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The Synthesis of Head-to-Tail Sulfono-Cyclic γ-AApeptides

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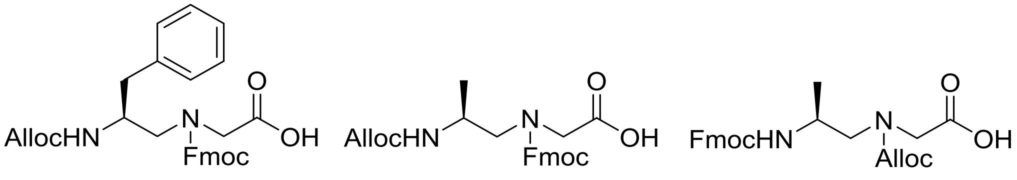
1. General information

All Fmoc protected/unprotected α-amino acids were purchased from Chem-Impex International, Inc. 2-Chlorotrityl (CTC) resin (0.98 mmol/g, 200-400 mesh) was purchased from AAPPTec LLC. All the other solvents and reagents were purchased from either Sigma-Aldrich or Fisher Scientific. NMR data for γ-AApeptide building blocks were analyzed on a Varian UnityInova400 spectrometer. Masses of AApeptide building blocks and cyclic sulfono-γ-AApeptides were obtained on an Agilent 6540 liquid chromatography/quadrupole time-of-flight mass spectrometer. The X-ray diffraction data for **γ5** was collected on a Bruker D8 Venture PHOTON 100 CMOS system equipped with a Cu Kα INCOATEC Imus micro-focus source (λ = 1.54178 Å). Solid phase synthesis was conducted in peptide synthesis vessels on a Burrell Wrist-Action shaker. Cyclic sulfono-γ-AApeptides were analyzed and purified on a Waters Breeze 2 HPLC system, and then lyophilized on a Labcono lyophilizer.

2. Experimental details

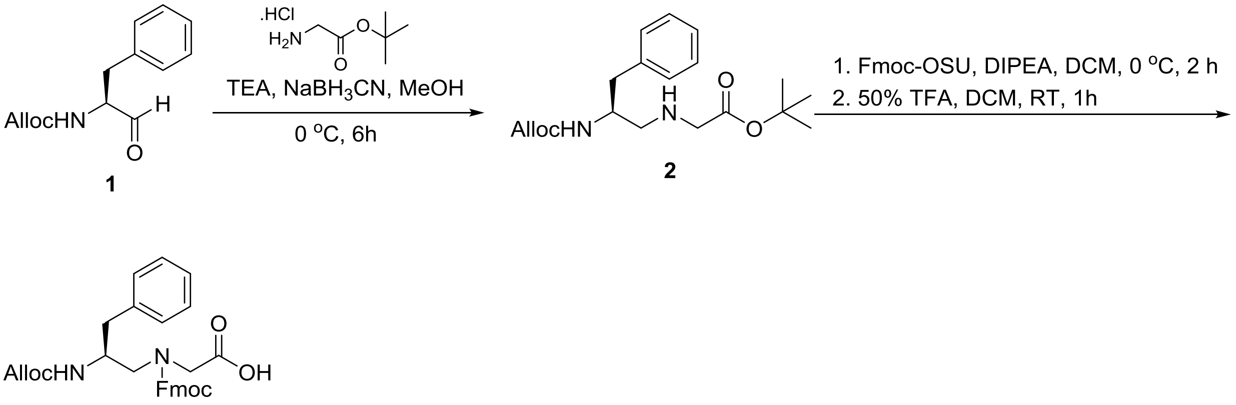
2.1 Synthesis of γ-AApeptide building blocks



γ−BB−1 γ−BB−2 γ−BB−3

Figure S1. γ-AApeptide building blocks used in the study.

2.1.1 Synthesis of Fmoc-N-Alloc γ-AApeptide building block **γ-BB-1**



1 **2**

γ−BB−1

Figure S2. Synthetic scheme of Fmoc-N-Alloc γ-AApeptide building block **γ-BB-1**.

Synthesis of compound **2**

A solution of compound **1**[1] (4.38 g, 15 mmol) in 100 mL methanol was stirred in an ice bath. A solution of Gly-OtBu.HCl (2.52 g, 15 mmol) and triethylamine (2.09 mL, 15mmol) in 20 mL methanol was added to the reaction flask. The reaction was allowed to stir at 0 °C for one hour, followed by the addition of acetic acid (4 mL, 3% v/v) and sodium cyanoborohydride (1.88 g, 30 mmol). The reaction was stirred at 0 °C for two more hours. After completion, methanol was removed by vacuum. The resulting slurry was dissolved in saturated sodium bicarbonate solution (150 mL) and dichloromethane (150 mL). The mixture was transferred to a separatory funnel. The bottom layer was collected and washed with water (150 mL), then brine solution (150 mL). The organic layer was collected, dried over sodium sulfate, and concentrated in vacuo. The pure product **2** was obtained as white foam with a yield of 75% after flash column with hexane/ethyl acetate (2:1) as eluent. TLC analysis showed an Rf value of 0.1 in 1:1 hexane/ethyl acetate solution. Compound **2** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26 (t, *J* = 8 Hz, 2H), 7.22 (d, *J* = 8 Hz, 1H), 7.16 (d, *J* = 8 Hz, 2H), 6.11 (d, *J* = 8 Hz, 1H), 5.84-5.75 (m, 1H), 5.20 (d, *J* = 16 Hz, 1H), 5.13 (d, *J* = 12 Hz, 1H), 4.50-4.38 (m, 2H), 4.20 (t, *J* = 8 Hz, 1H), 3.75-3.65 (m, 2H), 3.38-3.08 (m, 2H), 2.98-2.78 (m, 2H), 1.39 (s, 9H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.1, 156.6, 136.3, 132.5, 129.0, 128.7, 127.0, 117.5, 84.5, 65.9, 50.2, 49.7, 47.7, 38.8, 27.8 ppm. HR-ESI: $[M+H]^+$ calc: 349.2122, found: 349.2120.

Synthesis of **γ-BB-1**

A solution of **2** (3.9 g, 11.2 mmol) and N, N-diisopropylethylamine (2.15 mL, 12.3 mmol) in 50 mL dichloromethane was stirred in an ice bath. A solution of Fmoc-OSU (4.15 g, 12.3 mmol) in 20 mL dichloromethane was added dropwise. The reaction was allowed to stir at 0 °C for two hours. After completion, solvent was removed by vacuum. The slurry was used directly in the next reaction.

The slurry was treated with 20 mL 1:1 dichloromethane/trifluoroacetic acid mixture for 1 h at room temperature. After completion, solvent was removed by vacuum. Trifluoroacetic acid was removed completely by co-evaporation with dichloromethane (20 mL x 5). Pure **γ-BB-1** was obtained as white solid with a yield of 95% after flash column with hexane/ethyl acetate (1:1) as eluent. TLC analysis showed an Rf value of 0.1 in a hexane/ethyl acetate 1:1 solution. **γ-BB-1** ^{1}H NMR ($CDCl_3$, 400 MHz) δ (two rotamers) 9.73 (s, 1H), 7.76-7.69 (m, 2H), 7.55-7.48 (m, 2H), 7.38-7.17 (m, 9H), 5.83-5.69 (m, 1H), 5.25-5.03 (m, 2H), 4.64-4.79 (m, 4H), 4.22-4.00 (m, 2H), 3.88-3.57 (m, 1H), 3.29-3.81 (m, 2H), 2.79-2.37 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ (two rotamers) 173.4, 156.9, 156.5, 156.4, 156.0, 143.8, 143.7, 143.6, 141.4, 141.2, 137.3, 137.1, 132.6, 129.4, 129.2, 129.0, 128.5, 127.7, 127.4, 127.1, 126.6, 124.9, 124.6, 119.9, 117.6, 117.5, 117.4, 68.2, 67.2, 65.5, 51.6, 51.5, 51.1, 48.9, 47.1, 38.7, 38.5 ppm. HR-ESI: $[M+H]^+$ calc: 515.2177, found: 515.2165.

2.1.2 Synthesis of N-Alloc γ-AApeptide building block **γ-BB-2**

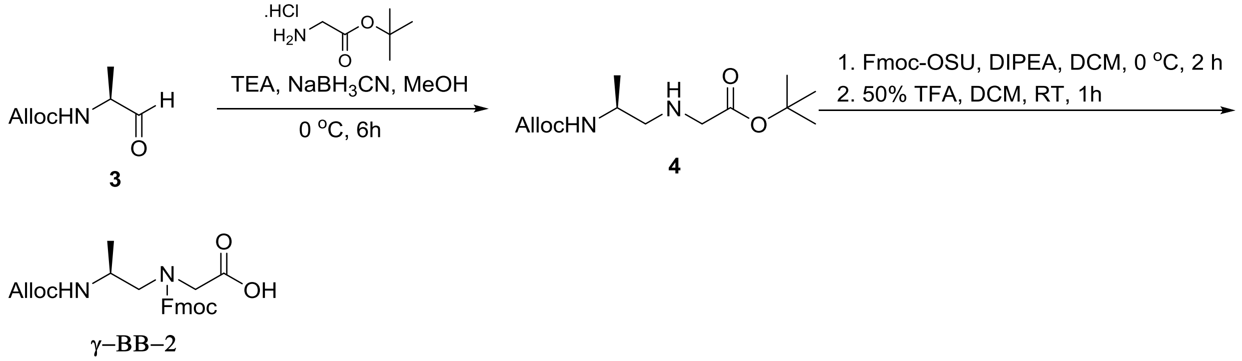


Figure S3. Synthetic scheme of N-Alloc γ-AApeptide building block **γ-BB-2**.

Compound **4** and **γ-BB-2** were prepared from **3**[2] following similar procedure as **γ-BB-1**.

Compound **4** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.01 (d, *J* = 8 Hz, 1H), 5.92-5.82 (m, 1H), 5.29 (d, *J* = 16 Hz, 1H), 5.20 (d, *J* = 12 Hz, 1H), 4.60-4.44 (m, 2H), 4.16-4.11 (m, 1H), 3.80 (s, 2H), 3.32-3.14 (m, 2H), 1.46 (s, 9H), 1.29 (d, *J* = 8 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.2, 156.8, 132.5, 117.8, 84.7, 66.1, 52.4, 47.9, 44.4, 27.8, 18.5 ppm. HR-ESI: $[M+H]^+$ calc: 273.1809, found: 273.1820.

γ-BB-2 ^{1}H NMR ($CDCl_3$, 400 MHz) δ (two rotamers) 9.99 (s, 1H), 7.76-7.70 (m, 2H), 7.56-7.49 (m, 2H), 7.41-7.24 (m, 4H), 5.88-5.80 (m, 1H), 5.38-5.09 (m, 2H), 4.66-4.40 (m, 4H), 4.22-4.15 (m, 1H), 4.03-3.89 (m, 2H), 3.56-2.89 (m, 2H), 1.14-0.80 (m, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ (two rotamers) 173.7, 156.7, 143.7, 143.5, 141.4, 141.3, 132.5, 124.6, 120.0, 119.9, 117.7, 68.3, 67.4, 65.8, 53.2, 52.5, 49.1, 48.9, 47.1, 46.3, 45.4, 18.4, 17.9 ppm. HR-ESI: $[M+H]^+$ calc: 439.1864, found: 439.1867.

2.1.3 Synthesis of N-Alloc γ-AApeptide building block **γ-BB-3**

γ-BB-3 was synthesized following previous reported procedure.[3]

2.2 Synthesis of cyclic sulfono-γ-AApeptides

2.2.1 Synthesis of **γ1**

2-Chlorotrityl chloride (CTC) resin (102 mg, 100 μmol) was swelled in 2 mL DCM for 15 min. The first attachment was conducted by adding building block **γ-BB-1** (77 mg, 150 μmol) and DIPEA (26 μL, 150 μmol) to the beads in the reaction vessel, which was allowed to shake at room temperature for two hours. After that, the reaction solution was drained, followed by washing with DMF (2 mL x3) and DCM (2 mL x3). The unreacted residues were capped with 2 mL methanol for 30 min. Again, the beads were washed with DCM (2 mL x3) and DMF (2 mL x3). The Fmoc group was removed by treating beads with 20% piperidine/DMF (v/v) solution for 10 min (x2) at room temperature. The solution was drained, followed by washing with DMF (2 mL x3) and DCM (2 mL x3). N-modification was achieved by reacting beads with methanesulfonyl chloride (23 μL, 300 μmol) and DIPEA (52 μL, 300 μmol) for 30 min (x2) at room temperature. The solution was then drained. After washing with DMF (2 mL x3) and DCM (2 mL x3), the beads were treated with $Pd(PPh_3)_4$ (12 mg, 10 μmol) and $Me_2NH.BH_3$ (35 mg, 600 μmol) in 2 mL dichloromethane for 10 min (x2) to remove Alloc protecting group.[4] After the reaction, the solution was drained. The beads were washed with DCM (2 mL x3), 0.2% TFA/DCM (2 mL 60s x3), DCM (2 mL x3), 5% DIPEA/DCM (2 mL 60s x3), and DCM (2 mL x3).

For the next coupling reaction, building block **γ-BB-3** (88 mg, 200 μmol), DIC (63 μL, 400 μmol), and HOBt (61 mg, 400 μmol) were pre-mixed in 2 mL DMF for 5 min before getting transferred to the reaction vessel. The reaction was shaken at room temperature for 2 hours, and the solution was drained. The beads were washed with DMF (2 mL x3) and DCM (2 mL x3) and the coupling reaction was repeated. The beads were washed with DMF (2 mL x3) and DCM (2 mL x3). The N-Alloc was removed following the same conditions discussed above: $Pd(PPh_3)_4$ (12 mg, 10 μmol) and $Me_2NH.BH_3$ (35 mg, 600 μmol) in 2 mL dichloromethane for 10 min (x2). After the reaction, the solution was drained. The beads were washed with DCM (2 mL x3), 0.2% TFA/DCM (2 mL 60s x3), DCM (2 mL x3), 5% DIPEA/DCM (2 mL 60s x3), and DCM (2 mL x3). After that, the beads were treated with phenylmethanesulfonyl chloride (57 mg, 300 μmol) and DIPEA (52 μL, 300 μmol) for 30 min (x2) at room temperature for the second N^α-modification. After washing with DCM (2 mL x3) and DMF (2 mL x3), N-terminal Fmoc group was removed with 20% piperidine/DMF (v/v) solution for 10 min (x2) at room temperature.

The previous reaction cycle was repeated until the desired sequence was obtained. The linear sulfono-γ-AApeptide was cleaved from resin with 4 mL cleavage cocktail (acetic acid : TFE : DCM = 1:1:8). After cleavage for 2 h, the solution was collected. The remaining beads were washed with 2 mL cleavage cocktail solution for three times. All the solution was combined and concentrated by vacuum. Acetic acid was completely removed by co-evaporation with hexane.

