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Electronic Supplementary Information

Organic and Biomolecular Chemistry

N-Hydroxy peptides: Solid-phase synthesis and β-sheet propensity

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EXPERIMENTAL METHODS

Solution-phase synthesis general notes. Unless stated otherwise, reactions were performed in flame dried glassware under a positive pressure of argon or nitrogen gas using dry solvents. Commercial grade reagents and solvents were used without further purification except where noted. Anhydrous solvents were purchased directly from chemical suppliers. Thin-layer chromatography (TLC) was performed using silica gel 60 F254 pre-coated plates (0.25 mm). Flash chromatography was performed using silica gel (60 µm particle size). Reaction progress was judged by TLC analysis (single spot/two solvent systems) using a UV lamp, CAM (ceric ammonium molybdate), ninhydrin, or basic $KMnO_4$ stain(s) for detection purposes. NMR spectra were recorded on a 400, 500, or 800 MHz spectrometer. Proton chemical shifts are reported as δ values relative to residual signals from deuterated solvents (D_2O, $CDCl_3$, CD_3OD, or DMSO-d_6).

Benzyl (cyanomethyl)-L-alaninate (2). A mixture of L-alanine benzyl ester (HCl salt, 3.00 g, 13.9 mmol) and DIEA (7.26 mL, 41.7 mmol) in MeCN was treated with bromoacetonitrile (1.07 mL, 15.3 mmol) dropwise over 10 min at rt. The reaction was stirred for 36 h at 40 ºC prior to the removal of MeCN. The residue was dissolved in DCM and washed with sat. aq. $NaHCO_3$. The organic layer was collected, and the aq phase was extracted with additional DCM. The combined organic layers were dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. Purification by silica gel flash chromatography (20% EtOAc/hexanes), gave **2** as a colorless oil (2.55 g, 84% yield). ^1H NMR (400 MHz, DMSO-d_6) δ 7.52 – 7.23 (m, 5H), 5.14 (s, 2H), 3.82 – 3.34 (m, 4H), 1.24 (d, J = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.4, 135.9, 128.5, 128.1, 127.9, 118.7, 65.9, 54.9, 34.7, 17.9; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{12}H_{14}N_2NaO_2$ 241.0947, found 241.0954.

H-hAla-OBn (3). Compound **2** (2.99g, 13.7 mmol) was immediately dissolved in DCM and cooled to 0º C. To the cooled mixture, 70% mCPBA (7.37g, 32.8 mmol) was added in two portions over 30 min. The solution was allowed to warm to rt and stirred for 1.5 h. The reaction flask was then cooled to 0º C prior to the addition of sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$ and the resulting slurry stirred for an additional 30 min until two layers were observed. The organic layer was collected, and the aq phase was extracted with additional DCM. The combined organic layers were dried over anhydrous Na_2SO_4. Concentration on a rotary evaporator provided the crude nitrone intermediate as a yellow oil. A mixture of the crude nitrone and hydroxylamine hydrochloride (4.76 g, 68.5 mmol) was dissolved in MeOH and stirred 18 h at 60º C. The solution was concentrated to remove MeOH. The residue was dissolved in DCM and washed with sat. aq. $NaHCO_3$. The organic layer was collected, and the aq phase was extracted with additional DCM. The combined organic layers were dried over anhydrous Na_2SO_4. Purification by silica gel flash chromatography (5%-40% EtOAc/hexanes) gave **3** as a colorless oil (2.01g, 72% yield over 2 steps) ^1H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.19 (m, 5H), 6.60 – 6.30 (br s, 2H), 5.19 – 4.99 (m, 2H), 3.70 (q, J = 7.1 Hz, 1H), 1.18 (d, J = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7, 135.6, 128.7, 128.42, 128.2, 66.9, 60.3, 14.6; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{10}H_{14}NO_3$ 196.0950, found 196.0968.

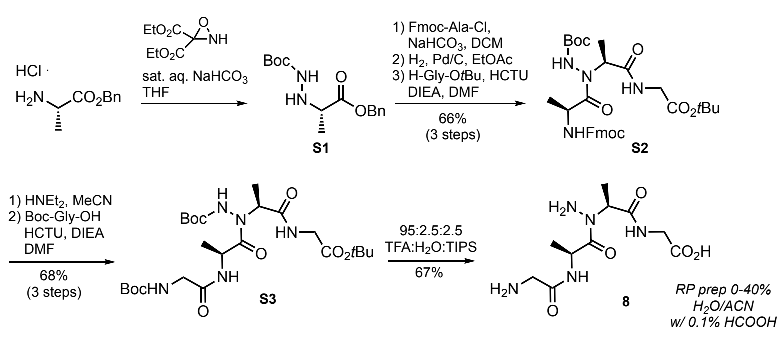
Fmoc-Ala-hAla-Gly-O*t*Bu (4). A solution of Fmoc-Ala-Cl[1] (3.80 g, 11.5 mmol) in DCM was added to a solution of **3** (1.50 g, 7.68 mmol) and $NaHCO_3$ (6.45 g, 76.8 mmol) in DCM. The reaction was stirred for 6 h at rt prior to the removal of DCM. The residue was diluted with EtOAc and washed with 1M aq HCl, sat. aq $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (20%-60% EtOAc/hexanes). The purified dipeptide was immediately dissolved in EtOAc and treated with Pd/C (10% wt, 1.20 g) and stirred under H_2 atmosphere at rt for 8 h. The reaction mixture was filtered through celite and concentrated. This crude material was then dissolved in DMF and treated with H-Gly-OtBu (1.44 g, 8.58 mmol), NMM (1.89 mL, 17.16 mmol), and HCTU (2.13 g, 5.15 mmol) and stirred for

18 h. The reaction was diluted with EtOAc and washed with 1M aq HCl, sat. aq $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. Purification by silica gel flash chromatography (20%-60% EtOAc/hexanes) gave **4** as a white solid (2.44 g, 62% yield over 3 steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.91-8.66 (br s, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.33-7.24 (m, 2H), 6.94 (br s, 1H), 5.87 (d, *J* = 8.1 Hz, 1H), 5.31 (m, 1H), 4.98 (t, *J* = 7.4 Hz, 1H), 4.41 – 4.26 (m, 2H), 4.21 (m, 1H), 4.04 – 3.82 (m, 2H), 1.64 – 1.37 (m, 15H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.9, 172.4, 169.1, 156.3, 143.9, 141.4, 127.9, 127.2, 125.2, 125.2, 120.1, 83.1, 67.3, 54.2, 47.2, 42.2, 28.1, 18.3, 14.4; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{27}H_{34}N_3O_9$ 512.2391, found 512.2368.

Boc-Gly-Ala-hAla-Gly-OtBu (6). Compound **4** (348 mg, 681 μmol) was dissolved in a 3:1 solution of diethylamine and MeCN and stirred for 1 h. The reaction was concentrated, and the crude material was taken up in DMF. Boc-Gly-OH (0.30 g, 710 μmol), HCTU (708 mg, 1.71 mmol), and NMM (374 μL, 3.40 mmol) were added, and the reaction was stirred for 18 h. The reaction was concentrated, and the crude material was purified on silica gel via flash chromatography. The purified tetrapeptide (89 mg, 0.15 mmol) was dissolved in a 20% piperidine/DMF solution and stirred for 1.5 h. The reaction was concentrated, then diluted with EtOAc and washed with 1M HCl. The organic layer was dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (60%-100% EtOAc/hexanes), yielding an off-white solid (206 mg, 68% yield over 3 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 – 8.68 (br s, 1H), 7.58 (d, *J* = 7.6 Hz, 1H), 7.45 (br s, 1H), 5.47 (br s, 1H), 5.38 – 5.26 (m, 1H), 5.26 – 5.10 (m, 1H), 4.02 – 3.77 (m, 4H), 1.56 – 1.38 (m, 26H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.1, 172.1, 169.5, 168.7, 156.4, 82.7, 80.5, 54.1, 46.0, 44.0, 42.0, 28.4, 28.1, 18.1, 14.8; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{19}H_{35}N_4O_8$ 447.2449, found 447.2449.

H-Gly-Ala-hAla-Gly-OH (7). Compound **6** (58 mg, 0.13 mmol) was dissolved in 3:1 TFA/DCM at 0 ºC and allowed to warm to room temperature while stirring over 4 h. Upon completion, the reaction was concentrated, and the crude material purified via preparative RP-HPLC (0-40% MeCN in H_2O, linear gradient with 0.1% TFA modifier) to provide **7** as a while solid after lyophilization (21 mg, 55% yield): ^{1}H NMR (400 MHz, D_2O with DSS standard) δ 5.22 – 4.92 (m, 2H), 4.00 – 3.72 (m, 4H), 1.64 – 1.29 (m, 6H); ^{13}C NMR (126 MHz, D_2O with DSS standard) δ 177.7, 176.9, 175.7, 169.5, 58.9, 49.8, 44.7, 43.2, 18.3, 15.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{10}H_{19}N_4O_6$ 291.1299, found 291.1291.

Synthesis of H-Gly-Ala-aAla-Gly-OH



H-aAla-OBn (S1). Benzyl L-alaninate (HCl salt, 2.00 g, 5.69 mmol) was suspended in a mixture of 20 mL THF and 20 mL sat aq $NaHCO_3$, before being treated with 2-(*tert*-butyl) 3,3-diethyl 1,2-oxaziridine-2,3,3-tricarboxylate (1.65 g, 5.69 mmol) dropwise over 5 min. The reaction was left to stir for 2.5 h then diluted with sat aq $NaHCO_3$ and DCM. The organic layer was collected, and the aq layer extracted with additional DCM. The organic layers were dried over anhydrous Na_2SO_4. Purification by silica gel flash chromatography (25% EtOAc/hexanes) gave **S1** as a pale-yellow oil (1.44 g, 86% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.15 (m, 5H), 6.35 (br s, 1H), 5.07 (q, *J* = 12.4, 1.7 Hz, 2H), 4.16 (br s, 1H), 3.70 (br s, 1H), 1.34 (s, 9H), 1.24 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7, 156.4, 135.6, 128.6, 128.4, 128.2, 80.7, 66.8, 58.5, 28.3, 15.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{15}H_{23}N_2O_4$ 295.1652, found 295.1641.

Fmoc-Ala-aAla-Gly-OtBu (S2). A solution of Fmoc-Ala-Cl[1] (5.90 g, 17.7 mmol) in DCM was added to a solution of **S1** (2.60 g, 8.87 mmol) and $NaHCO_3$ (6.45 g, 76.8 mmol) in DCM. The reaction was stirred for 6 h at rt prior to the removal of DCM. The residue was diluted with EtOAc and washed with 1M aq HCl, sat. aq $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (20%-60% EtOAc/hexanes). The purified dipeptide was dissolved in EtOAc and treated with Pd/C (10% wt, 1.20 g) and stirred under H_2 atmosphere at rt for 8 h. The reaction mixture was filtered through celite and concentrated. This crude material was immediately dissolved in DMF and treated with H-Gly-OtBu (1.44 g, 8.58 mmol), NMM (1.89 mL, 17.16 mmol), and HCTU (2.13 g, 5.15 mmol) and stirred for 18 h. The reaction was diluted with EtOAc and washed with 1M aq HCl, sat. aq $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. Purification by silica gel flash chromatography (20%-60% EtOAc/hexanes) gave **S2** as a white solid (3.58 g, 66% yield over 3 steps); ^{1}H NMR (500 MHz, MeOD, mixture of rotamers) δ 7.79 (d, *J* = 7.6 Hz, 2H), 7.70 – 7.60 (m, 2H), 7.44 – 7.22 (m, 4H), 4.76 – 4.51 (m, 2H), 4.38 – 4.23 (m, 2H), 4.24 – 4.14 (m, 1H), 4.00 – 3.67 (m, 2H), 1.63 – 1.25 (m, 24H); ^{13}C NMR (126 MHz, MeOD) δ 177.9, 176.7, 175.0, 173.6, 170.5, 169.9, 158.9, 158.2, 157.6, 157.5, 145.2, 145.1, 142.5, 128.8, 128.2, 128.1, 126.2, 120.9, 83.6, 82.9, 82.9, 82.7, 68.0, 60.2, 59.3, 42.8, 28.5, 28.3, 18.2, 17.3, 14.5, 13.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{32}H_{43}N_4O_8$ 611.3075, found 611.3068.

Boc-Gly-Ala-aAla-Gly-OtBu (S3). Compound **S2** (3.30 g, 5.40 mmol) was dissolved in a 3:1 solution of diethylamine and MeCN and stirred for 1 h. The reaction was concentrated, and the crude material was taken up in DMF. Boc-Gly-OH (2.37 g, 13.5 mmol), HCTU (5.58 g, 13.5 mmol), and NMM (1.48 mL, 13.5 mmol) were added, and the reaction was stirred for 18 h. The reaction was concentrated, and the crude material was purified on silica gel via flash chromatography (60%-100%), resulting in an off-white solid (5.01 g, 68% yield over 2 steps): ^{1}H NMR (400 MHz, MeOD, mixture of rotamers) δ 4.87 (m, 0.75H), 4.80 (m 0.25H), 4.69 (m, 0.25H), 4.55 (q, *J* = 7.4 Hz, 0.75H), 4.00 – 3.61 (m, 4H), 1.61 – 1.43 (m, 27H), 1.42 – 1.28 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6, mixture of rotamers) δ 174.3, 173.8, 170.8, 170.6, 168.3, 168.2, 167.9, 167.7, 156.4, 155.5, 155.2, 155.0, 81.0, 80.2, 80.1, 78.7, 77.5, 57.6, 55.3, 54.4, 44.5, 44.2, 42.7, 42.5, 41.1, 40.9, 17.9, 17.5, 16.9, 13.5, 13.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{24}H_{44}N_5O_9$ 546.3134, found 546.3116.

H-Gly-Ala-aAla-Gly-OH (8). Compound **S3** (0.15 g, 0.34 mmol) was dissolved in 3:1 TFA/DCM at 0 ºC and allowed to warm to room temperature while stirring over 4 h. Upon completion, the reaction was concentrated and the crude material purified via preparative RP-HPLC (0-40% MeCN in H_2O, linear gradient with 0.1% TFA modifier) to provide **8** as a while solid after lyophilization (65 mg, 67% yield): ^{1}H NMR (500 MHz, D_2O, with DSS standard, mixture of rotamers) δ 5.26 (q, *J* = 7.2 Hz, 1H), 5.05 (q, *J* = 7.3 Hz, 1H), 3.90 – 3.69 (m, 4H), 1.55 (d, *J* = 6.6 Hz, 0.25H), 1.48 (d, *J* = 7.0 Hz, 2.75H), 1.43 – 1.36 (m, 3H); ^{13}C NMR (126 MHz, D_2O, with DSS standard, mixture of rotamers) δ 176.7, 173.9, 173.3, 166.6, 55.3, 47.2, 41.2, 40.4, 15.9, 12.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{10}H_{20}N_5O_5$ 290.1459, found 290.1458.

H-(*O*-allyl)hPhe-OMe (13). A mixture of methyl (*R*)-2-hydroxy-3-phenylpropanoate **12** (2.00 g, 11.1 mmol), and 2,6-lutidine (3.84 mL, 33.3 mmol) was cooled to 0 ºC. The solution was treated dropwise with trifluoromethanesulfonic anhydride (2.25 mL, 13.3 mmol) and stirred for 1.5 h. The reaction was cooled again to 0 ºC before addition of a solution of *O*-allylhydroxylamine (free amine, 1.62 g, 22.2 mmol) in DCM. The solution was allowed to warm to rt and stirred for 18 h. The reaction was concentrated and the crude material was purified by silica gel flash chromatography (5% - 40% EtOAc/hexanes), yielding a colorless oil (1.88 g, 72% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.13 (m, 5H), 5.94 (ddt, *J* = 17.3, 10.4, 6.0 Hz, 1H), 5.38 – 5.17 (m, 2H), 4.28 (m, 2H), 4.00 (t, *J* = 7.1 Hz, 1H), 3.74 (s, 3H), 3.00 (d, *J* = 7.1 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7, 136.9, 134.3, 129.3, 128.7, 127.1, 118.2, 75.4, 65.2, 52.2, 35.9; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{13}H_{18}NO_3$ 236.1281, found 236.1290.

Fmoc-Lys(Boc)-(*O*-allyl)hPhe-OMe (14). A solution of N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^6-(*tert*-butoxycarbonyl)-L-lysine (6.70 g, 14.3 mmol) in DCM was treated with 1-chloro-*N,N,*2-trimethyl-1-propenylamine (2.83 mL, 21.4 mmol) and stirred for 5 min. This solution was then transferred into a flask containing a mixture of **13** (2.81 g, 11.9 mmol) and $NaHCO_3$ (9.99 g, 119 mmol) dissolved in DCM. The reaction was stirred for 18 h and quenched with water. The organic layer was collected and the aq phase extracted with additional DCM. The combined organic layers were dried over anhydrous Na_2SO_4. Purification by silica gel flash chromatography (15%-75% EtOAc/hexanes) gave **14** as an off-white solid (6.37 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 7.4 Hz, 2H), 7.43 (t, *J* = 7.4 Hz, 2H), 7.37 – 7.18 (m, 7H), 5.91 (ddt, *J* = 16.7, 11.3, 6.1 Hz, 1H), 5.48 – 5.28 (m, 3H), 5.14 (dd, *J* = 9.6, 6.1 Hz, 1H), 4.77 – 4.60 (m, 2H), 4.46 – 4.33 (m, 4H), 4.24 (t, *J* = 7.2 Hz, 1H), 3.76 (s, 3H), 3.51 (dd, *J* = 14.9, 6.2 Hz, 1H), 3.27 (dd, *J* = 14.8, 6.7 Hz, 1H), 3.11 (br s, 2H), 1.75 (br s, 2H), 1.60 – 1.32 (m, 13H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 175., 169.5, 156.3, 156.2, 144.2, 144.0, 141.5, 141.5, 137.0, 130.9, 129.1, 128.8, 127.9, 127.3, 127.1, 125.4, 121.1, 120.2, 120.2, 78.2, 67.2, 62.4, 52.9, 51.6, 47.4, 40.5, 34.4, 32.1, 29.7, 28.7, 22.6; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{39}H_{48}N_3O_8$ 686.3459, found 686.3455.

Fmoc-Lys(Boc)-(*O*-allyl)hPhe-OH (15). A solution of **14** (2.37 g, 3.46 mmol) in EtOAc was treated with lithium iodide (2.31 g, 17.3 mmol). The reaction was refluxed for 18 h at 80 ºC. The reaction was diluted with EtOAc, washed with 1M aq HCl, sat aq $Na_2S_2O_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (40%-100% EtOAc/hexanes), resulting in an off-white solid (1.76 g, 76% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (bs, 2H), 7.77 (d, *J* = 7.6 Hz, 2H), 7.68 – 7.45 (m, 2H), 7.40 (t, 2H), 7.35 – 7.27 (m, 3H), 7.27 – 7.17 (m, 4H), 5.98 – 5.83 (m, 1H), 5.40 – 5.28 (m, 2H), 5.23 (bs, 1H), 4.72 (td, *J* = 8.4, 4.6 Hz, 1H), 4.53 – 4.25 (m, 4H), 4.21 (t, *J* = 7.3 Hz, 1H), 3.52 (dd, *J* = 15.2, 6.0 Hz, 1H), 3.29 (dd, *J* = 15.2, 10.0 Hz, 1H), 3.14 – 2.96 (m, 2H), 1.81 – 1.67 (m, 1H), 1.55 – 1.24 (m, 13H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.8, 171.8, 156.6, 143.9, 143.8, 141.4, 136.6, 130.6, 128.8, 128.7, 127.8, 127.2, 127.2, 127.0, 125.3, 121.0, 120.0, 120.0, 78.2, 67.3, 62.3, 51.8, 47.1, 33.7, 31.5, 29.2, 28.4, 22.4; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{38}H_{46}N_3O_8$ 672.3279, found 672.3284.

***tert*-butyl (cyanomethyl)-L-valinate (20).** A mixture of L-valine *tert*-butyl ester (HCl salt, 3.00 g, 14.3 mmol) and DIEA (7.48 mL, 42.9 mmol) in MeCN was treated with bromoacetonitrile (1.10 mL, 15.7 mmol) dropwise over 10 min at rt. The reaction was stirred for 24 h at 40 ºC prior to the removal of MeCN. The residue was dissolved in DCM and washed with sat. aq. $NaHCO_3$. The organic layer was collected, and the aq phase was extracted with additional DCM. The combined organic layers were dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. The crude material was purified by silica gel flash chromatography (20% EtOAc/hexanes), resulting in a colorless oil (2.65 g, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 – 3.43 (m, 2H), 2.99 (d, *J* = 4.9 Hz, 1H), 2.03 – 1.87 (m, 2H), 1.46 (s, 9H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.9, 117.8, 82.0, 66.5, 36.9, 31.7, 28.2, 19.3, 17.8; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{11}H_{21}N_2O_2$ 213.1598, found 213.1599

H-hVal-OtBu (21). Compound **20** (2.50 g, 11.78 mmol) was dissolved in DCM and cooled to 0º C. To the cooled mixture, 70% mCPBA (6.33 g, 28.3 mmol) was added in two portions over 30 min. The solution was allowed to warm to rt and stirred for 1.5 h. The reaction flask was then cooled to 0 ºC prior to the addition of sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$ and the resulting slurry stirred for an additional 30 min until two layers were observed. The organic layer was collected, and the aq phase was extracted with additional DCM. The combined organic layers were dried over anhydrous Na_2SO_4. Concentration on a rotary evaporator provided the crude nitrone intermediate as a yellow oil. A mixture of the nitrone and hydroxylamine hydrochloride (4.09 g, 58.9 mmol) was dissolved in MeOH and stirred 18 h at 60º C. The solution was concentrated to remove MeOH. The residue was dissolved in DCM and washed with sat. aq. $NaHCO_3$. The organic layer was collected, and the aq phase was extracted with additional DCM. The combined organic layers were dried over anhydrous Na_2SO_4. Purification by silica gel flash chromatography (10%-60% EtOAc/hexanes) gave **19** as a colorless oil (1.74 g, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.13 (br s, 2H), 3.37 (d, *J* = 6.3 Hz, 1H), 2.03 – 1.87 (m, 1H), 1.50 (s, 9H), 0.98 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.7, 81.6, 71.8, 29.1, 28.2, 19.3; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_9H_{20}NO_3$ 190.1438, found 190.1442.

Fmoc-Ala-hVal-OtBu (S4). A solution of Fmoc-Ala-Cl[1] (3.60 g, 11.1 mmol) in DCM was added to a solution of H-hVal-O*t*Bu (1.40 g, 7.40 mmol) and $NaHCO_3$ (6.23 g, 74.0 mmol) in DCM. The reaction was stirred for 6 h at rt prior to the removal of DCM. The residue was diluted with EtOAc and washed with 1M aq HCl, sat. aq $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. Purification by silica gel flash chromatography (30% EtOAc/hexanes) gave **S5** as a white solid (3.24 g, 91% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.65 – 7.57 (m, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.32 (t, 2H), 5.65 (d, *J* = 8.1 Hz, 1H), 5.06 – 4.95 (m, 1H), 4.90 (d, *J* = 7.6 Hz, 1H), 4.36 (d, *J* = 7.3 Hz, 2H), 4.22 (t, *J* = 7.2 Hz, 1H), 2.46 – 2.30 (m, 1H), 1.65 (br s, 1H), 1.50 (s, 9H), 1.41 (d, *J* = 6.9 Hz, 3H), 1.10 – 0.97 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.9, 171.8, 156.0, 144.2, 141.5, 128.9, 127.3, 125.4, 120.2, 83.7, 67.3, 63.4, 47.4, 47.2, 29.4, 28.3, 19.8, 19.7, 18.7; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{27}H_{35}N_2O_6$ 483.2490, found 483.2492.

Fmoc-Ala-(*O*-allyl)hVal-OH (22). A mixture of compound **S4** (0.20 g, 0.41 mmol), triphenylphosphine (270 mg, 1.0 mmol), and allyl alcohol (71 µL, 1.0 mmol) was treated with DIAD (0.22 mL, 1.0 mmol) dropwise over 10 min at 0 ºC. The solution was allowed to warm to rt and stirred for 45 min. The volatiles were removed under reduced pressure and the crude material purified via flash chromatography over silica gel (2%-25% EtOAc/hexanes). This purified product was immediately dissolved in a 95:2.5:2.5 TFA/DCM/TIPS at 0 ºC and allowed to warm to room temperature while stirring over 4 h. Upon completion, the reaction was concentrated, and the crude material purified by silica gel flash chromatography (40%-100% EtOAc/hexanes) to give **22** as an off-white solid (0.13 g, 66% yield over 2 steps); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 6.5 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.5, 1.2 Hz, 2H), 5.96 (m, 1H), 5.50 – 5.36 (m, 3H), 4.84 – 4.75 (m, 1H), 4.60 – 4.49 (m, 2H), 4.48 – 4.32 (m, 2H), 4.23 (t, *J* = 7.1 Hz, 1H), 4.01 (d, *J* = 10.7 Hz, 1H), 2.65 – 2.47 (m, 1H), 1.42 (d, *J* = 7.0 Hz, 3H), 1.11 – 0.98 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.4, 171.1, 156.0, 144.1, 143.9, 141.5, 130.4, 127.9, 127.3, 125.4, 125.4, 122.2, 120.2, 77.9, 71.1, 67.3, 48.1, 47.3, 27.5, 20.1, 19.7, 18.3; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{26}H_{31}N_2O_6$ 467.2177, found 467.2172.

