Cyclizing Pentapeptides: Mechanism and Application of Dehydrophenylalanine as a Traceless Turn-Inducer

Diane N. Le,[†] Jan Riedel,[†] Natalia Kozlyuk, Rachel W. Martin, Vy M. Dong*

Department of Chemistry, University of California, Irvine, California, 92697-2025, United States

*dongv@uci.edu

Supporting Information

Table of Contents

1. General considerations

Commercial reagents were purchased from Sigma Aldrich, Strem, Acros Organics, ChemImpex, TCI and/or Alfa Aesar and used without further purification. Reaction progresses were monitored using a combination of LC/MS analysis[1] and thin-layer chromatography (TLC) on EMD Silica Gel 60 F254 plates. Visualization of the developed plates was performed under UV light (254 nm) and with $KMnO_4$ stain. Organic solutions were concentrated under reduced pressure on a Büchi rotary evaporator. ^{1}H and ^{13}C spectra were recorded on a Bruker DRX400, Bruker DRX500, Bruker DRX500 with TCI (three channel inverse) cryoprobe or a Bruker AVANCE600 spectrometer. ^{1}H NMR spectra were internally referenced to the residual solvent signal (δ 7.26 for $CDCl_3$, δ 2.50 for DMSO, δ 8.74 for Pyr) or to TMS. ^{13}C NMR spectra were internally referenced to the residual solvent signal (δ 77.16 for $CDCl_3$, δ 39.52 for DMSO, δ 150.4 for Pyr). Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (Hz), integration. Data for ^{13}C NMR are reported in terms of chemical shift (δ ppm). High resolution mass spectra (HRMS) were obtained on a Waters LCT Premier spectrometer (using ESI-TOF). Infrared (IR) spectra were obtained on a Nicolet iS5 FT-IR spectrometer with an iD5 ATR, and are reported in terms of frequency of absorption (cm^{-1}). CD spectroscopy was performed on a Jasco J-810 spectropolarimeter. Column chromatography was performed with Silicycle Silia-P Flash Silica Gel, using either glass columns or a Teledyne Isco Combiflash Rf 200 automated purification system. Preparative reverse phase HPLC (RP-HPLC) was performed on a Beckman (Agilent Zorbax 80 SB C_{18} column, 50 x 4.6 mm; solvent A: H_2O/0.1% TFA, solvent B: CH_3CN/0.1% TFA). Solvents were purchased from Fisher Chemical and were purified according to standard procedures.[2]

[1] Waters 2795 Separations Module equipped with Symmetry® C18 column (3.5 μm particle size; 4.6 × 75 mm), Waters micromass ZQ mass spectrometer and Waters 2996 Photodiode Array Detector

[2] Armarego, W. L. F.; Chai, C. L. L. *Purification of Laboratory Chemicals*, 5th ed.; Butterworth-Heinemann: New York, 2003.

2. Typical procedures for synthesis of linear pentapeptides

i) Synthesis of Gly-Tyr-Ala-ΔPhe-Ala **3**

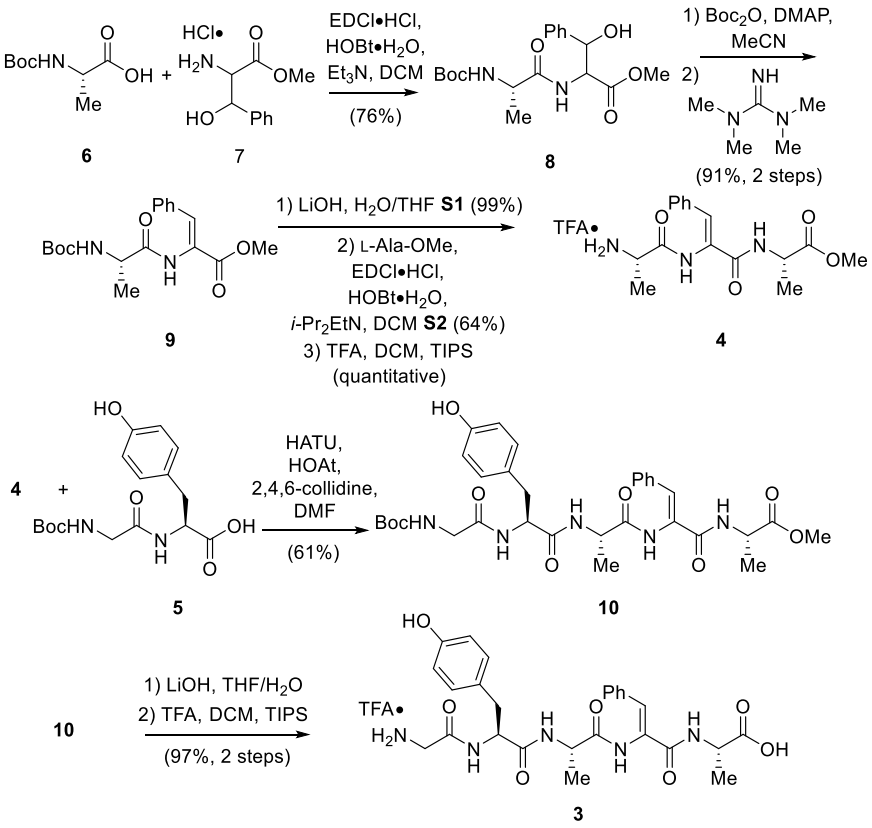


Figure S1. Synthesis of linear precursor **3**.

Representative peptide coupling with EDCI (Method A): To a round bottom flask equipped with a stir bar was added Boc-L-alanine **6** (5.00 g, 26.4 mmol), DL-(β-OH)–Phe–OMe **7** (6.13 g, 26.4 mmol), HOBt·H_2O (4.29 g, 31.7 mmol), and DCM (100 mL). The mixture was cooled to 0 °C and Et_3N (9.16 mL, 66.1 mmol) was subsequently added. EDCI·HCl (6.08 g, 31.7 mmol) was added in portions and the reaction gradually warmed to rt and stirred for 22 h. The reaction mixture was transferred to a separatory funnel and was washed with 100 mL sat. $NaHCO_3$ (aq), 100 mL 10% $KHSO_4$ (aq), and 100 mL brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The unpurified reaction mixture was then purified by column chromatography (eluting with 20:1 DCM/MeOH) to afford dipeptide **8** as a white solid (7.4 g, 76%).

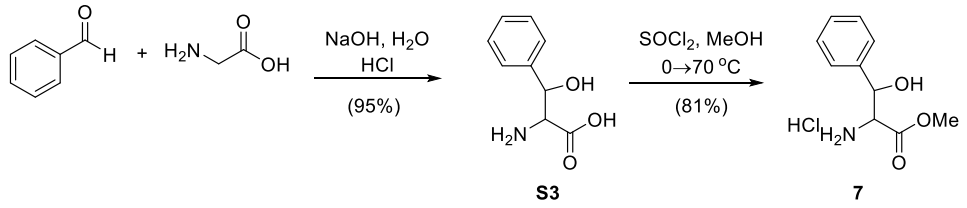
Representative elimination to form dehydroamino acid (Method B): The procedure was adapted from Suárez.[3] To a round bottom flask equipped with a stir bar was added dipeptide **8** (7.40 g, 20.2 mmol), DMAP (244 mg, 2.00 mmol) and MeCN (60 mL). The mixture was cooled to 0 °C and Boc_2O (4.63 g, 21.2 mmol) was quickly added. After disappearance of starting material analyzed via LC-MS, tetramethylguanidine (0.77 mL, 6.1 mmol) was added. After 12 h, the reaction mixture was concentrated under reduced pressure and then purified by column chromatography (eluting with 20:1 DCM/MeOH) to afford the unsaturated dipeptide **9** as a white solid (6.4 g, 91%, 2 steps).

Representative hydrolysis procedure (Method C): To a round bottom flask equipped with a stir bar was added methyl ester **9** (6.40 g, 18.4 mmol), THF (90 mL), and H_2O (90 mL). The mixture was cooled to 0 °C and 1M LiOH (aq) (19 mL, 19 mmol) was subsequently added. The reaction gradually warmed to rt and stirred for 14 h. The reaction mixture was acidified with 10% $KHSO_4$ (aq), and the THF was concentrated under reduced pressure. The reaction mixture was transferred to a separatory funnel where it was extracted with ethyl acetate (3 x 100 mL). The organic layer was washed with 100 mL brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford carboxylic acid **S1** as a colorless oil (6.1 g, 99%).

Representative Boc deprotection (Method D): To a round bottom flask equipped with a stir bar was added Boc-protected amine **S2** (638 mg, 1.52 mmol), triisopropylsilane (0.33 mL, 1.6 mmol), and DCM (15 mL). The reaction mixture was cooled to 0 °C and TFA (1.17 mL, 15.2 mmol) was slowly added. The reaction slowly warmed to rt and stirred for 24 h. The DCM was concentrated under reduced pressure and to the mixture was added toluene to form a TFA azeotrope, which was subsequently concentrated under reduced pressure. The reaction mixture was further dried on the high vacuum and subsequently triturated with Et_2O to afford amine **4** in quantitative yield which was used without further purification.

[3] Monteiro, L. S.; Andrade, J. J.; Suárez, A. C. *Eur. J. Org. Chem.* **2011**, *2011*, 6764.

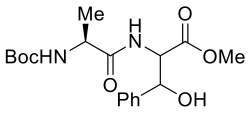
Preparation of DL-(β-OH)–Phe–OMe 7 (Method E):



To a stirring solution of NaOH (60 g, 1.5 mol) in water (250 mL) at rt was added glycine (75 g, 1 mol). The solution was stirred for 10 min and benzaldehyde (215 mL, 2.1 mol) was added and the solution was stirred for 20 min. The solution became a beige emulsion and the solid was broken apart. Concentrated HCl (aq) (130 mL) was added slowly and the mixture stirred until the beige solid was consumed to give a clear yellow solution. Shortly, beige precipitates were observed and the mixture was cooled to 0 °C. The beige solid was collected via vacuum filtration and the solid was washed with Et_2O. The solid was dried *in vacuo* to give **S3** an off-white solid (172 g, 95%). Characterization data was consistent with those previously reported.[4]

To a round bottom flask equipped with a stir bar was added **S3** (25 g, 138 mmol) and anhydrous MeOH (190 mL) under N_2 and the mixture was cooled to 0 °C. Thionyl chloride (22.5 mL, 310 mmol) was subsequently added and the reaction stirred overnight for 16 h at 70 °C. The reaction mixture was concentrated under reduced pressure, redissolved in DCM, and subsequently concentrated again (2x). The resulting white solid was washed with Et_2O to afford DL-(β-OH)–Phe–OMe **7** was an off-white solid (26.0 g, 81%). Characterization data was consistent with those previously reported.[5]

Methyl 2-((*S*)-2-((tert-butoxycarbonyl)amino)propanamido)-3-hydroxy-3-phenylpropanoate (8)

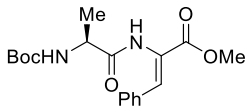


The product was prepared by method A using Boc alanine **6** (5.00 g, 26.4 mmol) and purified by column chromatography (eluting with 90:10 DCM/MeOH) to afford a white solid (7.4 g, 61%, 1:1 dr). ^{1}H NMR (499 MHz, DMSO) δ 7.89 (d, *J* = 9.1 Hz, 1H), 7.76 (d, *J* = 8.9 Hz, 1H), 7.45 – 7.32 (m, 4H), 7.31 – 7.24 (m, 4H), 7.25 – 7.19 (m, 2H), 7.00 (d, *J* = 8.0 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 5.92 (d, *J* =

[4] Shiraiwa, T.; Saijoh, R.; Suzuki, M.; Yoshida, K.; Nishimura, S.; Nagasawa, H. *Chem. Pharm. Bull.* **2003**, *51*, 1363.
[5] Miyata, O.; Asai, H.; Naito, T. *Chem. Pharm. Bull*. **2005**, *53*, 355.

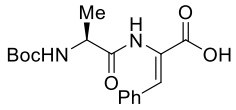
4.8 Hz, 2H), 5.14 (dd, *J* = 4.9, 3.2 Hz, 1H), 5.12 – 5.06 (m, 1H), 4.55 (ddd, *J* = 12.7, 9.0, 3.2 Hz, 2H), 4.10 – 3.84 (m, 2H), 3.65 (s, 3H), 3.61 (s, 3H), 1.39 (s, 9H), 1.38 (s, 9H), 1.02 (d, *J* = 7.2 Hz, 3H), 0.94 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.96, 172.91, 170.61, 154.88, 154.82, 141.57, 141.47, 127.76, 127.73, 127.25, 127.16, 126.38, 126.14, 109.52, 78.15, 77.97, 72.22, 72.11, 58.05, 52.02, 51.99, 49.78, 49.29, 28.21, 18.30, 17.96. IR (ATR): 3337, 3013, 2978, 1660, 1498, 1365, 1168 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{18}H_{26}N_2O_6Na$ $[M+Na]^+$: 389.1689, found: 389.1687.

Methyl (*S*,*Z*)-2-(2-((tert-butoxycarbonyl)amino)propanamido)-3-phenylacrylate (9)



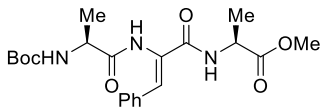
The product was prepared by method B using methyl ester **8** (7.40 g, 20.2 mmol) and purified by column chromatography (eluting with 93:7 DCM/MeOH) to afford the product as a white solid (6.4 g, 91%, 2 steps). ^{1}H NMR (400 MHz, DMSO) δ 9.56 (s, 1H), 7.77 – 7.64 (m, 2H), 7.43 – 7.30 (m, 3H), 7.26 (s, 1H), 7.03 (d, *J* = 7.2 Hz, 1H), 4.21 – 4.05 (m, 1H), 3.70 (d, *J* = 1.4 Hz, 3H), 1.41 (s, 9H), 1.26 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.13, 165.41, 155.23, 133.31, 132.11, 130.23, 129.47, 128.49, 125.92, 78.05, 52.16, 49.82, 28.24, 17.40. IR (ATR): 3291, 3005, 2979, 1673, 1490, 1249, 1162 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{18}H_{24}N_2O_5Na$ $[M+Na]^+$: 371.1583, found: 371.1588. $[\alpha]^{24}_D$ +66 (*c* = 0.46, MeOH).

(*S*,*Z*)-2-(2-((tert-butoxycarbonyl)amino)propanamido)-3-phenylacrylic acid (S1)



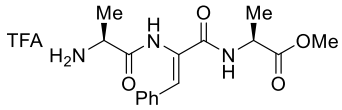
The product was prepared by method C using unsaturated dipeptide **9** (6.40 g, 18.4 mmol) and obtained as a white solid (6.0 g, 99%). ^{1}H NMR (500 MHz, DMSO) δ 12.68 (s, 1H), 9.39 (s, 1H), 7.76 – 7.55 (m, 2H), 7.42 – 7.32 (m, 3H), 7.28 (s, 1H), 7.02 (d, *J* = 7.3 Hz, 1H), 4.21 – 4.05 (m, 1H), 1.40 (s, 9H), 1.25 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.66, 166.30, 155.21, 133.68, 131.83, 130.14, 129.19, 128.39, 126.52, 78.03, 49.83, 28.25, 17.52. IR (ATR): 3281, 2980, 1685, 1539, 1162 cm^{-1} HRMS (ESI-TOF) *m/z* calc'd for $C_{17}H_{22}N_2O_5Na$ $[M+Na]^+$: 357.1426, found: 357.1419. $[\alpha]^{25}_D$ +70 (*c* = 0.25, MeOH).

Methyl ((*Z*)-2-((*S*)-2-((tert-butoxycarbonyl)amino)propanamido)-3-phenylacryloyl)-L-alaninate (S2)



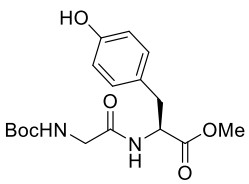
The product was prepared by method A using carboxylic acid **S1** (3.80 g, 11.4 mmol), L-alanine methyl ester (1.60 g, 11.4 mmol), and *i*-Pr_2EtN (7.90 mL, 45.6 mmol) and purified by column chromatography (eluting with 85:15 DCM/Acetone) to afford the product as a white solid (3.0 g, 64%). ^{1}H NMR (499 MHz, DMSO) δ 9.57 (s, 1H), 7.93 (d, *J* = 6.8 Hz, 1H), 7.65 – 7.49 (m, 2H), 7.42 – 7.31 (m, 3H), 7.25 (s, 1H), 7.21 (m, 1H), 4.44 – 4.34 (m, 1H), 4.08 – 3.99 (m, 1H), 3.64 (s, 3H), 1.39 (s, 9H), 1.34 (d, *J* = 7.2 Hz, 3H), 1.23 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.75, 172.71, 164.34, 155.71, 133.83, 130.04, 129.58, 128.84, 128.47, 128.39, 78.35, 51.86, 50.06, 48.23, 28.19, 16.93, 16.71. IR (ATR): 3291, 3017, 2981, 1751, 1685, 1530, 1163 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{21}H_{29}N_3O_6Na$ $[M+Na]^+$: 442.1954, found: 442.1946. $[\alpha]^{25}_D$ –41 (*c* = 0.25, MeOH).

Methyl ((Z)-2-((S)-2-aminopropanamido)-3-phenylacryloyl)-L-alaninate (4)



The product was prepared by method D using tripeptide **S2** (5.60 g, 13.3 mmol) and obtained as a white solid in quantitative yield. ^{1}H NMR (499 MHz, DMSO) δ 9.95 (s, 1H), 8.46 (d, *J* = 7.1 Hz, 1H), 8.22 – 8.14 (m, 2H), 7.60 – 7.52 (m, 2H), 7.49 – 7.39 (m, 2H), 7.38 – 7.33 (m, 1H), 7.16 (s, 1H), 4.41 (p, *J* = 7.2 Hz, 1H), 4.11 – 4.01 (m, 1H), 3.65 (s, 3H), 1.44 (d, *J* = 7.0 Hz, 3H), 1.35 (d, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.95, 169.08, 164.31, 133.67, 129.40, 129.08, 128.91, 128.55, 128.34, 51.91, 48.47, 48.12, 16.87, 16.42. IR (ATR): 2996, 1740, 1660, 1519, 1137 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{16}H_{21}N_3O_4H$ $[M+H]^+$: 320.1610, found: 320.1620. $[\alpha]^{26}_D$ +90 (*c* = 0.31, MeOH).

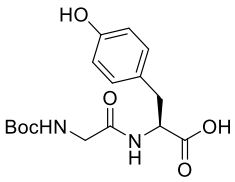
Methyl (tert-butoxycarbonyl)glycyl-L-tyrosinate (S3)



Dipeptide **S3** was prepared according to method A using Boc-Gly-OH (7.10 g, 40.6 mmol), and L-Tyrosine methyl ester (7.20 g, 36.9 mmol) and purified by column chromatography to afford the product as a white solid (11.8 g, 82% yield). The characterization data was in agreement

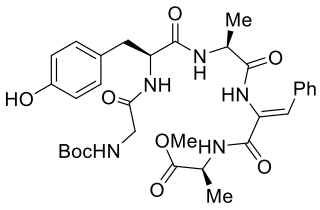
with literature.[6]

(tert-butoxycarbonyl)glycyl-L-tyrosine (5)



Dipeptide carboxylic acid **5** was prepared according to method C using methyl ester **S3** (11.8 g, 33.4 mmol) to afford the product as a white solid (10.9 g, 96%). The characterization data was in agreement with literature.[6]

Methyl ((*Z*)-2-((*S*)-2-((*S*)-2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-(4-hydroxyphenyl)propanamido)propanamido)-3-phenylacryloyl)-L-alaninate (10)

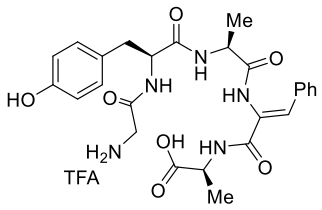


To a round botton flask equipped with a stir bar was added carboxylic acid **5** (4.10 g, 12.2 mmol), amine **4** (4.80 g, 11.1 mmol), HATU[7] (5.00 g, 13.3 mmol), HOAt (0.452 g, 3.32 mmol) and DMF (42 mL). The reaction mixture was cooled to 0 °C and 2,4,6-collidine (3.70 mL, 27.7 mmol) was added. The reaction warmed to rt and stirred for 24 h. The solvent was removed under reduced pressure and the product was purified by column chromatography (eluting with 90:10 DCM/MeOH, increasing in 0.5% increments) to afford the product as a white solid (4.32 g, 61%). ^{1}H NMR (500 MHz, DMSO) δ 9.66 (s, 1H), 9.16 (s, 1H), 8.47 (d, *J* = 5.1 Hz, 1H), 8.00 (d, *J* = 6.3 Hz, 1H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.61 – 7.53 (m, 2H), 7.44 – 7.37 (m, 2H), 7.38 – 7.32 (m, 1H), 7.27 (s, 1H), 6.98 (d, *J* = 8.2 Hz, 2H), 6.94 (t, *J* = 6.2 Hz, 1H), 6.60 (d, *J* = 8.4 Hz, 2H), 4.48 (td, *J* = 8.4, 4.3 Hz, 1H), 4.40 – 4.18 (m, 2H), 3.64 (s, 3H), 3.54 (dd, *J* = 16.8, 6.1 Hz, 1H), 3.43 (dd, *J* = 16.8, 6.0 Hz, 1H), 2.88 (dd, *J* = 14.1, 4.3 Hz, 1H), 2.67 (dd, *J* = 14.1, 8.6 Hz, 1H), 1.36 (s, 9H), 1.31 (d, *J* = 7.1 Hz, 3H), 1.26 (d, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.10, 172.16, 171.74, 169.06, 164.27, 155.84, 155.80, 133.82, 130.25, 130.22, 129.58, 128.94, 128.51, 128.40, 127.33, 114.84, 78.15, 53.39, 51.86, 49.24, 48.52, 43.26, 36.74, 28.17, 16.66, 16.49. IR (ATR): 3305, 2974, 1656, 1514, 1160 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{32}H_{41}N_5O_9Na$ $[M+Na]^+$: 662.2802, found: 662.2817. $[\alpha]^{26}_D$ – 82 (*c* = 0.25, MeOH).

[6] Naskar, J.; Drew, M. G. B.; Deb, I.; Das, S.; Banerjee, A. *Org. Lett*. **2008**, *10*, 2625.

[7] Other uronium salts such as HBTU have previously been used to couple unprotected Boc-Tyr-OH with H-Val-OMe, see: Mak, C. C.; Brik, A.; Lerner, D. L.; Elder, J. H.; Morris, G. M.; Olson, A. J.; Wong, C.-H. *Bioorg. Med. Chem*. **2003**, *11*, 2025.

((*Z*)-2-((*S*)-2-((*S*)-2-(2-aminoacetamido)-3-(4-hydroxyphenyl)propanamido)propanamido)-3-phenylacryloyl)-L-alanine (3)

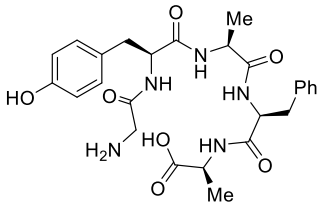


To a round bottom flask equipped with a stir bar was added methyl ester **10** (4.20 g, 6.60 mmol), THF (30 mL), and H_2O (30 mL). The mixture was cooled to 0 °C and 1M LiOH (aq) (7.26 mL, 7.26 mmol) was subsequently added. The reaction gradually warmed to rt and stirred for 48 h. The reaction mixture was acidified with 10% $KHSO_4$ (aq) and the THF was concentrated under reduced pressure. The reaction mixture was transferred to a separatory funnel where it was extracted with ethyl acetate (3 x 100 mL). The organic layer was washed with 100 mL brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford carboxylic acid **S4** which was used in the next step without further purification.

To a round bottom flask equipped with a stir bar was added carboxylic acid **S4** (6.60 mmol), and DCM (60 mL). The reaction mixture was cooled to 0 °C and TFA (5.1 mL, 66 mmol) was slowly added. The reaction slowly warmed to rt and stirred for 14 h. The DCM was concentrated under reduced pressure and to the mixture was added toluene to form a TFA azeotrope, which was subsequently concentrated under reduced pressure. The reaction mixture was further dried on the high vacuum and subsequently triturated with Et_2O to afford unsaturated peptide **3** (4.0 g, 97% 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 9.62 (s, 1H), 9.22 (s, 1H), 8.56 (d, *J* = 5.7 Hz, 1H, N–$H_{Ala(term)}$), 8.52 (d, *J* = 8.4 Hz, 1H, N–H_{Tyr}), 7.91 (d, *J* = 6.9 Hz, 3H, N–$H_{Ala(int)+Gly}$), 7.57 (d, *J* = 7.7 Hz, 2H), 7.42 – 7.30 (m, 3H), 7.22 (s, 1H), 7.04 (d, *J* = 8.1 Hz, 2H), 6.64 (d, *J* = 8.0 Hz, 2H), 4.56 (td, *J* = 9.2, 3.7 Hz, 1H), 4.39 – 4.22 (m, 2H), 3.55 (d, *J* = 16.2 Hz, 1H), 3.43 (d, *J* = 16.2 Hz, 1H), 2.93 (dd, *J* = 14.2, 3.8 Hz, 1H), 2.59 (dd, *J* = 14.1, 10.1 Hz, 1H), 1.36 (d, *J* = 7.3 Hz, 3H), 1.31 (d, *J* = 7.1 Hz, 3H).^{13}C NMR (126 MHz, DMSO) δ 174.22, 172.30, 171.59, 165.91, 164.30, 156.09, 134.02, 130.22, 129.90, 129.71, 129.02, 128.81, 128.64, 127.69, 115.13, 54.36, 49.29, 48.38, 40.19, 36.94, 17.25, 16.84. IR (ATR): 3270, 1656, 1515, 1172 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{26}H_{31}N_5O_7H$ $[M+H]^+$: 526.2302, found: 526.2303. $[\alpha]^{26}_D$ –20 (*c* = 0.23, MeOH).

*ii) Synthesis of Gly-Tyr-Ala-Phe-Ala **11***

Glycyl-L-tyrosyl-L-alanyl-L-phenylalanyl-L-alanine (11)



Linear pentapeptide **11** was manually synthesized via Fmoc solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride (CTC) resin (substitution 1.1 mmol/g). The resin (0.360 g, 0.400 mmol) was swelled in anhydrous DCM (20 mL) for 45 min. The beads were washed once with DMF (peptide grade). DMF (10 mL), Fmoc-Ala-OH (500 mg, 1.60 mmol) and *i*Pr_2EtN (2.5 mL) were added and allowed to mix under N_2 for 15 minutes. The coupling with Fmoc-Ala-OH was repeated again. After the double coupling, the beads were washed with DMF and the Fmoc group was deprotected with 20% 4-methylpiperidine in DMF (20 mL) for 15 minutes. This deprotection was repeated again. The resin was washed three times with DMF (15 mL) and a Kaiser test was performed to determine presence of deprotected amine. The beads were washed once with *N*-methyl-2-pyrrolidone (15 mL). To begin the next coupling, NMP (10 mL), Fmoc-Phe-OH (620 mg, 1.6 mmol), HBTU (610 mg, 1.6 mmol), and *i*Pr_2EtN (2.5 mL) were added and the resin bubbled under N_2 for 30 min. The completion of the coupling was monitored by the Kaiser test. If incomplete, the coupling step was repeated again. The resin was washed three times with DMF (15 mL). The deprotection of the Fmoc group was repeated as above two times. To elongate the peptide, the coupling and deprotection steps were performed with Fmoc-Ala-OH, Fmoc-Tyr-OH, and Fmoc-Gly-OH. After the last amino acid was deprotected, the beads were washed three times with DMF. To the resin was added 40 mL cleavage solution (95% TFA, 2.5% H_2O, 2.5% TIPS), and the resin bubbled under N_2 for 3 h. A new receiving flask was replaced on the peptide synthesizer and the TFA was drained into the new flask. The TFA solution was separated into 4 conical vials and cold ether (–20 °C) was added to precipitate the peptide. The vials were centrifuged (3000 rpm, 0–4 °C) for 20 minutes. The remaining TFA and ether solution was decanted from the conical vials and the peptide precipitate was concentrated. The peptide was dissolved in 18 mL (10% MeCN/H_2O), filtered through a 0.20 micron filter, and purified by RP-HPLC on a C_{18} column (eluting with MeCN and H_2O containing 0.1% TFA, linear gradient 10–35% MeCN over 30 min). The pure fractions were combined, concentrated under reduced pressure, and then lyophilized to afford the product as a white powder (136 mg, 64%). ^{1}H NMR (400 MHz, DMSO) δ 9.19 (s, 1H), 8.51 (d, *J* = 8.4 Hz, 1H, N–H_{Tyr}), 8.29 (d, *J* = 2.3 Hz, 1H N–

$H_{Ala(int)}$), 8.27 (d, *J* = 2.2 Hz, 1H, N–$H_{Ala(term)}$), 7.86 (d, *J* = 8.2 Hz, 1H, N–H_{Phe}), 7.28 – 7.12 (m, 5H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.63 (d, *J* = 8.5 Hz, 2H), 4.52 (td, *J* = 8.8, 4.4 Hz, 2H), 4.31 – 4.09 (m, 2H), 3.50 (d, *J* = 16.3 Hz, 1H), 3.36 (d, *J* = 16.2 Hz, 2H), 3.05 (dd, *J* = 14.0, 4.2 Hz, 1H), 2.83 (ddd, *J* = 16.4, 12.6, 6.2 Hz, 2H), 2.56 (dd, *J* = 14.1, 10.3 Hz, 1H), 1.28 (d, *J* = 7.3 Hz, 3H), 1.16 (d, *J* = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.03, 171.78, 170.58, 165.72, 157.78, 155.87, 137.62, 130.13, 129.32, 128.00, 127.59, 126.24, 114.93, 54.16, 53.40, 48.26, 47.59, 40.08, 37.44, 37.04, 18.23, 17.29. IR (ATR): 3284, 3040, 2928, 1676, 1654, 1636, 1500 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{26}H_{33}N_5O_7Na$ $[M+Na]^+$: 550.2278 found: 550.2266. $[\alpha]^{27}_D$ – 35 (*c* = 0.26, MeOH).

iii) Synthesis of Gly-ΔTyr-Ala-ΔPhe-Ala ***12***

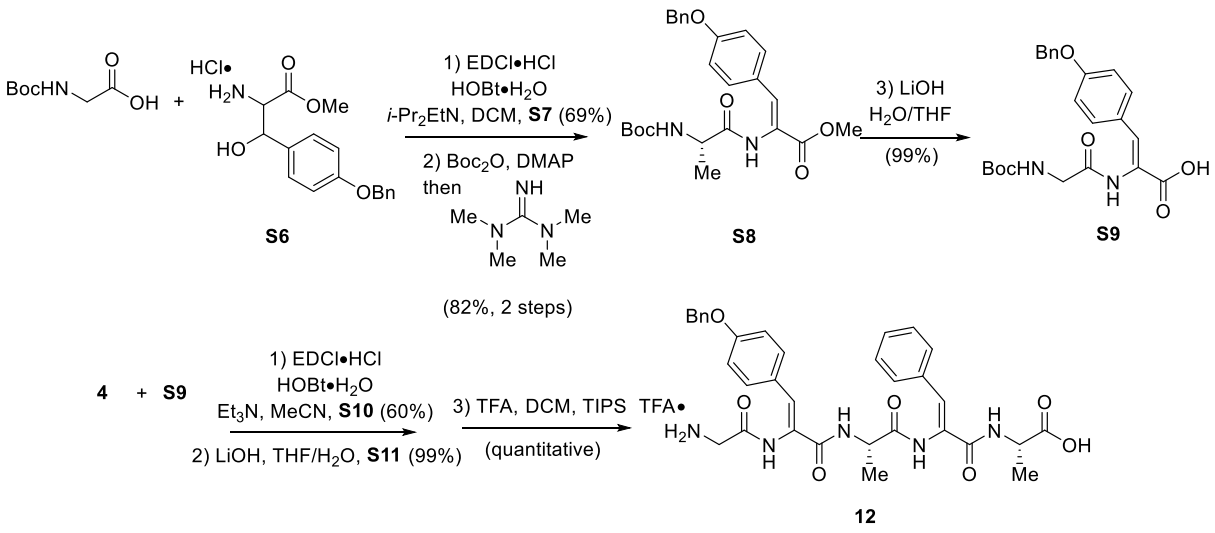
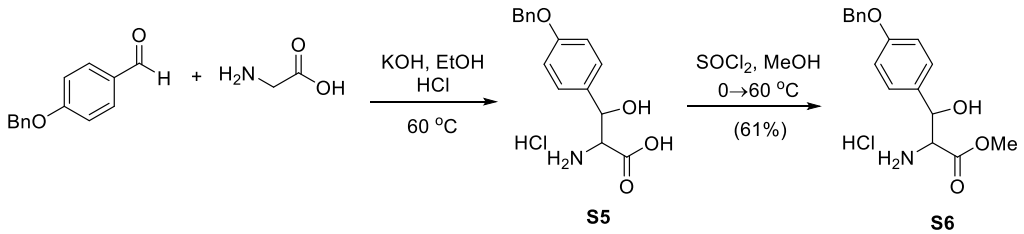
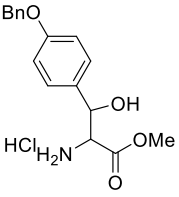


Figure S2. Synthesis of linear precursor **12**.



Methyl 2-amino-3-(4-(benzyloxy)phenyl)-3-hydroxypropanoate hydrochloride (S6)

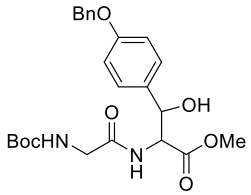


To a round bottom flask equipped with a stir bar was added glycine (3.0 g, 40 mmol), KOH (4.5 g, 80 mmol), and EtOH (100 mL). The reaction mixture

stirred for 10 min and changed from a cloudy to clear solution. 4-Benzoxybenzylaldehyde (17 g, 80 mmol) was added followed by additional portion of EtOH (40 mL). The reaction mixture stirred for another 30 min at rt before it was heated to 60 °C and stirred overnight. The resulting solution became vicious. The EtOH was concentrated under reduced pressure and then H_2O (40 mL) followed by concentrated HCl (8 mL) was added. The cloudy mixture underwent vacuum filtration and the solid was washed with DCM and Et_2O to afford **S5** as a light tan solid which was used in the next step without further purification.

To a round bottom flask equipped with a stir bar under N_2 was added **S5** (7.90 g, 24.4 mmol) and 49 mL anhydrous MeOH. The reaction mixture was cooled to 0 °C and $SOCl_2$ (3.54 mL, 48.8 mmol, 2 equiv) was added dropwise. The reaction mixture stirred for 30 min at 0 °C and then at 60 °C for 24 h. The MeOH was concentrated under reduced pressure. DCM was added to redissolve the compound and then concentrated under reduced pressure. This process was repeated once more with DCM. The solid was then triturated with Et_2O to afford the product as a light tan solid (8.3 g, 61%, 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 8.43 (s, 2H), 7.44 (d, *J* = 7.2 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.33 (d, *J* = 7.2 Hz, 1H), 7.30 (d, *J* = 8.6 Hz, 2H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.46 (d, *J* = 4.3 Hz, 1H), 5.11 (s, 2H), 5.04 – 4.93 (m, 1H), 4.11 (d, *J* = 5.6 Hz, 1H), 3.60 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.96, 158.16, 137.05, 131.56, 128.45, 127.84, 127.74, 127.64, 114.64, 70.71, 69.16, 58.61, 52.62. IR (ATR): 3243, 3013, 2955, 2933, 1753, 1504, 1244 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{17}H_{19}NO_4Na$ $[M+Na]^+$: 324.1212, found: 324.1214.

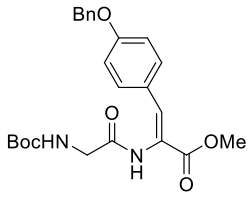
Methyl 3-(4-(benzyloxy)phenyl)-2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-hydroxypropanoate (S7)



The product was prepared by method A using Boc-Gly-OH (3.00 g, 17.1 mmol) and **S6** (6.37 g, 18.8 mmol) and purified by column chromatography (eluting with 4:1 DCM/Acetone) to afford the product as a white solid (5.4 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.30 (m, 5H), 7.26 (d, 2H), 6.93 (dd, *J* = 10.8, 4.0 Hz, 2H), 5.20 (d, *J* = 3.4 Hz, 1H), 5.13 (s 1H), 5.04 (s, 2H), 4.81 (dd, *J* = 8.8, 3.4 Hz, 1H), 3.84 – 3.73 (m, 2H), 3.72 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.92, 169.84, 158.82, 156.13, 136.95, 131.93, 128.73, 128.15, 127.66, 127.26, 114.96, 80.50, 73.54, 70.11, 58.19, 52.83, 44.26, 28.42. IR (ATR): 3356, 3014, 2978,

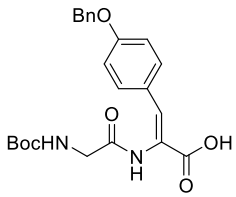
1735, 1672, 1509, 1253, 1167 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{30}N_2O_7Na$ $[M+Na]^+$: 481.1951, found: 481.1958.

Methyl (*Z*)-3-(4-(benzyloxy)phenyl)-2-(2-((tert-butoxycarbonyl)amino)acetamido)acrylate (S8)

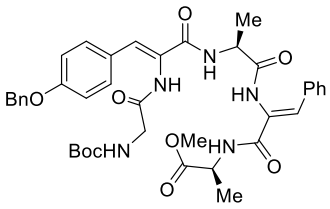


The product was prepared by method B using dipeptide **S7** (4.70 g, 10.3 mmol) and purified by column chromatography (eluting with 7:3 hexanes/ethyl acetate) to afford the product as a white solid (3.7 g, 82%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.50–7.31 (m, 7H), 6.96 (d, *J* = 8.8 Hz, 2H), 5.24 (s, 1H), 5.07 (s, 2H), 3.94 (s, 1H), 3.82 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.54, 165.85, 160.07, 156.34, 136.59, 133.89, 132.04, 128.80, 128.29, 127.64, 126.33, 121.33, 115.14, 80.68, 70.14, 52.76, 44.99, 28.45. IR (ATR): 3370, 3248, 3009, 2978, 1757, 1667, 1595, 1504, 1248 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{24}H_{28}N_2O_6Na$ $[M+Na]^+$: 463.1845, found: 463.1850.

(*Z*)-3-(4-(benzyloxy)phenyl)-2-(2-((tert-butoxycarbonyl)amino)acetamido)acrylic acid (S9)



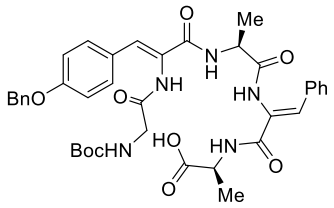
The product prepared by method C using methyl ester **S8** (3.70 g, 8.40 mmol) and was obtained as a white solid (3.5 g, 99%). ^{1}H NMR (500 MHz, DMSO) δ 9.28 (s, 1H), 7.57 (d, *J* = 8.4 Hz, 2H), 7.44 (m, 2H), 7.39 (m, 2H), 7.33 (m, 1H), 7.22 (s, 1H), 7.08 (t, *J* = 5.6 Hz, 1H), 6.98 (d, *J* = 8.7 Hz, 2H), 5.14 (s, 2H), 3.67 (d, *J* = 5.8 Hz, 2H), 1.39 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 168.83, 166.58, 158.82, 155.89, 136.86, 131.78, 128.79, 128.72, 128.49, 127.91, 127.67, 126.74, 114.67, 78.07, 69.21, 43.45, 28.23. IR (ATR): 3256, 3005, 2977, 2927, 1691, 1662, 1506, 1182 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{23}H_{26}N_2O_6Na$ $[M+Na]^+$: 449.1689, found: 449.1691.



Methyl ((*Z*)-2-((*S*)-2-((*Z*)-3-(4-(benzyloxy)phenyl)-2-(2-((tert-butoxycarbonyl)amino)acetamido)acrylamido)propanamido)-3-phenylacryloyl)-*L*-alaninate (S10) The product was obtained using method A using carboxylic acid **S9** (3.0 g, 7.0 mmol), amine **4** (3.0 g, 7.0 mmol), Et_3N (2.90 mL, 21.1 mmol), and MeCN (70 mL) as solvent and purified via column chromatography (eluting with 90:10 DCM/MeOH) to afford the product as a light yellow

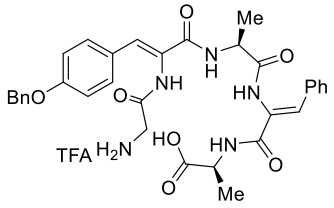
solid (3.04 g, 60%). ^{1}H NMR (500 MHz, DMSO) δ 9.58 (s, 1H), 9.53 (d, *J* = 11.6 Hz, 1H), 8.18 (d, *J* = 4.9 Hz, 1H), 8.04 (d, *J* = 6.5 Hz, 1H), 7.58 (d, *J* = 6.3 Hz, 2H), 7.55 (d, *J* = 8.7 Hz, 2H), 7.44 (d, *J* = 7.2 Hz, 2H), 7.39 (t, *J* = 7.3 Hz, 4H), 7.37 – 7.30 (m, 3H), 7.19 – 7.13 (m, 2H), 7.01 (d, *J* = 9.1 Hz, 2H), 5.15 (s, 2H), 4.36 (p, *J* = 7.1 Hz, 1H), 4.32 – 4.24 (m, 1H), 3.76 – 3.66 (m, 2H), 3.65 (s, 3H), 1.39 (s, 9H), 1.38 – 1.33 (m, 6H). ^{13}C NMR (126 MHz, DMSO) δ 172.98, 172.35, 169.86, 165.90, 164.08, 158.85, 156.25, 136.82, 133.72, 131.56, 130.74, 129.65, 129.52, 128.98, 128.51, 128.48, 128.20, 127.91, 127.65, 126.39, 126.05, 114.89, 78.38, 69.22, 51.90, 50.15, 48.39, 43.91, 28.21, 16.82, 16.18. IR (ATR): 3261, 3018, 2982, 2942, 1739, 1716, 1654, 1618, 1600, 1505, 1163 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{39}H_{45}N_5O_9Na$ $[M+Na]^+$: 750.3115, found: 750.3097. $[\alpha]^{25}_D$ –32 (*c* = 0.30, MeOH).

((*Z*)-2-((*S*)-2-((*Z*)-3-(4-(benzyloxy)phenyl)-2-(2-((tert-



butoxycarbonyl)amino)acetamido)acrylamido)propanamido)-3-phenylacryloyl)-*L*-alanine (S11) The product was obtained using method C from methyl ester **S10** (2.92 g, 4.01 mmol) as a light yellow solid in quantitative yield. ^{1}H NMR (400 MHz, DMSO) δ 9.58 (s, 1H), 9.55 (s, 1H), 8.14 (d, *J* = 5.3 Hz, 1H), 7.88 (d, *J* = 6.7 Hz, 1H), 7.58 (d, *J* = 7.3 Hz, 2H), 7.55 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 6.9 Hz, 2H), 7.43 – 7.36 (m, 4H), 7.34 (dd, *J* = 7.1, 1.8 Hz, 2H), 7.30 (s, 1H), 7.21 (t, *J* = 5.3 Hz, 1H), 7.18 (s, 1H), 7.01 (d, *J* = 8.9 Hz, 2H), 5.14 (s, 2H), 4.40 – 4.28 (m, 1H), 4.25 – 4.14 (m, 1H), 3.79 – 3.63 (m, 2H), 1.39 (s, 9H), 1.37 – 1.29 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 174.02, 172.29, 169.94, 165.67, 163.74, 158.85, 156.28, 136.86, 133.84, 131.59, 130.33, 129.66, 129.52, 128.88, 128.59, 128.51, 128.44, 127.93, 127.67, 126.50, 126.12, 114.89, 78.37, 69.25, 49.96, 48.69, 43.95, 28.23, 17.37, 16.41. IR (ATR): 3266, 3018, 2982, 2928, 1685, 1654, 1600, 1514, 1244, 1181 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{38}H_{43}N_5O_9Na$ $[M+Na]^+$: 736.2958, found: 736.2947. $[\alpha]^{27}_D$ +18 (*c* = 0.25, MeOH).

