SUPPORTING INFORMATION

Click Chemistry as a Route to Cyclic Tetrapeptide Analogs: Synthesis of *cyclo*-[Pro-Val-ψ(triazole)-Pro-Tyr]

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Experimental Methods

General: The following general procedures were used in all reactions unless otherwise noted. Oxygen- and moisture-sensitive reactions were carried out using standard Schlenk techniques under a nitrogen atmosphere. Similarly sensitive liquids and solutions were transferred via gas-tight syringe or cannula. Reactions were stirred with an oven-dried, Teflon-covered magnetic stir bar. Removal of solvents was accomplished by evaporation on a Buchi rotary evaporator (water bath 40 °C). Tetrahydrofuran and diethyl ether were freshly distilled from sodium/benzophenone. Dry DMF and CH_2Cl_2 were freshly distilled from CaH_2. All commercially available reagents were used as received, unless indicated otherwise.

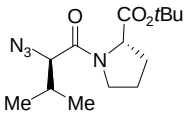
Chromatography: Analytical TLC chromatography was performed on 250 µm silica gel 60 plates with 254 nm fluorescent indicator. Chromatographic purification refers to flash chromatography[1] using the indicated solvent (mixture) and Acros silica gel (0.035–0.070 mm). Analytical reverse phase HPLC was carried out on C_{18} columns using gradients between 95 : 5 : 0.01 (water / acetonitrile / formic acid) and 5 : 95 : 0.01 (water / acetonitrile / formic acid).

Physical and Spectroscopic Measurements: NMR spectra were recorded in Fourier Transform mode on a Bruker ARX 400 (^{1}H at 400 MHz, ^{13}C at 100 MHz) magnetic resonance spectrophotometer. ^{1}H NMR spectra are reported as chemical shifts in parts per million (ppm) downfield from a tetramethylsilane internal standard (0.00 ppm). Spin multiplicity is described by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, and br = broad. Coupling constants (*J*) are reported in Hertz (Hz). ^{13}C NMR spectra are reported as chemical shifts in ppm with the solvent resonance as the internal standard ($CDCl_3$: 77.07 ppm; MeOD: 49.00 ppm) and were recorded with complete heterodecoupling as APT (attached proton test) spectra. Infrared spectra were obtained from $CDCl_3$ solutions on a Bruker IFS 28 Fourier Transform spectrometer (FTIR) and are reported in wavenumbers (cm^{-1}). Fast Atom Bombardment (FAB) mass spectrometry was carried out using a JEOL JMS SX/SX 102A four-sector mass spectrometer, coupled to a JEOL MS-MP9021D/UPD system program. Samples were loaded in a matrix solution (3-nitrobenzyl alcohol) on a stainless steel probe and bombarded with xenon atoms with an energy of 3keV. During the high resolution FAB-MS measurements a resolving power of 10,000 (10% valley definition) was used. MALDI-TOF mass spectra were recorded on a Micromass TofSpec 2EC (Micromass, Whyttenshawe, UK). Samples (50 pmol) were dissolved in EtOAc, mixed with a concentrated solution of DHB in EtOAc and spotted directly on the stainless steel MALDI target. Silver nitrate was used for the ionization of the molecules.

Abbreviations: DHB, 2,5-dihydroxybenzoic acid, DIPEA, diisopropylethylamine; EDC, 1-(3-(dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride; HATU, *O*-(7-azabenzotriazole-1-yl)-

1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, *N*-hydroxybenzotriazole; MALDI-TOF: matrix assisted laser desorption/ionization–time of flight; MeOD, methanol-d_4; PE, petroleum ether; PyAOP, (7-azabenzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium hexafluorophosphate

(*S*)-*tert*-butyl 1-((*S*)-2-azido-3-methylbutanoyl)pyrrolidine-2-carboxylate (5)



To a 100 mL round-bottomed flask equipped with a $CaCO_3$ drying tube and charged with azido valine **7**[2] (0.740 g, 5.17 mmol, 1 equiv) in freshly distilled CH_2Cl_2 (20 mL) was added EDC (1.09 g, 5.69 mmol, 1.1 equiv) and HOBt (0.734 g, 5.43 mmol, 1.05 equiv). L-proline *t*-butyl ester (0.927 g, 5.43 mmol, 1.05 equiv) was then added in freshly distilled CH_2Cl_2 (5 mL). After 16 h, this solution was diluted with $CHCl_3$ (40 mL) and washed with H_2O (1 × 70 mL), satd aq $NaHCO_3$ (1 × 70 mL), and 1N $HCl_{(aq)}$ (1 × 70 mL). The combined organics were then dried over Na_2SO_4, filtered, and concentrated in vacuo to yield azido valine-proline *t*-butyl ester **5** (1.447 g, 3.10 mmol, 60%) as a light yellow solid. This solid was carried on without further purification.

Data for compound **5**:

^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.52–4.31 (m, 1H, Pro C**H**$_\alpha$), 3.78–3.59 (m, 2H, Pro NC**H**$_2$), 3.39–3.19 (m, 1H, Val C**H**$_\alpha$), 2.35–1.97 (m, 5H, Pro NCH$_2$C**H**$_2$; Pro CH$_\alpha$C**H**$_2$; Val CH$_\alpha$C**H**), 1.48 (s, 9H, C(C**H**$_3$)$_3$), 1.12 (d, *J* = 6.7 Hz, 3H, one of two Val CH(C**H**$_3$)$_2$), 1.07 (d, *J* = 6.7 Hz, 3H, one of two Val CH(C**H**$_3$)$_2$) ppm.

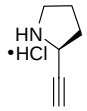
^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.77, 170.59, 168.70, 168.35, 82.60, 81.25, 65.99, 65.48, 60.00, 59.62, 47.01, 46.40, 42.74, 31.26, 30.71, 30.19, 29.48, 28.87, 27.76, 27.64, 24.58 22.06, 19.01, 18.98, 18.90, 18.76 ppm.

IR 3448, 2974, 2876, 2079, 1736, 1654, 1432, 1392, 1368, 1258, 1226, 1154, 1093, 1044, 1014, 979, 953, 882, 847 cm^{-1}.

