A flow-based transition-metal-catalysed hydrogenolysis approach to facilitate peptide side-chain deprotection

Electronic Supporting Information

Maria Menti,[a] Janice R. Aldrich-Wright,[b,c] and Christopher P. Gordon[b,c,d]

1. General Considerations

Protected amino acids, PS-2ClTrt-Cl (loading 0.9 mmol/g) and (2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylammonium hexafluorophosphate) (HCTU) were purchased from Auspep and used as received to synthesise the linear peptide sequences as previously published. All other reagents and reactants were purchased from standard suppliers and utilised without purification unless otherwise stated. PS-carbodiimide was purchased from biotage (loading 1.27 mmol/g). All solvents were purchased from ChemSupply and distilled under reduced pressure, with the exception of HPLC grade solvents being used as received. HPLC solvents were degassed before use. Deuterated solvents were purchased from Cambridge Isotope Laboratories and stored in a desiccator.

All flow hydrogenation methods were carried out on a ThalesNano H-Cube ProTM continuous flow reactor. The choice of catalyst (ThalesNano CatCarts®), and reaction parameters including temperature (°C) H_2 pressure (bar), flow rate (mL/min), H_2 production (%), and number of cycles are defined in each individual experiment.

All ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 25 °C on a Varian 'Mercury' 300 MHz spectrometer or a Bruker 'Avance' 400 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) and are measured in reference to the designated deuterated solvent. Coupling constants (J) are recorded in Hz and significant multiplicities described by singlet (s), doublet (d), doublet of doublets (dd), doublet of triplets (dt), triplet (t), quadruplet (q), broad (br s), multiplet (m). Spectra were assigned using appropriate gCOSY sequences.

Analytical RP-HPLC analysis was performed using an Agilent instrument comprising six modules; 1260 Bin Pump, 1260 HIP Degasser, 1260 ALS, 1260 TCC, 1260 DAD and 1260 FC-AS. Analytical RP-HPLC was performed using Phenomenex Onyx Monolithic reversed-phase C18 column (4.6 x 100 mm). A: 0.06% TFA in H_2O and solvent B: 0.06% TFA in CH_3CN:H_2O (9:1), flow rate of 1.0 mL/min, gradient 10-100 (%B), curve = 6, over 15.0 mins, and detection at 214 and 254 nm. For peptide purification semi-preparative RP-HPLC was performed using a Waters 2525 binary gradient pump equipped with a water 2487 dual λ absorbance detector and a Chromolith®SemiPrep RP-18e 100-10 mm column. A flow rate of 10 mL/min was used with solvent A: 0.06% TFA in water and solvent B: 0.06% TFA in CH_3CN:H_2O (90:10). Gradient 10-75 (%B) over 15 mins, curve = 6, with UV detection at 214 nm and 254 nm.

Mass spectrometry data was obtained at the Western Sydney University Mass Spectrometry Facility. Low-resolution mass spectrometry (LRMS) experiments were performed using a Waters 'Xevo TQ Triple Quadrupole' Mass Spectrometer *via* the ESI method. Spectra were recorded in positive ion mode from analyte solutions injected into a 0.1% formic acid in methanol solution flowing at 0.1 mL/min. A capillary voltage of 3.0 kV, cone voltage of 30 V, desolvation temperature of 300 °C and desolvation flow rate of 500 L/h. Spectra were recorded over 1 min with an *m/z* range of 100 – 2000. High resolution mass spectra (HRMS) were obtained on a Waters 'Xevo Quadrupole-Time of Flight' spectrometer via the ESI method and were within 5.00 ppm of the acceptable limit. The instrument was fitted with an ESI probe and mass-corrected sample spectra were recorded in positive mode, using leucine encephalin (200 $pg.mL^{-1}$ in 50% aqueous acetonitrile + 0.1% formic acid) as a lockmass reference.

2. General Methods

2.1. General Method 1 – Linear Peptide Synthesis

The linear sequences were synthesised using an adapted literature method,[1] where PS-2ClTrt-Cl (200 mg, loading = 0.97 mmol/g) was placed in an Omnifit™ column (one fixed end, one variable end) and swelled with DCM (4.0 mL, 0.5 h). The adjustable end was fixed within 5 mm of the swollen resin. The column was placed inline with a syringe pump, and acetyl chloride (3 M in DCM, 10 mL) was flowed through (1.0 mL/min) at room temperature. The resin was washed with DCM (20 mL). The first amino acid solution (AA_1) (Fmoc-AA-OH 4 eq., DIPEA 8 eq., in DCM (10mL)) was flowed through (1.0 mL/min) at 40 °C. To ensure all unreacted -Cl sites on the resin were hydrolysed, MeOH (10 mL) was flowed through (5.0 mL/min). The column was then placed on a thermostatically controlled heating block (60 °C) in-line with the HPLC driven flow synthesiser established in literature,[2] (UV detector zeroed to DMF at 290 nm) The resin was washed with solution A (100% DMF, 5.0 mL/min) until the UV absorbance read <0.1 AU. The resin was subsequently Fmoc-deprotected using solution B (1:1 DMF:Piperidine, 5.0 mL/min) until the detector reached maximum absorption and returned to <0.1 AU. The resin was then washed with solution A (2 min, 5.0 mL/min). The amino acid solution (Fmoc-AA-OH 4 eq., HCTU 4 eq., DIPEA 8 eq., DMF 1.0 mL) was injected into the sample loop of the reactor and flowed through the Omnifit™ column (2.0 mL/min) until the UV detector reached maximum absorbance and subsequently returned to <0.1 AU. This was followed by Fmoc-deprotection with solution B until the detector reached maximum detection and returned to <0.1 AU. The resin was then washed with solution A (2 min, 5.0 mL/min). The above steps of AA solution injection (step 1), Fmoc-deprotection (step 2) and washing (step 3) were repeated for all additional AA's in the sequence. Once the final Fmoc-deprotection was achieved, the column was removed from the heating block and the resin was washed with DCM (2 × 5 mL), MeOH (2 × 5 mL), and Et_2O (2 × 10 mL) and then dried *in vacuo*.

2.2. General Method 2 – Cbz Hydrogenation

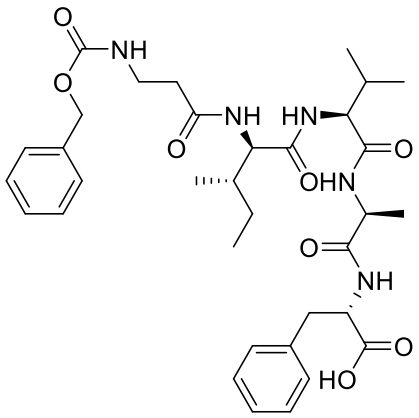
A solution of the linear Cbz protected peptide sequence in MeOH was flowed through the H-cube Pro™ continuous flow reactor under a 10% $Pd/CaCO_3$ catalyst at 40 °C, 1.0 mL/min, 1 atm, 25% H_2 production for 1 cycle. The collected eluent was concentrated *in vacuo* to afford each peptide as the free amine.

2.3. General Method 3 – PS-Carbodiimide Mediated Cyclisation

Cyclisations were performed using a previously reported flow synthesiser according to literature.[3] The reaction column was initially charged with dry carbodiimide functionalised resin (2 molar equivalents). Following resin swelling, which was effected with DCM, the reaction column was placed in-line where a continuous stream of neat DCM was flowed through the resin bed at 0.5 mL/min and 60 °C. Upon stabilisation of UV absorbance, the Rheodyne® injection loop was loaded with a solution of a linear peptide (0.02 M, 1.0 mL, 10% DMF:DCM). Following the injection of coupling solution into the continuous DCM stream (0.5 mL/min) the column eluent was monitored *via* UV and upon absorbance detection, the reaction stream was collected. Collection of the column eluent continued until UV absorbance of the solvent stream returned to baseline.

3. Characterisation Data for Compounds 7-26

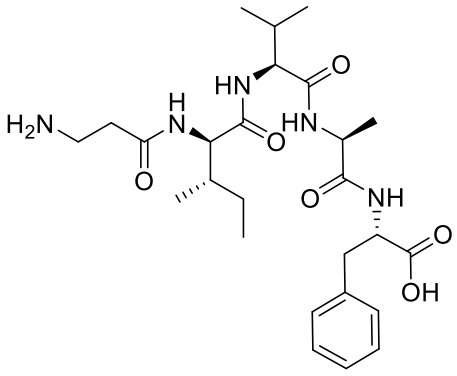
(3-(((benzyloxy)carbonyl)amino)propanoyl)-L-alloisoleucyl-L-valyl-L-alanyl-L-phenylalanine (8)



Chemical Formula: $C_{34}H_{47}N_5O_8$
Molecular Weight: 653.78

Compound **8** was synthesised using General Method 1, with Fmoc-L-Phe-OH as AA_1 (0.3012 g, 7.76 mmol), Fmoc-L-Ala-OH as AA_2 (0.2308 g, 7.76 mmol), Fmoc-L-Val-OH as AA_3 (0.2560 g, 7.76 mmol), Fmoc-L-Ile-OH as AA_4 (0.2666 g, 7.76 mmol), and Z-β-Ala-OH as AA_5 (0.1684 g, 7.76 mmol). The dried resin was added to a scintillation vial, to which a cleavage cocktail (TFA:TIPS:DCM 1.5:0.5:98) was added and allowed to cleave for 2 h. The cleaved peptide solution was filtered and H_2O (10 mL) was added to the filtrate. The organic solvent was concentrated *in vacuo* and the aqueous suspension was lyophilised to afford compound **8** as a white solid (89%, 114 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (d, J = 7.81 Hz, 1H), 7.96 (d, J = 8.48 Hz, 1H), 7.88 (d, J = 7.53 Hz, 1H), 7.72 (d, J = 8.48 Hz, 1H), 7.41 – 7.16 (m, 10H), 5.00 (s, 2H), 4.44 – 4.39 (m, 1H), 4.33 – 4.27 (m, 1H), 4.21 – 4.12 (m, 2H), 3.22 – 3.16 (m, 2H), 3.06 – 3.02 (m, 1H), 2.93 – 2.87 (m, 1H), 2.38 – 2.29 (m, 2H), 1.96 – 1.89 (m, 1H), 1.73 – 1.66 (m, 1H), 1.43 – 1.36 (m, 1H), 1.16 (d, J = 6.88, 2H), 0.99 (m, 6H), 0.81 – 0.71 (m, 10H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.12, 172.38, 171.54, 170.71, 156.44, 137.77, 137.64, 129.54, 128.61, 128.19, 128.12, 126.87, 65.65, 57.82, 57.44, 53.77, 48.33, 37.73, 37.11, 36.70, 35.95, 30.88, 24.82, 19.68, 19.64, 18.76, 18.43, 15.84, 11.41, 10.11. HRMS (ESI$^+$); for $C_{34}H_{47}N_5O_8Na$ calculated 676.3322, found 676.3295. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 9.82 min.

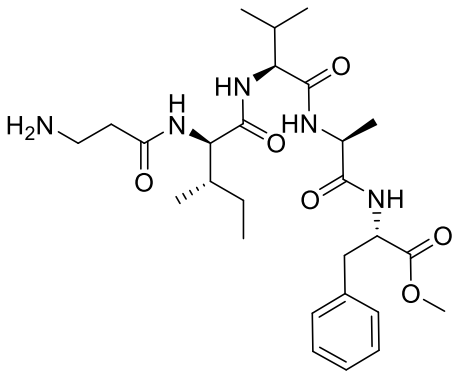
(3-aminopropanoyl)-L-alloisoleucyl-L-valyl-L-alanyl-L-phenylalanine (9)



Chemical Formula: $C_{26}H_{41}N_5O_6$
Molecular Weight: 519.64

A solution of **8** in MeOH was flowed through the H-cube Pro™ continuous flow reactor under a 10% $Pd/CaCO_3$ catalyst at 40 °C, 1.0 mL/min, 1 atm, 25% H_2 production for 1 cycle. The collected eluent was concentrated *in vacuo*, prior to the addition of H_2O. The suspension was lyophilised to afford **9** as a white solid (84% 32.7 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, 1H, J = 8.29 Hz), 8.09 (d, 1H, J = 7.60 Hz), 7.89 (d, 1H, J = 7.60 Hz), 7.83 (d, 1H, J = 8.98 Hz), 7.38 (br s, 2H), 7.28 – 7.17 (m, 5H), 4.44 – 4.39 (m, 1H), 4.33 – 4.32 (m, 2H), 4.14 (t, 1H, J = 7.56 Hz), 3.07 – 2.87 (m, 4H), 1.94 (quin, 1H, J = 6.78 Hz), 1.75 – 1.67 (m, 1H), 1.45 – 1.39 (m, 1H), 1.16 (d, 3H, J = 6.78 Hz), 0.99 (d, 2H, J = 7.40 Hz), 0.83 – 0.78 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.12, 172.36, 171.38, 170.67, 156.43, 137.79, 129.54, 128.61, 126.87, 57.91, 57.41, 53.78, 48.33, 37.07, 35.86, 32.32, 30.80, 24.82, 19.66, 18.81, 18.52, 18.31, 15.83, 12.53, 11.51. HRMS (ESI$^+$); for $C_{26}H_{41}N_5O_6$ calculated 520.3135, found 520.3119. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 6.44 min.

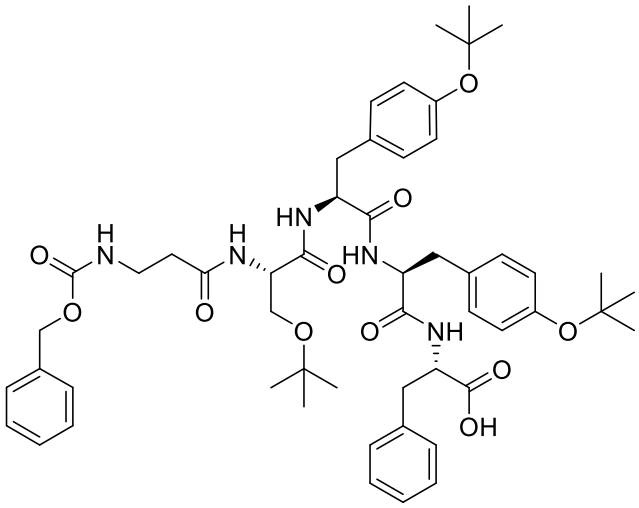
methyl (3-aminopropanoyl)-L-alloisoleucyl-L-valyl-L-alanyl-L-phenylalaninate (10)



Chemical Formula: $C_{27}H_{43}N_5O_6$
Molecular Weight: 533.67

A solution of **8** in 9:1 $CHCl_3$:MeOH was flowed through the H-cube Pro™ continuous flow reactor under a 10% Pd/C catalyst at 80 °C, 0.5 mL/min, 1 atm, 100% H_2 production for 1 cycle. The collected eluent was concentrated *in vacuo*, prior to the addition of H_2O. The suspension was lyophilised to afford **10** as a white solid (90%, 36.7 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24, (d, 1H, *J* = 7.92 Hz), 8.18 (d, 1H, *J* = 9.02 Hz), 7.90 (d, 1H, *J* = 6.90 Hz), 7.84 (d, 1H, *J* = 9.02 Hz), 7.69 (s, 2H), 7.29 – 7.25 (m, 2H), 7.22 – 7.19 (m, 3H), 4.45 (q, 1H, *J* = 7.86 Hz), 4.33 – 4.22 (m, 2H), 4.14 (t, 1H, *J* = 8.00 Hz), 3.58 (s, 3H), 3.04 – 2.89 (m, 4H), 1.98 – 1.91 (m, 4H), 1.96 – 1.91 (m, 1H), 1.74 – 1.66 (m, 1H), 1.44 – 1.38 (m, 1H), 1.16 (d, 3H, *J* = 6.59 Hz), 1.10 – 1.03 (m, 1H), 0.86 – 0.79 (m, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.58, 172.21, 171.38, 170.69, 169.84, 137.43, 129.48, 128.71, 127.04, 57.90, 57.36, 53.99, 52.29, 48.23, 37.09, 37.01, 35.85, 32.29, 24.81, 19.62, 18.74, 18.55, 15.83, 11.52. HRMS (ESI$^+$); for $C_{27}H_{44}N_5O_6$ calculated 534.3292, found 534.3333. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 7.42 min.

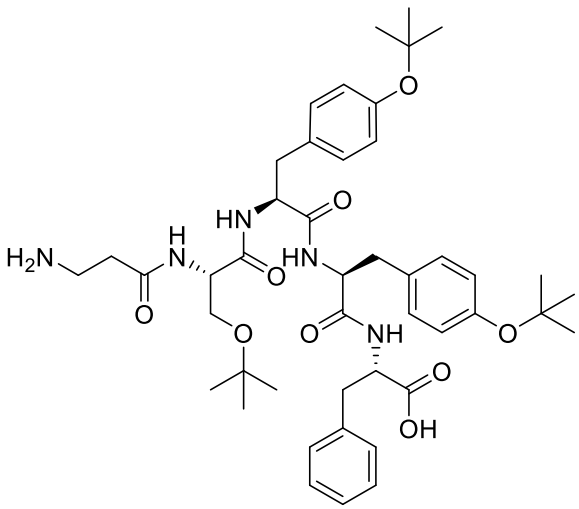
((*S*)-2-((*S*)-2-((*S*)-2-(3-(((benzyloxy)carbonyl)amino)propanamido)-3-(tert-butoxy)propanamido)-3-(4-(tert-butoxy)phenyl)propanamido)-3-(4-(tert-butoxy)phenyl)propanoyl)-L-phenylalanine (11)



Chemical Formula: $C_{53}H_{69}N_5O_{11}$
Molecular Weight: 952.16

Compound **5** was synthesised using General Method 1, with Fmoc-L-Phe-OH as AA_1 (0.3012 g, 7.76 mmol), Fmoc-L-Tyr(*t*Bu)-OH as AA_2 (0.3573 g, 7.76 mmol), Fmoc-L-Tyr(*t*Bu)-OH as AA_3 (0.3573 g, 7.76 mmol), Fmoc-L-Ser(*t*Bu)-OH as AA_4 (0.2982 g, 7.76 mmol), and Z-β-Ala-OH as AA_5 (0.1684 g, 7.76 mmol). The dried resin was added to a scintillation vial, to which a cleavage cocktail (TFA:TIPS:DCM 1.5:0.5:98) was added and allowed to cleave for 2 h. The cleaved peptide solution was filtered and H_2O (10 mL) was added to the filtrate. The organic solvent was concentrated *in vacuo* and the aqueous suspension was lyophilised to afford compound **11** as a white solid (76%, 120 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 8.27 (d, 1H, *J* = 8.20 Hz), 8.07 (d, 1H, *J* = 8.20 Hz), 7.91 (d, 1H, *J* = 8.20 Hz), 7.78 (d, 2H, *J* = 8.43 Hz), 7.23 – 7.17 (m, 11H), 7.11 (d, 2H, *J* = 8.93 Hz), 7.01 (d, 2H, *J* = 8.93 Hz), 6.82 (d, 2H, *J* = 8.44 Hz), 6.76 (d, 2H, *J* = 8.44 Hz), 5.00 (s, 2H), 4.58 – 4.41 (m, 3H), 4.26 (q, 1H, *J* = 6.45 Hz), 3.20 (q, 2H, *J* = 7.19 Hz), 3.09 – 3.05 (m, 1H), 2.99 – 2.89 (m, 2H), 2.88 – 2.82 (m, 1H), 2.76 – 2.60 (m, 2H), 2.31 (t, 2H, *J* = 7.90 Hz), 1.27 (s, 9H), 1.23 (s, 9H), 1.04 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.06, 171.39, 170.86, 170.81, 169.83, 156.44, 153.84, 153.79, 137.78, 137.62, 132.64, 132.53, 130.13, 129.57, 128.79, 128.65, 128.20, 128.15, 126.89, 123.74, 123.63, 77.97, 73.31, 65.65, 61.98, 53.89, 35.93, 29.00, 27.55, 19.70, 18.31, 12.53, 10.11. HRMS (ESI$^+$); for $C_{53}H_{70}N_5O_{11}$ calculated 952.5072, found 952.5110. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 14.22 min.

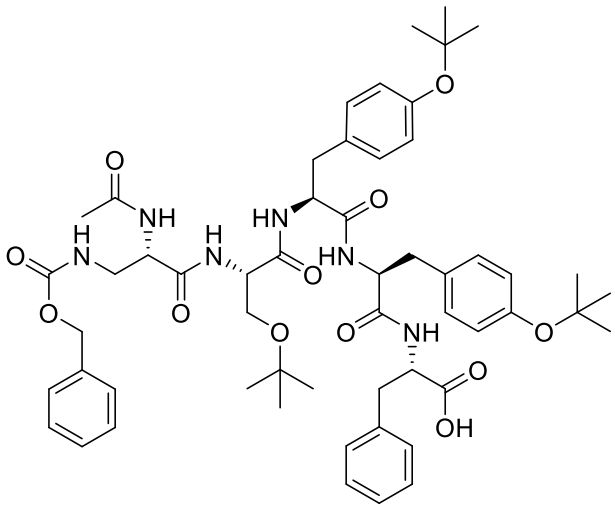
((*S*)-2-((*S*)-2-((*S*)-2-(3-aminopropanamido)-3-(tert-butoxy)propanamido)-3-(4-(tert-butoxy)phenyl)propanamido)-3-(4-(tert-butoxy)phenyl)propanoyl)-L-phenylalanine (12)



Chemical Formula: $C_{45}H_{63}N_5O_9$
Molecular Weight: 818.03

A solution of **11** in MeOH was flowed through the H-cube Pro™ continuous flow reactor under a 10% $Pd/CaCO_3$ catalyst at 40 °C, 1.0 mL/min, 1 atm, 25% H_2 production for 1 cycle. The collected eluent was concentrated *in vacuo* to afford **12** as a white solid (92%, 94.6 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, 1H, J = 7.54 Hz), 8.25 (d, 1H, J = 8.34 Hz), 7.99 (d, 1H, J = 8.34 Hz), 7.93 – 7.91 (m, 1H), 7.26 – 7.15 (m, 5H), 7.12 (d, 2H, J = 8.34 Hz), 6.98 (d, 2H, J = 8.34 Hz), 6.82 (d, 2H, J = 8.34 Hz), 6.77 (d, 2H, J = 8.34 Hz), 4.41 – 4.32 (m, 2H), 4.21 – 4.18 (m, 2H), 3.15 – 3.08 (m, 2H), 3.03 – 2.89 (m, 4H), 2.84 – 2.65 (m, 3H), 1.25 (s, 18H), 1.06 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 176.58, 170.97, 170.83, 170.24, 169.75, 153.84, 132.60, 130.11, 129.84, 128.37, 123.81, 123.64, 77.49, 73.42, 29.00, 27.60. HRMS (ESI$^+$); for $C_{45}H_{64}N_5O_9$ calculated 818.4704, found 818.4686. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 10.50 min.

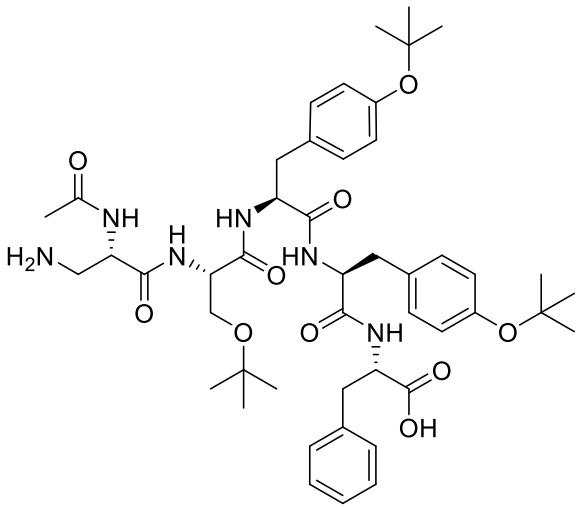
((*S*)-2-((*S*)-2-((*S*)-2-((*S*)-2-acetamido-3-(((benzyloxy)carbonyl)amino)propanamido)-3-(tert-butoxy)propanamido)-3-(4-(tert-butoxy)phenyl)propanamido)-3-(4-(tert-butoxy)phenyl)propanoyl)-L-phenylalanine (13)



Chemical Formula: $C_{55}H_{72}N_6O_{12}$
Molecular Weight: 1009.21

Compound **13** was synthesised using General Method 1, with Fmoc-L-Phe-OH as AA_1 (0.3012 g, 7.76 mmol), Fmoc-L-Tyr(*t*Bu)-OH as AA_2 (0.3573 g, 7.76 mmol), Fmoc-L-Tyr(*t*Bu)-OH as AA_3 (0.3573 g, 7.76 mmol), Fmoc-L-Ser(*t*Bu)-OH as AA_4 (0.2982 g, 7.76 mmol), Fmoc-L-Dap(Z)-OH as AA_5 (0.3581 g, 7.76 mmol). After the final Fmoc deprotection, acetic anhydride (85 μL, 7.76 mmol) and DIPEA (15.5 mmol) was added to the Omnifit column and left for 0.5 h, prior to resin washing. The dried resin was added to a scintillation vial, to which a cleavage cocktail (TFA:TIPS:DCM 1.5:0.5:98) was added and allowed to cleave for 2 h. The cleaved peptide solution was filtered and H_2O (10 mL) was added to the filtrate. The organic solvent was concentrated *in vacuo* and the aqueous suspension was lyophilised to afford compound **13** as a white solid (87%, 170 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 8.26 – 8.23 (m, 1H), 8.05 (d, 1H, *J* = 8.79 Hz), 8.01 (d, 1H, *J* = 8.08 Hz), 7.79 (d, 1H, *J* = 8.09 Hz), 7.75 (d, 1H, *J* = 8.09 Hz), 7.38 – 7.15 (m, 12H), 7.12 (d, 2H, *J* = 8.44 Hz), 7.01 (d, 2H, *J* = 8.08 Hz), 6.82 (d, 2H, *J* = 8.44), 6.76 (d, 2H, *J* = 8.09 Hz), 5.02 (s, 2H), 4.57 – 4.52 (m, 1H), 4.49 – 4.43 (m, 1H), 4.40 – 4.34 (m, 1H), 4.23 – 4.17 (m, 1H), 3.29 – 3.21 (m, 2H), 2.99 – 2.84 (m, 4H), 2.77 – 2.66 (m, 2H), 1,87 (s, 3H), 1.25 – 1.21 (m, 18H), 1.05 – 1.00 (m, 10H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.05, 141.38, 169.61, 153.84, 137.77, 137.51, 132.48, 130.13, 129.58, 128.77, 128.65, 128.11, 126.89, 123.75, 123.63, 77.99, 73.38, 54.05, 53.89, 37.23, 29.00, 27.49, 19.70, 10.11. HRMS (ESI$^+$); for $C_{55}H_{73}N_6O_{12}$ calculated 1009.5286, found 1009.5287. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 14.10 min.

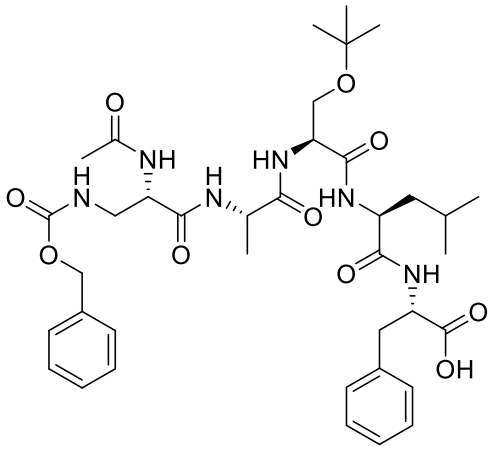
((*S*)-2-((*S*)-2-((*S*)-2-((*S*)-2-acetamido-3-aminopropanamido)-3-(tert-butoxy)propanamido)-3-(4-(tert-butoxy)phenyl)propanamido)-3-(4-(tert-butoxy)phenyl)propanoyl)-L-phenylalanine (14)



Chemical Formula: $C_{47}H_{66}N_6O_{10}$
Molecular Weight: 875.08

A solution of **13** in MeOH (0.5 mL/min) was flowed through the H-cube Pro™ continuous flow reactor under a 10% $Pd/CaCO_3$ catalyst at 40 °C, 1.0 mL/min, 1 atm, 25% H_2 production for 1 cycle. The collected eluent was concentrated *in vacuo* to afford **14** as a white solid (93%, 135 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (d, 1H, J = 7.94 Hz), 8.18 – 8.11 (m, 2H), 8.07 – 8.04 (m, 1H), 7.29 – 7.17 (m, 6H), 7.13 (d, 2H, J = 8.36 Hz), 7.00 (d, 2H, J = 8.36 Hz), 6.84 (d, 2H, J = 8.78 Hz), 6.77 (d, 2H, J = 8.78 Hz), 4.62 – 4.53 (m, 1H), 4.52 – 4.33 (m, 3H), 4.22 (q, 1H, J = 5.93 Hz), 3.13 – 3.05 (m, 2H), 3.00 – 2.89 (m, 3H), 2.85 – 2.66 (m, 3H), 1.90 (s, 3H), 1.25 (s, 18H), 1.06 (s, 9H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.22, 170.78, 170.61, 169.59, 169.54, 153.87, 153.84, 138.11, 132.77, 132.38, 130.14, 129.69, 128.57, 126.76, 123.79, 123.67, 78.02, 73.54, 61.69, 54.54, 53.97, 37.35, 29.00, 27.54, 23.15. HRMS (ESI$^+$); for $C_{47}H_{67}N_6O_{10}$ calculated 875.4919, found 875.4929. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 10.62 min.

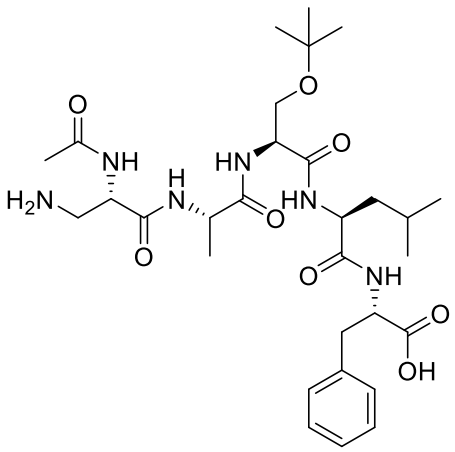
***N*-(((S)-2-acetamido-3-(((benzyloxy)carbonyl)amino)propanoyl)-L-alanyl)-*O*-(tert-butyl)-L-seryl-L-leucyl-L-phenylalanine (15)**



Chemical Formula: $C_{38}H_{54}N_6O_{10}$
Molecular Weight: 754.88

Compound **15** was synthesised using General Method 1, with Fmoc-L-Phe-OH as AA_1 (0.3012 g, 7.76 mmol), Fmoc-L-Leu-OH as AA_2 (0.2748 g, 7.76 mmol), Fmoc-L-Ser(*t*Bu)-OH as AA_3 (0.2982 g, 7.76 mmol) Fmoc-L-Ala-OH as AA_4 (0.2421 g, 7.76 mmol), Fmoc-L-Dap(Z)-OH as AA_5 (0.3581 g, 7.76 mmol). After the final Fmoc deprotection, acetic anhydride (85 μL, 7.76 mmol) and DIPEA (15.5 mmol) was added to the Omnifit column and left for 0.5 h, prior to resin washing. The dried resin was added to a scintillation vial, to which a cleavage cocktail (TFA:TIPS:DCM 1.5:0.5:98) was added and allowed to cleave for 2 h. The cleaved peptide solution was filtered and H_2O (10 mL) was added to the filtrate. The organic solvent was concentrated *in vacuo* and the aqueous suspension was lyophilised to afford compound **15** as a white solid (85%, 122 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 (s, 1H), 8.27 (d, 1H, J = 8.10 Hz), 8.08 (d, 1H, J = 7.43 Hz), 7.97 (d, 1H, J = 8.10 Hz) 7.96 (d, 1H, J = 8.10 Hz), 7.49 (d, 1H, J = 8.10 Hz), 7.39 – 7.29 (m, 5H), 7.26 – 7.18 (m, 5H), 7.14 – 7.13 (m, 1H), 5.02 (s, 2H), 4.44 – 4.39 (m, 1H), 4.38 – 4.29 (m, 3H), 4.27 – 4.22 (m, 1H), 3.48 – 3.44 (m, 2H), 3.30 – 3.20 (m, 2H), 3.06 – 3.01 (m, 1H), 2.92 – 2.84 (m, 1H), 1.96 – 1.90 (m, 1H), 1.84 (s, 3H), 1.21 (d, 3H, J = 7.28 Hz), 1.06 (s, 9H), 1.01 – 0.97 (m, 2H), 0.82 (d, 3H, J = 6.83 Hz), 0.79 (d, 3H, J = 6.83 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.19, 172.58, 171.07, 169.87, 169.64, 156.69, 137.86, 137.56, 129.43, 128.78, 128.64, 128.18, 128.09, 126.88, 73.29, 65.81, 57.32, 53.86, 53.69, 53.21, 48.65, 37.08, 31.74, 27.54, 23.07, 19.70, 19.55, 18.58, 18.31, 18.15, 12.53, 10.11. HRMS (ESI$^+$); for $C_{38}H_{55}N_6O_{10}Na$ calculated 777.3799, found 777.3789. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 9.62 min.

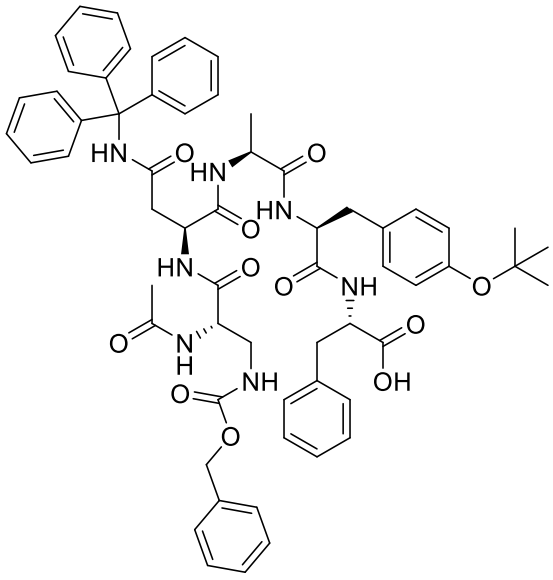
_N_-(((S)-2-acetamido-3-aminopropanoyl)-L-alanyl)-_O_-(tert-butyl)-L-seryl-L-leucyl-L-phenylalanine (16)



Chemical Formula: $C_{30}H_{48}N_6O_8$
Molecular Weight: 620.75

A solution of **15** in MeOH was flowed through the H-cube Pro™ continuous flow reactor under a 10% $Pd/CaCO_3$ catalyst at 40 °C, 1.0 mL/min, 1 atm, 25% H_2 production for 1 cycle. The collected eluent was concentrated *in vacuo* to afford **16** as a white solid (91%, 90.2 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 – 8.42 (m, 1H), 8.31 – 8.25 (m, 1H), 8.17 (d, 1H, *J* = 8.20 Hz), 7.81 (d, 1H, *J* = 8.20 Hz), 7.28 (d, 1H, *J* = 6.75 Hz), 7.20 – 7.08 (m, 5H), 4.32 – 4.21 (m, 3H), 4.13 (q, 1H, *J* = 6.68 Hz), 3.97 (q, 1H, *J* = 5.83 Hz), 3.08 – 3.03 (m, 1H), 2.94 – 2.88 (m, 1H), 2.84 – 2.76 (m, 1H), 2.74 – 2.63 (m, 1H), 2.55 (s, 3H), 1.62 – 1.52 (m, 1H), 1.44 – 1.41 (m, 2H), 1.09 (s, 9H), 0.89 (d, 3H, *J* = 6.60 Hz), 0.81 (d, 2H, *J* = 6.60 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.37, 172.64, 171.34, 170.84, 139.42, 130.08, 129.68, 128.69, 127.99, 126.00, 73.29, 62.00, 55.59, 55.09, 54.20, 54.16, 49.22, 49.05, 44.12, 12.56, 37.66, 27.63, 24.42, 23.52, 22.96, 21.93, 18.06. HRMS (ESI^+); for $C_{30}H_{49}N_6O_8$ calculated 621.3612, found 621.3609. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 7.06 min.

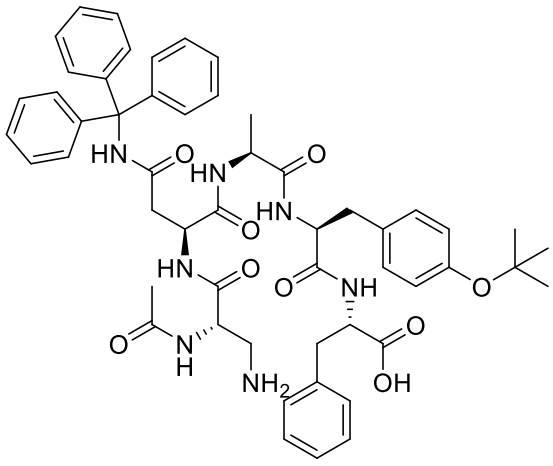
((*S*)-2-((*S*)-2-((*S*)-2-((*S*)-2-acetamido-3-(((benzyloxy)carbonyl)amino)propanamido)-4-oxo-4-(tritylamino)butanamido)propanamido)-3-(4-(tert-butoxy)phenyl)propanoyl)-L-phenylalanine (17)



Chemical Formula: $C_{61}H_{67}N_7O_{11}$
Molecular Weight: 1074.25

Compound **17** was synthesised using General Method 1, with Fmoc-L-Phe-OH as AA_1 (0.3012 g, 7.76 mmol), Fmoc-L-Tyr(*t*Bu)-OH as AA_2 (0.3573 g, 7.76 mmol), Fmoc-L-Ala-OH as AA_3 (0.2421 g, 7.76 mmol), Fmoc-L-Asn(trt)-OH as AA_4 (0.4640 g, 7.76 mmol), Fmoc-L-Dap(Z)-OH as AA_5 (0.3581 g, 7.76 mmol). After the final Fmoc deprotection, acetic anhydride (85 μL, 7.76 mmol) and DIPEA (15.5 mmol) was added to the Omnifit column and left for 0.5 h, prior to resin washing. The dried resin was added to a scintillation vial, to which a cleavage cocktail (TFA:TIPS:DCM 1.5:0.5:98) was added and allowed to cleave for 2 h. The cleaved peptide solution was filtered and H_2O (10 mL) was added to the filtrate. The organic solvent was concentrated *in vacuo* and the aqueous suspension was lyophilised to afford compound **17** as a white solid (89%, 185 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.71 (s, 1H), 8.69 (s, 1H), 8.32 (d, 1H, *J* = 7.78 Hz), 8.06 (d, 1H, *J* = 7.30 Hz), 7.94 (d, 1H, *J* = 7.30 Hz), 7.88 (d, 1H, *J* = 8.76 Hz), 7.82 (d, 1H, *J* = 7.30 Hz), 7.39 – 7.10 (m, 26H), 7.04 (d, 2H, *J* = 8.26 Hz), 6.78 (d, 2H, *J* = 8.76 Hz), 5.02 (s, 2H), 4.55 (q, 1H, *J* = 7.05 Hz), 4.44 – 4.36 (m, 3H), 4.15 (quin, 1H, *J* = 6.87 Hz), 3.31 – 3.23 (m, 2H), 3.07 – 3.01 (m, 2H), 2.93 – 2.86 (m, 2H), 2.68 – 2.64 (m, 2H), 1.89 (s, 3H), 1.22 (s, 9H), 1.07 – 1.04 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.03, 172.03, 171.28, 170.09, 169.75, 156.71, 153.73, 145.11, 137.77, 137.50, 132.84, 130.14, 129.55, 129.02, 128.78, 128.67, 128.15, 127.93, 126.90, 126.80, 123.76, 77.99, 69.88, 28.33, 23.06, 19.71, 12.29. HRMS (ESI^+); for $C_{61}H_{68}N_7O_{11}$ calculated 1074.4977, found 1074.4996. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 13.80 min.

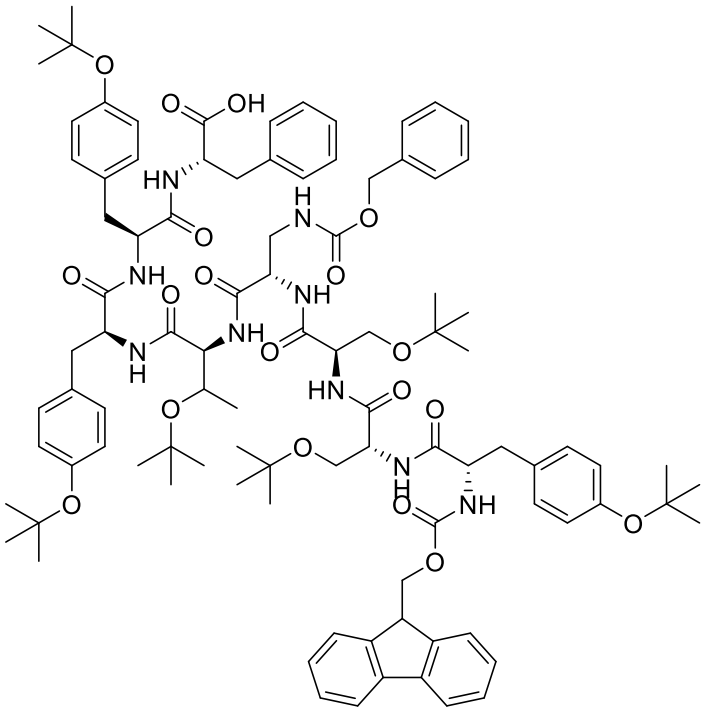
((*S*)-2-((*S*)-2-((*S*)-2-((S)-2-acetamido-3-aminopropanamido)-4-oxo-4-(tritylamino)butanamido)propanamido)-3-(4-(tert-butoxy)phenyl)propanoyl)-L-phenylalanine (18)



Chemical Formula: $C_{53}H_{61}N_7O_9$
Molecular Weight: 940.11

A solution of **17** in MeOH was flowed through the H-cube Pro™ continuous flow reactor under a 10% $Pd/CaCO_3$ catalyst at 40 °C, 1.0 mL/min, 1 atm, 25% H_2 production for 1 cycle. The collected eluent was concentrated *in vacuo* to afford **18** as a white solid (95%, 154 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.77 – 8.72 (m, 1H), 8.24 (d, 1H, J = 7.98 Hz), 8.07 (d, 1H, J = 5.99 Hz), 7.93 (d, 1H, J = 8.49 Hz), 7.66 – 7.60 (m, 1H), 7.27 – 7.14 (m, 20H), 7.01 (d, 2H, J = 8.49 Hz), 6.80 (d, 2H, J = 8.49 Hz), 4.68 – 4.63 (m, 1H), 4.60 – 4.55 (m, 1H), 4.03 – 3.97 (m, 2H), 3.07 – 2.95 (m, 4H), 2.91 – 2.83 (m, 4H), 1.88 (s, 9H), 1.04 (d, 3H, J = 8.04 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.16, 171.15, 170.81, 170.34, 170.07, 169.93, 153.72, 145.08, 133.06, 130.06, 129.71, 129.03, 128.43, 127.98, 126.83, 123.79, 78.03, 69.98, 28.38, 27.58, 23.09, 17.99. HRMS (ESI^+); for $C_{53}H_{62}N_7O_9$ calculated 940.4609, found 940.4611. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 11.27 min.

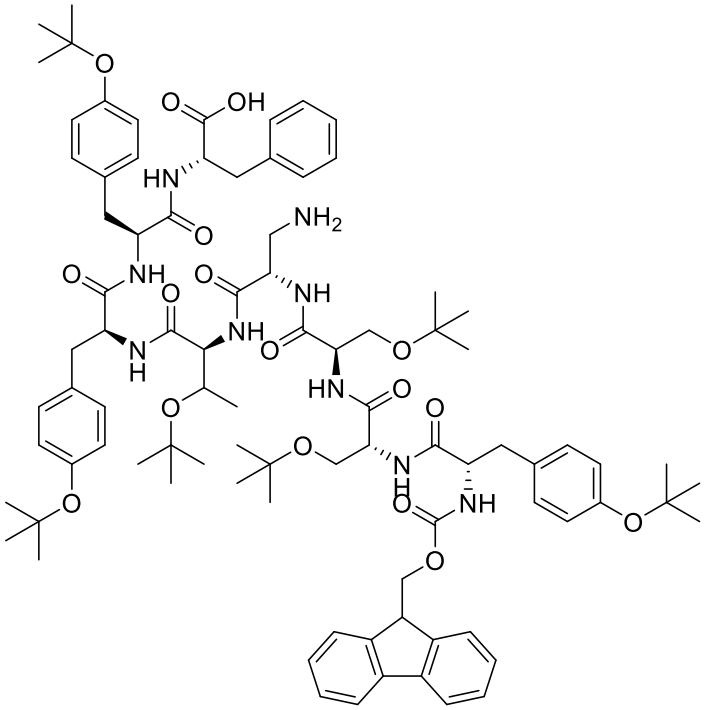
((*S*)-2-((*S*)-2-((*S*)-2-((*S*)-2-((2*S*,3*S*)-2-((*S*)-2-((*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(tert-butoxy)phenyl)propanamido)-3-(tert-butoxy)propanamido)-3-(tert-butoxy)butanamido)-3-(((benzyloxy)carbonyl)amino)propanamido)-3-(tert-butoxy)propanamido)-3-(4-(tert-butoxy)phenyl)propanamido)-3-(4-(tert-butoxy)phenyl)propanoyl)-L-phenylalanine (19)



Chemical Formula: $C_{96}H_{125}N_9O_{19}$
Molecular Weight: 1709.10

Compound **19** was synthesised using General Method 1, with Fmoc-L-Phe-OH as AA_1 (0.3012 g, 7.76 mmol), Fmoc-L-Tyr(*t*Bu)-OH as AA_2 (0.3573 g, 7.76 mmol), Fmoc-L-Tyr(*t*Bu)-OH as AA_3 (0.3573 g, 7.76 mmol), Fmoc-L-Ser(*t*Bu)-OH as AA_4 (0.2982 g, 7.76 mmol), Fmoc-L-Dap(Z)-OH as AA_5 (0.3581 g, 7.76 mmol), Fmoc-L-Thr(*t*Bu)-OH as AA_6 (0.3091 g, 7.76 mmol, Fmoc-L-Ser(*t*Bu)-OH as AA_7 (0.2982 g, 7.76 mmol), and Fmoc-L-Tyr(*t*Bu)-OH as AA_8 (0.3573 g, 7.76 mmol). The dried resin was added to a scintillation vial, to which a cleavage cocktail (TFA:TIPS:DCM 1.5:0.5:98) was added and allowed to cleave for 2 h. The cleaved peptide solution was filtered and H_2O (10 mL) was added to the filtrate. The organic solvent was concentrated *in vacuo* and the aqueous suspension was lyophilised to afford compound **19** as a white solid (85%, 280 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (s, 1H), 8.30 – 8.25 (m, 2H), 8.09 (d, 1H, *J* = 8.38 Hz), 7.94 (d, 1H, *J* = 8.38 Hz), 7.87 (d, 2H, *J* = 8.38 Hz), 7.78 (d, 2H, *J* = 8.38 Hz), 7.69 – 7.67 (m, 2H), 7.59 (d, 1H, *J* = 8.38 Hz), 7.39 (t, 2H, *J* = 7.34), 7.34 – 7.17 (m, 16H), 7.11 (d, 2H, *J* = 7.34 Hz), 7.04 (d, 2H, *J* = 7.34 Hz), 6.84 – 6.77 (m, 4H), 6.44 (d, 2H, *J* = 7.34 Hz), 5.01 (s, 2H), 4.56 – 4.35 (m, 6H), 4.31 – 4.24 (m, 2H), 4.15 – 4.08 (m, 3H), 3.99 – 3.95 (m, 1H), 3.61 – 3.56 (m, 1H), 3.52 – 3.48 (m, 1H), 3.10 – 3.02 (m, 1H), 2.98 – 2.83 (m, 3H), 2.79 – 2.67 (m, 3H), 1.27 – 0.92 (m, 58H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.05, 153.84, 144.18, 141.12, 137.76, 132.59, 130.27, 130.13, 129.56, 128.76, 128.64, 128.08, 127.48, 123.71, 123.66, 120.51, 77.95, 73.52, 73.38, 53.89, 37.21, 28.94, 28.25, 27.57, 27.47, 19.71, 10.11. HRMS (ESI$^+$); for $C_{96}H_{126}N_9O_{19}$ calculated 1708.9170, found 1708.9315. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 22.05 min.

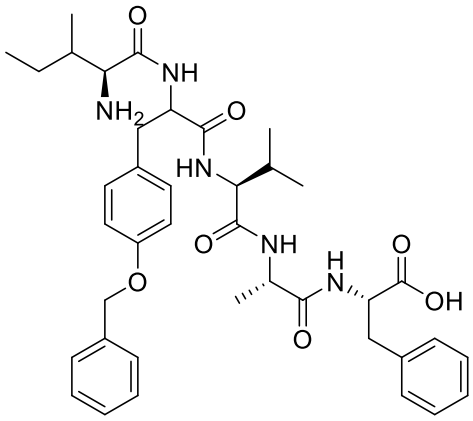
((*S*)-2-((*S*)-2-((*S*)-2-((*S*)-2-((2*S*,3*S*)-2-((*S*)-2-((*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(tert-butoxy)phenyl)propanamido)-3-(tert-butoxy)propanamido)-3-(tert-butoxy)butanamido)-3-aminopropanamido)-3-(tert-butoxy)propanamido)-3-(4-(tert-butoxy)phenyl)propanamido)-3-(4-(tert-butoxy)phenyl)propanoyl)-L-phenylalanine (20)



Chemical Formula: $C_{88}H_{119}N_9O_{17}$
Molecular Weight: 1574.97

A solution of **19** in MeOH was flowed through the H-cube Pro™ continuous flow reactor under a 10% $Pd/CaCO_3$ catalyst at 40 °C, 1.0 mL/min, 1 atm, 25% H_2 production for 1 cycle. The collected eluent was concentrated *in vacuo* to afford **20** as a white solid (85%, 199 mg). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.54 – 8.45 (m, 1H), 8.35 – 8.23 (m, 1H), 8.19 (br s, 1H), 7.89 – 7.84 (m, 2H), 7.72 – 7.62 (m, 3H), 7.52 – 7.41 (m, 3H), 7.33 – 7.07 (m, 16H), 6.90 – 6.74 (m, 6H), 4.47 – 4.29 (m, 6H), 4.20 – 4.09 (m, 4H), 3.98 – 3.92 (m, 2H), 3.19 – 3.10 (m, 2H), 2.81 – 2.71 (m, 6H), 2.23 – 2.18 (m, 2H), 1.27 – 1.08 (m, 58H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.04, 144.18, 141.12, 137.76, 132.59, 130.27, 130.13, 129.56, 128.76, 128.64, 128.08, 127.48, 123.71, 123.66, 120.51, 77.95, 73.52, 73.38, 53.89, 37.21, 28.94, 28.25, 27.57, 27.47, 19.71, 10.11.HRMS (ESI$^+$); for $C_{88}H_{120}N_9O_{17}$ calculated 1574.8802, found 1574.8795. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 15.37 min.

