UNIVERSITAT DE BARCELONA

Indole arylation in tryptophan residues: development of new chemical methodologies, synthetic studies and biological evaluation of modified peptides

Lorena Mendive Tapia

Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Electronic Supplementary Information

Synthesis and Biological Evaluation of a Postsynthetically Modified Trp-Based Diketopiperazine

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Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

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Supporting Information

General experimental information.

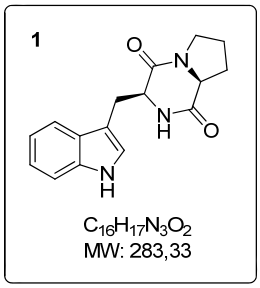
Unless stated otherwise, all reactions were carried out under argon atmosphere in dried glassware. Commercially available reactants were used without further purification. Thin-layer chromatography was performed on pre-coated Merk silica gel 60 F254 plates and visualized under a UV lamp. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 400 (at 400 MHz and 100 MHz respectively). Unless otherwise quoted, NMR spectra were recorded in $CDCl_3$ solution with TMS as an internal reference. Data for ^{1}H-NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, integration and coupling constants (Hz). Data for ^{13}C-NMR spectra are reported in terms of chemical shift (δ ppm). Signals were assigned as far as possible by means of two-dimensional NMR spectroscopy: ^{1}H-^{1}H-COSY, ^{1}H-^{13}C- COSY (HSQC: Heteronuclear Single Quantum Coherence) and long-range ^{1}H-^{13}C-COSY (HMBC: Heteronuclear Multiple Bond Connectivity). IR spectra were recorded using a Thermo Nicolet Nexus spectrometer and are reported in frequency of absortion (cm^{-1}). High Resolution Mass Spectrometry was performed by the University of Barcelona Mass Spectrometry Service.

Abbreviations

Abbreviation used for amino acids and designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* 247, 977-983 (1982). The following additional abbreviations are used: ACN: acetonitrile, DMF: *N,N*-dimethylformamide, DCM: dichloromethane, Fmoc: 9*H*-fluorenylmethyloxycarbonyl, DIPEA: *N,N*-diisopropylethylamine, DIPCDI: *N,N'*-diisopropylcarbodiimide, Et_2O: diethyl ether, HOAt: 1-hydroxy-7-azabenzotriazole, HOBt: Hydroxybenzotriazole, TFA: trifluoroacetic acid, TIS: triisopropylsilane. RP-HPLC: reverse-phase high performance liquid chromatography, HRMS: high-resolution mass spectrometry, NMR: nuclear magnetic resonance, SPPS: solid-phase peptide synthesis, IR: infrared spectroscopy.

Experimental procedures and characterization data for compounds

Solid-Phase Synthesis of Brevianamide F (1)



Aminomethyl-polystyrene resin (0.37 $mmolg^{-1}$, 5 g, 1.85 mmol) was introduced into a polypropylene syringe fitted with a porous polystyrene frit and was washed successively with DCM (10×30s), TFA (40% v/v) in DCM (1×1 min and 2×10 min), DCM (5×30s), DIEA (5% v/v) in DCM (6×2 min), DCM (5×30s), DMF (5×30s) and DCM (5×30s). 4-[(3,4-Dihydro-2*H*-pyran-2-yl)methoxy]benzoic acid (1.30 g, 5.55 mmol), DIPCDI (0.85 mL, 5.49 mmol) and ethyl cyanoglyoxyl-2-oxime (0.7 g, 5.55 mmol) in DCM (40 mL) were then added and the mixture was

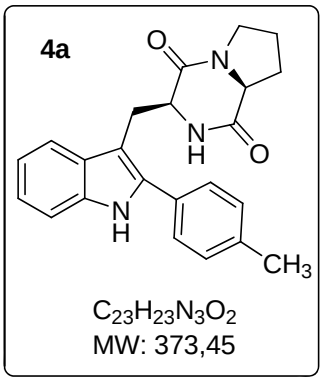
allowed to stand for 1 h at rt with occasional manual stirring. The resin was then washed with DCM (5×30s). Fmoc-Trp-OAl (1.3 g, 2.79 mmol) and PPTS (1.1 g, 4.38 mmol) in DCE were then added to the handle-resin and the suspension was shaken at 80 °C for 16 h in an Advanced Chemtech PLS 4x4 organic synthesizer. After cooling to rt the aminoacyl-resin was washed successively with DCM (5×30s), DMF (5×30s) and MeOH (5×30s). Spectrophotometric quantification of the dibenzofulvene-piperidine adduct indicated a 76% yield for amino acid coupling. After washing with DMF (5×30s) and DCM (5×30s) this resin was placed under Ar and $Pd(PPh_3)_4$ (0.86 g, 0.74 mmol) and $PhSiH_3$ (11 mL, 89.24 mmol) in DCM (40 mL) were added. The mixture was shaken for 30 min at rt, filtered and washed with DCM (8×30s). A second treatment with $Pd(PPh_3)_4$ and $PhSiH_3$ in DCM was then carried out. After filtration the resin was washed successively with DCM (8×30s), diethyl dithiocarbamate (5% v/v) in DMF (2×5 min), DMF (5×1 min), DCM (5×30s) and DMF (5×30s). The resin was then treated with H-Pro-OMe·HCl (0.72 g, 5.57 mmol), PyBOP (2.90 g, 5.57 mmol) and DIEA (3 mL, 17.22 mmol) in DMF (40 mL) for 60 min with occasional manual stirring. The resin was washed with DCM (5×30s) and DMF (5×30s). This coupling reaction and washing cycle was then repeated twice using the same quantities of reagents and solvents. The resulting resin was treated with piperidine (20% v/v) in DMF (2×10 min), was washed with DMF (5×30s) and DCM (5×30s) and dried. Cleavage of the product from the resin was brought about by treatment with TFA/*m*DMB/DCM (5:5:90 v/v) (3×10 min) and the collected washings were submitted to solvent removal. The crude product was washed with hexanes and the remaining solid was centrifuged (10 min at 6000 rpm) and filtered, affording **1** as a foamy white solid (0.37 g, 71 %).

^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.37 (m, 1H), 1.64 (m, 2H), 1.95 (m, 1H), 3.08 (dd, *J* = 14.9, 5.7 Hz, 1H), 3.26 (m, 2H), 3.39 (m, 1H), 4.04 (bt, *J* = 8.3 Hz, 1H), 4.29 (bt, *J* = 4.9 Hz, 1H), 6.95 (bt, *J* = 7.5 Hz, 1H), 7.05 (bt, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 2.1 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.72 (s, 1H), 10.9 (s, 1H) ppm. **^{13}C-NMR** (100 MHz, DMSO-d_6): δ 21.9, 25.8, 27.7, 44.6, 55.3, 58.4, 109.3, 111.2, 118.2, 118.7, 120.9, 124.4, 127.4, 136.0, 165.5, 169.1 ppm. **IR** (KBr, cm^{-1}) ν = 3290.93, 3262, 2873.42, 1675.84, 1654.62, 1620.88, 1457.92, 1451.17, 1428.99, 1341.25, 1301.72, 1242.9, 1223.61, 1109.83, 738.60, 693.28, 681.71, 642.18, 563.11, 431.98 cm^{-1}. **HRMS (ESI)** calcd for $C_{16}H_{18}N_3O_2$ $(M+H)^+$ 284.1399, found 284.1394.

General procedure for the C2 arylation of the indole residue in brevianamide F:

Unless stated otherwise, Brevianamide F (1 equiv), aryl iodide (3 equiv), $AgBF_4$ (1 equiv), 2-nitrobenzoic acid (1.5 equiv) and $Pd(OAc)_2$ (5 % equiv) were placed in a microwave reactor vessel in a 1:1 mixture of DMF:PBS (total volume of 1200 µL). The mixture was heated under microwave irradiation (80 W) at 80°C for 15 min. When detailed, a second irradiation cycle (15 min) was performed, adding extra $AgBF_4$ (1 equiv) and $Pd(OAc)_2$ (5 % equiv). Ethyl acetate (60 mL) was added and the resulting suspension was filtered through Celite. The filtrate was successively washed with aqueous saturated solutions of NH_4Cl_{sat} (3x20 mL), $NaHCO_{3\ sat}$ (3x20 mL) and brine (3x20 mL), then the organic phase was dried over Na_2SO_4, filtered and the solvent was removed under vacuum. Unless otherwise quoted, the crude extract was purified by flash chromatography on silica gel (hexane/ethyl acetate) to obtain **4** as a pure product.

(3S,8aS)-3-((2-(*p*-Tolyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4a)

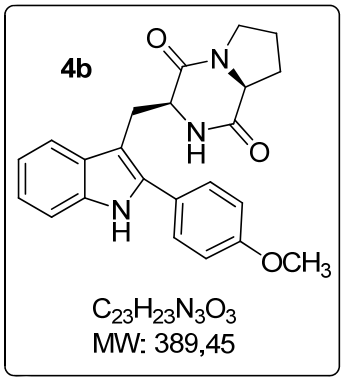


$C_{23}H_{23}N_3O_2$
MW: 373,45

Compound **4a** was prepared according to the general procedure using 1-iodo-4-methylbenzene (492.8 mg, 2.26 mmol). The crude product was purified by flash chromatography on silica using methyl *tert*-butyl ether (MTBE) to obtain **4a** as a white solid (235.3 mg, 84 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.26 (s, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.28 (s, 1H), 7.23 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.19 – 7.13 (m, 1H), 5.46 (s, 1H), 4.36 (d, *J* = 9.3 Hz, 1H), 3.98 (s, 1H), 3.88 (dd, *J* = 15.2, 3.7 Hz, 1H), 3.58 (tt, *J* = 11.5, 10.3 Hz, 2H), 3.21 (dd, *J* = 15.2, 11.6 Hz, 1H), 2.40 (s, 3H), 2.31 – 2.24 (m, 1H), 2.00 (dd, *J* = 14.3, 7.8 Hz, 2H), 1.91 – 1.82 (m, 1H) ppm. **^{13}C-NMR** (100 MHz, $CDCl_3$): 169.33, 165.74, 138.21, 136.75, 136.04, 129.79, 129.23, 128.32, 128.21, 122.66, 120.12, 118.39, 111.35, 105.67, 59.10, 54.54, 45.35, 28.14, 25.50, 22.57, 21.23 ppm. **IR** (Film, cm^{-1}) ν =3365.84, 3282.56, 3051.96, 2975.09, 2949.47, 2923.84, 2879.00, 1668.33, 1463.35, 1424.91, 1303.20, 1258.36, 1104.63, 1014.95, 912.46, 829.18, 739.50, 656.23 cm^{-1}. **HRMS** (ESI) m/z calcd 374,1790 ($C_{23}H_{24}N_3O_2$) found 374.1873 $(M+H)^+$.

(3S,8aS)-3-((2-(4-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4b)

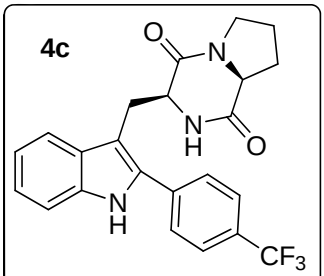


$C_{23}H_{23}N_3O_3$
MW: 389,45

Compound **4b** was prepared according to the general procedure using 1-iodo-4-methylbenzene (528.9 mg, 2.26 mmol). The crude product was purified by flash chromatography on silica using methyl *tert*-butyl ether (MTBE) to obtain **4b** as a white solid (251.2 mg, 86 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.05 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.38 – 7.30 (m, 3H), 7.16 (dt, *J* = 14.9, 7.2 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 5.45 (s, 1H), 4.29 (d, *J* = 8.6 Hz, 1H), 3.90 (t, *J* = 7.8 Hz, 1H), 3.80 (dd, *J* = 15.1, 3.7 Hz, 1H), 3.74 (s, 3H), 3.51 (dt, *J* = 20.6, 6.5 Hz, 2H), 3.13 (dd, *J* = 15.1, 11.4 Hz, 1H), 2.20 (dd, *J* = 15.1, 8.9 Hz, 1H), 1.93 (dd, *J* = 16.9, 7.8 Hz, 2H), 1.84 – 1.74 (m, 1H) ppm. **^{13}C-NMR** (100 MHz, $CDCl_3$): 169.33, 165.74, 159.50, 136.68, 136.02, 129.64, 128.30, 124.47, 122.49, 120.04, 118.31, 114.48, 111.36, 105.29, 59.08, 55.30, 54.52, 45.34, 28.12, 25.48, 22.56 ppm. **IR** (Film, cm^{-1}) ν = 3359.43, 3288.97, 3058.36, 2949.47, 2930.25, 2891.81, 2827.76, 1674.73, 1508.19, 1456.94, 1431.32, 1309.61, 1283.99, 1245.55, 1175.09, 1111.03, 1021.35, 835.59, 745.91 cm^{-1}. **HRMS** (ESI) m/z calcd 390,1739 ($C_{23}H_{24}N_3O_3$) found 390.1825 $(M+H)^+$.

(3S,8aS)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4c)

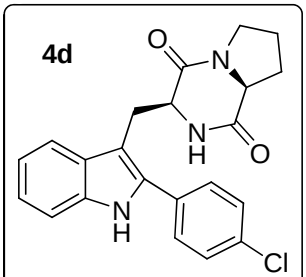


$C_{23}H_{20}F_3N_3O_2$
MW: 427,42

Compound **4c** was prepared according to the general procedure using 1-iodo-4-(trifluoromethyl)benzene (333 µL, 2.26 mmol). The crude product was purified by flash chromatography on silica using methyl *tert*-butyl ether (MTBE) to obtain **4c** as a white solid (236.4 mg, 73%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 1H), 7.69 (q, *J* = 8.6 Hz, 4H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.32 – 7.27 (m, 1H), 7.19 (t, *J* = 7.5 Hz, 1H), 5.41 (s, 1H), 4.38 (dd, *J* = 11.5, 2.8 Hz, 1H), 4.01 (t, *J* = 7.5 Hz, 1H), 3.91 (dd, *J* = 15.3, 3.9 Hz, 1H), 3.67 – 3.52 (m, 2H), 3.20 (dd, *J* = 15.3, 11.5 Hz, 2H), 2.29 (dt, *J* = 10.2, 7.0 Hz, 1H), 2.05 – 1.95 (m, 2H), 1.88 (dt, *J* = 9.0, 7.9 Hz, 1H) ppm. **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 169.38, 165.44, 136.46, 135.71, 134.93, 129.89 (q, J = 32.7 Hz),128.47, 128.08, 125.96, 125.93, 123.88 (q, J = 272.2 Hz), 123.53, 120.56, 118.75, 111.66, 107.24, 59.11, 54.43, 45.41, 28.18, 25.72, 22.50 ppm. **IR** (Film, cm^{-1}) ν = 3365.84, 3269.75, 3058.36, 2930.25, 2872.60, 1668.33, 1617.08, 1424.91, 1335.23, 1168.68, 1123.84, 1059.79, 1008.54, 912.46, 841.99, 733.10 cm^{-1}. **HRMS** (ESI) m/z calcd 428,1508 ($C_{23}H_{21}F_3N_3O_2$) found 428.1574 $(M+H)^+$.

(3S,8aS)-3-((2-(4-Chlorophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4d)

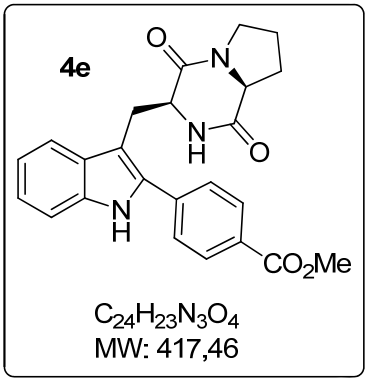


$C_{22}H_{20}ClN_3O_2$
MW: 393,87

Compound **4d** was prepared according to the general procedure using 1-chloro-4-iodobenzene (540.3 mg, 2.26 mmol). The crude product was purified by flash chromatography on silica using methyl *tert*-butyl ether (MTBE) to obtain **4d** as a white solid (213.7 mg, 72 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.52 – 7.47 (m, 2H), 7.46 – 7.39 (m, 3H), 7.28 (dd, *J* = 7.1, 1.1 Hz, 1H), 7.25 (dd, *J* = 3.1, 0.8 Hz, 1H), 7.20 – 7.15 (m, 1H), 5.42 (s, 1H), 4.36 (dd, *J* = 11.5, 2.5 Hz, 1H), 4.00 (t, *J* = 7.4 Hz, 1H), 3.87 (dd, *J* = 15.2, 3.8 Hz, 1H), 3.58 (tdd, *J* = 11.9, 10.4, 5.8 Hz, 2H), 3.17 (dd, *J* = 15.2, 11.5 Hz, 1H), 2.35 – 2.25 (m, 1H), 2.03 – 1.94 (m, 2H), 1.92 – 1.83 (m, 1H) ppm. **^{13}C-NMR** (100 MHz, $CDCl_3$): 169.36, 165.51, 136.16, 135.36, 134.29, 130.56, 129.53, 129.33, 128.16, 123.20, 120.46, 118.61, 111.45, 106.52, 59.13, 54.42, 45.40, 28.19, 25.63, 22.55 ppm. **IR** (Film, cm^{-1}) ν = 3365.84, 3282.56, 3051.96, 2930.25, 2872.60, 1668.33, 1488.97, 1456.94, 1431.32, 1303.20, 1264.77, 1091.81, 1008.54, 829.18, 733.10 cm^{-1}. **HRMS** (ESI) m/z calcd 393,1244 ($C_{22}H_{20}ClN_3O_2$) found 394.1321 $(M+H)^+$.

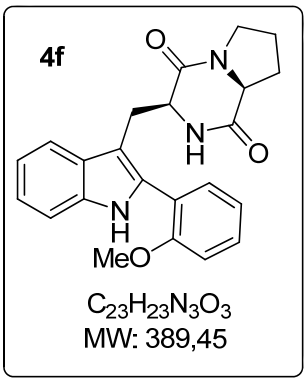
Methyl 4-(3-(((3S,8aS)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indol-2-yl)benzoate (4e)



Compound **4e** was prepared according to the general procedure using methyl 4-iodobenzoate (592.2 mg, 2.26 mmol). The crude product was purified by flash chromatography on silica using methyl *tert*-butyl ether (MTBE) to obtain **4e** as a white pale solid (298.3 mg, 95 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.40 (s, 1H), 8.12 (d, *J* = 8.3 Hz, 2H), 7.65 (d, *J* = 8.3 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 1H), 7.29 (d, *J* = 7.2 Hz, 1H), 7.18 (t, *J* = 7.4 Hz, 1H), 5.40 (s, 1H), 4.37 (d, *J* = 8.7 Hz, 1H), 3.99 (t, *J* = 7.8 Hz, 1H), 3.95 (s, 3H), 3.91 (d, *J* = 3.8 Hz, 1H), 3.67 – 3.52 (m, 2H), 3.23 (dd, *J* = 15.2, 11.5 Hz, 1H), 2.33 – 2.24 (m, 1H), 2.03 – 1.95 (m, 2H), 1.87 (dt, *J* = 15.4, 7.8 Hz, 1H) ppm. **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 169.35, 166.52, 165.43, 136.53, 136.33, 135.12, 130.41, 129.62, 128.32, 127.94, 123.63, 120.65, 118.79, 111.48, 107.68, 59.15, 54.45, 52.28, 45.40, 28.23, 25.73, 22.58 ppm. **IR** (Film, cm^{-1}) ν = 3378.27, 3282.17, 3064.34, 2949.02, 2878.55, 2840.11, 1718.96, 1667.71, 1603.64, 1430.66. 1276.90, 1110.33, 1007.83, 854.07, 770.78, 738.75, 687.50 cm^{-1}. **HRMS** (ESI) m/z calcd 418,1689 ($C_{23}H_{24}N_3O_3$) found 418.1768 $(M+H)^+$.

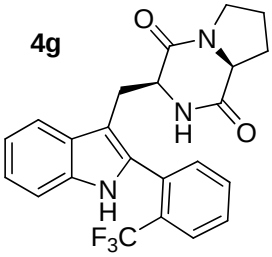
(3S,8aS)-3-((2-(2-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4f)



Compound **4f** was prepared according to the general procedure using 2-iodoanisole, 98 % (300.8 µL, 2.26 mmol). The crude product was purified by flash chromatography on silica using 100 % ethyl acetate to obtain **4f** as a pale solid (204.9 mg, 70 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1H), 7.61 (d, *J* = 8.1 Hz, 1H), 7.46 – 7.34 (m, 3H), 7.26 – 7.22 (m, 1H), 7.19 – 7.12 (m, 1H), 7.06 (dd, *J* = 12.1, 4.7 Hz, 2H), 6.03 (s, 1H), 4.40 (d, *J* = 9.4 Hz, 1H), 4.00 (t, *J* = 8.1 Hz, 1H), 3.89 (s, 3H), 3.79 (dd, *J* = 15.1, 3.5 Hz, 1H), 3.56 (dt, *J* = 12.3, 8.9 Hz, 2H), 2.89 (dd, *J* = 15.1, 11.8 Hz, 1H), 2.32 – 2.22 (m, 1H), 1.99 (ddd, *J* = 23.5, 13.1, 7.5 Hz, 2H), 1.91 – 1.80 (m, 1H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.40, 166.14, 157.42, 136.16, 133.69, 132.05, 130.62, 127.41, 122.66, 121.05, 120.77, 119.90, 118.61, 111.26, 111.26, 108.01, 59.30, 55.73, 54.41, 45.47, 28.19, 25.77, 22.85 ppm. **IR** (Film, cm^{-1}) ν = 3301.78, 3051.96, 2955.87, 2879.00, 2834.16, 1661.92 cm^{-1}. **HRMS** (ESI) m/z calcd 390.1812 ($C_{23}H_{23}N_3O_3$) found 390.1817 $(M+H)^+$.

(3S,8aS)-3-((2-(2-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4g)

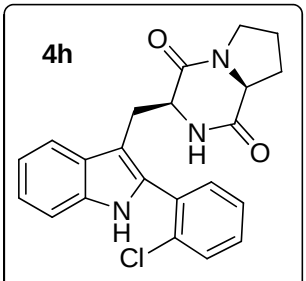


$C_{23}H_{20}F_3N_3O_2$
MW: 427,42

Compound **4g** was prepared according to the general procedure using 1-(trifluoromethyl)-2-iodobenzene, 99 % (321.4 µL, 2.26 mmol). A second irradiation cycle was performed. The crude product was purified by flash chromatography on silica using 100 % ethyl acetate to obtain **4g** as a pale solid (146.2 mg, 45%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (s, 1H), 7.83 (d, *J* = 7.5 Hz, 1H), 7.67 – 7.55 (m, 3H), 7.51 (d, *J* = 7.4 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.31 – 7.23 (m, 1H), 7.19 (t, *J* = 7.4 Hz, 1H), 5.50 (s, 1H), 4.28 (d, *J* = 11.0 Hz, 1H), 3.98 (t, *J* = 7.6 Hz, 1H), 3.70 (dd, *J* = 15.2, 3.5 Hz, 1H), 3.62 – 3.41 (m, 2H), 2.89 (dd, *J* = 15.0, 11.9 Hz, 1H), 2.35 – 2.21 (m, 1H), 1.98 (dd, *J* = 16.2, 7.9 Hz, 2H), 1.84 (dd, *J* = 18.4, 10.1 Hz, 1H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.30, 165.72, 136.01, 133.71, 133.47, 132.28, 130.44, 130.15, 129.86, 129.47, 127.36, 126.90, 123.43, 120.66, 118.76, 111.52, 108.56, 59.28, 54.53, 45.52, 28.36, 25.78, 22.75. **IR** (Film, cm^{-1}) ν = 3385.05, 3276.16, 3051.96, 2962.28, 2917.44, 2879.00, 2846.98, 1668.33 cm^{-1}. **HRMS** (ESI) m/z calcd 428.1580 ($C_{23}H_{20}F_3N_3O_2$) found 428.1587 $(M+H)^+$.

(3S,8aS)-3-((2-(2-Chlorophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4h)

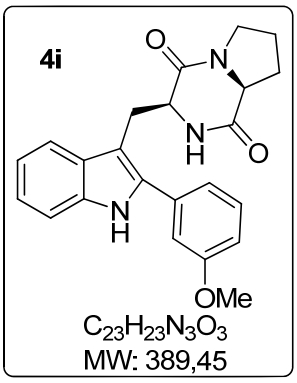


$C_{22}H_{20}ClN_3O_2$
MW: 393,87

Compound **4h** was prepared according to the general procedure using 1-chloro-2-iodobenzene (276.6 µL, 2.26 mmol). The crude product was purified by flash chromatography on silica using 100 % ethyl acetate to obtain **4h** as a pale solid (161.3 mg, 54 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 7.7 Hz, 1H), 7.46 – 7.33 (m, 4H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.19 (t, *J* = 7.4 Hz, 1H), 5.56 (s, 1H), 4.31 (d, *J* = 11.2 Hz, 1H), 3.99 (t, *J* = 7.8 Hz, 1H), 3.75 (dd, *J* = 15.1, 3.7 Hz, 1H), 3.66 – 3.45 (m, 2H), 2.91 (dd, *J* = 15.1, 11.8 Hz, 1H), 2.34 – 2.18 (m, 1H), 2.03 – 1.91 (m, 2H), 1.85 (dd, *J* = 18.4, 10.2 Hz, 1H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.33, 165.76, 136.19, 134.42, 134.03, 132.67, 131.13, 130.55, 130.24, 127.29, 127.26, 123.23, 120.35, 118.80, 111.58, 108.19, 59.25, 54.41, 45.47, 28.28, 25.85, 22.72 ppm. **IR** (Film, cm^{-1}) ν = 3372.24, 3282.56, 3051.96, 2975.09, 2955. 87, 2930.25, 2879.00, 1668.33 cm^{-1}. **HRMS** (ESI) m/z calcd 394.1317 ($C_{22}H_{20}ClN_3O_2$) found 394.1323 $(M+H)^+$.

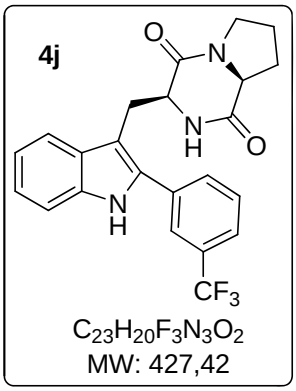
(3S,8aS)-3-((2-(2-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4i)



Compound **4i** was prepared according to the general procedure using 3-iodoanisole, 99 % (272.6 µL, 2.26 mmol). The crude product was purified by flash chromatography on silica using 100 % ethyl acetate to obtain **4i** as a pale solid (214.4 mg, 73 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.42 – 7.35 (m, 2H), 7.27 (d, *J* = 1.1 Hz, 1H), 7.25 – 7.22 (m, 1H), 7.19 – 7.12 (m, 2H), 7.10 – 7.06 (m, 1H), 6.95 – 6.91 (m, 1H), 5.45 (s, 1H), 4.36 (d, *J* = 9.0 Hz, 1H), 3.99 (t, *J* = 7.5 Hz, 1H), 3.89 (dd, *J* = 15.2, 3.8 Hz, 1H), 3.85 (s, 3H), 3.65 – 3.50 (m, 2H), 3.27 – 3.21 (m, 1H), 3.18 (s, 1H), 2.33 – 2.21 (m, 1H), 2.01 – 1.78 (m, 3H) ppm. **^{13}C NMR** (100 MHz, CDC_3): δ 169.41, 165.77, 160.10, 136.47, 136.09, 133.55, 130.38, 128.43, 123.10, 120.77, 120.43, 118.66, 113.99, 113.99, 111.44, 106.44, 59.24, 55.45, 54.69, 45.48, 28.32, 25.71, 22.70 ppm. **IR** (Film, cm^{-1}) ν = 3365.84, 3282.56, 3058.36, 2962.28, 2930.25, 2879.00, 2834.16, 1668.33 cm^{-1}. **HRMS** (ESI) m/z calcd 390.1812 ($C_{23}H_{23}N_3O_3$) found 390.1818 $(M+H)^+$.

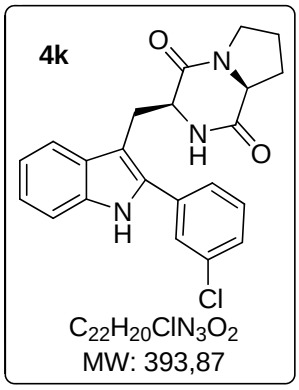
(3S,8aS)-3-((2-(3-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4j)



Compound **4j** was prepared according to the general procedure using 3-iodobenzotrifluoride, 99 % (333.3 µL, 2.26 mmol). The crude product was purified by flash chromatography on silica using 100 % ethyl acetate to obtain **4j** as a pale solid (164.7 mg, 51 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (s, 1H), 7.81 (s, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.68 – 7.56 (m, 3H), 7.45 – 7.41 (m, 1H), 7.31 – 7.26 (m, 1H), 7.22 – 7.16 (m, 1H), 5.45 (s, 1H), 4.45 – 4.34 (m, 1H), 4.02 (t, *J* = 7.6 Hz, 1H), 3.89 (dt, *J* = 9.2, 4.6 Hz, 1H), 3.68 – 3.50 (m, 2H), 3.20 – 3.13 (m, 1H), 2.34 – 2.23 (m, 1H), 2.03 – 1.81 (m, 3H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.47, 165.53, 136.41, 135.02, 133.13, 131.69, 131.68 (q, *J* = 32.5 Hz), 129.87, 128.27, 125.05, 125.05, 123.92 (q, *J* = 272.6 Hz), 123.71, 120.81, 118.92, 111.64, 107.42, 59.30, 54.61, 45.54, 28.40, 25.95, 22.68 ppm. **IR** (Film, cm^{-1}) ν = 3372.24, 3269.75, 3064.77, 2962.28, 2930.25, 2879.00, 1668.33 cm^{-1}. **HRMS** (ESI) m/z calcd 428.1580 ($C_{23}H_{20}F_3N_3O_2$) found 428.1589 $(M+H)^+$.

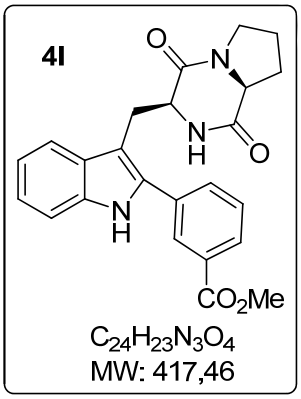
(3S,8aS)-3-((2-(3-Chlorophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4k)



Compound **4k** was prepared according to the general procedure using 3-chloroiodobenzene, 98 % (286.3 µL, 2.26 mmol). The crude product was purified by flash chromatography on silica using 100 % ethyl acetate to obtain **4k** as a pale solid (211.9 mg, 71 %).

^{1}H NMR (400 MHz, CDC_3): δ 8.32 (s, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.55 (t, *J* = 1.5 Hz, 1H), 7.48 – 7.34 (m, 4H), 7.28 – 7.25 (m, 1H), 7.20 – 7.15 (m, 1H), 5.42 (s, 1H), 4.39 (dd, *J* = 11.6, 2.6 Hz, 1H), 4.01 (t, *J* = 7.5 Hz, 1H), 3.89 (dd, *J* = 15.2, 3.8 Hz, 1H), 3.59 (tdd, *J* = 11.8, 10.3, 5.8 Hz, 2H), 3.24 – 3.14 (m, 1H), 2.34 – 2.24 (m, 1H), 2.06 – 1.80 (m, 3H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.49, 165.63, 136.26, 135.29, 135.12, 134.02, 130.65, 128.63, 128.37, 128.32, 126.60, 123.64, 120.79, 118.93, 111.55, 107.29, 59.33, 54.61, 45.57, 28.41, 25.81, 22.75 ppm. **IR** (Film, cm^{-1}) ν = 3365.84, 3269.75, 3051.96, 2962.28, 2923.84, 2872.60, 1668.33 cm^{-1}. **HRMS** (ESI) m/z calcd 394.1317 ($C_{22}H_{20}ClN_3O_2$) found 394.1325 $(M+H)^+$.

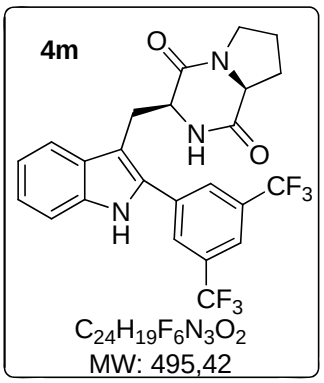
Methyl 3-(3-(((3S,8aS)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indol-2-yl)benzoate (4l)



Compound **4l** was prepared according to the general procedure using methyl-3-iodobenzoate, 97 % (306.2 mg, 1.13 mmol). The crude product was purified by flash chromatography on silica using 100 % ethyl acetate to obtain **4l** as a pale solid (62.6 mg, 40 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.39 (s, 1H), 8.23 (s, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.56 (t, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.29 (d, *J* = 7.1 Hz, 1H), 7.18 (t, *J* = 7.5 Hz, 1H), 5.45 (s, 1H), 4.39 (d, *J* = 8.9 Hz, 1H), 4.00 (t, *J* = 7.8 Hz, 1H), 3.95 (s, 3H), 3.91 (dd, *J* = 15.3, 3.7 Hz, 1H), 3.69 – 3.50 (m, 2H), 3.21 (dd, *J* = 15.8, 11.0 Hz, 1H), 2.35 – 2.23 (m, 1H), 2.03 – 1.81 (m, 3H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.48, 166.63, 165.64, 136.28, 135.51, 132.71, 132.60, 131.36, 129.56, 129.51, 129.31, 128.40, 123.58, 120.77, 118.90, 111.54, 107.21, 59.33, 54.66, 52.55, 45.57, 28.42, 25.85, 22.75 ppm. **IR** (Film, cm^{-1}) ν = 3372.24, 3282.56, 3058.36, 2949.47, 2923.84, 2885.41, 2846.98, 1725.98, 1661.92 cm^{-1}. **HRMS** (ESI) m/z calcd 418.1761 ($C_{24}H_{23}N_3O_4$) found 418.1770 $(M+H)^+$.

(3S,8aS)-3-((2-(3,5-bis(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4m)

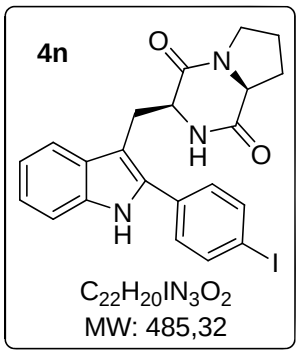


$C_{24}H_{19}F_6N_3O_2$
MW: 495,42

Compound **4m** was prepared according to the general procedure using 3,5-bis(trifluoromethyl)iodobenzene (409.4 µL, 2.26 mmol). A second irradiation cycle was performed. The crude product was purified by flash chromatography on silica using 80-95 % ethyl acetate to obtain **4m** as a pale solid (48.4 mg, 13 %).

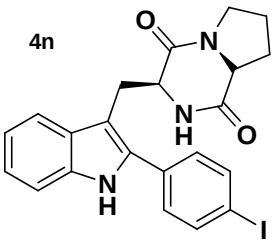
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.04 – 8.00 (m, 2H), 7.87 (s, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.32 – 7.15 (m, 2H), 5.50 (s, 1H), 4.50 – 4.37 (m, 1H), 4.03 (t, *J* = 7.5 Hz, 1H), 3.90 (dd, *J* = 15.3, 4.1 Hz, 1H), 3.68 – 3.48 (m, 2H), 3.15 (dd, *J* = 15.3, 11.2 Hz, 1H), 2.31 (ddd, *J* = 13.0, 10.9, 6.8 Hz, 1H), 2.02 – 1.80 (m, 3H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.43, 165.22, 136.69, 134.50, 133.39, 132.64 (q, *J* = 33.6 Hz), 128.28, 128.25, 128.08, 124.28, 123.16 (q, *J* = 273.1 Hz), 121.10, 119.14, 111.79, 108.58, 59.30, 54.61, 45.57, 28.52, 26.34, 22.59 ppm. **IR** (Film, cm^{-1}) ν = 3376.92, 3263.35, 3064.77, 2962.28, 2930.25, 2891.81, 1674.73 cm^{-1}. **HRMS** (ESI) m/z calcd 496.1454 ($C_{24}H_{19}F_6N_3O_2$) found 496.1458 $(M+H)^+$.

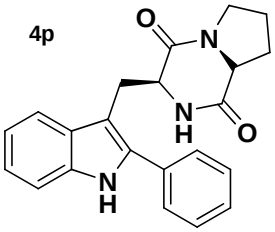
(3S,8aS)-3-((2-(4-Iodophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4n)

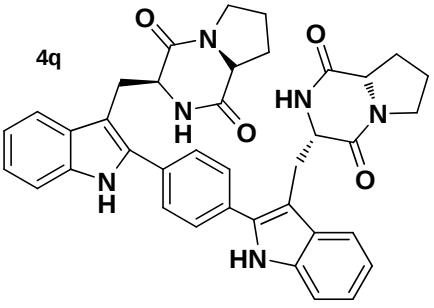


$C_{22}H_{20}IN_3O_2$
MW: 485,32

Compound **4n** was prepared using 1,4-diiodobenzene, 99 % (748.6 mg, 2.27 mmol). Brevianamide F (2 equiv), aryl iodide (3 equiv), $AgBF_4$ (2 equiv), 2-nitrobenzoic acid (3 equiv) and $Pd(OAc)_2$ (10 % equiv) were placed in a microwave reactor vessel in a 1:1 mixture of DMF:PBS (total volume of 2400 µL). Analysis by RP-HPLC-ESMS showed a mixture of compounds **4n**, **4p** and **4q** in a 1.1:1:2 range. Ethyl acetate (100 mL) was added and the resulting suspension was filtered through Celite. The filtrate was successively washed with aqueous saturated solutions of NH_4Cl_{sat} (3x30 mL), $NaHCO_{3\ sat}$ (3x30 mL) and brine (3x30 mL).



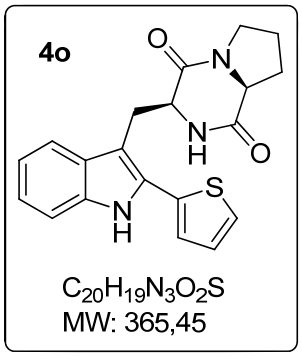




The crude product was purified by flash chromatography on silica using 80-90 % ethyl acetate to obtain **4n** as a pale solid (53.1 mg, 7.2%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64 (s, 1H), 7.76 – 7.71 (m, 2H), 7.59 (t, *J* = 7.2 Hz, 1H), 7.39 (t, *J* = 6.9 Hz, 1H), 7.29 – 7.22 (m, 3H), 7.20 – 7.10 (m, 1H), 5.41 (s, 1H), 4.35 (dd, *J* = 11.5, 2.7 Hz, 1H), 4.04 – 3.92 (m, 1H), 3.90 – 3.79 (m, 1H), 3.68 – 3.46 (m, 2H), 3.16 (dd, *J* = 15.2, 11.5 Hz, 1H), 2.35 – 2.23 (m, 1H), 2.03 – 1.77 (m, 3H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.49, 165.63, 138.42, 136.30, 135.49, 131.72, 130.01, 128.38, 123.44, 120.67, 118.78, 111.56, 106.87, 94.27, 59.28, 54.54, 45.54, 28.35, 25.77, 22.72 ppm. **IR** (Film, cm^{-1}) ν = 3365.84, 3282.56, 3058.36, 2962.28, 2917.44, 2879.00, 1655.52 cm^{-1}. **HRMS** (ESI) m/z calcd 486.0673 ($C_{22}H_{20}IN_3O_2$) found 486.0683 $(M+H)^+$.

(3S,8aS)-3-((2-(2-Iodothiophene)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4o)



Compound **4o** was prepared using 2-iodothiophene, 98 % (255.4 µL, 1.13 mmol). A second irradiation cycle was performed. Ethyl acetate (140 mL) was added and the resulting suspension was filtered through Celite. The filtrate was successively washed with aqueous saturated solutions of NH_4Cl_{sat} (3x60 mL), $NaHCO_{3\ sat}$ (3x60 mL) and brine (3x60 mL). The crude product was purified by flash chromatography on silica using 100 % ethyl acetate to obtain **4o** as a pale solid (23.7 mg, 8.6 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (s, 1H), 7.58 (d, *J* = 7.9 Hz, 1H), 7.41 – 7.37 (m, 2H), 7.30 (dd, *J* = 3.6, 1.1 Hz, 1H), 7.27 (d, *J* = 1.1 Hz, 1H), 7.26 – 7.23 (m, 1H), 7.19 – 7.12 (m, 2H), 5.58 (s, 1H), 4.43 (d, *J* = 11.9 Hz, 1H), 4.04 (t, *J* = 7.4 Hz, 1H), 3.92 (dd, *J* = 15.3, 3.7 Hz, 1H), 3.73 – 3.51 (m, 2H), 3.29 (dd, *J* = 15.3, 11.5 Hz, 1H), 2.39 – 2.24 (m, 1H), 2.10 – 1.99 (m, 2H), 1.96 – 1.75 (m, 1H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.59, 165.66, 136.15, 133.64, 130.15, 128.67, 128.09, 126.14, 125.80, 123.54, 120.76, 118.54, 111.35, 107.37, 59.31, 54.92, 45.57, 28.43, 25.99, 22.76 ppm. **IR** (Film, cm^{-1}) ν = 3365.84, 3276.16, 3109.61, 2949.47, 2917.44, 2879.00, 2846.98, 1661.92 cm^{-1}. **HRMS** (ESI) m/z calcd 366.1271 ($C_{20}H_{19}N_3O_2S$) found 366.1275 $(M+H)^+$.

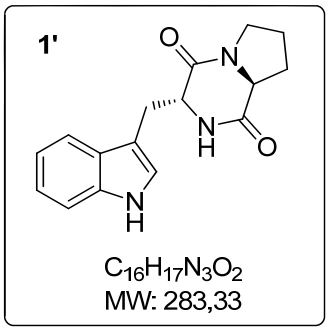
Synthesis of arylated diketopiperazines 4c'-4c'''

These compounds were prepared by arylation of the corresponding cycloTrp-Pro derivatives **1**, which in turn were synthesized by condensation of suitable Trp and Pro precursors.

General procedure for the synthesis of the diketopiperazines 1'-1'''

Z-Trp-OH (L or D) (1 equiv), H-Pro-OMe·HCl (L or D) (1 equiv), HOBt (1 equiv) and EDC·HCl (1 equiv) were suspended in ACN (30 mL). Then, DIEA (1.5 equiv) was added and the mixture was stirred during 2 h at rt. The solvent was removed and ethyl acetate (30 mL) was added. The organic phase was washed successively with a saturated solution of $NaHCO_3$ (2 × 30 mL), 5 % HCl (2 × 30 mL) and brine (2 × 30 mL) and the resulting organic solution was dried with $MgSO_4$. After filtration and solvent removal, the residue was dissolved in MeOH (60 mL) and was added 10% Pd/C (10 mol %). Then this mixture was stirred vigorously under H_2 at rt overnight. Finally, the corresponding product **1c'-1c'''**was obtained by filtration through Celite and the solvent was removed under vacuum.

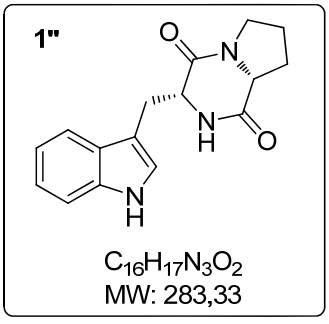
(3*R*,8a*S*)-3-((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (1')



Compound **1'** was prepared according to the general procedure using Z-D-Trp-OH (2.11 g, 6.24 mmol) and H-L-Pro-OMe·HCl (1.03 g, 6.24 mmol). After filtration through Celite the solvent was removed affording a foamy white solid (**1'**) (1.42, 80 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H), 7.54 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.33 – 7.24 (m, 1H), 7.12 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.05 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 6.24 (d, *J* = 4.3 Hz, 1H), 4.16 (dt, *J* = 5.8, 4.1 Hz, 1H), 3.54 – 3.39 (m, 1H), 3.34 (dd, *J* = 14.6, 5.9 Hz, 1H), 3.16 – 3.03 (m, 2H), 2.73 (dd, *J* = 10.8, 6.4 Hz, 1H), 2.05 – 1.92 (m, 1H), 1.81 – 1.72 (m, 1H), 1.67 – 1.55 (m, 1H), 1.43 – 1.27 (m, 1H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.86, 165.81, 136.48, 127.30, 124.51, 122.78, 120.16, 119.21, 111.55, 109.65, 58.67, 58.18, 45.39, 30.95, 29.25, 21.85 ppm. **IR** (Film, cm^{-1}) ν = 3269.75, 3077.58, 2923.84, 2879.00, 1649.11, 1456.94, 1341.64, 1296.80, 1091.81, 906.05, 726.69 cm^{-1}. **HRMS (ESI)** calcd 284.13935 ($C_{16}H_{18}N_3O_2$), found 284.13904 $(M+H)^+$.

(3*R*,8a*R*)-3-((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (1'')

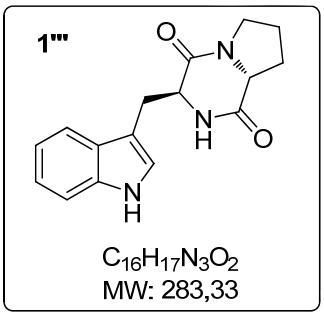


Compound **1'** was prepared according to the general procedure using Z-D-Trp-OH (1.74 g, 5.14 mmol) and H-D-Pro-OMe·HCl (0.85 g, 5.14 mmol). After filtration through Celite the solvent was removed affording a foamy white solid (**1'**) (1.20 g, 83 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.52 (dt, *J* = 8.0, 0.9 Hz, 1H), 7.36 – 7.27 (m, 1H), 7.21 – 7.11 (m, 1H), 7.09 – 6.99 (m, 2H), 5.71 (s, 1H), 4.35 – 4.23 (m, 1H), 4.05 – 3.94 (m, 1H), 3.69 (ddd, *J* = 15.0, 3.8, 1.1 Hz, 1H), 3.62 – 3.44 (m, 2H), 2.91 (dd, *J* = 15.1,

10.8 Hz, 1H), 2.31 – 2.18 (m, 1H), 1.97 – 1.88 (m, 2H), 1.87 – 1.76 (m, 1H) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 169.66, 165.83, 136.97, 127.02, 123.64, 123.07, 120.29, 118.80, 111.86, 110.22, 59.53, 54.89, 45.72, 28.61, 27.15, 22.92 ppm. **IR** (Film, cm^{-1}) ν = 3282.56, 3051.96, 2955.87, 2917.44, 2872.60, 1655.52, 1456.94, 1424.91, 1341.64, 1296.80, 1528.36, 1219.93, 1098.22, 1008.54, 912.46, 803.56, 739.50 cm^{-1}. **HRMS (ESI)** calcd 284.13935 ($C_{16}H_{18}N_3O_2$), found 284.13905 $(M+H)^+$.

(3*S*,8a*R*)-3-((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (1''')

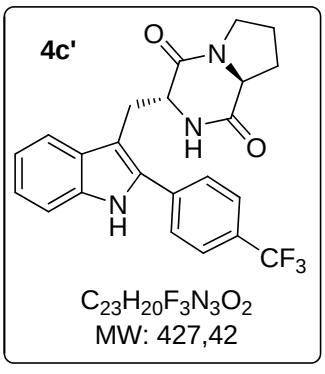


$C_{16}H_{17}N_3O_2$
MW: 283,33

Compound **1'** was prepared according to the general procedure using Z-L-Trp-OH (0.82 g, 2.43 mmol) and H-D-Pro-OMe·HCl (0.41 g, 2.45 mmol). After filtration through Celite the solvent was removed affording a foamy white solid (**1'**) (0.56 g, 81 %).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (s, 1H), 7.54 (ddt, *J* = 7.9, 1.4, 0.7 Hz, 1H), 7.33 – 7.24 (m, 1H), 7.09 (dddd, *J* = 28.2, 8.1, 7.1, 1.1 Hz, 2H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.12 (d, *J* = 3.8 Hz, 1H), 4.20 – 4.10 (m, 1H), 3.53 – 3.38 (m, 1H), 3.34 (dd, *J* = 14.6, 6.0 Hz, 1H), 3.16 – 3.03 (m, 2H), 2.74 (dd, *J* = 10.9, 6.3 Hz, 1H), 2.04 – 1.94 (m, 1H), 1.81 – 1.72 (m, 1H), 1.65 – 1.57 (m, 1H), 1.41 – 1.27 (m, 1H).ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 169.86, 165.81, 136.48, 127.30, 124.51, 122.78, 120.16, 119.21, 111.55, 109.65, 58.67, 58.18, 45.39, 30.95, 29.25, 21.85 ppm. **IR** (Film, cm^{-1}) ν = 3295.37, 3064.77, 2917.44, 2878.60, 1668.33, 1450.53, 1341.64, 1290.39, 1258.36, 1187.90, 1104.63, 1008.54, 906.05, 803.56, 739.50, 656.23 cm^{-1}. **HRMS (ESI)** calcd 284.13935 ($C_{16}H_{18}N_3O_2$), found 284.13919 $(M+H)^+$.

(3*R*,8a*S*)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (4c')



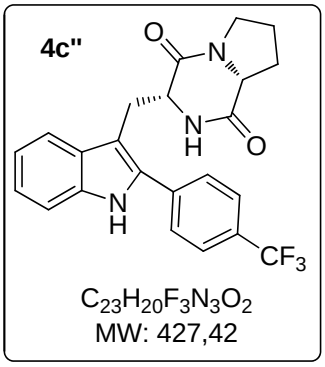
$C_{23}H_{20}F_3N_3O_2$
MW: 427,42

Compound **4c'** was prepared according to the general procedure using 1-iodo-4-(trifluoromethyl)benzene (340 µL, 2.26 mmol). The crude product was purified by flash chromatography on silica using 100 % ethyl acetate to obtain **4c'** as a pale solid (135.4 mg, 42 %).

^{1}H-NMR (400 MHz, $CDCl_3$): 8.90 (s, 1H), 7.53 (s, 1H), 7.50 (d, *J* = 5.4 Hz, 4H), 7.30 (s, 1H), 7.14 (t, *J* = 7.6 Hz, 1H), 7.04 (t, *J* = 7.6 Hz, 1H), 5.79 (s, 1H), 4.05 (q, *J* = 7.0, 5.5 Hz, 1H), 3.36 (dt, *J* = 21.1, 7.5 Hz, 2H), 3.26 (dd, *J* = 14.6, 5.4 Hz, 1H), 3.04 (t, *J* = 10.9 Hz, 1H), 2.75 (dd, *J* = 10.7, 6.5 Hz, 1H), 1.91 (p, *J* = 6.5, 6.0 Hz, 1H), 1.79 – 1.67 (m, 1H), 1.56 (qd, *J* = 11.5, 6.7 Hz, 1H), 1.38 – 1.24 (m, 1H) ppm. **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 168.82, 165.52,

136.30, 136.20, 135.95, 130.32, 129.99, 129.67, 129.01, 128.34, 125.93, 125.88, 125.41, 123.34, 122.71, 120.45, 119.44, 111.46, 107.47, 58.31, 57.82, 45.36, 29.30, 29.00, 21.73 ppm. **IR** (Film, cm^{-1}) ν = 3231.32, 3058.36, 2936.65, 2872.60, 1668.33 cm^{-1}. **HRMS** (ESI) m/z calcd 428.15804 ($C_{23}H_{21}F_3N_3O_2$), found 428.15815 $(M+H)^+$.

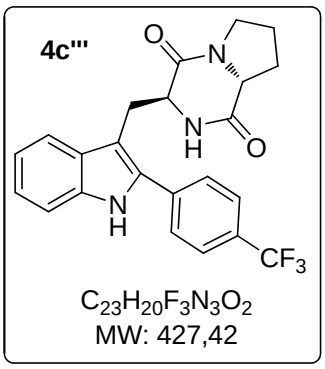
(3*R*,8a*R*)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (4c'')



Compound **4c''** was prepared according to the general procedure using 1-iodo-4-(trifluoromethyl)benzene (340 µL, 2.26 mmol). The crude product was purified by flash chromatography on silica using 92 % ethyl acetate to obtain **4c''** as a pale solid (168.0 mg, 52 %).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 1H), 7.63 (t, *J* = 8.1 Hz, 4H), 7.55 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.36 (dt, *J* = 8.2, 0.8 Hz, 1H), 7.25 – 7.17 (m, 1H), 7.12 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H), 5.34 (s, 1H), 4.42 – 4.20 (m, 1H), 3.98 – 3.90 (m, 1H), 3.84 (dd, *J* = 15.3, 3.8 Hz, 1H), 3.61 – 3.43 (m, 2H), 3.17 – 3.09 (m, 1H), 2.26 – 2.17 (m, 1H), 1.98 – 1.86 (m, 2H), 1.85 – 1.74 (m, 1H) ppm. **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 169.53, 165.53, 136.43, 135.80, 134.93, 130.55, 130.23, 128.60, 128.37, 126.42, 126.38, 126.35, 126.31, 125.39, 123.93, 122.69, 120.98, 119.03, 111.64, 107.91, 59.34, 54.57, 45.58, 28.41, 25.88, 22.75 ppm. **IR** (Film, cm^{-1}) ν = 3365.84, 3269.75, 3058.36, 2949.47, 2879.00, 1661.92 cm^{-1}. **HRMS** (ESI) m/z calcd 428.15804 ($C_{23}H_{21}F_3N_3O_2$), found 428.15807 $(M+H)^+$.

(3*S*,8a*R*)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (4c''')



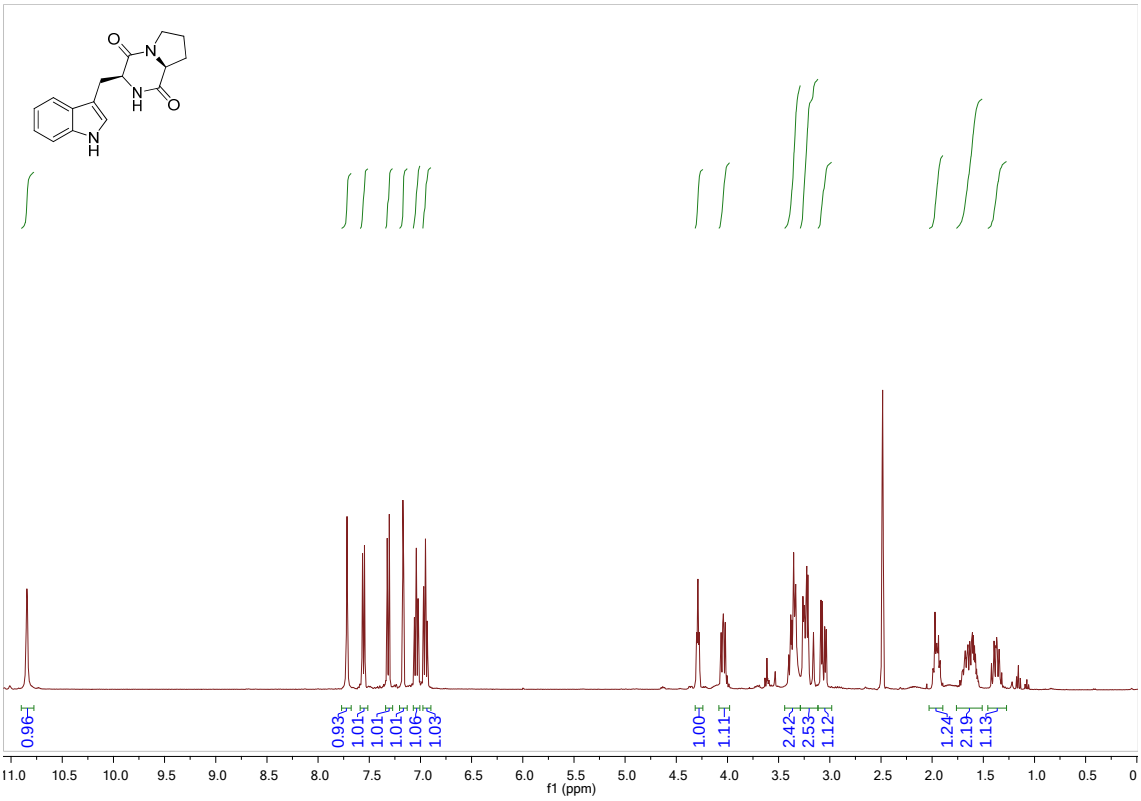
Compound **4c'''** was prepared according to the general procedure using 1-iodo-4-(trifluoromethyl)benzene (170 µL, 1.13 mmol). The crude product was purified by flash chromatography on silica using 97 % ethyl acetate to obtain **4c'''** as a pale solid (89.8 mg, 56 %).

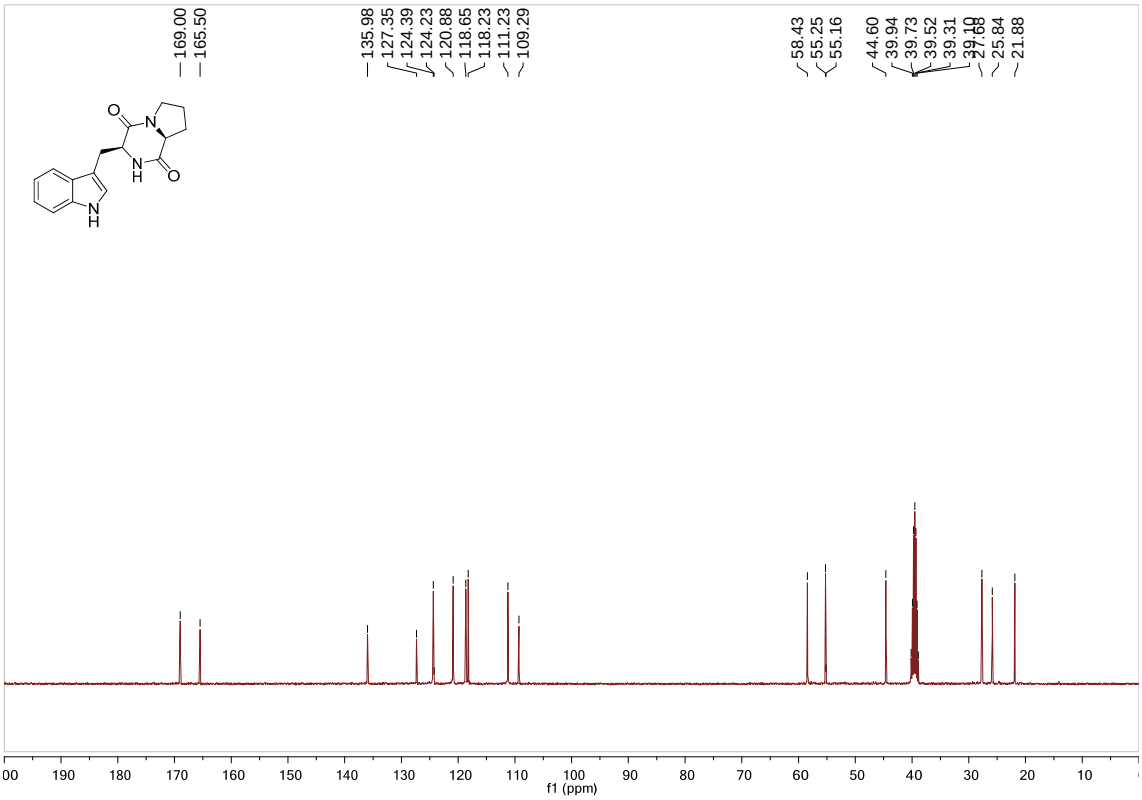
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H), 7.64 – 7.60 (m, 2H), 7.59 – 7.52 (m, 3H), 7.34 – 7.30 (m, 1H), 7.21 – 7.16 (m, 1H), 7.11 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 5.46 – 5.32 (m, 1H), 4.19 – 4.09 (m, 1H), 3.48 – 3.36 (m, 2H), 3.32 (dd, *J* = 14.7, 4.9 Hz, 1H), 3.08 (ddd, *J* = 12.1, 9.4, 2.7 Hz, 1H), 2.71 (dd, *J* = 10.8, 6.4 Hz, 1H), 2.00 – 1.91 (m, 1H), 1.83 – 1.71 (m, 1H), 1.68 – 1.56 (m, 1H), 1.41 – 1.26 (m, 1H) ppm. **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 168.35, 165.07,

135.85, 135.79, 135.70, 130.28, 129.95, 128.71, 128.03, 125.85, 125.82, 125.78, 125.74, 123.25, 120.34, 119.26, 111.00, 107.32, 58.16, 57.51, 45.06, 29.04, 28.75, 21.42 ppm. **IR** (Film, cm^{-1}) ν = 3244.13, 2949.47, 2872.60, 1668.33 cm^{-1}. **HRMS** (ESI) m/z calcd 428.15804 ($C_{23}H_{21}F_3N_3O_2$), found 428.15780 $(M+H)^+$.

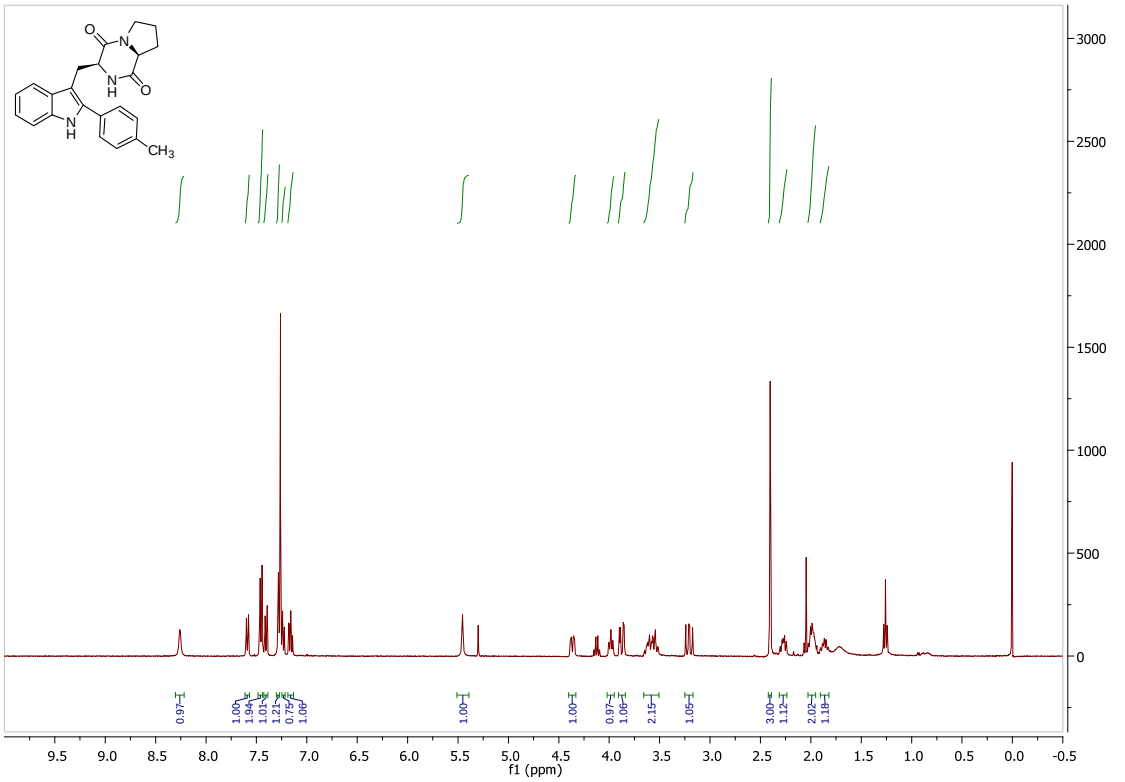
Spectroscopic data

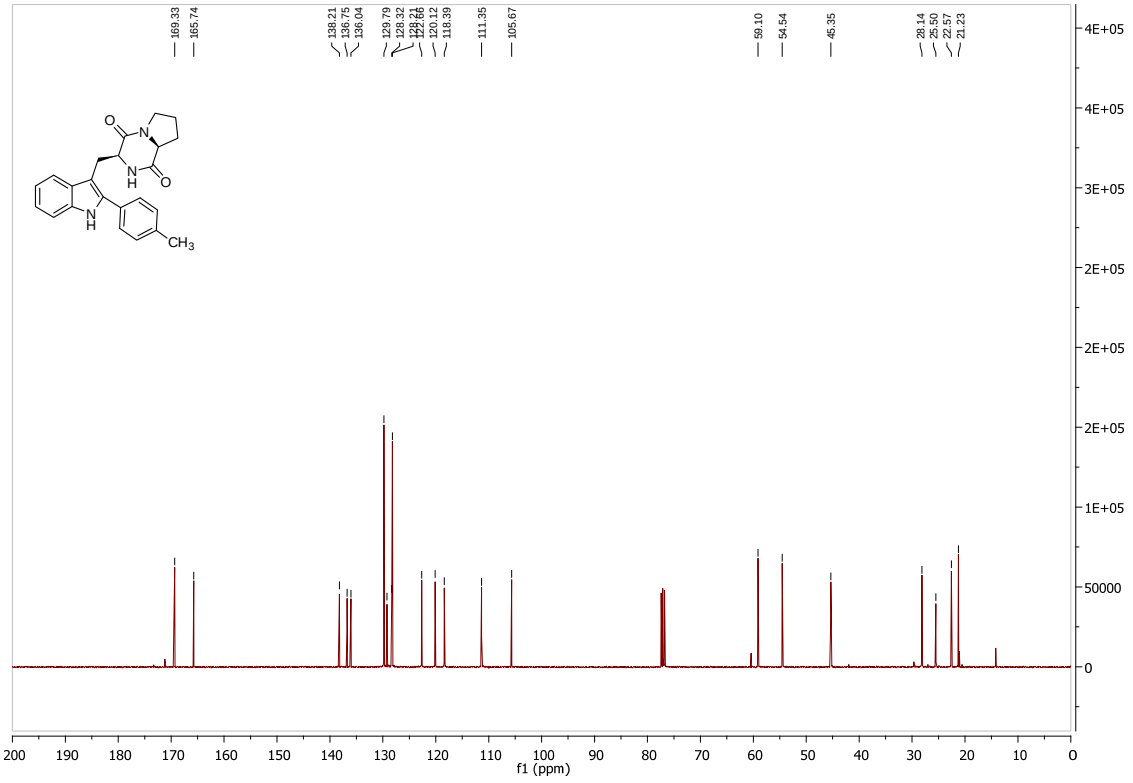
Brevianamide F (1)



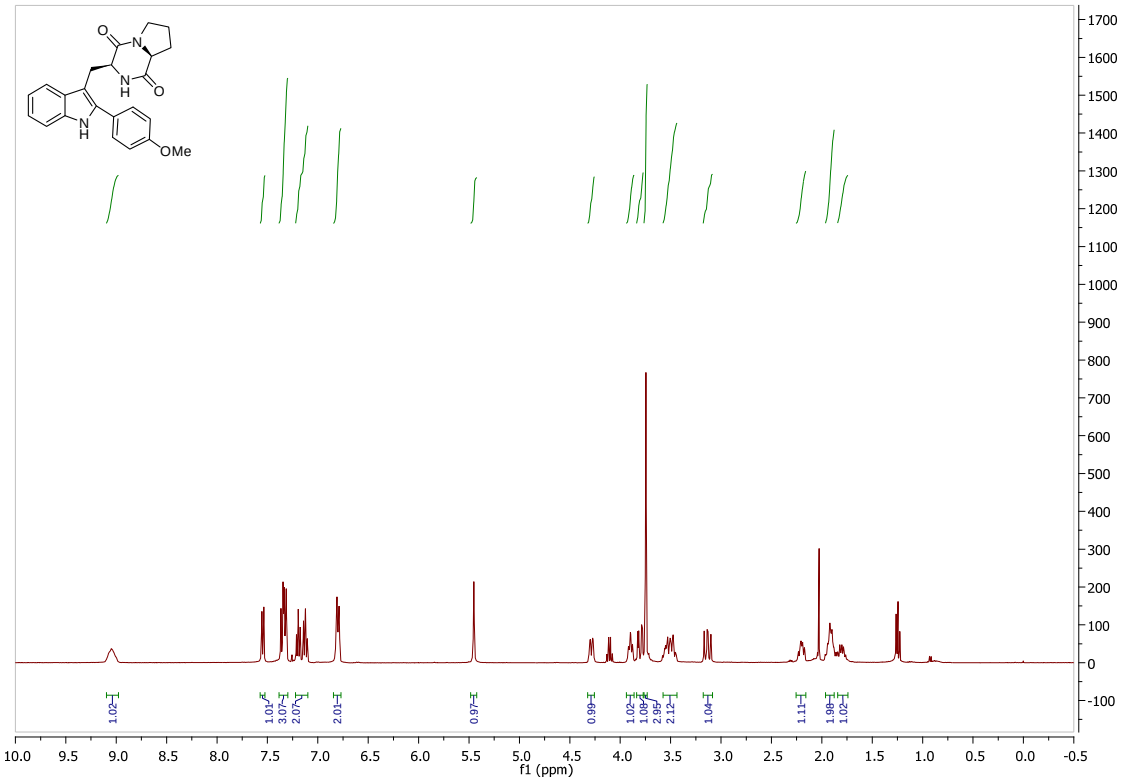


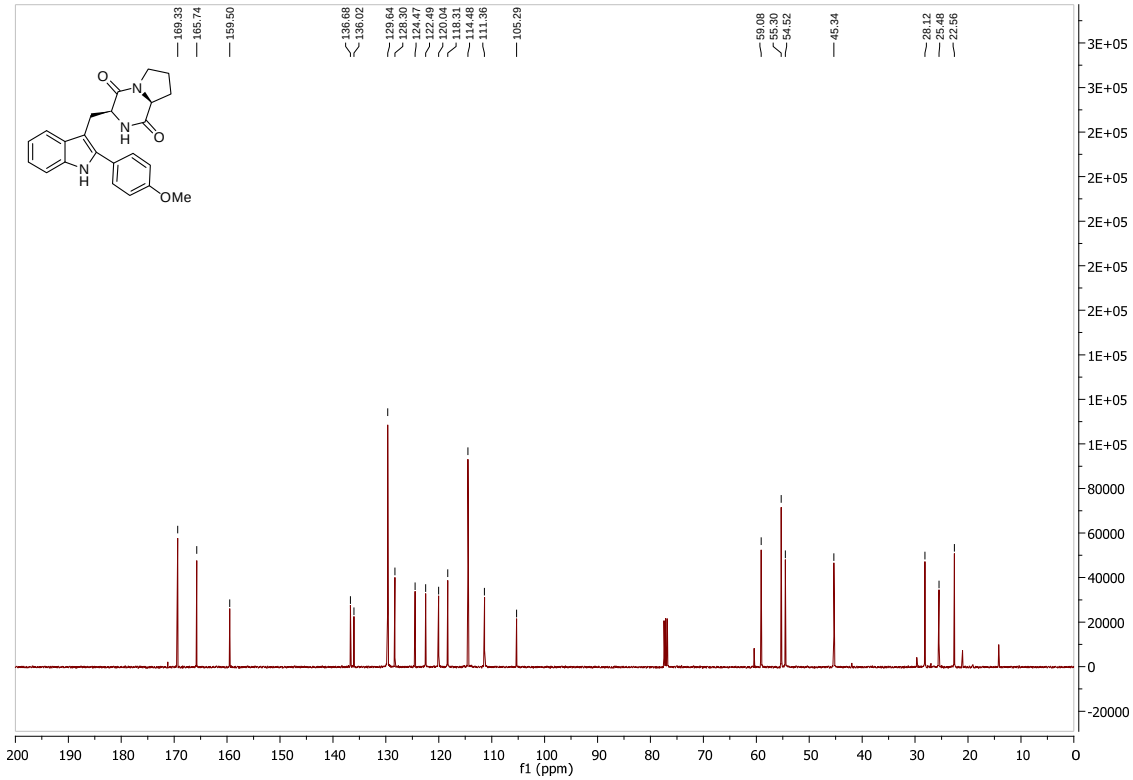
(3S,8aS)-3-((2-(*p*-Tolyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4a)



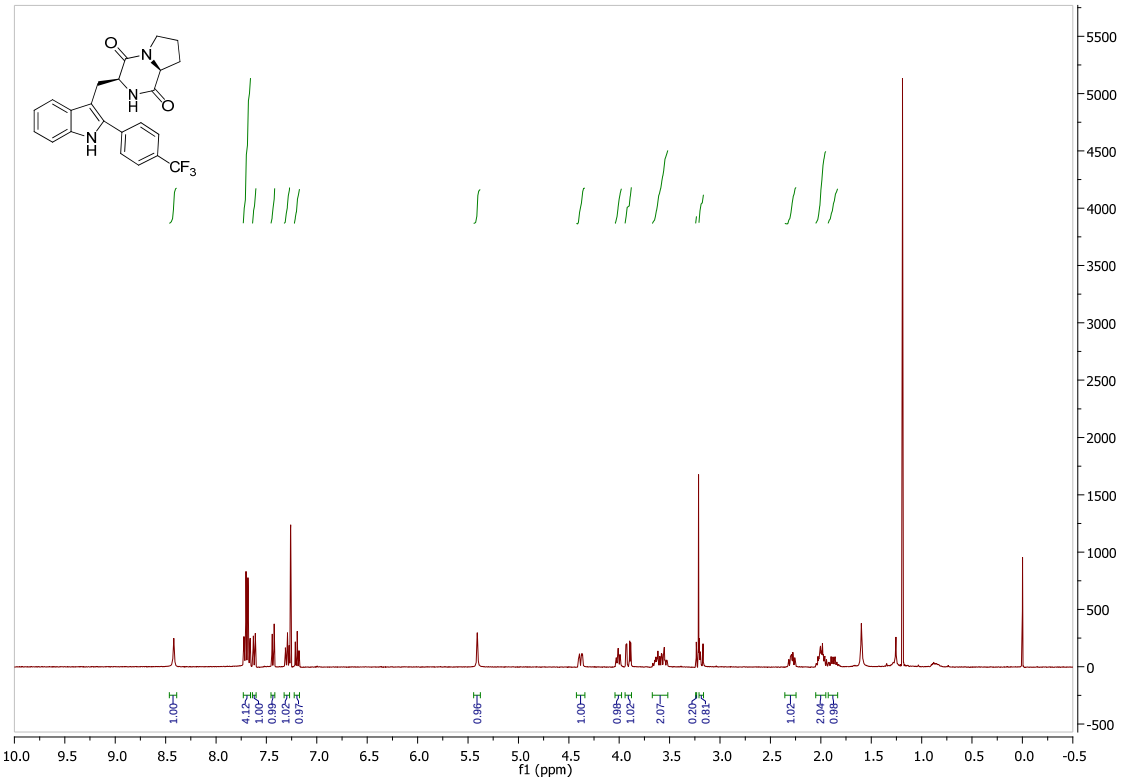


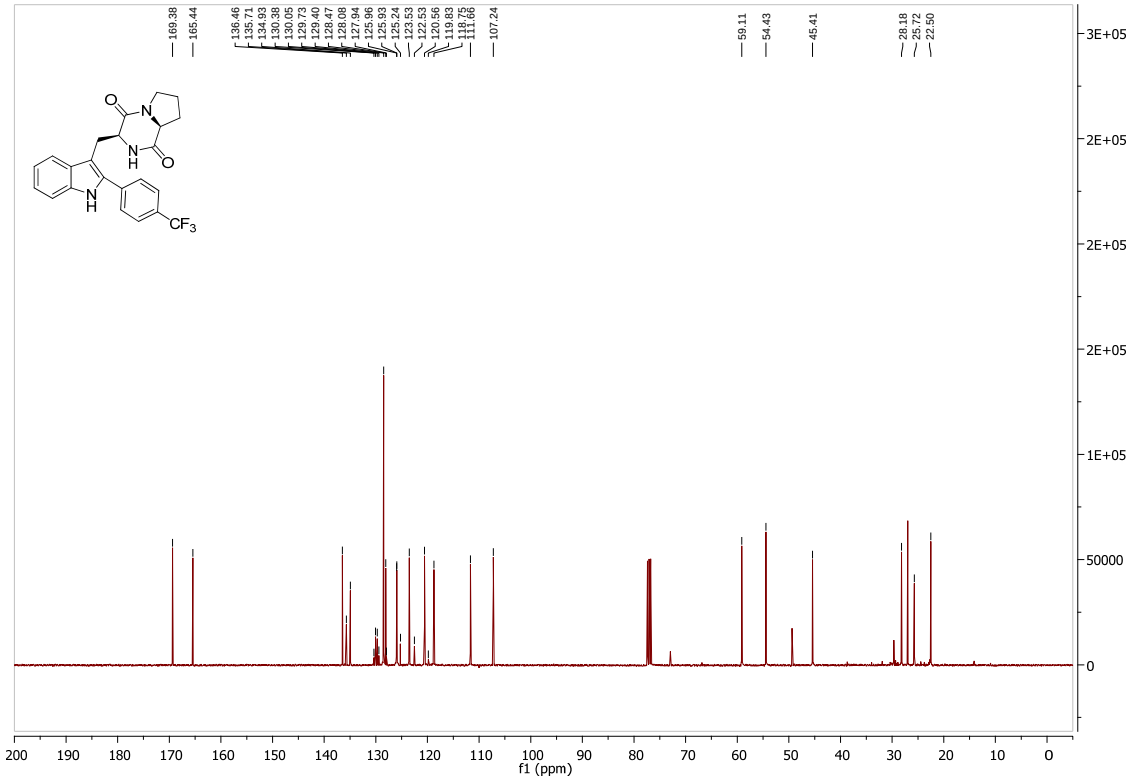
(3S,8aS)-3-((2-(4-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4b)



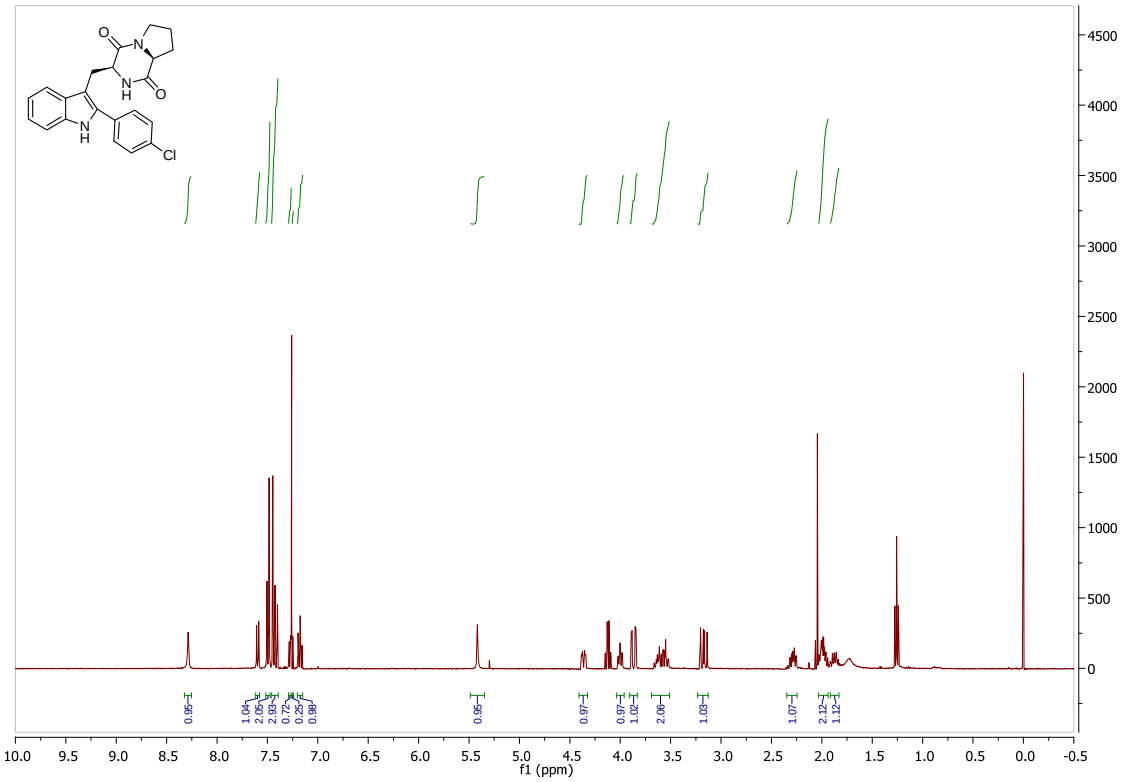


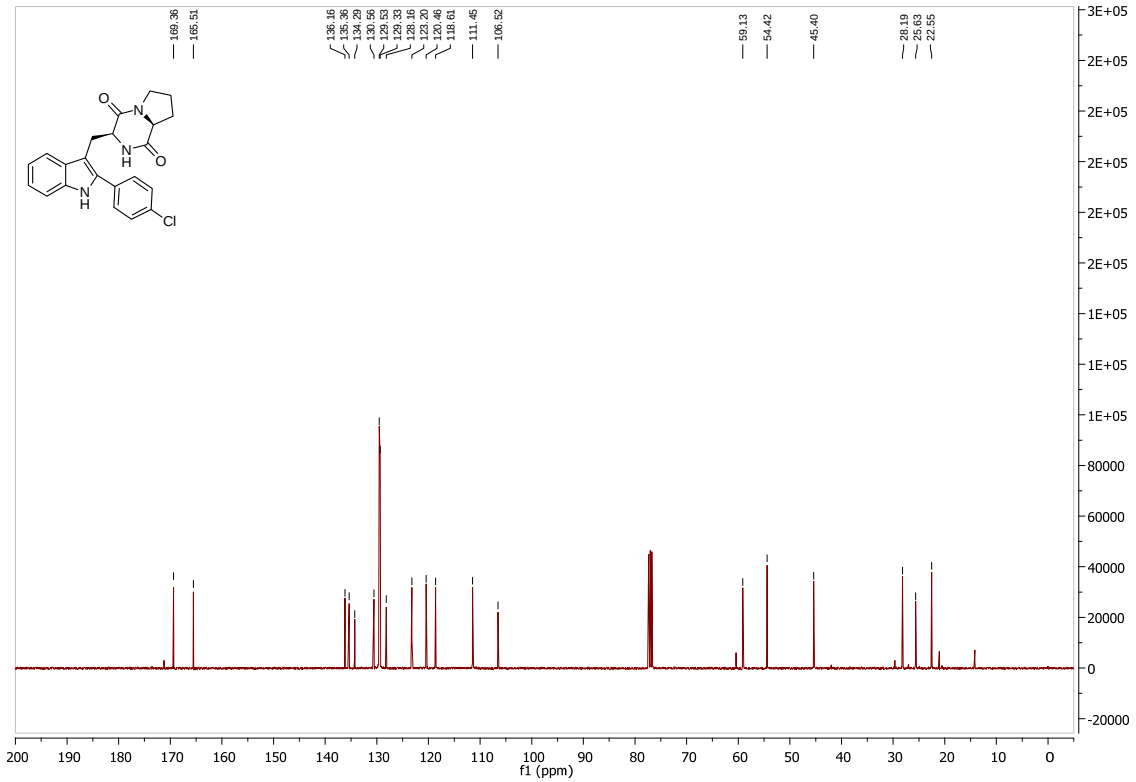
(3S,8aS)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4c)



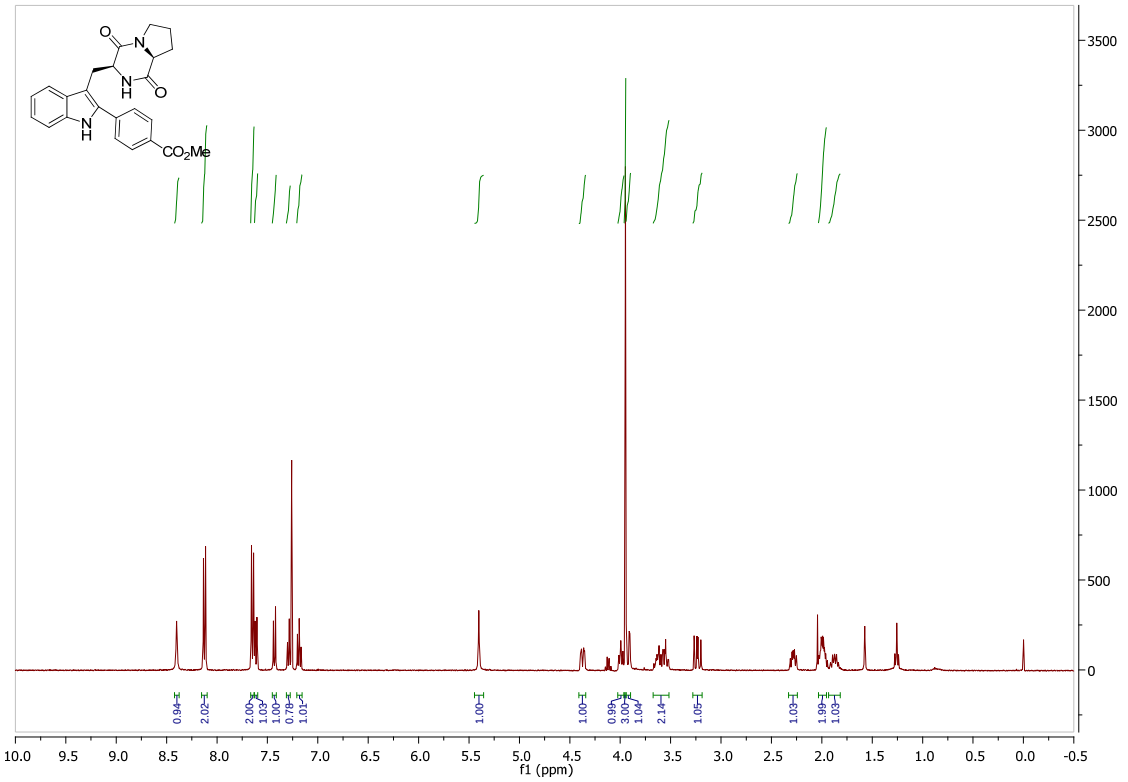


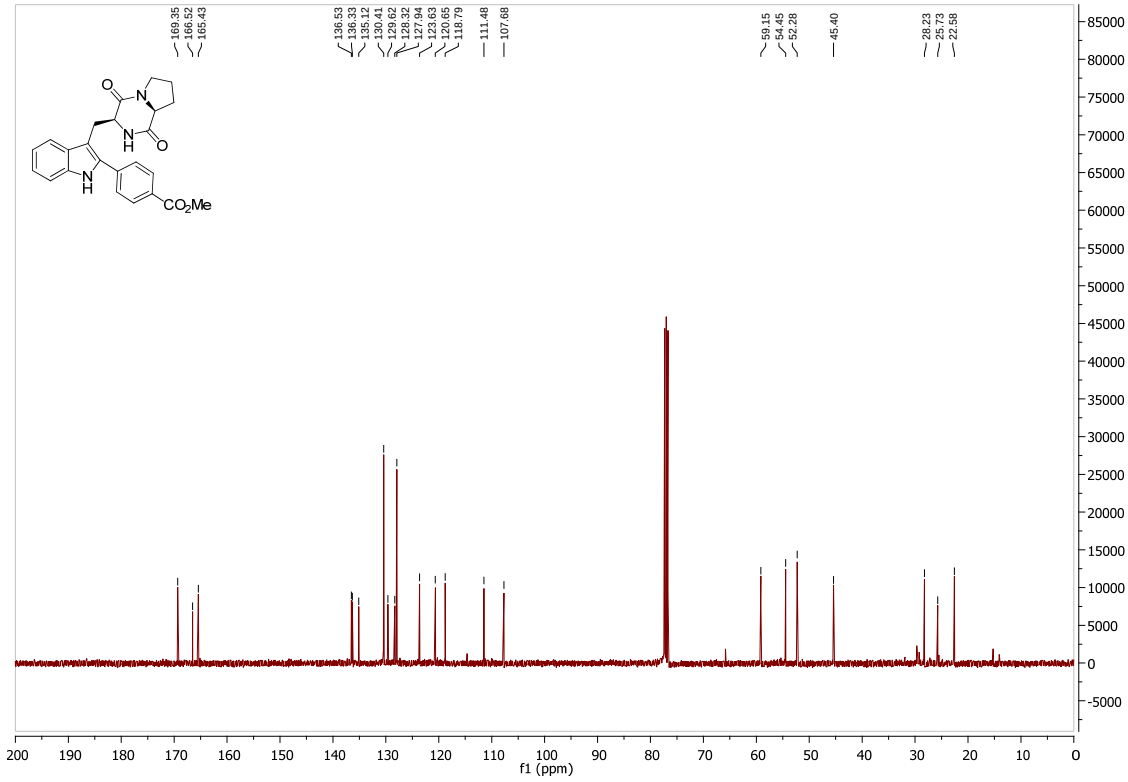
(3S,8aS)-3-((2-(4-Chlorophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4d)



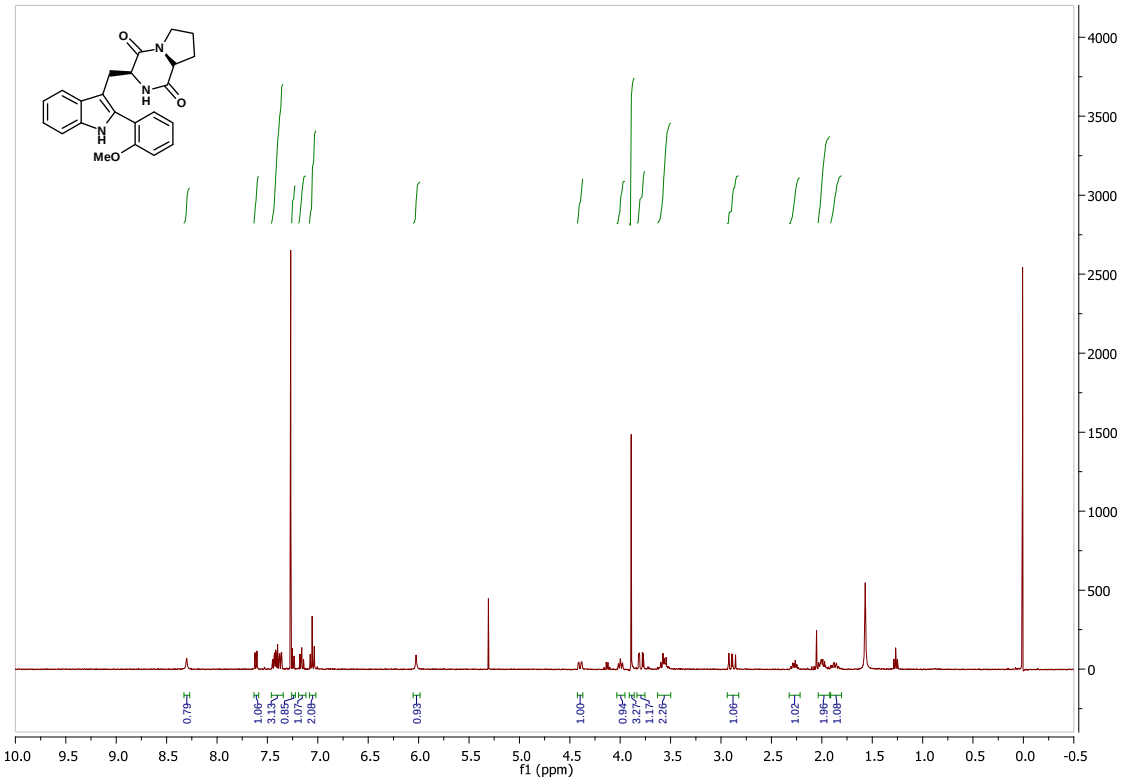


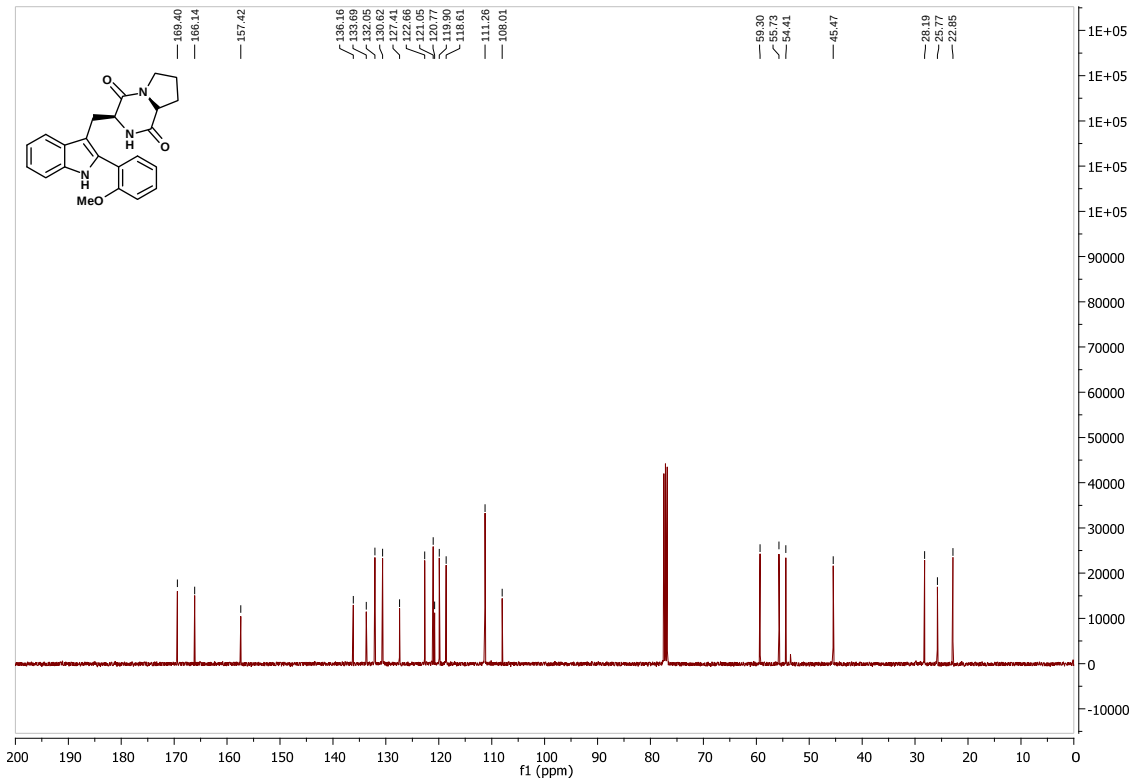
Methyl 4-(3-(((3S,8aS)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indol-2-yl)benzoate (4e)



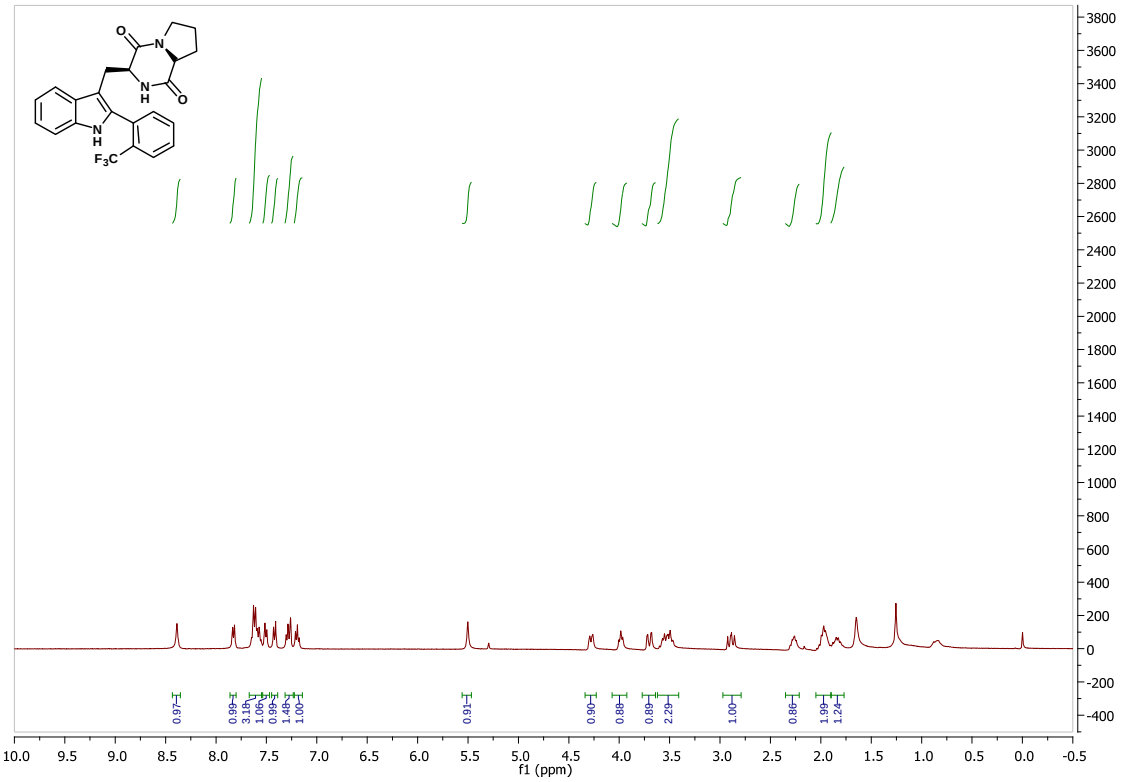


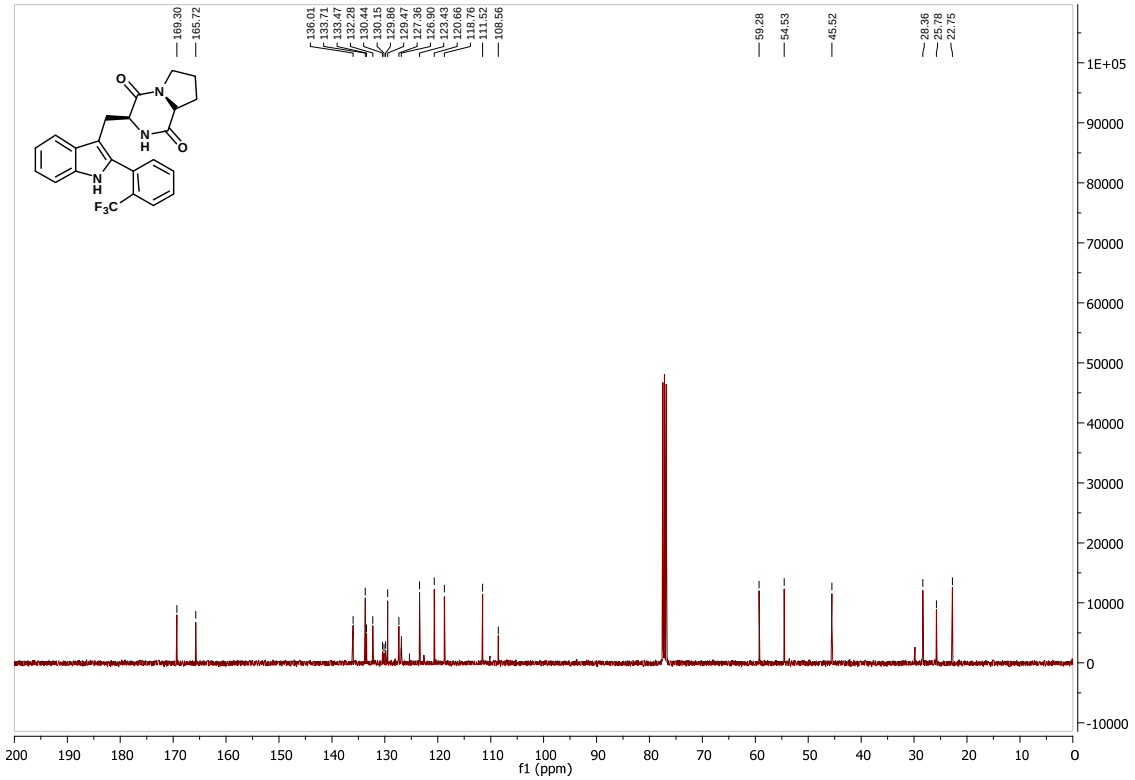
(3S,8aS)-3-((2-(2-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4f)



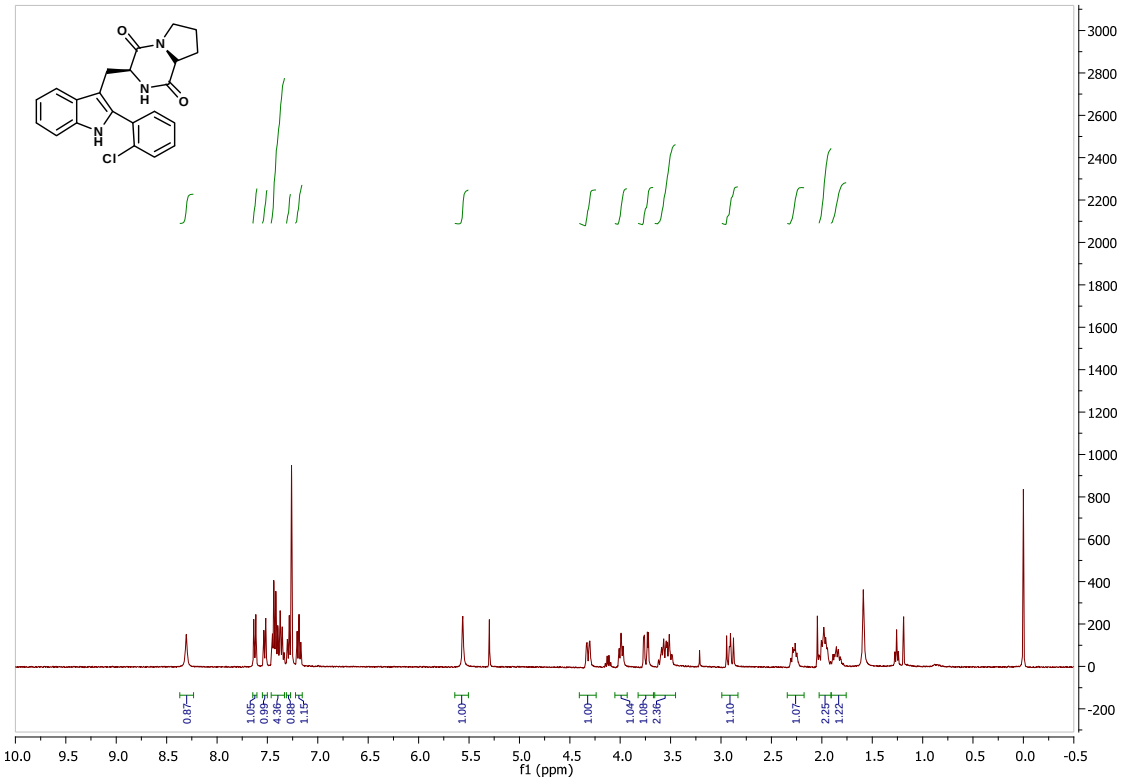


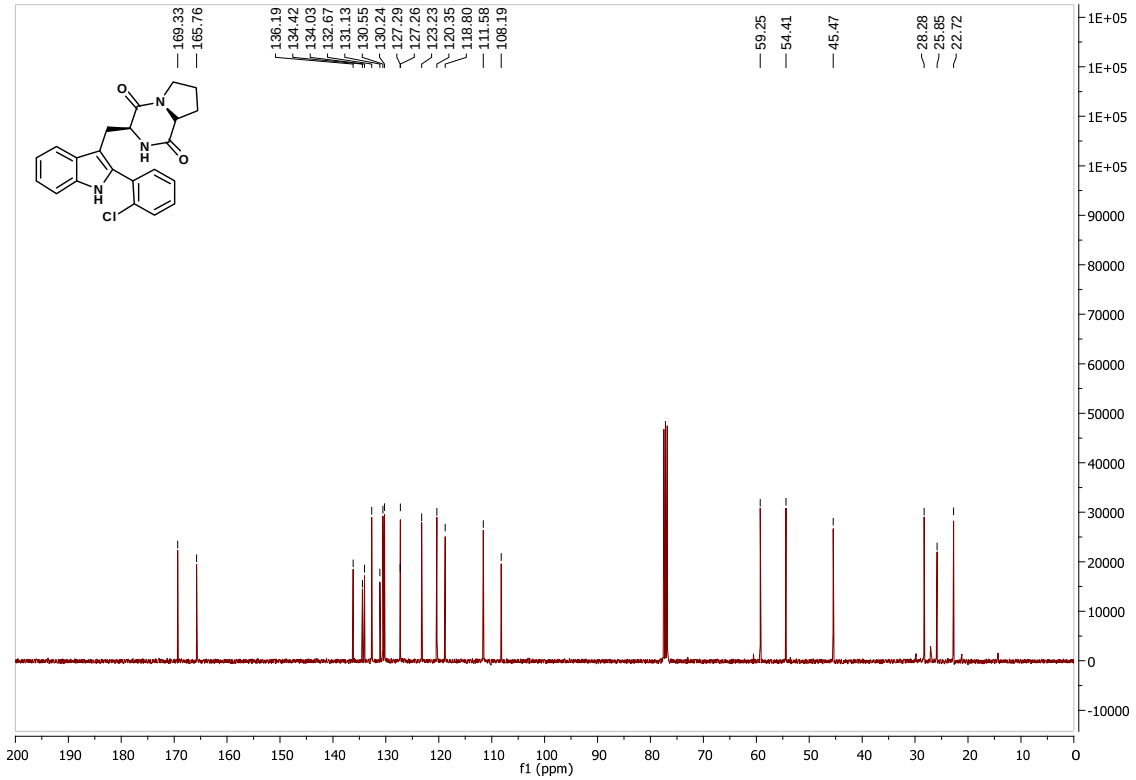
(3S,8aS)-3-((2-(2-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4g)



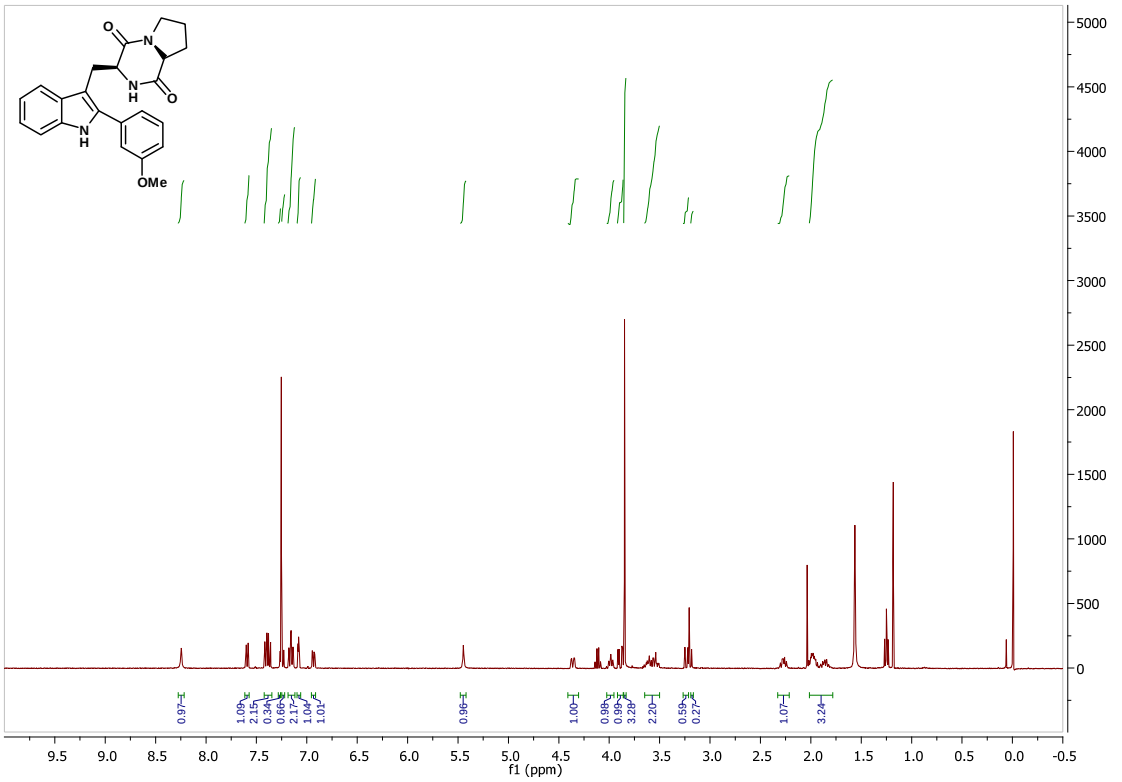


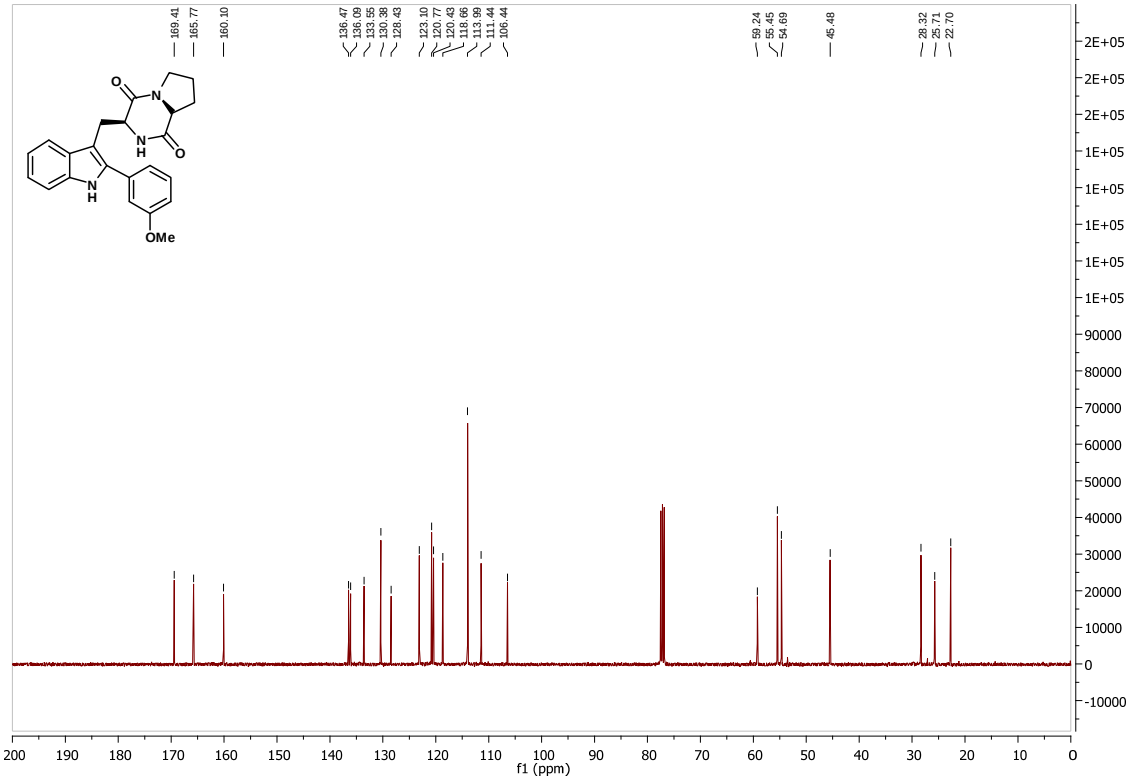
(3S,8aS)-3-((2-(2-Chlorophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4h)



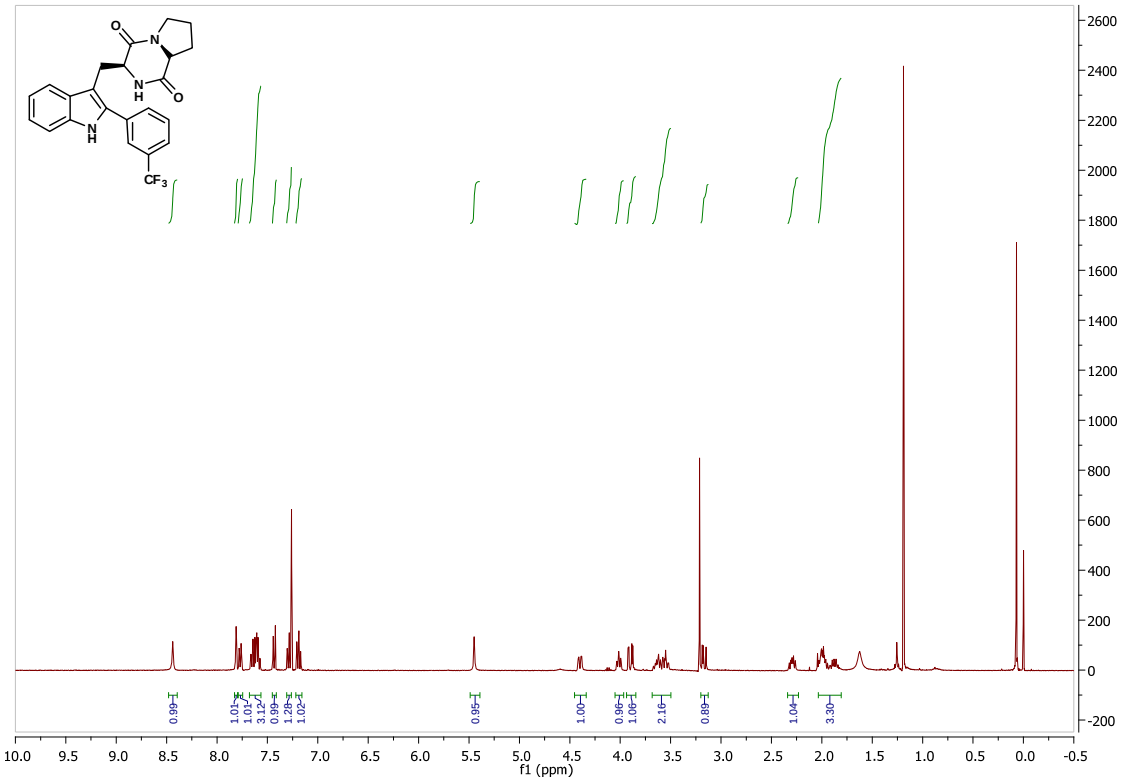


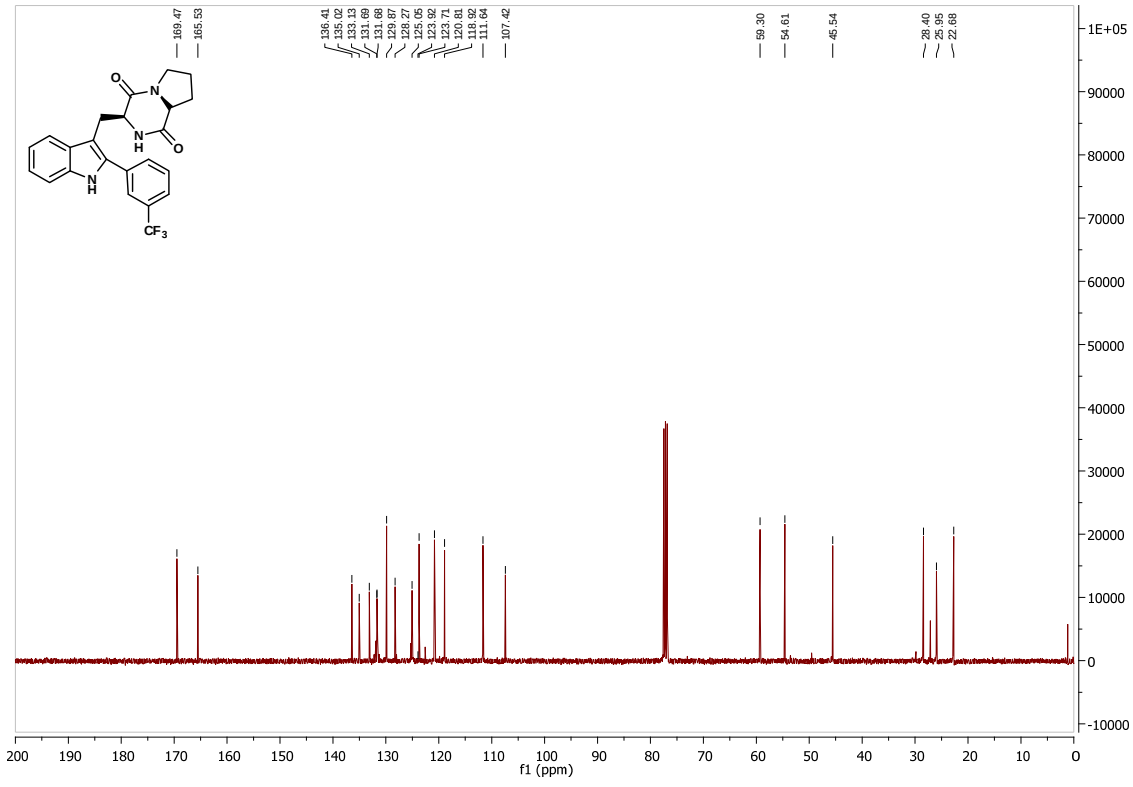
(3S,8aS)-3-((2-(3-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4i)





(3S,8aS)-3-((2-(3-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4j)





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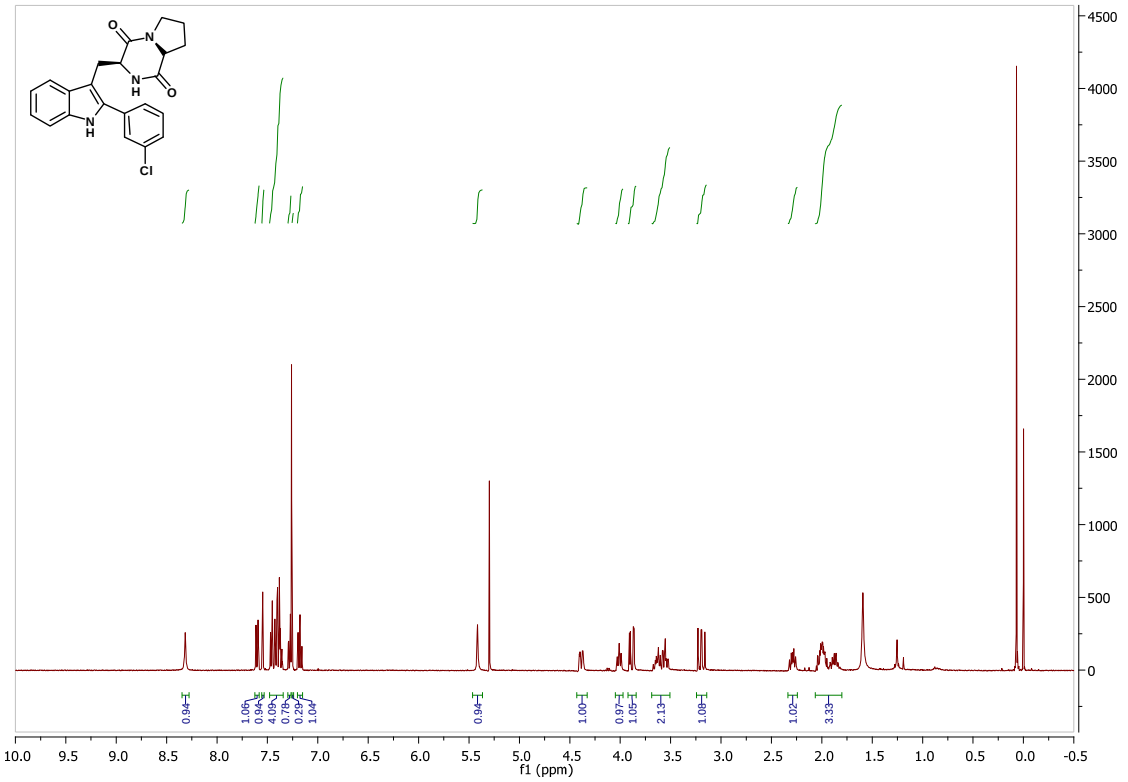
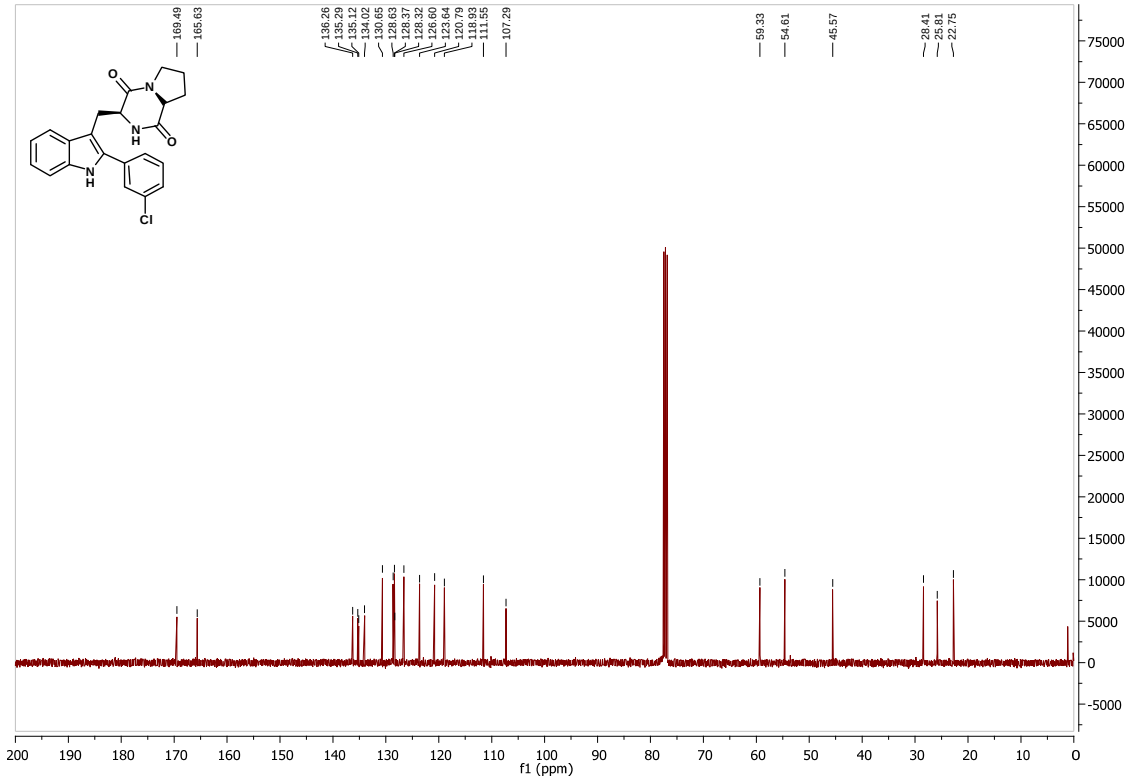
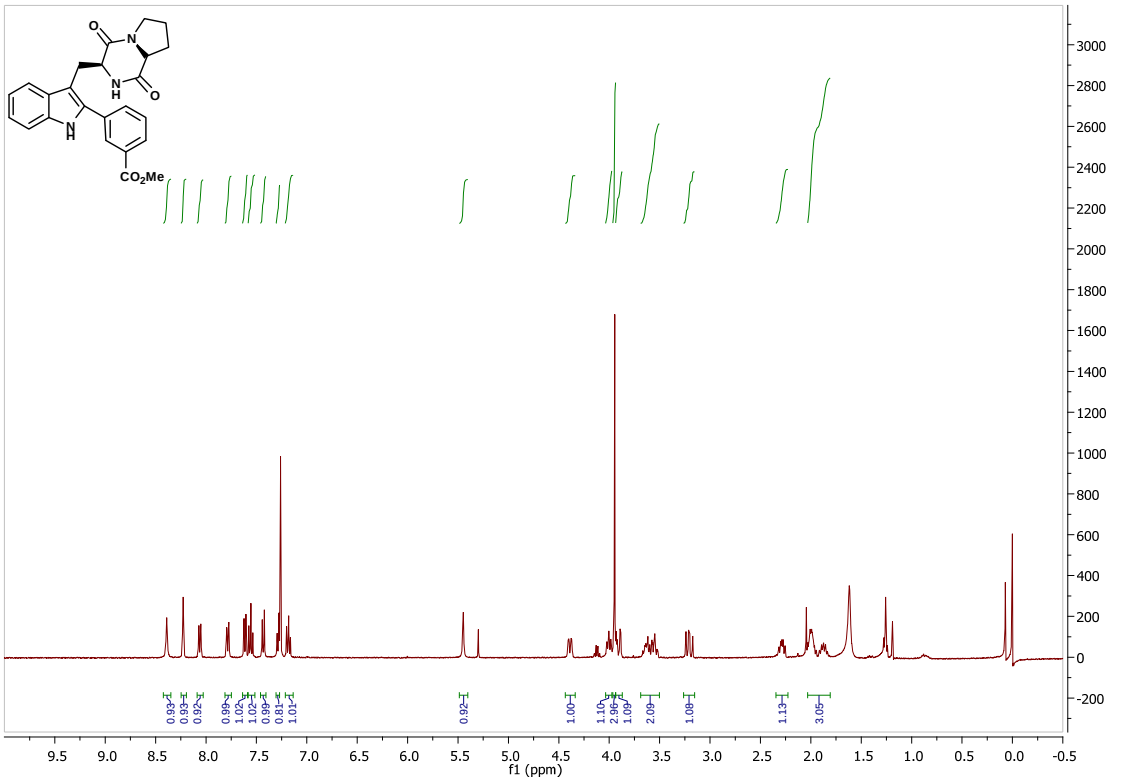
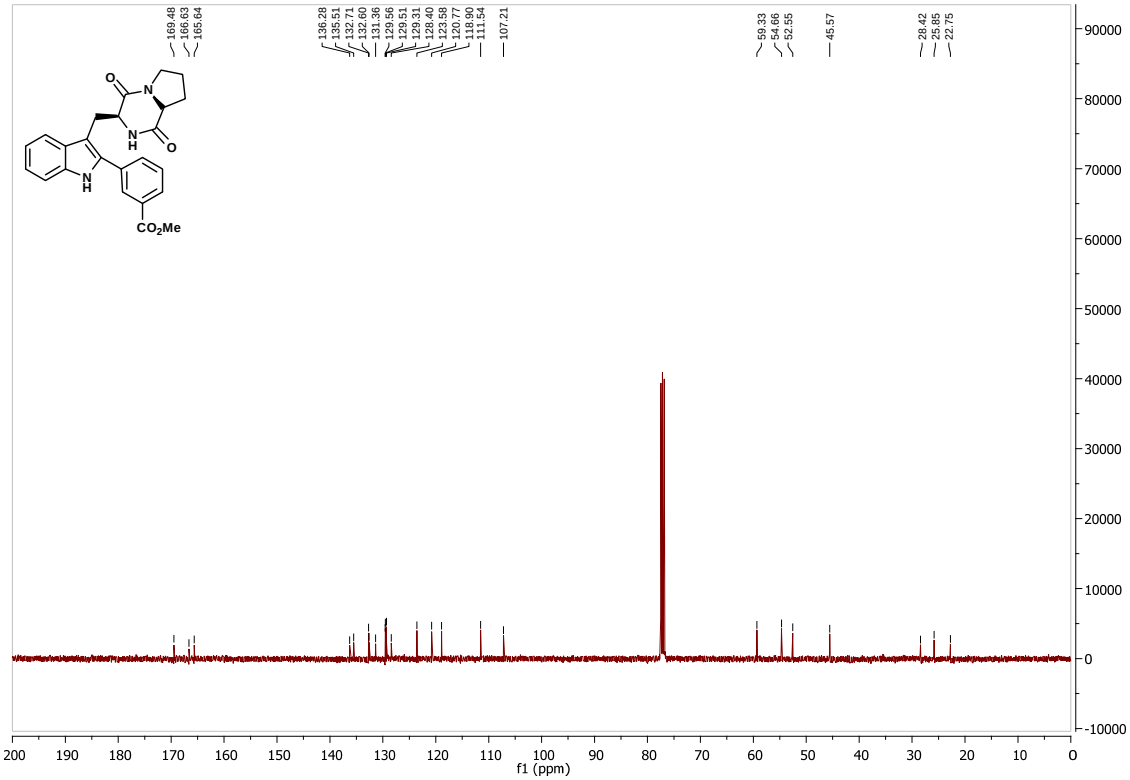


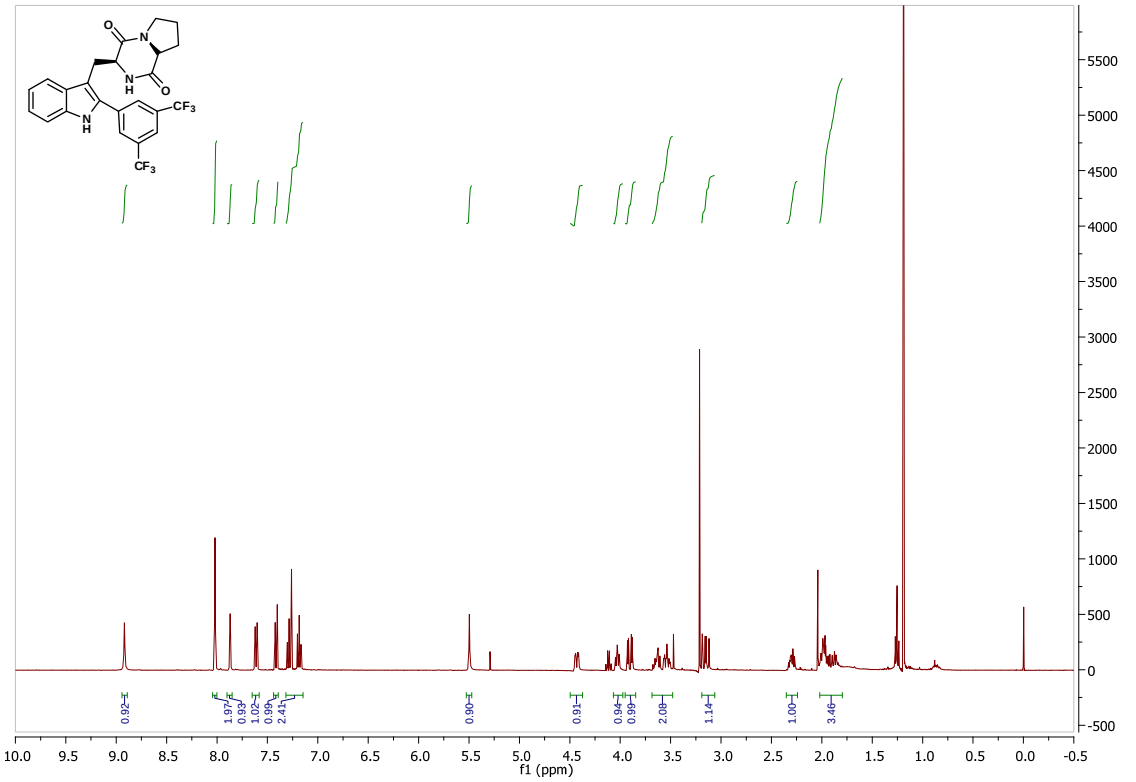


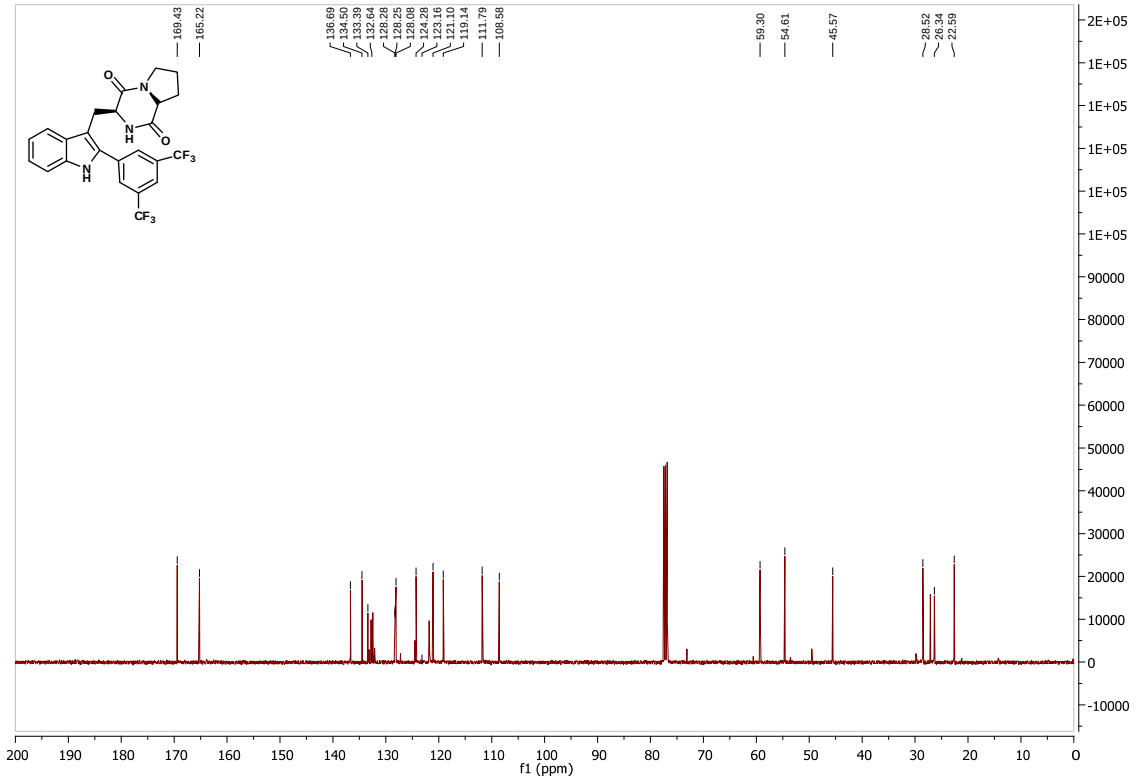
Methyl 3-(3-(((3S,8aS)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indol-2-yl)benzoate (4l)



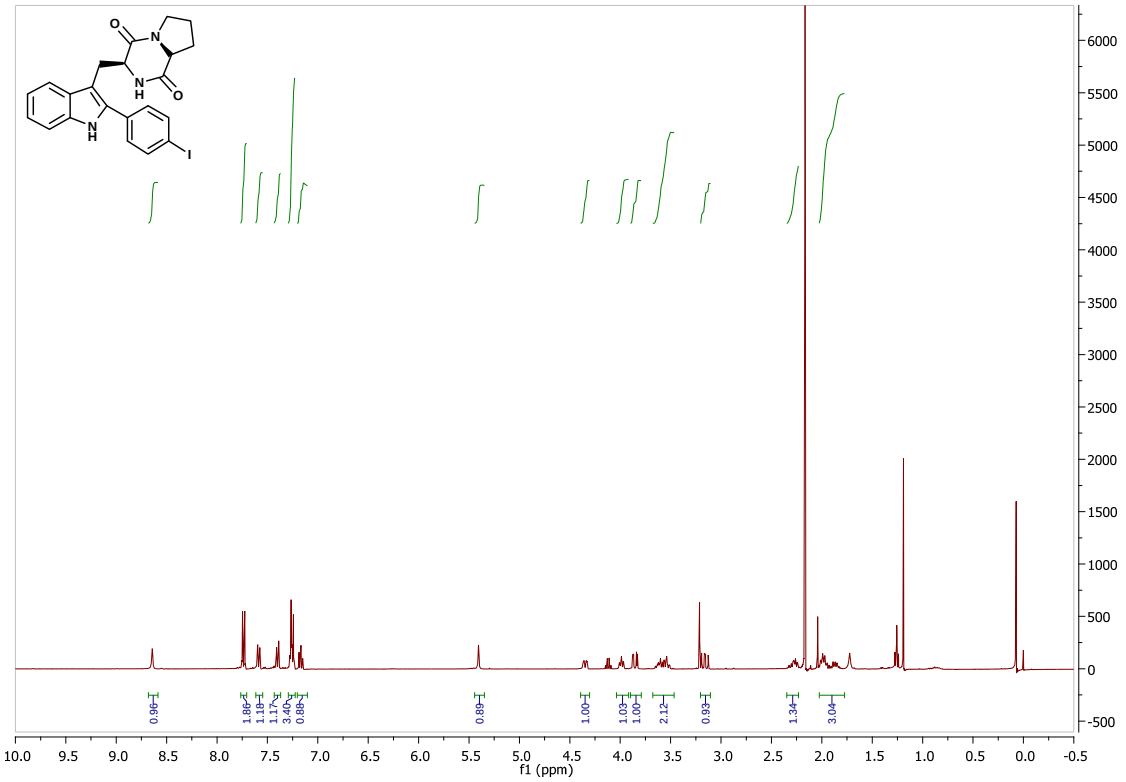


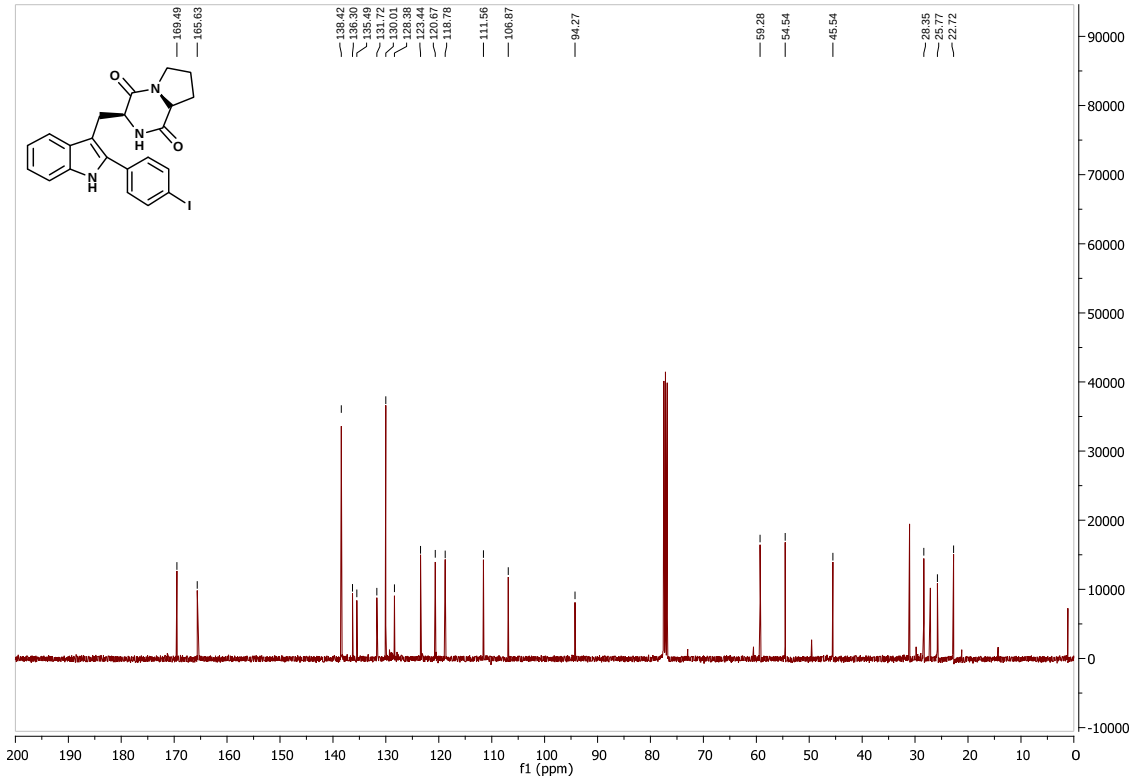
(3S,8aS)-3-((2-(3,5-bis(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4m)



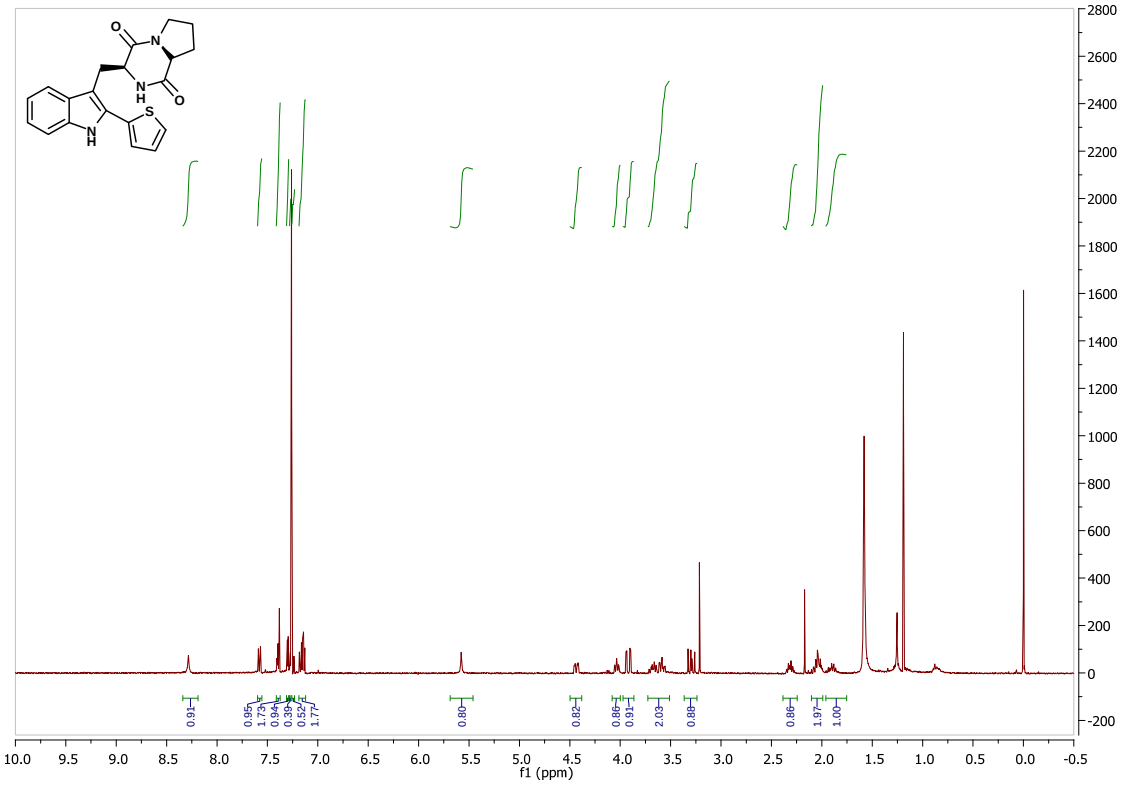


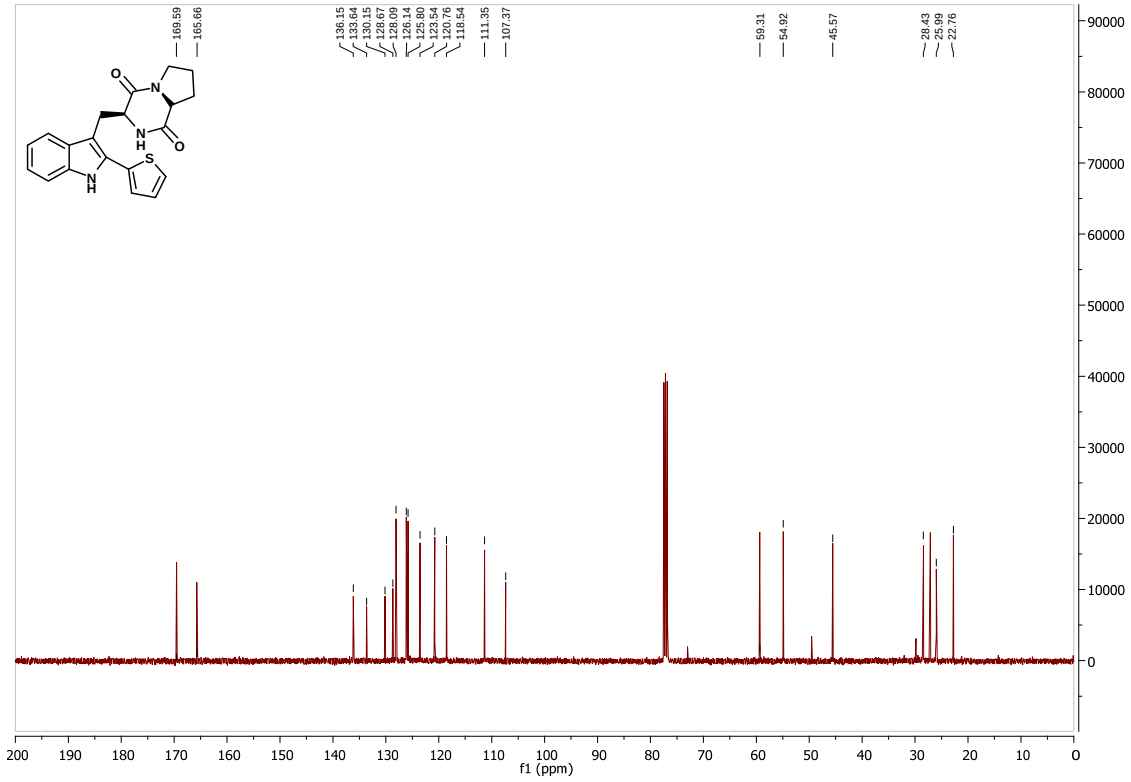
(3S,8aS)-3-((2-(4-Iodophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4n)



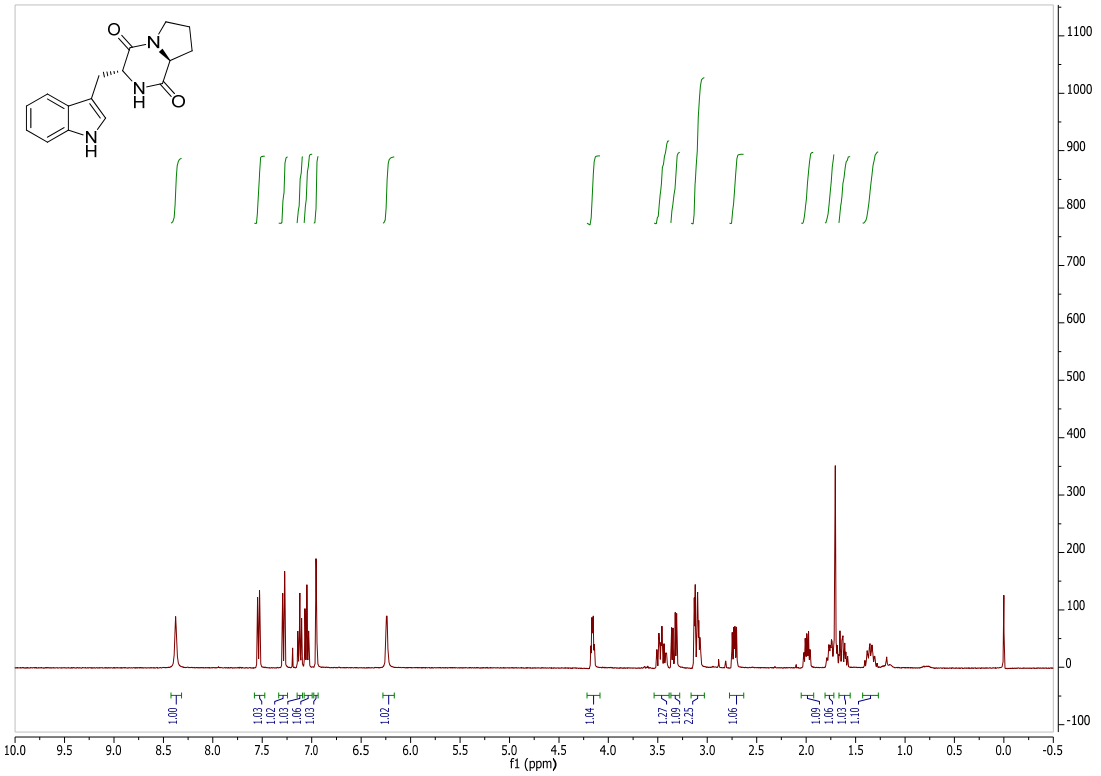


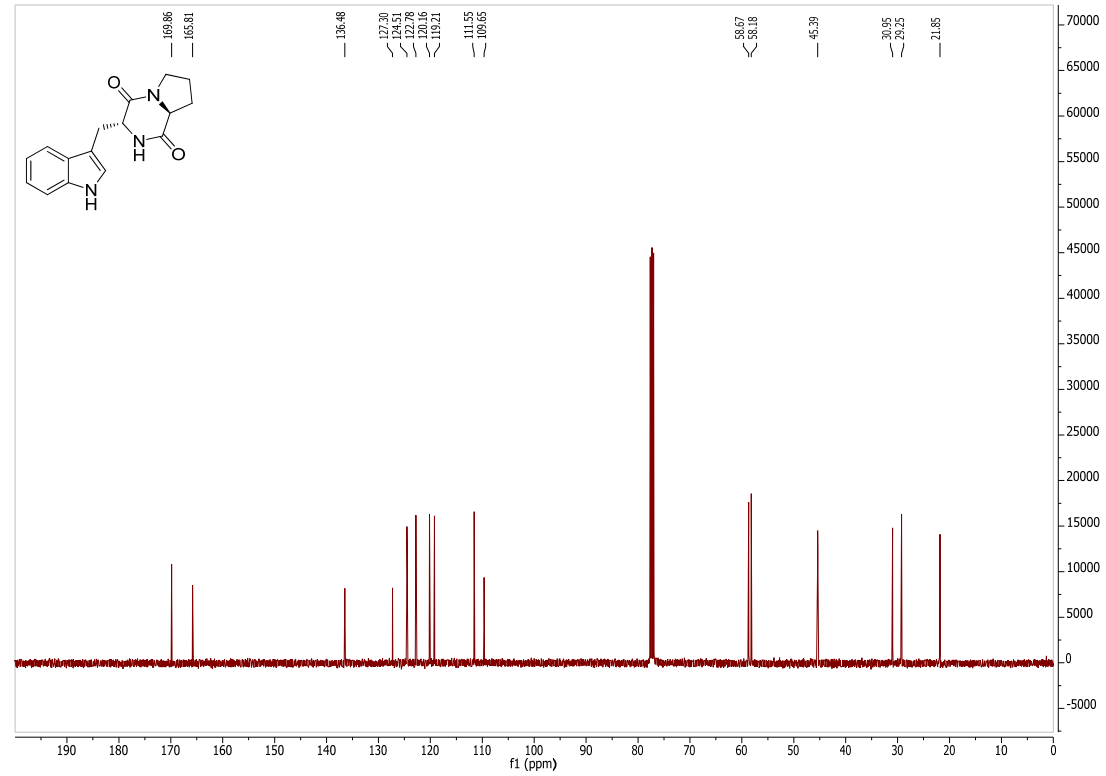
(3S,8aS)-3-((2-(2-Iodothiophene)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4o)





(3*R*,8a*S*)-3-((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (1')





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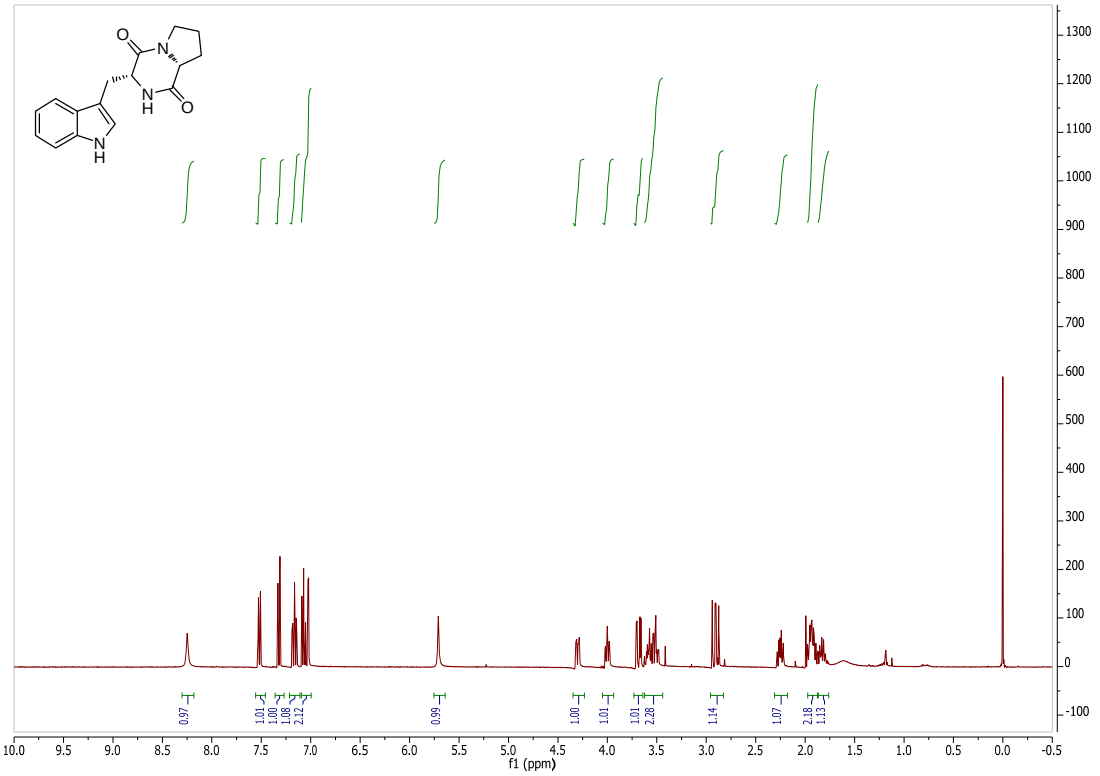

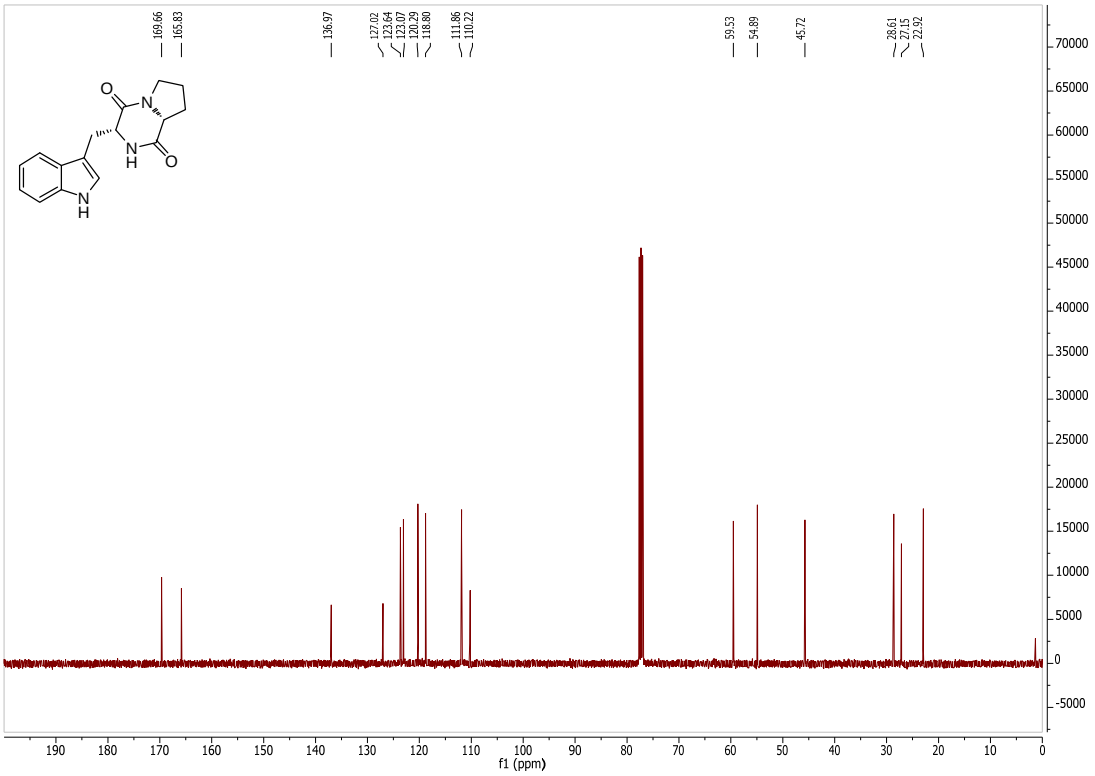

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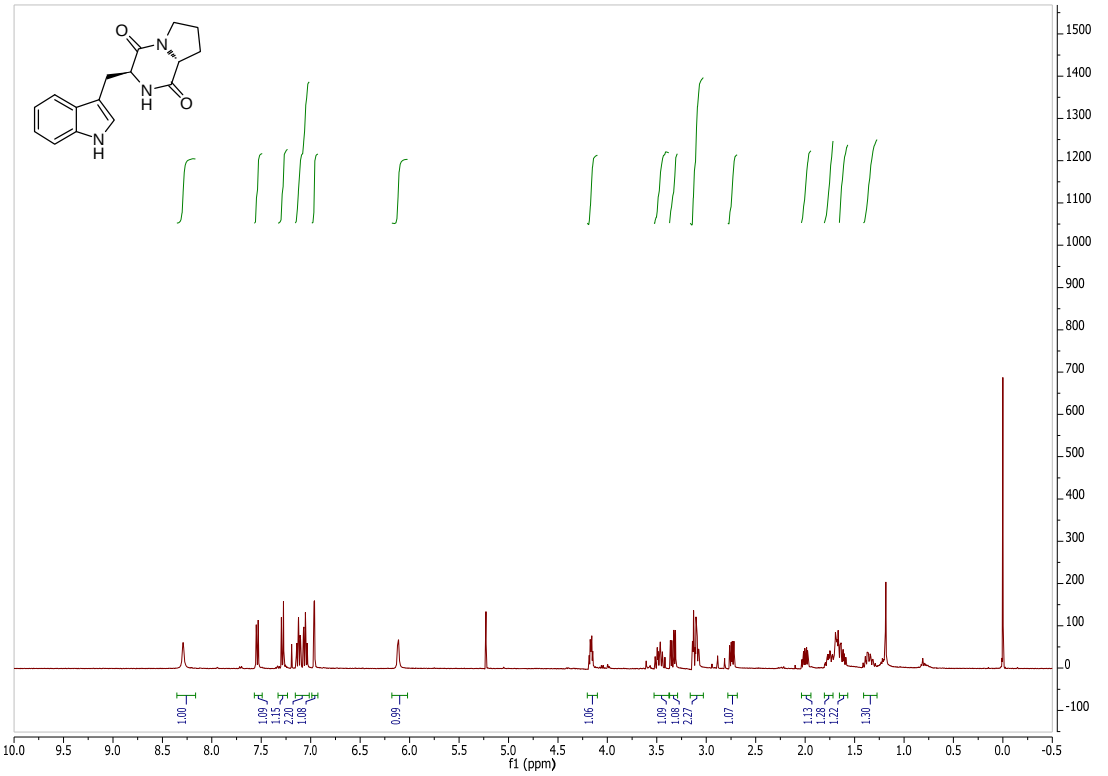
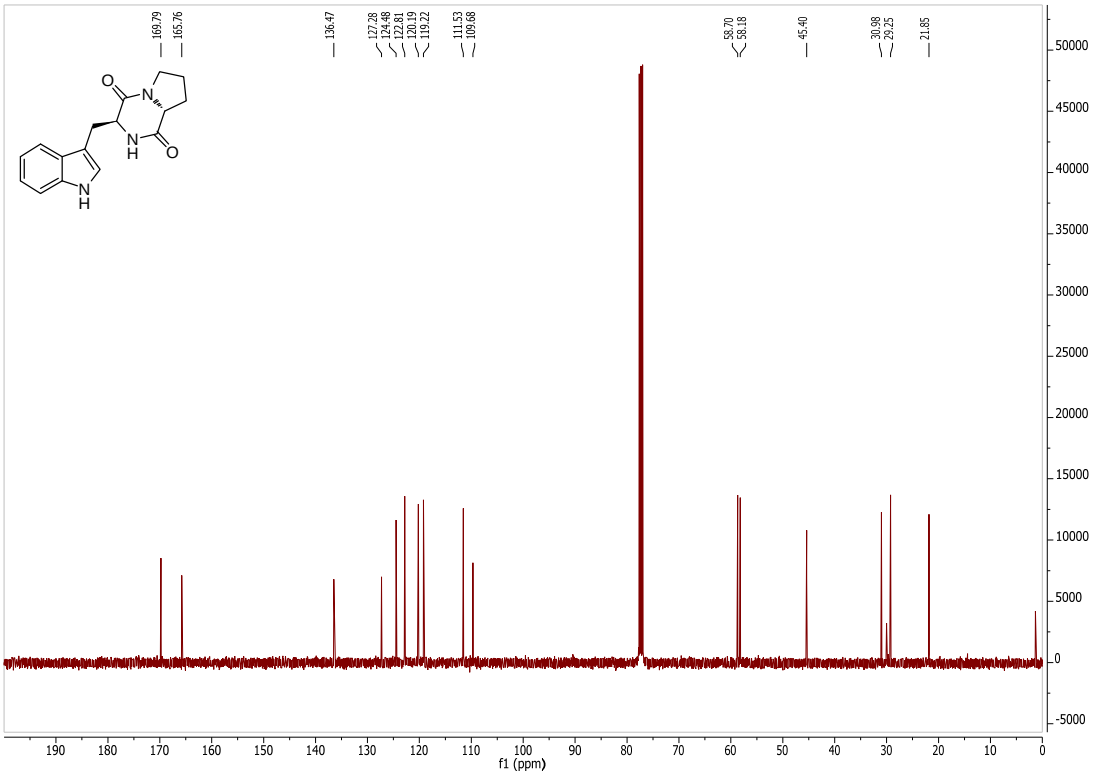
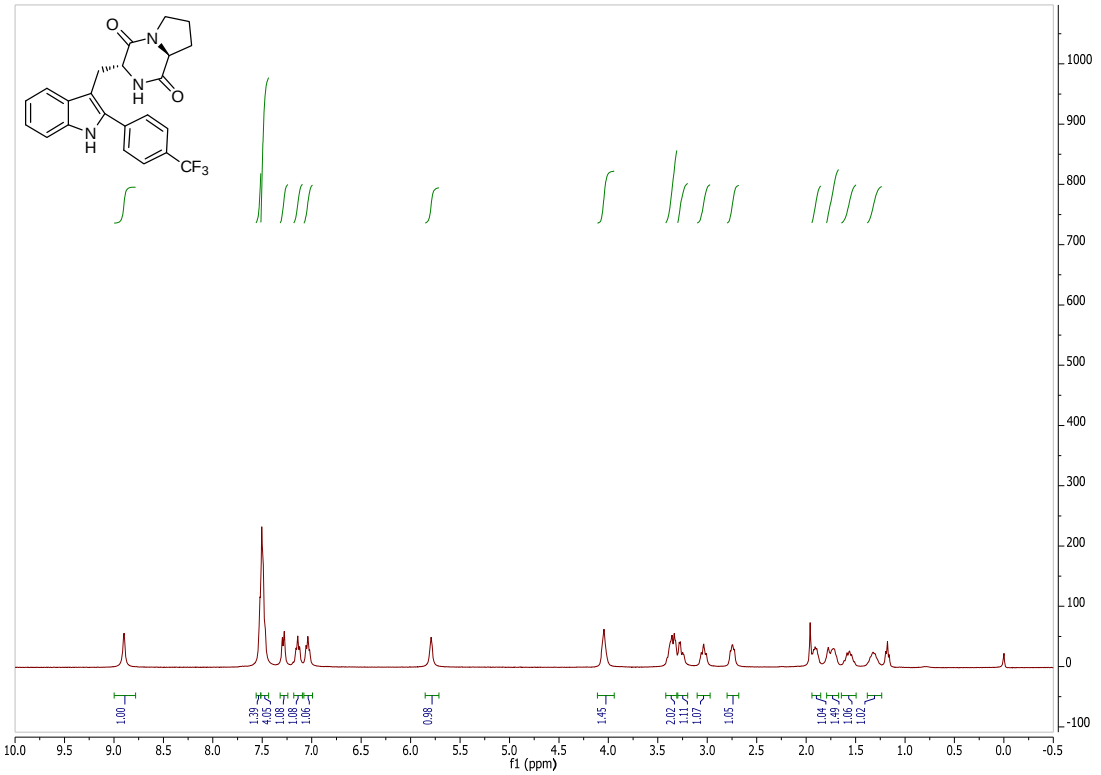
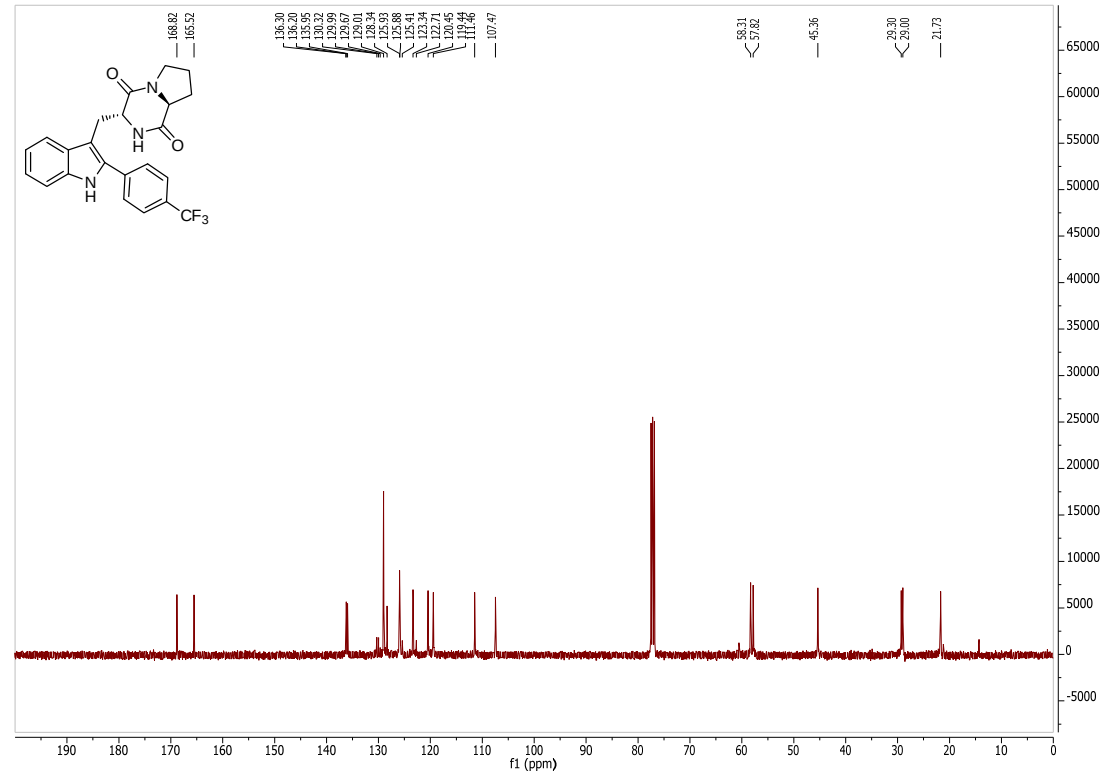


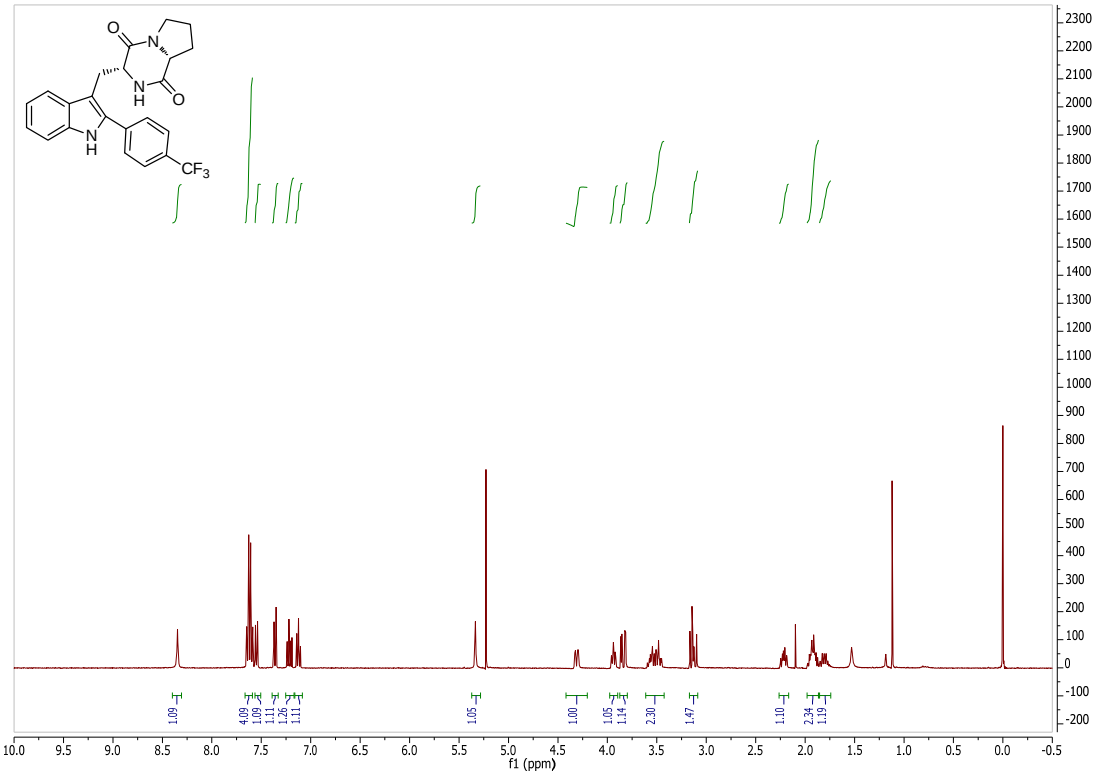


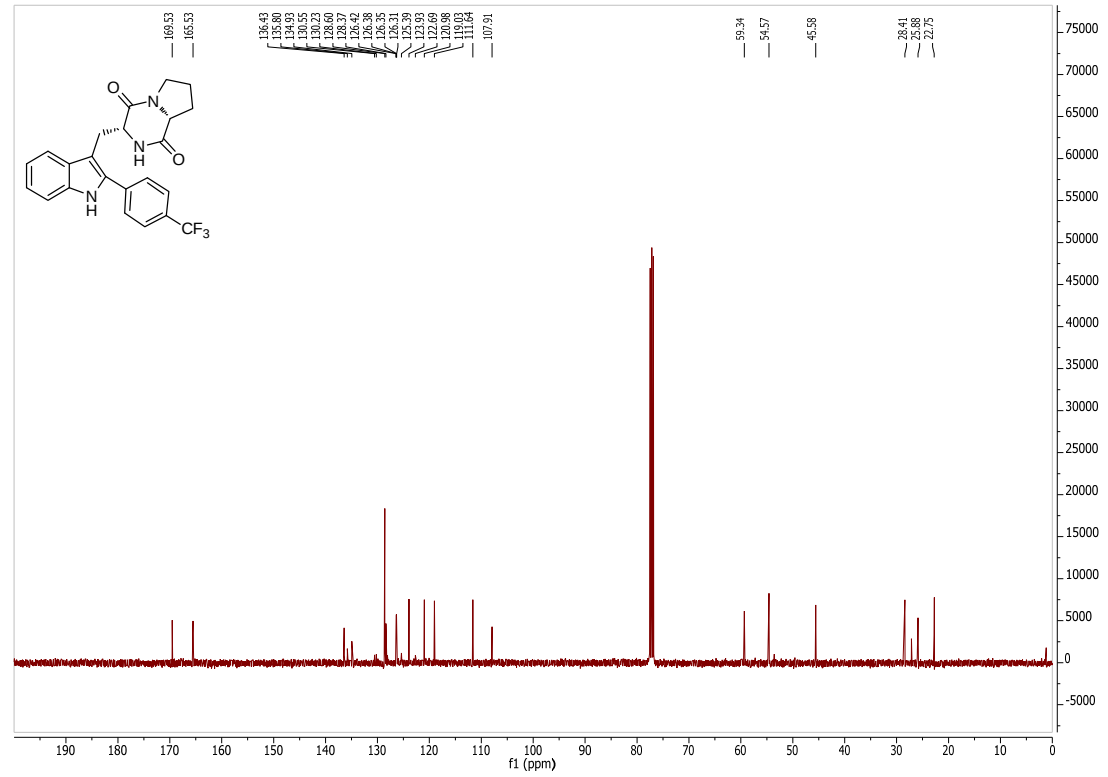
(3*R*,8a*S*)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (4c')



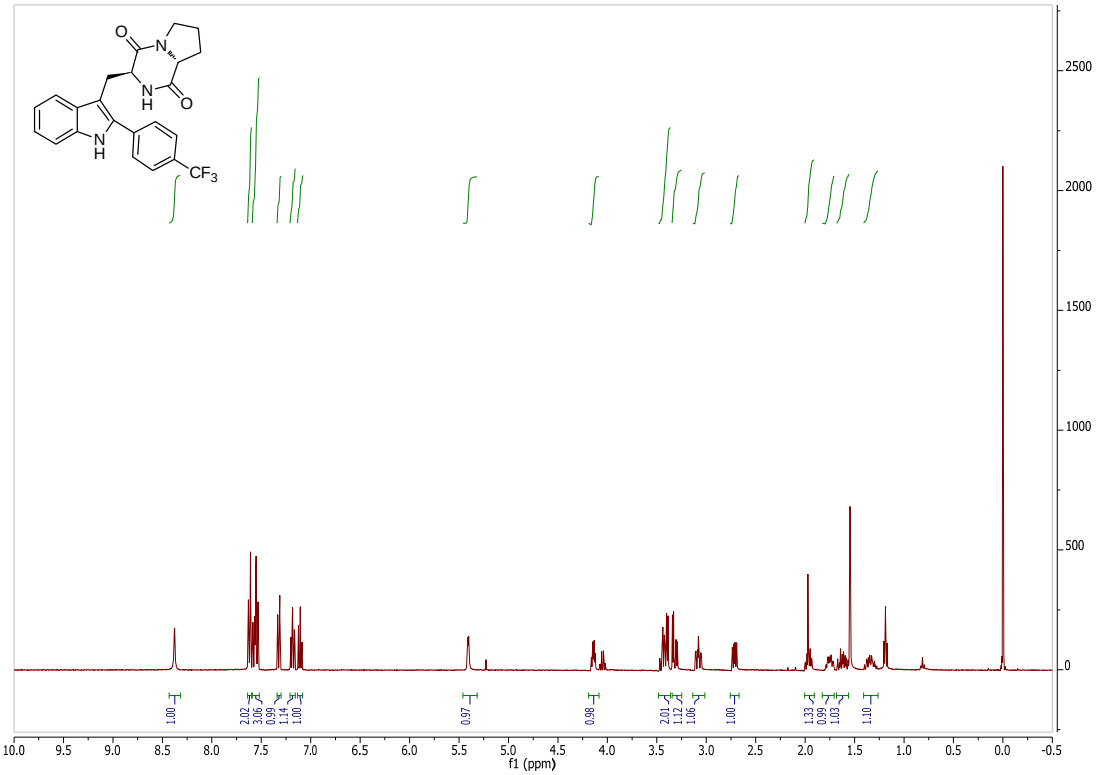


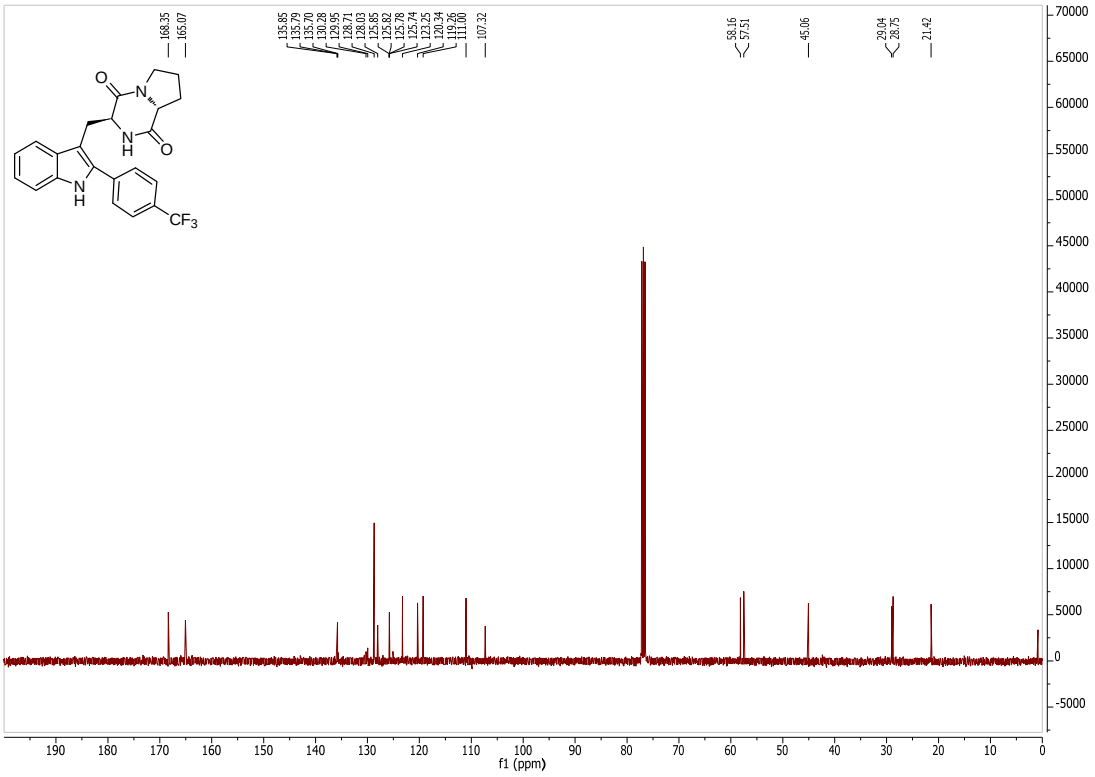
(3*R*,8a*R*)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (4c'')





(3*S*,8a*R*)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (4c’’’)





Biological Evaluation.

In vitro cell growth inhibitory activity

The synthesized compounds **4a-e** were evaluated for their antiproliferative activities against four human cancer cell lines: cervical adenocarcinoma HeLa cells, lung carcinoma A-549 cells, breast adenocarcinoma SK-BR-3 cells and colon adenocarcinoma HT-29 cells. In the assays the cells were exposed to the compounds *in vitro* and IC_{50} values calculated from dose-response relationships following quantitation of the surviving cells by the standard MTT test. The IC_{50} values are summarized in **tables 1 and 2**.

Cytotoxicity assay compounds 4a-4e

The four human cell lines were obtained from the American Type Culture Collection (ATCC). The HeLa cervical adenocarcinoma cells were grown in DMEM, A-549 lung carcinoma cells in F-12K Medium and SK-BR-3 breast adenocarcinoma and HT-29 colon adenocarcinoma cells in McCoy's 5a Medium Modified, all of them supplemented with 10% fetal bovine serum (FBS), 2 mM *L*-glutamine and antibiotics. Cells were subcultured twice a week and maintained at 37 ºC in a humidified atmosphere containing 5% CO_2.

Normally growing cells were plated at $5x10^3$ cells/well into 96-well plates and incubated for 24 h at 37 ºC to allow attachment to the plate surface. Samples were then added for inicial screening at 200 µM, 100 µM and 50 µM dissolved in a DMSO-PBS vehicle (less than 1% in culture medium). Any drug showing <50% cell survival at 200 µM was further tested using appropriate drug concentrations to determine its IC_{50}. Drugs were run in triplicate or greater and control wells contained appropriate percentages of vehicle.

After 72 h exposure, the antitumor effect was measured using a solution of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which is bioreduced by viable cells into formazan. The formed cristals were solubilized using DMSO and the amount of formazan was measured by reading the absorbance at 570 nm. The amount of formazan present was proportional to the number of living cells in culture. The absorbance of wells containing only the MTT reagent (the plate blank) was subtracted from all wells.

The IC_{50} values were determined by dose response curve analysis and statistical analysis using GraphPad Prism software version 5.0a.

Table 1. Cytotoxicity evaluation (IC_{50}, μM ± SD[1]) of compounds **4a**-**e** and a reference compound (brevianamide F, **1**) against selected tumor cell lines.

Cell line	4a	4b	4c	4d	4e	1
HeLa	135.0 ± 9.0	157.2 ± 12.7	25.8 ± 4.2	52.2 ± 9.2	>200	>200
A-549	149.7 ± 24.4	>200	104.6 ± 33.2	167.2 ± 26.3	>200	>200
SK-BR-3	>200	>200	127.2 ± 43.7	>200	>200	>200
HT-29	>200	191.3 ± 24.4	80.4 ± 16.2	152.1 ± 32.9	>200	>200

[1] SD: standard deviation. All experiments were independently performed at least three times.

Cytotoxicity assay compounds 4a-o

The human cell lines were obtained from the American Type Culture Collection (ATCC). The HeLa cervical adenocarcinoma cells were grown in DMEM and HT-29 colon adenocarcinoma cells in McCoy's 5a Medium Modified, both of them supplemented with 10% fetal bovine serum (FBS), 2 mM *L*-glutamine and antibiotics. Cells were subcultured twice a week and maintained at 37 ºC in a humidified atmosphere containing 5% CO_2.

