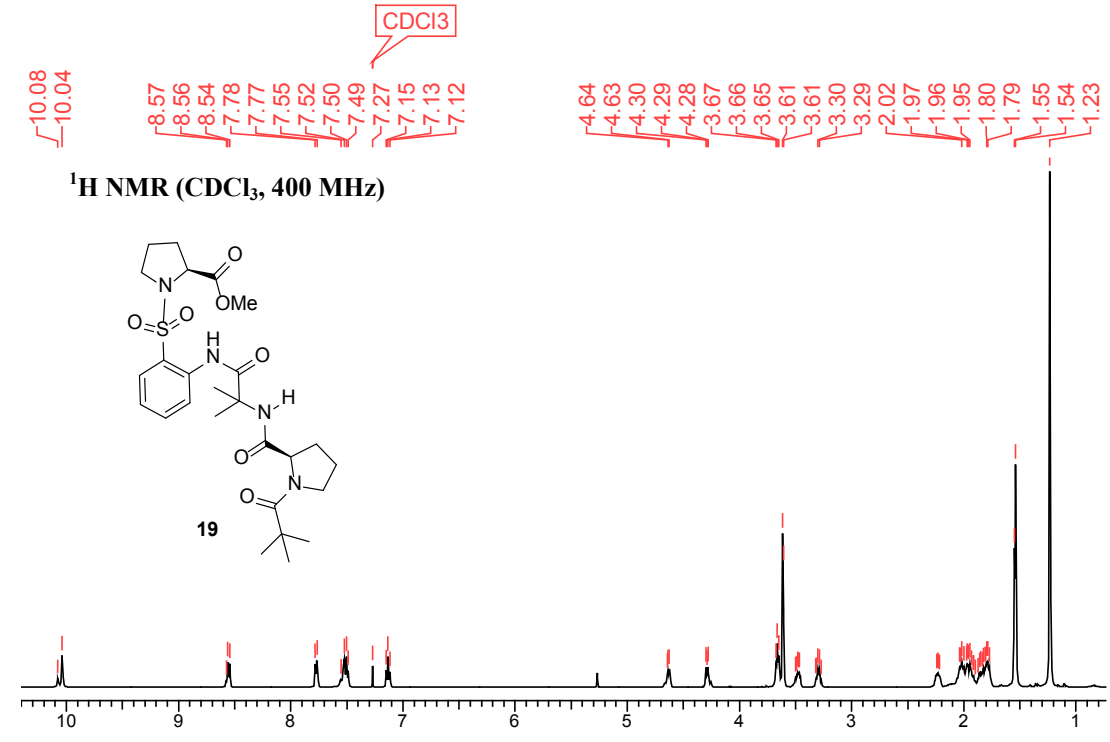
15

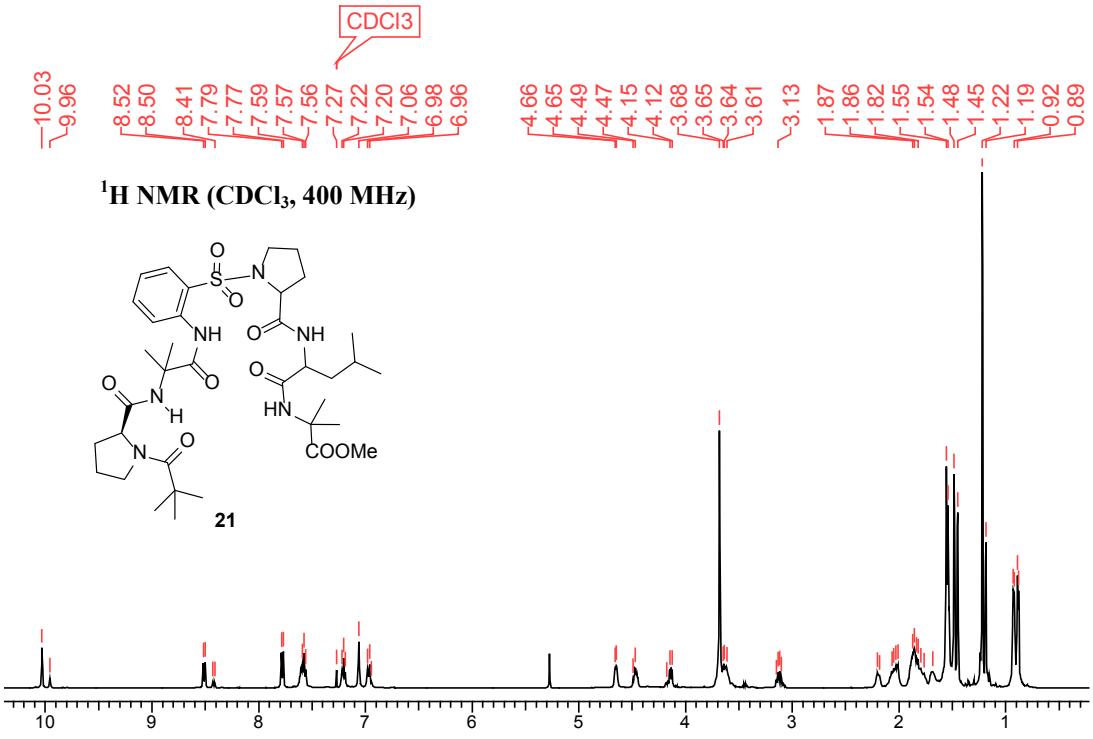


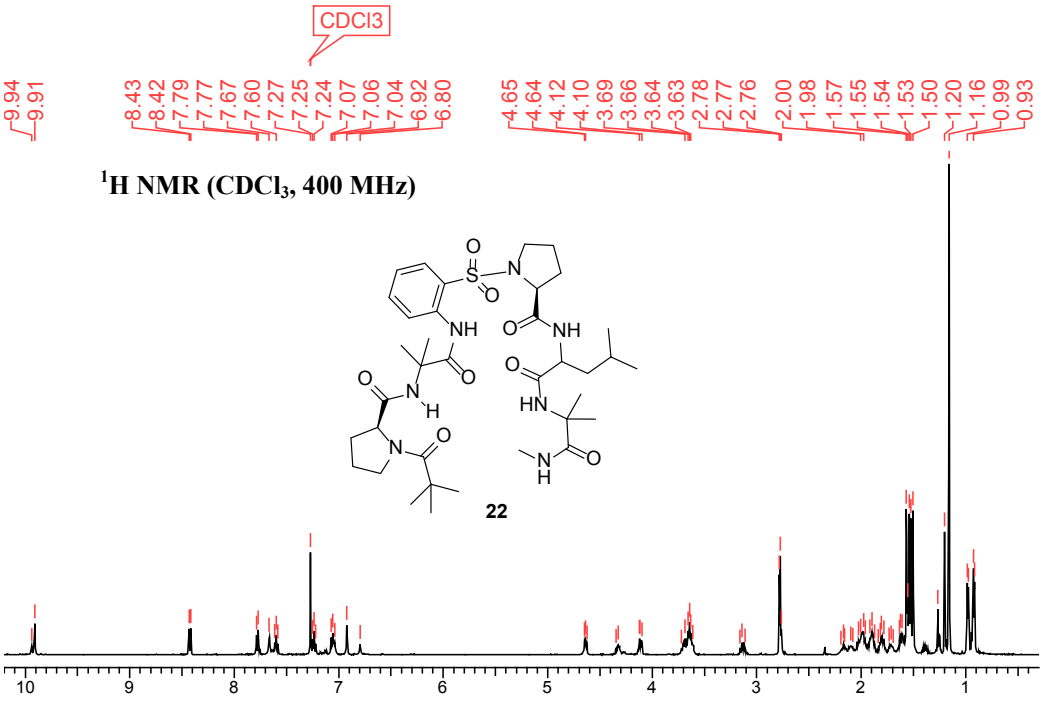
^{1}H NMR ($CDCl_3$, 400 MHz)

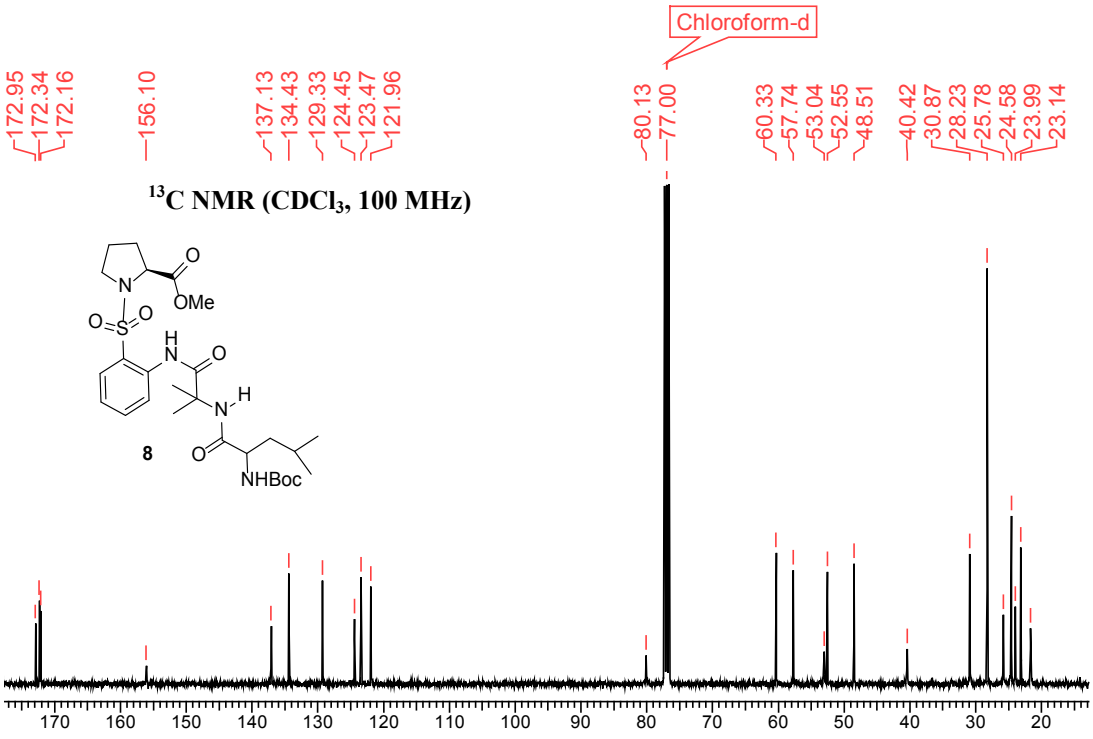
17

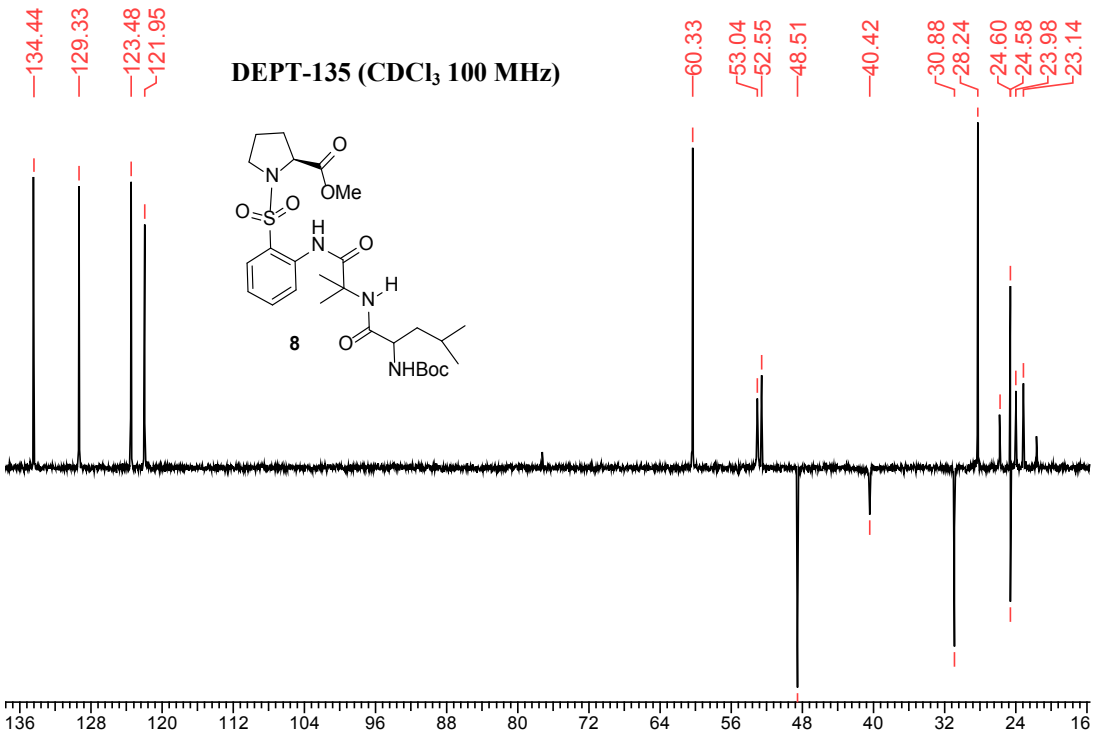

CDCl3
9.93
8.50
8.49
7.78
7.78
7.77
7.61
7.27
7.25
7.24
7.22
7.22
7.20
6.82
6.81
5.17
4.27
4.15
4.13
3.64
3.63
3.17
3.15
2.78
2.77
2.76
2.06
1.97
1.60
1.57
1.51
1.50
1.49
1.42
0.98
0.97
0.93
1H NMR (CDCl3, 400 MHz)
BocHN
18

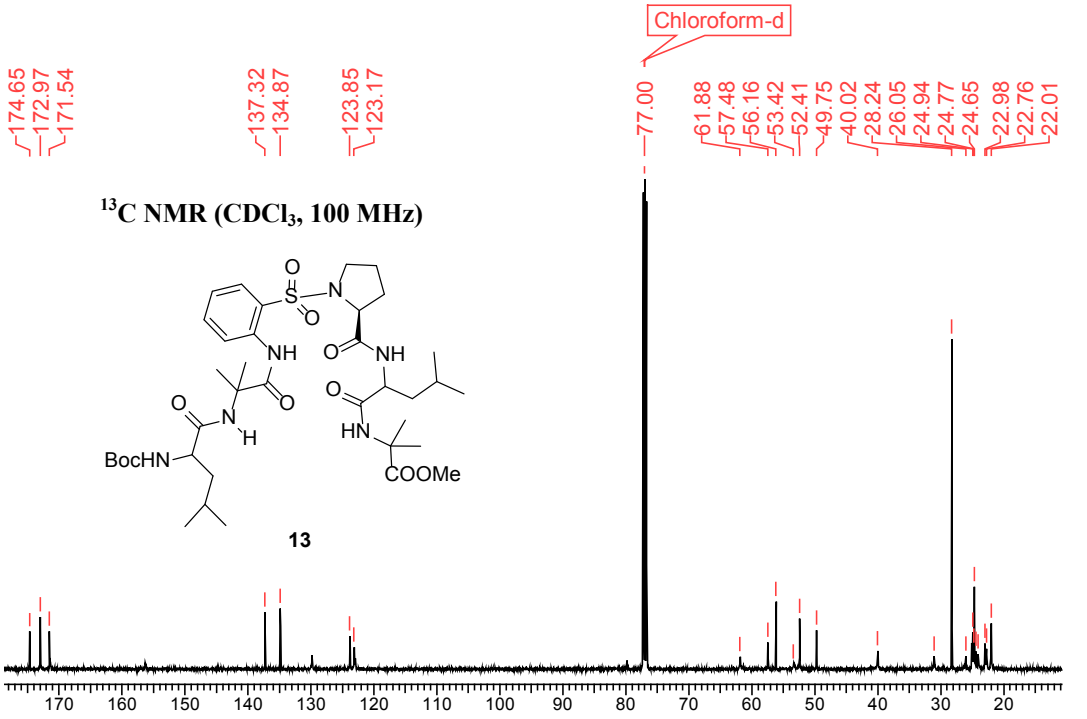

CDCl3
10.08
10.04
8.57
8.56
8.54
7.78
7.77
7.55
7.52
7.50
7.49
7.27
7.15
7.13
7.12
4.64
4.63
4.30
4.29
4.28
3.67
3.66
3.65
3.61
3.61
3.30
3.29
2.02
1.97
1.96
1.95
1.80
1.79
1.55
1.54
1.23
1H NMR (CDCl3, 400 MHz)
OMe
19