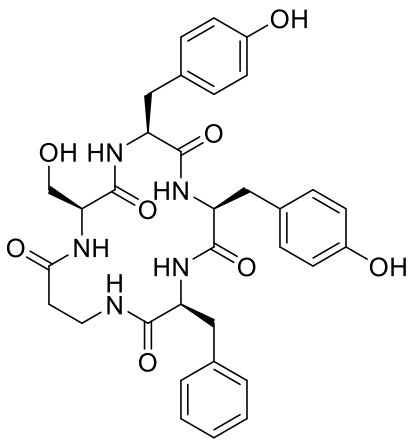
(2-((2*S*)-2-amino-3-methylpentanamido)-3-(4-(benzyloxy)phenyl)propanoyl)-L-valyl-L-alanyl-L-phenylalanine (21)



Chemical Formula: $C_{39}H_{51}N_5O_7$
Molecular Weight: 701.87

Compound **21** was synthesised using General Method 1, with Fmoc-L-Phe-OH as AA_1 (0.3012 g, 7.76 mmol), Fmoc-L-Ala-OH as AA_2 (0.2242 g, 7.76 mmol), Fmoc-L-Val-OH as AA_3 (0.2624 g, 7.76 mmol), Fmoc-L-Tyr(OBzl)-OH as AA_4 (0.3564 g, 7.76 mmol), and Fmoc-L-Ile-OH as AA_5 (0.2675 g, 7.76 mmol). The dried resin was added to a scintillation vial, to which a cleavage cocktail (TFA:TIPS:DCM 1.5:0.5:98) was added and allowed to cleave for 2 h. The cleaved peptide solution was filtered, and H_2O (10 mL) was added to the filtrate. The organic solvent was concentrated *in vacuo* and the aqueous suspension was lyophilised to afford compound **21** as a white solid (78%, 120 mg). ^{1}H NMR (400 MHz, DMSO-d_6) 8.50 (d, 1H, J = 8.61 Hz), 8.15 (d, 1H, J = 7.31 Hz), 8.08 (d, 1H, J = 8.66 Hz), 8.01 – 7.94 (m, 8H), 7.46 – 7.31 (m, 5H), 7.28 – 7.15 (m, 8H), 6.90 (d, 2H, J = 8.66 Hz), 5.05 (s, 2H), 4.71 – 4.63 (m, 1H), 4.48 – 4.40 (m, 1H), 4.24 – 4.17 (m, 1H), 3.07 – 3.01 (m, 2H), 2.95 – 2.89 (m, 3H), 2.78 – 2.66 (m, 2H), 1.97 – 1.90 (m, 1H), 1.84 – 1.78 (m, 1H), 1.45 – 1.39 (m, 1H), 1.19 (d, 2H, J = 7.11 Hz), 0.99 (d, 8H, J = 6.01 Hz), 0.89 (d, 3H, J = 7.38 Hz), 0.84 – 0.78 (m, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.22, 173.11, 172.46, 171.13, 170.61, 168.29, 158.58, 158.26, 157.42, 137.98, 137.76, 137.66, 130.71, 130.11, 129.55, 129.47, 128.90, 128.61, 128.21, 128.08, 126.31, 114.83, 69.56, 57.84, 56.96, 54.62, 53.76, 48.33, 37.12, 37.00, 36.83, 36.26, 31.24, 31.08, 23.81, 22.66, 19.56, 18.74, 18.30, 15.09, 12.52, 11.65. HRMS (ESI$^+$) for $C_{49}H_{59}N_9O_{14}$ *m/z* 702 (M + H, 100%); HRMS (ESI$^+$); calculated 702.3867, found; 702.3867 RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 8.69 min

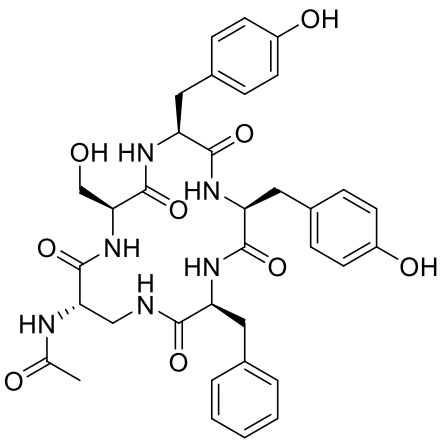
(3*S*,6*S*,9*S*,12*S*)-3-benzyl-6,9-bis(4-hydroxybenzyl)-12-(hydroxymethyl)-1,4,7,10,13-pentaazacyclohexadecane-2,5,8,11,14-pentaone (23)



Chemical Formula: $C_{33}H_{37}N_5O_8$
Molecular Weight: 631.69

Compound **23** was synthesised using general procedure 2 with a 10% DMF:DCM (2 mL) solution containing **12** (100 mg, 0.127 mmol). The sequence was deprotected with a TFA:TIPS:DCM cleavage cocktail (98:1.5:0.5) and evaporated to dryness to afford AIP analogue **23** (77 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (d, 1H, J = 13.22 Hz), 7.89 (d, 1H, J = 7.68 Hz), 7.86 (d, 1H, J = 7.68 Hz), 7.78 (d, 1H, J = 7.23 Hz), 7.40 – 7.36 (m, 1H), 7.27 – 7.22 (m, 2H), 7.20 – 7.14 (m, 3H), 6.92 (d, 2H, J = 8.14 Hz), 6.89 (d, 2H, J = 8.14 Hz), 6.65 (d, 4H, J = 7.68 Hz), 4.21 (q, 1H, J = 7.68 Hz), 4.14 – 4.07 (m, 2H), 4.04 (q, 1H, J = 7.68 Hz), 3.58 – 3.53 (m, 1H), 3.51 – 3.47 (m, 3H), 3.12 – 3.03 (m, 2H), 2.89 (dd, 2H, J = 14.51 Hz, 8.96 Hz), 2.86 – 2.57 (m, 5H), 2.58 – 2.53 (m, 1H), 2.51 – 2.49 (m, 1H), 2.42 – 2.24 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.95, 171.20, 170.74, 170.57, 170.24, 156.34, 156.23, 139.12, 130.49, 129.52, 128.59, 128.13, 127.94, 126.52, 115.49, 115.36, 61.61, 57.29, 56.92, 56.22, 55.77, 36.57, 36.20, 35.59, 34.95. MS (ESI$^+$) for $C_{33}H_{38}N_5O_8$ *m/z* 632 (M + H, 100%), 654 (M + Na, 100%); HRMS (ESI$^+$); calculated 632.2720, found 632.2711; RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 6.81 min.

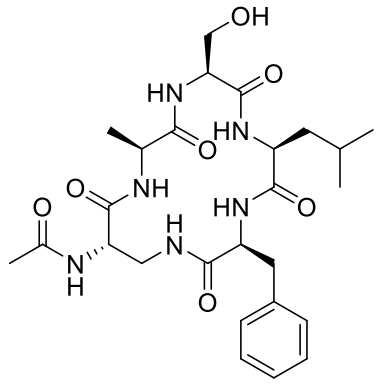
***N*-((3*S*,6*S*,9*S*,12*S*,15*S*)-3-benzyl-6,9-bis(4-hydroxybenzyl)-12-(hydroxymethyl)-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentaazacyclohexadecan-15-yl)acetamide (7)**



Chemical Formula: $C_{35}H_{40}N_6O_9$
Molecular Weight: 688.74

Compound **7** was synthesised using general method 2 with a 10% DMF:DCM (2 mL) solution containing **14** (110 mg, 0.127 mmol). The sequence was deprotected with a TFA:TIPS:DCM cleavage cocktail (98:1.5:0.5) and evaporated to dryness to afford AIP analogue **7** (83 mg, 90%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20 (s, 2H), 8.44 (d, 1H, *J* = 6.37 Hz) 8.04 (d, 1H, *J* = 7.96), 7.95 (d, 1H, *J* = 7.74 Hz), 7.82 – 7.75 (m, 2H), 7.27 – 7.16 (m, 5H), 6.87 (t, 4H, *J* = 7.97 Hz), 6.61 (d, 4H, *J* = 7.97 Hz), 5.15 (t, 1H, *J* = 4.84 Hz), 4.45 – 4.36 (m, 1H), 4.34 – 4.25 (m, 1H), 4.22 – 4.17 (m ,1H), 3.95 – 3.87 (m, 1H), 3.82 (q, 1H, *J* = 6.60 Hz), 3.65 – 3.50 (m, 2H), 3.28 (d, 1H, *J* = 4.85 Hz), 3.19 (d, 2H, *J* = 4.41 Hz), 3.00 – 2.98 (m, 2H), 2.79 – 2.66 (m, 2H), 1.83 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.42, 171.23, 171.21, 171.10, 170.93, 169.47, 156.27, 156.23, 138.93, 130.48, 130.20, 129.59, 128.71, 128.56, 128.15, 126.58, 115.51, 61.77, 57.74, 57.17, 56.03, 55.60, 51.92, 37.50, 35.39, 35.19, 22.85. MS (ESI$^+$) for $C_{35}H_{41}N_6O_9$ *m/z* 689 (M + H, 100%); HRMS (ESI$^+$); calculated 689.2935, found 689.2935; RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 6.84 min.

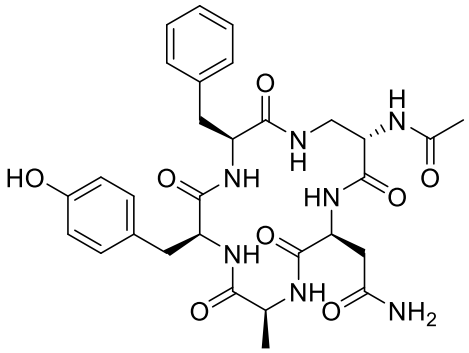
***N*-((3*S*,6*S*,9*S*,12*S*)-3-benzyl-9-(hydroxymethyl)-6-isobutyl-12-methyl-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentaazacyclohexadecan-15-yl)acetamide (24)**



Chemical Formula: $C_{26}H_{38}N_6O_7$
Molecular Weight: 546.63

Compound **24** was synthesised using general procedure 2 with a 10% DMF:DCM (2 mL) solution containing **16** (69.3 mg, 0.127 mmol). The sequence was deprotected with a TFA:TIPS:DCM cleavage cocktail (98:1.5:0.5), and evaporated to dryness to afford AIP analogue **24** (66 mg, 90%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.52 (d, 1H, *J* = 6.54 Hz), 8.12 (d, 1H, *J* = 7.85 Hz), 7.89 – 7.84 (m, 2H), 7.47 (d, 1H, *J* = 8.51 Hz), 7.29 – 7.17 (m, 5H), 7.04 – 7.01 (m, 1H), 4.38 – 4.28 (m, 3H), 3.95 (dd, 1H, *J* = 10.78, 8.04 Hz), 3.80 (q, 1H, *J* = 6.20 Hz), 3.69 – 3.66 (m, 1H), 3.06 – 3.00 (m, 1H), 2.98 – 2.92 (m, 1H), 1.85 (s, 3H), 1.37 – 1.32 (m, 2H), 1.27 (d, 3H, *J* = 7.46 Hz), 0.76 (t, 6H, *J* = 6.99 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 181.21, 178.66, 178.63, 178.45, 177.68, 176.31, 145.91, 136.33, 135.54, 133.56, 66.85, 62.81, 62.34, 60.63, 58.71, 44.24, 31.55, 30.24, 29.75, 28.59, 24.41. MS (ESI$^+$) for $C_{26}H_{39}N_6O_{37}$ *m/z* 547 (M + H, 100%); HRMS (ESI$^+$); calculated 547.2880, found 547.2881; RP-HPLC C18 150 × 4.6 mm, 10-100% B in 45 min, t_R 14.15 min.

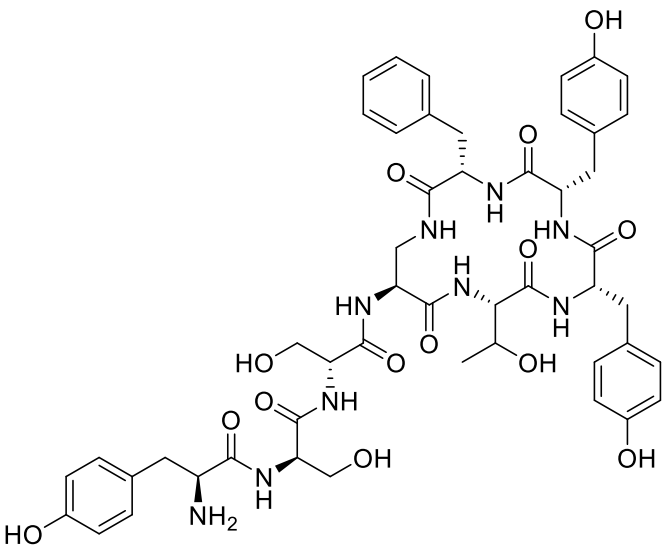
2-((2*S*,5*S*,8*S*,11*S*,15*S*)-15-acetamido-11-benzyl-8-(4-hydroxybenzyl)-5-methyl-3,6,9,12,16-pentaoxo-1,4,7,10,13-pentaazacyclohexadecan-2-yl)acetamide (25)



Chemical Formula: $C_{30}H_{37}N_7O_8$
Molecular Weight: 623.67

Compound **25** was synthesised using general procedure 2 with a 10% DMF:DCM (2 mL) solution containing **18** (69.3 mg, 0.127 mmol). The sequence was deprotected with a TFA:TIPS:DCM cleavage cocktail (98:1.5:0.5), and evaporated to dryness to afford AIP analogue **25** (68 mg, 94%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.24 (d, 1H, J = 4.38 Hz), 8.02 – 7.99 (m, 1H), 7.89 (d, 1H, J = 7.42 Hz), 7.84 (s, 1H), 7.81 (d, 1H, J = 8.43 Hz), 7.61 (d, 1H, J = 8.76 Hz), 7.36 (d, 1H, J = 9.10 Hz), 7.32 – 7.14 (m, 5H), 6.87 (d, 2H, J = 8.42 Hz), 6.60 (d, 2H, J = 8.09 Hz), 4.58 – 4.48 (m, 2H), 4.34 – 4.28 (m, 1H), 4.13 – 4.08 (m, 1H), 3.83 – 3.77 (m, 1H), 3.72 – 3.68 (m, 1H), 3.02 – 2.96 (m, 2H), 2.83 – 2.77 (m, 2H), 2.72 (d, 1H, J = 4.05 Hz), 2.67 (d, 1H, J = 4.05 Hz), 2.30 – 2.47 (m, 1H), 1.86 (s, 3H), 1.09 (d, 3H, J = 7.71 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 173.13, 172.08, 171.73, 170.99, 169.31, 156.13, 138.81, 129.99, 129.92, 128.50, 126.46, 115.34, 56.38, 55.02, 52.37, 52.06, 49.59, 36.46, 35.53, 22.79, 16.71. MS (ESI$^+$) for $C_{30}H_{38}N_7O_8$ *m/z* 646 (M + Na, 100%); HRMS (ESI$^+$); calculated 624.2782, found 624.2780; RP-HPLC C18 150 × 4.6 mm, 10-100% B in 45 min, t_R 14.15 min.

(2*S*,3*S*)-2-((*S*)-2-((*S*)-2-amino-3-(4-hydroxyphenyl)propanamido)-3-hydroxypropanamido)-*N*-((3*S*,6*S*,9*S*,12*S*,15*S*)-3-benzyl-6,9-bis(4-hydroxybenzyl)-12-(hydroxymethyl)-2,5,8,11,14-pentaoxo-1,4,7,10,13-pentaazacyclohexadecan-15-yl)-3-hydroxybutanamide (26)



Chemical Formula: $C_{49}H_{59}N_9O_{14}$
Molecular Weight: 998.06

Compound **26** was synthesised using general method 2 with a 10% DMF:DCM (2 mL) solution containing **20** (199 mg, 0.127 mmol). The sequence was deprotected with a TFA:TIPS:DCM cleavage cocktail (98:1.5:0.5), and Fmoc deprotected with a 50% Piperidine in DMF solution, prior to purification by RP-HPLC to afford AIP analogue **26** (105 mg, 80%). ^{1}H NMR (300 MHz, DMSO-d_6) 9.20 (br s, 1H), 9.07 (br s, 1H), 8.57 (d, 1H, J = 9.56 Hz), 8.35 (d, 1H, J = 6.15 Hz), 7.94 (d, 1H, J = 7.52 Hz), 7.80 – 7.72 (m, 5H), 7.45 (br s, 1H), 7.30 – 7.08 (d, 2H, J = 7.52 Hz), 6.92 (d, 2H, J = 7.52 Hz), 6.87 (d, 2H, J = 7.52 Hz), 6.70 (d, 2H, J = 7.52 Hz), 6.67 – 6.63 (m, 3H), 5.06 (br s, 1H), 4.77 (br s, 1H), 4.54 – 4.46 (m, 2H), 4.39 – 4.34 (m, 1H), 4.27 – 4.19 (m, 2H), 4.12 (br s, 1H), 4.03 (q, 1H, J = 4.89 Hz), 3.98 – 3.94 (m, 1H), 3.87 (q, 1H), 3.73 – 3.64 (m, 4H), 3.67 – 3.58 (m, 1H), 3.09 (d, 1H, J = 7.34 Hz), 3.04 (d, 1H, J = 5.30 Hz), 2.98 (dd, 1H, J = 13.66, 9.50 Hz), 2.88 (d, 2H, J = 3.67 Hz), 2.85 – 2.79 (m, 2H), 2.76 – 2.70 (m, 1H), 1.08 (d, 3H, J = 7.42 Hz). ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.50, 171.39, 171.32, 171.25, 170.27, 169.89, 168.77, 156.98, 156.28, 156.24, 138.85, 131.03, 130.54, 130.20, 129.65, 128.77, 128.62, 128.08, 126.67, 125.23, 115.78, 115.49, 115.46, 65.16, 61.70, 58.55, 57.41, 55.93, 55.61, 55.29, 55.18, 54.05, 54.01, 20.33. HRMS (ESI$^+$) for $C_{49}H_{59}N_9O_{14}$ *m/z* 998 (M + H, 100%); HRMS (ESI$^+$); calculated 998.4260, found; 998.4222 RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 6.67 min.

4. Spectral and Chromatographic Data for Compounds 7-26

Spectral and Chromatographic Data for Compound 8

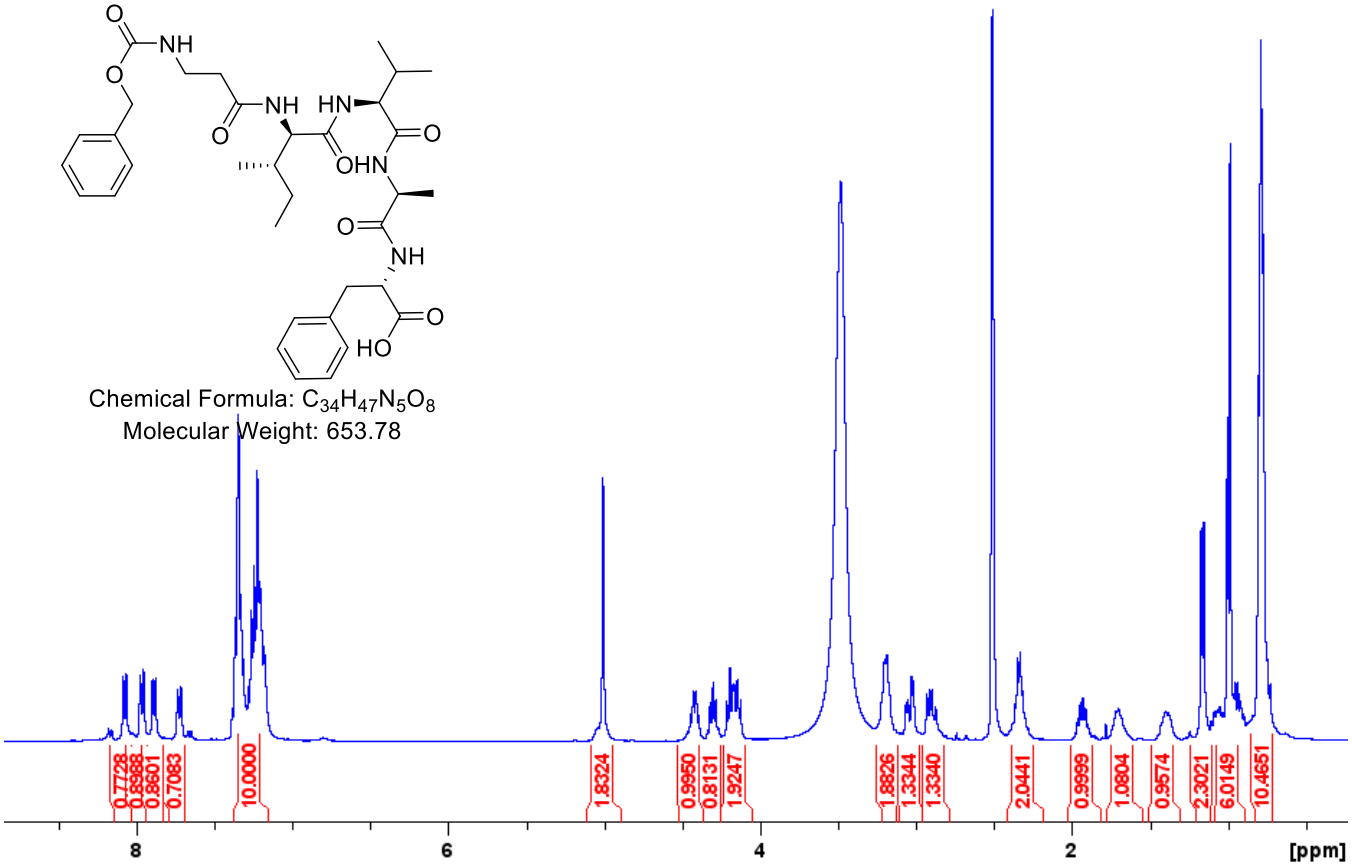


Figure S. 1. ^{1}H NMR spectrum for compound **8** in DMSO-d_6.

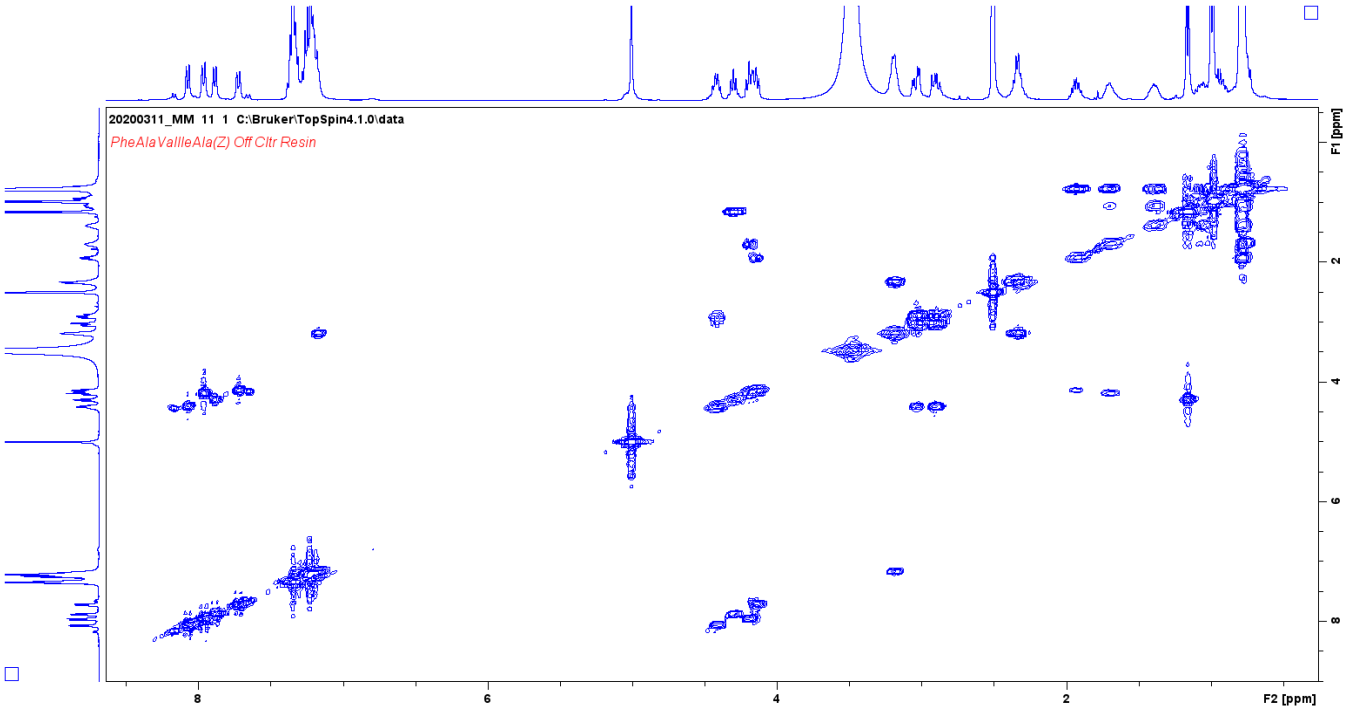


Figure S. 2. COSY NMR spectrum of compound **8** in DMSO-d_6.

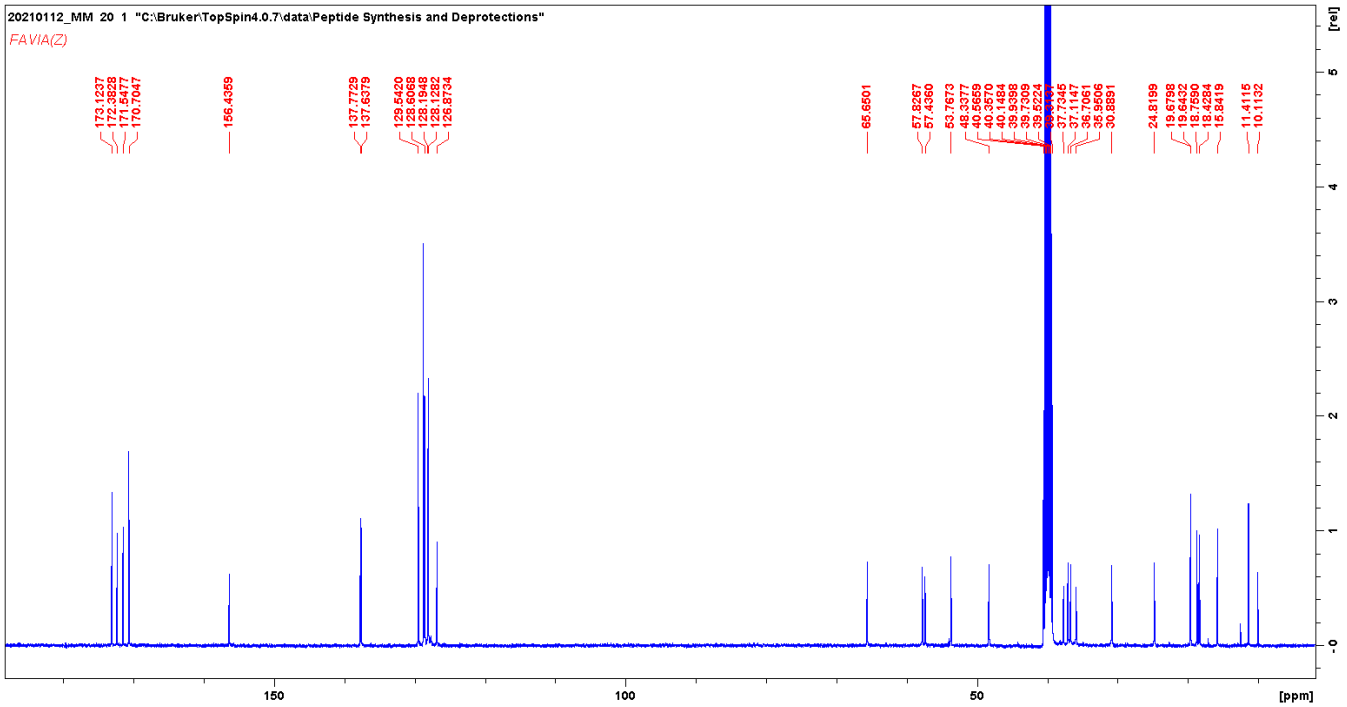


Figure S. 3. ^{13}C NMR spectrum of compound **8** in DMSO.

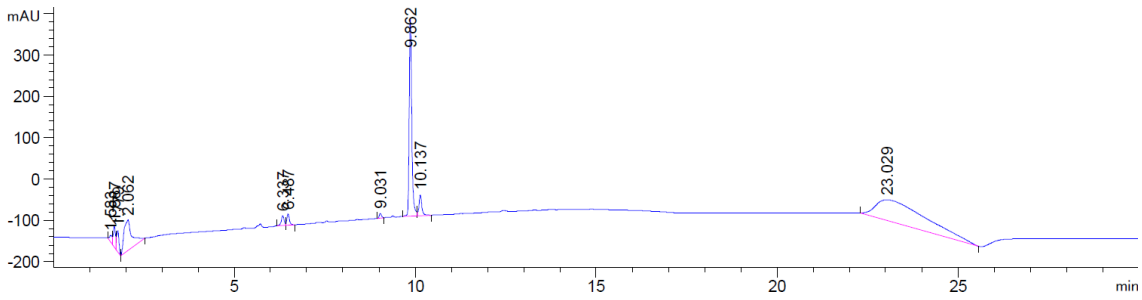


Figure S. 4. RP-HPLC chromatogram for compound **8** at 214 nm.

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Monoisotopic Mass, Even Electron Ions
4 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 34-38 H: 47-48 N: 3-6 O: 6-9 Na: 0-1

Mass	Calc. Mass	mDa	PPM	DBE	Formula	C	H	N	O	Na
676.3295	676.3322	-2.7	-4.0	13.5	C34 H47 N5 O8 Na	34	47	5	8	1

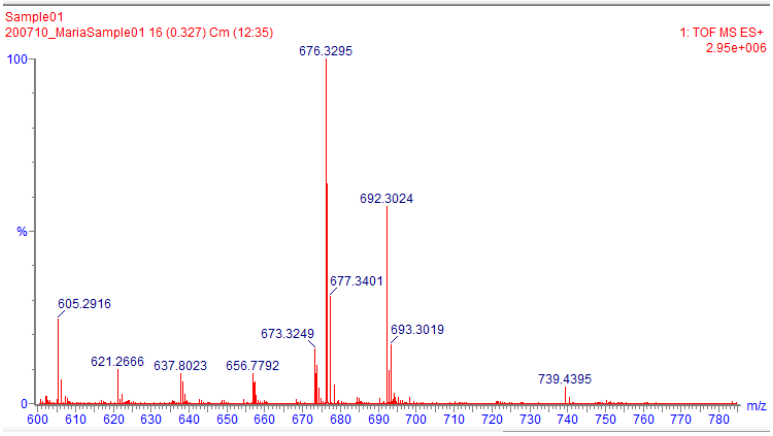


Figure S. 5. HRMS (ESI+) spectrum for compound **8**.

Spectral and Chromatographic Data for Compound 9

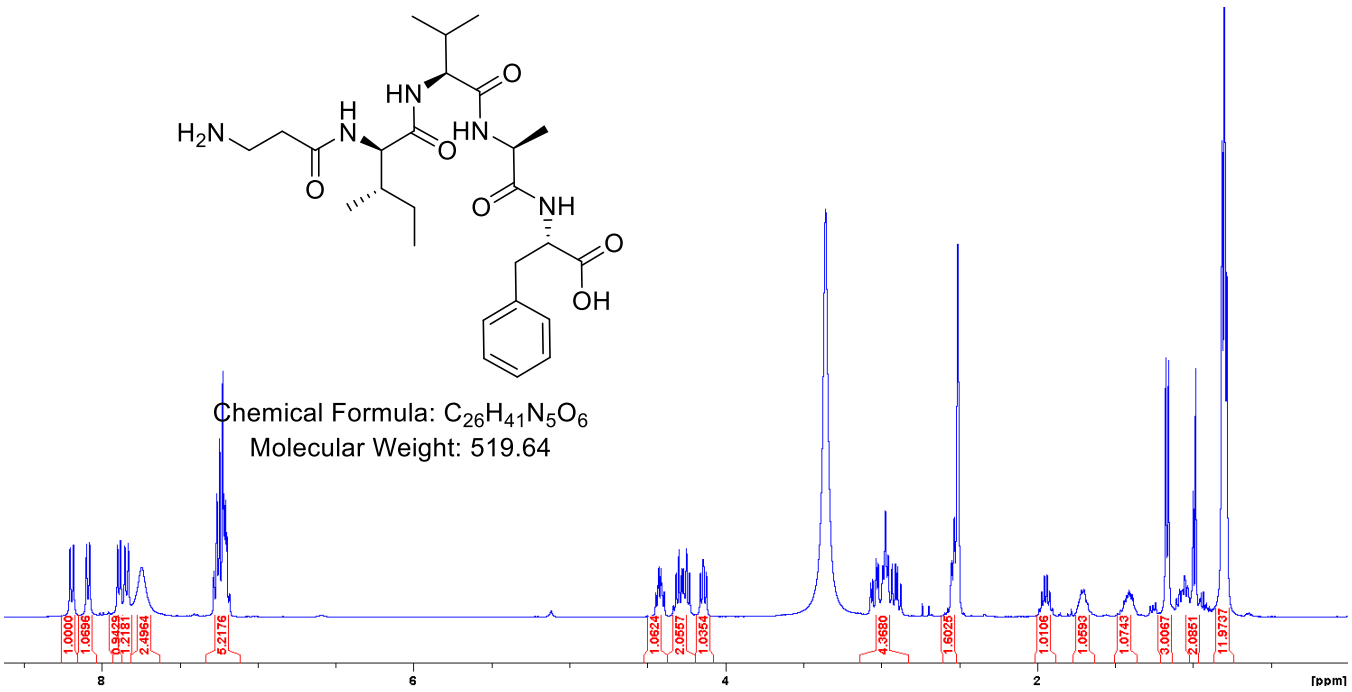


Figure S. 6. ^{1}H NMR spectrum for compound **9** in DMSO-d_6.

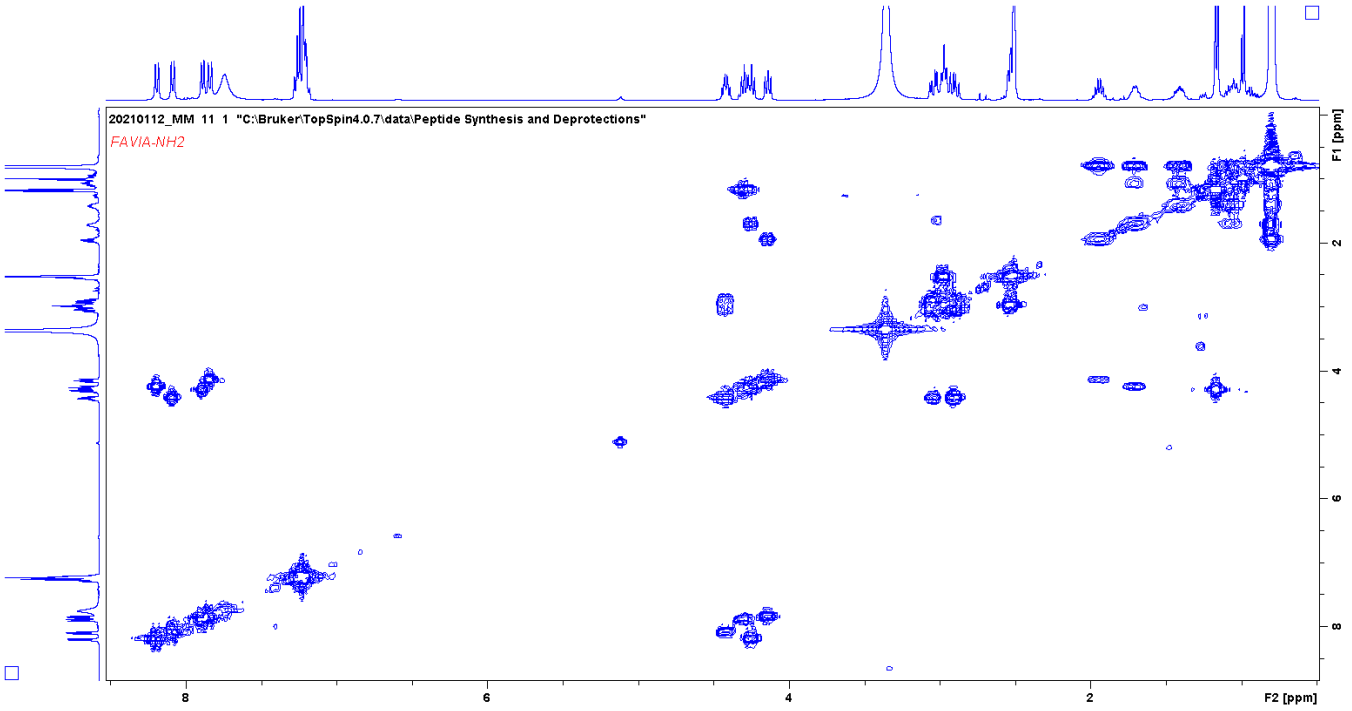


Figure S. 7. COSY NMR spectrum of compound **9** in DMSO-d_6.

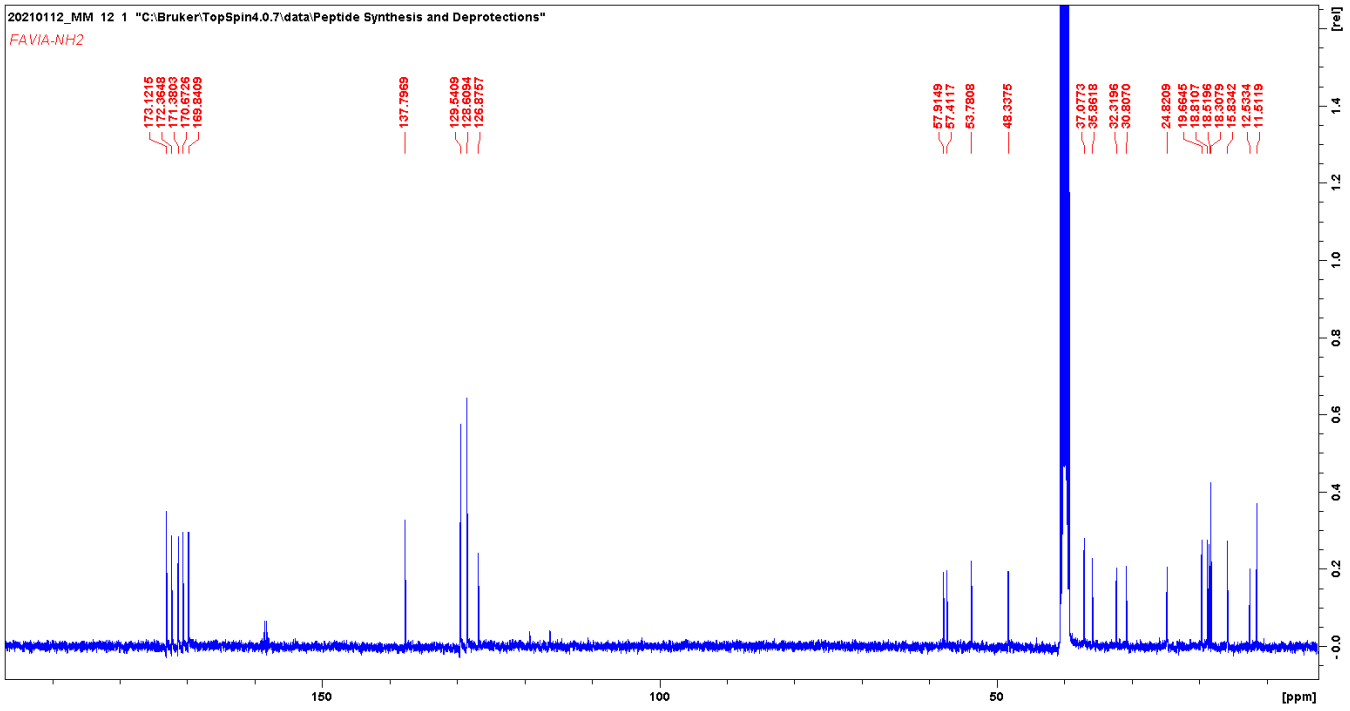


Figure S. 8. ^{13}C NMR spectrum of compound **9** in DMSO.

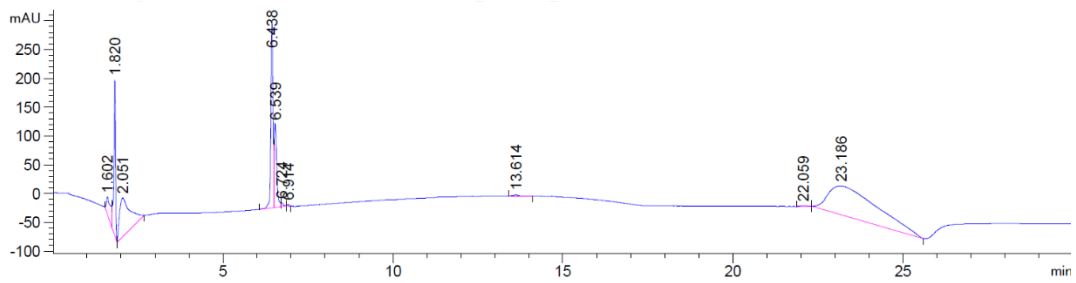


Figure S. 9. RP-HPLC chromatogram for compound **9** at 214 nm.

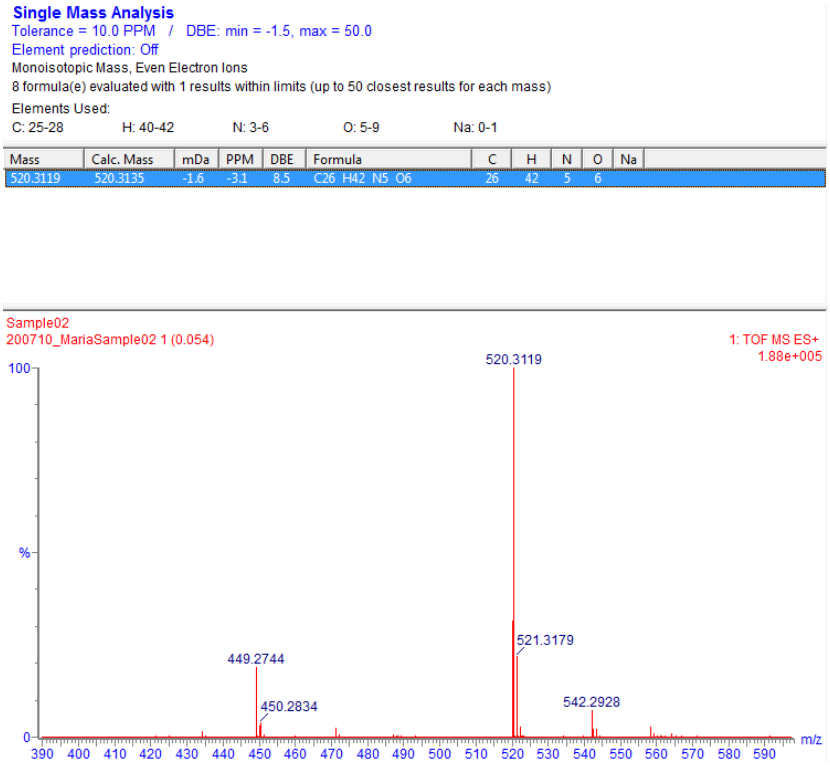


Figure S. 10. HRMS (ESI+) spectrum for compound **9**.

Spectral and Chromatographic Data for Compound 10

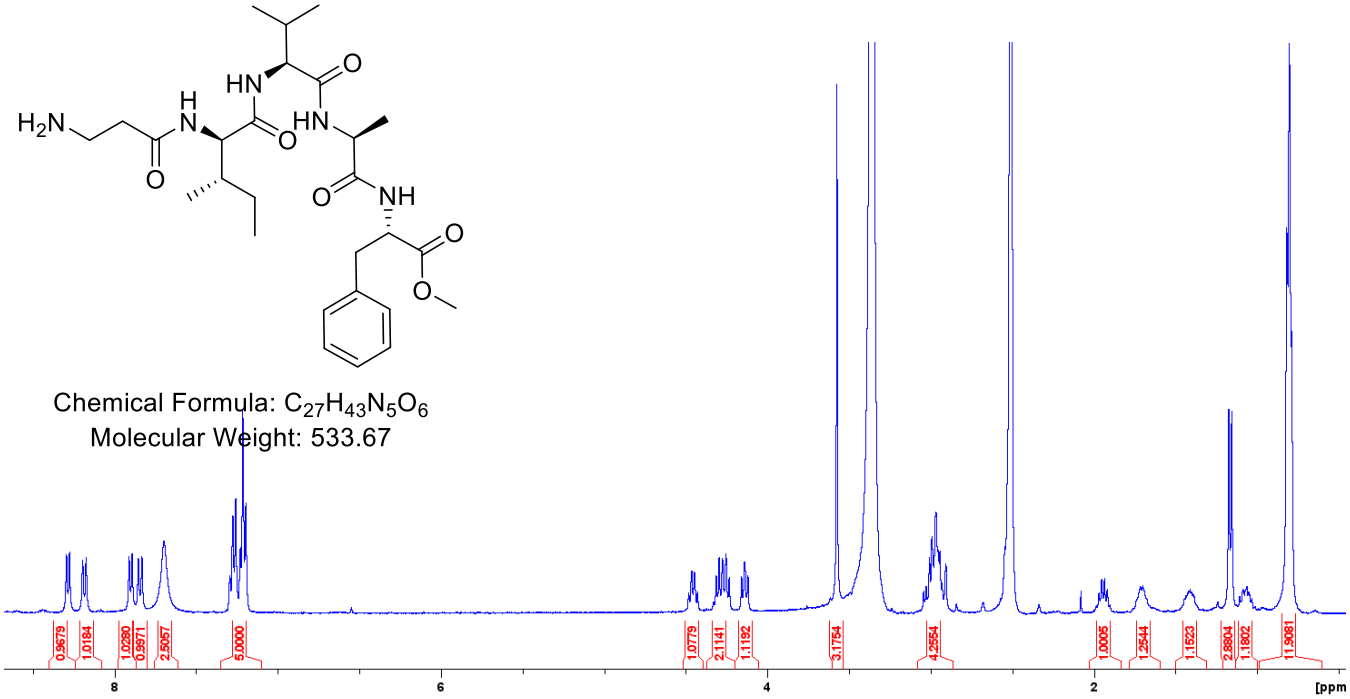


Figure S. 11. ^{1}H NMR spectrum for compound **10** in DMSO-d_6.

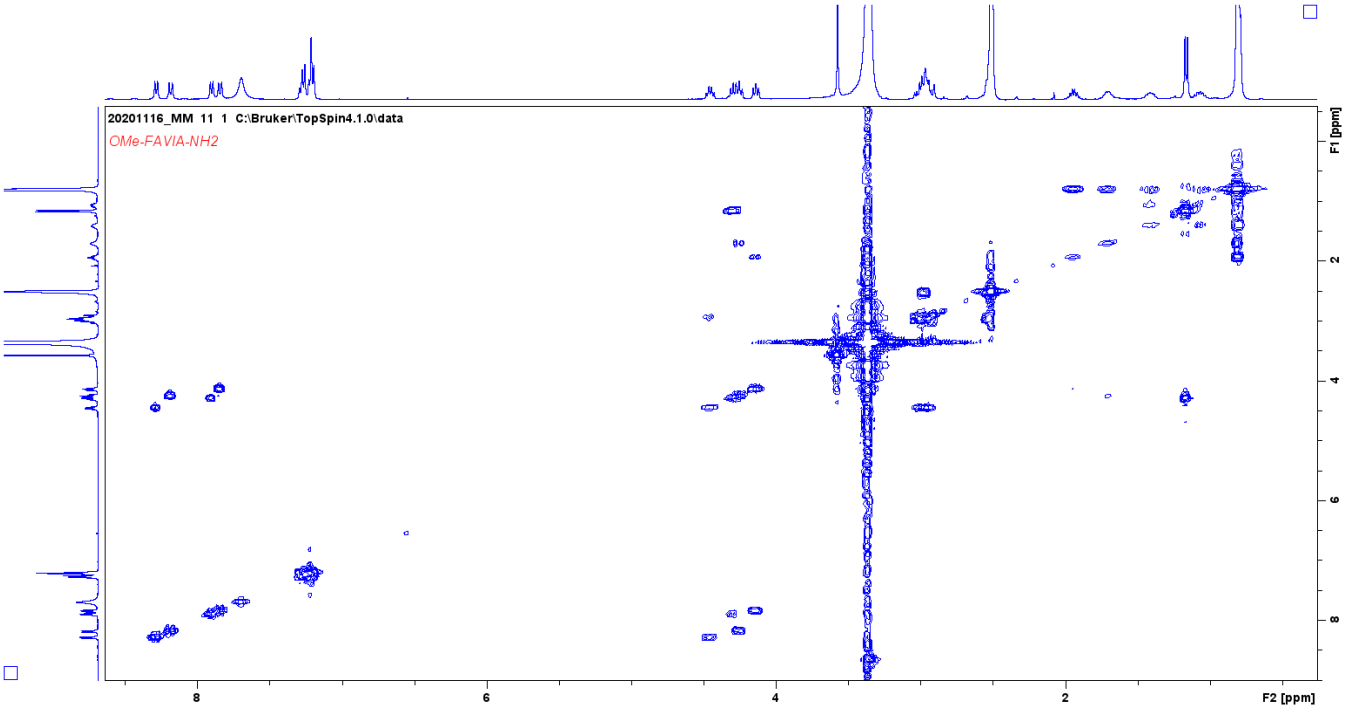


Figure S. 12. COSY NMR spectrum of compound **10** in DMSO-d_6.

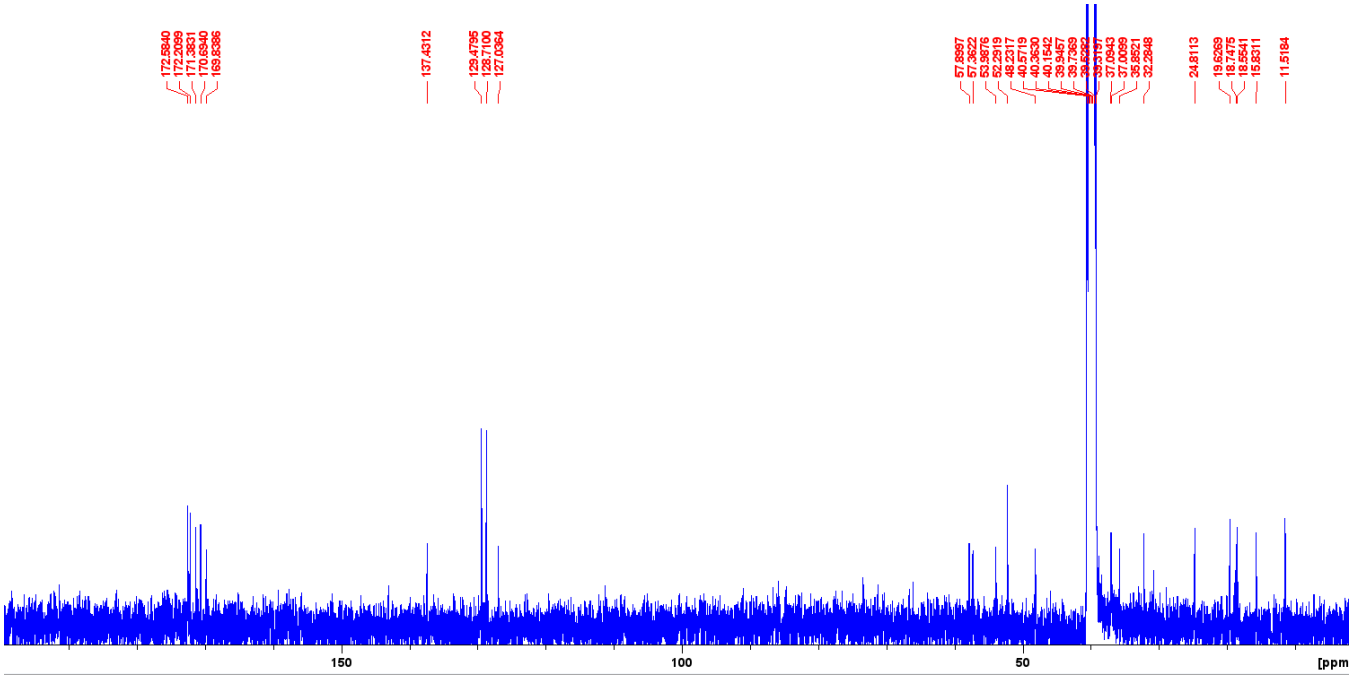


Figure S. 13. ^{13}C NMR spectrum of compound **10** in DMSO.

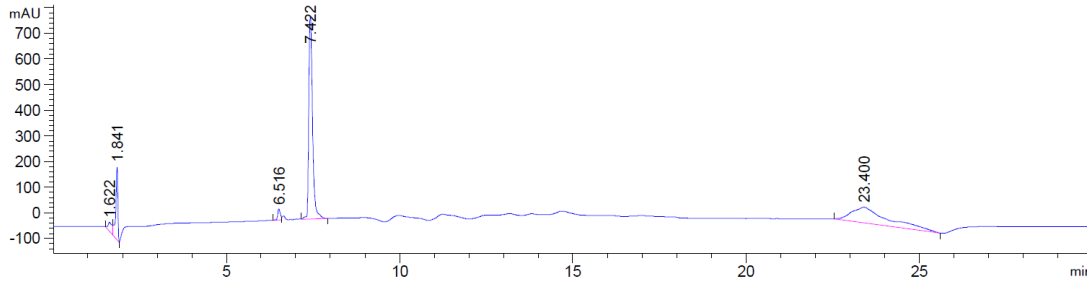


Figure S. 14. RP-HPLC chromatogram for compound **10** at 214 nm.