In the cyclization reaction, crude linear sulfono-γ-AApeptide was dissolved in 200 mL DCM (~0.5 mM), followed by the addition of coupling reagents: TBTU (96 mg, 300 μmol), HOBt (46 mg, 300 μmol), and DMAP (61 mg, 500 μmol). The reaction was allowed to stir at room temperature for overnight. After completion of reaction, the solution was washed with 0.1 M HCl (100 mL x3), water (100 mL), and brine (100 mL). The DCM layer was collected, dried over anhydrous sodium sulfate, and concentrated by vacuum. Crude compound **γ1** was dissolved in 10 mL water/acetonitrile (1:1) solution and filtered through a Whatman filter unit (0.45 μm) before HPLC analysis. Pure **γ1** was obtained as white powder after preparative HPLC and lyophilization.

2.2.2 Synthesis of **γ2-7**.

Following similar method as **γ1**, **γ2-7** (all white powder) were synthesized and characterized by HRMS (Table S1). **γ6** and **γ7** were treated with 2 mL DCM/TFA (1:1) for 1 h, before HPLC analysis and purification.

γ1 ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 8.23 (d, *J* = 8 Hz, 1H), 8.13-8.07 (m, 2H), 7.38-7.33 (m, 15H), 7.23-7.17 (m, 5H), 4.54-4.37 (m, 6H), 4.03-3.95 (m, 3H), 3.94-3.75 (m, 8H), 3.20-2.99 (m, 8H), 2.97 (s, 3H), 1.04 (d, *J* = 8 Hz, 9H) ppm.

γ2 ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 7.60 (d, *J* = 8 Hz, 1H), 7.43 (d, *J* = 8 Hz, 1H), 7.41-7.36 (m, 5H), 7.29-7.22 (m, 5H), 4.64-4.49 (m, 2H), 4.09-3.84 (m, 4H), 3.67-3.51 (m, 3H), 3.26-3.13 (m, 4H), 3.00 (s, 3H), 1.00 (d, *J* = 8 Hz, 3H) ppm.

γ3 ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 8.30 (d, *J* = 8 Hz, 1H), 8.05 (t, *J* = 8 Hz, 2H), 7.37 (s, 10H), 7.26-7.19 (m, 5H), 4.55-4.42 (m, 4H), 3.96-3.89 (m, 3H), 3.86-3.69 (m, 6H), 3.30-3.06 (m, 6H), 3.02 (s, 3H), 1.05 (t, *J* = 8 Hz, 6H) ppm.

γ4 ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 8.23 (d, *J* = 8 Hz, 2H), 8.16 (q, *J* = 8 Hz, 2H), 7.35 (s, 20H), 7.23-7.12 (m, 5H), 4.52-4.35 (m, 8H), 4.10-4.00 (m, 5H), 3.96-3.80 (m, 10H), 3.25-3.03 (m, 10H), 2.98 (s, 3H), 1.00 (t, *J* = 8 Hz, 12H) ppm.

γ5 ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 7.89 (d, *J* = 8 Hz, 3H), 7.83-7.79 (m, 6H), 7.67-7.64 (m, 6H), 3.90 (d, *J* = 16 Hz, 3H), 3.71-3.61 (m, 9H), 3.23-3.06 (m, 6H), 0.98 (d, *J* = 8 Hz, 9H) ppm.

γ6 ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 7.68 (s, 6H), 7.56 (s, 2H), 7.41-7.38 (m, 10H), 4.65 (q, *J* = 16 Hz, 4H), 3.96 (d, *J* = 16 Hz, 2H), 3.73-3.68 (m, 4H), 3.18-3.12 (m, 4H), 2.75-2.71 (m, 4H), 1.51-1.46 (m, 4H), 1.33-1.24 (m, 8H) ppm.

γ7 ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 8.53-8.07 (m, 3H), 7.68 (s, 9H), 7.38-7.29 (m, 15H), 4.61-4.41 (m, 4H), 4.20-4.08 (m, 3H), 3.94-3.72 (m, 6H), 3.19-3.11 (m, 6H), 2.75-2.71 (m, 6H), 1.49-1.27 (m, 18H) ppm.

Table S1. Cyclization of sulfono-γ-AApeptides with varying ring sizes.

compound	ring size	yield^a (%)	mass^b
γ1	4	95	1073.3595
γ2	2	90	537.1838
γ3	3	85	805.2720
γ4	5	85	1341.4466
γ5	3	75	865.1080
γ6	2	54	651.2999
γ7	3	50	976.4464

^{a}Yields were determined by analytical HPLC traces (Figure S5). ^{b}[M+1]$^+$ determined by ESI.

2.3 Crystallization study.

5 mg of **γ5** was dissolved in 500 µL chloroform. The solution was filtered through a 4 MM PVDF syringe filter (0.45 µm) and stored in a 2 mL vial, which was then covered with aluminum foil and placed in a sealed 20 mL vial containing 1 mL pentane. A block shaped crystal (dimension 0.3 mm x 0.3 mm x 0.2 mm) was obtained by pentane diffusion into the chloroform solution after 5 days at room temperature.

The X-ray diffraction data was measured on a Bruker D8 Venture PHOTON 100 CMOS diffractometer equipped with a Cu Kα INCOATEC Imus micro-focus source (λ = 1.54178 Å). Indexing was performed using APEX2 (Difference Vectors method). Data integration and reduction were performed using SaintPlus 6.01. Absorption correction was performed by multi-scan method implemented in SADABS. Space groups were determined using XPREP implemented in APEX2. The structure was solved using SHELXS-97 (direct methods) and refined using SHELXL-2013 (full-matrix least-squares on F2) contained in APEX2, WinGX v1.70.01 and OLEX2. All non-hydrogen atoms of the product were refined anisotropically. Hydrogen atoms of –CH, -NH, -CH$_2$ and -CH$_3$ groups were placed in geometrically calculated positions and included in the refinement process using riding model with isotropic thermal parameters: Uiso(H) = 1.2(1.5)Ueq(-CH,-NH, -CH$_2$(-CH$_3$)). Occupancies of disordered chloroform molecules have been refined as free variables or were fixed. Heavily disordered molecules of chloroform and trifluoroacetic acid (presence confirmed by F NMR studies) have been refined using restraints and isotropically in case of atoms with low occupancy. Crystal data and refinement conditions are shown in Table S2.

Table S2. Crystal data and structure refinement for γ5.

Identification code	**γ5**
Empirical formula	$C_{70.15}H_{81.65}Cl_{15.44}F_{1.5}N_{12}O_{19}S_6$
Moiety formula	$2(C_{33}H_{39}Cl_3N_6O_9S_3)$, $3.15(CHCl_3)$, $0.5\ (C_2HO_2F_3)$'
Formula weight	2165.20
Temperature/K	100(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	17.1143(4)
b/Å	20.8803(6)
c/Å	26.8416(6)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	9591.9(4)
Z	4
ρ_{calc}g/cm^3	1.499
μ/mm^{-1}	5.886
F(000)	4442.0
Crystal size/mm^3	0.09 × 0.02 × 0.02
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	7.446 to 136.394
Index ranges	$-20 \leq h \leq 20$, $-24 \leq k \leq 20$, $-32 \leq l \leq 32$
Reflections collected	50593
Independent reflections	17264 [R_{int} = 0.0936, R_{sigma} = 0.1036]
Data/restraints/parameters	17264/42/1169
Goodness-of-fit on F^2	1.016
Final R indexes [I>=2σ (I)]	R_1 = 0.0677, wR_2 = 0.1595
Final R indexes [all data]	R_1 = 0.1073, wR_2 = 0.1828
Largest diff. peak/hole / e Å^{-3}	0.55/-0.40
Flack parameter	0.058(9)

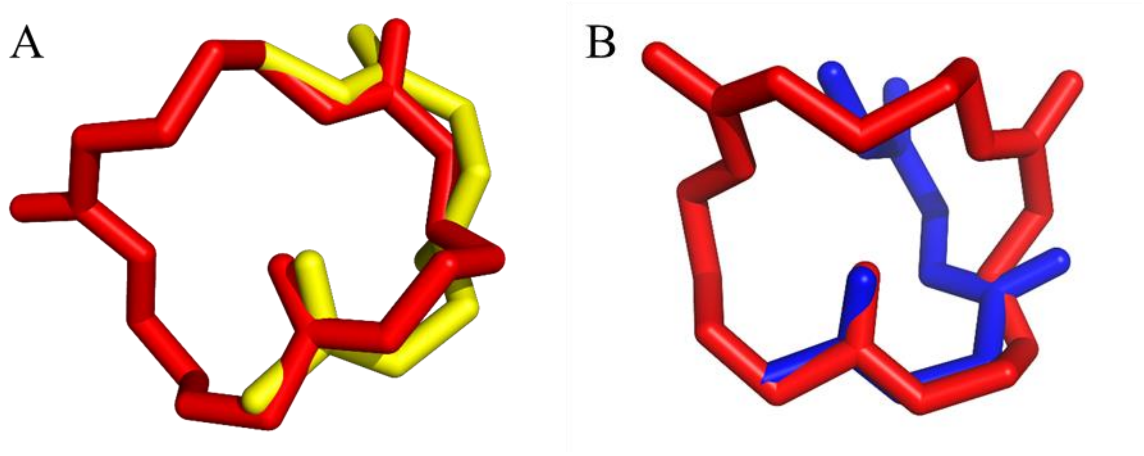
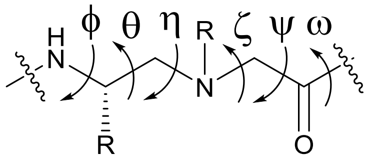


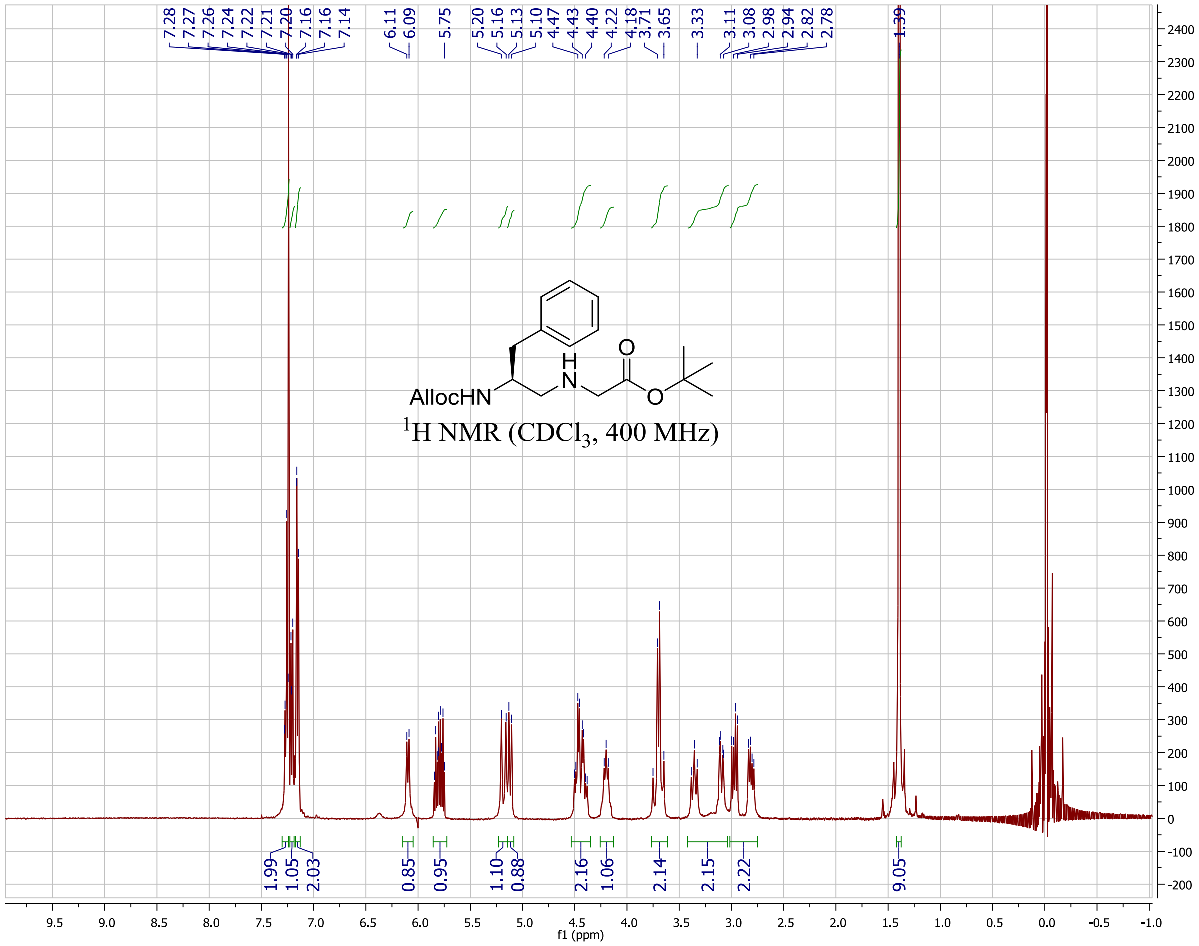
Figure S4. (**A**) Overlay of **γ5** (red) with a turn region of a type II β turn (yellow) and (**B**) a turn region of a type I β turn (blue).