HMRS (FAB) Calculated for $C_{14}H_{25}N_4O_3$ (MH^+): 297.1928
Found: 297.1917

$[\alpha]_D^{20}$ –2.13 (*c* 1.97, $CHCl_3$)

(*S*)-2-ethynylpyrrolidine hydrochloride (9)



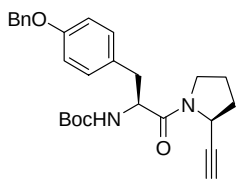
To a 100 mL round-bottomed flask charged with Boc-protected alkyne **8**[3] (3.10 g, 15.86 mmol, 1 equiv) in Et_2O (48 mL) was added concentrated $HCl_{(aq)}$ (4.1 mL, 47.59 mmol, 3 equiv). After 24 h, TLC indicated consumption of the starting materials, so the volatiles were evaporated in vacuo to yield deprotected amino alkyne **9** (2.23 g) as a sticky, red oil. This oil was carried on without further purification.

Data for compound **9**:

^{1}H NMR (MeOD, 400 MHz) δ 4.42 (m, 1H, Pro **H**$_\alpha$), 3.37 (m, 3H, NC**H**$_2$ and CC**H**), 2.40 (m, 1H, one of two NCH$_2$CH$_2$C**H**$_2$), 2.20 (m, 1H, one of two NCH$_2$CH$_2$C**H**$_2$), 2.10 (m, 2H, NCH$_2$C**H**$_2$CH$_2$) ppm.

IR 3388, 3230, 2957, 2739, 2548, 2125, 1646, 1455, 1408, 1195, 1050, 1020, 934 cm^{-1}.

***tert*-butyl (*S*)-3-(4-(benzyloxy)phenyl)-1-((*S*)-2-ethynylpyrrolidin-1-yl)-1-oxopropan-2-ylcarbamate (6)**



To a 100 mL round-bottomed flask equipped with a $CaCO_3$ drying tube and charged with deprotected amino alkyne **9** (1.676 g, 12.73 mmol, 1 equiv) in freshly distilled CH_2Cl_2 (20 mL) was added DIPEA (2.2 mL, 12.73 mmol, 1 equiv). After 10 min of stirring, this red solution was added to a 250 mL oven-dried flask equipped with a $CaCO_3$ drying tube and charged with Boc-Tyr(Bn)-OH (4.97 g, 13.37 mmol, 1.05 equiv), EDC (2.44 g, 12.73 mmol, 1 equiv), HOBt (1.72 g, 12.73

mmol, 1 equiv) and freshly distilled CH_2Cl_2 (15 mL). After 16 h of stirring at room temperature, this solution was diluted with $CHCl_3$ (90 mL) and washed with H_2O (1 × 100 mL), satd aq $NaHCO_3$ (1 × 100 mL), and 1N $HCl_{(aq)}$ (1 × 100 mL). The organics were then dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a red solid. The product was purified via flash chromatography (6:4 PE:EtOAc) to afford Boc-protected tyrosine-proline alkyne **6** (3.48 g, 7.77 mmol, 61%) as a white solid.

Data for compound **6**:

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38 (m, 5H, OCH_2**Ph**), 7.17 (m, 2H, Tyr Ar**H**), 6.89 (d, *J* = 8.4 Hz, 2H, Tyr Ar**H**), 5.43 (m, 1H, N**H**), 5.06 (s, 2H, OC**H**$_2$Ph), 4.74 (m, 1H, Tyr **H**$_\alpha$), 4.50 (m, 1H, Pro **H**$_\alpha$), 3.55–2.71 (m, 4H, Tyr $CH_\alpha C$**H**$_2$ and Pro NC**H**$_2$), 2.29 (m, 1H, CC**H**), 2.04–1.83 (m, 4H, Pro NCH_2C**H**$_2$ and Pro $CH_\alpha C$**H**$_2$), 1.43 (s, 9H, C(C**H**$_3$)$_3$) ppm.

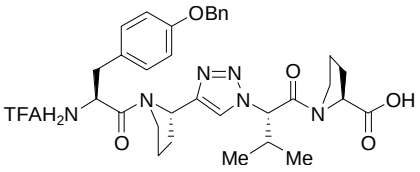
^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.59, 157.27, 154.75, 154.25, 136.77, 136.65, 130.53, 130.09, 128.61, 128.25, 128.18, 127.52, 127.00, 114.57, 114.45, 82.53, 81.67, 79.03, 72.31, 70.22, 69.45, 53.61, 53.22, 47.43, 47.12, 45.60, 45.20, 39.84, 38.81, 33.32, 31.55, 28.05, 24.36, 22.17 ppm.

IR 3429, 3302, 2978, 2876, 1711, 1644, 1612, 1583, 1511, 1446, 1391, 1366, 1295, 1242, 1174, 1110, 1024, 907, 863, 824 cm^{-1}.

HMRS (FAB) Calculated for $C_{27}H_{33}N_2O_4$ (MH^+): 449.2442
Found: 449.2438

$[\alpha]_D^{20}$ –53.8 (*c* 1.02, $CHCl_3$)

(*S*)-1-((*S*)-2-(4-((*S*)-1-((*S*)-2-amino-3-(4-(benzyloxy)phenyl)propanoyl)pyrrolidin-2-yl)-1H-1,2,3-triazol-1-yl)-3-methylbutanoyl)pyrrolidine-2-carboxylic acid triflic acid salt (3):



A 25 mL round-bottomed flask containing MeCN (5 mL) and THF (1 mL) was degassed with argon for thirty minutes. Azido valine-proline *t*-butyl ester **5** (0.296 mmol, 1.00 mmol, 1 equiv) and Boc-protected tyrosine-proline alkyne **6** (0.448 g, 1.00 mmol, 1 equiv) were added, followed by DIPEA (0.35 mL, 2.00 mmol, 2 equiv), 2,6-lutidine (0.23 mL, 2.00 mmol, 2 equiv), and CuI (0.019 g, 0.1 mmol, 0.1 equiv). After 16 h stirring under argon, the reaction was quenched by addition of satd aq NH_4Cl (10 mL), and the solution became a cloudy white. The volatiles were evaporated in vacuo, and the resulting aqueous slurry was extracted with CH_2Cl_2 (3 × 20 mL). The combined organics were washed with satd aq NH_4Cl (3 × 20 mL), 0.5 M $KHSO_4$ (1 × 20 mL), H_2O (1 × 20 mL), and brine (1 × 20 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was then purified via flash chromatography (4:6 PE:EtOAc) to afford a white solid. This triazole product was dissolved in TFA (4 mL) and $CHCl_3$ (4mL) and stirred for five hours. The solvents were then removed in vacuo and subsequently coevaporated with toluene (2 × 10 mL) and $CHCl_3$ (2 × 10 mL) to afford triazole-substituted tetrapeptide **3** (0.460 g, 0.742 mmol, 74%) as a white solid. This solid was carried on without further purification.