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Monoisotopic Mass, Even Electron Ions
9 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 26-28 H: 40-44 N: 3-6 O: 5-9 Na: 0-1

Mass	Calc. Mass	mDa	PPM	DBE	Formula	C	H	N	O	Na
534.3333	534.3292	4.1	7.7	8.5	C27 H44 N5 O6	27	44	5	6	

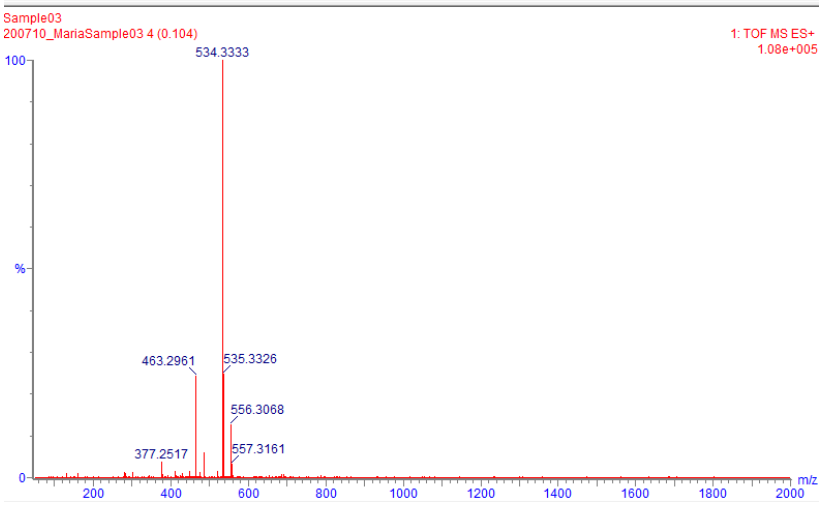


Figure S. 15. HRMS (ESI+) spectrum for compound **10**.

Spectral and Chromatographic Data for Compound 11

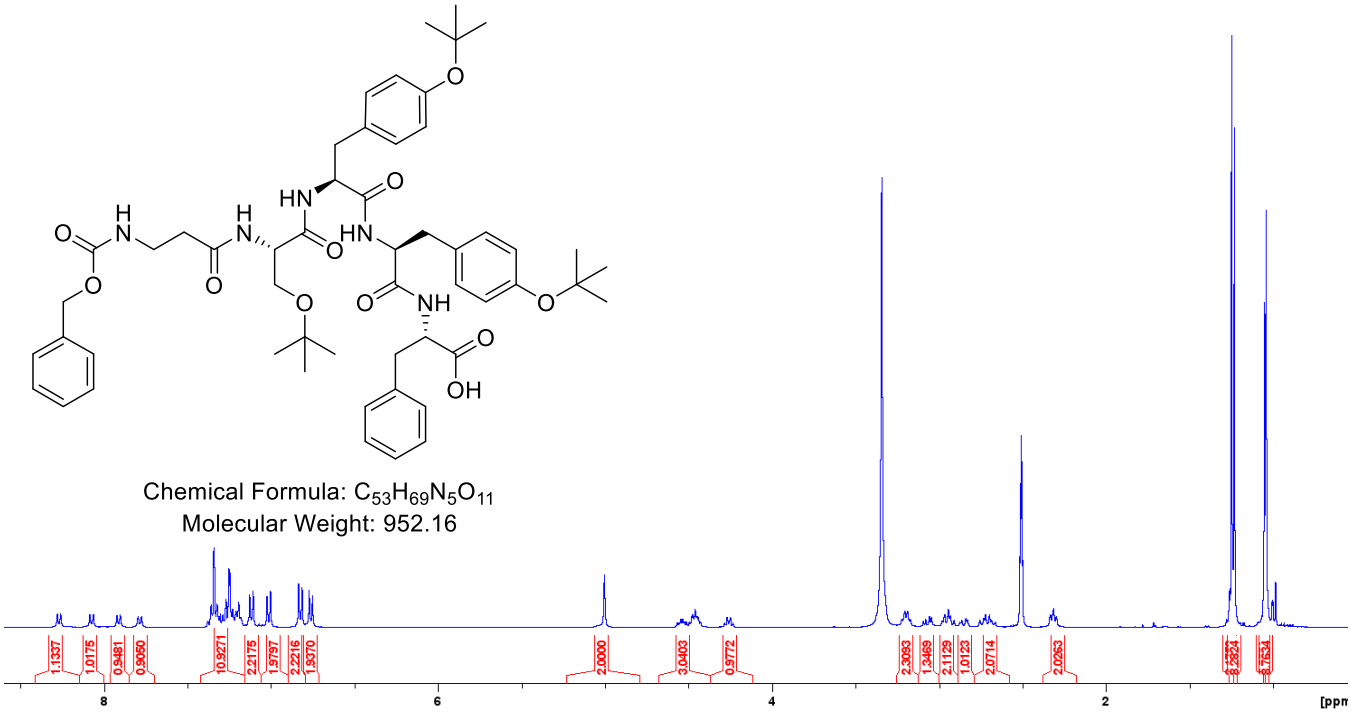


Figure S. 16. ^{1}H NMR spectrum for compound **11** in DMSO-d$_6$.

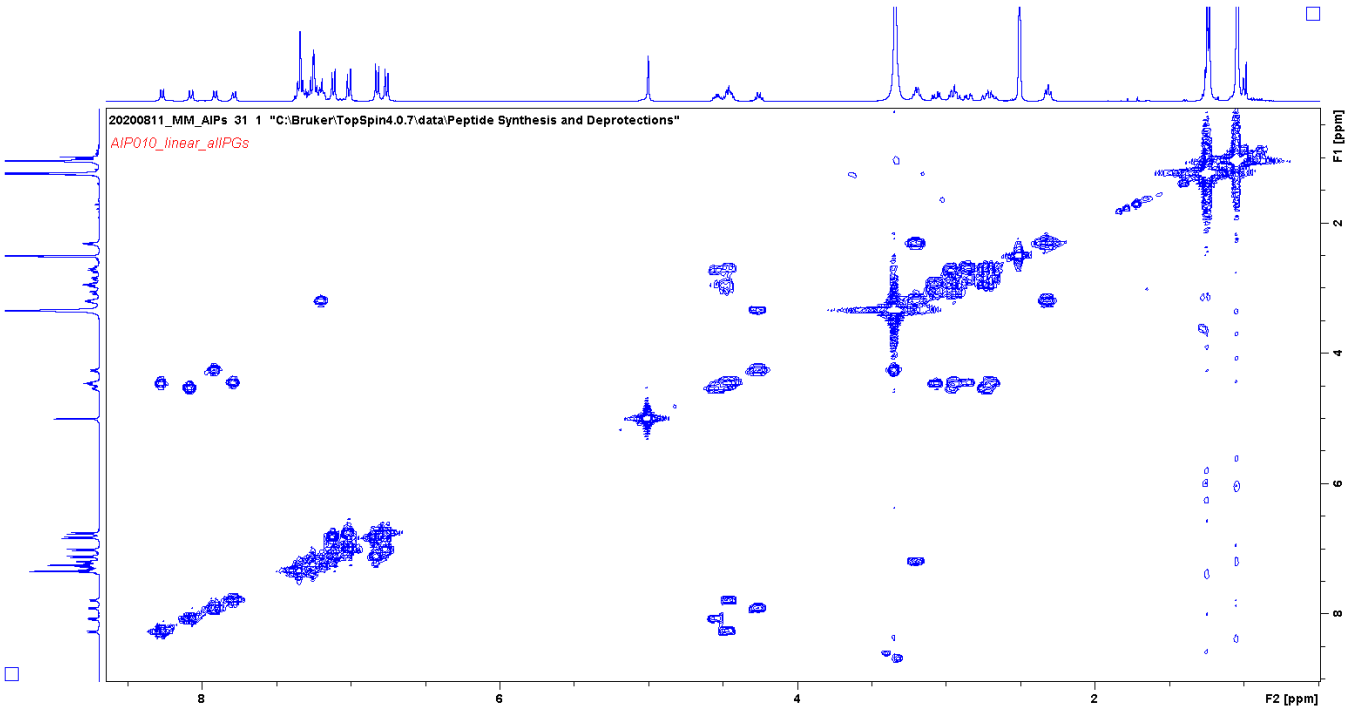


Figure S. 17. COSY NMR spectrum of compound **11** in DMSO-d$_6$.

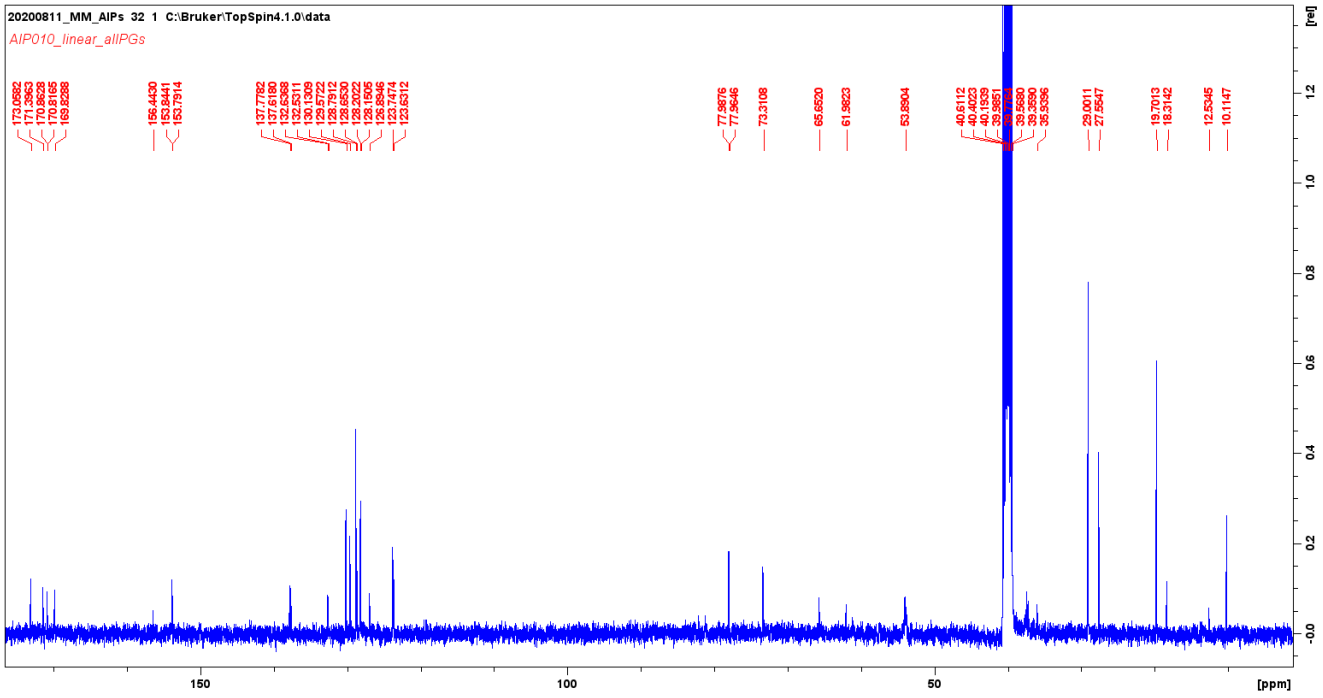


Figure S. 18. ^{13}C NMR spectrum of compound **11** in DMSO.

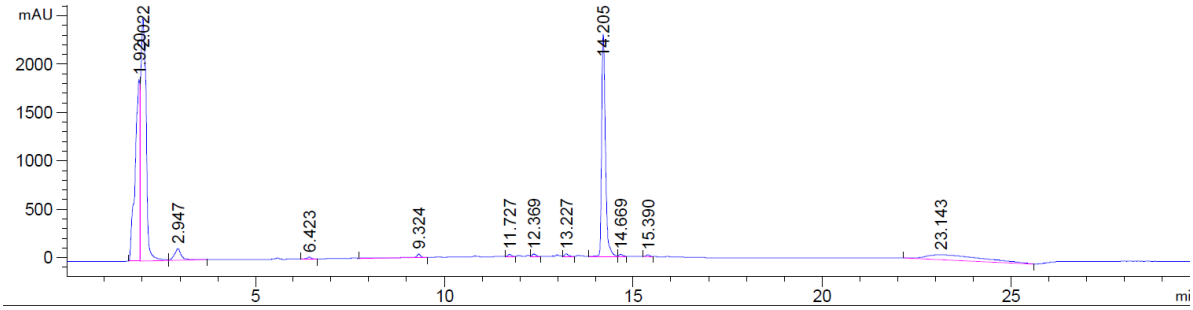


Figure S. 19. RP-HPLC chromatogram for compound **11** at 214 nm.

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
15 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Na
952.5110	952.5072	3.8	4.0	21.5	C53 H70 N5 O11	330.2	0.472	62.35	53	70	5	11	
	952.5184	-7.4	-7.8	21.5	C52 H70 N7 O10	330.7	0.977	37.65	52	70	7	10	

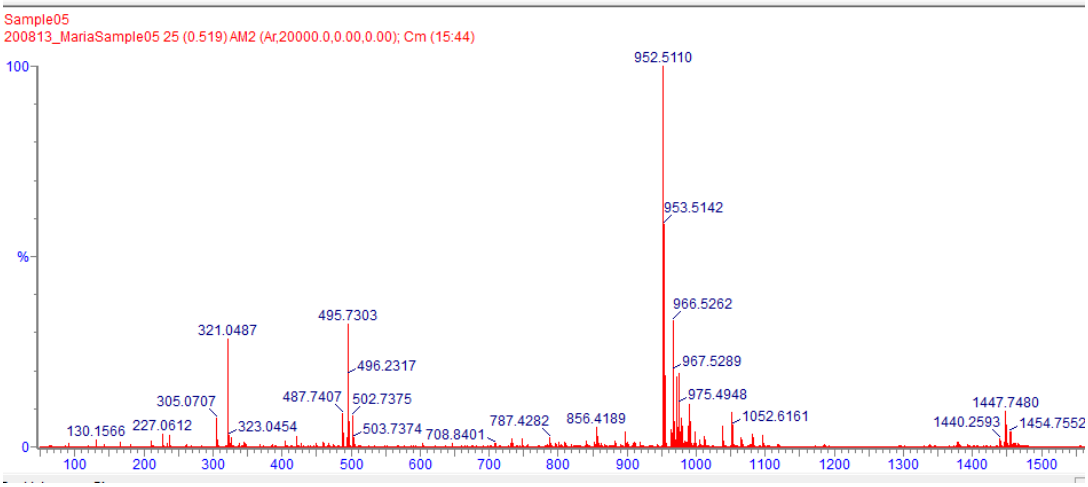


Figure S. 20. HRMS (ESI+) spectrum for compound **11**.

Spectral and Chromatographic Data for Compound 12

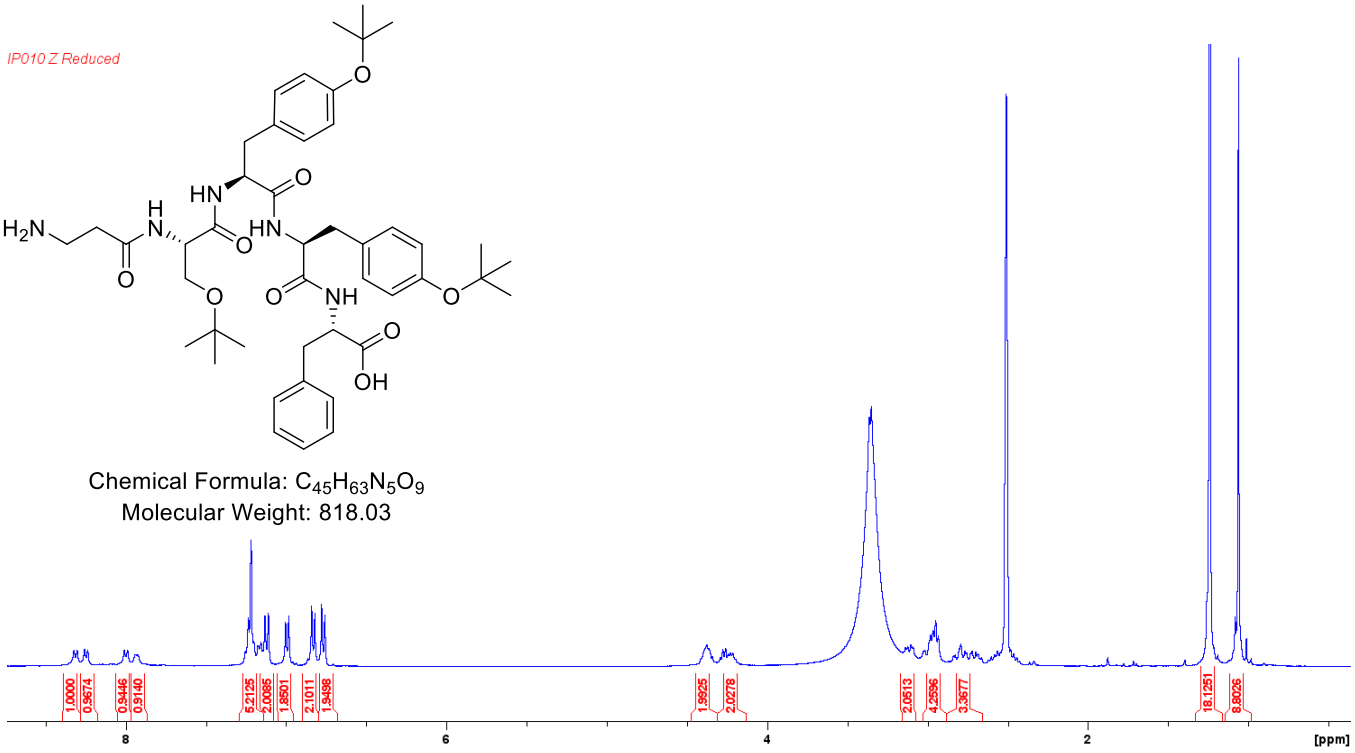


Figure S. 21. ^{1}H NMR spectrum for compound **12** in DMSO-d_6.

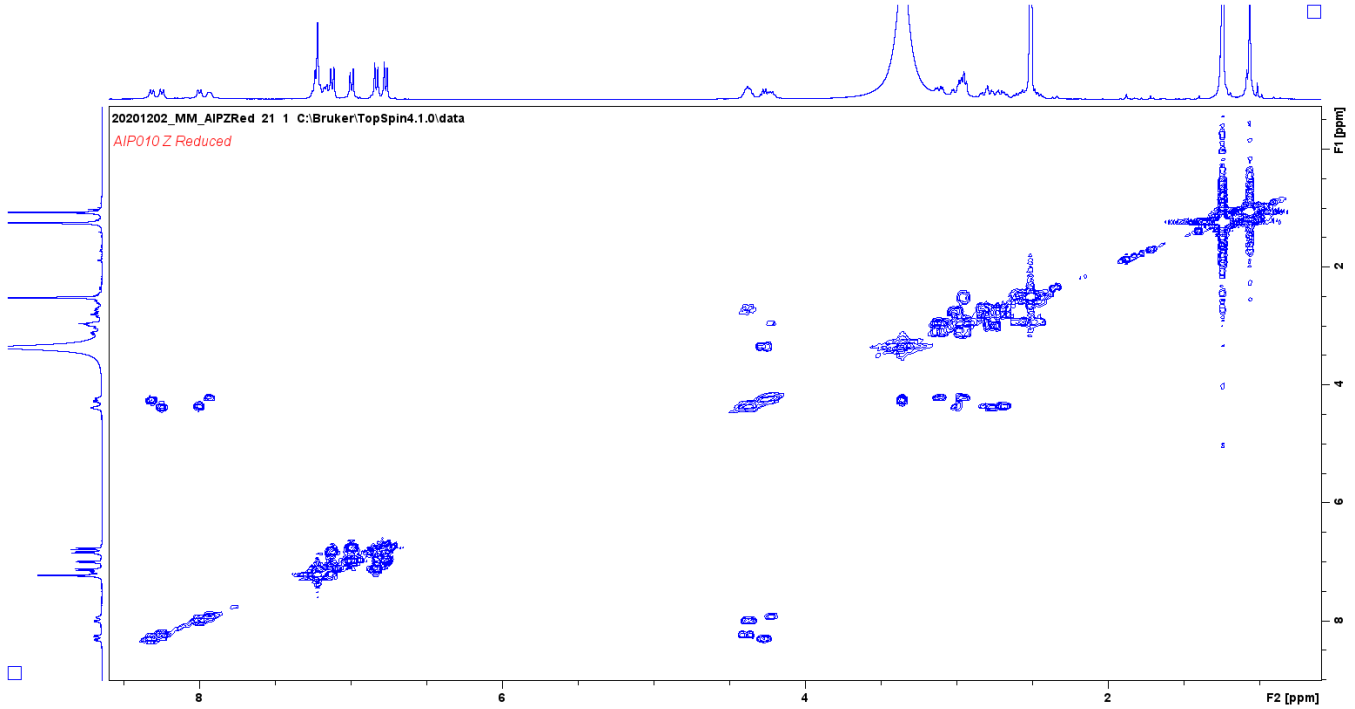


Figure S. 22. COSY NMR spectrum of compound **12** in DMSO-d_6.

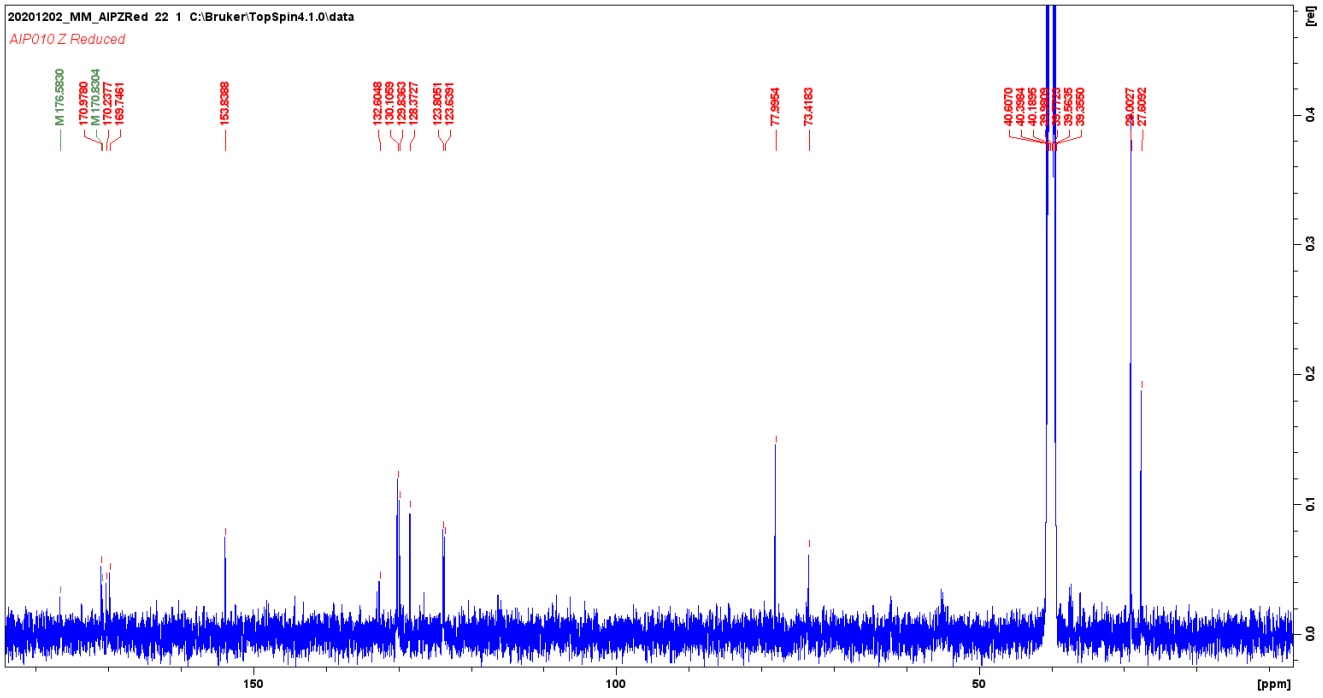


Figure S. 23. ^{13}C NMR spectrum of compound **12** in DMSO.

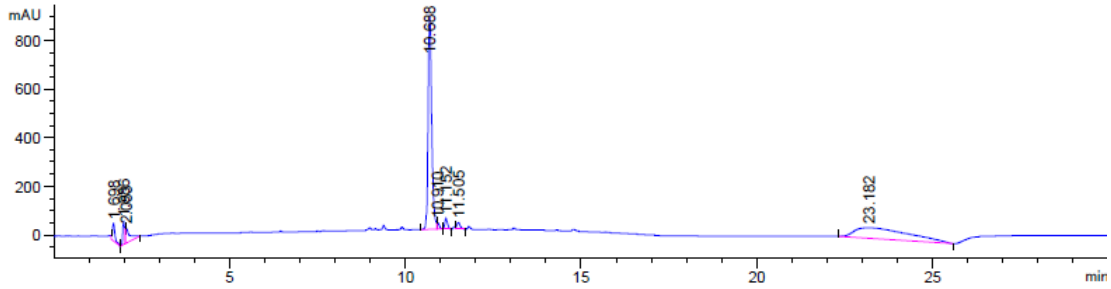


Figure S. 24. RP-HPLC chromatogram for compound **12** at 214 nm.

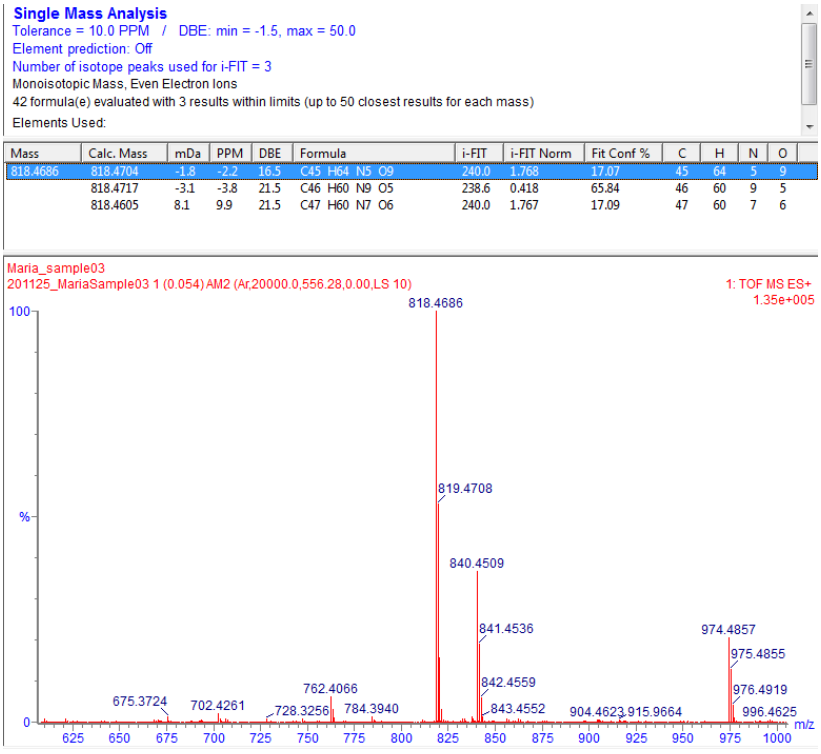


Figure S. 25. HRMS (ESI+) spectrum for compound **12**.

Spectral and Chromatographic Data for Compound 13

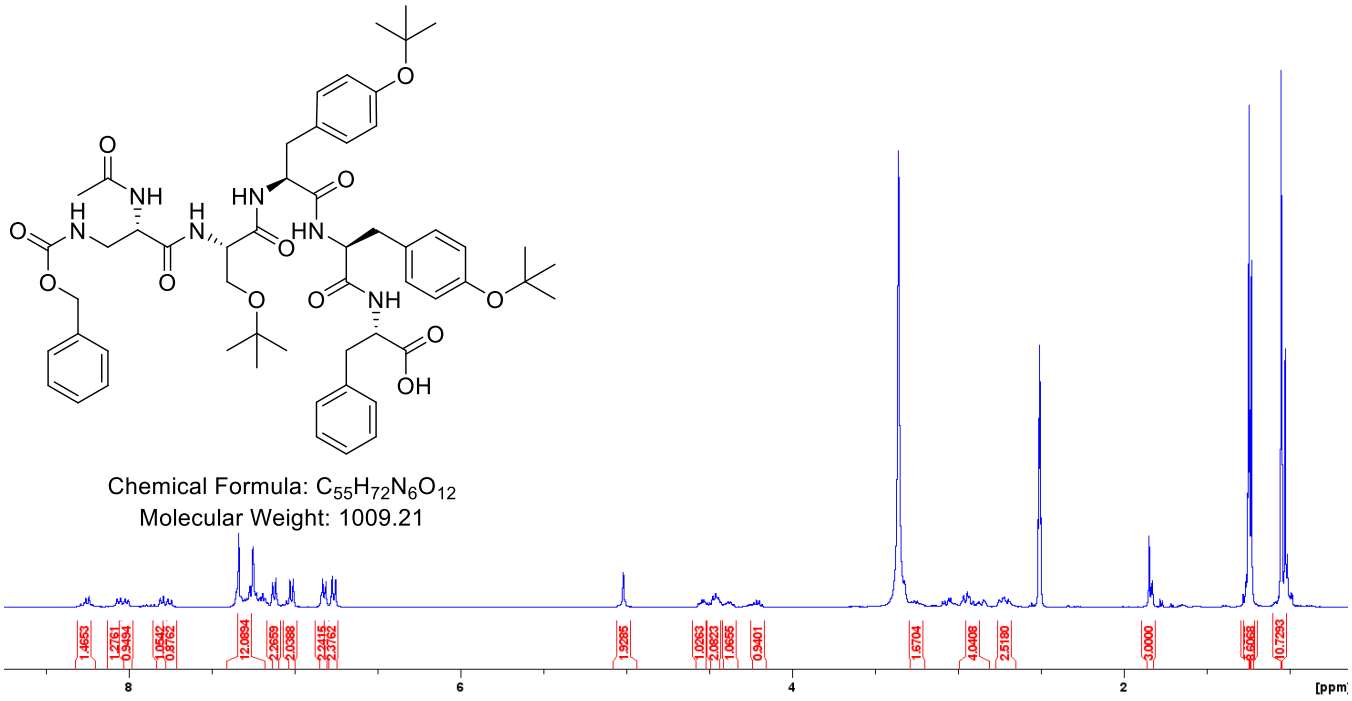


Figure S. 26. ^{1}H NMR spectrum for compound **13** in DMSO-d$_6$.

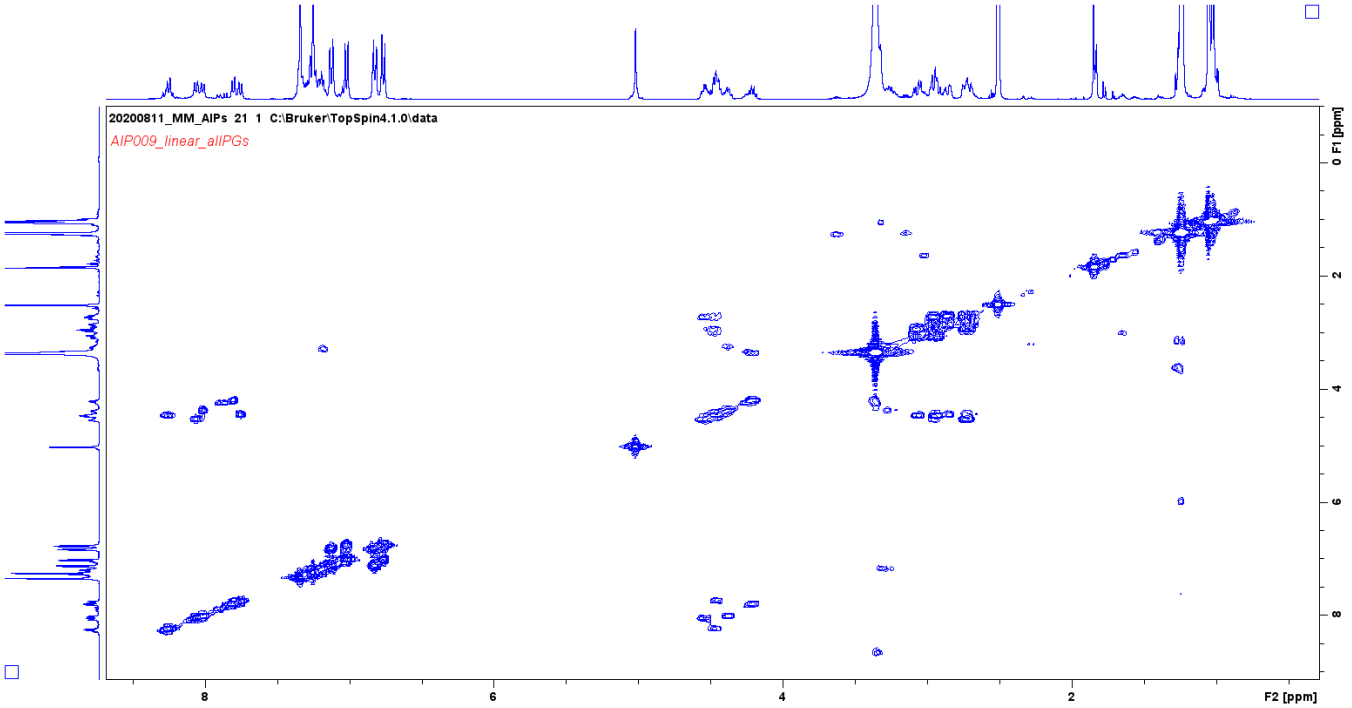


Figure S. 27. COSY NMR spectrum of compound **13** in DMSO-d$_6$.

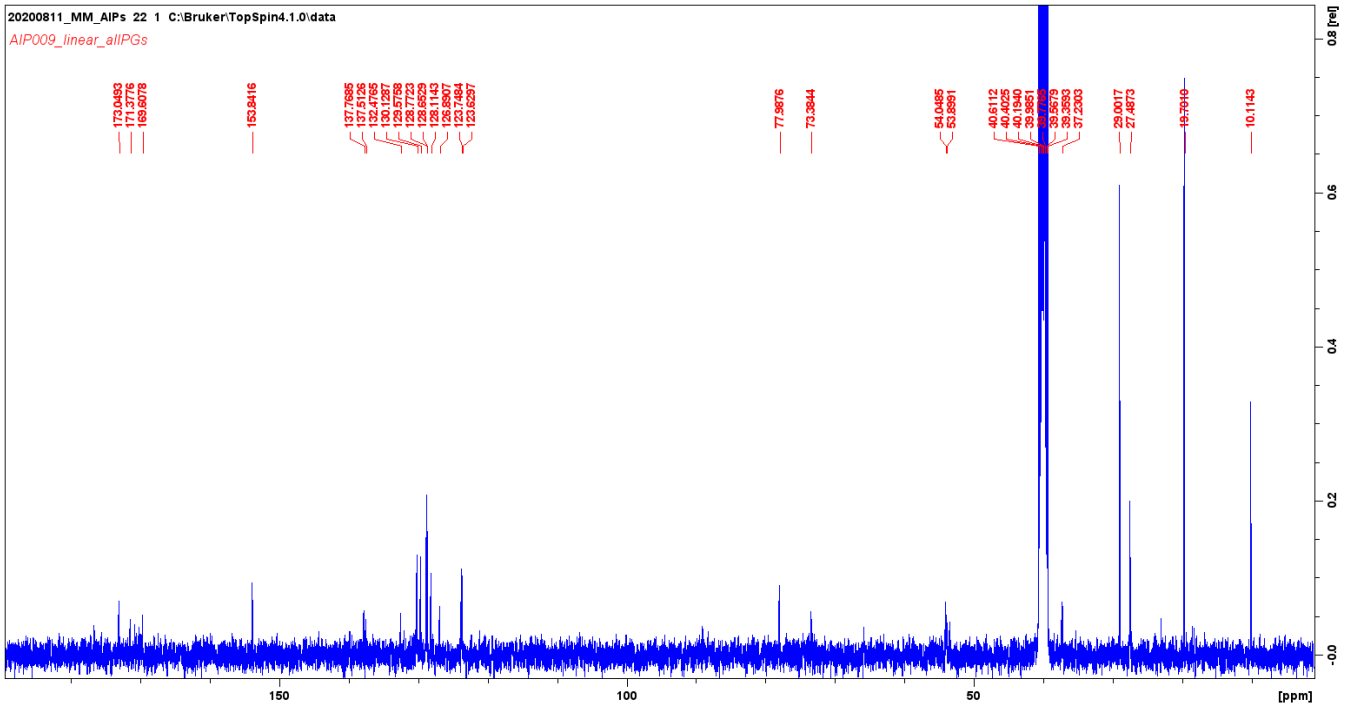


Figure S. 28. ^{13}C NMR spectrum of compound **13** in DMSO.

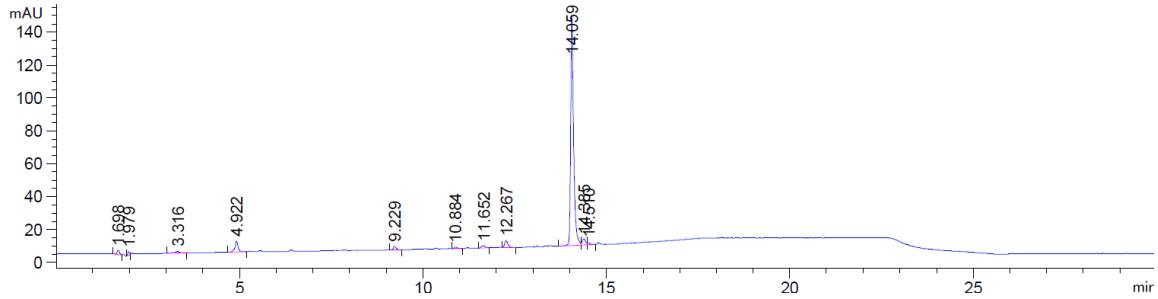


Figure S. 29. RP-HPLC chromatogram for compound **13** at 214 nm.

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Monoisotopic Mass, Even Electron Ions
3 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 55-55 H: 70-75 N: 6-7 O: 10-15 Na: 0-1

Mass	Calc. Mass	mDa	PPM	DBE	Formula	C	H	N	O	Na
1009.5287	1009.5286	0.1	0.1	22.5	C55 H73 N6 O12	55	73	6	12	

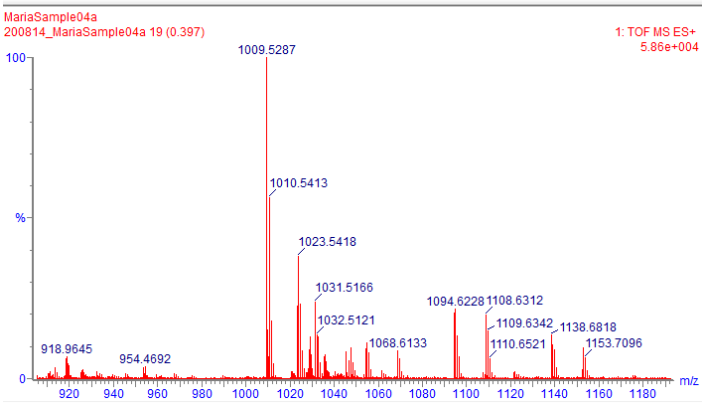


Figure S. 30. HRMS (ESI+) spectrum for compound **13**.

Spectral and Chromatographic Data for Compound 14

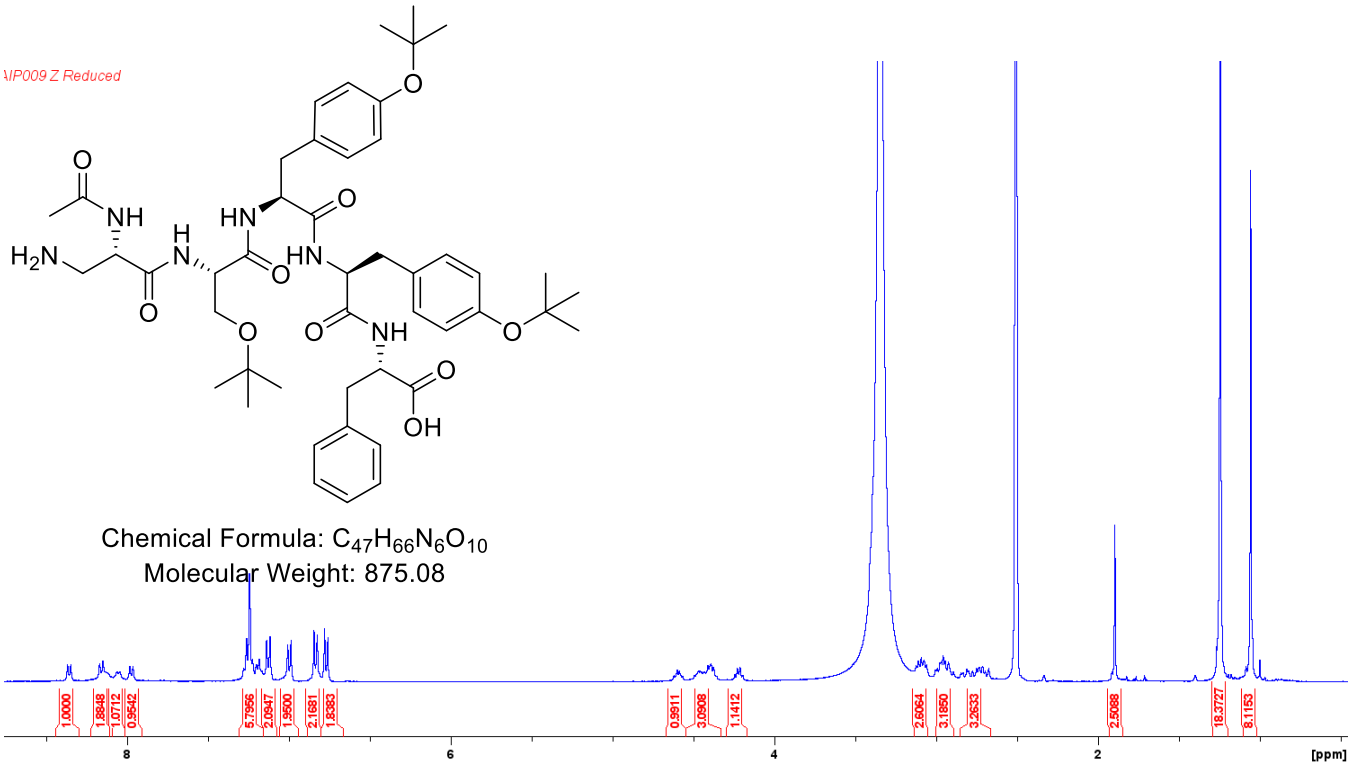


Figure S. 31. ^{1}H NMR spectrum for compound **14** in DMSO-d_6.

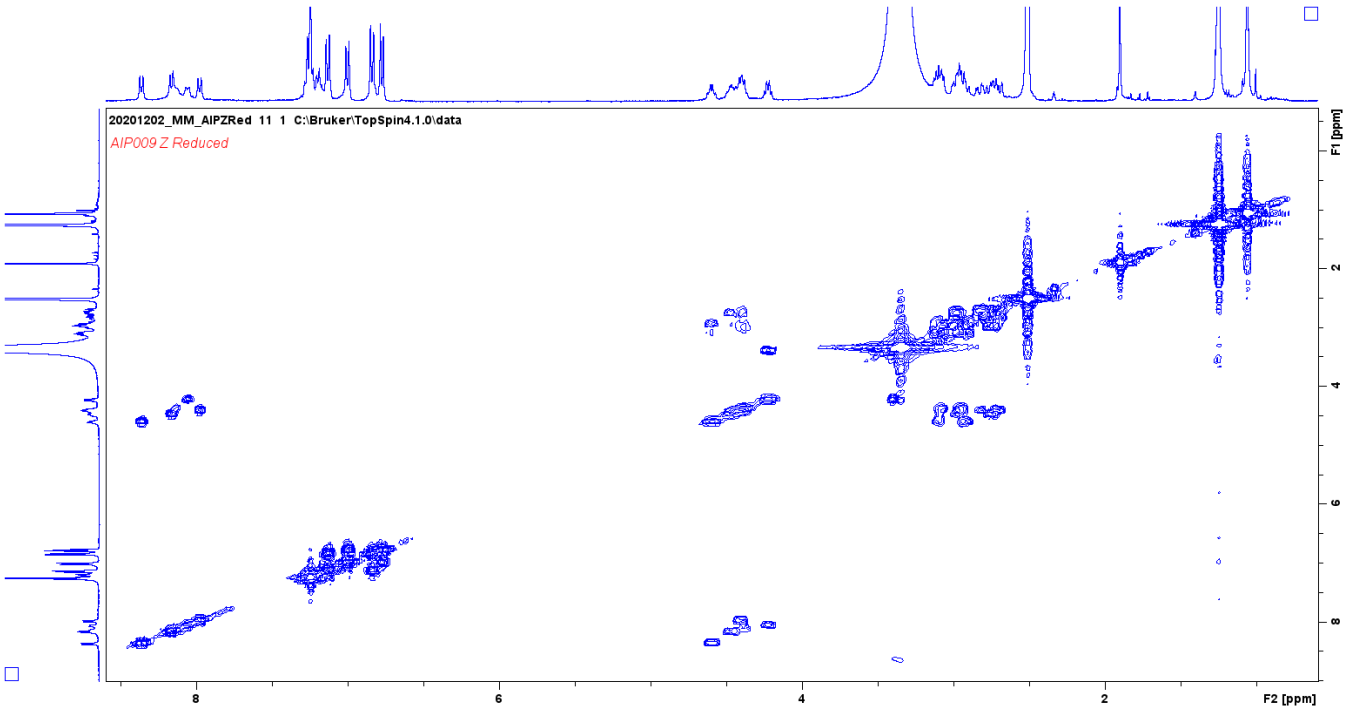


Figure S. 32. COSY NMR spectrum of compound **14** in DMSO-d_6.

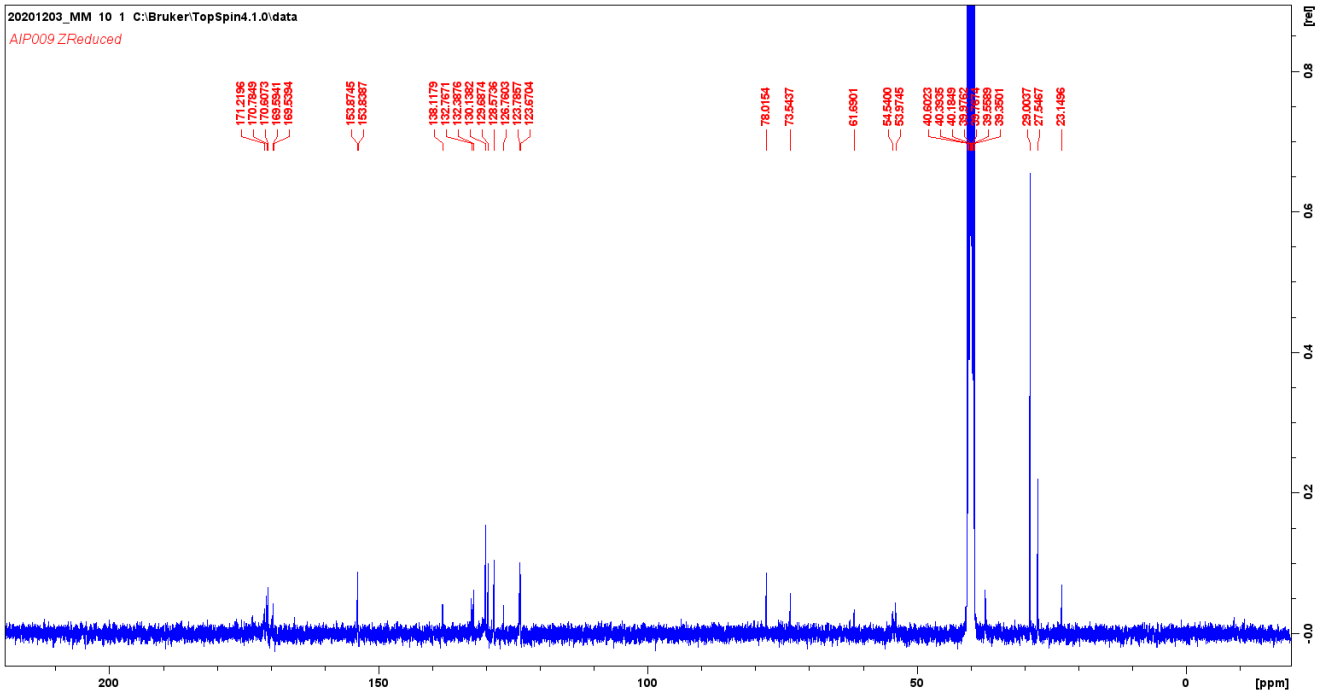


Figure S. 33. ^{13}C NMR spectrum of compound **14** in DMSO.

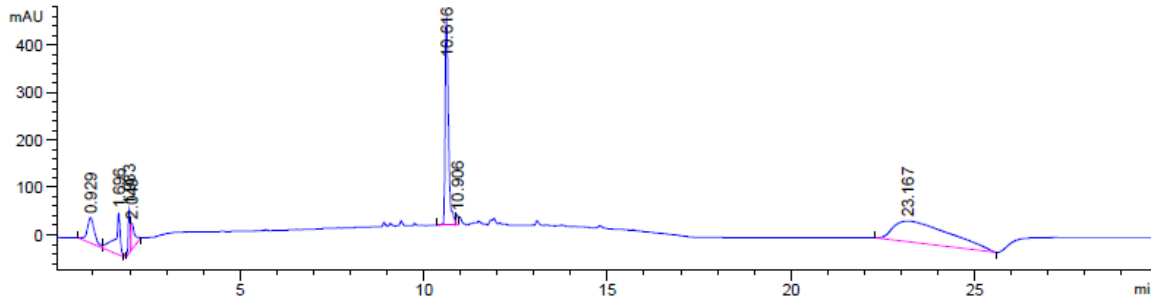


Figure S. 34. RP-HPLC chromatogram for compound **14** at 214 nm.

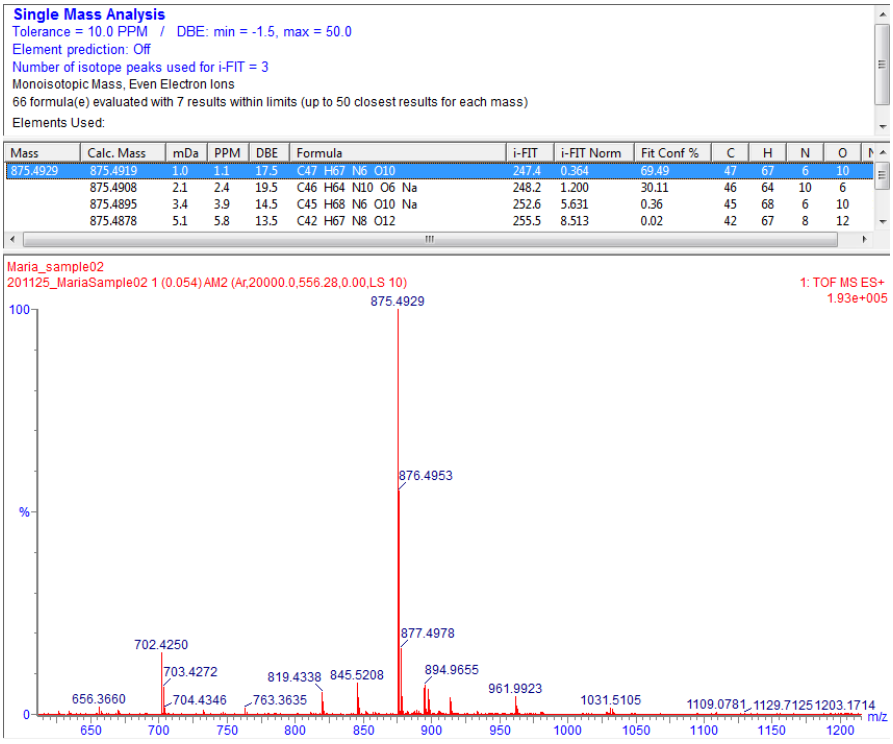


Figure S. 35. HRMS (ESI+) spectrum for compound **14**.

