Electronic Supporting Information

Site-selective post-modification of short α/γ hybrid foldamers: a powerful approach for molecular diversification towards biomedical applications

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1. General Remarks

Unless otherwise stated, the chemicals and reagents were obtained from following commercial sources. Ethyl acetate, dichloromethane, hexane, tetrahydrofuran, chloroform, acetone, sodium sulphate, methanol, potassium bisulphate from Rankem; silica gel, triethyl amine, boc-anhydride, methyl salicylate from Lobachemie; (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), from Sisco Research Laboratories; benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) from Tokyo Chemical Industry (TCI) and lithium hydroxide monohydrate, diisopropyl azodicarboxylate (DIAD), triphenyl phosphine (PPh_3), tert-butyl bromoacetate, diethylhydroxymethyl phosphonate, *N*-Z-ethanolamine, 2-(Boc-amino)ethyl bromide and celite from Sigma Aldrich. Dry solvents were prepared as per the standard procedures. Analytical thin layer chromatography was done on precoated silica gel plates (Kieselgel $60F_{254}$, Merck). Unless otherwise stated column chromatographic purifications were done with 230-400 mesh silica gel. NMR spectra were recorded in $CDCl_3$ on Bruker Ultra shield plus NMR spectrometer 500-MHz or Bruker Avance spectrometer 400 MHz. All chemical shifts are reported in δ ppm downfield to TMS referencing with $CDCl_3$ at 7.26 for proton and 77.0 for carbon or DMSO-d_6 at 2.50 for proton and 39.52 for carbon. NMR peak multiplicities are given as singlet (s), broad singlet (bs), doublet (d), triplet (t), doublet of doublet (dd), quartet (q), and multiplet (m). IR spectra were recorded using attenuated total reflectance-fourier transform infrared (ATR-FTIR) Perkin Elmer spectrometer. Melting point was determined with Stuart SMP40 automatic melting point apparatus. HRMS measurement was done by Electrospray ionization (ESI) on Xevo XS QTOF mass spectrometer waters ACQUITY UHPLC Milford USA. Matrix-Assisted Laser desorption/ionization (MALDI-TOF) was recorded on Bruker Autoflex speed using matrix 2,3-dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxycinnamic acid (CHCA). UV- studies were done with Shimadzu UV-1800 spectrophotometer using a standard 2 mL quartz cuvette and quantification of % drug loaded was studied using multi-well plate reader spectrophotometer (Synergy HT, BioTek) by taking absorbance at 288 nm. Fluorescence studies were carried out using Shimadzu RF-6000 Spectro fluorophotometer in methanol using a standard quartz cuvette of 2 mL capacity. Particle size analysis (PSA) studies were carried out using Malvern Nano-S90 and zeta potential was determined by MALVERN Nano-S. Field emission scanning electron microscopy (FE-SEM) studies were performed in JEOL JSM7600F using silicon wafer. High-resolution transmission electron microscopy (HR-TEM) studies were performed using

JEOL JEM2100 TEM, Tokyo, Japan using Formvar/carbon supported copper grid (200 mesh). Circular dichroism (CD) studies were done on J815 (Jasco) at 0.2 mM in acetonitrile. Confocal images were taken on TCS SP8 (Leica) microscope. Atomic Force Microscopy (AFM) studies were done on Bruker Nano wizard Sense AFM. Conformation analysis by computational studies was performed at the B3LYP/6-31G* level of theory using Gaussian09 software package.

2. General Synthetic Procedure

Procedure A: Ester hydrolysis

Hydrolysis of ester was performed in THF-H_2O mixture (1:1) with lithium hydroxide monohydrate (1.5 equiv.) at room temperature. The reaction was monitored by TLC. After completion of the reaction (~12 h), THF was removed under reduced pressure and ethyl acetate was added. The mixture was acidified with 1M $KHSO_4$ and organic layer was separated, washed with water, brine and finally dried over anhydrous sodium sulphate. Solvent was removed under reduced pressure to yield acid which was taken to the next reaction without further purification.

Procedure B: Boc-deprotection

To a stirred solution of Boc protected amine in THF cooled with ice cold water, dry HCl gas (generated *in situ* by NaCl/H_2SO_4) was bubbled for 20 min. The reaction was monitored by TLC. After completion of the reaction, ethyl acetate was added, and the mixture was basified with $NaHCO_3$. The organic layer was separated, washed with water, and brine and finally dried over anhydrous sodium sulphate. Solvent was removed under reduced pressure to yield amine which was taken to the next reaction without further purification.

Procedure C: Coupling with HBTU

To a stirred solution of amine (1 equiv.) in dry THF under nitrogen atmosphere at 0 ^{o}C, was added acid (1.1 equiv.), DIPEA (1.5 equiv.) and finally HBTU (1.3 equiv.) portion wise. After 15 min, the ice bath was removed, and the reaction mixture was stirred at room temperature for 12 h. Progress of the reaction was monitored by TLC. After completion of the reaction based on TLC, THF was evaporated under reduced pressure and mixture was diluted with ethyl acetate. The crude mixture was washed with $KHSO_4$ solution, $NaHCO_3$ solution, water, and finally with brine solution and organic layer was dried over anhydrous sodium sulphate and crude mixture was purified by column chromatography.

Procedure D: Coupling with PyBOP

To a stirred solution of acid (1 equiv.) in dichloromethane under nitrogen atmosphere, PyBOP (1.3 equiv.) and DIPEA (2.5 equiv.) were added sequentially at 0 ^{o}C. After 15 min added amine (1.1 equiv.) in dry DCM (35 mL) and the reaction was stirred overnight at rt. After completion of reaction based on TLC reaction mixture was diluted with DCM, washed with 1M $KHSO_4$ solution, saturated $NaHCO_3$ solution, water and finally with brine. The

organic layer was dried over anhydrous Na_2SO_4, and solvent was removed under reduced pressure. Crude product was purified with column chromatography.

Procedure E: *O*-alkylation

To a stirred solution of hydroxy compound (1 equiv.), side chain (1.2 equiv.), PPh_3 (1.5 equiv.) in THF in a round bottom flask at 0 ^oC under nitrogen added DIAD (1.5 equiv.) dropwise over 10 min and reaction mixture was stirred 0 ^oC for 45 min. The reaction was then stirred at room temperature for 90 min, and then at 50 ^oC for 16 h. The mixture was cooled to room temperature and THF was removed under reduced pressure yielding a yellow viscous oily liquid. Triphenyl phosphine oxide (PPh_3O) was removed by crystallization from a mixture of diethyl ether hexane mixture in 2:1 ratio and crude product was purified by column chromatography to afford mono *O*-alkylated product.

Procedure F: *O*-alkylation

To a stirred solution of hydroxy compound (1 equiv.), side chain (2.5 equiv.), PPh_3 (3 equiv.) in THF in a round bottom flask at 0 ^oC under nitrogen added DIAD (3 equiv.) dropwise over 10 min and reaction mixture was stirred 0 ^oC for 45 min. The reaction was then stirred at room temperature for 90 min, and then at 50 ^oC for 16 h. The mixture was cooled to room temperature and THF was removed under reduced pressure afforded a yellow viscous oily liquid. Triphenyl phosphine oxide (PPh_3O) was removed by crystallization from a mixture of diethyl ether hexane mixture in 2:1 ratio and crude product was purified by column chromatography to afford di *O*-alkylated product.

Procedure G: *O*-alkylation

A solution of the hydroxy compound (1 equiv.) K_2CO_3 (2 equiv.), and side chain (1 equiv.) in DMF was stirred for 12 hours at rt. Progress of the reaction was monitored by TLC and mixture was filtered and washed with ethyl acetate. Filtrate obtained was transferred into a separating funnel, washed with $KHSO_4$, and the organic layer was separated. The aqueous layer was back extracted (twice) with ethyl acetate and the combined organic layer was washed with water, brine and dried over anhydrous sodium sulphate. The crude product obtained after removal of solvent under reduced pressure was purified by column chromatography to obtain mono *O*-alkylated product.

Procedure H: *O*-alkylation

A solution of the hydroxy compound (1 equiv.) K_2CO_3 (4 equiv.), and side chain (2 equiv.) in DMF was stirred for 12 hours at rt. Progress of the reaction was monitored by TLC and mixture was filtered and washed with ethyl acetate. Filtrate obtained was transferred into a separating funnel, washed with $KHSO_4$, and the organic layer was separated. The aqueous layer was back extracted (twice) with ethyl acetate and the combined organic layer was washed with water, brine and dried over anhydrous sodium sulphate. The crude product obtained after removal of solvent under reduced pressure was purified by column chromatography to obtain di *O*-alkylated product.

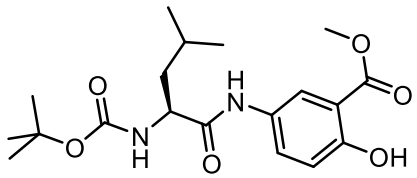
Procedure I: Debenzylation

To a round bottom flask containing *O*-Bn protected compound (1 equiv.) in ethyl acetate under nitrogen atmosphere, was added Pd-C (10% wt./wt.) dissolved in ethyl acetate and reaction mixture was stirred for 12 hours under hydrogen atmosphere at balloon pressure. Reaction was monitored by TLC and after completion of the reaction, the mixture was filtered over celite, and residue was washed thrice with ethyl acetate and thrice with THF. After removal of the solvent under reduced pressure product was purified by column chromatography.

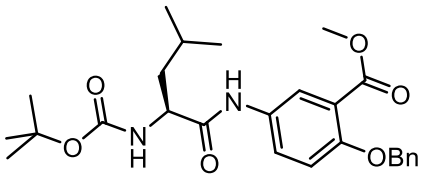
Procedure J: *N*-acylation

To a stirred solution of amine (1 equiv.). in dry THF at 0 ^oC added DIPEA (2 equiv.) and acetyl chloride (1 equiv.) and stirred at rt for one hour. Mixture was dissolved in ethyl acetate and washed with $KHSO_4$ solution, brine and water and dried over anhydrous sodium sulphate. After removal of the solvent under reduced pressure product was purified by column chromatography.

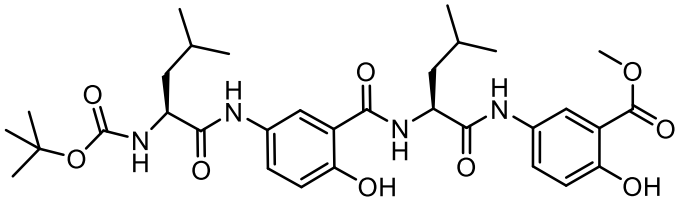
3. Naming of peptides



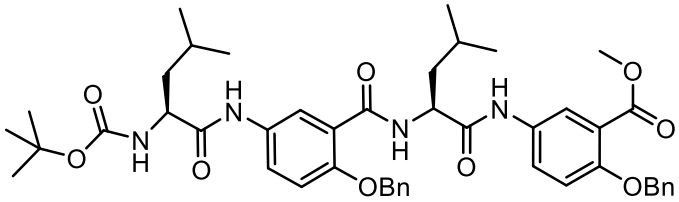
Boc-Leu-(5-ASA)-COOMe (17)



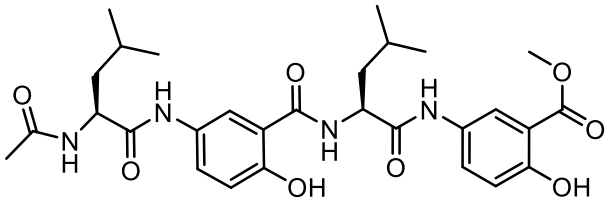
Boc-Leu-[*O*-Bn (5-ASA)]-COOMe (18)



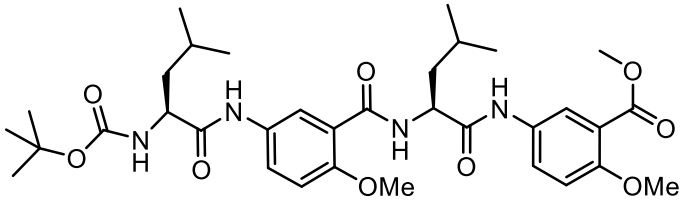
Boc-Leu-[5-ASA]-Leu-[5-ASA]-COOMe (1)



Boc-Leu-[*O*-Bn (5-ASA)]-Leu-[*O*-Bn (5-ASA)]-COOMe (2)



Acyl-Leu-[5-ASA]-Leu-[5-ASA]-COOMe (3)



Boc-Leu-[*O*-methyl (5-ASA)]-Leu-[*O*-methyl (5-ASA)]-COOMe (4)

4. Experimental Procedures

Circular dichroism studies

Peptides at 0.2 mM concentration were prepared in HPLC grade acetonitrile. The CD spectrum was taken with JASCO J-815. Wavelength range: 200-360 nm; scan rate: 50 nm/min; band width: 2 nm, data pitch: 0.1 nm, DIT: 4 sec, accumulations: 3.

Computational studies

Quantum mechanical calculations were performed at the B3LYP/6-31G* level of theory using Gaussian09 software package. All the optimized geometries are stationary points on the potential energy surface as confirmed by vibrational frequency calculation at the same level. Different conformers of tetrapeptide **1** differing in their energies relative to d (figure S18 a-d). were obtained using calculation done on above said package. Optimized minimum energy conformers of tetrapeptides **1** and **4** are given in Fig. S19 a-b).

2D-Nuclear Magnetic Resonance spectroscopy

Two-dimensional NMR experiments were performed in 400 MHz-Bruker Avance or 500 MHz Bruker Ultrashield plus spectrometer. The number of scans in 2D-NMR homonuclear experiments (COSY, TOCSY, and ROESY) and heteronuclear experiments (HMBC and HSQC) are 32 and 16, respectively.

UV-Visible spectroscopy

UV-visible absorption studies were carried out using Shimadzu UV-1800 spectrophotometer in methanol using 2 mL standard quartz cuvette from 190–600 nm with 0.2 nm stepping at continuous scanning rate. Quantification of loaded drug in nanosphere was done using multi-well plate reader spectrophotometer (Synergy HT, BioTek).

Fluorescence spectrophotometry studies

The studies were carried out using SHIMADZU RF-6000 Spectro fluorophotometer using 2 mL standard quartz cuvette. To find the critical aggregation concentration the stock solution of oligomer was made at 4 mM concentration by dissolving required amount in methanol and lower concentrations were prepared by dilution with methanol. Stepping rate at 1 nm with continuous scanning at excitation and emission band width of 0.1 nm.

SEM and FE-SEM studies

Stock solution of the oligomer was made at 1 mM in methanol and was first sonicated for 20 min. Oligomers at different concentrations (1 mM, 0.8 mM, 0.5mM, 0.2mM, and 0.09 mM) were prepared by diluting the stock solution with methanol and milli Q water to get final methanol: water solvent ratio (1:1, and 1:4). The solution was sonicated for 30 min. A 5µL

aliquot of peptide solution was drop casted on a silicon wafer and dried in a vacuum desiccator for 12 h. After coating with platinum, FE-SEM images were taken at 5 kV using JEOL JSM7600F instrument. SEM images were taken with SEM EV018 Zeiss instrument.

HR-TEM studies

Working concentration 0.2 mM was prepared by diluting the 1mM stock solution with methanol and milli Q water to get final methanol: water solvent ratio (1:4). The solution was sonicated for 30 minutes. A 5µL aliquot of peptide solution (0.2 mM, 20:80 MeOH-water) was taken on Formvar/carbon supported copper grid (200 mesh). It was air dried for 20 min and excess of solution was removed with a filter paper. Staining was done with 5µL of 1% uranyl acetate solution (prepared in 1:4 methanol-water ratio) and after 5 min, excess staining solution was removed using filter paper. Grid was allowed to dry for 12h. HR-TEM images of the dried samples were taken using JEOL JEM2100 TEM, Tokyo, Japan at 85 kV for tetrapeptide **1** and 85 kV and 150 kV for tetrapeptide **1**.

Atomic Force Microscopy

Peptide working solution (0.2 mM, 20:80 MeOH-water mixture) was sonicated for 30 min and a 5µL aliquot of peptide solution was taken on a clean mica sheet mounted on glass slide. The sample was dried under vacuum for 12 h. AFM images were taking using Bruker nanowizard sense instrument.

Fluorescence microscopic studies

Cellular intake studies were done with fluorescence microscope after treating triple-negative breast cancer cells (MDA-MB-231) with peptide **14a**. The live cell samples were imaged using Zeiss confocal laser scanning microscope 780 (CLSM) using its camera mode with excitation range 450-490 nm and emission range 515-565 nm. Images were captured using a 20X objective lens.

Confocal microscopy

Stock solution of oligomer **2** was made at 2 mM in methanol. 400 mL of peptide sample was mixed with 10 mL of 2mM dye (RhB in water and CF in methanol) and the final volume was made to 2mL using milli Q water. Final concentrations of peptide and dye were 0.4 mM and 10 mM, respectively. The solution was sonicated for 30 min. The resulting solution was aged for 12 h at 4 ^{o}C and centrifuged at 4 ^{o}C (10000 rpm, 30 min). Removed the supernatant and the residue obtained was washed with 20:80 methanol-water mixture (x2). Dispersed the residue in methanol water (20:80 v/v) to final concentration 0.4 mM. The solution was sonicated for 30 min. A 5µL aliquot of the solution was drop casted on a clean dry glass slide

and allowed to dry for 1 hour. Sample was covered with clean dry coverslip and allowed to dry for one hour. Images were taken using TCS SP8 (Leica) confocal microscope.

Drug encapsulation and release study

a) Fluorescence spectrophotometric titration study

Drug encapsulation study was carried with two peptides: (a) tetrapeptide **1** using rhodamine B and anticancer drug silibinin (b) cationic peptide **13a** with drug silibinin. Stock solution of the peptide **1** (2 mM) was prepared in methanol. Rhodamine B stock solution (2 mM) was prepared in milli Q water. Drug silibinin (2 mM) was prepared in methanol. Aliquot 200 μL of the peptide was taken in different vials and required volume of drug/dye solution was added to each vial and then diluted with water and methanol to (volume 2 mL) get final peptide concentration 0.2 mM in 20:80 methanol water medium. The vials were sonicated for 30 min for assembly and encapsulation. For the drug encapsulation study with tetrapeptide **13a**, stock solutions of the peptide and drug were prepared at 40 mM concentration in DMSO. Aliquot 25 μL of peptide stock solution was taken in different vials and mixed with required amount of drug solution and diluted with milli Q water and DMSO to get the final volume 5mL and final peptide concentration 0.2 mM (water-DMSO ratio 99:1). The vials were sonicated for 30 min for assembly and encapsulation. Fluorescence emission spectra were recorded with 5 nm and 3 nm excitation and emission band width at a stepping rate of 0.2 nm.

b) UV spectroscopy

First, a standard curve was made with different concentration of drug (20-180 μM) in methanol-water mixture (20:80 v/v). Taken 200 μL of the drug solutions in 96 well-plate and measured optical density at 288 nm using multi-well plate reader spectrophotometer (Synergy HT, BioTek). (Note: UV absorbance of the solution did not change with centrifugation indicating the drug did not undergo aggregation at this condition). Using the measured values, a standard curve was plotted (R^2 = 0.9956, Fig S32). To calculate the drug loading efficiency, from the peptide stock solution (2 mM) in methanol, aliquot 400 μL of the solution was taken in a 15 mL centrifuge tube and required volume of drug solution (200 μL, 2 mM) was added to the tube. It was then diluted with water and methanol (final volume 4 mL, concentration 0.2 mM, in 20:80 methanol water medium). The solution was sonicated for 30 min for assembly and encapsulation. The solution was stored at 4 ^{o}C for 12 h and centrifuged at 10000 rpm for 30 min. After centrifugation, optical density of the supernatant was measured using multi-well plate reader spectrophotometer.

Loading efficiency (LE) was calculated using following relation.[3]

LE = weight of drug added(w1) - weight of unloaded drug(w2) / weight of drug added(w1)

$$LE = \frac{w1-w2}{w1}$$

For **PN1**, LE was found to be 70 %.

Drug release Study

Drug release study with **PN1** and **PN13a** using anticancer drug silibilin was performed as follows. From the peptide **1** stock solution (2 mM) in methanol, aliquot 200 μL of the solution was taken into Eppendorf tube and required volume of drug solution (100 μL, 2 mM) was added to the tube. It was then diluted with water and methanol (final volume 2 mL, concentration 0.2 mM, in 20:80 methanol water medium). The solution was sonicated for 30 min for assembly and encapsulation. The solution was stored at 4 ^{o}C for 12h. For studies with peptide **13a**, stock solutions of peptide and drug were prepared at 40 mM in DMSO. Aliquot 25 μL of peptide stock solution was taken and 12.5 μL of drug solution in DMSO was added and diluted with milli Q water and DMSO to get the final volume 5mL (final peptide concentration 200 μM, water-DMSO ratio 99:1). The vials were sonicated for 30 min for assembly and encapsulation and aged for 12 h at 4 ^{o}C. Added 20 μL of 0.2 X acetate buffer solution (pH = 5) in each tube and incubated at 37 ^{o}C.[4] Fluorescence emission spectra was recorded at different time intervals (excitation band width 1.5 nm, 3 nm emission band width, stepping rate of 0.2 nm).

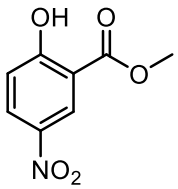
MTT assay

To access the biocompatibility of the peptides, peptide nanoparticles (PN) and drug loaded peptide nanoparticles (PND), the MDA-MB-231 cells were used for the in vitro cell viability study.[5] To conduct this experiment, 6 x 10^3 cells were seeded in 96 well culture plate. Post 24 hours of incubation, cells were treated for example, here with peptides (**1**, **2**, **4**, and **13a**) at required concentrations. Post 24 and 48 hours of incubation the treatment was removed and working concentration of MTT was added. After 4 hours of MTT incubation, the formazan crystals were dissolved using DMSO. The absorbance was measured at 570 nm using multi-mode plate reader (Synergy H1, BioTek, USA). The cell viability is directly proportional to the absorbance measured. Thus, the % cell viability of the treated group was calculated

against the viability of control group cells (cells incubated with culture media and DMSO equivalent to that in highest treatment concentration). The graph was plotted using GraphPad prism software and IC_{50} value was derived from it. The same method was used for peptide nanoparticles and drug loaded nanoparticles.

5. Synthetic Procedures

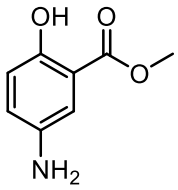
5-nitro-SA-COOMe (15)



15

Following the reported procedure[1] **15** was obtained from salicylic acid (7 mL, 54 mmol, 1 equiv.), acetic acid (6.2 mL, 108 mmol, 2 equiv.), nitric acid (22.5 mL, 538 mmol, 10 equiv.). Off-white solid (4.00 g, 37%); R_f: 0.5 (eluent: 20% ethyl acetate in hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 11.45 (s, 1H), 8.80 (s, 1H), 8.35–8.33 (d, J = 9.05 Hz, 1H), 7.10–7.08 (d, J = 9.17 Hz, 1H), 4.03 (s, 3H).

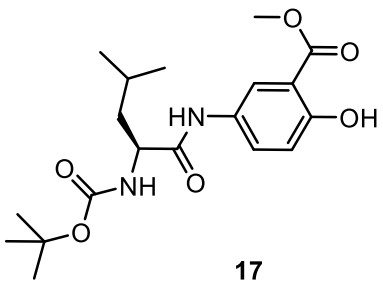
5-ASA-COOMe (16)



16

Following reported procedure[2] compound **16** was obtained from **15** (3.0 g, 15.21 mmol, 1 equiv.), $SnCl_2.2H_2O$ (17.17 g, 76.09 mmol, 5 equiv.) . Brown solid (2.14 g, 90%); R_f: 0.45 (eluent: ethyl acetate/hexane 70:30 v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.21 (s, 1H), 7.15 **(**s, 1H), 6.89–6.87 **(**d, J = 8.81 Hz, 1H), 6.83–6.82 (d, J = 8.72 Hz, 1H), 3.92 (s, 3H), 3.45 (bs, 2H).

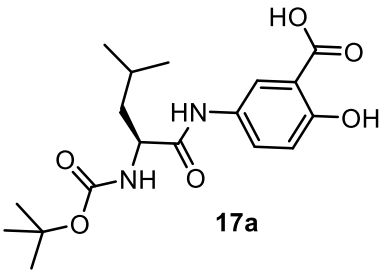
Boc-Leu-(5-ASA)-COOMe (17)



17

Following the general procedure C compound **17** was obtained from **16** (1.5 g, 8.98 mmol, 1 equiv.), Boc-Leucine (2.28 g, 9.87 mmol, 1.1 equiv.), DIPEA (2.5 mL, 14.8 mmol, 1.5 equiv.) and HBTU (4.43 g, 11.68 mmol, 1.3 equiv.). White puffy solid (2.91 g, 85%); R_f: 0.75 (eluent: ethyl acetate/hexane 7:3 v/v); m.p. 128 ^{o}C; $[\alpha]_D^{24}$: -35.4±0.2^o (c = 1, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.51 (s, 1H), 9.03 **(**s, 1H**),** 8.01 (s, 1H), 7.35–7.33 (d, J = 8.07 Hz, 1H), 6.77–6.75 (d, J = 8.46 Hz, 1H), 5.32–5.31 (d, J = 7.07 Hz, 1H**)**, 4.37–4.36 (m, 1H), 3.85 (s, 3H), 1.76–1.72 (m, 1H), 1.66–1.61 (m, 2H), 1.44 (s, 9H), 0.96–0.95 (d, J = 6.44 Hz, 3H) 0.93–0.92 (d, J = 6.33 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.5, 170.1, 157.9, 156.5, 129.8, 127.7, 120.6, 117.4, 111.5, 80.3, 53.7, 52.1, 41.2, 28.3, 24.7, 23.0, 21.6; IR (neat) $\nu_{max.}$ (cm^{-1}): 3290, 3100, 2955, 2867, 1680, 1660, 1528, 1490; ESI-MS: calculated for: $C_{19}H_{29}N_2O_6$: 381.2020 $(M+H)^+$; found: 381.2016.

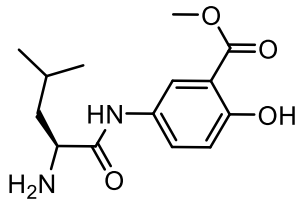
Boc Leu-(5-ASA)-COOH (17a)



17a

Following the general procedure A, compound **17a** was obtained from **17** (1 g, 2.63 mmol, 1 equiv.), lithium hydroxide monohydrate (0.165 g, 3.94 mmol, 1.5 equiv.). White solid (0.9 g, 93%); R_f: 0.20 (eluent: ethyl acetate/hexane 70:30 v/v); m.p. 159 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.79 (s, 1H), 9.56 (s, 1H**)**, 7.94–7.93 (d, J = 6.02 Hz, 1H), 7.38 (s, 1H), 6.79–6.78 (d, J = 8.73 Hz, 1H), 6.20 **(**bs, 1H**)**, 4.61 (bs, 1H), 1.87 (m, 2H) 1.67 (m, 1H), 1.30 (s, 9H), 1.03–1.01 (d, J =5.70 Hz, 3H), 0.96–0.95 (d, J = 5.01 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.3, 172.6, 158.7, 157.4, 129.1, 129.0, 122.0, 116.7, 111.8, 80.2, 53.9, 41.1, 28.3, 24.8, 23.4, 21.1; IR (neat) ν_{max} (cm^{-1}): 3302, 3105, 3070, 2962, 2920, 1778, 1670, 1535, 1500, 1442, 1273, 1172; ESI MS: calculated for $C_{18}H_{27}N_2O_6$: 367.1864 $(M+H)^+$; found; 367.1865.

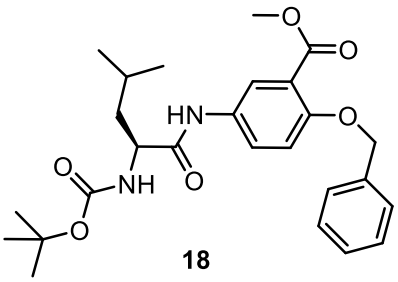
NH_2-Leu-(5-ASA)-COOMe (17b)



17b

General procedure B; **17** (1 g, 2.63 mmol, 1 equiv.). Yellow waxy liquid (0.7 g, 95%); R_f: 0.20 (eluent: ethyl acetate/hexane 60:40 v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.27 (s, 1H), 8.13 (s, 1H), 7.75 (s, 1H), 7.37–7.35 (d, J = 8.4 Hz, 1H), 6.84–6.82 (d, J = 8.6 Hz, 1H), 4.30 (s, 1H) 4.13–4.12 (s, 1H) 3.89, (s, 3H), 1.76 **(**m, 1H), 1.67 (m, 2H), 0.91–0.90 (d, J = 5.1 Hz, 3H), 0.89–0.88 (d, J = 4.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.3, 170.3, 158.1, 129.7, 127.9, 120.5, 117.8, 112.0, 53.7, 52.3, 43.7, 25.0, 23.3, 21.4; IR (neat) $\nu_{max.}$ (cm^{-1}): 3398, 3321, 3147, 3082, 2947, 2877, 1681, 1624, 1577, 1527, 1489, 1292, 1219, 1180; ESI-MS: calculated for $C_{14}H_{21}N_2O_4$: 281.1496$(M+H)^+$; found; 281.1492.

Boc-Leu-[*O*-Bn (5-ASA)]-COOMe (18)

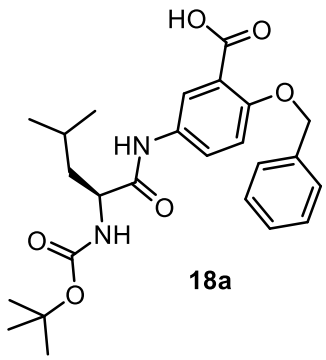


18

Compound **18** was synthesized following the general procedure G using **17** (4.6 g, 12.09 mmol, 1 equiv.) potassium carbonate (3.34 g, 24.2 mmol, 2 equiv.), benzyl bromide (1.43 mL, 12.09 mmol, 1 equiv.). Puffy solid (5.31 g, 95%), R_f: 0.5 (eluent: hexane/ethyl acetate 70:30 v/v); m.p. 88 ^{o}C; $[\alpha]_D^{24}$: -10.0 ± 0.3^{o} (c = 1, acetonitrile); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.38 (s, 1H), 7.87 (s, 1H), 7.69–7.68 (d, J = 7.82 Hz, 1H), 7.48–7.46 (d,

J = 7.32 Hz, 2H), 7.39–7.36 (t, *J* = 7.32 Hz, 2H) 7.32–7.29 (t, *J* = 7.29 Hz, 1H), 6.95–6.94 (d, *J* = 8.64 Hz, 1H), 5.15 (s, 2H), 4.95–4.93 (d, *J* = 7.17 Hz, 1H), 4.23 (s, 1H), 3.89 (s, 3H), 1.78–1.71 (m, 2H), 1.58–1.55 (m 1H), 1.46 (s, 9H), 0.98–0.95 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*: 171.2, 166.0, 156.4, 154.6, 136.7, 131.1, 128.4, 127.6, 126.8, 125.0, 123.2, 120.6, 114.5, 80.4, 70.9, 53.7, 51.9, 41.0, 28.3, 24.7, 23.0, 21.7; IR (neat) $\nu_{max.}$ (cm^{-1}): 3312, 2959, 1673, 1498, 1228, 1160; MALDI-TOF: (matrix: DHB) calculated for $C_{26}H_{34}N_2O_6Na$: 493.230 $(M+Na)^+$; found: 493.234; calculated for $C_{26}H_{34}N_2O_6K$: 509.204 $(M+K)^+$; found: 509.202.

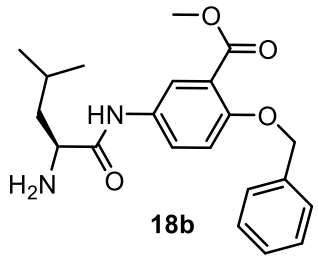
Boc-Leu-[*O*-Bn (5-ASA)]-COOH (18a)



18a

Following the general procedure A, compound **18a** was obtained from **18** (1 g, 2.13 mmol, 1 equiv.), lithium hydroxide monohydrate (0.133 g, 3.19 mmol, 1.5 equiv.). White puffy solid (0.90 g, 92%); R_f: 0.20 (eluent: ethyl acetate/hexane 70:30 v/v); ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 8.76 (s, 1H), 8.20 (s, 1 H), 8.03 (d, *J* = 2.77 Hz, 1H), 7.45–7.38 (m, 5H), 7.09–7.07 (d, *J* = 9.06 Hz, 1H), 5.26 (s, 2H), 5.06 (s, 1H), 4.34 (s, 1H), 1.78–1.72 (m, 2H), 1.60–1.56 (m, 1H), 1.46 (s, 9H), 0.98–0.95 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*:171.3, 166.1, 156.1, 153.8, 134.8, 132.9, 128.9, 128.8, 127.8 ,126.4, 124.4, 118.3, 114.0, 80.7, 72.3, 53.9, 41.2, 28.3, 24.8, 22.9, 21.9; IR (neat) $\nu_{max.}$ (cm^{-1}): 3340, 2955, 2927, 1702, 1662, 1518, 1255, 1168; MALDI-TOF: (matrix: DHB) calculated for $C_{25}H_{32}N_2O_6Na$: 479.215 $(M+Na)^+$; found: 479.212, calculated for $C_{25}H_{32}N_2O_6K$: 495.189 $(M+K)^+$; found: 495.190.

H_2N-Leu-[*O*-Bn (5-ASA)]-COOMe (18b)

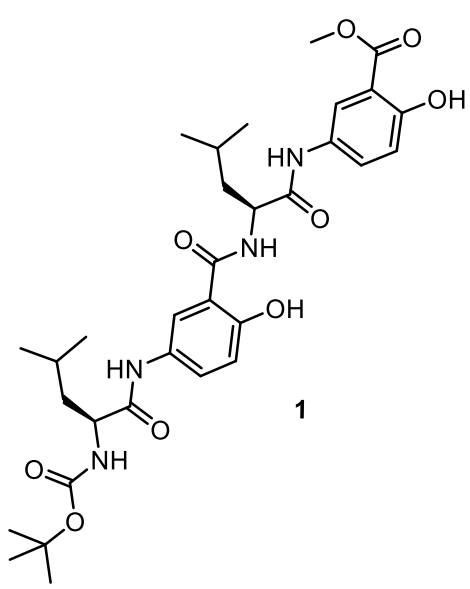


18b

Following the general procedure B, compound **18b** was obtained from **18** (1 g, 2.13 mmol, 1 equiv.). Yellowish waxy liquid (0.74 g, 95%); R_f: 0.45 (eluent: ethyl acetate/hexane 70:30 v/v); ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 9.63 (s, 1H), 7.92 (d, *J* = 2.67 Hz, 1H), 7.83–7.81 (dd, *J* = 8.94, 2.65 Hz, 1H), 7.47–7.46 (d, *J* = 7.45 Hz, 2H), 7.38–7.35 (m, 2H), 7.31–7.28 (m, 1H), 6.96–6.94 (d, *J* = 9.00 Hz, 1H), 5.13 (s, 2H), 3.88 (s, 3H), 3.57–3.54 (m, 1H), 2.81 (bs, 2H), 1.86–1.71 (m, 3H), 0.97–0.96 (d, *J* = 6.35 Hz, 3H), 0.94–0.93 (d, *J* = 6.13 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 166.3, 154.5, 136.7, 131.2, 128.6, 128.5, 127.7, 126.9, 126.8, 124.7, 122.8, 120.8, 114.9, 71.1, 53.7, 52.1, 43.7, 24.9, 23.3, 21.4; IR (neat) $\nu_{max.}$ (cm^{-1}): 3271, 2943, 2866, 1720,

1662, 15.4, 1446, 1242, 1207; ESI MS calculated for: $C_{21}H_{26}N_2O_4[M]^+$: 370.1893 found: 370.1917.

Boc-Leu-[5-ASA]-Leu-[5-ASA]-COOMe (1)



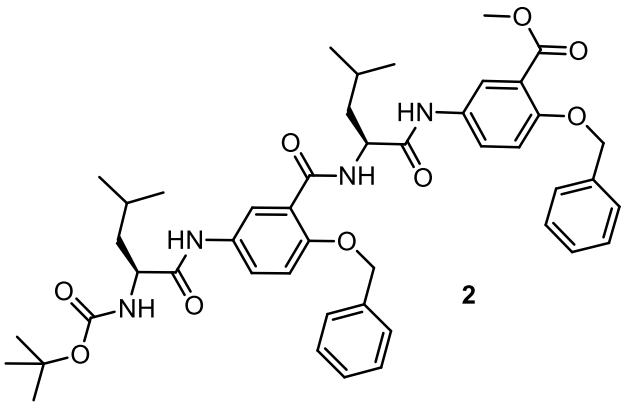
1

Following general procedure D, compound **1** was obtained from **17a** (2.0 g, 5.46 mmol, 1 equiv.), PyBOP (3.695 g, 7.1 mmol, 1.3 equiv.), DIPEA (2.4 mL, 13.65 mmol, 2.5 equiv.), **17b** (1.53 g, 5.46 mmol, 1 equiv.). White puffy solid **1** (1.1 g, 32 %); R_f: 0.30 (eluent: hexane/ethyl acetate 70:30 v/v); m.p. 125 ^oC; $[\alpha]_D^{24}$: -8.6.4 ± 0.3^o (c = 1, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$) δ: 11.59 (s, 1H), 10.47 (s, 1H), 9.78 (s, 1H), 8.70 (s, 1H), 8.02 (s, 1H), 7.99–7.98 (d, J = 8.21 Hz, 1H), 7.63–7.61 (d, J = 8.69 Hz, 1H), 7.56–7.54 (d, J = 8.75 Hz, 1H), 6.94–6.92 (d, J = 8.89 Hz, 1H), 6.75 (s, 1H), 6.73–6.71 (d, J = 8.88 Hz, 1H), 5.41–5.39 (d, J = 6.37 Hz, 1H), 4.89–4.88 (m, 1H), 4.44–4.43 (m, 1H), 3.94 (s, 3H), 1.99–1.93 (m, 1H), 1.91–1.88 (m, 1H), 1.79–1.67 (m, 4H), 1.17 (s, 9H), 1.06–1.05 (d, J = 6.40 Hz, 3H), 1.03–0.99 (m, 6H) 0.96–0.94 (d, J = 6.25 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.3, 170.4, 170.0, 169.7, 158.3, 157.8, 157.0, 129.6, 129.0, 128.4, 128.0, 127.6, 126.8, 121.6, 118.8, 117.8, 117.8, 114.1, 111.9, 80.9, 53.9, 52.9, 52.4, 41.6, 40.5, 28.0, 24.8, 24.7, 23.0, 22.9, 21.9, 21.5; IR (neat) ν_{max} (cm^{-1}): 3300, 2955, 2870, 1665, 1494, 1220, 1160; MALDI-TOF: (matrix: DHB) calculated for $C_{32}H_{44}N_4O_9Na$: 651.300 $(M+Na)^+$; found: 651.309; calculated for $C_{32}H_{44}N_4O_9K$: 667.274 $(M+K)^+$; found: 667.277.

Boc-Leu-[5-ASA]-Leu-[5-ASA]-COOMe (1) by debenzylation of 2

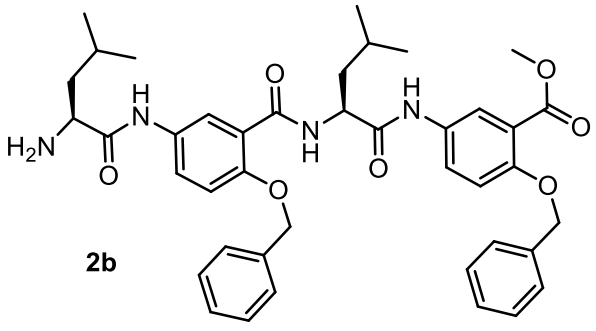
Compound **1** was also synthesized following general procedure I from **2** (1.1 g, 1.36 mmol, 1 equiv.), Pd-C (10%, 0.110 g) yielded white puffy solid (0.81 g, 94%). Complete characterization of the compound is given above.

Boc-Leu-[*O*-Bn (5-ASA)]-Leu-[*O*-Bn (5-ASA)]-COOMe (2)



Following the general procedure D, compound **2** was obtained using **18a** (1.50 g, 3.285 mmol, 1 equiv.), **18b** (1.22 g, 3.285 mmol, 1 equiv.), PyBOP (2.22 g, 4.27 mmol, 1.3 equiv.), DIPEA (1.43 mL, 8.21 mmol, 2.5 equiv.). White puffy solid **2** (2.35 g, 88%); R_f: 0.30 (eluent: hexane/ethyl acetate/DCM 65:30:5 v/v); m.p. 102 ^{o}C; $[\alpha]_D^{24}$: -10.6 ± 0.2^{o} (*c* = 1, acetonitrile); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.56 (s, 1H), 9.29 (s, 1H), 8.29–8.28 (d, *J* = 5.74 Hz, 1H), 7.95 (s, 1H), 7.90–7.88 (d, *J* = 8.95 Hz, 1H), 7.85 (s, 1H), 7.64–7.62 (d, *J* = 8.79 Hz, 1H), 7.49–7.35 (m, 9H), 7.30–7.29 (d, *J* = 7.32 Hz, 1H), 6.91–6.87 (m, 2H), 5.33–5.32 (d, *J* = 7.27 Hz, 1H), 5.13 (s, 2H), 5.10–5.08 (d, *J* = 10.13 Hz, 1H), 5.05–5.03 (d, *J* = 9.81 Hz, 1H), 4.65–4.61 (m, 1H), 4.49 (m, 1H), 3.86 (s, 3H), 1.74–1.48 (m, 5H), 1.44 (s, 9H), 1.40–1.38 (m, 1H), 0.93–0.92 (d, *J* = 5.74 Hz, 3H), 0.89–0.88 (d, *J* = 5.15 Hz, 3H), 0.78–0.77 (d, *J* = 6.28 Hz, 3H), 0.72–0.71 (d, *J* = 6.28 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.9, 170.5, 166.1, 165.2, 156.4, 154.5, 153.7, 136.9, 135.1, 131.6, 131.5, 128.9, 128.6, 128.5, 127.7, 126.8, 125.3, 124.0, 123.3, 123.2, 120.3, 120.3, 114.5, 112.6, 80.4, 71.7, 71.0, 53.7, 52.0, 51.8, 41.8, 40.2, 28.4, 24.7, 22.8, 21.8, 21.0, 20.7, 20.7; IR (neat) $\nu_{max.}$ (cm^{-1}): 3303, 3065, 2958, 1641, 1494, 1216, 1162; MALDI-TOF: (matrix: DHB) calculated for: $C_{46}H_{56}N_4O_9Na$: 831.394 $(M+Na)^+$; found: 831.390; calculated for: $C_{46}H_{56}N_4O_9K$: 847.367 $(M+K)^+$; found: 847.363.

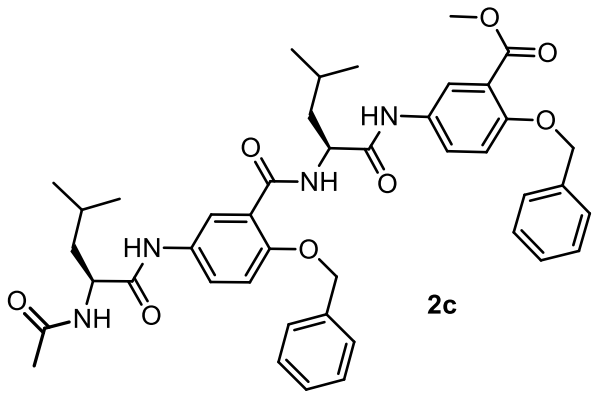
Leu-[*O*-Bn (5-ASA)]-Leu-[*O*-Bn (5-ASA)]-COOMe (2b)



Following the general procedure B compound **2b** was obtained from **2** (0.9 g, 1.11 mmol, 1 equiv.). White puffy solid (0.762 g, 97%); R_f: 0.25 (eluent: ethyl acetate); m.p. 79 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.25 (s, 1H), 8.41–8.40 (d, *J* = 7.46 Hz, 1H) 8.10–8.09 (d, *J* = 2.47 Hz, 1H), 8.04–8.02 (m, 1H), 7.90–7.87 (dd, *J* = 8.87, 2.34 Hz, 1H), 7.75–7.73 (dd, *J* = 8.94, 2.02 Hz, 1H), 7.57–7.56 (d, *J* = 6.85 Hz, 2H), 7.50–7.48 (d, *J* = 7.47 Hz, 2H), 7.41–7.37 (m, 6H), 733–7.29 (m, 2H), 7.22–7.20 (d, *J* = 9.08 Hz, 1H), 5.28–5.2 (m, 2H), 5.18 (s, 2H), 3.82, (s,

3H), 3.50–3.39 (m, 4H), 1.76–1.73 **(**m, 1H), 1.47–1.34 (m, 5H), 0.92**–**0.91 (d, *J* = 6.55 Hz, 3H), 0.90**–**0.88 (d, *J* = 6.53 Hz, 3H), 0.83**–**0.83 (d, *J* = 5.67 Hz, 3H), 0.76**–**0.74 (d, *J* = 5.66 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 174.2, 171.2, 166.2, 164.6, 153.8, 152.7, 137.5, 136.5, 133.0, 132.4, 129., 129.0, 128.9, 128.8, 128.7, 128.1, 127.6, 127.5, 125.0, 124.0, 122.5, 122.3, 120.6, 115.2, 114.3, 71.3, 70.4, 54.1, 52.8, 52.4, 44.2, 41.5, 24.8, 24.7, 23.6, 23.3, 22.4, 22.0; IR (neat) $\nu_{max.}$ (cm^{-1}): 3275, 3068, 2955, 2871, 1733, 1638, 1537, 1223; MALDI-TOF (matrix used DHB): calculated for $C_{41}H_{48}N_4O_7Na$: 731.341 $(M+Na)^+$; found 731.337, calculated for $C_{41}H_{48}N_4O_7K$: 747.315 $(M+K)^+$; found: 747.317.

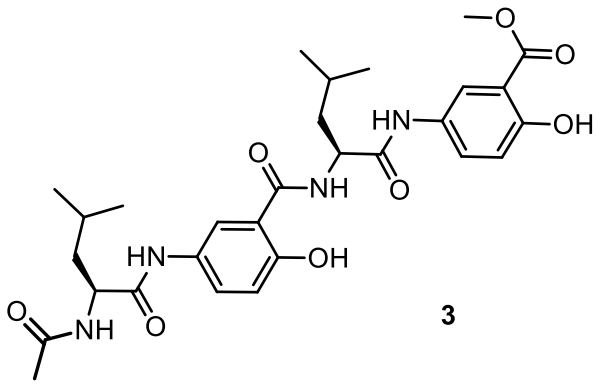
Acyl-Leu-[*O*-Bn (5-ASA)]-Leu-[*O*-Bn (5-ASA)]-COOMe (2c)



2c

Following the general procedure J, compound **2c** was obtained using **2b** (0.7 g, 0.98 mmol, 1 equiv.), DIPEA (350 μL, 1.98 mmol, 2 equiv.), acetyl chloride (70 μL, 0.98 mmol, 1 equiv.). Whitish solid (0.702 g, 95%); R_f: 0.25 (eluent: DCM/methanol 95:5 v/v); m.p. 116 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 9.95 (s, 1H), 9.56 (s, 1H), 8.53–8.51 (d, *J* = 7.10 Hz, 1H), 8.03–8.02 (d, *J* = 2.57 Hz, 1H), 7.97–7.95 (d, *J* = 8.87 Hz, 1H), 7.88–7.87 (d, *J* = 2.68 Hz, 1H), 7.73–7.71 (dd, *J* = 8.95, 2.51 Hz, 1H), 7.48–7.46 (d, *J* = 7.17 Hz, 4H), 7.42–7.35 (m, 6H), 7.30–7.29 (d, *J* = 7.35 Hz, 1H), 6.95–6.94 (d, *J* = 9.02 Hz, 1H), 6.88–6.86 (d, *J* = 9.07 Hz, 1H), 6.80–6.77 (m, 1H), 5.18–5.16 (d, *J* = 10.57 Hz, 1H), 5.13 (s, 2H), 5.11–5.09 (d, *J* = 10.64 Hz, 1H), 4.91–4.87 (m, 1H), 4.81–4.77 (m, 1H), 3.85 (s, 3H), 2.09 (s, 3H), 1.71–1.48 **(**m, 6H), 0.90**–**0.89 (d, *J* = 6.16 Hz, 3H), 0.87–0.85 (d, *J* = 5.85 Hz, 3H), 0.75**–**0.73 (d, *J* = 6.44 Hz, 3H), 0.69–0.68 (d, *J* = 6.47 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 171.5, 170.7, 170.4, 166.5, 165.0, 154.4, 153.7, 136.8, 135.2, 131.8, 131.7, 128.9, 128.8, 128.4, 128.4, 127.7, 126.8, 126.4, 125.2, 124.2, 123.1, 120.7, 120.4, 114.6, 113.0, 71.7, 71.0, 53.3, 52.5, 52.0, 42.0, 41.0, 24.8, 24.6, 23.2, 22.8, 22.8, 22.2, 21.8; IR (neat) $\nu_{max.}$ (cm^{-1}): 3299, 3088, 2926, 2855, 1695, 1635, 1495, 1413, 1214; MALDI-TOF (matrix used DHB): calculated for $C_{43}H_{50}N_4O_8Na$ 773.352 $(M+Na)^+$; found 773.358, calculated for $C_{43}H_{50}N_4O_8K$: 789.326 $(M+K)^+$; found: 789.332.

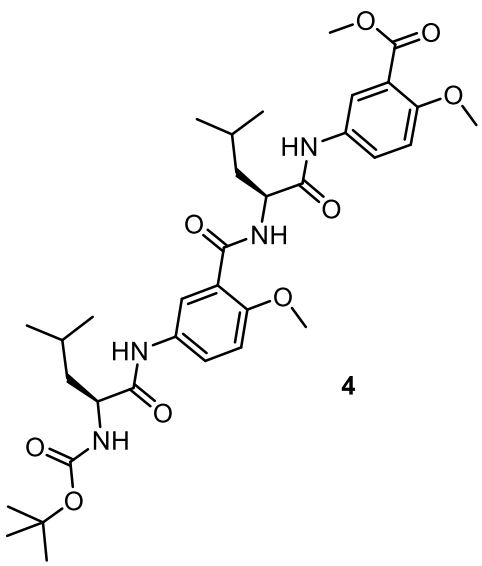
Acyl-Leu-[5-ASA]-Leu-[5-ASA]-COOMe (3)



3

Following the general procedure I, compound **3** was obtained from **2c** (0.650 g, 0.87 mmol, 1 equiv.), Pd-C (0.065 g, 10% wt/wt). White crystalline solid (0.45 g, 91%); R_f: 0.25 (eluent: ethyl acetate hexane, 80:20 v/v); m.p. 113 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$/DMSO-d_6 (2:1)) δ: 10.54 (s, 1H), 9.85 (s, 1H), 9.62 (s, 1H), 8.27–8.26 (d, J = 8.09 Hz, 1H), 8.19 (d, J = 2.55 Hz, 1H), 7.94 (d, J = 2.18 Hz, 1H), 7.68–7.67 (dd, J = 8.96, 2.55 Hz, 1H), 7.64–7.62 (d, J = 8.35 Hz, 1H), 7.59–7.57 (dd, J = 8.86, 2.27 Hz, 1H), 6.90–6.88 (d, J = 8.95 Hz, 1H), 6.84–6.82 (d, J = 8.86 Hz, 1H), 4.86–4.83 (m, 1H), 4.68–4.63 (m, 1H), 3.93 (s, 3H), 3.05 (s, 1H), 1.97 (s, 3H), 1.83–1.62 (m, 6H), 0.99–0.94 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$/DMSO-d_6 (2:1)) δ: 171.2, 170.7, 170.6, 169.8, 168.7, 157.5, 156.7, 129.9, 129.1, 128.0, 126.8, 120.6, 118.9, 117.3, 117.1, 114.1, 111.3, 52.1, 51.9, 40.9, 40.7, 24.41, 24.35, 22.60, 22.57, 22.4, 21.5, 21.4; IR (neat) $\nu_{max.}$ (cm^{-1}): 3286, 2957, 1676, 1642, 1561, 1491, 1293, 1243, 1197, 1085; MALDI-TOF (matrix used DHB): calculated for $C_{29}H_{38}N_4O_8Na$: 593.258 $(M+Na)^+$, found 593.250; calculated for $C_{29}H_{38}N_4O_8K$: 609.232 $(M+K)^+$; found: 609.22.

Boc-Leu-[*O*-methyl (5-ASA)]-Leu-[*O*-methyl (5-ASA)]-COOMe (4)

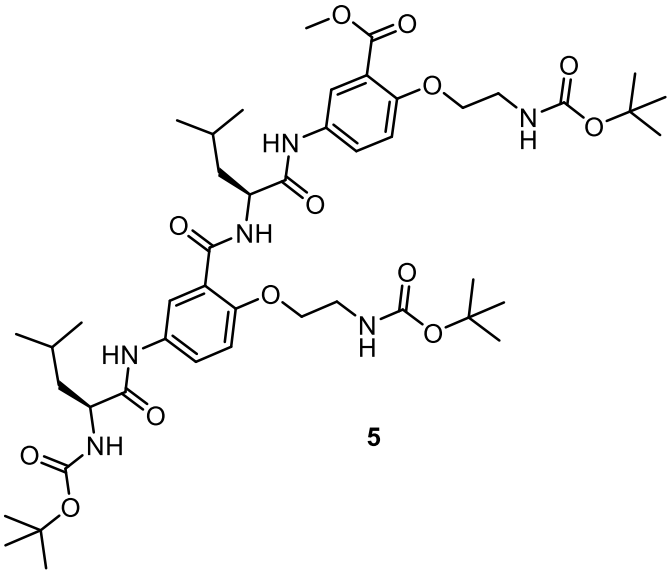


4

Following general procedure H, compound **4** was obtained from **1** (125 mg, 0.198 mmol, 1 equiv.), MeI (25 μL, 0.4 mmol, 2 equiv.), K_2CO_3 (109 mg, 0.8 mmol, 4 equiv.), Off white puffy solid **(**120 mg, 92%) R_f: 0.45 (eluent: hexane/ethyl acetate 55:45 v/v); mp: 112 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.53 (s, 1H), 9.31 **(**s, 1H), 8.31–8.30 (d, J = 5.93 Hz, 1H), 7.90 (s, 2 H), 7.85 (s, 1 H), 7.68–7.67 (d, J = 8.12 Hz, 1H), 6.84–6.82 (d, J = 8.21 Hz, 2H), 5.24–5.22 **(**d, J = 8.16 Hz, 1H**)**, 4.82 (m, 1H), 4.50 (m, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H) 1.83–1.65 **(**m, 6H), 1.44 **(**s, 9H); 1.00–0.88 **(**m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.7, 170.6, 166.1, 165.2, 156.3, 155.5, 154.1, 131.6, 131.0, 126.0, 125.6, 123.8,

123.4, 120.5, 119.4, 112.3, 111.7, 80.2, 56.2, 53.7, 53.4, 51.9, 41.9, 40.0, 28.3, 25.0, 24.7, 23.1, 22.8, 22.1, 21.8; IR (neat) ν_{max} cm^{-1}: 3295, 2957, 2929, 1665, 1487, 1286, 1225, 1161; MALDI-TOF (matrix used: DHB): calculated for $C_{34}H_{48}N_4O_9Na$ 679.331 $(M+Na)^+$; found: 679.329; calculated for $C_{34}H_{48}N_4O_9K$: 695.305 $(M+K)^+$; found: 695.309.

