Controlling Glycocalyx Remodelling: Structural and Biological Functions of Polyamide Based Proteoglycan Mimetics

A thesis submitted towards partial fulfilment of the requirement of

BS-MS Dual Degree Program

By

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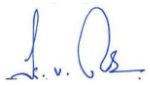
CERTIFICATE

This is to certify that this dissertation entitled "***Controlling Glycocalyx Remodelling: Structural and Biological Functions of Polyamide Based Proteoglycan Mimetics*"** towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents work carried out by Haritha A S at Indian Institute of Science Education and Research under the supervision of **Dr. Raghavendra Kikkeri**, Associate Professor, Department of Chemistry, during the academic year 2019-2020.

Dr. Raghavendra Kikkeri



Committee:

Dr. Raghavendra Kikkeri

Prof. Ramakrishna G Bhat

This thesis is dedicated to family & friends

DECLARATION

I hereby declare that the matter embodied in the report entitle "***Controlling Glycocalyx Remodelling: Structural and Biological Functions of Polyamide Based Proteoglycan Mimetics***" are the results of the work carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research, Pune, under the supervision of **Dr. Raghavendra Kikkeri** and the same has not been submitted elsewhere for any other degree

Haritha A S

Date: 29-03-2020

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ABBREVIATIONS

NMR	Nuclear Magnetic Resonance
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
Hz	Hertz
δ	Chemical Shift
ppm	Parts per million
$(Boc)_2O$	Di-tert-butyl bicarbonate
Et_3N	Triethylamine
DCM	Dichloromethane
RT	Room temperature
h	Hours
DIAD	Diisopropyl azodicarboxylate
PPh_3	Triphenyl Phosphine
H_2	Hydrogen
$Pd(OH)_2/C$	Palladium hydroxide on Charcoal
MeOH	Methanol
Cbz-Cl	Benzyl chloroformate
$NaHCO_3$	Sodium bicarbonate
THF	Tetrahydrofuran
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
HOBt	Hydroxybenzotriazole
DMF	Dimethyl formamide
TFA	Trifluoro acetic acid
ACN	Acetonitrile
Fmoc-Cl	Fluorenylmethyloxycarbonyl chloride
PFP	Pentafluorophenol
DIC	N,N′-Diisopropylcarbodiimide
DMAPN	3-(dimethylamino)propionitrile
NaH	Sodium hydride

NaN_3	Sodium azide
LiOH	Lithium hydroxide
NMP	N-Methyl-2-pyrrolidone
HBTU	N,N,N',N'-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate.
TIPS	Triisopropyl silane
PhOH	Phenol
HCl	Hydrochloric acid
ml	Milli litre
J	Coupling constant
EtOAc	Ethyl acetate
eq	Equivalents
mol	mole
mmol	Milli mole
H_2O	Water
g	Gram
µl	Micro litre
TLC	Thin Layer Chromatography
DEPT	Distortionless enhancement by polarization transfer
HRMS	High resolution mass spectrometry

ABSTRACT

The cell surface glycans serve as recognition elements for many biological events, ranging from cell-cell to cell-pathogen interaction, immune cells activation to organ development. Hence, cell surface engineering is a stimulating biomedical and biotechnology research. Herein we report a versatile approach to mimic cell surface glycans on a peptide backbone. Our method is based on solid-state peptide synthesis of alternative conjugation of *N*-*O*-methyloxyamine composed amino acid and pegylated spacer to form polypeptide chain with the well-defined sequence. By incorporating fluorescent probe and phospholipids at one of the peptide chains, a functional cell-surface ligand was developed. These ligands are treated with carbohydrate scaffolds to obtain desire multivalent glycolipids. By varying the length of polypeptide chain, a comprehensive library of cell-surface anchoring glycoprobes was developed. We hypothesized that the balance between hydrophilic/hydrophobic moieties in the glycoprobes regulates the residence time of the molecules on cell surface. Using specific sugar scaffold on these glycoprobes, we proposed to demonstrated epithelial to mesenchymal transitions (EMTs) and its potential applications in wound healing.

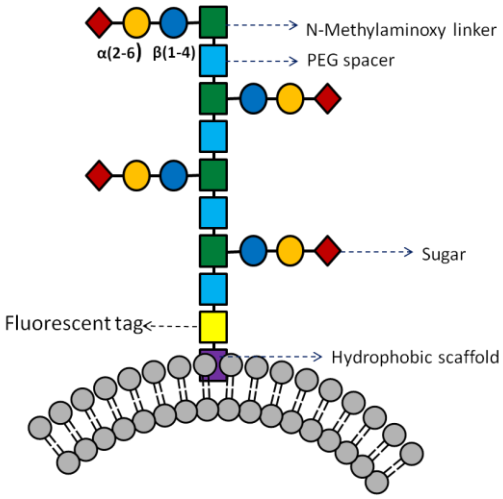


Figure 1: Schematic representation of proposed glycopeptides

INTRODUCTION

Cell surface glycoproteins and glycolipids are poised to mediate a wide range of biological events, including attachment of viruses and bacteria infection, immunomudulation and embryogenesis. Consequently, understanding the structure-function relationship of cell surface glycans will provide essential insights into cellular activities. However, the heterogeneous and complexity of cell surface glycans restricted molecular level details of glycans functions. Tailoring the cell membrane with specific glycan may enable a better understanding of carbohydrate epitope-based cell signalling and functions.

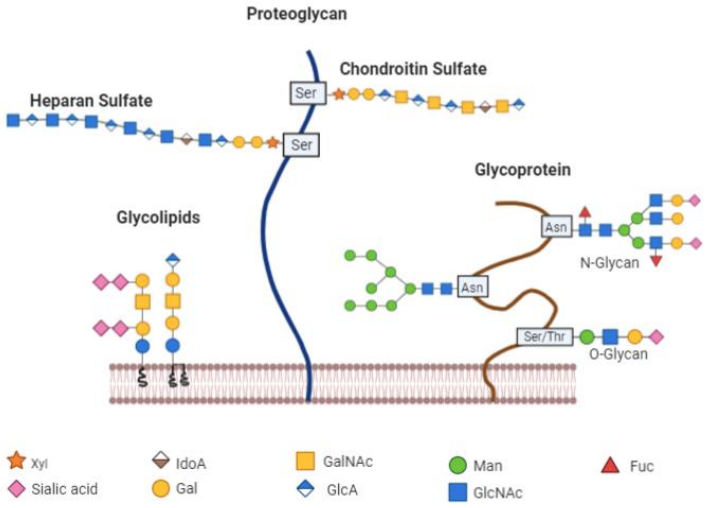


Figure 2: Major Glycan classes in the cell surface. Schematic representation of different sugar expression patterns such as glycoprotein, glycolipid and proteoglycans on the cell surface.

Many chemical modifications methods have been reported for cell surface engineering. These methods utilize either biosynthetic pathway of sialic acid or via lipid-based anchoring of glycans on plasma membrane[4, 6, 7, 9]. Among them, the lipid-based method is advantageous for generating multivalent carbohydrate probes, as they amplify the weak carbohydrate-protein interaction on cell-surface and enhance the potency of cellular responses. Using such strategy, extracellular mucin, proteoglycan structures were mimicked and expressed on the cell surface to modulate cell signalling. However, recent studies of the multivalent carbohydrate-protein interactions demonstrated that the special arrangement of carbohydrate units and the orientation of ligands on cell-surface are crucial to improve the efficacy of

carbohydrate mediated biological responses. Hence, there is a great deal of interest in developing a new architecture for cell-surface carbohydrate engineering for effective control over cellular events

With the goal of creating a new cell surface engineering molecules, we present glycopeptides based cell surface anchoring molecules that is structurally well-defined, synthetically facile and also accommodate carbohydrate ligands at different special arrangement. By incorporating fluorescent tag and lipid substrate at one of the polypeptide chains, these glycoprobes were anchored on cell surface and visualized. It is expected that the special arrangement and orientation of carbohydrate ligands in these glycoprobes synergistically influence specific carbohydrate-protein interaction and activate specific cellular events. Two different series of sialic acid conjugated glycolipids were produced. One groups of molecule contain Neu5Acα(2-6)Galβ(1-4)glc modified glycolipids (GL-26-4). The second group of molecule consists of Neu5Acα(2-3)Galβ(1-4)glc bearing glycolipids (GL-23-4) respectively. The fluorescent nature of glycolipid was employed to visualize the cell surface engineering, retention time and finally epithelial to Mesenchymal transition

EMT is a fundamental, reversible and continuous process by which epithelial cells lose their closely packed brick structural properties and become more motile and visa versa[20]. EMT plays an essential role in pathological and physiological processes in our body, including wound healing, tissue regeneration, cancer cell metastasis[11,12]. EMT can be characterized by the upregulation of N-cadherin, vimetin and α-smooth muscle actin or downregulation of epithelial cell adhesion receptors for example E-cadherins[10]. EMT process regulated by a wide range of physical and biological cues. Among them, transforming growth factor-beta (TGF-β) is one of the primary inducers of EMT[5]. TGF-β signalling can occur through canonical SMAD signalling pathways as well as non-canonical pathways, including the MAP kinase, JNK and PI3K pathways[2,15]. In canonical SMAD signalling, TGF-β binds to the TGF-β receptor I via sialylated ganglosides (GM3, GM2) and form complex with TGF-β receptor II transphosphorylates. This leads to phosphorylation of serine residues in ``cytoplasmic R-Smads, Smad2 and Smad 3 and activates EMT. This suggests that level of sialylated gangliosides on cell surfaces control EMT transitions. Herein, we report cell-surface engineering with a new set of glycolipids that target GM3-mediated TGF-β activation to induce EMT transitions.

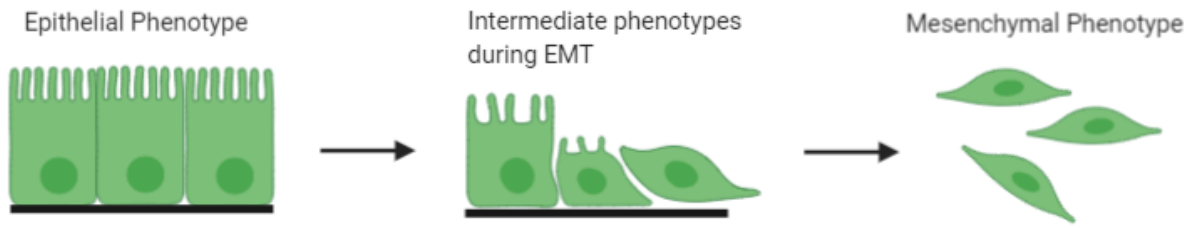


Figure 3: Progression from normal epithelium to invasive mesenchymal carcinoma. Schematic representation of EMT transition- polarized epithelial cells undergo changes in phenotype into mobile mesenchymal cells.

The main objective of this work was to mimic the proteoglycans with defined number of sugar to alter the cellular events, particularly TGF mediated EMT pathway.

The particular aims are:

1. Design and synthesis of glycopolymers for cell surface engineering
2. Evaluate the role of these scaffolds on EMT pathway

RESULTS AND DISCUSSION

Design of Peptide 1

Our strategy focused on the design and synthesis of well-defined glycopeptides carrying fluorescent probe, sugar units and hydrophobic moieties to alter cellular functions. To simulate the proteoglycans, we first synthesized *N*-Methylaminoxy linker with aspartic acid amino acid backbone, PEG spacer, pyrene-based fluorescent tag, and cholesterol-based hydrophobic scaffold. Then these building blocks were used assembled to synthesize protein **1** using Solid phase peptide synthesis methods[16].

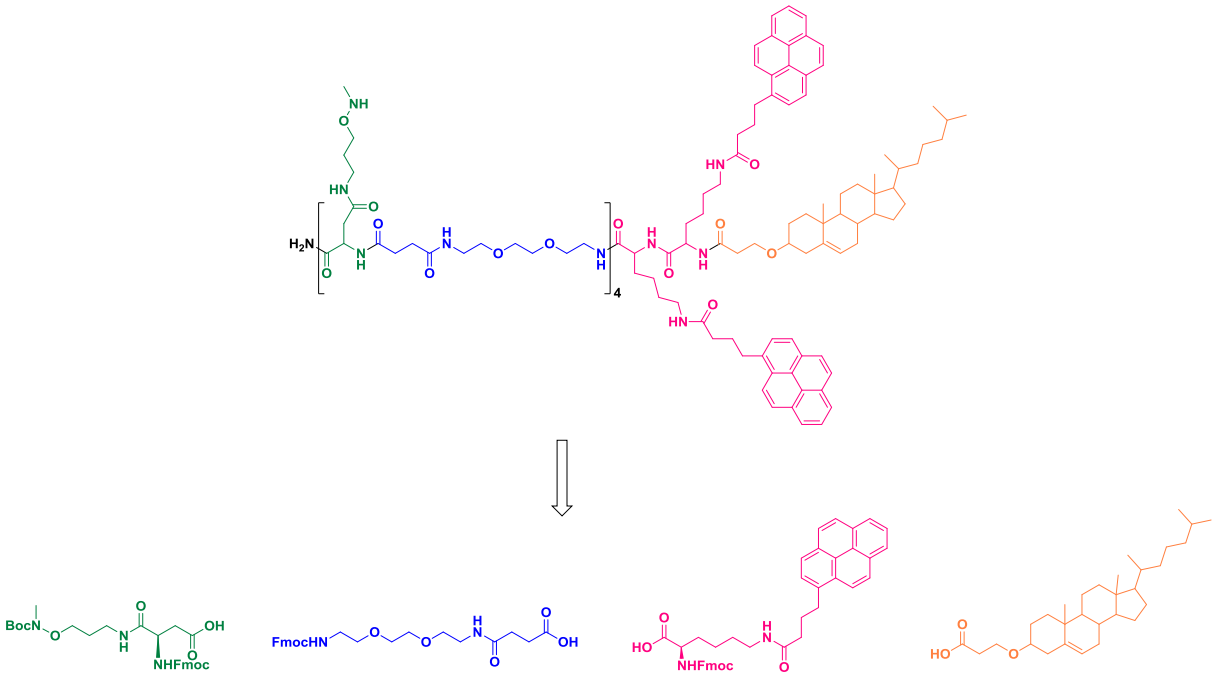
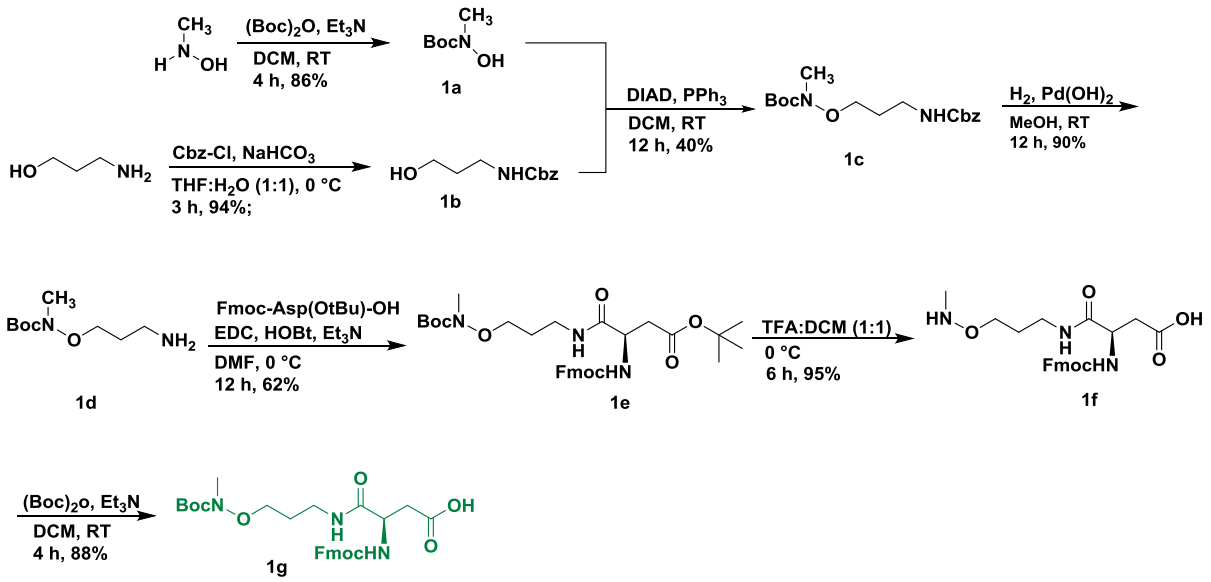


Figure 4: Target molecules for peptide 1

. We have employed several elegant and high yielding synthetic strategies for the synthesis of building blocks[1, 3, 16, 17, 22]. Techniques like NMR (^{1}H, ^{13} C, DEPT), MALDI-TOF and HRMS were used for the characterization of compounds.

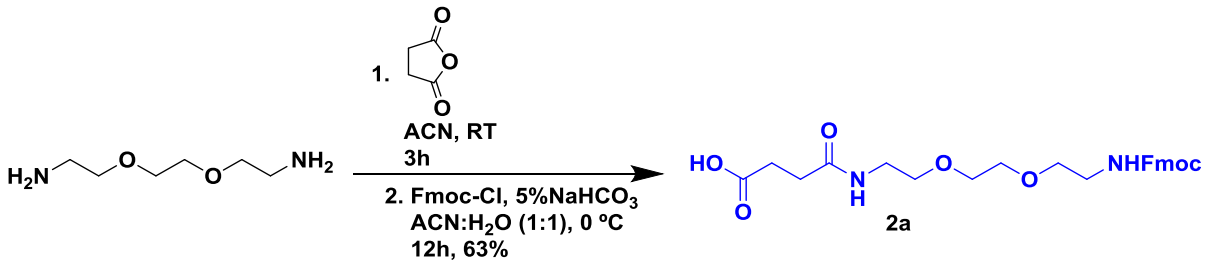
Synthesis of *N*-Methylaminoxy Linker



Scheme 1: Synthesis of L-aspartic acid linker

The aspartic acid linker **1g** was synthesized from the commercially available *N*-Methyl hydroxyl amine in 7 steps. In the first step, *N*- methyl hydroxyl amine was protected with Boc group using $(Boc)_2O$ and triethylamine in DCM at 0°C to get **1a** as brown liquid. 3- Aminopropanol was protected using Cbz group using Cbz-Cl in THF-water (1:1) mixture in the presence of $NaHCO_3$ at 0°C to yield **1b** as white powder. The **1a** was subjected to Mitsunobu reaction in the presence of **1b**, DIAD and triphenyl phosphine yielded 40% of desired linker compound **1d**. Finally, deprotection of Cbz group in the presence of $Pd(OH)_2/C$ in MeOH followed by peptide coupling reaction with Fmoc-Asp(OtBu)-OH yielded 62% compound **1e**. After that deprotection of tert-butyl group on **1e** was carried out, this led to Boc deprotection to yield compound 1f. Finally, without purification **1f** was taken in further step for Boc protection of amine using $(Boc)_2O$ to get the final building block **1g** with 88% yield (**Scheme 1**)

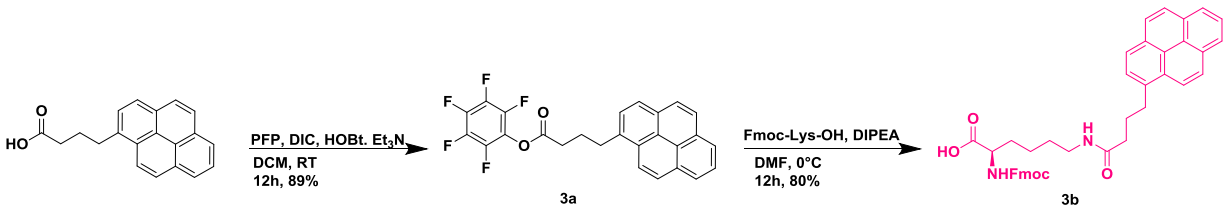
Synthesis of PEG Spacer



Scheme 2: Synthesis of PEG spacer

Commercially available 2, 2'-(ethylenedioxy)bisethylamine was used to prepare the required building block **2a** in 2 steps. 2, 2'-(ethylenedioxy)bisethylamine was dissolved in ACN and succinic anhydride was added. Waxy substance was formed in the organic solvent after 3h. After decanting the organic layer the waxy substance was taken for the next step without purification. Fmoc protection of amine in ACN-H_2O (1:1) in the presence of 5%$NaHCO_3$ and Fmoc-Cl to get the desired compound **2a** with 63% yield (**Scheme 2**).