Fmoc-Lys(Boc)-(*O*-allyl)hPhe α-methyl benzamide (23). To a solution of **15** (150 mg. 220 μmol) in 2 mL DMF was added *N*-methyl morpholine (48 μL, 440 μmol), (*R*)-α-methylbenzylamine (85 μL, 660 μmol), followed by HCTU (110 mg, 0.27 mmol) and the reaction mixture was allowed to stir at rt for 18 h. The reaction was diluted with EtOAc and washed with 1M aq HCl, sat. aq $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. Purification by silica gel flash chromatography (25% EtOAc/hexanes) gave **23** as an off-white solid (0.13 g, 75% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.65 – 7.48 (m, 2H), 7.46 – 7.38 (m, 2H), 7.33 (t, *J* = 7.5 Hz, 4H), 7.26 – 7.11 (m, 9H), 5.85 (ddt, *J* = 16.7, 10.4, 6.2 Hz, 2H), 5.42 (bs, 1H), 5.30 – 5.20 (m, 2H), 5.07 (t, *J* = 7.3 Hz, 1H), 4.76 – 4.49 (m, 3H), 4.46 – 4.30 (m, 4H), 4.27 – 4.14 (m, 1H), 3.55 – 3.40 (m, 2H), 3.13 – 2.99 (bs, 2H), 1.43 (m, 18H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.7, 168.6, 156.7, 156.3, 144.0, 144.0, 143.4, 141.5, 137.1, 130.6, 129.5, 128.7, 128.7, 128.0, 127.4, 127.3, 127.3, 127.1, 126.2, 125.3, 125.3, 121.6, 120.2, 120.2, 67.5, 67.3, 52.1, 49.3, 47.4, 34.5, 29.7, 28.7, 22.7, 22.3; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{46}H_{55}N_4O_7$ 775.4065, found 775.4059.

Solid-phase peptide synthesis. Solid-phase peptide synthesis was carried out using CEM – Liberty Blue peptide synthesizer on Fmoc-capped polystyrene rink amide MBHA resin (100-200 mesh, 0.05-0.15 mmol scale). The following amino acid derivatives suitable for Fmoc SPPS were used: Fmoc-Gly-OH, Fmoc-Glu(*t*Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Ala-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Pro-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Val-OH, Fmoc-(*N*-Me)-Val-OH, Boc-Gly-OH. Dry resin was washed with DMF 3x and allowed to swell in DMF for 2 h prior to use. All reactions were carried out using gentle agitation. Fmoc deprotection steps were carried out by treating the resin with a solution of 20% piperidine/DMF (15 min x 2). Coupling of Fmoc-protected amino acids as well as (N^2-Boc)-hydrazino acids was effected using 5 equiv. HCTU (0.5 M in DMF), 10 equiv. NMM (1.0 M in DMF), and 5 equiv. of the carboxylic acid in DMF at 50 ºC (20 min). After each reaction the resin was washed with DMF 2x, DCM 2x. Peptides were cleaved from the resin by incubating with gentle stirring in 2 mL of 95:2.5:2.5 TFA:H_2O:TIPS at rt for 2 h. The cleavage mixture was filtered and the resin was rinsed with an additional 1 mL of cleavage solution. The filtrate was treated with 8 mL of cold Et_2O to induce precipitation. The mixture was centrifuged and the supernatant was removed. The remaining solid was washed 2 more times with Et_2O and dried under vacuum. Peptides were analyzed and purified on C12 RP-HPLC columns (preparative: 4μ, 90Å, 250 x 21.2 mm; analytical: 4μ, 90Å, 150 x 4.6 mm) using linear gradients of MeCN/H_2O (with 0.1% formic acid), then lyophilized to afford white powders. All peptides were characterized by LCMS (ESI), HRMS (ESI-TOF), and ^{1}H NMR. Analytical HPLC samples for all purified peptides were prepared as 1 mM in H_2O containing 20 mM phosphate buffer at pH 7.0. Linear gradients of MeCN in H_2O (0.1% TFA) were run over 20 minutes and spectra are provided for λ = 280 nm.

H-Gly-Ala-(*N*-Me)Ala-Gly-NH$_2$ (9). The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). The pure peptide was obtained in 52% yield. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{11}H_{21}N_4O_5$ 289.1506, found 289.1505.

H-Gly-Ala-Ala-Gly-NH$_2$ (10). The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). The pure peptide was obtained in 68% yield. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{10}H_{19}N_4O_5$ 275.1350, found 275.1354

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-hPhe-Ala-Val-Thr-Glu-NH$_2$ (11b). The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). The pure peptide was obtained in 8% yield. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{80}H_{115}N_{20}O_{25}$ 1755.8337, found 1755.8324.

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-hVal-Thr-Glu-NH$_2$ (11c). The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). The pure peptide was obtained in 5% yield. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{80}H_{115}N_{20}O_{25}$ 1755.8337, found 1755.8334.

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-(*O*-All)hPhe-Ala-Val-Thr-Glu-NH$_2$ (11d). The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). The pure peptide was obtained in 5% yield. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{83}H_{119}N_{20}O_{25}$ 1795.8649, found 1795.8613.

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-(*O*-All)hVal-Thr-Glu-NH$_2$ (11e). The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). The pure peptide was obtained in 4% yield. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{83}H_{119}N_{20}O_{5}$ 1795.8649, found 1795.8652.

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-hPhe-Ala-hVal-Thr-Glu-NH$_2$ (11f). The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). The pure peptide was obtained in 4% yield. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{80}H_{115}N_{20}O_{26}$ 1771.8286, found 1771.8274.

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-(*O*-All)hPhe-Ala-(*O*-All)hVal-Thr-Glu-NH$_2$ (11g). The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). The pure peptide was obtained in 4% yield. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{86}H_{123}N_{20}O_{26}$ 1851.8912, found 1851.8914.

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-(*N*-Me)Val-Thr-Glu-NH$_2$ (11l). The crude peptide was purified by preparative scale RP-HPLC using a 5-40% MeCN/H_2O gradient (with 0.1% formic acid). The pure peptide was obtained in 11% yield. HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{81}H_{117}N_{20}O_{24}$ 1753.8544, found 1753.8539.

NMR acquisition parameters for all peptides. Purified peptides were dissolved in D_2O (50 mM phosphate buffer, pH=6.3, uncorrected) containing 50 mM 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as a standard. Final peptide concentration was 1 mM, determined by mass. For each peptide, 1D, GCOSY, TOCSY, and NOESY data were collected at 4 ºC on a 500 MHz Bruker ASCEND 11.74 T, narrow bore 54 mm, BOSS-3 36 shim system, BSMS shim and digital lock control units with a 5 mm direct detect SMART probe ($^{1}H/^{13}C/^{15}N$ with Z axis PFG), or an 800 MHz AVANCE II with UltraStabilized and UltraShield 18.79 T, 54 mm bore, BOSS-2 34 shim system and a 5 mm broadband (BBO) 15N-31P, 1H decoupling, Z-axis PFG. The 1D, GCOSY, TOCSY and ROESY experiments used solvent suppression with a 2 second solvent presaturation. The TOCSY used a mixing time of 80 ms, and the ROESY had a mixing time of 200 ms. In the f2 direction, the TOCSY and ROESY had 2048 complex points collected, and in the f1 direction, 256 complex points were collected. Bruker TopSpin 4.0 or Mestrenova 10.0 software was used to process the data, and Gaussian functions were used before Fourier transformation.

Thermodynamic analysis. The degree of folding for hairpin peptides was determined following the method of Griffiths-Jones et al.[2] For each peptide, the β-sheet population is measured using the Hα, Hα'diastereotopic separation observed for the Gly10 turn residue. The folded population as a percentage ($\%_f$) was calculated using the following equation:

$$\%_f = (\Delta\delta\mathrm{Gly}_{obs} - \Delta\delta\mathrm{Gly}_{U}) / (\Delta\delta\mathrm{Gly}_{F} - \Delta\delta\mathrm{Gly}_{U}) * 100$$

where $\Delta\delta\mathrm{Gly}_{obs}$ is the diastereotopic separation in the peptide of interest, $\Delta\delta\mathrm{Gly}_{F}$ is the diastereotopic separation of assumed, fully folded peptide, and $\Delta\delta\mathrm{Gly}_{U}$ is the separation of the assumed, fully unfolded peptide as previously reported.[3]

ΔG values were calculated using the following equation:

$$\Delta G = -\mathrm{RT}\ \ln(K_f)$$

where $K_f = \%_f/(100-\%_f)$. $\Delta\Delta G$ values are reported relative to native parent peptide **11a** where $\Delta\Delta G_{fold} = \Delta G_{cald} - \Delta G_{\mathbf{11a}}$.

TABULATED ^{1}H NMR DATA FOR ALL PEPTIDES

hPhe mutant (11b)

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-hPhe-Ala-Val-Thr-Glu-NH_2

	α	β	Others
Gly	3.85, 3.73		
Glu	4.52	2.04, 1.88	γ CH_2 2.26, 2.21
Trp	4.85	3.18, 3.12	δ_1 CH 7.22; ε_3 CH 7.44; ζ_2 CH 7.40; ζ_3 7.23; η_2 CH 7.14
Ala	4.56	1.33	
Tyr	3.79	2.54	δ CH 6.61; ε CH 6.60
Asn	4.94	3.23, 2.49	
Pro	4.06	2.41	γ CH_2 2.06, 2.03; δ CH_2 3.82, 3.77
Ala	4.24	1.44	
Thr	4.41	4.26	γ CH_3 1.10
Gly	4.056, 3.801		
Lys	5.18	2.01	γ CH_2 1.48, 1.38; δ CH_2 1.74, 1.66; ε CH_2 3.02
hPhe	5.28	3.32, 2.48	δ CH 7.08; ε CH 7.33; ζ CH 7.25
Ala	4.46	1.32	
Val	4.12	1.72	γ CH_3 0.77, 0.73
Thr	4.39	4.19	γ CH_3 1.20
Glu	4.28	2.08, 1.93	γ CH_2 2.27

hVal mutant (11c)

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-hVal-Thr-Glu-NH_2

	α	β	Others
Gly	3.86, 3.70		
Glu	4.53	2.03, 1.87	γ CH_2 2.20
Trp	4.90	3.26, 3.16	δ_1 CH 7.20; ε_3 CH 7.46; ζ_2 CH 7.38; ζ_3 CH 7.48; η_2 CH 7.09
Ala	4.54	1.33	
Tyr	3.76	2.53	δ CH 6.58; ε CH 6.51
Asn	4.93	3.25, 2.47	
Pro	4.06	2.40	γ CH_2 2.03; δ CH_2 3.77
Ala	4.24	1.42	
Thr	4.42	4.20	γ CH_3 1.20
Gly	4.013, 3.792		
Lys	4.61	1.85	γ CH_2 1.33; δ CH_2 1.69, 1.60; ε CH_2 2.99
Phe	4.69	2.80, 2.37	δ CH 7.01; ε CH 7.33; ζ CH 7.23
Ala	5.01	1.34	
hVal	4.75	2.25	γ CH_3 0.85, 0.76
Thr	4.39	4.25	γ CH_3 1.10
Glu	4.29	2.09, 1.95	γ CH_2 2.29

*(O-*All)hPhe mutant (11d)

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-(*O*-All)Phe-Ala-Val-Thr-Glu-NH_2

	α	β	Others
Gly	3.83, 3.71		
Glu	4.45	2.00, 1.85	γ CH_2 2.20
Trp	4.73	3.04	δ_1 CH 7.22; ε_3 CH 7.46; ζ_2 CH 7.34; ζ_3 7.23; η_2 CH 7.12
Ala	4.47	1.30	
Tyr	4.17	2.87, 2.71	δ CH 6.94; ε CH 6.72
Asn	4.95	3.15, 2.54	
Pro	4.17	2.40	γ CH_2 2.05; δ CH_2 3.81, 3.72
Ala	4.28	1.44	
Thr	4.41	4.29	γ CH_3 1.14
Gly	4.066, 3.858		
Lys	5.00	1.80	γ CH_2 1.35; δ CH_2 1.66, 1.63; ε CH_2 2.96
(*O*-All)hPhe	5.21	3.22, 2.98	δ CH 7.10; ε CH 7.34; ζ CH 7.29; *O*-All CH_2 4.32; *O*-All CH 5.99; *O*-All =CH_2 5.48, 5.40
Ala	4.36	1.28	
Val	4.02	1.67	γ CH_3 0.76, 0.70
Thr	4.35	4.16	γ CH_3 1.18
Glu	4.26	2.05, 1.93	γ CH_2 2.26

(*O*-All)hVal mutant (11e)

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-(*O*-All)hVal-Thr-Glu-NH_2

	α	β	Others
Gly	3.85, 3.70		
Glu	4.49	2.02, 1.86	γ CH_2 2.20
Trp	4.81	3.21, 3.14	δ_1 CH 7.23; ε_3 CH 7.44; ζ_2 CH 7.39; ζ_3 7.19; η_2 CH 7.08
Ala	4.55	1.34	
Tyr	3.94	2.60	δ CH 6.60; ε CH 6.56
Asn	4.94	3.21, 2.49	
Pro	4.09	2.40	γ CH_2 2.03; δ CH_2 3.77
Ala	4.25	1.43	
Thr	4.39	4.26	γ CH_3 1.10
Gly	4.019, 3.810		
Lys	4.59	1.89	γ CH_2 1.33; δ CH_2 1.68, 1.62; ε CH_2 2.99
Phe	4.76	2.92, 2.71	δ CH 7.24; ε CH 7.34; ζ CH 7.12
Ala	4.92	1.32	
(*O*-All)hVal	4.56	2.28	γ CH_3 0.82, 0.69; *O*-All CH_2 4.32; *O*-All CH 5.99; *O*-All =CH_2 5.48, 5.40
Thr	4.34	4.15	γ CH_3 1.17
Glu	4.28	2.07, 1.92	γ CH_2 2.26

hPhe, hVal mutant (11f)

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-hPhe-Ala-hVal-Thr-Glu-NH_2

	α	β	Others
Gly	3.87, 3.71		
Glu	4.57	2.21	γ CH_2 2.04, 1.88
Trp	4.98	3.27, 3.14	δ_1 CH 7.21; ε_3 CH 7.46; ζ_2 CH 7.39; ζ_3 7.20; η_2 CH 7.10
Ala	4.50	1.31	
Tyr	3.61	2.48	δ CH 6.59; ε CH 6.58
Asn	4.93	3.25, 2.41	
Pro	4.03	2.42	γ CH_2 2.04; δ CH_2 3.78
Ala	4.23	1.44	
Thr	4.43	4.20	γ CH_3 1.18
Gly	4.056, 3.796		
Lys	5.17	2.06	γ CH_2 1.45, 1.39; δ CH_2 1.75, 1.66; ε CH_2 3.03
hPhe	5.19	3.21, 2.03	δ CH 6.90; ε CH 7.32; ζ CH 7.25
Ala	4.97	1.31	
hVal	4.80	2.26	γ CH_3 0.86, 0.81
Thr	4.42	4.26	γ CH_3 1.09
Glu	4.30	2.08, 1.94	γ CH_2 2.30

(*O*-All)hPhe, (*O*-All)hVal (11g)

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-(*O*-All)hPhe-Ala-(*O*-All)hVal-Thr-Glu-NH_2

	α	β	Others
Gly	3.83		
Glu	4.41	1.96, 1.82	γ CH_2 2.15
Trp	4.68	3.05, 3.03	δ_1 CH 7.21; ε_3 CH 7.45; ζ_2 CH 7.42; ζ_3 7.23; η_2 CH 7.13
Ala	4.41	1.15	
Tyr	4.23	2.90, 2.70	δ CH 6.96; ε CH 6.72
Asn	4.95	3.14, 2.51	
Pro	4.16	2.38	γ CH_2 2.03; δ CH_2 3.79, 3.66
Ala	4.28	1.44	
Thr	4.41	4.30	γ CH_3 1.14
Gly	4.071, 3.868		
Lys	4.94	1.76	γ CH_2 1.41, 1.31; δ CH_2 1.64, 1.52; ε CH_2 2.95
(*O*-All)hPhe	5.17	3.32, 3.02	δ CH 7.11; ε CH 7.30; ζ CH 7.27; *O*-All CH_2 4.65, *O*-All CH 6.07, *O*-All =CH_2 5.52, 5.45
Ala	4.85	1.29	
(*O*-All)hVal	4.52	2.28	γ CH_3 0.85, 0.73; *O*-All CH_2 4.47, 4.35; *O*-All CH 5.91; *O*-All =CH_2 5.42, 5.32
Thr	4.31	4.14	γ CH_3 1.16
Glu	4.27	2.06, 1.93	γ CH_2 2.26

(*N*-Me) Val (11l)

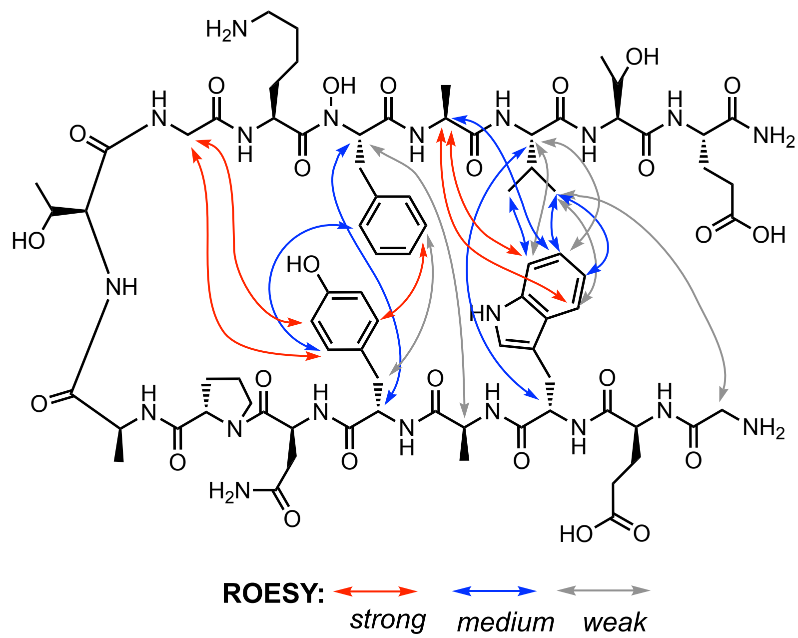
H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-(*N*-Me)Val-Thr-Glu-NH_2

	α	β	Others
Gly	3.84, 3.69		
Glu	4.51	2.02, 1.87	γ CH_2 2.20
Trp	4.78	3.22, 3.16	δ_1 CH 7.22; ε_3 CH 7.45; ζ_2 CH 7.42; ζ_3 7.20; η_2 CH 7.10
Ala	4.56	1.34	
Tyr	3.86	2.58	δ CH 6.61; ε CH 6.57
Asn	4.92	3.19, 2.51	
Pro	4.08	2.39	γ CH_2 2.03; δ CH_2 3.80, 3.73
Ala	4.25	1.43	
Thr	4.39	4.25	γ CH_3 1.11
Gly	4.016, 3.803		
Lys	4.58	1.84	γ CH_2 1.32, 1.39; δ CH_2 1.69, 1.61; ε CH_2 2.98
Phe	4.76	2.85, 2.62	δ CH 7.12; ε CH 7.34; ζ CH 7.24
Ala	4.86	1.30	
(*N*-Me)Val	4.68	2.06	γ CH_3 0.78, 0.48; *N*-Me CH_3 3.06
Thr	4.34	4.18	γ CH_3 1.19
Glu	4.29	2.08, 1.95	γ CH_2 2.29

SUMMARY OF INTER-STRAND NOEs FOR β-HAIRPIN PEPTIDES

hPhe mutant

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-hPhe-Ala-Val-Thr-Glu-NH_2 **(11b)**

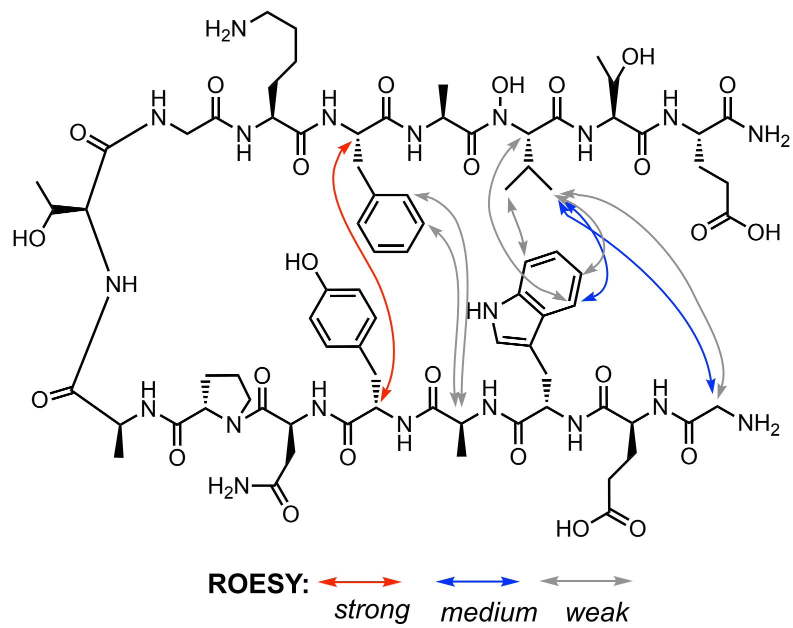


Gly1 α1 - Val14 γ (w)
Gly1 α2 - Val14 γ (w)
Trp3 α - Val14 α (m)
Trp3 ε3 - Val14 γ (w)
Trp3 ζ2 - Val14 γ (m)
Trp3 ζ3 - Val14 γ (m)
Trp3 η2 - Val14 γ (m)
Ala4 α - hPhe12 α (w)
Tyr5 α - hPhe12 α (m)
Tyr5 β - hPhe12 ε (w)
Tyr5 δ - hPhe12 δ (m)
Tyr5 δ - hPhe12 ε (s)
Tyr5 ε - Pro7 β (m)
Tyr5 ε - Pro7 δ (s)
Asn7 α - Pro7 δ1 (m)
Asn7 α - Pro7 δ2 (m)
Gly10 α - Tyr5 δ (s)
Gly10 α - Tyr5 ε (s)
Ala13 α - Trp3 ζ2 (s)
Ala13 α - Trp3 ζ3 (s)
Ala13 α - Trp3 η2 (m)
Val14 α - Trp3 ζ2 (w)
Val14 α - Trp3 η2 (w)

hVal mutant

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-hVal-Thr-Glu-NH_2 **(11c)**

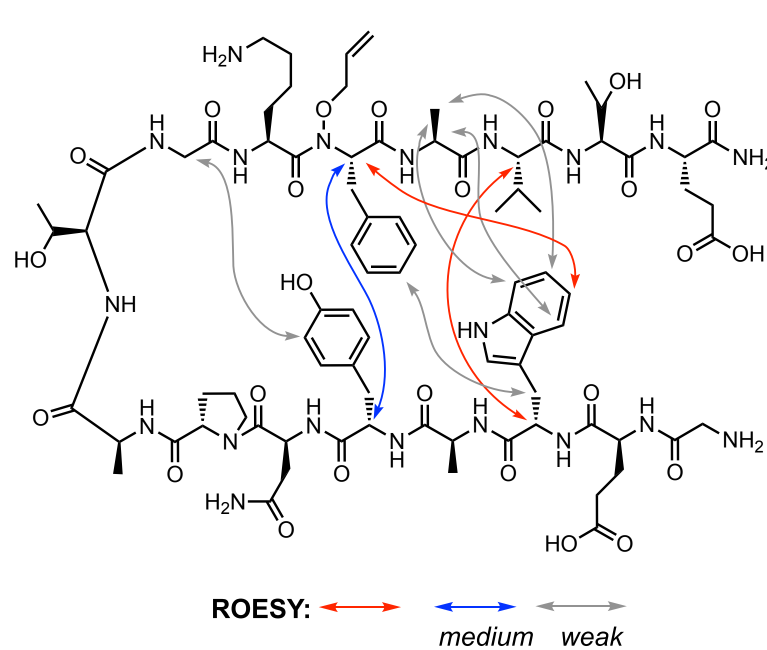
Gly1 α1 - hVal14 γ (m)
Gly1 α2 - hVal14 γ (w)
Trp3 ε3 - hVal14 γ (m)
Trp3 ζ2 - hVal14 γ (w)
Trp3 η2 - hVal14 γ (w)
Ala4 α - Phe12 δ (w)
Ala4 α - Phe12 ε (w)
Tyr5 α - Phe12 α (s)
Tyr5 δ - Pro7 β (s)
Tyr5 δ - Pro7 β1 (s)
Tyr5 δ - Pro7 δ (s)
Tyr5 δ - Pro7 γ (s)
Tyr5 ε - Pro7 β (w)
Tyr5 ε - Pro7 δ (s)
Tyr5 ε - Pro7 γ (s)
Asn6 α - Pro7 γ (w)
Asn6 α - Pro7 δ (s)
Pro7 α - Tyr5 δ (s)
Pro7 α - Tyr5 ε (s)
Phe12 α - Ala13 β (w)
hVal14 α - Trp3 ε3 (w)