Data for compound **3**:

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.23–7.61 (m, 4H), 7.44–6.88 (m, 9H), 5.23–4.99 (m, 2H) 4.79 (m, 1H), 4.51–4.07 (m, 3H), 3.97–3.59 (m, 4H), 3.35 (m, 1H), 3.02 (m, 1H), 3.73–1.57 (m, 9H), 1.31–1.01 (m, 3H), 0.81–0.65 (m, 3H) ppm.

^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.38, 169.19, 169.00, 168.91, 168.46, 167.51, 195.85, 159.79, 159.71, 159.51, 138.11, 132.41, 132.14, 131.90, 130.42, 130.03, 130.00, 129.93. 129.81, 129.61, 129.46, 129.44, 129.28, 129.06, 128.87, 128.79, 128.69, 127.13, 126.99, 126.68, 122.99, 116.98, 116.88, 116.75, 71.33, 71.29, 71.01, 69.39, 69.11, 68.94, 61.97,

61.40, 61.33, 56.08, 55.70, 55.44, 55.15, 55.00, 54.44, 49.80, 48.59, 48.52, 48.28, 48.13, 47.95, 38.80, 38.75, 34.92, 34.75, 34.52, 33.69, 33.19, 32.75, 31.08, 30.12, 26.58, 26.47, 25.40, 23.83, 23.66, 23.18, 30.71, 20.45, 19.75, 19.51, 19.25 ppm.

IR 2967, 2359, 1729, 1650, 1512, 1456, 1371, 1181, 1140, 1048, 1023, 911, 836 cm^{-1}.

Attempted Cyclization of Triazole-substituted Tetrapeptide 3 via EDC/HOBt-Mediated Coupling

To a 250 mL round-bottomed flask equipped with a $CaCO_3$ drying tube and charged with triazole-substituted tetrapeptide **3** (0.072 g, 0.100 mmol, 1 equiv) in freshly distilled CH_2Cl_2 (100 mL) was added was added DIPEA (0.034 mL, 0.100 mmol, 1 equiv). After 10 min of stirring, EDC (0.0191 g, 0.100 mmol, 1 equiv) and HOBt (0.0135 g, 0.100 mmol, 1 equiv) were added. After 16 h, this solution was quenched by addition of H_2O (50 mL) and concentrated in vacuo to remove the volatiles. The resulting aqueous slurry was diluted with $CHCl_3$ (50 mL), and the layers were separated. The organic layer was then washed with satd aq $NaHCO_3$ (1 × 50 mL), 1N $HCl_{(aq)}$ (1 × 50 mL), and H_2O (1 × 50 mL). The combined organics were dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a yellow solid (0.075 g). MALDI-TOF analysis indicated that the isolated products were dimers and higher oligomers.

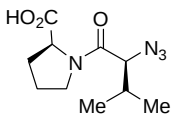
Attempted Cyclization of Triazole-substituted Tetrapeptide 3 via HATU-Mediated Coupling

To a 250 mL round-bottomed flask equipped with a $CaCO_3$ drying tube and charged with triazole-substituted tetrapeptide **3** (0.035 g, 0.0499 mmol, 1 equiv) in freshly distilled DMF (75 mL) was added was added HATU (0.019 mg, 0.100 mmol, 1 equiv) and DIPEA (0.035 mL, 0.200 mmol, 4 equiv). After 16 h, this solution was concentrated in vacuo and diluted with EtOAc (50 mL). The organic layers was then washed 0.5 M $KHSO_4$ (1 × 50 mL), H_2O (1 × 50 mL), and brine (1 × 50 mL). The combined organics were then dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a yellow solid (0.028 g). MALDI-TOF analysis indicated that the isolated products were dimers and higher oligomers.

Attempted Cyclization of Triazole-substituted Tetrapeptide 3 via PyOAP-Mediated Coupling

To a 250 mL round-bottomed flask equipped with a septum and $N_{2(g)}$ inlet and charged with triazole-substituted tetrapeptide **3** (0.0726 g, 0.103 mmol, 1 equiv) in freshly distilled CH_2Cl_2 (100 mL) was added was added DIPEA (0.034 mL, 0.206 mmol, 2 equiv). After 10 min of stirring, PyAOP (0.0521 g, 0.103 mmol, 1 equiv) were added. After 16 h, this solution was concentrated in vacuo to yield a yellow solid (0.082 g). MALDI-TOF analysis indicated that the isolated products were dimers and higher oligomers.

(*S*)-1-((*S*)-2-azido-3-methylbutanoyl)pyrrolidine-2-carboxylic acid (11):



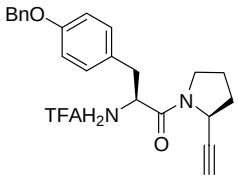
To a 100 mL round-bottomed flask charged with *t*Bu-protected valine-proline azide **5** (1.32 g, 4.45 mmol, 1 equiv) was added TFA (20 mL) and $CHCl_3$ (5 mL), and the mixture was stirred at rt. After 1 h, reaction progress was assessed by TLC. Since the starting material had been consumed, the mixture was concentrated in vacuo and subsequently coevaporated with $CHCl_3$ (4 × 10 mL) and toluene (2 × 10 mL) to afford valine-proline azide **11** (1.215 g, 4.15 mmol, quantitative) as a black oil. This oil was carried on without further purification.

Data for compound **11**:

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.80 (br s, 1H, CO_2**H**), 4.70–4.55 (m, 1H, Pro C**H**$_\alpha$), 3.68 (t, *J* = 7.3 Hz, 2H, Pro NC**H**$_2$), 3.48–3.39 (m, 1H, Val C**H**$_\alpha$), 2.39–2.01 (m, 5H, Pro NCH_2C**H**$_2$; Pro $CH_\alpha C$**H**$_2$; Val $CH_\alpha C$**H**$_2$), 1.12 (d, *J* = 6.6 Hz, 3H, one of two Val $CH(C$**H**$_3)_2$), 1.03 (d, *J* = 6.7 Hz, 3H, one of two Val $CH(C$**H**$_3)_2$) ppm.

IR 2973, 2881, 2550, 2099, 1786, 1723, 1626, 1602, 1448, 1346, 1218, 1155, 1047, 974, 910, 847 cm^{-1}.