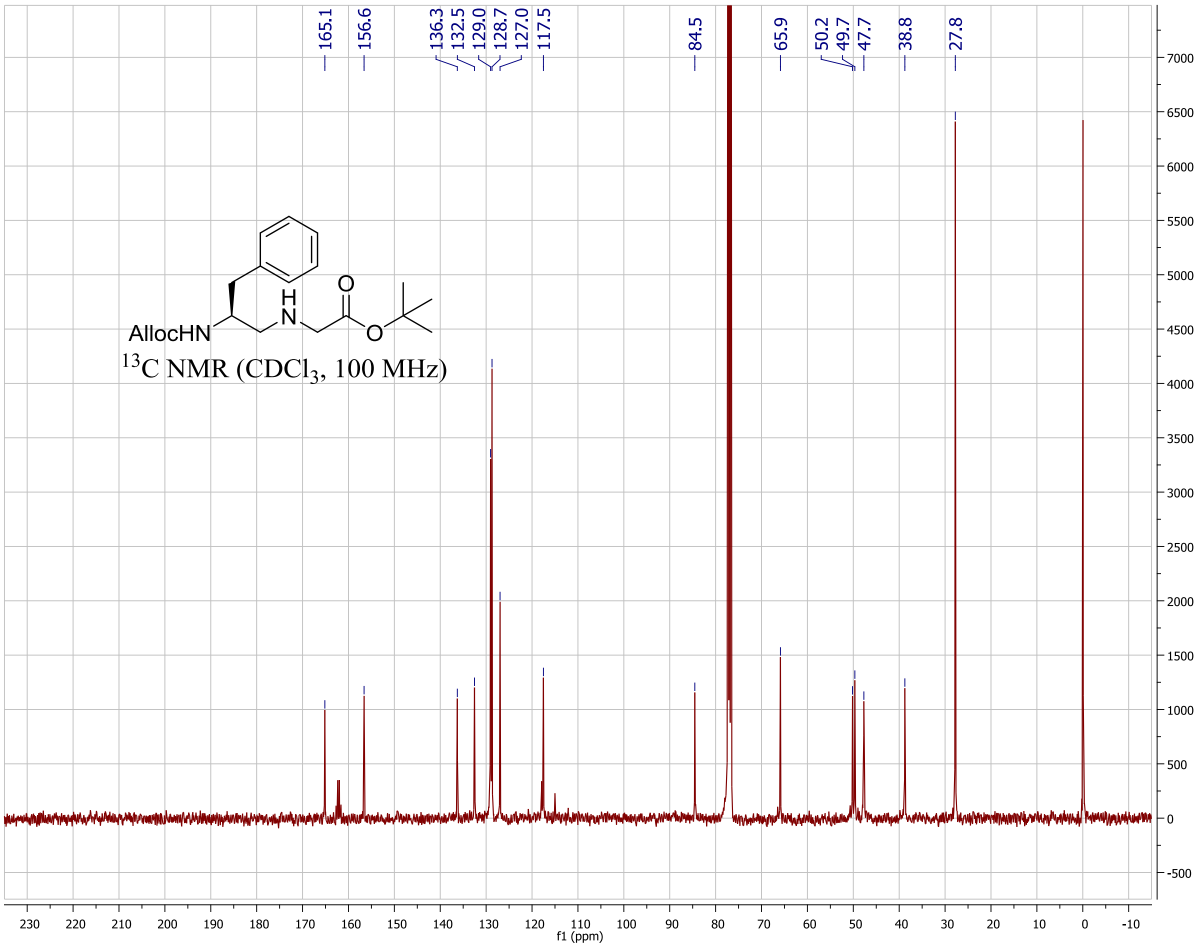
Table S3. Dihedral angle measurement for γ5.

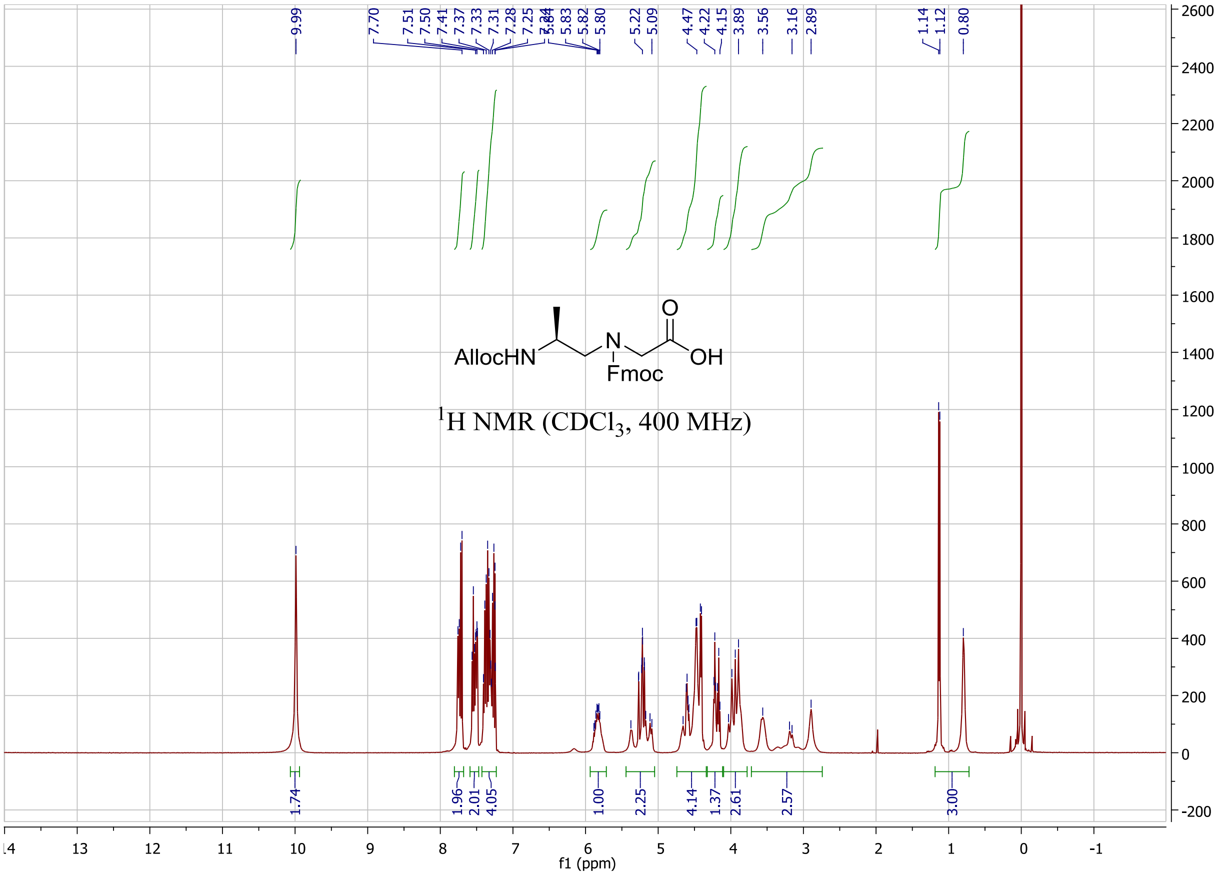


subunit	ϕ	θ	η	ζ	ψ	ω
1	-154.7°	48.0°	-134.7°	70.9°	174.9°	173.6°
2	-83.2°	74.9°	99.8°	-113.1°	6.0°	-171.4°
3	-142.3°	-68.1°	-86.8°	124.1°	3.5°	-179.8°

3. NMR spectra of γ-AApeptide building blocks and cyclic γ-AApeptides γ1-7




165.1
156.6
136.3
132.5
129.0
128.7
127.0
117.5
84.5
65.9
50.2
49.7
47.7
38.8
27.8
AllocHN
H
N
O
O
^{13}C NMR (CDCl$_3$, 100 MHz)
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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7.51
7.50
7.41
7.37
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7.31
7.28
7.25
7.24
5.84
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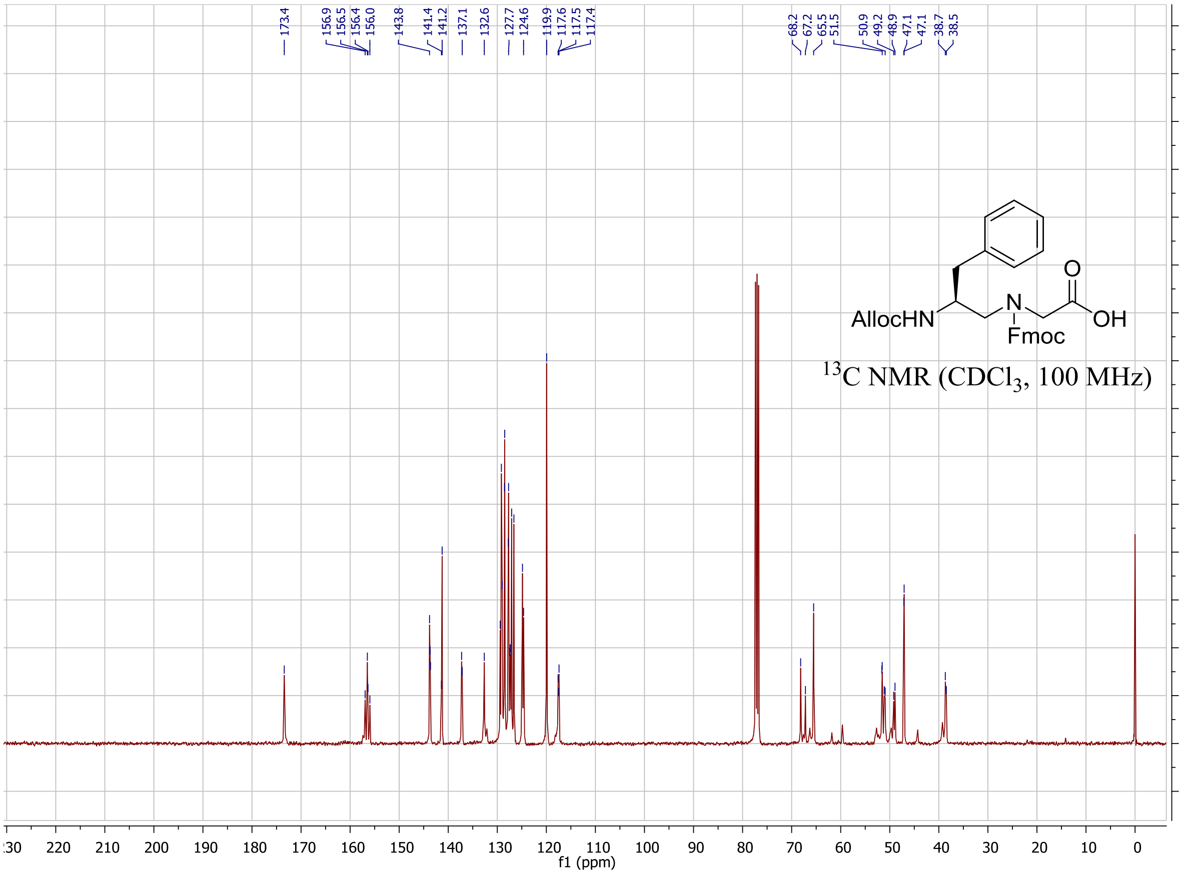
5.82
5.80
5.22
5.09
4.47
4.22
4.15
3.89
3.56
3.16
2.89
1.14
1.12
0.80
AllocHN
N
Fmoc
O
OH
^{1}H NMR (CDCl$_3$, 400 MHz)
1.74
1.96
2.01
4.05
1.00
2.25
4.14
1.37
2.61
2.57
3.00
f1 (ppm)