Normally growing cells were plated at $1x10^4$ cells/well into 96-well plates and incubated for 24 h at 37 ºC to allow attachment to the plate surface. Samples were then added in ten different concentrations dissolved in a DMSO-PBS vehicle (less than 1% in culture medium) to determine their IC_{50}. Drugs were run in triplicate or greater and control wells contained appropriate percentages of vehicle.

After 24 h exposure, the antitumor effect was measured using a solution of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which is bioreduced by viable cells into formazan. The formed cristals were solubilized using DMSO and the amount of formazan was measured by reading the absorbance at 570 nm. The amount of formazan present was proportional to the number of living cells in culture. The absorbance of wells containing only the MTT reagent (the plate blank) was subtracted from all wells.

The IC_{50} values were determined by dose response curve analysis and statistical analysis using GraphPad Prism software version 5.0a.

Table 2. Cytotoxicity evaluation (IC_{50}, μM ± SD[1]) of compounds **4a**-**o** and a reference compound (puromycin) against selected tumor cell lines.

Compound	HeLa cells	HT-29 cells
4a	160.2 ± 9.2	214.9 ± 10.0
4b	193.8 ± 10.7	205.3 ± 7.0
4c	62.0 ± 11.5	118.5 ± 7.7
4d	81.8 ± 12.0	184.3 ± 7.8
4e	208.7 ± 17.8	204.1 ± 10.5
4f	82.2 ± 12.0	143.0 ± 15.7
4g	82.4 ± 18.6	153.7 ± 12.4
4h	66.7 ± 17.4	144.2 ± 13.2
4i	172.1 ± 25.8	200.3 ± 9.9
4j	255.4 ± 11.8	187.8 ± 6.9
4k	174.6 ± 18.5	200.3 ± 12.7
4l	190.3 ± 19.6	177.6 ± 14.2
4m	160.3 ± 23.7	64.2 ± 15.8
4n	146.6 ± 13.3	170.8 ± 8.6
4o	170.1 ± 11.5	177.6 ± 15.2
Puromycin	0.7 ± 0.1	3.6 ± 1.2

[1] SD: standard deviation. All experiments were independently performed at least three times.

Cell lines and Culture Conditions

Human lung carcinoma (A549) and human mammary adenocarcinoma (MDA-MB-231) cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Human oral squamous carcinoma cells (Cal27) were kindly provided by Dr. Silvio Gutkind (Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, NIH, Bethesda, USA). A549 and Cal27 cells were cultured in DMEM medium and MDA-MB-231 cells in DMEM:F12 medium (1:1, Biological Industries, Beit Haemek, Israel) supplemented with 10% heat-inactivated foetal bovine serum (FBS; Life Technologies, Carlsbad, CA), 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L- glutamine, all from Biological Industries. Cells were grown at 37ºC in a 5% CO_2 atmosphere.

Cell Viability Assay for compounds 4c-4c'''

Cells (1 x 10^5 cells/mL) were seeded in 96-well plates and allowed to grow for 24 h. Afterwards, they were treated with all compounds at different concentrations, ranging from 1.56 to 200 µM, to calculate the inhibitory concentration of 50% of cell population (IC_{50}). Cell viability was determined by MTT assay. After a 72h-treatment, 10 µM of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) was added to each well for an additional 4 h. DMSO was added in control cells. Media was aspirated and the blue MTT formazan precipitate was dissolved in 100 µl of DMSO. The absorbance at 570 nm was measured on a multiwell plate reader. Cell viability was expressed as a percentage of control cells, and data are shown as the mean value ± S.D. of three independent experiments performed in duplicate. IC_{50} values were calculated with GraphPad Prism 5 software. The IC_{50} values are summarized in **table 3**.

Table 3. Cell viability assay (IC_{50}, µM ± SD[1]) for compounds **4c-4c'''** at 72h treatment (µM).

Compound	MDA-MB-231	Cal27	A549
4c	161.27 ± 10.53	182.14 ± 7.7	179.61 ± 8.34
4c'	>200 µM	>200 µM	>200 µM
4c''	161.35 ± 8.24	137.72 ± 29.20	185.92 ± 12.75
4c'''	>200 µM	>200 µM	>200 µM

Cell Cycle Analysis for compounds 4c and 4c''

Cells (2.5 x 10^4 cells/mL) were seeded in 6-well plates and allowed to grow for 24 h. Then, FBS depletion was performed during 24 h to synchronize the cell culture. Cells were treated with IC_{50} values of different compounds for 6, 24 and 48 h in the presence of FBS. After treatment, cells were trypsinized, collected and centrifuged at 300g for 5 min. Cells were washed with PBS and centrifuged again at the same conditions. Then, cells were resuspended in 100 µl PBS and added drop-wise into a tube containing 1 ml of ice cold 70% ethanol while vortexing. Cells were kept at -20ºC at least overnight. Ethanol-fixed cells (400 µl) were centrifuged at 300 g for 5 min, washed with PBS and centrifuged again. Cell pellet was resuspended in 200 µl of Muse™ Cell Cycle Reagent (Millipore, Billerica, MA) and incubated for 30 min at room temperature protected from light. FACS analysis was performed using a Muse™ Cell Analyzer cytometer (Millipore). The results for cell cycle analysis are summarized in **tables 4 and 5**, respectively.

Table 4. Cell cycle analysis of compound **4c**.

Compound 4c	Go/G1	S	G2/M
0h	64.37 ± 3.11	16.92 ± 0.67	18.67 ± 2.72
6h	47.30 ± 0.42	23.70 ± 0.00	29.00 ± 0.42
24h	60.30 ± 0.28	18.05 ± 0.35	21.65 ± 0.07
48h	61.22 ± 0.73	17.65 ± 0.66	21.07 ± 0.63

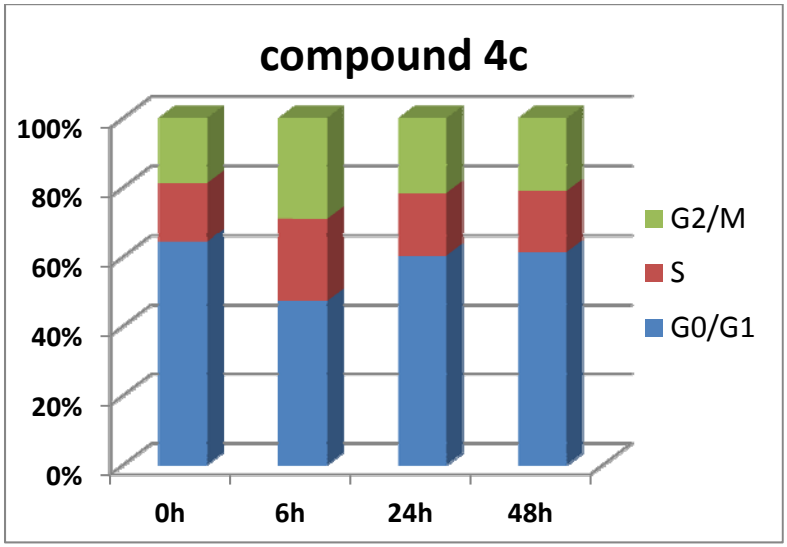
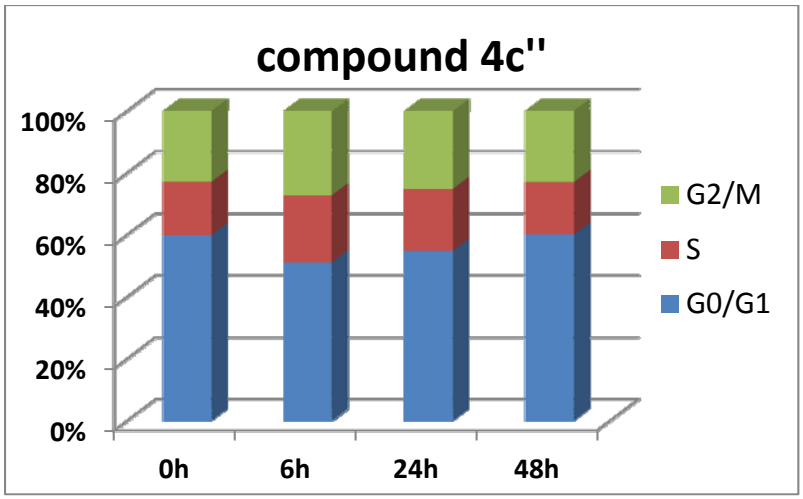


Table 5. Cell cycle analysis of compound **4c''**.

Compound 4c''	Go/G1	S	G2/M
0h	60.40 ± 0.94	17.30 ± 1.86	23.00 ± 0.61
6h	51.10 ± 0.42	21.65 ± 0.49	27.25 ± 0.07
24h	54.80 ± 1.13	20.05 ± 0.07	25.10 ± 1.13
48h	60.15 ± 0.89	16.95 ± 0.48	22.87 ± 0.63



Supporting Information

Synthesis of C-2 Arylated Tryptophan Amino Acids and Related Compounds through Palladium Catalyzed C-H Activation

Sara Preciado, Lorena Mendive-Tapia, Fernando Albericio and Rodolfo Lavilla**

Table of Contents

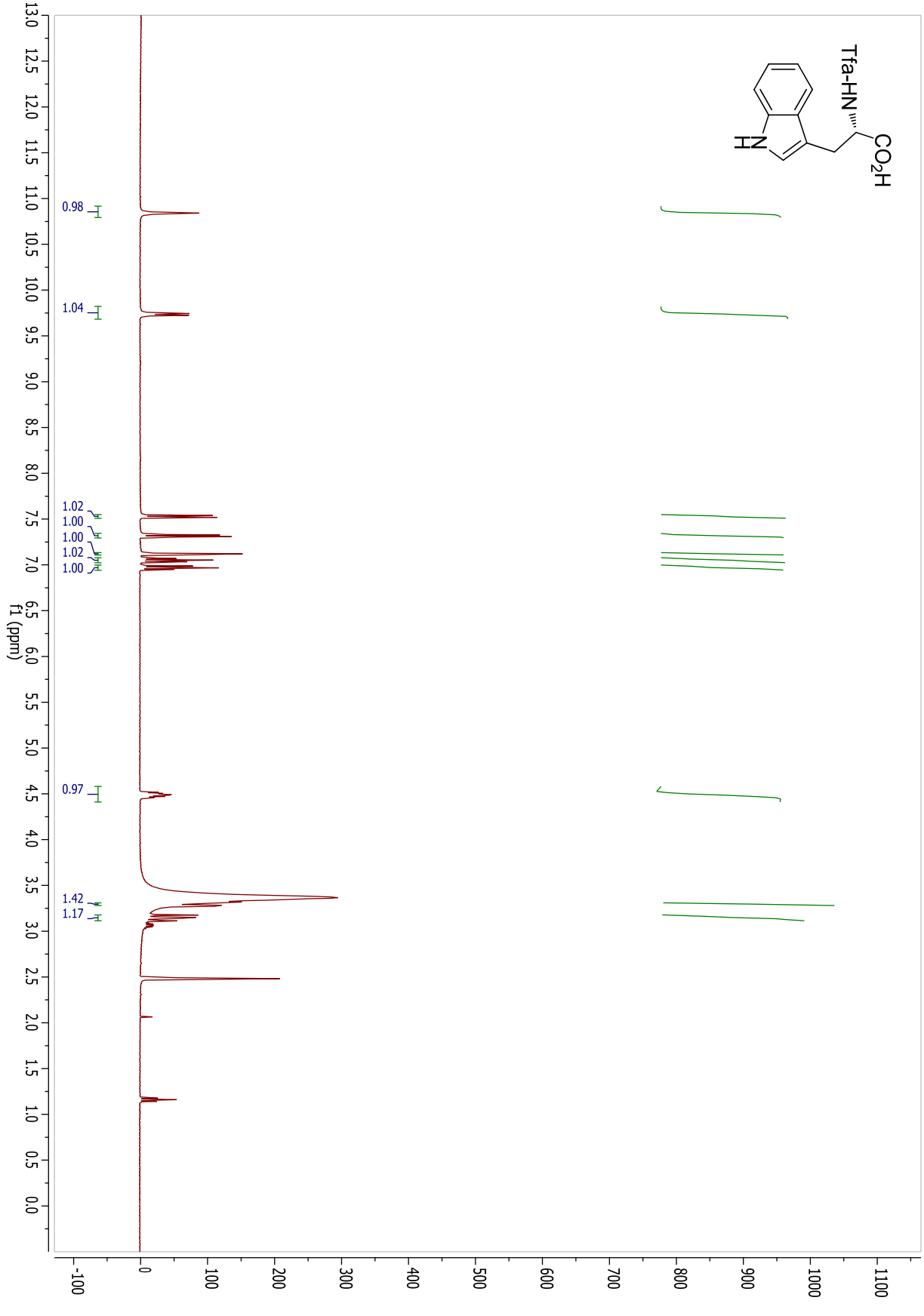
Supporting Information

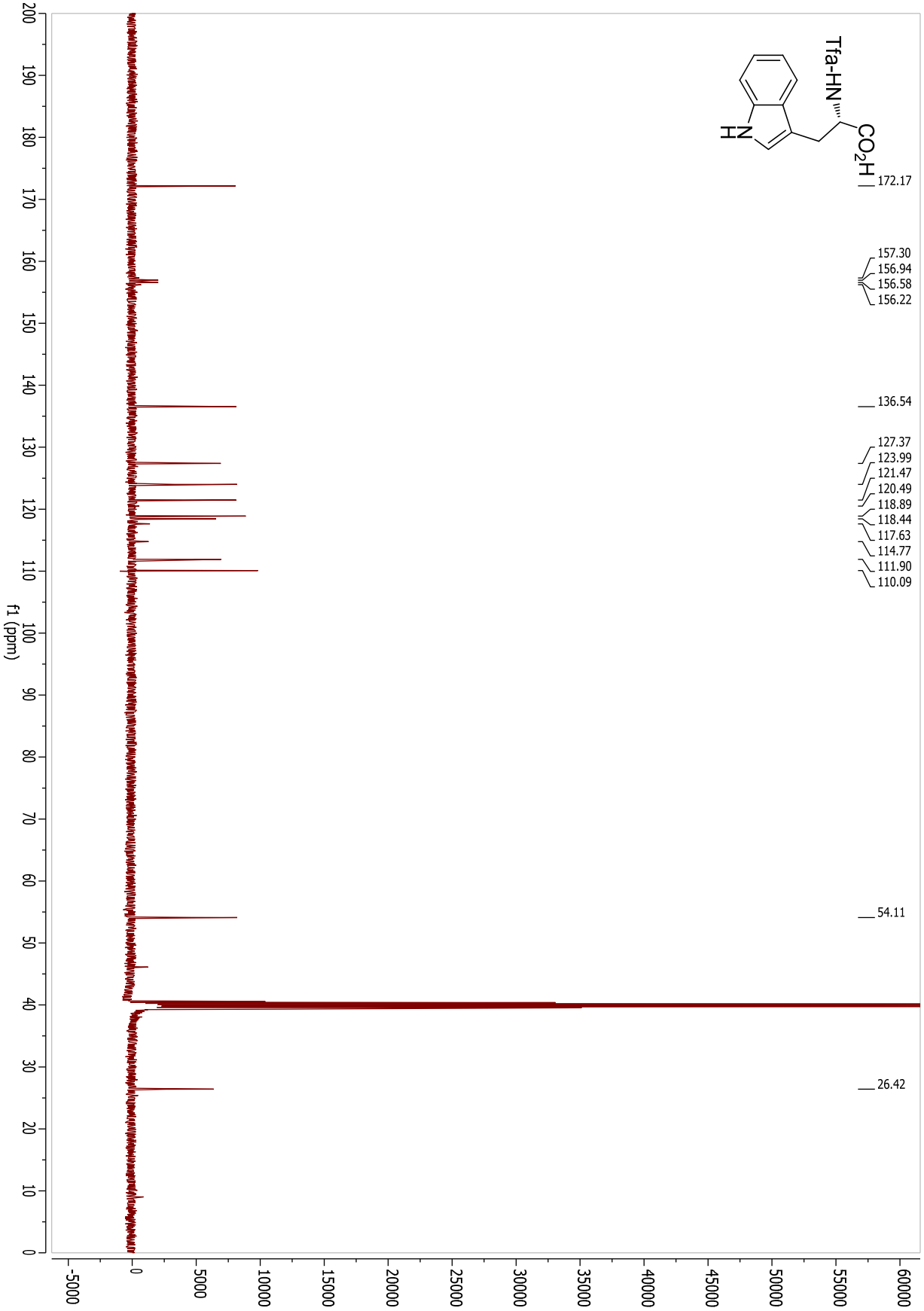
Abbreviations

Abbreviation used for amino acids and designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* 247, 977-983 (1982). The following additional abbreviations are used: DMF: *N*,*N*-dimethylformamide, DCM: dichloromethane, Fmoc: 9*H*-fluorenylmethyloxycarbonyl, Tfa: trifluoroacetyl, TFA: trifluoroacetic acid, SPPS: solid phase peptide synthesis, DIPEA: N,N-diisopropylethylamine, DIPCDI: N,N-diisopropylcarbodiimide, RP-HPLC-ESMS: reverse-phase high performance liquid chromatography electrospray mass spectrometry, HRMS: high-resolution mass spectrometry, NMR: nuclear magnetic resonance, SPPS: solid-phase peptide synthesis, IR: infrared spectroscopy.

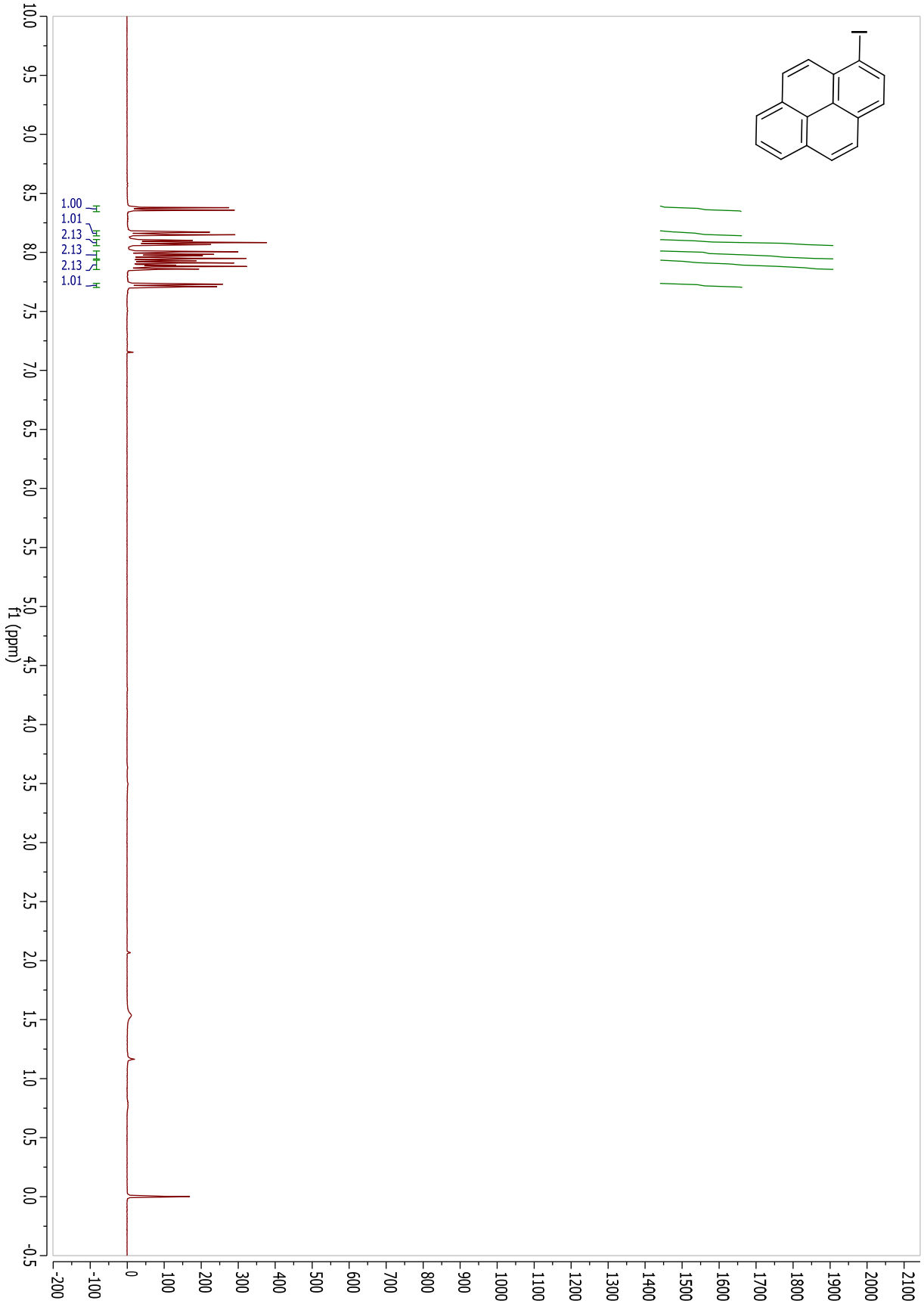
Spectroscopic data

(S)-3-(1*H*-Indol-3-yl)-2-(2,2,2-trifluoroacetamido)propanoic acid (1a)

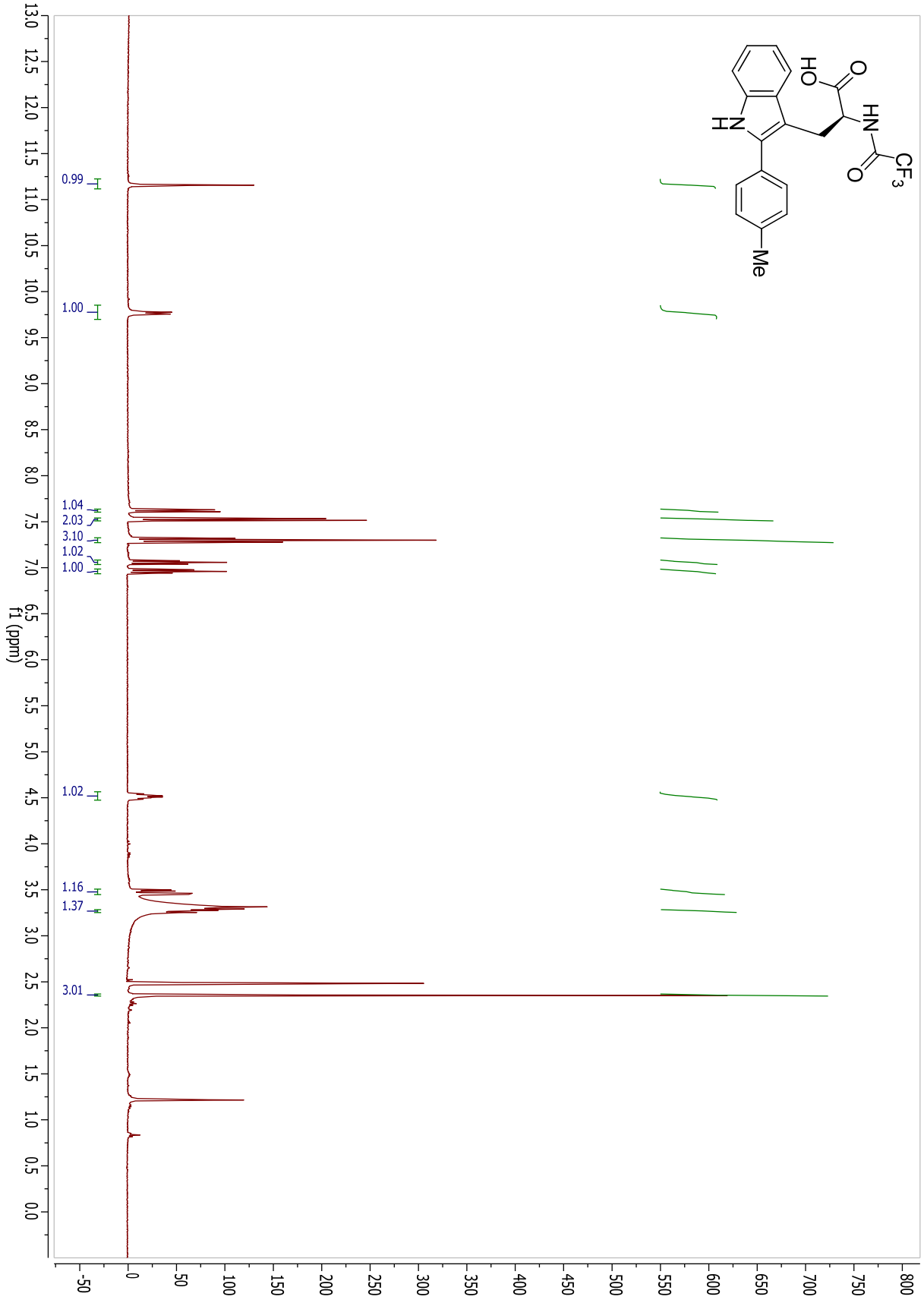


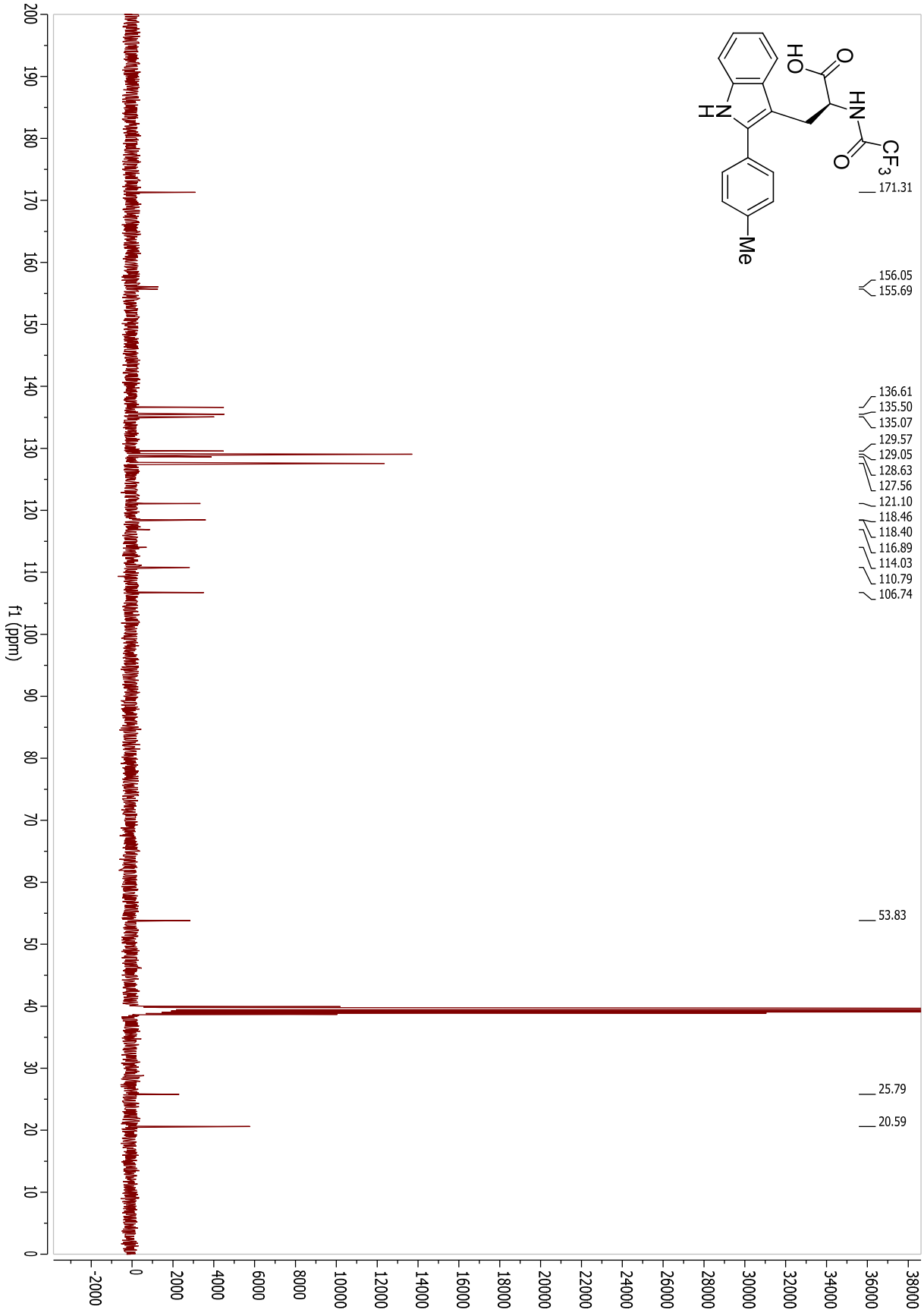

Tfa-HN
CO2H
N
H
172.17
157.30
156.94
156.58
156.22
136.54
127.37
123.99
121.47
120.49
118.89
118.44
117.63
114.77
111.90
110.09
54.11
26.42
f1 (ppm)

1-Iodopyrene (2b)

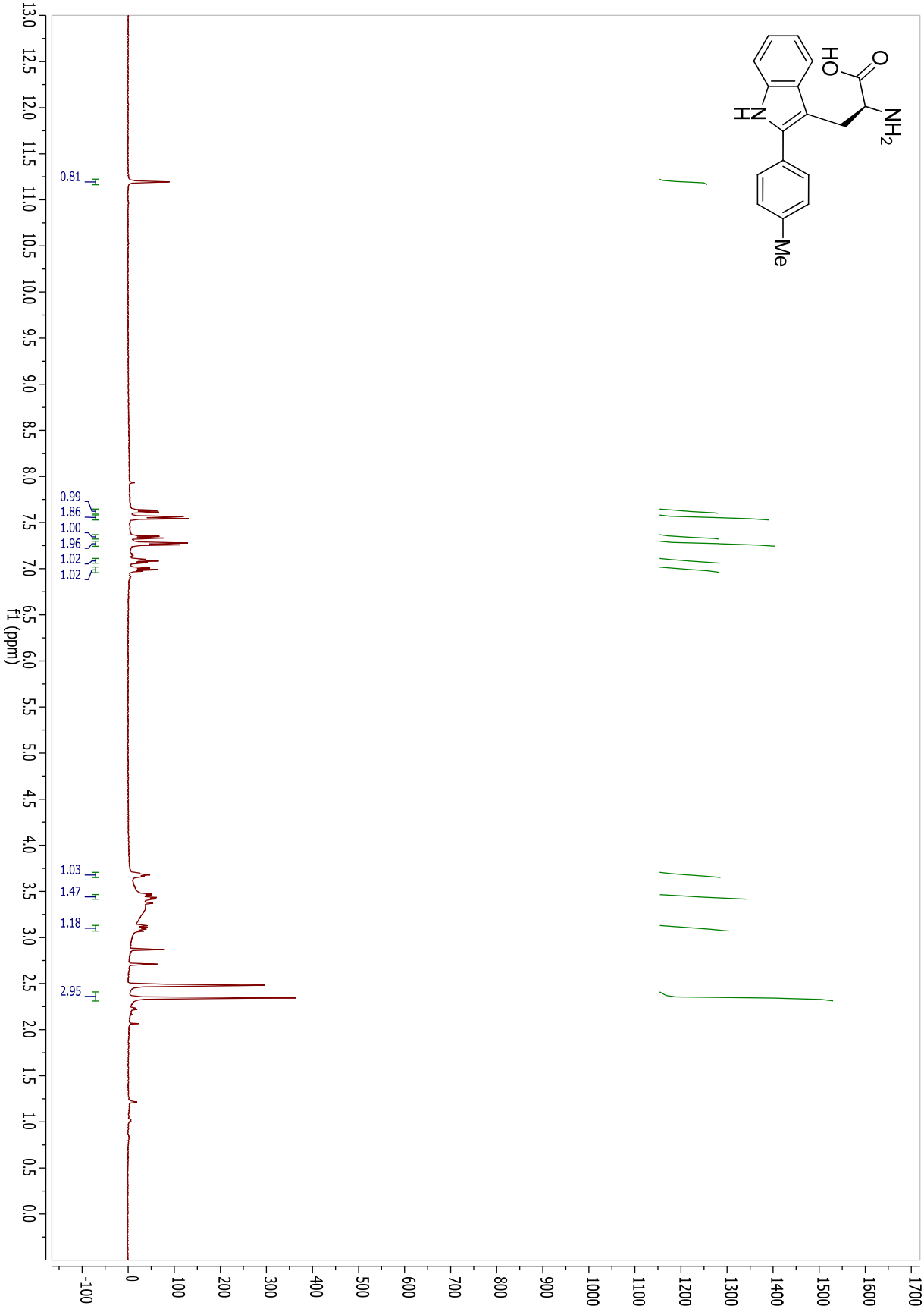


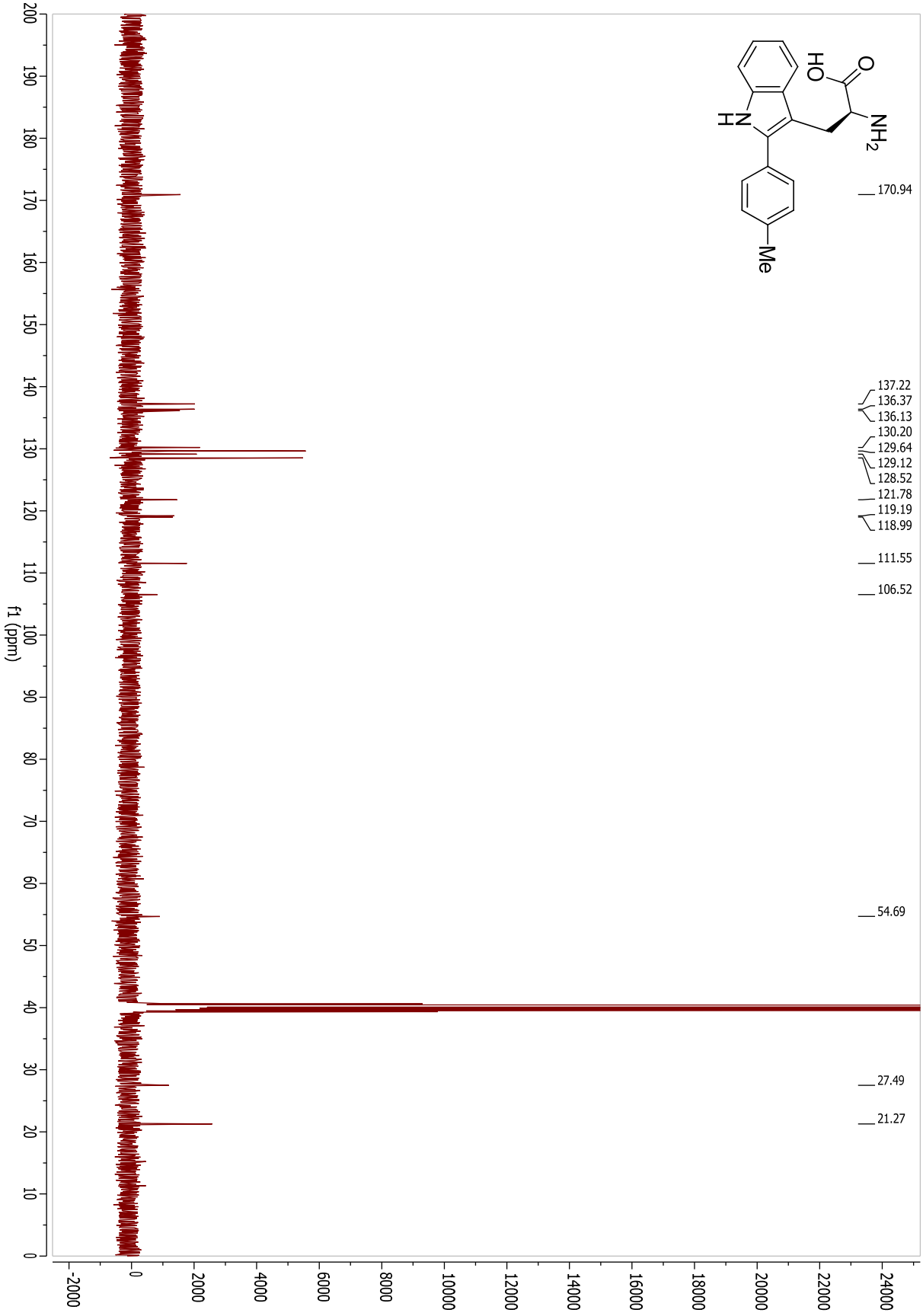
(S)-3-(2-phenyl-1*H*-indol-3-yl)-2-(2,2,2-trifluoroacetamido)propanoic acid (3)



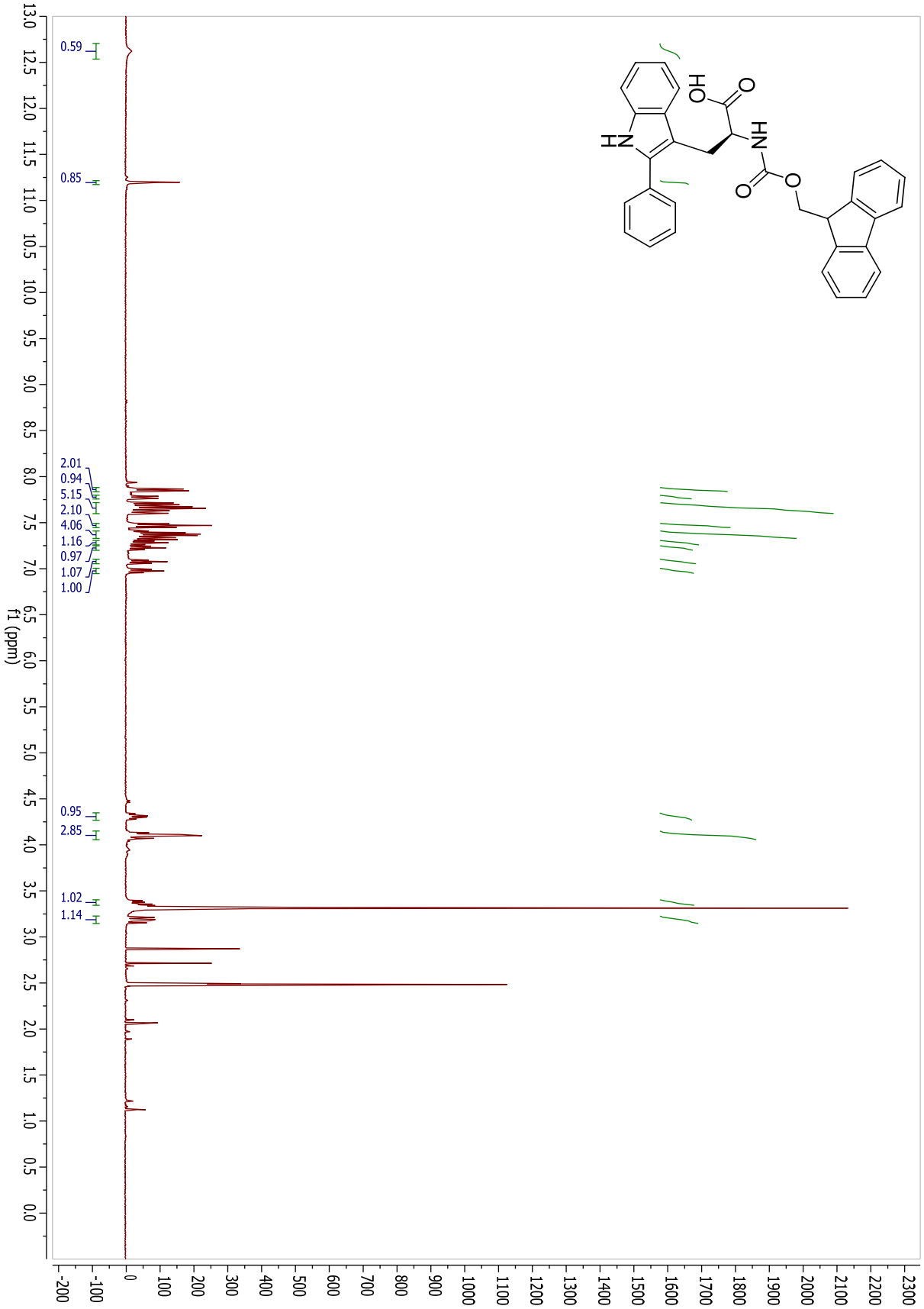

171.31
156.05
155.69
136.61
135.50
135.07
129.57
129.05
128.63
127.56
121.10
118.46
118.40
116.89
114.03
110.79
106.74
53.83
25.79
20.59
f1 (ppm)

(S)-2-amino-3-(2-*p*-tolyl-1*H*-indol-3-yl)propanoic acid (4)

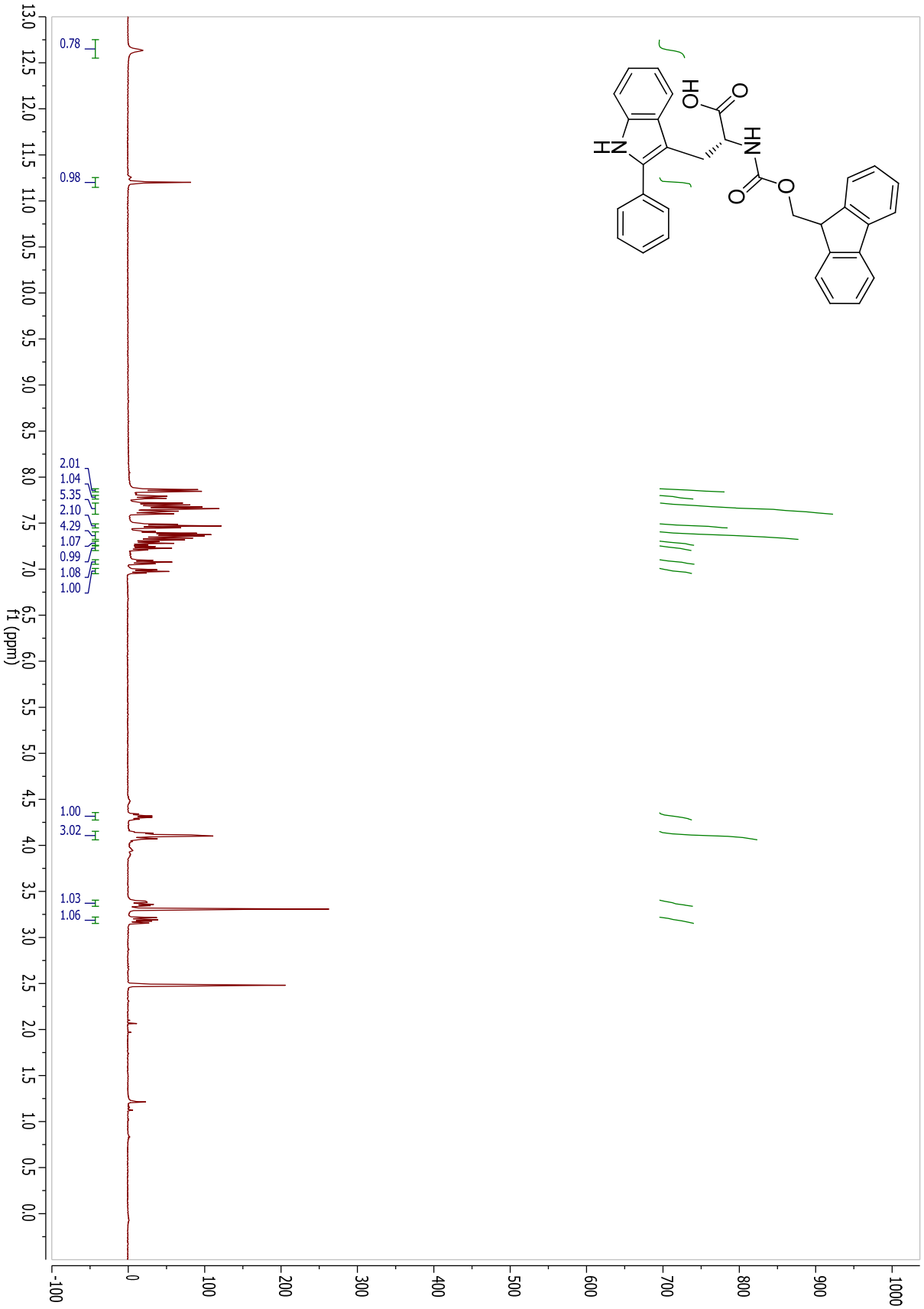


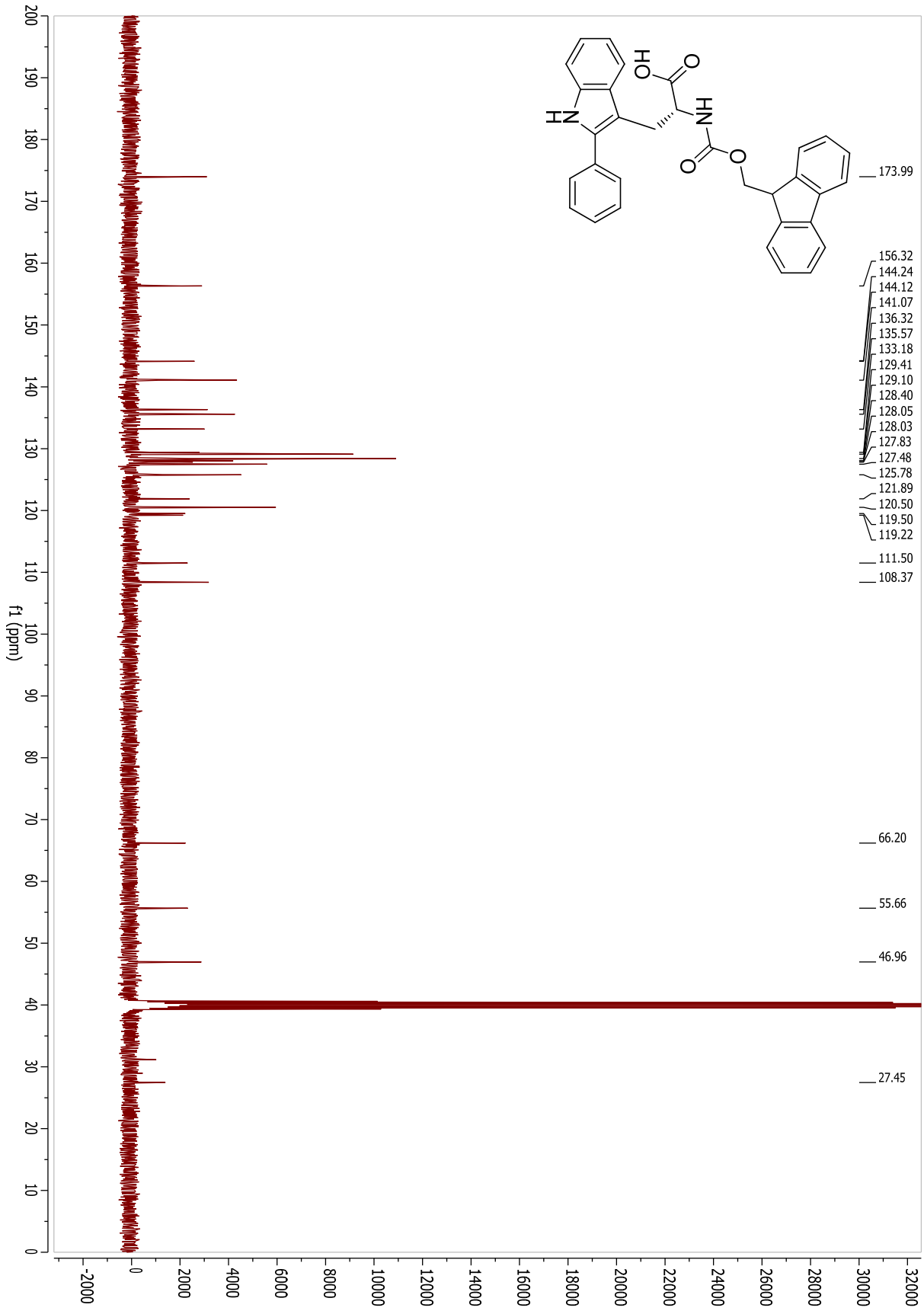

170.94
137.22
136.37
136.13
130.20
129.64
129.12
128.52
121.78
119.19
118.99
111.55
106.52
54.69
27.49
21.27
f1 (ppm)

(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-phenyl-1*H*-indol-3-yl)propanoic acid (5a)

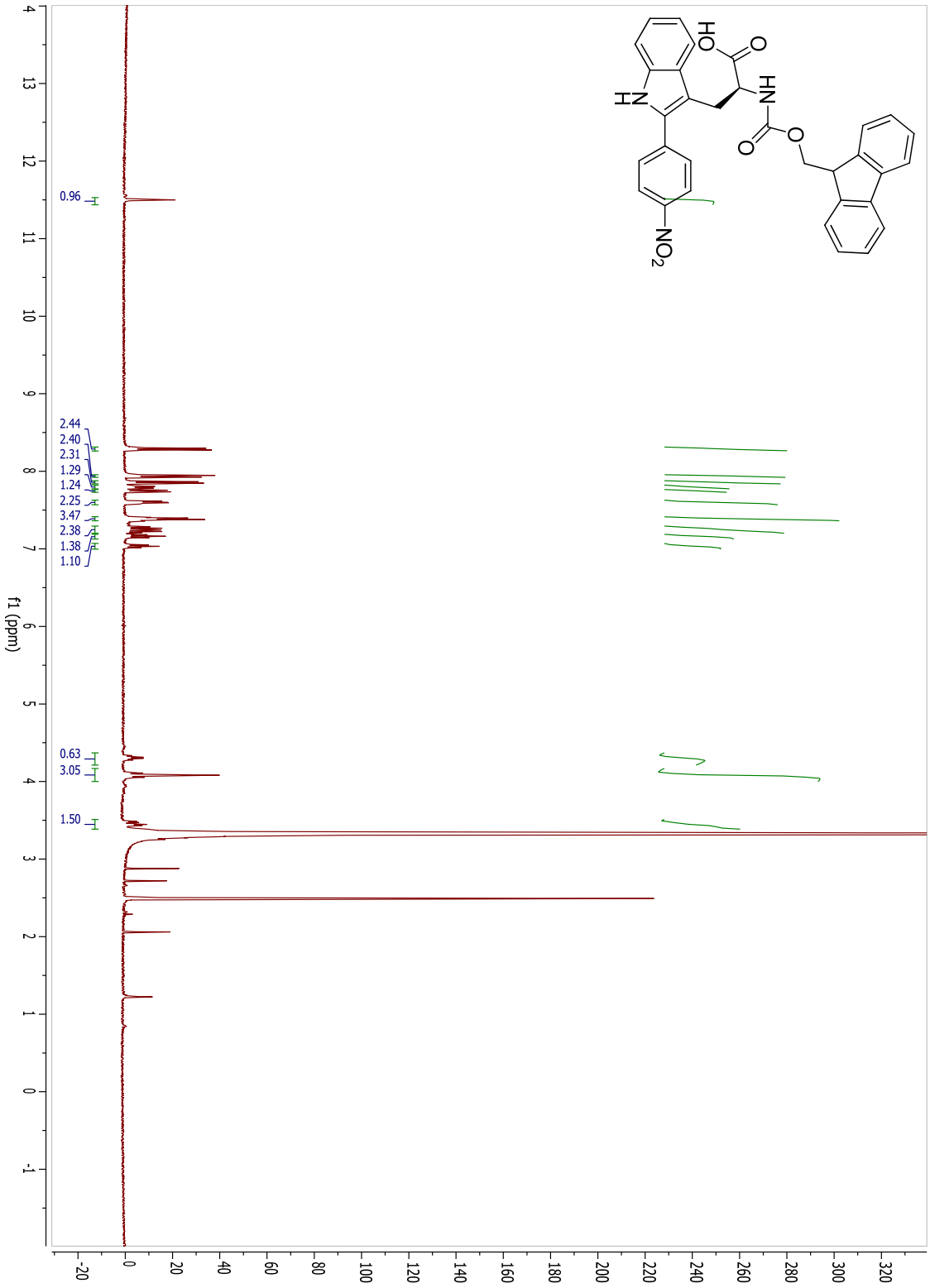


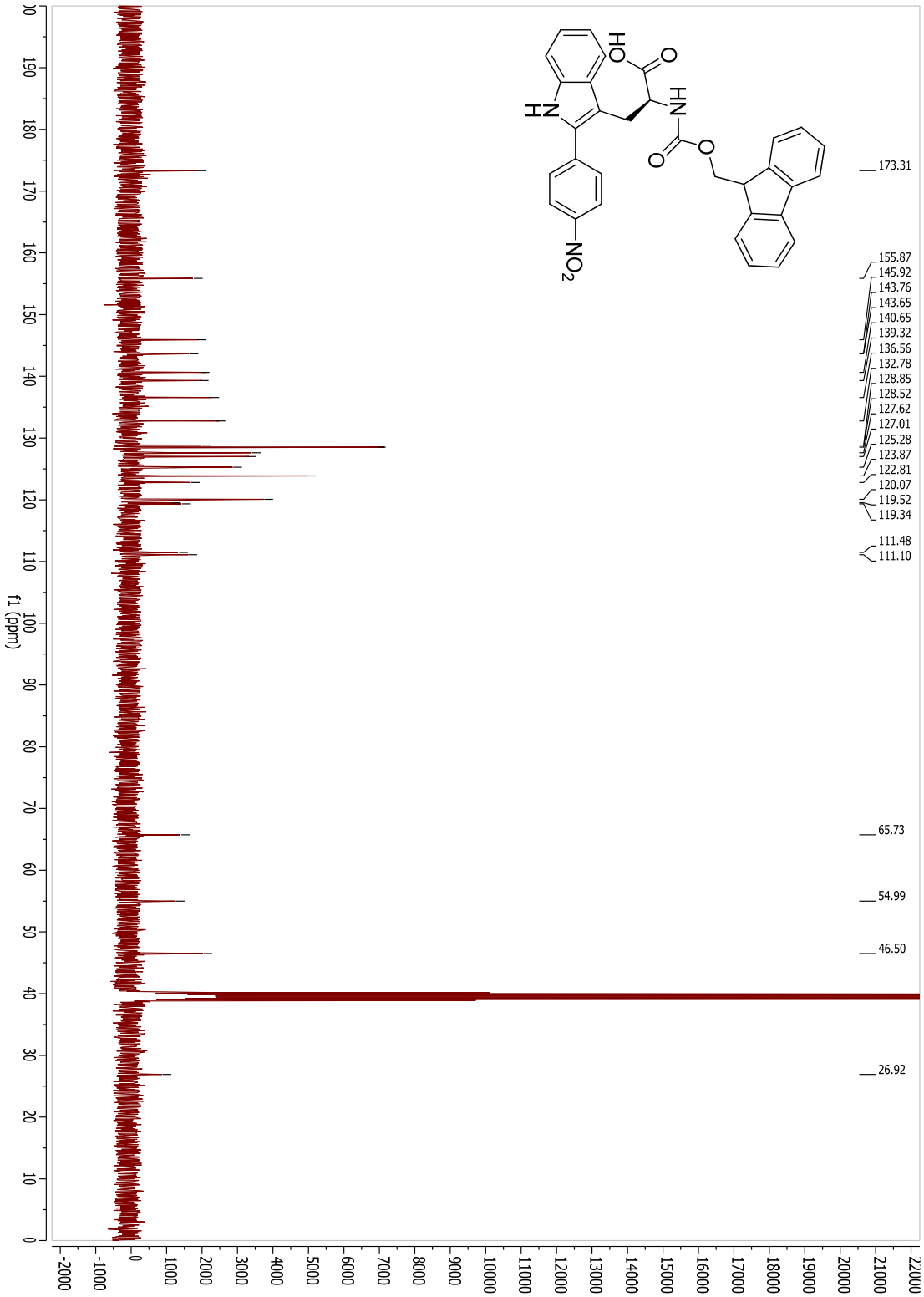
(*R*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-phenyl-1*H*-indol-3-yl)propanoic acid (5a')



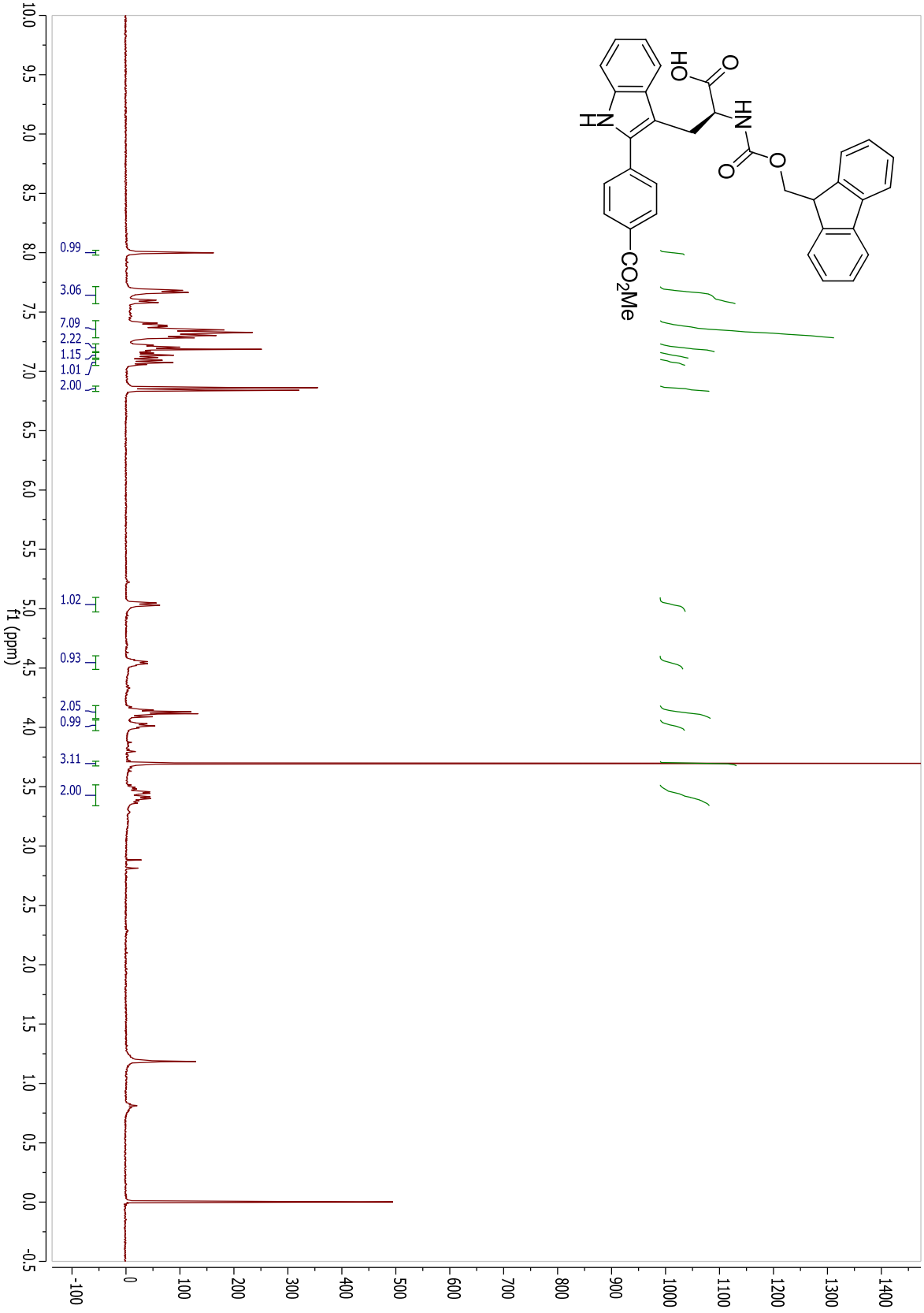

f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-2000
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
22000
24000
26000
28000
30000
32000
HO
O
HN
O
O
HN
173.99
156.32
144.24
144.12
141.07
136.32
135.57
133.18
129.41
129.10
128.40
128.05
128.03
127.83
127.48
125.78
121.89
120.50
119.50
119.22
111.50
108.37
66.20
55.66
46.96
27.45

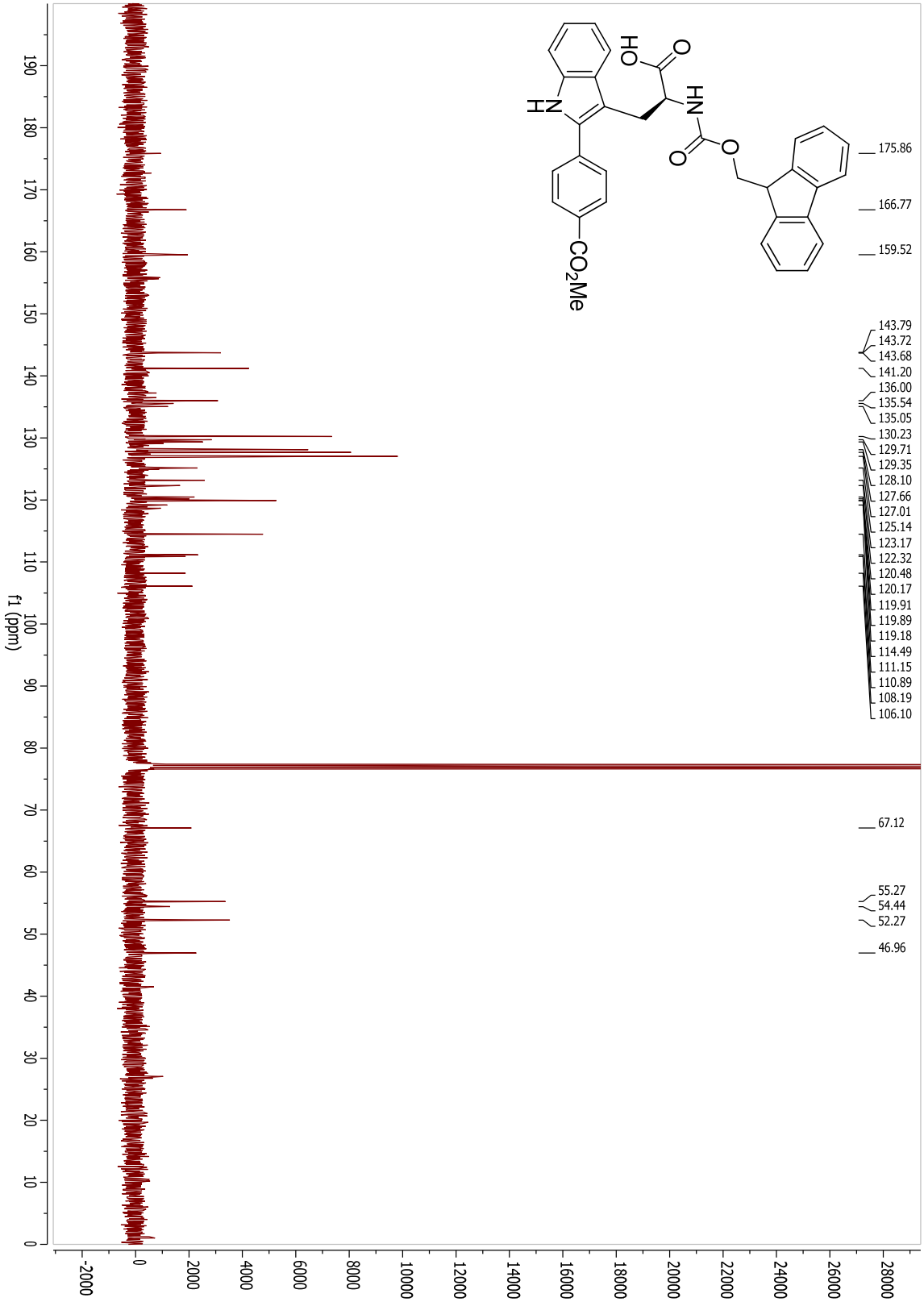
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-(4-nitrophenyl)-1*H*-indol-3-yl)propanoic acid (5b)



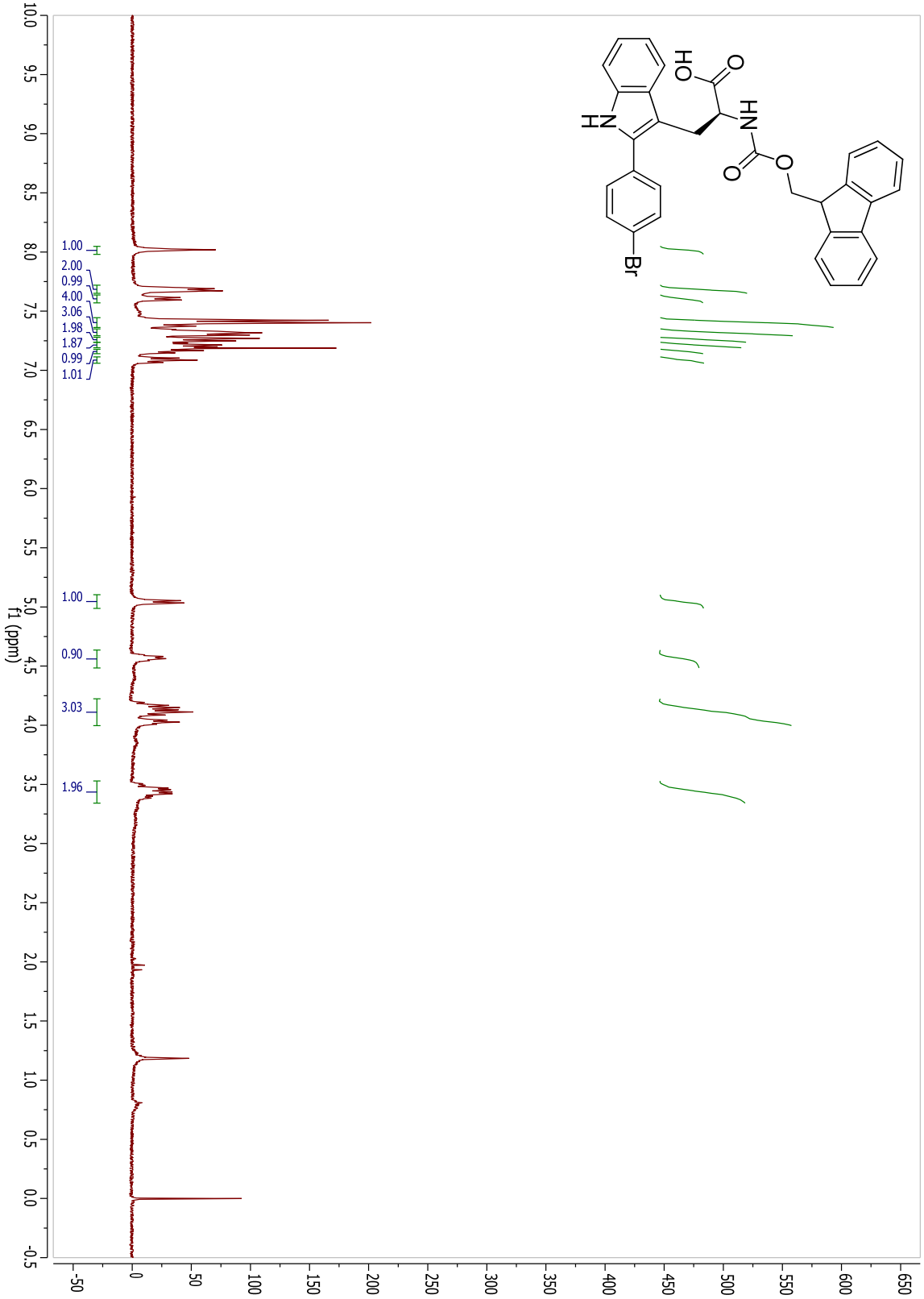

HO
O
HN
O
O
HN
NO2
173.31
155.87
145.92
143.76
143.65
140.65
139.32
136.56
132.78
128.85
128.52
127.62
127.01
125.28
123.87
122.81
120.07
119.52
119.34
111.48
111.10
65.73
54.99
46.50
26.92
f1 (ppm)

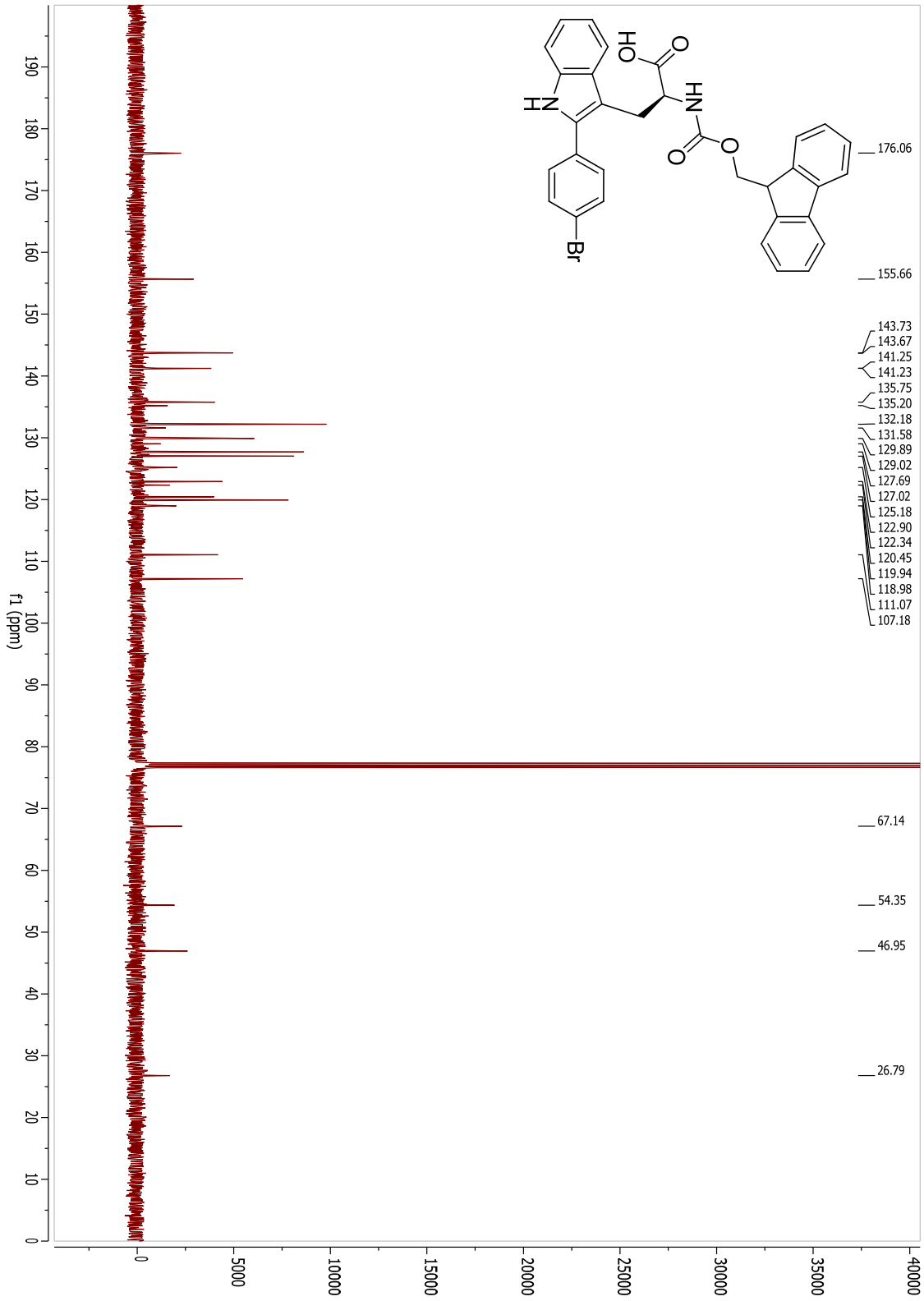
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-(4-(methoxycarbonyl)phenyl)-1*H*-indol-3-yl)propanoic acid (5c)



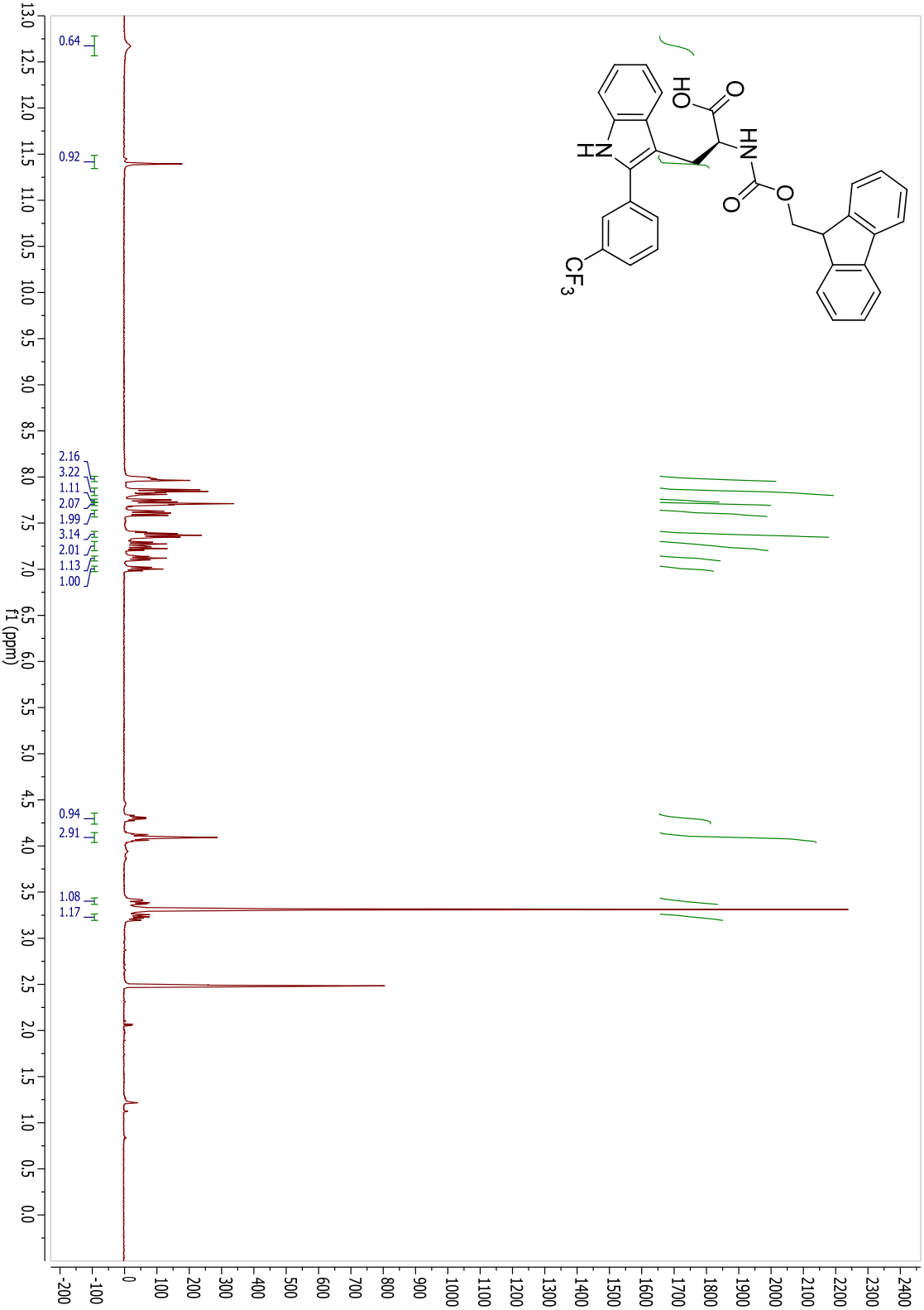

175.86
166.77
159.52
143.79
143.72
143.68
141.20
136.00
135.54
135.05
130.23
129.71
129.35
128.10
127.66
127.01
125.14
123.17
122.32
120.48
120.17
119.91
119.89
119.18
114.49
111.15
110.89
108.19
106.10
67.12
55.27
54.44
52.27
46.96
f1 (ppm)

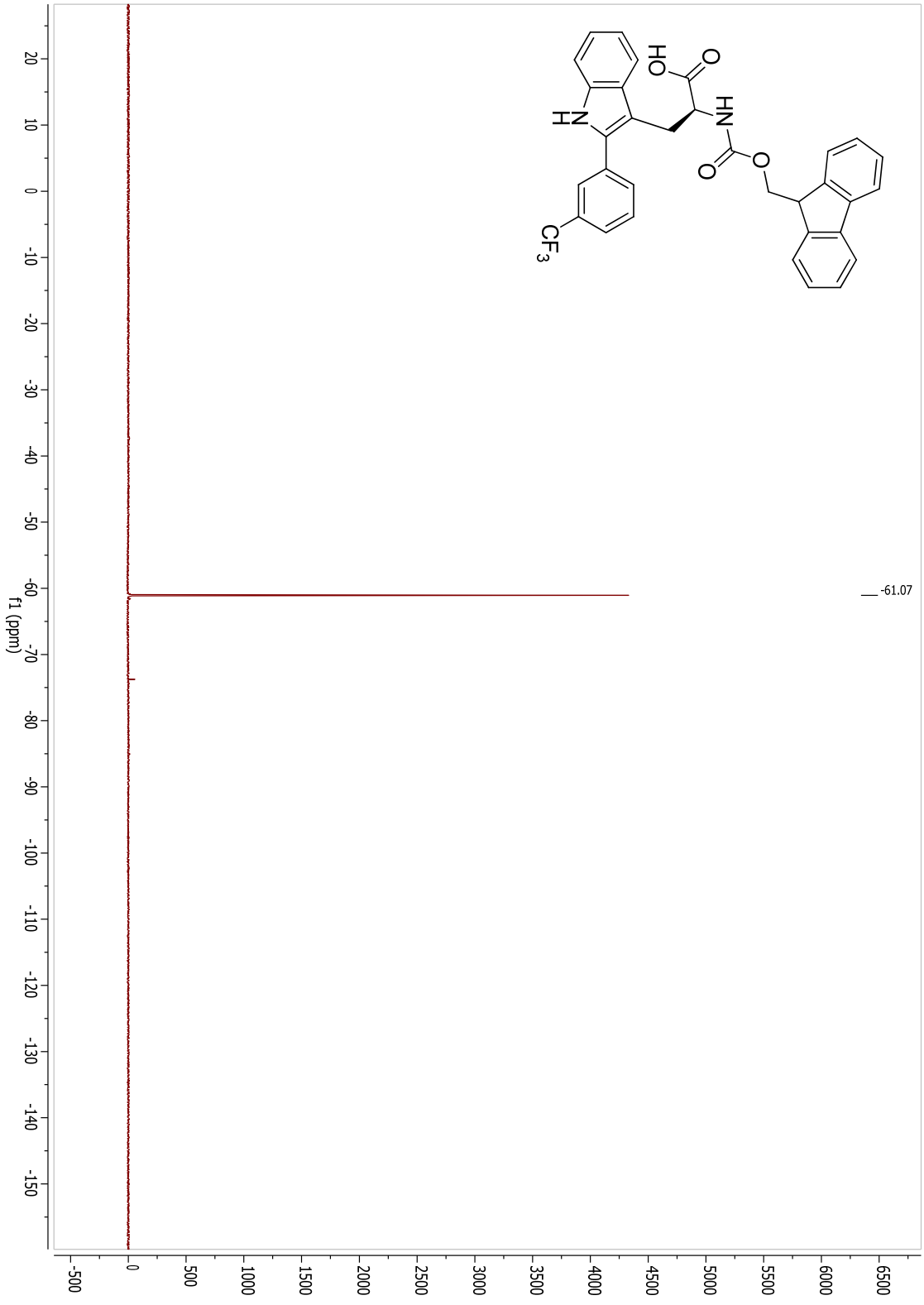
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-(4-bromophenyl)-1*H*-indol-3-yl)propanoic acid (5d)

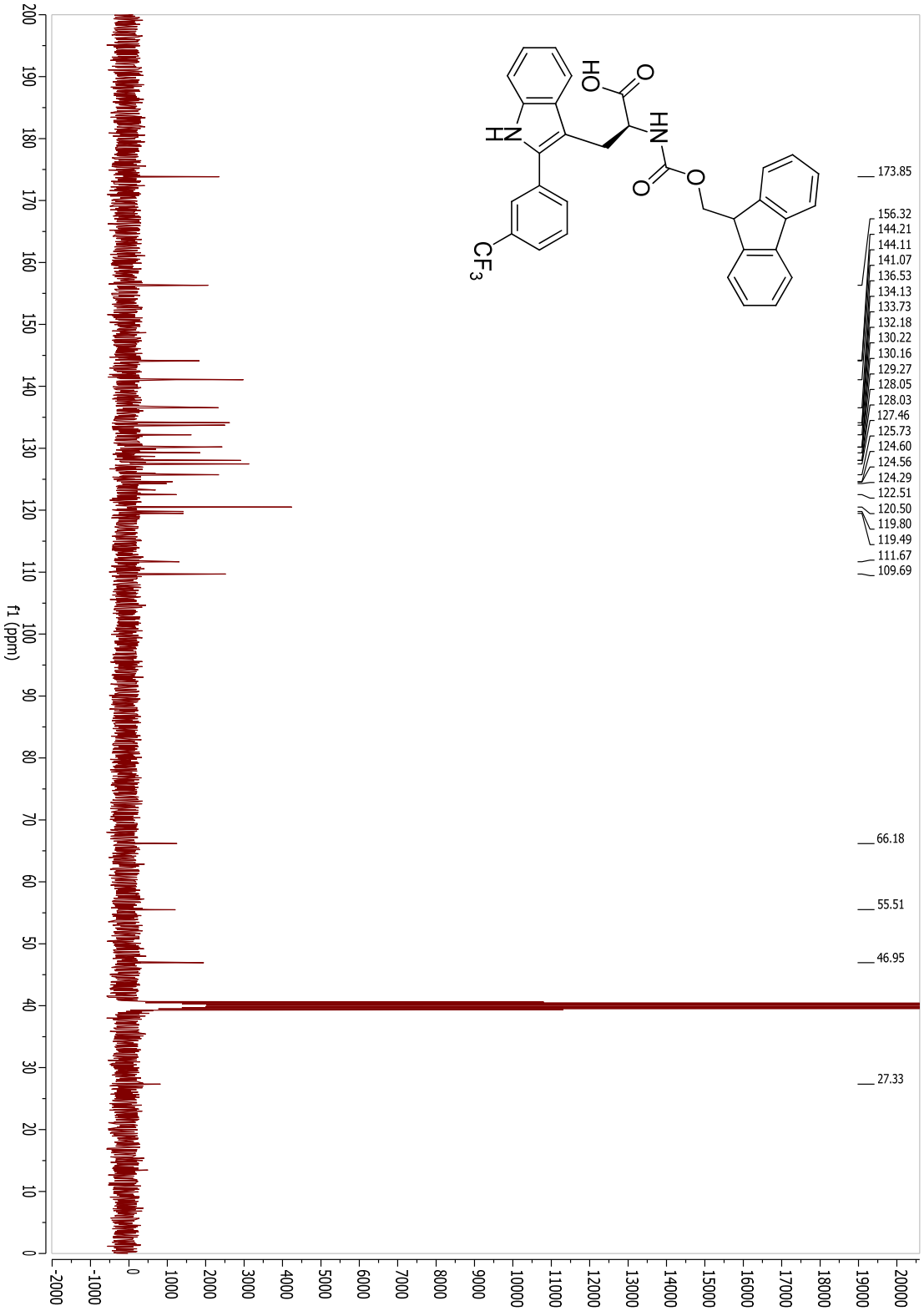



f1 (ppm)
176.06
155.66
143.73
143.67
141.25
141.23
135.75
135.20
132.18
131.58
129.89
129.02
127.69
127.02
125.18
122.90
122.34
120.45
119.94
118.98
111.07
107.18
67.14
54.35
46.95
26.79
HO
O
HN
O
O
HN
Br

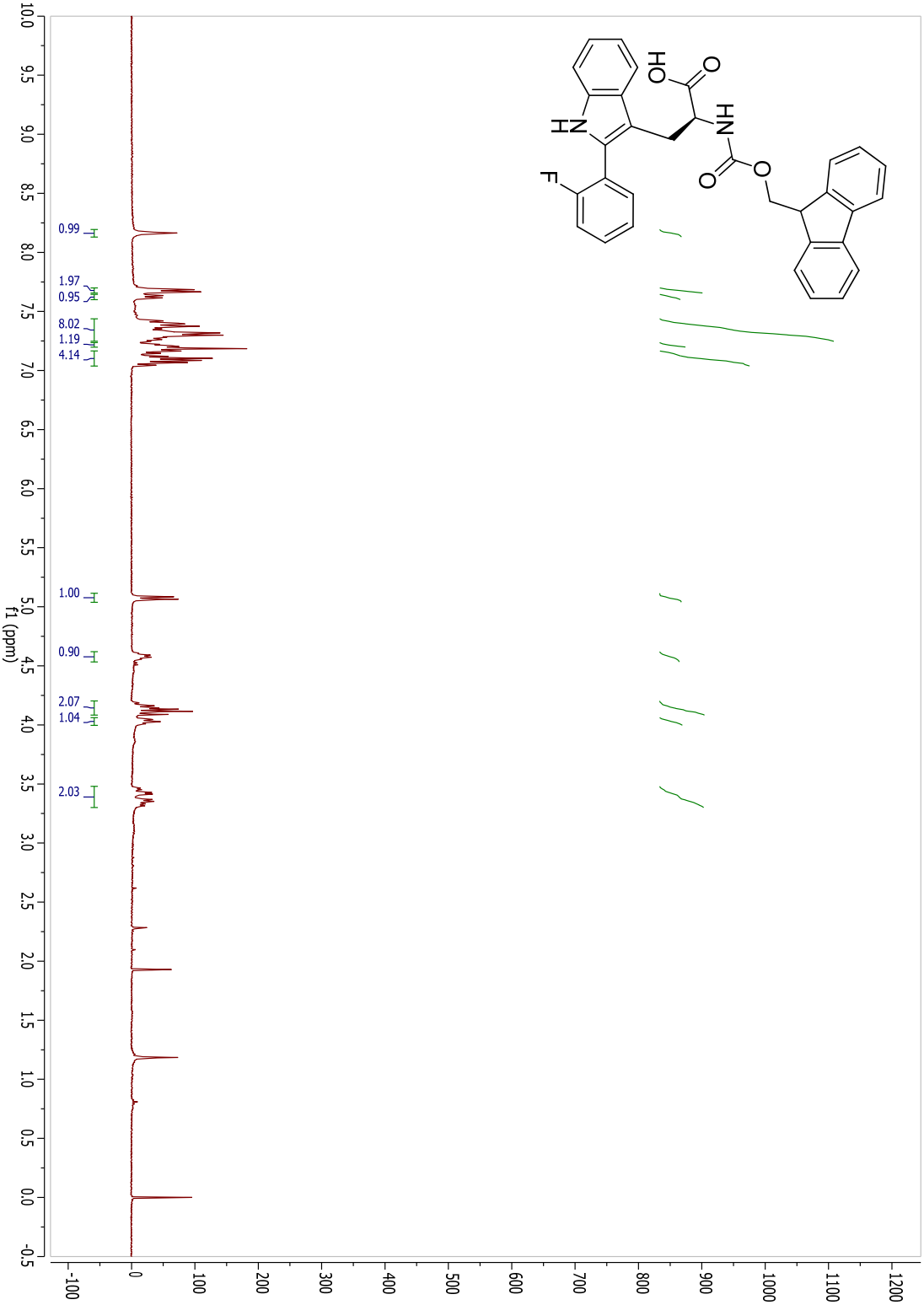
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-(3-(trifluoromethyl)phenyl)-1*H*-indol-3-yl)propanoic acid (5e)

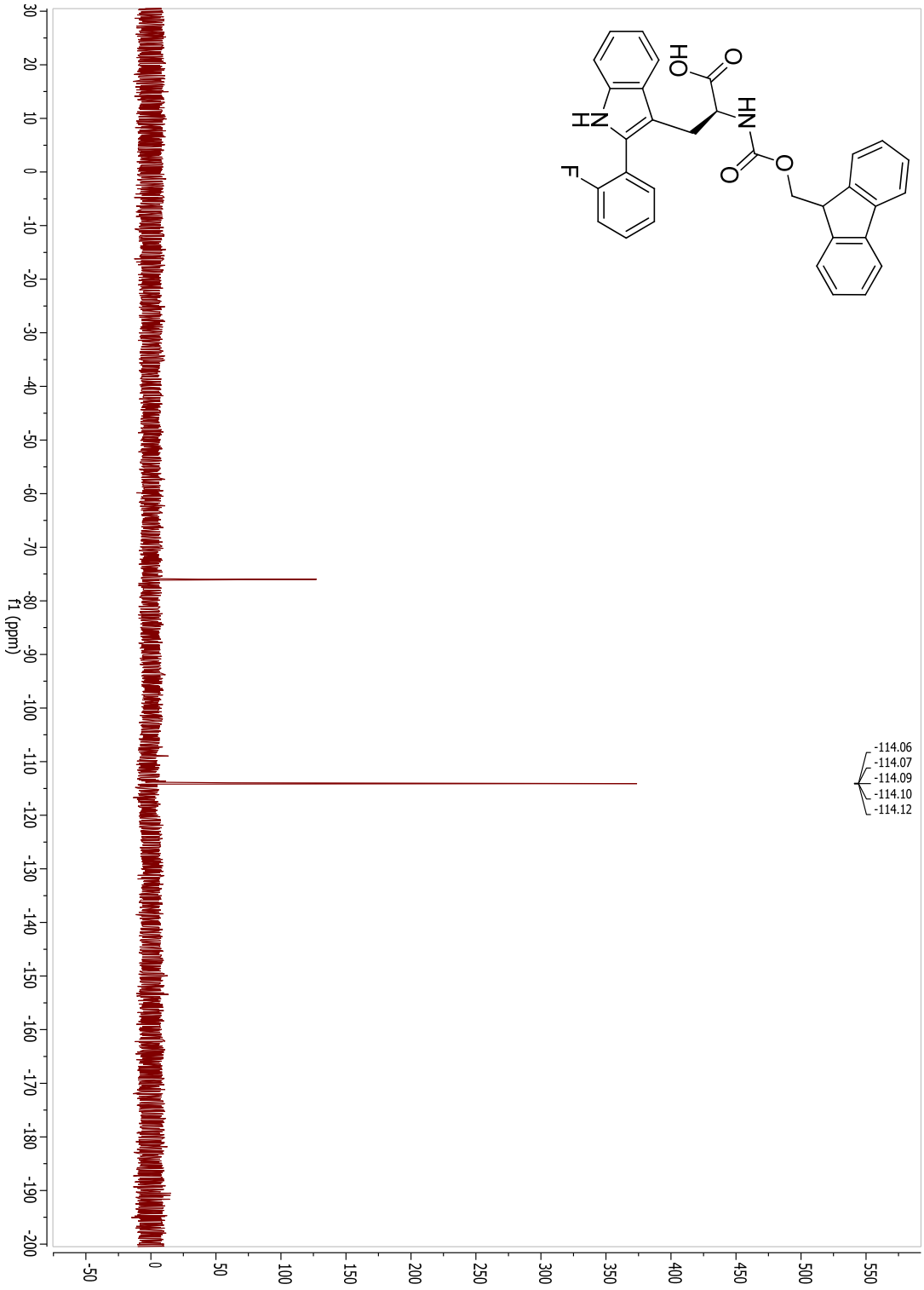


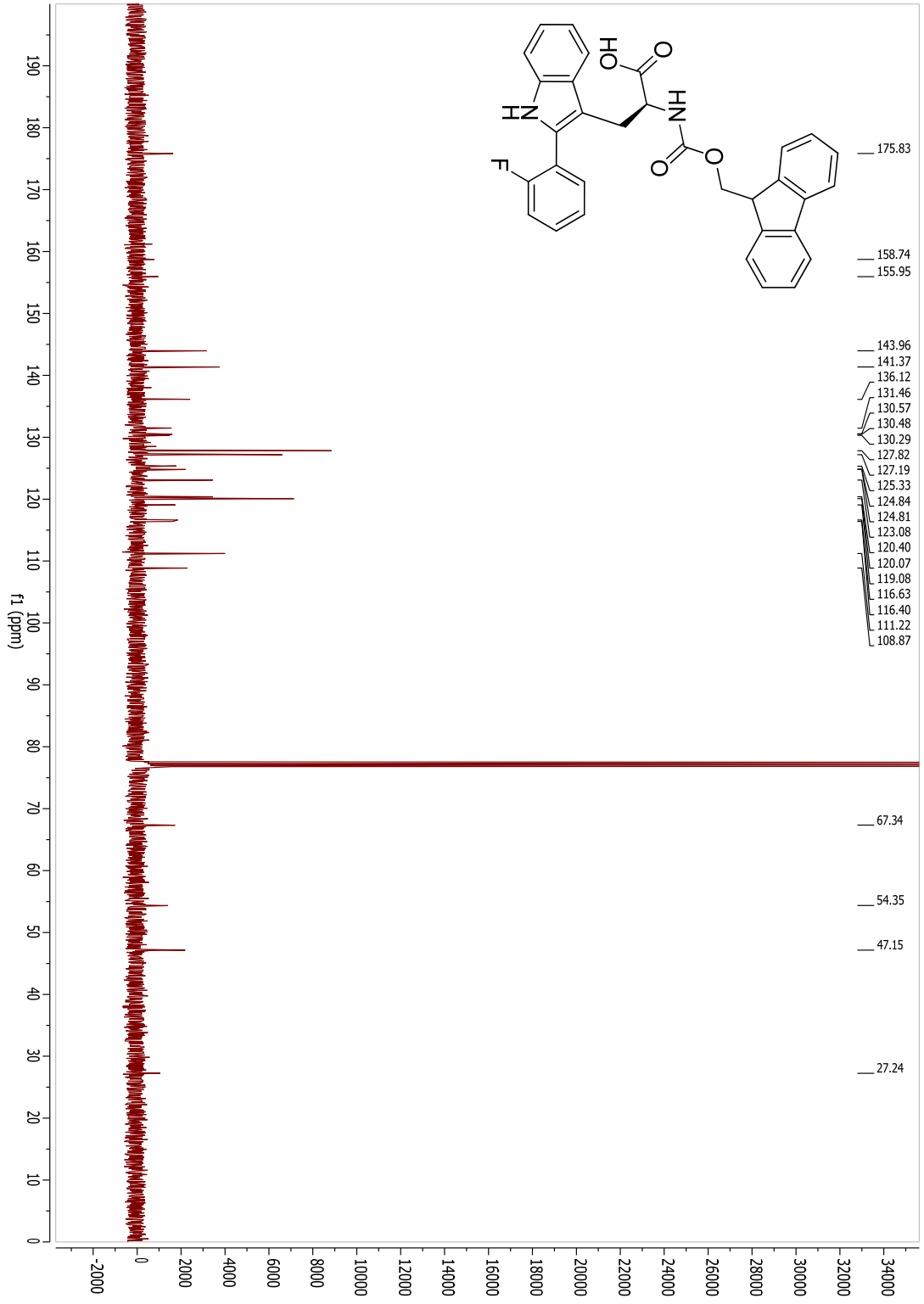

-61.07
f1 (ppm)


HO
O
HN
O
O
N
H
CF3
173.85
156.32
144.21
144.11
141.07
136.53
134.13
133.73
132.18
130.22
130.16
129.27
128.05
128.03
127.46
125.73
124.60
124.56
124.29
122.51
120.50
119.80
119.49
111.67
109.69
66.18
55.51
46.95
27.33
f1 (ppm)

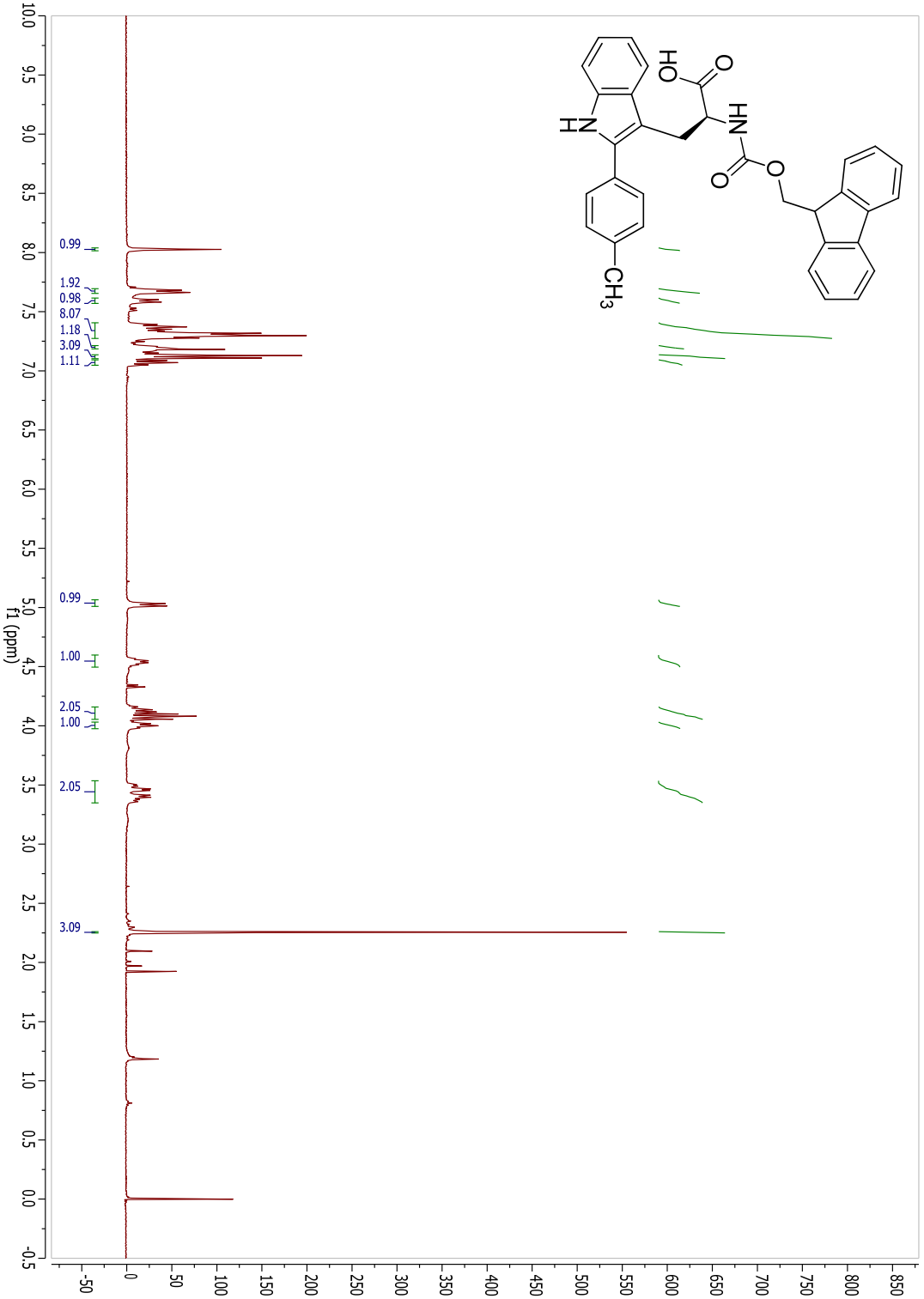
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-(2-fluorophenyl)-1*H*-indol-3-yl)propanoic acid (5f)

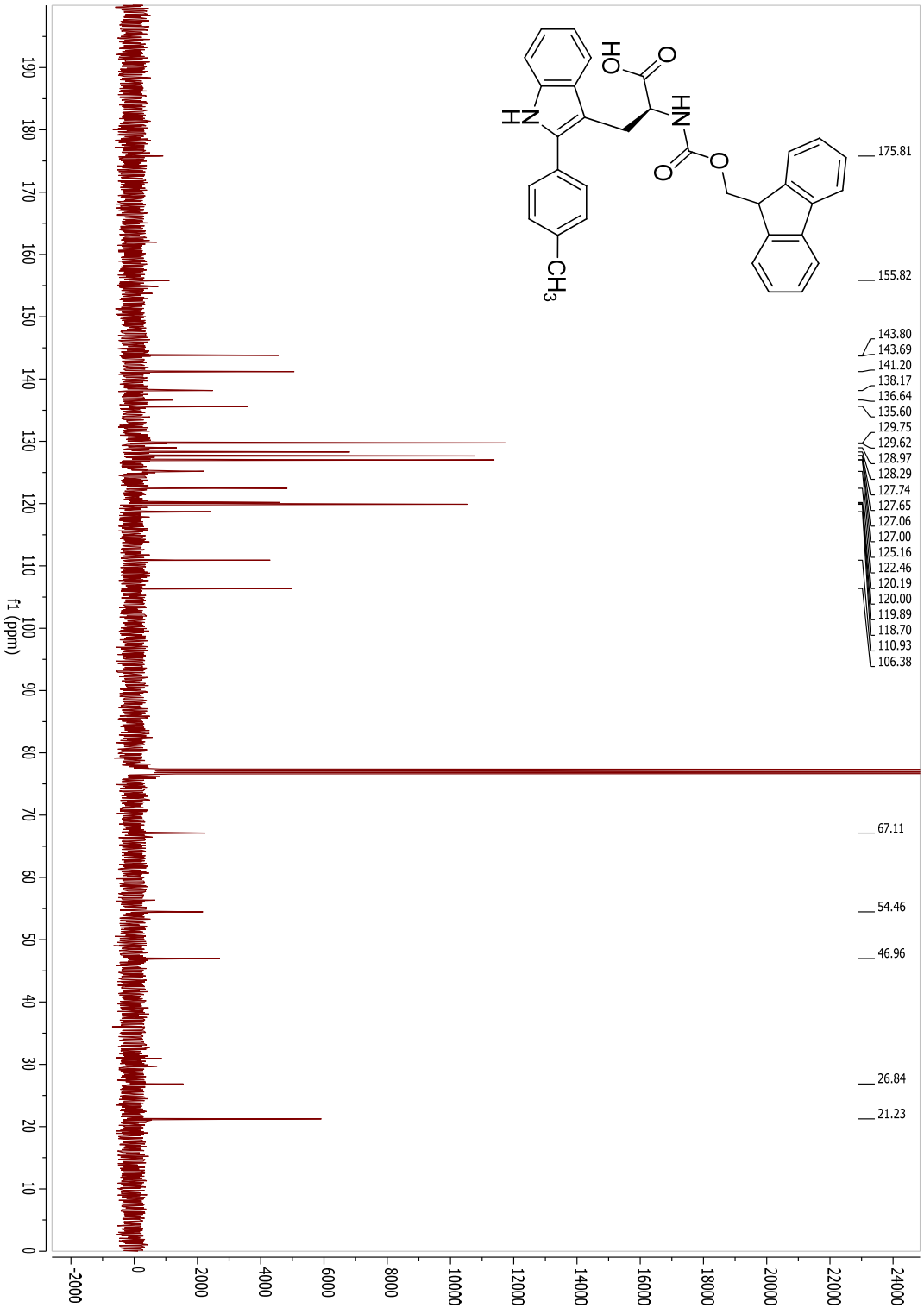



f1 (ppm)
-114.06
-114.07
-114.09
-114.10
-114.12

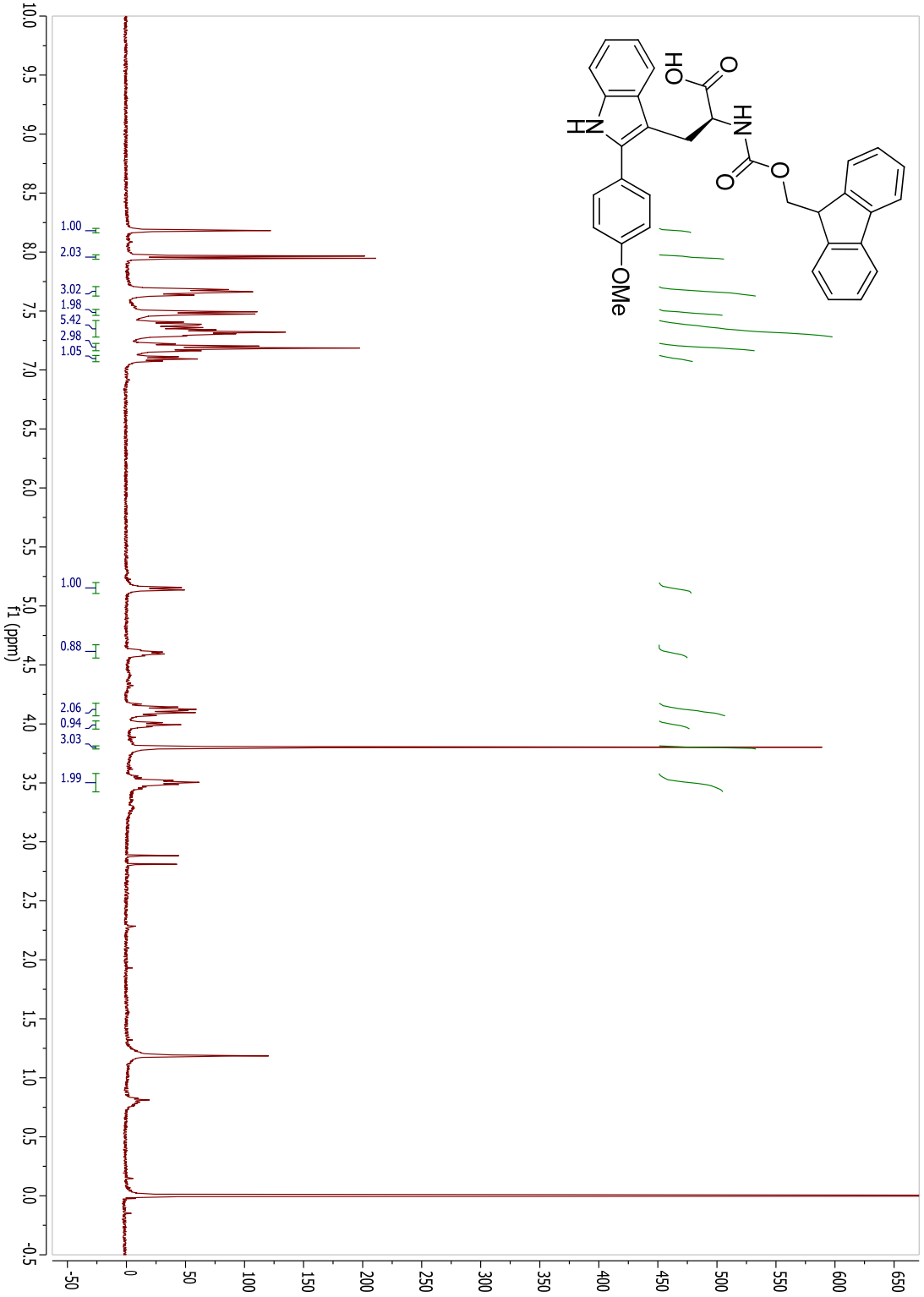

175.83
158.74
155.95
143.96
141.37
136.12
131.46
130.57
130.48
130.29
127.82
127.19
125.33
124.84
124.81
123.08
120.40
120.07
119.08
116.63
116.40
111.22
108.87
67.34
54.35
47.15
27.24
f1 (ppm)

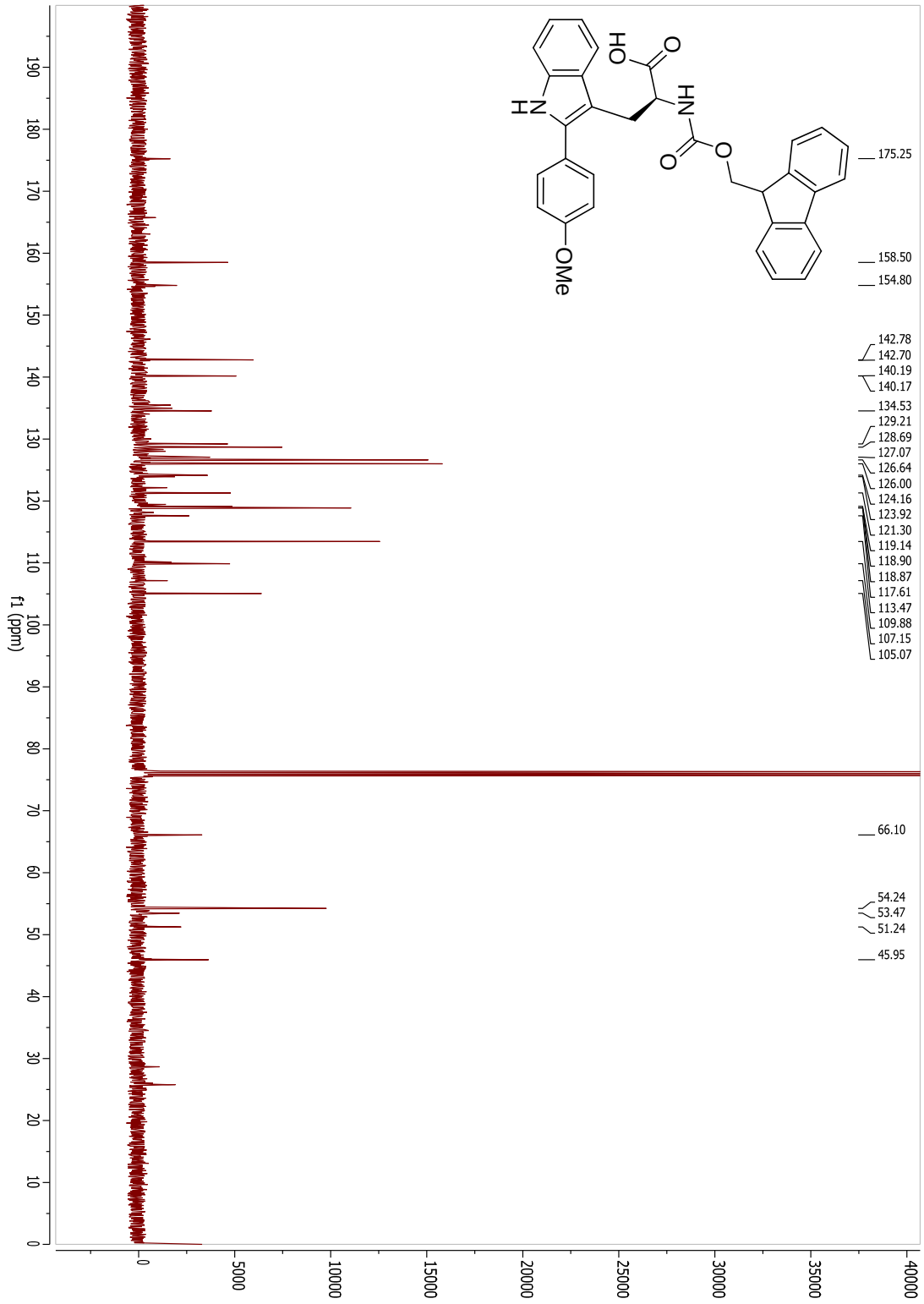
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-(*p*-tolyl)-1*H*-indol-3-yl)propanoic acid (5g)



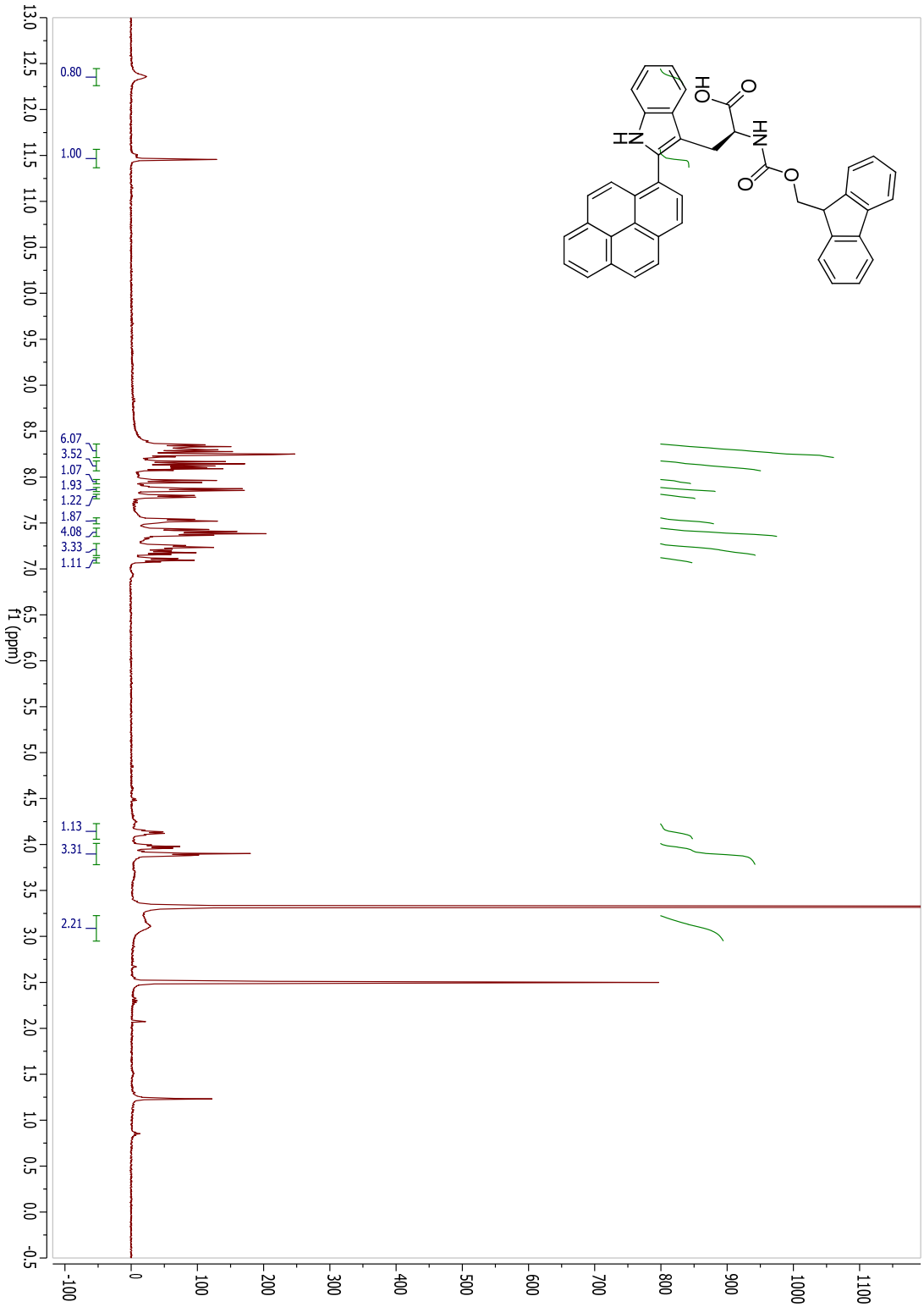

175.81
155.82
143.80
143.69
141.20
138.17
136.64
135.60
129.75
129.62
128.97
128.29
127.74
127.65
127.06
127.00
125.16
122.46
120.19
120.00
119.89
118.70
110.93
106.38
67.11
54.46
46.96
26.84
21.23
f1 (ppm)

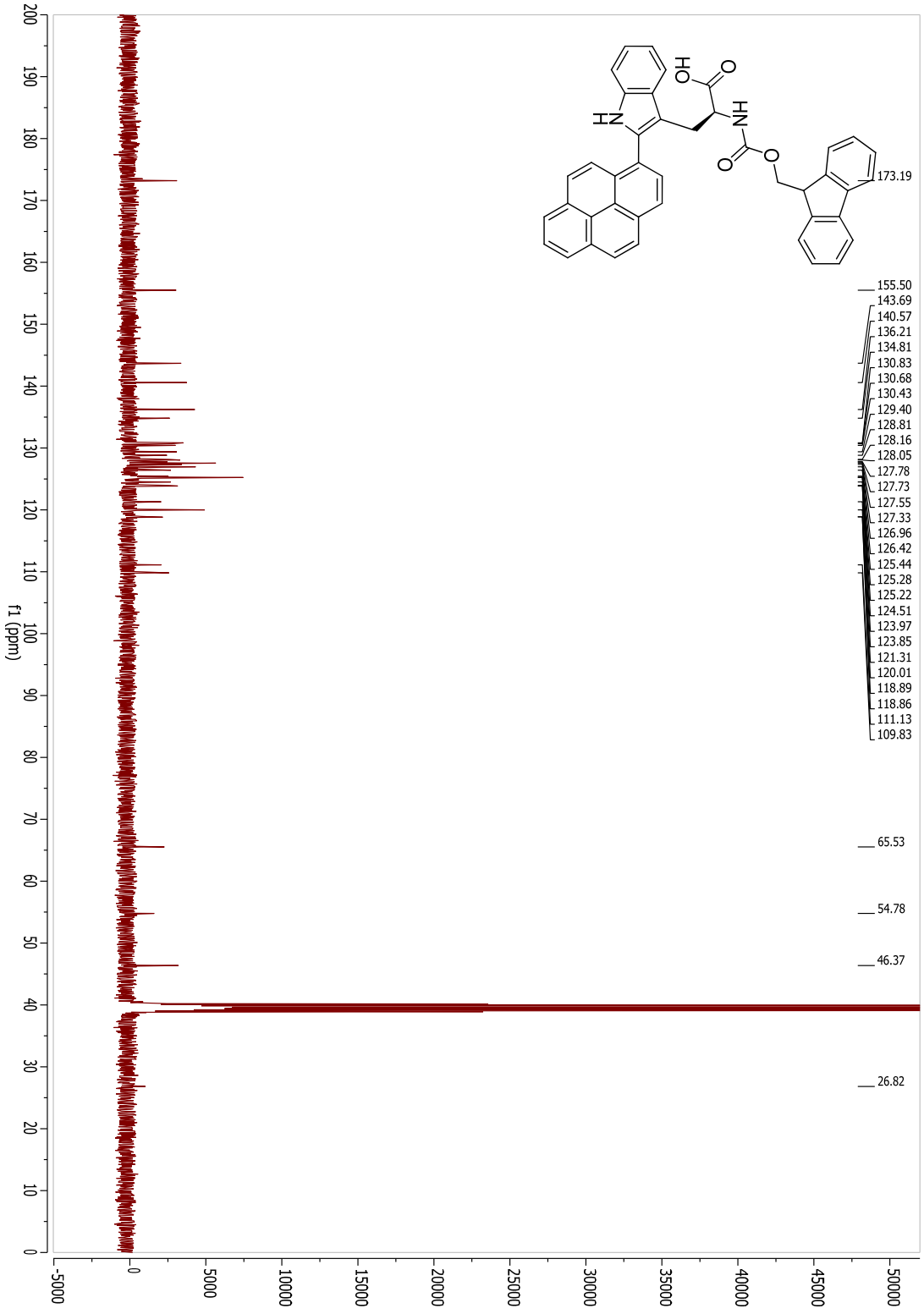
(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-(4-methoxyphenyl)-1*H*-indol-3-yl)propanoic acid (5h)



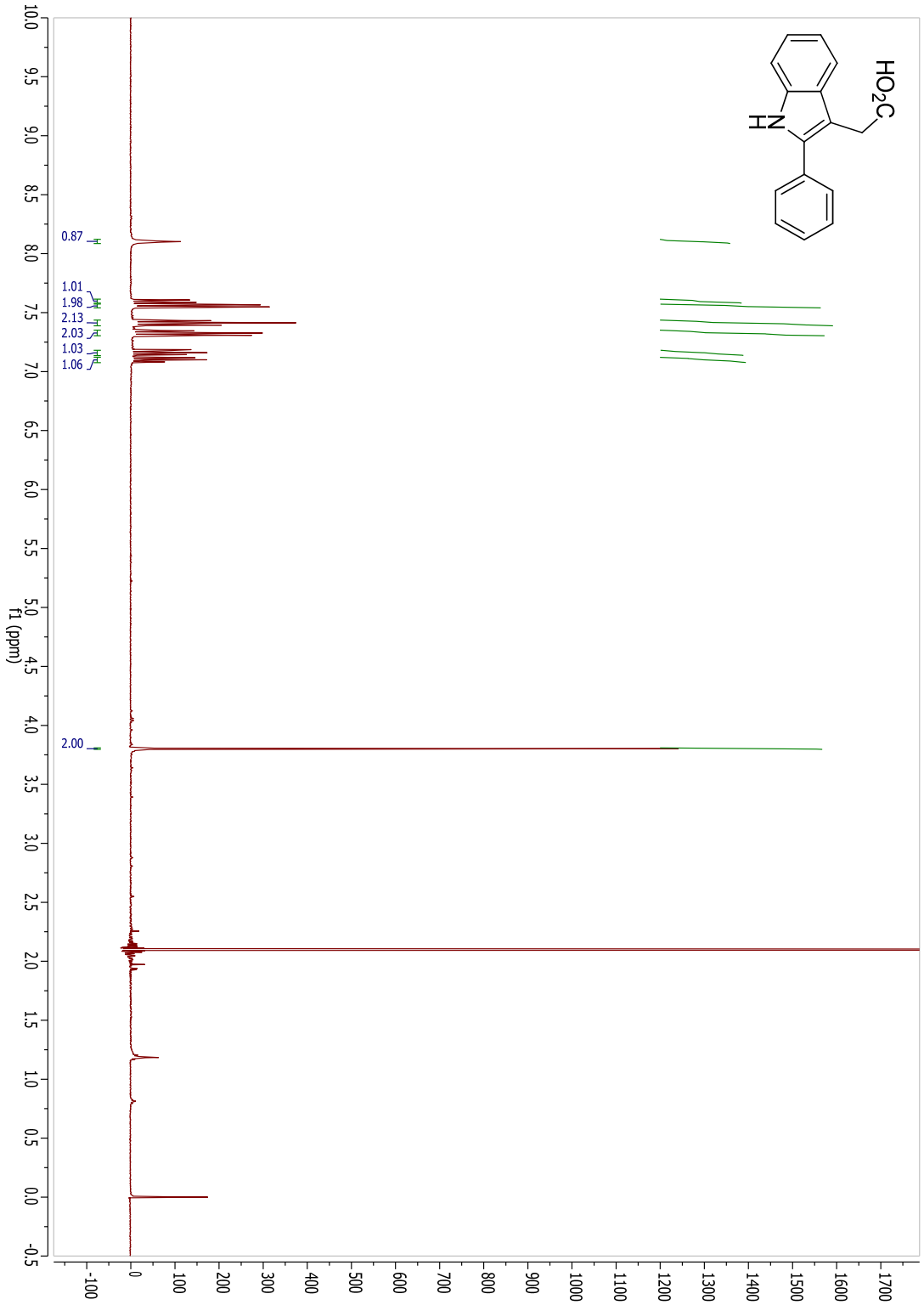

HO
O
HN
O
O
HN
OMe
175.25
158.50
154.80
142.78
142.70
140.19
140.17
134.53
129.21
128.69
127.07
126.64
126.00
124.16
123.92
121.30
119.14
118.90
118.87
117.61
113.47
109.88
107.15
105.07
66.10
54.24
53.47
51.24
45.95
f1 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
0
5000
10000
15000
20000
25000
30000
35000
40000

(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-(pyren-1-yl)-1*H*-indol-3-yl)propanoic acid (5i)

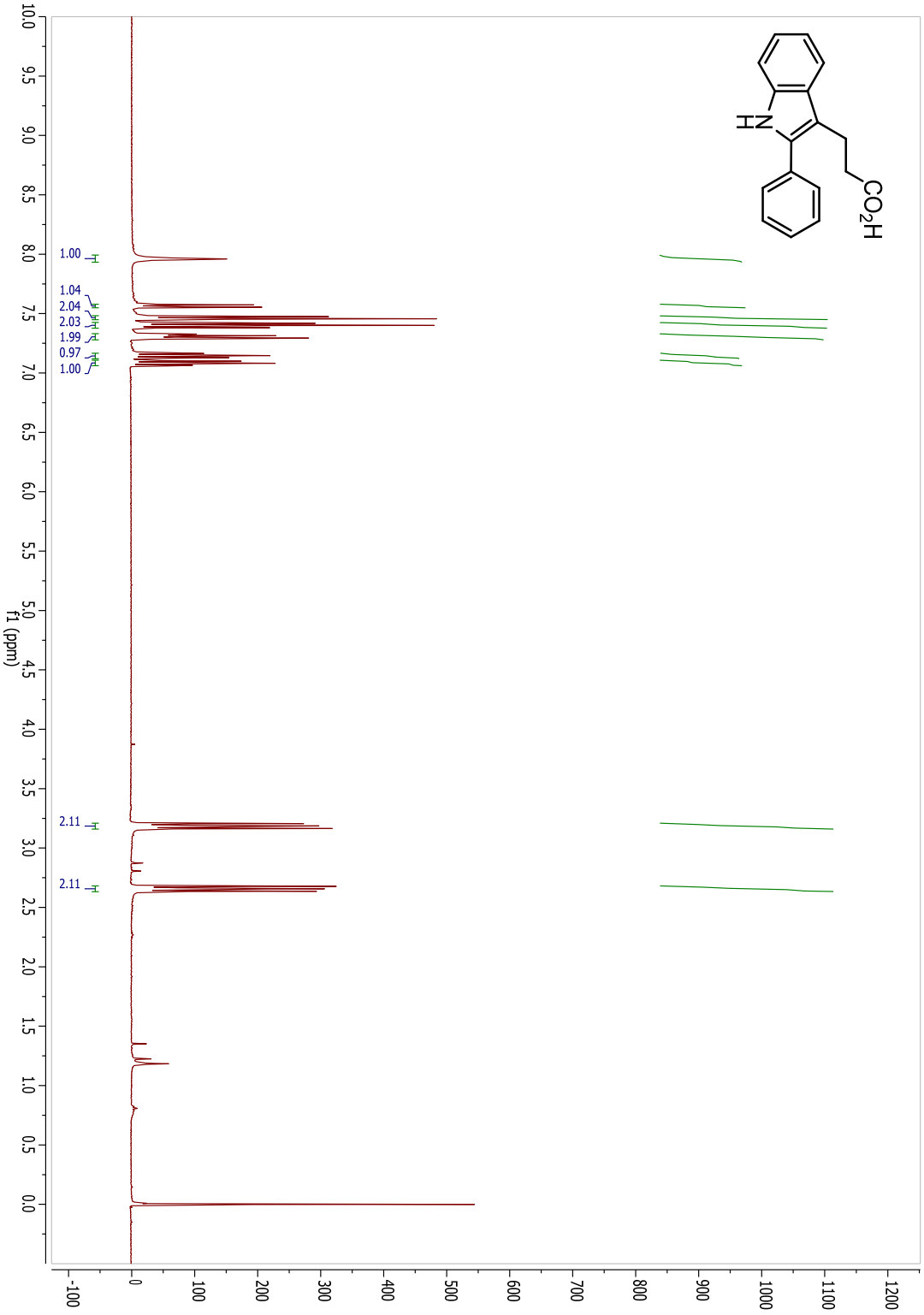



173.19
155.50
143.69
140.57
136.21
134.81
130.83
130.68
130.43
129.40
128.81
128.16
128.05
127.78
127.73
127.55
127.33
126.96
126.42
125.44
125.28
125.22
124.51
123.97
123.85
121.31
120.01
118.89
118.86
111.13
109.83
65.53
54.78
46.37
26.82
f1 (ppm)

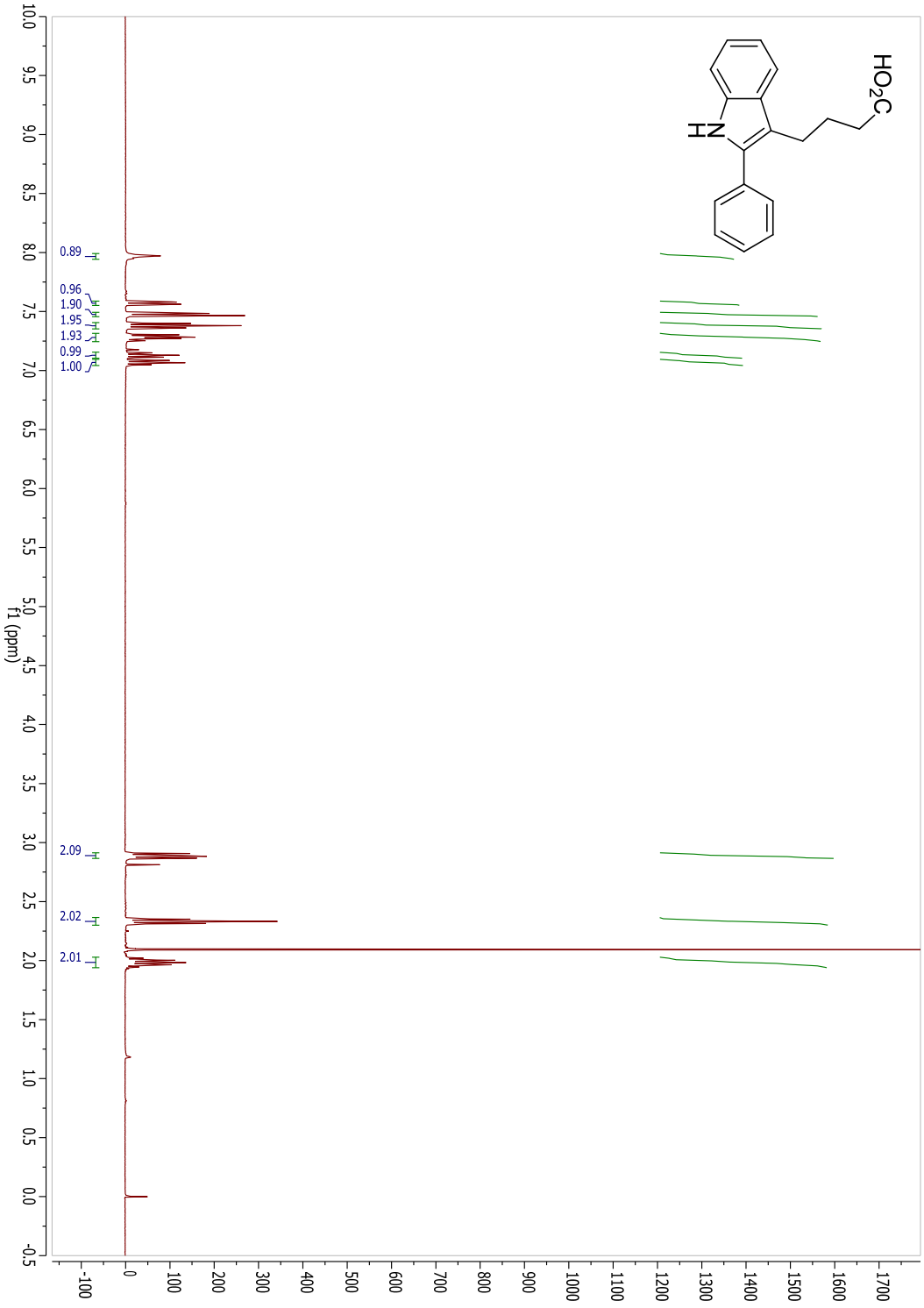
2-(2-Phenyl-1*H*-indol-3-yl)acetic acid (8)



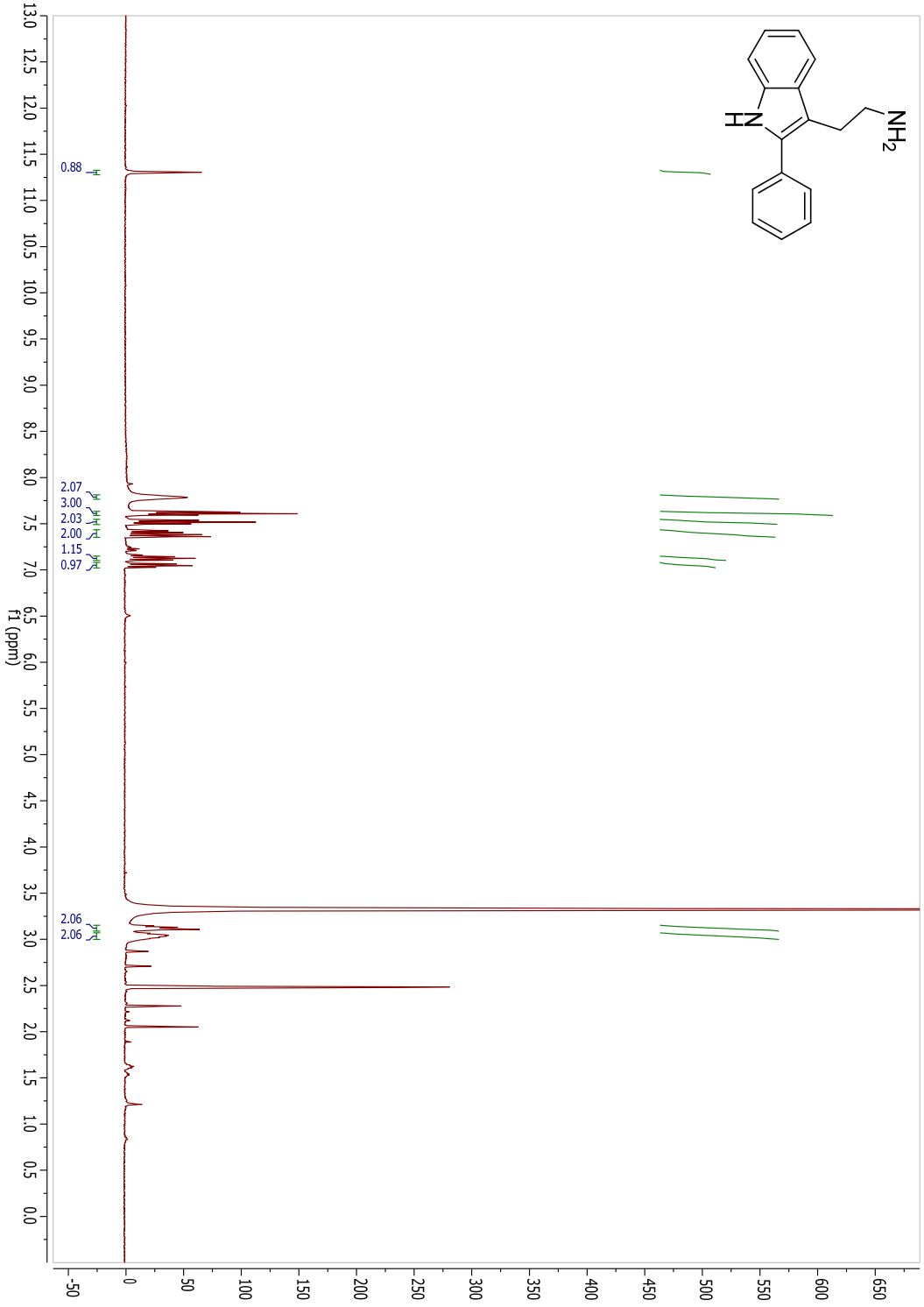
3-(2-Phenyl-1*H*-indol-3-yl)propanoic acid (9)



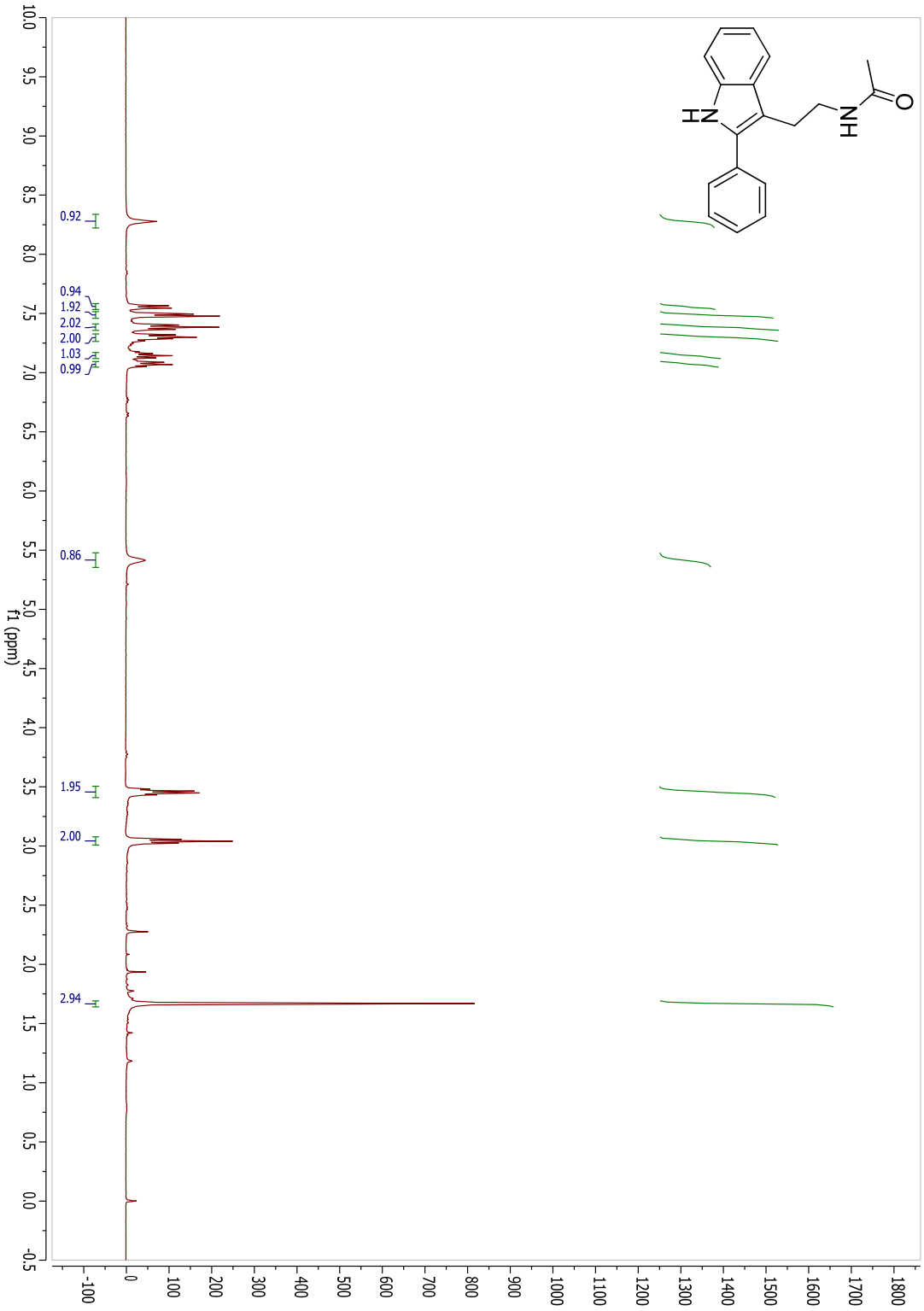
4-(2-Phenyl-1*H*-indol-3-yl)butanoic acid (10)



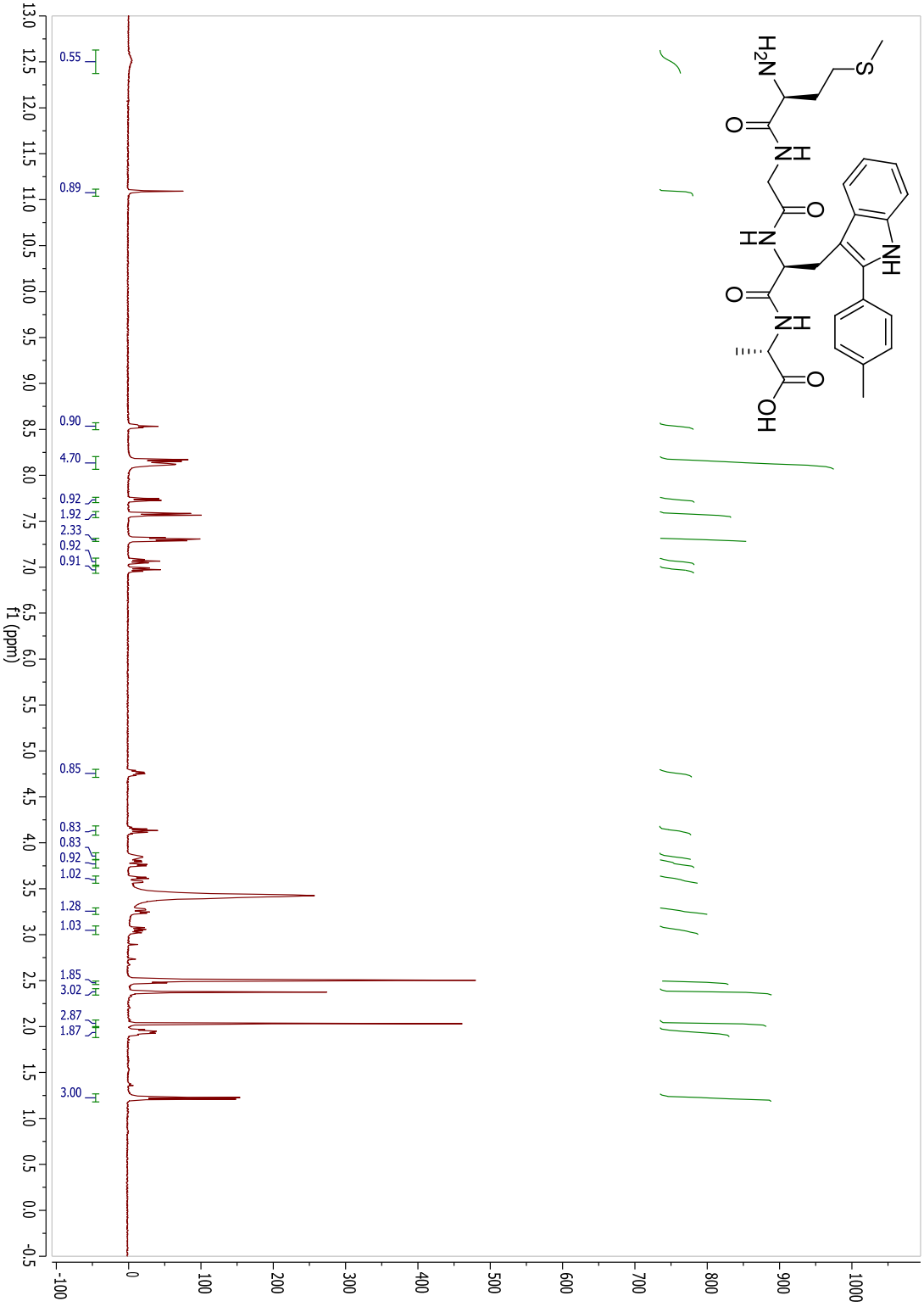
2-(2-Phenyl-1*H*-indol-3-yl)ethanamine (11)

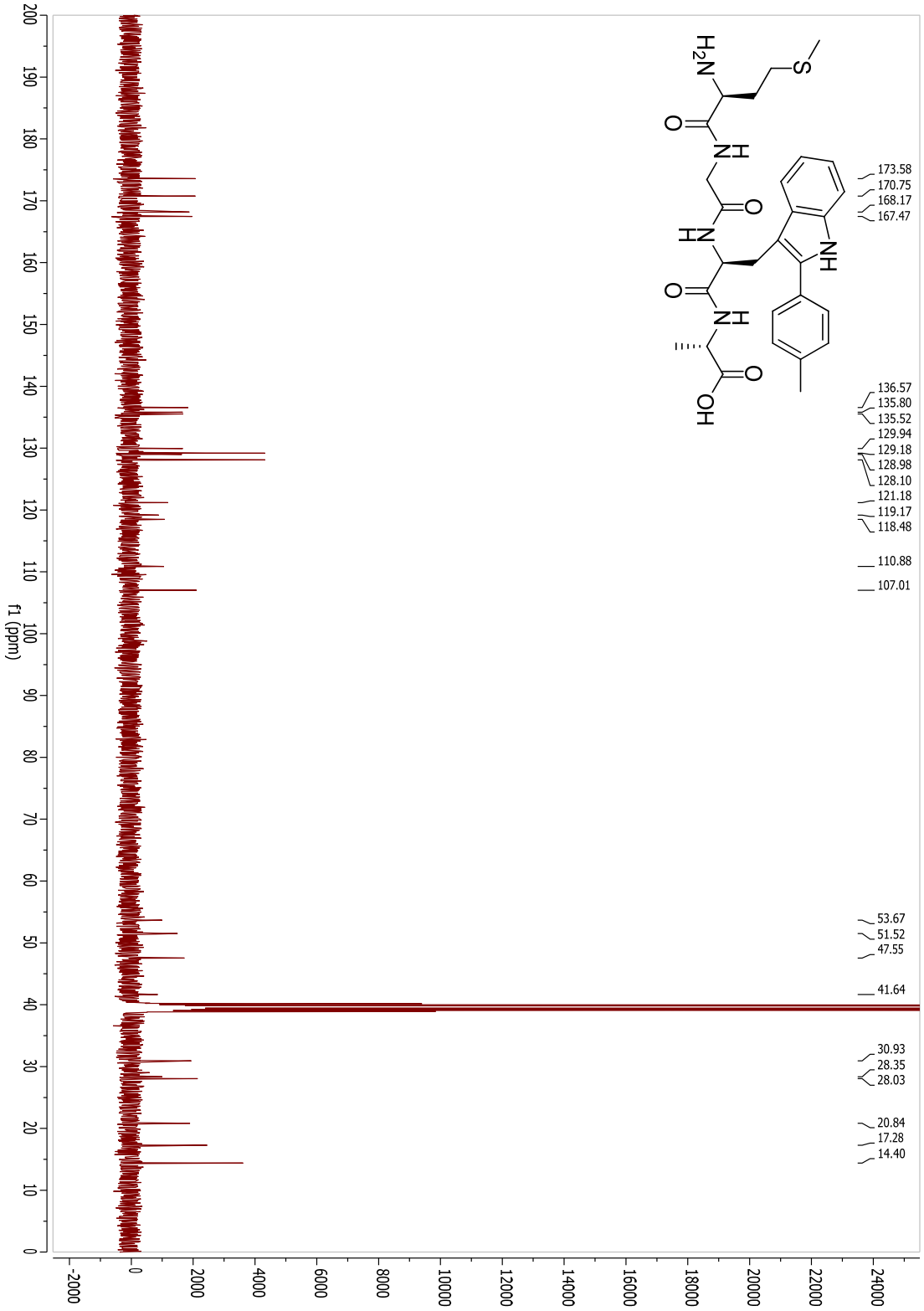


***N*-(2-(2-Phenyl-1*H*-indol-3-yl)ethyl)acetamide (12)**



H-Met-Gly-Trp(C2-*p*-methylphenyl)-Ala-OH (6)

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167.47
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129.18
128.98
128.10
121.18
119.17
118.48
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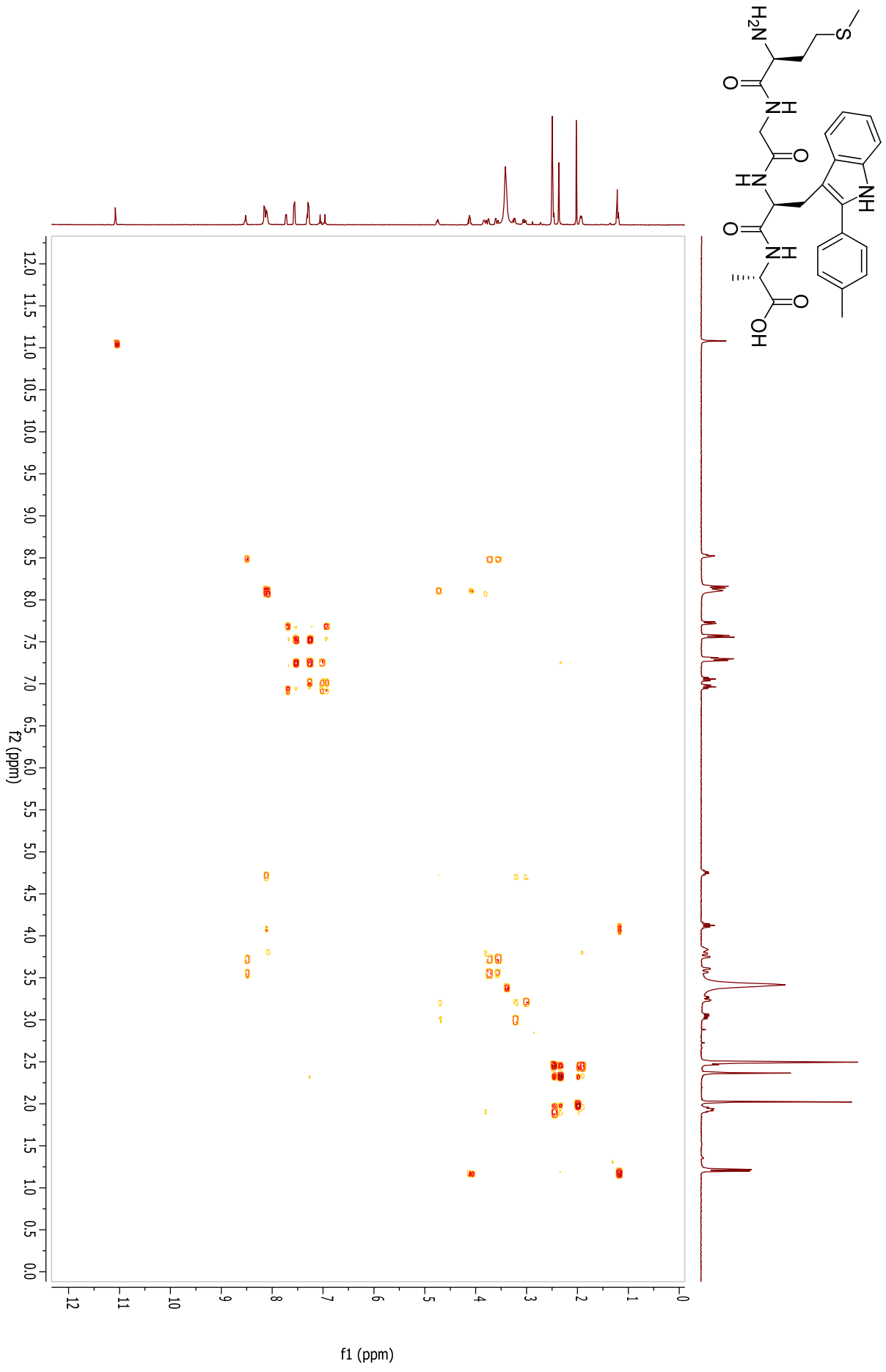
107.01
53.67
51.52
47.55
41.64
30.93
28.35
28.03
20.84
17.28
14.40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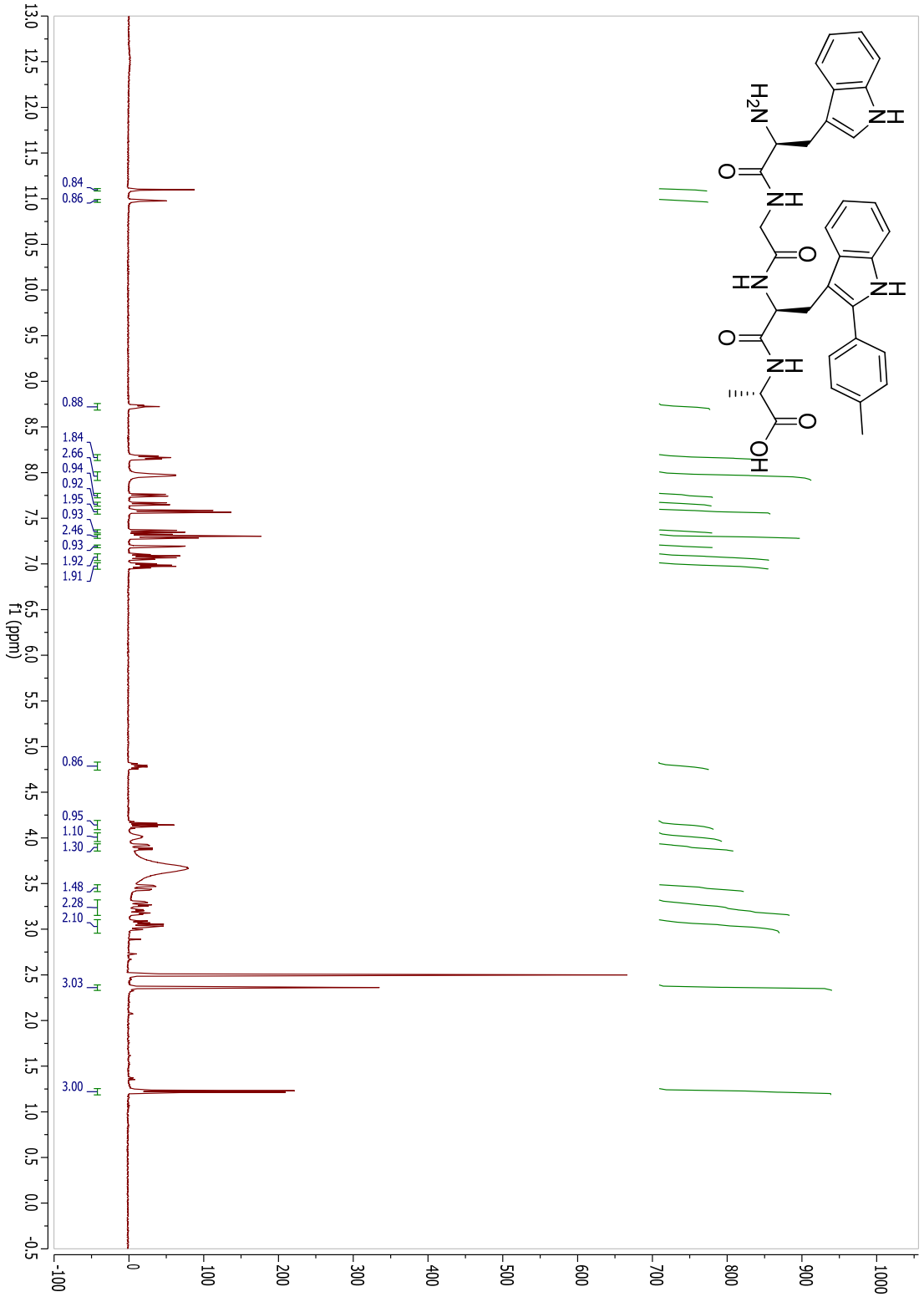
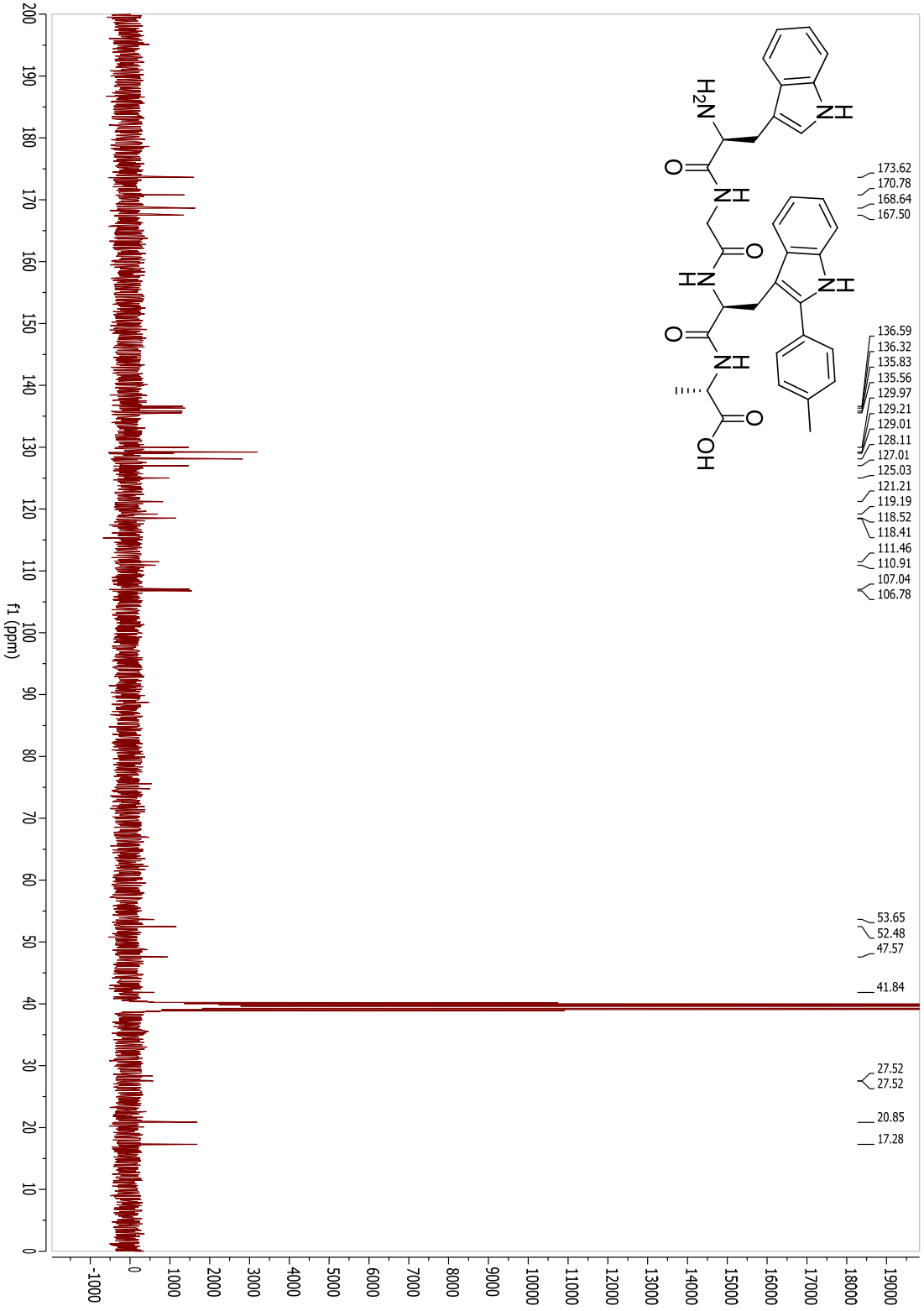
f1 (ppm)

H-Trp-Gly-Trp(C2-*p*-methylphenyl)-Ala-OH (7)

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127.01
125.03
121.21
119.19
118.52
118.41
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47.57
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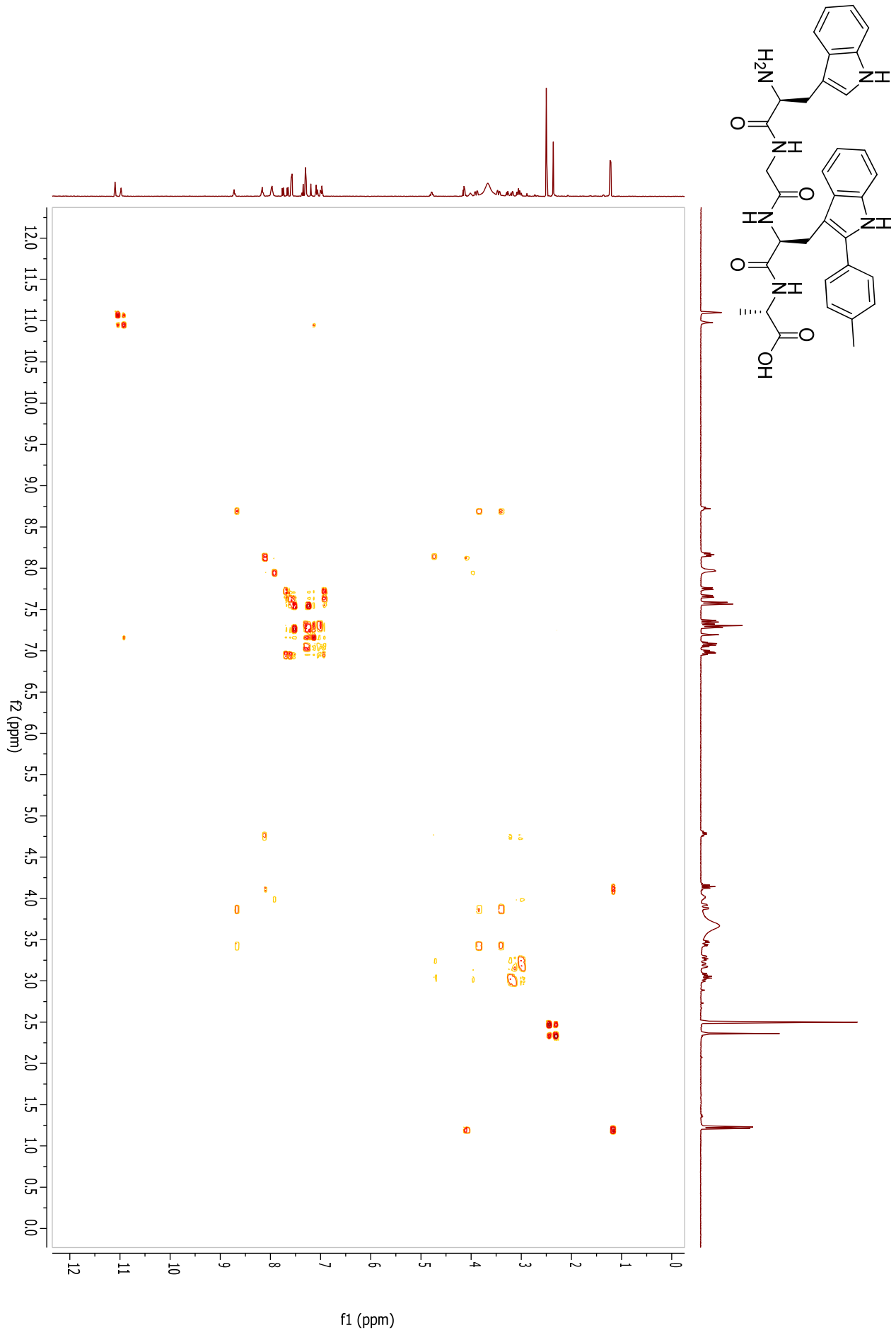
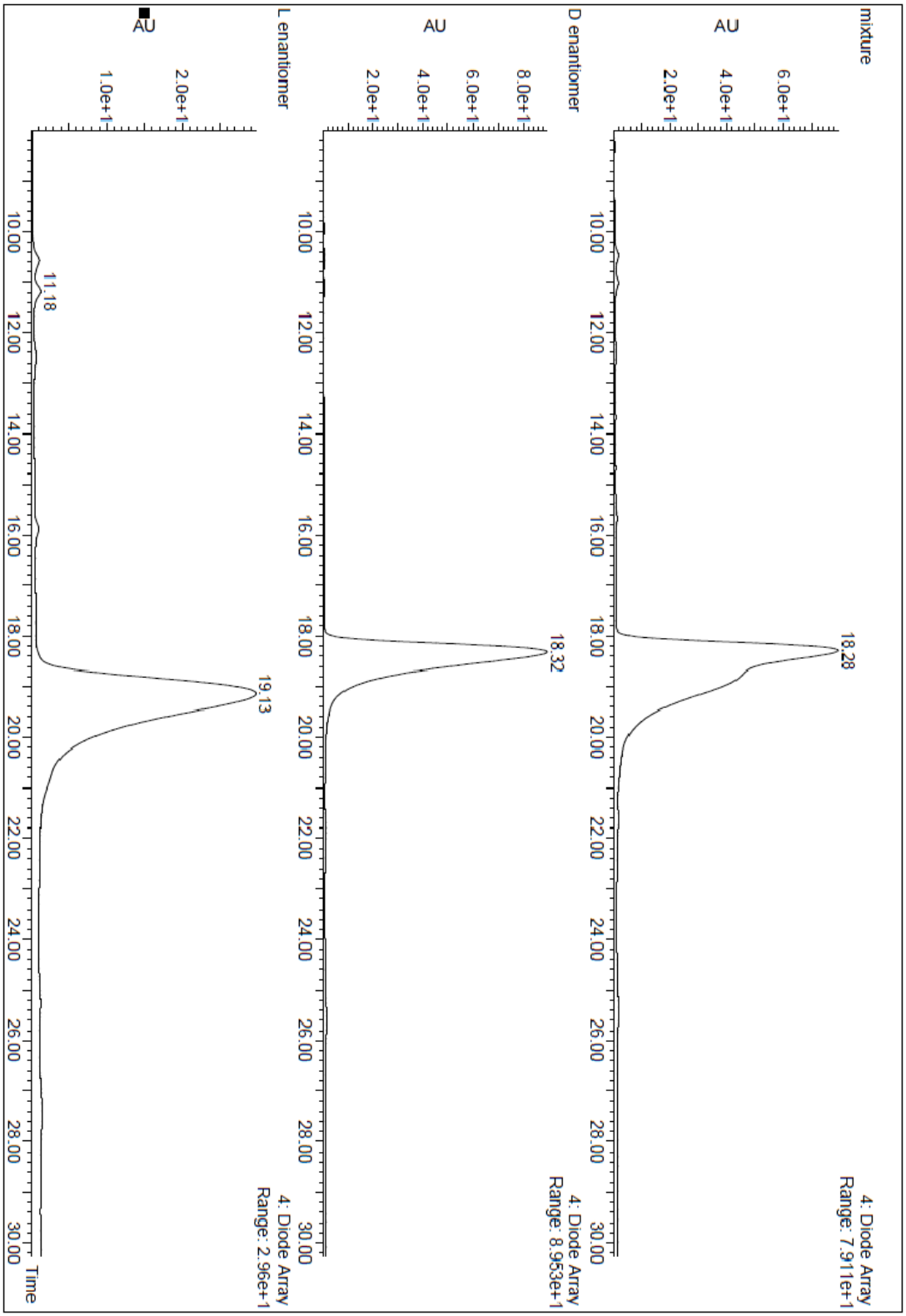
27.52
27.52
20.85
17.28


f2 (ppm)
f1 (ppm)

Chromatographic analysis of 5a and 5a'.

Chiral column HPLC:



Electronic Supplementary Information

Enhanced antimicrobial activity of a peptide derived from human lysozyme by arylation of its tryptophan residues

Rodrigo González, Lorena Mendive-Tapia, María B. Pastrian, Fernando Albericio, Rodolfo Lavilla Osvaldo Cascone and Nancy B. Iannucci

General experimental information

Commercially available reactants were used without further purification. Reactions were monitored by HPLC-MS at 220 nm using a HPLC Waters Alliance HT comprising a pump (Edwards RV12) with degasser, an autosampler and a diode array detector. Flow from the column was split to a MS spectrometer. The MS detector was configured with an eletrospray ionization source (micromass ZQ4000) and nitrogen was used as the nebulizer gas. Data acquisition was performed with MassLynx software. Microwave reactions were carried out in 10 mL sealed glass tubes in a focused mono-mode microwave oven ("Discover" by CEM Corporation) featured with a surface sensor for internal temperature determination. Cooling was provided by compressed air ventilating the microwave chamber during the reaction. When stated, the final crude was purified via flash column chromatography Combi Flash ISCO RF provided with dual UV detection. ^{1}H NMR spectrum was recorded on a Varian Mercury 400 at 400 MHz in DMSO solution with TMS as an internal reference. Data for ^{1}H-NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, integration and coupling constants (Hz). Multiplicities are referred by the following abbreviations: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, td = triplet of doublets, ddd = double doublet of doublets and m = multiplet. IR spectra were recorded using a Thermo Nicolet Nexus spectrometer and are reported in frequency of absortion (cm^{-1}). High Resolution Mass Spectrometry was performed by the University of Barcelona Mass Spectrometry Service.

Abbreviations

Abbreviation used for amino acids and designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* 247, 977-983 (1982). The following additional abbreviations are used: DMF: *N,N*-dimethylformamide, ACN: acetonitrile, Fmoc: 9*H*-fluorenylmethyloxycarbonyl, TFA: trifluoroacetic acid, Trp/W: tryptophan, Phe/F: phenylalanine, Arg/R: arginine, Ala/A: alanine, W, tryptophan, Val/V: valine, Asn/N: asparagine, RP-HPLC-ESMS: reverse phase-high performance liquid chromatography-electro spray mass spectrometry, NMR: nuclear magnetic resonance, SPPS: solid-phase peptide synthesis, IR: infrared spectroscopy.

Reagents and microorganisms

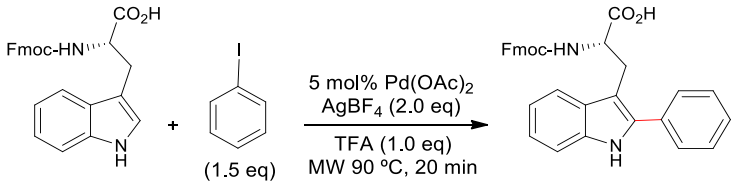
Nα-Fmoc-tryptophan, iodobenzene, palladium (II) acetate and N,N-dimethylformamide anhydrous (DMF) were from Aldrich, Fmoc-Rink-Amide AM resin was from Iris-Biotech. O-benzotriazole-N,N,N’,N’-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and 2-(1H-7-

azabenzotriazol-1-yl)-1,1,3,3,-tetramethyl uronium hexafluorophosphate (HATU) were from Fluorochem. N,N-dimethylformamide was from Panreac AppliChem. N,N-diisopropylethylamine (DIEA) and silicagel were from Merck Biosciences.

Müller-Hinton culture medium was from Oxoid. Bacterial strains *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 12228 were from The American Type Culture Collection (Manassas, VA, USA).

Tryptophan arylation

The arylation of the indolic C2 of tryptophan was carried out by activation of this carbon catalyzed by palladium as described by Ruiz-Rodríguez et al,[1] and Preciado-Gallego et al.[2] Briefly, the Nα-Fmoc-tryptophan, activated with $Pd(OAc)_2$ was treated with iodobenzene in a microwave at 90° C and purified by flash chromatography on silicagel as described by Preciado-Gallego et al. (2013).[3] The product was identified by nuclear magnetic resonance (NMR) and electrospray mass spectrometry (ESMS) and the yield was 56 %.

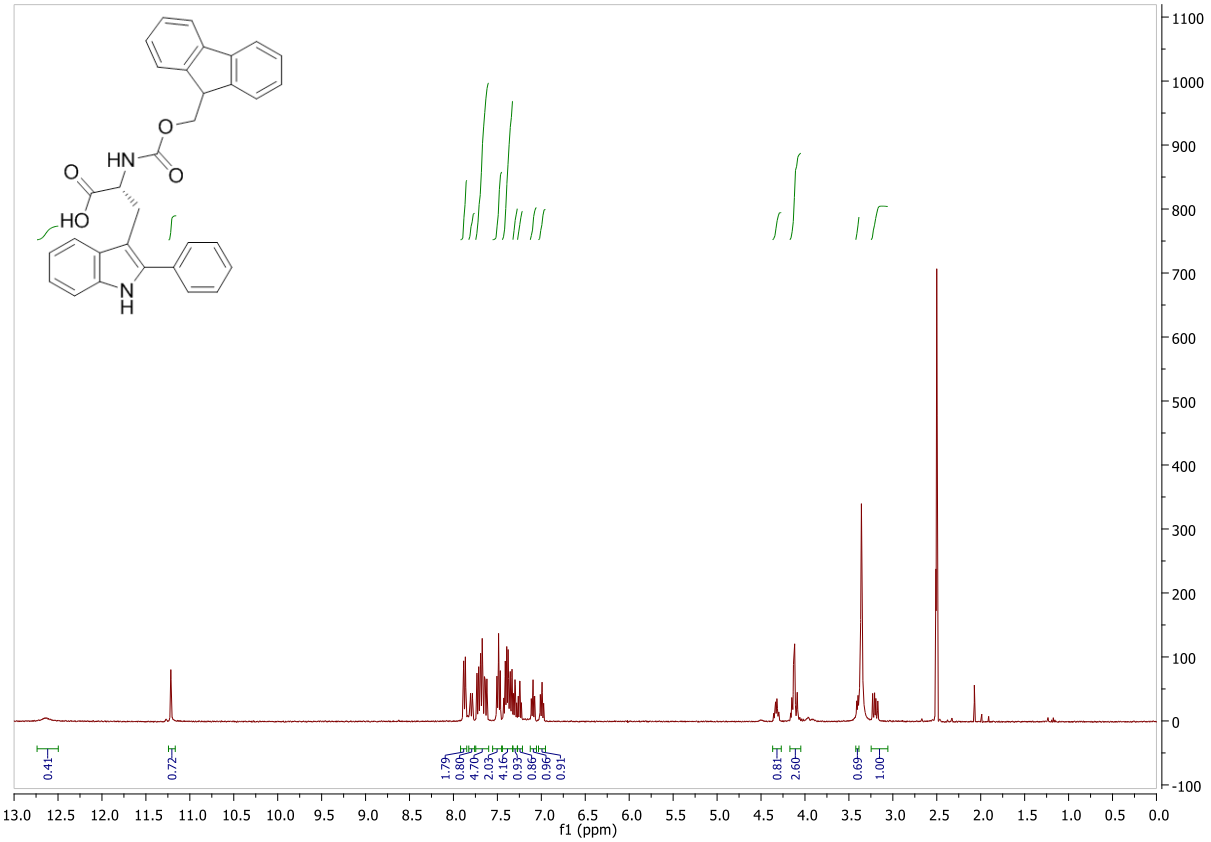


Fmoc-Trp-OH **Fmoc-Trp(C2-Ph)-OH**

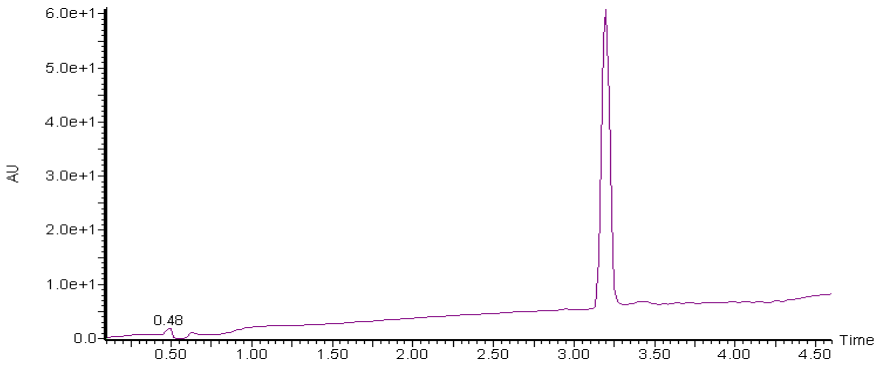
Fmoc-Trp(C2-Ph)-OH. Fmoc-Trp-OH (0.5 g, 1.17 mmol, 1 eq.), iodobenzene (1.5 eq.), $AgBF_4$ (2 eq.), TFA (1.0 eq.) and $Pd(OAc)_2$ (5 % mol) were placed in a microwave reactor vessel in dry DMF (2 mL). The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. This microwave process was performed up to six times and all the reaction mixtures were collected altogether. Ethyl acetate (20 mL) was added and the resulting suspension was filtered through Celite and the solvent was removed under vacuum. The crude extract was purified by flash column chromatography on silica gel (hexane/ethyl acetate). Fractions containing the arylated tryptophan were collected and the solvent was removed under reduced pressure. Finally, the residue was dissolved in ACN/H_2O and lyophilized for 24 h to yield the pure product Fmoc-Trp(C2-Ph)-OH as a solid (1.98 g, 56 %). **^{1}H-NMR** (400 MHz, DMSO): δ 12.62 (s, 1H), 11.20 (s, 1H), 7.88 – 7.84 (m, 2H), 7.78 (d, *J* = 8.6 Hz, 1H), 7.72 – 7.60 (m, 5H), 7.47 (t, *J* = 7.7 Hz, 2H), 7.41 – 7.32 (m, 4H), 7.28 (td, *J* = 7.4, 1.1 Hz, 1H), 7.23 (td, *J* = 7.5, 1.1 Hz, 1H), 7.10 – 7.05 (m, 1H), 6.97 (ddd, *J* = 8.0, 6.9, 1.0 Hz, 1H), 4.31 (td, *J* = 8.6, 5.8 Hz, 1H), 4.15 – 4.06 (m, 3H), 3.40 – 3.34 (m, 1H), 3.18 (dd, *J* = 14.6, 8.7 Hz, 1H) ppm. **IR** (Film, cm^{-1}) v = 3372.24, 3321.00, 3051.96, 2955.87, 1693.95 cm^{-1}. **RP-HPLC-ESMS**: m/z (%): 503.22 $(M+H)^+$. $[\alpha]_D^{20}$ -5.9 (*c* 0.48, MeOH).

(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-phenyl-1*H*-indol-3-yl)propanoic acid

^{1}H NMR



(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-3-(2-phenyl-1*H*-indol-3-yl)propanoic acid RP-HPLC chromatogram.

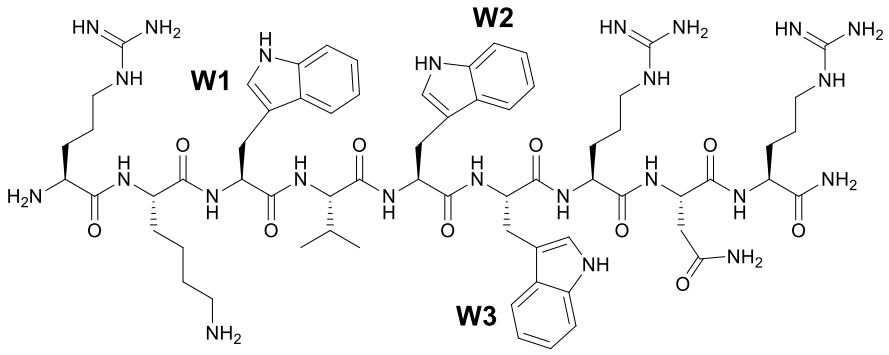


Gradient was 5-100% of B. Solvent A (0.1 % FA in H_2O) and solvent B (0.05 % FA in ACN), in 3.5 min. Elution was monitored at 254 nm and the flow rate was 1.6 mL/min.

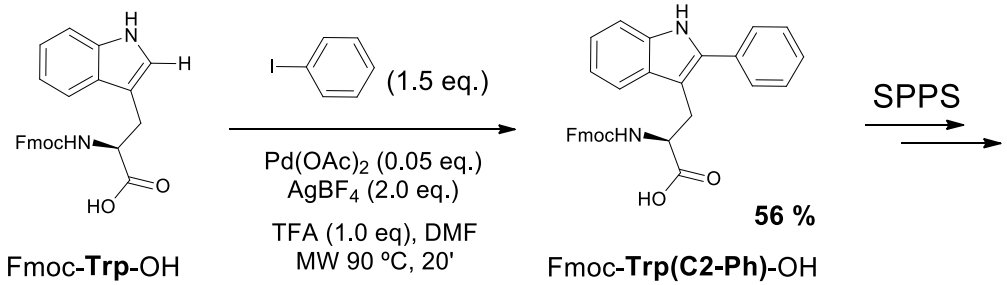
Synthesis of arylated peptides

The preparation of 2-aryl-Nα-Fmoc-tryptophan was performed as described from 4-iodobenzene and Nα-Fmoc-tryptophan (Preciado et al., 2013).[1] The resulting arylated amino acid was used in solid-phase peptide synthesis without any special requirement.

The solid-phase method was performed according to Kates and Albericio, (2000).[4] Fmoc chemistry and Fmoc-Rink-Amide AM resin were used. O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and N,N-diisopropylethylamine (DIEA) were used as coupling reagents for arginine, alanine, tryptophan, valine and asparagine, while 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3,-tetramethyl uronium hexafluorophosphate (HATU) and DIEA were used for aryl tryptophan. After side-chains removal, peptides were cleaved from the resin with trifluoroacetic acid (TFA)/water/triisopropylsilane (TIS). Purification of peptides was performed by RP-HPLC. Identification was carried out by MALDI-MS and MALDI-MS/MS.