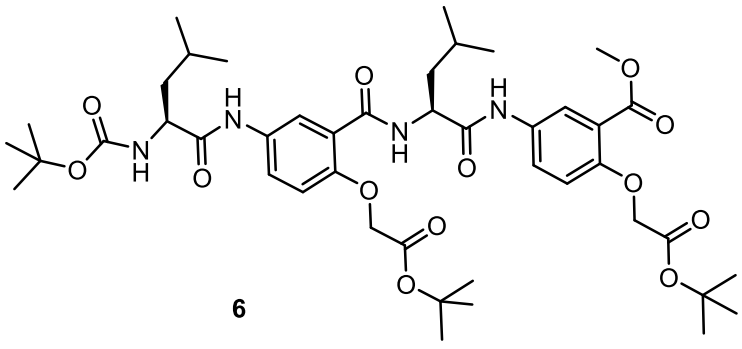
Boc-Leu-[*O*-NBocethylamine (5-ASA)]-Leu-[*O*-NBocethylamine (5-ASA)]-COOMe (5)



5

Following general procedure H, compound **5** was obtained from **1** (100 mg, 0.16 mmol, 1 equiv.), 2-(Boc-amino) ethyl bromide (71 mg, 0.32 mmol, 2 equiv.), K_2CO_3 (88 mg, 0.64 mmol, 4 equiv.). White puffy solid (93 mg, 64%); R_f: 0.35 (eluent: ethyl acetate/hexane 65:35 v/v); m.p. 57 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.64 (s, 1H), 9.46 (s, 1H), 8.96 (s, 1H), 8.00–7.95 (m, 3H), 7.84 (s, 1H), 6.90–6.89 (m, 2H), 6.68 (s, 1H), 5.53 (s, 1H), 5.46 (s, 1H), 5.14–5.13 (d, J = 6.25 Hz, 1H), 4.48 (bs, 1H), 4.21–4.08 (m, 4H), 3.91 (s, 3H), 3.63–3.54 (m, 4H), 1.78–1.61 (m, 6H), 1.44 (s, 9H), 1.39 (s, 18H), 0.93–0.82 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.7, 171.3, 166.7, 164.5, 162.5, 156.4, 156.0, 155.0, 153.7, 131.3, 127.0, 126.8, 124.1, 120.5, 120.2, 114.7, 112.6, 79.9, 79.4, 79.1, 69.0, 68.9, 53.7, 53.1, 52.3, 42.7, 42.0, 40.2, 39.9, 28.39, 28.35, 28.3, 24.8, 23.1, 22.9, 22.7, 22.2; IR (neat) $\nu_{max.}$ (cm^{-1}): 3317, 2958, 2930, 1682, 1496, 1250, 1163; MALDI-TOF (matrix used: DHB): calculated for $C_{46}H_{70}N_6O_{13}Na$: 937.489 $(M+Na)^+$; found 937.497, calculated for $C_{46}H_{70}N_6O_{13}K$: 953.463 $(M+K)^+$; found: 953.467.

Boc-Leu-[*O*-*tert*-butylacetate (5-ASA)]-Leu-[*O*-*tert*-butylacetate (5-ASA)]-COOMe (6)

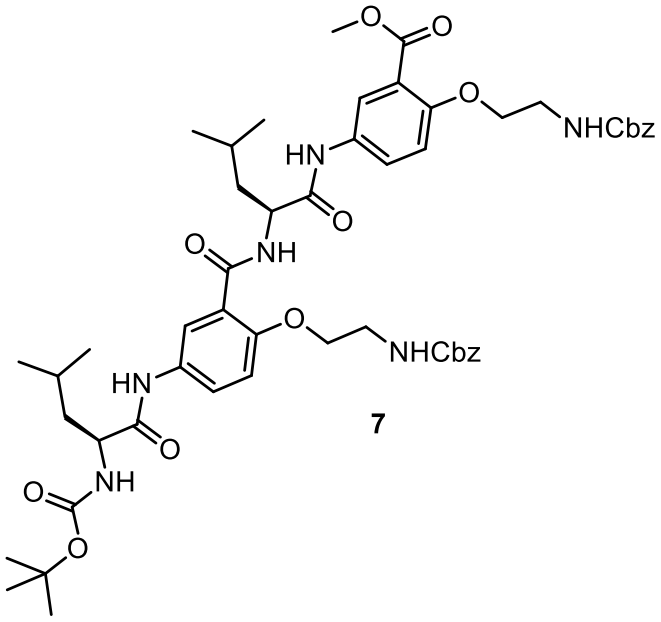


6

Following general procedure H, compound **6** was obtained from **1** (100 mg, 0.16 mmol, 1 equiv.), *tert*-butyl bromoacetate (47 μL, 2 equiv., 0.32 mmol) and K_2CO_3 (88 mg, 0.64 mmol, 4 equiv.). White puffy solid (102 mg, 75%); R_f: 0.45 (eluent: hexane/ethyl acetate 1:1 v/v; m.p.

72 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.62 (s, 1H), 9.33 (s, 1H), 9.04–9.03 (d, J = 6.17 Hz, 1H), 7.95–7.94 (d, J = 2.60 Hz, 1H), 7.84 (d, J = 2.58 Hz, 1H), 7.81–7.79 (d, J = 7.54 Hz, 1H), 7.68–7.66 (d, J = 11.37 Hz, 1H), 6.74–6.72 (d, J = 8.85 Hz, 2H), 5.22–5.20 (d, J = 8.55 Hz, 1H), 4.78–4.74 (m, 1H), 4.59–4.58 (m, 2H), 4.54 (s, 2H), 4.50 (bs, 1H), 3.84 (s, 3H), 2.07–2.01 (m, 2H), 1.86 (m, 1H), 1.73–1.66 (m, 3H), 1.50 (s, 9H), 1.46 (s, 9H), 1.45 (s, 9H), 1.01–1.00 (d, J = 6.44 Hz, 3H), 0.94–0.93 (d, J = 6.45 Hz, 3H), 0.91–0.90 (d, J = 6.05 Hz, 3H), 0.88–0.87 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.7, 171.1, 167.7, 167.0, 165.7, 165.1, 156.3, 153.7, 152.5, 139.2, 132.5, 132.0, 126.4, 124.8, 124.6, 123.1, 120.6, 114.5, 112.6, 82.9, 82.1, 80.3, 67.2, 66.1, 54.2, 53.6, 51.9, 41.9, 40.1, 29.6, 28.3, 28.0, 24.7, 23.1, 22.7, 22.6, 21.8, 21.5; IR (neat) $\nu_{max.}$ cm^{-1}: 3313, 2958, 2923, 1674, 1496, 1214; MALDI-TOF (matrix used DHB): calculated for $C_{44}H_{64}N_4O_{13}Na$: 879.436 $(M+Na)^+$; found: 879.441, calculated for $C_{44}H_{64}N_4O_{13}K$: 895.410 $(M+K)^+$; found: 895.414.

Boc-Leu-[*O*-*N*-Cbz ethylamine (5-ASA)]-Leu-[*O*-*N*-Cbz ethylamine (5-ASA)]-COOMe (7)

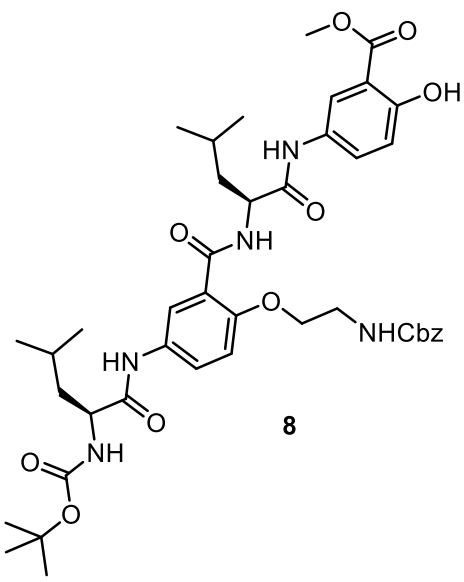


7

Following general procedure F compound **7** was obtained from **1** (150 mg, 0.24 mmol, 1 equiv.), PPh_3 (187 mg, 0.72 mmol, 3 equiv.), *N*-Z-ethanolamine (116 mg, 0.60 mmol, 2.5 equiv.), and DIAD (138 μL, 0.72 mmol, 3 equiv.). White puffy solid, (103 mg, 54%); R_f: 0.25 (eluent: hexane/ethyl acetate 55:45 v/v; m.p. 59 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.62 (s, 1H), 9.42 (s, 1H), 8.96 (s, 1H), 8.03–7.93 (m, 3H), 7.80 (s, 1H), 7.35–7.24 (m, 10H), 6.92–6.90 (d, J = 7.00 Hz, 1H), 6.60–6.58 (d, J = 7.36 Hz, 1H), 5.87 (bs, 1H), 5.45 (bs, 1H), 5.15–5.06 (m, 5H), 4.48 (bs, 1H), 4.24–4.17 (m, 2H), 3.98 (m, 2H), 3.83 (s, 3H), 3.73–3.60 (m, 5H), 1.73–1.63 (m, 6H), 1.41 (s, 9H), 0.92–0.85 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.7, 171.4, 166.6, 164.4, 156.9, 156.5, 155.9, 154.9, 153.6, 136.7, 136.5, 131.7, 131.2, 128.4, 128.3, 128.0, 127.9, 127.8, 127.6, 126.9, 124.0, 123.9, 120.5, 120.0, 114.8, 112.6, 79.9, 68.8, 68.6, 66.6, 66.4, 53.7, 53.1, 52.3, 42.8, 41.9, 40.6, 40.3, 28.3, 24.8, 24.7, 23.0, 22.9, 22.6, 22.1; IR (neat) $\nu_{max.}$ cm^{-1}: 3305, 2955, 2925, 1699, 1495, 1251; MALDI-TOF (matrix used: DHB): Calculated for $C_{52}H_{66}N_6O_{13}Na$:

1005.458 $(M+Na)^+$; found 1005.460, calculated for $C_{52}H_{66}N_6O_{13}K$: 1021.431 $(M+K)^+$; found: 1021.437.

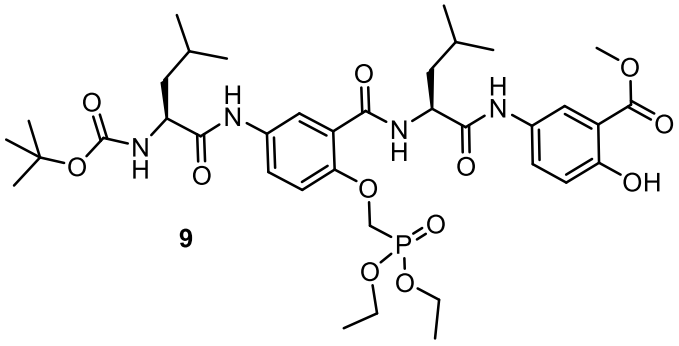
Boc-Leu-[*O*-*N*-Cbz ethylamine (5-ASA)]-Leu-[5-ASA]-COOMe (8)



8

Following general procedure E compound **8** was obtained from **1** (100 mg, 0.16 mmol, 1 equiv.), PPh_3 (62 mg, 0.24 mmol, 1.5 equiv.), DIAD (46 μL, 0.24 mmol, 1.5 equiv.), and *N*-Z-ethanolamine (37 mg, 0.19 mmol, 1.2 equiv.). White puffy solid (76 mg, 60%); R_f: 0.5 (eluent: hexane/ethyl acetate 3:2 v/v); m.p. 62 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.57 (s, 1H), 9.36 (s, 1H), 9.21 (s, 1H), 8.83–8.82 (d, J = 8.09 Hz, 1H), 7.94–7.88 (m, 3H), 7.62–7.61 (d, J = 7.59 Hz, 1H), 7.36–7.35 (m, 1H), 7.25 (m, 3H), 7.03 (s, 1H), 6.88–6.87 (d, J = 7.52 Hz, 1H), 6.76–6.75 (d, J = 8.21 Hz, 1H), 5.30 (m, 1H), 5.06 (m, 3H), 4.50 (m, 1H), 4.24 (m, 1H), 4.14–4.12 (m, 1H), 3.82 (s, 3H), 3.66 (m, 2H), 1.80–1.56 (m, 6H), 1.40 (s, 9H), 0.97–0.87 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.7, 171.4, 170.0, 164.5, 158.5, 157.0, 156.2, 153.7, 136.5, 131.5, 129.5, 129.2, 128.5, 128.3, 127.9, 127.6, 127.0, 124.2, 122.1, 120.6, 117.7, 112.6, 111.8, 80.1, 68.6, 66.5, 53.7, 52.9, 52.2, 42.7, 42.0, 40.6, 28.3, 24.8, 23.1, 22.9, 22.7, 22.2; IR (neat) $\nu_{max.}$ cm^{-1}: 3359, 3265, 2963, 2923, 1708, 1640, 1520, 1491, 1366, 1293, 1164; MALDI-TOF (with matrix DHB): Calculated for $C_{42}H_{55}N_5O_1Na$: 828.379 $(M+Na)^+$, Found 828.374; calculated for $C_{42}H_{55}N_5O_{11}K$: 844.353 $(M+K)^+$, Found: 844.349.

Boc-Leu-[*O*-methyl diethoxyphosphonate (5-ASA)]-Leu-[5-ASA]-COOMe (9)

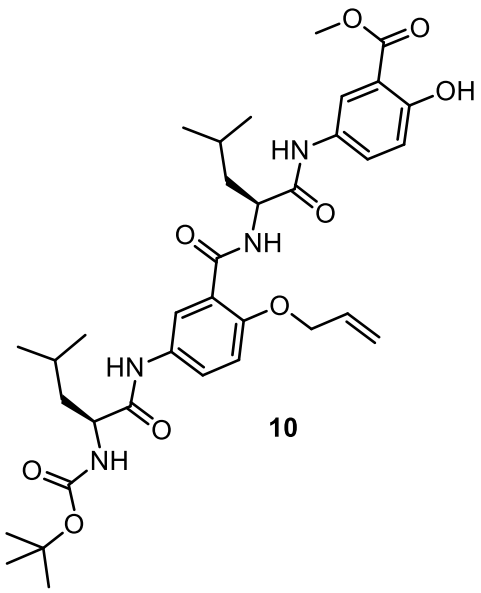


9

Following general procedure E compound **9** was obtained from **1** (100 mg, 0.16 mmol, 1 equiv.), PPh_3 (63 mg, 0.24 mmol, 1.5 equiv.), diethyl(hydroxymethyl)phosphonate (28 μL, 0.19 mmol, 1.2 equiv.), DIAD (46 μL, 0.24 mmol, 1.5 equiv.). White puffy solid (77 mg, 62%); R_f: 0.35 (eluent: ethyl acetate/hexane 3:2 v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.52 (s, 1H), 9.78 (s, 1H), 9.44 (s, 1H), 8.28–8.26 (d, J = 6.18 Hz, 1H), 8.01 (s, 1H), 7.94 (d, J = 2.31 Hz, 1H), 7.82–7.81 (d,

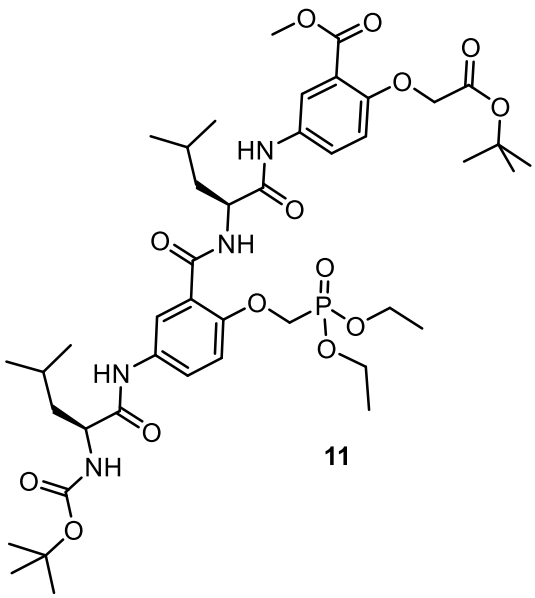
J = 8.18 Hz, 1H) 7.41–7.39 (d, *J* = 8.35 Hz, 1H), 6.88–6.87 (d, *J* = 8.98 Hz, 1H), 6.76–6.74 (d, *J* = 8.87 Hz, 1H), 5.47–5.45 (d, *J* = 8.50 Hz, 1H), 4.73–4.72 (m, 1H), 4.52–4.51 (m, 1H), 4.45–4.19 (m, 6H), 3.84 (s, 3H), 1.80–1.55 (m, 6H), 1.45 (s, 9H), 1.40–1.33 (m, 6H), 1.02–1.01 (d, *J* = 5.48 Hz, 3H), 0.95–.094 (d, *J* = 5.68 Hz, 3H), 0.89–0.87 (d, *J* = 6.45 Hz, 3H), 0.85–0.84 (d, *J* = 6.21 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 171.8, 170.5, 170.4, 164.6, 157.7, 156.4, 153.3, 153.2, 132.4, 130.2, 128.2, 126.3, 124.4, 121.0, 120.6, 117.3, 112.3, 111.7, 80.2, 63.2, 63.0, 61.6, 54.1, 53.6, 52.2, 41.8, 40.6, 28.3, 24.8, 24.7, 22.9, 22.8, 22.1, 21.7, 16.4, 16.4; MALDI-TOF (with matrix CHCA): calculated for $C_{37}H_{55}N_4O_{12}PNa$: 801.344 $(M+Na)^+$; Found 801.346; calculated for $C_{37}H_{55}N_4O_{12}PK$: 817.318 $(M+K)^+$, found: 817.321.

Boc-Leu-[*O*-2-propenyl (5-ASA)]-Leu-[5-ASA]-COOMe (10)



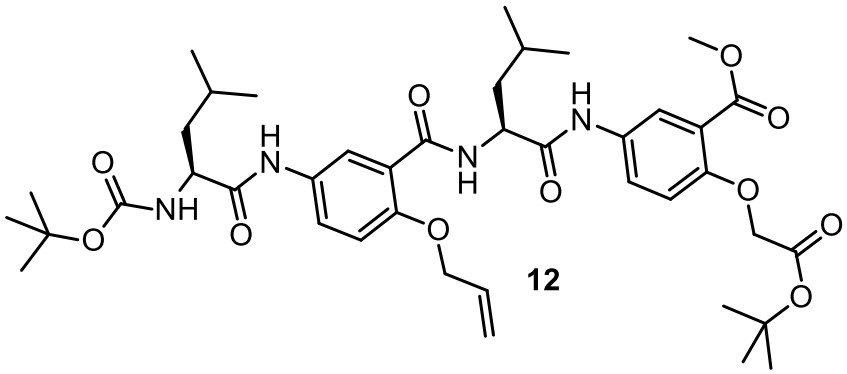
General procedure G, **1** (100 mg, 0.16 mmol, 1 equiv.), K_2CO_3 (44 mg, 0.32 mmol, 2 equiv.) and allyl bromide (14 μL, 0.16 mmol, 1 equiv.). White puffy solid (84 mg, 80%); R_f: 0.35 (eluent: hexane/ethyl acetate 3:2 v/v); ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 10.57 (s, 1H), 9.78 (s, 1H), 9.38 (s, 1H), 8.37–8.35 (d, *J* = 6.39 Hz, 1H), 8.05–8.04 (d, *J* = 2.58 Hz, 1H), 7.94 (d, *J* = 2.76 Hz, 1H), 7.83–7.81 (d, *J* = 8.67 Hz, 1H), 7.37–7.35 (dd, *J* = 8.85 Hz, 2.12 Hz, 1H), 6.81–6.80 (d, *J* = 8.92 Hz, 2H), 6.12–6.05 (m, 1H), 5.50–5.46 (dd, *J* = 17.20 Hz, 1.22 Hz, 1H), 5.40–5.38 (dd, *J* = 10.40 Hz, 0.93 Hz, 1H), 5.24–5.22 (d, *J* = 8.76 Hz, 1H), 4.76–4.72 (q, *J* = 7.08 Hz, 1H), 4.61 (m, 2H), 4.55–4.54 (m, 1H), 3.86 (s, 3H), 1.82–1.62 (m, 6H), 1.45 (s, 9H), 1.01–0.99 (d, *J* = 6.38 Hz, 3H), 0.95–0.94 (d, *J* = 6.30 Hz, 3H), 0.91–0.90 (d, *J* = 6.48 Hz, 3H), 0.87–0.86 (d, *J* = 6.39 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 171.7, 170.5, 170.4, 165.2, 157.8, 156.4, 153.4, 131.74, 131.71, 130.1, 128.2, 126.2, 124.1, 121.1, 120.4, 120.1, 117.4, 112.8, 111.7, 80.4, 70.3, 53.7, 53.6, 52.2, 42.0, 40.8, 28.3, 24.85, 24.76, 23.2, 22.8, 21.80, 21.77; MALDI-TOF: calculated for $C_{35}H_{48}N_4O_9Na$: 691.331$(M+Na)^+$, found 691.326; calculated for $C_{35}H_{48}N_4O_9K$: 707.305 $(M+K)^+$, found: 707.301.

Boc-Leu-[*O*-methyl diethoxyphosphonate (5-ASA)]-Leu-[*O-tert*-butylacetate (5-ASA)]-COOMe (11)



Using general procedure G, compound **11** was obtained from **9** (35 mg, 0.045 mmol, 1 equiv.), K_2CO_3 (12.42 mg, 0.089 mmol, 2 equiv.), *tert*-butyl bromoacetate (66 μL, 0.045 mmol, 1 equiv.), White puffy solid (36 mg, 90%) R_f: 0.35 (eluent: ethyl acetate/hexane 3:2 v/v); m.p. 54 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.43 (s, 1H), 9.38 (s, 1H), 8.34–8.33 (d, J = 6.43 Hz, 1H), 7.91–7.84 (m, 3H), 7.72–7.71 (d, J = 7.84 Hz, 1H), 6.88–6.86 (d, J = 8.83 Hz, 1H), 6.75–6.73 (d, J = 9.01 Hz, 1H), 5.26–5.24 (d, J = 7.93 Hz, 1H), 4.78–7.77 (m, 1H), 4.54 (s, 2H), 4.43–4.32 (m, 3H), 4.28–4.18 (m, 4H), 3.83 (s, 3H), 1.81–1.68 (m, 6H), 1.46 (s, 9H), 1.43 (s, 9H), 1.38–1.36 (t, J = 7.07 Hz, 3H), 1.33–1.31 (t, J = 7.07 Hz, 3H), 1.00–0.99 (d, J = 5.42 Hz, 3H), 0.94–0.91 (m, 6H), 0.89–0.88 (d, J = 5.85 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.5, 170.6, 167.7, 166.0, 164.7, 153.7, 153.3, 153.2, 132.64, 132.55, 126.1, 125.0, 124.3, 123.2, 121.1, 120.8, 114.6, 113.0, 82.3, 80.5, 67.2, 63.25, 63.20, 62.1, 54.0, 53.8, 52.0, 41.5, 40.5, 28.4, 28.1, 24.9, 24.8, 23.0, 22.9, 22.1, 21.9, 16.5, 16.4; MALDI-TOF (with matrix DHB): calculated for $C_{43}H_{65}N_4O_{14}PNa$: 915.412 $(M+Na)^+$, found 915.418; calculated for $C_{43}H_{65}N_4O_{14}PK$ 931.386 $(M+K)^+$, found: 931.391.

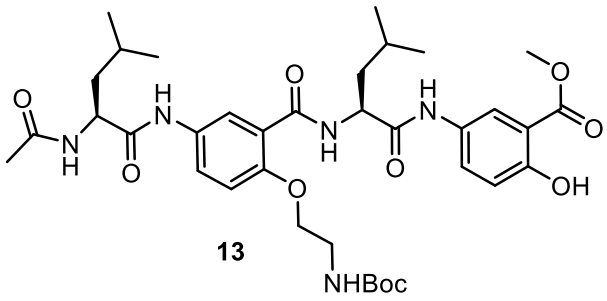
Boc-Leu-[*O*-2-propenyl (5-ASA)]-Leu-[*O-tert*-butylacetate [5-ASA]-COOMe (12)



Following general procedure G compound **12** was obtained from **9** (35 mg, 0.053 mmol, 1 equiv.), K_2CO_3 (15 mg, 0.106 mmol, 2 equiv.) and *tert*-butyl bromoacetate (8 μL 0.053 mmol, 1 equiv.,). White puffy solid (44 mg, 92%); R_f: 0.35 (eluent: hexane/ethyl acetate 3:2 v/v); m.p. 88 ºC; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.78 (s, 1H), 9.44 (s, 1H), 8.37–8.36 (d, J = 6.58 Hz, 1H), 7.89 (d, J = 2.25 Hz, 1H), 7.86–7.85 (d, J = 2.39 Hz,

1H), 7.83–7.81 (d, *J* = 8.90 Hz, 1H), 7.65–7.63 (dd, *J* = 8.84 Hz, 1.97 Hz, 1H), 6.82–6.80 (d, *J* = 9.00 Hz, 1H), 6.72–6.70 (d, *J* = 9.00 Hz, 1H), 6.12–6.04 (m, 1H), 5.48–5.44 (dd, *J* = 17.21, 1.25 Hz, 1H), 5.38–5.35 (dd, *J* = 10.41 Hz, 0.92 Hz, 1H), 5.33–5.31 (d, *J* = 8.68 Hz, 1H), 4.80–4.76 (m, 1H), 4.64–4.58 (m, 2H), 4.53 (s, 3H), 3.84 (s, 3H), 1.78–1.62 (m, 6H), 1.46 (s, 9H), 1.43 (s, 9H), 0.98–0.97 (d, *J* = 6.30 Hz, 3H), 0.93–0.92 (d, *J* = 6.23 Hz, 3H), 0.91–0.89 (d, *J* = 6.47 Hz, 3H), 0.87–0.86 (d, *J* = 6.34 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 171.6, 170.6, 167.6, 165.8, 165.3, 156.3, 153.8, 153.3, 132.2, 131.9, 131.7, 126.2, 125.1, 124.0, 123.2, 120.7, 120.6, 119.8, 114.6, 113.0, 82.2, 80.3, 70.3, 67.2, 53.7, 53.5, 52.0, 41.9, 40.8, 28.3, 28.0, 24.81, 24.77, 23.1, 22.8, 21.8; IR (neat) $\nu_{max.}$ cm^{-1}: 3308, 3077, 2955, 2929, 1716, 1669, 1643, 1532, 1495, 1368, 1299, 1251; MALDI-TOF: calculated for $C_{41}H_{58}N_4O_{11}K$: 821.373 $(M+K)^+$, found: 821.377.

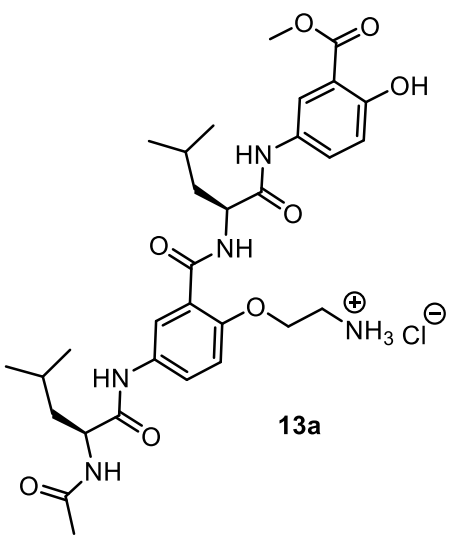
Acyl-Leu-[*O*-*N*-Boc ethylamine [5-ASA]-Leu-[5-ASA]-COOMe (13)



13

General procedure E; compound **3** (137 mg, 0.24 mmol, 1 equiv.), PPh_3 (94.56 mg, 0.36 mmol, 1.5 equiv.), Nboc-ethanolamine (44 μL, 0.28 mmol, 1.2 equiv.) and DIAD (70 μL, 0.36 mmol, 1.5 equiv.). White solid (90 mg, 53%); R_f: 0.25 (eluent: ethyl acetate/hexane 3:1 v/v); m.p. 205 °C; ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 10.50 (s, 1H), 10.24 (s, 1H), 10.01 (s, 1H), 9.12–9.11 (d, *J* = 7.78 Hz, 1H), 8.04–8.01 (m, 2H), 7.91 (d, *J* = 2.06 Hz, 1H), 7.85–7.83 (d, *J* = 8.19 Hz, 1H), 7.33 (bs, 1H), 6.97–6.95 (m, 1H), 6.93–6.91 (d, *J* = 8.97 Hz, 1H), 6.85–6.84 (d, *J* = 8.99 Hz, 1H), 5.27 (m, 1H), 5.00–4.99 (m, 1H), 4.17 (m, 1H), 4.06 (m, 1H), 3.86 (s, 3H), 3.67–3.57 (m, 2H), 2.12 (s, 3H), 1.83–1.72 (m, 6H), 1.39 (s, 9H), 1.05–1.02 (m, 6H), 0.94–0.91 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 171.8, 171.1, 170.8, 170.0, 164.1, 158.0, 156.4, 154.2, 130.7, 130.3, 128.5, 128.2, 125.3, 120.7, 120.4, 117.8, 112.1, 111.8, 79.0, 68.9, 52.8, 52.7, 52.3, 43.4, 42.2, 40.1, 28.4, 25.0, 24.8, 23.3, 23.0, 22.9, 22.9, 22.5; IR (neat) $\nu_{max.}$ cm^{-1}: 3295, 2958, 2920, 2850, 1689, 1650, 1635, 1548, 1493, 1367, 1290; MALDI-TOF (matrix used DHB): calculated for $C_{36}H_{51}N_5O_{10}Na$: 736.352 $(M+Na)^+$, found 736.361; calculated for $C_{36}H_{51}N_5O_{10}K$: 752.326 $(M+K)^+$, found: 752.335.

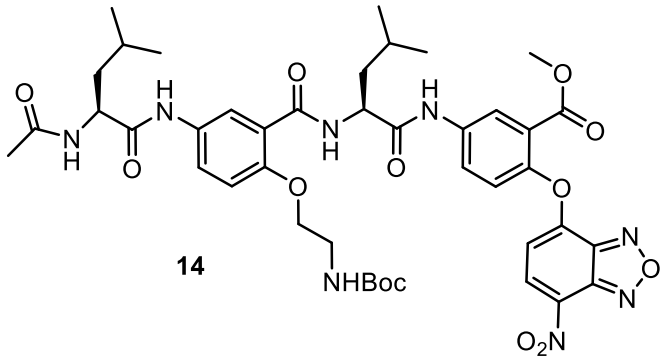
Acyl-Leu-[*O*-ethylamine [5-ASA]-Leu-[5-ASA]-COOMe (13a, HCl salt)



13a

With slight modification in general procedure B with **13** (40 mg, 0.056 mmol, 1 equiv.). The HCl salt as white solid was obtained after washing with chilled diethyl ether (three times) and filtration (32 mg, 93%); R_f: 0.1 (eluent: ethyl acetate); m.p. 165 ^oC; IR (neat) $\nu_{max.}$ cm^{-1}: 3266, 3068, 2956, 2934, 1636, 1533, 1490, 1439, 1291, 1215, 1087; MALDI-TOF (with matrix DHB): calculated for $C_{31}H_{44}N_5O_8Na$: 637.307 $(M+Na)^+$, found 637.304.

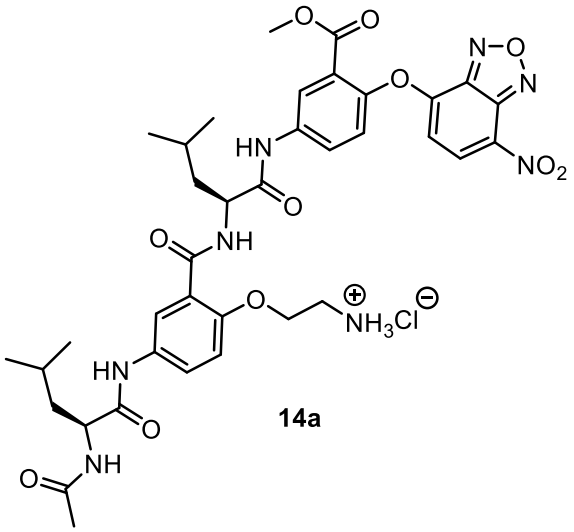
Acyl-Leu-[*O*-*N*-Boc ethylamine [5-ASA]-Leu-*O*-NBD [5-ASA]-COOMe (14)



14

Following general procedure E compound **14** was obtained from **13** (60 mg, 0.084 mmol, 1 equiv.), K_2CO_3 (23.3 mg, 0.36 mmol, 2 equiv.), NBD chloride (20 mg, 0.10 mmol, 1 equiv.). Brown solid (60 mg, 80%); R_f: 0.25 (eluent: ethyl acetate/hexane 3:1 v/v); ^1H NMR (500 MHz, $CDCl_3$) δ: 10.53 (s, 1H), 10.03 (s, 1H), 9.17–9.15 (d, J = 7.37 Hz, 1H), 8.46–8.40 (m, 1H), 7.99–7.87 (m, 2H), 7.29–7.27 (d, J = 9.02 Hz, 1H), 6.91–6.89 (d, J = 9.03 Hz, 1H), 6.79 (m, 1H), 6.39–6.38 (d, J = 8.33 Hz, 1H), 5.35 (m, 1H), 5.04 (m, 1H), 4.22 (m, 1H), 4.14–4.10 (m, 1H), 3.71–3.57 (m, 5H), 2.19 (s, 3H), 1.83–1.71 (m, 6H), 1.42–1.38 (m, 9H), 1.05–0.93 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 172.1, 171.7, 171.0, 170.1, 164.5, 163.9, 156.4, 154.9, 154.3, 147.3, 144.9, 144.2, 137.8, 133.4, 130.9, 130.7, 130.7, 125.9, 125.2, 124.0, 123.7, 123.4, 120.3, 117.9, 112.4, 107.2, 79.3, 69.0, 62.1, 60.4, 53.1, 52.7, 44.9, 43.2, 42.4, 40.2, 28.5, 25.0, 24.9, 23.4, 23.3, 23.0, 22.9, 226; MALDI-TOF (matrix used DHB): calculated for $C_{42}H_{52}N_8O_{13}Na$: 899.354 $(M+Na)^+$; Found 899.358.

Acyl-Leu-[*O*-ethylamine [5-ASA]-Leu-*O*-NBD [5-ASA]-COOMe (14a, HCl salt)



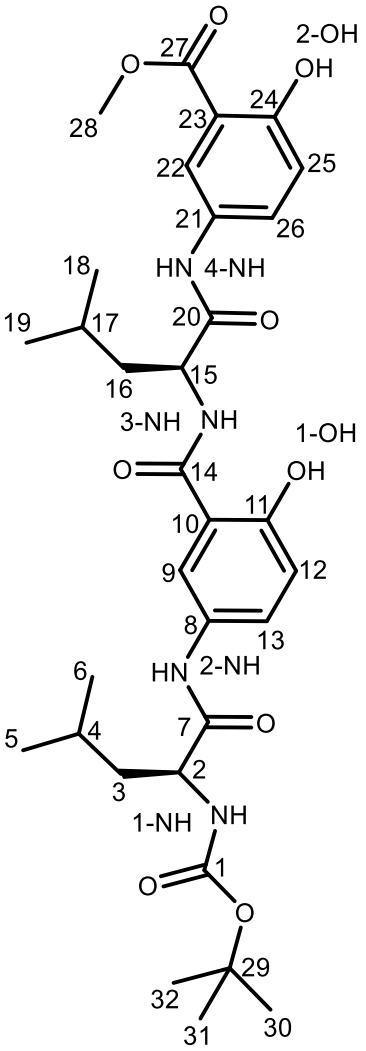
14a

With slight modification in general procedure B compound **14a** was obtained from **14** (60 mg, 0.078 mmol, 1 equiv.). The HCl salt obtained was washed with chilled diethyl ether (three times) and after filtration yielded brown solid (51 mg, 85%); R_f: 0.1 (eluent: ethyl acetate); m.p. 126 ^{o}C; IR (neat) $\nu_{max.}$ cm^{-1}: 3385, 3264, 3060, 2957, 2927, 2872, 1724, 1640, 1514, 1493, 1334, 1267, 1210, 1091; MALDI-TOF (matrix used DHB): calculated for $C_{37}H_{45}N_8O_{11}Na$: 800.309 $(M+Na)^+$, found 800.306; calculated for $C_{37}H_{44}N_8O_{11}K$: 815.276 $(M+K)^+$, found: 815.27.

6. 2D-NMR based solution-state conformational analysis

Table S1. COSY correlations in tetrapeptide **1**.

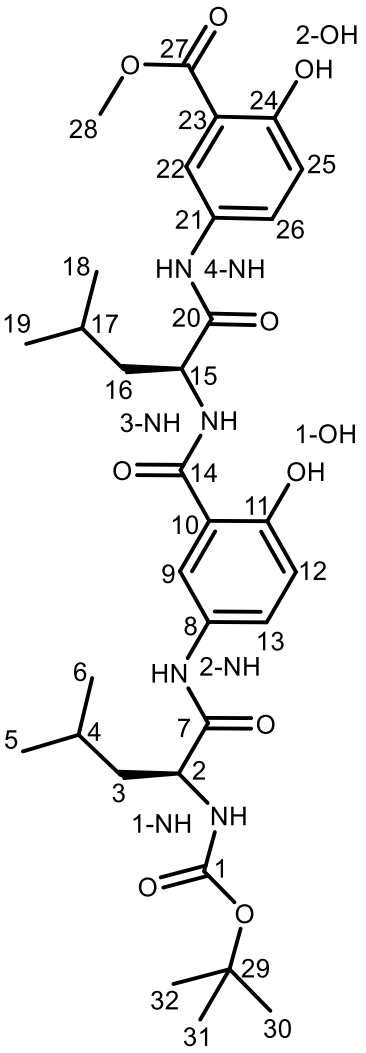
^{1}H (δ ppm)	^{1}H (δ ppm)
11.55 (1-OH)	
10.47 (2-OH)	
9.72 (2-NH)	
8.81 (4-NH)	
8.06-8.04 (3-NH)	4.92-4.88
8.01-8.00 (22-CH)	7.58-7.52
7.58-7.52 (26-CH)	8.01-8.00, 6.90-6.87
7.58-7.52 (13-CH)	6.81, 6.71-6.69
6.90-6.87 (25-CH)	7.58-7.52
6.81 (9-CH)	7.55-7.52
6.71-6.69 (12-CH)	7.55-7.52
5.52-5.50 (1-NH)	4.48-4.43
4.92-4.88 (15-CH)	8.06-8.04, 1.85
4.48-4.43 (2-CH)	5.52-5.50, 1.65
3.90 (28-CH)	
2.01-1.83 (16-CH)	4.92-4.88
1.72-1.70 (3-CH)	4.48-4.43, 1.04-0.94
1.83-1.74 (4 & 17-CH)	4.92-4.88
1.20 (30, 31 & 32-CH)	
1.04-0.99 (18 & 19-CH)	
1.00-0.94 (5 & 6-CH)	1.73



1

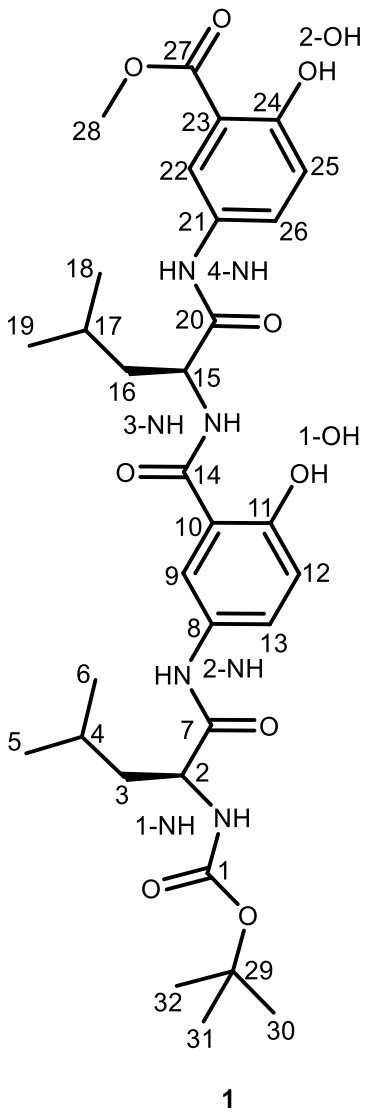
Table S2. ^{1}H -^{13}C HSQC assignments for tetrapeptide **1**.

^{1}H (δ ppm)	**^{13}C (δ ppm)**	
11.55 (1-OH)		
10.47 (2-OH)		
9.72 (2-NH)		
8.81 (4-NH)		
8.06-8.04 (3-NH)		
8.01-8.00 (22-CH)	121.6	
7.58-7.52 (26-CH)	129.0	
7.58-7.52 (13-CH)	127.6	
6.90-6.87 (25-CH)	117.8	
6.81 (9-CH)	118.8	
6.71-6.69 (12-CH)	117.8	
5.52-5.50 (1-NH)		
4.92-4.88 (15-CH)	52.9	
4.48-4.43 (2-CH)	53.9	
3.90 (28-CH)	52.4	
2.01-1.83 (16-CH)	40.5	
1.72-1.70 (3-CH)	41.6	
1.83-1.74 (4 & 17-CH)	24.8, 24.7	
1.20 (30, 31, & 32-CH)	28.0	
1.04-0.99 (18 & 19-CH)	23.0, 22.9	
1.00-0.94 (5 & 6-CH)	21.9, 21.5	



1

Table S3. ^{1}H-^{13}C HMBC assignments for tetrapeptide **1**.

^{1}H (δ ppm)	**^{13}C (δ ppm)**	
11.55 (1-OH)		
10.47 (2-OH)	111.9, 117.8, 129.0, 158.4	
9.72 (2-NH)	128.0, 173.4	
8.81 (4-NH)	121.6, 129.0, 170.4	
8.06-8.04 (3-NH)	169.8	
8.01-8.00 (22-CH)	129.0, 158.4, 170.0	
7.58-7.52 (26-CH)	121.6	
7.58-7.52 (13-CH)	118.8, 157.8, 111.9	
6.90-6.87 (25-CH)	111.9, 129.6, 158.3	
6.81 (9-CH)	127.6, 157.8, 169.7	
6.71-6.69 (12-CH)	114.1, 128.4, 157.0, 169.7	
5.52-5.50 (1-NH)	41.6, 53.9	
4.92-4.88 (15-CH)	24.7, 40.5, 170.0	
4.48-4.43 (2-CH)	24.8, 41.6, 157.0	
3.90 (28-CH)	170.0,	
2.01-1.83 (16-CH)	169.7, 22.9, 52.9	
1.72-1.70 (3-CH)		
1.83-1.74 (4 & 17-CH)	40.5, 41.6	
1.20 (30, 31 & 32-CH)	28.0, 80.9	
1.04-0.99 (18 & 19-CH)	40.5	
1.00-0.94 (5 & 6-CH)	41.6	

1

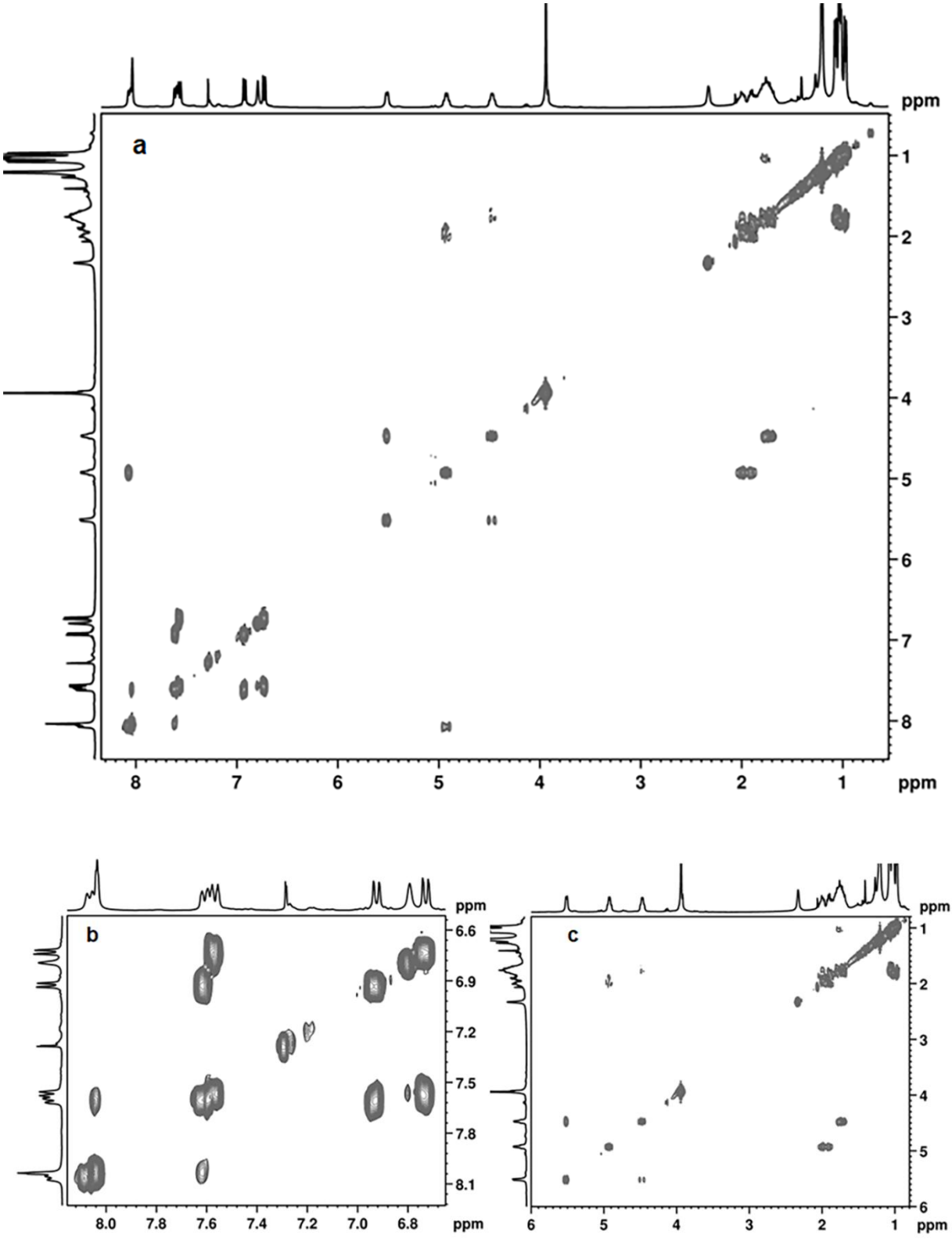


Fig S1. COSY spectrum of tetrapeptide **1**. (a) full spectrum, (b) aromatic region, and (c) aliphatic region.

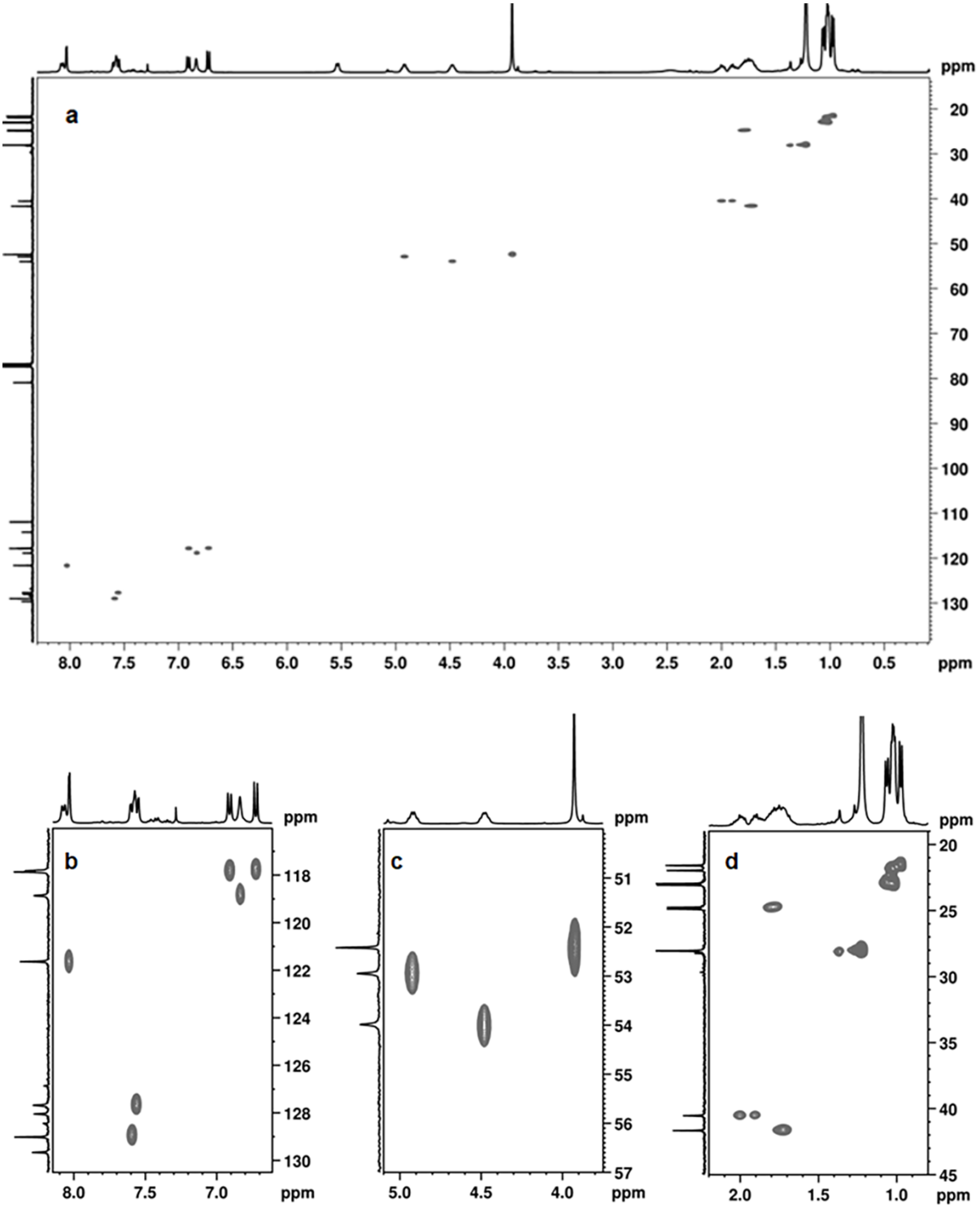


Fig S2. HSQC spectrum of tetrapeptide **1**. (a) full spectrum, (b) aromatic region, and (c and d) aliphatic regions.

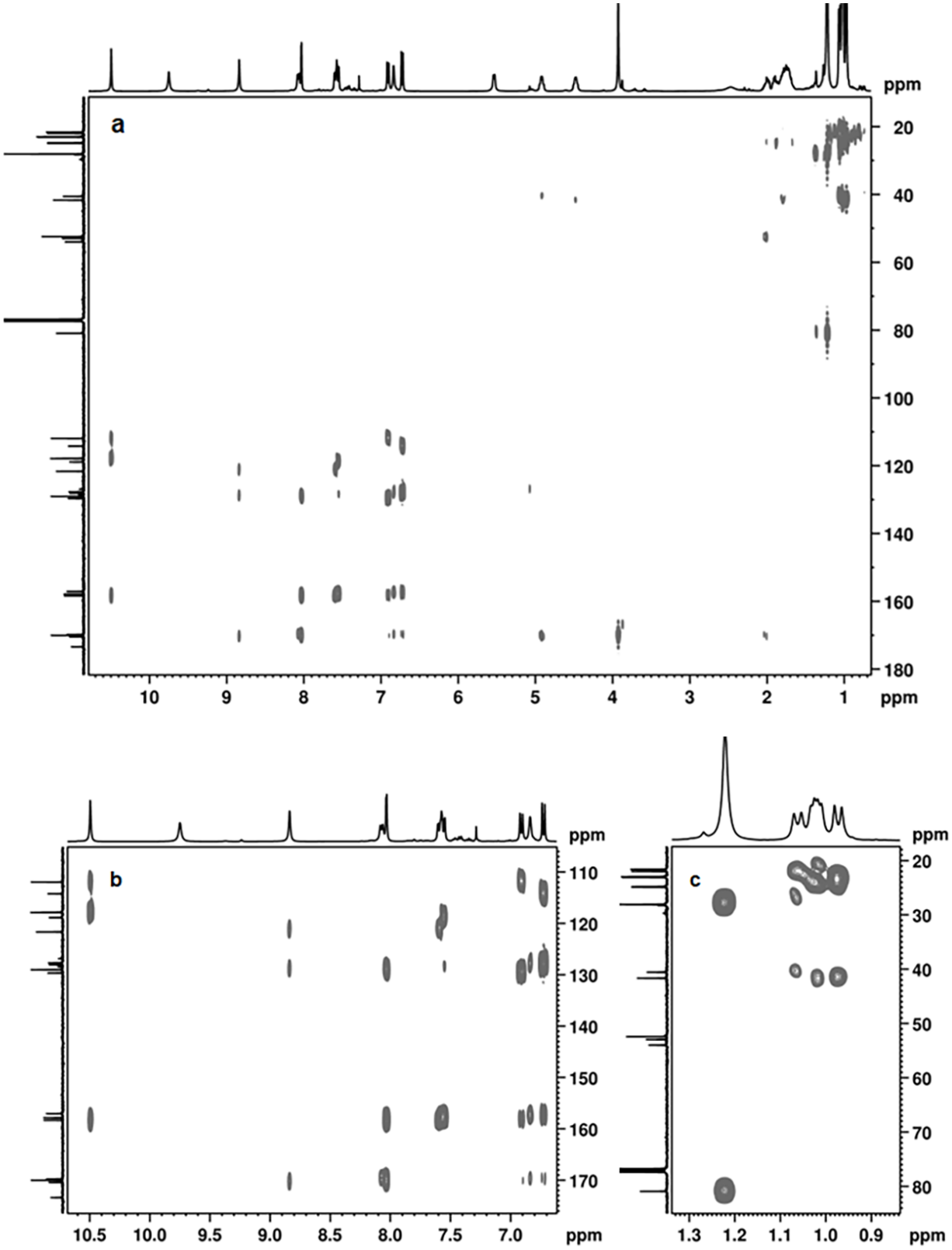


Fig S3. HMBC spectrum of tetrapeptide **1**. (a) full spectrum, (b) aromatic region, and (c) aliphatic region.

