Accepted Manuscript

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PII: S0040-4020(14)01177-6

DOI: 10.1016/j.tet.2014.08.014

Reference: TET 25914

To appear in: *Tetrahedron*

Received Date: 28 May 2014

Revised Date: 22 July 2014

Accepted Date: 8 August 2014

Please cite this article as: Balachandra C, Sharma NK, Synthesis and Conformational Analysis of New *Troponyl* Aromatic Amino Acid, *Tetrahedron* (2014), doi: 10.1016/j.tet.2014.08.014.

Graphical Abstract

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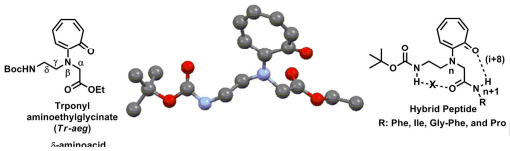
Graphical Abstract



Tetrahedron
journal homepage: www.elsevier.com

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ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Keyword_1 Tropolone
Keyword_2 Aaminotropone
Keyword_3 Conformational analysis
Keyword_4 Aminoethylglycine (aeg)
Keyword_5 δ-amino acid, hybrid Peptide

ABSTRACT

Synthetic peptides are in huge demand in expansion of potential peptide mimics, which may have improved or comparable function as natural one. With these concerns, phenyl bearing aromatic amino acids and peptides has extensively explored, because phenyl residue has high probability in forming stable secondary structure, owing to the presence of an extra stabilizing factor as π-π non-covalent interactions. Apart from phenyl bearing benzenoid aromatic amino acids, a few non-benzenoid aromatic derivatives such as tropolone and related compounds are also occurred in nature, but troponyl containing amino acids and peptides are very poorly understood. Tropolonyl derivatives also contain carbonyl functional group, which may play an important role to provide stable conformation in peptide. Herein we report the synthesis, and conformational analysis of rationally designed *new* unnatural *δ*-amino acid, troponyl aminoethyl glycine (*Tr-aeg*), which contains troponyl residue as side chain in flexible aminoethylglycine (*aeg*) amino acid backbone. We also demonstrate the role of troponyl carbonyl of *Tr-aeg* residue in hydrogen bonding with adjacent amide NH of their hybrid *di/tri*-peptides with NMR methods and DFT calculations. In future, *Tr-aeg* amino acid would be a potential building block in development of promisable peptide mimics.

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Introduction

Conformational analysis of a molecule defines the type of structural organization, which explain the functional behavior of that molecule.[1] In case of macromolecules, the conformation of building blocks has crucial role in controlling the overall functionality of molecules.[2] For an instance, the function of protein and enzyme relay on favorable conformation of their building block such as amino acid residue. The side chain functionality of that amino acid residue having stable conformation is also responsible in modulation of non-covalent interactions for being functional respective protein/enzymes.[3,4] Due to variable side chain of amino acids residue, various non-covalent interactions are present in functional protein and enzymes. Among them, hydrogen bonding, one of the important non-covalent interactions, plays an important role to acquire the well defined structural organization in protein/enzymes.[4,5] To improve the structural and functional properties, many synthetic peptide mimics have been synthesized from natural and unnatural amino acids and nicely explored.[6] Some of these peptide mimics have shown exceptional functional properties and then considered as therapeutics drug candidate.[7] For ideal peptide mimic, the presence of following secondary structural elements-helices, turns and sheets are required in target peptide to adopt protein-like conformations and folding pattern.[5,8] To meet these requirements, many unnatural synthetic peptides from non-natural amino acid, including backbone expanded amino acids such as *β*-,[8-10] *γ*-,[11] and *δ*-amino acid[12,13] were synthesized and studied extensively to find the interesting structurally folding behaviors. Some of these synthetic peptides have shown promiseable folding behavior with significant secondary structural elements. It is also learnt that the substituent of synthetic amino acids (*β*-, *γ*- and *δ*-amino acid) analogues also play an important role in acquiring secondary structure by restricting their allowed conformational space. For an instance, *β*-alanine peptide reportedly forms random structures in solution phase, while sheet like packing in its solid state.[14] However, the substituted ring constrained *β*-amino acid analogues, containing cycloalkane ring, also facilitate the formation of helical type of secondary structure formation in solid state.[15] Interestingly, the synthetic peptides, even with four appropriate residues, reportedly form stable secondary structures and inhibit protein-protein interactions and other biological events.[10,16] Some of the synthetic peptides have also shown high stability towards enzyme degradation at cellular level. Recently, many new synthetic aromatic amino acids have been synthesized by positioning of chain propagating groups, amino and carboxyl group, on or with aromatic frameworks to generate conformationally constrained *β*-turn type of peptide foldamers.[17,18] The structural analysis of phenyl bearing aromatic, benzenoid, peptides has revealed that π-π non-covalent interactions provide extra stabilities in formation of their secondary structure. So far many benzenoid aromatic compounds are known, but a very few of them have been utilized in development of synthetic aromatic amino acids and their peptides. In nature, non-benzenoid aromatic compounds such as tropolone (**1**) and related compounds are also available (Figure 1).[19,20] Tropolone related bioactive natural products as *β*-thuzaplicinol (hinokitiol),[21] and *α*-manicil,[22] are studied in details. Other tropolonyl derivatives including *α*-hydroxy tropolone and 2-aminotropone analogues are also considered as target drugs molecules.[23] In addition, tropolone derivatives contain metal chelating properties especially with Zn and Cu metal ion.[24-26] Very recently, new electronic properties such as proton transfer within tropolone (**1**) molecule have been observed *via* intramolecular hydrogen bonding.[27-30] Owing to many remarkable features, the tropolone and related compounds would be employed in synthetic peptides, to examine the conformational stability of their peptides. To expand the synthetic peptides repertoire, we rationally designed an *δ*-amino acid, troponyl aminoethylglycine (*Tr-aeg*), from tropolone and aminoethylglycine (*aeg*) *δ*-amino acid backbone derivative (Figure 1). In this report, we describe the synthesis of rationally designed new *Tr-aeg* amino acid where troponyl motif linked covalently at *aeg* backbone. We also demonstrate the role of troponyl carbonyl group of *Tr-aeg*, in hydrogen bonding with amide N-H of peptide containing *Tr-aeg* residue by NMR techniques. So far, there is no such report, which describes the role of troponyl carbonyl group in conformational stability of peptide *via* hydrogen bonding with adjacent amide N-H.

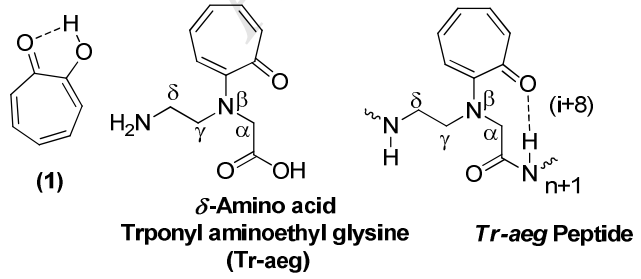
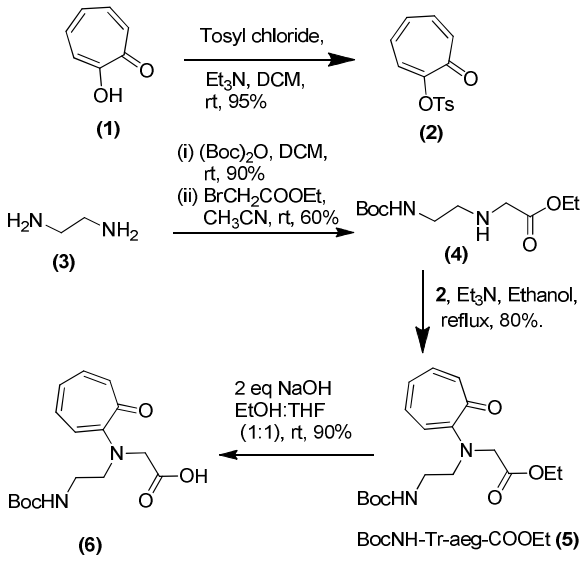


Figure 1. Chemical structure of tropolone (**1**) and troponyl amino acid (*Tr-aeg*) and *Tr-aeg* peptide

Results and discussions

The synthesis of *newly* designed *δ*-amino acid, *Tr-aeg*, was planned from commercially available materials: tropolone (**1**) and ethylenediamine (**3**) (Scheme 1). Herein, the hydroxyl functional group of tropolone (**1**) was derivatized into 2-tosyloxy tropolonyl intermediate (**2**) after treatment with tosylchloride by following literature procedure.[31] And ethylenediamine (**3**) was modified to *N*-Boc-aminoethylglycinate (*aeg*) (**4**) backbone by using known synthetic procedure.[32] Subsequently, the troponylation reactions performed at *N*-atom of *aeg* backbone (**4**) by refluxing with reactive tropolonyl tosylate intermediates (**2**), under mild basic

Scheme 1. Synthesis of *Tr-aeg* amino acid monomer



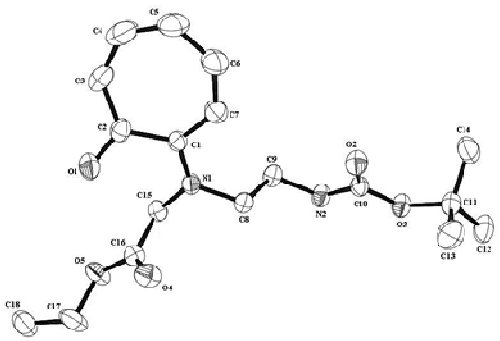


Figure 2. ORTEP diagram of δ-aminoacid monomer **5**

conditions, in Et_3N. After completion this reaction, mixture was purified by column chromatographic method and then characterized by NMR ($^1H/^{13}C$) and HRMS (ESI-Tof) analysis.† These analytical data suggest the formation of desired compound as *Tr-aeg* protected amino acid derivative, *BocNH-Tr-aeg-COOEt*, (**5**). The characterization data (NMR and HRMS spectra) of newly synthesized compound **5** are provided separately as Supplementary Information (SI). Moreover, the single crystal X-ray analysis was performed to crystalline solid form of *Tr-aeg* derivative **5**. Finally, X-ray studies confirm the chemical structure of **5** and its ORTEP diagram is depicted in Figure 2, whiles its crystal packing pattern is provided in SI. X-ray data of **5** as "*.cif*" file, are submitted to the Cambridge structural database (CSD) with CCDC No. 975954. Our structural studies demonstrate that troponyl ring of *Tr-aeg* derivative (**5)** is non-planar, possible owing to ring puckering. Tropnyl carbonyl substituent C(2)-O(3) of **5,** therefore, is not in same plane of troponyl ring. Due to ring puckering, non-planarity character in seven member closed ring compounds have also been noticed previously.[33,34] Our structural analysis results also reveal the orientation of troponyl carbonyl group toward the *C*'-end of *Tr-aeg* amino acid derivative (**5)**, not to the *N*'-end. After conformation analysis of derivative **5**, the role of its troponyl carbonyl group in peptide folding was planned to examine. That is why, a few hybrid peptides, combination of synthetic *Tr-aeg* and natural α-amino acid, planned to synthesize. The ester group of *Tr-aeg* derivative (**5**), therefore, was hydrolyzed into carboxylic acid functionalized *Tr-aeg* derivative (**6**) by treatment with aq. NaOH (2.0M) followed by neutralization with HCl (scheme 1). This carboxylic acid functionalized derivative **6** was coupled with amino group of methyl ester derivative of following α-amino acids- L-phenylalanine (Phe), L-proline (Pro), and L-isoleucine, under peptide coupling reagents, EDC/Et_3N (Scheme 2). Thus hybrid dipeptides **7/8/9** were achieved from **6** and respective methyl ester derivative of Phe/Pro/Ilu with moderate yield ca. 45%. For control studies, without containing troponyl residue, dipeptide **10** (Boc-NH-Gly-Phe-COOMe) from α-amino acid derivatives: *N*-Boc-glycine acid and phenylalanine (Phe) ester amine. With silica gel column purification method, purified hybrid peptides (**7/8/9/10**) were characterized by ^1H-/^{13}C-NMR/HRMS (ESI-Tof) techniques. The characterization data (NMR and HRMS spectra) are provided in SI. Furthermore, the hybrid *tri*-peptide (**11)** was also prepared from *Tr-aeg* derivative (**6)** and dipeptide (**10)** and then characterized by similar techniques. The characterization data of *tri*-peptide **11** are provided in SI. After successful synthesis and characterizing of hybrid *di*-/*tri*-peptides, we planned to examine the role of troponyl carbonyl group in hydrogen bonding with adjacent amide N-H or carbamate N-H within peptides (**7/8/9/11**). Thus, the conformational studies of hybrid peptides were essential. In this report, we used NMR methods to find most favorable conformation and orientation of troponyl residue within peptide. So that, 2D-NMR (COSY, NOESY, and HSQC) spectra of synthesized hybrid peptides (**7/8/9/11**) were recorded, which are provided in SI. In case of hybrid peptide **7**, the ^1H-^1H COSY spectrum analysis assigns chemical shift (δ) of all protons, while ^1H-^{13}C-HSQC spectrum analysis furnishes chemical shift (δ) of all $CH/CH_2/CH_3$ type of carbons in peptide **7**. After proton and Carbon analysis, ^1H-^1H NOESY 2D NMR spectrum is used to extract non vicinal coupling protons partner in peptide **7**. The NOE analysis results show a spatial interaction of troponyl ring C(7)-H proton (t7H), phenyl (ortho) protons or both with adjacent amide N-H of phenylalanine residue in dipeptide **7**. Amazingly, no NOE interaction observes for t7H proton with

Scheme 2. Synthesis of hybrid Peptides

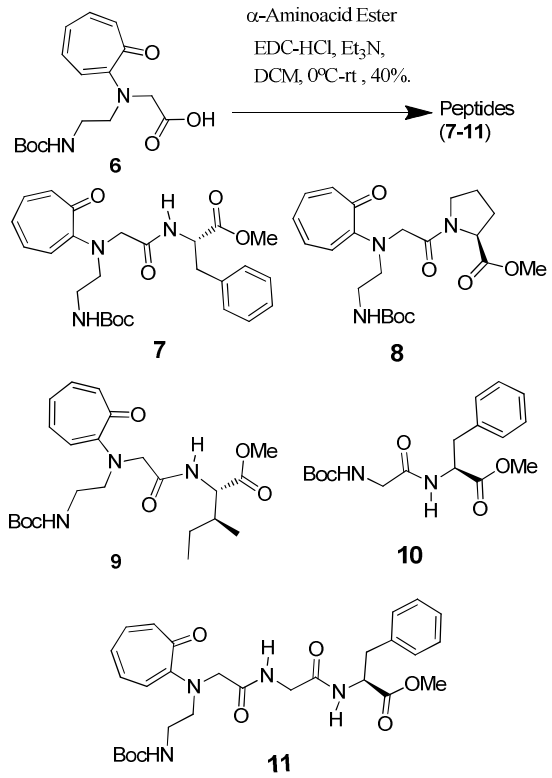


Table1. Chemical shift value of amide NH and troponyl C=O

Entry	Compound	Carbamate N-H (ppm)	Amide N-H (ppm)	Troponyl Carbonyl ($_{C=O}$) group (ppm)
1	5	5.6	no	181.7
2	7	5.5	7.7	182.5
3	8	5.8	no	181.7
4	9	5.6	7.9	183.2
5	10	5.1	6.6	no tr-aeg
6	11	5.5	7.9*	182.7

***Amide (i+1)th residue**

carbamate N-H in dipeptide **7**. In addition, the NOE interactions of troponyl ring C(3)-H proton (t3H) with aminoethyl protons met_1/met_2 (γH/δH,) are noticeably observed. These 2D-NMR analysis support the troponyl ring conformation in peptide **7**, where troponyl carbonyl group pointing to the *C'-end* of peptide (**7**), not to the *N*'-end. Hence, tropnyl ring of peptide **7** possibly has similar conformation as of *Tr-aeg* derivative (**5**). Similar sets of 2D-NMR analysis completed within other hybrid peptides **8/11**. Their results show that the troponyl residue protons (t3H) of peptides (**8/11)** have similar NOE interaction with aminoethyl protons γH/δH, which also support similar kind of troponyl carbonyl group conformation as of *di*-peptide **7**. After conformational analysis, the role of troponyl carbonyl group of synthesized hybrid peptides in hydrogen bonding examined with adjacent amide NH by following NMR titration method. According to this method, the chemical shift (proton resonance)

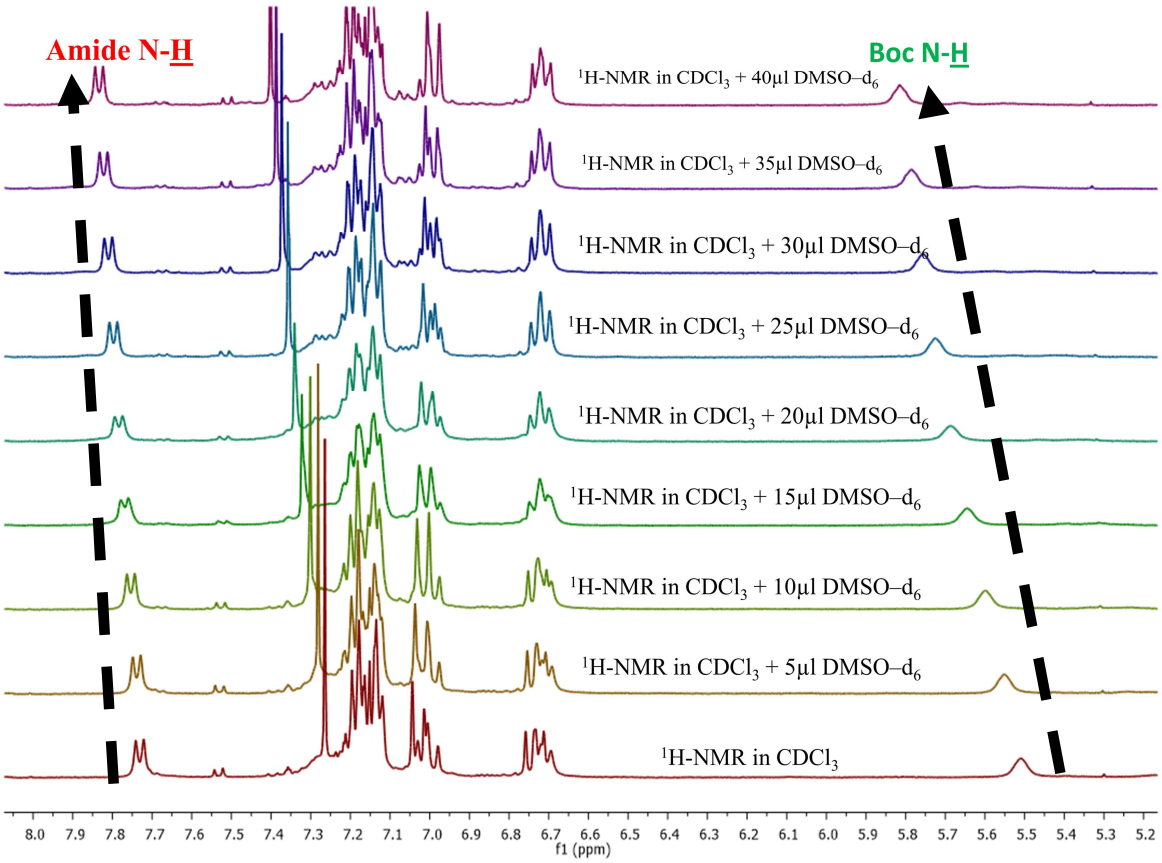


Figure 3. DMSO-d6 titration profile of dipeptide (**7**)

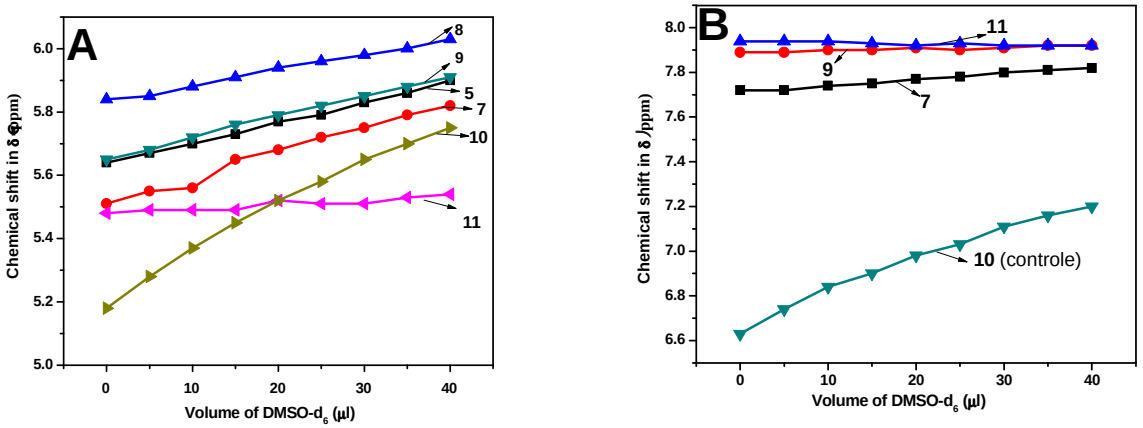


Figure. 4 NMR titration profile: (A) Boc-NH at N'- end of **5/7/8/9/10/11**; (B) Adjacent amide NH at C'-end of **7/9/11/10**

of intramolecular hydrogen bonded proton (amide N-H) in $CDCl_3$ reportedly exhibit slight downfield/upfield shift (deshielded/shielded region) or unchanged with sequential addition of (DMSO-d_6), which is strong hydrogen bond acceptor.[35] Whereas the chemical shift of exposed or non-hydrogen bonded amide N-H protons exhibit significant downfield shift (desheilded region) with sequential addition of DMSO-d_6 to the same NMR sample, because of hydrogen bonding with strong hydrogen bond acceptor solvent DMSO-d_6. We also performed similar NMR titration experiments with synthesized peptides, before that the chemical shift (δ) of amide NH, The carbamate NH, and troponyl carbonyl (C=O) of *di/tri* peptides (**7-11**) were extracted from their respective spectrum and given in table 1. Thereafter, a series of proton spectra of hybrid/control peptides (**7-11**) were recorded with volume wise sequential addition of DMSO-d_6 (see SI). For a representation, herein only extended regions (5-8 ppm) of sequential ^{1}H-NMR spectra of hybrid *di*-peptide **7** are depicted (Figure 3). Further in, the NMR titration profiles as plot of chemical shift (ppm) *vs* DMSO-d_6 volume (μL) are generated for both carbamate NH (Figure 4A)/ amide NH (Figure 4B) of hybrid peptides **7** and other peptides (**8-11**). The titration profile of hybrid peptide **7** indicates the significant downfield shift (deshielding effect) in cabamate N-H proton peak (δ 5.5-5.67, Figure 4A), while very little downfield shift (deshielding effect) in amide N-H peak (δ 7.8-7.74, Figure 4B). These NMR titration results for peptide 7 advocate that only amide N-H is involved in intramolecular hydrogen bonding even though one carbamate N-H is available. In case of control peptide **10**, NMR titration profiles for both carbamate (Figure 4A) and amide-NH (Figure 4B) show rapid downfield shift (deshielding effect) with sequential addition of DMSO-d_6, such as δ +0.47 ppm (aprox) downfield shift of amide N-H with addition of 40 μL of DSO-d_6 (see SI, Table S13). This titration results clearly show that no intramolecular hydrogen bonding is formed in non-hybrid dipeptide (**10)**. The NMR titration experimental results performed with other hybrid peptides **8/9/11** with addition of DMSO-D_6 in $CDCl_3$ NMR sample. The NMR titration profiles for amide/carbamate N-H of other hybrid peptides **8/9/11** are provided in Figure 4. The titration profiles for carbamte N-H of **8/9/11** show a similar shielding effect trend as of hybrid peptide 7 (Figure 4A). The titration profiles for amide N-H of peptides **9/11** exhibit desshielding effect (downfield shift) tendency in amide NH of **9/11** as similar to dipeptide **7** (Figure 4B). Structurally hybrid peptide (**8**) does not contain amide N-H so there is no amide NH titration profile observed. Overall the comaparative NMR titration studies of hybrid *di/tri*-peptide (**7/9/11)** with control peptide **10** are strongly suggested that only adjacent amide N-H's, rather than carbamate NH, of **7/9/11** are only involved in intramolecular hydrogen bonding. This intramolecular hydrogen bonding participation by amide N-H of hybrid peptide (**7/9/11**) may take place with troponyl carbonyl group of *Tr-aeg* residue of peptide (**7/9/11**), even though the presence of their carbamate carbonyl group. In further the involvement of troponyl carbonyl group of hybrid peptide were investigated in hydrogen bonding with adjacent amide NH. We, therefore, carefully studied the ^{13}C-NMR spectra of hybrid peptide (**7/8/9/11)** and *Tr-aeg* derivative **5,** especially to troponyl carbonyl carbon peak (SI). The chemical shift values of troponyl carbonyl carbon of *Tr-aeg* containg compounds **5**/**7**/**8**/**9**/**11** are given in Table 1. Herein the deshielding effects are observed in troponyl carobonyl carbon NMR peak of hybrid peptide **7/9/11**, which is marginally higher than that of **8** (*Tr-aeg-Pro*) and **5** (*Tr-aeg*). These significant changes in ^{13}C-NMR peak of hybrid peptide **7/9/11** also substantially suggest the involvement of troponyl carbonyl (C=O) in hydrogen bonding, possibly with adjacent amide N-H (n+1). In further search of troponyl carbonyl group (C=O) participation in hydrogen bonding with amide adjacent N-H, once again solvent dependent ^{13}C-NMR spectra of hybrid peptide **11** were recorded in following solvent system**:** aprotic polar solvents $CDCl_3$ (100%), DMSO-d_6 (100%) and mixture of both solvent $CDCl_3$:DMSO-d_6 (3:1, 1:1). All these spectra are provide in SI, while extended regions (δ 70-200 ppm) of ^{13}C-NMR spectra of peptide **11** is given in Figure 5, where the chemical shift all carbonyl groups of that peptide are appeared. The chemical shift of troponyl carbon peak (ca ~180 ppm) of hybrid peptide **11** is more desheilded in $CDCl_3$, in respect to DMSO-d_6, which also indicates the formation of intramolecular hydrogen bonding by troponyl carbonyl is better in $CDCl_3$ in comparison to DMSO-d_6. However the chemical shift of troponyl carbonyl carbon NMR peak in mixture of solvent $CDCl_3$:DMSO-d_6 (3:1 and 1:1) are almost same as in DMSO-d_6. This would be possible due to disturbance of intramolecular hydrogen bonding, between troponyl carbonyl and amide N-H, in $CDCl_3$ with DMSO-d_6. Because DMSO-d_6 is also known as strong hydrogen bond acceptor solvent and form strong intermolecular hydrogen bonding with exposed amide N-H.[36] However the chemical shift of ^{13}C-NMR peak (~δ 126.0 ppm) for Boc carbonyl group remain same in both $CDCl_3$ and DMSO-d_6 solvent (Figure 5). Hence the results of ^{13}C-NMR studies further support the involvement of troponyl carbonyl group in hydrogen bonding with amide N-H aprotic polar solvent as $CDCl_3$.