(*S*)-2-amino-3-(4-(benzyloxy)phenyl)-1-((*S*)-2-ethynylpyrrolidin-1-yl) propan-1-one triflic acid salt (12)



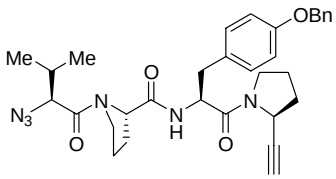
To a 25 mL round-bottomed flask charged with Boc-protected tyrosine-proline alkyne **6** (1.990 g, 4.45 mmol, 1 equiv) was added TFA (5 mL) and $CHCl_3$ (5 mL), and the mixture was stirred at rt. After 2 h, reaction progress was assessed by TLC. Since the starting material had been consumed, the mixture was concentrated in vacuo, and upon Et_2O (5 mL) addition, a white solid formed. The solid was filtered and washed in Et_2O (2 × 5 mL) to afford tyrosine-proline TFA-salt **12** (1.915 g, 4.14 mmol, 93%). This solid was carried on without further purification.

Data for compound **12**:

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.61–7.32 (m, 5H, OCH_2**Ph**), 7.20 (m, 2H, Tyr Ar**H**), 6.91 (d, *J* = 8.3 Hz, 2H, Tyr Ar**H**), 5.06 (s, 2H, O**C**$\mathbf{H}_2$Ph), 4.67 (m, 1H, Tyr $\mathbf{H}_\alpha$), 4.26 (m, 1H, Pro $\mathbf{H}_\alpha$), 3.56–3.00 (m, 4H, Tyr $CH_\alpha C\mathbf{H}_2$ and Pro N**C**$\mathbf{H}_2$), 2.47 (m, 1H, CC**H**), 2.02–1.65 (m, 4H, Pro $NCH_2C\mathbf{H}_2$ and Pro $CH_\alpha C\mathbf{H}_2$) ppm.

IR 3288, 2880, 2287, 1660, 1612, 1510, 1454, 1365, 1329, 1242, 1202, 1175, 1135, 1040, 1026, 912, 861, 835 cm^{-1}.

(*S*)-1-((*S*)-2-azido-3-methylbutanoyl)-*N*-((*S*)-3-(4-(benzyloxy)phenyl)-1-((*S*)-2-ethynylpyrrolidin-1-yl)-1-oxopropan-2-yl) pyrrolidine-2-carboxamide (4):



To a 100 mL round-bottomed flask equipped with a $CaCO_3$ drying tube and charged with tyrosine-proline alkyne **6** (1.915 g, 4.15 mmol, 1 equiv) in freshly distilled CH_2Cl_2 (5 mL) was added DIPEA (0.75 mL, 4.15 mmol, 1 equiv). After 10 min of stirring, this solution was added to a 250 mL oven-dried flask equipped with a $CaCO_3$ drying tube and charged with proline-valine azide **5** (1.215 g, 4.45 mmol, 1.07 equiv), EDC (0.795 g, 4.15 mmol, 1 equiv), HOBt (0.560 g, 4.15 mmol, 1 equiv) and freshly distilled CH_2Cl_2 (10 mL). After 16 h, this black solution was diluted with $CHCl_3$ (50 mL) and washed with H_2O (1 × 100 mL), satd aq $NaHCO_3$ (1 × 100 mL), 1N $HCl_{(aq)}$ (1 × 100 mL), and H_2O (1 × 100 mL). The combined organics were then dried over Na_2SO_4, filtered, and concentrated in vacuo to yield a brown solid. The product was purified via flash chromatography (100% EtOAc) to afford azido valine-proline-tyrosine-proline alkyne **4** (1.664 g, 2.90 mmol, 70%) as a white solid.

Data for compound **4**:

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44–7.30 (m, 5H), 7.28–7.12 (m, 2H), 6.89–6.87 (m, 2H) 5.05 (m, 3H), 4.71 (m, 1H), 4.62 (m, 1H), 3.61–2.68 (m, 8H), 2.37–1.66 (m, 9H), 1.12 (m, 3H), 1.07–0.99 (m, 3H) ppm.

^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.43, 170.15, 170.06, 169.56, 169.52, 168.99, 157.60, 157.50, 157.47, 136.91, 136.84, 136.77, 130.84, 130.70, 130.40, 130.31, 128.62, 128.35, 128.06, 127.75, 127.69, 127.22, 126.91, 114.72, 114.62, 82.51, 81.75, 72.29, 70.46, 70.31, 69.66, 69.64, 65.93, 65.52, 60.65, 60.06, 60.19, 59.61, 53.02, 52.88, 52.46, 52.37, 47.60, 47.55, 47.34, 45.99, 45.83, 45.59, 39.50, 38.54, 38.07, 33.54, 33.44, 31.96, 31.86, 30.64, 30.27, 30.24, 28.04, 28.35, 24.82, 24.75, 24.53, 22.33, 22.21, 20.87, 19.14, 19.11, 18.97, 18.78 ppm.

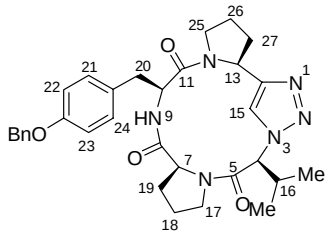
IR 3297, 2971, 2875, 2096, 1645, 1510, 1447, 1386, 1299, 1238, 1178, 1109, 1023, 911, 837 cm^{-1}.

HMRS (FAB) Calculated for $C_{32}H_{39}N_6O_4$ (MH^+): 571.3035

Found: 571.3032

$[\alpha]_D^{20}$ –41.0 (*c* 1.40, $CHCl_3$)

Bn-protected cyclic peptide 10



To a 250 mL round-bottomed flask charged with alkyne azide **4** (0.111 g, 0.195 mmol, 1 equiv) in toluene (195 mL) was added DBU (87 μL, 0.584 mmol, 3 equiv). The solution was degassed with argon for thirty minutes and then heated to reflux while flushing with argon. At reflux, CuBr (5.6 mg, 0.039 mmol, 0.2 equiv) was added, and the solution was stirred at reflux under argon for 16 h. The mixture was then cooled to rt and poured through a 2 in pad of Celite. The Celite pad was washed with MeOH (3 × 25 mL). The filtrate was concentrated in vacuo to provide a blue-green oil. The product was purified via flash chromatography (3% MeOH in CH_2Cl_2) to afford Bn-protected cyclic peptide **10** (0.0782 g, 0.136 mmol, 70% yield) as a white solid.