173.4
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156.4
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141.4
141.2
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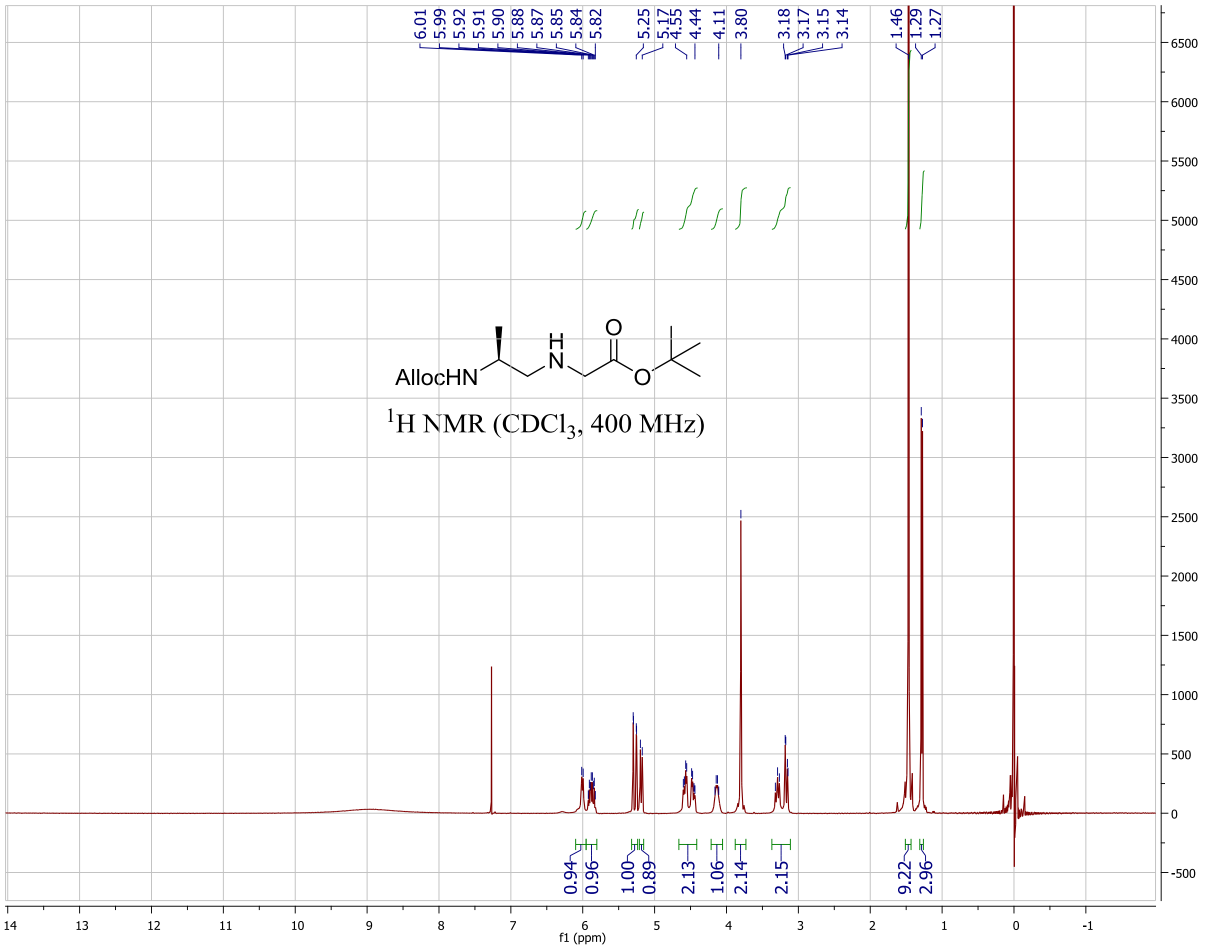
117.6
117.5
117.4
68.2
67.2
65.5
51.5
50.9
49.2
48.9
47.1
47.1
38.7
38.5
O
AllocHN
N
OH
Fmoc
^{13}C NMR (CDCl$_3$, 100 MHz)
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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5.91
5.90
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5.87
5.85
5.84
5.82
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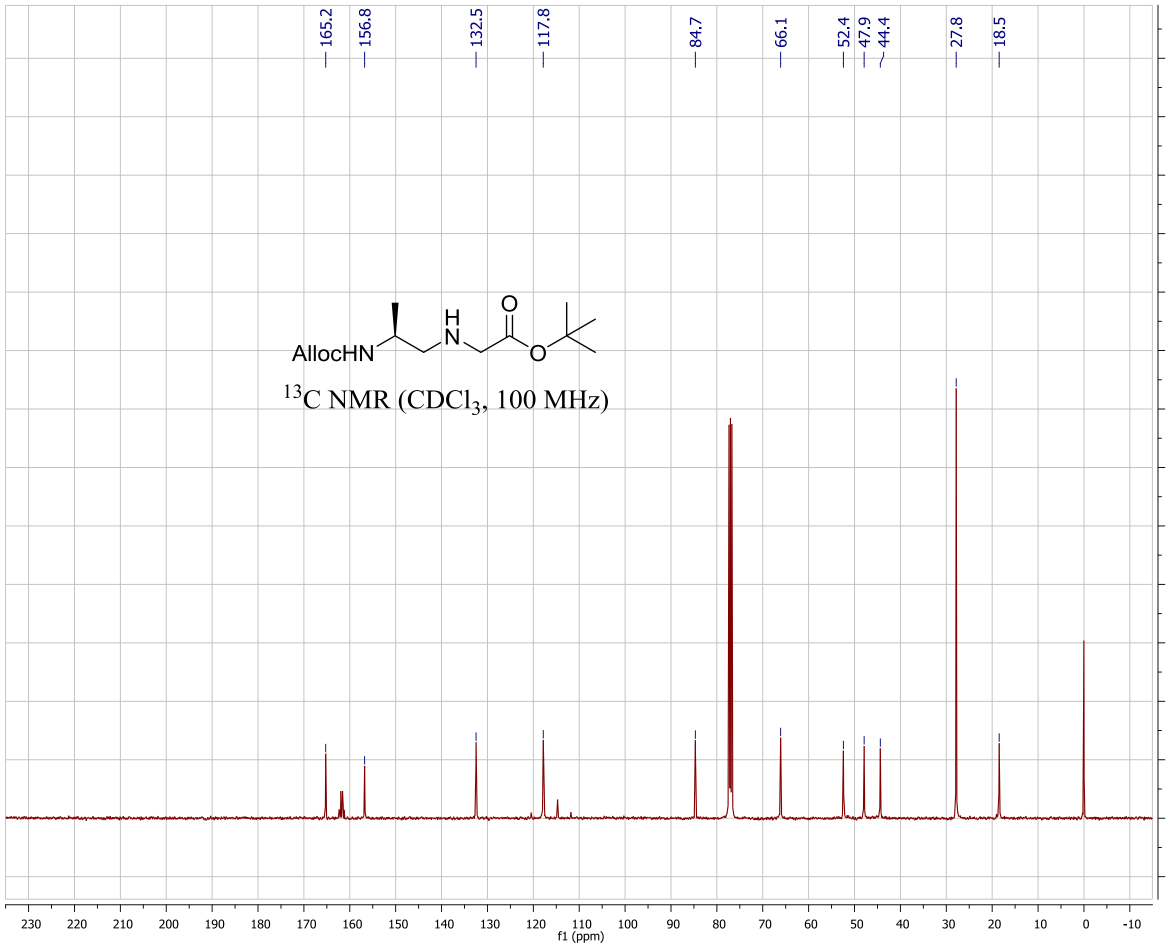
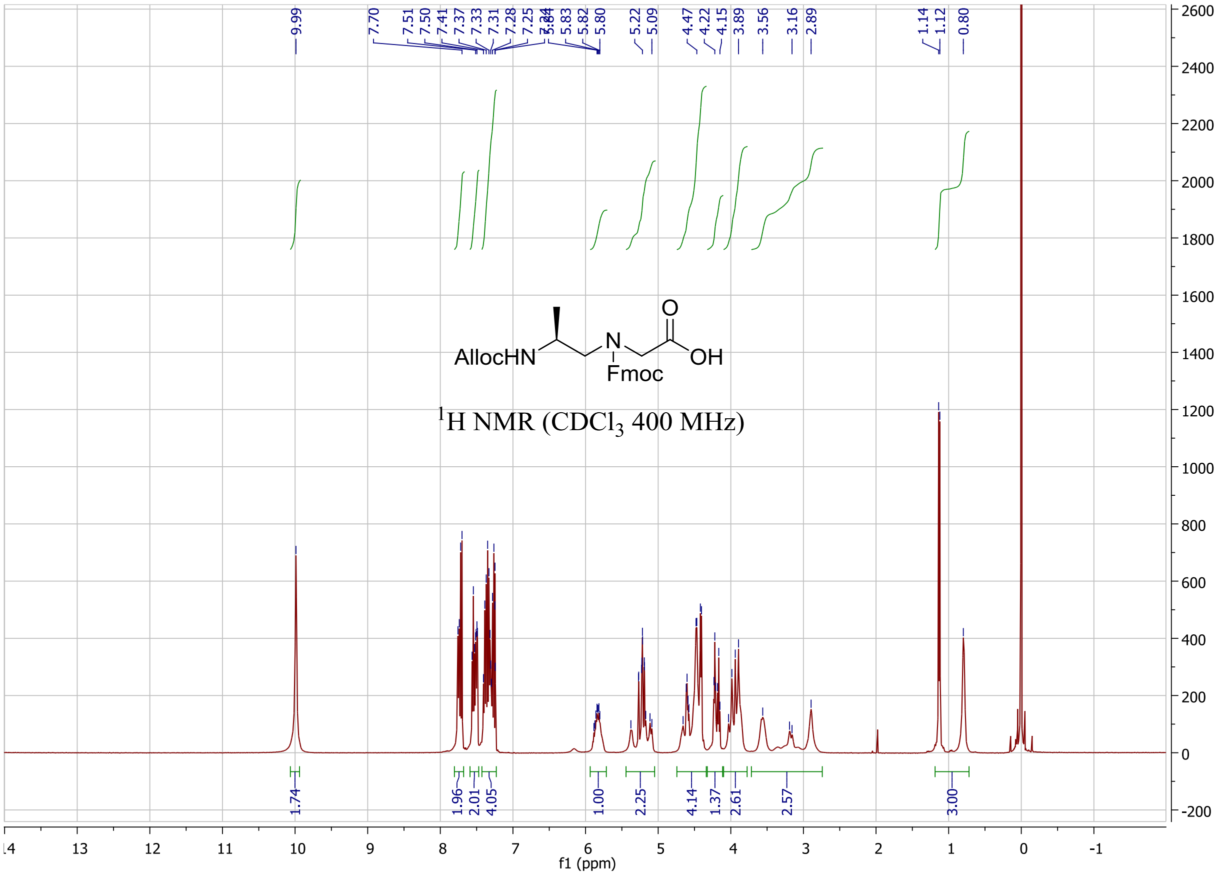
4.44
4.11
3.80
3.18
3.17
3.15
3.14
1.46
1.29
1.27
AllocHN
H
N
O
O
^{1}H NMR (CDCl$_3$, 400 MHz)
0.94
0.96
1.00
0.89
2.13
1.06
2.14
2.15
9.22
2.96
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