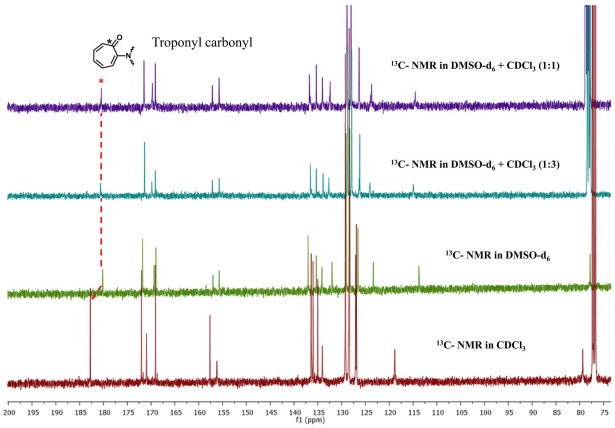


Figure 5. ^{13}C-NMR of tripeptide **11** (Expanded region) in DMSO-d6 and $CDCl_3$

To end with, a DFT calculation was performed with one of hybrid dipeptide **9** to acquire geometrically optimized structure in gas phase by using with B97 TURBOMOLE software.[37,38] As resultant, the optimized structure of **9** is depicted in Fig. 6., which also indicates the conformation of troponyl carbonyl group as pointing to the *C'-end* of peptide **9**, like monomer **5** and other peptides. The theoretical studies realted file as ".pdb" for peptide **9** is provided separately (see SI). In addition, the theoretically optimized structure of **9**, show following strong non-covalent hydrogen bond interaction: C=O----H-πC (2.41A); πC----H-C(2.47Å); C=O----H-C (2.05Å); and C=O----H-N (2.08Å). These interactions further support the formation of hydrogen bonding

between Troponyl carbonyl and adjacent, (i+1)th amide NH. Then the formation of 8-membered ring hydrogen bond motif is reasonably possible, which may provide the interesting helical structure in longer peptide. The theoretical folding pattern of hybrid peptide **9** also predicted from backbone dihedral angles ϕ (phi) and ψ (psi) of peptide **9.** Since peptide backbone dihedral as ϕ and ψ are being used to generate Ramachandran plot, ϕ *vs* ψ, which predict the secondary structure of peptide and protein.[39] With geometrically optimized structure of hybrid peptide **9**, the backbone dihedral angles as ϕ = -76.0°, θ1 = 170.3°; θ2= 89.3°; θ3=81.9°; ψ=92.7° are calculated and then generate a Ramachandran plot with help of software GNUPLOT 4.7 (SI). The coordinate (-76.0°, 92.7°) of ramachandran plot for peptide **9** indicates the formation of *β*-sheet or *β*-turns type of secondary structure, which is really provide encouraging information about development of conformationally defined useful peptide mimics.

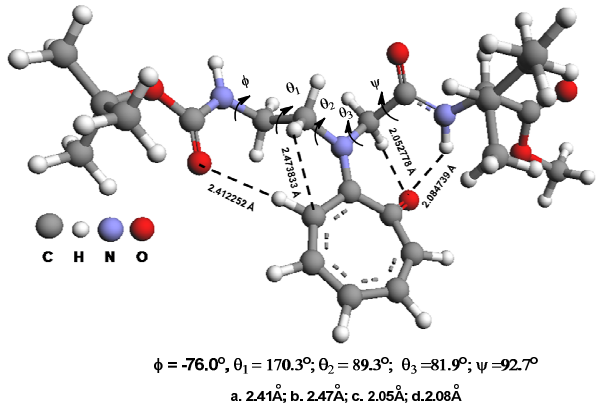
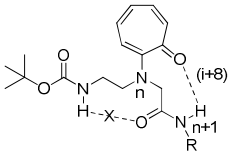


Figure 6. Energy minimized structure of *di*-peptide **9** from DFT calculations

Conclusions

In summary, we have successfully synthesized *new* unnatural δ-amino acid (*Tr-aeg*) containing troponyl residue and then have been used in synthesis of hybrid *di-tri*-peptides with α-amino acid. With experimental and theoretical studies, we have also shown the role of troponyl carbonyl group in intramolecular hydrogen bonding with adjacent amide NH. As resultant, the formation of 8-membered ring type of intramolecular hydrogen bond motif and *β*-sheet or *β*-turns type of secondary structure are observed. Our future works are in progress to synthesize and study the role of troponyl carbonyl group in homologous/heterologous long peptides containing *Tr-aeg* unit in development of potential peptide mimics.



Experimental Procedure

Synthesis of 2-tosyloxy tropone (**2**). From tropolone by following literature procedure.[15]

*Synthesis of aminoethylglycine (aeg) backbone (**4**).* From tropolone by following literature procedure.[19]

*Synthetic procedure of Tr-aeg monomer (**5**)*: A solution of 2-tosyloxy tropone (**2**) (1.2g, 4.34 mmol) in ethanol (25 mL) was refluxed in presence of Ethyl (2-N-Boc-aminoethyl) glycinate (**4**) (3.2 g, 13.04 mmol) for 48 hours. After completion of reaction, which was monitored by thin layer chromatography (TLC), reaction mixture was cooled down to room temperature and then concentrated to dryness under vacuum. The dried solid reaction residue was re-dissolved in DCM (50 mL) and washed with water (50ML) ,at least three times, followed by brine solution (10 mL) using separating flask. The DCM layer was kept over Na_2SO_4 for 30 min and then concentrated to dryness under vacuum. The concentrated reaction mixture was subject for purification by column chromatographic methods. Then The major component of reaction residue was purified with solvent system ethylacetate:hexane (1:3) and then characterized as desired product **5** (1.2 g, 80%) by ^{1}H/^{13}C-NMR and mass spectrometric method. ^{1}H NMR (400 MHz, CDCl3) δ (ppm) 7.12 – 6.96 (m, 2H), 6.92 (d, J = 11.8 Hz, 1H), 6.75–6.55 (m, 2H), 5.60 (s, 1H), 4.20 (s and q, 4H), 3.59 (t, J = 6.1 Hz, 2H), 3.45 – 3.31 (m, 2H), 1.40 (s, 9H), 1.26 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ (ppm) 181.69, 170.41, 157.93, 156.21, 135.62, 133.92, 133.78, 125.10, 115.79, 79.37, 61.15, 53.57, 52.37, 37.57, 28.33, 14.11. HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd. for $C_{18}H_{26}N_2O_5$ 351.1914, found 351.1921.

Acknowledgment

CB thank to UGC & NISER for research fellowship. This studied was supported by Grant-in Aid from NISER, Department of Atomic Energy (DAE)-India. We also thank Dr. Himanshu. Biswal for DFT calculation.

Supplementary Information

Electronic Supplementary Information (ESI) available: [All NMR (^{1}H, ^{13}C, 2D NMR and solvent dependent NMR) and mass spectra of all new compounds: monomer (**5** & **6**) and peptide (**7-11**) are provided]. See DOI: 10.1039/c000000x/

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Synthesis and Conformational Analysis of New *Troponyl* Aromatic Amino Acid

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Contents

1. General:

*Materials and instrumentation***:** All required materials were obtained from commercial suppliers and used without further purification**.** Dry dichloromethane was freshly prepared by distilling over KOH and Calcium hydride sequentially. Reactions were monitored by thin layer chromatography, visualized by UV and Ninhydrin. Column chromatography was performed in 100-200 mesh silica. NMR spectra were recorded on Bruker AV-400 (^{1}H: 400 MHz, ^{13}C: 100.6 MHz). ^{1}H and ^{13}C{1H} NMR chemical shifts were recorded in ppm downfield from tetramethyl silane. Splitting patterns are abbreviated as: s, Singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; dq, doublet of quartet; m, multiplet. Mass spectra were obtained from Bruker micrOTOF-Q II Spectrometer.

2. Experimental procedure:

1. Syntheses of Ethyl-(2-N-Boc-aminoethyl)troponyl)glycinate (5): To a solution of 2-tosyloxy tropone (**2**) (1.2g, 4.34 mmol) in ethanol (25 mL) was added Ethyl (2-*N*-Boc-aminoethyl) glycinate (**4**) (3.2 g, 13.04 mmol) and refluxed. Reaction was monitored using thin layer chromatography (TLC) and found the completion of reaction after two days. After cooling to room temperature, reaction mixture was concentrated under vacuum on rota vapour. The reaction residue was redissolved in DCM (50 mL) and washed with water (50mL) three times and then with brine solution (10 mL) by using separating flask. The organic layer was kept over Na_2SO_4 for 30 min and then concentrated to dryness under vacuum. The concentrated residue was subjected for purification on silica gel by column chromatographic methods. The major component of reaction residue was purified with solvent mixture Ethylacetate:Hexane (1:3) and characterized as desired product (1.2 g, 80%) by ^{1}H/^{13}C-NMR and Mass spectrometric method. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.12 – 6.96 (m, 2H), 6.92 (d, J = 11.8 Hz, 1H), 6.75–6.55 (m, 2H), 5.60 (s, 1H), 4.20 (s and q, 4H), 3.59 (t, J = 6.1 Hz, 2H), 3.45-3.31 (m, 2H), 1.40 (s, 10H), 1.26 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ (ppm) 181.69, 170.41, 157.93, 156.21, 135.62, 133.92, 133.78, 125.10, 115.79, 79.37, 61.15, 53.57, 52.37, 37.57, 28.33, 14.11. HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd. for $C_{18}H_{26}N_2O_5$ 351.1914, found 351.1922. Summary of X-ray data of monomer **5** is deposited to the Cambridge Structural Database (CSD) and their deposition number CCDC 975954. From X-ray analysis revealed the unit cell parameters: a 11.1125(4) b 15.3001(7) c 12.2394(5) P21/c and molecular formula $C_{18}H_{26}N_2O_5$ of monomer **5.**

2. Syntheses of dipeptide (7): A solution of *N*-(2-amioethyl)troponyl)glycine (**6**) (200 mg, 0.62 mmol) in anhydrous dichloromethane (10 mL) was cooled to 0 ^{o}C, and then EDC-HCl (142 mg, 0.744 mmol) was added, stirred for 5 min at 0^{o}C. Then L-Phenylalanine methyl ester hydrochloride (160 mg, 0.744 mmol) and Triethylamine (0.26 mL, 1.86 mmol) was added together. This reaction mixture was continued to stirrer at room temperature (rt) for overnight. After completion of reaction, the reaction mixture was concentrated to dryness under reduced pressure. Concentrated reaction residue was re-dissolved in DCM (30 mL) and then washed with water thrice (3*30mL) followed by saturated sodium bicarbonate (20 mL) by following extraction method. The washed organic layers were combined together and concentrated under reduced pressure and then loaded on silicagel column for purification by EtOAc/Hexane to obtain desired product (**7**). The major component was isolated with EtOAc/Hexane (30:70) and characterized as desired product (120 mg, 40%) by ^{1}H/^{13}C-NMR and Mass spectrometric method. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.76 (d, *J* = 7.9 Hz, 1H), 7.24 – 7.06 (m, 7H), 7.05 – 6.92 (m, 2H), 6.72 (dd, *J* = 21.4, 13.0 Hz, 2H), 5.59 (s, 1H), 4.78 (dd, *J* = 14.0, 6.7 Hz, 1H), 3.93 (d, *J* = 16.7 Hz, 1H), 3.88 – 3.75 (m, 1H), 3.72 – 3.60 (m, 3H), 3.55 – 3.37 (m, 1H), 3.36 – 3.12 (m, 4H), 3.05 – 2.93 (m, 1H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 182.52, 171.78, 169.39, 157.22, 156.02, 135.87, 134.34, 133.79, 129.02, 128.91, 128.27, 126.75, 126.38, 118.09, 79.02, 55.60, 53.25, 52.12, 51.18, 37.65, 37.46, 28.19. HRMS (ESI-TOF) m/z: [M+H]+ calcd for $C_{26}H_{33}N_3O_6$ 484.2442, found 484.2486.

3. Syntheses of dipeptide (8): Similarly the dipeptide **8** was synthesized from *Tr-aeg* (**6**) and L-prolime methyl ester **.** (150 mg, 41%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 – 6.98 (m, 3H), 6.92 (dd, *J* = 11.7, 5.5 Hz, 1H), 6.78 (t, *J* = 11.6 Hz, 1H), 6.70 – 6.58 (m, 1H), 5.91 – 5.77 (m, 1H), 4.72 (d, *J* = 17.2 Hz, 1H), 4.49 (dd, *J* = 8.4, 4.0 Hz, 1H), 4.20 (t, *J* = 13.9 Hz, 1H), 3.90 – 3.81 (m, 1H), 3.79 (s, 1H), 3.74 (dd, *J* = 11.2, 5.9 Hz, 1H), 3.69 (s, 3H), 3.64 (dd, *J* = 12.7, 5.5 Hz, 1H), 3.55 (dt, *J* = 17.1, 8.8 Hz, 2H), 3.40 (d, *J* = 5.7 Hz, 3H), 2.35 – 2.27 (m, 1H), 2.27 – 2.15 (m, 2H), 2.13 – 1.94 (m, 4H), 1.44 (d, *J* = 19.3 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 181.71, 172.49, 167.89, 157.49, 156.37, 135.66, 134.02, 133.72, 124.99, 116.67, 79.07, 58.92, 53.37, 52.49, 52.26, 46.21, 37.87, 28.83, 28.42, 24.89, (cis and trans isomers are existing around

secondary amide bond in 1:4, not predicted due to overlapping signals). HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd. for $C_{22}H_{31}N_3O_6$ 456.2105, found 456.2123.

3. Syntheses of dipeptide (9): Similarly the dipeptide **9** was synthesized from *Tr-aeg* (**6**) and L-Isoleucice methyl ester. (45 mg 36%) ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm 7.90 (d, *J* = 8.2 Hz, 1H), 7.24 – 7.12 (m, 1H), 7.11 – 7.00 (m, 2H), 6.92 – 6.81 (m, 1H), 6.79 – 6.70 (m, 1H), 5.65 (s, 1H), 4.65 – 4.43 (q, 1H), 4.18 – 4.05 (m, 1H), 3.72 (d, *J* = 5.3 Hz, 1H), 3.69 (s, 3H), 3.63 – 3.50 (m, 1H), 3.47 – 3.24 (m, 4H), 2.32 (dd, *J* = 17.1, 9.6 Hz, 1H), 2.02 – 1.85 (m, 1H), 1.52 (dd, *J* = 1.0, 6.1 Hz, 1H), 1.47 – 1.34 (m, 9H), .21 – 1.12 (m, 1H), 0.96 – 0.82 (m, 6H). (cis and trans isomers are existing around carbamate amide bond in 1:4, not predicted due to overlapping signals). ^{13}C-NMR (101 MHz, $CDCl_3$) δ in ppm 183.22, 172.43, 169.79, 157.71, 156.27, 136.95, 136.41, 136.18, 135.01, 133.83, 132.73, 127.70, 119.33, 113.09, 79.21, 64.92, 56.65, 56.25, 52.06, 51.39, 37.81, 37.28, 28.33, 24.94, 22.64, 15.68, 14.24, 11.53. (cis and trans isomers are existing around carbamate amide bond in 1:4). HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd. for $C_{23}H_{35}N_3O_6$ 472.2418, found 472.2428.

4. Syntheses of dipeptide (10): Similarly the dipeptide 10 was synthesized from N-Boc glycine and L-Phenylalanine methyl ester. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.17 (m, 3H), 7.16 – 7.03 (m, 2H), 6.59 (s, 1H), 5.14 (s, 1H), 4.94 – 4.82 (m, 1H), 3.78 (dd, *J* = 18.7, 5.5 Hz, 2H), 3.71 (s, 3H), 3.20 – 3.06 (m, 2H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ in ppm 171.70, 169.12, 155.91, 135.61, 129.17, 128.56, 127.09, 80.16, 77.00, 53.04, 52.29, 44.10, 37.83, 28.22. HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd. for $C_{17}H_{24}N_2O_5$ 337.1758, found 337.1723.

5. Syntheses of tripeptide (11): Similarly the tripeptide 11 was synthesized from *Tr-aeg* (**6**) and NH_2-Gly-Phe-OMe**.** (138 mg,41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.27 – 7.16 (m, 3H), 7.15 – 7.01 (m, 4H), 6.94 (dd, *J* = 25.0, 10.4 Hz, 2H), 6.81 – 6.68 (m, 1H), 5.55 (s, 1H), 4.81 (dd, *J* = 13.4, 6.5 Hz, 1H), 4.08 (dd, *J* = 16.7, 6.2 Hz, 1H), 3.97 – 3.73 (m, 3H), 3.75 – 3.54 (m, 4H), 3.53 – 3.34 (m, 2H), 3.29 (d, *J* = 5.3 Hz, 2H), 3.09 (qd, *J* = 13.8, 6.4 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ in ppm 182.74, 172.02, 170.96, 169.07, 157.65, 156.18, 136.39, 136.00, 135.01, 134.05, 129.19, 128.48, 126.97, 118.83, 79.37, 56.85, 53.42, 52.13, 42.90, 37.58, 37.09, 28.30. HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd. for $C_{28}H_{36}N_4O_7$ 541.2657, found 541.2565.

3. ^{1}H-NMR spectrum of 2:

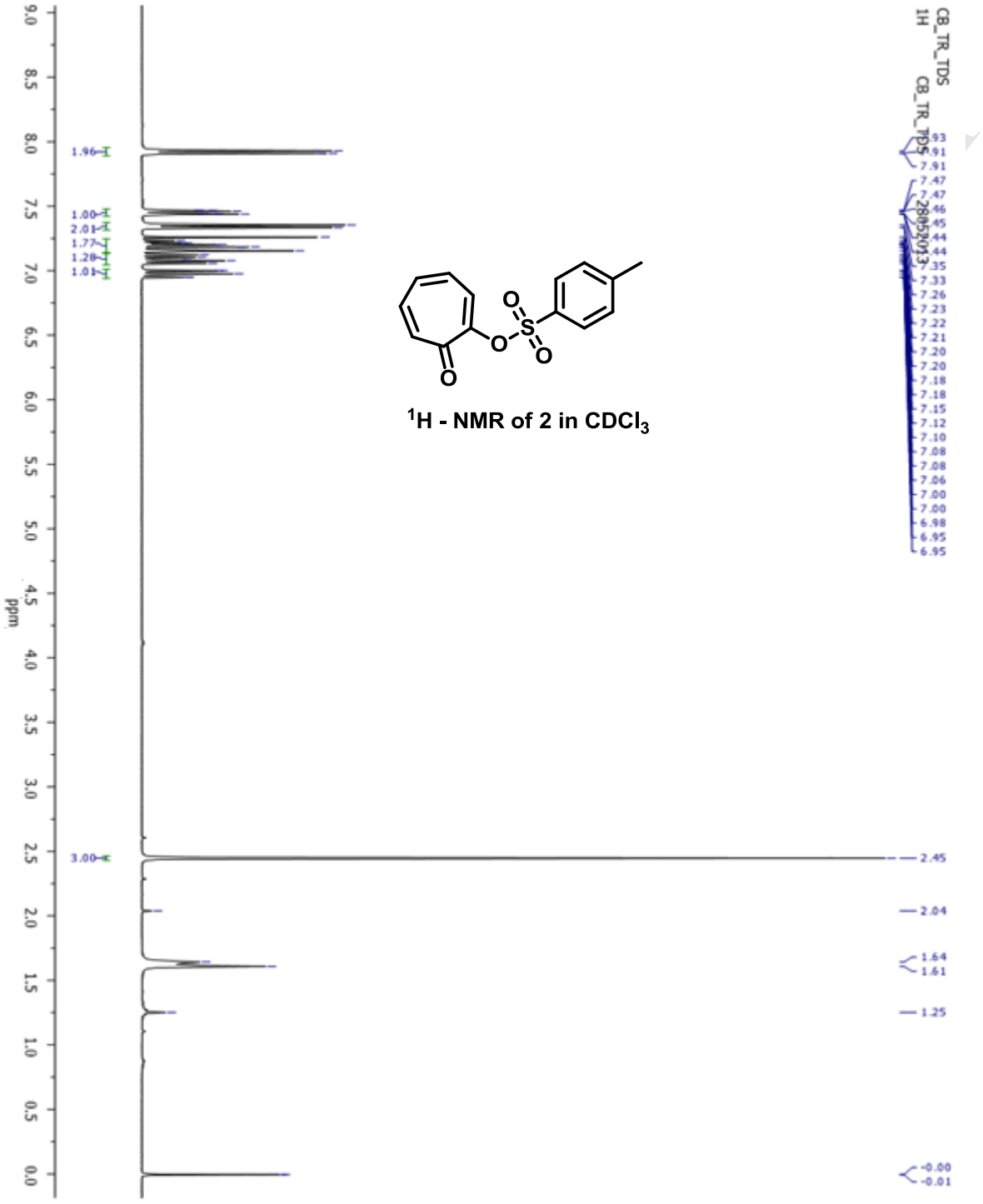

Figure S1: ^{1}H-NMR of 2–tosyloxy tropone (2)

4. ^{1}H-NMR spectrum of monomer 5:

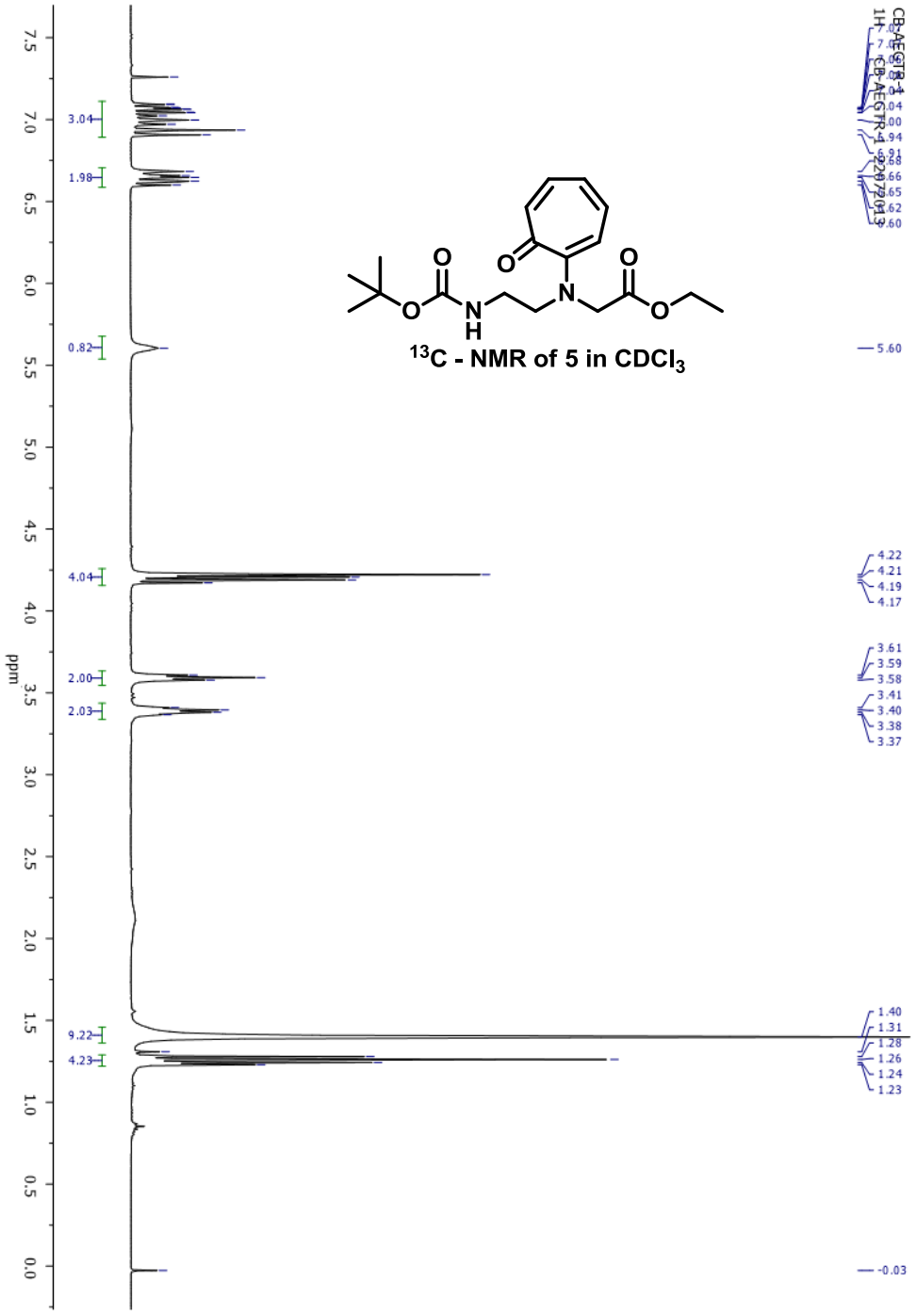

Figure S2: ^{1}H-NMR of monomer (Boc-*Tr*-*aeg*-OEt).

5. ^{13}C-NMR spectrum of monomer 5:

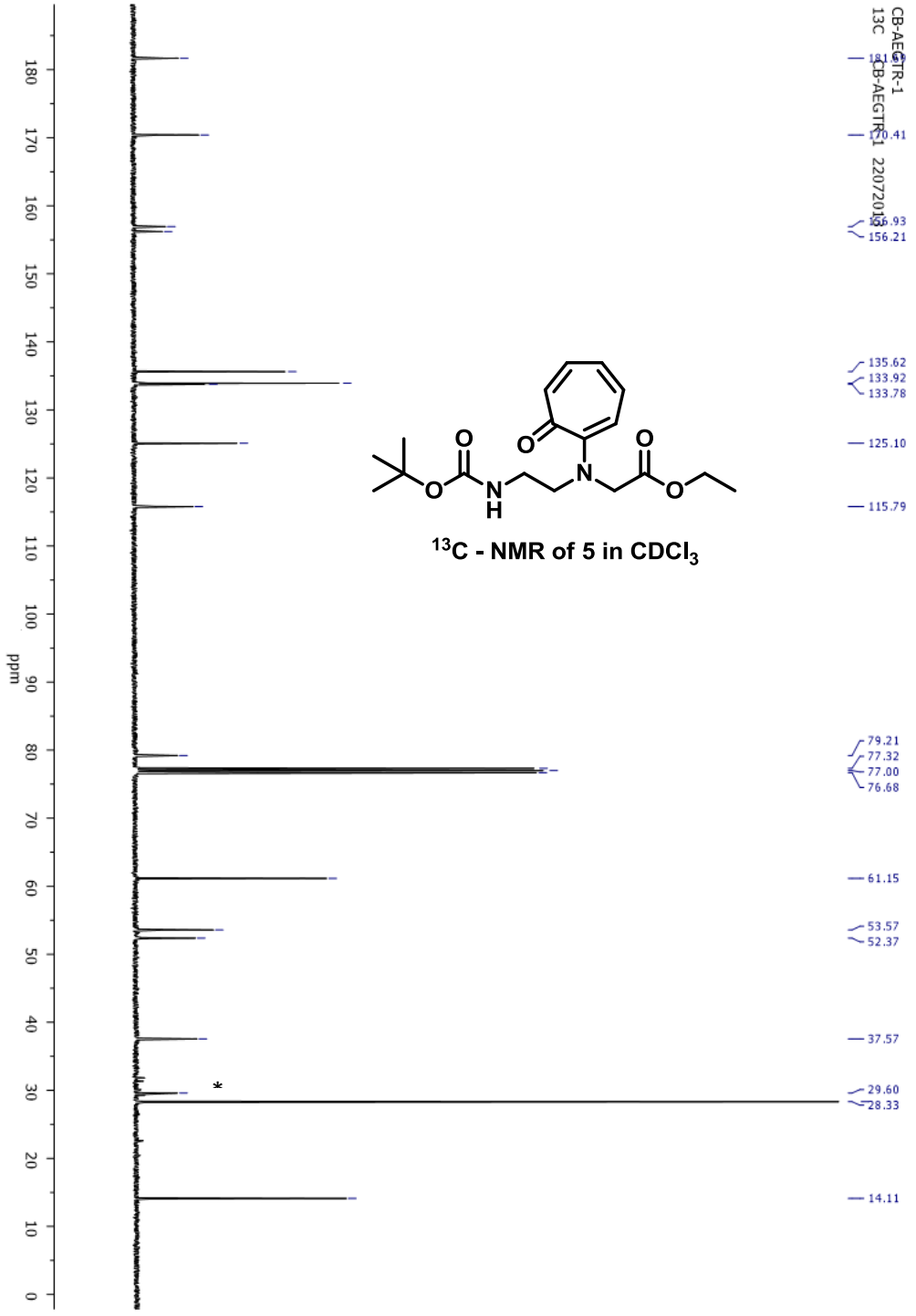

Figure S3: ^{13}C-NMR of monomer (Boc-*Tr*-*aeg*-OEt). (* hexane peaks)

6. Mass spectrum of dipeptide 5:

HRMS (ESI-MS–Tof)