((*Z*)-2-((*S*)-2-((*Z*)-2-(2-aminoacetamido)-3-(4-(benzyloxy)phenyl)acrylamido)propanamido)-



3-phenylacryloyl)-*L*-alanine (12) The product was obtained using method D using carboxylic acid **S11** (2.85 g, 3.99 mmol) as a yellow solid in quantitative yield. ^{1}H NMR (400 MHz, DMSO) δ 9.63 (s, 1H), 8.39 (d, *J* = 5.2 Hz, 1H), 7.96 (d, *J* = 6.9 Hz, 1H), 7.59 (d, *J* =

7.3 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.48 – 7.31 (m, 8H), 7.29 (s, 1H), 7.20 (s, 1H), 7.04 (d, *J* = 8.7 Hz, 2H), 5.16 (s, 2H), 4.44 – 4.16 (m, 2H), 3.80 (dd, *J* = 34.5, 16.3 Hz, 2H), 1.43 – 1.27 (m, 6H). ^{13}C NMR (126 MHz, DMSO) δ 174.00, 172.31, 166.19, 165.23, 164.00, 158.95, 136.77, 133.83, 131.47, 130.10, 129.77, 129.58, 129.50, 128.87, 128.53, 128.48, 127.93, 127.64, 126.11, 125.47, 115.00, 69.26, 50.02, 48.22, 40.71, 17.00, 16.33. IR (ATR): 3261, 3023, 2973, 1689, 1645, 1600, 1509, 1167 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{33}H_{35}N_5O_7Na$ $[M+Na]^+$: 636.2434, found: 636.2422. $[\alpha]^{25}_D$ +39 (*c* = 0.26, MeOH).

*iv) Synthesis of Gly-ΔTyr-Ala-Phe-Ala **S14***

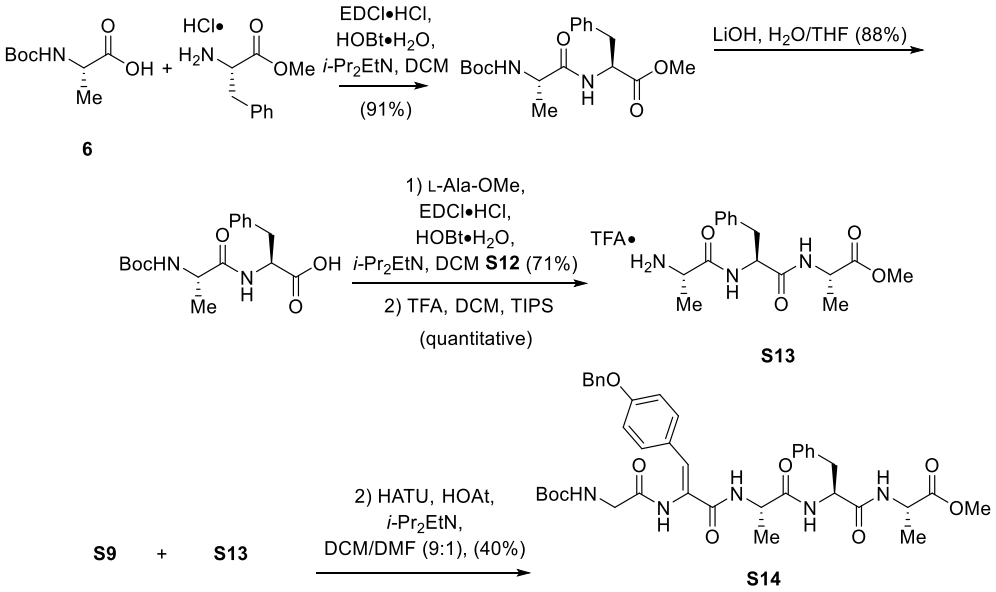
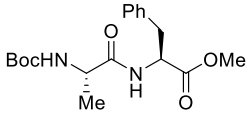


Figure S3. Synthesis of linear precursor **S14**.

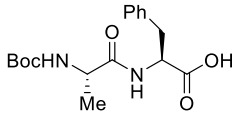
Methyl (tert-butoxycarbonyl)-L-alanyl-L-phenylalaninate



Boc-L-Ala-L-Phe-OMe was prepared according to method A using Boc-Ala-OH (3.00 g, 15.9 mmol), and L-phenylalanine methyl ester (3.76 g, 17.4 mmol) and purified by column chromatography (eluting with 2:1 hexanes/ethyl acetate) to afford the product as a white solid (5.1 g, 91% yield). The characterization data was in agreement with literature.[8]

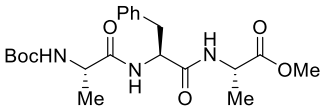
[8] Hardee, D. J.; Kovalchuke, L.; Lambert, T. H. *J. Am. Chem. Soc*. **2010**, *132*, 5002.

(tert-butoxycarbonyl)-L-alanyl-L-phenylalanine



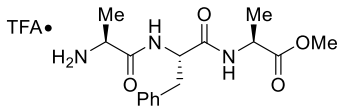
Boc-L-Ala-L-Phe-OH was prepared according to method C using Boc-L-Ala-L-Phe-OMe (5.00 g, 14.3 mmol) to afford the product as a white solid (4.2 g, 88%). The characterization data was in agreement with literature.[9]

Methyl (tert-butoxycarbonyl)-L-alanyl-L-phenylalanyl-L-alaninate (S12)



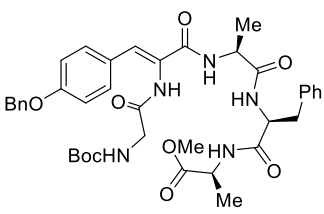
Tripeptide **S12** was prepared according to method A using Boc-L-Ala-L-Phe-OH (4.10 g, 12.5 mmol), and L-Alanine methyl ester (1.90 g, 13.7 mmol) and purified by column chromatography (eluting with 1:1 hexanes/ethyl acetate) to afford the product as a white solid (3.7 g, 71%). The characterization data was in agreement with literature.[10]

Methyl L-alanyl-L-phenylalanyl-L-alaninate (S13)



The product was prepared by method D using tripeptide **S12** (1.00 g, 2.37 mmol) and obtained as a white solid in quantitative yield. ^{1}H NMR (400 MHz, DMSO) δ 8.64 – 8.54 (m, 2H), 7.99 (d, *J* = 3.0 Hz, 2H), 7.32 – 7.25 (m, 4H), 7.25 – 7.16 (m, 1H), 4.58 (td, *J* = 9.8, 4.2 Hz, 1H), 4.29 (p, *J* = 7.2 Hz, 1H), 3.83 – 3.71 (m, 1H), 3.62 (s, 3H), 3.05 (dd, *J* = 14.0, 4.2 Hz, 1H), 2.79 (dd, *J* = 14.0, 10.0 Hz, 1H), 1.32 (d, *J* = 7.1 Hz, 3H), 1.30 (d, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.86, 170.73, 169.56, 137.56, 129.20, 128.18, 126.46, 54.07, 51.94, 47.95, 47.64, 37.29, 17.23, 16.90. IR (ATR): 3727, 3066, 2947, 1652, 1539, 1200, 1136 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{16}H_{23}N_3O_4H$ [M+H]$^+$: 322.1767, found: 322.1766. $[\alpha]^{24}_D$ –9 (*c* = 0.18, MeOH).

Methyl ((*Z*)-3-(4-(benzyloxy)phenyl)-2-(2-((tert-butoxycarbonyl)amino)acetamido)acryloyl)-L-alanyl-L-phenylalanyl-L-alaninate (S14)



To a round botton flask equipped with a stir bar was added carboxylic acid **S9** (500 mg, 1.17 mmol), amine **S13** (562 mg, 1.29 mmol), HOAt (0.452 g, 3.32 mmol), DCM (13.5 mL) and DMF (1.5 mL). The reaction mixture was cooled to 0 ^{o}C and *i*Pr$_2$EtN (0.611 mL, 3.51 mmol) was added. HATU (532

[9] Ray, S.; Das, A. K.; Banerjee, A. *Chem. Commun*. **2006**, 2816.

mg, 1.40 mmol) was added in portions and the reaction warmed to rt and stirred for 24 h. The solvent was removed under reduced pressure and the product was purified by column chromatography (eluting with 20:1 DCM/MeOH) to afford the product as a white solid (337 mg, 40%). ^{1}H NMR (500 MHz, DMSO) δ 9.73 (s, 1H), 8.06 (d, *J* = 6.5 Hz, 1H), 8.01 (d, *J* = 6.7 Hz, 1H), 7.82 (d, *J* = 8.6 Hz, 1H), 7.57 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 7.1 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.28 – 7.21 (m, 4H), 7.21 – 7.12 (m, 2H), 7.02 (d, *J* = 9.1 Hz, 3H), 5.16 (s, 2H), 4.42 (td, *J* = 9.8, 4.3 Hz, 1H), 4.23 (m, 1H), 4.16 (m, 1H), 3.76 (qd, *J* = 16.7, 5.7 Hz, 2H), 3.60 (s, 3H), 3.10 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.84 (dd, *J* = 13.8, 10.3 Hz, 1H), 1.37 (s, 9H), 1.27 (d, *J* = 7.3 Hz, 3H), 1.15 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.79, 171.91, 170.77, 170.41, 165.47, 158.73, 156.21, 137.96, 136.86, 131.52, 129.24, 128.49, 128.05, 128.01, 127.92, 127.67, 126.72, 126.43, 126.18, 114.89, 78.35, 69.22, 53.78, 51.84, 49.60, 47.71, 43.84, 36.98, 28.18, 17.12, 16.79. IR (ATR): 3272, 3081, 2984, 1640, 1539, 1515, 1455, 1228, 1173 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{39}H_{47}N_5O_9Na$ $[M+Na]^+$: 752.3271, found: 752.3300. $[\alpha]^{23}_D$ –42 (*c* = 0.17, MeOH).

v) Synthesis of Gly-Tyr-Ala-ΔCy-Ala ***S23***

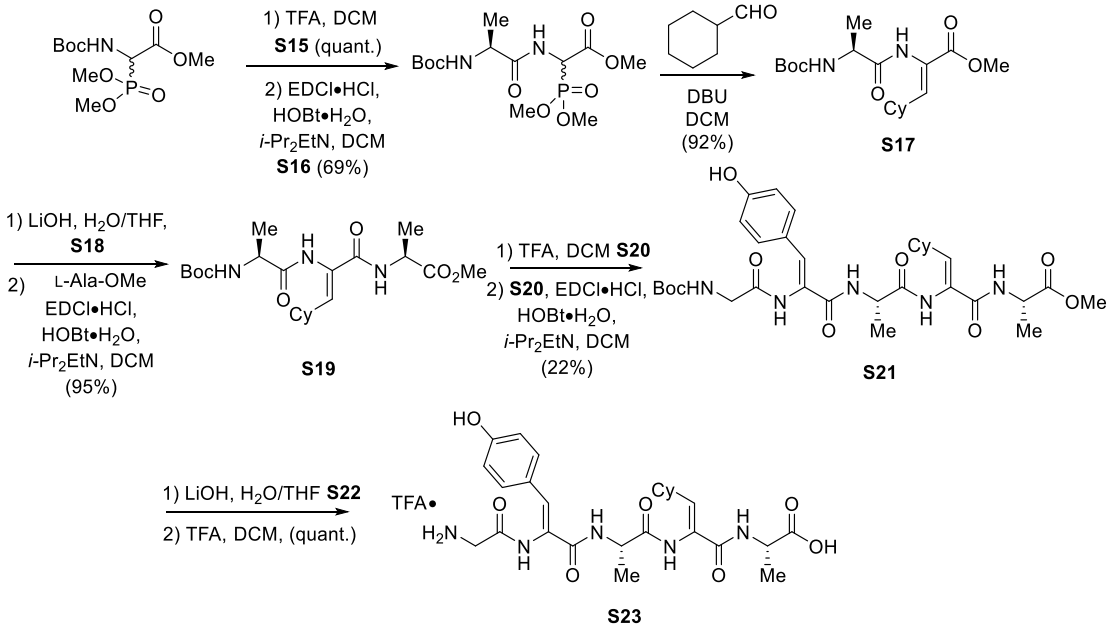
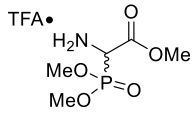


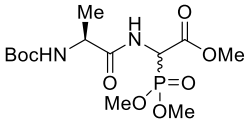
Figure S4. Synthesis of linear pentapeptide **S23**.

Methyl 2-amino-2-(dimethoxyphosphoryl)acetate (S15)



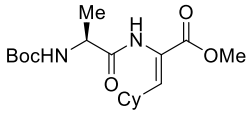
To a round bottom flask equipped with a stir bar was added methyl 2-((*tert*-butoxycarbonyl)amino)-2-(dimethoxyphosphoryl)acetate (10 g, 32 mmol) and DCM (340 mL). The reaction cooled to 0 ^{o}C and TFA was subsequently added dropwise. The reaction warmed to rt and stirred for 16 h. The DCM was concentrated under reduced pressure and toluene was added to form a TFA azeotrope, which was subsequently concentrated under reduced pressure. The unpurified reaction mixture was further dried on the high vacuum and subsequently triturated with Et_2O to afford the product in quantitative yield which was used without further purification. ^{1}H NMR (500 MHz, DMSO) δ 9.04 (s, 2H), 5.04 (d, *J* = 20.5 Hz, 1H), 3.83 – 3.73 (m, 9H). ^{13}C NMR (126 MHz, DMSO) δ 165.20 (d, *J* = 3.7 Hz), 54.58 (d, *J* = 6.5 Hz), 54.39 (d, *J* = 6.4 Hz), 53.63, 49.18 (d, *J* = 140.8 Hz). IR (ATR): 2975, 2869, 1761, 1218, 1140. HRMS (ESI-TOF) *m/z* calc'd for $C_5H_{12}NO_5PNa$ $[M+Na]^+$: 220.0351, found: 220.0355.

Methyl 2-((*S*)-2-((tert-butoxycarbonyl)amino)propanamido)-2-(dimethoxyphosphoryl)acetate (S16)



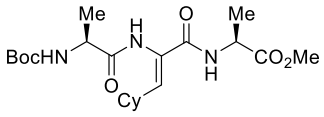
Dipeptide **S16** was prepared according to method A using **S15** (6.3 g, 32 mmol), and Boc-L-Ala-OH (6.36 g, 33.6 mmol) and purified by column chromatography to afford the product as a white solid (8.1 g, 69%, 1:1 dr). ^{1}H NMR (500 MHz, DMSO) δ 8.67 – 8.54 (m, 1H), 7.02 (dd, *J* = 13.1, 7.9 Hz, 1H), 5.27 – 4.80 (m, 1H), 4.26 – 3.95 (m, 1H), 3.83 – 3.50 (m, 9H), 1.37 (s, 9H), 1.17 (dd, *J* = 7.3, 3.1 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.43 , 173.37 , 173.17 , 173.12 , 167.03 (d, *J* = 2.7 Hz), 166.92 (d, *J* = 3.0 Hz), 155.06 , 155.00 , 78.08 , 78.05 , 54.04 (d, *J* = 6.6 Hz), 53.81 (d, *J* = 5.8 Hz), 53.78 (d, *J* = 6.8 Hz), 53.75 (d, *J* = 5.5 Hz), 52.88 , 52.86 , 49.66 (d, *J* = 147.2 Hz), 49.64 (d, *J* = 146.3 Hz), 49.32 , 28.19 , 18.08 , 17.86. IR (ATR): 3005, 2970, 2935, 1749, 1674, 1506, 1249, 1163, 1075. HRMS (ESI-TOF) *m/z* calc'd for $C_{13}H_{25}N_2O_8PNa$ $[M+Na]^+$: 391.1246, found: 391.1248.

Methyl (*S*,*Z*)-2-(2-((tert-butoxycarbonyl)amino)propanamido)-3-cyclohexylacrylate (S17)



Dipeptide **S17** was prepared according to an adapted procedure reported by Schmidt.[10,11] To a solution of **S16** (8.1 g, 22 mmol) in DCM (73 mL) at 0 ^{o}C was added DBU (4.0 mL, 26 mmol). After 10 min, cyclohexanecarbaldehyde (3 g, 26.7 mmol) was added and the reaction mixture stirred at rt for 24 h. The reaction mixture was transferred to a separatory funnel and washed with 100 mL sat. $NaHCO_3$ (aq), 100 mL 10% $KHSO_4$, and 100 mL brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The reaction mixture was then purified by column chromatography (eluting with 3:7 hexanes/ethyl acetate) to afford the product as a white solid (7.2 g, 92%). ^{1}H NMR (500 MHz, DMSO) δ 9.08 (s, 1H), 6.93 (d, *J* = 7.4 Hz, 1H), 6.27 (d, *J* = 10.0 Hz, 1H), 4.10 – 3.98 (m, 1H), 3.62 (s, 3H), 2.38 – 2.24 (m, 1H), 1.70 – 1.52 (m, 5H), 1.38 (s, 9H), 1.22 (d, *J* = 7.3 Hz, 4H), 1.19 – 1.02 (m, 4H). ^{13}C NMR (126 MHz, DMSO) δ 172.50, 164.85, 155.04, 142.22, 125.30, 77.95, 51.80, 49.62, 35.98, 31.06, 30.96, 28.20, 25.32, 25.04, 24.99, 17.90. IR (ATR): 2984, 2929, 2850, 1719, 1674, 1506, 1258, 1225, 1162. HRMS (ESI-TOF) *m/z* calc'd for $C_{18}H_{30}N_2O_5Na$ $[M+Na]^+$: 377.2052, found: 377.2048. $[\alpha]^{25}_D$ -5 (*c* = 0.55, MeOH).

Methyl ((*Z*)-2-((*S*)-2-((tert-butoxycarbonyl)amino)propanamido)-3-cyclohexylacryloyl)-L-alaninate (S19)



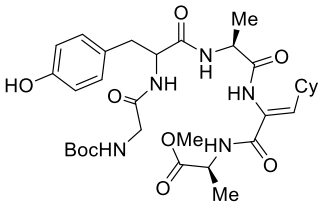
Dipeptide **S17 (**7.08 g, 20 mmol) was hydrolyzed to carboxylic acid **S18** according to general method C, which was obtained in quantitative yield and used without further purification.

Tripeptide **S19** was prepared according to method A using **S18** (20 mmol), and L-Ala methyl ester (3.1 g, 22 mmol) and purified by column chromatography to afford the product as a white solid (8.1 g, 95%). ^{1}H NMR (500 MHz, DMSO) δ 9.10 (s, 1H), 7.64 (d, *J* = 6.8 Hz, 1H), 7.13 (d, *J* = 5.5 Hz, 1H), 6.27 (d, *J* = 9.8 Hz, 1H), 4.31 (t, *J* = 7.1 Hz, 1H), 4.11 – 3.86 (m, 1H), 3.61 (s, 3H), 2.32 – 2.10 (m, 1H), 1.75 – 1.48 (m, 5H), 1.38 (s, 9H), 1.29 (d, *J* = 7.2 Hz, 3H), 1.25 – 0.99 (m, 8H). ^{13}C NMR (126 MHz, DMSO) δ 172.85, 172.67, 163.88, 155.56, 139.67, 127.41, 78.25, 51.87, 49.99, 47.98, 36.02, 31.24, 31.19, 28.19, 25.39, 25.18, 25.13, 17.33, 16.99. IR (ATR):

[10] Schmidt, U.; Griesser, H.; Leitenberger, V.; Lieberknecht, A.; Mangold, R.; Meyer, R.; Reidl, B. *Synthesis* **1992**, 487.
[11] Burk, M. J.; Johnson, N. B.; Lee, J. R. *Tetrahedron Lett.* **1999**, *40*, 6685.

3007, 2979, 2919, 2854, 1750, 1677, 1612, 1538, 1163. HRMS (ESI-TOF) *m/z* calc'd for $C_{21}H_{35}N_3O_6Na$ $[M+Na]^+$: 448.2424, found: 448.2419. $[\alpha]^{25}_D$ –46 (*c* = 0.28, MeOH).

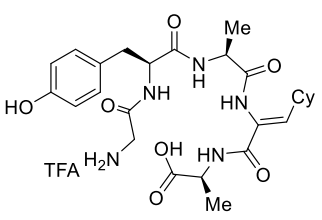
Methyl ((*Z*)-2-((2*S*)-2-(2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-(4-hydroxyphenyl)propanamido)propanamido)-3-cyclohexylacryloyl)-L-alaninate (S21)



Tripeptide **S19** (6.2 g, 15 mmol) was deprotected according to general procedure D to afford amine **S20** in quantitative yield which was used without further purification.

To a round botton flask equipped with a stir bar was added carboxylic acid **5** (4.7 g, 14 mmol), amine **S20** (15 mmol), HATU (5.3 g, 14 mmol), HOAt (0.50 g, 4.0 mmol) and DCM (49 mL). The reaction mixture was cooled to 0 °C and *i*Pr_2EtN (5.5 mL, 32 mmol) was added. The reaction warmed to rt and stirred for 24 h. The solvent was removed under reduced pressure and the product was purified by column chromatography (eluting with 95:5 ethyl acetate/acetone) to afford the product as a white solid (1.8 g, 22%). ^{1}H NMR (400 MHz, DMSO) δ 9.19 – 8.97 (m, 2H), 8.36 (d, *J* = 5.5 Hz, 1H), 7.73 (d, *J* = 6.6 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.60 (d, *J* = 7.4 Hz, 2H), 6.29 (d, *J* = 8.3 Hz, 1H), 4.59 – 4.39 (m, 1H), 4.33 – 4.17 (m, 2H), 3.61 (s, 3H), 3.56 – 3.48 (m, 1H), 3.48 – 3.37 (m, 1H), 3.38 – 3.33 (m, 1H), 2.93 – 2.84 (m, 1H), 2.75 – 2.61 (m, 1H), 2.22 (d, *J* = 11.3 Hz, 1H), 1.74 – 1.53 (m, 5H), 1.36 (s, 9H), 1.28 (d, *J* = 6.6 Hz, 3H), 1.23 (d, *J* = 7.2 Hz, 4H), 1.20 – 1.03 (m, 4H).^{13}C NMR (126 MHz, DMSO) δ 173.12, 171.98, 171.52, 169.07, 163.85, 155.82, 155.79, 139.61, 130.21, 127.41, 127.37, 114.84, 78.14, 59.79, 53.49, 51.83, 49.06, 48.19, 43.25, 36.06, 31.24, 28.16, 25.41, 25.15, 20.79, 17.25, 16.73, 14.12. IR (ATR): 3003, 2984, 2929, 2850, 1751, 1686, 1510, 1150. HRMS (ESI-TOF) *m/z* calc'd for $C_{32}H_{47}N_5O_9Na$ $[M+Na]^+$: 668.3271, found: 668.3270. $[\alpha]^{25}_D$ -33 (*c* = 0.25, MeOH).

((*Z*)-2-((*S*)-2-((*S*)-2-(2-aminoacetamido)-3-(4-hydroxyphenyl)propanamido)propanamido)-3-cyclohexylacryloyl)-L-alanine (S23)



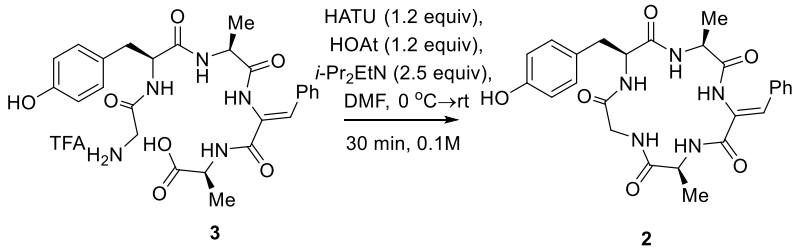
To a round bottom flask equipped with a stir bar was added methyl ester **S21** (0.820 g, 1.27 mmol), THF (6 mL), and H_2O (6 mL). The mixture was cooled to 0 °C and 1M LiOH (aq) (1.9 mL, 1.9 mmol)

was subsequently added. The reaction gradually warmed to rt and stirred for 48 h. The reaction mixture was acidified with 10% $KHSO_4$ (aq) and the THF was concentrated under reduced pressure. The reaction mixture was transferred to a separatory funnel where it was extracted with ethyl acetate (3 x 50 mL). The organic layer was washed with 50 mL brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford carboxylic acid **S22** which was used in the next step without further purification.

To a round bottom flask equipped with a stir bar was added carboxylic acid **S22** (1.27 mmol), and DCM (10 mL). The reaction mixture was cooled to 0 °C and TFA (0.73 mL, 9.5 mmol) was slowly added. The reaction slowly warmed to rt and stirred for 14 h. The DCM was concentrated under reduced pressure and to the mixture was added toluene to form a TFA azeotrope, which was subsequently concentrated under reduced pressure. The unpurified reaction mixture was further dried on the high vacuum and subsequently triturated with Et_2O to afford unsaturated peptide **S23** in quantitative yield. ^{1}H NMR (499 MHz, DMSO) δ 9.11 (s, 1H), 8.57 – 8.46 (m, 2H), 8.03 – 7.86 (m, 3H), 7.61 (d, *J* = 7.0 Hz, 1H), 7.03 (d, *J* = 8.6 Hz, 2H), 6.64 (d, *J* = 8.5 Hz, 2H), 6.26 (d, *J* = 9.8 Hz, 1H), 4.60 – 4.52 (m, 1H), 4.36 – 4.15 (m, 2H), 3.65 – 3.48 (m, 1H), 3.48 – 3.36 (m, 1H), 2.93 (dd, *J* = 14.2, 3.9 Hz, 1H), 2.59 (dd, *J* = 14.1, 10.0 Hz, 1H), 2.28 – 2.13 (m, 1H), 1.73 – 1.54 (m, 5H), 1.37 – 0.94 (m, 12H).^{13}C NMR (126 MHz, DMSO) δ 174.10, 171.91, 171.16, 165.68, 163.73, 158.39, 155.92, 139.03, 130.09, 127.61, 114.97, 54.15, 48.88, 47.90, 36.93, 36.10, 31.30, 27.09, 25.42, 25.41, 25.22, 25.17, 17.60, 17.19. IR (ATR): 3266, 3081, 2942, 2850, 1652, 1515, 1142. HRMS (ESI-TOF) *m/z* calc'd for $C_{26}H_{37}N_5O_7H[M+H]^+$: 532.2771, found: 532.2769. $[\alpha]^{26}_D$ –24 (*c* = 0.21, MeOH).

3. Typical procedures for macrocyclization

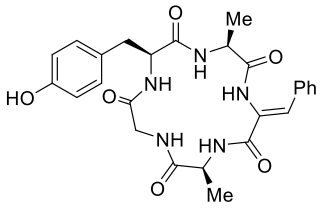
*i) Cyclization to Gly-Tyr-Ala-ΔPhe-Ala **2***



3 **2**

concentration (M)	selectivity (monomer:dimer)	yield
0.1	20:1	74%
0.05	39:1	81%

(3*S*,9*S*,12*S*)-6-((*Z*)-benzylidene)-12-(4-hydroxybenzyl)-3,9-dimethyl-1,4,7,10,13-pentaazacyclopentadecane-2,5,8,11,14-pentaone (2)



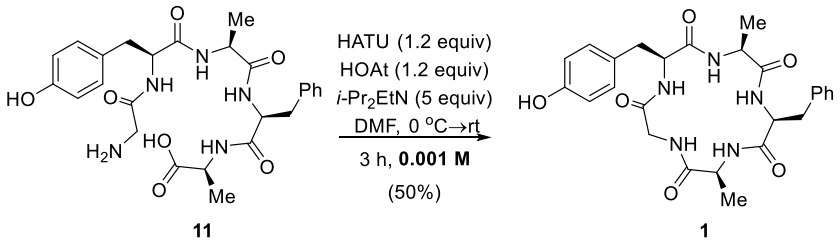
To a round bottom flask equipped with a stir bar was added peptide **3** (50 mg, 0.078 mmol), HOAt (13 mg, 0.094 mmol) and DMF (0.780 mL) and the mixture was stirred until homogenous. The reaction mixture was cooled to 0 °C and *i*Pr_2EtN (0.034 mL, 0.195 mmol) was added dropwise followed by the addition of HATU (36 mg, 0.094 mmol)[12] and it was subsequently warmed to rt and stirred for 30 min. DMF was then removed under reduced pressure and the product was purified by column chromatography (eluting with 93:7 ethyl acetate/MeOH) to afford the product as a white solid (29.4 mg, 74%) in 20:1 selectivity of monomer over cyclodimer. When the reaction was run on 0.05 M concentration from **3** (0.100 g, 0.156 mmol), the product was isolated as a white solid (63.7 mg, 81%) in 39:1 selectivity of monomer over cyclodimer. The selectivity was determined by LC-MS analysis of the unpurified reaction mixture. ^{1}H NMR (500 MHz, DMSO) δ 9.38 (s, 1H), 9.25 (s, 1H), 8.34 (d, *J* = 8.1 Hz, 1H), 8.19 (dd, *J* = 7.6, 3.3 Hz, 1H), 7.95 (t, *J* = 11.0 Hz, 1H), 7.64 (d, *J* = 8.7 Hz, 1H), 7.47 (d, *J* = 7.5 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.3 Hz, 1H), 7.10 (s, 1H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 2H), 4.48 – 4.34 (m, 3H), 4.15 (dd, *J* = 15.0, 7.7 Hz, 1H), 3.30 (dd, *J* = 15.1, 3.4 Hz, 1H), 2.81 (m, 2H), 1.27 (d, *J* = 7.0 Hz, 3H), 1.19 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 171.50, 170.88, 169.78, 169.03, 165.27, 156.01, 134.25, 130.05,

[12] Procedure modified from Nicolaou, K. C.; Safina, B. S.; Zak, M.; Estrada, A. A.; Lee, S. H. *Angew. Chem. Int. Ed* **2004**, *43*, 5087.

129.29, 128.93, 128.71, 128.48, 127.71, 127.03, 115.07, 56.04, 48.44, 48.27, 43.40, 37.45, 16.88, 16.87. IR (ATR): 3257, 3052, 2978, 1635, 1508, 1440, 1240 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{26}H_{29}N_5O_6Na$ $[M+Na]^+$: 530.2015, found: 530.2001. $[\alpha]^{26}_D$ –76 (*c* = 0.30, MeOH).

*ii) Cyclization to Gly-Tyr-Ala-Phe-Ala **1***

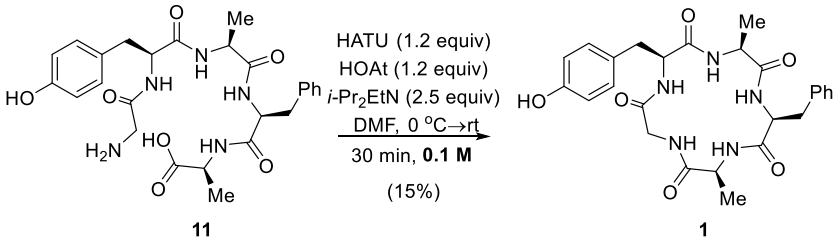
Preparation of authentic material of dichotomin E



11 **1**

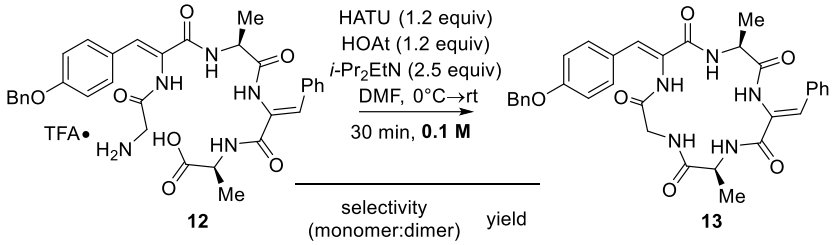
To a round bottom flask equipped with a stir bar was added peptide **11** (100 mg, 0.190 mmol), HOAt (31 mg, 0.228 mmol) and DMF (190 mL) and the mixture was stirred until homogenous. The reaction mixture was cooled to 0 °C and *i*-Pr_2EtN (0.166 mL, 0.950 mmol) was added dropwise followed by the addition of HATU (87 mg, 0.228 mmol) and it was subsequently warmed to rt and stirred for 30 min. DMF was then removed under reduced pressure. The peptide was dissolved in 10 mL 38% MeCN/H_2O, filtered through a 0.20 micron filter, and purified by RP-HPLC on a C_{18} column (eluting with MeCN and H_2O containing 0.1% TFA, linear gradient 20-70% MeCN over 45 min). The pure fractions were combined, and concentrated under reduced pressure to afford dichotomin E **1** as a colorless powder (47 mg, 50%). ^{1}H NMR (400 MHz, Pyr, 303 K) δ 9.73 (br t, *J* = 5.6 Hz, 1H), 9.34 (d, *J* = 7.1 Hz, 1H), 9.10 (d, *J* = 8.2 Hz, 1H), 9.06 (d, *J* = 8.0 Hz, 1H), 8.77 (s, 1H), 7.40 (d, *J* = 7.4 Hz, 2H), 7.28 (m, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.23 (m, 1H), 7.09 (d, *J* = 8.2 Hz, 2H), 5.15 (td, *J* = 8.4, 6.8 Hz, 1H), 4.93 – 4.85 (m, 1H), 4.84 – 4.77 (m, 1H), 4.58 (dd, *J* = 14.6, 6.8 Hz, 1H), 4.51 – 4.42 (m, 1H), 3.73 (dd, *J* = 14.4, 4.9 Hz, 1H), 3.54 (m, 2H), 3.40 (dd, *J* = 13.7, 6.8 Hz, 1H), 3.16 (dd, *J* = 13.9, 8.8 Hz, 1H), 1.62 (d, *J* = 6.8 Hz, 3H), 1.58 (d, *J* = 7.1 Hz, 3H). ^{1}H NMR (500 MHz, DMSO, 298 K) δ 9.21 (s, 1H), 8.55 (br t, *J* = 5.7 Hz, 1H), 8.08 (d, *J* = 7.4 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.28 (m, 2H), 7.21 (t, *J* = 7.5 Hz, 3H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 2H), 4.26 – 4.23 (m, 1H), 4.23 – 4.18 (m, 1H), 4.13 – 4.09 (m, 1H), 4.07 – 4.03 (m, 1H), 3.92 (dd, *J* = 14.4, 6.3 Hz, 1H), 3.28 (dd, *J* = 14.4, 5.2

Hz, 1H), 3.11 (dd, *J* = 13.4, 9.6 Hz, 1H), 3.04 (dd, *J* = 13.2, 5.1 Hz, 1H), 2.93 (dd, *J* = 14.0, 5.3 Hz, 1H), 2.71 (dd, *J* = 13.9, 9.5 Hz, 1H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.21 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, Pyr, 303 K) δ 174.15, 173.67, 173.20, 172.55, 171.43, 158.14, 138.96, 131.21, 130.17, 129.25, 128.73, 127.45, 116.69, 58.33, 57.13, 52.32, 50.38, 45.04, 37.96, 37.86, 17.81, 17.44. ^{13}C NMR (126 MHz, DMSO, 298 K) δ 172.52, 171.66, 170.81, 170.47, 169.24, 155.88, 137.73, 129.90, 129.12, 128.24, 127.66, 126.47, 115.03, 56.92, 55.72, 49.60, 48.30, 43.33, 36.07, 22.53, 17.46, 17.09. IR (ATR): 3281, 1648, 1512, 1442, 1207, 1160 cm^{-1}. IR (ATR): 3281, 1648, 1512, 1442, 1207, 1160 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{26}H_{31}N_5O_6Na$ [M+Na]$^+$: 532.2172 found: 532.2169. $[\alpha]^{25}_D$ +67 (*c* = 0.24, MeOH). For detailed analysis, see table S1 on page S30.



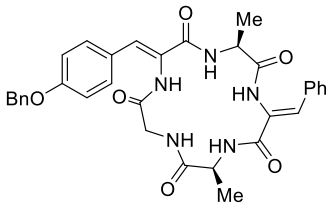
To a round bottom flask equipped with a stir bar was added peptide **11** (50 mg, 0.095 mmol), HOAt (15 mg, 0.11 mmol) and DMF (0.950 mL) and the mixture was stirred until homogenous. The reaction mixture was cooled to 0 °C and *i*-Pr_2EtN (0.042 mL, 0.238 mmol) was added dropwise followed by the addition of HATU (36 mg, 0.094 mmol) and it was subsequently warmed to rt and stirred for 30 min. DMF was then removed under reduced pressure. The peptide was dissolved in 10 mL 20% MeCN/H_2O, filtered through a 0.20 micron filter, and purified by RP-HPLC on a C_{18} column (eluting with MeCN and H_2O containing 0.1% TFA, linear gradient 15-60% MeCN over 45 min). The pure fractions were combined, and concentrated under reduced pressure to afford dichotomin E **1** as an off-white solid (7.2 mg, 15%). The cyclic dimer was also obtained as ~2:1 mixture of diastereomers (5.0 mg, 10%). MS (ESI) *m/z* calc'd for $C_{52}H_{62}N_{10}O_{12}H$ [M+H]$^+$: 1019.5 found: 1019.5.

*iii) Cyclization to Gly-ΔTyr-Ala-ΔPhe-Ala **13***



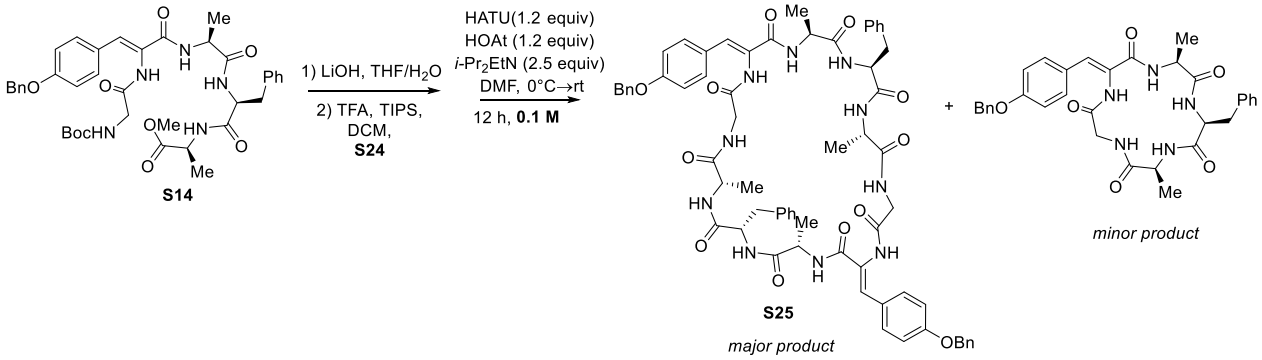
selectivity (monomer:dimer)	yield
26:1	84%

(3*S*,9*S*)-6-((*Z*)-benzylidene)-12-((*Z*)-4-(benzyloxy)benzylidene)-3,9-dimethyl-1,4,7,10,13-pentaazacyclopentadecane-2,5,8,11,14-pentaone (13)



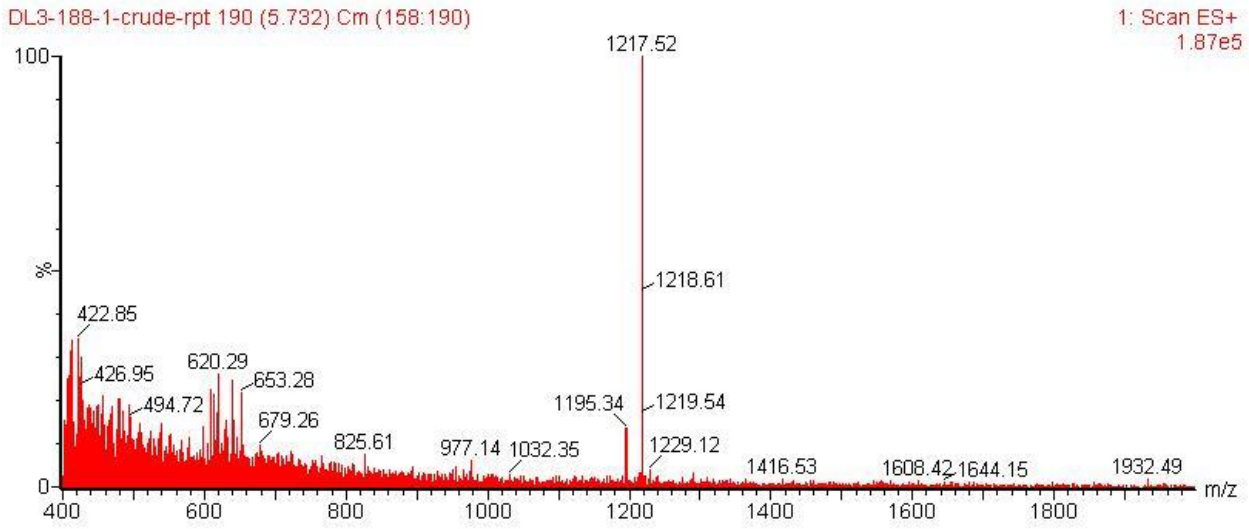
To a round bottom flask equipped with a stir bar was added peptide **12** (1.00 g, 1.37 mmol), HOAt (225 mg, 1.65 mmol) and DMF (13.7 mL) and the mixture was stirred until homogenous. The reaction mixture was cooled to 0 °C and *i*-Pr$_2$EtN (0.596 mL, 3.43 mmol) was added dropwise followed by the addition of HATU (627 mg, 1.65 mmol). The reaction mixture was subsequently warmed to rt and stirred for 30 min. DMF was then removed via vacuum distillation and the product was purified by column chromatography (eluting with 93:7 DCM/MeOH) to afford cyclic peptide **13** as a light yellow solid (684 mg, 84%) in 26:1 selectivity for the monomer over cyclodimer. The selectivity was determined by LC-MS analysis of the unpurified reaction mixture. ^{1}H NMR (500 MHz, DMSO) δ 9.47 (s, 1H), 9.31 (s, 1H), 8.20 (d, *J* = 7.5 Hz, 1H), 8.13 (s, 1H), 8.02 (d, *J* = 8.3 Hz, 1H), 7.51 (t, *J* = 7.4 Hz, 4H), 7.46 (d, *J* = 7.0 Hz, 2H), 7.43 – 7.37 (m, 4H), 7.33 (dt, *J* = 10.1, 7.2 Hz, 2H), 7.14 (s, 1H), 7.07 – 6.99 (m, 3H), 5.15 (s, 2H), 4.61 – 4.48 (m, 1H), 4.44 – 4.34 (m, 1H), 4.06 (dd, *J* = 15.6, 6.2 Hz, 1H), 3.84 (dd, *J* = 15.3, 4.2 Hz, 1H), 1.28 (d, *J* = 6.8 Hz, 6H).^{13}C NMR (126 MHz, DMSO) δ 172.04, 170.40, 167.97, 165.96, 165.19, 158.76, 136.85, 134.24, 131.41, 129.32, 129.08, 128.68, 128.49, 128.20, 128.11, 127.93, 127.73, 127.66, 126.71, 125.57, 114.76, 69.23, 49.31, 48.80, 43.37, 17.29, 17.10. IR (ATR): 3023, 2969, 2910, 1680, 1644, 1631, 1613, 1500, 1174 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{33}H_{33}N_5O_6Na$ $[M+Na]^+$: 618.2328, found: 618.2327. $[\alpha]^{25}_D$ +123 (*c* = 0.30, MeOH).

*iv) Cyclization to Gly-ΔTyr-Ala-Phe-Ala **S25***



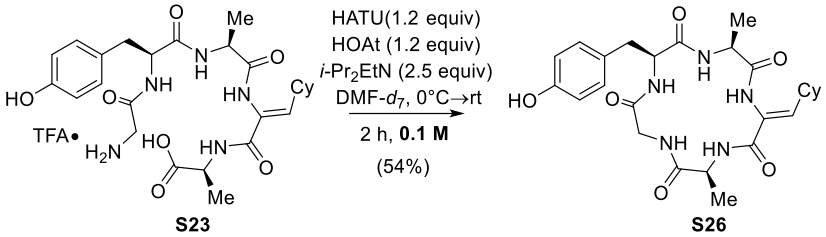
Peptide **S14** (226 mg, 0.310 mmol) underwent hydrolysis according to general method C. The corresponding carboxylic acid was used without further purification. The carboxylic acid was then subjected to TFA deprotection using general method D to afford **S24** and used in the next step without further purification.

To a round bottom flask equipped with a stir bar was added peptide **S24** (50 mg, 0.069 mmol), HOAt (11 mg, 0.083 mmol) and DMF (0.690 mL) and the mixture was stirred until homogenous. The reaction mixture was cooled to 0 °C and *i*-Pr_2EtN (0.030 mL, 0.17 mmol) was added dropwise followed by the addition of HATU (32 mg, 0.083 mmol). The reaction mixture was subsequently warmed to rt and stirred for 12 h. The reaction then was analyzed by LC-MS.