Data for compound **10**:

^{1}H NMR (MeOD, 400 MHz) δ 7.63 (s, 1H, C_{15}**H**), 7.28–7.12 (m, 5H, OCH_2**Ph**), 7.14 (d, *J* = 8.6 Hz, 2H, Tyr C_{21}**H** and C_{24}**H**), 6.88 (d, *J* = 8.7 Hz, 2H, Tyr C_{22}**H** and C_{23}**H**), 5.49 (d, *J* = 6.8 Hz, 1H, Val C_4**H**), 5.07 (m, 3H, OC**H**$_2$Ph and Tyr C_{10}**H**), 4.45 (m, 1H, Pro C_{13}**H**), 4.31 (m, 1H, Pro C_7**H**), 3.71 (m, 2H, one of two Pro C_{17}**H**$_2$ and one of two Pro C_{25}**H**$_2$), 3.59 (m, 1H, one of two Pro C_{25}**H**$_2$), 3.45 (m, 1H, one of two Pro C_{17}**H**$_2$), 2.96 (dd, *J* = 13.1 and 9.1 Hz, 1H, one of two Tyr C_{20}**H**$_2$), 2.82 (dd, *J* = 13.1 and 5.4 Hz, 1H, one of two Tyr C_{20}**H**$_2$), 2.64 (m, 1H, Val C_{16}**H**), 2.42 (m, 1H, one of two Pro C_{27}**H**$_2$), 2.04 (m, 2H, one of two Pro C_{27}**H**$_2$ and one of two Pro C_{19}**H**$_2$), 1.90 (m, 3H, Pro C_{26}**H**$_2$ and one of two Pro C_{18}**H**$_2$), 1.78 (m, 1H, one of two Pro C_{19}**H**$_2$), 1.68 (m, 1H, one of two Pro C_{18}**H**$_2$), 1.08 (d, *J* = 6.6 Hz, 3H, one of two Val C_{16}(C**H**$_3$)$_2$), 0.81 (d, *J* = 6.8 Hz, 3H, one of two Val C_{16}(C**H**$_3$)$_2$) ppm.

^{13}C NMR (MeOD, 100 MHz) δ 172.41, 170.33, 168.41, 158.96, 152.34, 138.82, 131.52, 130.80, 129.47, 128.82, 128.56, 124.07, 115.79, 71.52, 70.90, 68.81, 63.25, 56.50, 55.46, 37.55, 35.36, 33.01, 31.02, 23.31, 22.27, 20.76, 18.37 ppm.

IR 3305, 2966, 1649, 1612, 1507, 1444, 1414, 1380, 1230, 1179, 1106, 1012 cm^{-1}.

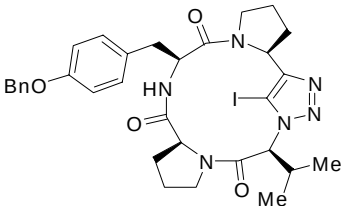
HMRS Calculated for $C_{32}H_{39}N_6O_4$ (MH^+): 571.3035

(FAB) Found: 571.3032

(ESI) Found: 571.31

$[\alpha]_D^{20}$ –85.9 (*c* 0.92, $CHCl_3$)

Iodotriazole 13



Data for compound **13**:

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44–7.31 (m, 5H), 7.19(d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 9.0 Hz, 2H), 5.30 (d, *J* = 7.0 Hz, 1H), 5.04 (s, 2H), 4.82 (dd, *J* = 5.5 and 1.5 Hz, 1H), 4.62 (d, *J* = 9.5 Hz, 1H), 4.57–4.51 (m, 1H), 4.30 (d, *J* = 8.5 Hz, 1H), 4.10–4.05 (m, 1H), 3.72–3.65 (m, 1H), 3.42–3.34 (m, 2H), 3.07–3.01 (m, 2H), 2.84 (dd, *J* = 13.0 and 4.5,

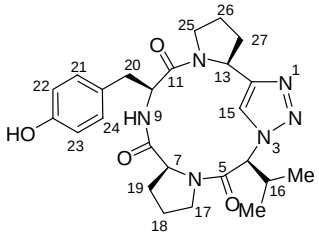
1H), 2.36–2.30 (m, 2H), 2.13–2.07 (m, 2H), 1.95–1.91 (m, 3H), 1.85–1.80 (m, 1H), 1.17 (d, J = 6.0 Hz, 3H), 0.96 (d, J = 6.5, 3H) ppm.

^{13}C NMR (MeOD, 100 MHz) δ 170.8, 168.2, 167.2, 157.4, 153.0, 137.1, 130.7, 129.5, 128.5, 127.9, 127.5, 114.5, 80.8, 69.9, 66.2, 62.4, 55.1, 54.4, 47.9, 47.1, 37.5, 33.2, 32.5, 30.5, 22.0, 21.9, 20.6, 18.9 ppm.

IR 3305, 2966, 1649, 1612, 1507, 1444, 1414, 1380, 1230, 1179, 1106, 1012 cm^{-1}.

MS (FAB) Calculated for $C_{32}H_{38}IN_6O_4$ (MH^+): 697.20
Found: 697.22
Calculated for $C_{32}H_{37}IN_6O_4Na$ (MNa^+): 719.18
(FAB) Found: 719.21

***Cyclo*-[Pro-Val-ψ(triazole)-Pro-Tyr] 2**



To a 50 mL round-bottomed flask charged with Bn-protected cyclic peptide **10** (13.7 mg, 0.024 mmol, 1 equiv) in methanol (5 mL) was added 10% Pd/C (7 mg). The resulting mixture was subjected to a three-cycle of vacuum-H_2 and was stirred at rt under a H_2 balloon for 16 h. The catalyst was then removed by filtration through Celite, and the filtrate concentrated in vacuo to afford cyclic peptide **2** (10.5 mg, 0.022 mmol, 93% yield) as a white solid.