CDCl3
10.03
9.96
8.52
8.50
8.41
7.79
7.77
7.59
7.57
7.56
7.27
7.22
7.20
7.06
6.98
6.96
4.66
4.65
4.49
4.47
4.15
4.12
3.68
3.65
3.64
3.61
3.13
1.87
1.86
1.82
1.55
1.54
1.48
1.45
1.22
1.19
0.92
0.89
1H NMR (CDCl3, 400 MHz)
21


CDCl3
9.94
9.91
8.43
8.42
7.79
7.77
7.67
7.60
7.27
7.25
7.24
7.07
7.06
7.04
6.92
6.80
4.65
4.64
4.12
4.10
3.69
3.66
3.64
3.63
2.78
2.77
2.76
2.00
1.98
1.57
1.55
1.54
1.53
1.50
1.20
1.16
0.99
0.93
1H NMR (CDCl3, 400 MHz)
22

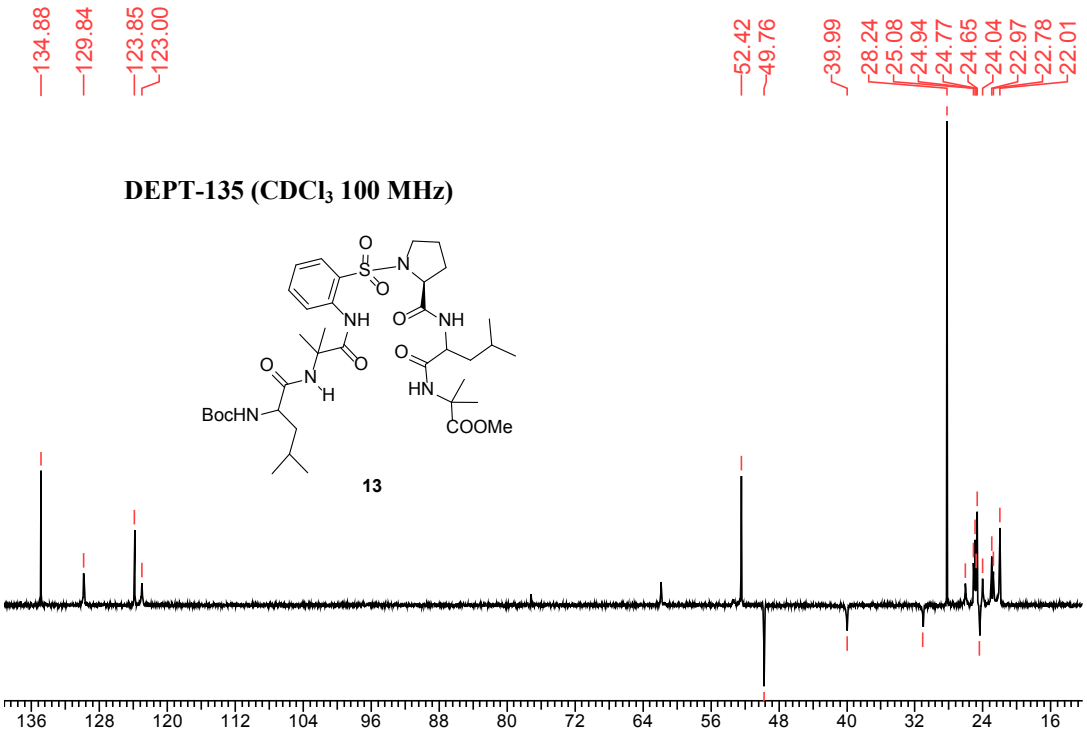

Chloroform-d
172.95
172.34
172.16
156.10
137.13
134.43
129.33
124.45
123.47
121.96
80.13
77.00
60.33
57.74
53.04
52.55
48.51
40.42
30.87
28.23
25.78
24.58
23.99
23.14
13C NMR (CDCl3, 100 MHz)
OMe
NHBoc
8
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20


134.44
129.33
123.48
121.95
DEPT-135 (CDCl3 100 MHz)
60.33
53.04
52.55
48.51
40.42
30.88
28.24
24.60
24.58
23.98
23.14
OMe
NHBoc
8
136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16



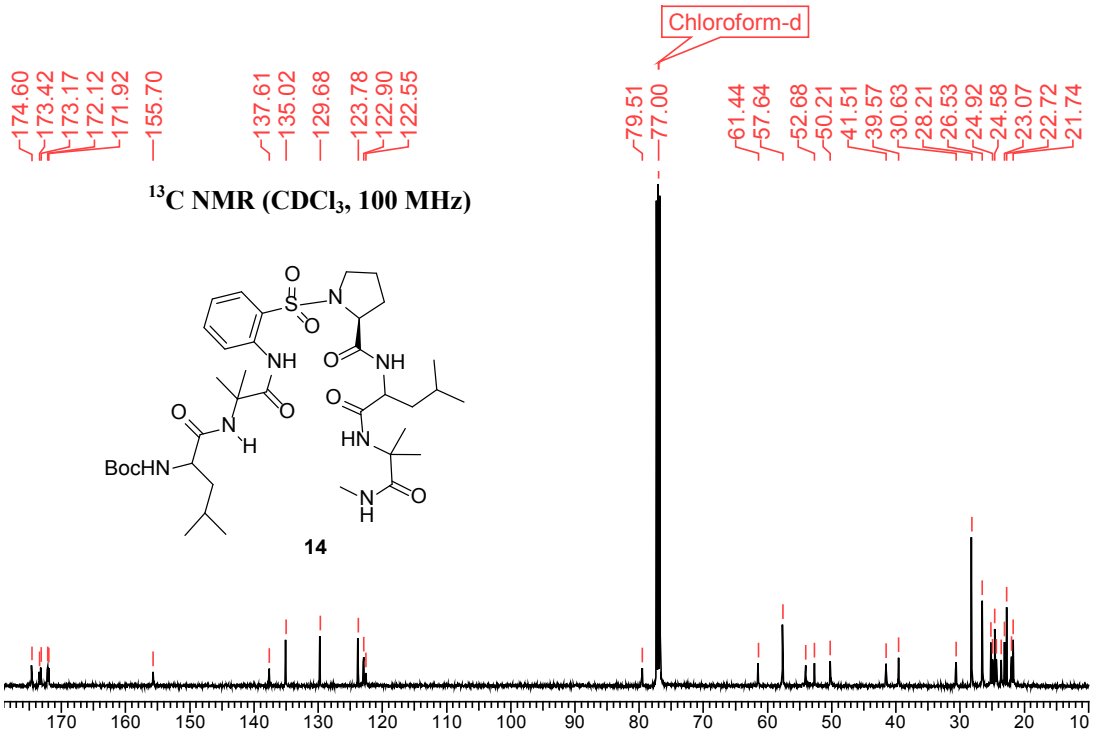
^{13}C NMR ($CDCl_3$, 100 MHz)

13



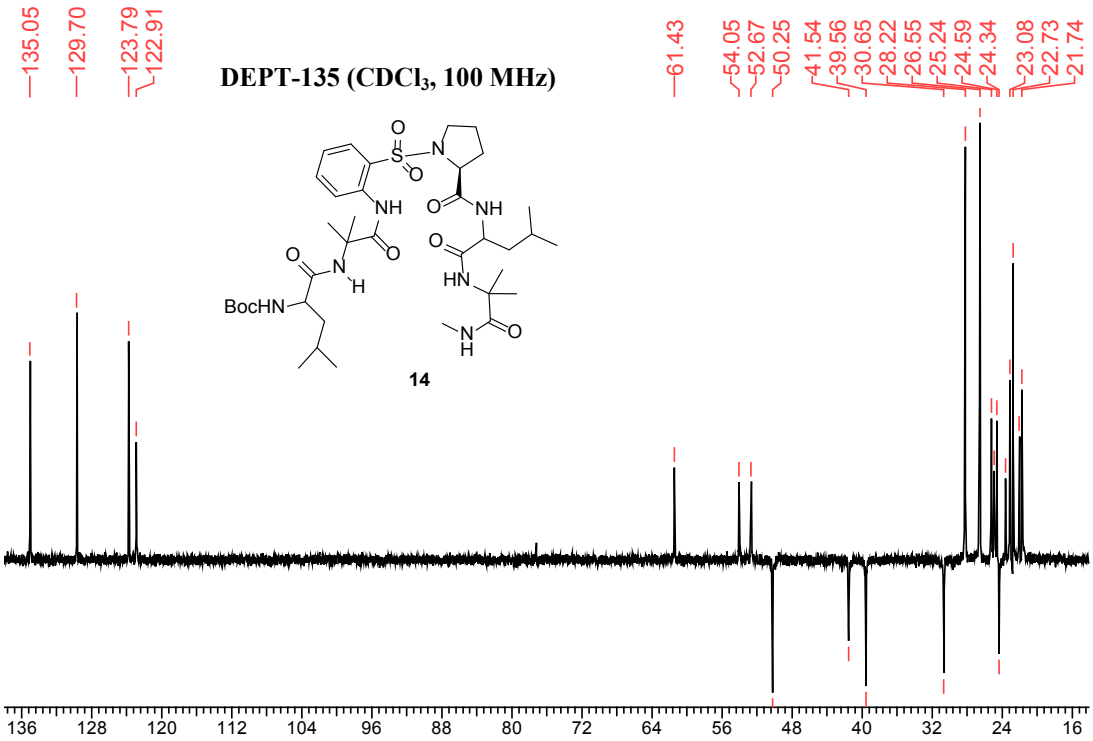
DEPT-135 ($CDCl_3$ 100 MHz)

13



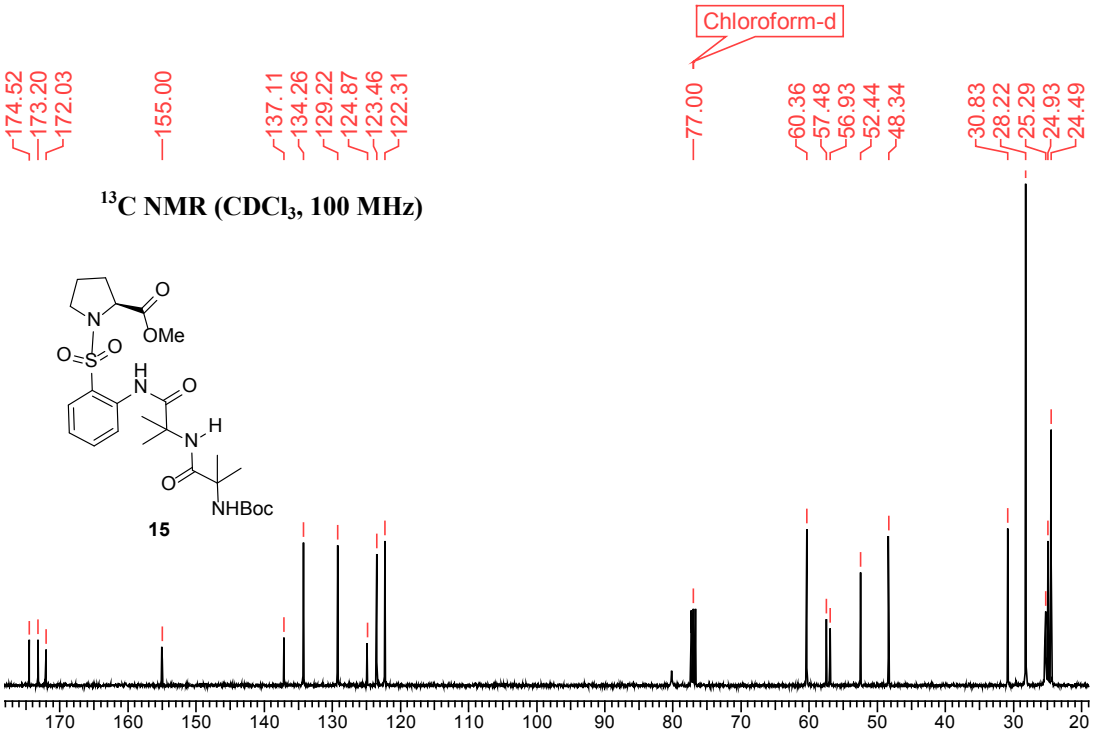
^{13}C NMR ($CDCl_3$, 100 MHz)

14



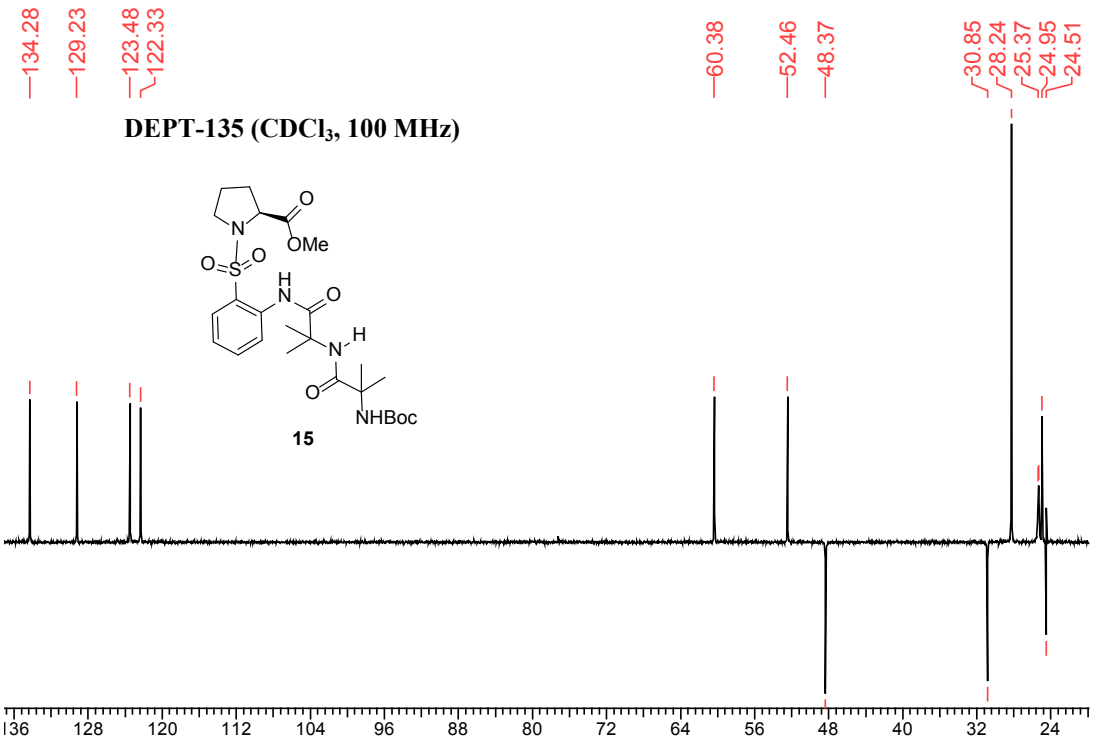
DEPT-135 ($CDCl_3$, 100 MHz)

14



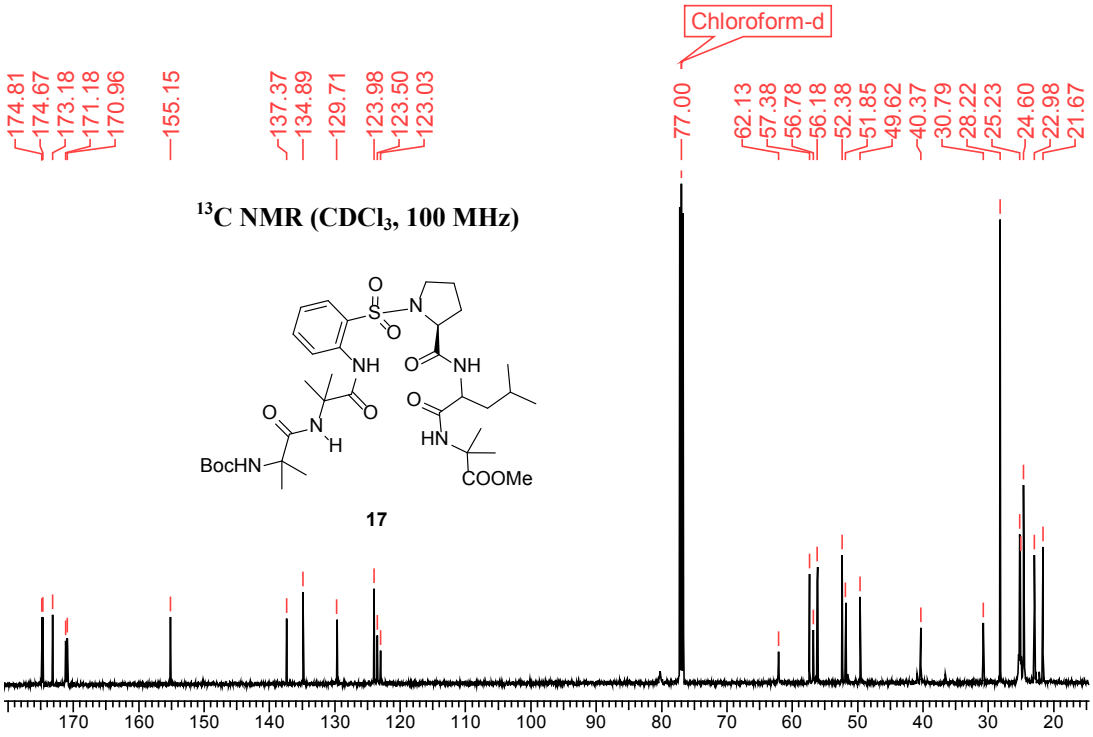
^{13}C NMR ($CDCl_3$, 100 MHz)