Spectral and Chromatographic Data for Compound 15

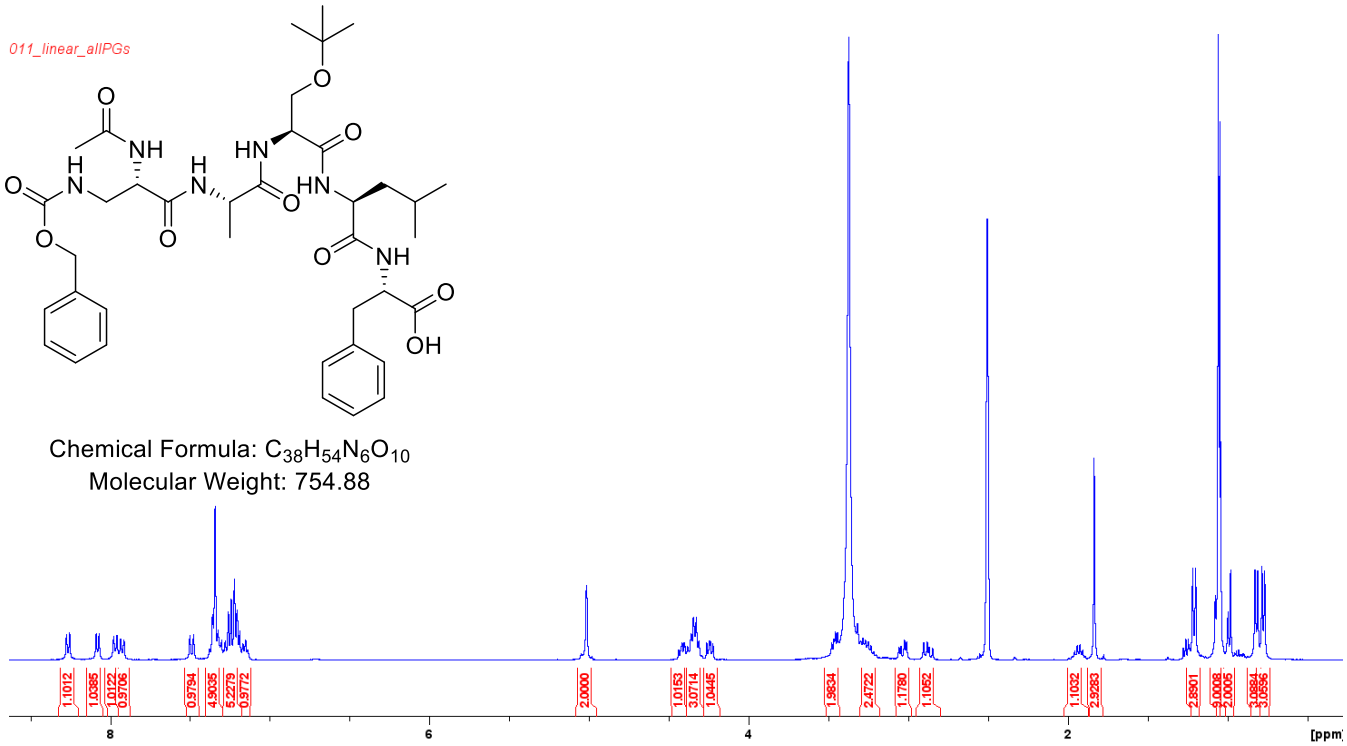


Figure S. 36. ^{1}H NMR spectrum for compound **15** in DMSO-d_6.

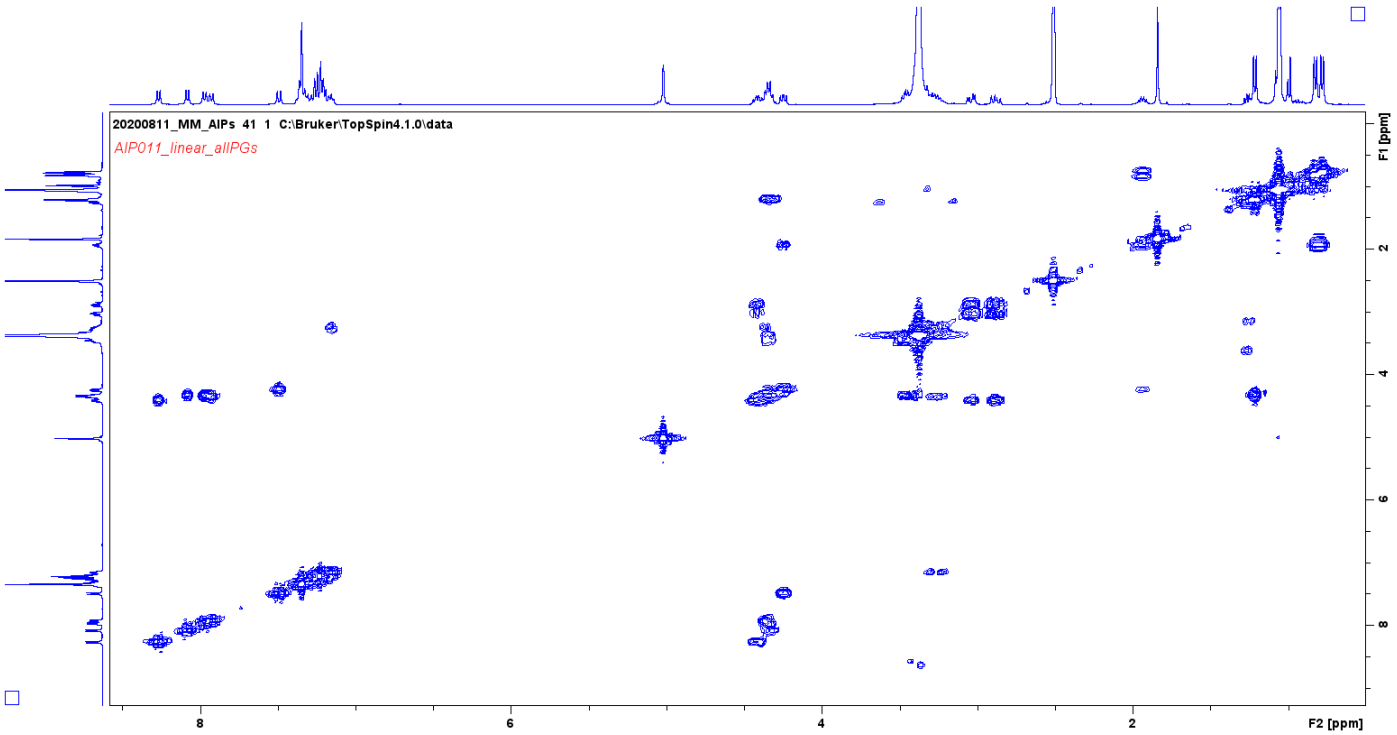


Figure S. 37. COSY NMR spectrum of compound **15** in DMSO-d_6.

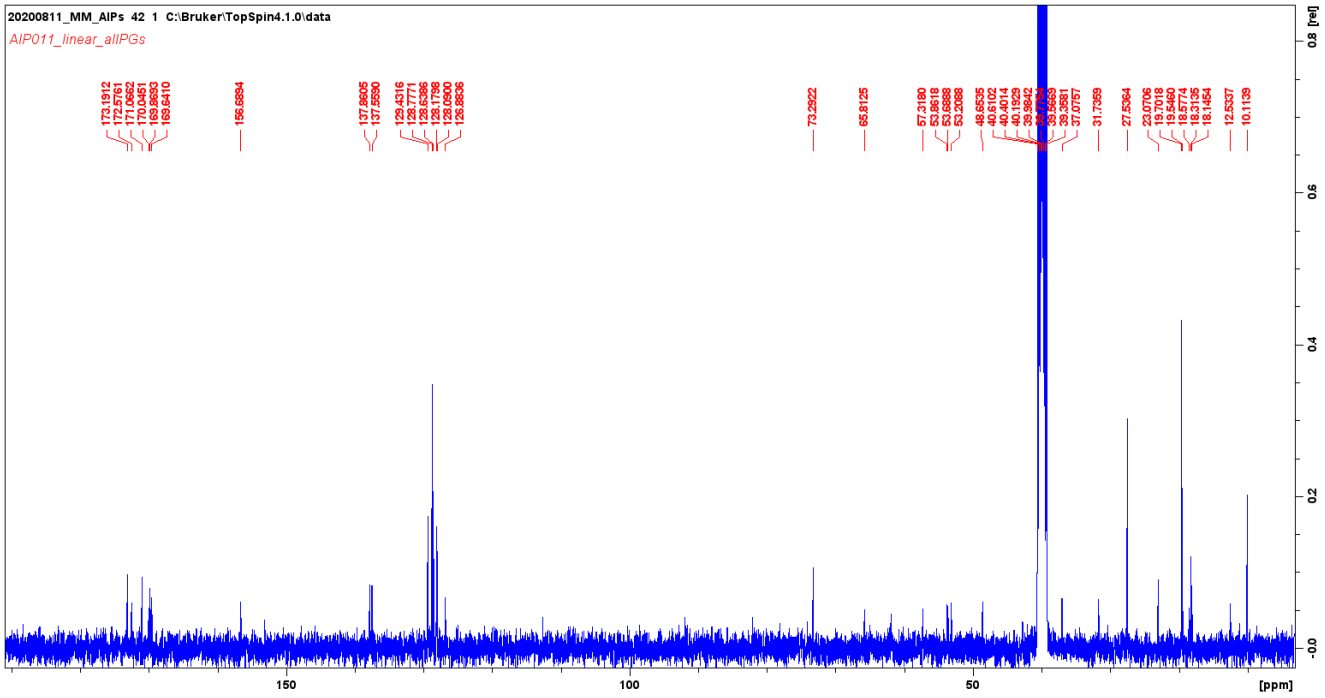


Figure S. 38. ^{13}C NMR spectrum of compound **15** in DMSO.

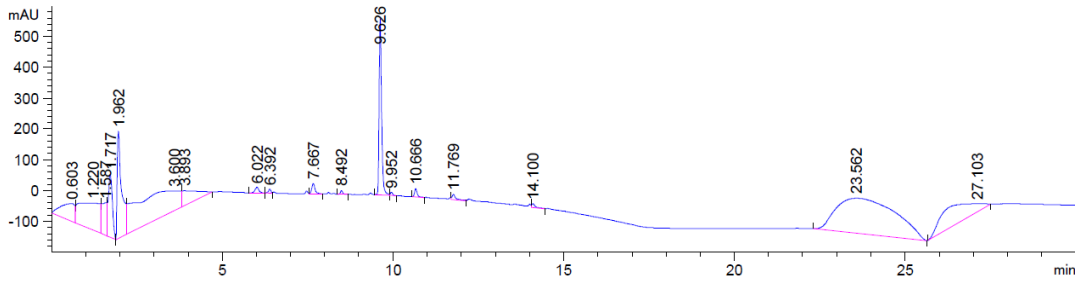


Figure S. 39. RP-HPLC chromatogram for compound **15** at 214 nm.

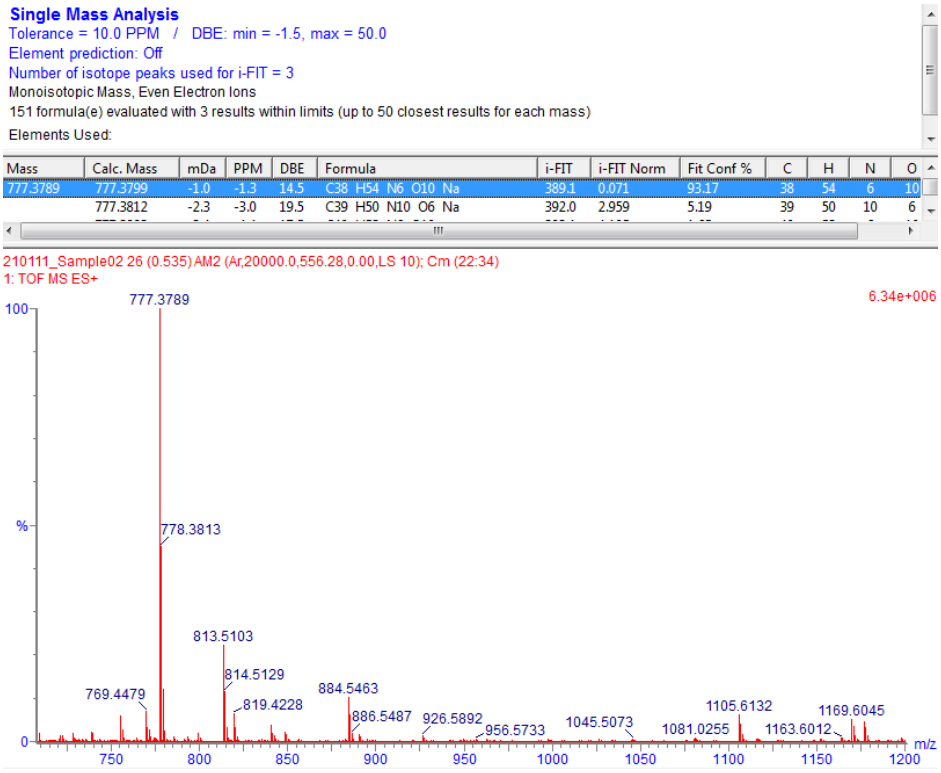


Figure S. 40. HRMS (ESI+) spectrum for compound **15**.

Spectral and Chromatographic Data for Compound 16

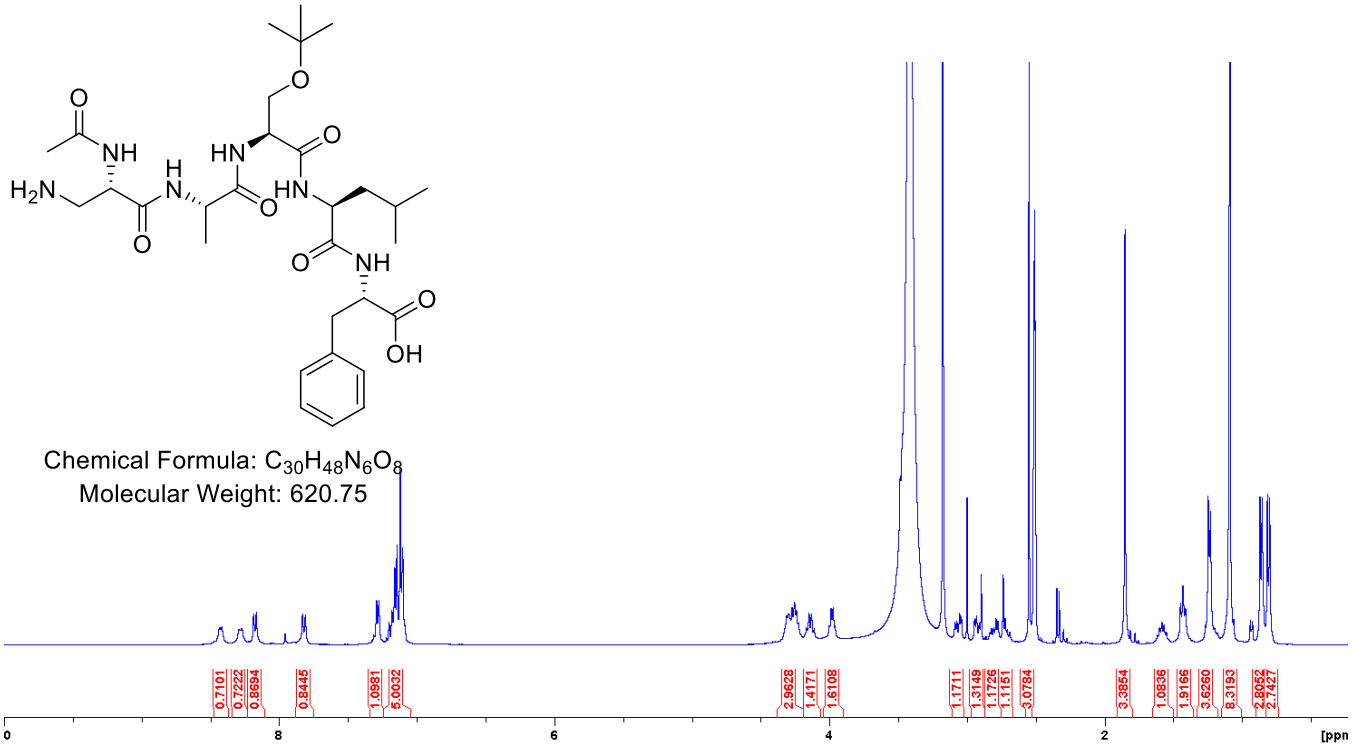


Figure S. 41. ^{1}H NMR spectrum for compound **16** in DMSO-d_6.

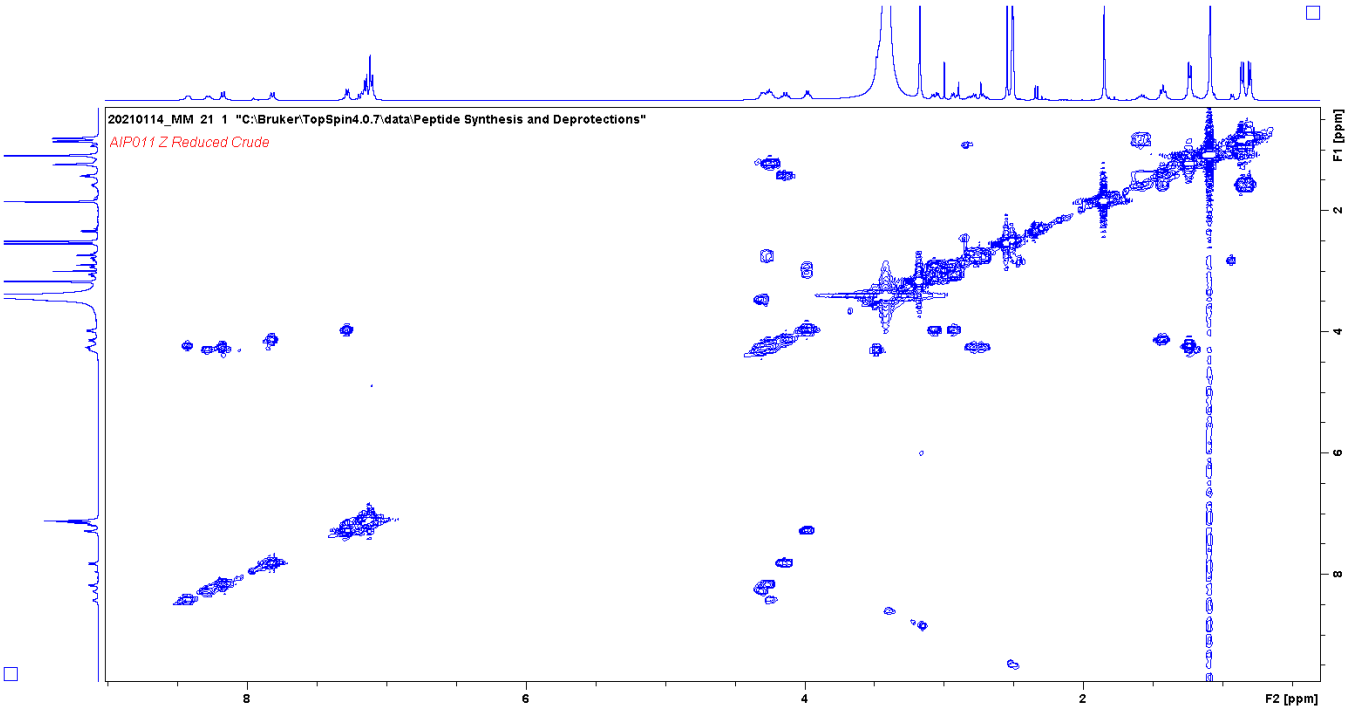


Figure S. 42. COSY NMR spectrum of compound **16** in DMSO-d_6.

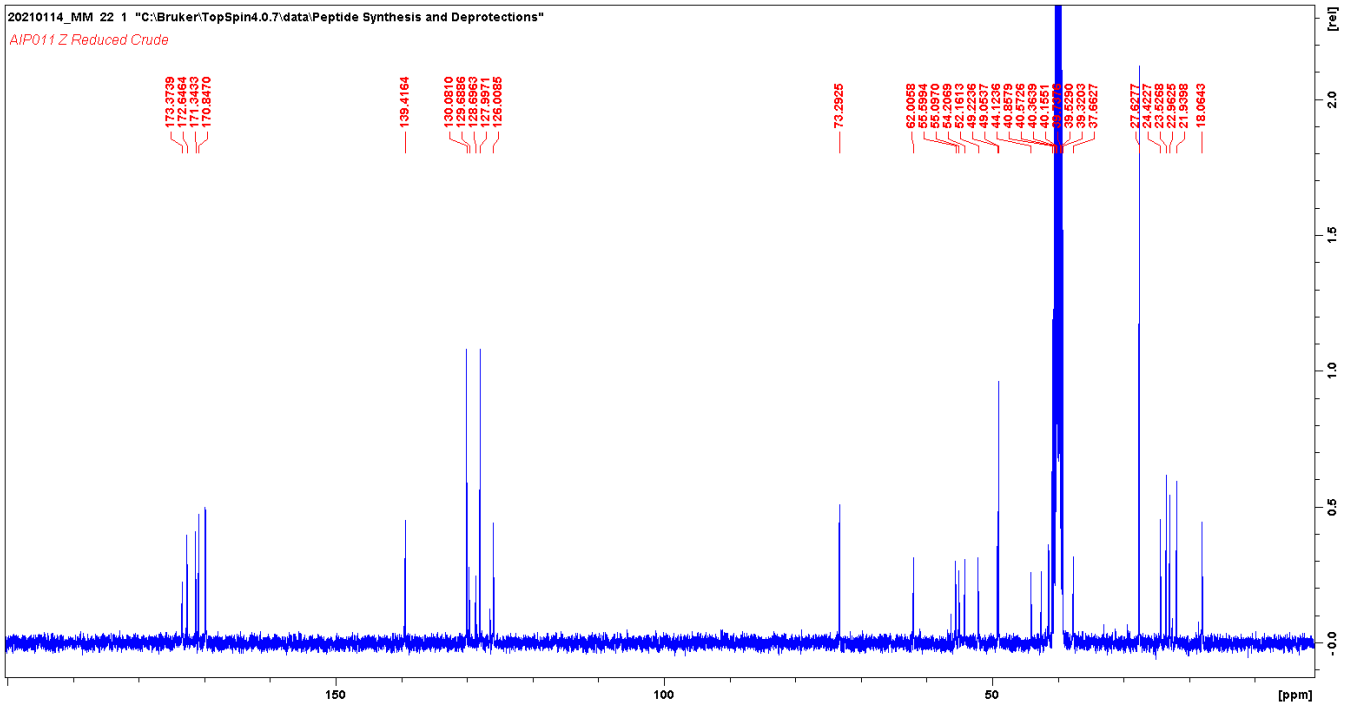


Figure S. 43. ^{13}C NMR spectrum of compound **16** in DMSO.

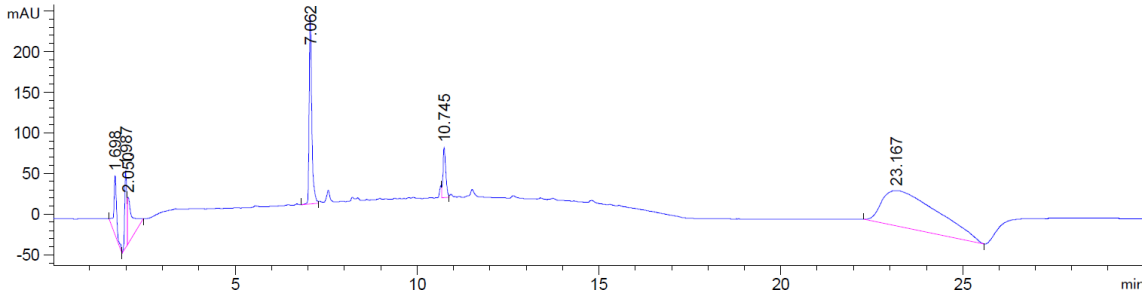


Figure S. 44. RP-HPLC chromatogram for compound **16** at 214 nm.

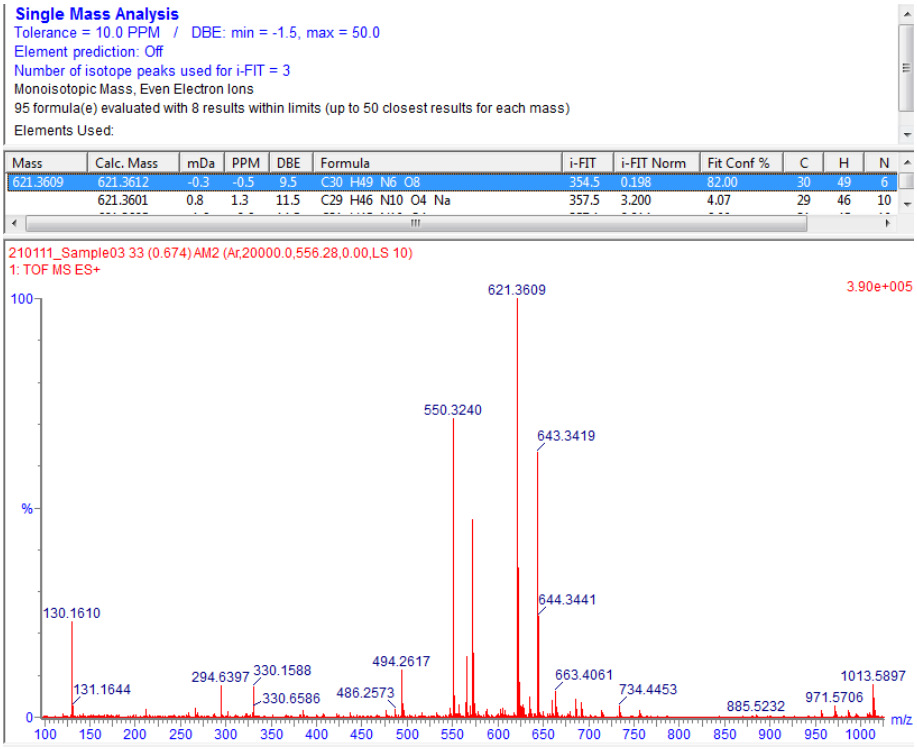


Figure S. 45. HRMS (ESI+) spectrum for compound **16**.

Spectral and Chromatographic Data for Compound 17

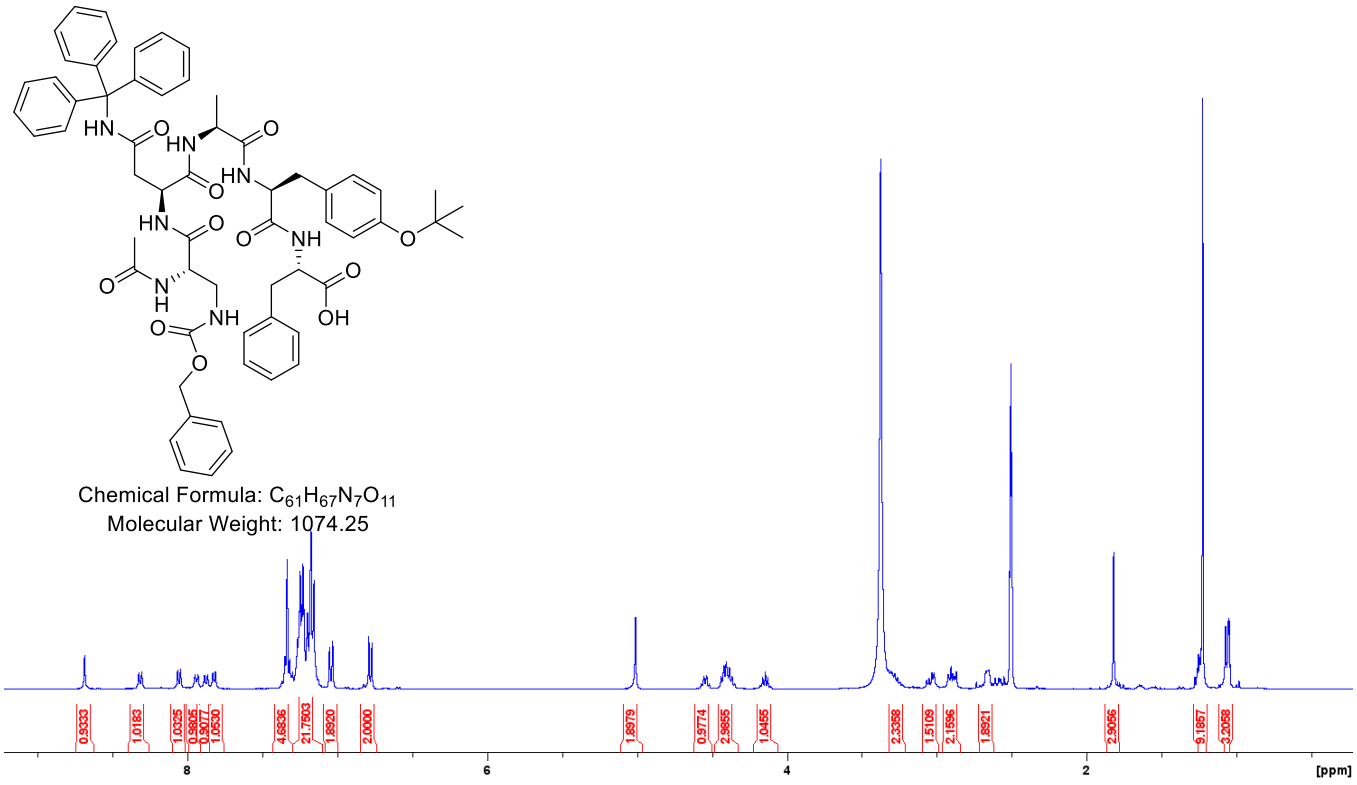


Figure S. 46. ^{1}H NMR spectrum for compound **17** in DMSO-d_6.

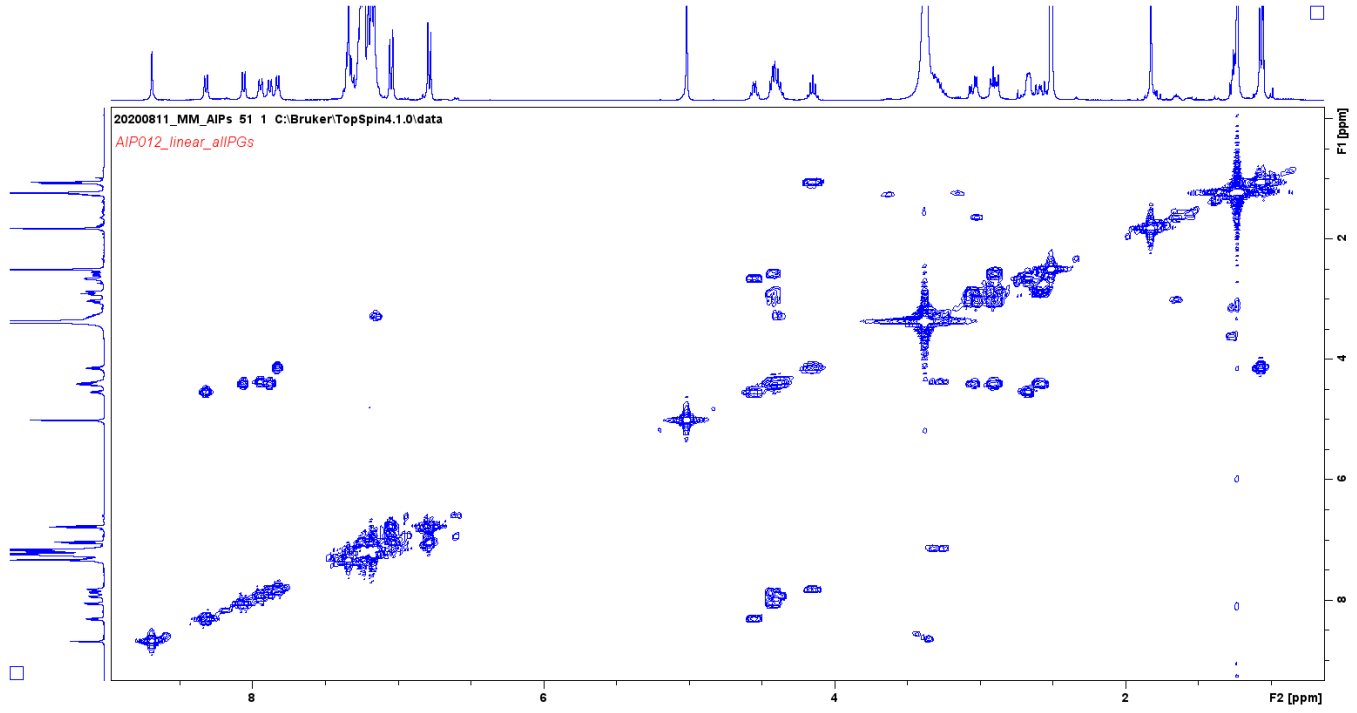


Figure S. 47. COSY NMR spectrum of compound **17** in DMSO-d_6.

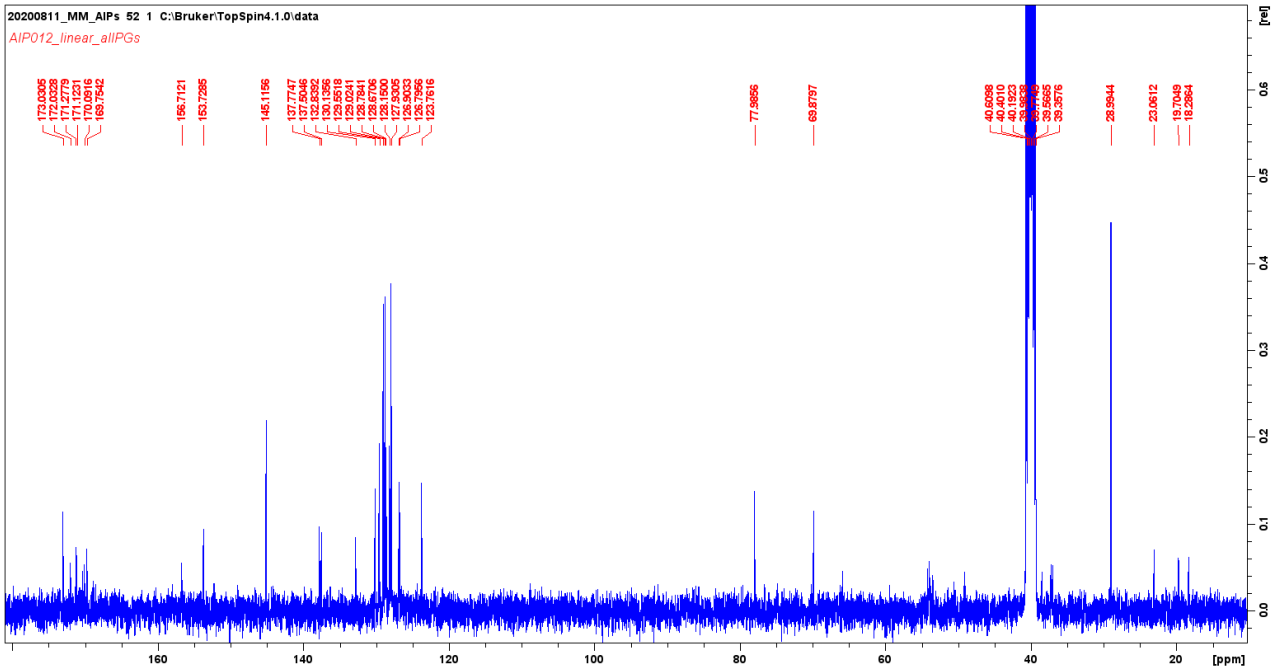


Figure S. 48. ^{13}C NMR spectrum of compound **17** in DMSO.

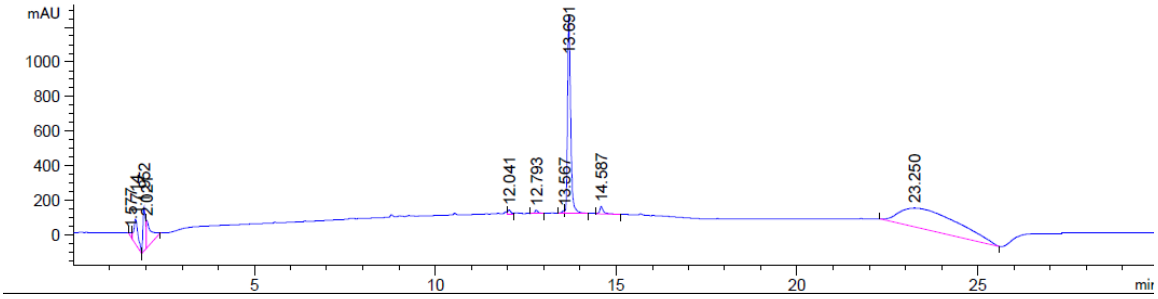


Figure S. 49. RP-HPLC chromatogram for compound **17** at 214 nm.

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Monoisotopic Mass, Even Electron Ions
10 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 60-62 H: 65-69 N: 4-8 O: 9-15 Na: 0-1

Mass	Calc. Mass	mDa	PPM	DBE	Formula	C	H	N	O	Na
1074.4996	1074.4977	1.9	1.8	31.5	C61 H68 N7 O11	61	68	7	11	

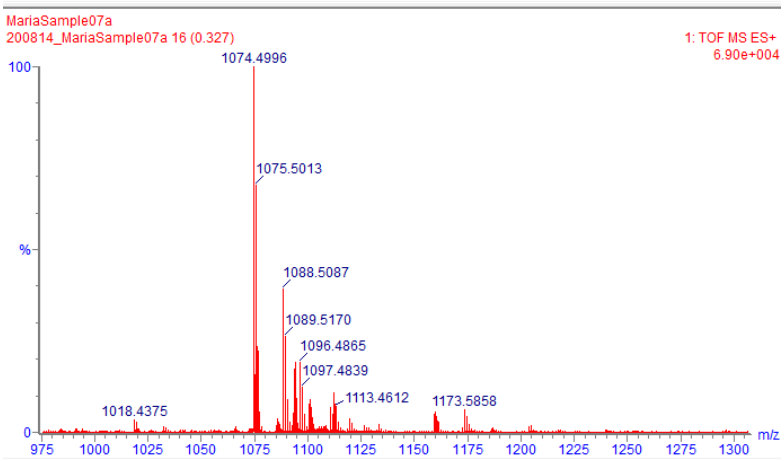


Figure S. 50. HRMS (ESI+) spectrum for compound **17**.

Spectral and Chromatographic Data for Compound 18

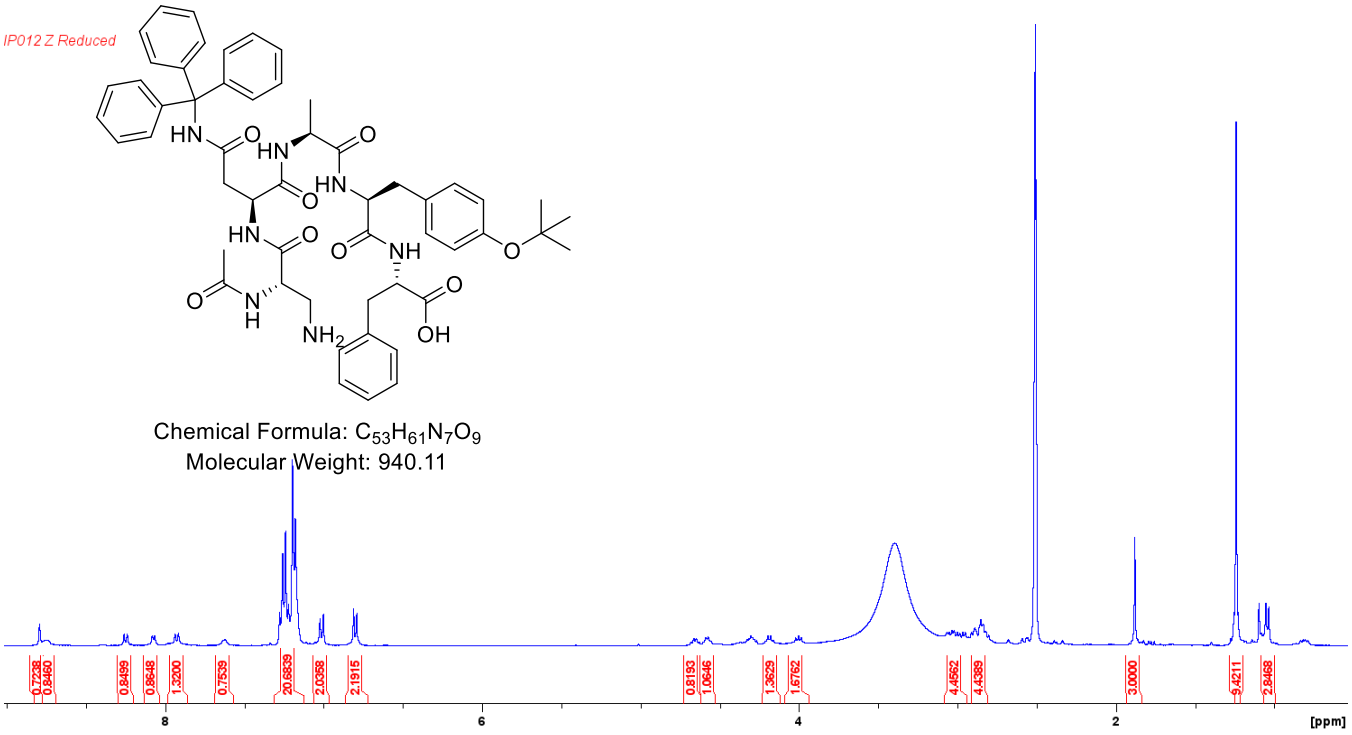


Figure S. 51. ^{1}H NMR spectrum for compound **18** in DMSO-d$_6$.

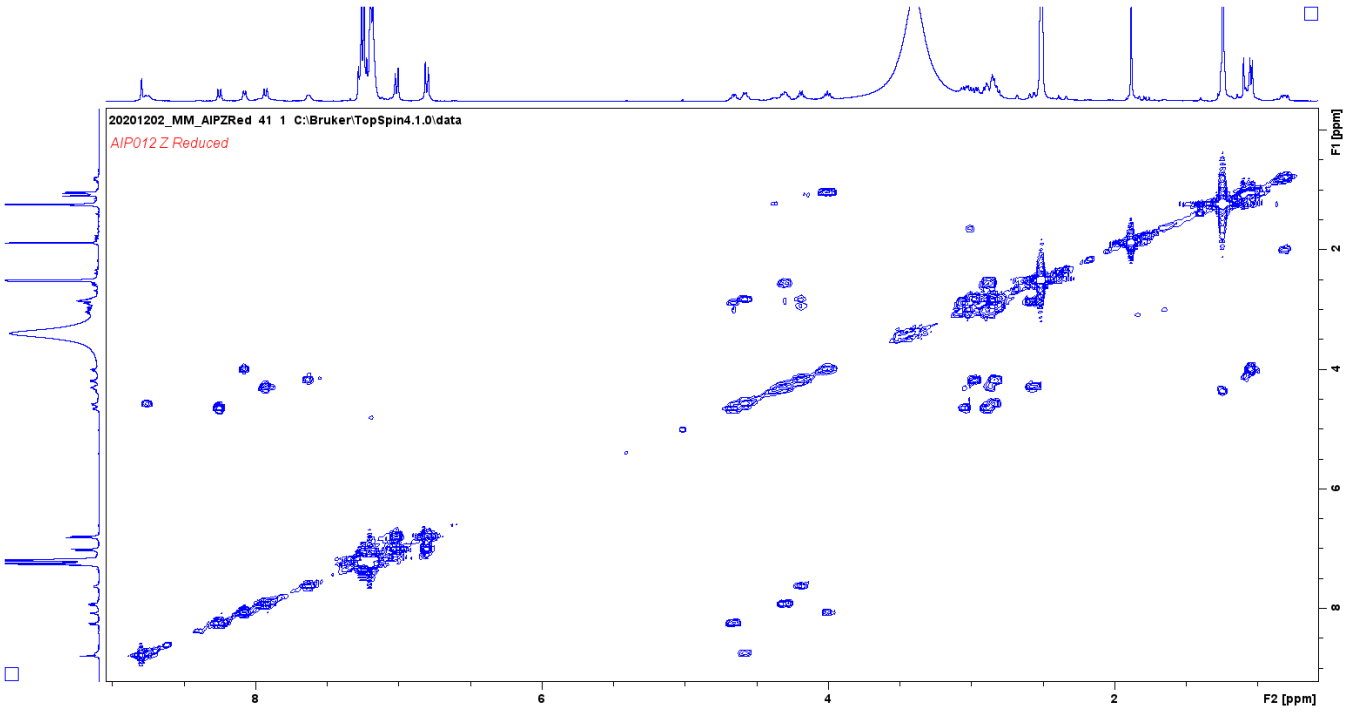


Figure S. 52. COSY NMR spectrum of compound **18** in DMSO-d$_6$.

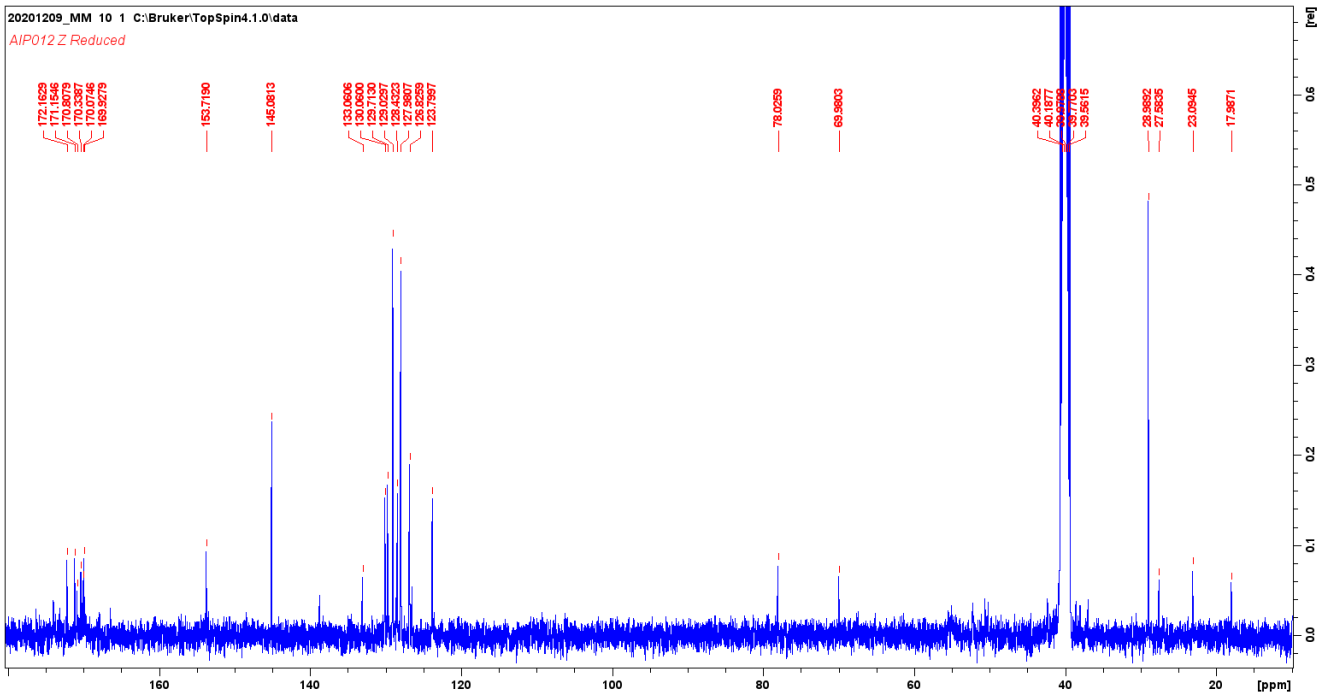


Figure S. 53. ^{13}C NMR spectrum of compound **18** in DMSO.

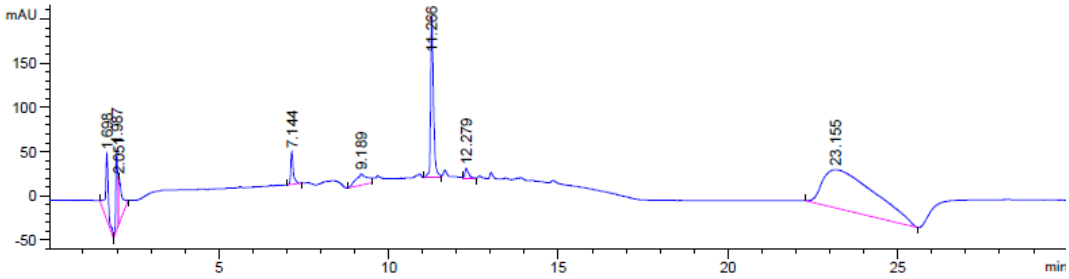


Figure S. 54. RP-HPLC chromatogram for compound **18** at 214 nm.

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
18 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
940.4611	940.4609	0.2	0.2	26.5	C53 H62 N7 O9	207.1	n/a	n/a	53	62	7	9

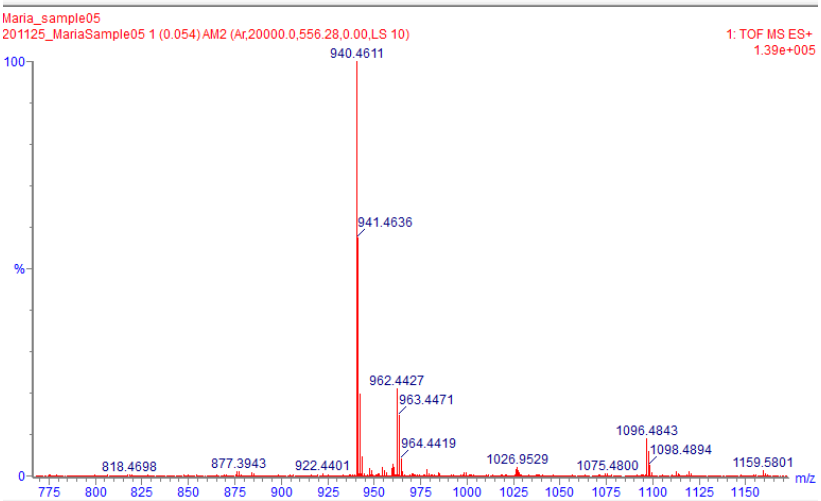


Figure S. 55. HRMS (ESI+) spectrum for compound **18**.

Spectral and Chromatographic Data for Compound 19

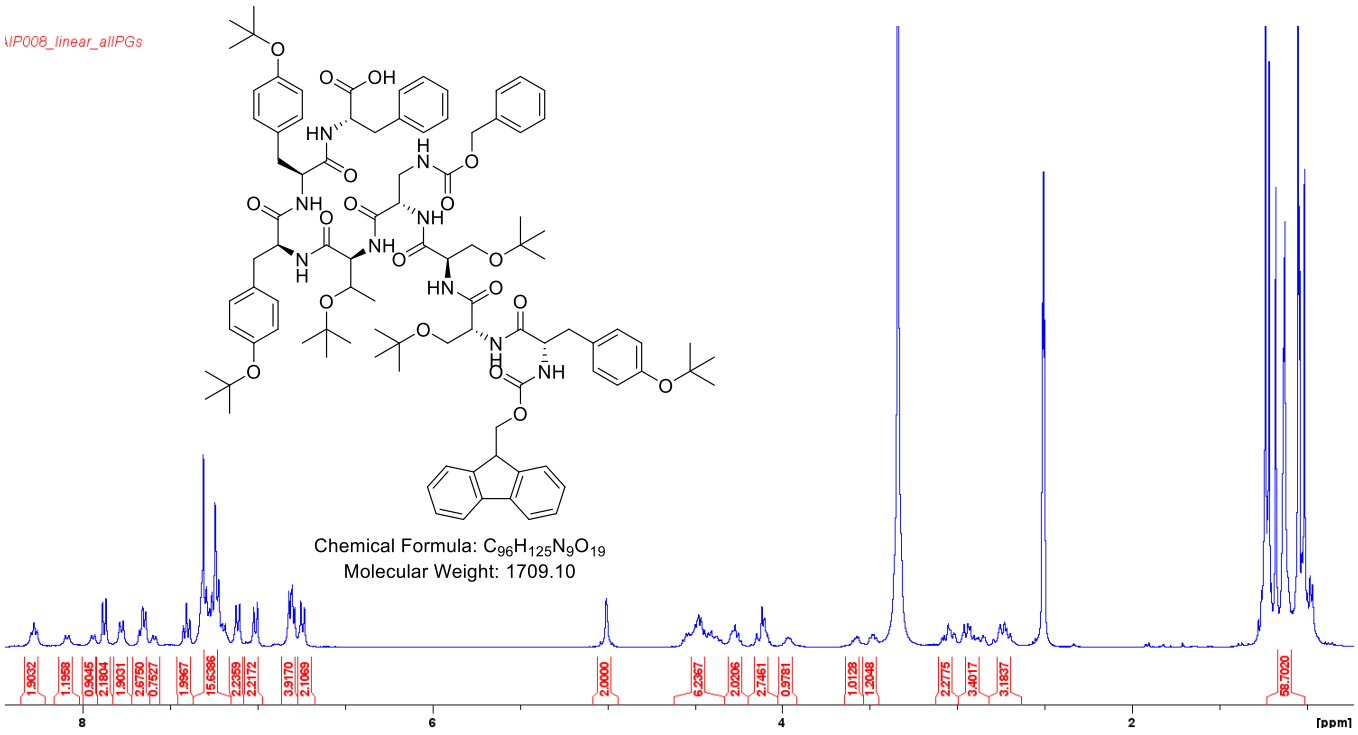


Figure S. 56. ^{1}H NMR spectrum for compound **19** in DMSO-d_6.