Data for compound **2**:

^{1}H NMR (MeOD, 400 MHz) δ 7.62 (s, 1H, C_{15}**H**), 7.03 (d, J = 8.4 Hz, 2H, Tyr C_{21}**H** and C_{24}**H**), 6.65 (d, J = 8.5 Hz, 2H, Tyr C_{22}**H** and C_{23}**H**), 5.47 (d, J = 6.8 Hz, 1H, Val C_4**H**), 5.06 (m, 1H, Tyr C_{10}**H**), 4.41 (m, 1H, Pro C_{13}**H**), 4.32 (m, 1H, Pro C_7**H**), 3.68 (m, 2H, one of two Pro C_{17}**H**$_2$ and one of two Pro C_{25}**H**$_2$), 3.57 (m, 1H, one of two Pro C_{25}**H**$_2$), 3.44 (m, 1H, one of two Pro C_{17}**H**$_2$), 2.91 (dd, J = 13.1 and 9.2 Hz, 1H, one of two Tyr C_{20}**H**$_2$), 2.78 (dd, J = 13.1 and 5.4 Hz, 1H, one of two Tyr C_{20}**H**$_2$), 2.64 (m, 1H, Val C_{16}**H**), 2.42 (m, 1H, one of two Pro C_{27}**H**$_2$), 2.10 (m, 1H, one of two Pro C_{19}**H**$_2$), 2.03 (m, 1H, one of two Pro C_{27}**H**$_2$), 1.91 (m, 3H, Pro C_{26}**H**$_2$ and one of two Pro C_{18}**H**$_2$), 1.79 (m, 1H, one of two Pro C_{19}**H**$_2$), 1.69 (m, 1H, one of two Pro C_{18}**H**$_2$), 1.07 (d, J = 6.7 Hz, 3H, one of two Val C_{16}(C**H**$_3$)$_2$), 0.78 (d, J = 6.8 Hz, 3H, one of two Val C_{16}(C**H**$_3$)$_2$) ppm.

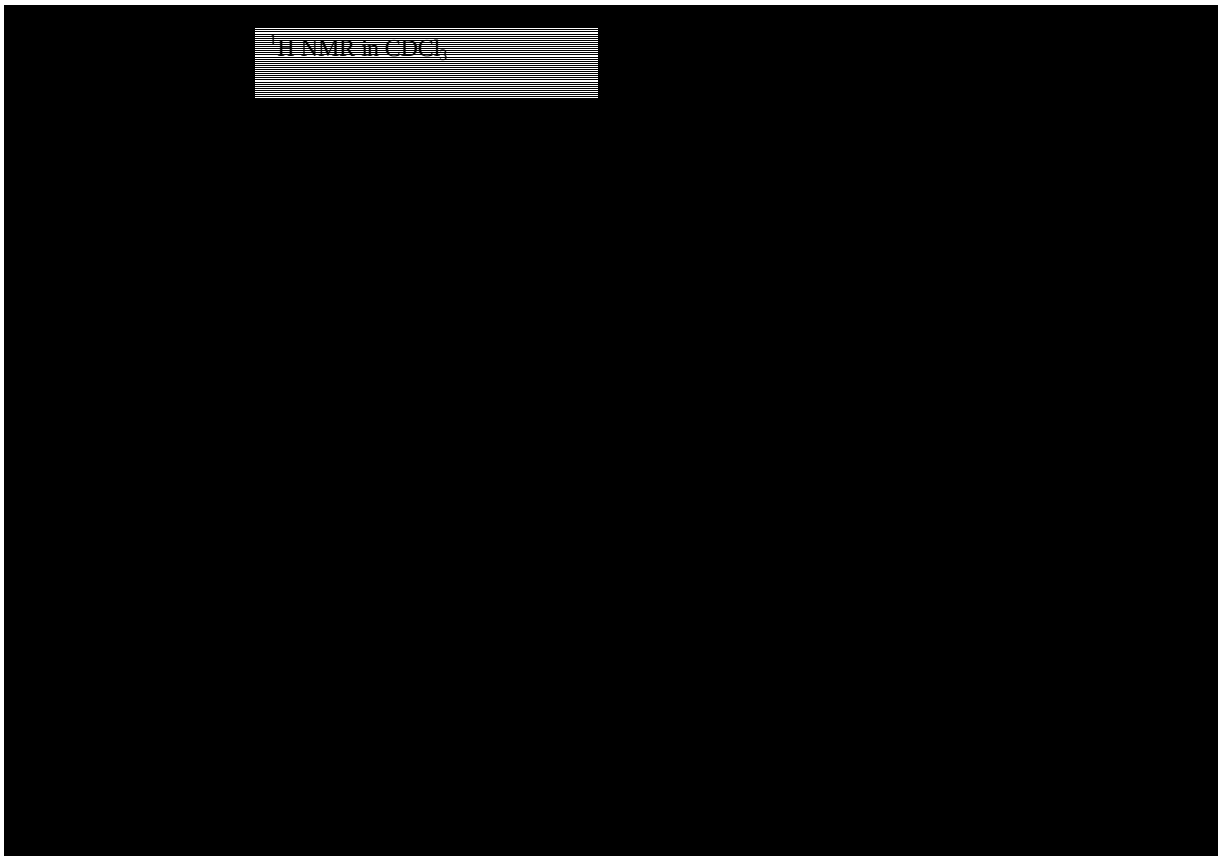
^{13}C NMR (MeOD, 100 MHz) δ 179.39, 170.37, 168.40, 157.17, 152.32, 131.47, 129.19, 124.09, 116.04, 68.78, 63.28, 56.51, 55.58, 49.70, 32.99, 31.01, 23.31, 22.26, 20.77 ppm.