15



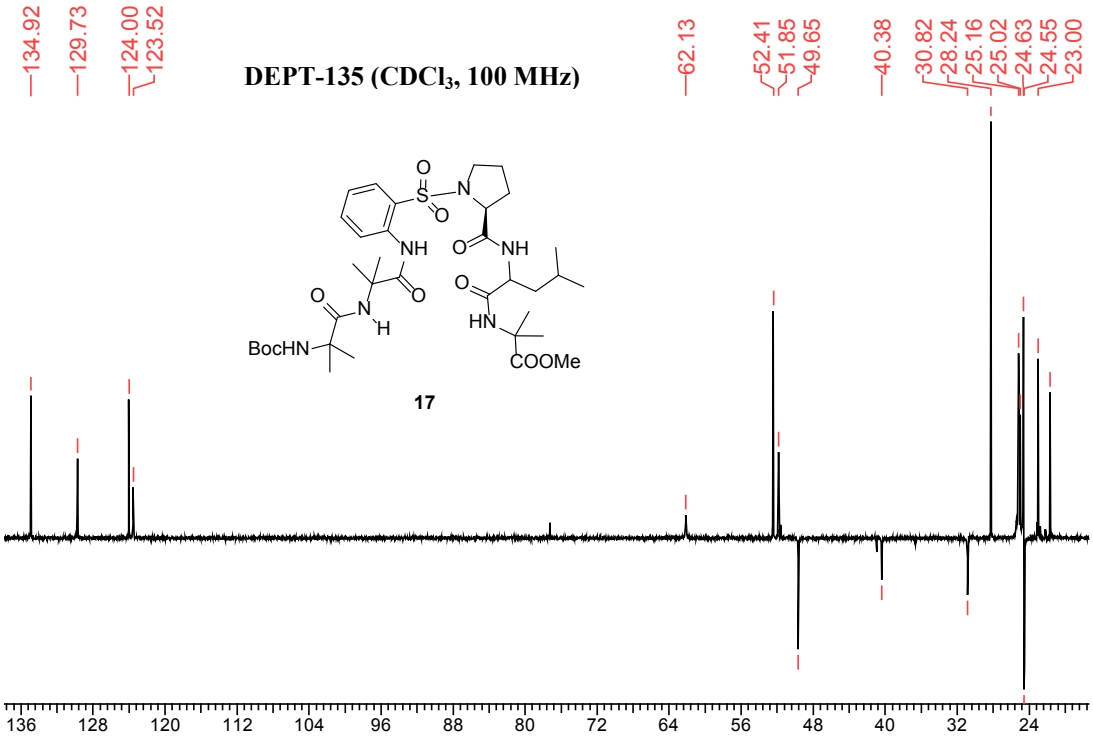
DEPT-135 ($CDCl_3$, 100 MHz)

15



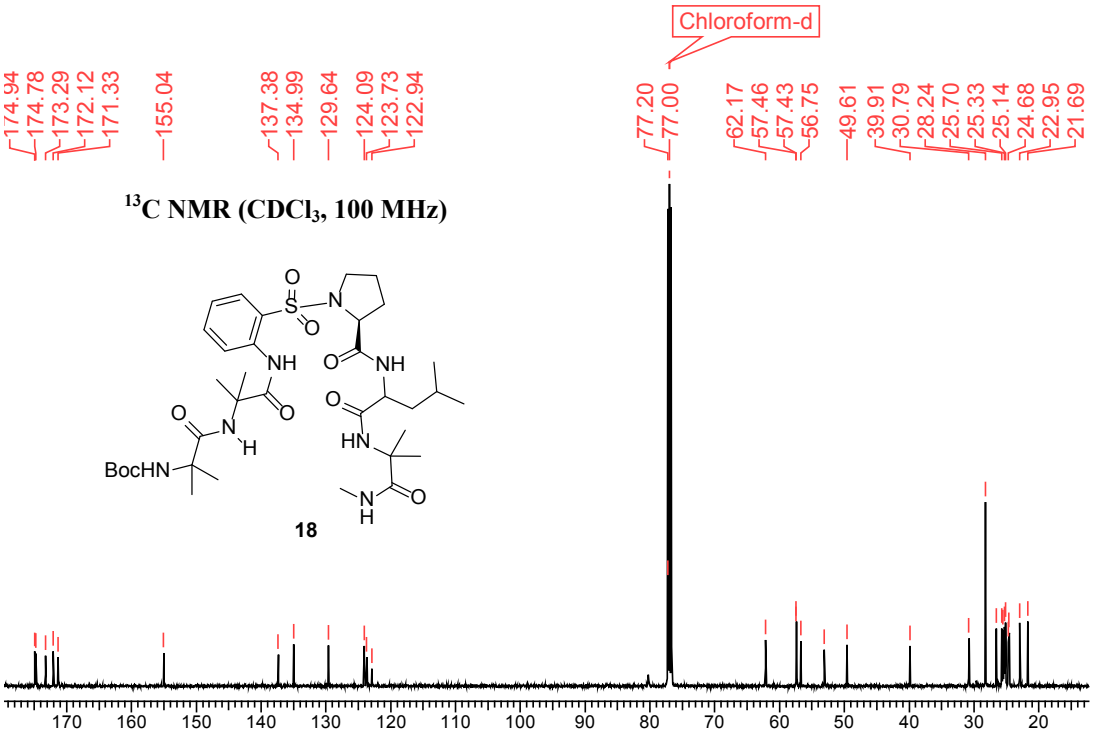
^{13}C NMR ($CDCl_3$, 100 MHz)

17



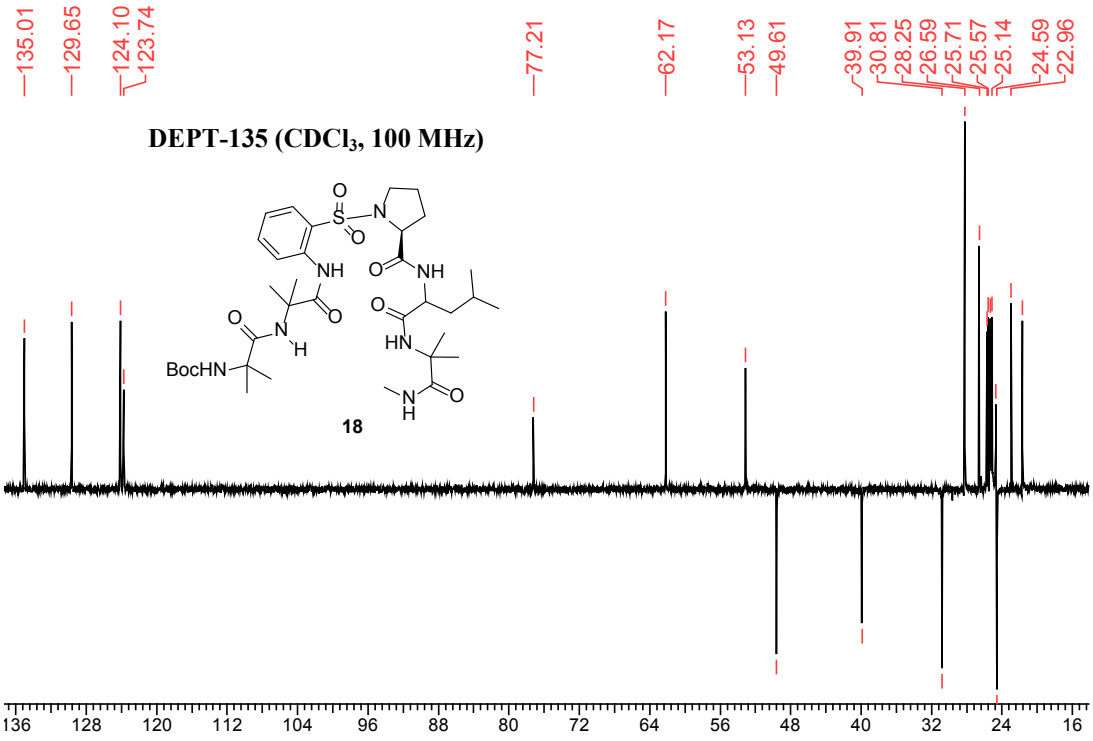
DEPT-135 ($CDCl_3$, 100 MHz)

17



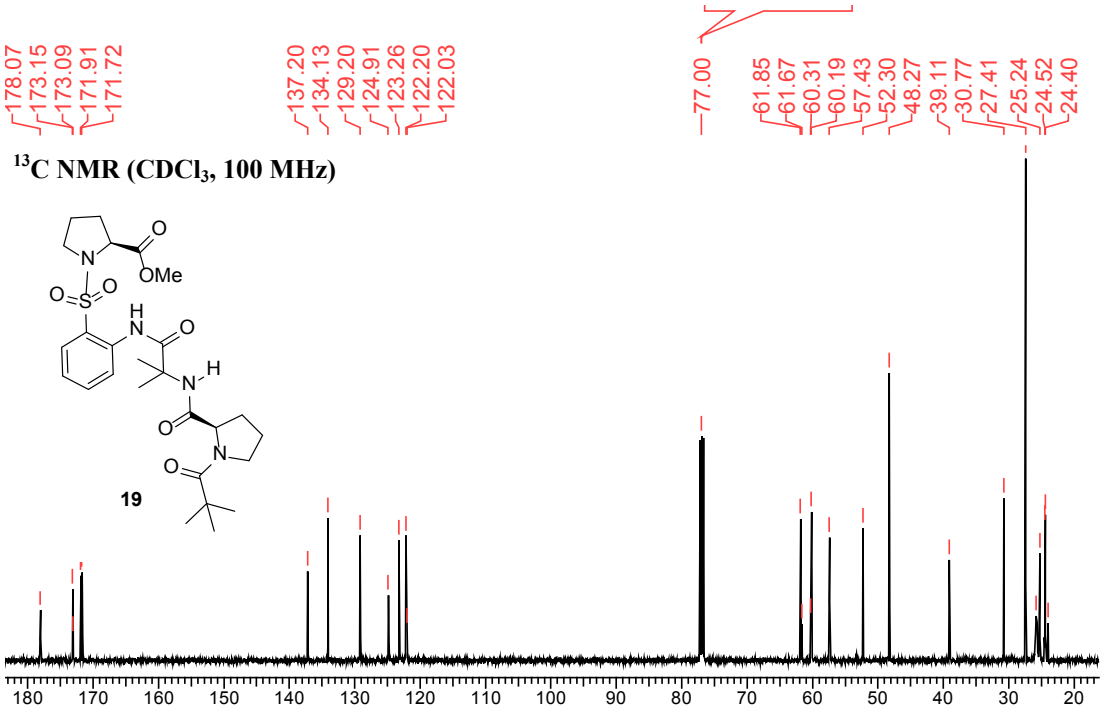
^{13}C NMR ($CDCl_3$, 100 MHz)

18



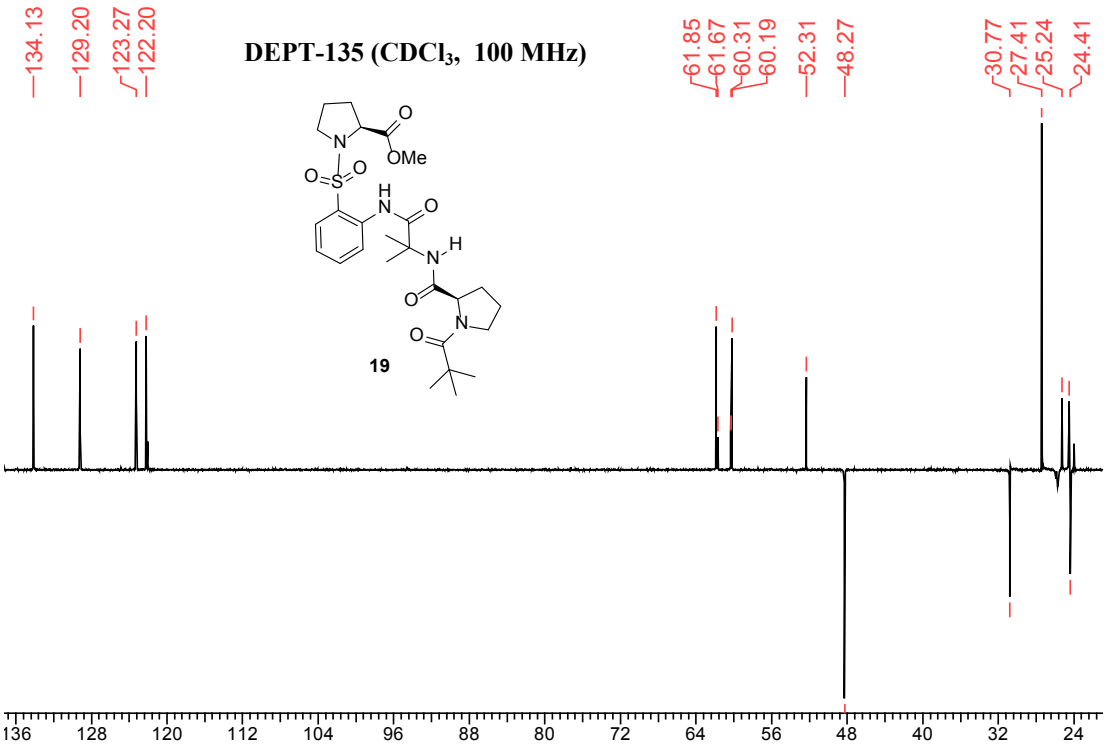
DEPT-135 ($CDCl_3$, 100 MHz)

18



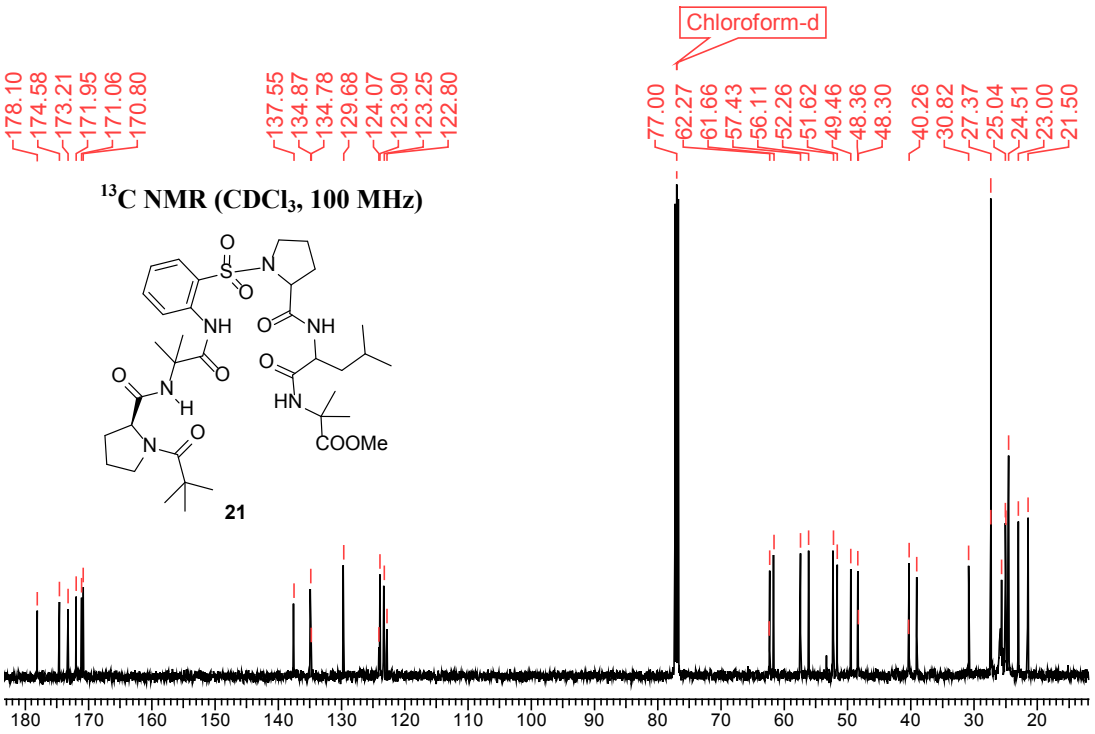
^{13}C NMR ($CDCl_3$, 100 MHz)