Native Peptide H-Arg-Lys-**Trp**-Val-**Trp**-**Trp**-Arg-Asn-Arg-NH_2 **(1)**



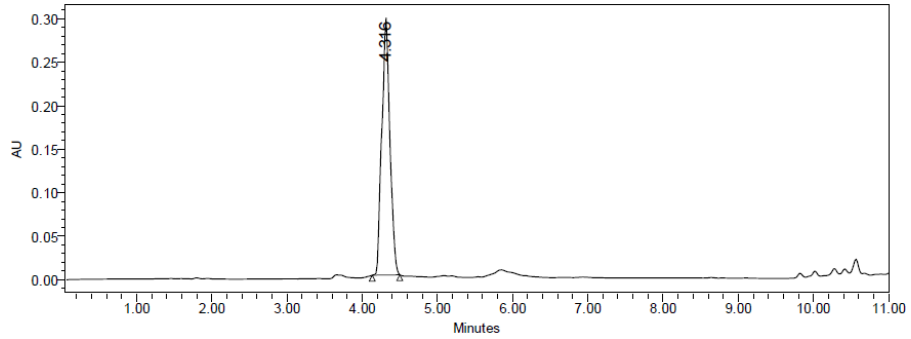
W1-Ar H-Arg-Lys-**Trp(C2-Ph)**-Val-**Trp**-**Trp**-Arg-Asn-Arg-NH_2 **(2)**

W2-Ar H-Arg-Lys-**Trp**-Val-**Trp(C2-Ph)**-**Trp**-Arg-Asn-Arg-NH_2 **(3)**

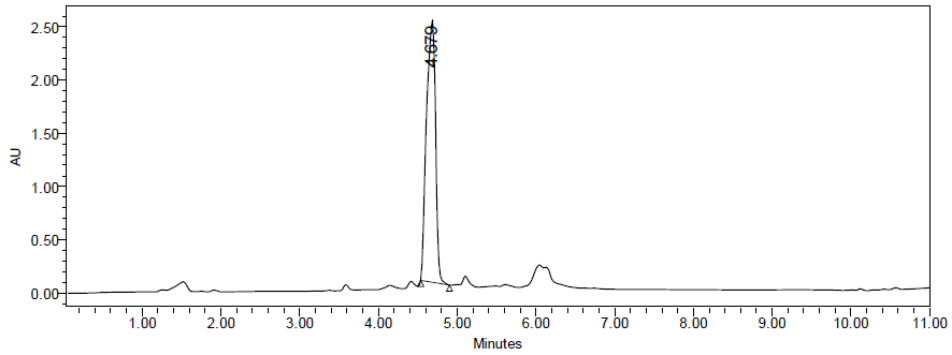
W3-Ar H-Arg-Lys-**Trp**-Val-**Trp**-**Trp(C2-Ph)**-Arg-Asn-Arg-NH_2 **(4)**

RP-HPLC chromatograms of crude arylated peptides 1-4

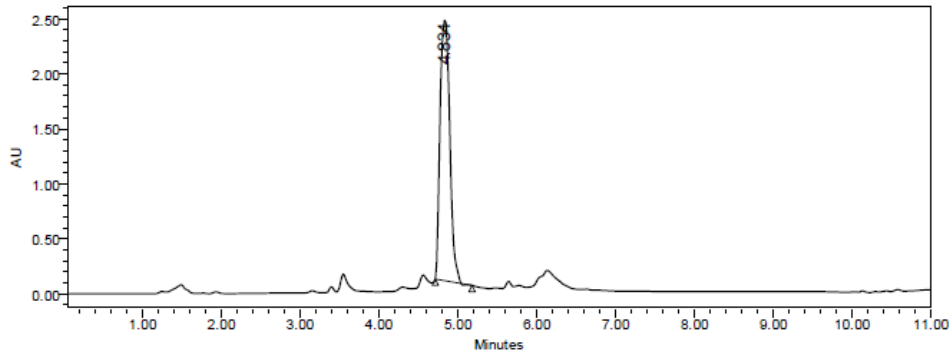
H-Arg-Lys-**Trp**-Val-**Trp**-**Trp**-Arg-Asn-Arg-NH_2 **(1)**



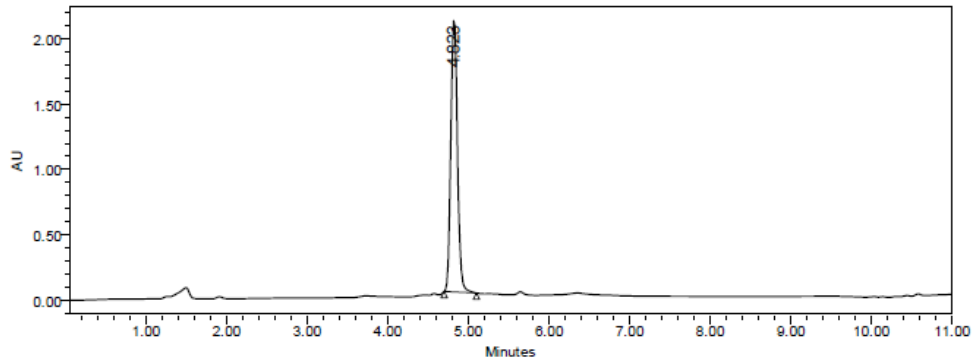
H-Arg-Lys-**Trp(C2-Ph)**-Val-**Trp**-**Trp**-Arg-Asn-Arg-NH_2 **(2)**



H-Arg-Lys-**Trp**-Val-**Trp(C2-Ph)**-**Trp**-Arg-Asn-Arg-NH_2 **(3)**



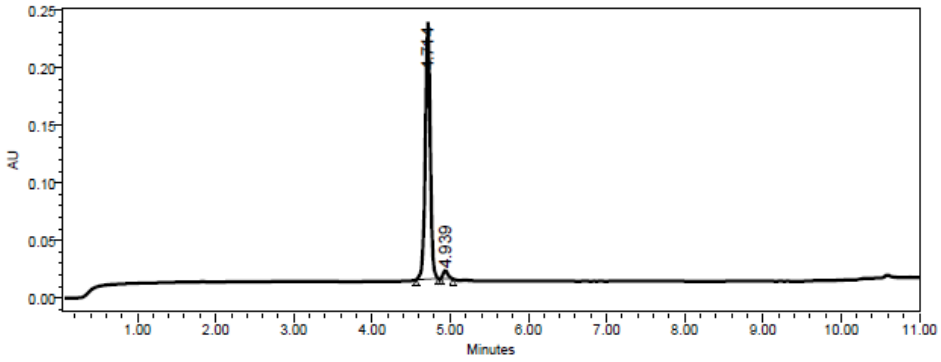
H-Arg-Lys-**Trp**-Val-**Trp**-**Trp(C2-Ph)**-Arg-Asn-Arg-NH_2 **(4)**



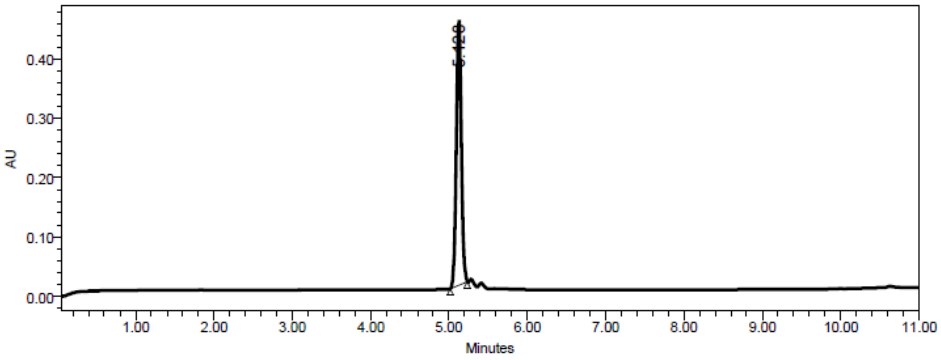
Gradient was 5-60% of B. Solvent A (0.045 % TFA in H_2O) and solvent B (0.036 % TFA in ACN), in 8 min. Elution was monitored at 220 nm and the flow rate was 1.0 mL/min.

RP-HPLC chromatograms of pure arylated peptides 1-4

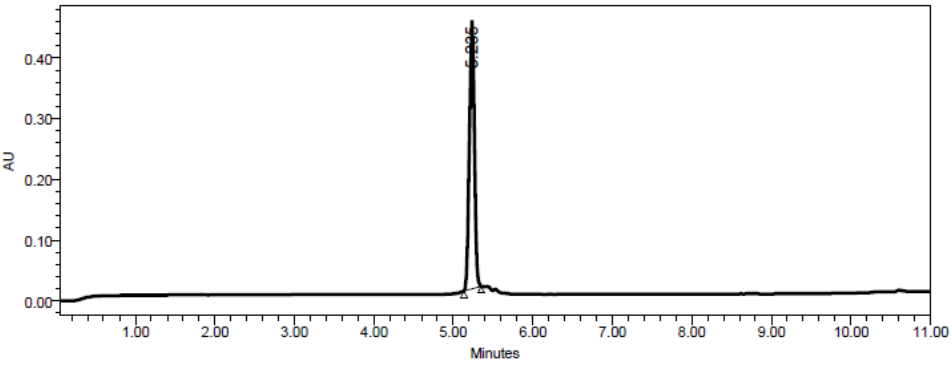
H-Arg-Lys-**Trp**-Val-**Trp**-**Trp**-Arg-Asn-Arg-NH_2 **(1)**



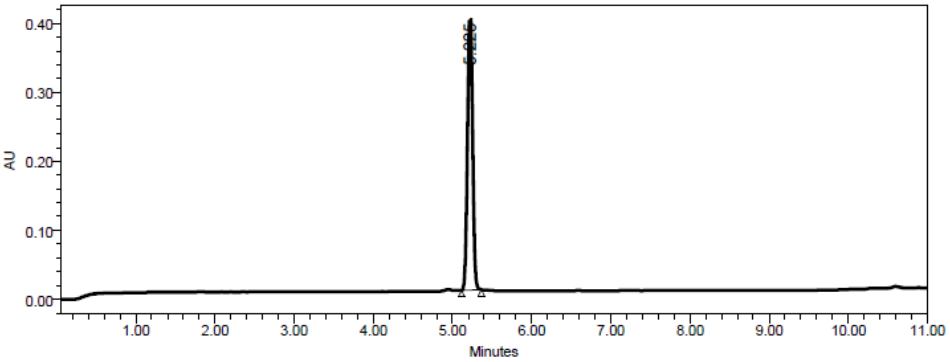
H-Arg-Lys-**Trp(C2-Ph)**-Val-**Trp**-**Trp**-Arg-Asn-Arg-NH_2 **(2)**



H-Arg-Lys-**Trp**-Val-**Trp(C2-Ph)**-**Trp**-Arg-Asn-Arg-NH_2 **(3)**



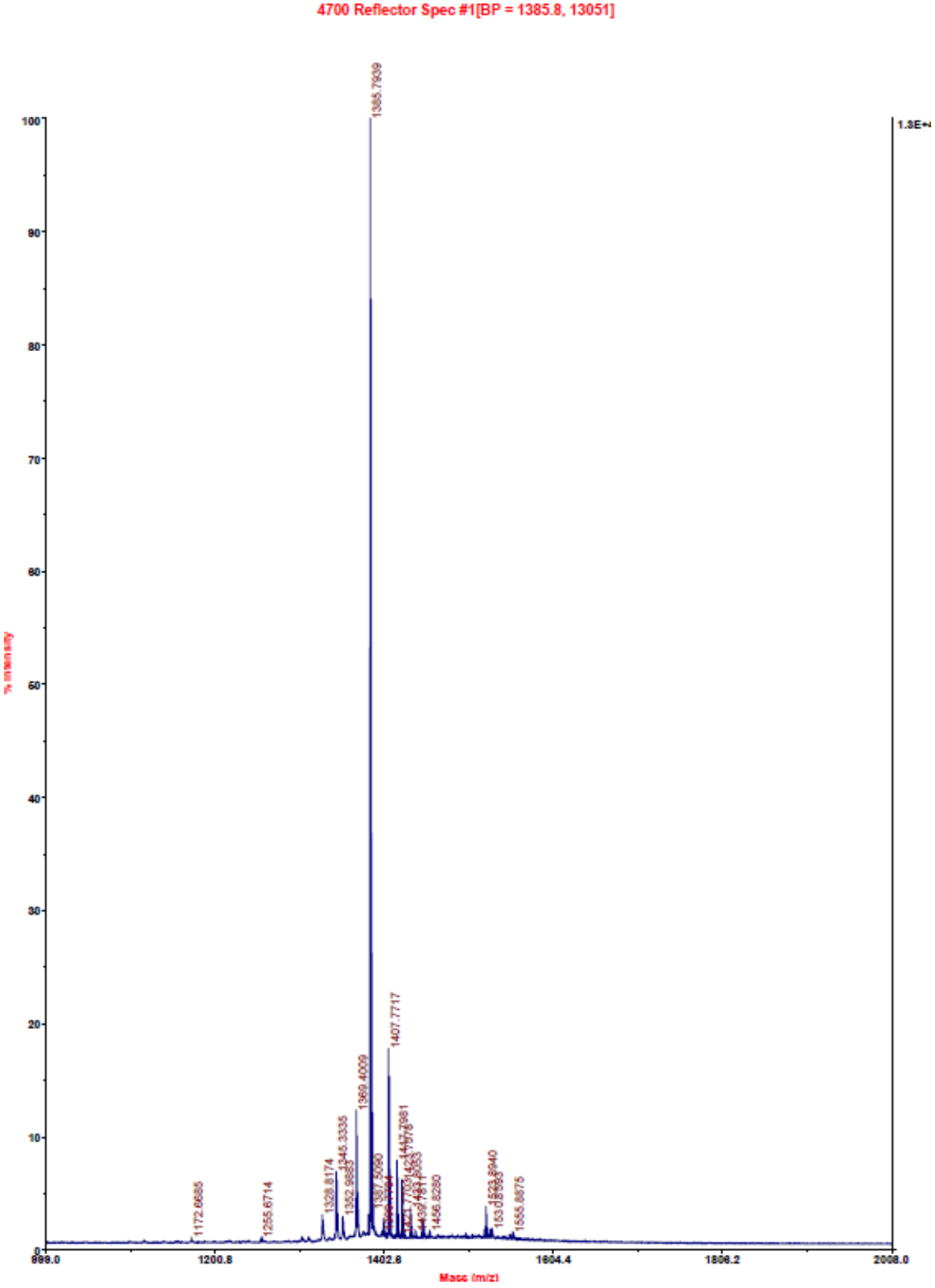
H-Arg-Lys-**Trp**-Val-**Trp**-**Trp(C2-Ph)**-Arg-Asn-Arg-NH_2 **(4)**



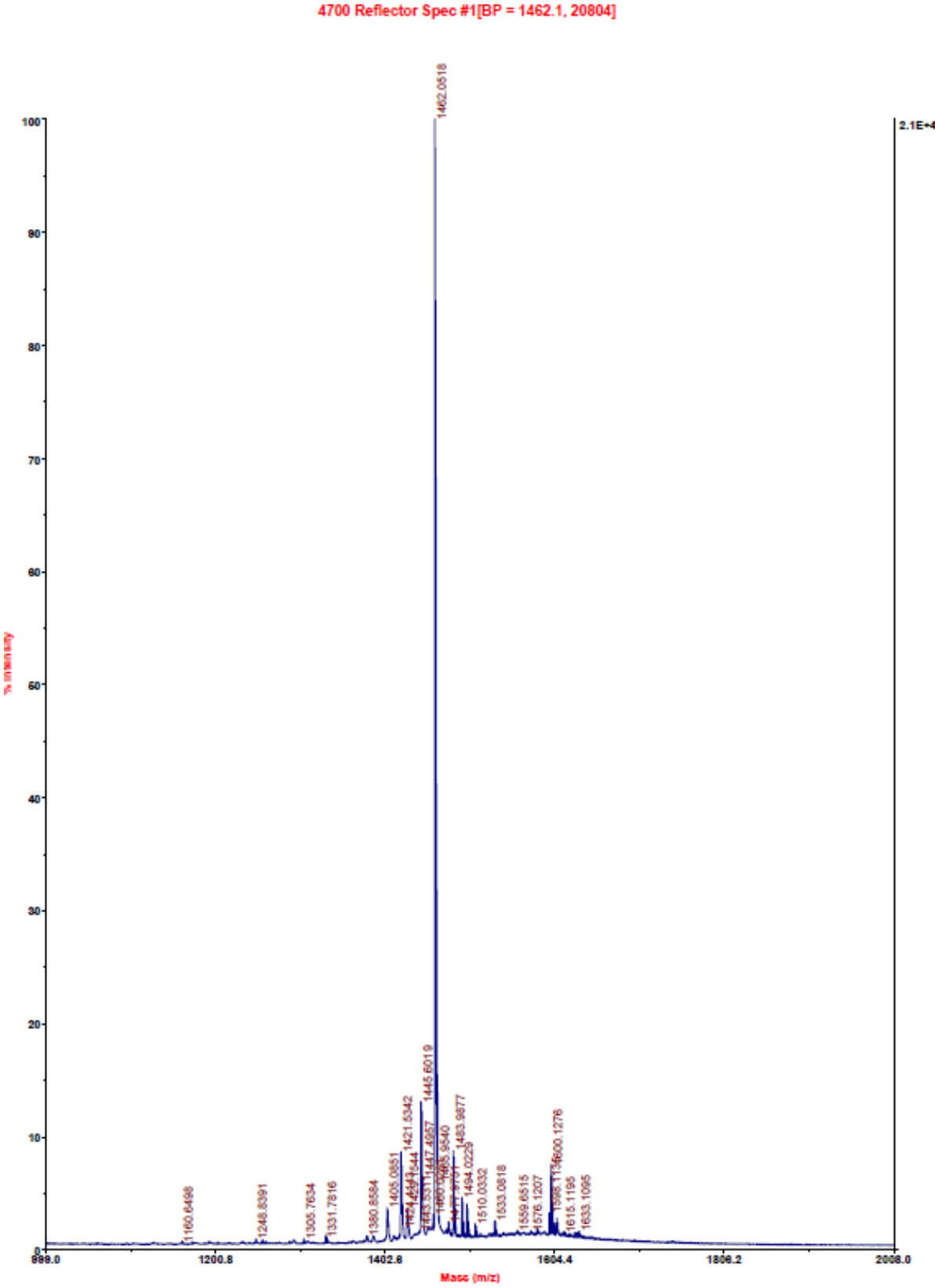
Gradient was 5-60% of B. Solvent A (0.045 % TFA in H_2O) and solvent B (0.036 % TFA in ACN), in 8 min. Elution was monitored at 254 nm and the flow rate was 1.0 mL/min.

MALDI spectra of arylated peptides 1-4

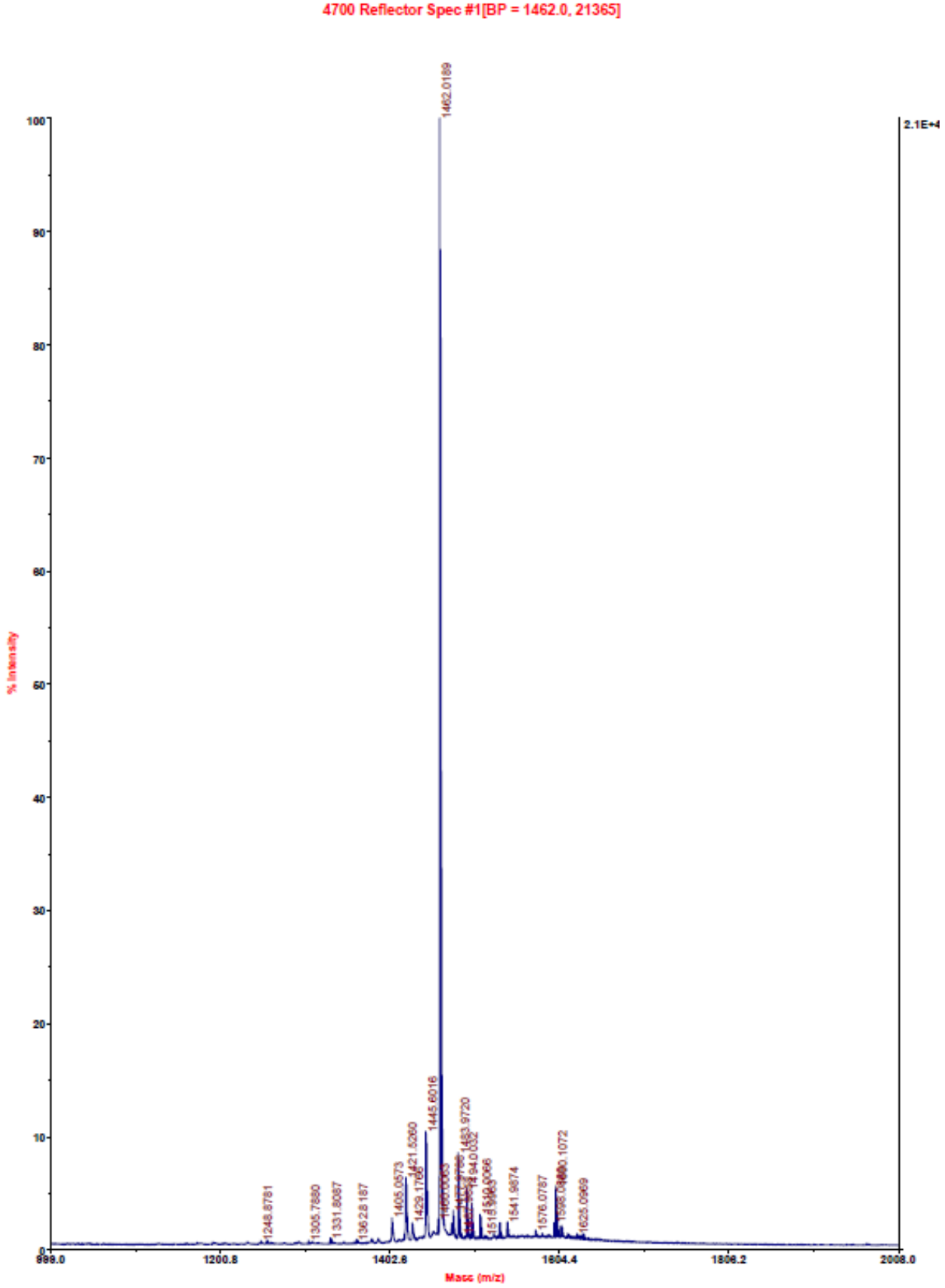
H-Arg-Lys-**Trp**-Val-**Trp**-**Trp**-Arg-Asn-Arg-NH_2 **(1)**



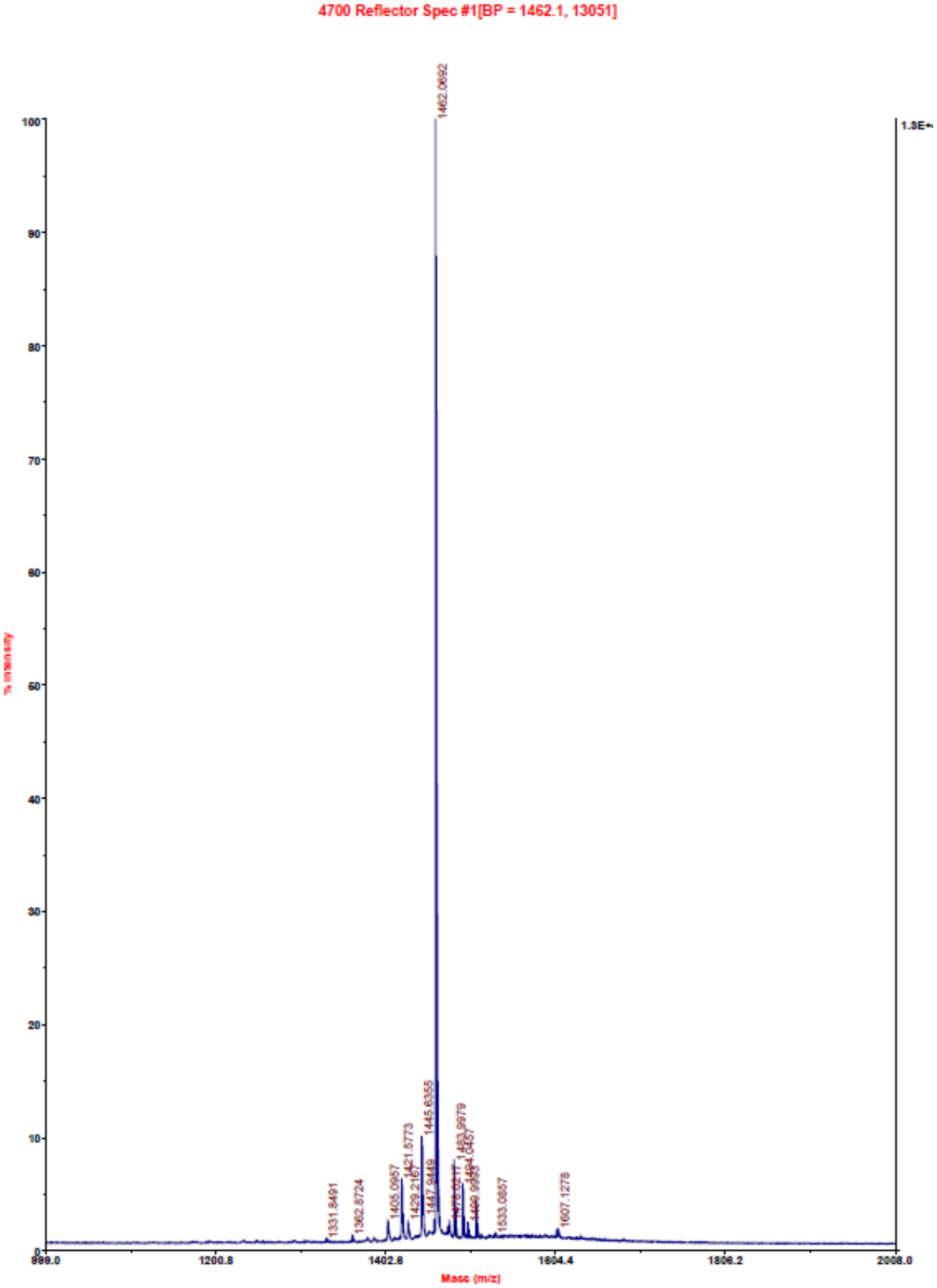
H-Arg-Lys-**Trp(C2-Ph)**-Val-**Trp**-**Trp**-Arg-Asn-Arg-NH_2 **(2)**



H-Arg-Lys-**Trp**-Val-**Trp(C2-Ph)**-**Trp**-Arg-Asn-Arg-NH_2 **(3)**



H-Arg-Lys-**Trp**-Val-**Trp**-**Trp(C2-Ph)**-Arg-Asn-Arg-NH_2 **(4)**



Detailed experimental procedures for the biological assays

Growth inhibition assay

Bacteria were grown in Müeller-Hinton broth until a density of 1 x 108 CFU/mL was reached. A final bacteria concentration of 1-5 x 10^5 CFU/mL was used.

Peptide solutions in concentrations of 2.5 to 0.1 mg/mL, were mixed with the same volume of 2X Müeller-Hinton medium and incubated at 37° C for 21 h in serial two-fold dilutions. Bacterial growth was determined by measuring the absorbance at 620 nm. Positive control (100 % growth) was performed in absence of peptide and negative control was carried out in absence of bacteria.

Minimum inhibitory concentration (MIC) determination

MIC determination was performed by the micro-dilution assay (Wiegand et al., 2008).[5] Briefly, decreasing amounts of peptides (from 0.5 mg to 0.5 µg) were incubated with a bacterial inoculum in exponential growing phase, in Müller-Hinton culture medium. After 12 h at 37° C, the absorbance at 600 nm was measured. A positive control without peptide (100 % growth) and a negative control without bacteria (0 % growth) were also carried out.

MIC is defined as the minimum concentration of peptide producing total inhibition of the growth under these conditions. Each peptide was assayed in triplicate.

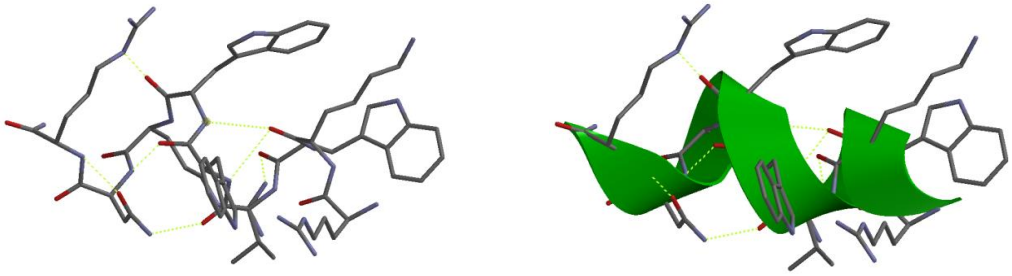
Hemolysis assay

It was performed on human red blood cells (RBC), basically as described by Helmerhorst et al. (1999).[6] Briefly, RBC from heparinized blood were washed three times with phosphate-buffered saline (PBS) and resuspended at 0.5 % in PBS. The peptide solution, in concentrations of 15, 50 and 125 µg/mL was added to equal volume of RBC suspension. After 1 h at 37° C, the mixture was centrifuged at 2800 rpm for 5 min and the absorbance of the supernatant at 414 nm was measured. PBS and Triton X-100 were the negative and positive controls, respectively. The hemolysis percentage was calculated by the formula:

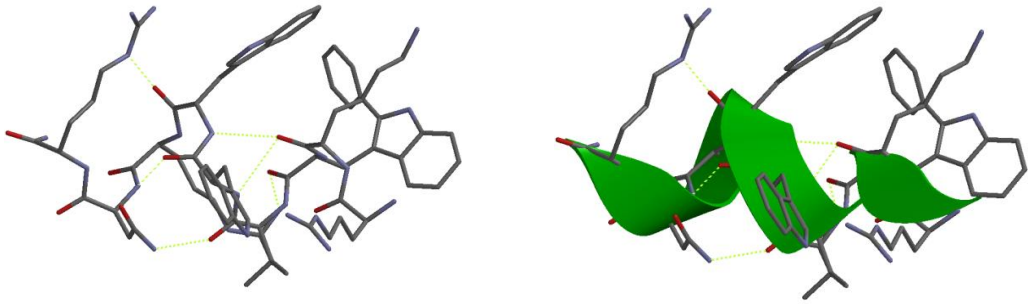
$$[(A_{peptide} - A_{PBS}) / A_{Triton} - A_{PBS})] \times 100$$

Minimized geometries of the native peptide and the arylated derivatives generated by the Spartan '14 suite (molecular mechanics, MMFF94)[7]

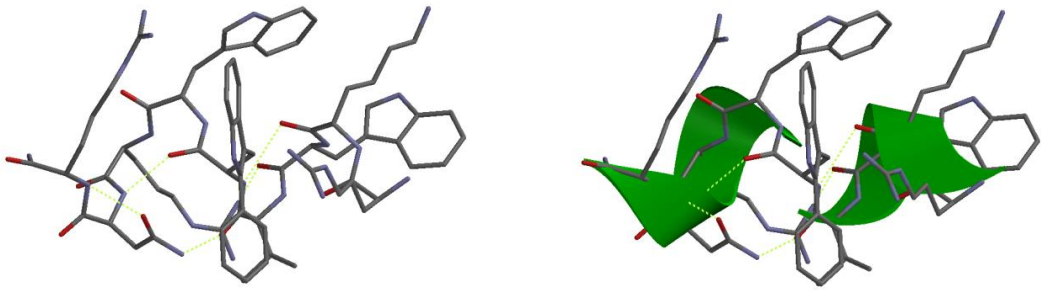
1- Native Peptide: RKWVWWRNR-NH_2



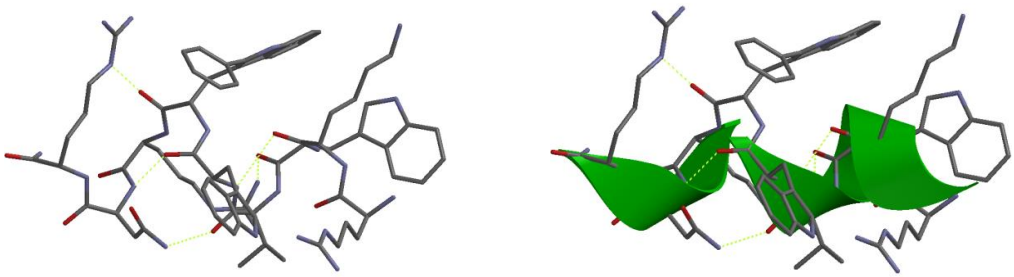
2- W1Ar: RK<u>W(Ar)</u>VWWRNR-NH_2



3-W2Ar: RKWV<u>W(Ar)</u>WRNR-NH_2



4- W3-Ar: RKWVW<u>W(Ar)</u>RNR-NH_2



Hydrogens omitted for clarity. Left section: hydrogen bonds shown in yellow. Right section: ribbons in green. No significant changes were observed when the optimization was done with semi-empirical methods.

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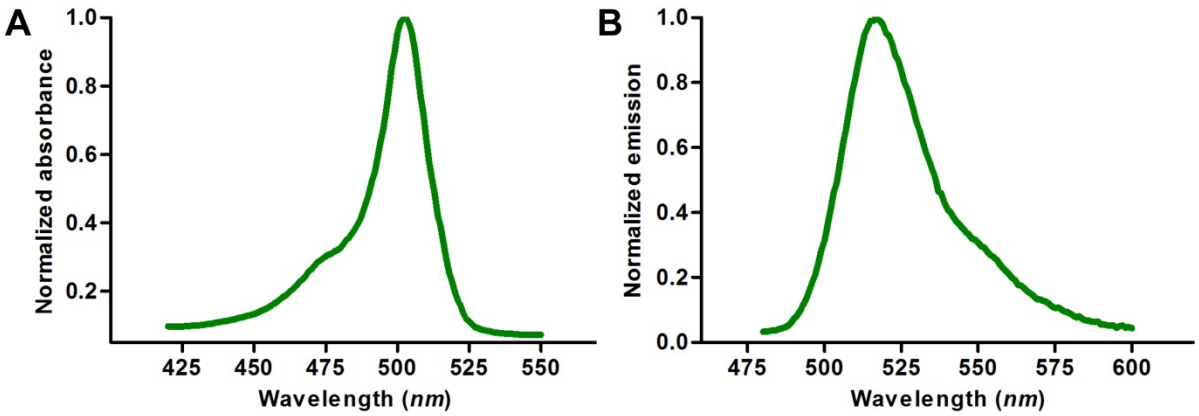
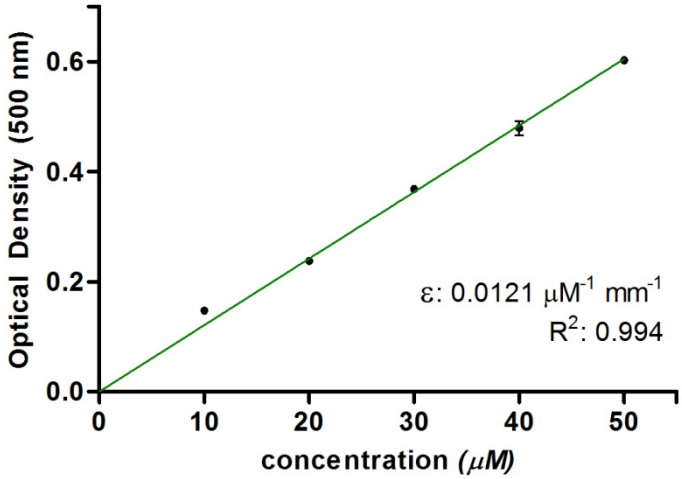
Supplementary Figures

Spectral properties of compound 3.

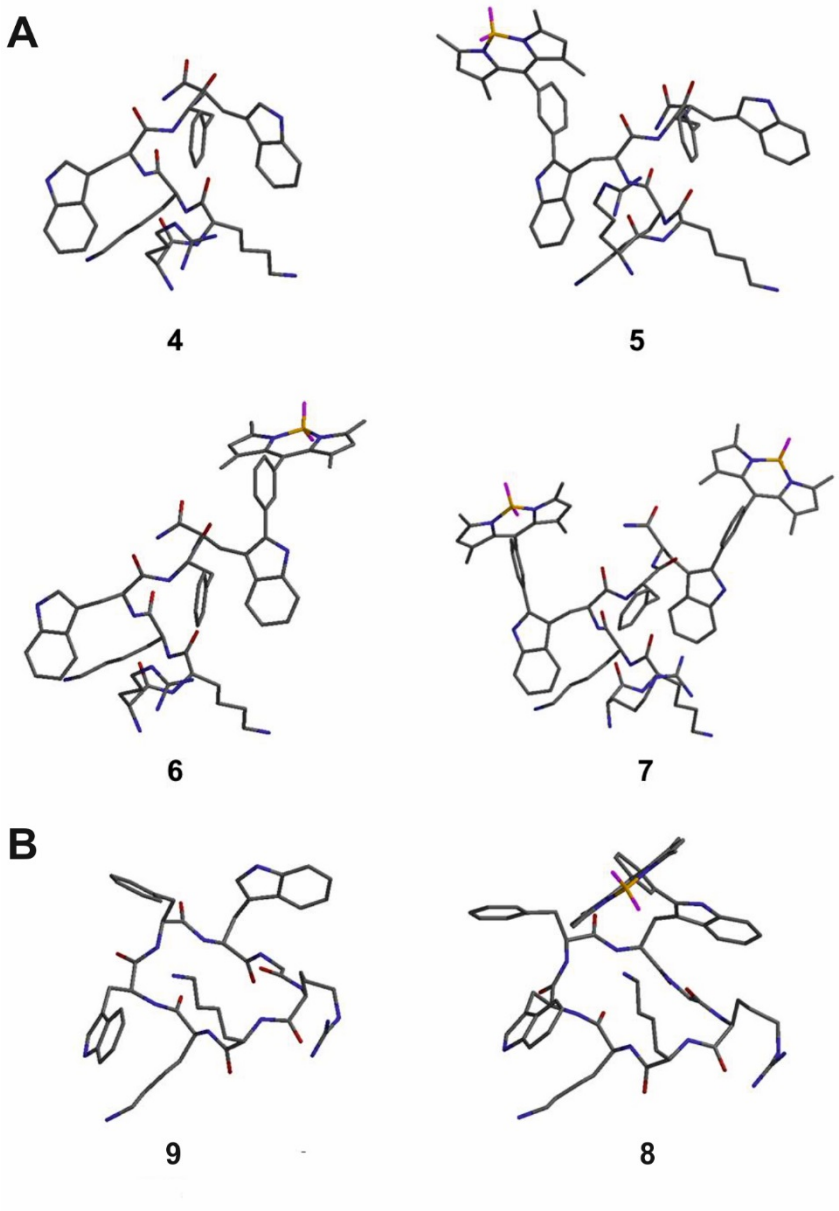


Supplementary Figure 1. Spectral characterisation of the amino acid 3. A) Absorbance and B) emission spectra ($\lambda_{exc.}$: 450 nm) of compound **3**.



Supplementary Figure 2. Determination of the extinction coefficient of the amino acid 3. Solutions of **3** were prepared in ethanol and their optical densities were measured at 500 nm in a NanoDrop 1000 spectrophotometer. Data represented as means ± s.e.m. (*n*=3).

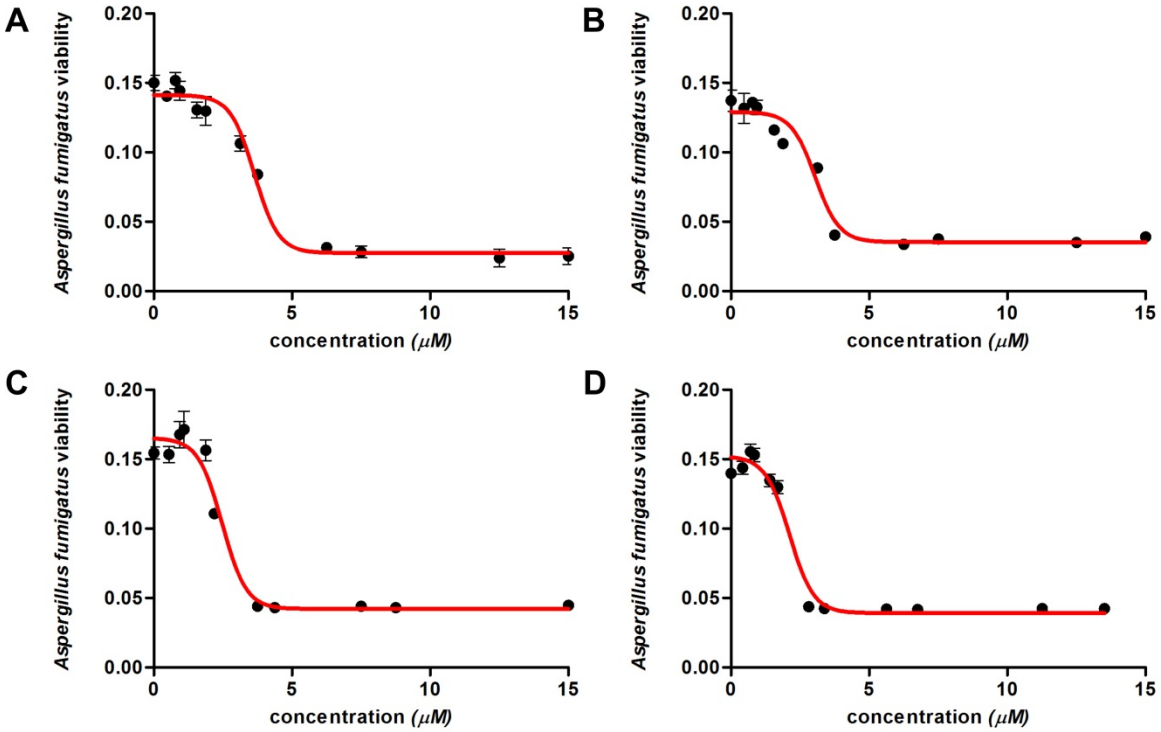
Molecular simulation models of labelled and non-labelled peptides.



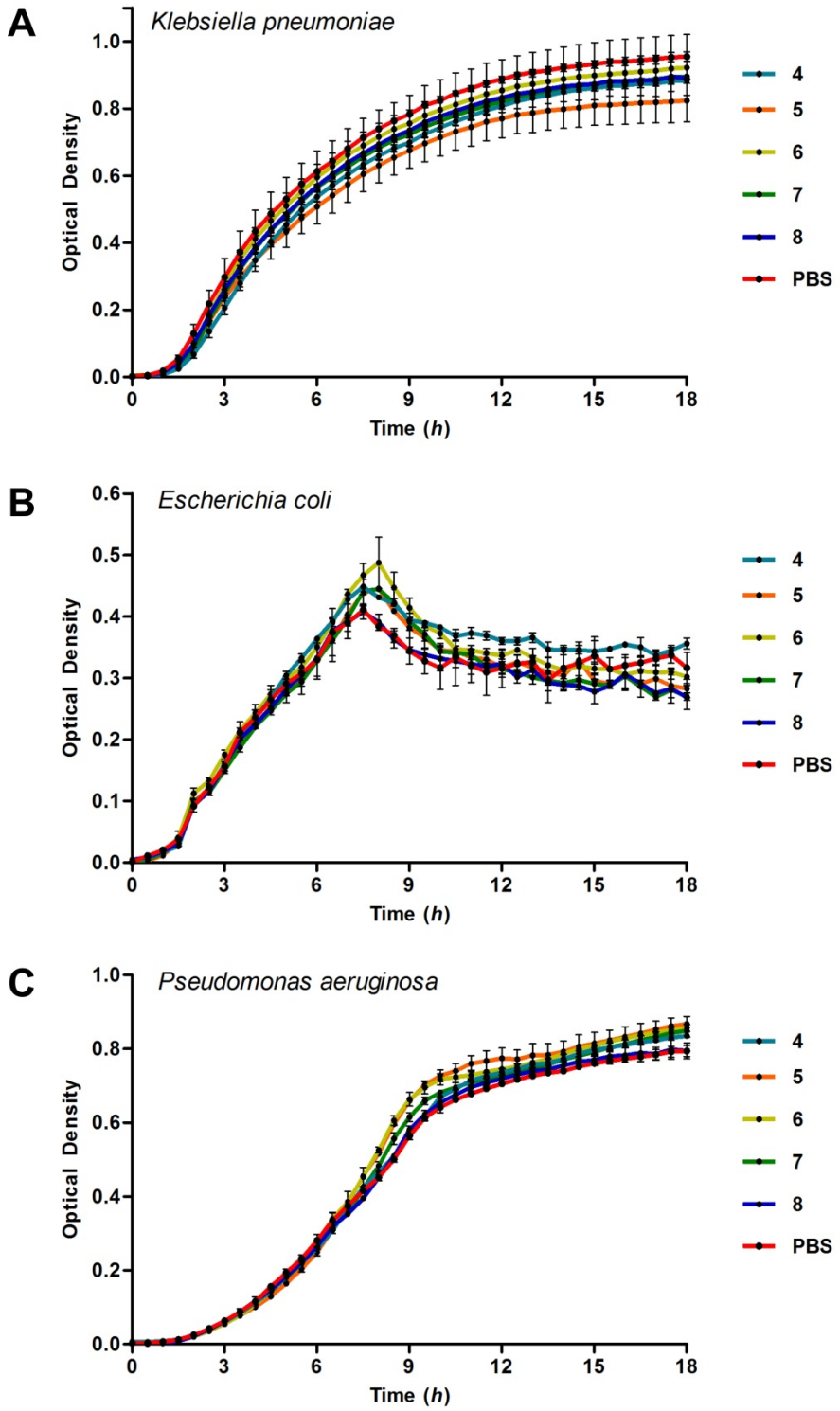
Supplementary Figure 3. A) Optimised geometries of fluorogenic linear peptides **5**-**7** and the corresponding non-labelled linear peptide **4**. B) Optimised geometries of the fluorogenic cyclic peptide **8** and the corresponding non-labelled cyclic peptide **9** (for structure see Supplementary Fig. 8). All simulations were generated by the Spartan '14 suite using Molecular Mechanics (MMFF94) and Semi-Empirical (AM1) methods.*

* Spartan'14 for Windows, Macintosh and Linux, version 1.1.4, wavefunction, inc. www.wavefun.com.

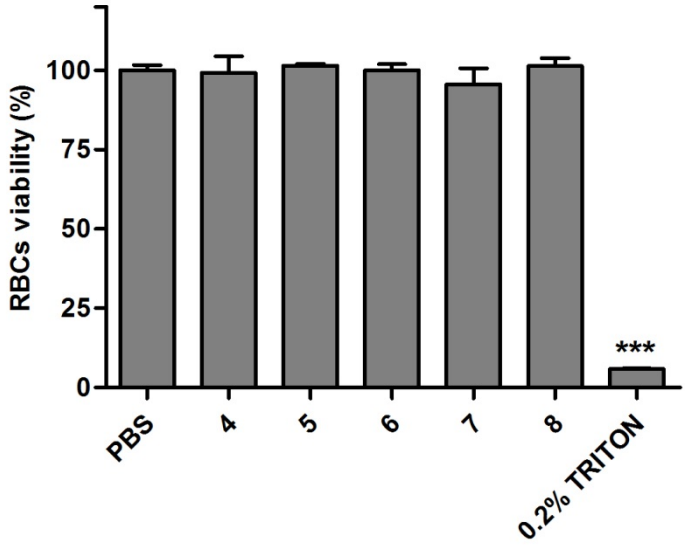
Antimicrobial and haemolysis assays.



Supplementary Figure 4. Determination of IC_{50} values of BODIPY-labelled peptides 5-8 in *A. fumigatus*. Cell viability plots and non-linear regressions for peptides **5** (A), **6** (B), **7** (C) and **8** (D). Peptides were incubated at different concentrations with *A. fumigatus* conidia to reach a final volume of 100 µL per well. The final conidia concentration was 5×10^5 cells/mL in 10% Vogel's medium. After 24 h incubation at 37 ºC in 96 well-plates, fungal growth was determined by measuring the optical density at 610 nm. The IC_{50} values were determined using four parameter logistic regression. Data represented as means ± s.e.m (*n*=3).

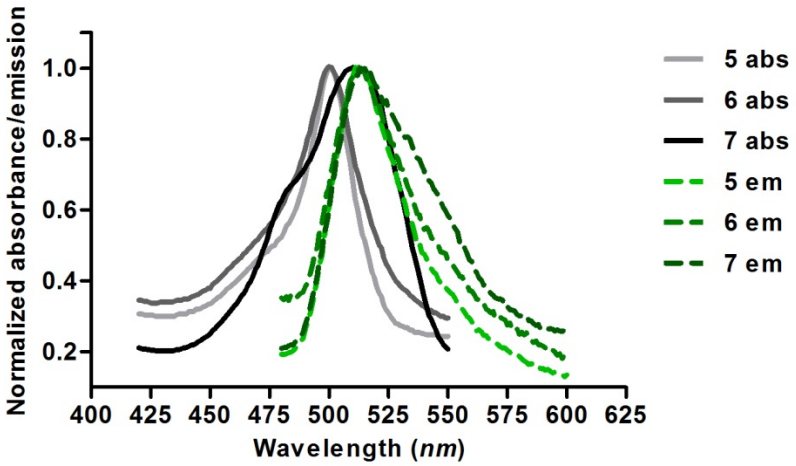


Supplementary Figure 5. Activity of peptides 4-8 in different bacterial strains. Peptides were added to the *Klebsiella pneumoniae* (A), *Escherichia coli* (B) and *Pseudomonas aeruginosa* (C), and their viability (optical density at 595 nm) was monitored at 37 ºC for 16 h. Data represented as means ± s.d. (*n*=3).



Supplementary Figure 6. Haemolytic activity of peptides 4-8 in human red blood cells (RBCs). Peptides **4**-**8** and Triton X-100 (0.2% as positive control) were incubated with human RBCs at 37 ºC and their viability was assessed after 1 h. Data represented as means ± s.d. (*n*=3). *** for $p < 0.001$ was determined as a statistically significant difference between the cell viabilities in PBS (phosphate buffer saline) and in Triton X-100.

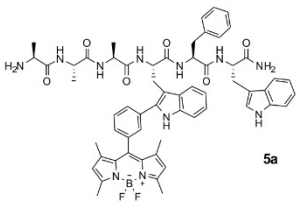
Spectral properties of peptides 5-7.



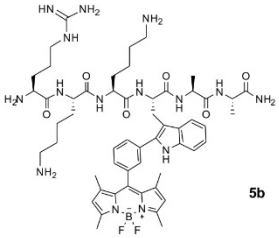
Supplementary Figure 7. Spectral characterisation of peptides 5, 6 and 7. Absorbance (solid lines) and emission (dashed lines) spectra ($\lambda_{exc.}$: 450 nm) of peptides **5**-**7** in PBS (phosphate buffer saline).

Characterisation of compounds 3, 5a, 5b and 9.

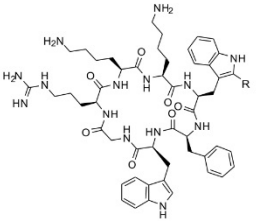
a



5a



5b

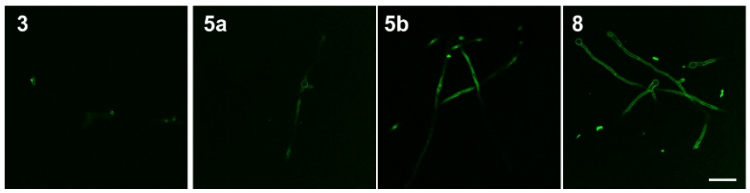


R: BODIPY (**8**)
R: H (**9**)

b

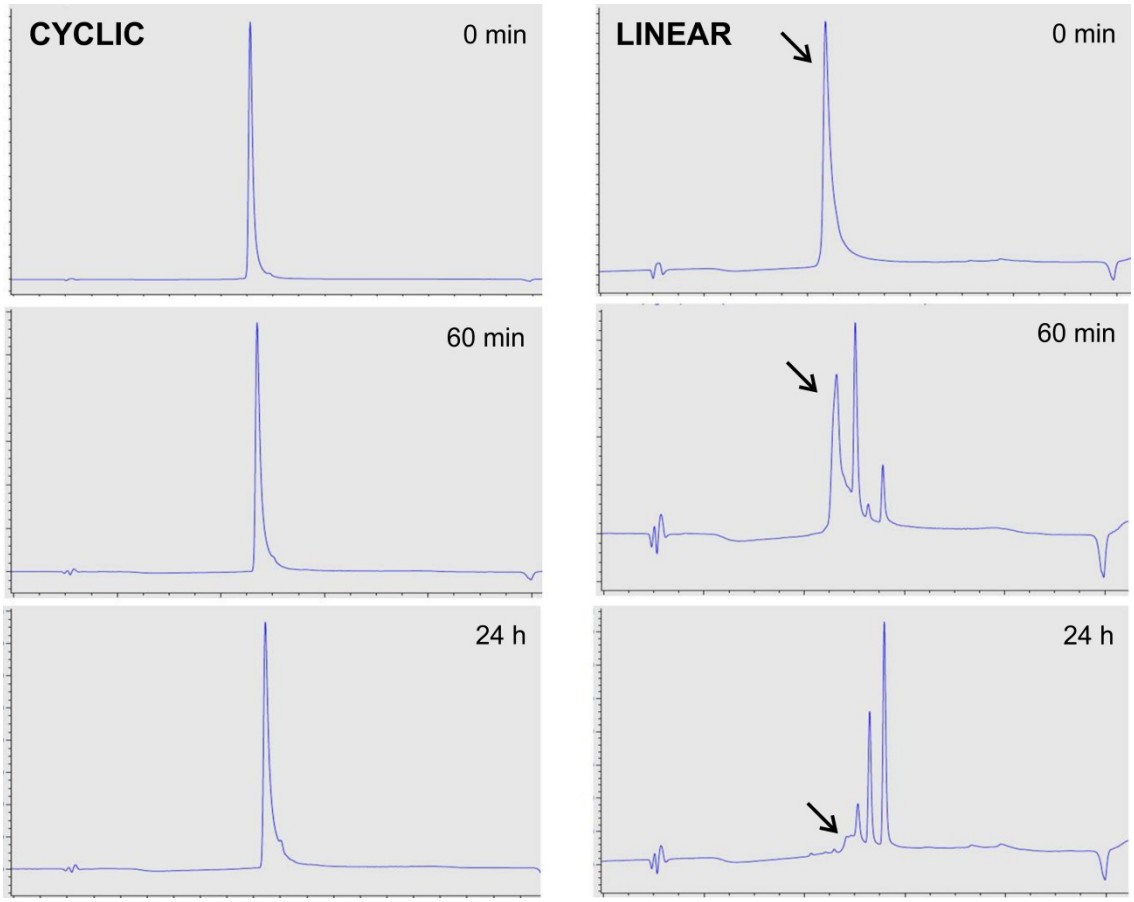
	Aspergillus fumigatus[1]	*Klebsiella pneumoniae*[2]	*Escherichia coli*[2]	*Pseudomonas aeruginosa*[2]	Human RBCs[3]
3	> 50	94%	>99%	>99%	99%
5a	> 50	70%	>99%	87%	99%
5b	4.6 ± 0.4	99%	>99%	94%	>99%
9	7.7 ± 0.7	97%	>99%	84%	99%

c

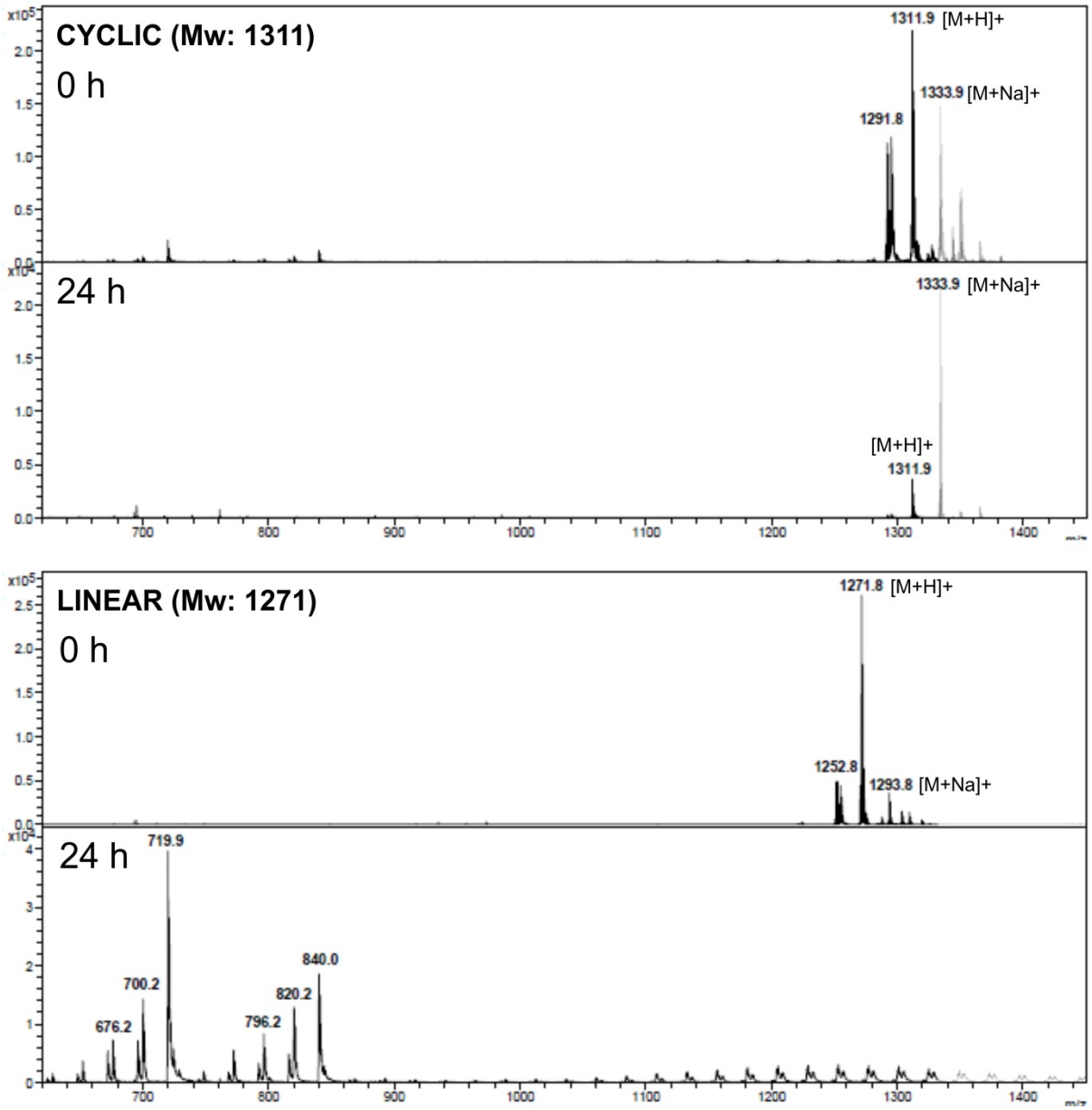


Supplementary Figure 8. Characterisation of the amino acid 3 and peptides 5a, 5b and 9 for live cell imaging of *A. fumigatus*. a) Chemical structures of fluorogenic linear (**5a**, **5b**) and cyclic peptides (**8**, **9**). b) Activity in *A. fumigatus*, several bacterial strains and in human red blood cells (RBCs).[1] IC_{50} (μM) values as means ± s.e.m. (*n*=3), [2] cell viability upon 16 h incubation with **3**, **5a**, **5b** or **9** at their respective IC_{50} concentrations or 20 μM for compounds **3** and **5a** (*n*=3), [3] cell viability upon 1 h incubation with **3**, **5a**, **5b** or **9** at their respective IC_{50} concentrations or 20 μM for compounds **3** and **5a** (*n*=3). c) Wash-free fluorescent live cell images of *A. fumigatus* at 37 ºC using confocal microscopy after incubation with peptides with **3**, **5a**, **5b** or **8** (all at 2 μM). Scale bar: 20 μm.

Stability assays.

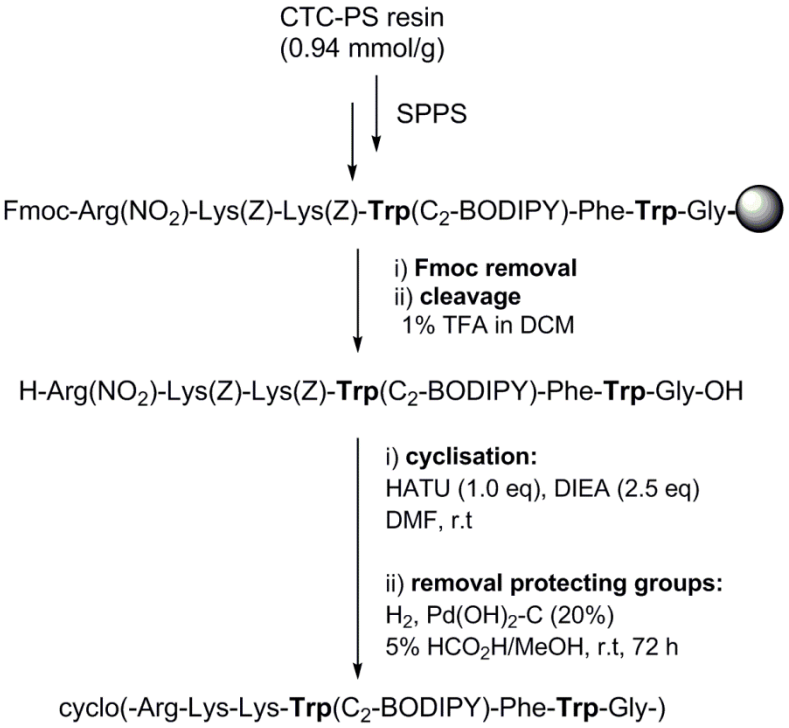


Supplementary Figure 9. Stability of cyclic (8) and linear (5) mono-BODIPY labelled peptides in human bronchoalveolar lavage samples from patients with acute respiratory distress syndrome. Peptides were incubated in human bronchoalveolar lavage samples at 37 ºC and analysed by HPLC at the time points indicated. Arrows point at the peaks corresponding to the intact linear peptide.

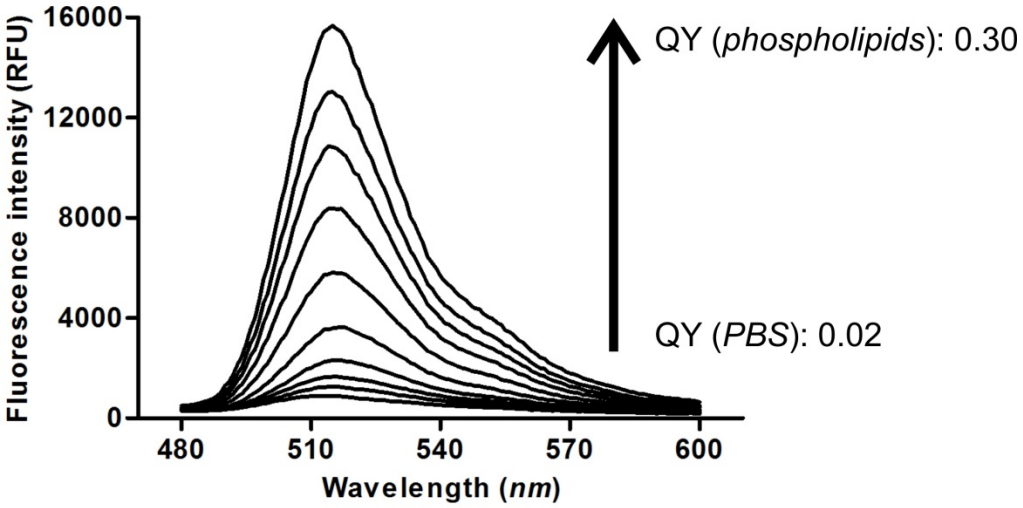


Supplementary Figure 10. MALDI analysis of cyclic (8) and linear (5) mono-BODIPY labelled peptides in bronchoalveolar lavage samples from patients with acute respiratory distress syndrome. Peptides were incubated in human bronchoalveolar lavage samples at 37 ºC and analysed by MALDI at the time points indicated.

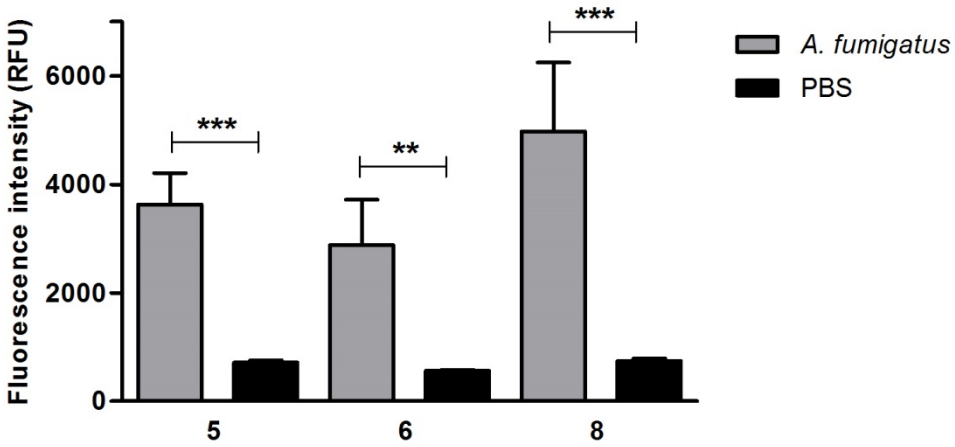
Synthesis and spectral properties of peptide 8.



Supplementary Figure 11. Synthetic scheme for the cyclic peptide **8**.

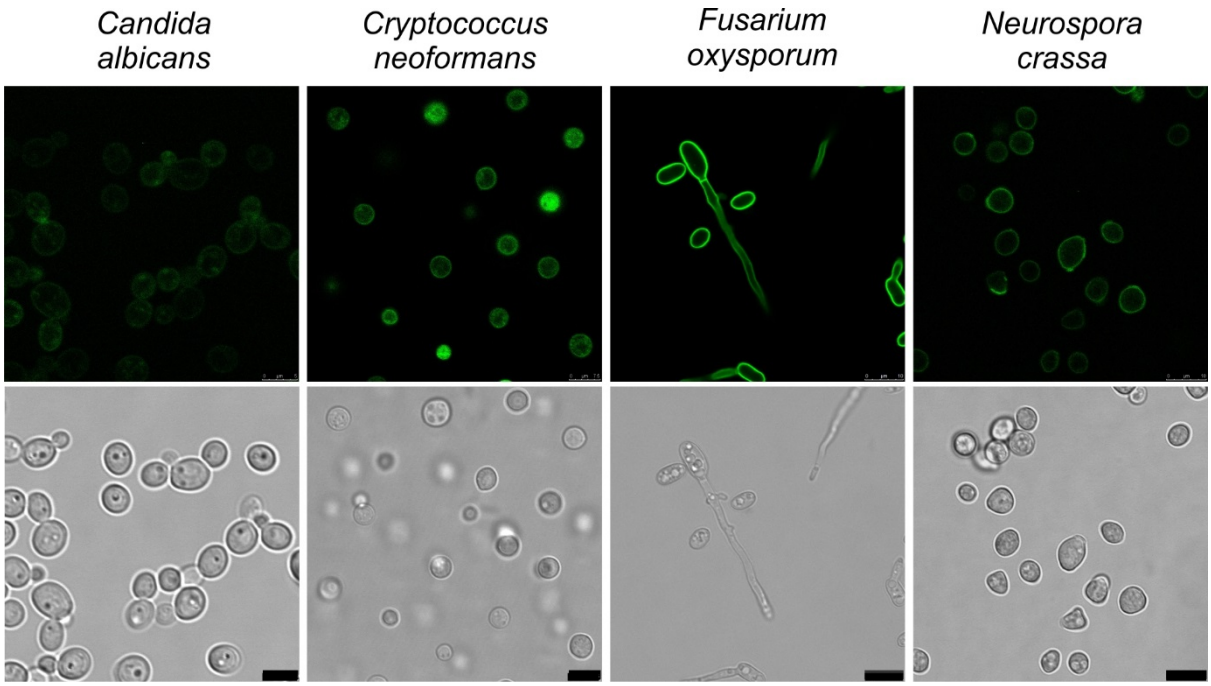


Supplementary Figure 12. Fluorogenic behaviour of the cyclic peptide 8 in phospholipid membranes. Spectra of peptide **8** were recorded after incubation with phosphatidylcholine:cholesterol (7:1) liposomes suspensions in phosphate buffer saline (PBS) ranging from 3.75 mg mL^{-1} to 0.004 mg mL^{-1} of PC in 2-fold serial dilutions, $\lambda_{exc.}$: 450 nm. Quantum yields were determined using fluorescein in basic ethanol as the reference (QY: 0.97).[1]



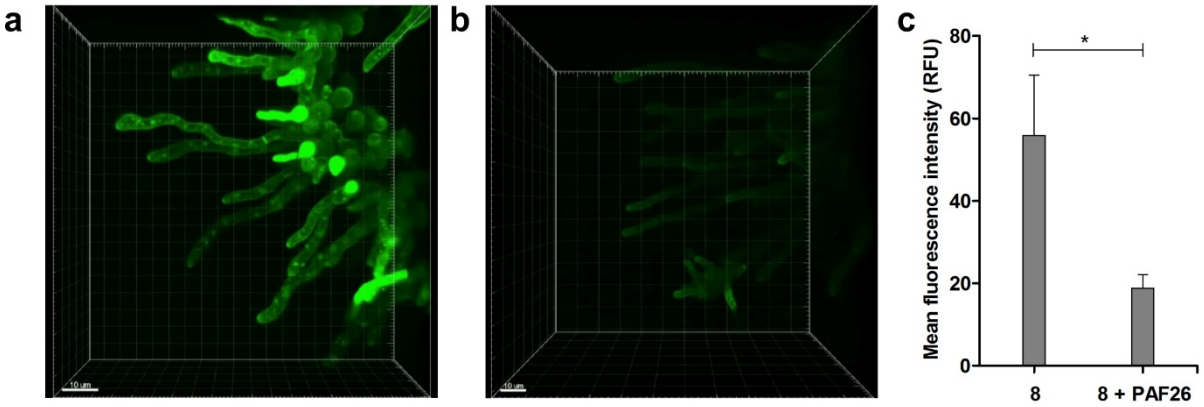
Supplementary Figure 13. Fluorescence emission of mono-BODIPY labelled peptides 5, 6 (linear) and 8 (cyclic) upon incubation with *A. fumigatus*. Peptides **5**, **6**, and **8** were incubated in PBS (phosphate buffer saline) alone or in suspensions of *A. fumigatus* in PBS ($\lambda_{exc.}$: 485 nm; $\lambda_{em.}$: 515 nm). Data represented as means ± s.d. (*n*=3). ** for $p < 0.01$ and *** for $p < 0.005$ were determined as statistically significant differences between the fluorescence emission values in PBS and in suspensions of *A. fumigatus* in PBS.

Confocal microscopy images of different fungal species.



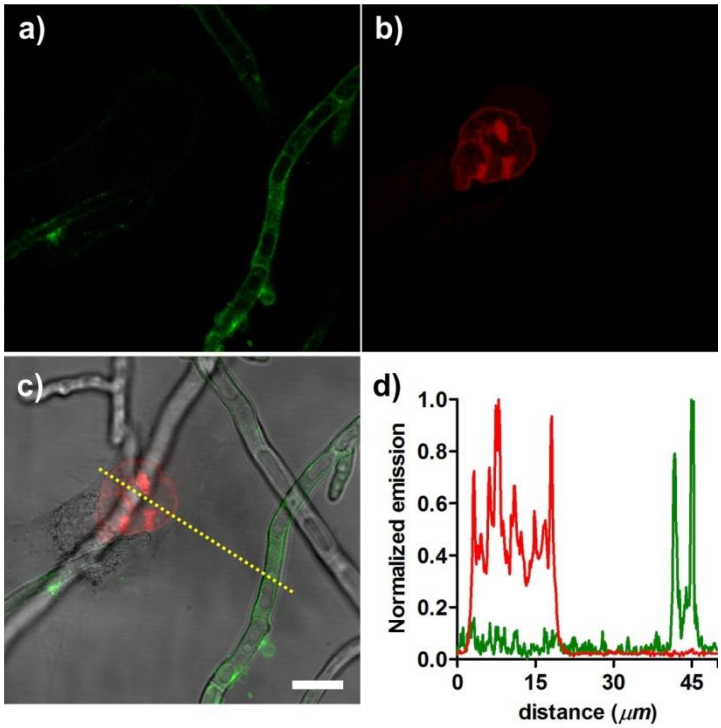
Supplementary Figure 14. Live cell imaging of fungal cells after incubation with the peptide 8. Peptide **8** was used at 2 μM for all fungi except *C. albicans* for which 10 μM was used. Fluorescence (*top*) and correspondinding brightfield (*bottom*) images were acquired under a confocal microscope at r.t.. Scale bars: *C. albicans*: 5 μm; *C. neoformans*: 7.5 μm; *F. oxysporum* and *N. crassa*: 10 μm.

Competition experiments between 8 and PAF26 (4).



Supplementary Figure 15. Fluorescence images of *A. fumigatus* after incubation with the peptide 8 without and with pre-treatment of PAF26 (4). Peptide **8** (2 μM) was incubated for 15 min with *A. fumigatus* that had not been pre-treated (a) or had been pre-treated with PAF26 (3 μM) for 30 min at 37°C (b). Images were captured under a confocal microscope at r.t. For 3D projections, see Supplementary Movies 1 and 2. Scale bars: 10 μm. c) Mean fluorescence intensity values were determined using the software Imaris and represented as means ± s.d. (*n*=3).* for $p < 0.05$ was determined as a statistically significant difference between PAF26 pre-treated and not pre-treated *A. fumigatus*.

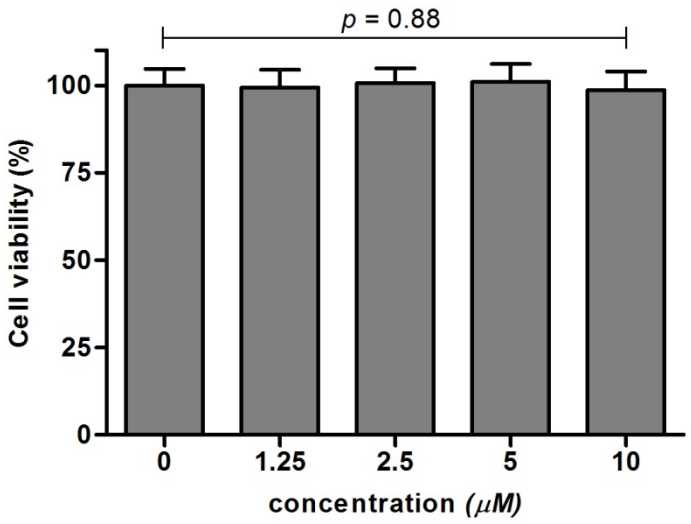
Confocal microscopy images of *Aspergillus fumigatus*.



Supplementary Figure 16. Live cell imaging of *A. fumigatus* in co-cultures with human lung A549 epithelial cells after treatment with the peptide 8. Peptide **8** (2 μM, green) and Syto82 (2.5 μM, red counterstain for human lung cells) were incubated in co-cultures and imaged without washing under a confocal microscope at 37 ºC. Fluorescence staining of **8** (a), Syto82 (b) and merged (c). (d) Plot profile analysis of the staining from **8** (green) and Syto82 (red) shown in the merged image. Scale bar: 10 μm.

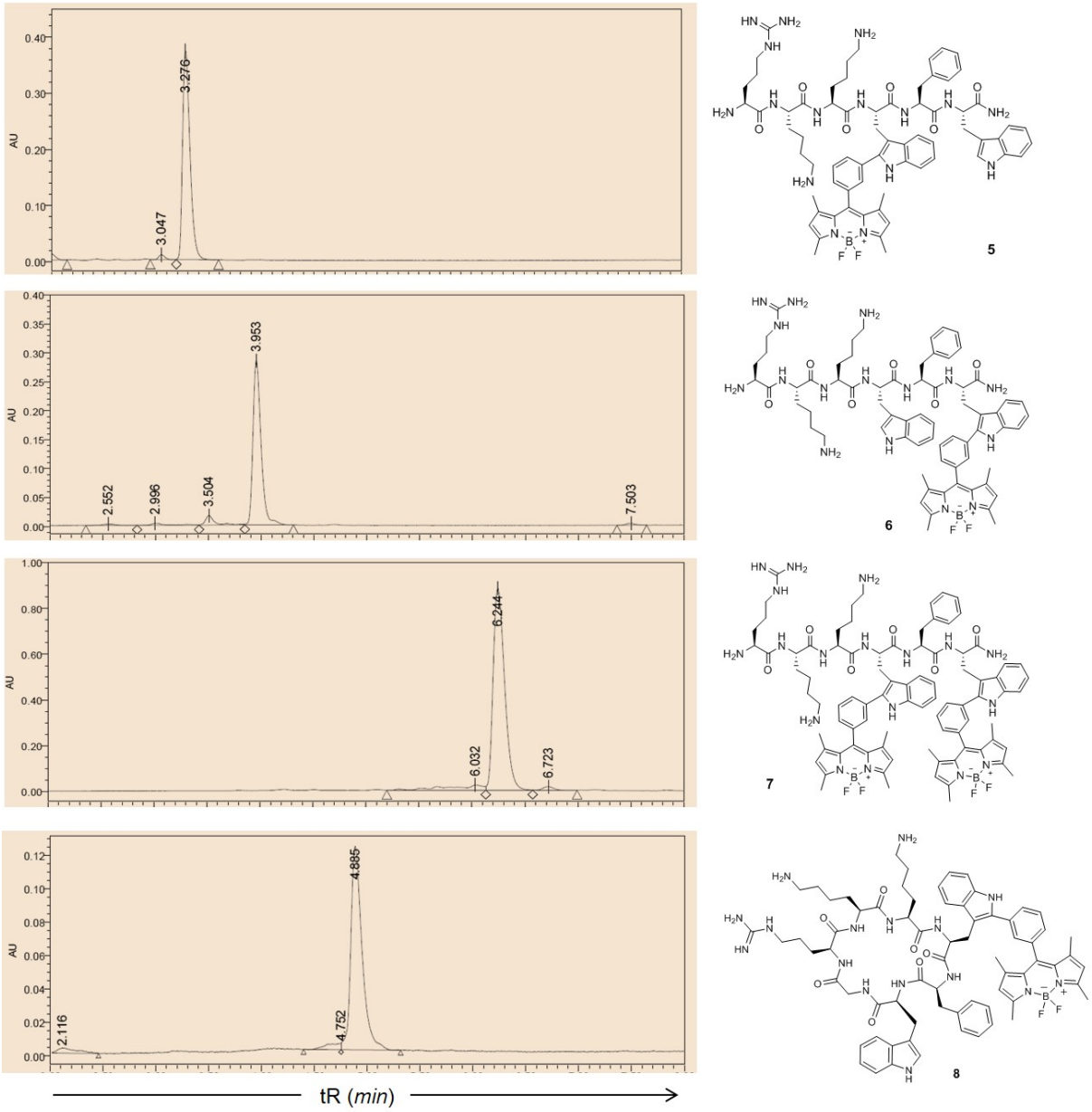
Cell viability assays.

Cell viability was determined with a TACS® MTT Cell Proliferation assay (Trevigen) according to the manufacturer's instructions. Briefly, human lung A549 epithelial cells were plated on 96-well plates the day before the experiment, reaching 90-95% confluence on the day of the experiment. The peptide **8** was added to the cells at different concentrations (0, 1.25, 2.5, 5 and 10 μM) and incubated at 37 ºC for 4 h. After 4 h, cells were washed, treated according to the manufacturer's instructions and their absorbance values (570 nm) were measured in a Synergy HT spectrophotometer (Biotek). Cell viability data was normalised to the proliferation of cells without addition of the peptide **8**.

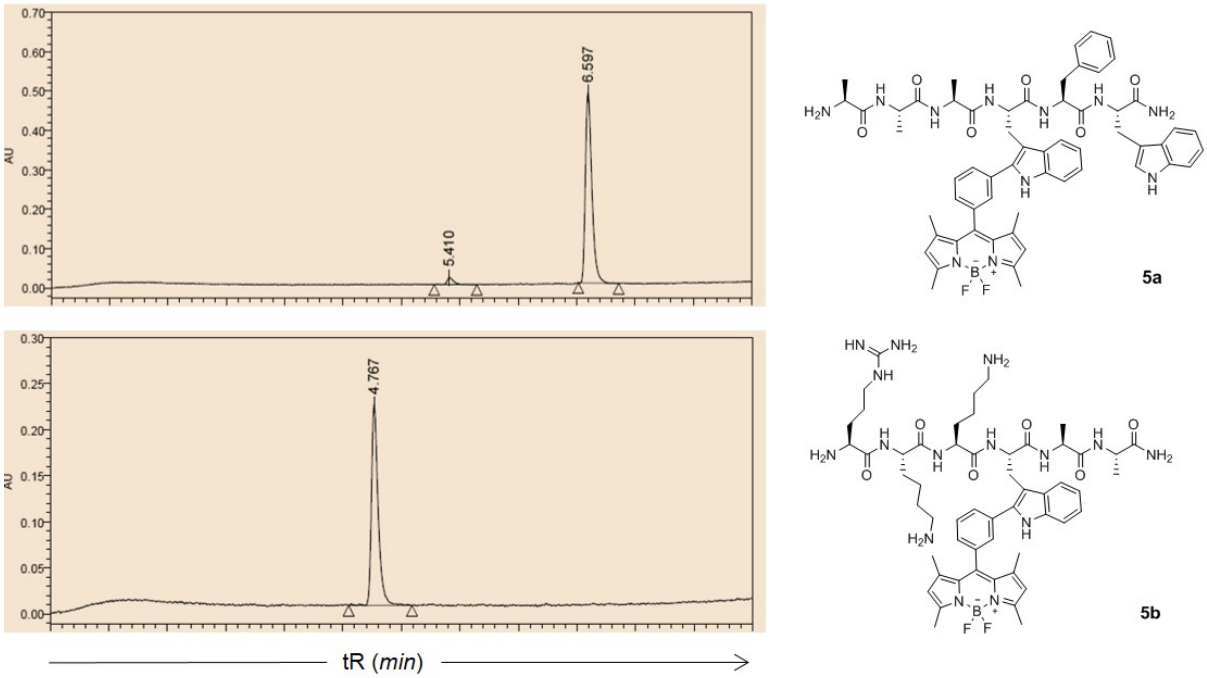


Supplementary Figure 17. Cell proliferation assays. Viability of human lung A549 epithelial cells after incubation with different concentrations of the peptide **8**. Data represented as means ± s.d. (*n*=4). No significant differences ($p > 0.05$) were determined between the control and any of the treatments.

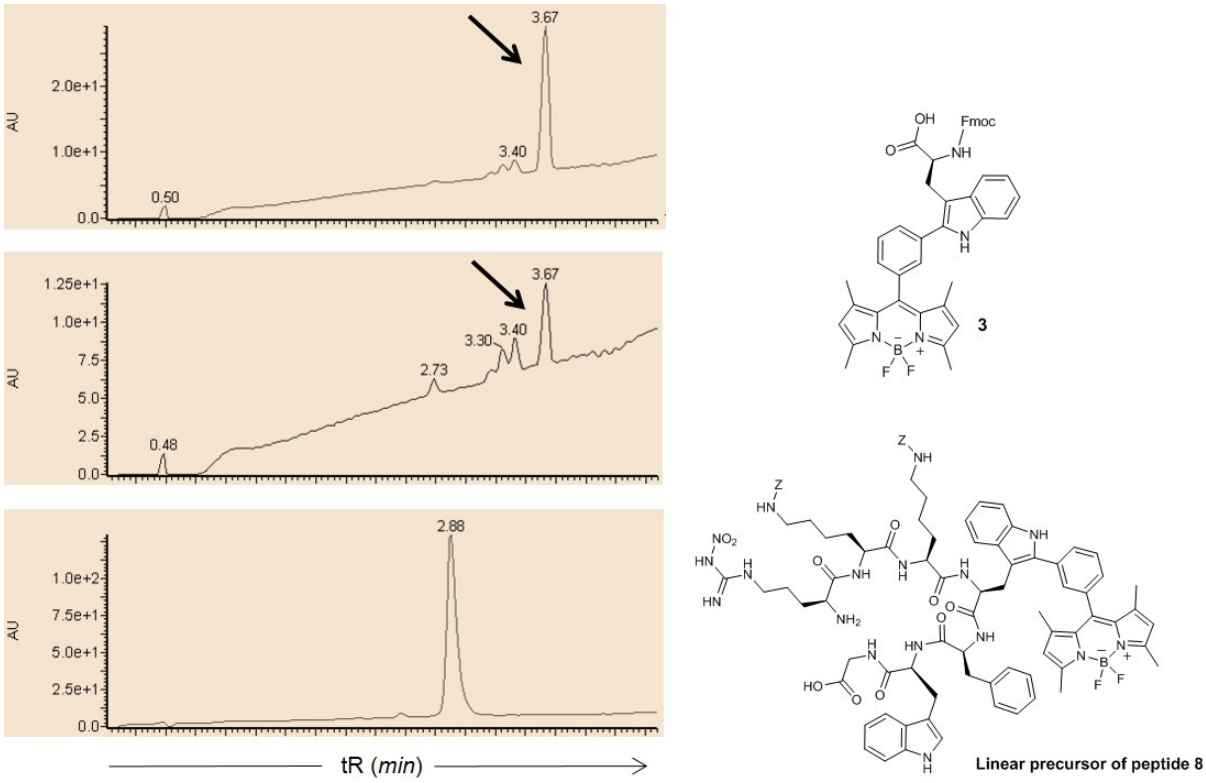
Chemical characterisation data.



Supplementary Figure 18. HPLC analysis of BODIPY-labelled peptides **5**-**8**.

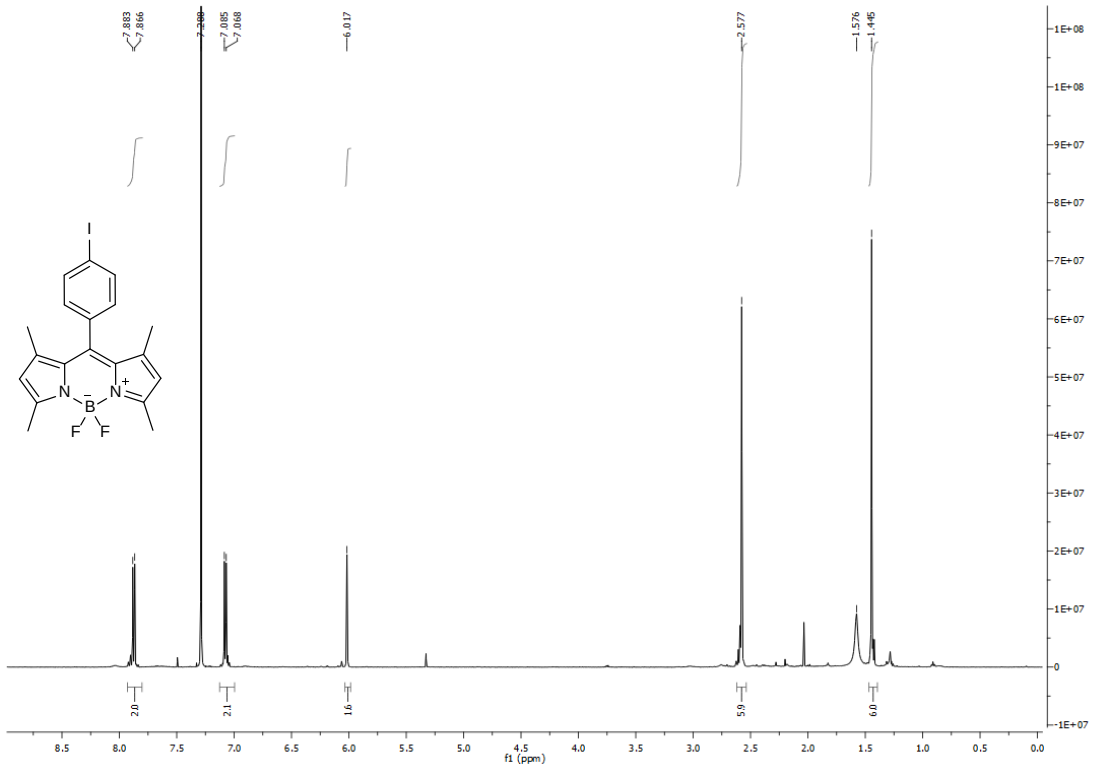


Supplementary Figure 19. HPLC analysis of BODIPY-labelled peptides **5a** and **5b.**

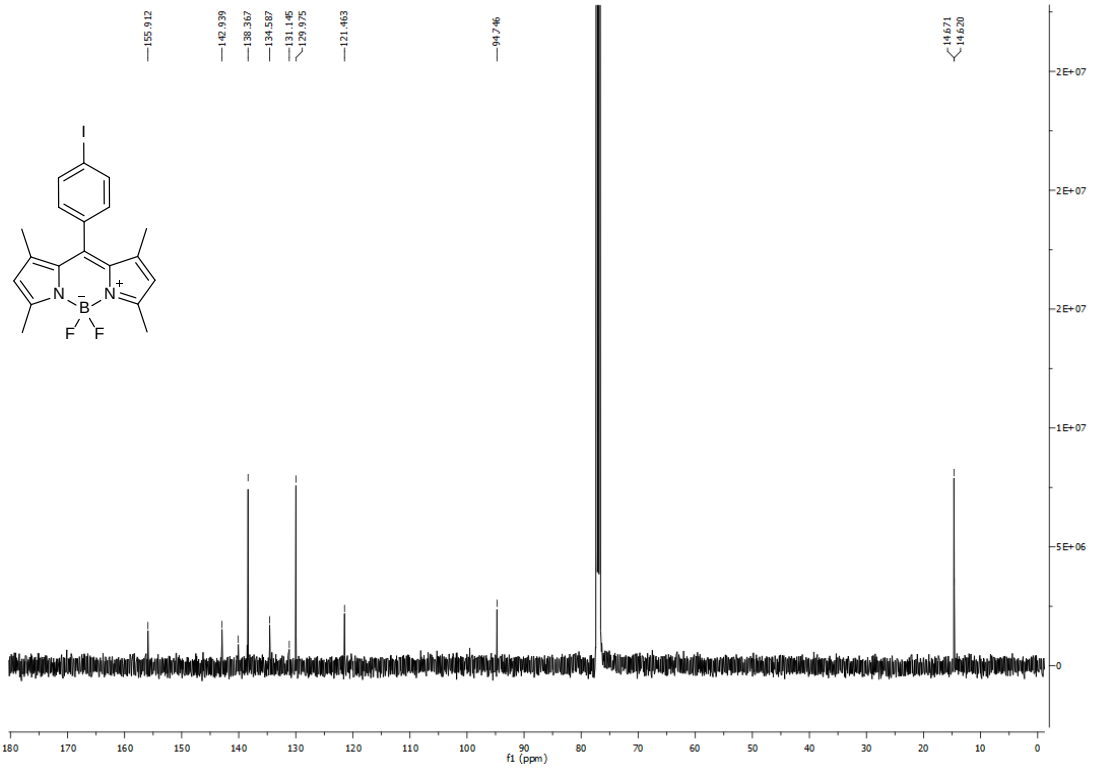


Supplementary Figure 20. Stability studies of BODIPY derivatives. HPLC analysis of Fmoc-Trp(C_2-BODIPY)-OH (**3**) in TFA:DCM (1:99) at r.t. after 10 min (83% purity) (*top panel*), Fmoc-Trp(C_2-BODIPY)-OH (**3**) in in TFA:DCM (1:9) at r.t. after 30 min (46% purity) (*mid panel*), crude BODIPY-labelled linear precursor of the cyclic peptide **8** directly after cleavage from the resin with TFA:DCM (1:99) at r.t. (*bottom panel*) (98% purity). Black arrows in top and mid panels point at the peak corresponding to the amino acid **3**.

^{1}H NMR ($CDCl_3$)

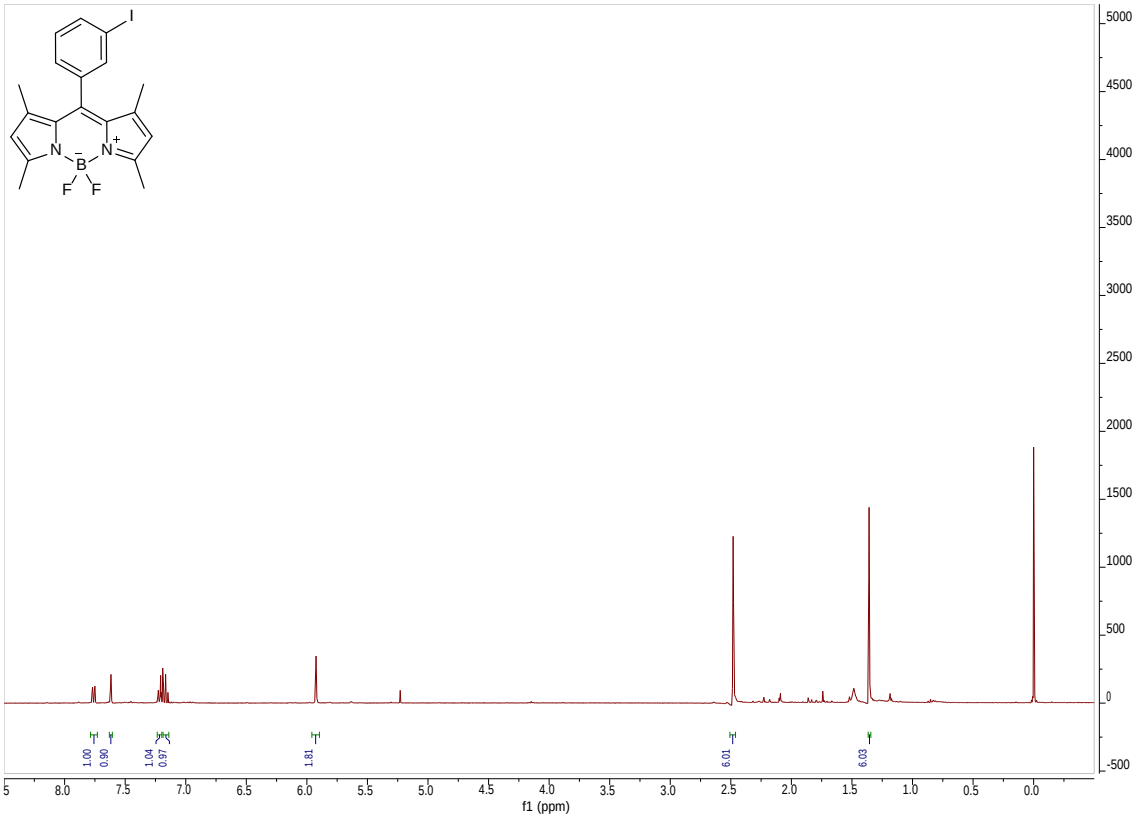


^{13}C NMR ($CDCl_3$)

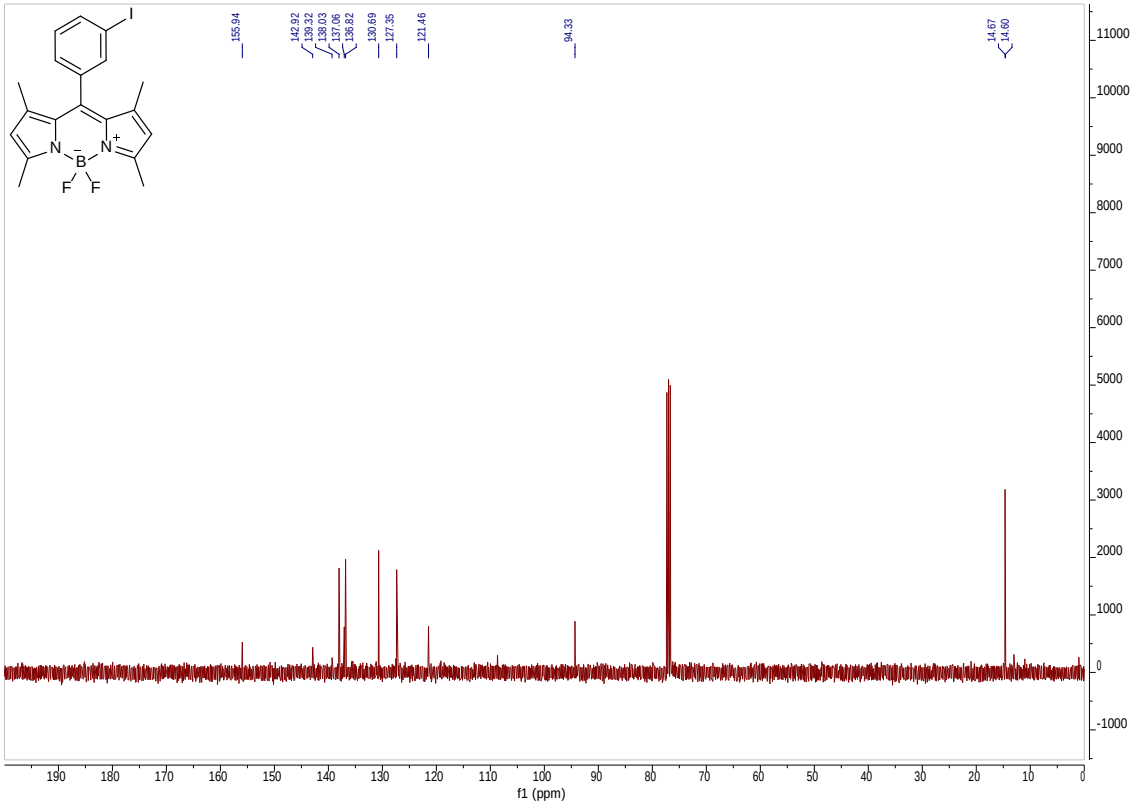


Supplementary Figure 21. NMR characterisation of 4,4-difluoro-8-(4-iodophenyl)-3,5-dimethyl-4-bora-*3a,4a*-diaza-*s*-indacene (**1**).

^{1}H NMR ($CDCl_3$)

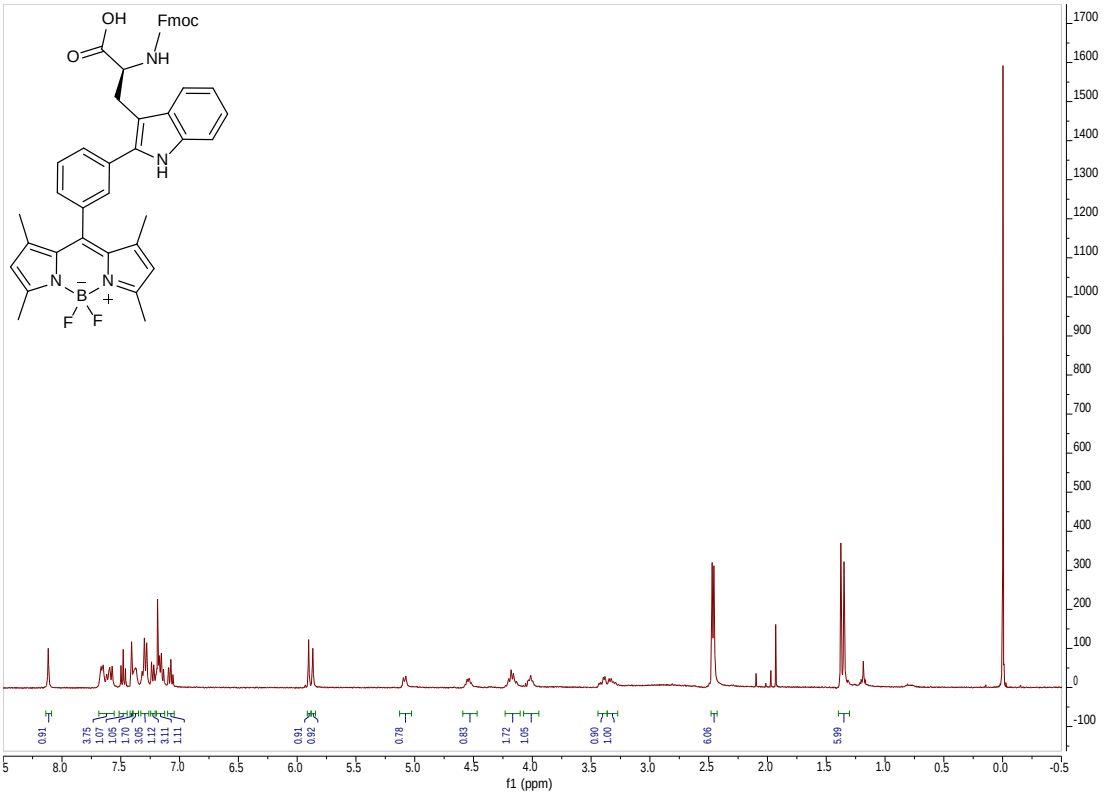


^{13}C NMR ($CDCl_3$)

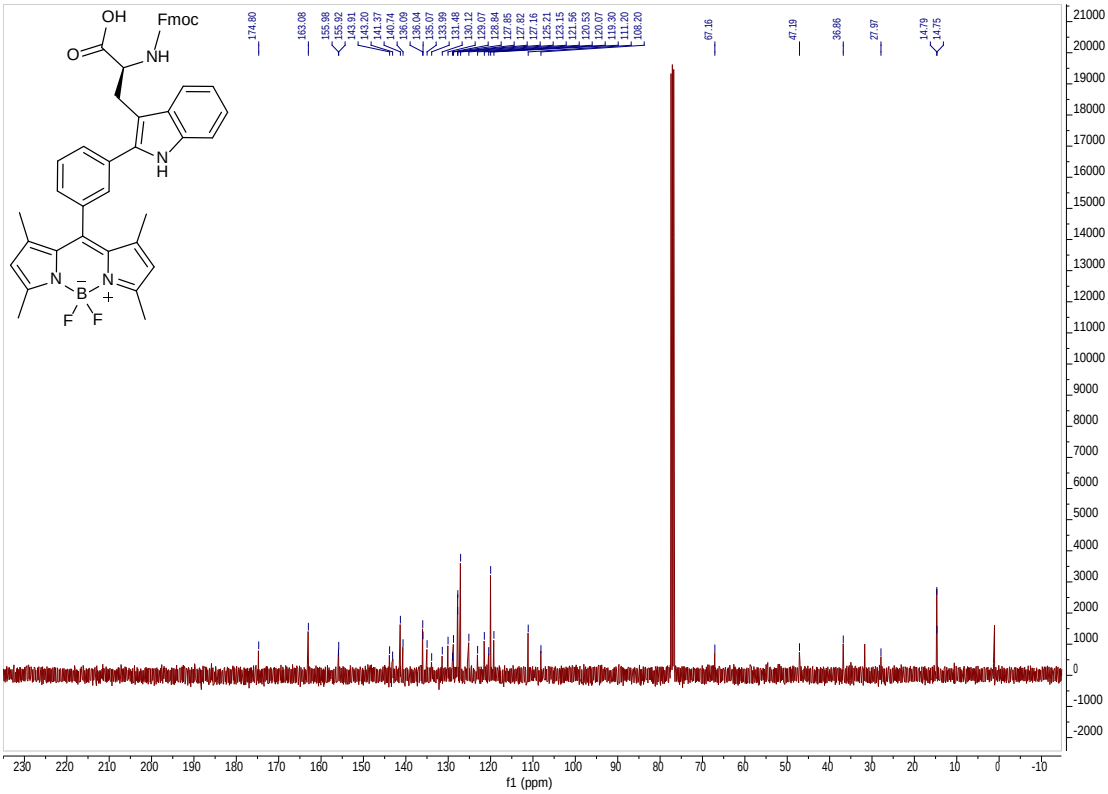


Supplementary Figure 22. NMR characterisation of 4,4-difluoro-8-(3-iodophenyl)-3,5-dimethyl-4-bora-*3a,4a*-diaza-*s*-indacene (**2**).

^{1}H NMR ($CDCl_3$)

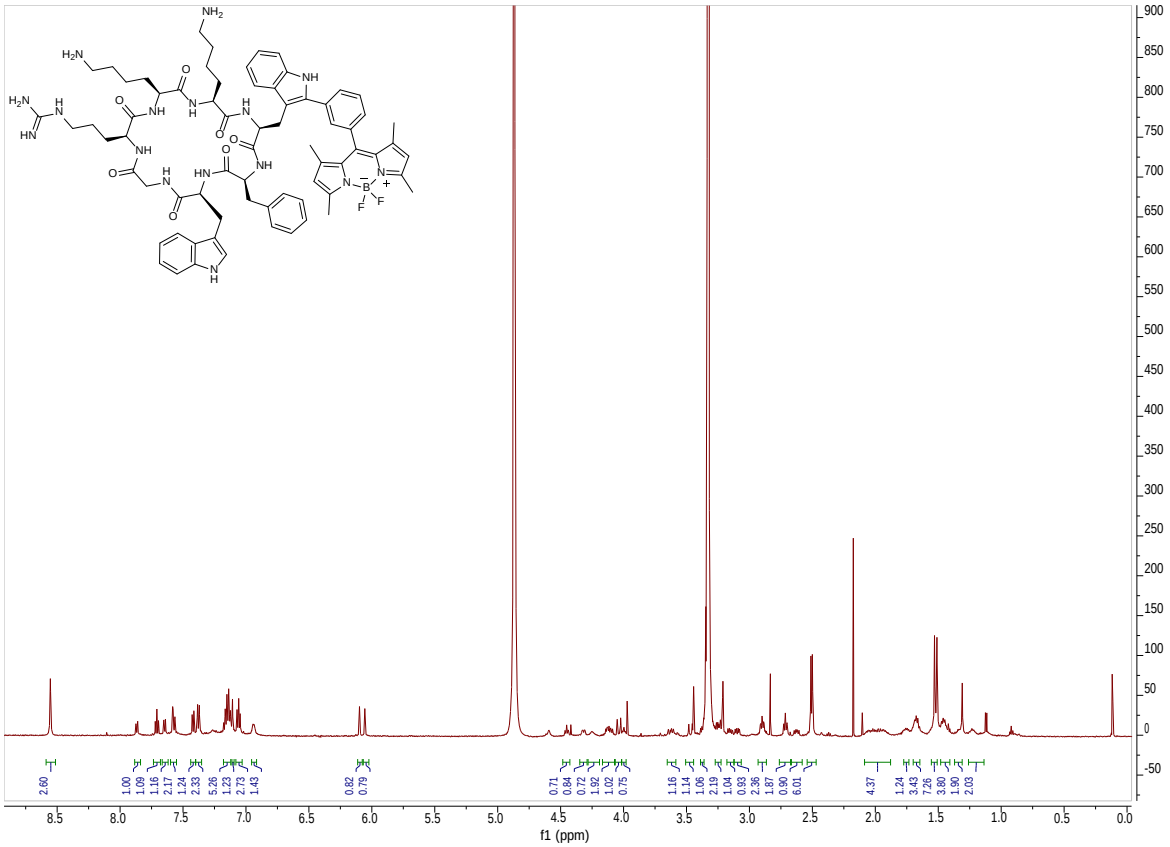


^{13}C NMR ($CDCl_3$)

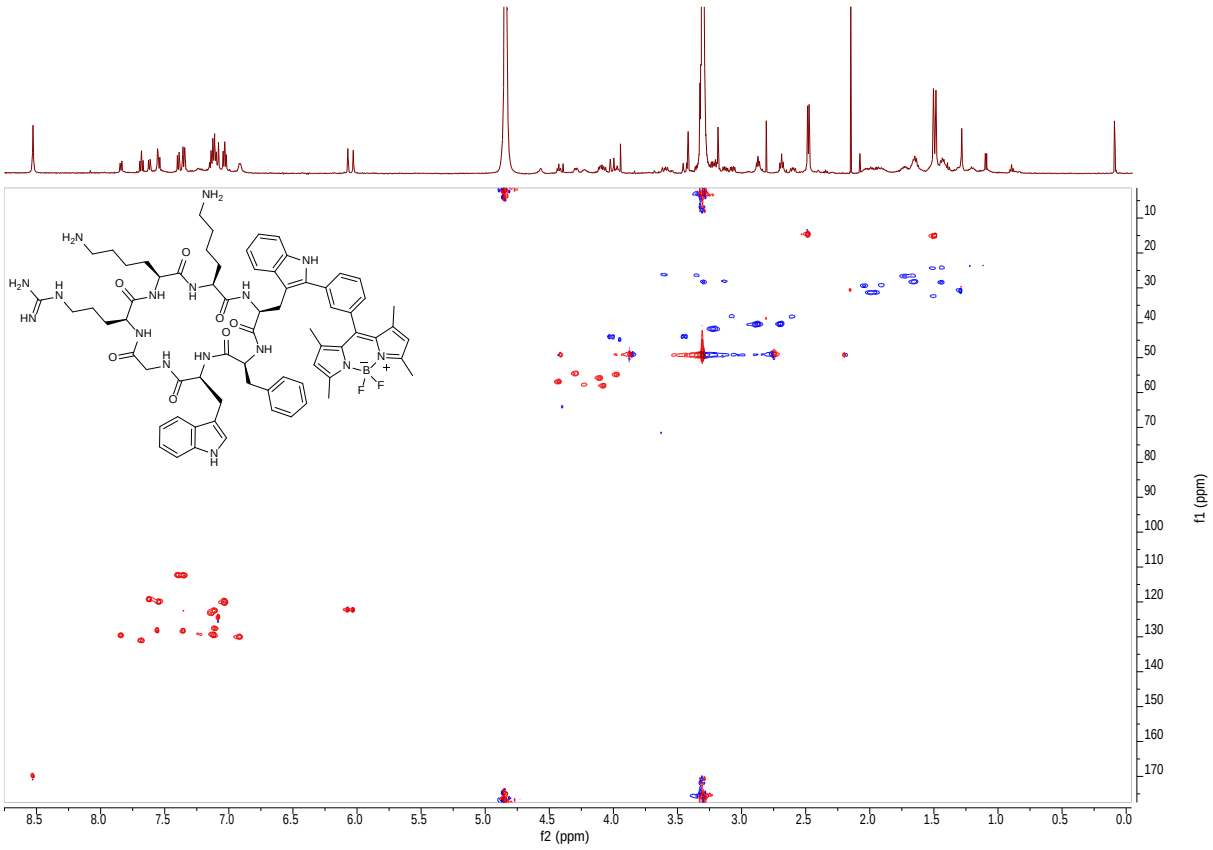


Supplementary Figure 23. NMR characterisation of Fmoc-Trp(C_2-BODIPY)-OH (**3**).

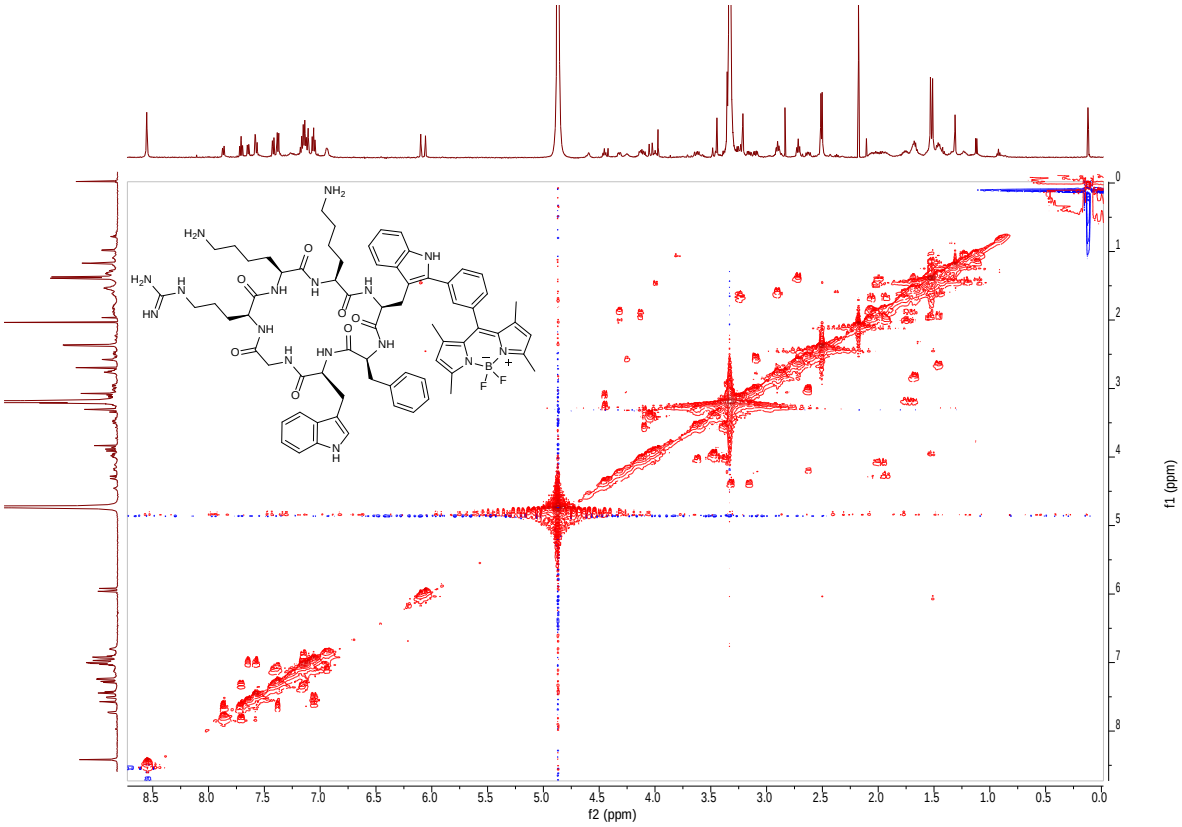
^{1}H NMR (MeOD)



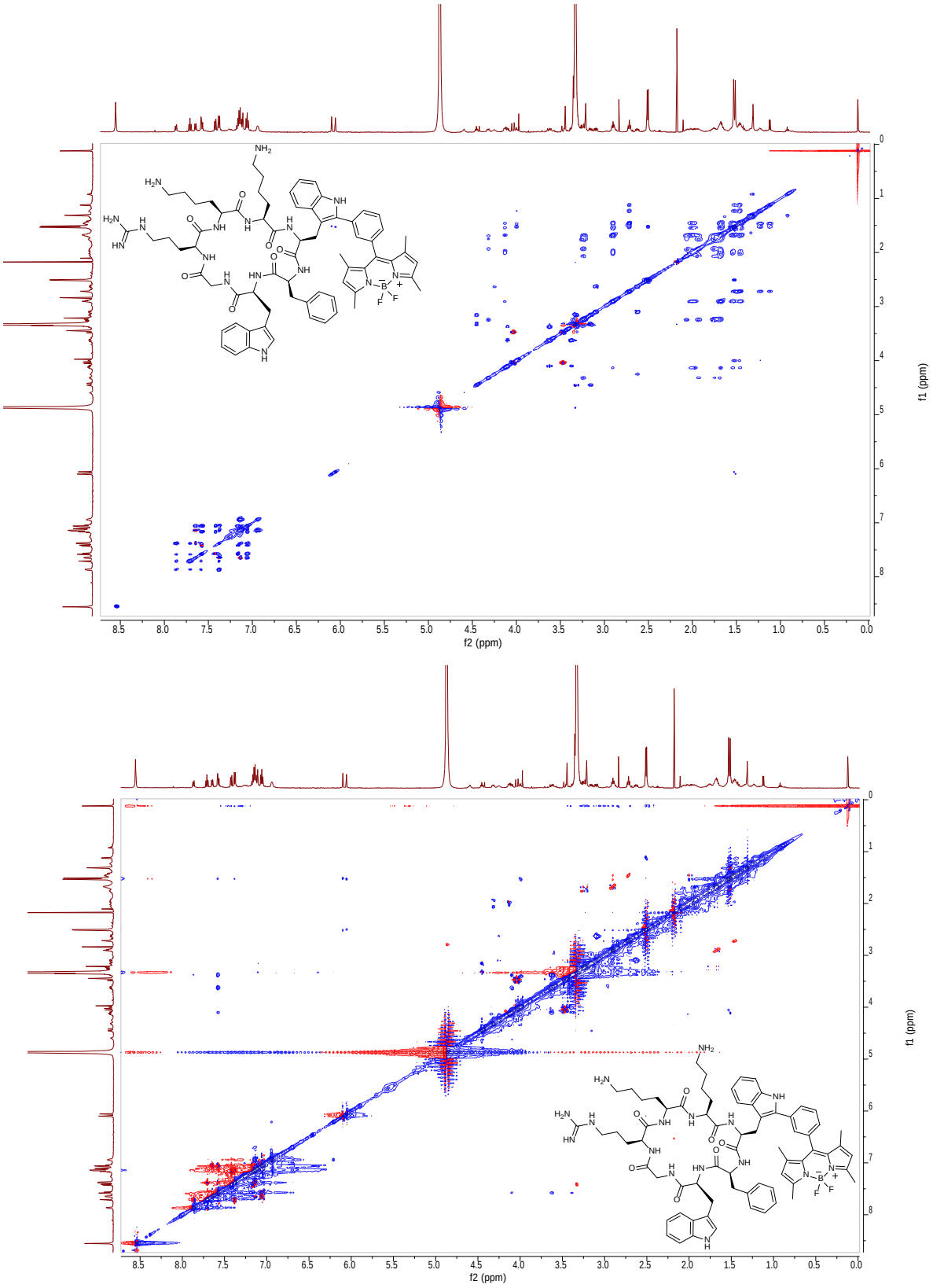
HSQC NMR (MeOD)



COSY NMR (MeOD)

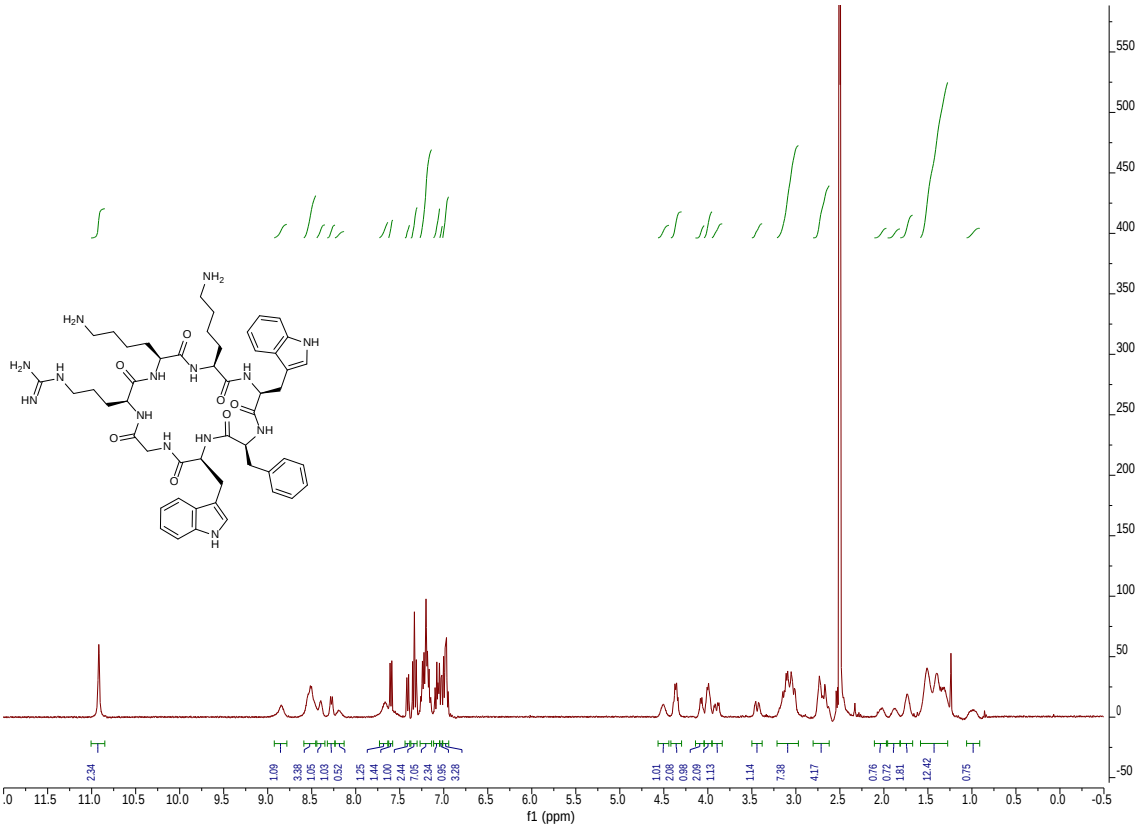


TOCSY NMR (MeOD)

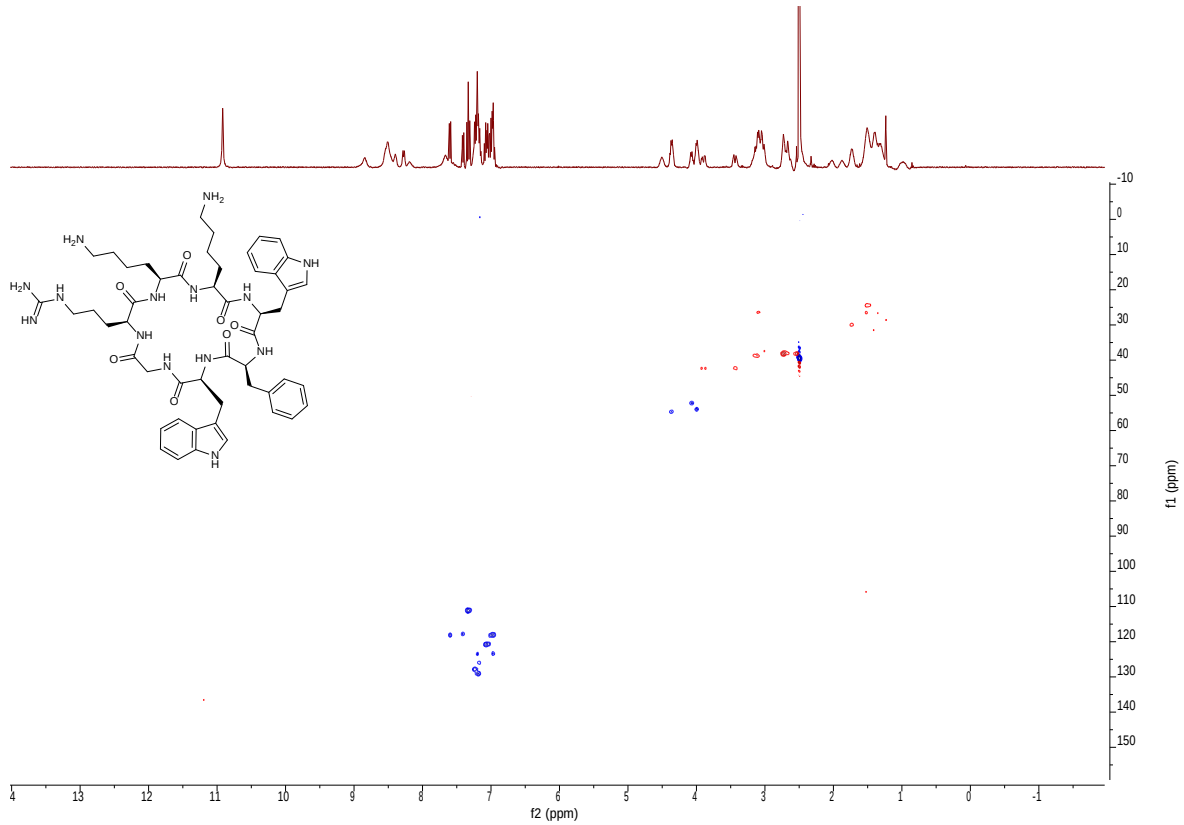


Supplementary Figure 24. NMR characterisation of cyclo(Arg-Lys-Lys-Trp(C_2-BODIPY)-Phe-Trp-Gly) (**8**).

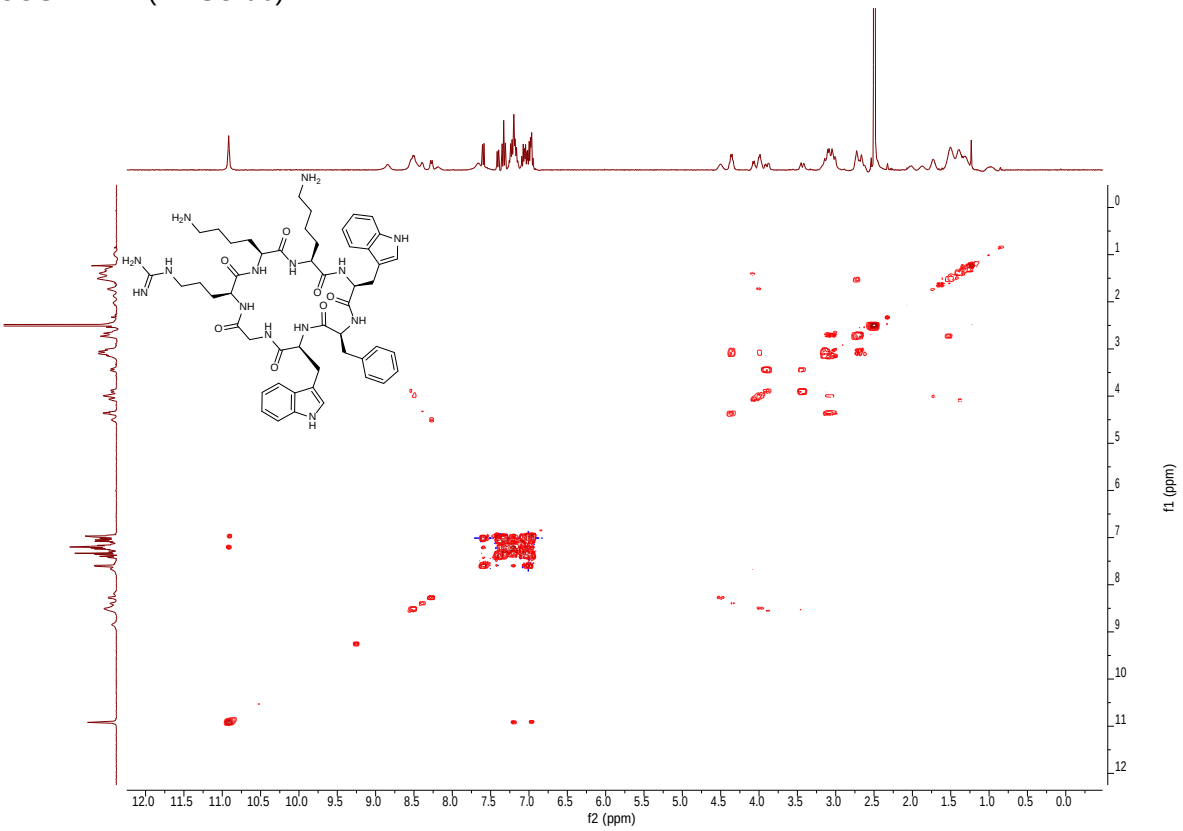
^{1}H-NMR (DMSO-*d6*)



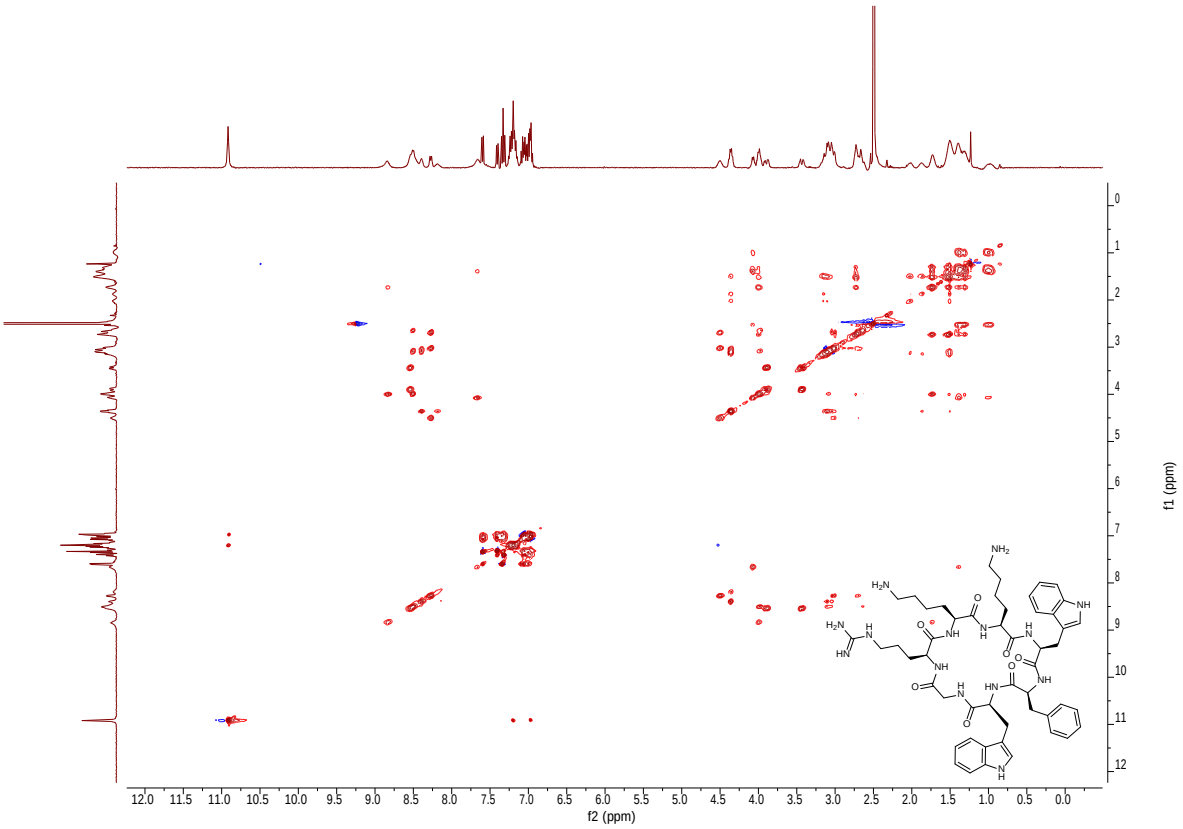
HSQC NMR (DMSO-*d6*)



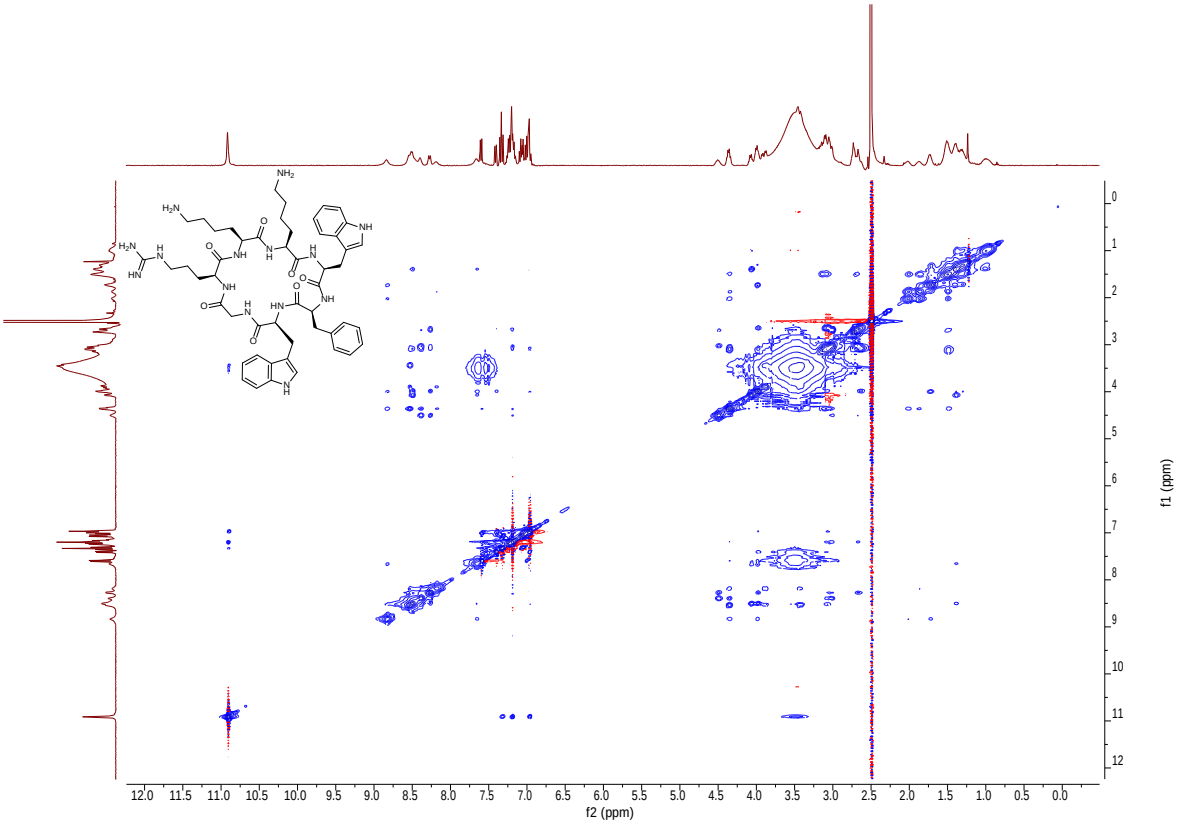
COSY NMR (DMSO-*d6*)



TOCSY NMR (DMSO-*d6*)



NOESY NMR (DMSO-*d6*)



Supplementary Figure 25. NMR characterisation of cyclo(Arg-Lys-Lys-Trp-Phe-Trp-Gly) (**9**).

Supplementary Discussion

Considerations about the influence of the aryl iodide substitution pattern.

Initially, we explored the interaction of Fmoc-Trp-OH with the *p*-iodophenyl-BODIPY **1**[2] under similar conditions reported for this type of reactions[3] [5 mol% $Pd(OAc)_2$, **1** (4.0 eq.), $AgBF_4$ (1.0 eq.) and *o*-nitrobenzoic acid (1.5 eq.) in DMF under microwave irradiation at 150 ºC for 5 min]. Under these conditions, no reaction occurred. We then decided to test the *m*-iodophenyl-BODIPY **2** under the same reaction conditions and we observed the formation of the desired product (**3**). Next, we performed the reaction of Fmoc-Trp-OH with **2** applying an optimised arylation protocol[4] [5 mol% $Pd(OAc)_2$, **2** (1.5 eq.), $AgBF_4$ (1.0 eq.) and TFA (1.5 eq.) in DMF under microwave irradiation at 80 ºC for 20 min], which afforded the expected product **3** in 74% yield. The derivative **3** was recovered as a stable orange solid and properly characterised.

A plausible explanation lies in the nature of the arylation mechanism. Considering that the process is likely to involve a Pd(II)/Pd(IV) catalytic cycle, the C-H activation step may take place first to yield indole-Pd(II) species, where the oxidative addition to the Ar-I bond should occur, to be followed by the reductive elimination leading to the biaryl release and the regeneration of the catalyst.[5,6] As the intermediate Pd(II) species are quite electrophilic, it is reasonable to think that electron-rich aryl halides will insert faster than deactivated derivatives. Therefore, BODIPY groups, which are considered electron-withdrawing, should exert a stronger deactivating effect in para position and a milder one in meta position, thus facilitating the insertion of Pd(II) species into the C-I bond in the latter case.[7] Recent experimental results in similar reactions seem to support this hypothesis.[8]

Supplementary Methods

General experimental information.

Unless stated otherwise, all reactions were carried out under argon in dried glassware. Commercially available reactants were used without further purification. Thin-layer chromatography was conducted on Merck silica gel 60 F254 sheets and visualized by UV (254 nm and 365 nm). Silica gel (particle size 35–70 μm) was used for flash column chromatography. HPLC analysis were performed on an Agilent 1100 separations module connected to a multiwavelength UV detection system using Discovery or Symmetry columns (C_{18}, 5 μm, 4.6 × 150 mm), unless otherwise stated. NMR spectra were recorded on 400 or 500 MHz spectrometers. Chemical shifts (δ) are reported in ppm. Multiplicities are referred by the following abbreviations: s = singlet, d = doublet, t = triplet, dd = double doublet, dt = double triplet and m = multiplet. HRMS (ESI positive) were obtained with a LTQ-FT Ultra (Thermo Scientific) mass spectrometer. MALDI analysis was performed on a Bruker Ultraflex mass spectrometer. All microwave reactions were carried out in 10 mL sealed glass tubes in a focused mono-mode microwave oven ("Discover" by CEM Corporation) featured with a surface sensor for internal temperature determination. Cooling was provided by compressed air ventilating the microwave chamber during the reaction. When stated, the final crude was purified via flash column chromatography CombiFlash ISCO RF provided with dual UV detection.

Experimental procedures and characterisation data.

4,4-Difluoro-8-(4-iodophenyl)-1,3,5,7-tetramethyl-4-bora-*3a,4a*-diaza-*s*-indacene (1).

4-iodobenzaldehyde (500 mg, 2.2 mmol) was dissolved in anhydride DCM (50 mL) under N_2. Then, 2,4-dimethylpyrrole (492 µL, 4.8 mmol) and 2 drops of TFA were added and the reaction was stirred overnight at r.t in N_2 atmosphere or until the consumption of the aldehyde was complete (TLC). DDQ (490 mg, 2.2 mmol) dissolved in DCM (20 mL) was added dropwise (10-15 min) to the reaction mixture and the reaction was stirred for 15 min at r.t. Finally, TEA (4 mL, 45 mmol) and BF_3OEt_2 (4 mL, 30 mmol) were added and the mixture stirred for 3 h. Workup was done by diluting with DCM (50 mL) and washing with H_2O (4 x 100 mL). The organic layers were combined, dried over sodium sulphate, filtered and concentrated under vacuum. The crude was purified via flash column chromatography using and DCM/hexane gradient on silica gel. The expected compound was isolated as a red amorphous solid (290 mg, 29%).

Characterisation data: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.87 (d, J = 8.5 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 6.02 (s, 2H), 2.58 (s, 6H), 1.45 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.9, 142.9, 138.3, 134.6, 131.1, 130.0, 121.5, 94.7, 14.7, 14.6; HRMS (m/z): $[M+H]^+$ calcd. for $C_{19}H_{18}BF_2IN_2$, 451.0654; found, 451.0651.

4,4-Difluoro-8-(3-iodophenyl)-1,3,5,7-tetramethyl -4-bora-*3a,4a*-diaza-*s*-indacene (2).

3-iodobenzaldehyde (500 mg, 2.2 mmol) was dissolved in anhydride DCM (50 mL) under N_2. Then, 2,4-dimethylpyrrole (492 µL, 4.8 mmol) and three drops of TFA were added and the reaction was stirred overnight at r.t in N_2 atmosphere or until the consumption of the aldehyde was complete (TLC). DDQ (490 mg, 2.2 mmol) dissolved in DCM (20 mL) was added dropwise (10-15 min) to the reaction mixture and the reaction was stirred for 15 min at r.t. Finally, TEA (4 mL, 45 mmol) and BF_3OEt_2 (4 mL, 30 mmol) were added and the mixture stirred for 3 h. Workup was done by diluting with DCM (50 mL) and washing with H_2O (4 x 100 mL). The organic layers were combined, dried over sodium sulfate, filtered and concentrated under vacuum. The crude was purified via flash column chromatography using and DCM/hexane gradient on silica gel. The expected compound was isolated as a red amorphous solid (401 mg, 41%).

Characterisation data: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (dt, J = 7.7, 1.5 Hz, 1H), 7.62 (t, J = 1.6 Hz, 1H), 7.24 – 7.20 (m, 1H), 7.19 – 7.14 (m, 1H), 5.92 (s, 2H), 2.48 (d, J = 1.3 Hz, 6H), 1.36 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.9, 142.9, 139.3, 138.0, 137.1, 136.8, 130.7, 127.3, 121.5, 94.3, 14.7, 14.6 (one quaternary carbon signal not seen); HRMS (m/z): $[M+H]^+$ calcd. for $C_{19}H_{18}BF_2IN_2$, 451.0654; found, 451.0651.

Fmoc-Trp(C_2-BODIPY)-OH (3).

Fmoc-Trp-OH (100 mg, 0.234 mmol), **2** (1.5 eq., 158 mg, 0.352 mmol), $AgBF_4$ (1.0 eq., 46 mg, 0.234 mmol), TFA (1.0 eq., 18 µL, 0.234 mmol) and $Pd(OAc)_2$ (0.05 eq., 2.6 mg, 0.0117 mmol) were placed in a microwave reactor vessel in 1.8 mL DMF. The mixture was heated under microwave irradiation (250 W) at 80°C for 20 min. EtOAc was added and the resulting suspension was filtered through Celite and concentrated under vacuum. The resulting crude was purified by flash column chromatography using and EtOAc/hexane gradient on silica gel. The expected adduct was isolated as a red solid (130 mg, 74%).

Characterisation data: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.69 – 7.56 (m, 4H), 7.48 (t, J = 7.7 Hz, 1H), 7.41 (t, J = 1.7 Hz, 1H), 7.37 (d, J = 4.9 Hz, 2H), 7.30 (t, J = 8.0 Hz, 3H), 7.25 – 7.21 (m, 1H), 7.20 – 7.13 (m, 3H), 7.07 (ddd, J = 8.0, 7.0, 1.1 Hz, 1H), 5.90 (s, 1H), 5.87 (s, 1H), 5.09 (d, J = 8.0 Hz, 1H), 4.55 (d, J = 7.5 Hz, 1H), 4.17 (q, J = 10.3, 9.4 Hz, 2H), 4.01 (s, 1H), 3.44 – 3.37 (m, 1H), 3.37 – 3.28 (m, 1H), 2.47 (s, 3H), 2.46 (s, 3H), 1.38 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.8, 163.1, 156.0, 155.9, 143.9, 143.2, 141.4, 140.7, 136.1, 136.0, 135.1, 133.9, 131.5, 130.1, 129.1, 128.8, 127.9, 127.8, 127.2, 125.2, 123.2, 121.6, 120.5, 120.1, 119.3, 111.2, 108.2, 67.2, 47.2, 36.9, 28.0, 14.8, 14.7; HRMS (m/z): $[M+Na]^+$ calcd. for $C_{45}H_{39}BF_2N_4O_4$, 771.2930; found, 771.2925.

General procedures for SPPS. All peptides were manually synthesized in polystyrene syringes fitted with a polyethylene porous disc using Fmoc-based SPPS. Solvents and soluble reagents were removed by suction. The Fmoc group was removed with piperidine-DMF (1:4) (1 × 1 min, 2 × 5 min). Peptide synthesis transformations and washings were performed at r.t.

Resin loading (only for 2-chlorotrityl polystyrene resin). Fmoc-AA-OH (1 eq.) was attached to the resin (1 eq.) with DIPEA (3 eq.) in DCM at r.t for 10 min and then DIPEA (7.0 eq.) for 40 min. The remaining trityl groups were capped adding 0.8 µL MeOH/mg resin for 10 minutes. After that, the resin was filtered and washed with DCM (4 x 1 min), DMF (4 x 1 min). The loading of the resin was determined by titration of the Fmoc group. Peptide elongation. After the Fmoc group was removed, the resin was washed with DMF (4 x 1 min), DCM (3 x 1 min), DMF (4 x 1 min). Unless otherwise noted, standard coupling procedures used DIC (3 eq.) and OxymaPure (3 eq.) in DMF for 1 h and 5-min of pre-activation. The completion of the coupling was monitored by the Kaiser test.[9] Then, the resin was filtered and washed with DCM (4 x 1 min) and DMF (4 x 1 min). Final cleavage (for Sieber amide and for 2-chlorotrityl polystyrene resins). The resin bound peptide was treated for 5 times with 1% TFA in DCM (1 min in each treatment) and washed with DCM. The combined filtered mixtures were poured over DCM and evaporated under vacuum. Then, the residue was dissolved in ACN:H_2O and liophilised.

General procedures for linear peptides 4-7. Syntheses were performed on Sieber amide resin (0.69 mmol/g). Amino acid **3** (1.5 eq.) was incorporated with a 5-min pre-activation using DIC (1.5 eq.) and OxymaPure (1.5 eq.) in DMF for 1h. For Fmoc-Arg-OH (3 eq.), we performed several treatments (up to 9) with DIC (3 eq.) and HOBt (3 eq.) in DMF for 15 min without pre-activation. The rest of amino acids (Fmoc-AA-OH, 3 eq.) were incorporated with a 5-min pre-activation with DIC (3 eq.) and OxymaPure (3 eq.) in DMF for 1 h. Peptides were purified by semi-preparative RP-HPLC (XBRIDGE™ BEH 130,

C_{18}, 5 µM OBD 19×50 mm column). Mobile phase: ACN (0.1% HCOOH)/H_2O (0.1% HCOOH); flow rate: 20 mL/min. Pure fractions were lyophilised furnishing the corresponding peptides.

H-Arg-Lys-Lys-Trp-Phe-Trp-NH_2 (4). Synthesised as previously reported.[10]

White powder (5.0 mg).

Characterisation data: HPLC: t_R: 3.37 min (95% purity); HRMS (m/z): $[M+H]^+$ calcd. for $C_{49}H_{67}N_{13}O_7$, 950.5365; found, 950.5364.

H-Arg-Lys-Lys-Trp(C_2-BODIPY)-Phe-Trp-NH_2 (5).

Red powder (3.6 mg).

Characterisation data: HPLC: t_R: 3.28 min (90% purity); HRMS (m/z): $[M+H]^+$ calcd. for $C_{68}H_{85}BF_2N_{16}O_6$,1271.6977; found, 1271.6947.

H-Ala-Ala-Ala-Trp(C_2-BODIPY)-Phe-Trp-NH_2 (5a).

Red powder (20 mg).

Characterisation data: HPLC: t_R: 6.60 min (96% purity). HRMS (m/z): $[M+H]^+$ calcd. for $C_{59}H_{64}BF_2N_{11}O_6$: 1072.5175; found, 1072.5183.

H-Arg-Lys-Lys-Trp(C_2-BODIPY)-Ala-Ala-NH_2 (5b).

Red powder (7 mg).

Characterisation data: HPLC: t_R: 4.77 min (99% purity). HRMS (m/z): $[M+H]^+$ calcd. for $C_{54}H_{76}BF_2N_{15}O_6$: 1080.6237; found, 1080.6250.

H-Arg-Lys-Lys-Trp-Phe-Trp(C_2-BODIPY)-NH_2 (6).

Red powder (1.8 mg).

Characterisation data: HPLC: t_R: 3.95 min (90% purity); HRMS (m/z): $[M+H]^+$ calcd. for $C_{68}H_{85}BF_2N_{16}O_6$, 1271.6977; found: 1271.6937.

H-Arg-Lys-Lys-Trp(C_2-BODIPY)-Phe-Trp(C_2-BODIPY)-NH_2 (7).

Red powder (16.8 mg).

Characterisation data: HPLC: t_R: 6.24 min (91% purity); HRMS (m/z): $[M+H]^+$ calcd. for $C_{87}H_{103}B_2F_4N_{18}O_6$, 1593.8449; found, 1593.8532.

Cyclo(Arg-Lys-Lys-Trp(C_2-BODIPY)-Phe-Trp-Gly) (8). The synthesis was performed on 91 mg of 2-chlorotrityl polystyrene resin (0.94 mmol/g). Amino acid **3** (1.5 eq.) was incorporated with PyBOP (1.5 eq.), HOBt (1.5 eq.) and DIPEA (2.0 eq.) in DMF for 1 h. Other Fmoc amino acids (3 eq.) were incorporated with a 5 min pre-activation with DIC (3 eq.) and OxymaPure (3 eq.) in DMF for 1 h. After cleavage as described above, the protected linear peptide (119 mg, 0.073 mmol) was dissolved in 1.3 mL of DMF (0.055 M). DIPEA (2.5 eq., 32 µL, 0.182 mmol) and HATU (1.0 eq., 28 mg, 0.073 mmol) were added. The solution was stirred at r.t. until the cyclisation was complete (approx. 2 h). The cyclic peptide was precipitated by adding H_2O to the solution. The precipitate was washed with H_2O, decanted and dried, obtaining 109 mg of crude protected cyclic peptide (87% yield). The crude protected macrocycle (108 mg, 0.067 mmol) and 20% $Pd(OH)_2$-C (54 mg) were dissolved in 5% HCOOH/MeOH (10.8 mL), previously purged with Ar. Then, the reaction flask was flushed again with Ar, evacuated and filled with H_2. The reaction mixture was stirred under H_2 for 72 h (H_2 balloons were refilled periodically during the reaction along with re-addition of $Pd(OH)_2$-C (4 times). The catalyst was removed by filtration

and the filtrate was evaporated to afford 46 mg of the crude deprotected peptide. The final peptide was purified by PoraPak Rxn RP 60 cc reverse phase column. Mobile phase: ACN (0.1% HCOOH)/H_2O (0.1% HCOOH). Pure fractions were lyophilised furnishing the corresponding peptide.
Red powder (30 mg).
Characterisation data: ^{1}H NMR (600 MHz, CD_3OD): δ 8.55 (s, 3H), 7.89 – 7.84 (m, 1H), 7.71 (t, J = 7.7 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.60 – 7.55 (m, 2H), 7.42 (dt, J = 8.1, 0.9 Hz, 1H), 7.40 – 7.36 (m, 2H), 7.18 – 7.12 (m, 5H), 7.11 (s, 1H), 7.06 (m, 3H), 6.94 (m, 1H), 6.10 (s, 1H), 6.06 (s, 1H), 4.45 (t, J = 7.4 Hz, 1H), 4.32 (d, J = 10.5 Hz, 1H), 4.25 (m, 1H), 4.17 – 4.07 (m, 2H), 4.04 (d, J = 16.5 Hz, 1H), 4.00 (m, 1H), 3.63 (dd, J = 14.9, 9.5 Hz, 1H), 3.51 – 3.45 (m, 1H), 3.38 (m, 1H), 3.30 (1H), 3.27 – 3.23 (m, 2H), 3.15 (dd, J = 14.2, 7.5 Hz, 1H), 3.10 (dd, J = 14.0, 5.9 Hz, 1H), 2.90 (td, J = 8.6, 4.2 Hz, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.63 (dd, J = 14.2, 8.5 Hz, 1H), 2.51 (s, 3H), 2.50 (s, 3H), 2.09 – 1.88 (m, 4H), 1.76 (m, 1H), 1.67 (m, 3H), 1.52 (m, 7H), 1.48 – 1.41 (m, 4H), 1.37 (m, 2H), 1.26 – 1.14 (m, 2H);
HPLC: $t_{R:}$ 4.45 min (98% purity); HRMS (m/z): $[M+H]^+$ calcd. for $C_{70}H_{86}BF_2N_{16}O_7$, 1311.6926; found, 1311.6864.

Cyclo(Arg-Lys-Lys-Trp-Phe-Trp-Gly) (9). The synthesis was performed on 500 mg of 2-chlorotrityl polystyrene resin (1.0 mmol/g). Fmoc amino acids (3 eq.) were incorporated with a 5-min pre-activation with DIC (3 eq.) and OxymaPure (3 eq.) in DMF for 1 h. After cleavage as described above, the fully protected linear peptide (300 mg, 0.18 mmol) was dissolved in 3.5 mL of DMF (0.052 M). DIPEA (2.5 eq., 79 µL, 0.18 mmol) and HATU (1.0 eq., 69 mg, 0.18 mmol) were added. The solution was stirred at r.t until the cyclisation was complete (2 h). The cyclic peptide was precipitated by adding H_2O to the solution and the precipitate was washed with H_2O, decanted and dried. The protected linear peptide was dissolved in TFA:TIS:H_2O (95:2.5:2.5) and the solution was stirred at r.t. for 2 h. The resulting solution was evaporated under vacuum, washed with Et_2O, dissolved in ACN:H_2O and liophilised to afford 231 mg of the crude peptide (72% yield). 145 mg of peptide were purified by PoraPak Rxn RP 60 cc reverse phase column. Mobile phase: ACN (0.1% HCOOH)/H_2O (0.1% HCOOH). Pure fractions were lyophilised furnishing the corresponding pure cyclic peptide.
White powder (51 mg).
Characterisation data: ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.92 (s, 2H), 8.92 – 8.78 (m, 1H), 8.59 – 8.45 (m, 3H), 8.44 – 8.35 (m, 1H), 8.27 (d, J = 8.4 Hz, 1H), 8.23 – 8.13 (m, 1H), 7.73 – 7.63 (m, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.41 (d, J = 7.9 Hz, 1H), 7.38 – 7.29 (m, 1H), 7.26 – 7.13 (m, 7H), 7.11 – 7.04 (m, 2H), 7.02 (dd, J = 3.9, 1.1 Hz, 1H), 7.01 – 6.94 (m, 3H), 4.56 – 4.44 (m, 1H), 4.36 (q, *J* = 6.9 Hz, 2H), 4.14 – 4.04 (m, 1H), 4.00 (q, J = 6.7 Hz, 2H), 3.90 (dd, J = 16.9, 6.0 Hz, 1H), 3.50 – 3.38 (m, 1H), 3.21 – 2.97 (m, 7H), 2.80 – 2.62 (m, 4H), 2.10 – 1.97 (m, 1H), 1.95 – 1.81 (m, 1H), 1.81 – 1.67 (m, 2H), 1.58 – 1.27 (m, 12H), 1.06 – 0.91 (m, 1H); HPLC: $t_{R:}$ 1.55 min (98% purity); HRMS (m/z): $[M]^+$ calcd. for $C_{51}H_{68}N_{14}O_7$, 988.5395; found, 988.5387.

Supplementary References

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Oxidative transformations on tryptophan-based diketopiperazines through cross dehydrogenative couplings

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Abbreviations

Abbreviation used for amino acids and designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in J. Biol. Chem. 247, 977-983 (1982). The following additional abbreviations are used: ACN: acetonitrile, Ala: alanine, Asp: aspartic acid, BQ: 1,4-benzoquinone, CD: circular dichroism, DCM: dichloromethane, DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone, DIEA: N,N-diisopropylethylamine, DKP: diketopiperazine, DLP: Dilauroyl peroxide, DMF: N,N-dimethylformamide, DMSO: dimethyl sulfoxide, DTBP: Di-*tert*-butyl peroxide, Gly: glycine, HBTU: *o*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate, HPLC-MS: high performance liquid chromatography mass spectrometry, HRMS(ESI): high-resolution mass spectrometry (electrospray ionization), Fmoc: 9H-fluorenylmethyloxycarbonyl, Leu: leucine, Lys: lysine, MW: microwave, NMR: nuclear magnetic resonance, Phe: phenylalanine, PIFA: bis(trifluoroacetoxy)iodobenzene, Pro: proline, TBH: *tert*-Butyl hydroperoxide, TFA: trifluoroacetic acid, Trp: tryptophan, Z: benzyloxylcarbonyl.

General experimental information

Reactions were monitored by HPLC-MS using a HPLC Waters Alliance HT comprising a pump (Edwards RV12) with degasser, an autosampler and a diode array detector. Flow from the column was split to a MS spectrometer. The MS detector was configured with an eletrospray ionization source (micromass ZQ4000) and nitrogen was used as the nebulizer gas. Data acquisition was performed with MassLynx software. Unless otherwise stated, yields are for the isolated pure compound. All microwave reactions were carried out in 10 mL sealed glass tubes in a focused mono-mode microwave oven ("Discover" by CEM Corporation) featured with a surface sensor for internal temperature determination. Cooling was provided by compressed air ventilating the microwave chamber during the reaction. When stated, the final crude was purified via flash column chromatography Combi Flash ISCO RF provided with dual UV detection.

NMR spectra of peptides in DMSO-d_6 were acquired with either a Varian Mercury 400 MHz or a Bruker DMX-500 MHz spectrometer. The spectra were referenced relative to the residual DMSO signal (^{1}H, 2.50 ppm; ^{13}C, 39.5 ppm). Chemical shifts (δ) are reported in ppm. Multiplicities are referred by the following abbreviations: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, ddd: doublet of doublet of doublets, dt = doublet of triplets, td: triplet of doublets, and m = multiplet. HRMS (ESI positive) were obtained with a LTQ-FT Ultra (Thermo Scientific) mass Spectrometer.

CD spectroscopy. Circular dichroism (CD) measurements were performed using a Jasco J-815 spectrophotometer. The spectra were recorded from 300 to 190 nm using a 1.0 mm path-length quartz cuvette at 2 nm bandwidth, 50 nm·min^{-1} scan speed, 0.5 s response time, 0.2 nm data pitch and four-scan accumulations. The background signal of the buffer alone was subtracted for each spectrum. CD spectra were converted from raw ellipticity (θ, mdeg) to molar ellipticity ([θ], deg·cm^{-2}·dmol^{-1}). All the samples were dissolved in MeOH at 0.3 mM final concentration.

Chiral chromatography. The chiral chromatography was performed in a chiral HPLC column (Chiralpak ia, Amylose tris(3,5-dimethylphenylcarbamate) immobilized on 5 µm silica gel, 250 ×

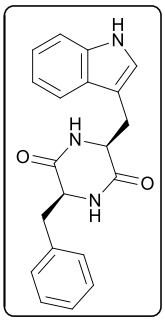
4.6 mm), at a flow rate of 1 mL·min^{-1} and a linear gradient of ACN (+0.036% TFA) into H_2O (+0.045% TFA) from 50% to 70% ACN for 30 min.

Experimental procedures and peptide characterization

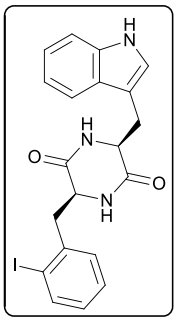
General procedure for the synthesis of 2,5-Diketopiperazines 1a-j

Unless stated otherwise, all DKPs were synthesized using the following procedure. Fmoc-AA-OH (1.0 eq), H-Trp-OMe·HCl (1.0 eq), HBTU (1.0 eq) and DIEA (2.0 eq) were dissolved in DMF and the solution was stirred at r.t. for 24 h followed by evaporation under vacuum. The resulting suspension was dissolved in ethyl acetate and washed with saturated aqueous solution of $NaHCO_3$ (×5). Then, the organic phase was dried over Na_2SO_4, filtered and the solvent was removed under vacuum obtaining the desired dipeptide. The resulting white solid was suspended in 20% piperidine/ACN and stirred for 16 h. The resulting suspension was concentrated under vacuum and washed with diethyl ether (×5). The white solid obtained was dried to yield the corresponding pure product (66-93% isolated yields). Cyclo(Pro-Trp) stereoisomers **1f-h** were prepared according to a previously published procedure on brevianamide arylation disclosed by the group.[1]

Cyclo(Phe-Trp) (**1a**). Compound **1a** was prepared using Fmoc-Phe-OH (2.32 g, 5.99 mmol, 1.0 eq), following the general procedure for the synthesis of DKPs to obtain the desired product as a white solid (1.86 g, 93%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.87 (s, 1H), 7.89 (d, *J* = 2.7 Hz, 1H), 7.68 (d, *J* = 1.7 Hz, 1H), 7.47 (d, *J* = 7.9 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.15 (m, 3H), 7.05 (t, *J* = 7.5 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 2.6 Hz, 1H), 6.69 (dd, *J* = 6.7 Hz, 2H), 3.96 (m, 1H), 3.84 (m, 1H), 2.79 (dd, *J* = 14.5, 4.4 Hz, 1H), 2.53 (m, 1H), 2.43 (d, *J* = 4.6 Hz, 1H), 1.84 (dd, *J* = 13.4, 7.0 Hz, 1H) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ 166.8, 166.2, 136.5, 136.0, 129.7, 128.0, 127.5, 126.4, 124.4, 120.9, 118.8, 118.4, 111.3, 108.8, 55.6, 55.3, 29.7 ppm.

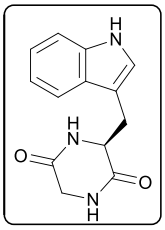


Cyclo(2-I-Phe-Trp) (**1b**). Compound **1b** was prepared using Fmoc-Phe(2-I)-OH (1.50 g, 2.93 mmol, 1.0 eq), H-Trp-OMe·HCl (730 mg, 2.93 mmol, 98%, 1.0 eq), HBTU (1.10 g, 2.93 mmol, 1.0 eq), DIEA (1.0 mL, 5.86 mmol, 2.0 eq) which were dissolved in DMF (3.3 mL). The pale yellow solution was stirred at r.t. for 24 h. The resulting solution was precipitated over 10 mL of cold water and centrifugated (RPM: 2000, t: 5 min, T= 4 °C). Precipitation cycles in cold water and centrifugation were repeated 8 times obtaining the desired adduct (2.6 g, 89%). The white solid was suspended in 20% piperidine/ACN (15 mL) and stirred for 22 h and the resulting solution was precipitated over 10 mL of cold water and centrifugated (RPM: 2000, t: 5 min, T= 4 °C). Precipitation cycles in cold water and centrifugation were repeated 2 times. The crude was purified by flash chromatography on silica using DCM/MeOH:DCM (1:5) to yield the pure product **1b** as a white solid (1.0 g, 85% yield). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.97 (d, *J* = 2.4 Hz, 1H), 8.21 (d, *J* = 2.7 Hz, 1H), 7.66 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.62 (dd, *J* = 7.5, 1.3 Hz, 1H), 7.44 (d, *J* = 3.2 Hz, 1H), 7.29 (d, *J* = 7.6 Hz, 1H), 7.11 (d, *J* = 2.3 Hz, 1H), 7.13-7.02 (m, 2H), 6.94 (td, *J* = 7.5, 1.3 Hz, 1H), 6.82 (td, *J* = 7.6, 1.7

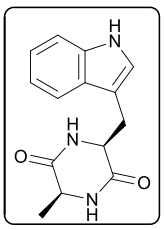


Hz, 1H), 5.56 (dd, *J* = 7.6, 1.7 Hz, 1H), 4.11 (m, 1H), 3.69 (dt, *J* = 9.3, 3.9 Hz, 1H), 3.27 (dd, *J* = 14.4, 3.8 Hz, 1H), 2.97 (dd, *J* = 14.3, 4.6 Hz, 1H), 2.44 (dd, *J* = 13.3, 4.4 Hz, 1H), 1.12 (dd, *J* = 13.4, 9.9 Hz, 1H) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ 166.9, 166.1, 138.9, 138.6, 136.0, 131.8, 128.5, 127.8, 124.9, 121.1, 119.3, 118.6, 111.5, 108.6, 100.6, 55.7, 53.4, 45.2, 29.2 ppm. **HRMS (ESI)**: (M: $C_{20}H_{18}O_2N_3I$) m/z calcd. 460.0516, found 460.0519 $(M+H)^+$.

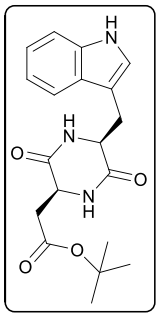
Cyclo(Gly-Trp) (**1c**). Compound **1c** was prepared using Fmoc-Gly-OH (1.78 g, 5.99 mmol, 1.0 eq), following the general procedure for the synthesis of DKPs to obtain the desired product as a white solid (1.23 g, 85%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.92 (s, 1H), 8.09 (d, *J* = 1.9 Hz, 1H), 7.76 (br s, 1H), 7.54 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.33 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.08 – 7.01 (m, 2H), 6.95 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 4.02 (m, 1H), 3.31 (dd, *J* = 17.2, 2.8 Hz, 1H), 3.24 (dd, *J* = 14.4, 4.7 Hz, 1H), 3.02 (dd, *J* = 14.4, 4.6 Hz, 1H), 2.79 (d, *J* = 17.3 Hz, 1H) ppm.



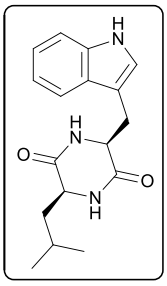
Cyclo(Ala-Trp) (**1d**). Compound **1d** was prepared using Fmoc-Ala-OH (1.87 g, 6.01 mmol, 1.0 eq), following the general procedure for the synthesis of DKPs to obtain the desired product as a white solid (1.57 g, 83%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.88 (s, 1H), 8.00 (s, 1H), 7.90 (s, 1H), 7.56 (d, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.08 – 6.98 (m, 2H), 6.94 (t, *J* = 7.4 Hz, 1H), 4.10 (m, 1H), 3.59 (q, *J* = 6.5 Hz, 1H), 3.24 (dd, *J* = 14.4, 4.1 Hz, 1H), 3.01 (dd, *J* = 14.4, 4.6 Hz, 1H), 0.42 (d, *J* = 7.0 Hz, 3H) ppm.



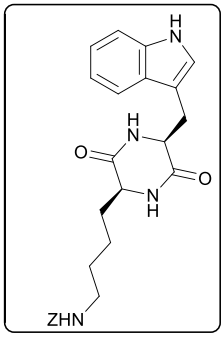
Cyclo[Asp(^{t}Bu)-Trp] (**1e**). Compound **1e** was prepared using Fmoc-Asp(^{t}Bu)-OH (2.47 g, 6.00 mmol, 1.0 eq), following the general procedure for the synthesis of DKPs to obtain the desired product as a pale yellow solid (2.18 g, 66%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.92 (s, 1H), 7.98 (s, 1H), 7.76 (s, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.10 (d, *J* = 2.3 Hz, 1H), 7.05 (t, *J* = 7.1 Hz, 1H), 6.94 (t, *J* = 7.1 Hz, 1H), 4.16 (t, *J* = 4.2 Hz, 1H), 3.97 (t, *J* = 6.2 Hz, 1H), 3.22 (dd, *J* = 14.5, 4.9 Hz, 1H), 3.08 (dd, *J* = 14.6, 4.5 Hz, 1H), 1.95 (dd, *J* = 16.4, 5.5 Hz, 1H), 1.50 (dd, *J* = 16.4, 6.9 Hz, 1H), 1.32 (s, *J* = 4.3 Hz, 9H) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ 169.1, 167.3, 166.6, 135.9, 127.5, 124.5, 120.9, 118.8, 118.4, 111.2, 108.6, 80.1, 55.1, 51.0, 44.3, 27.7, 23.1 ppm.



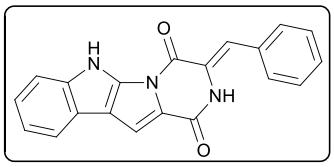
Cyclo(Leu-Trp) (**1i**). Compound **1i** was prepared using Fmoc-Leu-OH (710 mg, 2.01 mmol, 1.0 eq), following the general procedure for the synthesis of DKPs to obtain the desired product as a white solid (470 mg, 78%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.90 (s, 1H), 8.04 (s, 1H), 7.94 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.02 (m, 2H), 6.92 (t, *J* = 7.4 Hz, 1H), 4.09 (m, 1H), 3.46 – 3.36 (m, 1H), 3.26 (dd, *J* = 14.3, 3.8 Hz, 1H), 2.98 (dd, *J* = 14.3, 4.5 Hz, 1H), 1.19 (m, 1H), 0.64 (m, 1H), 0.53 (d, *J* = 6.5 Hz, 3H), 0.42 (d, *J* = 6.6 Hz, 3H), -0.01 (m, 1H) ppm.



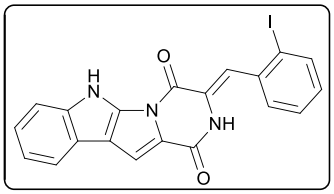
Cyclo[Lys(z)-Trp] (**1j**). Compound **1j** was prepared using Fmoc-Lys(z)-OH (1.00 g, 1.99 mmol, 1.0 eq), following the general procedure for the synthesis of DKPs to obtain the desired product as a white solid (725 mg, 93%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.85 (s, 1H), 8.02 (d, *J* = 1.5 Hz, 1H), 7.91 (d, *J* = 1.4 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.41 – 7.26 (m, 6H), 7.09 – 6.98 (m, 3H), 6.92 (t, *J* = 7.4 Hz, 1H), 4.99 (s, 2H), 4.10 (m, 1H), 3.55 – 3.46 (m, 1H), 3.24 (dd, *J* = 14.4, 4.2 Hz, 1H), 3.00 (dd, *J* = 14.4, 4.7 Hz, 1H), 2.71 (q, *J* = 6.9 Hz, 2H), 1.07 – 0.89 (m, 3H), 0.68 – 0.47 (m, 3H) ppm.



(Z)-3-benzylidene-2,3-dihydro-1*H*-pyrazino[1',2':1,5]pyrrolo[2,3-*b*]indole-1,4(6*H*)-dione (2a). Compound **2a** (150 mg, 0.450 mmol, 1.0 eq) and DDQ (306 mg, 1.35 mmol, 3.0 eq) were dissolved in 100 mL of DMF and the solution was stirred at 120 °C for 24h. Then, the resulting crude was concentrated under vacuum, dissolved in ethyl acetate and washed with saturated aqueous solution of $NaHCO_3$ (3 x 20 mL); the aqueous solution was back-extracted with ethyl acetate. Then, the organic layers were combined, dried over Na_2SO_4, filtered and concentrated under vacuum (17% yield estimated by HPLC-MS conversion). An analytically pure sample was obtained by via flash column chromatography on Celite using H_2O (0.1% formic acid)/ACN (0.1% formic acid). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 12.31 (s, 1H), 9.89 (s, 1H), 7.82 (d, *J* = 7.7 Hz, 1H), 7.68 (d, *J* = 7.4 Hz, 2H), 7.59 – 7.51 (m, *J* = 11.9 Hz, 2H), 7.48 (t, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 1H), 7.26 (t, *J* = 7.6 Hz, 1H), 7.18 (t, *J* = 7.3 Hz, 1H), 7.13 (s, 1H) ppm. **HRMS (ESI)**: (M: $C_{20}H_{13}O_2N_3$) m/z calcd 328.10805, found 328.10800 $(M+H)^+$.



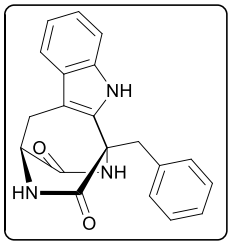
(Z)-3-(2-iodobenzylidene)-2,3-dihydro-1*H*-pyrazino[1',2':1,5]pyrrolo[2,3-*b*]indole-1,4(6*H*) dione (2b). Compound **2a** (150 mg, 0.327 mmol, 1.0 eq) and DDQ (222 mg, 0.980 mmol, 3.0 eq) were dissolved in 3 mL of DMF and placed in a microwave reactor vessel. The mixture was heated under microwave irradiation (250W) at 120 °C for 20 min. The resulting crude was dissolved in ethyl acetate and washed with saturated aqueous solution of $NaHCO_3$ (4 x 20 mL); the aqueous solution was back-extracted with ethyl acetate. Then, the organic layers were combined, dried over Na_2SO_4, filtered and concentrated under vacuum (24% yield estimated by HPLC-MS conversion). An analytically pure sample was obtained by via flash column chromatography on Celite using H_2O (0.1% formic acid)/ACN (0.1% formic acid). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 12.33 (s, 1H), 10.05 (s, 1H), 7.98 (d, *J* = 7.6 Hz, 1H), 7.82 (d, *J* = 7.9 Hz, 1H), 7.61 (d, *J* = 7.4 Hz, 1H), 7.57 (d, *J* = 8.2 Hz, 1H), 7.54 (s, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.27 (t, *J* = 7.2 Hz, 1H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.13 (t, *J* = 7.6 Hz, 1H), 6.99 (s, 1H) ppm. **HRMS (ESI)**: (M: $C_{20}H_{12}O_2N_3I$) m/z calcd 454.00470, found 454.00468 $(M+H)^+$.



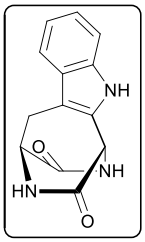
General procedure for the oxidative cross-dehydrogenative coupling (CDC) of DKPs 1b-j

Unless stated otherwise, cyclo(AA-Trp) (0.2 mmol, 1.0 eq) and $Cu(OCOCF_3)_2$ (4.0 eq) were placed in a microwave reactor vessel and dissolved in DMF (1 mL). Afterwards, TFA (4.0 eq) was added to the solution and the mixture was heated under microwave irradiation (250 W) at 120 °C for 30 min. The resulting crude was diluted in ethyl acetate, filtered through Celite and evaporated under vacuum. Then, the crude was dissolved again in ethyl acetate and washed with NaCl (×3); the aqueous solution was back-extracted. Then, all the organic layers were mixed, dried over Mg_2SO_4, filtered and concentrated under vacuum. The crude was purified by flash chromatography on silica using DCM/DCM:MeOH (8:2) to yield the pure products **1b-j**.

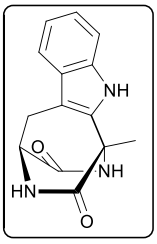
Oxidized cyclo(Phe-Trp) (**4b**). Compound **4b** was prepared using compound **1b** (67 mg, 0.201 mmol, 1.0 eq), following the general procedure for the synthesis of oxidized DKPs to obtain the pure product **4b** as a yellow solid (30% yield estimated by HPLC-MS conversion). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 11.28 (s, 1H), 8.59 (s, 1H), 8.35 (d, *J* = 4.0 Hz, 1H), 7.55 (d, *J* = 7.1 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 2H), 7.23 (t, *J* = 7.3 Hz, 1H), 7.14 (t, *J* = 7.2 Hz, 1H), 7.03 (t, *J* = 7.2 Hz, 1H), 4.02 (m, 1H), 3.91 (d, *J* = 13.7 Hz, 1H), 3.32 (1H), 3.15 (dd, *J* = 17.0, 1.8 Hz, 1H), 2.99 (dd, *J* = 17.0, 3.9 Hz, 1H) ppm. **HRMS (ESI)**: (M: $C_{20}H_{17}O_2N_3$) m/z calcd 332.13935, found 332.13922 $(M+H)^+$.



Oxidized cyclo(Gly-Trp) (**4c**). Compound **4c** was prepared using compound **1c** (50 mg, 0.206 mmol, 1.0 eq), following the general procedure for the synthesis of oxidized DKPs to obtain the pure product **4c** as a pale brown solid (16.6 mg, 30%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.33 (s, 1H), 9.05 (d, *J* = 4.9 Hz, 1H), 8.43 (d, *J* = 3.9 Hz, 1H), 7.43 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 7.01 (t, *J* = 7.4 Hz, 1H), 4.47 (dd, *J* = 5.1, 1.9 Hz, 1H), 4.11 – 4.05 (m, 1H), 3.14 (dd, *J* = 17.0, 2.5 Hz, 1H), 2.97 (dd, *J* = 17.0, 4.5 Hz, 1H) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ 171.7, 169.3, 134.6, 131.7, 127.7, 121.8, 119.2, 118.0, 111.5, 105.8, 54.5, 52.3, 27.3 ppm. **HRMS (ESI)**: (M: $C_{13}H_{11}O_2N_3$) m/z calcd 242.09240, found 242.09228 $(M+H)^+$.



Oxidized cyclo[Asp(^{t}Bu)-Trp] (**4d**). Compound **4d** was prepared using compound **1e** (73 mg, 0.204 mmol, 1.0 eq), following the general procedure for the synthesis of oxidized DKPs to obtain the pure product **4d** as a brown-orange solid (15.2 mg, 43%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.02 (s, 1H), 8.91 (s, 1H), 8.42 (d, *J* = 5.1 Hz, 1H), 7.42 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.1, 1H), 7.12 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.01 (ddd, *J* = 7.9, 7.1, 1.1 Hz, 1H), 4.07 (m, 1H), 3.14 (dd, *J* = 16.9, 2.5 Hz, 1H), 2.97 (dd, *J* = 16.9, 4.6 Hz, 1H), 1.69 (s, 3H) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ 171.8, 170.8, 134.6, 134.3, 127.9, 121.9, 119.2, 117.9, 111.7, 106.2, 54.6, 54.5, 27.5, 17.1 ppm. **HRMS (ESI)**: (M: $C_{14}H_{13}O_2N_3$) m/z calcd 256.1086, found 256.1085 $(M+H)^+$.



Oxidized cyclo(Pro$_L$-Trp$_L$) (**4f**). Compound **4f** was prepared using compound **1f** (58 mg, 0.205 mmol, 1.0 eq), following the general procedure for the synthesis of oxidized DKPs to obtain the pure product **4f** as a pale brown solid (34.8 mg, 60%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.20 (s, 1H), 8.46 (d, *J* = 5.1 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 8.1, 1H), 7.13 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.01 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 4.16 (td, *J* = 4.9, 2.3 Hz, 1H), 3.46 – 3.35 (m, 2H), 3.14 (dd, *J* = 17.1, 2.4 Hz, 1H), 3.00 (dd, *J* = 17.1, 4.8 Hz, 1H), 2.80 (ddd, *J* = 13.4, 6.3, 4.2 Hz, 1H), 2.48 – 2.41 (m, 1H), 2.04 – 1.89 (m, 2H) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ 170.8, 167.6, 134.7, 131.7, 128.0, 122.0, 119.2, 117.9, 111.6, 106.9, 64.1, 55.0, 44.5, 28.4, 27.2, 23.0 ppm. $[\alpha]_D^{25}$ +24.6 (c 0.5, MeOH). **HRMS (ESI)**: (M: $C_{16}H_{15}O_2N_3$) m/z calcd 282.1243, found 282.1243 $(M+H)^+$.

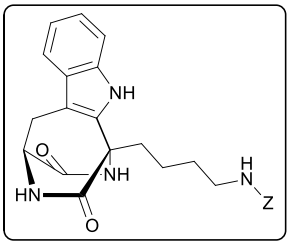
Oxidized cyclo(Pro$_D$-Trp$_D$) (**4g**). Compound **4g** was prepared using compound **1g** (58 mg, 0.205 mmol, 1.0 eq), following the general procedure for the synthesis of oxidized DKPs to obtain the pure product **4g** as a brown-orange solid (41.0 mg, 71%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.20 (s, 1H), 8.46 (d, *J* = 5.1 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 1H), 7.36 (dt, *J* = 8.1, 0.8 Hz, 1H), 7.13 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.01 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H), 4.16 (td, *J* = 4.9, 2.4 Hz, 1H), 3.46 – 3.35 (m, *J* = 9.7, 8.0, 5.6 Hz, 2H), 3.14 (dd, *J* = 17.1, 2.3 Hz, 1H), 3.00 (dd, *J* = 17.1, 4.8 Hz, 1H), 2.80 (ddd, *J* = 13.6, 6.3, 4.2 Hz, 1H), 2.48 – 2.42 (m, 1H), 2.02 – 1.88 (m, 2H) ppm. $[\alpha]_D^{25}$ -26.0 (c 0.5, MeOH). **HRMS (ESI)**: (M: $C_{16}H_{15}O_2N_3$) m/z calcd 282.1243, found 282.1241 $(M+H)^+$.

Oxidized cyclo(Pro$_L$-Trp$_D$) (**4h**). Compound **4h** was prepared using compound **1h** (58 mg, 0.205 mmol, 1.0 eq), following the general procedure for the synthesis of oxidized DKPs to obtain the pure product **4h** as a brown-orange solid (9.6 mg, 17%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.20 (s, 1H), 8.46 (d, *J* = 5.1 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.13 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.01 (ddd, *J* = 7.9, 7.2, 0.9 Hz, 1H), 4.16 (td, *J* = 4.9, 2.3 Hz, 1H), 3.43 – 3.35 (m, 2H), 3.14 (dd, *J* = 17.1, 2.3 Hz, 1H), 3.00 (dd, *J* = 17.1, 4.8 Hz, 1H), 2.80 (ddd, *J* = 13.4, 6.3, 4.2 Hz, 1H), 2.48 – 2.43 (m, *J* = 8.0, 5.4 Hz, 1H), 2.03 – 1.88 (m, 2H) ppm. **HRMS (ESI)**: (M: $C_{16}H_{15}O_2N_3$) m/z calcd 282.1243, found 282.1242 $(M+H)^+$.