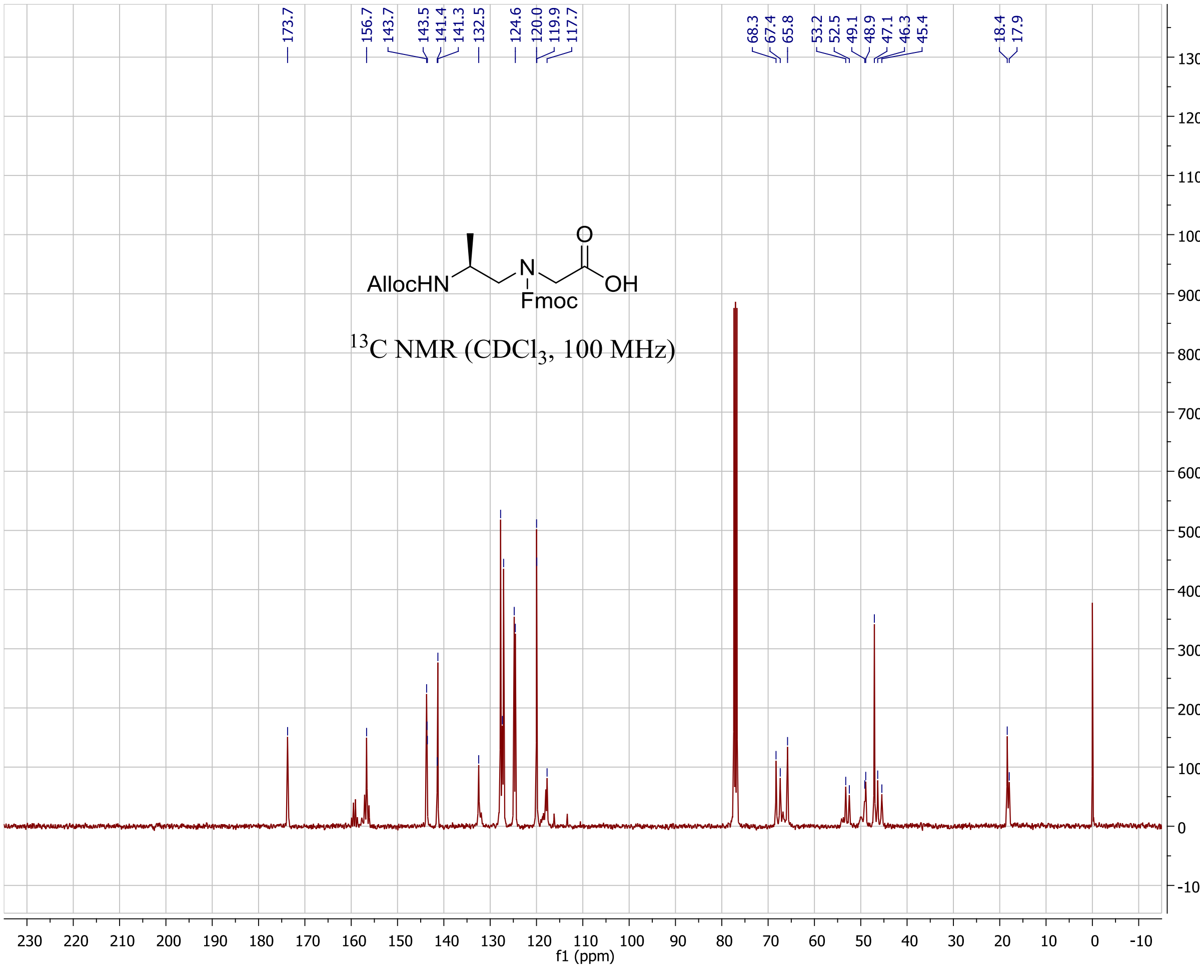


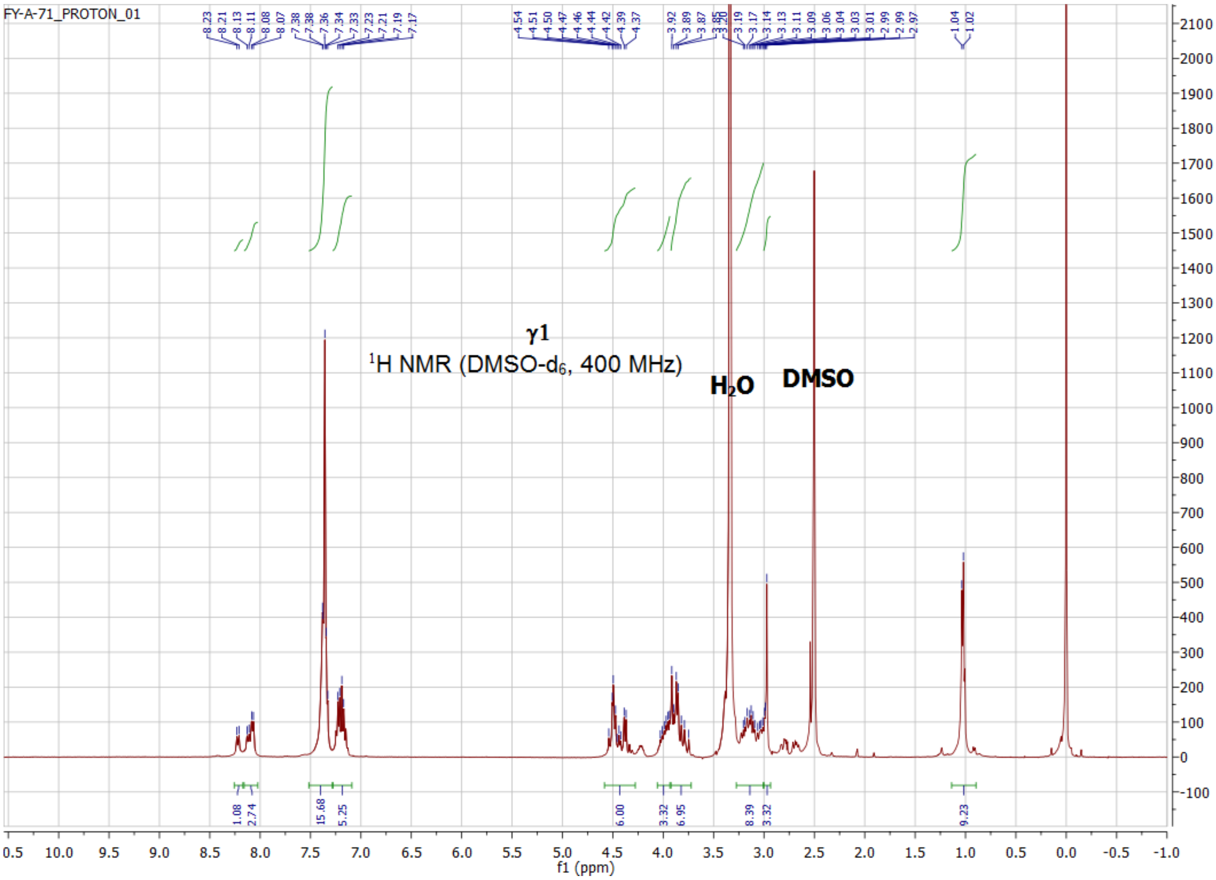
AllocHN

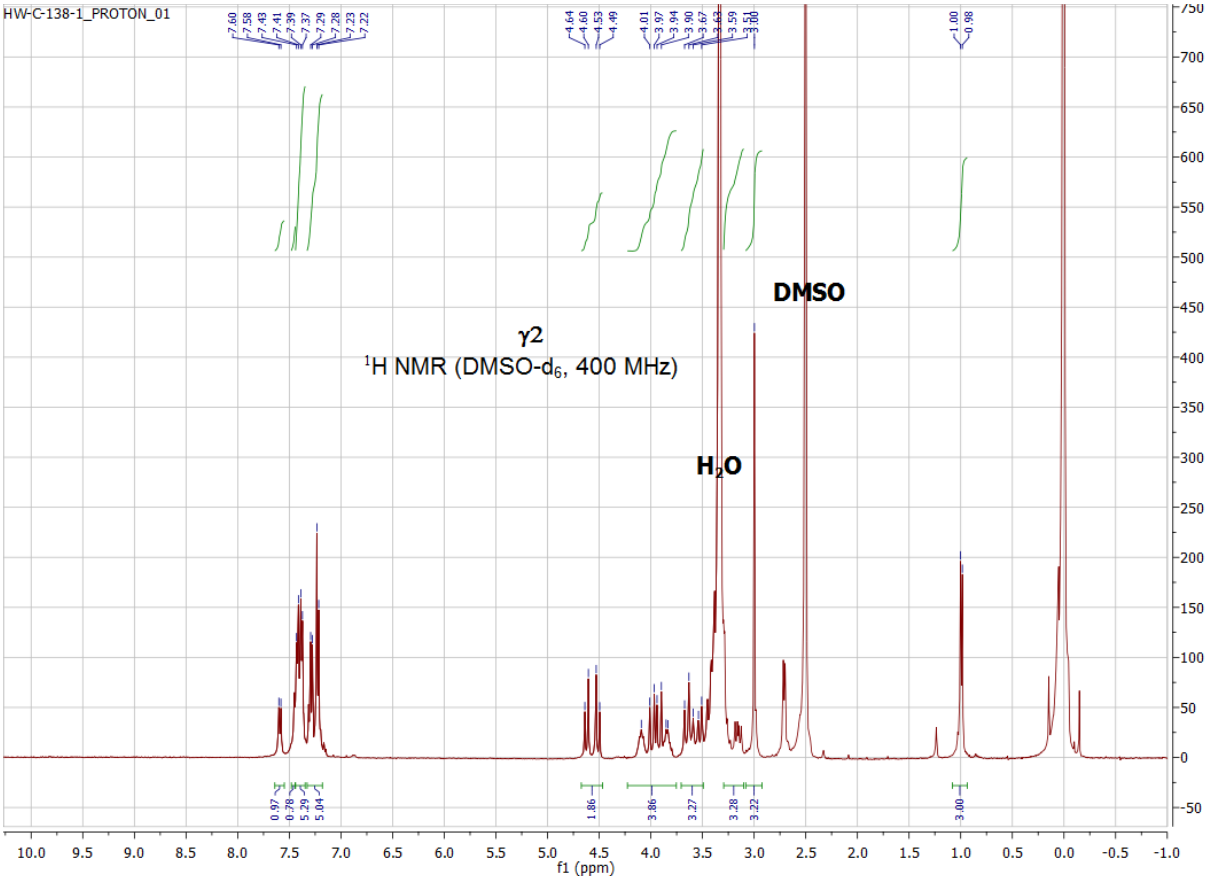
^{13}C NMR (CDCl$_3$, 100 MHz)



^{1}H NMR (CDCl$_3$ 400 MHz)

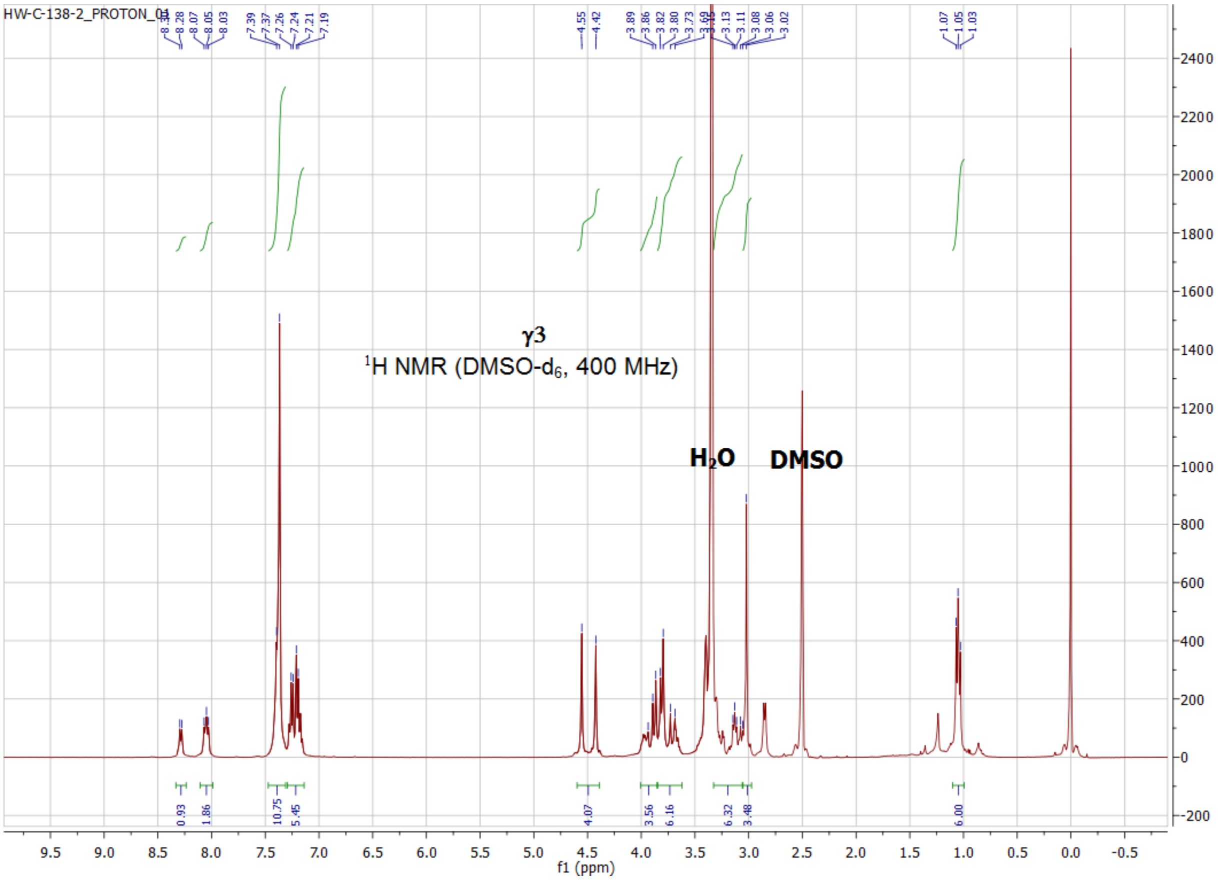

173.7
156.7
143.7
143.5
141.4
141.3
132.5
124.6
120.0
119.9
117.7
68.3
67.4
65.8
53.2
52.5
49.1
48.9
47.1
46.3
45.4
18.4
17.9
AllocHN
N
Fmoc
O
OH
^{13}C NMR (CDCl$_3$, 100 MHz)
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

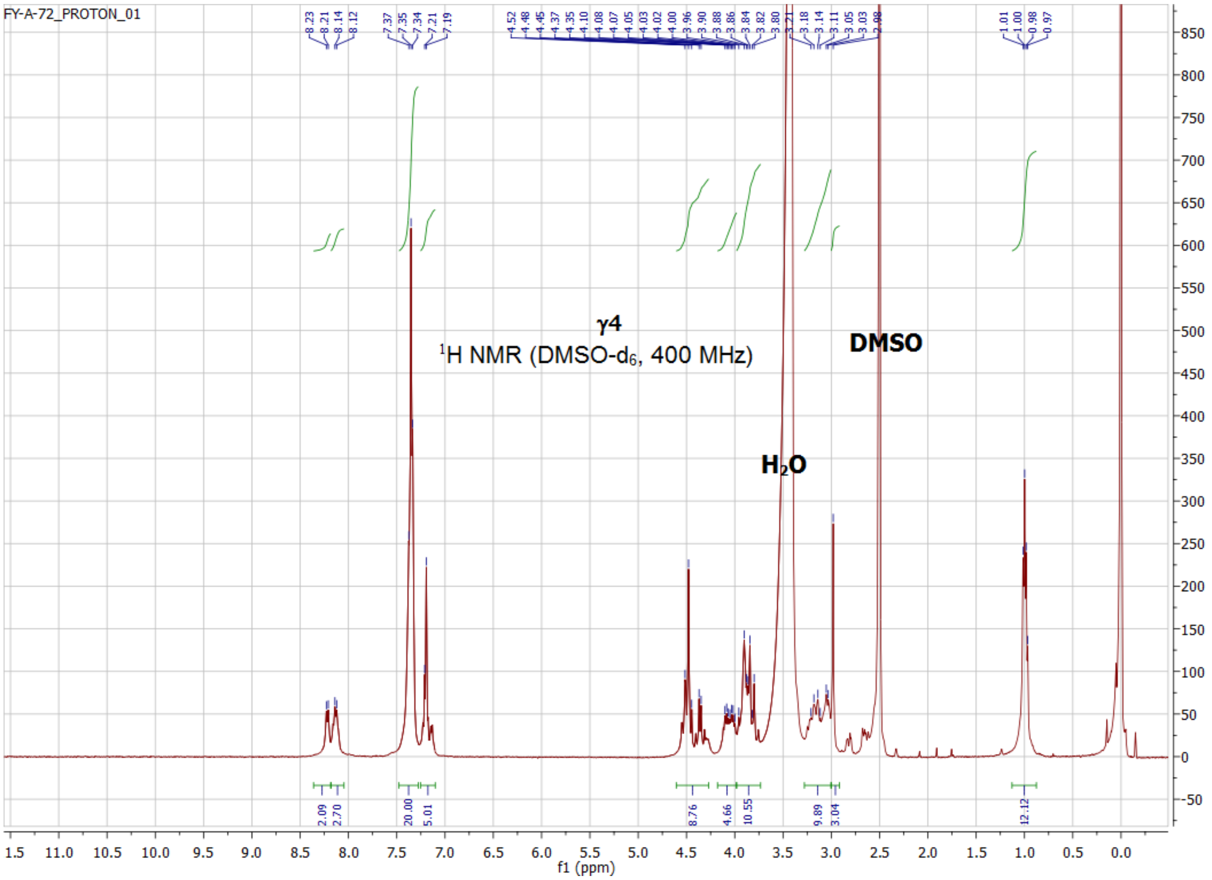

FY-A-71_PROTON_01
γ1
^{1}H NMR (DMSO-d$_6$, 400 MHz)
H$_2$O
DMSO
8.23 8.21 8.13 8.11 8.08 8.07 7.38 7.36 7.34 7.33 7.23 7.21 7.19 7.17
4.54 4.51 4.50 4.47 4.46 4.44 4.42 4.39 4.37
3.92 3.89 3.87 3.85 3.20 3.19 3.17 3.14 3.13 3.11 3.09 3.06 3.04 3.03 3.01 2.99 2.97
1.04 1.02
1.08 2.74 15.68 5.25 6.00 3.32 6.95 8.39 3.32 9.23
f1 (ppm)