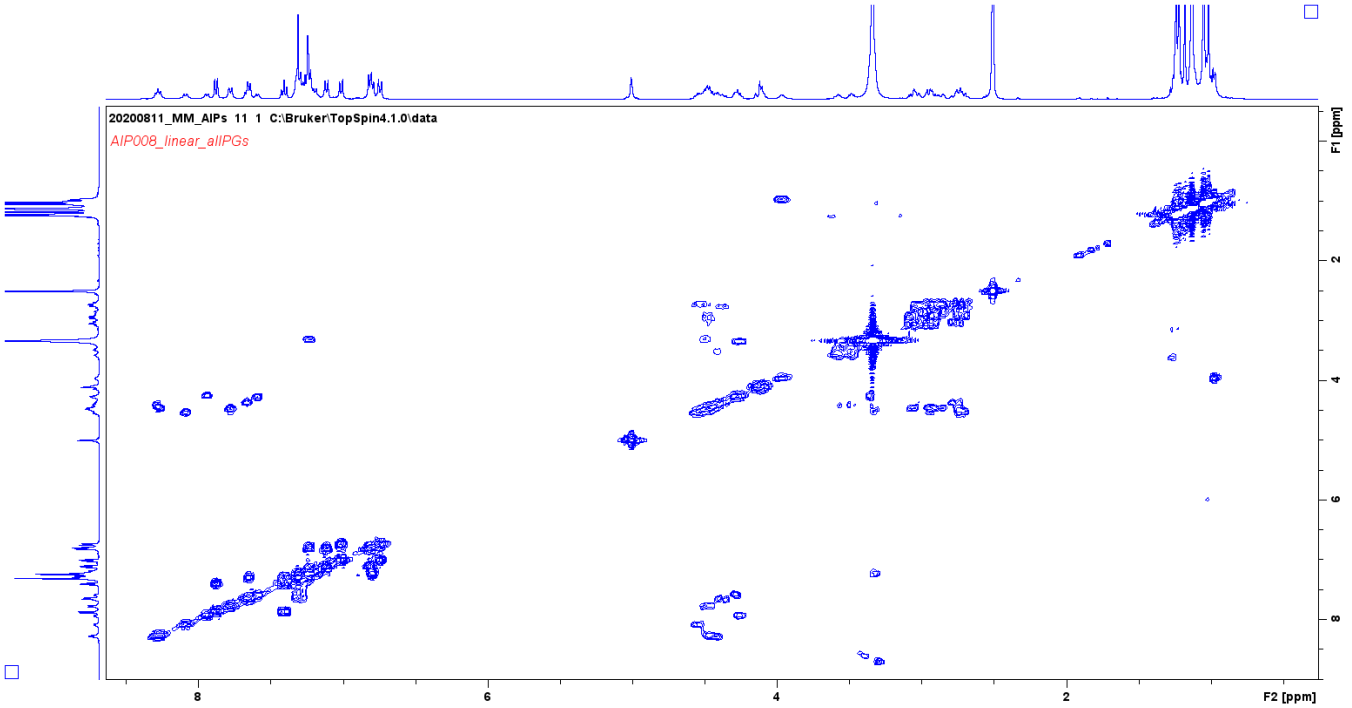


Figure S. 57. COSY NMR spectrum of compound **19** in DMSO-d_6.

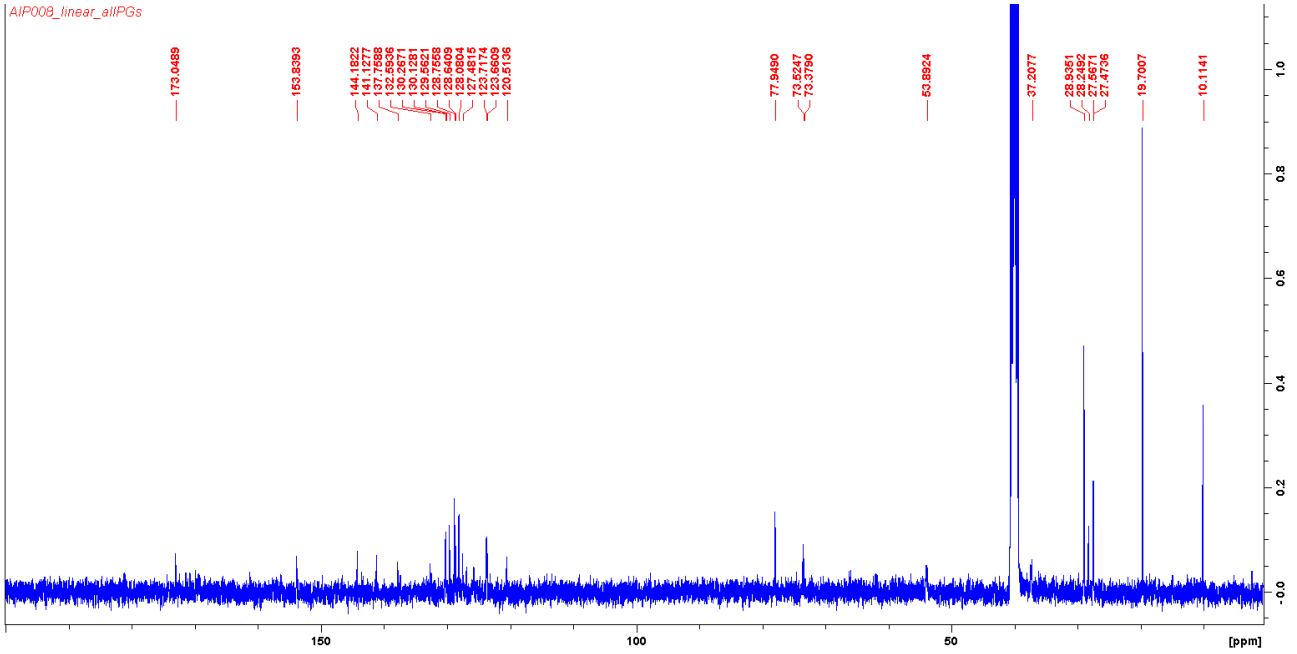


Figure S. 58. ^{13}C NMR spectrum of compound **19** in DMSO.

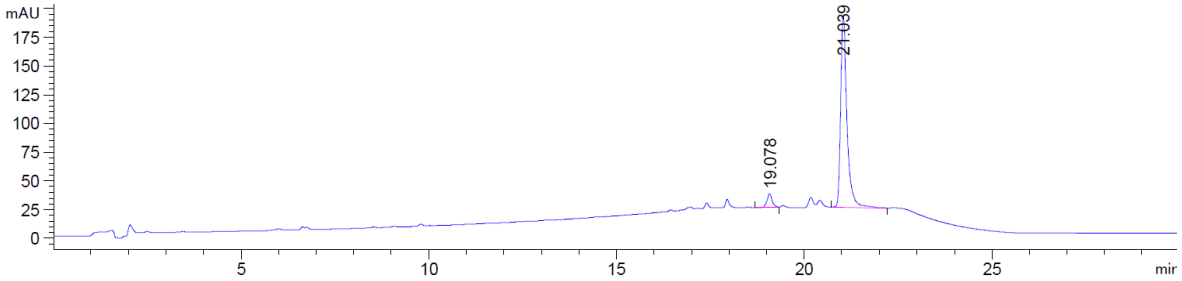


Figure S. 59. RP-HPLC chromatogram for compound **19** at 214 nm.

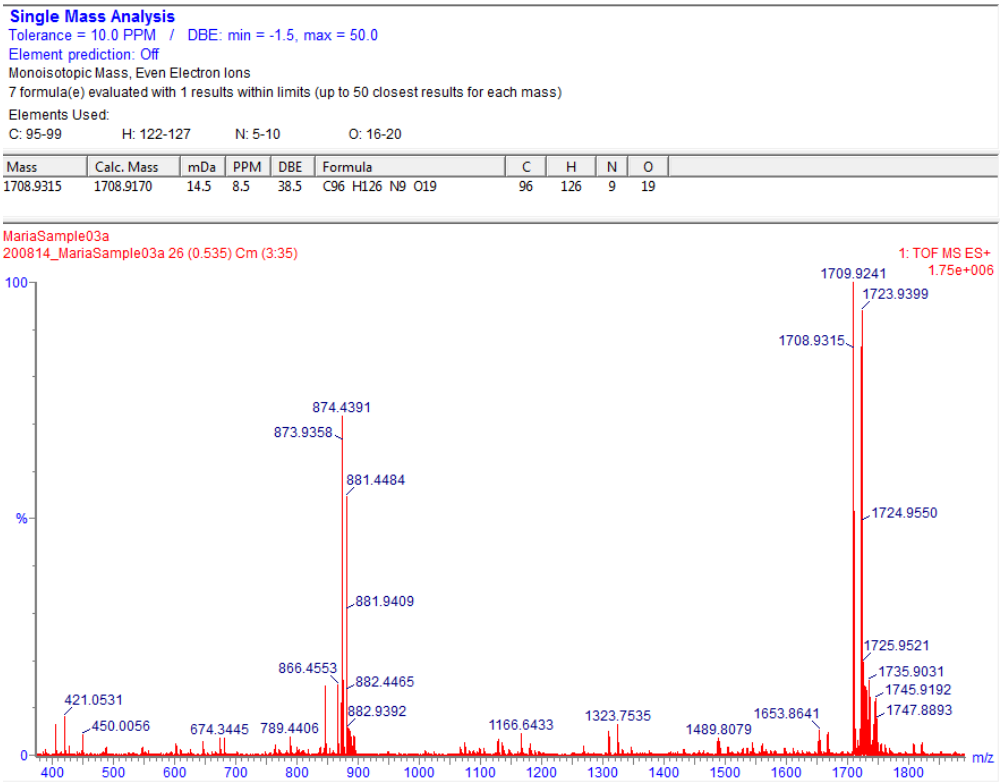


Figure S. 60. HRMS (ESI+) spectrum for compound **19**.

Spectral and Chromatographic Data for Compound 20

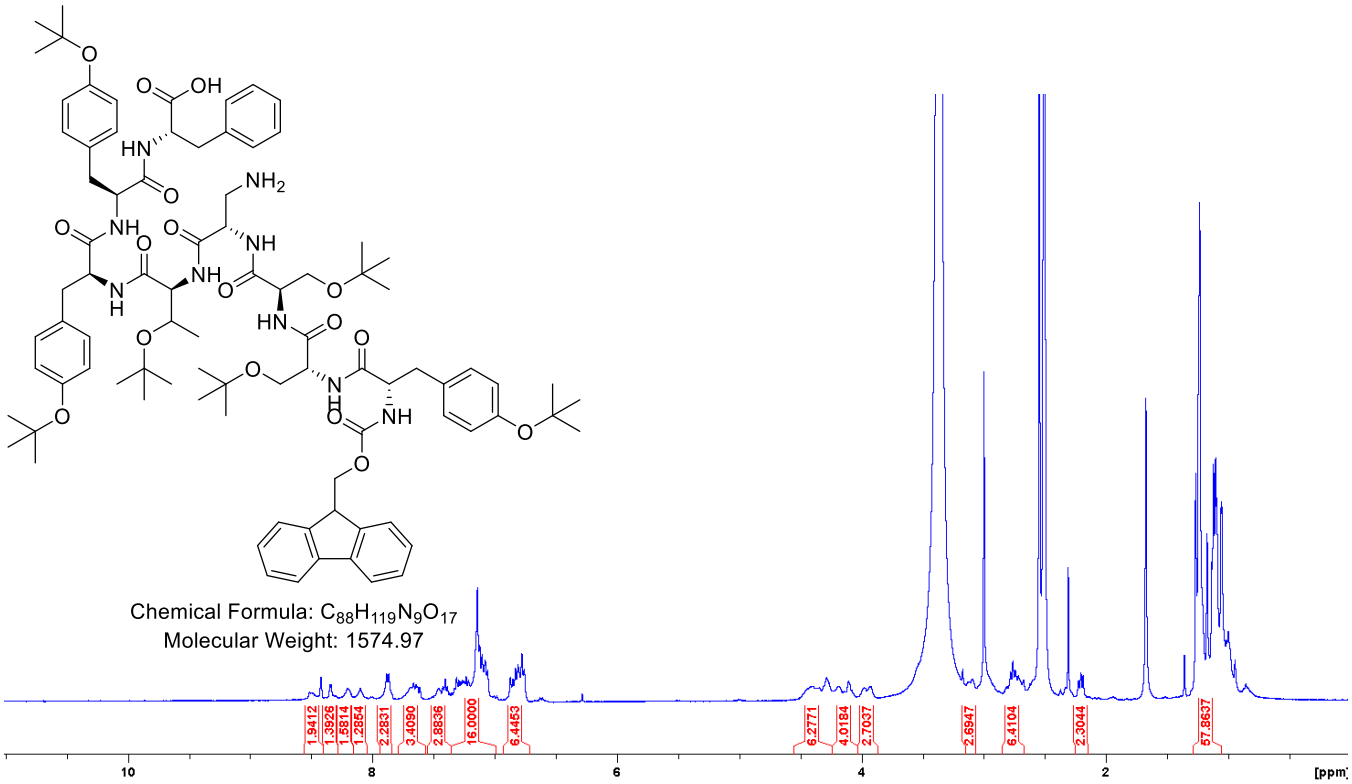


Figure S. 61. ^{1}H NMR spectrum for compound **20** in DMSO-d$_6$.

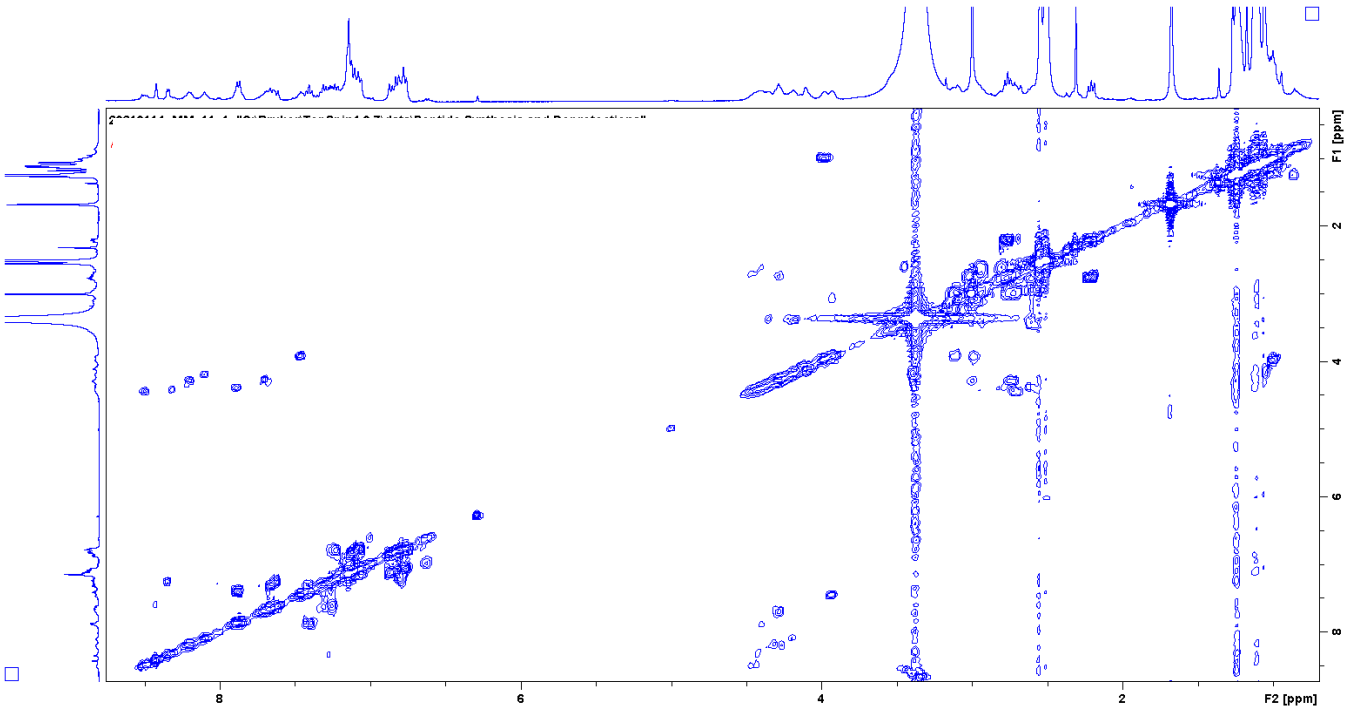


Figure S. 62. COSY NMR spectrum of compound **20** in DMSO-d$_6$.

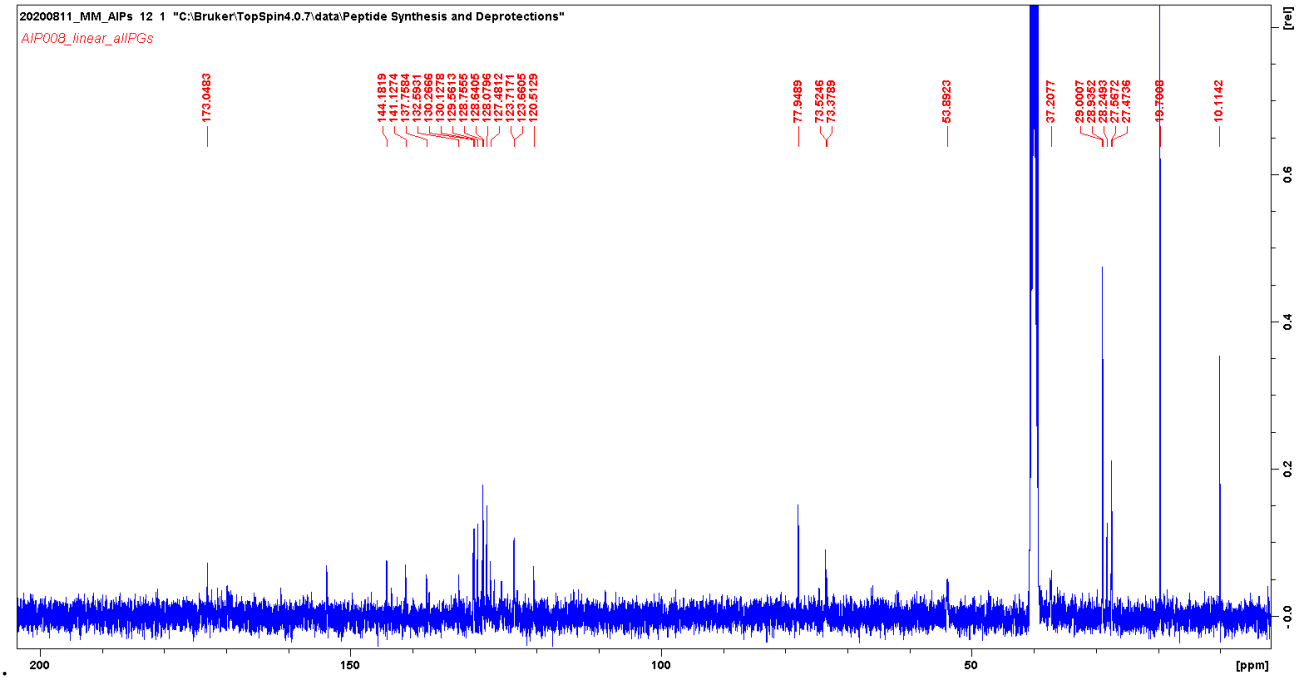


Figure S. 63. ^{13}C NMR spectrum of compound **20** in DMSO.

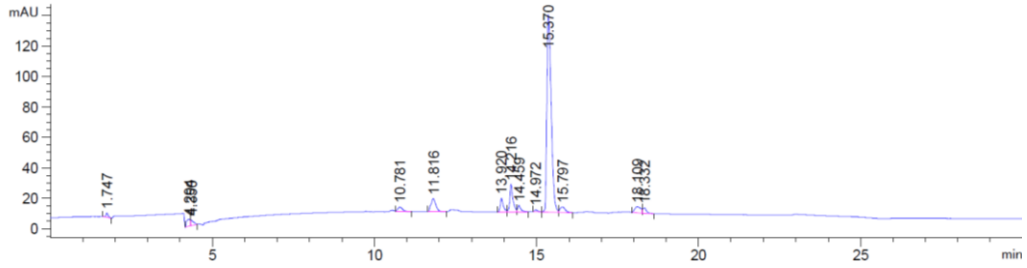


Figure S. 64. RP-HPLC chromatogram for compound **20** at 214 nm.

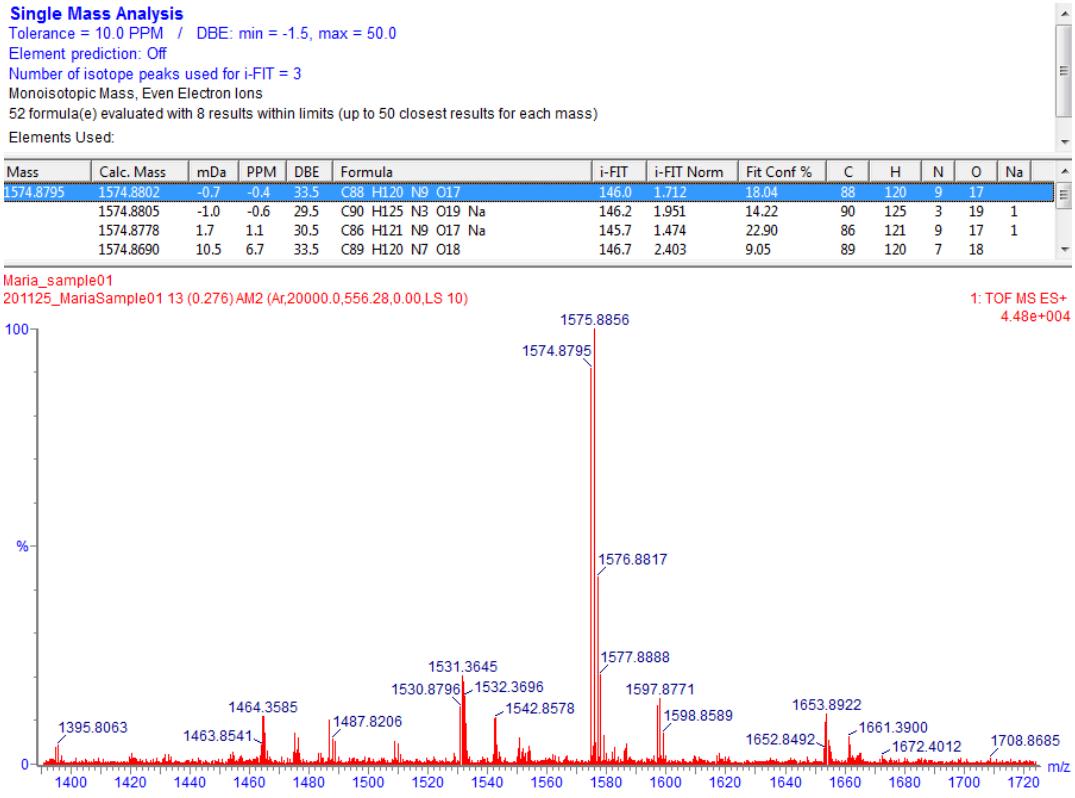


Figure S. 65. HRMS (ESI+) spectrum for compound **20**.

Spectral and Chromatographic Data for Compound 21

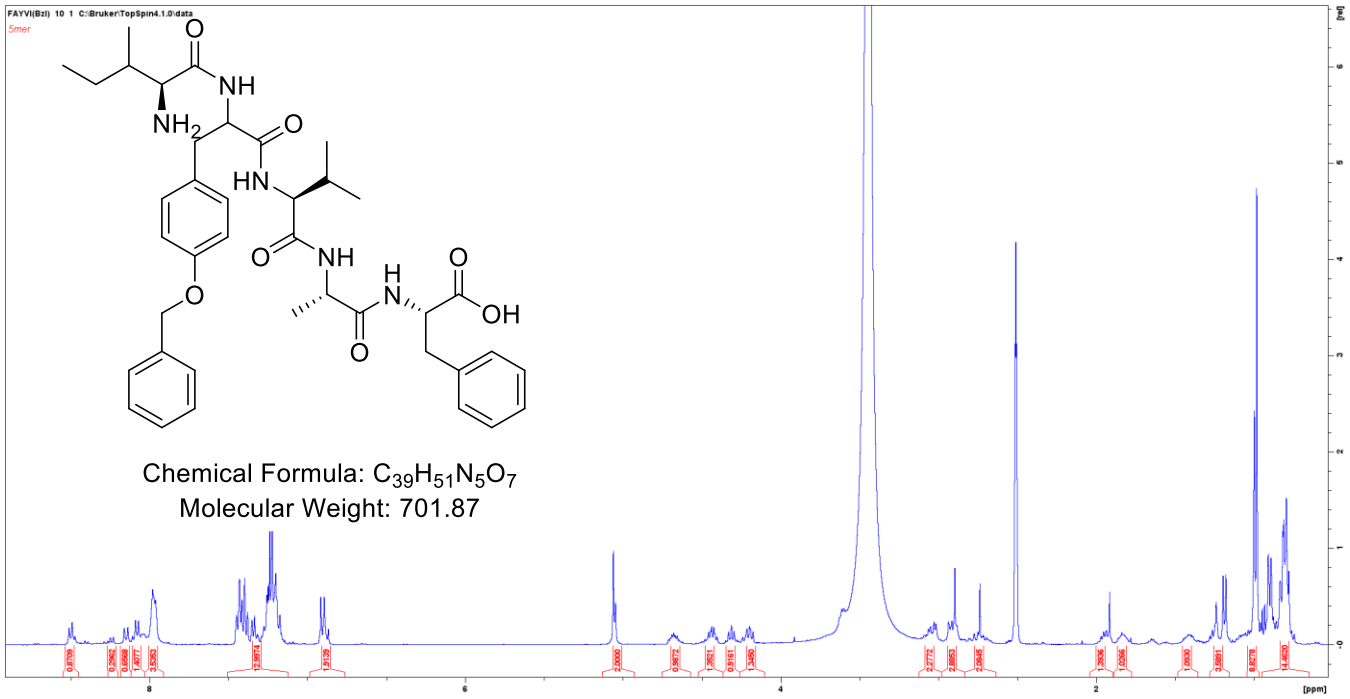


Figure S. 66. ^{1}H NMR spectrum for compound **21** in DMSO-d_6.

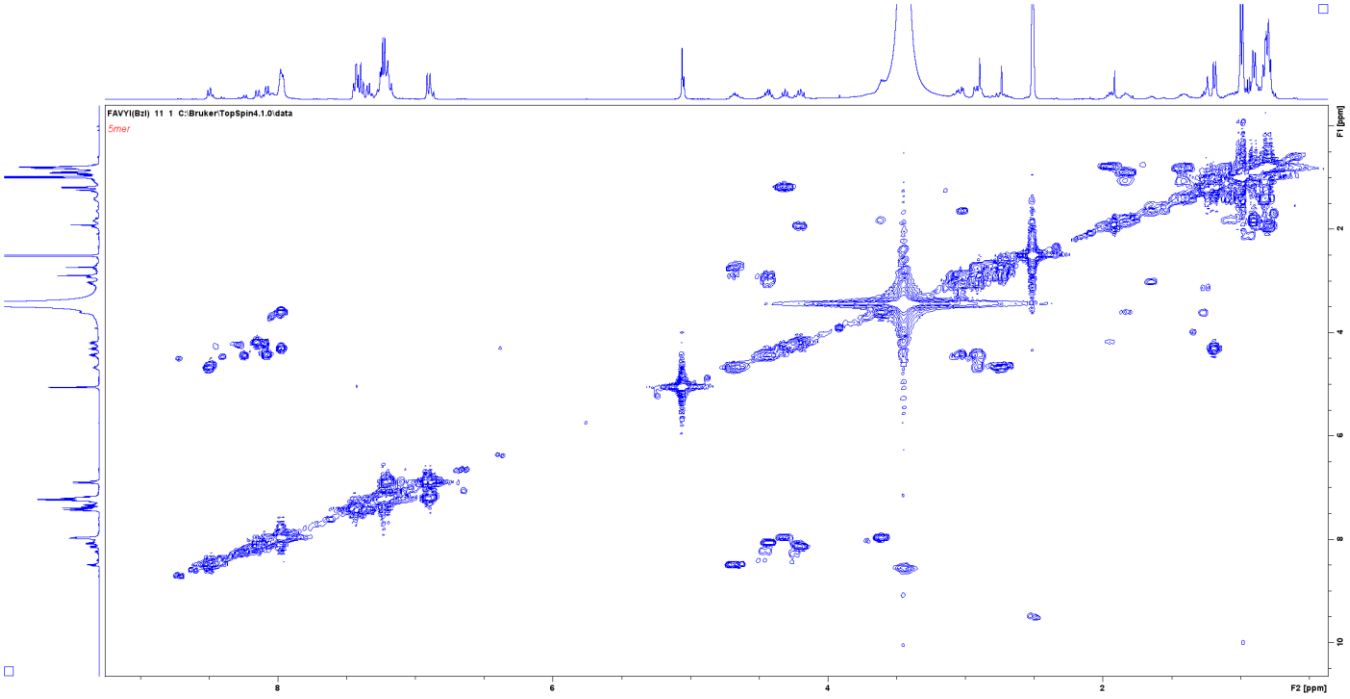


Figure S. 67. COSY NMR spectrum of compound **21** in DMSO-d_6.

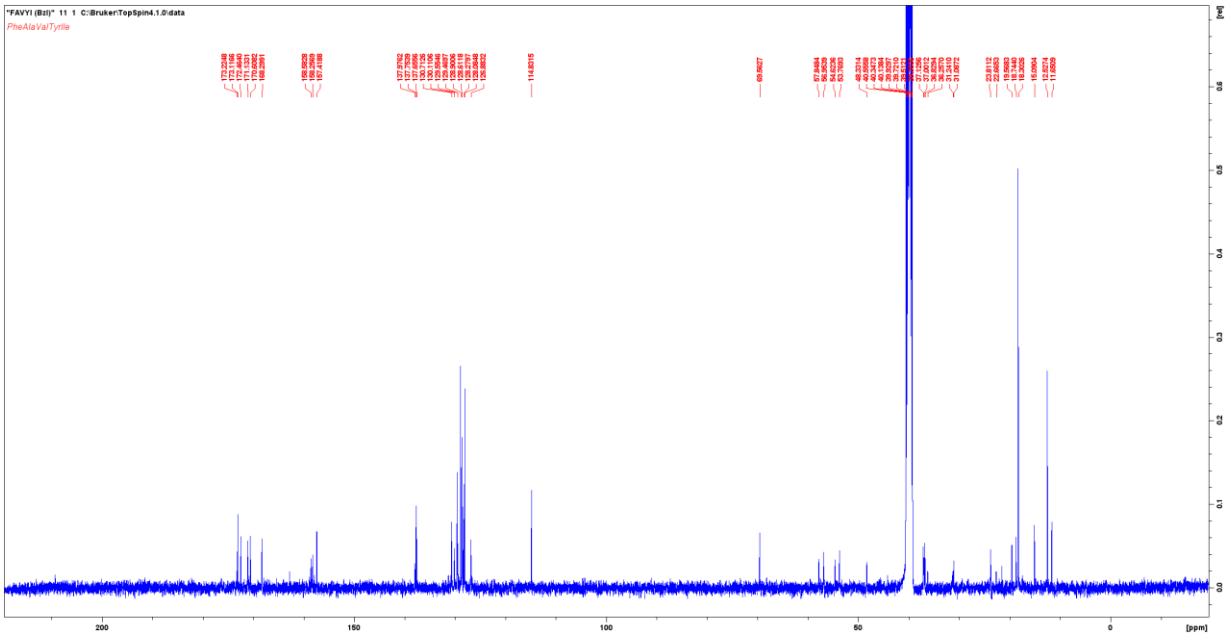


Figure S. 68. ^{13}C NMR spectrum of compound **21** in DMSO.

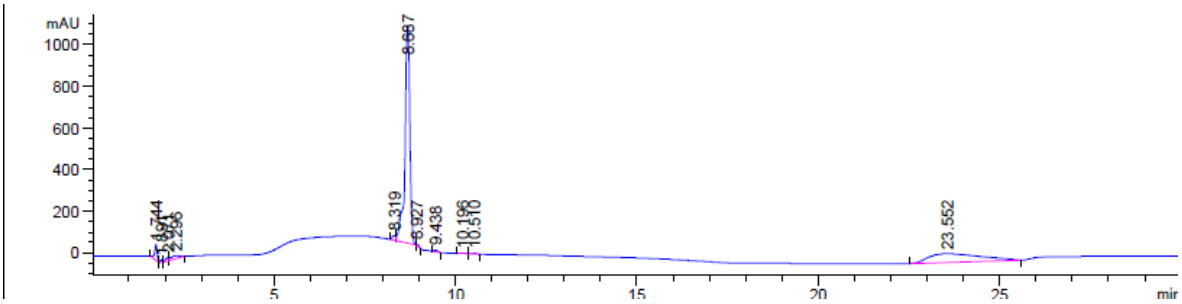


Figure S. 69. RP-HPLC chromatogram for compound **21** at 214 nm.

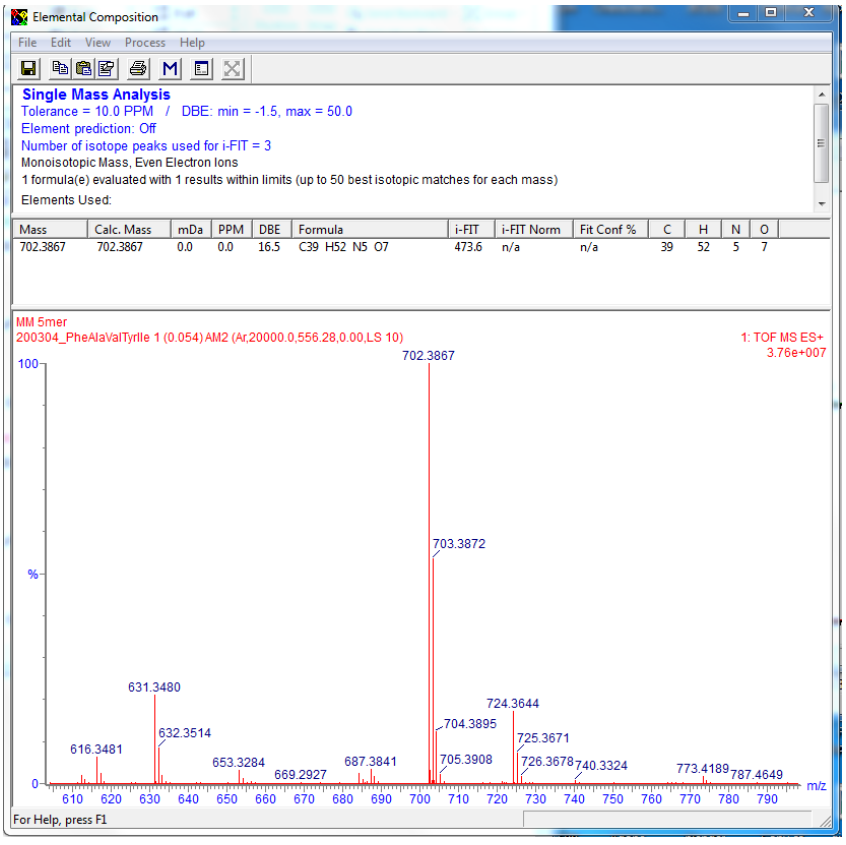


Figure S. 70. HRMS (ESI+) spectrum for compound **21**.

Preliminary chromatographic Data for Compound 22

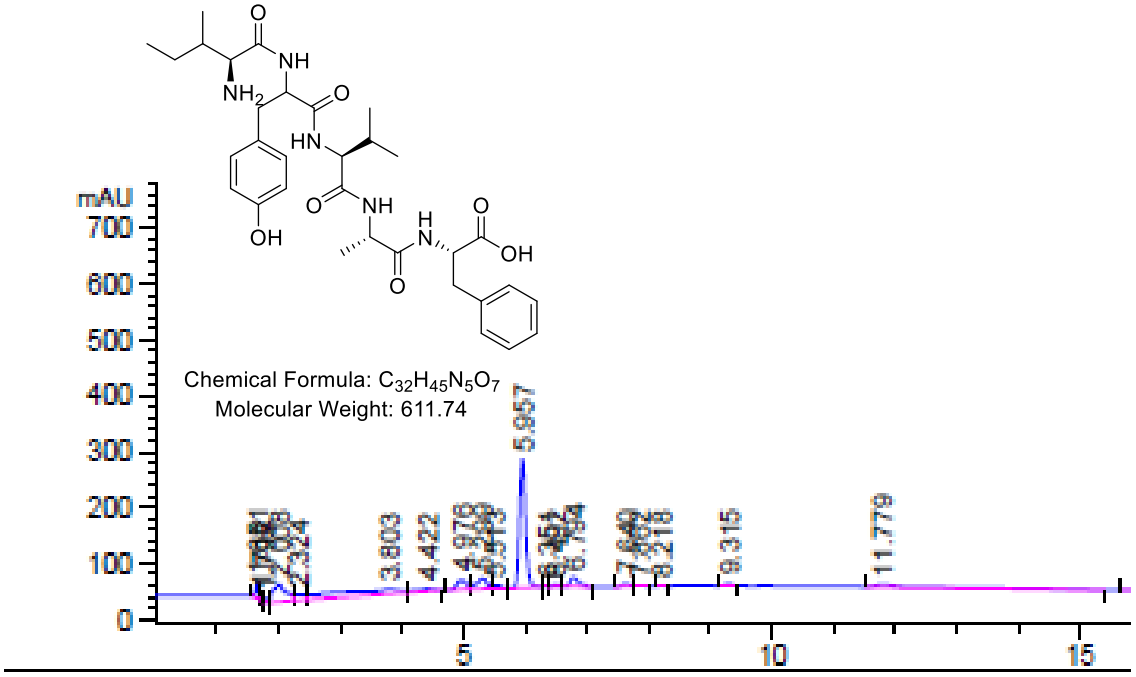


Figure S. 71. RP-HPLC chromatogram for compound **22** at 214 nm.

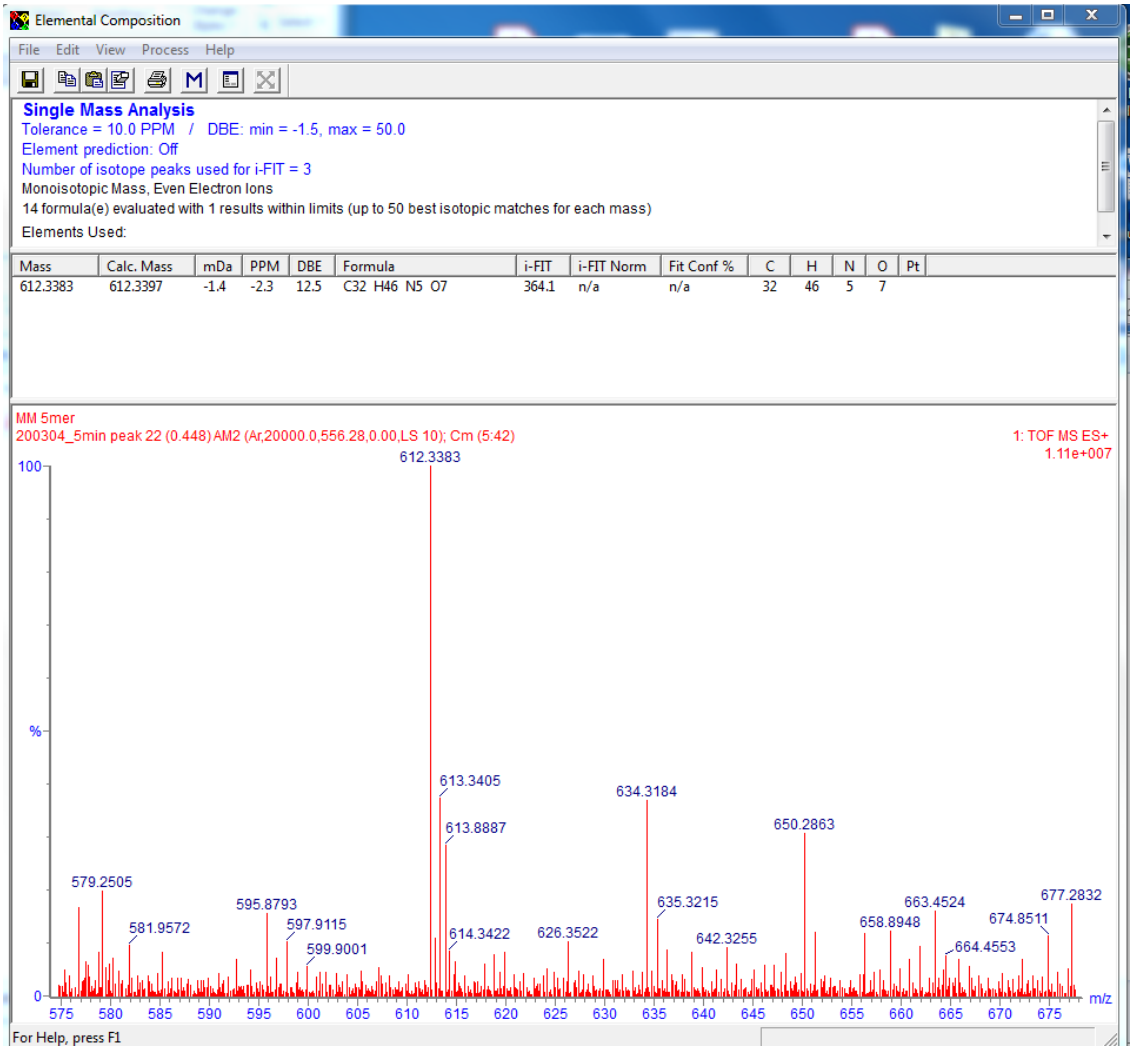


Figure S. 72. HRMS (ESI+) spectrum for compound **22**.

Spectral and Chromatographic Data for Compound 23

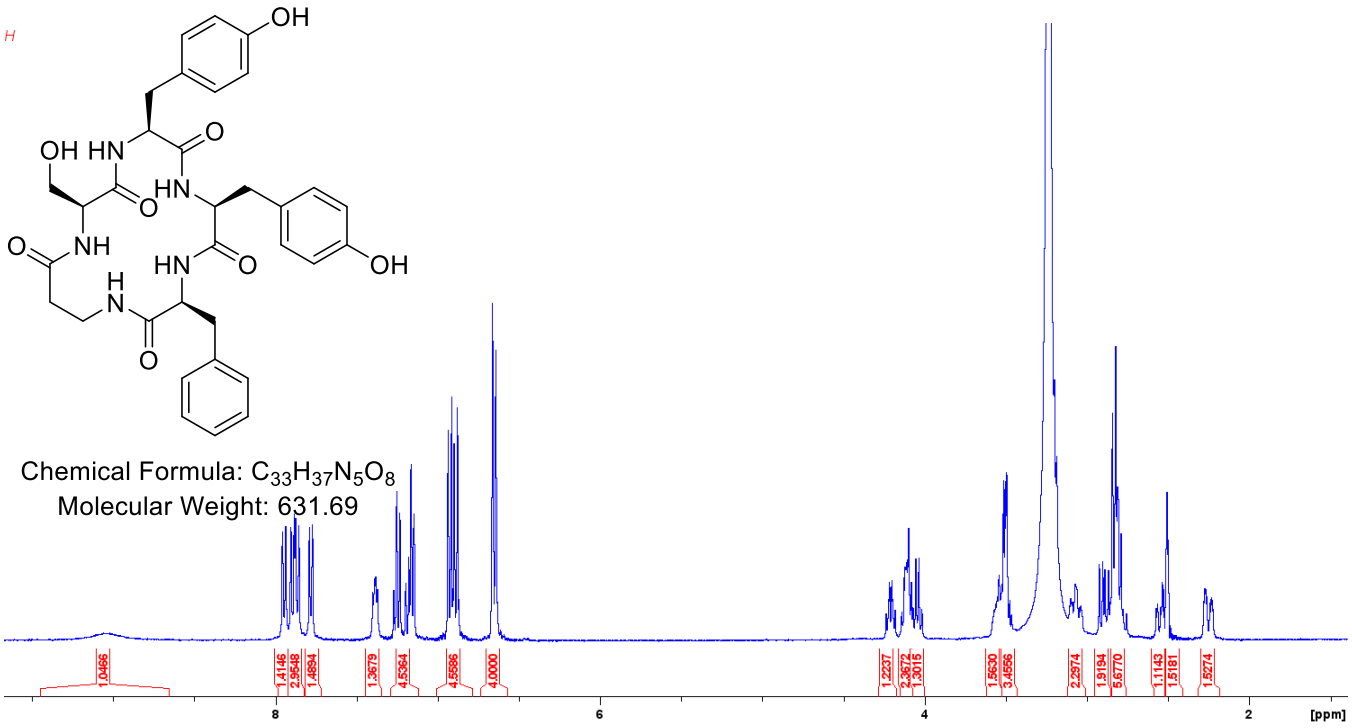


Figure S. 73. ^{1}H NMR spectrum for compound **23** in DMSO-d_6.

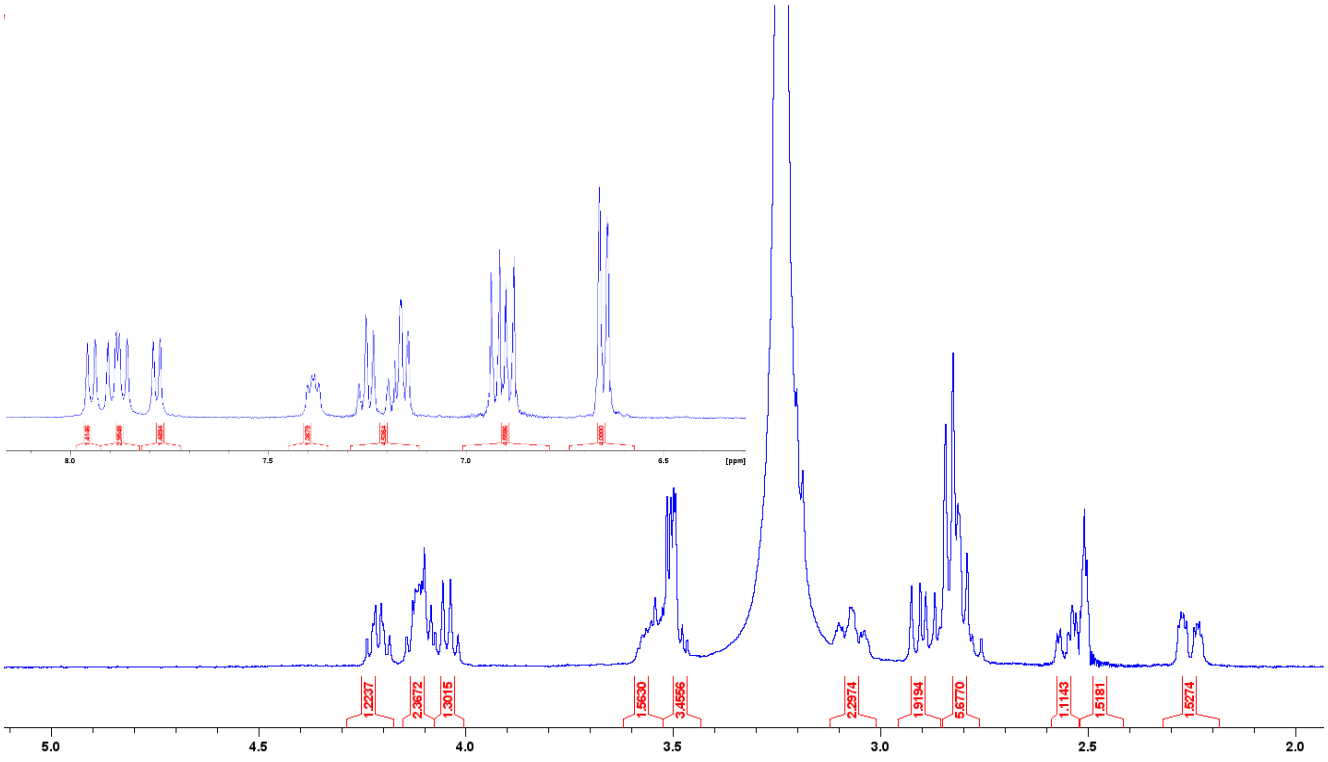


Figure S. 74. Expanded ^{1}H NMR spectrum for compound **23** (1 – 5 ppm), and an insert of 7 – 8 ppm.

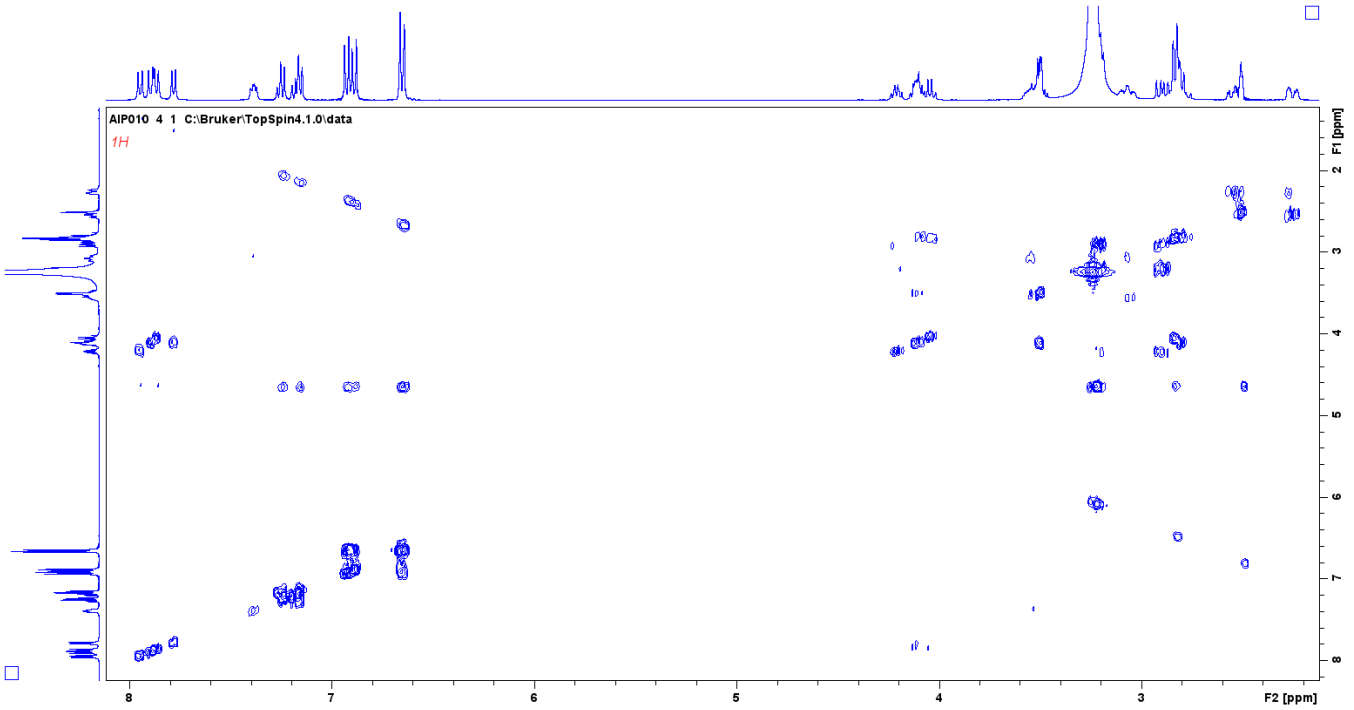


Figure S. 75. COSY NMR spectrum of compound **23** in DMSO-d_6.

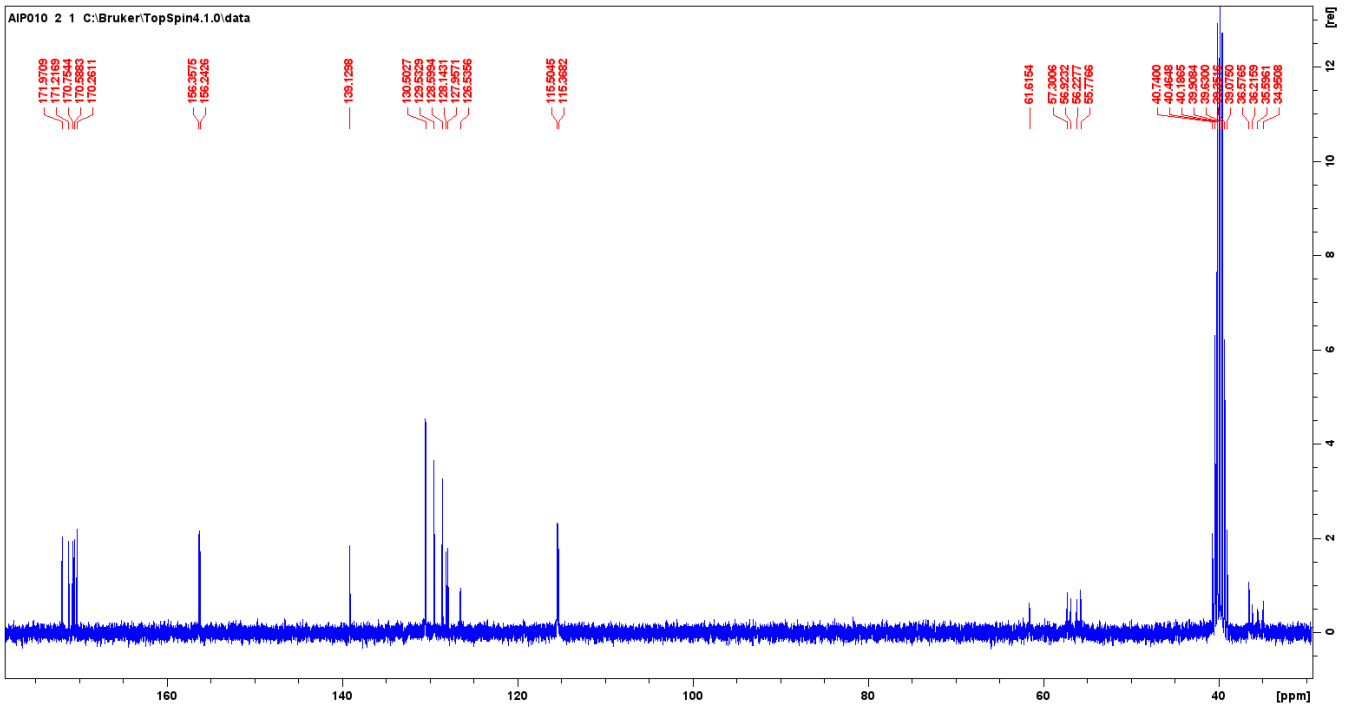


Figure S. 76. ^{13}C NMR spectrum of compound **23** in DMSO.