(*O*-All)hPhe mutant

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-(*O*-All)hPhe-Ala-Val-Thr-Glu-NH_2. **(11d)**

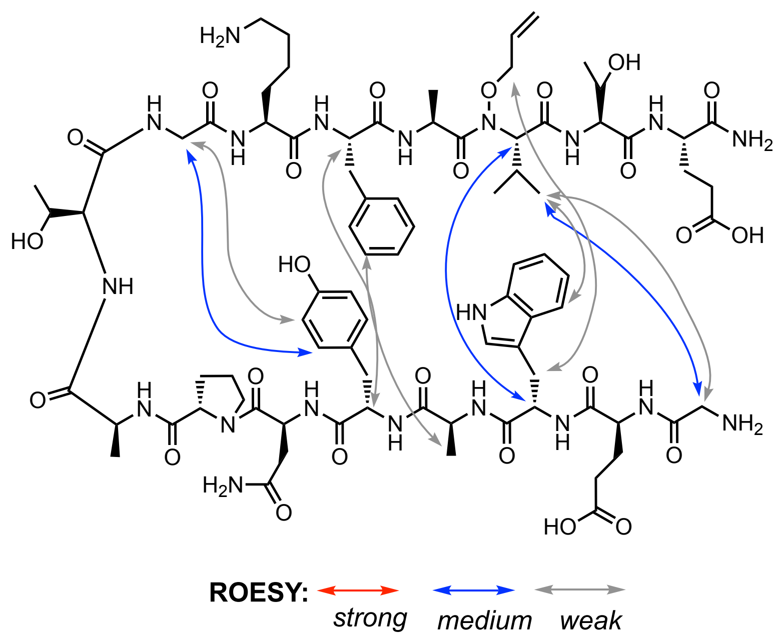
Trp3 α - Val14 α (s)
Trp3 β - (*O*-All)Phe12 ζ2 (w)
Trp3 ε3 - Ala13 β (w)
Trp3 ζ2 - Ala13 β (w)
Trp3 η2 - Ala13 β (w)
Tyr5 α - (*O*-All)Phe12 α (m)
Tyr5 δ - Pro7 β (s)
Tyr5 δ - Pro7 γ (s)
Tyr5 ε - Pro7 β (s)
Tyr5 ε - Pro7 γ (s)
Asn6 α - Pro7 δ1 (s)
Asn6 α - Pro7 δ2 (s)
Asn6 α - Pro7 γ (w)
Gly10 α - Tyr5 ε (w)
Lys11 β - (*O*-All)Phe12 CH_2 (w)
(*O*-All)Phe12 α - Trp3 η2 (s)



(*O*-All)hVal mutant

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-(*O*-All)hVal-Thr-Glu-NH_2 **(11e)**

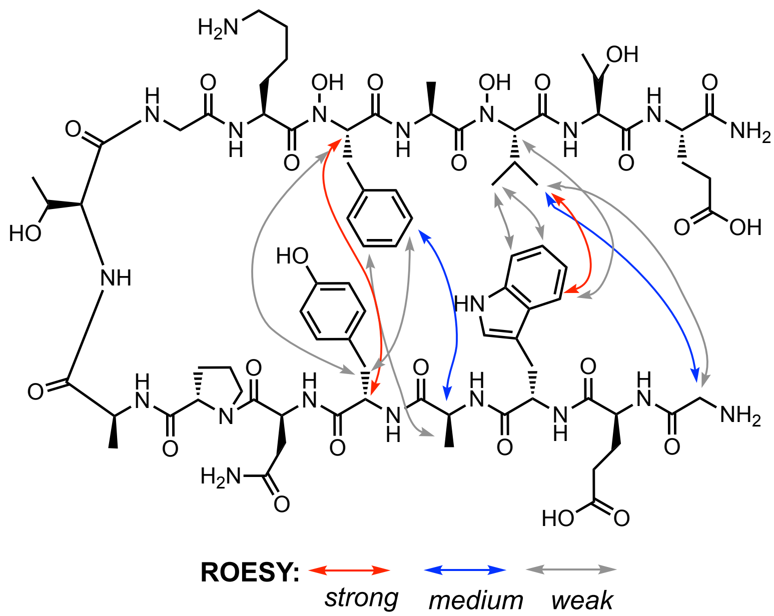
Gly1 α1 - (*O*-All)Val14 γ (w)
Gly1 α2 - (*O*-All)Val14 γ (m)
Trp3 α - (*O*-All)Val14 α (m)
Trp3 β - (*O*-All)Val CH_2 (w)
Trp3 ε3 - (*O*-All)Val14 γ (w)
Ala4 β - Phe12 ε (w)
Tyr5 α - Phe12 α (w)
Tyr5 δ - Pro7 β (s)
Tyr5 δ - Pro7 γ (s)
Tyr5 ε - Pro7 γ (s)
Asn6 α - Pro7 δ (m)
Asn6 α - Pro7 γ (m)
Pro7 α - Tyr5 δ (s)
Pro7 α - Tyr5 ε (s)
Gly10 α - Tyr5 δ (m)
Gly10 α - Tyr5 ε (w)
Ala13 β - (*O*-All)Val14 CH_2 (w)
Ala13 β - (*O*-All)Val14 CH (m)



hPhe, hVal mutant

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-hPhe-Ala-hVal-Thr-Glu-NH_2 **(11f)**

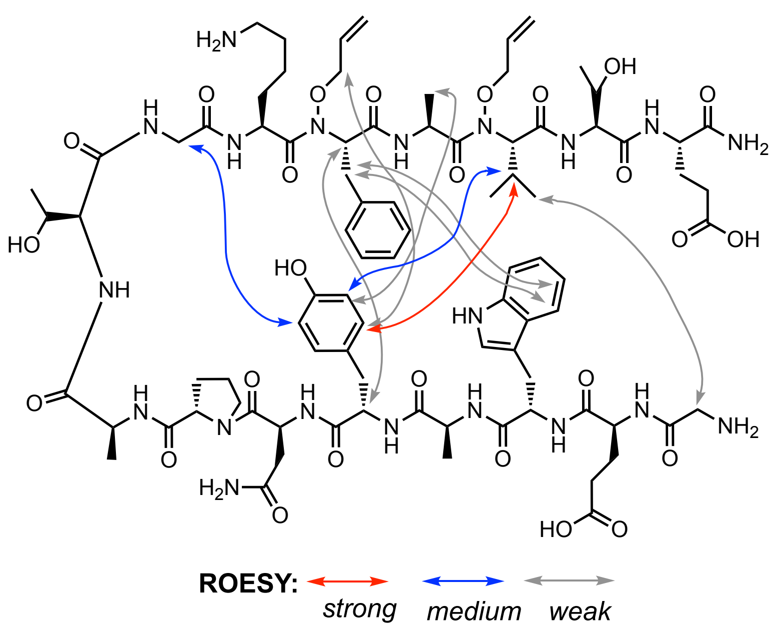
Gly1 α1 - hVal14 γ (m)
Gly1 21 - hVal14 γ (w)
Trp3 ε3 - hVal14 γ (s)
Trp3 ζ2 - hVal14 γ (w)
Trp3 η2 - hVal14 γ (w)
Ala4 α - Phe12 ε (m)
Ala4 β - Phe12 δ (w)
Tyr5 α - Phe12 α (s)
Tyr5 β - Phe12 ε (w)
Tyr5 δ - Pro7 α (s)
Tyr5 δ - Pro7 γ (s)
Tyr5 ε - Pro7 δ (s)
Asn6 α - Pro7 δ (s)
Asn6 α - Pro7 γ (w
hPhe12 α - Tyr5 β (w)
hVal14 α - Trp3 ε3 (w)



(*O*-All)hPhe, (*O*-All)hVal mutant

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-(*O*-All)hPhe-Ala-(*O*-All)hVal-Thr-Glu-NH_2 **(11g)**

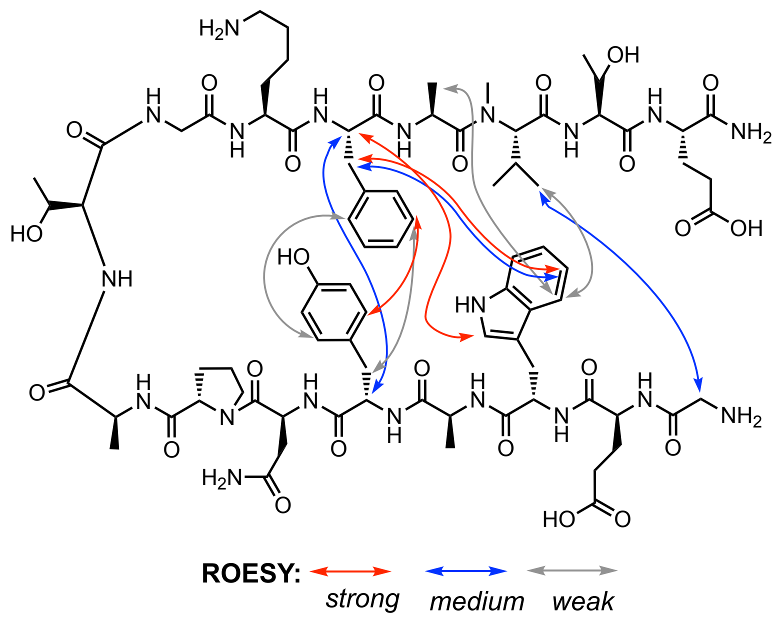
Gly1 α1 - (*O*-All)Val14 γ (w)
Trp3 ε3 - (*O*-All)Phe12 β (w)
Trp3 ζ3 - (*O*-All)Phe12 β (w)
Tyr5 α - (*O*-All)Phe12 α (w)
Tyr5 δ - Pro7 β (m)
Tyr5 δ - Pro7 γ (w)
Tyr5 δ - (*O*-All)Phe12 CH_2 (w)
Tyr5 δ - (*O*-All)Val14 β (s)
Tyr5 ε - Ala13 β (w)
Tyr5 ε - (*O*-All)Val14 β (m)
Asn6 β - Pro7 δ1 (m)
Asn6 β - Pro7 δ2 (w)
Asn6 β - (*O*-All)Phe12 δ (m)
Pro7 α - Tyr5 δ (s)
Gly10 α - Tyr5 ε (m)



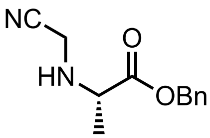
_N_-Me Val mutant

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-(*N*-Me)Val-Thr-Glu-NH_2 **(11l)**

Gly1 α - (*N*-Me)Val14 γ (m)
Trp3 ζ3 - Ala13 β (w)
Trp3 ζ3 - (*N*-Me)Val14 γ (w)
Trp3 η2 - Phe12 β1 (s)
Trp3 η2 - Phe12 β2 (m)
Tyr5 α - Phe12 α (m)
Tyr5 β - Phe12 ε (w)
Tyr5 δ - Pro7 β1 (s)
Tyr5 δ - Pro7 β2 (m)
Tyr5 δ - Phe12 δ1 (s)
Tyr5 δ - Phe12 δ2 (w)
Asn6 α - Phe12 γ (w)
Asn6 α - Phe12 δ1 (m)
Asn6 α - Phe12 δ2 (m)
Pro7 α - Tyr5 δ1 (s)
Pro7 α - Tyr5 δ2 (w)
Phe12 α - Trp3 δ1 (s)
Ala13 α - (*N*-Me)Val CH_3 (m)

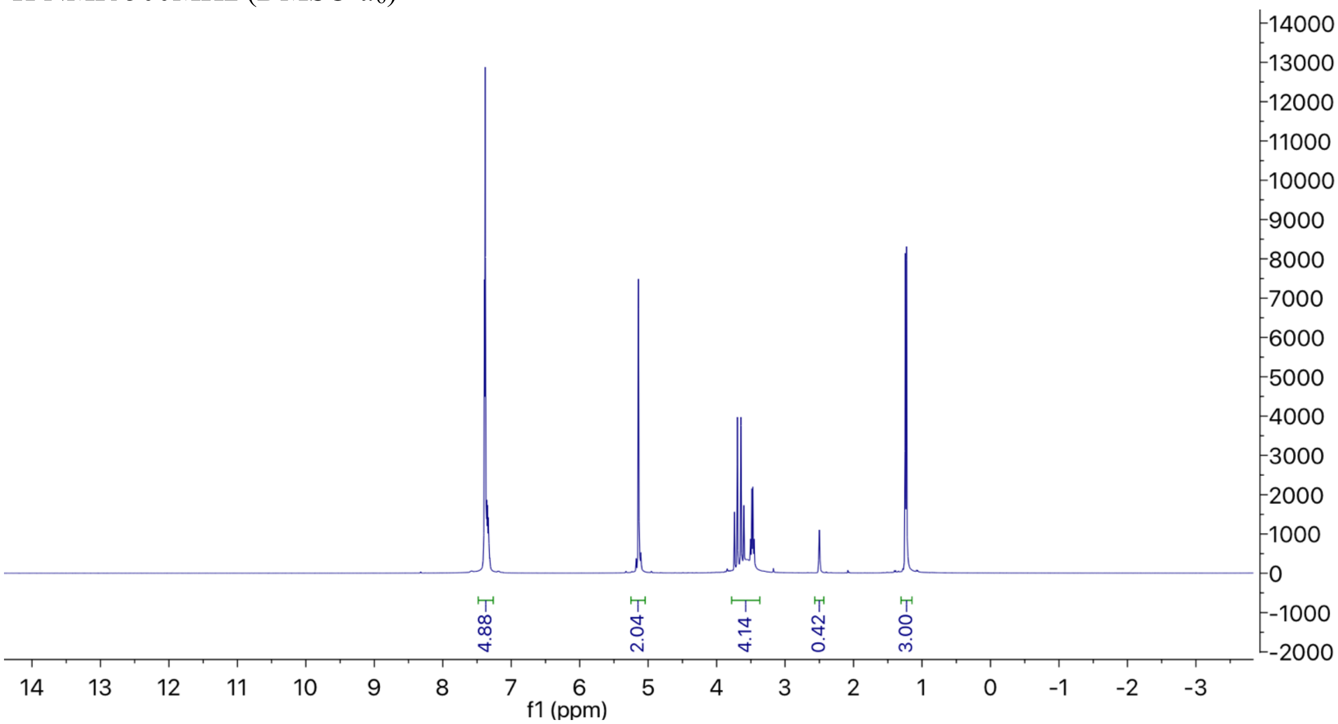


^{1}H and ^{13}C NMR SPECTRA FOR SMALL MOLECULES

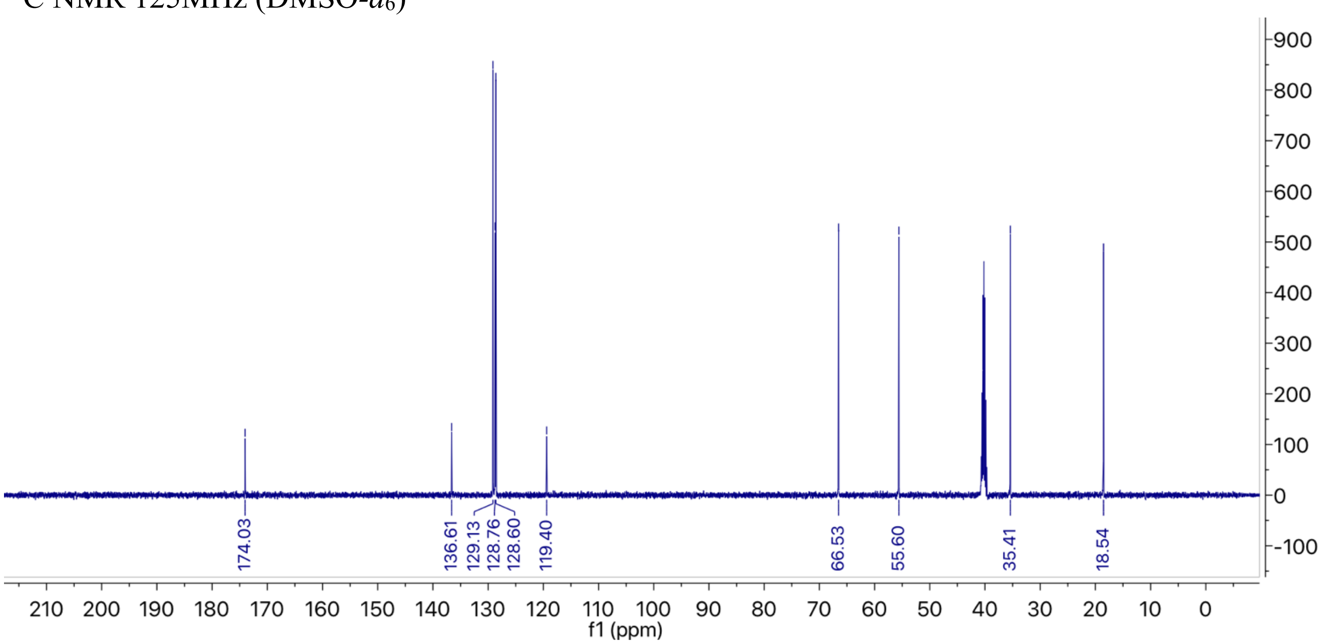


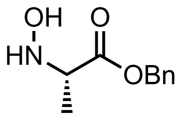
2

^{1}H NMR 500MHz (DMSO-d_6)



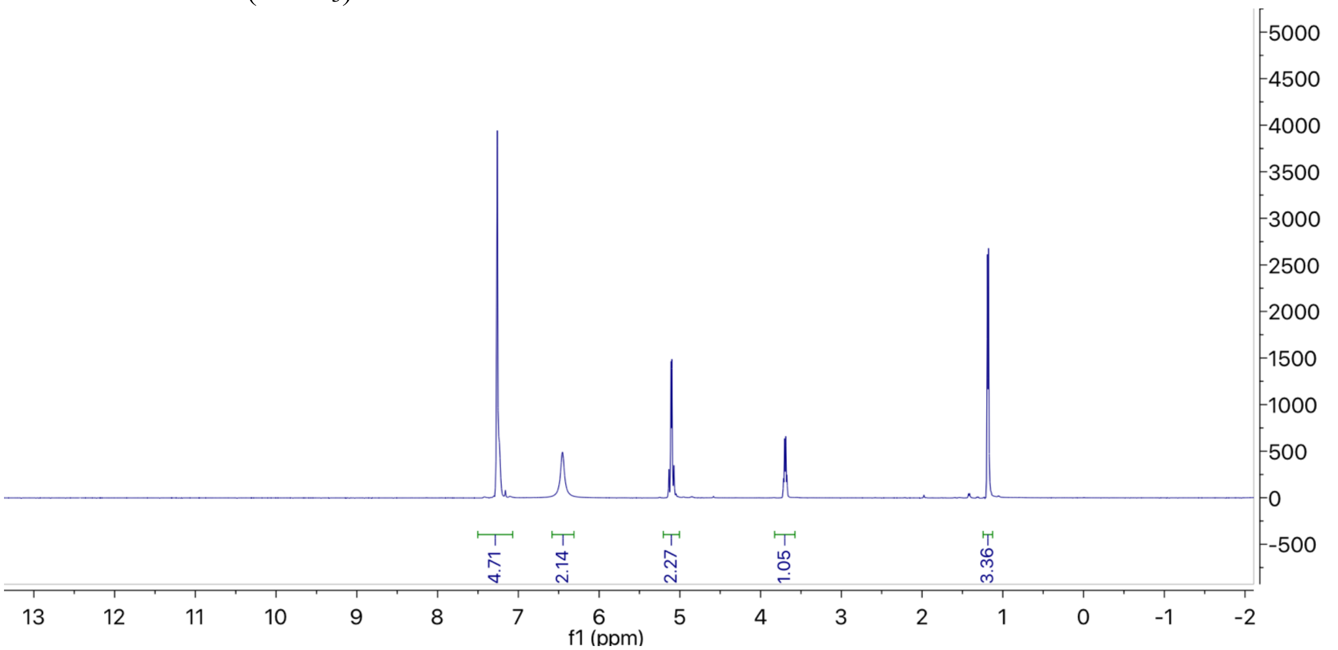
^{13}C NMR 125MHz (DMSO-d_6)



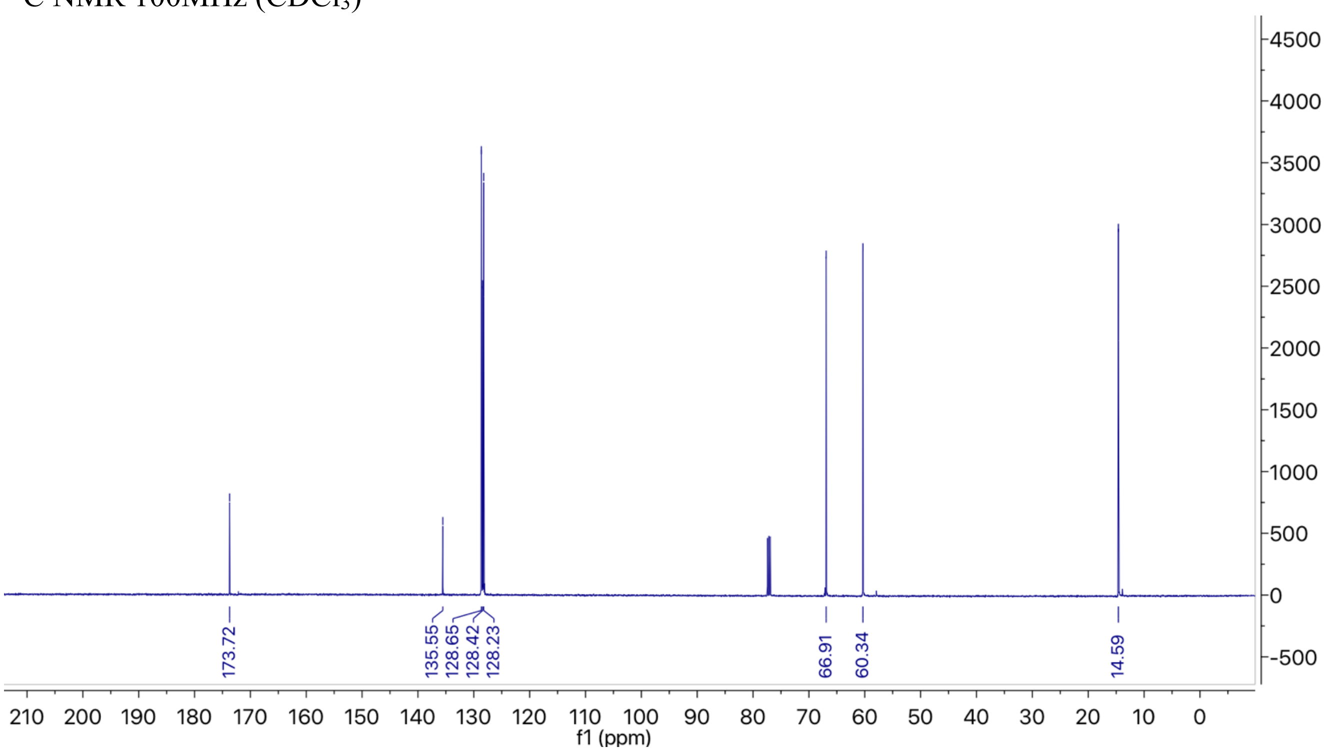


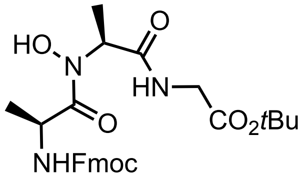
3

[1]H NMR 400MHz ($CDCl_3$)