Oxidized cyclo(Leu-Trp) (**4i**). Compound **4i** (180 mg, 0.601 mmol, 1.0 eq) and $Cu(OCOCF_3)_2$ (6.0 eq) were placed in a microwave reactor vessel and dissolved in DMF (3 mL). Afterwards, TFA (4.0 eq) was added to the solution and the mixture was heated at 200 °C for 18 h. The resulting crude was diluted in ethyl acetate, filtered through Celite and evaporated under vacuum. Then, the crude was dissolved again in ethyl acetate and washed with NaCl (×3) and 5% aqueous LiCl (×3); the aqueous solution was back-extracted. Then, all the organic layers were mixed, dried over Mg_2SO_4, filtered and concentrated under vacuum. The crude was purified by flash chromatography on silica using DCM/DCM:MeOH (8:2) to yield the pure product **4i** as a pale yellow solid (36.4 mg, 20%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.02 (s, 1H), 8.68 (s, 1H), 8.27 (d, *J* = 4.5 Hz, 1H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.99 (t, *J* = 7.5 Hz, 1H), 4.07 (m, 1H), 3.13 (dd, *J* = 17.0, 2.4 Hz, 1H), 2.95 (dd, *J* = 17.1, 4.5 Hz, 1H), 2.41 (dd, *J* = 15.5, 9.4 Hz, 1H), 2.02 (m, 2H), 1.04 (d, *J* =

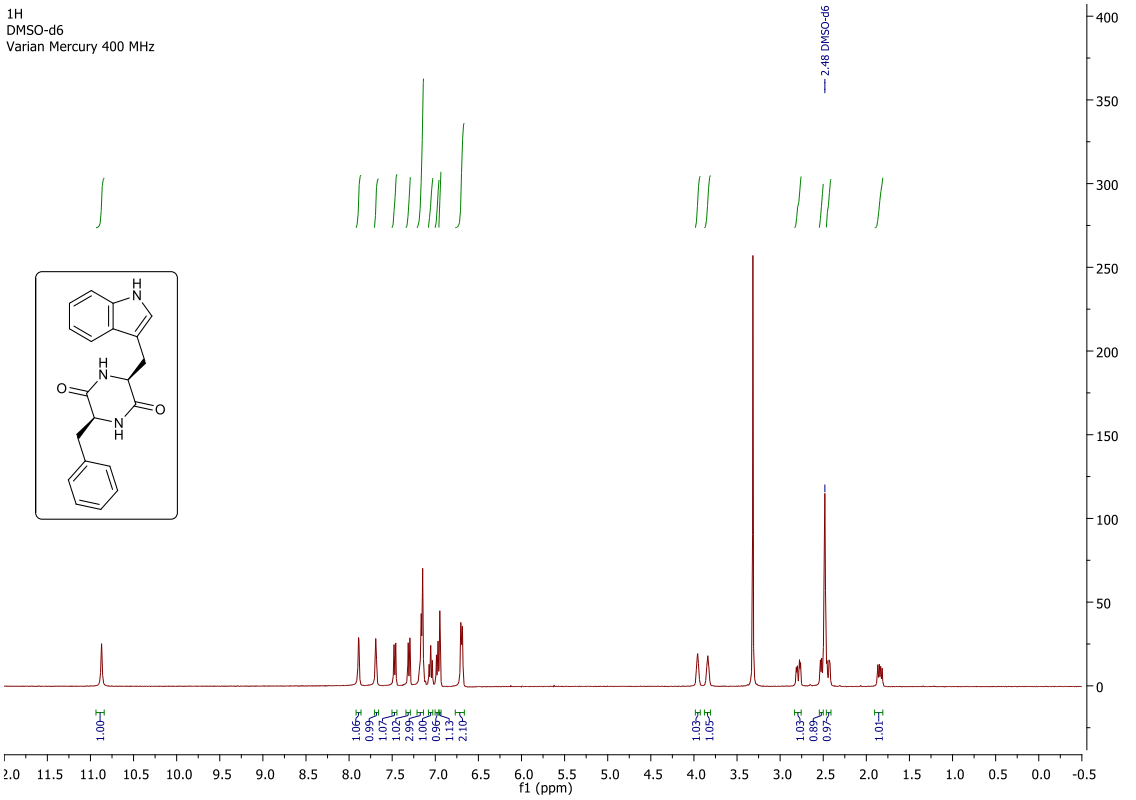
6.3 Hz, 3H), 0.95 (d, *J* = 6.5 Hz, 3H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 171.9, 169.7, 135.0, 134.6, 127.8, 121.8, 119.2, 117.8, 111.7, 105.7, 57.6, 54.1, 36.2, 27.9, 24.1, 23.9, 23.0 ppm. **HRMS (ESI)**: (M: $C_{17}H_{19}O_2N_3$) m/z calcd 298.1556, found 298.1559 $(M+H)^+$.

Oxidized cyclo[Lys(z)-Trp] (**4j**). Compound **4j** was prepared using compound **1j** (90 mg, 0.201 mmol, 1.0 eq) and $Cu(OSO_2CF_3)_2$ (4.0 eq) instead of $Cu(OCOCF_3)_2$, following the general procedure for the synthesis of oxidized DKPs. The crude was purified by flash chromatography on silica using hexane/ethyl acetate to yield the pure product **4j** as a yellow solid (31.5 mg, 32%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 11.03 (s, 1H), 8.79 (s, 1H), 8.37 (d, *J* = 4.7 Hz, 1H), 7.41 (d, *J* = 7.9 Hz, 1H), 7.39 – 7.26 (m, 7H), 7.11 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.00 (ddd, *J* = 8.0, 7.1, 1.0 Hz, 1H), 5.03 (s, 2H), 4.06 (m, 1H), 3.19 – 3.04 (m, 3H), 2.95 (dd, *J* = 17.1, 4.6 Hz, 1H), 2.41 – 2.30 (m, 1H), 2.06 – 1.91 (m, 1H), 1.60 – 1.43 (m, 4H) ppm. **HRMS (ESI)**: (M: $C_{25}H_{26}O_4N_4$) m/z calcd 447.2032, found 447.2036 $(M+H)^+$.

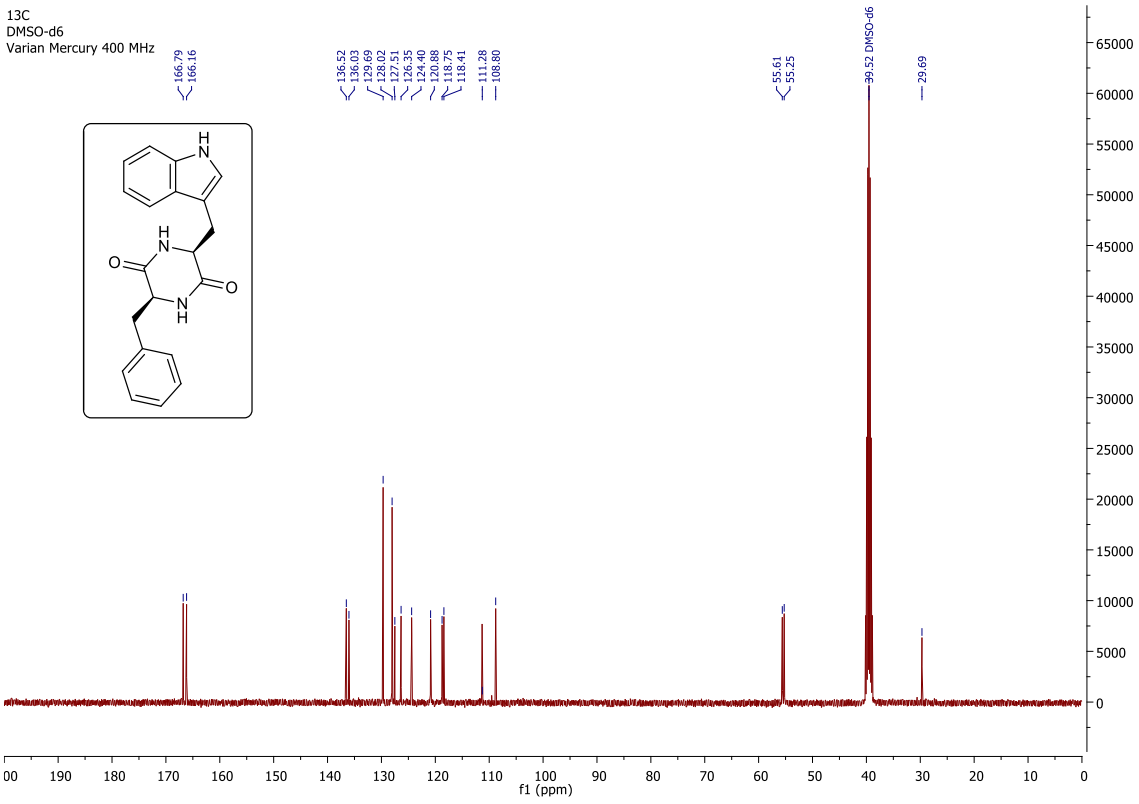


NMR spectra of compounds 1-4

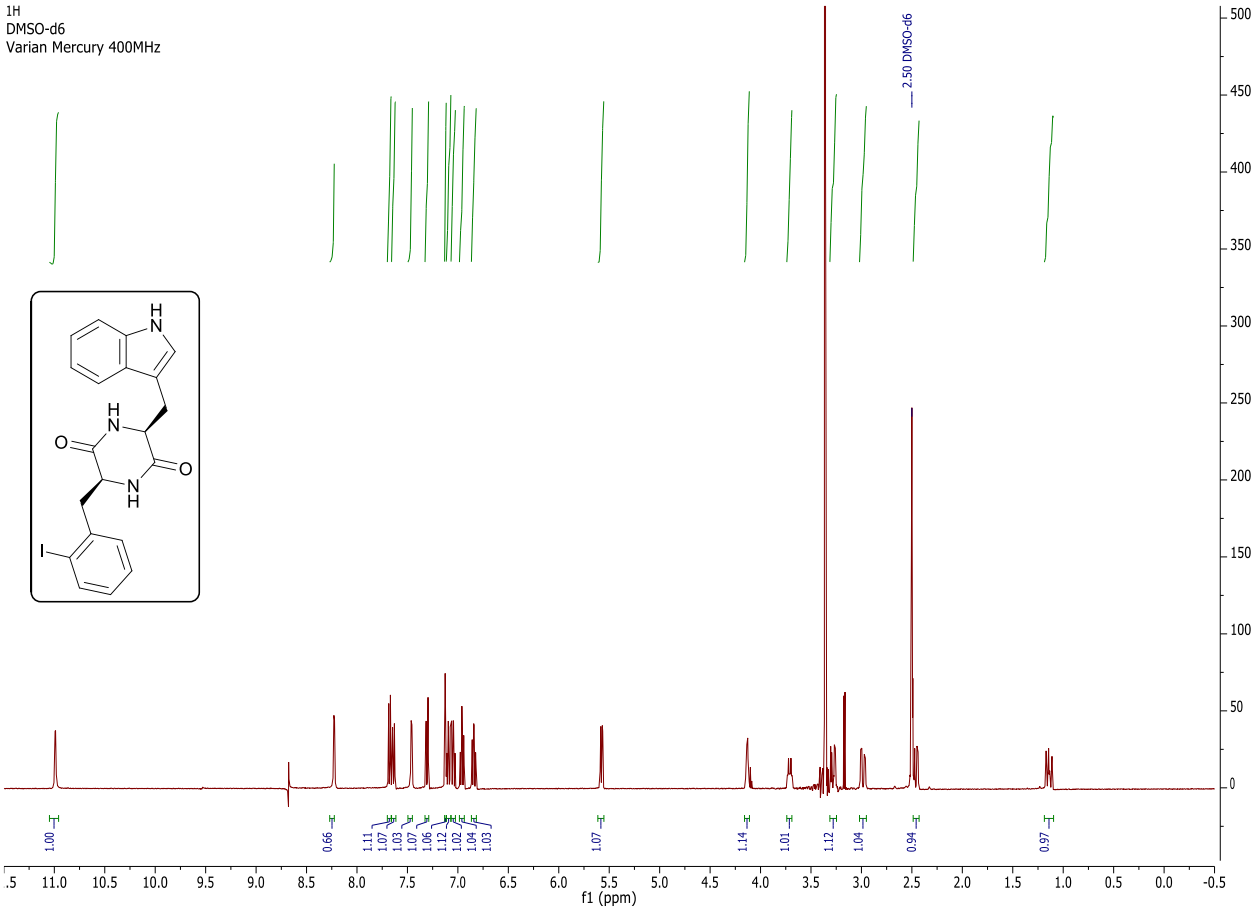
Cyclo(Phe-Trp) (1a) ^{1}H NMR



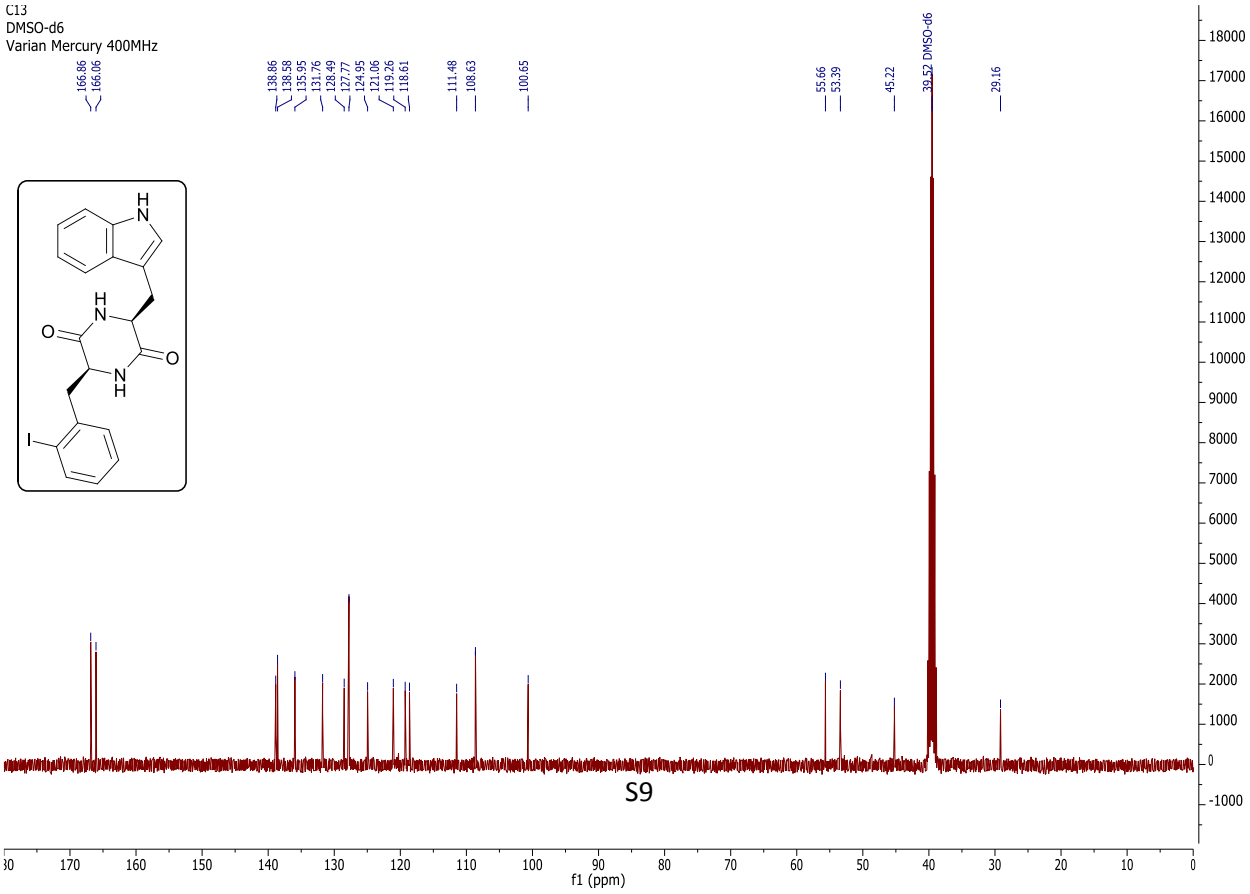
Cyclo(Phe-Trp) (1a) ^{13}C NMR



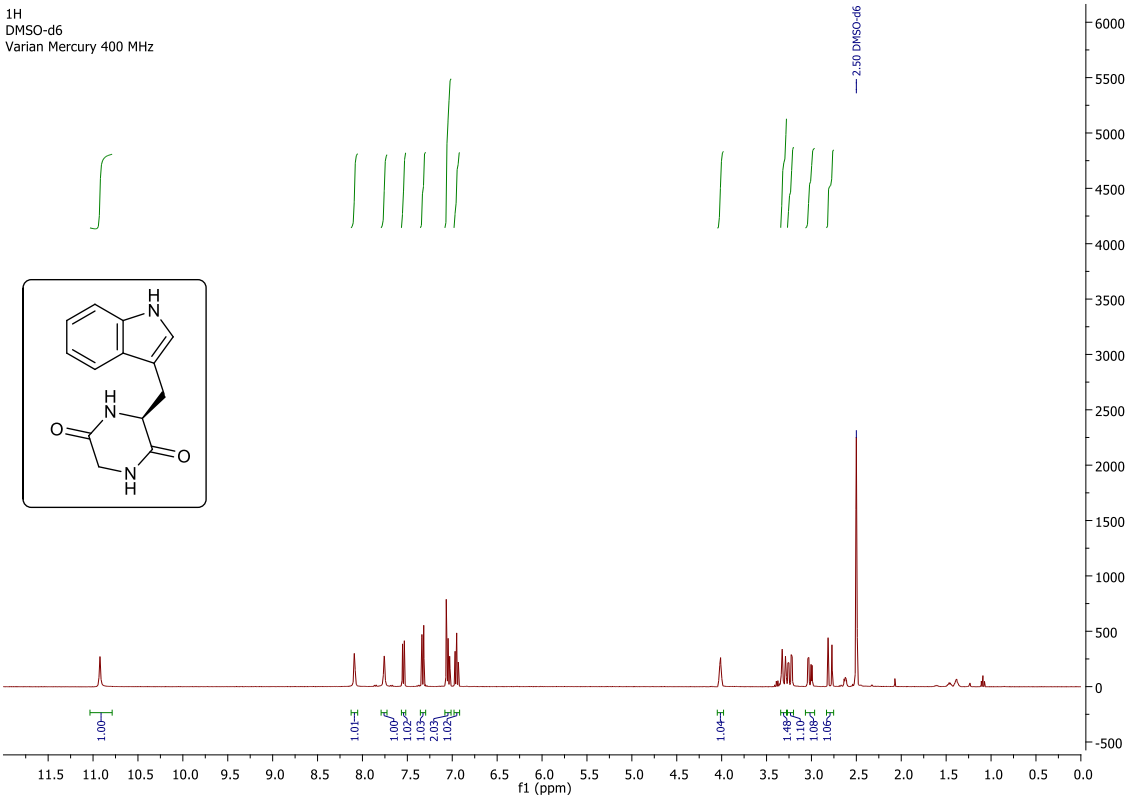
Cyclo(2-I-Phe-Trp) (1b) ^{1}H NMR



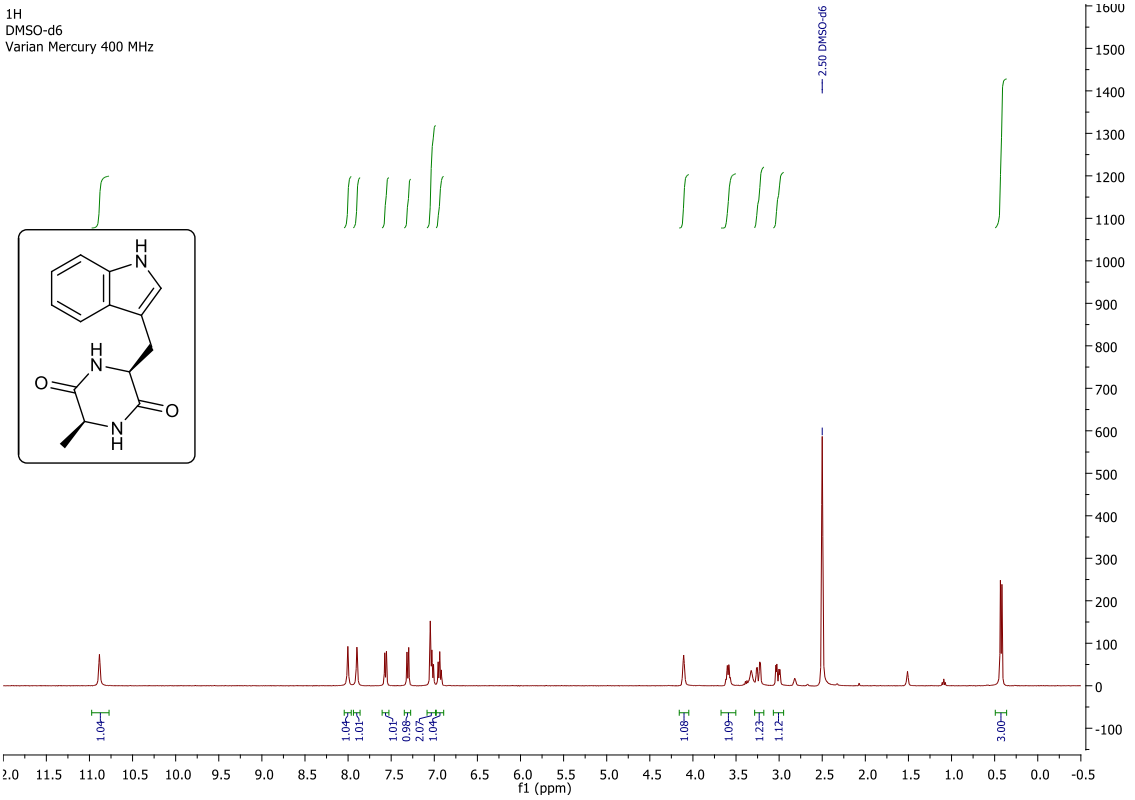
Cyclo(2-I-Phe-Trp) (1b) ^{13}C NMR



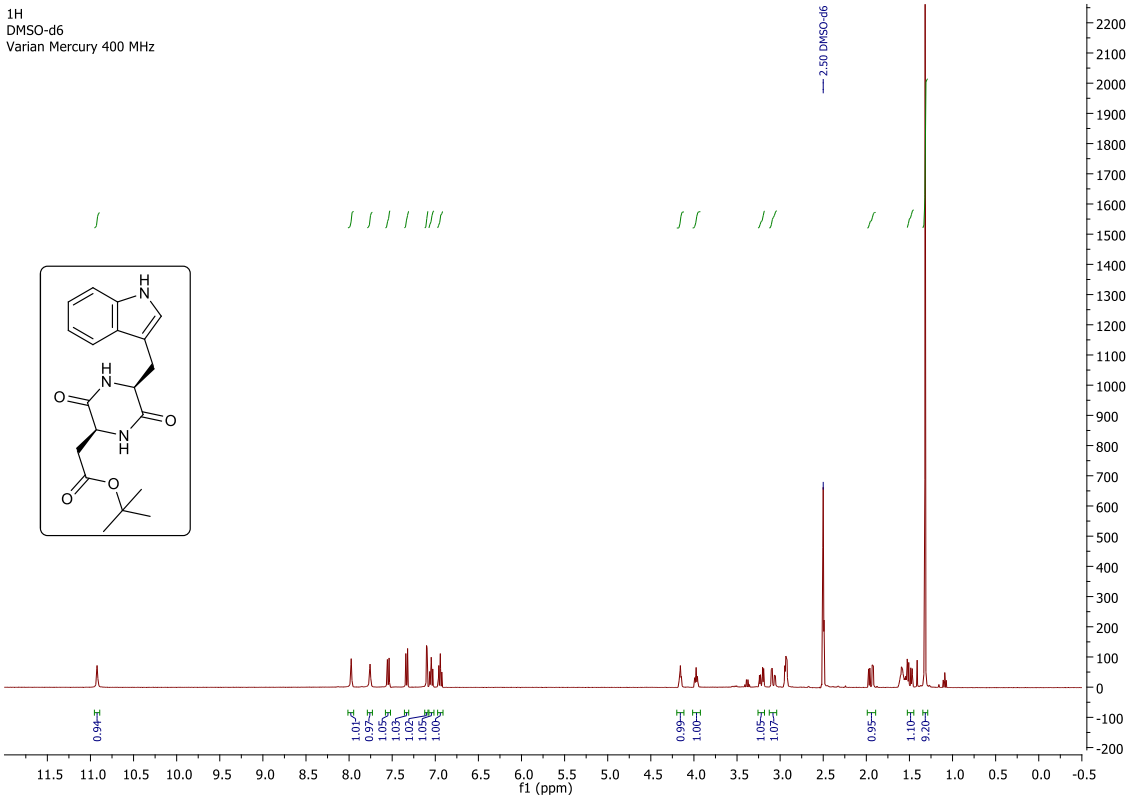
Cyclo(Gly-Trp) (1c) ^{1}H NMR



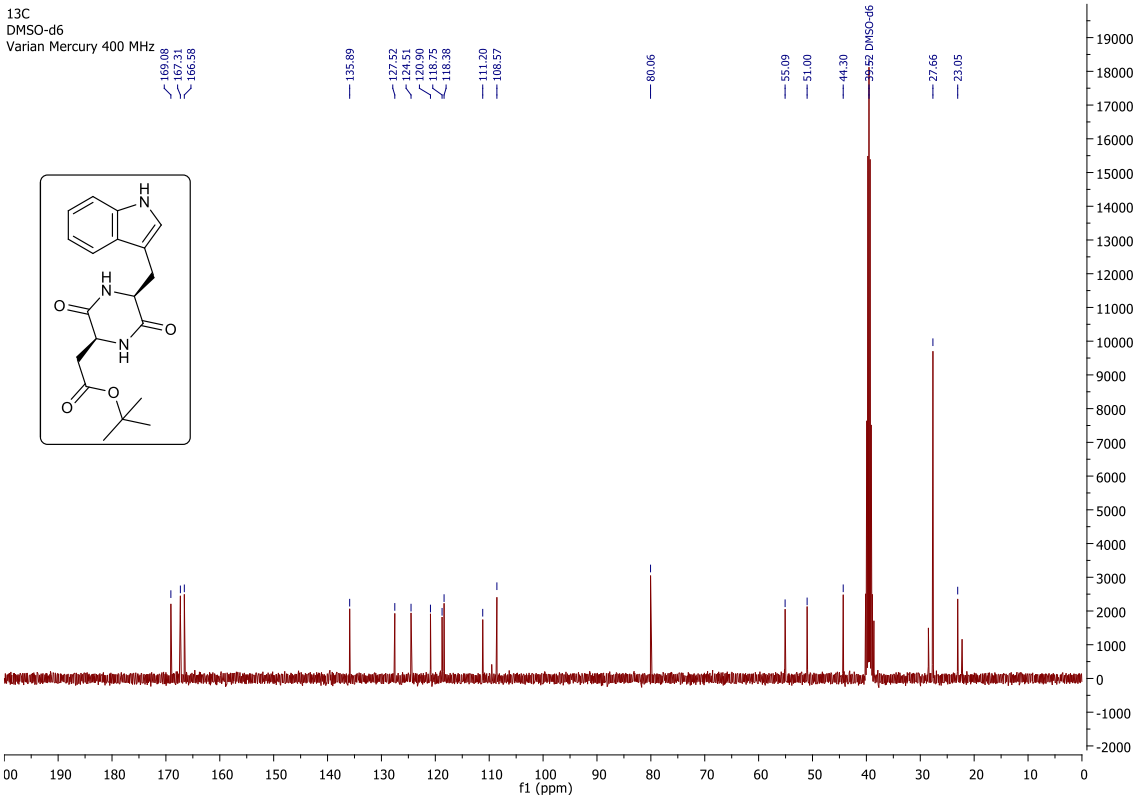
Cyclo(Ala-Trp) (1d) ^{1}H NMR



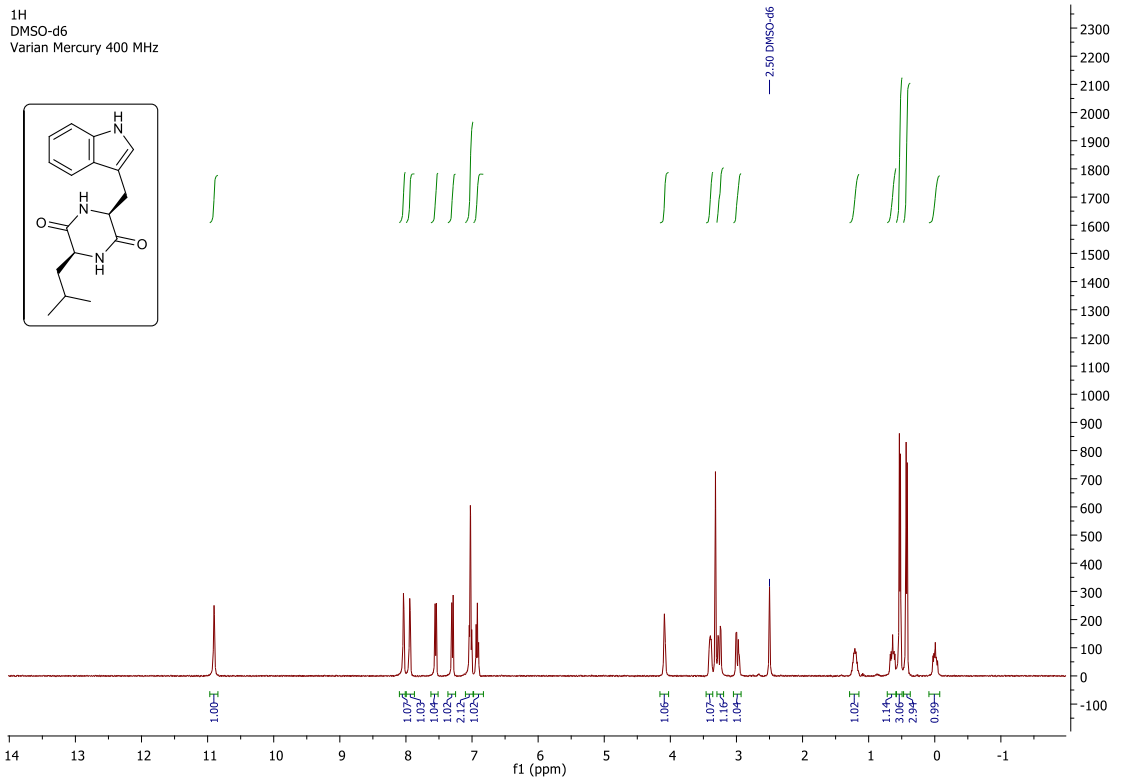
Cyclo[Asp(ᵗBu)-Trp] (1e) ^{1}H NMR



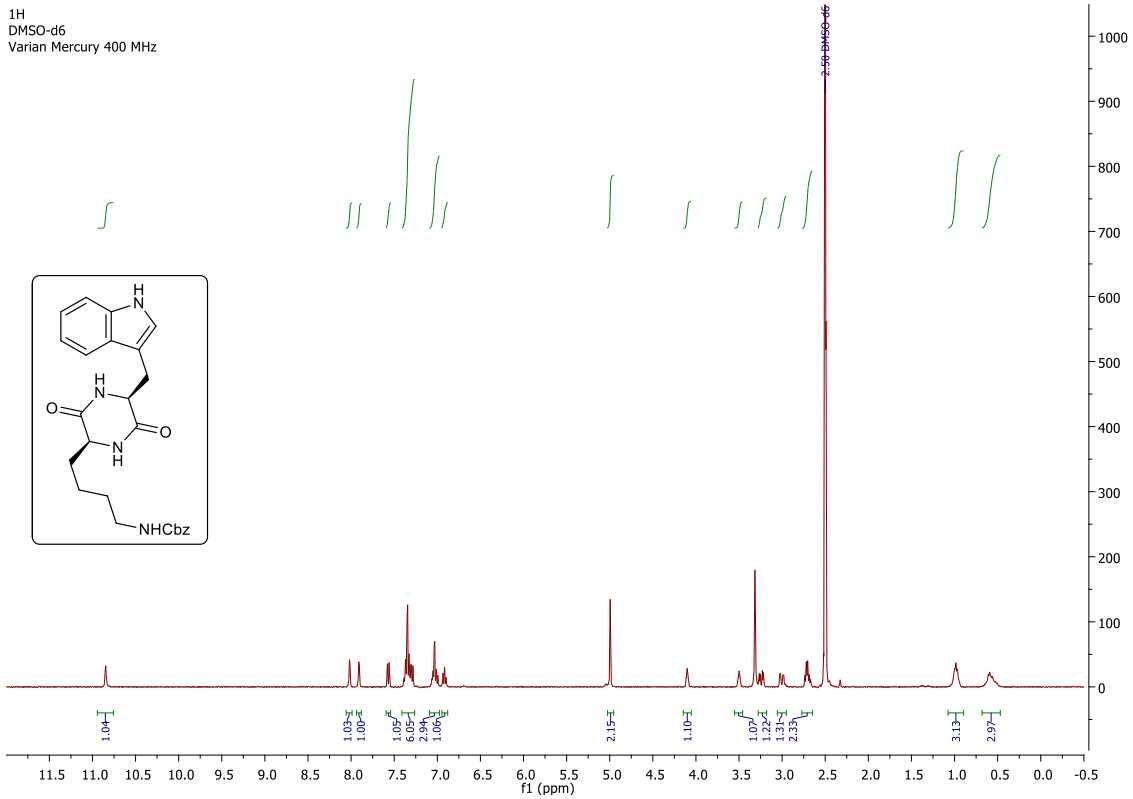
Cyclo[Asp(ᵗBu)-Trp] (1e) ^{13}C NMR



Cyclo(Leu-Trp) (1i) ^{1}H NMR

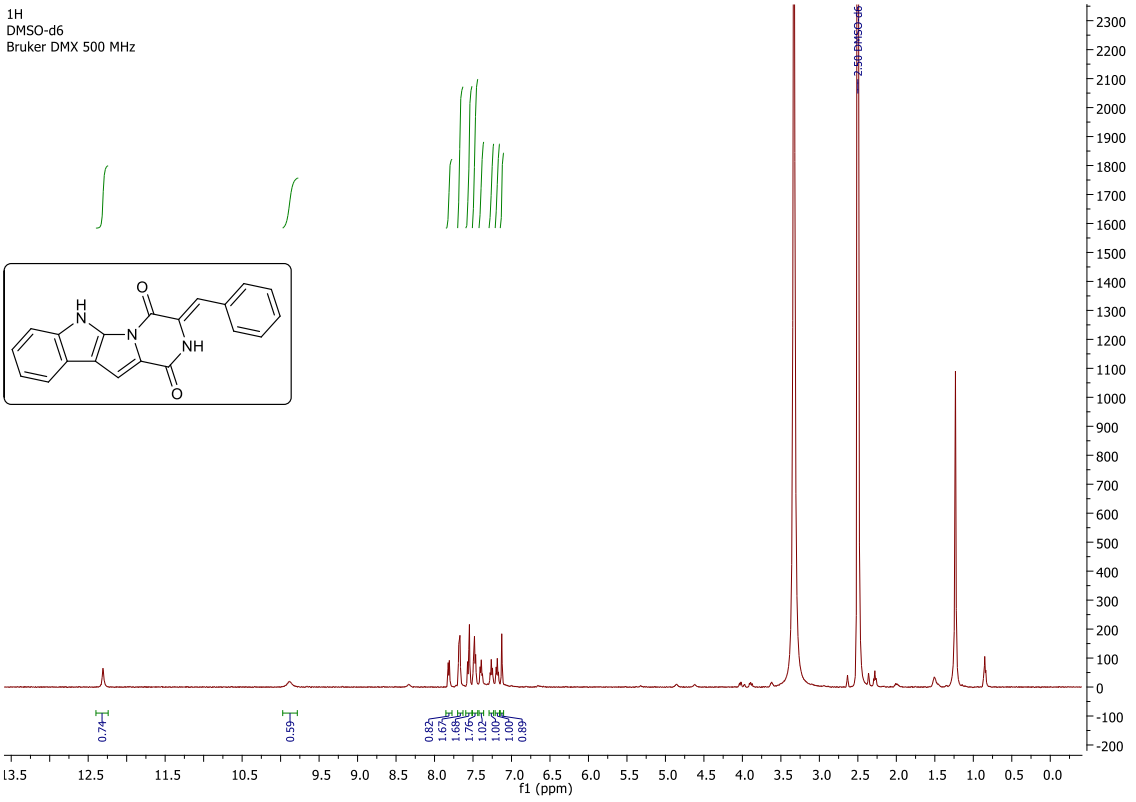


Cyclo[Lys(z)-Trp] (1j) ^{1}H NMR



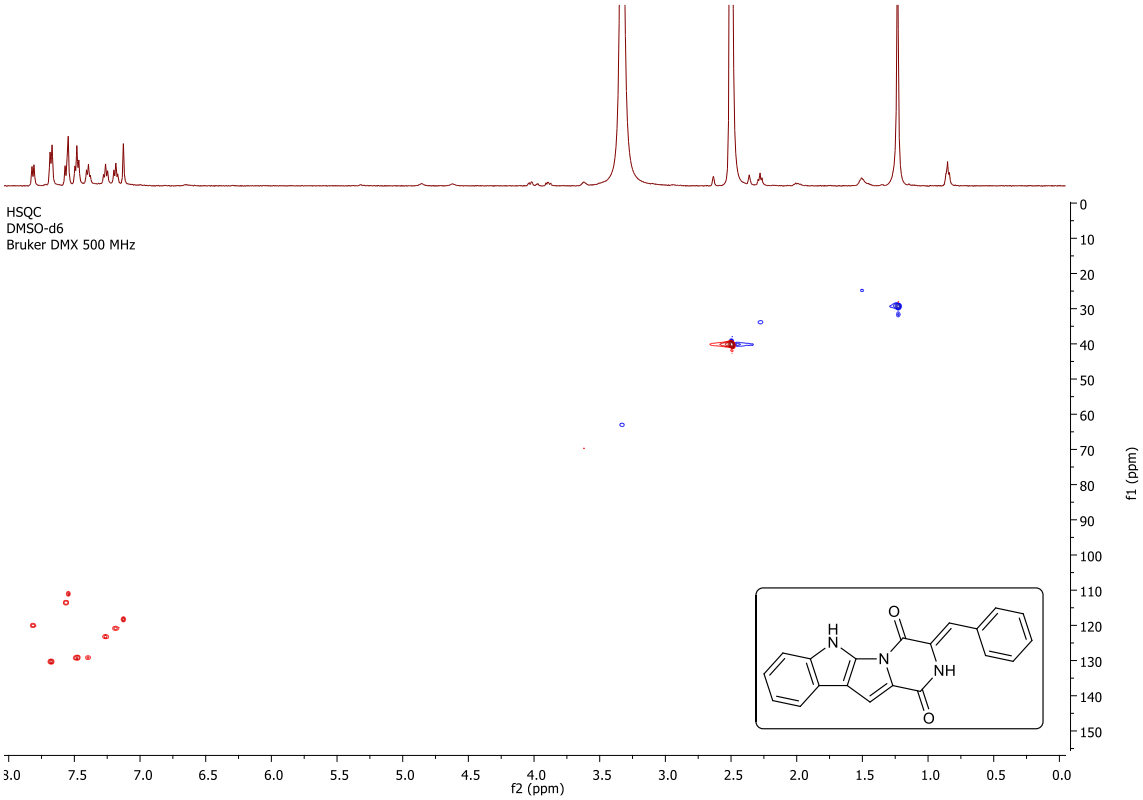
(Z)-3-benzylidene-2,3-dihydro-1*H*-pyrazino[1',2':1,5]pyrrolo[2,3-*b*]indole-1,4(6*H*)-dione (2a)

^{1}H NMR



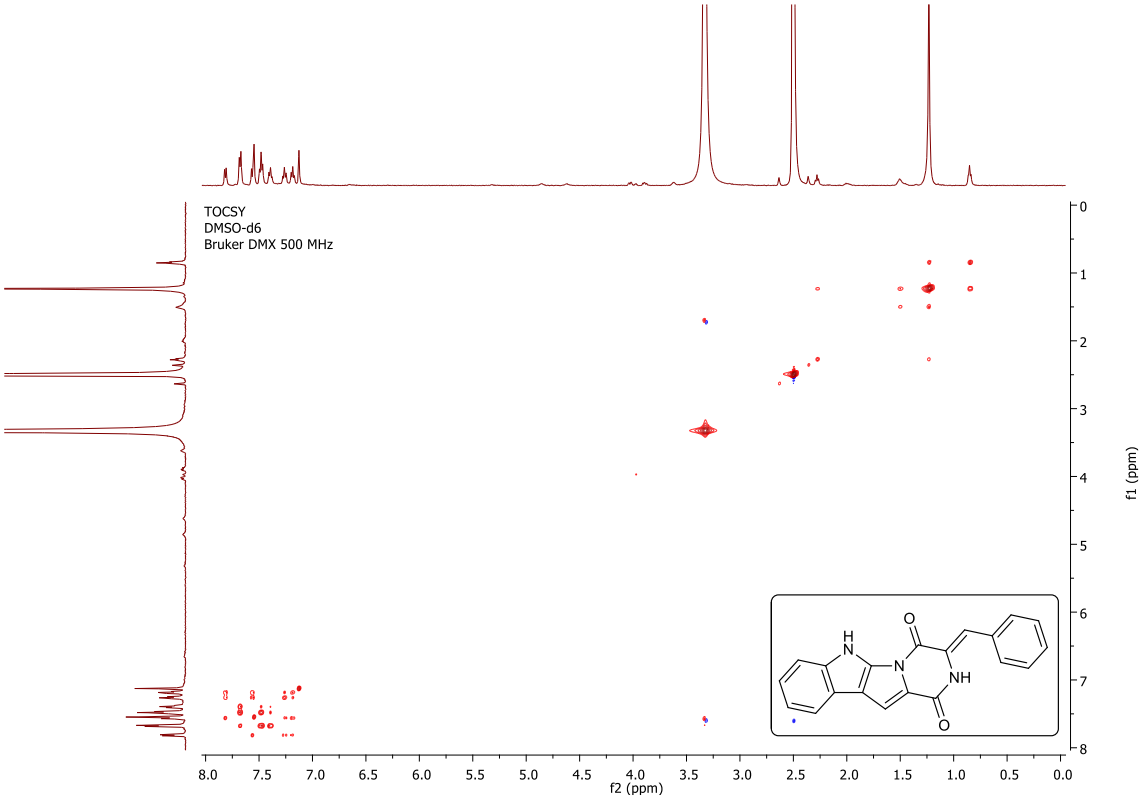
(Z)-3-benzylidene-2,3-dihydro-1*H*-pyrazino[1',2':1,5]pyrrolo[2,3-*b*]indole-1,4(6*H*)-dione (2a)

^{1}H-^{13}C HSQC NMR



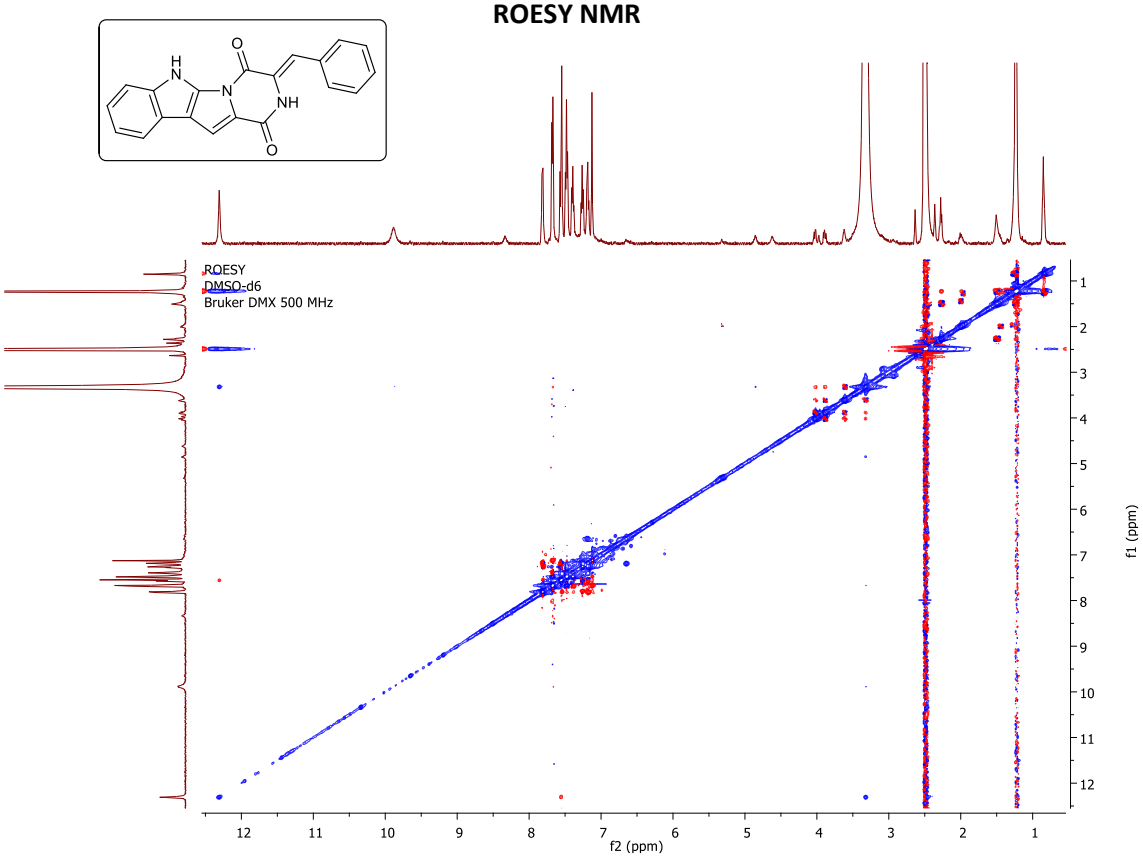
(Z)-3-benzylidene-2,3-dihydro-1*H*-pyrazino[1',2':1,5]pyrrolo[2,3-*b*]indole-1,4(6*H*)-dione (2a)

TOCSY NMR

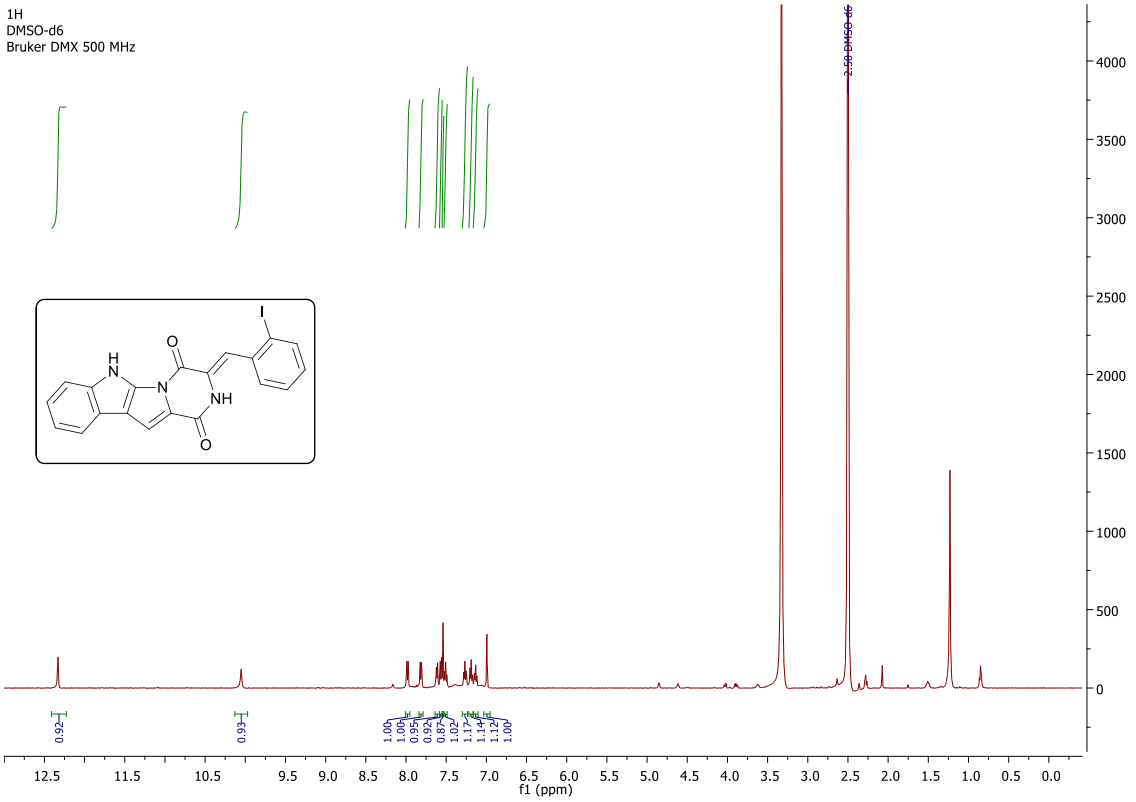


(Z)-3-benzylidene-2,3-dihydro-1*H*-pyrazino[1',2':1,5]pyrrolo[2,3-*b*]indole-1,4(6*H*)-dione (2a)

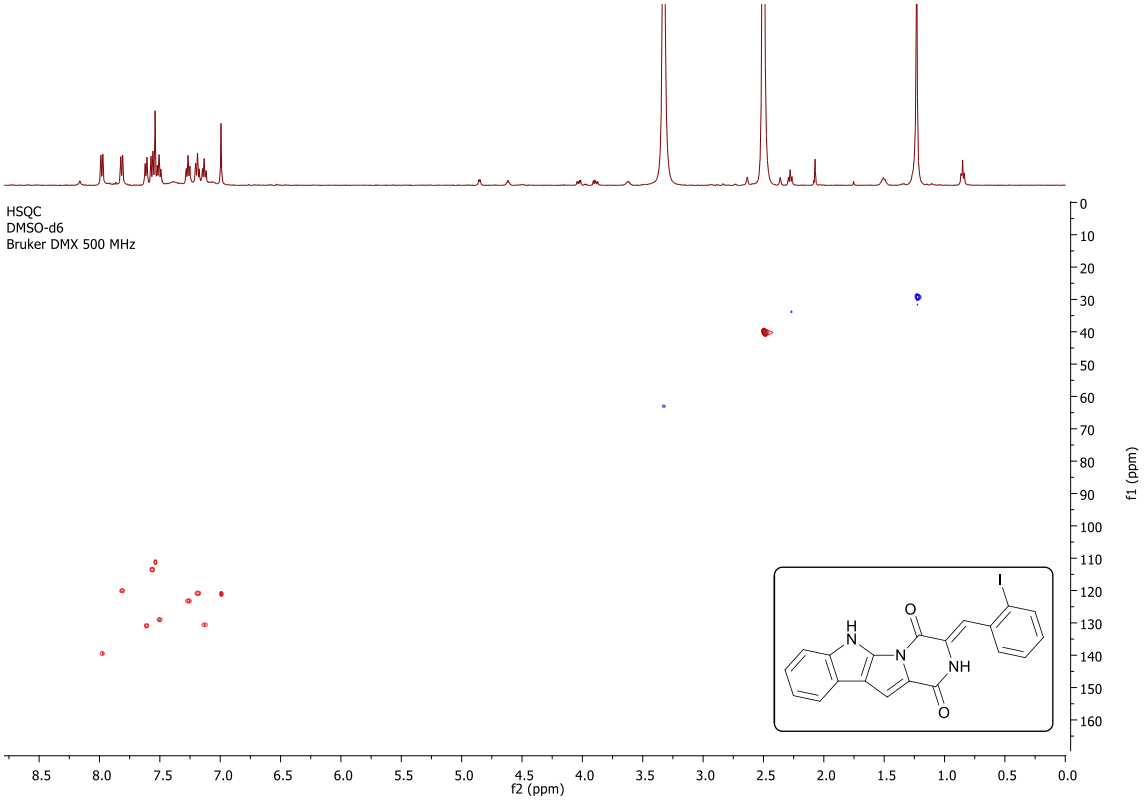
ROESY NMR



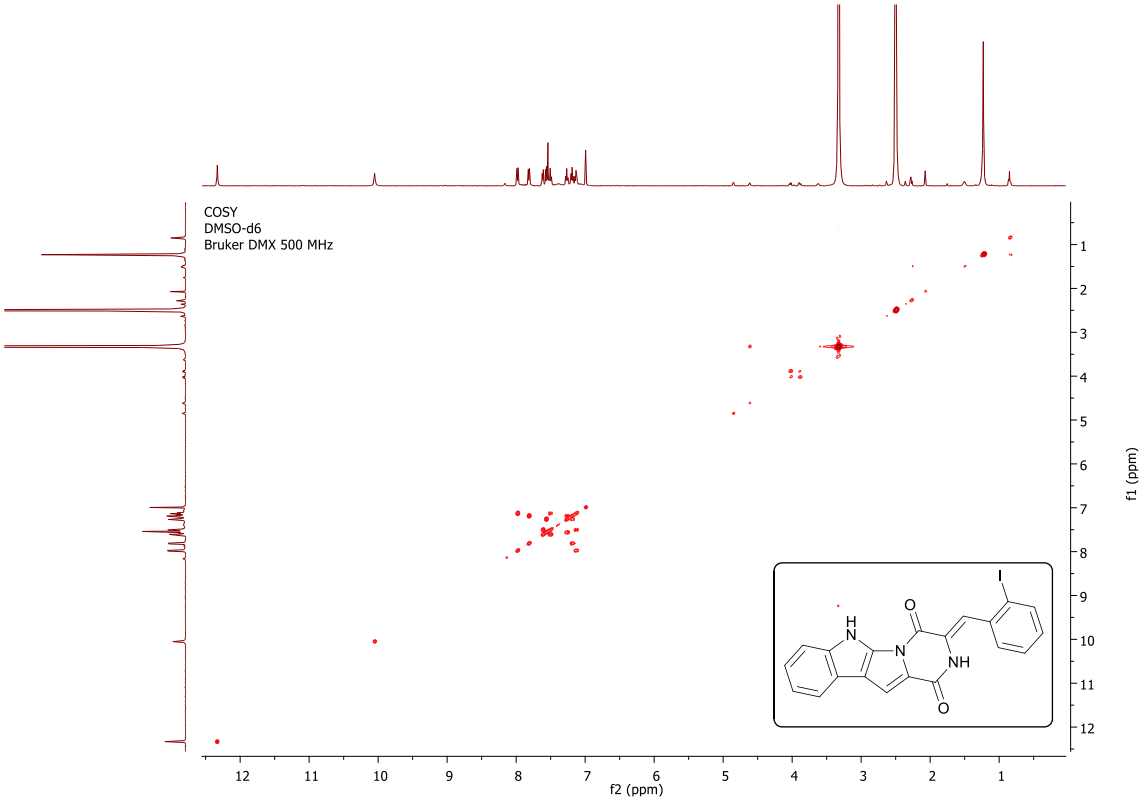
(Z)-3-(2-iodobenzylidene)-2,3-dihydro-1H-pyrazino[1',2':1,5]pyrrolo[2,3-b]indole-1,4(6H)-dione (2b) ^{1}H NMR



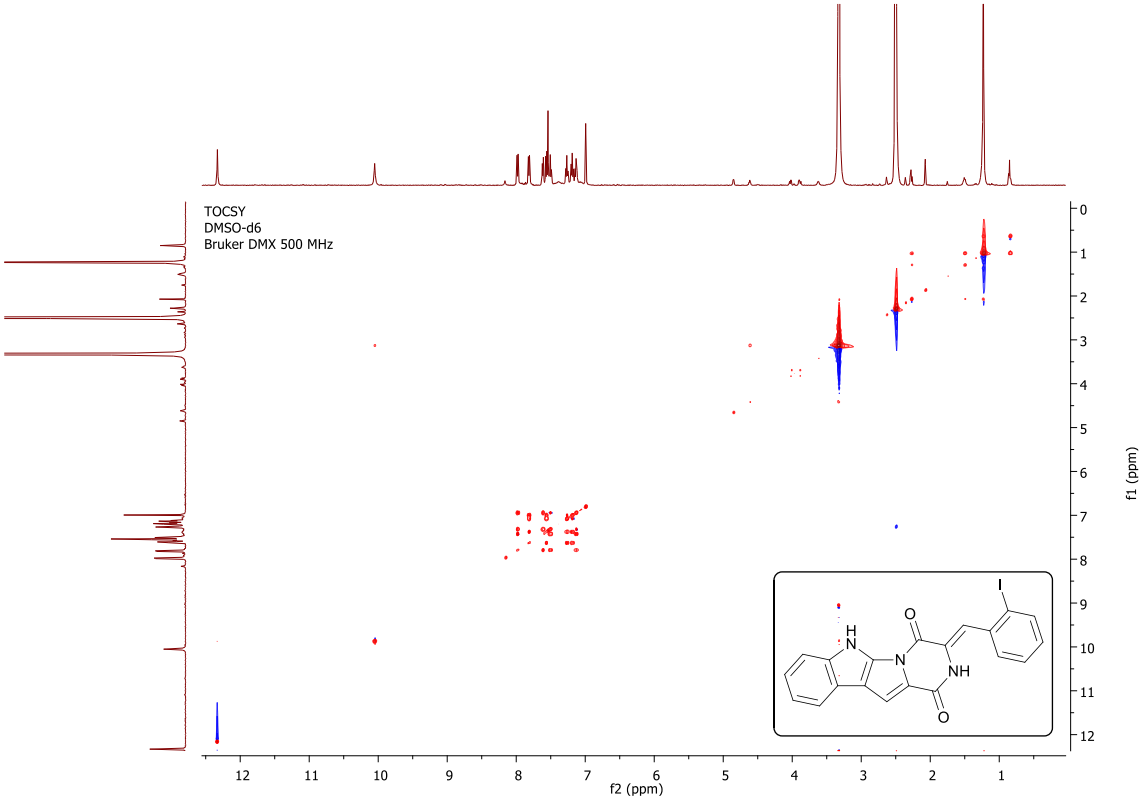
(Z)-3-(2-iodobenzylidene)-2,3-dihydro-1H-pyrazino[1',2':1,5]pyrrolo[2,3-b]indole-1,4(6H)-dione (2b) ^{1}H-^{13}C HSQC NMR



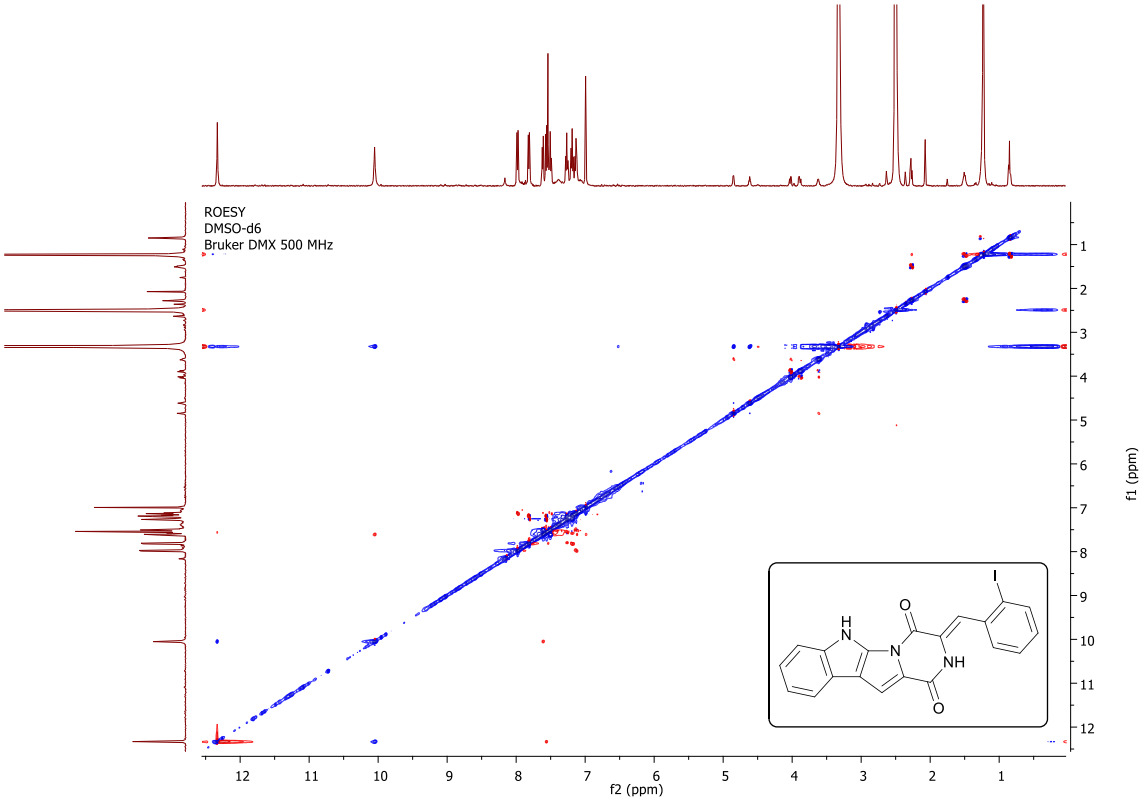
(Z)-3-(2-iodobenzylidene)-2,3-dihydro-1H-pyrazino[1',2':1,5]pyrrolo[2,3-b]indole-1,4(6H)-dione (2b) COSY NMR



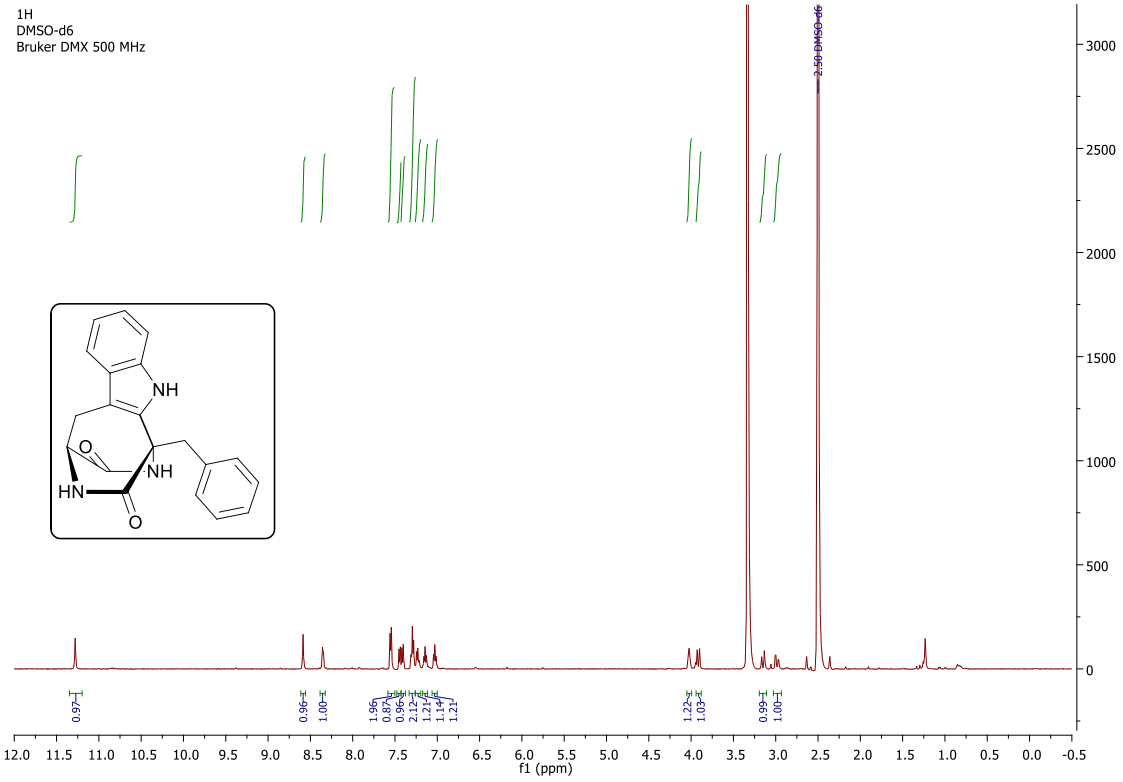
(Z)-3-(2-iodobenzylidene)-2,3-dihydro-1H-pyrazino[1',2':1,5]pyrrolo[2,3-b]indole-1,4(6H)-dione (2b) TOCSY NMR



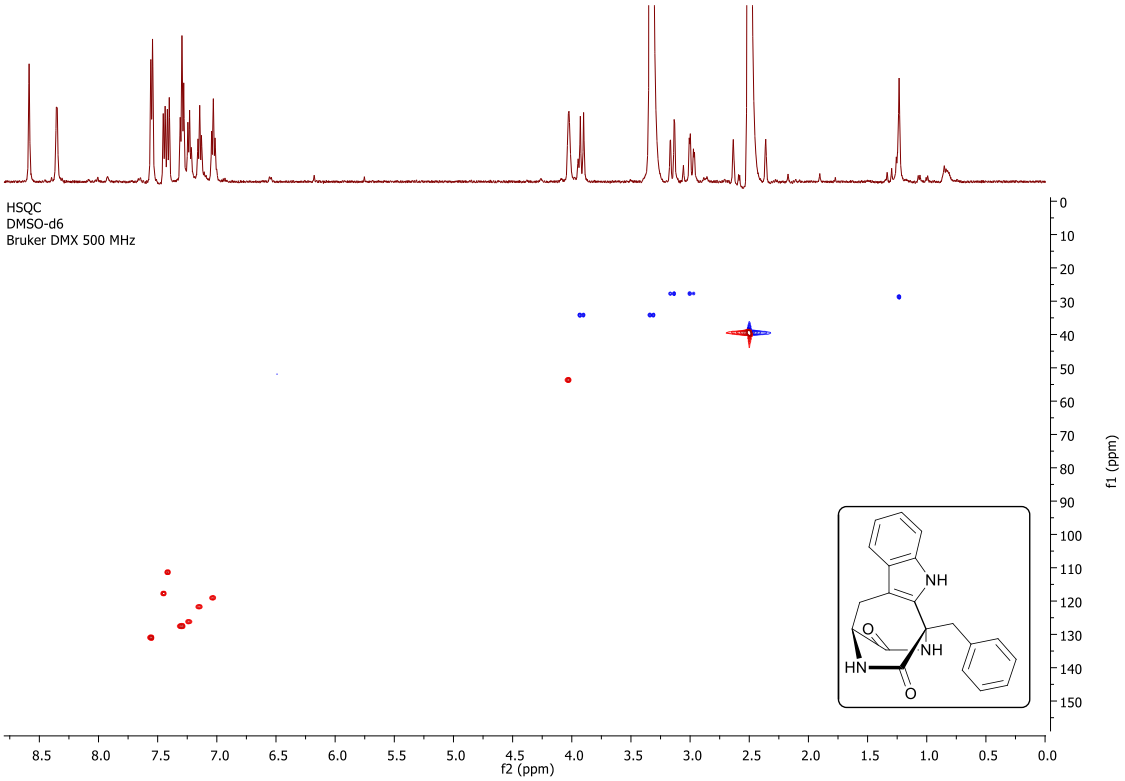
(Z)-3-(2-iodobenzylidene)-2,3-dihydro-1H-pyrazino[1',2':1,5]pyrrolo[2,3-b]indole-1,4(6H)-dione (2b) ROESY NMR



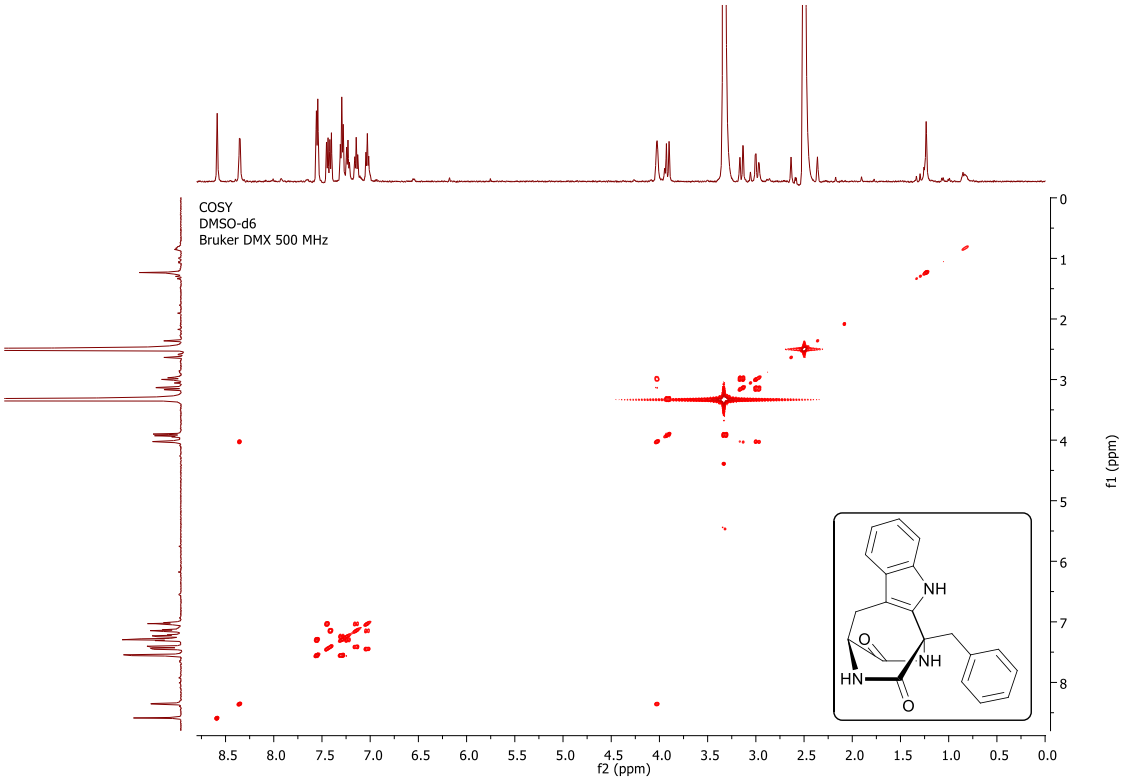
Oxidized cyclo(Phe-Trp) (4b) ^{1}H NMR



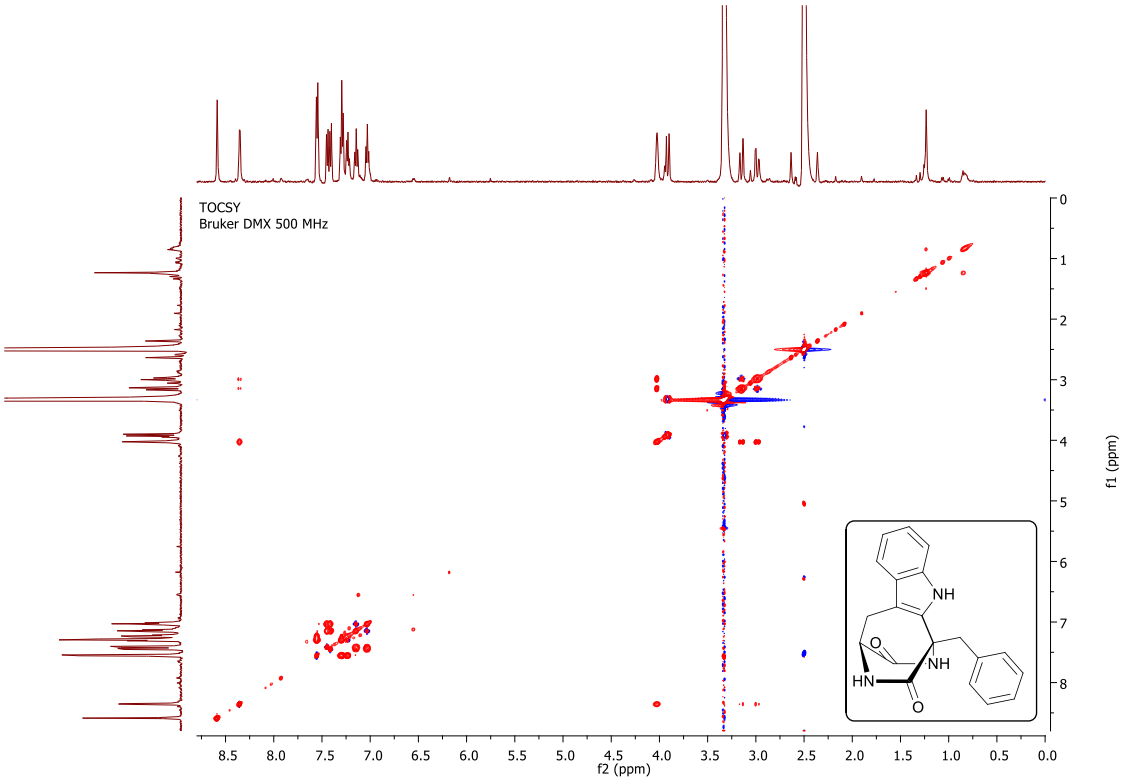
Oxidized cyclo(Phe-Trp) (4b) ^{1}H-^{13}C HSQC NMR



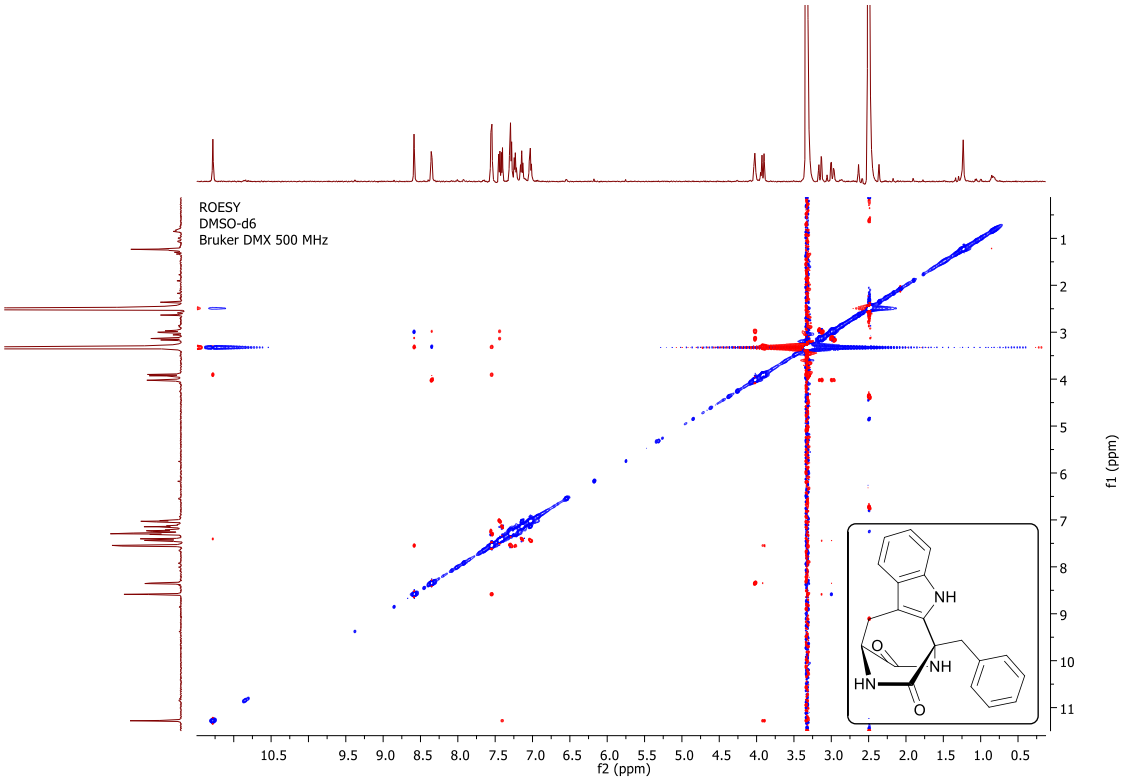
Oxidized cyclo(Phe-Trp) (4b) COSY NMR



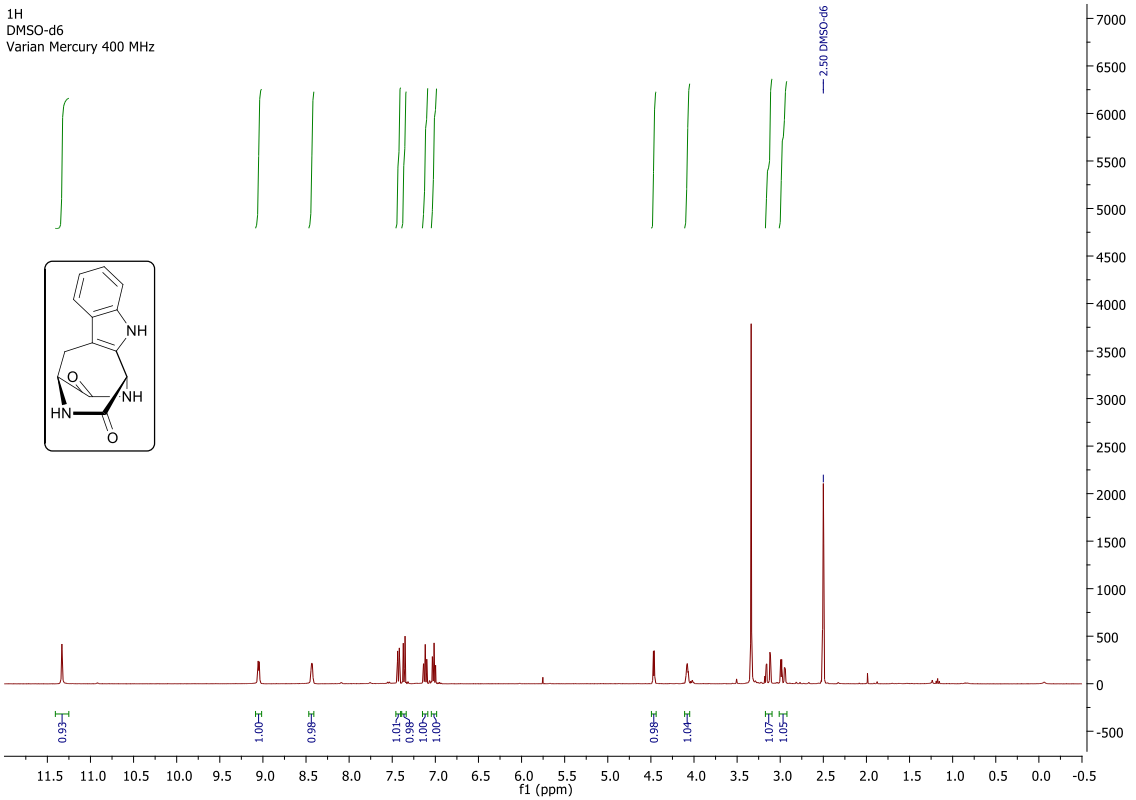
Oxidized cyclo(Phe-Trp) (4b) TOCSY NMR



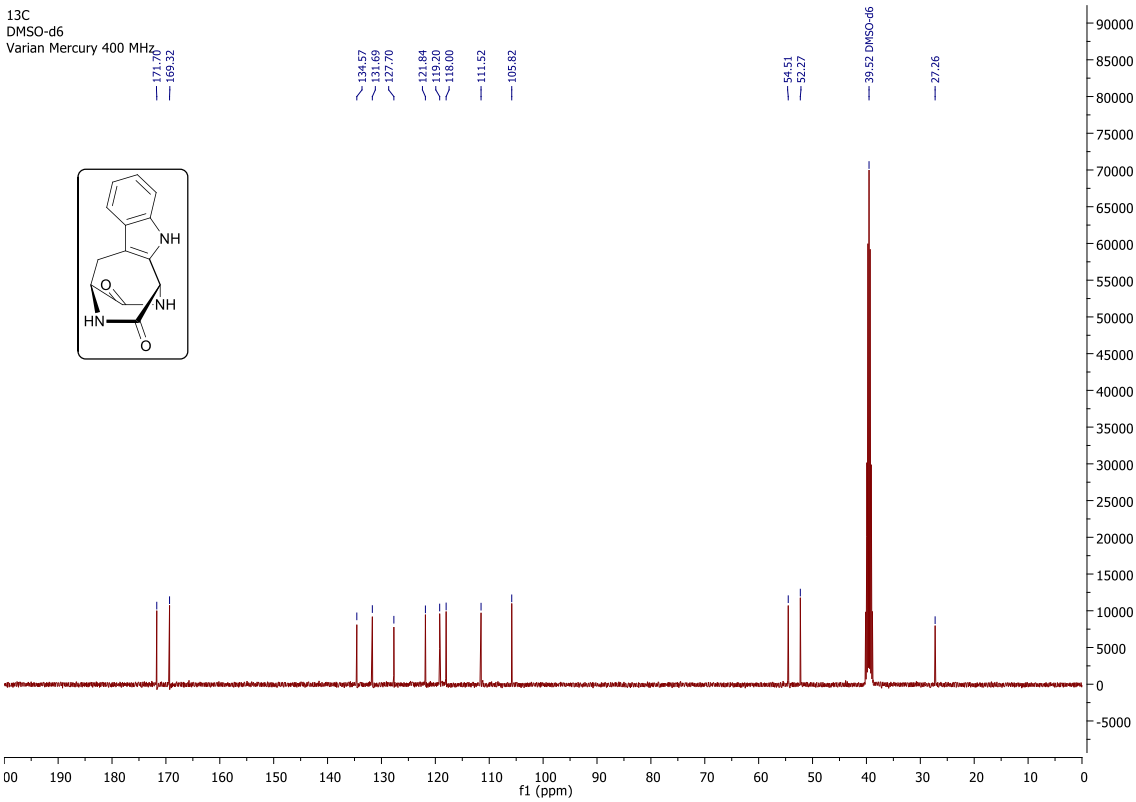
Oxidized cyclo(Phe-Trp) (4b) ROESY NMR



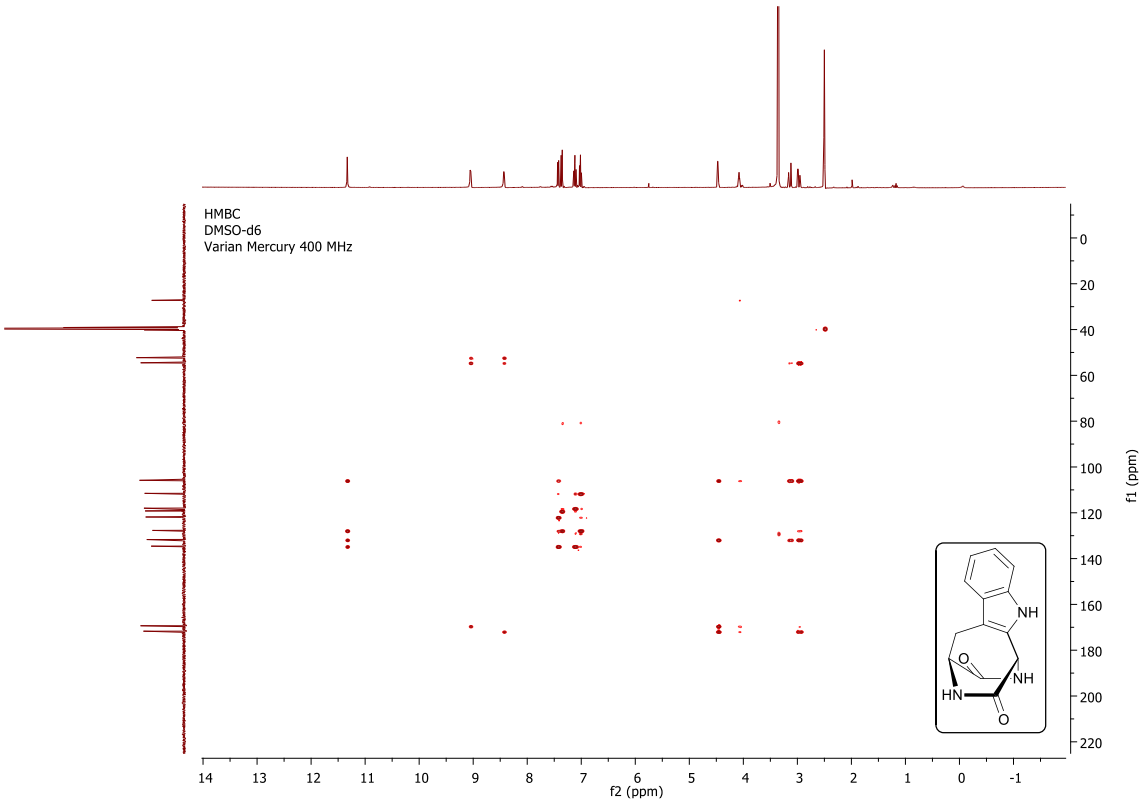
Oxidized cyclo(Gly-Trp) (4c) ^{1}H NMR



Oxidized cyclo(Gly-Trp) (4c) ^{13}C NMR



Oxidized cyclo(Gly-Trp) (4c) ^{1}H-^{13}C HMBC NMR



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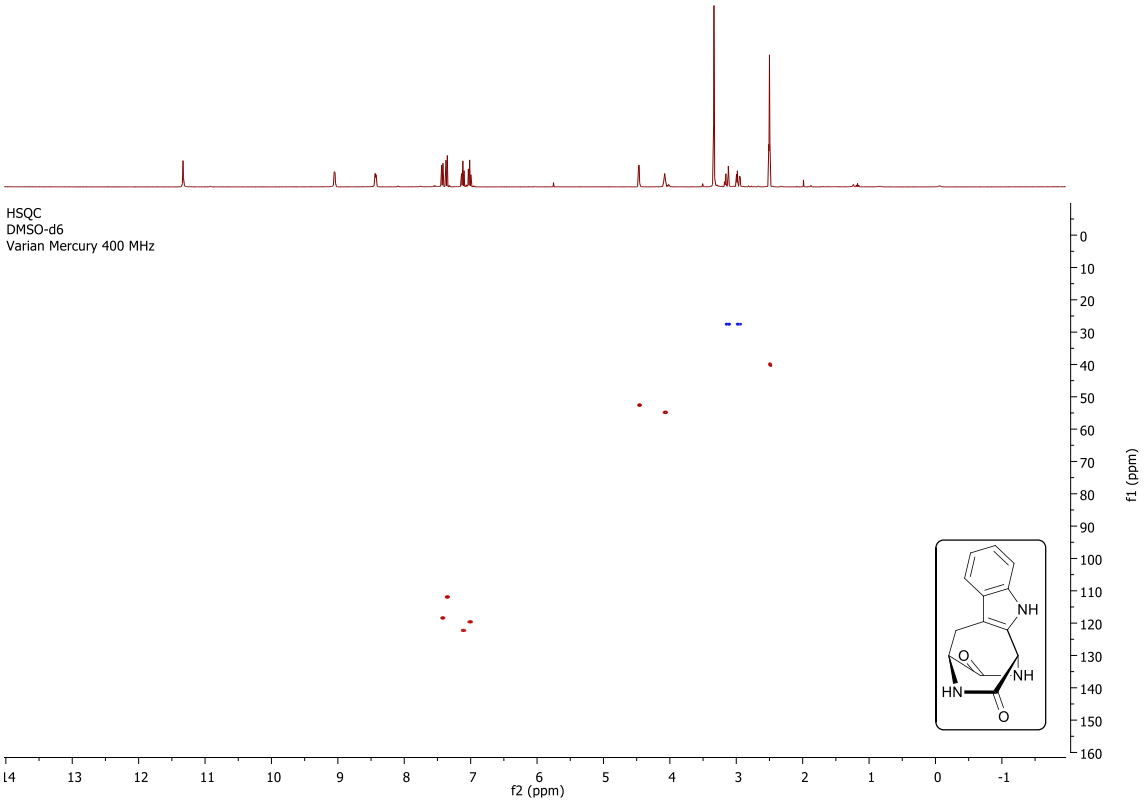

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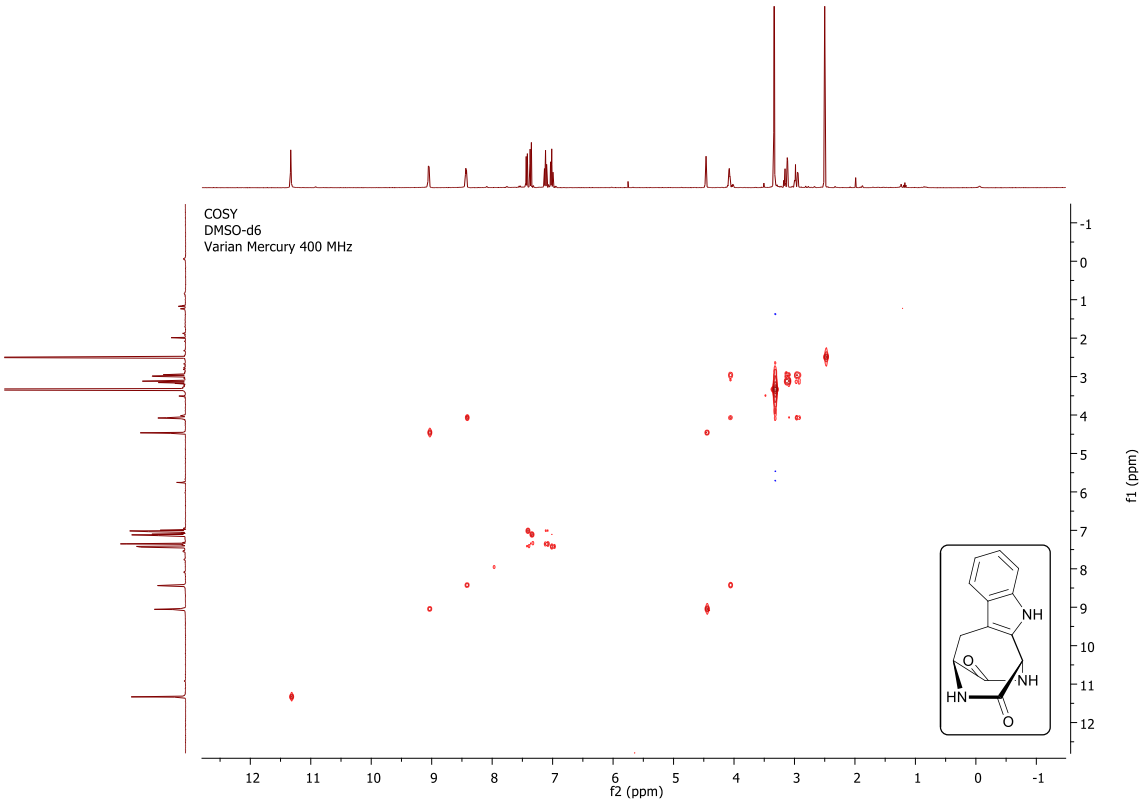
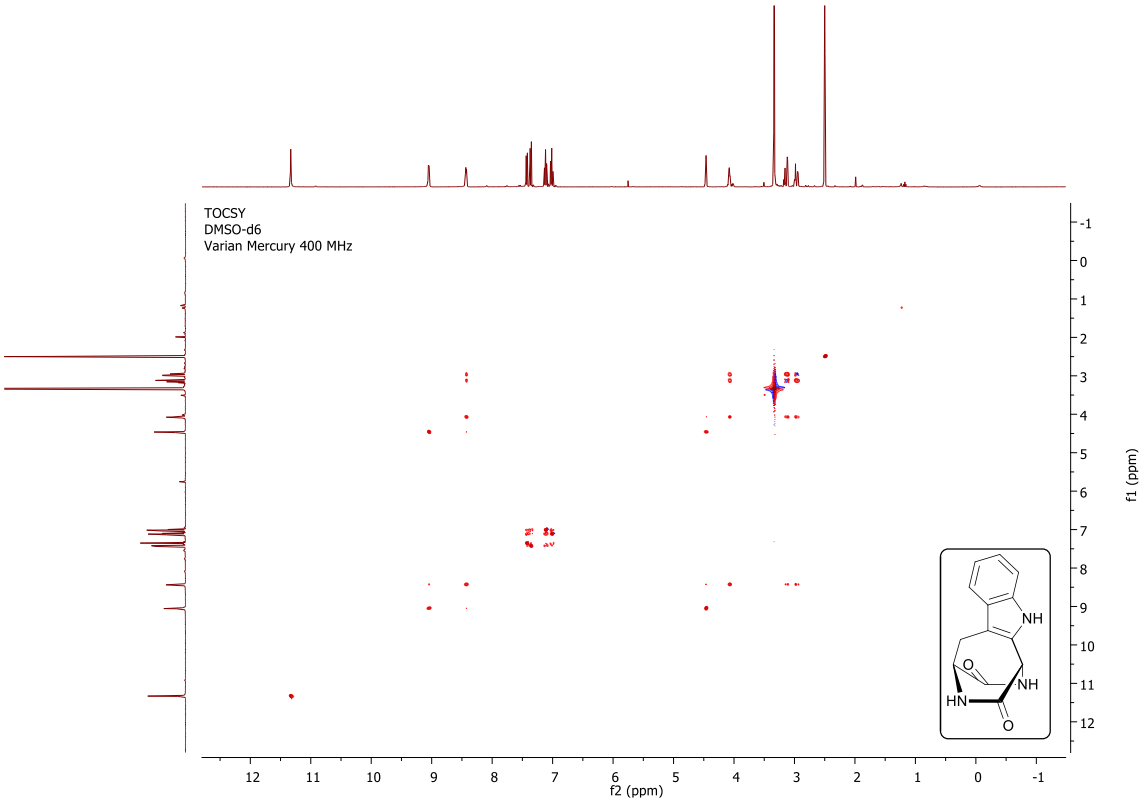
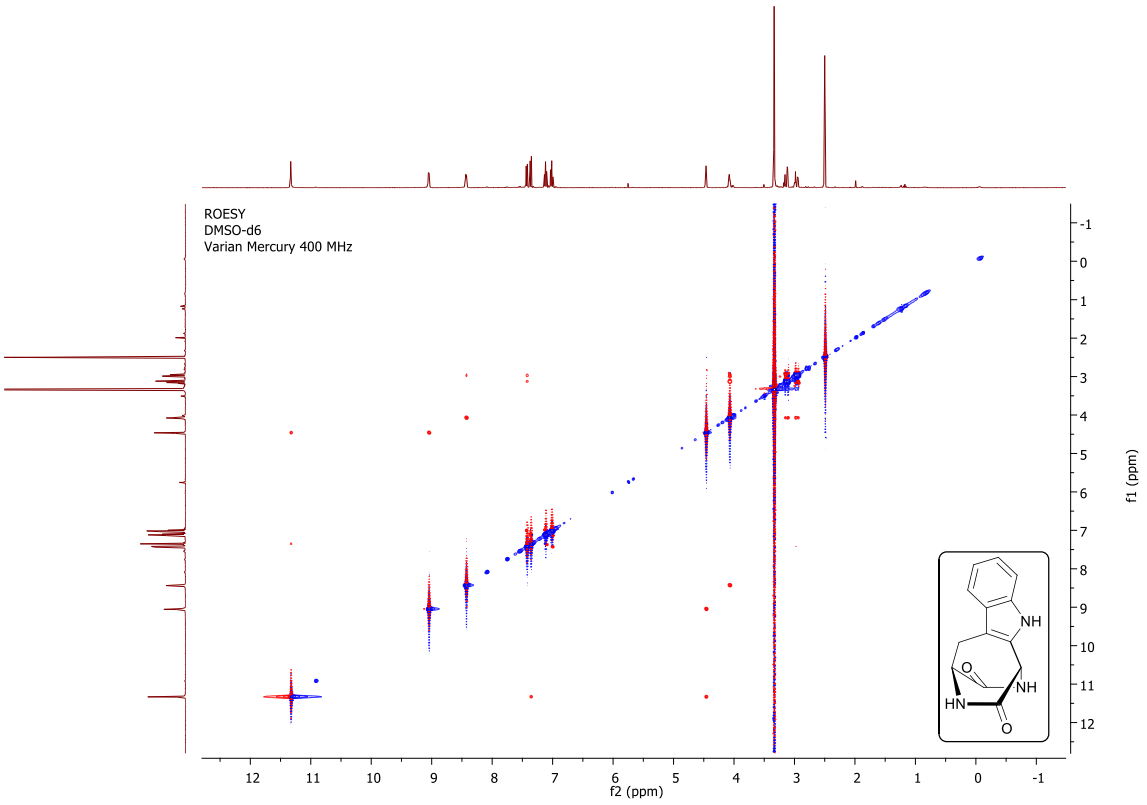


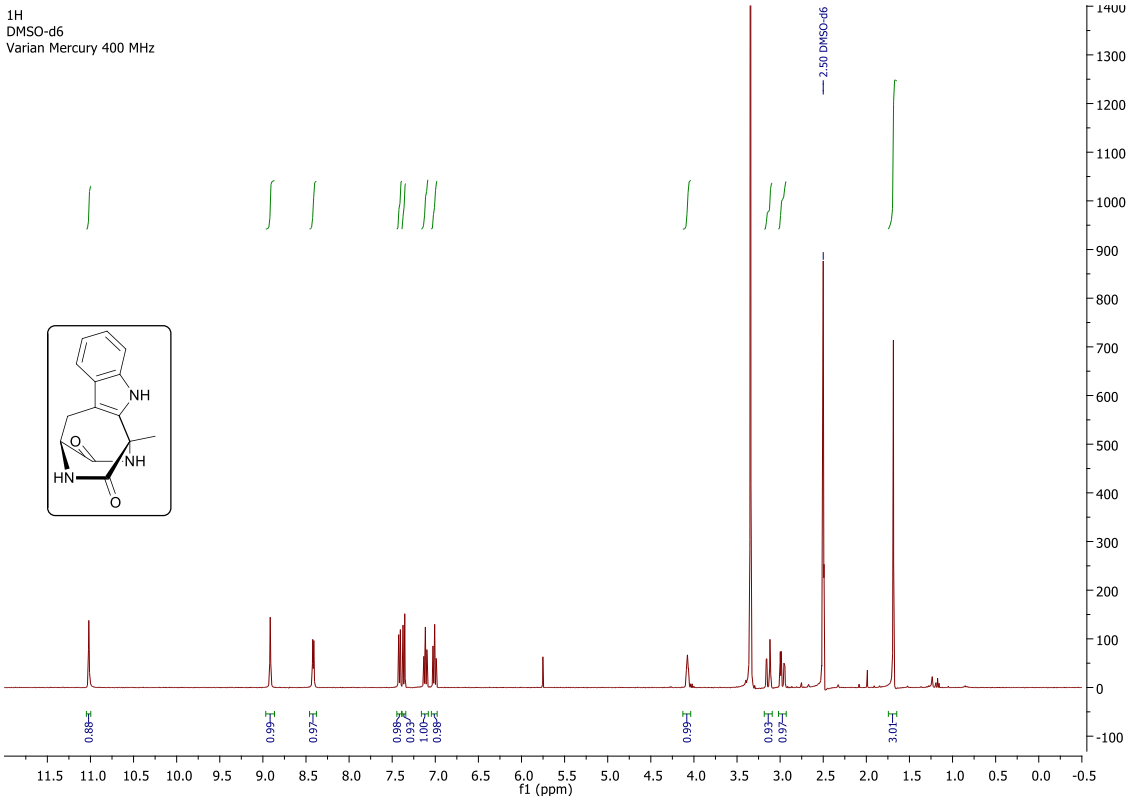
Oxidized cyclo(Gly-Trp) (4c) TOCSY NMR



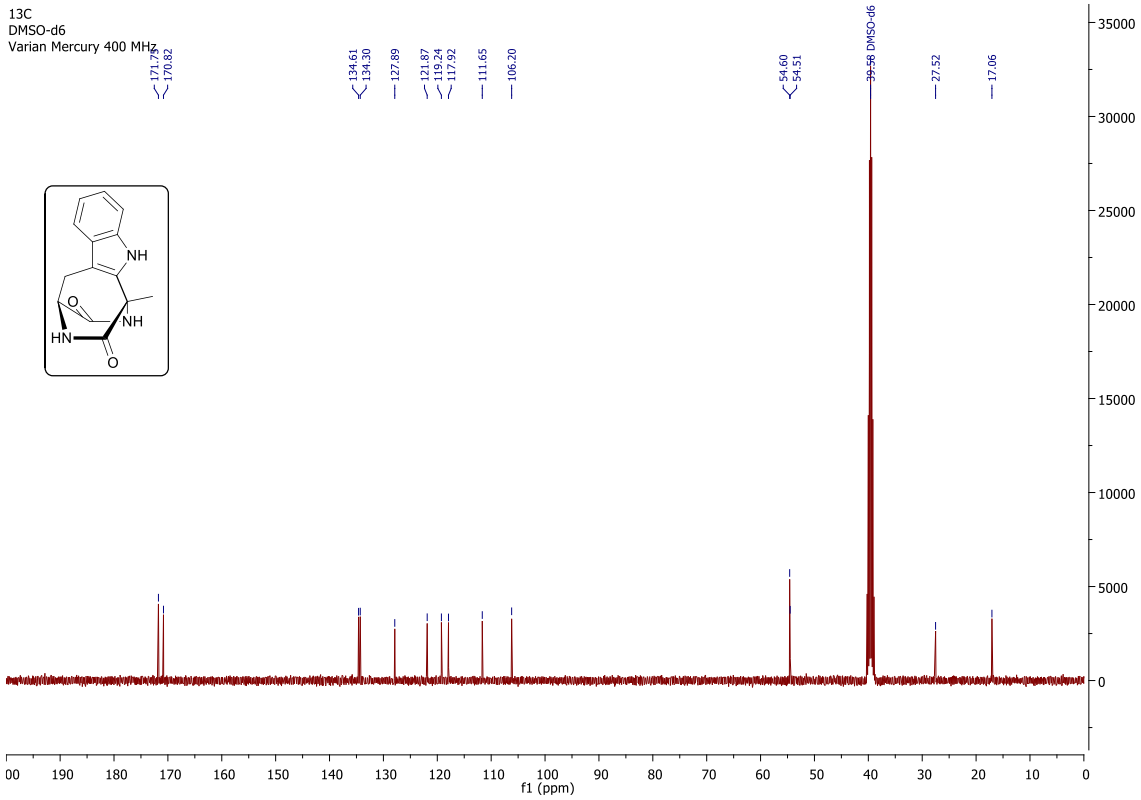
Oxidized cyclo(Gly-Trp) (4c) ROESY NMR



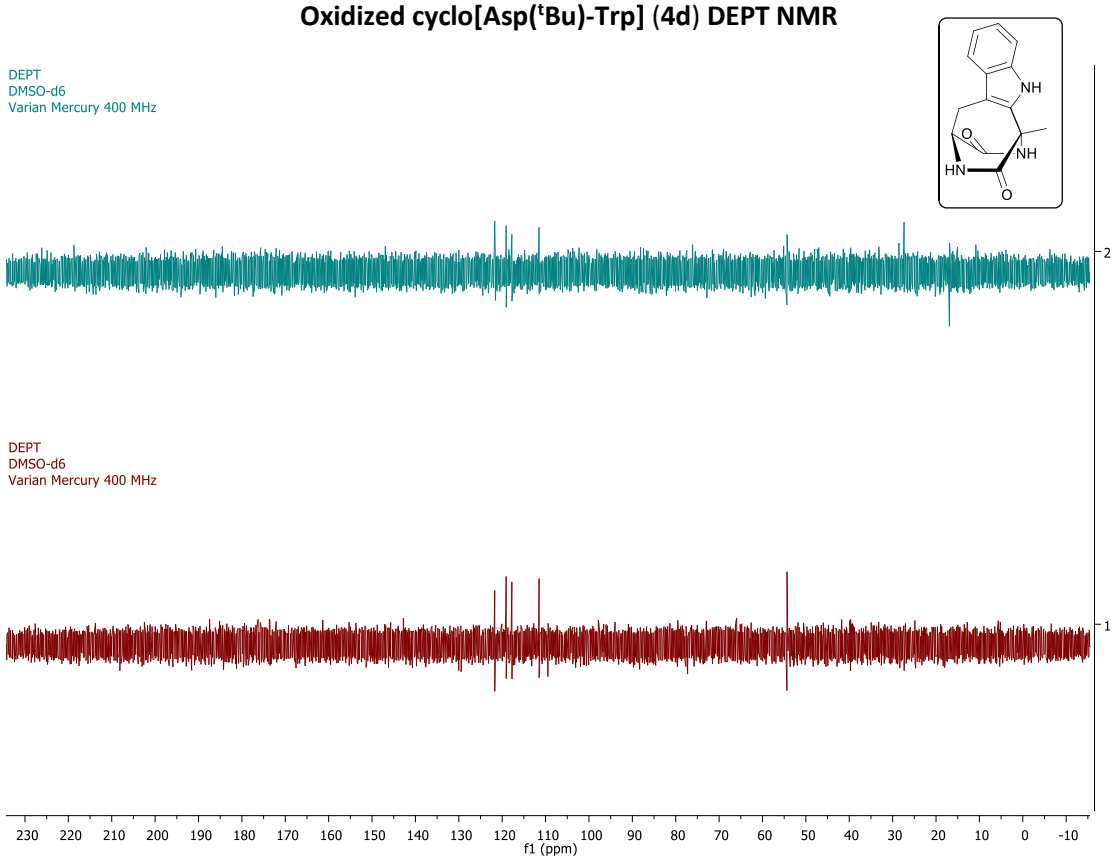
Oxidized cyclo[Asp(^{t}Bu)-Trp] (4d) ^{1}H NMR



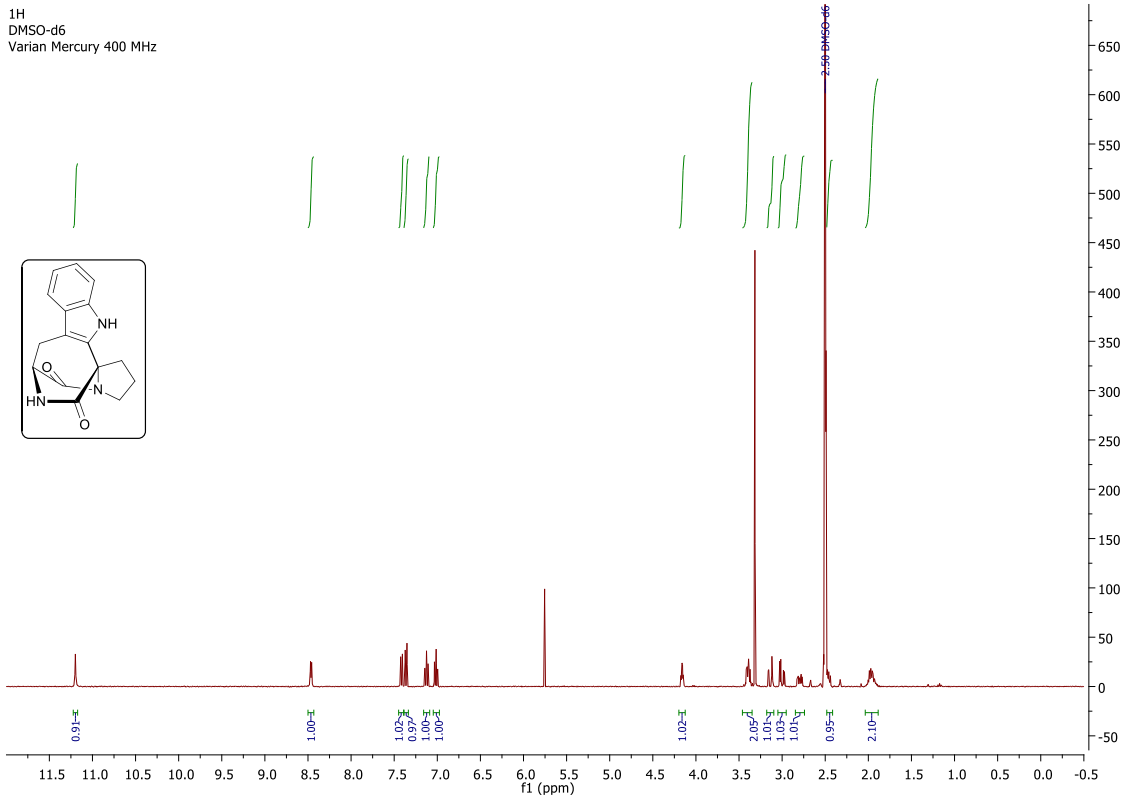
Oxidized cyclo[Asp(ᵗBu)-Trp] (4d) ^{13}C NMR



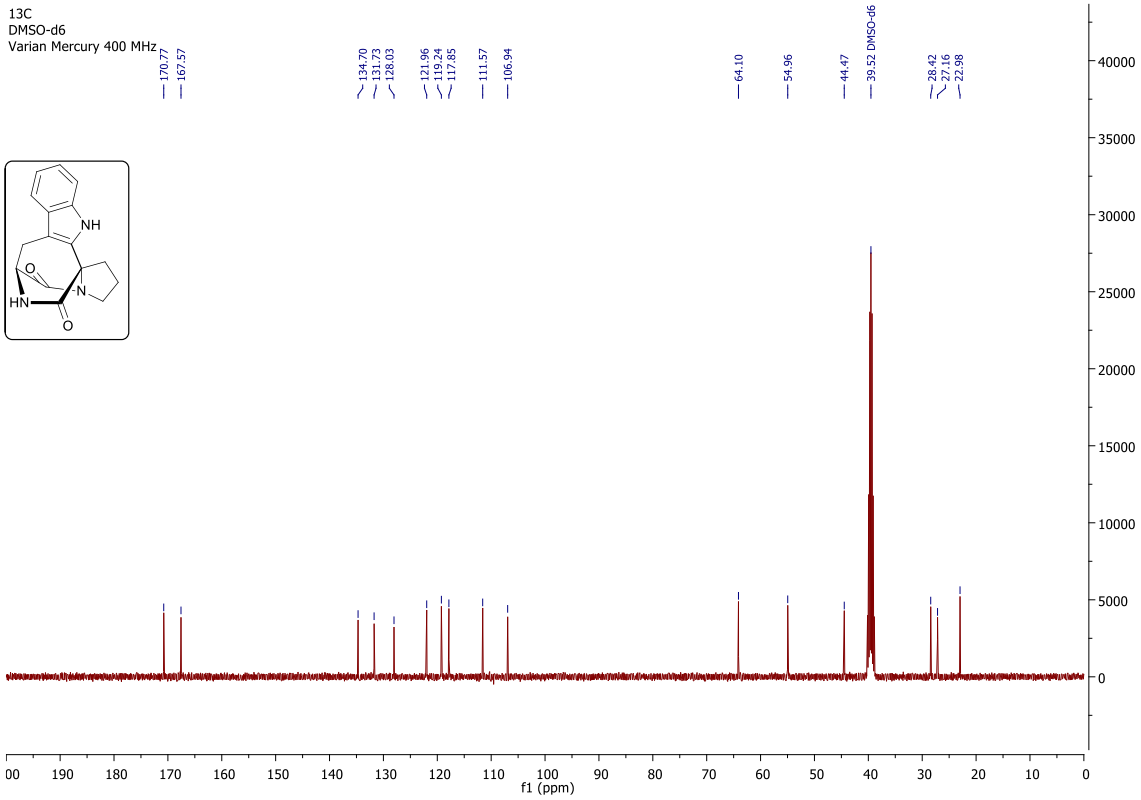
Oxidized cyclo[Asp(ᵗBu)-Trp] (4d) DEPT NMR



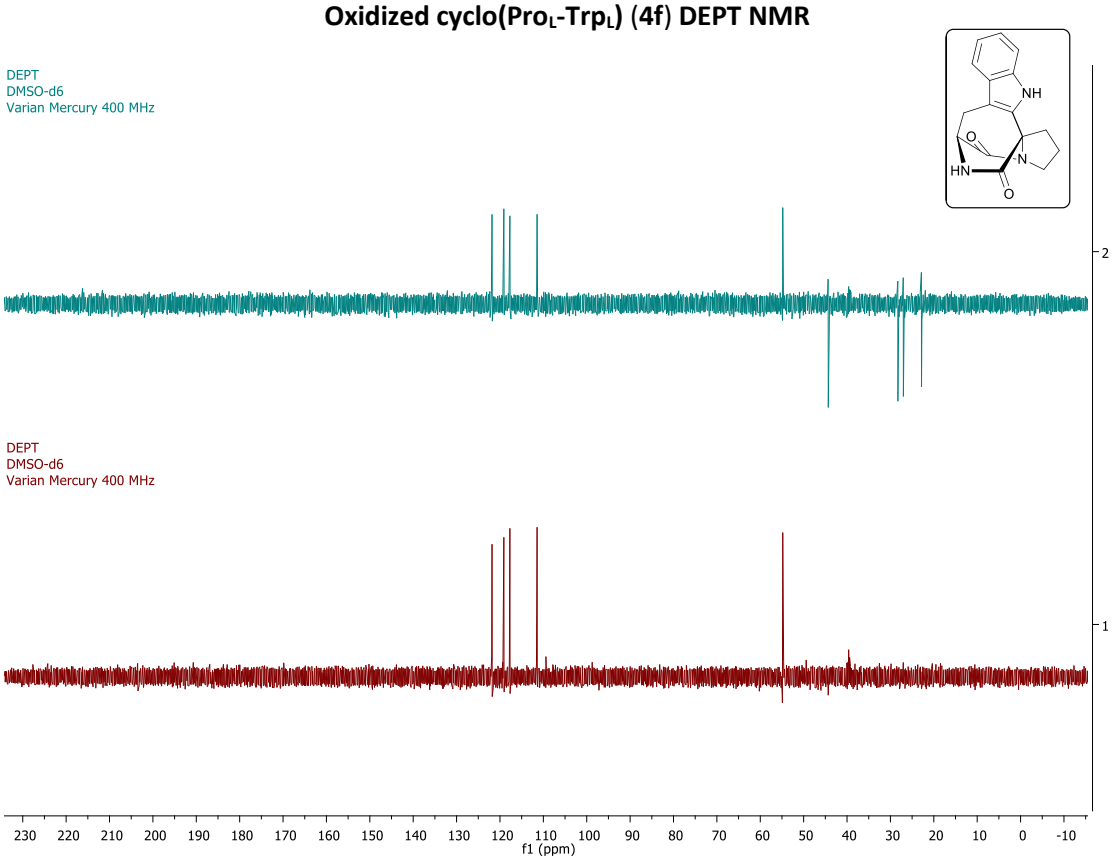
Oxidized cyclo(Pro$_L$-Trp$_L$) (4f) ^{1}H NMR



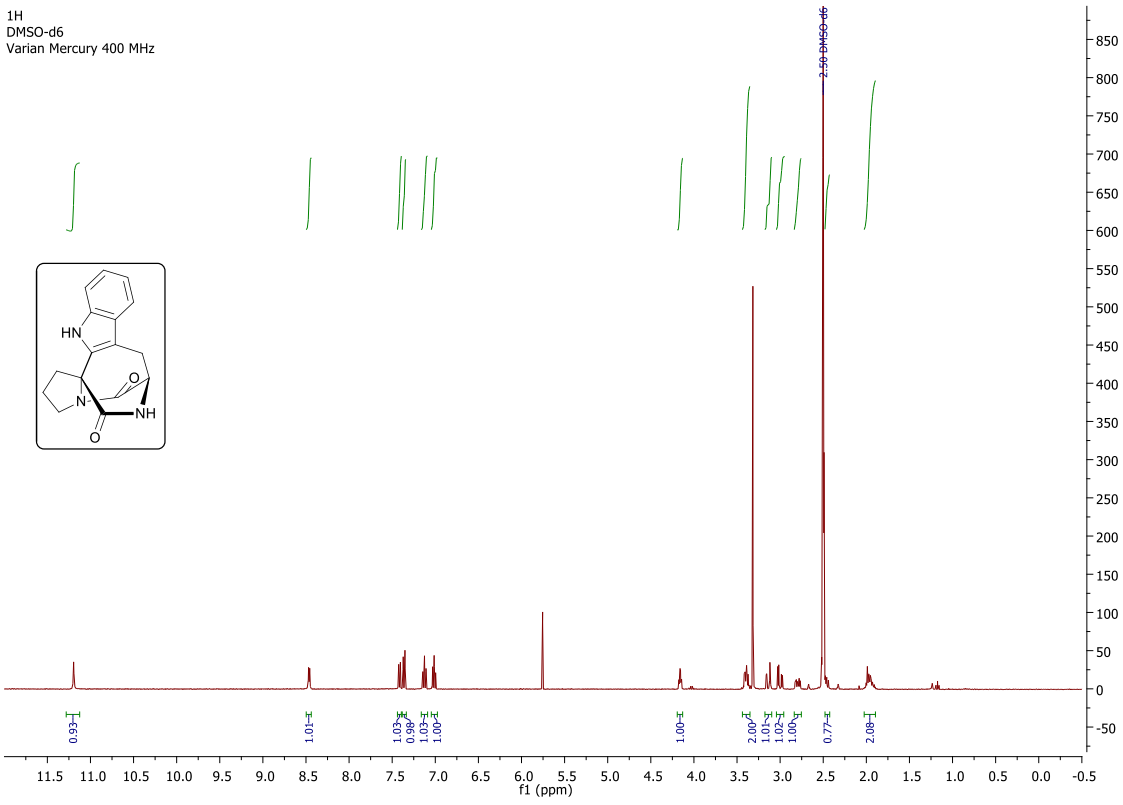
Oxidized cyclo(Pro$_L$-Trp$_L$) (4f) ^{13}C NMR



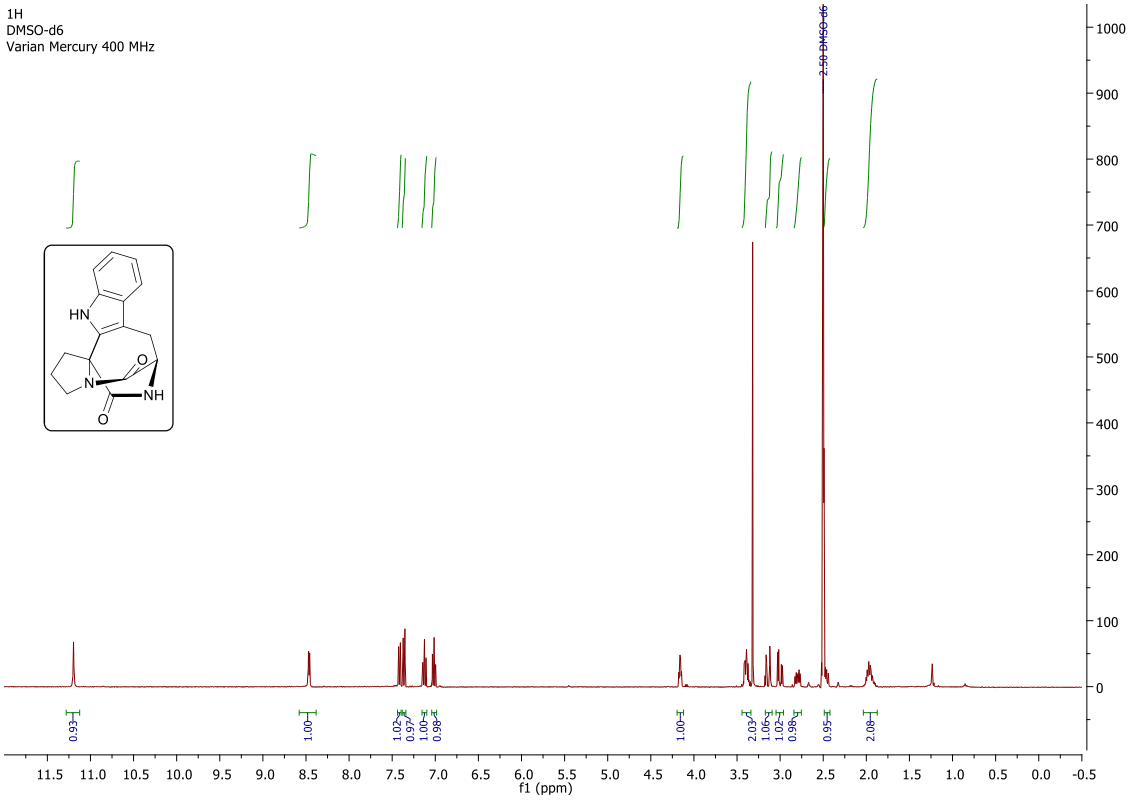
Oxidized cyclo(Pro$_L$-Trp$_L$) (4f) DEPT NMR



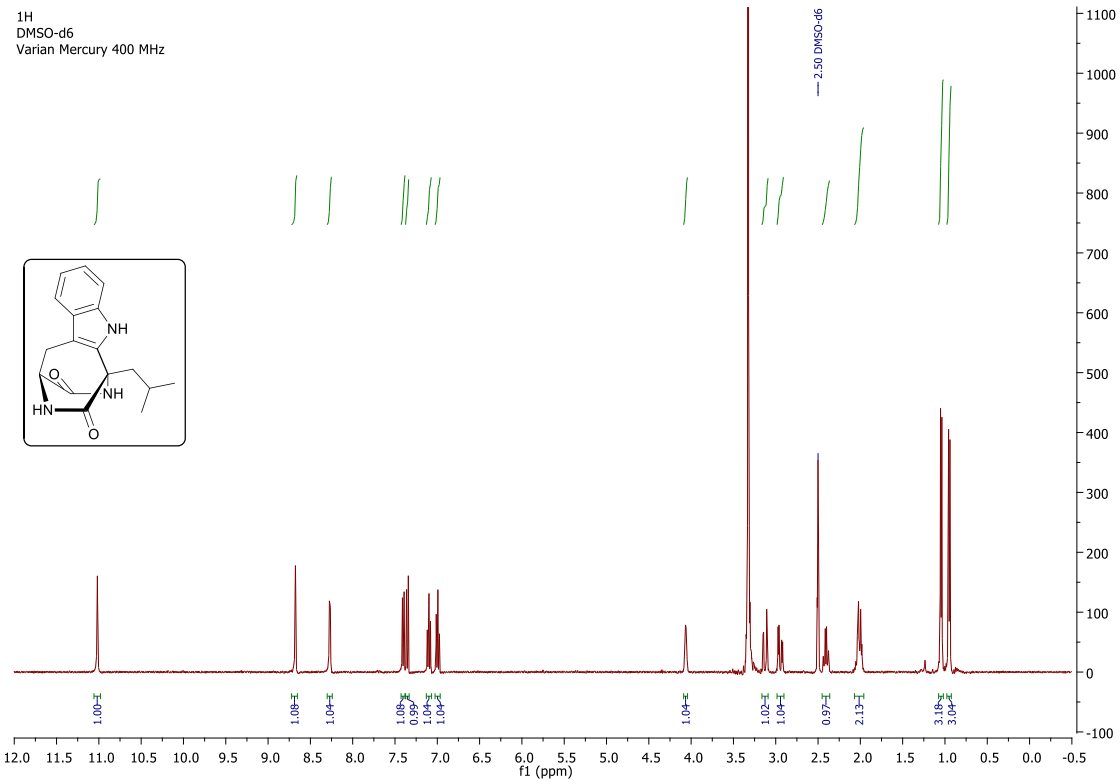
Oxidized cyclo(Pro$_D$-Trp$_D$) (4g) ^{1}H NMR



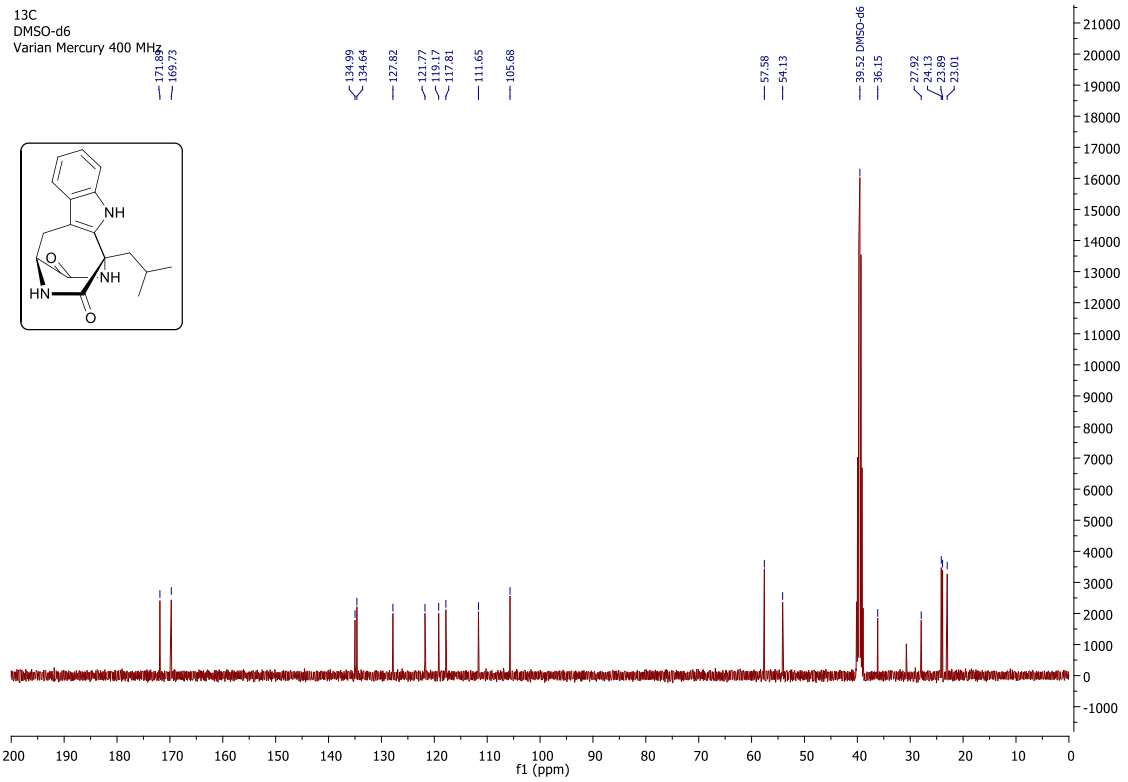
Oxidized cyclo(Pro$_L$-Trp$_D$) (4h) ^{1}H NMR



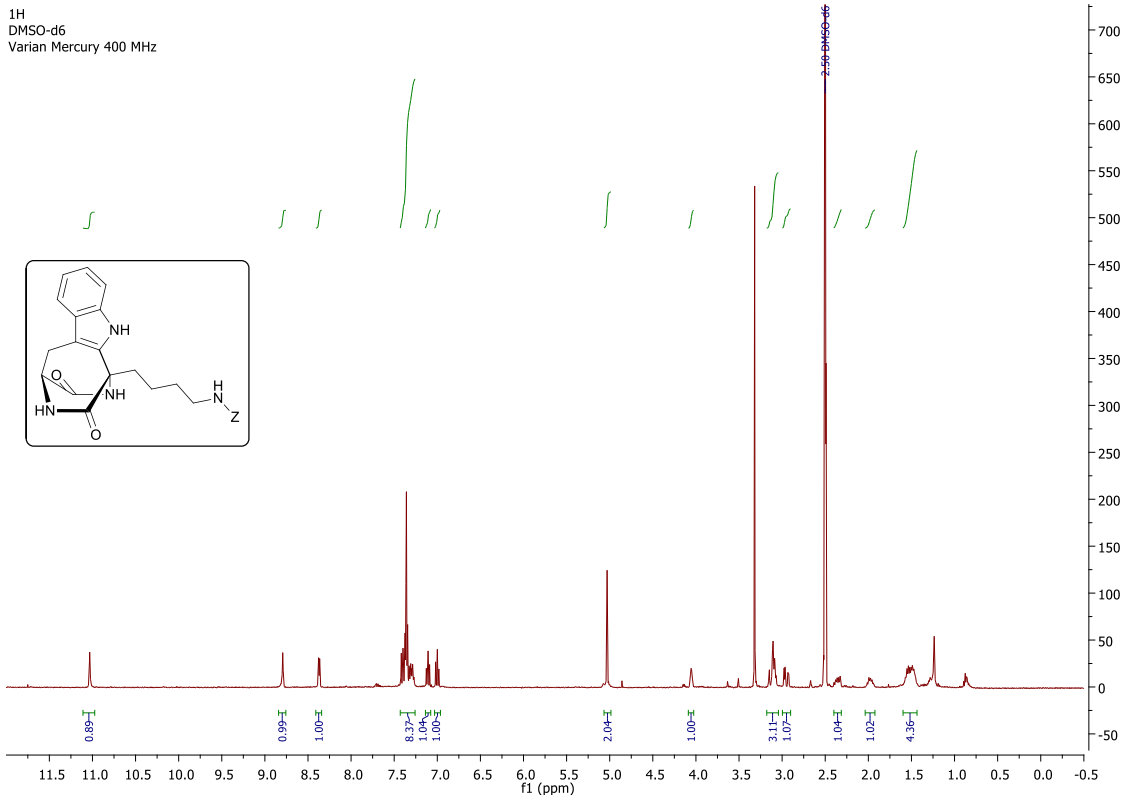
Oxidized cyclo(Leu-Trp) (4i) ^{1}H NMR



Oxidized cyclo(Leu-Trp) (4i) ^{13}C NMR

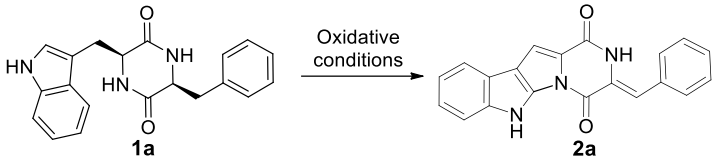


Oxidized cyclo[Lys(z)-Trp] (4j) ^{1}H NMR



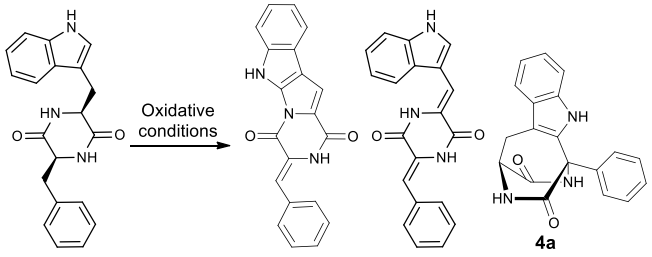
Oxidative screening experiments. Supplementary tables

Table S1. First oxidative screening upon c(Phe-Trp) diketopiperazine **1a**.



T (°C)	Oxidant (eq)	Solvent	Time (h)	**2a** (HPLC-MS
120	TBH (3.0)	1-buthanol	26	-
RT	$FeCl_3$(3.0)	CH_2Cl_2	24	-
RT	PIFA (3.0)	DMSO	23	-
120	BQ (3.0)	DMF	25	-
reflux	DLP (3.0)	CH_2Cl_2:H_2O (1:0.2)	30	-
170	DLP (3.0)	DMSO	24	< 5
77	MnO_2 (100)	AcOEt	20	< 5
70	DTBP (3.0)	$CHCl_3$:DMF (1:0.1)	18	-
140	DTBP (4.0)	DMF	48	< 5
140	DTBP:$FeCl_3$ (4.0:0.2)	DMF	24	-
120	DDQ:$FeCl_3$ (3.0:0.1)	DMF	37	16
80	DDQ (3.0)	^{t}BuOH	27	-
120	DDQ (3.0)	DMF	39	25

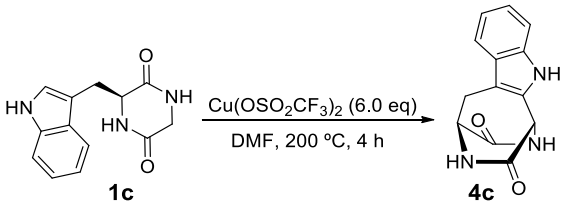
Table S2. Oxidative screening upon c(Phe-Trp) diketopiperazine **1a**.[a]



Entry	Oxidant (eq)	Additive (eq)	Time	**2a**[b]	**3a**	**4a**
1[b]	$PhI(OAc)_2$ (1.1)	-	4 h	-	-	-
2	$PdCl_2$ (3.0)	2,5-lutidine (6.0) Ag_2CO_3 (3.0)	4 h	-	19	5
3	$CuCl_2$ (6.0)	-	4 h	-	-	-
4	$Cu(OAc)_2$ (6.0)	-	4 h	14	59	3
5	$Cu(OCOCF_3)_2$ (6.0)	-	4 h	1	42	28
6	$Cu(OSO_2CF_3)_2$ (6.0)	-	4 h	-	4	71
7	$Cu(OAc)_2$ (6.0)	DDQ (1.0)	4 h	3	45	4
8	$Mn(OAc)_3$ (6.0)	DDQ (1.0)	4 h	-	12	4
9	$Mn(OAc)_3$ (6.0)	$Cu(OAc)_2$ (1.0)	4 h	-	6	20
10	MnO_2 (6.0)	$Cu(OAc)_2$ (1.0)	4 h	-	-	-
11	$Cu(OAc)_2$ (6.0)	TFA (3.0)	4 h	-	32	31
12	$Cu(OAc)_2$ (6.0)	2,5-lutidine (6.0)	4 h	1	44	4

[a] 30 mg of DKP (0.09 mmol), C:0.2-0.3M, DMF, T: 200 °C, [b] T: r.t, HFIP. [b] Values are given in (%).

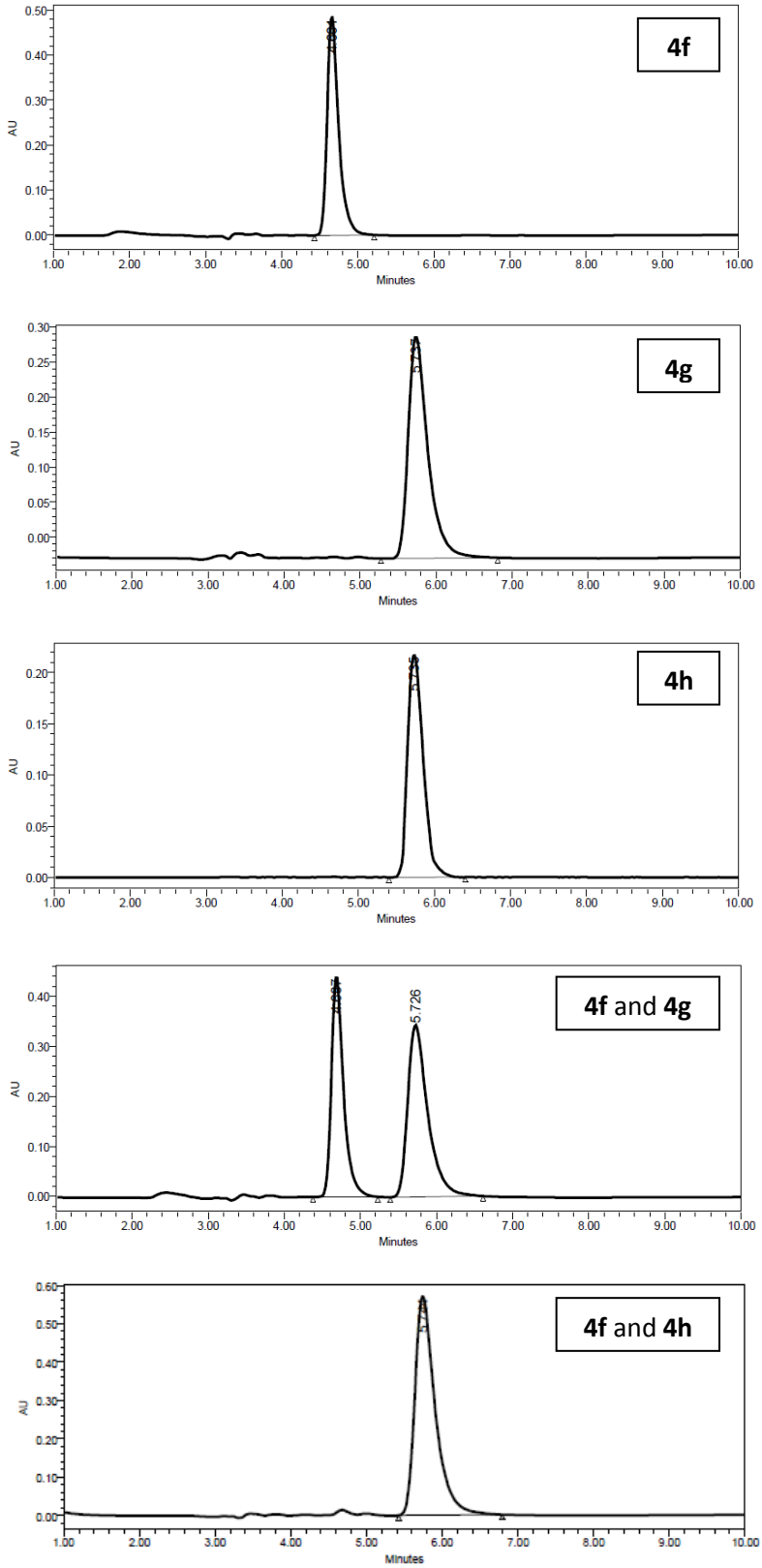
Table S3. Optimization of Cu(II)-based oxidation upon c(Gly-Trp) diketopiperazine **1c**.[a]

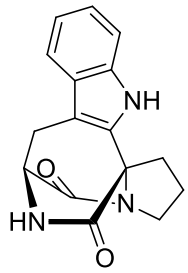


T (°C)	Oxidant (eq)	Additive (eq)	Solvent	Time	Product (%)[b]
200	$Cu(OCOCF_3)_2$ (6.0)	-	DMF	4 h	90
200	$Cu(OAc)_2$ (6.0)	-	DMF	4 h	75
200	$Cu(OSO_2CF_3)_2$ (6.0)	-	DMF	4 h	40
200	$Cu(OCOCF_3)_2$ (6.0)	-	DMF	2 h	74
200	$Cu(OCOCF_3)_2$ (4.0)	-	DMF	4 h	70
150	$Cu(OCOCF_3)_2$ (6.0)	-	DMF	16 h	68
150 (MW)	$Cu(OCOCF_3)_2$ (6.0)	-	DMF	30 min	49
150 (MW)	$Cu(OCOCF_3)_2$ (2.0)	TFA (4.0)	DMF	30 min	66
150 (MW)	$Cu(OCOCF_3)_2$ (2.0)	-	DMF	30 min	54
120 (MW)	$Cu(OCOCF_3)_2$ (2.0)	TFA (4.0)	DMF	30 min	69
120 (MW)	$Cu(OCOCF_3)_2$ (4.0)	TFA (4.0)	DMF	30 min	92
120 (MW)	$Cu(OCOCF_3)_2$ (4.0)	TFA (2.0)	DMF	30 min	86

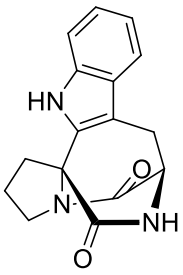
[a] 30 mg of DKP (0.12 mmol) , C: 0.2 M. [b] Conversion estimated by HPLC-MS. [c] C: 0.4 M.

Chiral chromatography of oxidized DKPs 4f-h

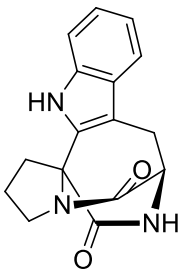




L-Pro-L-Trp (4f)



D-Pro-D-Trp (4g)



L-Pro-D-Trp (4h)

Figure S1. Chiral chromatograms of a) **4f**, b) **4g**, c) **4h**, d) co-elution of **4f** and **4g**, b) co-elution of **4g** and **4h**. Linear gradient of ACN (+0.036% TFA) into H_2O (+0.045% TFA) from 50% to 70% ACN for 30 min.

Proposed mechanism for the decarboxylation of Asp-containing DKP

Asp(^{t}Bu)-containing DKP **1e** underwent decarboxylation to yield the oxidized c(Ala-Trp) **4e**. Since no traces of decarboxylated starting DKP **1e** were detected by HPLC-MS analysis, it was proposed a mechanism where the initial DKP undergoes decarboxylation once the dehydrogenative C-C bond is formed to yield compound **4e** (Fig. S2). Incidentally, use of alternative non-acid labile protecting groups for Asp (*i.e.* -Allyl, -Bzl) was detrimental for the outcome of the CDC reaction.

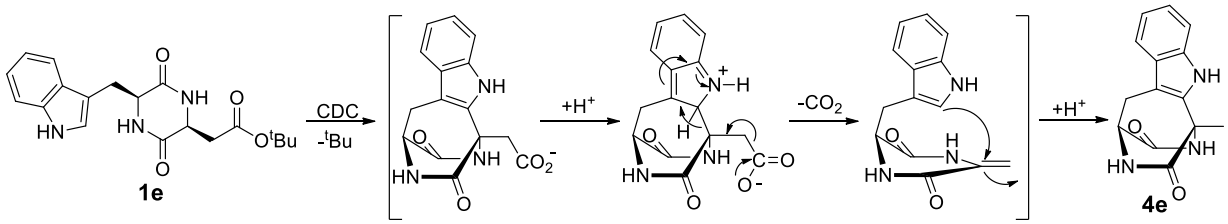


Figure S2. Plausible mechanism for the decarboxylation of compound **4e**.

Proposed 1,4-hydride shift within iminium forms for the CDC

The high conversion observed for different DKPs suggests a high α-C selectivity for the C-C bond formation, which is remarkable taking into consideration that two N-iminium intermediates are possible. This may be attributed to an hypothetical 1,4-hydride shift within the two iminium forms. The following mechanism proposal was based on other 1,4-hydride shifts reported in the literature[2–5] as well as hydrogen tunneling and relay mechanisms[6,7] (Fig. S3). Moreover, the cyclization via the nucleophilic attack of the indole ring to the N-iminium of Trp residue would arise a highly strained four-member cycle.

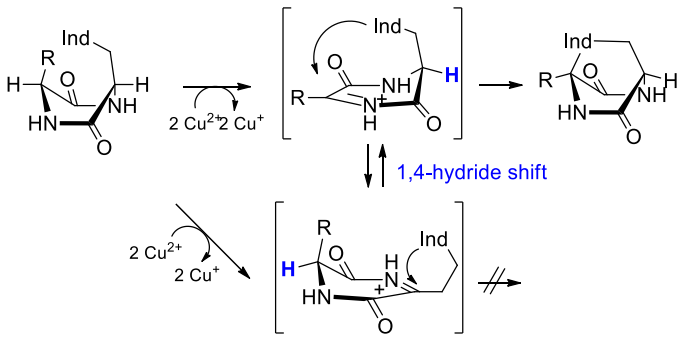


Figure S3. Hypothetical 1,4-hydride shift within iminium intermediates generated during CDC.

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Electronic Supplementary Information

A Trp-BODIPY cyclic peptide for fluorescence labelling of apoptotic bodies

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Materials and Methods

Fmoc-amino acids were obtained from Iris Biotech GmbH (Fmoc-Gly-OH, Fmoc-Ile-OH, Fmoc-Phe-OH), Chem-Impex International [Fmoc-Asp-OAllyl, Fmoc-Gln-OH, Fmoc-His(Mmt)-OH] and Sigma-Aldrich [Fmoc-Arg(NO_2)-OH]. Resins and HBTU were obtained from Iris Biotech GmbH. HOBt was purchased from Carbosynth. Pd-based catalysts and bases were obtained from Sigma-Aldrich. All reagents were used without further purification unless otherwise stated. All microwave reactions were carried out in 10 mL sealed glass tubes in a focused mono-mode microwave oven ("Discover" by CEM Corporation) featured with a surface sensor for internal temperature determination. Cooling was provided by compressed air ventilating the microwave chamber during the reaction. Spectroscopic data and quantum yield data were measured on a Synergy HT spectrophotometer (Biotek), and the data analysis was performed using GraphPad Prism 5.0.

Reactions were monitored by HPLC-MS at 220 nm using a HPLC Waters Alliance HT comprising a pump (Edwards RV12) with degasser, an autosampler, a YMC-Pack ODS-AQ, 50 × 4.6 mm, S-3 µm column and a diode array detector. Eluents: H_2O (0.1% FA) and ACN (0.1% FA). Flow: 1.6 mL·min^{-1}. The MS detector was configured with an electrospray ionization source (Micromass ZQ4000) and nitrogen was used as the nebulizer gas. Data acquisition was performed with MassLynx software. HRMS (ESI positive) were obtained in a LTQ-FT Ultra (Thermo Scientific) mass spectrometer. NMR spectra were recorded on Bruker Avance-III 600 MHz spectrometer in DMSO-d_6 at 308 K. Chemical shifts (δ) are reported in ppm. Multiplicities are referred by the following abbreviations: s = singlet, d = doublet, t = triplet, dd = double

doublet, ddd = double double doublet, dt = double triplet, q= quartet and m = multiplet.

General procedures for SPPS

All peptides were manually synthesized in polystyrene syringes fitted with a polyethylene porous disc using Fmoc-based SPPS. Solvents and soluble reagents were removed by suction. The Fmoc group was removed with piperidine: DMF (1: 4) (1 × 1 min, 2 × 5 min). Peptide synthesis transformations and washings were performed at r.t.

Resin loading. Fmoc-Asp-OAllyl (1 eq.) was attached to the resin (1 eq.) with DIPEA (3 eq.) in DCM at r.t. for 10 min and then DIPEA (7 eq.) for 40 min. The remaining trityl groups were capped adding 0.8 µL MeOH mg^{-1} resin for 10 min. The resin was filtered and washed with DCM (4 × 1 min), DMF (4 × 1 min). The loading of the resin was determined by titration of the Fmoc group.

Peptide elongation. After the Fmoc group was removed with piperidine: DMF (1: 4) (1 × 1 min, 2 × 5 min), the resin was washed with DMF (4 × 1 min), DCM (3 × 1 min), DMF (4 × 1 min). Unless otherwise noted, standard coupling procedure with DIC (3 eq.) and OxymaPure (3 eq.) in DMF for 1 h and 5 min of pre-activation was carried out. The completion of the coupling was monitored with the Kaiser test. Then, the resin was filtered and washed with DCM (4 × 1 min) and DMF (4 × 1 min) and was ready for the elongation with the next Fmoc amino acid.

Final cleavage. The resin bound peptide was treated repeated times with TFA: DCM (1: 99) for 1 min in each treatment and washed with DCM. The combined filtered mixtures were poured over DCM and evaporated under vacuum. The residue was precipitated in Et_2O, dissolved in ACN: H_2O and liophilised.

Experimental Section

Chemical synthesis.

cLac-1. Starting from 750 mg of *2*-chlorotrityl PS resin (0.3 mmol g^{-1}). Amino acid coupling. Fmoc-AA-OH (3 eq.) were incorporated with a 5 min pre-activation with DIC (3 eq.) and OxymaPure (3 eq.) in DMF for 1 h. Fmoc-AA-OH: Fmoc-Asp-OAllyl, Fmoc-Gly-OH, Fmoc-Arg(NO_2)-OH, Fmoc-Gln-OH, Fmoc-Ile-OH, Fmoc-His(Mmt)-OH, Fmoc-Phe-OH, Fmoc-Trp(Boc)-OH. Peptide cyclisation. The C-terminal allyl ester group of the aspartic acid was removed after addition of the last amino acid with $Pd(PPh_3)_4$ (26 mg, 0.023 mmol, 0.1 eq.) and N-methylmorpholine (244 µL, 2.25 mmol, 10 eq.) in THF for 1 h at r.t. (3 × 15 min). The head-to-tail cyclization was performed by removal of the *N*-terminal Fmoc group before addition of PyAOP (352 mg, 0.674 mmol, 3 eq.), HOAt (92 mg, 0.674 mmol, 3 eq.) and DIPEA (235 µL, 1.35 mmol, 6 eq.) in DMF at r.t for 1 h. Peptide cleavage. The resin bound peptide was treated repeated times with the TFA cocktail obtaining 210 mg of cyclic peptide crude (87% purity by HPLC-MS). Removal of nitro group. The crude protected peptide (210 mg, 0.153 mmol) and 20% $Pd(OH)_2$-C (105 mg) were dissolved in HCO_2H: DMF: H_2O (5: 47.5: 47.5) (10 mL) and the reaction flask was flushed with Ar, evacuated and filled with H_2. The reaction mixture was stirred under balloon pressure of H_2 for 48 h (H_2 was refilled periodically during the reaction along with re-addition of $Pd(OH)_2$-C (2 X). The catalyst was removed through filtration with Celite and the filtrate was evaporated *in vacuo* to afford 164 mg of the crude peptide (83% purity by HPLC-MS, 75% yield). Peptide purification. A highly pure fraction of the totally deprotected cyclic peptide was obtained by semi-preparative RP-HPLC (XBRIDGE™, C_{18}, 5 µM OBD 19 × 150 mm column.

The pure fractions were lyophilised rendering the pure peptide as a white solid (>99% purity by HPLC-MS).

^{1}H NMR (600 MHz, DMSO-d_6): δ 10.70 (s, 1H), 8.61 (m, 1H), 8.46 – 8.31 (m, 3H), 8.23 (s, 1H), 8.18 (s, 1H), 8.15 (s, 1H), 8.06 (s, 1H), 7.99 (s, 1H), 7.88 (s, 1H), 7.71 – 7.59 (m, 1H), 7.57 – 7.51 (m, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.31 – 7.27 (m, 3H), 7.25 – 7.20 (m, 4H), 7.07 (s, 1H), 7.03 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 6.96 (ddd, *J* = 7.9, 6.9, 1.1 Hz, 1H), 6.83 (s, 1H), 6.74 (s, 1H), 4.57 (q, *J* = 7.1 Hz, 1H), 4.42 (q, *J* = 6.3 Hz, 2H), 4.33 (s, 1H), 4.27 (td, *J* = 8.3, 5.0 Hz, 1H), 4.18 (q, *J* = 7.8, 7.1 Hz, 1H), 4.10 (t, *J* = 7.4 Hz, 1H), 3.91 (dd, *J* = 16.9, 6.1 Hz, 1H), 3.79 – 3.68 (m, 9H), 3.65 – 3.61 (m, 1H), 3.55 – 3.50 (m, 1H), 3.11 – 3.03 (m, 2H), 2.99 (dd, *J* = 14.7, 4.6 Hz, 2H), 2.96 – 2.87 (m, 3H), 2.81 (dd, *J* = 15.0, 9.2 Hz, 1H), 2.76 – 2.65 (m, 1H), 2.46 (1H), 2.15 – 2.00 (m, 2H), 1.99 – 1.88 (m, 1H), 1.83 – 1.70 (m, 3H), 1.65 – 1.55 (m, 1H), 1.54 – 1.42 (m, 2H), 1.40 – 1.27 (m, 1H), 1.07 – 0.94 (m, 1H), 0.80 – 0.75 (m, 6H) ppm. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{59}H_{80}O_{16}N_{20}$, 1324.6056; found, 1324.6046.

cLac-BODIPY. Starting from 750 mg of *2*-chlorotrityl PS resin (0.3 mmol g^{-1}). Amino acid coupling. Fmoc-Trp(BODIPY)-OH[1] (1.5 eq.) was incorporated with HBTU (1.2 eq.), HOBt (1.2 eq.) and DIEA (2.4 eq.) in DMF for 1 h. The other amino acids Fmoc-AA-OH (3 eq.) were incorporated with a 5 min pre-activation with DIC (3 eq.) and OxymaPure (3 eq.) in DMF for 1 h. Fmoc-AA-OH: Fmoc-Asp-OAllyl, Fmoc-Gly-OH, Fmoc-Arg(NO_2)-OH, Fmoc-Gln-OH, Fmoc-Ile-OH, Fmoc-His(Mmt)-OH, Fmoc-Phe-OH. Peptide cyclisation. The C-terminal allyl ester group of the aspartic acid was removed after addition of the last amino acid with $Pd(PPh_3)_4$ (26 mg, 0.023 mmol, 0.1 eq.) and N-methylmorpholine (244 µL, 2.25 mmol, 10 eq.) in THF for 1 h at r.t (3 x 15 min). The head-to-tail

cyclization was performed by removal of the *N*-terminal Fmoc group before addition of DIC (96 µL, 0.548 mmol, 3 eq.) and HOBt (75 mg, 0.548 mmol, 3 eq.) in DMF at r.t. for 5 h. Peptide cleavage. The resin bound peptide was treated repeated times with the TFA cocktail obtaining 69 mg of cyclic peptide crude (60% purity by HPLC-MS). Removal of nitro group. The crude protected peptide (69 mg, 0.041 mmol) and 20% $Pd(OH)_2$-C (34 mg) were dissolved in HCO_2H: DMF: H_2O (5: 47.5: 47.5) (10 mL) and the reaction flask was flushed with Ar, evacuated and filled with H_2. The reaction mixture was stirred under balloon pressure of H_2 for 32 h (H_2 was refilled periodically during the reaction along with re-addition of $Pd(OH)_2$-C (2 X). The catalyst was removed through filtration with Celite and the filtrate was evaporated *in vacuo* to afford 64 mg of the crude peptide (70% purity by HPLC-MS, 69% yield). Peptide purification. A highly pure fraction of the totally deprotected cyclic peptide was obtained by semi-preparative RP-HPLC (XBRIDGE^{TM}, C_{18}, 5 µM OBD 19 × 150 mm column. The pure fractions were lyophilised rendering the corresponding peptide as a red solid (>99% purity by HPLC-MS).

^{1}H NMR (600 MHz, DMSO-d_6): δ 11.27 (s, 1H), 8.37 – 7.99 (m, 7H), 7.90 – 7.83 (m, 1H), 7.66 – 7.59 (m, 2H), 7.43 – 7.36 (m, 1H), 7.34 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.32 – 7.26 (m, 2H), 7.26 – 7.18 (m, 3H), 7.15 (t, *J* = 7.2 Hz, 3H), 7.07 (t, *J* = 7.4 Hz, 1H), 6.92 (t, *J* = 8.3 Hz, 1H), 6.79 (s, 1H), 6.19 (d, *J* = 4.5 Hz, 2H), 4.63 – 4.44 (m, 2H), 4.40 – 4.29 (m, 1H), 4.28 – 4.18 (m, 3H), 4.17 – 4.05 (m, 1H), 3.87 – 3.51 (m, 14H), 3.18 – 3.11 (m, 2H), 3.09 – 3.04 (m, 2H), 2.96 – 2.89 (m, 1H), 2.56 (m, 1H), 2.47 – 2.45 (m, 6H), 2.18 – 2.06 (m, 2H), 1.95 (s, 1H), 1.86 – 1.65 (m, 3H), 1.60 – 1.47 (m, 3H), 1.43 (d, *J* = 9.4 Hz, 6H), 1.40 – 1.31 (m, 1H), 1.29 – 1.24 (m, 2H), 1.04 (m, 1H), 0.81 – 0.73 (m, 6H) ppm. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{78}H_{97}O_{16}N_{22}BF_2$, 1646.7509; found, 1646.7509.

Spectral characterisation.

PS and PC stock solutions were prepared by solubilisation in EtOH at a concentration of 1 mg mL^{-1}. 96-well plates were filled with 100 µL of different PS/PC solutions to form films after evaporation of EtOH at r.t. overnight. PS/PC-coated plates were incubated with **cLac-BODIPY** (5 µM) for 1 h at 37 °C in the dark. Quantum yields were determined by measuring the integrated emission area of the fluorescence spectra and comparing it to the area measured for fluorescein in 0.1 M NaOH when excited at 450 nm (QY: 0.92).[2] Quantum yields were calculated using the equation:

$$\Phi_{sample} = \Phi_{reference}\left(\frac{F^{sample}}{F^{reference}}\right)\left(\frac{Abs^{reference}}{Abs^{sample}}\right)\left(\frac{\eta^{sample}}{\eta^{reference}}\right)^2$$

where F represents the area of fluorescent emission, η is the refractive index of the solvent, and Abs is absorbance at the excitation wavelength selected (i.e. 450 nm). Emission was integrated between 480 and 600 nm.

Surface pressure measurements.

DOPS, eggPG and Liss-Rho-DOPE were purchased from Avanti Polar Lipids (Alabaster, Alabama, U.S.A.). eggPC was purchased from Lipid Products (South Nutfield, UK). Surface pressure assays were performed with a DeltaPi-4 tensiometer (Kibron, Helsinki, Finland). Langmuir lipid monolayers at the air-water interface were used as a model membrane system to study lipid-peptide interaction. Surface pressure experiments were carried out with a DeltaPi-4 at 22°C with constant stirring. The aqueous phase consisted of 1.25 mL of 25 mM HEPES, 150 mM NaCl (pH 7.4). Lipids dissolved in chloroform: methanol (2: 1), eggPC, DOPS and eggPG were spread gently over the surface until the desired initial surface pressure was attained. The peptides were injected with a micropipette through a hole connected to the subphase. The increment in

surface pressure was recorded until a stable signal was obtained. Values of critical pressure (πc) were determined by linear regression using the values over the saturation pressure (πs).

Confocal microscopy.

Electroformation of Giant Unilamellar Vesicles. GUV formation took place with aid of a TG330 function generator (Thurlby Thandar Instruments, Huntingdon, UK). GUVs were prepared by electroformation on a pair of platinum (Pt) wires by a method first developed by Angelova and Dimitrov,[3,4] modified as described previously.[5] Lipid stock solutions were prepared in 2:1 (v/v) chloroform/methanol at 0.2 mg mL^{-1}. Labelling was carried out by pre-mixing the fluorescent probes with the lipids in organic solvent. We used Liss-Rho-DOPE as marker for lipid membranes. The average concentration of individual fluorescent probes in each sample was 0.2 mol%. 2.5 μL lipid mixtures containing the fluorescent probes were deposited on Pt wires. The Pt wires were placed under vacuum for 2 h to completely remove the organic solvent. The sample was covered to avoid light exposure and allowed to precipitate onto the Pt wires for 5 min. One side of the chamber was then sealed with a coverslip. 500 μL assay buffer, prepared with high-purity water (Millipore SuperQ) heated at 37ºC was added to the chamber until it covered the Pt wires and connected to a TG330 function generator. AC field was applied in three steps, all of them performed at 37ºC: 1), frequency 500 Hz, amplitude 220 mV (35 V/m) for 5 min; 2), frequency 500 Hz, amplitude 1900 mV (313 V/m) for 20 min; 3), frequency 500 Hz, amplitude 5.3 V (870 V/m) for 90 min. The temperatures used for GUV formation correspond to those at which the different membranes display a single fluid phase.

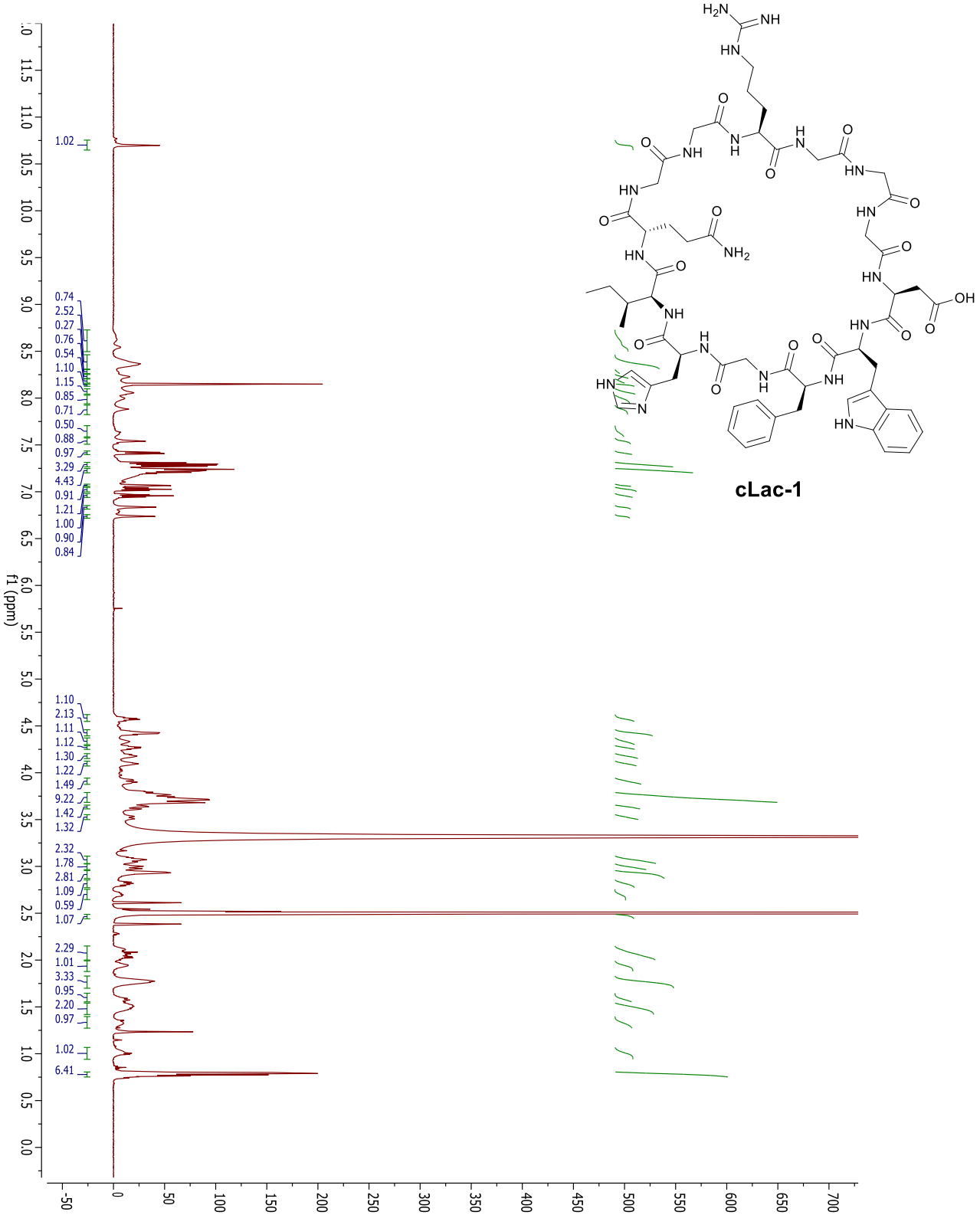
Confocal microscopy. Nikon D-eclipse C1 confocal system (Nikon corporation, Tokyo, Japan) was used for GUV imaging, treating the images using the software EZ-C1 3.20 (Nikon Inc., Melville, N.Y.). The excitation wavelengths were 488 nm for **cLac-BODIPY** and 561 nm for Liss-Rho-DOPE. Fluorescence emission was retrieved at 500-530 for **cLac-BODIPY** and at 573-613 for Liss-Rho-PE. cLac-BODIPY was used at 3.3 μg mL^{-1} to study the labelling of the GUVs. All experiments were performed at 22 ºC.

Flow cytometry.

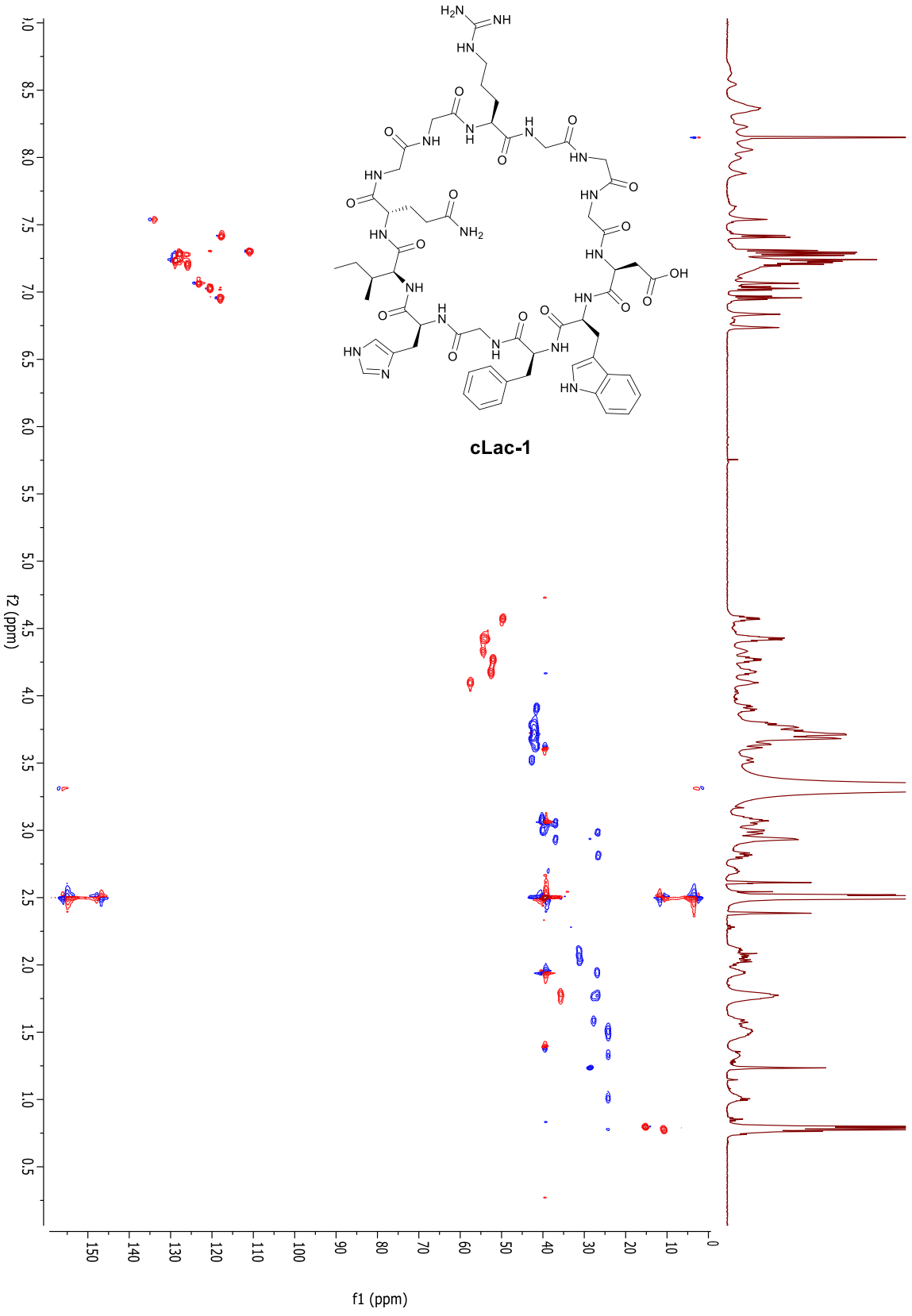
Subcellular material was obtained from supernatants of the human Burkitt lymphoma cell line BL2 undergoing UV-induced apoptosis. Exponentially growing cells were centrifuged and resuspended in culture medium passed through a 0.1 μm PVDF filter (Millipore) then exposed to a UV-B dose of 300 mJ per cm^2. After reculture for 5 h, cells were centrifuged at 25 xg for 1 h at 4 ^oC, and the supernatant was filtered through a 5 μm mesh filter (PluriSelect Life Sciences, Liepzig, Germany) to exclude residual cells. Binding of **cLac-BODIPY** and PE-labelled Annexin V (Invitrogen) was analysed by flow cytometry after excitation with a 488 nm laser using emission filters at 530$\pm$30 nm and 574$\pm$26 nm respectively, with appropriate electronic compensation for channel spillover. Data acquisition was carried out using a dual laser Attune acoustic focusing cytometer (Thermo Fisher Scientific Inc, Waltham, MA, USA) with post-acquisition data analysis using Flowjo software (Flowjo, Ashland, OR, USA).