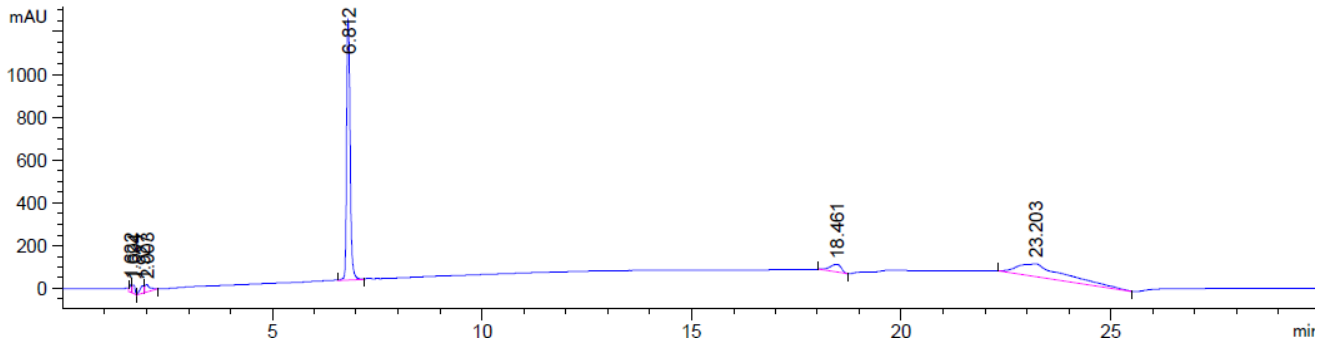


Figure S. 77. RP-HPLC chromatogram for compound **23** at 214 nm.

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
48 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
632.2711	632.2720	-0.9	-1.4	17.5	C33 H38 N5 O8	326.1	0.212	80.93	33	38	5	8
	632.2662	4.9	7.7	26.5	C40 H34 N5 O3	331.0	5.104	0.61	40	34	5	3
	632.2761	-5.0	-7.9	21.5	C38 H38 N3 O6	329.6	3.701	2.47	38	38	3	6
	632.2648	6.3	10.0	21.5	C39 H38 N O7	330.3	4.408	1.22	39	38	1	7
	632.2608	10.3	16.3	17.5	C34 H38 N3 O9	327.8	1.912	14.77	34	38	3	9

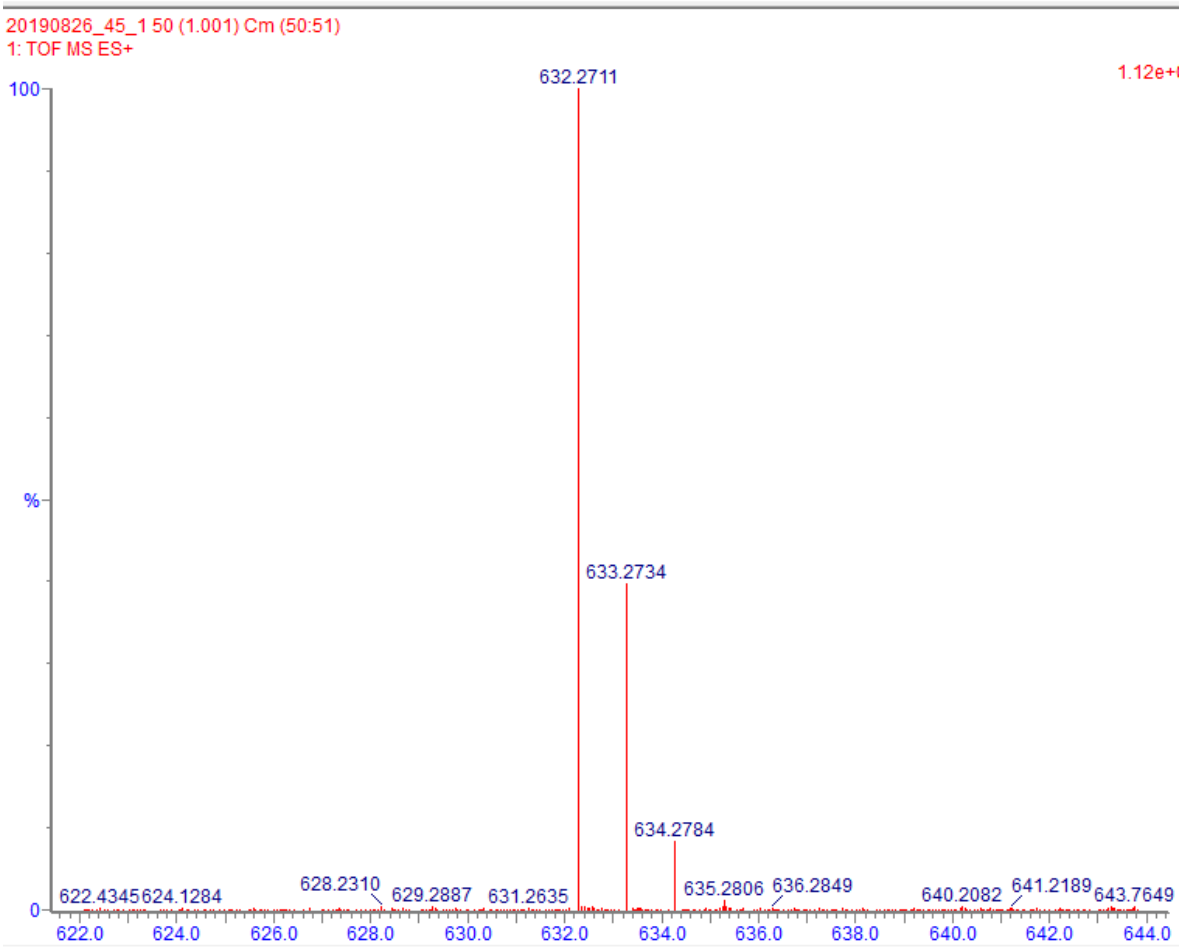


Figure S. 78. HRMS (ESI+) spectrum for compound **23**.

Spectral and Chromatographic Data for Compound 7

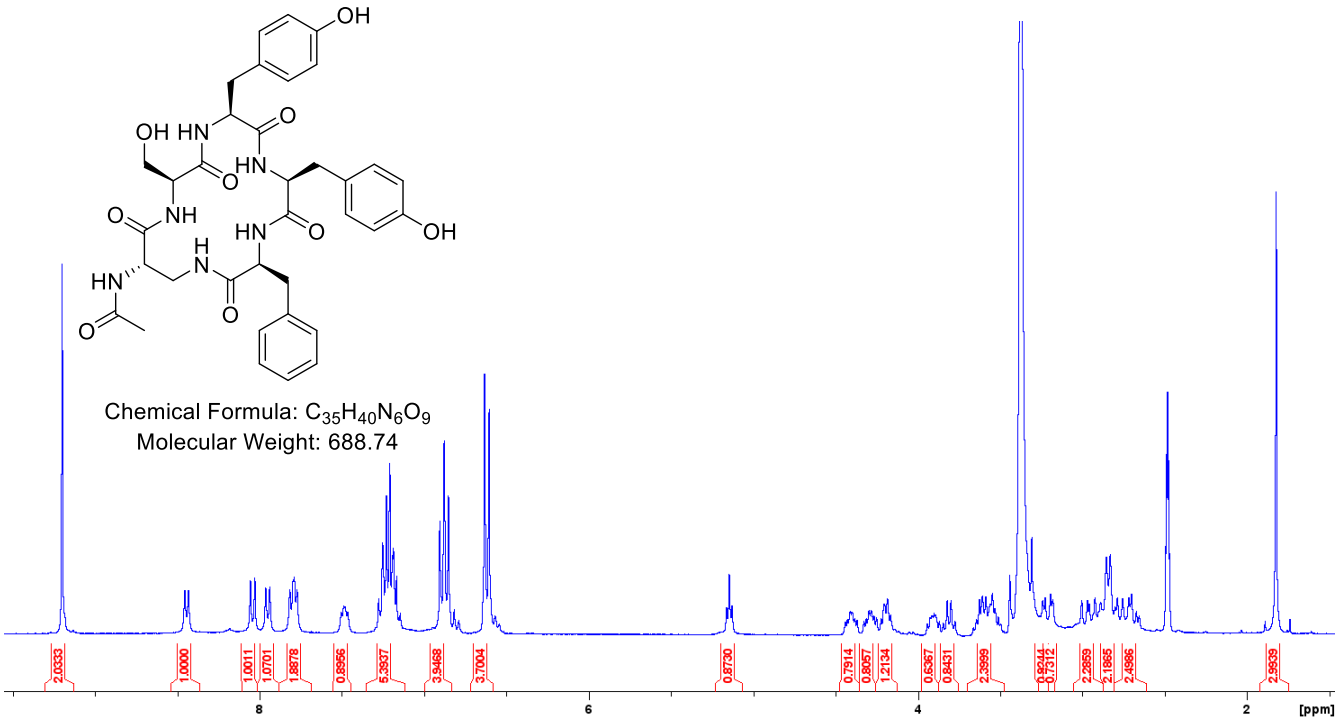


Figure S. 79. ^{1}H NMR spectrum for compound **7** in DMSO-d_6.

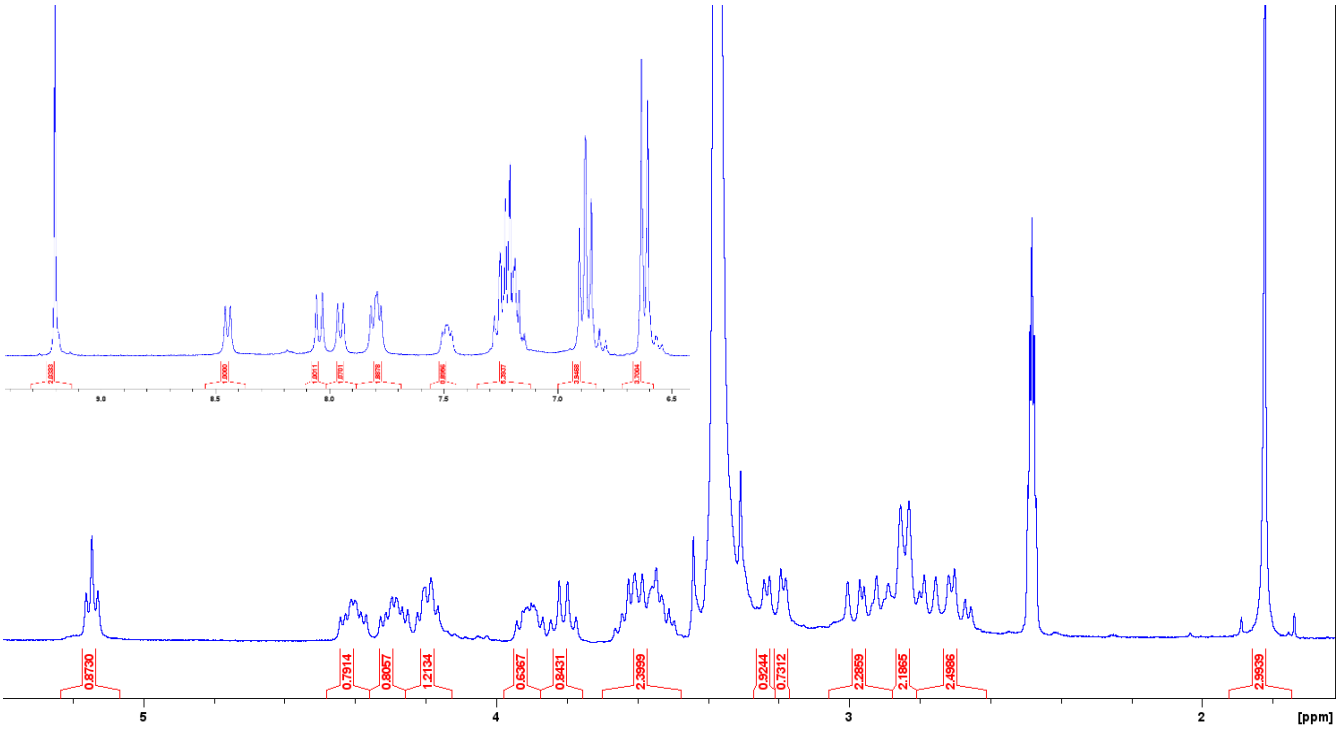


Figure S. 80. Expanded ^{1}H NMR spectrum for compound **7** (1 – 5 ppm), and an insert of 7 – 8 ppm.

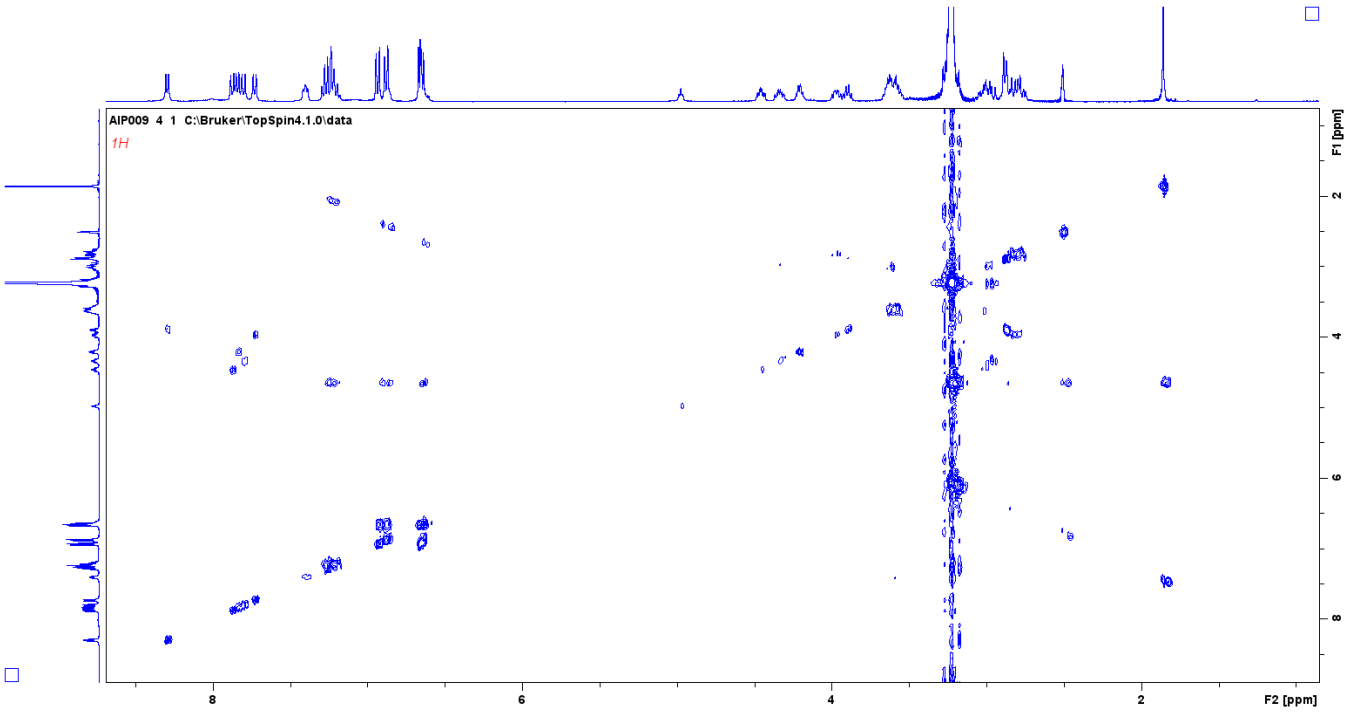


Figure S. 81. COSY NMR spectrum of compound **7** in DMSO-d_6.

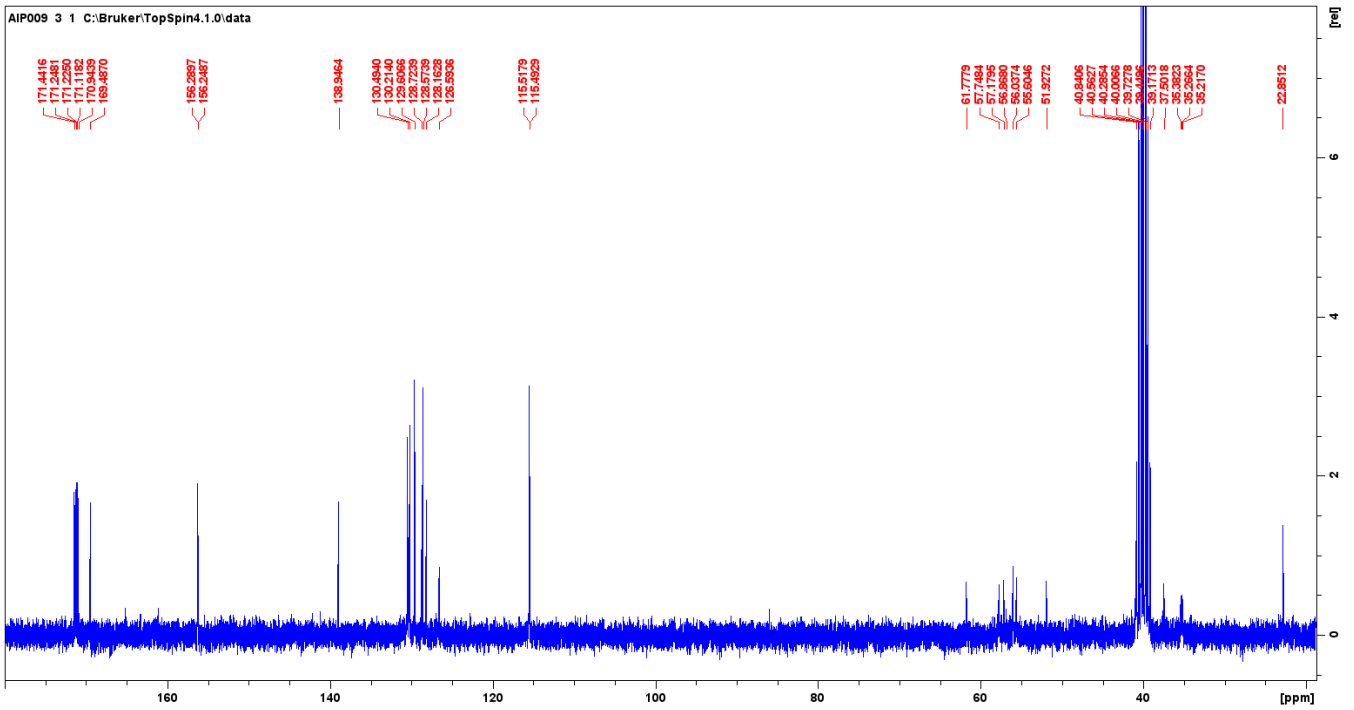


Figure S. 82. ^{13}C NMR spectrum of compound **7** in DMSO.

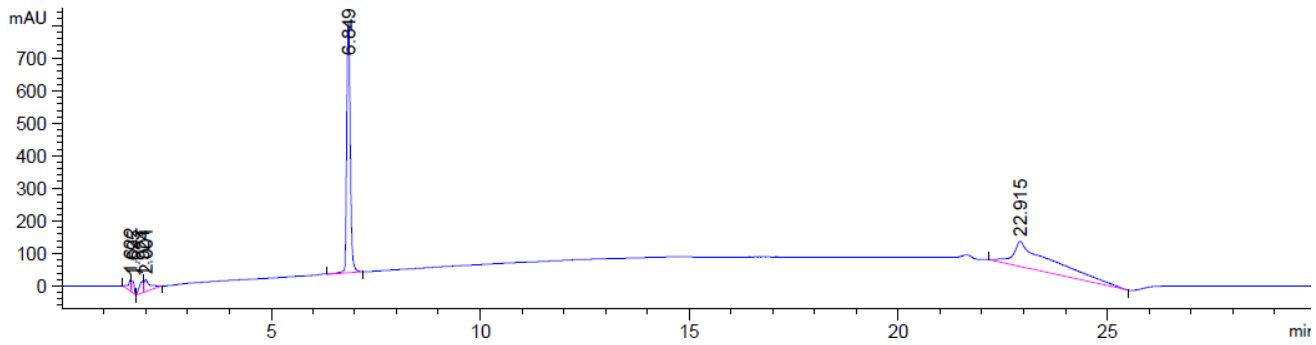


Figure S. 83. RP-HPLC chromatogram for compound **7** at 214 nm.

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
40 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N
689.2935	689.2935	0.0	0.0	18.5	C35 H41 N6 O9	722.6	0.049	95.17	35	41	6
	689.2975	-4.0	-5.8	22.5	C40 H41 N4 O7	727.8	5.254	0.52	40	41	4
	689.2823	11.2	16.2	18.5	C36 H41 N4 O10	725.7	3.145	4.31	36	41	4

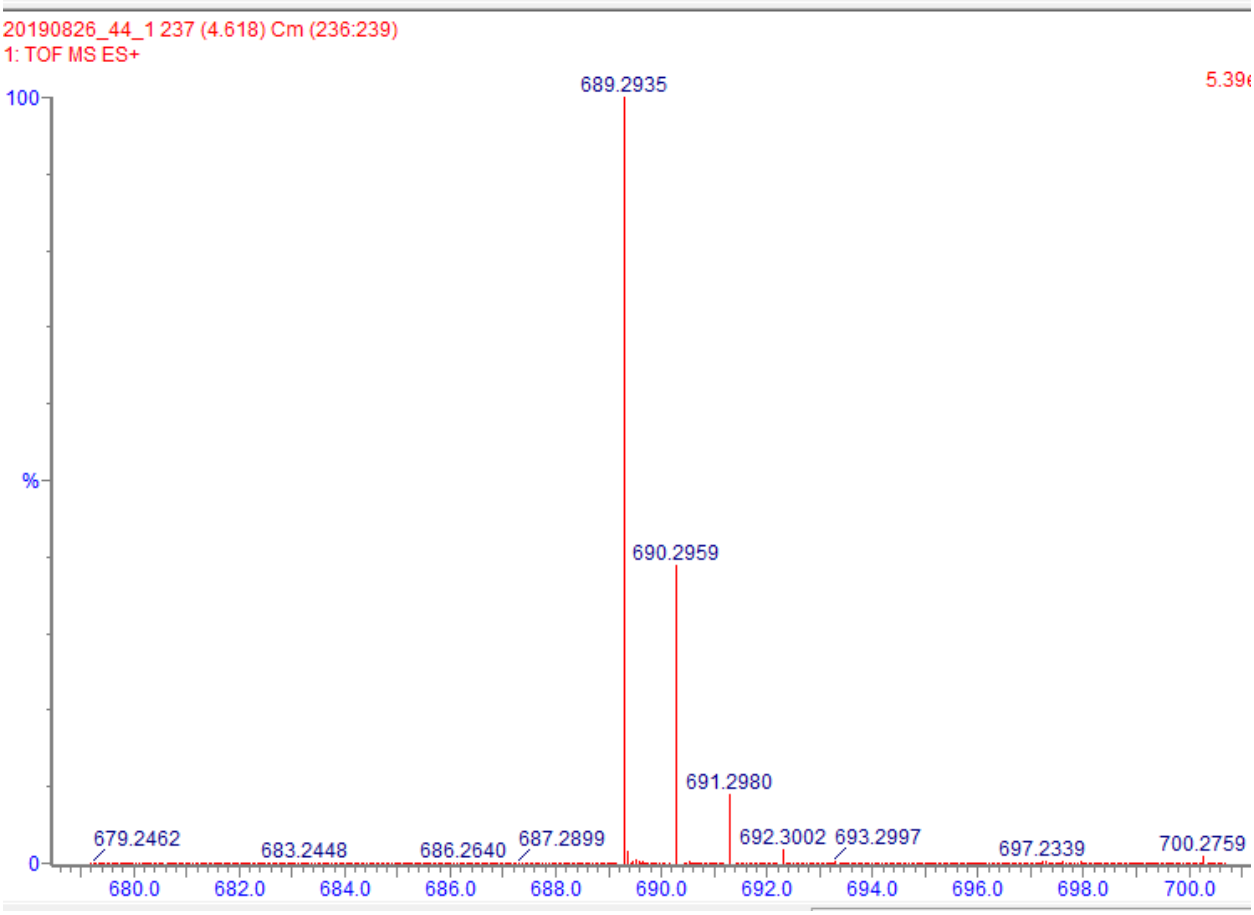


Figure S. 84. HRMS (ESI+) spectrum for compound **7**.

Spectral and Chromatographic Data for Compound 24

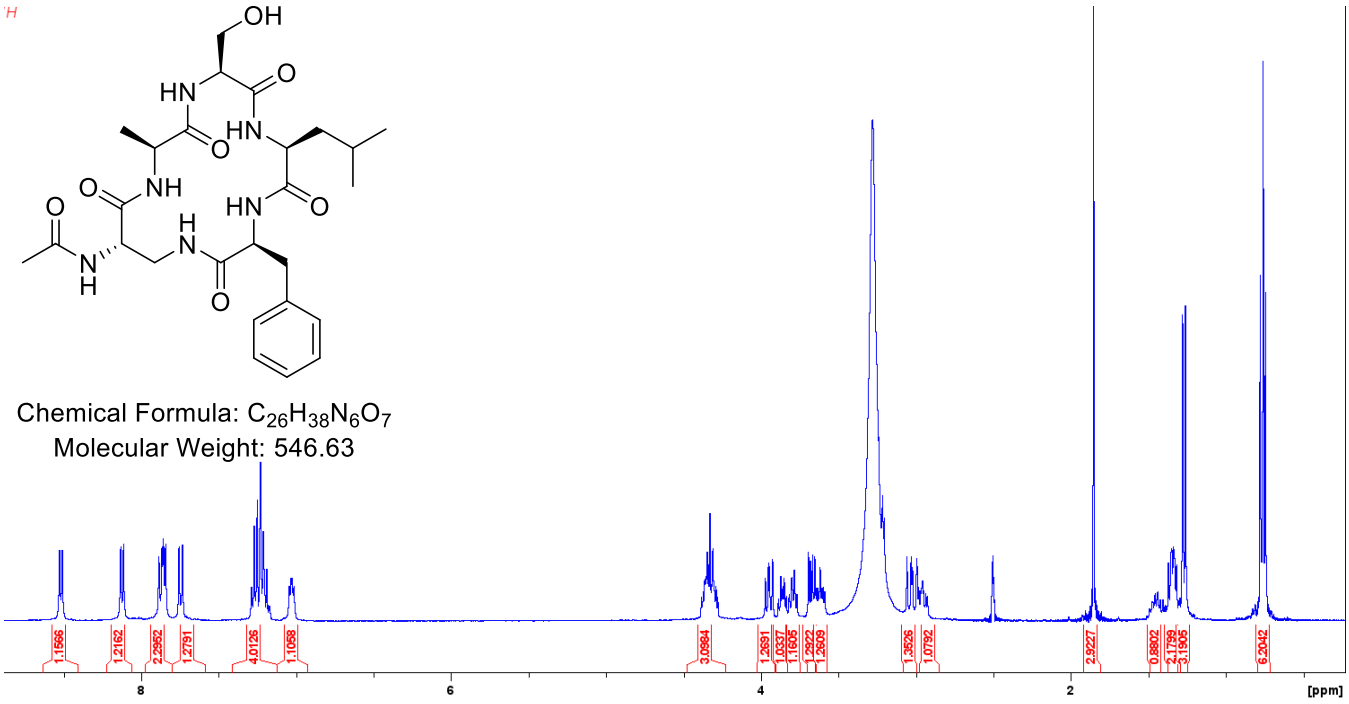


Figure S. 85. ^{1}H NMR spectrum for compound **24** in DMSO-d_6.

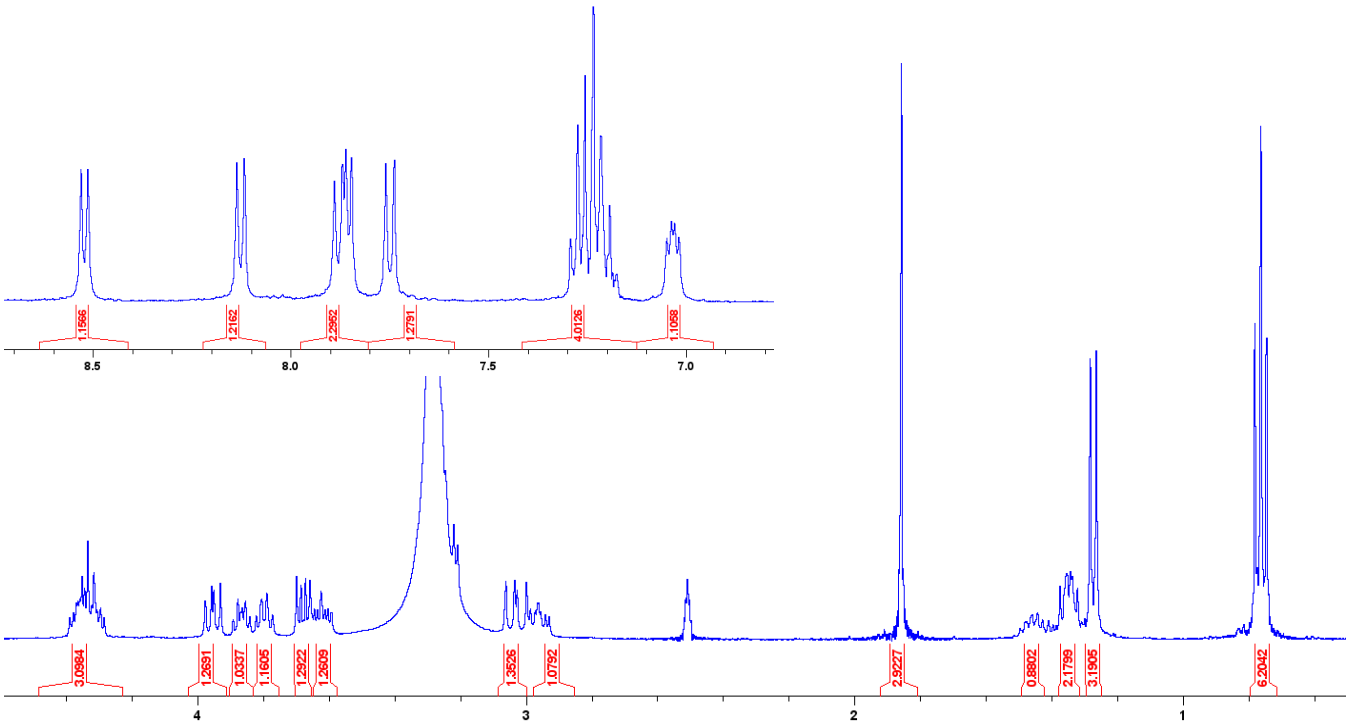


Figure S. 86. Expanded ^{1}H NMR spectrum for compound **24** (1 – 5 ppm), and an insert of 7 – 8 ppm.

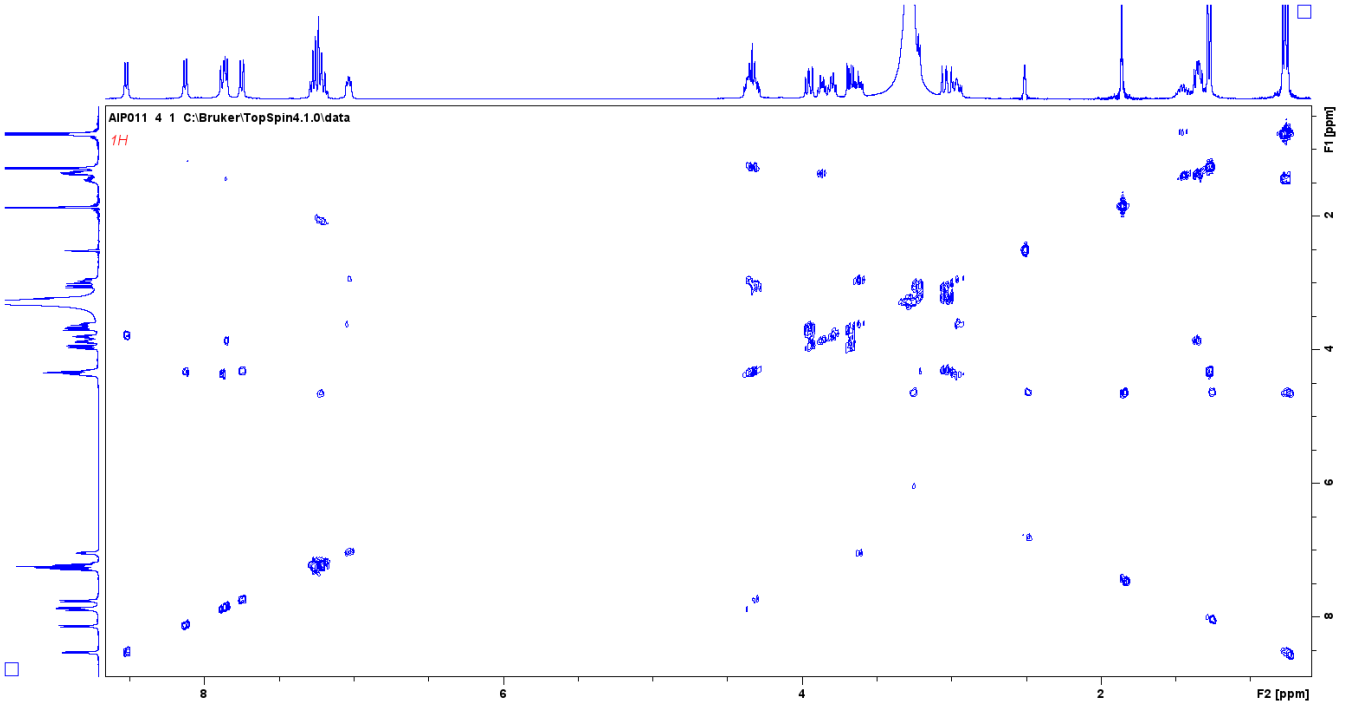


Figure S. 87. COSY NMR spectrum of compound **24** in DMSO-d_6.

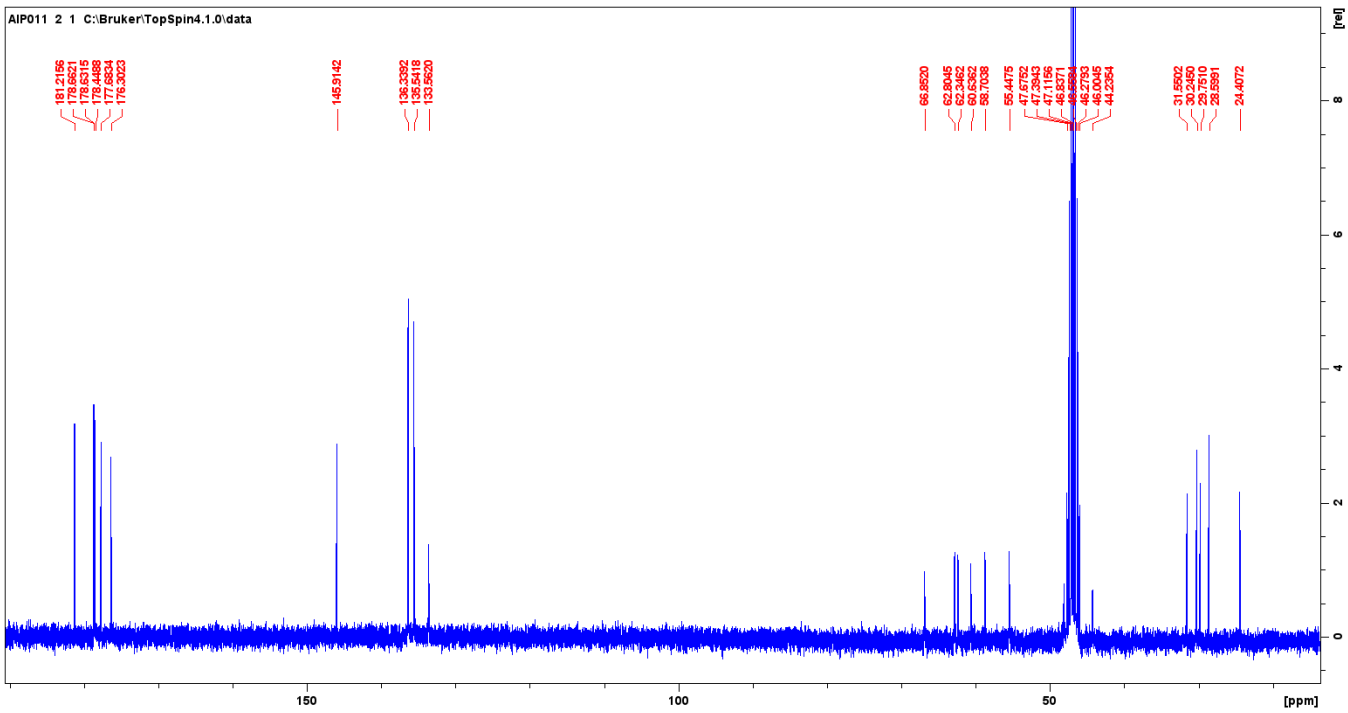


Figure S. 88. ^{13}C NMR spectrum of compound **24** in DMSO.

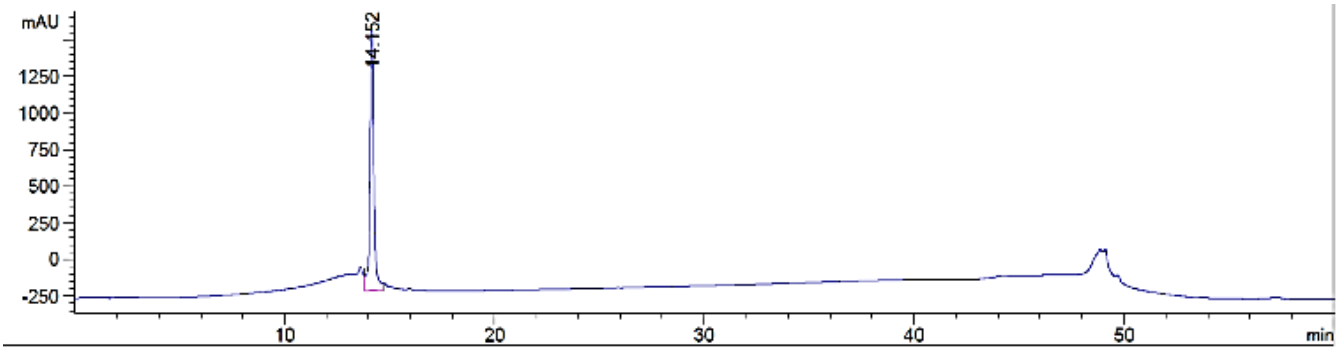


Figure S. 89. RP-HPLC chromatogram for compound **24** at 214 nm.

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
48 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
547.2881	547.2880	0.1	0.2	10.5	C26 H39 N6 O7	608.7	n/a	n/a	26	39	6	7

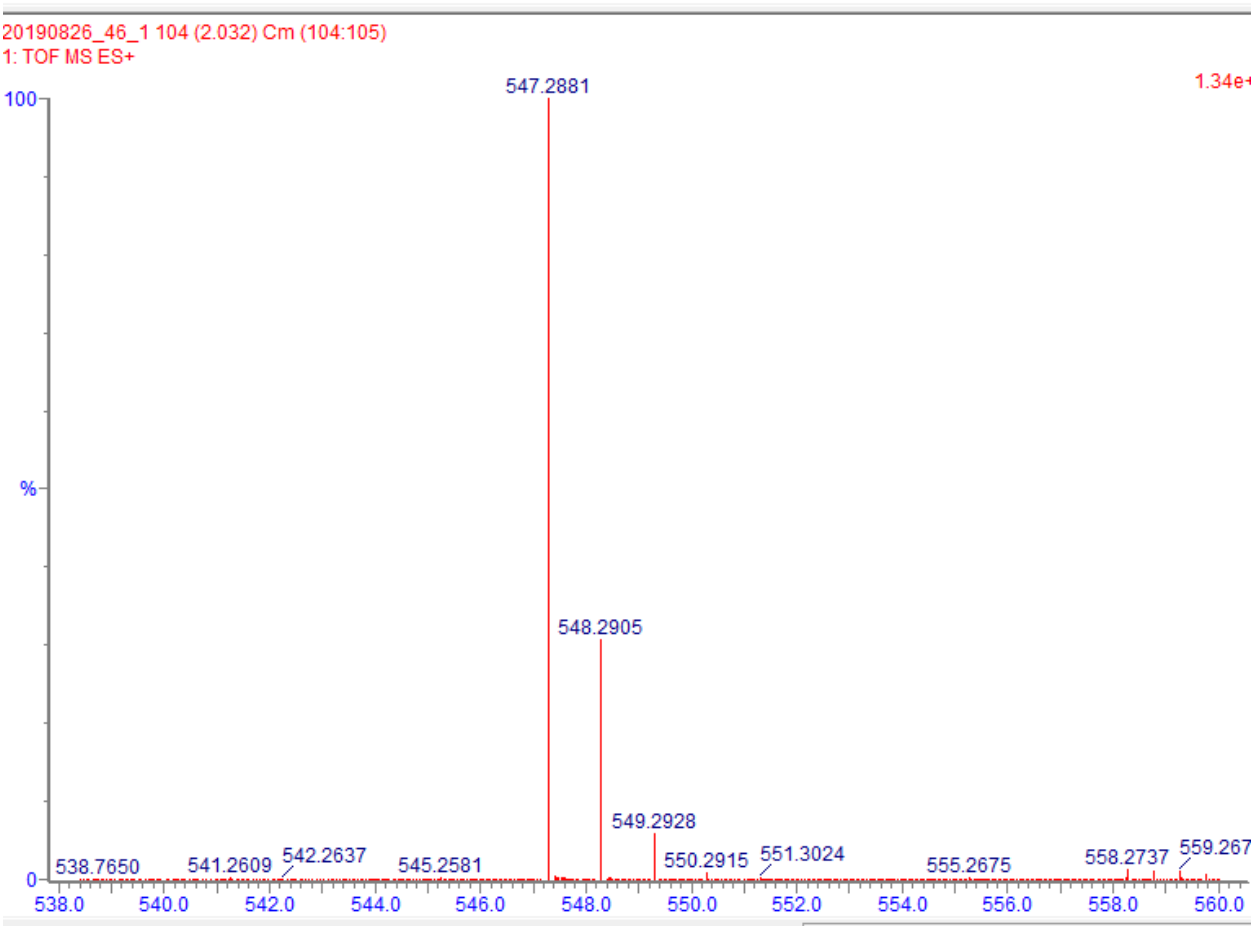


Figure S. 90. HRMS (ESI+) spectrum for compound **24**.

Spectral and Chromatographic Data for Compound 25

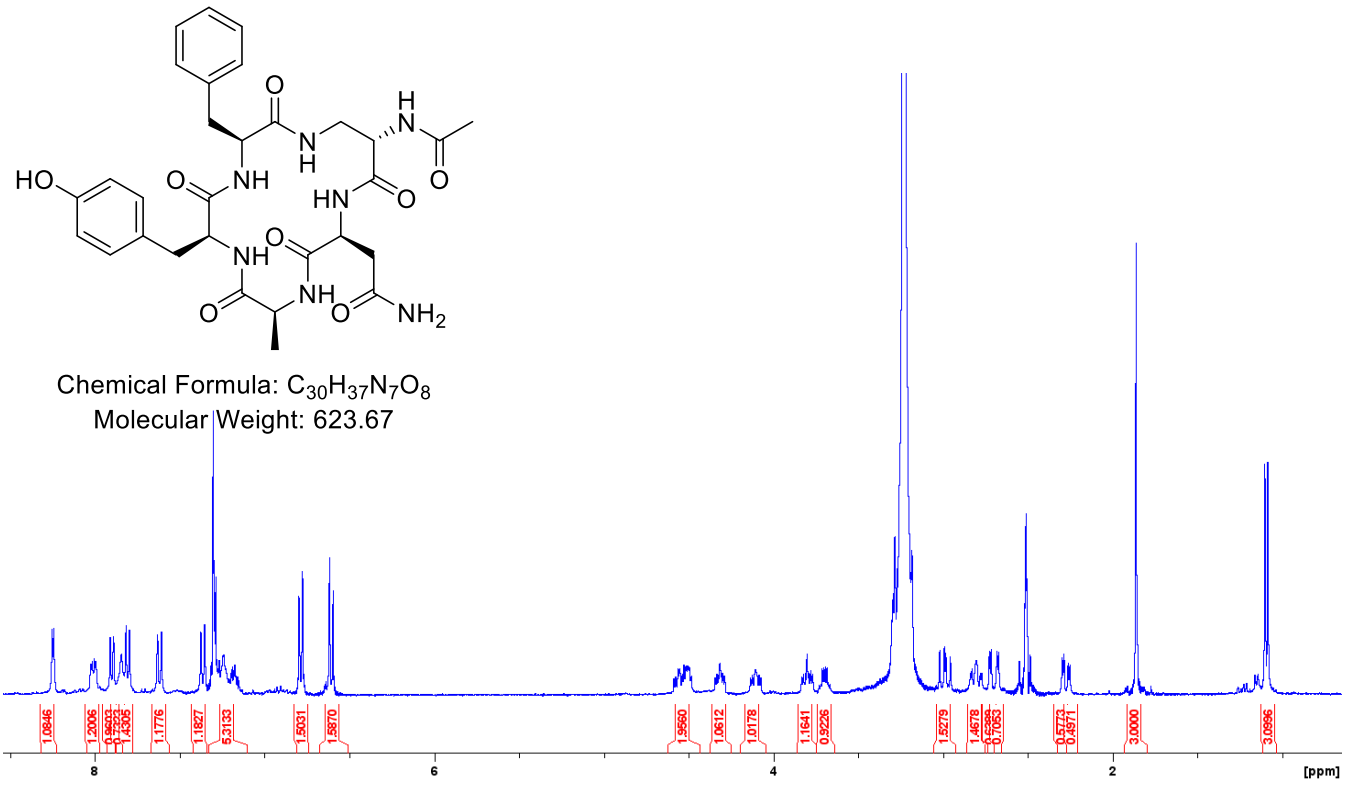


Figure S. 91. ^{1}H NMR spectrum for compound **25** in DMSO-d$_6$.

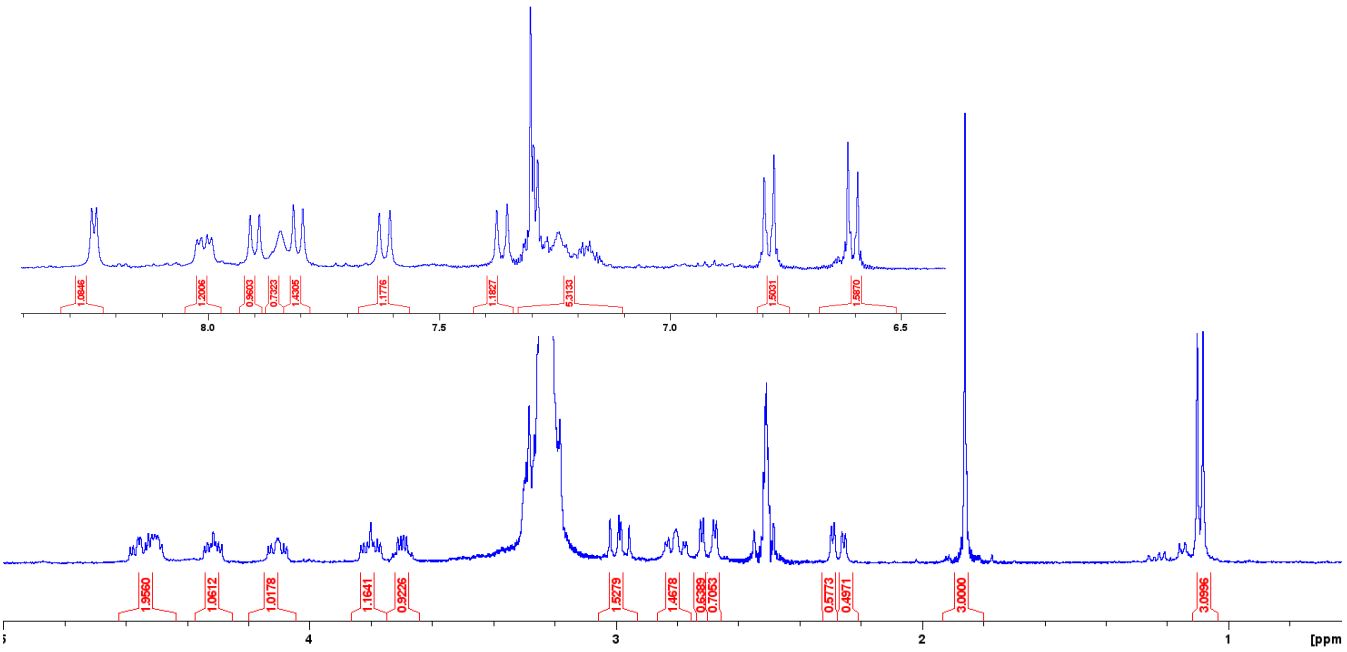


Figure S. 92. Expanded ^{1}H NMR spectrum for compound **25** (1 – 5 ppm), and an insert of 7 – 8 ppm.

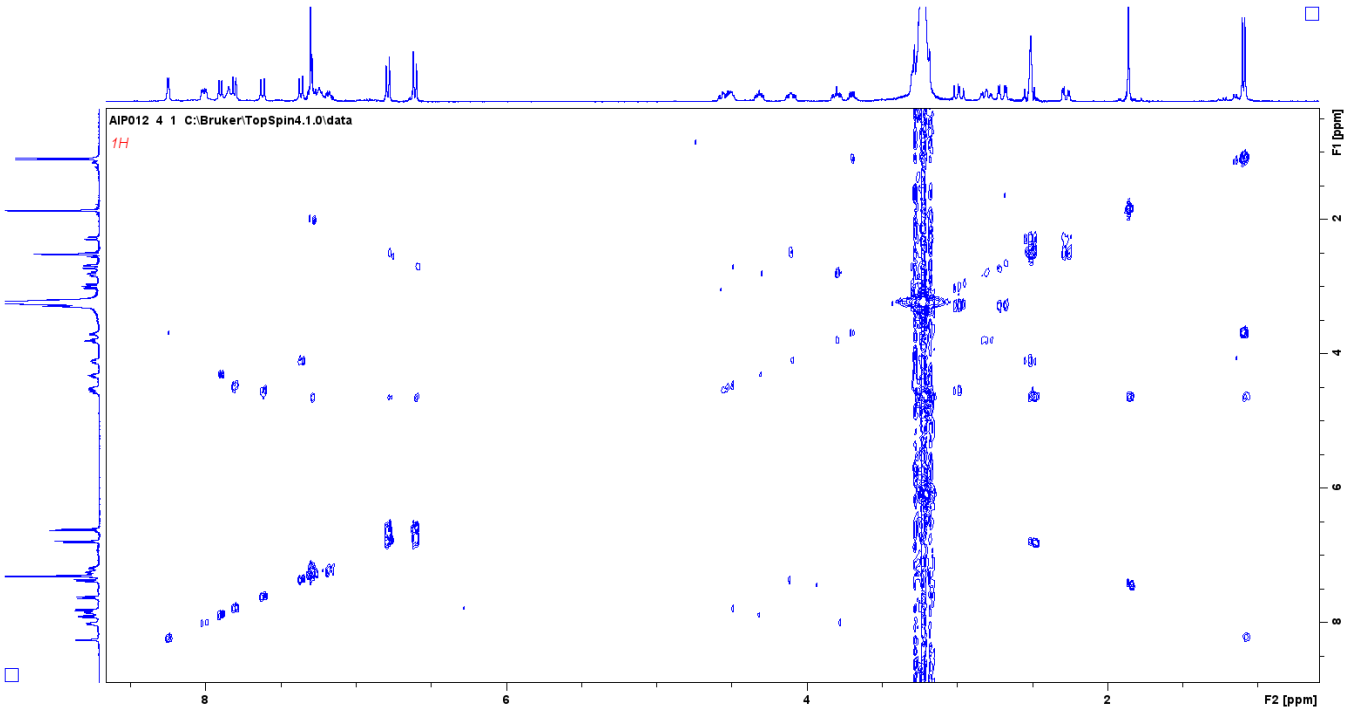


Figure S. 93. COSY NMR spectrum of compound **25** in DMSO-d_6.