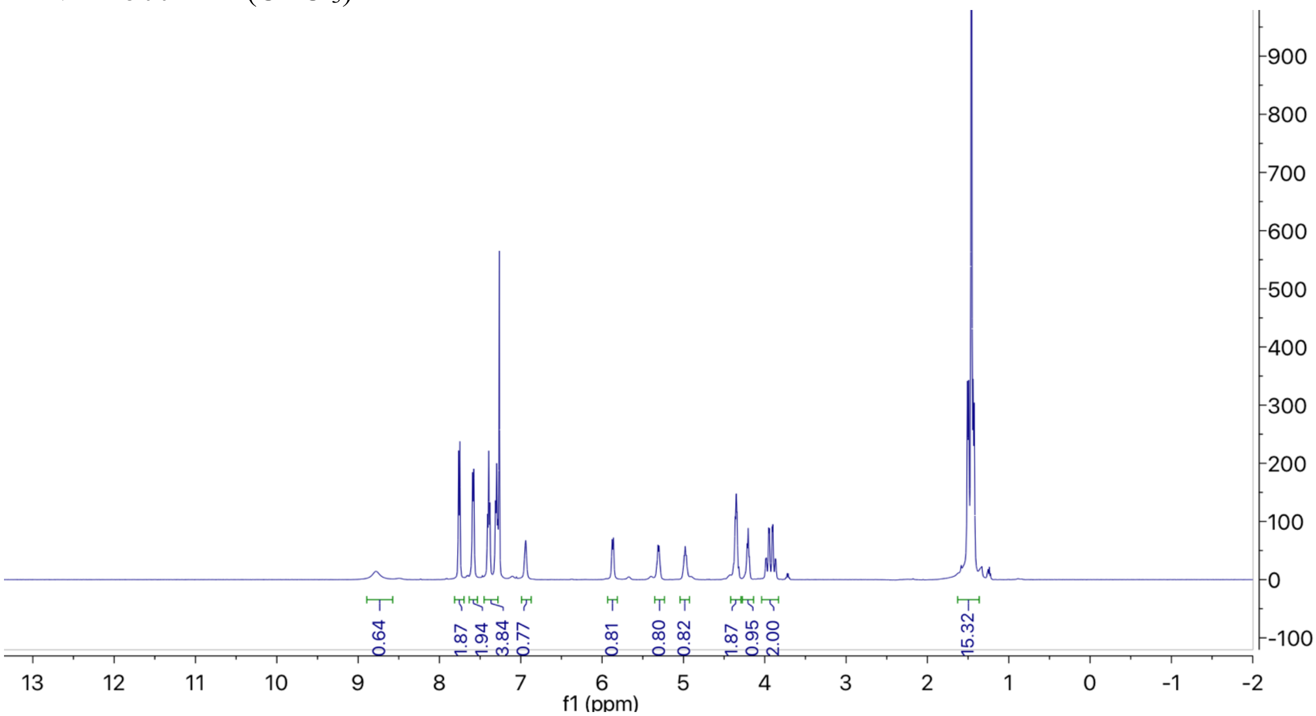
[13]C NMR 100MHz ($CDCl_3$)



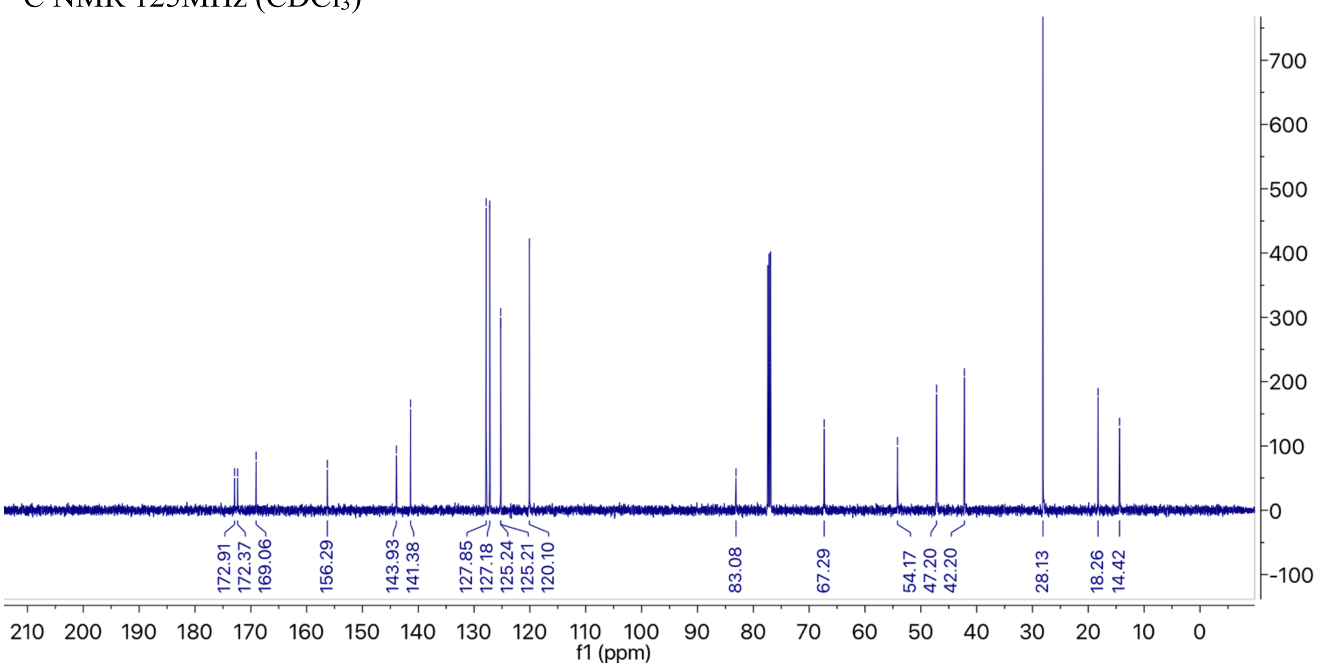


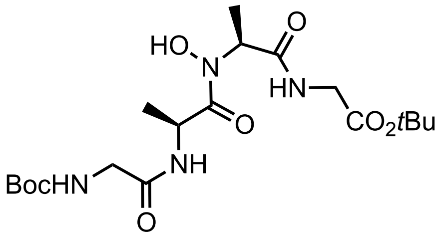
4

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



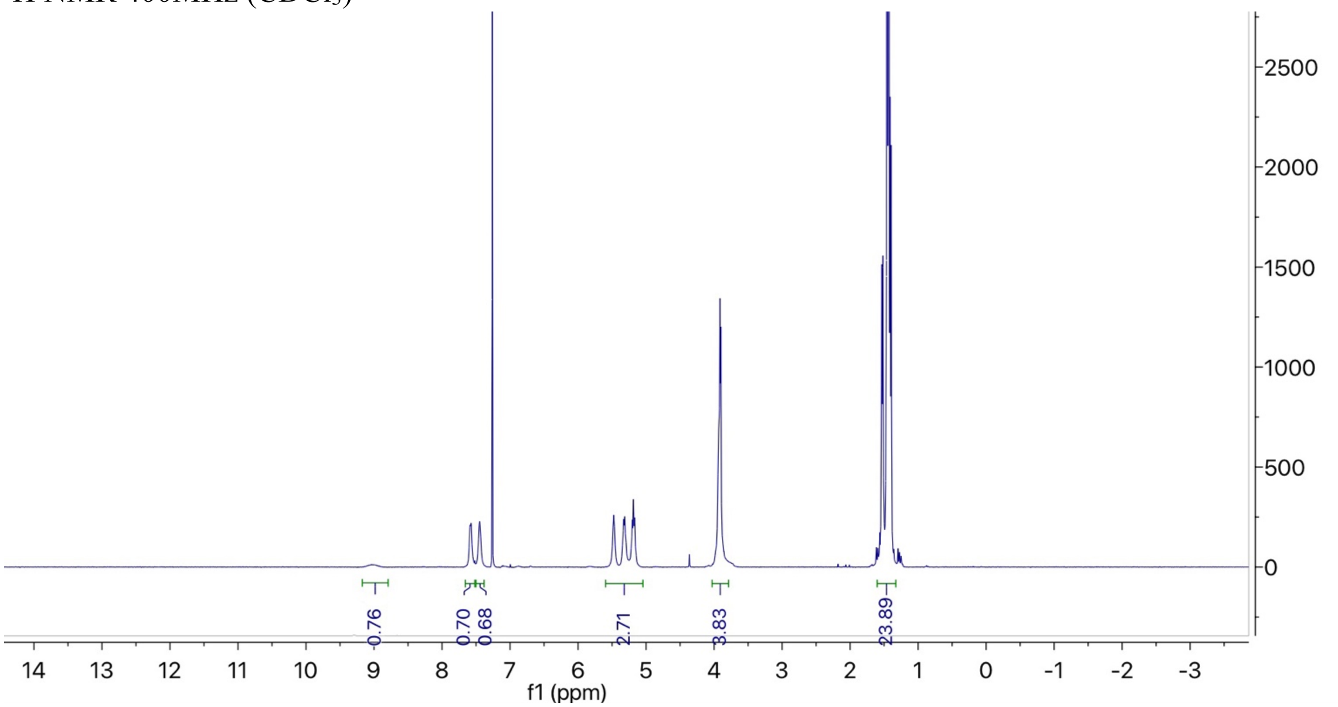
^{13}C NMR 125MHz ($CDCl_3$)



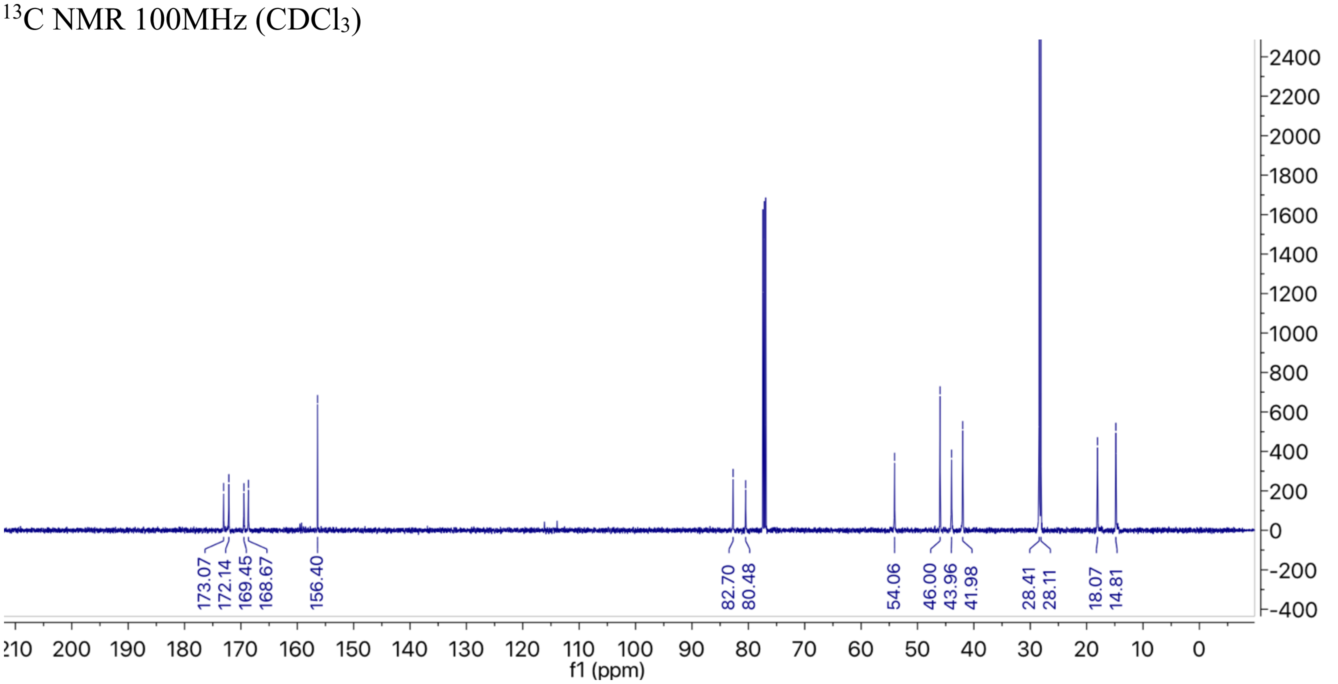


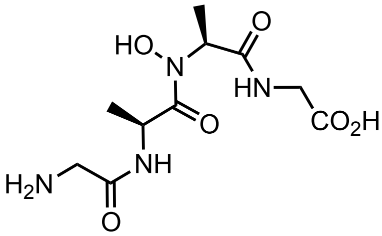
6

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



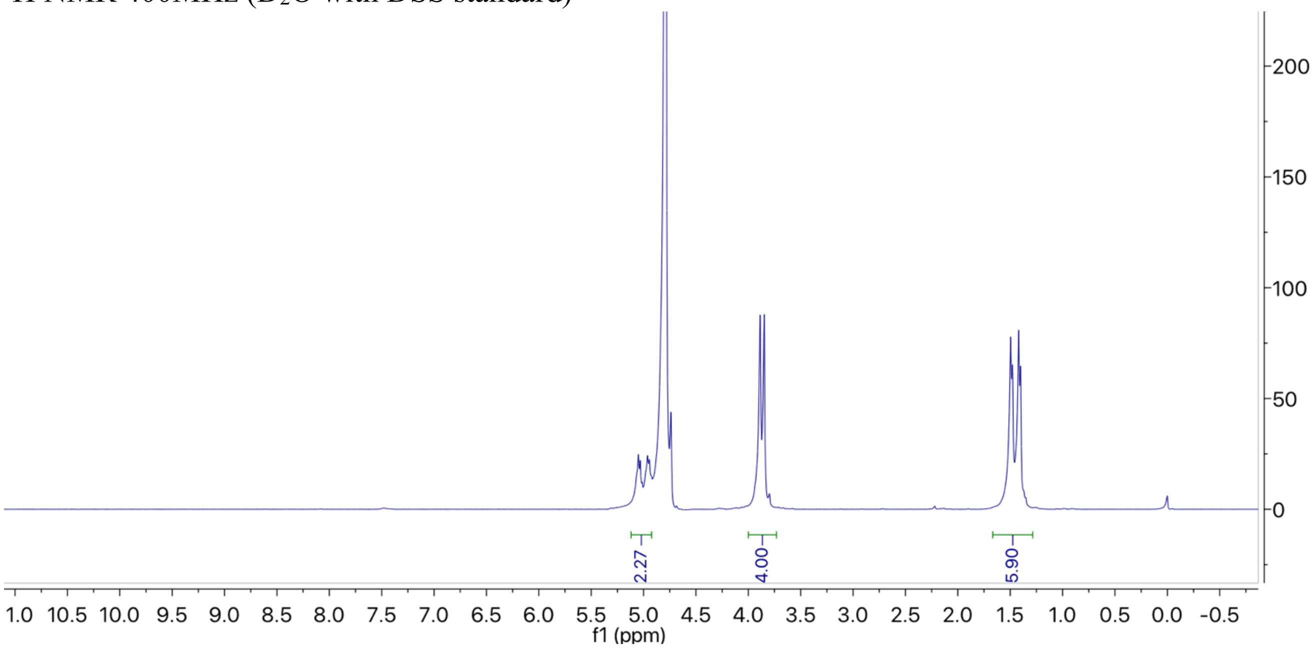
^{13}C NMR 100MHz ($CDCl_3$)



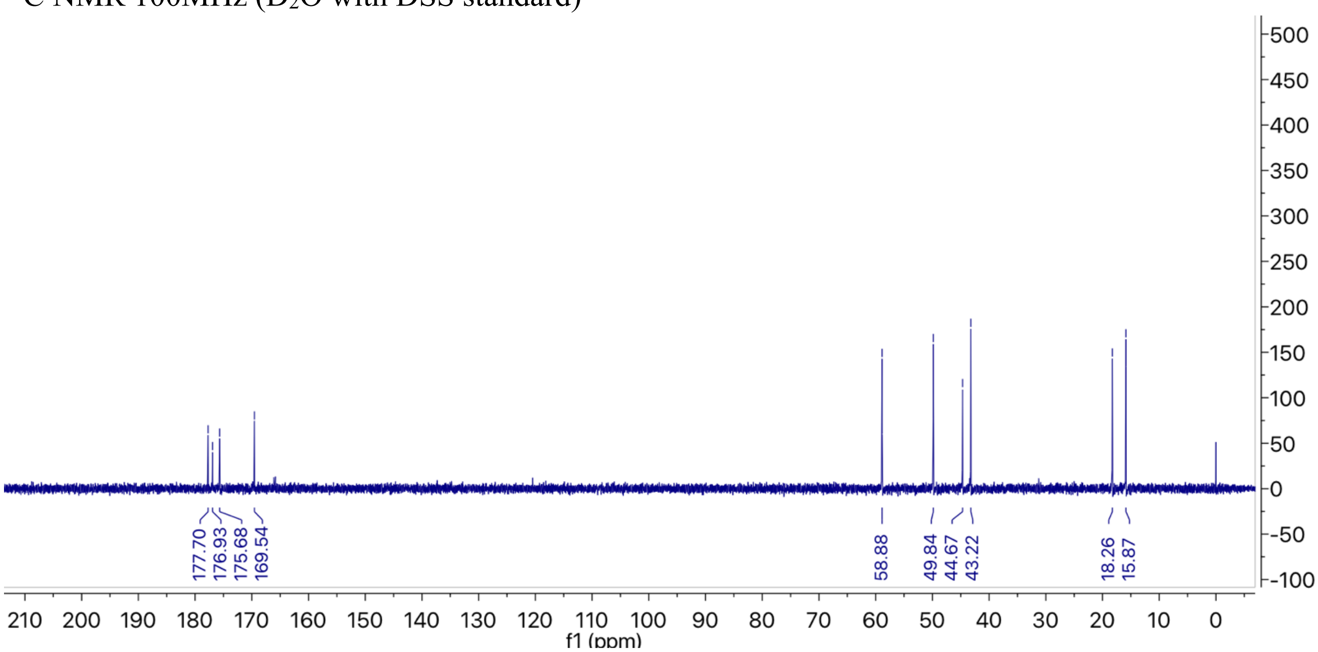


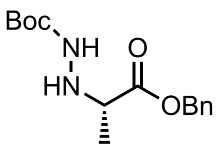
7

^{1}H NMR 400MHz (D_2O with DSS standard)



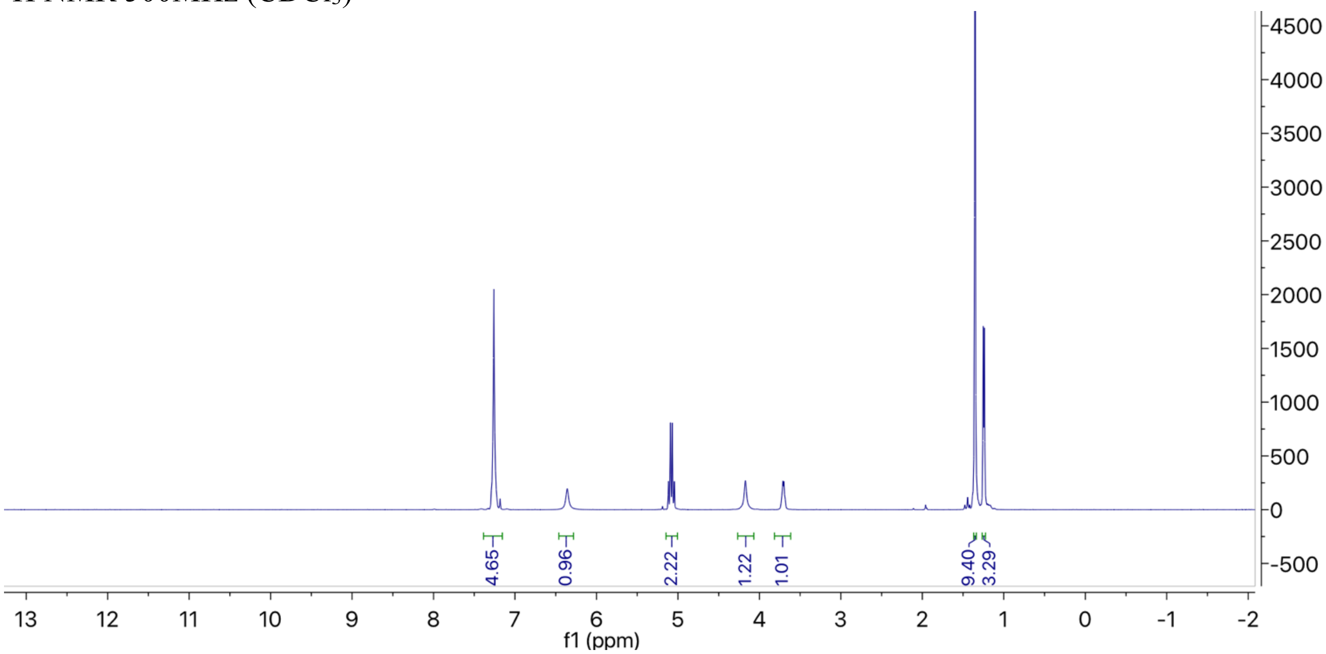
^{13}C NMR 100MHz (D_2O with DSS standard)



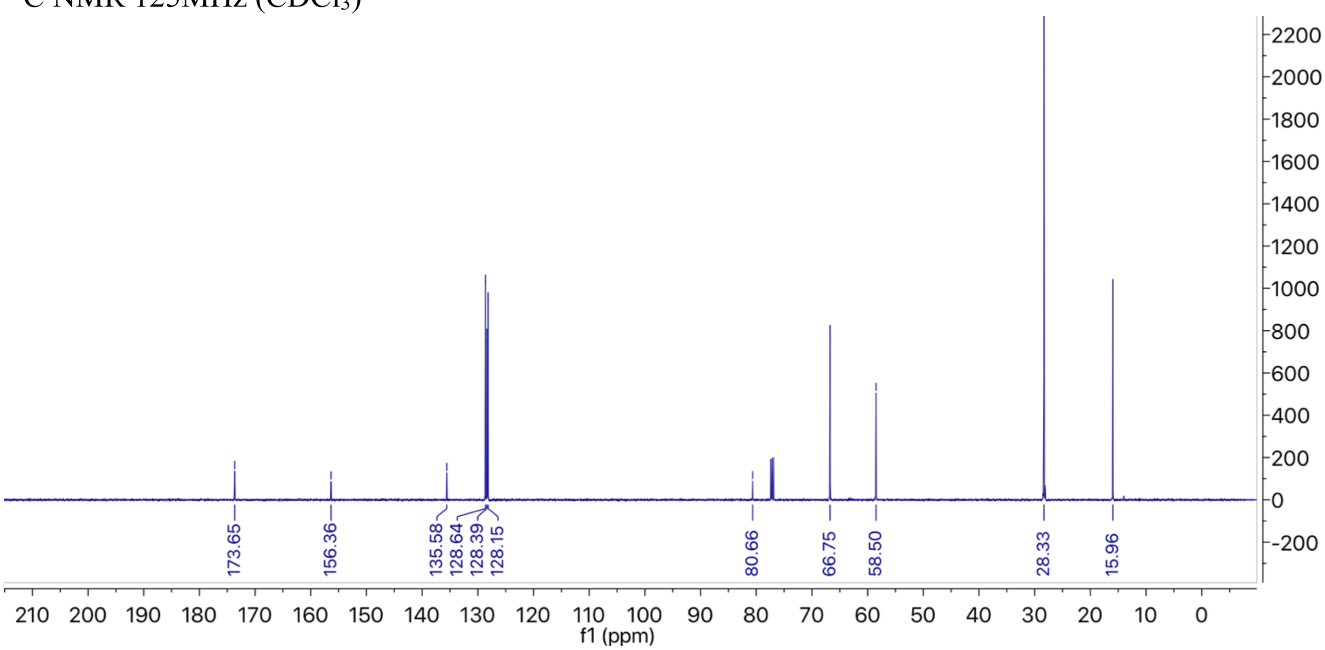


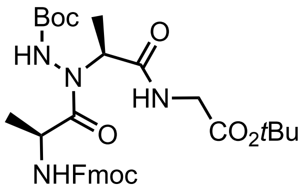
S1

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



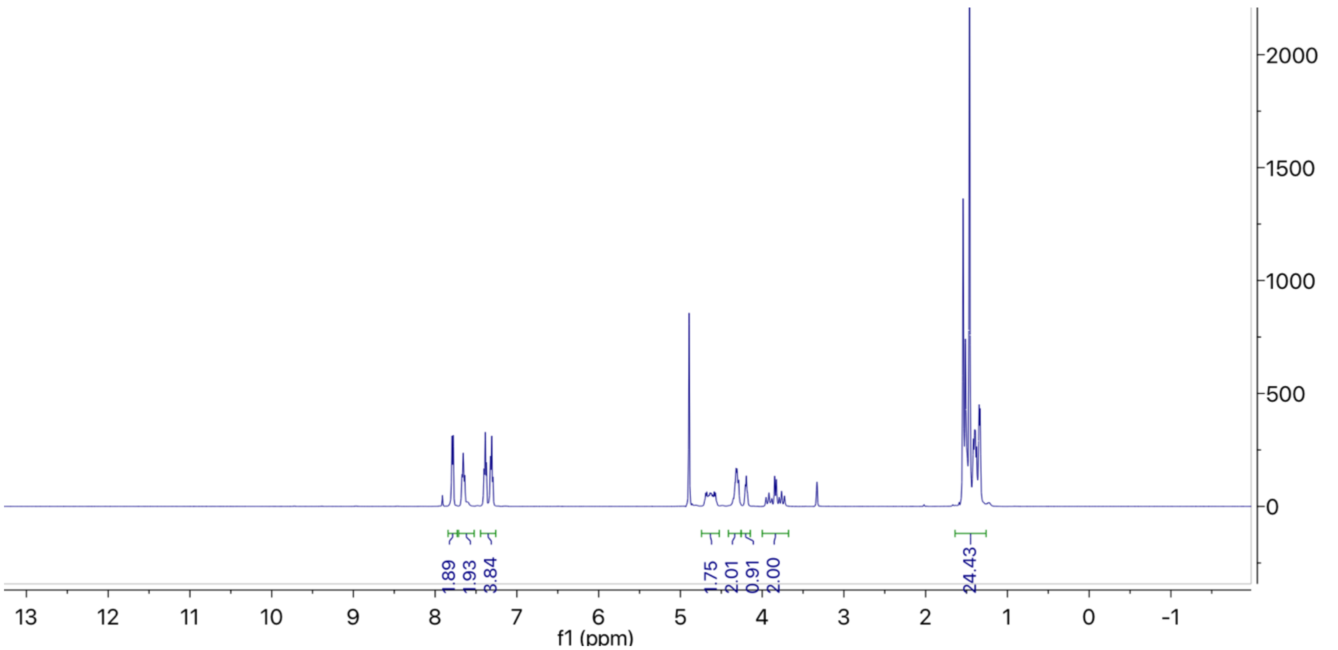
^{13}C NMR 125MHz ($CDCl_3$)