Display Report

Analysis Info

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Comment			

Acquisition Parameter

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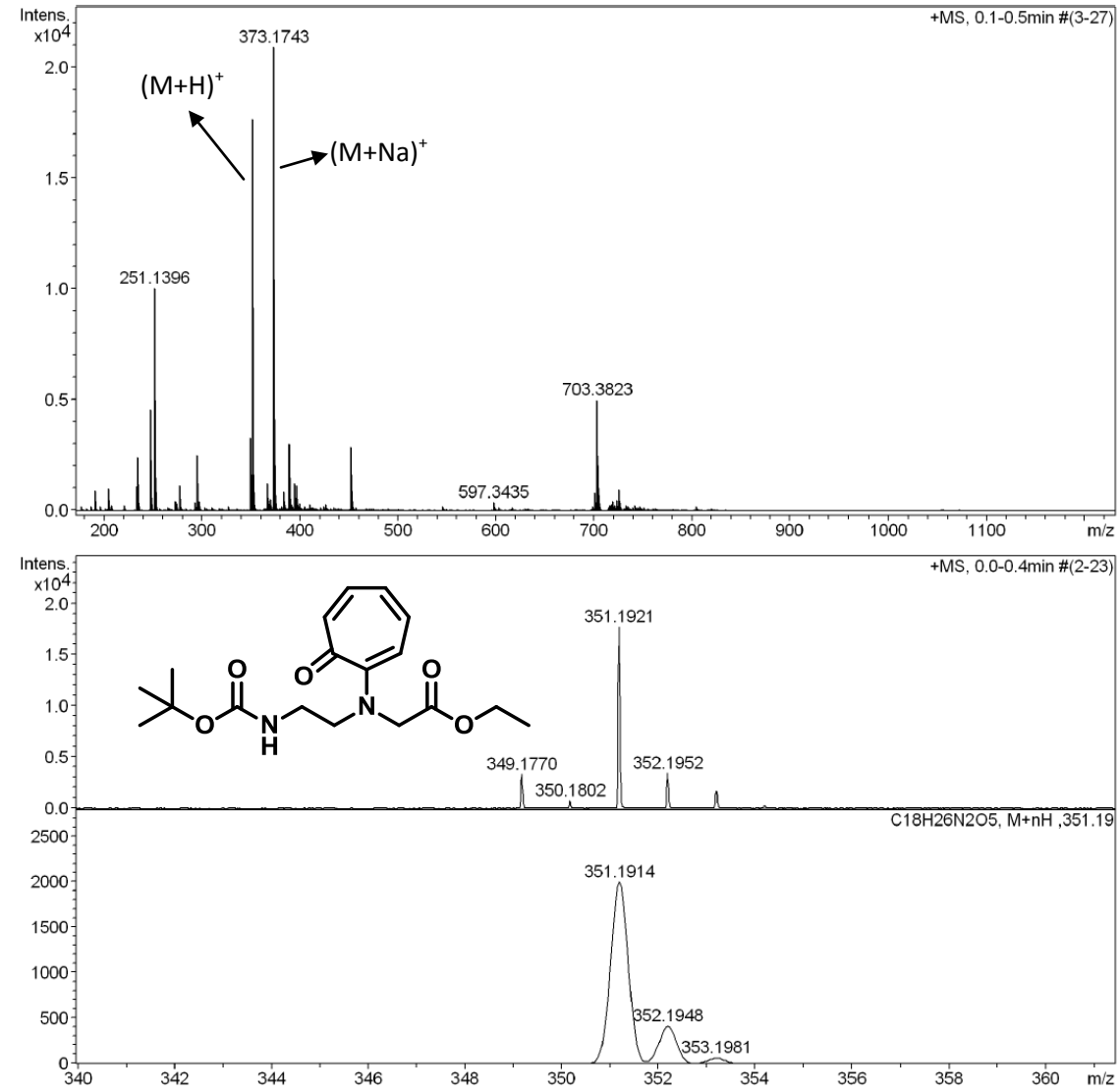

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7. ^{1}H-NMR spectrum of dipeptide 7:

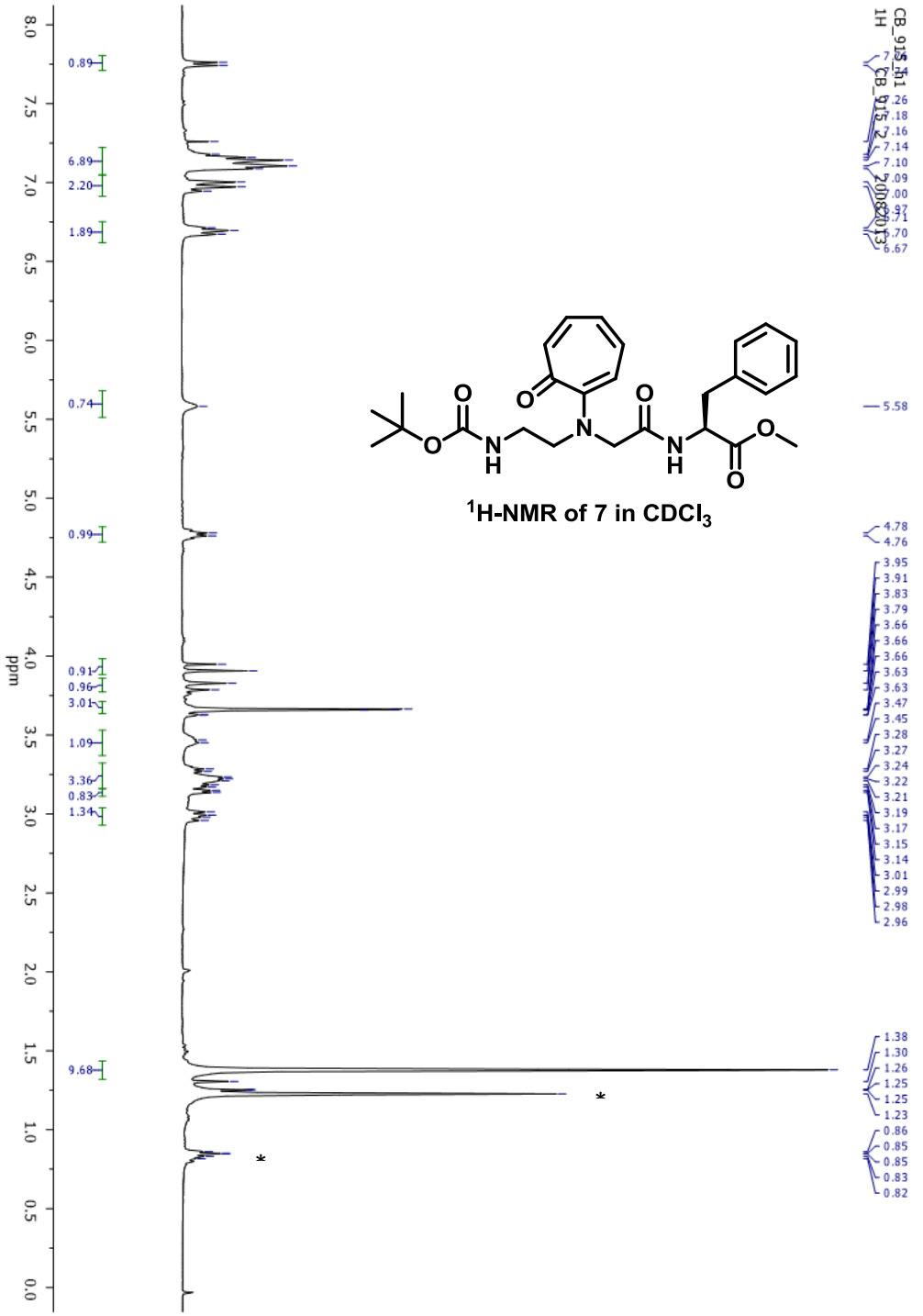

Figure S4: ^{1}H-NMR of dipeptide (Boc-*Tr*-*aeg*-Phe-OMe). (* hexane peaks)

8. ^{13}C-NMR spectrum of dipeptide 7:

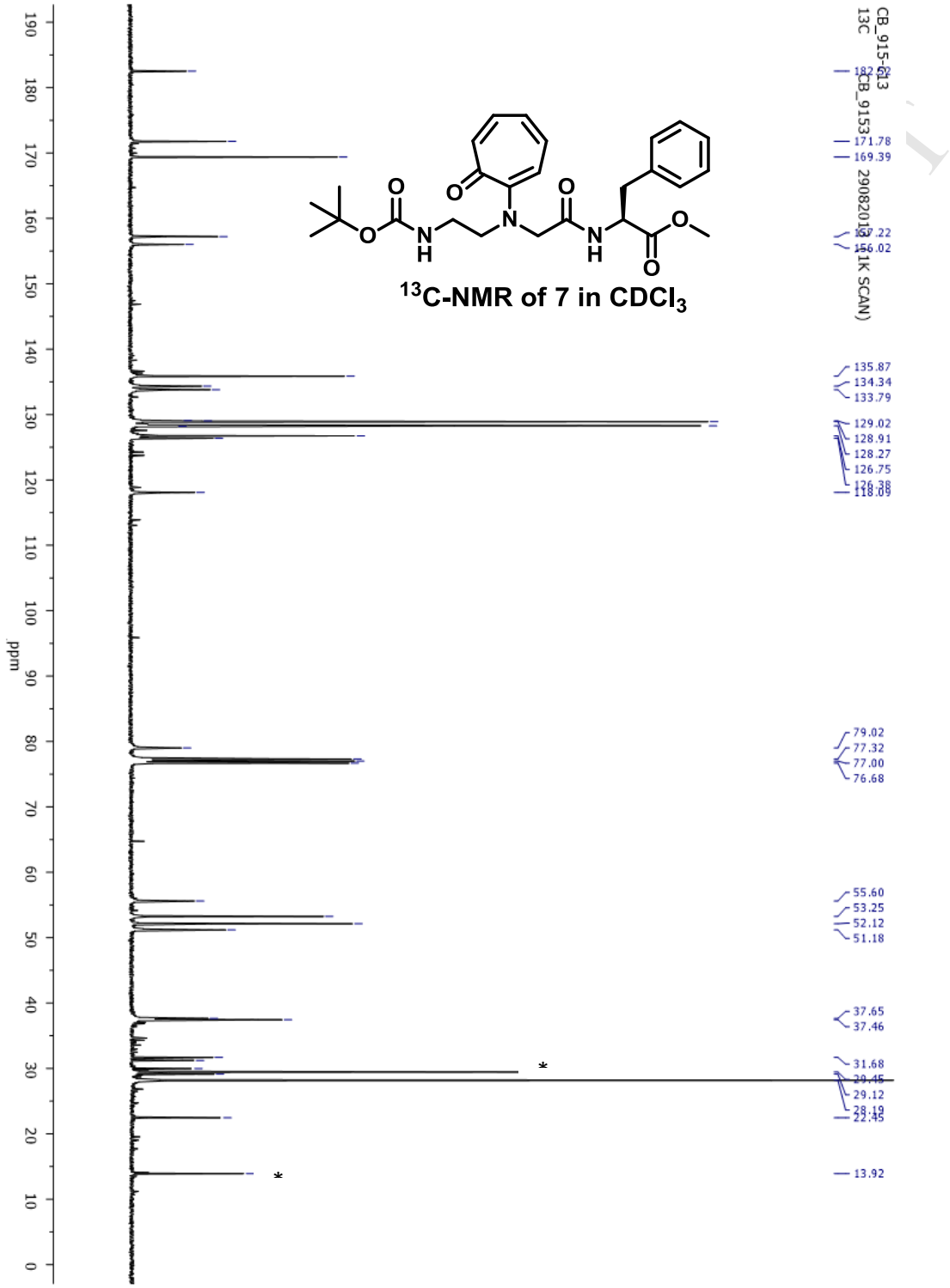

Figure S5: ^{13}C-NMR of dipeptide (Boc-*Tr*-*aeg*-Phe-OMe). (* hexane peaks)

9. Deuterium exchange ^{1}H-NMR experiment of dipeptide 7:

5 mg of dipeptide was dissolved in 0.5 ml of $CDCl_3$, to this three drops of D_2O was added and recorded NMR after 5 hrs.

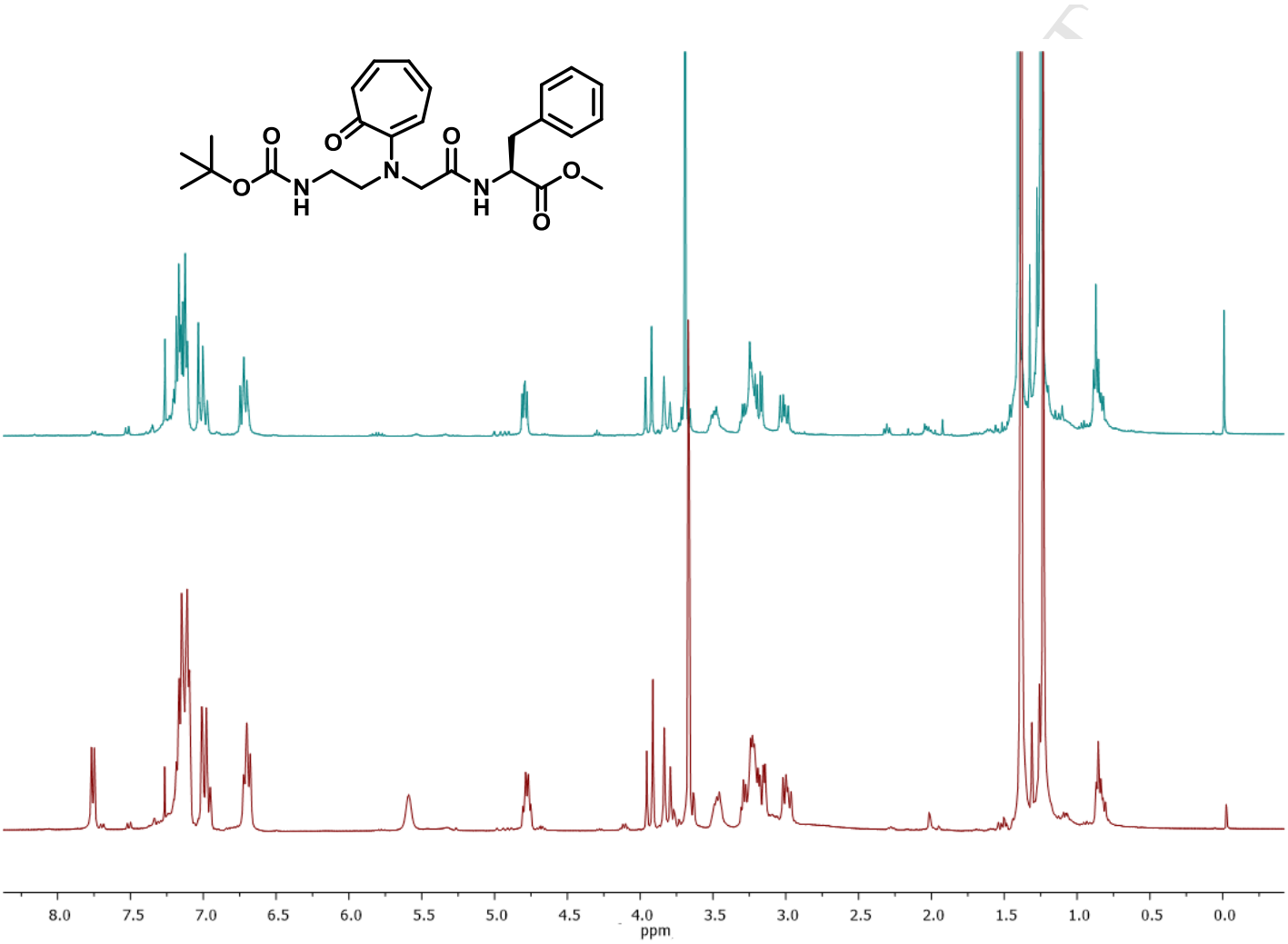

Figure S6: ^{1}H-NMR of dipeptide (Boc-*Tr*-*aeg*-Phe-OMe, **7**) in $CDCl_3$ and $CDCl_3$+D_2O.

10. Mass spectrum of dipeptide 7:

HRMS (ESI-MS–Tof) (7)

Display Report

Analysis Info

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Comment			

Acquisition Parameter

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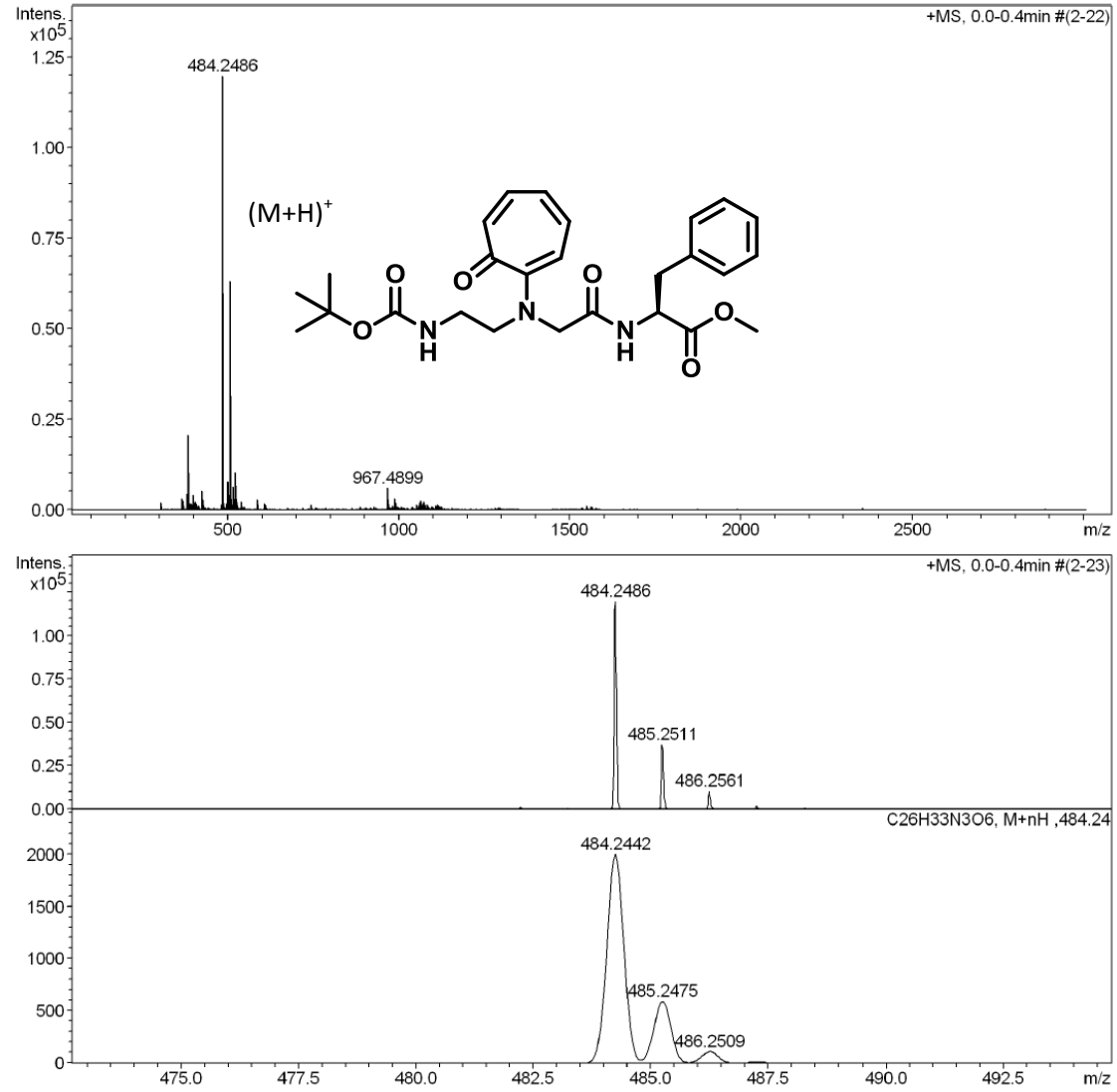

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11. ^{1}H-NMR spectrum of dipeptide 8:

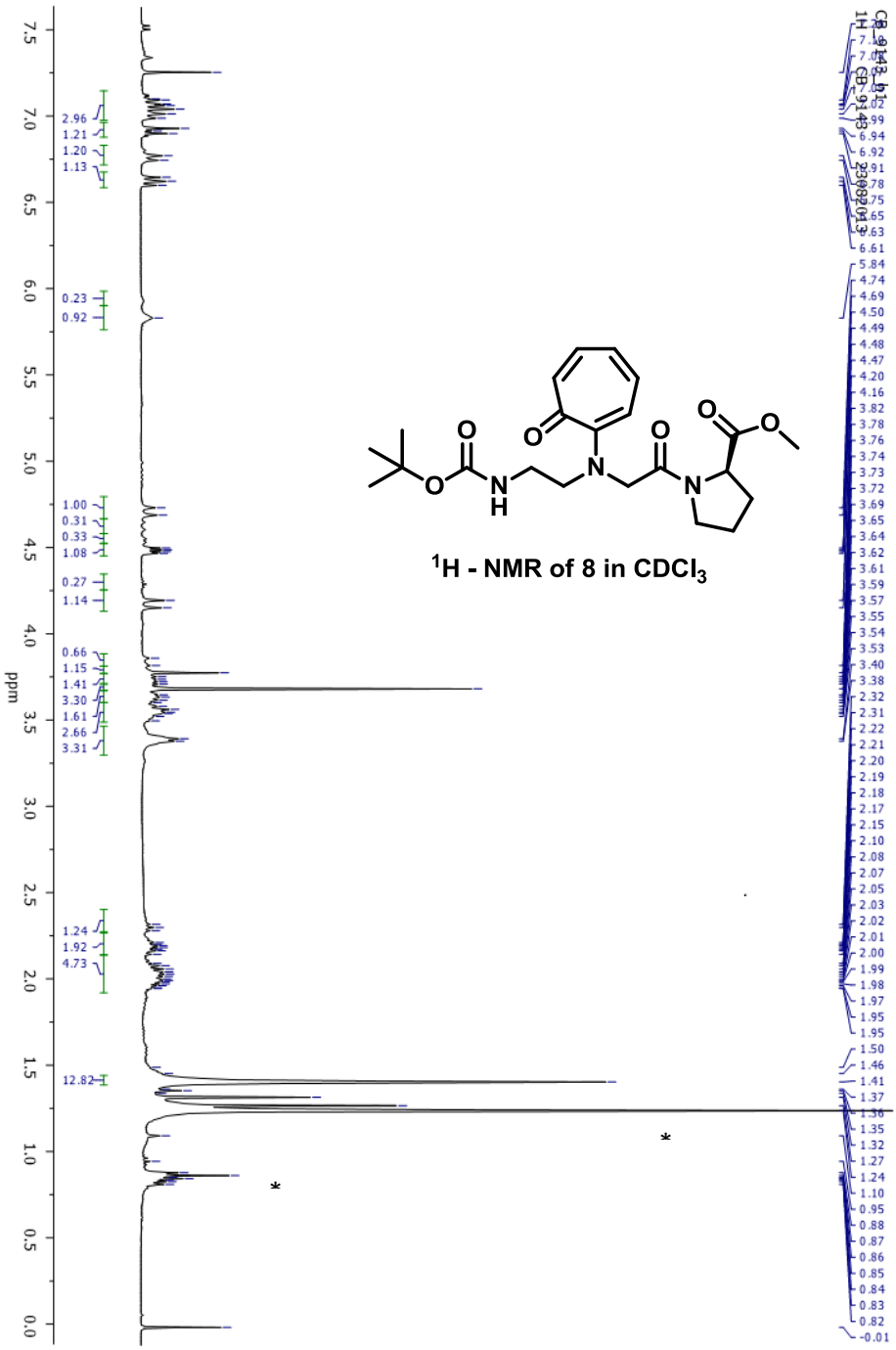
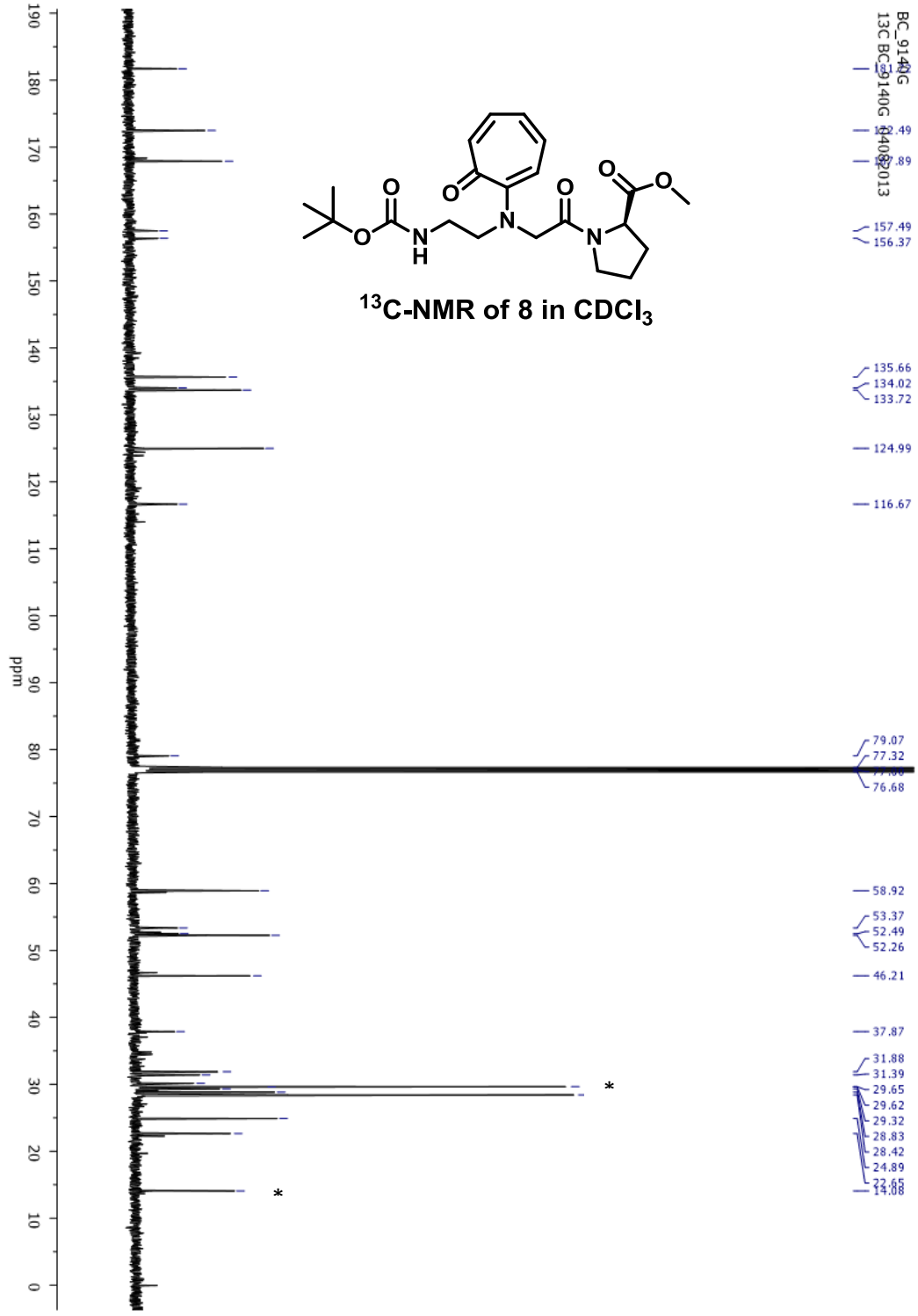


Figure S7: ^{1}H-NMR of dipeptide (Boc-*Tr*-*aeg*-Pro-OMe). (* hexane peaks)

12. ^{13}C-NMR spectrum of dipeptide 8:



^{13}C-NMR of 8 in $CDCl_3$

Figure S8: ^{13}C-NMR of dipeptide (Boc-*Tr*-*aeg*-Pro-OMe). (* hexane peaks)

13. Mass spectrum of dipeptide 8:

HRMS (ESI-MS–Tof)

Display Report

Analysis Info

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Sample Name	CH3CN	Instrument	micrOTOF-Q II 10337
Comment			