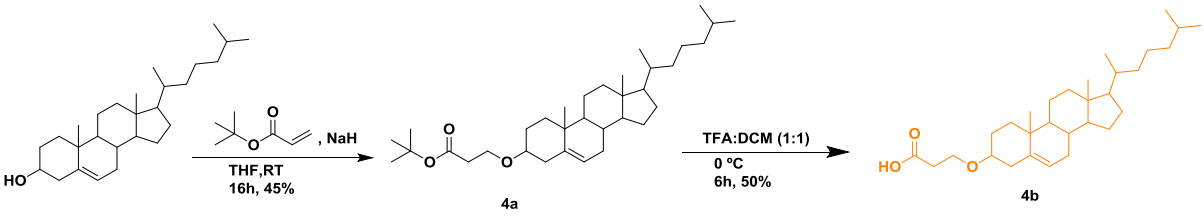
Synthesis of Pyrene Based Fluorophore



Scheme 3: Synthesis of pyrene based fluorophore

Commercially available Pyrene-1-butyric acid was used to prepare the required fluorophore **3b.** Active ester **3a** was synthesized using pentafluorophenol using DIC and HOBt as coupling agents in the presence of triethylamine in DCM. Then amide coupling with Fmoc-Lys-OH was done in the presence of DIPEA to yield compound **3b** as yellow solid (**Scheme 3**)

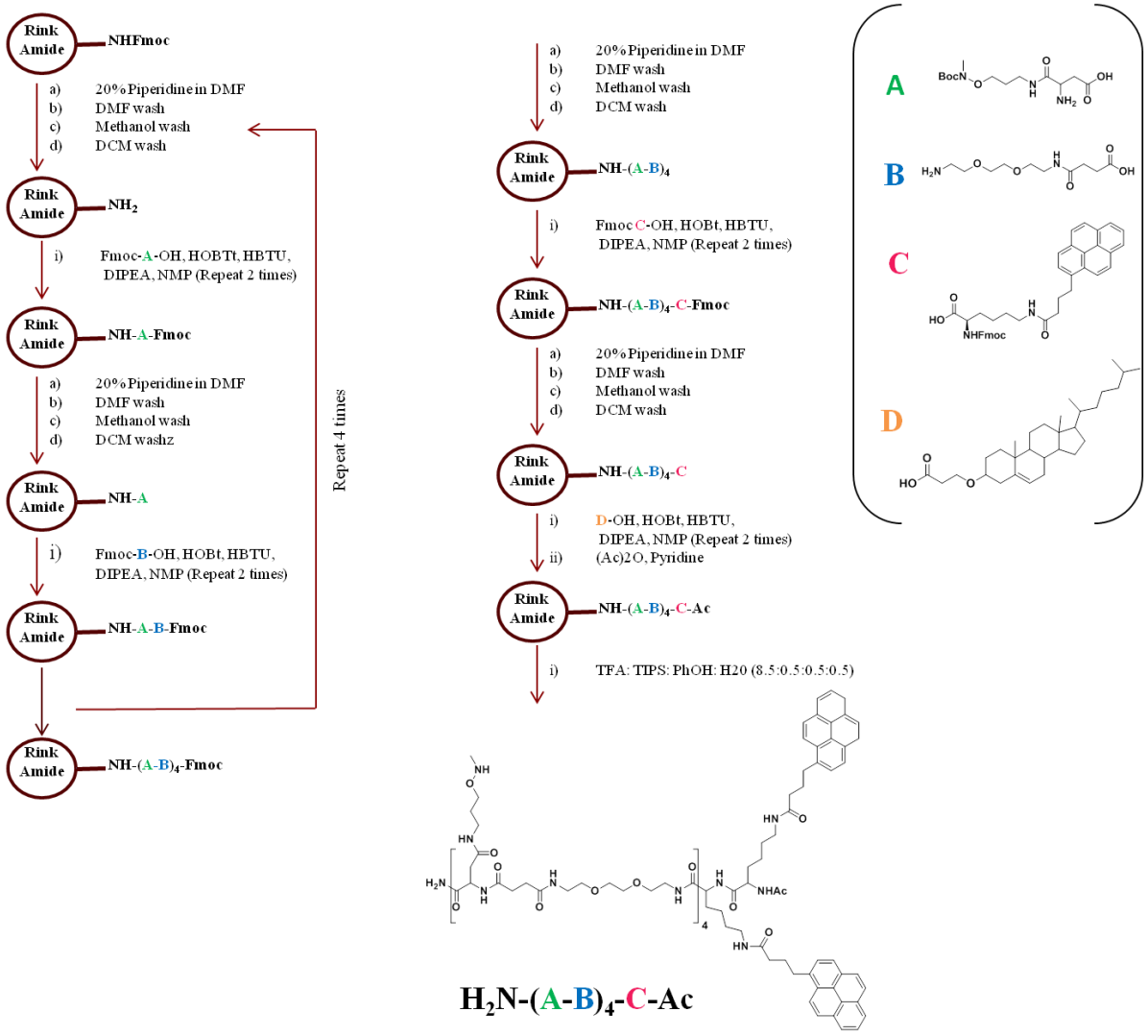
Synthesis of Cholesterol Building Block



Scheme 4: Synthesis of cholesterol building block

Cholesterol building block **4b** was synthesized from commercially available cholesterol. Cholesterol was dissolved in THF and 1,4 addition was carried out in the presence of NaH with tert-butyl acrylate to get **4a**. Then tert-butyl group was deprotected using 50% TFA-DCM (1:1) mixture to yield desired compound **4b** as white powder (**Scheme 4**)

Synthesis of Peptide 1



Scheme 5: Synthesis of peptide 1

Peptide **1** was synthesized using **1g**, **2a**, **3b** and **4b** as the building block by solid phase peptide method. Resin was deprotected using 20% piperidine in DMF and repeated again for complete deprotection. Coupling of **1g** to the resin was done twice using HOBt, HBTU and TEA in NMP to avoid the formation of mixtures of peptides. Fmoc was deprotected using 20% piperidine in DMF and repeated twice. The same procedure was followed for coupling of all monomers. But the coupling of last monomer **4b** was failed. This could be because of the steric hindrance of two bulky pyrene moieties in the peptide. The peptide cleavage from the resin was done using TFA: TIPS: PhOH: H_2O (98.5:0.5:0.5:0.5) mixture. The final product after purification using HPLC was obtained as white powder

Peptide 2

During synthesis of peptide **1** cholesterol based hydrophobic scaffold did not conjugate to the growing peptide chain. Possible reason for the failed conjugation can be the presence of bulky pyrene group that can mask the coupling by steric hindrance. Peptide 1 shows very less fluorescence intensity because of low quantum yield of pyrene. Pyrene has emission in the blue region which will merge with staining agents like DAPI and will make the cellular imaging difficult.

So, we changed our strategy by changing fluorescent tag with good quantum yield and greater chemical stability and hydrophobic scaffold with long hydrocarbon chain.

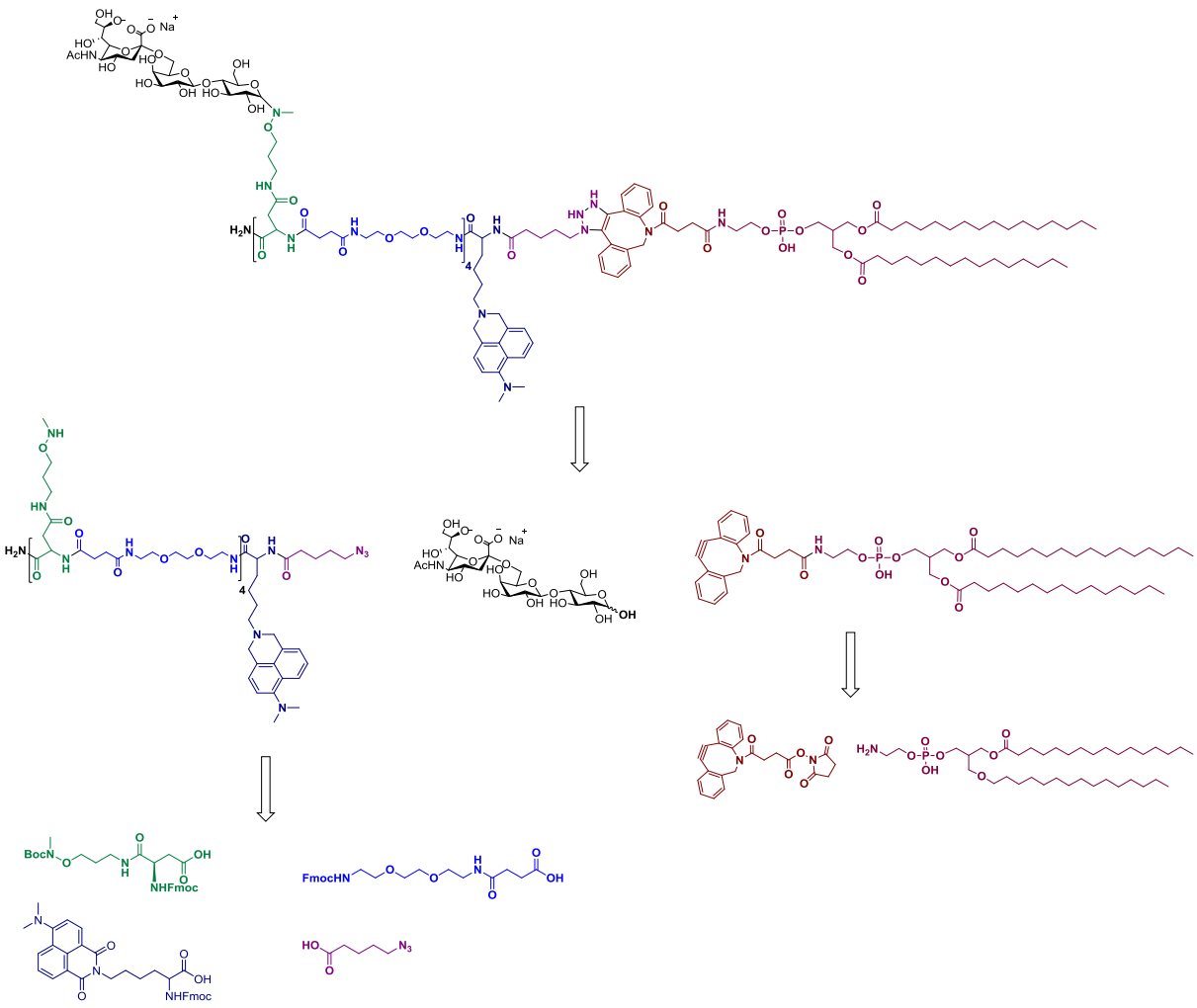
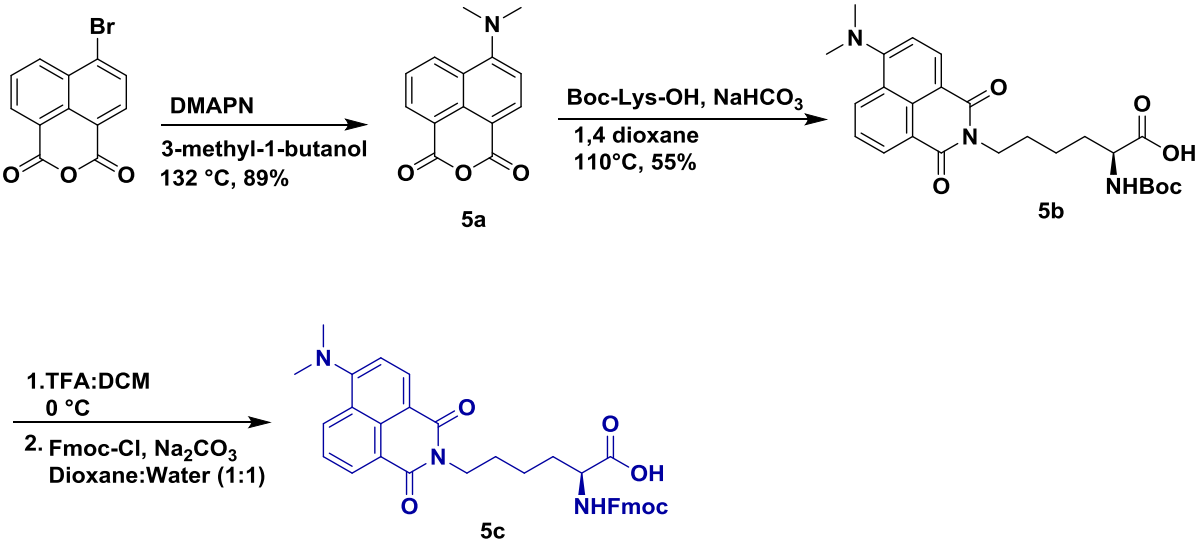


Figure 5: Target molecules for peptide 2

We synthesized N-Methylaminoxy linker with Aspartic acid amino acid backbone, PEG spacer, Aminonaphthyl-type fluorescent tag, and long hydrocarbon chain hydrophobic scaffold[1,3,8,14,16,17,19,22]. Aminonaphthyl-type fluorescent tag shows better chemical stability and quantum yield compared to pyrene based fluorescent tag.

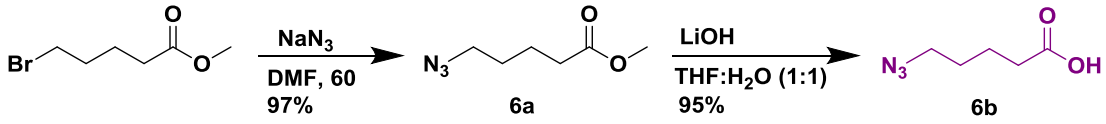
Synthesis of Aminonaphthyl Type Fluorophore



Scheme 6: Synthesis of naphthyl fluorophore

Aminonaphthy type fluorescent tag **5c** was synthesized from commercially available 4-Bromo-1,8- `naphthalic anhydride. 4-Bromo-1,8- `naphthalic anhydride was dissolved in 3-mathyl 1-butanol and refluxed at 132ºC .Then 3- dimethylamino-propionitrile was added and stirred for 12h to yield compound **5a** as orange crystal. Then Boc-Lys-OH in the presence of $NaHCO_3$ in 1,4 dioxane as the solvent reacted with compound **4a** to yields compound **5b**. Boc group was deprotected in the presence of TFA-DCM (1:4) mixture at 0ºC. Then Fmoc peotection was done using Fmoc-Cl in the presence of Na_2CO_3 in dioxane-water (1:1) mixture to yield compound **5c** as orange crystal (**Scheme 6**)

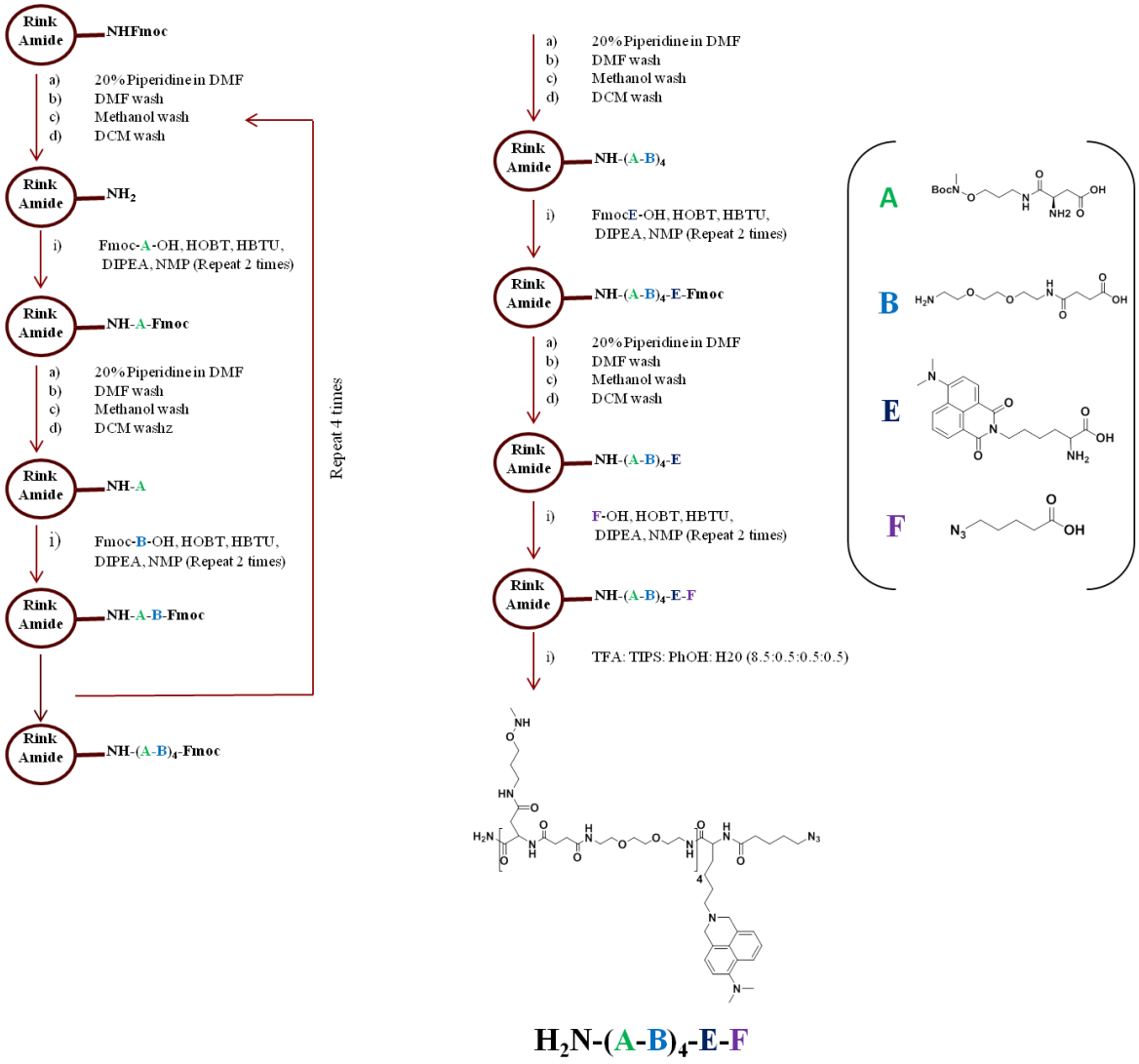
Synthesis of Azide linker



Scheme 7 : Synthesis of azide linker

The azide linker was synthesized from commercially available 5-methyl bromovalerate as the starting material. 5-methyl bromo valerate was dissolved in DMF and refluxed at 60 ºC in the presence of sodium azide to yield compound **6a.** Methyl was deprotected in presence of LiOH in THF-water (1:1) mixture to yield **6b** as colourless liquid (**Scheme 7**).