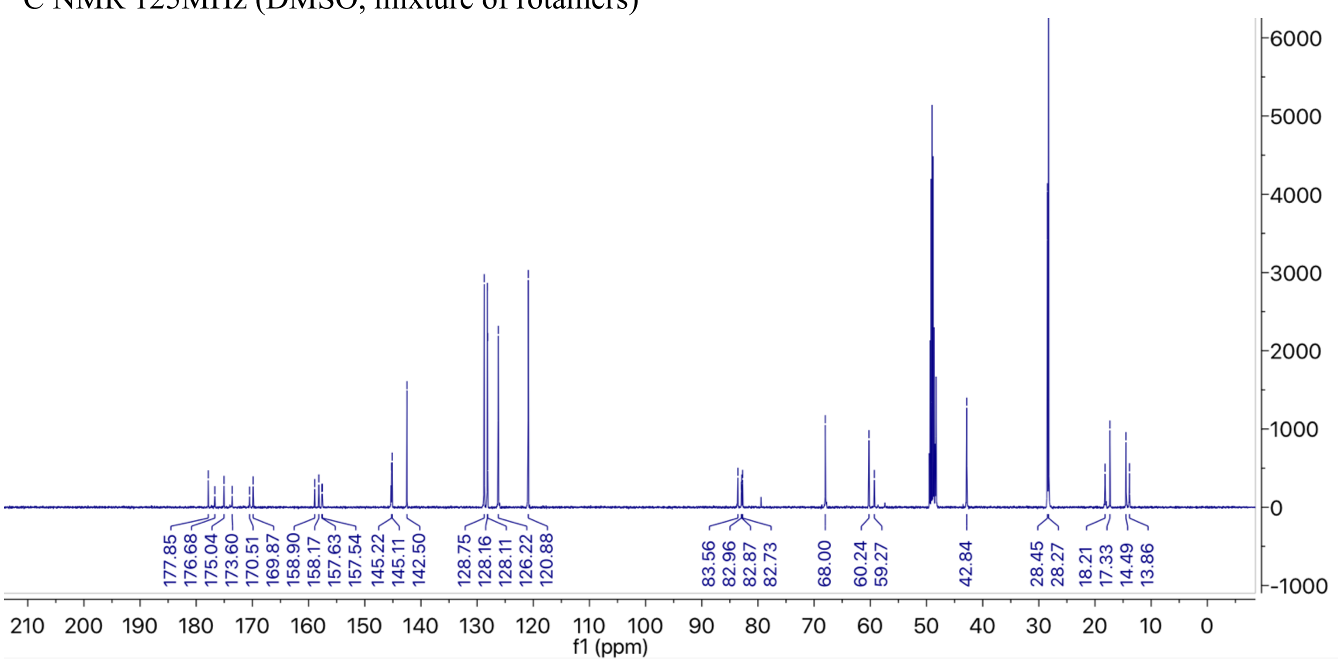


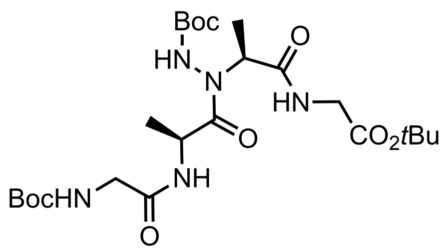
S2

[1]H NMR 400MHz (MeOD, mixture of rotamers)



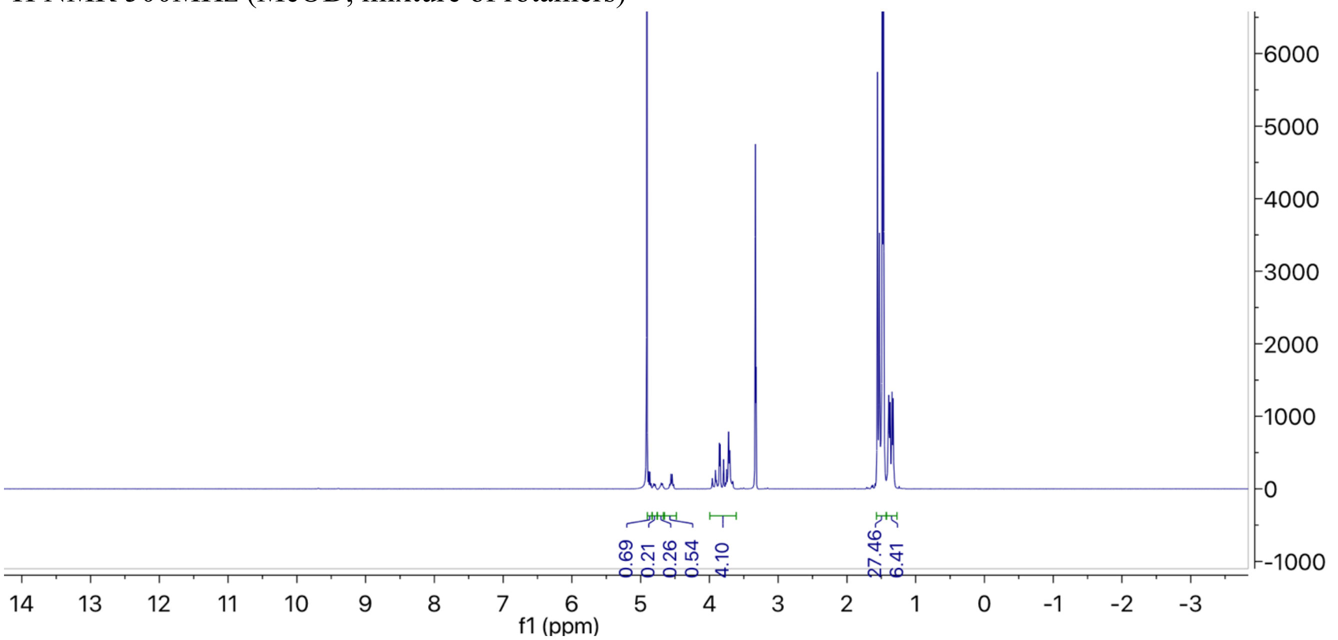
[13]C NMR 125MHz (DMSO, mixture of rotamers)



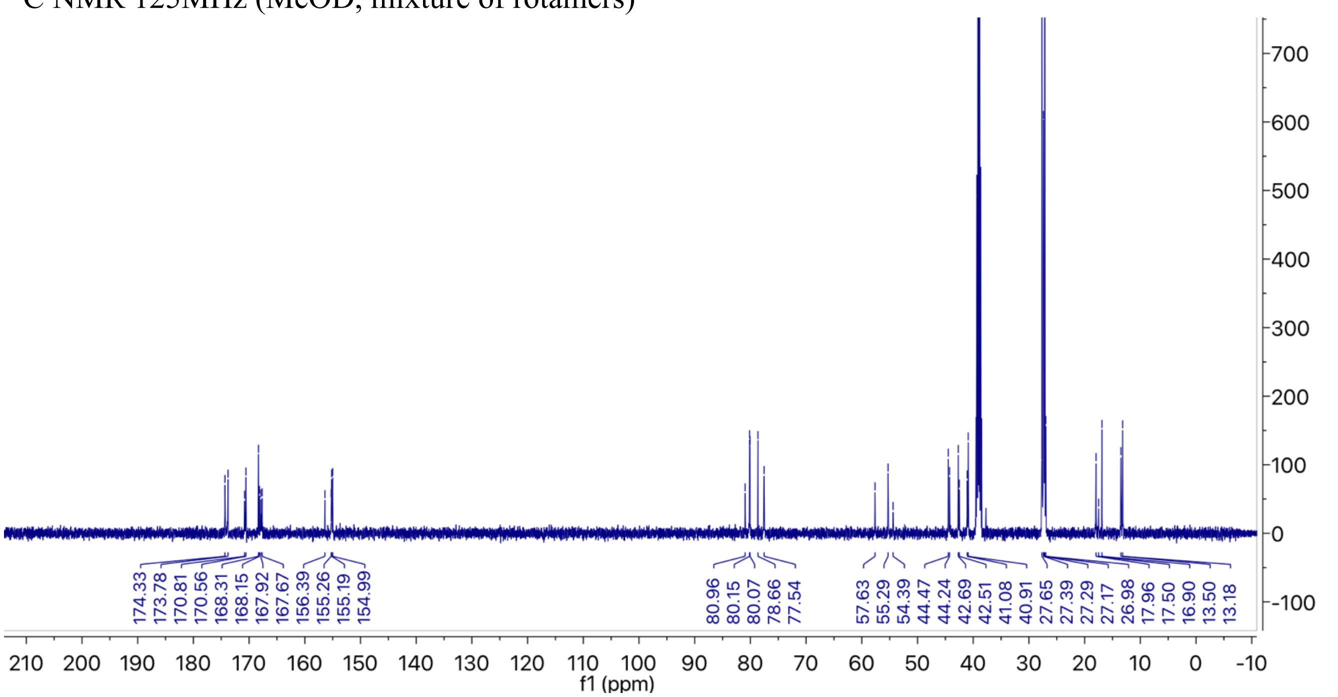


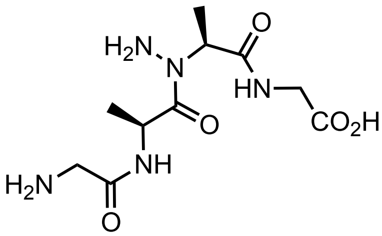
S3

^{1}H NMR 500MHz (MeOD, mixture of rotamers)



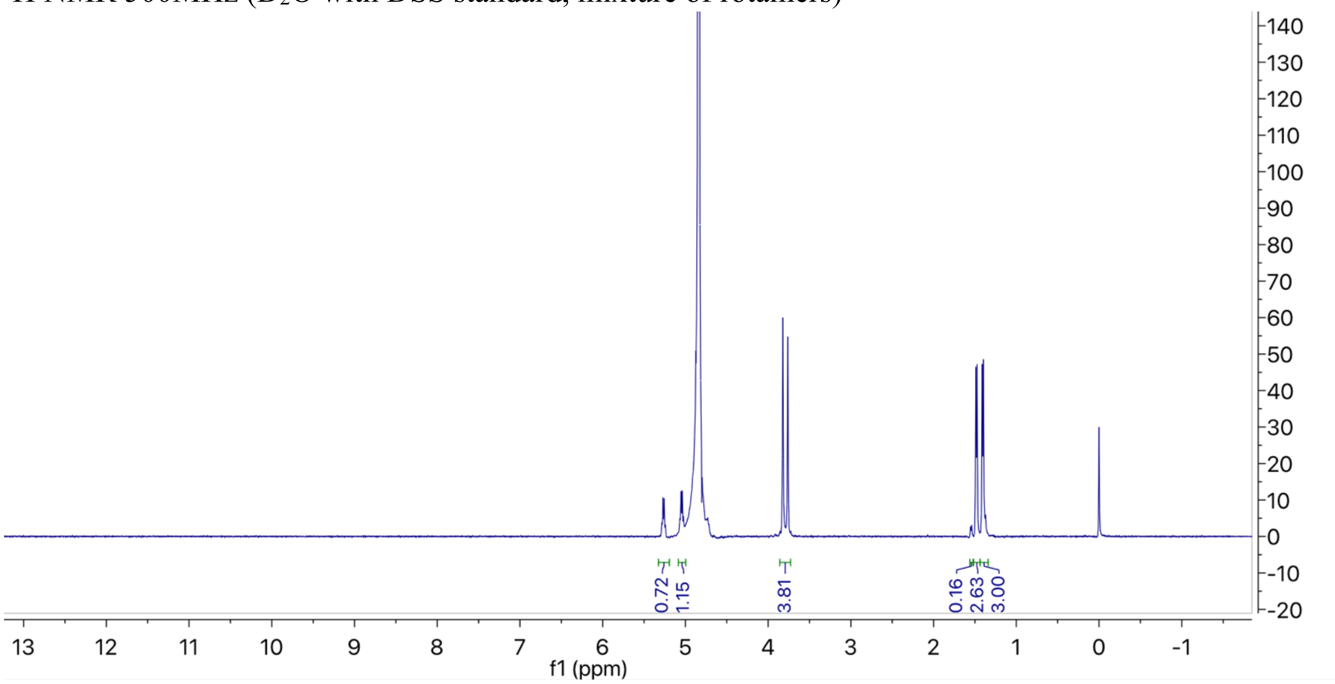
^{13}C NMR 125MHz (MeOD, mixture of rotamers)



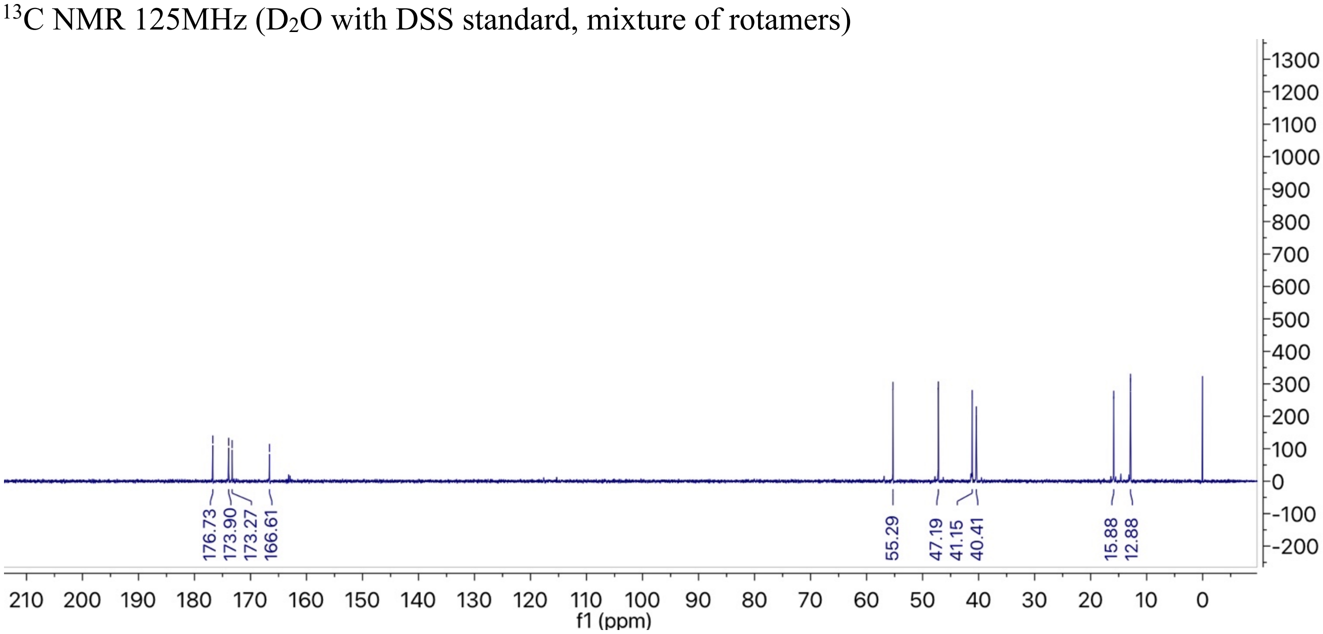


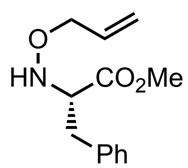
8

[1]H NMR 500MHz (D_2O with DSS standard, mixture of rotamers)



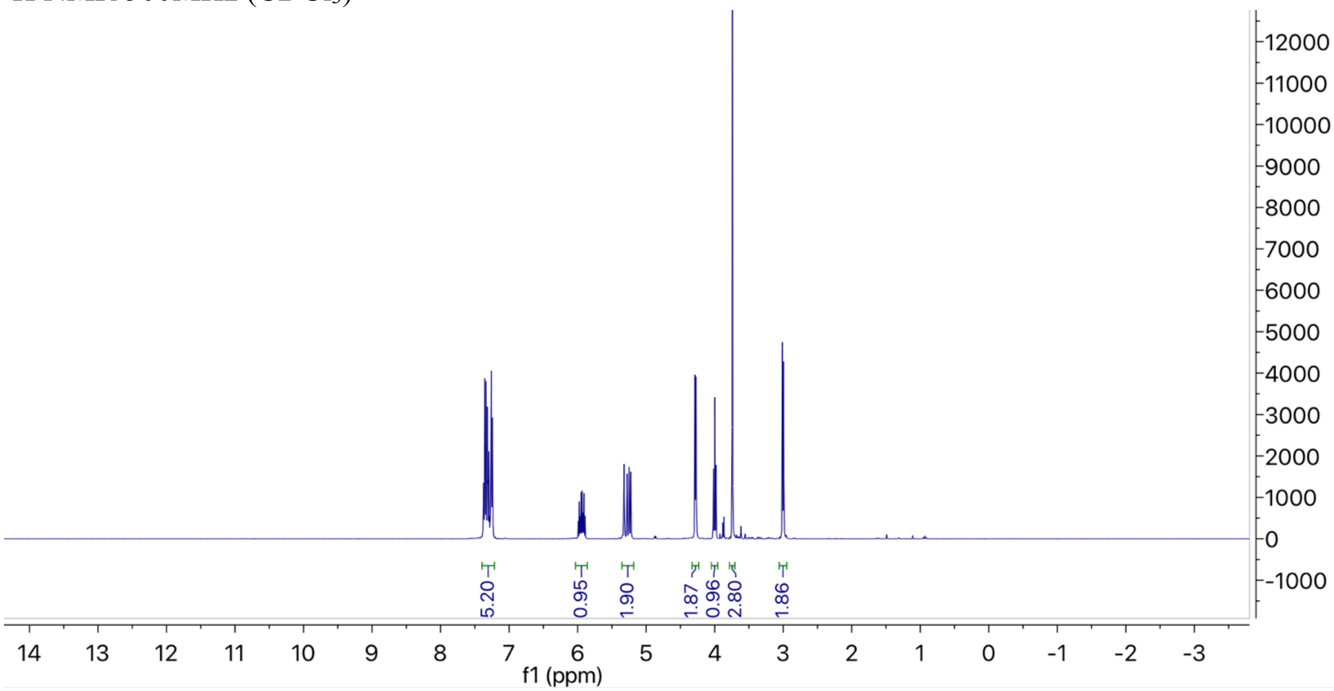
[13]C NMR 125MHz (D_2O with DSS standard, mixture of rotamers)



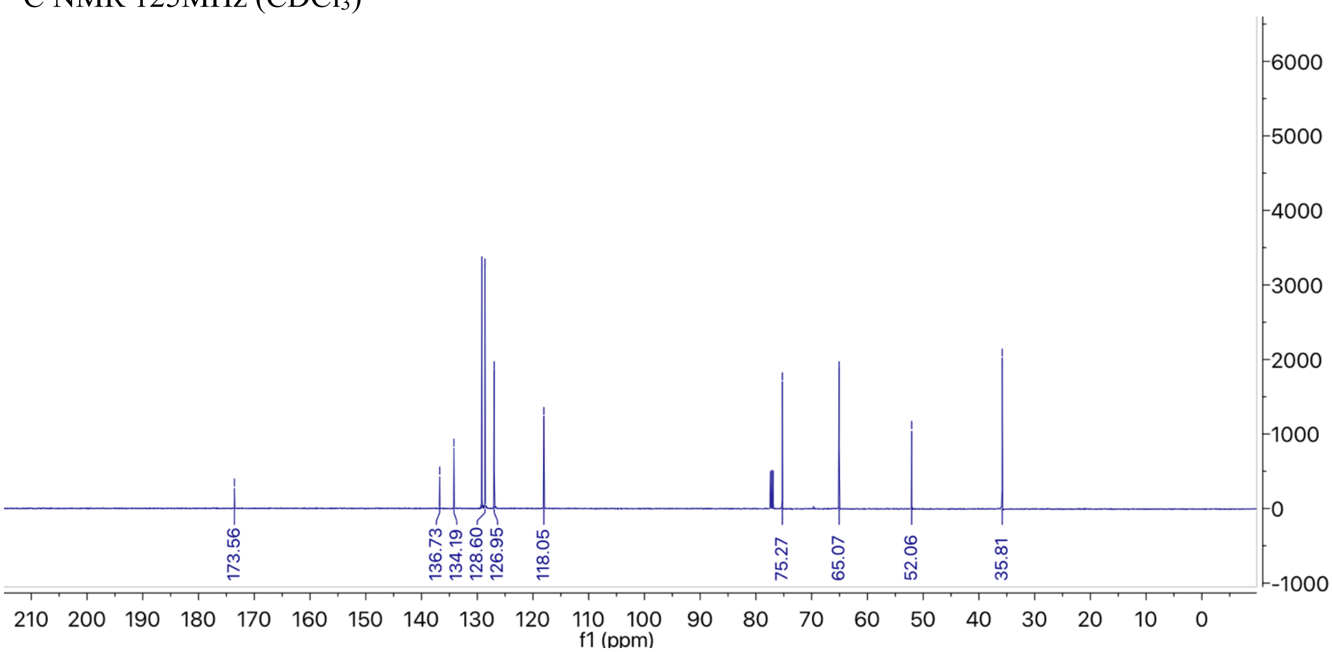


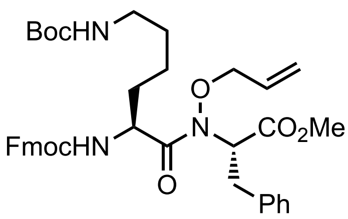
13

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



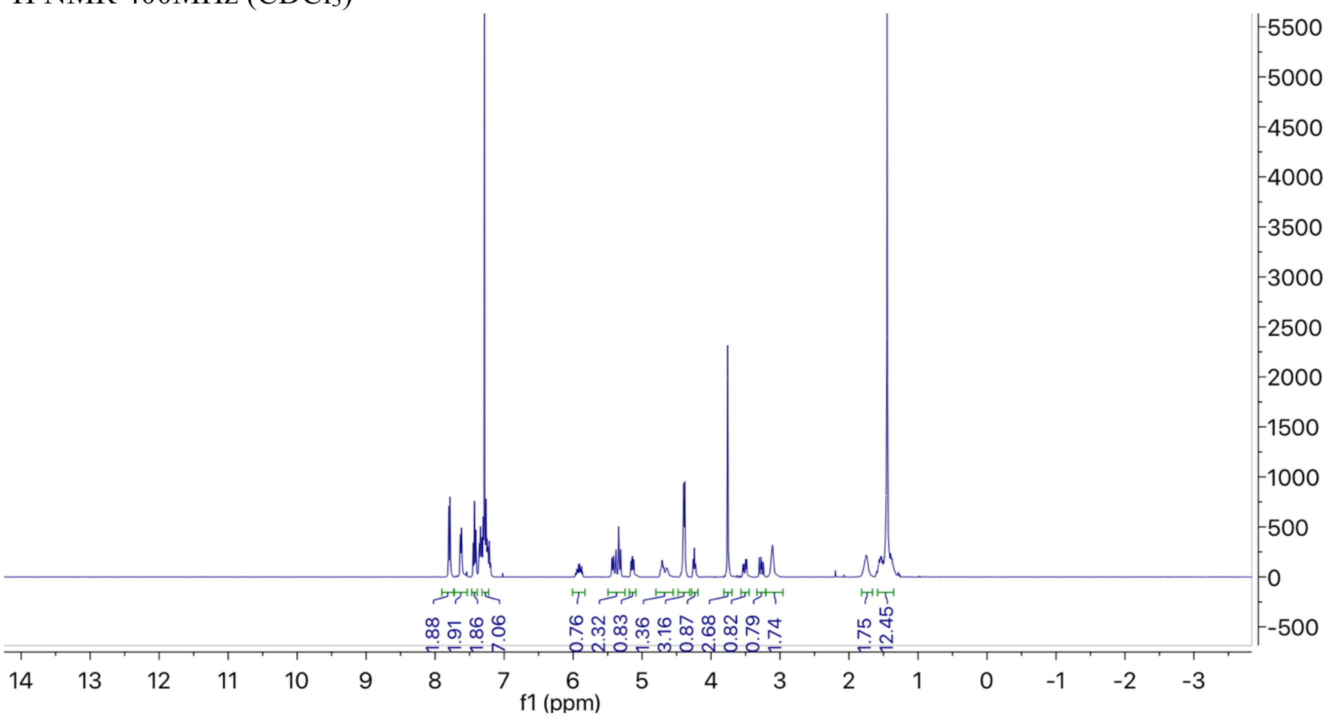
^{13}C NMR 125MHz ($CDCl_3$)