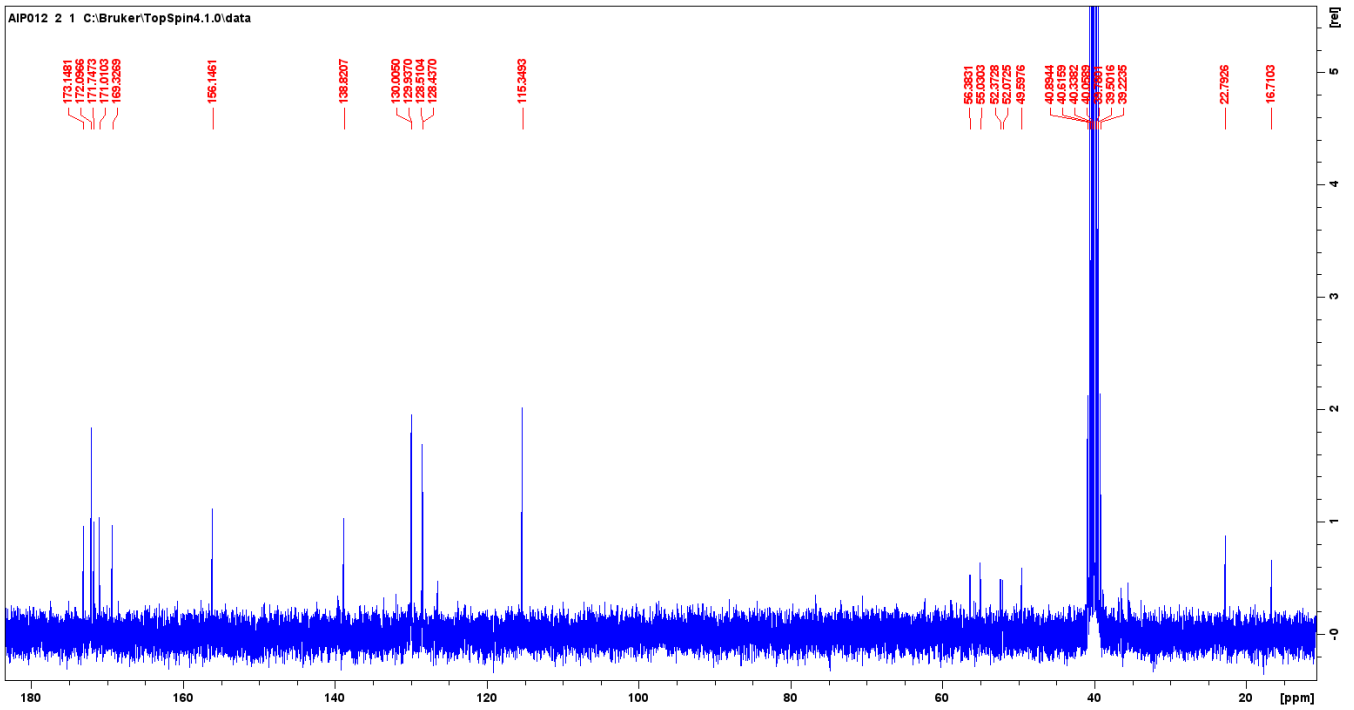


Figure S. 94. ^{13}C NMR spectrum of compound **25** in DMSO.

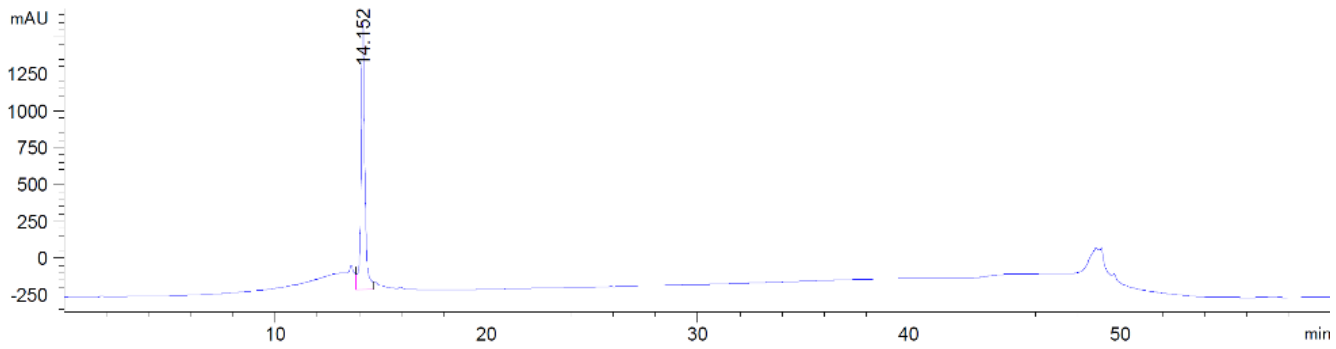


Figure S. 95. RP-HPLC chromatogram for compound **25** at 214 nm.

Single Mass Analysis

Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

71 formula(e) evaluated with 6 results within limits (all results (up to 1000) for each mass)

Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
624.2780	624.2782	-0.2	-0.3	15.5	C30 H38 N7 O8	312.8	0.399	67.10	30	38	7	8
	624.2822	-4.2	-6.7	19.5	C35 H38 N5 O6	315.7	3.266	3.82	35	38	5	6
	624.2723	5.7	9.1	24.5	C37 H34 N7 O3	317.2	4.738	0.88	37	34	7	3
	624.2710	7.0	11.2	19.5	C36 H38 N3 O7	316.4	3.963	1.90	36	38	3	7
	624.2862	-8.2	-13.1	23.5	C40 H38 N3 O4	318.8	6.326	0.18	40	38	3	4
	624.2670	11.0	17.6	15.5	C31 H38 N5 O9	313.8	1.342	26.13	31	38	5	9

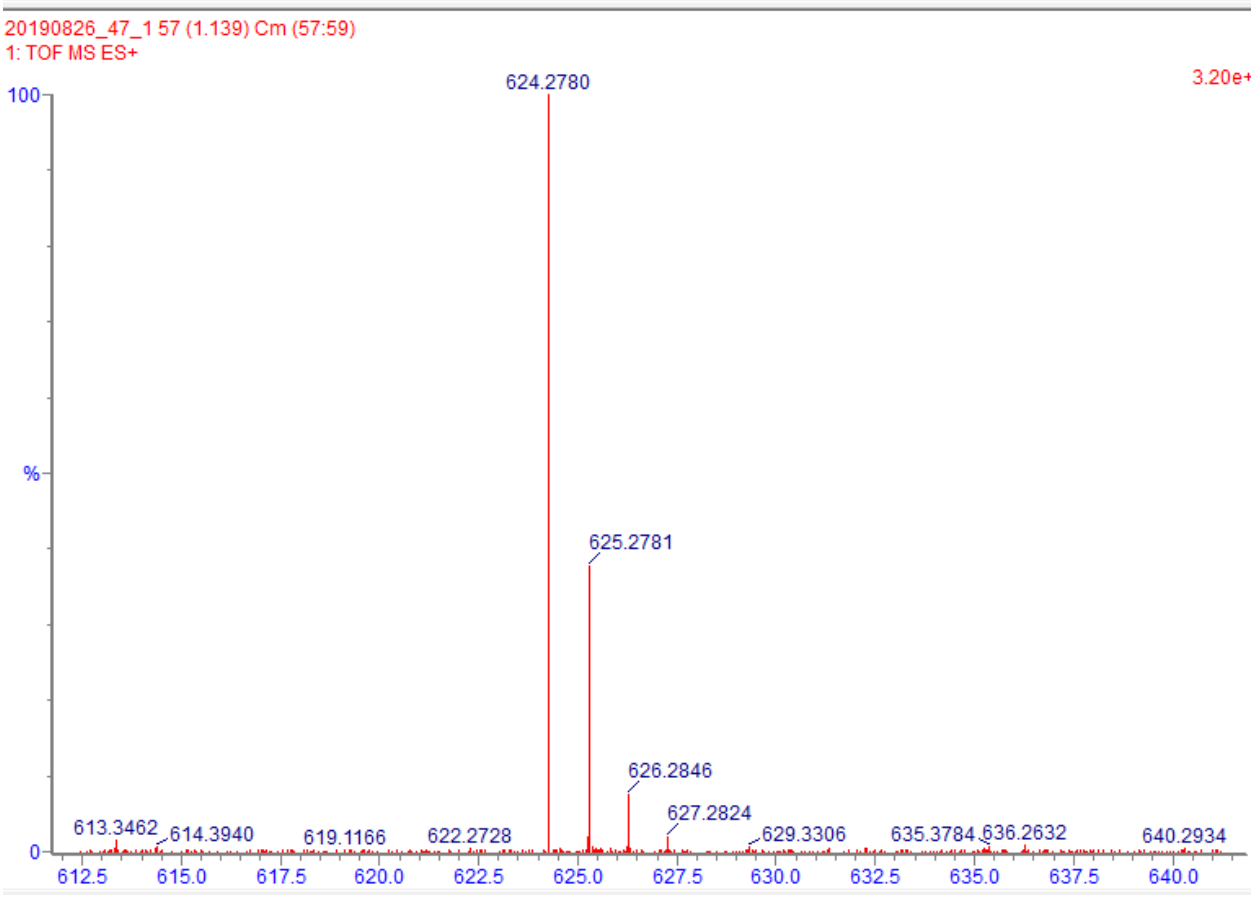


Figure S. 96. HRMS (ESI+) spectrum for compound **25**.

Spectral and Chromatographic Data for Compound 26

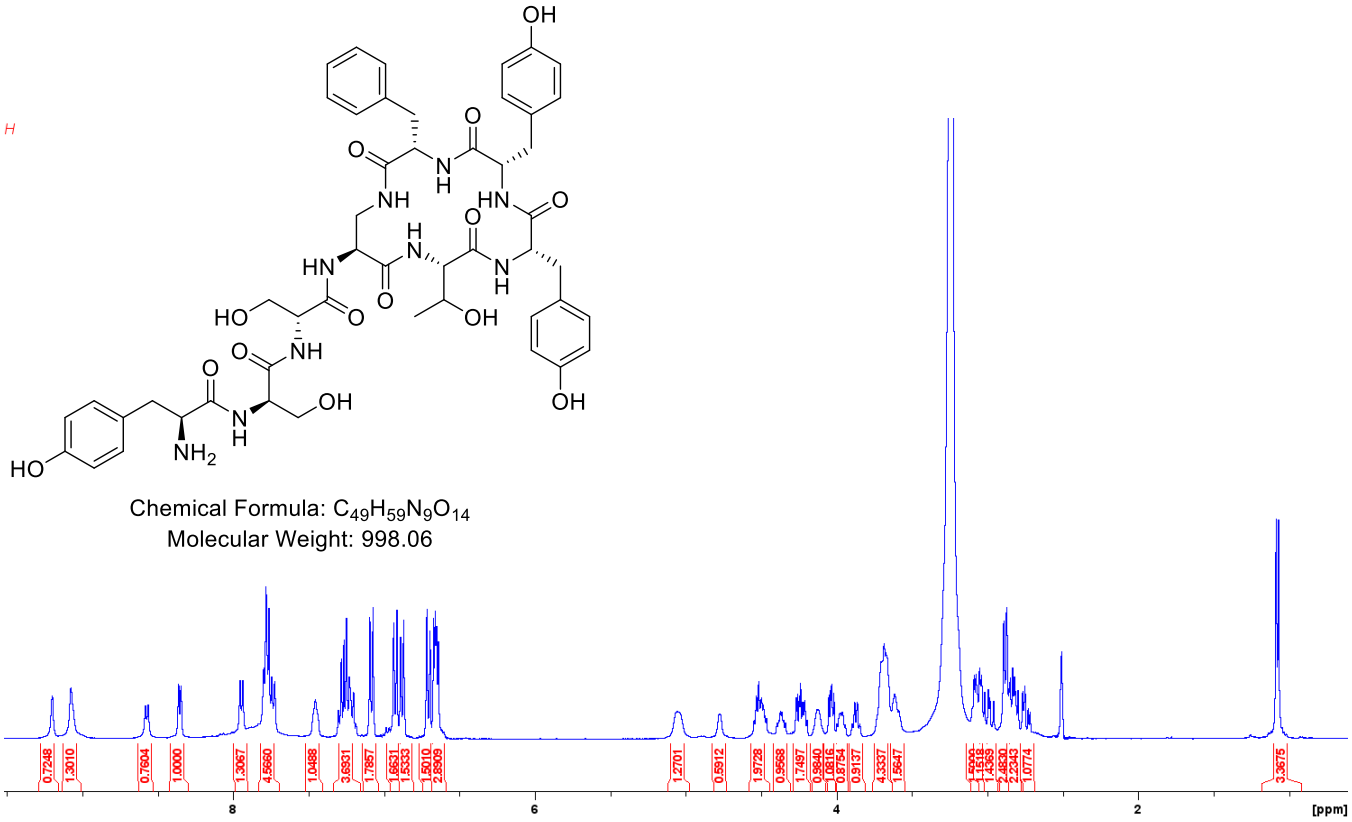


Figure S. 97. ^{1}H NMR spectrum for compound **26** in DMSO-d_6.

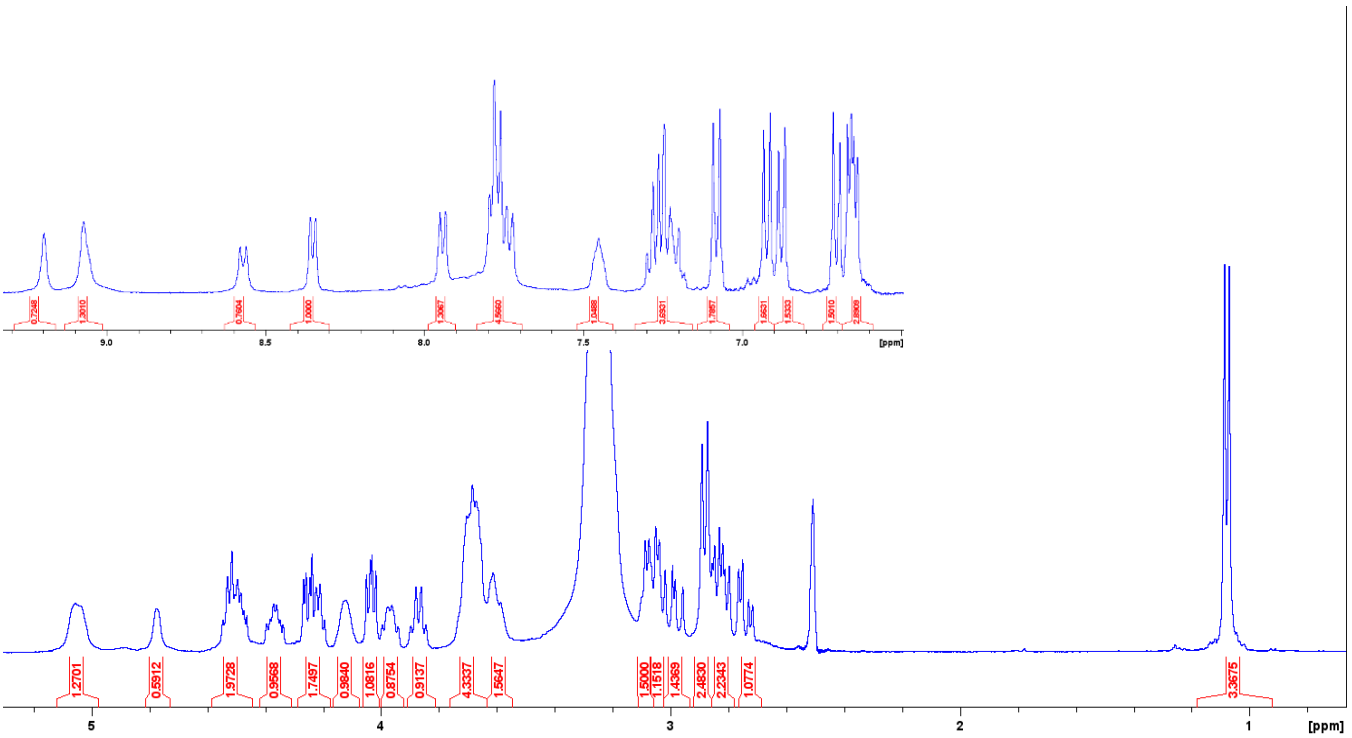


Figure S. 98. Expanded ^{1}H NMR spectrum for compound **26** (1 – 5 ppm), and an insert of 7 – 8 ppm.

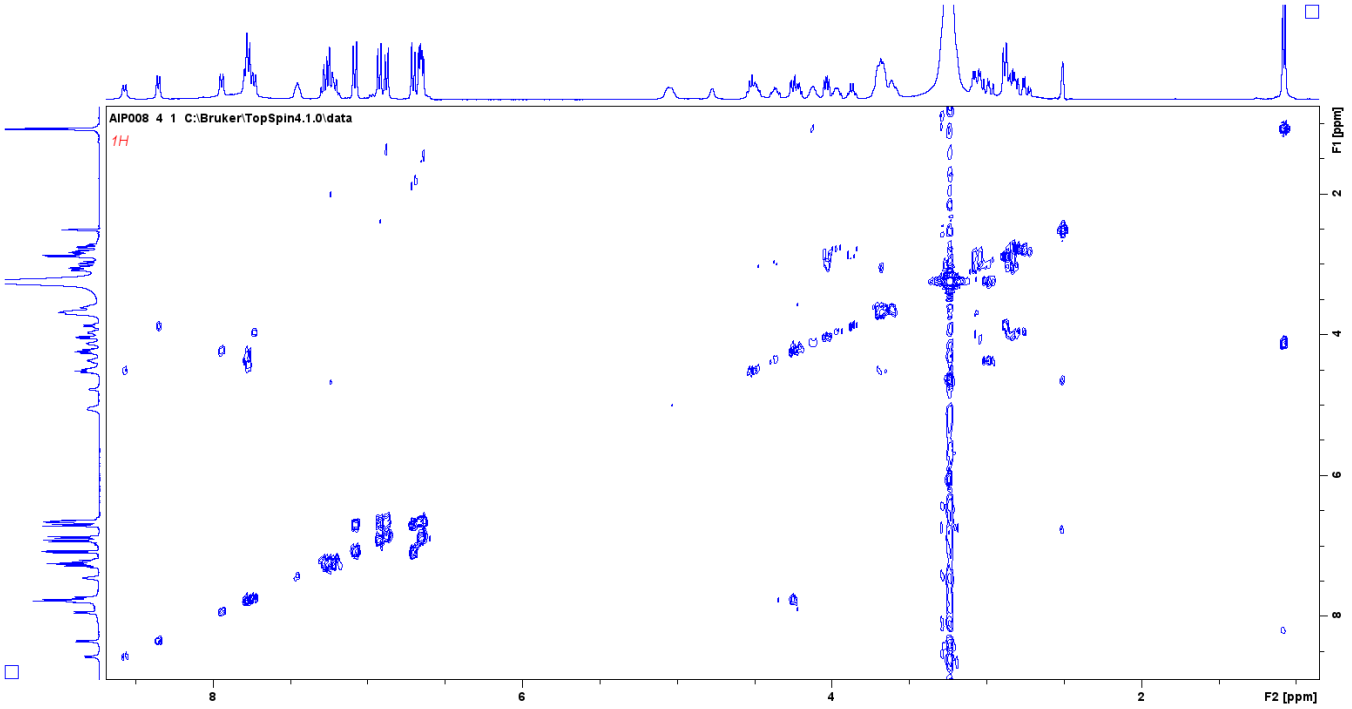


Figure S. 99. COSY NMR spectrum of compound **26** in DMSO-d_6.

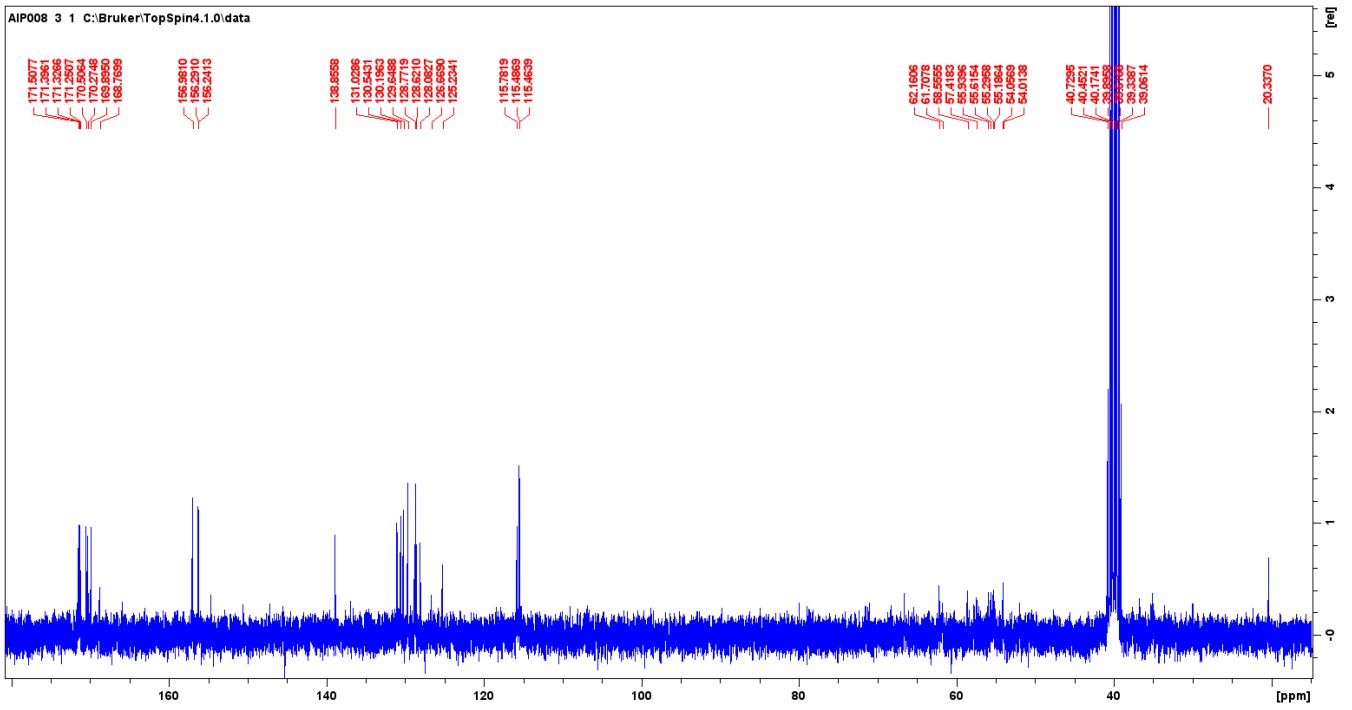


Figure S. 100. ^{13}C NMR spectrum of compound **26** in DMSO.

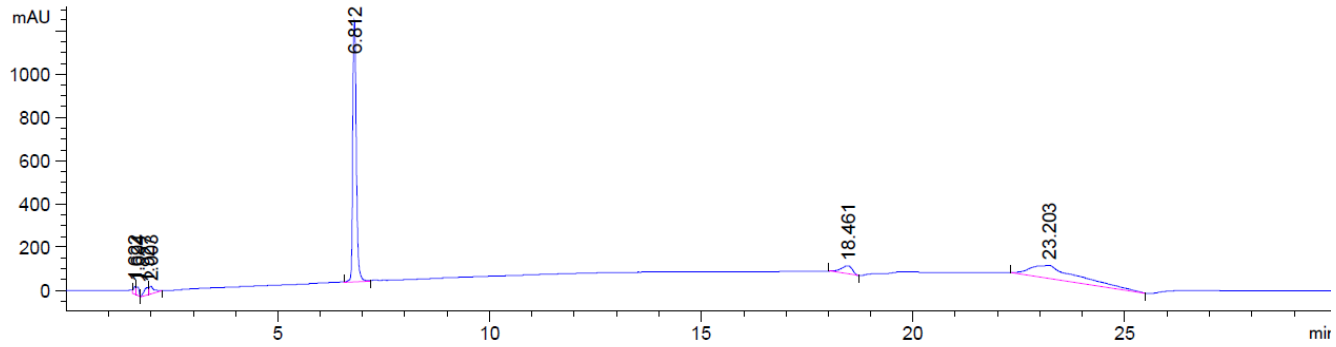


Figure S. 101. RP-HPLC chromatogram for compound **26** at 214 nm.

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Even Electron Ions
28 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O
998.4222	998.4260	-3.8	-3.8	24.5	C49 H60 N9 O14	223.6	0.032	96.85	49	60	9	14
	998.4147	7.5	7.5	24.5	C50 H60 N7 O15	227.0	3.459	3.15	50	60	7	15

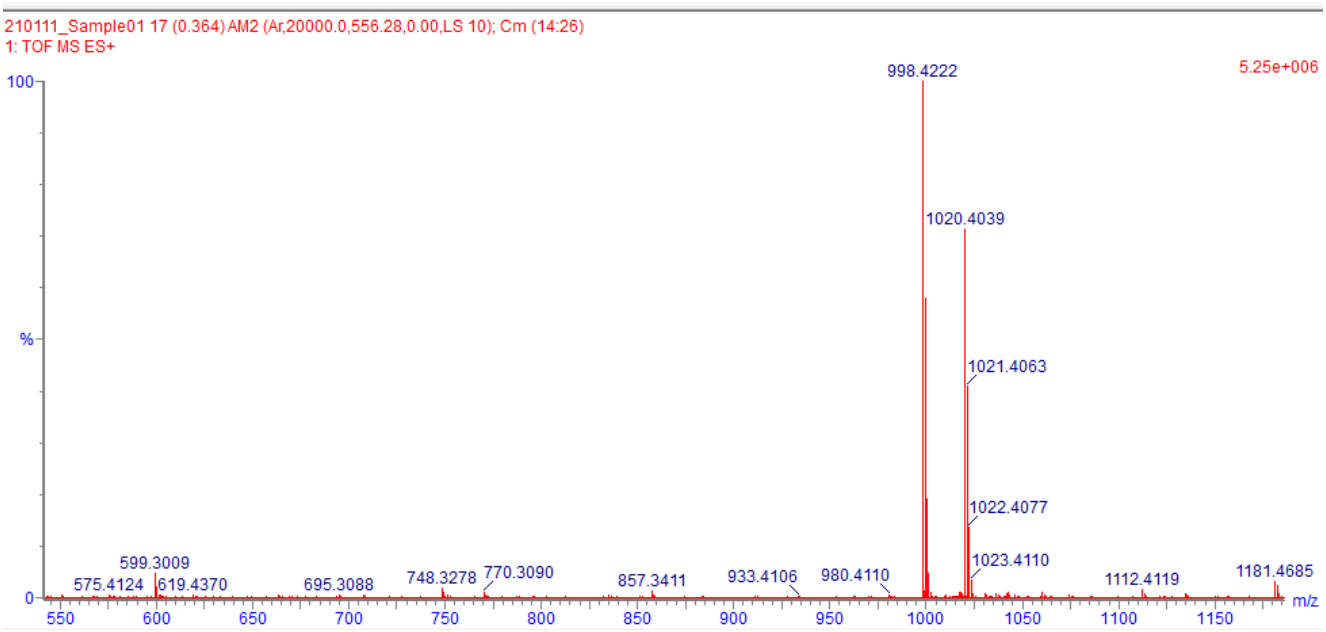


Figure S. 102. HRMS (ESI+) spectrum for compound **26**.

5. Chromatographic Data for Cbz Deprotection Optimisation Trials

5.1. Chromatographic Data for 10% Pd/C Cbz Hydrogenolysis of Compound 8

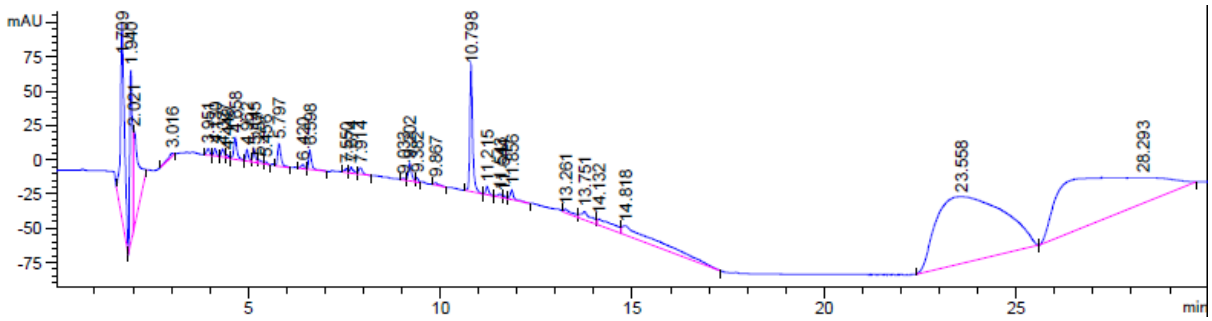


Figure S. 103. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under10% Pd/C, 20 °C, 0.5 mL/min

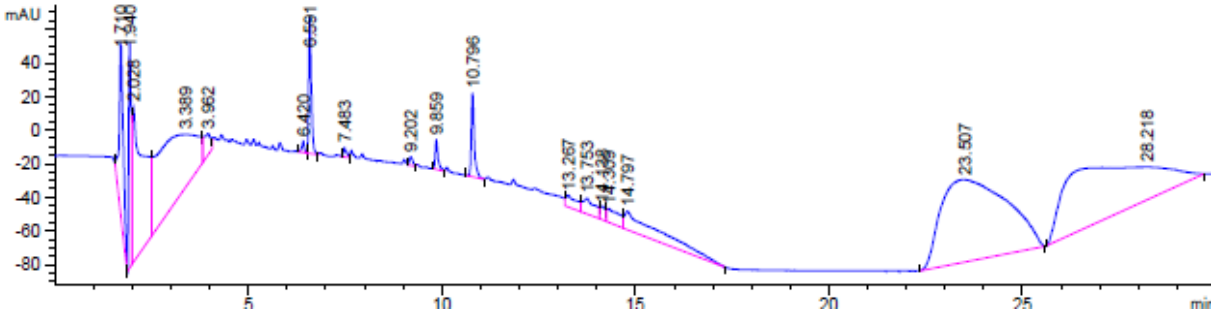


Figure S. 104. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 20 °C, 1.0 mL/min

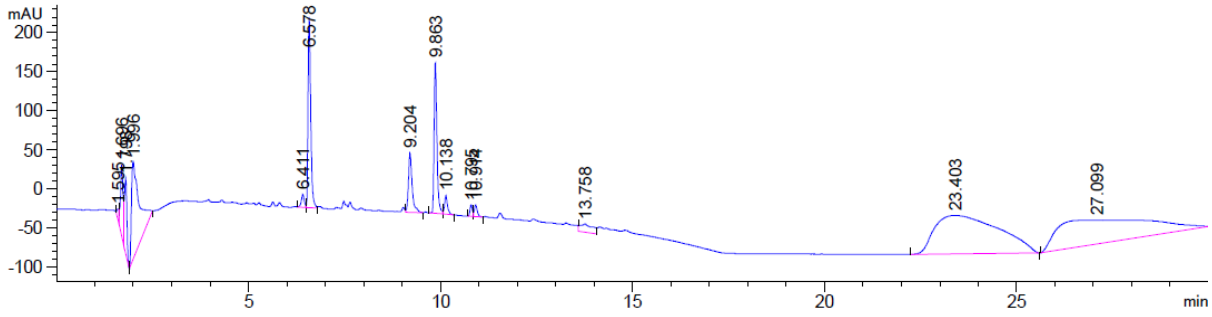


Figure S. 105. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 20 °C, 2.0 mL/min

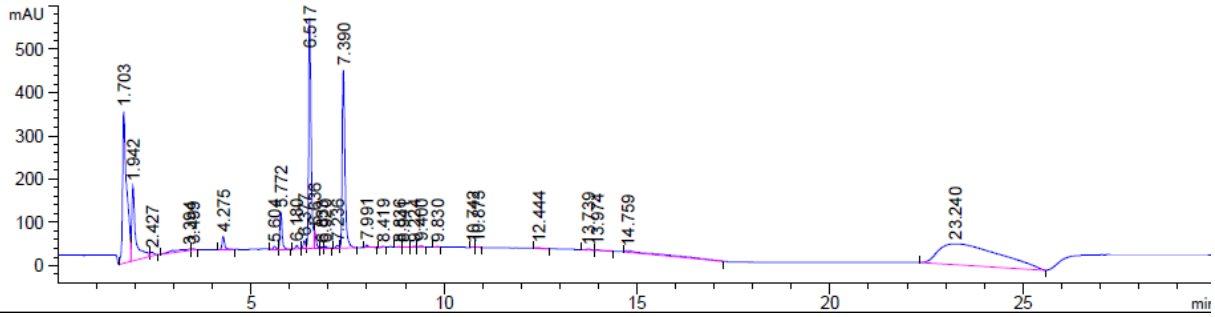


Figure S. 106. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 40 °C, 0.5 mL/min

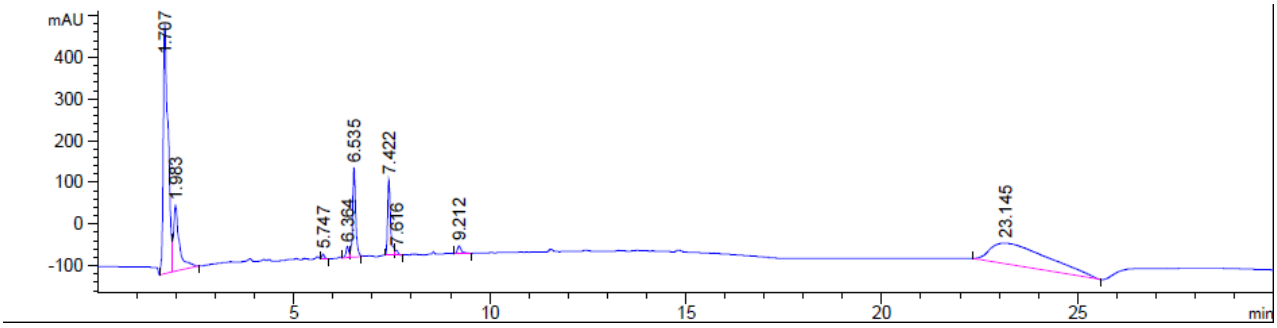


Figure S. 107. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 40 °C, 1.0 mL/min

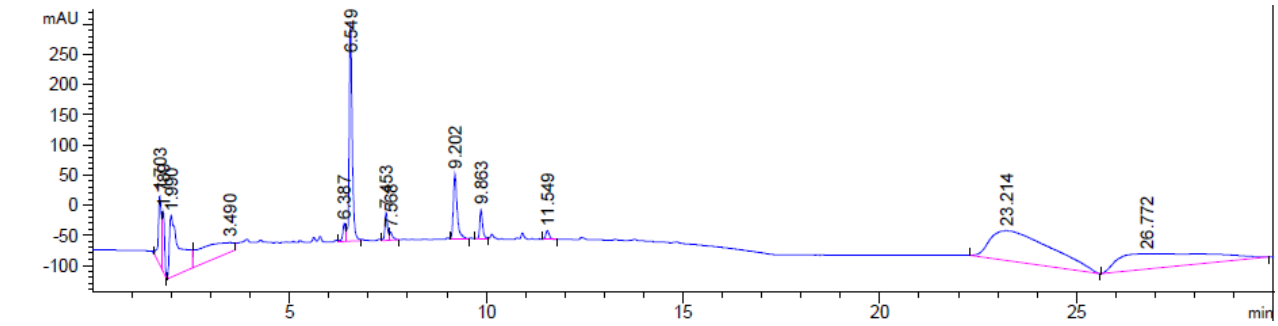


Figure S. 108. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 40 °C, 2.0 mL/min

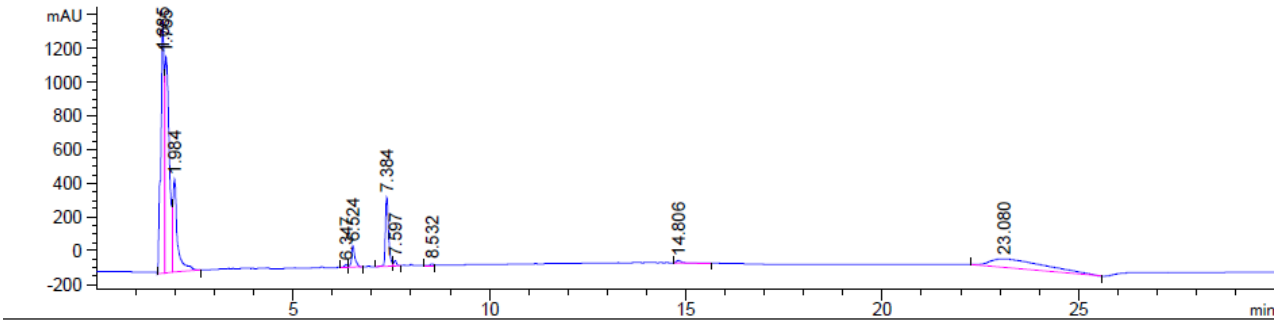


Figure S. 109. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 60 °C, 0.5 mL/min

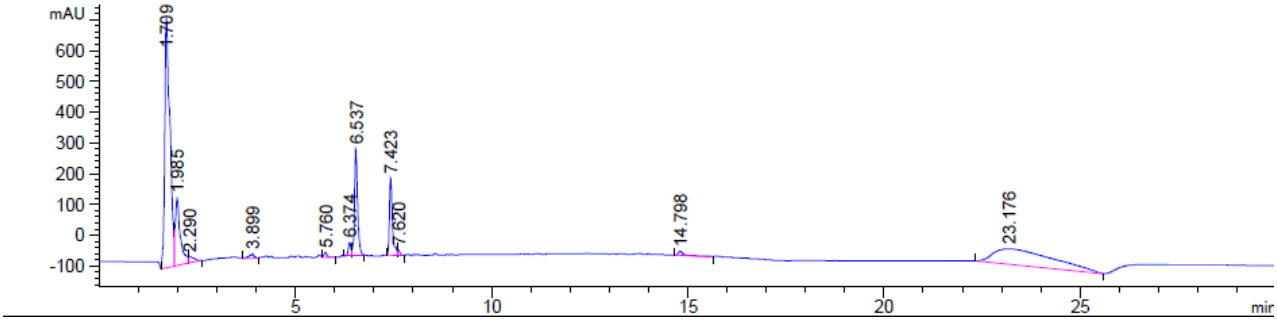


Figure S. 110. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 60 °C, 1.0 mL/min

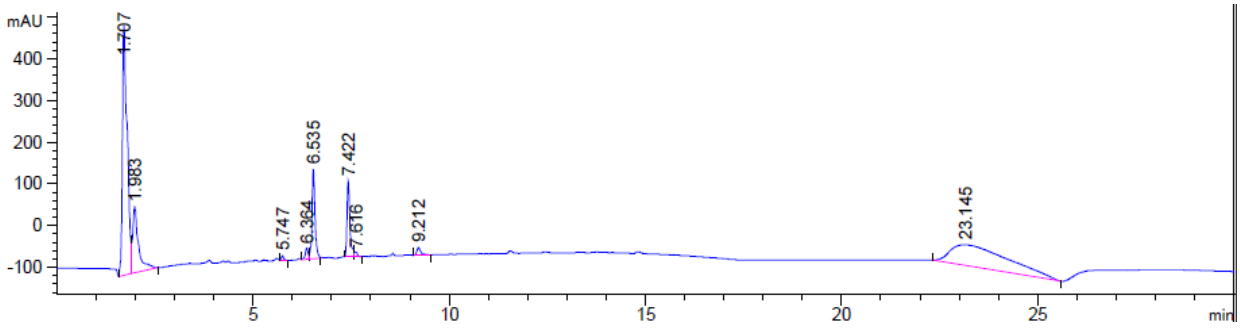


Figure S. 111. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 60 °C, 2.0 mL/min

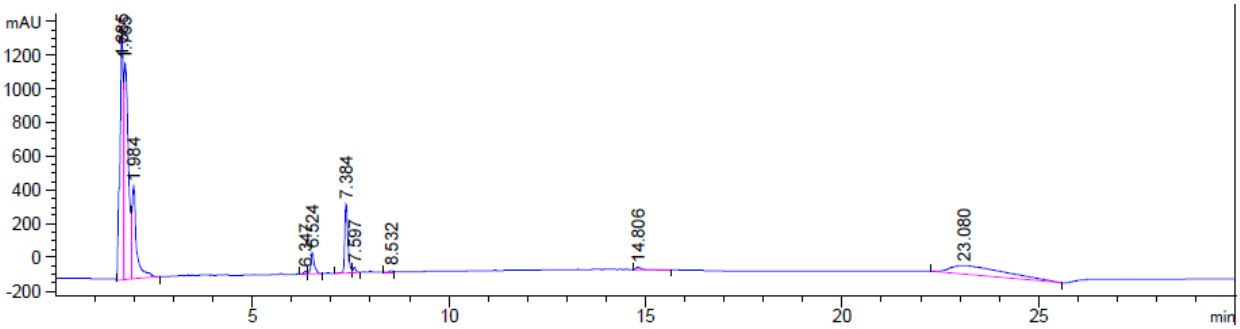


Figure S. 112. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 80 °C, 0.5 mL/min

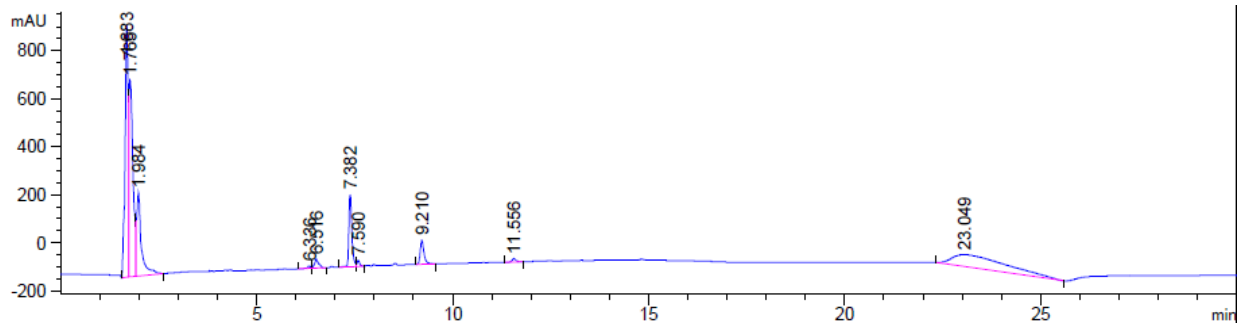


Figure S. 113. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 80 °C, 1.0 mL/min

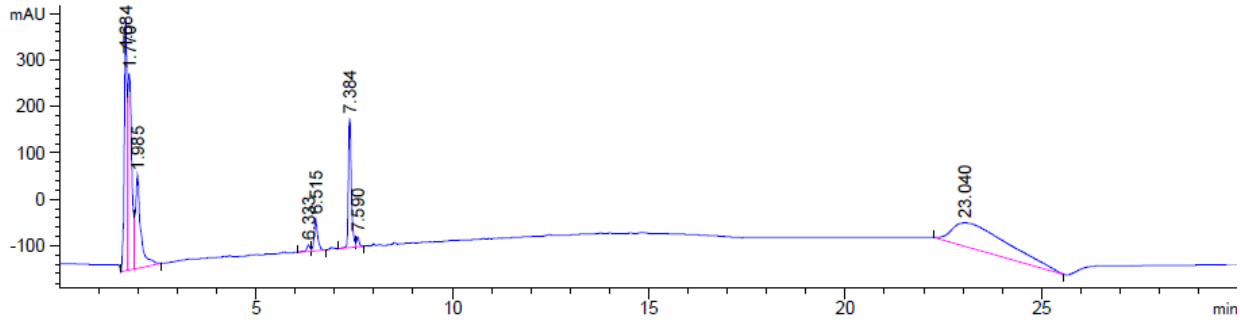


Figure S. 114. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/C, 80 °C, 2.0 mL/min

5.2 Chromatographic Data for Catalyst Trials for Cbz Hydrogenolysis of Compound 8

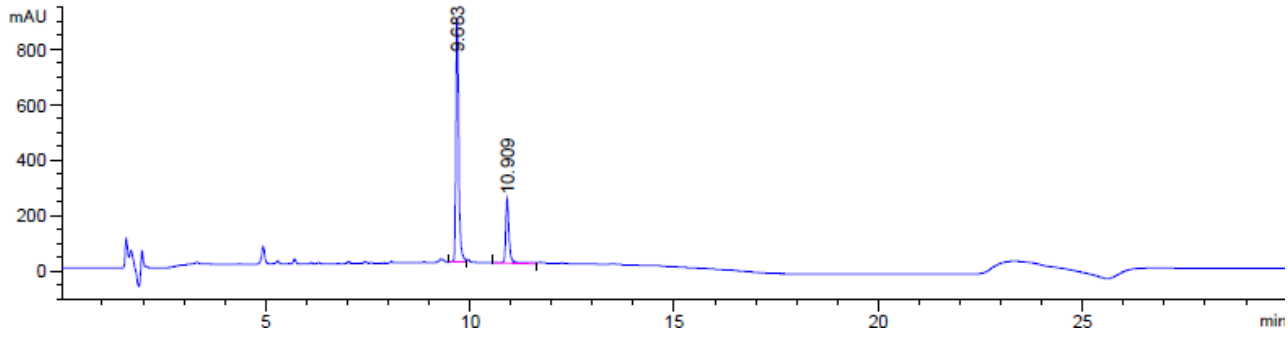


Figure S. 115. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under CuO/Al_2O_3, across all flow rates (0.5 – 2.0 mL/min), and temperatures (20 – 80 °C).

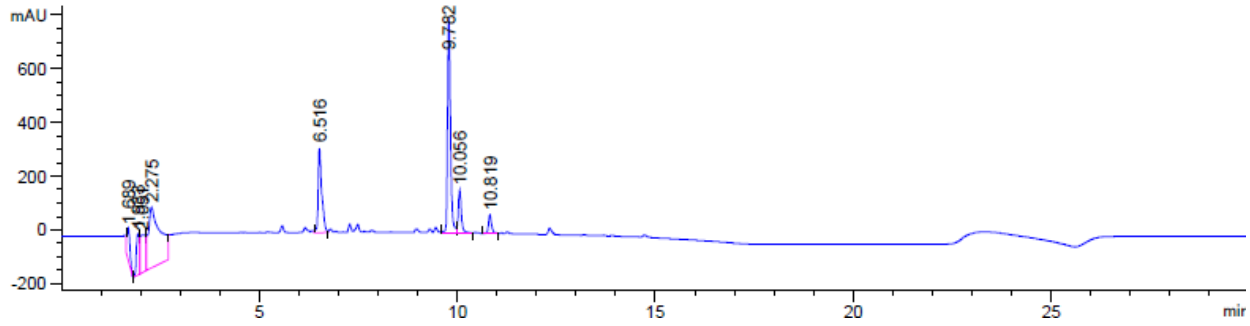


Figure S. 116. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% $Pd/CaCO_3$, 20 °C, 0.5 mL/min.

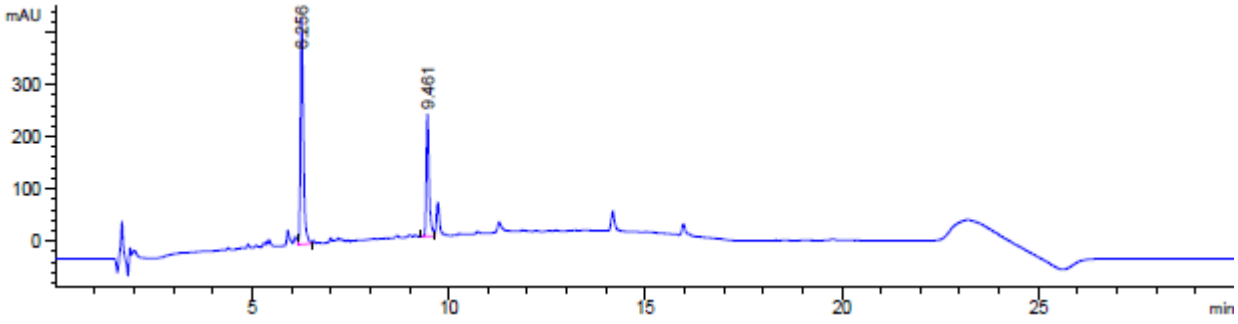


Figure S. 117. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% $Pd/CaCO_3$, 20 °C, 1.0 mL/min.

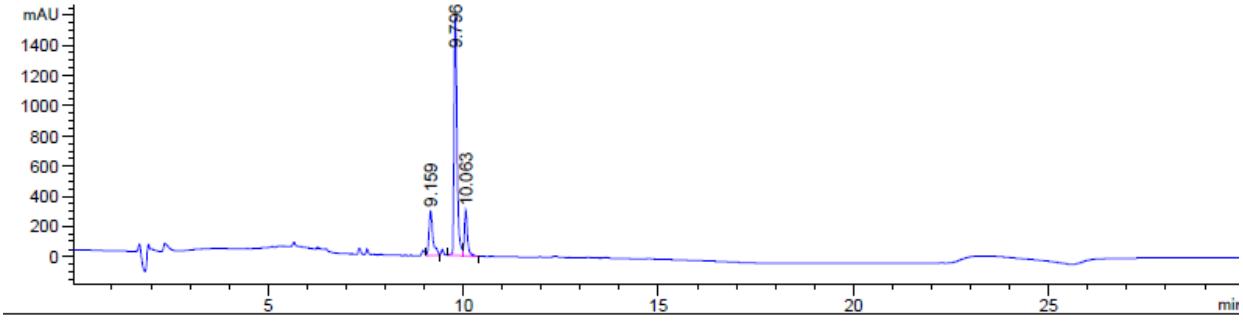


Figure S. 118. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% $Pd/CaCO_3$, 20 °C, 2.0 mL/min.

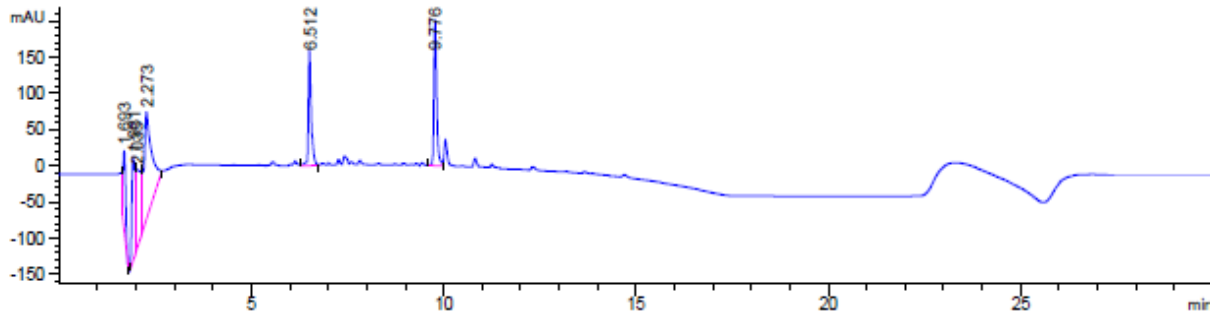


Figure S. 119. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/$CaCO_3$, 40 °C, 0.5 mL/min

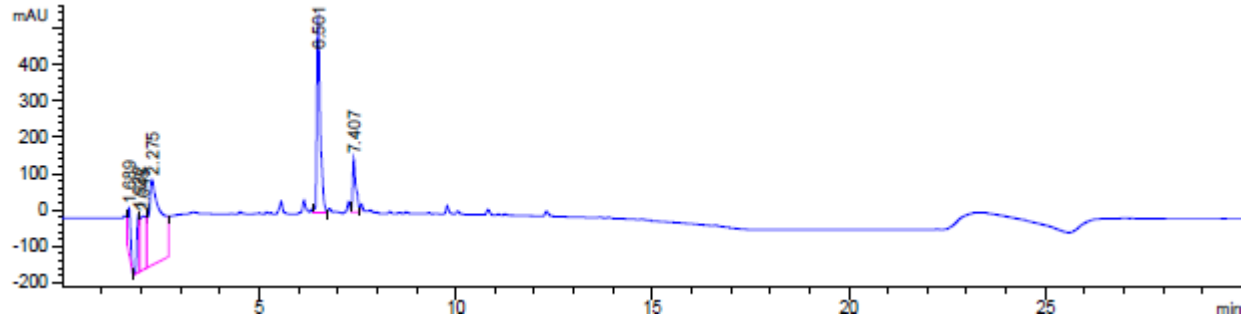


Figure S. 120. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/$CaCO_3$, 40 °C, 1.0 mL/min

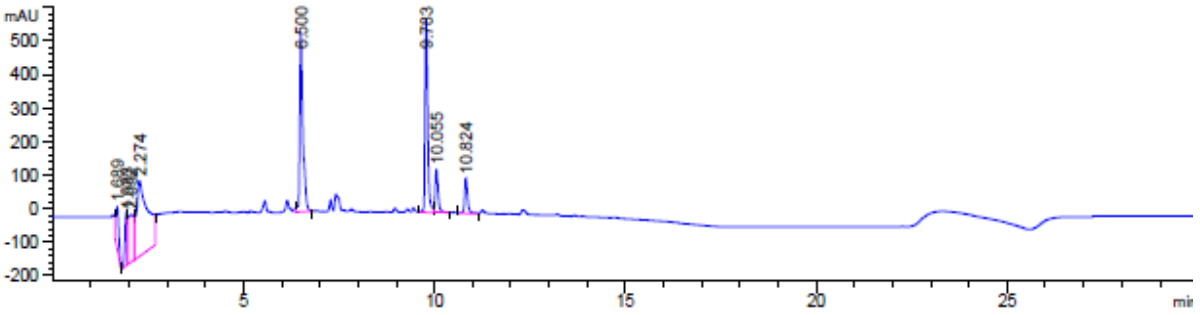


Figure S. 121. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/$CaCO_3$, 40 °C, 2.0 mL/min.