Synthesis of Peptide 2a



Scheme 8 : Synthesis of peptide 2a

Peptide 2a was synthesized using **1g**, **2a**, **4c** and **6b** as the building block by solidphase peptide method. Resin was deprotected using 20% pipeidine in DMF and repeated again for complete deprotection. Coupling of **1g** to the resin was done twice using HOBt, HBTU and TEA in NMP to avoid the formation of mixture. Fmoc was deprotected using 20% piperidine in DMF and repeated twice. The same procedure was followed for coupling of all monomers. The peptide cleavage from the

resin was done using TFA: TIPS: PhOH: H_2O (98.5:0.5:0.5:0.5) mixture. The final product after purification was obtained as orange powder (**Scheme 8**)

CONCLUSIONS

In summary, we have synthesized polypeptides scaffold composed of *N*-*O*-methyloxyamine, PEG spacer, fluorescent tag and lipid compositions. Using simple glycosylation strategy, we can incorporate different carbohydrate residue on these scaffolds and express them on cell surfaces. Currently, we are in the process of sialic acid trisaccharide conjugation and express them on cell surface to monitor the residence time. Finally, these compounds will be utilized to alter GM2/TGF-beta mediated EMT transitions.

EXPERIMENTAL PROCEDURE

Compound 1a

N-Methyl hydroxylamine (5.00 g, 59.84 mmol) was dissolved in dry DCM (300 mL). To this solution $(Boc)_2O$ (10.22 mL, 47.84 mmol) was added, then treated with triethylamine (8.23 mL, 59.81 mmol). The mixture was stirred for 4 h under inert atmosphere at room temperature. The crude product was washed with saturated $NaHCO_3$ solution (x3) and brine solution (x3). Organic phase was dried over anhydrous Na_2SO_4. The residue was purified by column chromatography (1:9 EtoAc-Hexane) gave compound **1a** as a brown liquid (7.08 g, 86%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.65 (s, 1H), 3.14 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 157.81, 81.88, 38.05, 28.41. HRMS (ESI) m/z calculated for $C_6H_{13}NO_3$: 147.0895; found: 148.0973.

Compound 1b

Propanol amine (5.00 g, 66.53 mmol) was dissolved methanol-water (100 mL, 9:1 v/v) and cooled to 0 ºC. Benzylchloroformate (13.42g, 79.12 mmol) and $NaHCO_3$ (13.52 g, 0.16 mmol) were added in to this solution. Then this solution was stirred for 3 h. Methanol was evaporated under reduced pressure. The crude product was dissolved in EtOAc and washed with brine solution (x3). Organic phase was dried over anhydrous Na_2SO_4. The residue was purified by column chromatography (1:1 EtOAc-Hexane) gave compound **1b** as a white powder (13.93 g, 94%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37 – 7.27 (m, 5H), 5.39 (s, 1H), 5.07 (s, 2H), 3.63 (q, J = 5.6 Hz, 2H), 3.29 (dd, J = 11.8, 5.8 Hz, 2H), 1.69– 1.63 (m, 2H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 157.35, 136.39, 128.54, 128.15, 128.07, 66.79, 59.57, 37.86, 32.42. HRMS (ESI) m/z calculated for $C_{11}H_{15}NO_3$: 209.1052; found: 210.1045.

Compound 1c

Compound **1a** (7.00 g, 47.51 mmol) was dissolved in anhydrous DCM (600 mL) cooled to 0 ºC. Triphenyl phosphine (14.96 g, 57.07 mmol) was added and stirred for 5 mins. Then DIAD (10.93 ml, 55.20 mmol) was added drop wise. Finally compound **1b** (9.90 g, 47.51 mmol) was dissolved in DCM (200 mL) and added to the reaction mixture drop wise. The reaction mixture was warmed to room temperature and stirred for 12 h. The residue was purified by column chromatography (2:8 EtOAc-Hexane) gave compound **1c** as a white solid (6.4 g, 40%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.37 – 7.29 (m, 5H), 5.66 (s, 1H), 5.10 (s, 2H), 3.90 (t, J = 5.7 Hz, 2H), 3.36 (q, J = 6.1 Hz, 2H), 3.08 (s, 3H), 1.79 (dt, J = 11.8, 6.0 Hz, 2H), 1.47 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 156.93, 156.68, 136.92, 128.55, 128.02, 81.70, 72.27, 66.51, 38.76, 36.53, 28.37, 28.14. HRMS (ESI) m/z calculated for $C_{17}H_{26}N_2O_5Na$: 361.1739; found: 361.1741 $[M + Na]^+$.

Compound 1d

Compound **1c** (6.40 g, 18.92 mmol) was dissolved in anhydrous methanol (40 mL). Hydrogenolysis was performed using $Pd(OH)_2$ on carbon (2.0 g) and H_2 gas for 24 h. After completion of reaction solution was filtered and concentrated under reduced pressure. The product was purified by column chromatography (3:7 EtOAc-Hexane) gave compound **1d** as a white solid (3.47 g, 90%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.01 (t, J = 5.2 Hz, 2H), 3.25 (t, J = 5.7 Hz, 2H), 3.09 (s, 3H), 2.11 – 2.06 (m, 2H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 157.86, 83.21, 73.02, 39.25, 36.75, 28.35, 24.91 . HRMS (ESI) m/z calculated for $C_9H_{20}N_2O_3$: 204.1474; found: 205.1552 $[M + H]^+$.

C**ompound 1e**

Fmoc-Asp(OtBu)-OH (7.24 g, 17.62 mmol) was dissolved in DMF (200 mL) and cooled to 0 ºC. EDC (3.7 g, 19.41 mmol) and HOBt (0.22 g, 1.6 mmol) were added and stirred for 10 mins. To this stirring solution compound **1d** (3.3 g, 16.02 mmol) was added dropwise and then triethylamine was added to adjust the pH to 8 . The

reaction mixture was warmed to room temperature and stirred for 12 h. After completion of reaction solvent was concentrated under reduced pressure. The residue was dissolved in EtOAc and extracted with brine(x3) and dried over Na_2SO_4. The crude was purified by column chromatography (6:4 EtOAc-Hexane) gave compound **1e**(6 g, 62%) as a yellow liquid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.75 (d, J = 7.5 Hz, 2H), 7.59 – 7.57 (m, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.31 – 7.28 (m, 2H), 6.09 (d, J = 8.5 Hz, 1H), 4.55 (dd, J = 13.8, 5.5 Hz, 1H), 4.42 (dd, J = 10.5, 7.3 Hz, 1H), 4.35 – 4.31 (m, 1H), 4.22 (t, J = 7.1 Hz, 1H), 3.90 (t, J = 5.6 Hz, 2H), 3.09 (s, 3H), 2.87 (dd, J = 16.6, 5.4 Hz, 1H), 2.70 (dd, J = 16.5, 5.6 Hz, 1H), 1.78 (dd, J = 11.4, 5.7 Hz, 2H), 1.47 (s, 9H), 1.43 (s, 9H).^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.78, 170.59, 157.05, 156.08, 143.93, 141.40, 127.84, 127.19, 125.33, 120.11, 81.92, 81.60, 71.95, 67.26, 51.64, 47.28, 38.08, 37.17, 36.65, 28.44, 28.17, 27.34. HRMS m/z calculated for $C_{32}H_{43}N_3O_8$: 597.3050; found: 598.3128 $[M + H]^+$.

Compound 1f

Compound **1e** (6.00 g, 10.05 mmol) was dissolved in TFA:DCM (100mL, 1:1 v/v) for 6h. The residue was purified by column chromatography (1:9 DCM-Methanol) gave compound **1f** (4.23 g, 95%) as colourless liquid. ^{1}H NMR (400 MHz, CD3OD) δ 7.72 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 2H), 7.25 (td, *J* = 7.5, 1.2 Hz, 2H), 4.42 – 4.34 (m, 2H), 4.26 (dd, *J* = 10.4, 7.0 Hz, 1H), 4.15 (t, *J* = 6.8 Hz, 1H), 3.88 (t, *J* = 6.0 Hz, 2H), 3.22 (d, *J* = 6.5 Hz, 2H), 2.77 (s, 3H), 2.75 – 2.62 (m, 2H), 1.78 – 1.71 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 174.03, 173.86, 158.36, 145.17, 142.58, 128.83, 128.16, 126.25, 120.95, 72.38, 68.15, 53.15, 36.98, 36.80, 36.27, 30.71, 28.93. HRMS m/z calculated for $C_{23}H_{27}N_3O_6$: 441.1900; found: 442.1978$[M + H]^+$.

Compound 1g

The vaccum dried Compound **1f** (4.23 g, 9.61 mmol) was dissolved in DMF and cooled to 0 ºC. Then $(Boc)_2O$ (2.96 g, 13.63 mmol) was added drop wise to this solution and basified with triethylamine. Reaction mixture was warmed to RT and kept for stirring for 5h. Once the reaction was completed, the solvent was

concentrated and product was extracted with ethyl acetate. The organic layer was washed with brine solution (x3) and dried over Na_2SO_4. Organic layer was concentrated under reduced pressure and purified by Column chromatography (2:8 Methanol: DCM) gave compound **1g** (4.33 g, 88%) as yellow liquid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 7.73 (d, *J* = 7.5 Hz, 2H), 7.57 (d, *J* = 7.3 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.29 (d, *J* = 7.4 Hz, 2H), 4.62 (dd, *J* = 13.8, 7.6 Hz, 1H), 4.34 (dt, *J* = 17.1, 9.9 Hz, 2H), 4.19 (t, *J* = 7.1 Hz, 1H), 3.87 (t, *J* = 5.3 Hz, 2H), 3.43 – 3.3 (m, 2H), 3.06 (s, 3H), 2.97 (dd, *J* = 16.7, 5.2 Hz, 1H), 2.82 – 2.76 (m, 1H), 1.77 (dd, *J* = 8.4, 5.2 Hz, 1H), 1.45 (s, 9H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 173.88, 171.03, 157.24, 156.19, 143.89, 141.40, 127.86, 127.23, 125.24, 120.11, 82.28, 72.17, 67.41, 51.45, 49.70, 47.23, 37.48, 37.16, 36.62, 30.76, 29.81, 28.41, 27.18, 17.75. HRMS (ESI) m/z calculated for $C_{28}H_{35}N_3O_8$: 541.2424; found: 542.2499 $[M + H]^+$.

Compound 2a

2, 2'-(ethylenedioxy)bisethylamine (2.92 mL, 20.0mmol) was dissolved in ACN (100 mL). To this solution Succinic anhydride (2.0 g, 20.0mmol) in ACN (50 mL) was added drop wise. Reaction mixture was kept stirring for 3h. Stirring was stopped after the formation of waxy substance and left standing for 1h. Solvent was decanted and the waxy substance was dissolved in ACN-H_2O (1:1) mixture and cooled to 0 ºC. Then Fmoc-Cl (6.73 g, 26.0mmol) in ACN (20mL) was added drop wise and pH was adjusted to 7-8 with 5% $NaHCO_3$. The reaction mixture was kept for stirring 16h. After completion of reaction, reaction mixture was concentrated and the remaining product was dissolved in 5% $NaHCO_3$. Aqueous layer was washed with EtOAc (x4), acidified to pH 2 with 1M HCl then extracted with EtOAc (x4). The combined organic layer was washed with water (x2) dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The product **2a** (6.03g, 64%) was obtained as clear oil and used without purification.^{1}H NMR (400 MHz, chloroform-*d*) δ 7.75 (d, *J* = 7.0 Hz, 2H), 7.57 (dd, *J* = 14.7, 7.4 Hz, 2H), 7.39 (dd, *J* = 10.7, 7.0 Hz, 2H), 7.30 (dd, *J* = 8.0, 5.7 Hz, 2H), 6.95 (s, 1H), 6.62 (s, 1H), 5.67 (s, 1H), 4.43 (dd, *J* = 22.7, 6.6 Hz, 2H), 4.22 (dt, *J* = 13.6, 6.6 Hz, 1H), 3.57 – 3.32 (m, 12H), 2.68 – 2.62 (m, 2H), 2.46 (dd, *J* = 14.3, 8.5 Hz, 2H).^{13}C NMR (100 MHz, chloroform-*d*) δ 173.04, 172.55, 158.12, 156.77, 143.98, 141.43, 127.82, 127.20, 125.13, 124.95, 120.12, 70.39,

70.25, 69.93, 69.70, 67.61, 47.22, 40.87, 39.45, 31.45, 30.26. HRMS (ESI) m/z calculated for $C_{25}H_{30}N_2O_7$: 470.2053; found: 493.1950 $[M + Na]^+$.

Compound 3a

Pyrene-1-butyric acid (1.00 g, 3.47 mmol) was dissolved in DCM (50 mL) and Pentafluorophenol (2.24 g, 12.15 mmol) was added. DIC (0.65 g, 5.20 mmol) and HOBt (0.05 g, 0.34 mmol) were added to the reaction mixture at 0 °C. Then Triethylamine was added to adjust the pH of the mixture to 8. The reaction mixture was stirrer at RT for 12h. After completion solvent was concentrated under reduced pressure and residue was purified by column chromatography (4:6 EtOAC: Hexane) to yield compound **3a** as white powder (1.4 g, 89%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.29 (d, J = 9.3 Hz, 1H), 8.20 – 8.13 (m, 4H), 8.05 – 7.99 (m, 3H), 7.88 (d, J = 7.8 Hz, 1H), 3.48 (t, J = 7.6 Hz, 2H), 2.78 (t, J = 7.2 Hz, 2H), 2.37 – 2.30 (m, 2H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 169.54, 134.96, 131.54, 130.99, 130.31, 128.88, 127.76, 127.59, 127.49, 127.02, 126.07, 125.27, 125.20, 125.09, 125.02, 123.14, 32.89, 32.46, 26.63. HRMS (ESI) m/z calculated for $C_{26}H_{15}F_5O_2$: 454.0992; found: 455.1125 $[M + H]^+$.

Compound 3b

Compound **3a** (1.40 g, 3.08 mmol) was dissolved in DMF (50 mL). To this stirring solution Fmoc-Lys-OH (1.54 g, 4.16 mmol) was added and basified with DIPEA (12 mL, 6.94 mmol). The reaction mixture was stirred for 16h. Solvent was concentrated under reduced pressure. The residue was diluted with EtOAc, washed with brine solution (x3) and dried over Na_2SO_4. The organic layer was concentrated under reduced pressure and purified by column chromatography (7:3 EtOAc: Hexane) to yield compound **3b** (1.33 g, 80%) as white powder. ^{1}H NMR (400 MHz, DMSO-d^6) δ 8.36 – 8.32 (m, 1H), 8.21 (dd, *J* = 25.3, 7.8 Hz, 2H), 8.10 (s, 2H), 8.03 (t, *J* = 7.6 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 1H), 7.85 (d, *J* = 6.6 Hz, 3H), 7.68 (d, *J* = 6.7 Hz, 2H), 7.37 (d, *J* = 5.0 Hz, 2H), 7.30 (d, *J* = 6.0 Hz, 2H), 4.24 – 4.17 (m, 3H), 3.89 (s, 1H), 3.06 (d, *J* = 4.9 Hz, 2H), 2.21 (d, *J* = 6.9 Hz, 2H), 2.02 – 1.98 (m, 2H), 1.67 (d, *J* = 46.2 Hz, 2H), 1.37 (d, *J* = 25.6 Hz, 4H), 1.22 – 1.15 (m, 1H). ^{13}C NMR (100 MHz, DMSO-

d^6) δ 171.70, 155.94, 143.88, 143.80, 140.70, 136.52, 130.88, 130.51, 129.28, 128.14, 127.59, 127.50, 127.43, 127.29, 127.19, 127.03, 126.47, 126.09, 125.27, 124.90, 124.76, 124.24, 124.15, 123.48, 121.38, 120.07, 79.19, 65.48, 59.76, 54.33, 46.69, 38.39, 32.28, 28.94, 27.58, 23.07, 20.81. HRMS (ESI) m/z calculated for $C_{28}H_{35}N_3O_8$: 541.2424; found: 542.2499 $[M + H]^+$.

Compound 4a

Cholesterol (1.5 g, 3.88 mmol) was dissolved in anhydrous THF (20 mL) and NaH (0.37 g, 15.54 mmol) was added. The reaction mixture was kept for stirring for 30 mins and tert-butyl acrylate (1.5 g, 11.65 mmol) was added drop wise. The reaction was kept stirring for 16h at RT. The solvent was concentrated under reduced pressure. The crude product was diluted with EtOAc, washed with 1N HCl (x2), brine (x2), dried over anhydrous Na_2SO_4. The residue was purified by column chromatography (0.3:9.7 EtOAC: Hexane) to yield compound **4a** (0.89 g, 45%) as white powder. ^{1}H NMR (400 MHz, chloroform-*d*) δ 5.33 (dd, *J* = 3.1, 2.1 Hz, 1H), 3.70 (t, *J* = 6.6 Hz, 2H), 3.20 – 3.12 (m, 1H), 2.47 (t, *J* = 6.6 Hz, 2H), 2.37 – 2.32 (m, 1H), 2.20 – 2.13 (m, 1H), 2.03 – 1.93 (m, 2H), 1.90– 1.77 (m, 3H), 1.62 (d, *J* = 3.6 Hz, 2H), 1.60 – 1.48 (m, 5H), 1.45 (s, 9H), 1.44 – 1.42 (m, 1H), 1.40 – 1.21 (m, 5H), 1.18 – 0.98 (m, 8H), 0.98 (s, 3H), 0.91 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 1.8 Hz, 3H), 0.87 (d, *J* = 1.8 Hz, 3H), 0.67 (s, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 171.27, 141.07, 121.69, 80.59, 79.38, 63.91, 56.92, 56.30, 50.34, 42.47, 39.94, 39.67, 39.20, 37.44, 37.01, 36.93, 36.34, 35.94, 32.09, 32.04, 28.52, 28.38, 28.26, 28.16, 24.44, 23.97, 22.97, 22.71, 21.21, 19.52, 18.87, 12.01. HRMS (ESI) m/z calculated for $C_{34}H_{58}O_3$: 514.4386; found: 537.4269 $[M + Na]^+$.

Compound 4b

Compound **4a** (0.89 g, 1.73 mmol) was dissolved in DCM (5 mL) and cooled to 0 °C. TFA (5 mL) was added and stirred for 4h. The solvent was concentrated under reduced pressure and purified by column chromatography (2:8 EtOAc: Hexane) to yield compound **4b** as white powder (0.39 g, 50%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 5.35 (d, *J* = 5.1 Hz, 1H), 3.76 (t, *J* = 6.2 Hz, 2H), 3.28 – 3.19 (m, 1H), 2.63 (t, *J* =

6.1 Hz, 2H), 2.36 (dd, *J* = 13.1, 2.9 Hz, 1H), 2.21 (t, *J* = 13.3 Hz, 1H), 2.00 (dd, *J* = 18.6, 9.3 Hz, 3H), 1.92 – 1.78 (m, 2H), 1.61 – 1.43 (m, 7H), 1.33 (dd, *J* = 15.3, 7.7 Hz, 3H), 1.25 (s, 3H), 1.21 – 1.02 (m, 8H), 1.00 (s, 3H), 0.91 (d, *J* = 6.5 Hz, 3H), 0.86 (dd, *J* = 6.6, 1.3 Hz, 6H), 0.67 (s, 3H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 172.25, 141.00, 121.76, 79.50, 63.51, 56.92, 56.30, 51.79, 50.33, 42.47, 39.93, 39.67, 39.16, 37.36, 37.01, 36.34, 35.94, 35.48, 32.09, 28.48, 28.38, 28.16, 24.44, 23.97, 22.97, 22.71, 21.21, 19.52, 18.87, 12.01. HRMS (ESI) m/z calculated for $C_{30}H_{50}O_3$: 458.3760; found: 458.3856

Peptide 1

The resin was pre-swellen overnight and the following steps were performed
Wash with DMF 4 x 5 mL.
20% piperidine in DMF 3 x 5 mL (10 mins for each) for deprotection of Fmoc group
Wash with DMF 3 x 5 mL, MeOH 3 x 5 mL, DCM 3 x 5 mL and DMF 3 x 5 mL
Test for complete deprotection (Chloranil test)
Coupling reaction with amino acid, DIPEA, HOBt and HBTU (3 eq.) in NMP (1 mL).
Recoupling with the same aminoacid, DIPEA, HOBt and HBTU in NMP (1 mL)
This cycle was repeated for every aminoacid

<u>General procedure for Fmoc deprotection</u>

20 % piperidine in DMF was added to the resin and the reaction mixture was kept for 15 min, drained and the piperidine treatment was repeated 3 times. Finally the resin was washed with DMF (3x), MeOH (3x) and DCM (3x).