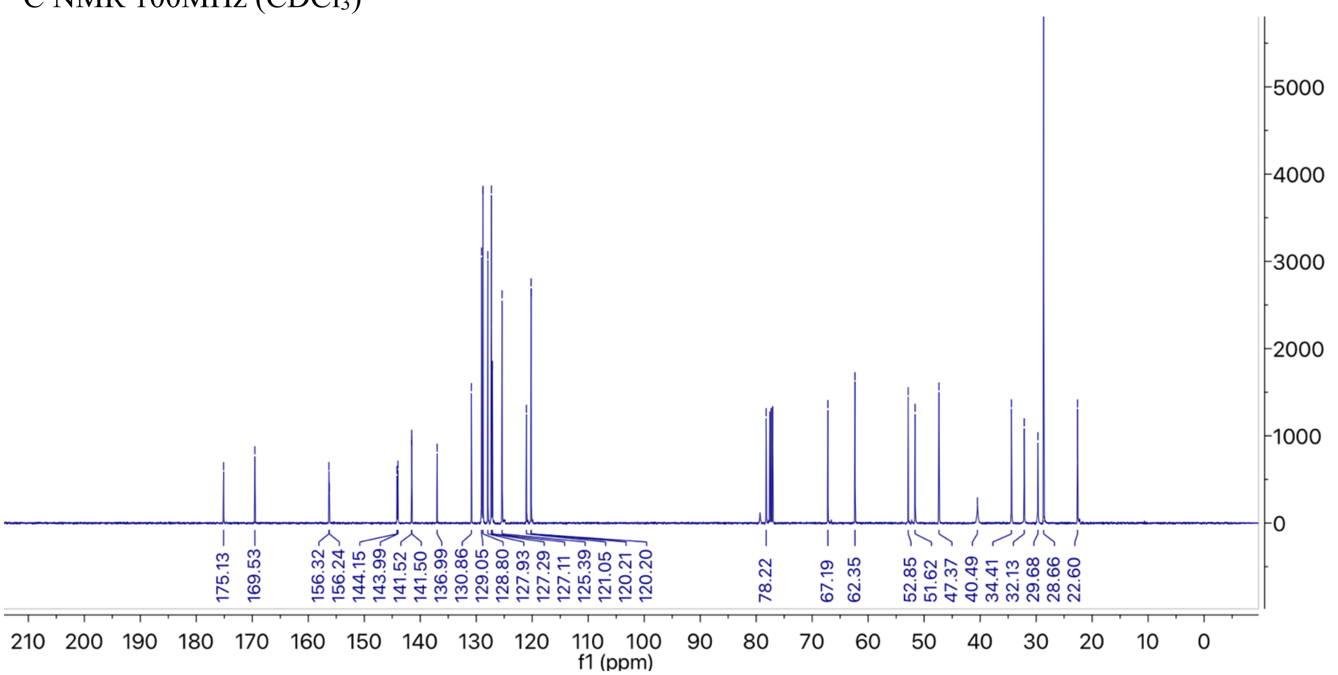


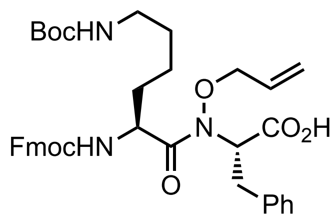
14

^1H NMR 400MHz ($CDCl_3$)



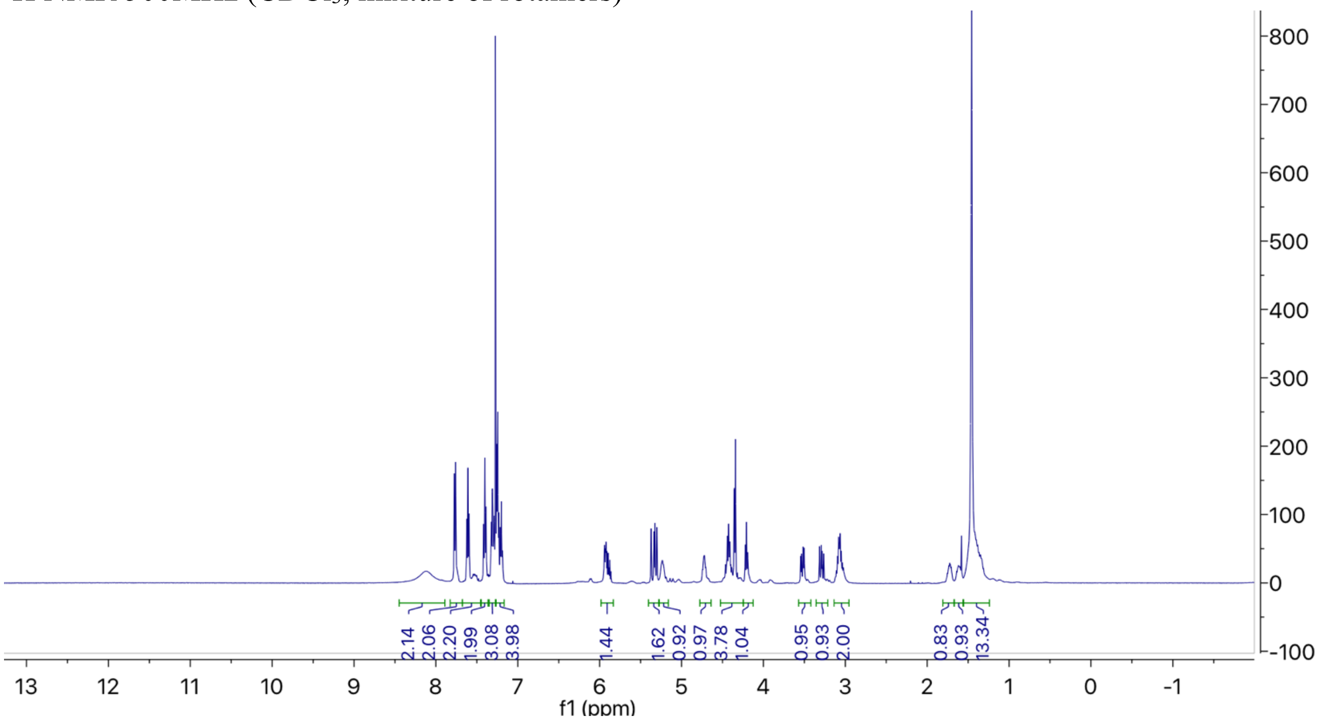
^{13}C NMR 100MHz ($CDCl_3$)



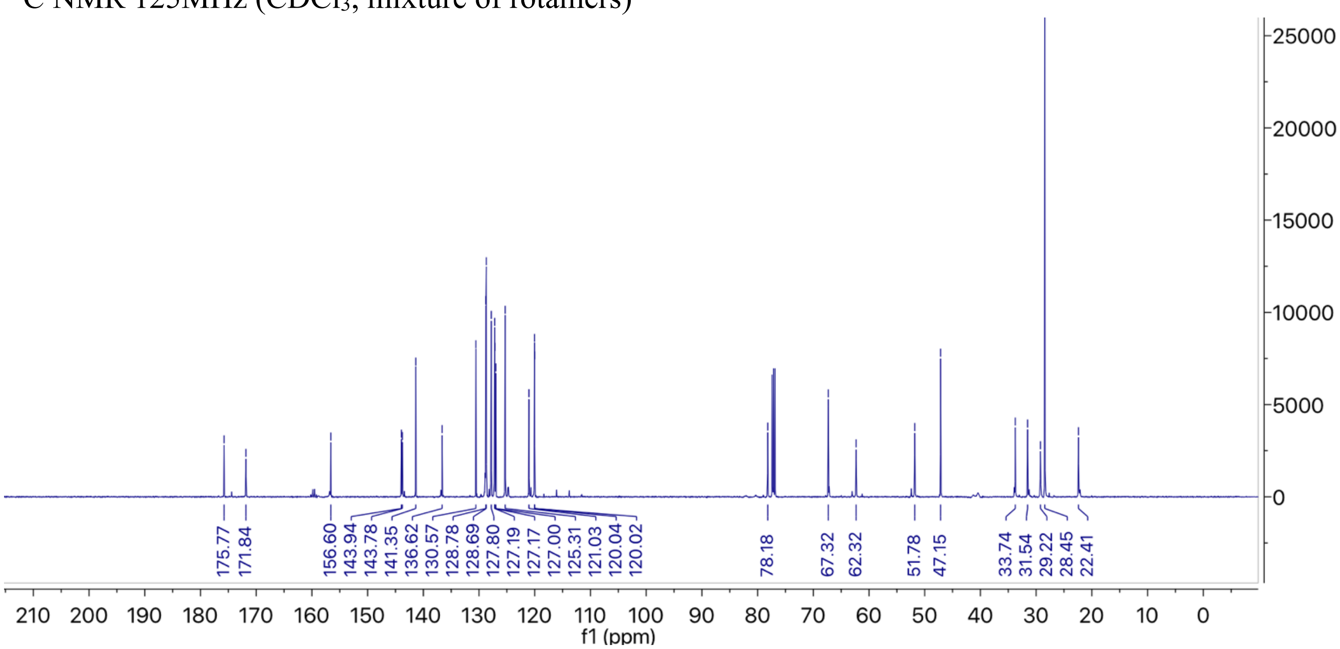


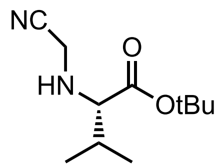
15

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



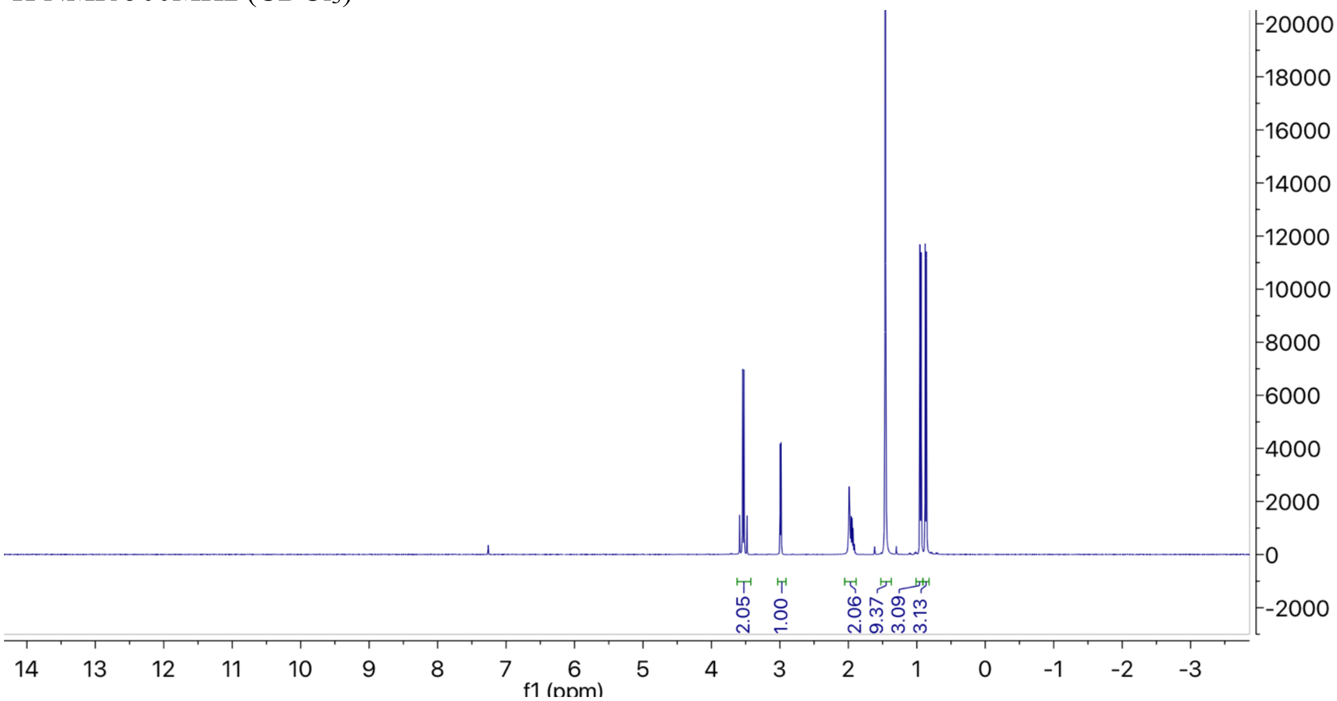
^{13}C NMR 125MHz ($CDCl_3$, mixture of rotamers)



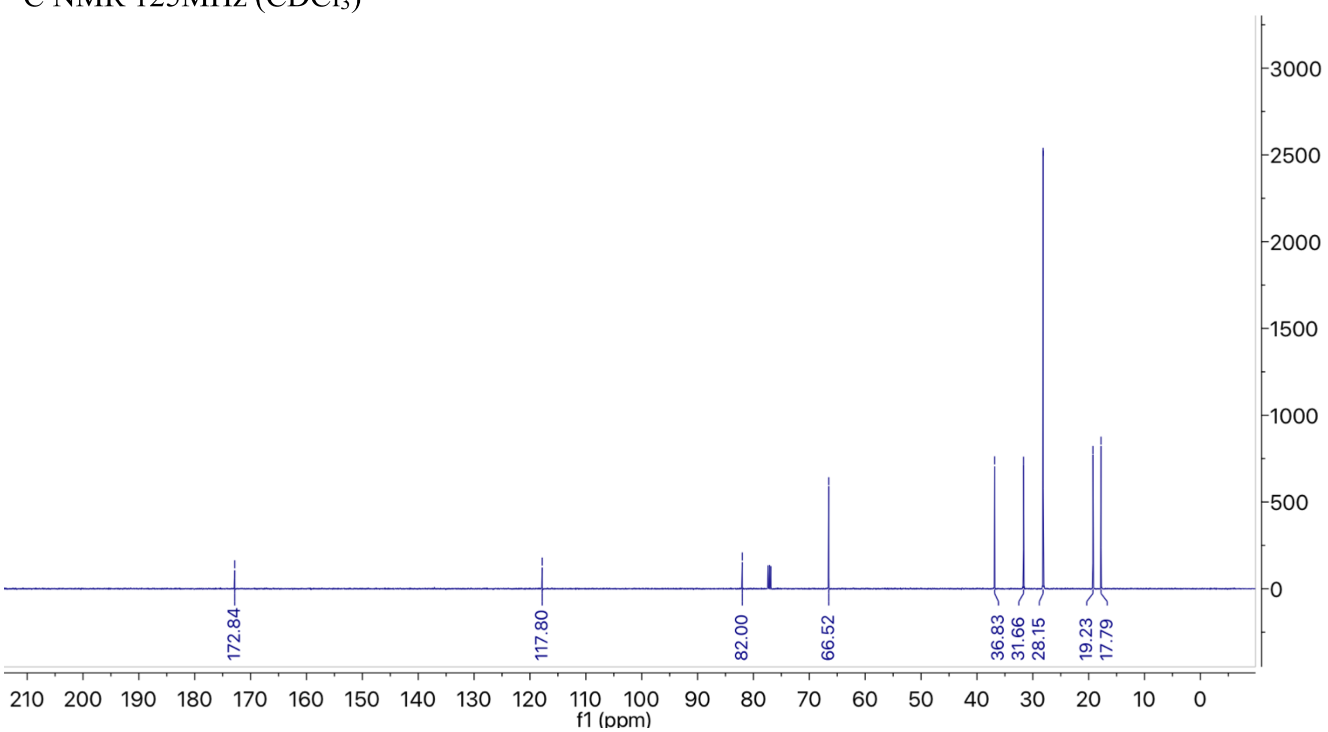


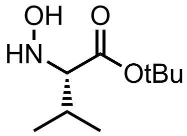
20

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



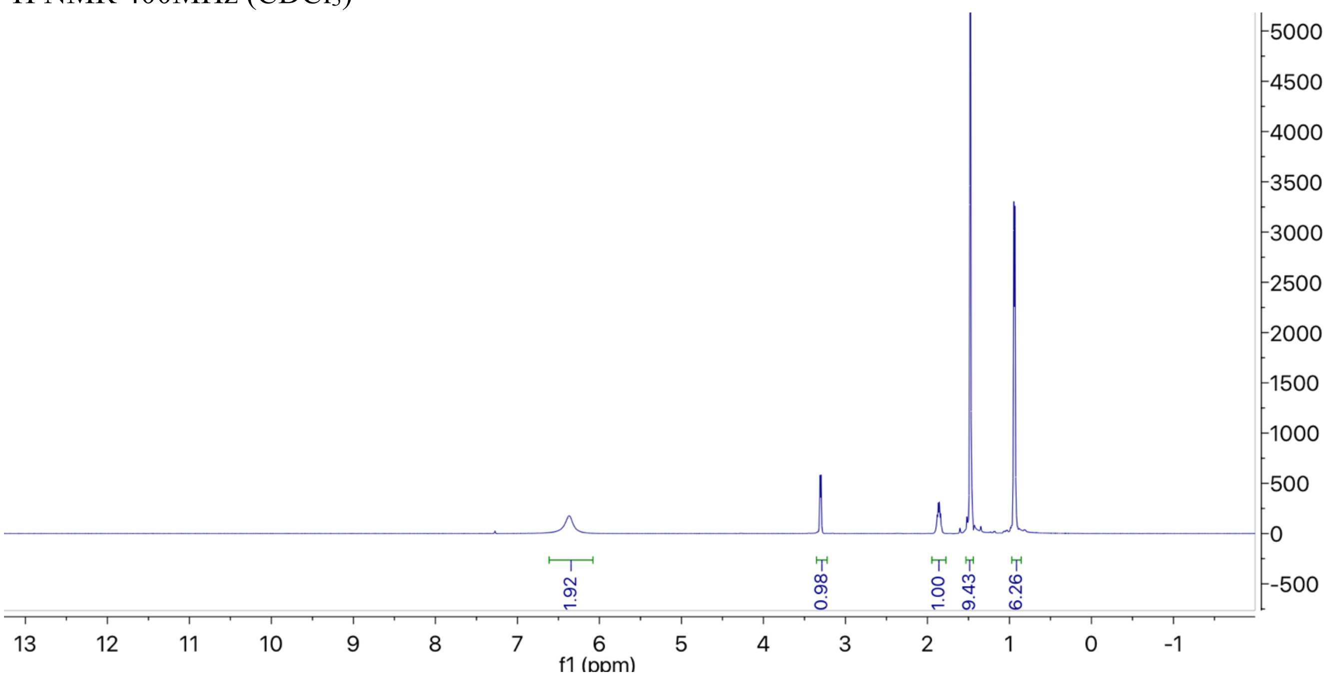
^{13}C NMR 125MHz ($CDCl_3$)



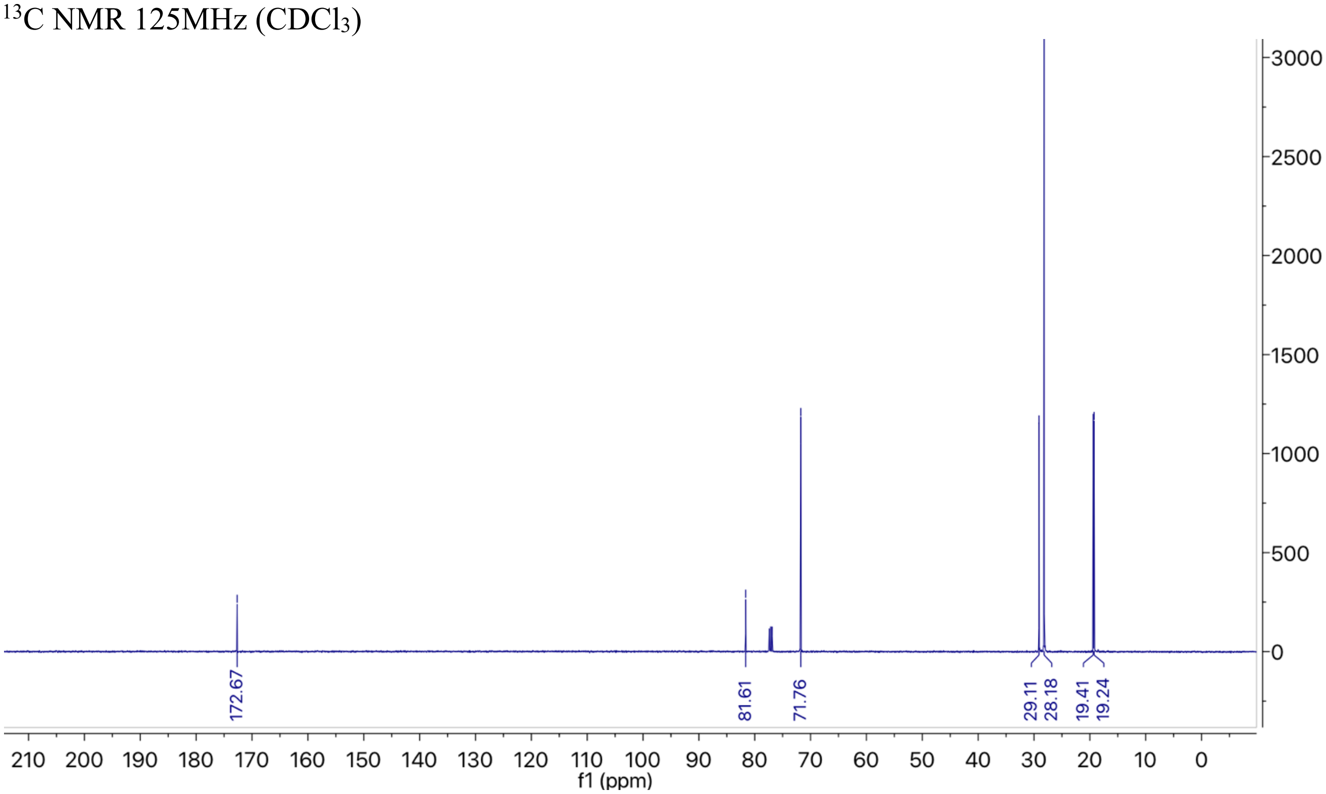


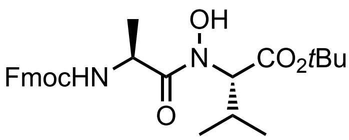
21

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



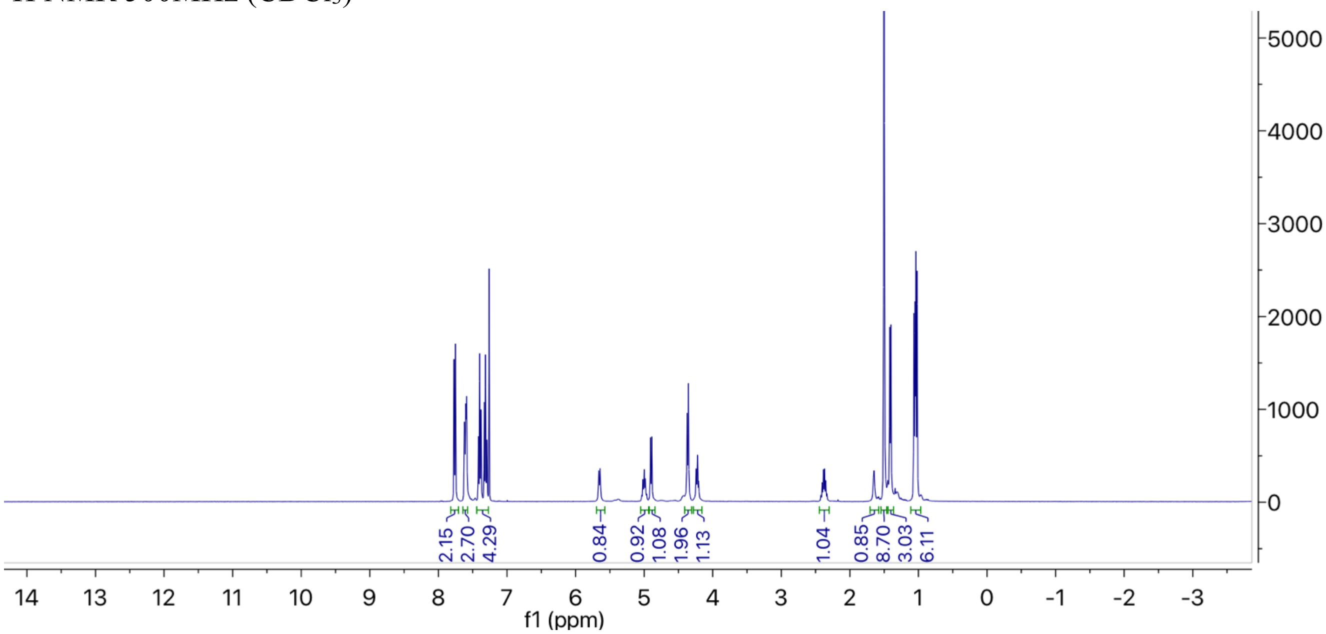
^{13}C NMR 125MHz ($CDCl_3$)