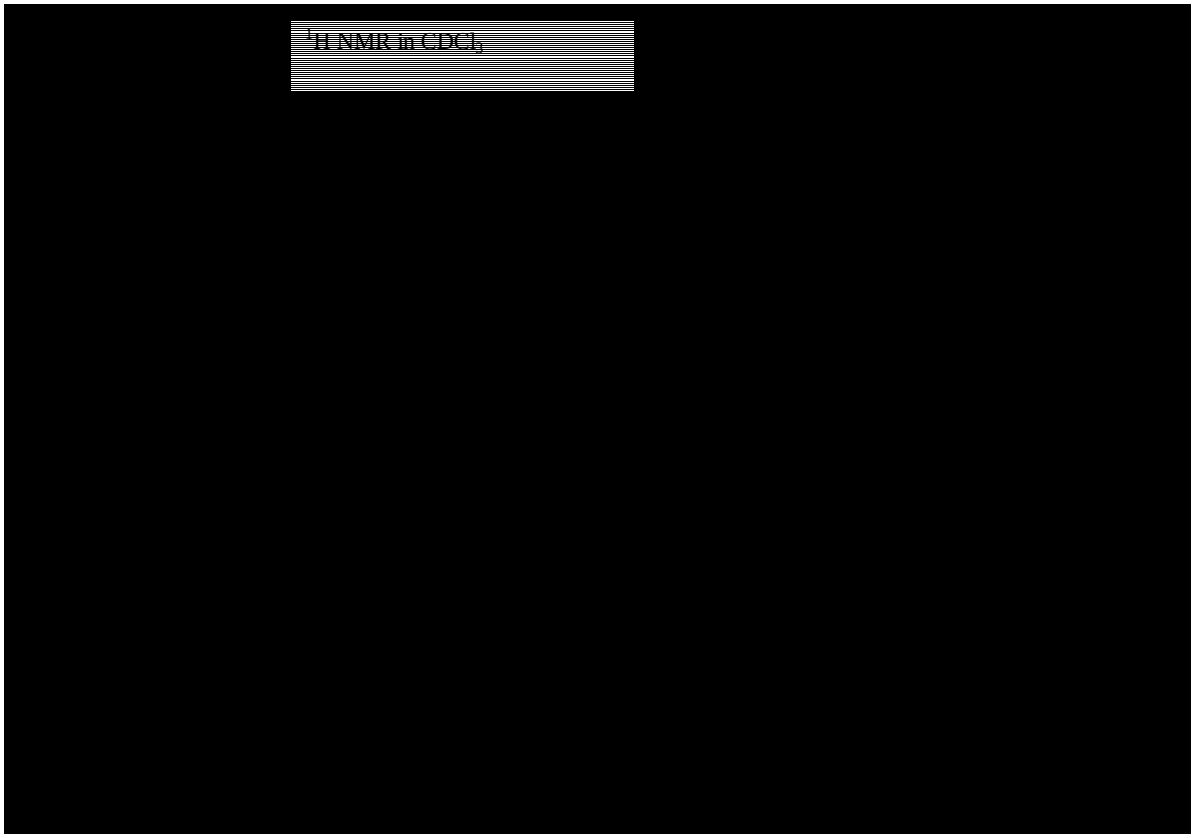
IR 3486, 2966, 2924, 2541, 1645, 1616, 1514, 1444, 1261, 1230, 1183, 1105, 1051, 974 cm^{-1}.

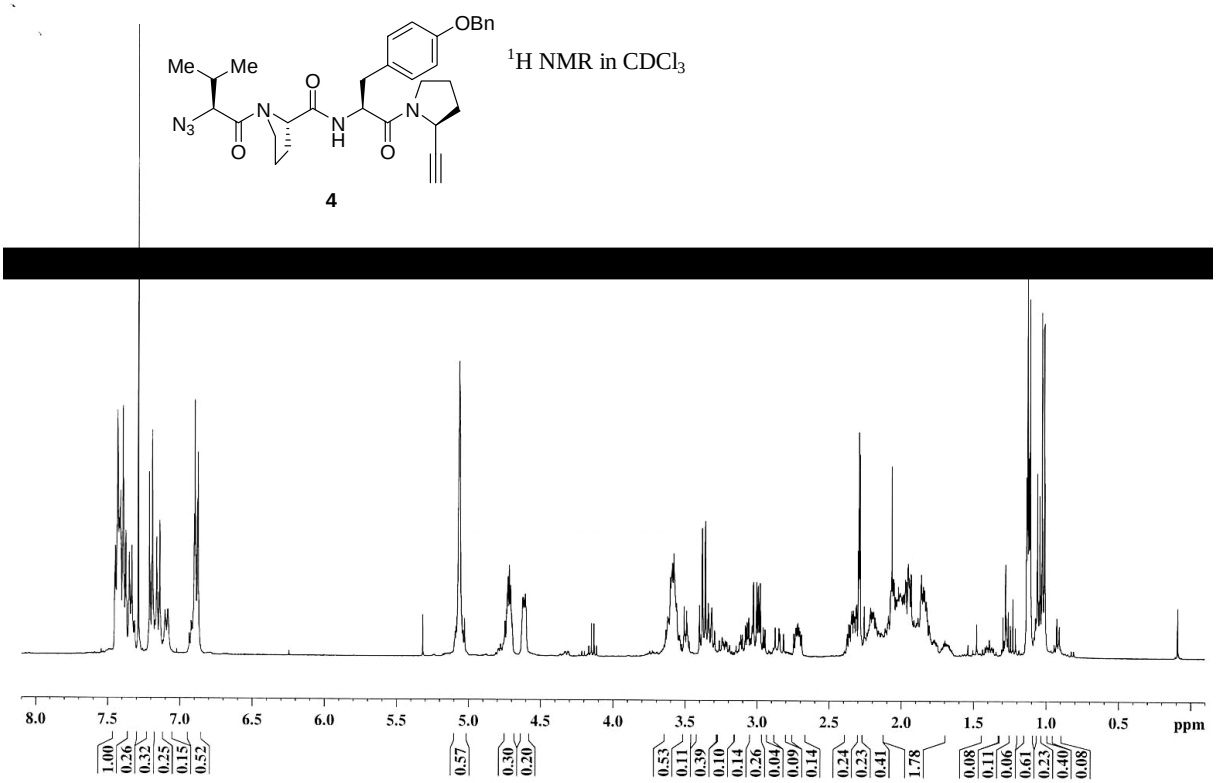
HMRS (FAB) Calculated for $C_{25}H_{33}N_6O_4$ (MH^+): 481.2565
Found: 481.2564

$[\alpha]_D^{20}$ –83.6 (*c* 0.95, $CHCl_3$)