NMR spectra

1H-NMR of **cLac-1**



HSQC-NMR of **cLac-1**



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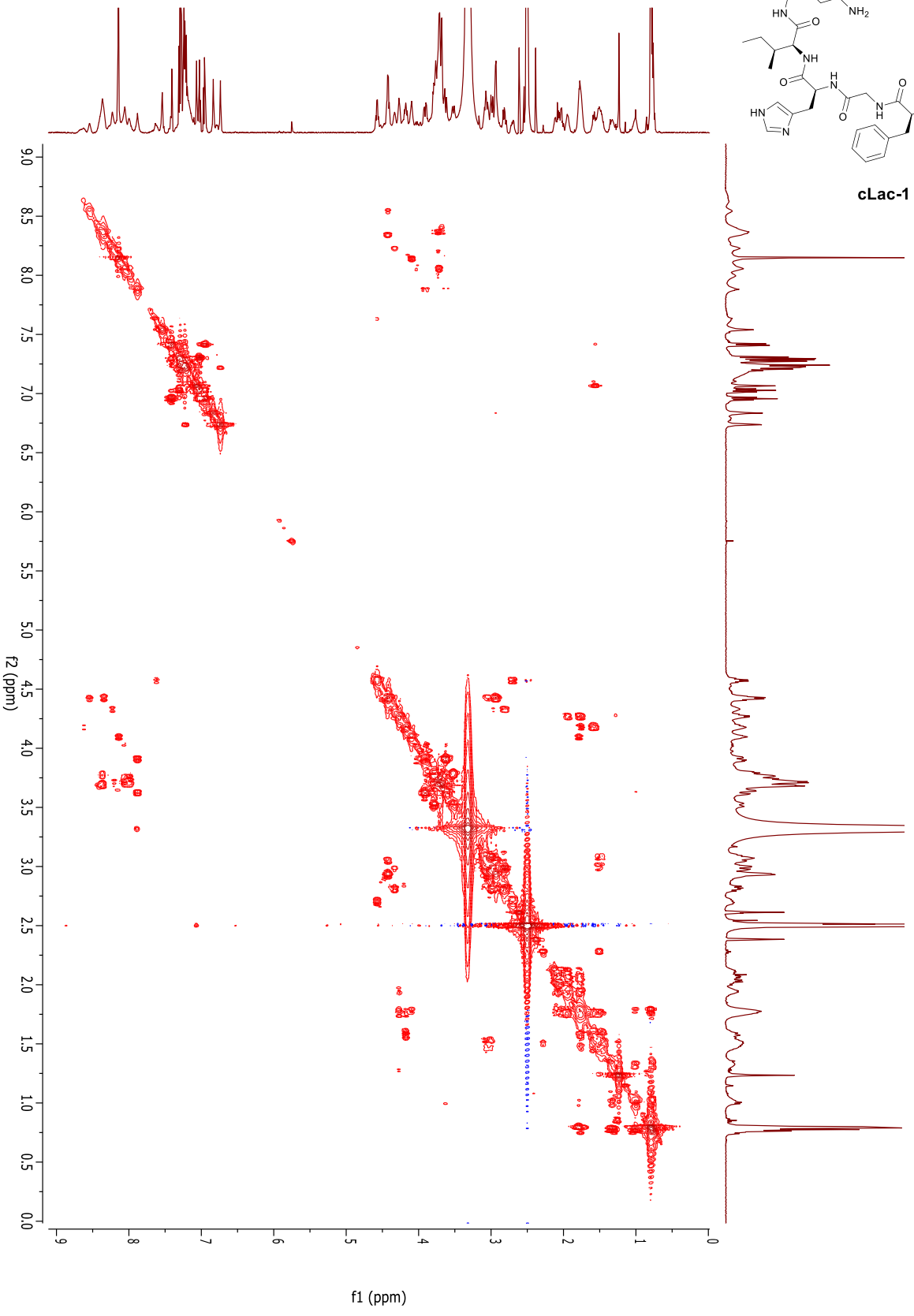
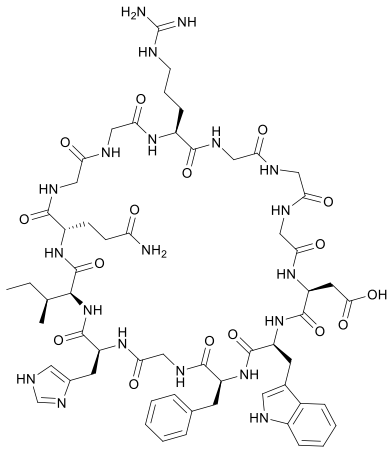
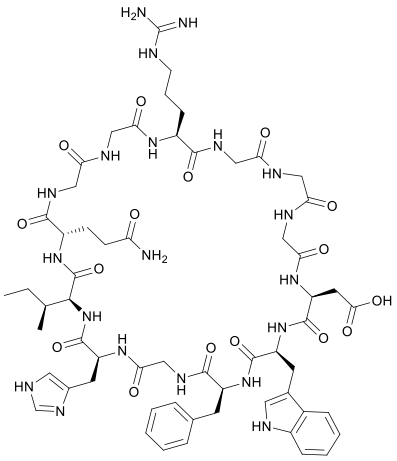




cLac-1

TOCSY-NMR of **cLac-1**



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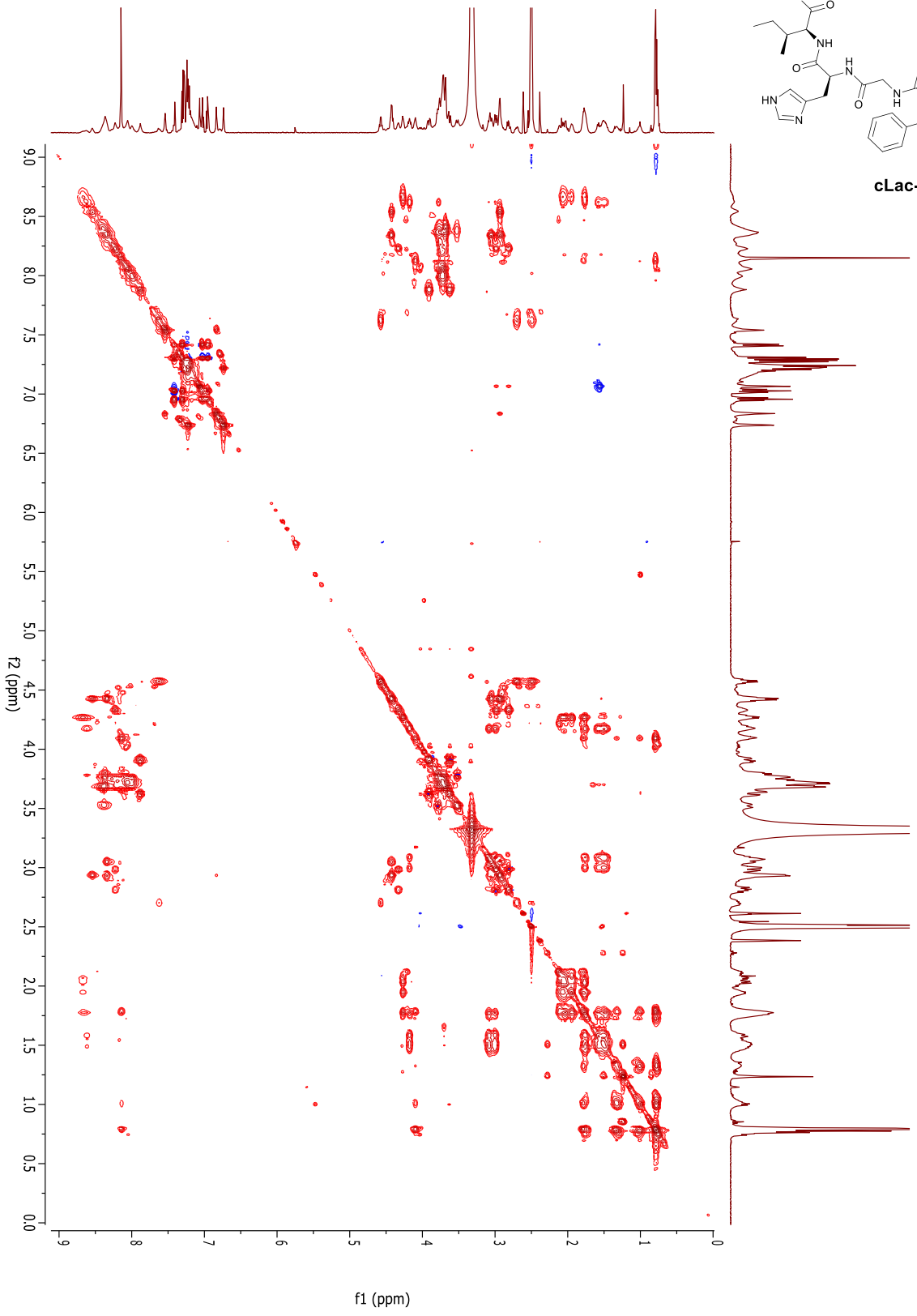

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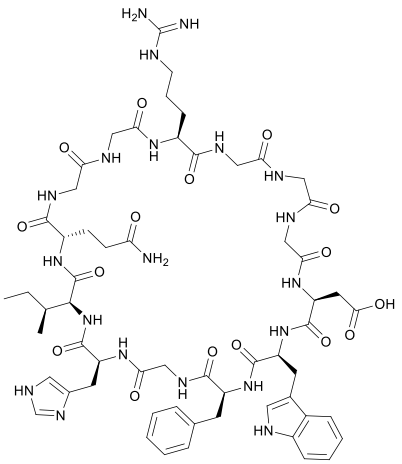

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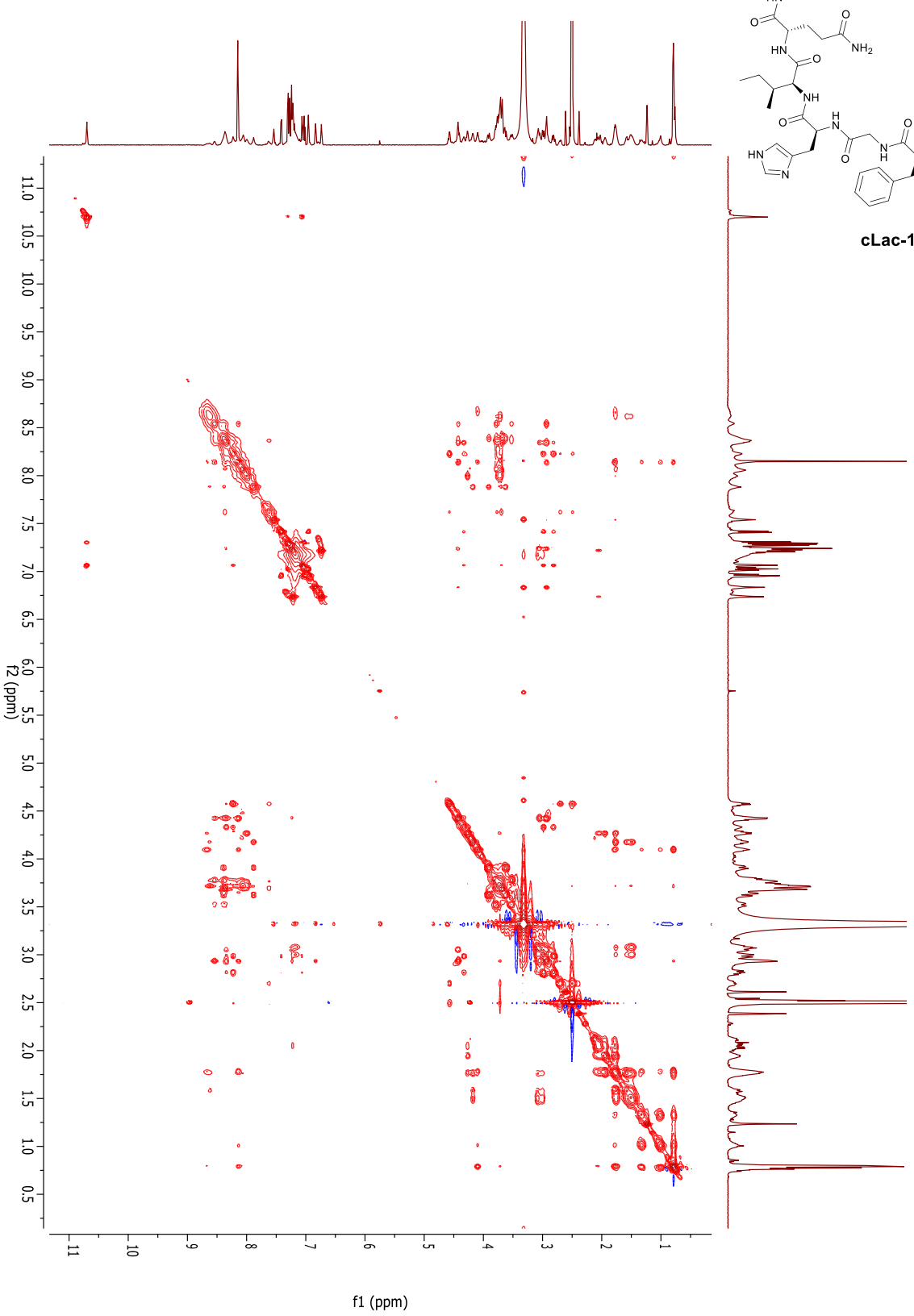
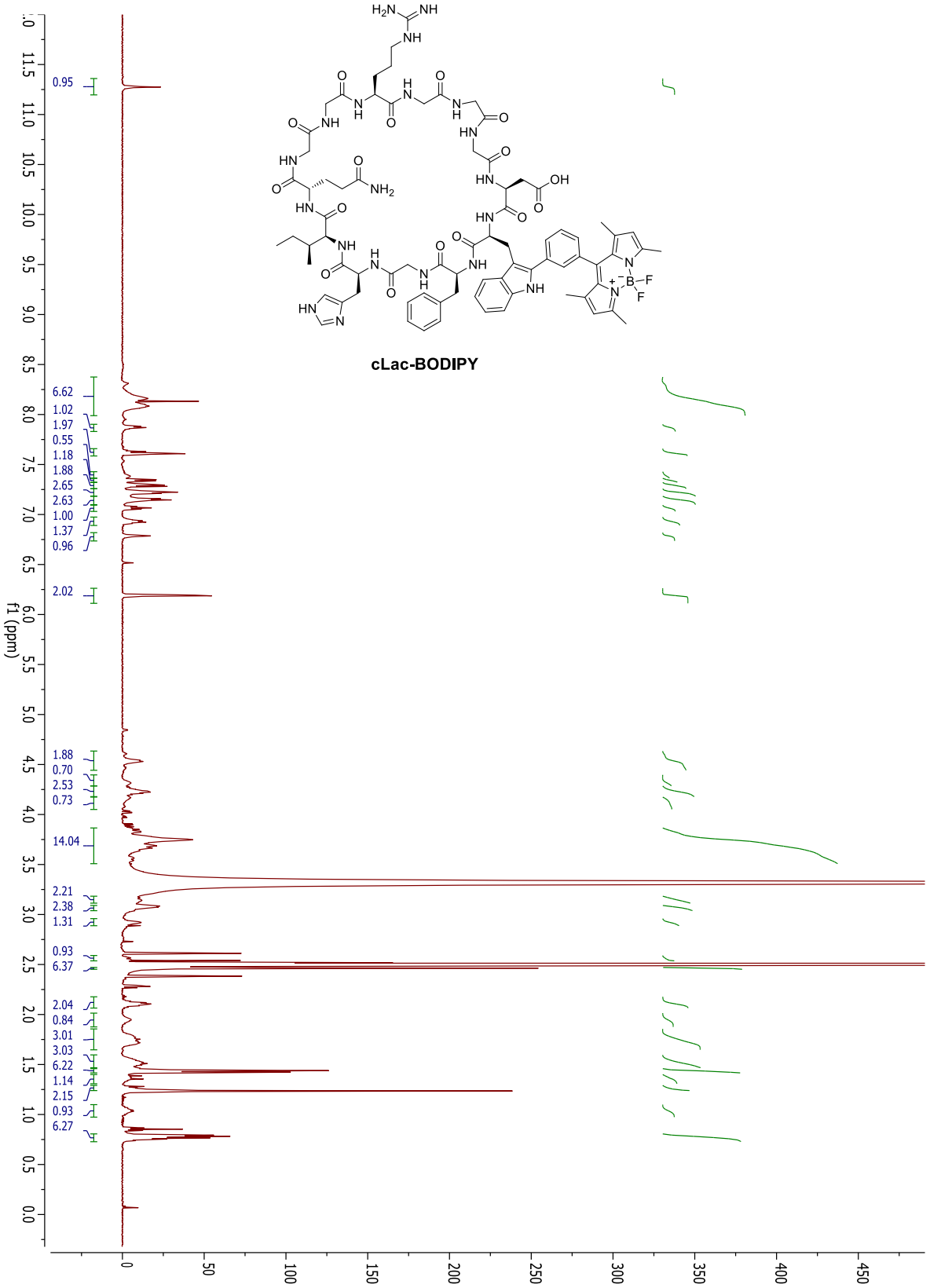
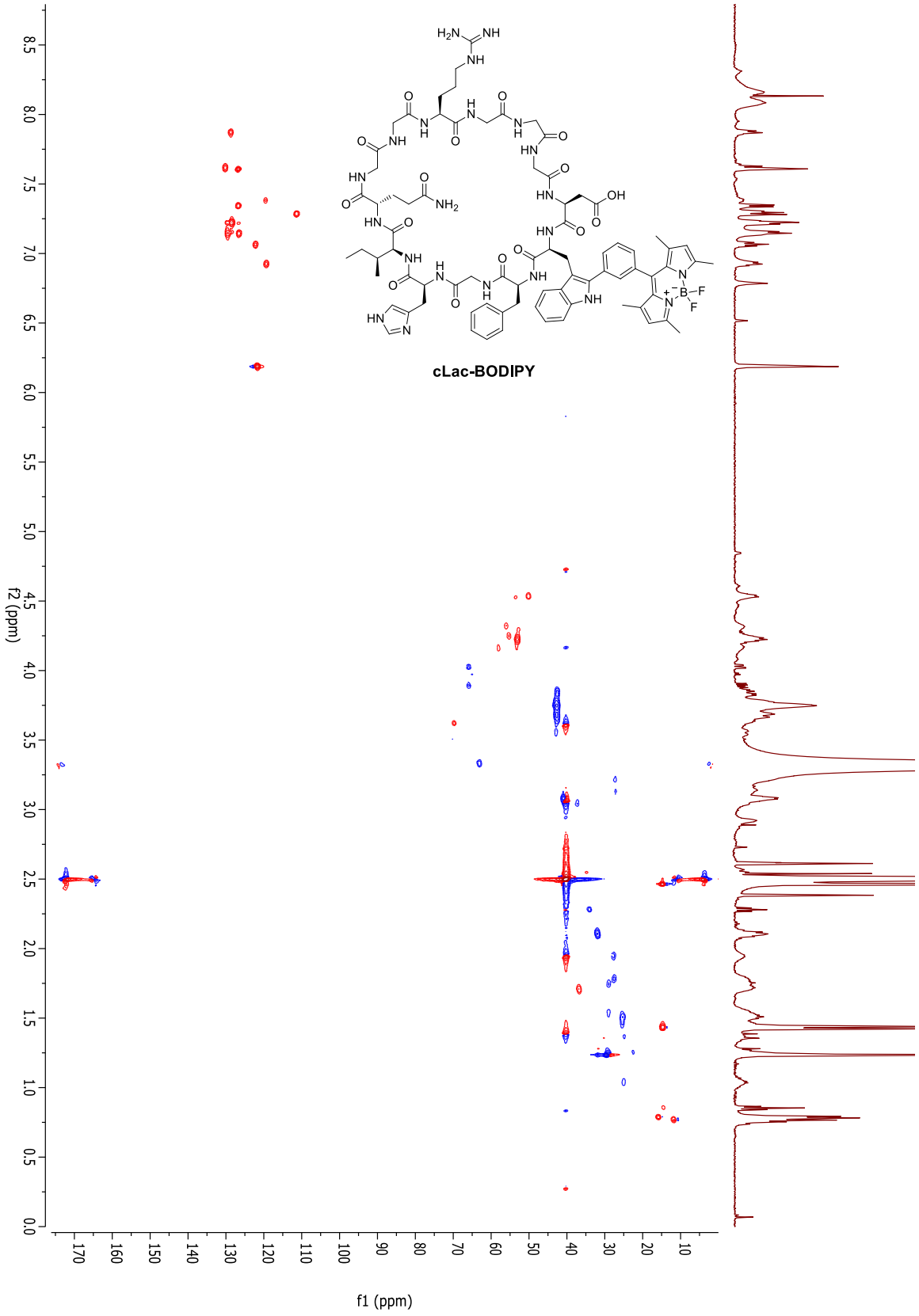


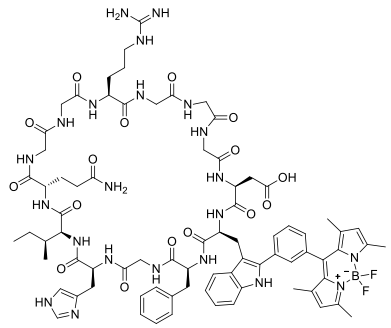
1H-NMR of **cLac-BODIPY**



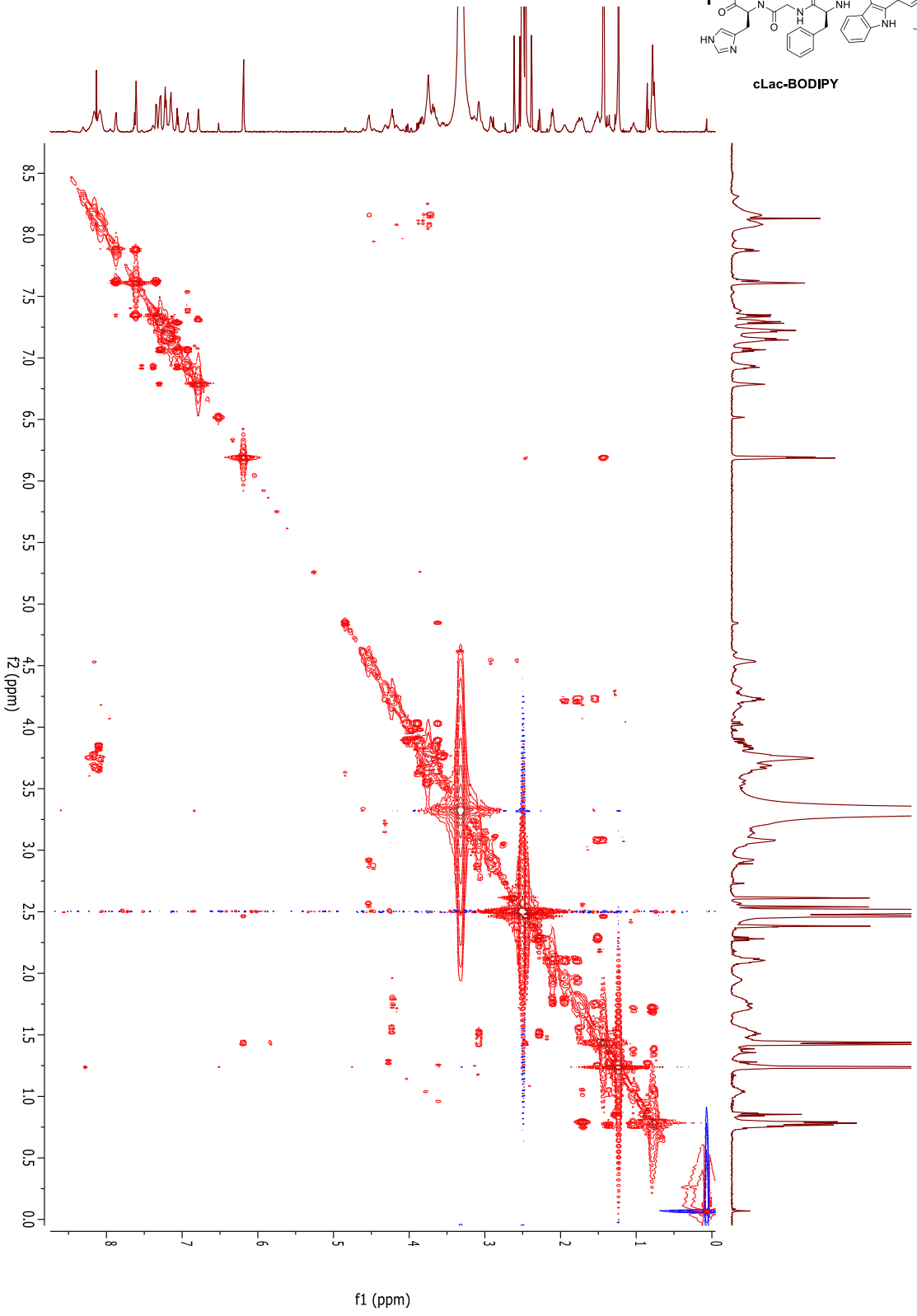
HSQC-NMR of **cLac-BODIPY**



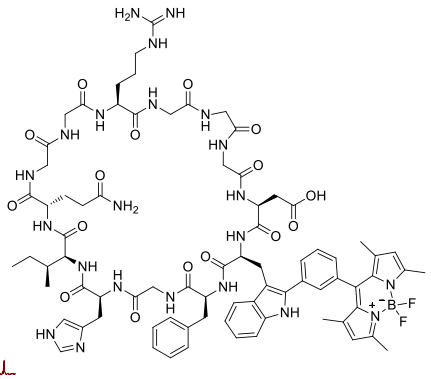
COSY-NMR of **cLac-BODIPY**



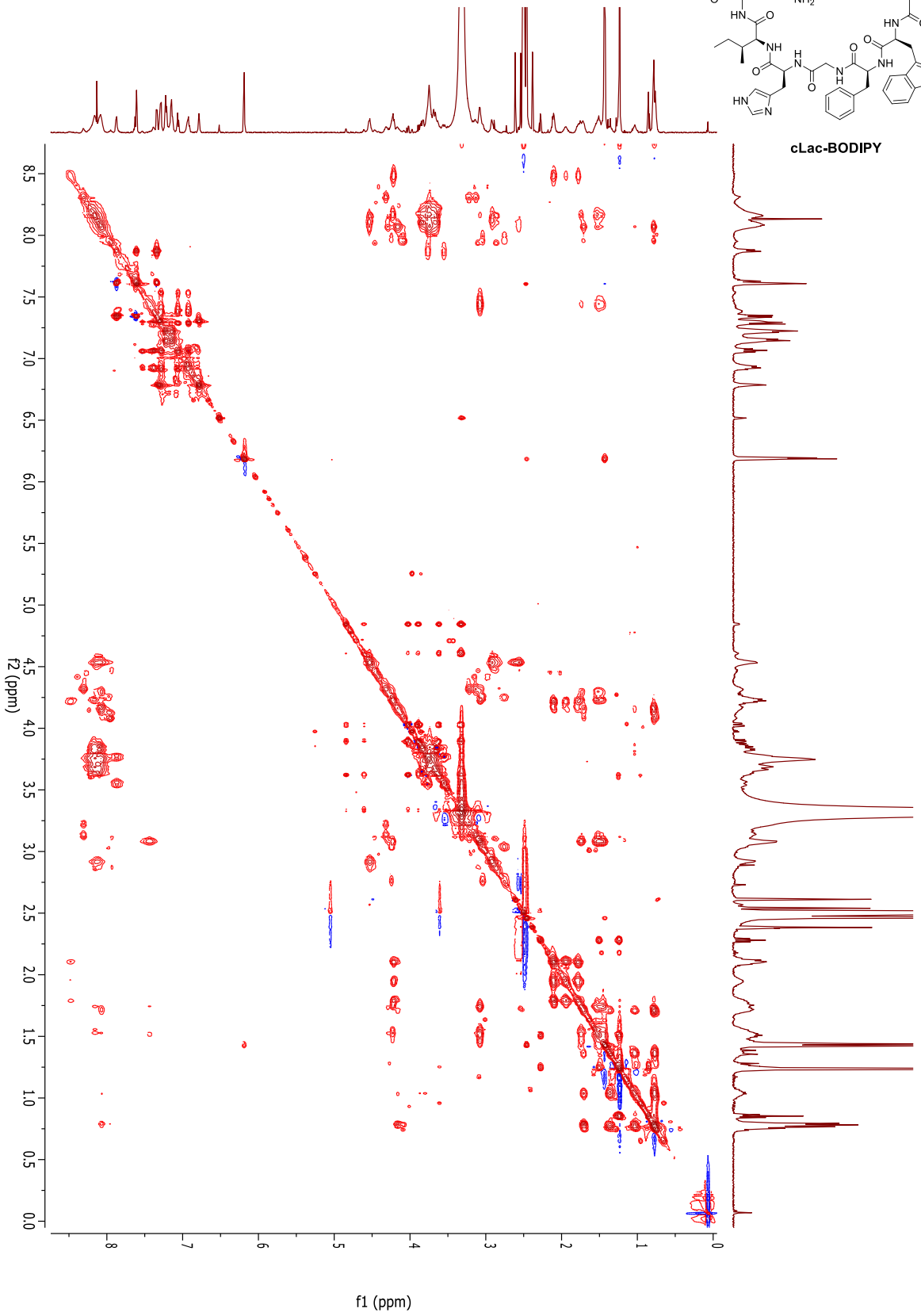
cLac-BODIPY



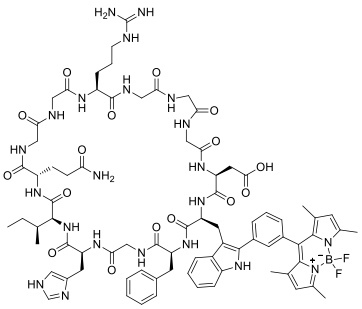
TOCSY-NMR of **cLac-BODIPY**



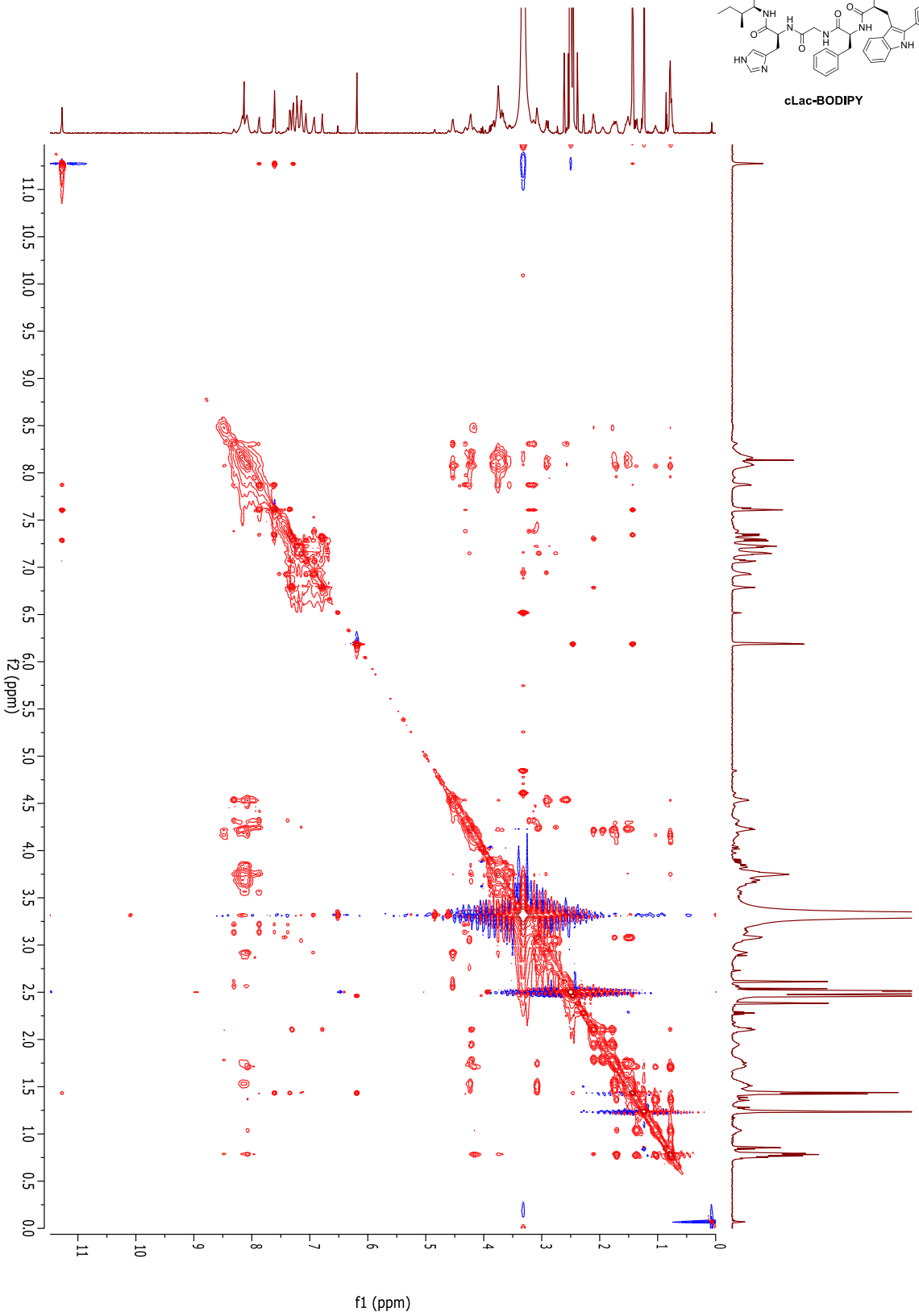
cLac-BODIPY



NOESY-NMR of **cLac-BODIPY**



cLac-BODIPY



Supplementary Figures

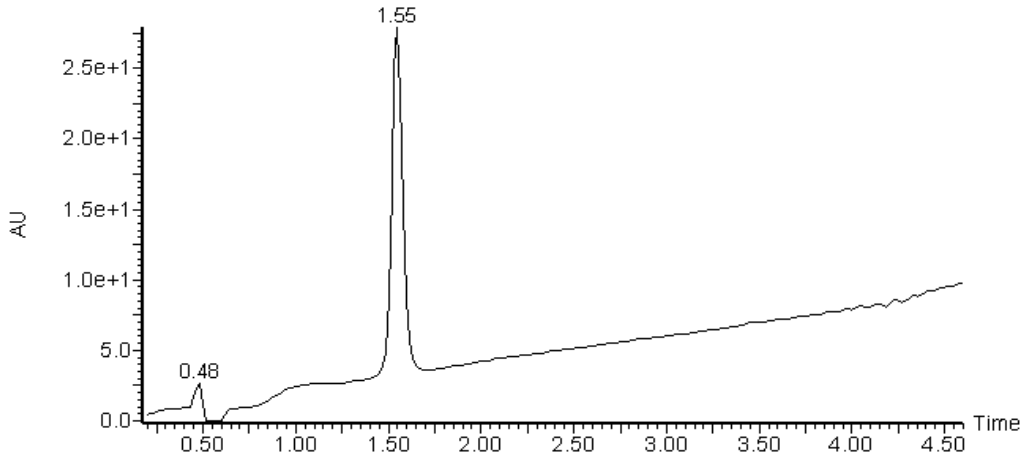


Fig. S1. HPLC trace of cLac-1. Eluents: H_2O (0.1% FA) and ACN (0.1% FA). Flow: 1.6 mL min^{-1}. Linear gradient from 5% to 100% ACN (0.1% FA) over 3.5 min. The plot corresponds to the integrated absorbance signal from 210 to 400 nm.

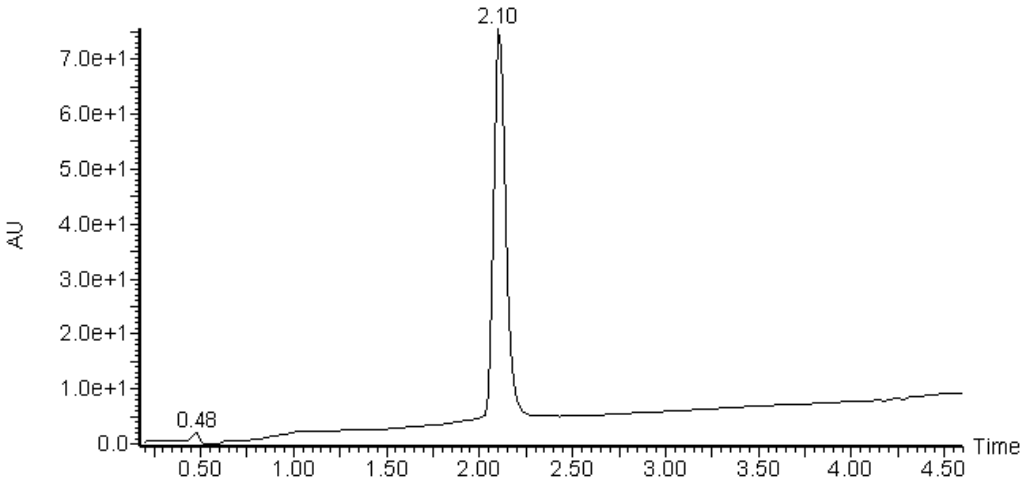


Fig. S2. HPLC trace of cLac-BODIPY. Eluents: H_2O (0.1% FA) and ACN (0.1% FA). Flow: 1.6 mL min^{-1}. Linear gradient from 5% to 100% ACN (0.1% FA) over 3.5 min. The plot corresponds to the integrated absorbance signal from 210 to 400 nm.

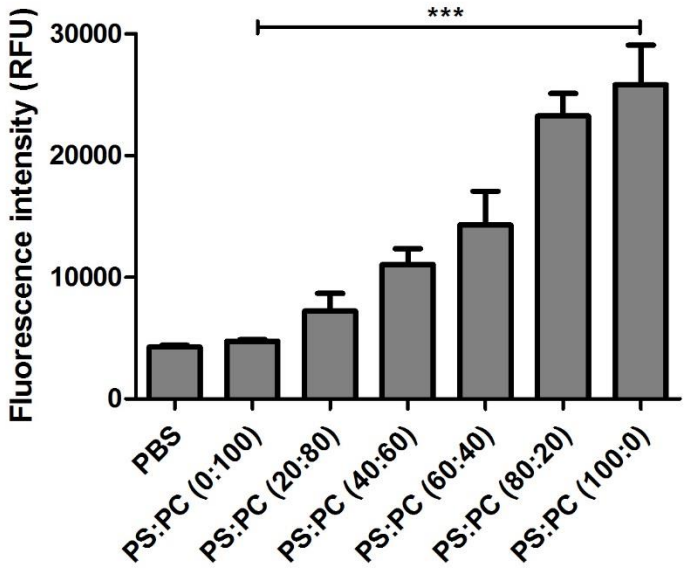


Figure S3. Fluorescence intensity values of **cLac-BODIPY** (5 μM) after incubation with different PS: PC films. Values are represented as means and error bars as SD (*n* = 3). *** for $p < 0.001$.

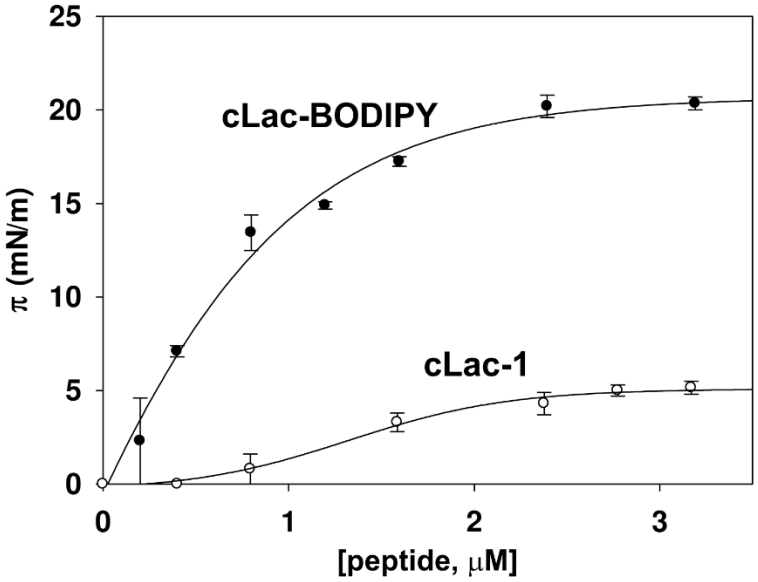


Figure S4. Determination of the tensioactivity of **cLac-1** and **cLac-BODIPY**. **cLac-BODIPY** shows high tensioactivity with a saturation pressure (π_s) of 20.4 mN m^{-1} and a saturation concentration (C_s) of 3 μM. The π_s for **cLac-1** is 5 mN m^{-1}. Values represented as means and error bars as SD (*n* = 3).

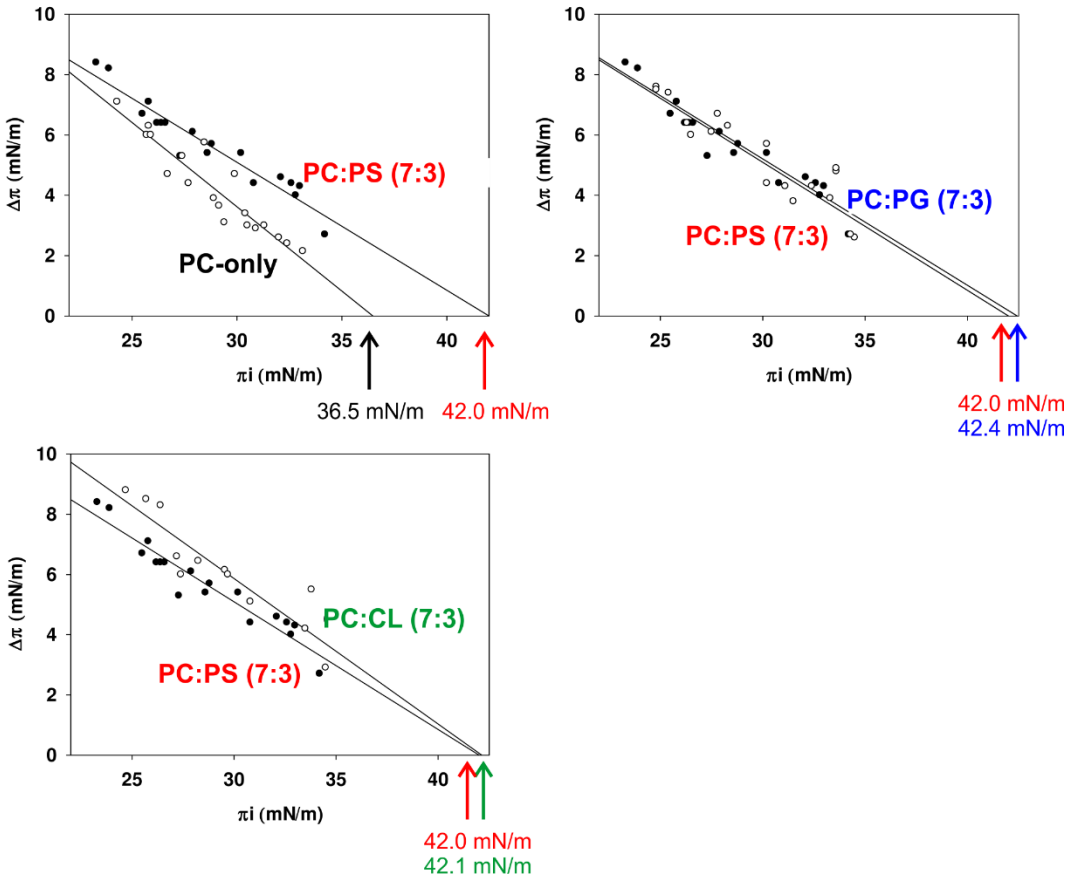


Figure S5. Quantitative binding assays of **cLac-BODIPY** to monolayers with variable lipid composition. Arrows point at the π_c values for every lipid composition. PC: phosphatidylcholine, PS: phosphatidylserine, PG: phosphatidylglicine, CL: cardiolipin. Values represented as means from *n* = 3.

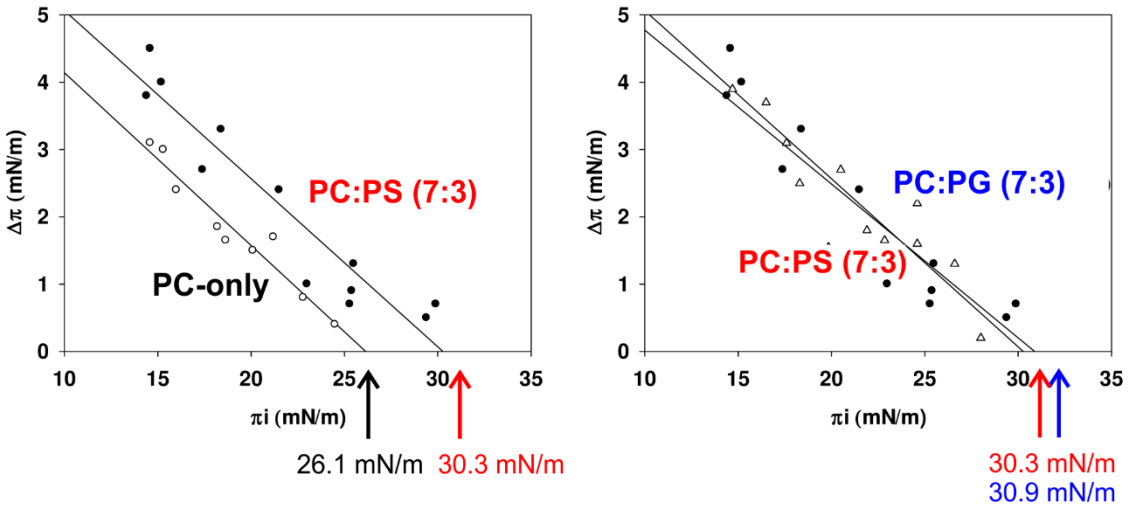


Figure S6. Quantitative binding assays of **cLac-1** to monolayers with variable lipid composition. Arrows point at the π_c values for every lipid composition. PC: phosphatidylcholine, PS: phosphatidylserine, PG: phosphatidylglicine. Values represented as means from *n* = 3.

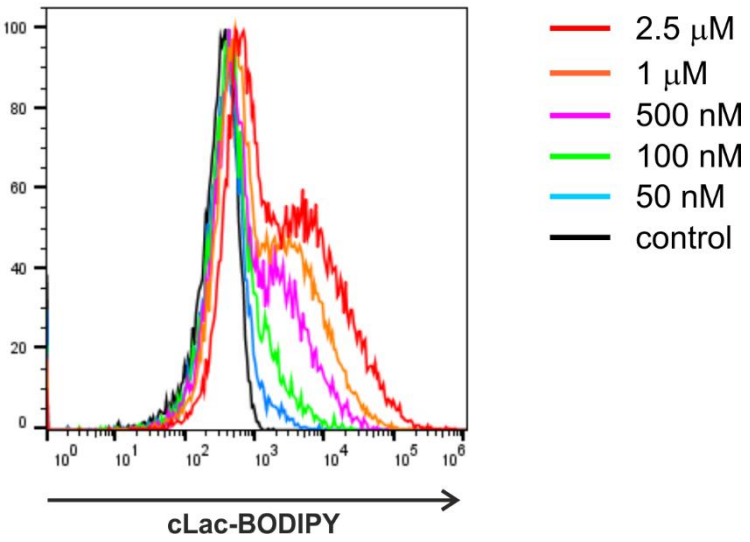


Figure S7. Flow cytometry analysis of the fluorescence labelling of apoptotic bodies after incubation with different concentrations of **cLac-BODIPY**.

References

[1] Mendive-Tapia, L.; Zhao, C.; Akram, A.R.; Preciado, S.; Albericio, F.; Lee, M.; Serrels, A.; Kielland, N.; Read, N. D.; Lavilla, R.; Vendrell, M. *Nat. Commun.* 2016, **7**, 10940.

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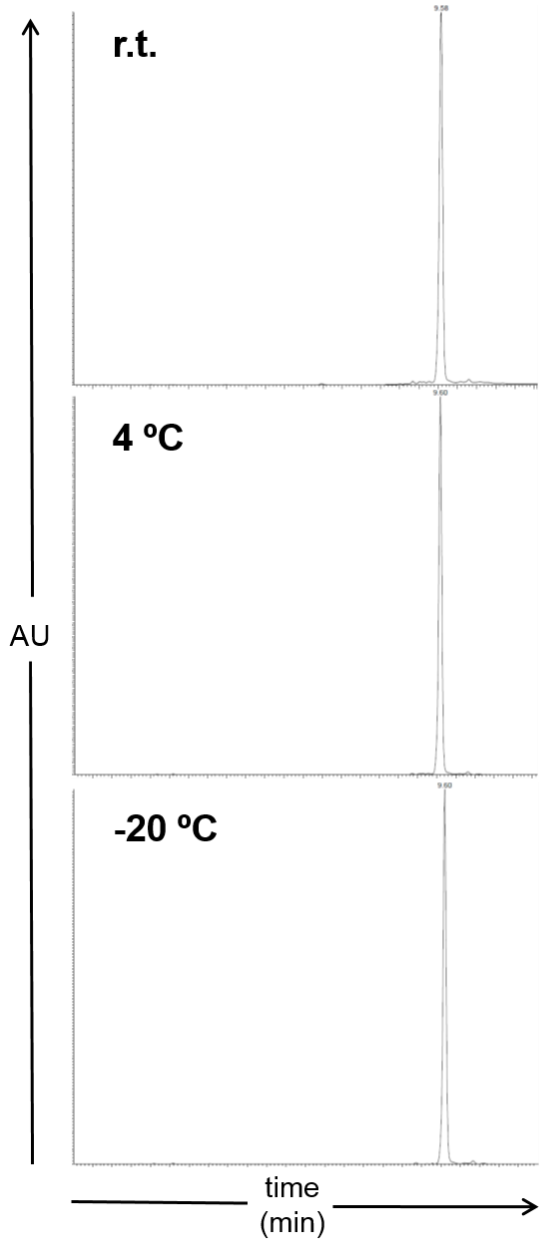
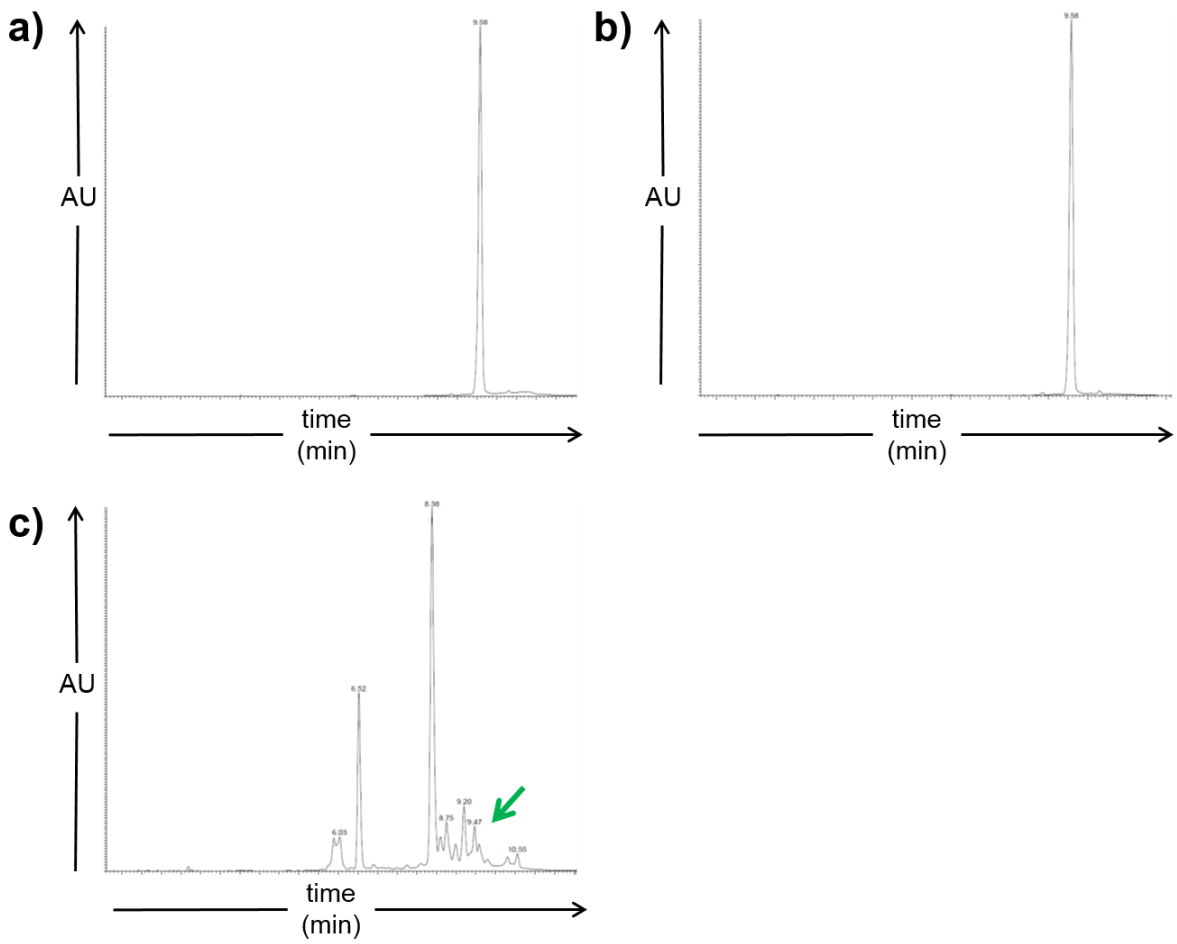
Supplementary Information



Supplementary Figure 1. Analysis of the long-term stability of the amino acid 1 when stored as a solid at different temperatures. HPLC-MS traces of the amino acid **1** after being stored in the dark for 4 months at r.t., 4 ºC, and -20 ºC. UV detection: 500 nm.



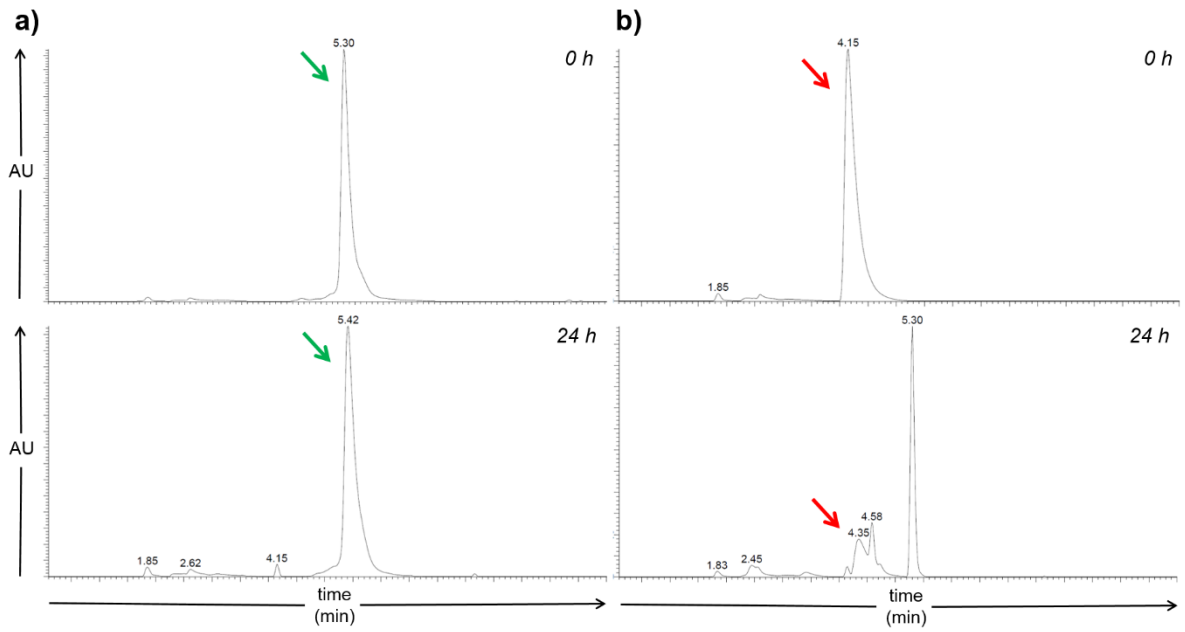
Supplementary Figure 2. Analysis of the long-term stability of the amino acid 1 when dissolved in organic solvents at different temperatures. HPLC-MS traces of the amino acid **1** after being stored in the dark for 4 months in: a) DCM at -20 ºC, b) MeOH at 4 ºC, c) DMF at r.t. In c), the green arrow points at the remaining amino acid **1** and the main peaks correspond to Fmoc-deprotection side products. UV detection: 500 nm.

Supplementary Table 1. Cellular activity of BODIPY-cPAF26 and PAF26 in different fungal species. Cell viability was measured after 16 h incubation with different concentrations of **BODIPY-cPAF26** or PAF26 peptides at 25 ˚C for *N. crassa* or 37 ˚C for *A. fumigatus*. IC_{50} values (μM) are represented as means ± s.d. (n=3).

	IC_{50} (*N. crassa*)	**IC_{50} (*A. fumigatus*)**
BODIPY-cPAF26	1.4 ± 0.1	2.2 ± 0.1
PAF26	3.7 ± 0.1	7.9 ± 0.3

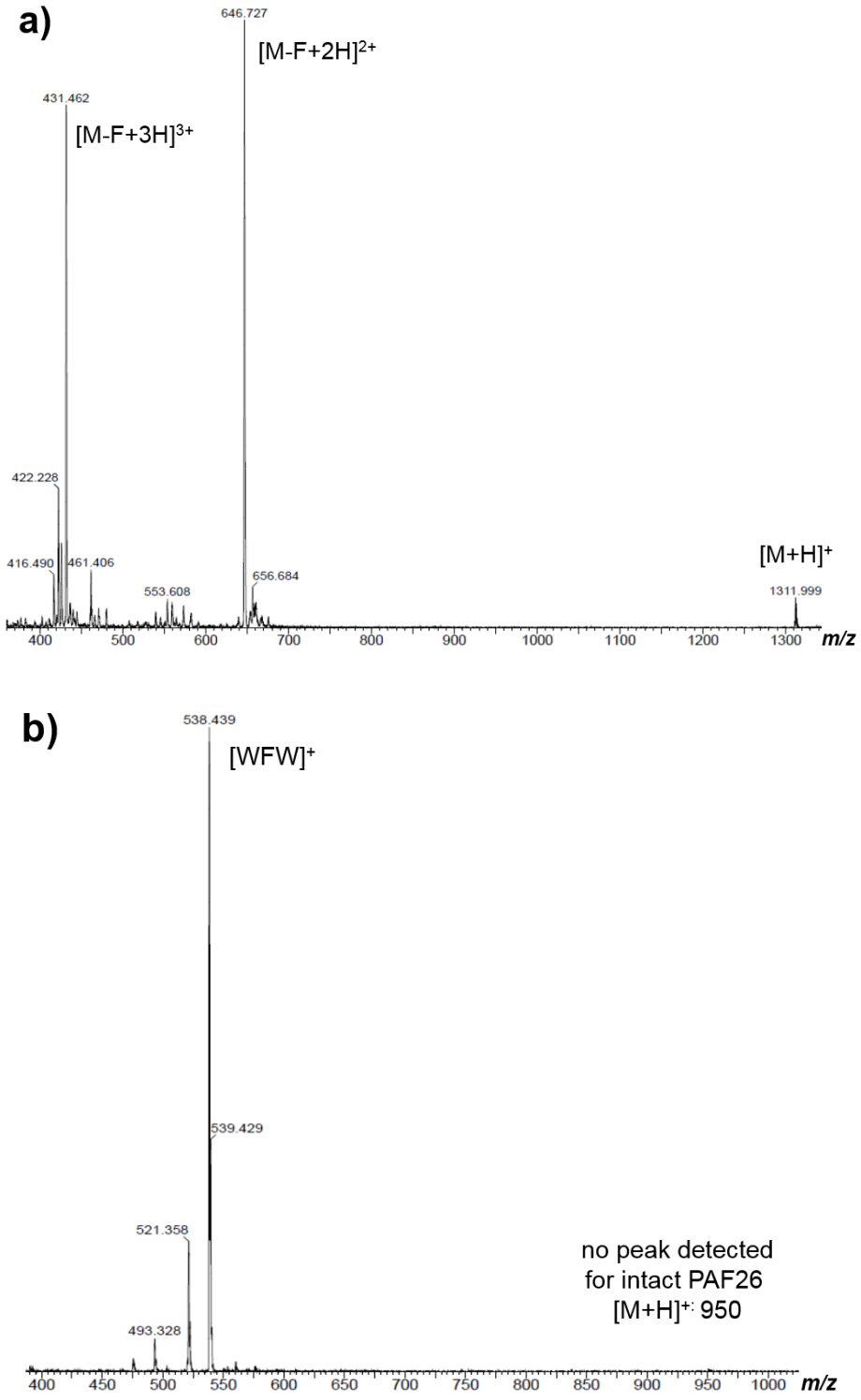
Supplementary Table 2. Fluorescence quantum yields of BODIPY-cPAF26 in PC: cholesterol (7: 1) liposome suspensions in PBS with increasing hydrophobicity. PBS alone was used as a negative control. Quantum yields were determined by comparing the integrated emission area of the fluorescence spectra to the emission area of fluorescein in basic EtOH (QY: 0.97), $\lambda_{exc.}$: 450 nm. Data represented as means ± s.d. (n=3).

PC (mg mL^{-1}) content on liposomes	**Quantum yield**
3.75	0.32 ± 0.04
1.88	0.27 ± 0.04
0.94	0.20 ± 0.02
0.47	0.14 ± 0.02
0.23	0.09 ± 0.01
0.12	0.06 ± 0.01
0.06	0.04 ± 0.01
0.03	0.02 ± 0.004
0.02	0.02 ± 0.003
0.007	0.01 ± 0.002
PBS	0.01 ± 0.002



Time (h)	Purity (% of intact peptide left)	
	BODIPY-cPAF26	Unlabeled PAF26
0.25	99%	83%
1	99%	79%
4	99%	73%
8	99%	63%
24	98%	20%

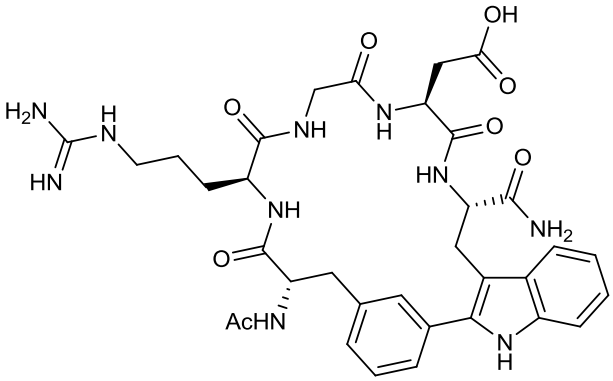
Supplementary Figure 3. Time-course analysis of the chemical integrity of BODIPY-cPAF26 and unlabeled linear PAF26 in proteolytic environments. HPLC traces of **BODIPY-cPAF26** (a) and unlabeled PAF26 (b) before incubation (*top*) and after incubation (*bottom*) at a concentration of 200 μM in a protease cocktail (1 mg L^{-1}). Green arrows point at the peaks of intact **BODIPY-cPAF26** and red arrows point at intact PAF26. UV detection: 280 nm. Purities were determined by integration of the peak areas in respective HPLC chromatograms at 280 nm.



Supplementary Figure 4. Electrospray analysis of BODIPY-cPAF26 and unlabeled PAF26 after 24 h incubation in a protease cocktail. Both peptides (200 μM) were incubated in 1 mg L^{-1} of the protease cocktail, and their respective mass spectra were recorded on a Waters Micromass ZQ mass spectrometer (ESI positive mode). a) MS analysis of **BODIPY-cPAF26** (exact mass: 1311 Da); b) MS analysis of unlabeled PAF26 (exact mass: 949 Da).

Supplementary Video 1. Time-course high-resolution imaging of *A. fumigatus* upon treatment with BODIPY-cPAF26. *A. fumigatus* were pre-treated with a cell membrane counterstain (*red*) and imaged under the confocal microscope. Cells were then treated with **BODIPY-cPAF26** (2 μM, *green*) and further imaged without any washing steps. The movie shows the rapid fluorogenic response of **BODIPY-cPAF26** upon interaction with the cell membrane of *A. fumigatus*. Scale bar: 5 μm.

Supplementary Figures



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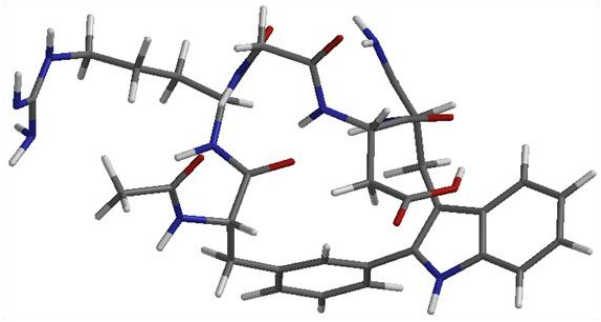

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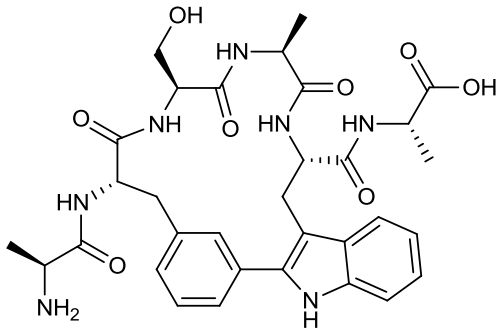

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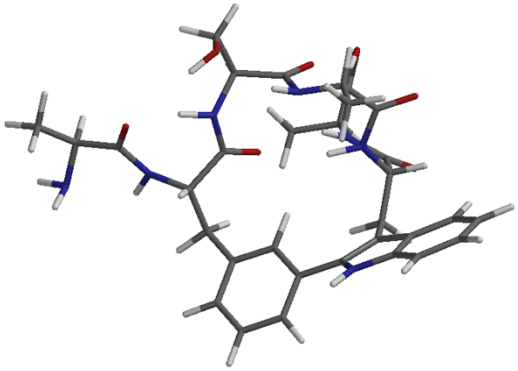

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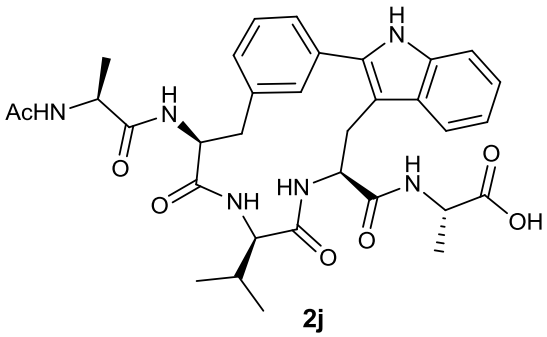

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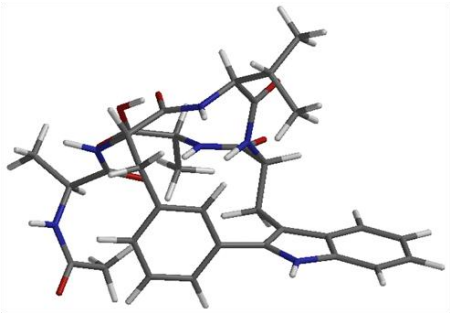
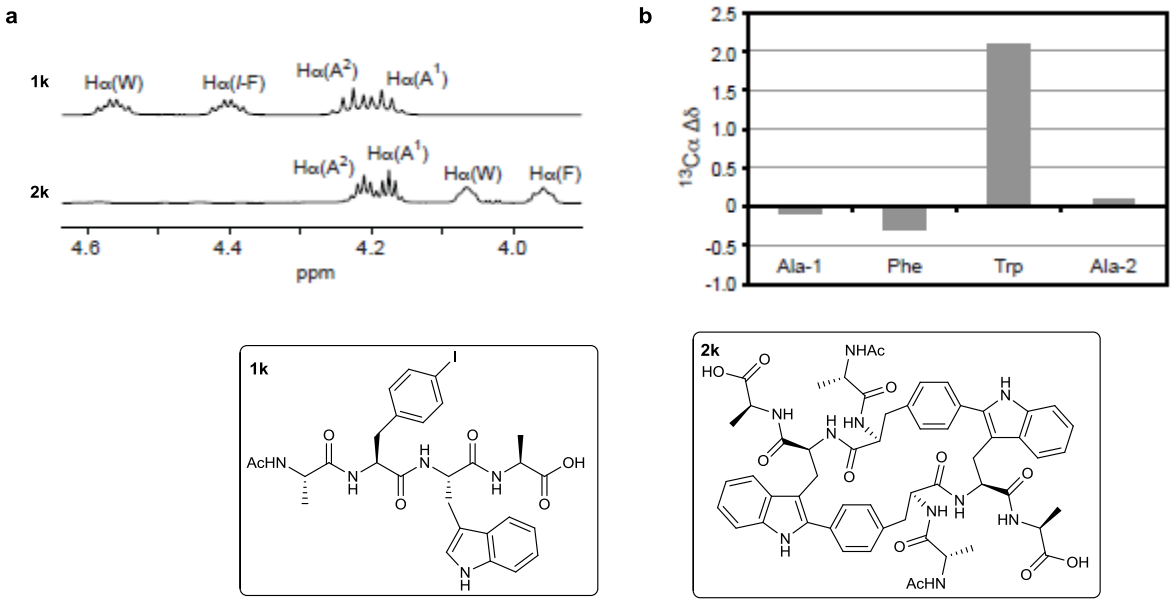
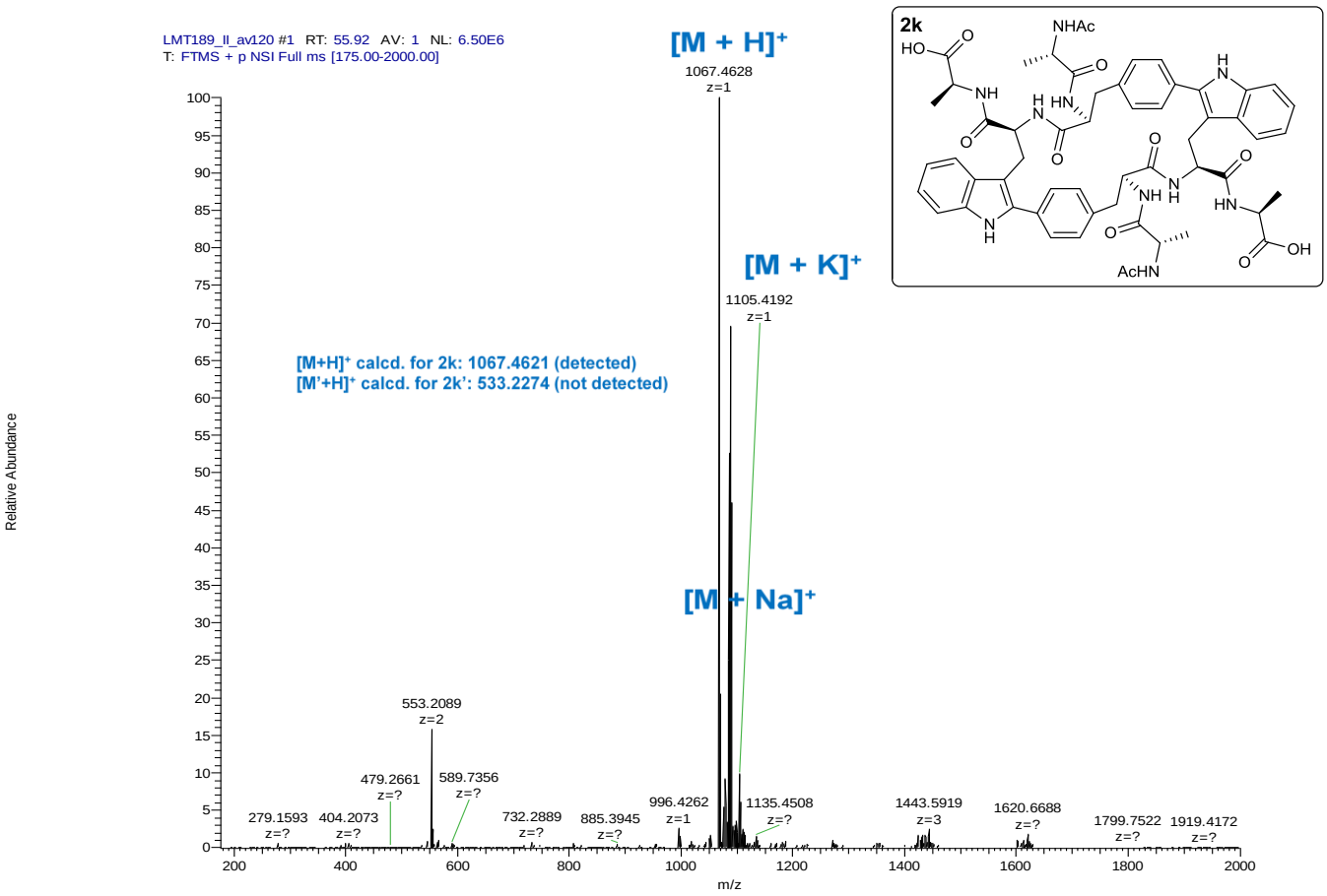


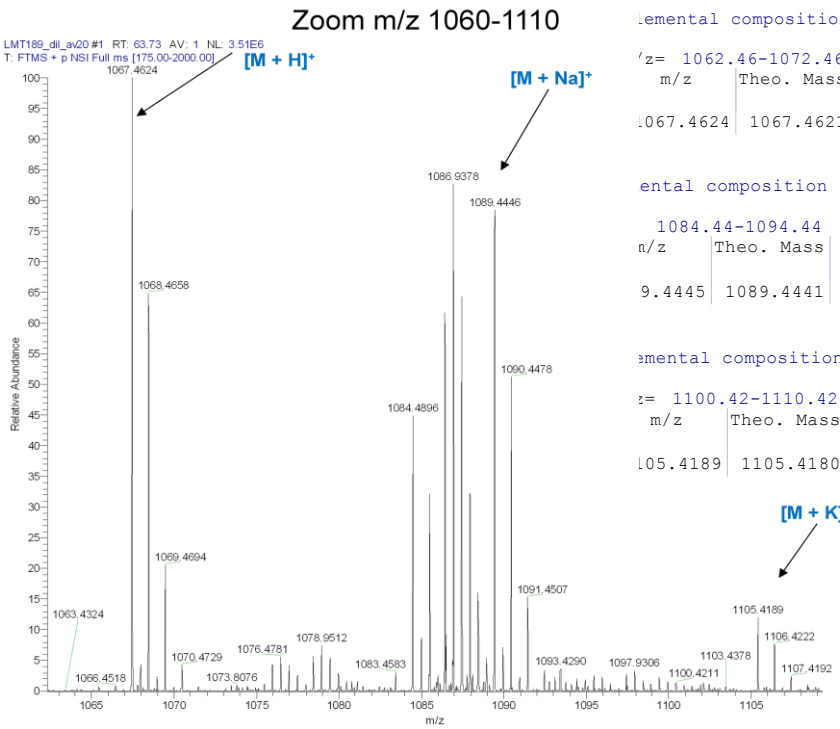
2j

Supplementary Figure 1 | Minimized geometries of compounds 2h-j generated by the Spartan '14 suite (molecular mechanics, MMFF94).[8]



Supplementary Figure 2 | Peptide NMR spectra comparison between compounds 2k and 1k. a, NMR H_{α} region of peptide **2k** and its linear precursor **1k**. **b,** Plot of the $^{13}C_{\alpha}$ chemical shift differences ($^{13}C_{\alpha}$ $\Delta\delta_{cyclic\text{-}linear}$) between cyclodimer **2k** and its linear counterpart **1k**. Temperature coefficients of the NH amide protons, $\Delta\delta/\Delta T$ (ppb/ºK), were -3.5 (A^1), -2.8 (*I*-F), -3.8 (W), -5.9 (A^2) and -4.1 (A^1), -5.6 (F), 0.7 (W), -5.3 (A^2) for peptide **1k** and **2k**, respectively.





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z= 1062.46-1072.46

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1067.4624	1067.4621	0.22	30.5	$^{12}C_{56}H_{63}O_{12}N_{10}$

[M + H]+

ental composition search on mass 1089.44

1084.44-1094.44

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
9.4445	1089.4441	0.42	30.5	$^{12}C_{56}H_{62}O_{12}N_{10}{}^{23}Na_1$

[M + Na]+

emental composition search on mass 1105.42

z= 1100.42-1110.42

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
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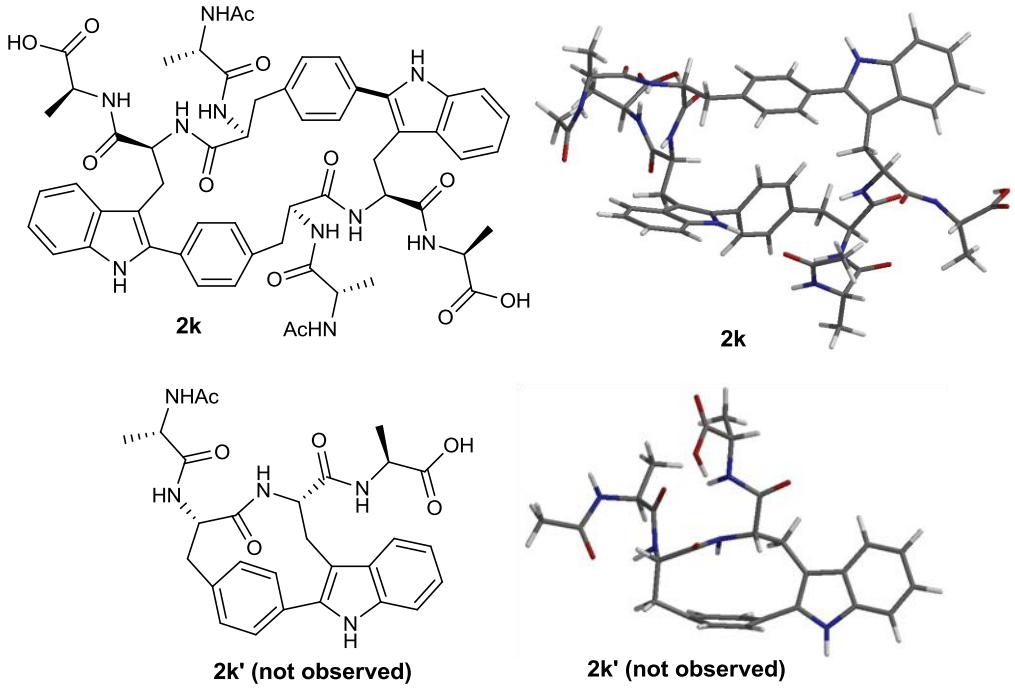
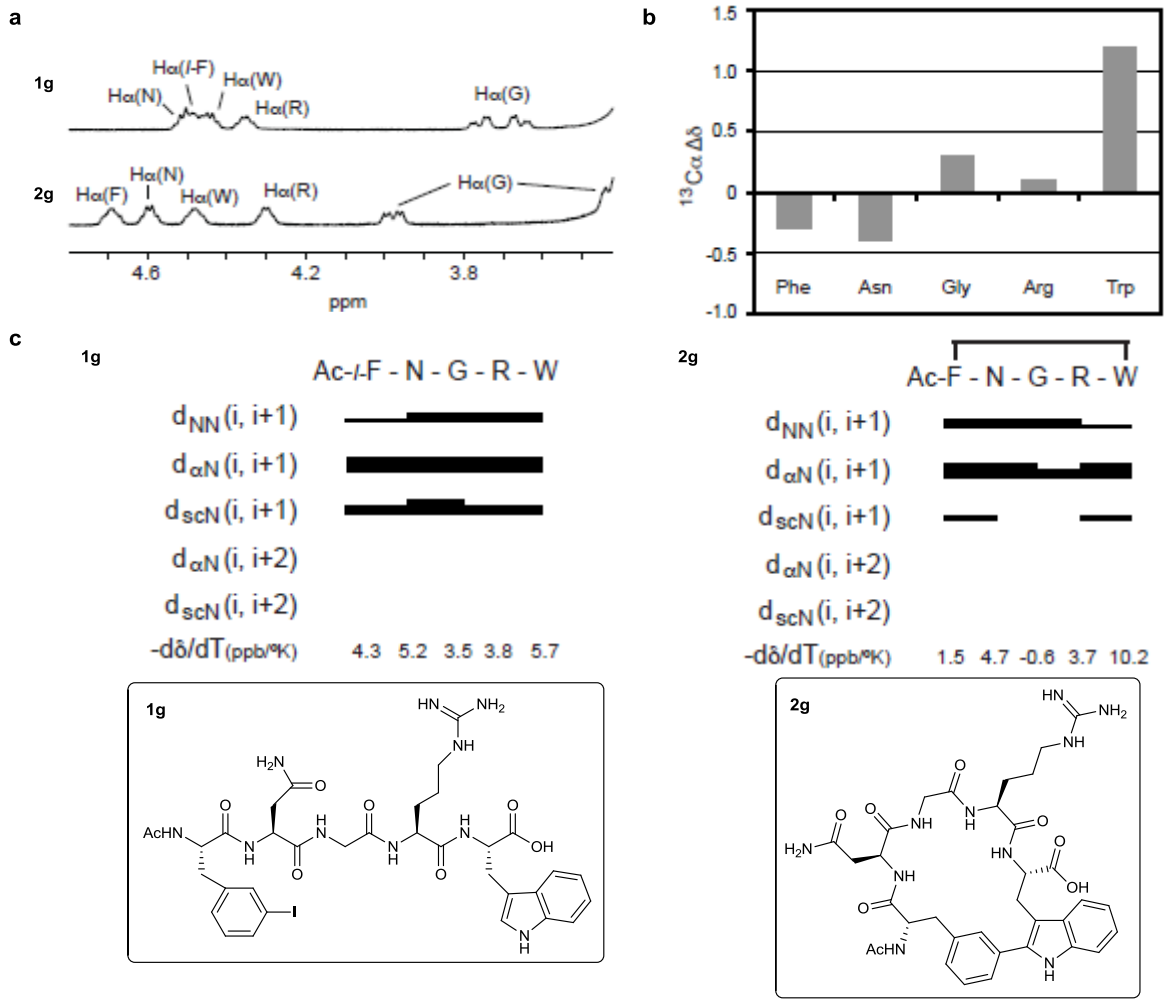
[M + K]+

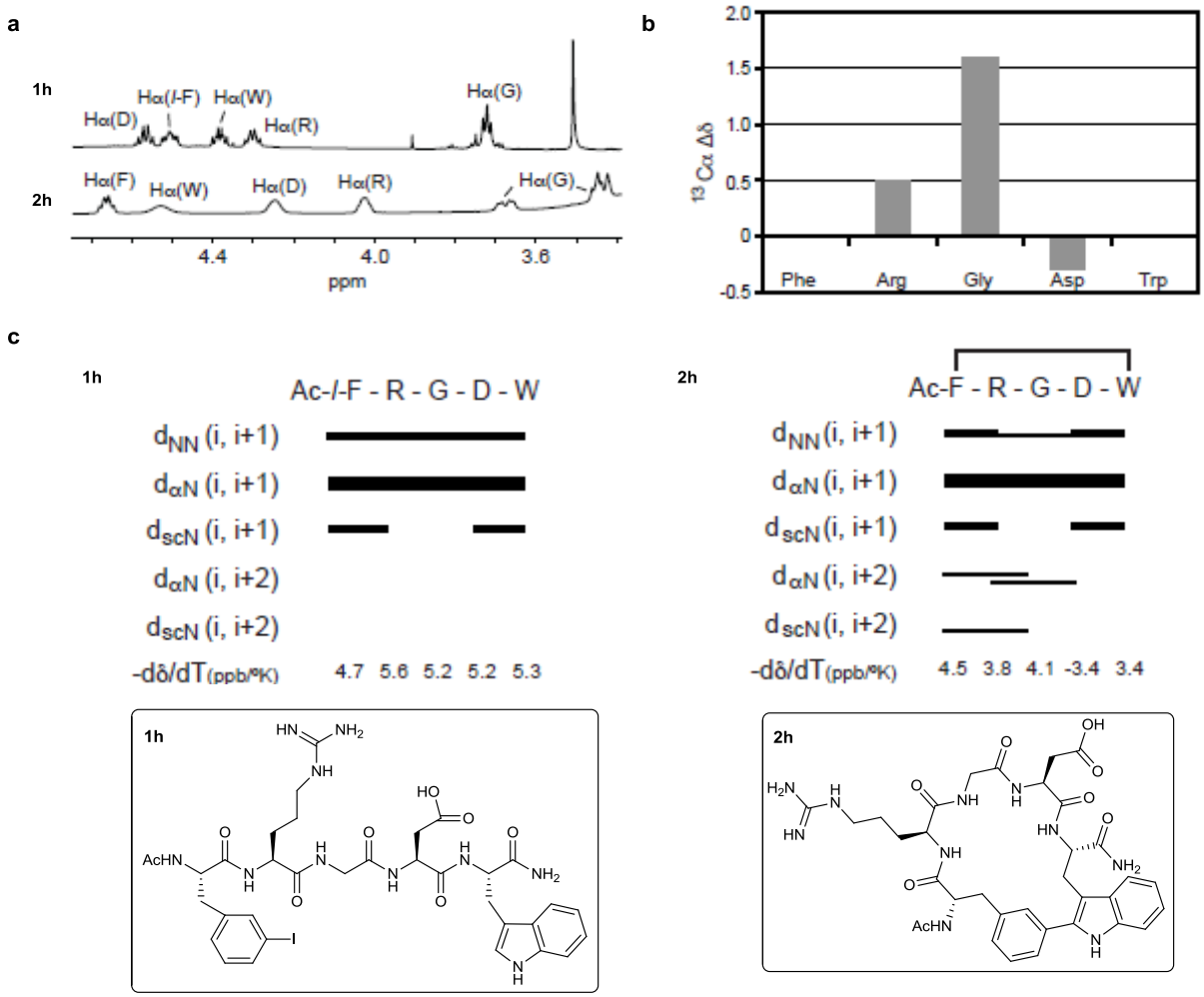
Supplementary Figure 3 | High resolution mass spectrometry analysis of compound 2k. Sample was reconstructed in 100 µL of ACN and diluted 1/100 in H_2O/ACN (1:1) 1% formic acid for MS analysis. **HRMS** (ESI) (m/z); found, 1067.4624. $[M+H]^+$ calcd. for compound **2k'** ($C_{28}H_{31}N_5O_6$), 533.2274; not found.



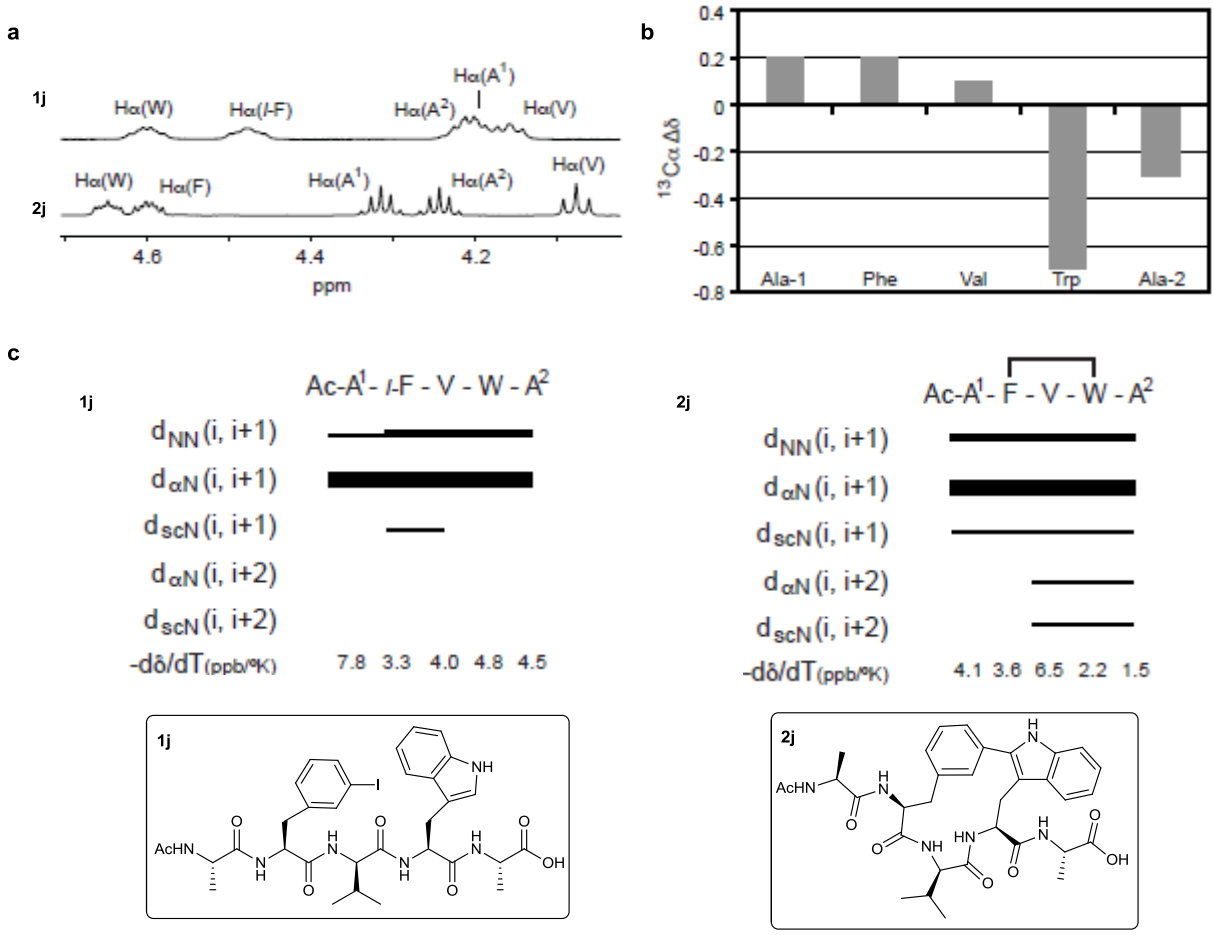
Supplementary Figure 4 ‖ Minimized geometries of compound 2k (left) and its monomeric analog 2k' (right) generated by the Spartan '14 suite.[8] Structure **2k** display planar phenyl rings in contrast with the constrained monomeric structure **2k'**, where a phenyl ring is severely distorted.



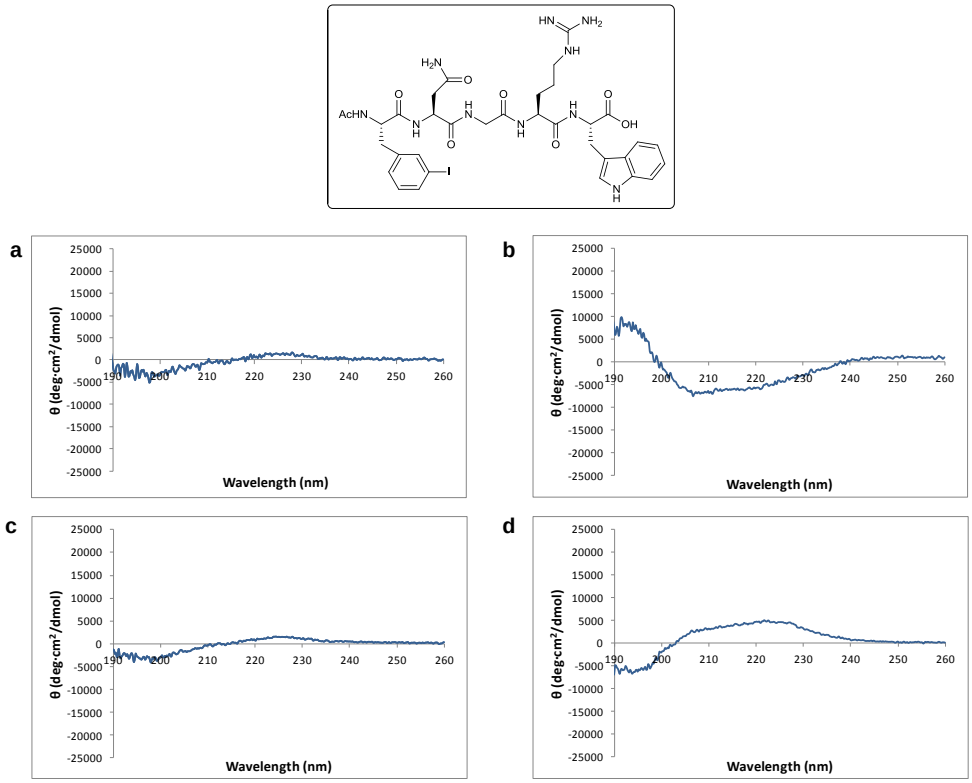
Supplementary Figure 5 | Peptide NMR spectra comparison between compounds 2g and 1g. a, NMR H_α region of peptide **2g** and its linear precursor **1g**. **b,** Plot of the $^{13}C_\alpha$ chemical shift differences ($^{13}C_\alpha$ $\Delta\delta_{cyclic\text{-}linear}$) between stapled peptide **2g** and its linear counterpart **1g**. **c,** Summary of NOE connectivities and temperature coefficients of the NH amide protons ($\Delta\delta/\Delta T$) of peptide **1g** (bottom left) and **2g** (bottom right). The thickness of the bars reflects the intensity of the NOEs, i.e. weak (▬), medium (▬) and strong (▬). *I*-F: *m*-iodophenylalanine.



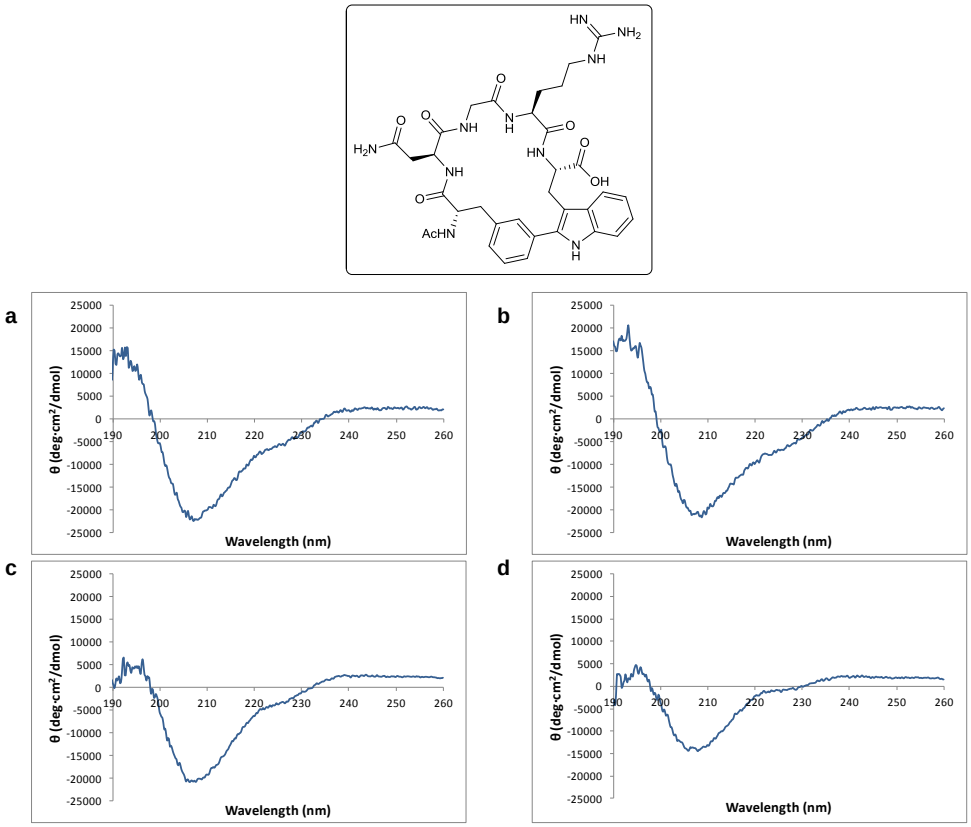
Supplementary Figure 6 ‖ Peptide NMR spectra comparison between compounds 2h and 1h. a, NMR H_α region of peptide **2h** and its linear precursor **1h**. **b,** Plot of the $^{13}C_\alpha$ chemical shift differences ($^{13}C_\alpha$ $\Delta\delta_{cyclic-linear}$) between stapled peptide **2h** and its linear counterpart **1h**. **c,** Summary of NOE connectivities and temperature coefficients of the NH amide protons ($\Delta\delta/\Delta T$) of peptide **1h** (bottom left) and **2h** (bottom right). The thickness of the bars reflects the intensity of the NOEs, i.e. weak (▬), medium (▬) and strong (▬). *I*-F: *m*-iodophenylalanine.



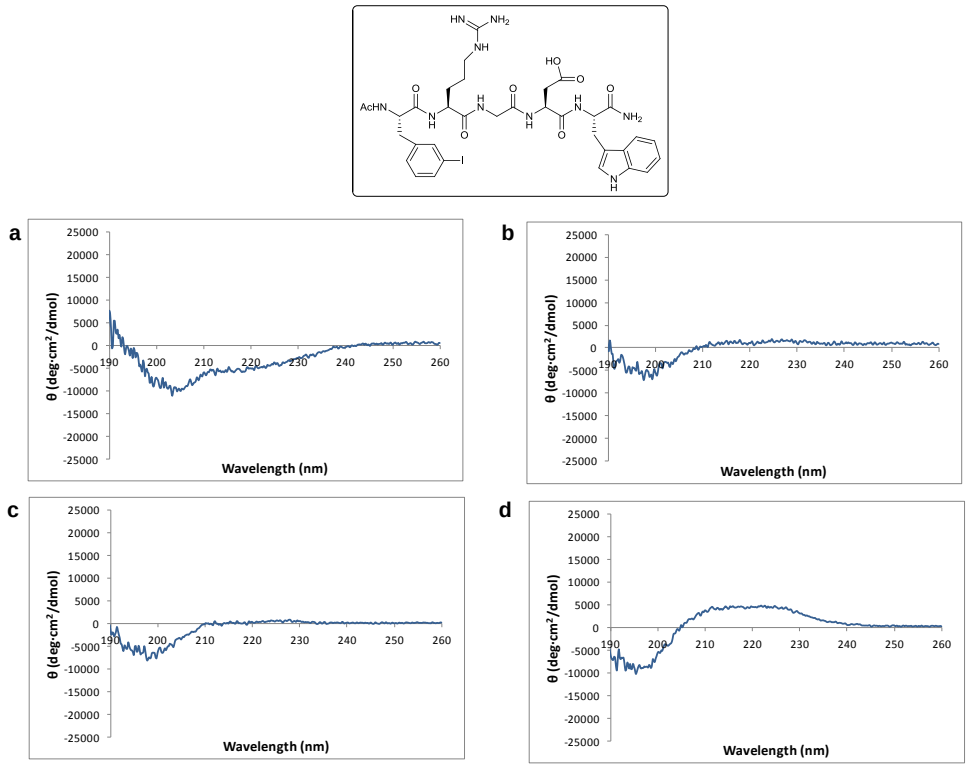
Supplementary Figure 7 ‖ Peptide NMR spectra comparison between compounds 2j and 1j. a, NMR H_α region of peptide **2j** and its linear precursor **1j**. **b,** Plot of the $^{13}C_\alpha$ chemical shift differences ($^{13}C_\alpha$ $\Delta\delta_{cyclic\text{-}linear}$) between stapled peptide **2j** and its linear counterpart **1j**. **c,** Summary of NOE connectivities and temperature coefficients of the NH amide protons ($\Delta\delta/\Delta T$) of peptide **1j** (bottom left) and **2j** (bottom right). The thickness of the bars reflects the intensity of the NOEs, i.e. weak (▬), medium (▬) and strong (▬). *I*-F: *m*-iodophenylalanine.



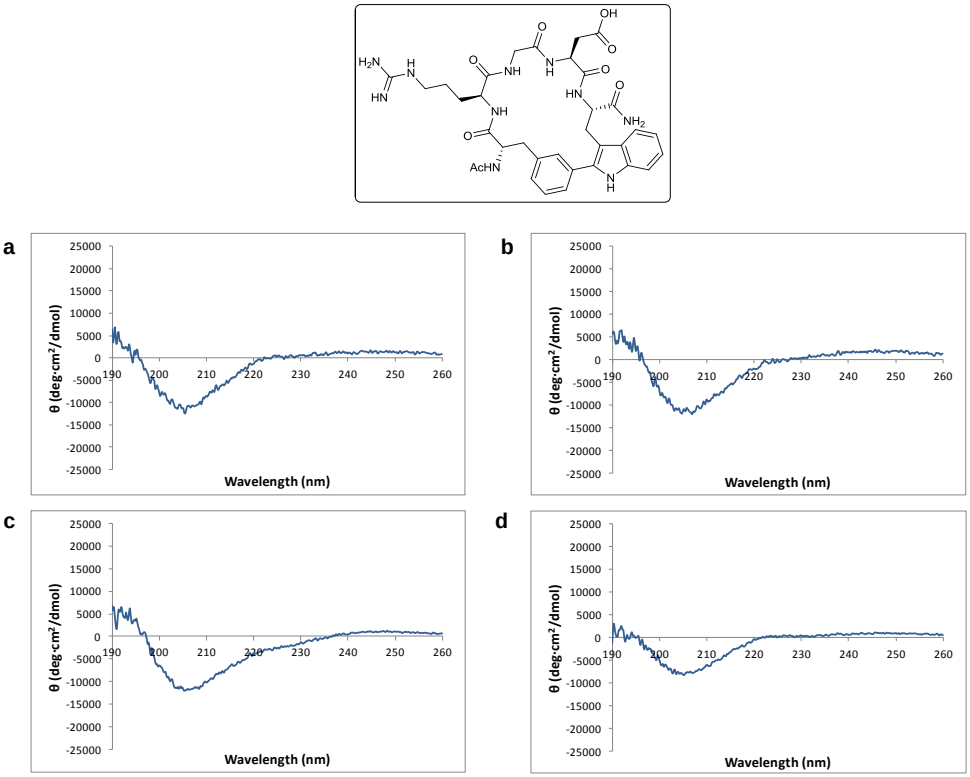
Supplementary Figure 8 | Circular dichroism spectra of 1g. **a,** At 100 µM in a buffer of 25 mM Na_2HPO_4 (pH 7). **b,** At 100 µM in a buffer of 25 mM Na_2HPO_4 (pH 7) (90%) and 10% of TFE. **c,** At 200 µM in a buffer of 25 mM Na_2HPO_4 (pH 7). **c,** At 200 µM in PBS (90%) and TFE (10%).



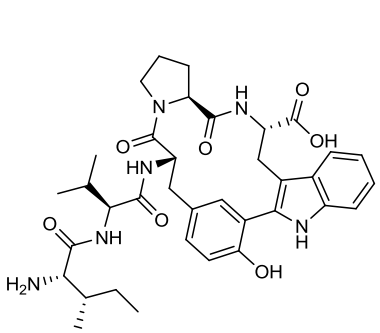
Supplementary Figure 9 | Circular dichroism spectra of 2g. **a,** At 100 µM in a buffer of 25 mM Na_2HPO_4 (pH 7). **b,** At 100 µM in a buffer of 25 mM Na_2HPO_4 (pH 7) (90%) and 10% of TFE. **c,** At 200 µM in a buffer of 25 mM Na_2HPO_4 (pH 7). **d,** At 200 µM in PBS (90%) and TFE (10%).

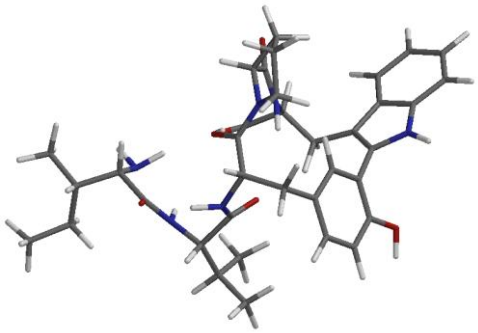


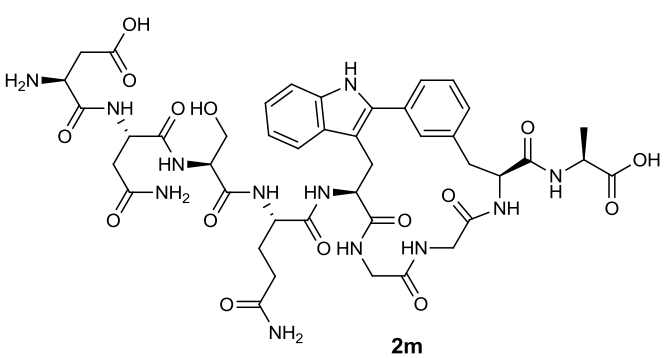
Supplementary Figure 10 | Circular dichroism spectra of 1h. **a,** At 100 µM in a buffer of 25 mM Na_2HPO_4 (pH 7). **b,** At 100 µM in a buffer of 25 mM Na_2HPO_4 (pH 7) (90%) and 10% of TFE. **c,** At 200 µM in a buffer of 25 mM Na_2HPO_4 (pH 7). **d,** At 200 µM in PBS (90%) and TFE (10%).

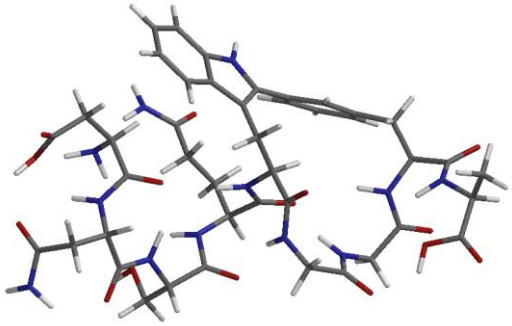


Supplementary Figure 11 | Circular dichroism spectra of 2h. **a,** At 100 µM in a buffer of 25 mM Na_2HPO_4 (pH 7). **b,** At 100 µM in a buffer of 25 mM Na_2HPO_4 (pH 7) (90%) and 10% of TFE. **c,** At 200 µM in a buffer of 25 mM Na_2HPO_4 (pH 7). **d,** At 200 µM in PBS (90%) and TFE (10%).

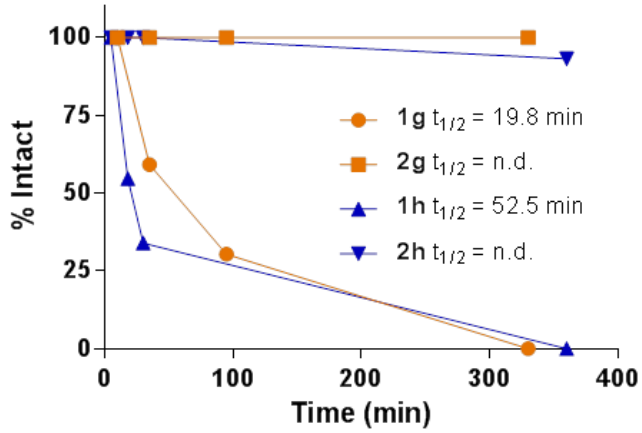




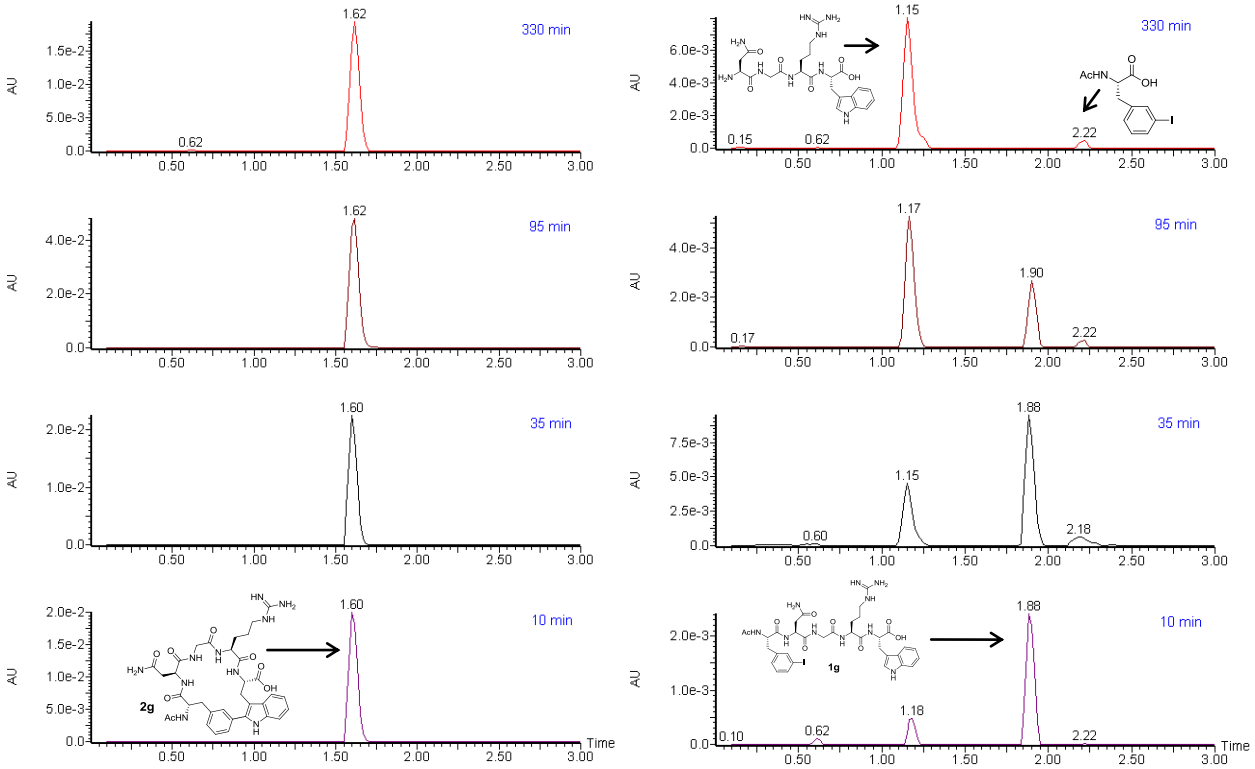




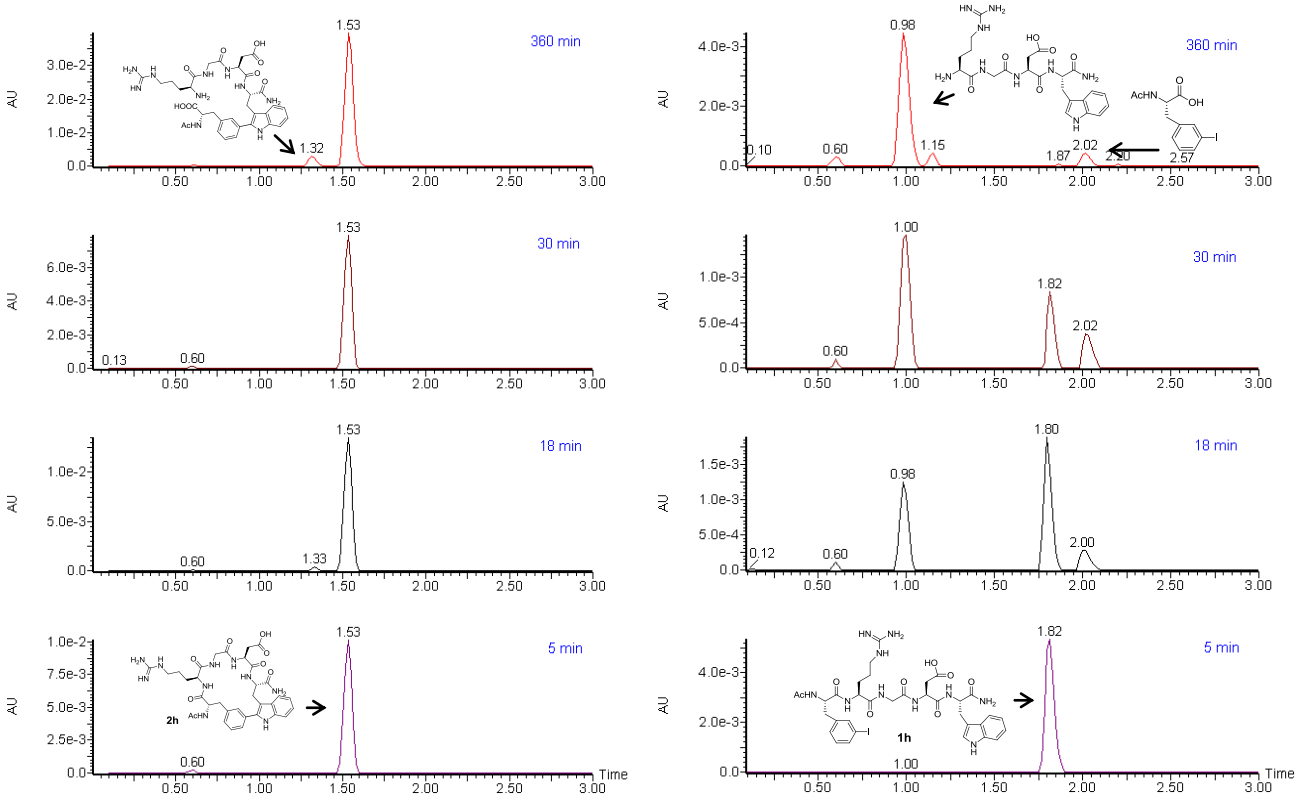
Supplementary Figure 12 | Minimized geometries of compounds 2l and 2m generated by the Spartan '14 suite (molecular mechanics, MMFF94).[8]



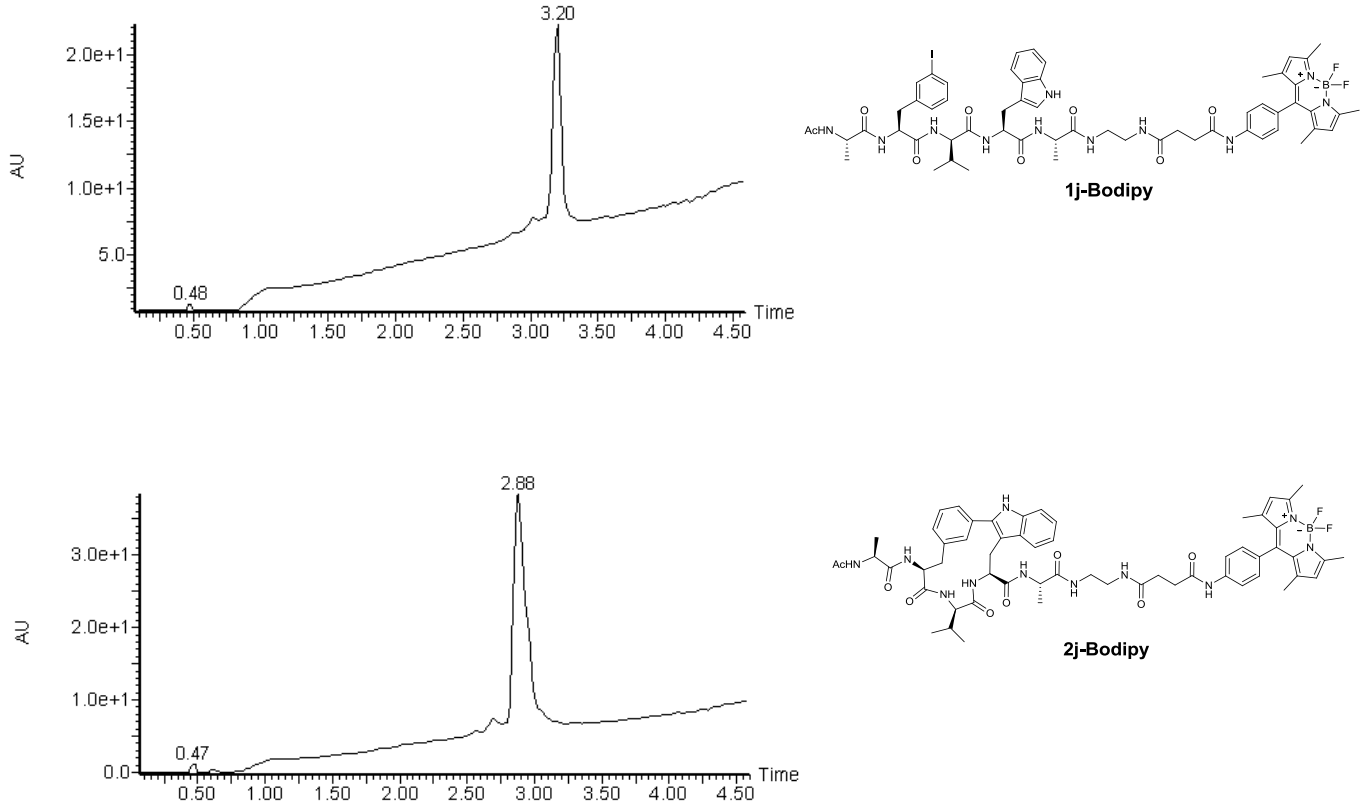
Supplementary Figure 13 | Proteolytic degradation assay of stapled peptides 2g and 2h and their linear precursors 1g and 1h. With respect to the linear compounds **1g** and **1h**, α-chymotrypsin cleavage products (from the hydrolysis on the *C*-terminal side of *l*-phenylalanine)[12] were observed, being the degradation complete after 6 h. Significant different behavior was observed for the corresponding stapled peptides **2g** and **2h,** which remained unaltered and only traces of the corresponding C-terminal hydrolysis of *l*-phenylalanine were detected.



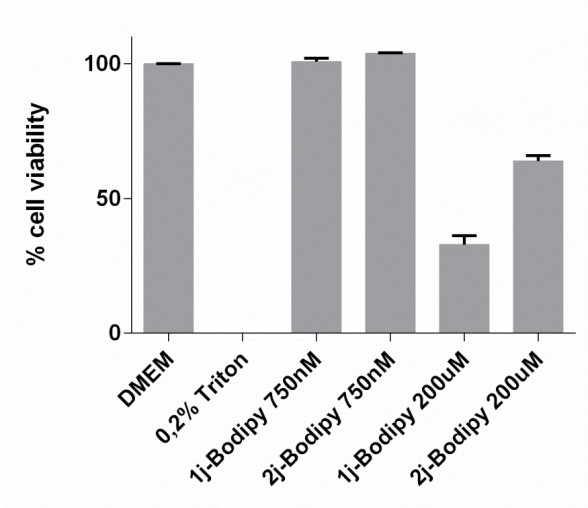
Supplementary Figure S14 | HPLC-MS chromatograms of stapled peptide 2g (left) and its linear precursor 1g (right) monitorized by at 300 and 280 nm, respectively.



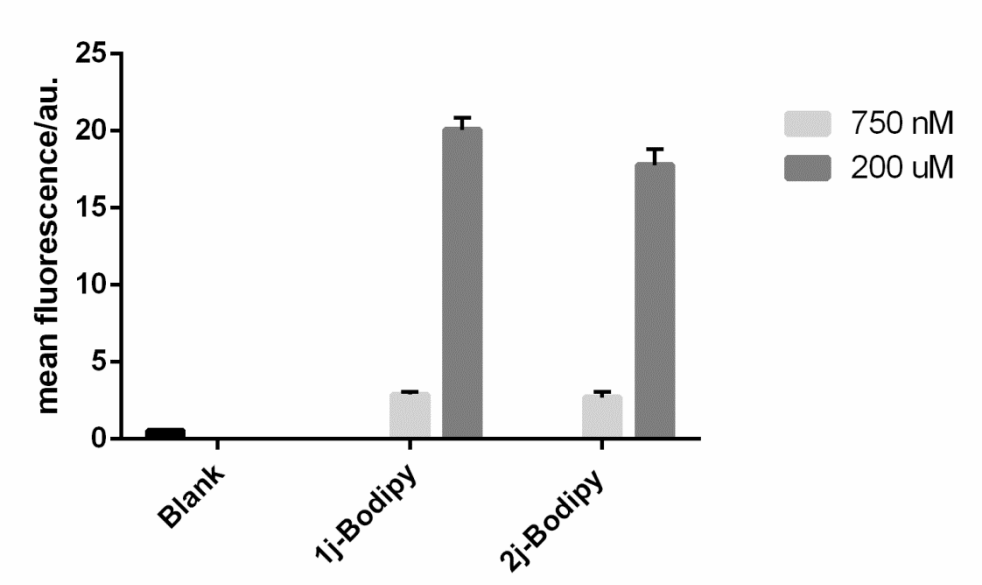
Supplementary Figure S15 | HPLC-MS chromatograms of stapled peptide 2h (left) and its linear precursor 1h (right) monitorized at 300 and 280 nm, respectively.



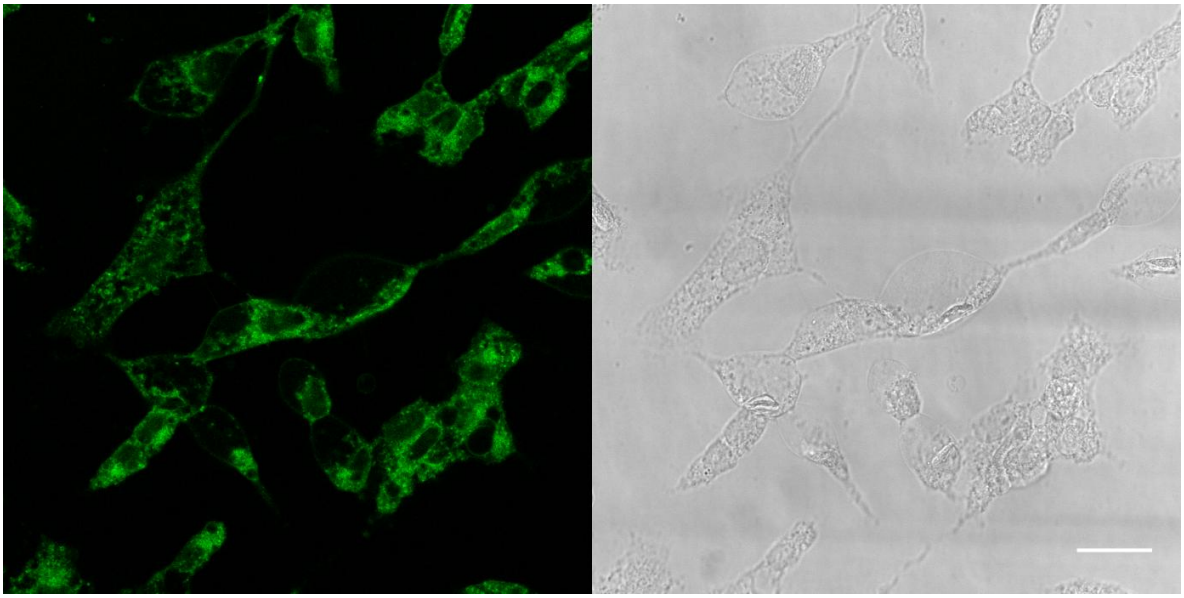
Supplementary Figure 16 ‖ HPLC-MS chromatograms of BODIPY-labelled peptides 1j-BODIPY (above) and 2j-BODIPY (down).



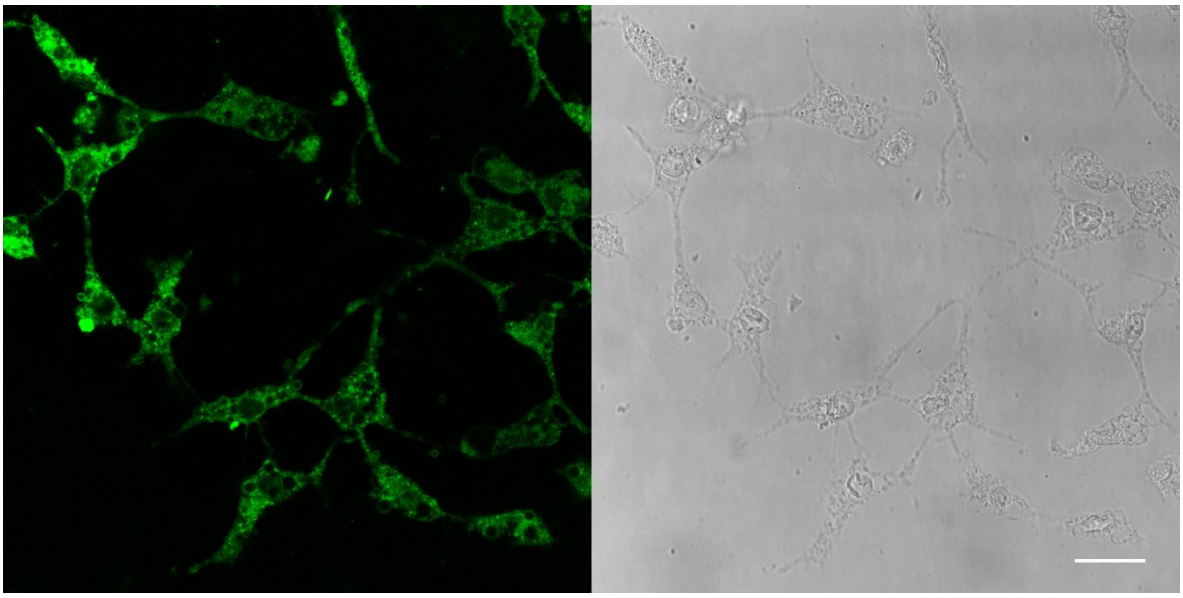
Supplementary Figure 17 | Cytotoxicity of 1j-Bodipy and 2j-Bodipy peptides using the MTT assay. The toxicity assay was carried out as described in the literature.[13] SH-SY5Y cells were transferred to 96-well plates (100 µL medium/well) at a density of 5000 cell/well. After 24 h, cells in triplicate were treated either with **1j-Bodipy** or **2j-Bodipy** (750 nM and 50 µM) or TRITON 0.2% as positive control for 24 h. Values are represented as means ± SD from three independent experiments.



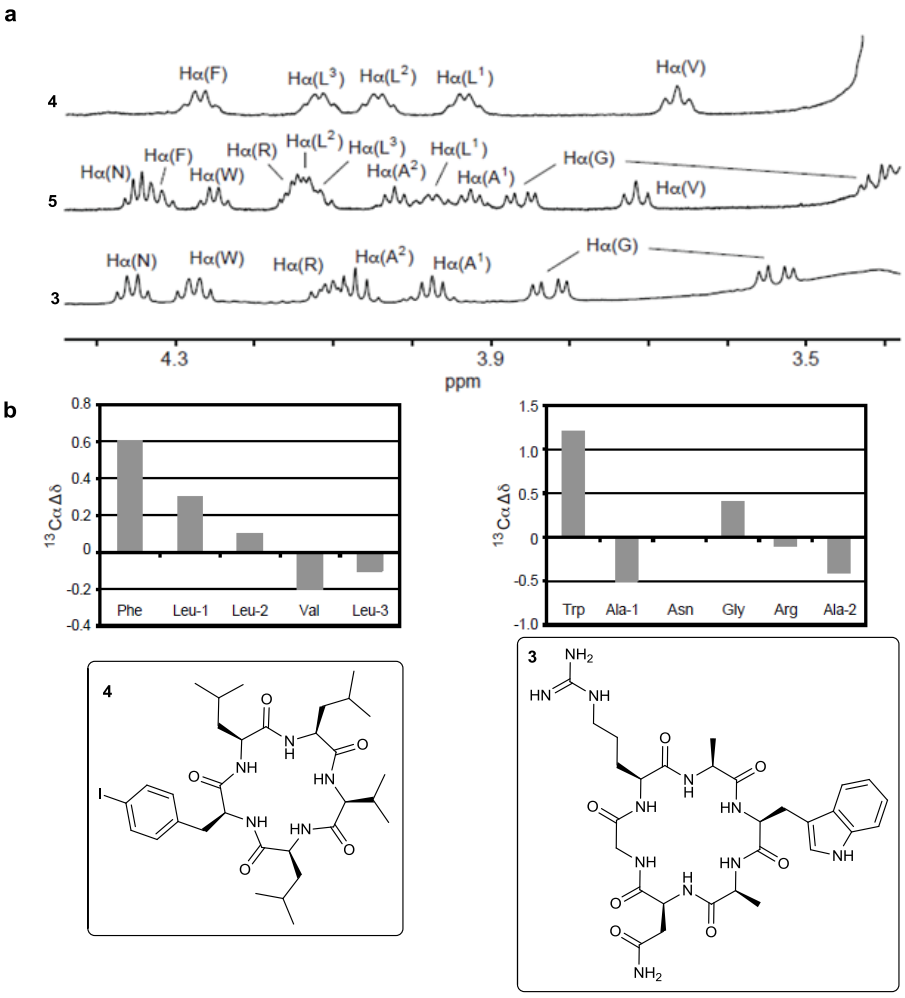
Supplementary Figure 18 | FACS analysis of SH-SY5Y cells upon incubation with 1j-Bodipy and 2j-Bodipy. For FACS analysis, SH-SY5Y cells were seeded on a plastic 24 well plate and cultured for 24 h. The culture medium was discarded, and cells were incubated for 30 min at 37 ºC under 5% CO_2 with fresh medium containing either **1j-Bodipy** or **2j-Bodipy**. Cells were then rinsed, treated with trypsin, collected, centrifuged at 4 ^{o}C, filtered and re-suspended in cold medium. Fluorescence sorting was performed with a Gallios Beckman Coulter flow cytometer.



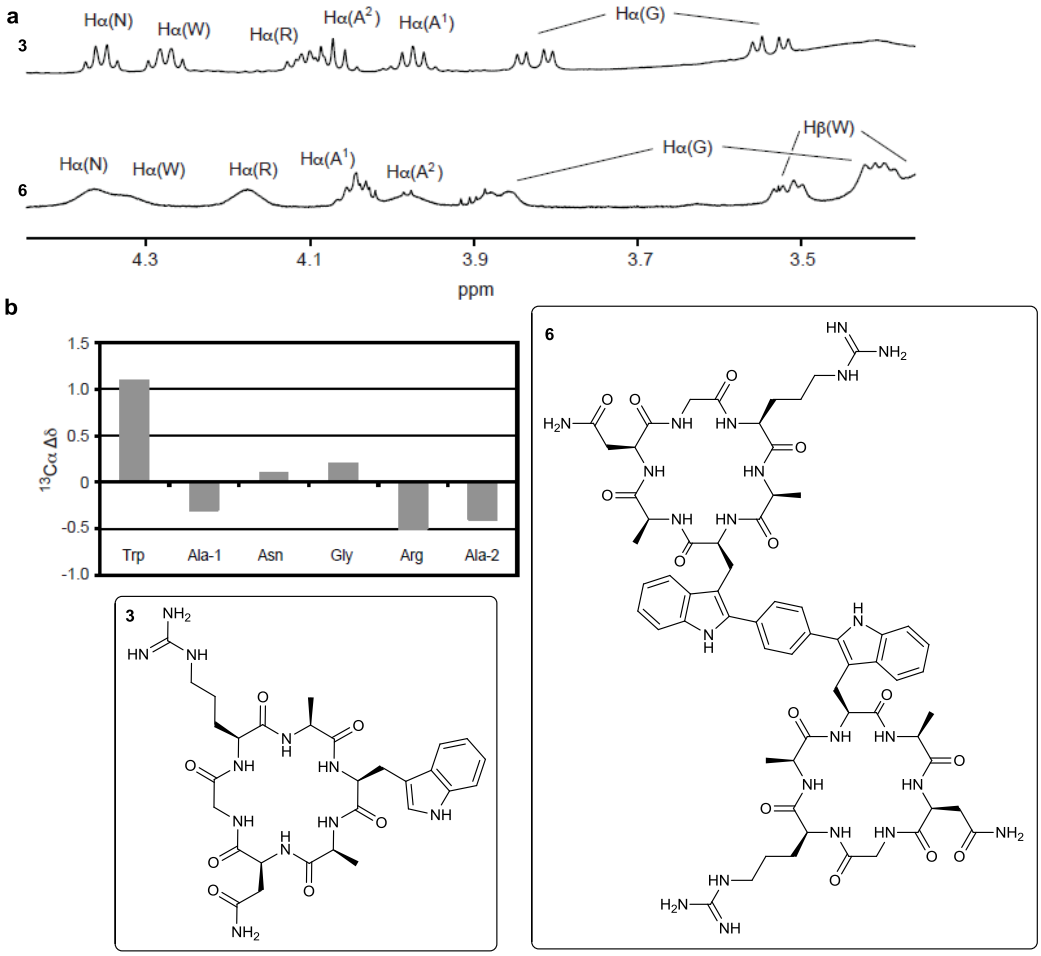
Supplementary Figure 19 | Spontaneous internalization of 1j-Bodipy in SH-SY5Y cells (scale bar, 25 μm).



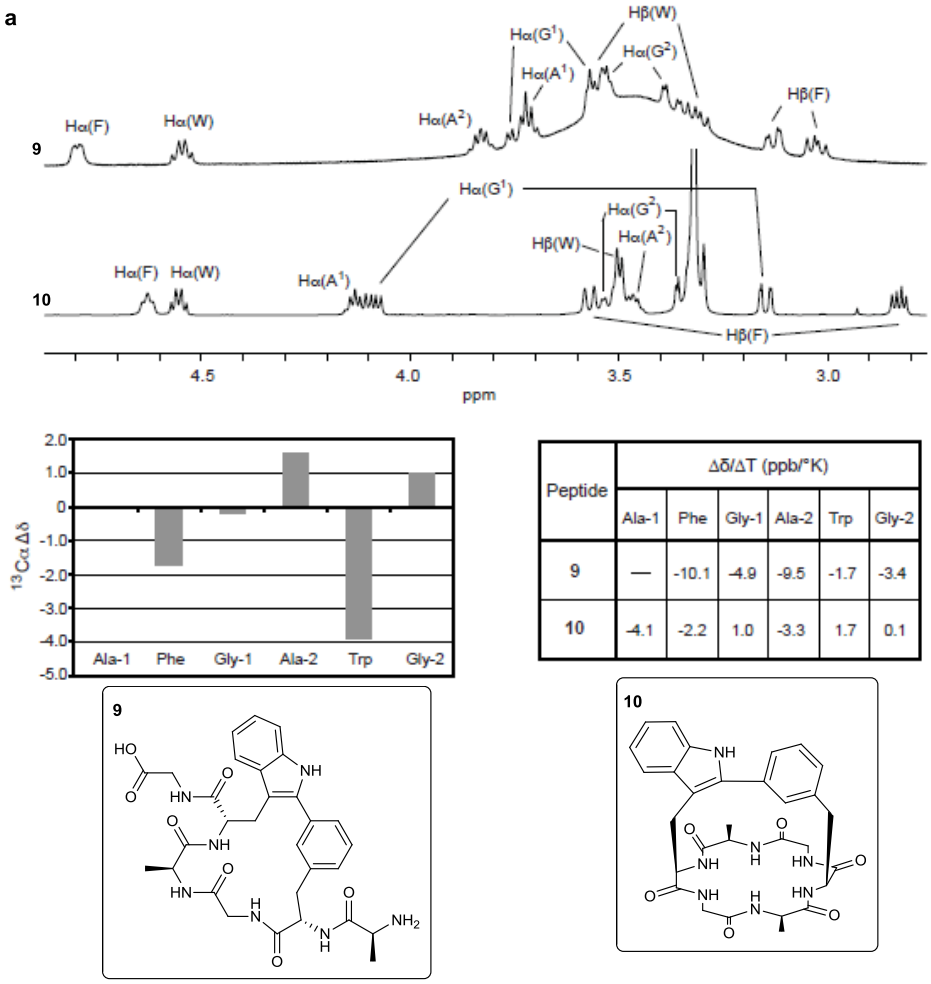
Supplementary Figure 20 | Spontaneous internalization of 2j-Bodipy in SH-SY5Y cells (scale bar, 25 μm).



Supplementary Figure 21 ‖ **Peptide NMR spectra comparison between compounds 3 and 4. a,** NMR H_α region of peptides **3, 4** and **5**. **b,** Plot of the $^{13}C_\alpha$ chemical shift differences between conjugated peptide **5** and its macrocyclic precursors **3** and **4** ($^{13}C_\alpha$ $\Delta\delta_{5\text{-}x}$, where x=3 or 4).

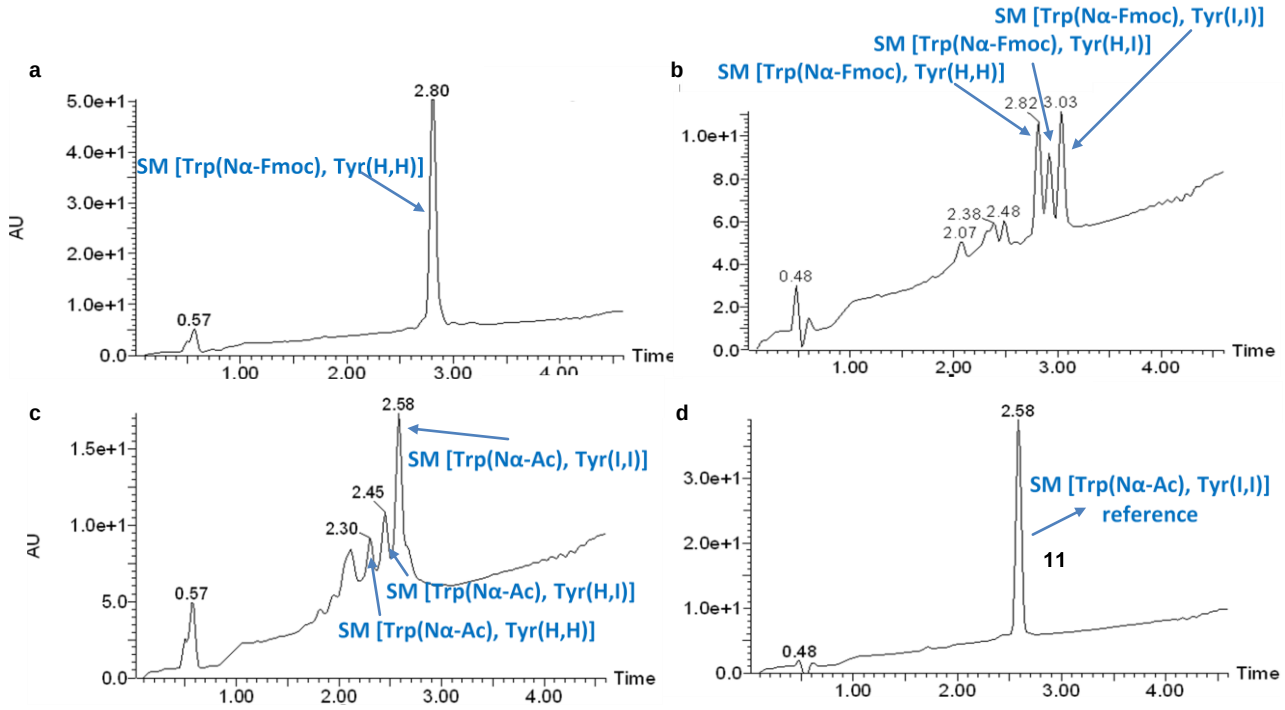


Supplementary Figure 22 ‖ Peptide NMR spectra comparison between compounds 3 and 6. a, NMR H_α region of peptides **3** and **6**. **b,** Plot of the $^{13}C_\alpha$ chemical shift differences between conjugated peptide **6** and its macrocyclic precursor **3** ($^{13}C_\alpha$ $\Delta\delta_{6\text{-}3}$).

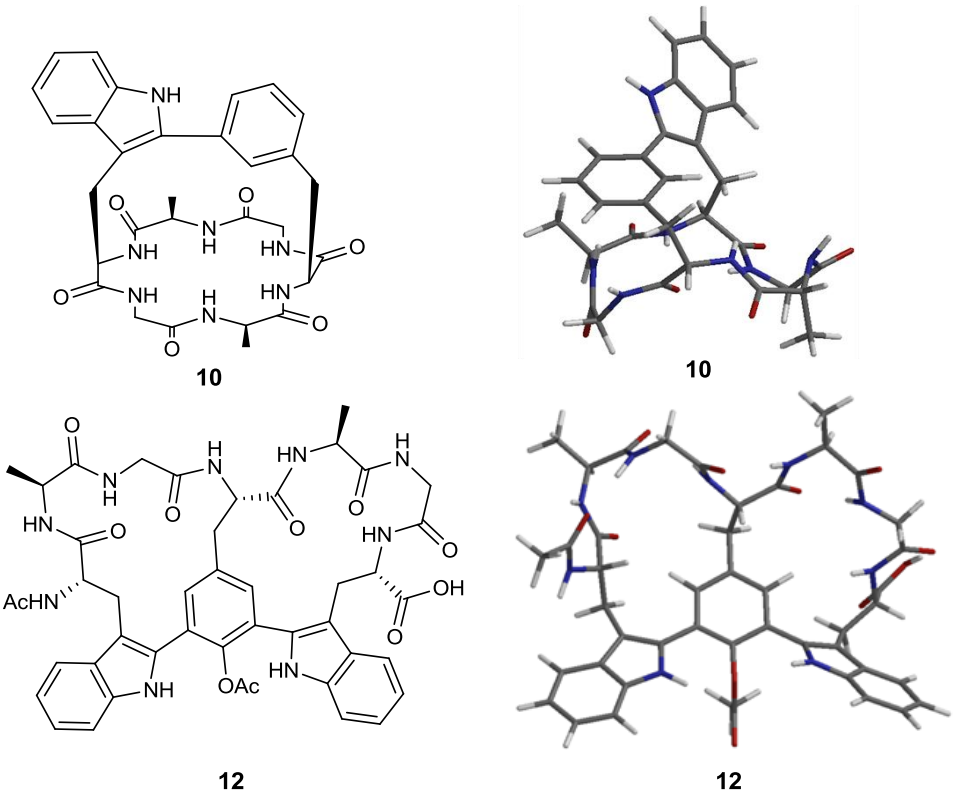


Peptide	Δδ/ΔT (ppb/°K)					
	Ala-1	Phe	Gly-1	Ala-2	Trp	Gly-2
9	—	-10.1	-4.9	-9.5	-1.7	-3.4
10	-4.1	-2.2	1.0	-3.3	1.7	0.1

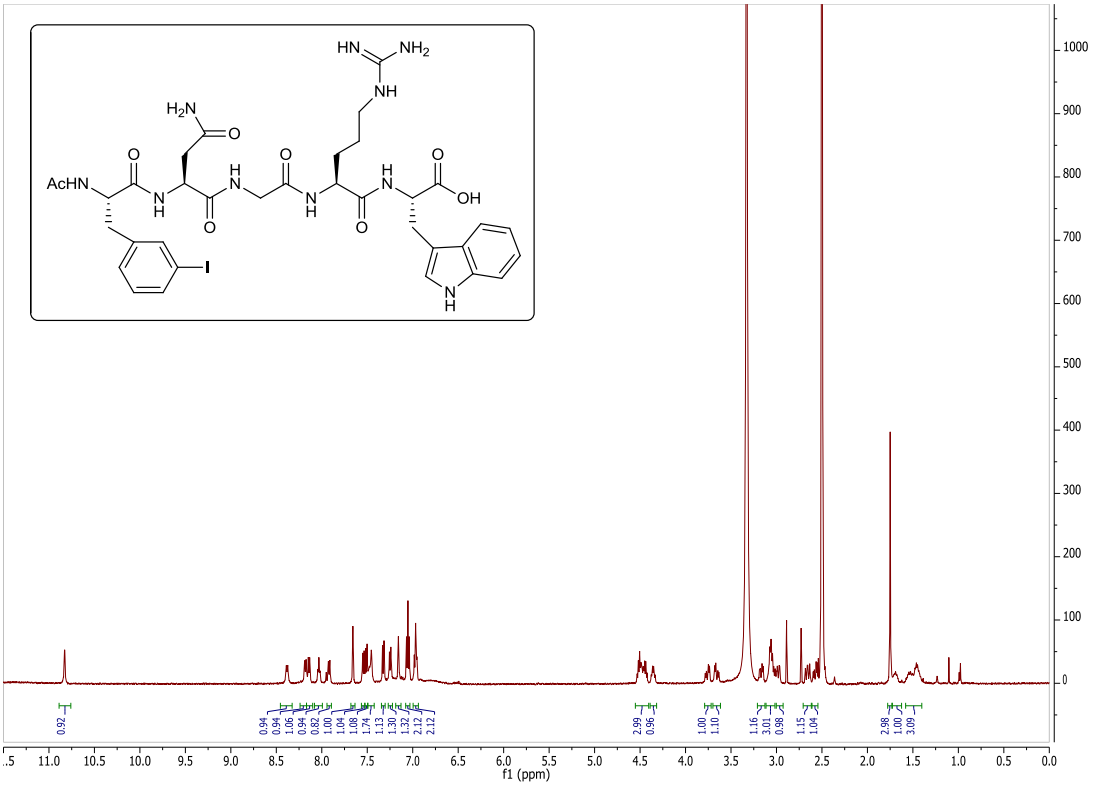
Supplementary Figure 23 | **Peptide NMR spectra comparison between compounds 9 and 10. a,** NMR H_α region of peptides **9** and **10**. **b,** Plot of the $^{13}C_\alpha$ chemical shift differences between peptides **9** and **10** ($^{13}C_\alpha$ $\Delta\delta_{10\text{-}9}$). **c,** Summary of temperature coefficients of the NH amide protons ($\Delta\delta/\Delta T$) of peptides **9** and **10**.



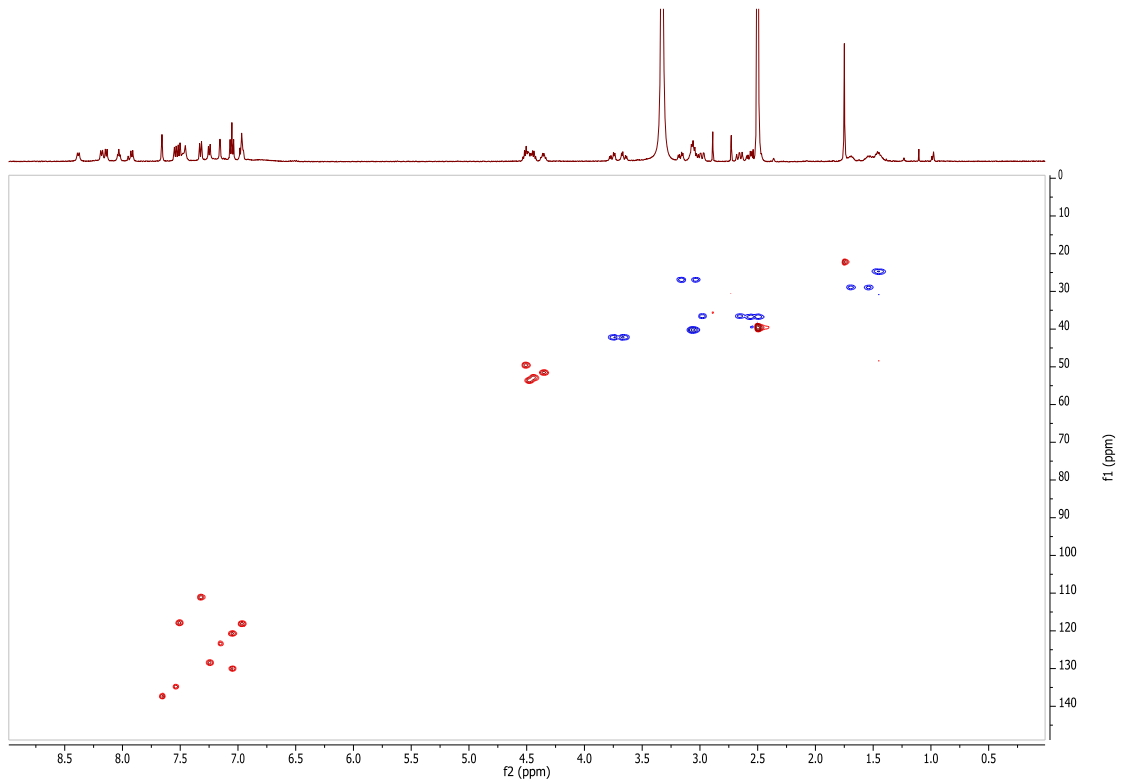
Supplementary Figure 24 ‖ HPLC-MS chromatograms relative to diiodination of the corresponding non-halogenated N-terminal Fmoc-protected linear sequence of 11 on resin. In a preliminary procedure the corresponding non-halogenated linear sequence of **11** anchored to TentaGel resin provided with HMPPA linker was treated with IPy_2BF_4 in DCM. Although this approach is not free from byproducts relative to the halogenation of the linker, it clearly shows the feasibility of the methodology.**a,** Non-halogenated linear precursor of compound **11** obtained through routine amide couplings. **b,** On-resin iodination of the Tyr residue to yield derivative N-terminal Fmoc-protected of **11** (41% conversion). Reaction conditions: IPy_2BF_4 (2.2 eq.), DCM, r.t, (1 x 10 min, 1 x 50 min). **c,** On-resin iodination of the Tyr residue followed by Fmoc protecting group removal and N-terminal acetilation. Reaction conditions: (i) IPy_2BF_4 (4.4 eq.), DCM, r.t, (1 x 1 h), (ii) piperidine-DMF (1:4) (1 × 1 min, 2 × 5 min), (iii) DIEA (10.0 eq.), acetic anhydride (10.0 eq.),DMF, r.t,, 30 min. **d,** HPLC-MS chromatogram reference of the halogenated compound **11**. All the peptides were cleaved from the resin with TFA-DCM (95:5), r.t, 1h to be analyzed by HPLC-MS. Gradient from 5 to 100% ACN (0,1% FA).



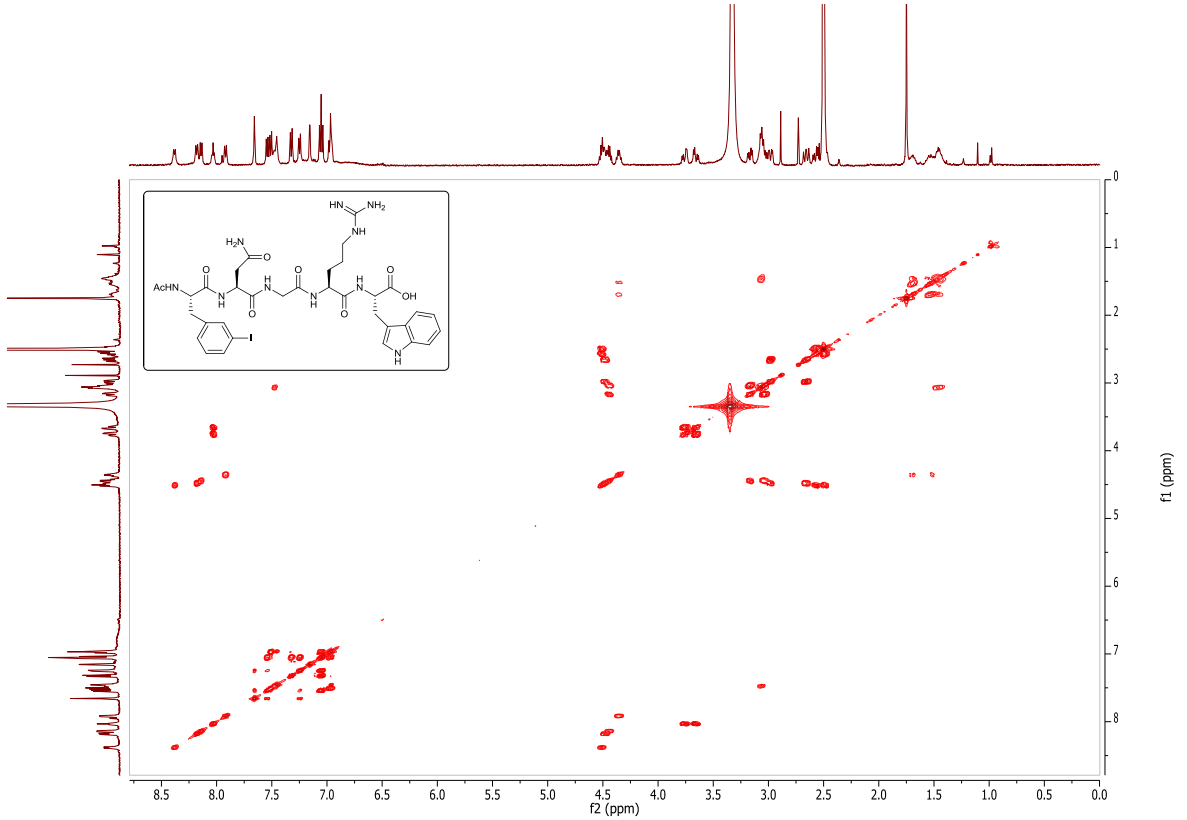
Supplementary Figure 25 | Minimized geometries of compounds 10 and 12 generated by the Spartan '14 suite.[8]



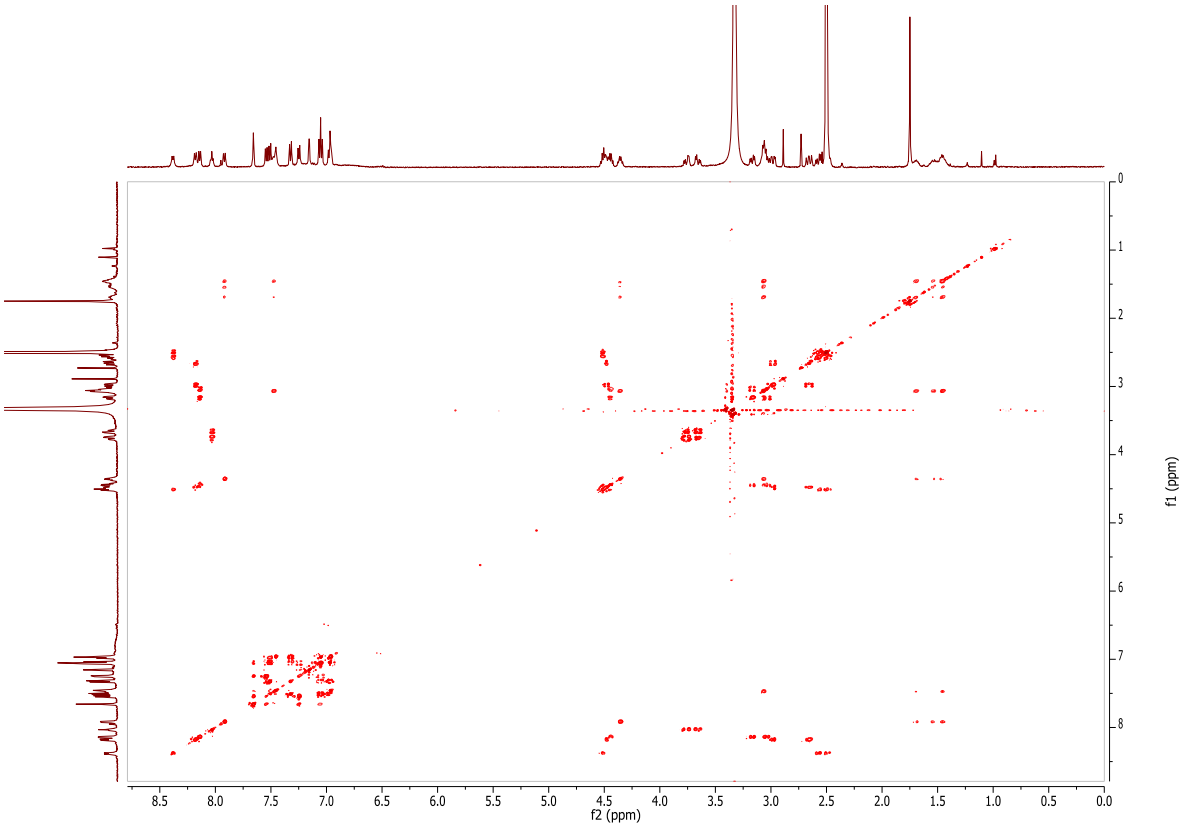
Supplementary Figure 26 ‖ ^{1}H NMR spectrum of compound Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-OH (1g).



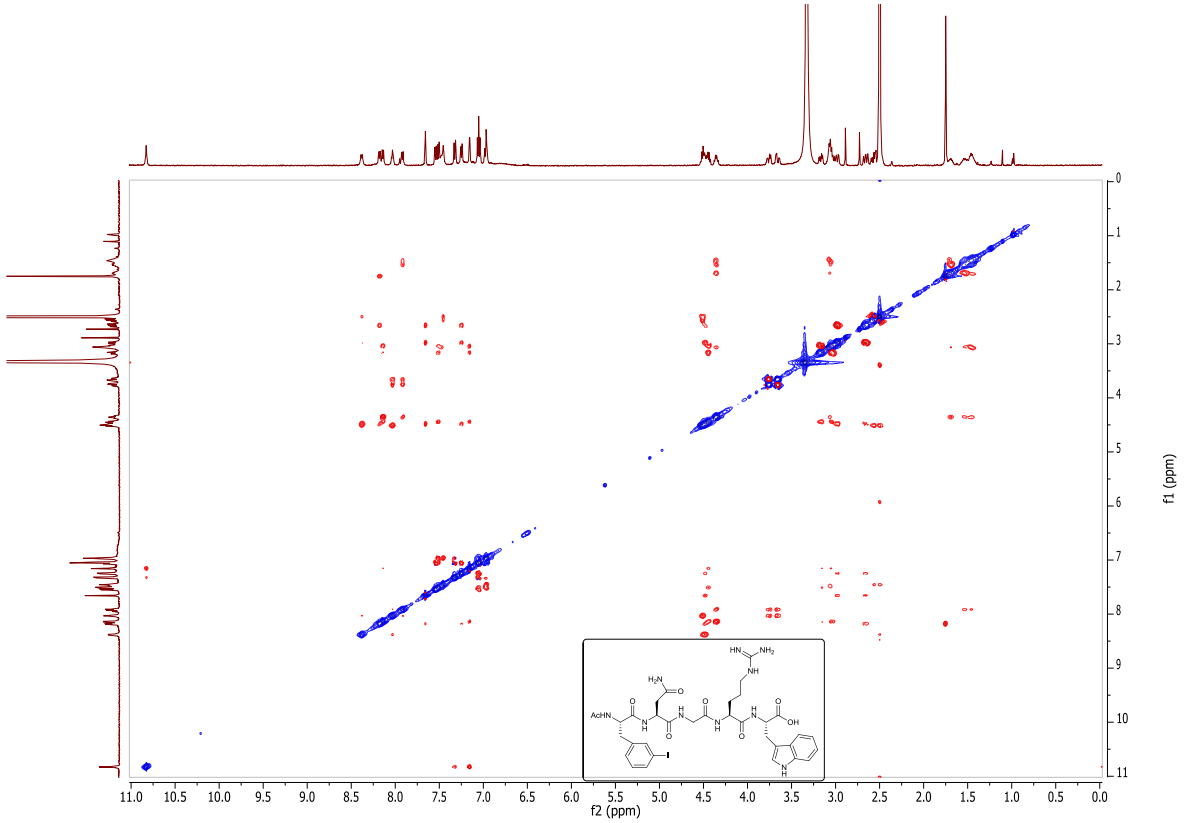
Supplementary Figure 27 ‖ ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-OH (1g).



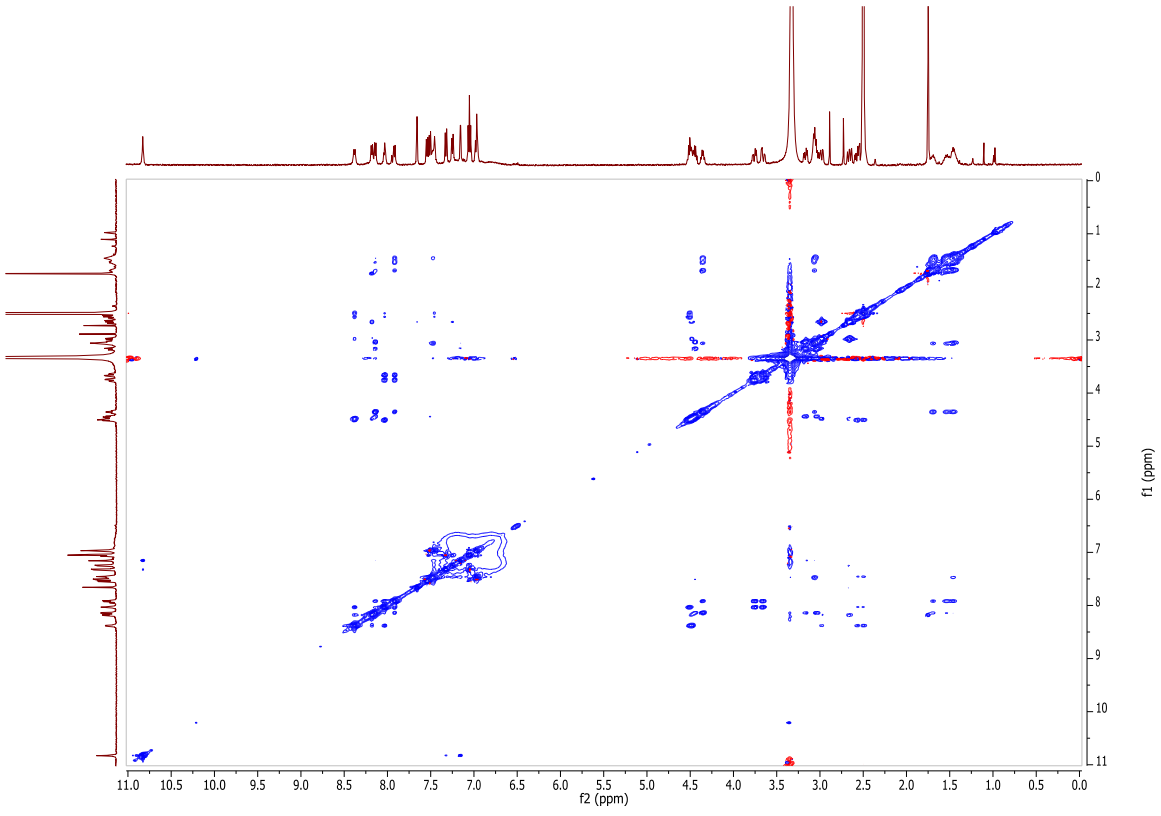
Supplementary Figure 28 | COSY NMR spectrum of compound Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-OH (1g).



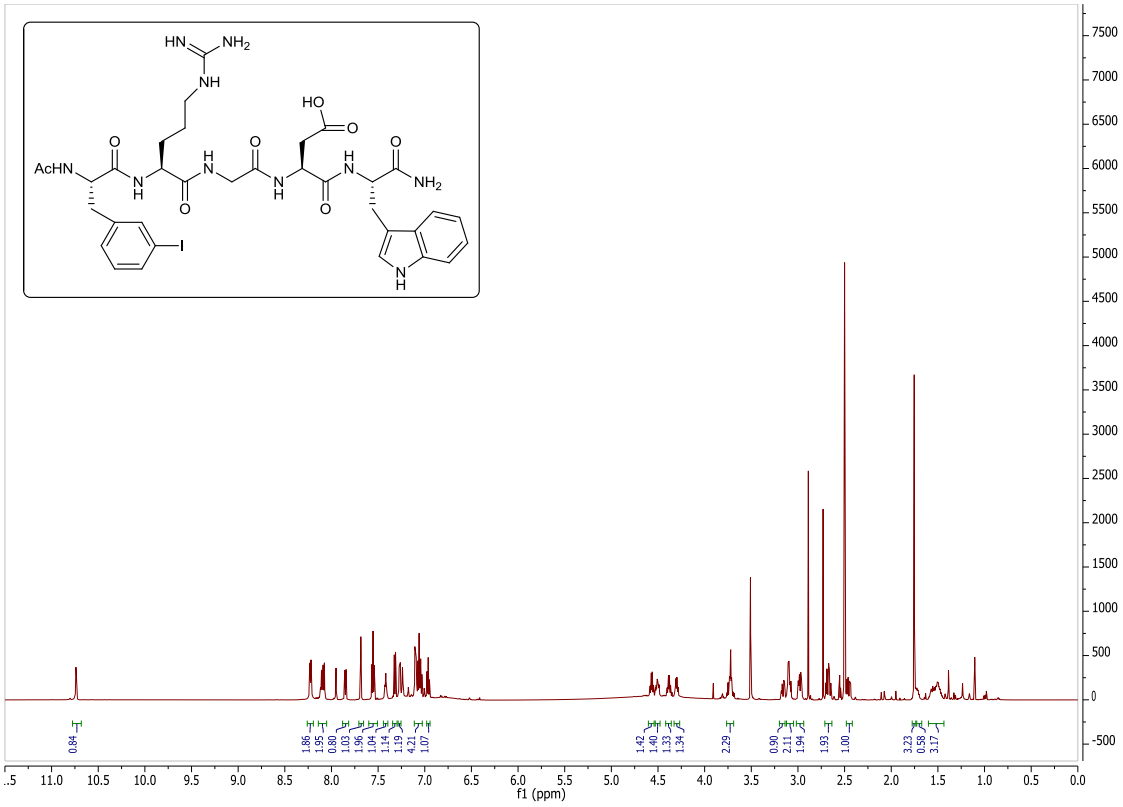
Supplementary Figure 29 | TOCSY NMR spectrum of compound Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-OH (1g).



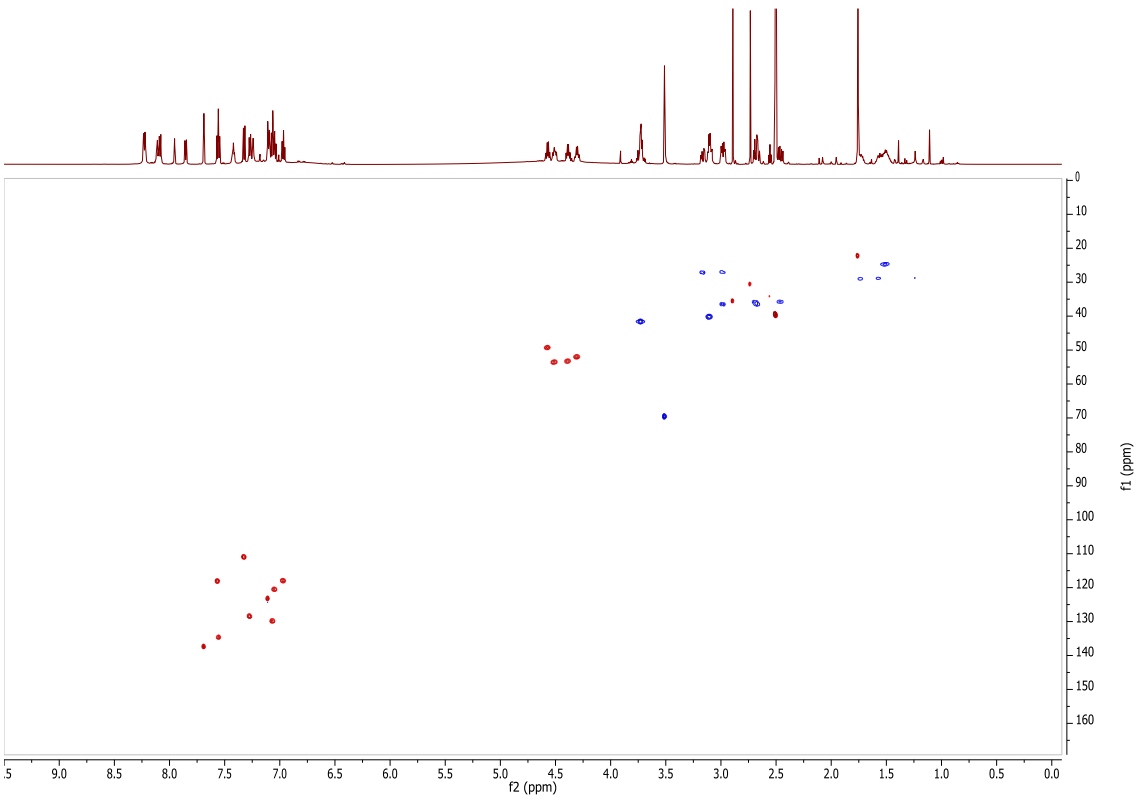
Supplementary Figure 30 | ROESY NMR spectrum of compound Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-OH (1g).



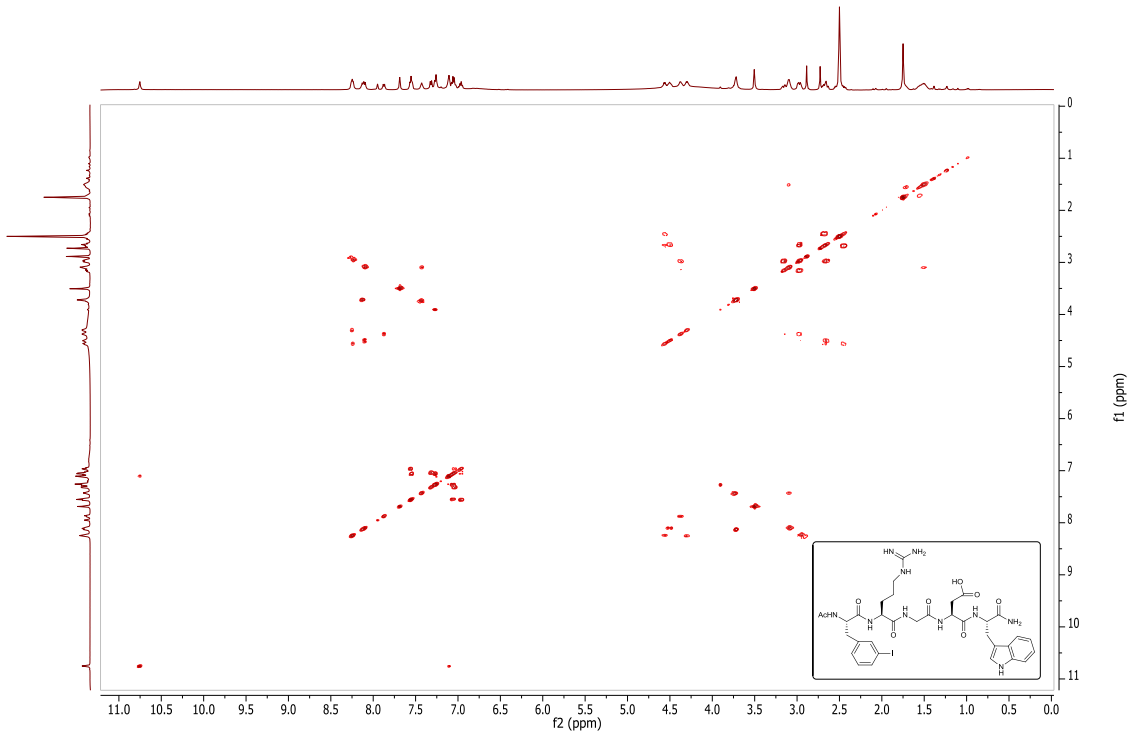
Supplementary Figure 31 | NOESY NMR spectrum of compound Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-OH (1g).



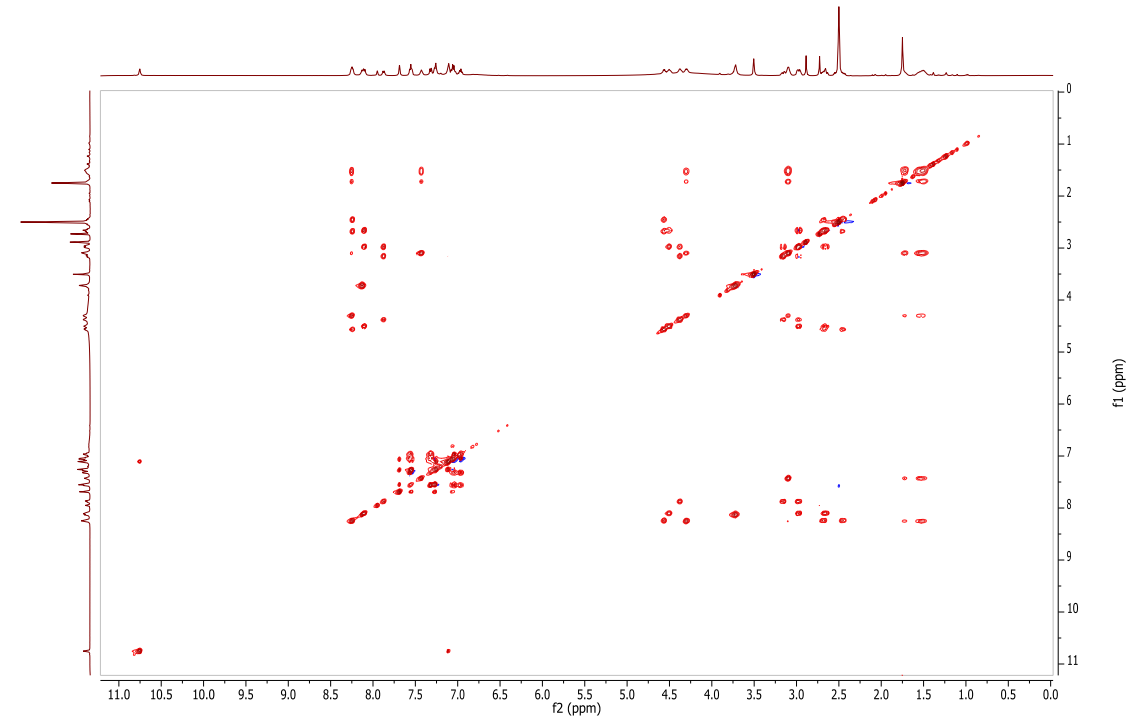
Supplementary Figure 32 ‖ ^{1}H NMR spectrum of compound Ac-*m*-I-Phe-Arg-Gly-Asp-Trp-OH (1h).



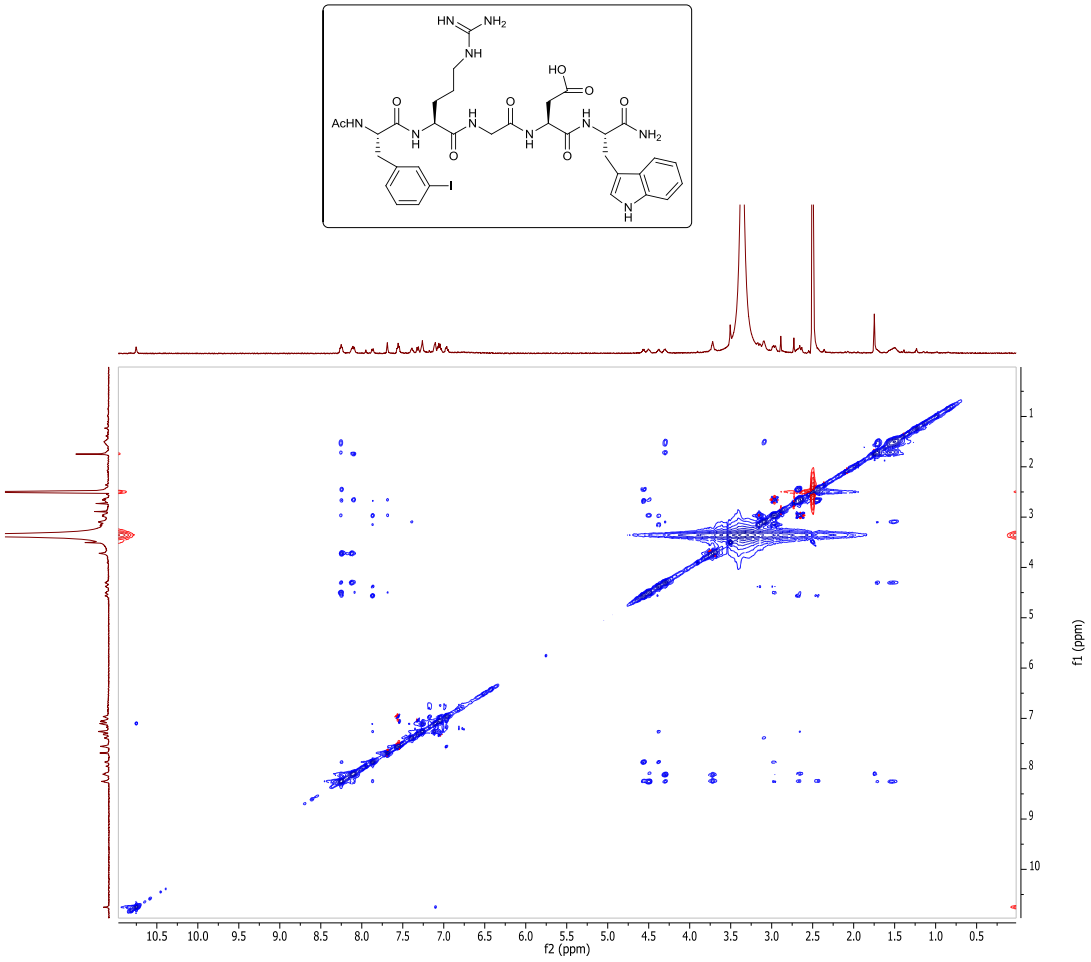
Supplementary Figure 33 ‖ ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-*m*-I-Phe-Arg-Gly-Asp-Trp-OH (1h).



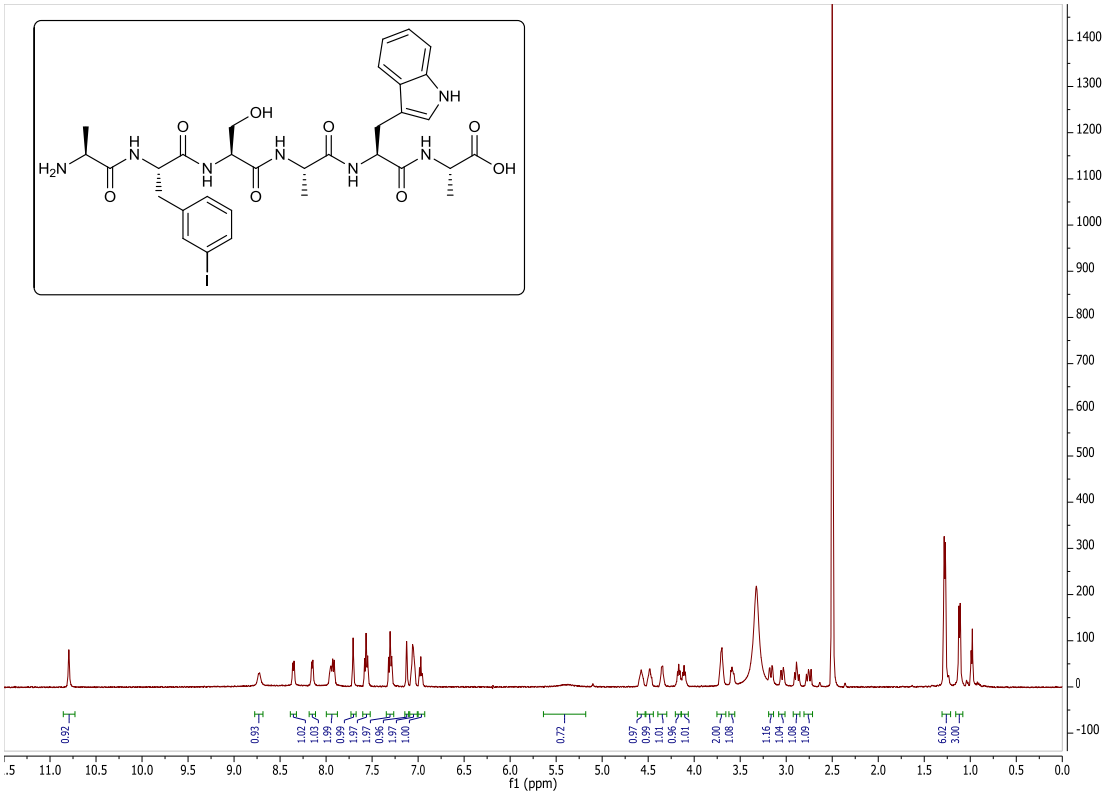
Supplementary Figure 34 | COSY NMR spectrum of compound Ac-*m*-I-Phe-Arg-Gly-Asp-Trp-OH (1h).



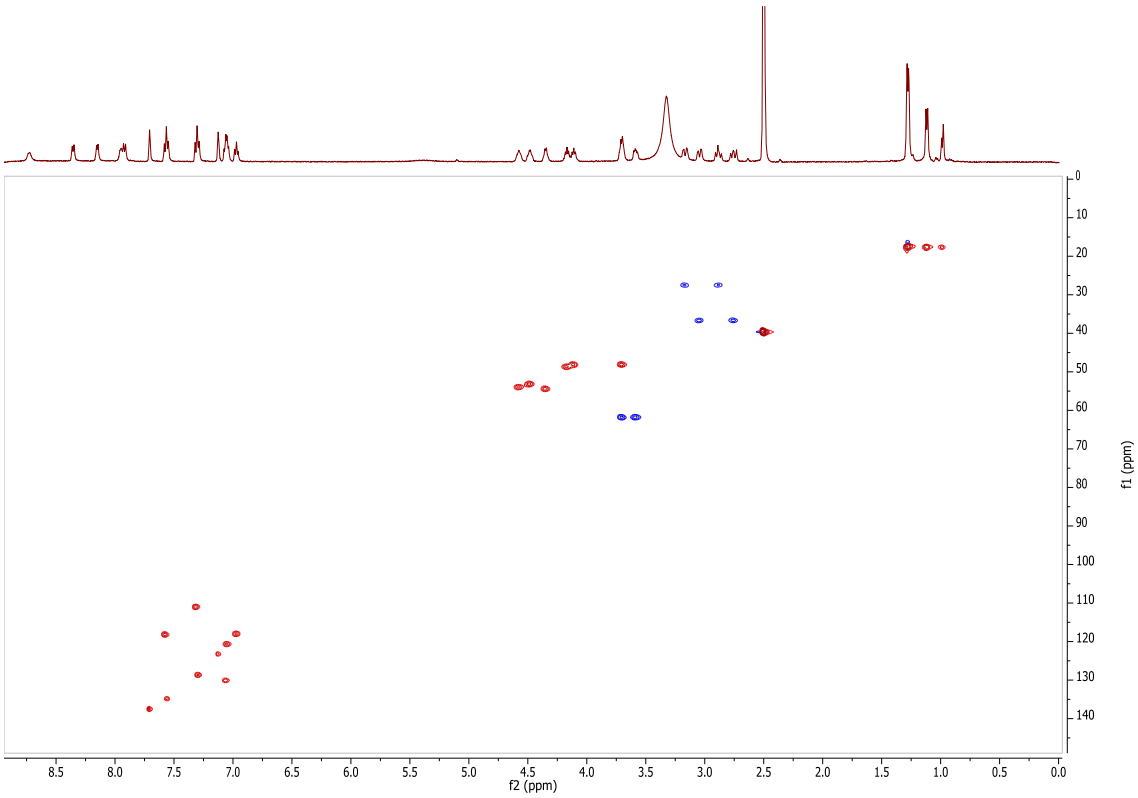
Supplementary Figure 35 | TOCSY NMR spectrum of compound Ac-*m*-I-Phe-Arg-Gly-Asp-Trp-OH (1h).



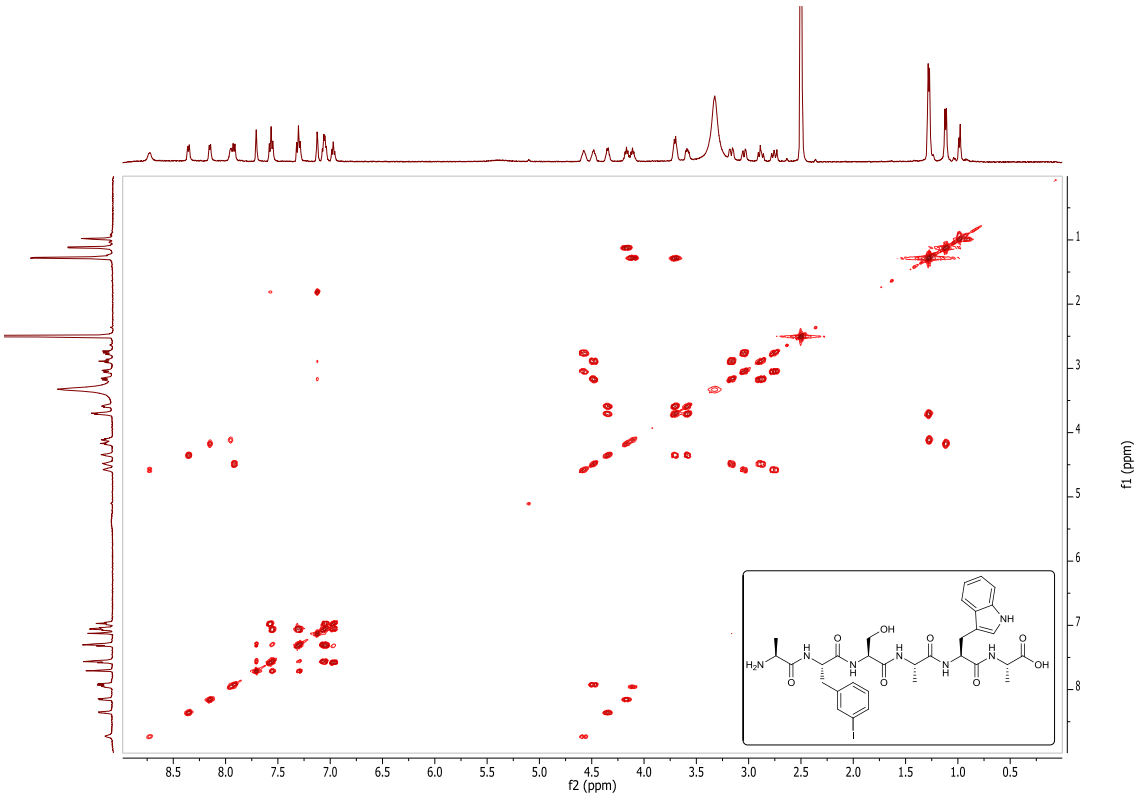
Supplementary Figure 36 | NOESY NMR spectrum of compound Ac-*m*-I-Phe-Arg-Gly-Asp-Trp-OH (1h).



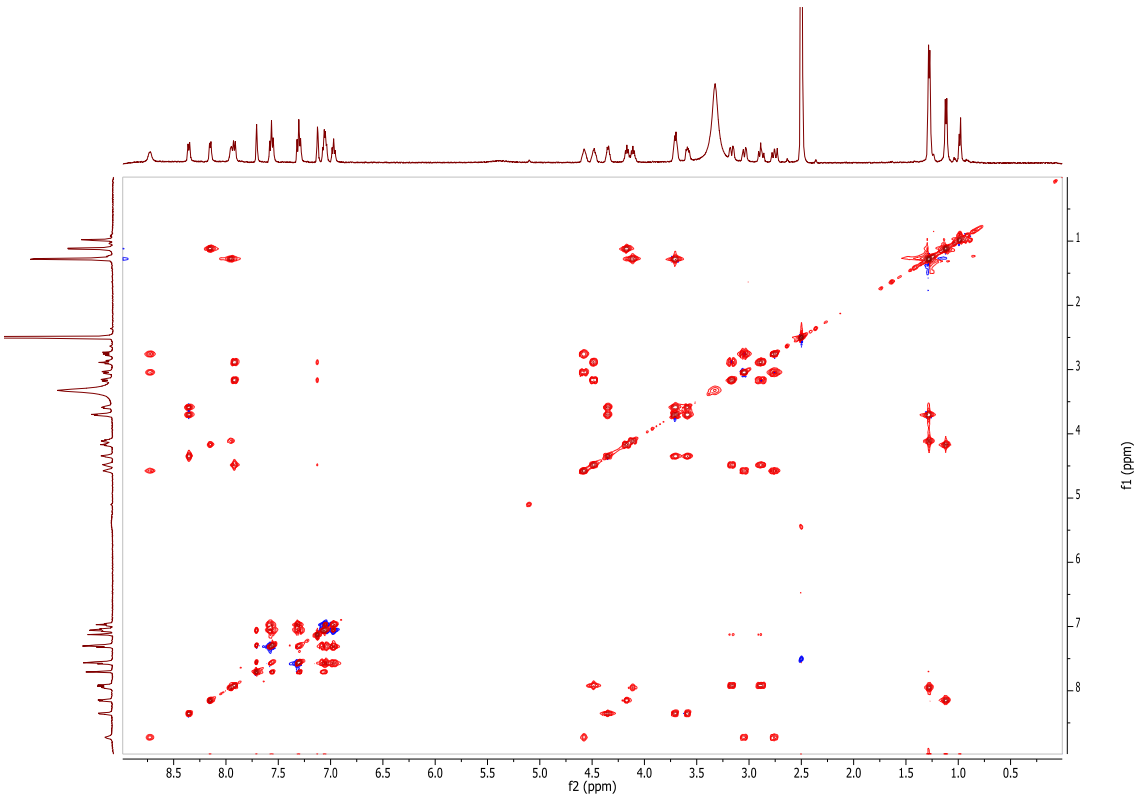
Supplementary Figure 37 | ^{1}H NMR spectrum of compound H-Ala-*m*-I-Phe-Ser-Ala-Trp-Ala-OH (1i).



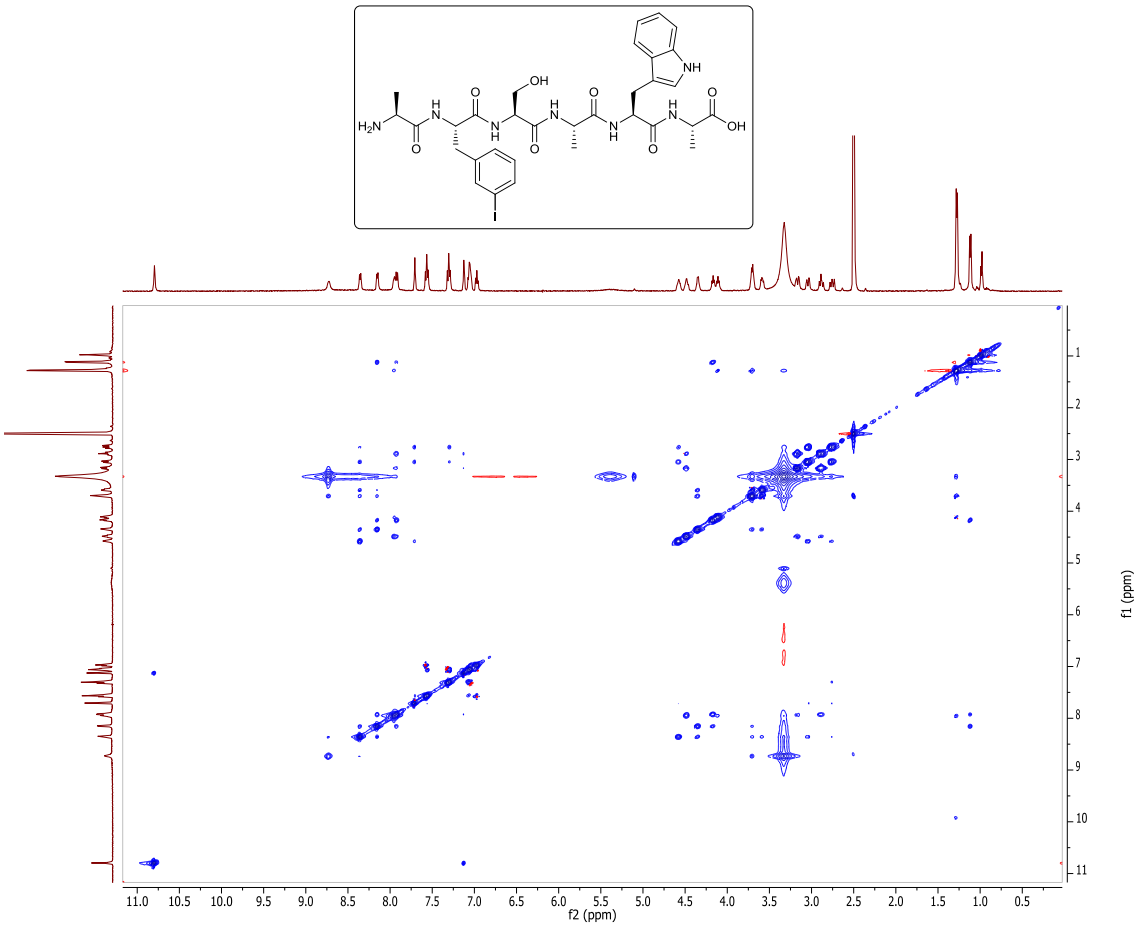
Supplementary Figure 38 | ^{1}H-^{13}C HSQC NMR spectrum of compound H-Ala-*m*-I-Phe-Ser-Ala-Trp-Ala-OH (1i).



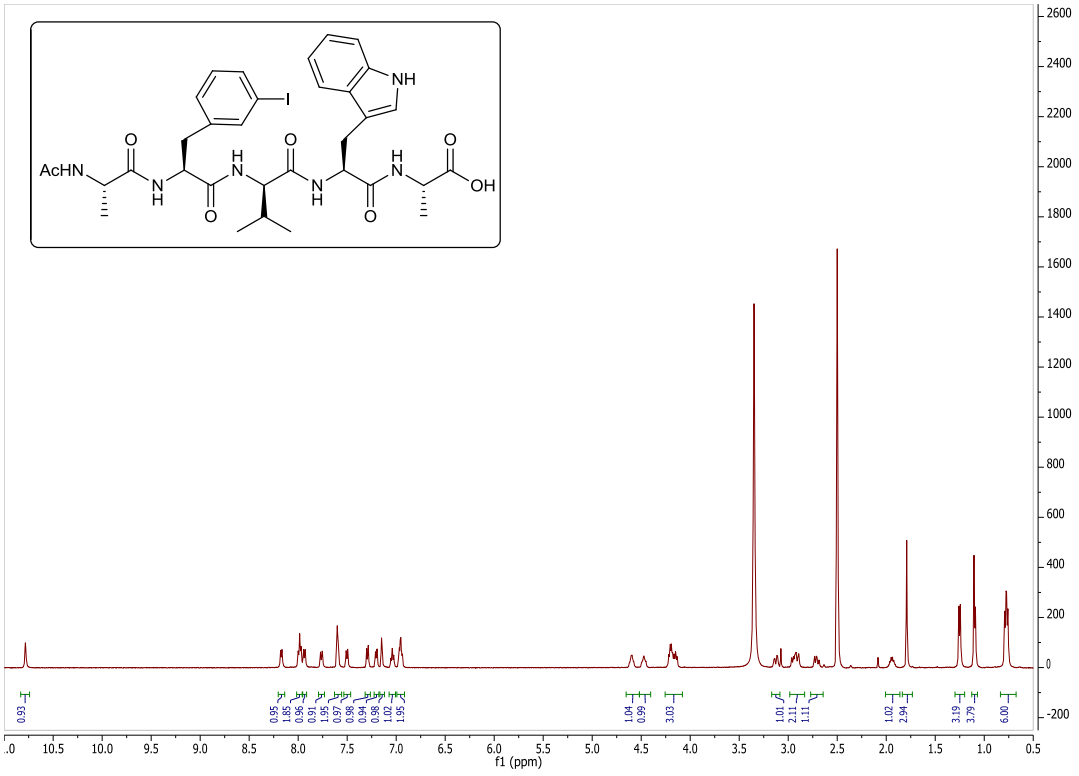
Supplementary Figure 39 | COSY NMR spectrum of compound H-Ala-*m*-I-Phe-Ser-Ala-Trp-Ala-OH (1i).