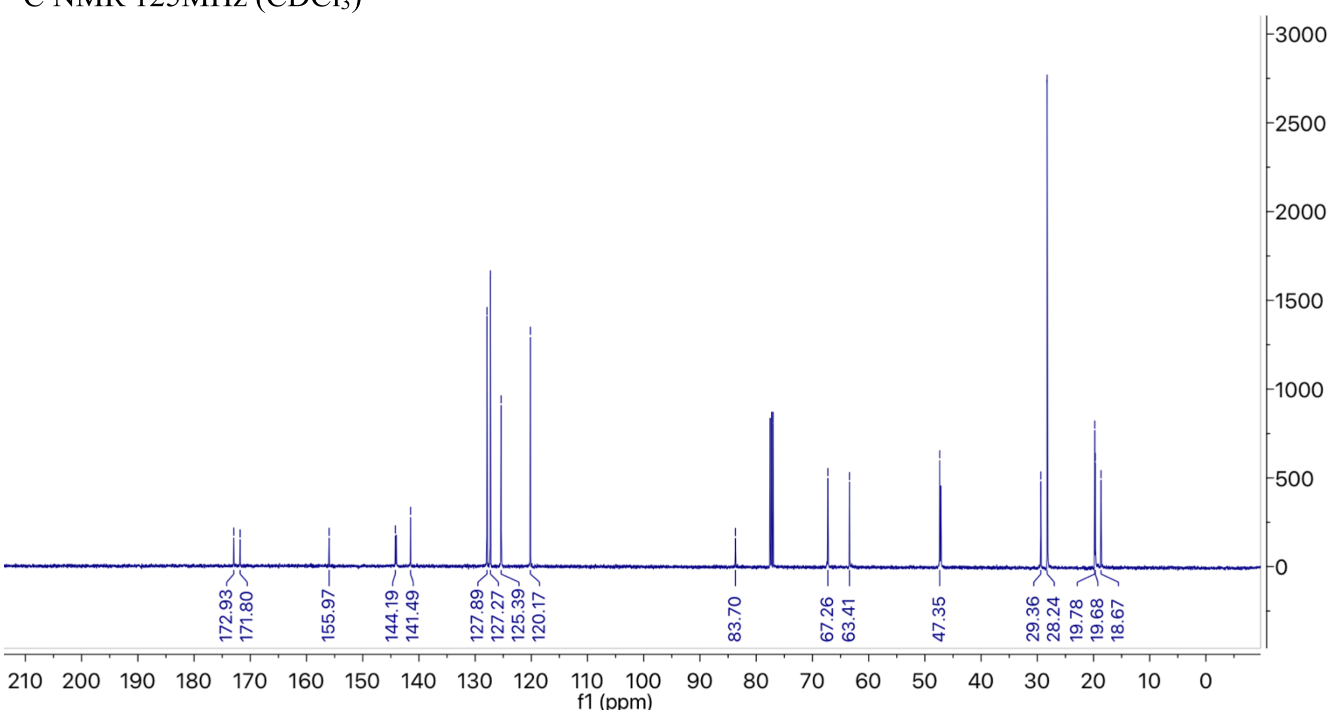


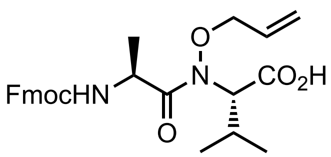
S4

[1]H NMR 500MHz ($CDCl_3$)



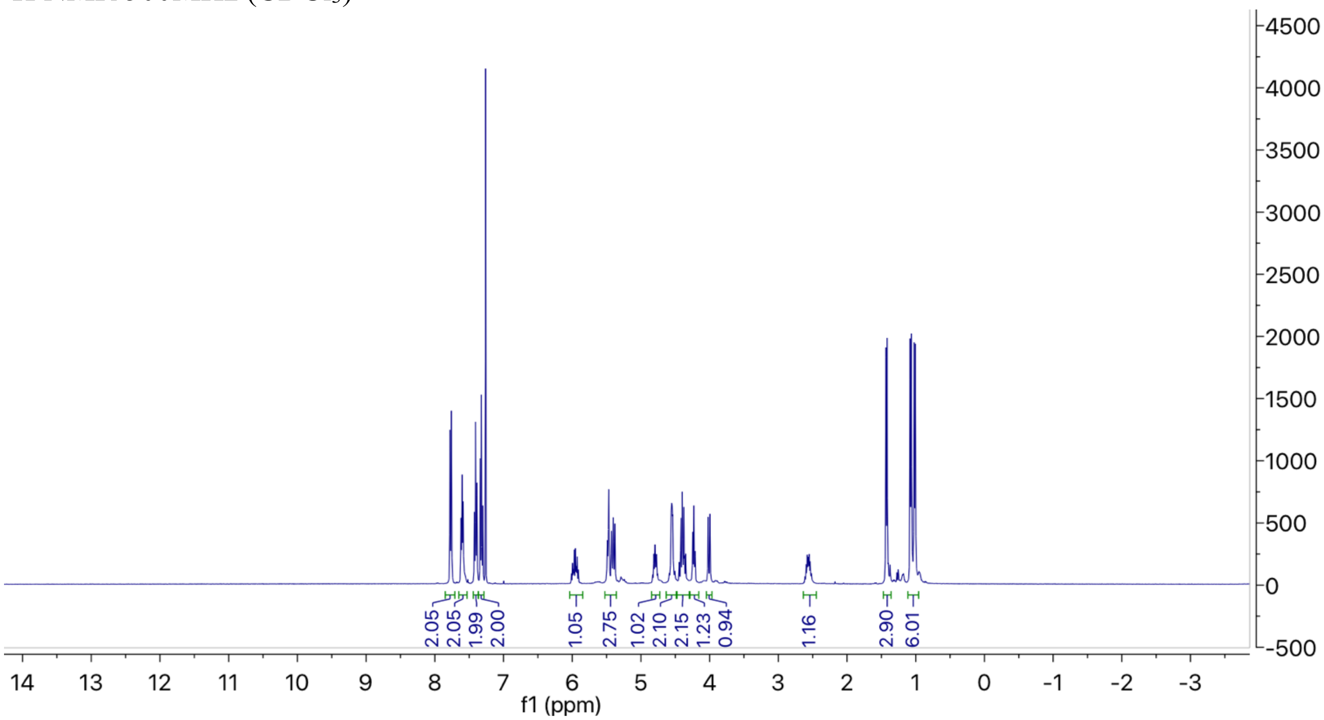
[13]C NMR 125MHz ($CDCl_3$)



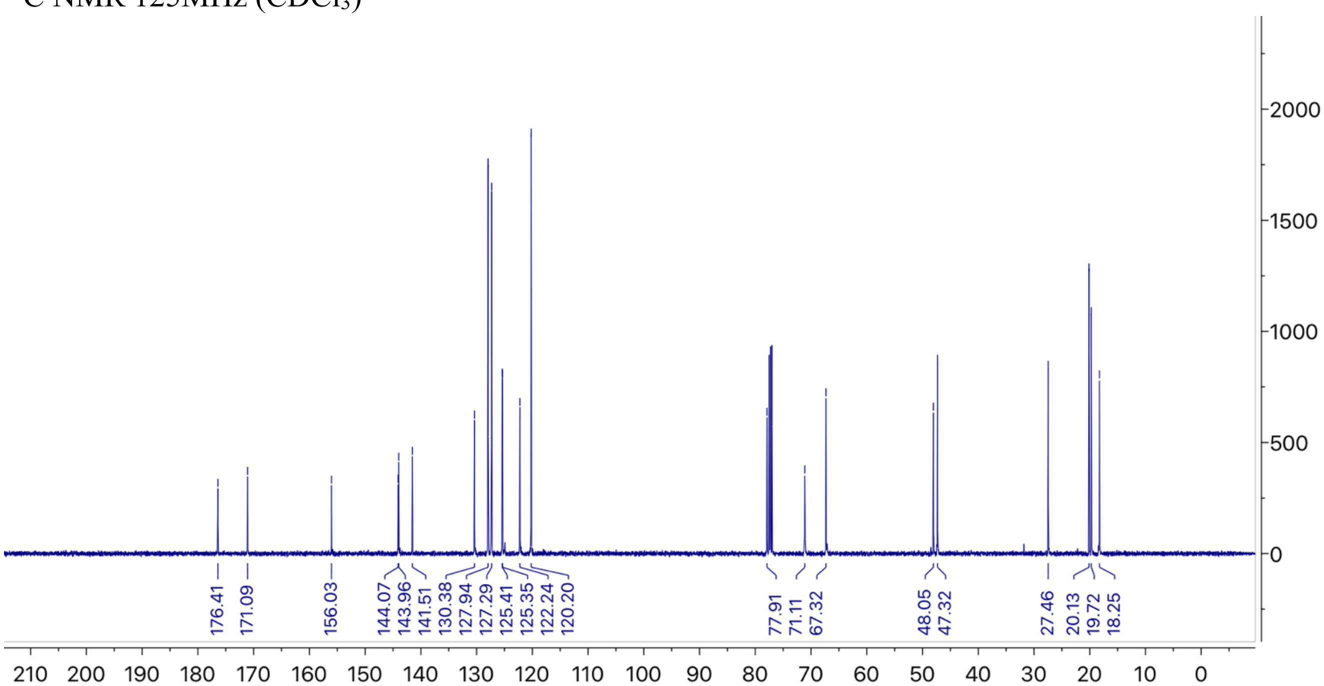


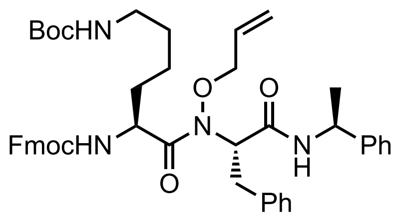
22

[1]H NMR 500MHz ($CDCl_3$)



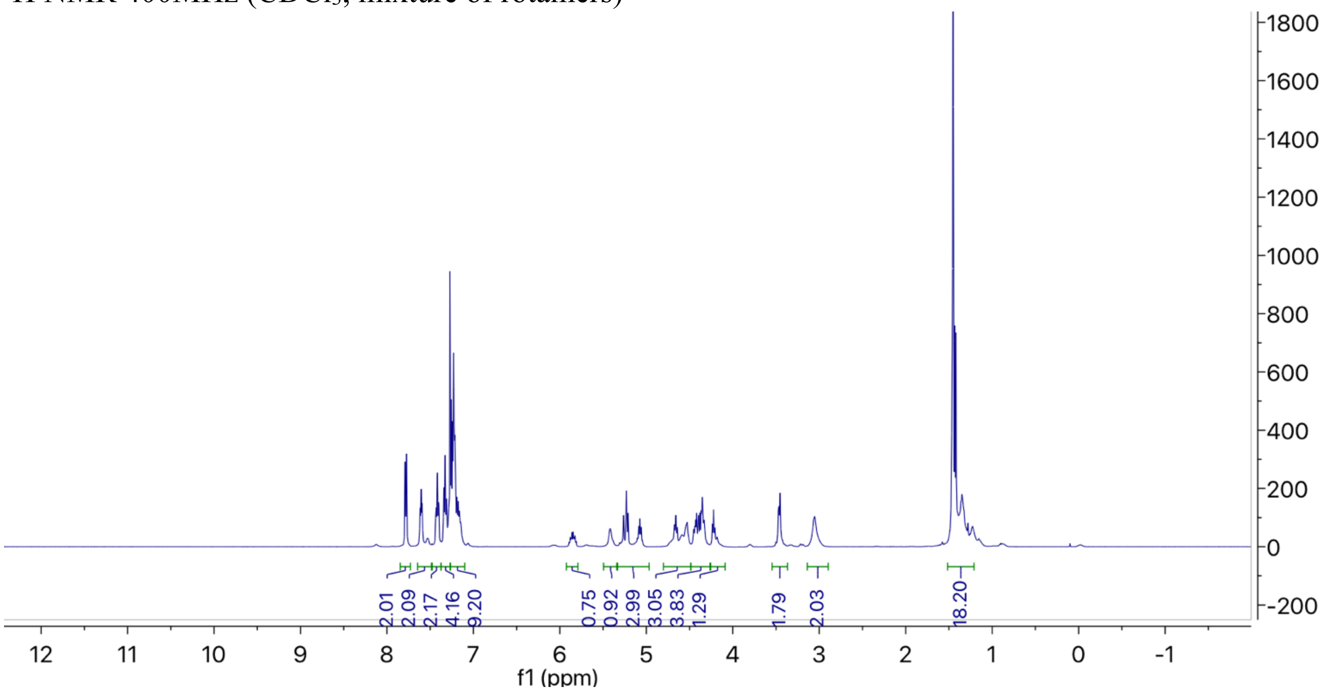
[13]C NMR 125MHz ($CDCl_3$)



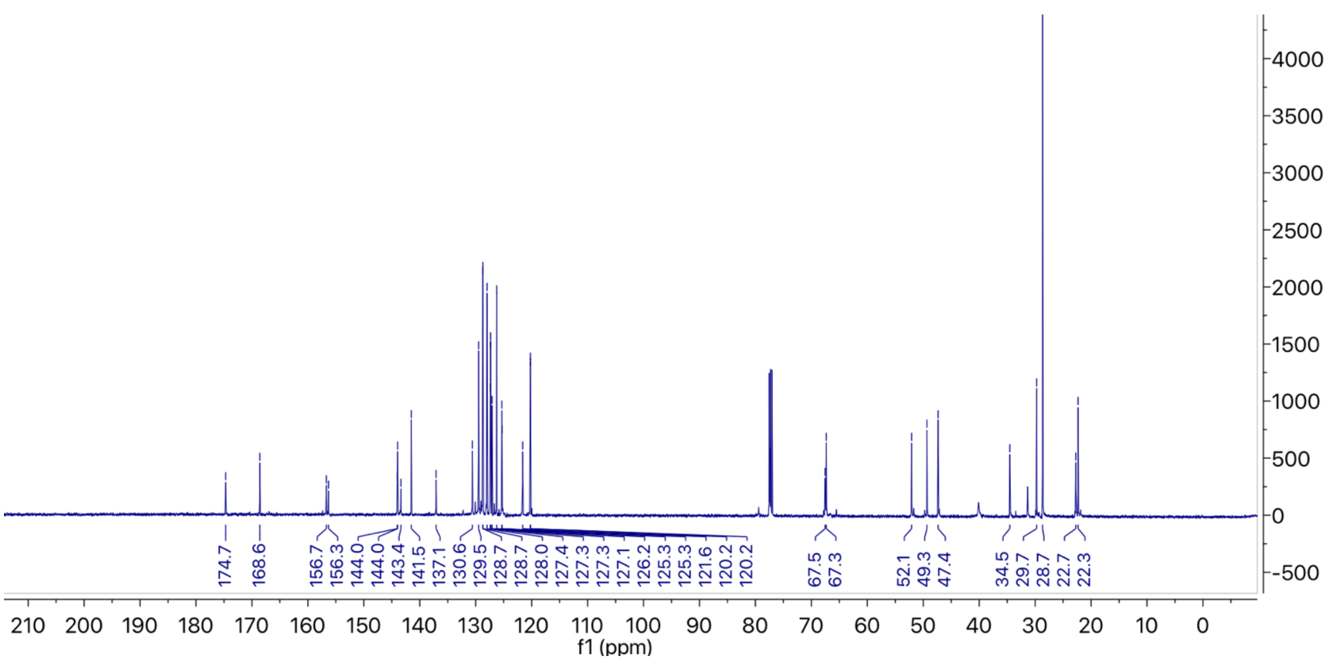


23

[1]H NMR 400MHz ($CDCl_3$, mixture of rotamers)



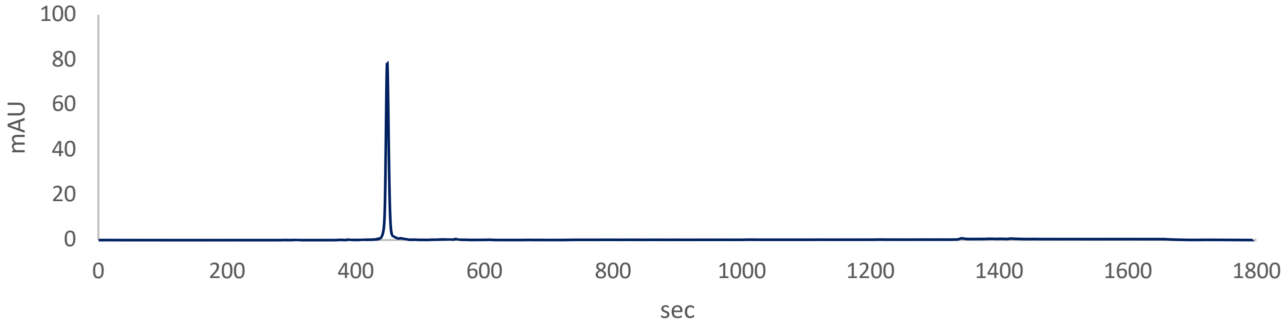
[13]C NMR 125MHz ($CDCl_3$, mixture of rotamers)



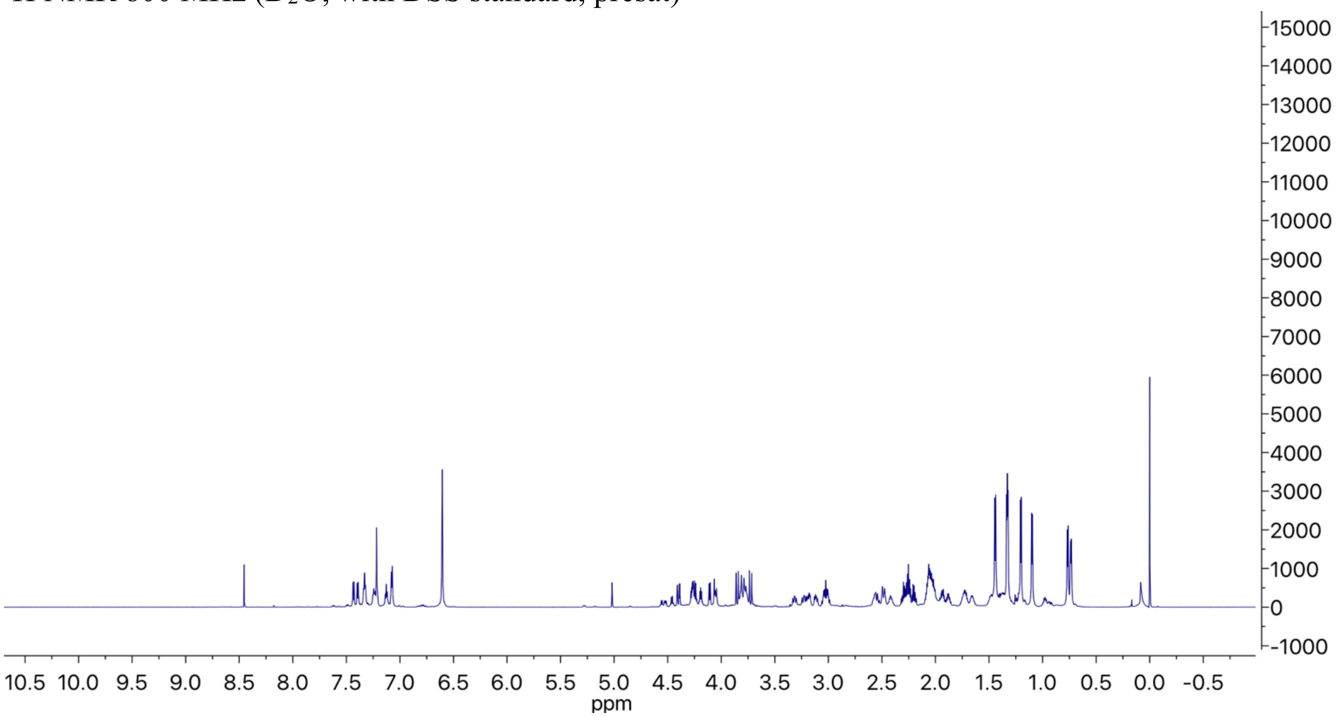
^{1}H NMR and RP-HPLC SPECTRA FOR ALL HAIRPIN PEPTIDES

H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-hPhe-Ala-Val-Thr-Glu-NH$_2$ (11b)

RP-HPLC 5-40% MeCN/H_2O (modified with 0.1% TFA)

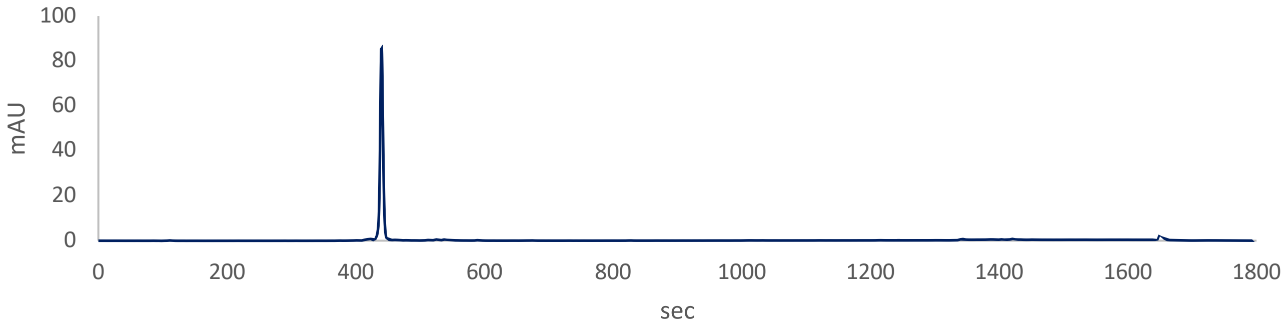


^{1}H NMR 800 MHz (D_2O, with DSS standard, presat)

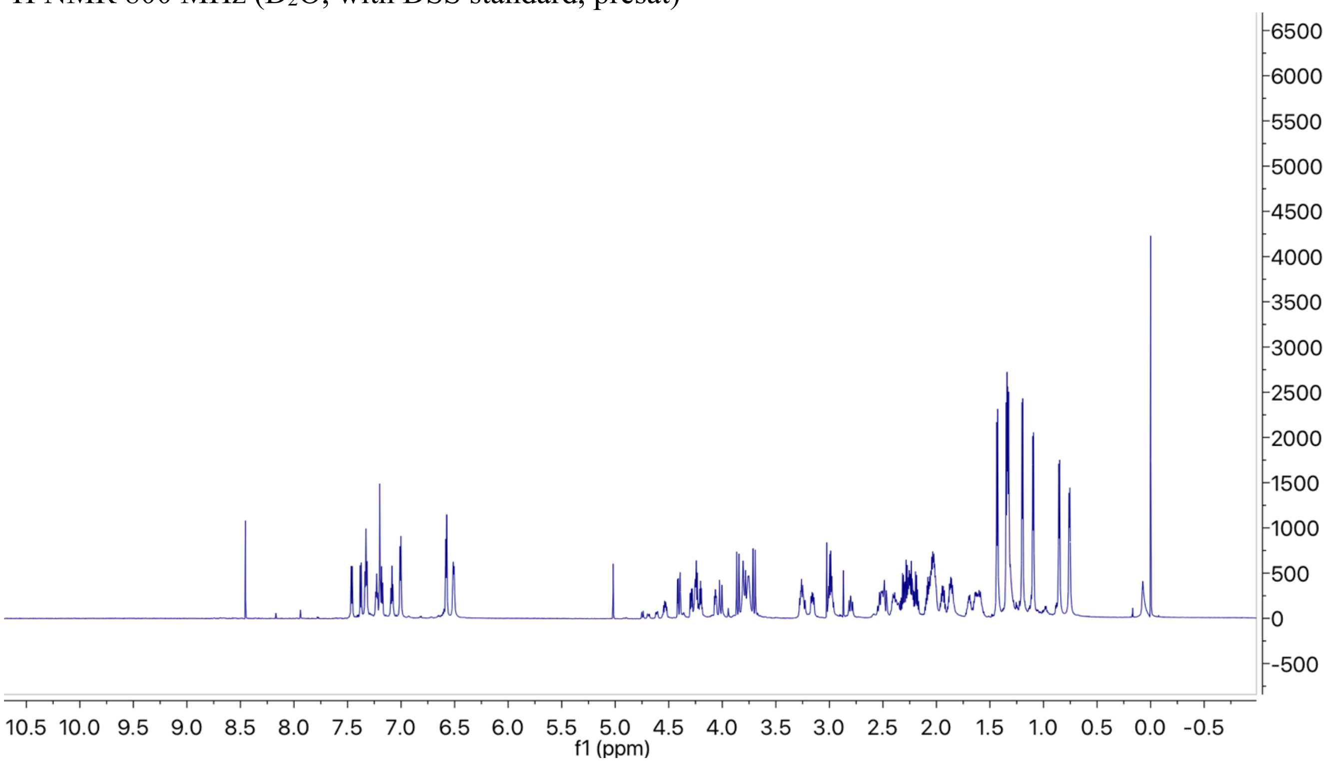


H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-hVal-Thr-Glu-NH_2 (11c)