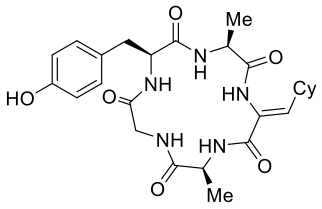


LC-MS analysis for the macrocyclization of **S14** showed that there was predominant cyclodimer formation over monomer formation. The cyclodimer mass is represented by major peaks, m/z = 1195.3 (M+H) and 1217.5 (M+Na).

v) Cyclization to Gly-Tyr-Ala-ΔCy-Ala ***S26***



(3*S*,9*S*,12*S*,*Z*)-6-(cyclohexylmethylene)-12-(4-hydroxybenzyl)-3,9-dimethyl-1,4,7,10,13-pentaazacyclopentadecane-2,5,8,11,14-pentaone (S26)

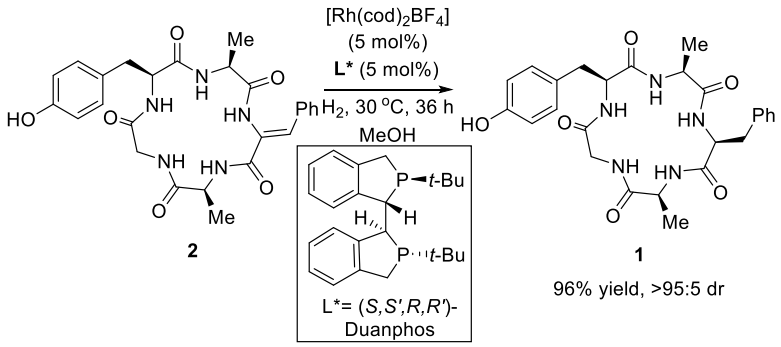


To a round bottom flask equipped with a stir bar was added peptide **S23** (52 mg, 0.10 mmol), HOAt (16 mg, 0.12 mmol) and DMF-d_7 (1 mL) and the mixture was stirred until homogenous. The reaction mixture was cooled to 0 °C and *i*Pr_2EtN (0.043 mL, 0.25 mmol) was added dropwise followed by the addition of HATU (45 mg, 0.12 mmol) and the reaction mixture was subsequently warmed to rt and stirred for 2 h. The reaction mixture was transferred to an NMR tube and after ^{1}H NMR analysis using 1,3,5-trimethoxybenzene as an internal standard, cyclic peptide **S26** was observed in 54% yield. ^{1}H NMR[13] (500 MHz, DMSO) δ 9.24 (s, 1H), 8.74 (s, 1H), 8.41 (d, *J* = 8.3 Hz, 1H), 8.19 – 8.06 (m, 1H), 7.65 (d, *J* = 8.7 Hz, 1H), 7.43 (d, *J* = 9.0 Hz, 1H), 6.98 (d, *J* = 8.3 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 6.18 (d, *J* = 10.2 Hz, 1H), 4.45 (q, *J* = 7.7 Hz, 1H), 4.43 – 4.29 (m, 2H), 4.20 (dd, *J* = 15.2, 8.5 Hz, 1H), 3.21 (dd, *J* = 15.2, 2.7 Hz, 1H), 2.80 (d, *J* = 7.3 Hz, 2H), 2.22 – 2.06 (m, 1H), 1.85 – 1.76 (m, 1H), 1.71 – 1.56 (m, 3H), 1.51 – 1.41 (m, 1H), 1.28 – 1.02 (m, 16H). ^{13}C NMR (126 MHz, DMSO) δ 171.41, 170.52, 169.71, 168.88, 164.48, 156.00, 139.14, 130.08, 126.85, 126.55, 115.05, 55.80, 48.07, 47.88, 43.41, 37.86, 36.56, 31.45, 30.50, 25.48, 25.15, 24.99, 16.84, 16.80. IR (ATR): 3289, 2919, 2854, 1645, 1510, 1224, 841. HRMS (ESI-TOF) *m/z* calc'd for $C_{26}H_{35}N_5O_6Na$ $[M+Na]^+$: 536.2485, found: 536.2471.

[13] Cyclodimer impurity could not be fully separated from the product after purification.

4. General procedure for hydrogenation

i) Hydrogenation of Gly-Tyr-Ala-ΔPhe-Ala ***2***



Dichotomin E (**1**)

In a N_2-filled glovebox, $[Rh(cod)_2BF_4]$ (0.40 mg, 0.0010 mmol, 5 mol%) and (*S,S',R,R'*)-Duanphos (0.40 mg, 0.0010 mmol, 5 mol%) were added to a ½ dr vial equipped with a stir bar containing pentapeptide **2** (10 mg, 0.020 mmol). MeOH (394 µL) was added and the vial was capped with a screwcap with a slitted rubber septum and taken outside of the glovebox. The vial was sonicated to ensure all contents were dissolved and then placed in the HEL CATalyst block and the head was screwed into place. The block was filled then purged three times with hydrogen, and then pressurized with hydrogen to 30 atm. The reaction stirred at 30 °C[14] and was stopped after 36 h. The solvent was concentrated under reduced pressure and the reaction mixture was triturated with DCM to afford dichotomin E as an off-white solid (9.6 mg, 96%). ^{1}H NMR (400 MHz, Pyr, 303 K) δ 9.73 (br t, *J* = 5.6 Hz, 1H), 9.34 (d, *J* = 7.1 Hz, 1H), 9.10 (d, *J* = 8.2 Hz, 1H), 9.06 (d, *J* = 8.0 Hz, 1H), 8.77 (s, 1H), 7.40 (d, *J* = 7.4 Hz, 2H), 7.28 (m, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.23 (m, 1H), 7.09 (d, *J* = 8.2 Hz, 2H), 5.15 (td, *J* = 8.4, 6.8 Hz, 1H), 4.93 – 4.85 (m, 1H), 4.84 – 4.77 (m, 1H), 4.58 (dd, *J* = 14.6, 6.8 Hz, 1H), 4.51 – 4.42 (m, 1H), 3.73 (dd, *J* = 14.4, 4.9 Hz, 1H), 3.54 (m, 2H), 3.40 (dd, *J* = 13.7, 6.8 Hz, 1H), 3.16 (dd, *J* = 13.9, 8.8 Hz, 1H), 1.62 (d, *J* = 6.8 Hz, 3H), 1.58 (d, *J* = 7.1 Hz, 3H). ^{1}H NMR (500 MHz, DMSO, 298 K) δ 9.21 (s, 1H), 8.55 (br t, *J* = 5.7 Hz, 1H), 8.08 (d, *J* = 7.4 Hz, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.28 (m, 2H), 7.21 (t, *J* = 7.5 Hz, 3H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 2H), 4.26 – 4.23 (m, 1H), 4.23 – 4.18 (m, 1H), 4.13 – 4.09 (m, 1H), 4.07 – 4.03 (m, 1H), 3.92 (dd, *J* = 14.4, 6.3 Hz, 1H), 3.28 (dd, *J* = 14.4, 5.2 Hz, 1H), 3.11 (dd, *J* = 13.4, 9.6 Hz,

[14] 30 °C was chosen as the reaction temperature for the hydrogenations to enhance substrate solubility and increase the overall conversion of starting material to product.

1H), 3.04 (dd, *J* = 13.2, 5.1 Hz, 1H), 2.93 (dd, *J* = 14.0, 5.3 Hz, 1H), 2.71 (dd, *J* = 13.9, 9.5 Hz, 1H), 1.23 (d, *J* = 6.7 Hz, 3H), 1.21 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, Pyr, 303 K) δ 174.15, 173.67, 173.20, 172.55, 171.43, 158.14, 138.96, 131.21, 130.17, 129.25, 128.73, 127.45, 116.69, 58.33, 57.13, 52.32, 50.38, 45.04, 37.96, 37.86, 17.81, 17.44. ^{13}C NMR (126 MHz, DMSO, 298 K) δ 172.52, 171.66, 170.81, 170.47, 169.24, 155.88, 137.73, 129.90, 129.12, 128.24, 127.66, 126.47, 115.03, 56.92, 55.72, 49.60, 48.30, 43.33, 36.07, 22.53, 17.46, 17.09. IR (ATR): 3281, 1648, 1512, 1442, 1207, 1160 cm^{-1}. IR (ATR): 3281, 1648, 1512, 1442, 1207, 1160 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{26}H_{31}N_5O_6Na$ $[M+Na]^+$: 532.2172 found: 532.2169. $[\alpha]^{25}_D$ +67 (*c* = 0.24, MeOH).

^{1}H NMR comparison of literature,[15] authentic material synthesized via SPPS, and synthetic material after hydrogenation is given below (Table S1). (Note: due to rapid proton exchange and other dynamic effects, the ^{1}H NMR spectrum of the synthetic sample of dichotomin E obtained via SPPS in pyridine–d_5 varies slightly with literature values. Therefore, characterization data was also obtained in DMSO–d_6. Dichotomin E has previously been synthesized by Tam,[16] however, no spectral data was reported and characterization of the natural product was confirmed by MALDI-MS).

[15] Morita, H.; Kayashita, T.; Shishido, A.; Takeya, K.; Itokawa, H.; Shiro, M. *Tetrahedron* **1996**, *52*, 1165.
[16] Zhang, L.; Tam, J. P. *J. Am. Chem. Soc.* **1999**, *121*, 3311.

Table S1. ^{1}H NMR data for dichotomin E, cyclo(Gly-Tyr-Ala$_1$-Phe-Ala$_2$)

	^{1}H NMR δ (multiplicity, *J* (Hz), integration)			
Proton	Literature Report (pyridine–d_5)[a] at 303 K	Synthetic Sample via SPPS (pyridine–d_5)[b] at 303 K	Synthetic Sample via SPPS (DMSO–d_6)[c] at 298 K	Synthetic Sample via Hydrogenation (DMSO–d_6)[d] at 298 K
CH_2 (Gly)	4.49 (dd, *J* = 14.5, 6.5 Hz, 1H) 3.82 (dd, *J* = 14.5, 5.1, 1H)	4.58 (dd, *J* = 14.6, 6.8 Hz, 1H) 3.73 (dd, *J* = 14.4, 4.9 Hz, 1H)	3.92 (dd, *J* = 14.4, 6.3 Hz, 1H) 3.28 (dd, *J* = 14.4, 5.3 Hz, 1H)	3.91 (dd, *J* = 14.7, 6.2 Hz, 1H) 3.28 (dd, *J* = 14.4, 5.5 Hz, 1H)
N–H (Gly)	9.74 (br t, 1H)	9.73 (br t, *J* = 5.6 Hz, 1H)	8.55 (br t, *J* = 5.8 Hz, 1H)	8.54 (br t, *J* = 5.7 Hz, 1H)
C_α–H (Tyr)	5.15 (dt, *J* = 8.6, 6.6 Hz, 1H)	5.15 (td, *J* = 8.4, 6.8 Hz, 1H)	4.24 (m, 1H)	4.24 (m, 1H)
CH_2 (Tyr)	3.23 (dd, *J* = 14.0, 8.6 Hz, 1H) 3.43 (dd, *J* = 14.0, 6.6 Hz, 1H)	3.16 (dd, *J* = 13.9, 8.8 Hz, 1H) 3.40 (dd, *J* = 13.7, 6.8 Hz, 1H)	2.71 (dd, *J* = 13.9, 9.5 Hz, 1H) 2.93 (dd, *J* = 14.0, 5.3 Hz, 1H)	2.72 (dd, *J* = 13.6, 9.3 Hz, 1H) 2.92 (dd, *J* = 13.7, 4.9 Hz, 1H)
C_{sp2}–H (Tyr)	7.28 (d, *J* = 8.4 Hz, 2H) 7.07 (d, *J* = 8.4 Hz, 2H)	7.29 (d, *J* = 8.0 Hz, 1H) 7.09 (d, *J* = 8.2 Hz, 2H)	7.01 (d, *J* = 8.4 Hz, 2H) 6.65 (d, *J* = 8.4 Hz, 2H)	7.01 (d, *J* = 8.5 Hz, 2H) 6.65 (d, *J* = 8.5 Hz, 2H)
N–H (Tyr)	9.01 (d, *J* = 8.6 Hz, 1H)	8.76 (1H)	8.04 (d, *J* = 8.4 Hz, 1H)	8.04 (d, *J* = 8.6 Hz, 1H)
C_α–H (Ala$_1$)	4.58 (br t, *J* = 7.0 Hz, 1H)	4.48 (m, 1H)	4.05 (m, 1H)	4.06 (m, 1H)
CH_3 (Ala$_1$)	1.57 (d, *J* = 7.1 Hz, 3H)	1.58 (d, *J* = 7.1 Hz, 1H)	1.21 (d, *J* = 6.8 Hz, 3H)	1.21 (d, *J* = 7.1 Hz, 3H)
N–H (Ala$_1$)	9.47 (d, *J* = 8.1 Hz, 1H)	9.34 (d, *J* = 7.1 Hz, 1H)	7.84 (d, *J* = 8.2 Hz, 1H)	7.89 (br s, 1H)
C_α–H (Phe)	4.87 (m, 1H)	4.82 (m, 1H)	4.10 (m, 1H)	4.11 (m, 1H)
CH_2 (Phe)	3.53 (m, 2H)	3.54 (m, 2H)	3.11 (dd, *J* = 13.4, 9.6 Hz, 1H) 3.04 (dd, *J* = 13.2, 5.1 Hz, 1H)	3.11 (dd, *J* = 13.4, 9.9 Hz, 1H) 3.04 (dd, *J* = 13.5, 5.4 Hz, 1H)
C_{sp2}–H (Phe)	7.39 (d, *J* = 7.3 Hz, 2H) 7.28 (t, *J* = 7.3 Hz, 2H) 7.23 (m, 1H)	7.40 (d, *J* = 7.4 Hz, 2H) 7.28 (m, 2H) 7.23 (m, 1H)	7.28 (m, 2H) 7.21 (m, 3H)	7.28 (m, 2H) 7.20 (m, 3H)
N–H (Phe)	9.30 (br s, 1H)	9.10 (d, *J* = 8.2 Hz, 1H)	8.08 (d, *J* = 7.4 Hz, 1H)	8.11 (br s, 1H)
C_α–H (Ala$_2$)	4.89 (m, 1H)	4.89 (m, 1H)	4.21 (m, 1H)	4.21 (m, 1H)
CH_3 (Ala$_2$)	1.61 (d, *J* = 6.9 Hz, 3H)	1.62 (d, *J* = 6.8 Hz, 1H)	1.23 (d, *J* = 6.7 Hz, 3H)	1.22 (d, *J* = 7.0 Hz, 3H)
N–H (Ala$_2$)	9.30 (br s, 1H)	9.06 (d, *J* = 8.0 Hz, 1H)	8.07 (d, *J* = 7.6 Hz, 1H)	8.09 (br s, 1H)

[a] Data reported by Shiro and recorded on a Bruker AM400 or AM500 spectrometer (not specified). Concentration was 15 mg/0.5 mL pyridine–d_5 [b] Data recorded on a Bruker DRX400 spectrometer. Concentration was 15 mg/0.5 mL pyridine–d_5 [c] Data recorded on a Bruker DRX500 with TCI (three channel inverse) cryoprobe. [d] Data recorded on a Bruker DRX400 spectrometer

Table S2. ^{13}C NMR data for dichotomin E, cyclo(Gly-Tyr-Ala$_1$-Phe-Ala$_2$)

	^{13}C NMR δ	
Carbon	Literature Report (pyridine–d_5)[a] at 303 K	Synthetic Sample via SPPS (pyridine–d_5)[b] at 303 K
CH_2 (Gly)	44.51	45.04
C=O (Gly)	171.02	171.43
C_α–H (Tyr)	56.83	57.13
C_β–H (Tyr)	37.36	37.86
C_γ-H (Tyr)	128.30	128.73
C_δ-H (Tyr)	130.73	131.21
C_ε-H (Tyr)	116.21	116.69
C_ζ-H (Tyr)	157.57	158.14
C=O (Tyr)	172.65	172.55
C_α–H (Ala$_1$)	51.75	52.32
CH_3 (Ala$_1$)	17.34	17.81
C=O (Ala$_1$)	173.23	173.20
C_α–H (Phe)	57.76	58.33
C_β–H (Phe)	37.63	37.96
C_γ-H (Phe)	138.42	138.96
C_δ-H (Phe)	129.71	130.17
C_ε-H (Phe)	128.76	129.25
C_ζ-H (Phe)	126.95	127.45
C=O (Phe)	173.92	174.15
C_α–H (Ala$_2$)	50.16	50.38
CH_3 (Ala$_2$)	17.34	17.44
C=O	173.92	173.67

[a] Data reported by Shiro and recorded on a Bruker AM400 or AM500 spectrometer (not specified). Concentration was 15 mg/0.5 mL pyridine–d_5. [b] Data recorded on a Bruker DRX500 with TCI (three channel inverse) cryoprobe. Concentration was 15 mg/0.5 mL pyridine–d_5.

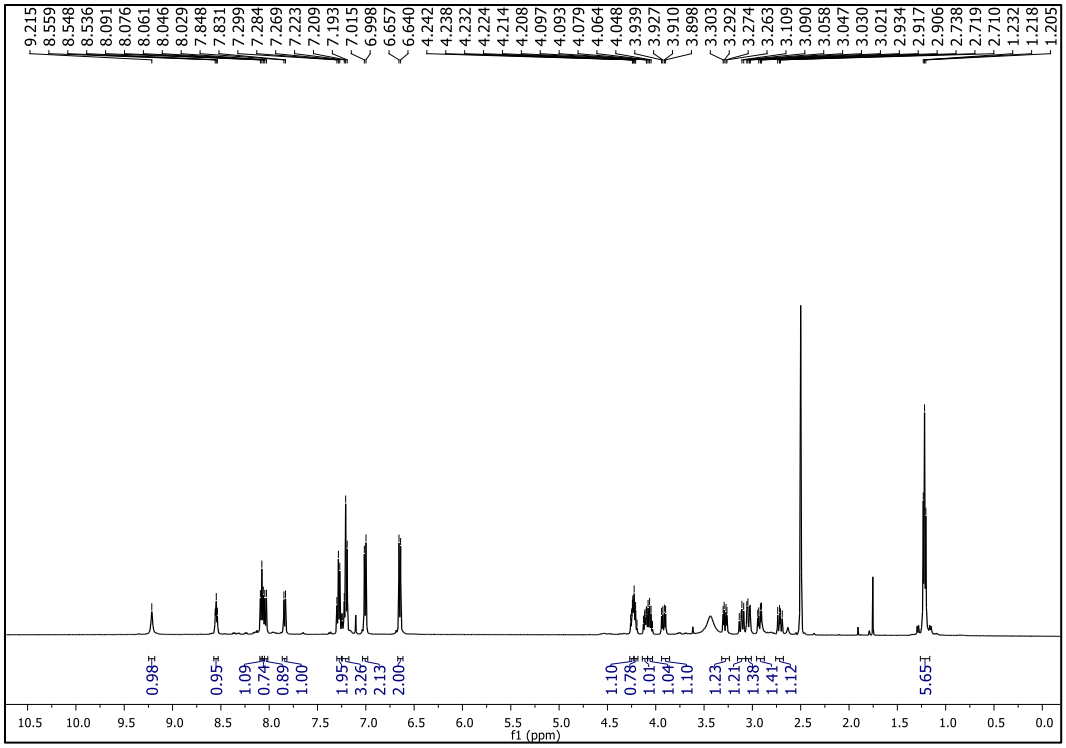


Figure S5. ^{1}H NMR (DMSO–d_6, 298 K) of synthetic sample of dichotomin E made via SPPS

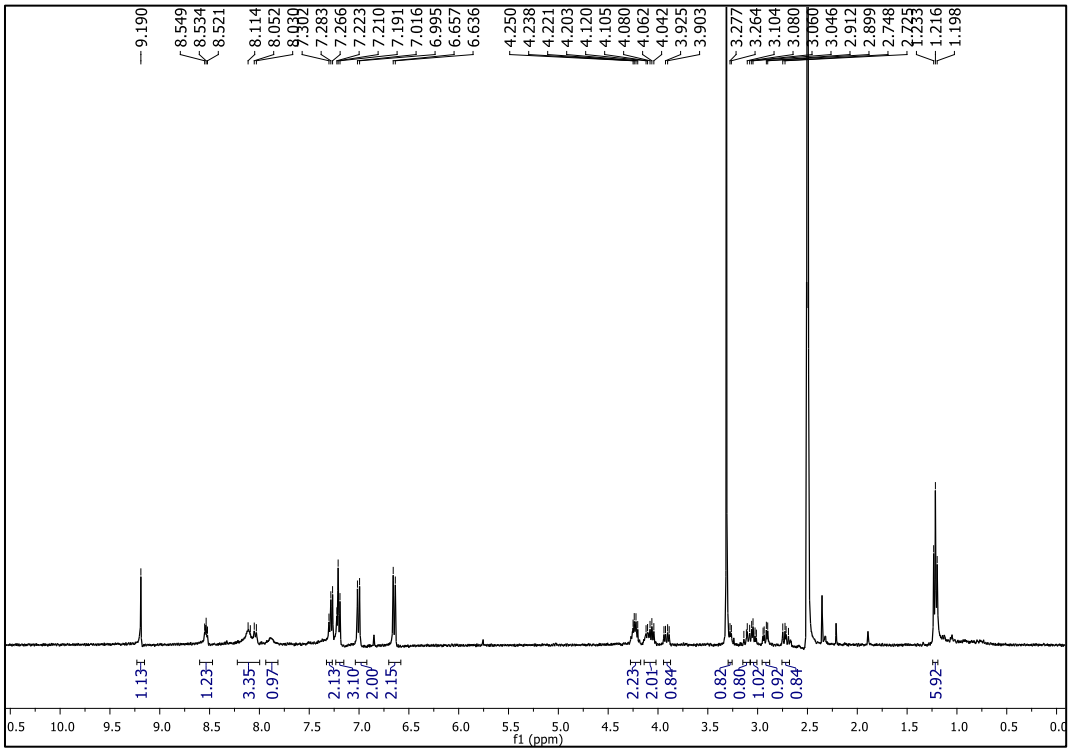
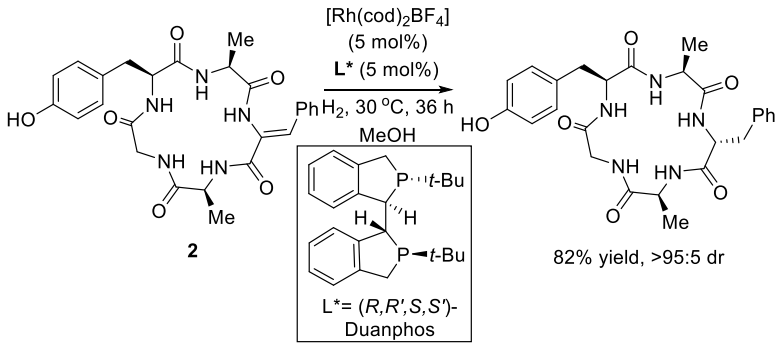
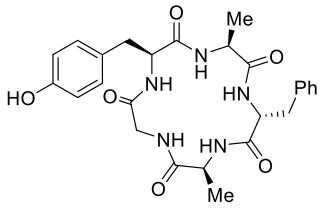


Figure S6. ^{1}H NMR (DMSO–d_6, 298 K) of synthetic sample of dichotomin E made via hydrogenation

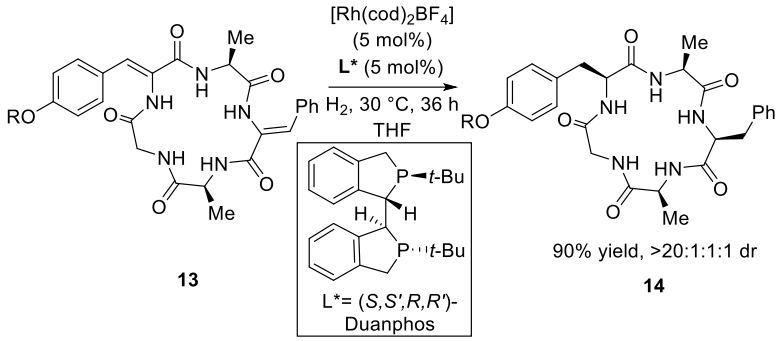


Dichotomin epimer (**1'**)

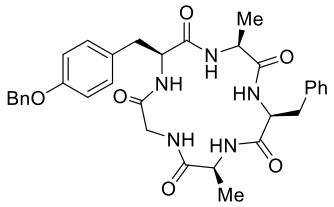


In a N_2-filled glovebox, $[Rh(cod)_2BF_4]$ (0.40 mg, 0.0010 mmol, 5 mol%) and (*R,R',S,S'*)-Duanphos (0.40 mg, 0.0010 mmol, 5 mol%) were added to a ½ dr vial equipped with a stir bar containing pentapeptide **2** (10 mg, 0.020 mmol). MeOH (394 µL) was added and the vial was capped with a screwcap with a slitted rubber septum and taken outside of the glovebox. The vial was then placed in the HEL CATalyst block and the head was screwed into place. The block was filled then purged three times with hydrogen, and then pressurized with hydrogen to 30 atm. The reaction stirred at 30 °C. The reaction was stopped after 36 h and the solvent was concentrated under reduced pressure. The reaction mixture was triturated with DCM to afford the product as an off-white solid (8.2 mg, 82%). ^{1}H NMR (500 MHz, DMSO) δ 9.22 (s, 1H), 8.60 (d, *J* = 7.7 Hz, 1H), 8.35 (d, *J* = 7.5 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.98 (t, *J* = 5.5 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.25 (dt, *J* = 13.9, 7.1 Hz, 4H), 7.19 (t, *J* = 7.0 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 2H), 4.40 (dd, *J* = 14.9, 7.8 Hz, 1H), 4.34 – 4.21 (m, 2H), 4.09 (ddd, *J* = 9.9, 7.5, 5.1 Hz, 1H), 3.73 (dd, *J* = 14.4, 4.9 Hz, 1H), 3.53 (dd, *J* = 14.3, 6.3 Hz, 1H), 2.98 (dd, *J* = 13.7, 6.6 Hz, 1H), 2.88 (dd, *J* = 14.0, 4.9 Hz, 1H), 2.75 (ddd, *J* = 24.1, 13.9, 9.2 Hz, 2H), 1.14 (d, *J* = 7.2 Hz, 3H), 1.06 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 172.47, 171.99, 170.97, 170.84, 169.38, 155.94, 137.81, 129.89, 129.13, 128.12, 127.62, 126.31, 115.04, 56.99, 54.43, 47.91, 47.79, 42.76, 35.85, 35.39, 18.02, 17.16. IR (ATR): 3271, 3086, 2984, 1638, 1539, 1443, 1372, 1233 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{26}H_{31}N_5O_6Na$ $[M+Na]^+$: 532.2172, found: 532.2151. $[\alpha]^{26}_D$ –14 (*c* = 0.39, MeOH).

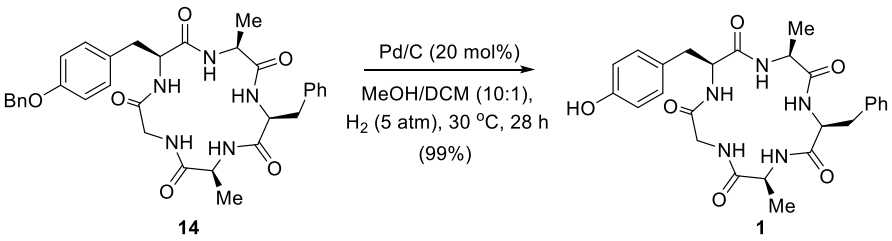
ii) Hydrogenation of Gly-ΔTyr-Ala-ΔPhe-Ala ***13***



(3*S*,6*S*,9*S*,12*S*)-6-benzyl-12-(4-(benzyloxy)benzyl)-3,9-dimethyl-1,4,7,10,13-pentaazacyclopentadecane-2,5,8,11,14-pentaone (14)



In a N_2-filled glovebox, $[Rh(cod)_2BF_4]$ (2 mg, 0.005 mmol, 5 mol%) and (*S,S',R,R'*)-Duanphos (0.8 mg, 0.002 mmol, 5 mol%) were added to a ½ dr vial equipped with a stir bar containing pentapeptide **13** (24 mg, 0.040 mmol). THF (0.8 mL) was added and the vial was capped with a screwcap with a slitted rubber septum and taken outside of the glovebox. The vial was sonicated to ensure all contents were dissolved and then placed in the HEL CATalyst block and the head was screwed into place. The block was filled then purged three times with hydrogen, and then pressurized with hydrogen to 30 atm. The reaction was stopped after 24 h and the solvent was removed under reduced pressure. The compound was then triturated with DCM to afford the product as a light tan solid (21.6 mg, 90%). ^{1}H NMR (400 MHz, DMSO) δ 8.56 (t, *J* = 5.7 Hz, 1H), 8.12 – 8.03 (m, 3H), 7.85 (d, *J* = 8.1 Hz, 1H), 7.44 (d, *J* = 7.0 Hz, 2H), 7.39 (t, *J* = 7.2 Hz, 2H), 7.35 – 7.30 (m, 1H), 7.28 (d, *J* = 6.9 Hz, 2H), 7.21 (t, *J* = 7.1 Hz, 3H), 7.14 (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 5.06 (s, 2H), 4.31 – 4.17 (m, 2H), 4.14 – 4.02 (m, 2H), 3.92 (dd, *J* = 14.3, 6.4 Hz, 1H), 3.29 – 3.24 (m, 1H), 3.13 (dd, *J* = 13.3, 9.4 Hz, 1H), 3.04 (dd, *J* = 13.7, 5.5 Hz, 1H), 2.99 (dd, *J* = 14.3, 4.7 Hz, 1H), 2.77 (dd, *J* = 13.6, 9.3 Hz, 1H), 1.22 (dd, *J* = 7.0, 4.0 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 172.58, 171.65, 170.83, 170.39, 169.27, 156.99, 137.73, 137.19, 130.00, 129.79, 129.11, 128.43, 128.24, 127.80, 127.70, 126.46, 114.51, 69.11, 56.98, 55.61, 49.57, 48.28, 43.34, 36.02, 35.98, 17.52, 17.08. IR (ATR): 3077, 3014, 2978, 1672, 1658, 1645, 1500, 1239 cm^{-1}. HRMS (ESI-TOF) *m/z* calc'd for $C_{33}H_{37}N_5O_6Na$ $[M+Na]^+$: 622.2642, found: 622.2639. $[\alpha]^{27}_D$ +114 (*c* = 0.19, DMF).



To a ½ dr vial equipped with a stir bar was added cyclic pentapeptide **14** (5 mg, 0.008 mmol) and 10% Pd/C (1.7 mg, 0.0016 mmol, 20 mol%). The contents were dissolved in MeOH (0.600 mL) and DCM (0.060 mL) and the vial was capped with a screwcap with a slitted rubber septum. The vial was sonicated to ensure all contents were dissolved and then placed in the HEL CATalyst block and the head was screwed into place. The block was filled then purged three times with hydrogen, and then pressurized with hydrogen to 5 atm. The reaction was warmed to 30 ^{o}C. After 28 h and the reaction mixture was filtered through a plug of celite to afford dichotomin E **1** as an off-white solid (4.1 mg, 99%). ^{1}H NMR of the product after debenzylation showed variation in chemical shift values. The product obtained after debenzylation was then spiked with authentic synthetic sample obtained via SPPS (Table S1) to afford the ^{1}H NMR spectrum of dichotomin E (Figure S7).

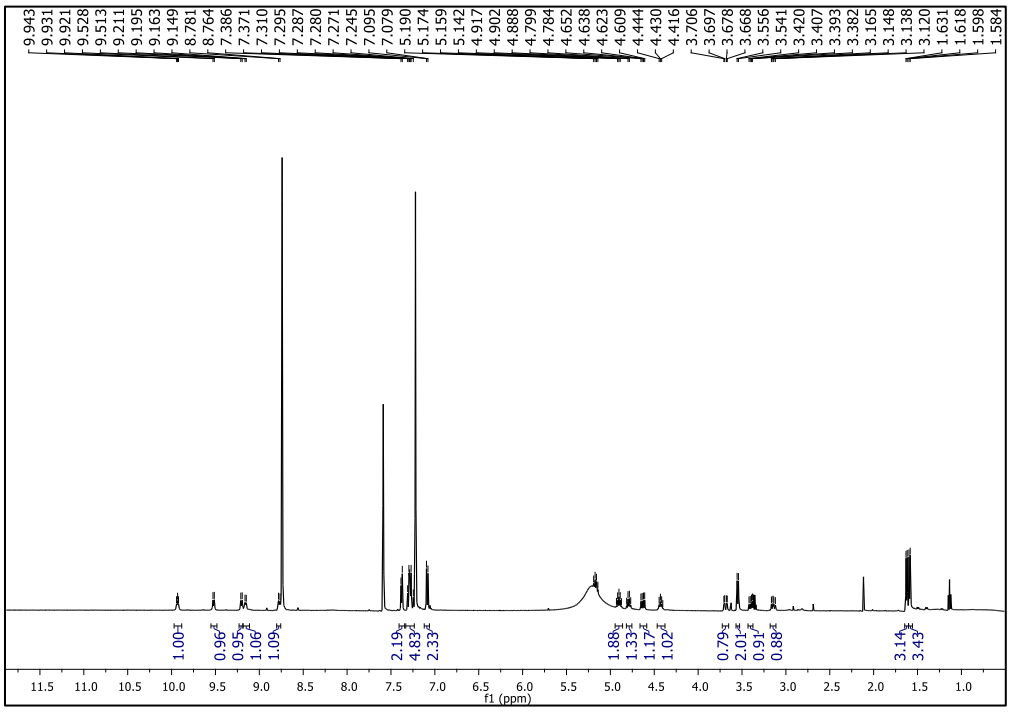


Figure S7. ^{1}H NMR spectrum (Pyr–d_5, 298 K) of synthetic sample of dichotomin E after debenzylation spiked with authentic material.

5. Circular dichroism spectroscopy

Peptide solutions with a concentration of 1.13 μM for pentapeptide **11**, 1.15 μM for pentapeptide **3** and 1.34 μM for pentapeptide **2** in MeOH were prepared. CD measurements were performed on a Jasco J-810 spectropolarimeter. Spectra were recorded at 296 K, with a 1 mm Starna quartz cell, over the wavelength range of 320-210 nm at 50 nm/min, with a bandwidth of 1.0 nm, response time of 1 s, resolution step width of 0.5 nm and sensitivity of 100 Mdeg. Each spectrum represents the average of 5 scans. Spectra were analysed using OriginLab data analysis software.

6. NMR analysis and molecular modeling

i) Variable temperature NMR experiment

^{1}H-NMR variable temperature experiments[17] were performed on a 500 MHz Bruker DRX500 spectrometer with a TCI cryoprobe. For each experiment, the temperature was increased by 5 K with 10 minute equilibration time between experiments. 8 scans were collected for each data point. The experiments were conducted in DMSO solvent. After obtaining the spectra, the δ (ppm) was plotted vs. T(K), and the Δδ/ ΔT was obtained for each N–H bond.

Table S3. Variable temperature data for unsaturated pentapeptide **3**

T (K)	δ (ppm) Tyr N–H	δ (ppm) Ala N–H (internal)	δ (ppm) ΔPhe N–H	δ (ppm) Ala N–H (terminal)
293	8.5345	7.9371	9.6430	8.5933
298	8.5144	7.9170	9.6164	8.5649
303	8.4947	7.8974	9.5897	8.5364
308	8.4753	7.8782	9.5633	8.5076
313	8.4562	7.8593	9.5385	8.4780

Table S4. Variable temperature data for saturated pentapeptide **11**

T (K)	δ (ppm) Tyr N–H	δ (ppm) Ala N–H (internal)	δ (ppm) Phe N–H	δ (ppm) Ala N–H (terminal)
293	8.5562	8.3481	7.8956	8.3254
298	8.5355	8.3086	7.8792	8.2886
303	8.5186	–	7.8458	–
308	8.4997	8.2645	7.8222	8.2436
313	8.4809	8.2390	7.7995	8.2133

[17] Kessler, H. *Angew. Chem. Int. Ed.* **1982**, *21*, 512.

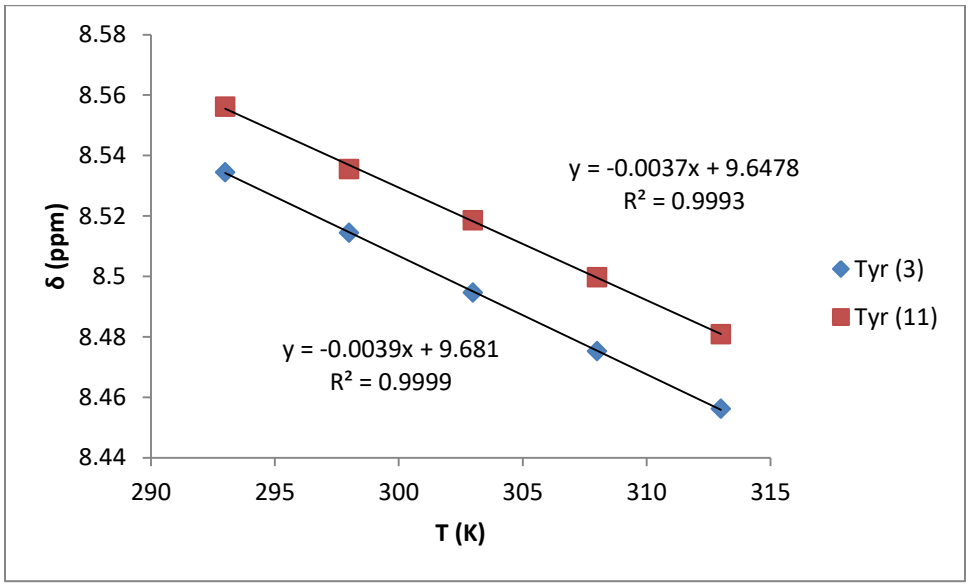


Figure S8. Variable temperature data comparing tyrosine N–H for unsaturated peptide 3 and saturated peptide 11

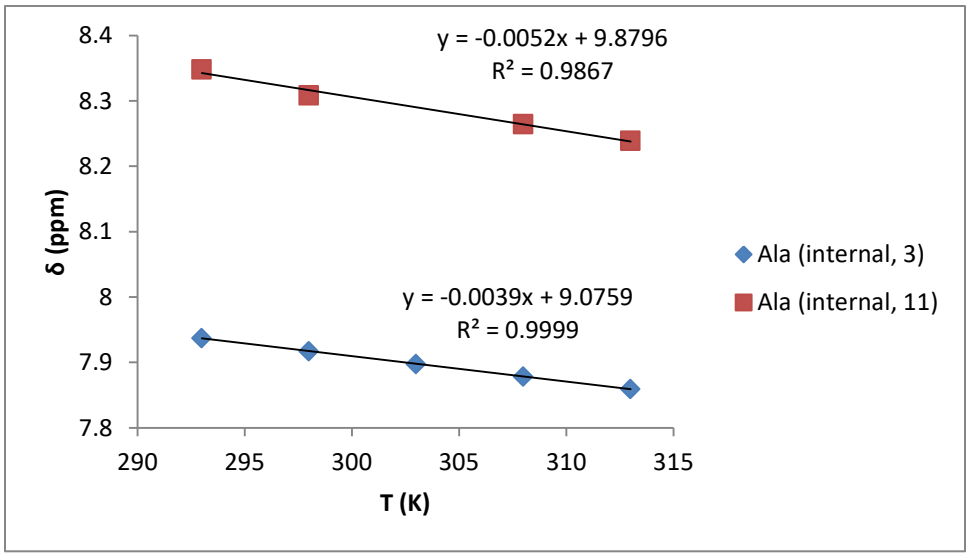


Figure S9. Variable temperature data comparing internal alanine N–H for unsaturated peptide 3 and saturated peptide 11

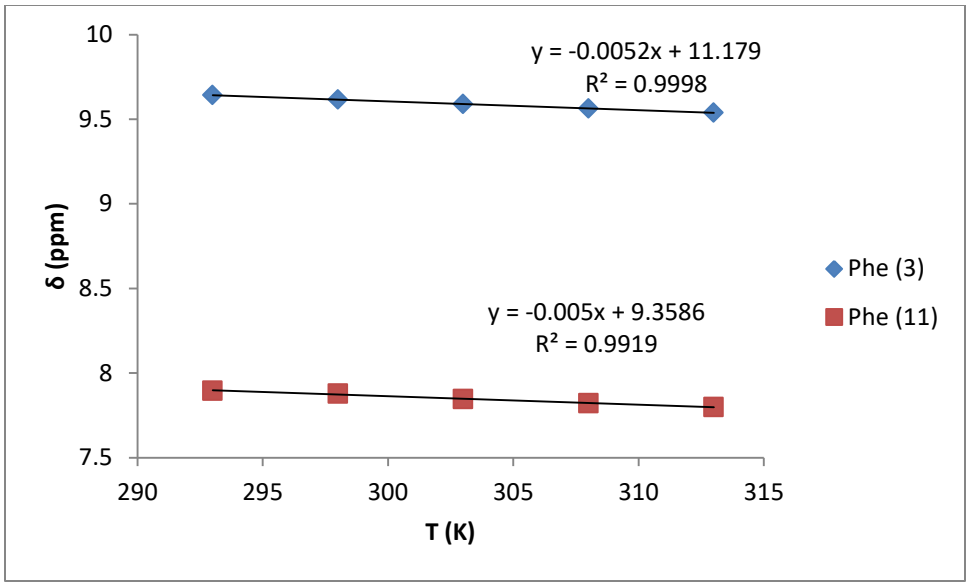


Figure S10. Variable temperature data comparing phenylalanine N–H for unsaturated peptide 3 and saturated peptide 11

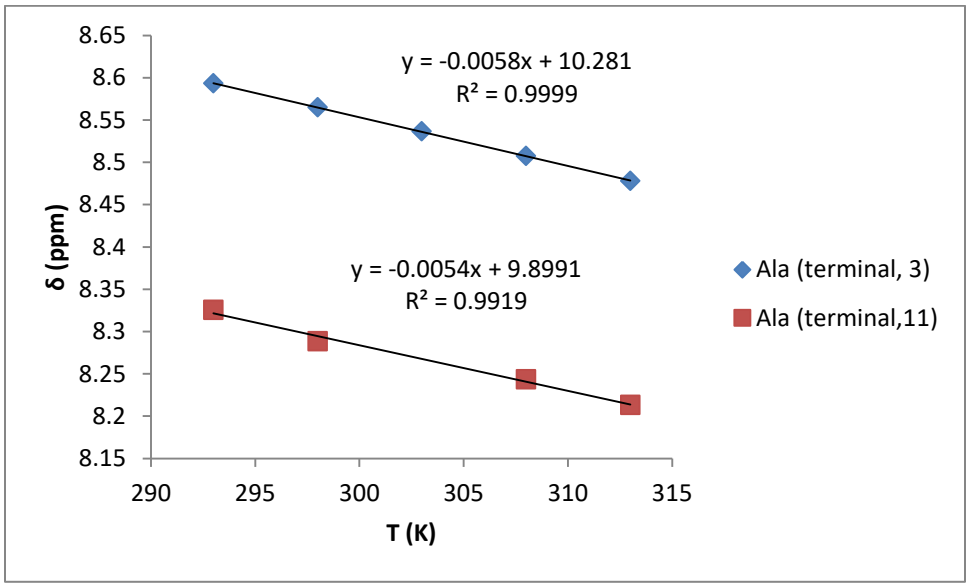
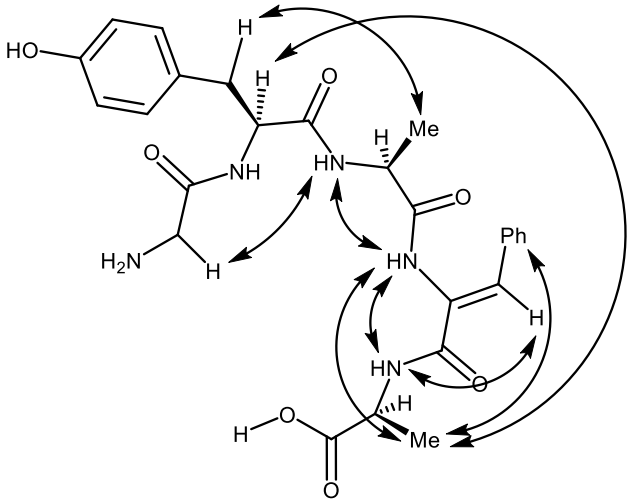
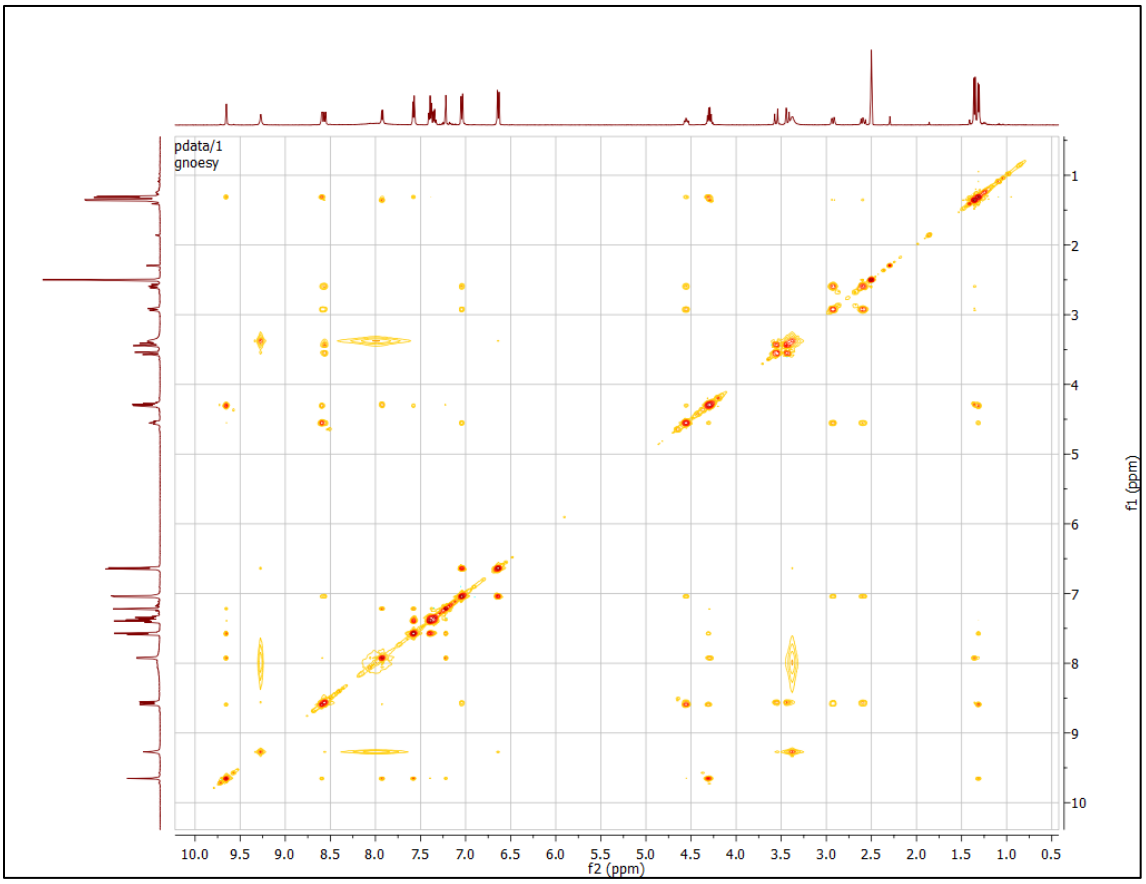