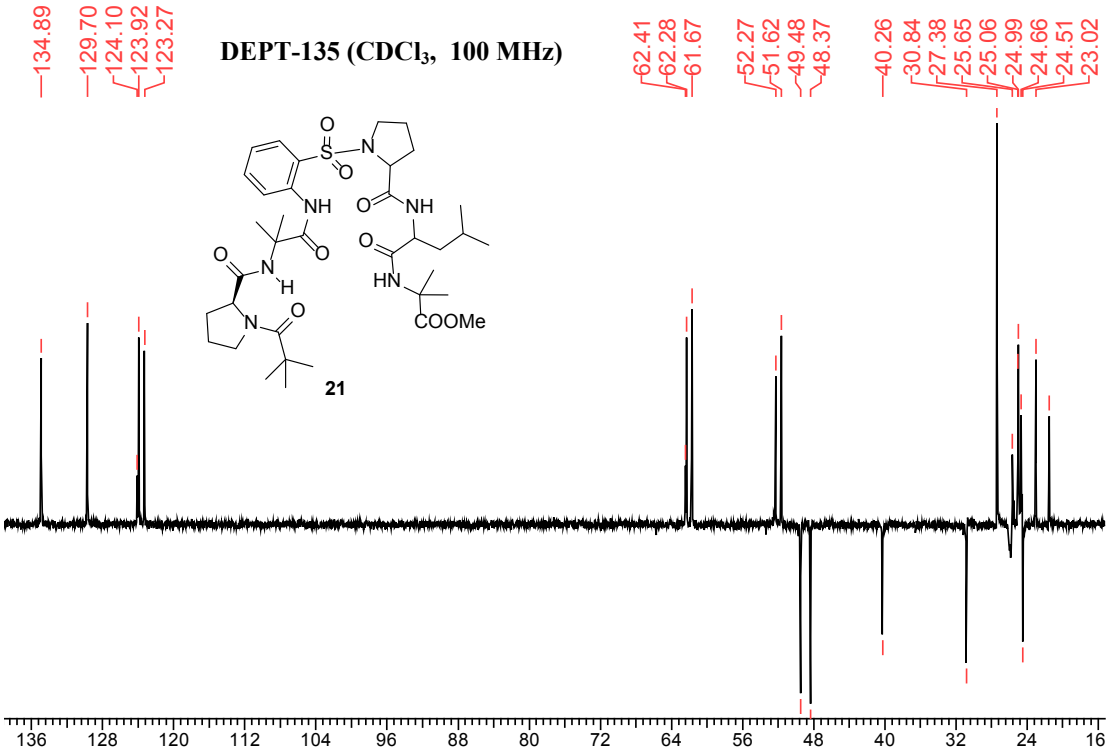
19

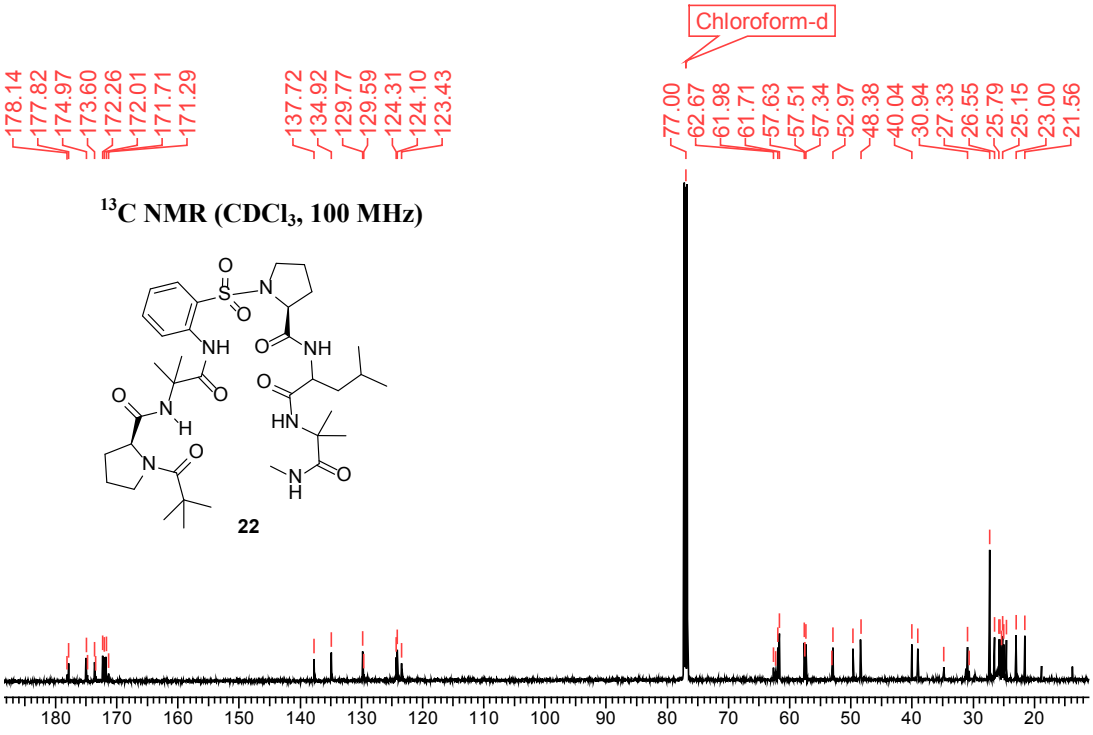


DEPT-135 ($CDCl_3$, 100 MHz)

19

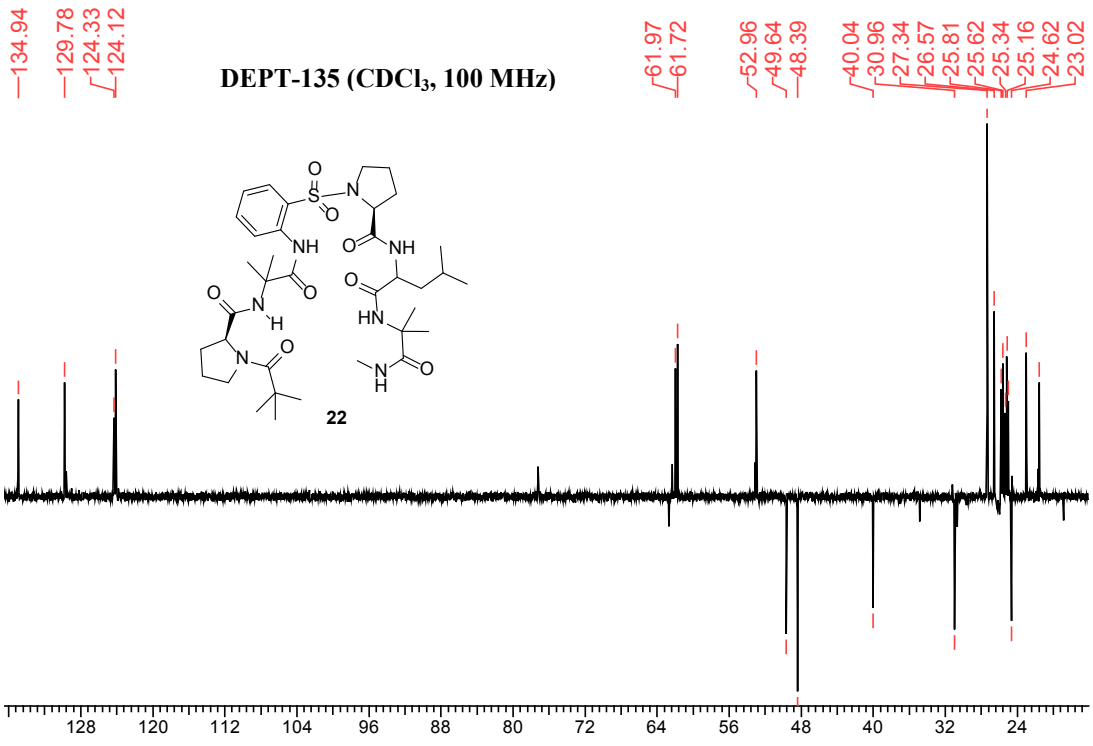

Chloroform-d
178.10
174.58
173.21
171.95
171.06
170.80
137.55
134.87
134.78
129.68
124.07
123.90
123.25
122.80
77.00
62.27
61.66
57.43
56.11
52.26
51.62
49.46
48.36
48.30
40.26
30.82
27.37
25.04
24.51
23.00
21.50
13C NMR (CDCl3, 100 MHz)
21
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20


134.89
129.70
124.10
123.92
123.27
DEPT-135 (CDCl3, 100 MHz)
62.41
62.28
61.67
52.27
51.62
49.48
48.37
40.26
30.84
27.38
25.65
25.06
24.99
24.66
24.51
23.02
21
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16



^{13}C NMR ($CDCl_3$, 100 MHz)

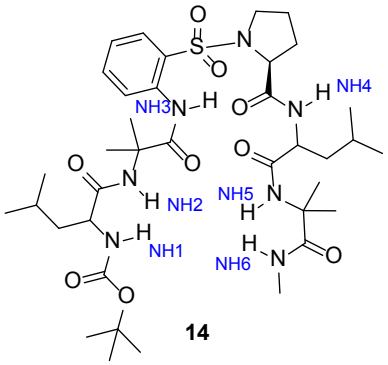
22



DEPT-135 ($CDCl_3$, 100 MHz)

22

Table 3.1 Titration Study of oligomer 14 (a) in $CDCl_3$ (10 mmol) with DMSO-d_6 (volume of DMSO-d6 added at each addition = 5 μl) and temperature variation study (b) (10 mmol, 400 MHz, $CDCl_3$)



14

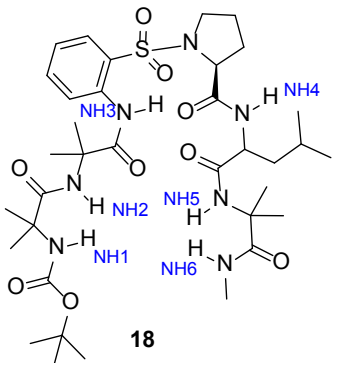
(a)

No	V DMSO-d6 (in μ lit)	Chemical Shift (in ppm)					
		NH6	NH5	NH4	NH3	NH2	NH1
1	0	7.03	6.68	7.31	10.13	7.66	5.21
2	5	7.04	6.90	7.35	10.16	7.75	5.23
3	10	7.05	7.01	7.37	10.17	7.82	5.24
4	15	7.05	7.13	7.40	10.17	7.88	5.25
5	20	7.05	7.21	7.41	10.16	7.90	5.26
6	25	7.04	7.28	7.43	10.14	7.92	5.26
7	30	7.06	7.32	7.44	10.11	7.93	5.27
8	35	7.06	7.34	7.44	10.09	7.93	5.27
9	40	7.05	7.35	7.44	10.08	7.93	5.28
10	45	7.03	7.37	7.44	10.07	7.93	5.29
11	50	7.00	7.38	7.44	10.02	7.91	5.30

(b)

Temperature (in K)	Chemical Shift (in ppm)					
	NH6	NH5	NH4	NH3	NH2	NH1
268	7.12	6.69	7.40	10.19	7.81	5.22
273	7.11	6.68	7.38	10.17	7.78	5.22
278	7.10	6.68	7.35	10.16	7.76	5.22
283	7.08	6.68	7.33	10.15	7.73	5.22
288	7.07	6.67	7.31	10.14	7.70	5.22
293	7.04	6.67	7.30	10.13	7.66	5.21
298	7.01	6.67	7.28	10.12	7.63	5.21
303	6.99	6.67	7.26	10.11	7.61	5.20
308	6.98	6.68	7.25	10.10	7.57	5.19
313	6.95	6.68	7.24	10.09	7.53	5.18
318	6.93	6.68	7.22	10.08	7.49	5.17
323	6.91	6.68	7.20	10.07	7.45	5.16

Table 3.2 Titration Study of oligomer 18 (a) in $CDCl_3$ (10 mmol) with DMSO-d_6 (volume of DMSO-d6 added at each addition = 5 μl) and temperature variation study (b) (10 mmol, 400 MHz, $CDCl_3$).



18

(a)

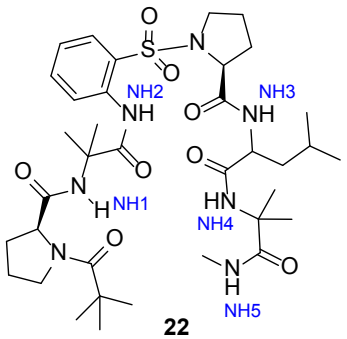
No	V DMSO-d6 (in μ lit)	Chemical Shift (in ppm)					
		NH6	NH5	NH4	NH3	NH2	NH1
1	0	6.80	6.76	7.15	9.92	7.34	5.11
2	5	6.84	6.82	7.21	9.92	7.33	5.19
3	10	6.86	6.94	7.27	9.91	7.32	5.29
4	15	6.87	6.99	7.32	9.90	7.30	5.35
5	20	6.87	7.04	7.37	9.88	7.29	5.41
6	25	6.87	7.06	7.39	9.87	7.28	5.44
7	30	6.87	7.08	7.42	9.84	7.27	5.49
8	35	6.87	7.10	7.44	9.82	7.26	5.52
9	40	6.86	7.12	7.45	9.79	7.24	5.56
10	45	6.85	7.13	7.47	9.78	7.23	5.58
11	50	6.83	7.14	7.48	9.74	7.21	5.63

(b)

Temperature (in K)	Chemical Shift (in ppm)					
	NH6	NH5	NH4	NH3	NH2	NH1
268	6.83	6.86	7.36	9.94	7.39	5.21
273	6.83	6.84	7.31	9.93	7.39	5.19
278	6.82	6.83	7.26	9.93	7.38	5.17
283	6.82	6.81	7.22	9.93	7.36	5.15
288	6.81	6.80	7.18	9.93	7.35	5.13
293	6.81	6.77	7.15	9.92	7.34	5.11
298	6.79	6.75	7.13	9.92	7.33	5.10
303	6.78	6.73	7.11	9.92	7.33	5.09
308	6.77	6.72	7.10	9.92	7.32	5.08
313	6.76	6.71	7.08	9.92	7.32	5.07
318	6.75	6.70	7.07	9.92	7.31	5.06
323	6.74	6.69	7.05	9.92	7.30	5.05

Table 3.3 Titration Study of oligomer 22 (a) in $CDCl_3$ (10 mmol) with DMSO-d_6 (volume of DMSO-d6 added at each addition = 5 μl) and temperature variation study (b) (10 mmol, 400 MHz, $CDCl_3$).