Acquisition Parameter

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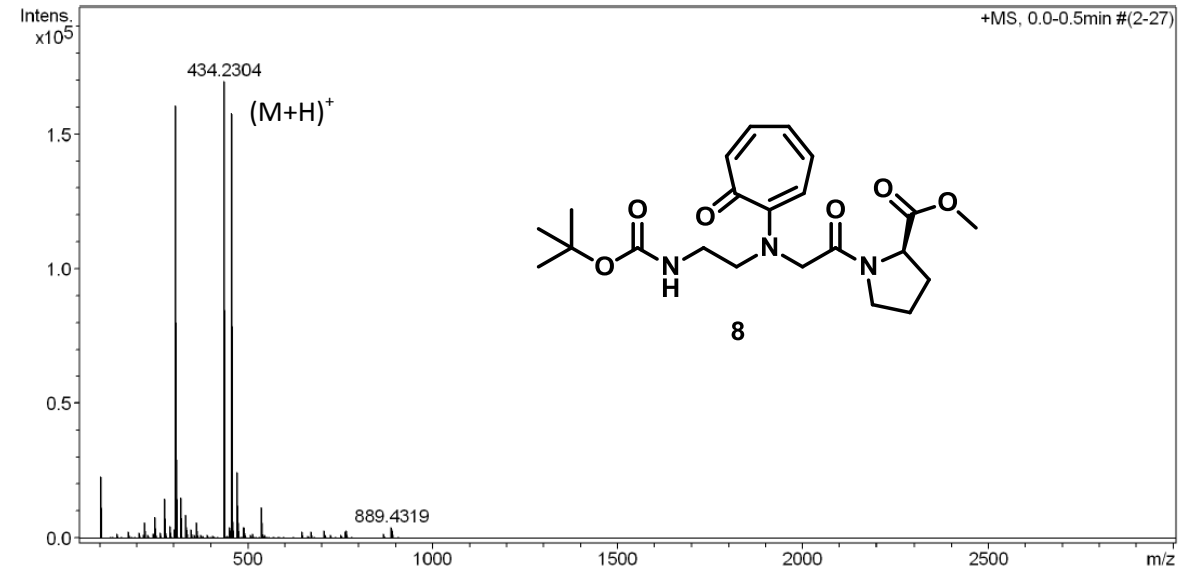
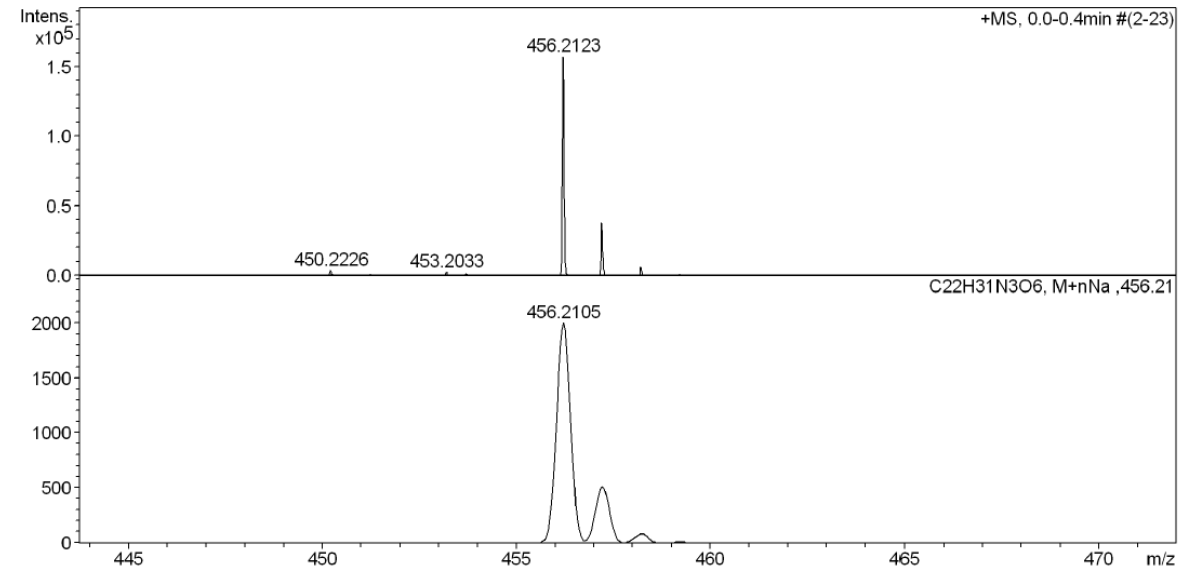
Scan End	3000 m/z	Set Collision Cell RF	130.0 Vpp	Set Divert Valve	Waste



8



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14. ^{1}H-NMR spectrum of dipeptide 9:

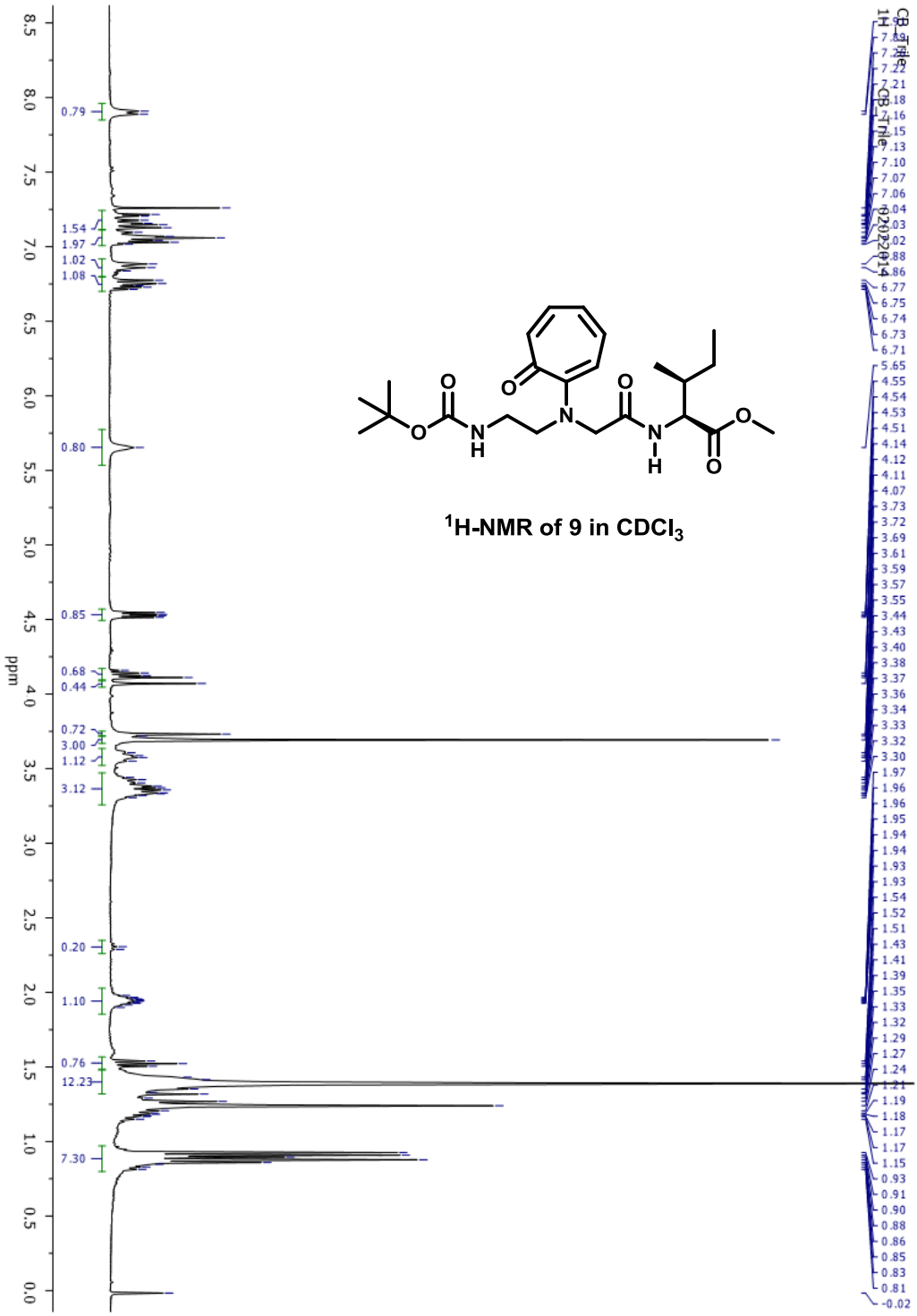

Figure S9: ^{1}H-NMR of dipeptide (Boc-Tr-aeg-Ile-OMe).

15. ^{13}C-NMR spectrum of dipeptide 9:

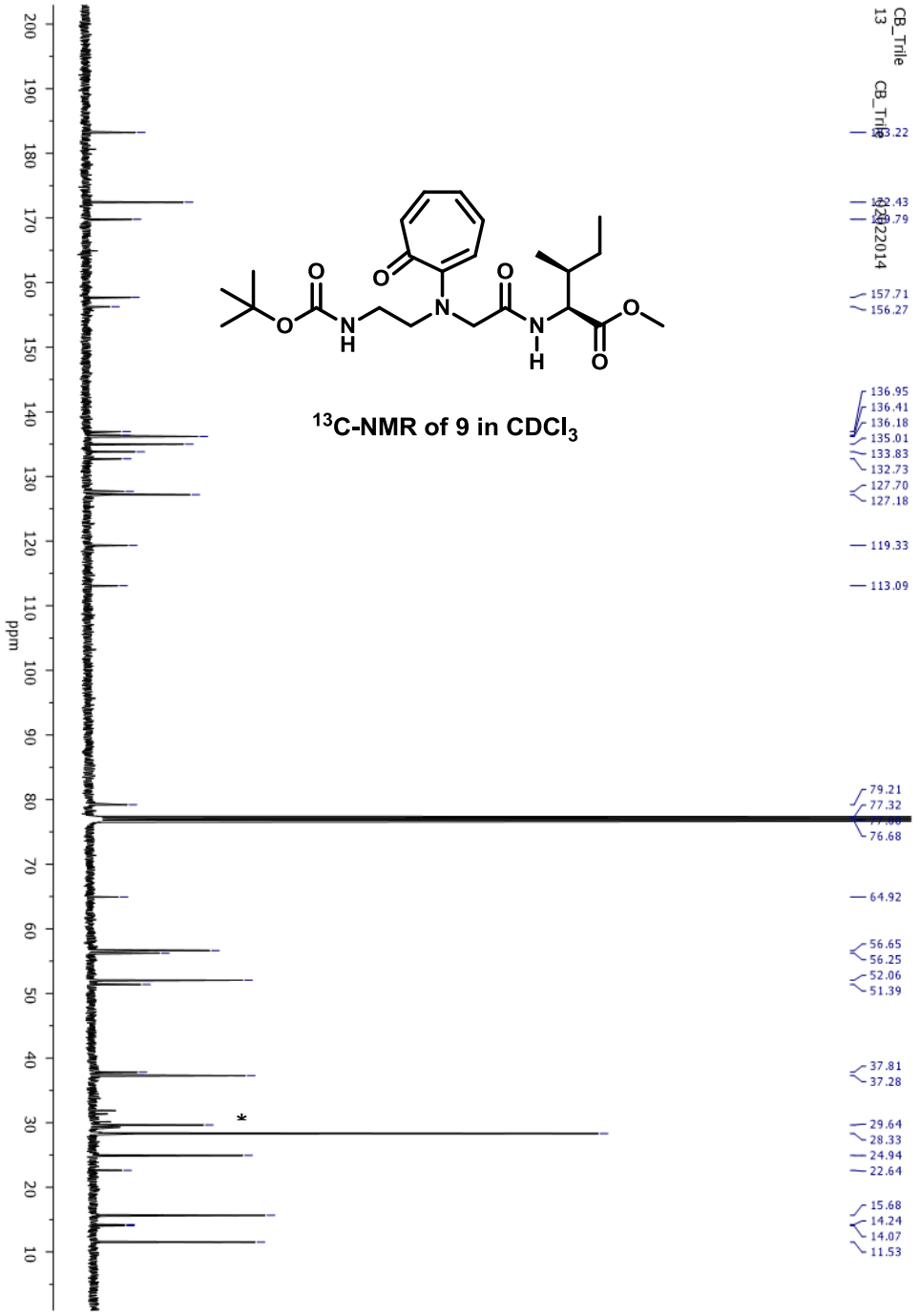

Figure S10: ^{13}C-NMR of dipeptide (Boc-Tr-aeg-Ile-OMe). (* hexane peaks)

16. Mass spectrum of dipeptide 9:

HRMS (ESI-MS–Tof)

Display Report

Analysis Info

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Method	Pso_tune_wide.m	Operator	A.S.Sahu
Sample Name		Instrument	micrOTOF-Q II 10337
Comment			

Acquisition Parameter

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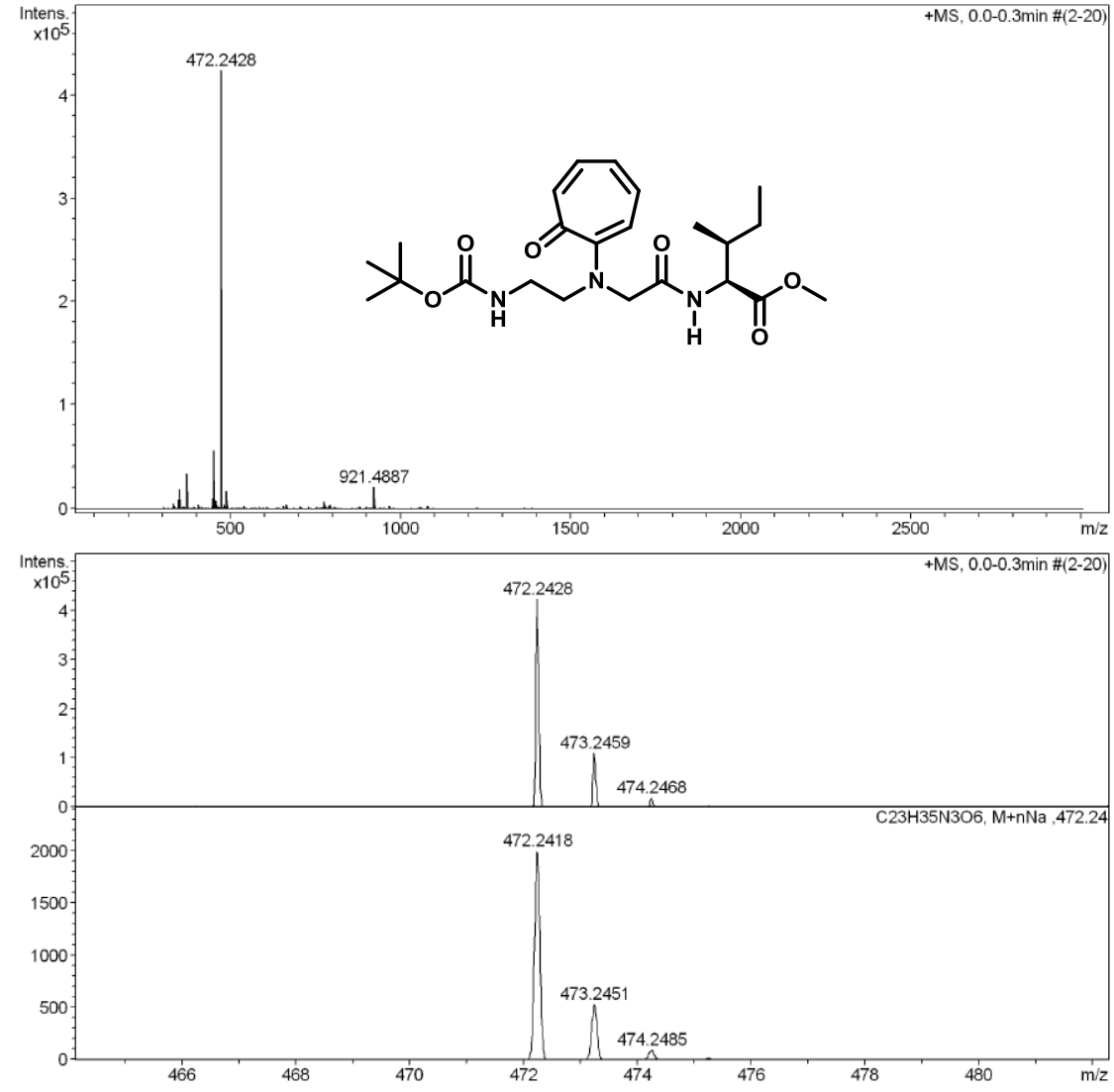

Bruker Compass DataAnalysis 4.0 printed: 2/4/2014 12:31:24 PM Page 1 of 1

17. ^{1}H-NMR spectrum of dipeptide 10:

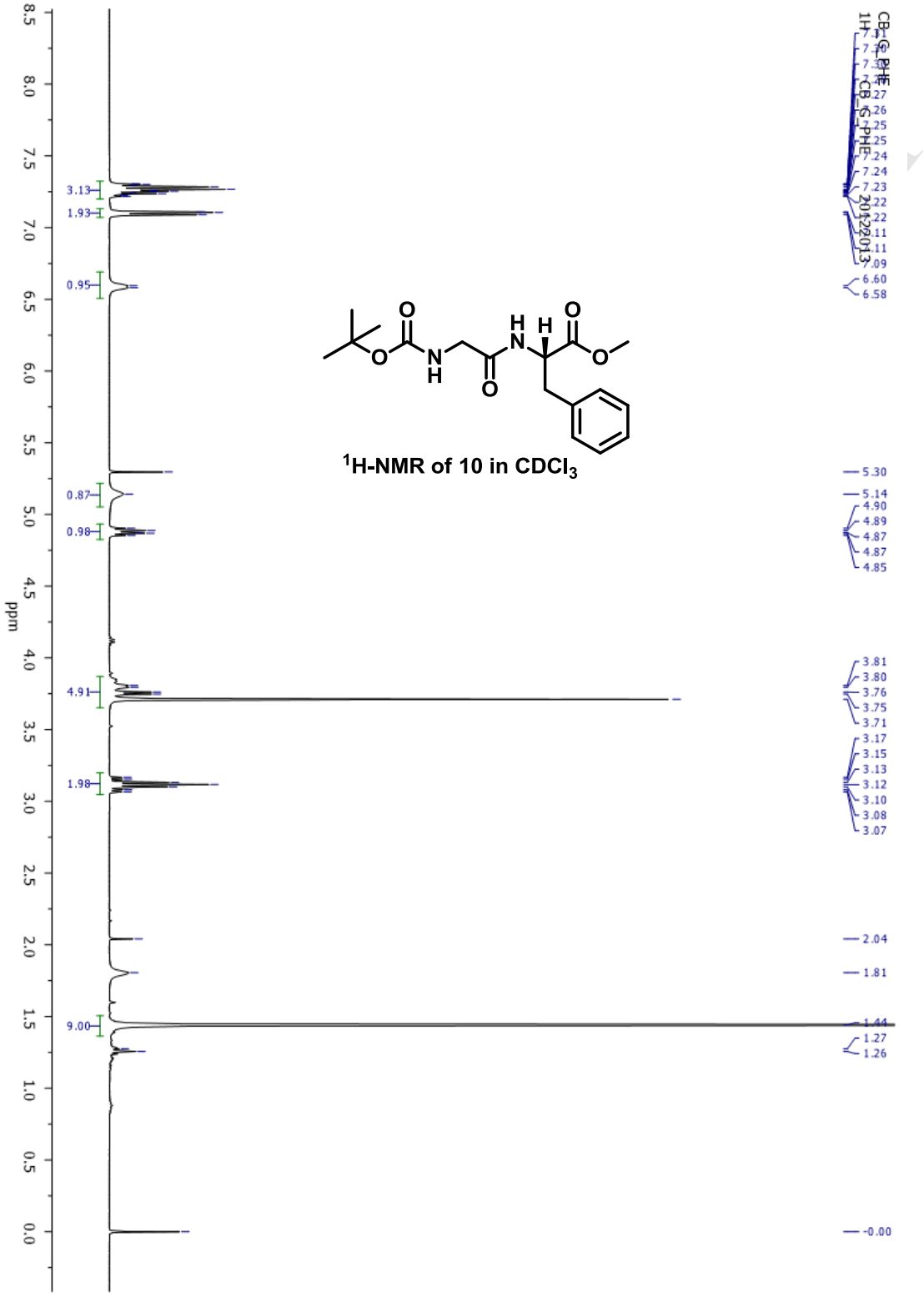

Figure S11: ^{1}H-NMR of dipeptide (Boc-Gly-Phe-OMe).

18. ^{13}C-NMR spectrum of dipeptide 10:

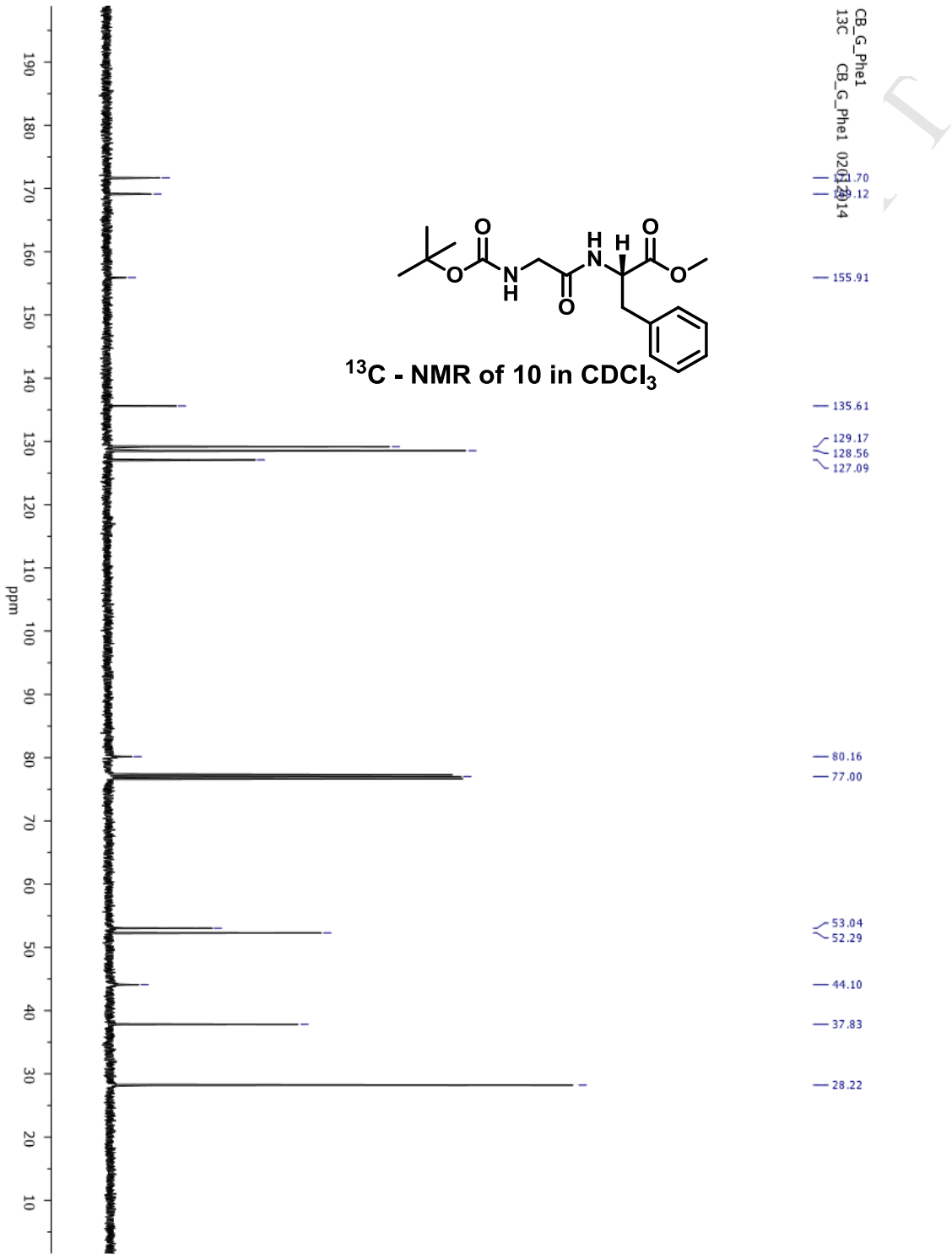

Figure S12: ^{13}C-NMR of dipeptide (Boc-Gly-Phe-OMe).