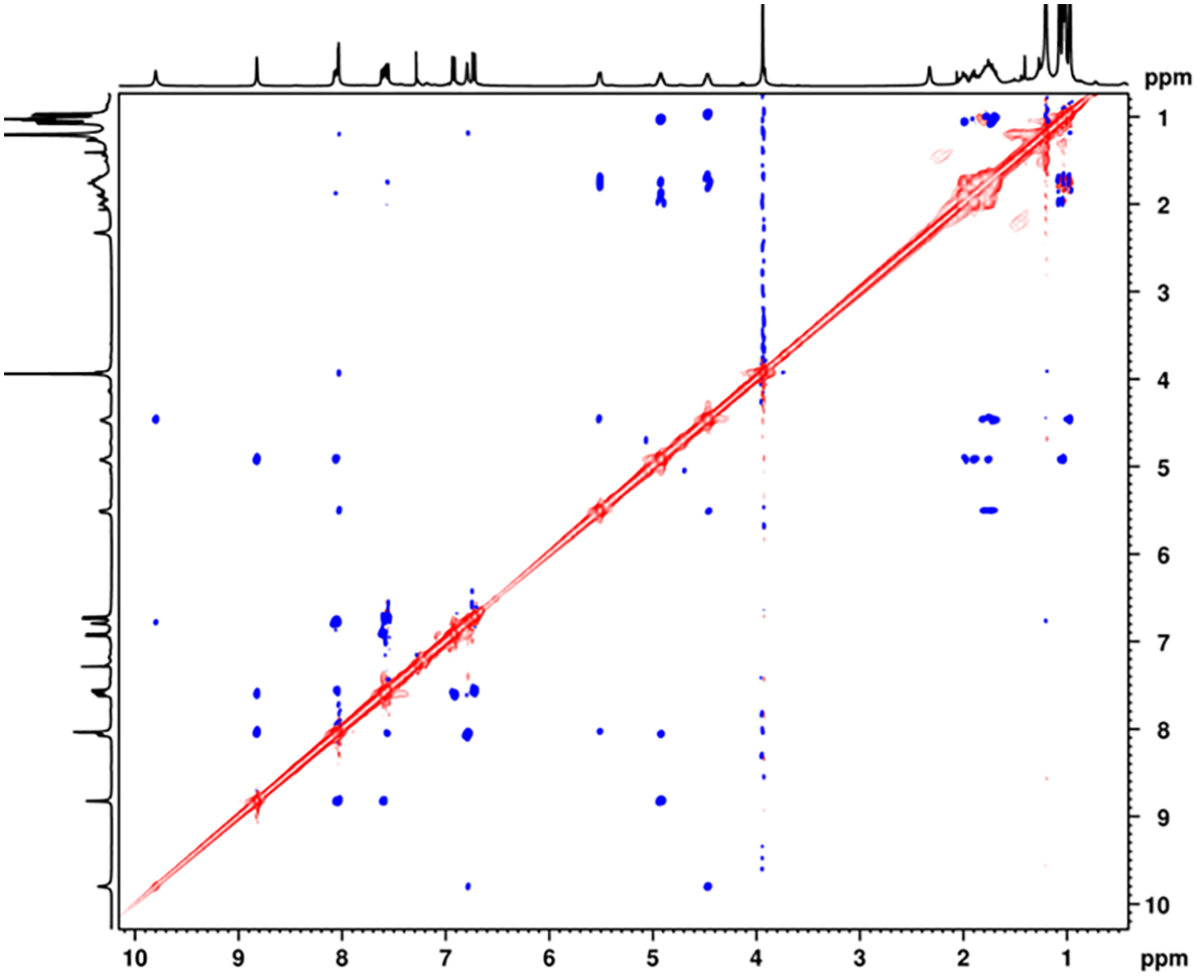
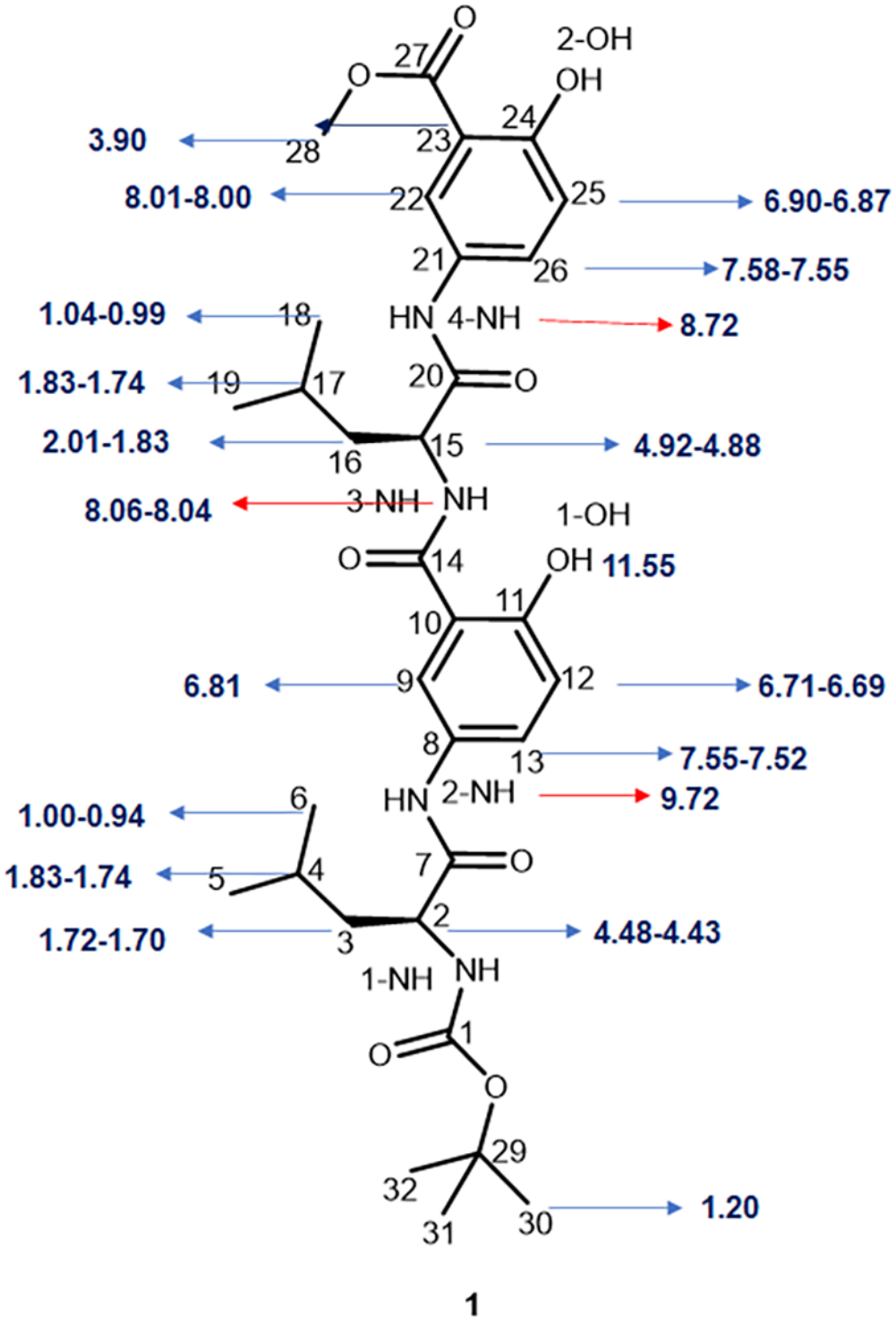


Fig S4. ROESY spectrum of tetrapeptide **1**.



1

Fig S5. Proton peak assignment of tetrapeptide **1**.

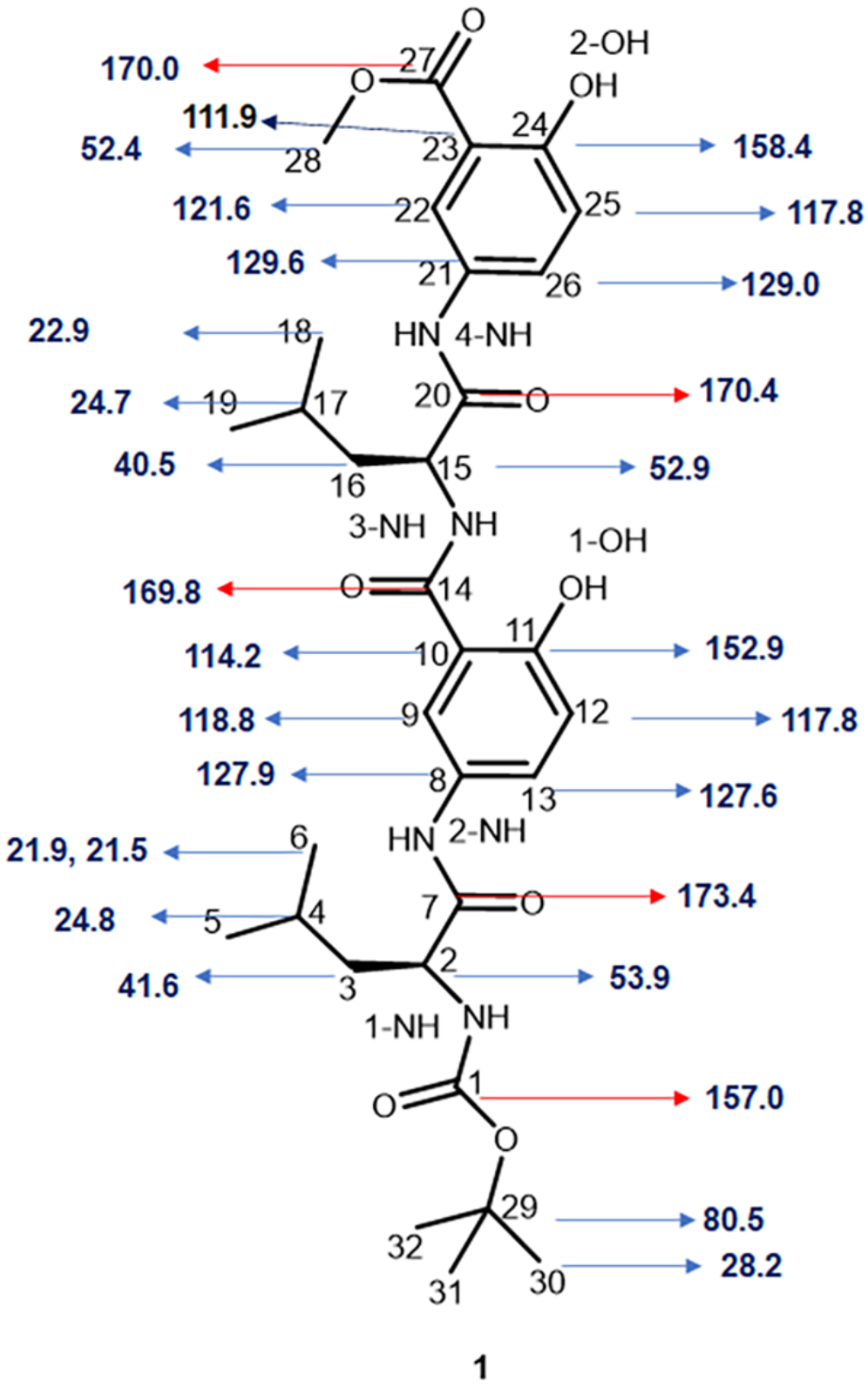


Fig S6. Carbon peak assignment of tetrapeptide **1**.

Table S4. COSY correlations in tetrapeptide **4**.

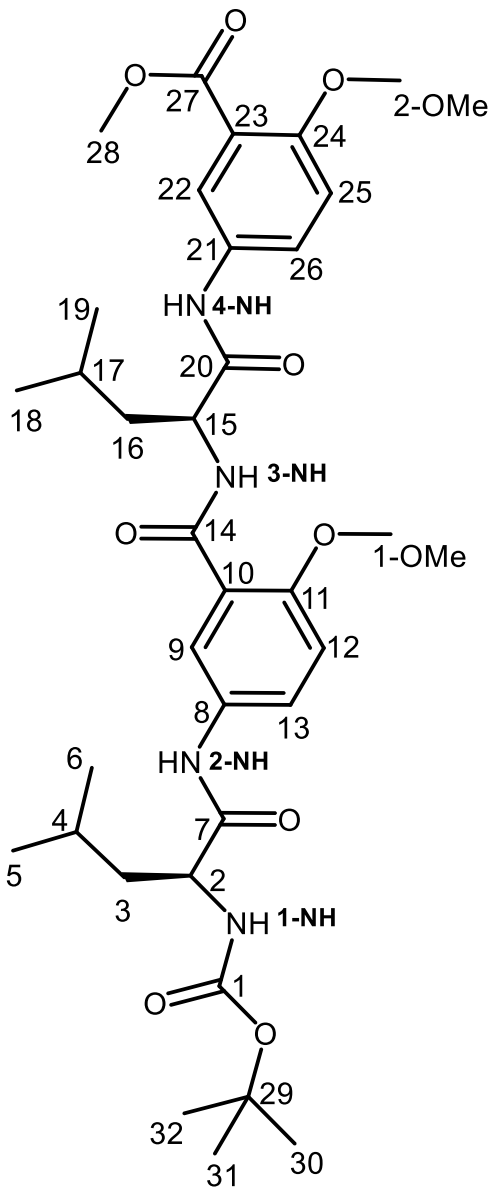
^{1}H (δ ppm)	**^{1}H (δ ppm)**	
9.77 (2-NH)		
9.42 (4-NH)		
8.31-8.29 (3-NH)	4.86-4.85	
7.90 (9-CH)	7.90	
7.89 (13-CH)	6.82-6.80	
7.89 (22-CH)	7.66-7.65	
7.66-7.65 (26-CH)	7.86, 6.82-6.80	
6.82-6.80 (25-CH)	7.90	
6.80-6.79 (12-CH)	7.66-7.65	
5.24-5.22 (1-NH)	4.57-4.56	
4.82 (15-CH)	8.31-8.29, 1.83-1.65	
4.50 (2-CH)	5.42-5.40, 1.83-1.65	
3.91 (1-OMe)		
3.84 (2-OMe)		
3.84 (28-CH)		
1.83-1.65 (16-CH)	4.86-4.85, 1.00-0.95	
1.83-1.65 (3-CH)	4.57-4.56, 0.92-0.88	
1.83-1.65 (17-CH)	1.00-0.95	
1.83-1.65 (4-CH)	0.92-0.88	
1.44 (32, 33 & 34-CH)		
1.00-0.95 (19 & 20-CH)	1.83-1.65	
0.92-0.88 (5 & 6-CH)	1.83-1.65	

Table S5. ^{1}H -^{13}C HSQC assignments for tetrapeptide **4**.

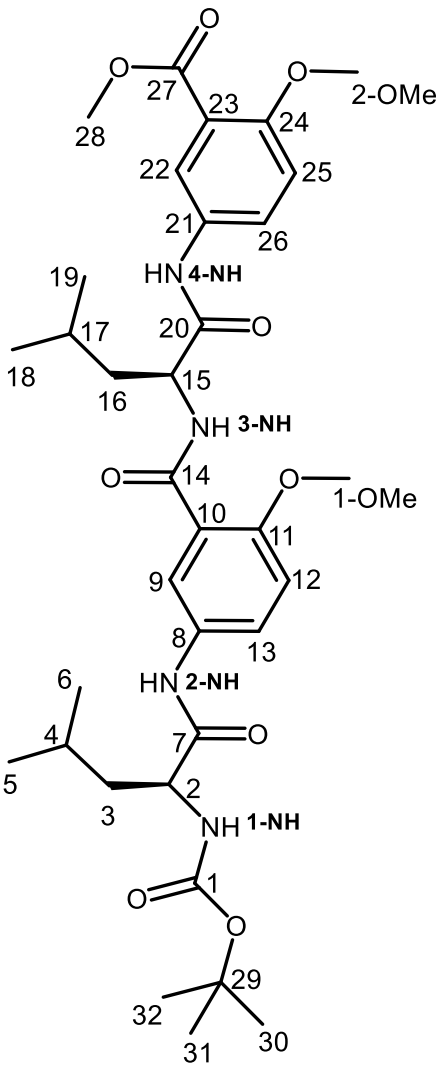
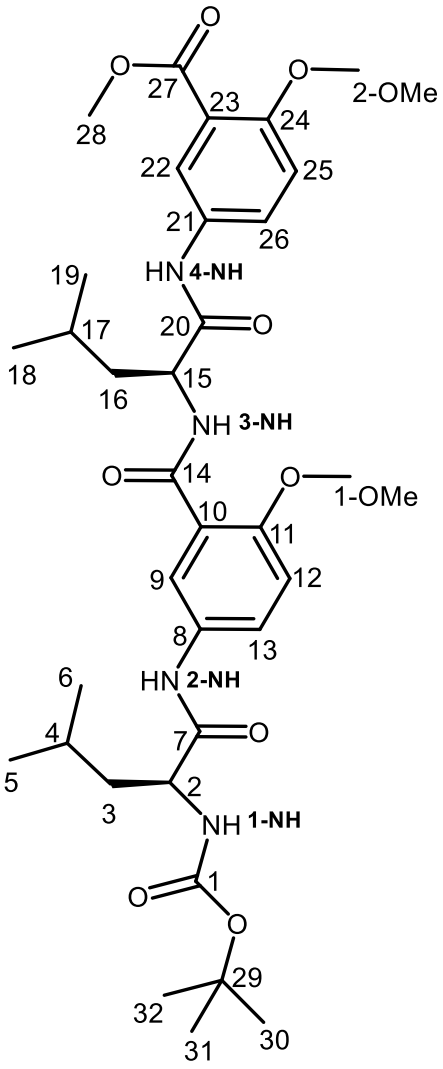
^{1}H (δ ppm)	**^{13}C (δ ppm)**	
9.77 (2-NH)		
9.42 (4-NH)		
8.31-8.29 (3-NH)		
7.90 (9-CH)	124.0	
7.89 (13-CH)	123.6	
7.89 (22-CH)	126.2	
7.66-7.65 (26-CH)	125.8	
6.82-6.80 (25-CH)	112.4	
6.80-6.79 (12-CH)	111.8	
5.24-5.22 (1-NH)		
4.82 (15-CH)	53.6	
4.50 (2-CH)	53.8	
3.91 (1-OMe)	56.3	
3.84 (2-OMe)	56.3	
3.84 (28-CH)	52.0	
1.83-1.65 (16-CH)	41.2	
1.83-1.65 (3-CH)	42.1	
1.83-1.65 (17-CH)	25.1	
1.83-1.65 (4-CH)	24.9	
1.44 (32, 33 & 34-CH)	28.4	
1.00-0.95 (19 & 20-CH)	23.3, 23.0	
0.92-0.88 (5 & 6-CH)	22.3, 21.9	

Table S6. ^{1}H -^{13}C HMBC assignments for tetrapeptide **4**.

^{1}H (δ ppm)	**^{13}C (δ ppm)**	
9.77 (2-NH)	171.8, 126.2	
9.42 (4-NH)	170.8, 131.2, 125.8, 123.6	
8.31-8.29 (3-NH)	165.4, 53.6, 41.2	
7.90 (9-CH)	165.4, 154.2, 131.2, 126.2	
7.89 (13-CH)	166.3, 155.7, 154.2, 131.2, 125.8, 124.0	
7.89 (22-CH)	155.7,123.6	
7.66-7.65 (26-CH)	166.2, 155.7, 131.2, 119.6	
6.82-6.80 (25-CH)	165.4, 154.2, 131.8, 120.6	
6.80-6.79 (12-CH)		
5.24-5.22 (1-NH)	53.8, 42.1	
4.82 (15-CH)	170.8, 165.4, 25.1, 41.2,	
4.50 (2-CH)	171.8, 156.4, 24.9, 42.1	
3.91 (1-OMe)	154.2	
3.84 (2-OMe,)	155.7	
3.84 (28-CH)	166.3	
1.83-1.65 (16-CH)	170.8, 25.1, 22.3	
1.83-1.65 (3-CH)	171.8, 24.9, 23.0, 21.9	
1.83-1.65 (17-CH)	25.1, 22.3	
1.83-1.65 (4-CH)	171.8, 24.9, 23.0, 21.9	
1.44 (32, 33 & 34-CH)	80.3, 28.4	
1.00-0.95 (19 & 20-CH)	41.2, 25.1, 22.3	
0.92-0.88 (5 & 6-CH)	42.1, 24.9, 230, 21.9	

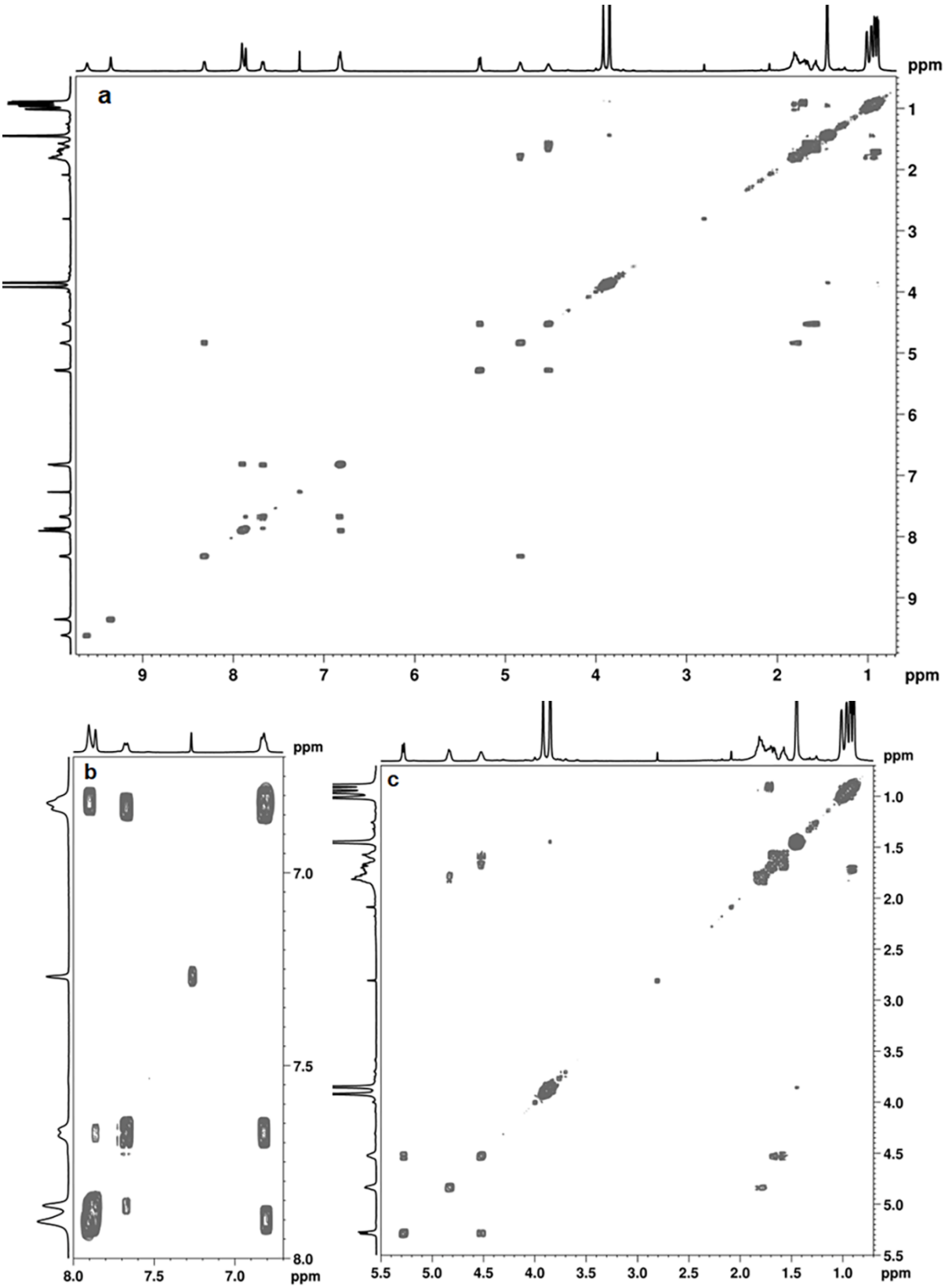


Fig S7. COSY spectrum of tetrapeptide **4**. (a) full spectrum, (b) aromatic region, and (c) aliphatic region.

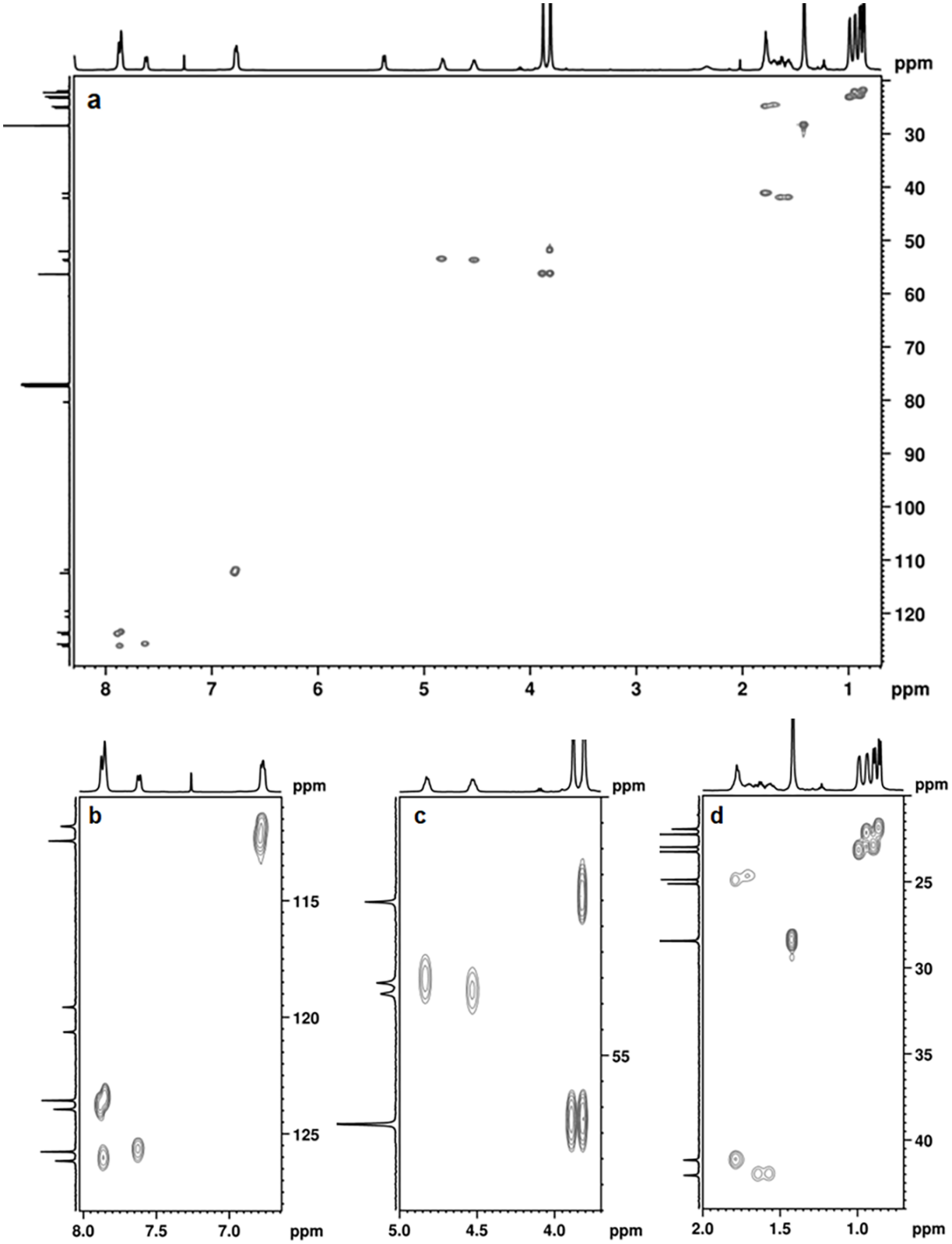


Fig S8. HSQC spectrum of tetrapeptide **4**. a) full spectrum, b) aromatic region, and c-d) aliphatic region.

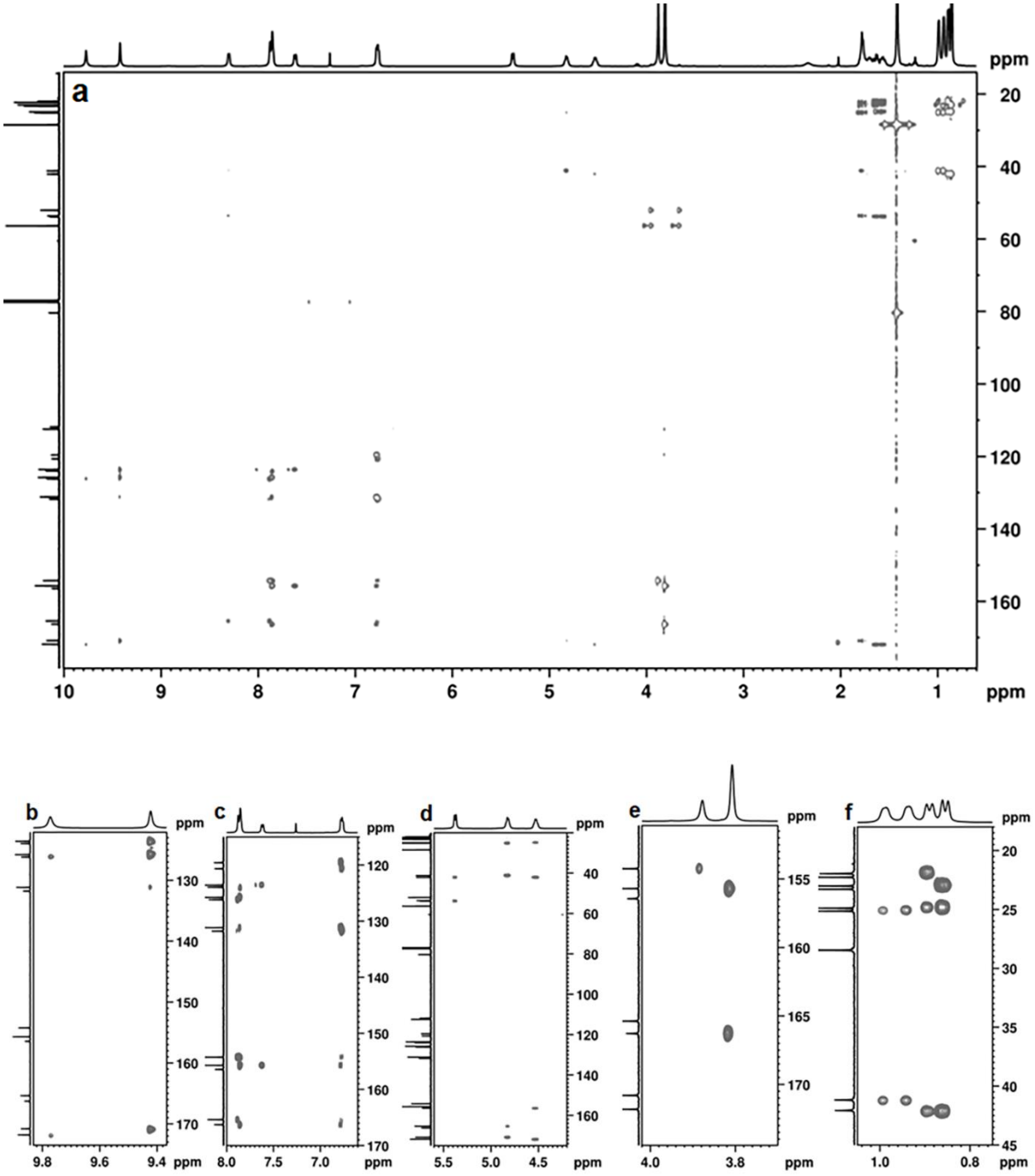


Fig S9. HMBC spectrum of tetrapeptide **4**. a) full spectrum, b-c) aromatic region, and d-f) aliphatic region.

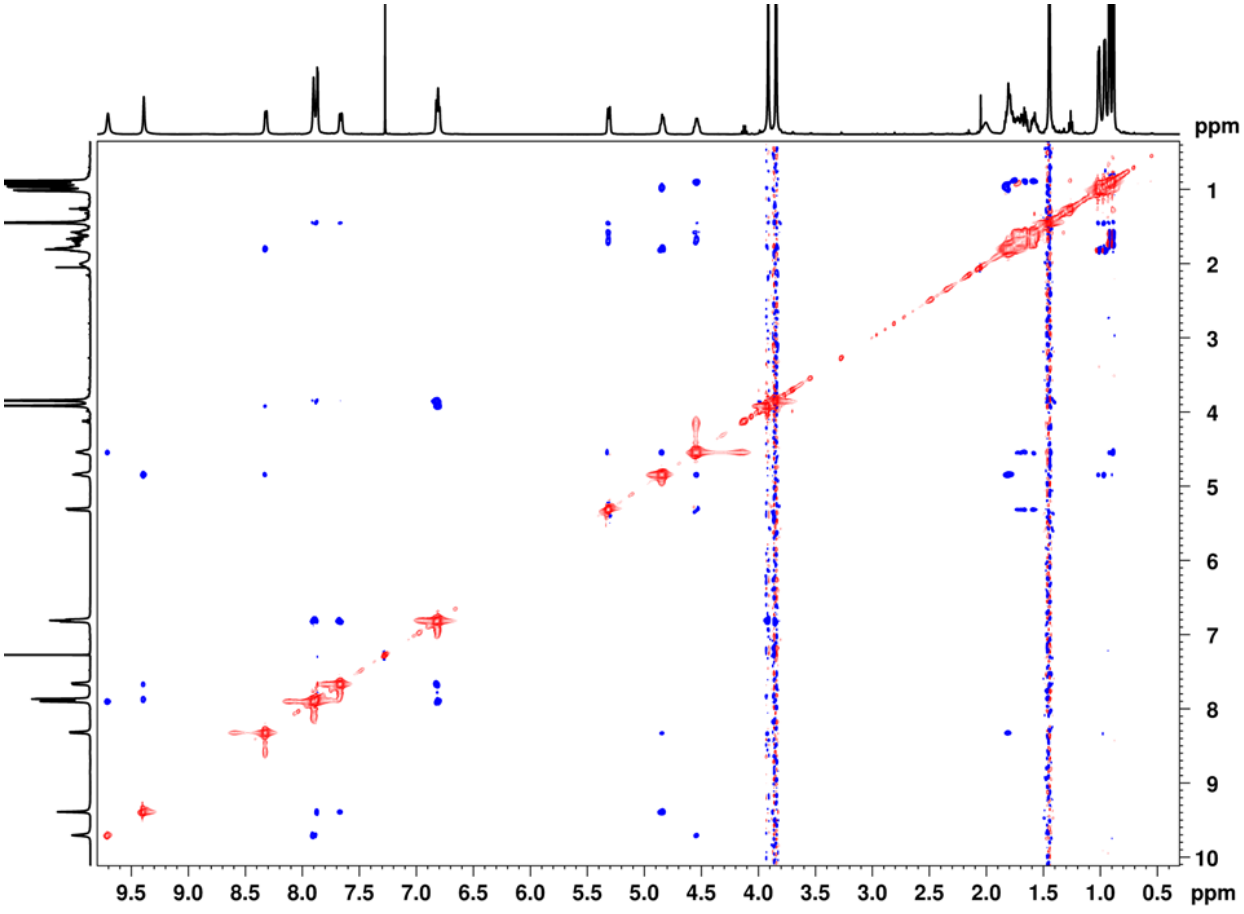


Fig S10. ROSEY spectrum of tetrapeptide **4**.

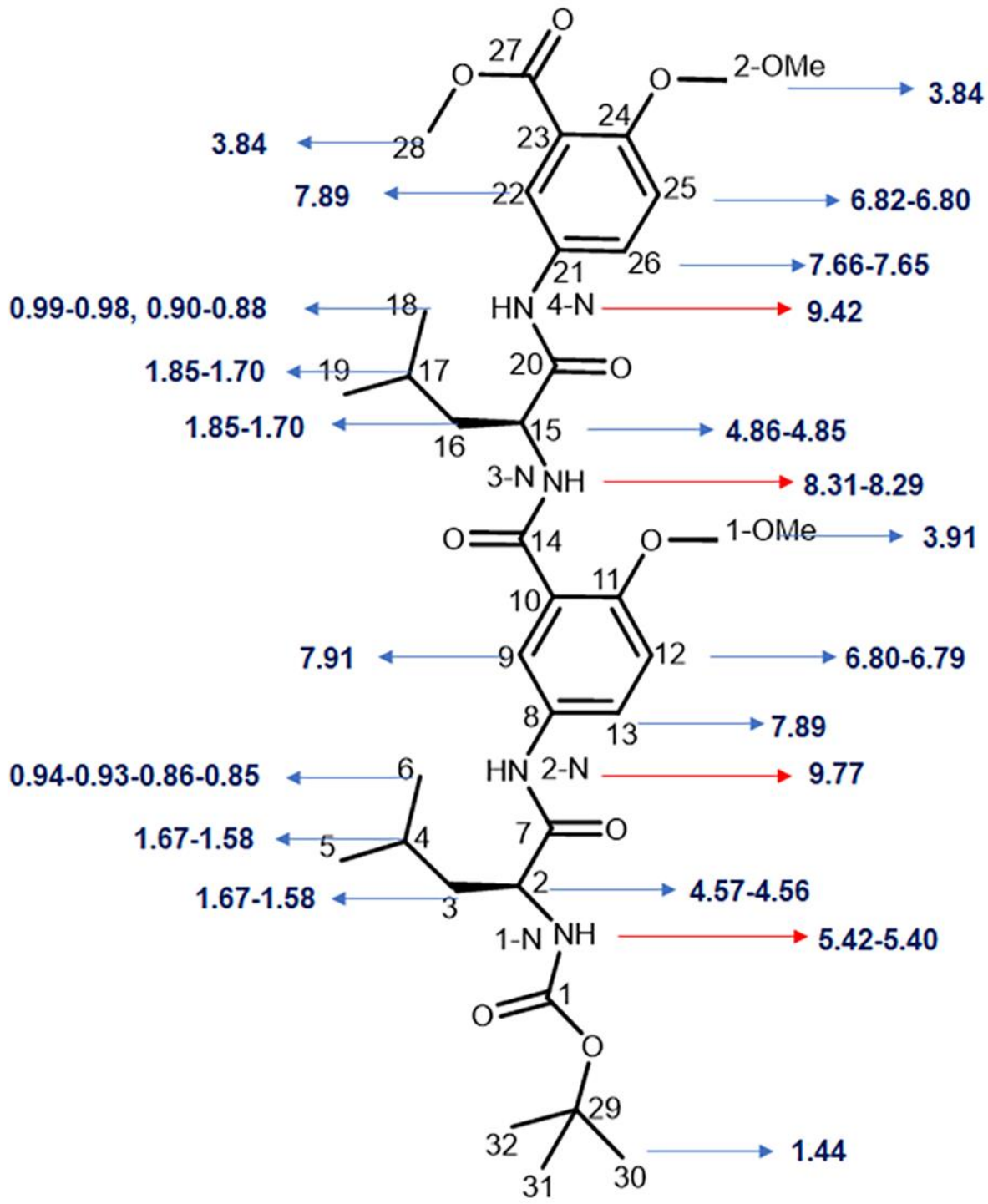


Fig S11. Proton peak assignment of tetrapeptide **4**.

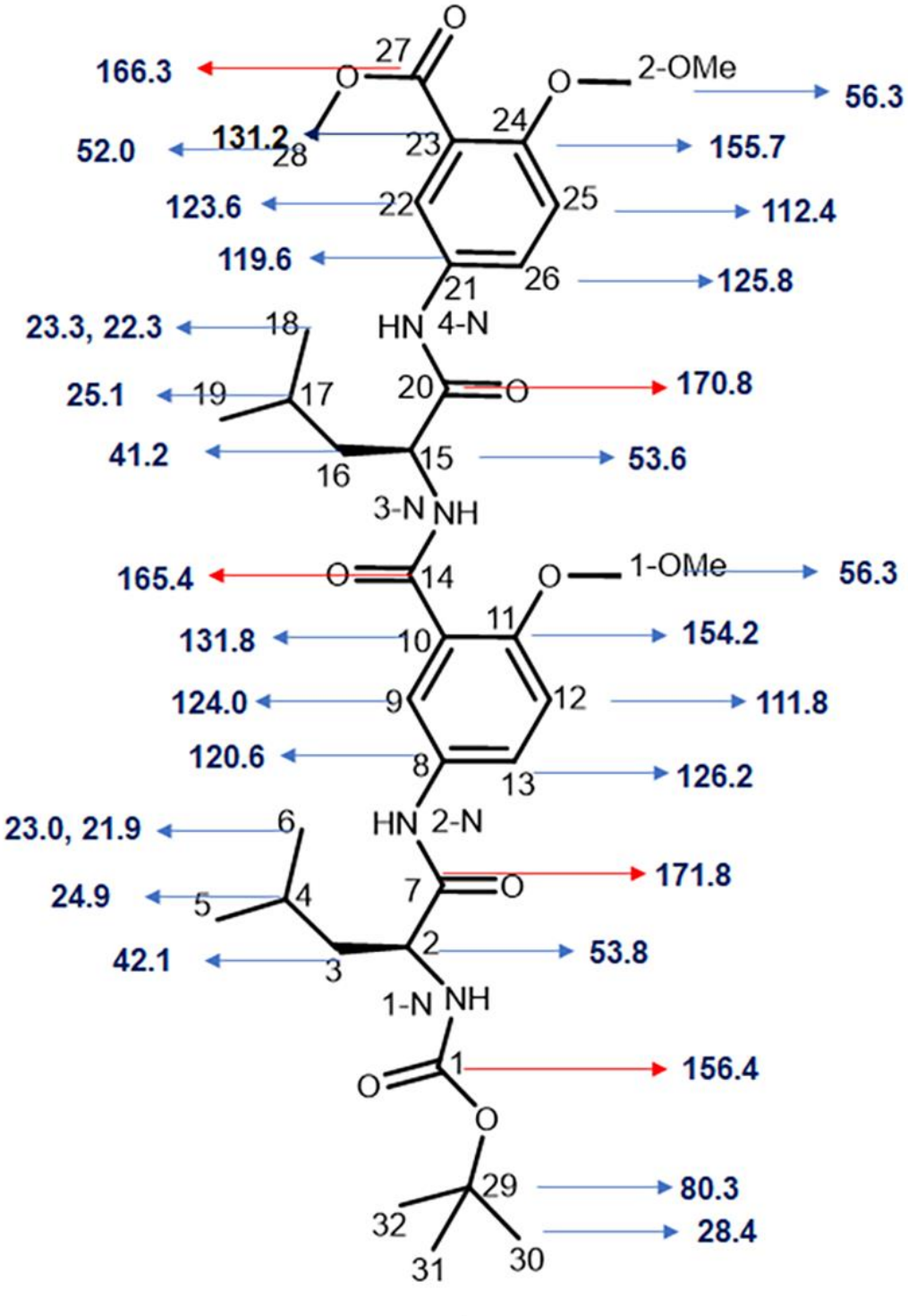


Fig S12. Carbon peak assignment of tetrapeptide **4**.

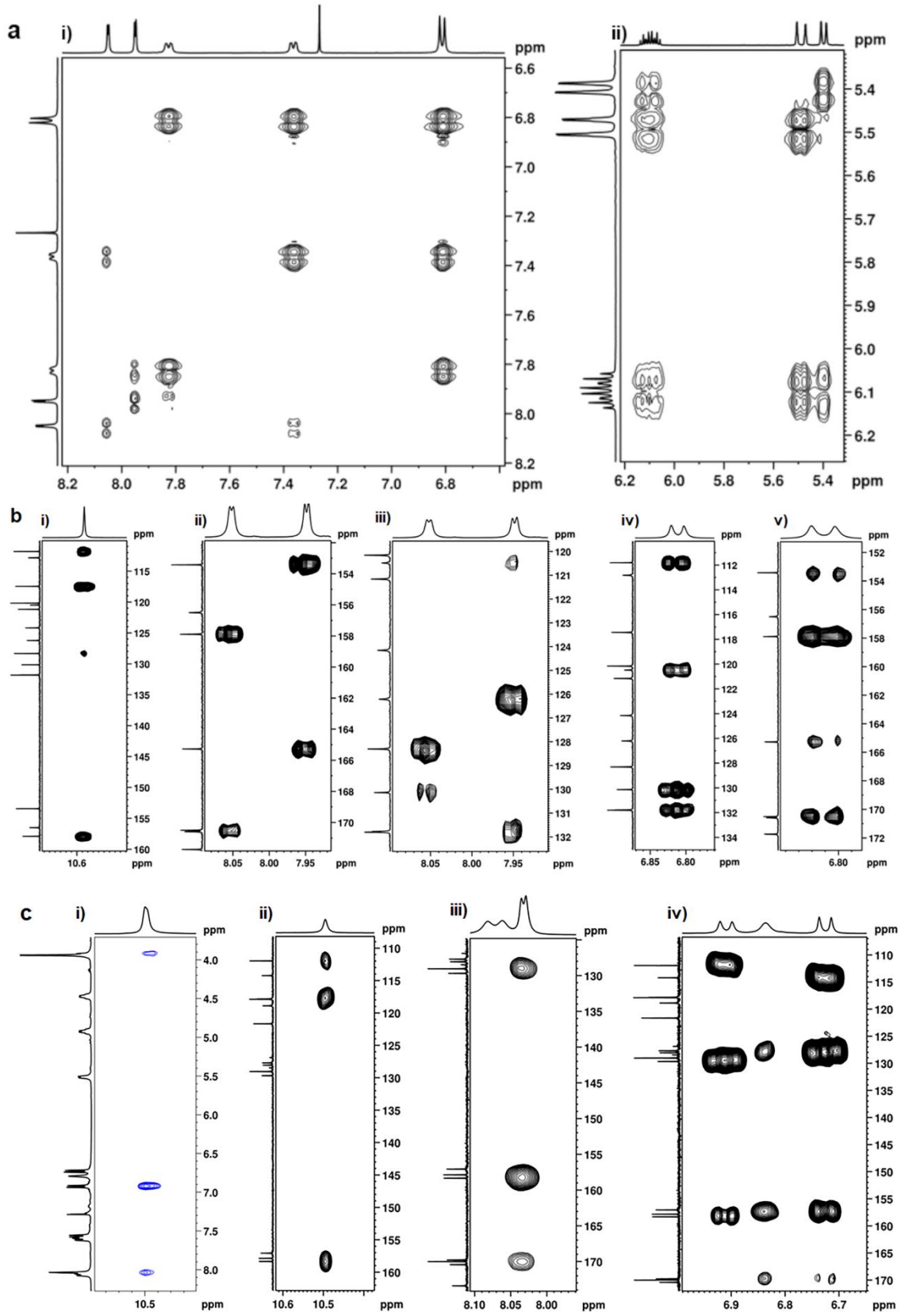
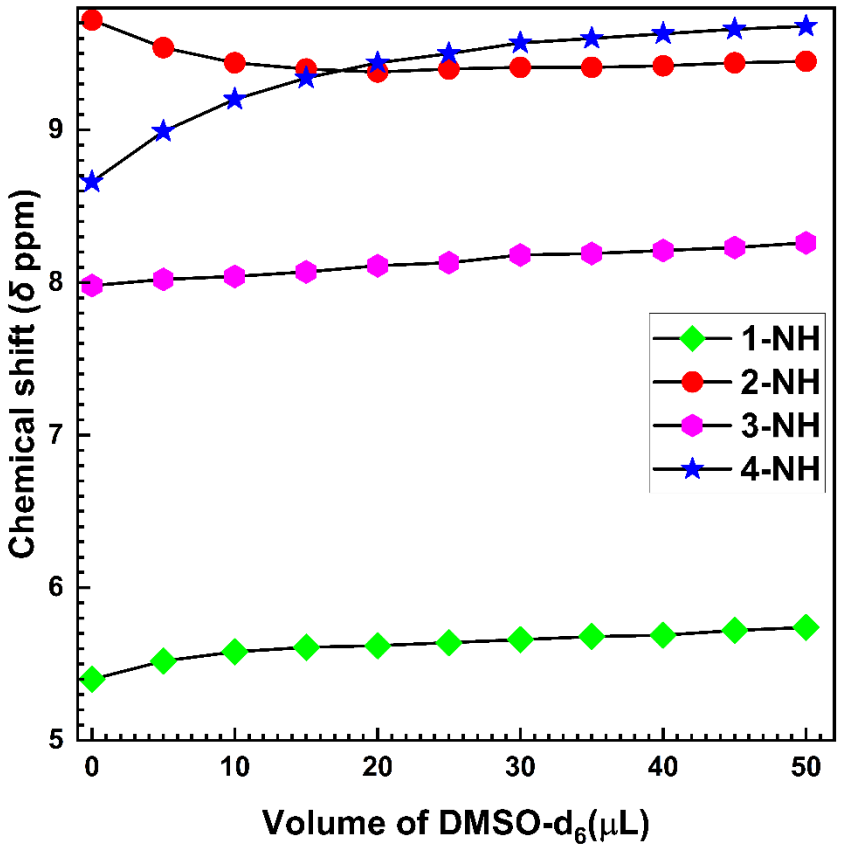


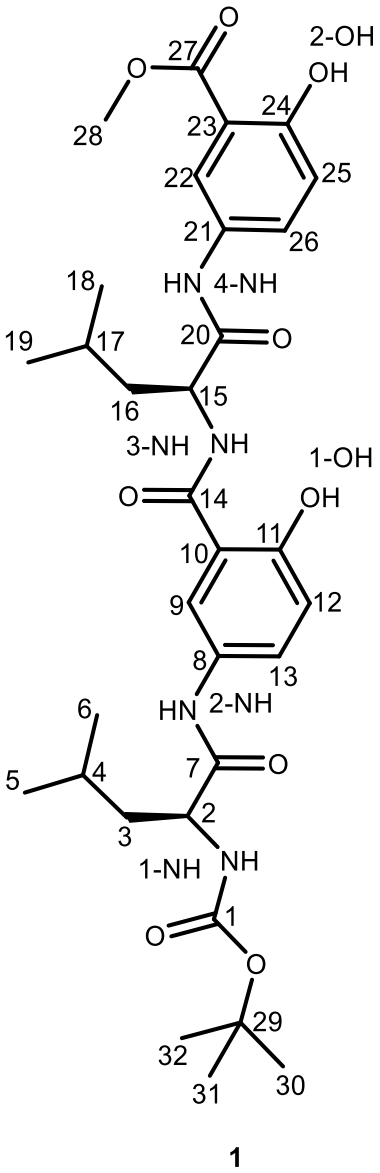
Fig S13. 2D-NMR of tetrapeptide **10**. a) COSY extracts, b) HMBC extracts (aromatic region) and c) (i) ROESY extract and (ii-iv) HMBC extracts.

7. ^{1}H-NMR titration study with DMSO-d_6

Table S7. Titration study of tetrapeptide **1** in $CDCl_3$ (10 mmol) with the addition of DMSO-d_6 (5 μL at each interval)

Volume of DMSO-d6 added (in μL)	Chemical Shift (in ppm)			
	1-NH	**2-NH**	**3-NH**	**4-NH**
0	5.4	9.72	7.98	8.66
5	5.52	9.54	8.02	8.99
10	5.58	9.44	8.04	9.2
15	5.61	9.4	8.07	9.34
20	5.62	9.38	8.11	9.44
25	5.64	9.4	8.13	9.5
30	5.66	9.41	8.18	9.57
35	5.68	9.41	8.19	9.6
40	5.69	9.42	8.21	9.63
45	5.72	9.44	8.23	9.66
50	5.74	9.45	8.26	9.68
Difference in δ ppm	**0.34**	**0.32**	**0.28**	**1.02**



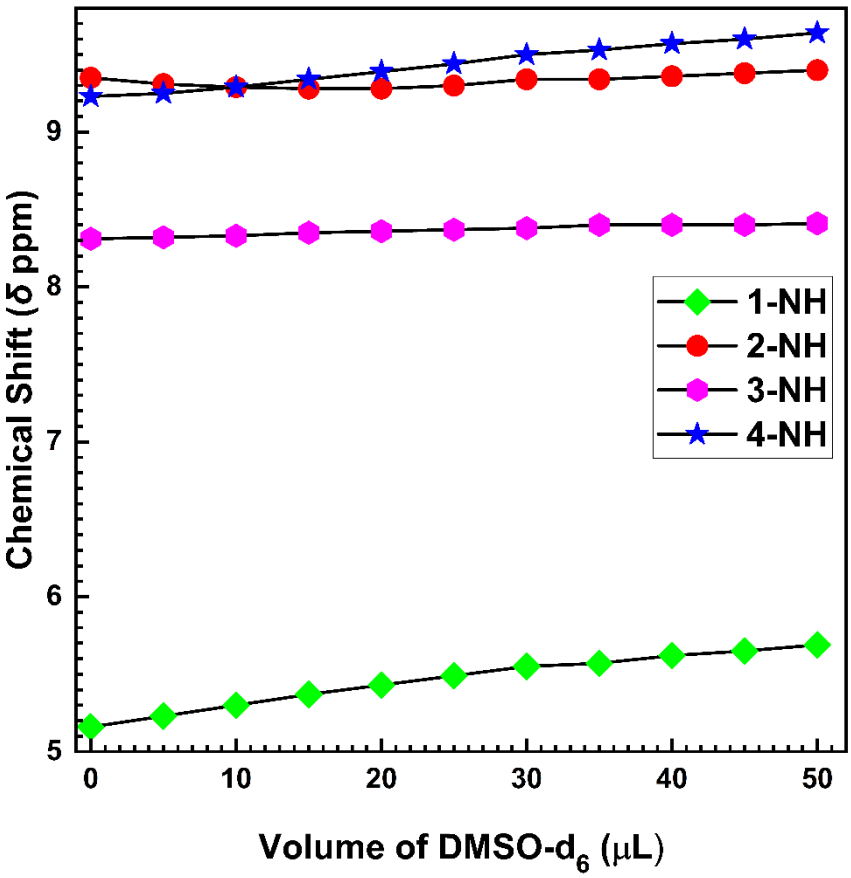


1

Fig S14. DMSO-d_6 titration study of tetrapeptide **1** in $CDCl_3$ (10 mM).