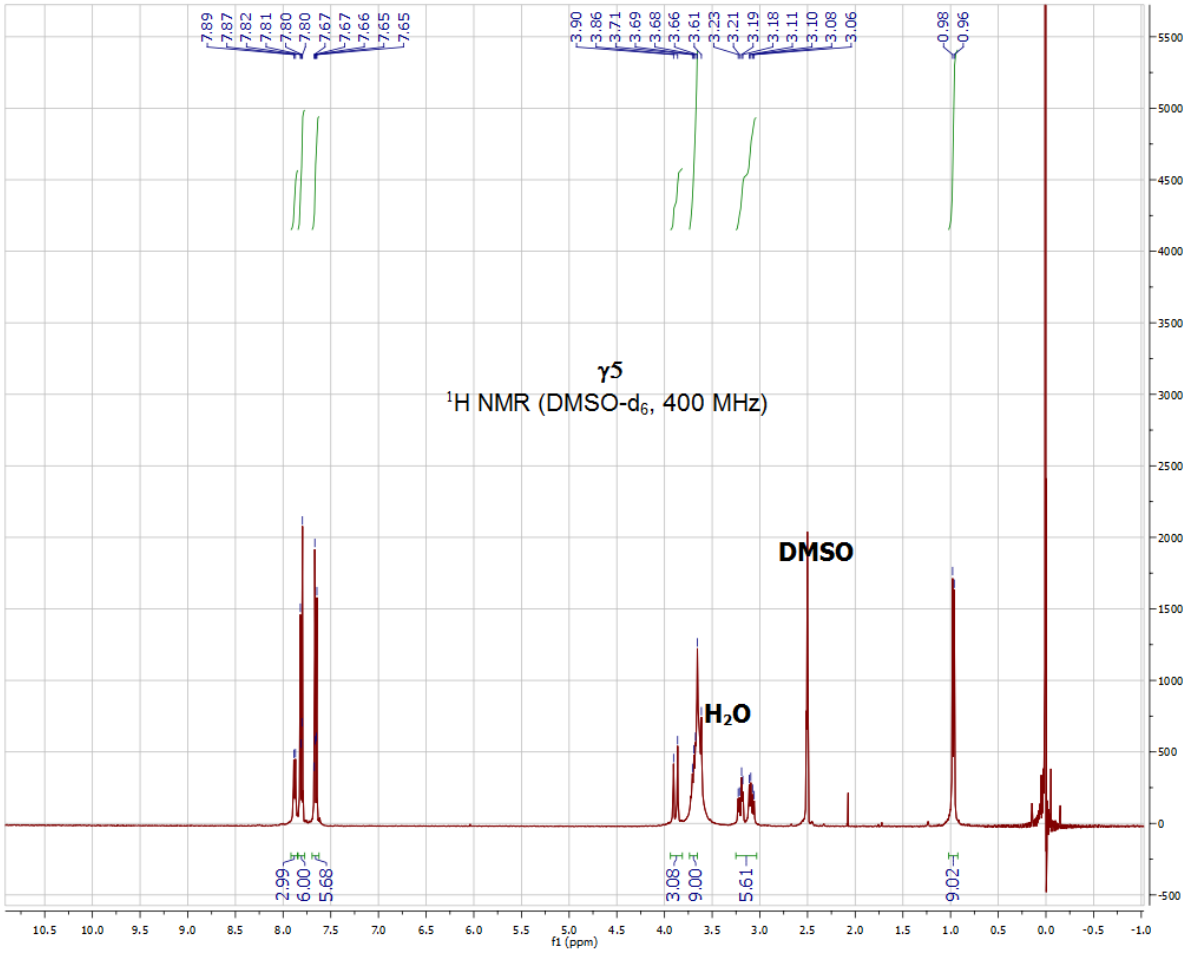


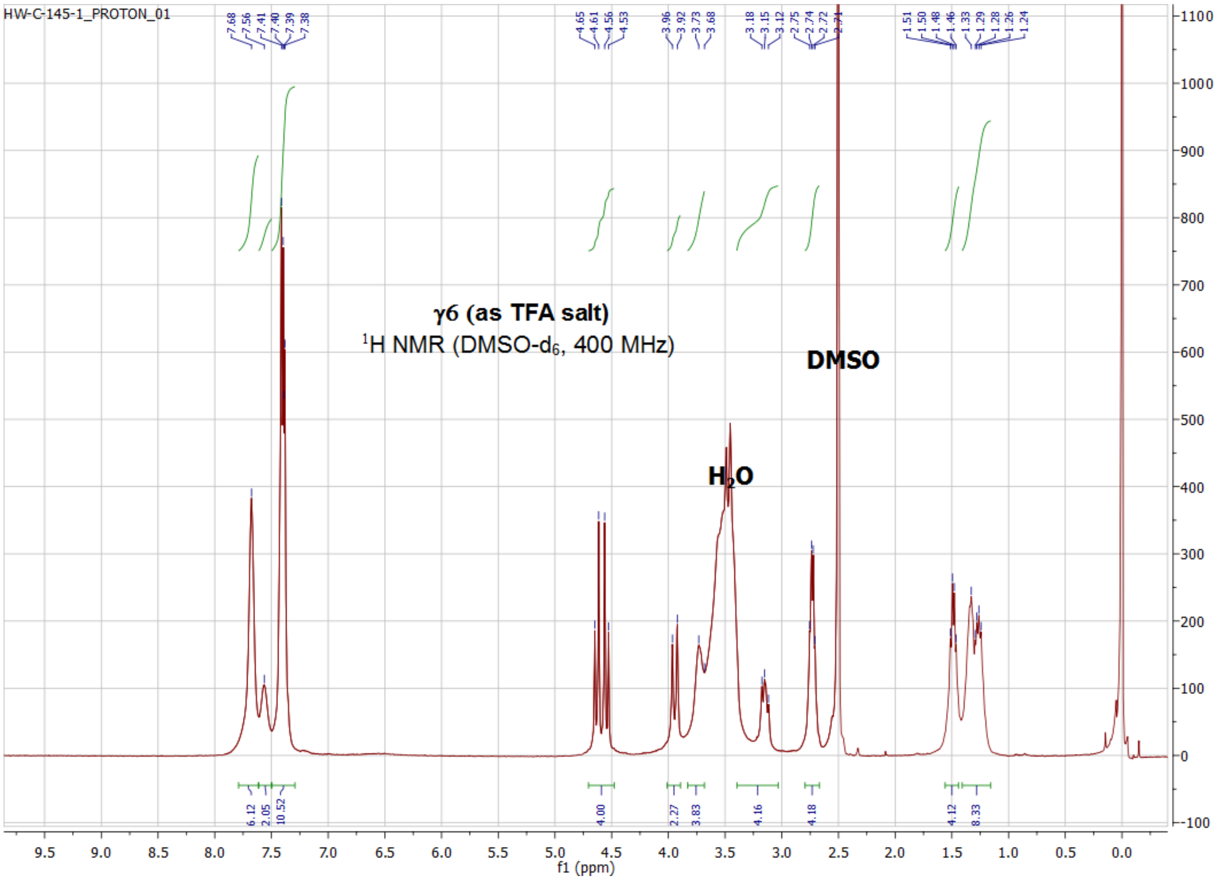
γ2

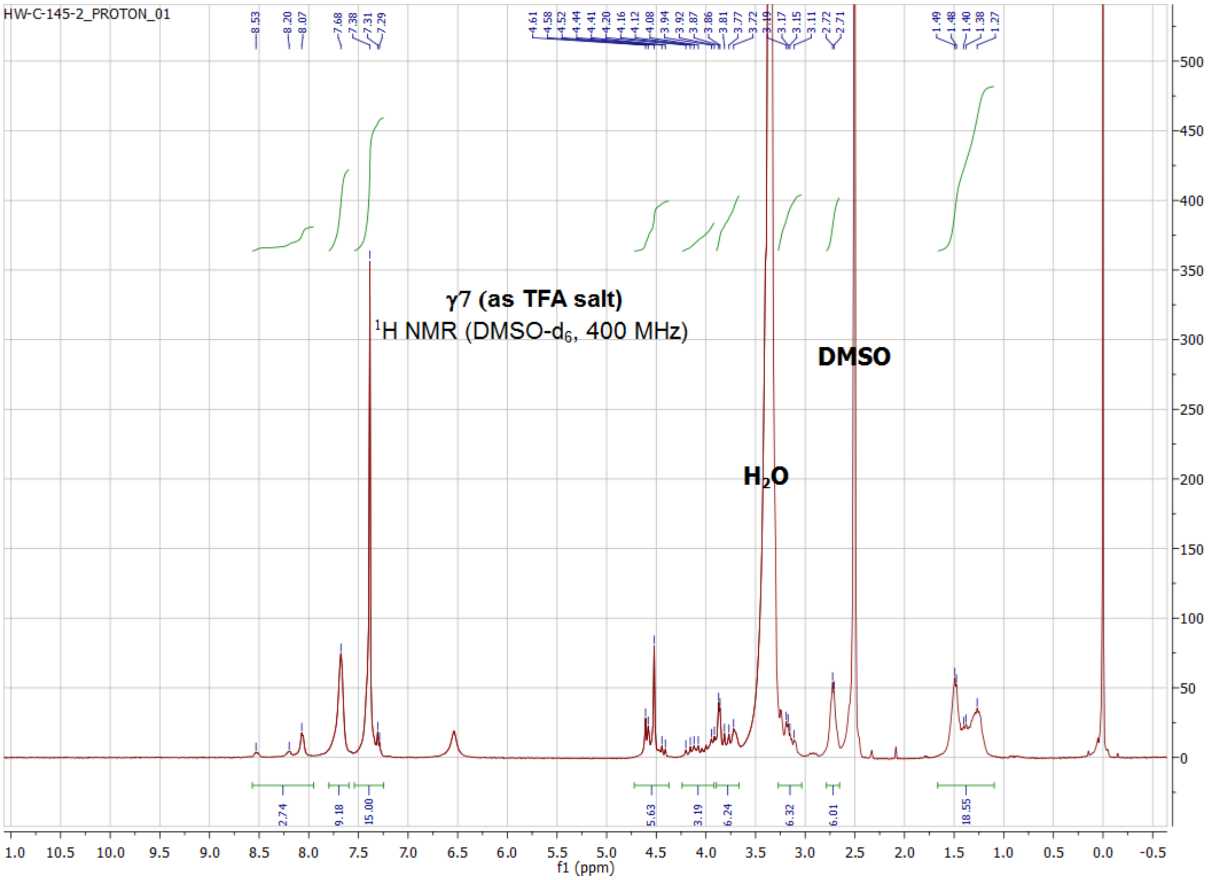
^{1}H NMR (DMSO-d_6, 400 MHz)


HW-C-138-2_PROTON_01
8.30
8.28
8.07
8.05
8.03
7.39
7.37
7.26
7.24
7.21
7.19
4.55
4.42
3.89
3.86
3.82
3.80
3.73
3.69
3.15
3.13
3.11
3.08
3.06
3.02
1.07
1.05
1.03
γ3
¹H NMR (DMSO-d6, 400 MHz)
H2O
DMSO
0.93
1.86
10.75
5.45
4.07
3.56
6.16
6.32
3.48
6.00
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
-200
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

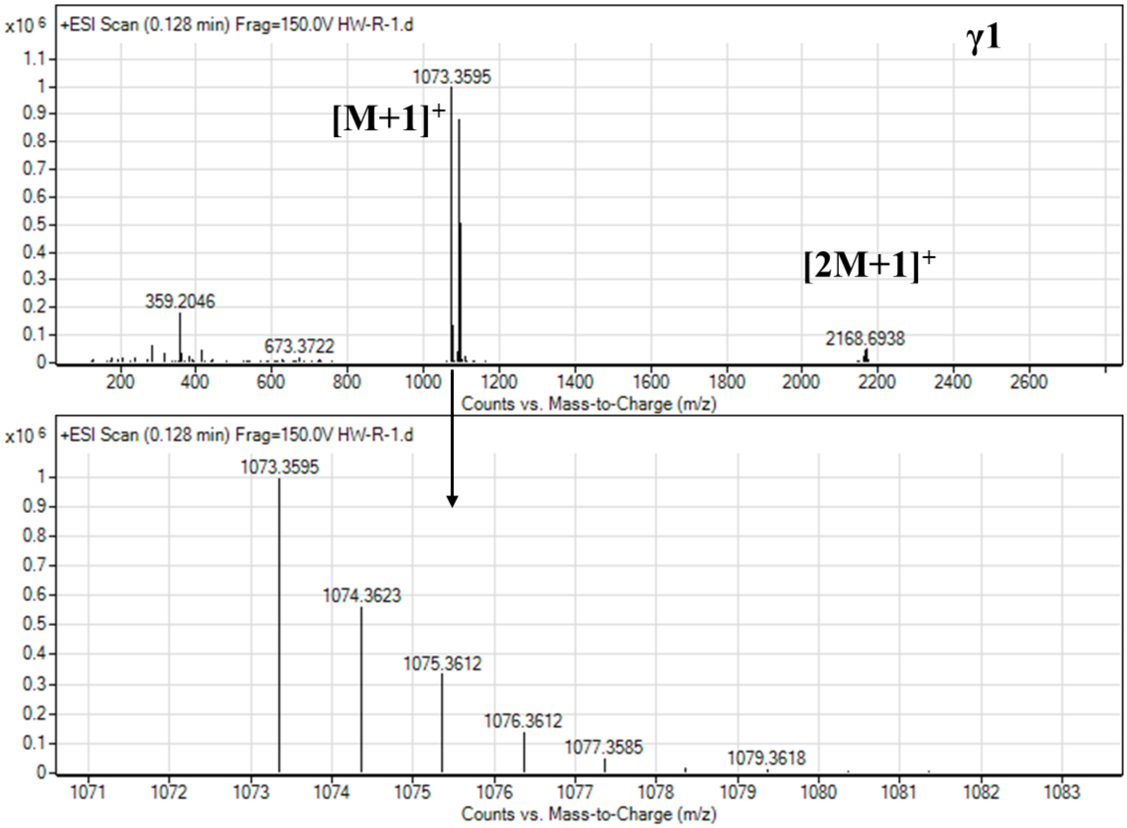

FY-A-72_PROTON_01
8.23
8.21
8.14
8.12
7.37
7.35
7.34
7.21
7.19
4.52
4.48
4.45
4.37
4.35
4.10
4.08
4.07
4.05
4.03
4.02
4.00
3.96
3.90
3.88
3.86
3.84
3.82
3.80
3.21
3.18
3.14
3.11
3.05
3.03
2.98
1.01
1.00
0.98
0.97
γ4
^{1}H NMR (DMSO-d_6, 400 MHz)
DMSO
H_2O
2.09
2.70
20.00
5.01
8.76
4.66
10.55
9.89
3.04
12.12
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
1.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

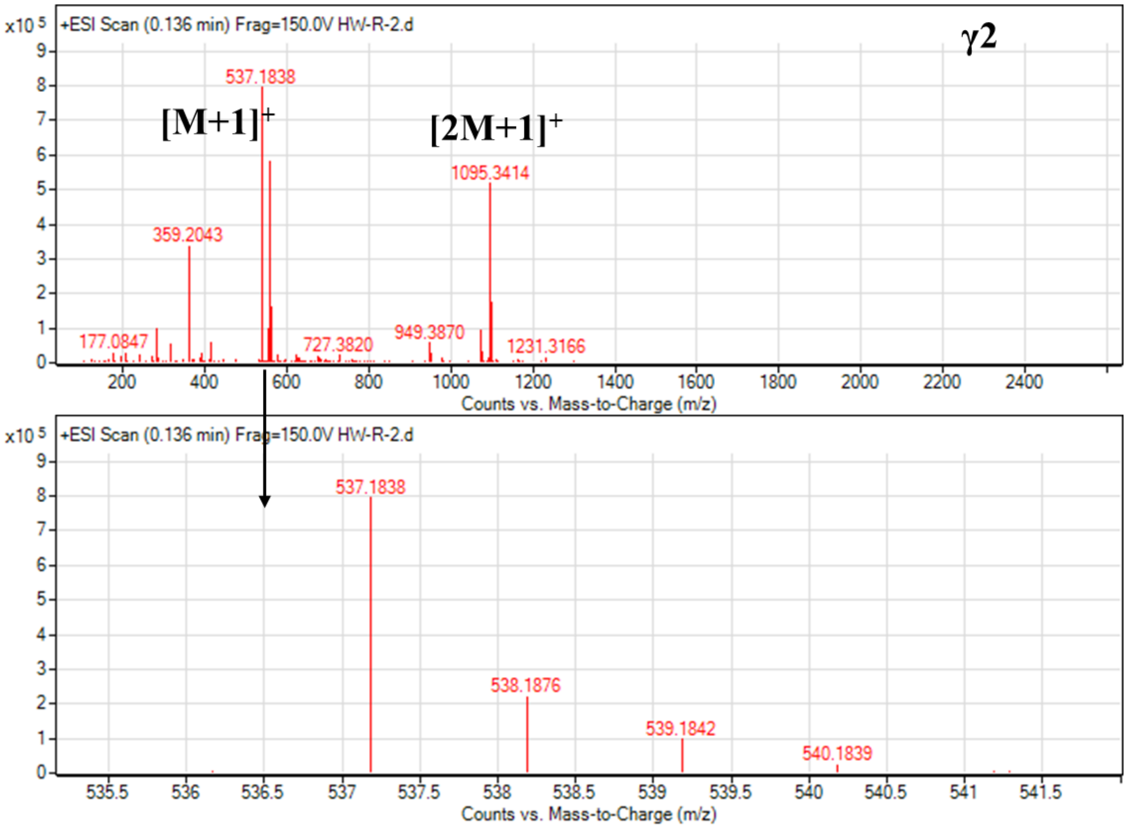

γ5
^{1}H NMR (DMSO-d_6, 400 MHz)
7.89
7.87
7.82
7.81
7.80
7.80
7.67
7.67
7.66
7.65
7.65
3.90
3.86
3.71
3.69
3.68
3.66
3.61
3.23
3.21
3.19
3.18
3.11
3.10
3.08
3.06
0.98
0.96
H$_2$O
DMSO
2.99
6.00
5.68
3.08
9.00
5.61
9.02
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500