Figure S11. Variable temperature data comparing terminal alanine N–H for unsaturated peptide 3 and saturated peptide 11

ii) 2D NOESY Spectroscopy

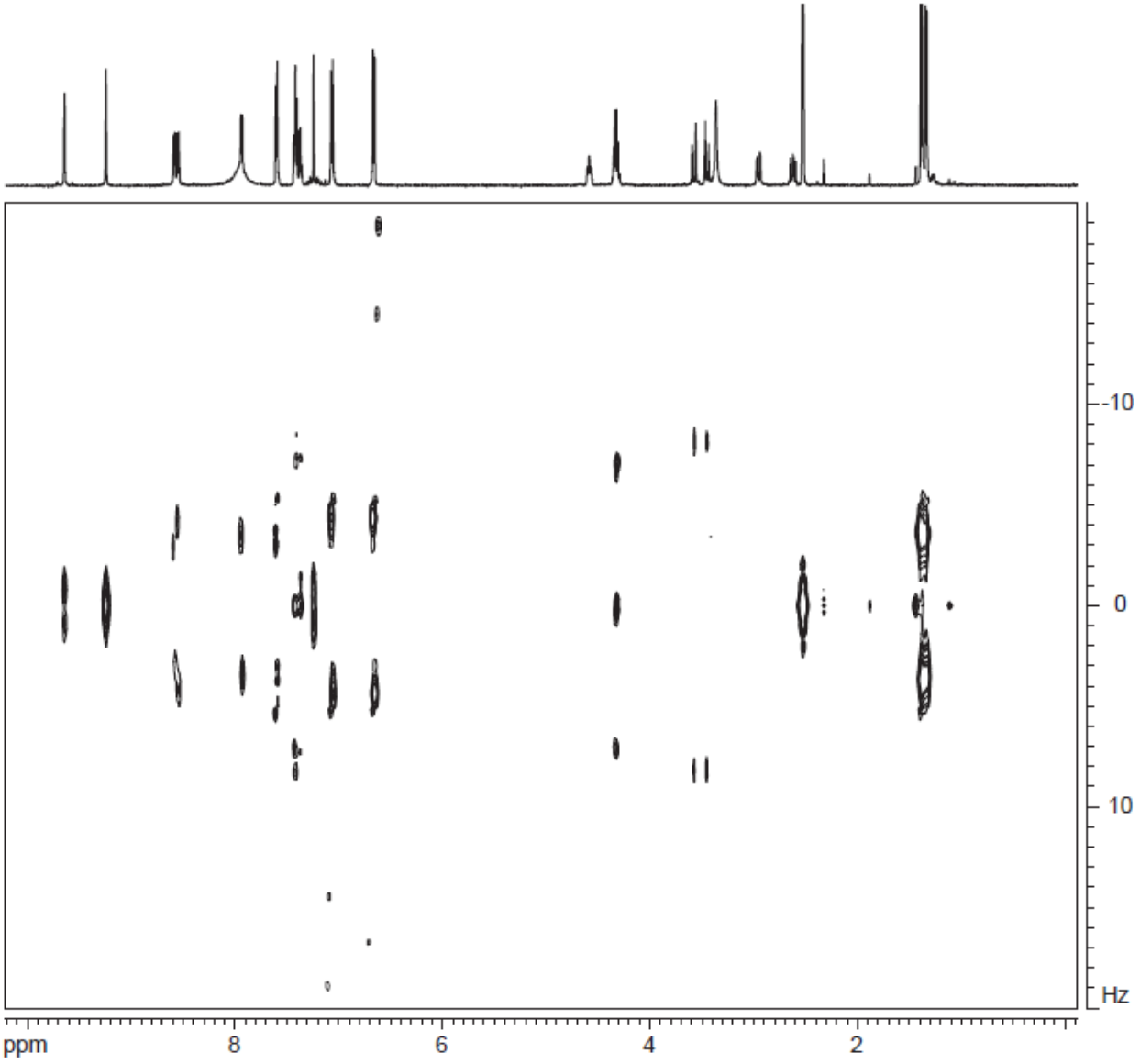
2D NOESY experiments were performed on a 500 MHz Bruker DRX500 spectrometer with a TCI cryoprobe with 2 number of scans, 800 ms mixing time, 2 s relaxation delay, and a spectral width of 8012.8 Hz.





iii) 2D J-resolved experiment

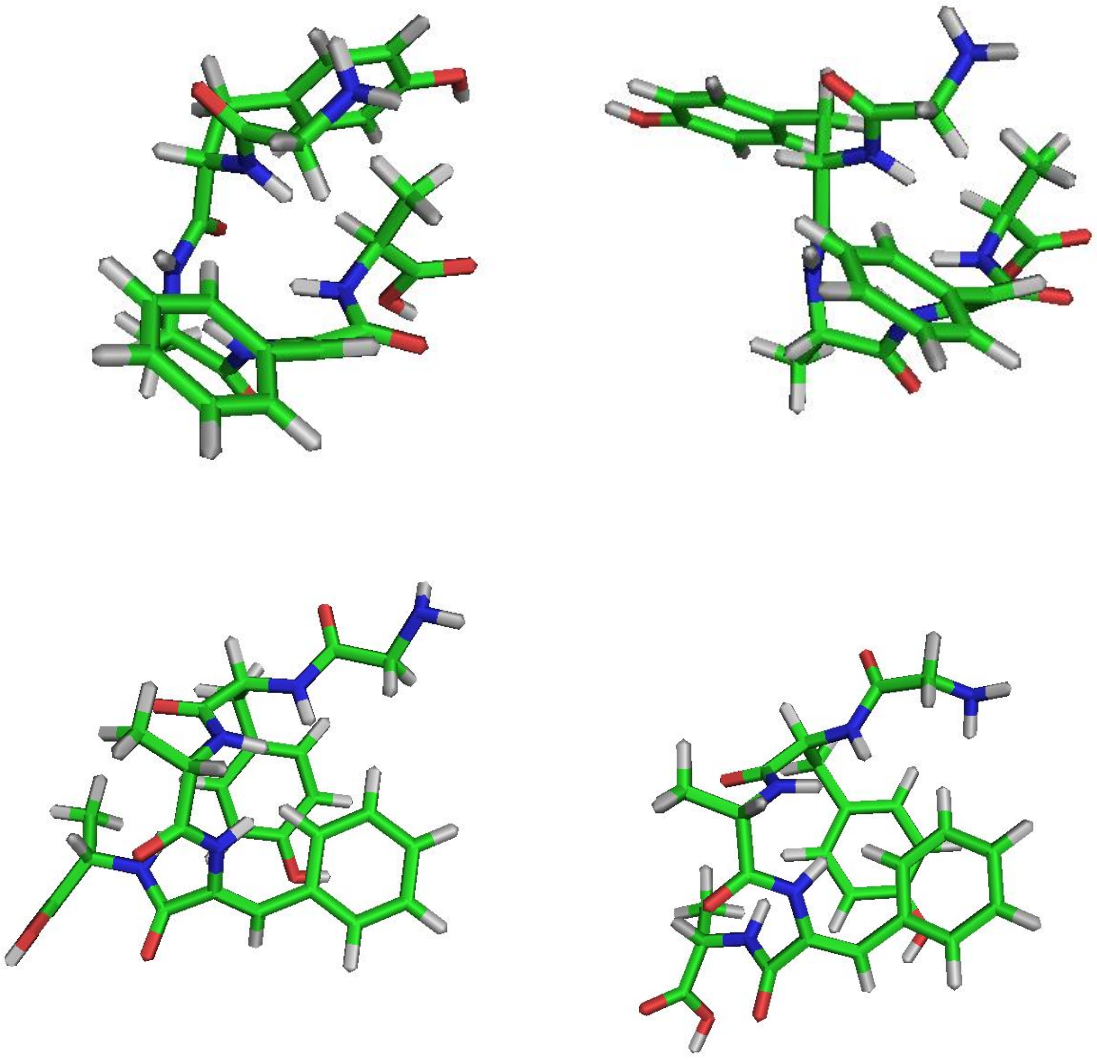
2D ^{1}H *J*-resolved experiments were performed on a 500 MHz Bruker DRX500 spectrometer with a TCI cryoprobe with 4 number of scans and 256 number of points in the indirect dimension and a spectral width of 5165.289 Hz. Using the Karplus curve[18] and the ^{3}J couplings measured for $H_NH\alpha$ of Tyr, Ala (internal) and Ala (terminal) from the *J*-resolved experiment for the unsaturated peptide, the following φ H_NH_α dihedral angles were obtained ± 152.58°, ± 140.6° or ± 12.99°, ± 132.47° or ± 29.83°, respectively.



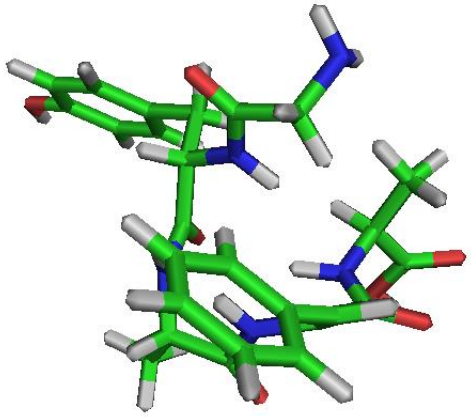
[18] Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870.

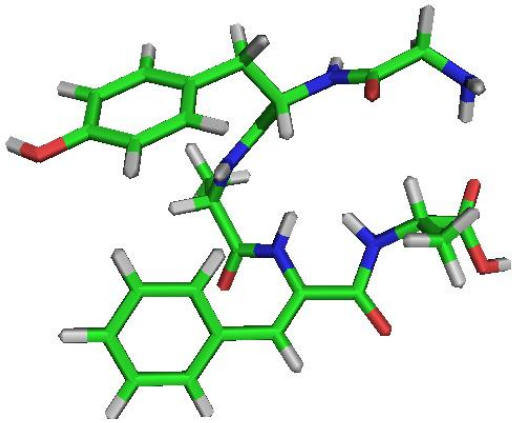
iv) Molecular modeling using Maestro

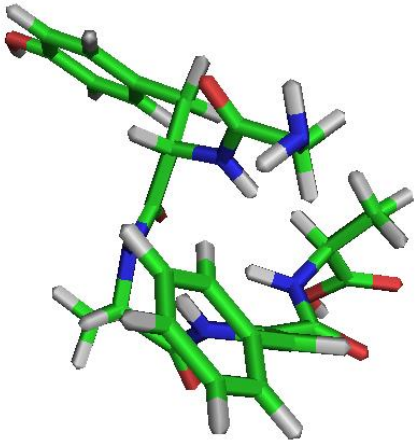
Structures with all experimentally feasible combinations of $H_NH\alpha$ ϕ dihedral angles for Tyr, Ala (internal) and Ala (terminal) were simulated in no solvent but with a dielectric constant corresponding to that of DMSO (47.6) using Maestro (Schrodinger, Inc.).[19] An error of ± 40° was added to every structure. Only weak and very weak distance restraints found from NOESY spectrum using a mixing time of 800 ms were also included in the simulations. The cross-peak volumes were classified as weak (upper distance constraint ≤ 5) and very weak (upper distance constraint ≤ 6). The lowest-energy 20 structures are displayed.

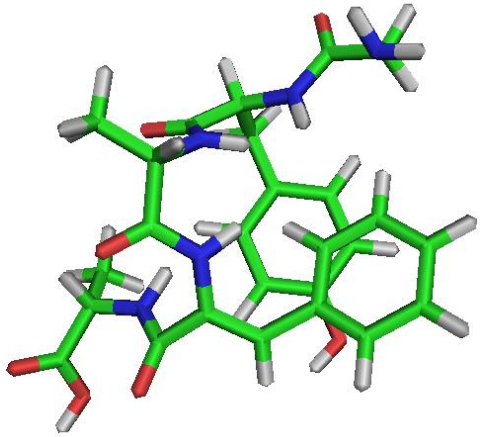


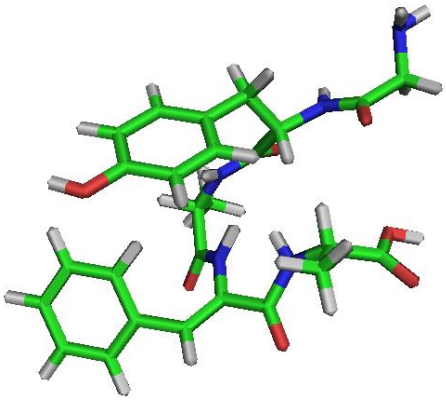
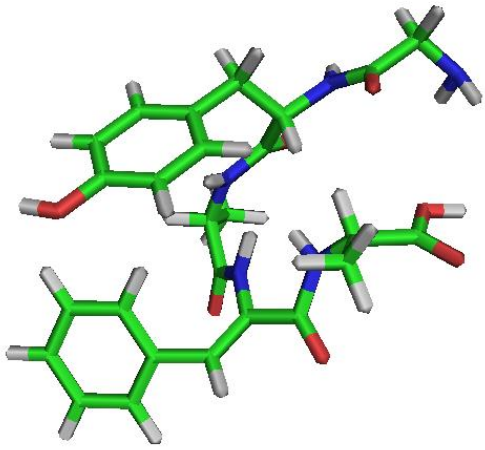
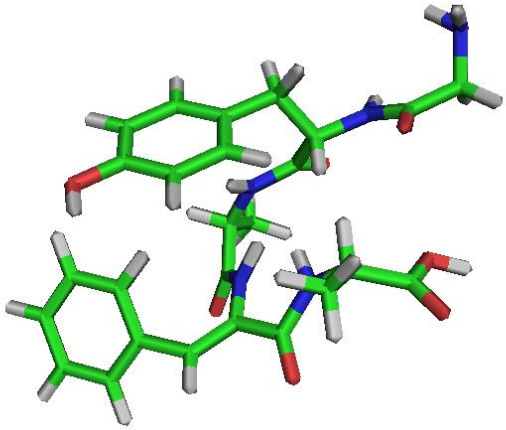
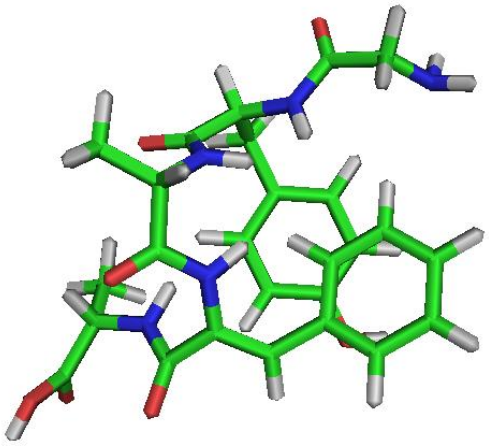
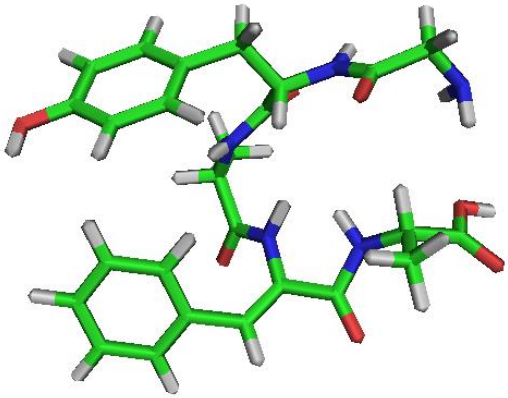
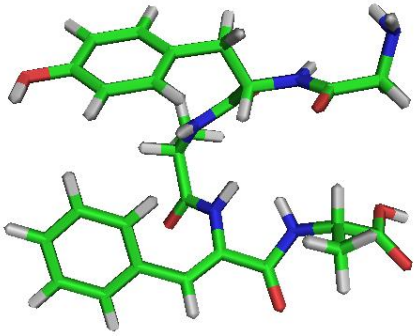
[19] Schrödinger Release 2015-2: Maestro, version 10.2, Schrödinger, LLC, New York, NY, 2015.

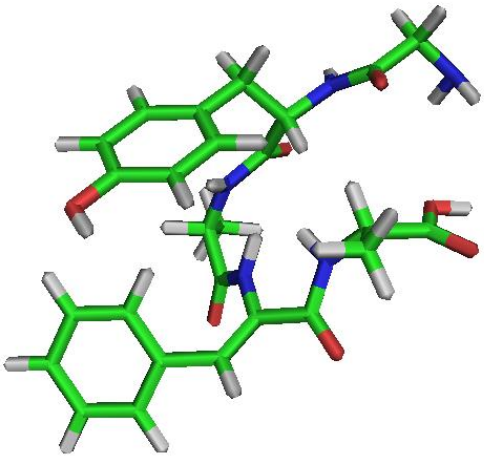


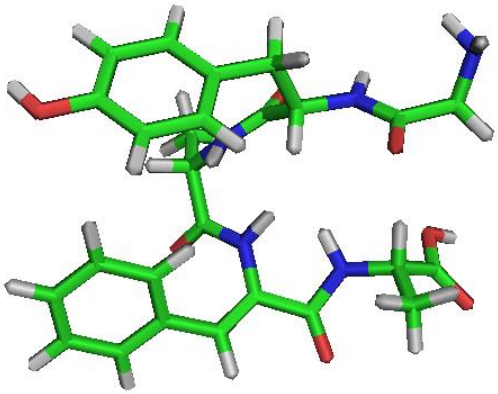


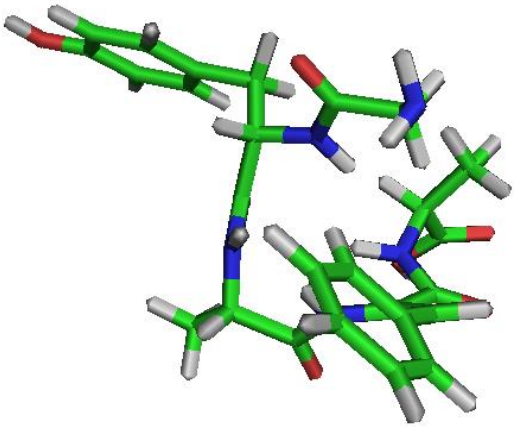


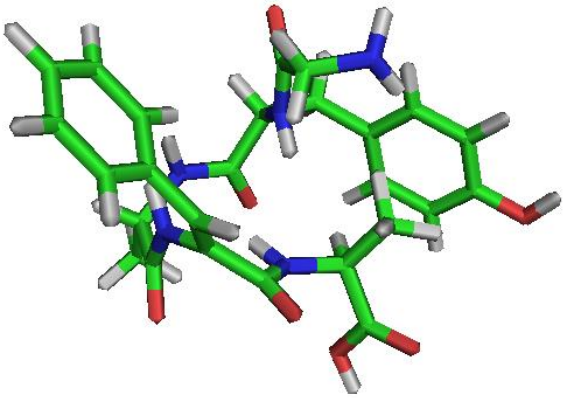


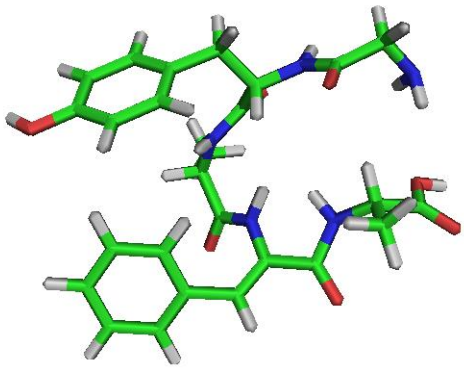









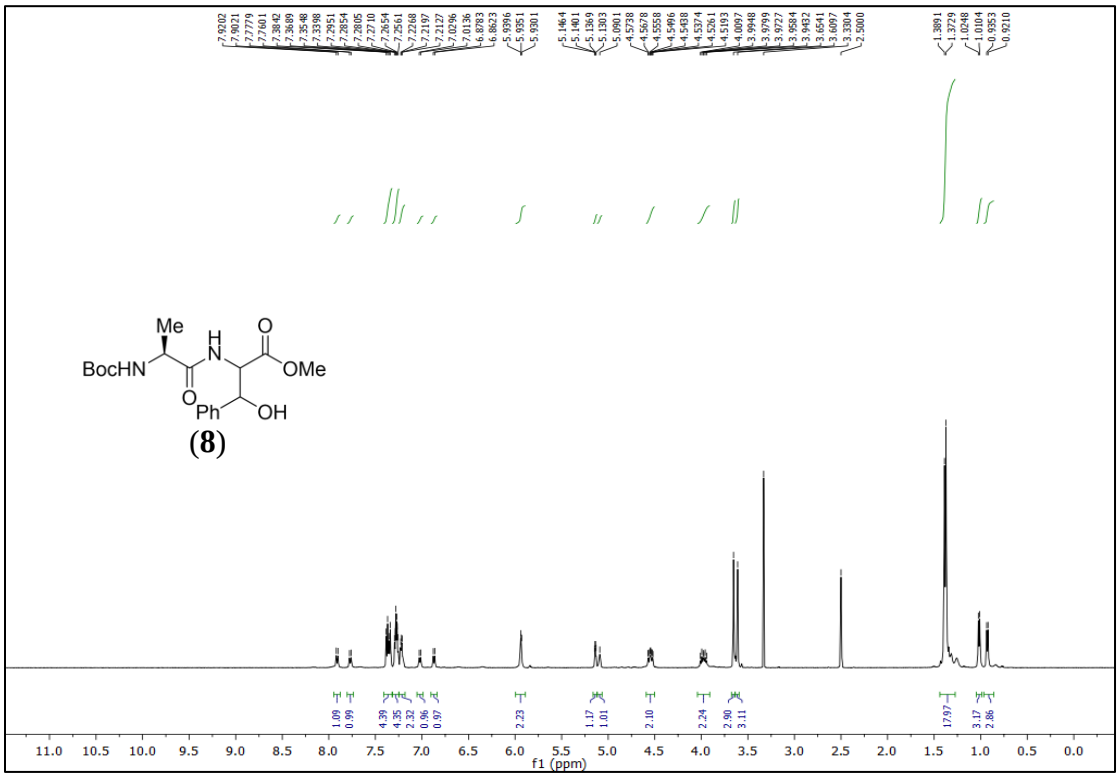


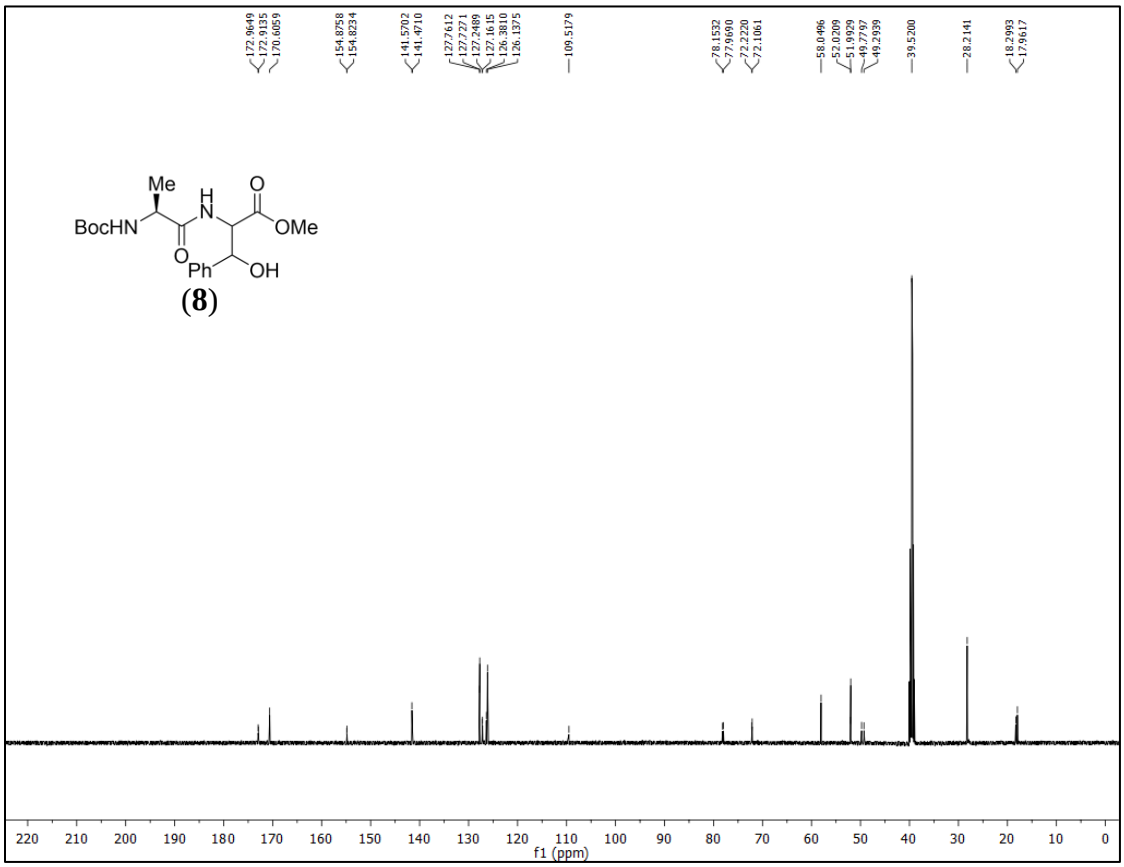


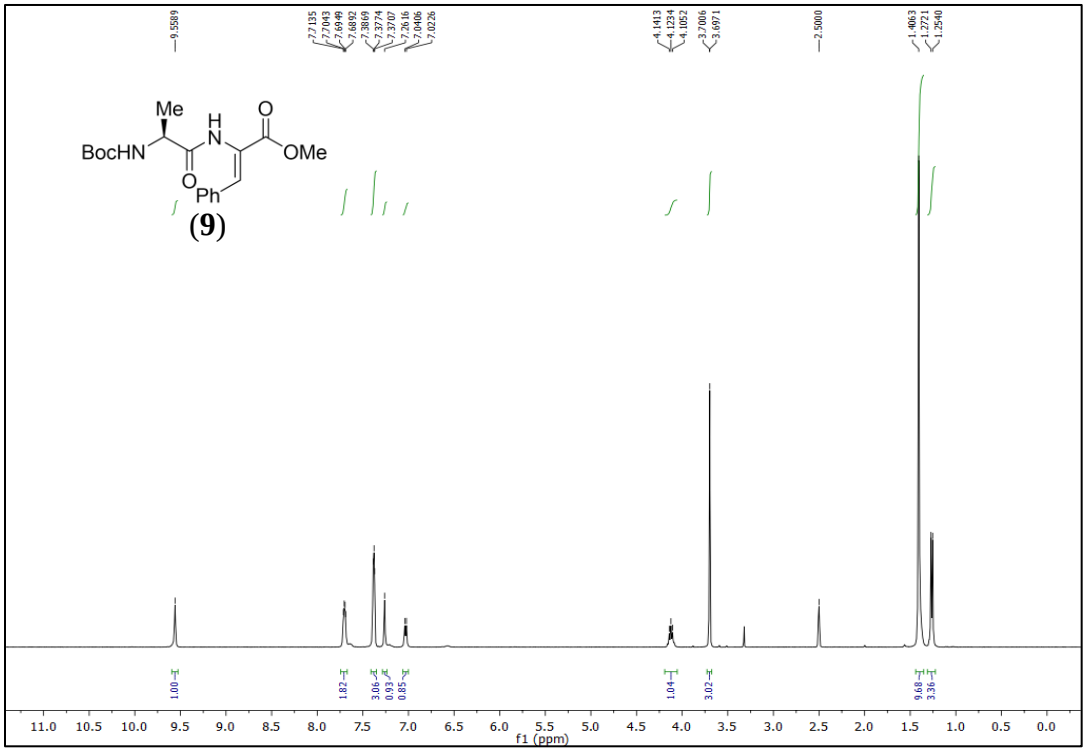


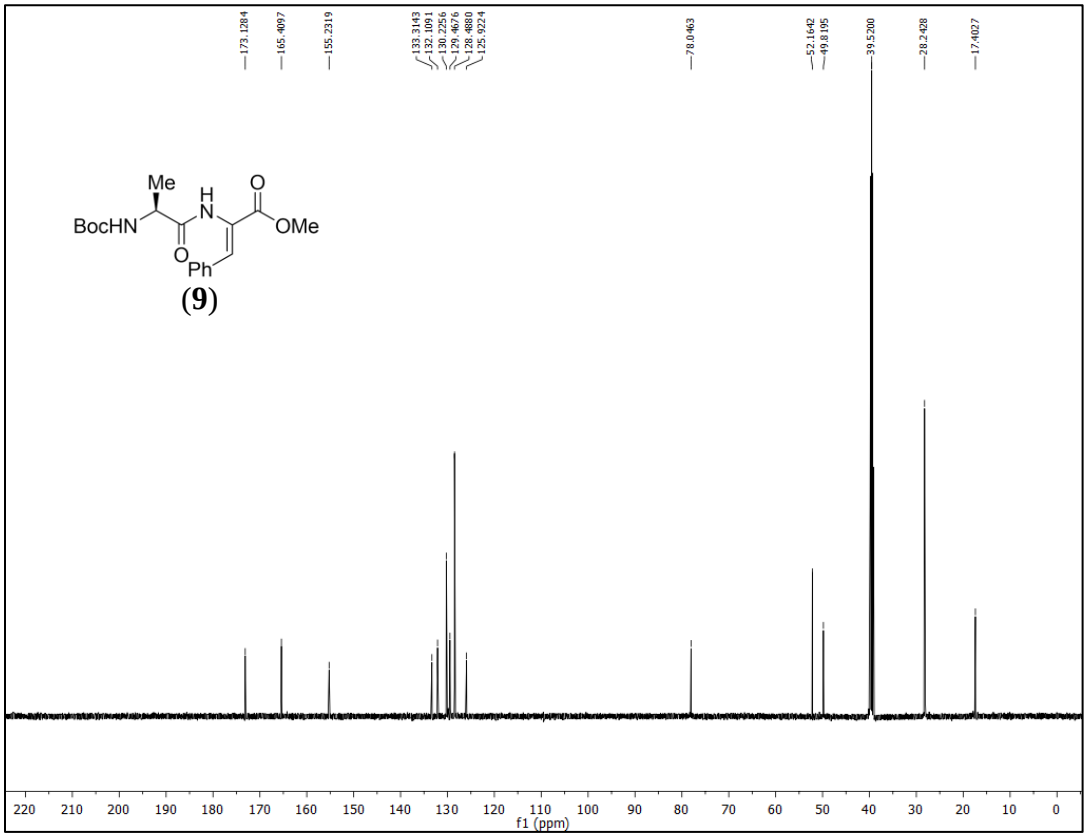


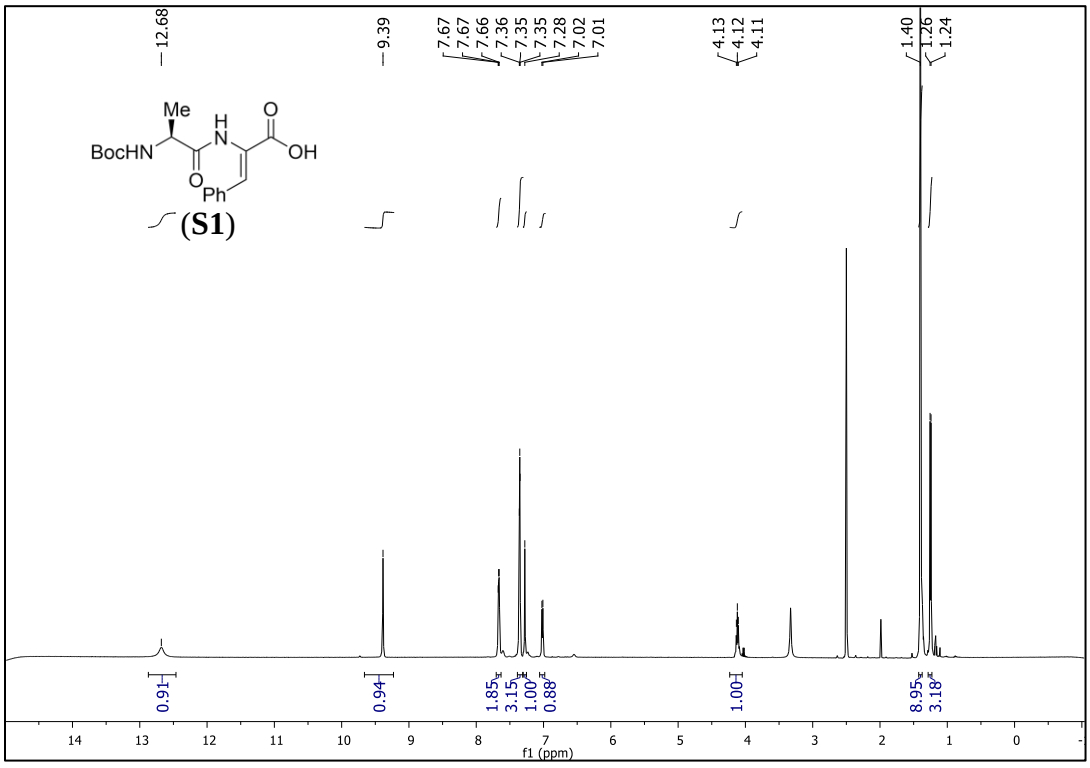
7) NMR Spectra

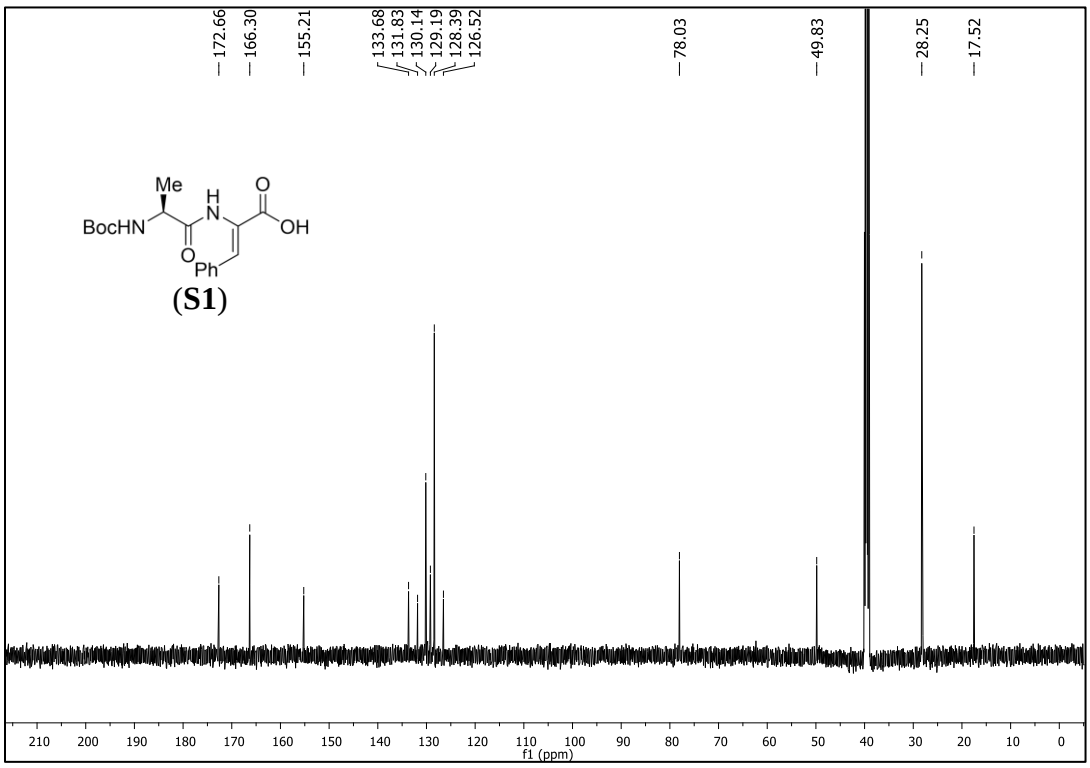


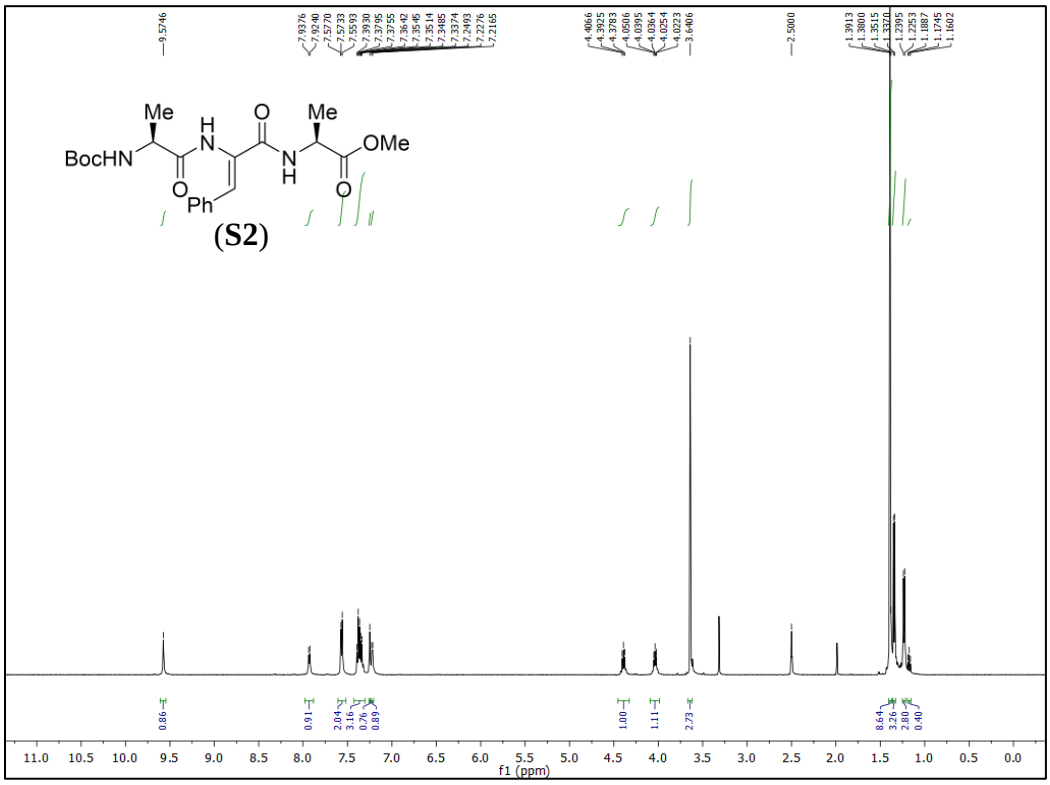


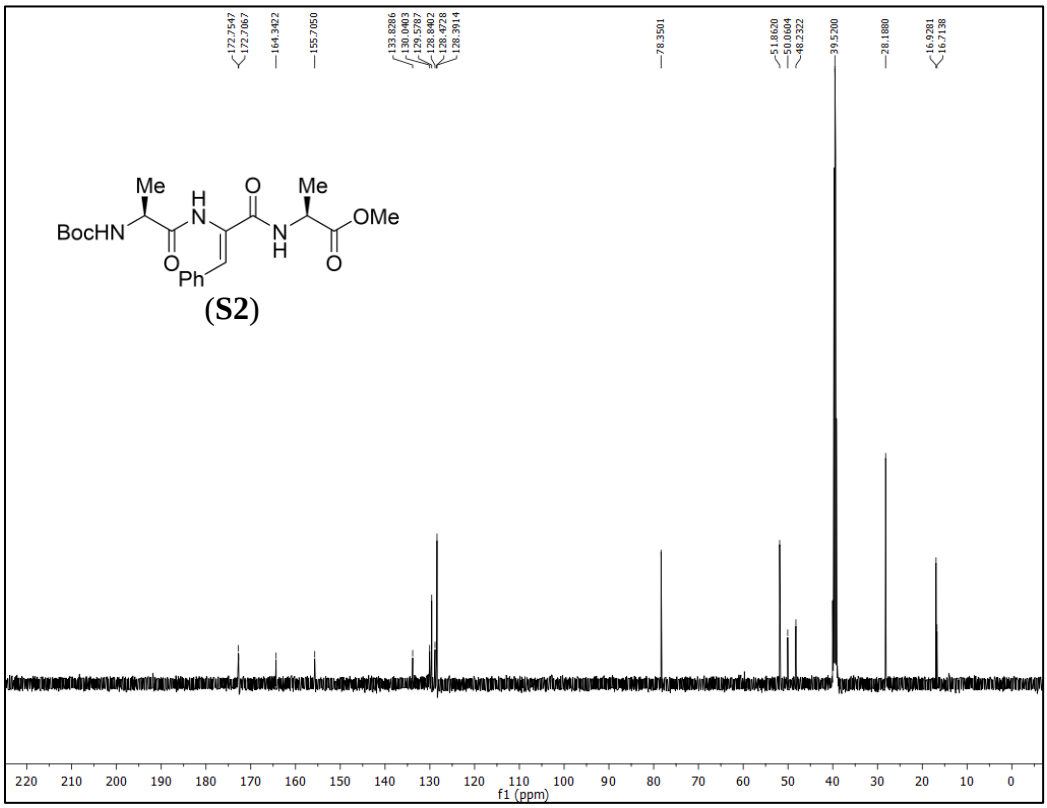

9.5589
7.7135
7.7043
7.6949
7.6892
7.3869
7.3774
7.3707
7.2616
7.0406
7.0226
4.1413
4.1234
4.1052
3.7006
3.6971
2.5000
1.4063
1.2721
1.2540
Me
H
N
O
BocHN
OMe
O
Ph
(9)
1.00
1.82
3.06
0.93
0.85
1.04
3.02
9.68
3.36
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

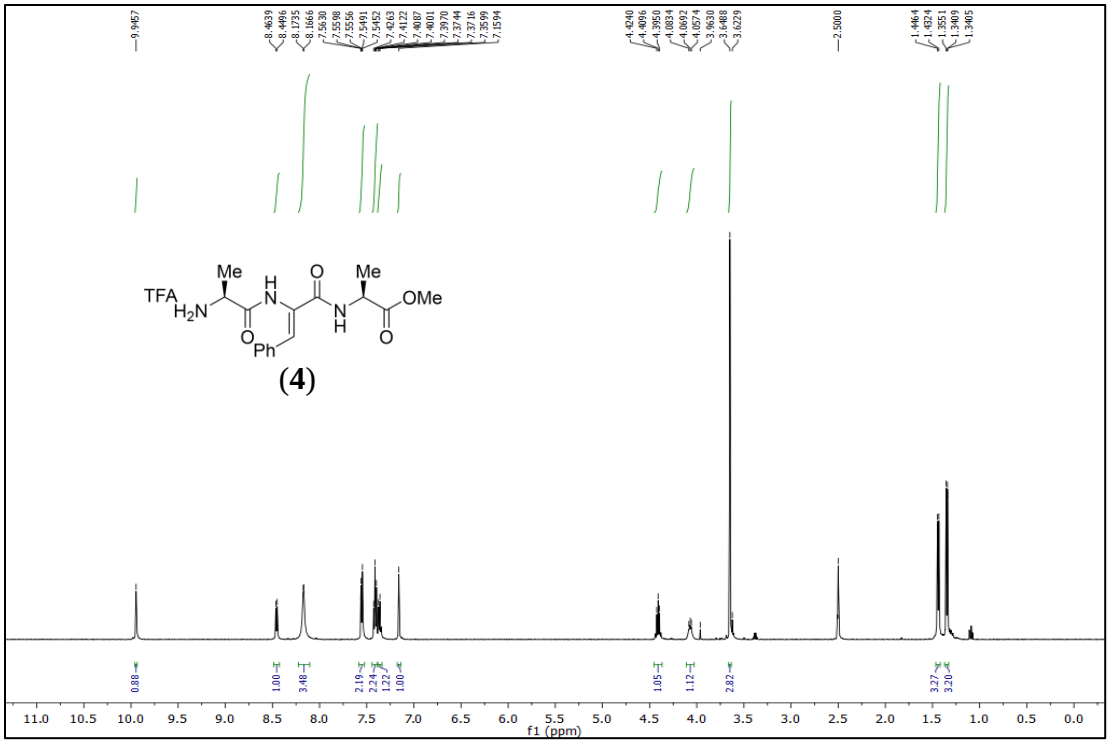

173.1284
165.4097
155.2319
133.3143
132.1091
130.2256
129.4676
128.4880
125.9224
78.0463
52.1642
49.8195
39.5200
28.2428
17.4027
Me
H
N
O
BocHN
OMe
O
Ph
(9)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