RP-HPLC 5-40% MeCN/H_2O (modified with 0.1% TFA)

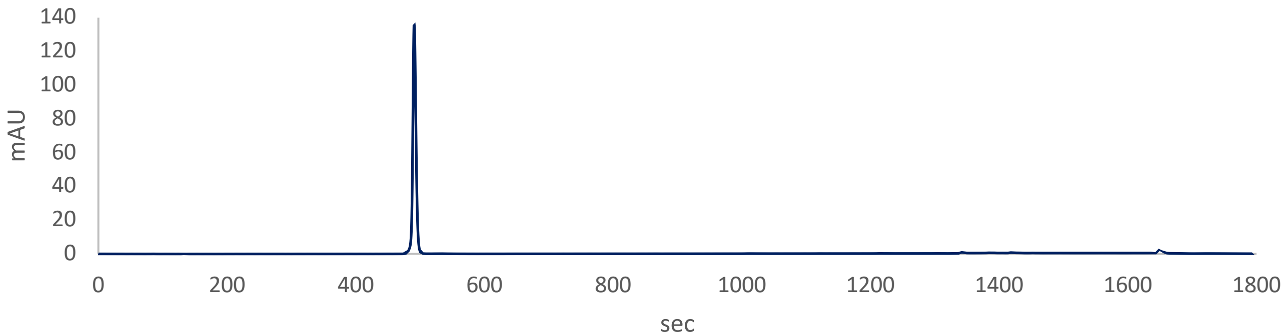


^{1}H NMR 800 MHz (D_2O, with DSS standard, presat)

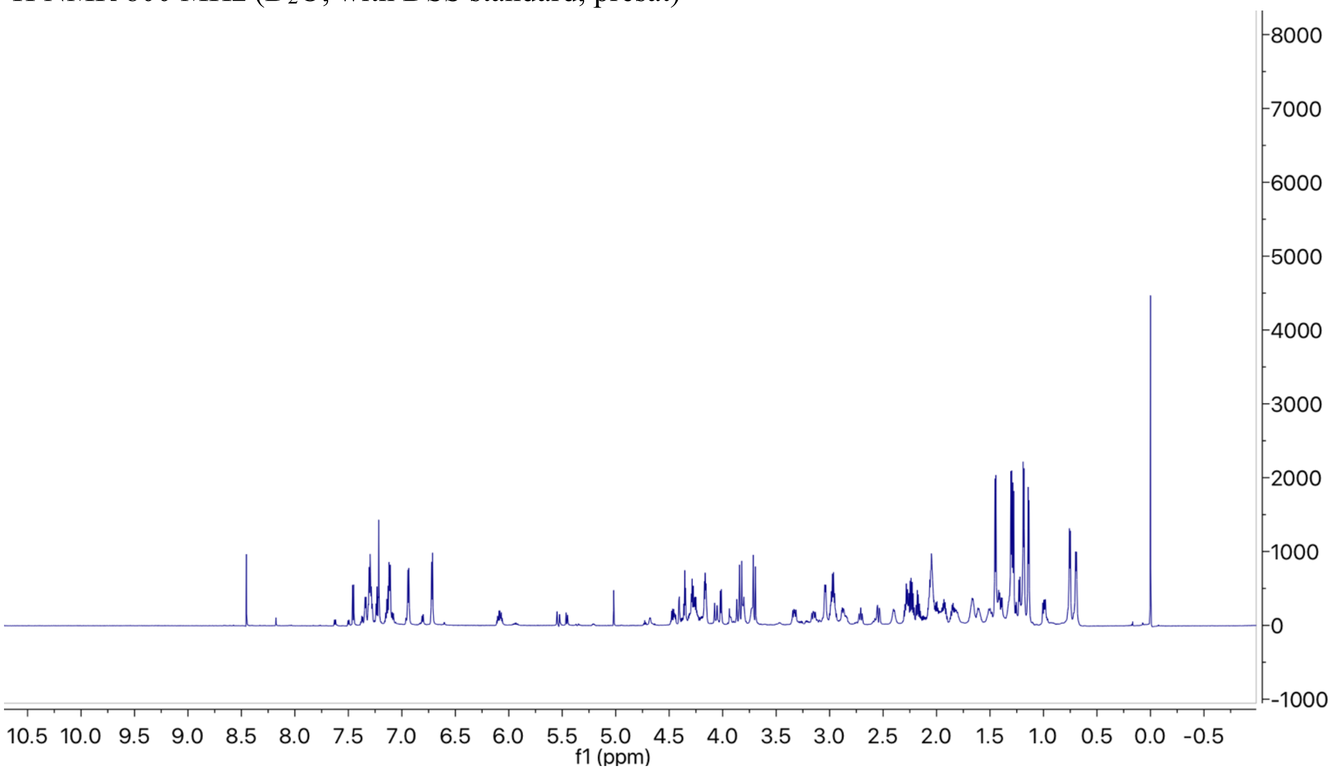


H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-(*O*-All)hPhe-Ala-Val-Thr-Glu-NH$_2$ (11d)

RP-HPLC 5-40% MeCN/H_2O (modified with 0.1% TFA)

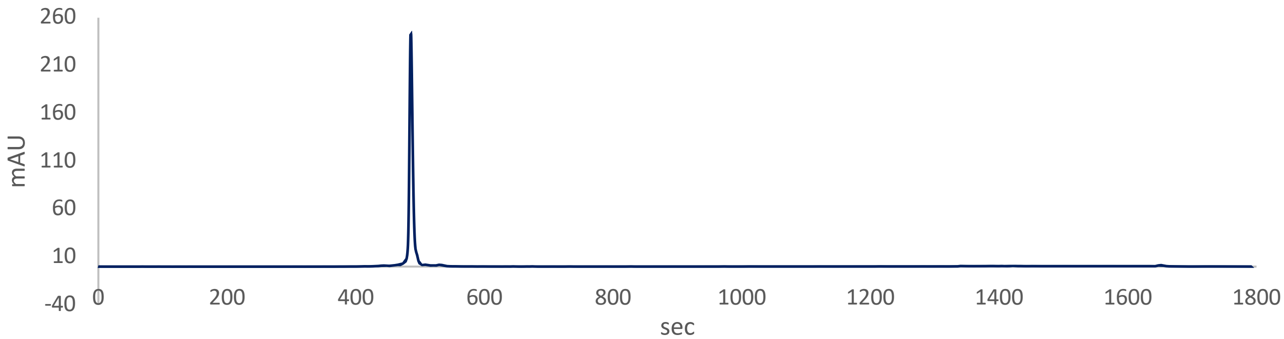


^{1}H NMR 800 MHz (D_2O, with DSS standard, presat)

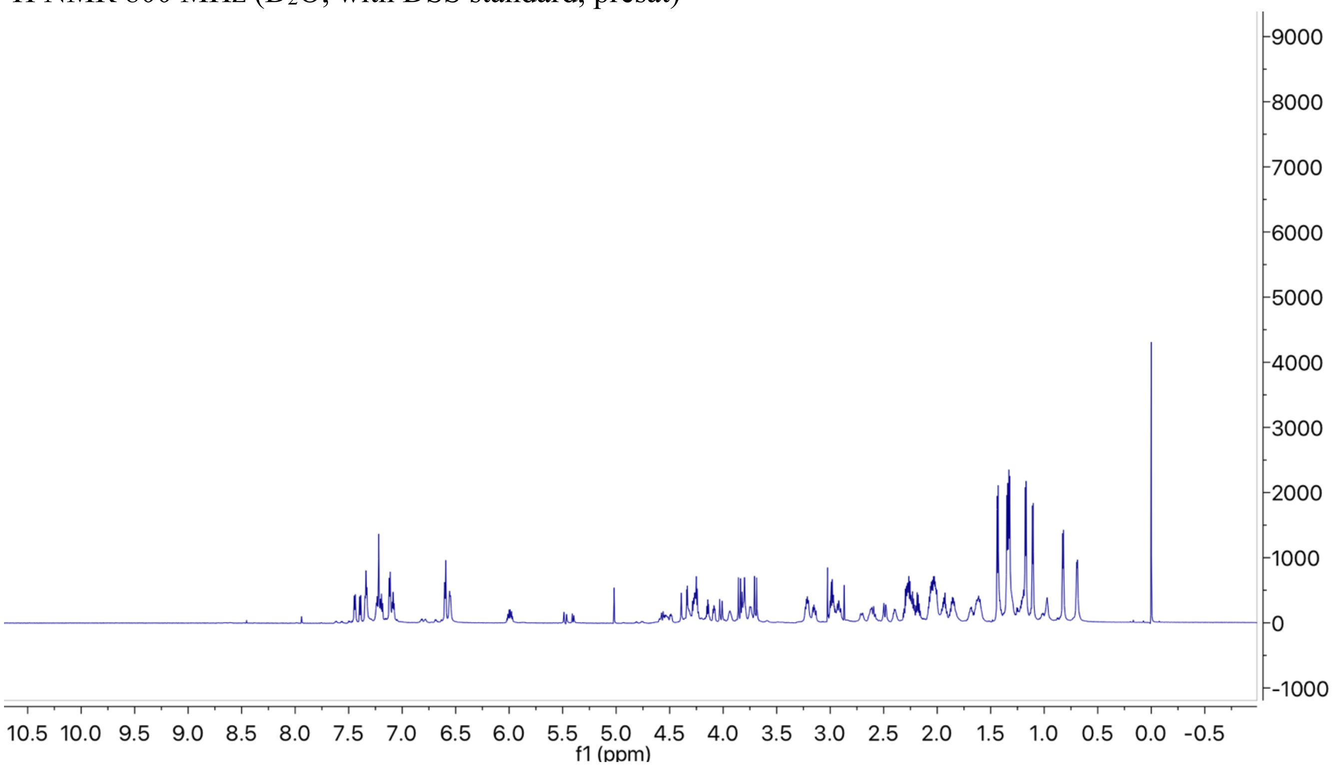


H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-Phe-Ala-(*O*-All)hVal-Thr-Glu-NH_2 (11e)

RP-HPLC 5-40% MeCN/H_2O (modified with 0.1% TFA)

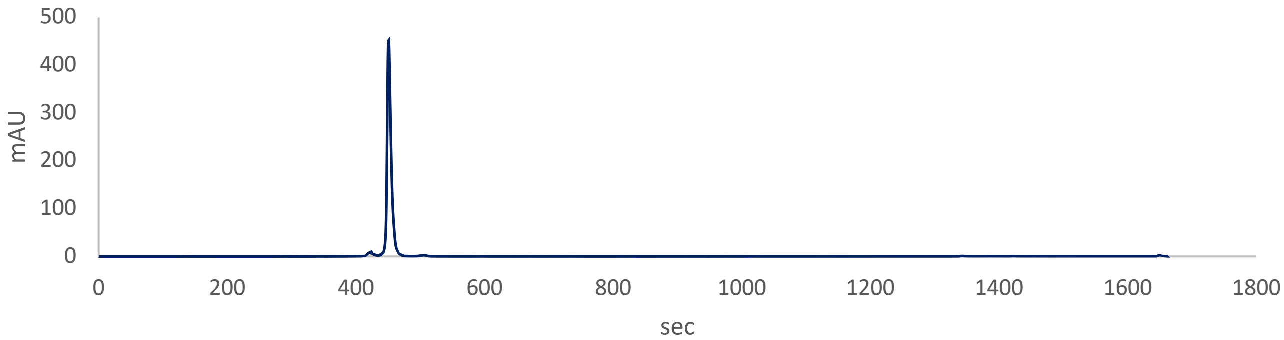


^{1}H NMR 800 MHz (D_2O, with DSS standard, presat)

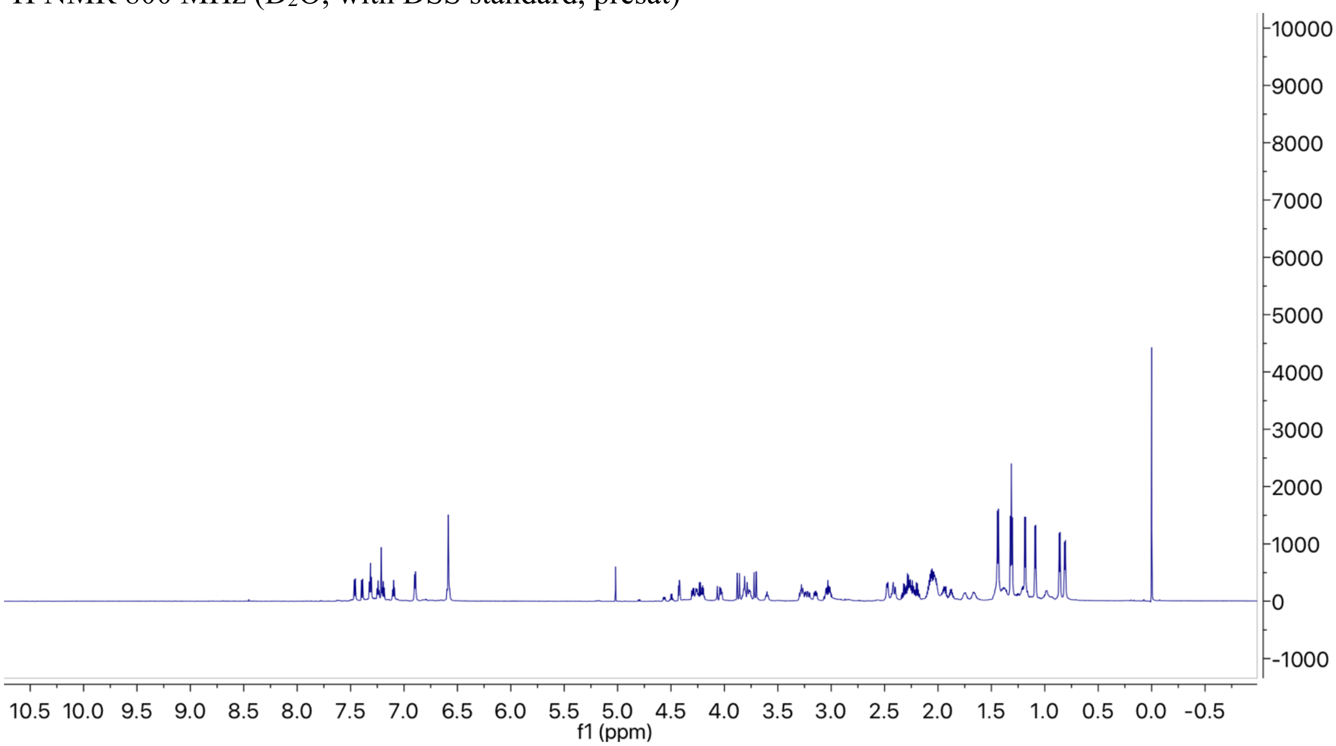


H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-hPhe-Ala-hVal-Thr-Glu-NH_2 (11f)

RP-HPLC 5-40% MeCN/H_2O (modified with 0.1% TFA)

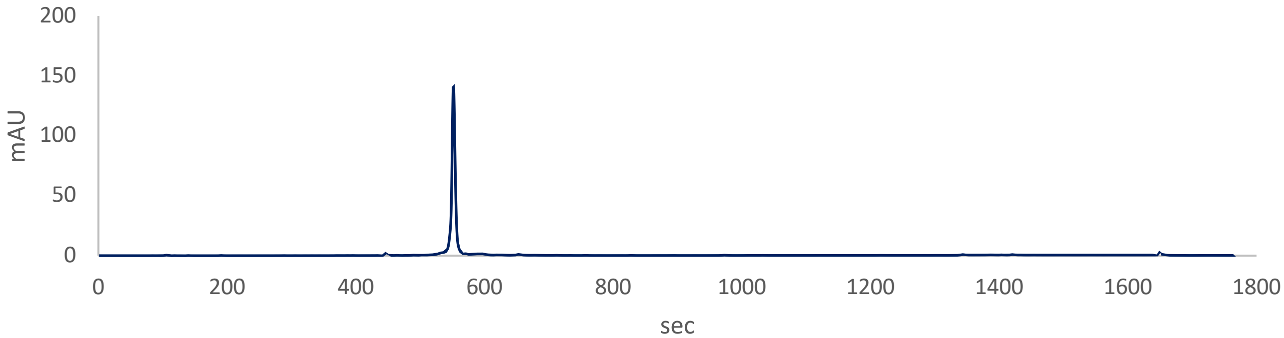


[1]H NMR 800 MHz (D_2O, with DSS standard, presat)

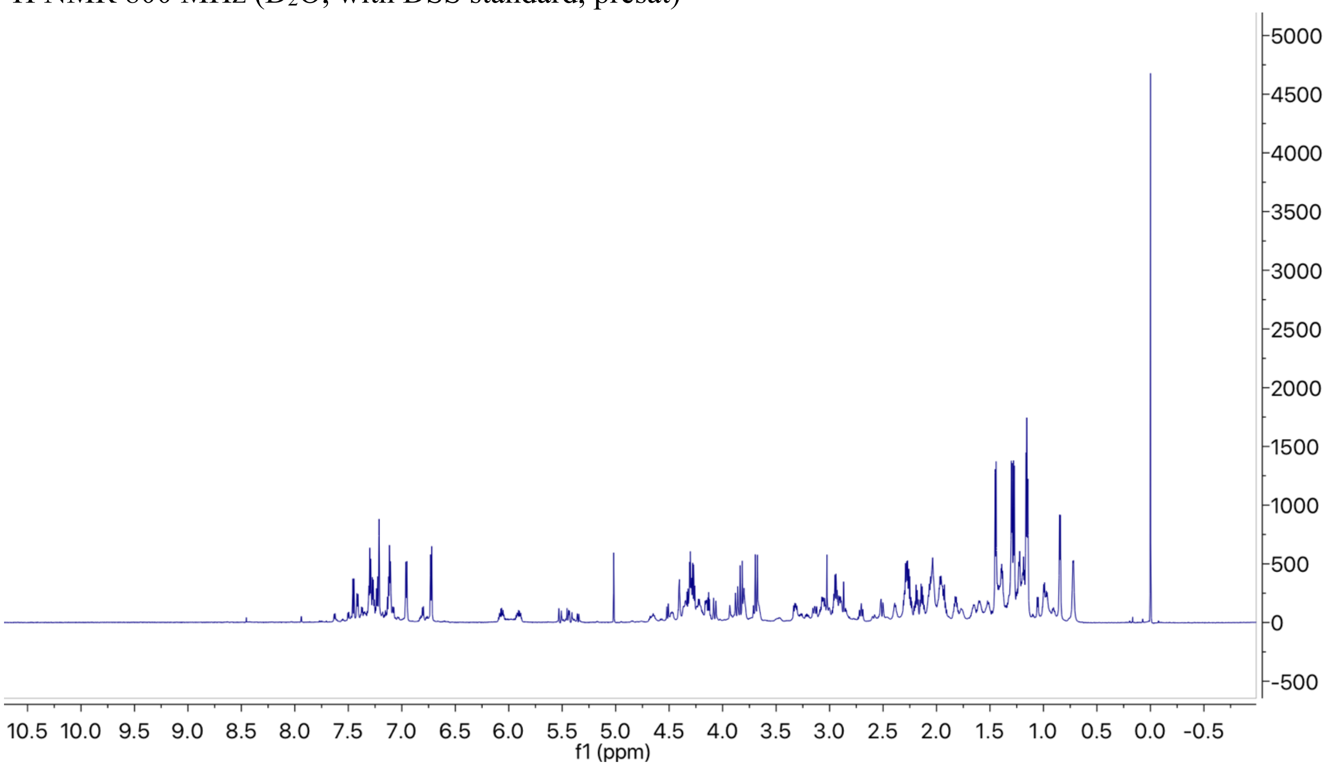


H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys-(*O*-All)hPhe-Ala-(*O*-All)hVal-Thr-Glu-NH_2 (11g)

RP-HPLC 5-40% MeCN/H_2O (modified with 0.1% TFA)

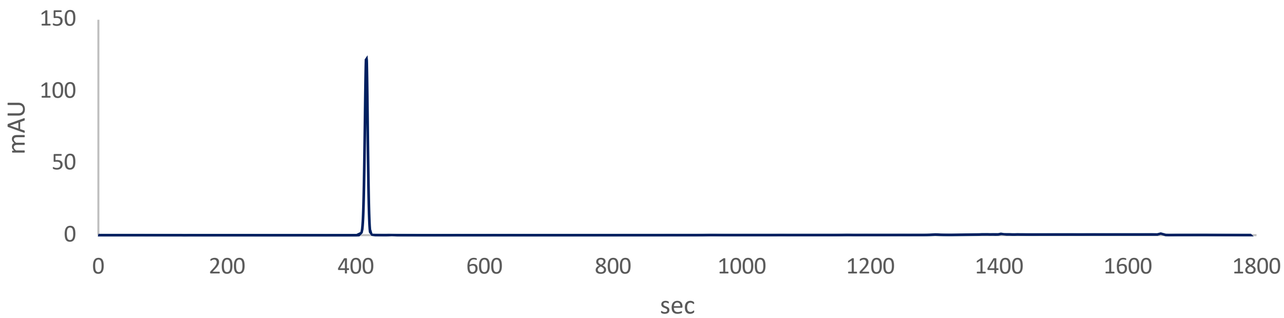


^1H NMR 800 MHz (D_2O, with DSS standard, presat)

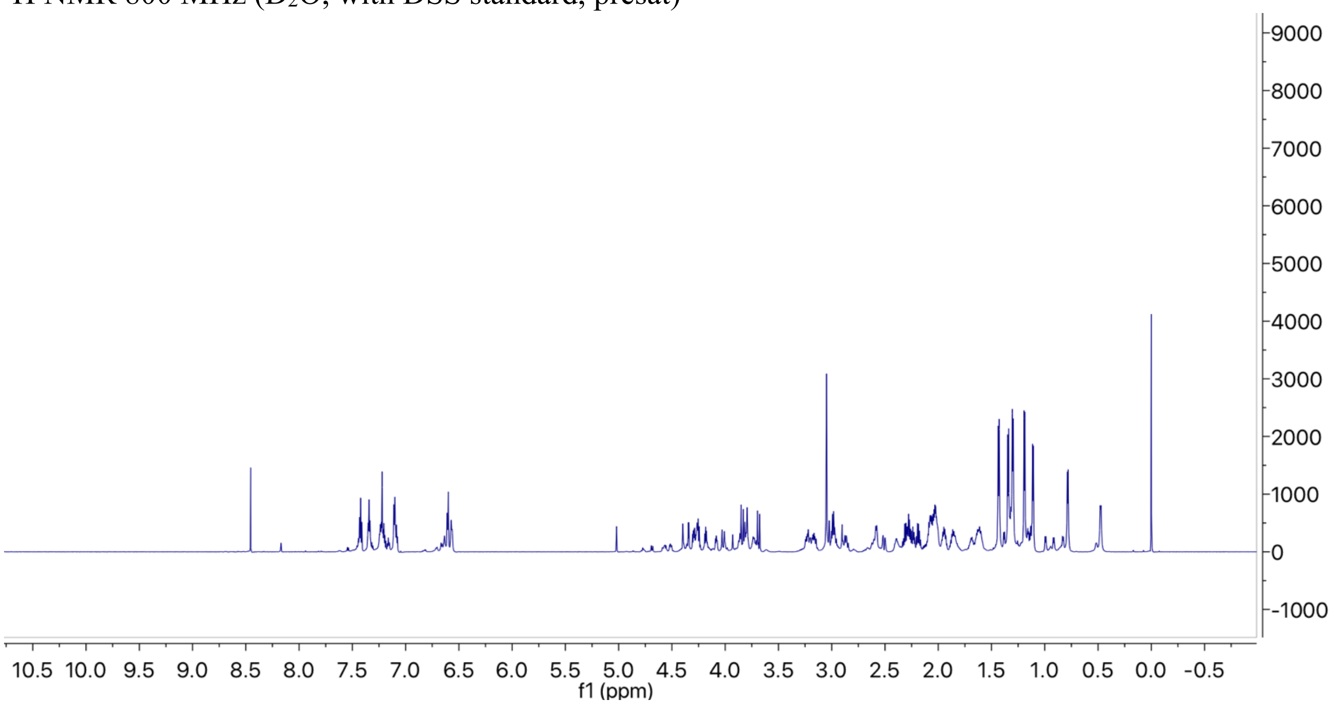


H-Gly-Glu-Trp-Ala-Tyr-Asn-Pro-Ala-Thr-Gly-Lys- Phe-Ala-(*N*-Me)Val-Thr-Glu-NH_2 (11l)

RP-HPLC 5-40% MeCN/H_2O (modified with 0.1% TFA)



[1]H NMR 800 MHz (D_2O, with DSS standard, presat)



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[2] Griffiths-Jones, S. R.; Maynard, A. J.; Searle, M. S. *J. Mol. Biol.* **1999**, *292*, 1051.

[3] Sarnowski, M. P.; Pedretty, K. P., Giddings, J.; Woodcock, H. Lee.; Del Valle, J. R. *Bioorg. Med. Chem.* **2018,** *26*, 1162