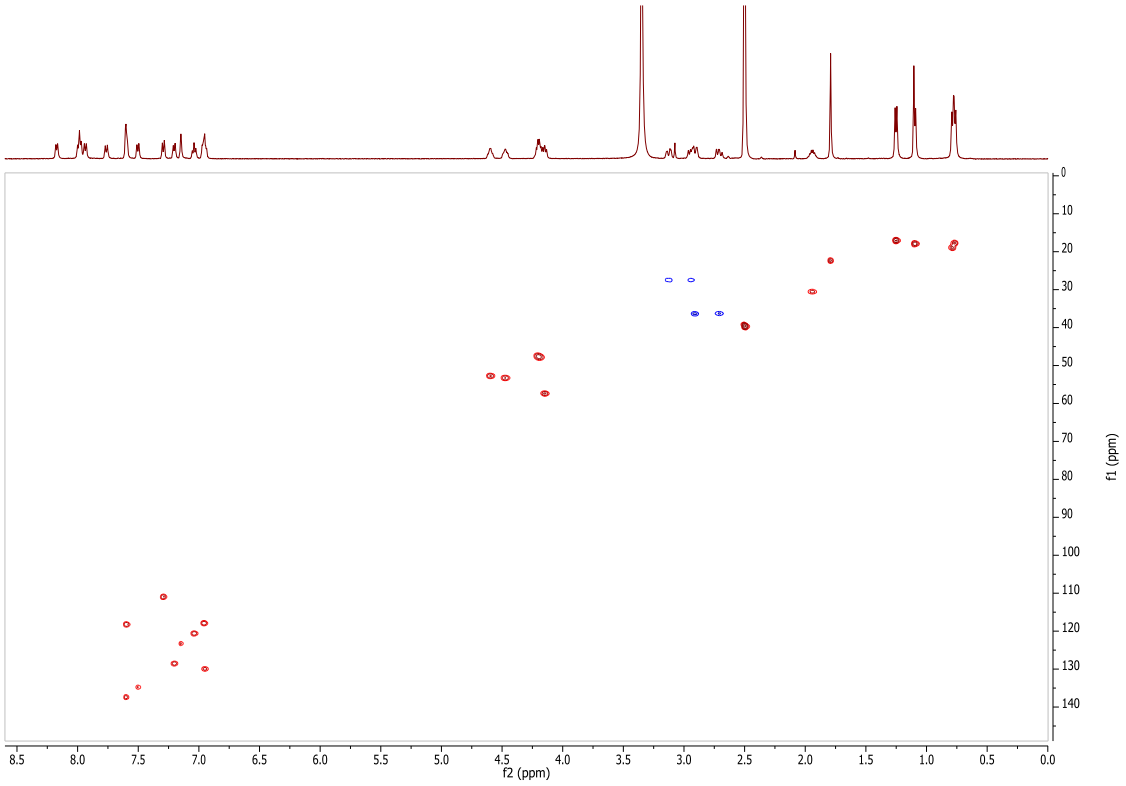
Supplementary Figure 40 | TOCSY NMR spectrum of compound H-Ala-*m*-I-Phe-Ser-Ala-Trp-Ala-OH (1i).



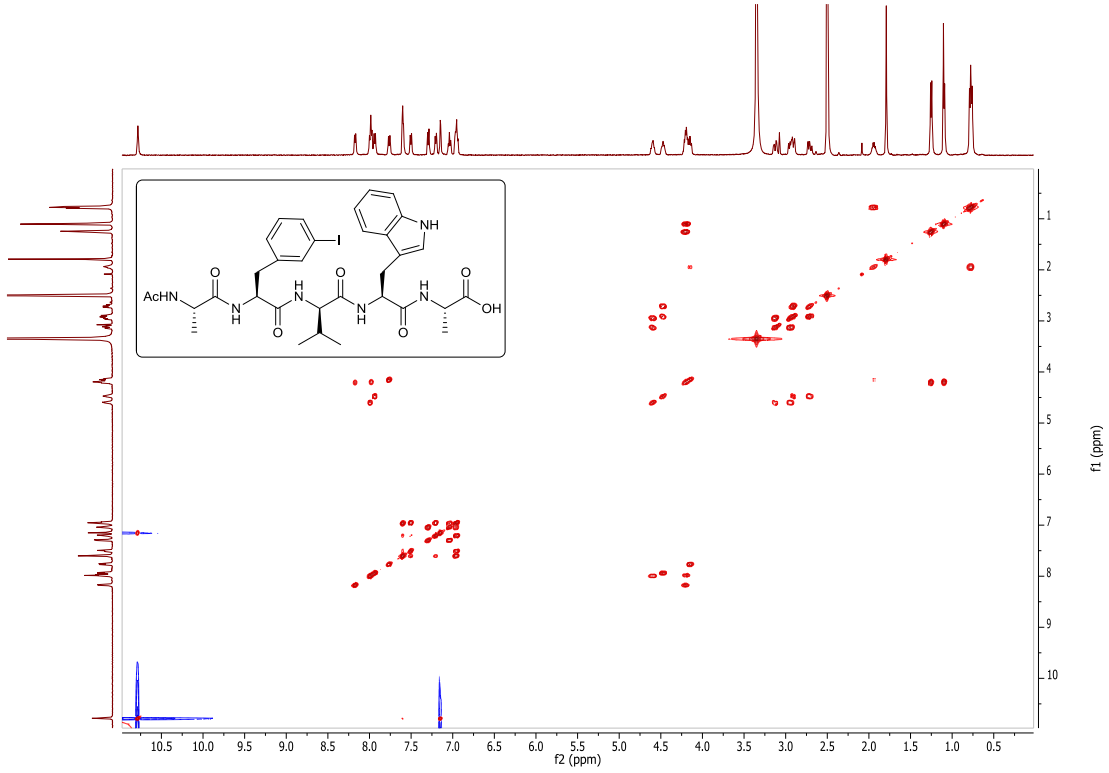
Supplementary Figure 41 | NOESY NMR spectrum of compound H-Ala-*m*-I-Phe-Ser-Ala-Trp-Ala-OH (1i).



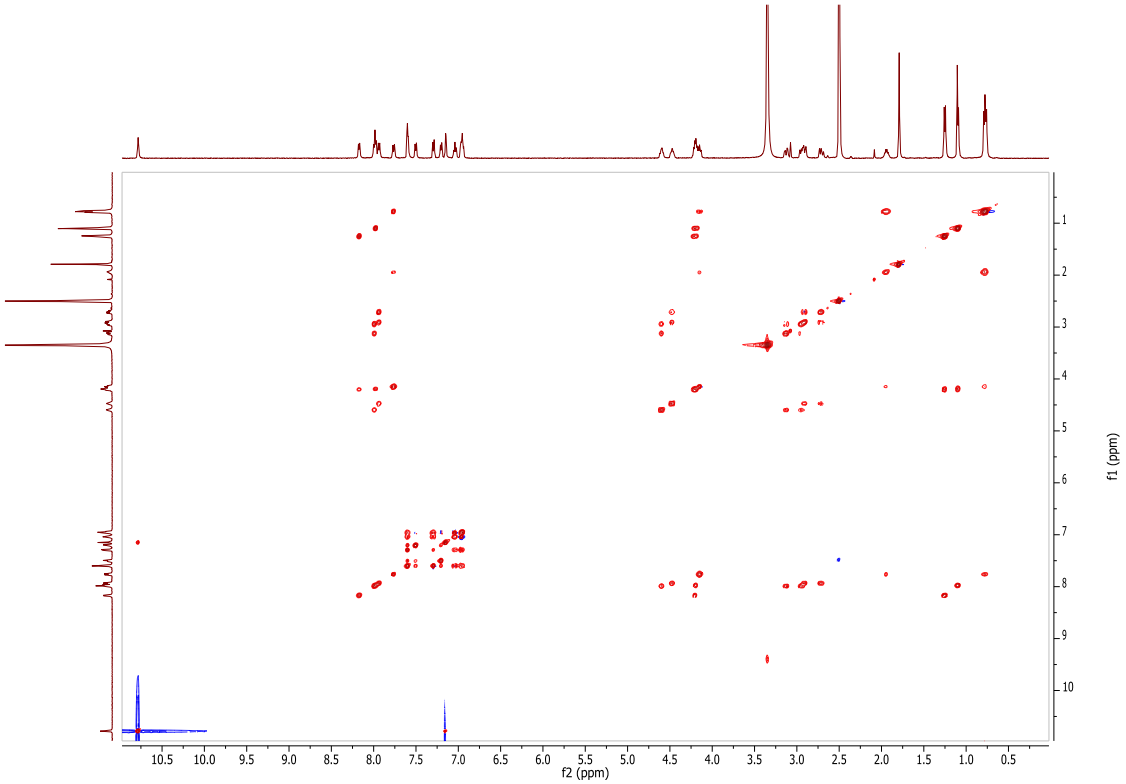
Supplementary Figure 42 | ^{1}H NMR spectrum of compound Ac-Ala-*m*-I-Phe-Val-Trp-Ala-OH (1j).



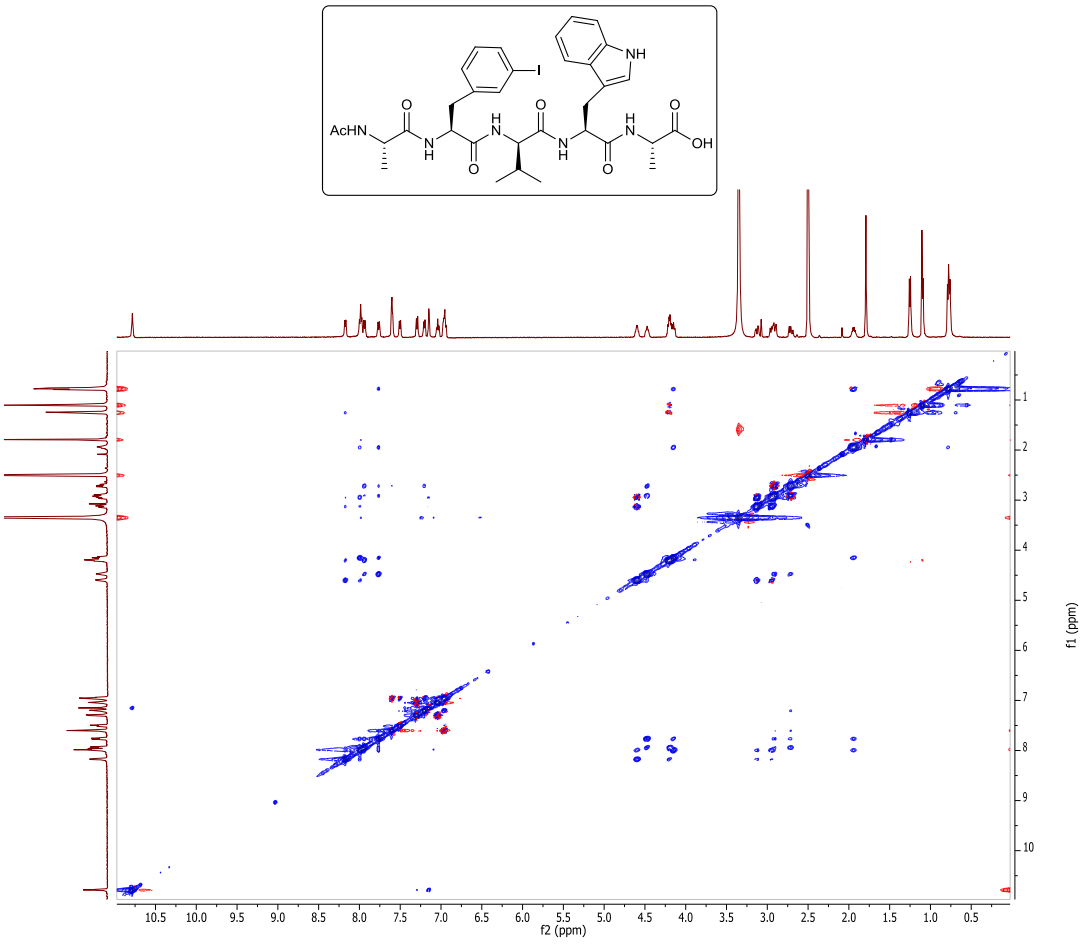
Supplementary Figure 43 | ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-Ala-*m*-I-Phe-Val-Trp-Ala-OH (1j).



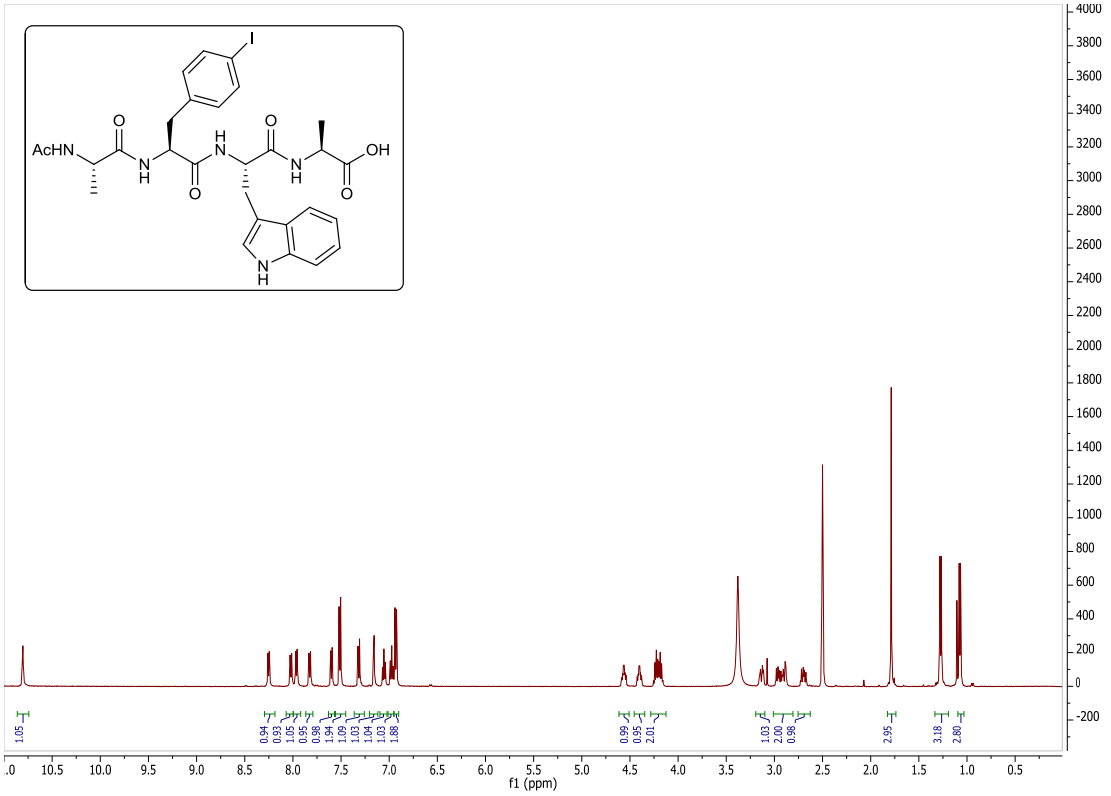
Supplementary Figure 44 | COSY NMR spectrum of compound Ac-Ala-*m*-I-Phe-Val-Trp-Ala-OH (1j).



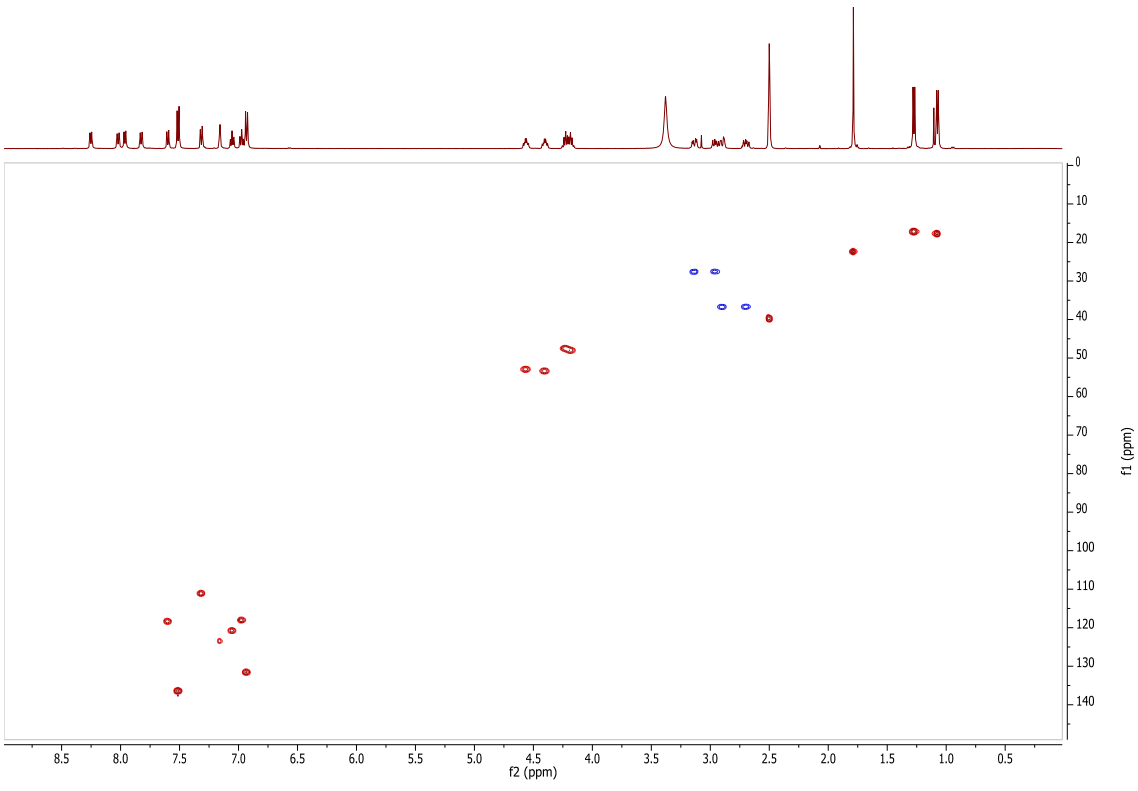
Supplementary Figure 45 | TOCSY NMR spectrum of compound Ac-Ala-*m*-I-Phe-Val-Trp-Ala-OH (1j).



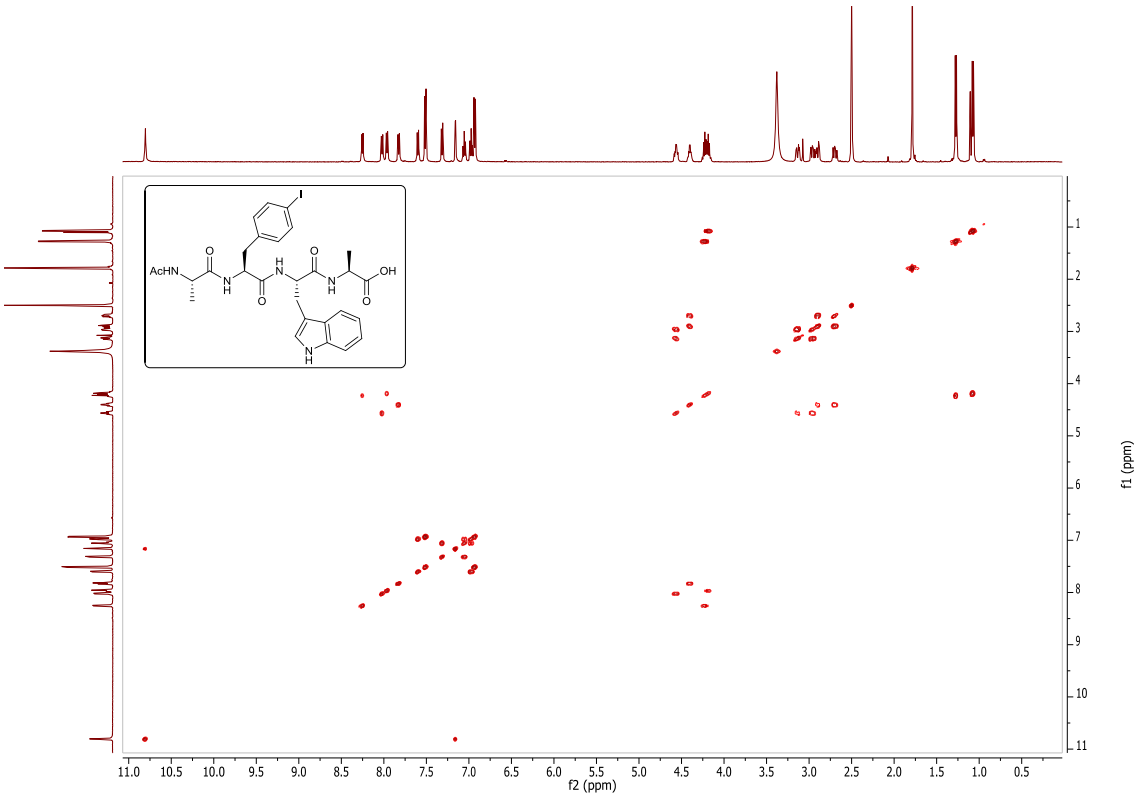
Supplementary Figure 46 | NOESY NMR spectrum of compound Ac-Ala-*m*-I-Phe-Val-Trp-Ala-OH (1j).



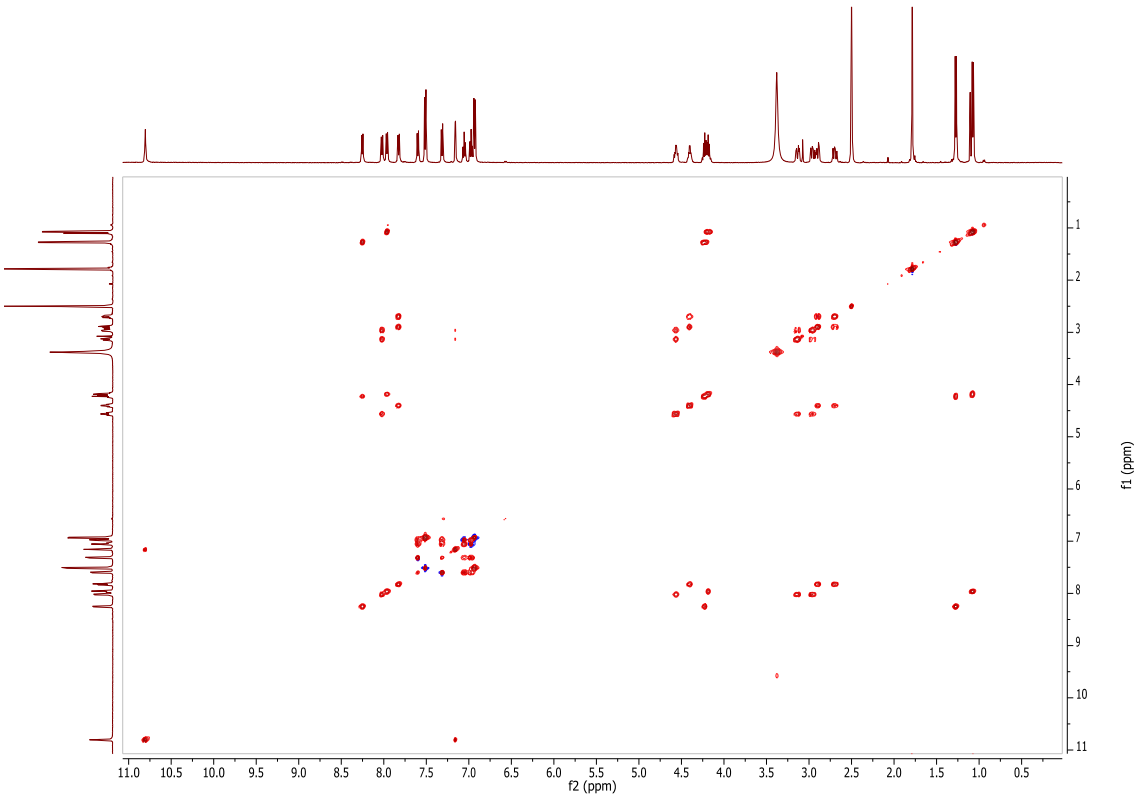
Supplementary Figure 47 ‖ ^{1}H NMR spectrum of compound Ac-Ala-*p*-I-Phe-Trp-Ala-OH (1k).



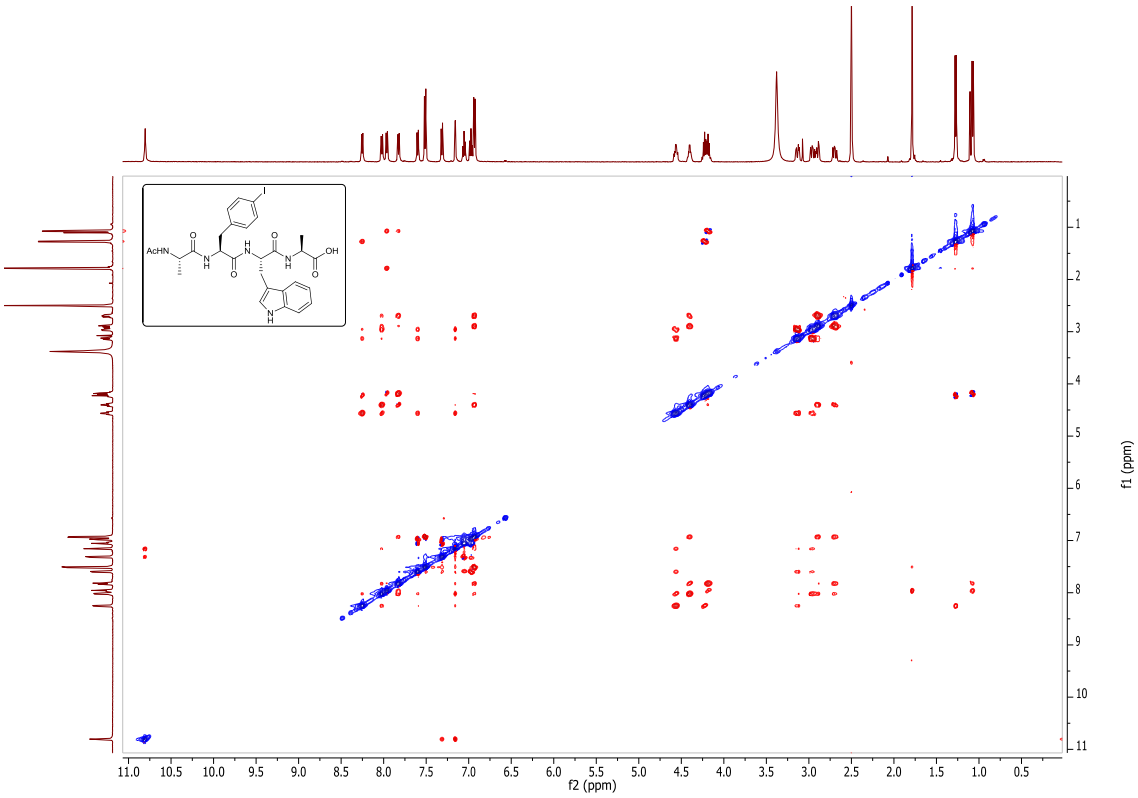
Supplementary Figure 48 ‖ ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-Ala-*p*-I-Phe-Trp-Ala-OH (1k).



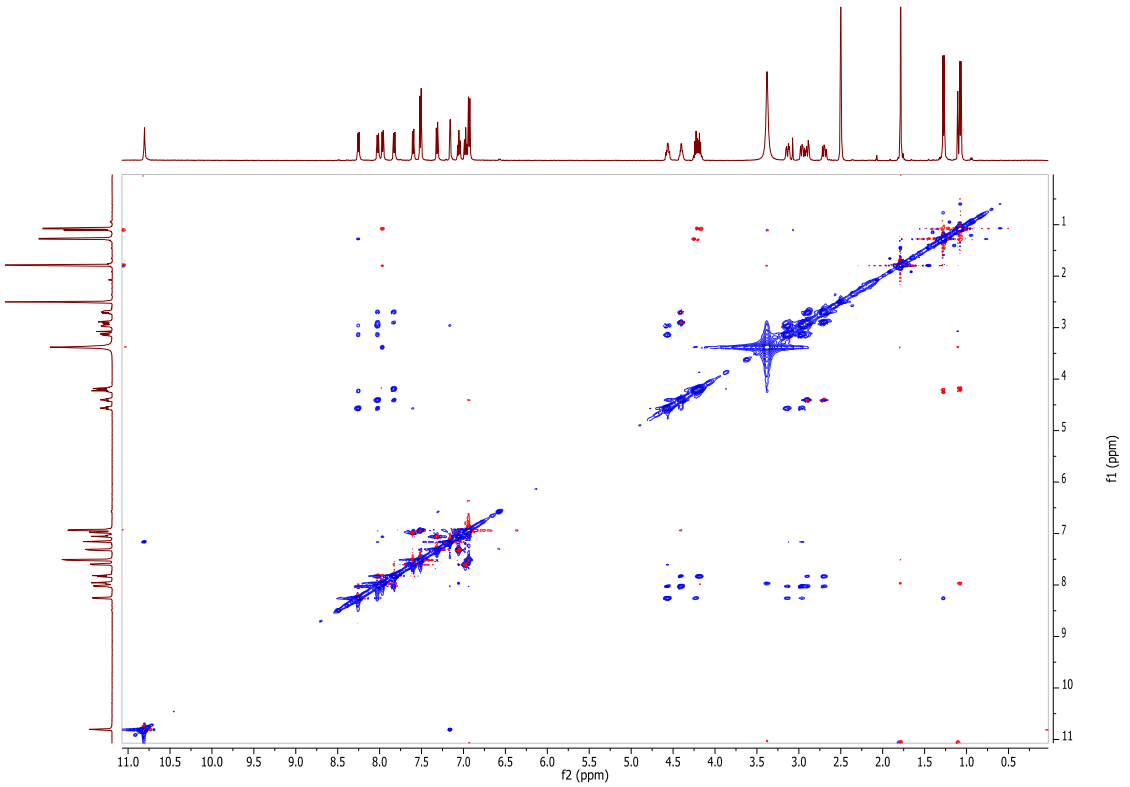
Supplementary Figure 49 | COSY NMR spectrum of compound Ac-Ala-*p*-I-Phe-Trp-Ala-OH (1k).



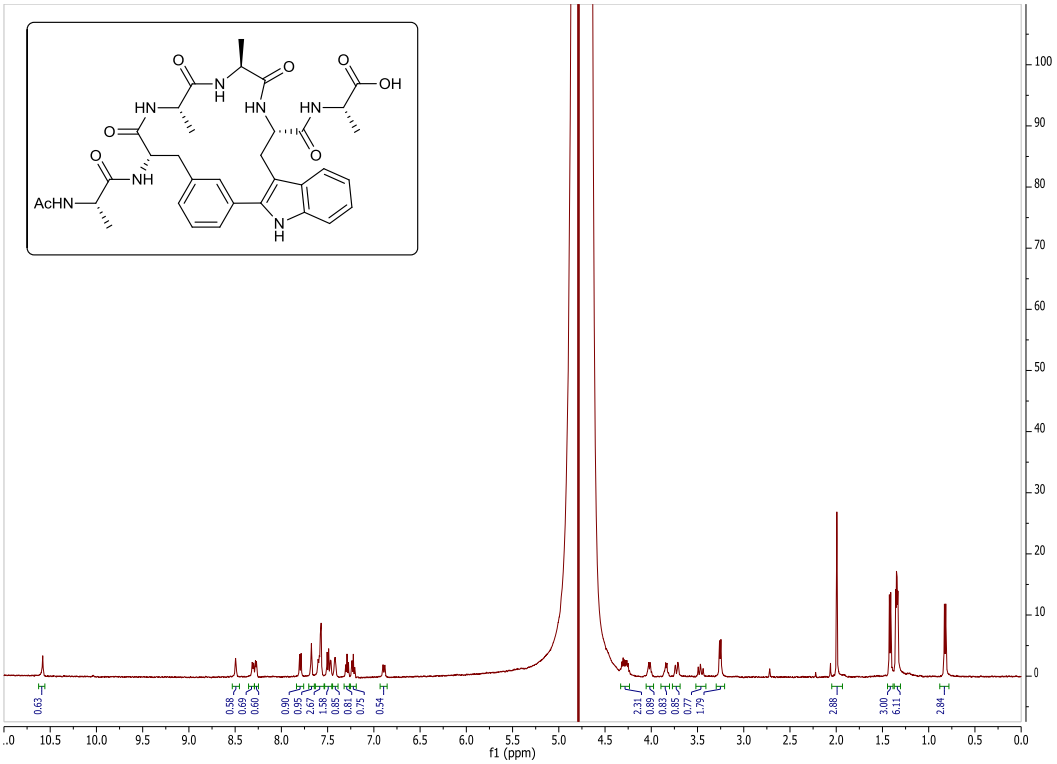
Supplementary Figure 50 | TOCSY NMR spectrum of compound Ac-Ala-*p*-I-Phe-Trp-Ala-OH (1k).



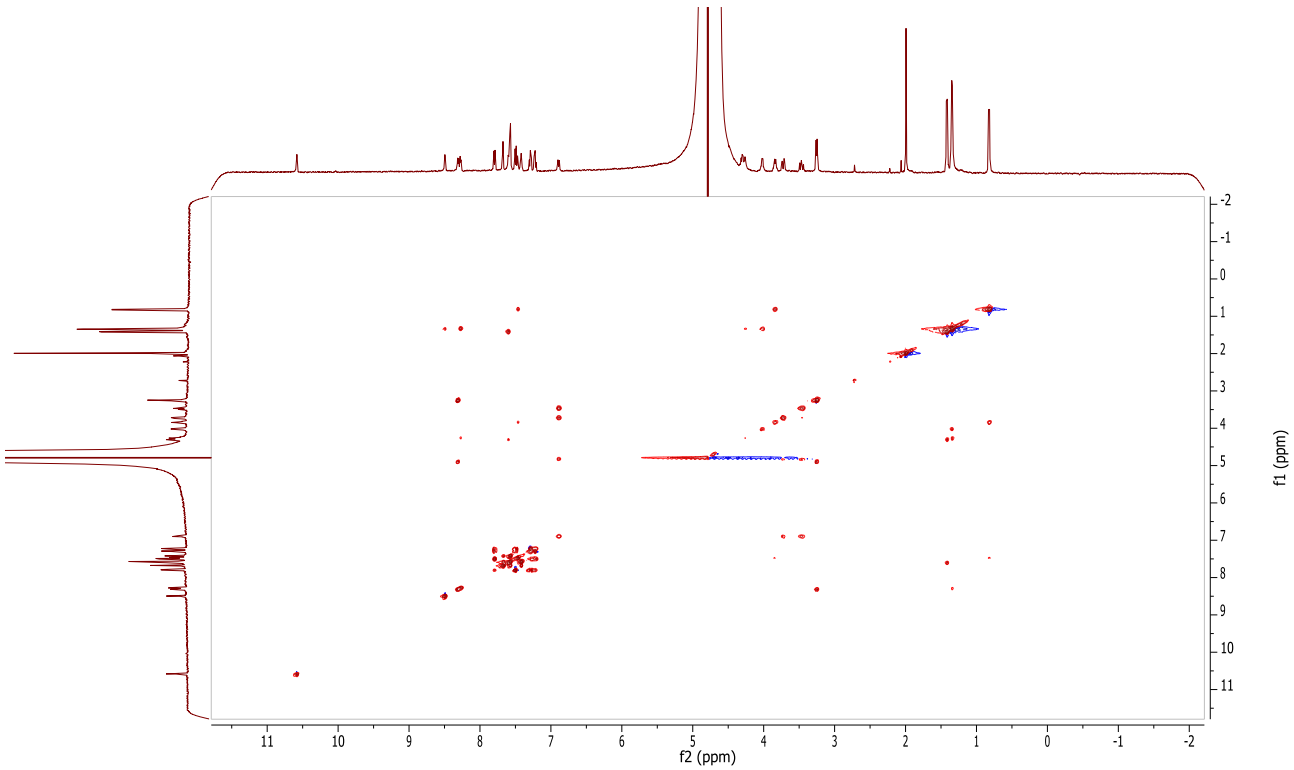
Supplementary Figure 51 | ROESY NMR spectrum of compound Ac-Ala-*p*-I-Phe-Trp-Ala-OH (1k).



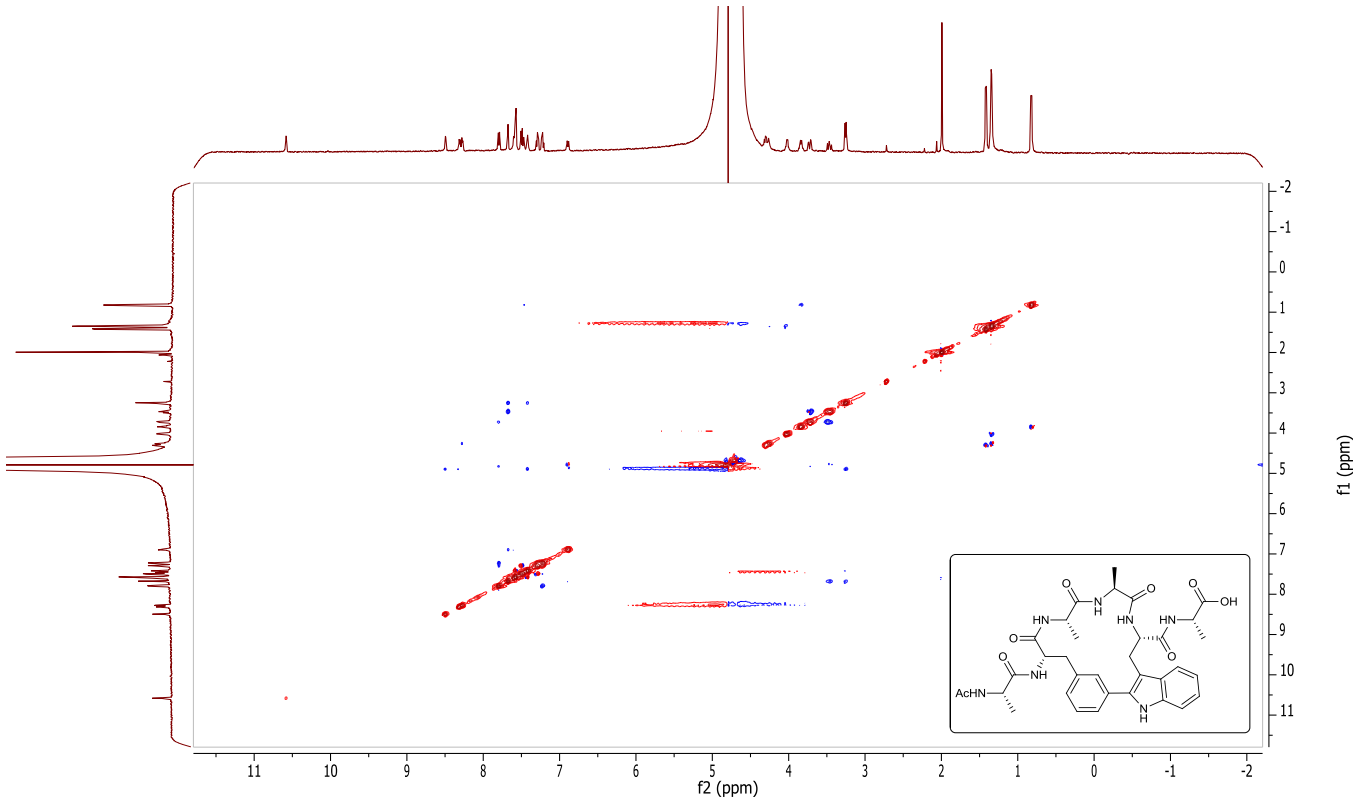
Supplementary Figure 52 | NOESY NMR spectrum of compound Ac-Ala-*p*-I-Phe-Trp-Ala-OH (1k).



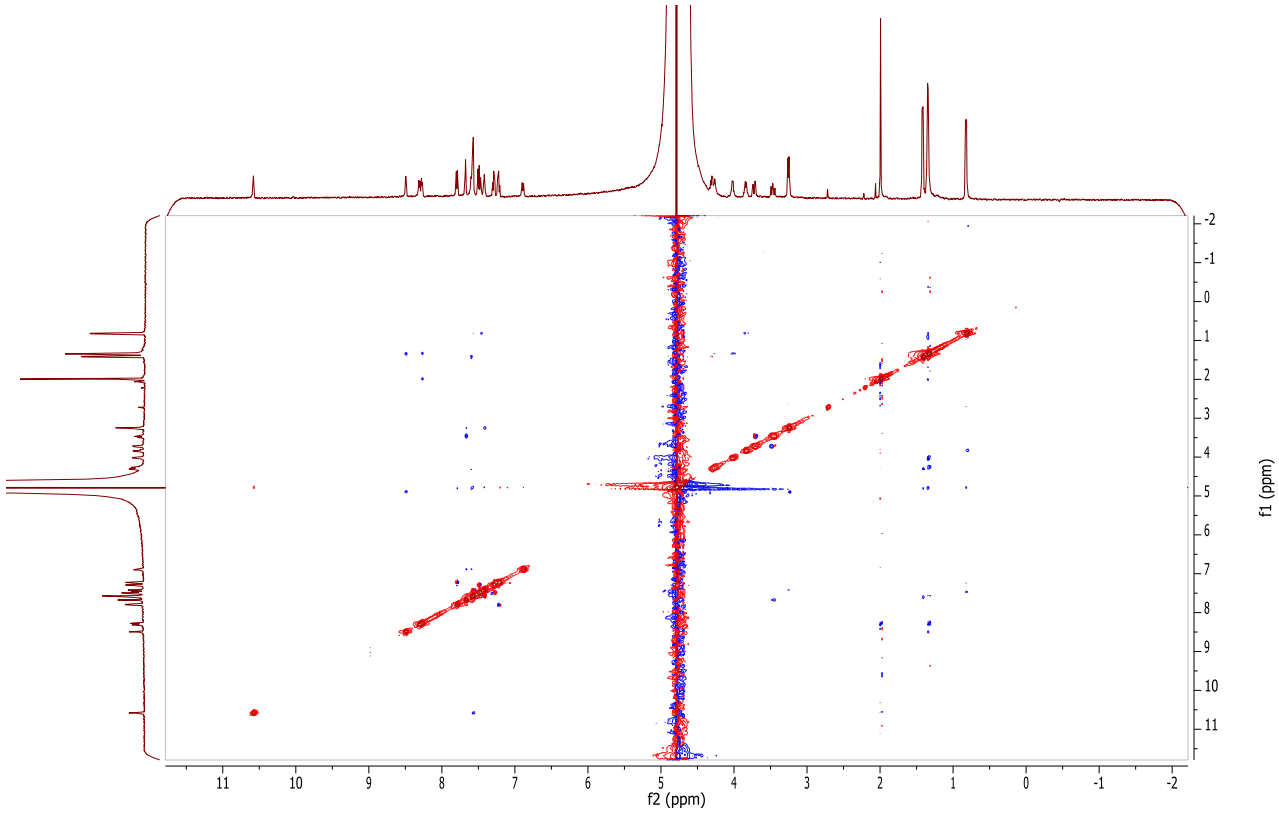
Supplementary Figure 53 | ^{1}H NMR spectrum of compound Ac-Ala-(Cyclo-*m*)-[Phe-Ala-Ala-Trp]-Ala-OH (2b).



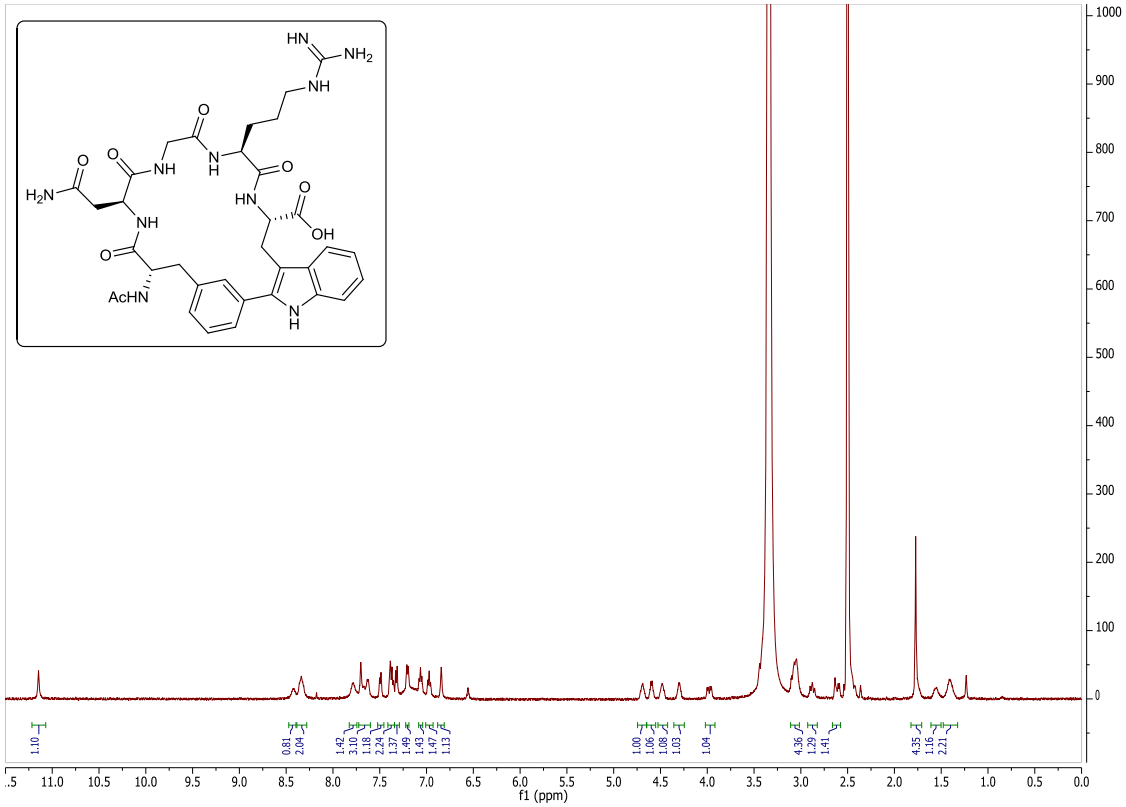
Supplementary Figure 54 | TOCSY NMR spectrum of compound Ac-Ala-(Cyclo-*m*)-[Phe-Ala-Ala-Trp]-Ala-OH (2b).



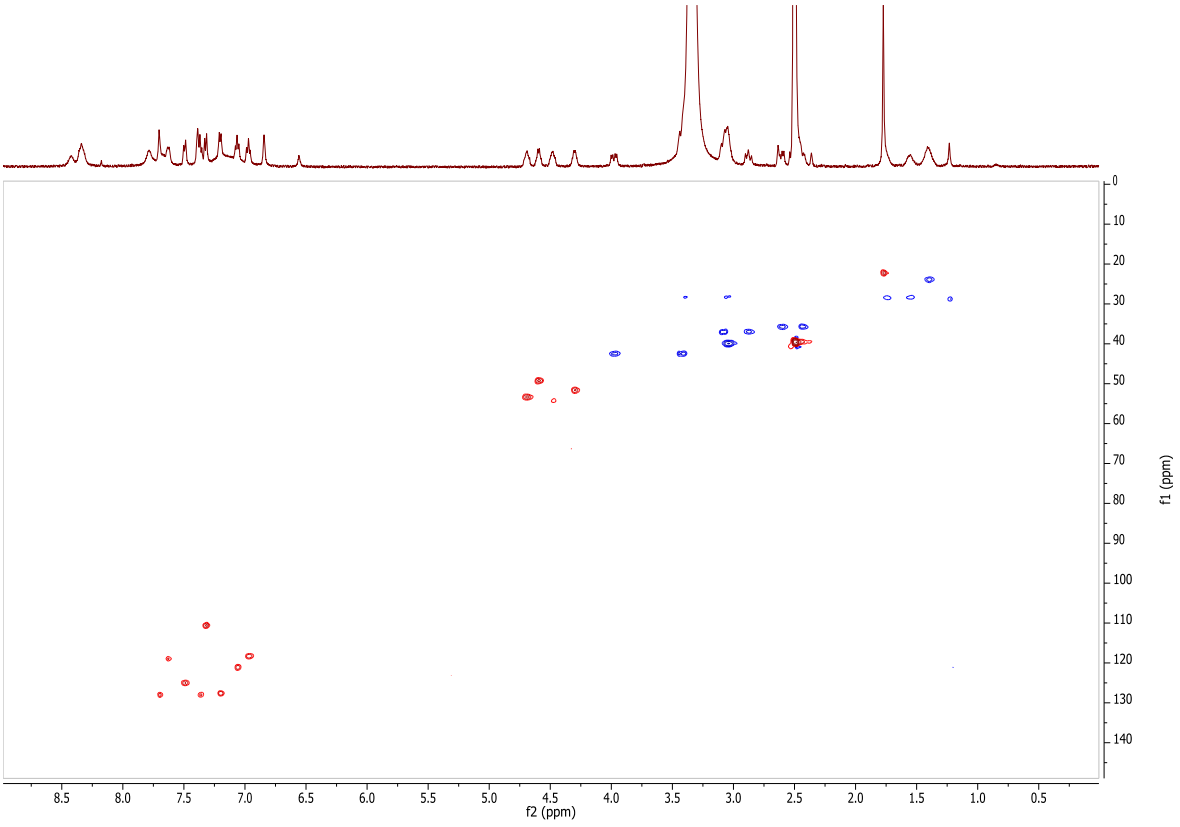
Supplementary Figure 55 | ROESY NMR spectrum of compound Ac-Ala-(Cyclo-*m*)-[Phe-Ala-Ala-Trp]-Ala-OH (2b).



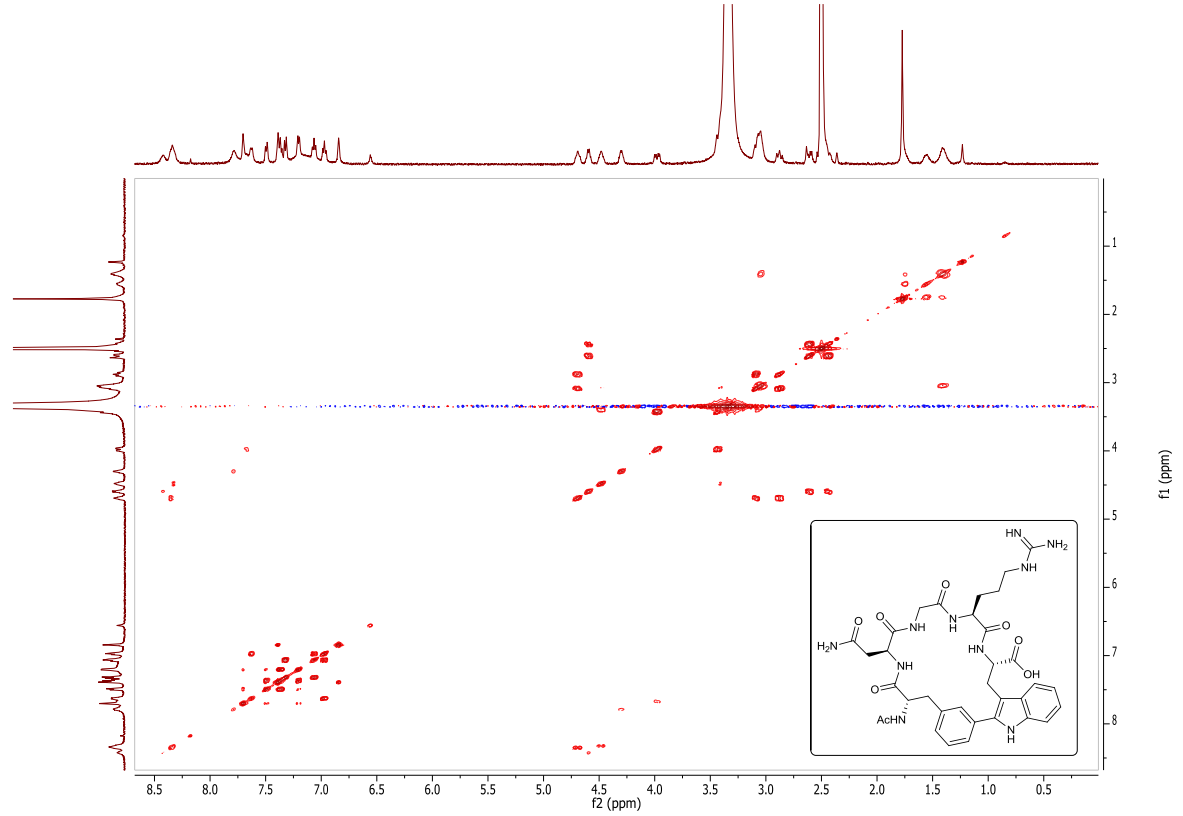
Supplementary Figure 56 | NOESY NMR spectrum of compound Ac-Ala-(Cyclo-*m*)-[Phe-Ala-Ala-Trp]-Ala-OH (2b).



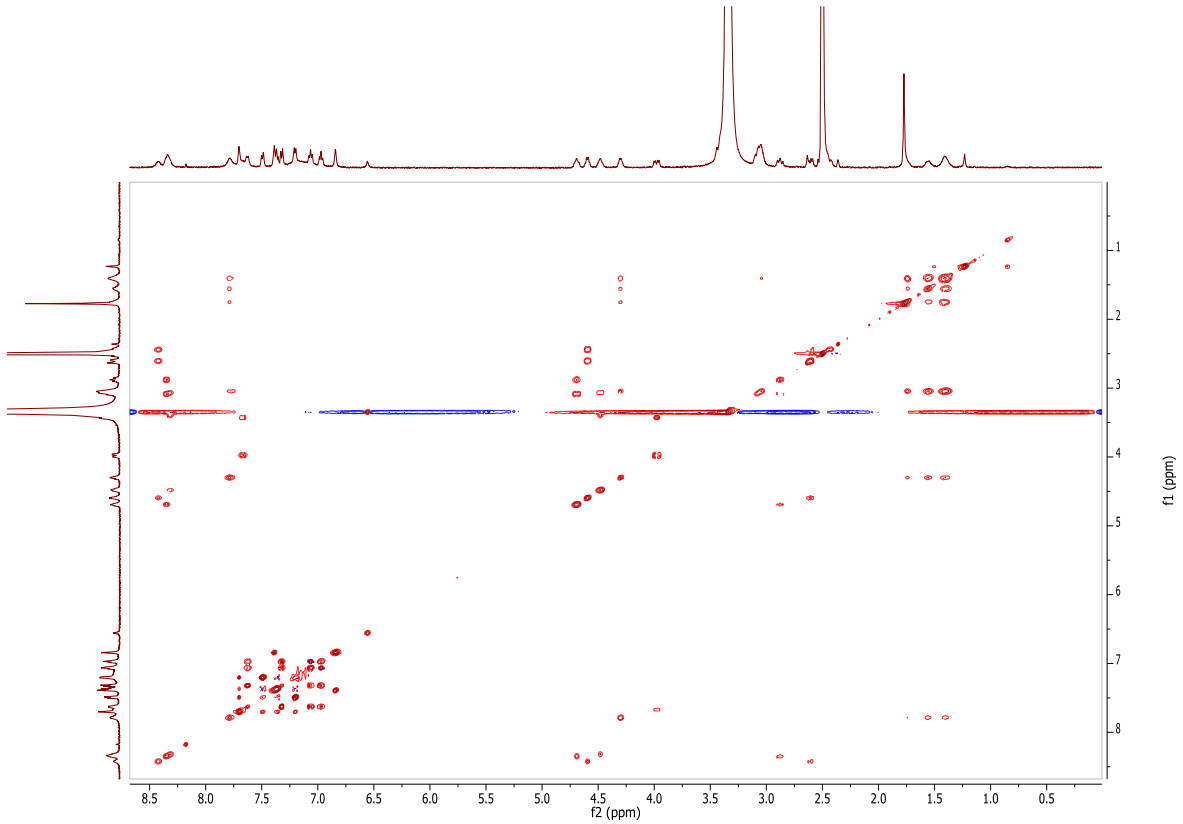
Supplementary Figure 57 ‖ ^{1}H NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Asn-Gly-Arg-Trp]-OH (2g).



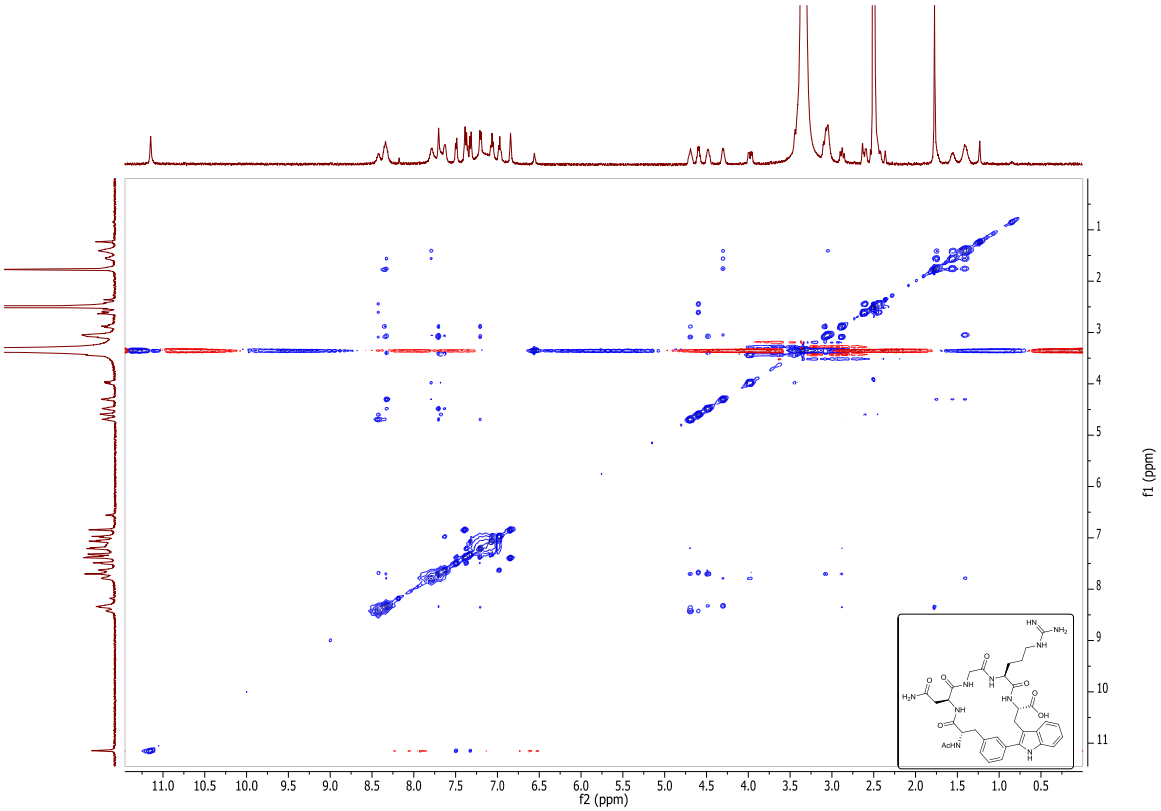
Supplementary Figure 58 ‖ ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Asn-Gly-Arg-Trp]-OH (2g).



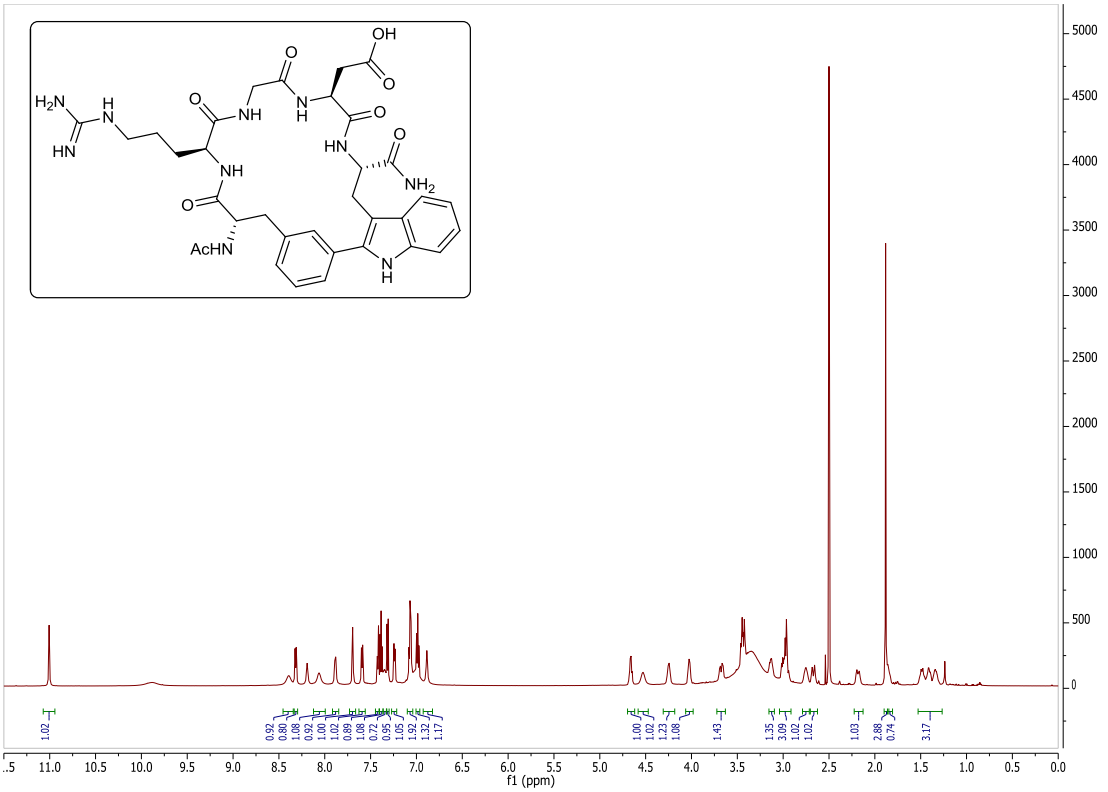
Supplementary Figure 59 | COSY NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Asn-Gly-Arg-Trp]-OH (2g).



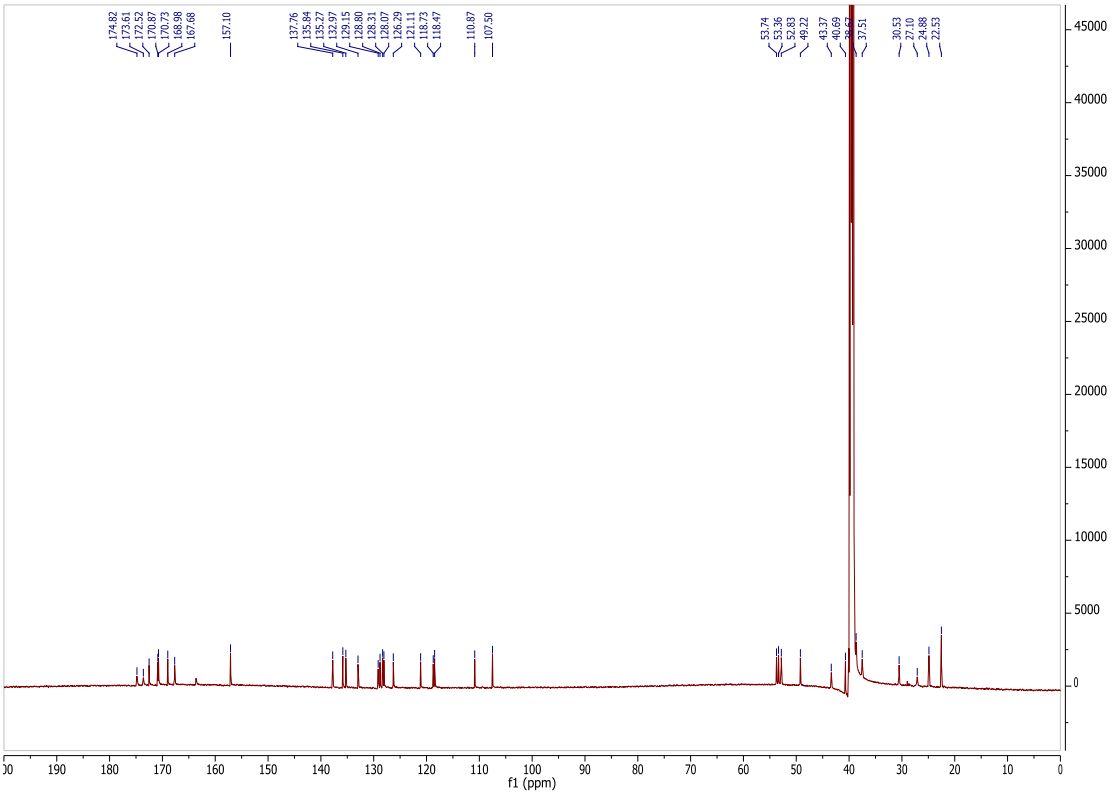
Supplementary Figure 60 | TOCSY NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Asn-Gly-Arg-Trp]-OH (2g).



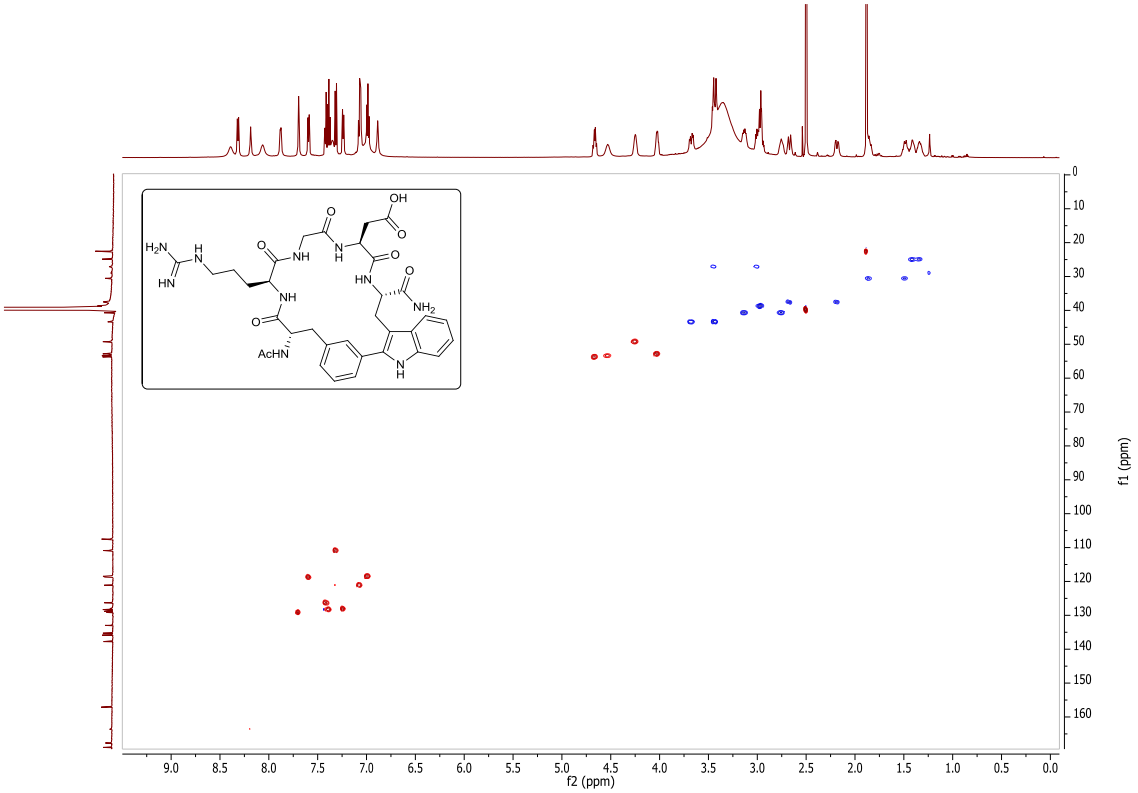
Supplementary Figure 61 | NOESY NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Asn-Gly-Arg-Trp]-OH (2g).



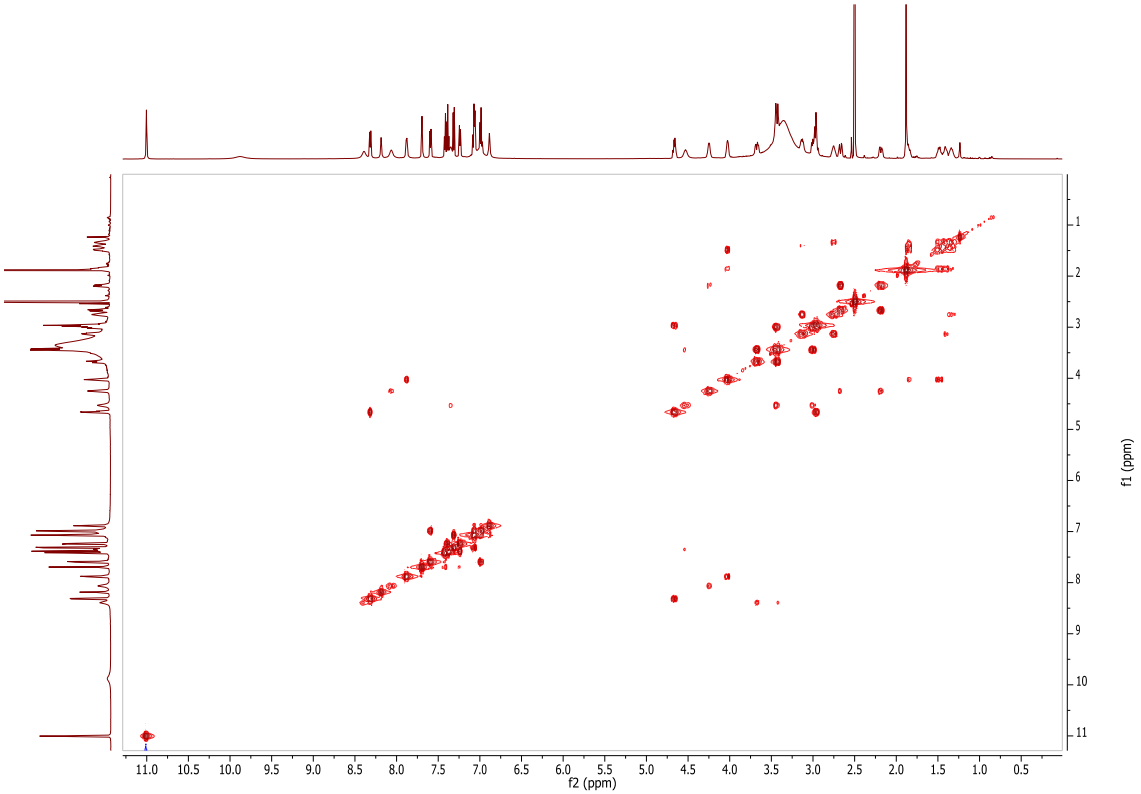
Supplementary Figure 62 | ^{1}H NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Arg-Gly-Asp-Trp]-NH_2 (2h).



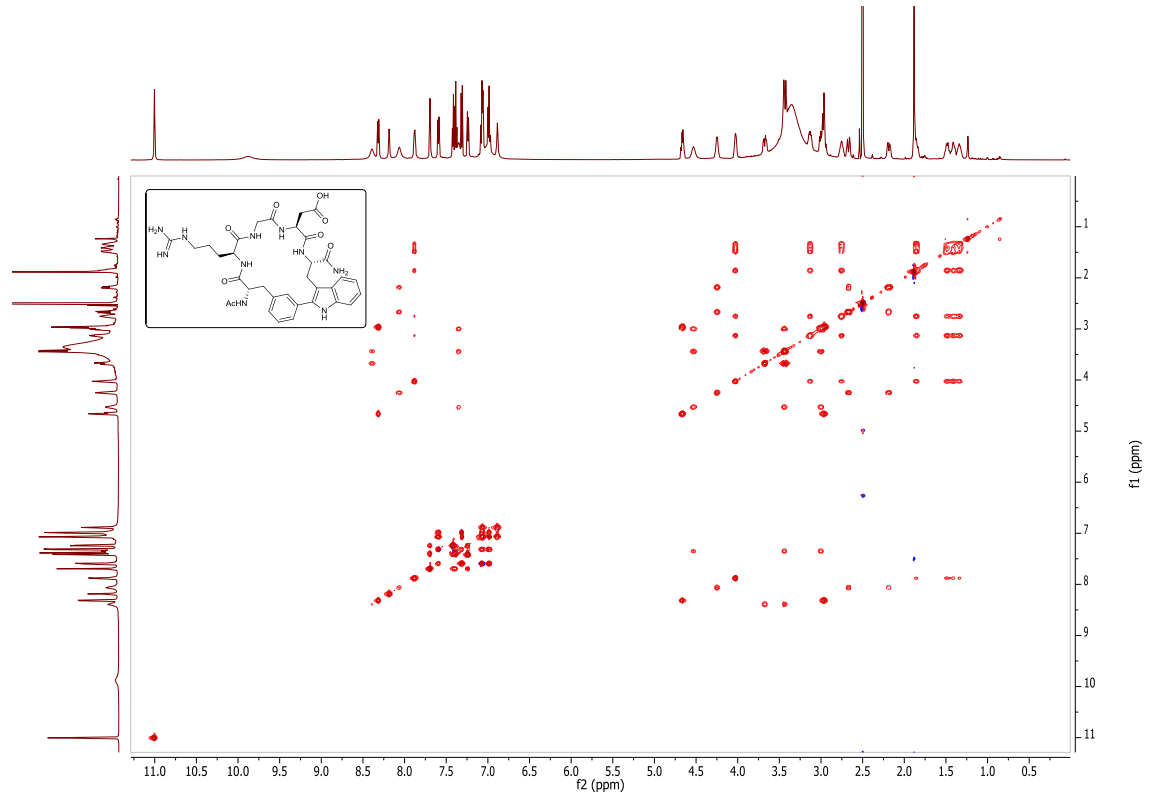
Supplementary Figure 63 | ^{13}C NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Arg-Gly-Asp-Trp]-NH_2 (2h).



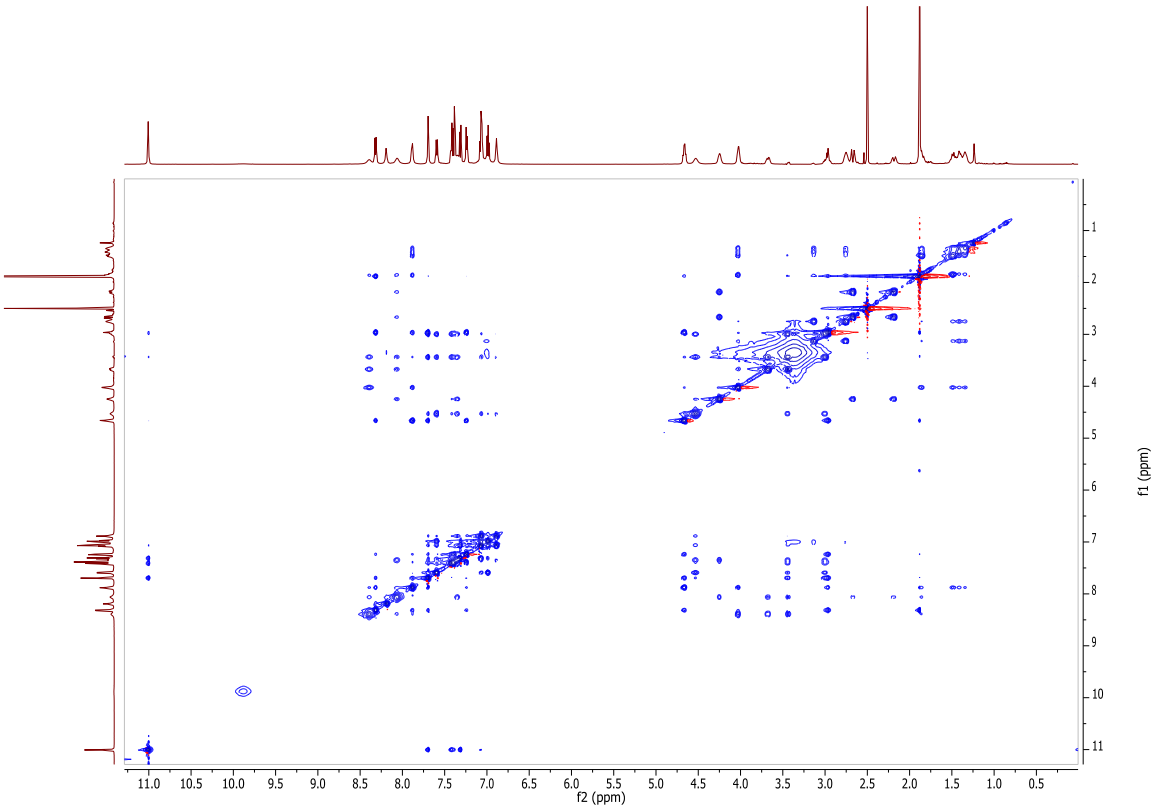
Supplementary Figure 64 | ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Arg-Gly-Asp-Trp]-NH_2 (2h).



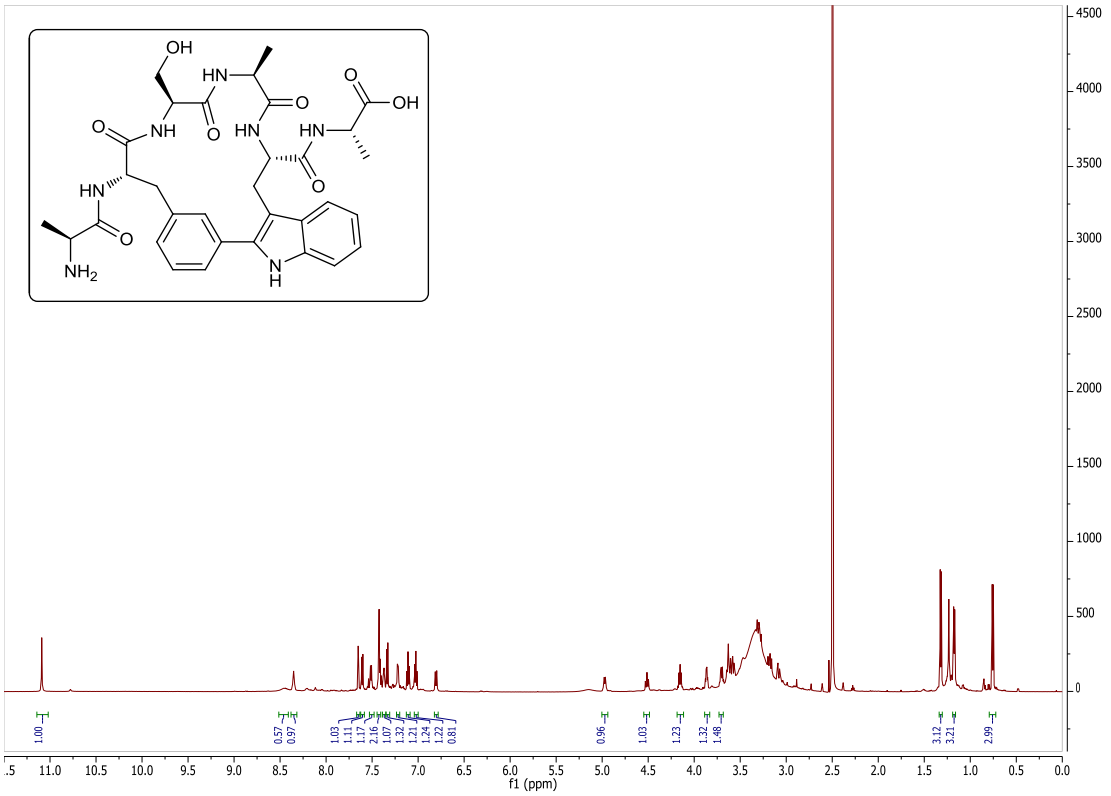
Supplementary Figure 65 | COSY NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Arg-Gly-Asp-Trp]-NH_2 (2h).



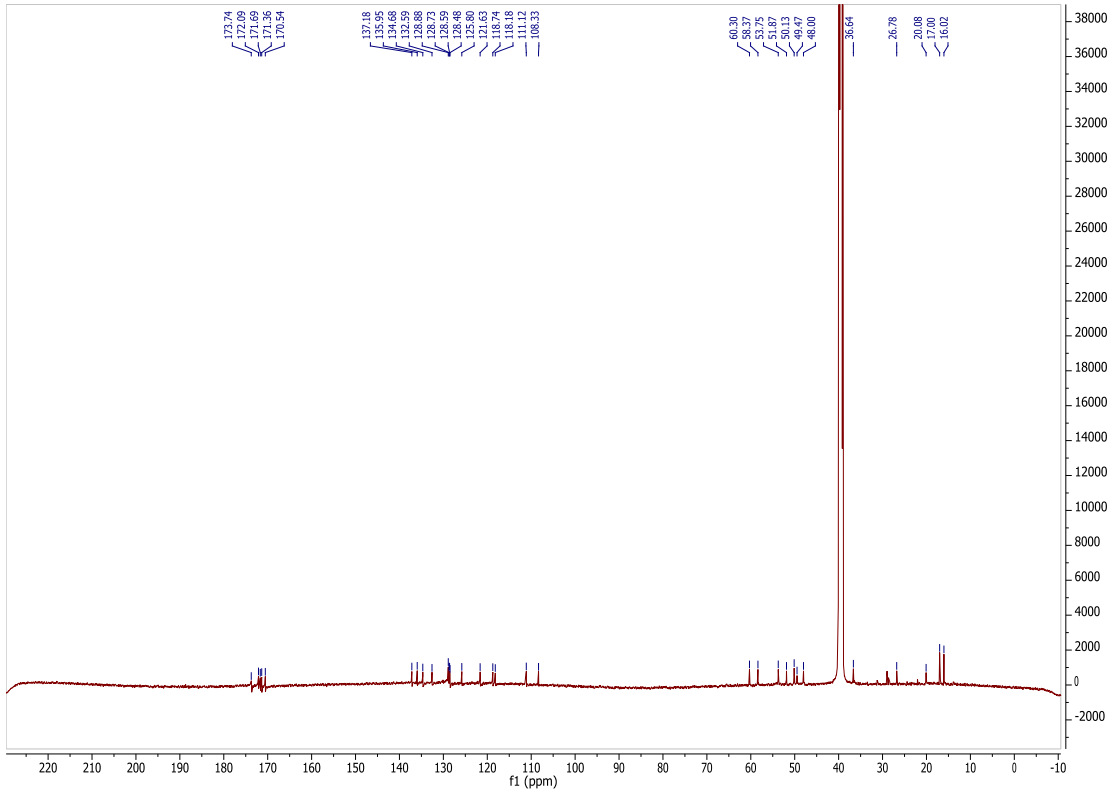
Supplementary Figure 66 | TOCSY NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Arg-Gly-Asp-Trp]-NH_2 (2h).



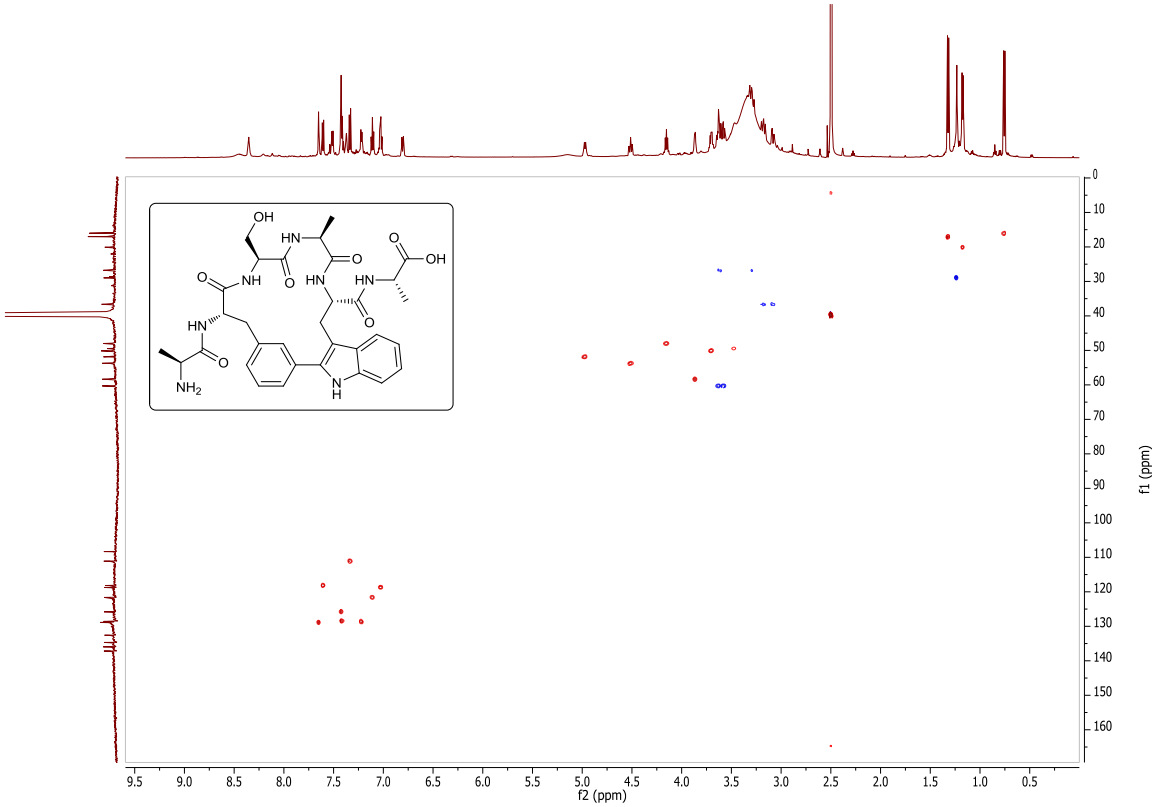
Supplementary Figure 67 | NOESY NMR spectrum of compound Ac-(Cyclo-*m*)-[Phe-Arg-Gly-Asp-Trp]-NH_2 (2h).



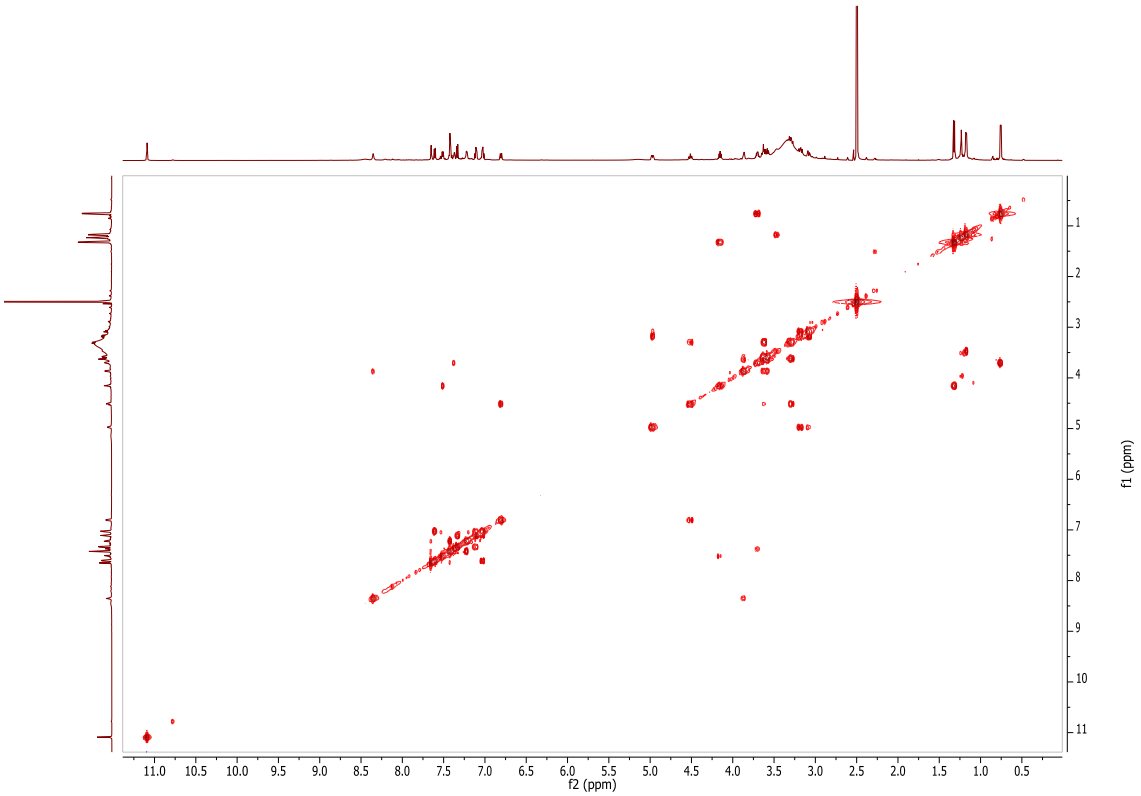
Supplementary Figure 68 ‖ ^{1}H NMR spectrum of compound H-Ala-(Cyclo-*m)*-[Phe-Ser-Ala-Trp]-Ala-OH (2i).



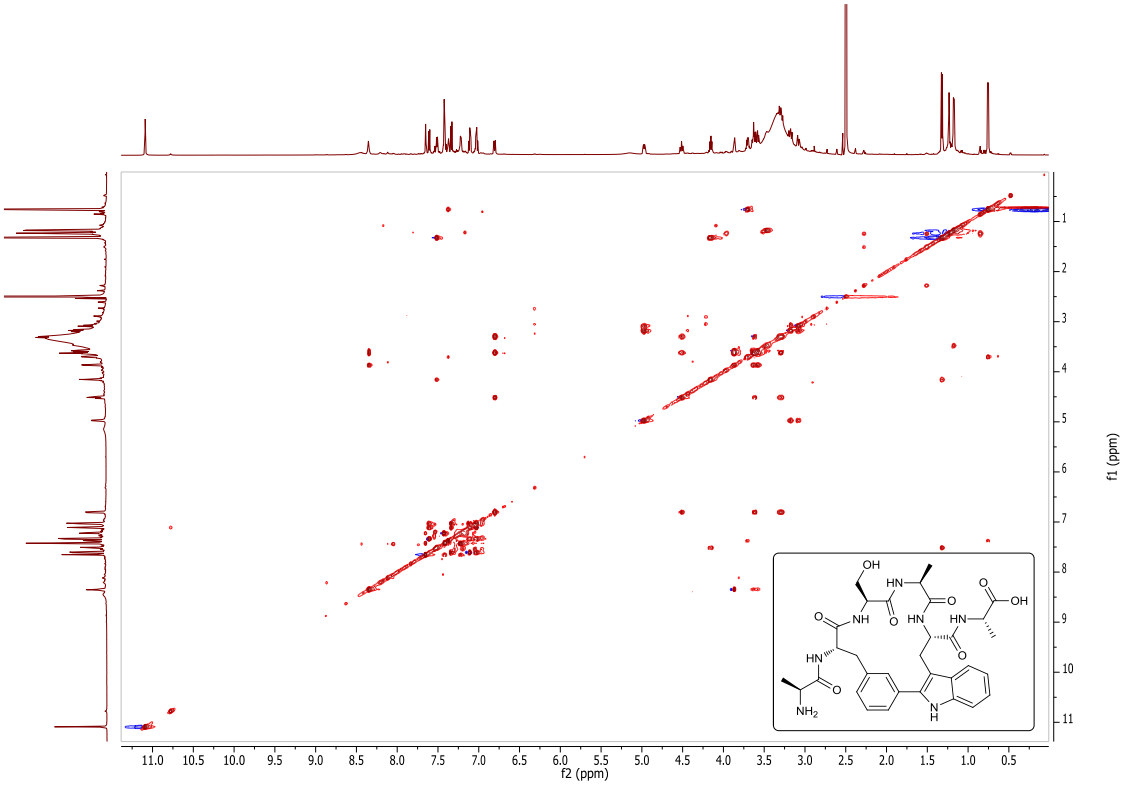
Supplementary Figure 69 ‖ ^{13}C NMR spectrum of compound H-Ala-(Cyclo-*m)*-[Phe-Ser-Ala-Trp]-Ala-OH (2i).



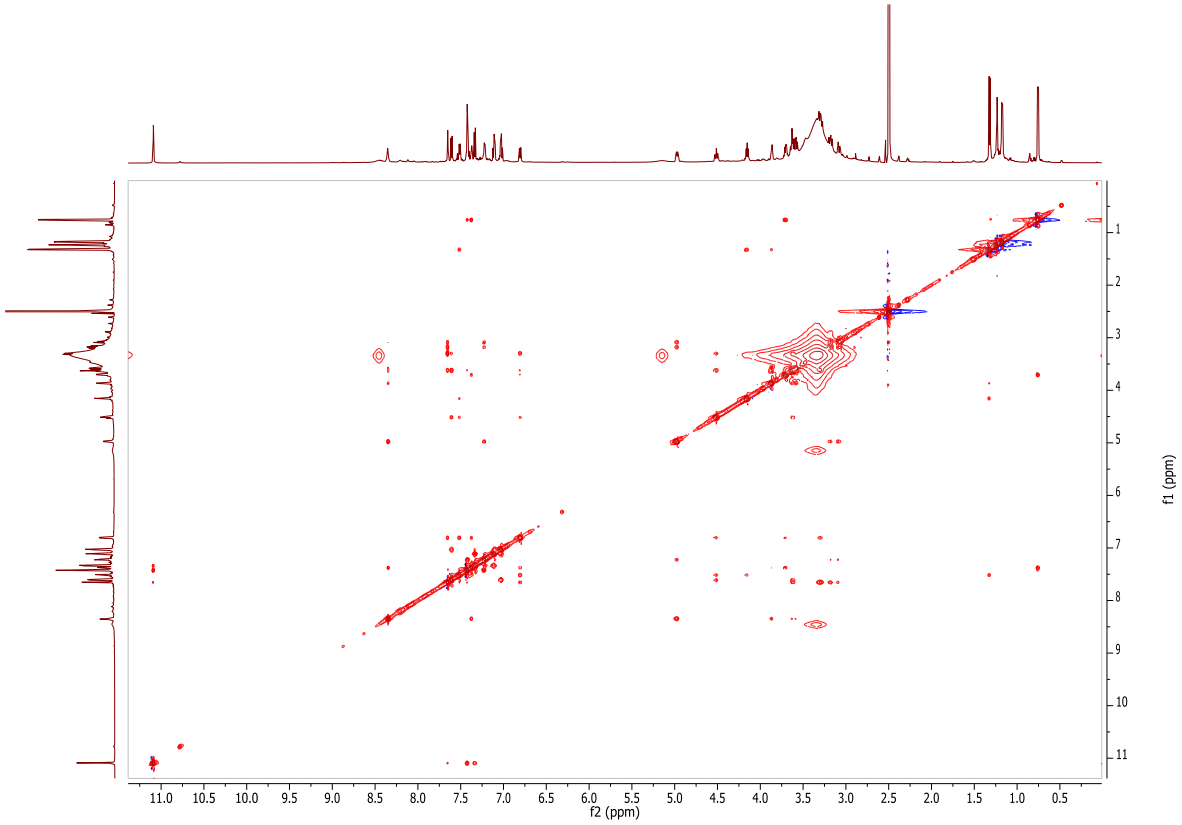
Supplementary Figure 70 | ^{1}H-^{13}C HSQC NMR spectrum of compound H-Ala-(Cyclo-*m)*-[Phe-Ser-Ala-Trp]-Ala-OH (2i).



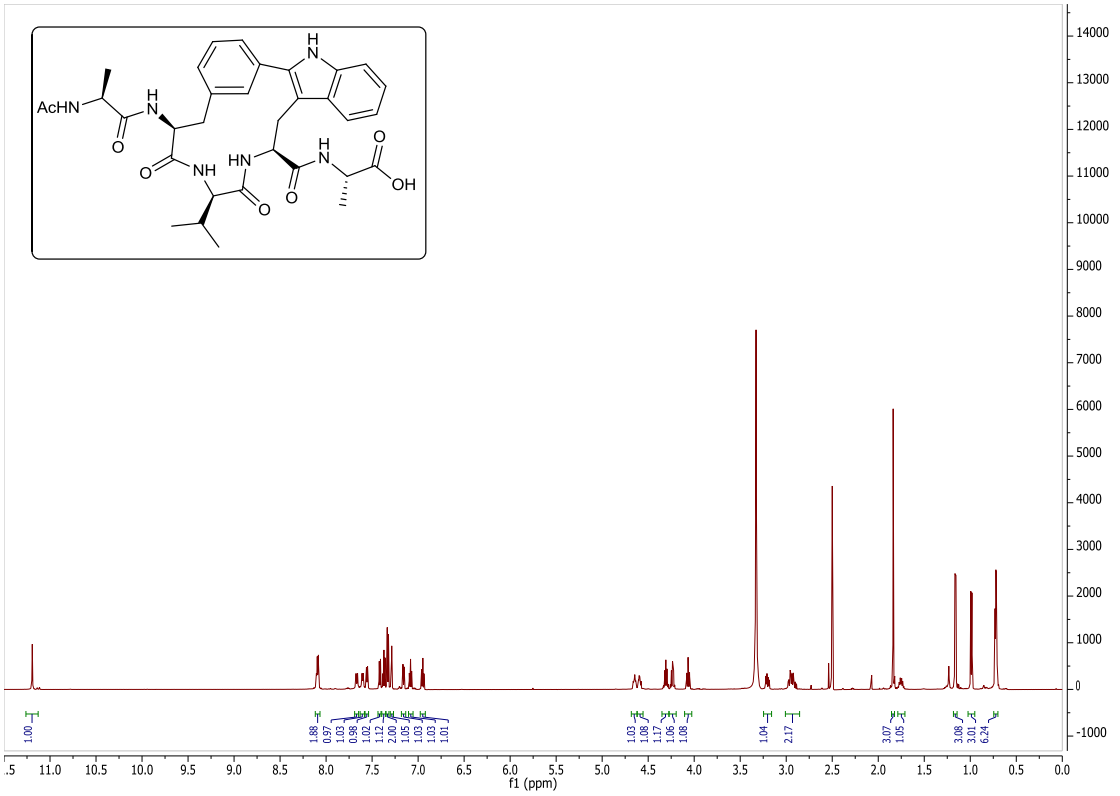
Supplementary Figure 71 | COSY NMR spectrum of compound H-Ala-(Cyclo-*m)*-[Phe-Ser-Ala-Trp]-Ala-OH (2i).



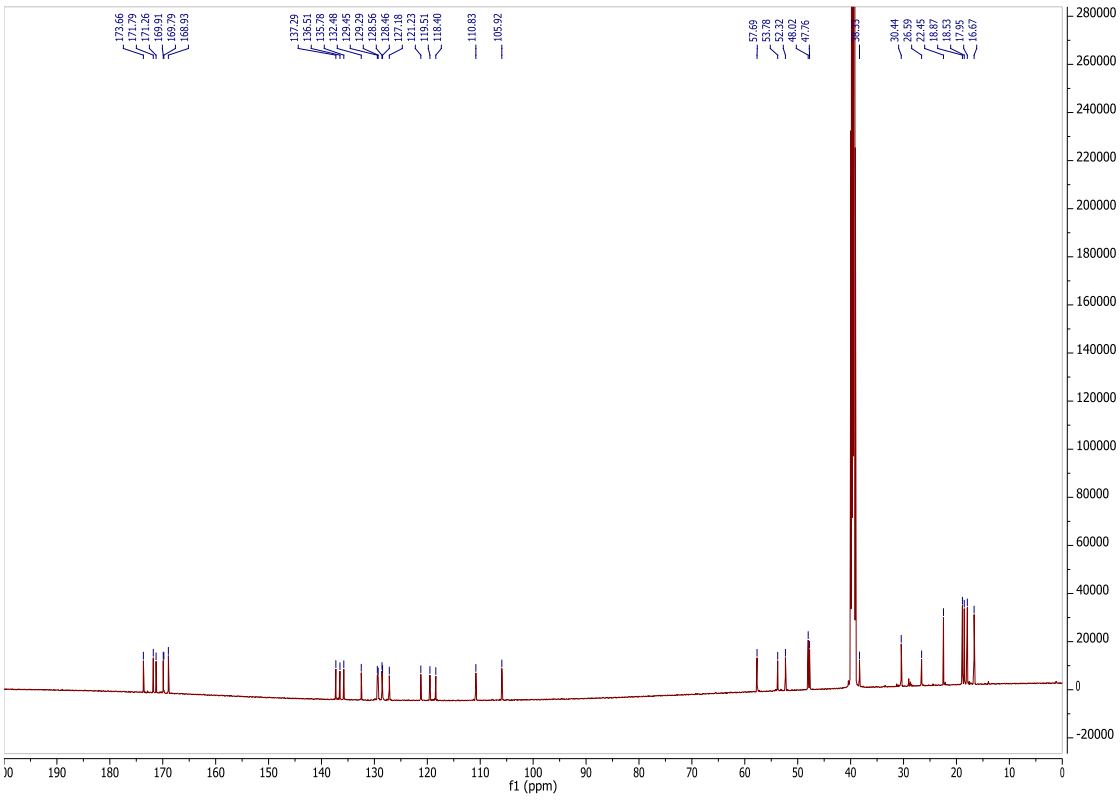
Supplementary Figure 72 | TOCSY NMR spectrum of compound H-Ala-(Cyclo-*m)*-[Phe-Ser-Ala-Trp]-Ala-OH (2i).



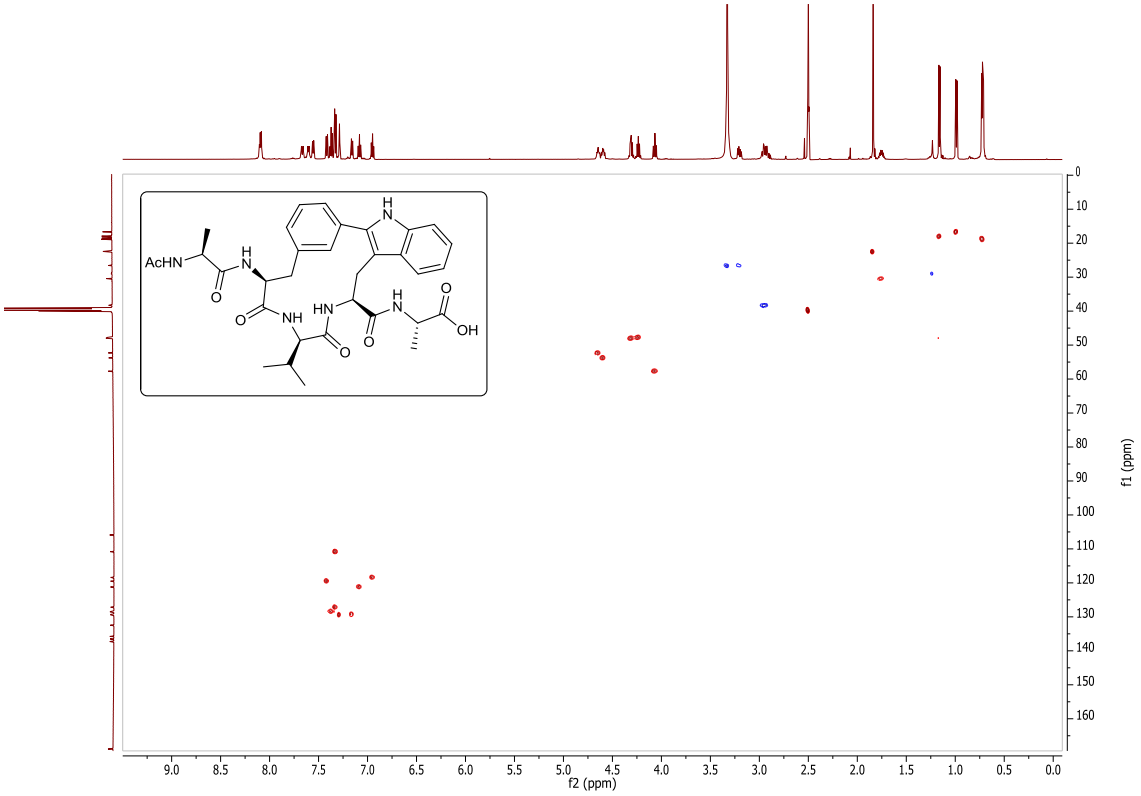
Supplementary Figure 73 | NOESY NMR spectrum of compound H-Ala-(Cyclo-*m)*-[Phe-Ser-Ala-Trp]-Ala-OH (2i).



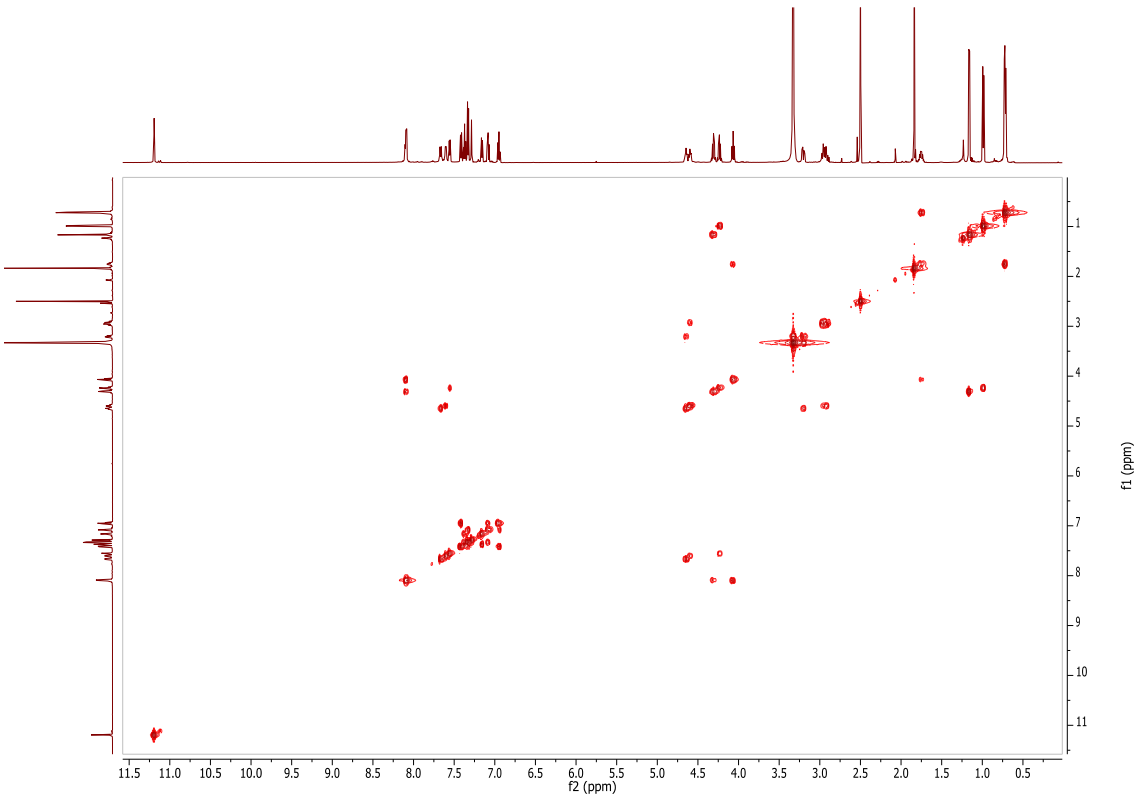
Supplementary Figure 74 | ^{1}H NMR spectrum of compound Ac-Ala-(Cyclo-*m)*-[Phe-Val-Trp]-Ala-OH (2j).



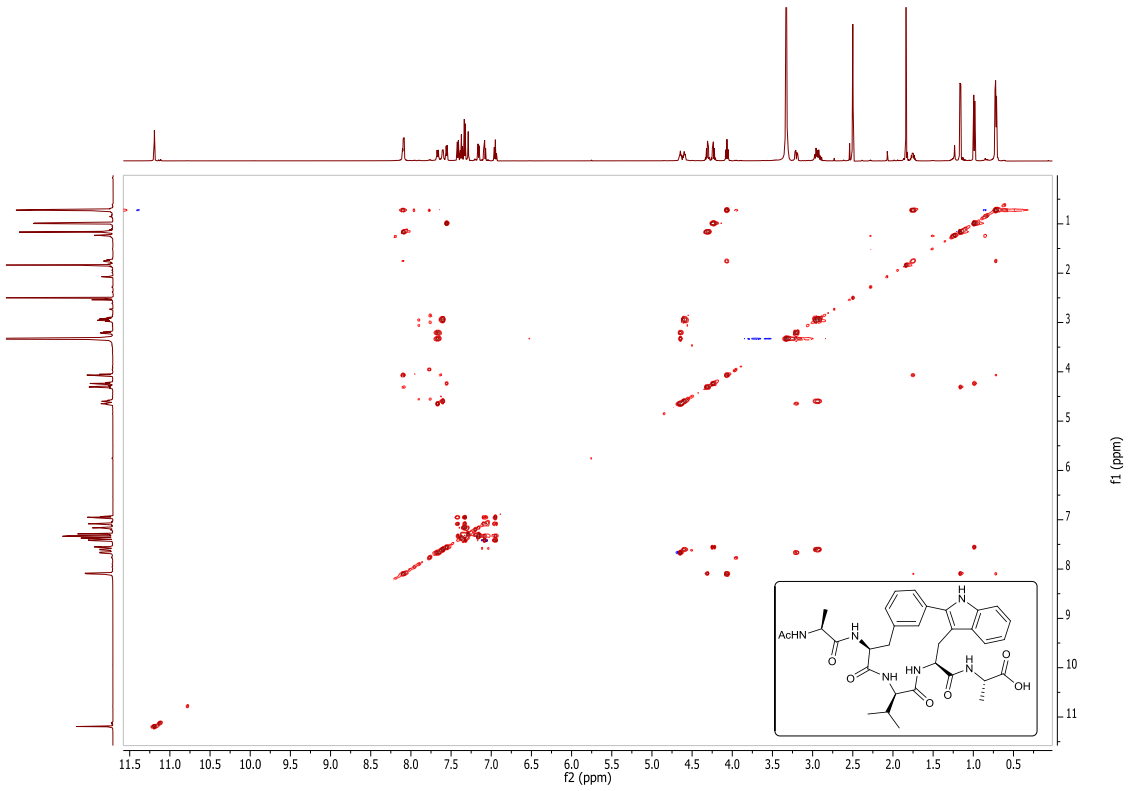
Supplementary Figure 75 | ^{13}C NMR spectrum of compound Ac-Ala-(Cyclo-*m)*-[Phe-Val-Trp]-Ala-OH (2j).



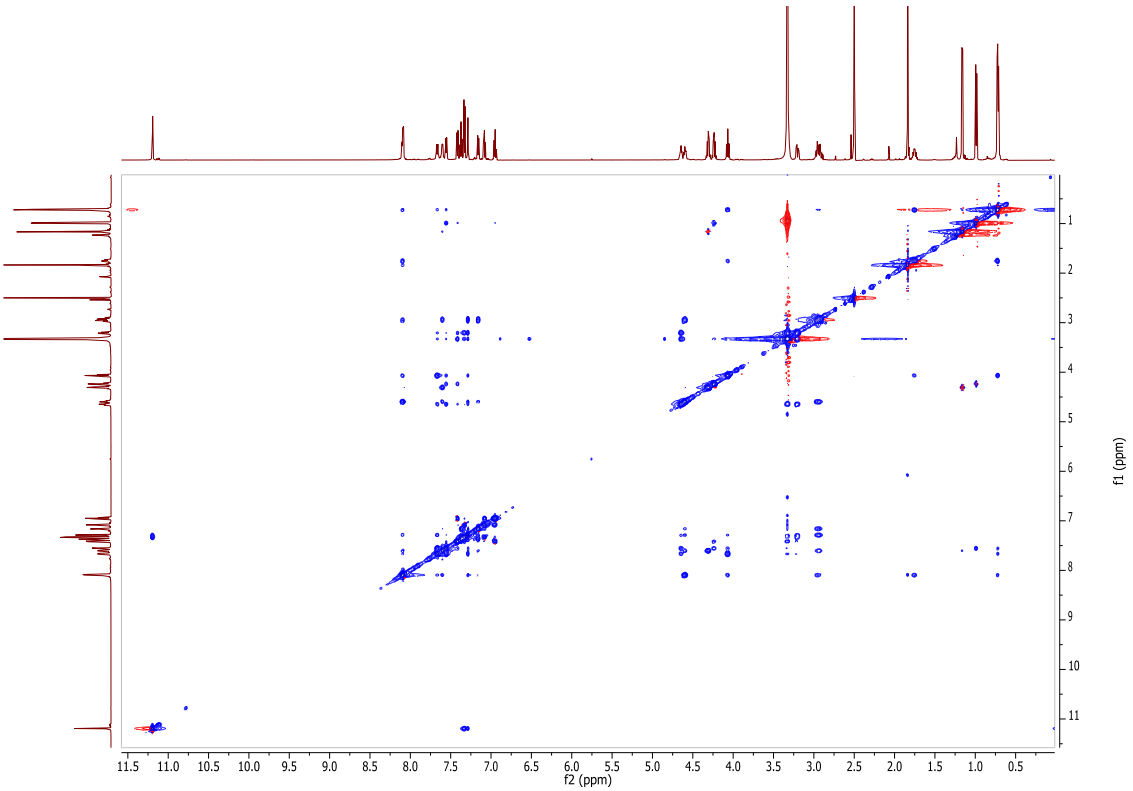
Supplementary Figure 76 | ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-Ala-(Cyclo-*m)*-[Phe-Val-Trp]-Ala-OH (2j).



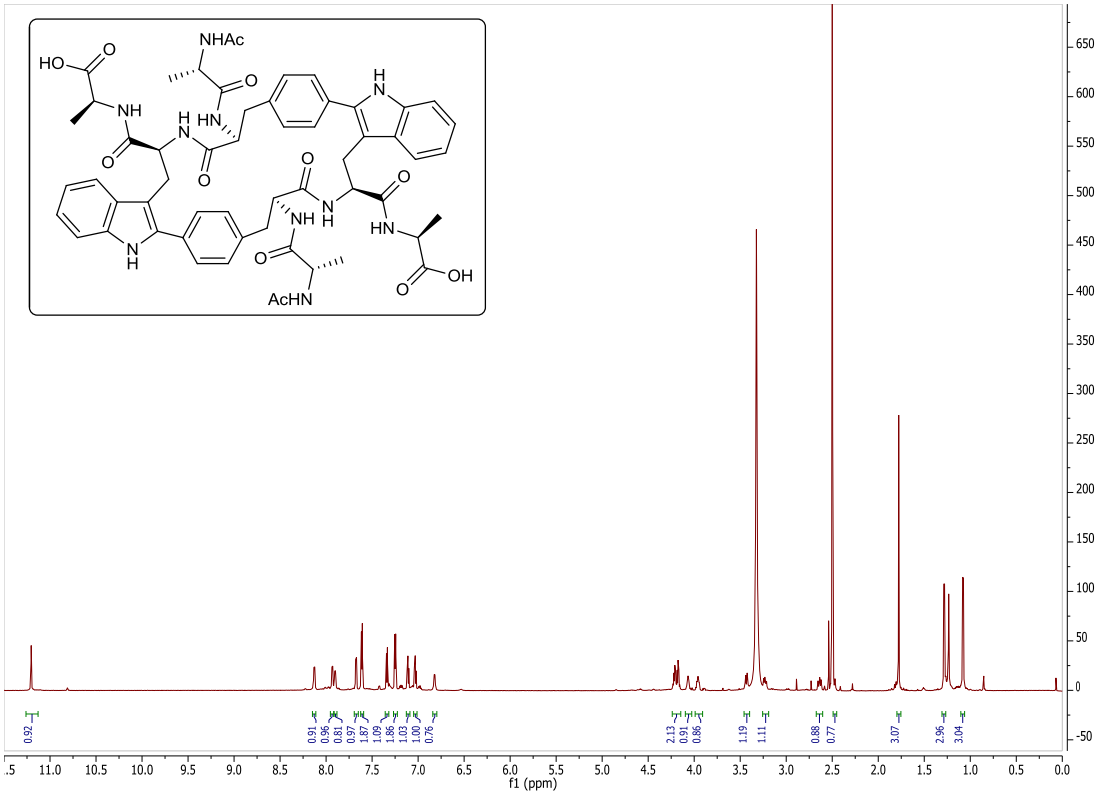
Supplementary Figure 77 | COSY NMR spectrum of compound Ac-Ala-(Cyclo-*m)*-[Phe-Val-Trp]-Ala-OH (2j).



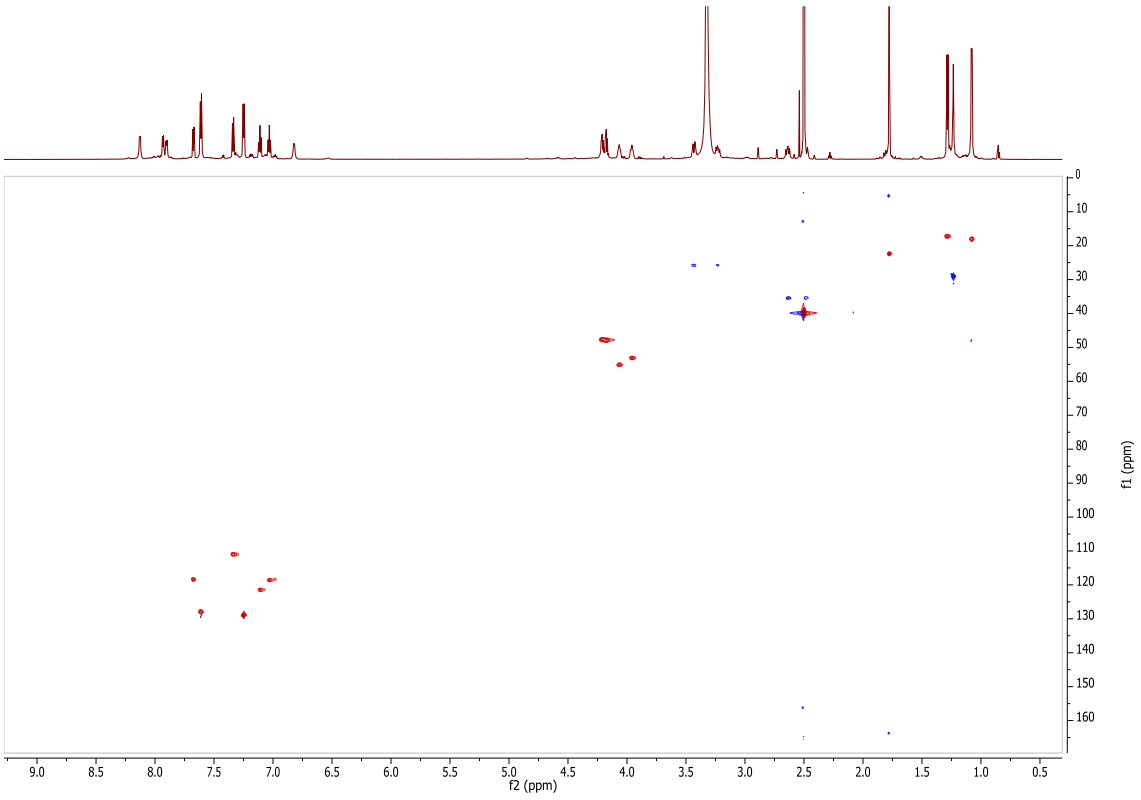
Supplementary Figure 78 | TOCSY NMR spectrum of compound Ac-Ala-(Cyclo-*m)*-[Phe-Val-Trp]-Ala-OH (2j).



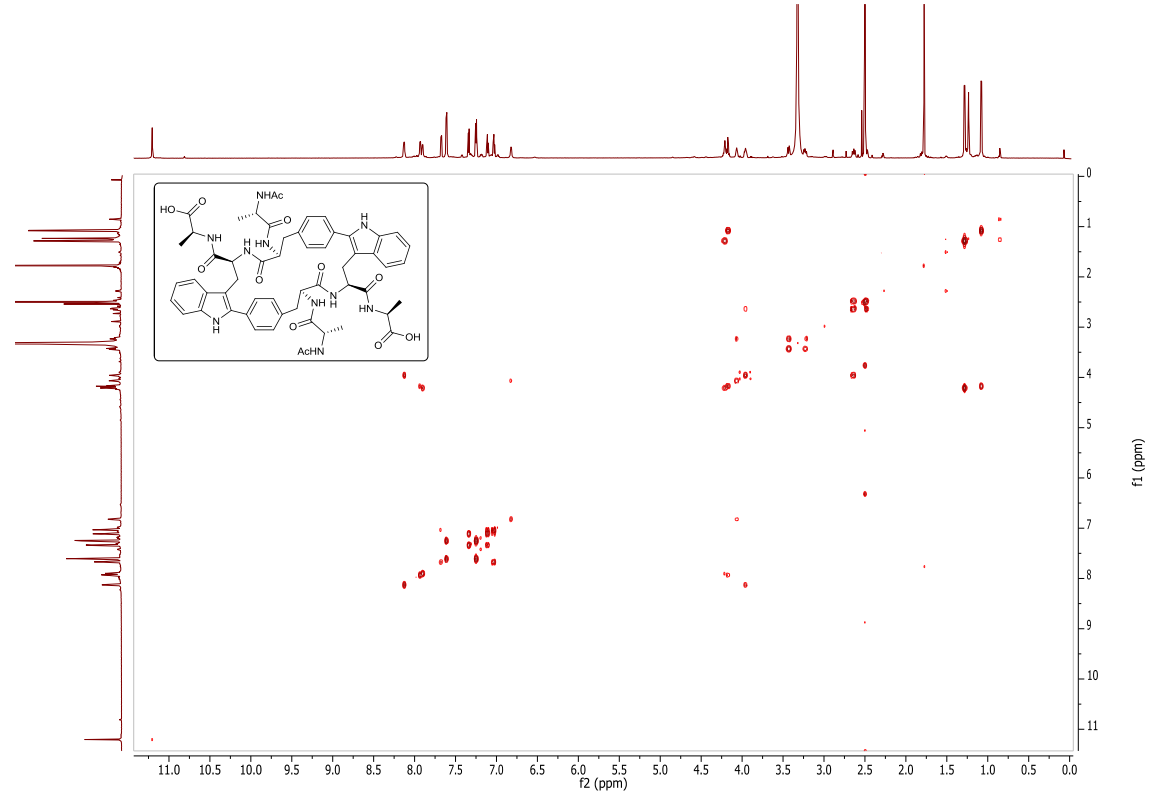
Supplementary Figure 79 | NOESY NMR spectrum of compound Ac-Ala-(Cyclo-*m)*-[Phe-Val-Trp]-Ala-OH (2j).



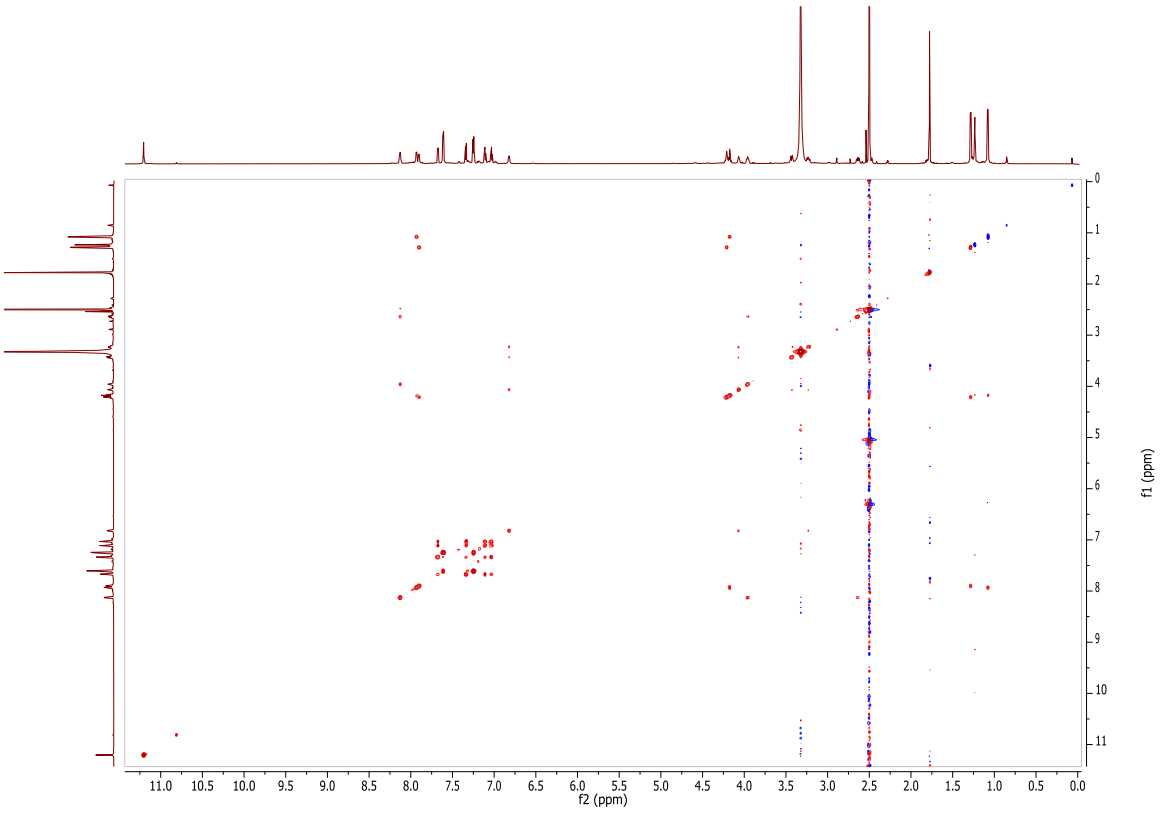
Supplementary Figure 80 ‖ ^{1}H NMR spectrum of compound (Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (2k).



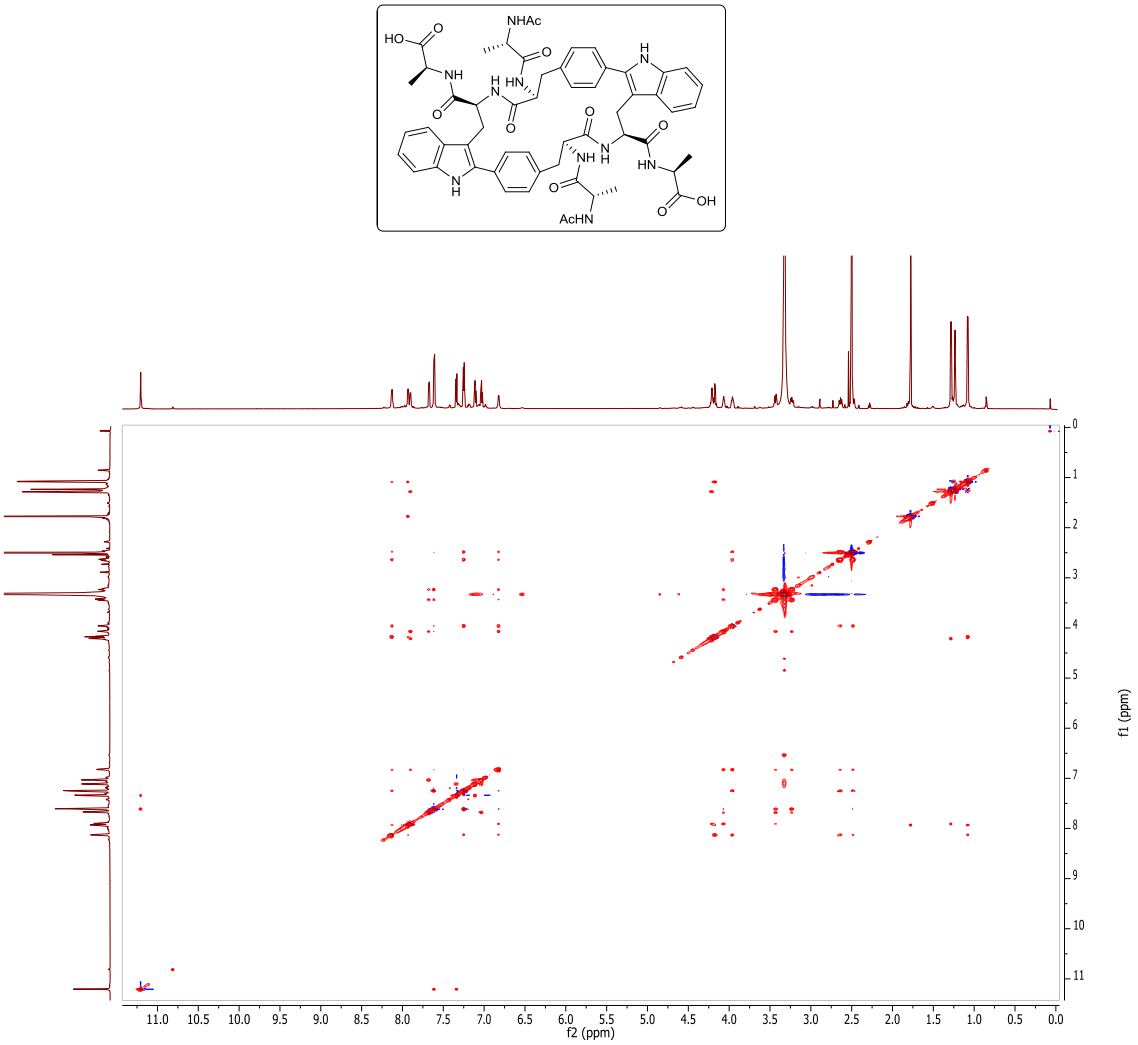
Supplementary Figure 81 ‖ ^{1}H-^{13}C HSQC NMR spectrum of compound (Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (2k).



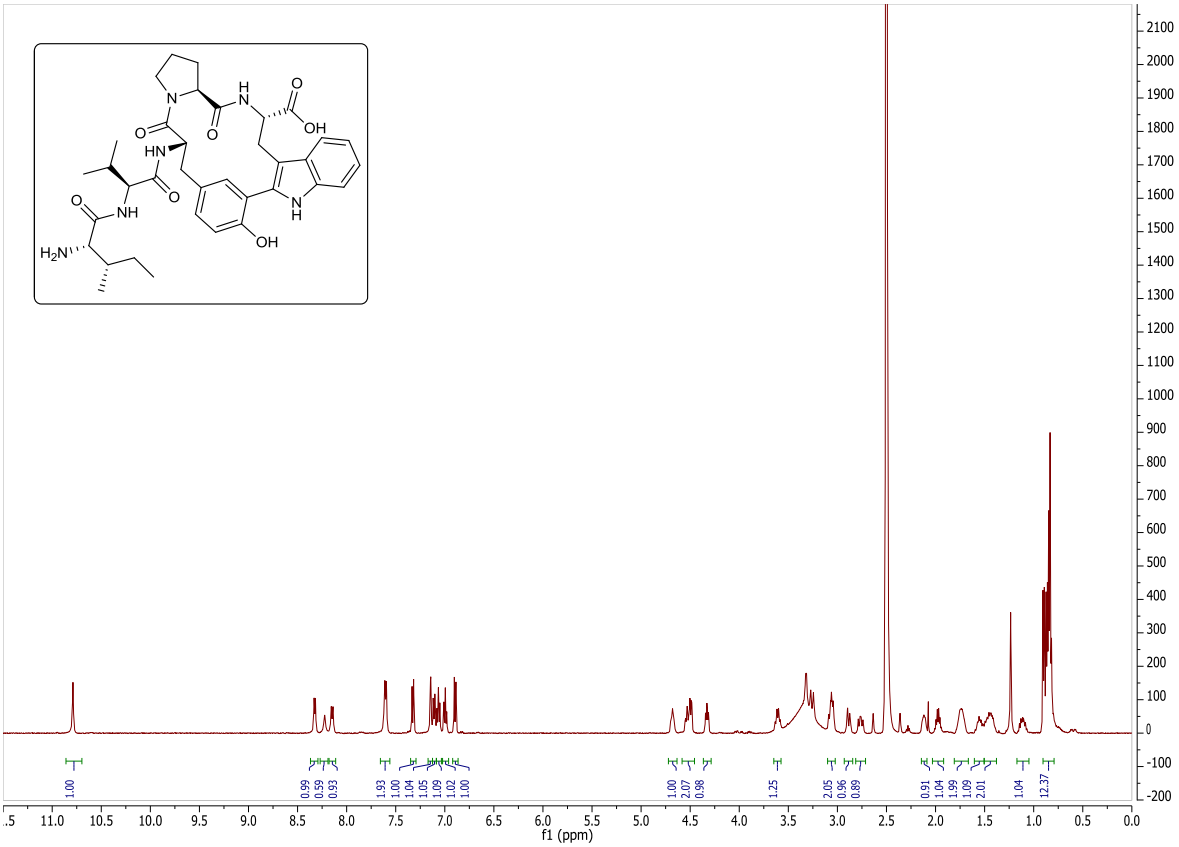
Supplementary Figure 82 | COSY NMR spectrum of compound (Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (2k).



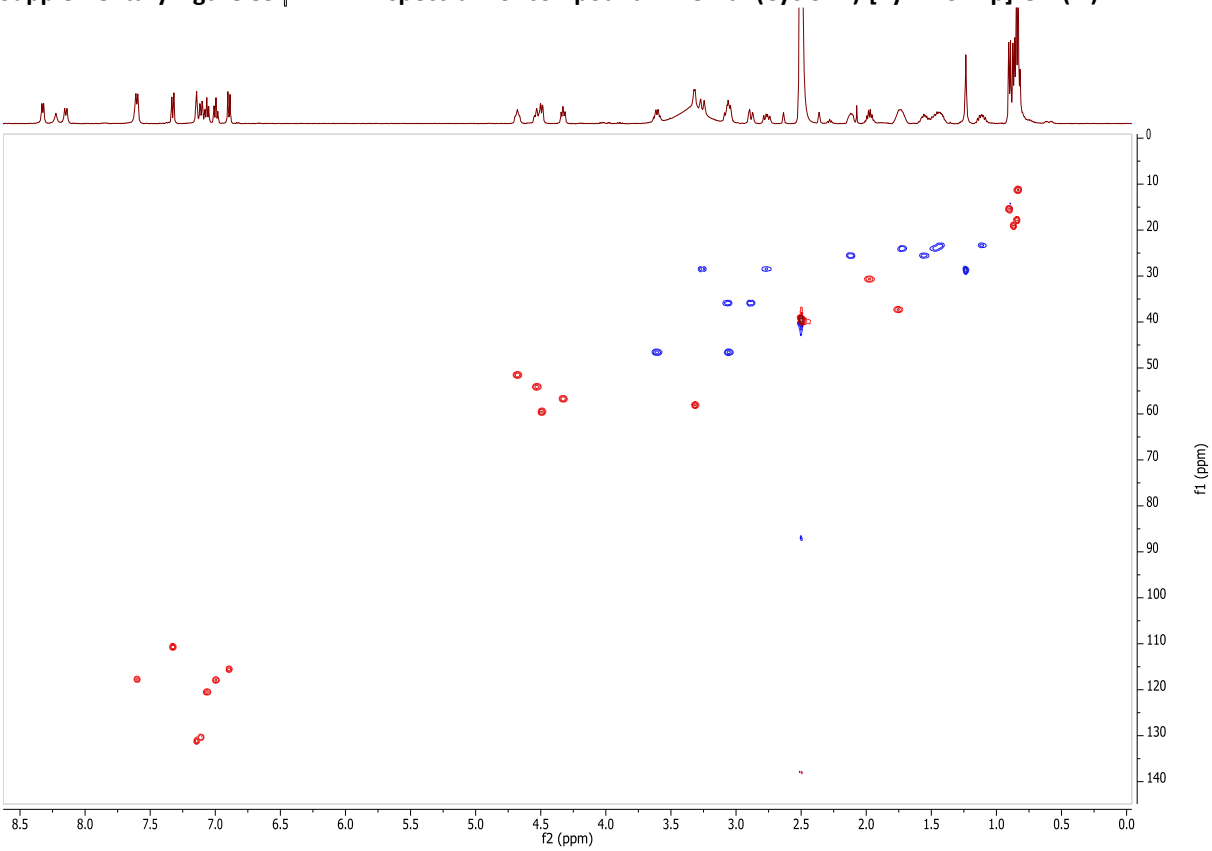
Supplementary Figure 83 | TOCSY NMR spectrum of compound (Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (2k).



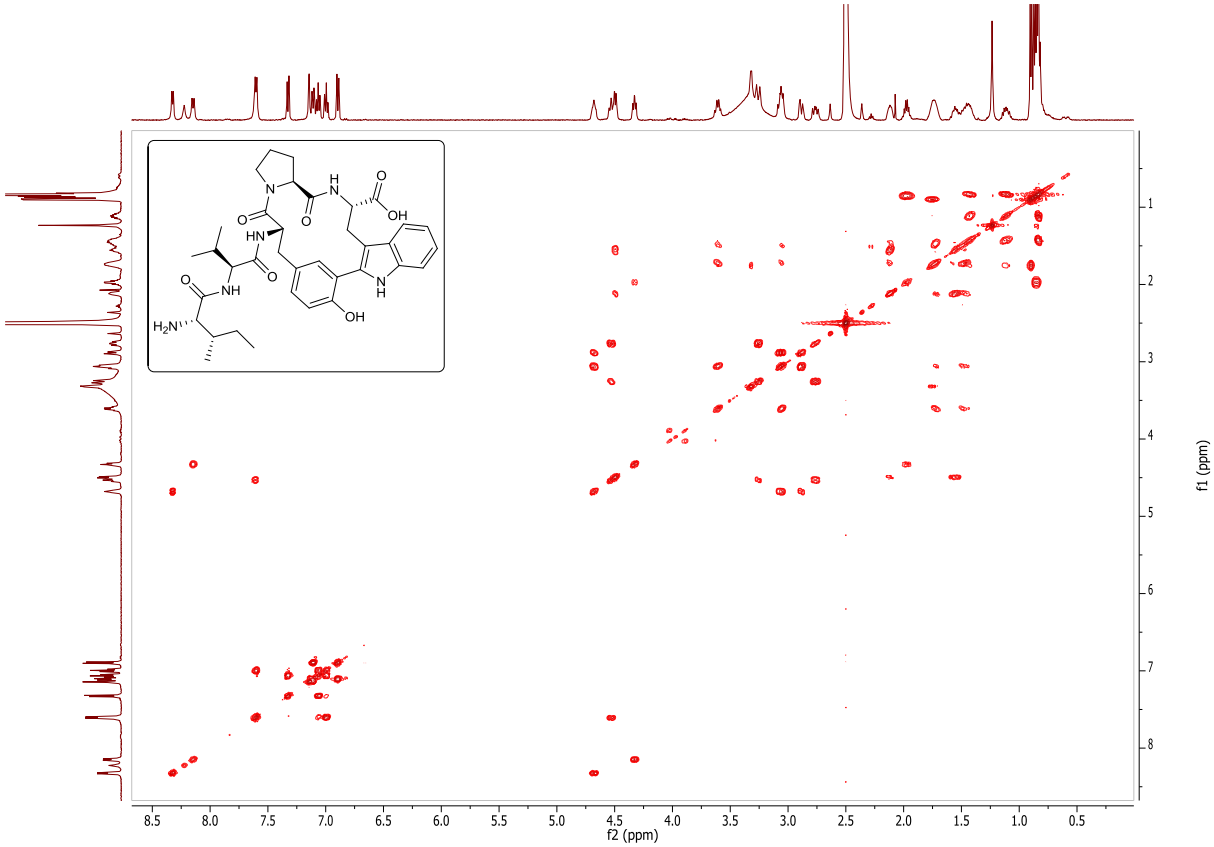
Supplementary Figure 84 | NOESY NMR spectrum of compound (Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (2k).



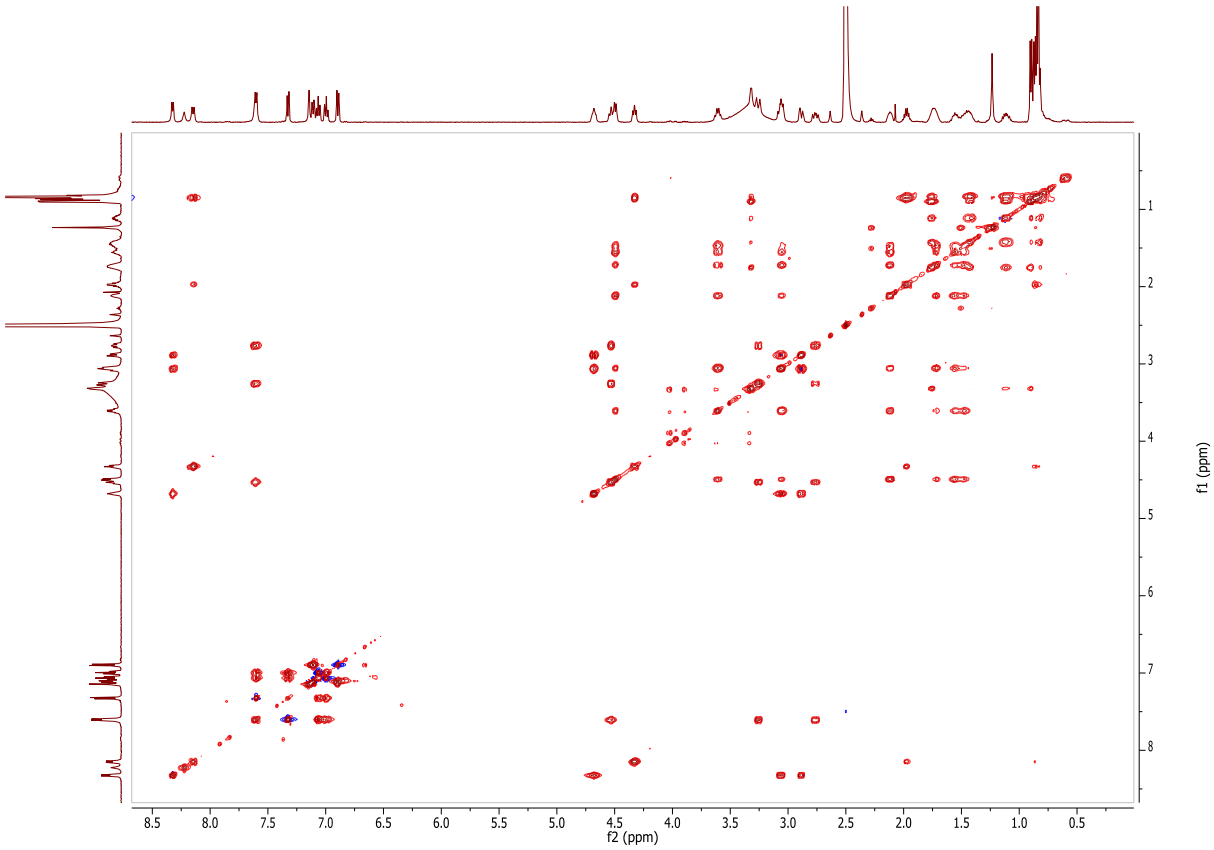
Supplementary Figure 85 | ^{1}H NMR spectrum of compound H-Ile-Val-(Cyclo-*m*)-[Tyr-Pro-Trp]-OH (2l).



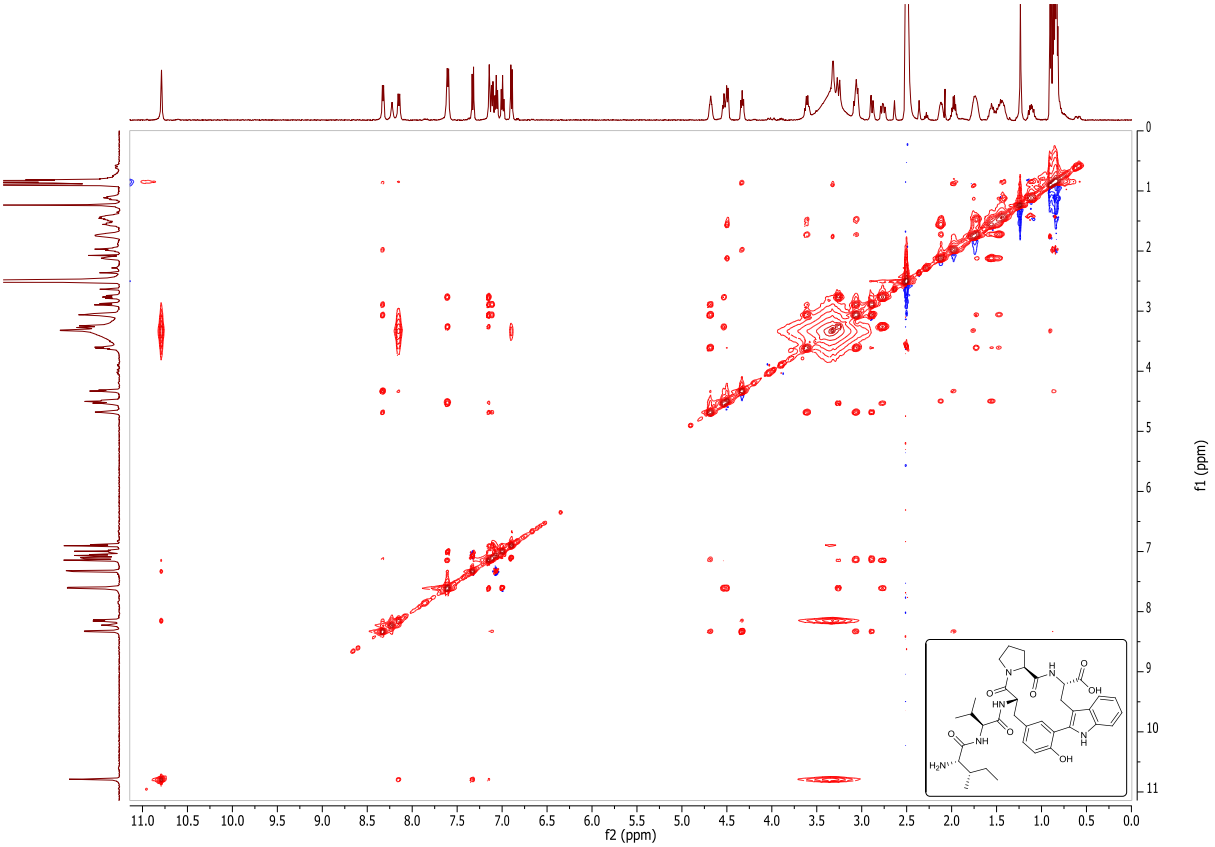
Supplementary Figure 86 | ^{1}H-^{13}C HSQC NMR spectrum of compound H-Ile-Val-(Cyclo-*m*)-[Tyr-Pro-Trp]-OH (2l).



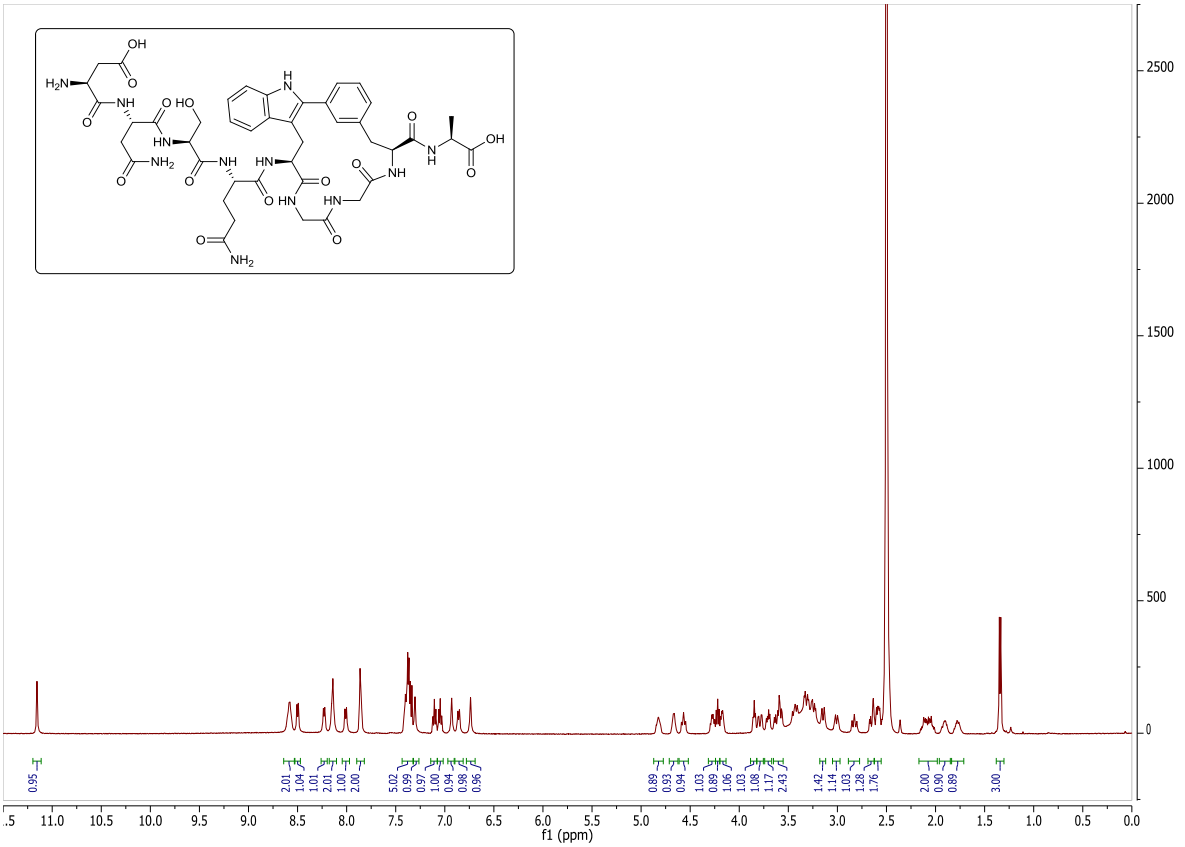
Supplementary Figure 87 | COSY NMR spectrum of compound H-Ile-Val-(Cyclo-*m*)-[Tyr-Pro-Trp]-OH (2l).



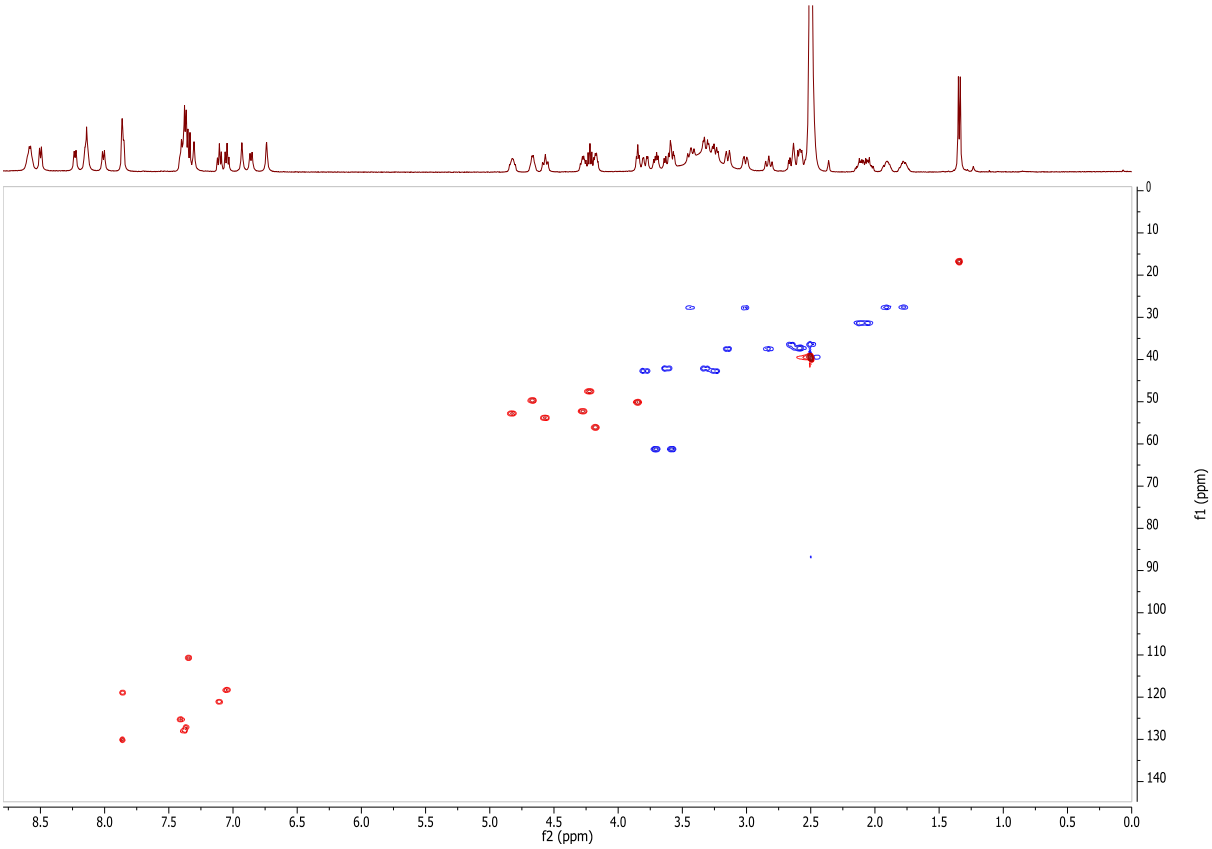
Supplementary Figure 88 | TOCSY NMR spectrum of compound H-Ile-Val-(Cyclo-*m*)-[Tyr-Pro-Trp]-OH (2l).



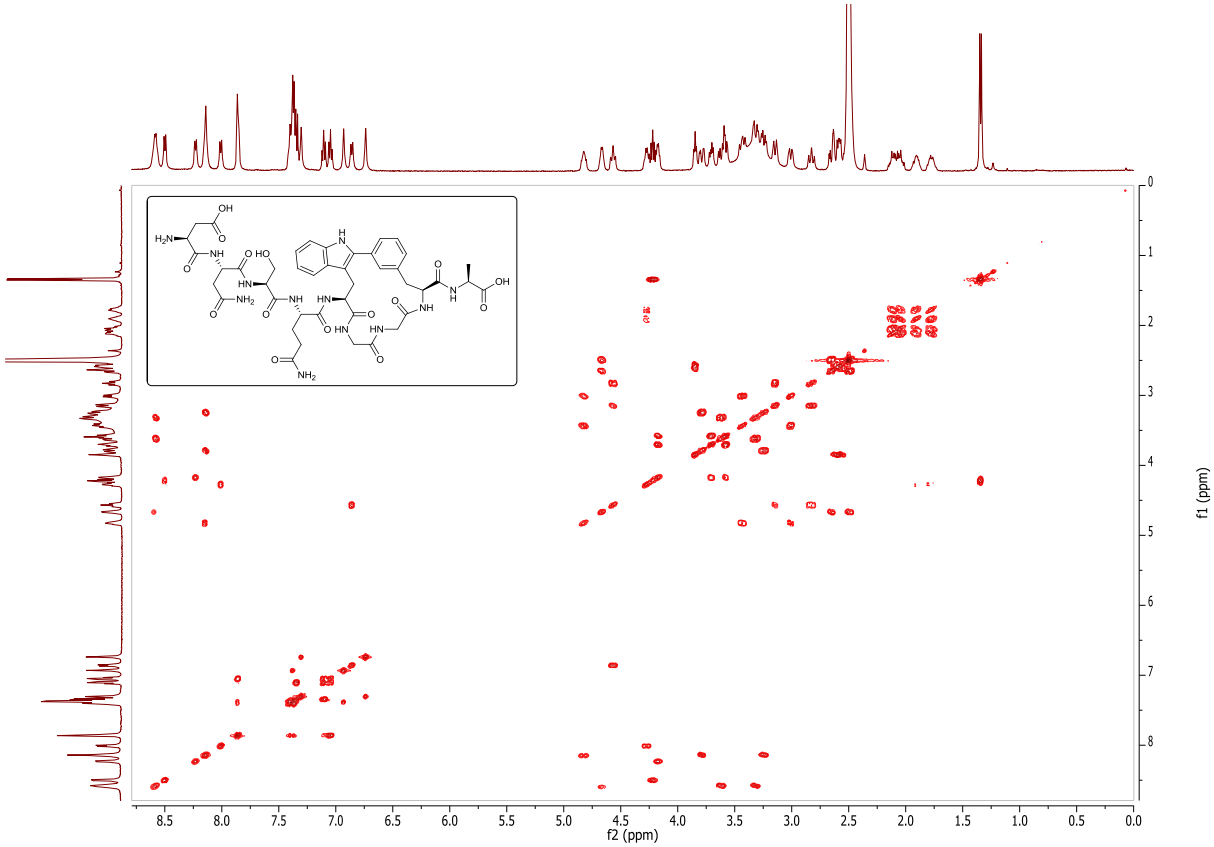
Supplementary Figure 89 | NOESY NMR spectrum of compound H-Ile-Val-(Cyclo-*m*)-[Tyr-Pro-Trp]-OH (2l).



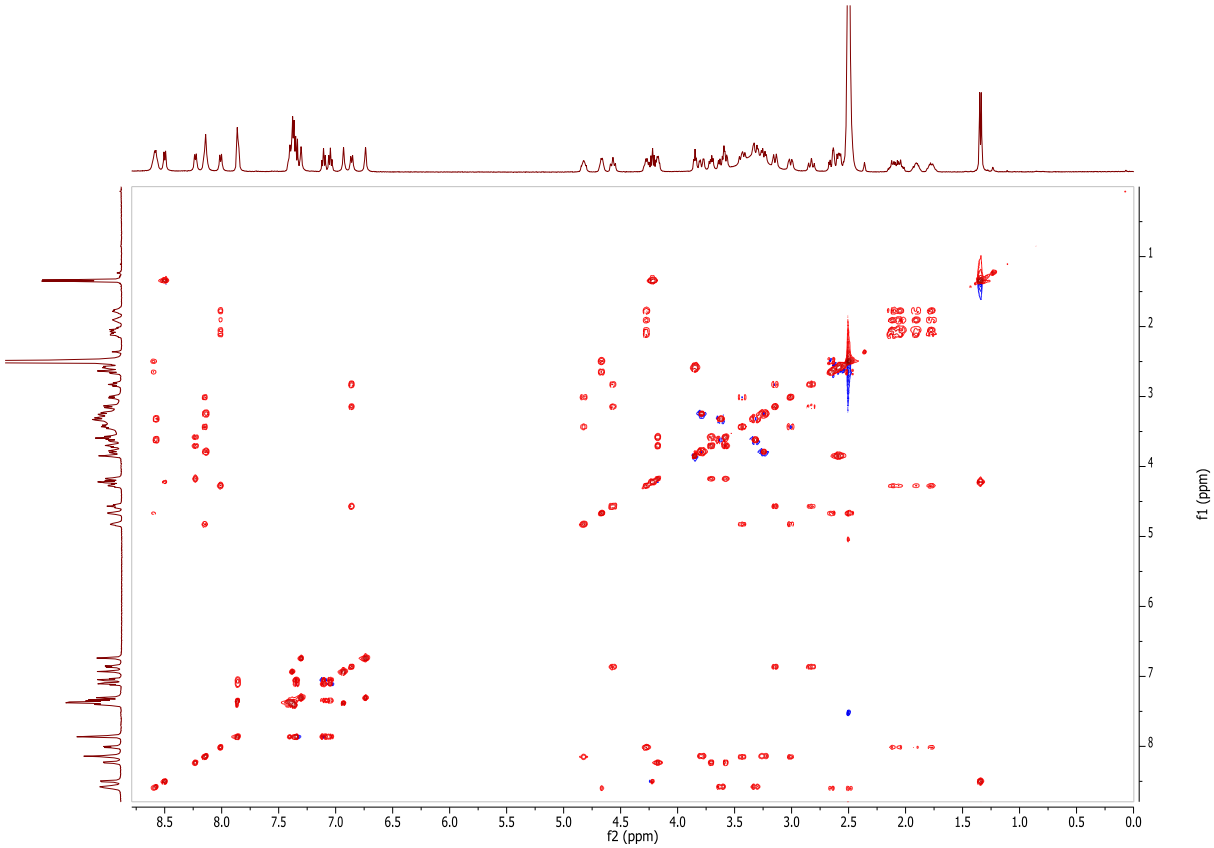
Supplementary Figure 90 ‖ ^{1}H NMR spectrum of compound H-Asp-Asn-Ser-Gln-(Cyclo-*m*)-[Trp-Gly-Gly-Phe]-Ala-OH (2m).



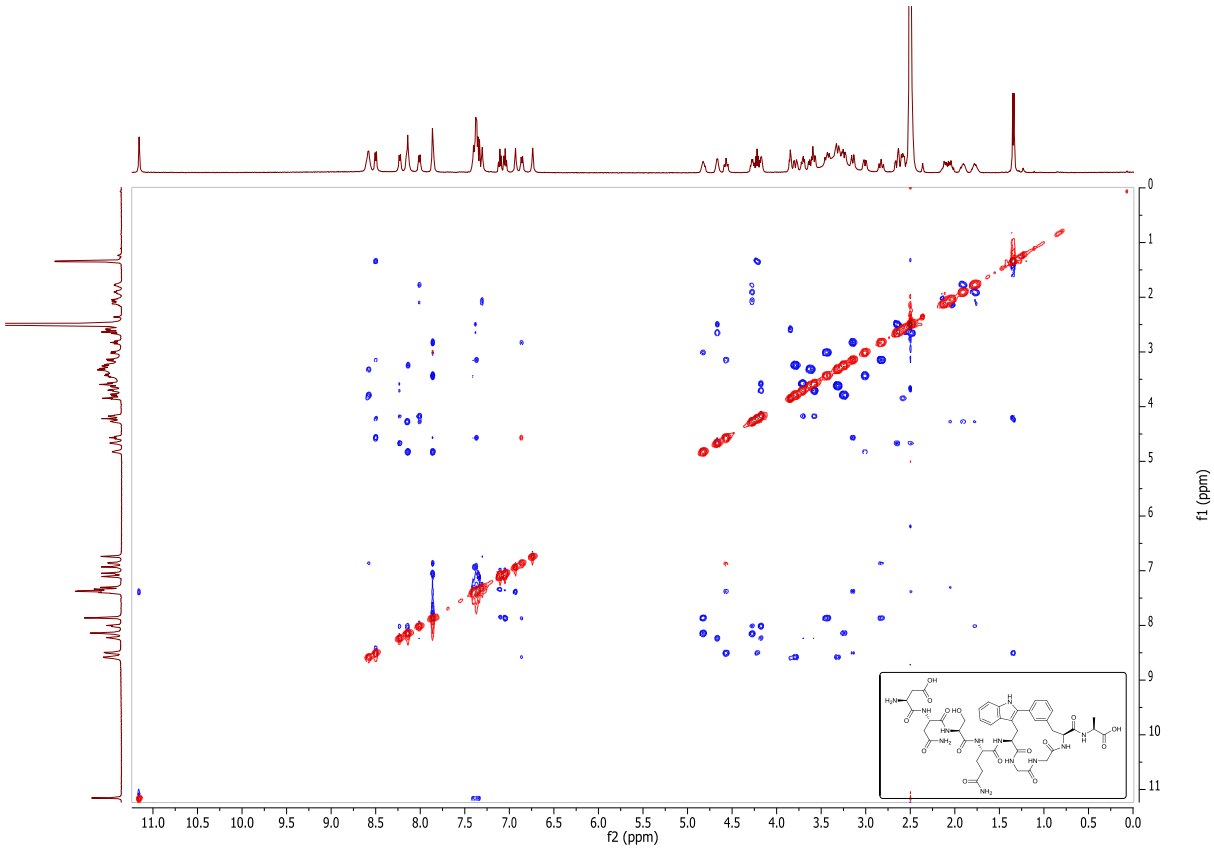
Supplementary Figure 91 ‖ ^{1}H-^{13}C HSQC NMR spectrum of compound H-Asp-Asn-Ser-Gln-(Cyclo-*m*)-[Trp-Gly-Gly-Phe]-Ala-OH (2m).



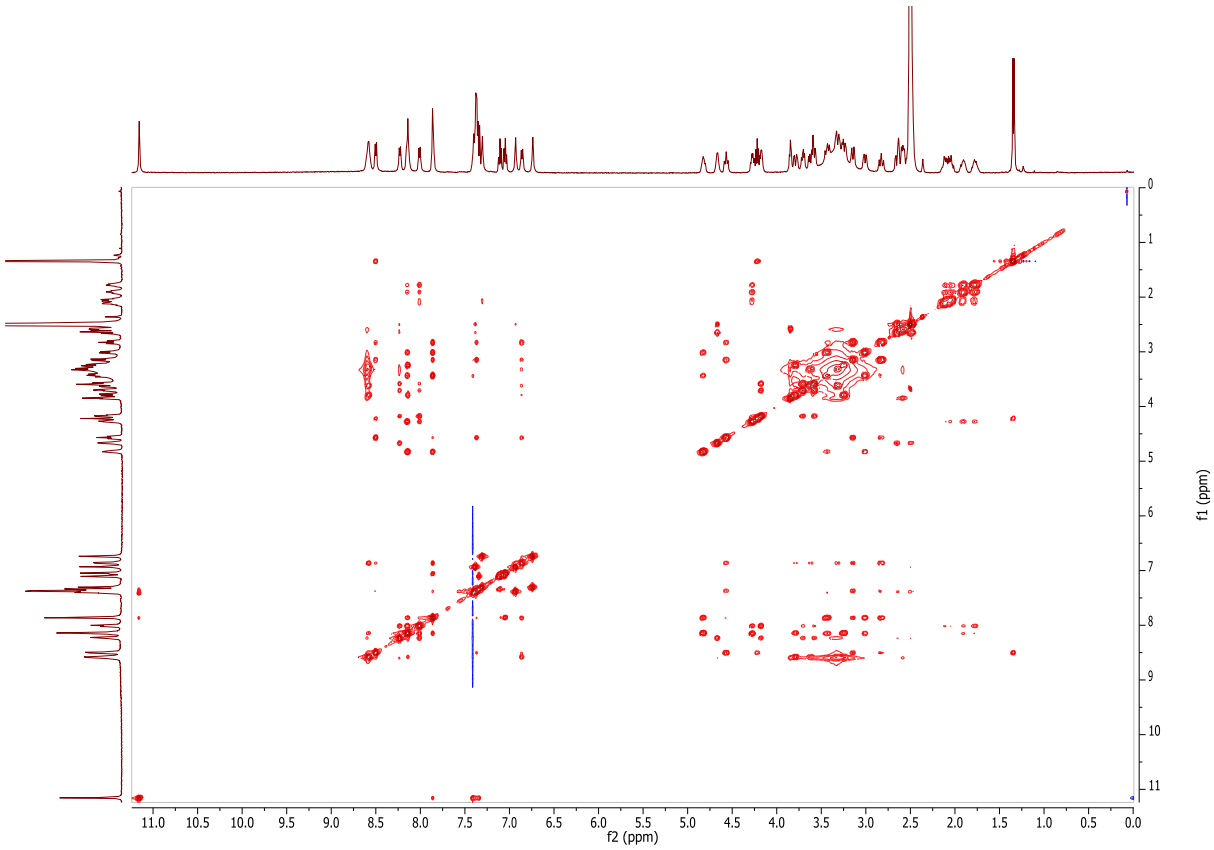
Supplementary Figure 92 | COSY NMR spectrum of compound H-Asp-Asn-Ser-Gln-(Cyclo-*m*)-[Trp-Gly-Gly-Phe]-Ala-OH (2m).



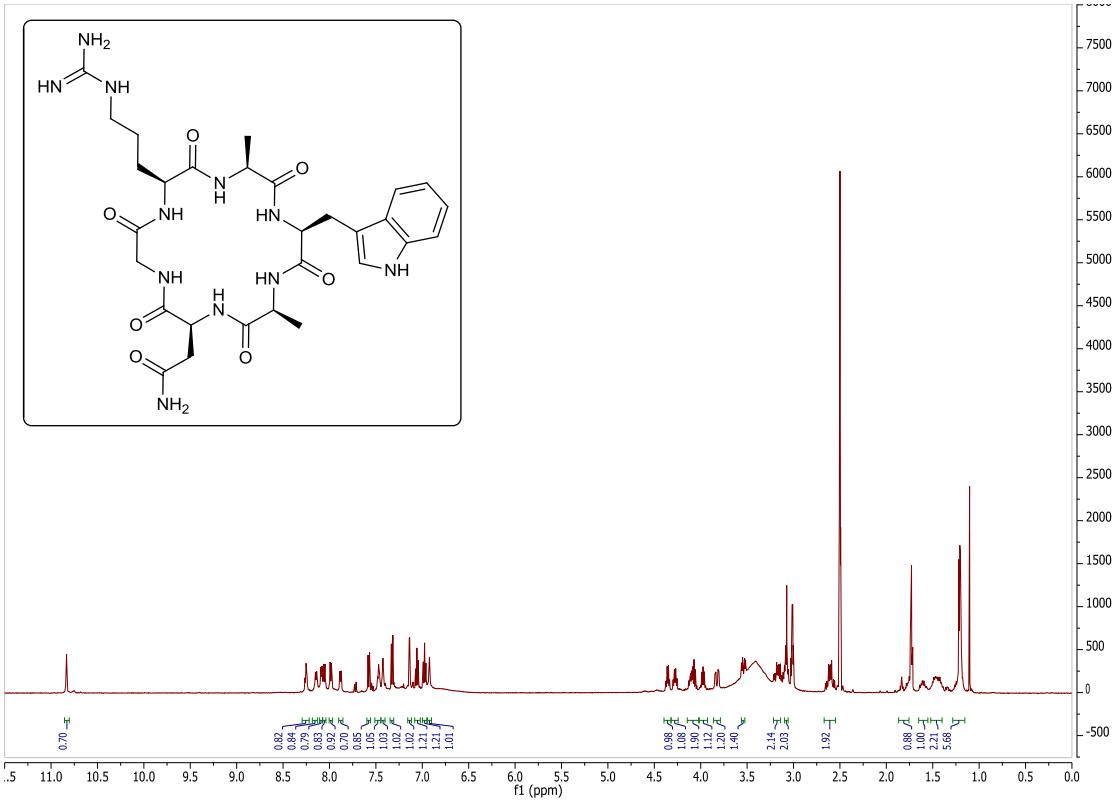
Supplementary Figure 93 | TOCSY NMR spectrum of compound H-Asp-Asn-Ser-Gln-(Cyclo-*m*)-[Trp-Gly-Gly-Phe]-Ala-OH (2m).



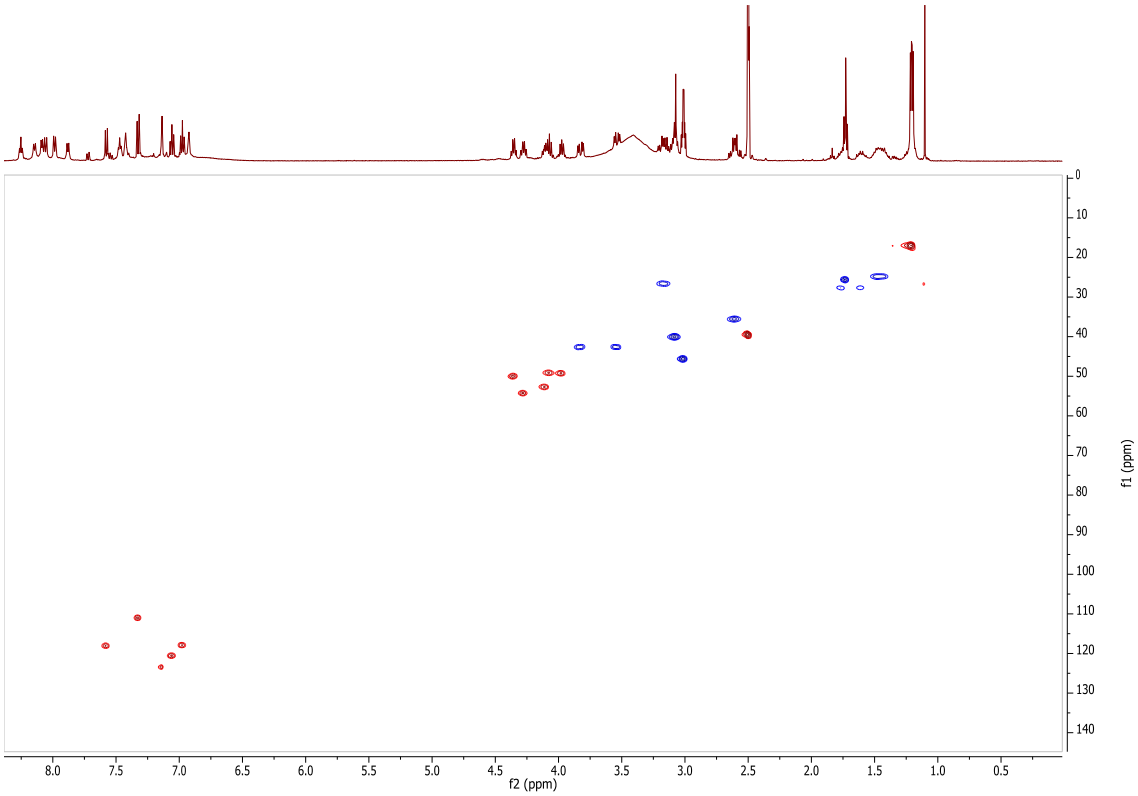
Supplementary Figure 94 | ROESY NMR spectrum of compound H-Asp-Asn-Ser-Gln-(Cyclo-*m*)-[Trp-Gly-Gly-Phe]-Ala-OH (2m).



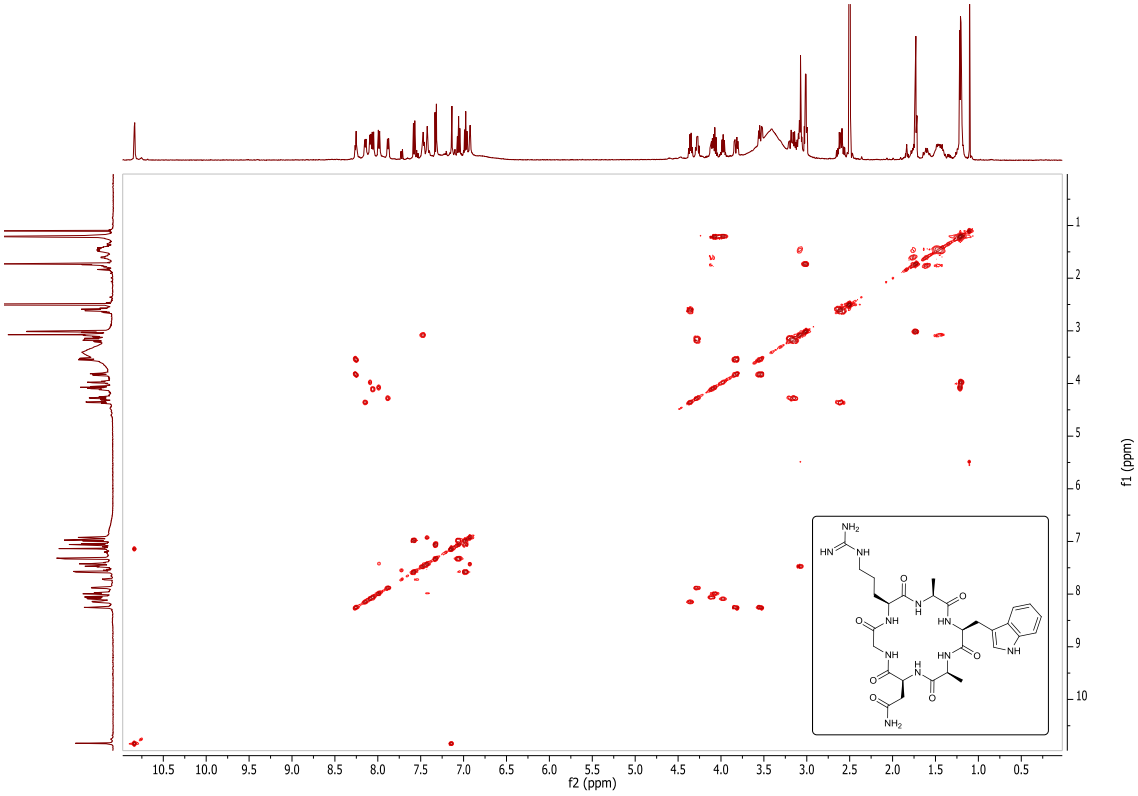
Supplementary Figure 95 | NOESY NMR spectrum of compound H-Asp-Asn-Ser-Gln-(Cyclo-*m*)-[Trp-Gly-Gly-Phe]-Ala-OH (2m).



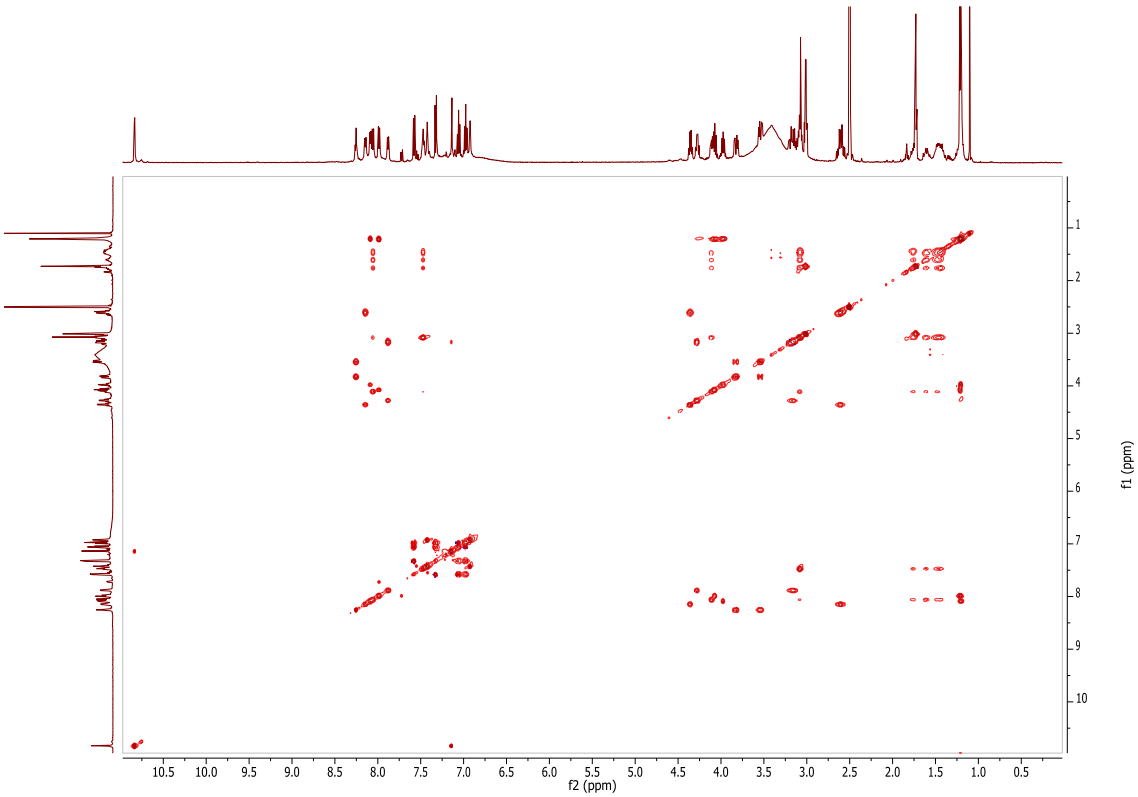
Supplementary Figure 96 | ^{1}H NMR spectrum of compound Cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-) (3).



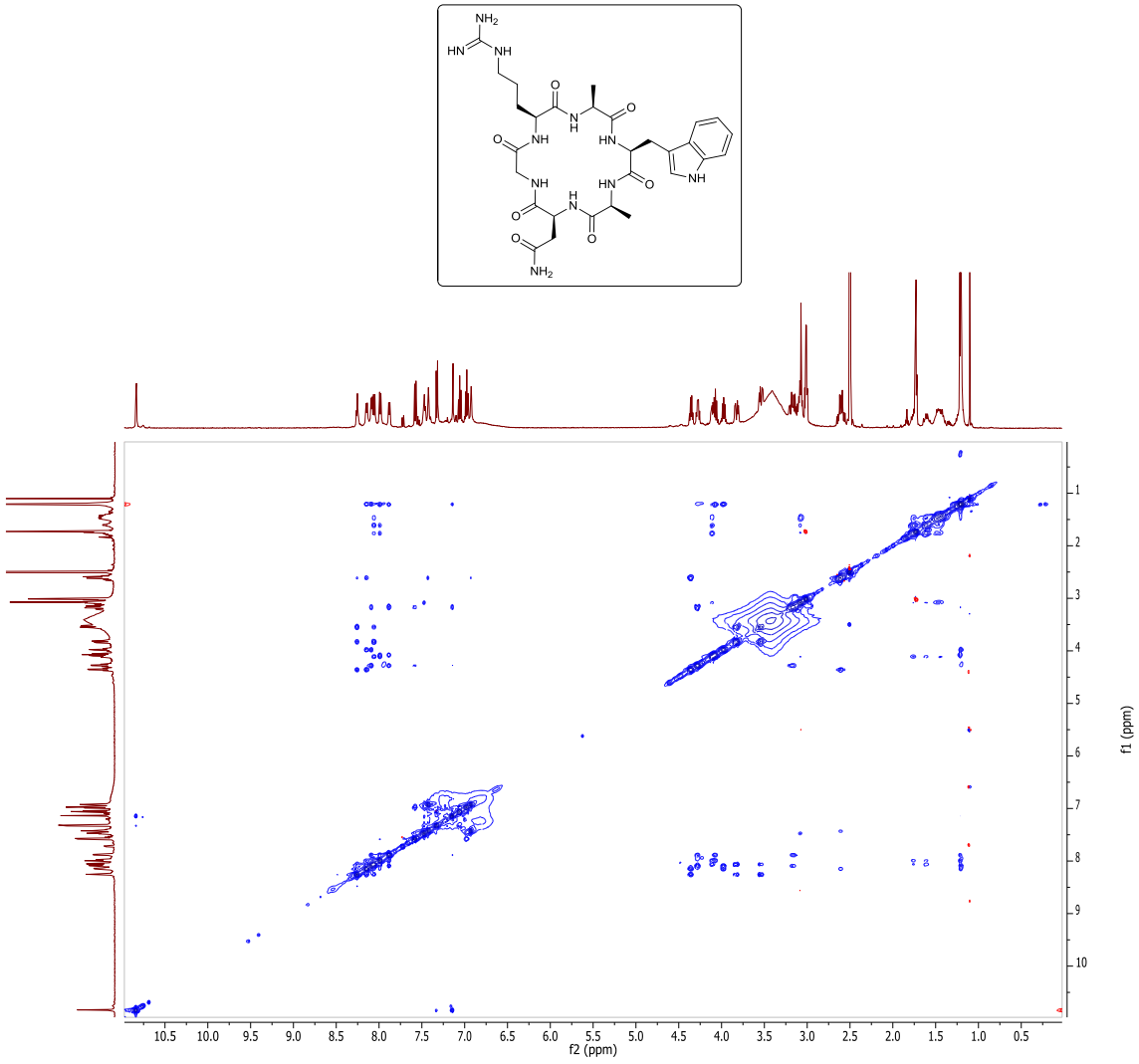
Supplementary Figure 97 | ^{1}H-^{13}C HSQ NMR spectrum of compound Cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-) (3).