19. Mass spectrum of dipeptide 10:

HRMS (ESI-Mass-Tof) of

Display Report

Analysis Info

Analysis Name	D:\Data\DEC-2013\NKS\20122013_NKS_CB-G_Phe.d	Acquisition Date	12/20/2013 2:35:18 PM
Method	Pos_tune_low.m	Operator	RAJKUMAR
Sample Name		Instrument	micrOTOF-Q II 10337
Comment			

Acquisition Parameter

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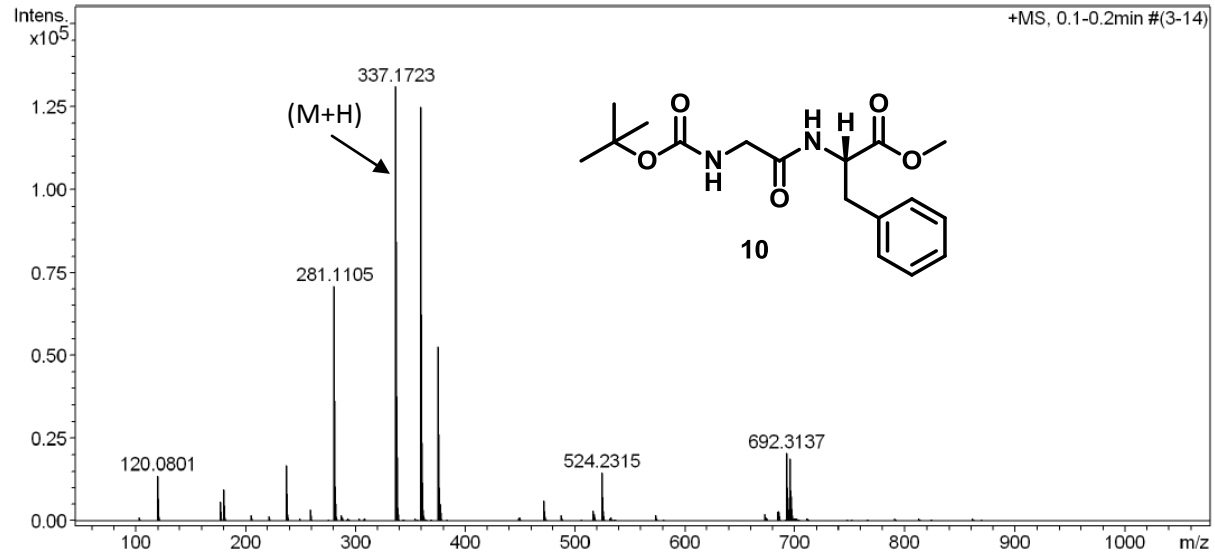
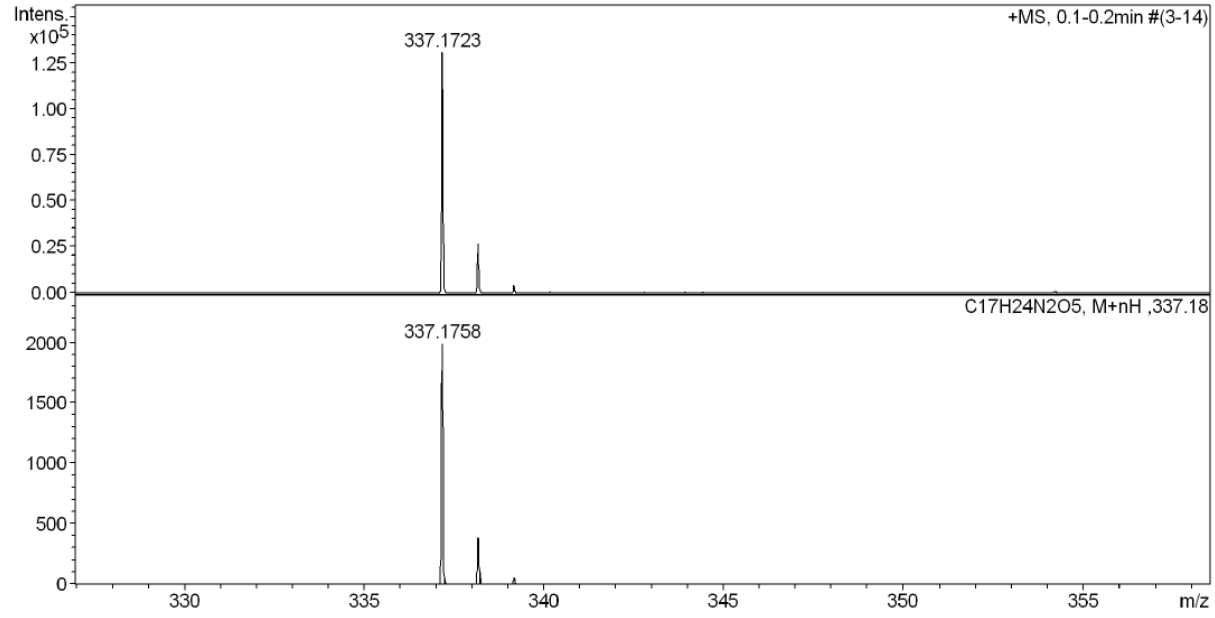
Scan End	3000 m/z	Set Collision Cell RF	130.0 Vpp	Set Divert Valve	Waste





20. ^{1}H-NMR spectrum of tripeptide 11:

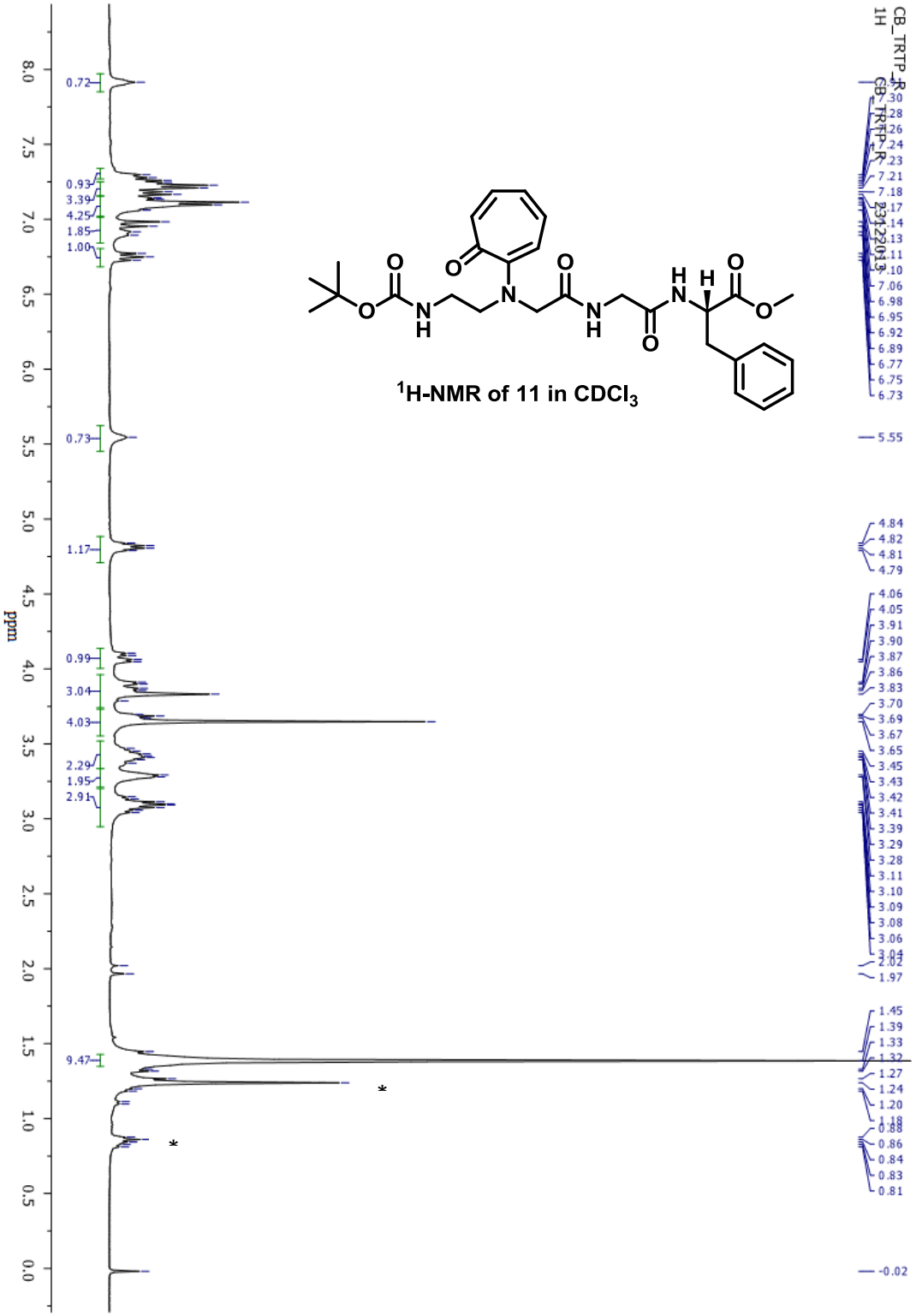


Figure S13: ^{1}H-NMR of tripeptide (Boc-*Tr-aeg*-Gly-Phe-OMe). (* hexane peaks)

21. ^{13}C-NMR spectrum of tripeptide 11:

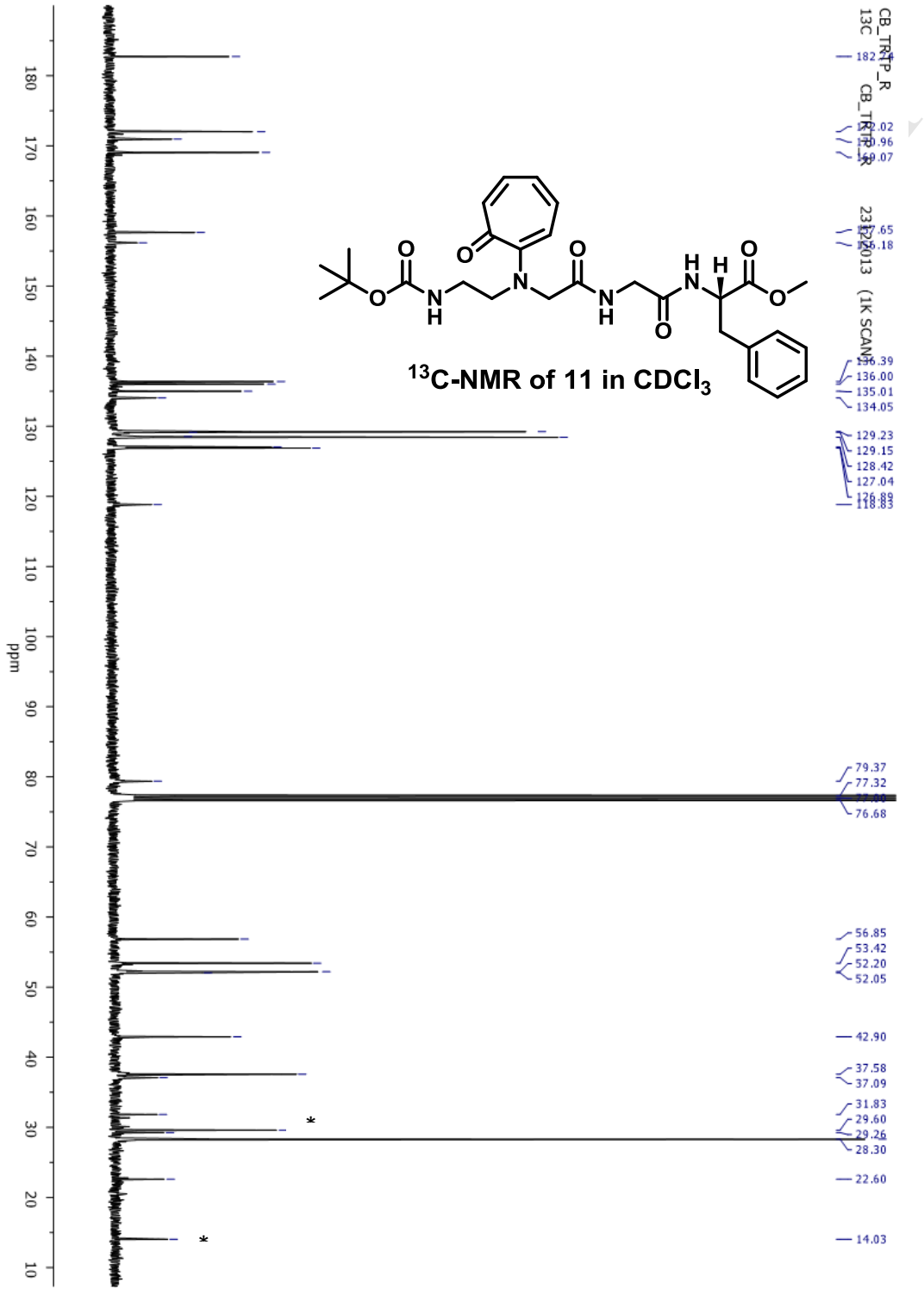


Figure S14: ^{13}C-NMR of tripeptide (Boc-*Tr*-*aeg*-Gly-Phe-OMe). (* hexane peaks).

22. Mass spectrum of tripeptide 11:

HRMS (ESI-Mass-Tof)

Display Report

Analysis Info

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Sample Name	< No Sample >	Instrument	micrOTOF-Q II 10337
Comment			

Acquisition Parameter

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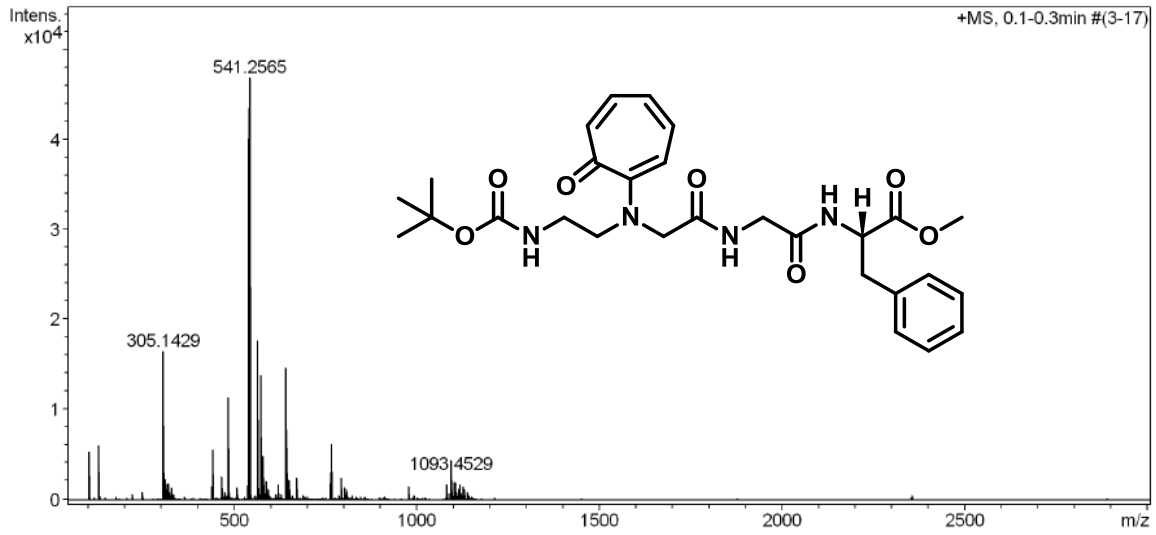

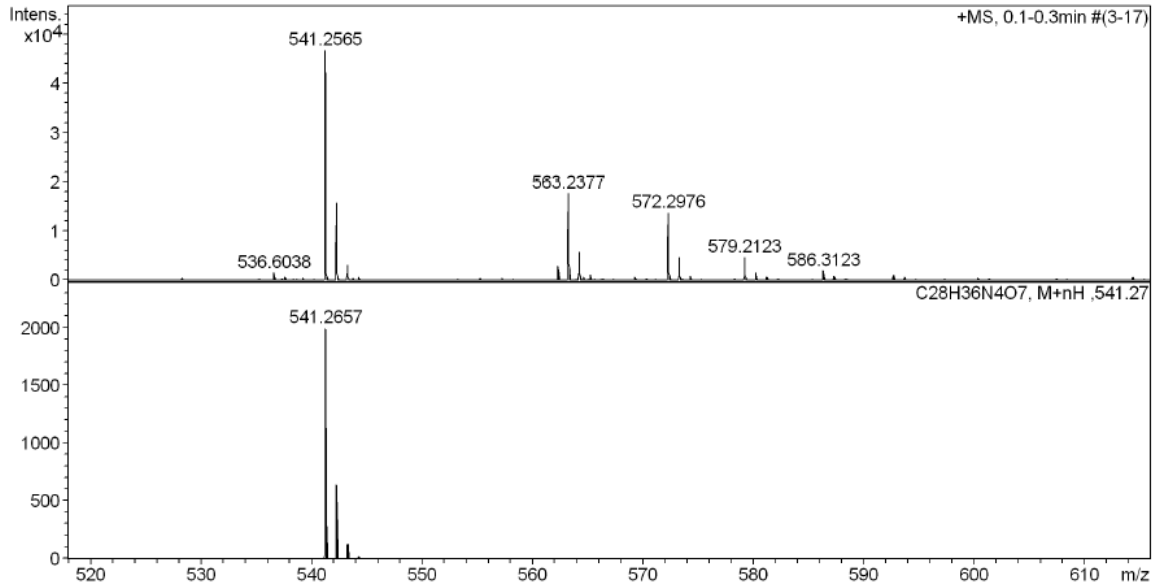

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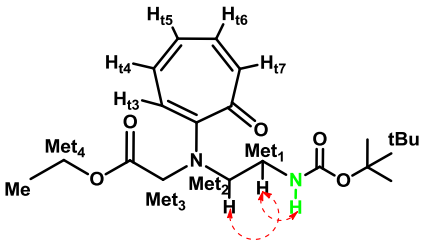
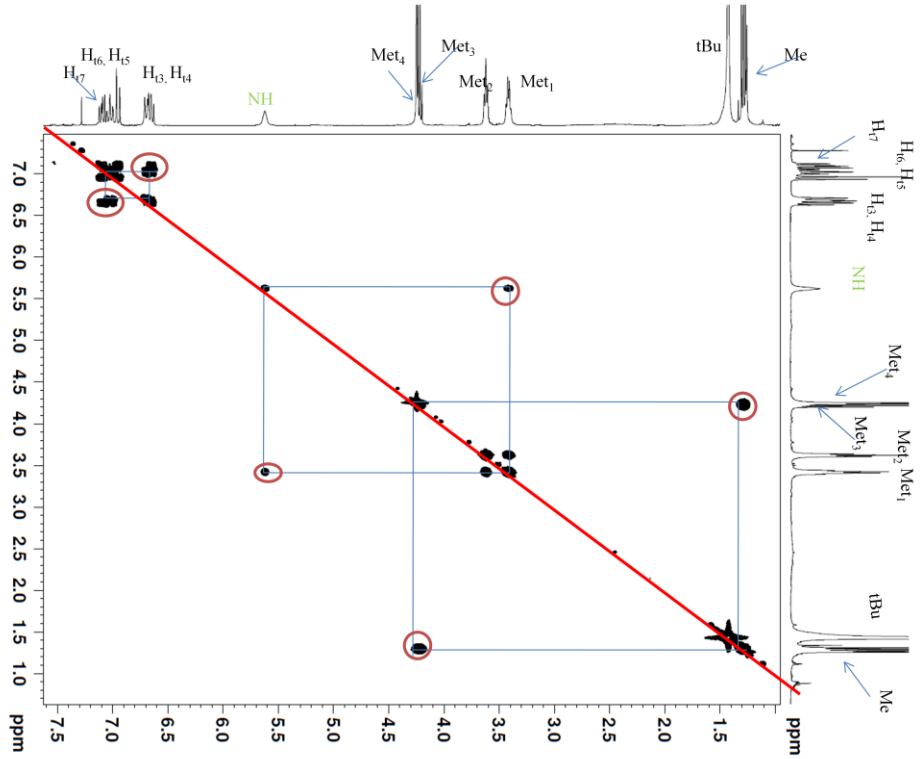
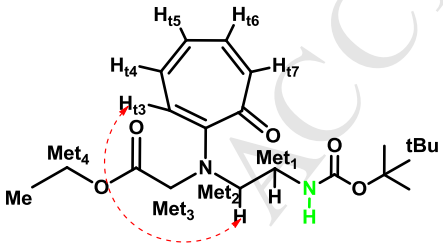
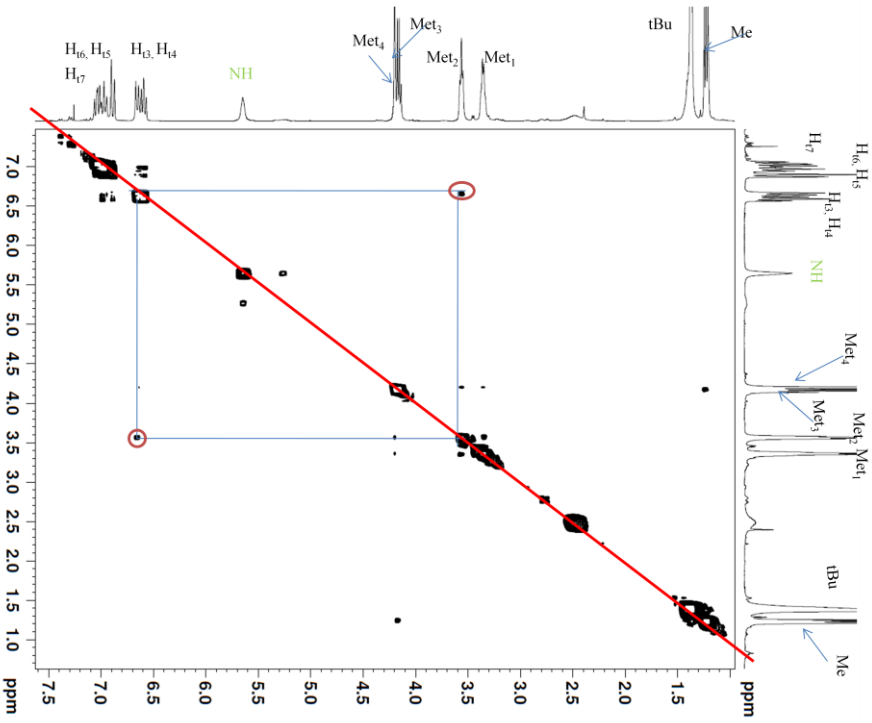
23. 1H-1H COSY and NOESY 2D NMR spectrum of monomer 5:

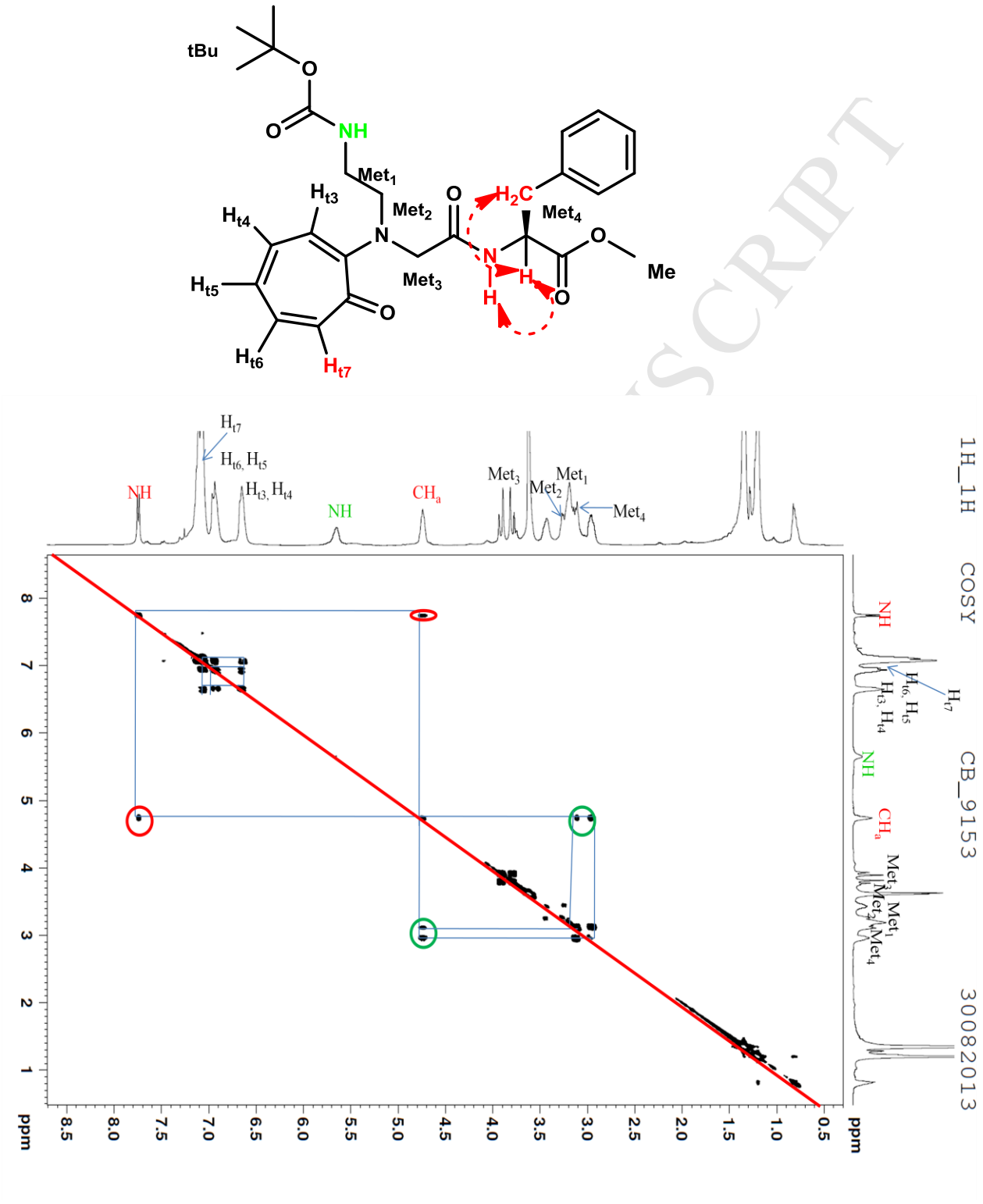








24. ^{1}H-^{1}H COSY 2D NMR spectrum of dipeptide 7:



25. ^{1}H-^{1}H NOESY 2D NMR spectrum of dipeptide 7:

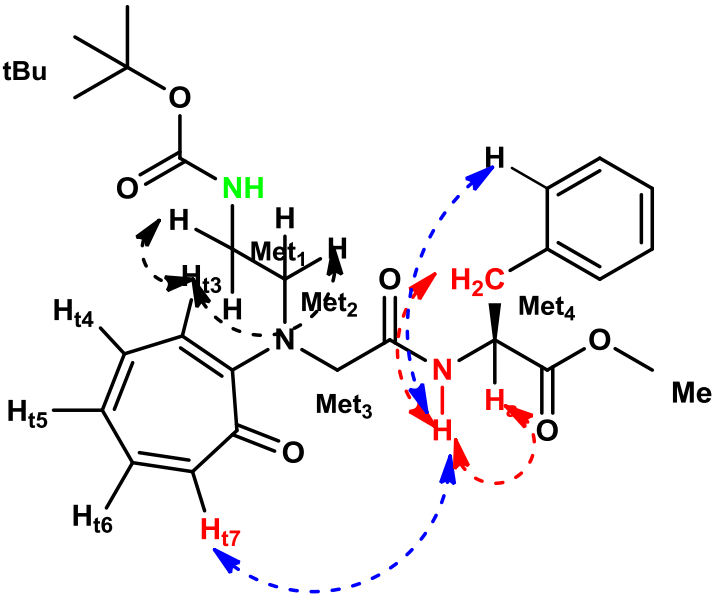
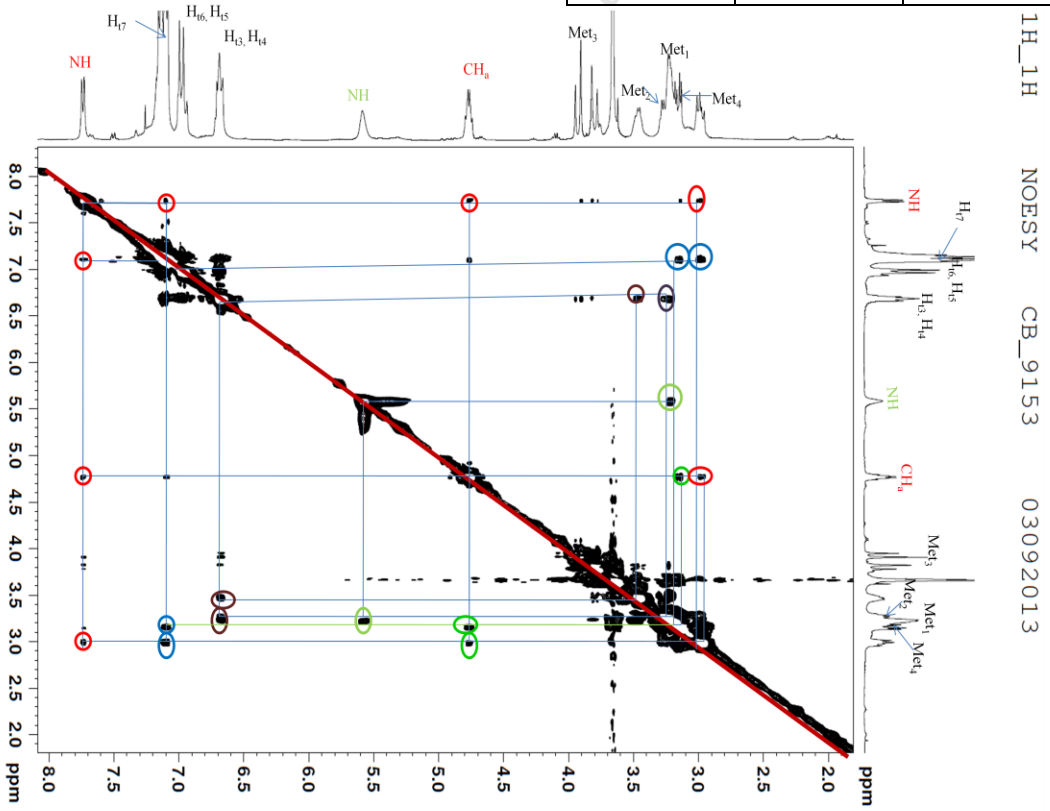
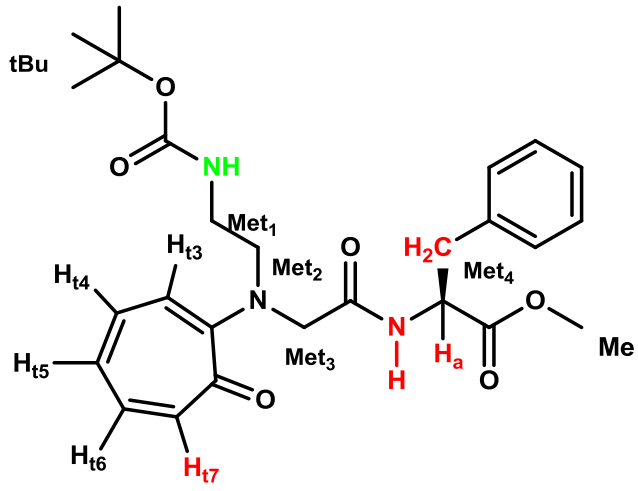


Table S1:

	COSY interaction	NOESY interaction
CH_{t3}		Met_1, Met_2
CH_{t4}		
CH_{t5}		
CH_{t6}		
CH_{t7}		NH
Phe		
Carbamate NH		Met_1
Amide NH	CH_a	Met_4, Met_3 (faint)
Met_1		
Met_2		
Met_3		
Met_4		
CH_a		
tBu		
Me		