<u>General procedure for peptide couplings on Rink Amide Resin</u>

Fmoc-A-OH (3 eq.), HBTU (3 eq.) and HOBt (3 eq.) dissolved in NMP followed by *i*Pr2NEt (3 eq.) were added to the amino-functionalized resin. The mixture was kept for 2 h and last 5 min bubbled with N_2 and washed with DMF (3x), MeOH (3x) DCM (3x) and DMF (3x). The loading value for peptide synthesis is taken as 0.38 The coupling reaction was repeated in NMP for better yield

<u>General procedure for cleavage of peptides from the solid support</u>

The dry peptide-resin (20 mg) was taken in round-bottomed flask to which of 95 % TFA in DCM (10 mL) and Triisopropylsilane (as scavengers) (2-3 drops) were added. The resulting mixture was kept for 2 h by gentle shaking. The mixture was filtered

through a sintered funnel and the resin was washed with 3 x 5 mL of above solution. The filtrate was collected in pear shape round-bottom flask and evaporated under reduced pressure. The resin was washed with MeOH (3 X 5 mL) and the washings were evaporated to dryness.

Compound 5a

4-Bromo-1,8- naphthalic anhydride (1.10 g, 4.00 mmol) was dissolved in 3 methyl butanol (28 mL) and the solution was stirred at 132 °C. 3- dimethylamino-propionitrile (1.57 g, 1.60 mmol) was added and stirred for 12h. After completion of reaction, The reaction mixture was allowed to reach RT. Formed crystals were filtered out and washed with water and hexane to yield Compound **5a** (0.85 g, 89%) as orange crystals. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.52 (d, *J* = 7.1 Hz, 1H), 8.46 (t, *J* = 6.2 Hz, 1H), 8.41 (d, *J* = 8.3 Hz, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.08 (d, *J* = 8.3 Hz, 1H), 3.18 (s, 6H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 161.78, 160.81, 157.99, 135.05, 133.22, 132.98, 125.04, 124.86, 119.22, 113.21, 109.37, 44.69. HRMS (ESI) m/z calculated for $C_{14}H_{11}NO_3$: 241.0739; found: 242.0820 $[M + H]^+$.

Compound 5b

Compound **5a** (0.86 g, 3.56 mmol) was kept in two neck round bottom flask fitted with water condenser. 1,4 Dioxane (100 mL) was added and mixture was heated and refluxed at 120°C for 1h. Boc-Lys-OH (0.73 g, 2.96 mmol) and $NaHCO_3$ (1.49 g, 17.8 mmol) was dissolved in 10 mL water and added to the reaction mixture. The reaction mixture was refluxed for 12h. After completion of reaction, the mixture was concentrated under reduced pressure and water was added. The aqueous phase was washed with Et_2O. Then the aqueous layer was acidified to pH 3 in ice bath using 10%HCl solution. The aqueous phase was extracted with DCM. The combined organic layer was dried over Na_2SO_4 . filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (1:1 EtOAc:Hexane) to yield compound **5b** (0.92 g, 55%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.54 (d, *J* = 7.3 Hz, 1H), 8.42 (dd, *J* = 17.2, 8.3 Hz, 2H), 7.62 (t, *J* = 7.9 Hz, 1H), 7.07 (d, *J* = 8.3 Hz, 1H), 5.37 (d, *J* = 7.3 Hz, 1H), 4.30 – 4.25 (m, 1H), 4.21 – 4.12

(m, 2H), 3.08 (s, 6H), 1.98 – 1.92 (m, 1H), 1.86 – 1.69 (m, 3H), 1.53 (d, *J* = 31.5 Hz, 2H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 164.92, 164.40, 157.18, 156.10, 133.02, 131.43, 131.34, 130.37, 125.31, 125.01, 123.05, 114.88, 113.43, 80.18, 53.52, 44.90, 39.61, 31.61, 31.08, 28.46, 27.74, 22.76. HRMS (ESI) m/z calculated for $C_{25}H_{31}N_3O_6$: 469.2213; found: 469.2202 $[M + H]^+$.

Compound 5c

Compound **5b** (0.92 g, 1.96 mmol) was dissolved in DCM (5 mL) and cooled to 0°C. Then TFA (5 mL) was added to this solution and stirred for 1h. After complete consumption of starting material, TFA was evaporated and re-dissolved in 10% Na_2CO_3 Solution. The mixture was cooled to 0°C and Fmoc-Cl in 1,4 dioxane was added. Reaction mixture was stirred for 12h. After completion of the reaction, 1,4-dioxane was evaporated and redissolved in water. Aqueous layer was extracted with diethylether and acidified with 10% HCl solution to pH 3. After that, aqueous layer was extracted with EtOAc. Organic Layer was combined, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified using column chromatography to yield compound **5c** (0.69 g, 60%) as orange crystals. ^{1}H NMR (400 MHz, chloroform-*d*) δ 8.55 (d, *J* = 7.1 Hz, 1H), 8.45 (d, *J* = 8.2 Hz, 1H), 8.37 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 7.1 Hz, 3H), 7.35 (t, *J* = 7.4 Hz, 2H), 7.24 (d, *J* = 7.2 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 4.46 – 4.23 (m, 3H), 4.17 (dd, *J* = 16.7, 9.5 Hz, 3H), 3.04 (s, 6H), 2.05 – 1.52 (m, 6H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 175.94, 165.00, 164.56, 157.21, 156.51, 144.07, 143.91, 141.35, 133.14, 131.50, 131.40, 130.38, 127.77, 127.21, 125.33, 125.21, 124.97, 122.96, 120.00, 114.66, 113.39, 67.29, 53.96, 47.27, 44.84, 39.49, 31.48, 29.83, 22.65.

Compound 6a

5-Bromovaleric acid (0.30 g, 1.5 mmol) was dissolved in anhydrous DMF (5 mL) and sodium azide (0.11 g, 1.69 mmol) was added. The reaction mixture was stirred at 60 °C for 12h. After completion of reaction the reaction mixture was extracted with EtOAc, washed with water (x3), brine solution (x3) and dried over Na_2SO_4. Organic

layer was collected and concentrated under reduced pressure to yield compound **6a** (0.23 g, 97%). ^{1}H NMR (400 MHz, chloroform-*d*) δ 3.63 (s, 3H), 3.26 (t, *J* = 6.6 Hz, 2H), 2.31 (t, *J* = 7.2 Hz, 2H), 1.71 – 1.64 (m, 2H), 1.59 (tt, *J* = 6.8, 4.6 Hz, 2H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 173.59, 51.59, 51.04, 33.39, 28.27, 22.08.). HRMS (ESI) m/z calculated for $C_6H_{11}N_3O2$: 157.0851; found: 158.0929 $[M + H]^+$.

Compound 6b

Compound **5a** (0.23 g, 1.46 mmol) was dissolved in THF: H_2O (10 mL, 1:1 v/v). Then LiOH (0.52 g, 2.19 mmol) was added to the reaction mixture and stirred for 30 mins. After completion of reaction, Reaction mixture was acidified into pH 2 and extracted with ethyl acetate. Organic layer was concentrated under reduced pressure to yield compound **6b** (0.20 g, 95%) as colourless liquid. ^{1}H NMR (400 MHz, chloroform-*d*) δ 3.30 (t, *J* = 6.5 Hz, 2H), 2.40 (t, *J* = 7.1 Hz, 2H), 1.72 (tdd, *J* = 7.3, 6.4, 2.6 Hz, 2H), 1.64 (tdd, *J* = 8.8, 6.7, 2.4 Hz, 2H). ^{13}C NMR (100 MHz, chloroform-*d*) δ 179.83, 51.14, 33.51, 28.26, 21.87. HRMS (ESI) m/z calculated for $C_5H_9N_3O_2$: 143.0695; found: 142.0614 $[M - H]^+$.

Peptide 2a

The resin was pre-swellen overnight and the following steps were performed

Wash with DMF 4 x 5 mL.
20% piperidine in DMF 3 x 5 mL (10 mins for each) for deprotection of Fmoc group
Wash with DMF 3 x 5 mL, MeOH 3 x 5 mL, DCM 3 x 5 mL and DMF 3 x 5 mL
Test for complete deprotection (Chloranil test)
Coupling reaction with amino acid, DIPEA, HOBt and HBTU (3 eq.) in NMP (1 mL).
Recoupling with the same aminoacid, DIPEA, HOBt and HBTU in NMP (1 mL)
This cycle was repeated for every aminoacid
<u>General procedure for Fmoc deprotection</u>
20 % piperidine in DMF was added to the resin and the reaction mixture was kept for 15 min, drained and the piperidine treatment was repeated 3 times. Finally the resin was washed with DMF (3x), MeOH (3x) and DCM (3x).

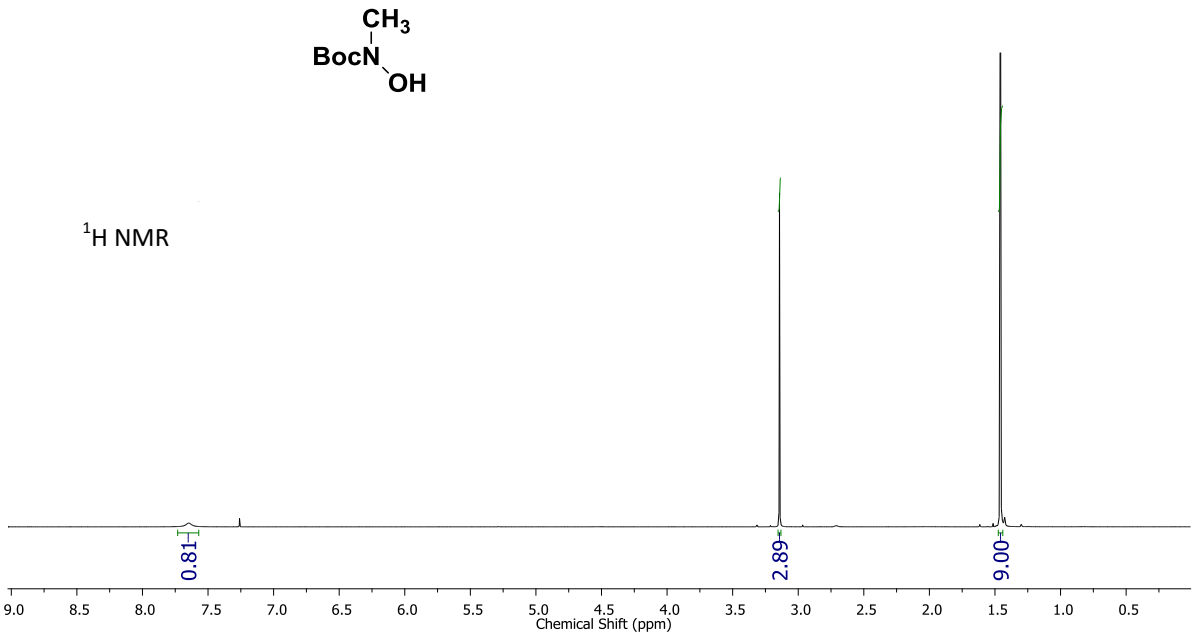
General procedure for peptide couplings on Rink Amide Resin

Fmoc-A-OH (3 eq.), HBTU (3 eq.) and HOBT (3 eq.) dissolved in NMP followed by *i*Pr2NEt (3 eq.) were added to the amino-functionalized resin. The mixture was kept for 2 h and last 5 min bubbled with N_2 and washed with DMF (3x), MeOH (3x) DCM (3x) and DMF (3x). The loading value for peptide synthesis is taken as 0.38 The coupling reaction was repeated in NMP for better yield

General procedure for cleavage of peptides from the solid support

The dry peptide-resin (20 mg) was taken in round-bottomed flask to which of 95 % TFA in DCM (10 mL) and Triisopropylsilane (as scavengers) (2-3 drops) were added. The resulting mixture was kept for 2 h by gentle shaking. The mixture was filtered through a sintered funnel and the resin was washed with 3 x 5 mL of above solution. The filtrate was collected in pear shape round-bottom flask and evaporated under reduced pressure. The resin was washed with MeOH (3 X 5 mL) and the washings were evaporated to dryness.

SPECTRAS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2.89
9.00
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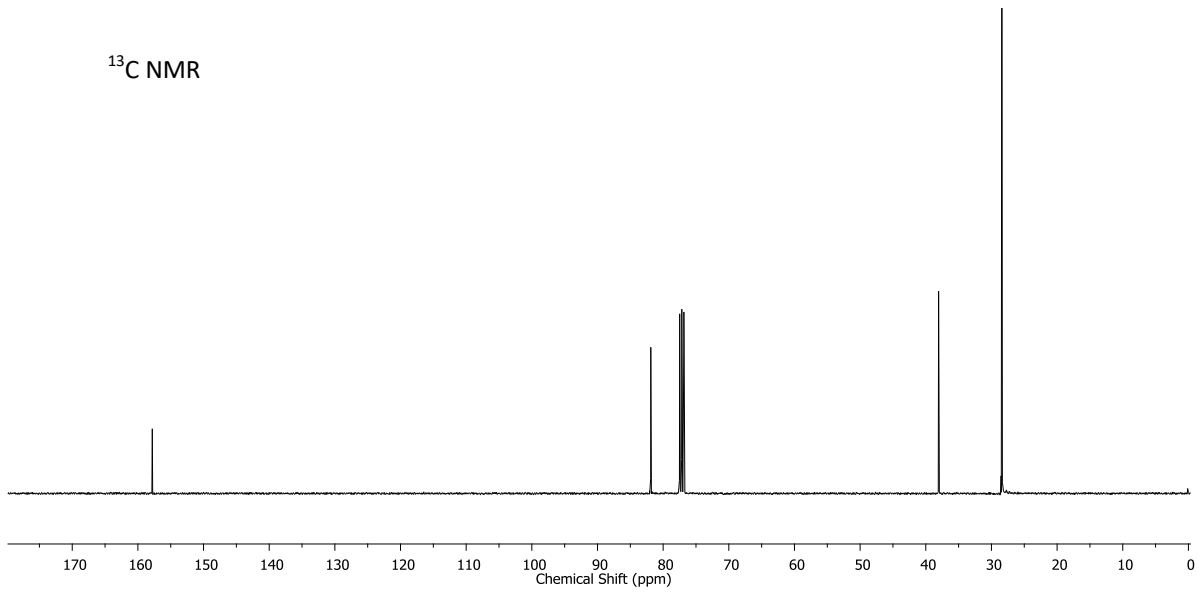
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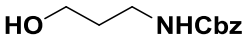
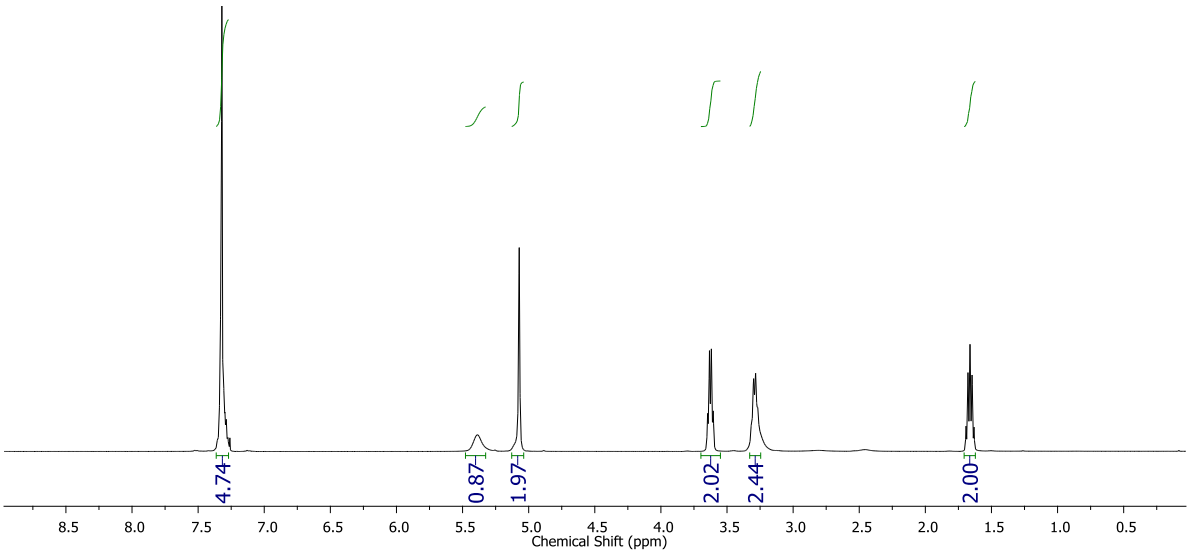
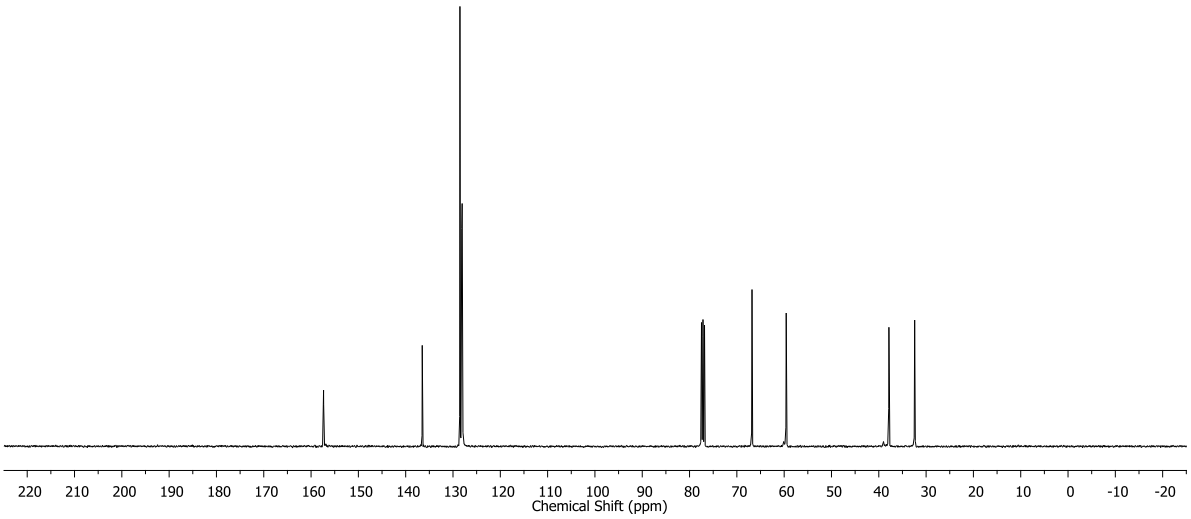
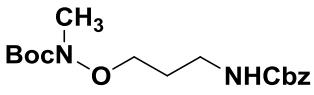
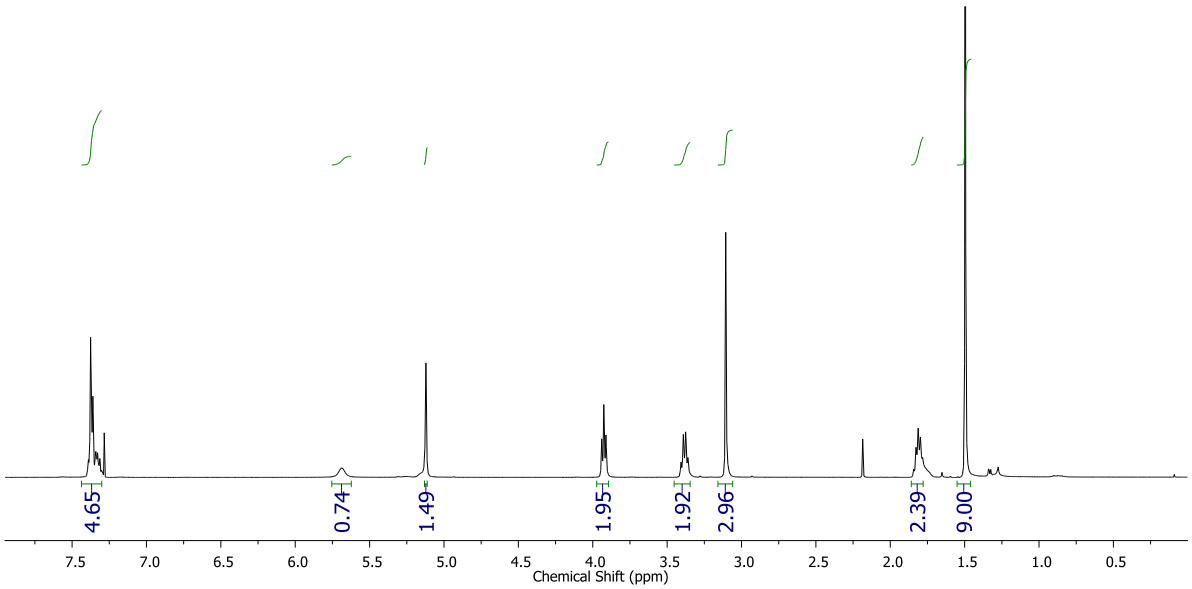
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Chemical Shift (ppm)