(a)



22

No	V DMSO-d6 (in μ lit)	Chemical Shift (in ppm)				
		NH5	NH4	NH3	NH2	NH1
1	0	7.07	6.91	7.03	9.91	7.67
2	5	7.05	6.96	7.12	9.89	7.66
3	10	7.03	7.01	7.19	9.88	7.65
4	15	7.00	7.05	7.28	9.85	7.64
5	20	6.98	7.08	7.35	9.83	7.63
6	25	6.96	7.10	7.40	9.80	7.62
7	30	6.95	7.12	7.44	9.78	7.61
8	35	6.93	7.13	7.46	9.74	7.60
9	40	6.91	7.14	7.49	9.71	7.59
10	45	6.89	7.15	7.50	9.68	7.58
11	50	6.88	7.16	7.52	9.66	7.56

(b)

Temperature (in K)	Chemical Shift (in ppm)				
	NH5	NH4	NH3	NH2	NH1
268	7.14	7.01	7.18	9.92	7.76
273	7.13	6.99	7.14	9.91	7.74
278	7.12	6.97	7.11	9.91	7.73
283	7.11	6.95	7.08	9.91	7.71
288	7.09	6.94	7.06	9.91	7.69
293	7.07	6.91	7.04	9.91	7.67
298	7.05	6.89	7.02	9.91	7.66
303	7.03	6.87	7.01	9.91	7.64
308	7.01	6.86	7.00	9.91	7.63
313	6.99	6.84	6.99	9.91	7.61
318	6.97	6.82	6.98	9.91	7.60
323	6.94	6.80	6.97	9.91	7.59

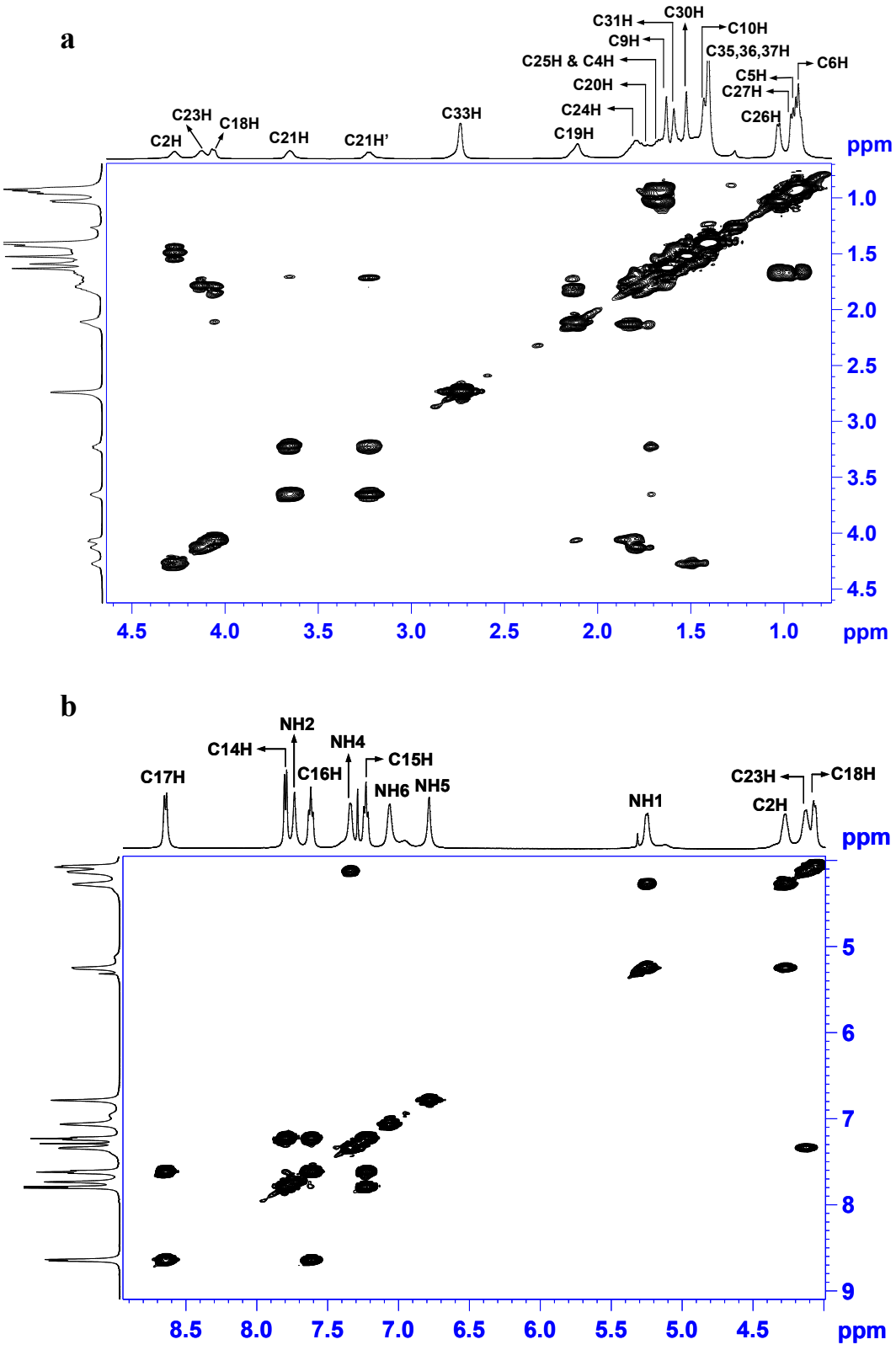


Fig. 3.10 Partial COSY spectrum (**a** and **b**) of oligomer **14** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

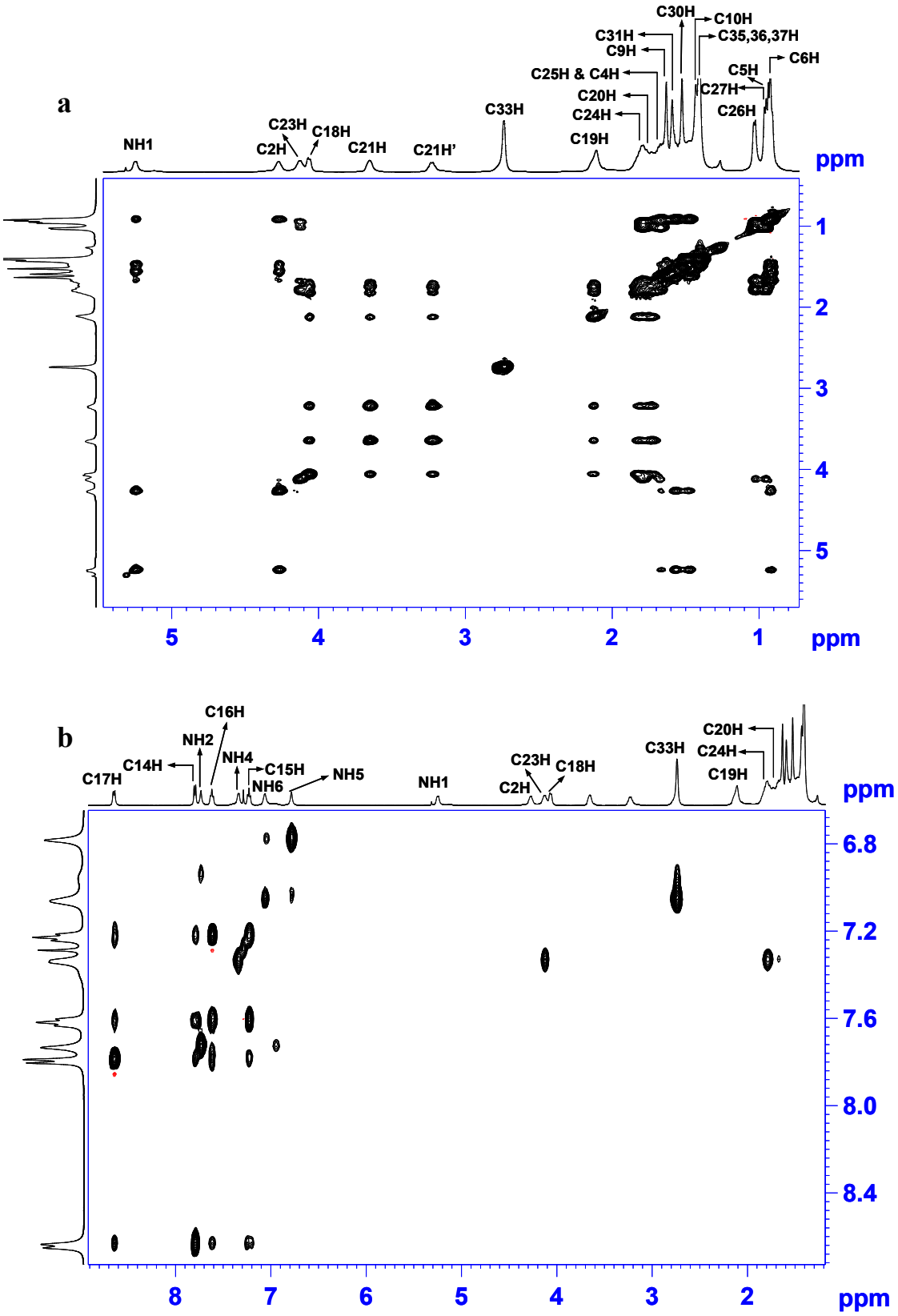


Fig. 3.11 Partial TOCSY spectrum (**a** and **b**) of oligomer **14** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

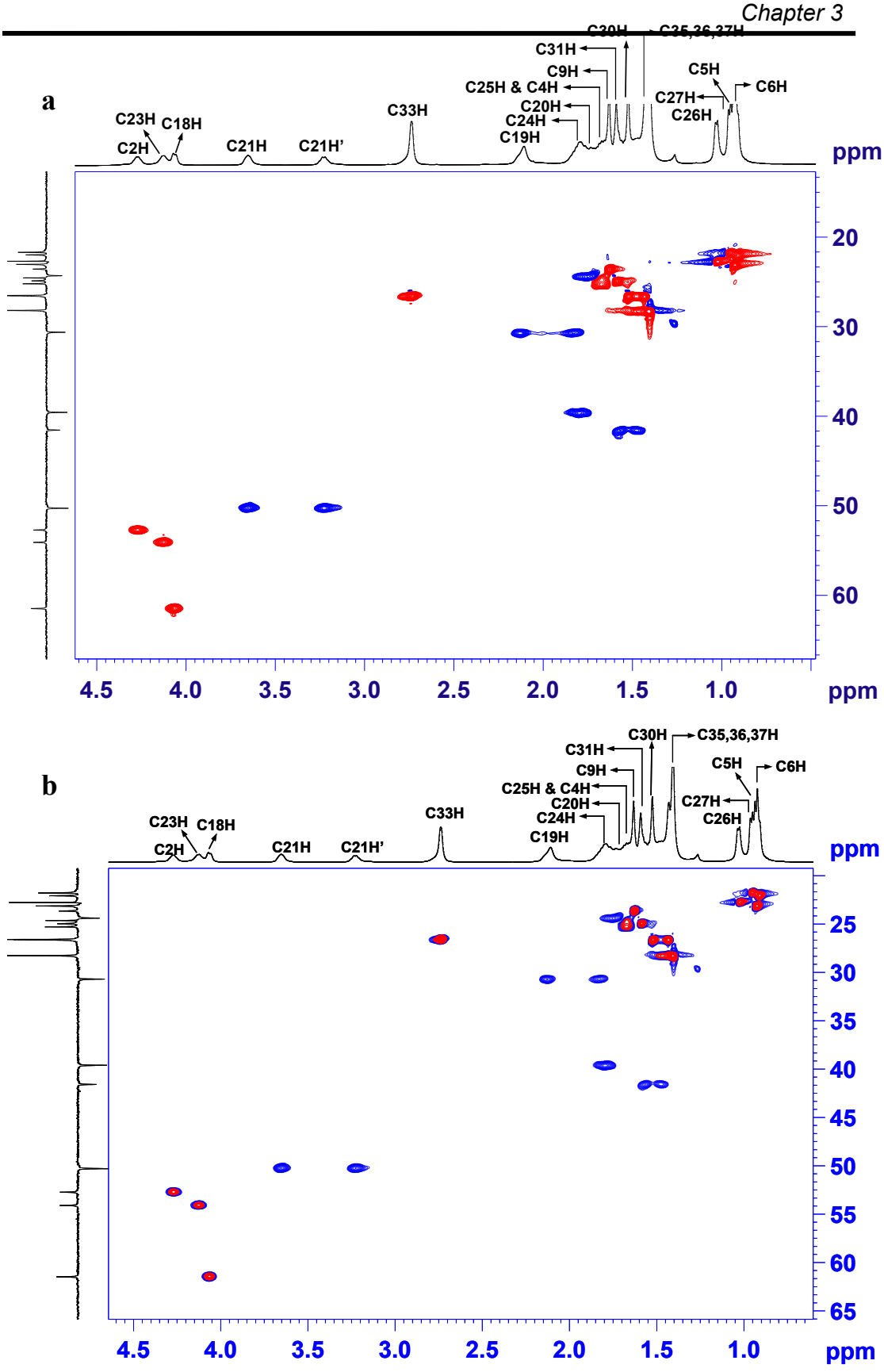


Fig. 3.12 Partial HSQC spectrum (**a** and **b**) of oligomer **14** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

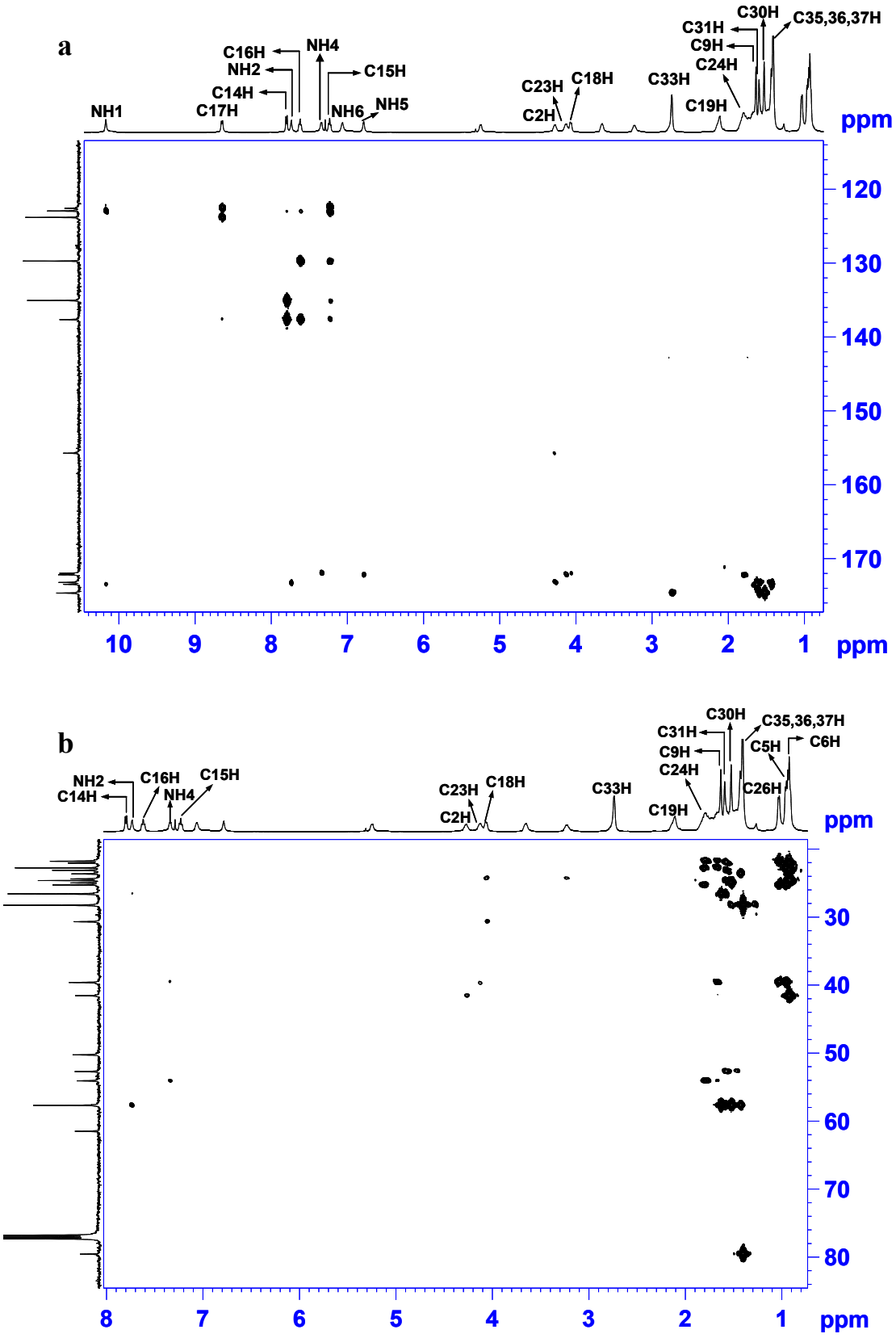


Fig. 3.13 Partial HMBC spectrum (**a** and **b**) of oligomer **14** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