Table S8. Titration Study of tetrapeptide **4** in $CDCl_3$ (10 mmol) with the addition of DMSO-d_6 (5 μL at each interval).

Volume of DMSO-d6 added (in μL)	**Chemical Shift (in ppm)**			
	1-NH	**2-NH**	**3-NH**	**4-NH**
0	5.16	9.35	8.31	9.23
5	5.23	9.31	8.32	9.25
10	5.3	9.29	8.33	9.29
15	5.37	9.28	8.35	9.34
20	5.43	9.28	8.36	9.39
25	5.49	9.3	8.37	9.44
30	5.55	9.34	8.38	9.5
35	5.57	9.34	8.4	9.53
40	5.62	9.36	8.4	9.57
45	5.65	9.38	8.4	9.6
50	5.69	9.4	8.41	9.64
Difference in δ ppm	**0.53**	**0.12**	**0.1**	**0.41**



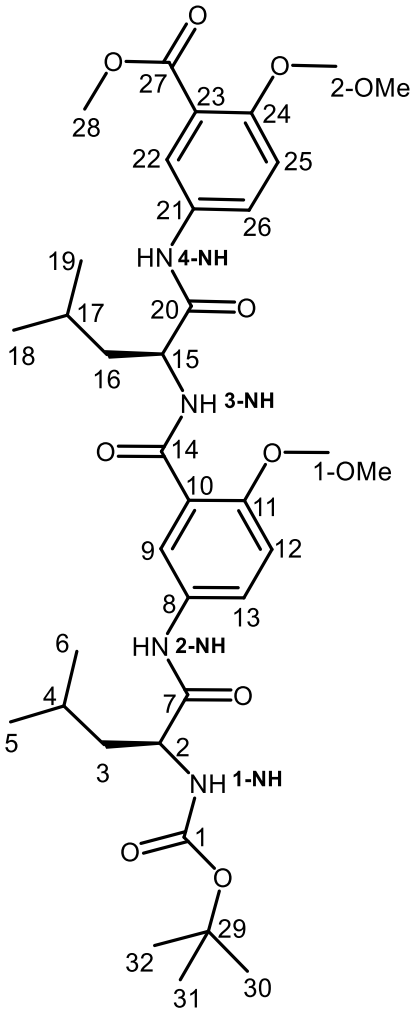


Fig S15. DMSO-d_6 titration study of tetrapeptide **4** in $CDCl_3$ (10 mM).

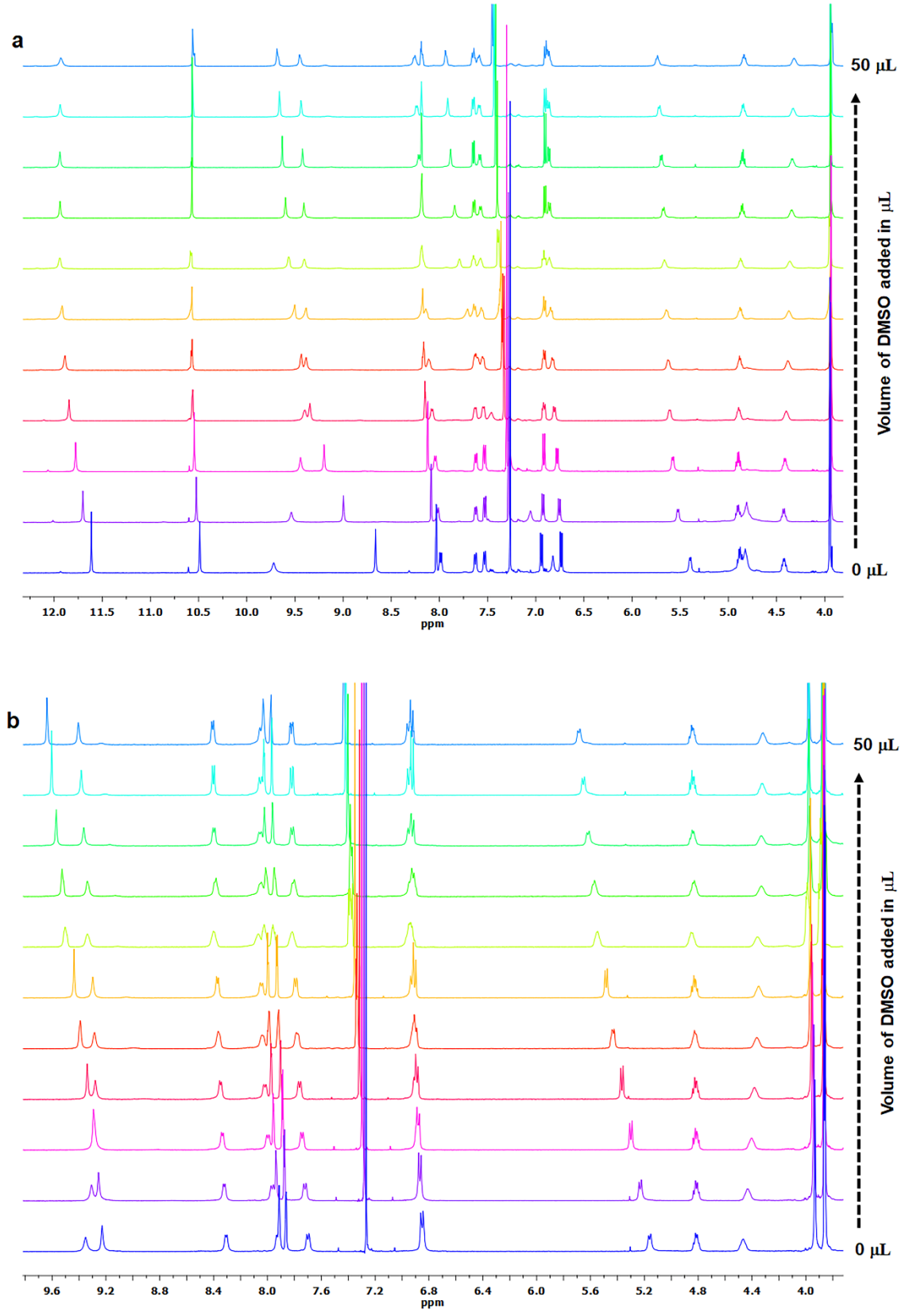


Fig S16. Stacked plot of ^{1}H NMR spectra of tetrapeptides in $CDCl_3$ at 10 mmol concentration with the addition of DMSO-d_6 (5 µL) at each interval. a) stacked plot of tetrapeptide **1**. b) stacked plot of tetrapeptide **4**.

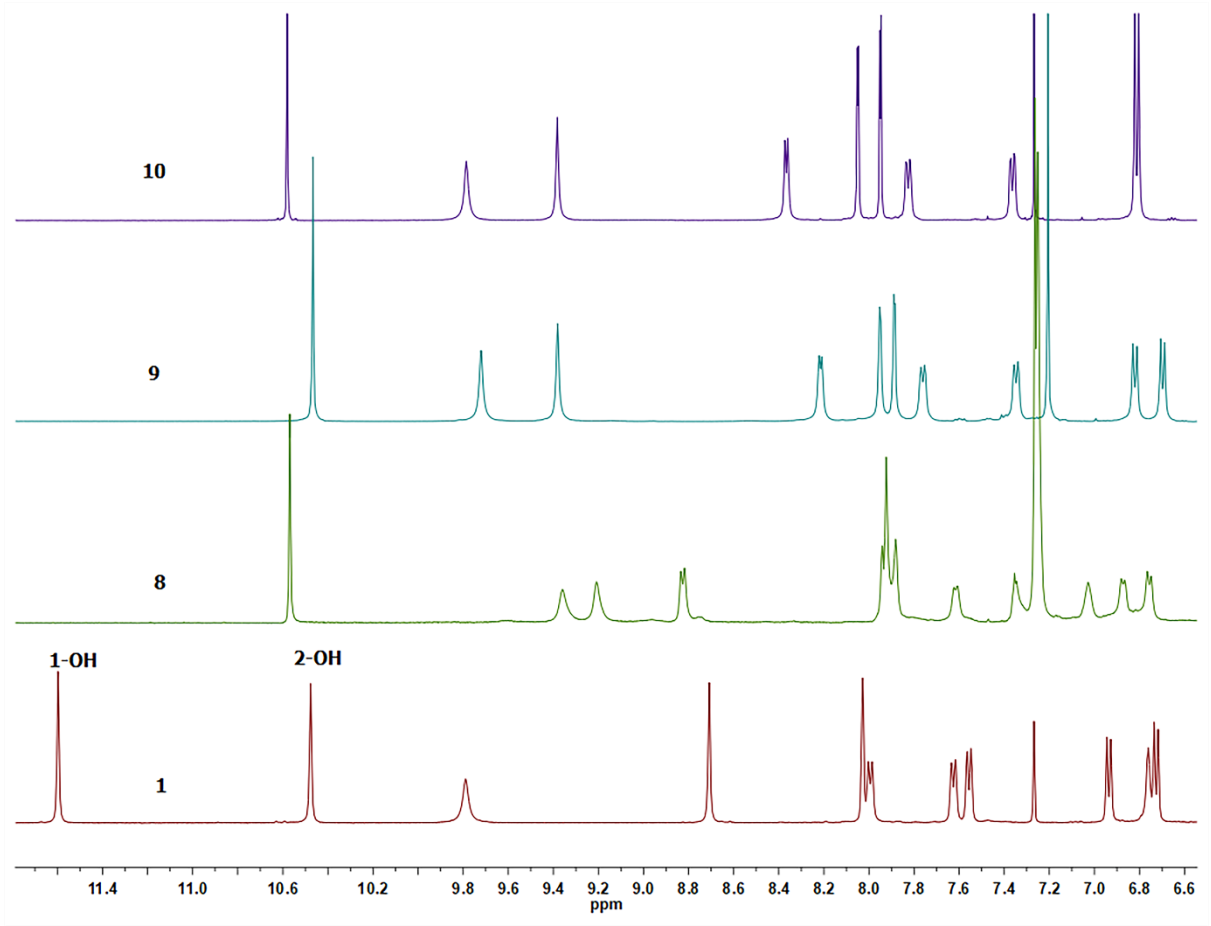


Fig S17. A stacked plot of ^{1}H NMR spectra of tetrapeptides **1** and monoalkylated derivatives **8**, **9**, and **10** showing downfield region.

8. Computational analysis

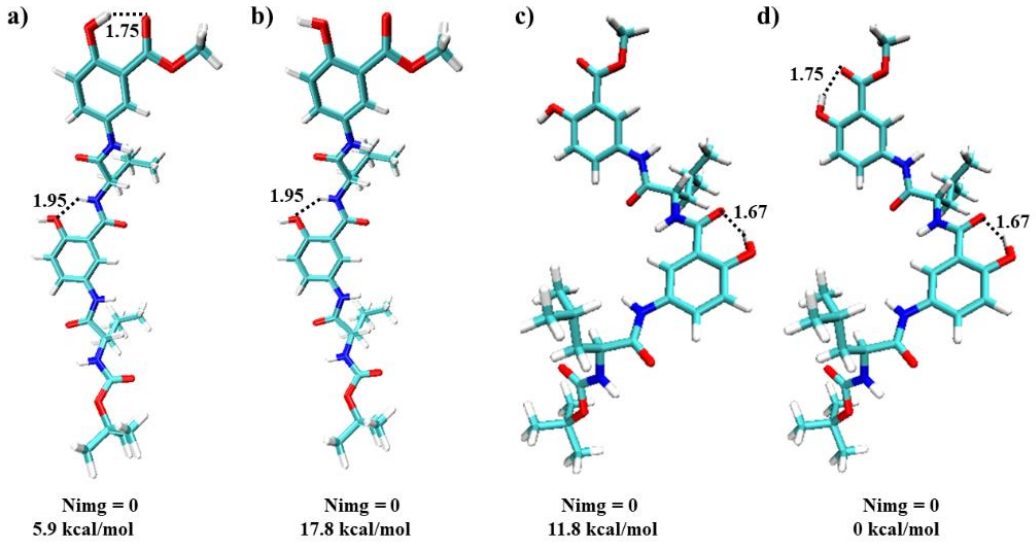


Fig S18. Different optimized conformers of peptide **1** (a-d) at the B3LYP/6-31G* level of theory using Gaussian09 software package differing in energy relative to structure d.

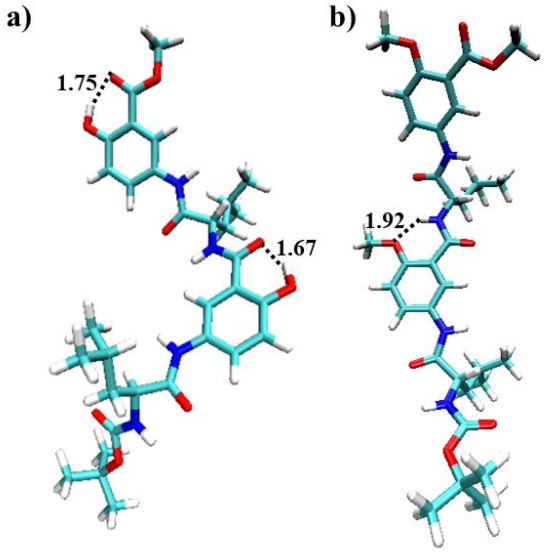


Fig S19. Optimized minimum energy conformers of peptides (a) **1** and (b) **4** at the B3LYP/6-31G* level of theory using Gaussian09 software package.

9. UV titration study

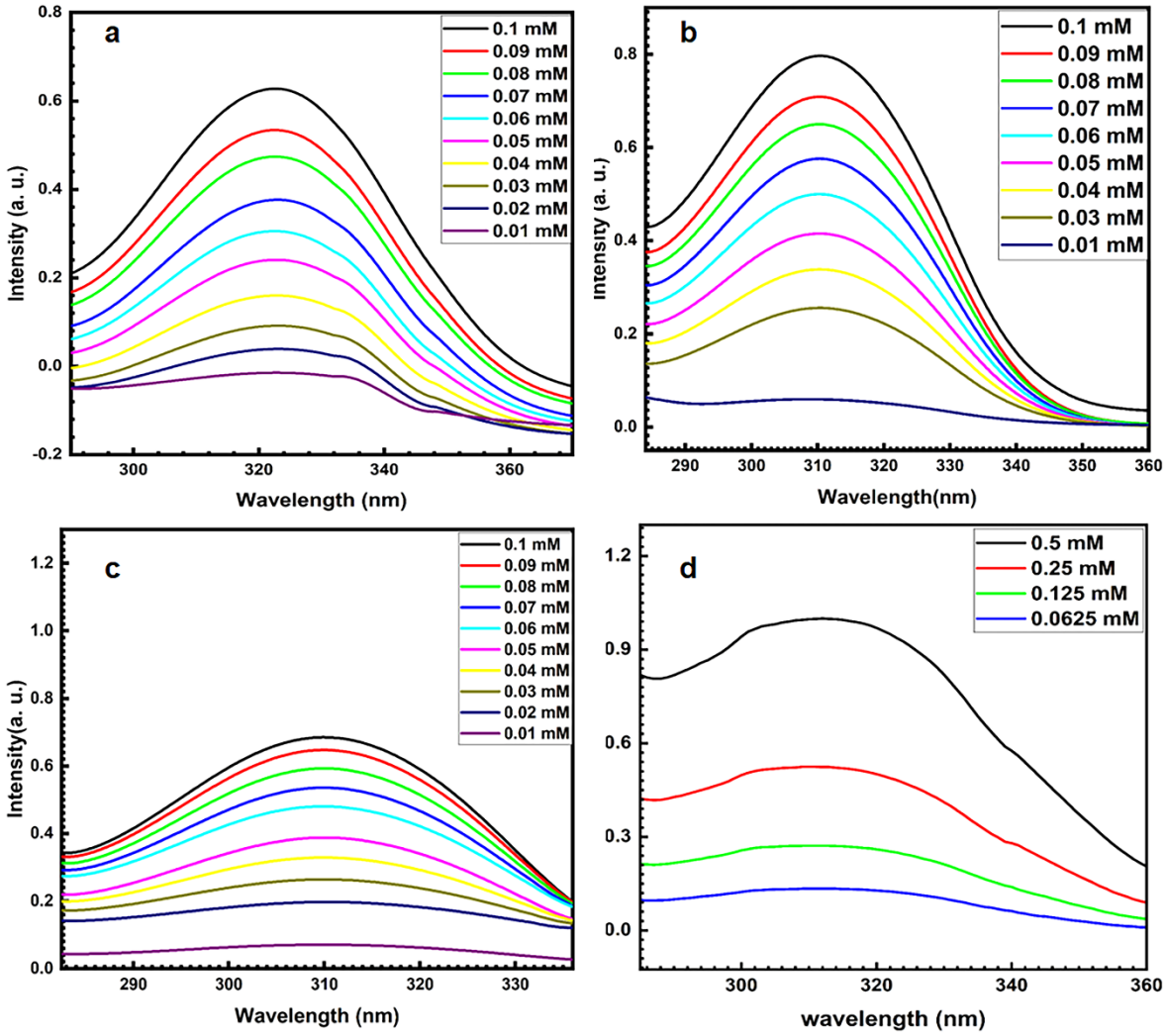


Fig S20. UV-Visible absorption spectra of tetrapeptides in methanol. a) **1**, b) **2**, c) **4**, and d) **13a**.

Table S9. λ_{max} for tetrapeptides **1**, **2**, **4**, and **13a**.

S. No.	Compound	λ_{max} (nm) in methanol
1.	1	322
2.	2	310
3.	4	310
4.	13a	310

10. Fluorescence spectrophotometric titration study

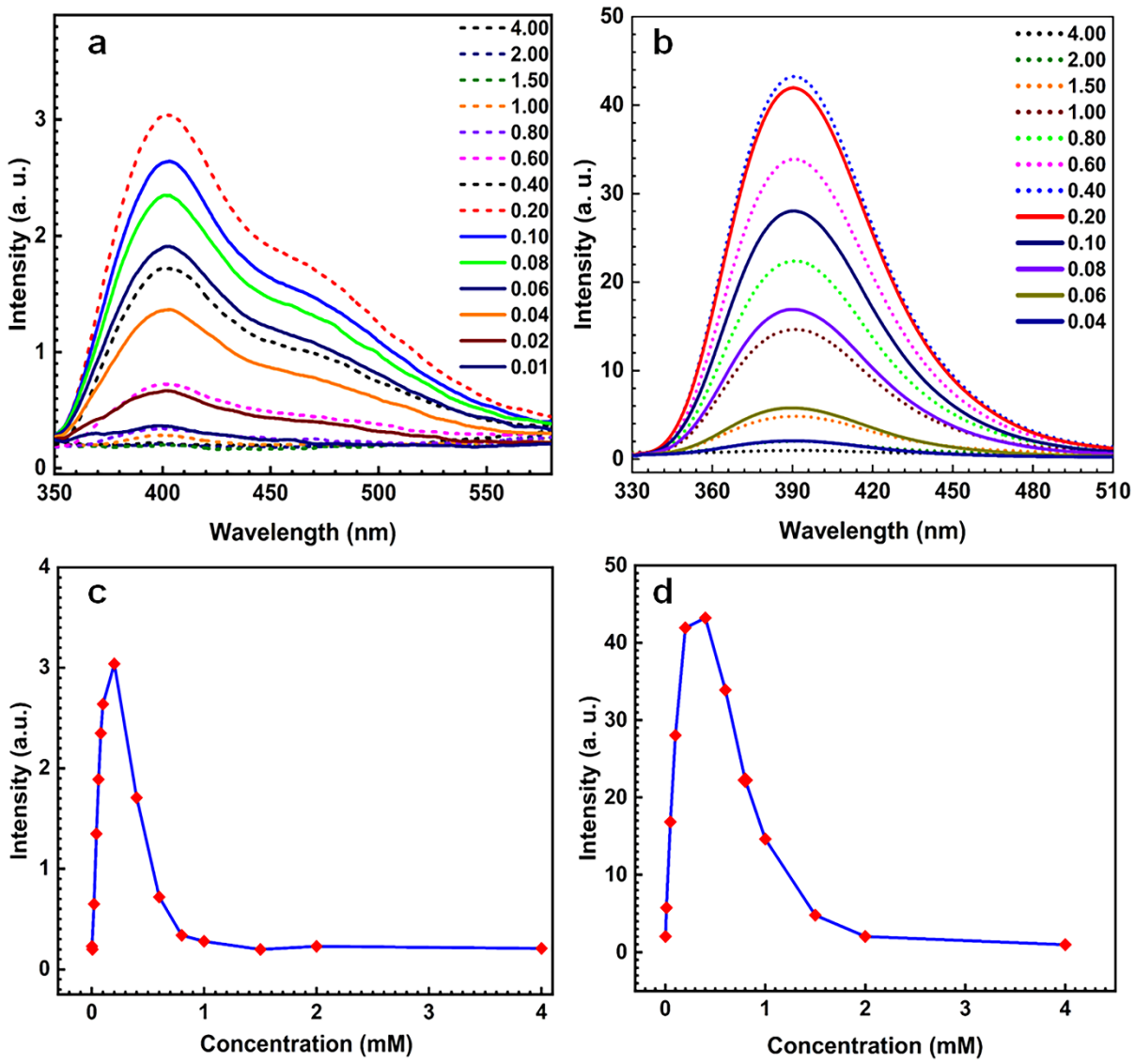


Fig. S21. Concentration-dependent fluorescence emission spectra of peptides in methanol. a) dipeptide **17**, b) dipeptide **18**, and c-d) A plot of λ_{max} emission *vs* concentration of **17** and **18** respectively.

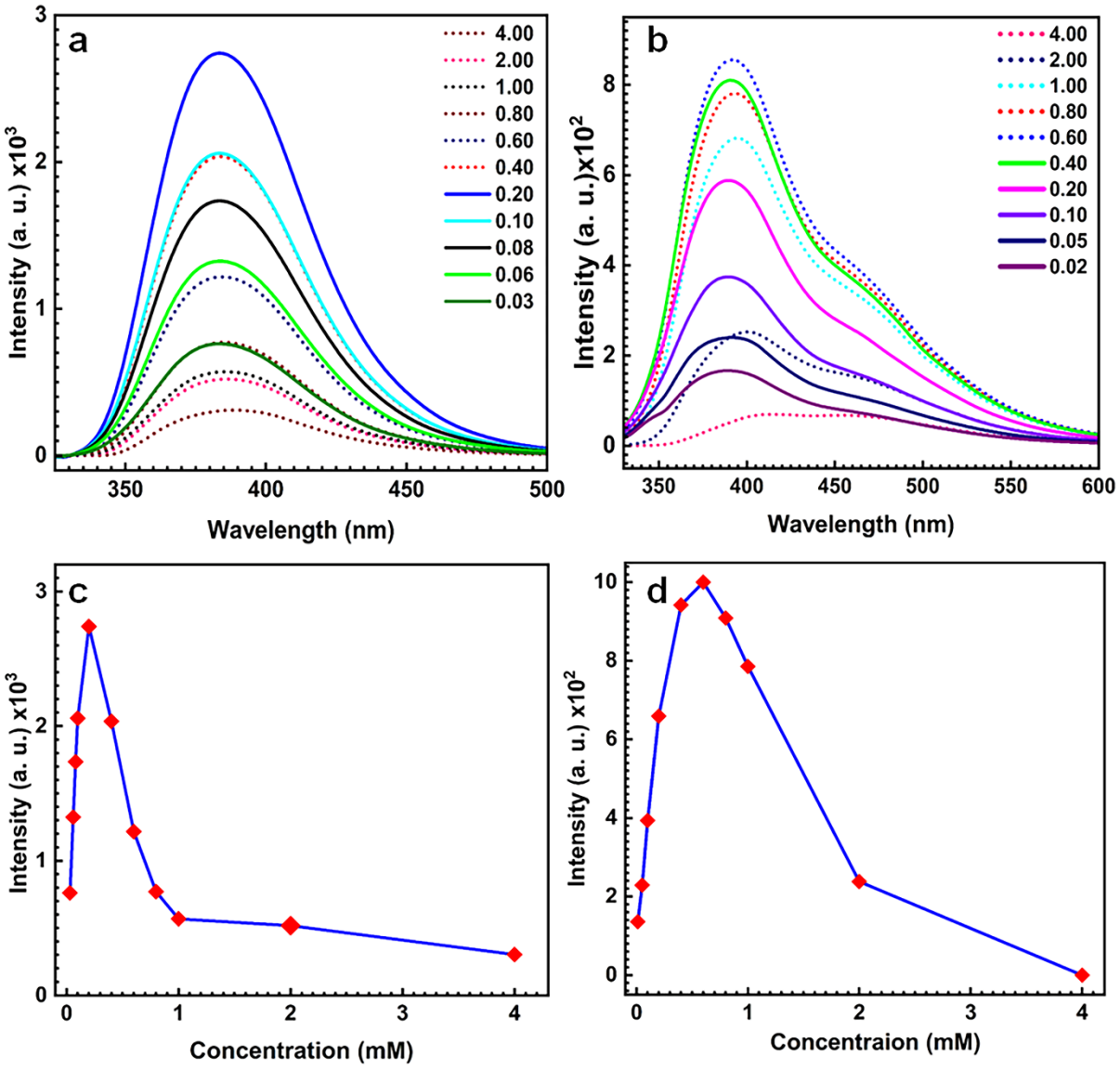


Fig. S22. Concentration-dependent fluorescence emission spectra of tetrapeptides in methanol. a) peptide **4**, b) peptide **13a**, and c-d) A plot of λ_{max} emission *vs* concentration of peptide **4** and **13a** respectively.

11. Particle size and distribution analysis

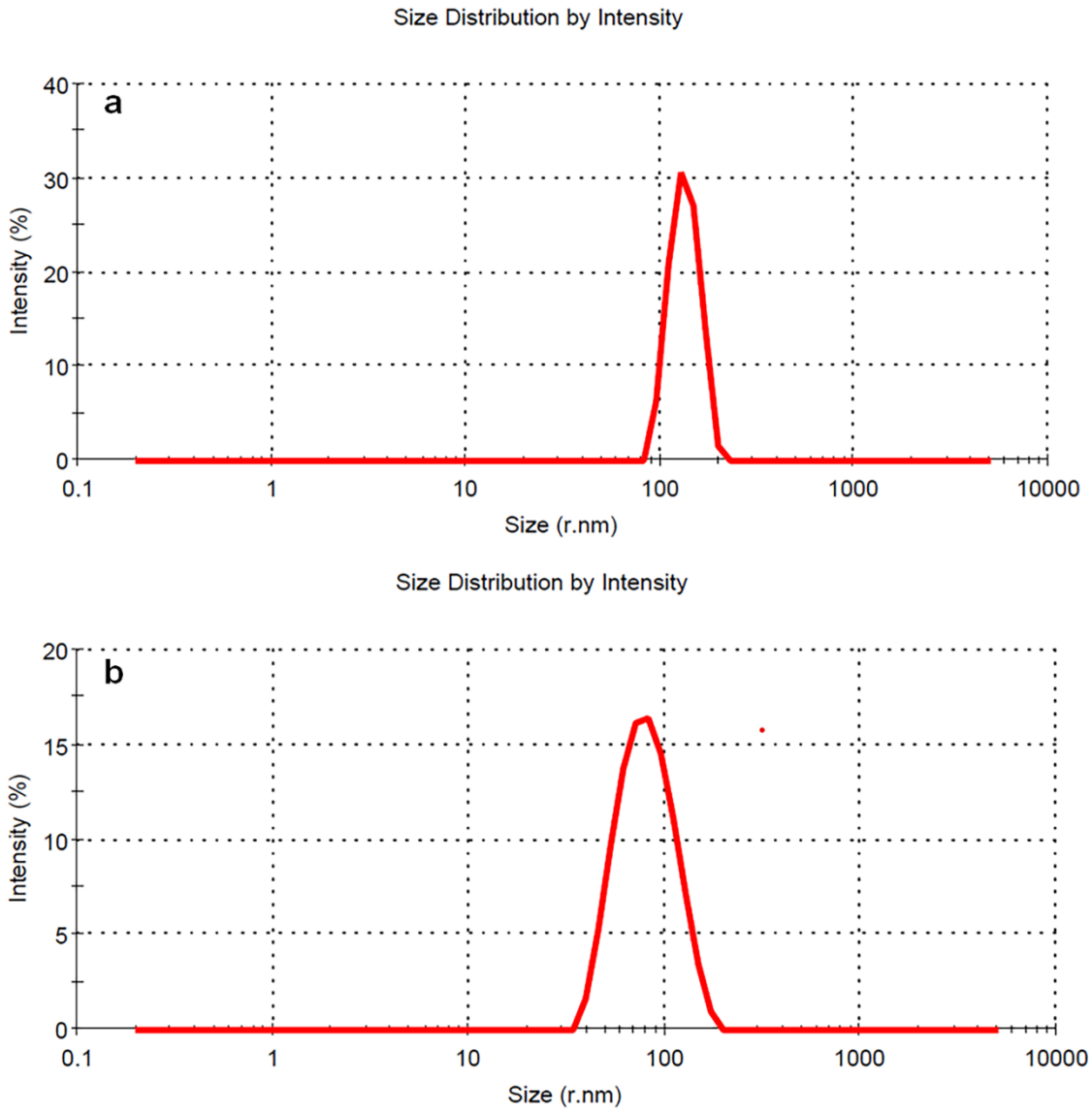


Fig S23. Size of particles formed by tetrapeptides. a) **1** at 0.2 mM concentration (methanol-water 1:4). b) **2** at 0.2 mM concentration (methanol-water 1:4).

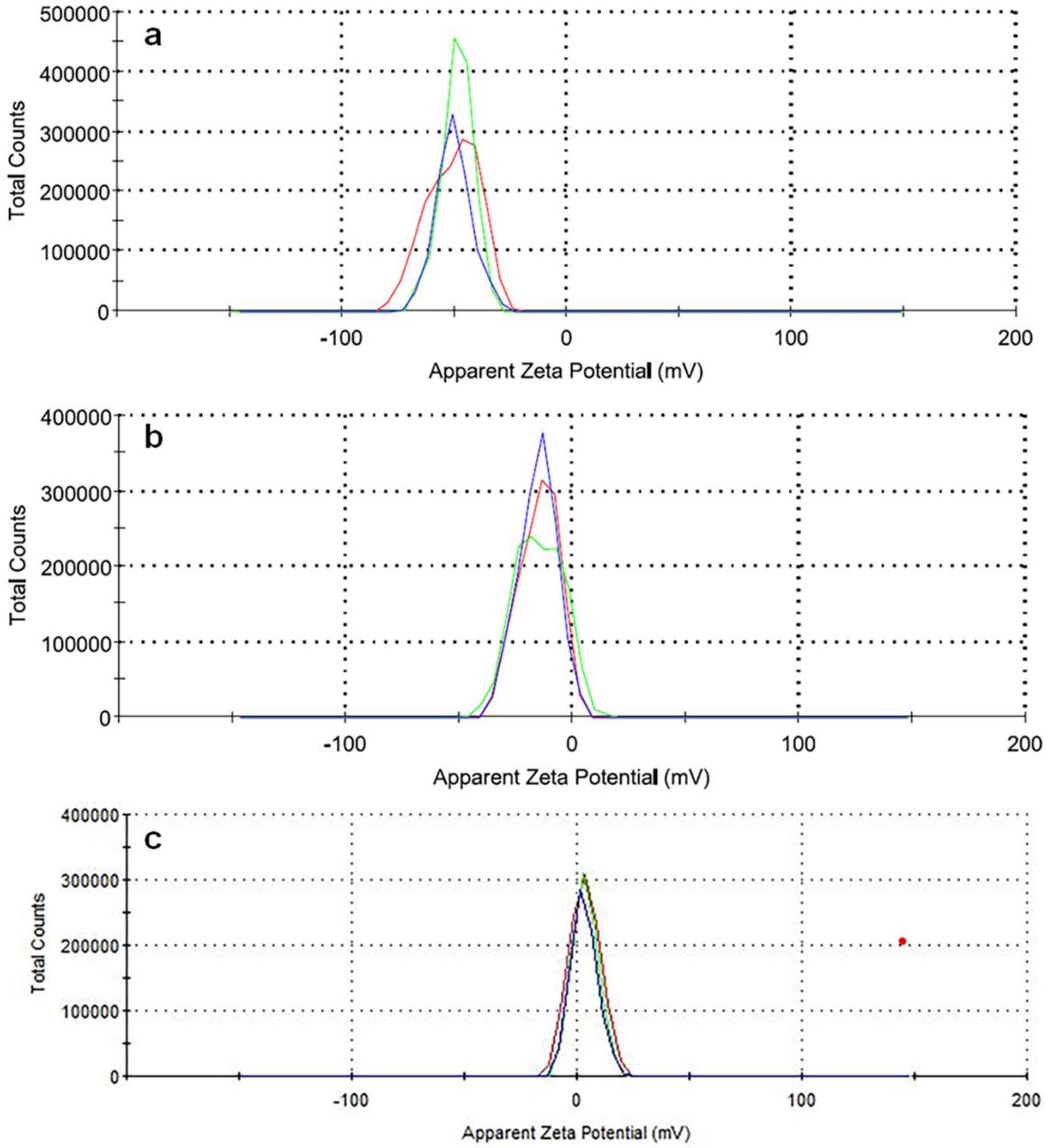


Fig S24. Zeta potential of tetrapeptides at 0.2 mM concentration in methanol-water 1:4. a) peptide **1**, b) peptide **2**, and c) peptide **13a**.

12. Morphology analysis

11.1 Field Emission Scanning Electron Microscopy (FE-SEM) study

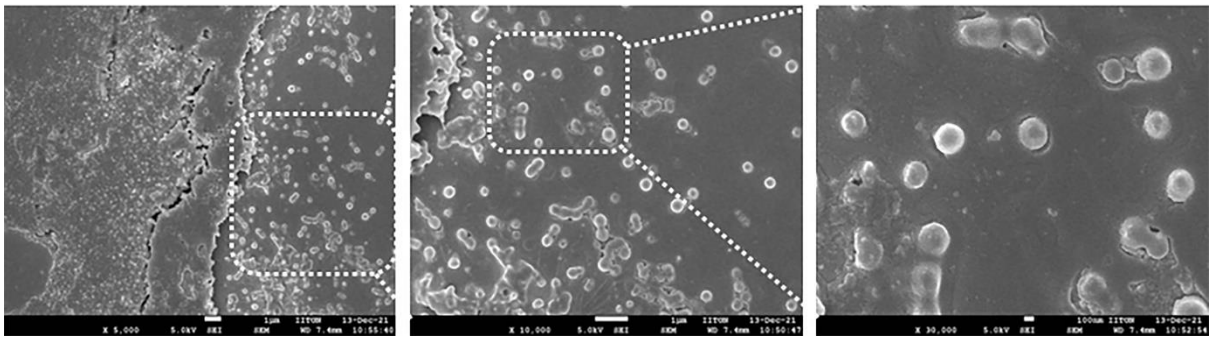


Fig S25. FE-SEM images of tetrapeptide **1**. 0.09 mM in methanol-water mixture (1:4).

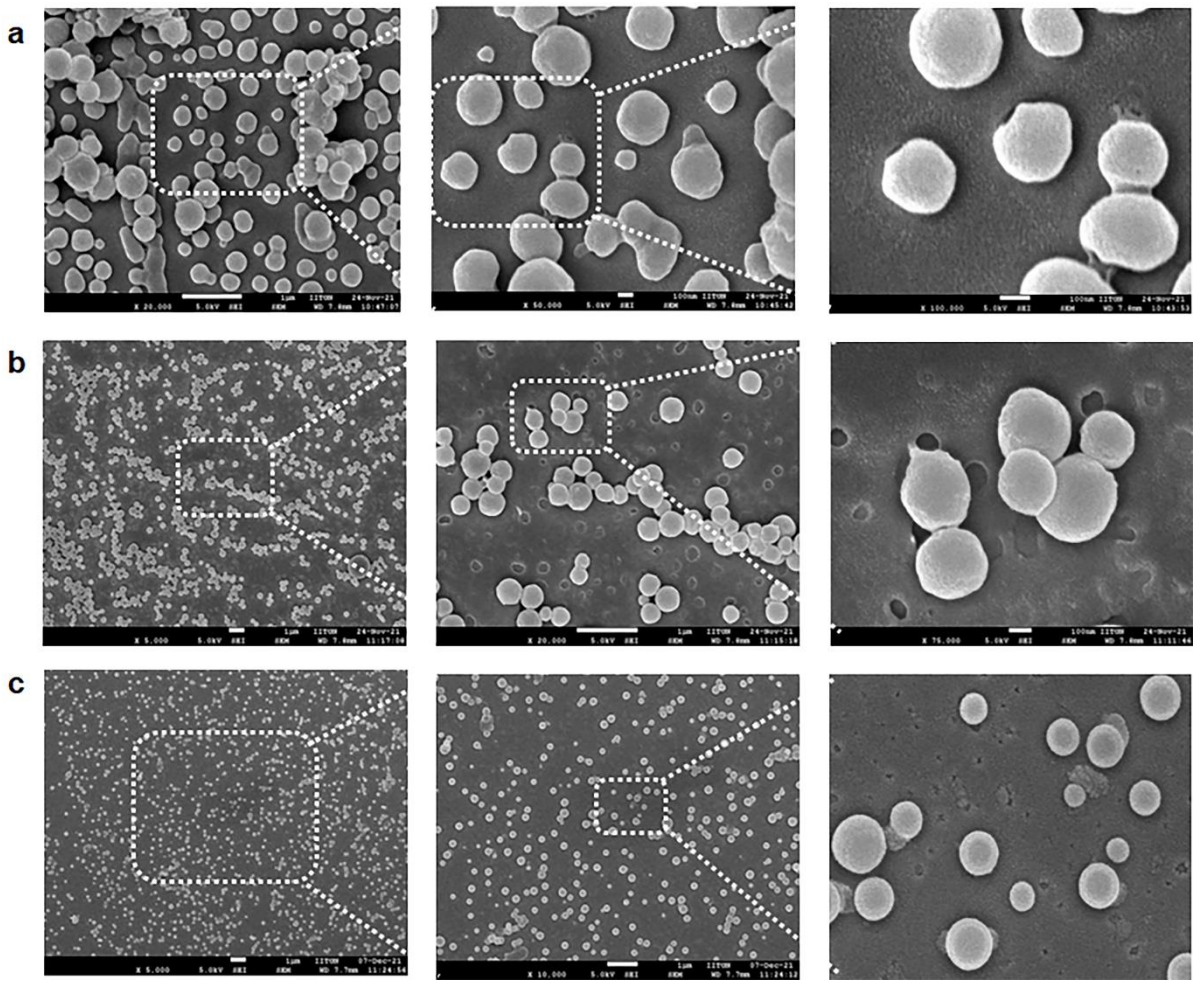


Fig S26. FE-SEM images of tetrapeptide **1**. a) 1 mM in methanol, b) 1 mM in methanol water ratio 1:1, and c) 0.2 mM in methanol-water mixture (1:4).

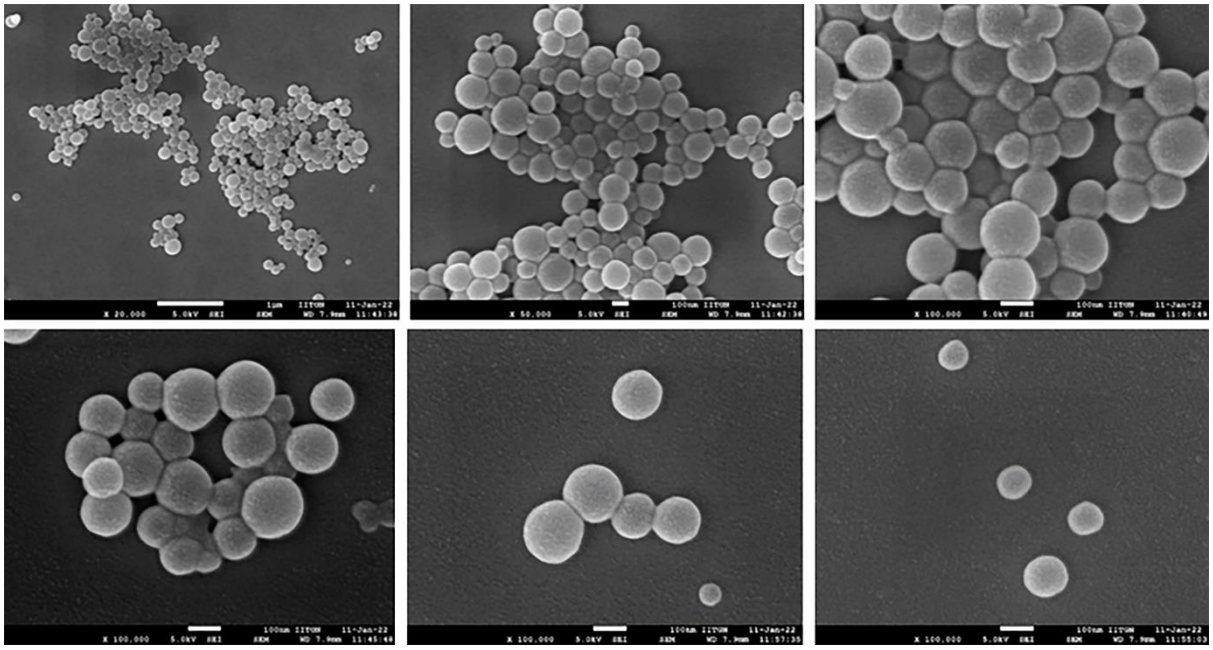


Fig S27. FESEM images of tetrapeptide **2** at 0.2 mM in methanol water mixture (1:4).

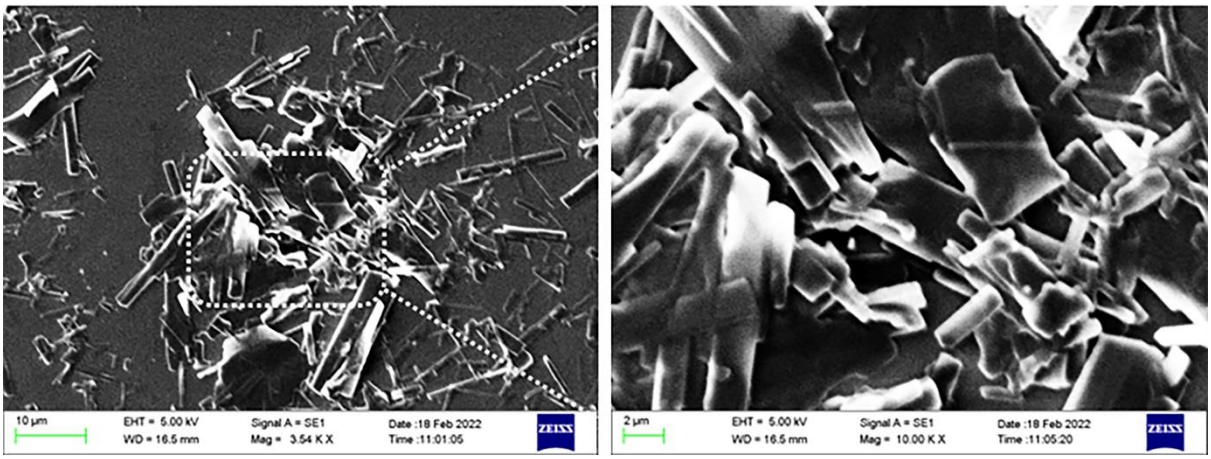


Fig S28. SEM images of dipeptide **18** at 0.2 mM in methanol water 1:4.

12.2 High Resolution Transmission Electron Microscopy (HR-TEM) study

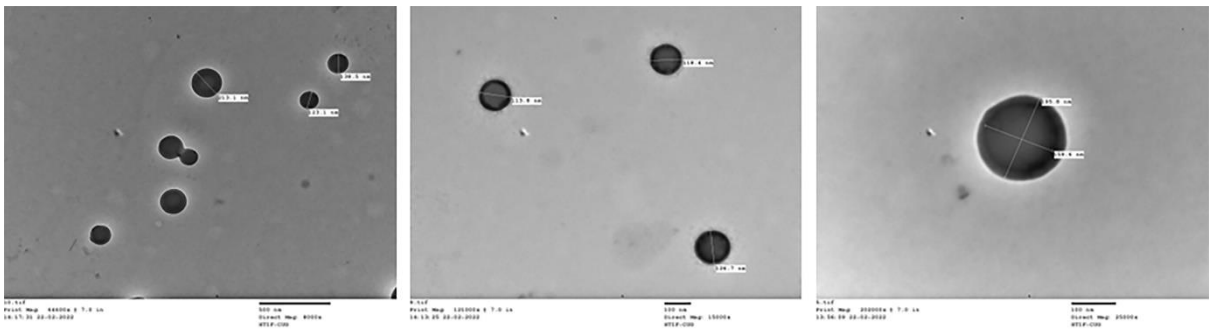


Fig S29. HR-TEM images of tetrapeptide **1** at 0.2 mM in methanol water 1:4.

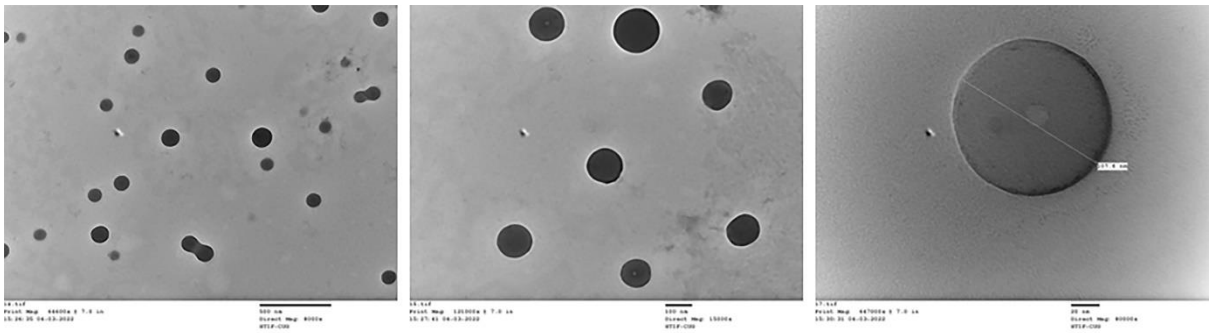


Fig S30. HR-TEM image of tetrapeptide **2** at 0.2 mM in methanol water 1:4.

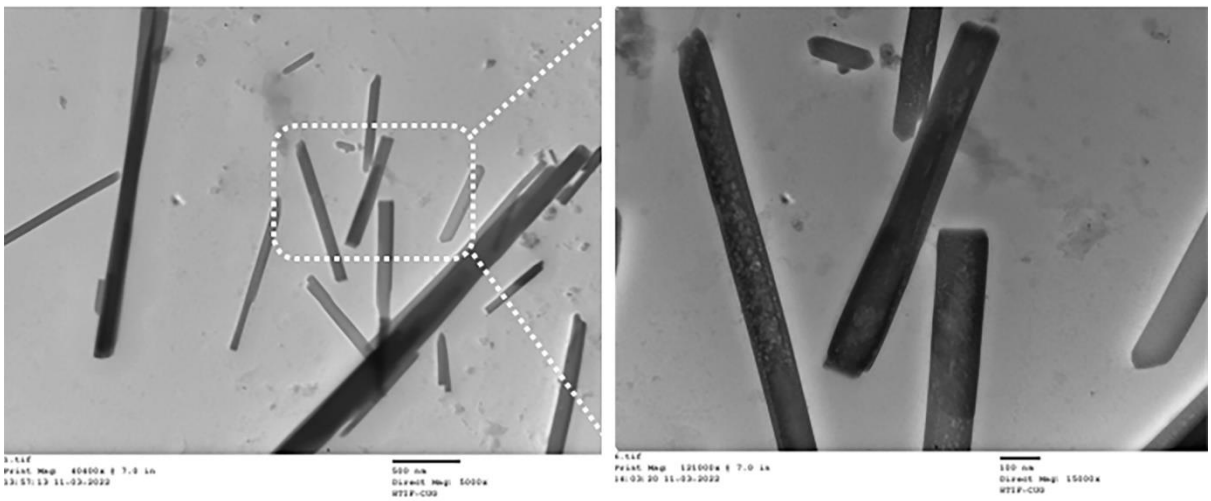


Fig S31. HR-TEM image of dipeptide **18** at 0.2 mM in methanol water 1:4.