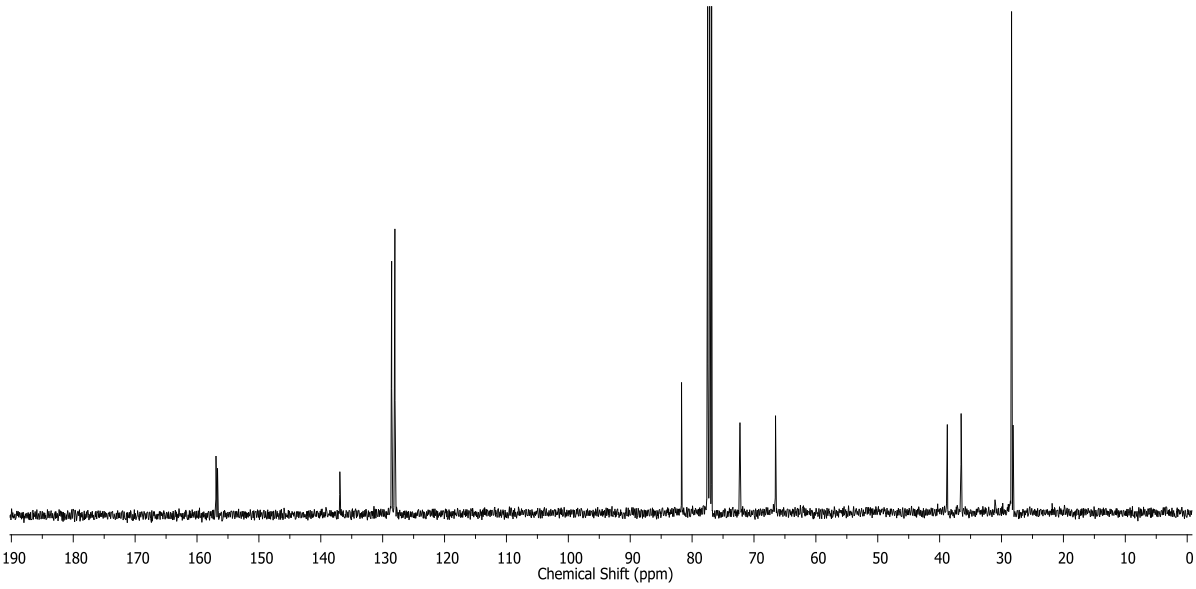

HO
NHCbz

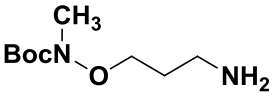

4.74
0.87
1.97
2.02
2.44
2.00
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
Chemical Shift (ppm)

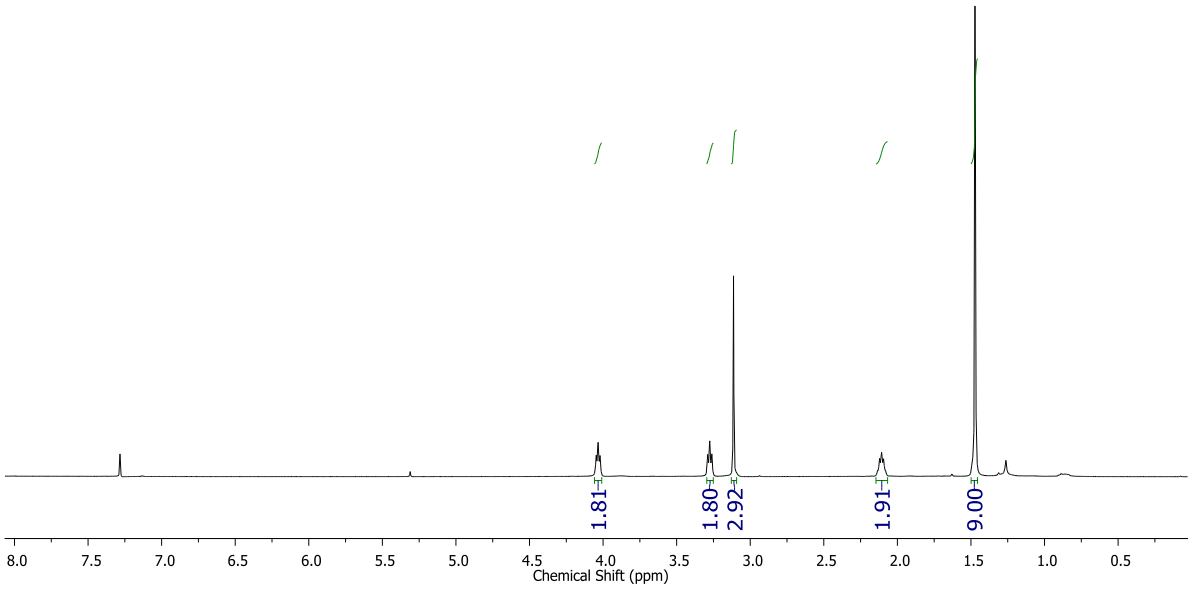

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
Chemical Shift (ppm)

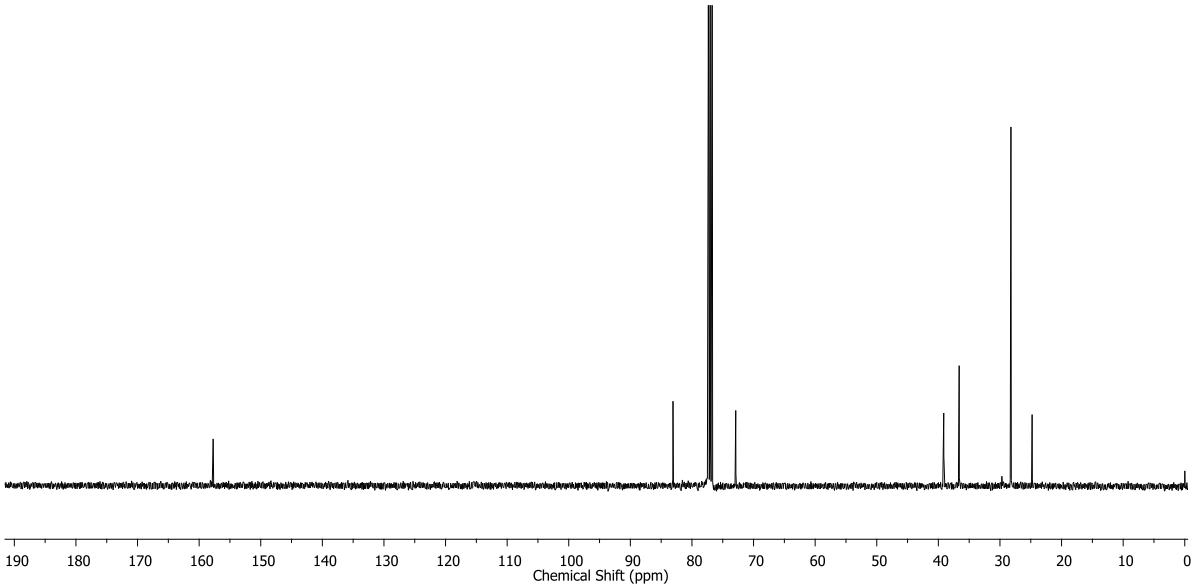

CH3
BocN
O
NHCbz

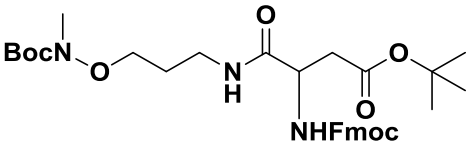

4.65
0.74
1.49
1.95
1.92
2.96
2.39
9.00
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
Chemical Shift (ppm)

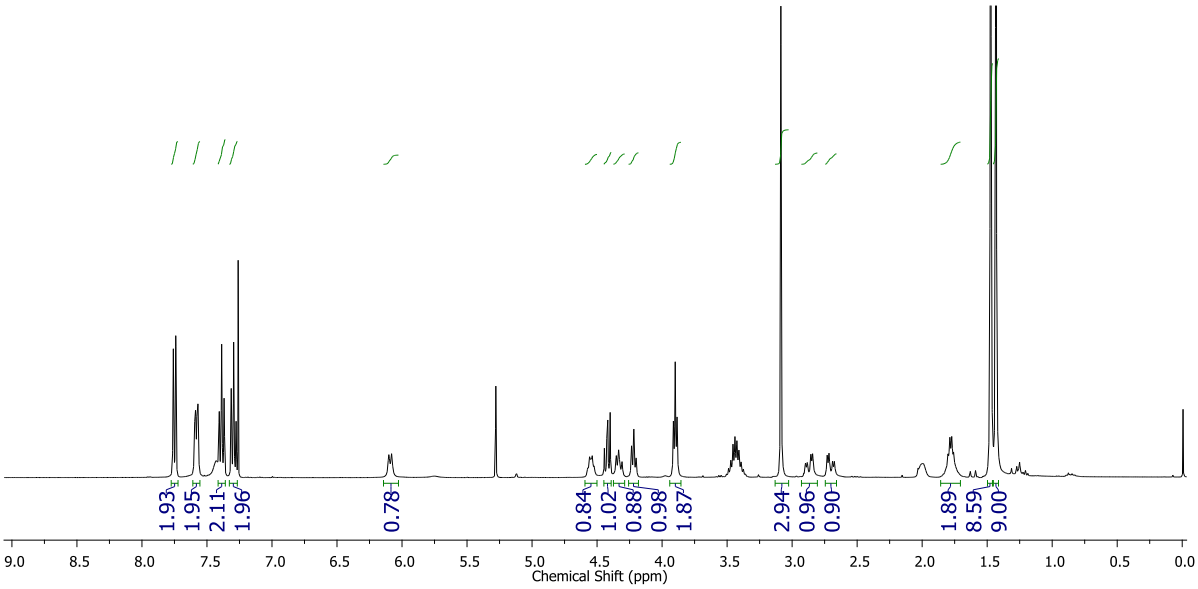

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Chemical Shift (ppm)

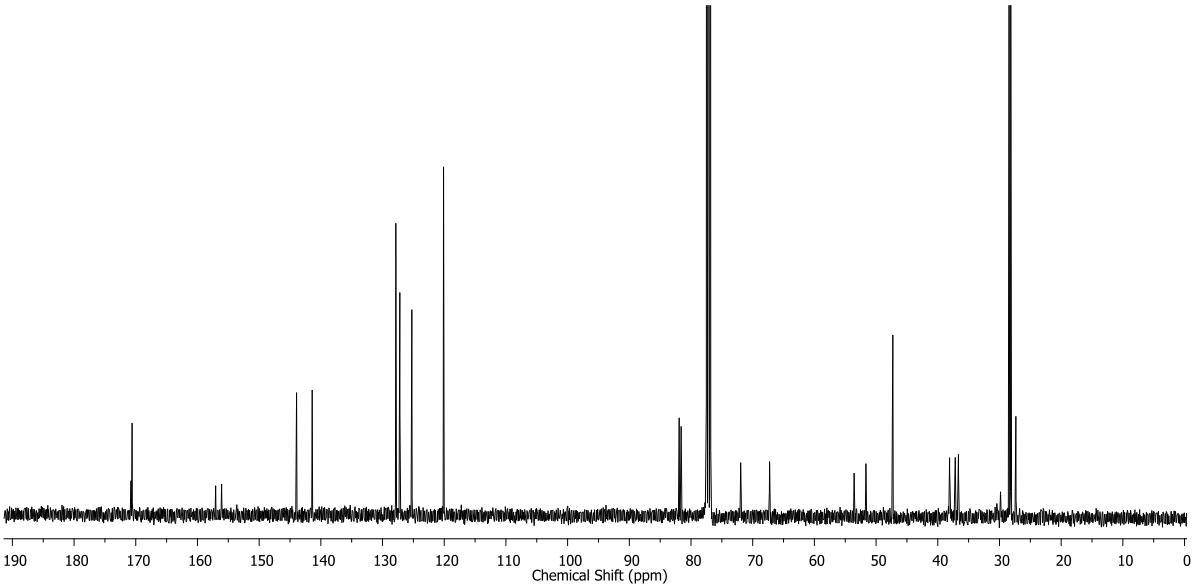

CH3
BocN
O
NH2

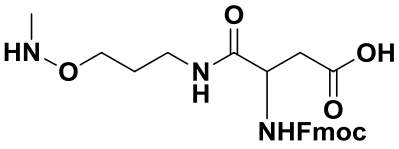

1.81
1.80
2.92
1.91
9.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)

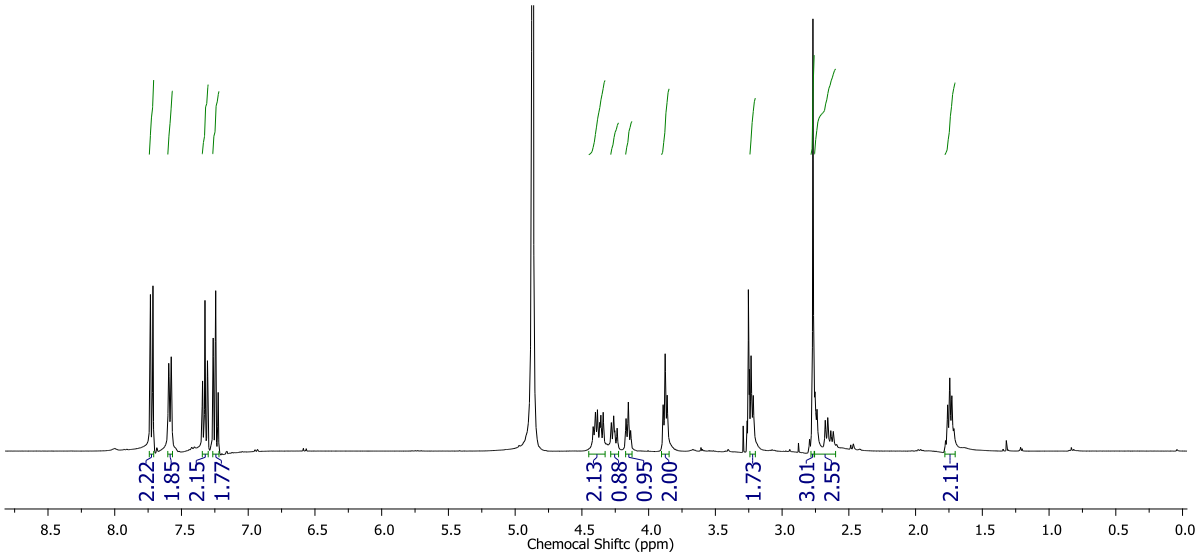

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Chemical Shift (ppm)

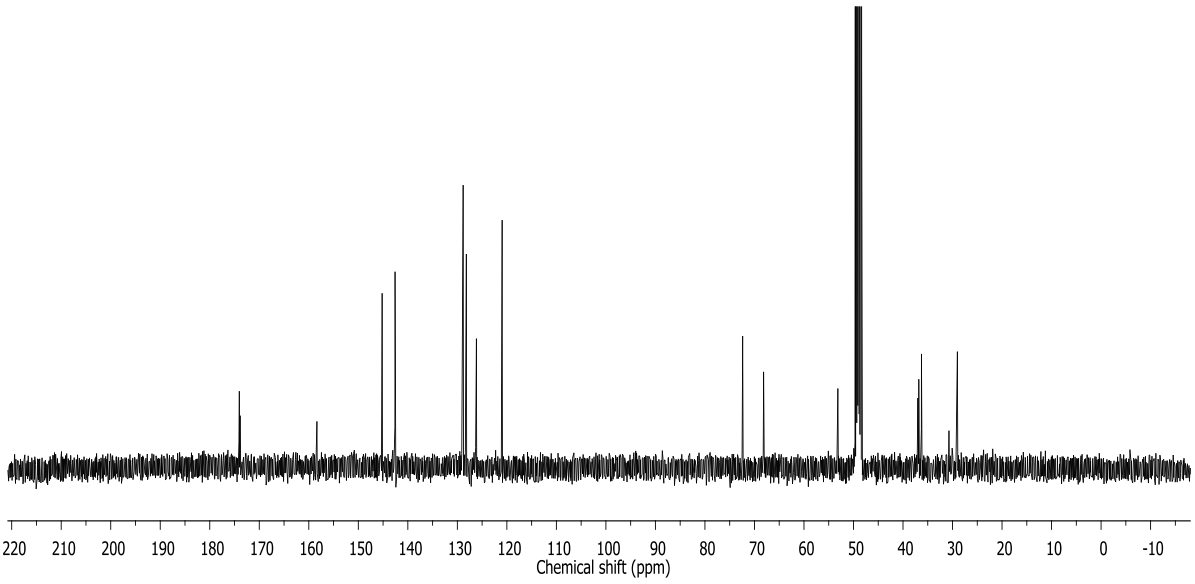

BocN
O
N
H
O
O
O
NHFmoc

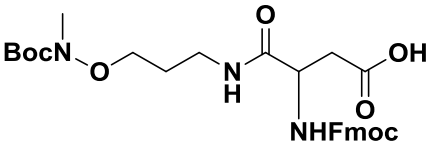

1.93
1.95
2.11
1.96
0.78
0.84
1.02
0.88
0.98
1.87
2.94
0.96
0.90
1.89
8.59
9.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemical Shift (ppm)