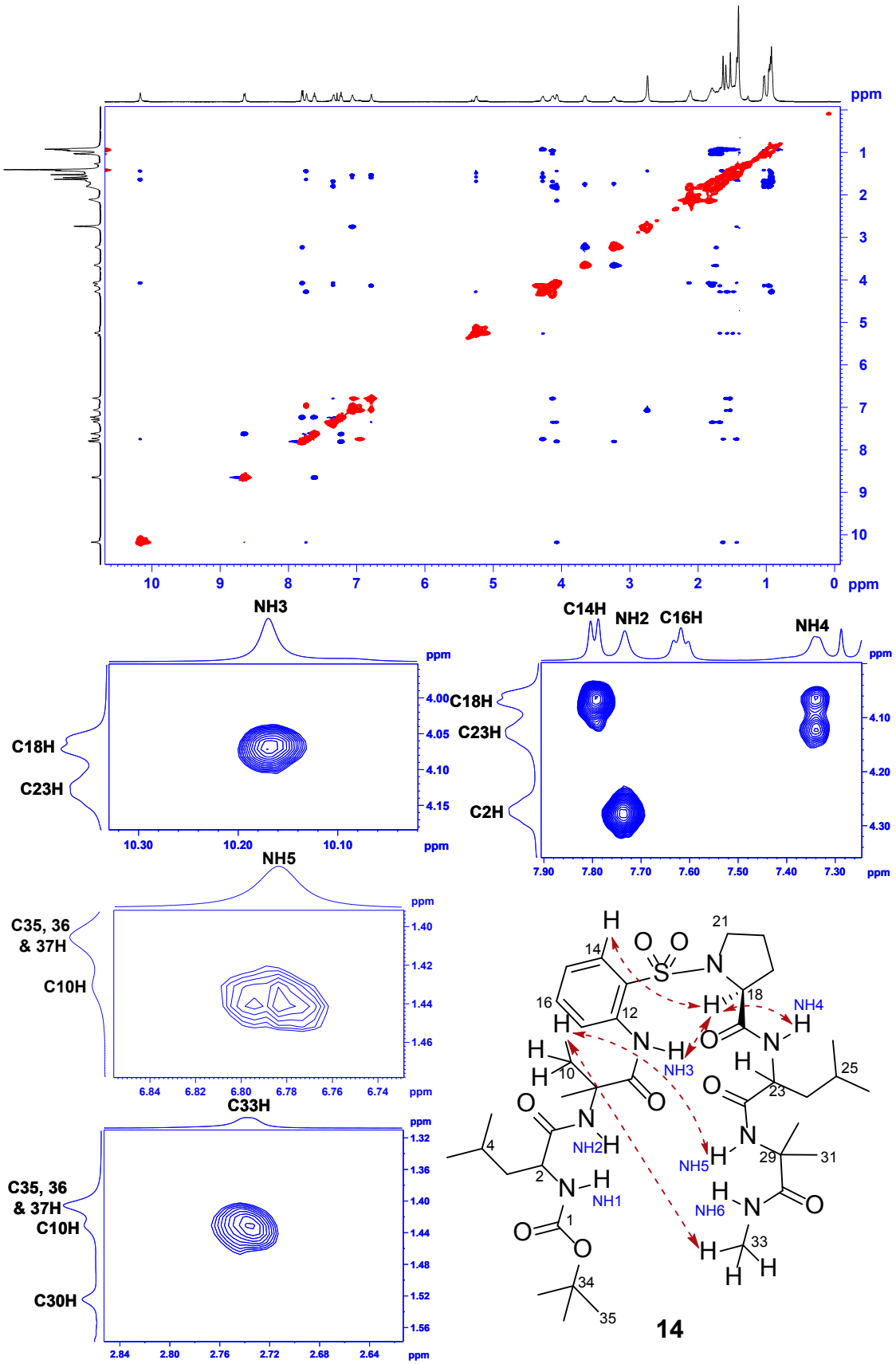


Fig. 3.14 NOESY spectrum and selected nOe excerpts of oligomer **14** (500 MHz, $CDCl_3$).

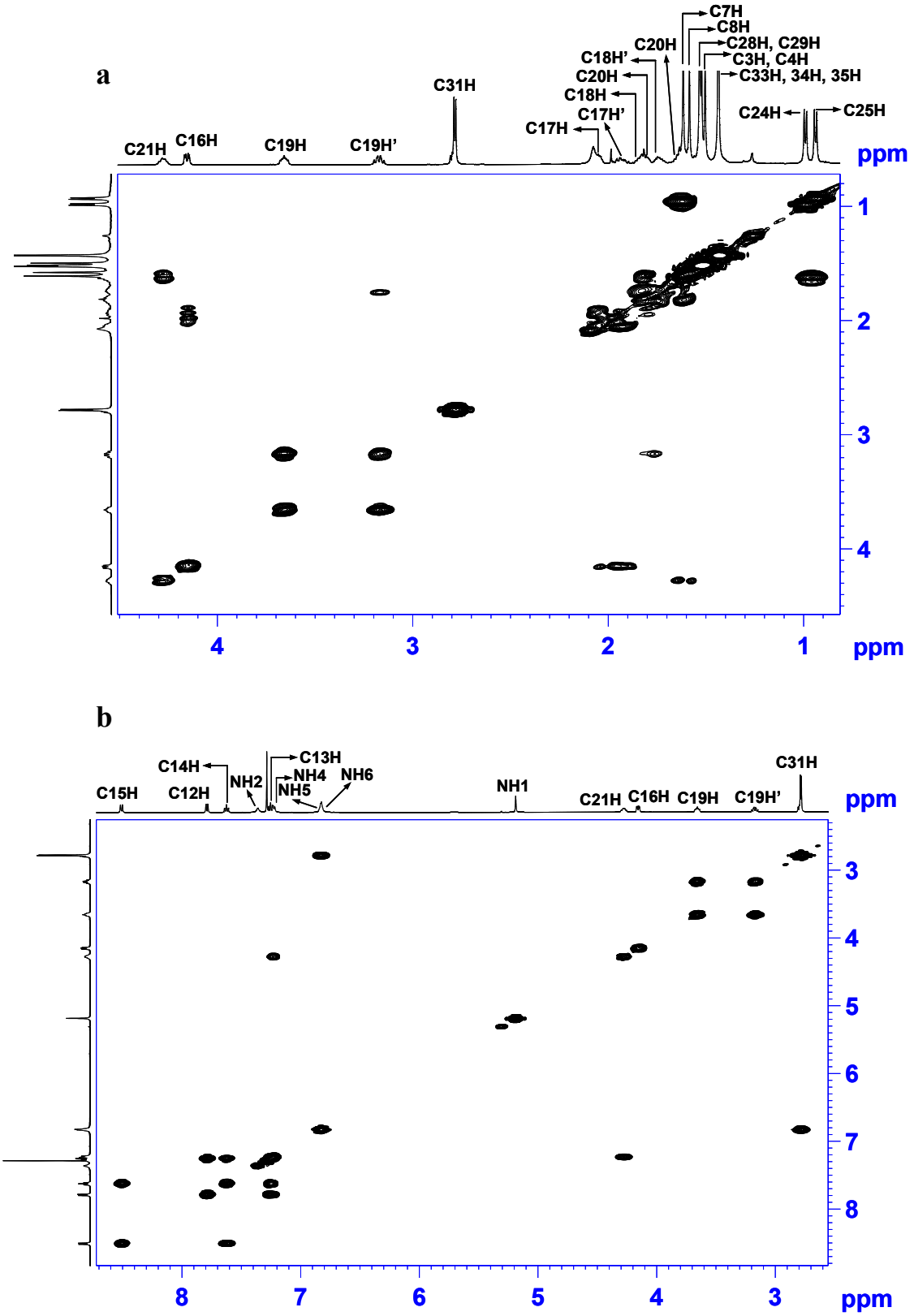


Fig. 3.15 Partial COSY spectrum (**a** and **b**) of oligomer **18** (500 MHz, $CDCl_3$).

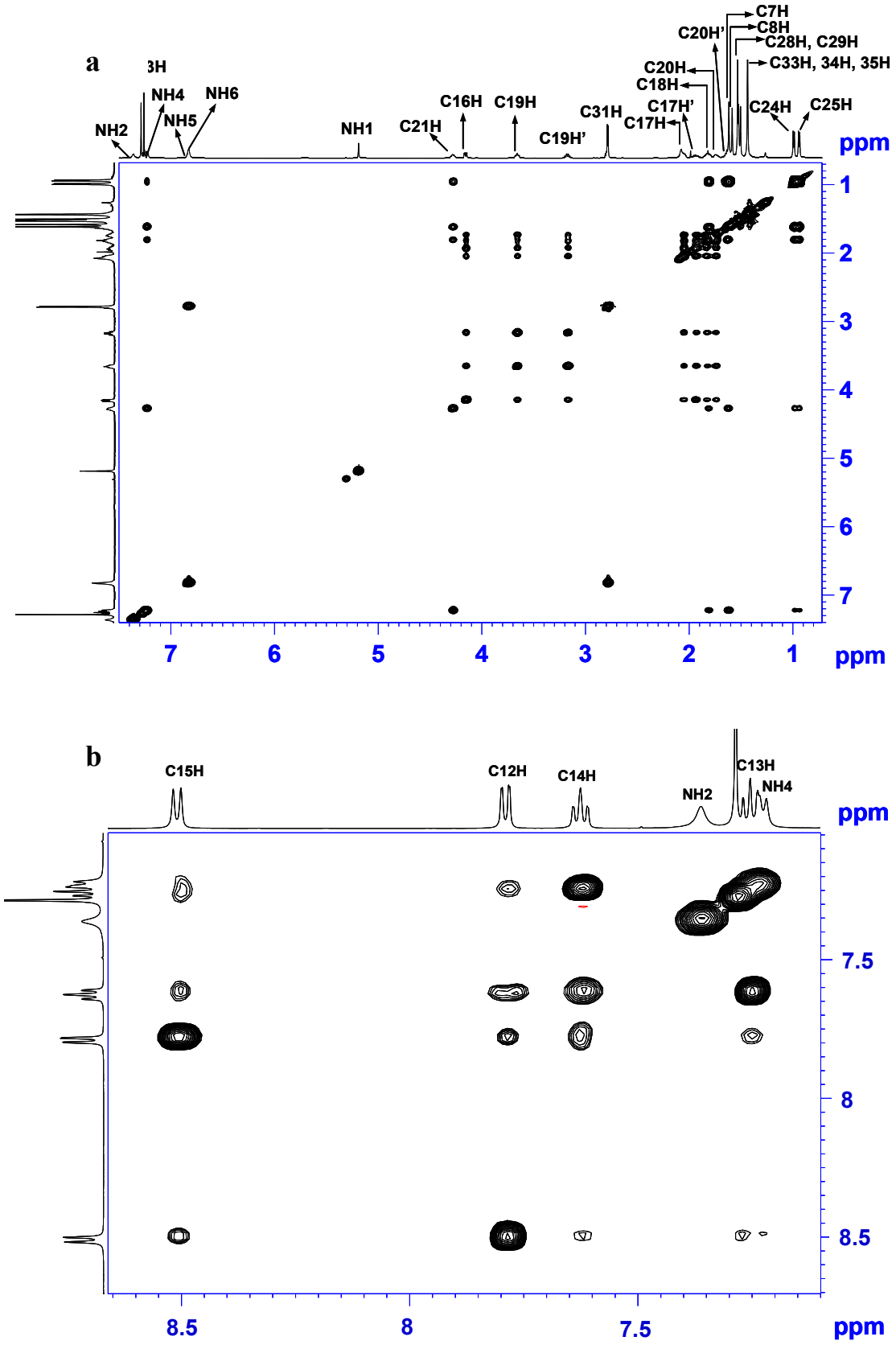


Fig. 3.16 Partial TOCSY spectrum (**a** and **b**) of oligomer **18** (500 MHz, $CDCl_3$).

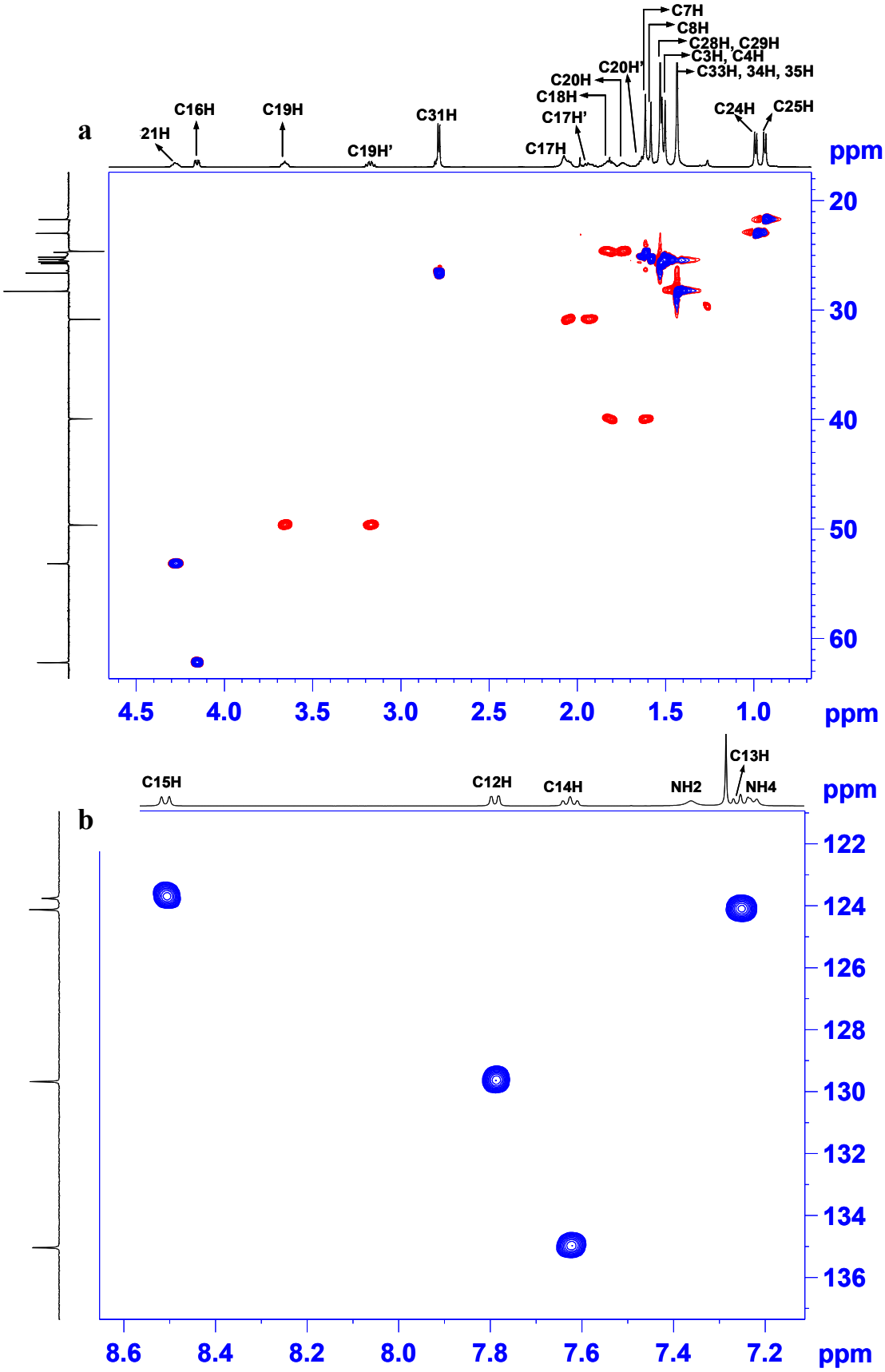


Fig. 3.17 Partial HSQC spectrum (**a** and **b**) of oligomer **18** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