13. MTT assay

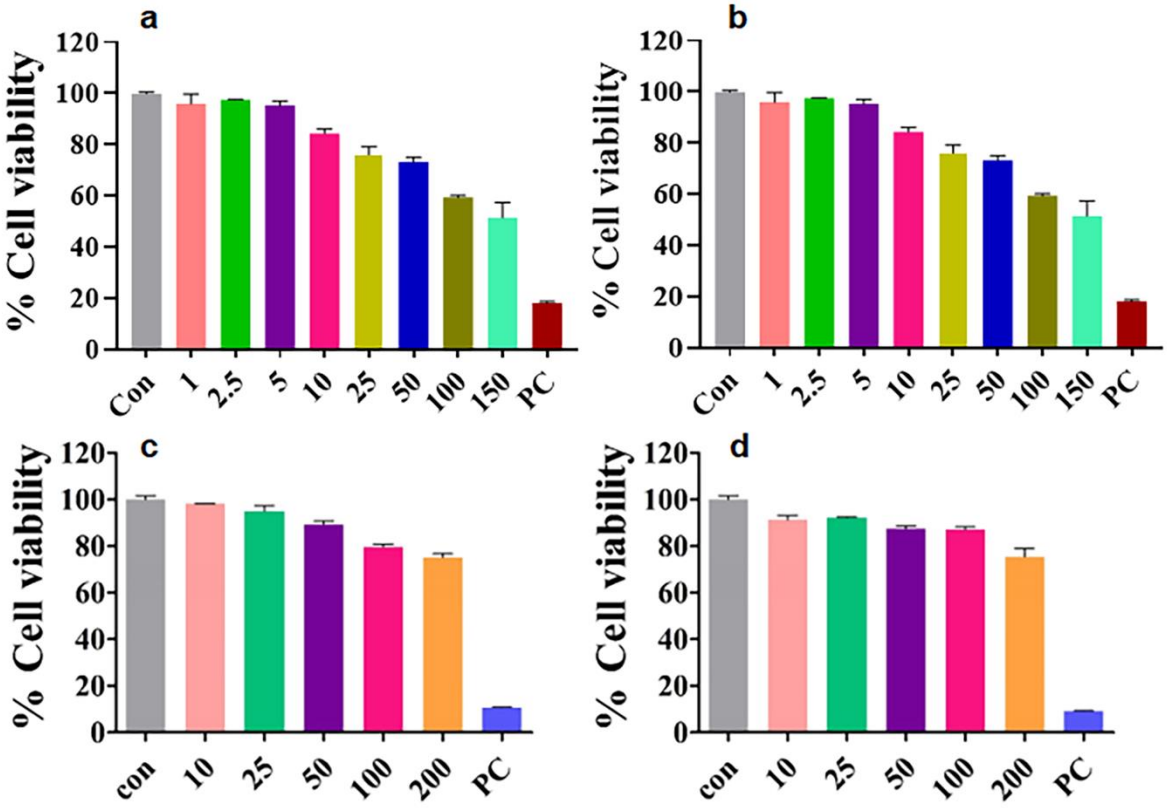
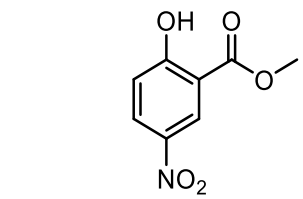


Fig S32. Cytotoxicity of peptide nanoparticles determined by MTT assay on triple-negative breast cancer cells (MDA-MB-231). a) **PN1**, b) **PND1**, c) **PN13a**, and d) **PND13a.**

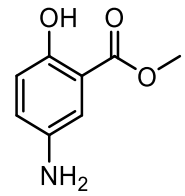
14. Spectra

^{1}H NMR (500 MHz, CDCl$_3$)



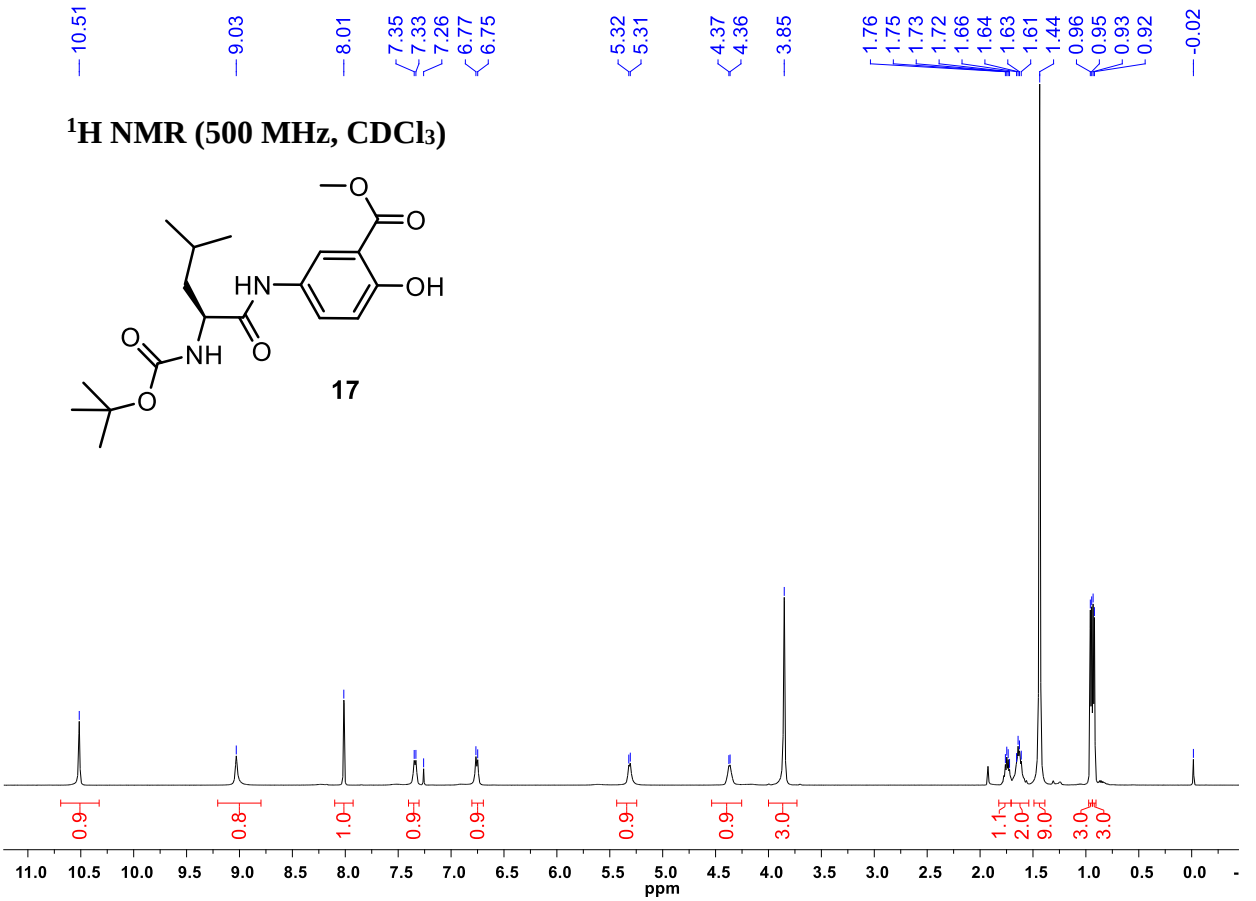
15

^{1}H NMR (500 MHz, CDCl$_3$)



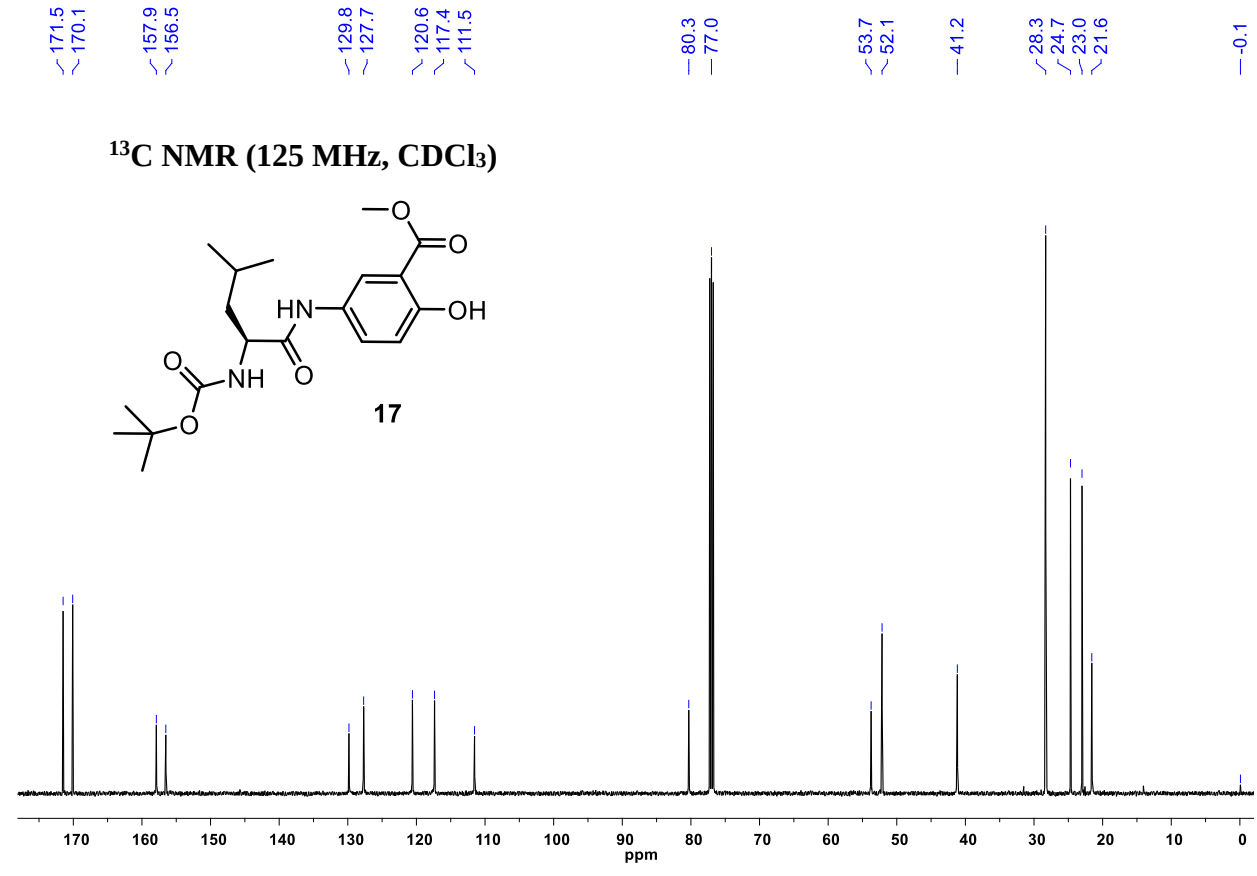
16

^{1}H NMR (500 MHz, $CDCl_3$)



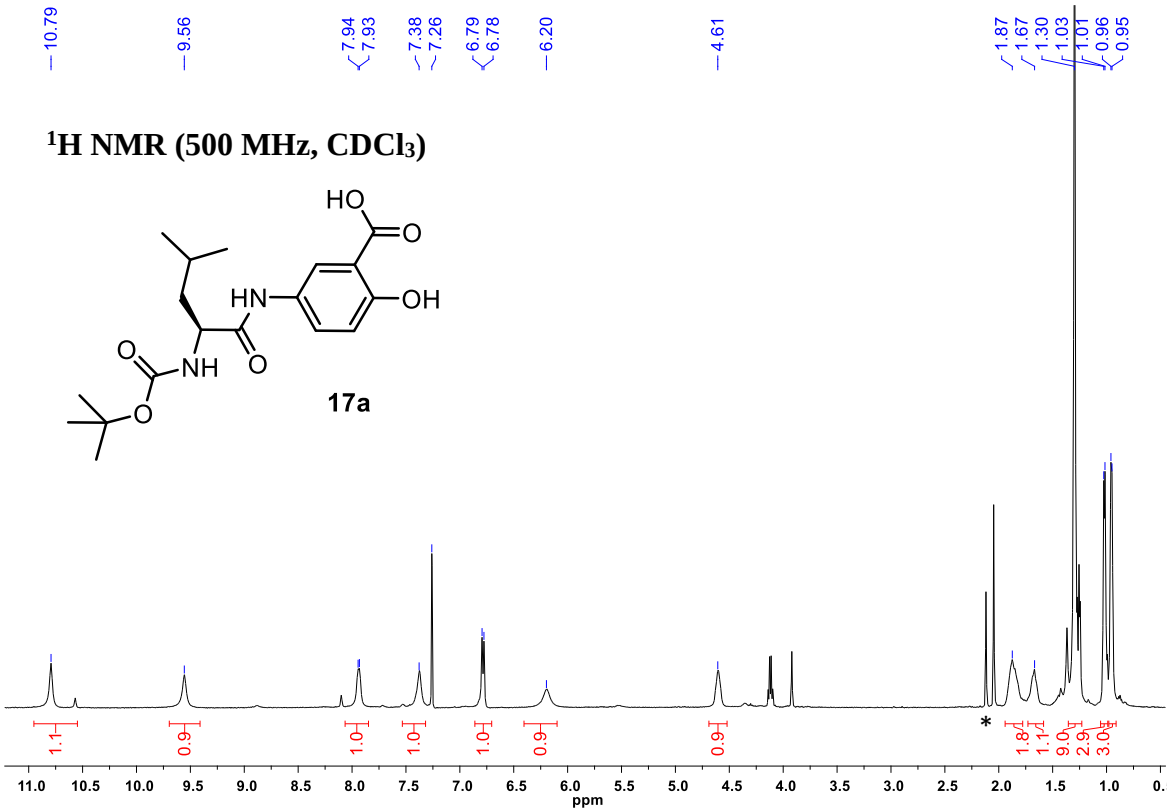
17

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



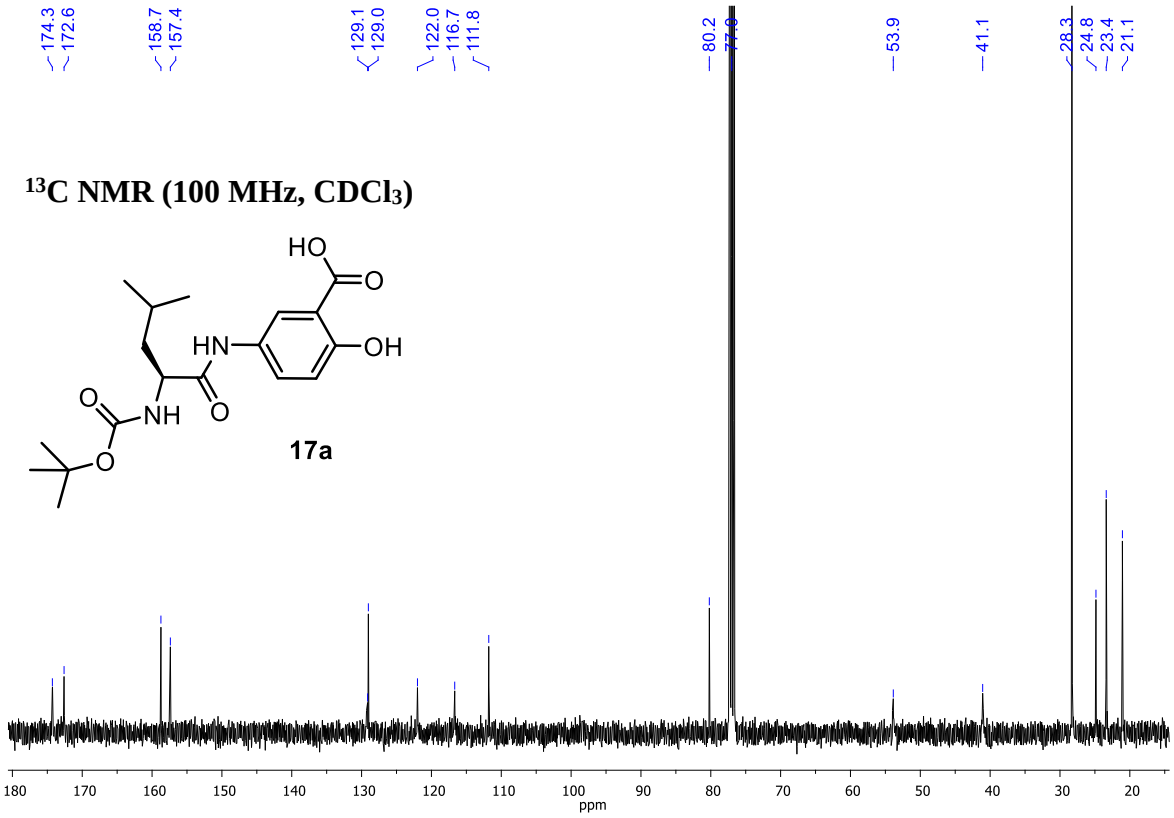
17

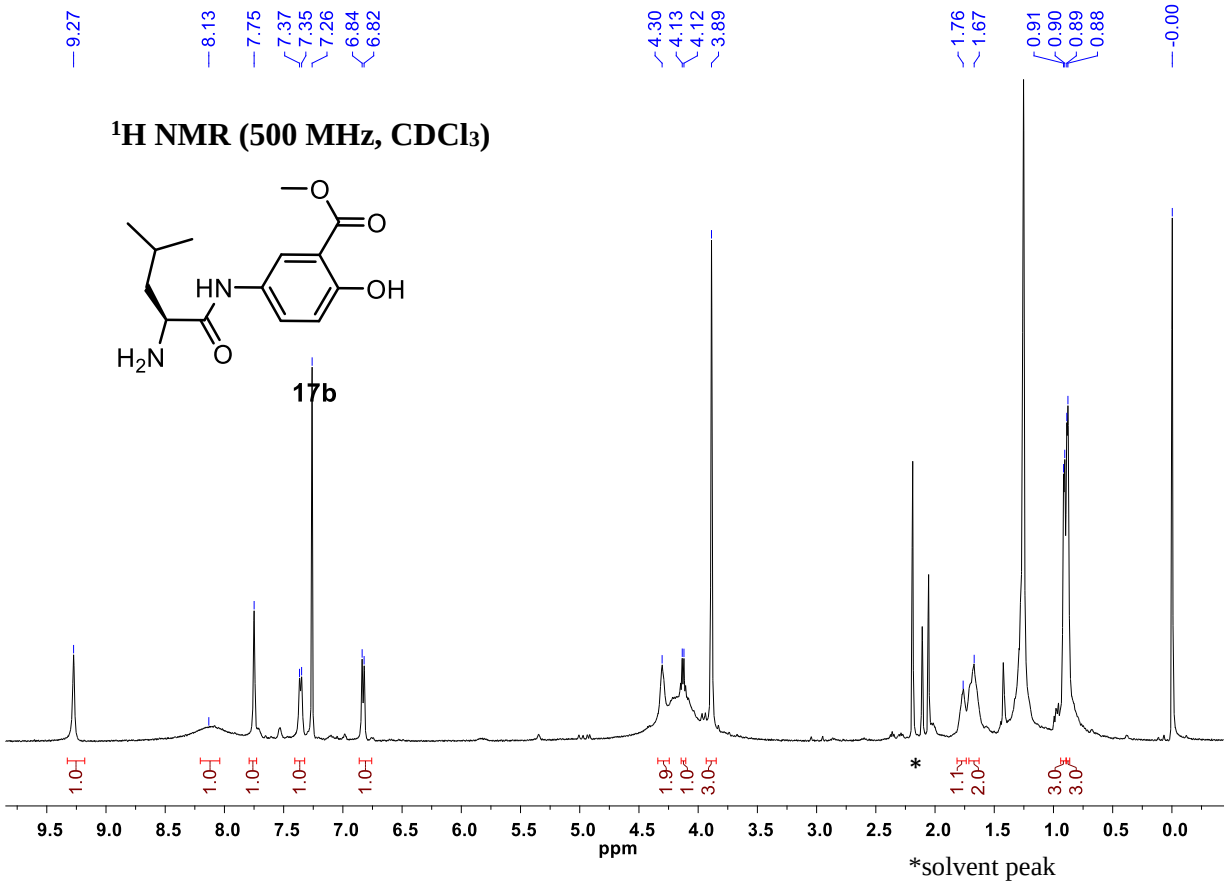
^{1}H NMR (500 MHz, $CDCl_3$)



*Solvent peak

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





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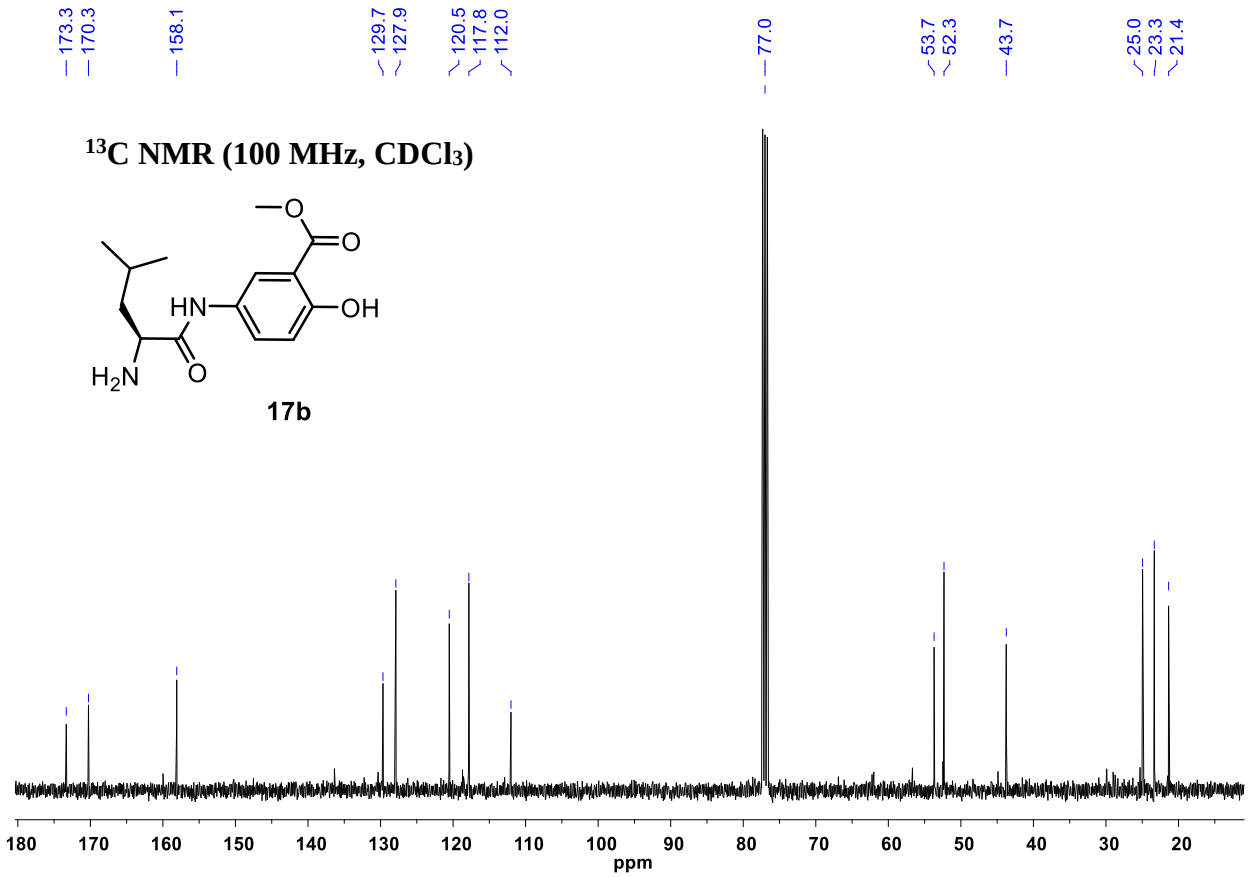
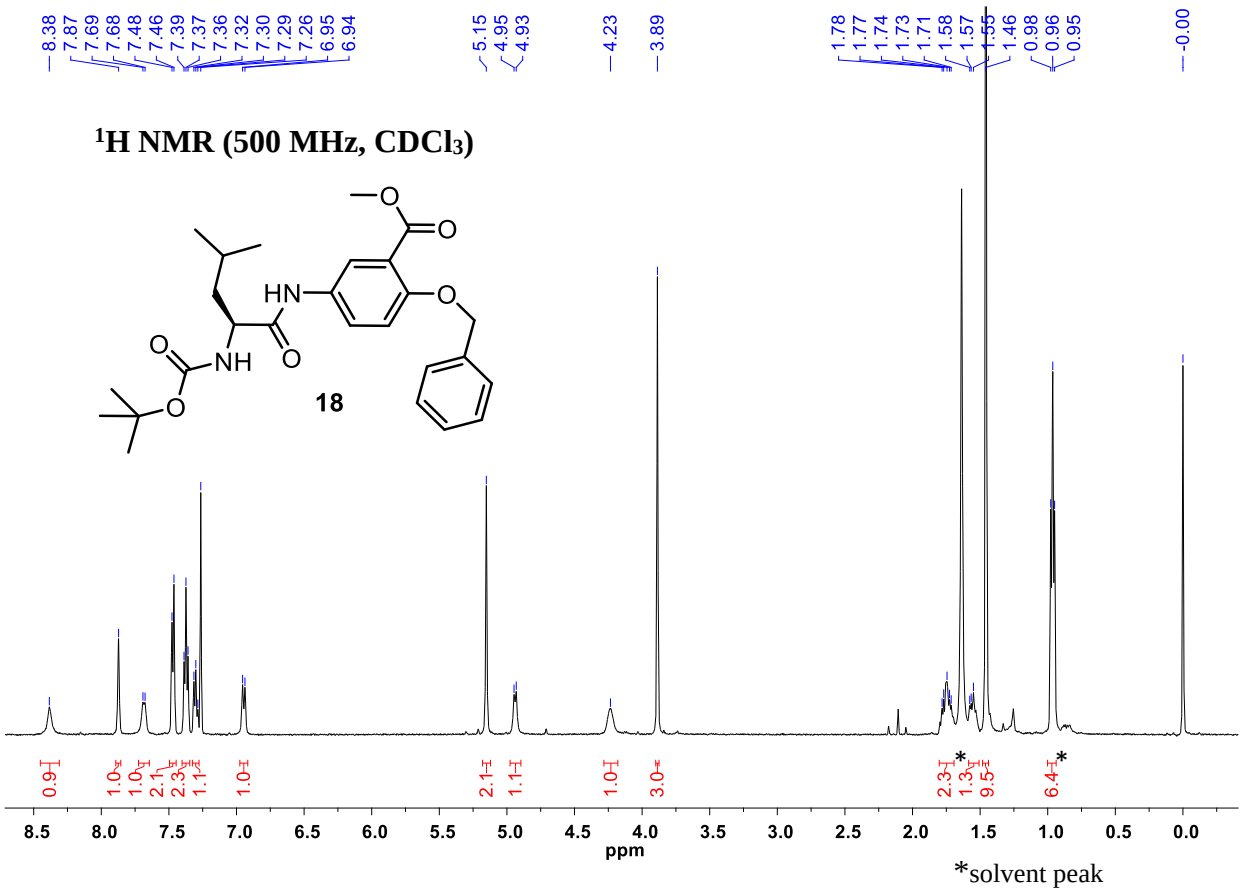
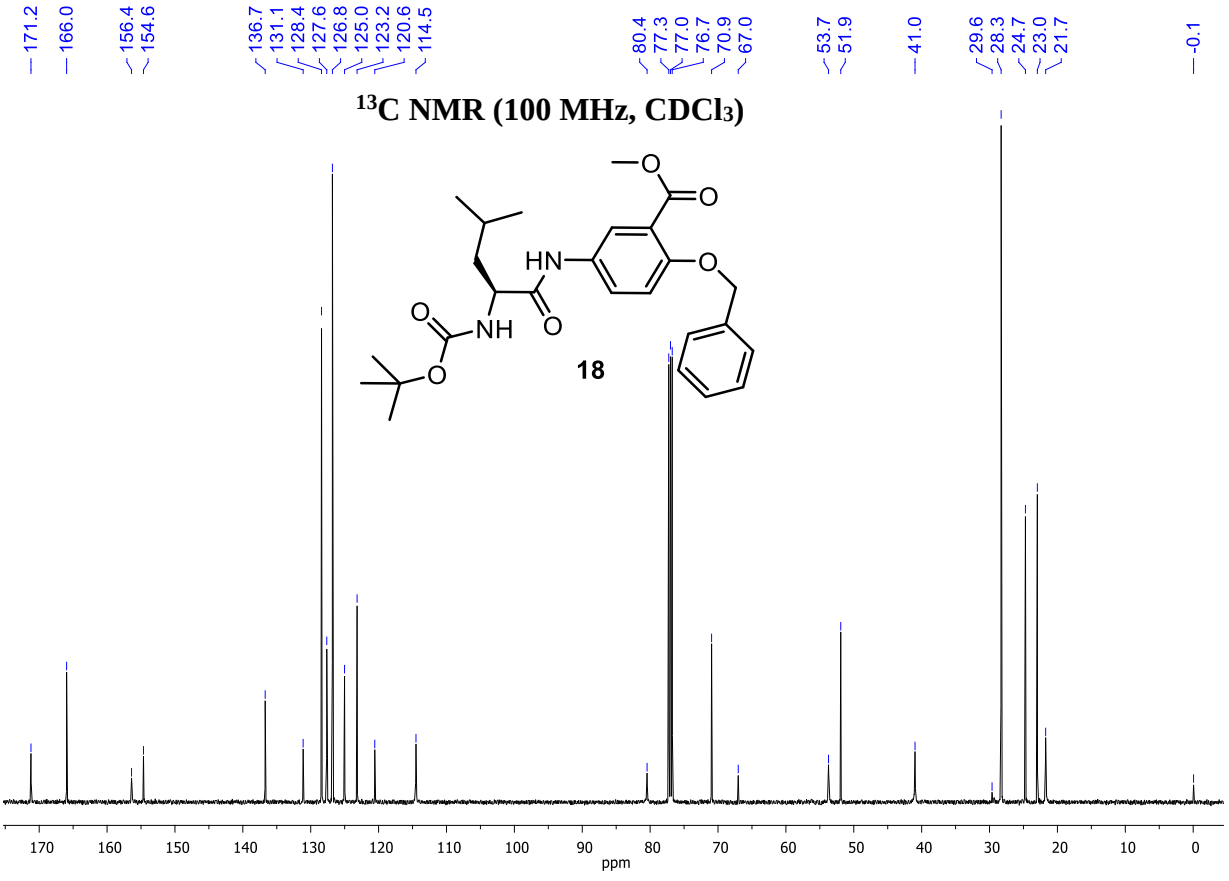


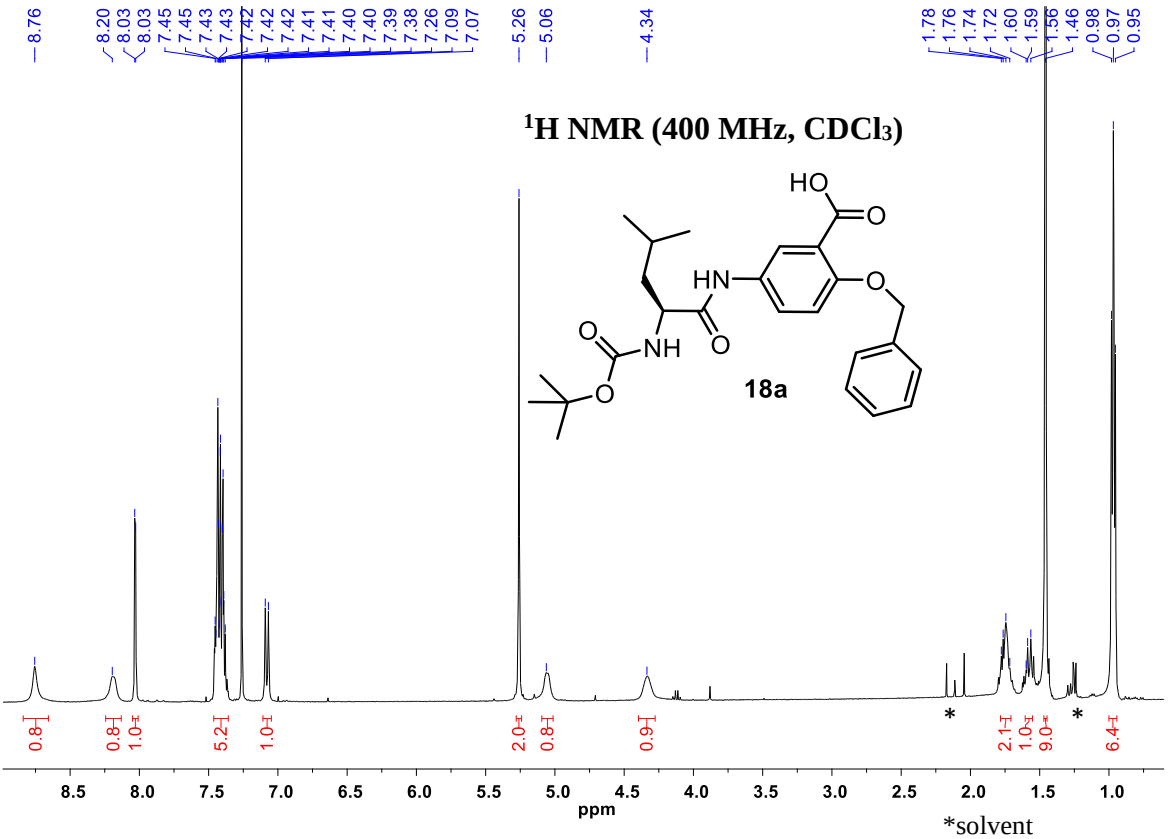


^{1}H NMR (500 MHz, $CDCl_3$)

*solvent peak



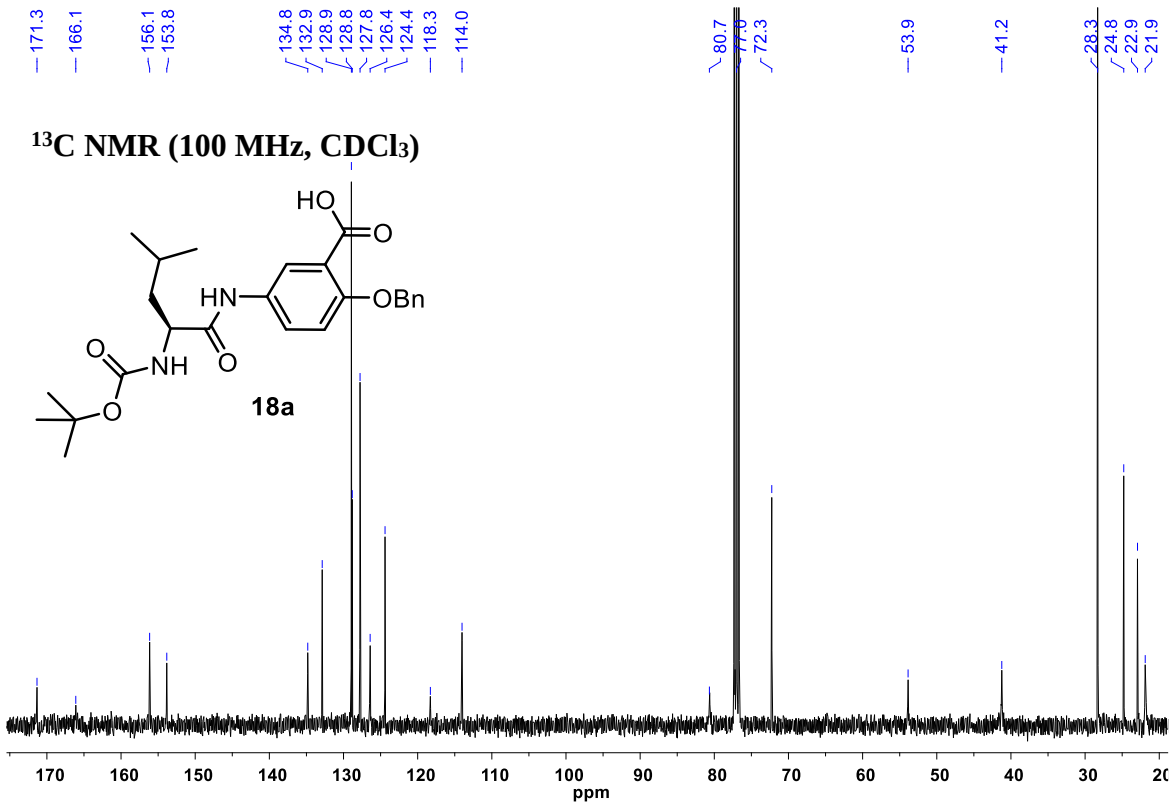
^{13}C NMR (100 MHz, $CDCl_3$)



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

18a

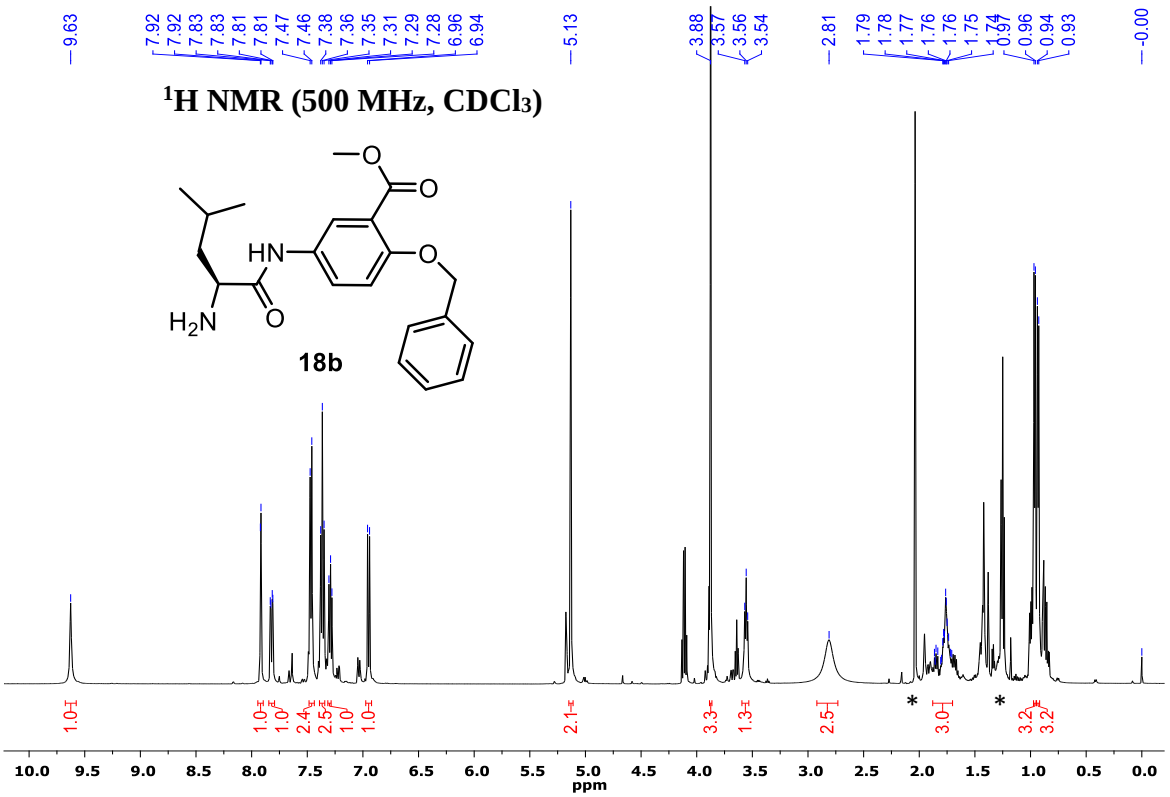
*solvent



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

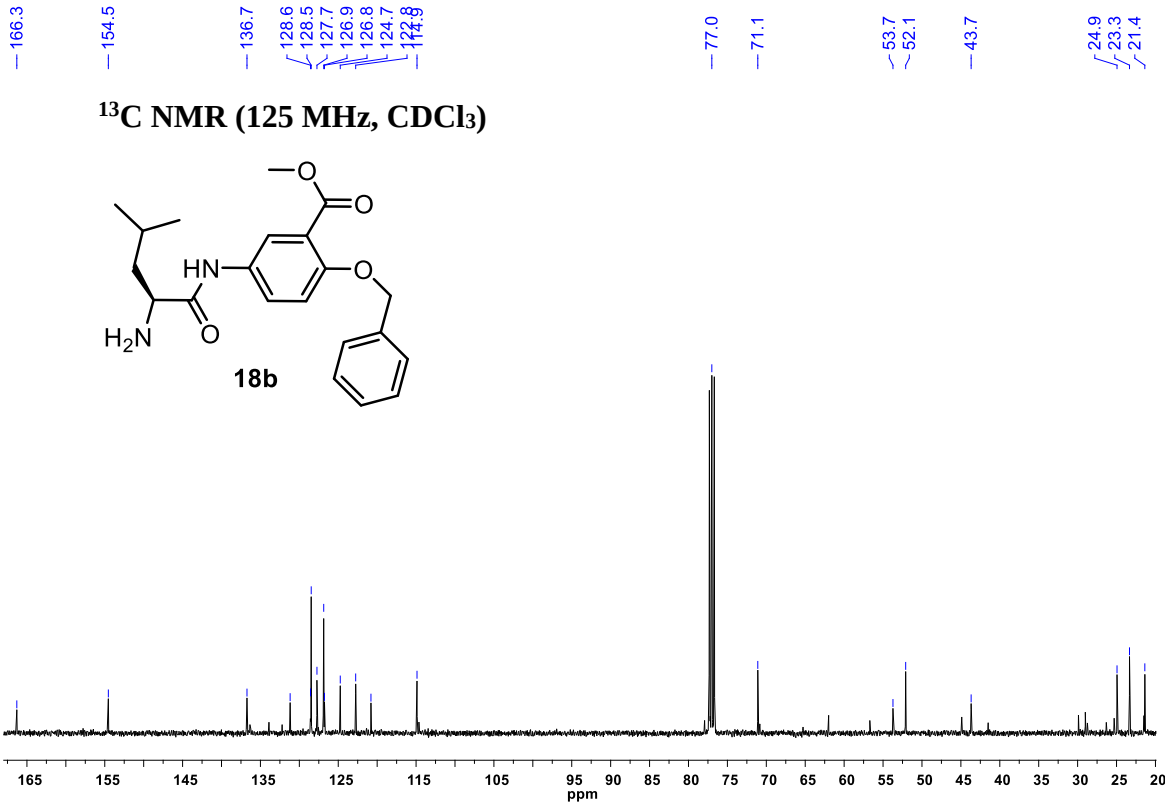
18a

^{1}H NMR (500 MHz, $CDCl_3$)

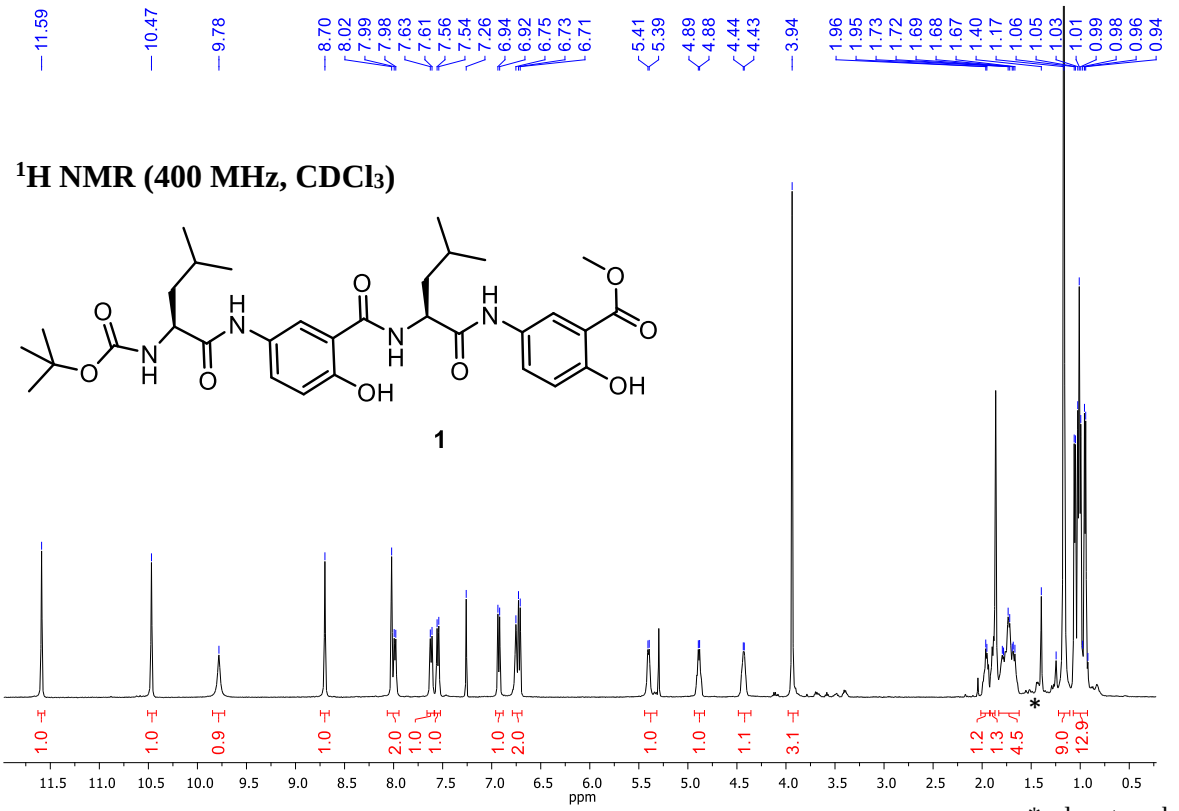


*solvent

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



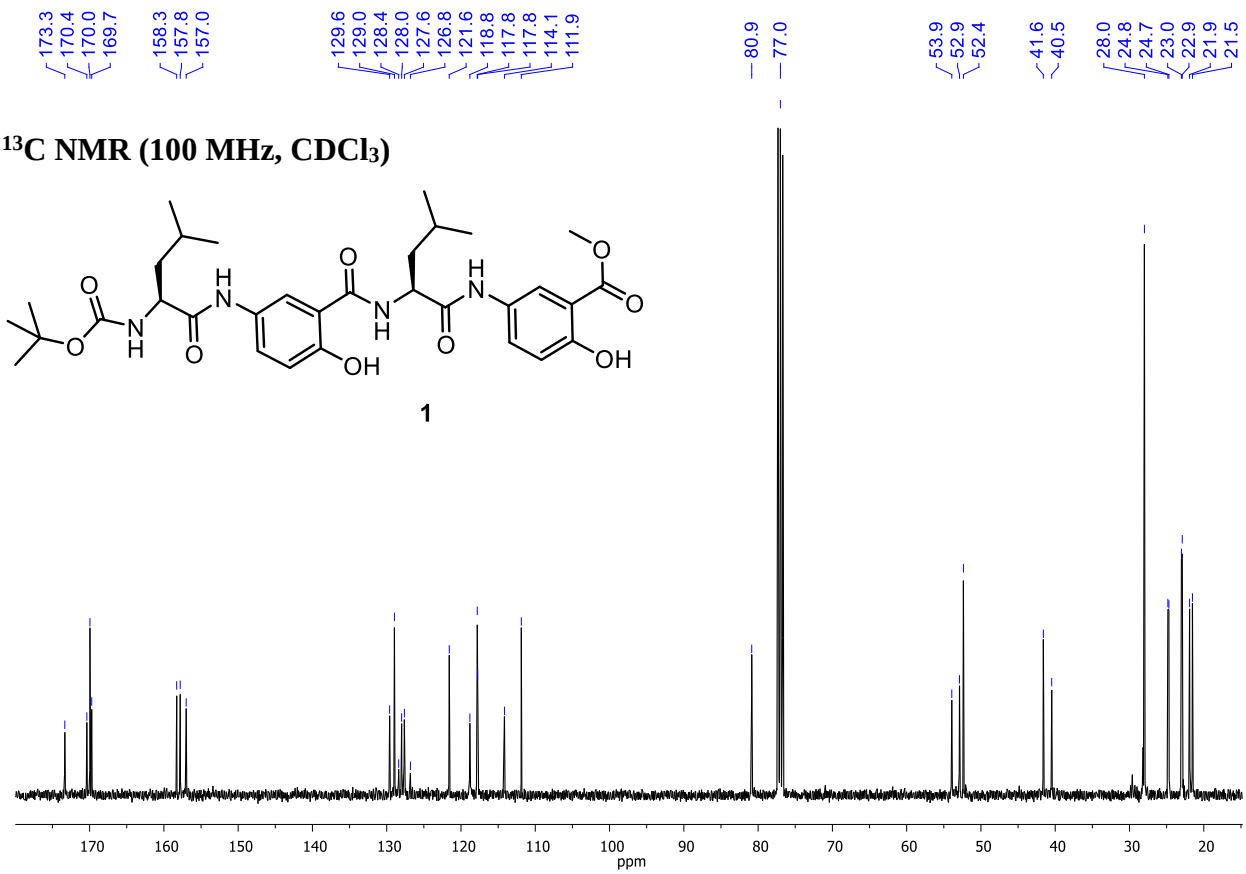
^{1}H NMR (400 MHz, $CDCl_3$)



1

*solvent peak

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



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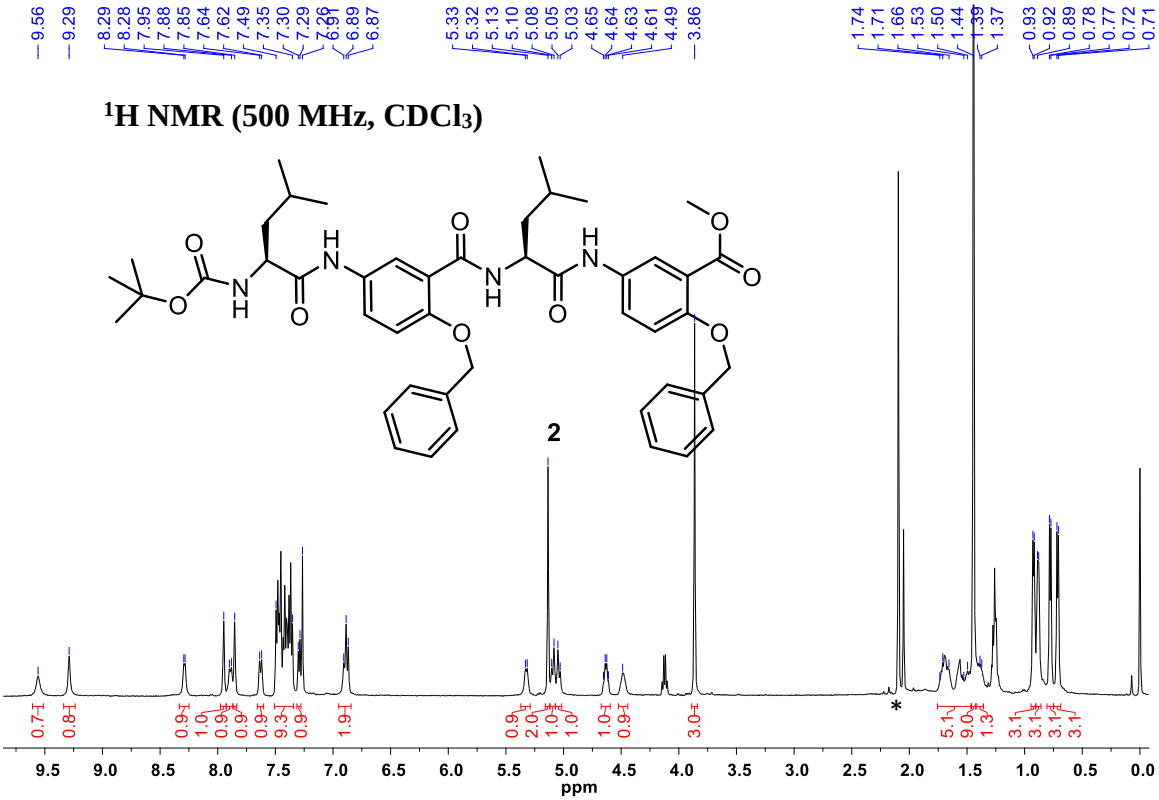
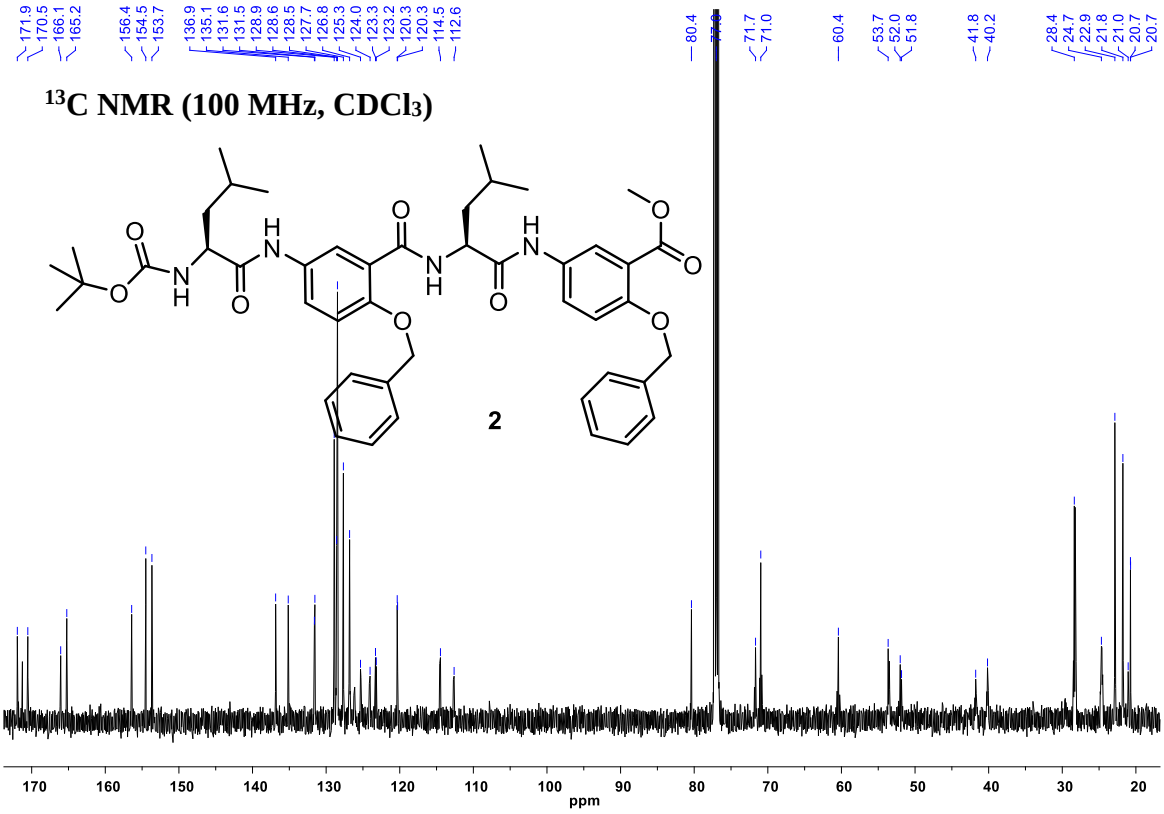
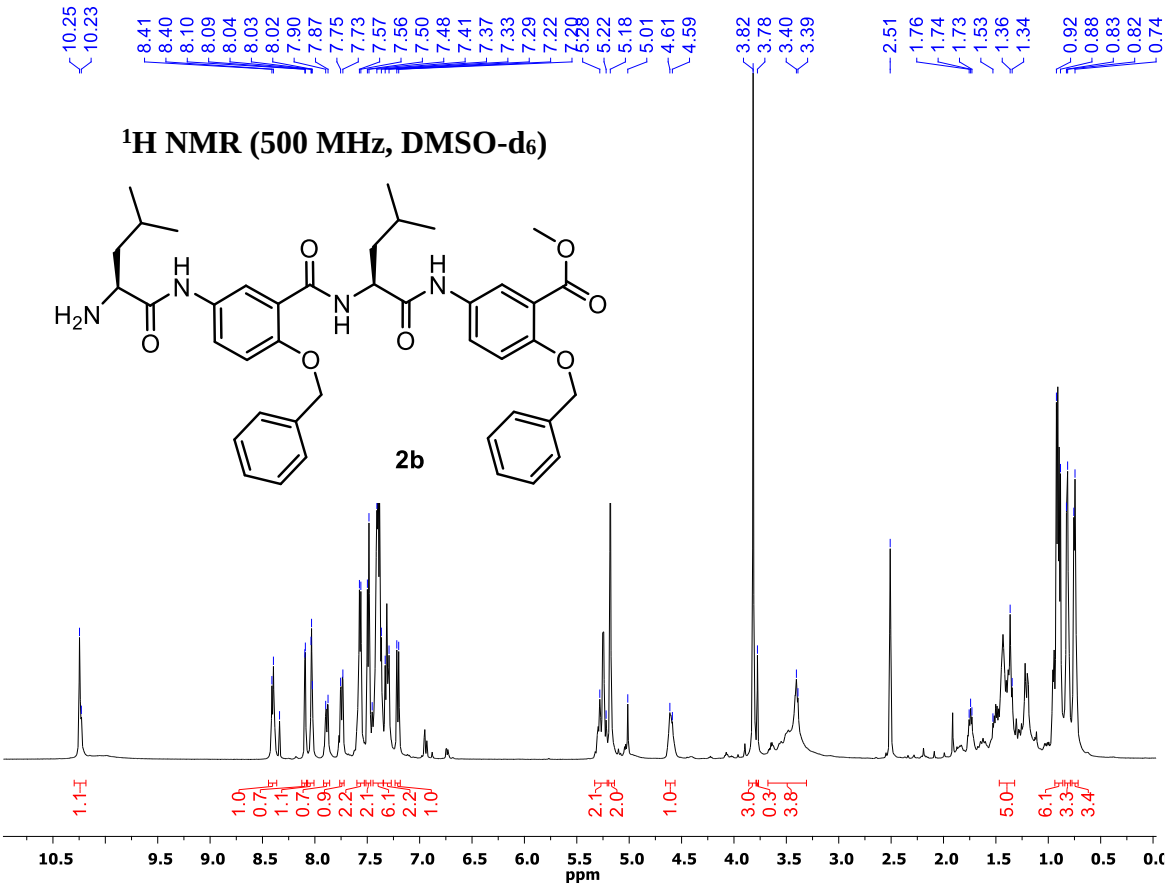


^{1}H NMR (500 MHz, $CDCl_3$)