NMR Characterization



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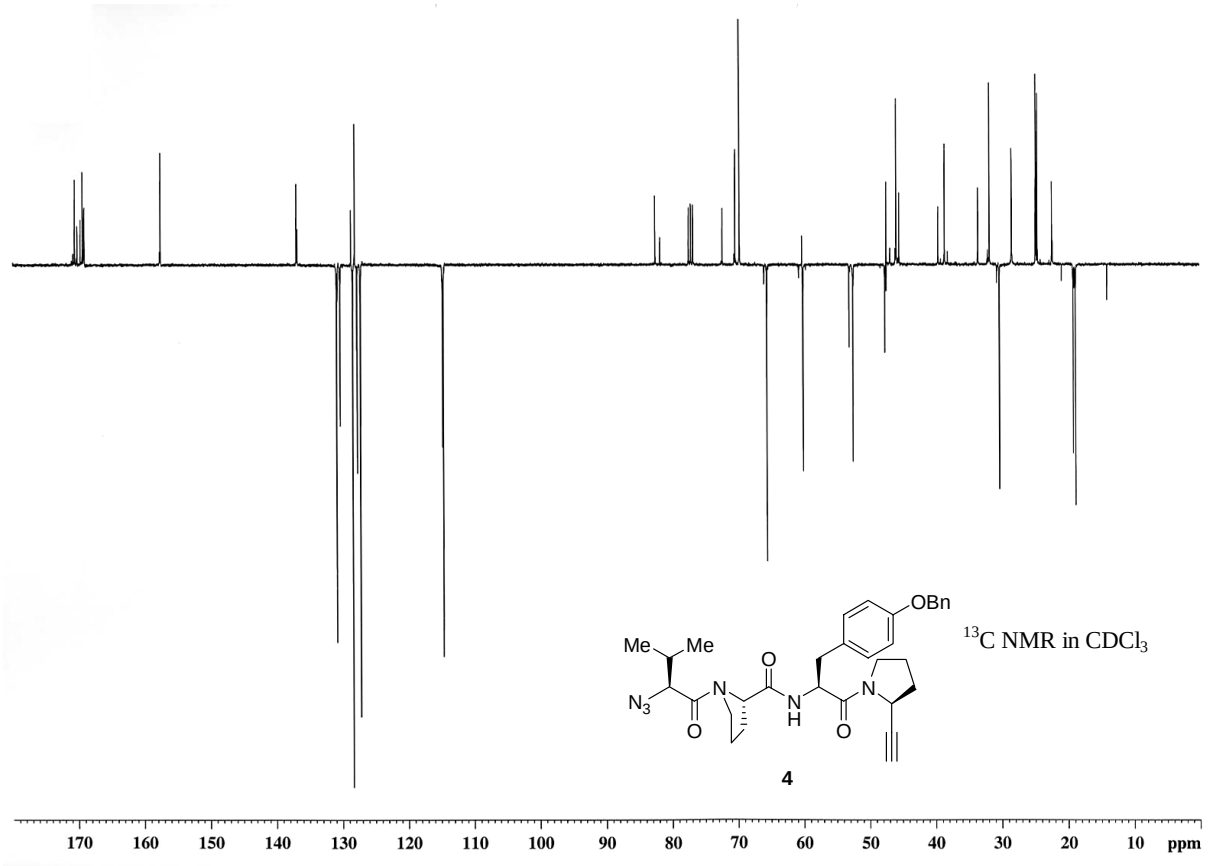
Me
N3
O
N
O
N
H
O
N
O
4
1H NMR in CDCl3
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00 0.26 0.32 0.25 0.15 0.52 0.57 0.30 0.20 0.53 0.11 0.39 0.10 0.14 0.26 0.04 0.09 0.14 0.24 0.23 0.41 1.78 0.08 0.11 0.06 0.61 0.23 0.40 0.08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N
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O
N
O
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13C NMR in CDCl3
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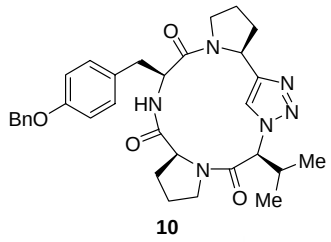
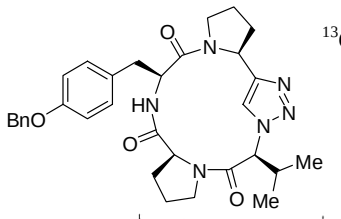


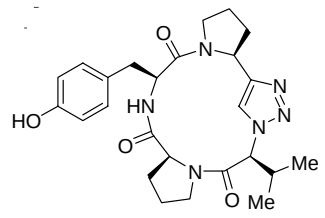
10

^{1}H NMR in MeOD



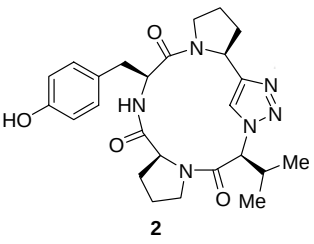
10

^{13}C NMR in MeOD



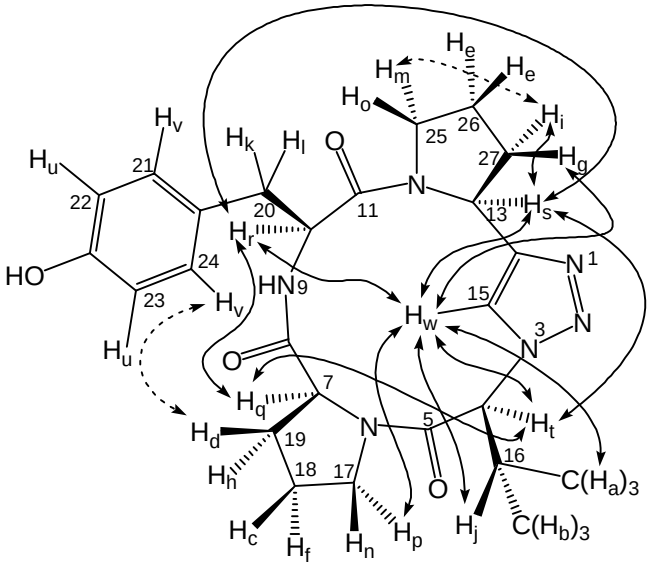
2

^{1}H NMR in MeOD



2

^{13}C NMR in MeOD



SI Figure 1. Important NOESY correlations in cyclic peptide **2** in MeOD. Solid and dashed lines indicate observed strong and weak NOESY cross-peaks, respectively. See SI Table 1 for peak assignments.

SI Table 1. ^{1}H NMR peak assignments of cyclic peptide **2** in MeOD. Peaks were assigned via H,H-COSY and C,H-COSY (HETCOR) experiments.

δ (ppm)	Corresponding proton(s)	Multiplicity	Integration
0.78	H_a	d	3H
1.07	H_b	d	3H
1.69	H_c	m	1H
1.79	H_d	m	1H
1.91	H_e and H_f	m	3H
2.03	H_g	m	1H
2.10	H_h	m	1H
2.42	H_i	m	1H
2.64	H_j	m	1H
2.78	H_k	dd	1H
2.91	H_l	dd	1H
3.44	H_m	m	1H
3.57	H_n	m	1H
3.68	H_o and H_p	m	2H
4.32	H_q	m	1H
4.41	H_r	m	1H
5.06	H_s	m	1H
5.47	H_t	d	1H
6.65	H_u	d	2H
7.03	H_v	d	2H
7.62	H_w	s	1H

[1] Still,W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
[2] Lundquist, J. T., IV; Pelletier, J. C. *Org. Lett.* **2001**, *3*, 781–783.
[3] Dickson, H. D.; Smith, S. C.; Hinkle, K. W. *Tetrahedron Lett.* **2004**, *45*, 5597–5599.