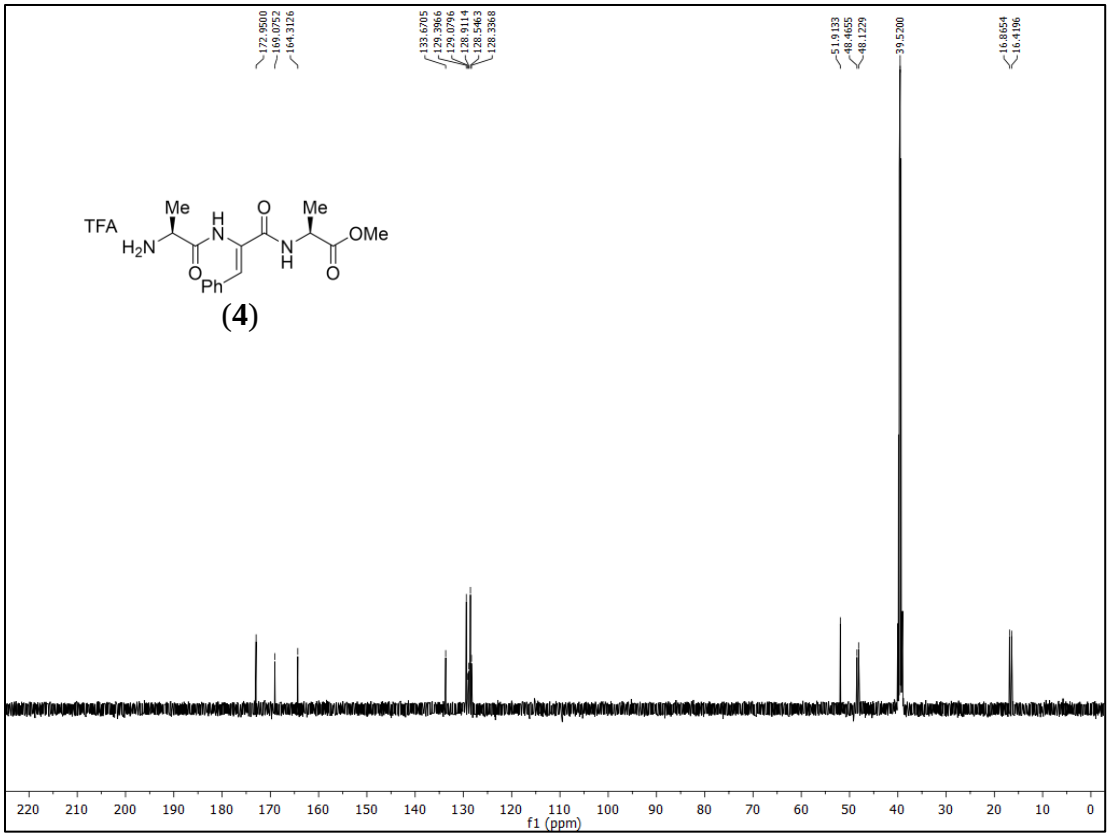

12.68
9.39
7.67
7.67
7.66
7.36
7.35
7.35
7.28
7.02
7.01
4.13
4.12
4.11
1.40
1.26
1.24
Me
H
N
O
BocHN
OH
O
Ph
(S1)
0.91
0.94
1.85
3.15
1.00
0.88
1.00
8.95
3.18
f1 (ppm)

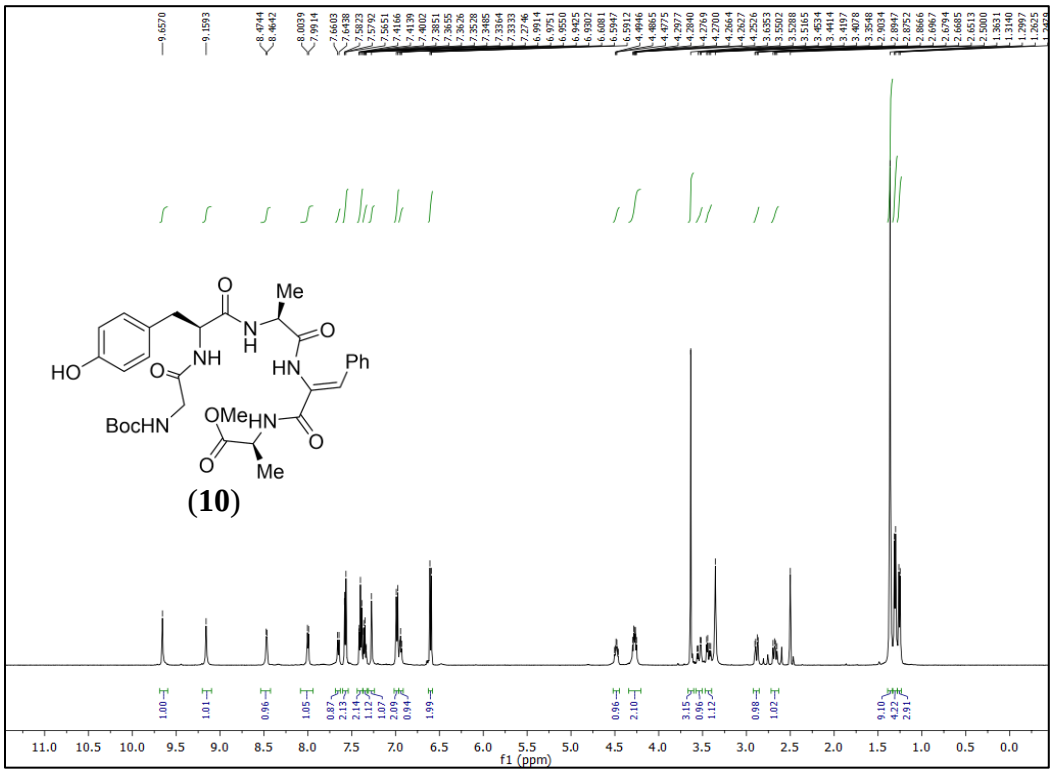

172.66
166.30
155.21
133.68
131.83
130.14
129.19
128.39
126.52
78.03
49.83
28.25
17.52
Me
H
N
O
BocHN
OH
O
Ph
(S1)
f1 (ppm)


(S2)
f1 (ppm)

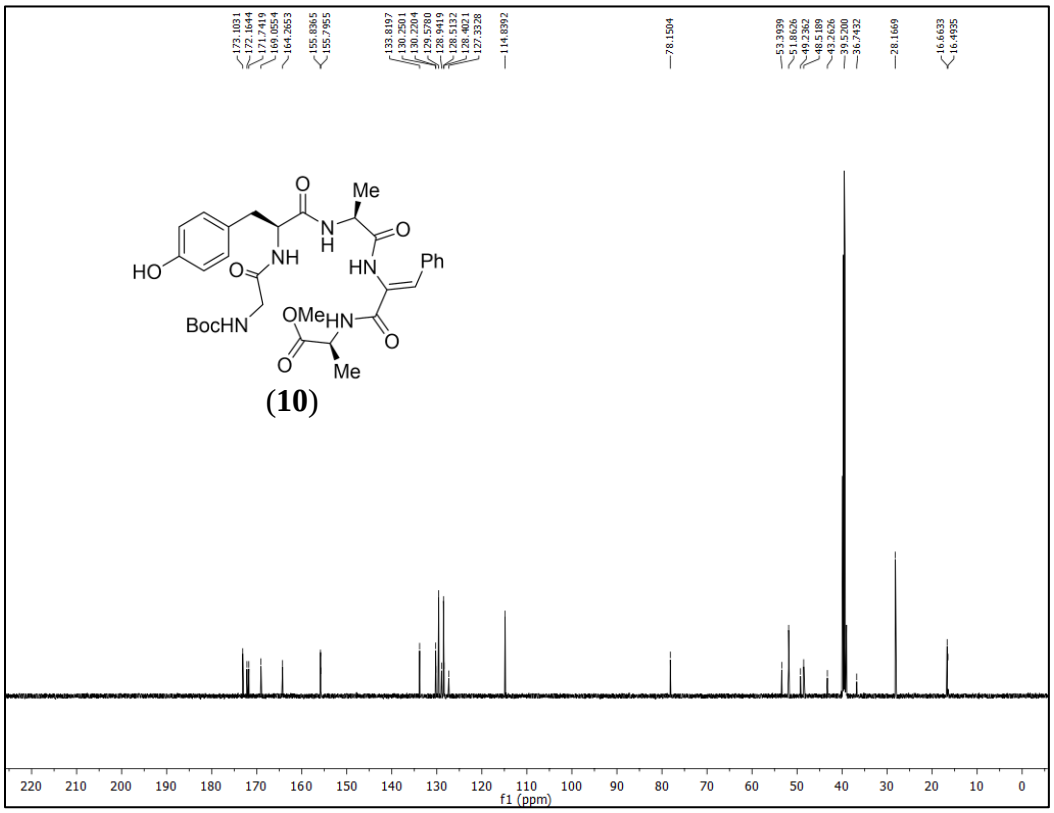

(S2)
f1 (ppm)


TFA
H2N
Me
H
N
O
Me
N
H
OMe
O
O
Ph
(4)
f1 (ppm)

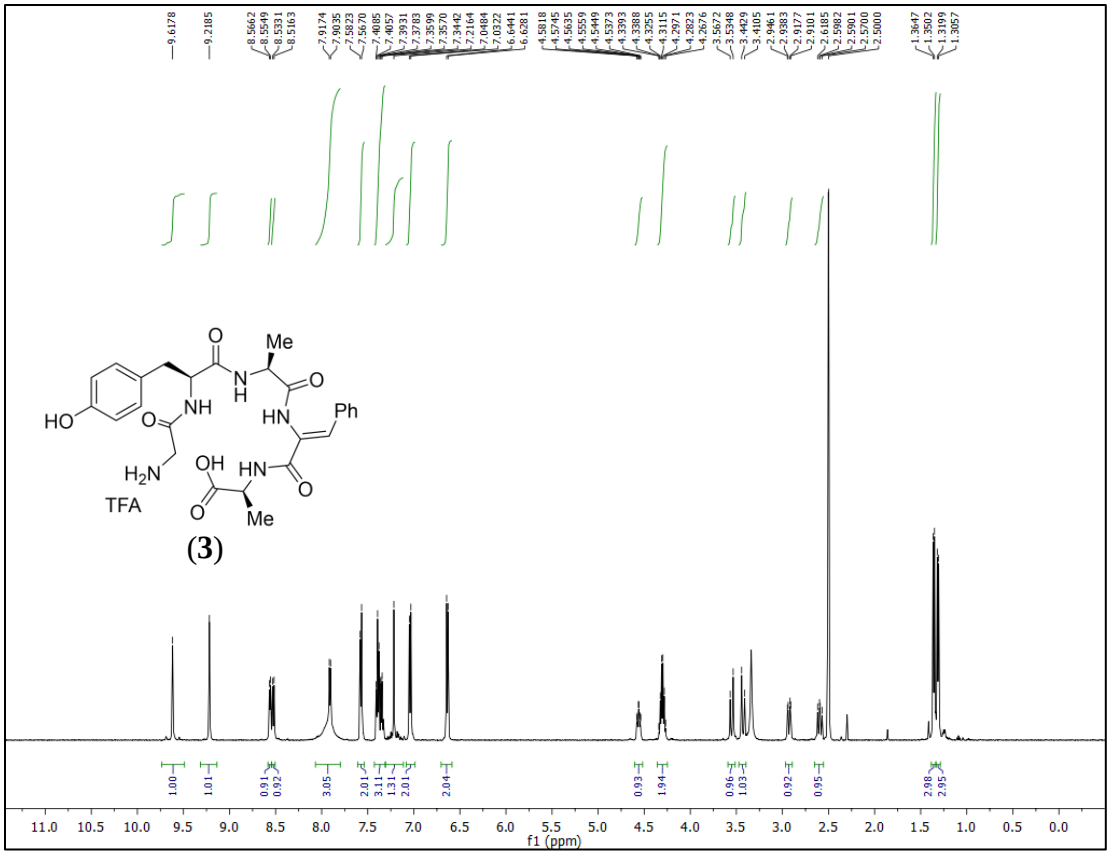

TFA
H2N
Me
H
N
O
Me
N
H
OMe
O
O
Ph
(4)
f1 (ppm)


HO
NH
O
N
H
Me
O
HN
Ph
BocHN
OMe
HN
O
O
Me
f1 (ppm)

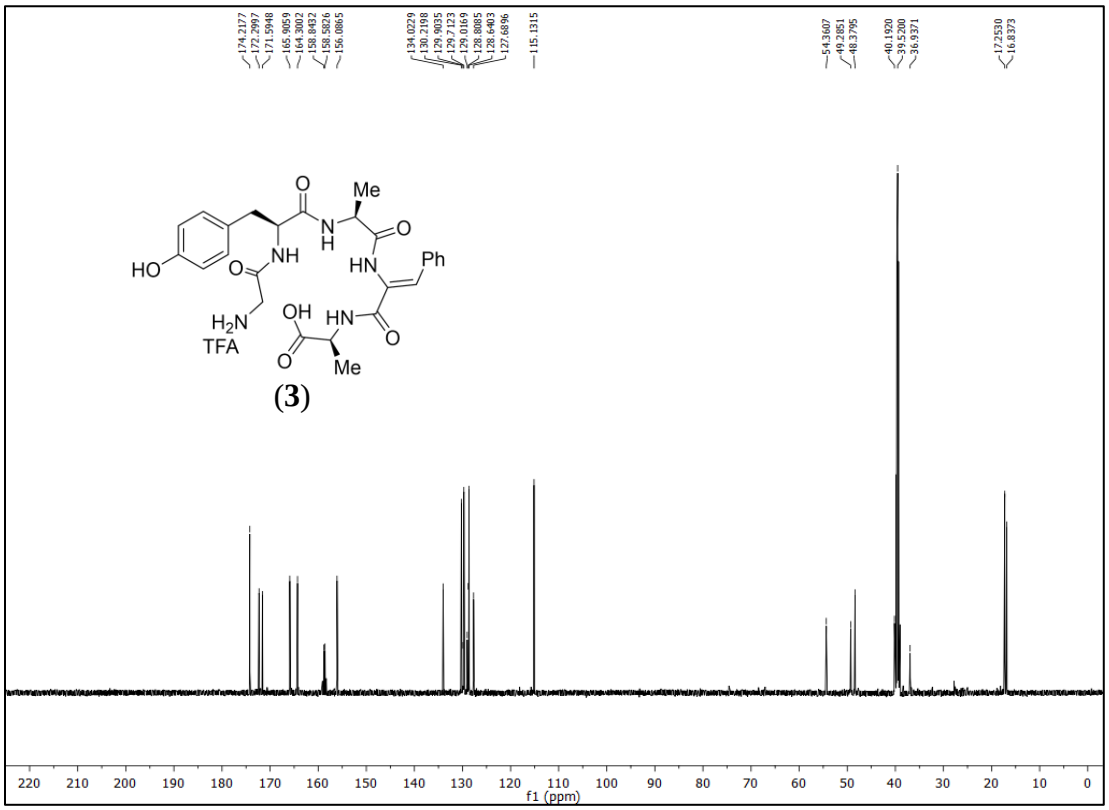
(**10**)


HO
NH
O
N
H
Me
O
HN
Ph
BocHN
OMe
HN
O
O
Me
f1 (ppm)

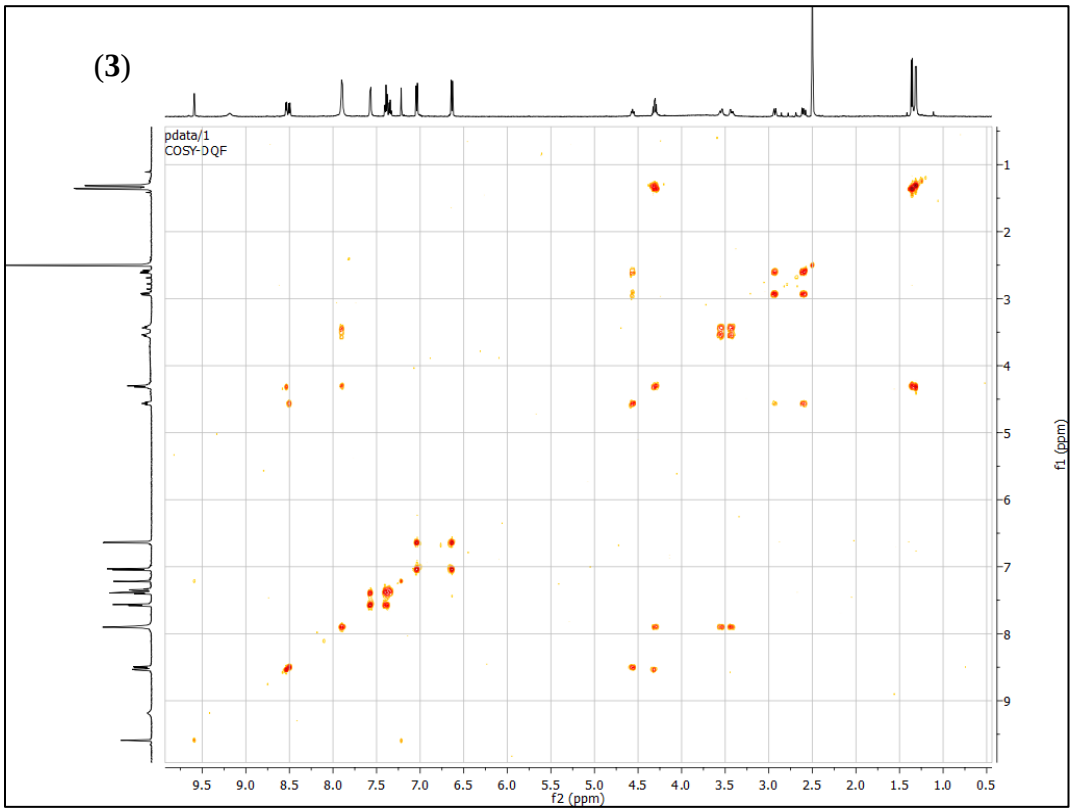
(**10**)

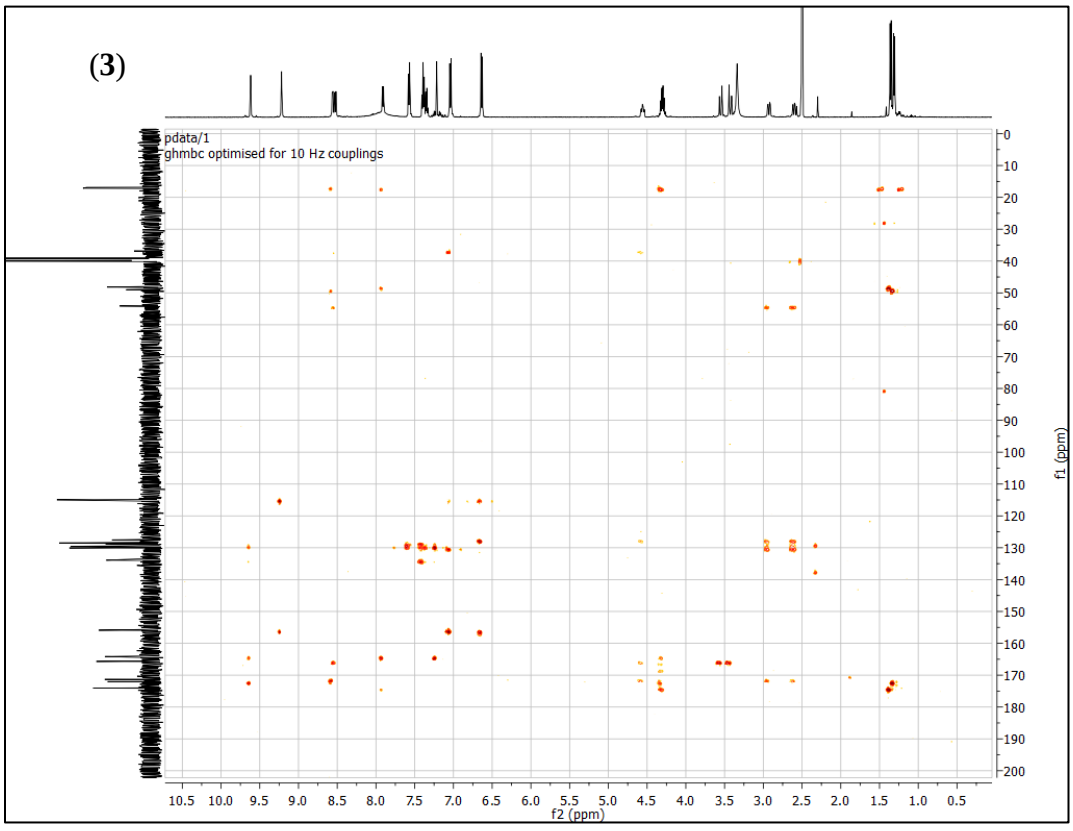

HO
O
N
H
Me
NH
HN
Ph
H2N
TFA
OH
HN
Me
f1 (ppm)

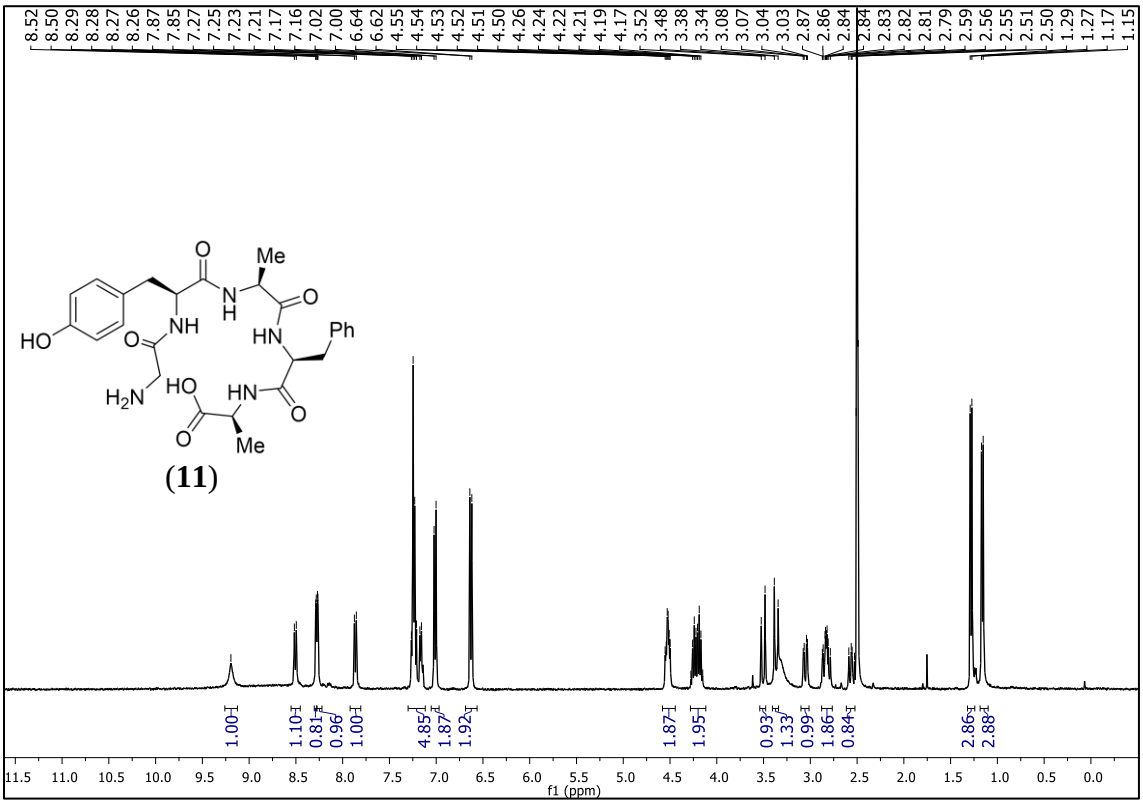
(3)


HO
O
N
H
Me
NH
HN
Ph
H2N
TFA
OH
HN
Me
f1 (ppm)

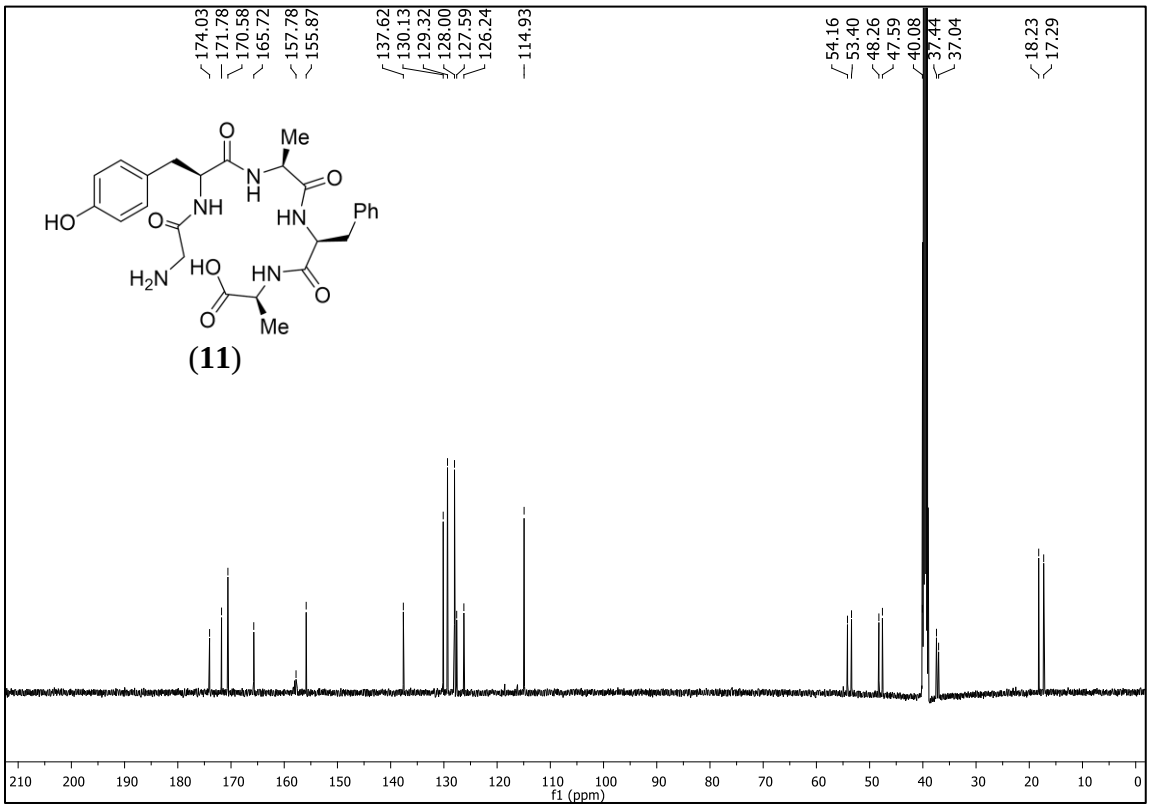
(3)


(3)
pdata/1
COSY-DQF
f2 (ppm)
f1 (ppm)

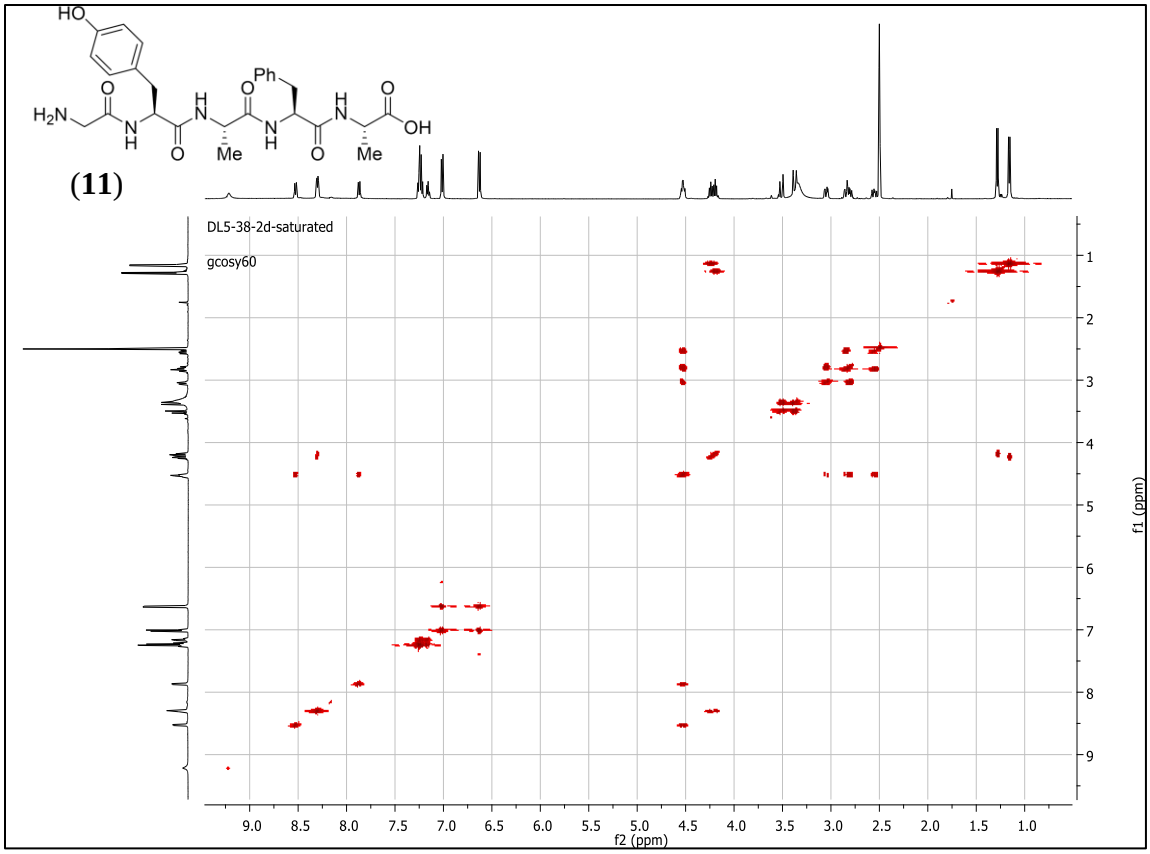

(3)
pdata/1
ghmbc optimised for 10 Hz couplings
f2 (ppm)
f1 (ppm)

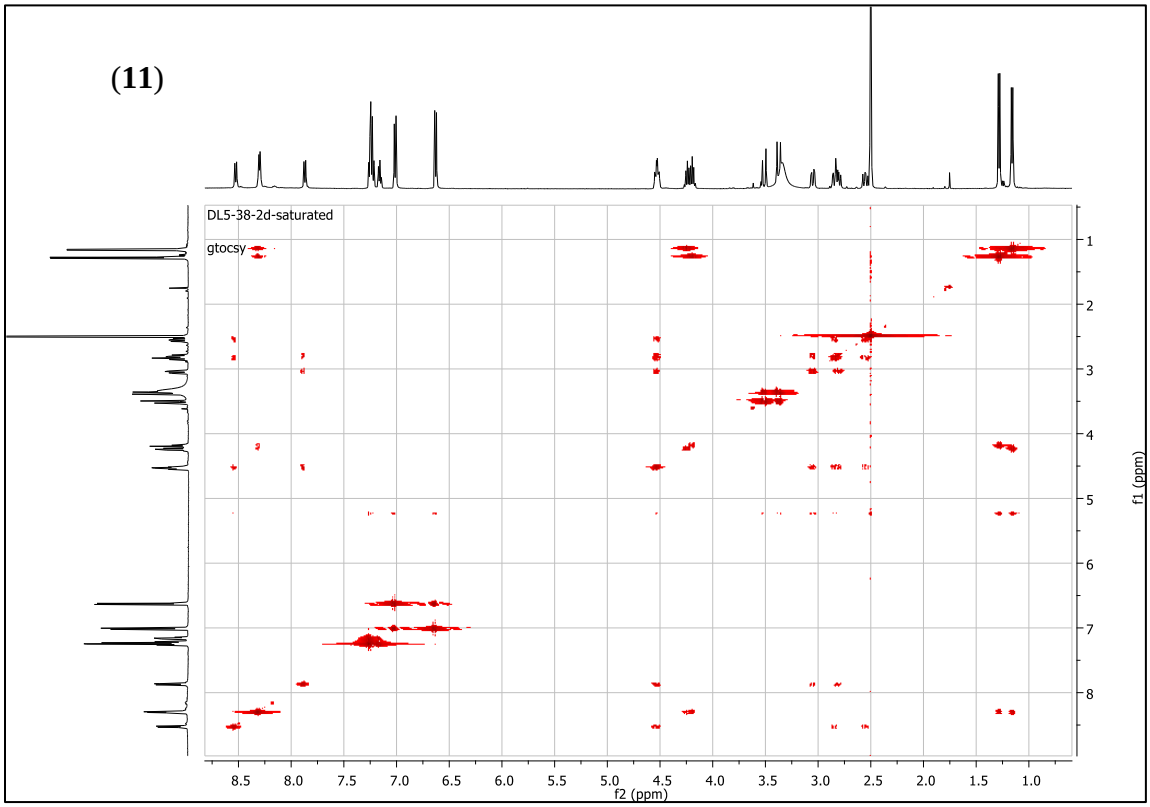

8.52 8.50 8.29 8.28 8.27 8.26 7.87 7.85 7.27 7.25 7.23 7.21 7.17 7.16 7.02 7.00 6.64 6.62 4.55 4.54 4.53 4.52 4.51 4.50 4.26 4.24 4.22 4.21 4.19 4.17 3.52 3.48 3.38 3.34 3.08 3.07 3.04 3.03 2.87 2.86 2.84 2.84 2.83 2.82 2.81 2.79 2.59 2.56 2.55 2.51 2.50 1.29 1.27 1.17 1.15
1.00 1.10 0.81 0.96 1.00 4.85 1.87 1.92 1.87 1.95 0.93 1.33 0.99 1.86 0.84 2.86 2.88
11.5 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)

(11)

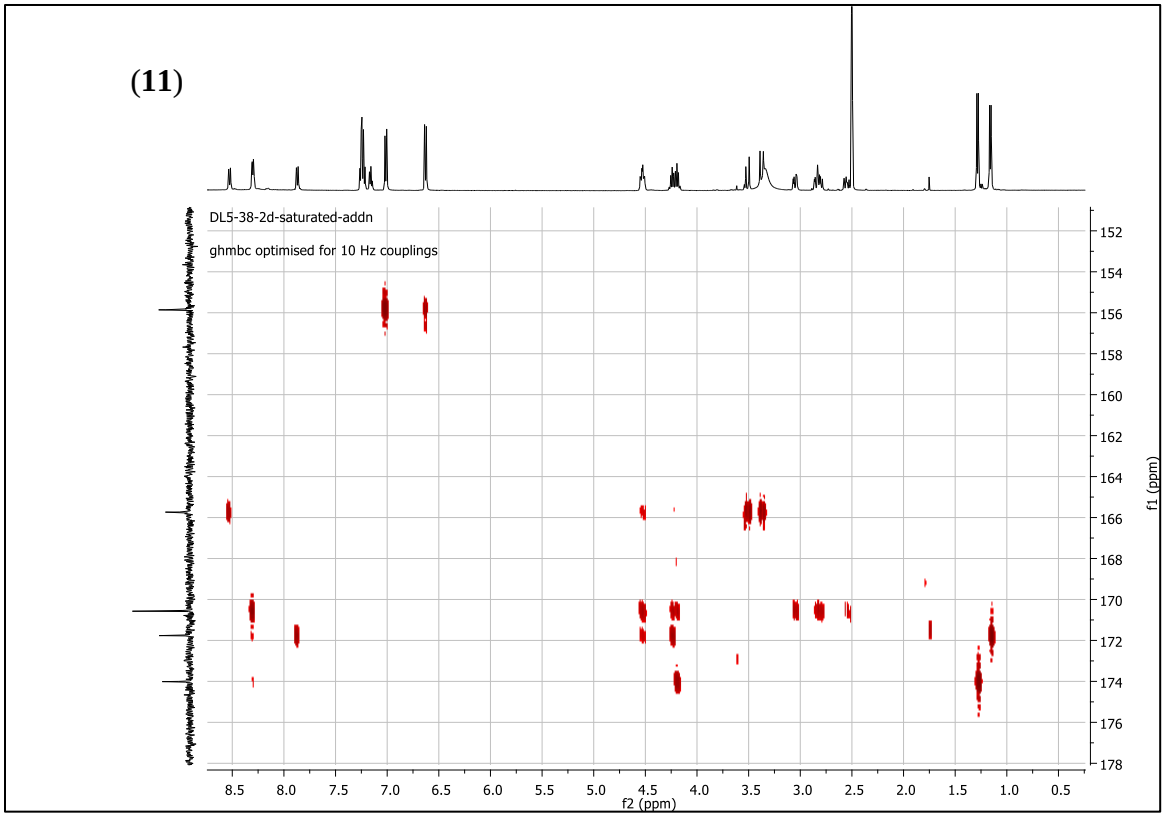

174.03 171.78 170.58 165.72 157.78 155.87 137.62 130.13 129.32 128.00 127.59 126.24 114.93 54.16 53.40 48.26 47.59 40.08 37.44 37.04 18.23 17.29
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

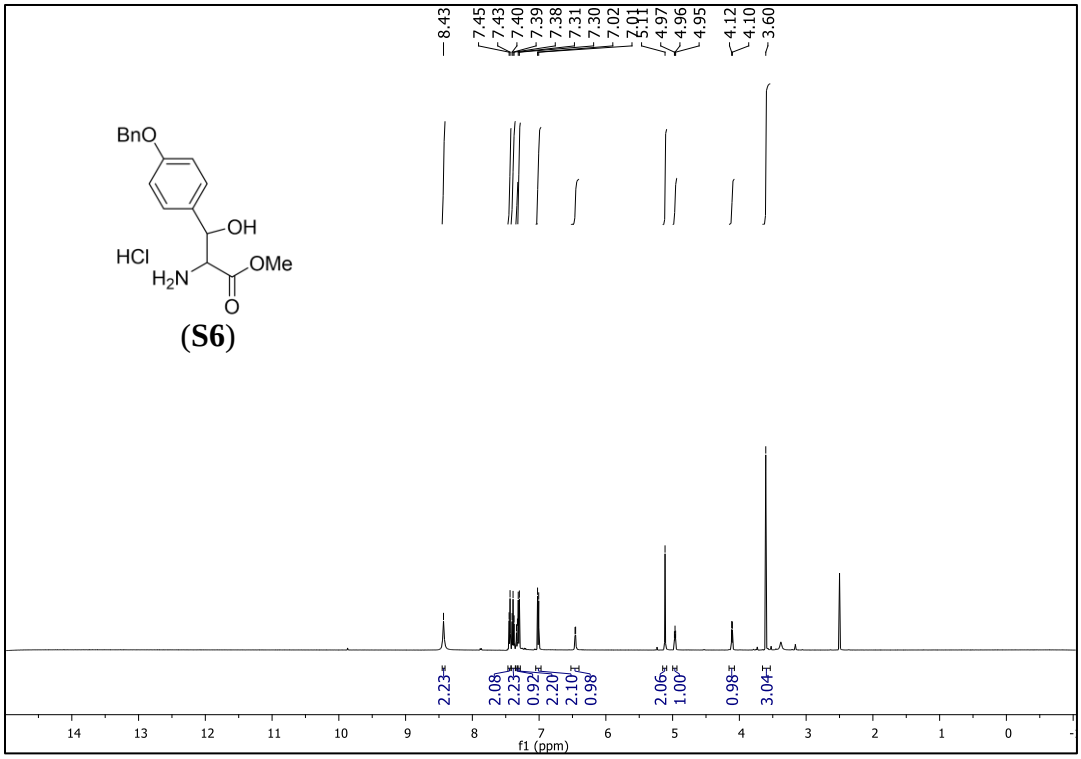
(11)


HO
Ph
H_2N
O
N
H
O
Me
OH
(11)
DL5-38-2d-saturated
gcosy60
f1 (ppm)
f2 (ppm)

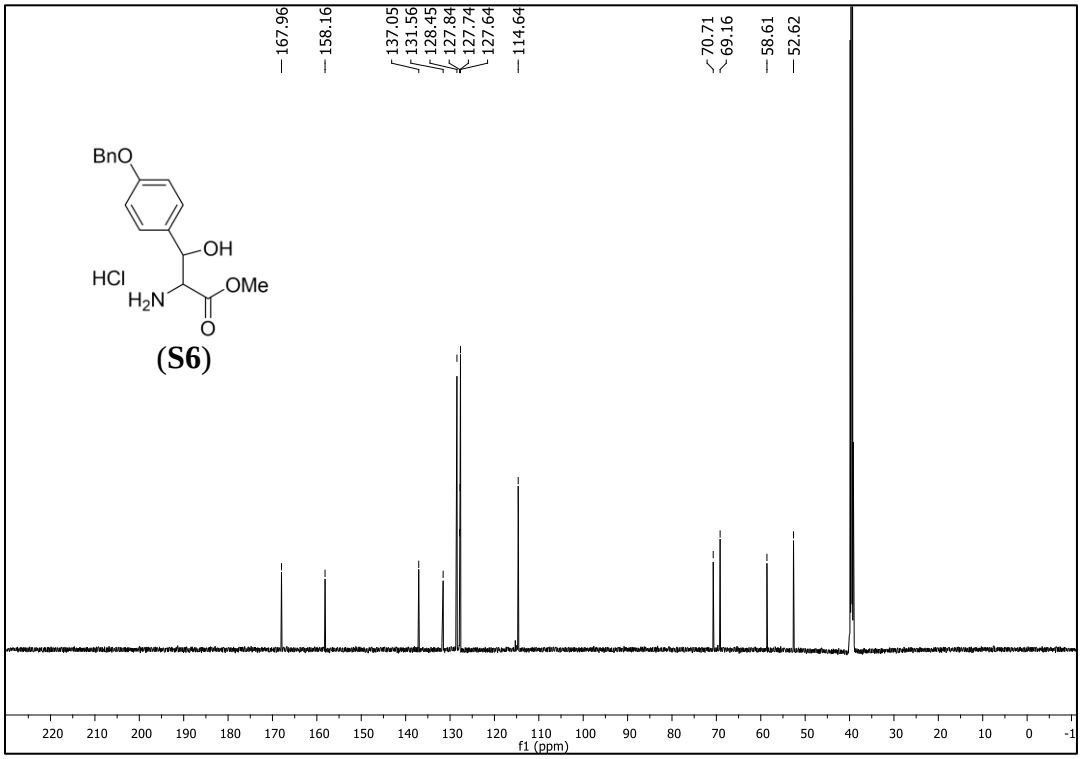

(11)
DL5-38-2d-saturated
gtocsy
f1 (ppm)
f2 (ppm)

(11)