*solvent peak



^{13}C NMR (100 MHz, $CDCl_3$)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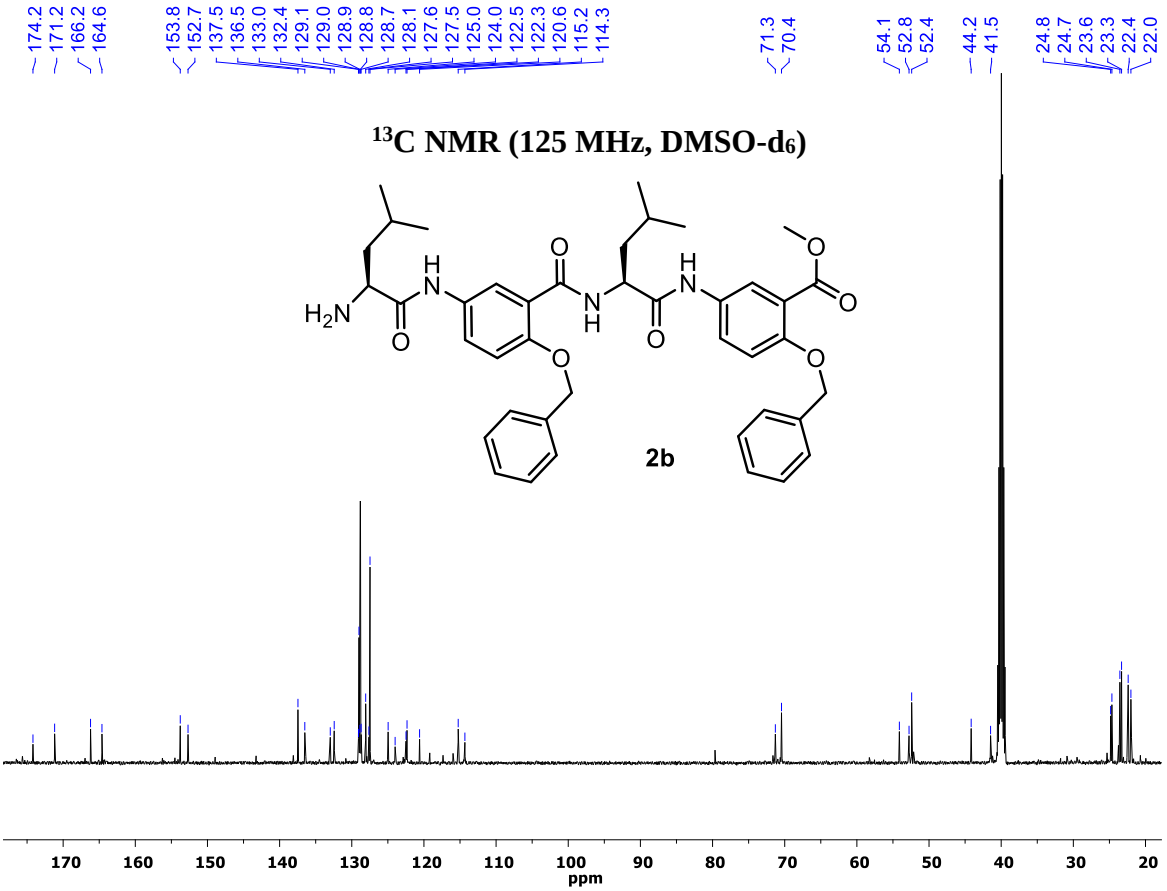
8.09
8.04
8.03
8.02
7.90
7.87
7.75
7.73
7.57
7.56
7.50
7.48
7.41
7.37
7.33
7.29
7.22
7.20
5.22
5.18
5.01
4.61
4.59
3.82
3.78
3.40
3.39
2.51
1.76
1.74
1.73
1.53
1.36
1.34
0.92
0.88
0.83
0.82
0.74
1H NMR (500 MHz, DMSO-d6)
2b
ppm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166.2
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152.7
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128.1
127.6
127.5
125.0
124.0
122.5
122.3
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115.2
114.3
71.3
70.4
54.1
52.8
52.4
44.2
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22.4
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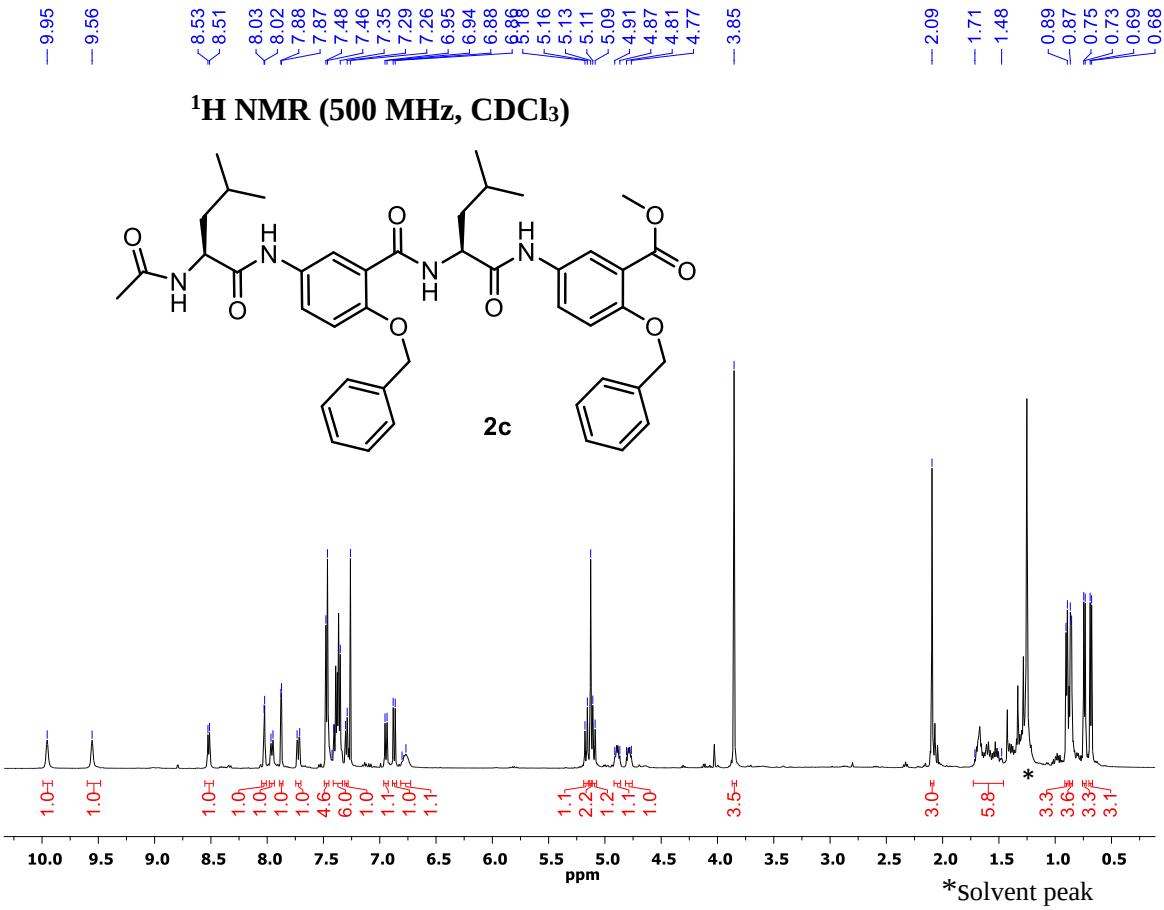
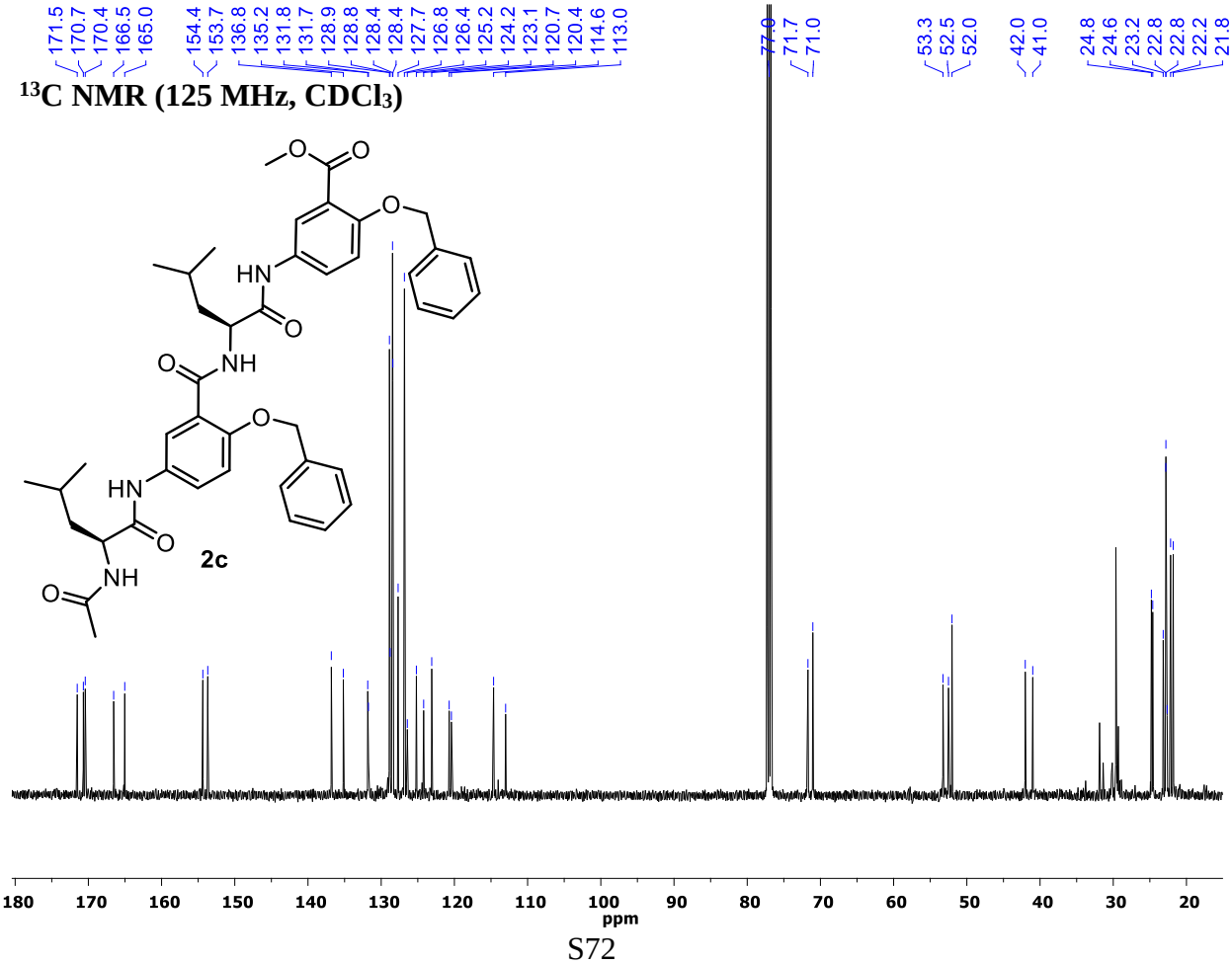
2b
ppm

^{1}H NMR (500 MHz, $CDCl_3$)

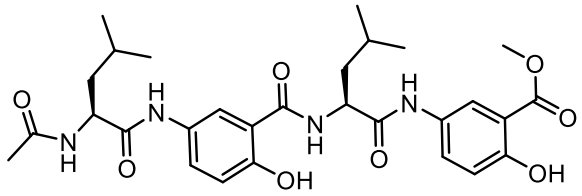


*Solvent peak

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



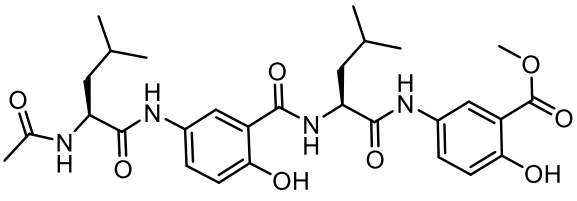
^{1}H NMR (500 MHz, $CDCl_3$/DMSO-d_6 2:1 v/v)



3

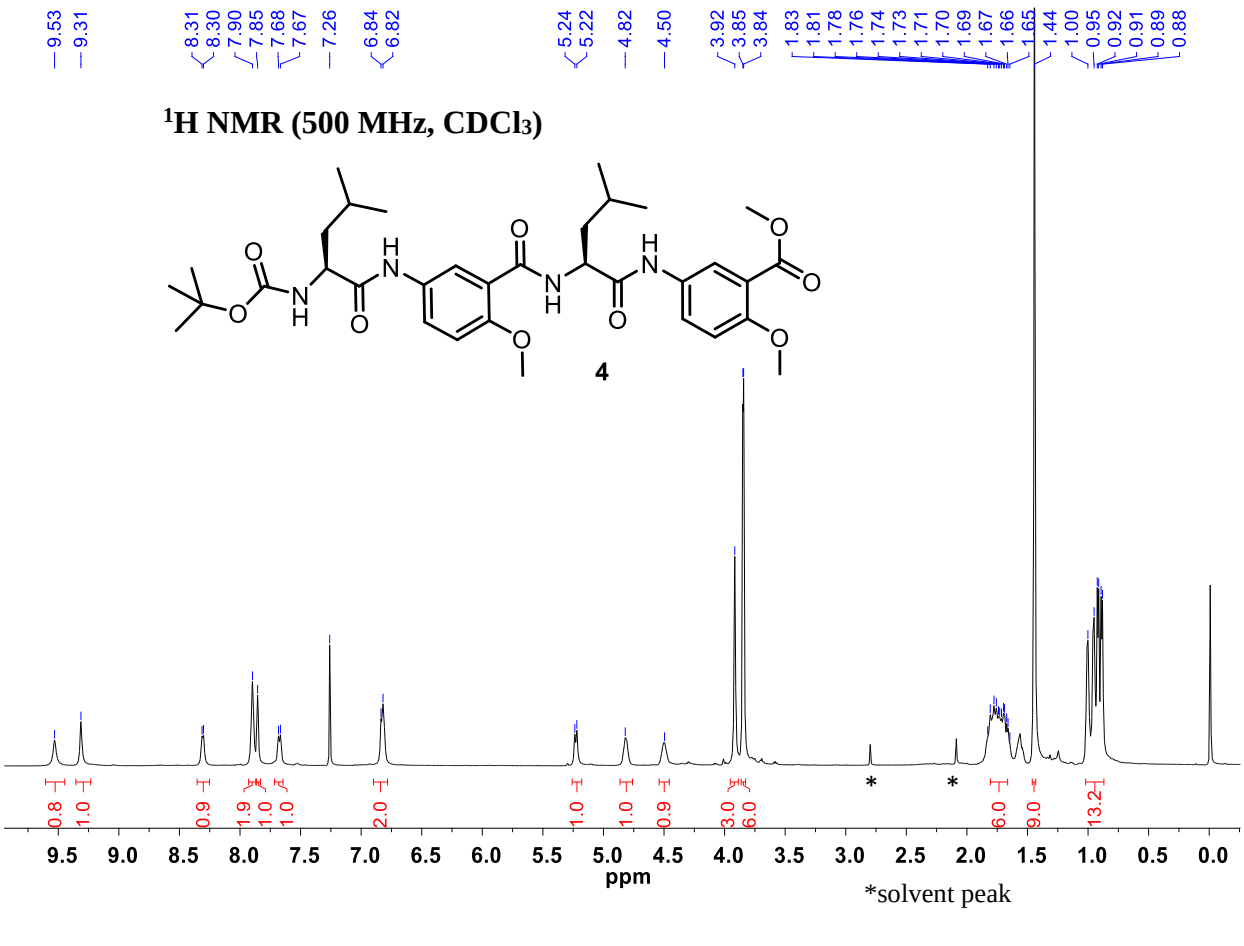
*solvent peak

^{13}C NMR (125 MHz, $CDCl_3$/DMSO-d_6 2:1 v/v)



3

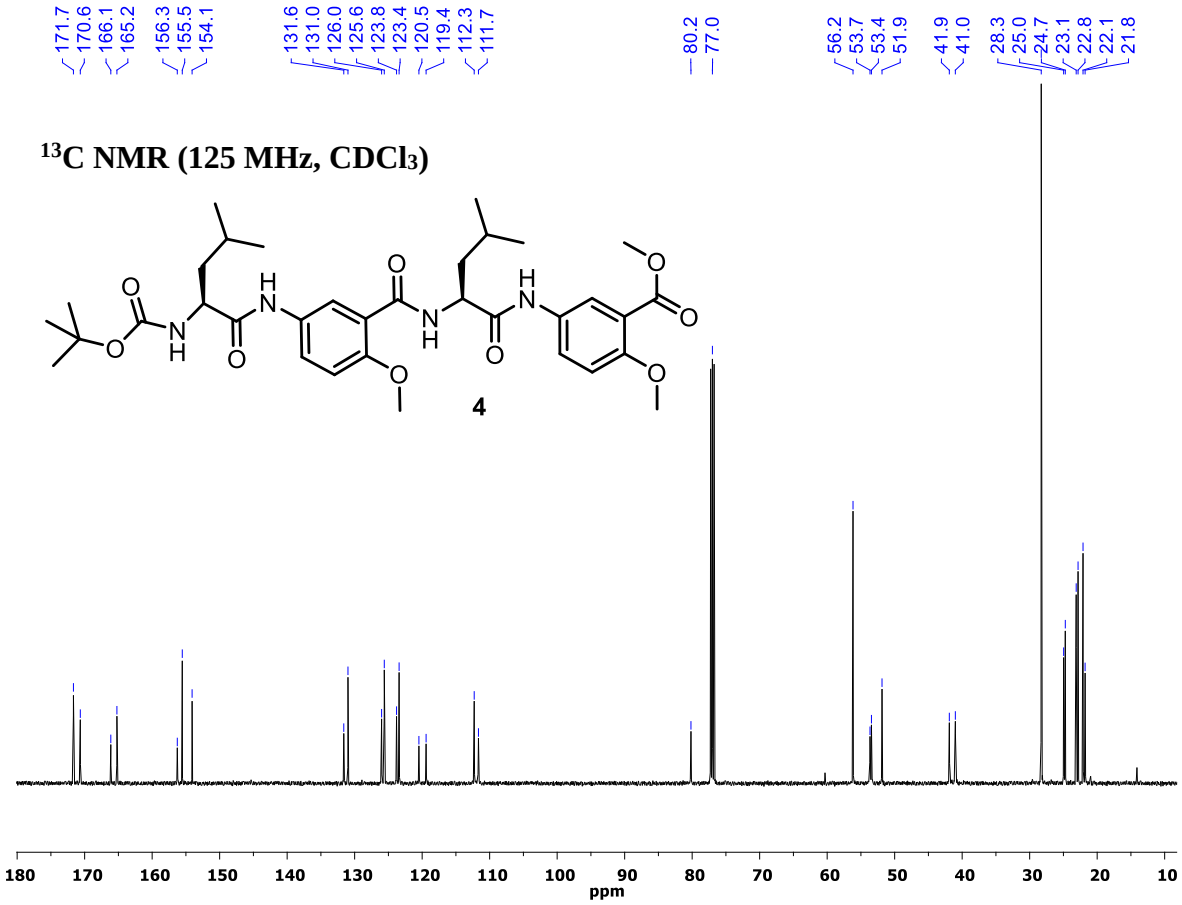
^{1}H NMR (500 MHz, $CDCl_3$)



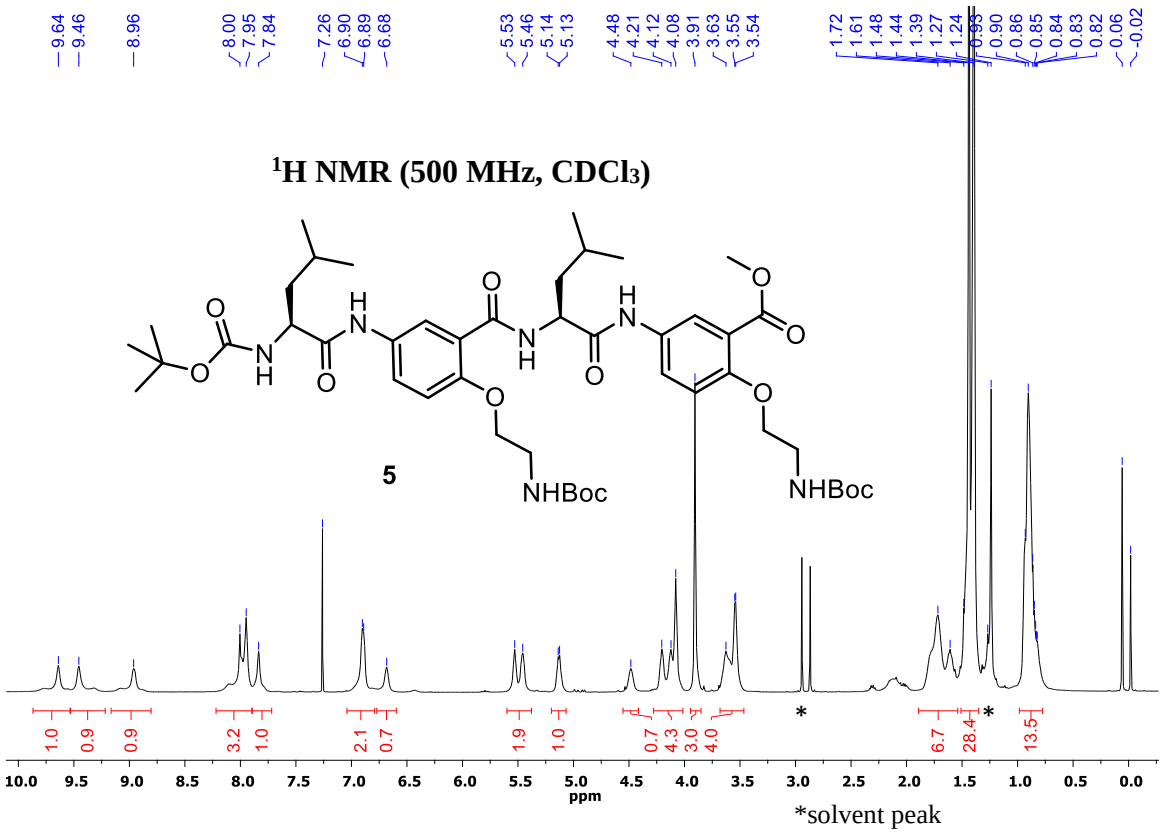
4

*solvent peak

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

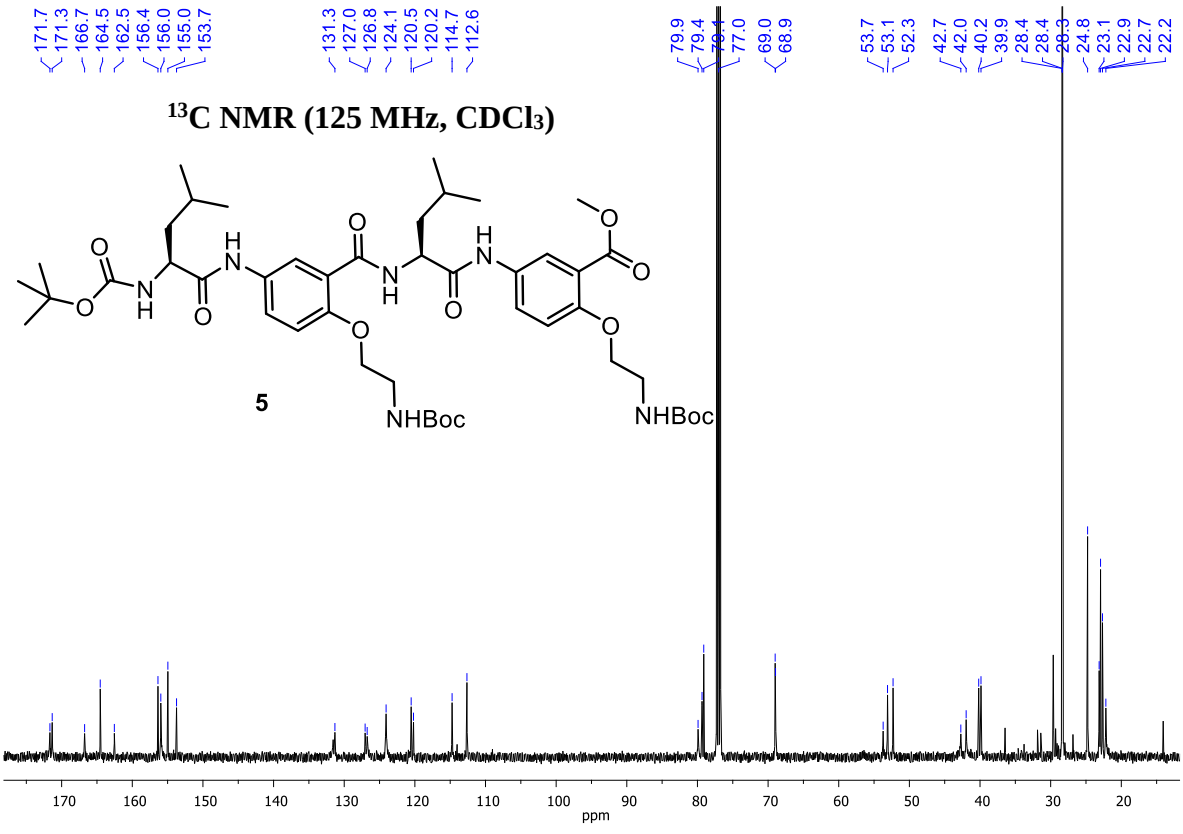


4



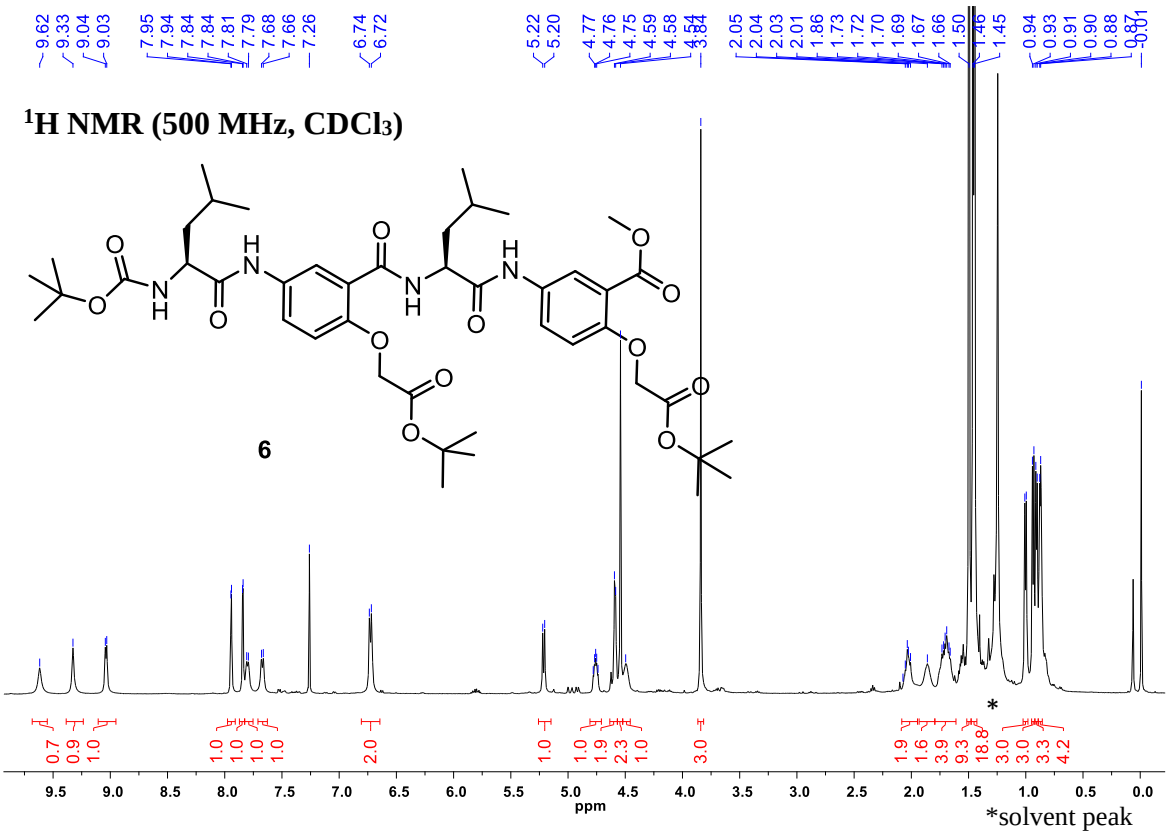
^{1}H NMR (500 MHz, $CDCl_3$)

*solvent peak



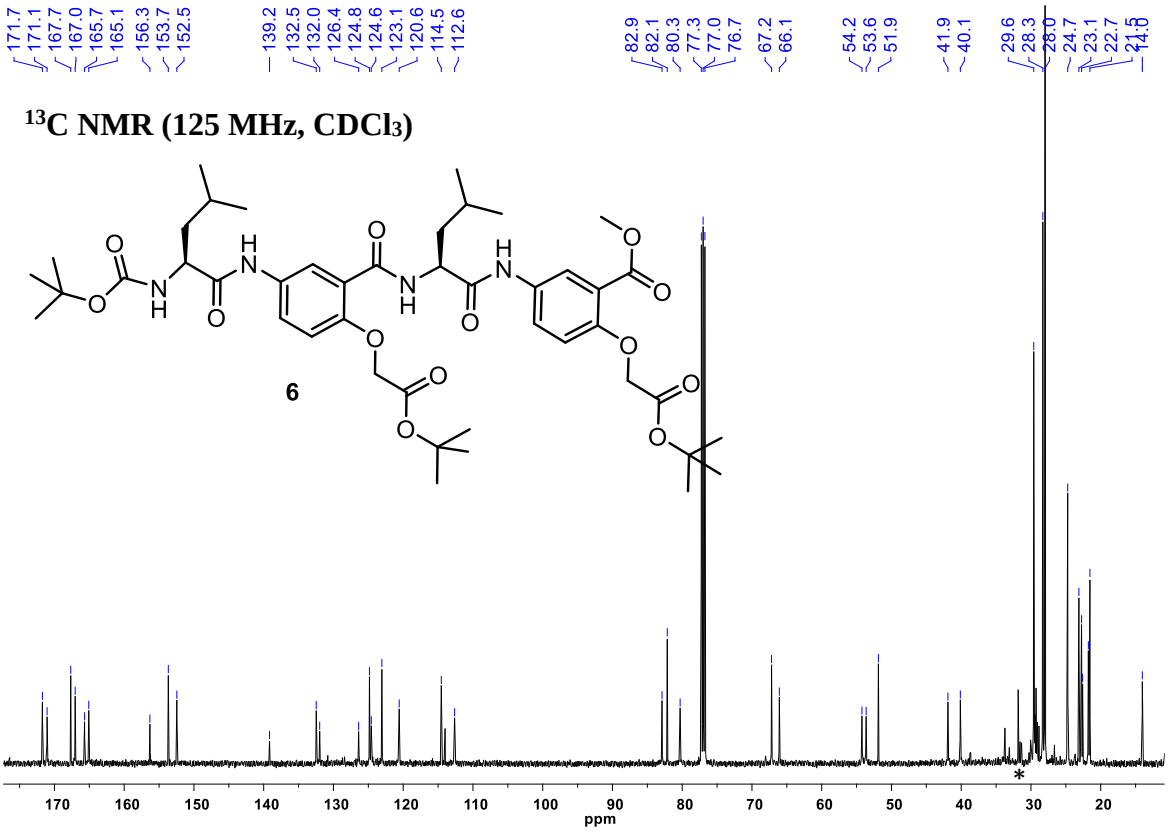
^{13}C NMR (125 MHz, $CDCl_3$)

^{1}H NMR (500 MHz, $CDCl_3$)

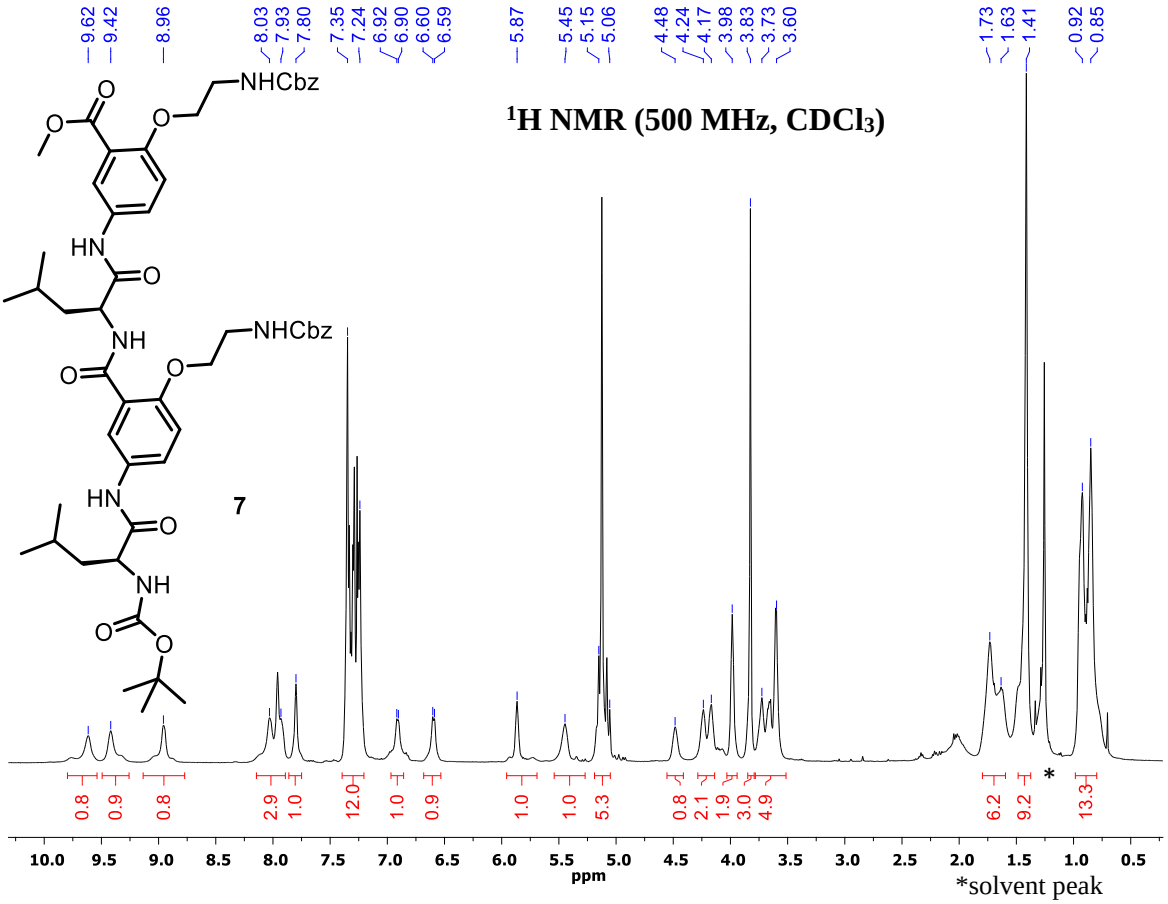


*solvent peak

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

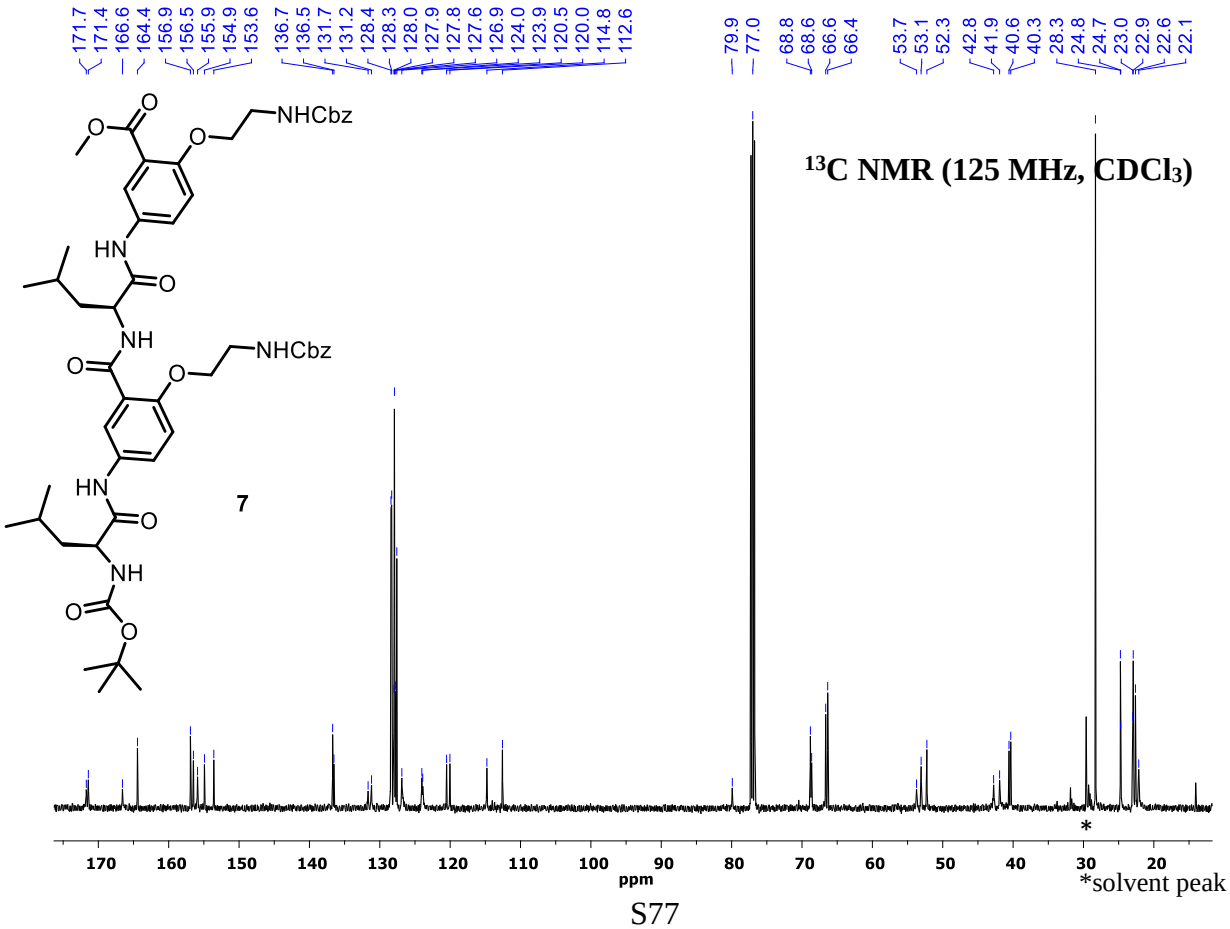


*solvent peak



^{1}H NMR (500 MHz, $CDCl_3$)

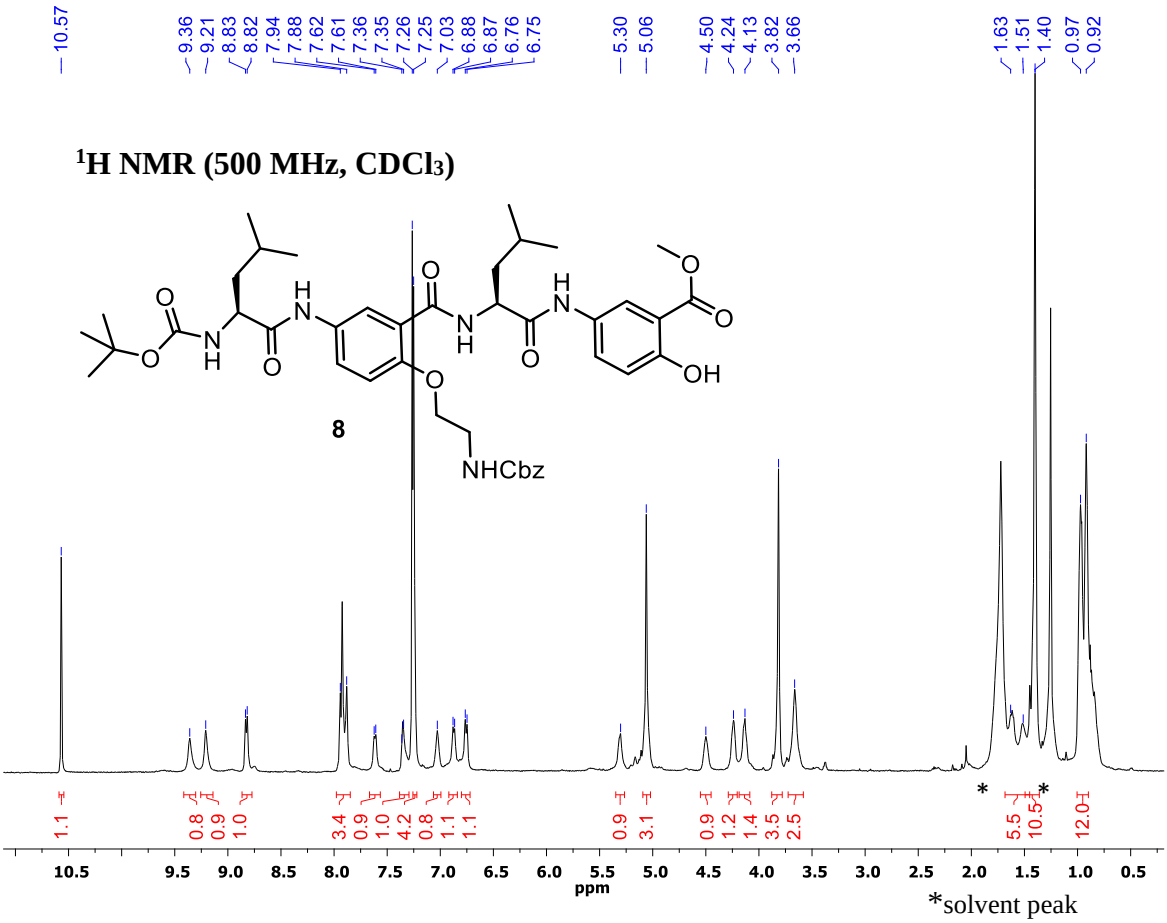
*solvent peak



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

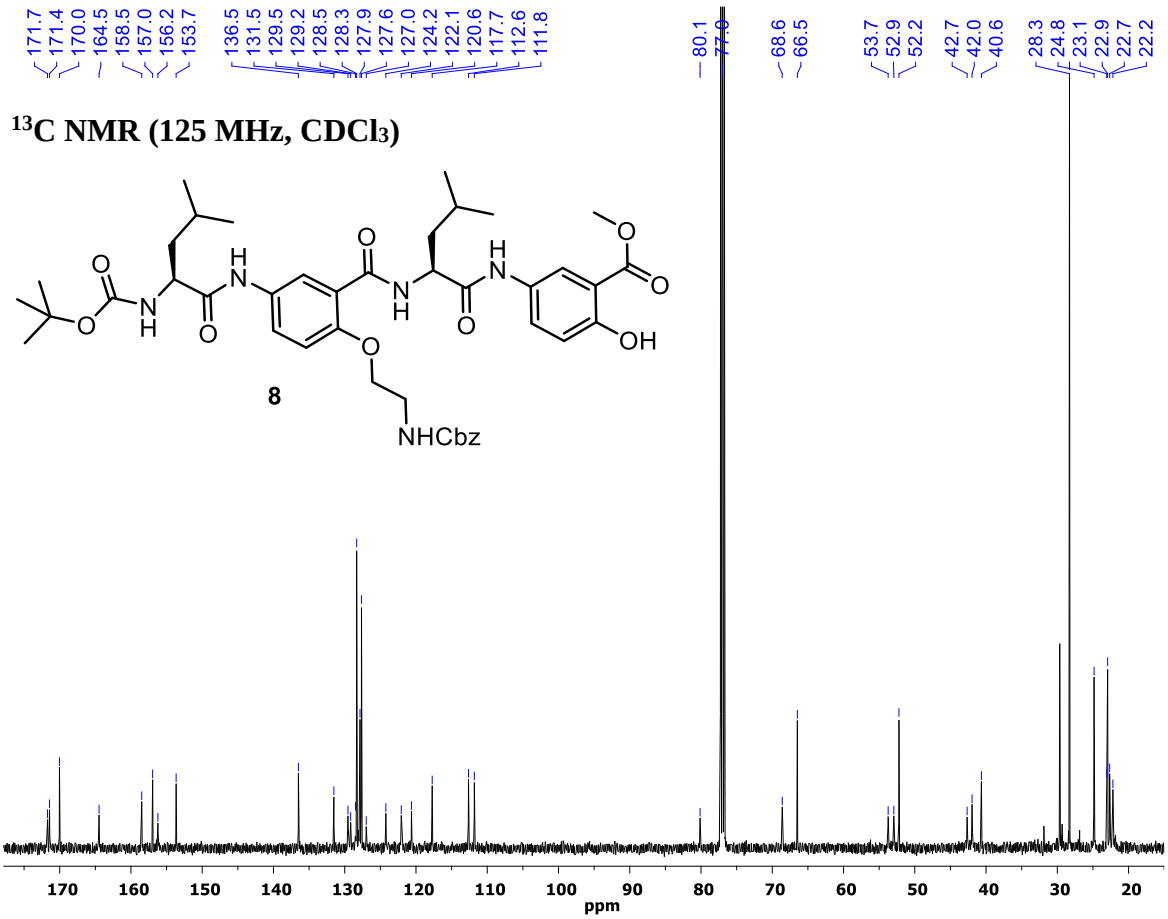
*solvent peak

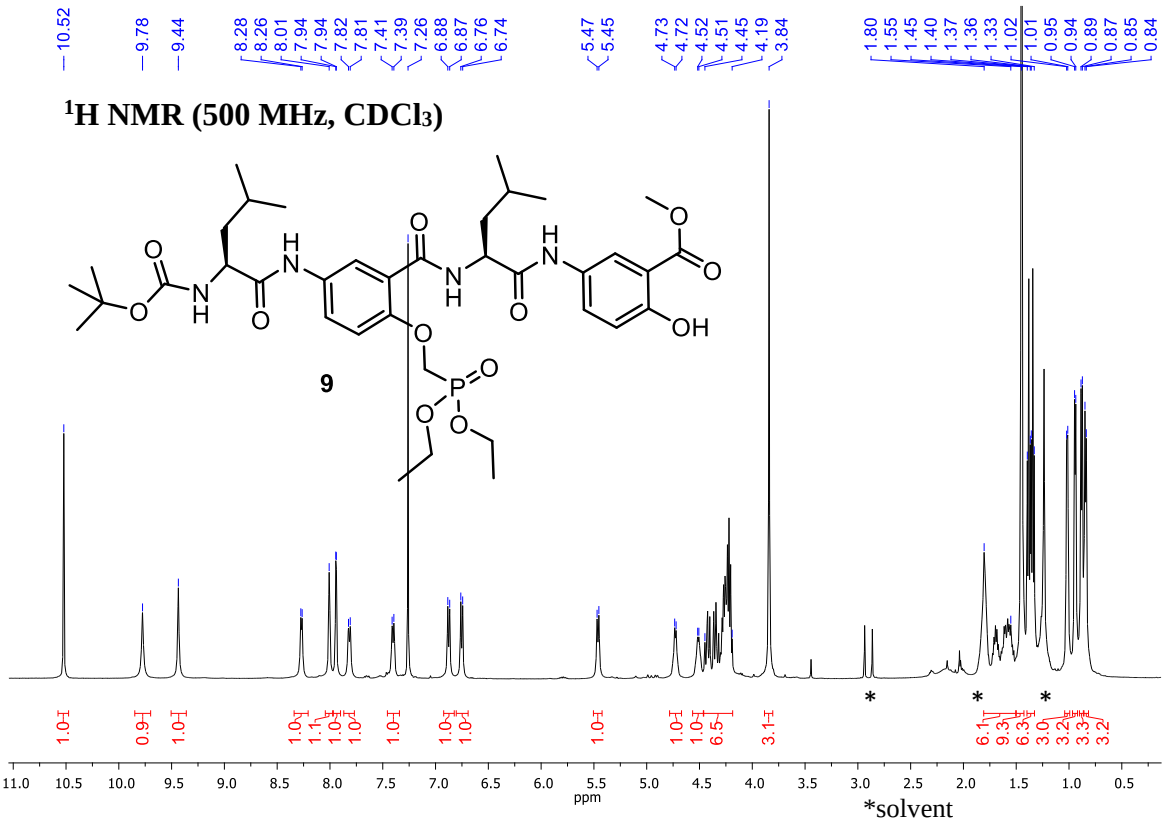
^{1}H NMR (500 MHz, $CDCl_3$)

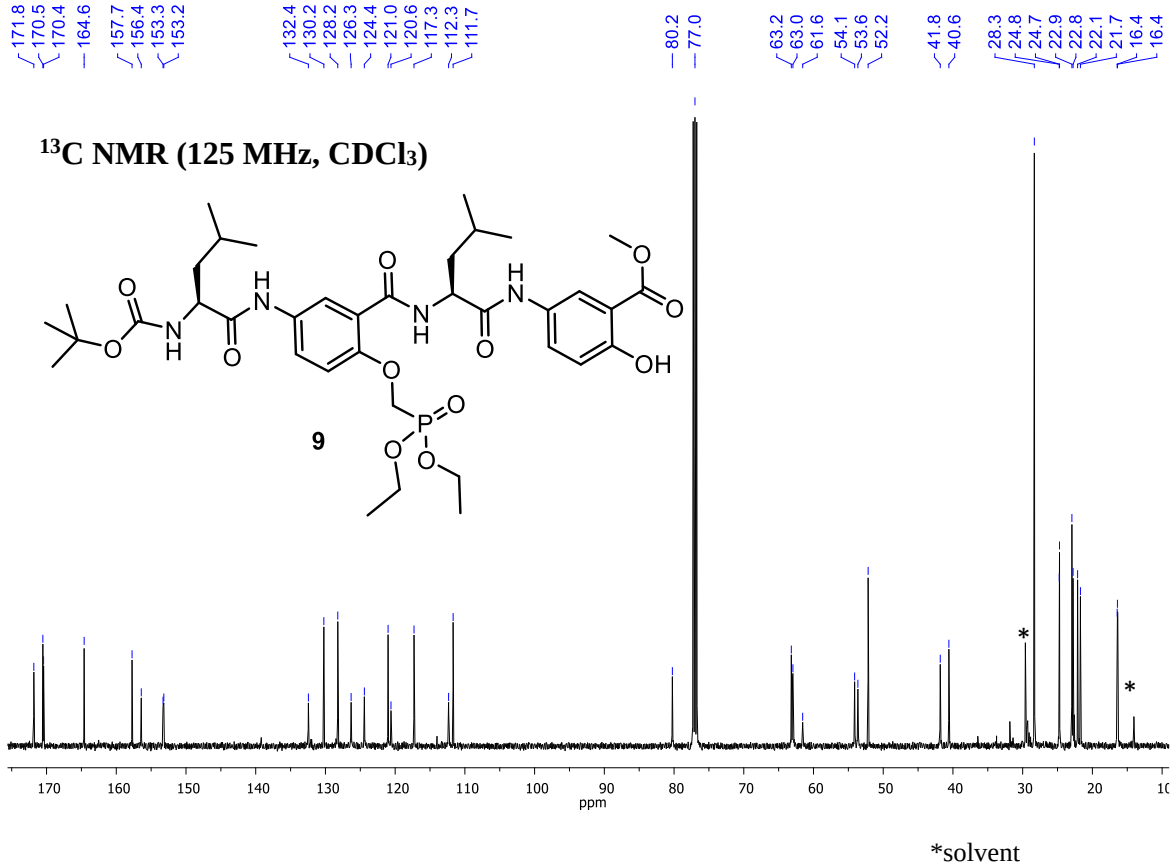


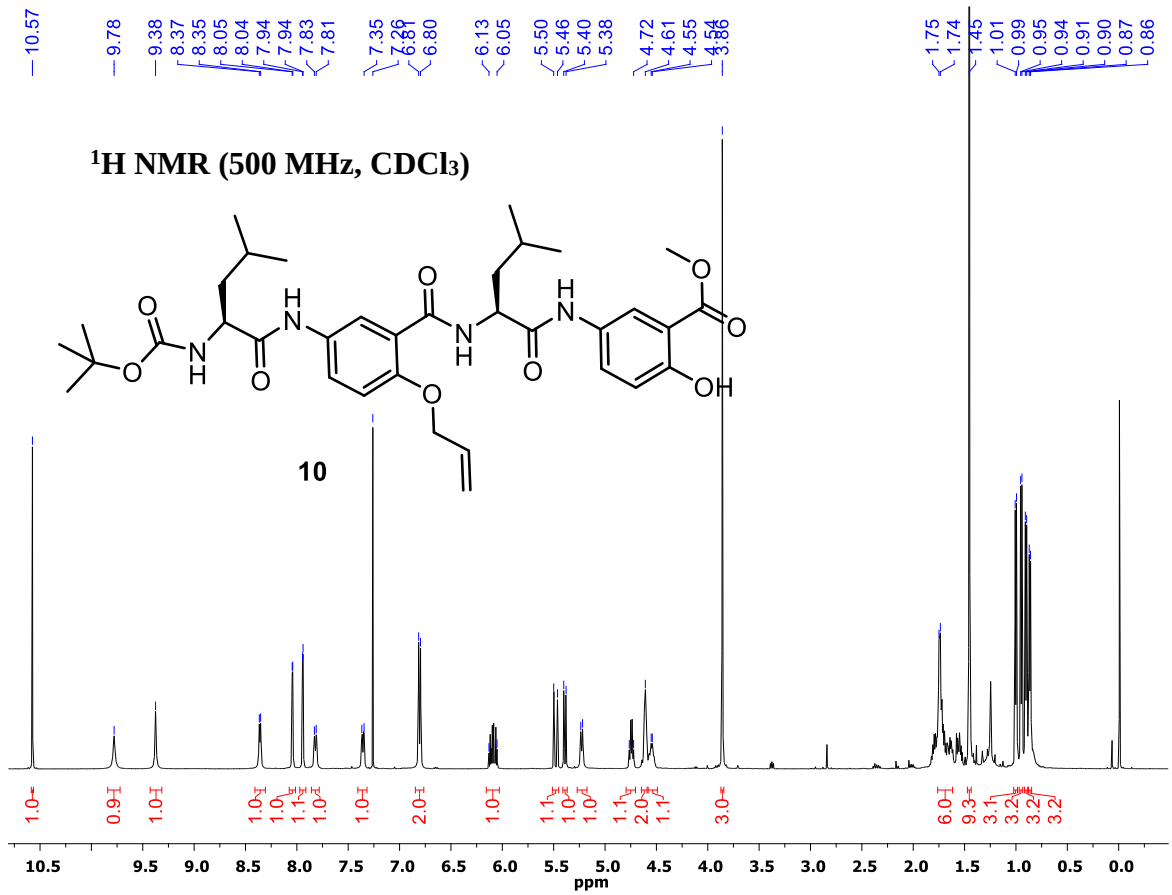
*solvent peak

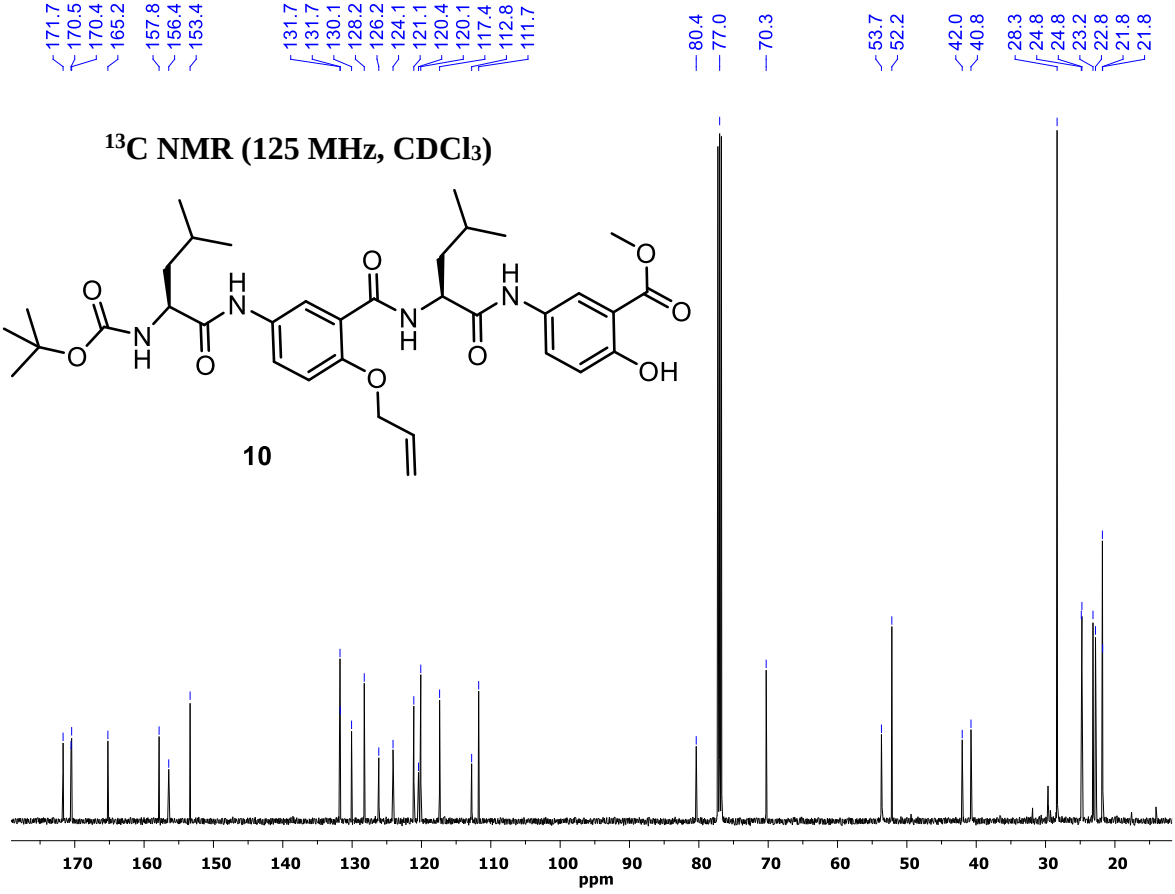
^{13}C NMR (125 MHz, $CDCl_3$)