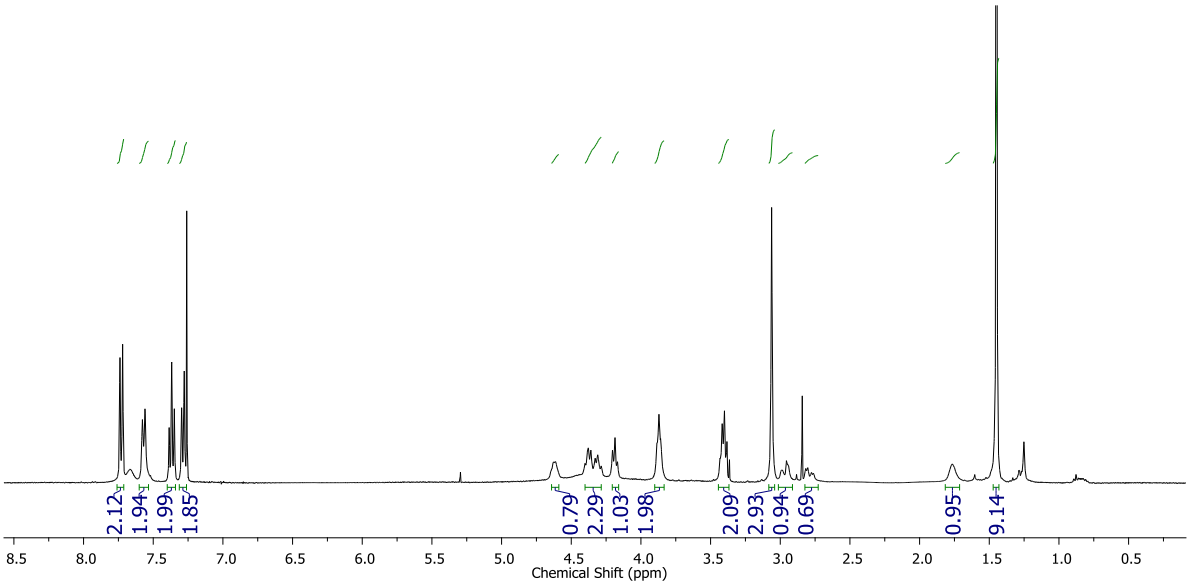

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Chemical Shift (ppm)

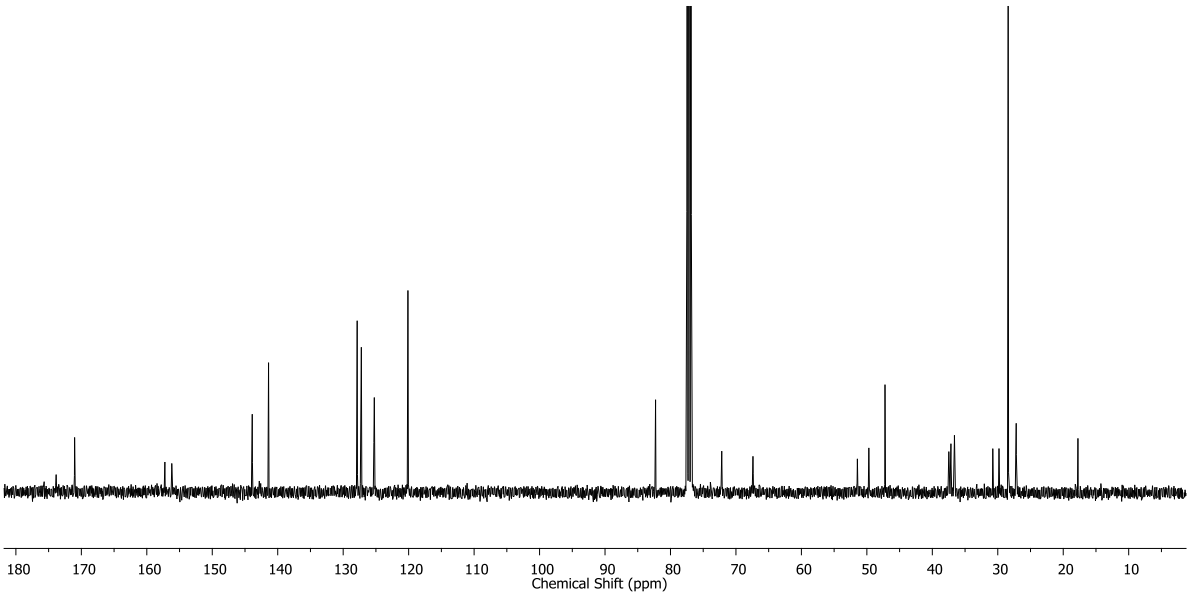

HN
O
N
H
O
OH
O
NHFmoc

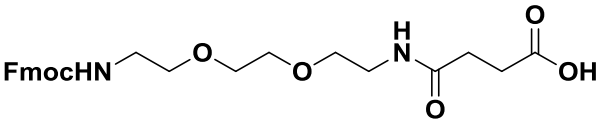

2.22
1.85
2.15
1.77
2.13
0.88
0.95
2.00
1.73
3.01
2.55
2.11
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemocal Shiftc (ppm)

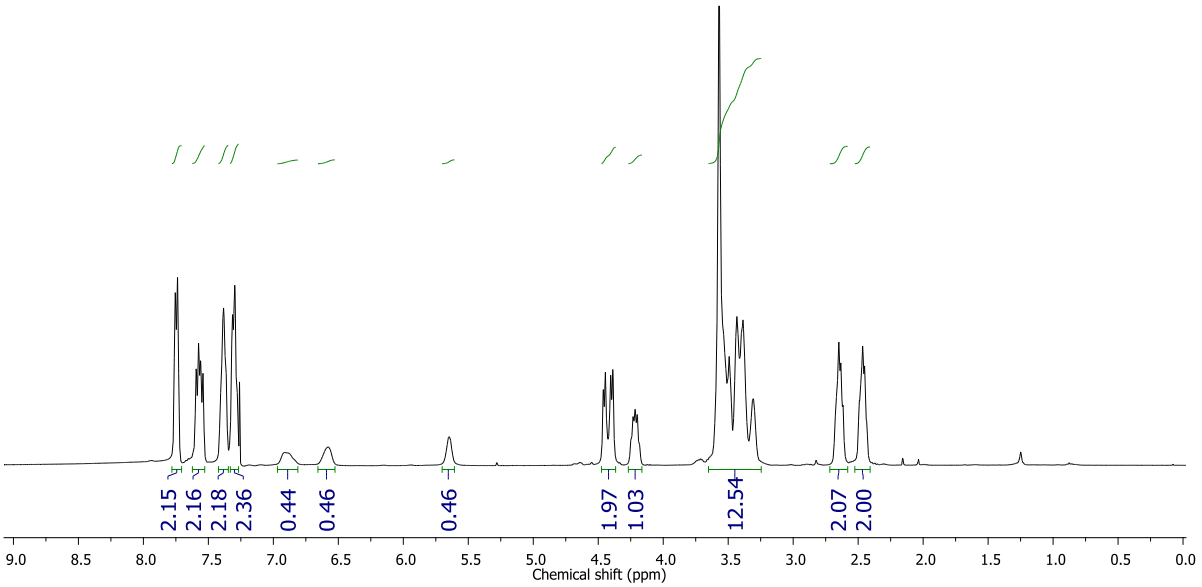

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
Chemical shift (ppm)


BocN
O
N
H
O
OH
O
NHFmoc

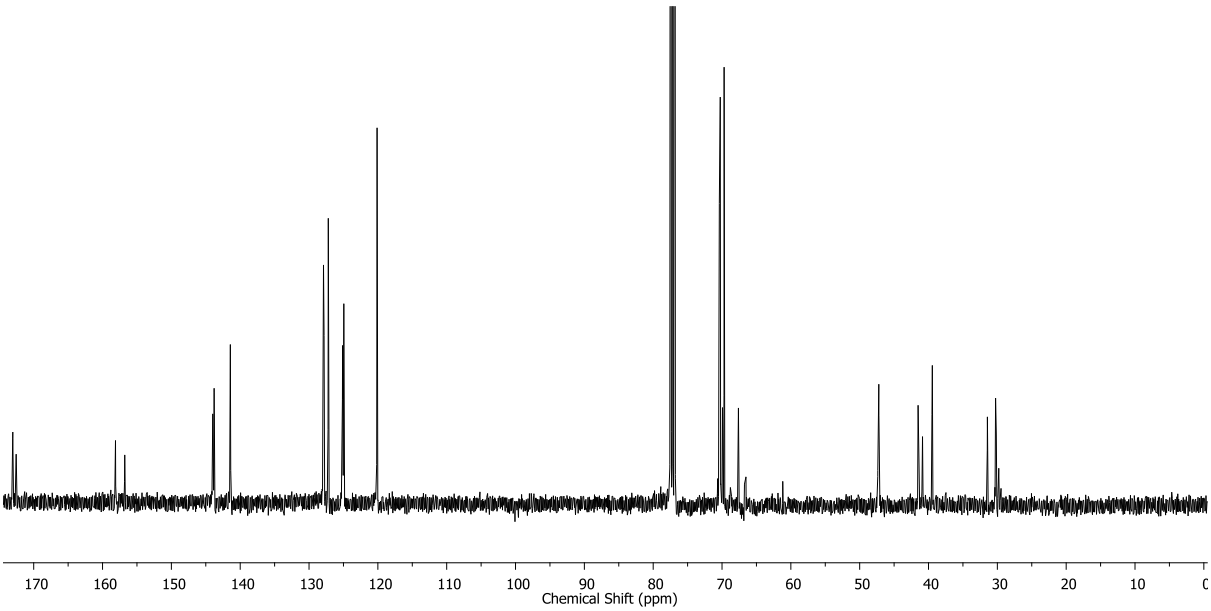

2.12
1.94
1.99
1.85
0.79
2.29
1.03
1.98
2.09
2.93
0.94
0.69
0.95
9.14
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)

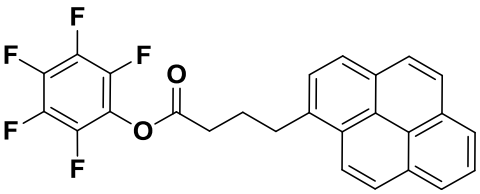

180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
Chemical Shift (ppm)

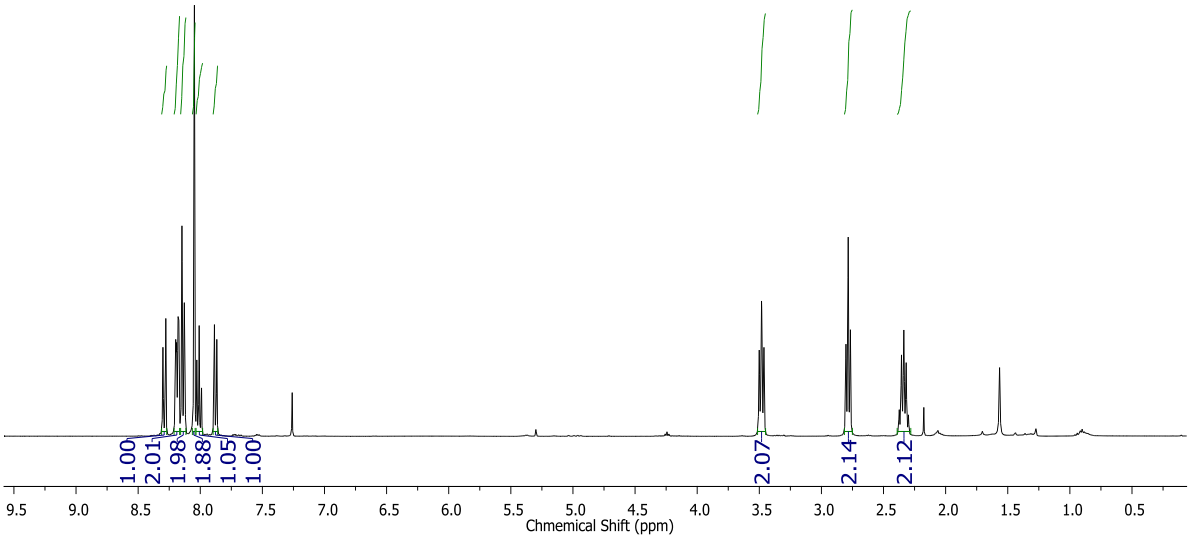

FmocHN
O
O
H
N
O
O
OH

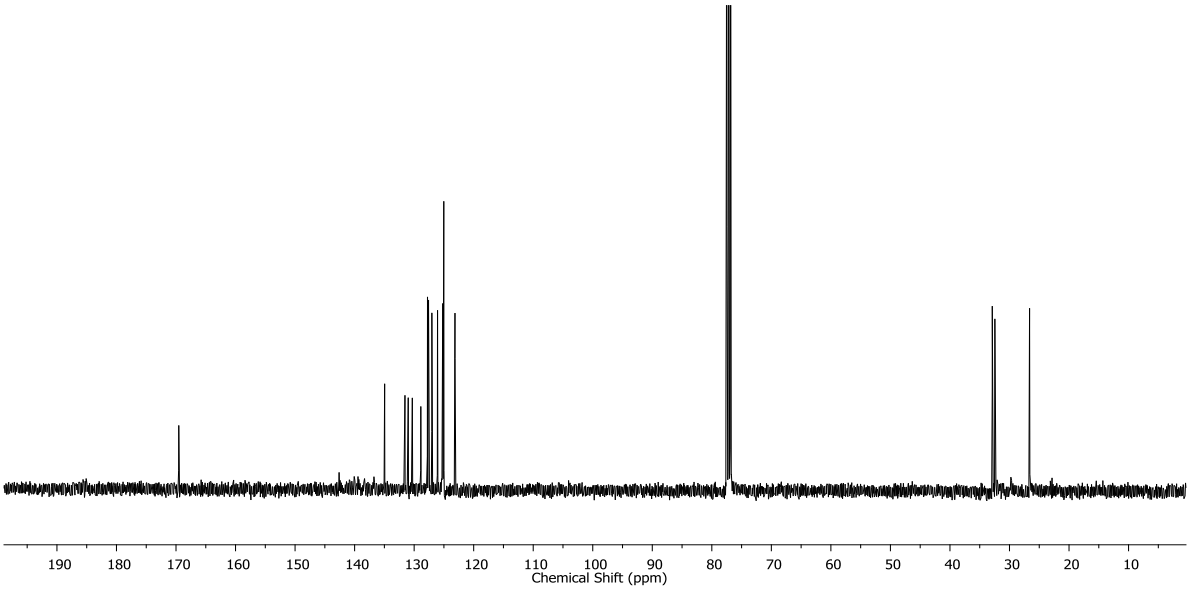

2.15
2.16
2.18
2.36
0.44
0.46
0.46
1.97
1.03
12.54
2.07
2.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemical shift (ppm)

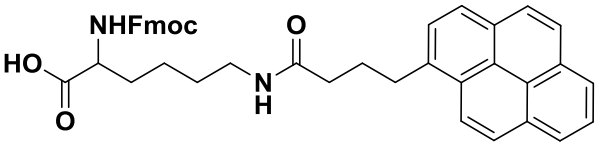
.

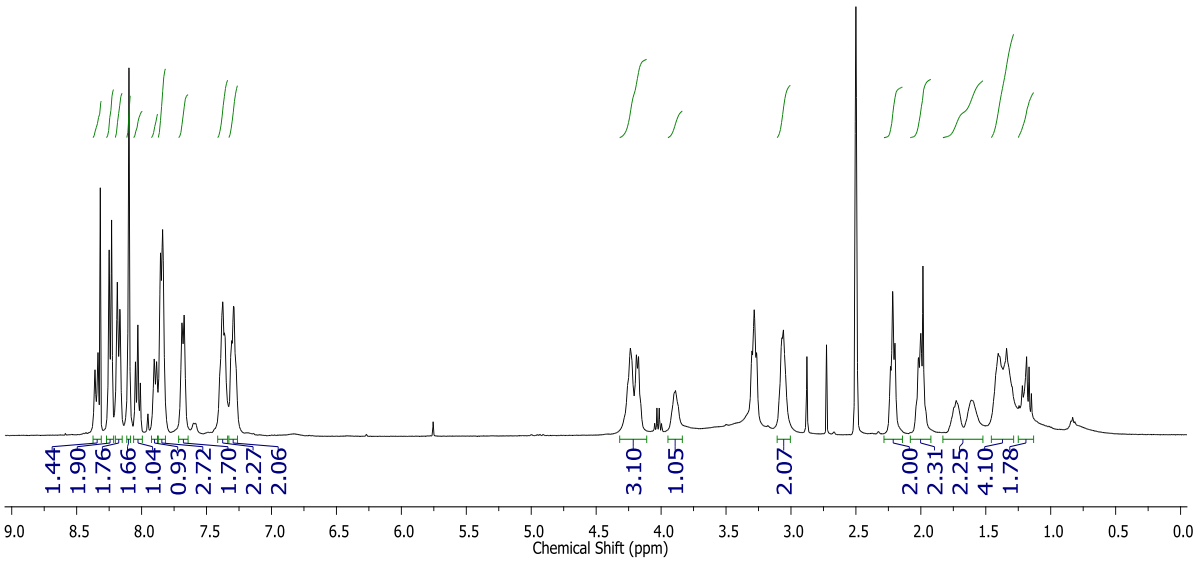

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
Chemical Shift (ppm)

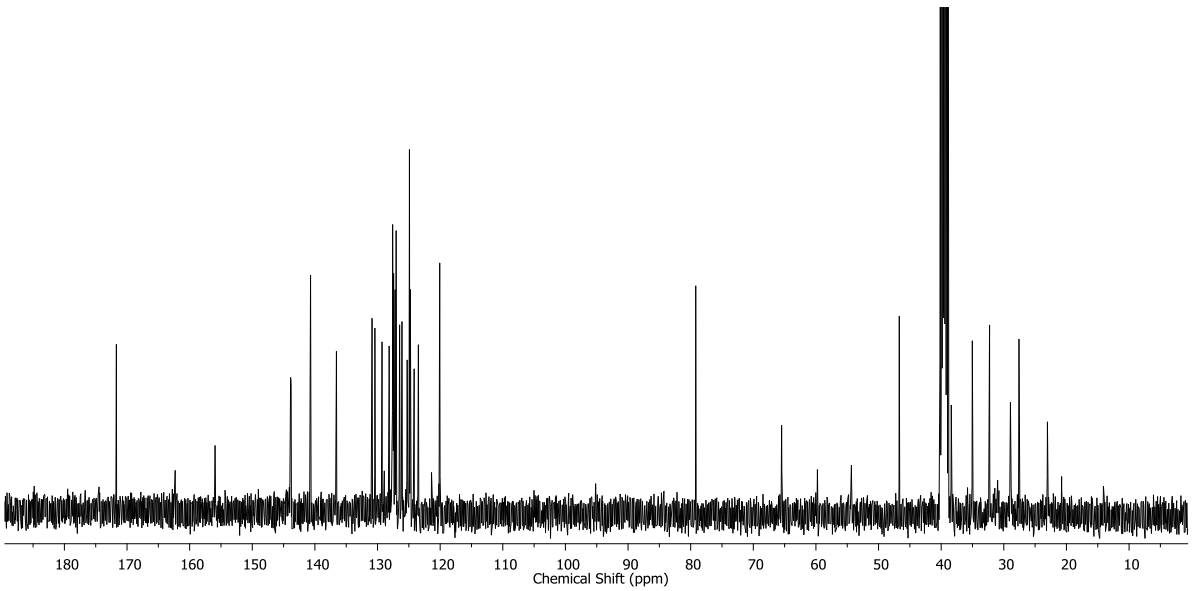

F
F
F
F
F
O
O

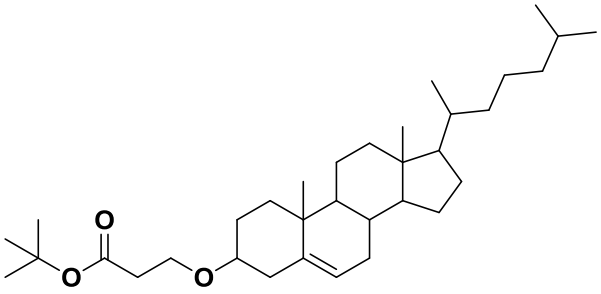

1.00
2.01
1.98
1.88
1.05
1.00
2.07
2.14
2.12
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
Chmemical Shift (ppm)