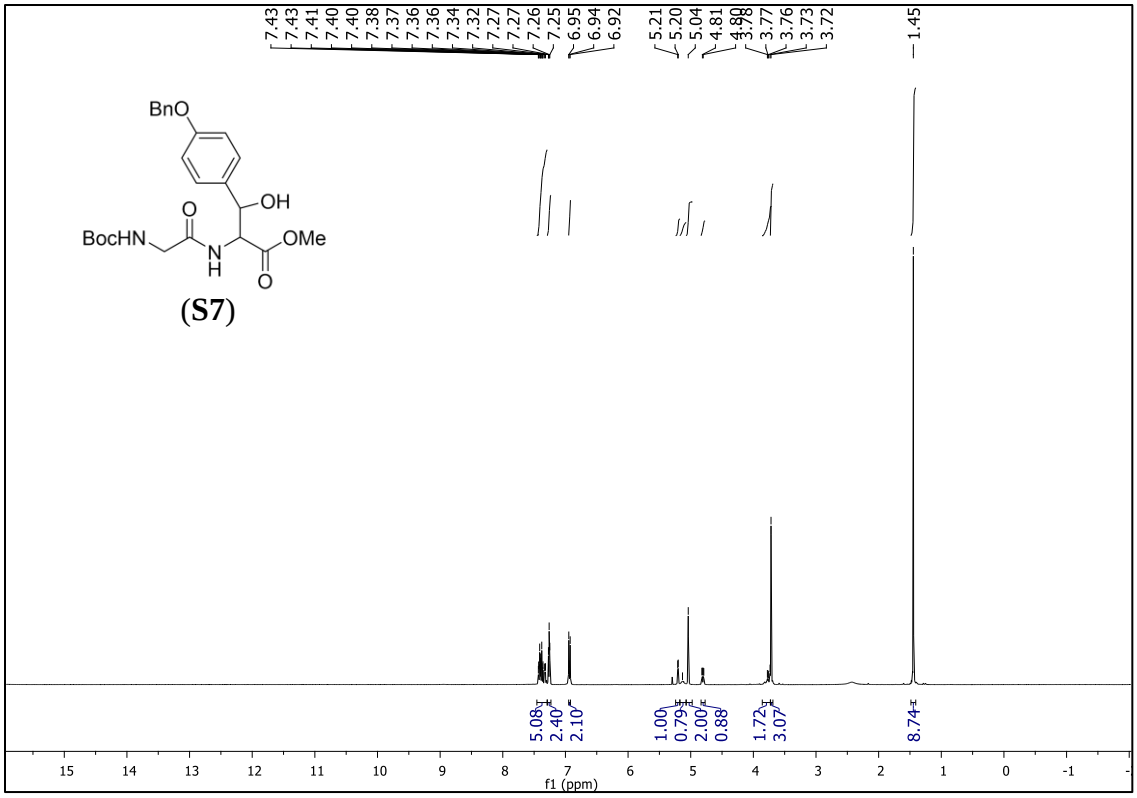

DL5-38-2d-saturated-addn
ghmbc optimised for 10 Hz couplings
f2 (ppm)
f1 (ppm)


8.43
7.45
7.43
7.40
7.39
7.38
7.31
7.30
7.02
7.01
5.11
4.97
4.96
4.95
4.12
4.10
3.60
BnO
OH
HCl
H2N
OMe
O
2.23
2.08
2.23
0.92
2.20
2.10
0.98
2.06
1.00
0.98
3.04
f1 (ppm)

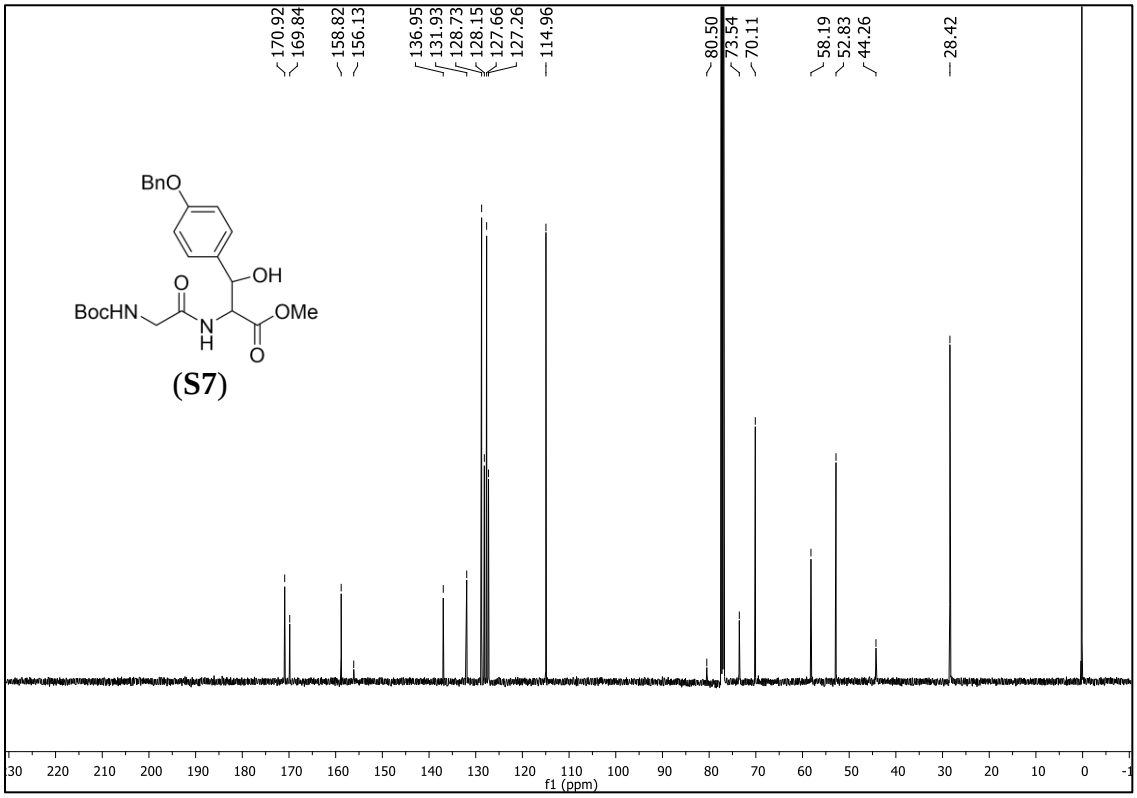
(**S6**)


167.96
158.16
137.05
131.56
128.45
127.84
127.74
127.64
114.64
70.71
69.16
58.61
52.62
BnO
OH
HCl
H2N
OMe
O
f1 (ppm)

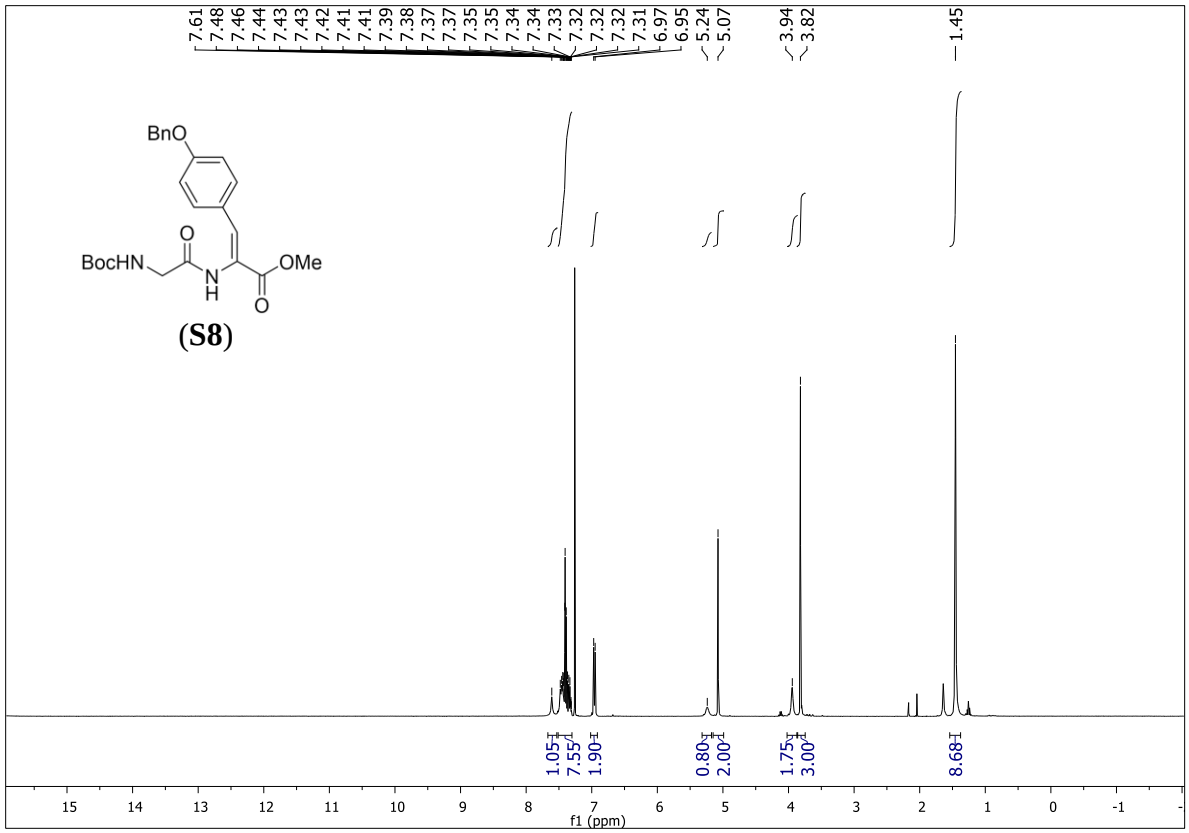
(**S6**)


7.43
7.43
7.41
7.40
7.40
7.38
7.37
7.36
7.36
7.34
7.32
7.27
7.27
7.26
7.25
6.95
6.94
6.92
5.21
5.20
5.04
4.81
4.80
3.78
3.77
3.76
3.73
3.72
1.45
BnO
OH
BocHN
O
N
H
OMe
O
5.08
2.40
2.10
1.00
0.79
2.00
0.88
1.72
3.07
8.74
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)

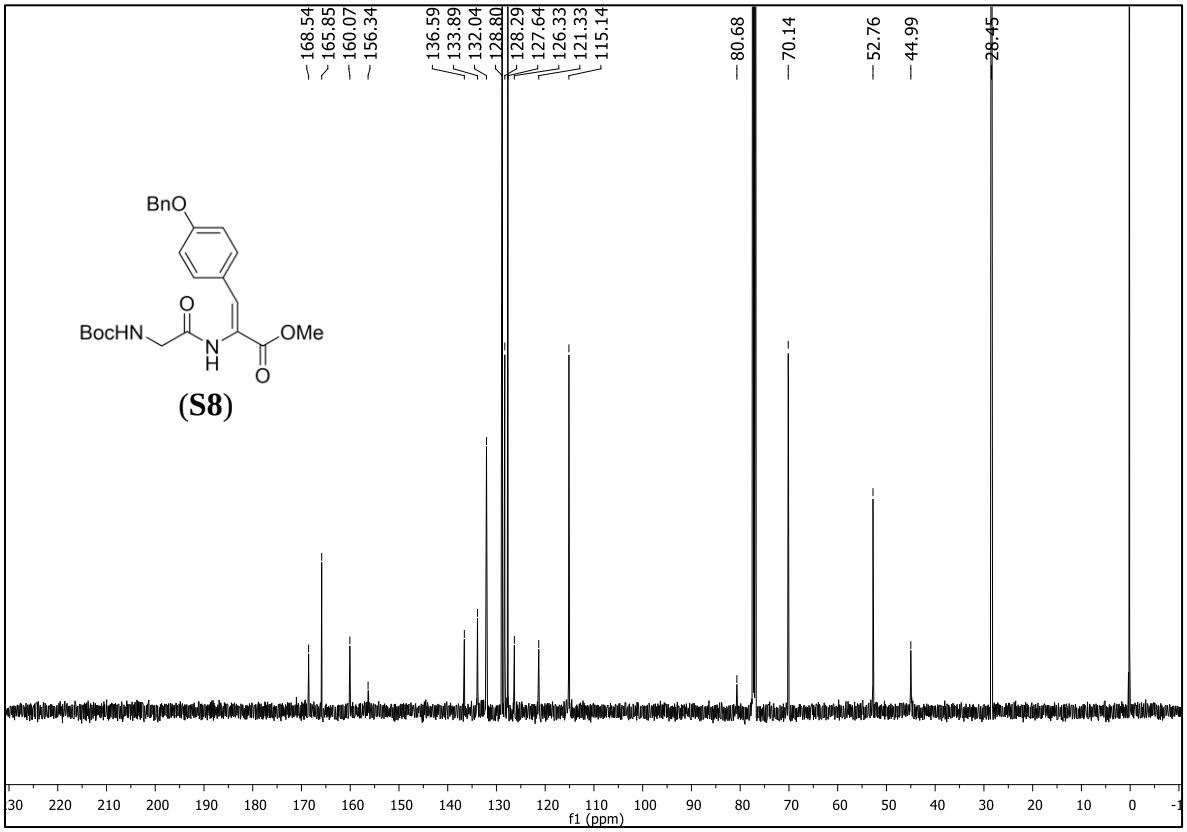
(S7)


170.92
169.84
158.82
156.13
136.95
131.93
128.73
128.15
127.66
127.26
114.96
80.50
73.54
70.11
58.19
52.83
44.26
28.42
BnO
OH
BocHN
O
N
H
OMe
O
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)

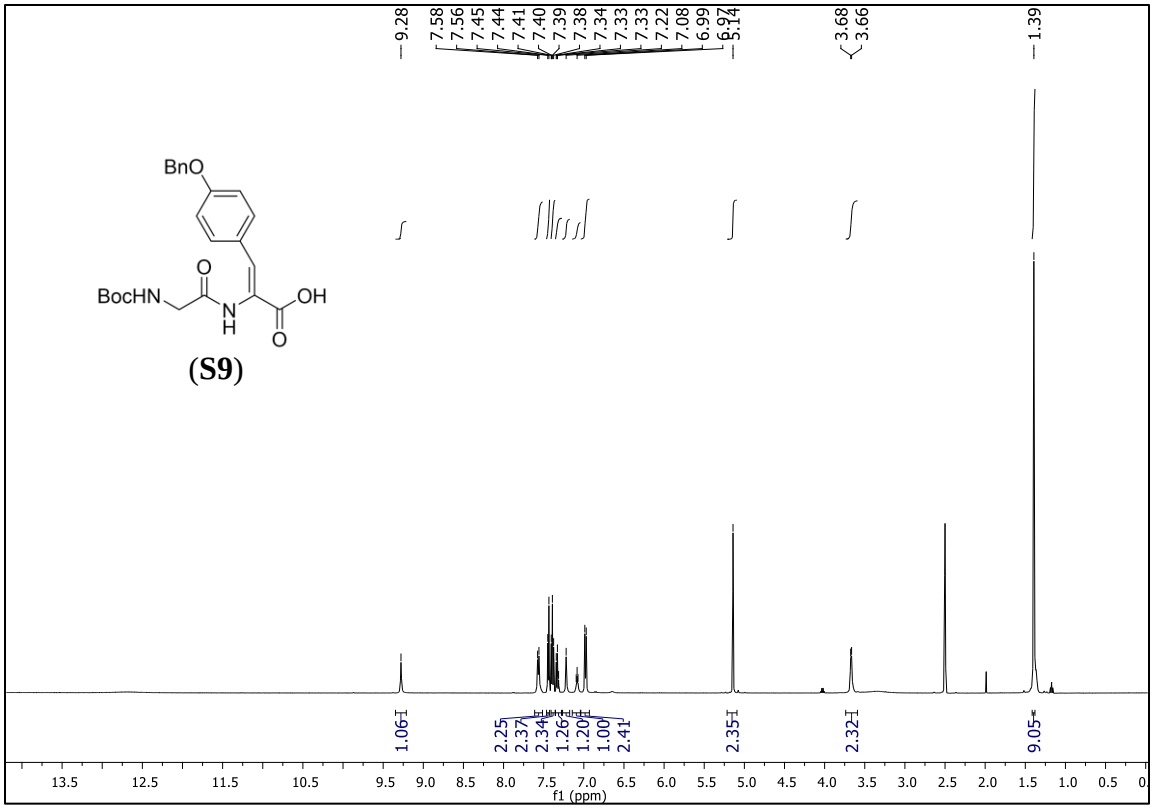
(S7)


7.61
7.48
7.46
7.44
7.43
7.43
7.42
7.41
7.41
7.39
7.38
7.37
7.37
7.35
7.35
7.34
7.34
7.33
7.32
7.32
7.32
7.31
6.97
6.95
5.24
5.07
3.94
3.82
1.45
BnO
BocHN
O
N
H
OMe
O
1.05
7.55
1.90
0.80
2.00
1.75
3.00
8.68
f1 (ppm)

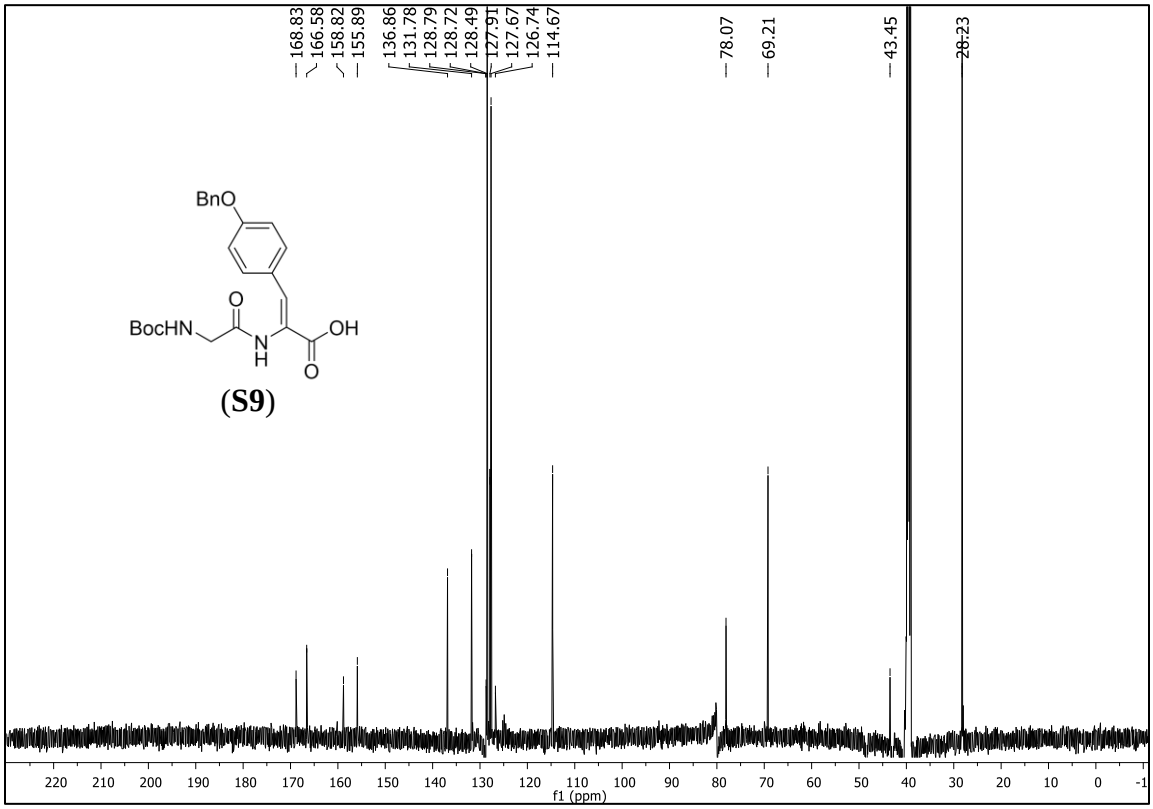
(S8)


168.54
165.85
160.07
156.34
136.59
133.89
132.04
128.80
128.29
127.64
126.33
121.33
115.14
80.68
70.14
52.76
44.99
28.45
BnO
BocHN
O
N
H
OMe
O
f1 (ppm)

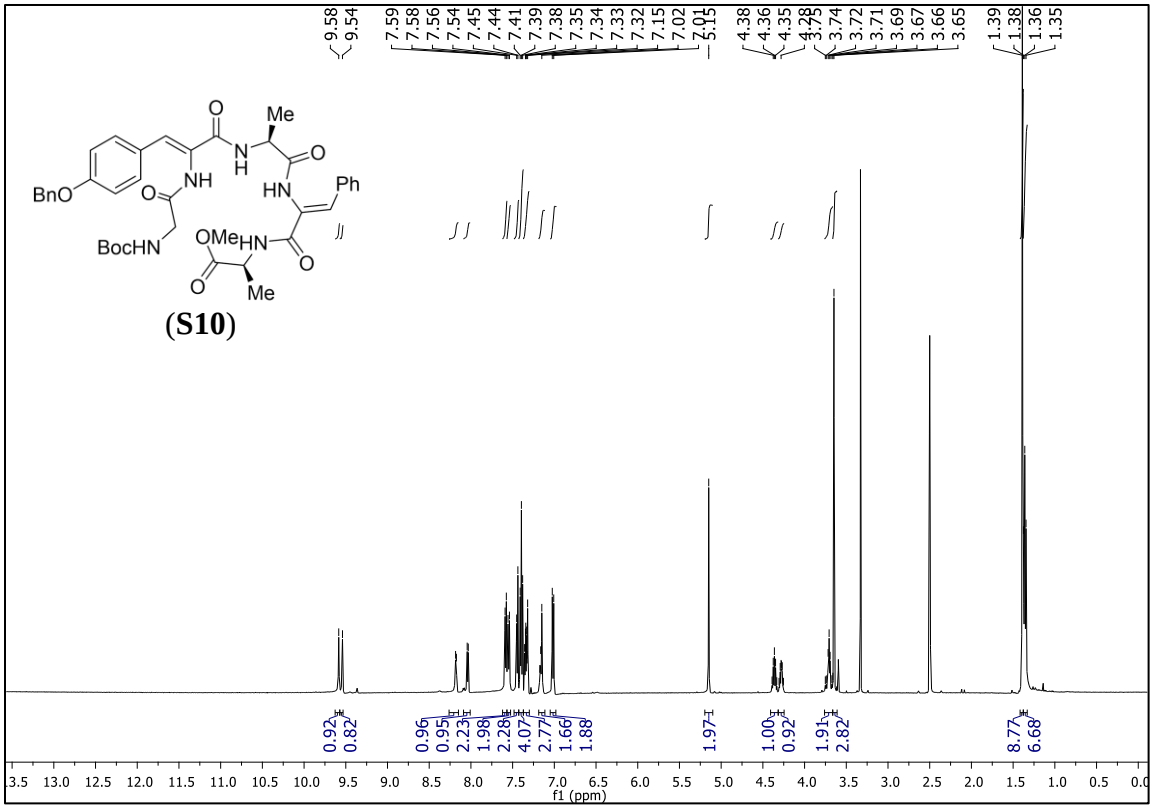
(S8)


9.28
7.58
7.56
7.45
7.44
7.41
7.40
7.39
7.38
7.34
7.33
7.33
7.22
7.08
6.99
6.97
5.14
3.68
3.66
1.39
BnO
BocHN
O
N
H
OH
O
1.06
2.25
2.37
2.34
1.26
1.20
1.00
2.41
2.35
2.32
9.05
13.5
12.5
11.5
10.5
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
f1 (ppm)

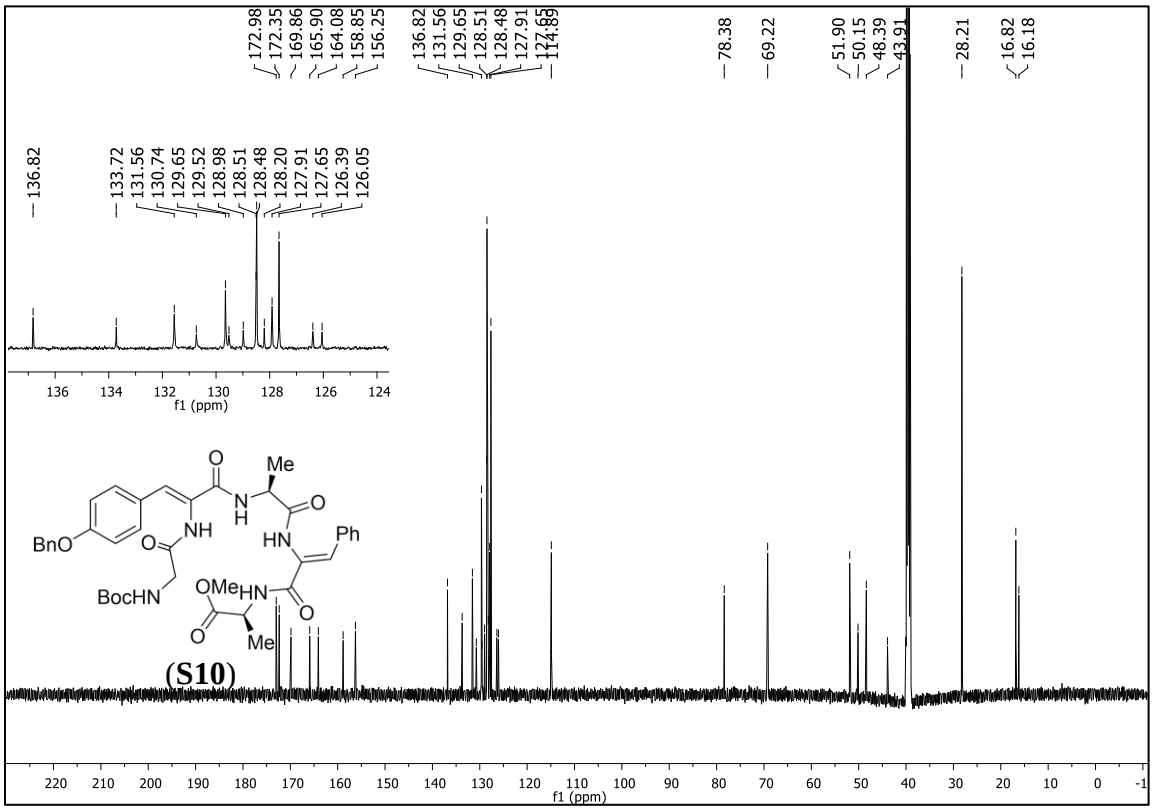
(**S9**)


168.83
166.58
158.82
155.89
136.86
131.78
128.79
128.72
128.49
127.91
127.67
126.74
114.67
78.07
69.21
43.45
28.23
BnO
BocHN
O
N
H
OH
O
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

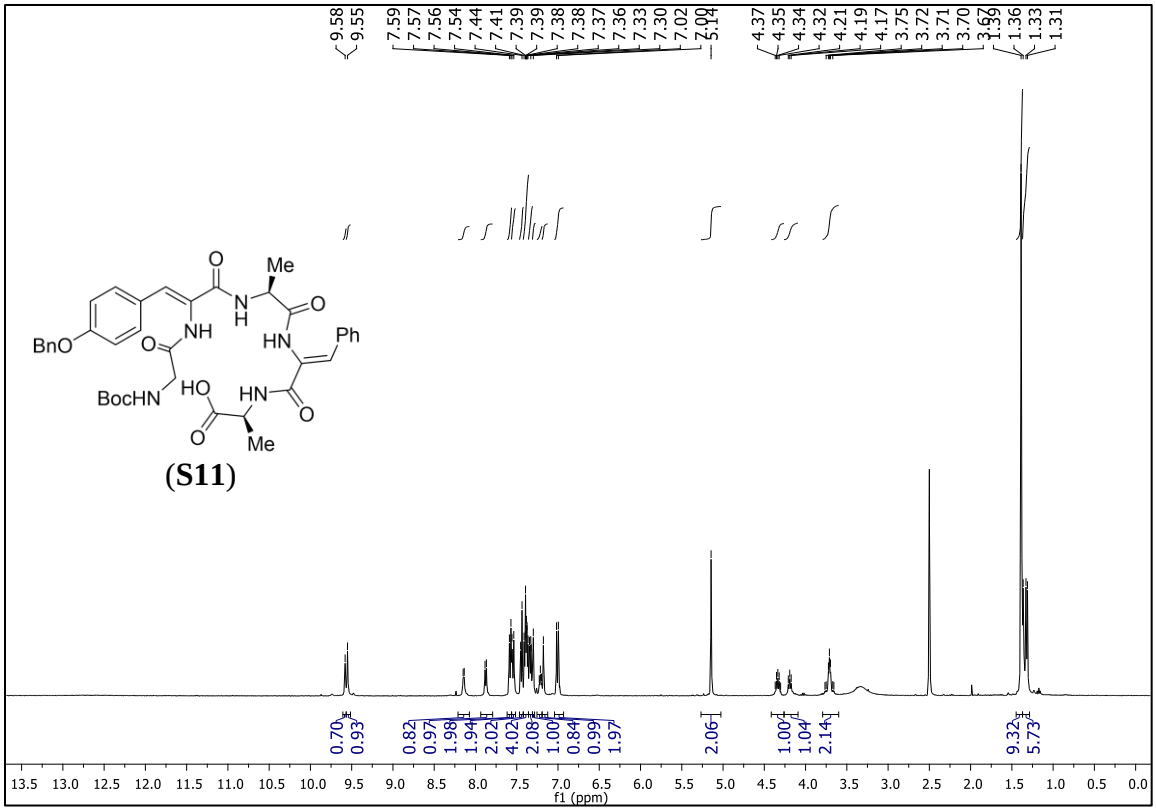
(**S9**)

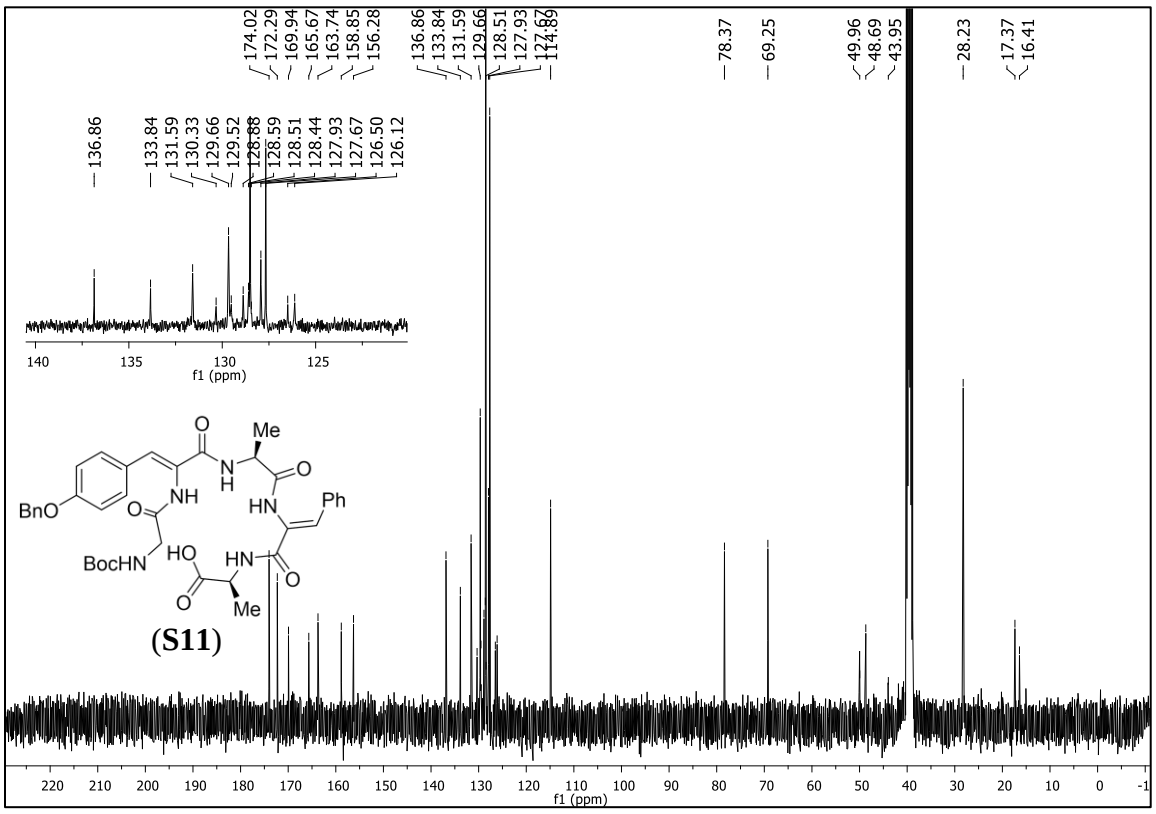

9.58
9.54
7.59
7.58
7.56
7.54
7.45
7.44
7.41
7.39
7.38
7.35
7.34
7.33
7.32
7.15
7.02
7.01
5.15
4.38
4.36
4.35
4.28
4.25
3.74
3.72
3.71
3.69
3.67
3.66
3.65
1.39
1.38
1.36
1.35
0.92
0.82
0.96
0.95
2.23
1.98
2.28
4.07
2.77
1.66
1.88
1.97
1.00
0.92
1.91
2.82
8.77
6.68
f1 (ppm)

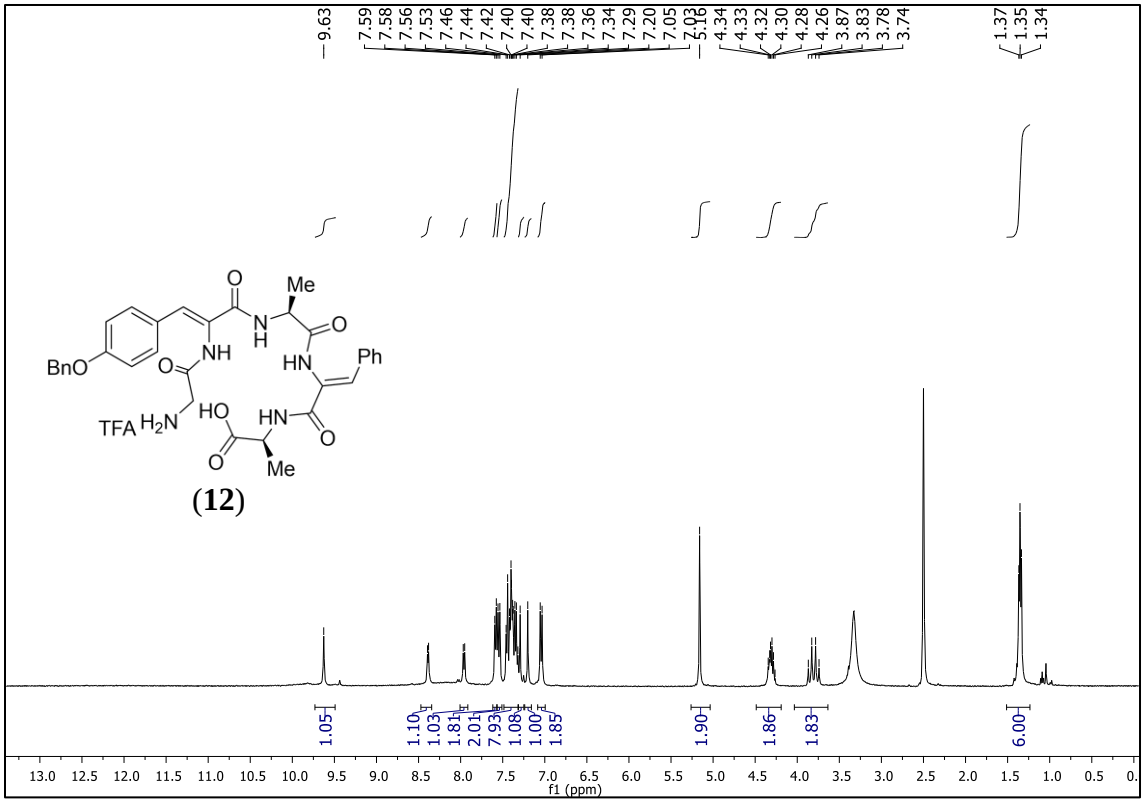
(**S10**)


172.98
172.35
169.86
165.90
164.08
158.85
156.25
136.82
131.56
129.65
128.51
128.48
127.91
78.38
69.22
51.90
50.15
48.39
43.91
28.21
16.82
16.18
136.82
133.72
131.56
130.74
129.65
129.52
128.98
128.51
128.48
128.20
127.91
127.65
126.39
126.05
f1 (ppm)

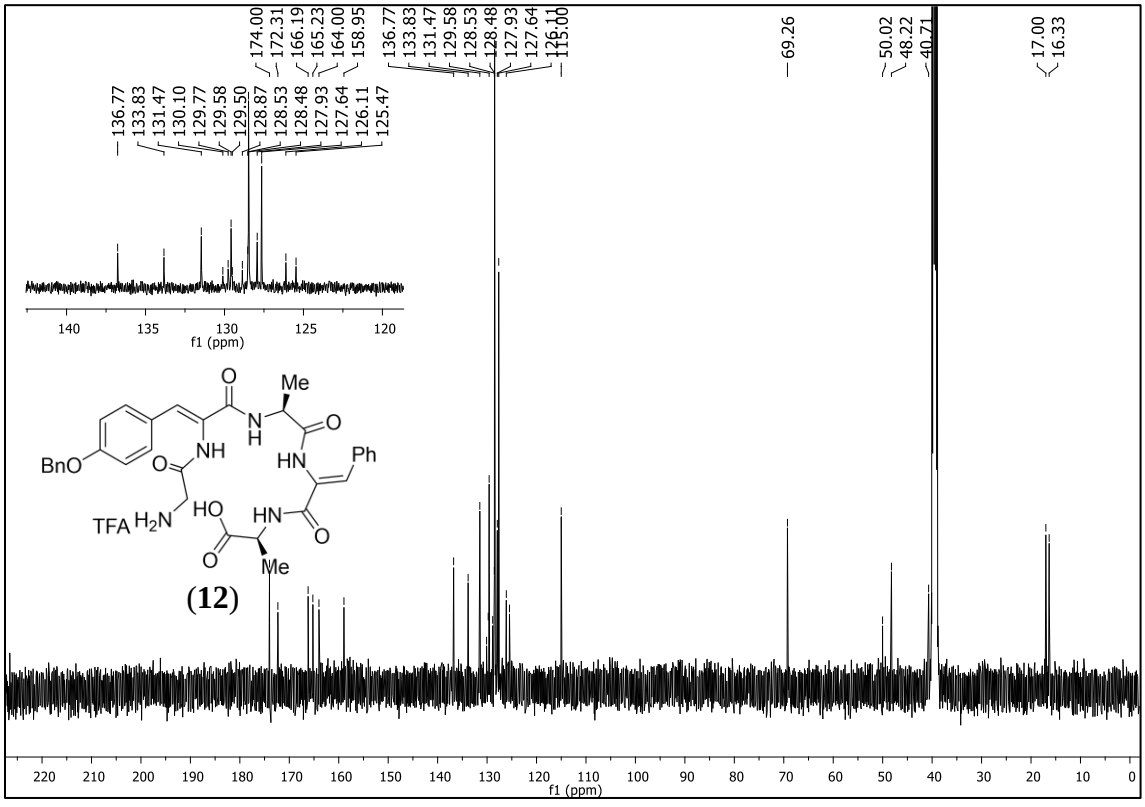
(**S10**)


(S11)

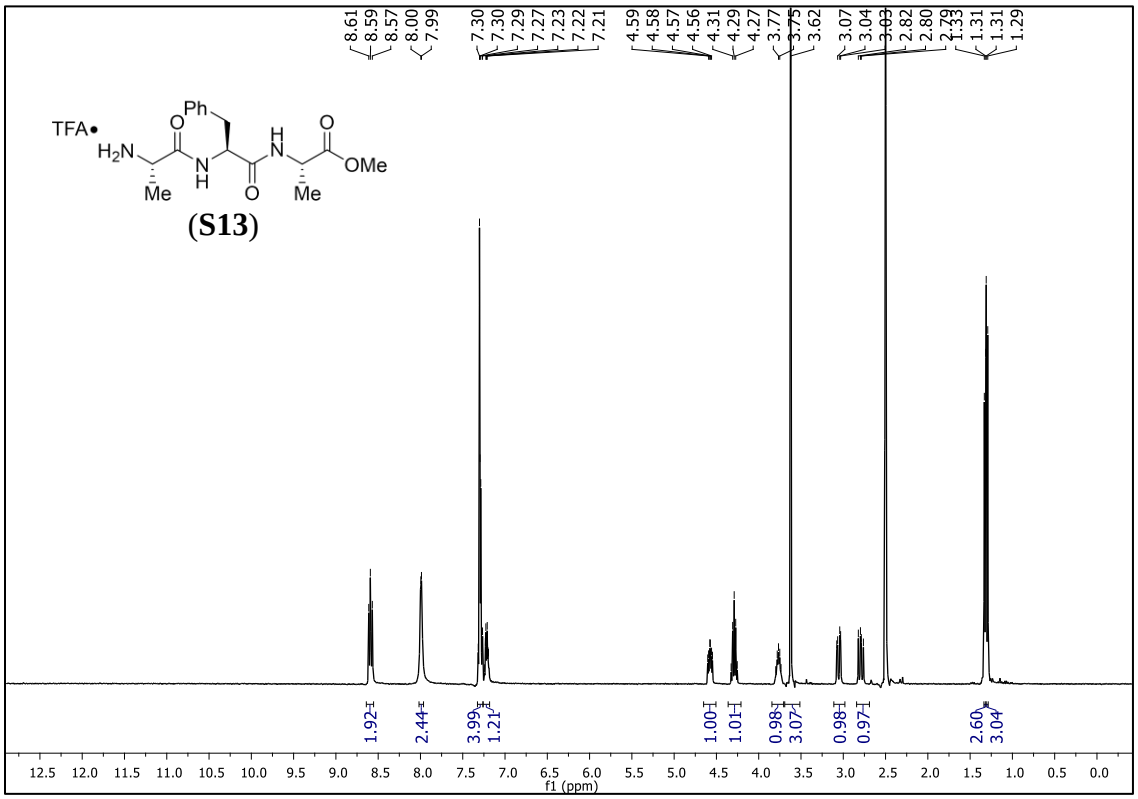

(S11)

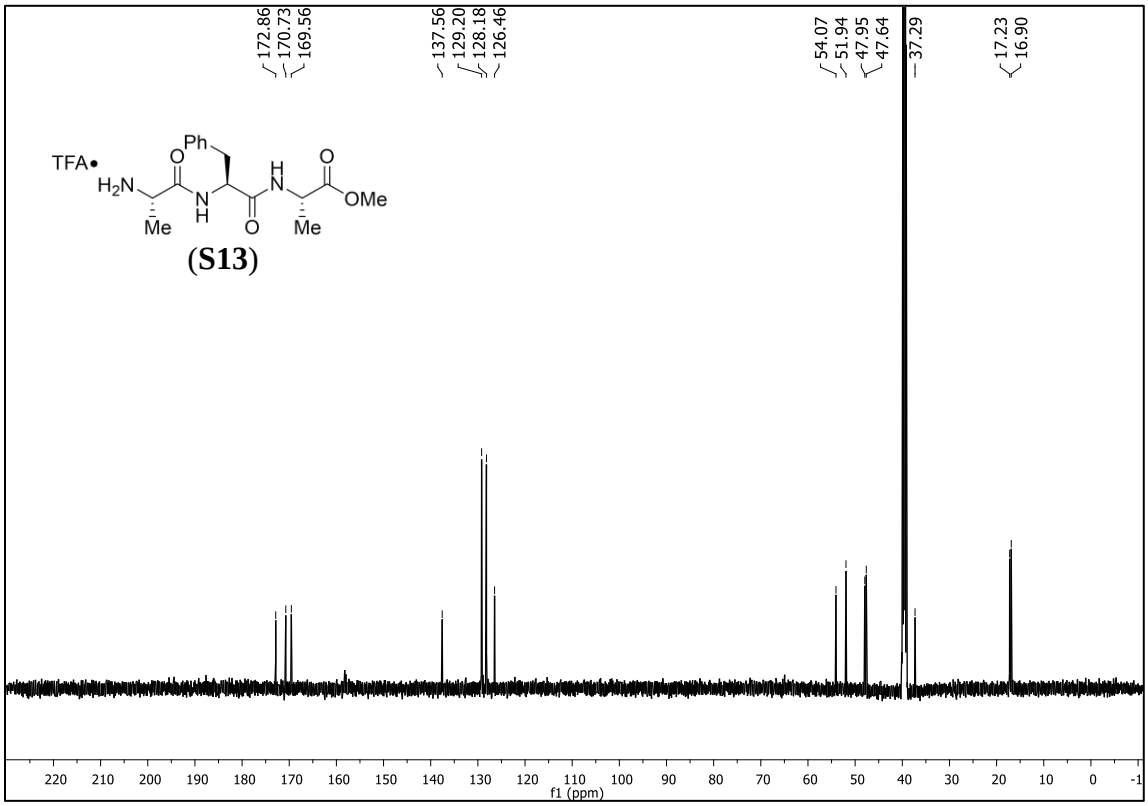

9.63
7.59
7.58
7.56
7.53
7.46
7.44
7.42
7.40
7.40
7.38
7.38
7.36
7.34
7.29
7.20
7.05
7.03
5.16
4.34
4.33
4.32
4.30
4.28
4.26
3.87
3.83
3.78
3.74
1.37
1.35
1.34
1.05
1.10
1.03
1.81
2.01
7.93
1.08
1.00
1.85
1.90
1.86
1.83
6.00
f1 (ppm)