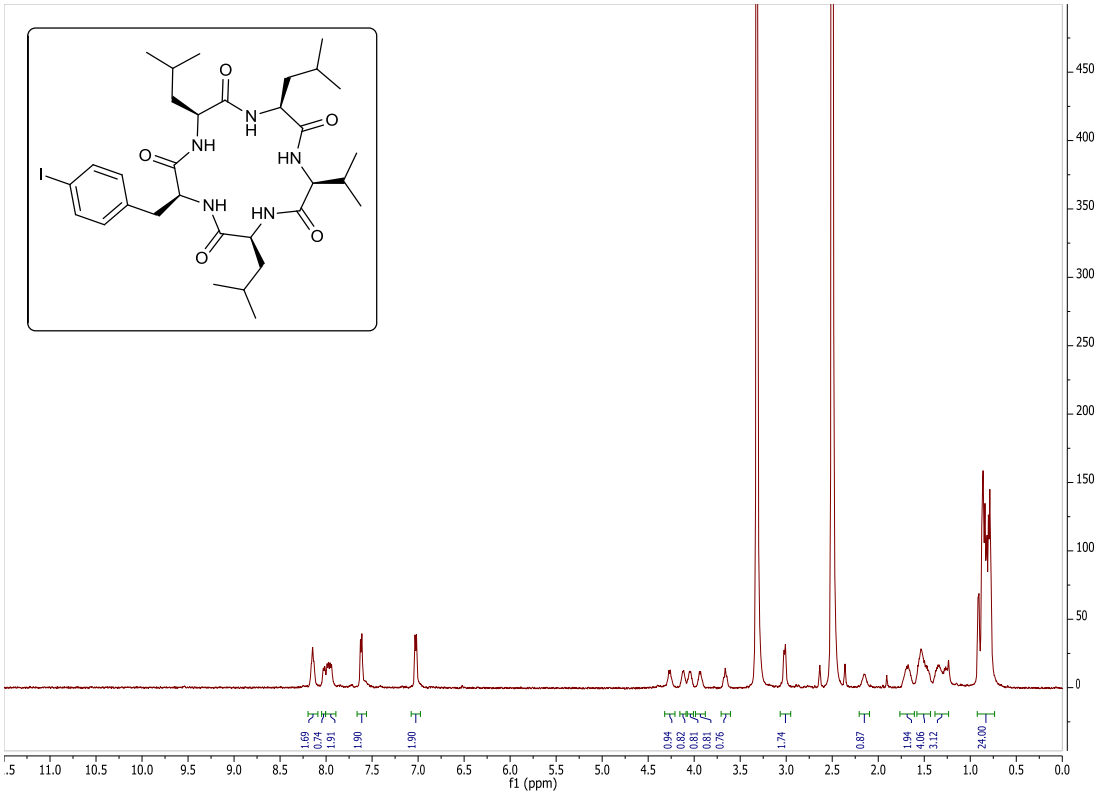
Supplementary Figure 98 | COSY NMR spectrum of compound Cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-) (3).



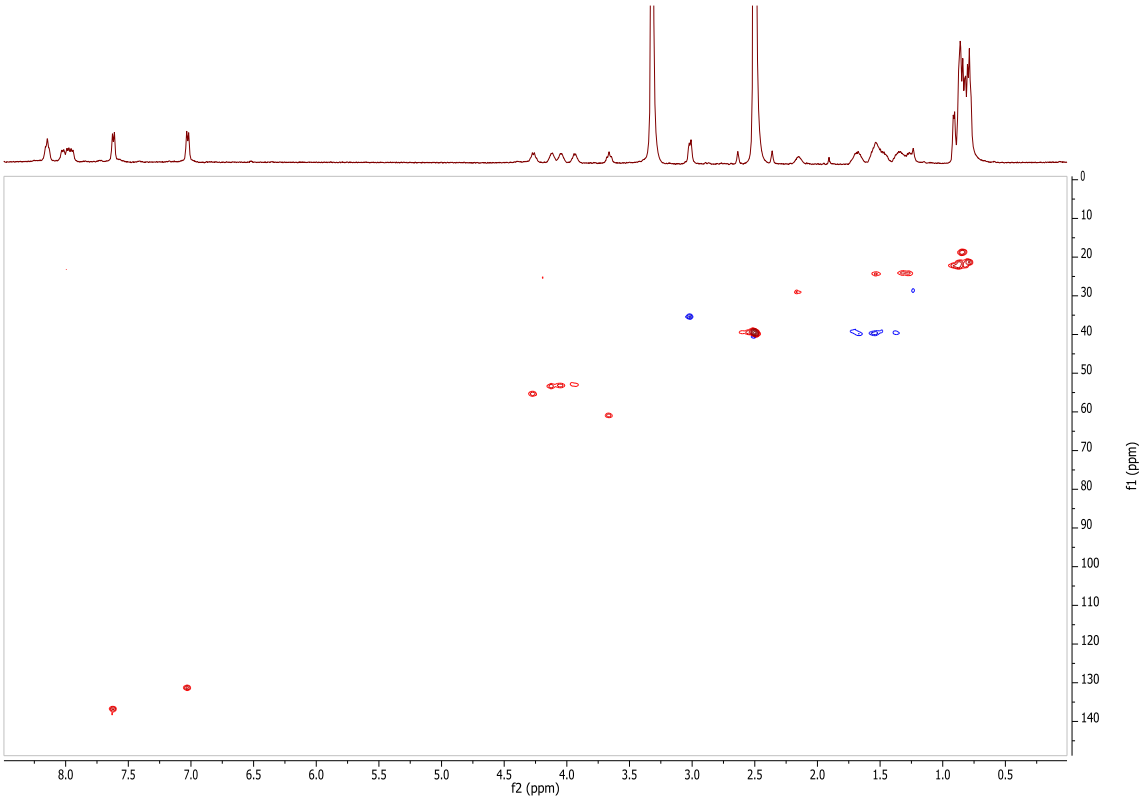
Supplementary Figure 99 | TOCSY NMR spectrum of compound Cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-) (3).



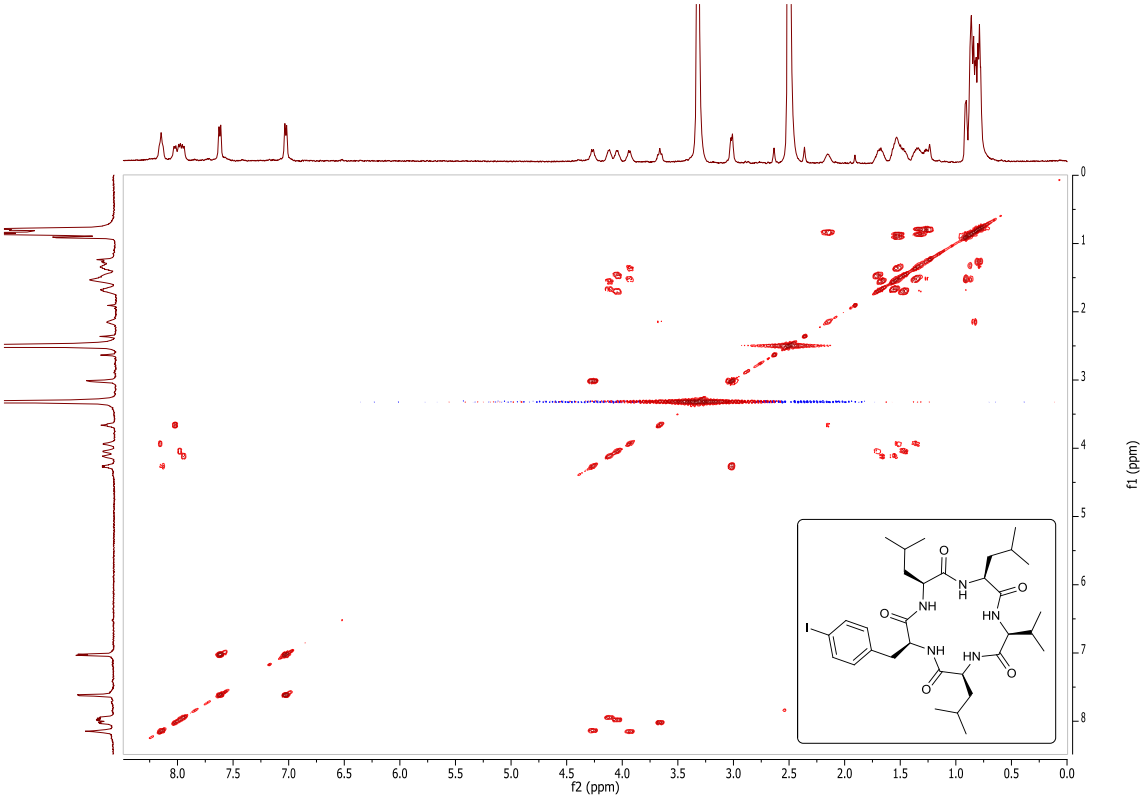
Supplementary Figure 100 | NOESY NMR spectrum of compound Cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-) (3).



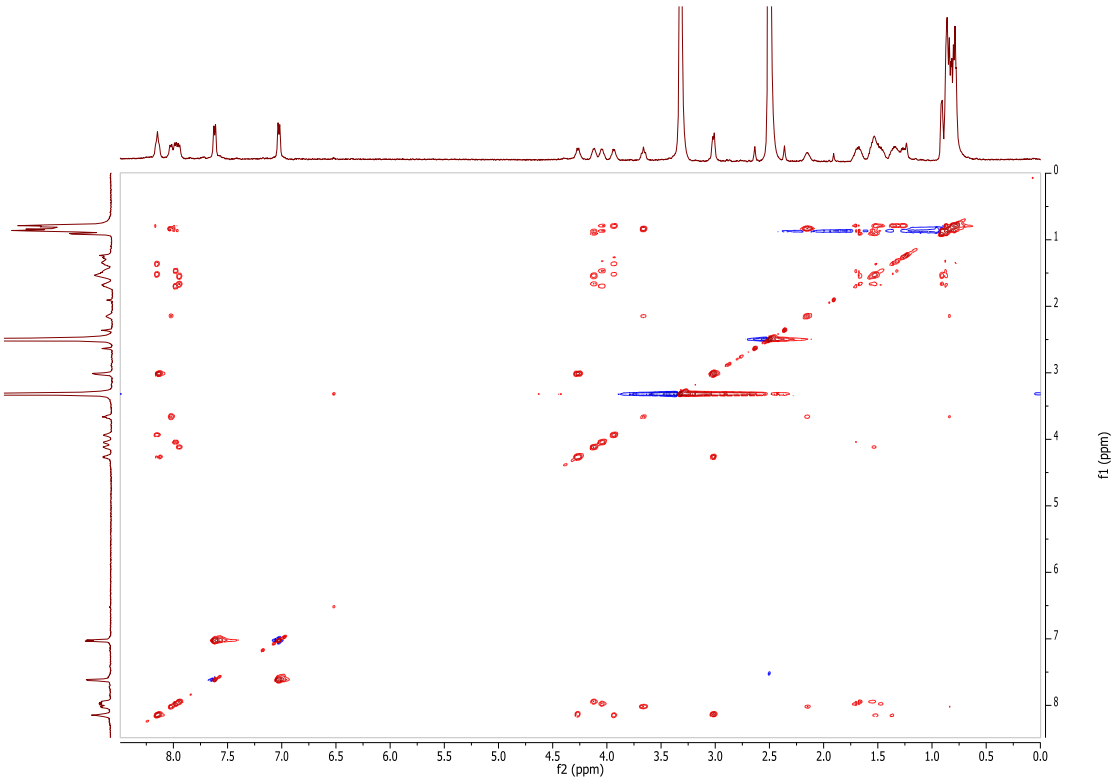
Supplementary Figure 101 | ^{1}H NMR spectrum of compound Cyclo(-Leu-Leu-Val-Leu-*p*-I-Phe-) (4).



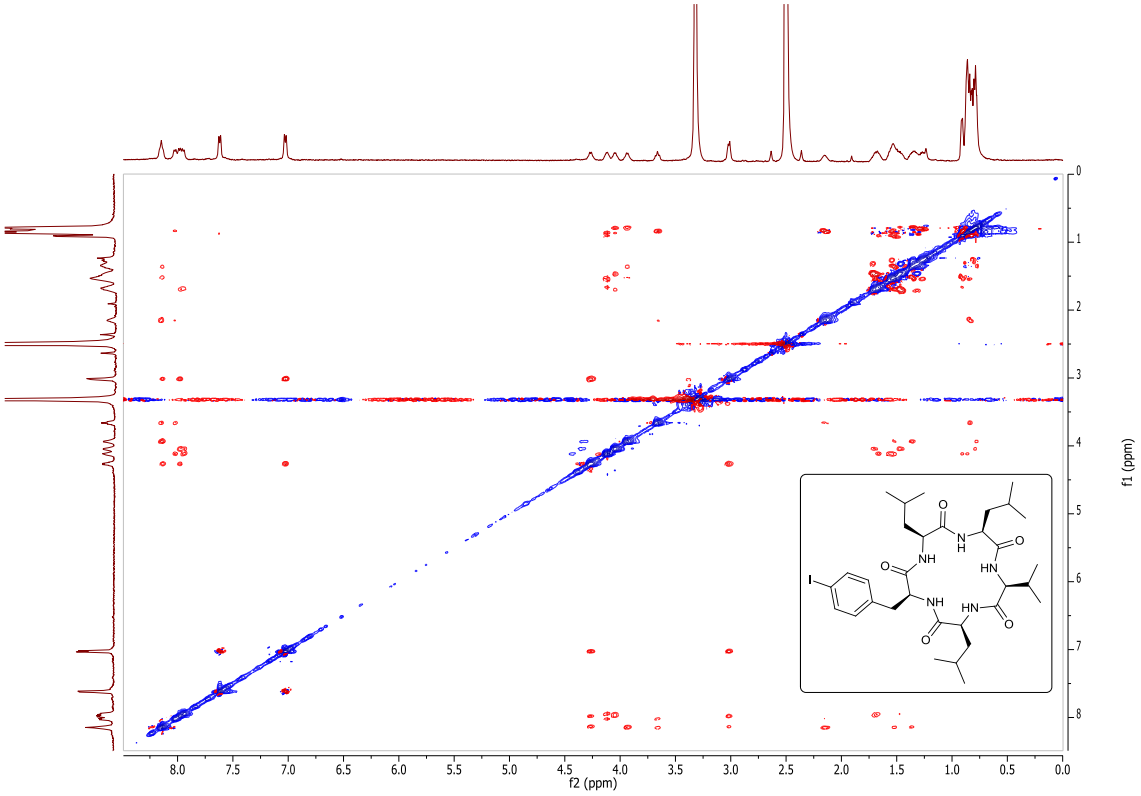
Supplementary Figure 102 | ^{1}H-^{13}C HSQC NMR spectrum of compound Cyclo(-Leu-Leu-Val-Leu-*p*-I-Phe-) (4).



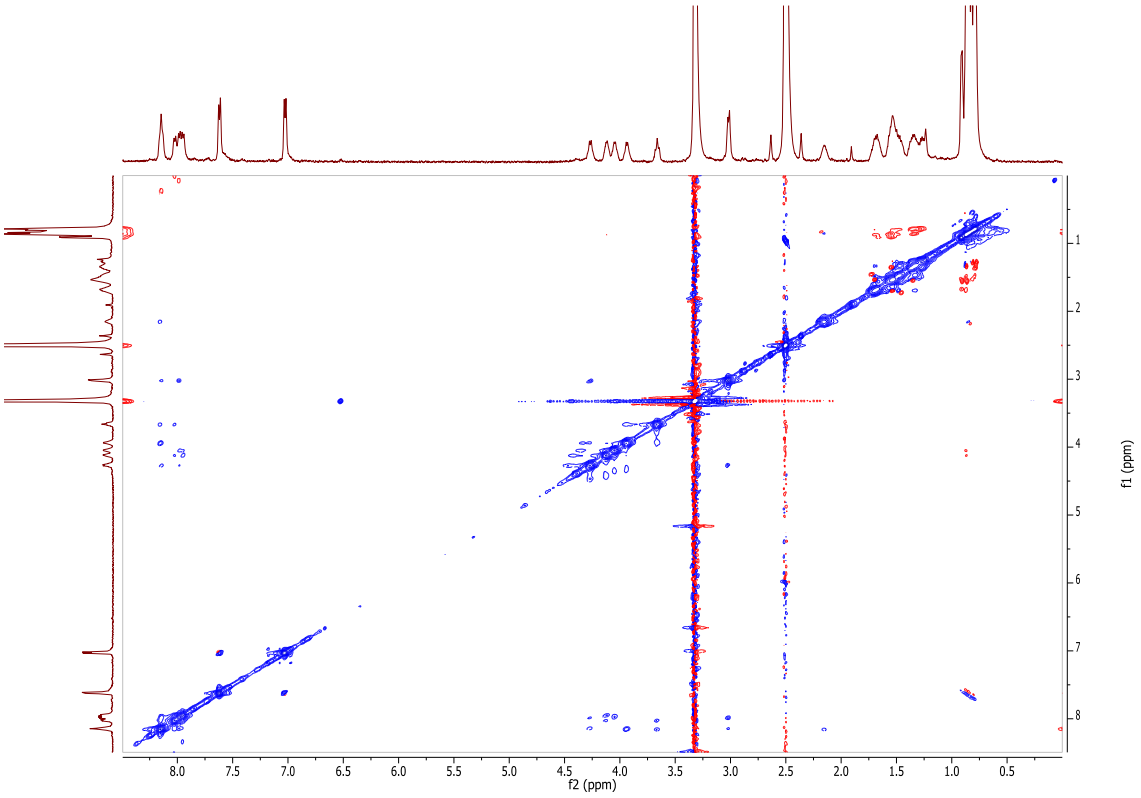
Supplementary Figure 103 | COSY NMR spectrum of compound Cyclo(-Leu-Leu-Val-Leu-*p*-I-Phe-) (4).



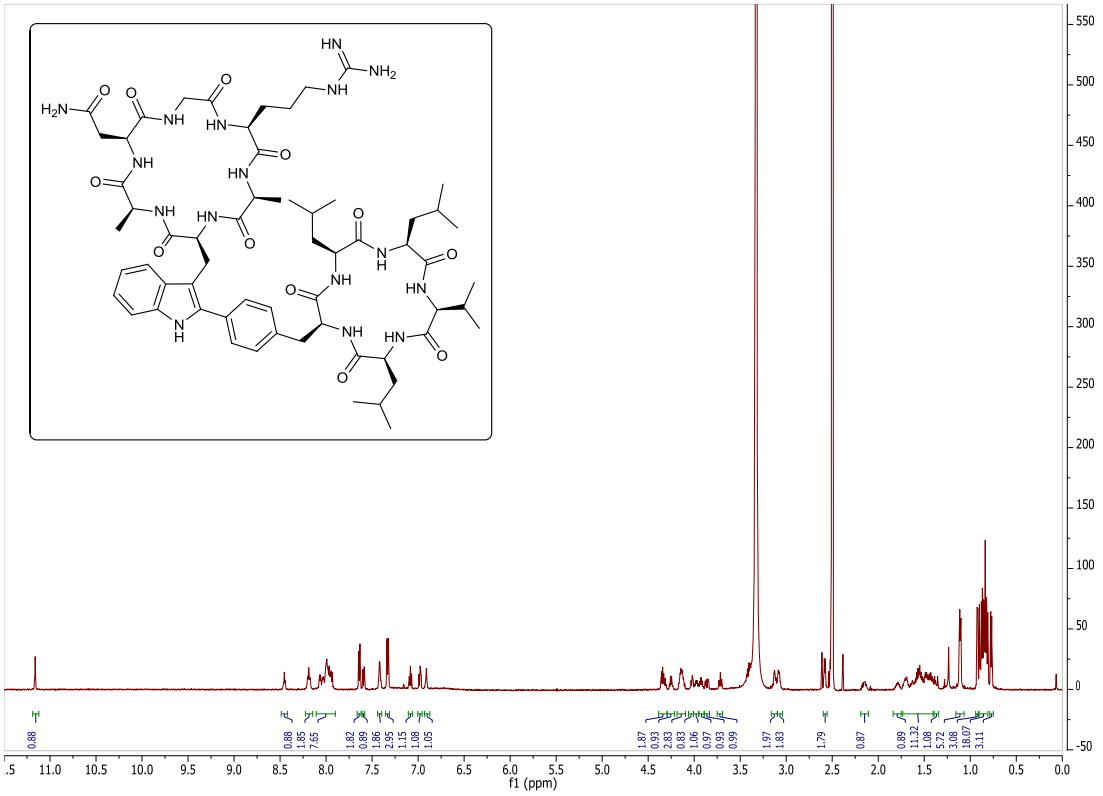
Supplementary Figure 104 | TOCSY NMR spectrum of compound Cyclo(-Leu-Leu-Val-Leu-*p*-I-Phe-) (4).



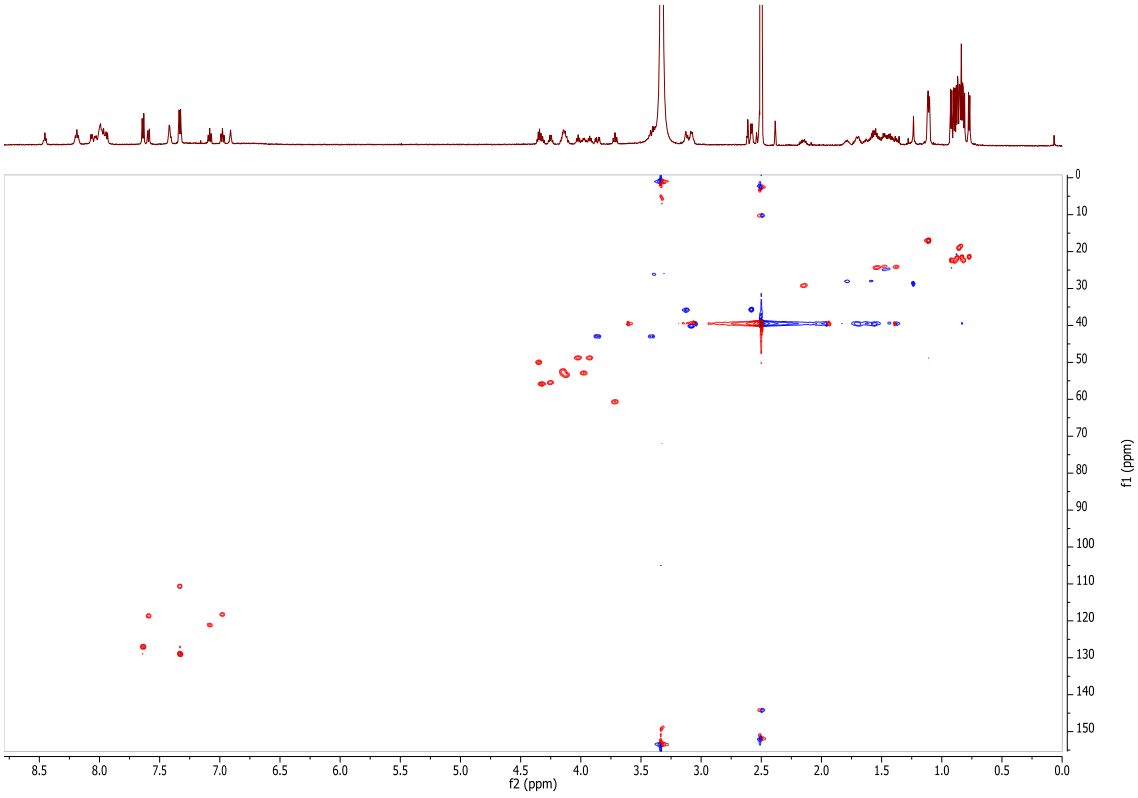
Supplementary Figure 105 | ROESY NMR spectrum of compound Cyclo(-Leu-Leu-Val-Leu-*p*-I-Phe-) (4).



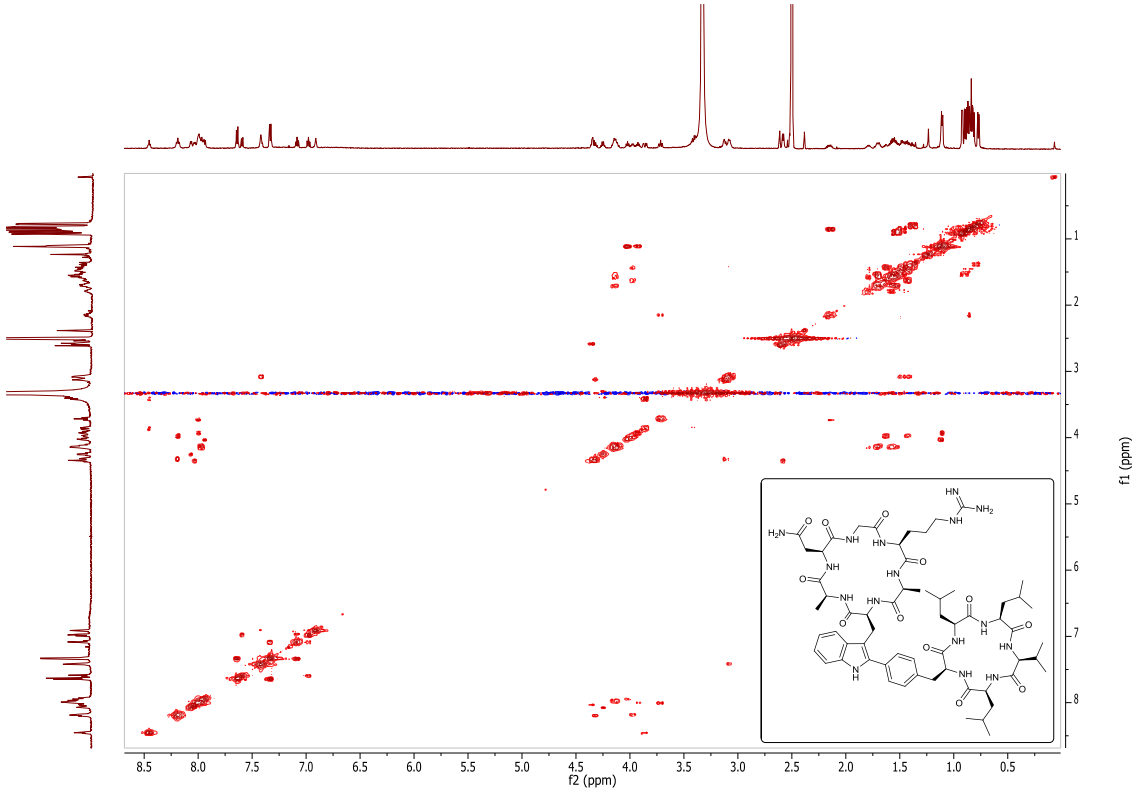
Supplementary Figure 106 | NOESY NMR spectrum of compound Cyclo(-Leu-Leu-Val-Leu-*p*-I-Phe-) (4).



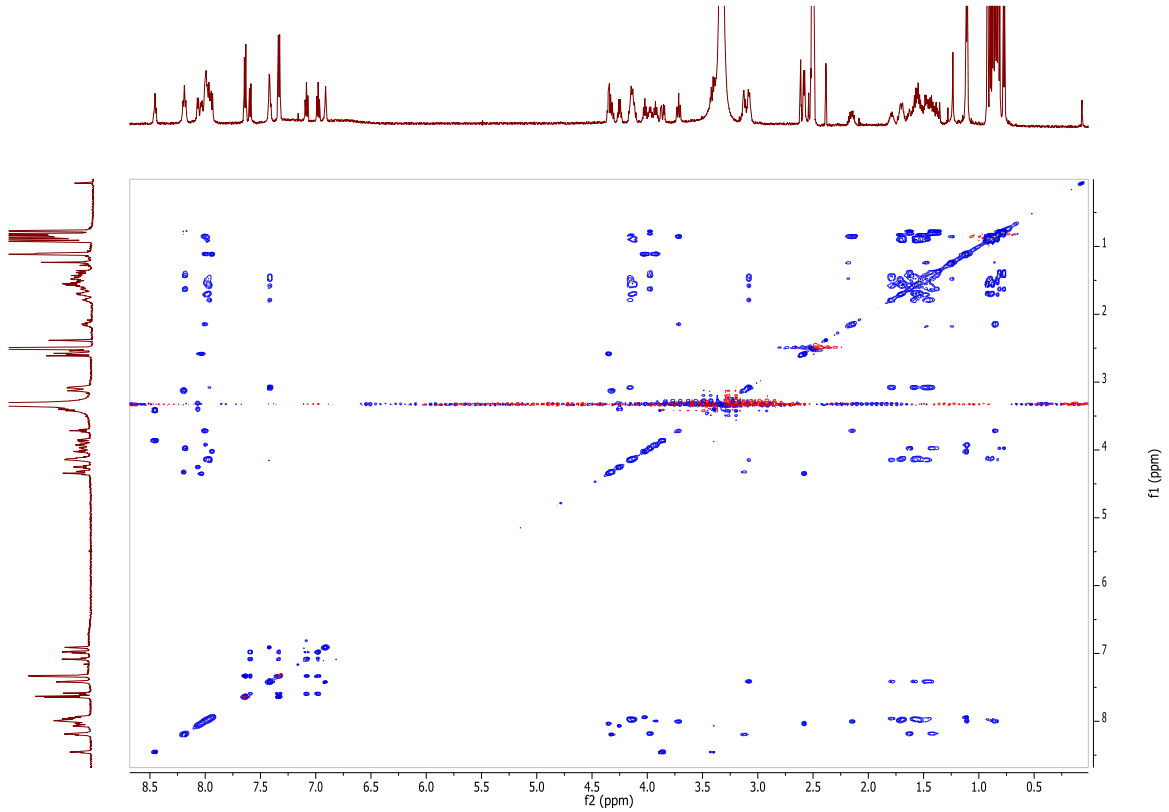
Supplementary Figure 107 | ^{1}H NMR spectrum of compound Cyclo(Ala-Asn-Gly-Arg-Ala-C2-Trp-)—Cyclo(C4-Phe-Leu-Leu-Val-Leu-) (5).



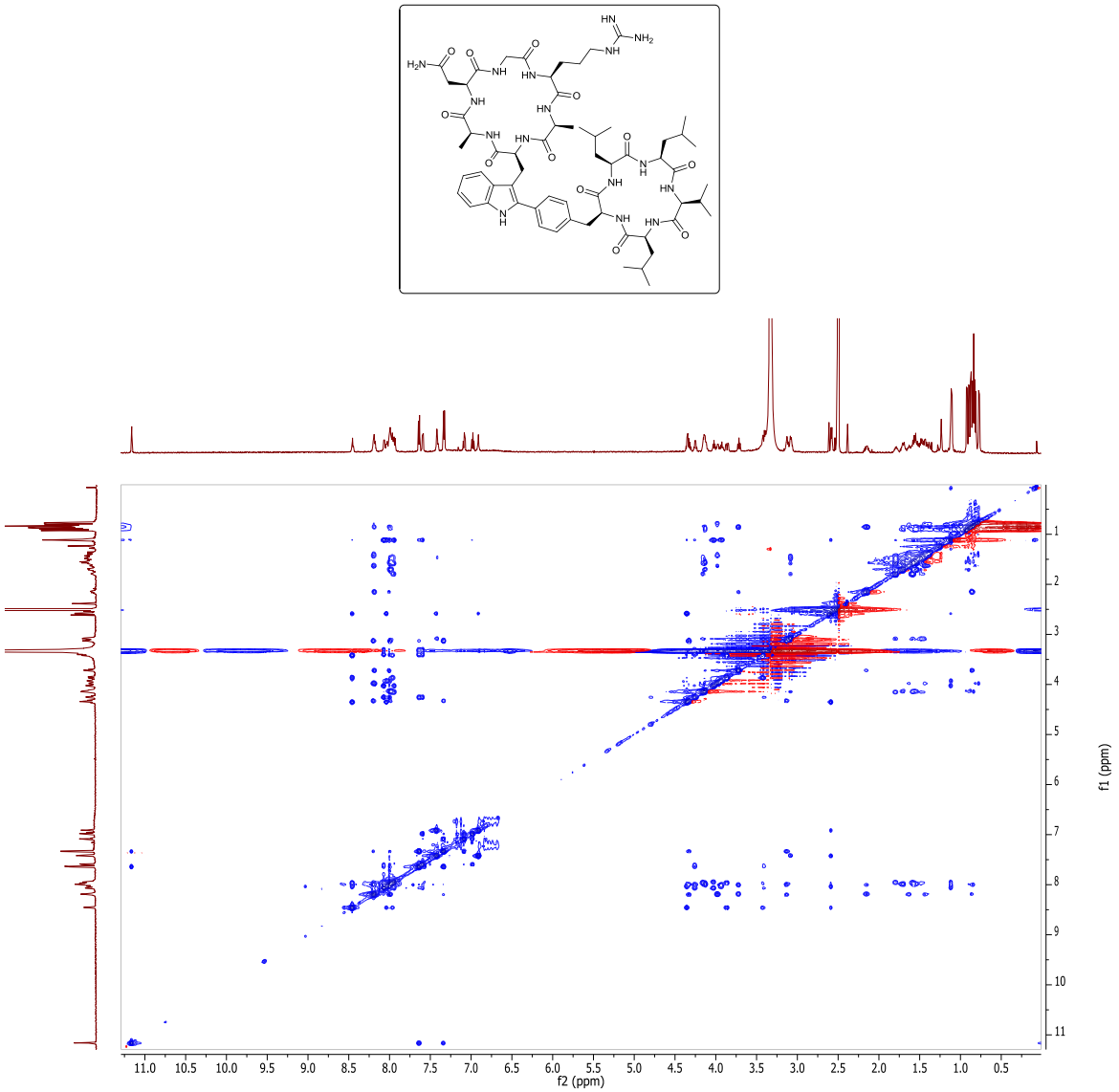
Supplementary Figure 108 | ^{1}H-^{13}C HSQC NMR spectrum of compound Cyclo(Ala-Asn-Gly-Arg-Ala-C2-Trp-)—Cyclo(C4-Phe-Leu-Leu-Val-Leu-) (5).



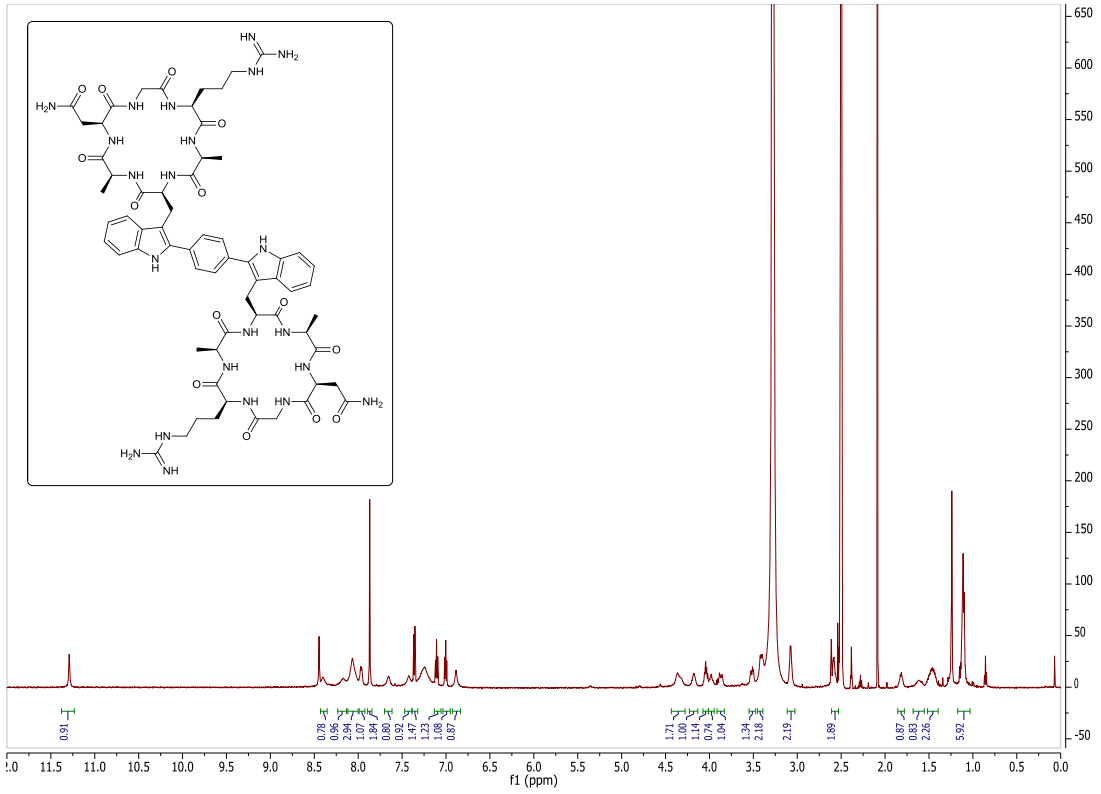
Supplementary Figure 109 | COSY NMR spectrum of compound Cyclo(Ala-Asn-Gly-Arg-Ala-C2-Trp-)—Cyclo(C4-Phe-Leu-Leu-Val-Leu-) (5).



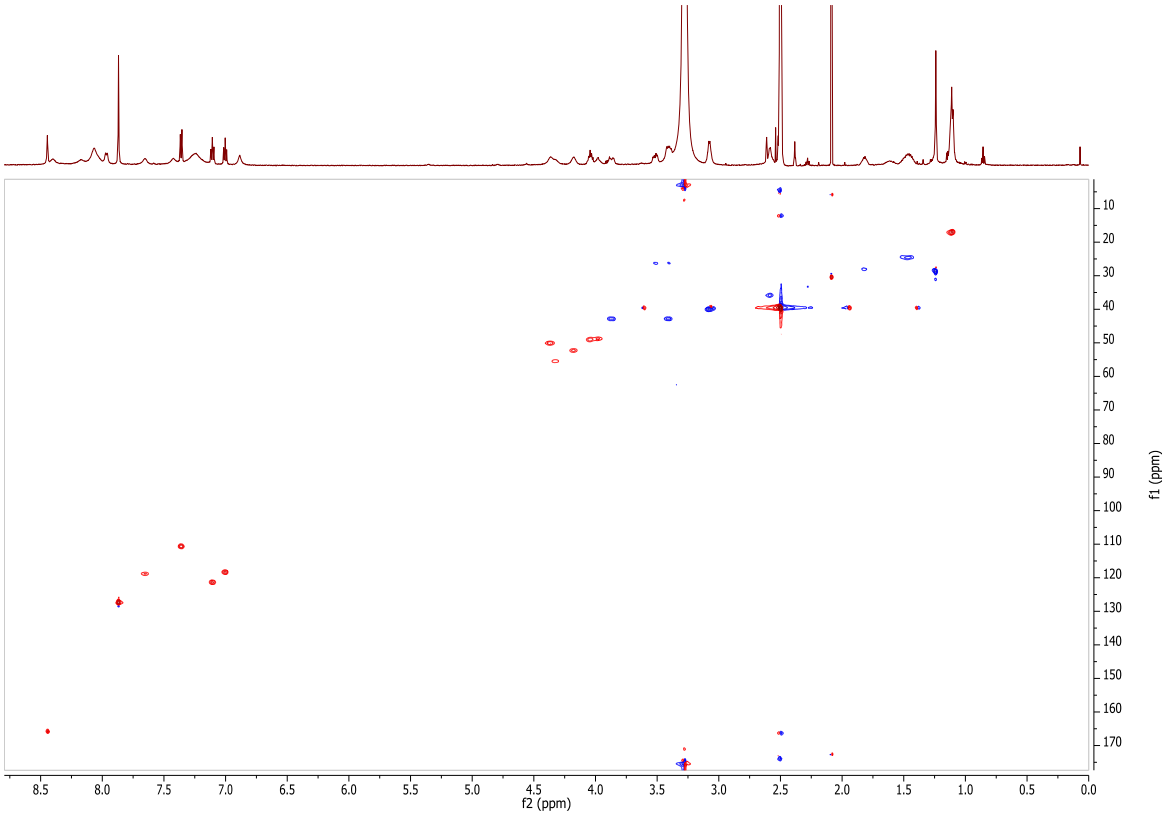
Supplementary Figure 110 | TOCSY NMR spectrum of compound Cyclo(Ala-Asn-Gly-Arg-Ala-C2-Trp-)—Cyclo(C4-Phe-Leu-Leu-Val-Leu-) (5).



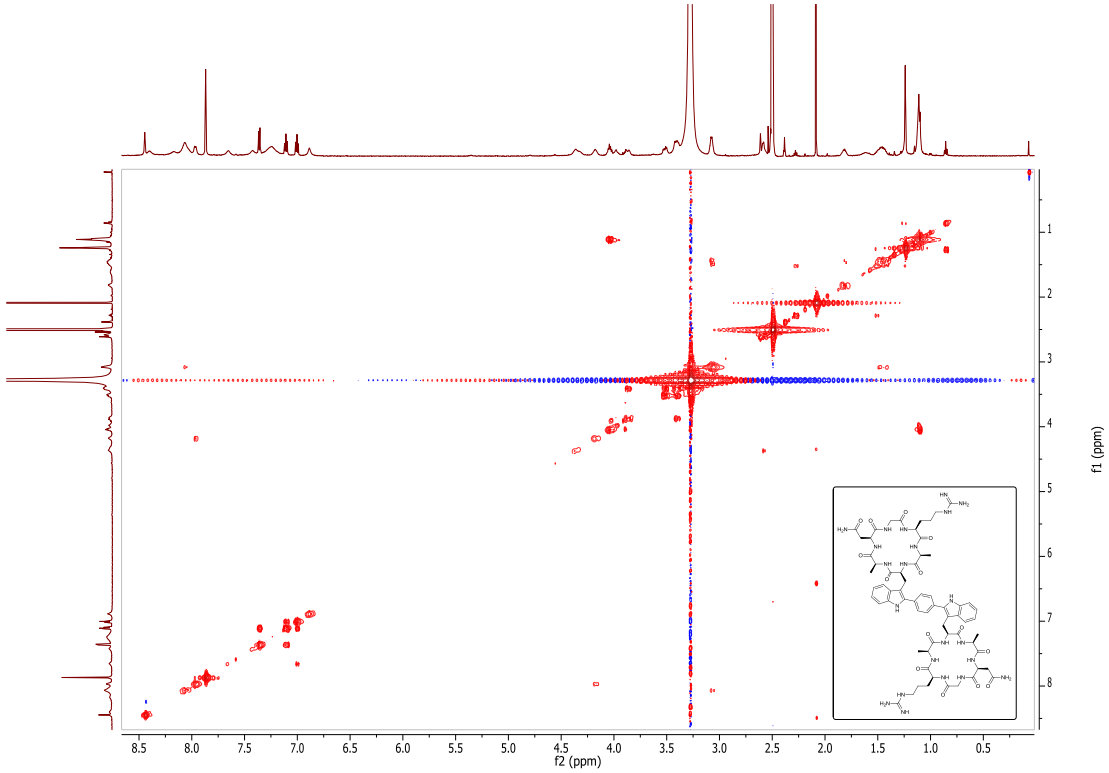
Supplementary Figure 111 | NOESY NMR spectrum of compound Cyclo(Ala-Asn-Gly-Arg-Ala-C2-Trp-)—Cyclo(C4-Phe-Leu-Leu-Val-Leu-) (5).



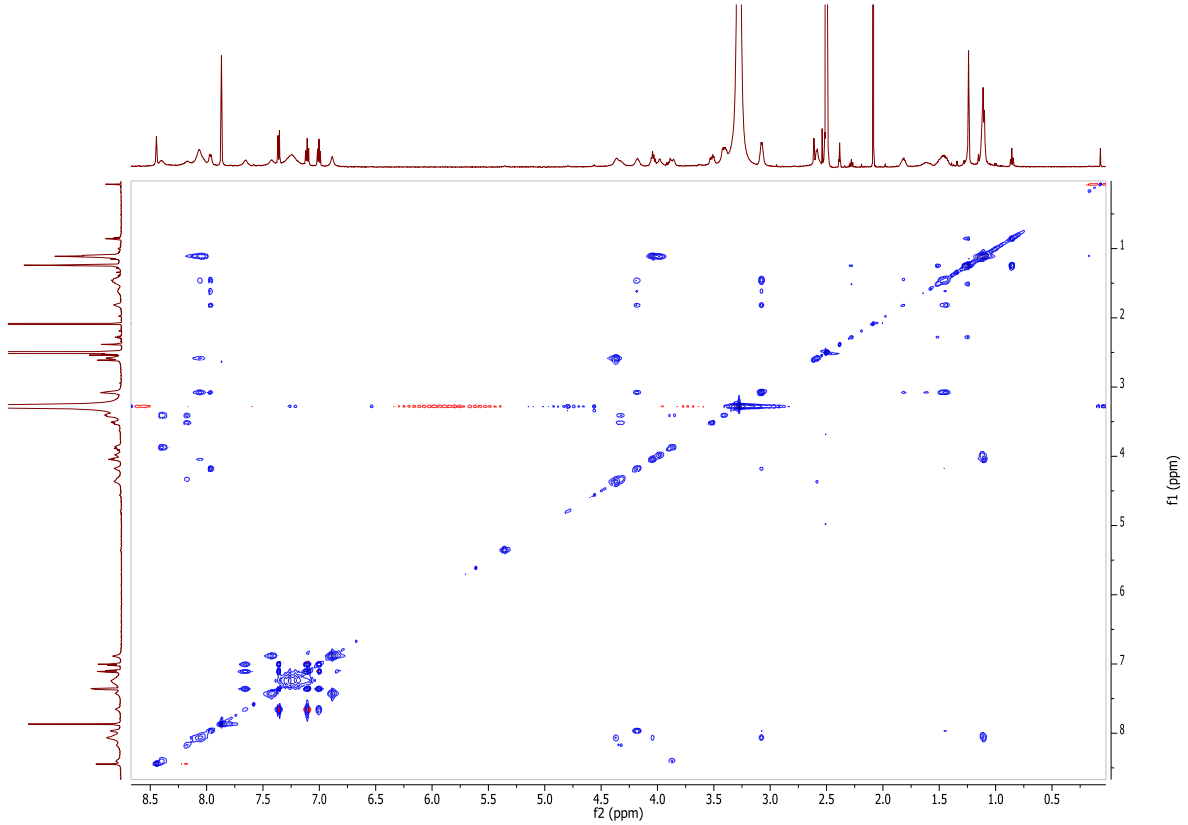
Supplementary Figure 112 | ^{1}H NMR spectrum of compound Bis[cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-)] adduct (6).



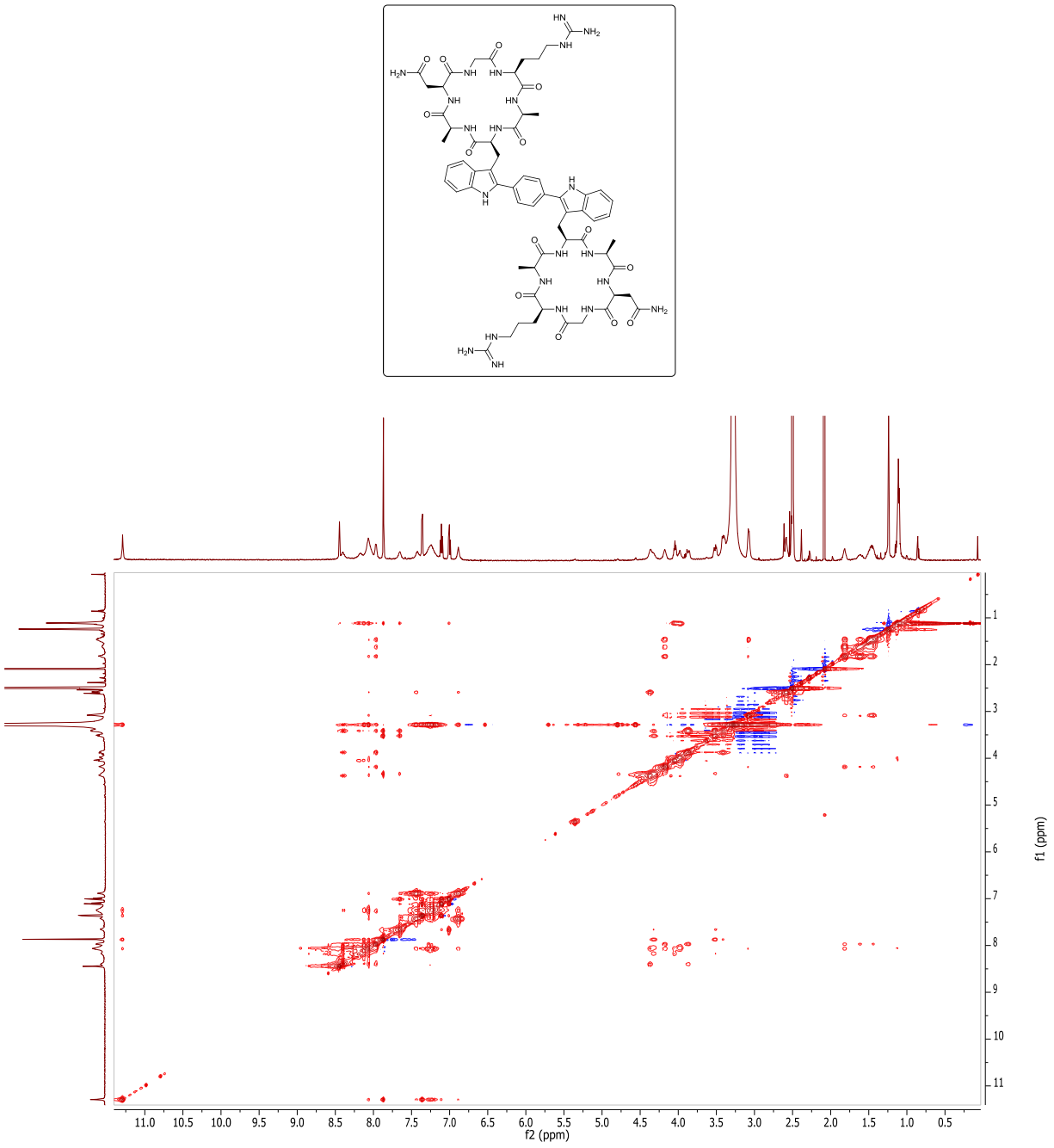
Supplementary Figure 113 | ^{1}H-^{13}C HSQC NMR spectrum of compound Bis[cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-)] adduct (6).



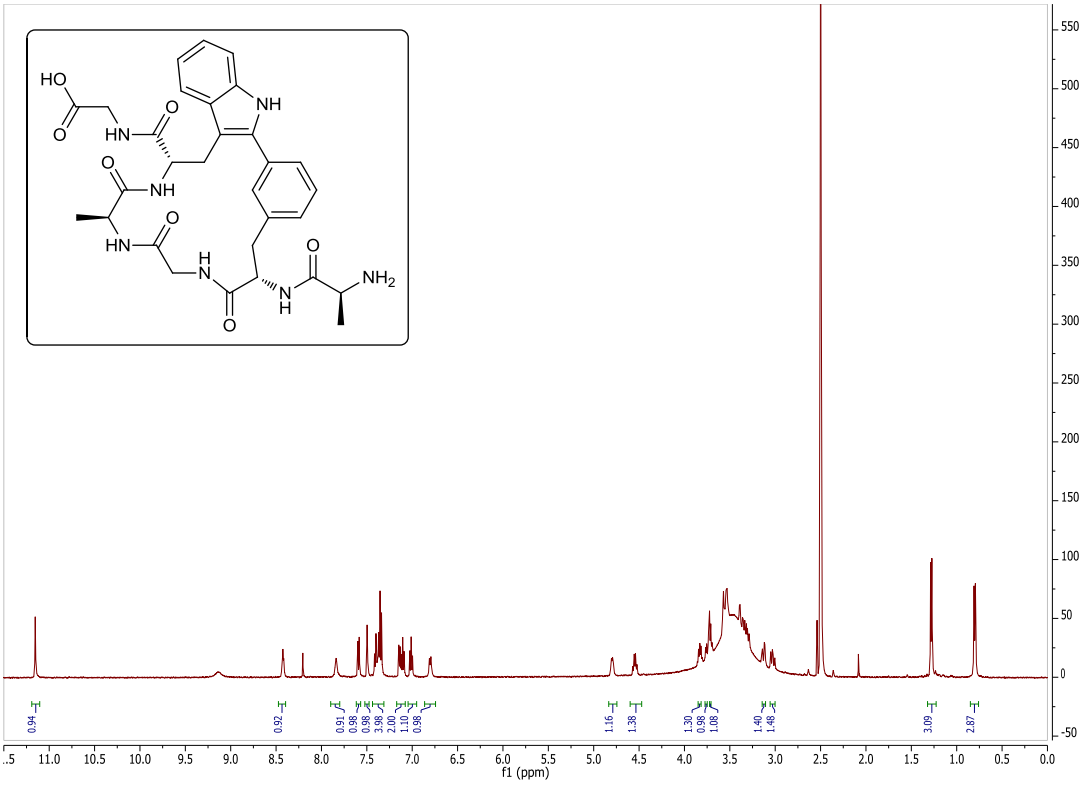
Supplementary Figure 114 | COSY NMR spectrum of compound Bis[cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-)] adduct (6).



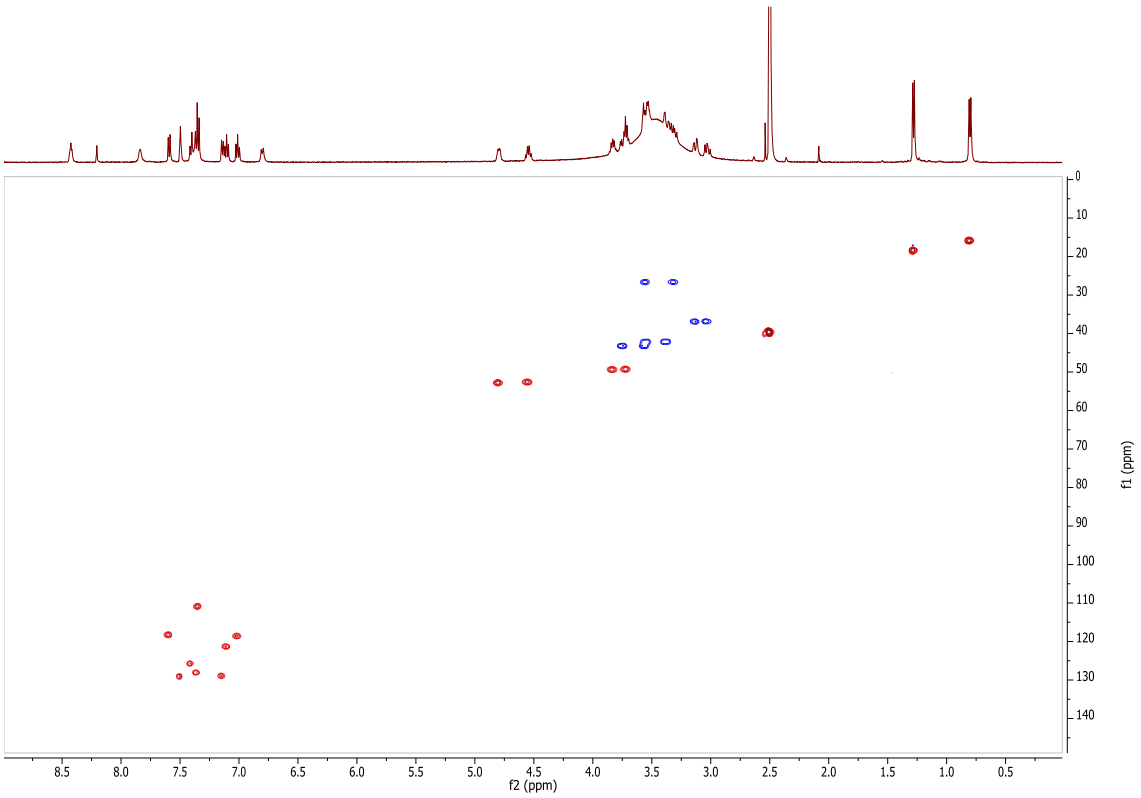
Supplementary Figure 115 | TOCSY NMR spectrum of compound Bis[cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-)] adduct (6).



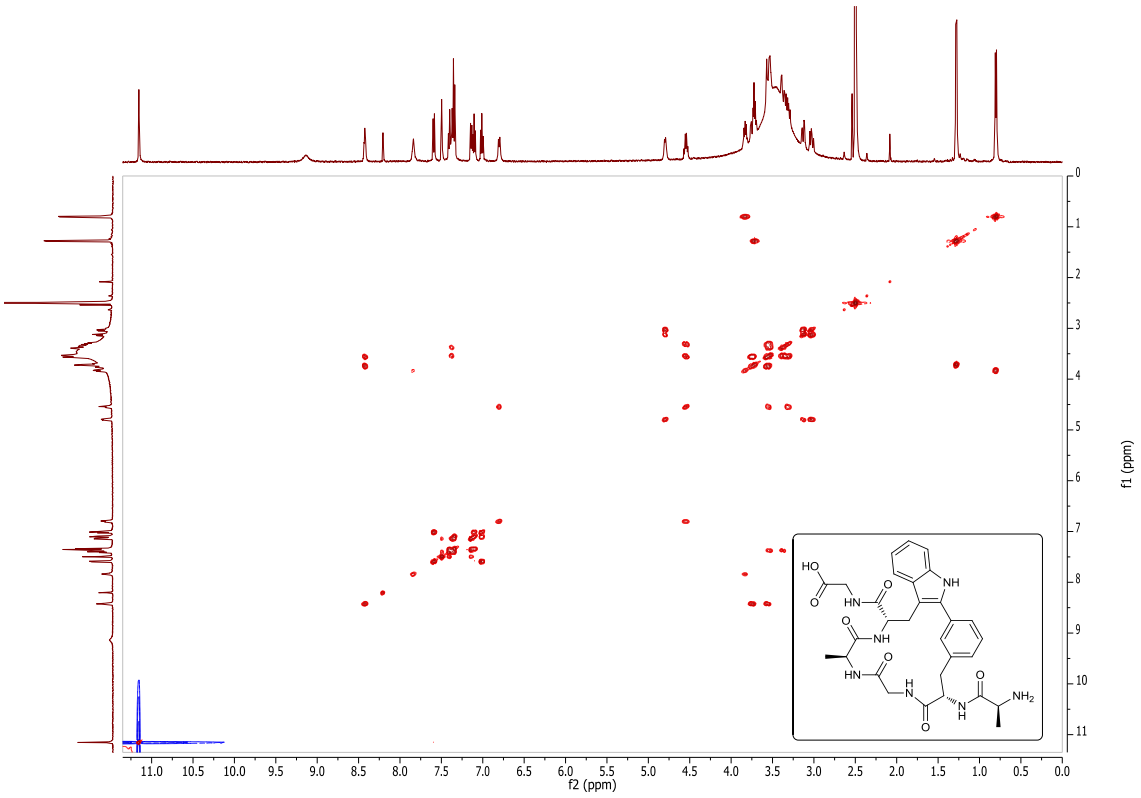
Supplementary Figure 116 | NOESY NMR spectrum of compound Bis[cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-)] adduct (6).



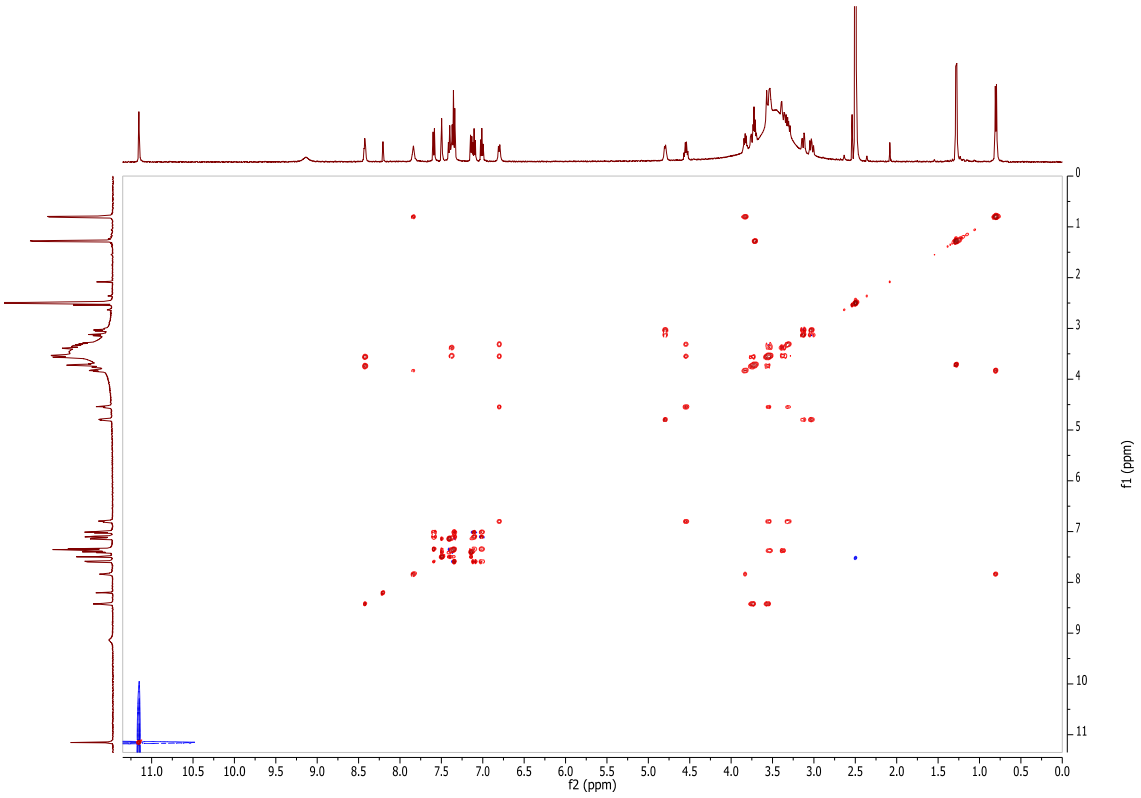
Supplementary Figure 117 ‖ ^{1}H NMR spectrum of compound H-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-OH (9).



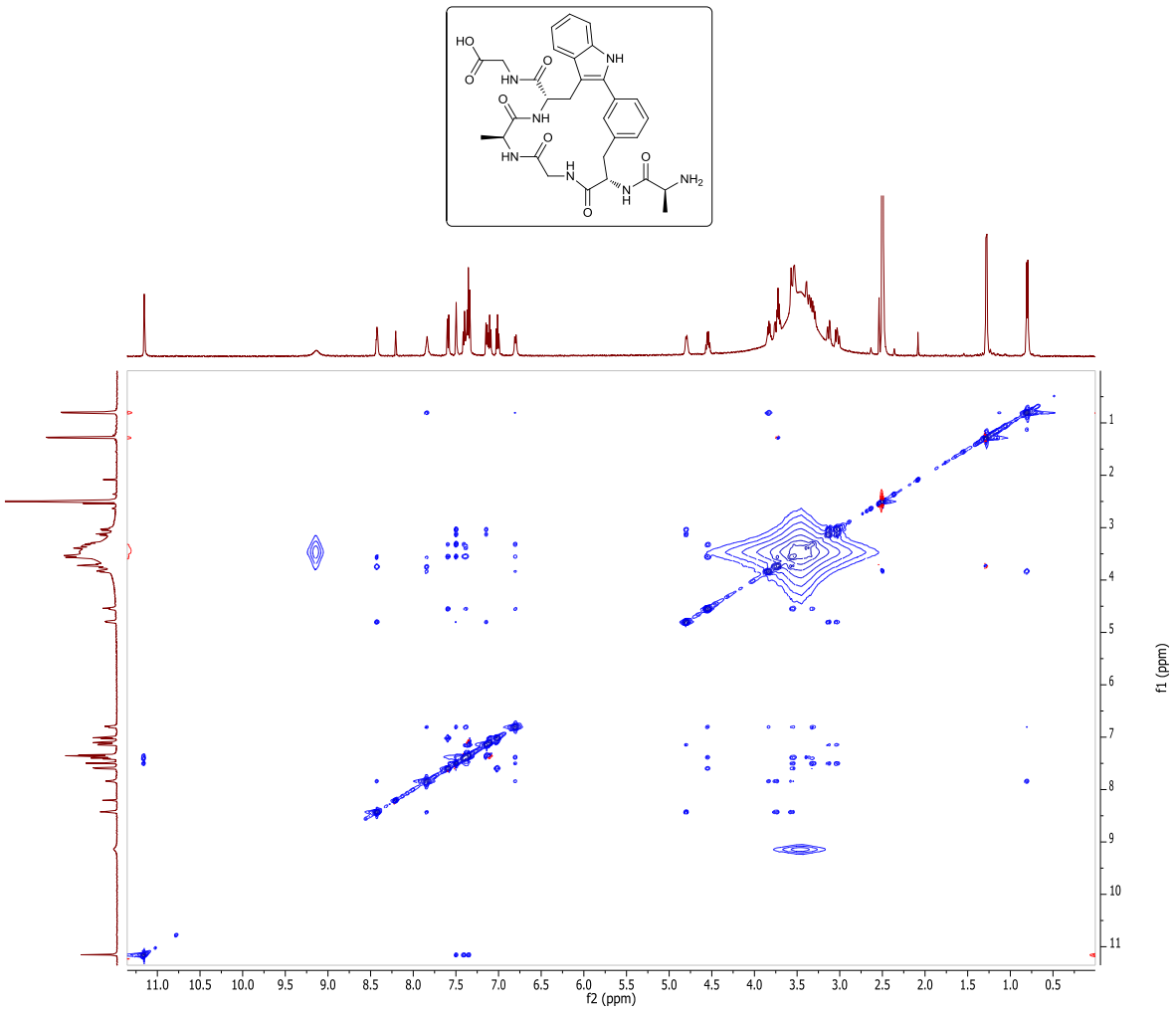
Supplementary Figure 118 ‖ ^{1}H-^{13}C HSQC NMR spectrum of compound H-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-OH (9).



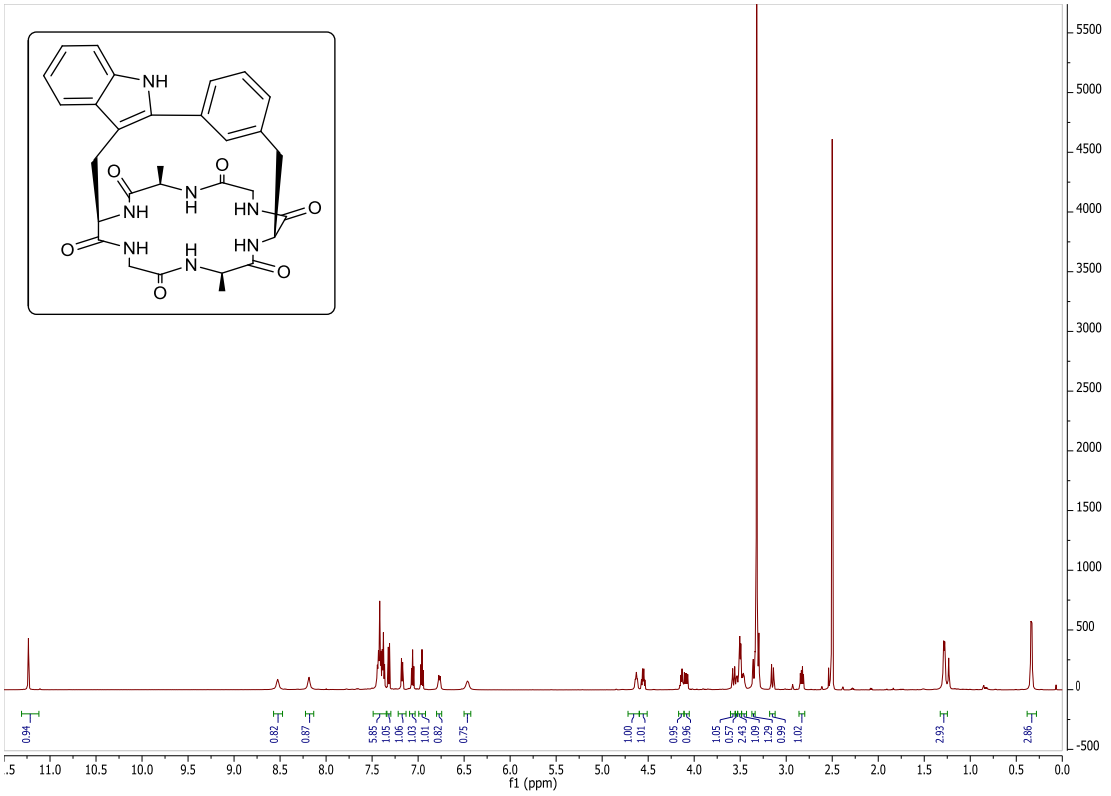
Supplementary Figure 119 | COSY NMR spectrum of compound H-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-OH (9).



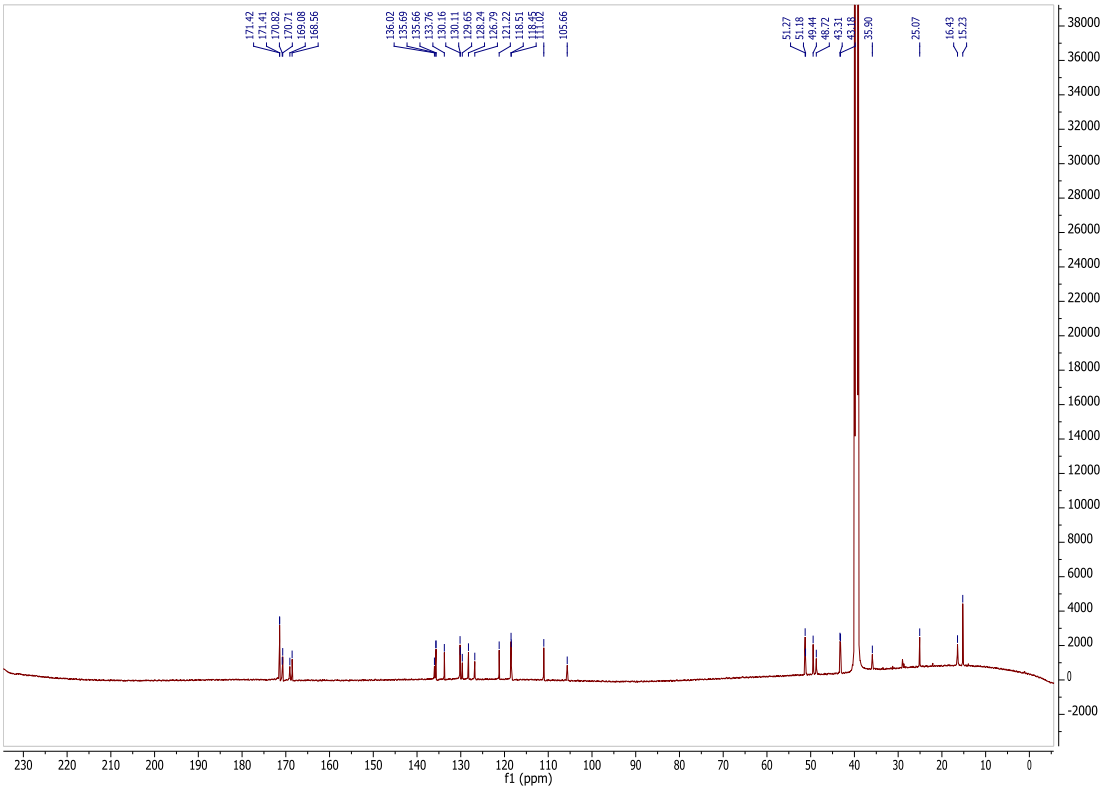
Supplementary Figure 120 | TOCSY NMR spectrum of compound H-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-OH (9).



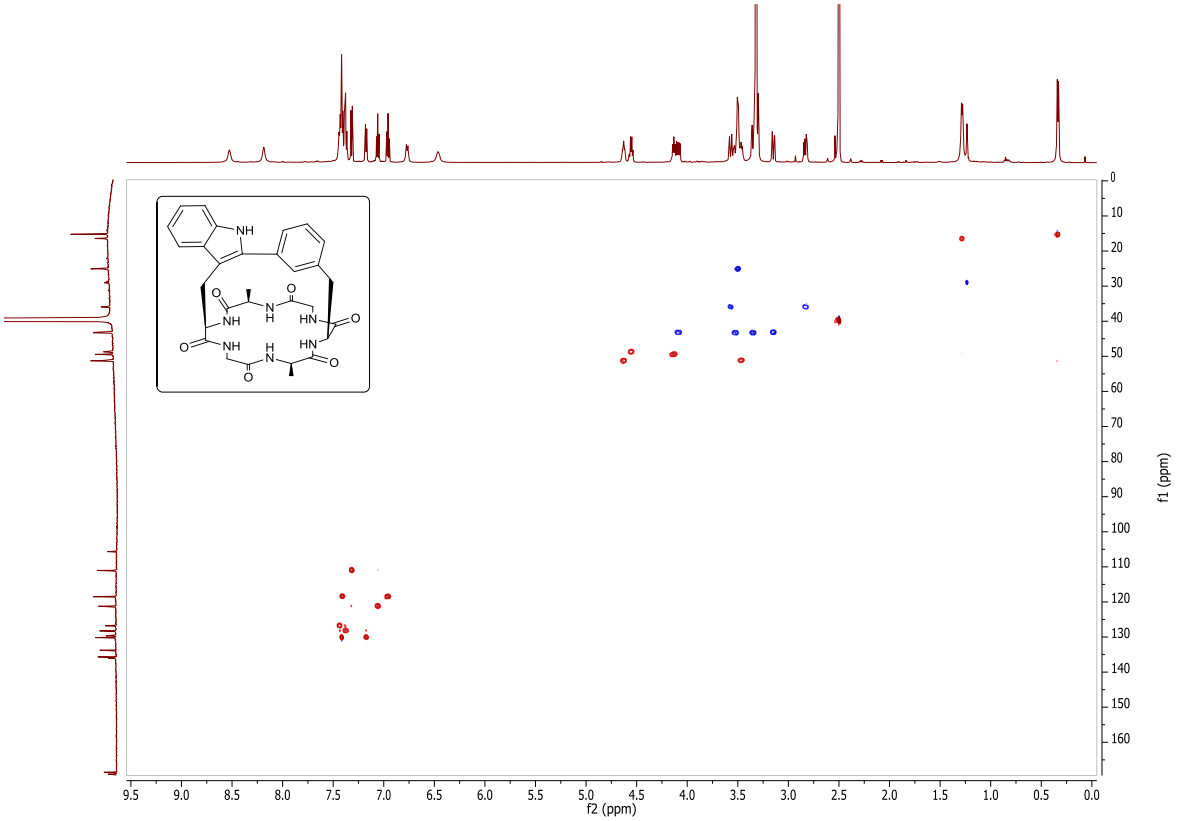
Supplementary Figure 121 | NOESY NMR spectrum of compound H-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-OH (9).



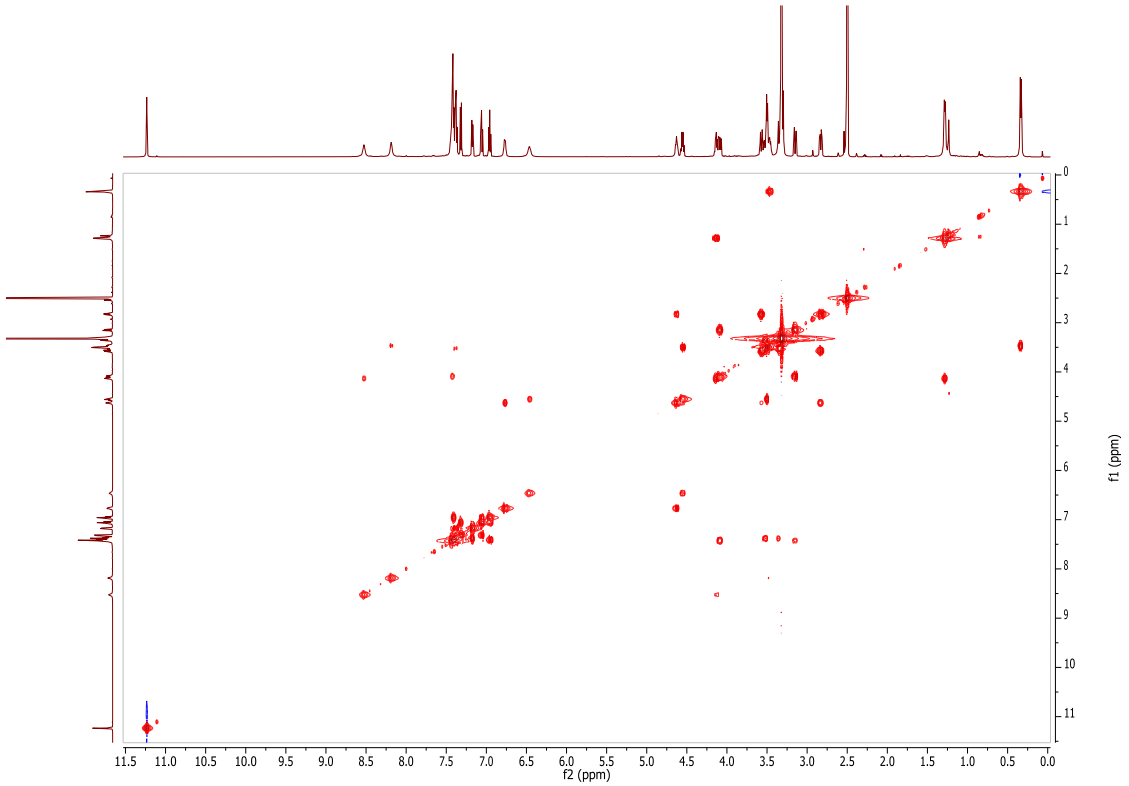
Supplementary Figure 122 | ^{1}H NMR spectrum of compound Cyclo[-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-] (10).



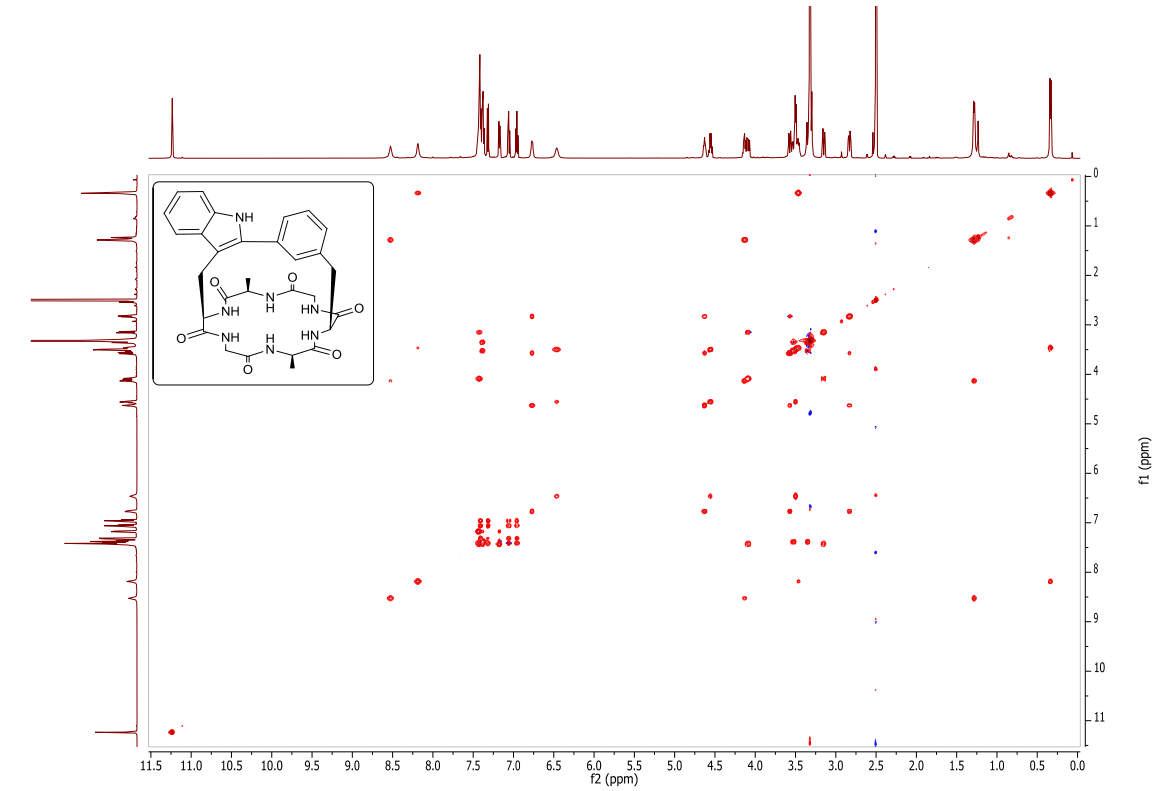
Supplementary Figure 123 | ^{13}C NMR spectrum of compound Cyclo[-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-] (10).



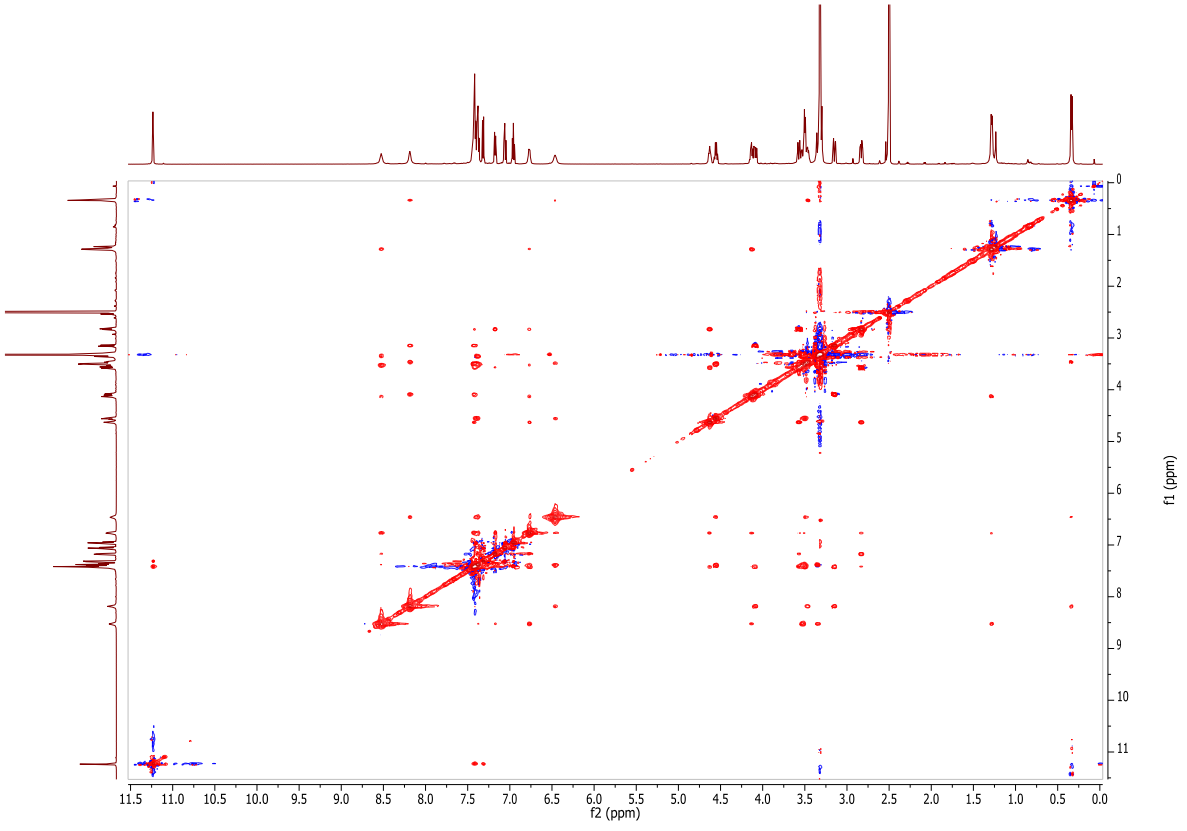
Supplementary Figure 124 | ^{1}H-^{13}C HSQC NMR spectrum of compound Cyclo[-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-] (10).



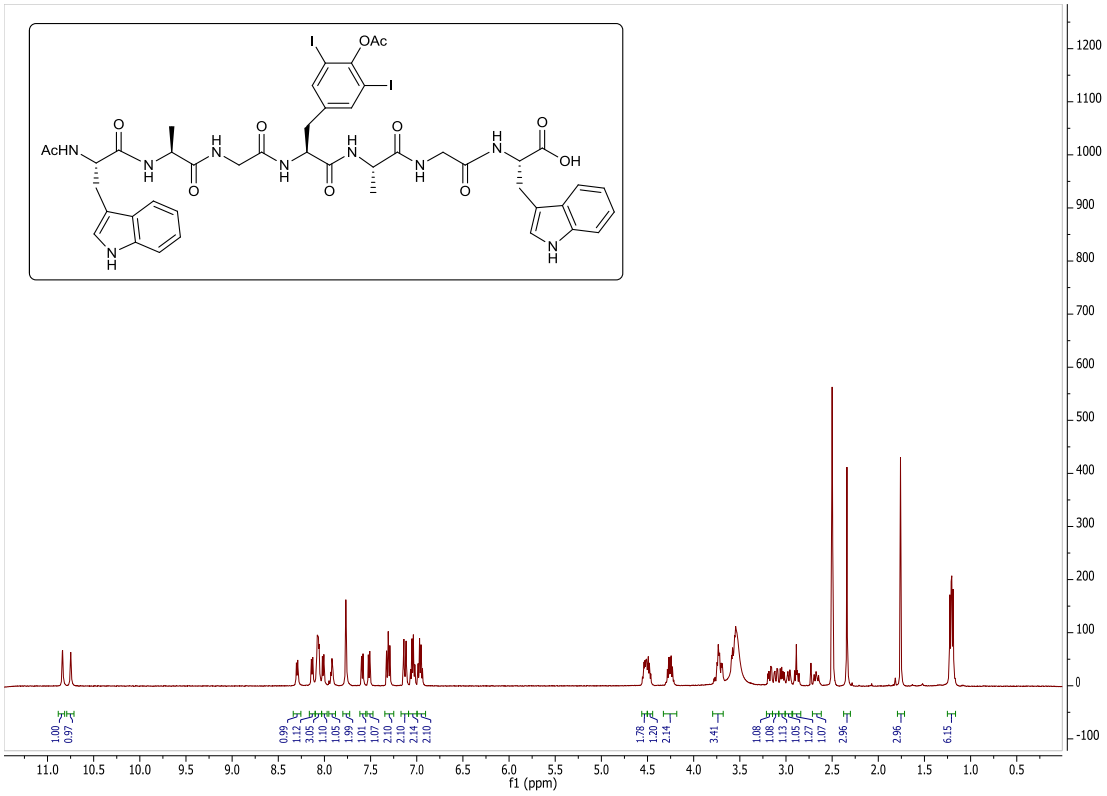
Supplementary Figure 125 | COSY NMR spectrum of compound Cyclo[-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-] (10).



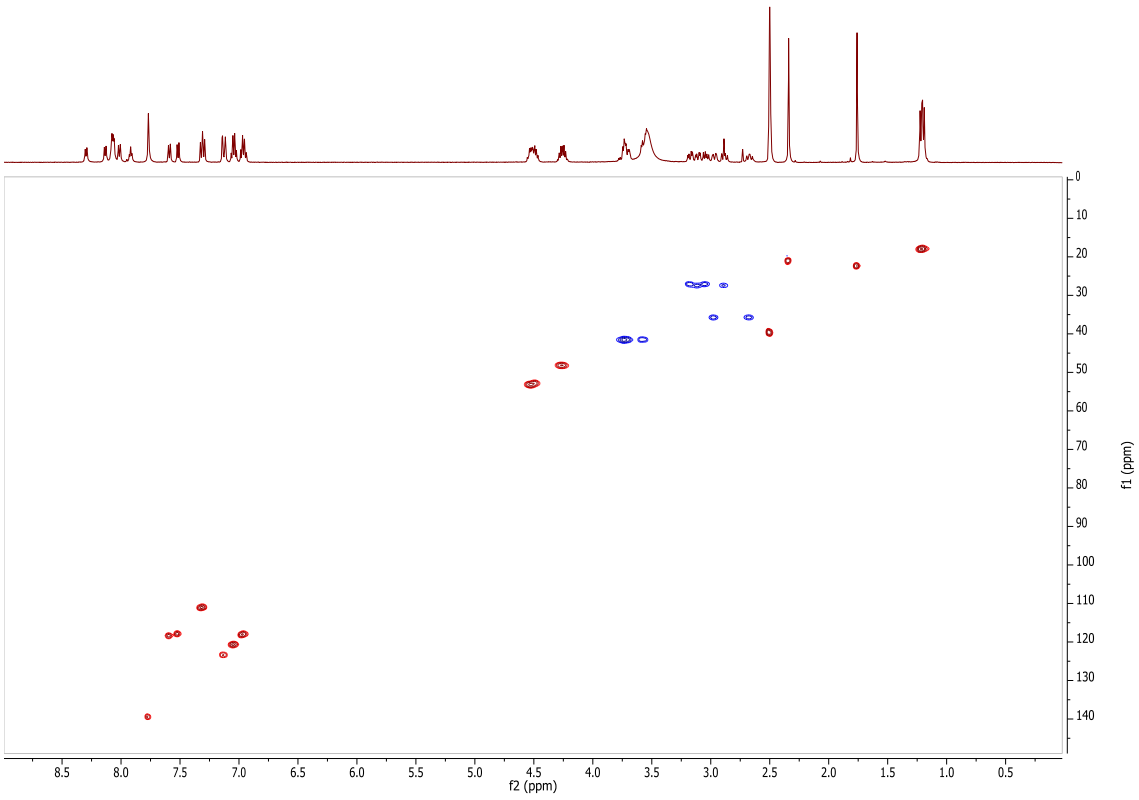
Supplementary Figure 126 | TOCSY NMR spectrum of compound Cyclo[-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-] (10).



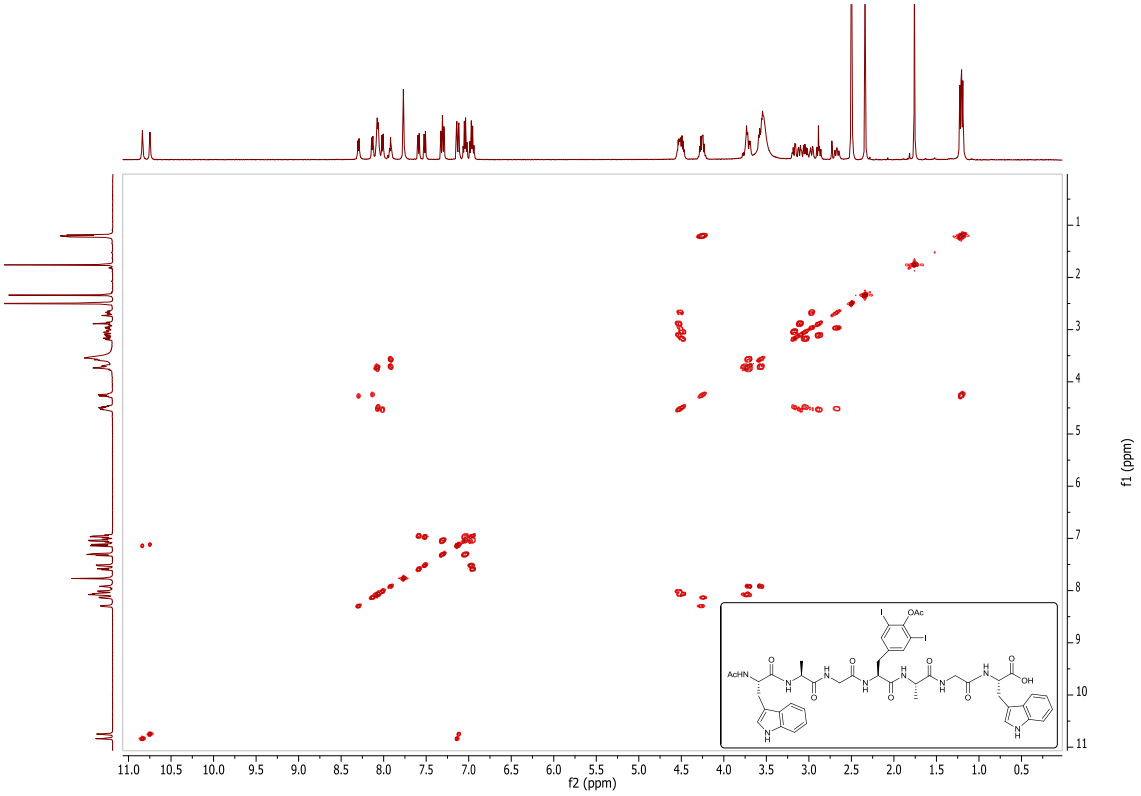
Supplementary Figure 127 | NOESY NMR spectrum of compound Cyclo[-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-] (10).



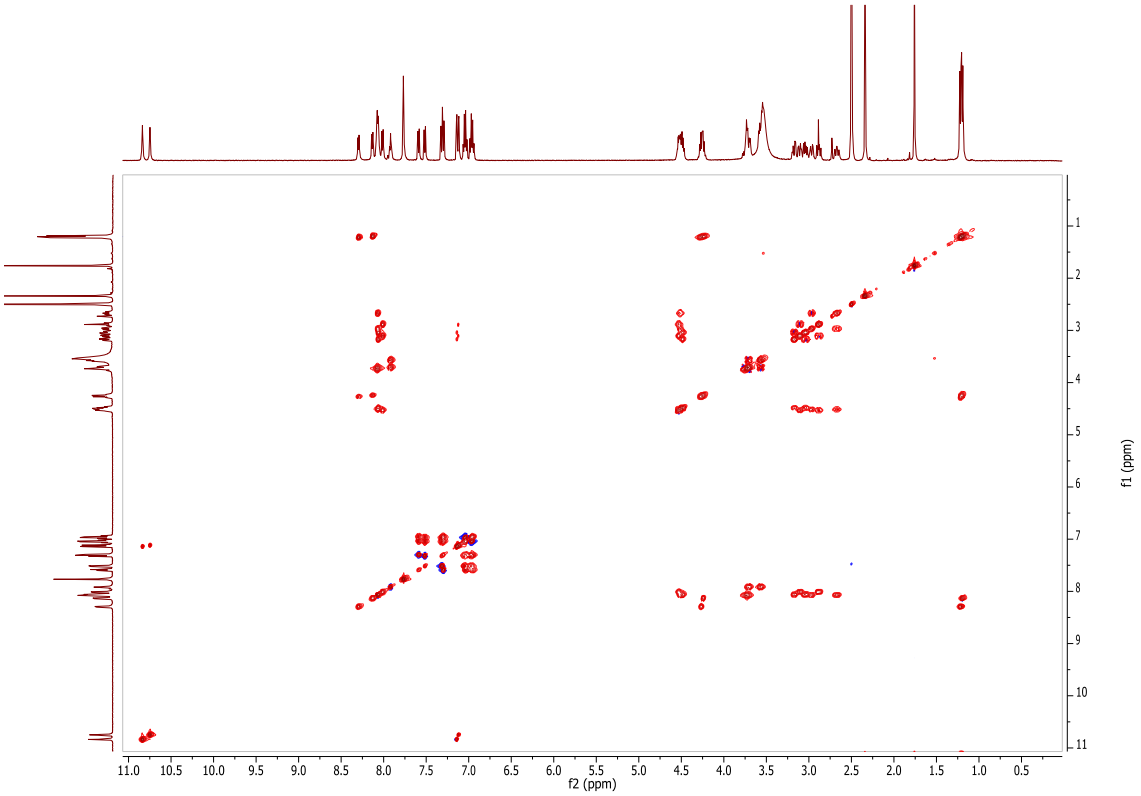
Supplementary Figure 128 | ^{1}H NMR spectrum of compound Ac-Trp-Ala-Gly-*3,5*-I,I-Tyr(OAc)-Ala-Gly-Trp-OH (11).



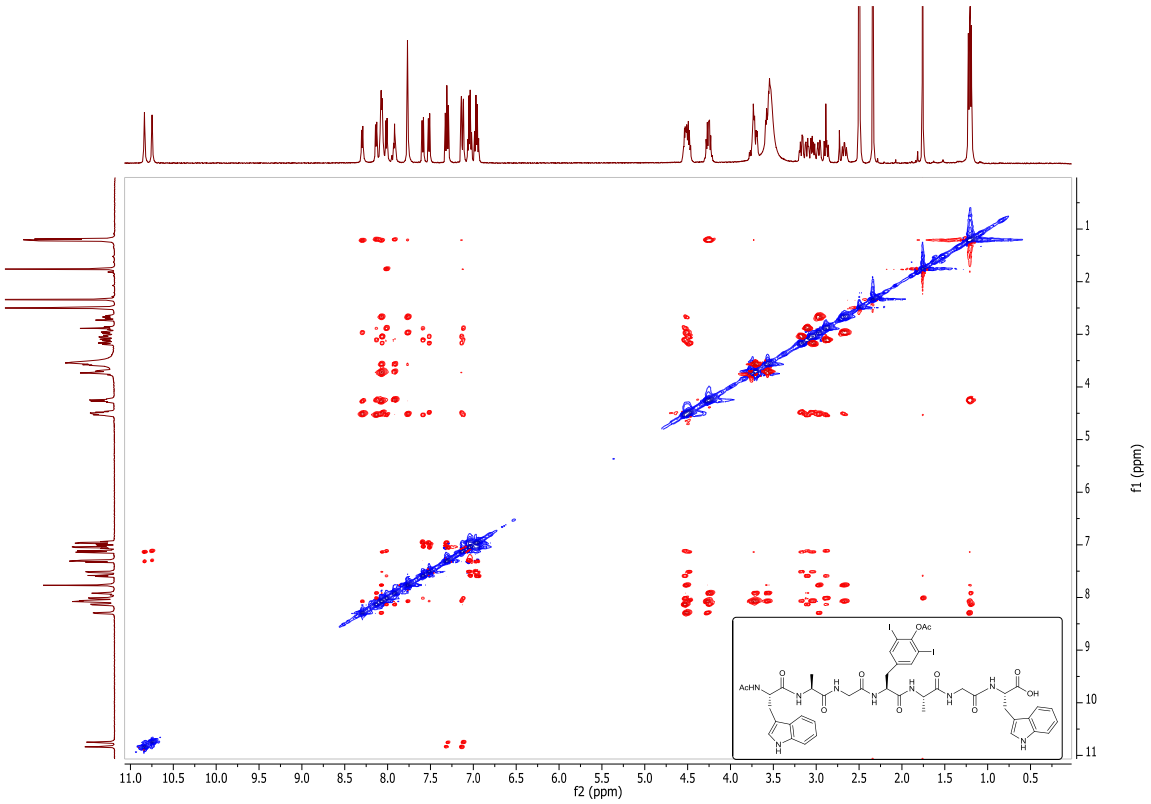
Supplementary Figure 129 | ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-Trp-Ala-Gly-*3,5*-I,I-Tyr(OAc)-Ala-Gly-Trp-OH (11).



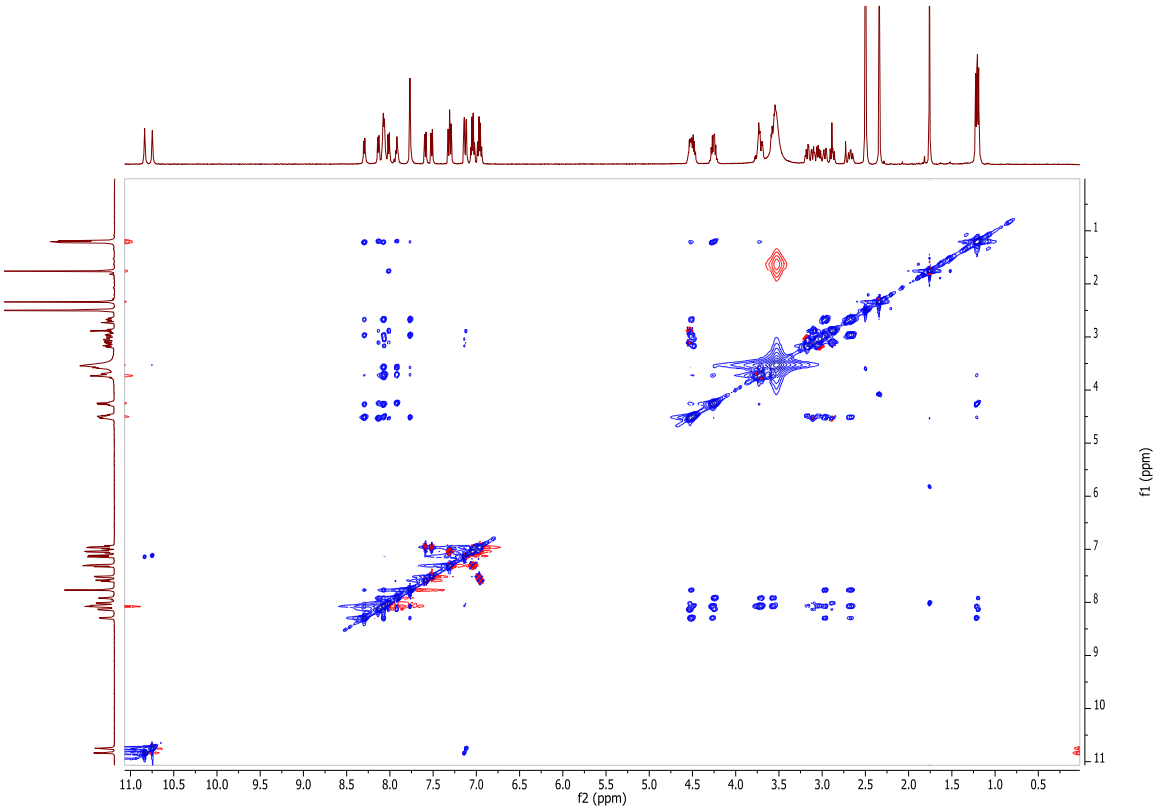
Supplementary Figure 130 | COSY NMR spectrum of compound Ac-Trp-Ala-Gly-*3,5*-I,I-Tyr(OAc)-Ala-Gly-Trp-OH (11).



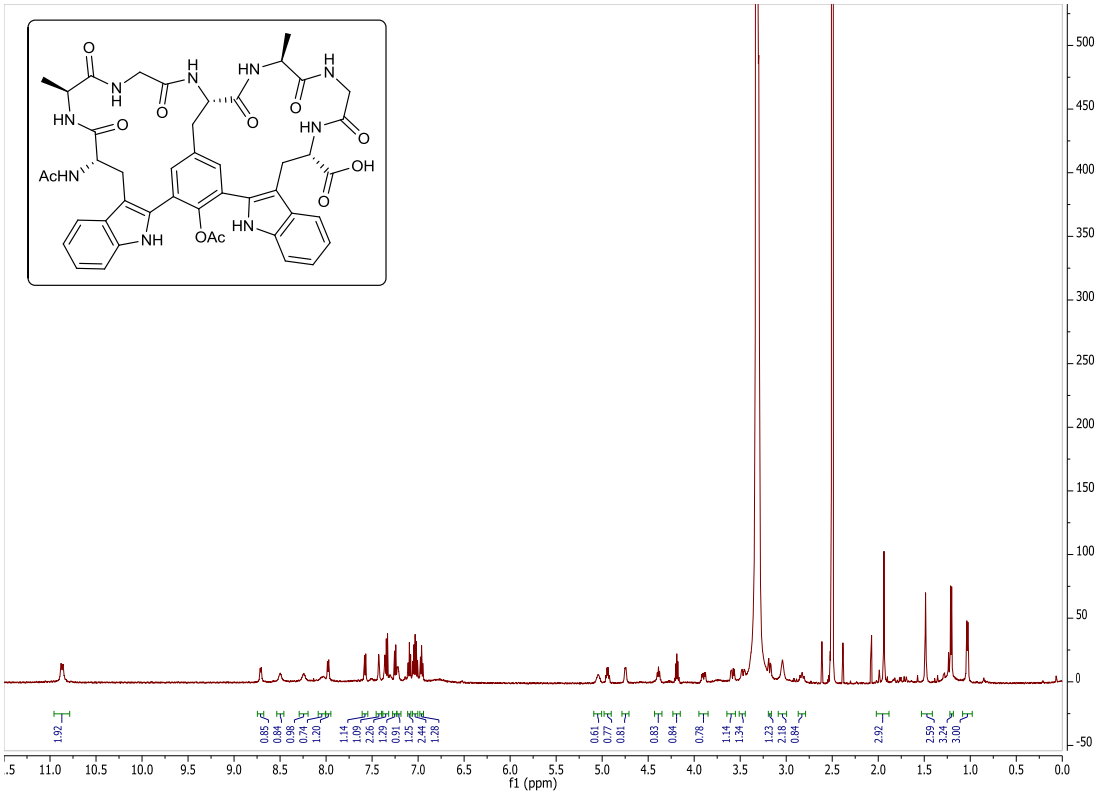
Supplementary Figure 131 | TOCSY NMR spectrum of compound Ac-Trp-Ala-Gly-*3,5*-I,I-Tyr(OAc)-Ala-Gly-Trp-OH (11).



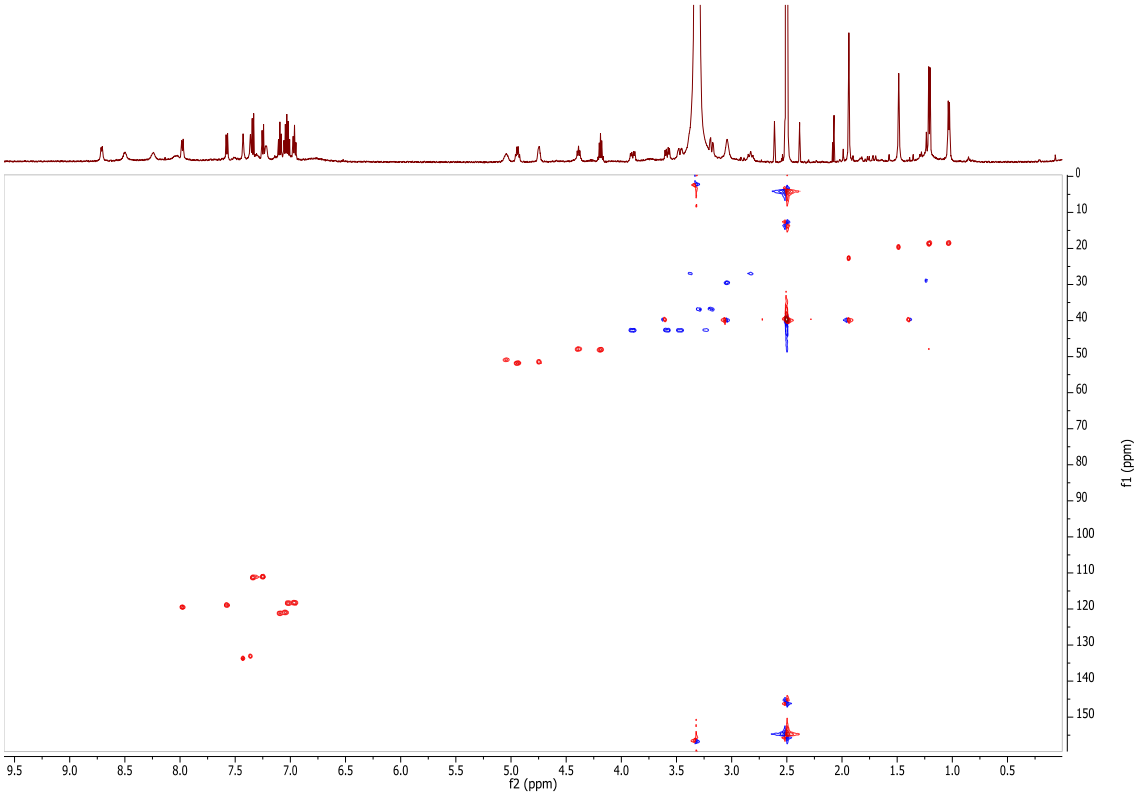
Supplementary Figure 132 | ROESY NMR spectrum of compound Ac-Trp-Ala-Gly-*3,5*-I,I-Tyr(OAc)-Ala-Gly-Trp-OH (11).



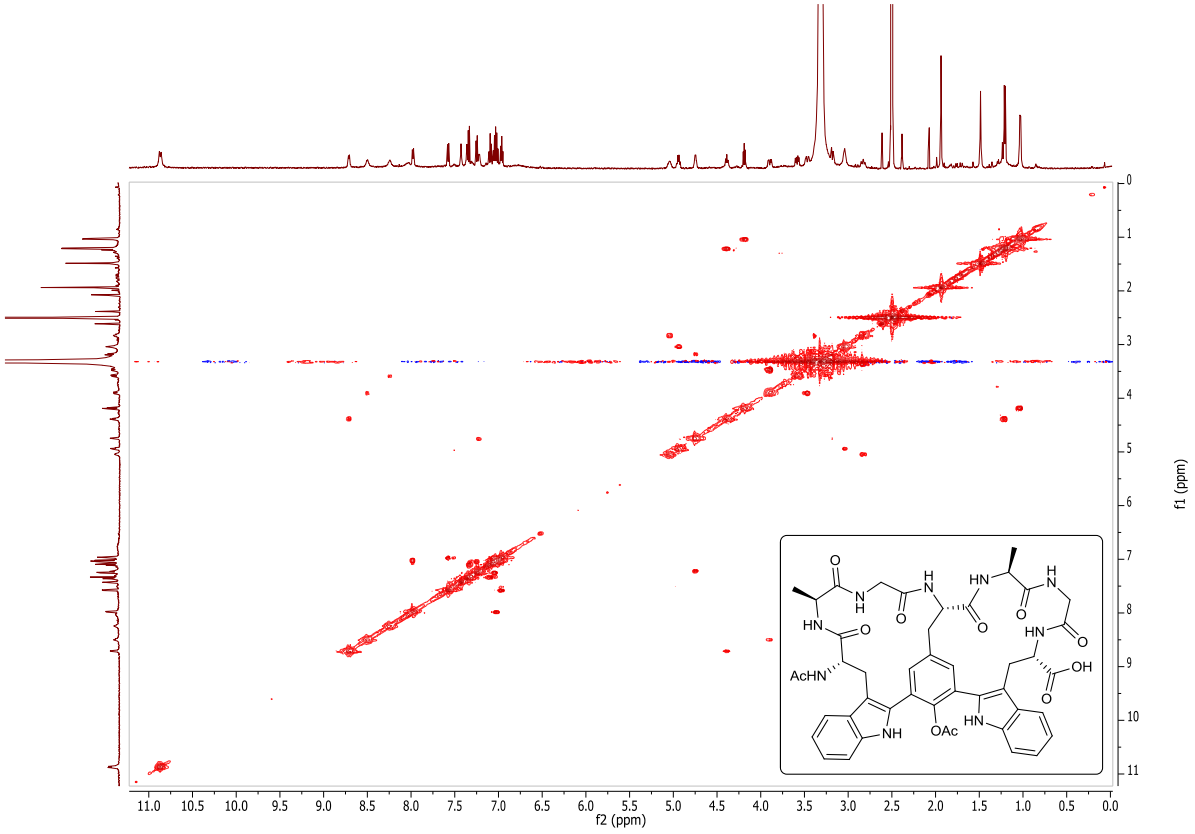
Supplementary Figure 133 | NOESY NMR spectrum of compound Ac-Trp-Ala-Gly-*3,5*-I,I-Tyr(OAc)-Ala-Gly-Trp-OH (11).



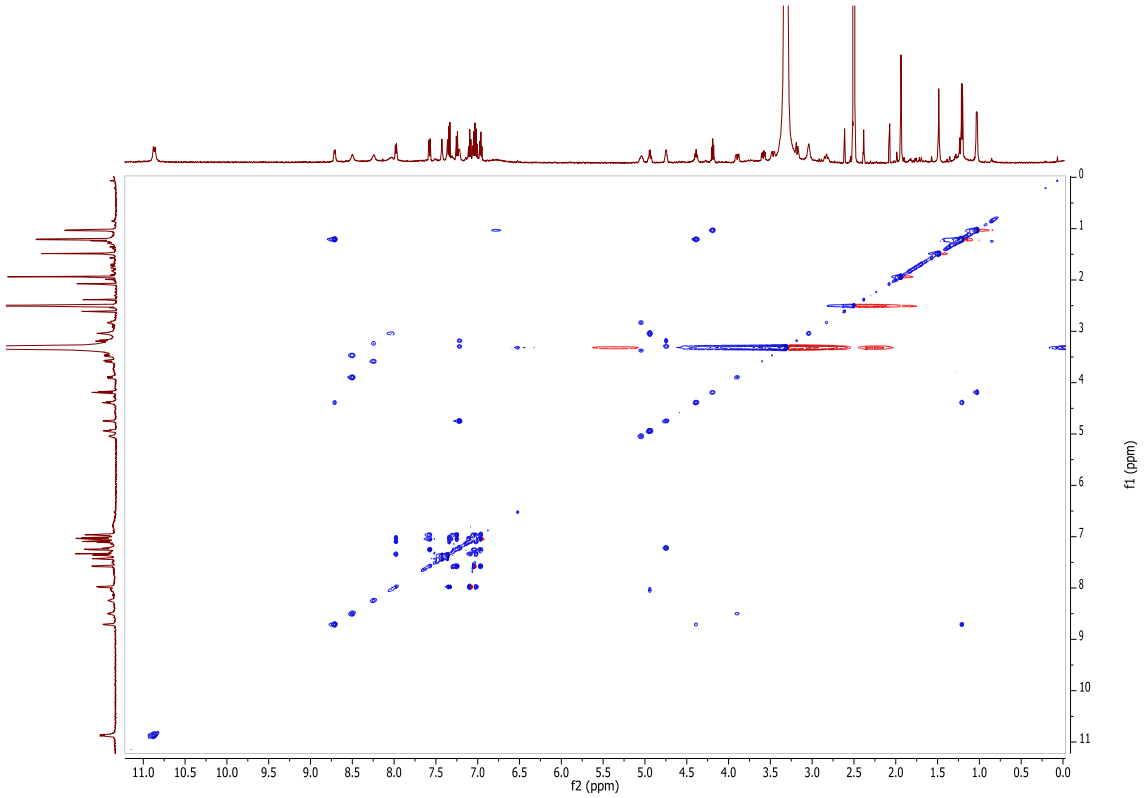
Supplementary Figure 134 | ^{1}H NMR spectrum of compound Ac-(bicyclo-*m,m*)-[Trp-Ala-Gly-Tyr(OAc)]-[Tyr(OAc)-Ala-Gly-Trp]-OH (12).



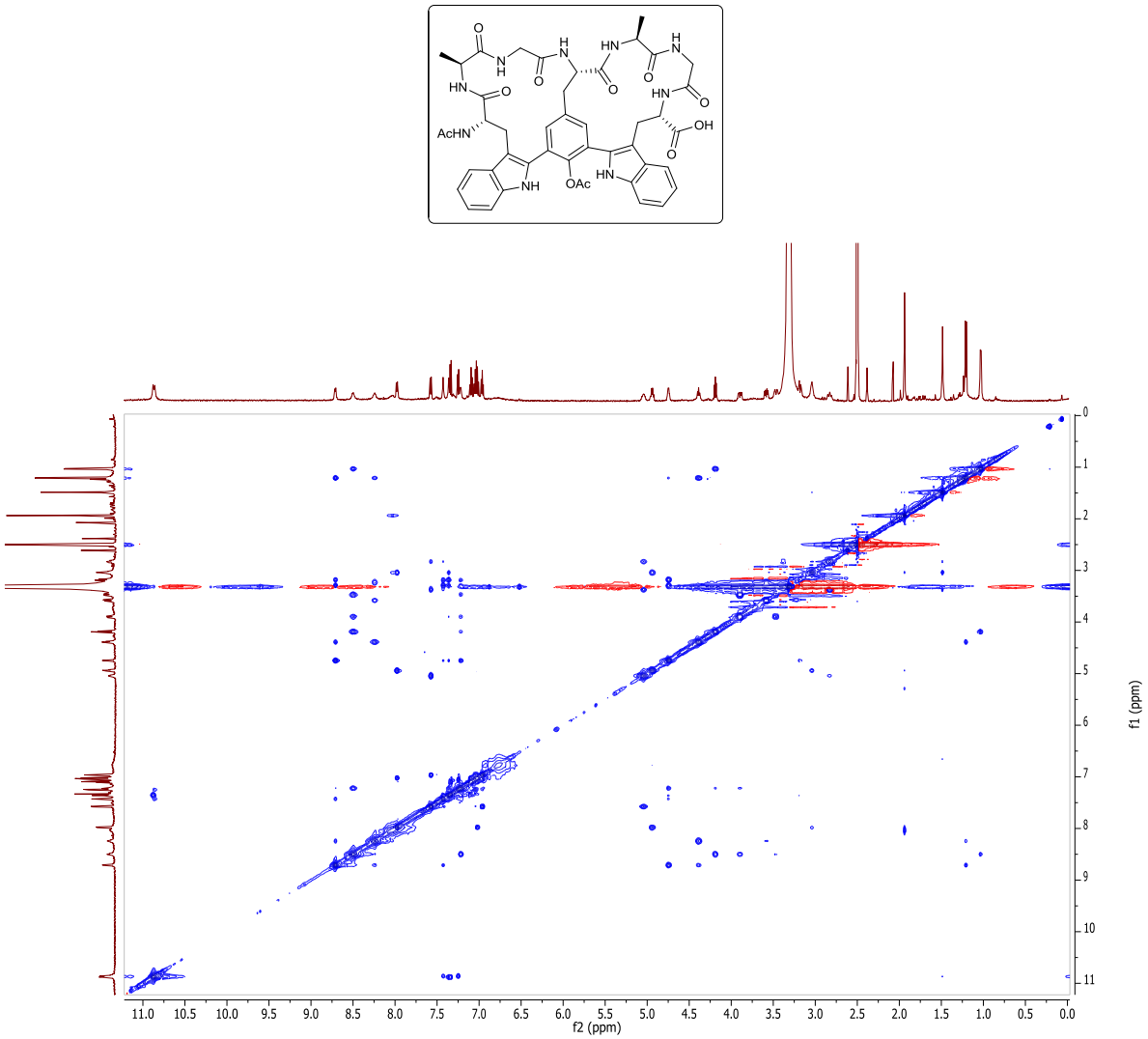
Supplementary Figure 135 | ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-(bicyclo-*m,m*)-[Trp-Ala-Gly-Tyr(OAc)]-[Tyr(OAc)-Ala-Gly-Trp]-OH (12).



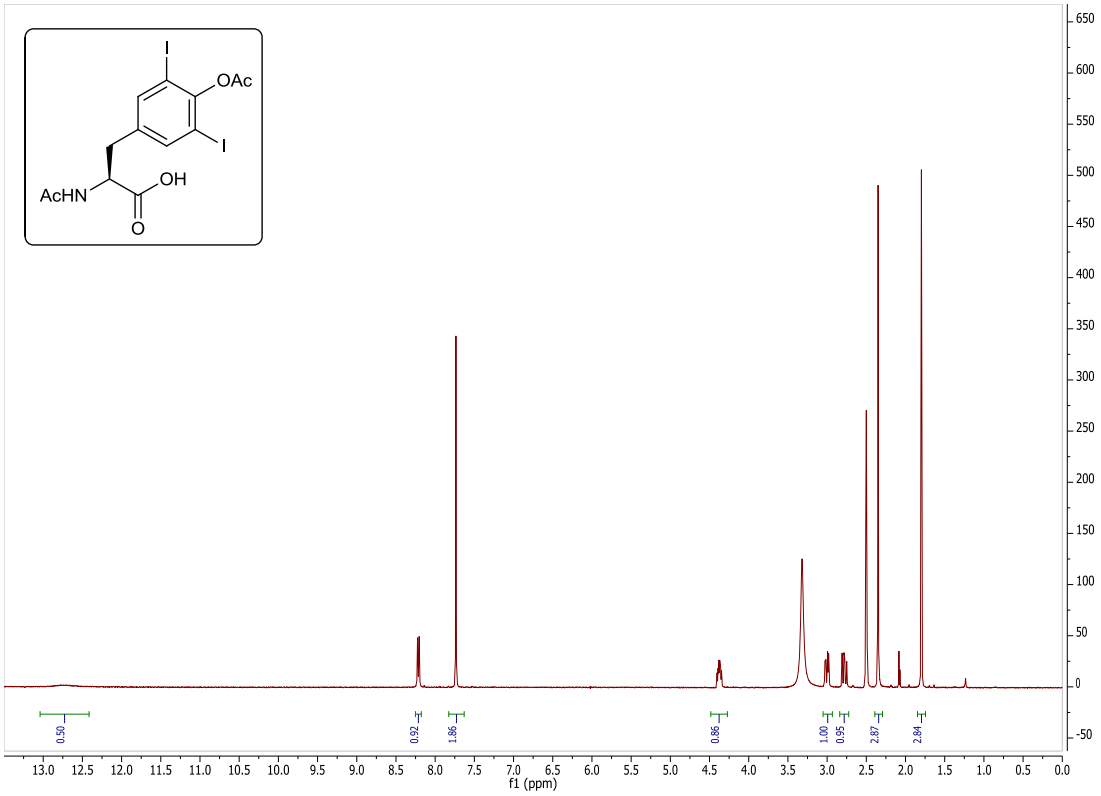
Supplementary Figure 136 | COSY NMR spectrum of compound Ac-(bicyclo-*m,m*)-[Trp-Ala-Gly-Tyr(OAc)]-[Tyr(OAc)-Ala-Gly-Trp]-OH (12).



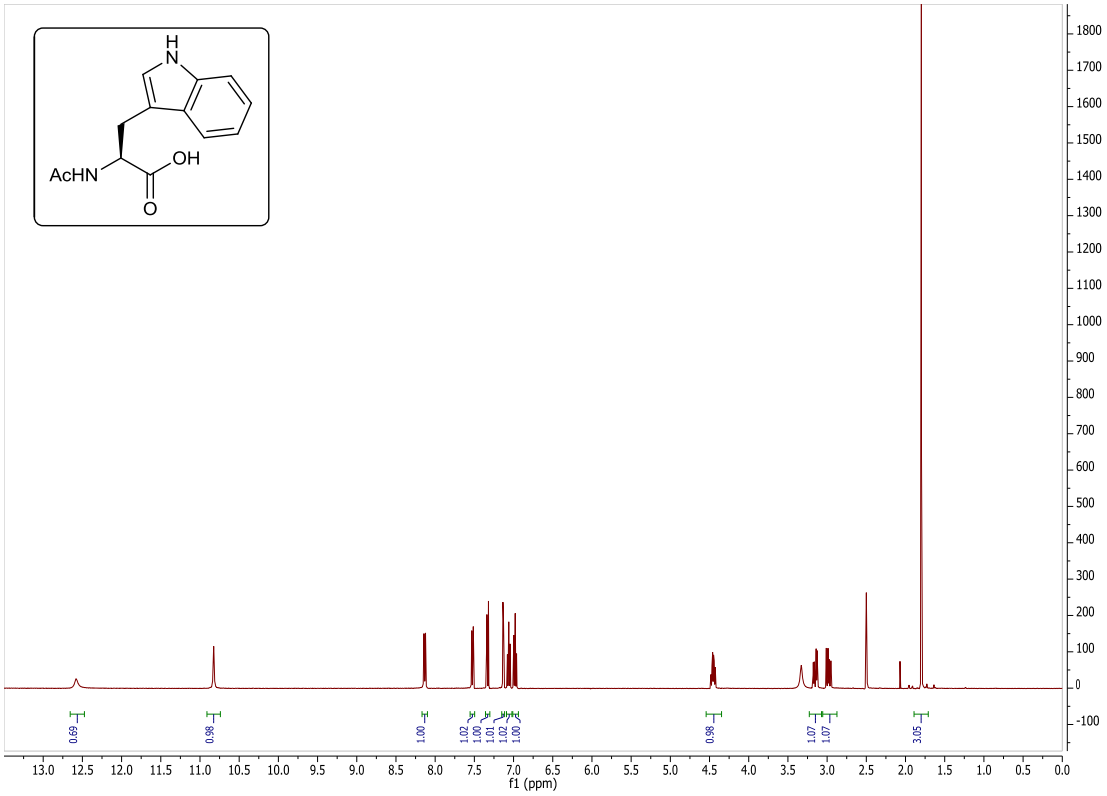
Supplementary Figure 137 | TOCSY NMR spectrum of compound Ac-(bicyclo-*m,m*)-[Trp-Ala-Gly-Tyr(OAc)]-[Tyr(OAc)-Ala-Gly-Trp]-OH (12).



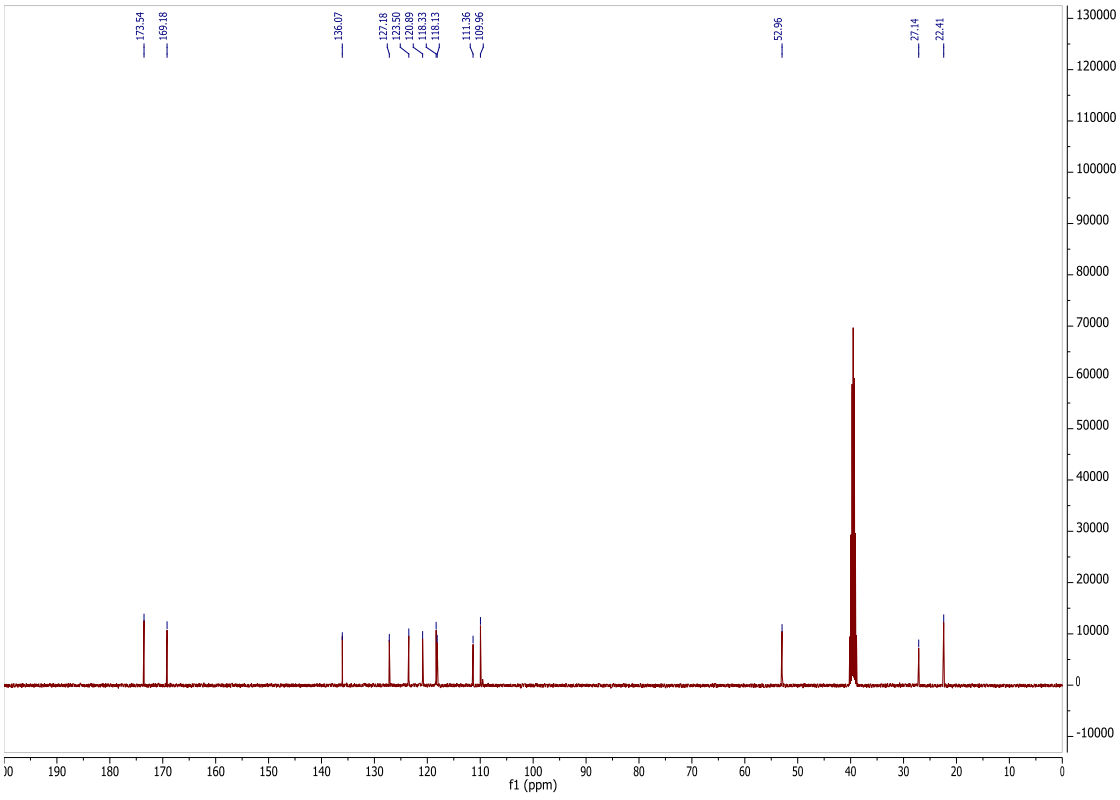
Supplementary Figure 138 | NOESY NMR spectrum of compound Ac-(bicyclo-*m,m*)-[Trp-Ala-Gly-Tyr(OAc)]-[Tyr(OAc)-Ala-Gly-Trp]-OH (12).



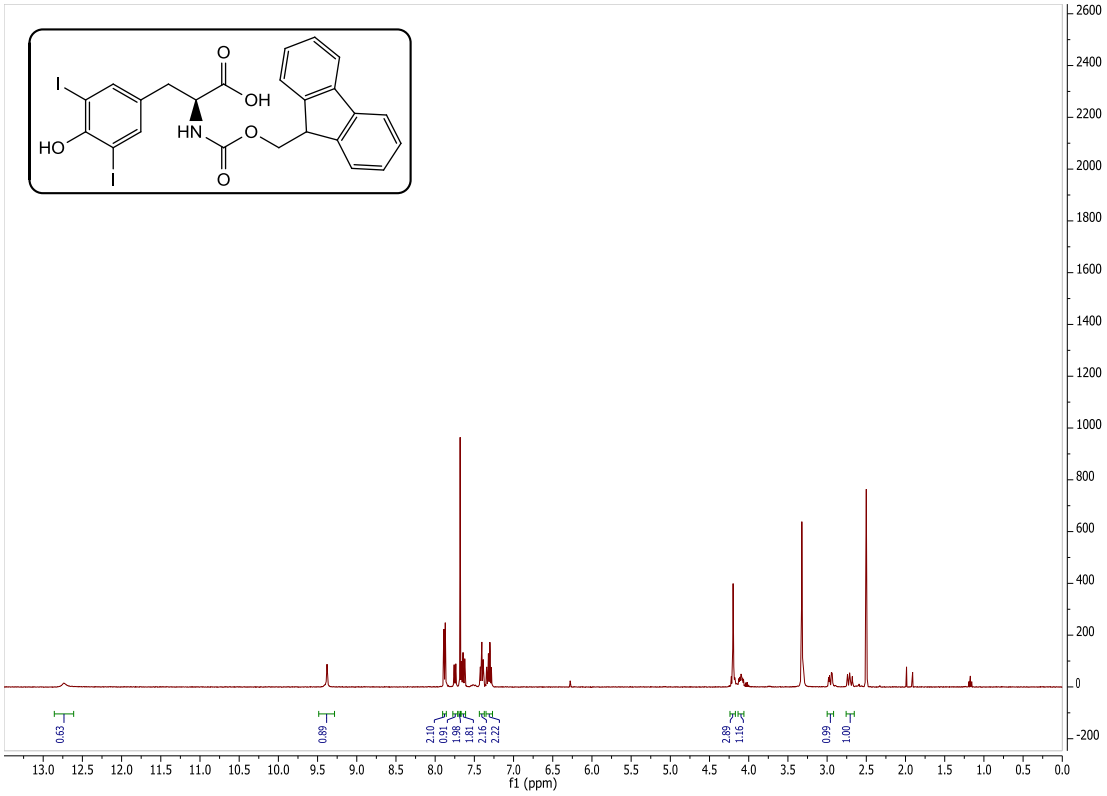
Supplementary Figure 139 ‖ ^{1}H NMR spectrum of compound Ac-*m,m'*-I,I-Tyr(OAc)-OH (13).



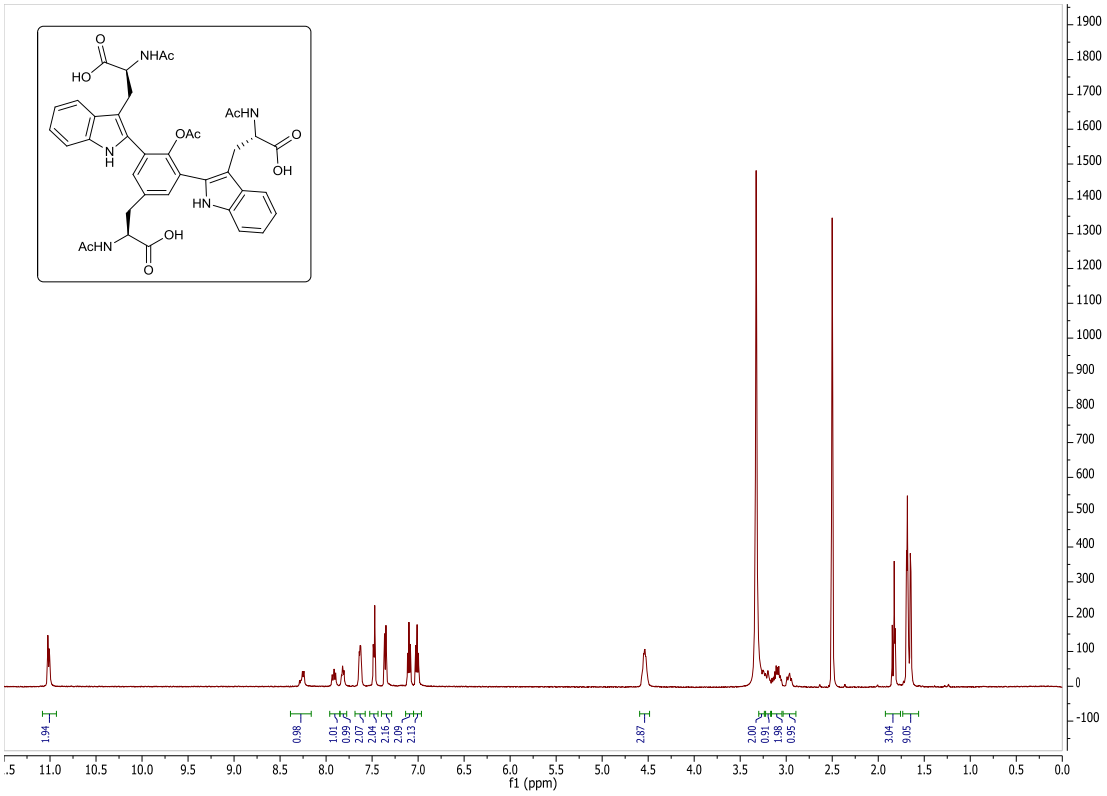
Supplementary Figure 140 | ^{1}H NMR spectrum of compound Ac-Trp-OH (14).



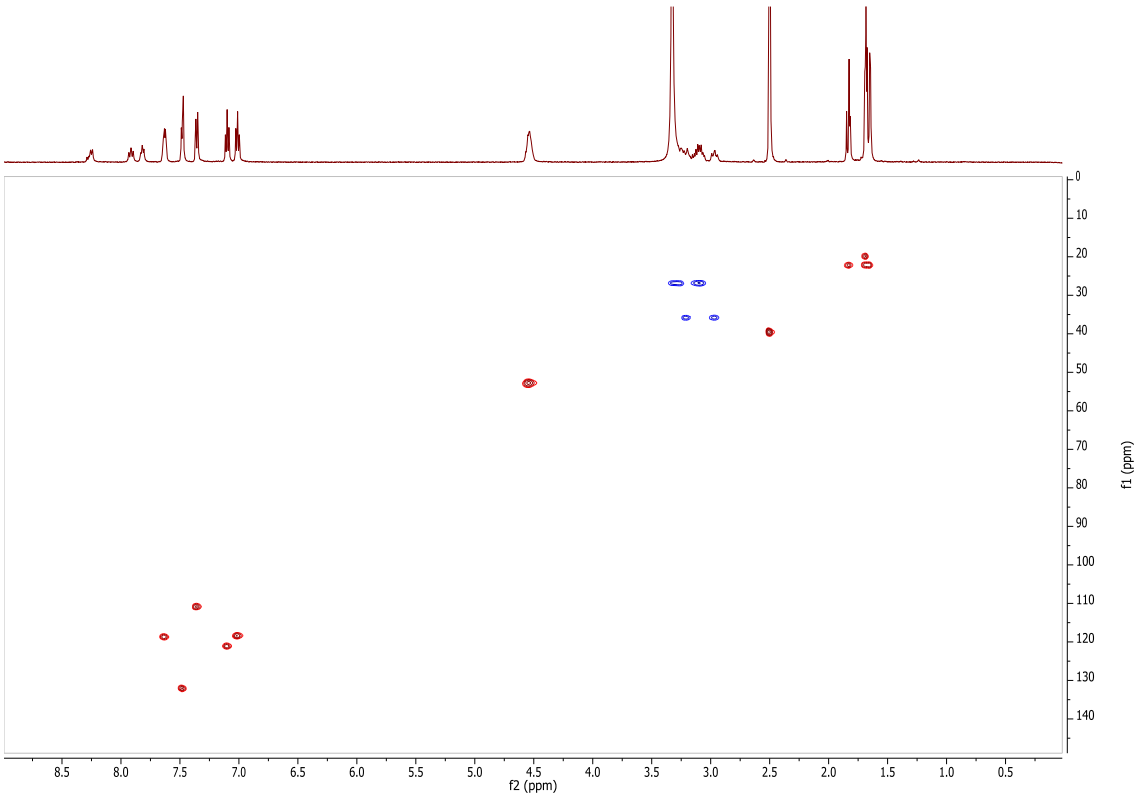
Supplementary Figure 141 | ^{13}C NMR spectrum of compound Ac-Trp-OH (14).



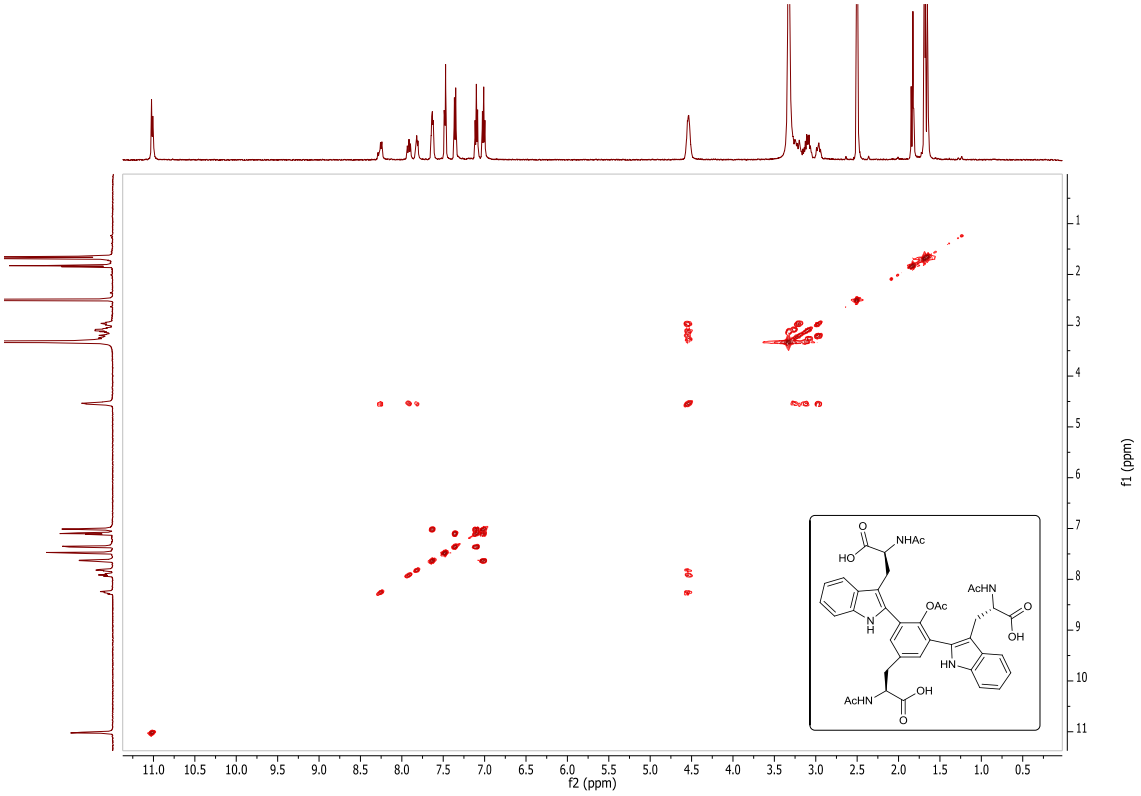
Supplementary Figure 142 | ^{1}H NMR spectrum of compound Fmoc-3,5-diiodo-L-Tyr-OH (15).



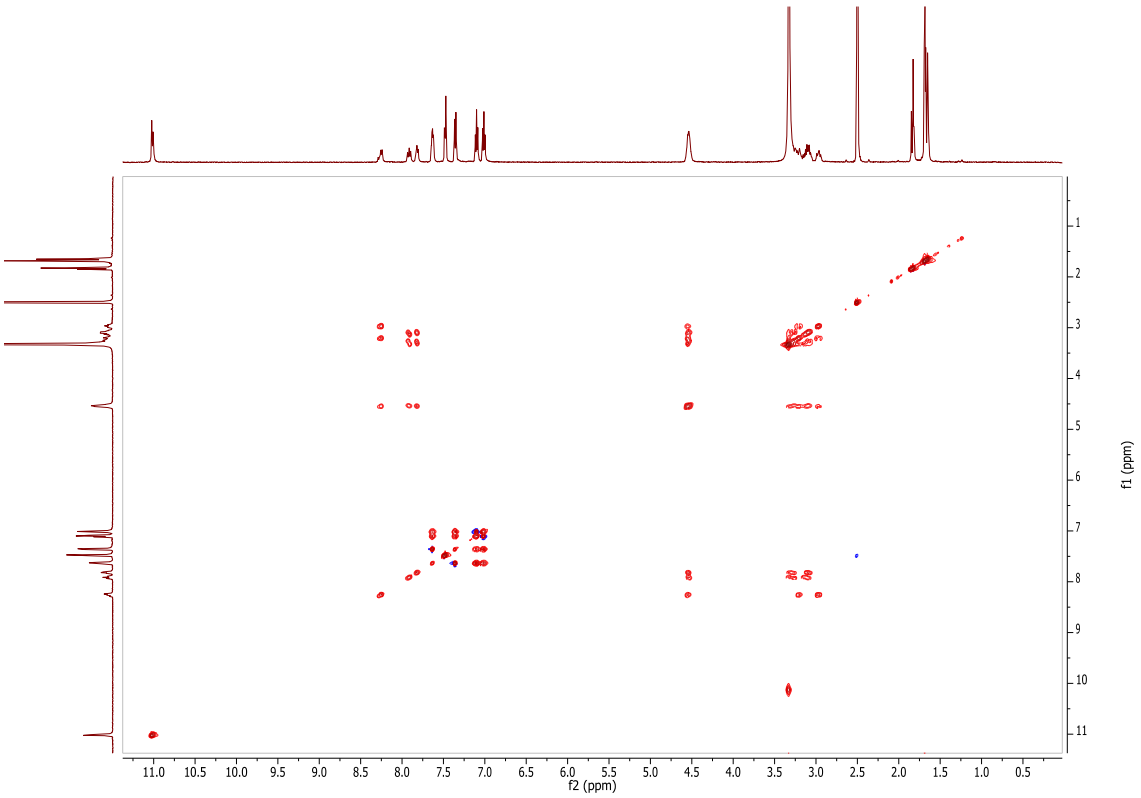
Supplementary Figure 143 | ^{1}H NMR spectrum of compound Ac-*3,5*-di-(Ac-Trp-OH)-Tyr(OAc)-OH (16).



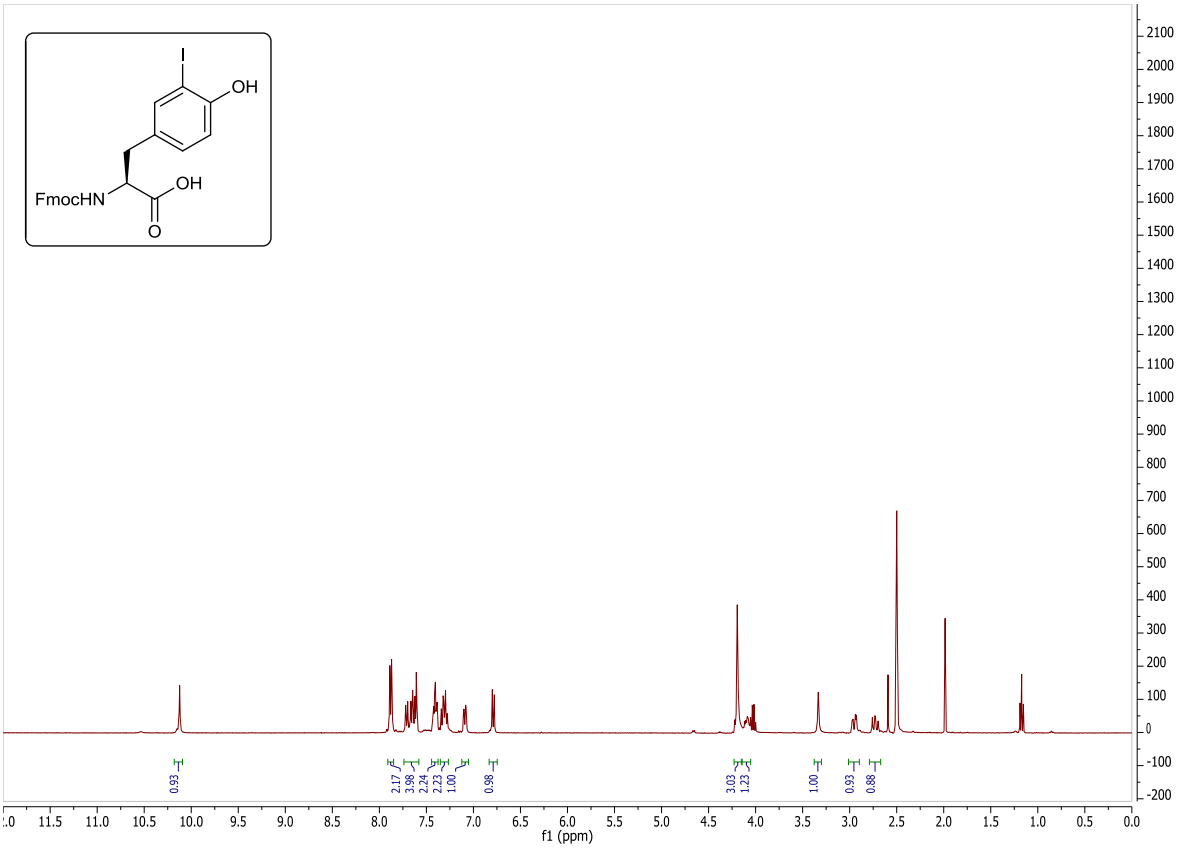
Supplementary Figure 144 | ^{1}H-^{13}C HSQC NMR spectrum of compound Ac-*3,5*-di-(Ac-Trp-OH)-Tyr(OAc)-OH (16).



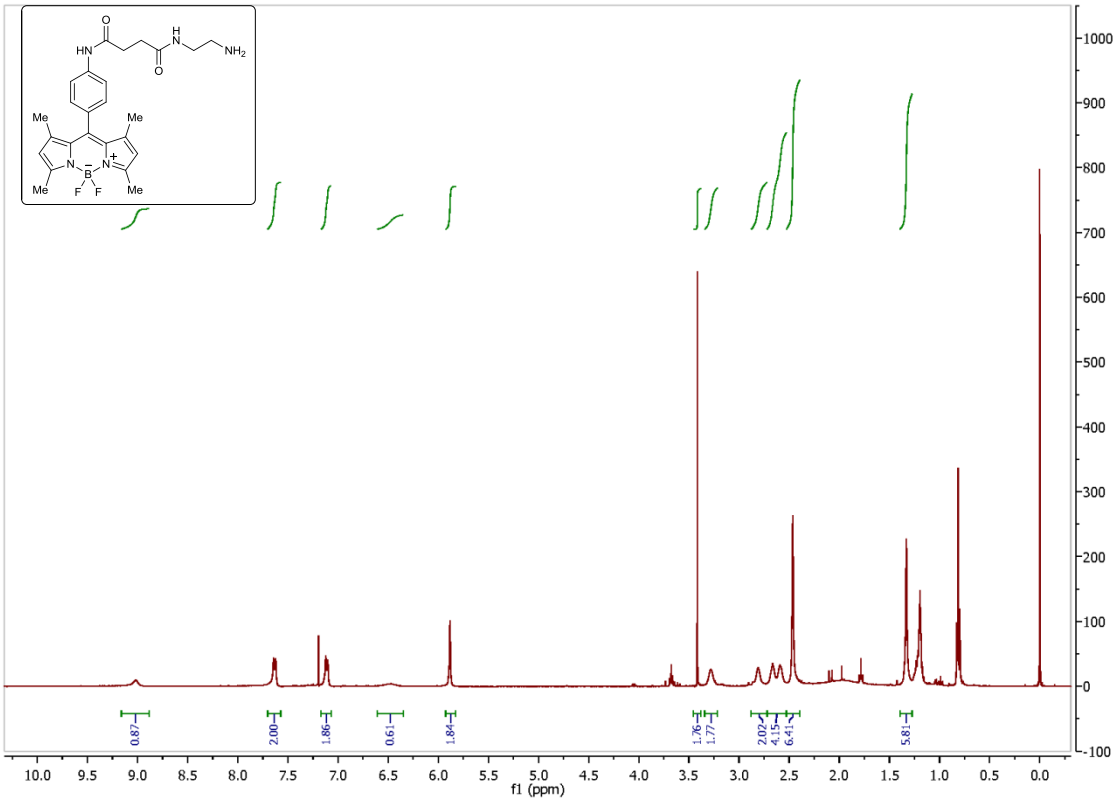
Supplementary Figure 145 | COSY NMR spectrum of compound Ac-*3,5*-di-(Ac-Trp-OH)-Tyr(OAc)-OH (16).



Supplementary Figure 146 | TOCSY NMR spectrum of compound Ac-*3,5*-di-(Ac-Trp-OH)-Tyr(OAc)-OH (16).

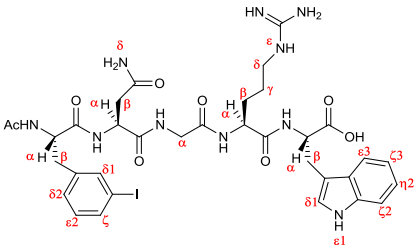


Supplementary Figure 147 | ^{1}H NMR spectrum of compound Fmoc-*3*-iodo-Tyr-OH (17).



Supplementary Figure 148 | ^{1}H NMR spectrum of compound 10-(4-(4-(2-aminoethylamino)-4-oxobutanamido)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:1',2'-*f*][1,3,2]diazaborinin-4-ium-5-uide (19).

Supplementary Tables

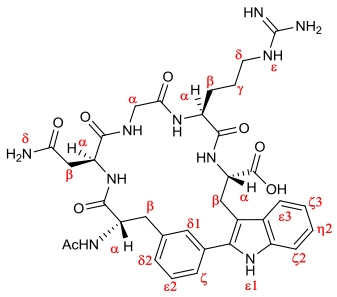


1g (^{1}H)	δ (ppm)												
AA	**NH**	**α**	**β**	**γ**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	8.18	4.47	2.98/ 2.66	-	7.66	7.25	7.05	7.54	-	-	-	-	-
Asn	8.38	4.51	2.57/ 2.50	-	7.46/6.96	-	-	-	-	-	-	-	-
Gly	8.03	3.76/ 3.66	-	-	-	-	-	-	-	-	-	-	-
Arg	7.92	4.36	1.68/ 1.54	1.46	3.07	-	-	-	7.46	-	-	-	-
Trp	8.14	4.45	3.17/ 3.07	-	7.16	-	-	-	10.83	7.32	7.05	6.97	7.51

Supplementary Table 1 | ^{1}H chemical shifts assignments of compound Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-OH (1g).

1g (^{13}C)	δ (ppm)										
AA	**α**	**β**	**γ**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	53.6	36.5	-	137.4	128.4	130.0	134.8	-	-	-	-
Asn	49.6	36.7	-	-	-	-	-	-	-	-	-
Gly	42.2	-	-	-	-	-	-	-	-	-	-
Arg	51.5	28.9	24.7	40.2	-	-	-	-	-	-	-
Trp	53.0	26.9	-	123.4	-	-	-	111.1	120.7	118.1	117.9

Supplementary Table 2 | ^{13}C chemical shifts assignments of compound Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-OH (1g).

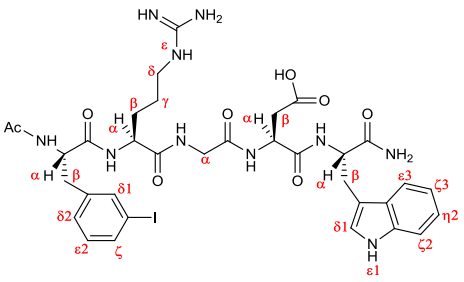


2g (^{1}H)	δ (ppm)												
AA	**NH**	**α**	**β**	**γ**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	8.36	4.68	3.09/2.88	-	7.70	7.20	7.37	7.49	-	-	-	-	-
Asn	8.42	4.60	2.62/2.44	-	7.38/6.85	-	-	-	-	-	-	-	-
Gly	7.67	3.98/3.43	-	-	-	-	-	-	-	-	-	-	-
Arg	7.79	4.30	1.74/1.55	1.41	3.04	-	-	-	-	-	-	-	-
Trp	8.33	4.48	3.42/3.06	-	-	-	-	-	11.15	7.32	7.06	6.97	7.63

Supplementary Table 3 | ^{1}H chemical shifts assignments of compound Ac-(Cyclo-*m*)-[Phe-Asn-Gly-Arg-Trp]-OH (2g).

2g (^{13}C)	δ (ppm)										
AA	**α**	**β**	**γ**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	53.4	37.0	-	128.0	127.6	127.9	125.0	-	-	-	-
Asn	49.2	35.7	-	-	-	-	-	-	-	-	-
Gly	42.5	-	-	-	-	-	-	-	-	-	-
Arg	51.6	28.4	23.9	39.9	-	-	-	-	-	-	-
Trp	54.2	28.2	-		-	-	-	110.6	121.1	118.3	119.0

Supplementary Table 4 | ^{13}C chemical shifts assignments of compound Ac-(Cyclo-*m*)-[Phe-Asn-Gly-Arg-Trp]-OH (2g).

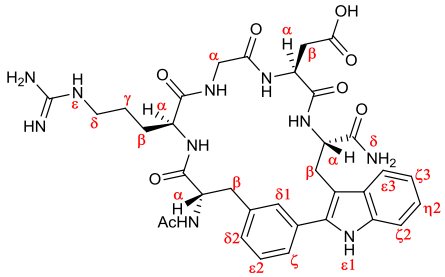


1h (^{1}H)	δ (ppm)												
AA	**NH**	**α**	**β**	**γ**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	8.10	4.51	2.98/2.67	-	7.69	7.27	7.05	7.55	-	-	-	-	-
Arg	8.23	4.31	1.72/1.57	1.51	3.10	-	-	-	7.42	-	-	-	-
Gly	8.10	3.72	-	-	-	-	-	-	-	-	-	-	-
Asp	8.23	4.57	2.69/2.46	-	-	-	-	-	-	-	-	-	-
Trp	7.85	4.39	3.16/2.98	-	7.10	-	-	-	10.74	7.32	7.05	6.97	7.55

Supplementary Table 5 | ^{1}H chemical shifts assignments of compound Ac-*m*-I-Phe-Arg-Gly-Asp-Trp-OH (1h).

1h (^{13}C)	δ (ppm)										
AA	**α**	**β**	**γ**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	53.5	36.5	-	137.3	128.4	129.8	134.6	-	-	-	-
Arg	52.0	28.9	24.5	40.1	-	-	-	-	-	-	-
Gly	41.5	-	-	-	-	-	-	-	-	-	-
Asp	49.2	35.8	-	-	-	-	-	-	-	-	-
Trp	53.1	27.1	-	123.2	-	-	-	110.9	120.5	117.9	118.0

Supplementary Table 6 | ^{13}C chemical shifts assignments of compound Ac-*m*-I-Phe-Arg-Gly-Asp-Trp-OH (1h).

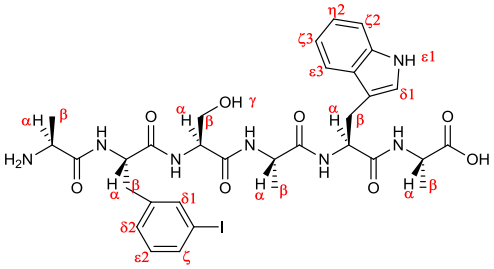


2h (^{1}H)	δ (ppm)												
AA	**NH**	**α**	**β**	**γ**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	8.32	4.66	2.97	-	7.69	7.24	7.39	7.42	-	-	-	-	-
Arg	7.88	4.03	1.84/ 1.49	1.41/ 1.34	3.14/ 2.75	-	-	-	-	-	-	-	-
Gly	8.39	3.68/3.43	-	-	-	-	-	-	-	-	-	-	-
Asp	8.06	4.25	2.67/2.18	-	-	-	-	-	-	-	-	-	-
Trp	7.35	4.53	3.43/2.97	-	7.07/6.89	-	-	-	11.00	7.31	7.07	6.99	7.59

Supplementary Table 7 | ^{1}H chemical shifts assignments of compound Ac-(Cyclo-*m*)-[Phe-Arg-Gly-Asp-Trp]-NH_2 (2h).

2h (^{13}C)	δ (ppm)										
AA	**α**	**β**	**γ**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	53.5	38.4	-	128.8	127.8	128.0	126.0	-	-	-	-
Arg	52.5	30.3	24.6	40.4	-	-	-	-	-	-	-
Gly	43.1	-	-	-	-	-	-	-	-	-	-
Asp	48.9	37.2	-	-	-	-	-	-	-	-	-
Trp	53.1	26.8	-	-	-	-	-	110.6	120.8	118.2	118.5

Supplementary Table 8 | ^{13}C chemical shifts assignments of compound Ac-(Cyclo-*m*)-[Phe-Arg-Gly-Asp-Trp]-NH_2 (2h).

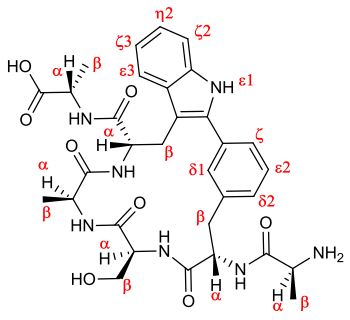


1i (^{1}H)	δ (ppm)												
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Ala1	-	3.70	1.31-1.21	-	-	-	-	-	-	-	-	-	-
***m-I*-Phe**	8.73	4.58	3.04/2.76	7.71	7.3	7.06	7.57	-	-	-	-	-	-
Ser	8.36	4.35	3.7/3.59	-	-	-	-	-	-	-	-	-	5.39
Ala2	8.15	4.17	1.12	-	-	-	-	-	-	-	-	-	-
Trp	7.94	4.48	3.17/2.88	7.12	-	-	-	10.8	7.30	7.06	6.97	7.57	-
Ala3	7.94	4.10	1.31-1.21	-	-	-	-	-	-	-	-	-	-

Supplementary Table 9 | ^{1}H chemical shifts assignments of compound H-Ala-*m*-I-Phe-Ser-Ala-Trp-Ala-OH (1i).

1i (^{13}C)	δ (ppm)										
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Ala1	48.0	17.4	-	-	-	-	-	-	-	-	-
***m-I*-Phe**	53.8	36.5	137.4	128.5	129.9	134.7	-	-	-	-	-
Ser	54.3	61.7	-	-	-	-	-	-	-	-	
Ala2	48.6	17.5	-	-	-	-	-	-	-	-	-
Trp	53.0	27.4	123.1	-	-	-	110.9	120.5	117.9	118.0	-
Ala3	48.0	17.4	-	-	-	-	-	-	-	-	-

Supplementary Table 10 | ^{13}C chemical shifts assignments of compound H-Ala-*m*-I-Phe-Ser-Ala-Trp-Ala-OH (1i).

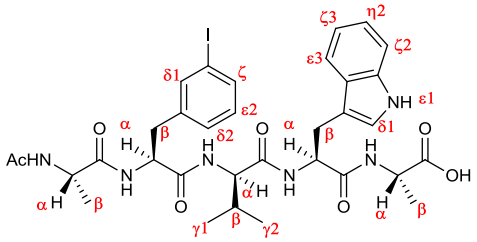


2i (^{1}H)	δ (ppm)											
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	-	3.48	1.17	-	-	-	-	-	-	-	-	-
Phe	8.46	4.97	3.18/3.08	7.65	7.22	7.42	7.42	-	-	-	-	-
Ser	8.35	3.87	3.63/3.58	-	-	-	-	-	-	-	-	-
Ala2	7.37	3.70	0.76	-	-	-	-	-	-	-	-	-
Trp	6.81	4.51	3.63/3.30	-	-	-	-	11.1	7.34	7.11	7.02	7.61
Ala3	7.51	4.15	1.32	-	-	-	-	-	-	-	-	-

Supplementary Table 11 | ^{1}H chemical shifts assignments of compound H-Ala-(Cyclo-*m)*-[Phe-Ser-Ala-Trp]-Ala-OH (2i).

2i (^{13}C)	δ (ppm)									
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	49.1	19.8	-	-	-	-	-	-	-	-
Phe	51.5	36.3	128.5	128.3	128.1	125.4	-	-	-	-
Ser	58.0	59.9	-	-	-	-	-	-	-	-
Ala2	49.8	15.7	-	-	-	-	-	-	-	-
Trp	53.4	26.6	-	-	-	-	110.8	121.3	118.4	117.8
Ala3	47.6	16.6	-	-	-	-	-	-	-	-

Supplementary Table 12 | ^{13}C chemical shifts assignments of compound H-Ala-(Cyclo-*m)*-[Phe-Ser-Ala-Trp]-Ala-OH (2i).

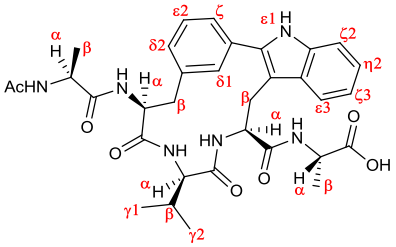


1j (^{1}H)	δ (ppm)												
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Ala1	7.99	4.19	1.10	-	-	-	-	-	-	-	-	-	-
***m-I*-Phe**	7.94	4.47	2.92/2.71	7.60	7.20	6.96	7.50	-	-	-	-	-	-
Val	7.76	4.15	1.94	-	-	-	-	-	-	-	-	-	0.77
Trp	7.99	4.60	3.13/2.92	7.15	-	-	-	10.78	7.29	7.04	6.96	7.60	-
Ala2	8.17	4.20	1.25	-	-	-	-	-	-	-	-	-	-

Supplementary Table 13 ‖ ^{1}H chemical shifts assignments of compound Ac-Ala-*m*-I-Phe-Val-Trp-Ala-OH (1j).

1j (^{13}C)	δ (ppm)										
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Ala1	47.5	17.9	-	-	-	-	-	-	-	-	-
***m-I*-Phe**	53.2	36.3	137.4	128.5	129.9	134.7	-	-	-	-	-
Val	57.3	30.5	-	-	-	-	-	-	-	-	18.9/17.7
Trp	52.7	27.4	123.3	-	-	-	110.9	120.6	117.9	118.2	-
Ala2	47.7	17.0	-	-	-	-	-	-	-	-	-

Supplementary Table 14 ‖ ^{13}C chemical shifts assignments of compound Ac-Ala-*m*-I-Phe-Val-Trp-Ala-OH (1j).

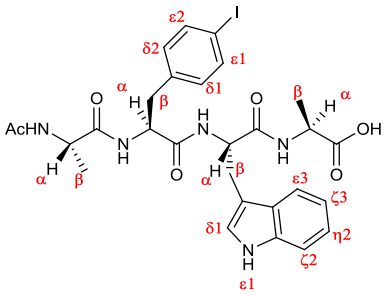


2j (^{1}H)	δ (ppm)												
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Ala1	8.09	4.31	1.16	-	-	-	-	-	-	-	-	-	-
***m-I*-Phe**	7.60	4.60	2.93	7.29	7.16	7.37	7.33	-	-	-	-	-	-
Val	8.09	4.07	1.75	-	-	-	-	-	-	-	-	-	0.72
Trp	7.67	4.65	3.34/3.20	-	-	-	-	11.19	7.33	7.08	6.95	7.42	-
Ala2	7.56	4.24	0.99	-	-	-	-	-	-	-	-	-	-

Supplementary Table 15 ‖ ^{1}H chemical shifts assignments of compound Ac-Ala-(Cyclo-*m)*-[Phe-Val-Trp]-Ala-OH (2j).

2j (^{13}C)	δ (ppm)										
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Ala1	47.7	17.7	-	-	-	-	-	-	-	-	-
***m-I*-Phe**	53.4	38.0	129.1	128.9	128.1	126.8	-	-	-	-	-
Val	57.4	30.1	-	-	-	-	-	-	-	-	18.5
Trp	52.0	26.2	-	-	-	-	110.5	120.9	118.0	119.2	-
Ala2	47.4	16.4	-	-	-	-	-	-	-	-	-

Supplementary Table 16 ‖ ^{13}C chemical shifts assignments of compound Ac-Ala-(Cyclo-*m)*-[Phe-Val-Trp]-Ala-OH (2j).

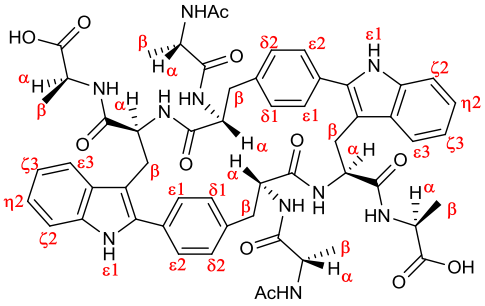


1k (^{1}H)	δ (ppm)										
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	7.96	4.19	1.07	-	-	-	-	-	-	-	-
***p-I*-Phe**	7.83	4.40	2.90/2.70	6.93	6.93	7.51	7.51	-	-	-	-
Trp	8.02	4.56	3.13/2.95	7.16	-	-	10.81	7.32	7.05	6.97	7.60
Ala2	8.25	4.23	1.27	-	-	-	-	-	-	-	-

Supplementary Table 17 | ^{1}H chemical shifts assignments of compound Ac-Ala-*p*-I-Phe-Trp-Ala-OH (1k).

1k(^{13}C)	δ (ppm)									
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	47.8	17.6	-	-	-	-	-	-	-	-
***p-I*-Phe**	53.2	36.6	131.4	131.4	136.3	136.3	-	-	-	-
Trp	52.8	27.5	123.3	-	-	-	111.0	120.6	117.9	118.2
Ala2	47.4	17.0	-	-	-	-	-	-	-	-

Supplementary Table 18 | ^{13}C chemical shifts assignments of compound Ac-Ala-*p*-I-Phe-Trp-Ala-OH (1k).

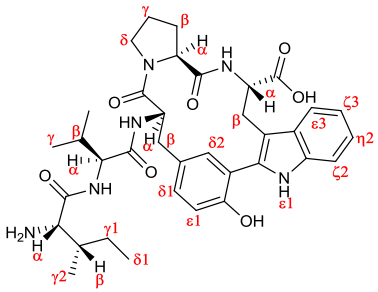


2k (^{1}H)	δ (ppm)										
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	7.93	4.18	1.08	-	-	-	-	-	-	-	-
***p-I*-Phe**	8.13	3.96	2.64/2.48	7.25	7.25	7.61	7.61	-	-	-	-
Trp	6.82	4.07	3.43/3.43	-	-	-	11.20	7.34	7.11	7.03	7.67
Ala2	7.90	4.21	1.28	-	-	-	-	-	-	-	-

Supplementary Table 19 | ^{1}H chemical shifts assignments of compound Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (2k).

2k(^{13}C)	δ (ppm)									
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	47.7	17.8	-	-	-	-	-	-	-	-
***p-I*-Phe**	52.9	35.2	128.8	128.8	127.9	127.9	-	-	-	-
Trp	54.9	25.55	-	-	-	-	110.9	121.4	118.5	118.3
Ala2	47.5	17.0	-	-	-	-	-	-	-	-

Supplementary Table 20 | ^{13}C chemical shifts assignments of compound Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (2k). Due to the symmetric nature of peptide **2k'**, both tetrapeptide moieties (A^1-F-W-A^2) are equivalent and have identical NMR signals.

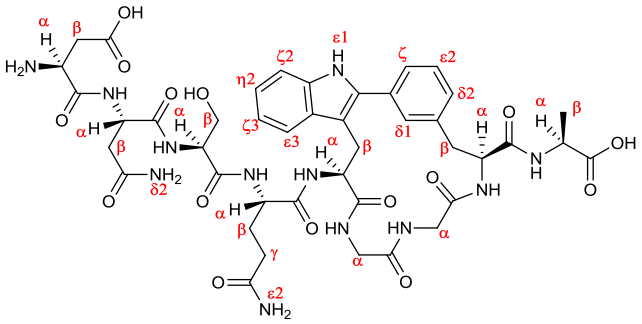


2l (^{1}H)												
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**	**γ2**
Ile	8.22	3.31	1.75	0.85	-	-	-	-	-	-	1.43/1.11	0.85
Val	8.15	4.33	1.97	-	-	-	-	-	-	-	0.85	-
***m-l*-Tyr**	8.32	4.68	3.07/2.89	7.11	7.14	6.89	-	-	-	-	-	-
Pro	-	4.49	2.12/1.56	3.61/3.06	-	-	-	-	-	-	1.72/1.47	-
Trp	7.60	4.53	3.26/2.77	-	-	10.79	7.32	7.06	6.99	7.60	-	-

Supplementary Table 21 ‖ ^{1}H chemical shifts assignments of compound H-Ile-Val-(Cyclo-*m*)-[Tyr-Pro-Trp]-OH (2l).

2l (^{13}C)											
AA	**α**	**β**	**δ1**	**δ2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**	**γ2**
Ile	58.1	37.3	-	-	-	-	-	-	-	23.3	-
Val	56.7	30.7	-	-	-	-	-	-	-	-	-
***m-l*-Tyr**	51.5	35.9	130.3	131.2	115.6	-	-	-	-	-	-
Pro	59.5	25.6	46.6	-	-	-	-	-	-	24.1	-
Trp	54.1	28.5	-	-	-	110.7	120.5	117.9	117.8	-	-

Supplementary Table 22 ‖ ^{13}C chemical shifts assignments of compound H-Ile-Val-(Cyclo-*m*)-[Tyr-Pro-Trp]-OH (2l). Based on NOE interactions and ^{13}C chemical shift differences of Pro, it was assigned a *trans* type configuration to this amino acid (^{13}C $\Delta\delta_{\beta-\alpha}$ = 1.6 and $^{1}H_{\alpha}$ – $^{1}H_{\delta}$ NOE correlation identified).[6,7]

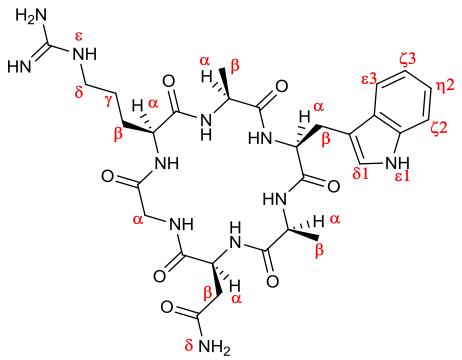


2m (^{1}H)	δ (ppm)											
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ/ ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Asp	-	3.85	2.59	-	-	-	-	-	-	-	-	-
Asn	8.58	4.67	2.65/2.50	-	7.38/6.93	-	-	-	-	-	-	-
Ser	8.23	4.18	3.71/3.58	-	-	-	-	-	-	-	-	-
Gln	8.01	4.27	1.91/1.78	-	-	7.30/6.74	-	-	-	-	-	2.12/2.06
Trp	8.15	4.83	3.45/3.01	-	-	-	11.16	7.86	7.05	7.11	7.35	-
Gly	8.15	3.79/3.24	-	-	-	-	-	-	-	-	-	-
Gly	8.58	3.62/3.32	-	-	-	-	-	-	-	-	-	-
***m-l*-Phe**	6.86	4.57	3.15/2.83	7.36	7.86	7.38	-	7.41	-	-	-	-
Ala	8.50	4.22	1.34	-	-	-	-	-	-	-	-	-

Supplementary Table 23 | ^{1}H chemical shifts assignments of compound H-Asp-Asn-Ser-Gln-(Cyclo-*m*)-[Trp-Gly-Gly-Phe]-Ala-OH (2m).

2m (^{13}C)	δ (ppm)										
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ/ ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Asp	50.1	37.3	-	-	-	-	-	-	-	-	-
Asn	49.8	36.5	-	-	-	-	-	-	-	-	-
Ser	56.0	61.2	-	-	-	-	-	-	-	-	-
Gln	52.3	27.5	-	-	-	-	-	-	-	-	31.4
Trp	52.7	27.7	-	-	-	-	118.9	118.3	121.1	110.7	-
Gly	42.7	-	-	-	-	-	-	-	-	-	-
Gly	42.1	-	-	-	-	-	-	-	-	-	-
***m-l*-Phe**	53.8	37.5	127.2	130.1	127.9	-	125.2	-	-	-	-
Ala	47.5	16.76	-	-	-	-	-	-	-	-	-

Supplementary Table 24 | ^{13}C chemical shifts assignments of compound H-Asp-Asn-Ser-Gln-(Cyclo-*m*)-[Trp-Gly-Gly-Phe]-Ala-OH (2m).

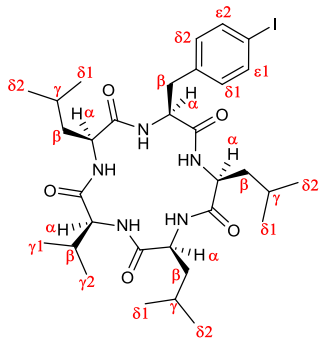


3 (^{1}H)	δ (ppm)									
AA	**NH**	**α**	**β**	**δ1**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Trp	7.88	4.28	3.18	7.14	10.83	7.32	7.06	6.97	7.58	-
Ala1	8.09	3.97	1.21	-	-	-	-	-	-	-
Asn	8.14	4.35	2.61	7.42/6.92	-	-	-	-	-	-
Gly	8.25	3.84/3.55	-	-	-	-	-	-	-	-
Arg	8.06	4.12	1.87/1.60	3.08	7.47	-	-	-	7.47	1.47
Ala2	7.99	4.08	1.21	-	-	-	-	-	-	-

Supplementary Table 25 | ^{1}H chemical shifts assignments of compound Cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-) (3).

3 (^{13}C)	δ (ppm)							
AA	**α**	**β**	**δ1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Trp	54.3	26.6	123.5	111.0	120.6	117.9	118.1	-
Ala1	49.3	17.0	-	-	-	-	-	-
Asn	50.0	35.6	-	-	-	-	-	-
Gly	42.6	-	-	-	-	-	-	-
Arg	52.7	27.7	40.1	-	-	-	-	24.8
Ala2	49.2	17.0	-	-	-	-	-	-

Supplementary Table 26 | ^{13}C chemical shifts assignments of compound Cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-) (3).

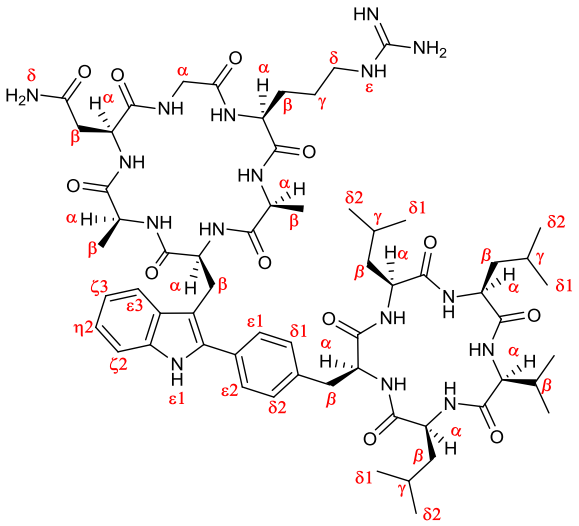


4 (^{1}H)	δ (ppm)					
AA	**NH**	**α**	**β**	**γ**	**δ**	**ε**
Leu1	8.15	3.94	1.52/1.35	1.28	0.83	-
***p-I*-Phe**	8.14	4.27	3.02	-	7.03	7.62
Leu2	7.98	4.05	1.72/1.48	1.33	0.83	-
Leu3	7.95	4.12	1.68/1.55	1.53	0.83	-
Val	8.02	3.66	2.15	0.83	-	-

Supplementary Table 27 | ^{1}H chemical shifts assignments of compound Cyclo(-Leu-Leu-Val-Leu-*p*-I-Phe-) (4).

4 (^{13}C)	δ (ppm)				
AA	**α**	**β**	**γ**	**δ**	**ε**
Leu1	53.0	39.5	24.1	-	-
***p-I*-Phe**	55.3	35.4	-	131.3	136.7
Leu2	53.2	39.2	24.1	-	-
Leu3	53.4	39.7	24.4	-	-
Val	60.9	29.0	-	-	-

Supplementary Table 28 | ^{13}C chemical shifts assignments of compound Cyclo(-Leu-Leu-Val-Leu-*p*-I-Phe-) (4).

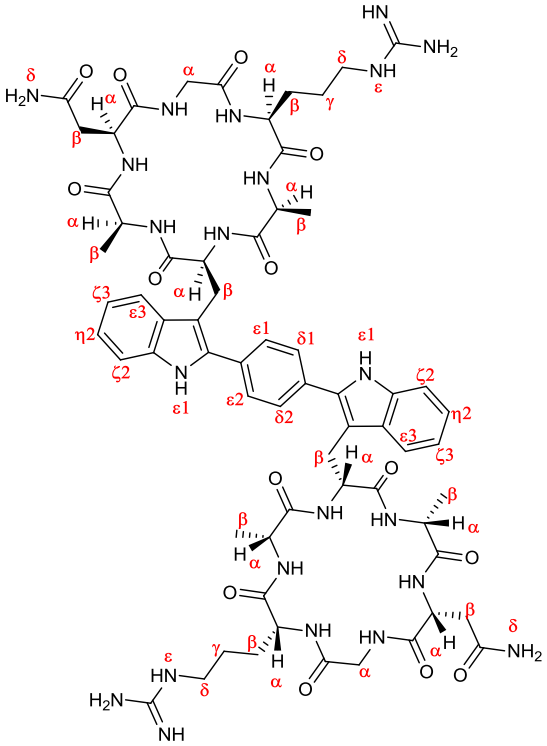


5 (^{1}H)				δ (ppm)								
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Trp	8.07	4.25	3.40/3.32	-	-	-	11.16	7.33	7.08	6.98	7.59	-
Ala1	8.00	3.92	1.11	-	-	-	-	-	-	-	-	-
Asn	8.04	4.35	2.58	7.42/6.91	-	-	-	-	-	-	-	-
Gly	8.45	3.86/3.41	-	-	-	-	-	-	-	-	-	-
Arg	7.98	4.15	1.79/1.59	3.08	-	-	7.42	-	-	-	-	1.47
Ala2	7.94	4.02	1.11	-	-	-	-	-	-	-	-	-
Leu1	8.18	3.97	1.63	0.85	0.85	-	-	-	-	-	-	1.37
***p-l*-Phe**	8.19	4.33	3.12	7.33	7.33	7.64	7.64	-	-	-	-	-
Leu2	7.98	4.13	1.56	0.85	0.85	-	-	-	-	-	-	1.47
Leu3	7.98	4.13	1.71	0.85	0.85	-	-	-	-	-	-	1.54
Val	8.00	3.72	2.15	-	-	-	-	-	-	-	-	0.85

Supplementary Table 29 | ^{1}H chemical shifts assignments of compound Cyclo(Ala-Asn-Gly-Arg-Ala-C2-Trp-)—Cyclo(C4-Phe-Leu-Leu-Val-Leu-) (5).

5 (^{13}C)	δ (ppm)										
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Trp	55.5	26.2	-	-	-	-	110.7	121.2	118.3	118.8	-
Ala1	48.8	17.0	-	-	-	-	-	-	-	-	-
Asn	50.0	35.7	-	-	-	-	-	-	-	-	-
Gly	43.0	-	-	-	-	-	-	-	-	-	-
Arg	52.6	28.1	40.1	-	-	-	-	-	-	-	24.9
Ala2	48.8	17.0	-	-	-	-	-	-	-	-	-
Leu1	52.9	39.4	-	-	-	-	-	-	-	-	24.2
***p-l*-Phe**	55.9	35.9	129.0	129.0	127.1	127.1	-	-	-	-	-
Leu2	53.5	39.6	-	-	-	-	-	-	-	-	24.1
Leu3	53.5	39.5	-	-	-	-	-	-	-	-	24.4
Val	60.7	29.2	-	-	-	-	-	-	-	-	-

Supplementary Table 30 | ^{13}C chemical shifts assignments of compound Cyclo(Ala-Asn-Gly-Arg-Ala-C2-Trp-)—Cyclo(C4-Phe-Leu-Leu-Val-Leu-) (5).