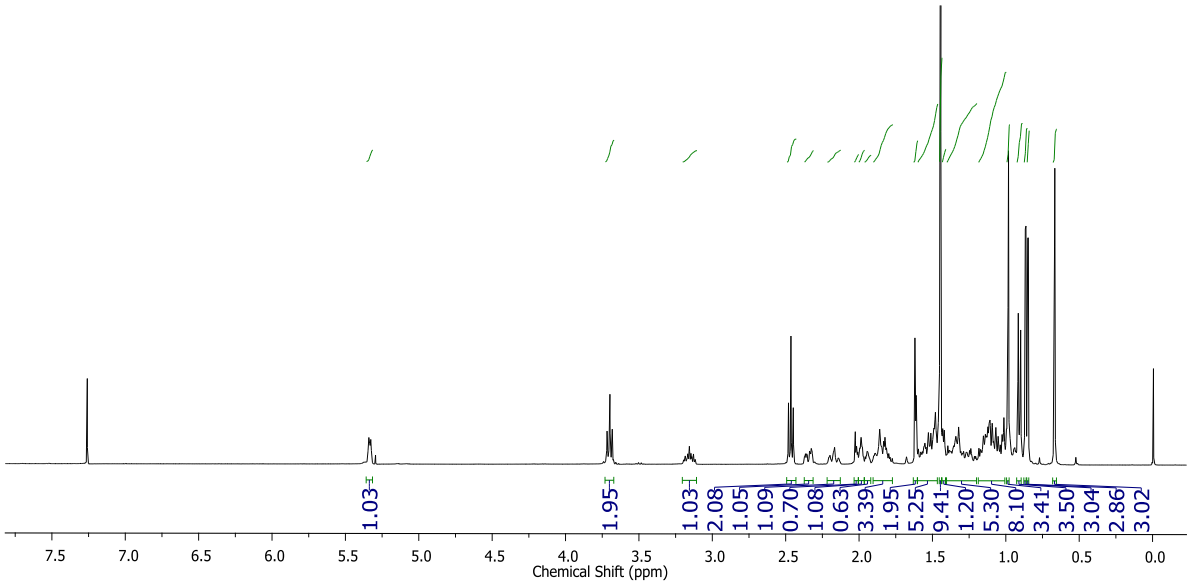

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
Chemical Shift (ppm)

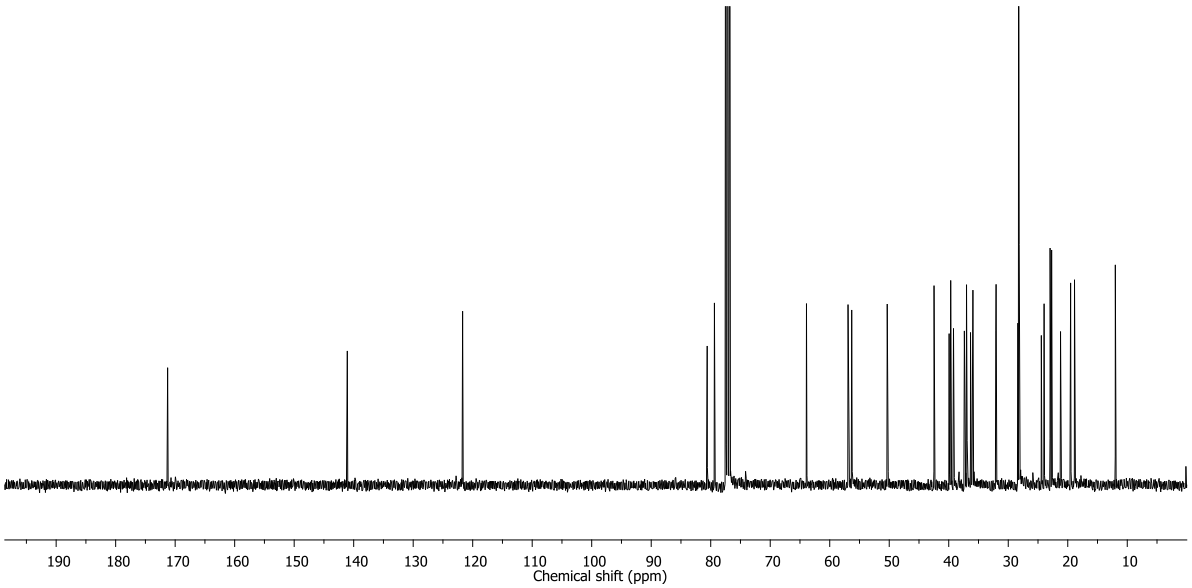

NHFmoc
O
HO
N
H
O

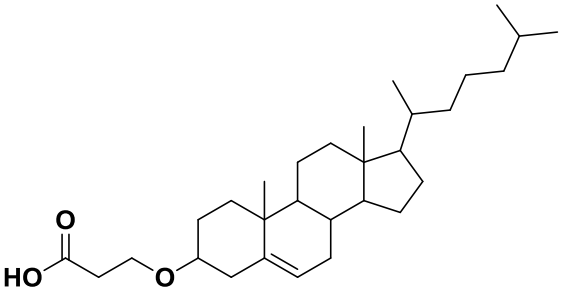

1.44
1.90
1.76
1.66
1.04
0.93
2.72
1.70
2.27
2.06
3.10
1.05
2.07
2.00
2.31
2.25
4.10
1.78
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Chemical Shift (ppm)

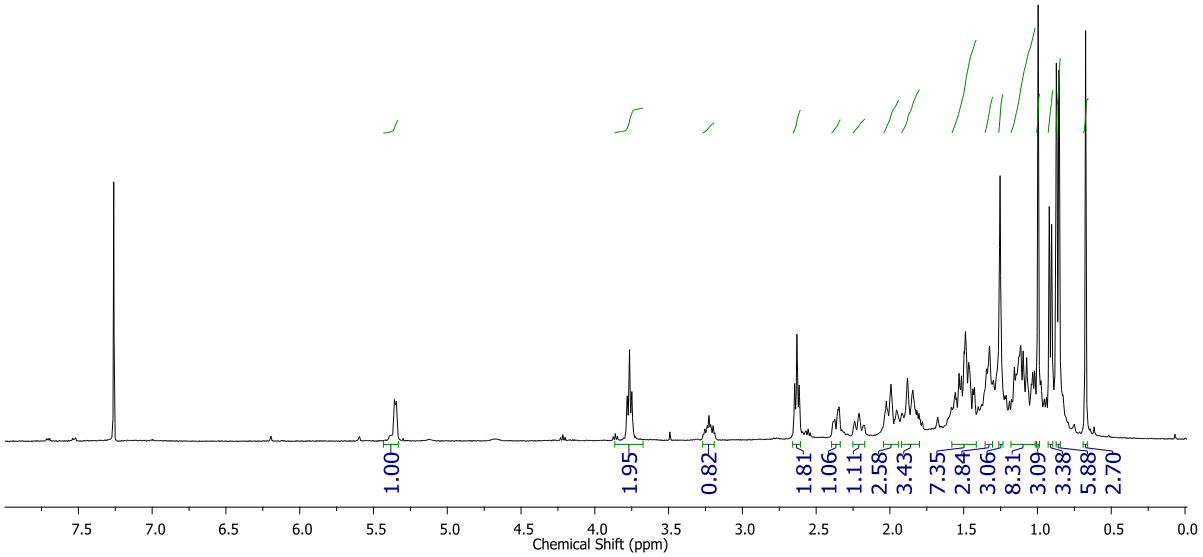

180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
Chemical Shift (ppm)

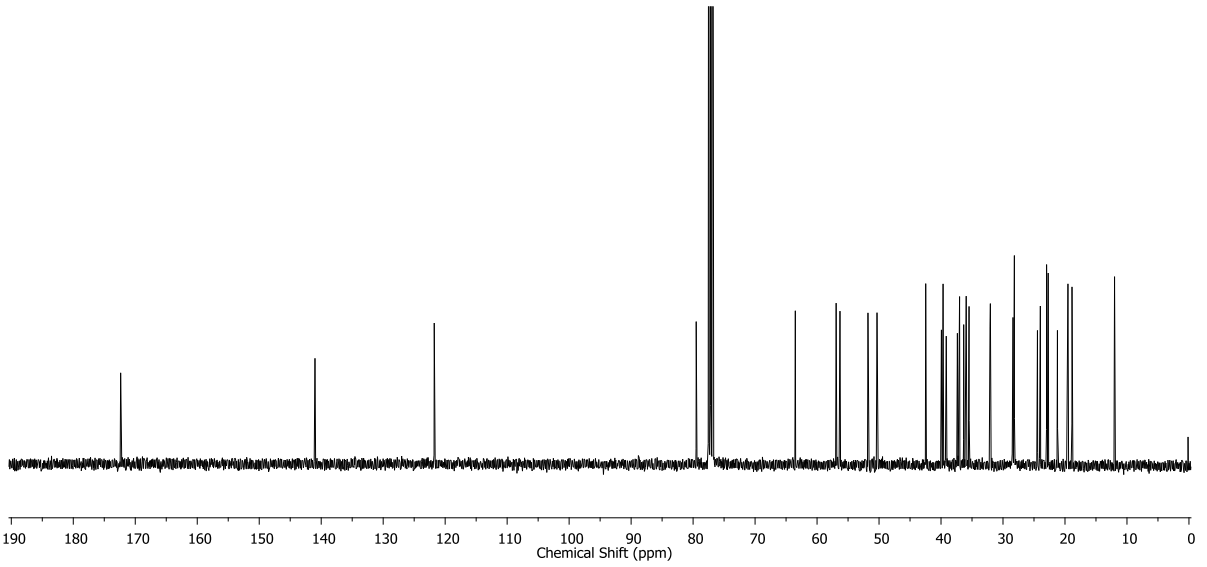

O
O
O

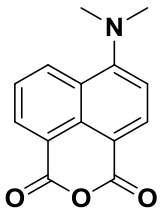

1.03
1.95
1.03
2.08
1.05
1.09
0.70
1.08
0.63
3.39
1.95
5.25
9.41
1.20
5.30
8.10
3.41
3.50
3.04
2.86
3.02
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemical Shift (ppm)

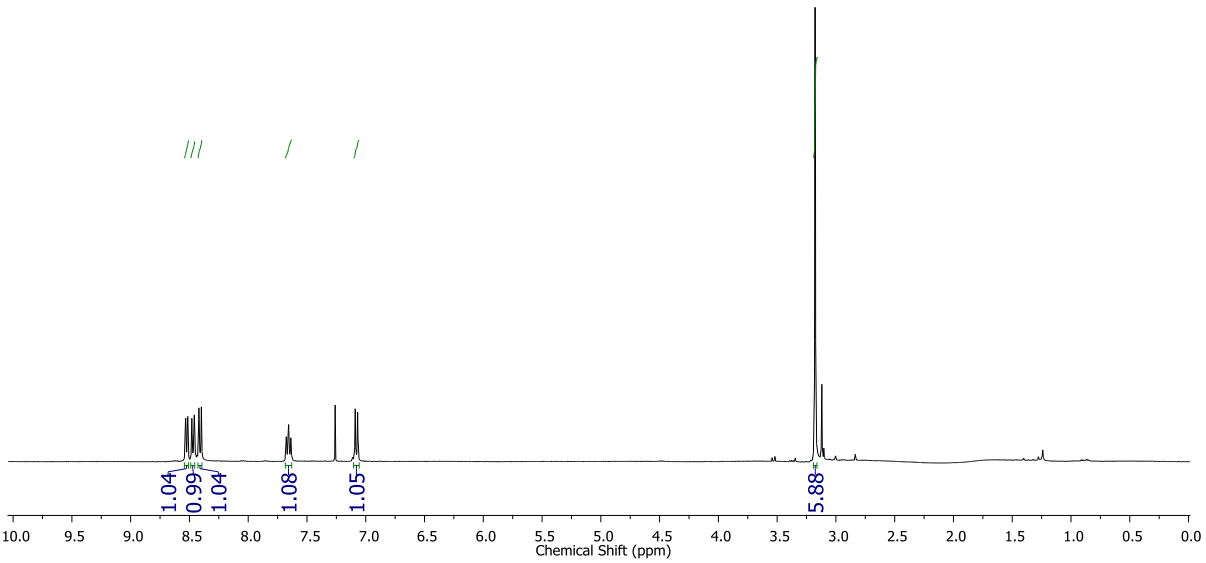

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
Chemical shift (ppm)

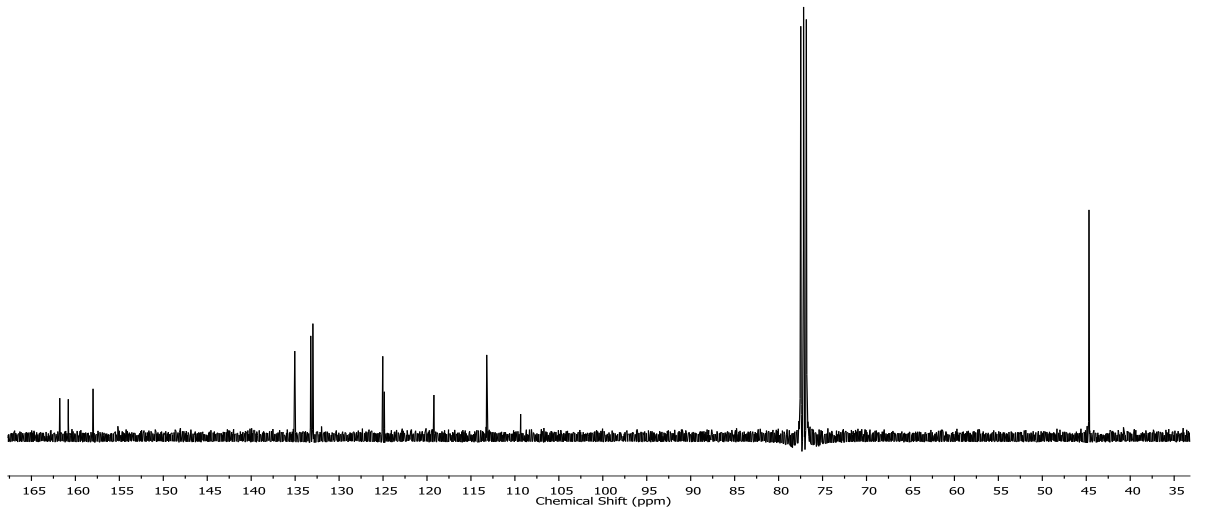

O
HO
O

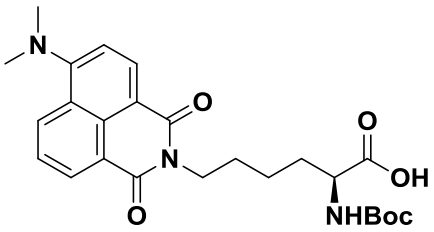

1.00
1.95
0.82
1.81
1.06
1.11
2.58
3.43
7.35
2.84
3.06
8.31
3.09
3.38
5.88
2.70
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemical Shift (ppm)

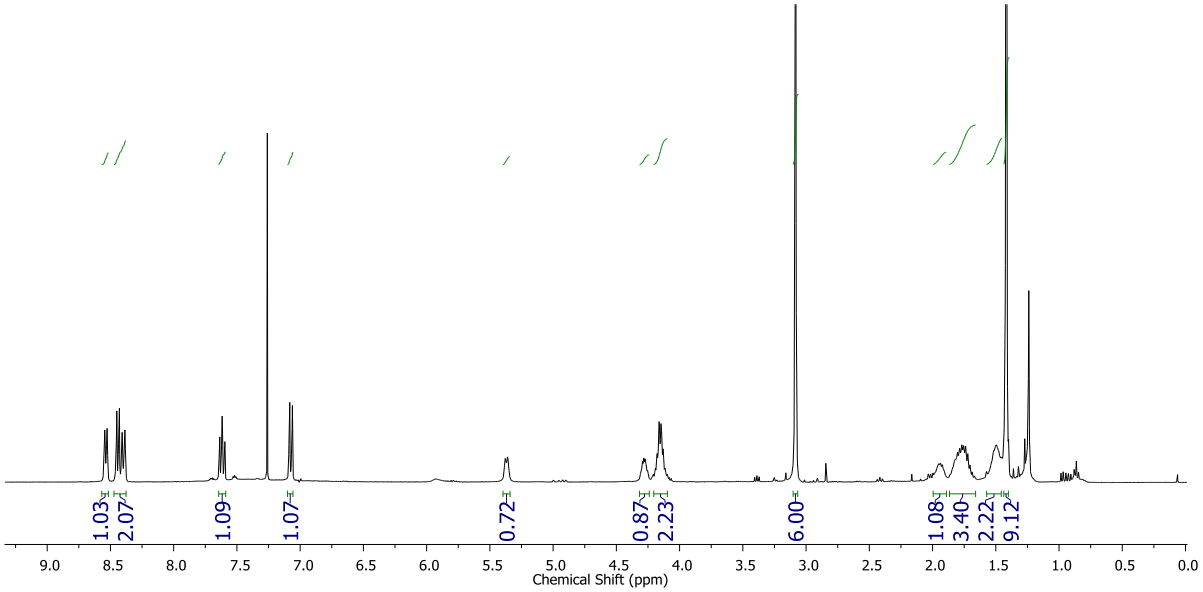

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Chemical Shift (ppm)