26. HSQC 2D NMR spectrum of dipeptide 7:



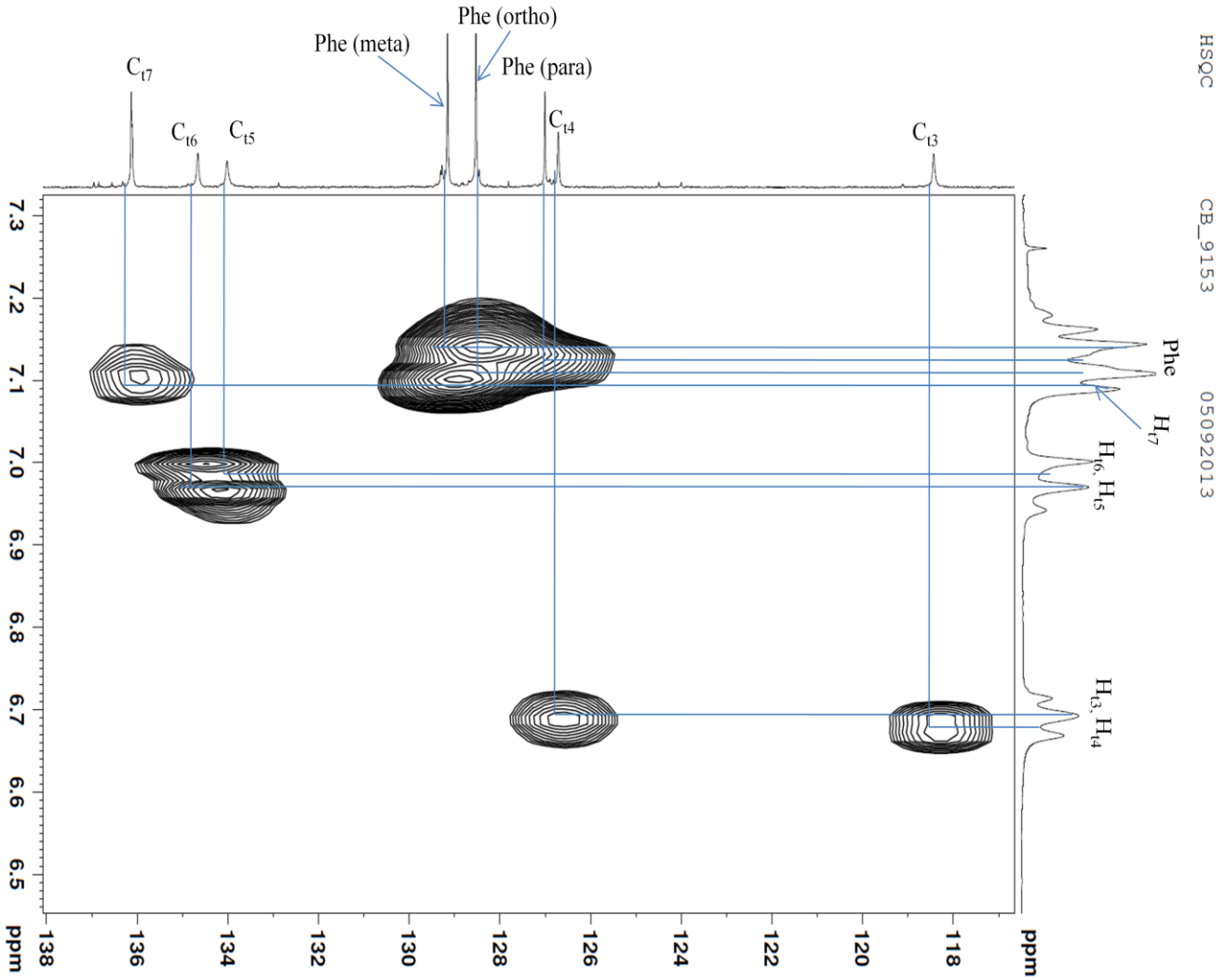
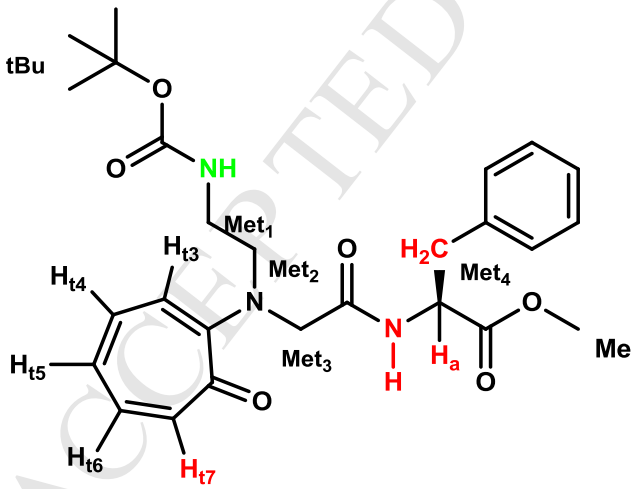
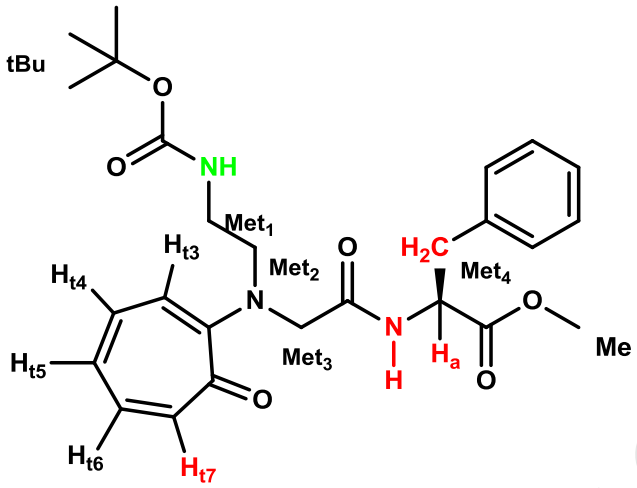
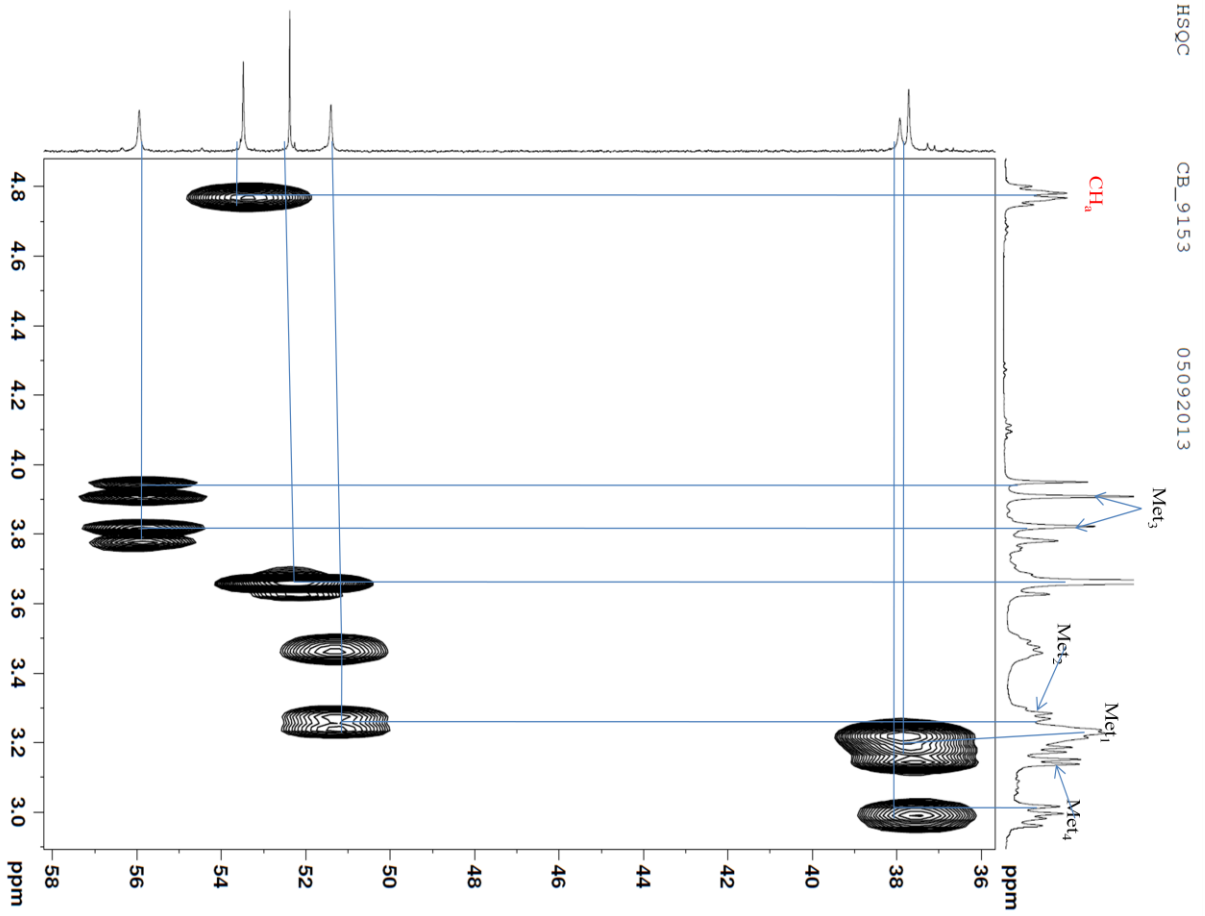


Table S2:

^{1}H NMR		^{13}C NMR
6.72	CH_{t3}	118.09
6.72	CH_{t4}	126.68
7.05 – 6.92 (m)	CH_{t5}	133.79
7.05 – 6.92 (m)	CH_{t6}	134.34
7.24 - 7.06 (m)	CH_{t7}	135.87
7.24 – 7.06 (m)	Phe (meta)	129.02
7.24 – 7.06 (m)	Phe (ortho)	128.91
7.24 – 7.06 (m)	Phe (para)	126.75
5.59 (s)	Carbamate NH	
7.76 (d)	Amide NH	
3.55 – 3.37, 3.36 – 3.12, (m)	Met_1	37.46
3.36 – 3.12, (m)	Met_2	51.18
3.93, 3.81 (d)	Met_3	55.60
3.05 – 2.93 (m), 3.36 – 3.12 (m)	Met_4	37.65
4.78 (dd)	CH_a	53.25
1.38 (s)	tBu, C_{tBu}	28.19
3.7 (s)	Me, C_{Me}	52.12




tBu
O
O
NH
Met1
Ht3
Ht4
Met2
O
H2C
Met4
N
N
O
Ht5
Met3
H
Ha
O
Me
O
Ht6
Ht7


HSQC
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05092013
CHa
Met3
Met2
Met1
Met4
ppm
ppm
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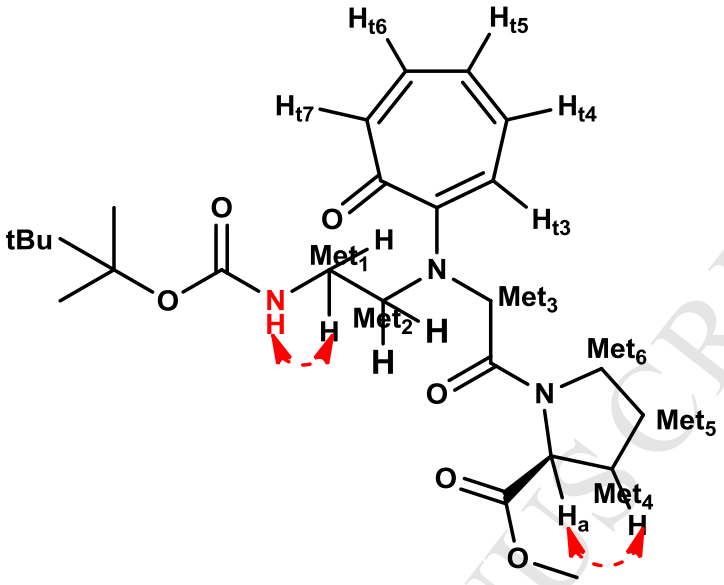
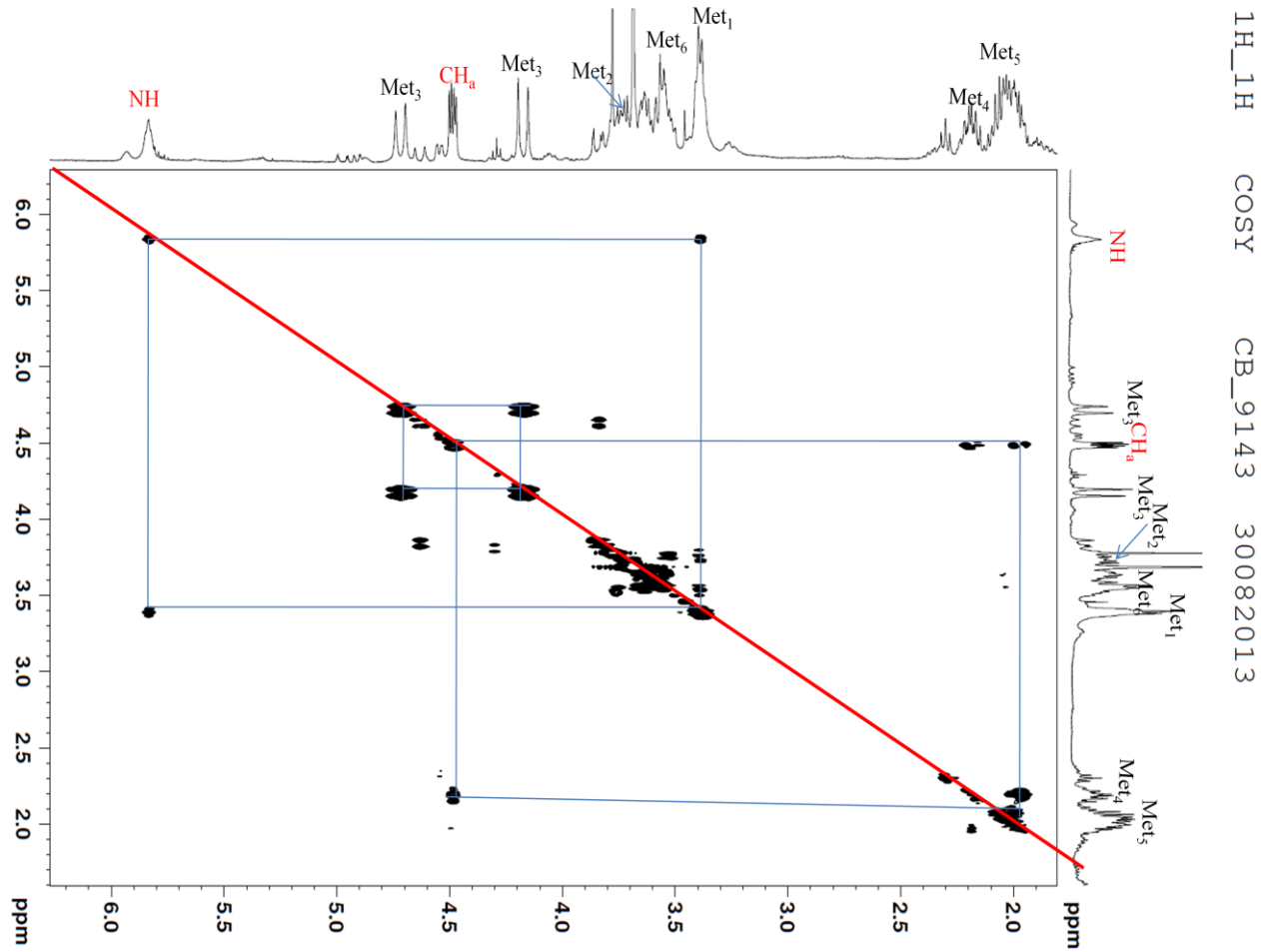
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
58
56
54
52
50
48
46
44
42
40
38
36

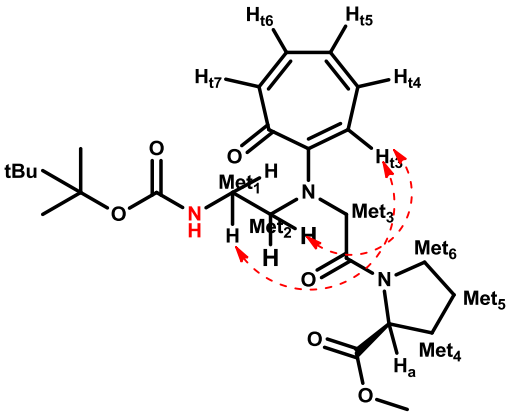
27.^{1}H-^{1}H COSY 2D NMR spectrum of dipeptide 8:



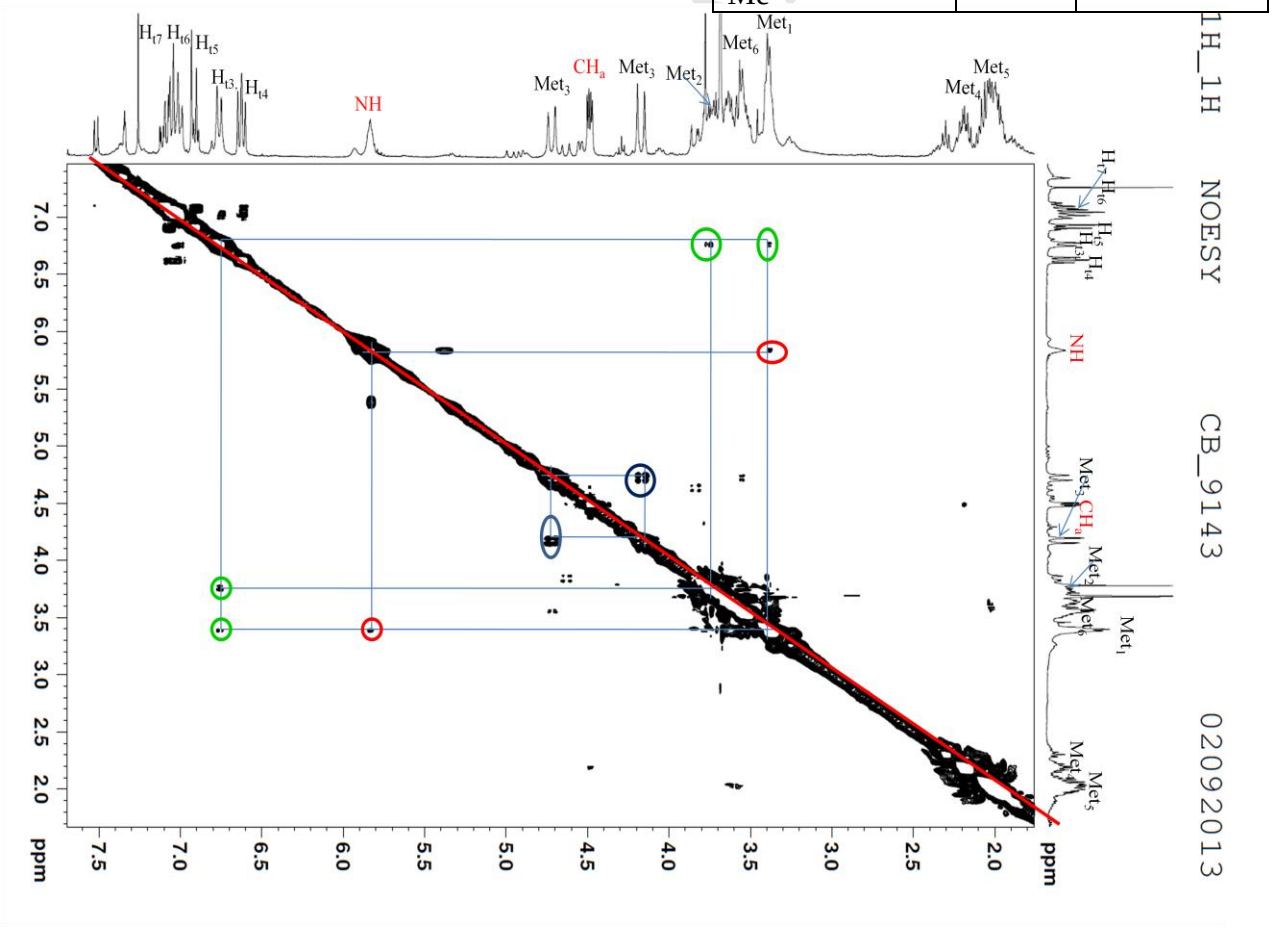


28. ^{1}H-^{1}H NOESY 2D NMR spectrum of dipeptide 8:

Table S3:

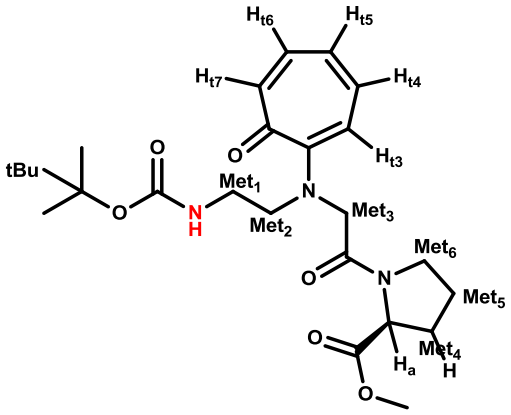


	COSY interaction	NOESY interaction
CH_{t3}		Met_1, Met_2
CH_{t4}		
CH_{t5}		
CH_{t6}		
CH_{t7}		
Carbamate NH	Met_1	Met_1
Met_1		
Met_2		
Met_3		
Met_4		
Met_5		
Met_6		
CH_a	Met_4	
tBu		
Me		

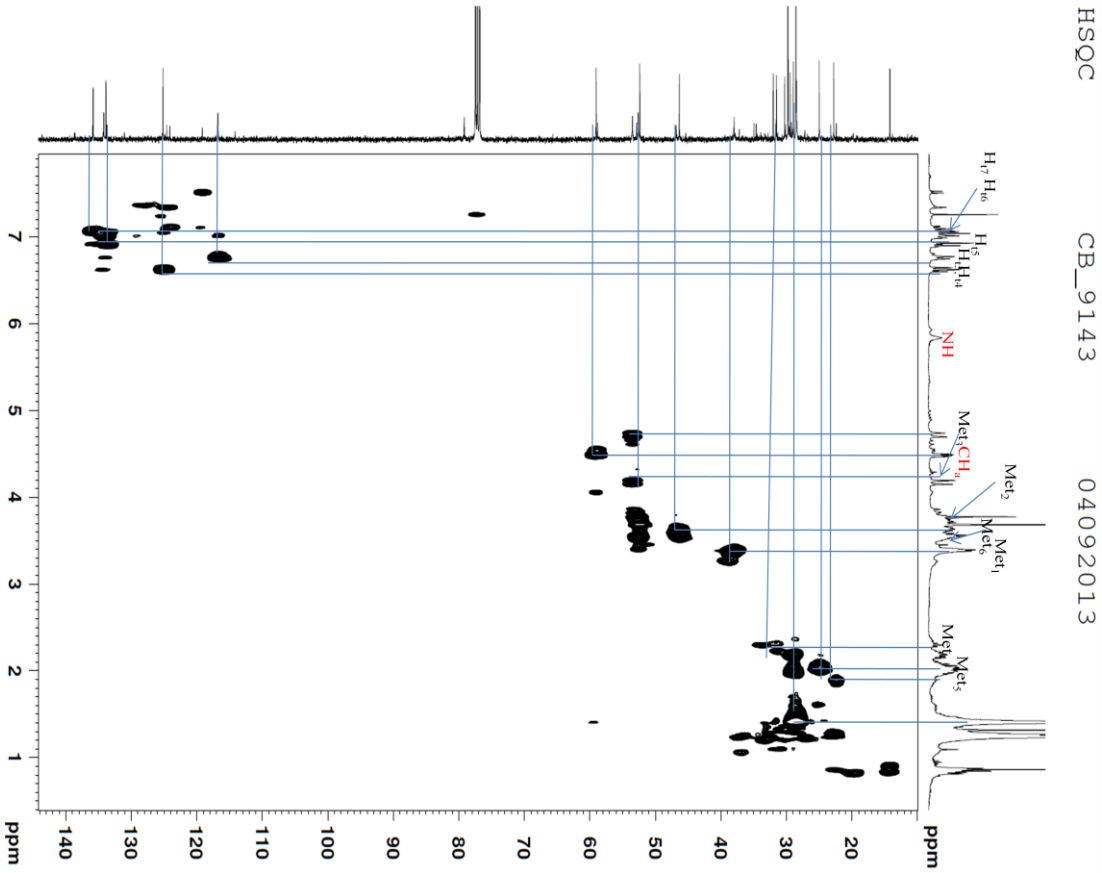


29. HSQC 2D-NMR spectrum of Dipeptide 8:

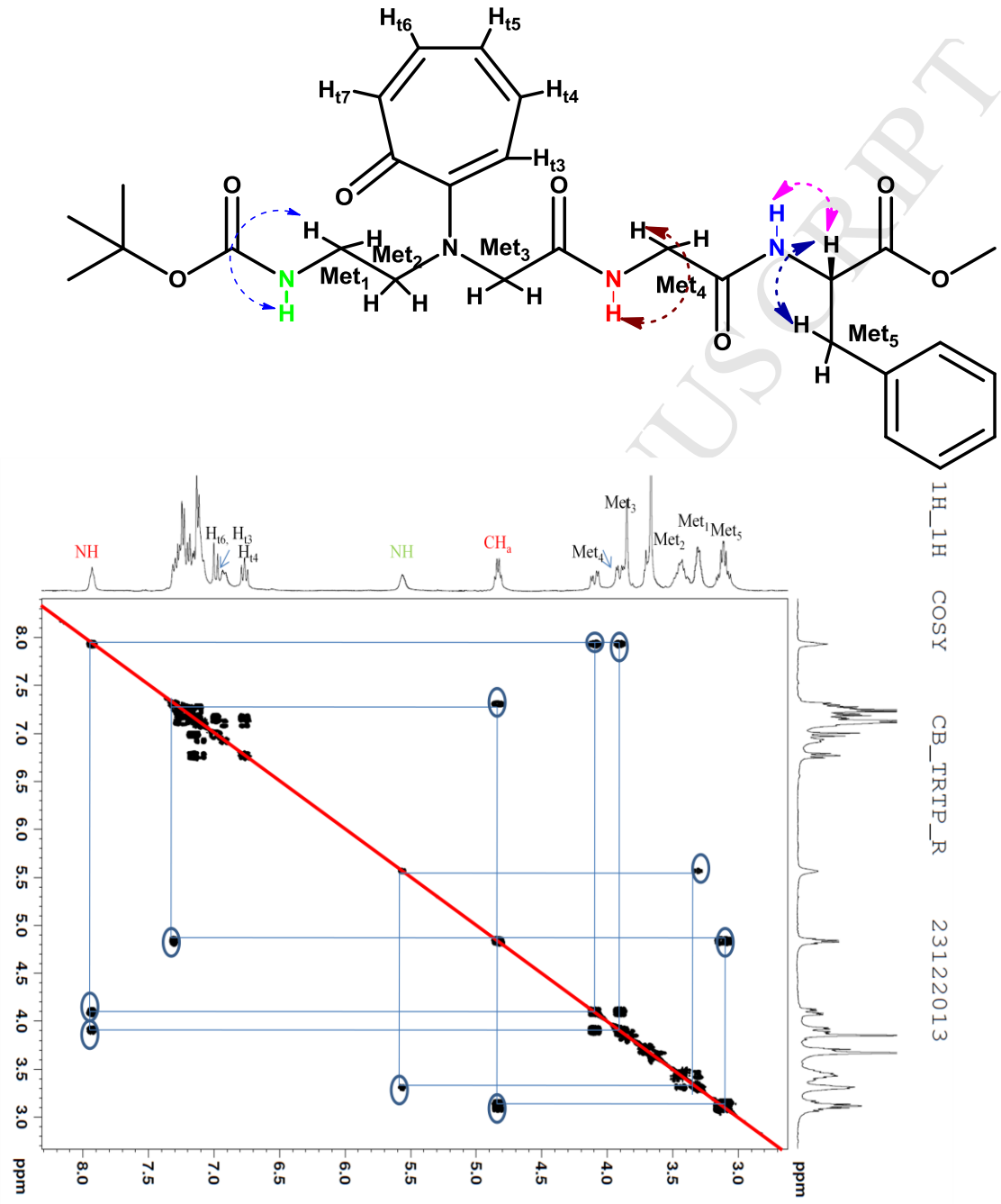
TableS4:



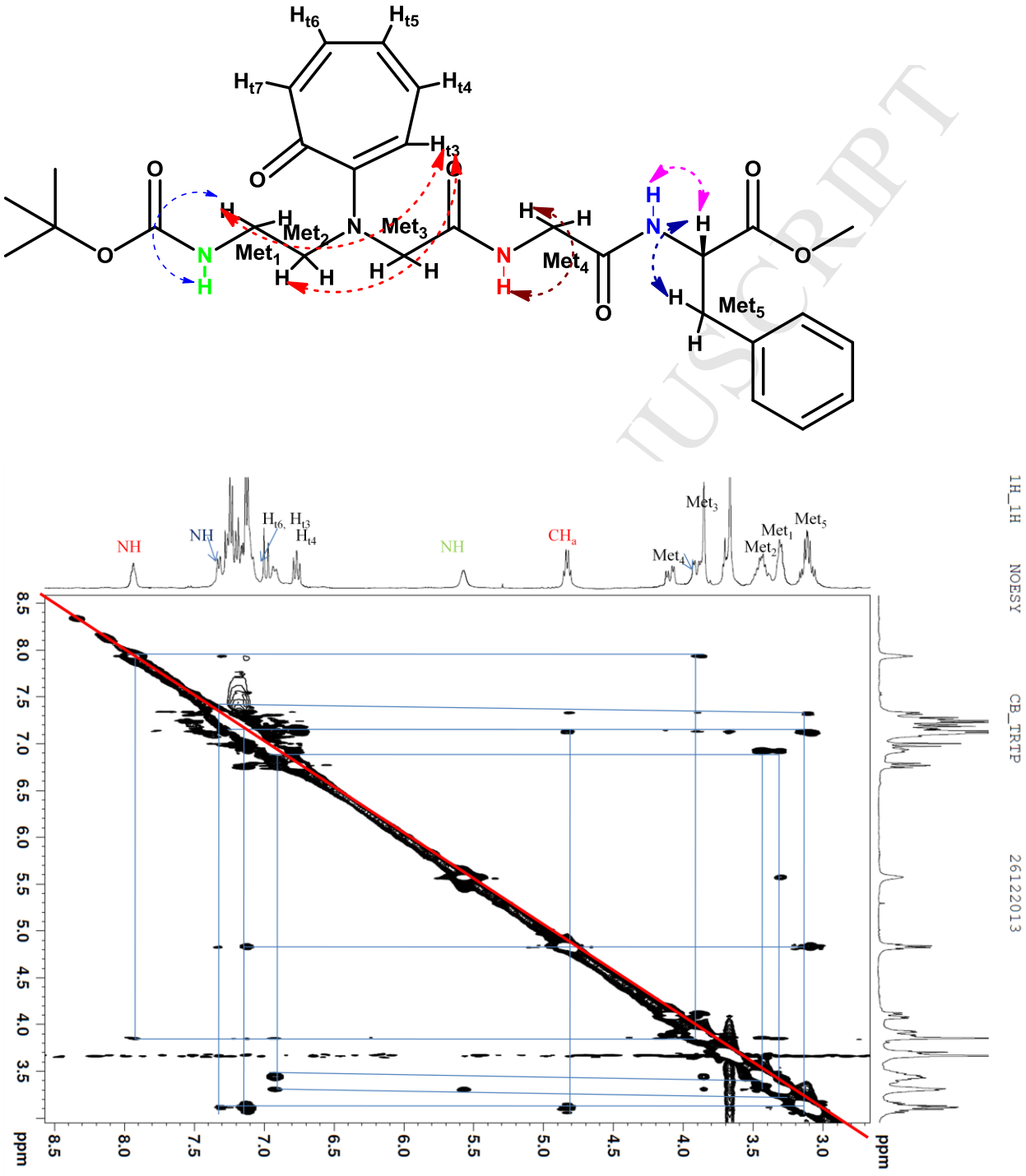
^{1}H NMR		^{13}C NMR
6.70 – 6.58	CH_{t3}	116.67
6.78	CH_{t4}	124.99
6.92 (m)	CH_{t5}	133.72
7.15 – 6.98 (m)	CH_{t6}	134.02
7.15 – 6.98(m)	CH_{t7}	135.66
5.84 (s)	Carbamate NH	
3.40 (d)	Met_1	37.87
3.74, 3.64 (dd)	Met_2	52.49
4.72, 4.18 (d)	Met_3	53.37
2.41 – 2.27, 2.27 – 2.19	Met_4	28.83
2.14 – 1.93	Met_5	24.89
3.55	Met_6	46.21
4.49 (dd)	CH_a	58.92
1.41 (s)	tBu	28.42
3.7 (s)	Me	52.49



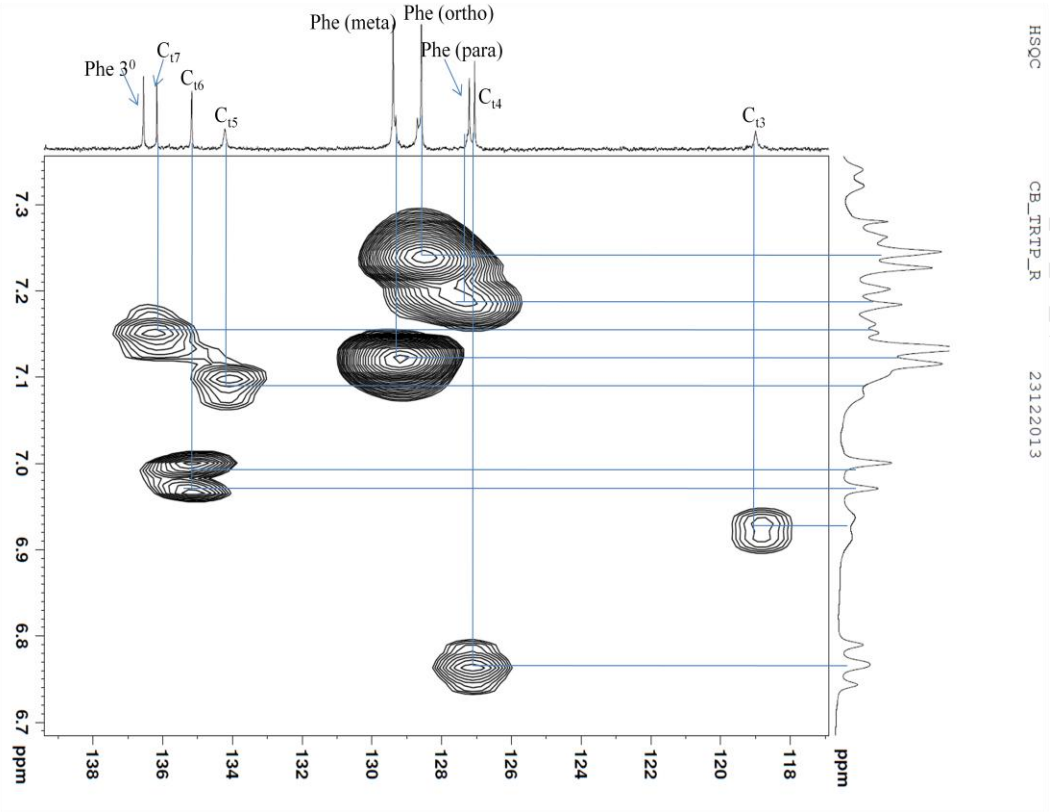
30. ^{1}H-^{1}H COSY 2D-NMR spectrum of tripeptide 11:



31. ^{1}H-^{1}H NOESY 2D NMR spectrum of tripeptide 11:



32. HSQC 2D NMR sptectrum of tripeptide 11:



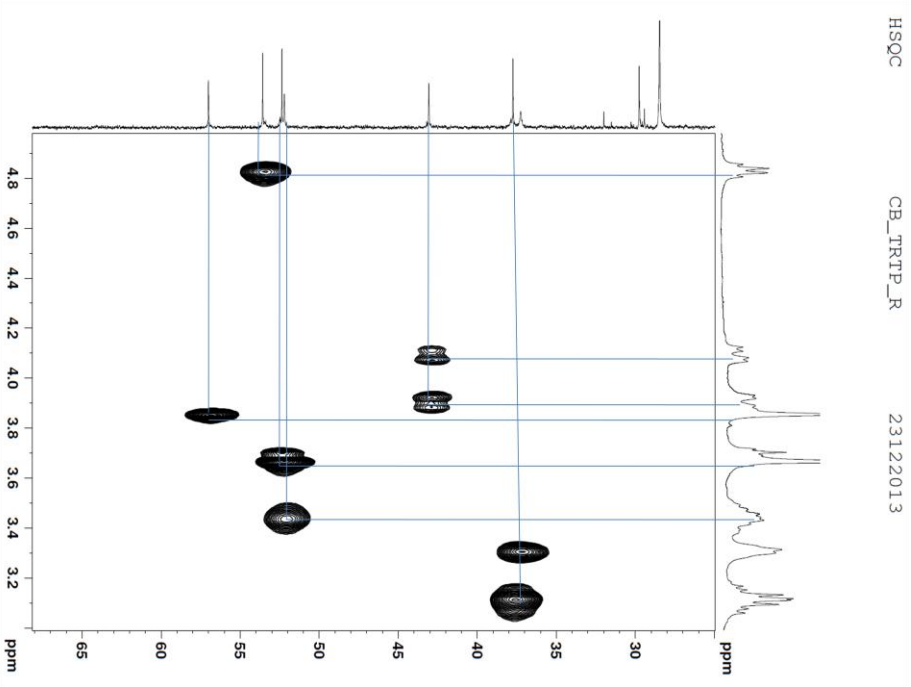


Table S5: COSY and NOESY assignements of tripeptide **11**:

	COSY interaction	NOESY interaction
CH_{t3}		Met_1, Met_2
CH_{t4}		
CH_{t5}		
CH_{t6}		
CH_{t7}		
Phe		
Carbamate NH	Met_1	Met_1
Gly NH	Met_4	Met_4
Phe NH	CH_a	CH_a
Met_1		CH_{t3}
Met_2		CH_{t3}
Met_3		
Met_4		
Met_5		Phe(meta)
CH_a	Met_5	Met_5
tBu		
Me		

Table S6: HSQC assignements of tripeptide **11**:

^{1}H NMR		^{13}C NMR
6.81-6.68	CH_{t4}	126.89
6.94	CH_{t3}, CH_{t6}	118.83, 135.01
7.15 – 7.01	CH_{t7}, CH_{t5}	136.00,134.06
7.15 – 7.01	Phe (meta)	129.23
7.27 – 7.16 (m, 3H)	Phe (ortho)	128.42
7.27 – 7.16 (m, 3H)	Phe (para)	127.04
5.49 (s)	Carbamate NH	
7.29 (d)	Phe (i+2) NH	
7.91 (t)	Gly (i+1) NH	
3.29 (d)	Met_1	37.09
3.53 – 3.34 (m)	Met_2	52.06
3.97 – 3.73 (m)	Met_3	56.85
4.08 (dd),3.97 – 3.73 (m)	Met_4	42.90
3.09 (qd)	Met_5	37.93
4.81 (dd)	CH_a	53.42
1.39 (s)	tBu	28.30
3.75 – 3.54 (m)	Me	52.20

33. NMR titration studies of 5/7/8/9/10/11:

NMR titration studies of dipeptide (Boc-*Tr*-*aeg*-Phe-OMe**, 7)** in $CDCl_3$ with DMSO–d6**:**

(5 mg of dipeptide was dissolved in $CDCl_3$, NMR was recorded at 297 K with out DMSO-d6 and 5 µl of DMSO–d6 was added at each addition and recorded NMR. All spectra were calibrated to tetramethy silane. Changes in chemical shift values of Nitrogen attached protons are given in **TableS7**)

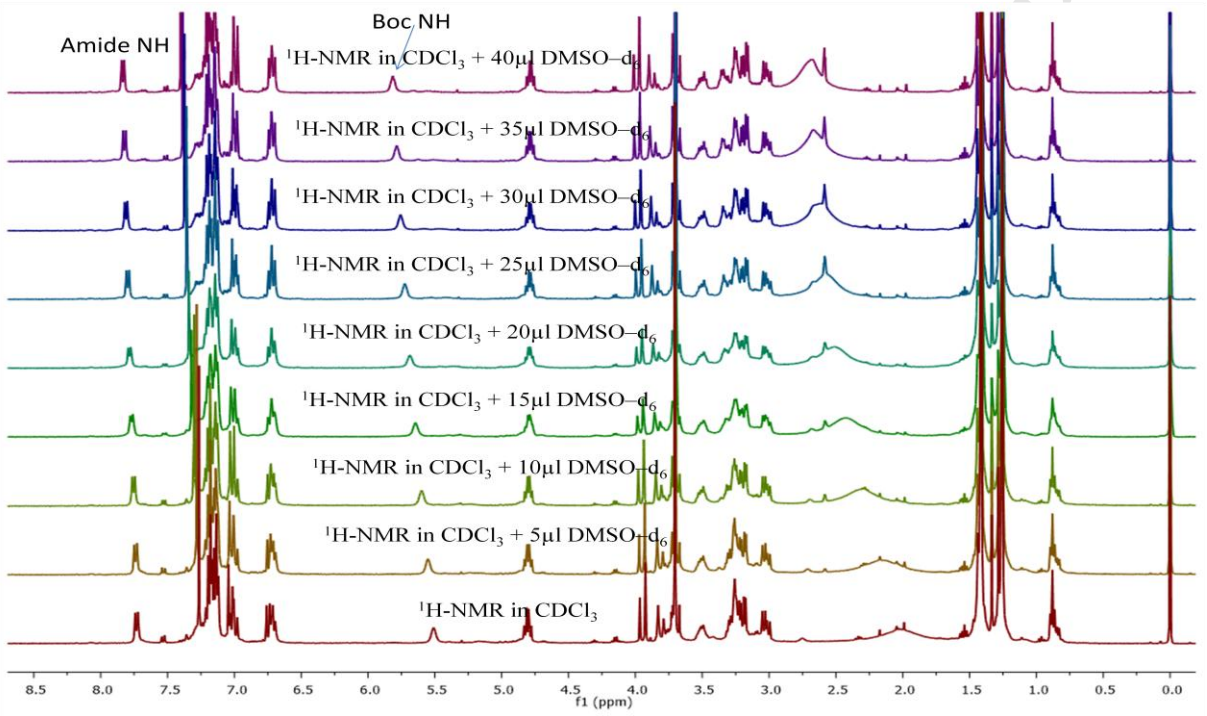
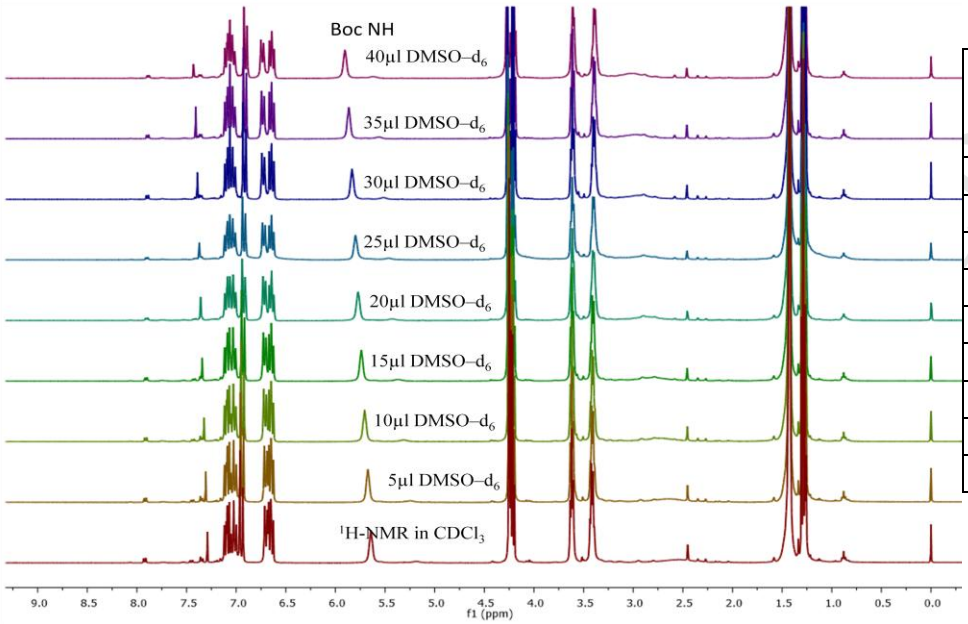


Table S7:

No	Volume of DMSO – d6 (in µl)	δNH	δNH
1	0	7.72	5.51
2	5	7.72	5.55
3	10	7.74	5.56
4	15	7.75	5.65
5	20	7.77	5.68
6	25	7.78	5.72
7	30	7.80	5.75
8	35	7.81	5.79
9	40	7.82	5.82

NMR titration studies of monomer (Boc-*Tr*-*aeg*-OEt, **5**) in $CDCl_3$ with DMSO-d6:

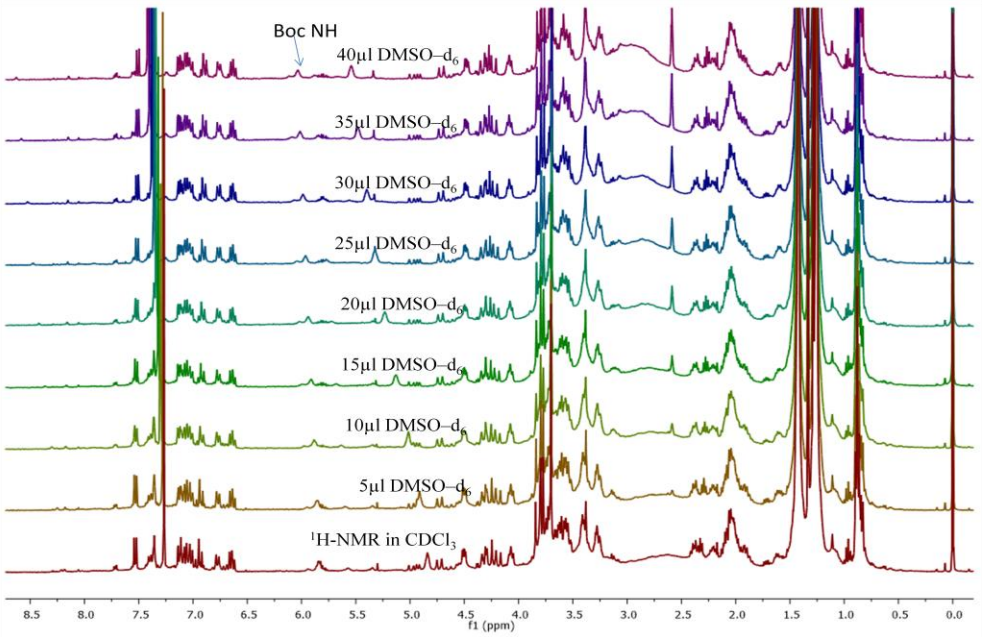
(5 mg of dipeptide was dissolved in $CDCl_3$, NMR was recorded at 297 K with out DMSO-d6 and 5µl of DMSO-d6 was added at each addition and recorded NMR. All spectra were calibrated to tetramethy silane. Changes in chemical shift values of Nitrogen attached protons are given in **Table S8**)



No	Volume of DMSO-d6 (in µl)	δNH (monomer)
1	0	5.64
2	5	5.67
3	10	5.70
4	15	5.73
5	20	5.77
6	25	5.79
7	30	5.83
8	35	5.86
9	40	5.90

NMR titration studies of dipeptide (Boc-*Tr*-*aeg*-Pro-OMe**, 8**) in $CDCl_3$ with DMSO-d6:

(5 mg of dipeptide was dissolved in $CDCl_3$, NMR was recorded at 297 K without DMSO-d6 and 5 µl of DMSO-d6 was added at each addition and recorded NMR. All spectra were calibrated to tetramethy silane. Changes in chemical shift values of Nitrogen attached protons are given in **TableS9)**



TableS9:

No	Volume of DMSO–d6 (in µl)	δNH (dipeptide,14)
1	0	5.84
2	5	5.85
3	10	5.88
4	15	5.91
5	20	5.94
6	25	5.96
7	30	5.98
8	35	6.01
9	40	6.03

NMR Titration studies of tripeptide (Boc-*Tr-aeg*-Gly-Phe-OMe, **9**) in $CDCl_3$ with DMSO-d6:

(8mg of tripeptide was dissolved in $CDCl_3$, NMR was recorded at 297K without DMSO-d6 and 5µl of DMSO-d6 was added at each addition and recorded NMR. All spectra were calibrated to tetramethy silane. Changes in chemical shift values of Nitrogen attached protons are given in **TableS10**)

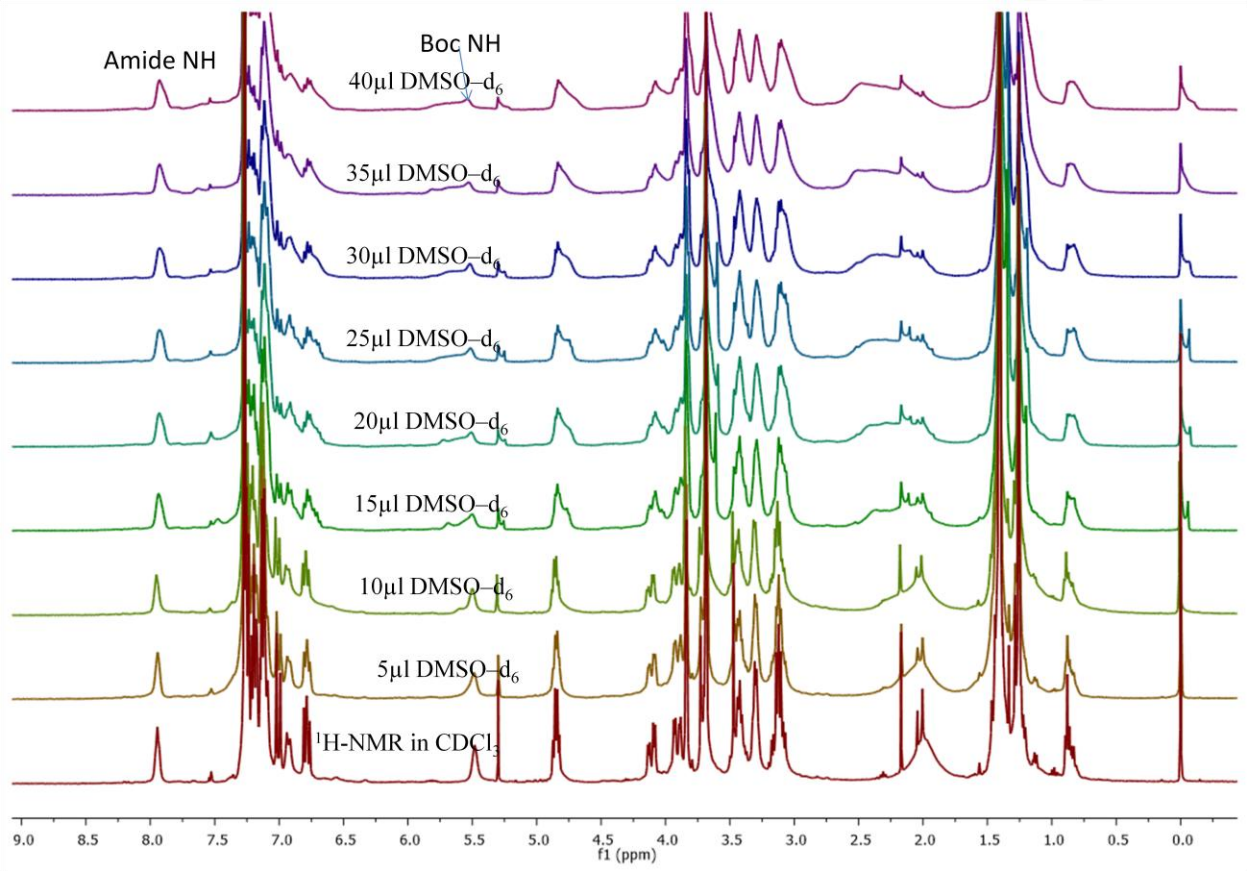


Table S10:

No	Volume of DMSO – d6 (in µl)	δNH	δNH
1	0	7.94	5.48
2	5	7.94	5.49
3	10	7.94	5.49
4	15	7.93	5.49
5	20	7.92	5.52
6	25	7.93	5.53
7	30	7.92	5.53
8	35	7.92	5.53
9	40	7.92	5.54

NMR titration studies of dipeptide (Boc-*Tr-aeg*-Ile-OMe**, 9**) in $CDCl_3$ with DMSO-d6**:**

(5 mg of dipeptide was dissolved in $CDCl_3$, NMR was recorded at 297 K with out DMSO-d6 and 5 µl of DMSO-d6 was added at each addition and recorded NMR. All spectra were calibrated to tetramethy silane. Changes in chemical shift values of Nitrogen attached protons are given in **TableS11**)

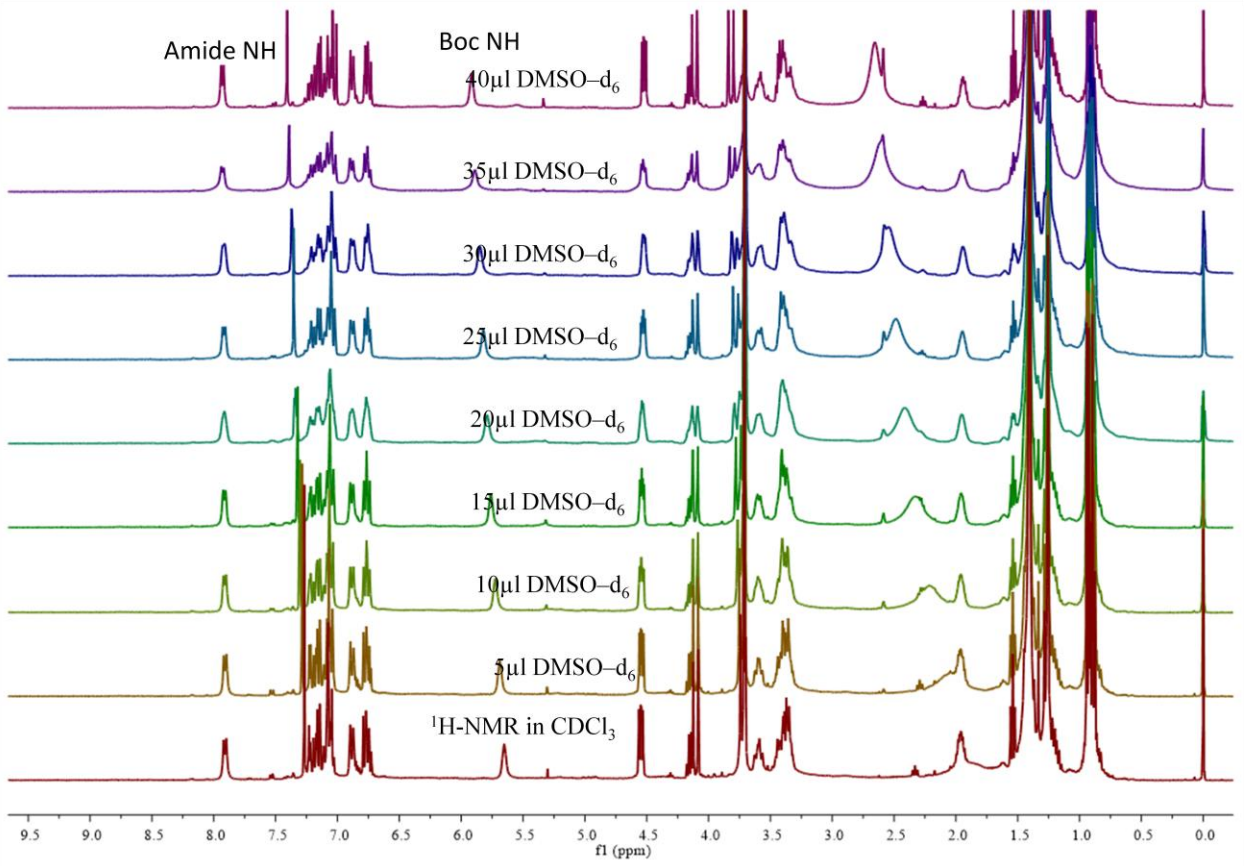


Table S11:

No	Volume of DMSO – d6 (in µl)	δNH	δNH
1	0	7.89	5.65
2	5	7.89	5.68
3	10	7.90	5.72
4	15	7.90	5.76
5	20	7.91	5.79
6	25	7.90	5.82
7	30	7.91	5.85
8	35	7.92	5.88
9	40	7.92	5.91

NMR titration studies of dipeptide (Boc-Gly-Phe-OMe, Controle **10**) in $CDCl_3$ with DMSO-d6:

(5 mg of dipeptide was dissolved in $CDCl_3$, NMR was recorded at 297 K with out DMSO-d6 and 5 µl of DMSO-d6 was added at each addition and recorded NMR. All spectra were calibrated to tetramethy silane. Changes in chemical shift values of Nitrogen attached protons are given in **TableS12**)

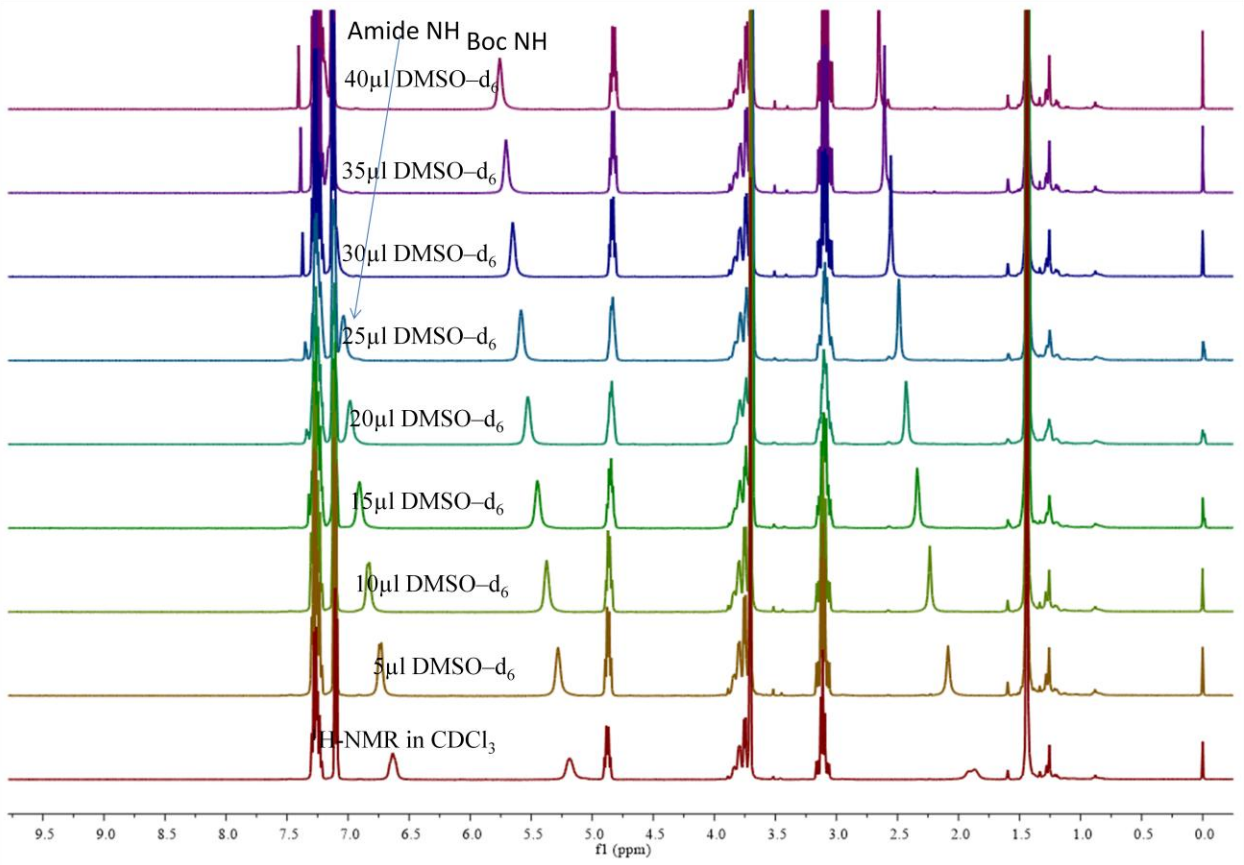


Table S12:

No	Volume of DMSO – d6 (in µl)	δNH	δNH
1	0	6.63	5.18
2	5	6.74	5.28
3	10	6.84	5.37
4	15	6.90	5.45
5	20	6.98	5.52
6	25	7.03	5.58
7	30	7.11	5.65
8	35	7.16	5.70
9	40	7.20	5.75

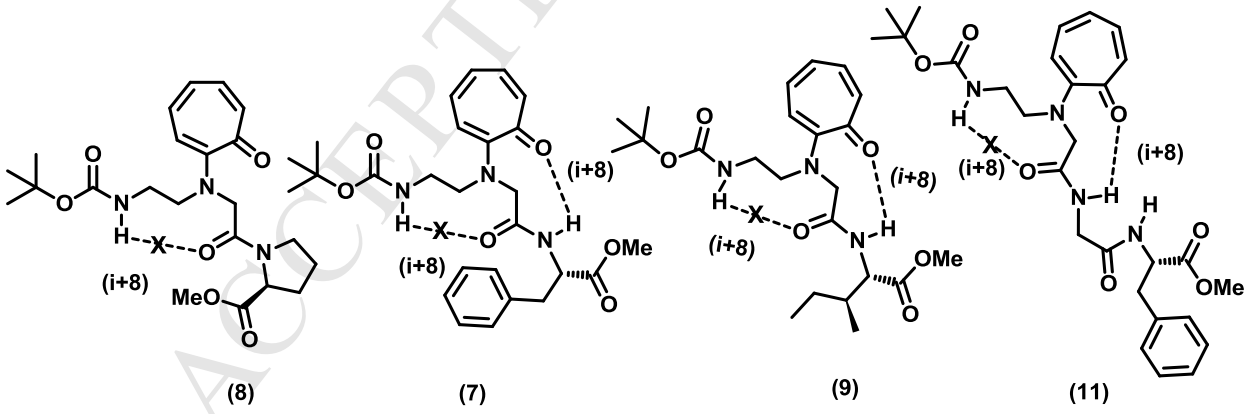
34. ^{1}H-NMR titration results of monomer (5) and peptides (7/8/9/10/11) with DMSO-d_6:

Table S13: NMR titration results

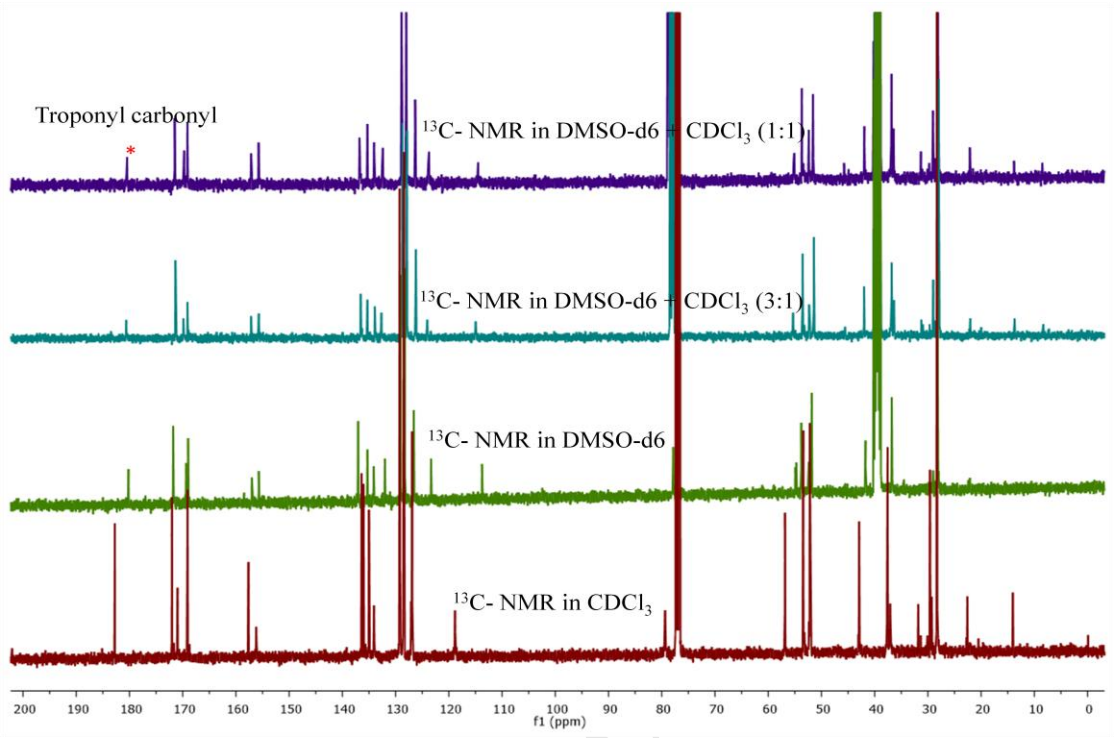
Sr. No	dipeptide/ Monomer	Type of NH	only $CDCl_3$ (ppm)	40.0 µL DMSO-d_6 in $CDCl_3$ (ppm)	Δ(ppm)*
1	**7**	Phe (i+1) Amide NH	7.72	7.82	+0.10
2	**7**	Carbamate NH	5.51	5.82	+0.31
3	**11**	Gly (i+1) Amide NH	7.94	7.92	-0.02
#4	**11**	Phe (i+2) Amide NH	7.29	Not predicted	
5	**11**	Carbamate NH	5.48	5.54	+0.06
6	**8**	Carbamate NH	5.84	6.03	+0.19
7	**5**	Carbamate NH	5.64	5.90	+0.26
8	**9**	Ile (i+1) Amide NH	7.89	7.92	+0.03
9	**9**	Carbamate NH	5.65	5.91	+0.26
10	**10 (controle)**	Phe (i+1) Amide NH	6.63	7.20	+0.57
11	**10 (controle)**	Carbamate NH	5.18	5.75	+0.57

*Δ: difference between column 5 and 4, #: Exact chemical shift change with the addition of DMSO-d6 is not determined due to overlap of signal with aromatic region but found that it is showing upfield shifting.

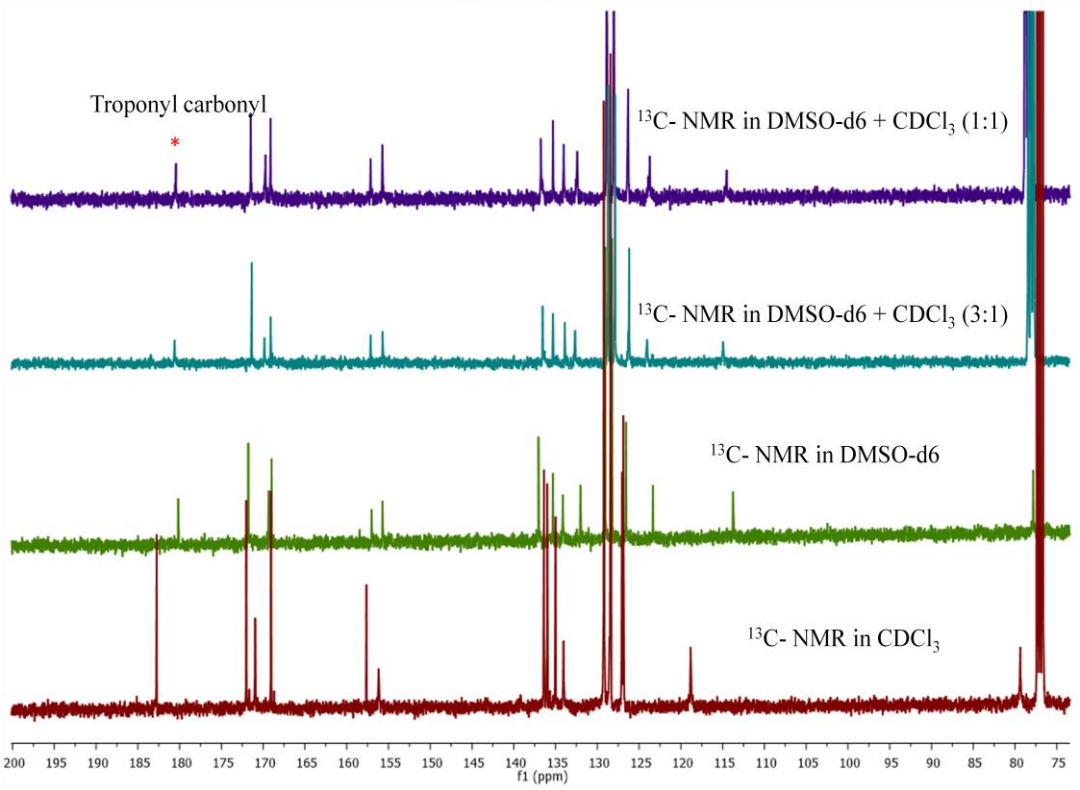
35. Proposed Hydrogen bonding in peptides



36. ^{13}C-NMR of tripeptide (11) in $CDCl_3$ and DMSO-d6 solvent pairs:



Expanded region of above given spectra:



37. ^{13}C-NMR and ^{1}H-NMR of monomer (5) and peptide (7/8/9/10/11):

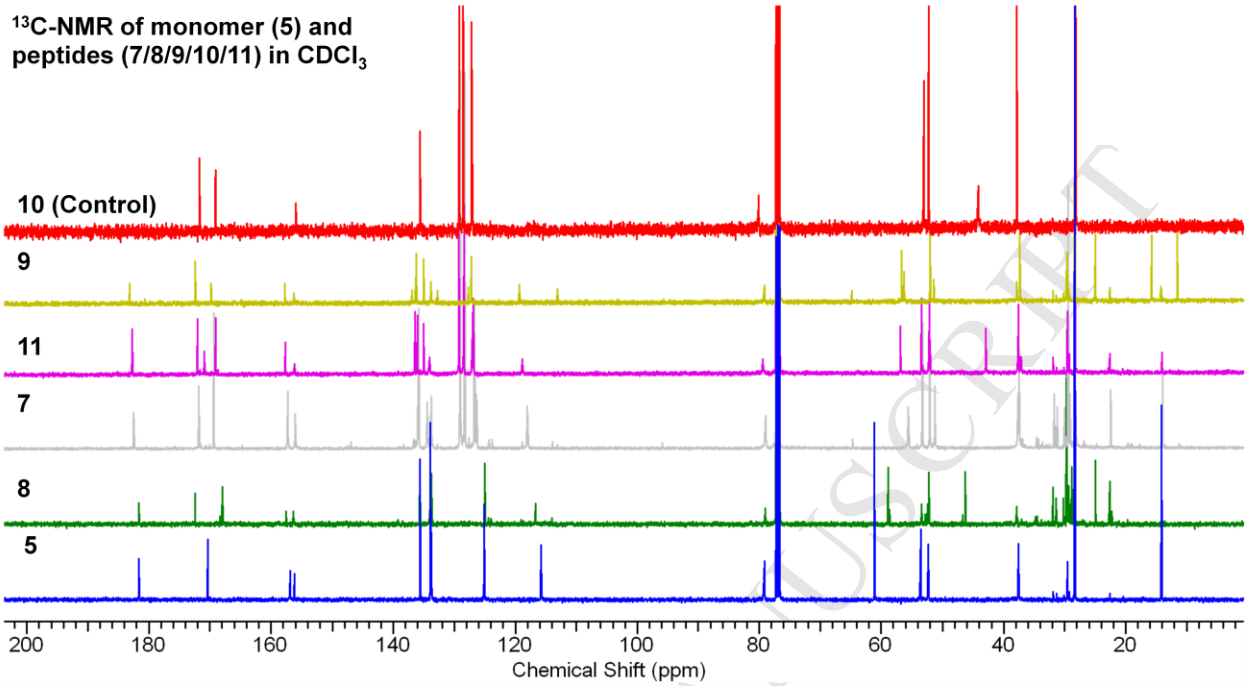


Figure S15: Stacked ^{13}C-NMR of *Tr-aeg* monomer and peptides (5/7/8/9/10/11), Which is showing down field shifting of tropone carbonyl.

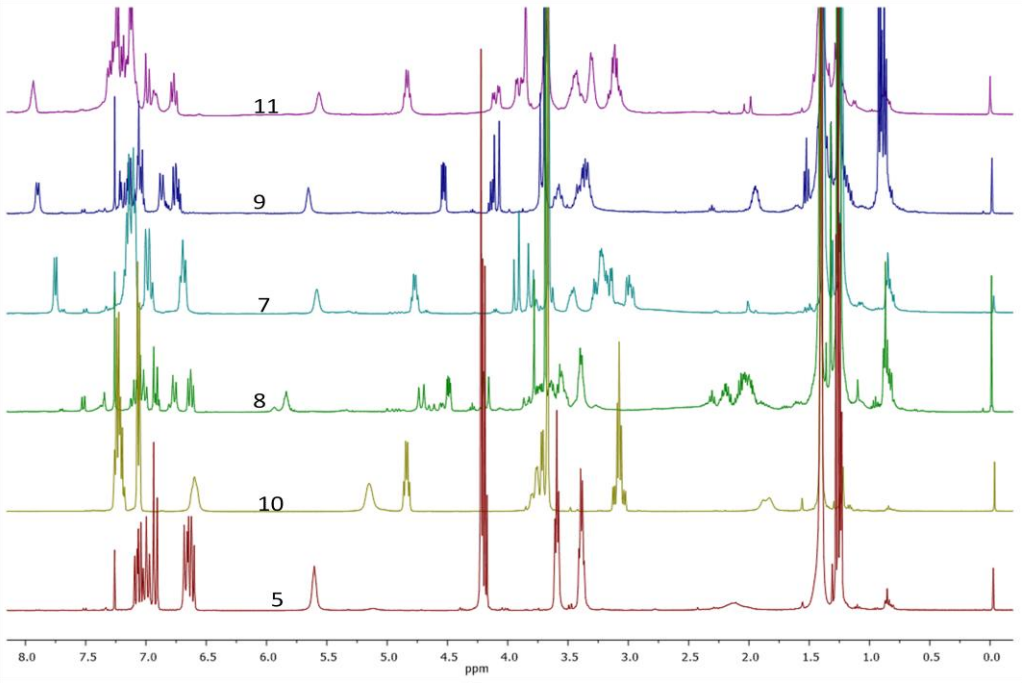


Figure S16: Stacked ^{1}H-NMR of *Tr-aeg* monomer and peptides (5/7/8/9/10/11) in $CDCl_3$, Which is showing down field shifting of i+1 amide NH with respect to control (10) amide NH.

38. Crystal data of monomer 5:

Table S12. Crystal data and structure refinement for agtr : (CCDC file No:975954)

Identification code	agtr
Empirical formula	$C_{18}H_{26}N_2O_5$
Formula weight	350.41
Temperature	296(2) K
Wavelength	0.71073 A
Crystal system,	Monoclinic
space group	P2(1)/c
Unit cell dimensions	a = 11.1125(4)Å $\alpha = 90^0$.
	b = 15.3001(7)Å $\beta = 112.346^0$.
	c = 12.2394(5) Å $\gamma = 90^0$.
Volume	1924.70(14) $Å^3$
Z	4
Calculated density	1.209 Mg/m^3
Absorption coefficient	0.088 mm^{-1}
F(000)	752
Crystal size	0.06 x 0.041 x 0.032 mm
Theta range for data collection	2.49 to 26.77^0.
Limiting indices	-14<=h<=14, -19<=k<=19, -15<=l<=14
Reflections collected / unique	25584 / 4071 [R(int) = 0.0609]
Completeness to theta	26.77 99.2 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7454 and 0.6864
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4071 / 0 / 230
Goodness-of-fit on F^2	1.013
Final R indices [I>2sigma(I)]	R1 = 0.0484, wR2 = 0.1217
R indices (all data)	R1 = 0.0990, wR2 = 0.1457
Largest diff. peak and hole	0.247 and -0.199 $e.Å^{-3}$

Table S14: Hydrogen bonds for agtr**:**

Donor --- H....Acceptor	[ARU]	D - H	H...A	D...A	D - H...A
N(2) --H(2) ..O(4)	[4554.01]	0.86	2.30	2.981 (2)	136

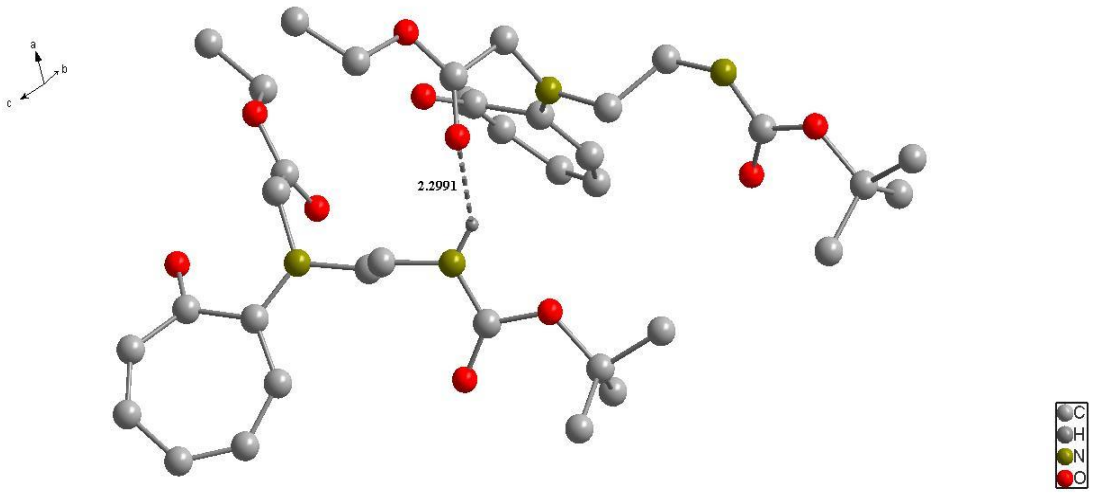
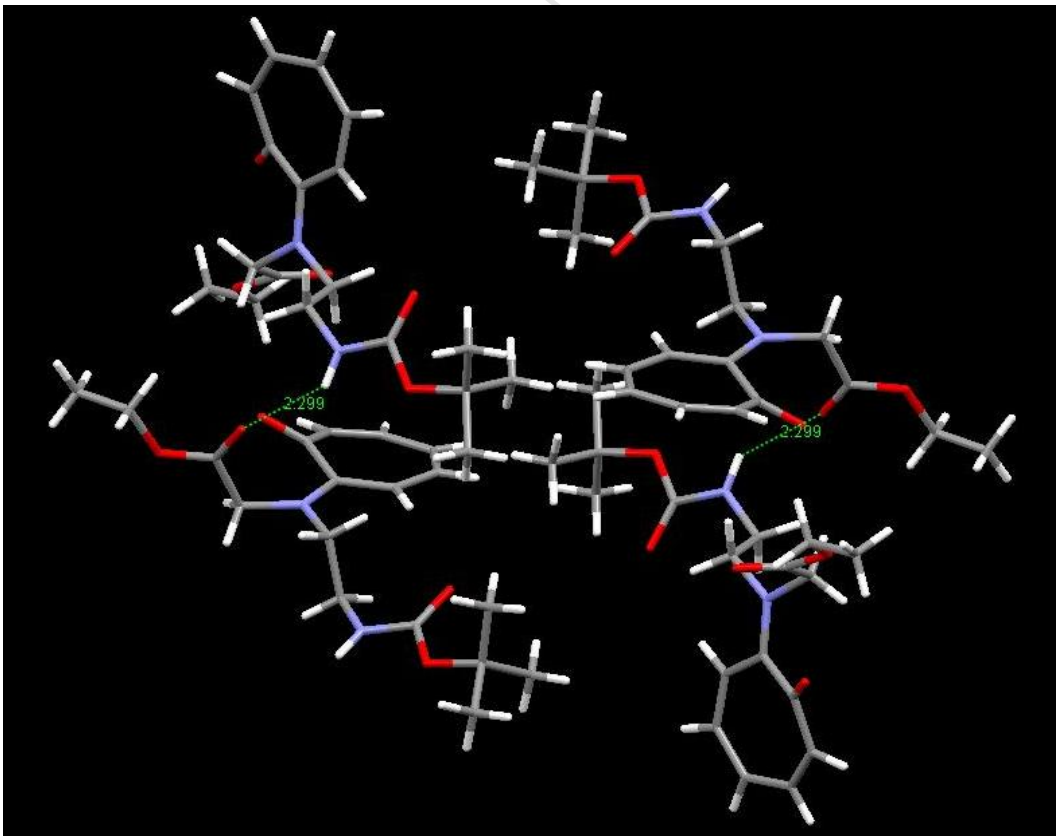
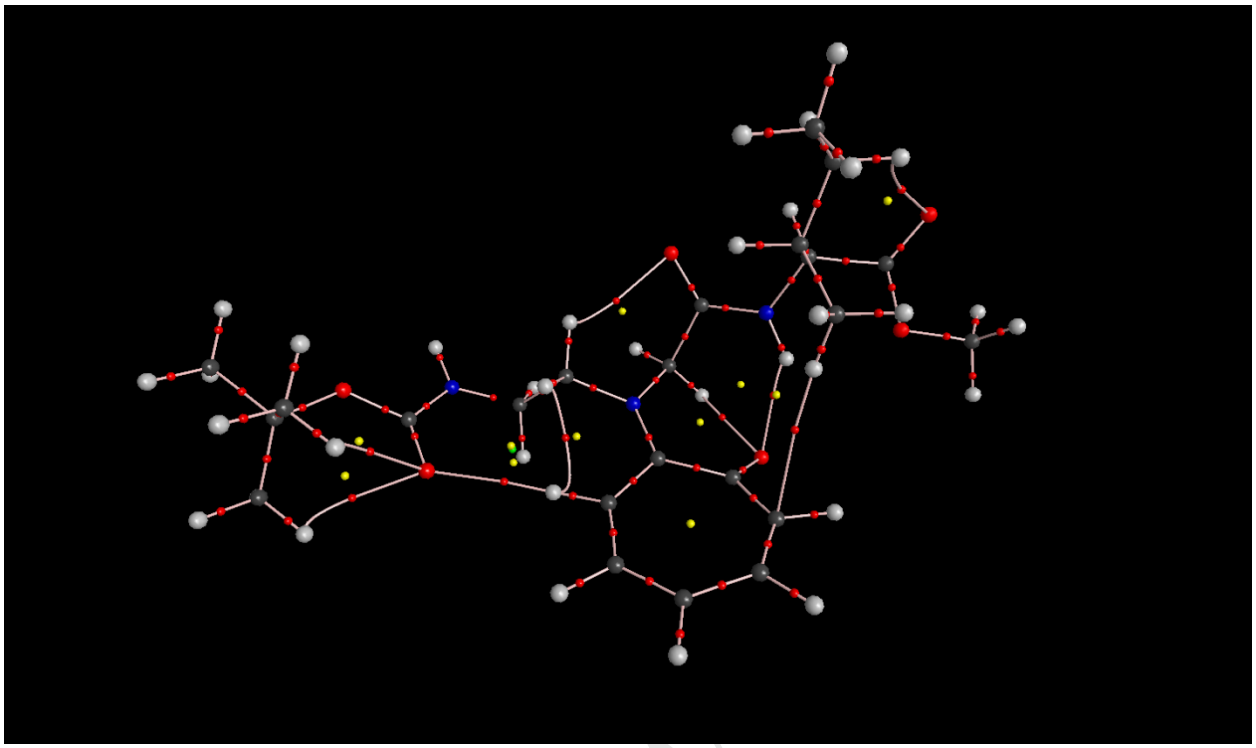


Figure S17: A molecular Packing diagram of *Tr-aeg* monomer. All Hydrogen atoms are omitted for clarity. One intermolecular hydrogen bond is shown by dashed line.



39. DFT calculated density map



40. Theoretical Ramachandran Plot