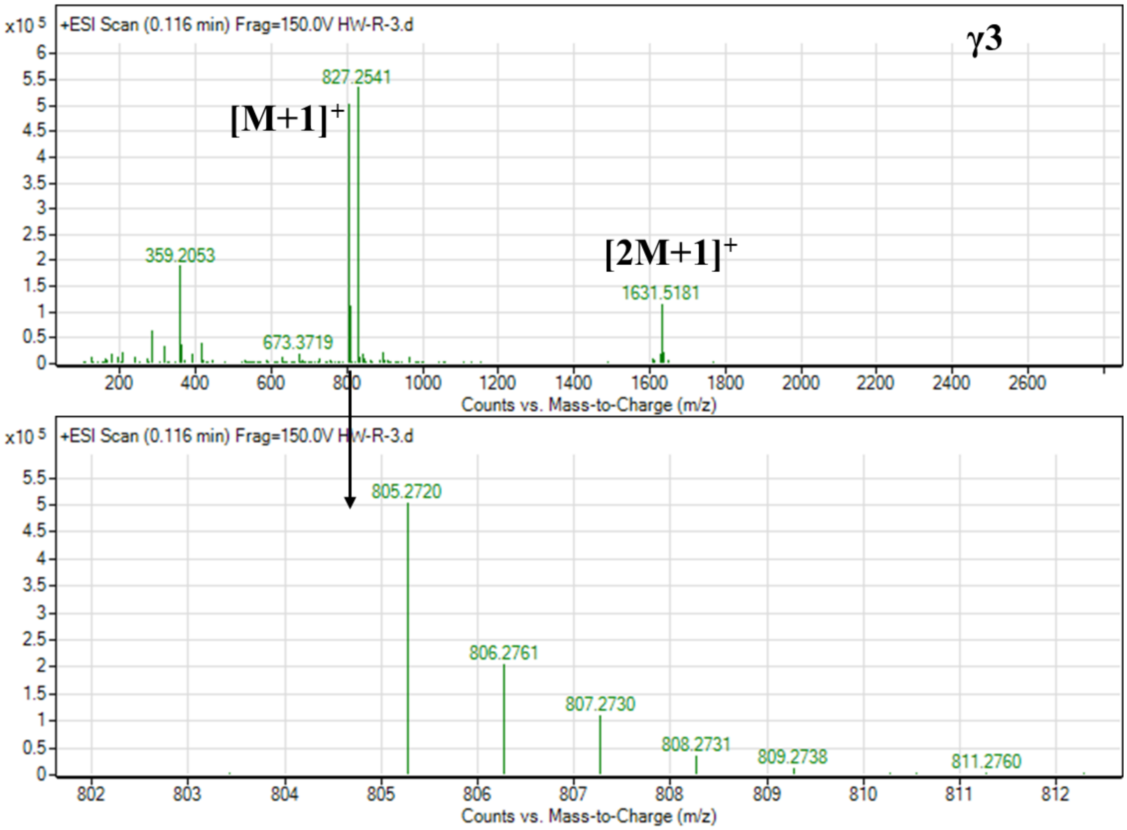

HW-C-145-1_PROTON_01
7.68
7.56
7.41
7.40
7.39
7.38
4.65
4.61
4.56
4.53
3.96
3.92
3.73
3.68
3.18
3.15
3.12
2.75
2.74
2.72
2.71
1.51
1.50
1.48
1.46
1.33
1.29
1.28
1.26
1.24
γ6 (as TFA salt)
^{1}H NMR (DMSO-d$_6$, 400 MHz)
DMSO
H$_2$O
6.12
2.05
10.52
4.00
2.27
3.83
4.16
4.18
4.12
8.33
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
1100
1000
900
800
700
600
500
400
300
200
100
0
-100

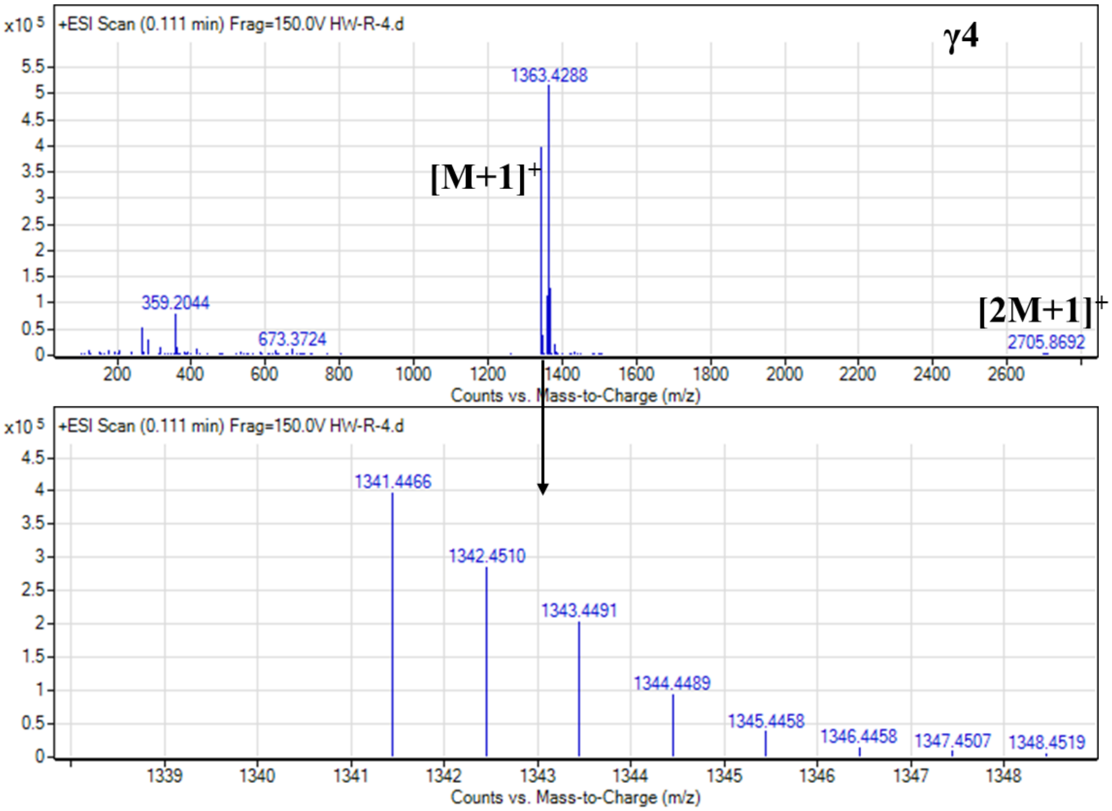
HW-C-145-2_PROTON_01
γ7 (as TFA salt)
^{1}H NMR (DMSO-d$_6$, 400 MHz)
DMSO
H$_2$O
8.53
8.20
8.07
7.68
7.38
7.31
7.29
4.61
4.58
4.52
4.44
4.41
4.20
4.16
4.12
4.08
3.94
3.92
3.87
3.86
3.81
3.77
3.72
3.19
3.17
3.15
3.11
2.72
2.71
1.49
1.48
1.40
1.38
1.27
2.74
9.18
15.00
5.63
3.19
6.24
6.32
6.01
18.55
500
450
400
350
300
250
200
150
100
50
0
1.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

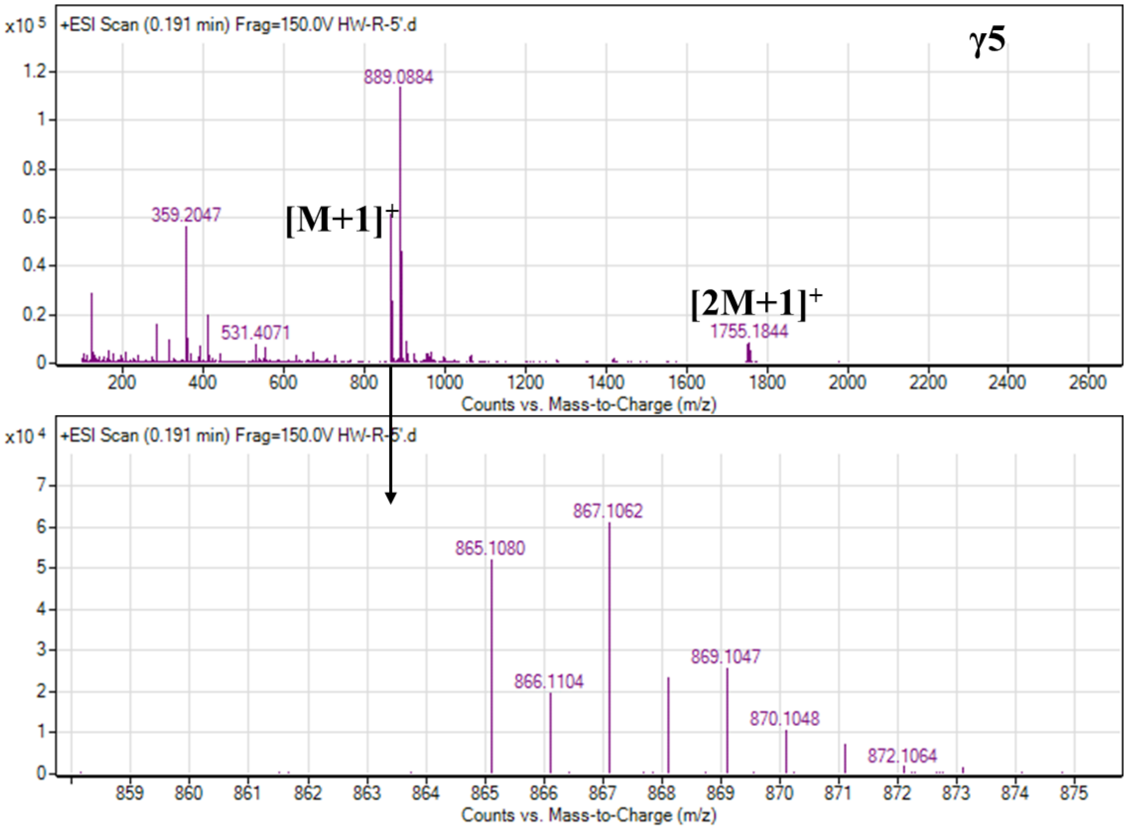
4. Mass spectra of cyclic γ-AApeptides γ1-7

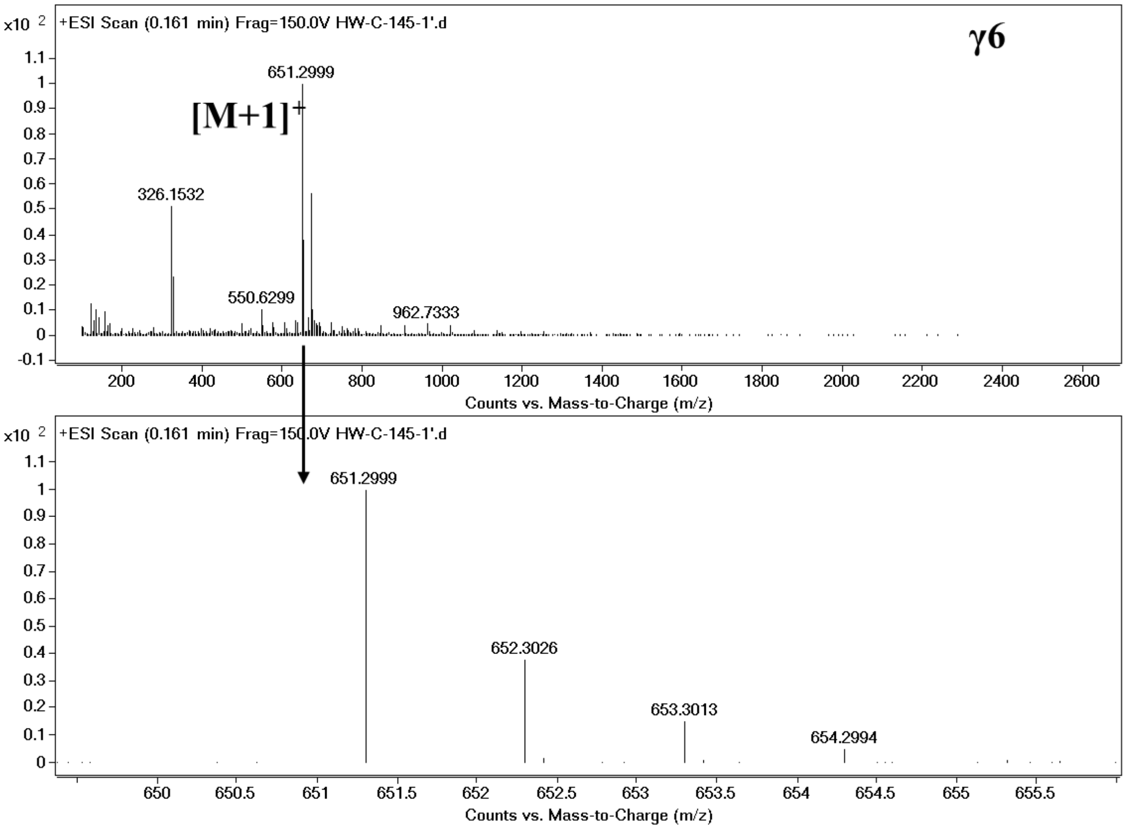


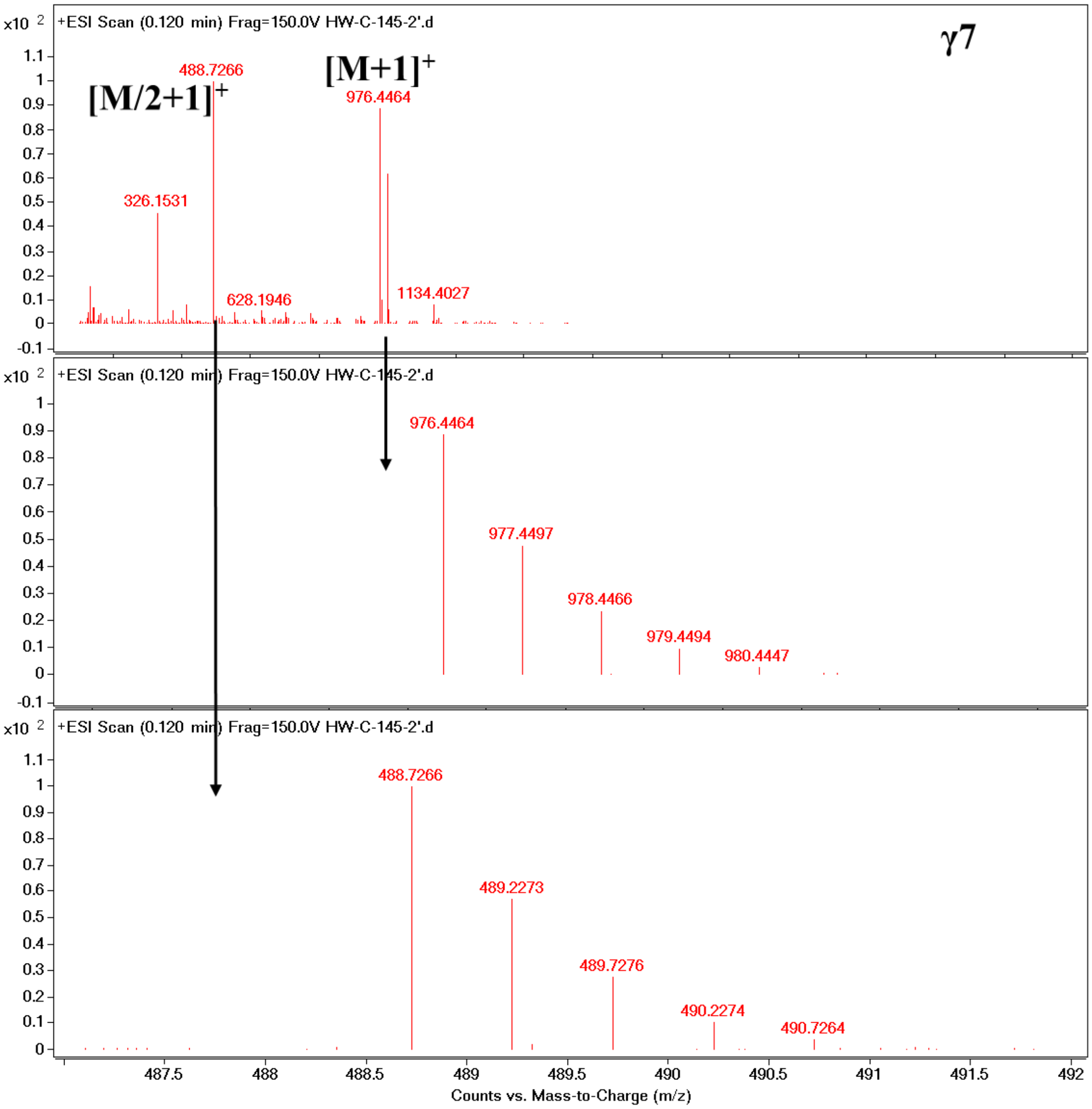



γ3
+ESI Scan (0.116 min) Frag=150.0V HW-R-3.d
x10 5
[M+1]+
827.2541
359.2053
673.3719
[2M+1]+
1631.5181
Counts vs. Mass-to-Charge (m/z)
805.2720
806.2761
807.2730
808.2731
809.2738
811.2760
802
803
804
805
806
807
808
809
810
811
812
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600