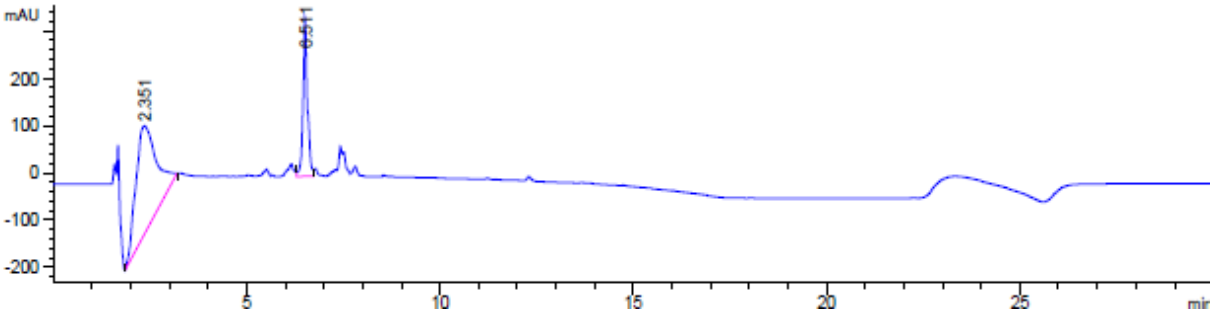


Figure S. 122. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/$CaCO_3$, 60 °C, 0.5 mL/min

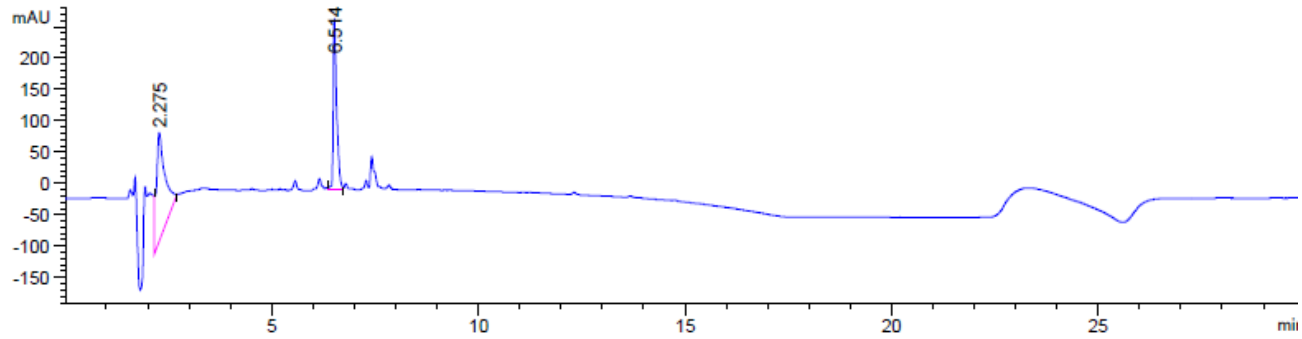


Figure S. 123. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/$CaCO_3$, 60 °C, 1.0 mL/min

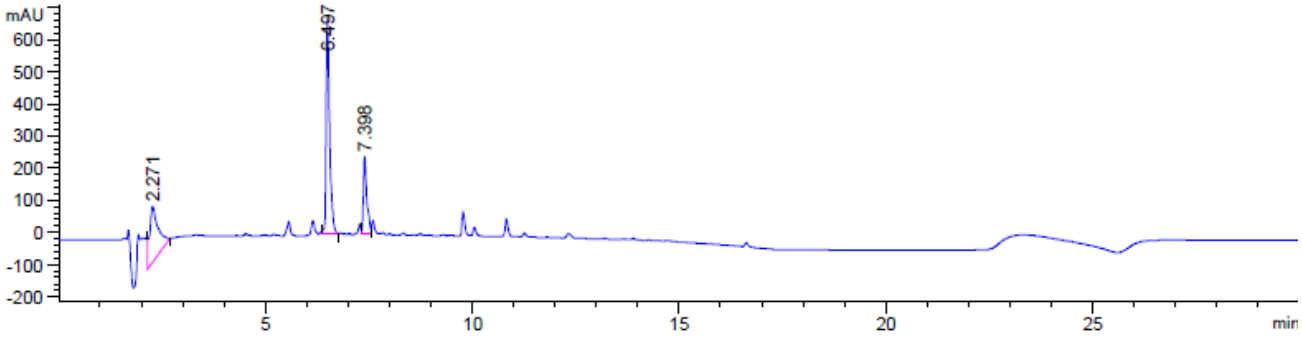


Figure S. 124. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/$CaCO_3$, 60 °C, 2.0 mL/min

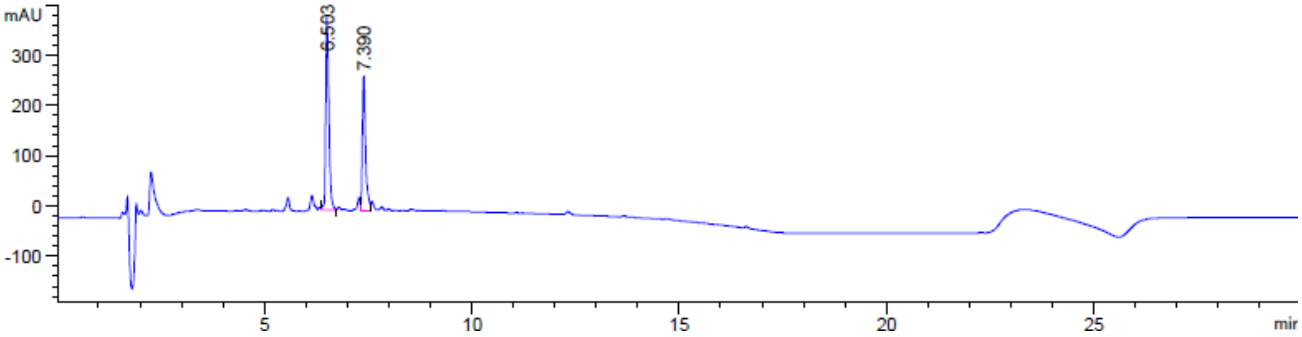


Figure S. 125. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/$CaCO_3$, 80 °C, 0.5 mL/min

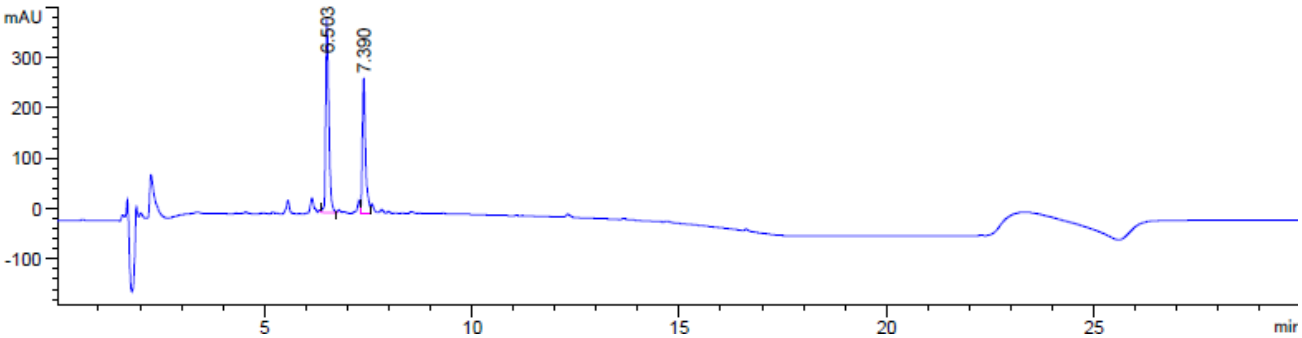


Figure S. 126. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/$CaCO_3$, 80 °C, 1.0 mL/min

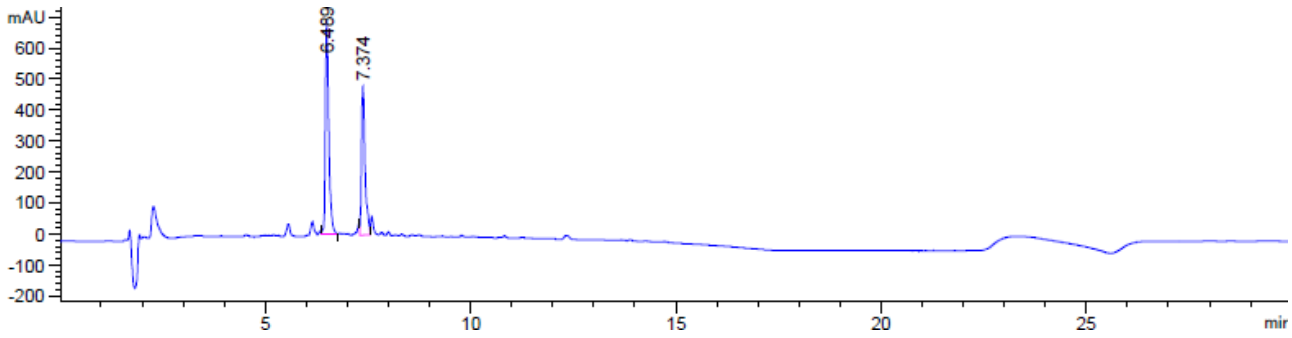


Figure S. 127. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% $Pd/CaCO_3$, 80 °C, 1.0 mL/min

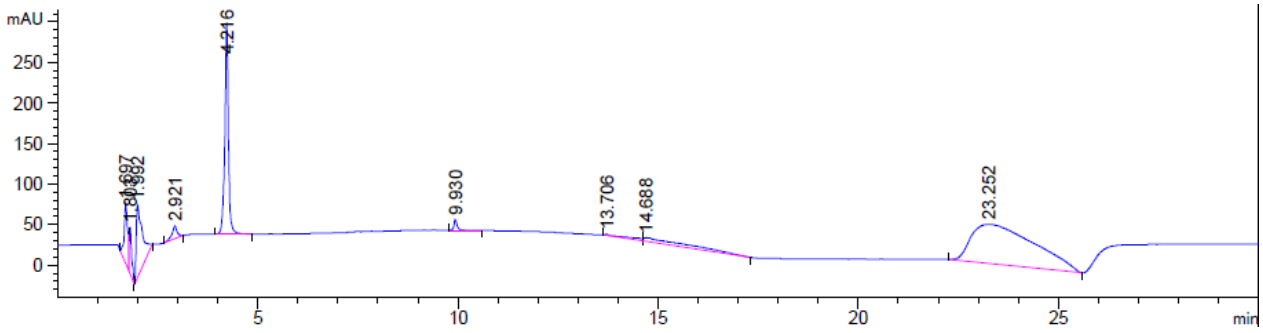


Figure S. 128. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 20 °C 0.5 mL/min

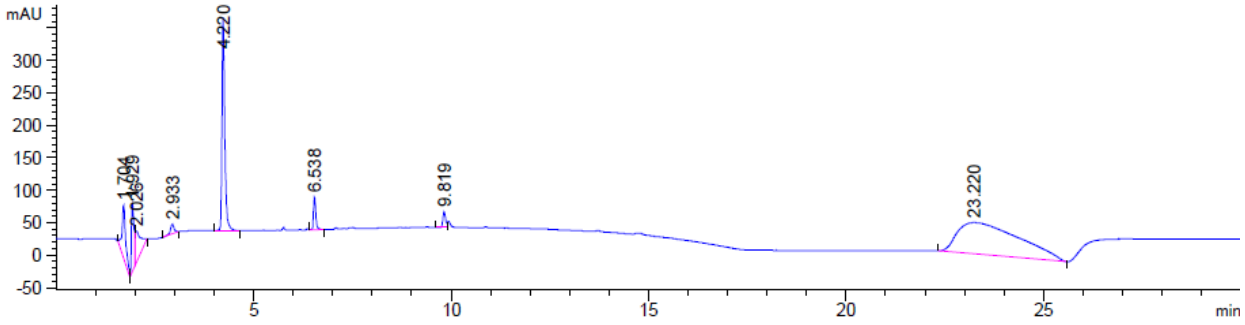


Figure S. 129. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 20 °C 1.0 mL/min

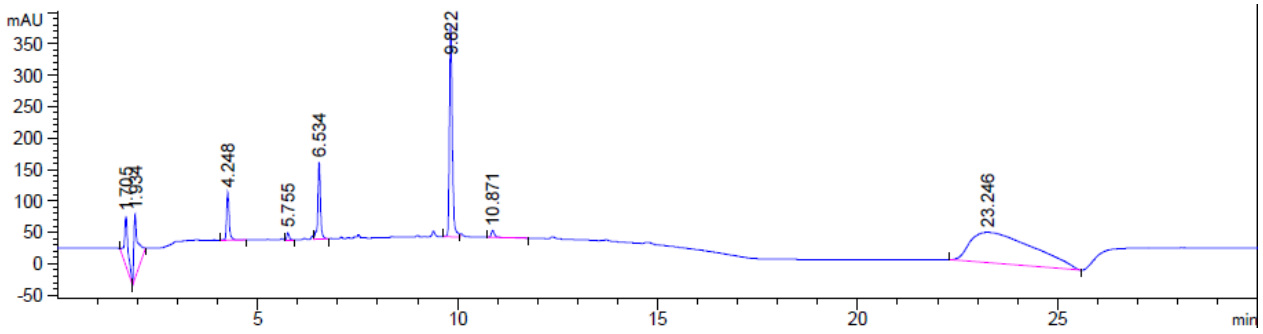


Figure S. 130. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 20 °C 2.0 mL/min

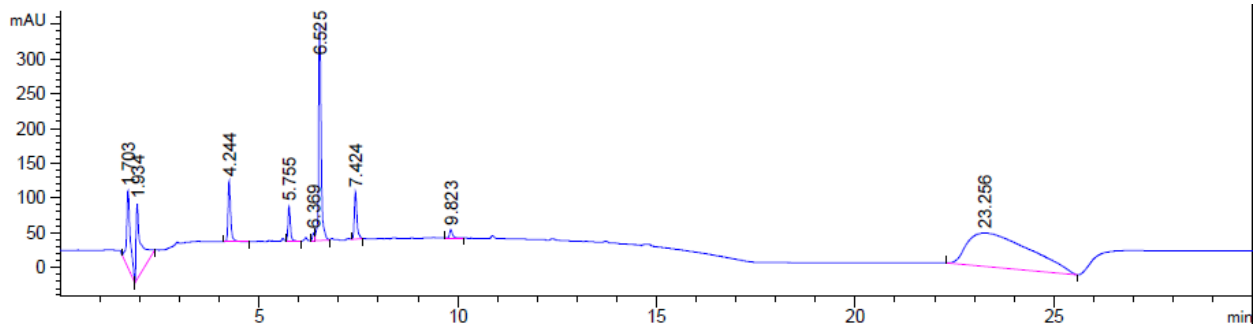


Figure S. 131. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 40 °C 0.5 mL/min

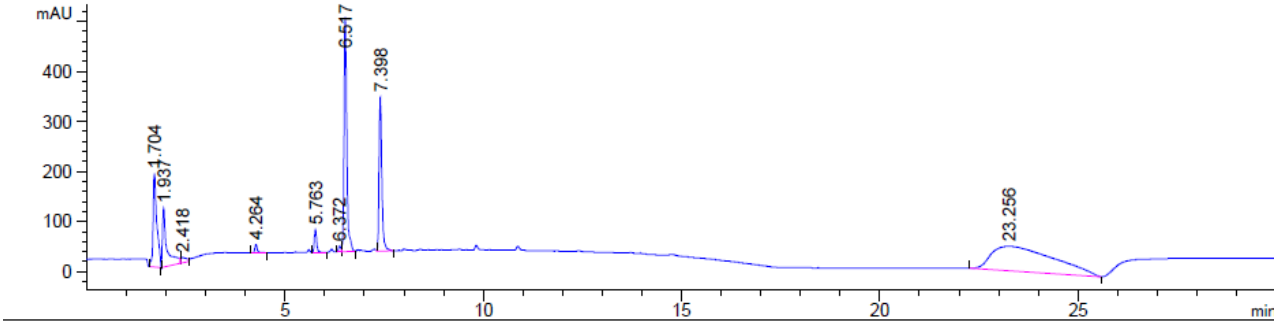


Figure S. 132. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 40 °C 1.0 mL/min

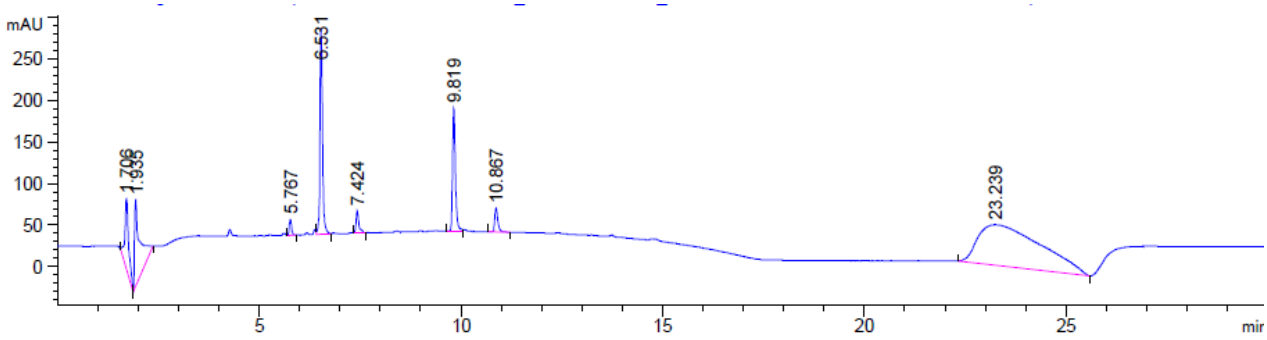


Figure S. 133. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 40 °C 2.0 mL/min

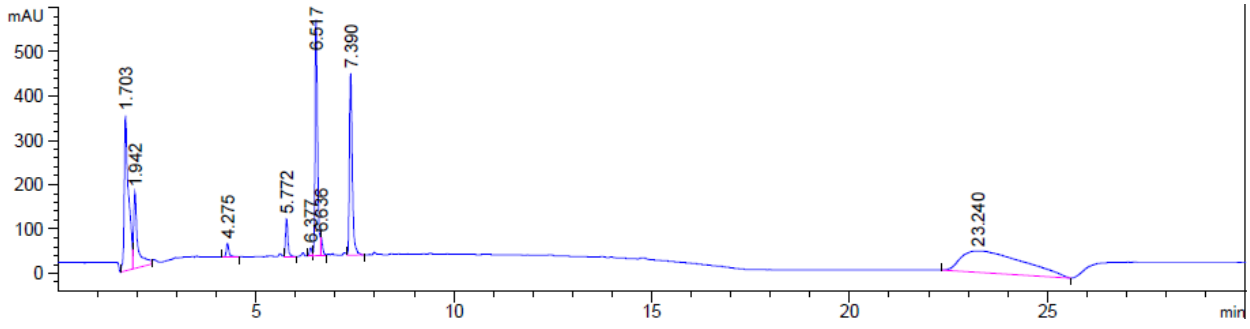


Figure S. 134. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 60 °C 0.5 mL/min

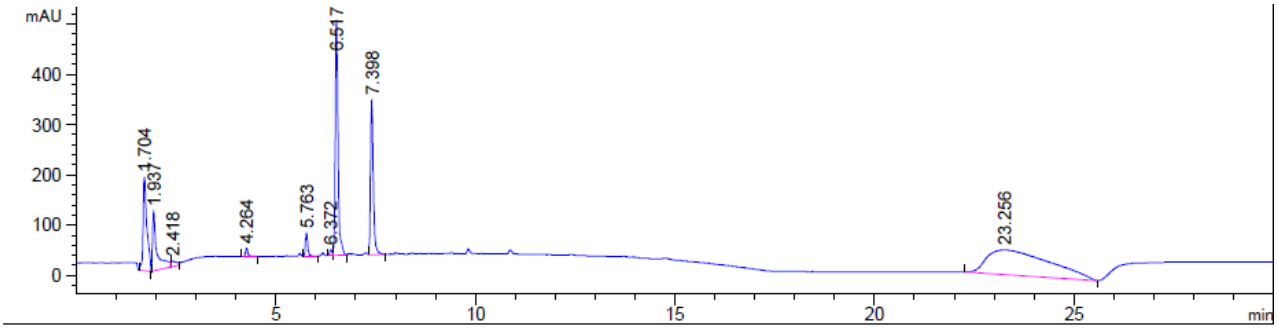


Figure S. 135. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 60 °C 1.0 mL/min

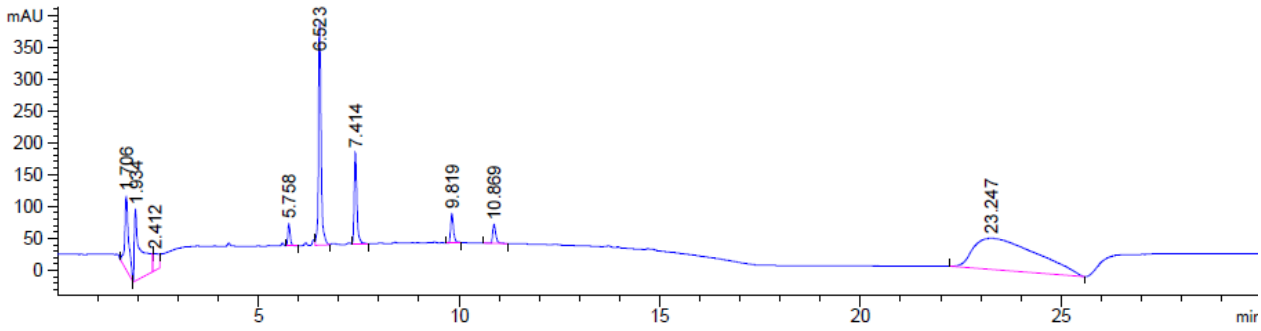


Figure S. 136. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 60 °C 2.0 mL/min

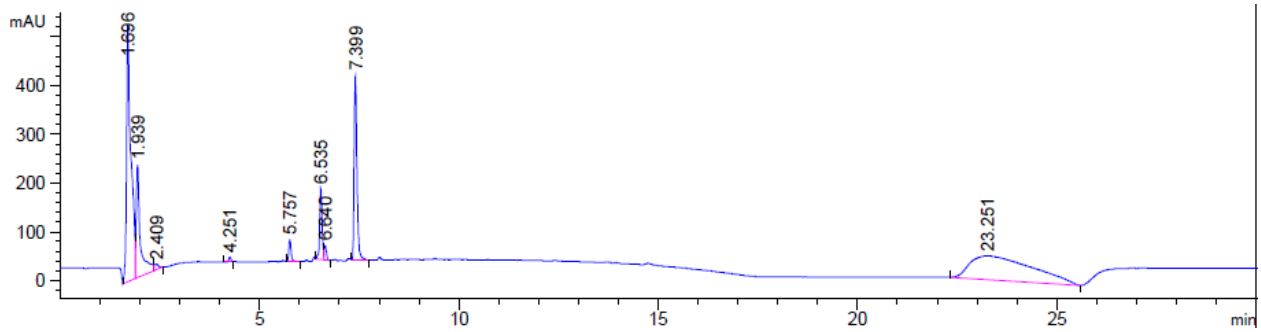


Figure S. 137. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 80 °C 0.5 mL/min

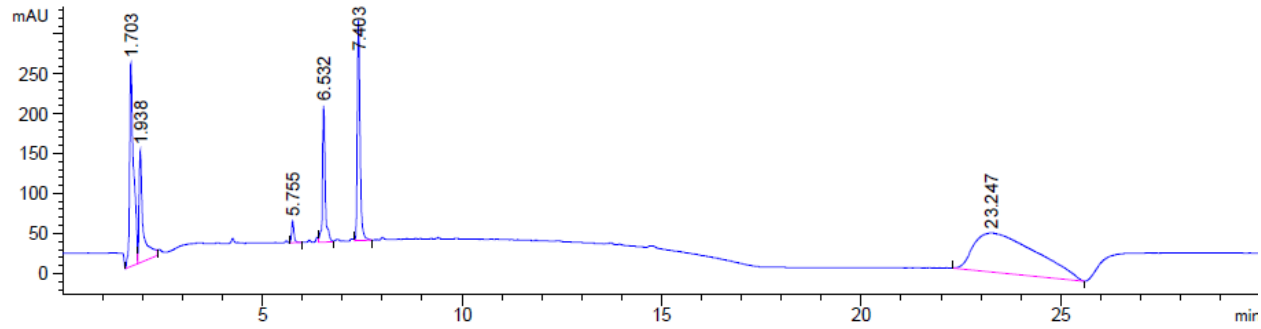


Figure S. 138. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 80 °C 1.0 mL/min

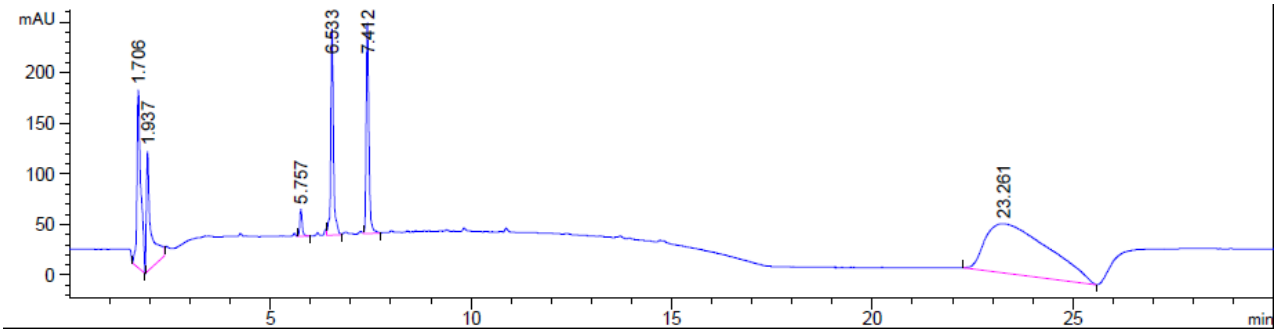


Figure S. 139. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 80 °C 2.0 mL/min

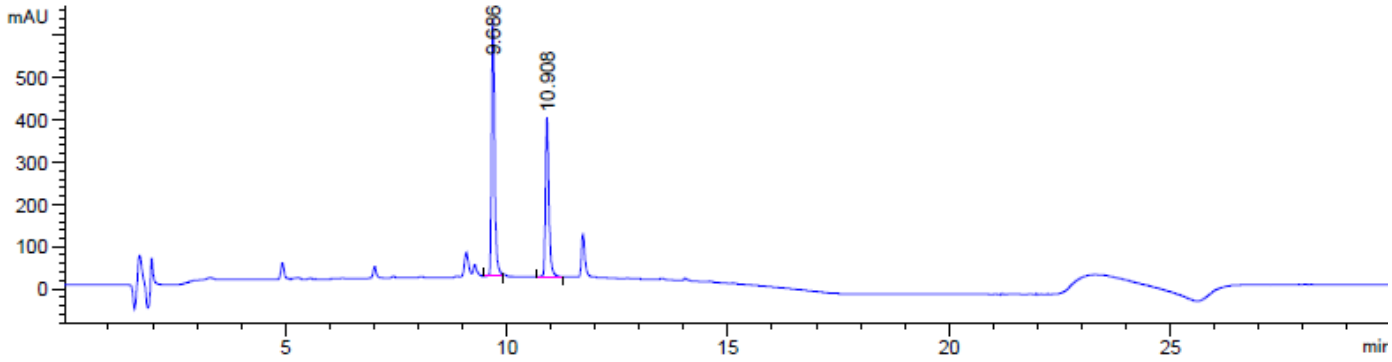


Figure S. 140. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under Nickel Sponge, across all flow rates (0.5 – 2.0 mL/min), and temperatures (20 – 80 °C)

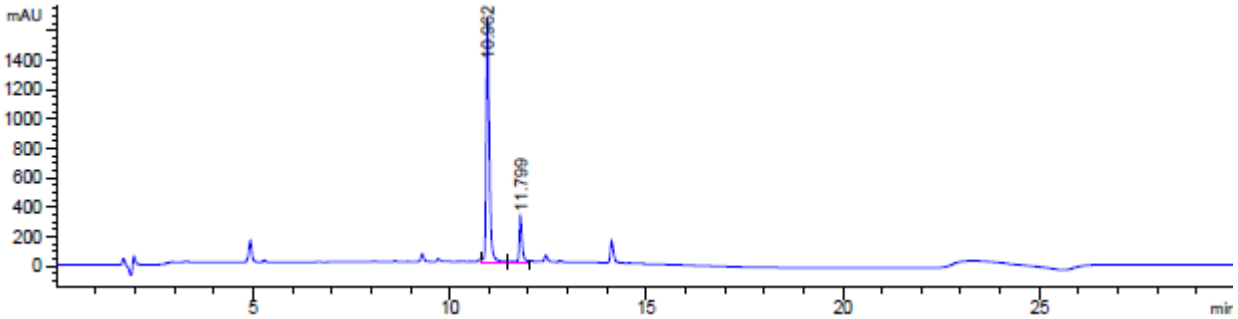


Figure S. 141. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under Raney Copper, across all flow rates (0.5 – 2.0 mL/min), and temperatures (20 – 80 °C).

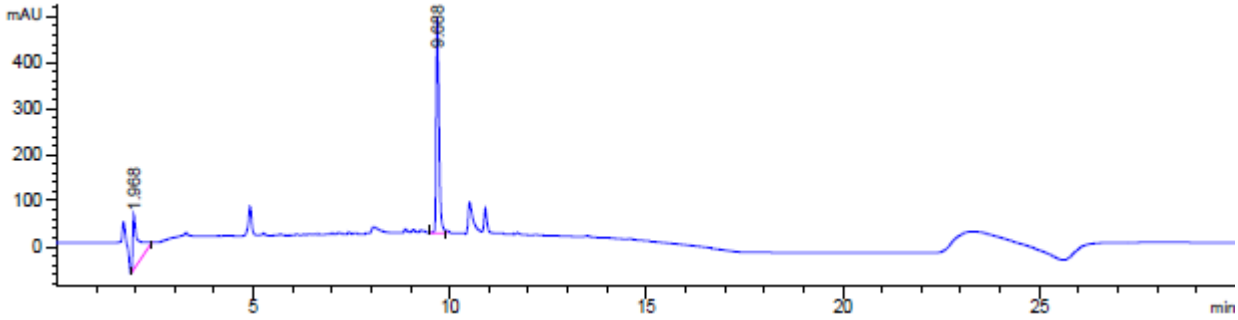


Figure S. 142. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under Raney Nickel, across all flow rates (0.5 – 2.0 mL/min), and temperatures (20 – 80 °C).

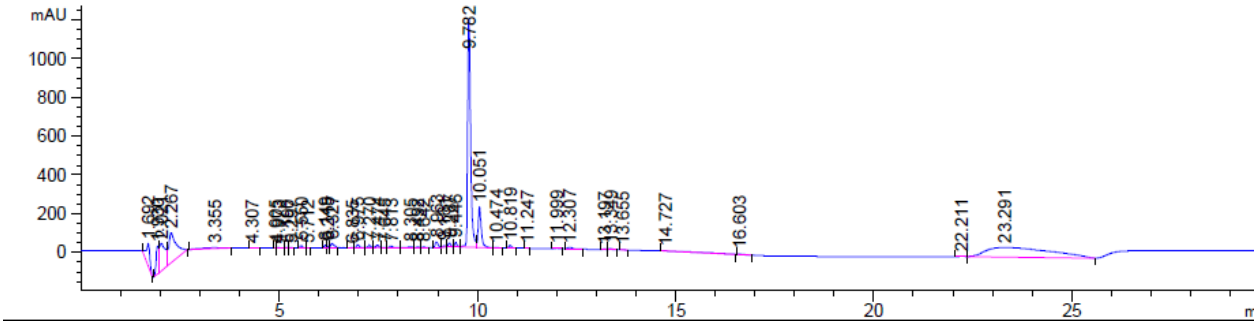


Figure S. 143. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under Rh(COD)(dppf), across all flow rates (0.5 – 2.0 mL/min), and temperatures (20 – 80 °C).

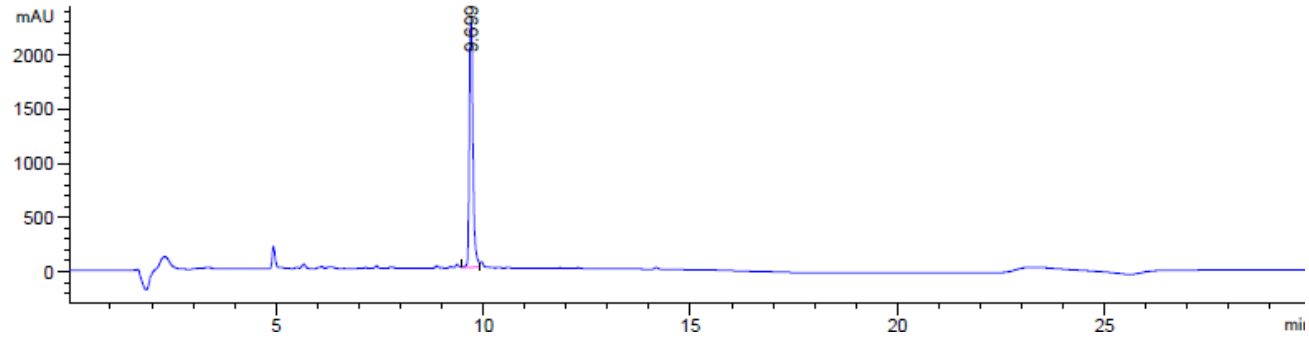


Figure S. 144. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under Fibrecat1001, across all flow rates (0.5 – 2.0 mL/min), and temperatures (20 – 80 °C).

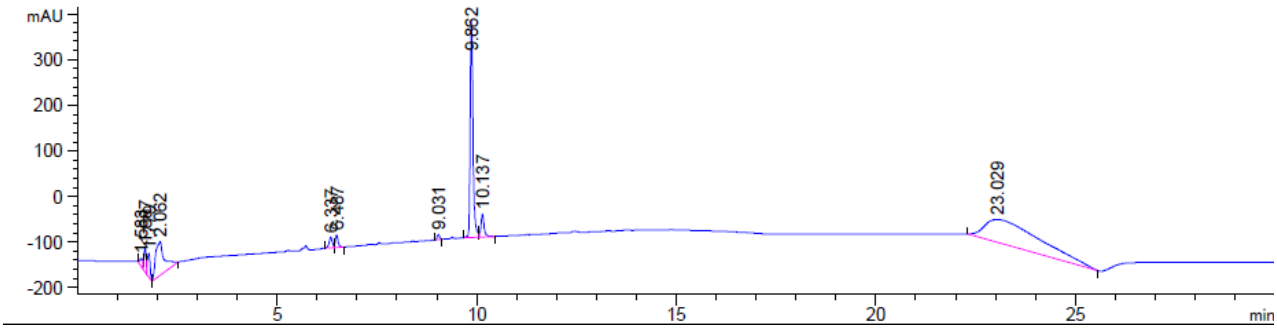


Figure S. 145. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under Pd(II) Encat, across all flow rates (0.5 – 2.0 mL/min), and temperatures (20 – 80 °C).

5.3 Chromatographic Data for Differing H_2 Saturation Trials for Cbz Hydrogenolysis of Compound 8

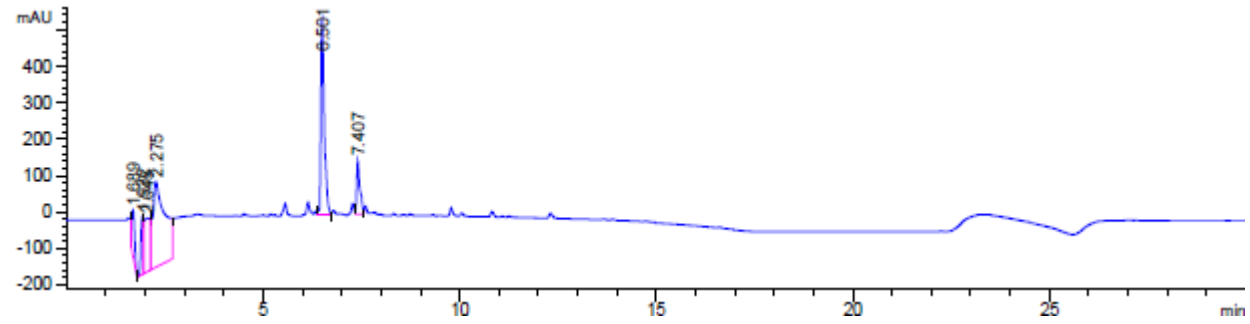


Figure S. 146. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% $Pd/CaCO_3$, 40 °C, 1.0 mL/min, 100% H_2

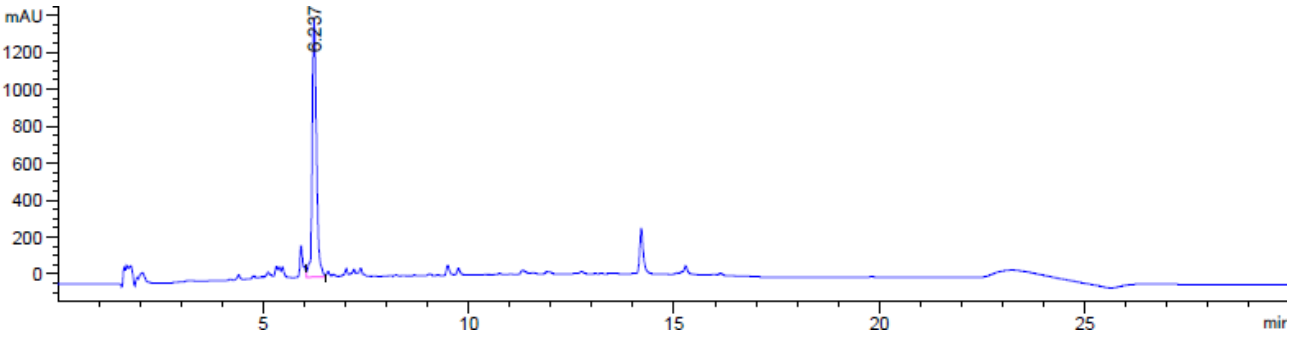


Figure S. 147. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% $Pd/CaCO_3$, 40 °C, 1.0 mL/min, 75% H_2

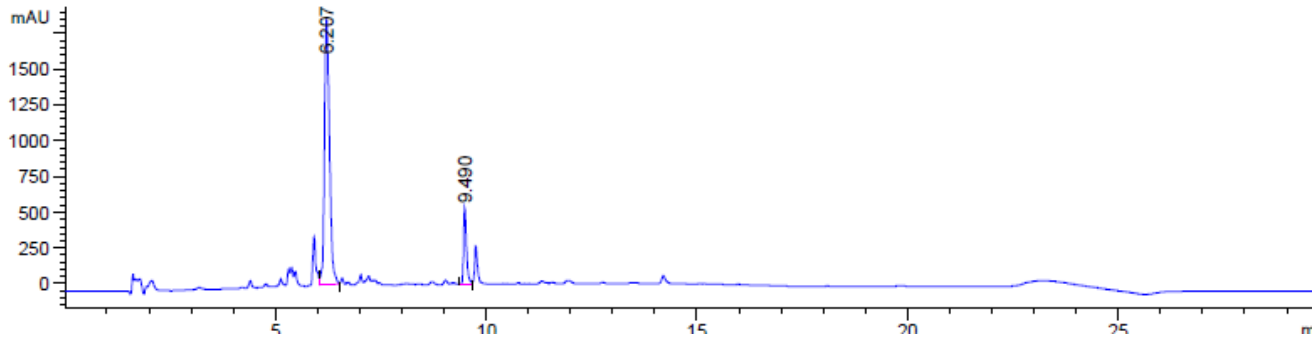


Figure S. 148. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% $Pd/CaCO_3$, 40 °C, 1.0 mL/min, 50% H_2

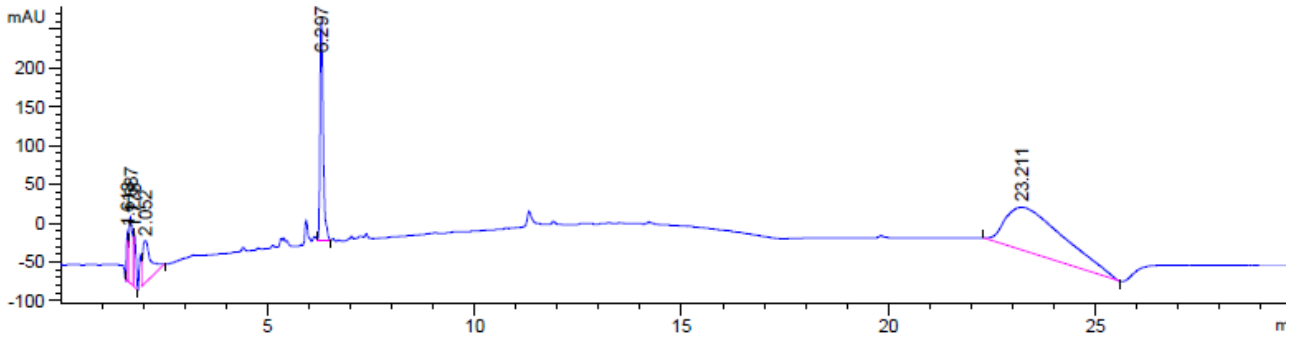


Figure S. 149. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 10% $Pd/CaCO_3$, 40 °C, 1.0 mL/min, 25% H_2

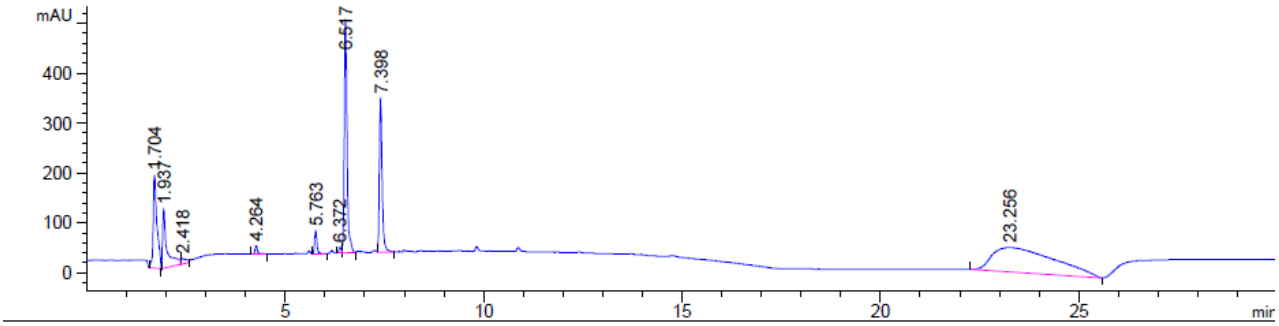


Figure S. 150. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 40 °C, 1.0 mL/min, 100% H_2

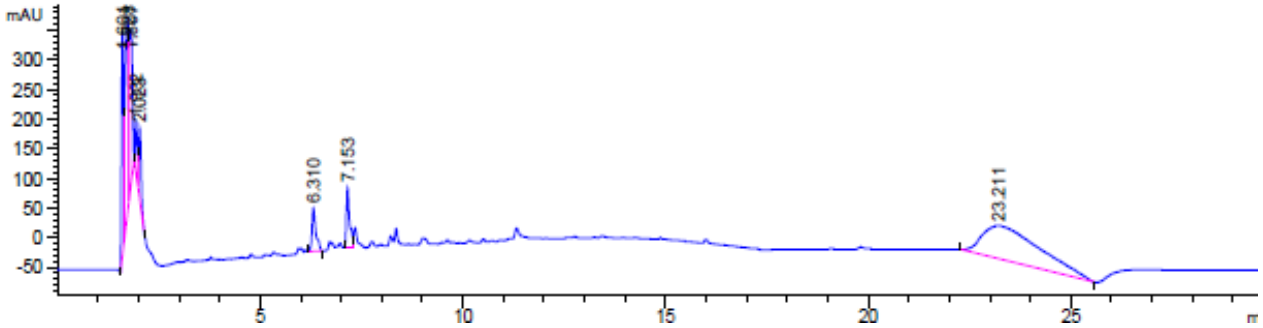


Figure S. 151. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 40 °C, 1.0 mL/min, 75% H_2

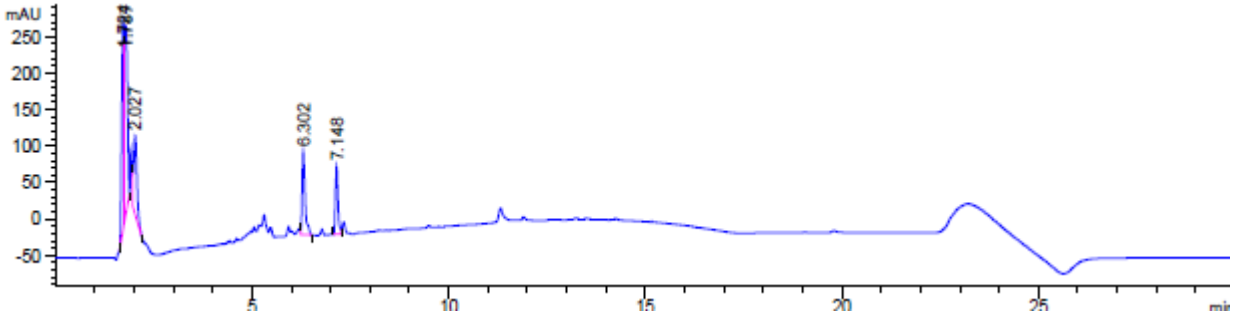


Figure S. 152. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 40 °C, 1.0 mL/min, 50% H_2

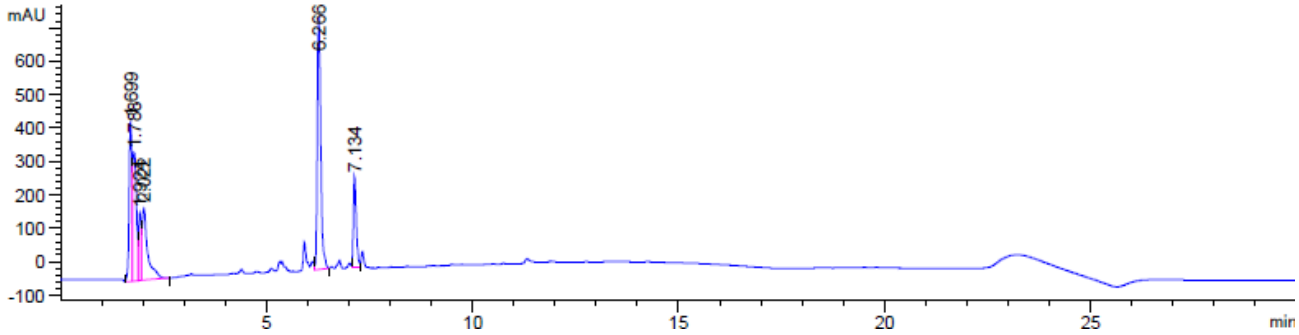


Figure S. 153. HPLC chromatogram for Cbz hydrogenolysis of compound **8** under 20% $Pd(OH)_2/C$, 40 °C, 1.0 mL/min, 25% H_2

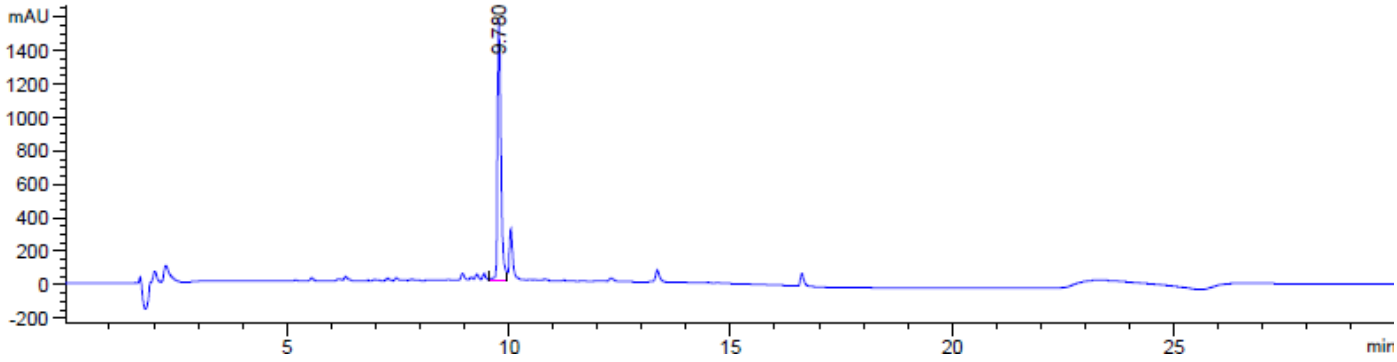


Figure S. 154. HPLC Chromatogram for Cbz hydrogenolysis of compound **8** under 10% Pd/$CaCO_3$ in a 9:1 CHCl3:MeOH solvent system, with acid doping and catalyst washing.

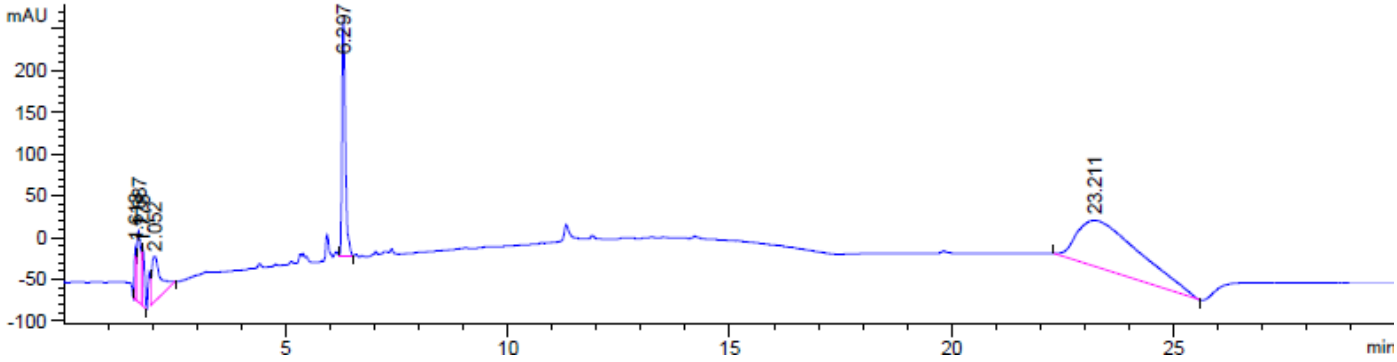


Figure S. 155. HPLC Chromatogram for Cbz hydrogenolysis of compound **8** under the optimised conditions of 10% Pd/$CaCO_3$, in MeOH, 40 °C, 1.0 mL/min.

1. L. K. Spare, M. Menti, D. G. Harman, J. R. Aldrich-Wright and C. P. Gordon, *Reaction Chemistry & Engineering*, 2019, **4**, 1309-1317.
2. L. K. Spare, V. Laude, D. G. Harman, J. R. Aldrich-Wright and C. P. Gordon, *Reaction Chemistry & Engineering*, 2018, **3**, 875-882.
3. C. Dankers, J. Tadros, D. G. Harman, J. R. Aldrich-Wright, T. V. Nguyen and C. P. Gordon, *ACS Combinatorial Science*, 2020, **22**, 255-267.