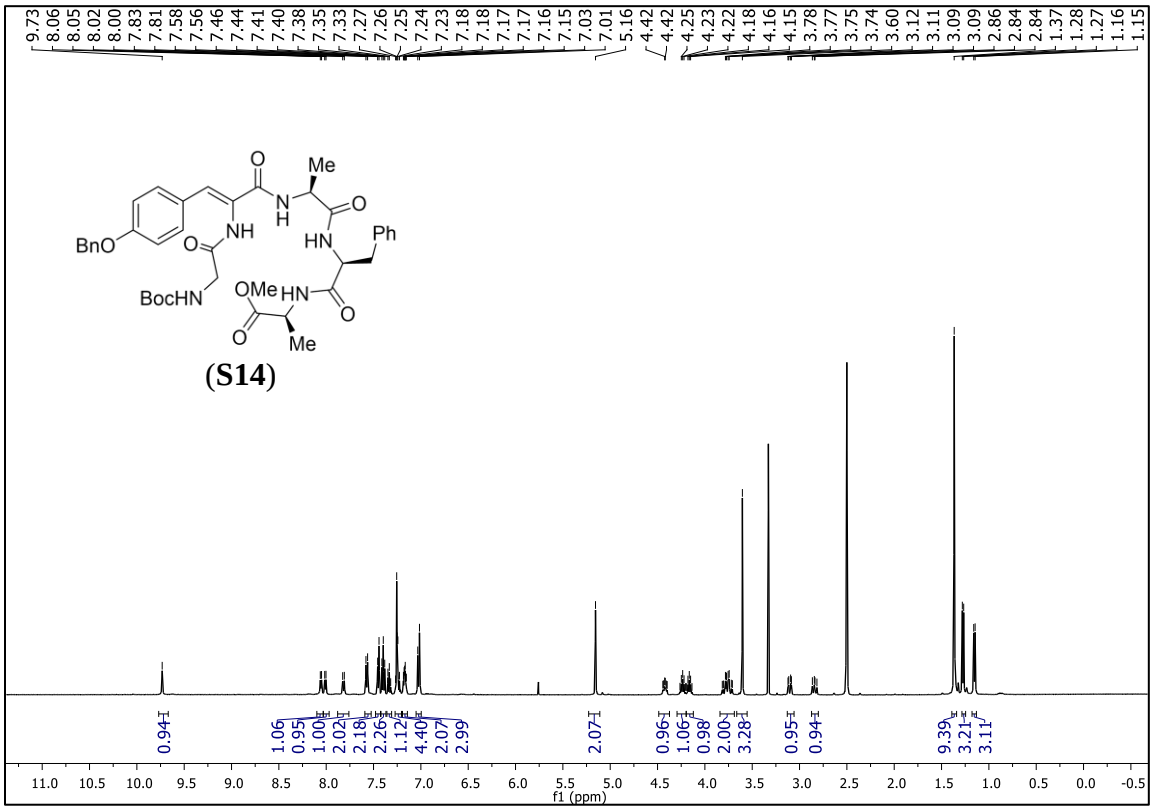
(12)


174.00
172.31
166.19
165.23
164.00
158.95
136.77
133.83
131.47
129.58
128.53
128.48
127.93
127.64
126.11
125.47
69.26
50.02
48.22
40.71
17.00
16.33
f1 (ppm)

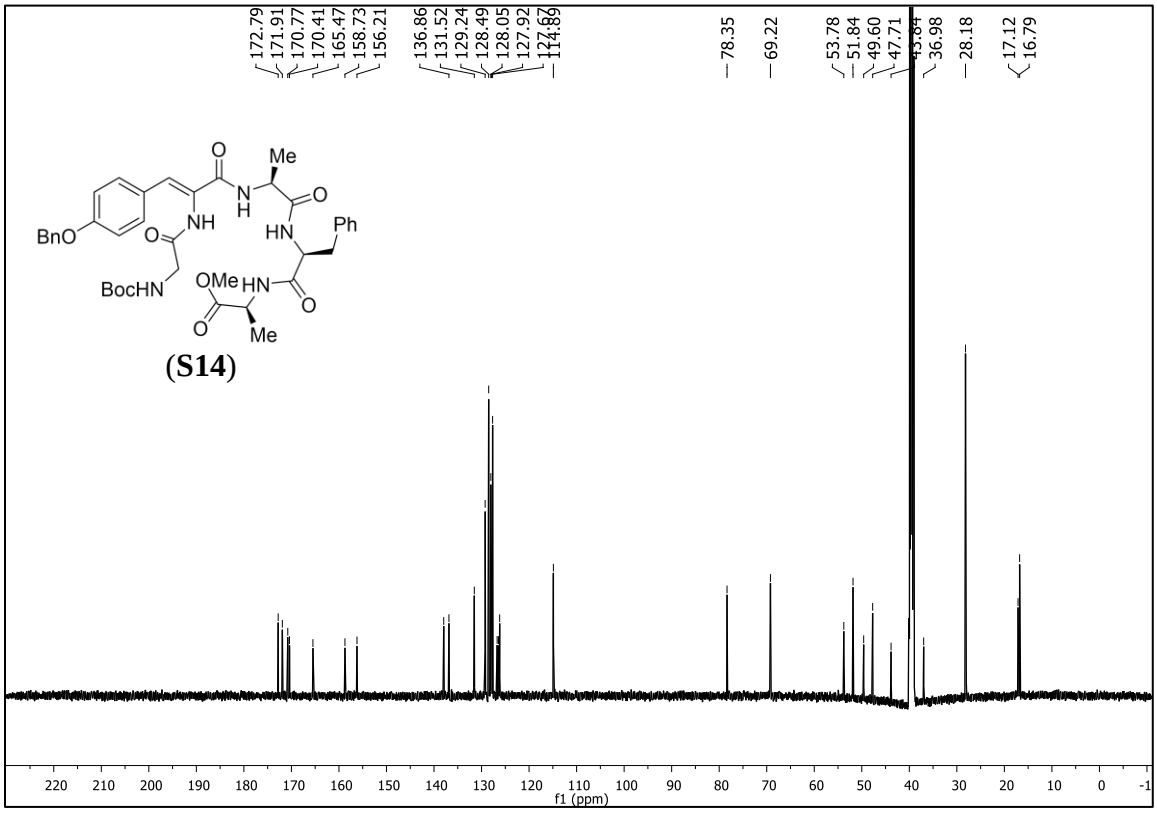
(12)


TFA•
Ph
O
H2N
N
H
O
N
H
O
OMe
Me
Me
(S13)
8.61
8.59
8.57
8.00
7.99
7.30
7.30
7.29
7.27
7.23
7.22
7.21
4.59
4.58
4.57
4.56
4.31
4.29
4.27
3.77
3.75
3.62
3.07
3.04
3.03
2.82
2.80
2.79
2.33
1.31
1.31
1.29
1.92
2.44
3.99
1.21
1.00
1.01
0.98
3.07
0.98
0.97
2.60
3.04
f1 (ppm)

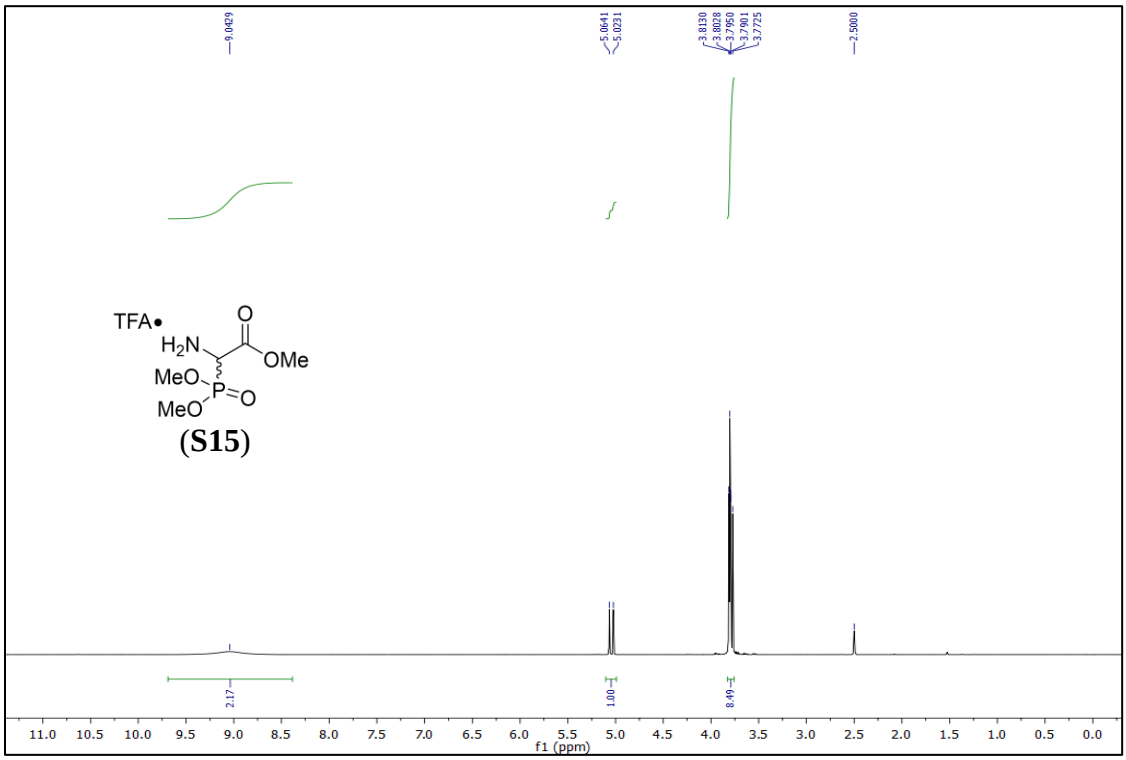

TFA•
Ph
O
H2N
N
H
O
N
H
O
OMe
Me
Me
(S13)
172.86
170.73
169.56
137.56
129.20
128.18
126.46
54.07
51.94
47.95
47.64
37.29
17.23
16.90
f1 (ppm)


9.73 8.06 8.05 8.02 8.00 7.83 7.81 7.58 7.56 7.46 7.44 7.41 7.40 7.38 7.35 7.33 7.27 7.26 7.25 7.24 7.23 7.18 7.18 7.17 7.17 7.16 7.15 7.03 7.01 5.16 4.42 4.42 4.25 4.23 4.22 4.18 4.16 4.15 3.78 3.77 3.75 3.74 3.60 3.12 3.11 3.09 3.09 2.86 2.84 2.84 1.37 1.28 1.27 1.16 1.15
Me
O
N
H
O
NH
O
HN
Ph
BnO
BocHN
OMe
HN
O
O
Me
0.94 1.06 0.95 1.00 2.02 2.18 2.26 1.12 4.40 2.07 2.99 2.07 0.96 1.06 0.98 2.00 3.28 0.95 0.94 9.39 3.21 3.11
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 -0.5
f1 (ppm)

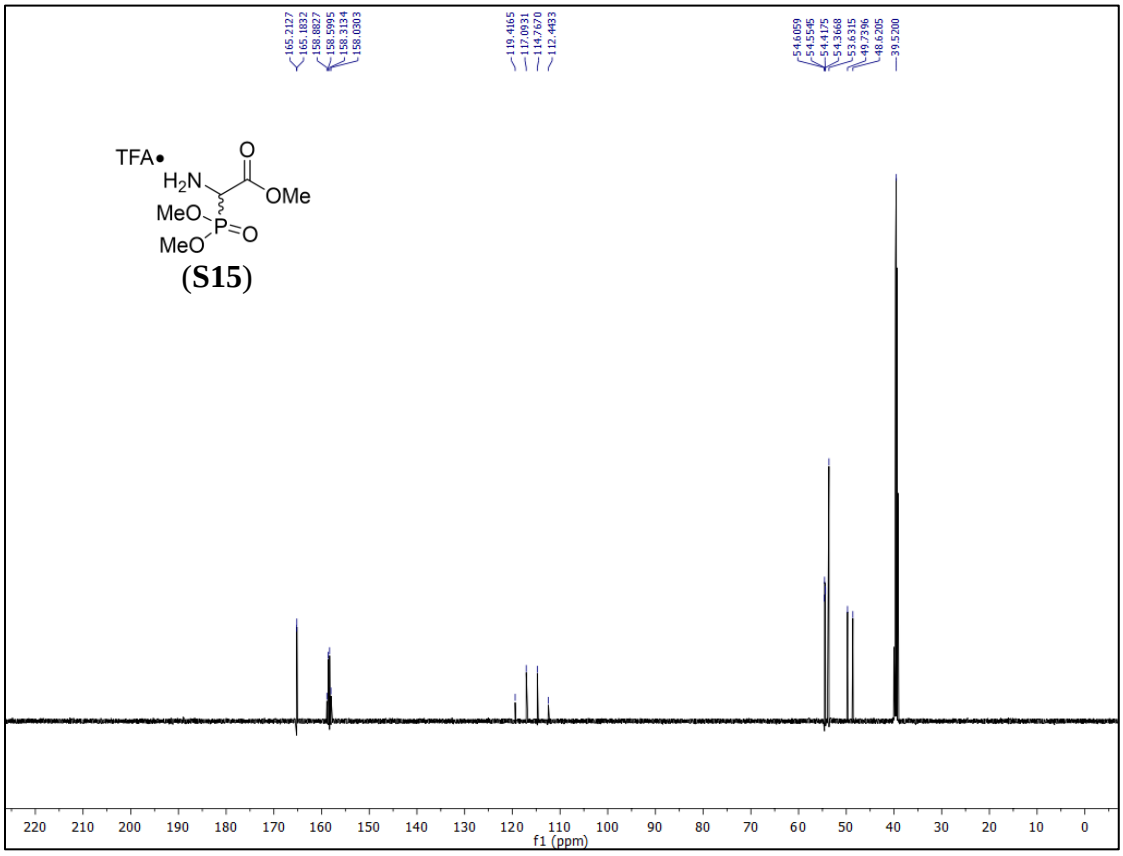
(**S14**)


172.79 171.91 170.77 170.41 165.47 158.73 156.21 136.86 131.52 129.24 128.49 128.05 127.92 78.35 69.22 53.78 51.84 49.60 47.71 36.98 28.18 17.12 16.79
Me
O
N
H
O
NH
O
HN
Ph
BnO
BocHN
OMe
HN
O
O
Me
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)

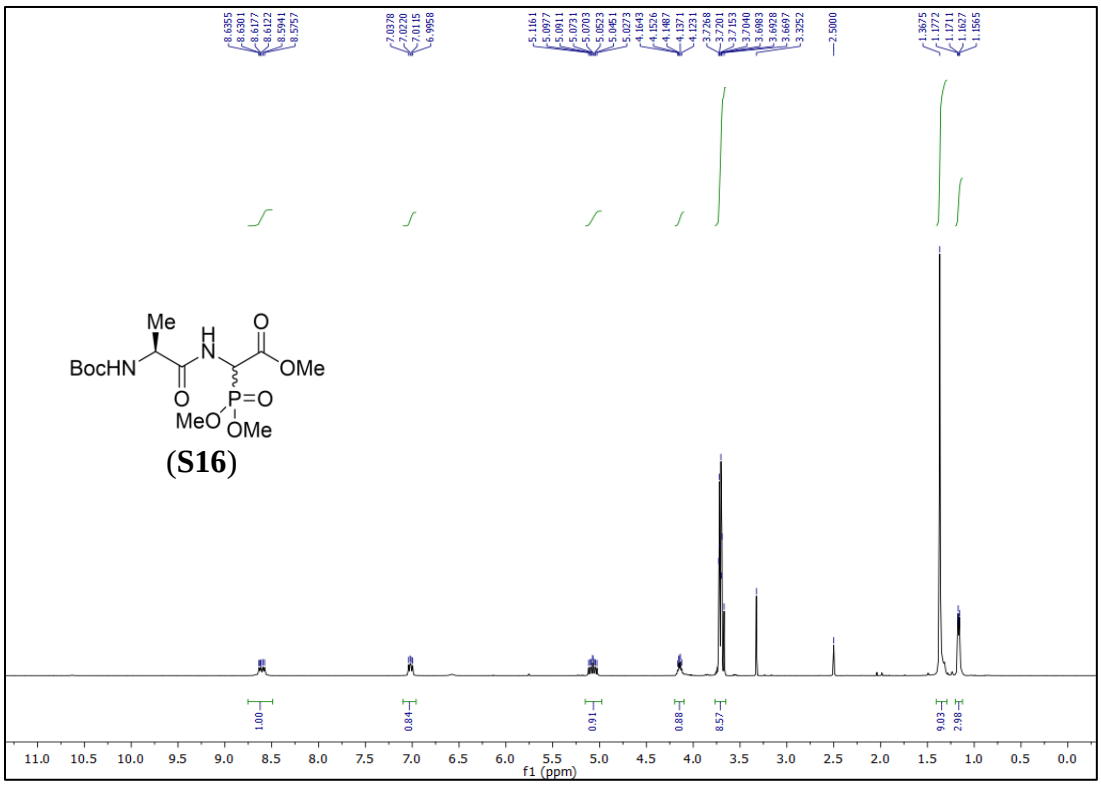
(**S14**)


9.0429
5.0641
5.0231
3.8130
3.8028
3.7950
3.7901
3.7725
2.5000
TFA•
H2N
O
OMe
MeO
P
O
MeO
2.17
1.00
8.49
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)

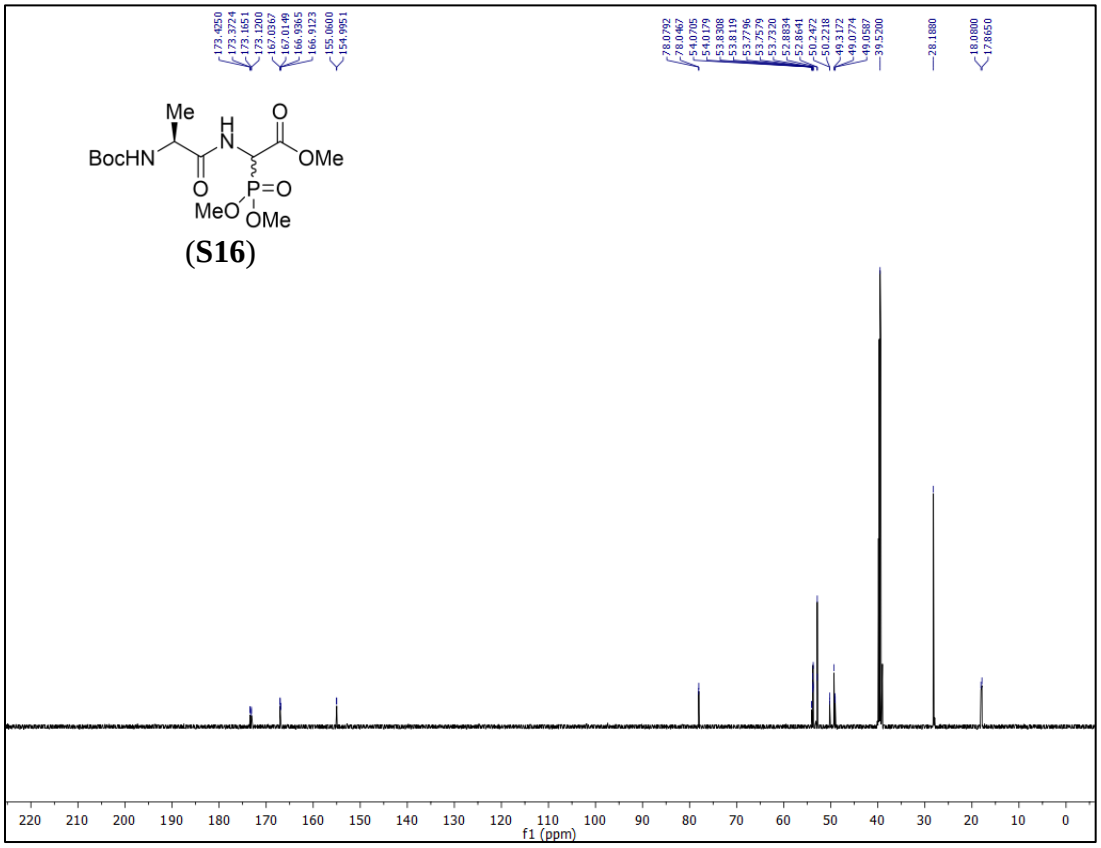
(S15)


165.2127
165.1832
158.8827
158.5995
158.3134
158.0303
119.4165
117.0931
115.7670
112.9433
54.6059
54.5546
54.4175
54.3668
53.6315
49.7396
48.6205
39.5200
TFA•
H2N
O
OMe
MeO
P
O
MeO
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)

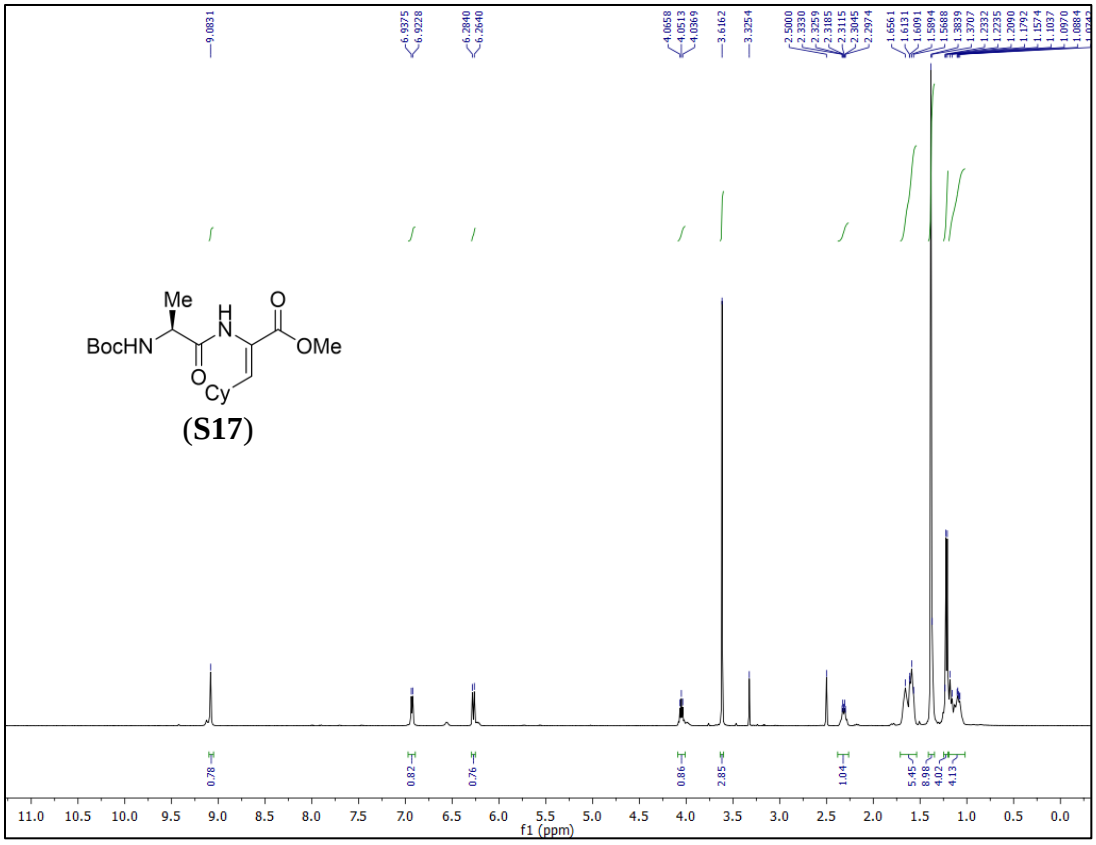
(S15)



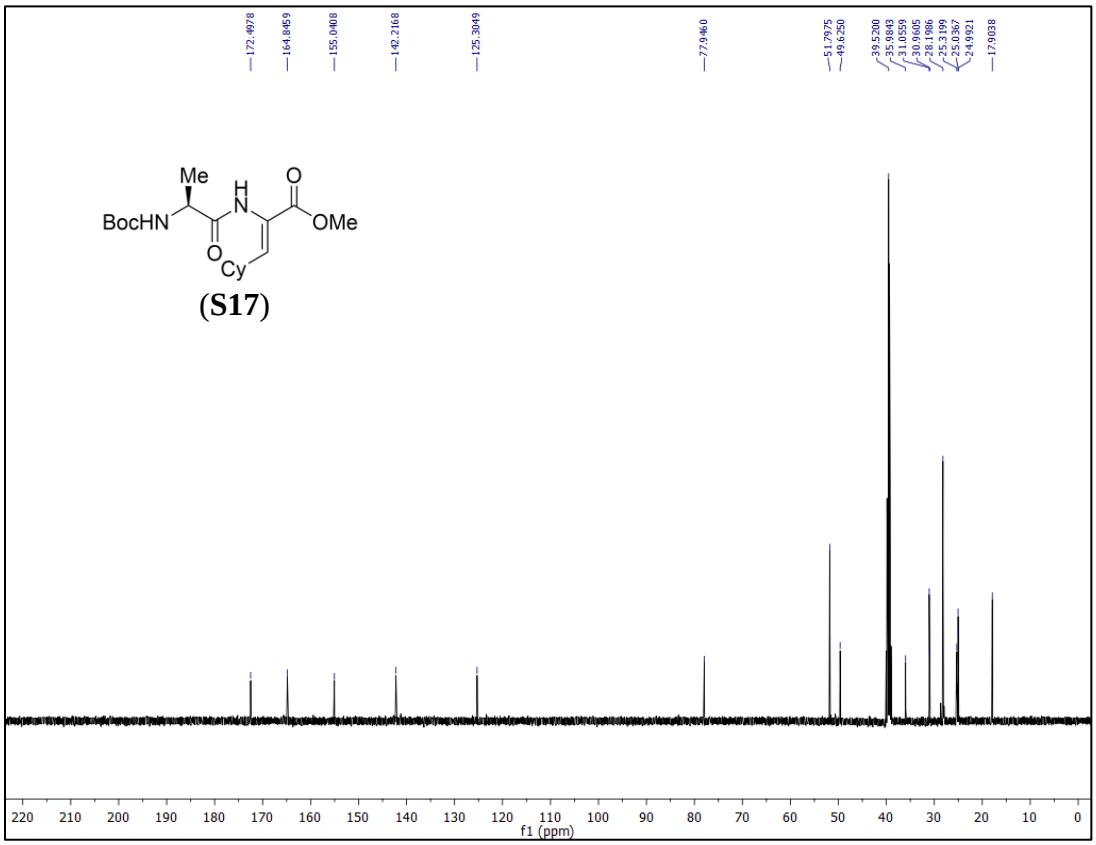
(**S16**)



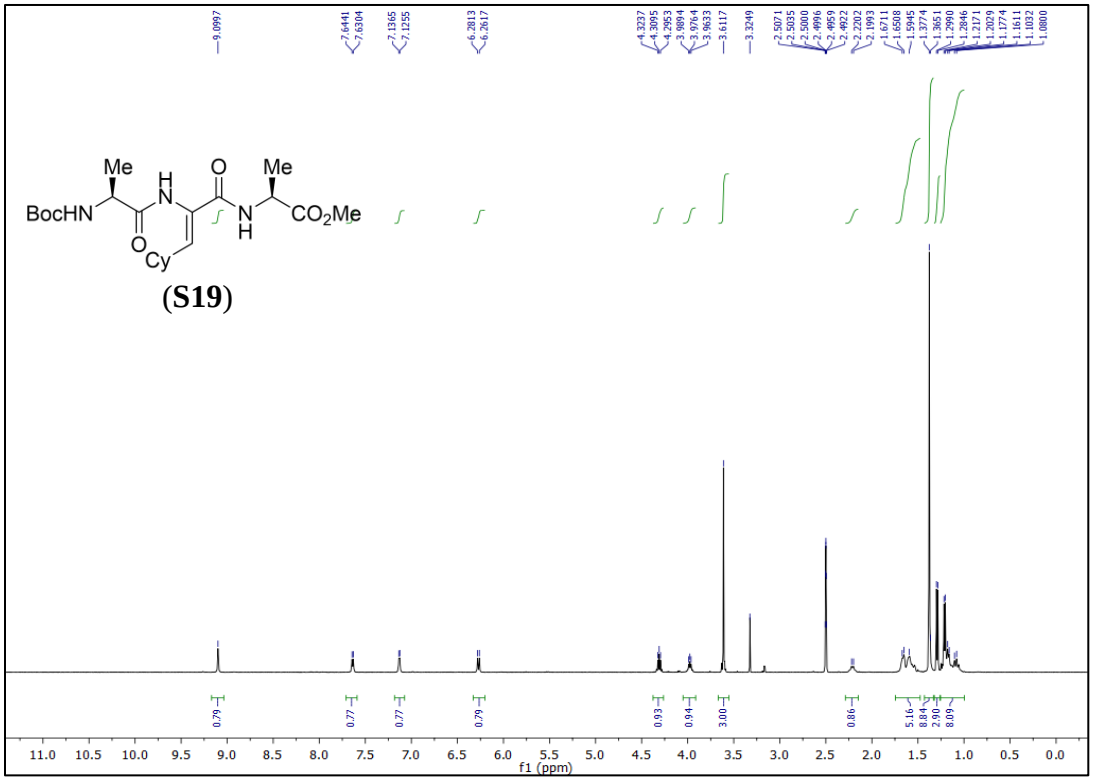
(**S16**)



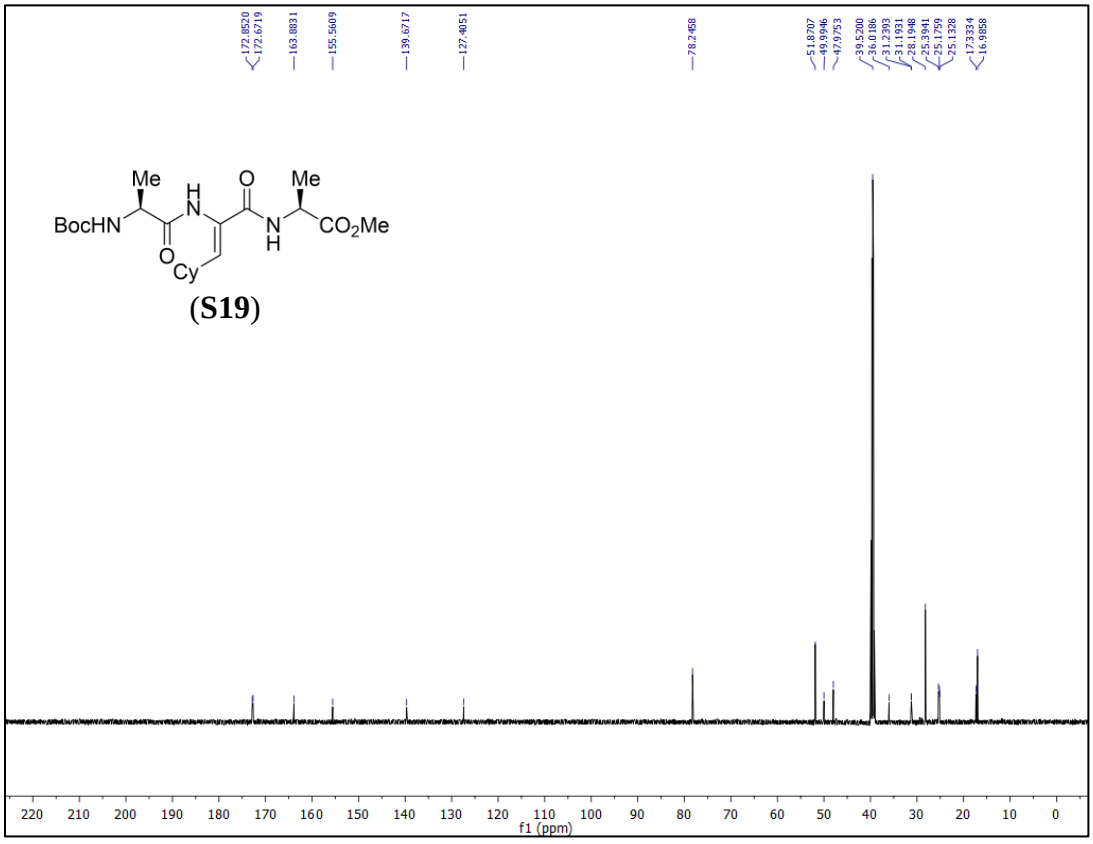
(S17)



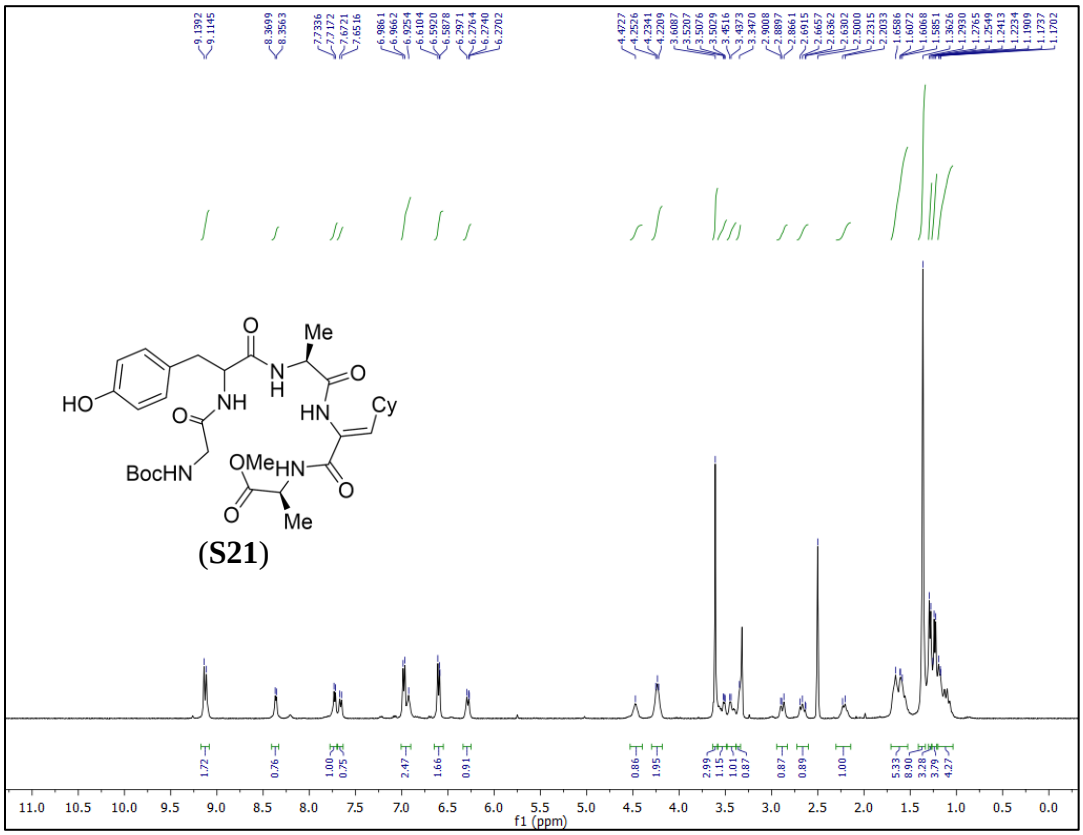
(S17)

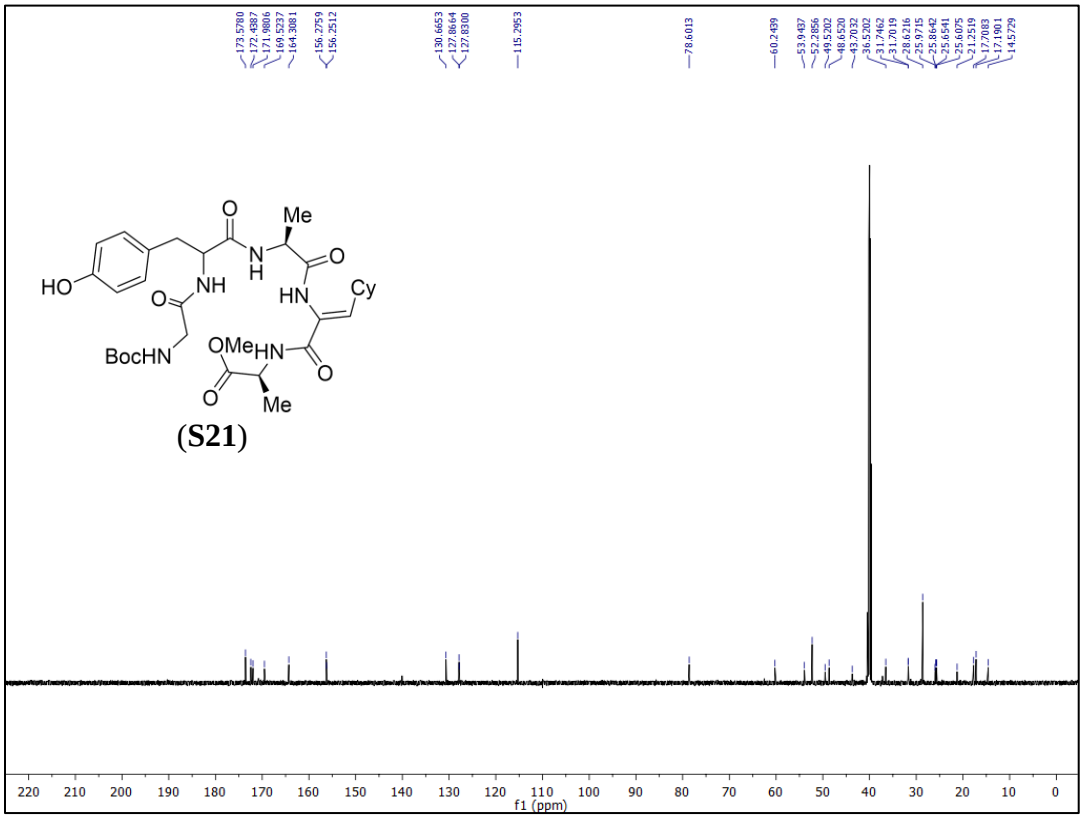


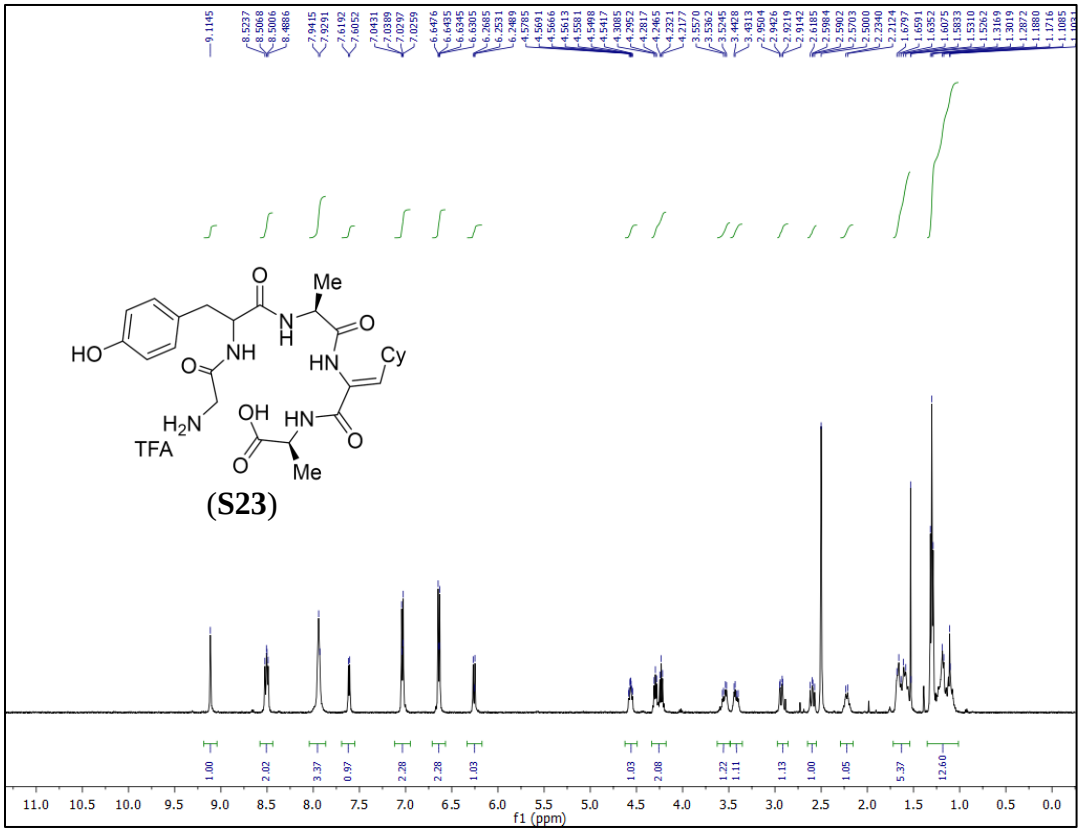
(S19)



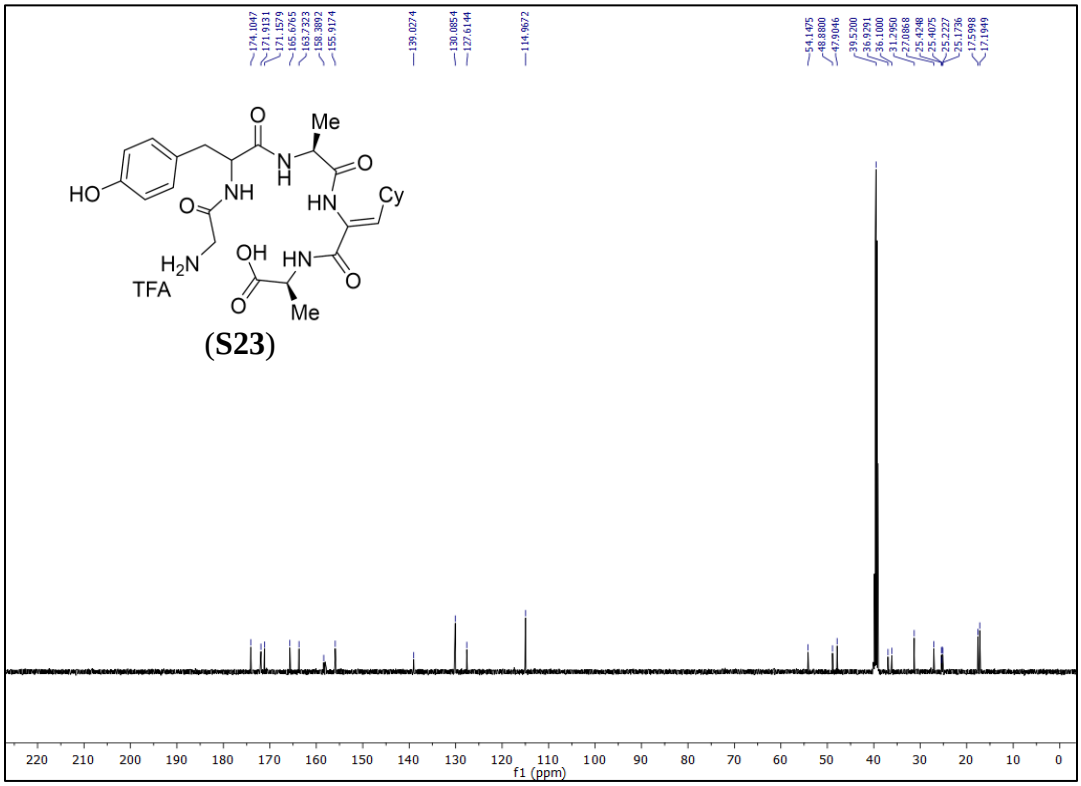
(S19)


HO
NH
O
BocHN
OMe
HN
Me
Cy
(S21)
f1 (ppm)

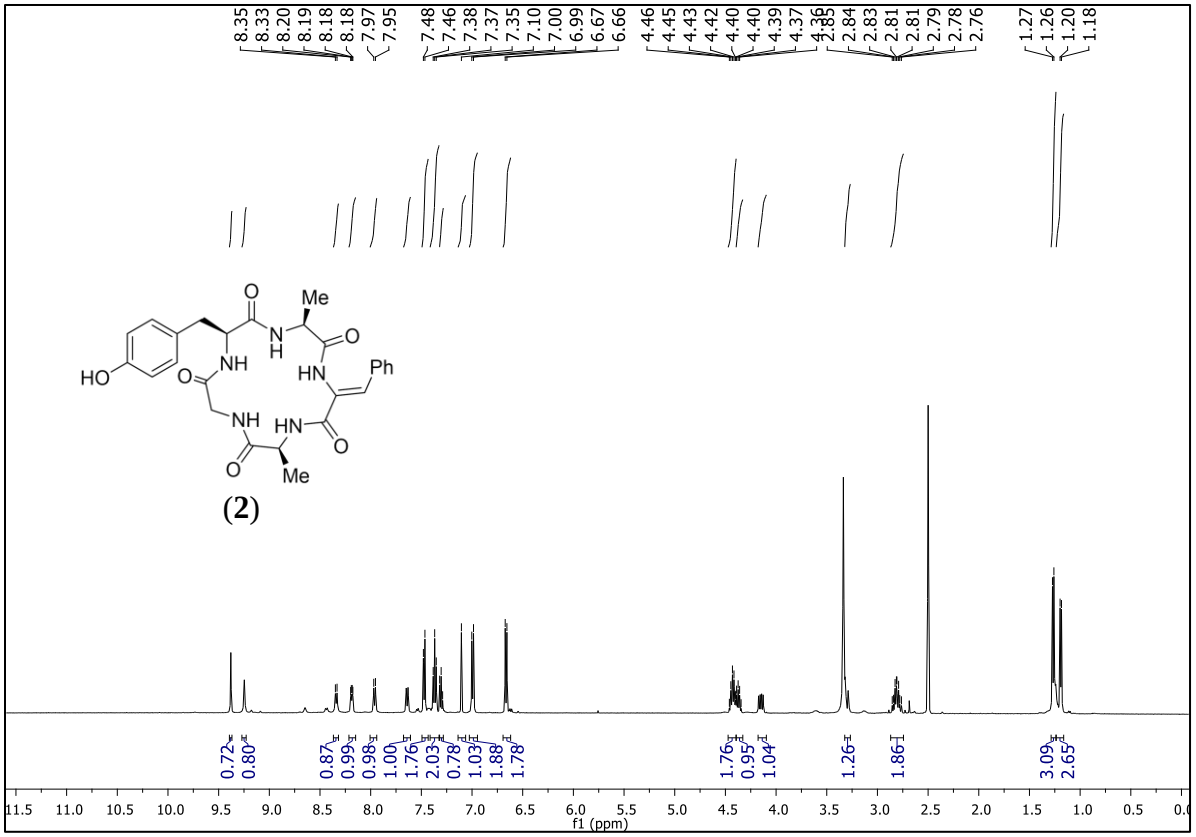

HO
NH
O
BocHN
OMe
HN
Me
Cy
(S21)
f1 (ppm)