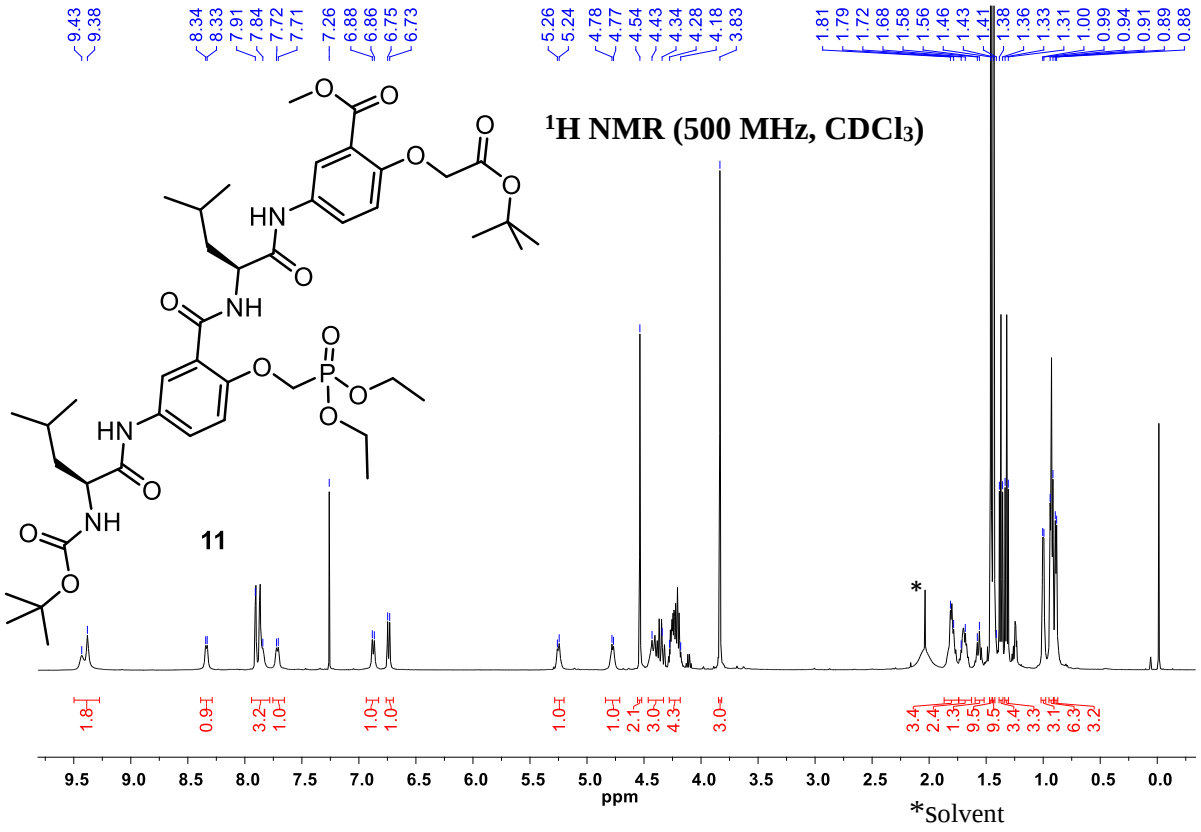


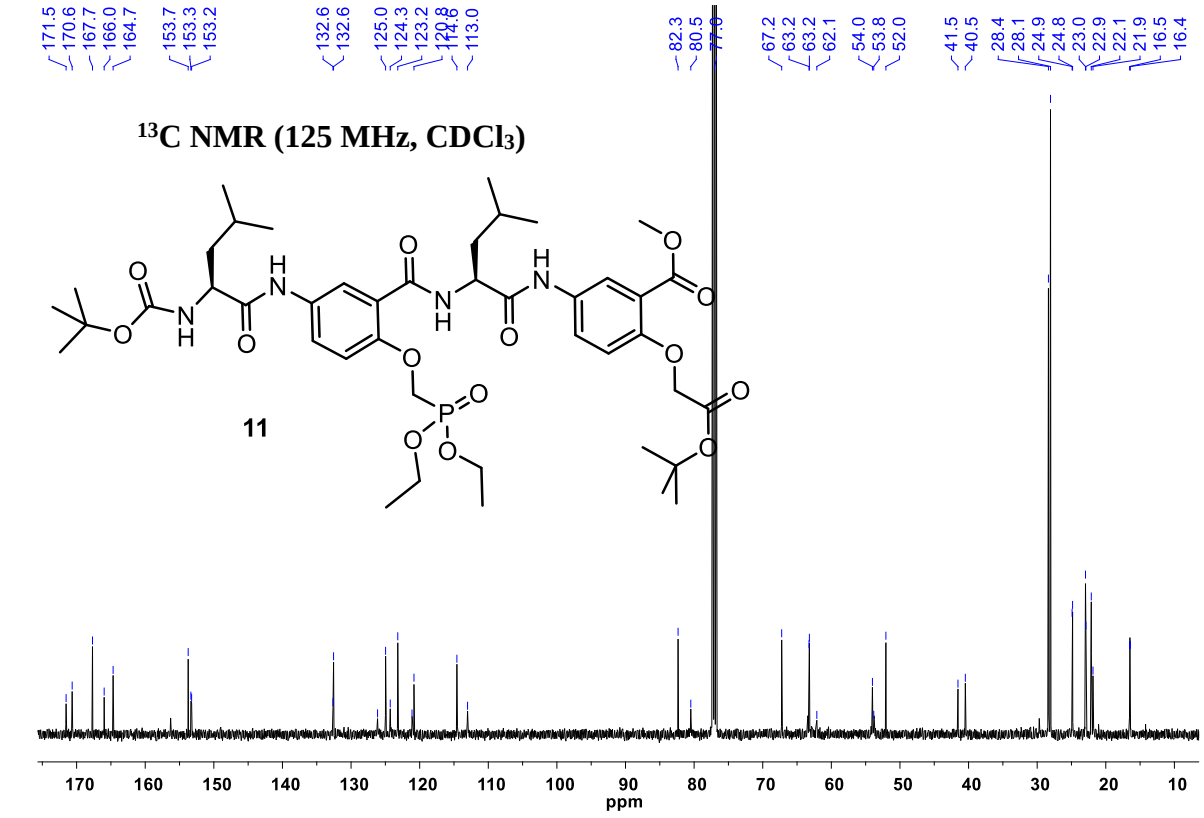

^{1}H NMR (500 MHz, CDCl$_3$)
9
ppm
*solvent


^{13}C NMR (125 MHz, CDCl$_3$)
9
ppm
*solvent

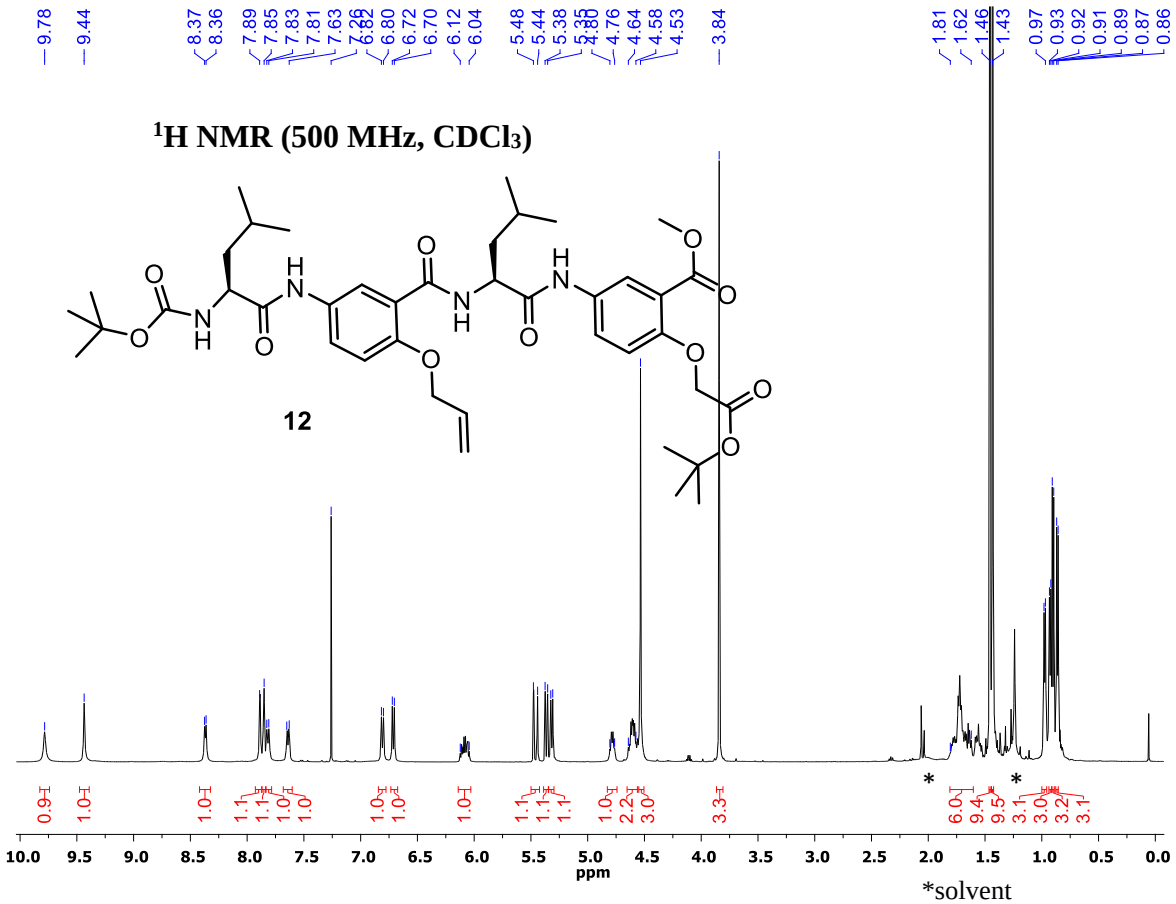

10.57
9.78
9.38
8.37
8.35
8.05
8.04
7.94
7.83
7.81
7.35
6.80
6.13
6.05
5.50
5.46
5.40
5.38
4.72
4.61
4.55
4.54
3.86
1.75
1.74
1.45
1.01
0.99
0.95
0.94
0.91
0.90
0.87
0.86
1H NMR (500 MHz, CDCl3)
10
ppm


171.7
170.5
170.4
165.2
157.8
156.4
153.4
131.7
131.7
130.1
128.2
126.2
124.1
121.1
120.4
120.1
117.4
112.8
111.7
80.4
77.0
70.3
53.7
52.2
42.0
40.8
28.3
24.8
24.8
23.2
22.8
21.8
21.8
13C NMR (125 MHz, CDCl3)
10
ppm


9.43
9.38
8.34
8.33
7.91
7.84
7.72
7.71
7.26
6.88
6.86
6.75
6.73
5.26
5.24
4.78
4.77
4.54
4.43
4.34
4.28
4.18
3.83
1.81
1.79
1.72
1.68
1.58
1.56
1.46
1.43
1.41
1.38
1.36
1.33
1.31
1.00
0.99
0.94
0.91
0.89
0.88
1H NMR (500 MHz, CDCl3)
11
*
1.8
0.9
3.2
1.0
1.0
1.0
1.0
2.1
3.0
4.3
3.0
3.4
2.4
1.3
9.5
9.5
3.4
3.3
3.1
6.3
3.2
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
ppm
*Solvent

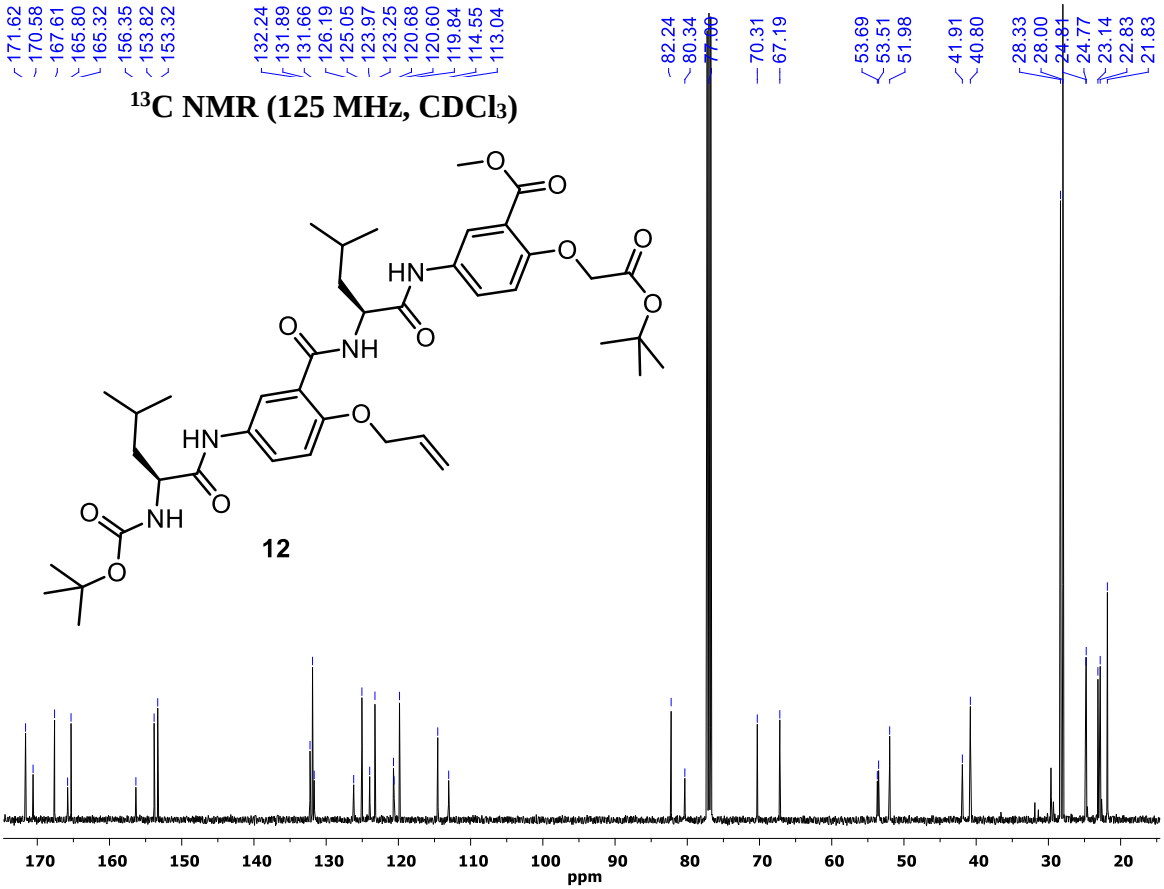

171.5
170.6
167.7
166.0
164.7
153.7
153.3
153.2
132.6
132.6
125.0
124.3
123.2
113.0
82.3
80.5
67.2
63.2
63.2
62.1
54.0
53.8
52.0
41.5
40.5
28.4
28.1
24.9
24.8
23.0
22.9
22.1
21.9
16.5
16.4
13C NMR (125 MHz, CDCl3)
11
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm

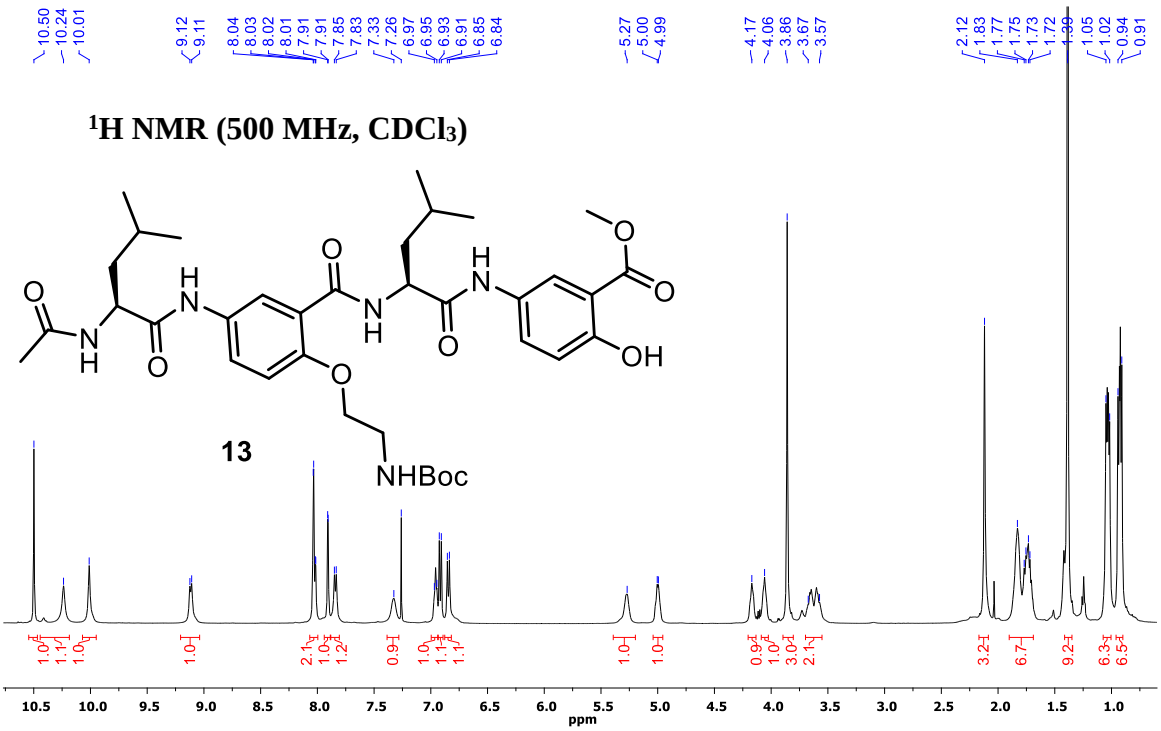
^{1}H NMR (500 MHz, $CDCl_3$)

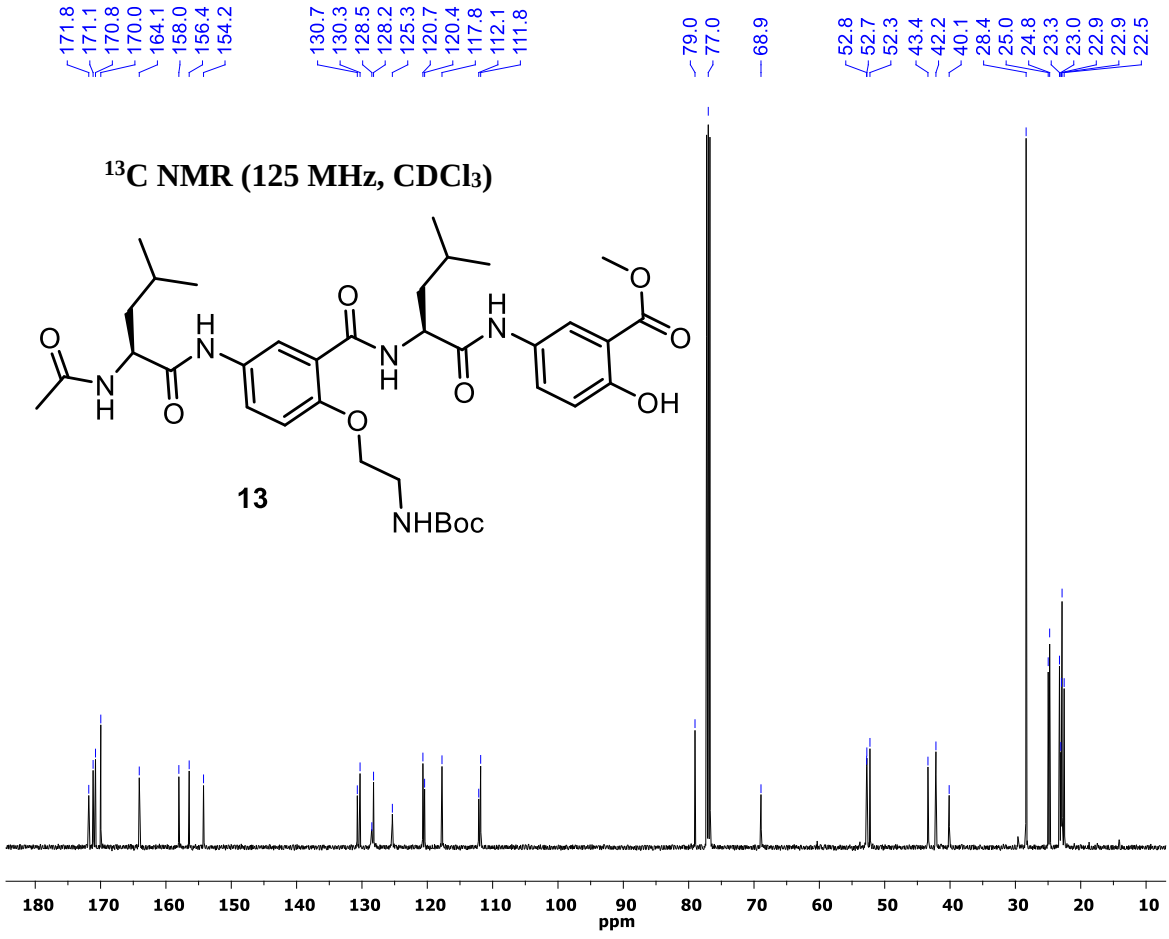


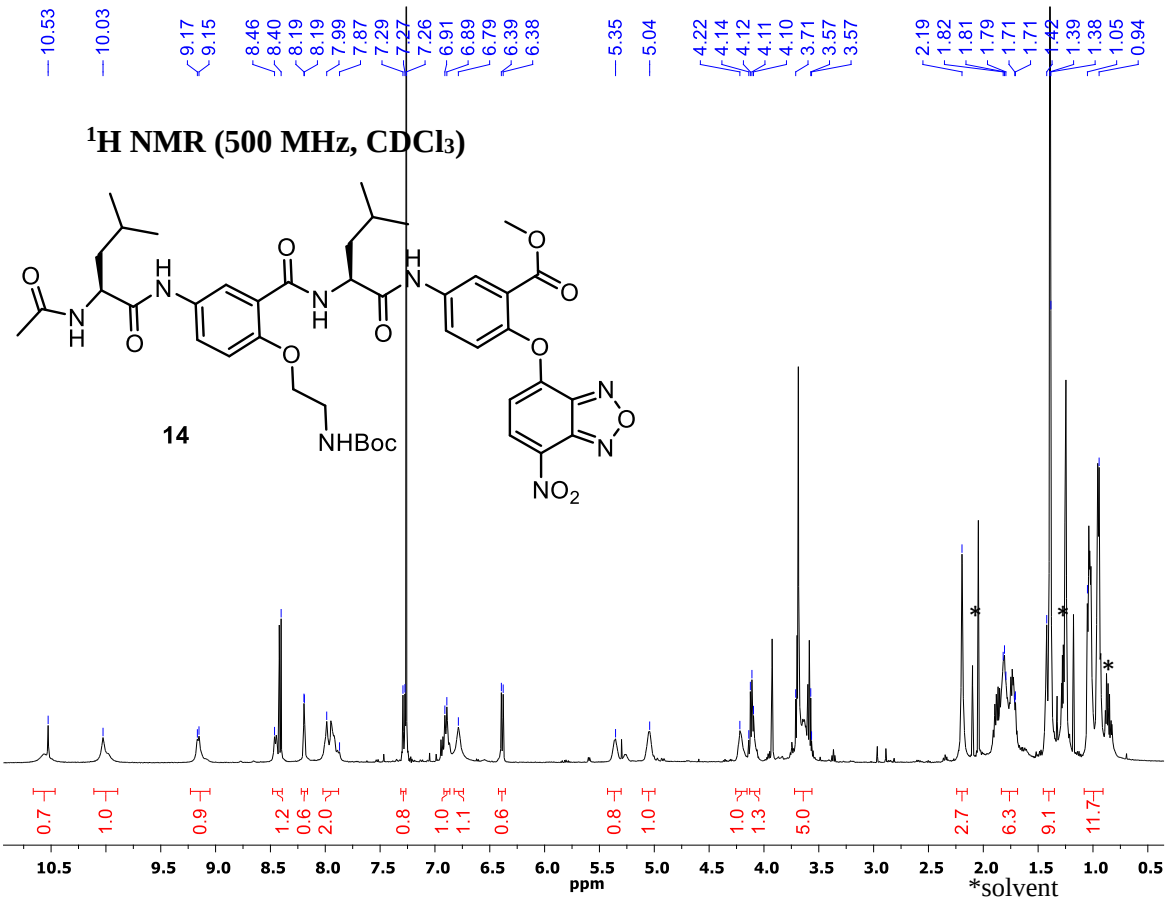
*solvent

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

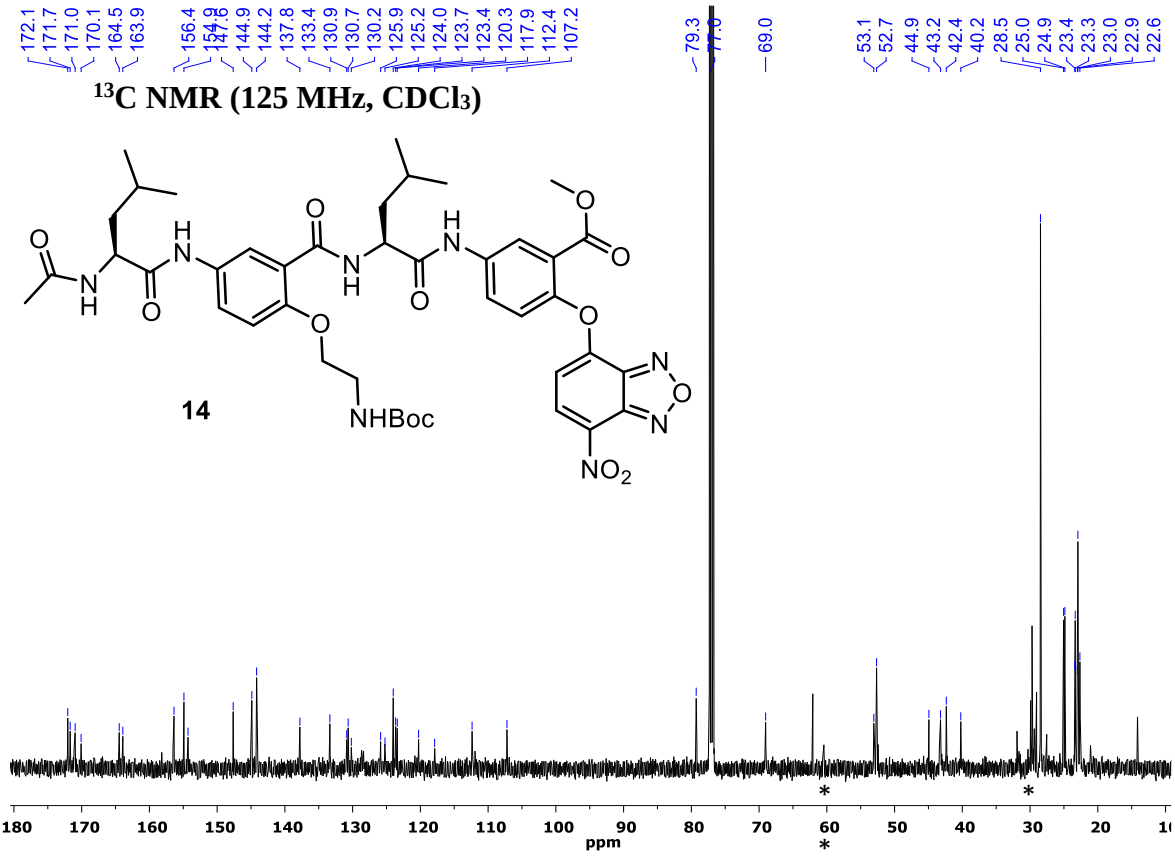



10.50
10.24
10.01
9.12
9.11
8.04
8.03
8.02
8.01
7.91
7.91
7.85
7.83
7.33
7.26
6.97
6.95
6.93
6.91
6.85
6.84
5.27
5.00
4.99
4.17
4.06
3.86
3.67
3.57
2.12
1.83
1.77
1.75
1.73
1.72
1.50
1.05
1.02
0.94
0.91
1H NMR (500 MHz, CDCl3)
13
NHBoc
OH
ppm


171.8
171.1
170.8
170.0
164.1
158.0
156.4
154.2
130.7
130.3
128.5
128.2
125.3
120.7
120.4
117.8
112.1
111.8
79.0
77.0
68.9
52.8
52.7
52.3
43.4
42.2
40.1
28.4
25.0
24.8
23.3
23.0
22.9
22.9
22.5
13C NMR (125 MHz, CDCl3)
13
NHBoc
OH
ppm

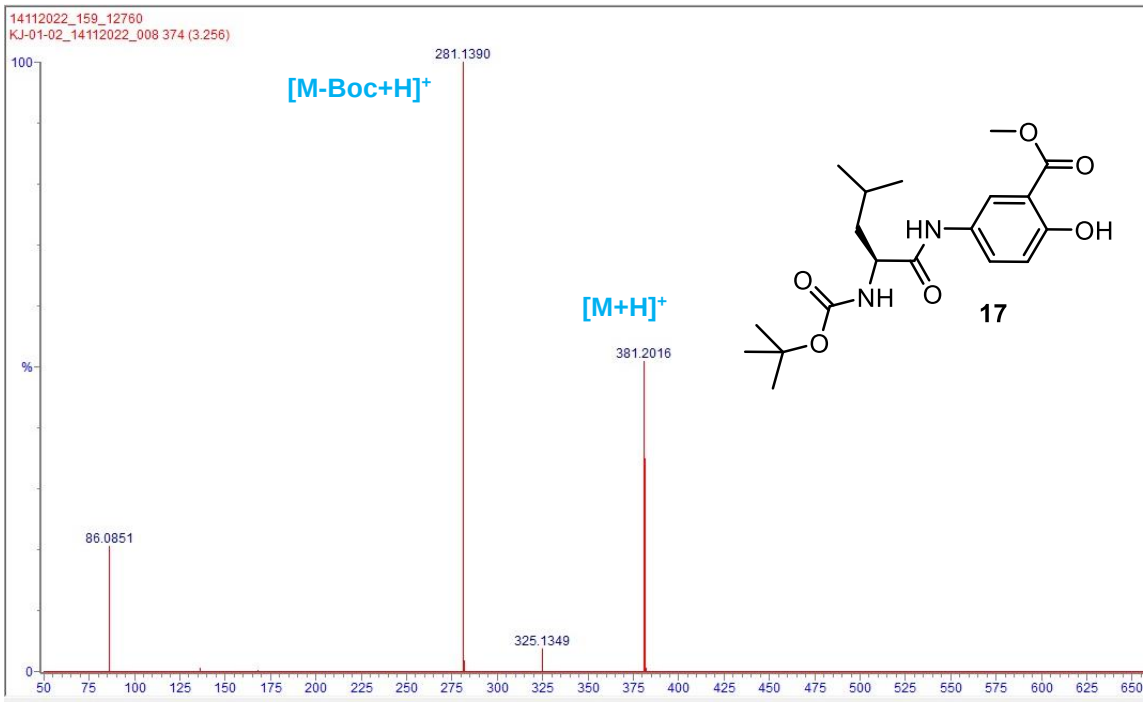


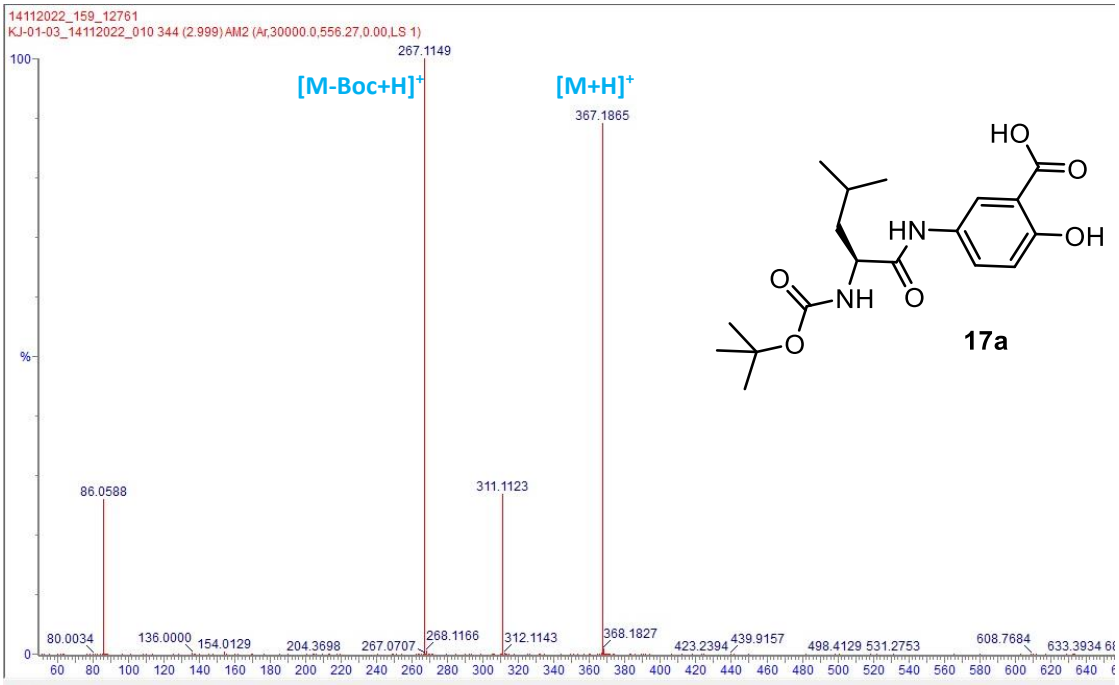
^{1}H NMR (500 MHz, $CDCl_3$)

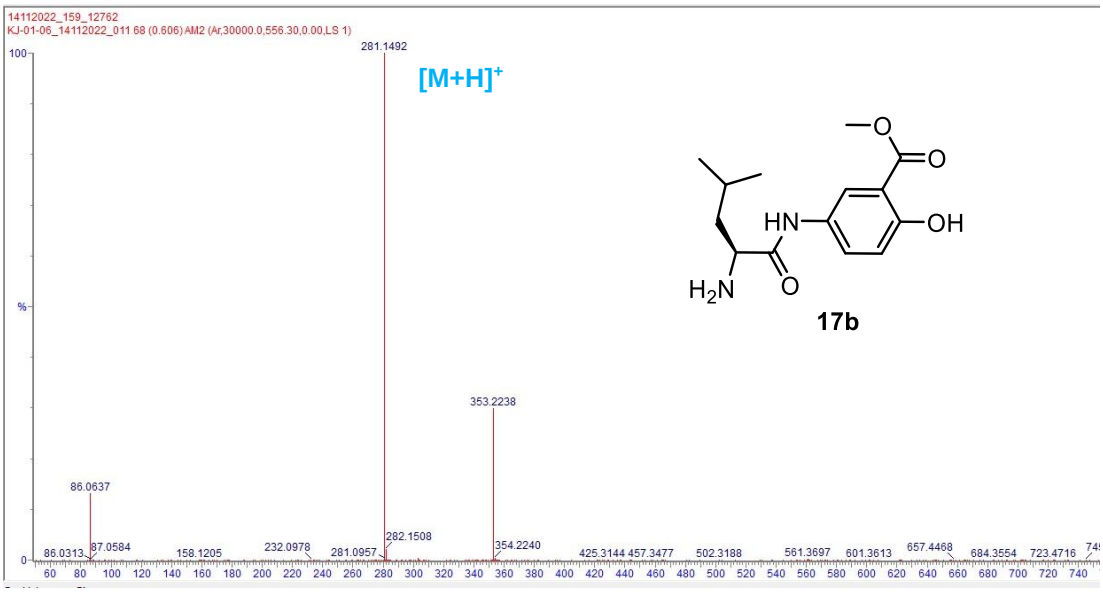


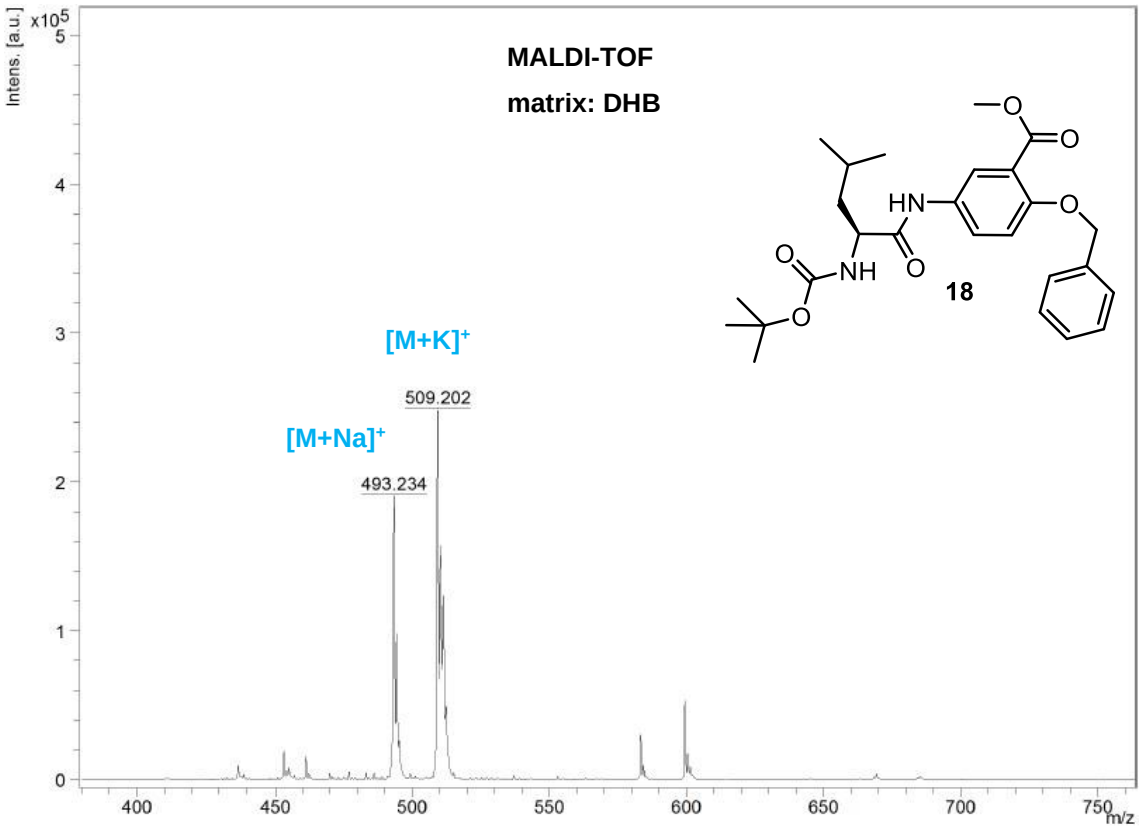
^{13}C NMR (125 MHz, $CDCl_3$)

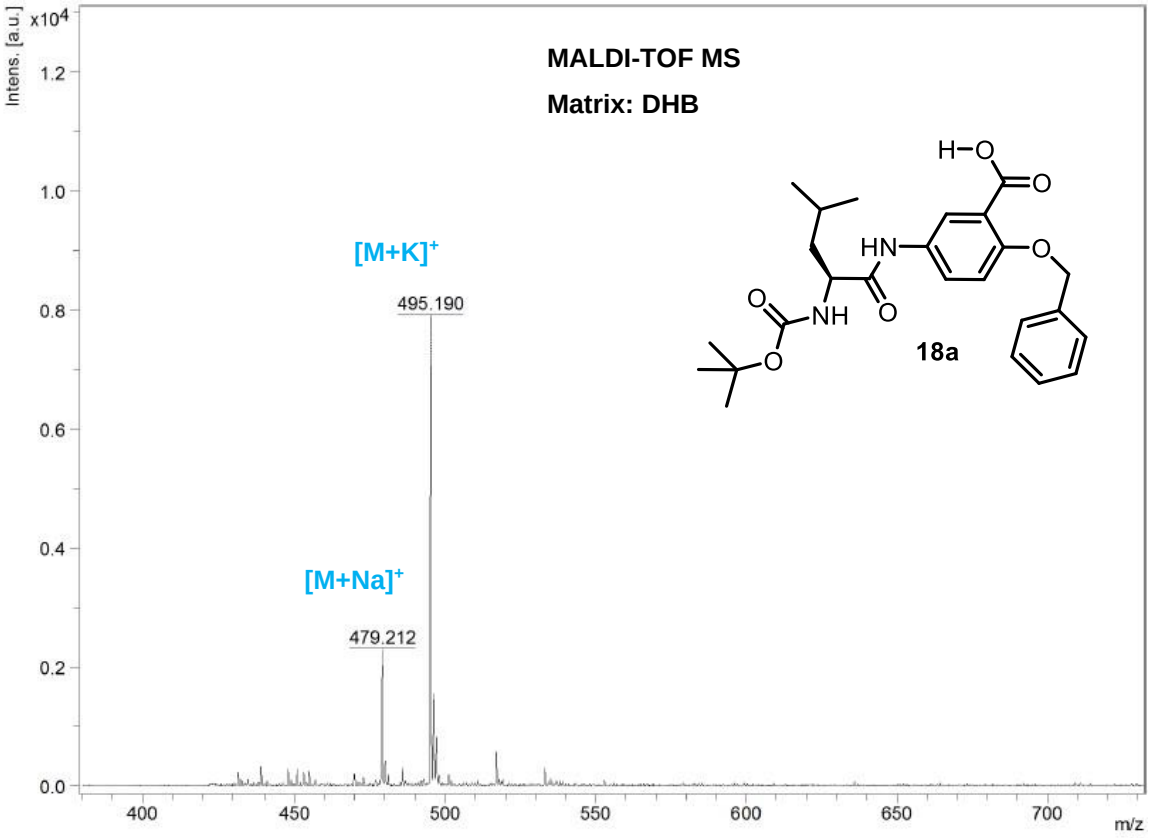
Mass spectrometry

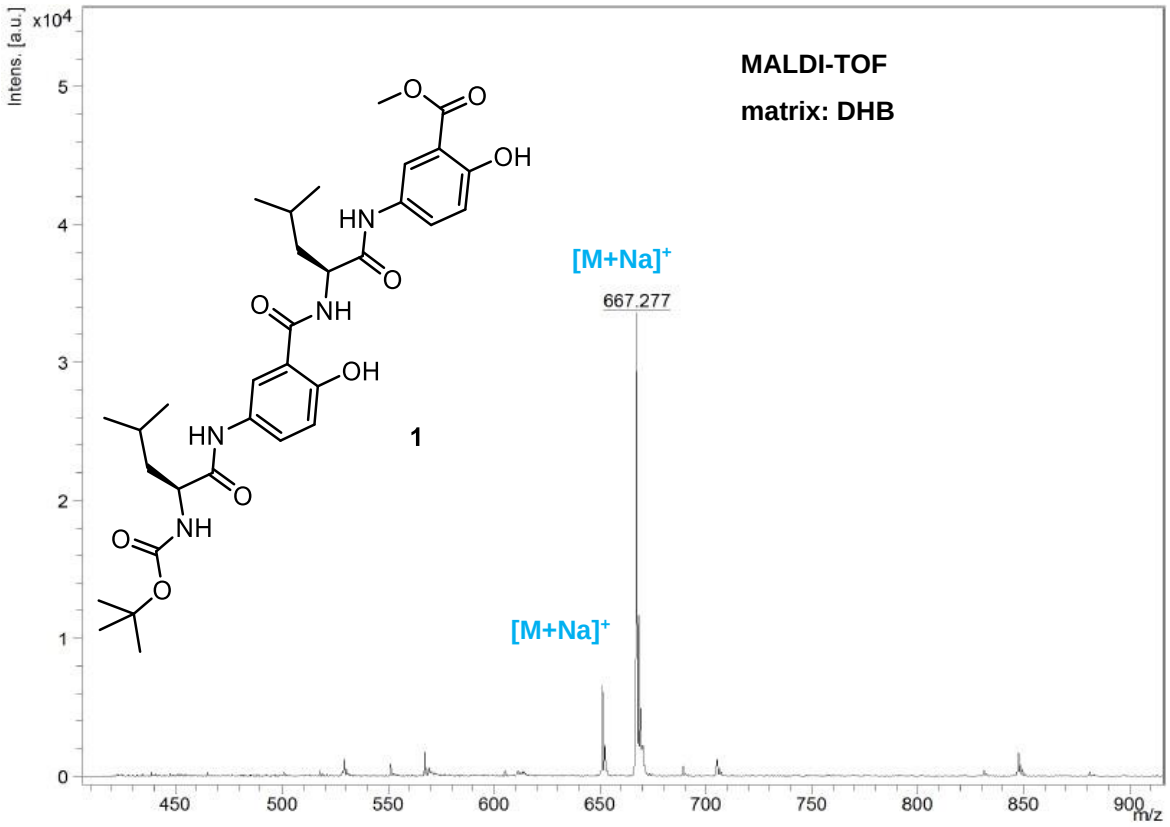


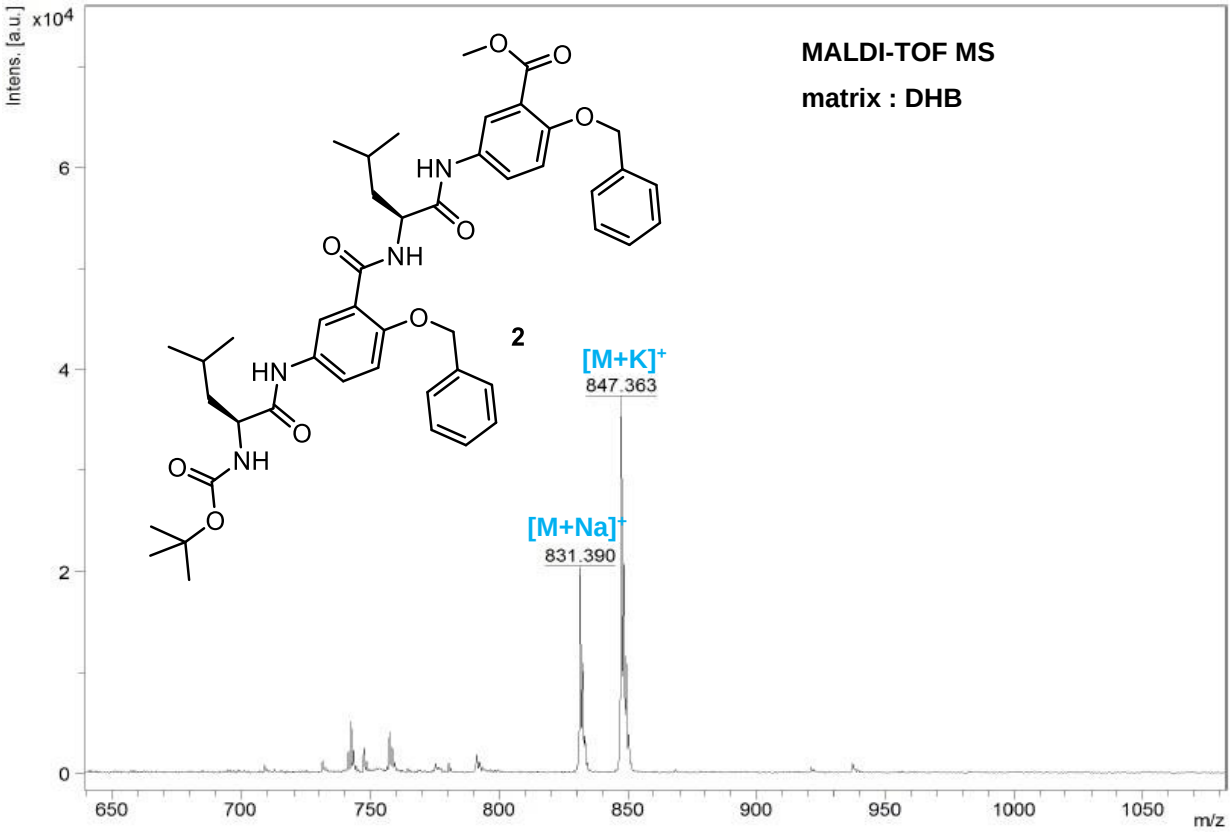


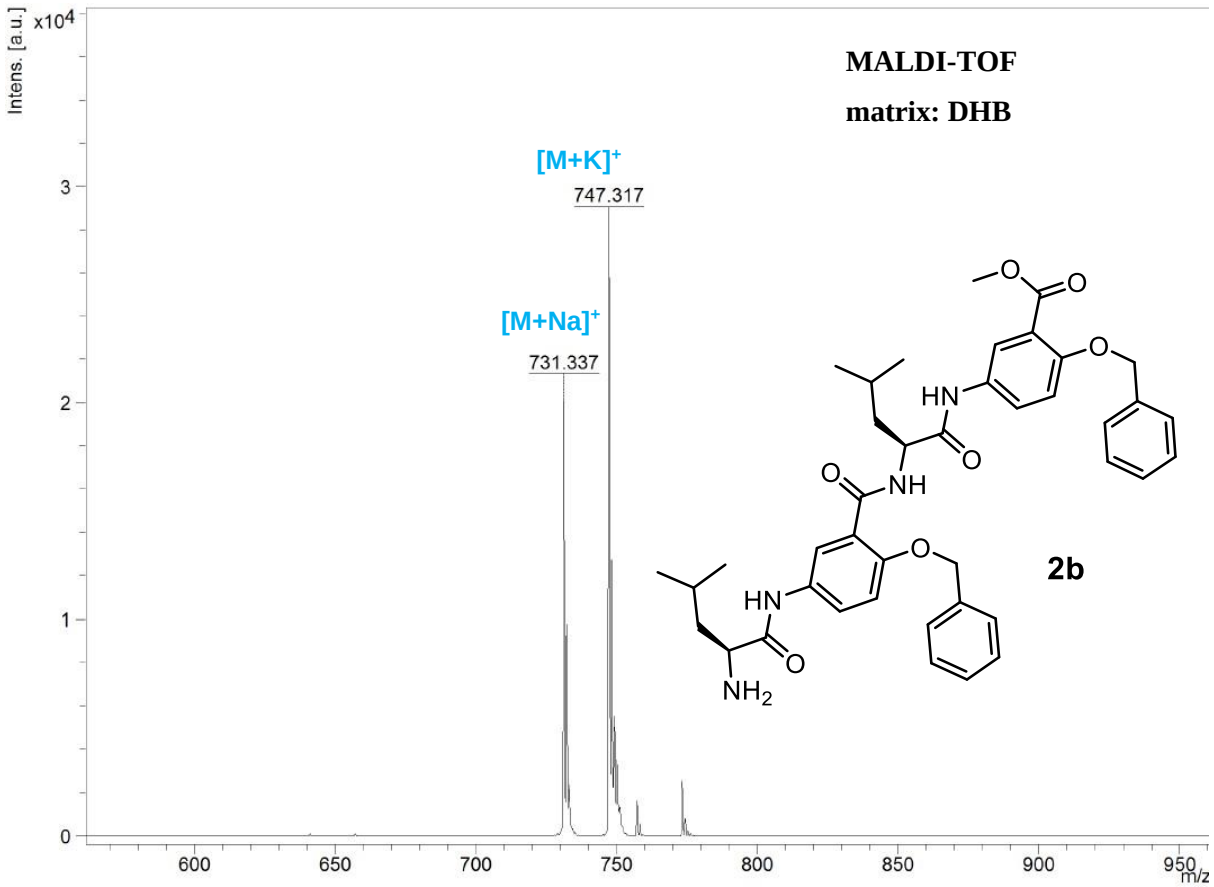

14112022_159_12762
KJ-01-06_14112022_011 68 (0.606) AM2 (Ar,30000.0,556.30,0.00,LS 1)
281.1492
[M+H]+
353.2238
86.0637
282.1508
354.2240
17b