γ4
+ESI Scan (0.111 min) Frag=150.0V HW-R-4.d
x10 5
1363.4288
[M+1]+
359.2044
673.3724
[2M+1]+
2705.8692
Counts vs. Mass-to-Charge (m/z)
1341.4466
1342.4510
1343.4491
1344.4489
1345.4458
1346.4458
1347.4507
1348.4519
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348

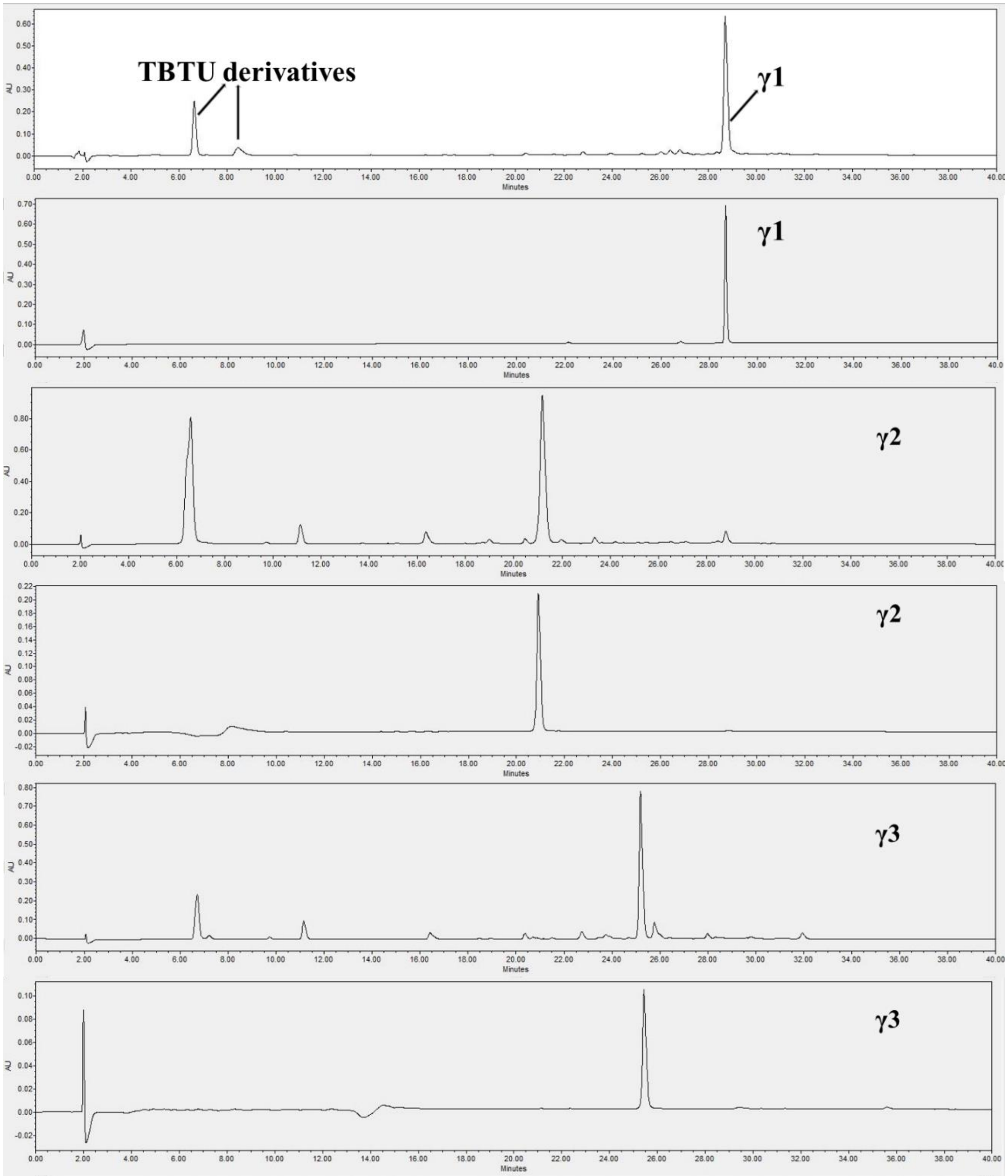

γ5
x10 5
+ESI Scan (0.191 min) Frag=150.0V HW-R-5'.d
889.0884
359.2047
[M+1]+
531.4071
[2M+1]+
1755.1844
Counts vs. Mass-to-Charge (m/z)
x10 4
+ESI Scan (0.191 min) Frag=150.0V HW-R-5'.d
867.1062
865.1080
869.1047
866.1104
870.1048
872.1064
Counts vs. Mass-to-Charge (m/z)

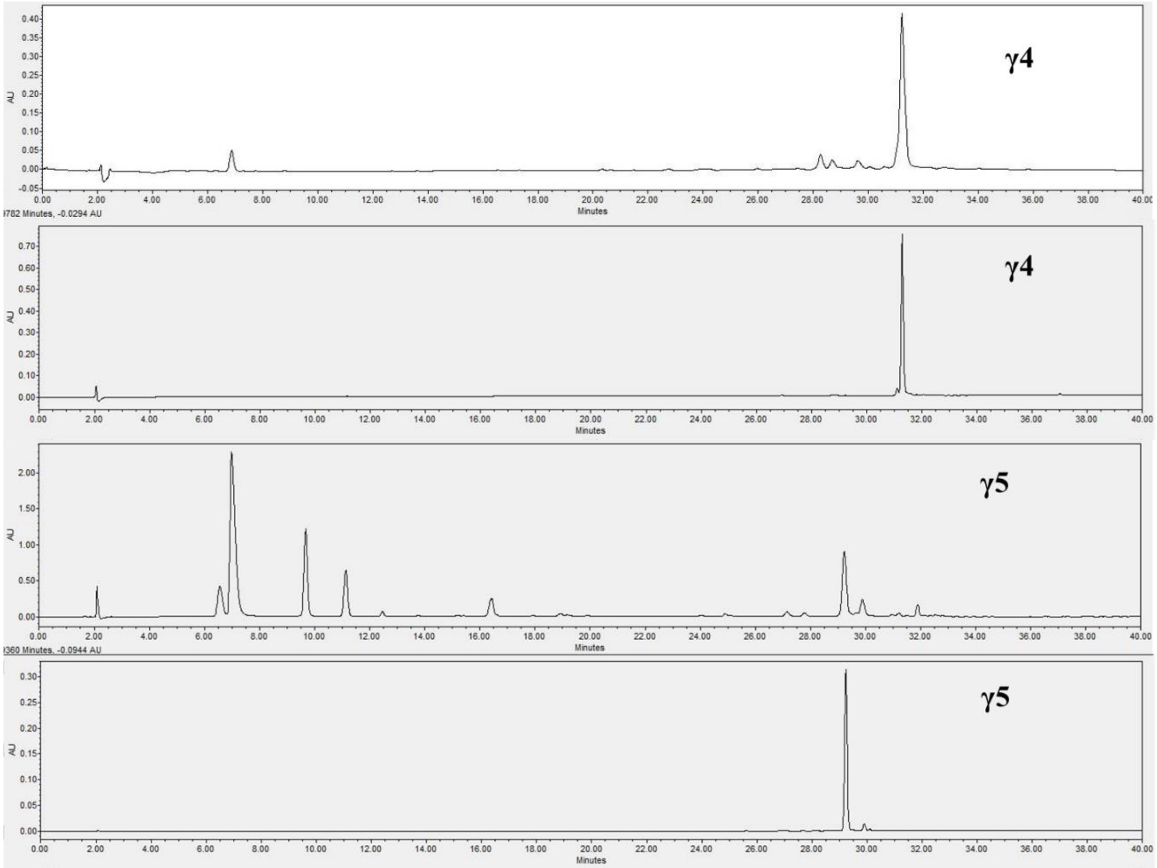

γ6
x10 2
+ESI Scan (0.161 min) Frag=150.0V HW-C-145-1'.d
651.2999
[M+1]+
326.1532
550.6299
962.7333
Counts vs. Mass-to-Charge (m/z)
x10 2
+ESI Scan (0.161 min) Frag=150.0V HW-C-145-1'.d
651.2999
652.3026
653.3013
654.2994
Counts vs. Mass-to-Charge (m/z)



5. HPLC traces

The analytical HPLC gradient system employed a program of 5 % to 100 % linear gradient of solvent B (0.1 % TFA in acetonitrile) in A (0.1 % TFA in water) over the duration of 40 min.


TBTU derivatives
γ1
γ1
γ2
γ2
γ3
γ3
AU
Minutes
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
38.00
40.00


γ4
AU
Minutes
782 Minutes, -0.0294 AU
γ4
AU
Minutes
γ5
AU
Minutes
360 Minutes, -0.0944 AU
γ5
AU
Minutes
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
38.00
40.00

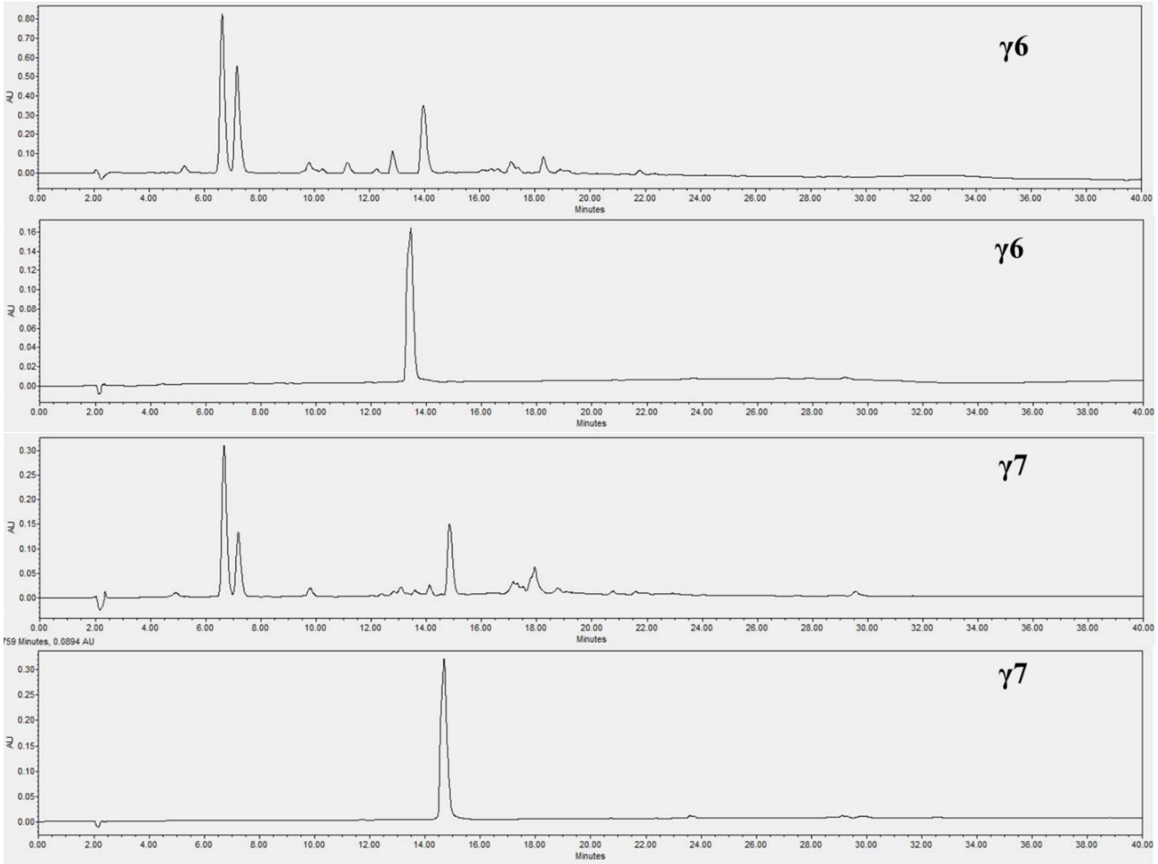


Figure S5. HPLC analysis of crude and pure cyclic sulfono-γ-AApeptides. (Peaks between 6-18 mins belong to coupling reagent derivatives, thus not counted when calculating purity)

References

(1) Hurevich, M.; Swed, A.; Joubran, S.; Cohen, S.; Freeman, N. S.; Britan-Rosich, E.; Briant-Longuet, L.; Bardy, M.; Devaux, C.; Kotler, M.; Hoffman, A.; Gilon, C. *Bioorgan. Med. Chem.* **2010**, *18*, 5754.

(2) Kim, H. O.; Carroll, B.; Lee, M. S. *Synth. Commun.* **1997**, *27*, 2505.

(3) Wu, H. F.; Teng, P.; Cai, J. F. *Eur. J. Org. Chem.* **2014**, *2014*, 1760.

(4) Gomez-Martinez, P.; Dessolin, M.; Guibe, F.; Albericio, F. *J. Chem. Soc., Perkin Trans. 1.* **1999**, 2871.