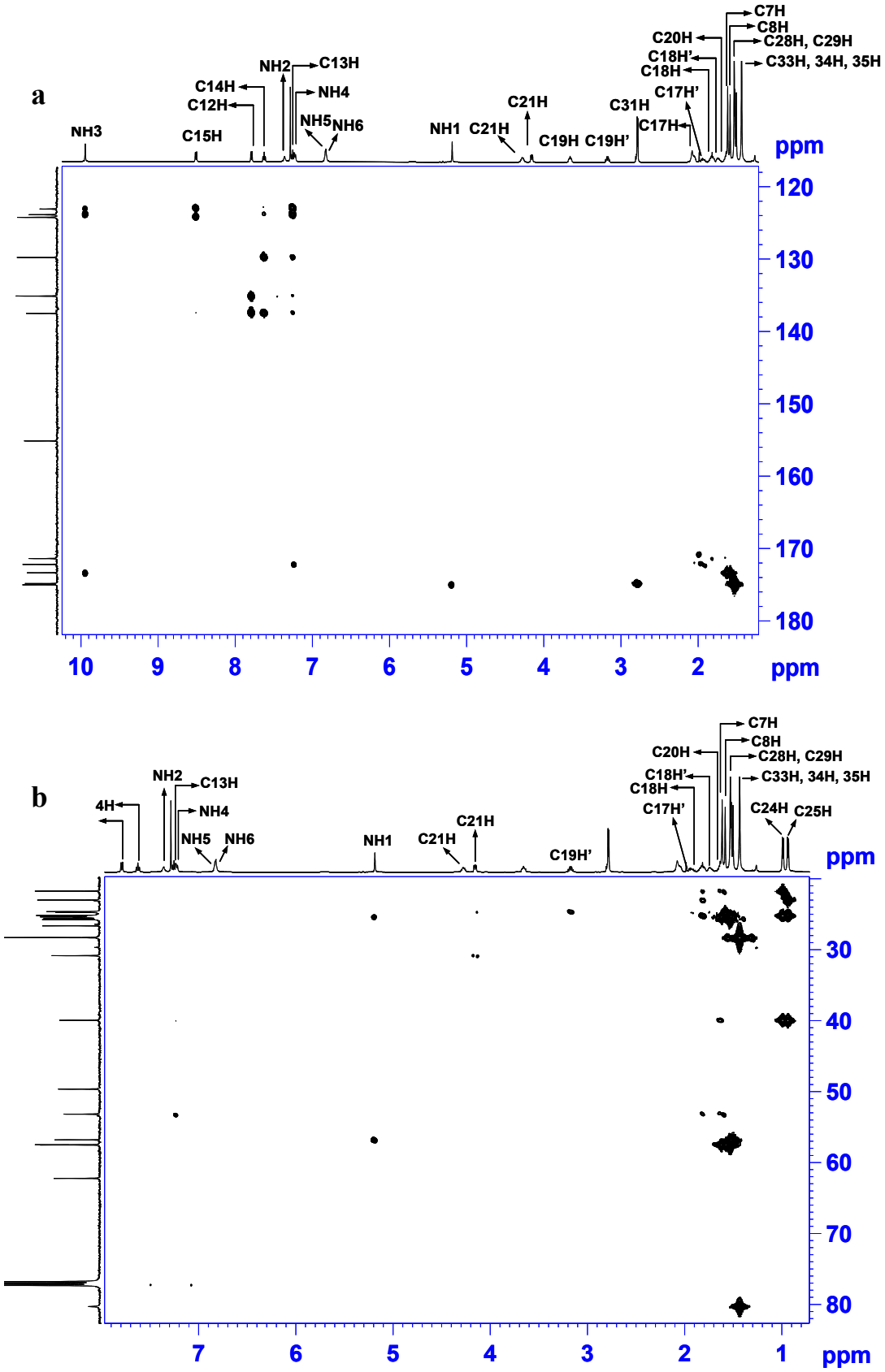


Fig. 3.18 Partial HMBC spectrum (**a** and **b**) of oligomer **18** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

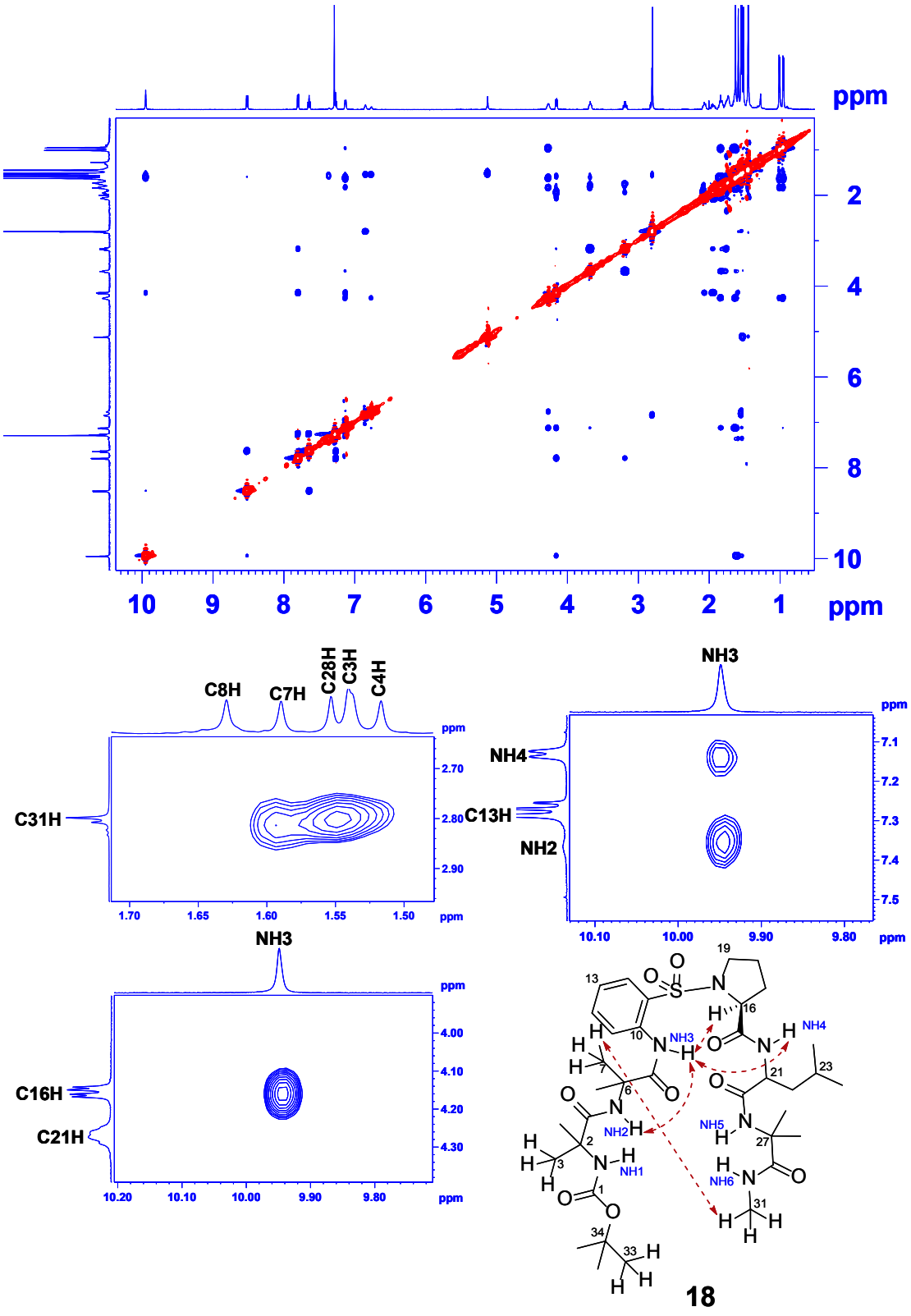


Fig. 3.19 NOESY spectrum and selected nOe excerpts of oligomer **18** (500 MHz, $CDCl_3$).

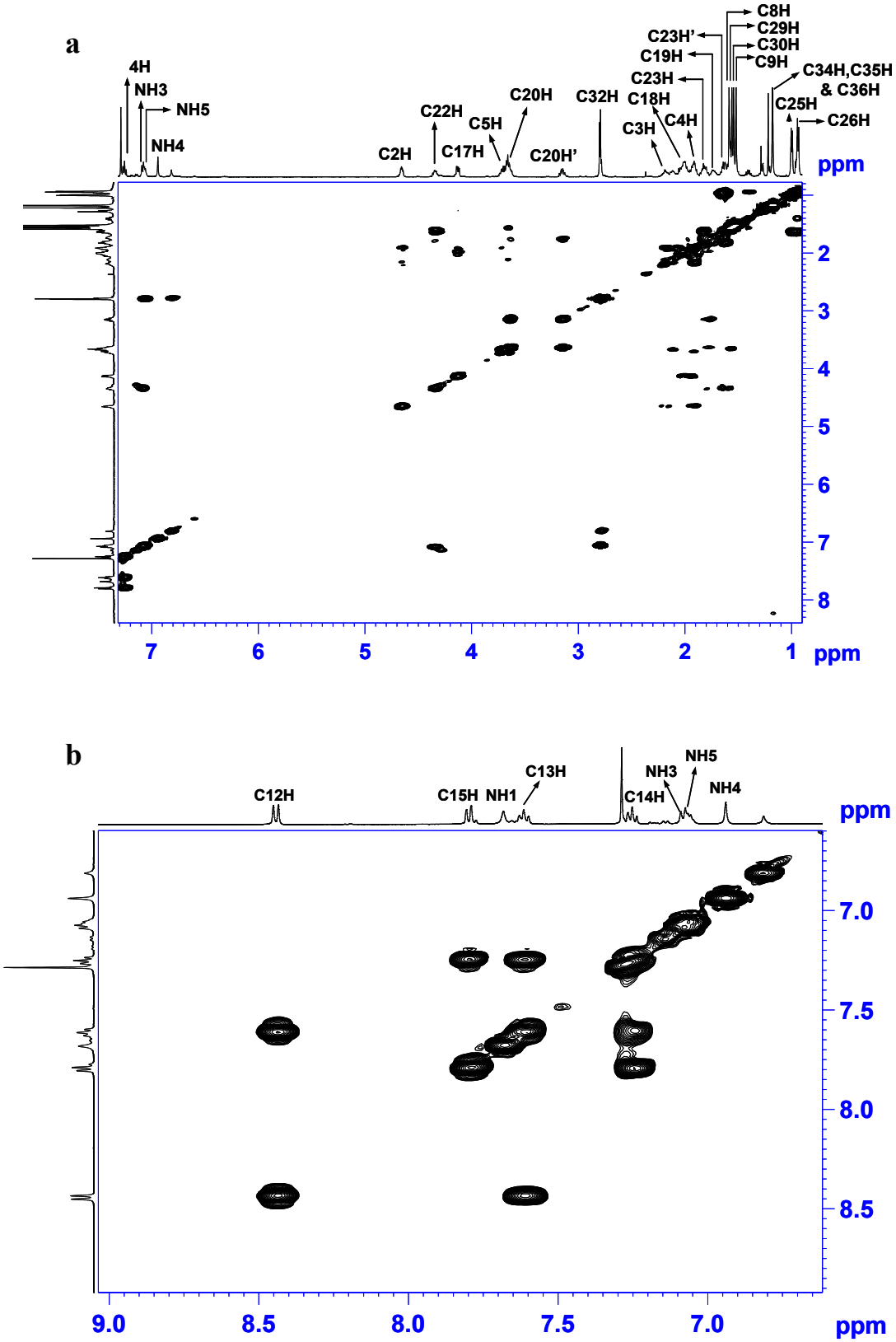


Fig. 3.20 Partial COSY spectrum (**a** and **b**) of oligomer **22** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

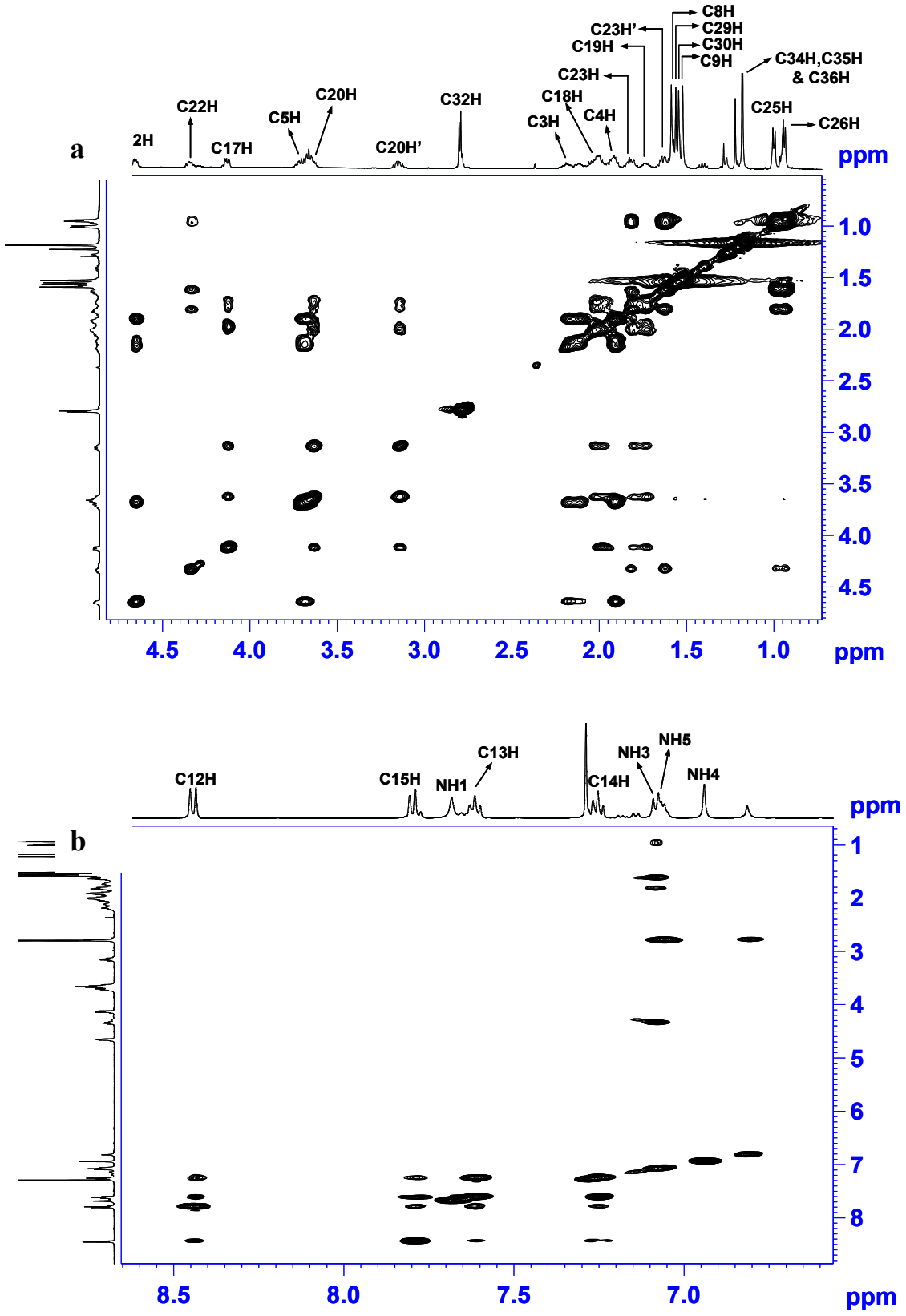


Fig. 3.21 Partial TOCSY spectrum (**a** and **b**) of oligomer **22** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