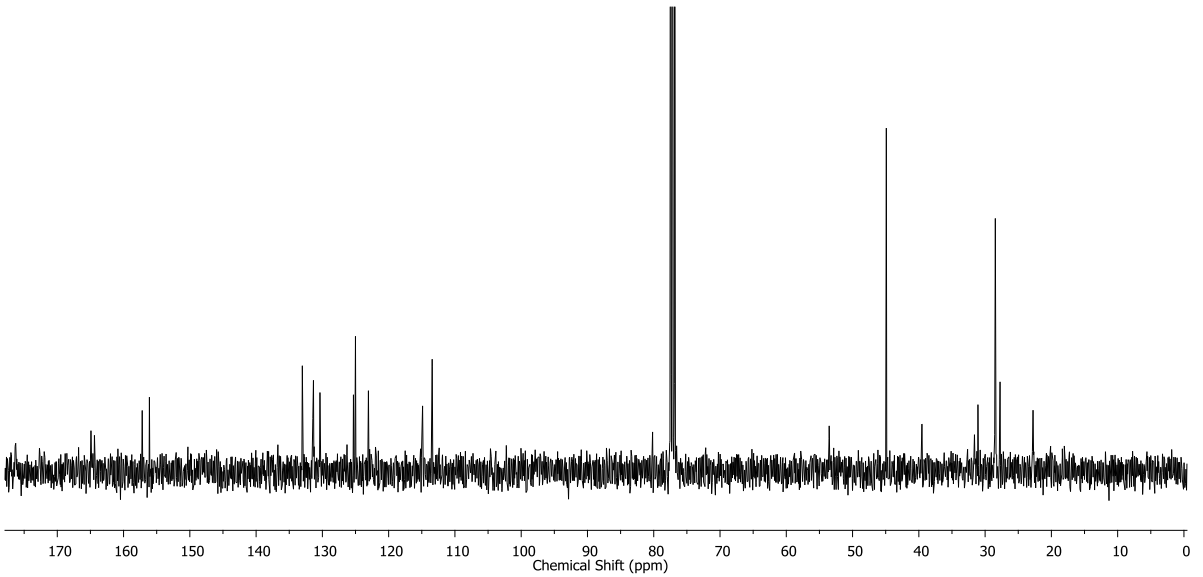

N
O
O
O

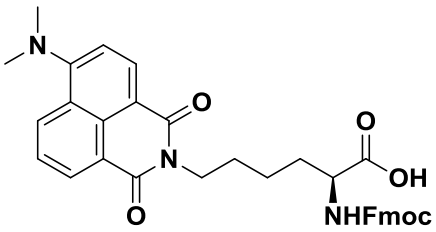

1.04
0.99
1.04
1.08
1.05
5.88
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemical Shift (ppm)

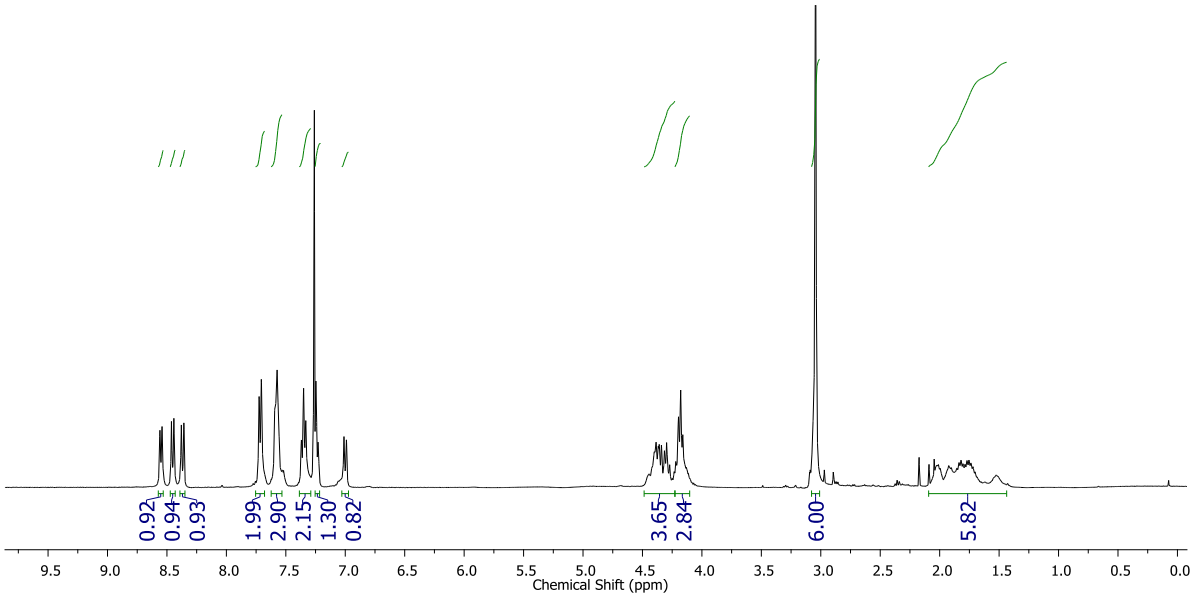

165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35
Chemical Shift (ppm)

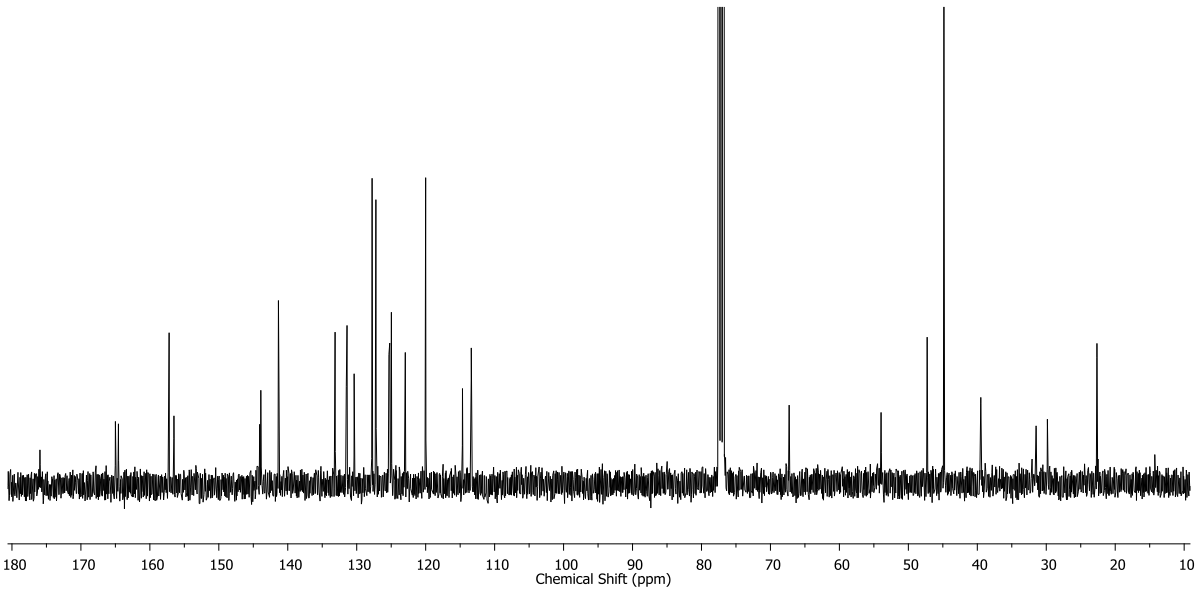

N
O
N
O
O
OH
NHBoc

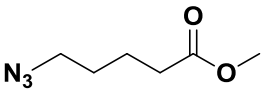

1.03
2.07
1.09
1.07
0.72
0.87
2.23
6.00
1.08
3.40
2.22
9.12
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemical Shift (ppm)

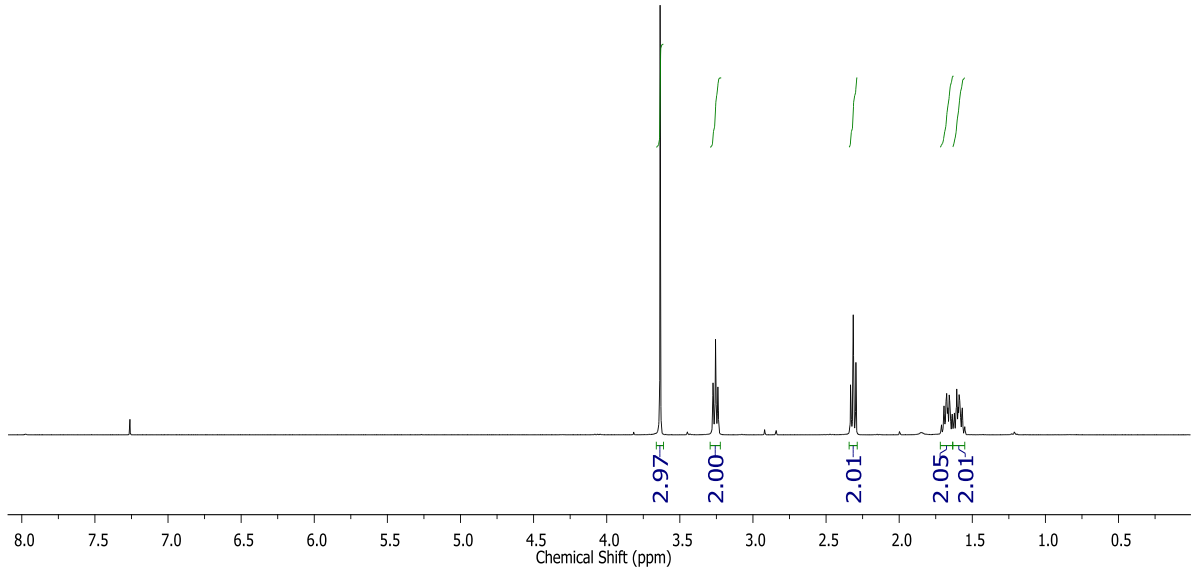

170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Chemical Shift (ppm)

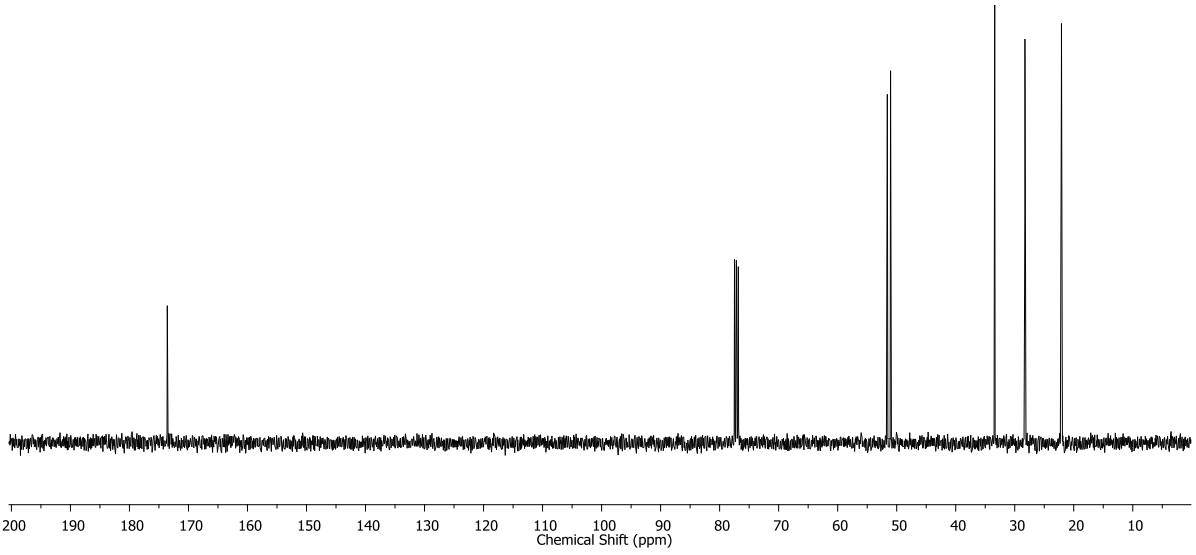

N
O
N
O
O
OH
O
NHFmoc

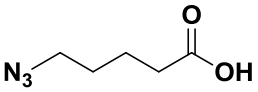

0.92
0.94
0.93
1.99
2.90
2.15
1.30
0.82
3.65
2.84
6.00
5.82
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Chemical Shift (ppm)

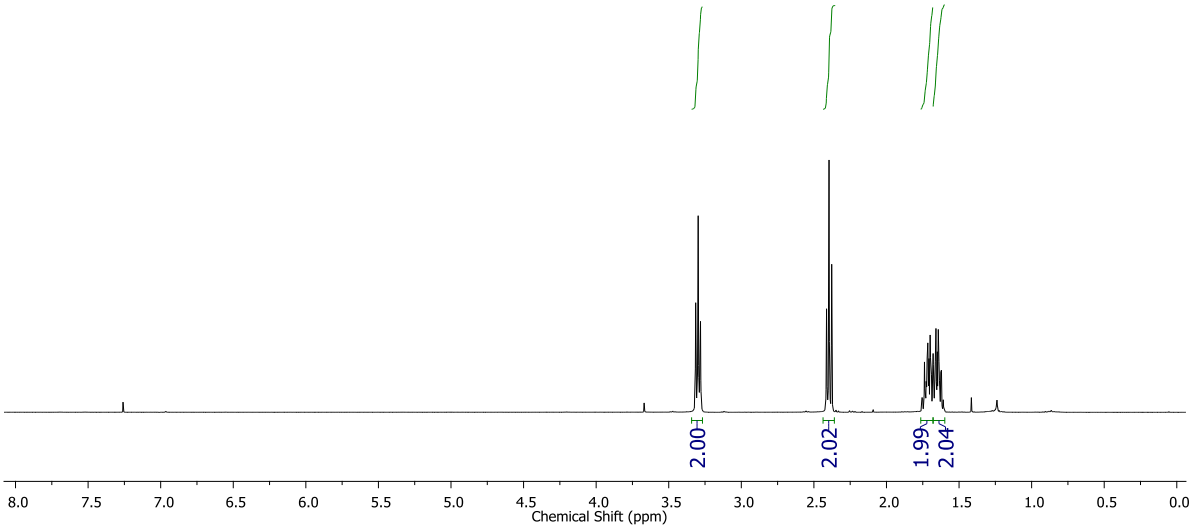

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
Chemical Shift (ppm)

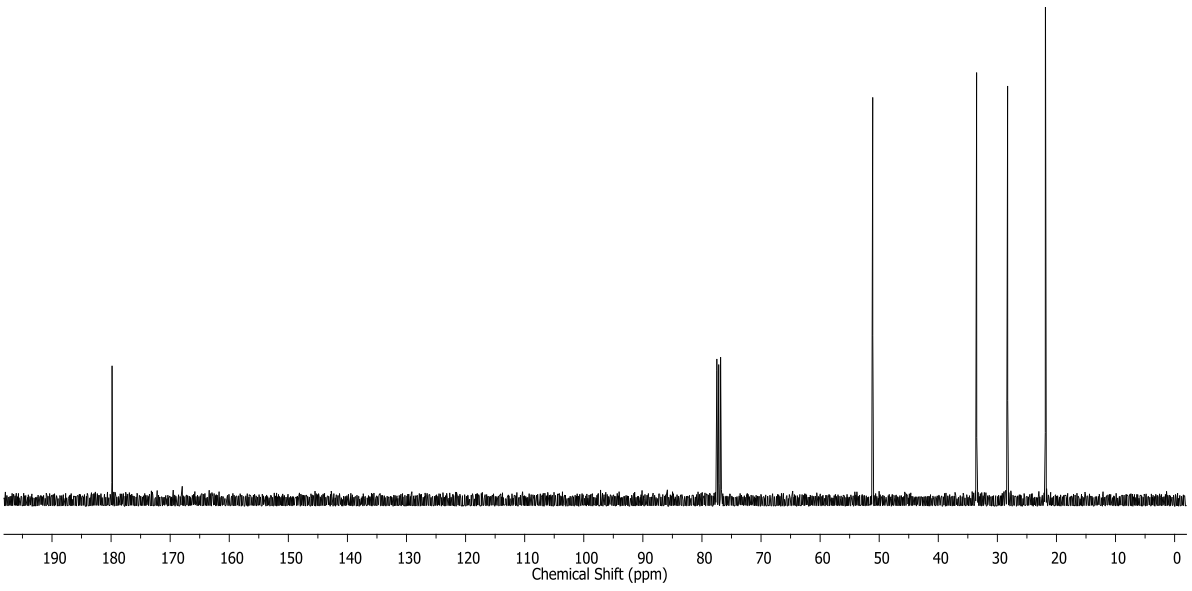

O
N3
O

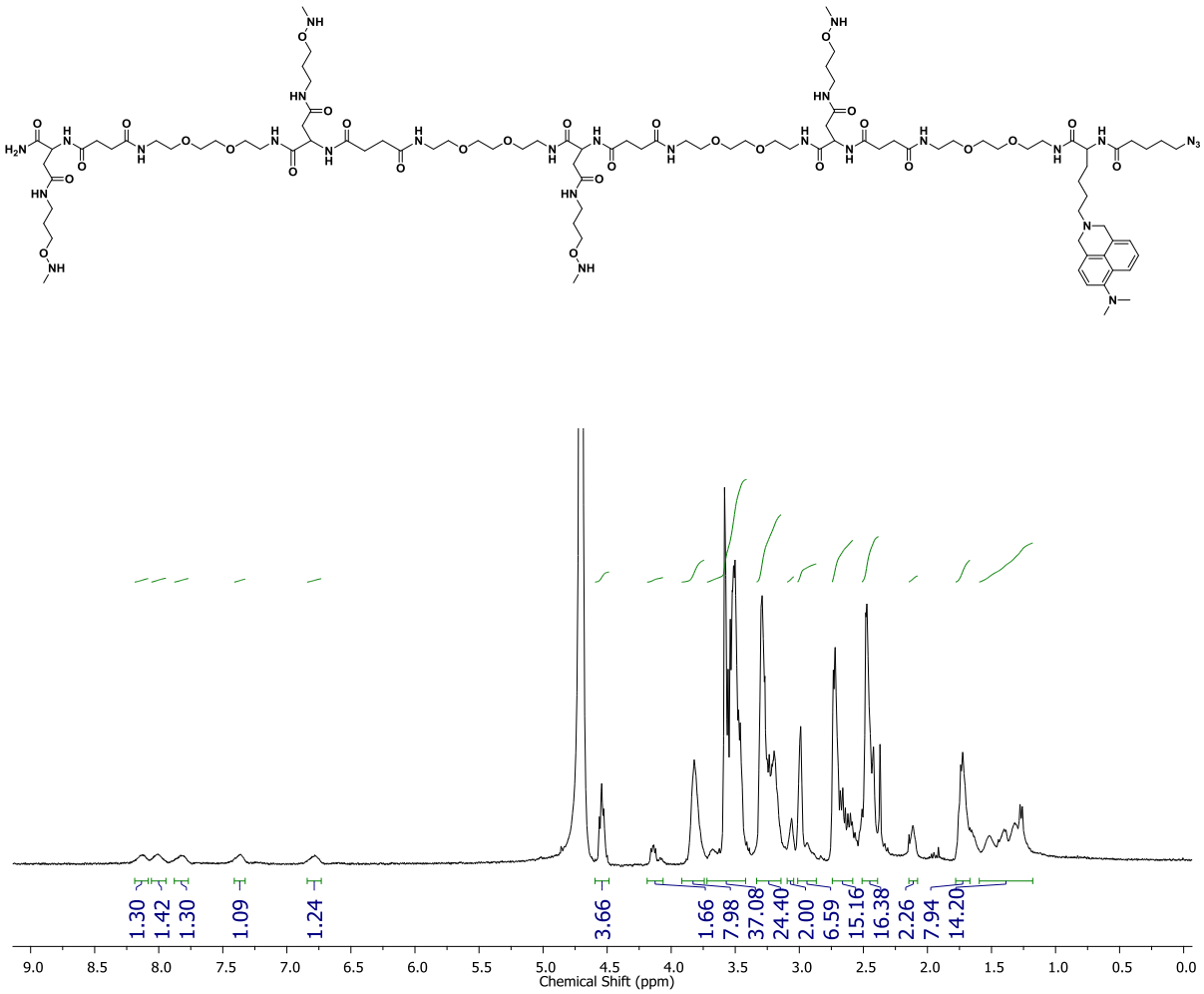

2.97
2.00
2.01
2.05
2.01
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)


200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
Chemical Shift (ppm)


O
N3
OH


2.00
2.02
1.99
2.04
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
Chemical Shift (ppm)


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Chemical Shift (ppm)


1.30
1.42
1.30
1.09
1.24
3.66
1.66
7.98
37.08
24.40
2.00
6.59
15.16
16.38
2.26
7.94
14.20
9.0
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
1.0
0.5
0.0
Chemical Shift (ppm)

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