HO
NH
O
Me
N
H
O
HN
Cy
H2N
TFA
OH
HN
O
Me
f1 (ppm)

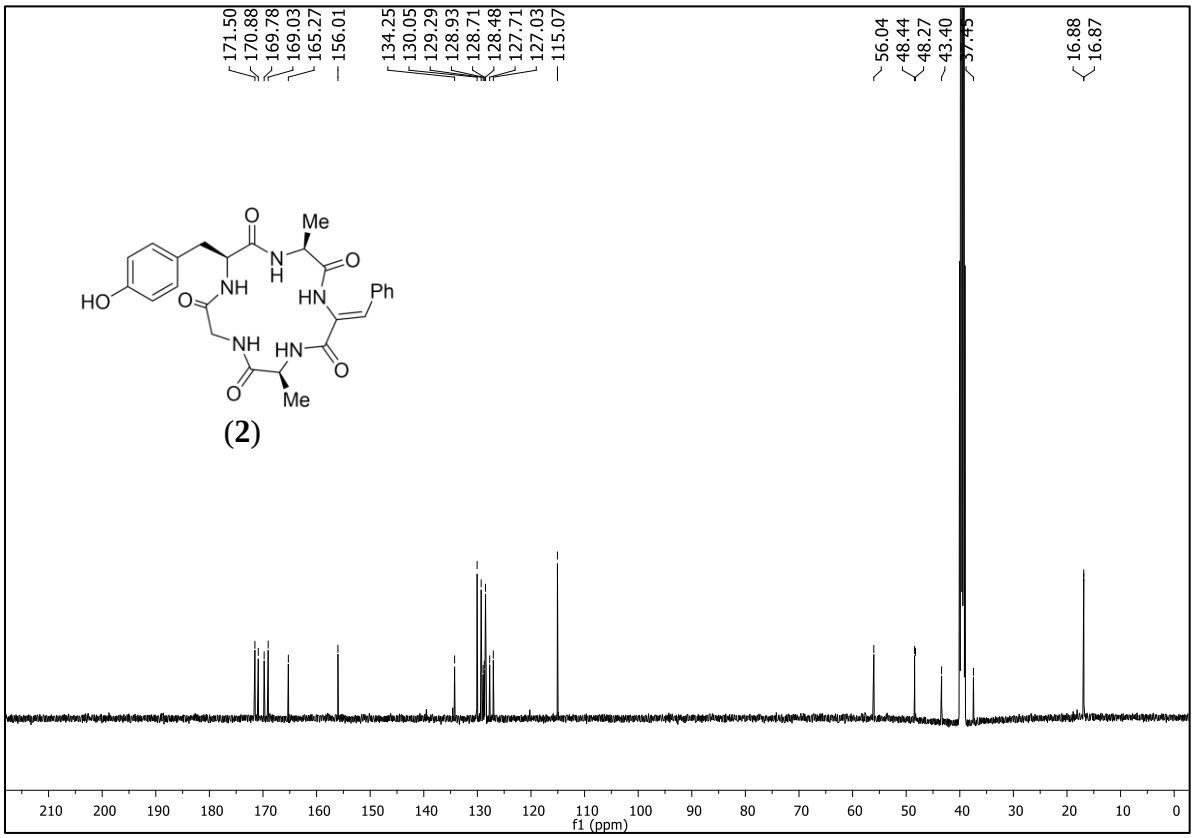
(S23)


HO
NH
O
Me
N
H
O
HN
Cy
H2N
TFA
OH
HN
O
Me
f1 (ppm)

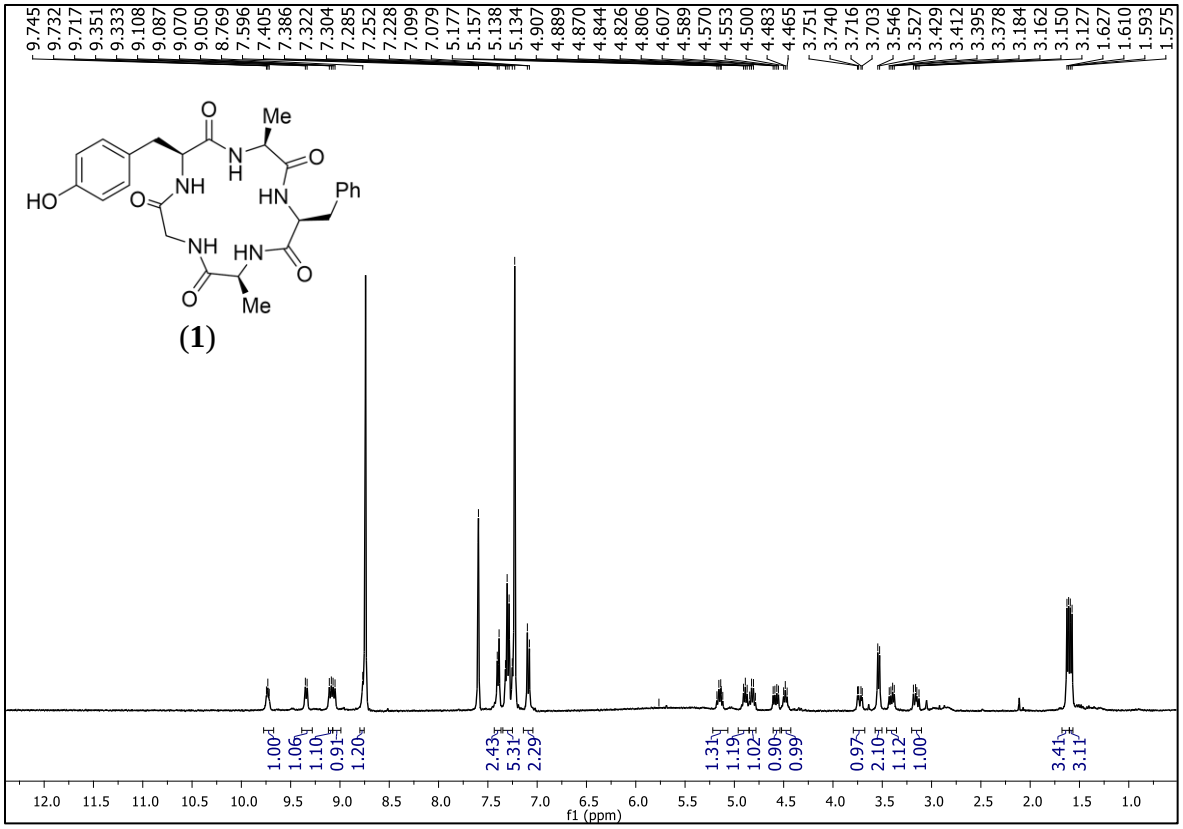
(S23)


8.35
8.33
8.20
8.19
8.18
8.18
7.97
7.95
7.48
7.46
7.38
7.37
7.35
7.10
7.00
6.99
6.67
6.66
4.46
4.45
4.43
4.42
4.40
4.40
4.39
4.37
4.36
2.85
2.84
2.83
2.81
2.81
2.79
2.78
2.76
1.27
1.26
1.20
1.18
HO
NH
HN
NH
HN
Me
Me
Ph
0.72
0.80
0.87
0.99
0.98
1.00
1.76
2.03
0.78
1.03
1.88
1.78
1.76
0.95
1.04
1.26
1.86
3.09
2.65
f1 (ppm)

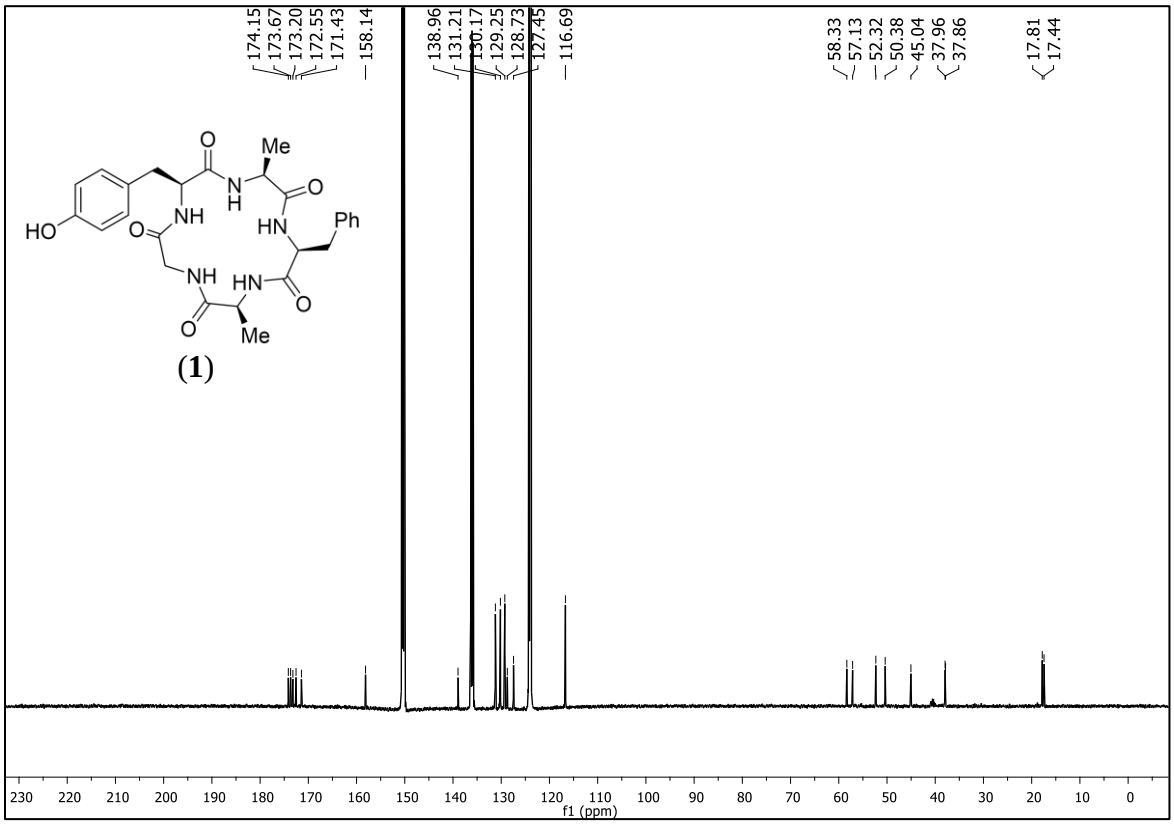
(2)


171.50
170.88
169.78
169.03
165.27
156.01
134.25
130.05
129.29
128.93
128.71
128.48
127.71
127.03
115.07
56.04
48.44
48.27
43.40
37.45
16.88
16.87
HO
NH
HN
NH
HN
Me
Me
Ph
f1 (ppm)

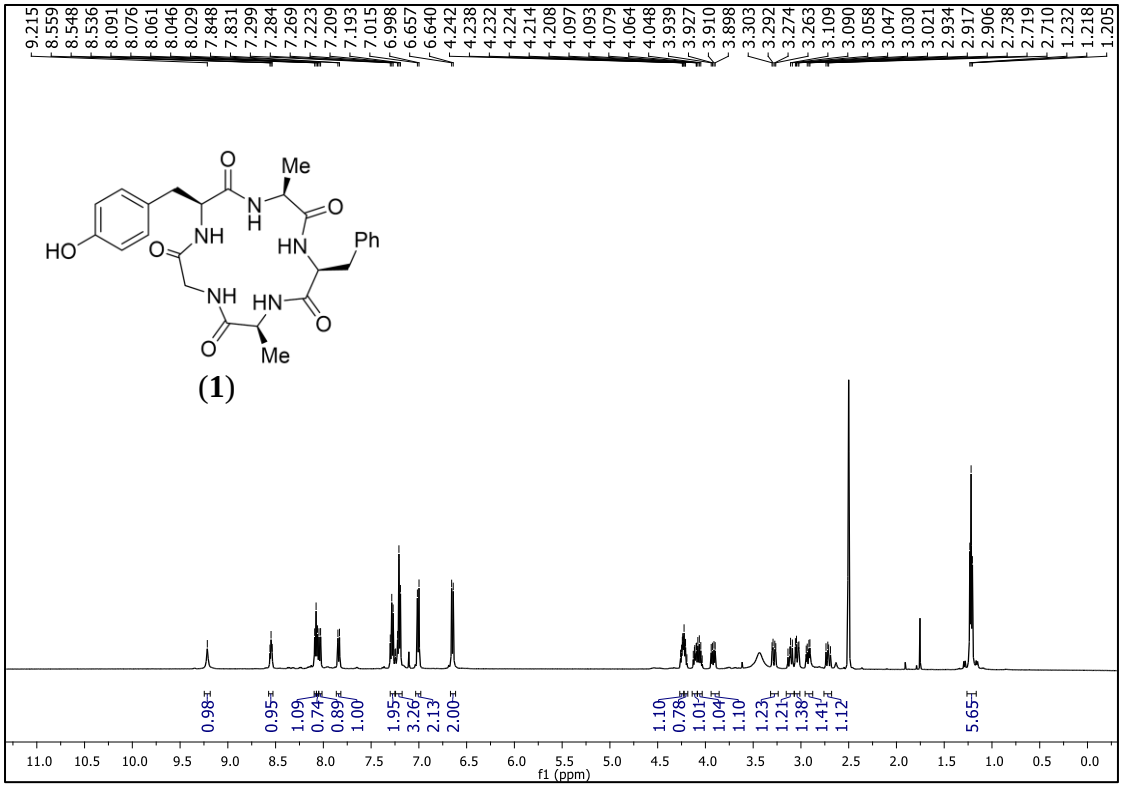
(2)


9.745 9.732 9.717 9.351 9.333 9.108 9.087 9.070 9.050 8.769 7.596 7.405 7.386 7.322 7.304 7.285 7.252 7.228 7.099 7.079 5.177 5.157 5.138 5.134 4.907 4.889 4.870 4.844 4.826 4.806 4.607 4.589 4.570 4.553 4.500 4.483 4.465 3.751 3.740 3.716 3.703 3.546 3.527 3.429 3.412 3.395 3.378 3.184 3.162 3.150 3.127 1.627 1.610 1.593 1.575
1.00 1.06 1.10 0.91 1.20 2.43 5.31 2.29 1.31 1.19 1.02 0.90 0.99 0.97 2.10 1.12 1.00 3.41 3.11
f1 (ppm)

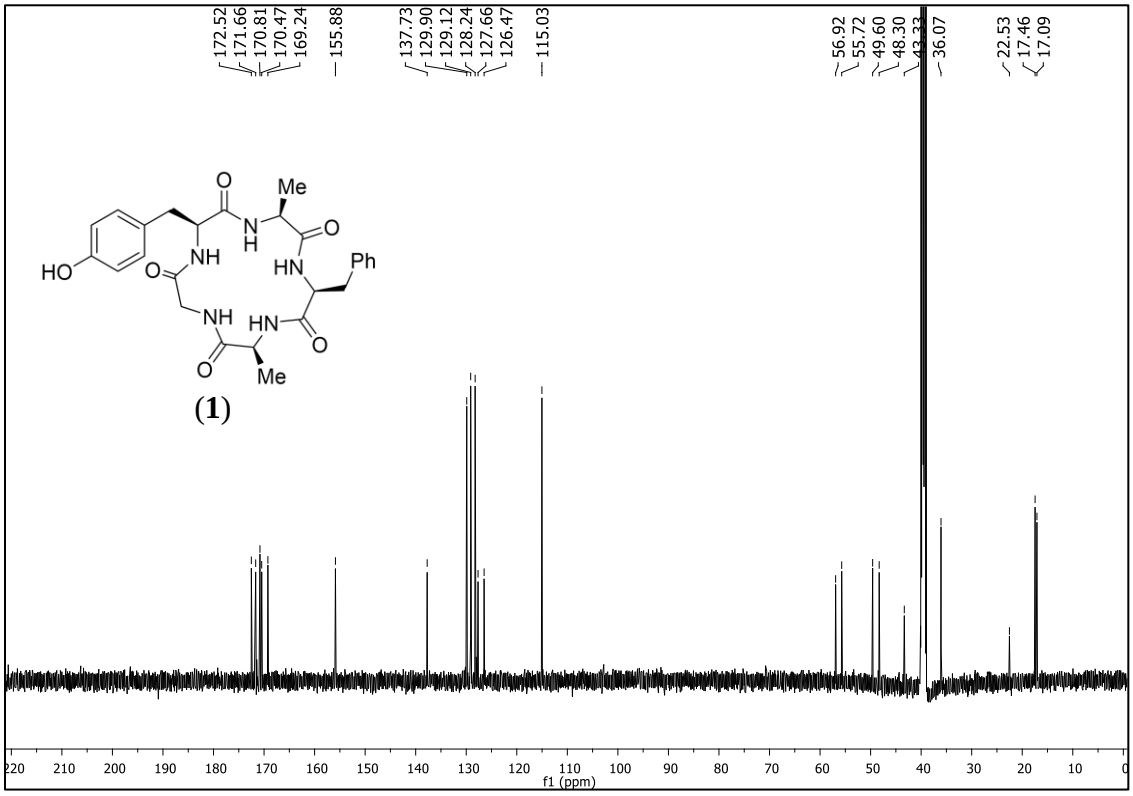
(1)


174.15 173.67 173.20 172.55 171.43 158.14 138.96 131.21 130.17 129.25 128.73 127.45 116.69 58.33 57.13 52.32 50.38 45.04 37.96 37.86 17.81 17.44
f1 (ppm)

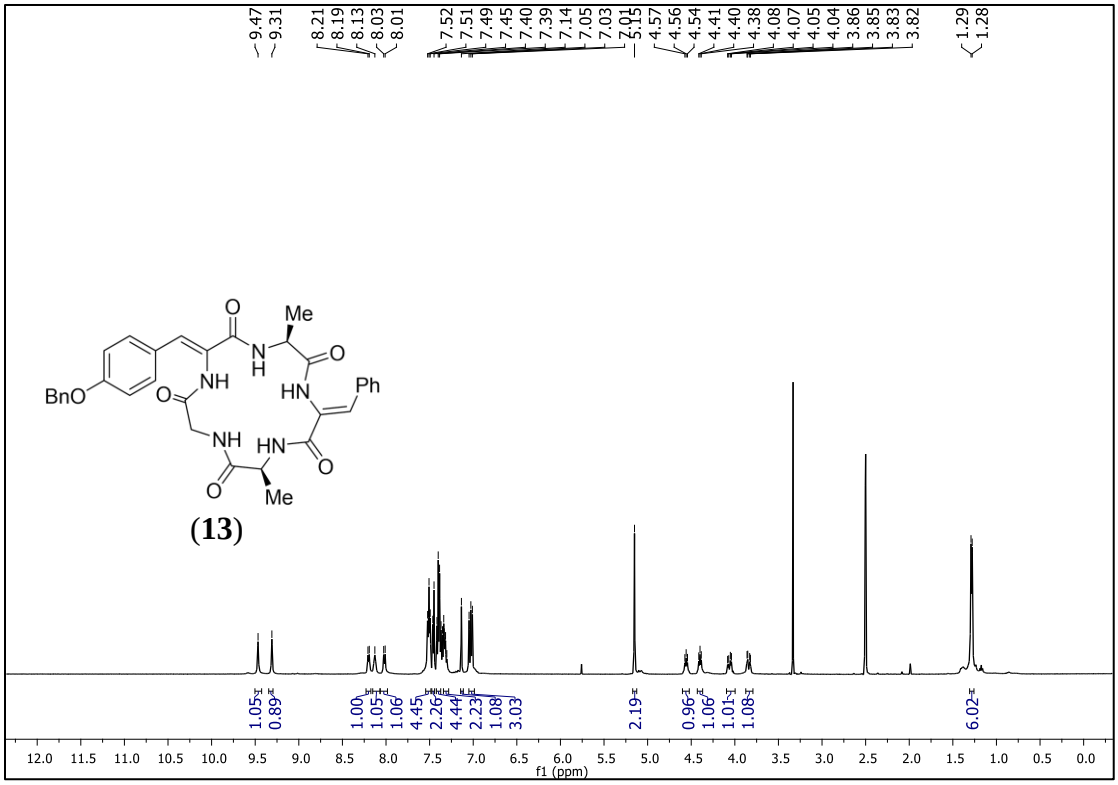
(1)

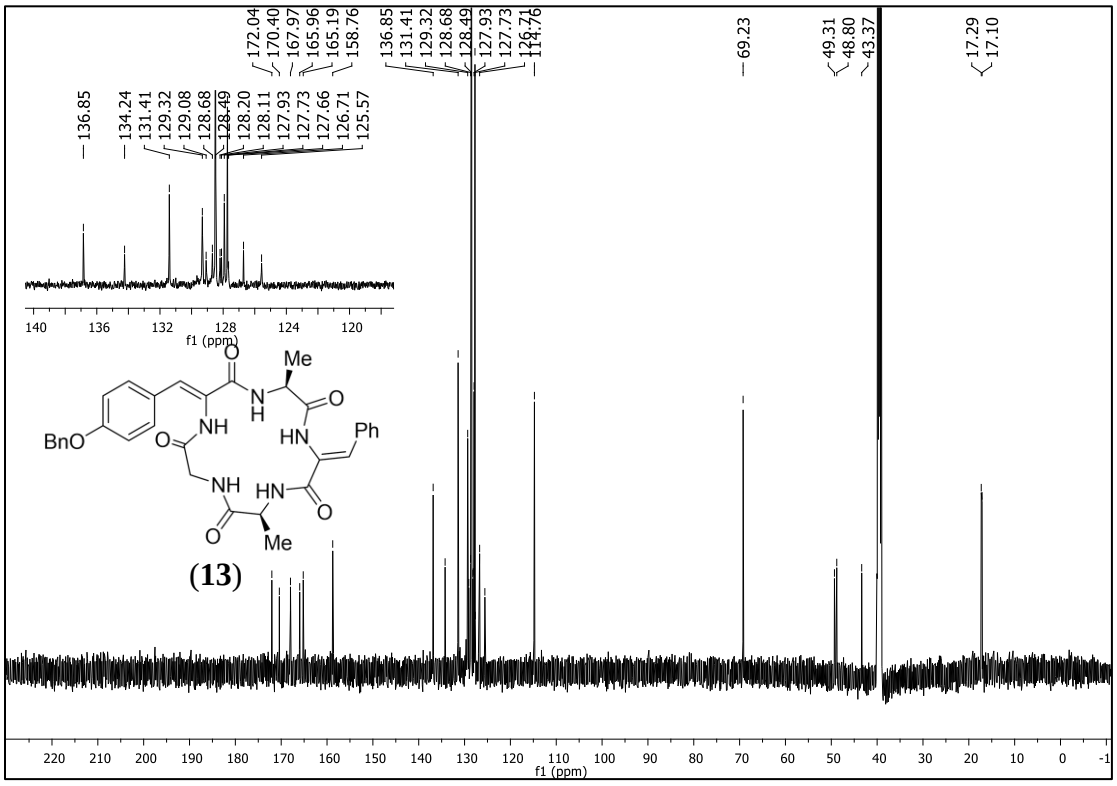

9.215
8.559
8.548
8.536
8.091
8.076
8.061
8.046
8.029
7.848
7.831
7.299
7.284
7.269
7.223
7.209
7.193
7.015
6.998
6.657
6.640
4.242
4.238
4.232
4.224
4.214
4.208
4.097
4.093
4.079
4.064
4.048
3.939
3.927
3.910
3.898
3.303
3.292
3.274
3.263
3.109
3.090
3.058
3.047
3.030
3.021
2.934
2.917
2.906
2.738
2.719
2.710
1.232
1.218
1.205
Me
O
N
H
O
HO
NH
HN
Ph
O
NH
HN
O
O
Me
0.98
0.95
1.09
0.74
0.89
1.00
1.95
3.26
2.13
2.00
1.10
0.78
1.01
1.04
1.10
1.23
1.21
1.38
1.41
1.12
5.65
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)

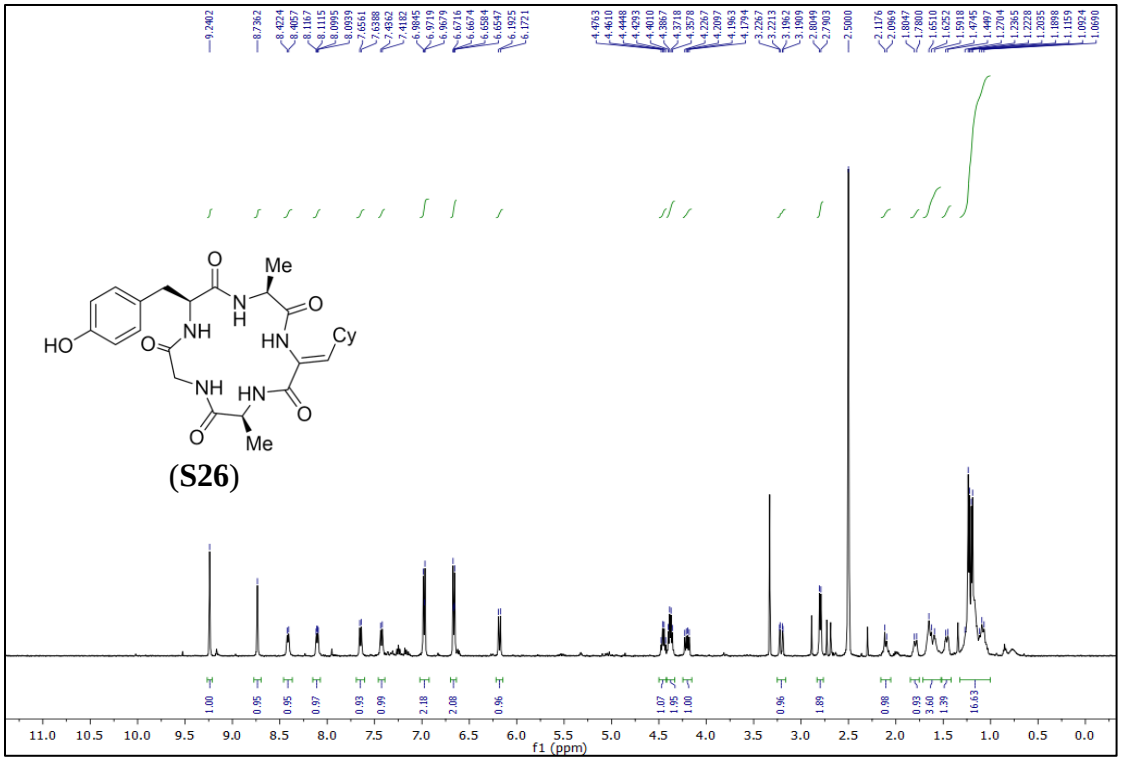
(**1**)


172.52
171.66
170.81
170.47
169.24
155.88
137.73
129.90
129.12
128.24
127.66
126.47
115.03
56.92
55.72
49.60
48.30
43.33
36.07
22.53
17.46
17.09
Me
O
N
H
O
HO
NH
HN
Ph
O
NH
HN
O
O
Me
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)

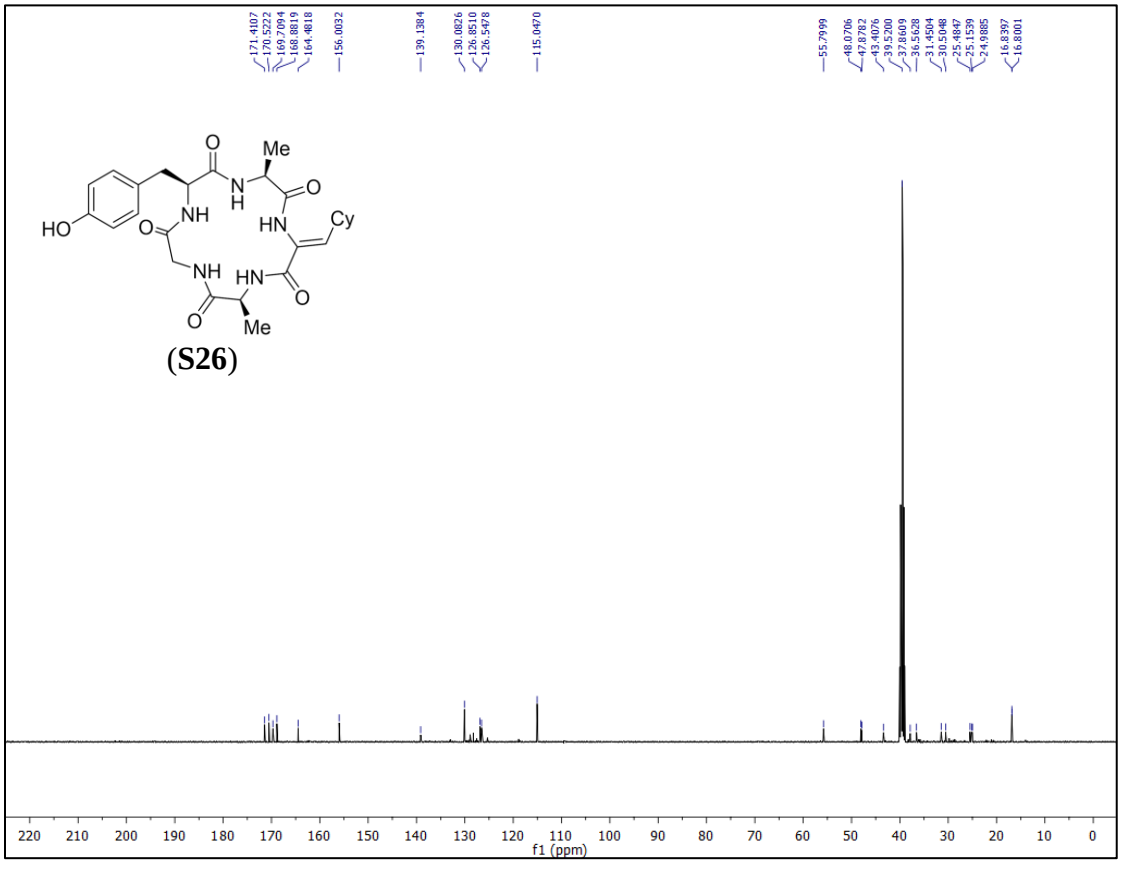
(**1**)


9.47
9.31
8.21
8.19
8.13
8.03
8.01
7.52
7.51
7.49
7.45
7.40
7.39
7.14
7.05
7.03
7.01
5.15
4.57
4.56
4.54
4.41
4.40
4.38
4.08
4.07
4.05
4.04
3.86
3.85
3.83
3.82
1.29
1.28
BnO
Me
Ph
(13)
1.05
0.89
1.00
1.05
1.06
4.45
2.26
4.44
2.23
1.08
3.03
2.19
0.96
1.06
1.01
1.08
6.02
f1 (ppm)

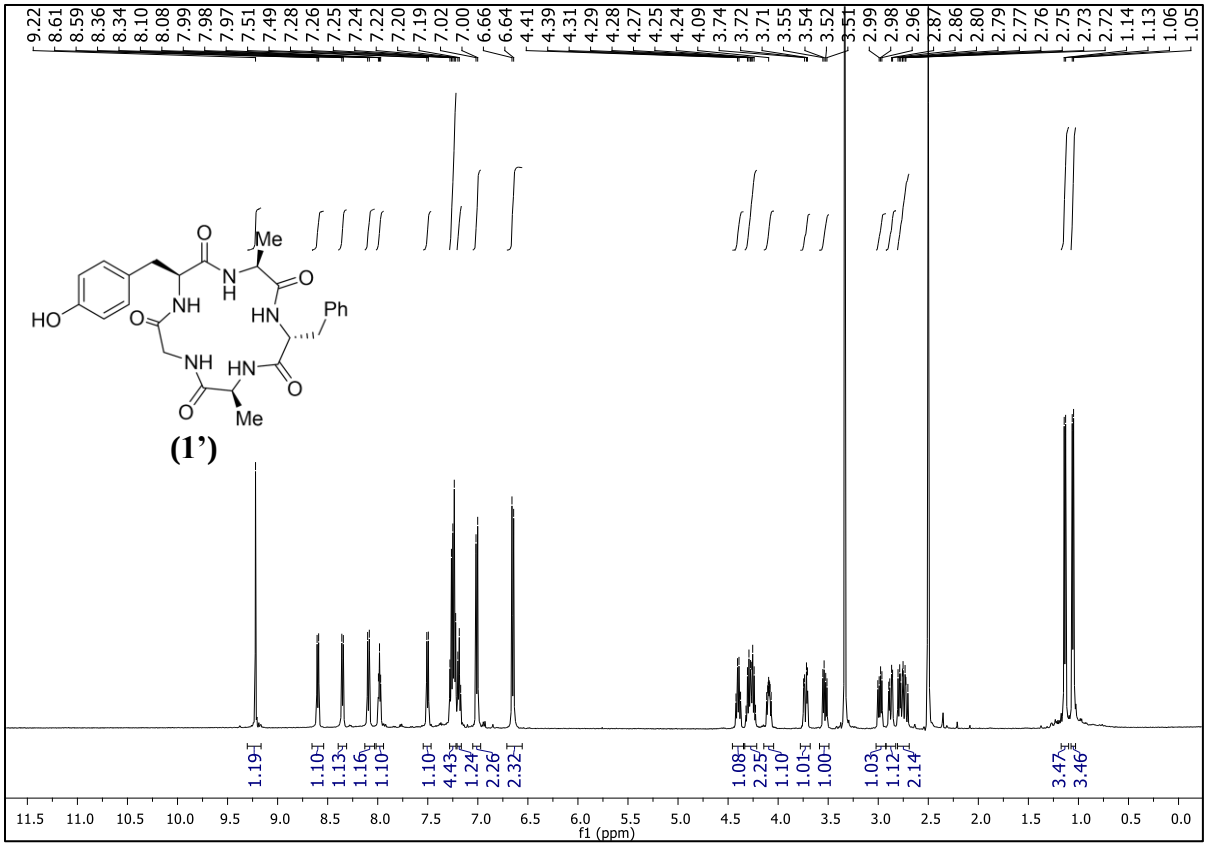

172.04
170.40
167.97
165.96
165.19
158.76
136.85
131.41
129.32
128.68
128.49
127.93
127.73
126.71
114.76
69.23
49.31
48.80
43.37
17.29
17.10
136.85
134.24
131.41
129.32
129.08
128.68
128.49
128.20
128.11
127.93
127.73
127.66
126.71
125.57
f1 (ppm)
BnO
Me
Ph
(13)
f1 (ppm)

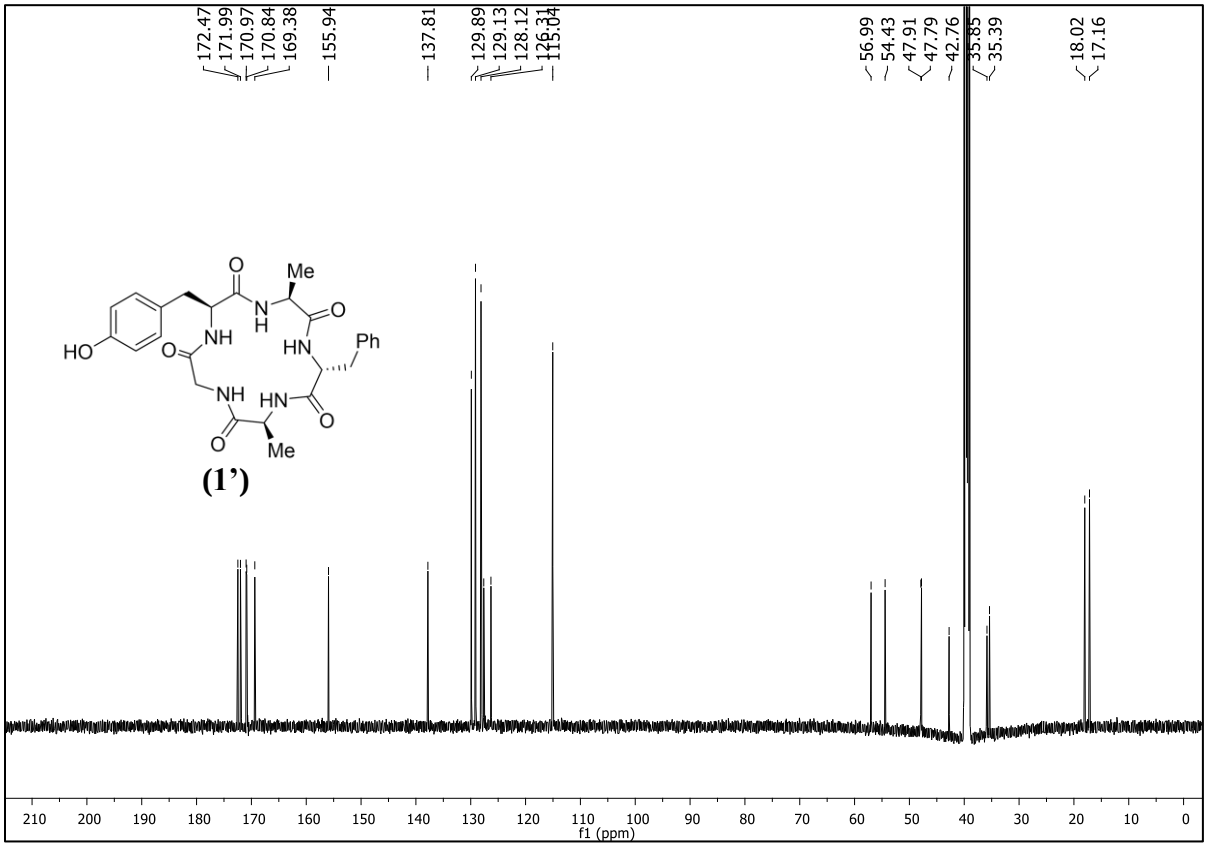


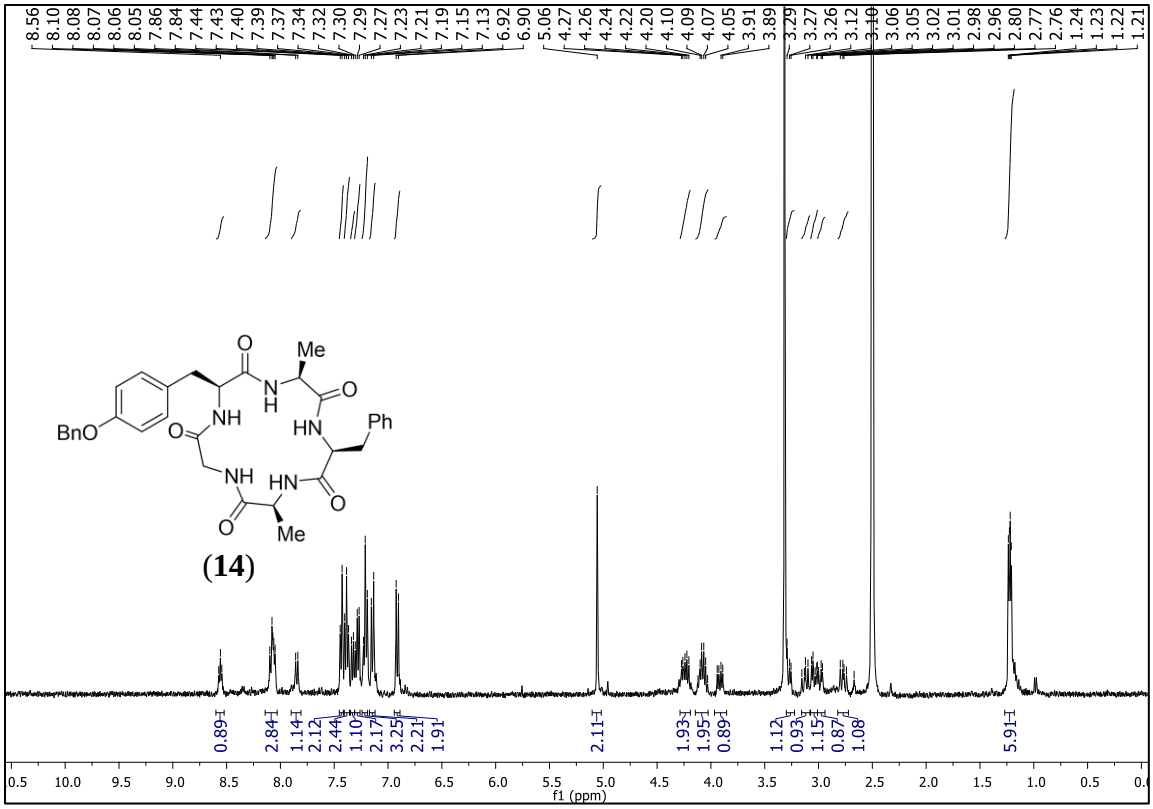
(**S26**)

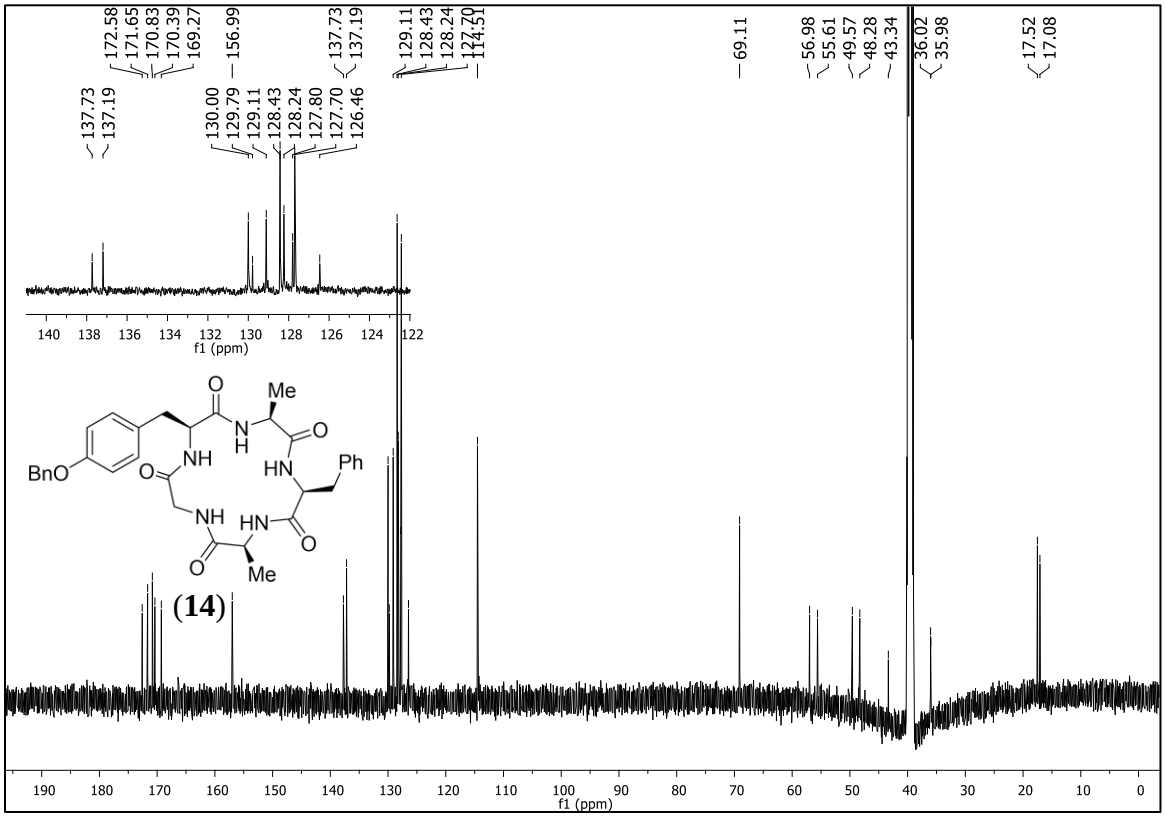


(**S26**)


(1')


(1')


(14)
f1 (ppm)


(14)
f1 (ppm)