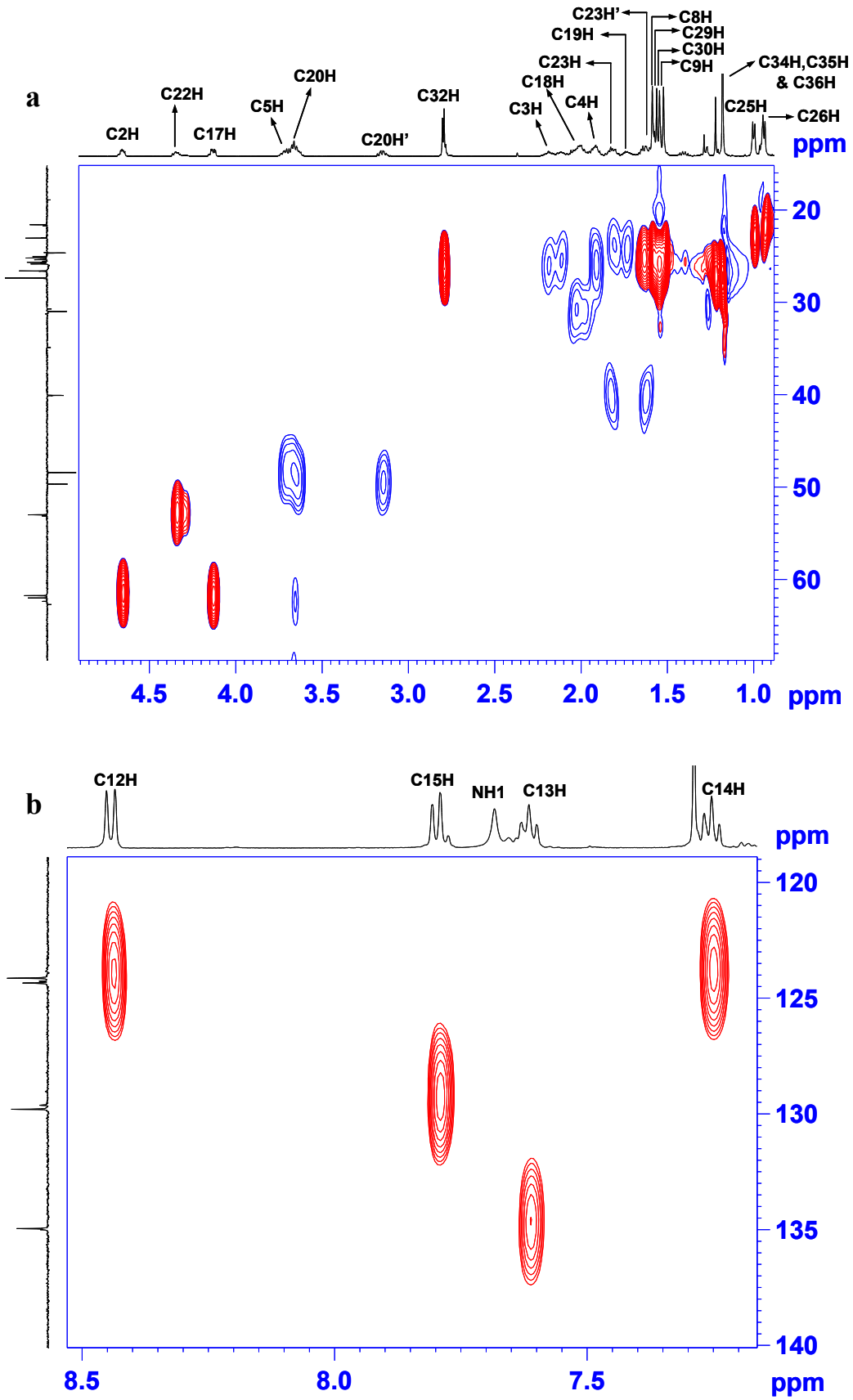


Fig. 3.22 Partial HSQC spectrum (**a** and **b**) of oligomer **22** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

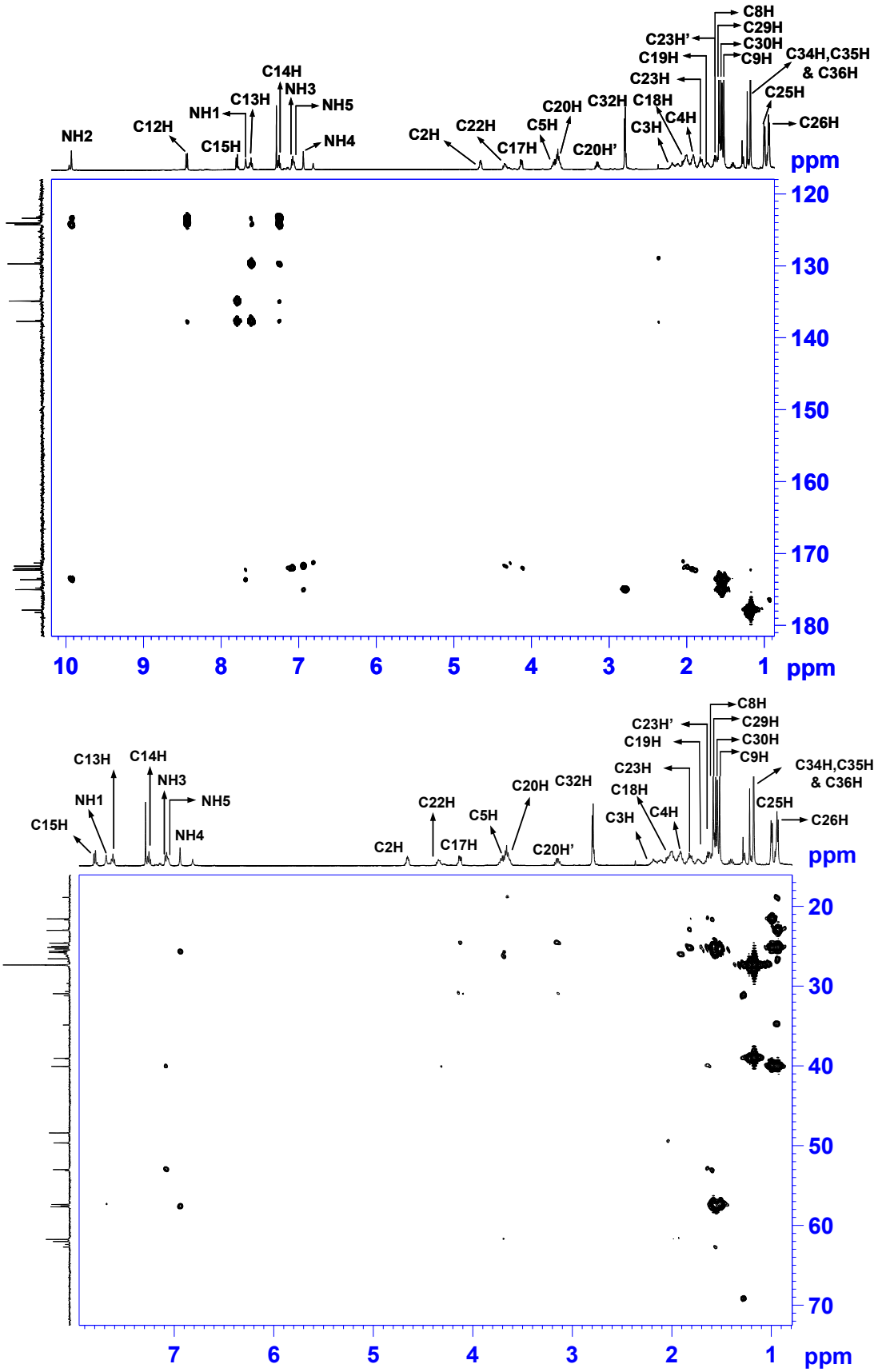


Fig. 3.23 Partial HMBC spectrum (**a** and **b**) of oligomer **22** (500 MHz, $CDCl_3$). For better view, aromatic and aliphatic regions are given separately.

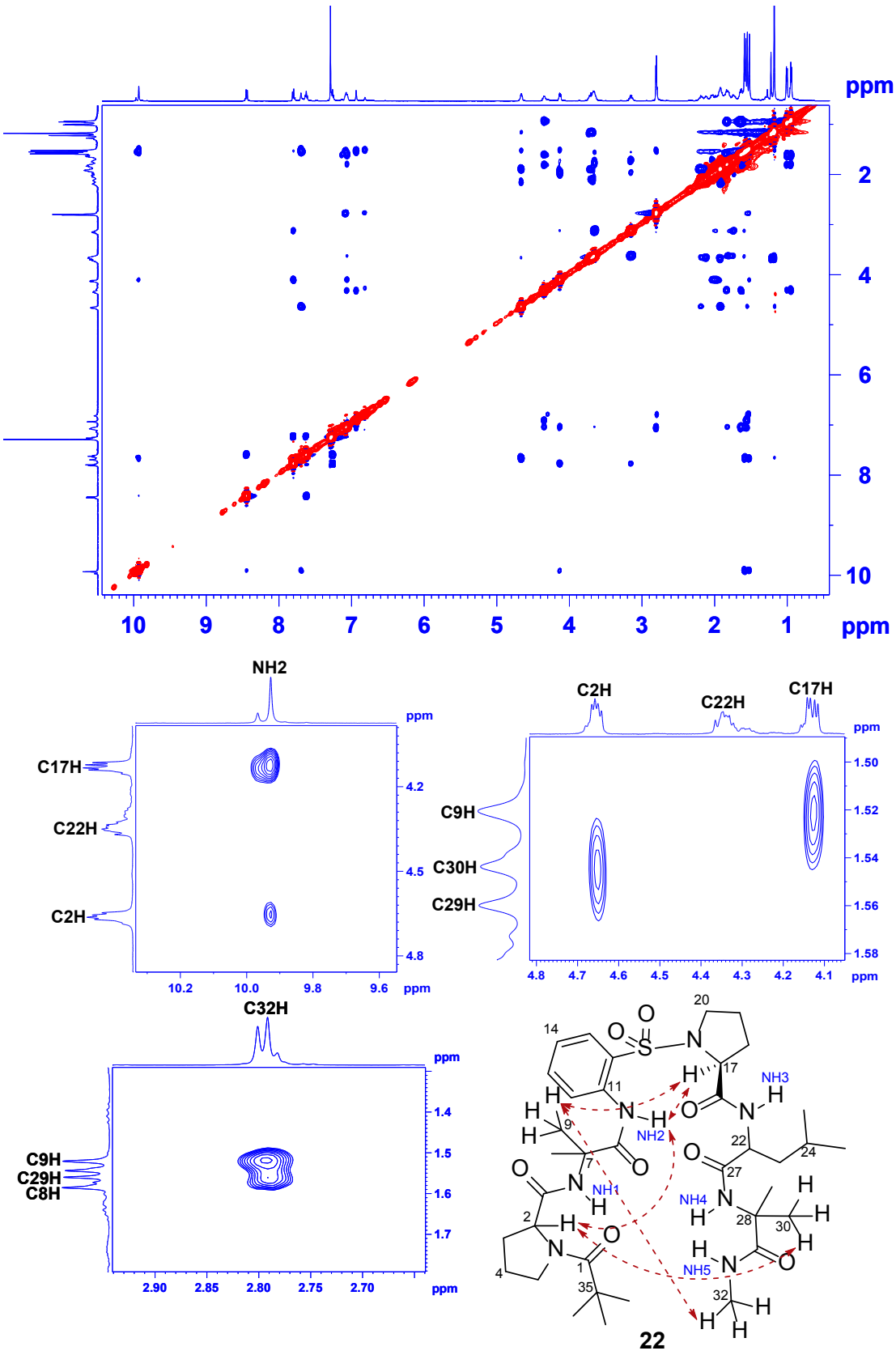


Fig. 3.24 NOESY spectrum and selected nOe excerpts of oligomer **14** (500 MHz, $CDCl_3$).

Table 3.4 nOe distances of oligomer **14**

Atom-I	Atom-II	Chemical Shift I	Chemical Shift II	Upper Bound	Lower bound
17	NH3	8.6426	10.1753	4.6600	3.8127
NH2	NH3	7.7484	10.1753	3.9600	3.2400
18	NH3	4.0767	10.1753	3.4667	2.8364
10	NH3	1.6359	10.1753	3.3916	2.7750
9	NH3	1.4413	10.1753	3.6283	2.9686
9	17	1.4413	8.6426	5.7867	4.7345
9	NH2	1.4413	7.7484	3.3658	2.7538
10	NH2	1.6359	7.7484	3.5904	2.9376
14	21A	7.795	3.2298	3.3091	2.7074
14	18	7.795	4.0767	3.2652	2.6715
2	NH2	4.2819	7.7484	3.1164	2.5498
NH1	NH2	5.255	7.7371	4.4330	3.6270
26	NH4	0.9574	7.3479	5.2692	4.3111
20	NH4	1.6728	7.3479	3.3833	2.7681
24	NH4	2.1251	7.3479	4.8980	4.0074
21B	NH4	3.6559	7.3479	4.8772	3.9905
18	NH4	4.0767	7.3479	3.6335	2.9729
23	NH4	4.1241	7.3479	3.3704	2.7576
NH5	NH4	6.791	7.3479	3.9683	3.2468
30	NH6	1.5255	7.0612	3.4542	2.8261
31	NH6	1.5939	7.0612	3.5290	2.8873
33	NH6	2.7406	7.0612	3.0122	2.4645
27	NH5	1.0363	6.7877	5.5280	4.5229
30	NH5	1.515	6.7877	3.3896	2.7733
31	NH5	1.5991	6.7877	3.3384	2.7314
25	NH5	1.8095	6.7877	4.7222	3.8636
23	NH5	1.8095	6.7877	3.2189	2.6336
6	NH1	0.9311	5.2464	5.3238	4.3558
35	NH1	1.415	5.2464	4.1666	3.4091
3	NH1	1.415	5.2464	3.6025	2.9475
2	NH1	4.2661	5.2464	3.8061	3.1141
2	6	4.2661	0.9153	2.9529	2.4160
23	26	4.1312	0.9574	3.0893	2.5276
9	18	1.4466	4.076	3.8016	3.1104
23	20	4.1338	1.6675	3.5087	2.8707
10	33	1.4308	2.7478	3.7432	3.0626
31	33	1.5307	2.7478	4.6416	3.7977

Table 3.5 nOe distance restraints used to determine the solution state structure of oligomer **18**.

Atom I	Atom II	Chemical shift I	Chemical shift II	Upper Bound	Lower Bound
15	NH3	8.5188	9.9472	4.1897	3.4279
16	NH3	4.1559	9.9472	3.5851	2.9333
7	NH3	1.6111	9.9472	2.7159	2.2221
8	NH3	1.46	9.9472	3.6211	2.9627
7	15	1.6111	8.5188	4.0429	3.3078
12	19’	7.8018	3.1893	3.3499	2.7409
12	19	7.8018	3.6719	5.4713	4.4765
7	NH2	1.6111	7.3691	3.2756	2.6800
23	NH4	1.6056	7.1375	2.7899	2.2827
17	NH4	2.0591	7.1375	4.3316	3.5440
24	NH4	0.9732	7.1375	4.1401	3.3874
28	NH6	1.5501	6.8519	3.0606	2.5041
25	NH5	1.5584	6.7576	3.1717	2.5950
31	NH6	2.8024	6.8519	2.9171	2.3867
21	NH6	4.2599	6.8519	4.4338	3.6277
21	NH5	4.2599	6.7576	3.5880	2.9356
19	NH4	3.6802	7.1375	4.0895	3.3459
16	NH4	4.1337	7.1375	3.2511	2.6600
21	NH4	4.2988	7.1375	3.3505	2.7413
3	NH1	1.5044	5.1239	2.6991	2.2084
16	17	4.1573	1.9897	2.6182	2.1422
16	7	4.1573	1.6111	3.4722	2.8409
29	31	1.5443	2.8024	3.5909	2.9380
8	31	1.46	2.8024	3.8454	3.0053

Table 3.6 nOe distance restraints used to determine the solution state structure of oligomer **22**.

Atom I	Atom II	Chemical Shift I	Chemical Shift II	Upper Bound	Lower Bound
16	NH2	8.4313	9.9262	4.6873	3.8350
17	NH2	4.1307	9.9262	3.8322	3.1355
8	NH2	1.585	9.9262	3.0018	2.4560
9	NH2	1.52123	9.9262	3.3628	2.7514
34	16	1.1733	8.4423	5.3222	4.3545
34’	16	1.585	8.4423	5.2548	4.2994
Piv	NH1	1.1733	7.673	5.0630	4.1424
9	NH1	1.52123	7.673	3.4275	2.8043
8	NH1	1.585	7.673	2.9332	2.3999
13	20	7.7978	3.1449	3.3920	2.7753
13	17	7.7978	4.1307	2.9032	2.3753
2	NH1	4.6507	7.6841	2.7462	2.2469
29	NH5	1.5416	7.0612	3.2115	2.6276
18	NH3	2.0183	7.0829	4.7169	3.8592
32	NH5	2.7983	7.0612	2.9141	2.3842
17	NH3	4.1199	7.0829	3.4999	2.8635
22	NH3	4.3365	7.0829	3.4895	2.8550
22	NH4	4.3365	6.9421	3.2346	2.6465
2	35	4.6566	1.1733	4.0426	3.3076
2	4	4.6566	1.5525	4.1170	3.3685
2	30	4.6507	1.5413	4.6066	3. 8100
22	26	4.337	0.211	2.9643	2.4254
9	17	1.5178	4.1258	3.9916	3.2658
29	32	1.5512	2.7935	3.8613	3.1592
8	32	1.5202	2.7935	3.6221	2.9635
5	35	3.725	1.2167	2.6951	2.2051
5’	35	3.66	1.2167	2.6854	2.1971
NH1	NH2	7.673	9.9262	3.5527	2.9068
2	NH2	4.6615	9.9262	4.9115	4.0185

3.6 References and Notes

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Erratum