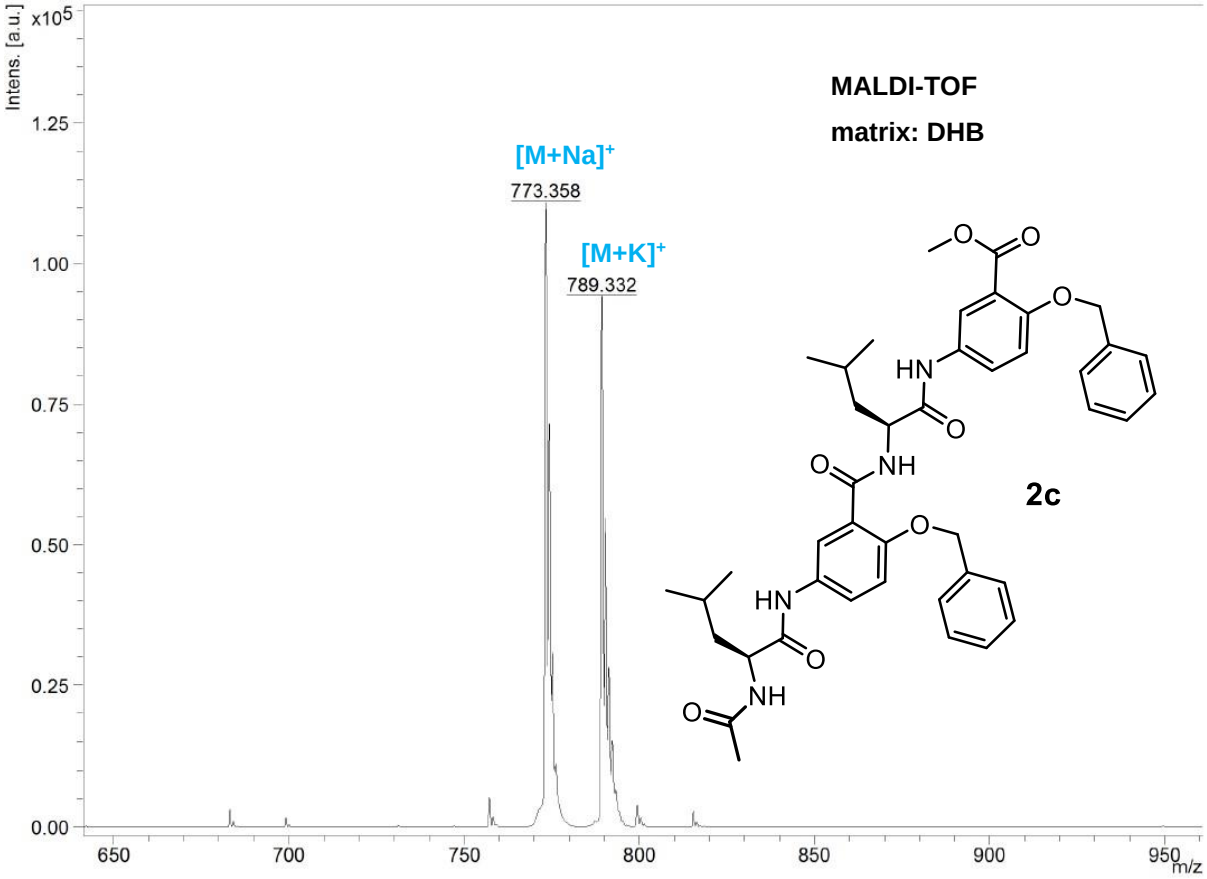

MALDI-TOF
matrix: DHB
Intens. [a.u.]
x10^5
[M+K]+
509.202
[M+Na]+
493.234
m/z
18

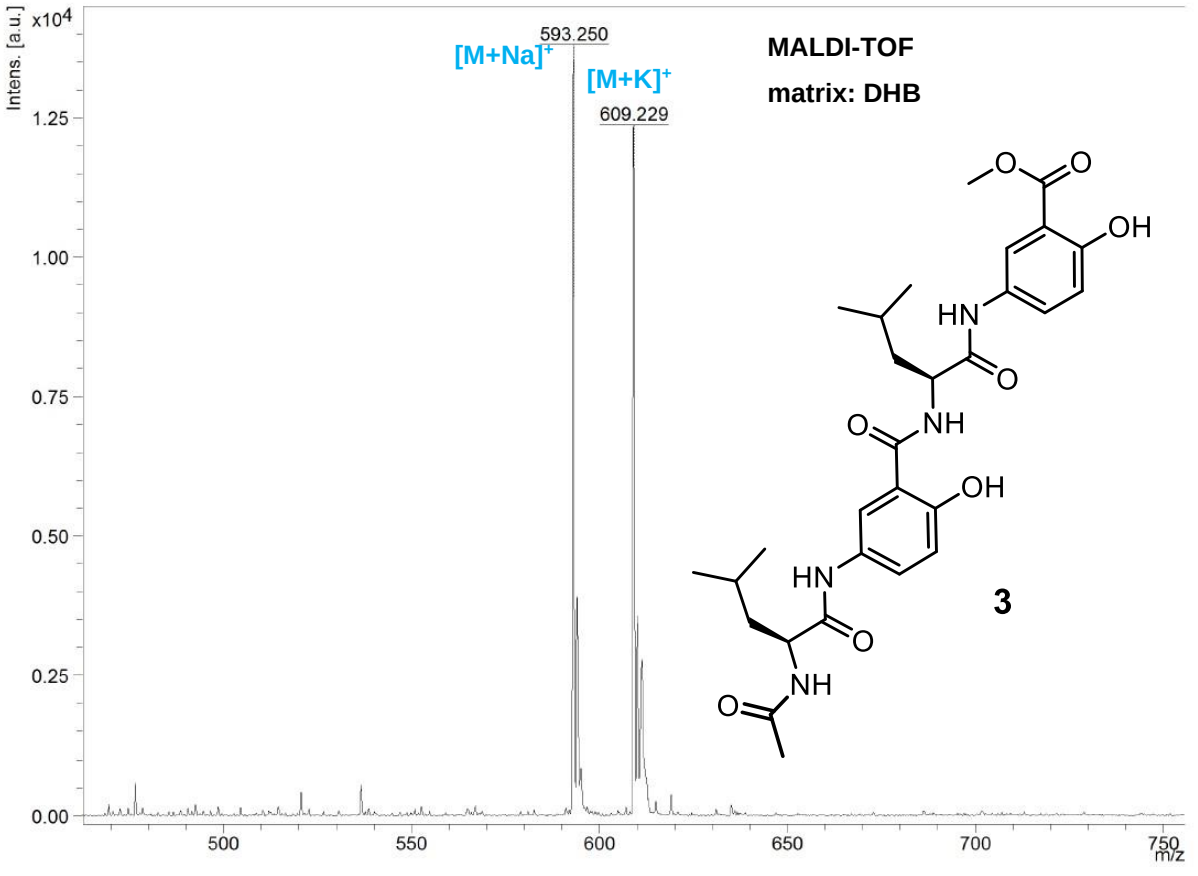

MALDI-TOF MS
Matrix: DHB
Intens. [a.u.]
x10^4
[M+K]+
495.190
[M+Na]+
479.212
18a
m/z

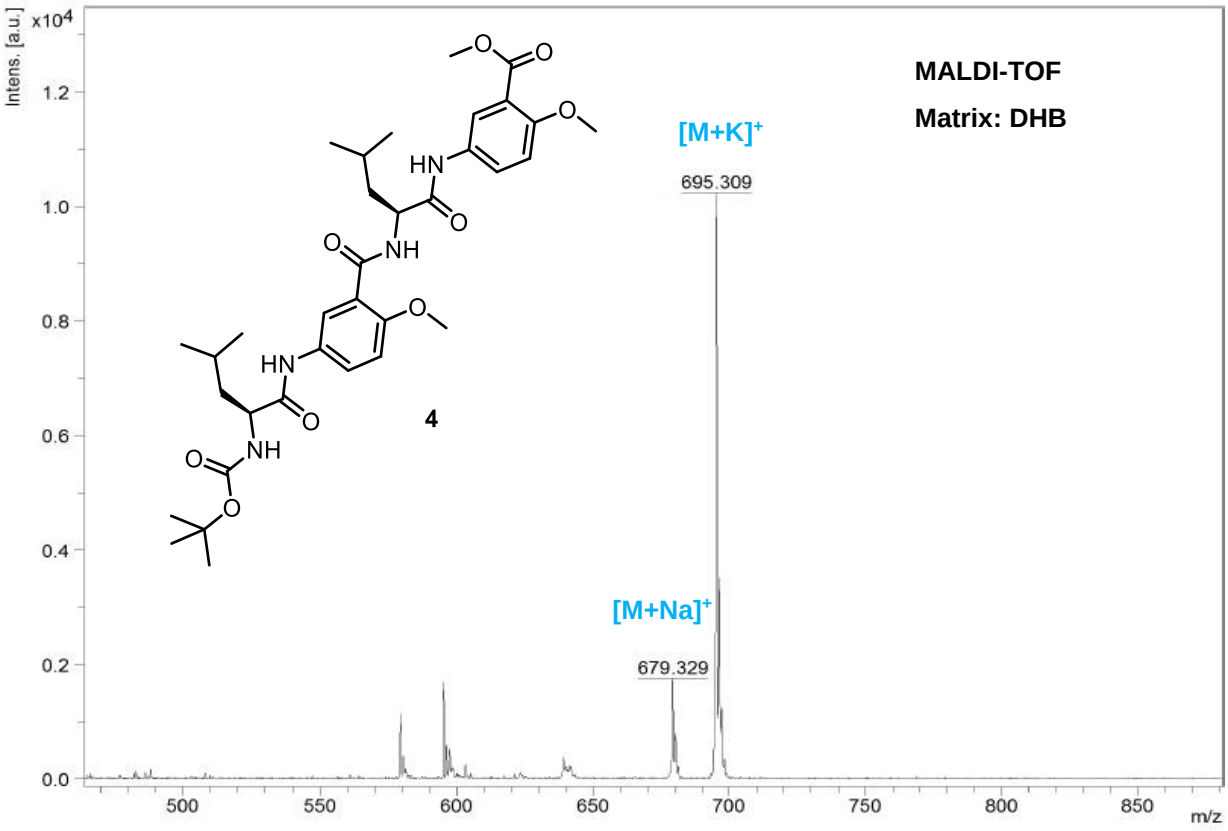

MALDI-TOF
matrix: DHB
Intens. [a.u.]
x10^4
[M+Na]+
667.277
[M+Na]+
1
m/z

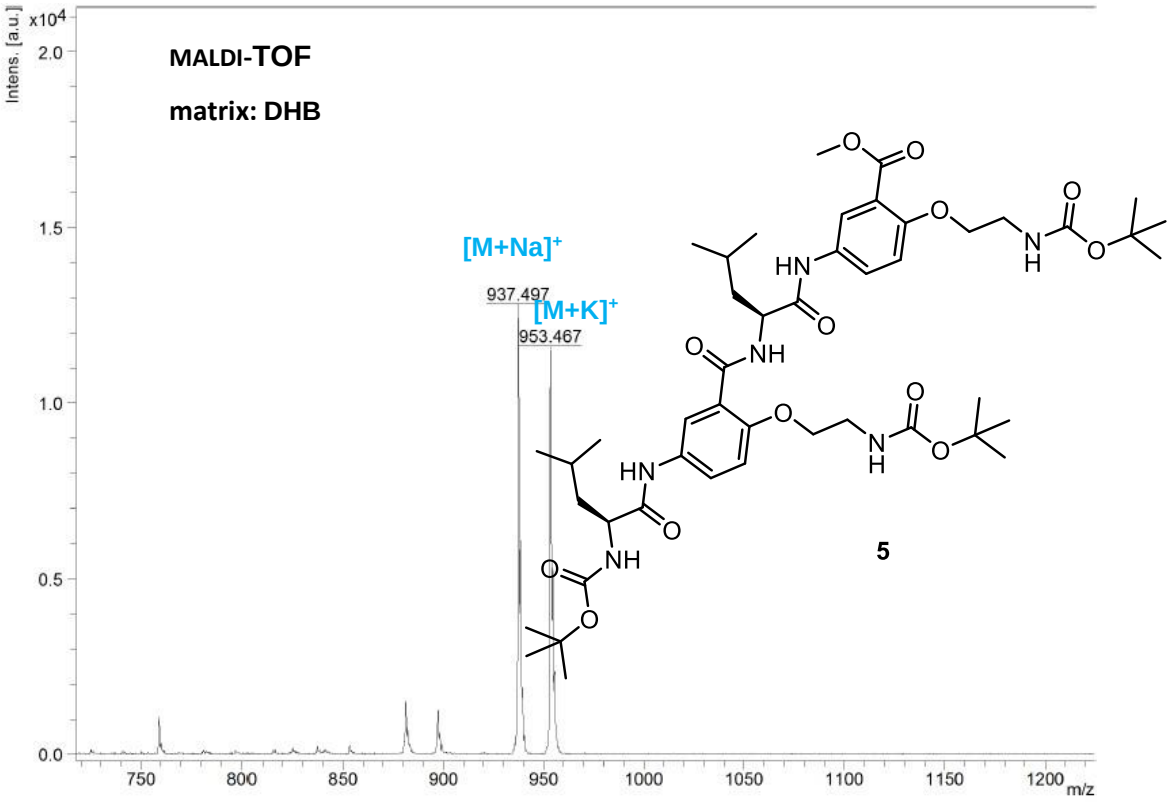

MALDI-TOF MS
matrix : DHB
2
[M+K]+
847.363
[M+Na]+
831.390
Intens. [a.u.]
x10^4
m/z

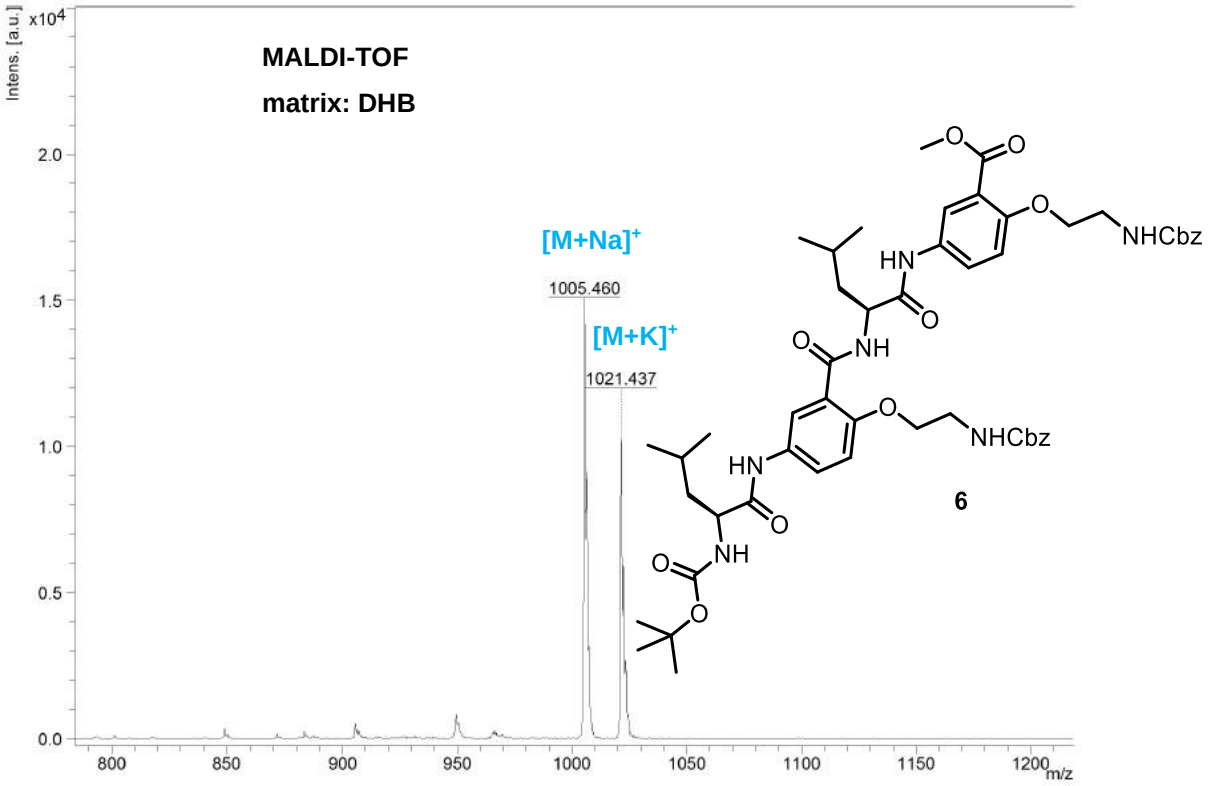

MALDI-TOF
matrix: DHB
[M+K]+
747.317
[M+Na]+
731.337
2b
Intens. [a.u.]
x10^4
m/z

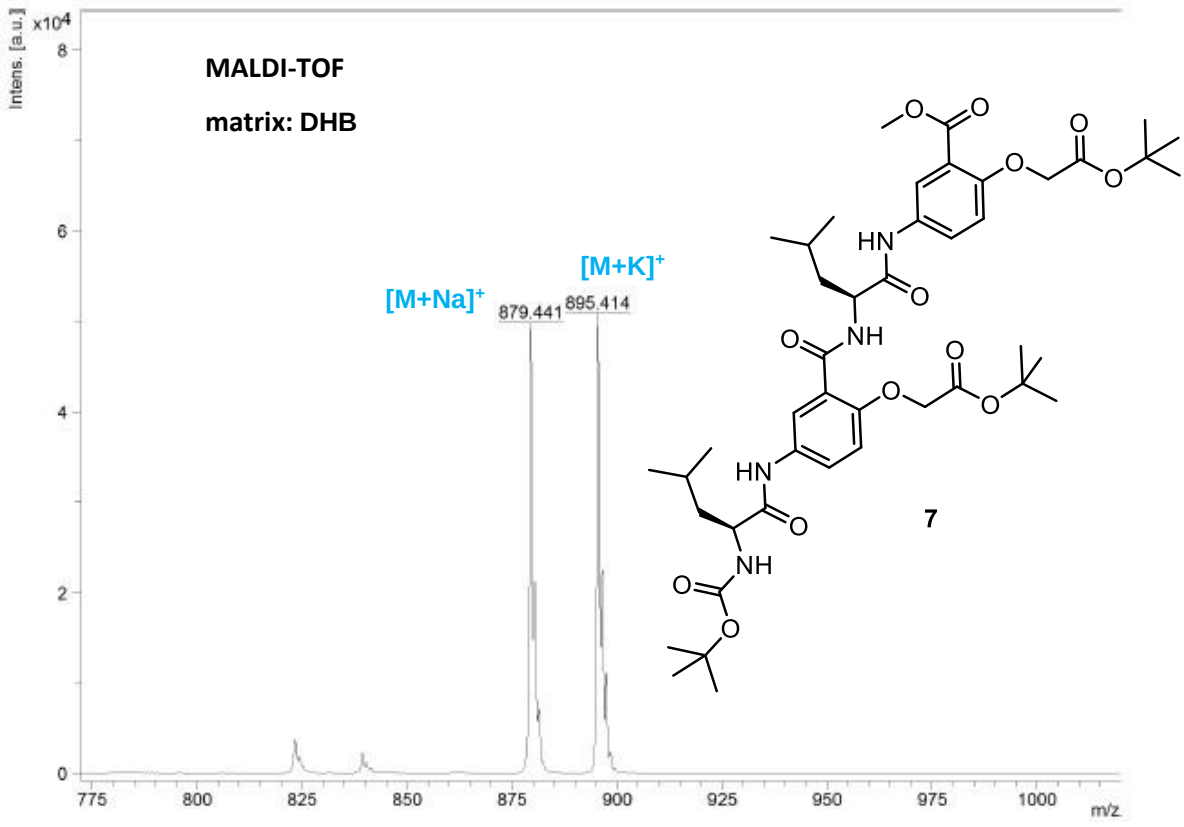

Intens. [a.u.]
x10^5
1.25
1.00
0.75
0.50
0.25
0.00
650
700
750
800
850
900
950
m/z
[M+Na]+
773.358
[M+K]+
789.332
MALDI-TOF
matrix: DHB
2c

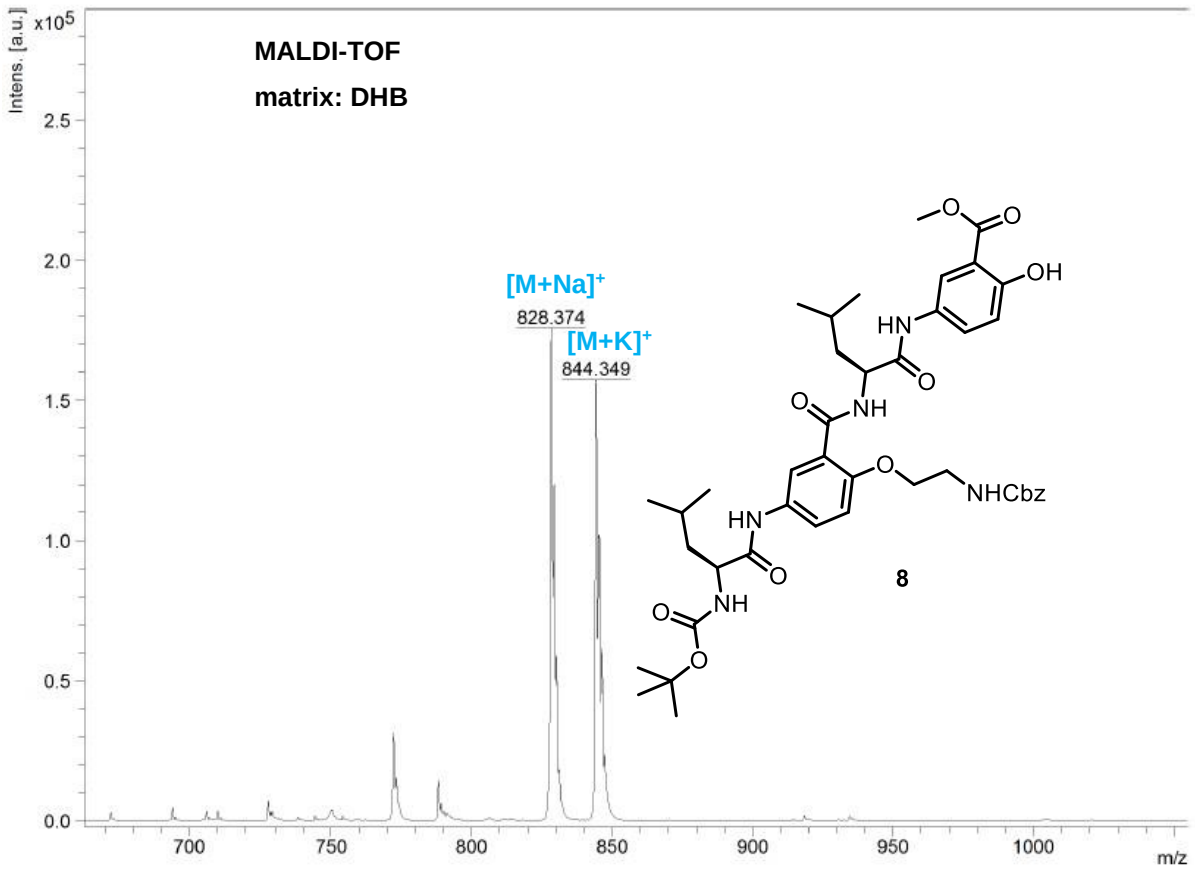

Intens. [a.u.]
x10^4
1.25
1.00
0.75
0.50
0.25
0.00
500
550
600
650
700
750
m/z
593.250
[M+Na]+
[M+K]+
609.229
MALDI-TOF
matrix: DHB
3

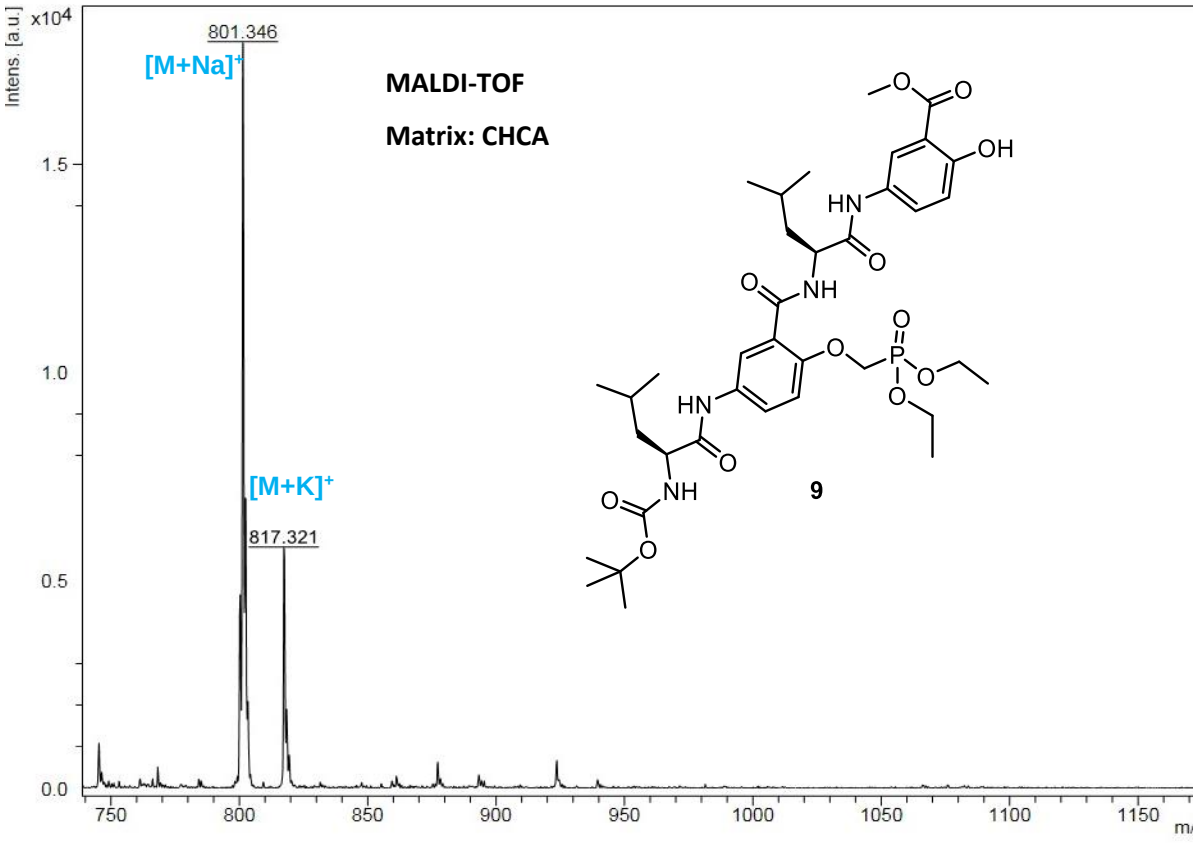

Intens. [a.u.]
x10^4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
500
550
600
650
700
750
800
850
m/z
MALDI-TOF
Matrix: DHB
[M+K]+
695.309
[M+Na]+
679.329
4

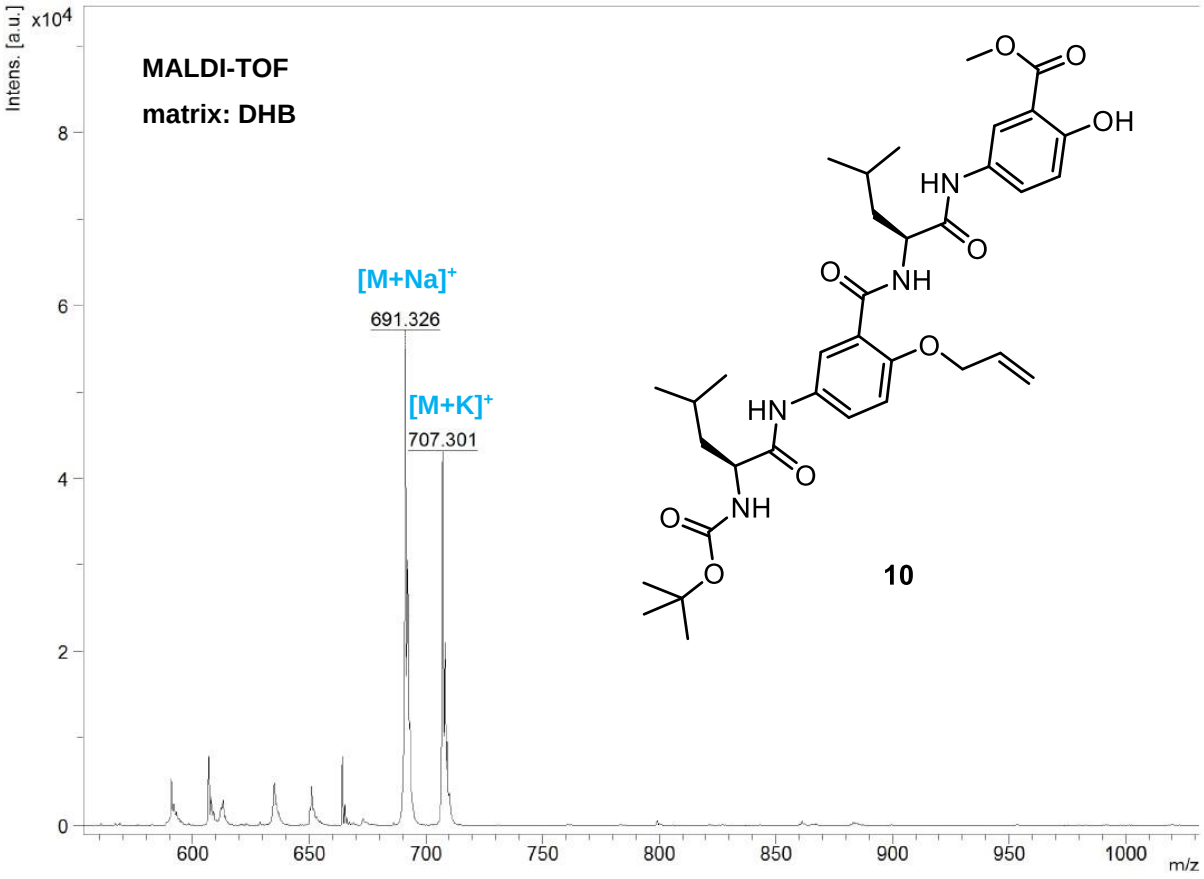

Intens. [a.u.]
x10^4
2.0
1.5
1.0
0.5
0.0
750
800
850
900
950
1000
1050
1100
1150
1200
m/z
MALDI-TOF
matrix: DHB
[M+Na]+
937.497
[M+K]+
953.467
5

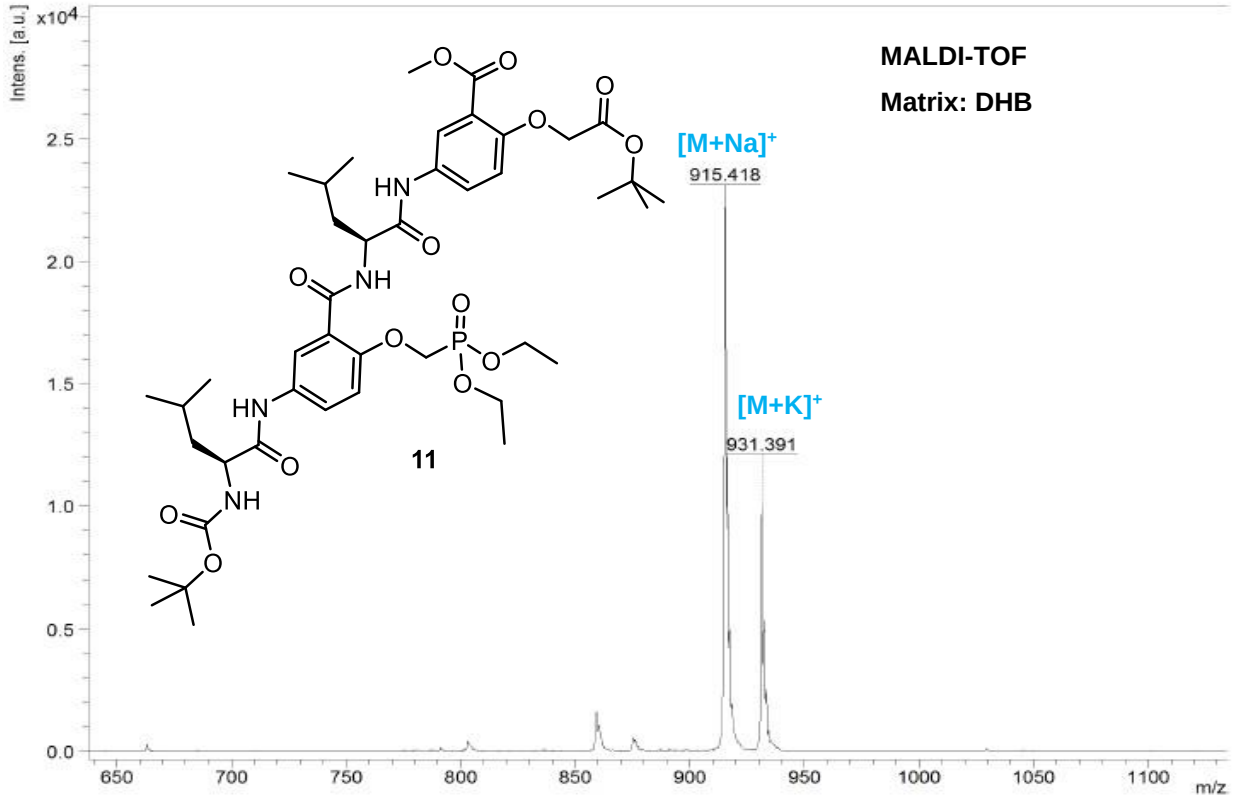

MALDI-TOF
matrix: DHB
[M+Na]+
1005.460
[M+K]+
1021.437
NHCbz
NHCbz
6
Intens. [a.u.]
x10^4
m/z

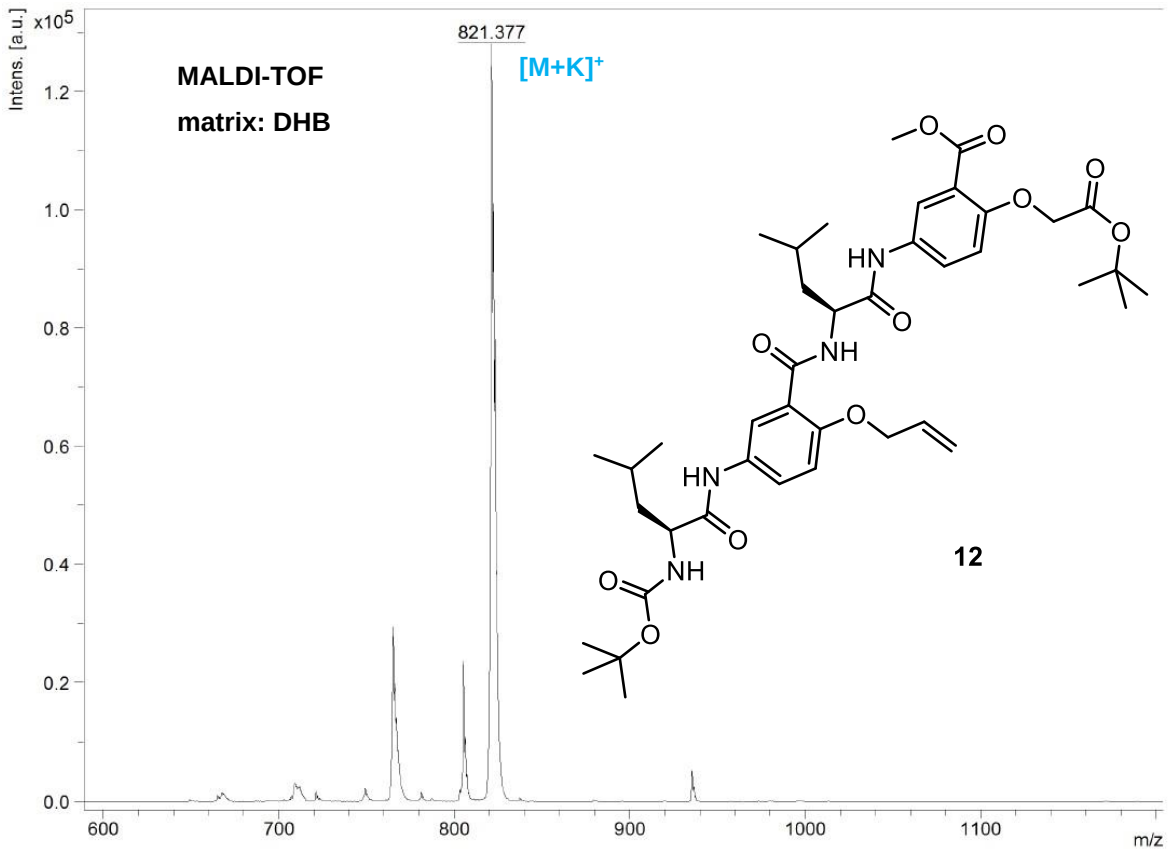

MALDI-TOF
matrix: DHB
[M+Na]+
879.441
[M+K]+
895.414
7
Intens. [a.u.]
x10^4
m/z

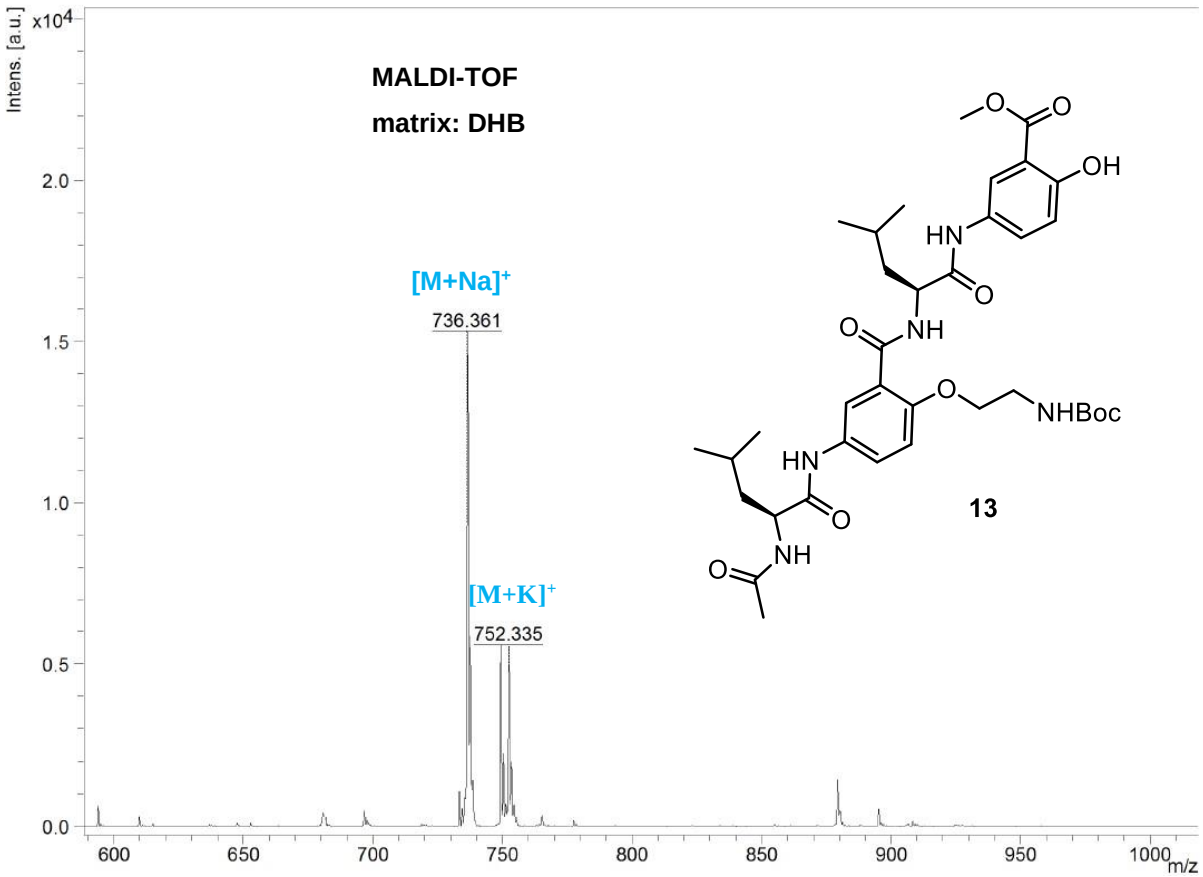

MALDI-TOF
matrix: DHB
Intens. [a.u.]
x10^5
2.5
2.0
1.5
1.0
0.5
0.0
[M+Na]+
828.374
[M+K]+
844.349
700
750
800
850
900
950
1000
m/z
OH
HN
NH
NHCbz
8

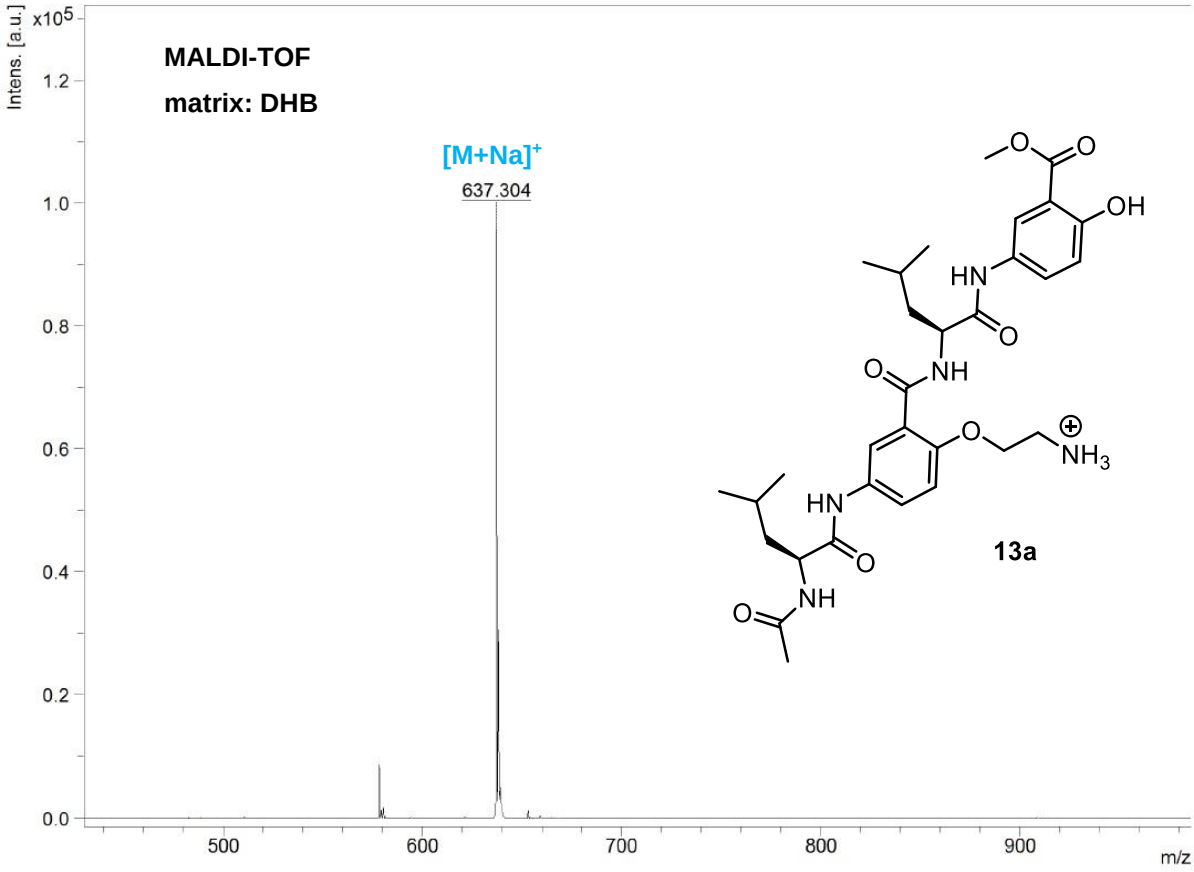

Intens. [a.u.]
x10^4
1.5
1.0
0.5
0.0
801.346
[M+Na]+
MALDI-TOF
Matrix: CHCA
[M+K]+
817.321
750
800
850
900
950
1000
1050
1100
1150
OH
HN
NH
9

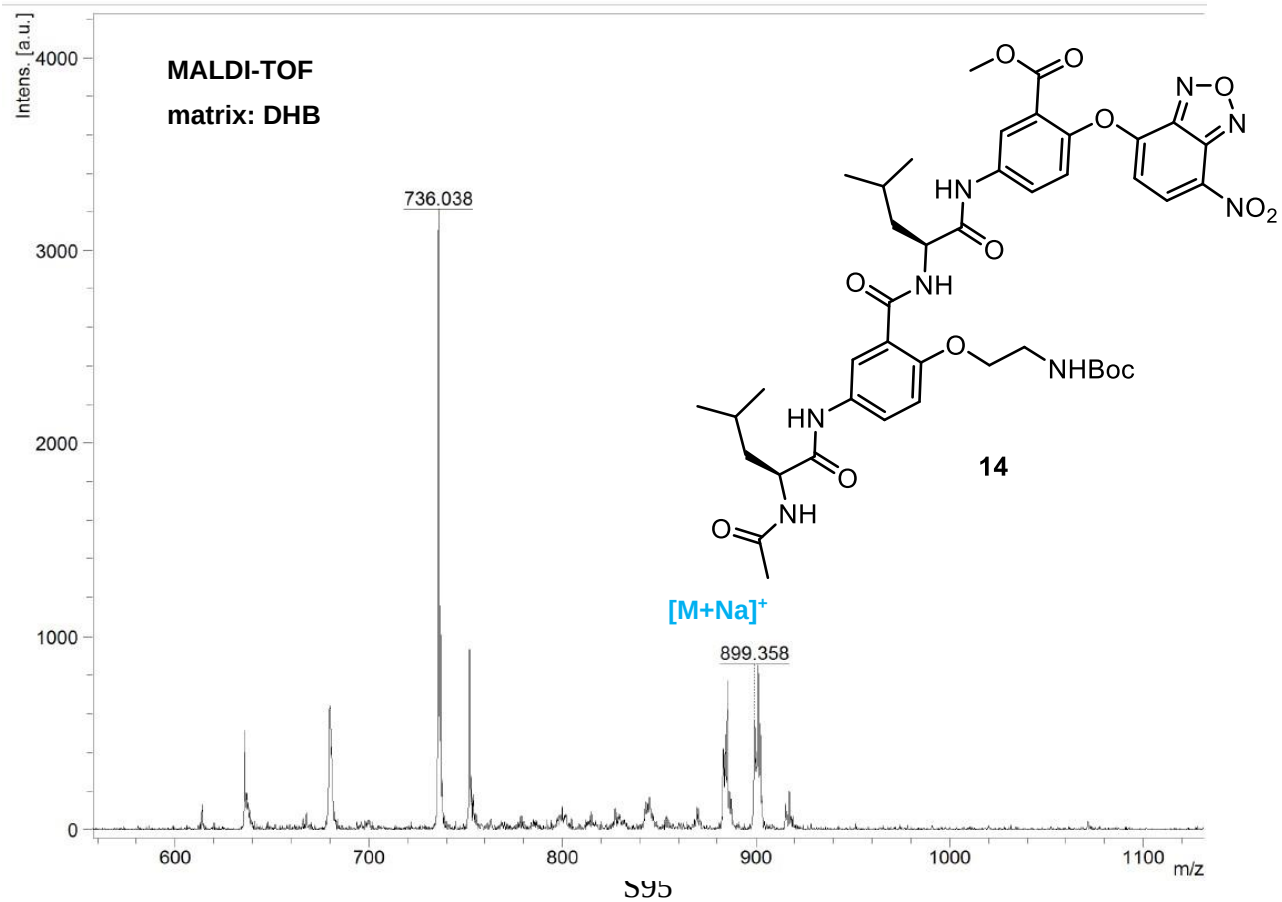

MALDI-TOF
matrix: DHB
[M+Na]+
691.326
[M+K]+
707.301
10
Intens. [a.u.]
x10^4
m/z

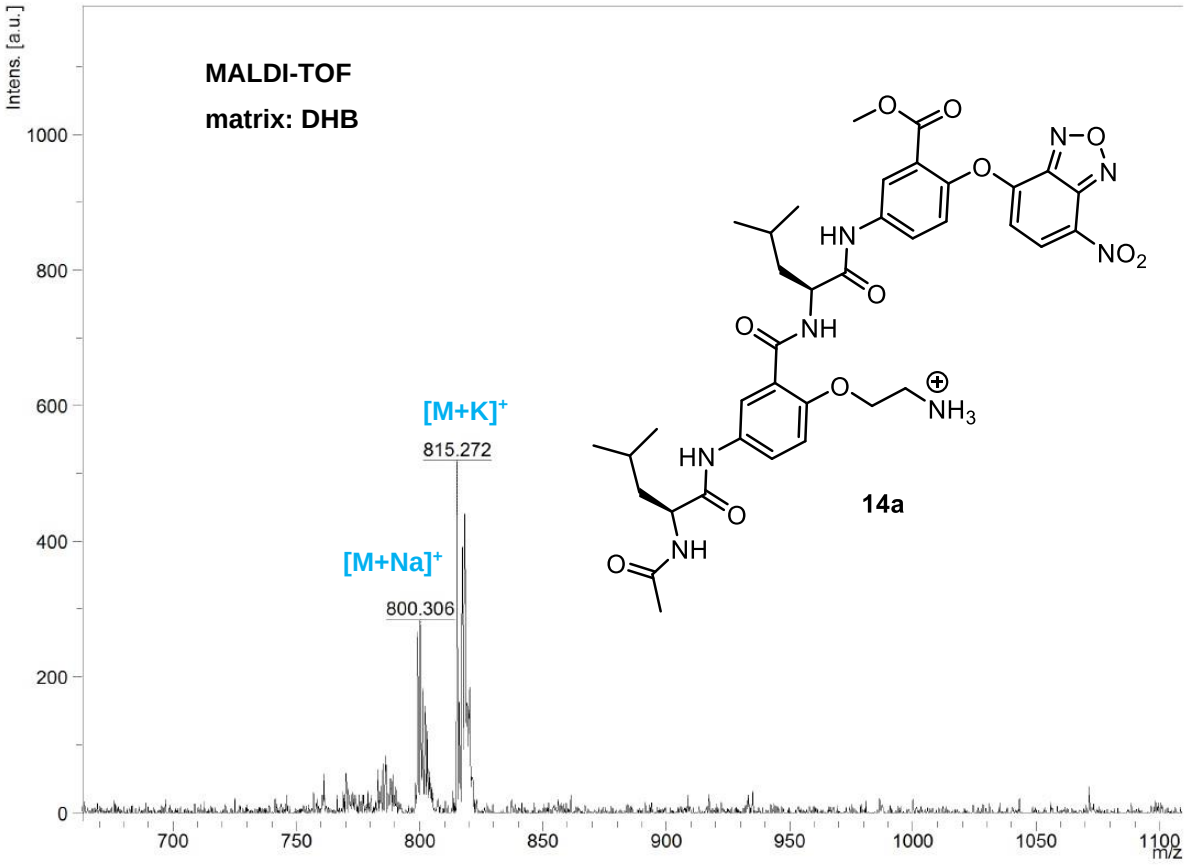

MALDI-TOF
Matrix: DHB
[M+Na]+
915.418
[M+K]+
931.391
11
Intens. [a.u.]
x10^4
m/z


MALDI-TOF
matrix: DHB
821.377
[M+K]+
Intens. [a.u.]
x10^5
1.2
1.0
0.8
0.6
0.4
0.2
0.0
600
700
800
900
1000
1100
m/z
12


MALDI-TOF
matrix: DHB
[M+Na]+
736.361
[M+K]+
752.335
Intens. [a.u.]
x10^4
2.0
1.5
1.0
0.5
0.0
600
650
700
750
800
850
900
950
1000
m/z
NHBoc
OH
13


MALDI-TOF
matrix: DHB
[M+Na]+
637.304
Intens. [a.u.]
x10^5
1.2
1.0
0.8
0.6
0.4
0.2
0.0
500
600
700
800
900
m/z
13a


MALDI-TOF
matrix: DHB
736.038
[M+Na]+
899.358
Intens. [a.u.]
4000
3000
2000
1000
0
600
700
800
900
1000
1100
m/z
14


MALDI-TOF
matrix: DHB
Intens. [a.u.]
1000
800
600
400
200
0
700
750
800
850
900
950
1000
1050
1100
m/z
[M+K]+
815.272
[M+Na]+
800.306
HN
O
NH
O
O
NH3
HN
O
NH
O
O
N–O
N
NO2
14a

15. References

1 R. Andreozzi, M. Canterino, V. Caprio, I. Di Somma and R. Sanchirico, *Org. Process Res. Dev.*, 2006, **10**, 1199–1204.

2 (a) F. D. Bellamy and K. Ou, *Tetrahedron Letters*, 1984, **25**, 839–842. (b) J. L. Yap, X. Cao, K. Vanommeslaeghe, K.-Y. Jung, C. Peddaboina, P. T. Wilder, A. Nan, A. D. MacKerell, W. R. Smythe and S. Fletcher, *Org. Biomol. Chem.*, 2012, **10**, 2928.

3 (a) K. Lee, Y. Choi, K. Kim, H.-J. Koo and J. Choi, *Applied Sciences*, 2019, **9**, 130. (b) Q. Liang, X. Sun, H. Raza, M. Aslam Khan, H. Ma and X. Ren, *Ultrasonics Sonochemistry*, 2021, **80**, 105830.

4 (a) A. K. Jangid, D. Pooja, P. Jain, N. Gupta, S. Ramesan and H. Kulhari, *RSC Adv.*, 2021, **11**, 13928–13939. (b) A. Patel, P. Heussen, J. Hazekamp and K. P. Velikov, *Soft Matter*, 2011, **7**, 8549.5 U. Modi, D. Kedaria and R. Vasita, *Macromol. Biosci.*, 2022, **22**, 2200196.

5 U. Modi, D. Kedaria and R. Vasita, *Macromol. Biosci.*, 2022, **22**, 2200196.