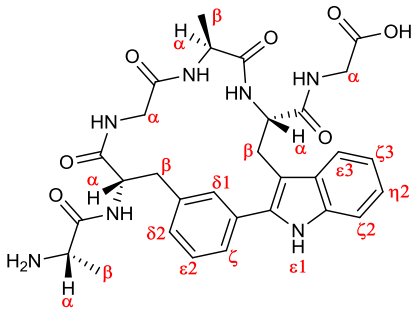


6 (^{1}H)	δ (ppm)											
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Trp	8.17	4.36	3.51/3.40	-	-	-	11.29	7.36	7.11	7.00	7.65	-
Ala1	8.06	4.04	1.11	-	-	-	-	-	-	-	-	-
Asn	8.06	4.36	2.63-2.56	7.42/6.89	-	-	-	-	-	-	-	-
Gly	8.40	3.86/3.42	-	-	-	-	-	-	-	-	-	-
Arg	7.97	4.18	1.81/1.62	3.08	-	-	-	-	-	-	-	1.47
Ala2	8.06	3.98	1.11	-	-	-	-	-	-	-	-	-
Ph	-	-	-	7.87	7.87	7.87	7.87	-	-	-	-	-

Supplementary Table 31 | ^{1}H chemical shifts assignments of compound Bis[cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-)] adduct (6).

6 (^{13}C)	δ (ppm)										
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ**
Trp	55.4	26.3	-	-	-	-	110.7	121.3	118.3	118.1	-
Ala1	49.0	17.0	-	-	-	-	-	-	-	-	-
Asn	50.1	35.8	-	-	-	-	-	-	-	-	-
Gly	42.8	-	-	-	-	-	-	-	-	-	-
Arg	52.2	28.0	39.9	-	-	-	-	-	-	-	24.6
Ala2	48.8	17.0	-	-	-	-	-	-	-	-	-
Ph	-	-	127.3	127.3	127.3	127.3	-	-	-	-	-

Supplementary Table 32 | ^{13}C chemical shifts assignments of compound Bis[cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-)] adduct (6).

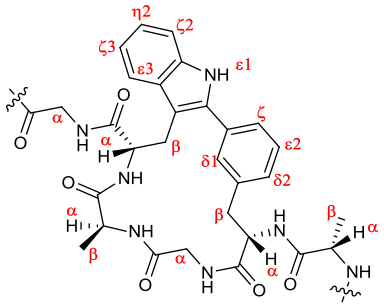


9 (^{1}H)				δ (ppm)								
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	-	3.71	1.28	-	-	-	-	-	-	-	-	-
***m-l*-Phe**	9.14	4.79	3.14/3.03	7.50	7.14	7.35	7.40	-	-	-	-	-
Gly1	8.43	3.76/3.57	-	-	-	-	-	-	-	-	-	-
Ala2	7.84	3.83	0.80	-	-	-	-	-	-	-	-	-
Trp	6.80	4.55	3.56/3.32	-	-	-	-	11.15	7.34	7.11	7.01	7.59
Gly2	7.38	3.55/3.38	-	-	-	-	-	-	-	-	-	-

Supplementary Table 33 | ^{1}H chemical shifts assignments of compound H-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-OH (9).

9 (^{13}C)					δ (ppm)					
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	49.2	18.3	-	-	-	-	-	-	-	-
***m-l*-Phe**	52.7	36.8	129.0	128.9	128.0	128.7	-	-	-	-
Gly1	43.1	-	-	-	-	-	-	-	-	-
Ala2	49.3	15.8	-	-	-	-	-	-	-	-
Trp	52.4	26.6	-	-	-	-	110.9	121.3	118.5	118.2
Gly2	42.1	-	-	-	-	-	-	-	-	-

Supplementary Table 34 | ^{13}C chemical shifts assignments of compound H-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-OH (9).

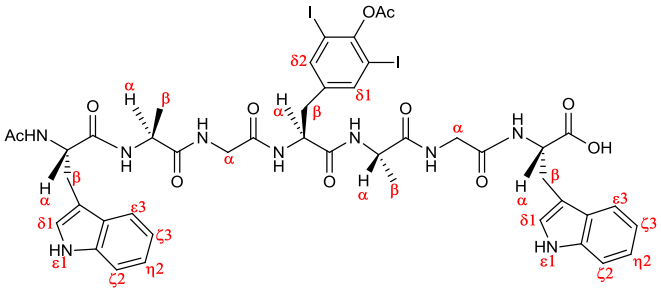


10 (^{1}H)	δ (ppm)											
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	8.53	4.14	1.28	-	-	-	-	-	-	-	-	-
***m-l*-Phe**	6.77	4.63	3.57/2.83	7.42	7.17	7.38	7.44	-	-	-	-	-
Gly1	7.39	4.09/3.15	-	-	-	-	-	-	-	-	-	-
Ala2	8.19	3.47	0.34	-	-	-	-	-	-	-	-	-
Trp	6.46	4.55	3.50	-	-	-	-	11.2	7.32	7.06	6.96	7.41
Gly2	7.38	3.54/3.35	-	-	-	-	-	-	-	-	-	-

Supplementary Table 35 | ^{1}H chemical shifts assignments of compound Cyclo[-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-] (10).

10 (^{13}C)	δ (ppm)									
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	49.2	16.2	-	-	-	-	-	-	-	-
***m-l*-Phe**	51.0	35.6	129.9	129.8	128.0	126.5	-	-	-	-
Gly1	42.9	-	-	-	-	-	-	-	-	-
Ala2	50.9	15.0	-	-	-	-	-	-	-	-
Trp	48.5	24.8	-	-	-	-	110.8	121.0	118.2	118.2
Gly2	43.1	-	-	-	-	-	-	-	-	-

Supplementary Table 36 | ^{13}C chemical shifts assignments of compound Cyclo[-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-] (10).

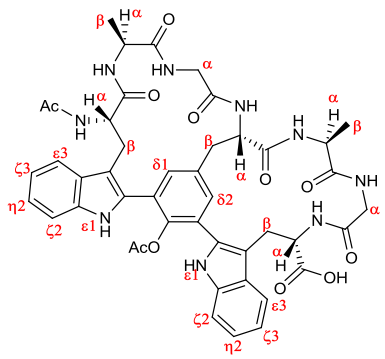


11 (^{1}H)					δ (ppm)					
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Trp1	8.01	4.53	3.11/2.89	7.12	-	10.75	7.29	7.05	6.94	7.52
Ala1	8.13	4.24	1.20	-	-	-	-	-	-	-
Gly1	7.92	3.70/3.56	-	-	-	-	-	-	-	-
Tyr	8.07	4.56-4.45*	2.97/2.67	7.77	7.77	-	-	-	-	-
Ala2	8.30	4.27	1.21	-	-	-	-	-	-	-
Gly2	8.08	3.73	-	-	-	-	-	-	-	-
Trp2	8.06	4.48	3.18/3.04	7.14	-	10.84	7.32	7.07	6.97	7.59

Supplementary Table 37 ‖ ^{1}H chemical shifts assignments of compound Ac-Trp-Ala-Gly-*3,5*-I,I-Tyr(OAc)-Ala-Gly-Trp-OH (11).

11 (^{13}C)					δ (ppm)			
AA	**α**	**β**	**δ1**	**δ2**	**ζ2**	**η2**	**ζ3**	**ε3**
Trp1	53.1	27.4	123.4	-	110.9	120.6	117.9	117.8
Ala1	48.1	17.9	-	-	-	-	-	-
Gly1	41.4	-	-	-	-	-	-	-
Tyr	53.1	35.7	139.4	139.4	-	-	-	-
Ala2	48.1	17.9	-	-	-	-	-	-
Gly2	41.5	-	-	-	-	-	-	-
Trp2	52.8	27.1	123.4	-	110.9	120.6	117.9	118.4

Supplementary Table 38 ‖ ^{13}C chemical shifts assignments of compound Ac-Trp-Ala-Gly-*3,5*-I,I-Tyr(OAc)-Ala-Gly-Trp-OH (11).



12 (^{1}H)	δ (ppm)									
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Trp1	8.03	4.94	3.04	-	-	10.87	7.34	7.09	7.03	7.98
Ala1	6.79	4.19	1.03	-	-	-	-	-	-	-
Gly1	8.50	3.90/3.47	-	-	-	-	-	-	-	-
Tyr	7.22	4.75	3.30/3.19	7.43	7.36	-	-	-	-	-
Ala2	8.71	4.39	1.21	-	-	-	-	-	-	-
Gly2	8.24	3.58/3.24	-	-	-	-	-	-	-	-
Trp2	6.79	5.04	3.38/2.83	-	-	10.87	7.25	7.03	6.96	7.58

Supplementary Table 39 | ^{1}H chemical shifts assignments of compound Ac-(bicyclo-*m,m*)-[Trp-Ala-Gly-Tyr(OAc)]-[Tyr(OAc)-Ala-Gly-Trp]-OH (12).

12 (^{13}C)	δ (ppm)							
AA	**α**	**β**	**δ1**	**δ2**	**ζ2**	**η2**	**ζ3**	**ε3**
Trp1	51.5	29.2	-	-	111.0	120.9	118.1	119.2
Ala1	47.8	18.2	-	-	-	-	-	-
Gly1	42.4	-	-	-	-	-	-	-
Tyr	51.2	36.6	132.8	133.4	-	-	-	-
Ala2	47.6	18.4	-	-	-	-	-	-
Gly2	42.4	-	-	-	-	-	-	-
Trp2	50.6	26.7	-	-	110.8	120.7	118.0	118.7

Supplementary Table 40 | ^{13}C chemical shifts assignments of compound Ac-(bicyclo-*m,m*)-[Trp-Ala-Gly-Tyr(OAc)]-[Tyr(OAc)-Ala-Gly-Trp]-OH (12).

Supplementary Methods

1. Abbreviations

Abbreviation used for amino acids and designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* 247, 977-983 (1982). The following additional abbreviations are used: ACN: acetonitrile, DMF: *N*,*N*-dimethylformamide, DCM: dichloromethane, Fmoc: 9*H*-fluorenylmethyloxycarbonyl, TFA: trifluoroacetic acid, PBS: phosphate buffered saline, SPPS: solid phase peptide synthesis, DIEA: *N*,*N*-diisopropylethylamine, DIPCDI: *N*,*N*-diisopropylcarbodiimide, HOBt: hydroxybenzotriazole, HBTU: *o*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate, TIS: triisopropylsilane, PyBOP: (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, PyAOP: (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, TBTU: *o*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate, TFE: 2,2,2-trifluoroethanol, PivOH: pivalic acid, DMAP: 4-(dimethylamino)pyridine, Trt: trityl, Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl, AB linker: 2-(4-hidroxymethylphenoxy)-propionic acid, IR: infrared spectroscopy, HPLC-MS: high performance liquid chromatography mass spectrometry, HRMS(ESI): high-resolution mass spectrometry (electrospray ionization), RP-HPLC; reversed phase-high performance liquid chromatography, NMR: nuclear magnetic resonance. HUVEC: human umbilica vein endothelial cell, Fb: fibrinogen, Vn: vitronectin.

2. General experimental information

Reactions were monitored by HPLC-MS at 220 nm using a HPLC Waters Alliance HT comprising a pump (Edwards RV12) with degasser, an autosampler and a diode array detector. Flow from the column was split to a MS spectrometer. The MS detector was configured with an eletrospray ionization source (micromass ZQ4000) and nitrogen was used as the nebulizer gas. Data acquisition was performed with MassLynx software. For compounds **2a-2c, 2l, 2m, 1j-BODIPY**, **5**, **6**, and **16**, yields are estimated from the integration of the peak areas in the HPLC-MS crude. Other yields are for the isolated pure compound. All microwave reactions were carried out in 10 mL sealed glass tubes in a focused mono-mode microwave oven ("Discover" by CEM Corporation) featured with a surface sensor for internal temperature determination. Cooling was provided by compressed air ventilating the microwave chamber during the reaction. When stated, the final crude was purified via flash column chromatography Combi Flash ISCO RF provided with dual UV detection.

NMR spectra of peptides in DMSO-d_6 were acquired with either a Bruker DMX-500 MHz spectrometer or Bruker Avance III 600 MHz and Bruker Avance 800 MHz spectrometers equipped with TCI cryoprobes. The spectra were referenced relative to the residual DMSO signal (^{1}H, 2.49 ppm; ^{13}C, 39.5 ppm). ^{1}H resonances were unequivocally assigned by two-dimensional NMR

experiments (COSY, TOCSY and NOESY and/or ROESY). Then, the ^{13}C resonances were straightforwardly assigned on the basis of the cross-correlations observed in the ^{1}H-^{13}C HSQC spectra. Mixing times for TOCSY spectra were 70 ms, for NOESY spectra 300-450 ms and for ROESY experiments were 200 ms. The temperature coefficients for the amide protons of each peptide were determined via ^{1}H spectra in the range 298-313 K with a step size of 5 K. Chemical shifts (δ) are reported in ppm. Multiplicities are referred by the following abbreviations: s = singlet, d = doublet, t = triplet, dd = double doublet, dt = double triplet, q = quartet, p = pentuplet and m = multiplet. HRMS (ESI positive) were obtained with a LTQ-FT Ultra (Thermo Scientific) mass Spectrometer. IR spectra were obtained on a Thermo Nicolet NEXUS.

CD Spectroscopy. Circular dichroism (CD) measurements were performed using a Jasco J-815 spectrophotometer. The spectra were recorded from 260 to 170 nm using a 1.0 mm path-length quartz cuvette at 2 nm bandwidth, 50 nm/min scan speed, 0.5 s response time, 0.2 nm data pitch and three accumulations. The background signal of the buffer alone was subtracted for each spectrum. CD spectra were converted from raw ellipticity (θ, mdeg) to mean molar ellipticity per residue ([θ], deg cm^2 $dmol^{-1}$).

All the samples were dissolved in a buffer of 25 mM Na_2HPO_4 (pH 7) at both 100 and 200 μM final peptide concentration. Additionally, new determinations were made in 10% of 2,2,2-trifluoroethanol (TFE) to increase the propensity to form secondary structures. To ensure no interference of peptide aromatic moiety on the spectra profiles, the previously reported 3-(2-Phenyl-1*H*-indol-3-yl)propanoic acid[1] was also analysed at identical conditions as for the tested compounds.

General procedure for SPPS.[2] All peptides were manually synthesized in polystyrene syringes fitted with a polyethylene porous disc using Fmoc-based SPPS. Solvents and soluble reagents were removed by suction. The Fmoc group was removed with piperidine-DMF (1:4) (1×1 min, 2×5 min). Peptide synthesis transformations and washes were performed at r.t.

Resin loading (only for 2-Chlorotrityl resin). Fmoc-XX-OH (1.0 eq.) was attached to the resin (1.0 eq.) with DIEA (3.0 eq.) in DCM at r.t for 10 min and then DIEA (7.0 eq.) for 40 min. The remaining trityl groups were capped adding 0.8 μL MeOH/mg resin for 10 minutes. After that, the resin was

filtered and washed with DCM (4 x 1 min), DMF (4 x 1 min). The loading of the resin was determined by titration of the Fmoc group.[2]

Peptide elongation. After the Fmoc group was eliminated, the resin was washed with DMF (4 x 1 min), DCM (3 x 1 min), DMF (4 x 1 min). The completion of the coupling was monitored with the ninhydrin (free primary amine) or chloranil (free secondary amine) tests.[3] Then, the resin was filtered and washed with DCM (4 x 1 min) and DMF (4 x 1 min) and the Fmoc group was eliminated.

Acetylation. When indicated, once the peptide was fully elongated, N-terminal acetylation was performed with acetic anhydride (10 eq.), DIEA (10 eq.) in DMF (30 min).

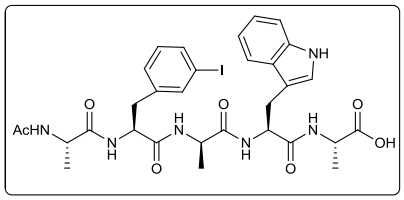
Final cleavage. The resin bound peptide was treated with the corresponding TFA cleavage cocktail. Then, the resin was washed with DCM and the combined eluates were evaporated under vacuum. Then, the residue was washed with Et_2O, dissolved in ACN:H_2O and lyophilized furnishing the corresponding peptide.

3. Experimental procedures and peptide characterization

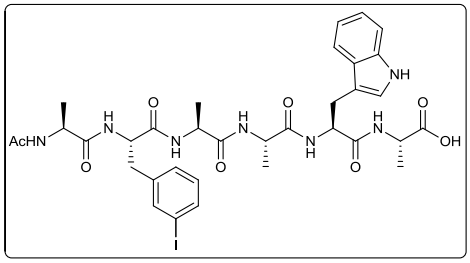
Synthesis and peptide characterization of linear peptides 1a-1f

2-Chlorotrityl resin (1 mmol/g). Amino acid coupling. Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and HOBt (3.0 eq.) in DMF for 1h. The N-terminal was acetilated, and then the resin bound peptide was treated with a 5% (v/v) TFA/DCM solution (5 x 1 min).

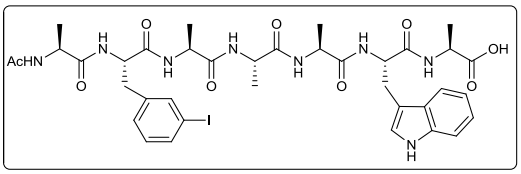
Ac-Ala-*m*-Phe-Ala-Trp-Ala-OH (1a). HRMS (ESI) (m/z): [M] calcd. for $C_{31}H_{37}IN_6O_7$, 732.1768; $[M+H]^+$ found, 733.1836.



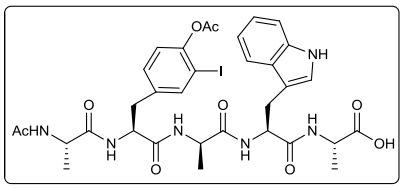
Ac-Ala-*m*-Phe-Ala-Ala-Trp-Ala-OH (1b). HRMS (ESI) (m/z): [M] calcd. for $C_{34}H_{42}IN_7O_8$, 803.2140; $[M+H]^+$ found, 804.2215.



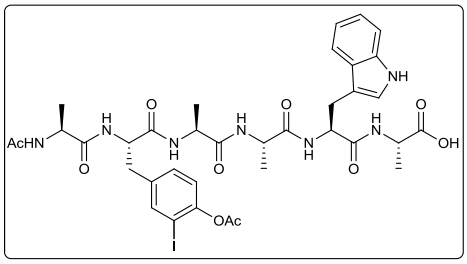
Ac-Ala-*m*-Phe-Ala-Ala-Ala-Trp-Ala-OH (1c). HRMS (ESI) (m/z): [M] calcd. for $C_{37}H_{47}IN_8O_9$, 874.2511; $[M+H]^+$ found, 875.2583.



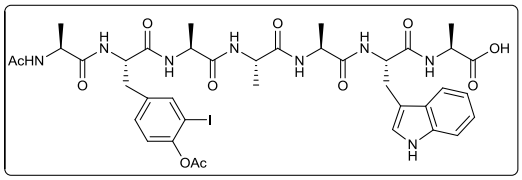
Ac-Ala-*m*-Tyr(OAc)-Ala-Trp-Ala-OH (1d). HPLC-MS (m/z): [M] calcd. for $C_{33}H_{39}IN_6O_9$, 790.18; $[M+H]^+$ found, 791.06.



Ac-Ala-*m*-Tyr(OAc)-Ala-Ala-Trp-Ala-OH (1e). HPLC-MS (m/z): [M] calcd. for $C_{36}H_{44}IN_7O_{10}$, 861.22; $[M+H]^+$ found, 862.15.

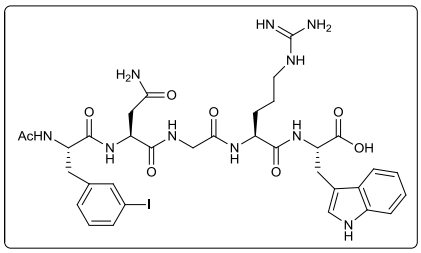


Ac-Ala-*m*-Tyr(OAc)-Ala-Ala-Ala-Trp-Ala-OH (1f). HPLC-MS (m/z): [M] calcd. for $C_{39}H_{49}IN_8O_{11}$, 932.26; $[M+H]^+$ found, 933.17.



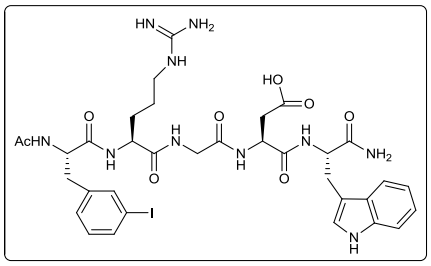
Synthesis and peptide characterization of linear peptides 1g-1k

Ac-*m*-I-Phe-Asn-Gly-Arg-Trp-OH (1g). 2-Chlorotrityl resin (0.94 mmol/g). Amino acid coupling. Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and OxymaPure (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Trp(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH. Fmoc-*m*-I-Phe-OH (1.5 eq.) was incorporated with HBTU (1.5 eq.), HOBt (1.5 eq.) and DIEA (3.0 eq.) in DMF for 1h. The resin bound peptide was treated with a 95% TFA, 2.5% TIS, 2.5% H_2O cocktail (1h). Pale solid (90-92% purity, estimated by HPLC-MS). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 10.83 (m, 1H), 8.38 (d, *J* = 7.7 Hz, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 8.14 (d, *J* = 7.5 Hz, 1H), 8.03 (t, *J* = 5.7 Hz, 1H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.66 (d, *J* = 1.7 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.51 (d, *J* = 7.9 Hz, 1H),

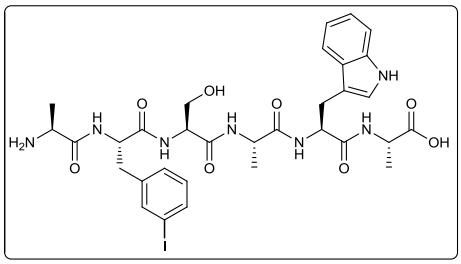


7.46 (m, 2H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.25 (d, *J* = 7.7 Hz, 1H), 7.16 (d, *J* = 2.4 Hz, 1H), 7.05 (t, *J* = 7.7 Hz, 2H), 7.00 – 6.94 (m, 2H), 4.55 – 4.41 (m, 3H), 4.36 (td, *J* = 8.4, 5.2 Hz, 1H), 3.76 (dd, *J* = 16.9, 5.9 Hz, 1H), 3.66 (dd, *J* = 16.8, 5.5 Hz, 1H), 3.17 (dd, *J* = 14.7, 5.5 Hz, 1H), 3.07 (m, 3H), 2.98 (dd, *J* = 13.7, 4.1 Hz, 1H), 2.66 (dd, *J* = 13.7, 10.3 Hz, 1H), 2.57 (dd, *J* = 15.5, 6.0 Hz, 1H), 2.50 (1H), 1.75 (s, 3H), 1.68 (m, 1H), 1.58 – 1.40 (m, 3H) ppm. **HPLC-MS** (m/z): $[M+H]^+$ calcd. for $C_{34}H_{43}IN_{10}O_8$, 847.7; found, 847.1.

Ac-*m*-I-Phe-Arg-Gly-Asp-Trp-OH (1h). H-Rink-Amide Chemmatrix resin (0.53 mmol/g). Amino acid coupling. Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and HOBt (3.0 eq.) in DMF for 1h (2h of coupling were carried out in the case of Fmoc-Trp(Boc)-OH). Fmoc-XX-OH: Fmoc-Trp(Boc)-OH, Fmoc-Asp(O*t*-Bu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-*m*-I-Phe-OH. The N-terminal was acetilated, and then the resin bound peptide was treated with a 95% TFA, 2.5% TIS, 2.5% H_2O cocktail (3h). Pale solid (>99% purity, estimated by HPLC-MS). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 10.74 (d, *J* = 2.4 Hz, 1H), 8.23 (dd, *J* = 7.9, 2.4 Hz, 2H), 8.14 – 8.05 (m, 2H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 1.7 Hz, 1H), 7.60 – 7.51 (m, 2H), 7.42 (m, 1H),7.32 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.27 (dt, *J* = 7.6, 1.3 Hz, 1H), 7.12 – 7.03 (m, 4H), 6.97 (ddd, *J* = 7.9, 6.9, 1.0 Hz, 1H), 4.57 (td, *J* = 7.7, 6.0 Hz, 1H), 4.51 (ddd, *J* = 10.5, 8.3, 4.0 Hz, 1H), 4.39 (td, *J* = 8.1, 5.0 Hz, 1H), 4.31 (td, *J* = 8.0, 5.6 Hz, 1H), 3.77 – 3.69 (m, 2H), 3.16 (dd, *J* = 14.7, 5.0 Hz, 1H), 3.10 (dt, *J* = 11.5, 5.7 Hz, 2H), 2.98 (ddd, *J* = 14.8, 6.6, 2.7 Hz, 2H), 2.71 – 2.64 (m, 2H), 2.46 (dd, *J* = 16.6, 7.7 Hz, 1H), 1.76 (s, 3H), 1.72 (q, *J* = 6.0, 4.3 Hz, 1H), 1.60 – 1.44 (m, 3H) ppm. **HPLC-MS** (m/z): $[M+H]^+$ calcd. for $C_{34}H_{43}IN_{10}O_8$, 847.7; found, 847.2.

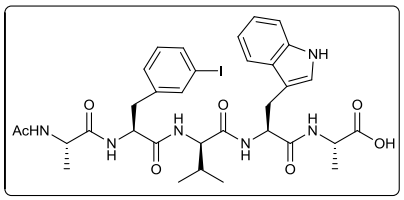


H-Ala-*m*-I-Phe-Ser-Ala-Trp-Ala-OH (1i). 2-Chlorotrityl resin (0.94 mmol/g). Amino acid coupling. Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and HOBt (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Ala-OH, Fmoc-



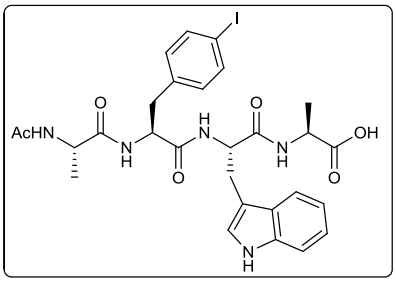
Trp(Boc)-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-*m*-I-Phe-OH. The resin bound peptide was treated with a 95% TFA, 2.5% TIS, 2.5% cocktail (1h). Pale solid (>99% purity, estimated by HPLC-MS). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 10.80 (d, *J* = 2.8 Hz, 1H), 8.73 (s, 1H), 8.36 (d, *J* = 7.7 Hz, 1H), 8.15 (d, *J* = 6.7 Hz, 1H), 7.94 (dd, *J* = 16.4, 7.4 Hz, 2H), 7.71 (s, 1H), 7.57 (t, *J* = 8.5 Hz, 2H), 7.30 (t, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 2.6 Hz, 1H), 7.06 (m, 2H), 6.97 (t, *J* = 7.5 Hz, 1H), 5.39 (s, 1H), 4.58 (m, 1H), 4.48 (td, *J* = 9.1, 4.5 Hz, 1H), 4.35 (q, *J* = 6.3 Hz, 1H), 4.17 (q, *J* = 7.0 Hz, 1H), 4.10 (q, *J* = 7.1 Hz, 1H), 3.70 (m, 2H), 3.59 (dd, *J* = 10.6, 5.7 Hz, 1H), 3.17 (dd, *J* = 14.7, 4.3 Hz, 1H), 3.04 (dd, *J* = 14.2, 3.8 Hz, 1H), 2.88 (dd, *J* = 14.8, 9.6 Hz, 1H), 2.76 (dd, *J* = 14.2, 10.7 Hz, 1H), 1.31 – 1.21 (m, 6H), 1.12 (d, *J* = 7.2 Hz, 3H) ppm. **HPLC-MS** (m/z): $[M+H]^+$ calcd. for $C_{32}H_{40}IN_7O_8$, 778.6; found, 778.2.

Ac-Ala-*m*-I-Phe-Val-Trp-Ala-OH (1j). 2-Chlorotrityl resin (1.0 mmol/g). Amino acid coupling.



Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and HOBt (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Ala-OH, Fmoc-Trp-OH, Fmoc-Val-OH. Fmoc-*m*-I-Phe-OH (1.5 eq.) was incorporated with PyBOP (1.5 eq.), HOBt (1.5 eq.) and DIEA (3.0 eq.) in DMF for 1h. The N-terminal was acetilated, and then the resin bound peptide was treated with a 5% (v/v) TFA/DCM solution (5 x 1 min). Pale solid (>99% purity, estimated by HPLC-MS). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 10.78 (d, *J* = 2.8 Hz, 1H), 8.17 (d, *J* = 7.1 Hz, 1H), 7.99 (t, *J* = 7.5 Hz, 2H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.76 (d, *J* = 8.7 Hz, 1H), 7.60 (m, 2H), 7.50 (d, *J* = 7.9 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.20 (d, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.96 (td, *J* = 7.6, 3.4 Hz, 2H), 4.60 (td, *J* = 8.5, 4.8 Hz, 1H), 4.47 (td, *J* = 9.3, 8.7, 3.9 Hz, 1H), 4.25 – 4.11 (m, 3H), 3.13 (dd, *J* = 14.9, 4.7 Hz, 1H), 2.92 (ddd, *J* = 19.8, 10.3, 5.4 Hz, 2H), 2.76 – 2.67 (m, 1H), 1.94 (h, *J* = 6.8 Hz, 1H), 1.79 (s, 3H), 1.25 (d, *J* = 7.3 Hz, 3H), 1.10 (d, *J* = 7.2 Hz, 3H), 0.77 (dd, *J* = 9.8, 6.6 Hz, 6H) ppm. **HPLC-MS** (m/z): $[M+H]^+$ calcd. for $C_{33}H_{41}IN_6O_7$, 761.6; found, 761.3.

Ac-Ala-*p*-I-Phe-Trp-Ala-OH (1k). 2-Chlorotrityl resin (1.0 mmol/g). Amino acid coupling. Fmoc-



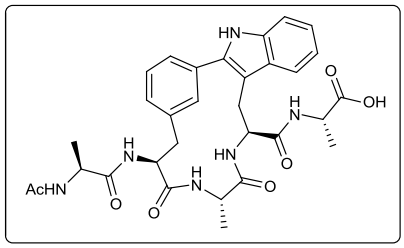
XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and HOBt (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Ala-OH, Fmoc-Trp-OH. Fmoc-*p*-I-Phe-OH (1.5 eq.) was incorporated with HBTU (1.5 eq.), HOBt (1.5 eq.) and DIEA (3.0 eq.) in DMF for 1h. The N-terminal was acetylated, and the resin bound peptide was treated with a 5% (v/v) TFA/DCM solution (5 x 1 min). Pale solid (>99% purity, estimated by HPLC-MS). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 10.81 (d, *J* = 2.5 Hz, 1H), 8.25 (d, *J* = 7.2 Hz, 1H), 8.02 (d, *J* = 8.1 Hz, 1H), 7.96 (d, *J* = 7.4 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.56 – 7.45 (m, 2H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.16 (d, *J* = 2.4 Hz, 1H), 7.05 (dd, *J* = 8.2, 6.9 Hz, 1H), 6.97 (t, *J* = 7.4, 6.9 Hz, 1H), 6.95 – 6.90 (m, 2H), 4.56 (td, *J* = 8.5, 4.7 Hz, 1H), 4.40 (td, *J* = 8.5, 4.6 Hz, 1H), 4.21 (dp, *J* = 21.4, 7.1 Hz, 2H), 3.13 (dd, *J* = 14.9, 4.7 Hz, 1H), 3.01 – 2.81 (m, 2H), 2.70 (dd, *J* = 13.9, 8.9 Hz, 1H), 1.79 (s, 3H), 1.27 (d, *J* = 7.3 Hz, 3H), 1.07 (d, *J* = 7.1 Hz, 3H). ppm. **HPLC-MS** (m/z): $[M+H]^+$ calcd. for $C_{28}H_{32}IN_5O_6$, 662.5; found, 662.2.

General procedure for the C-H activation process of peptides 2a-2f

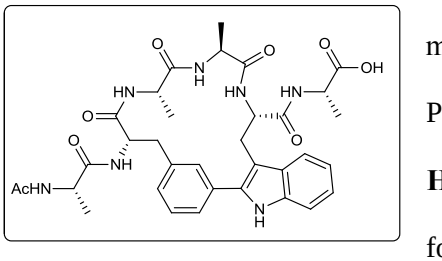
Unless stated otherwise, the linear peptide (50 mg), $AgBF_4$ (1.0 eq.), 2-nitrobenzoic acid (1.5 eq) and $Pd(OAc)_2$ (0.05 eq) were placed in a microwave reactor vessel in DMF or in a 1:1 mixture of DMF:PBS. The mixture was heated under microwave irradiation (80 W) at 80 °C for 15 min. Water was added and the resulting suspension was filtered through Celite. The filtrate was successively washed with Et_2O, and the aqueous phase was lyophilized. The residue was purified by semi-preparative RP-HPLC (XBRIDGE^{TM} Pref C18 5µM 19x100 mm column), using gradients of 25% of B to 60% B [solvent A (0.1% TFA in H_2O) and solvent B (0.07% TFA in ACN)], in 20 min, flux: 16 mL·min-1, detection at λ=220 nm.

Synthesis and peptide characterization of locked peptides 2a-2f

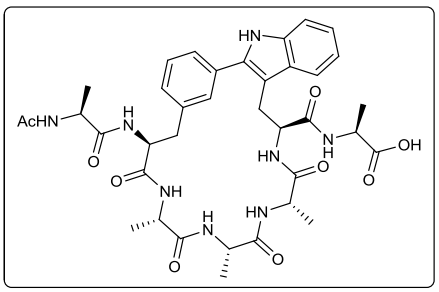
Ac-Ala-(Cyclo-*m*)-[Phe-Ala-Trp]-Ala-OH (2a). Starting from peptide **1a** (27 mg, 0.037 mmol) in a 1:1 mixture of DMF:PBS (total volume of 600 μL). Pale solid (38% conversion, estimated by HPLC-MS, 32% yield). **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{31}H_{36}N_6O_7$, 605.27182; found, 605.27400.



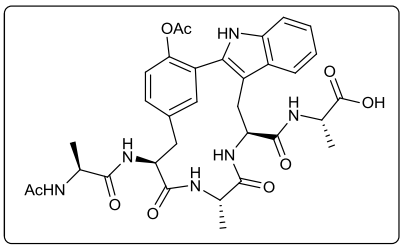
Ac-Ala-(Cyclo-*m*)-[Phe-Ala-Ala-Trp]-Ala-OH (2b). Starting from peptide **1b** (50 mg, 0.062 mmol) in a 1:1 mixture of DMF:PBS (total volume of 400 μL). Pale solid (total conversion, estimated by HPLC-MS, 26% yield). **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{34}H_{41}N_7O_8$, 676.30894; found, 676.31130. **^{1}H NMR** (500 MHz, D_2O): δ 10.58 (s, 1H), 8.49 (d, *J* = 3.2 Hz, 1H), 8.29 (m, 2H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.68 (s, 1H), 7.59 (dd, *J* = 11.3, 5.5 Hz, 3H), 7.48 (m, 2H), 7.44 – 7.39 (m, 1H), 7.29 (t, *J* = 7.5 Hz, 1H), 7.22 (t, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 9.5 Hz, 1H), 4.89 (1H), 4.82 (1H), 4.37 – 4.22 (m, 2H), 4.02 (dd, *J* = 7.3, 3.0 Hz, 1H), 3.84 (qd, *J* = 7.3, 3.6 Hz, 1H), 3.73 (dd, *J* = 15.2, 3.4 Hz, 1H), 3.47 (dd, *J* = 15.1, 11.5 Hz, 1H), 3.25 (d, *J* = 7.3 Hz, 2H), 1.99 (s, 3H), 1.42 (d, *J* = 7.3 Hz, 3H), 1.34 (m, 6H), 0.82 (d, *J* = 7.4 Hz, 3H) ppm.



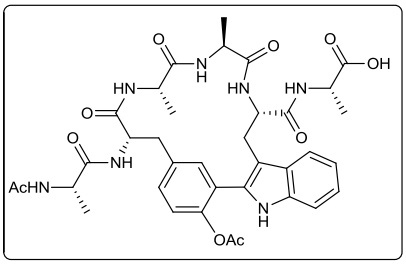
Ac-Ala-(Cyclo-*m*)-[Phe-Ala-Ala-Ala-Trp]-Ala-OH (2c). Starting from peptide **1c** (25 mg, 0.029 mmol) in a 1:1 mixture of DMF:PBS (total volume of 600 μL). Pale solid (total conversion, estimated by HPLC-MS, 54% yield). **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{37}H_{46}N_8O_9$, 747.34605; found, 747.34752.



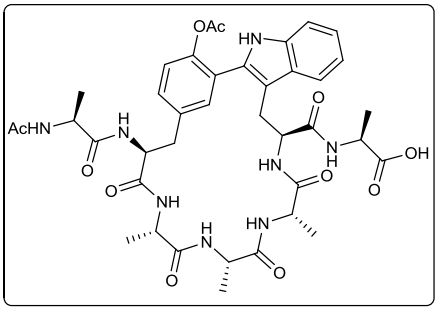
Ac-Ala-(Cyclo-*m*)-[Tyr(OAc)-Ala-Trp]-Ala-OH (2d). Starting from peptide **1d** (50 mg, 0.063 mmol) in a 1:1 mixture of DMF:PBS (total volume of 400 μL) (total conversion, estimated by HPLC-MS). **HPLC-MS** (m/z): $[M+H]^+$ calcd. for $C_{33}H_{38}N_6O_9$, 663.69; found, 663.14.



Ac-Ala-(Cyclo-*m*)-[Tyr(OAc)-Ala-Ala-Trp]-Ala-OH (2e). Starting from peptide **1e** (50 mg, 0.058 mmol) in a 1:1 mixture of DMF:PBS (total volume of 400 μL). (total conversion, estimated by HPLC-MS). **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{36}H_{43}N_7O_{10}$, 734.31442; found, 734.31681.



Ac-Ala-(Cyclo-*m*)-[Tyr(OAc)-Ala-Ala-Ala-Trp]-Ala-OH (2f). Starting from peptide **1f** (50 mg, 0.053 mmol) in a 1:1 mixture of DMF:PBS (total volume of 400 μL). (total conversion, estimated by HPLC-MS). **HPLC-MS** (m/z): $[M+H]^+$ calcd. for $C_{39}H_{48}N_8O_{11}$, 805.85; found, 805.34.

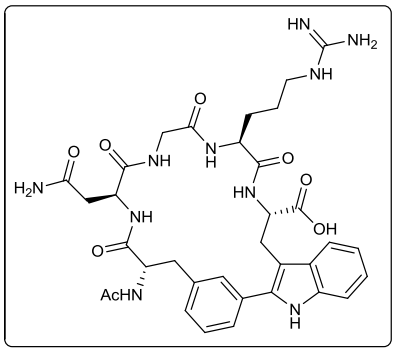


General procedure for the C-H activation process of peptides 2g-2k

Unless stated otherwise, the linear peptide (50 mg), $AgBF_4$ (2.0 eq.), trifluoroacetic acid (1.0 eq.) and $Pd(OAc)_2$ (0.05 eq.) were placed in a microwave reactor vessel in DMF. The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. The residue was filtered and purified by semi-preparative RP-HPLC (XBRIDGE™ BEH 130, C18, 5μM OBD 19x50 mm column) [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)], in 10 min, flux: 20 mL·min-1, detection at λ=220 nm.

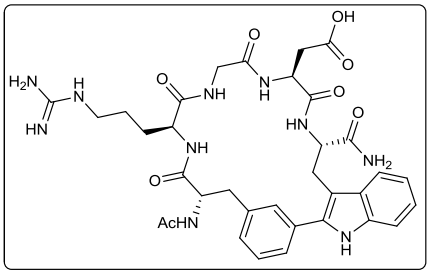
Synthesis and peptide characterization of locked peptides 2g-2m

Ac-(Cyclo-*m*)-[Phe-Asn-Gly-Arg-Trp]-OH (2g). Starting from peptide **1g** (186 mg, 0.220 mmol) (77% conversion, estimated by HPLC-MS). Semi-preparative RP-HPLC gradient: 15-30% of B. Pale solid (45.3 mg, 29%). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 11.15 (s, 1H), 8.42 (s, 1H), 8.35 (m, 2H), 7.79 (d, *J* = 8.2 Hz, 1H), 7.72 – 7.60 (m, 3H), 7.49 (d, *J* = 7.7 Hz, 1H), 7.38 (m, 2H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.20 (d, *J* = 7.7 Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.97 (t, *J* = 7.5 Hz, 1H), 6.85 (m, 1H), 4.68 (m, 1H), 4.60 (m, 1H), 4.48



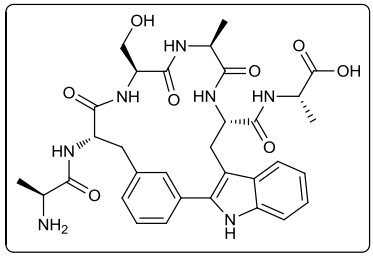
(m, 1H), 4.30 (m, 1H), 3.98 (dd, *J* = 16.4, 6.8 Hz, 1H), 3.43 (1H), 3,42 (1H), 3.14-2.99 (4H), 2.88 (t, *J* = 12.4 Hz, 1H), 2.66 – 2.58 (m, 1H), 2.44 (1H), 1.77 (m, 4H), 1.55 (m, 1H), 1.41 (m, 2H) ppm. **IR** (Film, cm^{-1}) ν = 3417.08, 3276.16, 3064.77, 2911.03, 1623.49, 1533.81 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{34}H_{42}N_{10}O_8$, 719.8; found, 720.1.

Ac-(Cyclo-*m*)-[Phe-Arg-Gy-Asp-Trp]-NH_2 (2h). Starting from peptide **1h** (190 mg, 0.224 mmol)



(70% conversion, estimated by HPLC-MS). Semi-preparative RP-HPLC gradient: 15-20% of B. Pale solid (28.9 mg, 18%). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 11.00 (s, 1H), 9.88 (s, 1H), 8.39 (s, 1H), 8.32 (d, *J* = 7.8 Hz, 1H), 8.06 (s, 1H), 7.88 (d, *J* = 5.5 Hz, 1H), 7.69 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.42 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.35 (s, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 7.4 Hz), 7.10 – 7.04 (m, 2H), 7.01 – 6.97 (t, *J* = 7.2 Hz, 1H), 6.89 (s, 1H), 4.66 (td, *J* = 7.9, 5.4 Hz, 1H), 4.53 (m, 1H), 4.25 (dt, *J* = 9.4, 4.4 Hz, 1H), 4.06 – 3.99 (m, 1H), 3.68 (dd, *J* = 16.7, 6.6 Hz, 1H), 3.46 – 3.42 (m, 2H), 3.14 (m, 1H), 3.04 – 2.93 (m, 3H), 2.75 (m, 1H), 2.67 (dd, *J* = 16.5, 4.0 Hz, 1H), 2.18 (dd, *J* = 16.9, 5.7 Hz, 1H), 1.88 (s, 3H), 1.86 – 1.82 (m, 1H), 1.49 (m, 1H), 1.41 (m, 1H), 1.34 (m, 1H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 174.82, 173.61, 172.52, 170.87, 170.73, 168.98, 167.68, 157.10, 137.76, 135.84, 135.27, 132.97, 129.15, 128.80, 128.31, 128.07, 126.29, 121.11, 118.73, 118.47, 110.87, 107.50, 53.74, 53.36, 52.83, 49.22, 43.37, 40.69, 38.67, 37.51, 30.53, 27.10, 24.88, 22.53 ppm. **IR** (Film): ν = 3423.49, 3269.75, 2923.84, 1649.11, 1629.89 1540.21 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{34}H_{42}N_{10}O_8$, 719.32598; found, 719.32718.

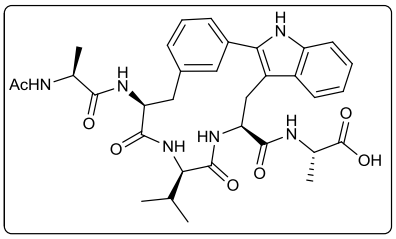
H-Ala-(Cyclo-*m*)-[Phe-Ser-Ala-Trp]-Ala-OH (2i). Starting from peptide **1i** (145 mg, 0.186



mmol). Additional microwave irradiation cycles were necessary to perform until obtain the desired product as the main peak by HPLC-MS (39% conversion, estimated by HPLC-MS). Semi-preparative RP-HPLC gradient: 12-32% of B. Pale solid (11.5 mg, 10%). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 11.09 (s, 1H), 8.46 (m, 1H), 8.35 (t, *J* = 4.7 Hz, 1H), 7.65 (s, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.53 – 7.48 (m, 1H), 7.45 – 7.39 (m, 2H), 7.39

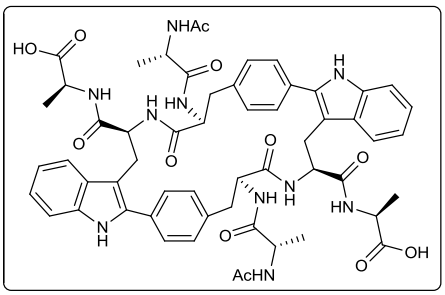
– 7.35 (m, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.22 (dt, *J* = 6.4, 1.9 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 7.04 – 7.00 (t, *J* = 7.6 Hz, 1H), 6.81 (d, *J* = 9.5 Hz, 1H), 4.97 (dd, *J* = 10.4, 3.6 Hz, 1H), 4.51 (ddd, *J* = 13.3, 8.5, 2.5 Hz, 1H), 4.15 (p, *J* = 7.3 Hz, 1H), 3.87 (dt, *J* = 5.9, 4.4 Hz, 1H), 3.70 (qd, *J* = 7.3, 4.1 Hz, 1H), 3.65 – 3.58 (m, 3H), 3.48 (1H), 3.30 (1H), 3.18 (dd, *J* = 13.6, 10.3 Hz, 1H), 3.08 (dd, *J* = 13.7, 3.5 Hz, 1H), 1.32 (d, *J* = 7.3 Hz, 3H), 1.17 (d, *J* = 6.9 Hz, 3H), 0.76 (d, *J* = 7.3 Hz, 3H). **^{13}C NMR** (151 MHz, DMSO-d_6): δ 173.74, 172.09, 171.69, 171.36, 170.54, 137.18, 135.95, 134.68, 132.59, 128.88, 128.73, 128.59, 128.48, 125.80, 121.63, 118.74, 118.18, 111.12, 108.33, 60.30, 58.37, 53.75, 51.87, 50.13, 49.47, 48.00, 36.64, 26.78, 20.08, 17.00, 16.02 ppm. **IR** (Film): ν = 3321.00, 2917.44, 3064.77, 2846.98, 1655.52, 1597.86 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{32}H_{39}N_7O_8$, 650.29329; found, 650.29471.

Ac-Ala-(Cyclo-*m*)-[Phe-Val-Trp]-Ala-OH (2j). Starting from peptide **1j** (207 mg, 0.374 mmol)

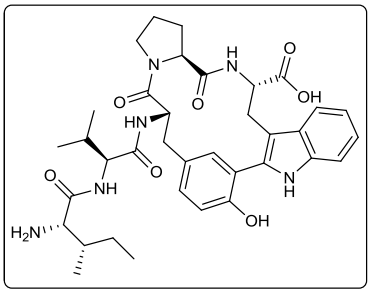


(71% conversion, estimated by HPLC-MS). Semi-preparative RP-HPLC gradient: 20-40% of B. Pale solid (55.2 mg, 32%). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 11.19 (s, 1H), 8.09 (m, 2H), 7.67 (d, *J* = 9.6 Hz, 1H), 7.60 (d, *J* = 7.3 Hz, 1H), 7.56 (d, *J* = 7.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.33 (dd, *J* = 7.9, 1.3 Hz, 2H), 7.29 (t, *J* = 1.7 Hz, 1H), 7.16 (dt, *J* = 7.5, 1.5 Hz, 1H), 7.08 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 6.95 (ddd, *J* = 7.9, 6.9, 1.1 Hz, 1H), 4.65 (ddd, *J* = 9.7, 6.4, 3.2 Hz, 1H), 4.60 (ddd, *J* = 8.8, 7.2, 4.4 Hz, 1H), 4.31 (p, *J* = 7.2 Hz, 1H), 4.24 (p, *J* = 7.3 Hz, 1H), 4.07 (t, *J* = 9.5 Hz, 1H), 3,34 (1H), 3.20 (dd, *J* = 14.7, 6.6 Hz, 1H), 3.01 – 2.85 (m, 2H), 1.84 (s, 3H), 1.75 (m, 1H), 1.16 (d, *J* = 7.1 Hz, 3H), 0.99 (d, *J* = 7.3 Hz, 3H), 0.72 (dd, *J* = 6.7, 4.3 Hz, 6H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 173.66, 171.79, 171.26, 169.91, 169.79, 168.93, 137.29, 136.51, 135.78, 132.48, 129.45, 129.29, 128.56, 128.46, 127.18, 121.23, 119.51, 118.40, 110.83, 105.92, 57.69, 53.78, 52.32, 48.02, 47.76, 38.33, 30.44, 26.59, 22.45, 18.87, 18.53, 17.95, 16.67 ppm. **IR** (Film, cm^{-1}) ν = 3295.37, 3051.96, 2962.28, 1642.70, 1617.08, 1514.59 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{33}H_{40}N_6O_7$, 633.30312; found, 633.30487.

(Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (2k). Starting from peptide **1k** (600 mg, 0.907 mmol) (60% conversion, estimated by HPLC-MS). Semi-preparative RP-HPLC gradient: 25-30% of B. Pale solid (3.8 mg, 1%). **^{1}H NMR** (800 MHz, DMSO-d_6): δ 11.20 (s, 1H), 8.13 (d, *J* = 7.7 Hz, 1H), 7.93 (d, *J* = 7.3 Hz, 1H), 7.90 (d, *J* = 7.0 Hz, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 2H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 7.7 Hz, 2H), 7.11 (t, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 6.82 (d, *J* = 6.9 Hz, 1H), 4.24 – 4.15 (dp, *J* = 28.2, 7.1 Hz, 2H), 4.07 (ddd, *J* = 10.4, 6.9, 3.8 Hz, 1H), 3.96 (m, 1H), 3.43 (dd, *J* = 15.1, 3.7 Hz, 1H), 3.23 (dd, *J* = 15.2, 9.4 Hz, 1H), 2.64 (dd, *J* = 15.1, 10.6 Hz, 1H), 2.48 (m, 1H), 1.78 (s, 3H), 1.28 (d, *J* = 7.3 Hz, 3H), 1.08 (d, *J* = 7.0 Hz, 3H) ppm. **IR** (Film, cm^{-1}) ν = 3404.27, 2911.03, 1655.52 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{56}H_{62}N_{10}O_{12}$, 1067.4621; found, 1067.4624.

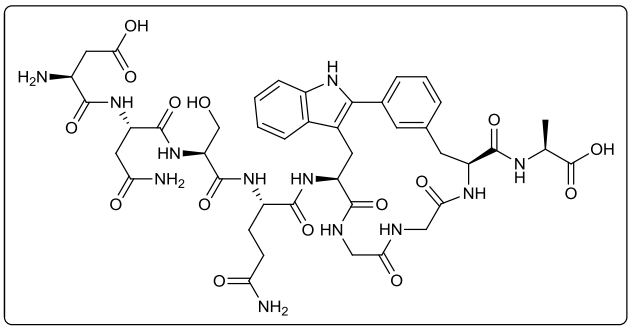


H-Ile-Val-(Cyclo-*m*)-[Tyr-Pro-Trp]-OH (2l). *AB linker incorporation for TentaGel S NH_2 resin.* AB linker (3.0 eq.) was attached to the resin (1.0 eq.) with DIPCDI (3.0 eq.), OxymaPure (3.0 eq.) in DMF at r.t for 1h. *First amino acid incorporation.* Fmoc-Trp-OH (4.0 eq.) was attached to the resin (1.0 eq.) with DIPCDI (2.0 eq.), DMAP (0.4 eq.) in DCM at r.t (1 x 2h, 1 x 16h). *End-capping of resin to block any remaining unreacted active resin sites.* Anhydride acetic (5.0 eq.) and DIEA (5.0 eq.) in DMF were added for 30 min. Peptide elongation. Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and OxymaPure (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Pro-OH, Fmoc-*3*-iodo-Tyr-OH, Fmoc-Val-OH, Fmoc-Ile-OH. *Stapled bond formation on solid-phase.* The resulting peptide anchored to the resin (67 mg, 0.065 mmol), $AgBF_4$ (13 mg, 0.065 mmol, 1.0 eq.), 2-nitrobenzoic acid (16 mg, 0.098 mmol, 1.5 eq.) and $Pd(OAc)_2$ (0.7 mg, 3.3 μmol, 0.05 eq.) were placed in a microwave reactor vessel in 900 μL of DMF. The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. Three more batches were carried out following the same procedure and were combined. The peptide anchored to the resin was treated with 1% DDC in DMF and after removing the Fmoc group it was cleaved from the resin with a 95% TFA, 2.5% TIS, 2.5% H_2O cocktail (1h). Pale solid (76.7 mg, 79% purity estimated by



HPLC-MS, 32% yield). A pure fraction was obtained by semi-preparative RP-HPLC (XBRIDGE, PrepC18, 5µM OBD™ 19x150 mm column) [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)], in 20 min, flux: 16 mL·min-1, detection at λ=220 nm (gradient: 20-25% of B). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 10.79 (s, 1H), 8.32 (d, *J* = 6.7 Hz, 1H), 8.22 (s, 1H), 8.15 (d, *J* = 8.9 Hz, 1H), 7.66 – 7.56 (m, 2H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.14 (d, *J* = 2.2 Hz, 1H), 7.11 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.99 (t, *J* = 7.4 Hz, 1H), 6.89 (d, *J* = 8.2 Hz, 1H), 4.68 (td, *J* = 8.0, 6.4, 3.6 Hz, 1H), 4.58 – 4.46 (m, 2H), 4.33 (dd, *J* = 8.7, 6.3 Hz, 1H), 3.61 (q, *J* = 8.5 Hz, 1H), 3.06 (td, *J* = 12.7, 11.0, 4.9 Hz, 2H), 2.88 (dd, *J* = 13.6, 3.3 Hz, 1H), 2.76 (dd, *J* = 14.8, 9.0 Hz, 1H), 2.14 – 2.09 (m, 1H), 1.98 (h, *J* = 6.7 Hz, 1H), 1.75 (tq, *J* = 14.0, 5.8, 4.5 Hz, 2H), 1.55 (dtd, *J* = 16.1, 11.9, 6.2 Hz, 1H), 1.50 – 1.38 (m, 2H), 1.23 (s, 2H), 1.11 (ddd, *J* = 13.4, 9.5, 7.0 Hz, 1H), 0.91 – 0.79 (m, 12H) ppm. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{36}H_{46}N_6O_7$, 675.35007; found, 675.35096.

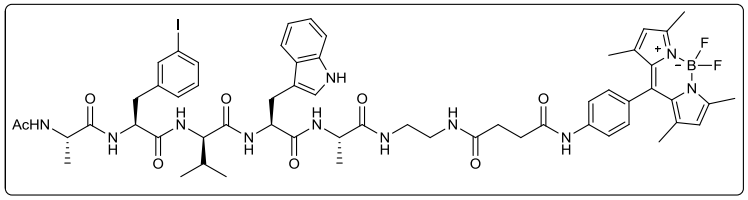
H-Asp-Asn-Ser-Gln-(Cyclo-*m*)-[Trp-Gly-Gly-Phe]-Ala-OH (2m). *AB linker incorporation for TentaGel S NH_2 resin.* AB linker (3.0 eq.) was attached to the resin (1.0 eq.) with DIPCDI (3.0 eq.), OxymaPure (3.0 eq.) in DMF at r.t for 1h. *First amino acid incorporation*. Fmoc-Trp-OH (4.0 eq.) was attached to the resin (1.0 eq.) with DIPCDI (2.0 eq.), DMAP (0.4 eq.) in DCM at r.t (1 x 2h, 1 x 16h). *End-capping of resin to block any remaining unreacted active resin sites.* Anhydride acetic (5.0 eq.) and DIEA (5.0 eq.) in DMF were added for 30 min. *Peptide elongation until fifth amino acid incorporation.* Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and OxymaPure (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Ala-OH, Fmoc-Gly-OH, Fmoc-Trp-OH. Fmoc-*m*-I-Phe-OH (1.5 eq.) was incorporated with HBTU (1.5 eq.), HOBt (1.5 eq.) and DIEA (3.0 eq.) in DMF for 1h. *Stapled bond formation on solid-phase.* The resulting peptide anchored to the resin (64 mg, 0.072 mmol), $AgBF_4$ (14 mg, 0.072 mmol, 1.0 eq.), 2-nitrobenzoic acid (18 mg, 0.108 mmol, 1.5 eq.) and $Pd(OAc)_2$ (0.8 mg, 3.6 µmol, 0.05 eq.) were placed in a microwave reactor vessel in 980 µL of DMF. The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. Three more batches



were carried out following the same procedure and were combined. *Peptide elongation until ninth amino acid incorporation.* The peptide anchored to the resin was treated with 1% DDC in DMF and Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and OxymaPure (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Gln(Trt)-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(O*t*-Bu)-OH. After removing the Fmoc group, the final peptide sequence was cleaved from the resin with a 95% TFA, 2.5% TIS, 2.5% H_2O cocktail (1h). Pale solid (86.3 mg, 85% purity estimated by HPLC-MS, 26% yield). A pure fraction was obtained by semi-preparative RP-HPLC (XBRIDGE, PrepC18, 5µM OBD^{TM} 19x150 mm column) [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)], in 20 min, flux: 16 mL·min-1, detection at λ=220 nm (gradient: 10-25% of B). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 11.16 (s, 1H), 8.58 (q, *J* = 6.7, 6.2 Hz, 2H), 8.50 (d, *J* = 7.2 Hz, 1H), 8.23 (d, *J* = 7.2 Hz, 1H), 8.15 (m, 2H), 8.01 (d, *J* = 7.9 Hz, 1H), 7.90 – 7.82 (m, 2H), 7.38 (m, 5H), 7.32 – 7.26 (m, 1H), 7.11 (t, *J* = 7.5 Hz, 1H), 7.05 (t, *J* = 7.4 Hz, 1H), 6.97 – 6.90 (m, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 6.78 – 6.69 (m, 1H), 4.87 – 4.77 (m, 1H), 4.67 (q, *J* = 6.2 Hz, 1H), 4.57 (ddd, *J* = 11.2, 8.3, 2.8 Hz, 1H), 4.27 (td, *J* = 8.8, 5.1 Hz, 1H), 4.22 (t, *J* = 7.3 Hz, 1H), 4.17 (q, *J* = 5.8 Hz, 1H), 3.85 (t, *J* = 5.8 Hz, 1H), 3.79 (dd, *J* = 15.8, 4.6 Hz, 1H), 3.71 (dd, *J* = 11.5, 5.8 Hz, 1H), 3.65 – 3.55 (m, 2H), 3.15 (d, *J* = 13.3 Hz, 1H), 3.01 (dd, *J* = 14.1, 4.5 Hz, 1H), 2.83 (dd, *J* = 13.7, 11.1 Hz, 1H), 2.58 (dd, *J* = 10.6, 5.9 Hz, 2H), 2.17 – 1.98 (m, 2H), 1.91 (ddt, *J* = 15.7, 10.7, 5.4 Hz, 1H), 1.77 (dtd, *J* = 14.8, 9.7, 5.3 Hz, 1H), 1.34 (d, *J* = 7.3 Hz, 3H) ppm. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{43}H_{54}N_{12}O_5$, 979.39044; found, 979.39270.

Synthesis and peptide characterization of peptides 1j-Bodipy and 2j-Bodipy

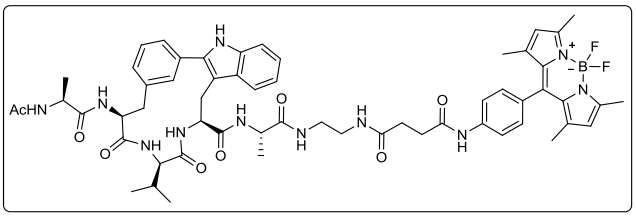
Ac-Ala-*m*-I-Phe-Val-Trp-Ala-*linker*-BODIPY (1j-Bodipy). The linear peptide sequence **1j** (16.9



mg, 0.022 mmol, 1.2 eq.), EDC·HCl (5.3 mg, 0.028 mmol, 1.5 eq.) and HOBt·H_2O (4.2 mg, 0.028 mmol, 1.5 eq.) were dissolved in 0.5 mL of DMF. Then, compound **19** (8.9 mg, 0.018 mmol, 1.0 eq.) dissolved in 1 mL of DMF and DIEA (9.7 µL, 0.055 mmol, 3.0 eq.) were added to give a final peptide concentration of 0.01 M and the solution was stirred for 16 h at r.t. Workup was done by removing the

DMF under vacuum, dissolving the crude in EtOAc and extracting with $NaHCO_{3sat}$. Organic layers were combined, dried over sodium sulfate, filtered and concentrated under vacuum to afford 18.0 mg of the crude peptide (58% yield estimated by HPLC-MS conversion). A highly pure fraction of the linear peptide was obtained by purification of 11.0 mg of crude in a PoraPak Rxn RP 60 cc reverse phase column (2 g) [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)]. Red solid (95% purity by HPLC-MS). **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{58}H_{69}BF_2IN_{11}O_8$, 1224.45091; found, 1224.45351.

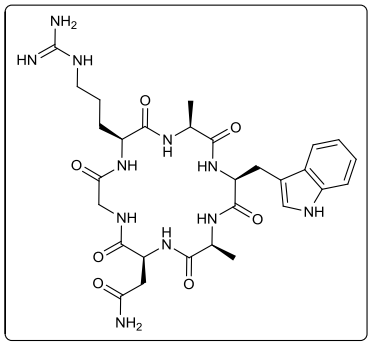
Ac-Ala-(Cyclo-*m)*-[Phe-Val-Trp]-Ala-OH-*linker*-BODIPY (2j-Bodipy). The staple peptide **2j**



(16.1 mg, 0.021 mmol, 1.2 eq.), EDC·HCl (5.1 mg, 0.026 mmol, 1.5 eq.) and HOBt·H_2O (4.1 mg, 0.026 mmol, 1.5 eq.) were dissolved in 0.6 mL of DMF. Then, compound **19** (8.5 mg, 0.018 mmol, 1.0 eq.) dissolved in 1 mL of DMF and DIEA (9.2 μL, 0.053 mmol, 3.0 eq.) were added to give a final peptide concentration of 0.01 M and the solution was stirred for 16 h at r.t. Workup was done by removing the DMF under vacuum and purifying the crude in a PoraPak Rxn RP 60 cc reverse phase column (2 g) [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)]. Red solid (10.2 mg, 97% purity by HPLC-MS, 53% yield). **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{58}H_{68}BF_2N_{11}O_8$, 1096.53862; found, 1096.54044.

Synthesis and characterization of compounds 3-19

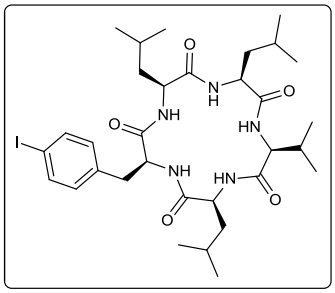
Cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-) (3). 2-Chlorotrityl resin (1.0 mmol/g). Amino acid coupling.



Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and OxymaPure (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ala-OH, Fmoc-Trp(Boc)-OH, Fmoc-Arg(Pbf)-OH. The resin bound peptide was treated with a 1% (v/v) TFA/DCM solution (5 x 1 min). Pale solid (94% purity, HPLC). Cyclization in solution. The free-amine free-acid protected linear peptide (1.5 g, 1.18 mmol) was dissolved in 395 mL of ACN/DMF (14:1) solution (0.003 M) and DIEA (6.0 eq.), PyBOP (3.0 eq.) and HOBt (3.0 eq.) were added. The solution was stirred at r.t until

the cyclization was complete (1h). Workup was done by extracting with NH_4Cl_{sat} and $NaHCO_{3sat}$. Organic layers were combined, dried over sodium sulfate, filtered and concentrated under vacuum. Then, the macrocycle was treated with a 95% TFA, 2.5% TIS, 2.5% H_2O cocktail (3h), washed with Et_2O, dissolved in ACN:H_2O and lyophilized furnishing the corresponding peptide. Pale solid (436 mg, 33%). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 10.83 (d, *J* = 2.4 Hz, 1H), 8.25 (t, *J* = 5.6 Hz, 1H), 8.14 (d, *J* = 7.3 Hz, 1H), 8.09 (d, *J* = 6.1 Hz, 1H), 8.06 (d, *J* = 8.1 Hz, 1H), 8.00 – 7.97 (d, *J* = 7.4 Hz, 1H), 7.88 (d, *J* = 6.9 Hz, 1H), 7.60 – 7.56 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 5.7 Hz, 1H), 7.42 (m, 1H), 7.32 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.14 (d, *J* = 2.4 Hz, 1H), 7.06 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 6.97 (ddd, *J* = 7.9, 6.9, 1.0 Hz, 1H), 6.92 (m, 1H),4.35 (q, *J* = 6.4 Hz, 1H), 4.32 – 4.24 (m, 1H), 4.15 – 4.02 (m, 2H), 3.97 (p, *J* = 7.0 Hz, 1H), 3.83 (dd, *J* = 16.1, 5.5 Hz, 1H), 3.56 – 3.52 (m, 1H), 3.18 (m, 2H), 3.10 – 3.06 (m, 2H), 2.67 – 2.55 (m, 2H), 1.87 – 1.76 (m, 1H), 1.60 (m, 1H), 1.52 – 1.40 (m, 2H), 1.28 – 1.15 (m, 6H) ppm. **IR** (Film, cm^{-1}) ν = 3276.16, 3186.48, 3051.96, 2917.44, 1642.70, 1508.19 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{29}H_{41}N_{11}O_7$, 656.32632; found, 656.32680.

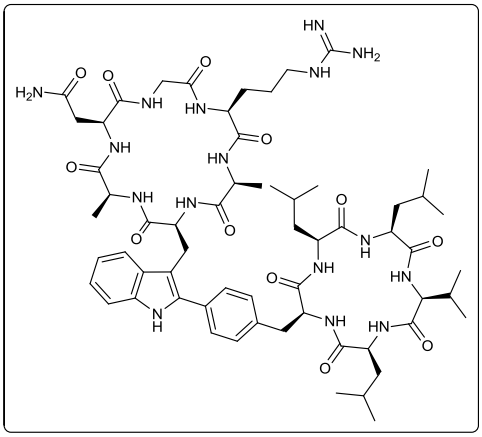
Cyclo(-Leu-Leu-Val-Leu-*p*-I-Phe-) (4). 2-Chlorotrityl resin (0.94 mmol/g). Amino acid coupling.



Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and OxymaPure (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-*p*-I-Phe-OH, Fmoc-Leu-OH, Fmoc-Val-OH. The resin bound peptide was treated with a 5% (v/v) TFA/DCM solution (5 x 2 min). Pale solid (85% purity, HPLC). Cyclization in solution. The free-amine free-acid linear peptide (119 mg, 0.163 mmol) was dissolved in 55 mL of ACN/DCM (1:9) solution (0.003 M) and DIEA (6.0 eq.), HATU (1.5 eq.) and TBTU (1.5 eq.) were added. The solution was stirred at r.t until the cyclization was complete (3h). Workup was done by extracting with NH_4Cl_{sat} and $NaHCO_{3sat}$. Organic layers were combined, dried over sodium sulfate, filtered and concentrated under vacuum. Then, the macrocycle was purified via flash column chromatography using and EtOAc/hexane gradient on silica gel furnishing the corresponding peptide. Pale solid (28.1 mg, 24%). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 8.15 (t, *J* = 7.7 Hz, 2H), 8.02 (d, *J* = 8.4 Hz, 1H), 8.00 – 7.89 (m, 2H), 7.62 (d, *J* = 7.7 Hz, 2H), 7.03 (d, *J* = 7.9 Hz, 2H), 4.27 (q, *J* = 8.0 Hz, 1H), 4.12 (q, *J* = 8.4, 6.2 Hz, 1H), 4.05 (m, 1H), 3.94 (q, *J* =

8.4 Hz, 1H), 3.66 (t, *J* = 9.5 Hz, 1H), 3.07 – 2.95 (m, 2H), 2.15 (m, 1H), 1.68 (m, 2H), 1.58 – 1.43 (m, 4H), 1.38 – 1.24 (m, 3H), 0.92 – 0.74 (m, 24H) ppm. **IR** (Film, cm^{-1}) ν = 3295.37, 3077.58, 2962.28, 1655.52, 1540.21 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{32}H_{50}IN_5O_5$, 712.29294; found, 712.29392.

Cyclo(Ala-Asn-Gly-Arg-Ala-C2-Trp-)—Cyclo(C4-Phe-Leu-Leu-Val-Leu-) (5). Macrocycle



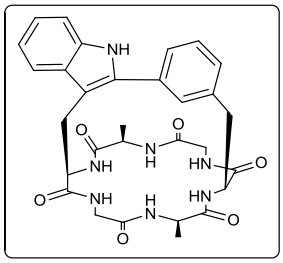
4 (40.0 mg, 0.056 mmol), macrocycle **3** (55.3 mg, 0.084 mmol, 1.5 eq.), $AgBF_4$ (43.8 mg, 0.225 mmol, 4.0 eq.), pivalic acid (5.7 mg, 0.056 mmol, 1.0 eq.) and $Pd(OAc)_2$ (1.4 mg, 0.077 mmol, 0.1 eq.) were placed in a microwave reactor vessel in 2 mL of PBS:DMF (1:1). The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. The irradiation cycle was repeated by adding a new portion of $Pd(OAc)_2$ and $AgBF_4$. The residue was filtered and partially purified in a PoraPak Rxn RP 60 cc reverse phase column (5 g) [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)]. Pale solid (1.63 mg, 2% yield estimated by HPLC-MS conversion). A pure fraction was obtained by analytic RP-HPLC (XBRIDGE™ BEH 130, C18, 5μM 10x100 mm column) [solvent A (0.045% TFA in H_2O) and solvent B (0.036% TFA in ACN)], in 30 min, flux: 3 mL·min-1, detection at λ=220 nm (gradient: 30-50% of B). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 11.16 (s, 1H), 8.45 (t, *J* = 5.9 Hz, 1H), 8.19-8.18 (t, *J* = 7.9 Hz, 2H), 8.11 – 7.90 (m, 8H), 7.64 (d, *J* = 8.1 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.44 – 7.40 (m, 2H), 7.35 – 7.31 (m, 3H), 7.11 – 7.06 (m, 1H), 7.00 – 6.96 (m, 1H), 6.91 (m, 1H), 4.39 – 4.29 (m, 2H), 4.25 (q, *J* = 7.0 Hz, 1H), 4.19 – 4.09 (m, 3H), 4.02 (m, 1H), 3.97 (q, *J* = 8.7 Hz, 1H), 3.92 (q, *J* = 6.6, 6.1 Hz, 1H), 3.86 (dd, *J* = 15.8, 5.8 Hz, 1H), 3.72 (t, *J* = 9.0 Hz, 1H), 3.41 (1H), 3.40-3.32 (2H) 3.12 (m, 2H), 3.10 – 3.04 (m, 2H), 2.58 (d, *J* = 6.2 Hz, 2H), 2.19 – 2.11 (m, 1H), 1.79 (m, 1H), 1.73 – 1.41 (m, 11H), 1.39 – 1.34 (m, 1H), 1.11 (dd, *J* = 7.1, 3.2 Hz, 6H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.90 – 0.81 (m, 18H), 0.77 (d, *J* = 6.3 Hz, 3H) ppm. **IR** (Film, cm^{-1}) ν = 3321.00, 3199.29, 2962.28, 2923.84, 1655.52, 1533.81 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{61}H_{91}N_{16}O_{12}$, 1239.69969; found, 1239.70241.

Bis[cyclo(-Arg-Ala-Trp-Ala-Asn-Gly-)] adduct (6). 1,4-diiodobenzene (35 mg, 0.106 mmol), macrocycle **3** (209 mg, 0.318 mmol, 3.0 eq.), $AgBF_4$ (124 mg, 0.637 mmol, 6.0 eq.), pivalic acid (16.3 mg, 0.159 mmol, 1.5 eq.) and $Pd(OAc)_2$ (9.5 mg, 0.042 mmol, 0.4 eq.) were placed in a microwave reactor vessel in 2 mL of PBS:DMF (1:1). The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. The crude was filtered and the workup was done by washing with AcOEt and then precipitating by adding ACN to the aqueous phase. The precipitated was washed with ACN, decanted and dried, obtaining 159 mg of crude (pale solid, 42%, yield estimated by HPLC-MS conversion). A pure fraction was obtained by semi-preparative RP-HPLC (Phenomenex Jupiter, C18, 10μM, 21.20x100 mm column, [solvent A (0.1% FA in H_2O) and solvent B (0.05% FA in ACN)], in 20 min, flux: 20 mL·min-1, detection at λ=220 nm (gradient: 15-20% of B). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 11.29 (s, 2H), 8.40 (s, 2H), 8.17 (s, 2H), 8.06 (m, 6H), 7.97 (d, *J* = 8.7 Hz, 2H), 7.87 (s, 4H), 7.65 (m, 2H), 7.42 (s, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.32 – 7.16 (m, 6H), 7.13 – 7.08 (m, 2H), 7.00 (t, *J* = 7.3 Hz, 2H), 6.89 (s, 2H), 4.36 (m, 4H), 4.18 (m, 2H), 4.09 – 4.02 (m, 2H), 3.98 (m, 2H), 3.86 (m, 2H), 3.56 – 3.48 (m, 2H), 3.44 – 3.39 (m, 4H), 3.08 (q, *J* = 6.4 Hz, 4H), 2.63 – 2.56 (m, 4H), 1.81 (dd, *J* = 13.4, 6.9 Hz, 2H), 1.62 (m, 2H), 1.47 (m, 4H), 1.11 (m, 12H) ppm. **IR** (Film, cm^{-1}) ν = 3314.59, 3199.29, 3051.96, 2911.03, 1661.92, 1533.81 cm^{-1}. **HRMS** (ESI) (m/z): [M] calcd. for $C_{64}H_{84}N_{22}O_{14}$, 1384.65373; found, 1384.65505.

H-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-OH (9). *AB linker incorporation for TentaGel S NH_2 resin*. AB linker (3.0 eq.) was attached to the resin (1.0 eq.) with DIPCDI (3.0 eq.), OxymaPure (3.0 eq.) in DMF at r.t for 1h. *First amino acid incorporation*. Fmoc-Gly-OH (4.0 eq.) was attached to the resin (1.0 eq.) with DIPCDI (2.0 eq.), DMAP (0.4 eq.) in DCM at r.t

(1 x 2h, 1 x 16h). *End-capping of resin to block any remaining unreacted active resin sites.*Anhydride acetic (5.0 eq.) and DIEA (5.0 eq.) in DMF were added for 30 min. *Peptide elongation.* Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and OxymaPure (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Trp-OH, Fmoc-Ala-OH, Fmoc-Gly-OH. Fmoc-*m*-I-Phe-OH (1.5 eq.) was incorporated with HBTU (1.5 eq.), HOBt (1.5 eq.) and DIEA (3.0 eq.) in DMF for 1h. *Stapled bond formation on solid-phase.* The resulting peptide **7** anchored to the resin (139 mg, 0.145 mmol), $AgBF_4$ (28 mg, 0.144 mmol, 1.0 eq.), 2-nitrobenzoic acid (36 mg, 0.215 mmol, 1.5 eq.) and $Pd(OAc)_2$ (1.6 mg, 7.1 μmol, 0.05 eq.) were placed in a microwave reactor vessel in 2 mL of DMF. The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. Eight more batches were carried out following the same procedure and were combined. The peptide **8** anchored to the resin was treated with 1% DDC in DMF and after removing the Fmoc group it was cleaved from the resin with a 95% TFA, 2.5% TIS, 2.5% H_2O cocktail (1h). (85% purity, estimated by HPLC-MS). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 11.15 (s, 1H), 8.43 (t, *J* = 5.1 Hz, 1H), 7.84 (s, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.50 (s, 1H), 7.41-7.35 (m, 4H), 7.17 – 7.08 (m, 2H), 7.01 (t, *J* = 7.4 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 4.83 – 4.74 (m, 1H), 4.55 (q, *J* = 8.2 Hz, 1H), 3.83 (m, 1H), 3.76 (m, 1H), 3.71 (m, 1H), 3.57 – 3.53 (m, 3H), 3.38-3.32 (m, 2H), 3.14 – 3.11 (m, 1H), 3.03 (dd, *J* = 13.5, 8.8 Hz, 1H), 1.28 (d, *J* = 6.9 Hz, 3H), 0.80 (d, *J* = 7.2 Hz, 3H) ppm. **IR** (Film, cm^{-1}) ν = 3288.97, 3051.96, 2923.84, 1649.11, 1527.40 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{30}H_{35}N_7O_7$, 606.26707; found, 606.26754.

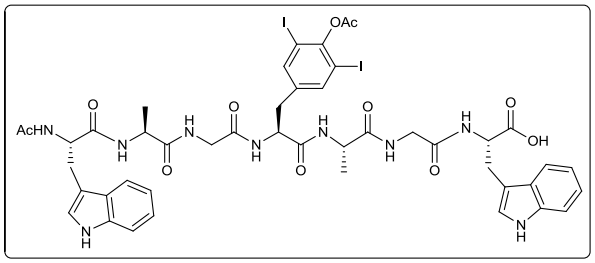
Cyclo[-Ala-(Cyclo-*m*)-[Phe-Gly-Ala-Trp]-Gly-] (10). The free-amine free-acid stapled peptide **9**



(92.2 mg, 0.152 mmol) was dissolved in 152 mL of DMF (0.001 M) and DIEA (6.0 eq.) and PyAOP (2.0 eq.) were added. The solution was stirred at r.t until the cyclization was complete (1.5h). DMF was removed under vacuum, and the crude was dissolved in EtOAc and extracted with NH_4Cl_{sat} and $NaHCO_{3sat}$. Organic layers were combined, dried over sodium sulfate, filtered and concentrated under vacuum. The crude was purified by semi-preparative RP-HPLC (XBRIDGE™ BEH 130, C18, 5μM OBD 19x50 mm column) [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)], in 10 min, flux: 20 mL·min-1, detection at λ=220 nm (gradient: 20-30% of B). Pale solid (16.0 mg, 18%).

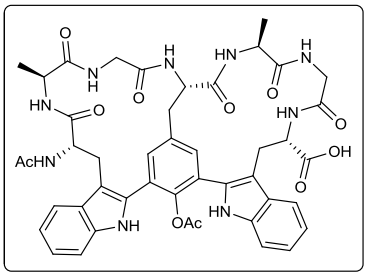
^{1}H NMR (600 MHz, DMSO-d_6): δ 11.23 (s, 1H), 8.53 (s, 1H), 8.19 (s, 1H), 7.49 – 7.35 (m, 6H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.17 (dt, *J* = 7.7, 1.4 Hz, 1H), 7.06 (ddd, *J* = 8.0, 6.9, 1.2 Hz, 1H), 6.96 (ddd, *J* = 8.0, 6.8, 1.0 Hz, 1H), 6.77 (d, *J* = 8.9 Hz, 1H), 6.46 (s, 1H), 4.72 – 4.60 (m, 1H), 4.55 (dt, *J* = 8.6, 6.7 Hz, 1H), 4.14 (m, 1H), 4.11 – 4.05 (m, 1H), 3.57 (dd, *J* = 13.4, 1.9 Hz, 1H), 3.54 (d, *J* = 4.9 Hz, 1H), 3.50 (m, 2H), 3.49 – 3.43 (m, 1H), 3.37 – 3.34 (m, 1H), 3.15 (dd, *J* = 14.1, 2.6 Hz, 1H), 2.83 (dd, *J* = 13.7, 6.7 Hz, 1H), 1.28 (d, *J* = 7.4 Hz, 3H), 0.34 (d, *J* = 7.3 Hz, 3H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 171.42, 171.41, 170.82, 170.71, 169.08, 168.56, 136.02, 135.69, 135.66, 133.76, 130.16, 130.11, 129.65, 128.24, 126.79, 121.22, 118.51, 118.45, 111.02, 105.66, 51.27, 51.18, 49.44, 48.72, 43.31, 43.18, 35.90, 25.07, 16.43, 15.23 ppm. **IR** (Film, cm^{-1}) ν = 3378.65, 3301.78, 3051.96, 2930.25, 1649.11, 1533.81 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{30}H_{33}N_7O_6$, 588.25651; found, 588.25770.

Ac-Trp-Ala-Gly-*3,5*-I,I-Tyr(OAc)-Ala-Gly-Trp-OH (11). 2-Chlorotrityl resin (0.8 mmol/g).



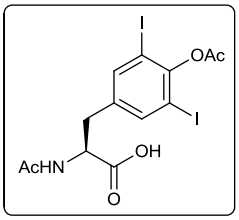
Amino acid coupling. Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and OxymaPure (3.0 eq.) in DMF for 1h. Fmoc-XX-OH: Fmoc-Gly-OH, Fmoc-Ala-OH, Fmoc-*m.m*-I,I-Tyr-OH, Fmoc-Trp-OH. The N-terminal was acetilated, and the resin bound peptide was treated with a 5% (v/v) TFA/DCM solution (5 x 2 min). Pale solid (>99% purity, estimated by HPLC-MS). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 10.84 (d, *J* = 2.5 Hz, 1H), 10.75 (d, *J* = 2.5 Hz, 1H), 8.30 (d, *J* = 7.2 Hz, 1H), 8.13 (d, *J* = 7.2 Hz, 1H), 8.10 – 8.03 (m, 3H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.92 (t, *J* = 5.7 Hz, 1H), 7.77 (s, 2H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.31 (dd, *J* = 9.7, 8.3 Hz, 2H), 7.13 (dd, *J* = 12.0, 2.4 Hz, 2H), 7.09 – 7.00 (m, 2H), 6.96 (dddd, *J* = 8.9, 7.8, 6.9, 1.1 Hz, 2H), 4.56 – 4.50 (m, 2H), 4.48 (dt, *J* = 7.8, 3.8 Hz, 1H), 4.33 – 4.18 (m, 2H), 3.79 – 3.68 (m, 3H), 3.56 (1H), 3.18 (dd, *J* = 14.7, 5.3 Hz, 1H), 3.11 (dd, *J* = 14.7, 4.7 Hz, 1H), 3.04 (dd, *J* = 14.6, 8.0 Hz, 1H), 2.97 (dd, *J* = 14.1, 3.8 Hz, 1H), 2.93 – 2.84 (m, 1H), 2.67 (dd, *J* = 13.9, 10.0 Hz, 1H), 2.34 (s, 3H), 1.76 (s, 3H), 1.21 (dd, *J* = 10.6, 7.0 Hz, 6H) ppm. **HPLC-MS** (m/z): $[M+H]^+$ calcd. for $C_{45}H_{49}I_2N_9O_{11}$,1146.7; found, 1146.9.

Ac-(bicyclo-*m,m*)-[Trp-Ala-Gly-Tyr(OAc)]-[Tyr(OAc)-Ala-Gly-Trp]-OH (12). The linear



peptide **11** (50 mg, 0.044 mmol), $AgBF_4$ (51 mg, 0.262 mmol, 6.0 eq.), pivalic acid (6.7 mg, 0.066 mmol, 1.5 eq.) and $Pd(OAc)_2$ (3.9 mg, 0.018 mmol, 0.4 eq.) were placed in a microwave reactor vessel in 500 µL of DMF. The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. Three more batches were carried out following the same procedure. All the crudes were filtered and combined (25% conversion, estimated by HPLC-MS). The crude was purified by semi-preparative RP-HPLC (XBRIDGE™ BEH 130, C18, 5µM OBD 19x50 mm column, [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)], in 10 min, flux: 20 mL·min-1, detection at λ=220 nm, gradient: 25-30% of B. **^{1}H NMR** (600 MHz, DMSO-d_6): δ 10.87 (d, *J* = 12.6 Hz, 2H), 8.71 (d, *J* = 8.0 Hz, 1H), 8.50 (s, 1H), 8.24 (s, 1H), 8.03 (m, 1H), 7.98 (d, *J* = 8.0 Hz, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 7.38 – 7.32 (m, 2H), 7.25 (d, *J* = 8.2 Hz, 1H), 7.22 (d, *J* = 7.0 Hz, 1H), 7.09 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 7.03 (dt, *J* = 15.7, 7.6 Hz, 2H), 6.96 (t, *J* = 7.8 Hz, 1H), 6.79 (2H), 5.04 (m, 1H), 4.94 (q, *J* = 7.4 Hz, 1H), 4.75 (m, 1H), 4.43 – 4.35 (m, 1H), 4.19 (p, *J* = 6.9 Hz, 1H), 3.90 (dd, *J* = 17.0, 7.1 Hz, 1H), 3.58 (dd, *J* = 16.7, 7.0 Hz, 1H), 3.51 – 3.44 (m, 1H), 3.38 (1H), 3.30 (1H), 3.24 (1H), 3.19 (m, 1H), 3.04 (s, 2H), 2.83 (t, *J* = 13.2 Hz, 1H), 1.94 (s, 3H), 1.49 (s, 3H), 1.21 (d, *J* = 7.2 Hz, 3H), 1.03 (d, *J* = 7.0 Hz, 3H) ppm. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{45}H_{47}N_9O_{11}$, 890.34678; found, 890.34796.

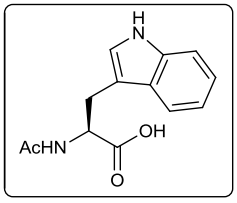
Ac-*m,m'*-I,I-Tyr(OAc)-OH (13). To a vigorously stirred suspension of commercially available H-



3,5-*I,I*-Tyr-OH·$2H_2O$ (2.17 g, 4.62 rnmol) in H_2O (14.2 rnL) at 0°C was added Et_3N (1.29 mL, 9.24 rnmol). Acetic anhydride (1.05 mL, 11.1 rnmol) was added dropwise at 0°C and Et_3N was added to maintain the pH between 6 and 8. After the addition was complete, the reaction was warmed to rt and stirred vigorously. After 5 min of reaction, a new portion of acetic anhydride and Et_3N was added. Once the reaction was completed (30 min), The solution was then carefully acidified to pH 2 with 1.2M $HCl_{(aq)}$. The white precipitate formed was dissolved in EtOAc and subsequent extractions with H_2O afforded the desired product as a white solid (2.41 g, 90%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 12.76 (s, 1H), 8.21 (d, *J* = 8.1 Hz, 1H),

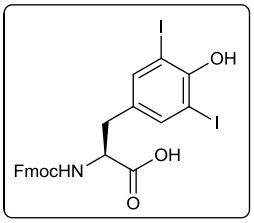
7.73 (s, 2H), 4.38 (ddd, *J* = 9.5, 8.0, 4.9 Hz, 1H), 3.00 (dd, *J* = 13.9, 4.9 Hz, 1H), 2.78 (dd, *J* = 13.9, 9.5 Hz, 1H), 2.35 (s, 3H), 1.80 (s, 3H) ppm.

Ac-Trp-OH (14).[4] To a solution of tryptophan (2.5 g, 12.2 mmol) and NaOH (0.588 g, 14.7 mmol)



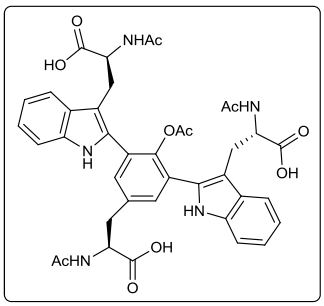
in 75 mL of H_2O was added acetic anhydride (15 mL, 159 mmol) and was stirred for 2 h, resulting in a white solid precipitate. The precipitate was filtered from the reaction mixture and rinsed with cold water. The residue was suspended in 50 mL of 0.2 M HCl, cooled, filtered and washed with cold water and dried to give a white solid (1.46 g, 48%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 12.58 (s, 1H), 10.91 – 10.74 (m, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.13 (d, *J* = 2.4 Hz, 1H), 7.06 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 6.98 (ddd, *J* = 7.9, 7.0, 1.1 Hz, 1H), 4.45 (ddd, *J* = 8.6, 7.8, 5.1 Hz, 1H), 3.15 (ddd, *J* = 14.6, 5.1, 0.9 Hz, 1H), 3.06 – 2.87 (m, 1H), 1.80 (s, 3H) ppm. **^{13}C NMR** (101 MHz): δ 173.54, 169.18, 136.07, 127.18, 123.50, 120.89, 118.33, 118.13, 111.36, 109.96, 52.96, 27.14, 22.41 ppm.

Fmoc-*3,5*-diiodo-Tyr-OH (15). To a suspension of 9.0 g (20.8 mmol) of H-*3,5*-I,I-Tyr-OH in 31.2



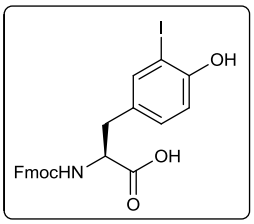
mL of 10% $NaHCO_3$ was added 7.7 g (22.9 mmol) of Fmoc-OSu in 63 mL of acetone. The resulting mixture was stirred at room temperature for 23 h and acetone was removed by rotary evaporation. Upon extraction with ether, the sodium salt of Fmoc-diiodotyrosine precipitated and was collected by filtration; the solid was washed thoroughly with H_2O then EtOAc and dried. This sodium salt was suspended in 60 mL of H_2O and acidified with 12M HC1. The free acid was extracted with EtOAc. The organic layers were combined, dried over sodium sulfate, filtered and concentrated under vacuum. White solid (11.4 g, 84%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 12.73 (s, 1H), 9.38 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 1H), 7.68 (s, 2H), 7.67 – 7.61 (m, 2H), 7.41 (tdd, *J* = 7.5, 2.6, 1.1 Hz, 2H), 7.31 (dtd, *J* = 9.7, 7.5, 1.2 Hz, 2H), 4.20 (m, 3H), 4.10 (ddd, *J* = 10.6, 8.4, 4.3 Hz, 1H), 2.96 (dd, *J* = 13.9, 4.4 Hz, 1H), 2.71 (dd, *J* = 13.8, 10.6 Hz, 1H) ppm.

Ac-*3,5*-di-(Ac-Trp-OH)-Tyr(OAc)-OH (16). Ac-3,5-diiodo-Tyr(OAc)-OH (100 mg, 0.087 mmol),



Ac-Trp-OH (286 mg, 1.16 mmol, 6.0 eq.), $AgBF_4$ (226 mg, 1.16 mmol, 6.0 eq.), pivalic acid (30 mg, 0.290 mmol, 1.5 eq.) and $Pd(OAc)_2$ (17 mg, 0.077 mmol, 0.4 eq.) were placed in a microwave reactor vessel in 1200 µL of DMF. The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. The residue was filtered and partially purified in a PoraPak Rxn RP 60 cc reverse phase column (5 g) [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)]. Pale solid (28.5 mg, 20% yield estimated by HPLC-MS conversion). A pure fraction was obtained by semi-preparative RP-HPLC (XBRIDGE^{TM}, C18, 5µM OBD 19x100 mm column, [solvent A (0.1% FA in H_2O) and solvent B (0.05% FA in ACN)], in 20 min, flux: 16 mL·min-1, detection at λ=220 nm (gradient: 25-30% of B). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 12.62 (s, 3H), 11.08 – 10.93 (m, 2H), 8.26 (dd, *J* = 15.5, 7.9 Hz, 1H), 7.91 (dd, *J* = 10.8, 8.0 Hz, 1H), 7.82 (t, *J* = 6.9 Hz, 1H), 7.63 (dt, *J* = 7.8, 3.8 Hz, 2H), 7.53 – 7.44 (m, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.10 (t, *J* = 7.5 Hz, 2H), 7.01 (t, *J* = 7.5 Hz, 2H), 4.54 (m, 3H), 3.26 (dd, *J* = 9.2, 6.2 Hz, 2H), 3.22 – 3.17 (m, 1H), 3.16 – 3.05 (m, 2H), 2.96 (m, 1H), 1.92 – 1.76 (m, 3H), 1.73 – 1.56 (m, 9H) ppm. **IR** (Film, cm^{-1}) ν = 3353.02, 3051.96, 2923.84, 1732.38, 1649.11, 1527.40 cm^{-1}. **HRMS** (ESI) (m/z): $[M+H]^+$ calcd. for $C_{39}H_{39}N_5O_{11}$, 754.27188; found, 754.27292.

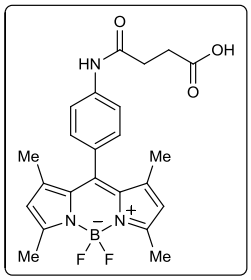
Fmoc-*3*-iodo-Tyr-OH (**17**)**.** To a suspension of 2.0 g (6.5 mmol) of H-*3*-I-Tyr-OH in 10.0 mL of



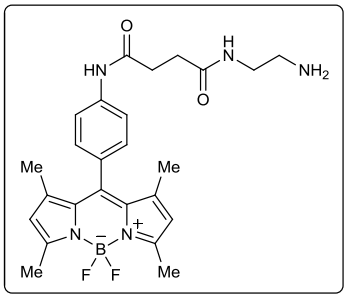
10% $NaHCO_3$ was added 2.4 g (7.2 mmol) of Fmoc-OSu in 20 mL of acetone. The resulting mixture was stirred at room temperature for 20 h and acetone was removed by rotary evaporation. Upon extraction with ether, the sodium salt of Fmoc-diiodotyrosine precipitated and was collected by filtration; the solid was washed thoroughly with H_2O and dried. This sodium salt was suspended in 20 mL of H_2O and acidified with 12M HC1. The free acid was extracted with EtOAc. The organic layers were combined, dried over sodium sulfate, filtered and concentrated under vacuum. The residue was recrystallized from EtOAc/hexane to give a white solid (2.8 g, 81%). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 10.12 (s, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.74 – 7.58 (m, 4H), 7.41 (dddd, *J* = 7.7, 5.0, 3.8, 2.0 Hz, 2H), 7.31 (dtd, *J* = 10.6, 7.4, 1.2

Hz, 2H), 7.09 (dd, *J* = 8.3, 2.1 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 4.19 (d, *J* = 4.3 Hz, 3H), 4.14 – 4.05 (m, 1H), 3.33 (s, 1H), 2.95 (dd, *J* = 13.8, 4.4 Hz, 1H), 2.73 (dd, *J* = 13.9, 10.5 Hz, 1H) ppm.

10-(4-(3-carboxypropanamido)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:1',2'-*f*][1,3,2]diazaborinin-4-ium-5-uide (18). Compound **18** was prepared as described in the literature.[5]



10-(4-(4-(2-aminoethylamino)-4-oxobutanamido)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:1',2'-*f*][1,3,2]diazaborinin-4-ium-5-uide (19). Bodipy derivative **18** (100 mg, 0.228 mmol) and ethylenediamine (76 μL, 1.14 mmol, 5.0 eq.) were dissolved in DCM (5 mL), EDC-HCl (52 mg, 0.271 mmol, 1.2 eq.) and HOBt (37 mg, 0.274 mmol, 1.2 eq.) were added, and the mixture was stirred for 16 h. Another portion of EDC-HCl (52 mg, 0.271 mmol, 1.2 eq.) and HOBt (37 mg, 0.274 mmol, 1.2 eq.) were added, and the reaction further stirred for 24 h. 10 ml of DCM were added, and the mixture was extracted $Na_2CO_{3aq.}$ (3 x 15 mL). The organic layers were combined, dried over Na_2SO_4, filtered and evaporated under vacuum. The residue was dissolved in MeOH and loaded into a SCX-2 isolute (500 mg) column, washed with MeOH (30 mL) and released with NH_3 solution (4M) in MeOH (10 mL). After removal of the solvents, amino bodipy derivative **19** was recovered as an orange solid (67 mg, 62%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.02 (s, 1H), 7.63 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 6.48 (s, 1H), 5.88 (s, 2H), 3.42 (s, 2H), 3.28 (s, 2H), 2.85 – 2.53 (m, 6H), 2.46 (s, 6H), 1.33 (s, 6H) ppm. **HPLC-MS** (m/z): [M] calcd. for $C_{25}H_{30}BF_2N_5O_2$, 481.25; [M] found, 481.78.



4. Biochemical and cellular studies

Proteolytic degradation assay

Stock solutions of α-chymotrypsin (from bovine pancreas type II, ≥ 40 U/mg) were freshly prepared in 1 mM HCl (placed on ice), and stock solutions of peptides (500 μM) were dissolved in DMSO. Reactions were conducted in glass vials by dilution of α-chymotrypsin solution into assay buffer (56 mMTris pH 7.8, 560 μM $CaCl_2$, 0.1 %*v* Tween-80) and subsequent addition of the peptide stock solution to give a final substrate concentration of 50 μM, 50 μg/mL α-chymotrypsin, and 10 %*v* DMSO. Aliquots were removed at different times and diluted 1:1 with H_2O containing 1 %*v* TFA, which resulted in a pH < 2. The rate of peptide degradation was monitorized by HPLC-MS analysis at the corresponding wavelength were the UV absorption was maximum. No loss was observed in control reactions which contained BSA instead of α-chymotrypsin.

Cell adhesion assays of RGD-containing compound 2h

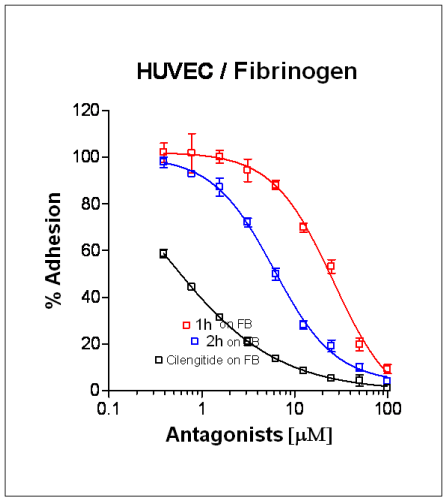
As the signaling peptides specifically bind to integrins (transmembrane receptors, directly involved in tumor metastasis and tumor-induced angiogenesis) acting as mild antagonists,[9] we tested wether the rigidified analog conserved this property. The inhibition of the cellular adhesion of the RGD-containing compound **2h** and its linear precursor **1h** were evaluated against Human Umbilical Vein Endothelial Cell cancer cell line, with coating of fibrinogen (Fb) and vitronectin (Vn) as integrin ligands and using cilengitide® as a positive control. The tested compounds selectively block αvβ3-mediated cell adhesion with Fb but do not have blocking activity of Vn against integrin αvβ5.

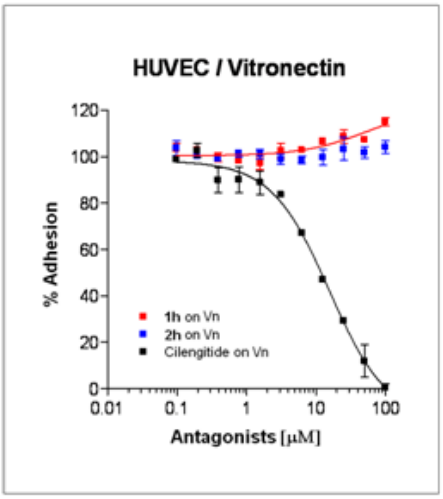
Non-tissue culture treated ELISA plates [NUNC, Maxisorp 442404] were coated ON at 4 ºC with the specific concentration of the ligand. Coating solution was discarded and wells were blocked with blocking solution (PBS + BSA 1.5%; 60 minutes at 37 ºC). Blocking solution was discarded by flicking and serial dilutions of the compounds were plated in quadruplicates. Immediately, harvested cells are plated at a given concentration (20000-25000 / well for HUVEC and DAOY and 50000 for HT-29) to the same plate. Plates were incubated for 90 minutes at 37 ºC/5%CO_2 to allow cell adhesion on the ligand. After then, non-adhered cells were removed and hexosaminidase substrate (N-acetyl-β-

D-glucosaminide) was added to each well and incubated for 3 hours (37ºC/5%CO_2 for VN and O/N for de FB). Optical density was read at 405 nm. The proliferation inhibition EC50 was calculated using the Prism-4 software based on the sigmoidal dose-response (variable slope) equation.

Each plate contains positive and negative controls and peptides are tested as duplicates. Each assay has been repeated at least twice and the adhesion inhibition EC_{50} is calculated, when possible, using the Prism-4 software based on the sigmoidal dose-response (variable slope) equation.

Cell adhesion inhibition curves for compounds **1h** and **2h** using Vn and Fb as ligands in HUVEC cell line.





HUVEC / Fb	EC_{50} (μM)
1h	**26**
2h	**6**
Cilengitide	**0.08**

HUVEC / Vn	EC_{50} (μM)
1h	**_**
2h	**_**
Cilengitide	**16**

Cytotoxicity determination of RGD-containing compound 2h

The citotoxicity of the conjugated peptide **5** and its macrocyclic precursors **3** and **4** were evaluated against three human cancer cell lines: lung carcinoma A549, breast cancer MCF-7 and MCF-10A cell lines. Citotoxicity experiments were performed following the methods indicated elsewhere.[10] Only the macrocycle **4** showed activity (IC_{50} <10 μM) in line with the expected activity for the parent compound (sansalvamide).[11]

Live imaging of SH-SY5Y cells upon incubation with 1j-Bodipy and 2j-Bodipy

For confocal microscopy analysis, cells were seeded ($4\div12\times10^3$ per cm^2) on glass chamber coverslips and cultured for 24 h before being incubated for 30 min in fresh medium containing **1j-Bodipy** (750 nM) or **2j-Bodipy** (750 nM). Cells were washed, and fresh medium was introduced to perform experiments with living cells in CO_2 and temperature-controlled conditions. Images were collected with a Leica SP5 Spectral confocal microscope attached to an inverted DMI 6000, using a 63×/1.3 Glyc HCX PL APO objective.

Supplementary References

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CHEMISTRY
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Supporting Information

Constrained Cyclopeptides: Biaryl Formation through Pd-Catalyzed C–H Activation in Peptides—Structural Control of the Cyclization vs. Cyclodimerization Outcome

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Table of contents

Abbreviations

Abbreviation used for amino acids and designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* 247, 977-983 (1982). The following additional abbreviations are used: FA: formic acid, ACN: acetonitrile, DMF: *N,N*-dimethylformamide, DCM: dichloromethane, Fmoc: 9*H*-fluorenylmethyloxycarbonyl, SPPS: solid phase peptide synthesis, DIEA: *N,N*-diisopropylethylamine, DIPCDI: *N,N*-diisopropylcarbodiimide, HOBt: hydroxybenzotriazole, HBTU: *o*-benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate, TIS: triisopropylsilane, IR: infrared spectroscopy, NMR: nuclear magnetic resonance, RP-HPLC: reverse-phase high performance liquid chromatography, HPLC-MS high performance liquid chromatography mass spectrometry, HRMS(ESI): high-resolution mass spectrometry (electrospray ionization).

General experimental information

Reactions were monitored by HPLC-MS at 220 nm using a HPLC Waters Alliance HT comprising a pump (Edwards RV12) with degasser, an autosampler and a diode array detector. Flow from the column was split to a MS spectrometer. The MS detector was configured with an eletrospray ionization source (micromass ZQ4000) and nitrogen was used as the nebulizer gas. Mass scans were acquired in positive ion mode, and linear gradients of ACN (+0.05% formic acid) into H_2O (+0.1% formic acid) were run at a flow rate of 1.6 mL·min^{-1} over 3.5 min. Data acquisition was performed with MassLynx software. High resolution mass spectrometry analyses were conducted on an LTQ-FT Ultra (Thermo Scientific) spectrometer with a NanoESI positive ionization. Data was acquired with Xcalibur software, vs.2.0SR2 (ThermoScientific) and elemental compositions from experimental exact mass monoisotopic values were obtained with a dedicated algorithm integrated in Xcalibur. All microwave reactions were carried out in 10 mL sealed glass tubes in a focused mono-mode microwave oven ("Discover" by CEM Corporation) featured with a surface sensor for internal temperature determination. Cooling was provided by compressed air ventilating the microwave chamber during the reaction.

When stated, final crudes were purified on a semi-preparative RP-HPLC provided of a RP-HPLC XBRIDGE^{TM} Prep C18, 5µM OBD 19 x 50 mm column, a Waters Delta 600 system comprising a sample manager (Waters 2700), a controller (Waters 600), a dual λ absorbance detector (Waters 2487), a fraction collector II, and a software system controller (MassLynx). Linear gradients of ACN (+0.05% FA) into H_2O (+0.1% FA) were run at a flow rate of 16 mL·min^{-1} over 20 min. Otherwise, the final crude was purified via flash column chromatography Combi Flash ISCO RF provided with dual UV detection. Normal mode: crude residue and silica media were dissolved in CH_2Cl_2, concentrated, and the resultant solid samples were eluded on a RediSep Rf silica column. Reverse mode: crude residues and Celite were dissolved in CH_2Cl_2, concentrated, and the resultant solid samples were eluded on a RediSep Rf GOLD C18 column.

NMR spectra of peptides in [D_6]DMSO were acquired with either a Varian Mercury 400 MHz, Bruker DMX-500 MHz or Bruker Avance III 600 MHz spectrometers equipped with TCI cryoprobes. The spectra were referenced relative to the residual DMSO signal (^{1}H, 2.49 ppm; ^{13}C, 39.5 ppm). For peptides **5a-c** and **7b-c**, ^{1}H resonances were unequivocally assigned by two-dimensional NMR experiments (COSY, TOCSY and NOESY and/or ROESY). Then, the ^{13}C resonances were straightforwardly assigned on the basis of the cross-correlations observed in the ^{1}H-^{13}C HSQC spectra. Mixing times for TOCSY spectra were 70 ms, for NOESY spectra 300-450 ms and for ROESY experiments were 200 ms. Chemical shifts (δ) are reported in ppm. Multiplicities are referred by the

following abbreviations: s = singlet, d = doublet, t = triplet, dd = double doublet, dt = double triplet, q = quartet and m = multiplet. HRMS (ESI positive) were obtained with a LTQ-FT Ultra (Thermo Scientific) mass Spectrometer. IR spectra were obtained on a Thermo Nicolet NEXUS.

General procedure for SPPS.[1] All peptides were manually synthesized in polystyrene syringes fitted with a polyethylene porous disc using Fmoc-based SPPS. Solvents and soluble reagents were removed by suction. The Fmoc group was removed with piperidine-DMF (1:4) (1×1 min, 2×5 min). Peptide synthesis transformations and washes were performed at r.t.

Resin loading (2-Chlorotrityl resin 1 mmol/g). Fmoc-XX-OH (1.0 eq.) was attached to the resin (1.0 eq.) with DIEA (3.0 eq.) in DCM at r.t for 10 min and then DIEA (7.0 eq.) for 40 min. The remaining trityl groups were capped adding 0.8 µL MeOH/mg resin for 10 min. After that, the resin was filtered and washed with DCM (4 x 1 min), DMF (4 x 1 min). The loading of the resin was determined by titration of the Fmoc group.[1]

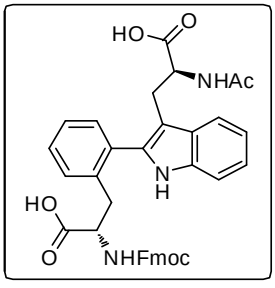
Peptide elongation. After the Fmoc group was eliminated, the resin was washed with DMF (4 x 1 min), DCM (3 x 1 min), DMF (4 x 1 min). Amino acid coupling. Fmoc-XX-OH (3.0 eq.) were incorporated with a 5-min pre-activation with DIPCDI (3.0 eq.) and OxymaPure (3.0 eq.) in DMF for 1h. To ensure the coupling of Fmoc-*x*-I-Phe (*x*: 2, 3 or 4), it was incorporated with HBTU (1.5 eq.), HOBt (1.5 eq.) and DIEA (3.0 eq.) in DMF for 1h. The completion of the coupling was monitored with the ninhydrin (free primary amine) test.[2] Then, the resin was filtered and washed with DCM (4 x 1 min) and DMF (4 x 1 min) and the Fmoc group was eliminated.

Acetylation. Once the peptide sequence was fully elongated, N-terminal acetylation was performed with acetic anhydride (10 eq.), DIEA (10 eq.) in DMF (30 min).

Final cleavage. The resin bound peptide was treated with a 95% TFA, 2.5% TIS, 2.5% H_2O cleavage cocktail (1h) and the eluates were evaporated under vacuum. Then, the residue was washed with Et_2O, dissolved in ACN:H_2O and lyophilized furnishing the corresponding deprotected peptide.

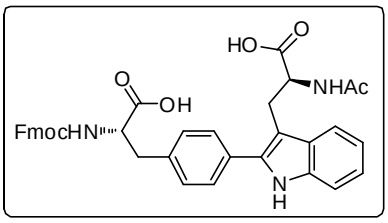
Experimental procedures and peptide characterization

[Ac-*C2*-Trp-OH)—(Fmoc-*o*-Phe-OH)] adduct (1a). Fmoc-Phe(2-I)-OH (50 mg, 0.097 mmol, 1.0 eq.), Ac-Trp-OH[3] (24 mg, 0.097 mmol, 1.0 eq.), $AgBF_4$ (38 mg, 0.195 mmol, 2.0 eq.), trifluoroacetic acid (7.5 µL, 0.195 mmol, 1.0 eq.) and $Pd(OAc)_2$ (4.4 mg, 0.012 mmol, 0.20 eq.) were placed in a microwave reactor vessel in DMF (0.60 mL). The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. Then, $Pd(OAc)_2$ (4.4 mg, 0.012 mmol, 0.20 eq.) and $AgBF_4$ (38 mg, 0.195 mmol, 2.0 eq.) were added again, and a second irradiation cycle was performed. The resulting suspension was filtered through Celite and the filtrate was evaporated under vacuum. The crude residue was purified by flash chromatography on SIlica using DCM/EtOH to obtain the final adduct **1b** as a pale solid (23 mg, 37%). A highly pure fraction was obtained by PoraPak Rxn RP 20 cc reverse phase column purification. Mobile phase: ACN (0.1% HCOOH)/H_2O (0.1% HCOOH). Pure fractions were lyophilised furnishing the final adduct **1a** as a pale solid (10 mg, 15%). **^{1}H NMR** (400 MHz, [D_6]DMSO): *δ* 12.50 (s, 2H), 11.01 (d, *J* = 9.3 Hz, 1H), 8.01 (t, *J* = 7.5 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.65 (t, *J* = 6.6 Hz, 4H), 7.40 (m, 4H), 7.31 (m, 5H), 7.07 (t, *J* = 7.5 Hz, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 4.58 – 4.43 (m, 1H), 4.19 (m, 5H), 3.15 – 3.06 (m, 1H), 2.89 (m, 2H), 2.85 – 2.73 (m, 1H), 1.68 (d, *J* = 12.5 Hz, 3H) ppm. **IR** (Film, cm^{-1}): v = 3406.75, 3322.83, 3062.38, 2955.31, 2920.58, 2848.23, 1719.61, 1708.04, 1661.74,



1528.62, 1450.48 cm^{-1}. **HPLC-MS**: t_R 2.83 min (gradient 5-100% ACN). **HRMS (ESI)**: (M: $C_{37}H_{33}N_3O_7$) m/z calcd. 632.23913, found 632.23916 $(M+H)^+$.

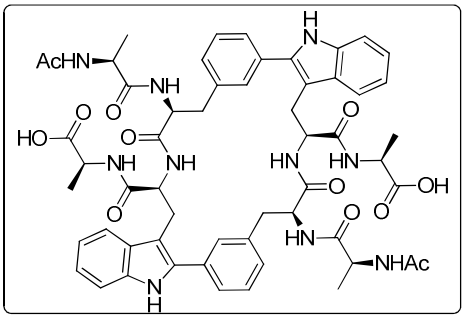
[Ac-*C2*-Trp-OH)—(Fmoc-*p*-Phe-OH)] adduct (1b). Fmoc-Phe(4-I)-OH (50 mg, 0.097 mmol, 1.0 eq.), Ac-Trp-OH (24 mg, 0.097 mmol, 1.0 eq.), $AgBF_4$ (38 mg, 0.195 mmol, 2.0 eq.), trifluoroacetic acid (7.5 µL, 0.195 mmol, 1.0 eq.) and $Pd(OAc)_2$ (4.4 mg, 0.012 mmol, 0.20 eq.) were placed in a microwave reactor vessel in DMF (0.60 mL). The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. The resulting suspension was filtered through Celite and the filtrate was evaporated under vacuum. The crude residue was purified by flash chromatography on SIlica using DCM/EtOH to obtain the final adduct **1b** as a pale solid (39 mg, 63%). **^{1}H NMR** (400 MHz, $[D_6]$DMSO): δ 11.17 (s, 1H), 8.21 (dd, *J* = 8.2, 3.6 Hz, 1H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.80 (d, *J* = 8.3 Hz, 1H), 7.67 (dd, *J* = 14.0, 7.1 Hz, 3H), 7.60 (d, *J* = 6.8 Hz, 2H), 7.39 (dd, *J* = 10.2, 6.0 Hz, 4H), 7.30 (m, 3H), 7.08 (dd, *J* = 11.1, 4.0 Hz, 1H), 6.99 (dd, *J* = 11.4, 4.4 Hz, 1H), 4.57 (dd, *J* = 15.0, 7.5 Hz, 1H), 4.25 (m, 4H), 3.35 – 3.26 (m, 1H), 3.19 – 3.08 (m, 2H), 3.01 – 2.91 (m, 1H), 1.75 (s, 3H) ppm. **^{13}C NMR** (101 MHz, $[D_6]$DMSO): δ 173.5, 173.3, 169.0, 162.3, 156.0, 143.7, 140.7, 137.2, 135.8, 135.0, 130.9, 129.4, 128.9, 127.7, 127.6, 127.1, 125.2, 121.4, 120.1, 118.9, 118.7, 111.0, 107.5, 107.4, 65.6, 55.5, 53.1, 46.6, 36.1, 27.4, 22.4 ppm. **IR** (Film, cm^{-1}): ν = 3406.75, 3325.72, 3056.59, 2952.41, 2917.68, 2848.23, 1728.30, 1664.63, 1525.72, 1450.48 cm^{-1}. **HPLC-MS**: t_R 2.82 min (gradient 5-100% ACN). **HRMS (ESI):** (M: $C_{37}H_{33}N_3O_7$) m/z calcd. 632.23913, found 632.23820 $(M+H)^+$.



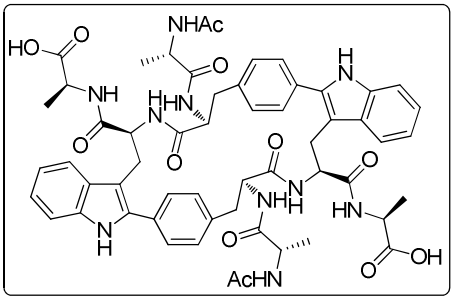
General procedure for the C-H activation process of peptides 3 and 5

Unless stated otherwise, the linear peptide, $AgBF_4$ (2.0 eq.), trifluoroacetic acid (1.0 eq.) and $Pd(OAc)_2$ (0.20 eq.) were placed in a microwave reactor vessel in DMF. The mixture was heated under microwave irradiation (250 W) at 90 °C for 20 min. The resulting suspension was filtered through Celite and the filtrate was evaporated under vacuum. The crude residue was purified by semi-preparative RP-HPLC (XBRIDGE^{TM} Prep C18, 5µM OBD 19 x 50 mm column) [solvent A (0.1% FA in H_2O) and solvent B (0.1% FA in ACN)], in 10 min, flux: 20 mL·min^{-1}, detection at λ=220 nm.

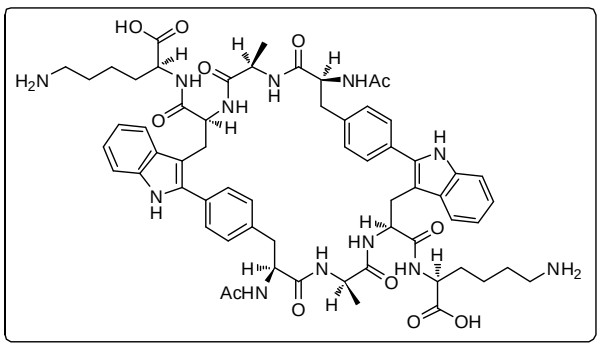
(Cyclo-*m,m)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (3). Starting from peptide **2** (200 mg, 0.302 mmol, 1.0 eq.), $AgBF_4$ (118 mg, 0.605 mmol, 2.0 eq.), trifluoroacetic acid (23 µL, 0.302 mmol, 1.0 eq.) and $Pd(OAc)_2$ (3.4 mg, 0.015 mmol, 0.20 eq.) were placed in a microwave reactor vessel in DMF (2.0 mL). Then, $Pd(OAc)_2$ (3.4 mg, 0.015 mmol, 0.20 eq.) and $AgBF_4$ (3.4 mg, 0.015 mmol, 0.20 eq.) were added again, and a second irradiation cycle was performed. The resulting suspension was filtered through Celite and the filtrate was precipitated in H_2O (40% conversion estimated by HPLC-MS, 28% yield estimated by HPLC-MS conversion). A crude fraction was purified by flash chromatography on Celite using H_2O (0.1% FA)/ACN (0.1% FA) to obtain the final dimeric compound **3**. Pale solid (10 mg, 13% isolated yield). **HPLC-MS**: t_R 2.28 min (gradient 5-100% ACN). **HRMS (ESI)** (m/z): [M] calcd. for $C_{56}H_{62}N_{10}O_{12}$, 1066.45487; found, 1067.45620.



(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (5a). Starting from peptide **4a** (600 mg, 0.907 mmol) (60% conversion estimated by HPLC-MS). An analytically pure sample was obtained by semi-preparative RP-HPLC purification. Gradient: 25-30% of B (pale solid). **^{1}H NMR** (800 MHz, [D_6]DMSO): δ 11.20 (s, 1H), 8.13 (d, *J* = 7.7 Hz, 1H), 7.93 (d, *J* = 7.3 Hz, 1H), 7.90 (d, *J* = 7.0 Hz, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.61 (d, *J* = 7.6 Hz, 2H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.25 (d, *J* = 7.7 Hz, 2H), 7.11 (t, *J* = 7.5 Hz, 1H), 7.03 (t, *J* = 7.5 Hz, 1H), 6.82 (d, *J* = 6.9 Hz, 1H), 4.24 – 4.15 (dp, *J* = 28.2, 7.1 Hz, 2H), 4.07 (ddd, *J* = 10.4, 6.9, 3.8 Hz, 1H), 3.96 (m, 1H), 3.43 (dd, *J* = 15.1, 3.7 Hz, 1H), 3.23 (dd, *J* = 15.2, 9.4 Hz, 1H), 2.64 (dd, *J* = 15.1, 10.6 Hz, 1H), 2.48 (m, 1H), 1.78 (s, 3H), 1.28 (d, *J* = 7.3 Hz, 3H), 1.08 (d, *J* = 7.0 Hz, 3H) ppm. **IR** (Film, cm^{-1}): ν = 3404.27, 2911.03, 1655.52 cm^{-1}. **HPLC-MS**: t_R 2.32 min (gradient 5-100% ACN). **HRMS (ESI)** (m/z): $[M+H]^+$ calcd. for $C_{56}H_{62}N_{10}O_{12}$, 1067.4621; found, 1067.4624.



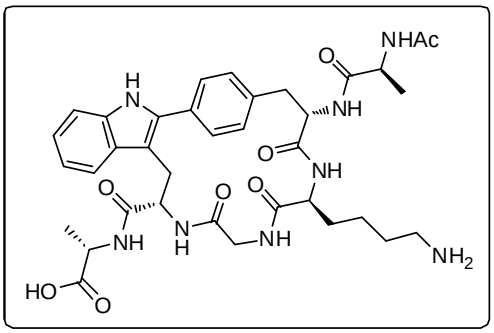
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Phe-Ala-Trp-Lys-OH) (5b). Starting from peptide **4b** (0.15 g, 0.209 mmol, 1.0 eq.), $AgBF_4$ (81 mg, 0.417 mmol, 2.0 eq.), trifluoroacetic acid (16 µL, 0.209 mmol, 1.0 eq.) and $Pd(OAc)_2$ (9.4 mg, 0.042 mmol, 0.20 eq.) were placed in a microwave reactor vessel in DMF (0.87 mL) (54% conversion estimated by HPLC-MS). A highly pure fraction was obtained by semi-preparative RP-HPLC purification. Gradient: 19-22% of B (pale solid). **^{1}H NMR** (500 MHz, [D_6]DMSO): δ 10.92 (s, 1H), 8.14 (s, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.49 (s, 1H), 7.43 (m, 2H), 7.34 – 7.26 (m, 4H), 7.08 (t, *J* = 7.6 Hz, 1H), 7.01 (m, 1H), 4.59 (m, 2H), 4.14 (m, 1H), 3.86 (m, 1H), 3.32 (1H), 3.13 (1H), 3.01 (1H), 2.81 (m, 1H), 2.66 (m, 2H), 1.81 (s, 3H), 1.63 (m, 1H), 1.53 (m, 1H), 1.46 (m, 2H), 1.26 (m, 2H), 1.08 (d, *J* = 6.9 Hz, 3H) ppm. **HPLC-MS**: t_R 1.42 min (gradient 20-60% ACN). **HRMS (ESI)**: (M: $C_{62}H_{76}O_{12}N_{12}$) m/z calcd 591.29390, found 591.29388 $[(M+2H)/2]^{2+}$.



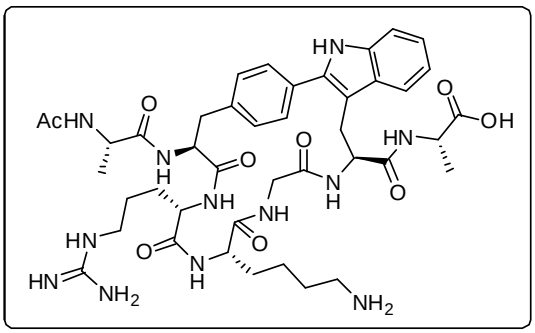
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Lys-Gly-Trp-Ala-OH) (5c). Starting from peptide **4c** (0.20 g, 0.236 mmol, 1.0 eq.), $AgBF_4$ (92 mg, 0.472 mmol, 2.0 eq.), trifluoroacetic acid (18 µL, 0.236 mmol, 1.0 eq.) and $Pd(OAc)_2$ (10 mg, 0.047 mmol, 0.20 eq.) were placed in a microwave reactor vessel in DMF (1.0 mL) (23% conversion estimated by HPLC-MS). A highly pure fraction was obtained by semi-preparative RP-HPLC purification. Gradient: 15-20% of B (pale solid). **^{1}H NMR** (500 MHz, [D_6]DMSO): δ 11.10 (s, 1H), 8.35 – 7.86 (m, 6H), 7.70 (m, 1H), 7.51 (d, *J* = 6.7 Hz, 2H), 7.37 – 7.24 (m, *J* = 8.4 Hz, 3H), 7.06 (t, *J* = 7.4 Hz, 1H), 6.99 (t, *J* = 7.2 Hz, 1H), 4.50 (m, 2H), 4.29 (m, 1H), 4.20 (m, 1H), 3.83 (m, 1H), 3.77 – 3.44 (m, *J* = 57.6 Hz, 2H), 3.43 (1H), 3.05 (m, 2H), 2.88 (m, 1H), 2.76 (m, 2H), 1.81 (s, *J* = 6.8 Hz, 3H), 1.68 (m, 1H), 1.58 – 1.45 (m, 3H), 1.37 – 1.27 (m, 2H), 1.19 (d, *J* = 6.6 Hz,

3H), 1.12 (d, *J* = 6.6 Hz, 3H) ppm. **HPLC-MS**: t_R 1.07 min (gradient20-60% ACN). **HRMS (ESI):** (M: $C_{72}H_{92}N_{16}O_{16}$) m/z calcd. 1436.68772, found 1436.68351 (M).

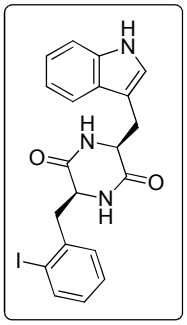
Ac-Ala-(Cyclo-*p*)-[Phe-Lys-Gly-Trp]-Ala-OH (5c'). Starting from peptide **4c** (0.20 g, 0.236 mmol, 1.0 eq.), $AgBF_4$ (92 mg, 0.472 mmol, 2.0 eq.), trifluoroacetic acid (18 µL, 0.236 mmol, 1.0 eq.) and $Pd(OAc)_2$ (10 mg, 0.047 mmol, 0.20 eq.) were placed in a microwave reactor vessel in DMF (1.0 mL) (51% conversion estimated by HPLC-MS). A highly pure fraction was obtained by semi-preparative RP-HPLC purification. Gradient: 15-20% of B (pale solid). **^{1}H NMR** (500 MHz, [D_6]DMSO): δ 11.05 (s, 1H), 8.35 (d, *J* = 8.5 Hz, 1H), 8.18 (d, *J* = 6. 2 Hz, 1H), 8.11 (m, 1H), 8.08 (d, *J* = 7.3 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.51 (d, *J* = 7.9 Hz, 2H), 7.36 – 7.26 (m, *J* = 7.3, 4.6 Hz, 3H), 7.07 (t, *J* = 8.1 Hz, 1H), 7.00 (m, 1H), 6.96 (t, *J* = 7.2 Hz, 1H), 6.40 (m, 1H), 4.68 (m, 1H), 4.48 (m, 1H), 4.34 (m, 1H), 4.19 (m, 1H), 4.14 (m, 1H), 3.56 (dd, *J* = 17.7, 6.8 Hz, 1H), 3.45 (m, 1H), 3.27 (1H), 3.26 (1H), 2.96 (m, 2H), 2.72 (m, 2H), 1.86 (s, 3H), 1.68 (m, 1H), 1.49-1.39 (m, 3H), 1.36 (m, 1H), 1.18 (m, 7H) ppm. **HPLC-MS**: t_R 1.00 min (gradient 20-60% ACN). **HRMS (ESI):** (M: $C_{36}H_{46}N_8O_8$) m/z calcd. 719.35114, found 719.35134 $(M+H)^+$.



Ac-Ala-(Cyclo-*p*)-[Phe-Arg-Lys-Gly-Trp]-Ala-OH (5d'). Starting from peptide **4d** (200 mg, 0.199 mmol, 1.0 eq.), $AgBF_4$ (79 mg, 0.399 mmol, 2.0 eq.), trifluoroacetic acid (16 µL, 0.199 mmol, 1.0 eq.) and $Pd(OAc)_2$ (9.1 mg, 0.040 mmol, 0.20 eq.) were placed in a microwave reactor vessel in DMF (2.0 mL). Then, $Pd(OAc)_2$ (49.1 mg, 0.040 mmol, 0.20 eq.) and $AgBF_4$ (79 mg, 0.399 mmol, 2.0 eq.) were added again, and a second irradiation cycle was performed (81% conversion estimated by HPLC-MS). An analytically pure sample was obtained by semi-preparative RP-HPLC purification. Gradient: 10-20% of B (pale solid)). **^{1}H NMR** (500 MHz, [D_6]DMSO): δ 10.89 (s, 1H), 8.36 (m, 1H), 8.02 (m, 3H), 7.64 (m, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.23 (d, *J* = 7.8 Hz, 2H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.99 (t, *J* = 7.9 Hz, 1H), 6.95(m, 1H), 4.56 (m, 1H), 4.30 (m, 2H), 4.22 (m, 1H), 4.04 – 3.89 (m, 2H), 3.79 (m, 1H), 3.56 (m, 1H), 3.24 (1H), 3.13 (2H), 3.12 (1H), 3.05 (1H), 2.94 (m, 2H), 2.78 (m, 2H), 1.84 (s, 3H), 1.77 (m, 1H), 1.71 – 1.47 (m, 7H), 1.40 (m, 2H), 1.23 (d, *J* = 7.2 Hz, 3H), 1.13 (d, *J* = 6.9 Hz, 3H) ppm. **HPLC-MS**: t_R 2.50 min (gradient 5-30% ACN). **HRMS (ESI):** (M: $C_{42}H_{58}N_{12}O_9$) m/z calcd. 875.44822, found 875.45014 $(M+H)^+$.

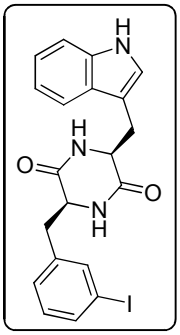


Cyclo(2-I-Phe-Trp) (6a). Compound **6a** was prepared using Fmoc-Phe(2-I)-OH (1.5 g, 2.93 mmol, 1.0 eq.), H-Trp-OMe.HCl (0.73 g, 2.93 mmol, 98%, 1.0 eq.), HBTU (1.1 g, 2.93 mmol, 1.0 eq.), DIEA (1.0 mL, 5.86 mmol, 2.0 eq.) which were dissolved in DMF (3.3 mL). The pale yellow solution was stirred at r.t. for 24 h. The resulting solution was precipitated over 10 mL of cold water and centrifugated (RPM: 2000, t: 5 min, T= 4 °C). Precipitation cycles in cold water and centrifugation were repeated 8 times obtaining the desired adduct (2.6 g, 89%). The white solid was suspended in 20% piperidine/ACN (15 mL) and stirred for 22 h and the resulting solution was

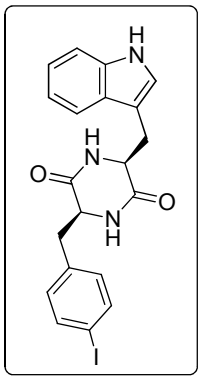


precipitated over 10 mL of cold water and centrifugated (RPM: 2000, t: 5 min, T= 4 °C). Precipitation cycles in cold water and centrifugation were repeated 2 times. The crude was purified by flash chromatography on silica using DCM/MeOH:DCM (1:5) to yield the pure product **6a** (1.0 g, 85% isolated yield). **^{1}H NMR** (400 MHz, [D_6]DMSO: δ 10.97 (d, *J* = 2.4 Hz, 1H), 8.21 (d, *J* = 2.7 Hz, 1H), 7.66 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.62 (dd, *J* = 7.5, 1.3 Hz, 1H), 7.44 (d, *J* = 3.2 Hz, 1H), 7.29 (m, 1H), 7.11 (d, *J* = 2.3 Hz, 1H), 7.07 (ddd, *J* = 8.1, 7.0, 1.4 Hz, 1H), 7.03 (ddd, *J* = 8.1, 7.0, 1.3 Hz, 1H), 6.94 (td, *J* = 7.5, 1.3 Hz, 1H), 6.82 (td, *J* = 7.6, 1.7 Hz, 1H), 5.56 (dd, *J* = 7.6, 1.7 Hz, 1H), 4.11 (m, 1H), 3.69 (dt, *J* = 9.3, 3.9 Hz, 1H), 3.27 (m, 1H), 2.97 (dd, *J* = 14.3, 4.6 Hz, 1H), 2.44 (dd, *J* = 13.3, 4.4 Hz, 1H), 1.12 (dd, *J* = 13.4, 9.9 Hz, 1H) ppm. **^{13}C NMR** (100 MHz, [D_6]DMSO): δ 166.9, 166.1, 138.9, 138.6, 136.0, 131.8, 128.5, 127.8, 124.9, 121.1, 119.3, 118.6, 111.5, 108.6, 100.6, 55.7, 53.4, 45.2, 29.2 ppm. **IR** (Film, cm^{-1}): ν = 3389.39, 3175.24, 3047.91, 2952.41, 2914.79, 2848.23, 1681.99, 1453.38 cm^{-1}. **HPLC-MS**: t_R 2.40 min (gradient 5-100% ACN). **HRMS (ESI):** (M: $C_{20}H_{18}O_2N_3I$) m/z calcd. 460.0516, found 460.0519 $(M+H)^+$.

Cyclo(3-I-Phe-Trp) (6b). Compound **6b** was prepared using Fmoc-Phe(3-I)-OH *(*0.22 g, 0.435 mmol, 1.0 eq.), H-Trp-OMe.HCl (0.11 g*,* 0.435 mmol, 98%, 1.0 eq.), HBTU (0.17 g, 0.435 mmol, 1.0 eq.), DIEA (0.15 mL, 0.871 mmol, 2.0 eq.) which were dissolved in 0.94 mL of DMF). The pale yellow solution was stirred at r.t. for 22 h followed by the evaporation under vacuum of the obtained suspension. The solid was dissolved in ethyl acetate and was washed with saturated aqueous solution of $NaHCO_3$ (3 x 20 mL). Then, the organic phase was dried over Na_2SO_4, filtered and the solvent was removed under vacuum obtaining the desired adduct (0.31 g, 99%). The white solid was suspended in 20% piperidine/ACN (10 mL) and stirred for 20 h. The resulting suspension was concentrated under vacuum and diethyl ether was added over the solid. The suspension was stirred for 10 min, filtered and washed with diethyl ether (3 x 10 mL). The white solid obtained was lyophilized to yield the pure product (0.16 g, 81% isolated yield). **^{1}H NMR** (400 MHz, [D_6]DMSO): δ 10.90 (m, 1H), 7.99 (d, *J* = 2.7 Hz, 1H), 7.79 (m, 1H), 7.50 (dd, *J* = 3.1, 1.7 Hz, 1H), 7.48 (m, 1H), 7.32 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.09 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.02 (m, 1H), 6.98 (m, 1H), 6.94 (t, *J* = 7.7 Hz, 1H), 6.87 (t, *J* = 1.7 Hz, 1H), 6.68 (dt, *J* = 7.7, 1.3 Hz, 1H), 3.99 (td, *J* = 4.9, 1.9 Hz, 1H), 3.79 (m, 1H), 2.82 (d, *J* = 4.6 Hz, 1H), 2.68 (m, 1H), 2.30 (dd, *J* = 13.4, 4.8 Hz, 1H), 1.61 (dd, *J* = 13.4, 7.3 Hz, 1H) ppm. **^{13}C NMR** (100 MHz, [D_6]DMSO): δ 167.3, 166.4, 139.8, 138.4, 136.4, 135.6, 130.6, 129.4, 127.9, 125.0, 121.6, 119.3, 119.1, 111.8, 109.2, 95.1, 55.8, 45.3, 30.1, 24.3 ppm. **IR** (Film, cm^{-1}): ν = 3345.98, 3172.35, 3050.80, 2961.09, 2914.79, 2868.49, 1679.10, 1462.06 cm^{-1}. **HRMS (ESI):** (M: $C_{20}H_{18}O_2N_3I$) m/z calcd. 460.0516, found 460.0518 $(M+H)^+$.

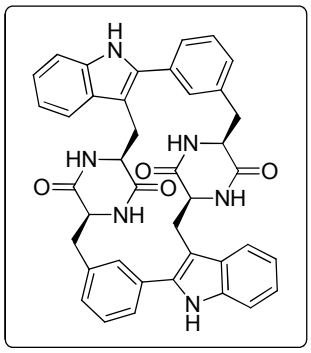


Cyclo(4-I-Phe-Trp) (6c). Compound **6c** was prepared using Fmoc-Phe(4-I)-OH (1.0 g, 1.95 mmol, 1.0 eq.), H-Trp-OMe.HCl (0.47 g*,* 1.95 mmol, 98%, 1.0 eq.), HBTU (0.7 g, 1.95 mmol, 1.0 eq.), DIEA (0.68 mL, 3.90 mmol, 2.0 eq.) which were dissolved in 1.5 mL of DMF. The pale yellow solution was stirred at 0 ºC for 24 h followed by precipitation over 10 mL of cold water and centrifugation (RPM: 2000, t: 5 min, T= 4 °C). Precipitation cycles in cold water and centrifugation were repeated 4 times obtaining the desired adduct (1.3 g, 97% isolated yield). The white solid was suspended in 20% piperidine/ACN (10 mL) and stirred for 30 min at r.t. The resulting suspension was precipitated over 10 mL of cold water and centrifuged (RPM: 2000, t: 5 min, T= 4 °C). Precipitation cycles in cold water and centrifugation were repeated 2 times. The white solid obtained was lyophilized to

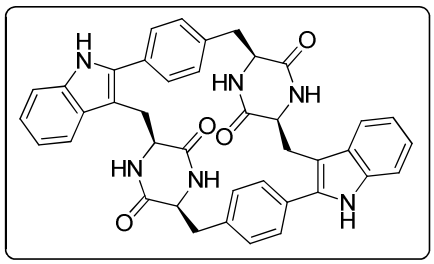


yield the pure product (0.82 g, 94% isolated yield). **^{1}H NMR** (400 MHz, [D_6]DMSO): δ 10.93 (s, 1H), 7.98 (s, 1H), 7.71 (s, 1H), 7.50 (dt, *J* = 7.9, 0.9 Hz, 1H), 7.45 (m, 2H), 7.31 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.07 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.01 (s, 1H), 6.99 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 6.34 (m, 2H), 4.01 (ddd, *J* = 4.7, 5.3, 2.3 Hz, 1H), 3.75 (ddd, *J* = 7.6, 4.8, 2.3 Hz, 1H), 2.85 (dd, *J* = 14.4, 4.7 Hz, 1H), 2.73 (dd, *J* = 14.4, 5.3 Hz, 1H), 2.30 (dd, *J* = 13.4, 4.8 Hz, 1H), 1.57 (dd, *J* = 13.4, 7.6 Hz, 1H) ppm. **^{13}C NMR** (100 MHz, [D_6]DMSO): δ 167.2, 166.5, 137.1, 136.8, 136.5, 132.4, 128.0, 125.0, 121.4, 119.4, 119.0, 111.8, 109.1, 92.7, 55.8, 55.7, 40.0, 30.1 ppm. **IR** (Film, cm^{-1}): ν = 3186.82, 3050.80, 2961.09, 2917.68, 2845.34, 1664.63, 1459.16 cm^{-1}. **HRMS (ESI):** (M: $C_{20}H_{18}O_2N_3I$) m/z calcd. 460.0516, found 460.0522 $(M+H)^+$. **IR** (Film, cm^{-1}): ν = 3408.41 (s), 1664.77 (s), 1459.39 (s) cm^{-1}.

(Cyclo-*m,m)*bis-[Phe-Trp]-cyclo(Phe-Trp) (7b). Compound **6b** (91 mg, 0.198 mmol), $AgBF_4$ (77 mg, 0.396 mmol, 2.0 eq.), trifluoroacetic acid (15.2 µL, 0.198 mmol, 1.0 eq.) and $Pd(OAc)_2$ (2.2 mg, 0.010 mmol, 0.05 eq.) were dissolved in 2 mL of DMF and placed in a microwave reactor vessel. The mixture was heated under microwave irradiation (250W) at 90 °C for 20 min. The resulting suspension was filtered through Celite and the filtrate was evaporated under vacuum (28% yield estimated by HPLC-MS conversion). An analytically pure sample was obtained by semi-preparative RP-HPLC. **^{1}H NMR** (500 MHz, [D_6]DMSO): δ 11.24 (s, 1H), 8.51 (s, 1H), 8.26 (s, 1H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.33 (m, 2H), 7.20 (d, *J* = 7.5 Hz, 1H), 7.08 (t, *J* = 7.6 Hz, 1H), 7.04 – 6.97 (m, 2H), 6.86 (s, 1H), 3.97 (s, 1H), 3.38(1H), 3.24 (m, 1H), 2.99 (m, 1H), 2.20 (m, 1H), 0.24 (m, 1H) ppm. **HPLC-MS**: t_R 2.38 min (gradient 5-100% ACN). **HRMS (ESI):** (M: $C_{40}H_{34}O_4N_6$) m/z calcd 663.2707, found 663.2714 $(M+H)^+$.

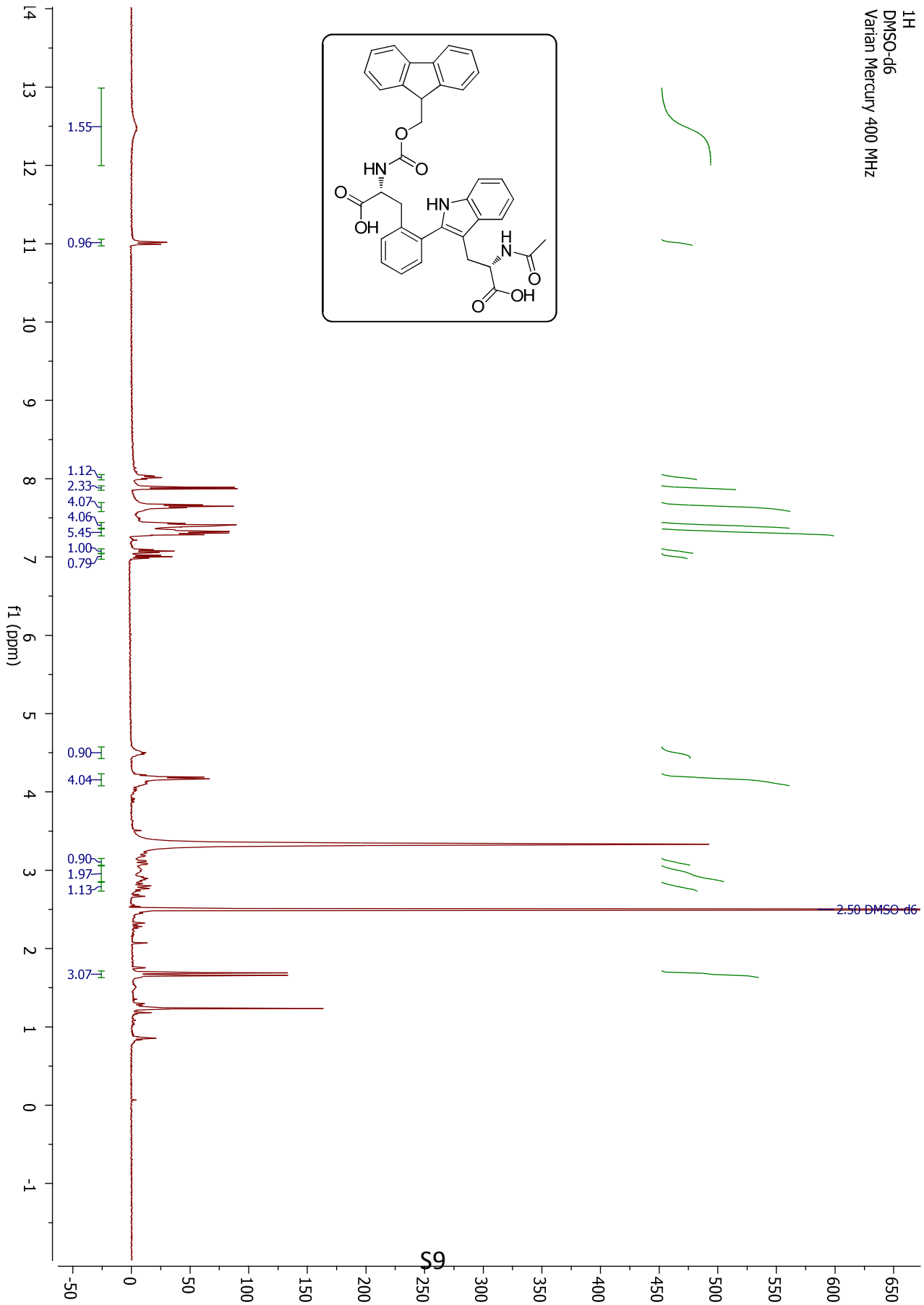


(Cyclo-*p,p)*bis-[Phe-Trp]-cyclo(Phe-Trp) (7c). Compound **6c** (80 mg, 0.175 mmol), $AgBF_4$ (68 mg, 0.350 mmol, 2.0 eq.), trifluoroacetic acid (13 µL, 0.175 mmol, 1.0 eq.) and $Pd(OAc)_2$ (2 mg, 0.009 mmol, 0.2 eq.) were dissolved in 2 mL of DMF and placed in a microwave reactor vessel. The mixture was heated under microwave irradiation (250W) at 90 °C for 1 h. Then, $Pd(OAc)_2$ (2.0 mg, 0.009 mmol, 0.2 eq.) and $AgBF_4$ (68 mg, 2.0 eq.) were added again, and a second irradiation cycle of 20 min was performed. The same procedure was repeated and both fractions were mixed. The resulting suspension was filtered through Celite and the filtrate was evaporated under vacuum (24% yield estimated by HPLC-MS conversion). An analytically pure sample was obtained by flash chromatography on Celite using H_2O (0.1% FA)/ACN (0.1% FA) (4.1 mg, 2% isolated yield). **^{1}H NMR** (800 MHz, [D_6]DMSO): δ 10.99 (s, 1H), 8.54 (d, *J* = 2.5 Hz, 1H), 8.32 (s, 1H), 7.58 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.38 (d, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.04 (m, 1H), 6.92 (m, 1H), 4.38 (dt, *J* = 5.3, 2.7 Hz, 1H), 3.78 (dt, *J* = 9.0, 2.9 Hz, 1H), 3.36 (m, 1H), 3.00 (dd, *J* = 13.3, 5.4 Hz, 1H), 2.70 (m, 1H), 1.33 (m, 1H) ppm. **HPLC-MS**: t_R 2.18 min (gradient 5-100% ACN). **HRMS (ESI)**: (M: $C_{40}H_{34}O_4N_6$) m/z calcd 663.2726, found 663.2714 $(M+H)^+$.

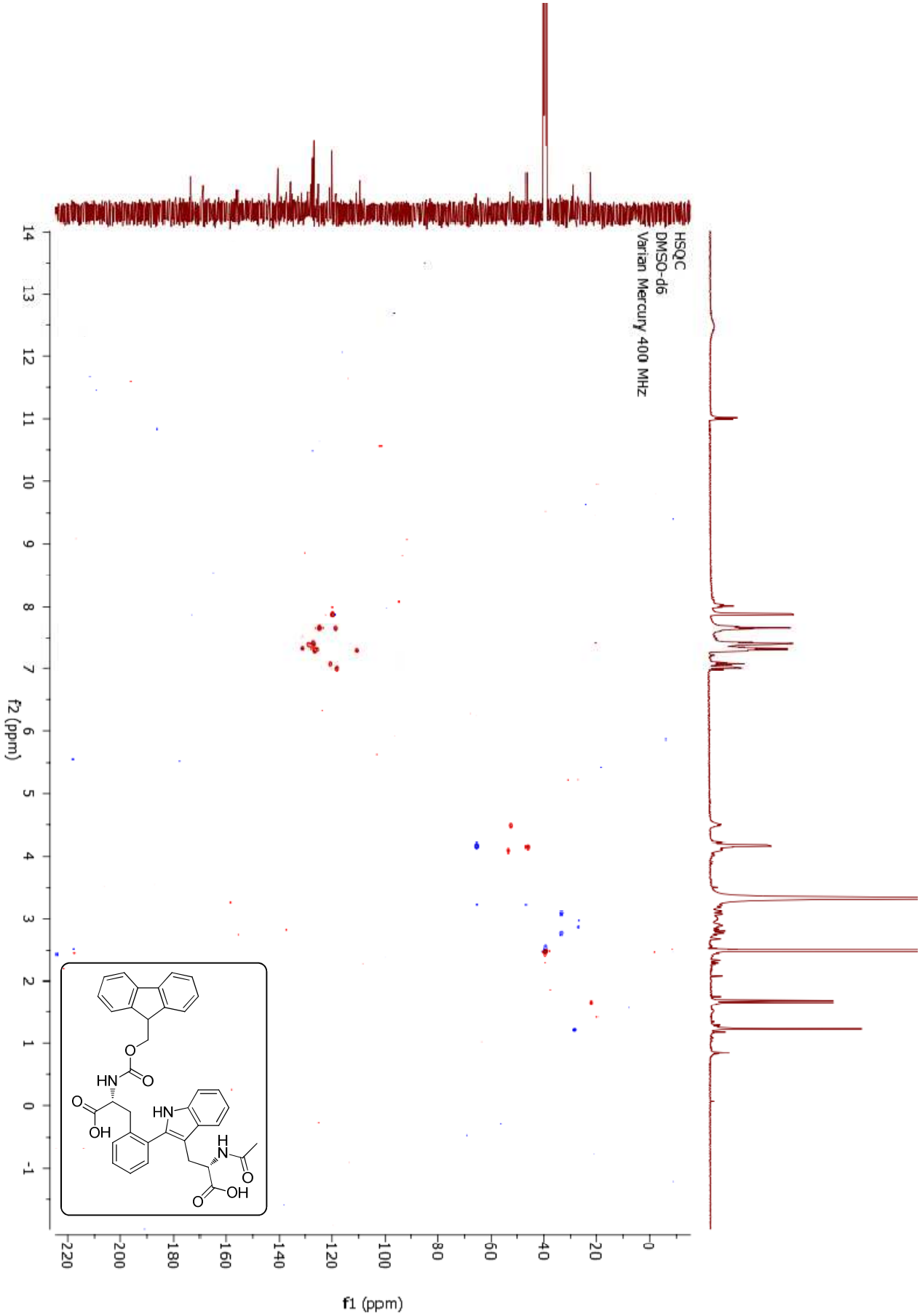


NMR spectra of compounds 1, 3, 5, 6 and 7

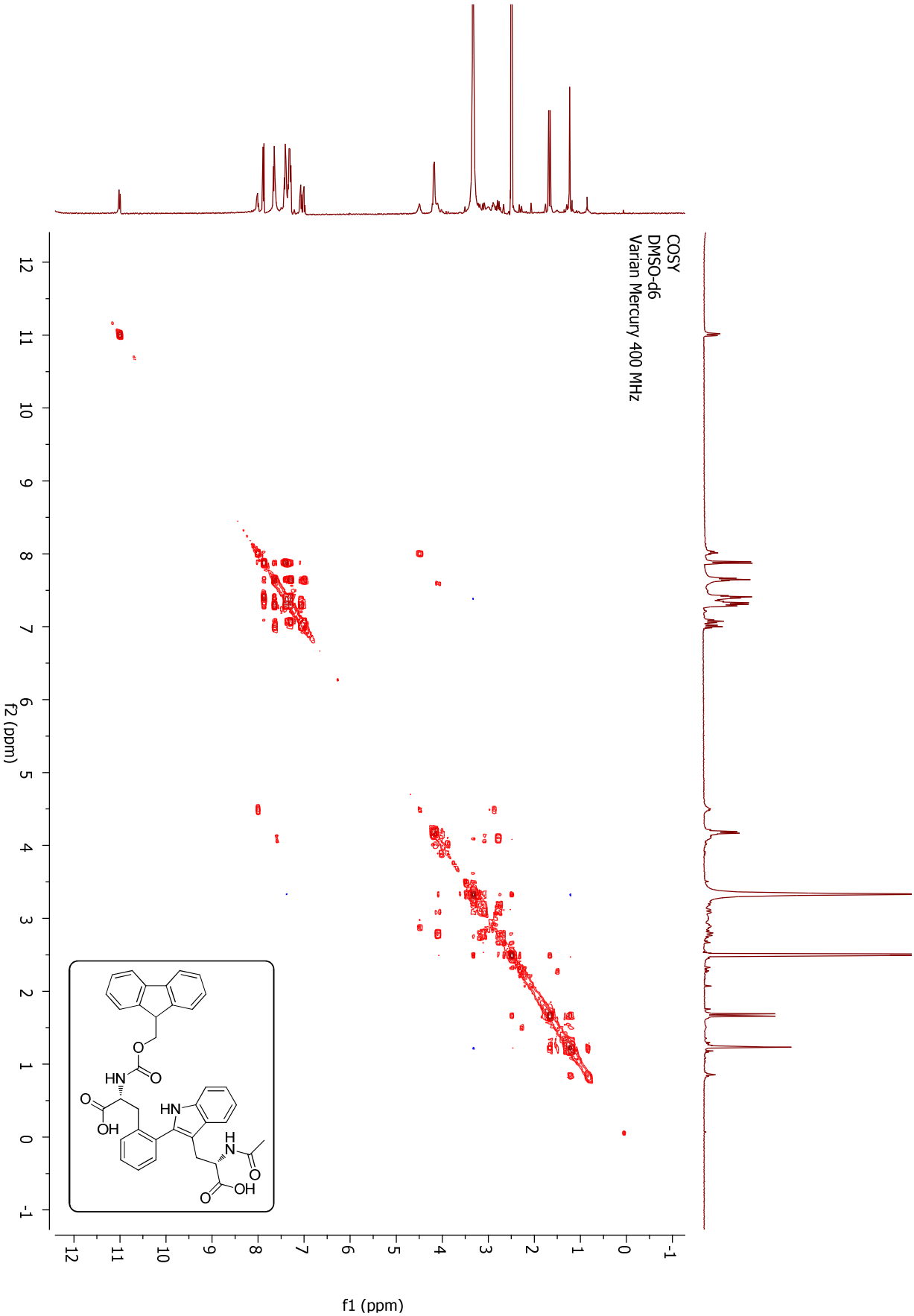
[Ac-*C2*-Trp-OH)—(Fmoc-*o*-Phe-OH)] adduct (1a) ^{1}H NMR



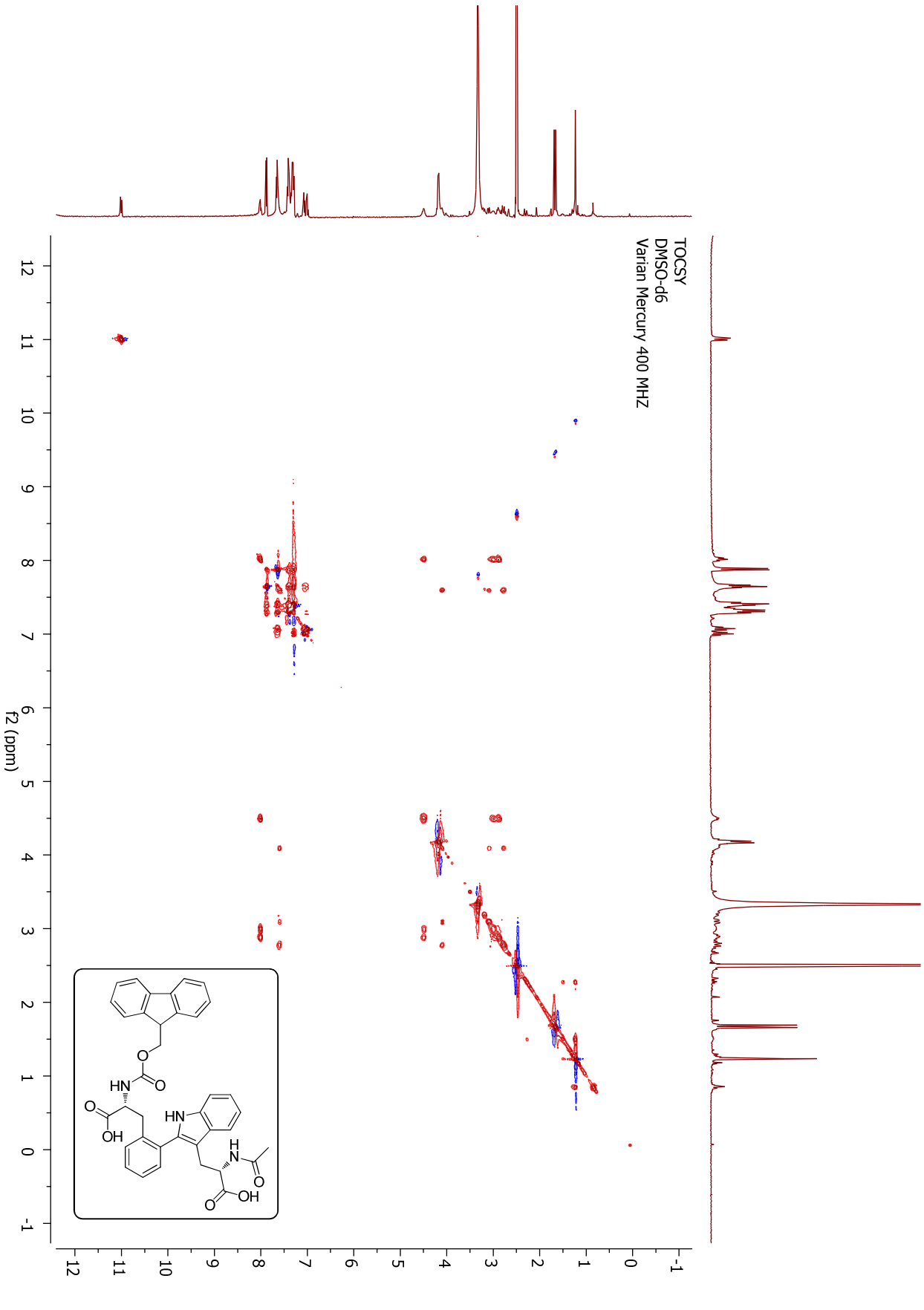
[Ac-*C2*-Trp-OH)—(Fmoc-*o*-Phe-OH)] adduct (1a) ^{1}H-^{13}C HSQC NMR



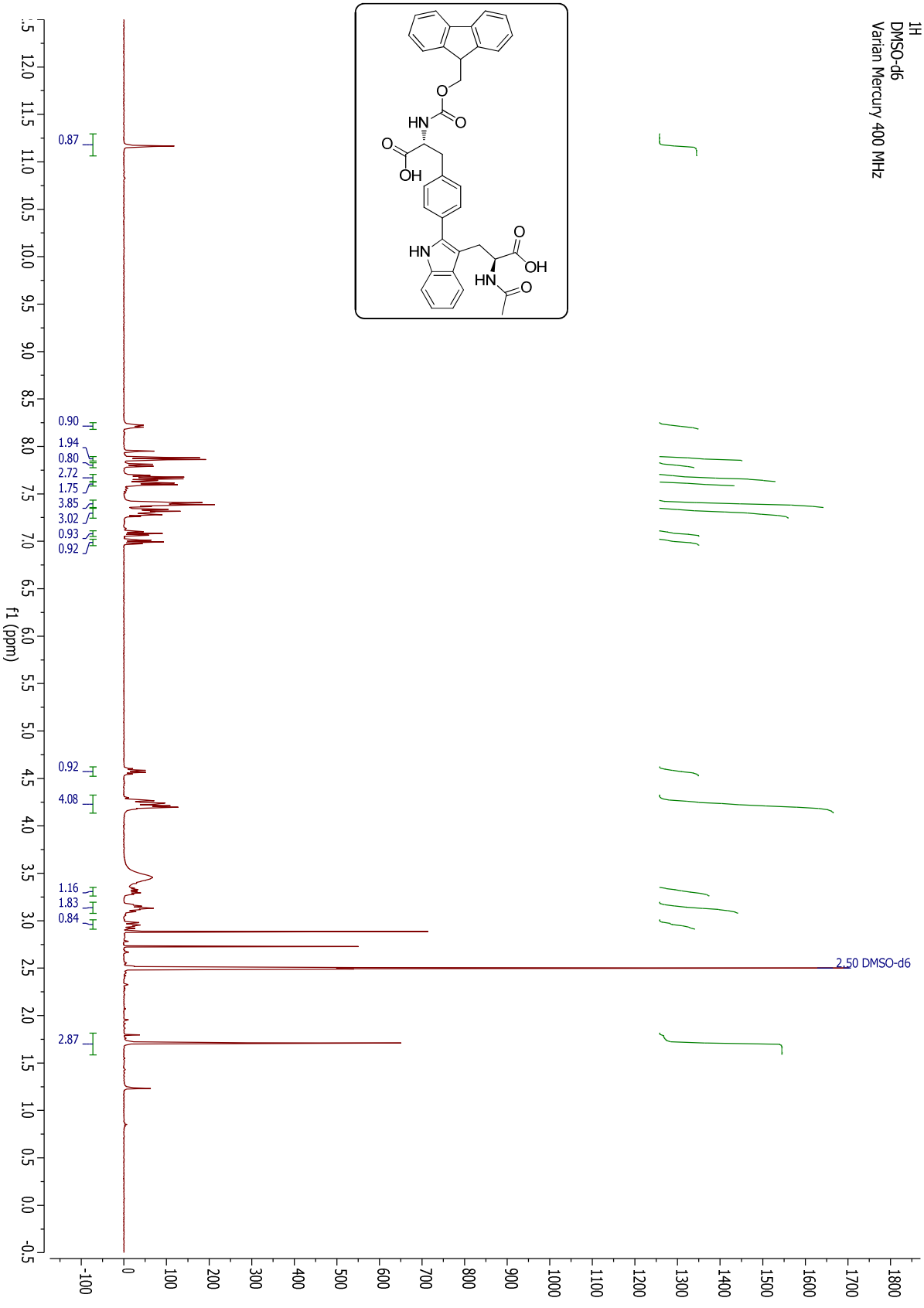
[Ac-*C2*-Trp-OH)—(Fmoc-*o*-Phe-OH)] adduct (1a) COSY NMR



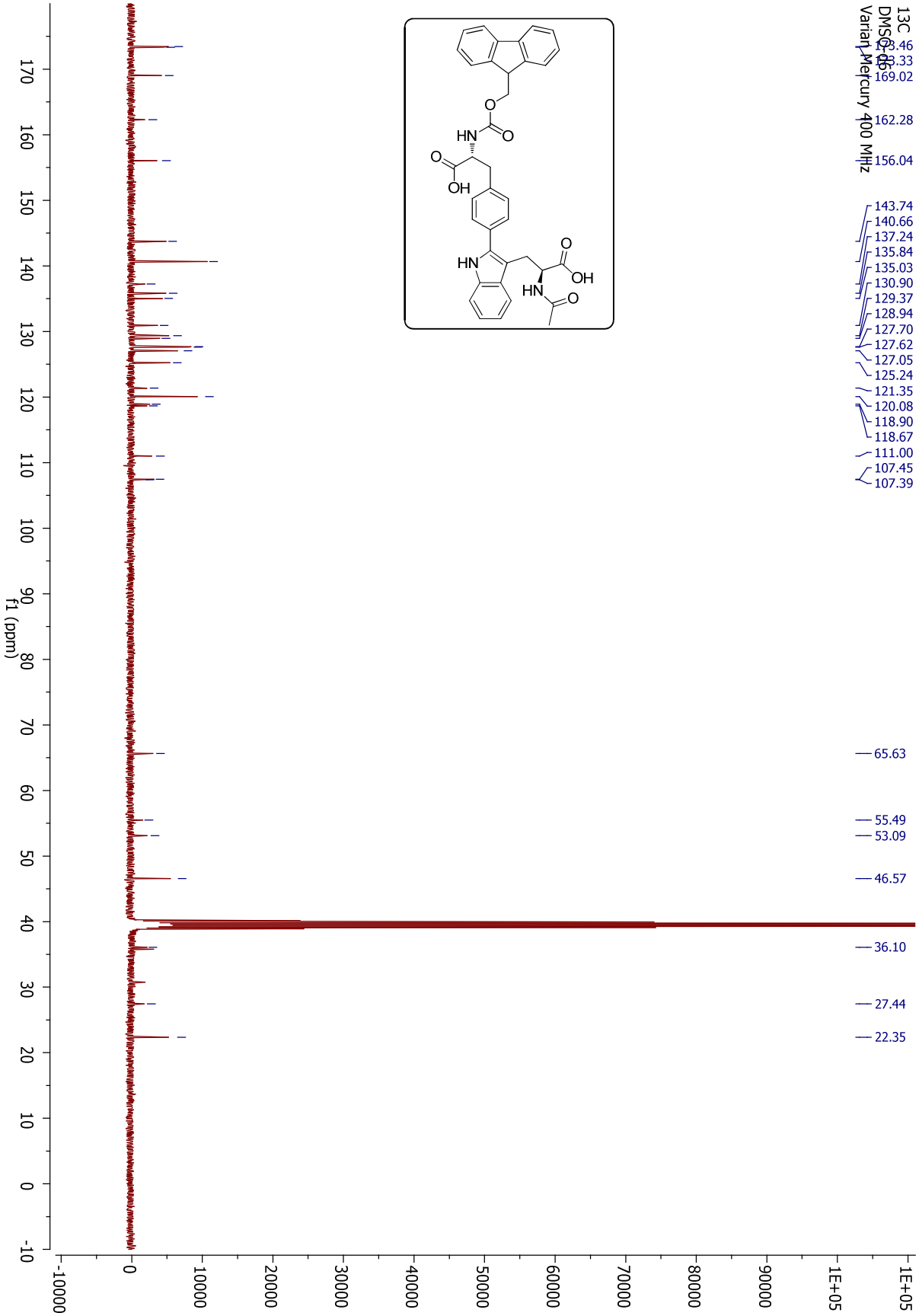
[Ac-*C2*-Trp-OH)—(Fmoc-*o*-Phe-OH)] adduct (1a) TOCSY NMR



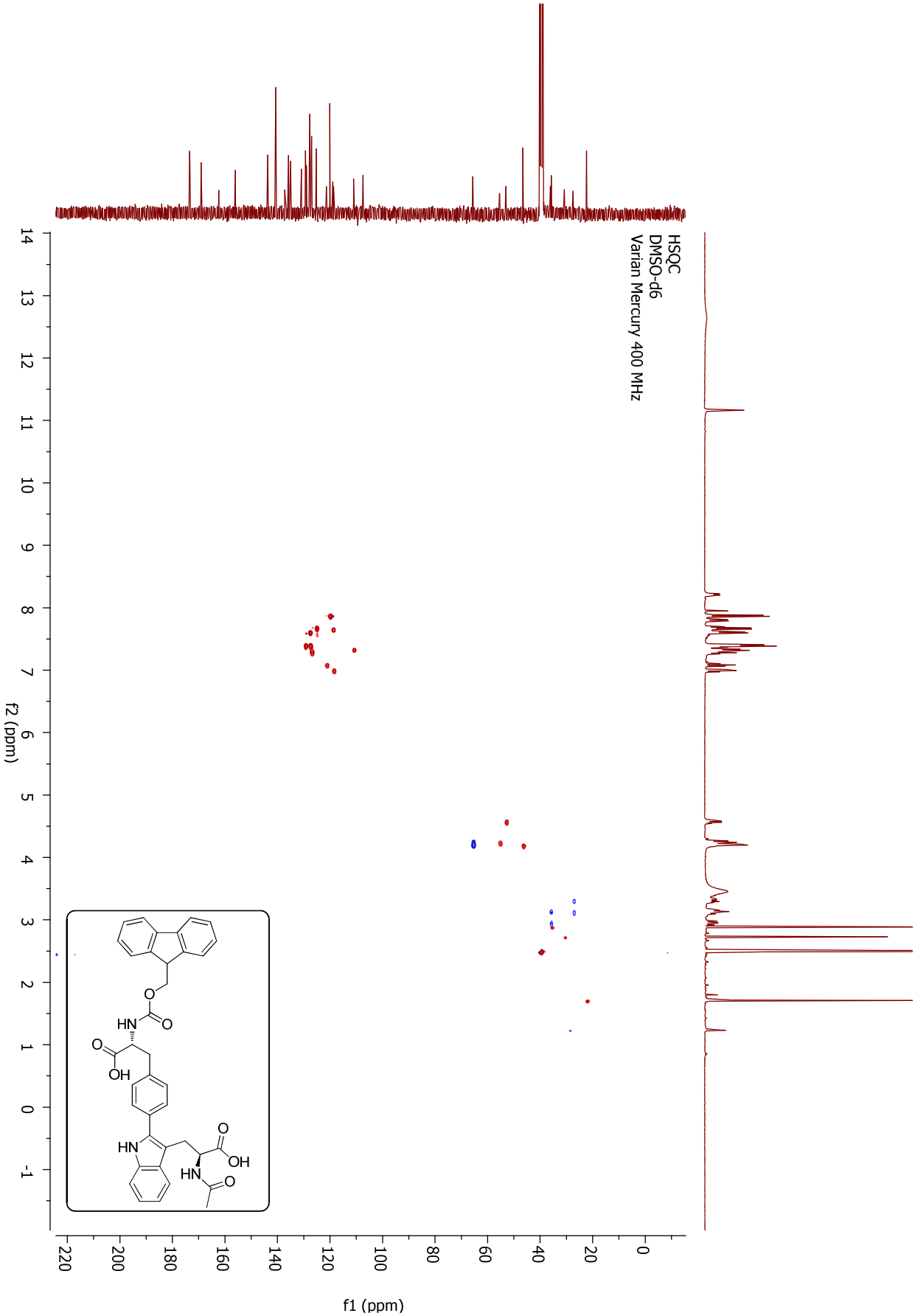
[Ac-*C2*-Trp-OH)—(Fmoc-*p*-Phe-OH)] adduct (1b) ^{1}H NMR



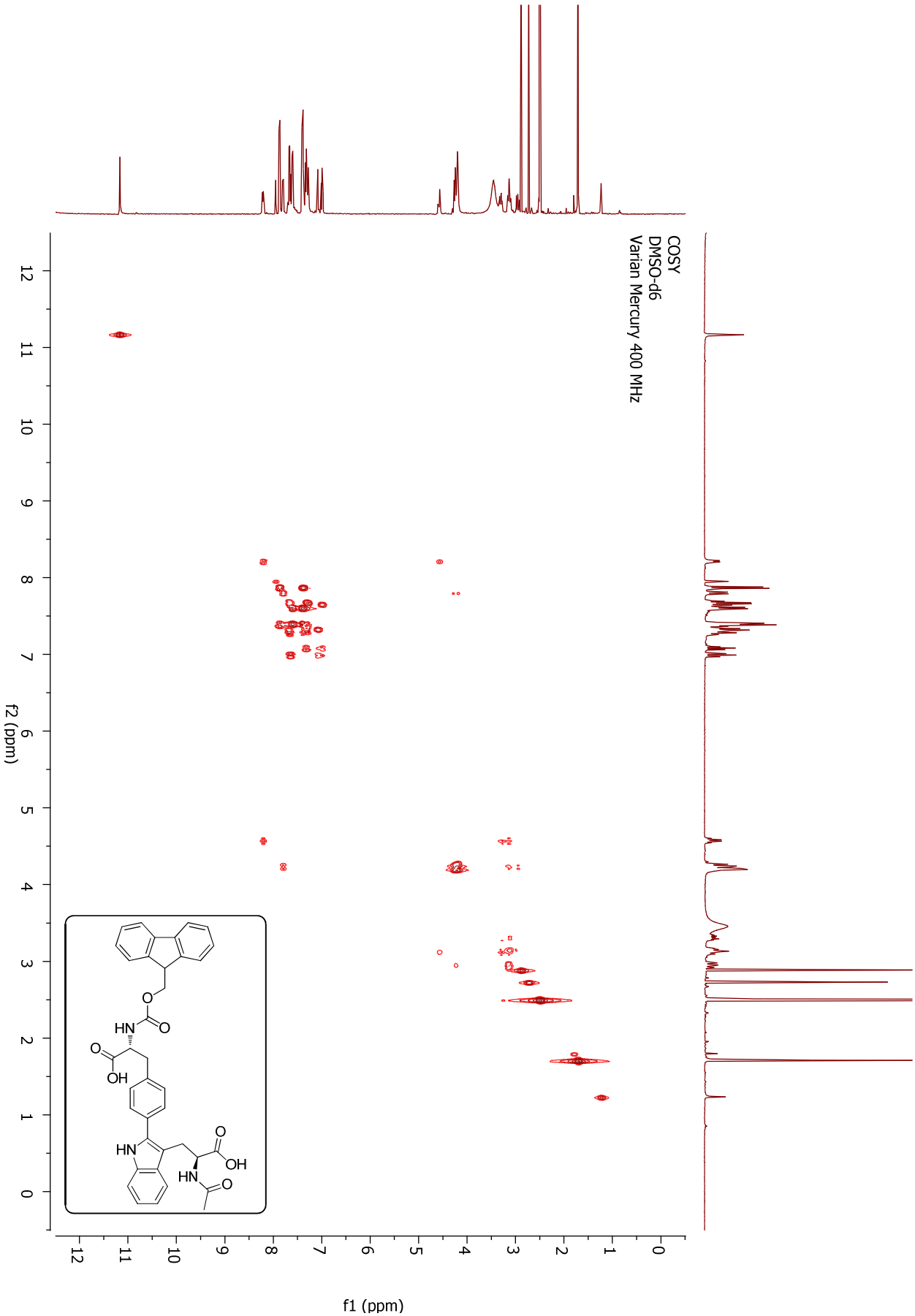
[Ac-*C2*-Trp-OH)—(Fmoc-*p*-Phe-OH)] adduct (1b) ^{13}C NMR



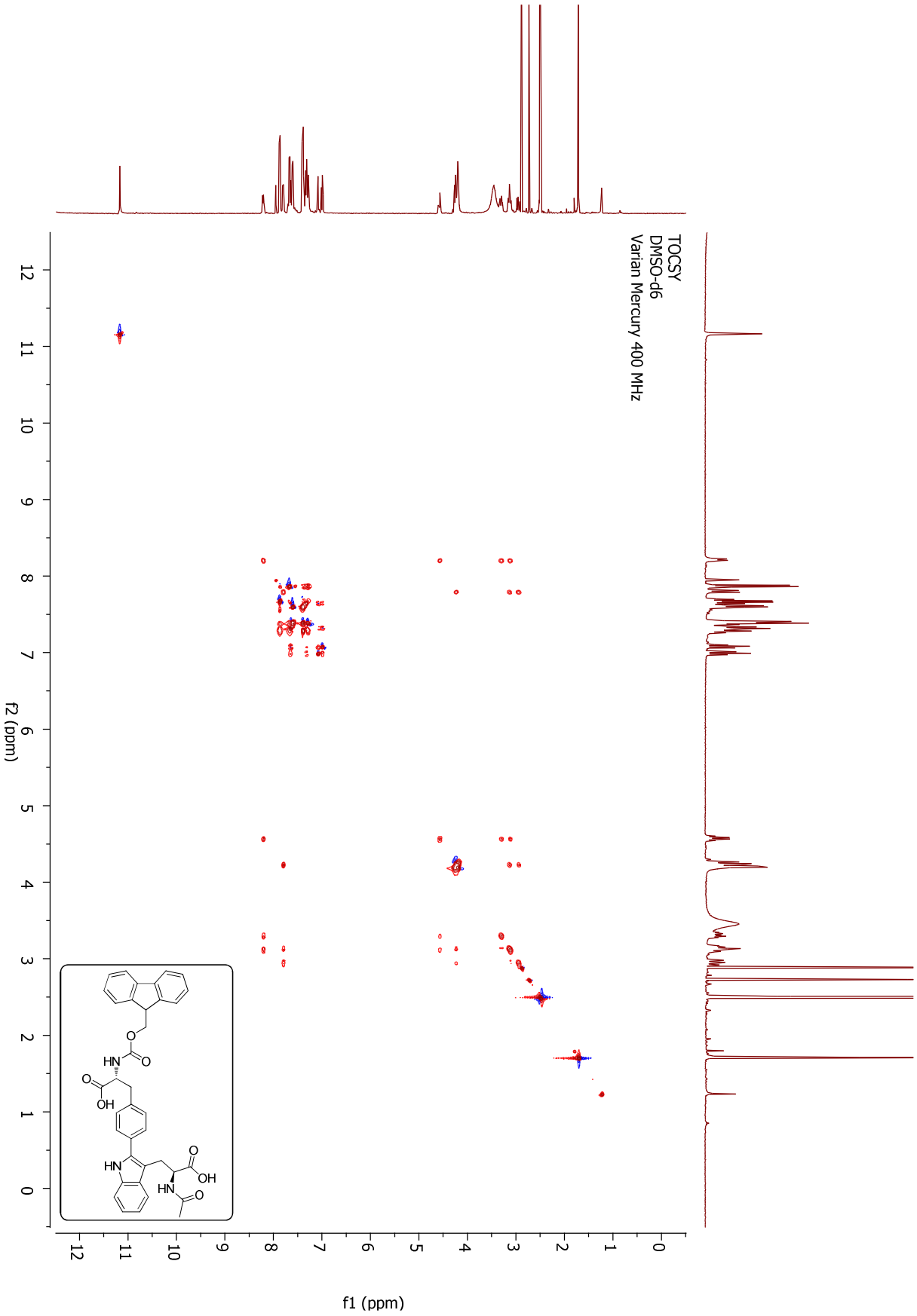
[Ac-*C2*-Trp-OH)—(Fmoc-*p*-Phe-OH)] adduct (1b) ^{1}H-^{13}C HSQC NMR



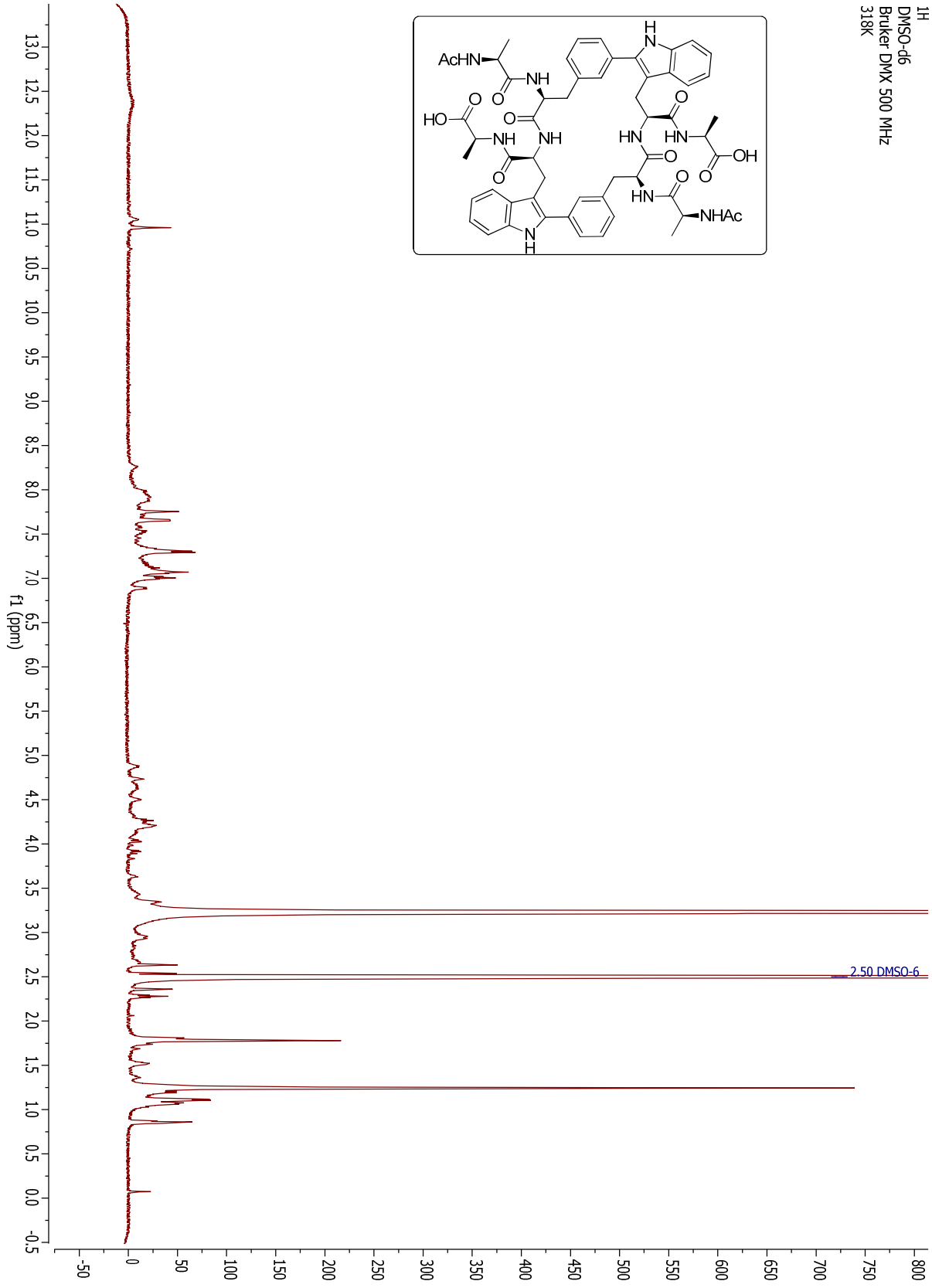
[Ac-*C2*-Trp-OH)—(Fmoc-*p*-Phe-OH)] adduct (1b) COSY NMR



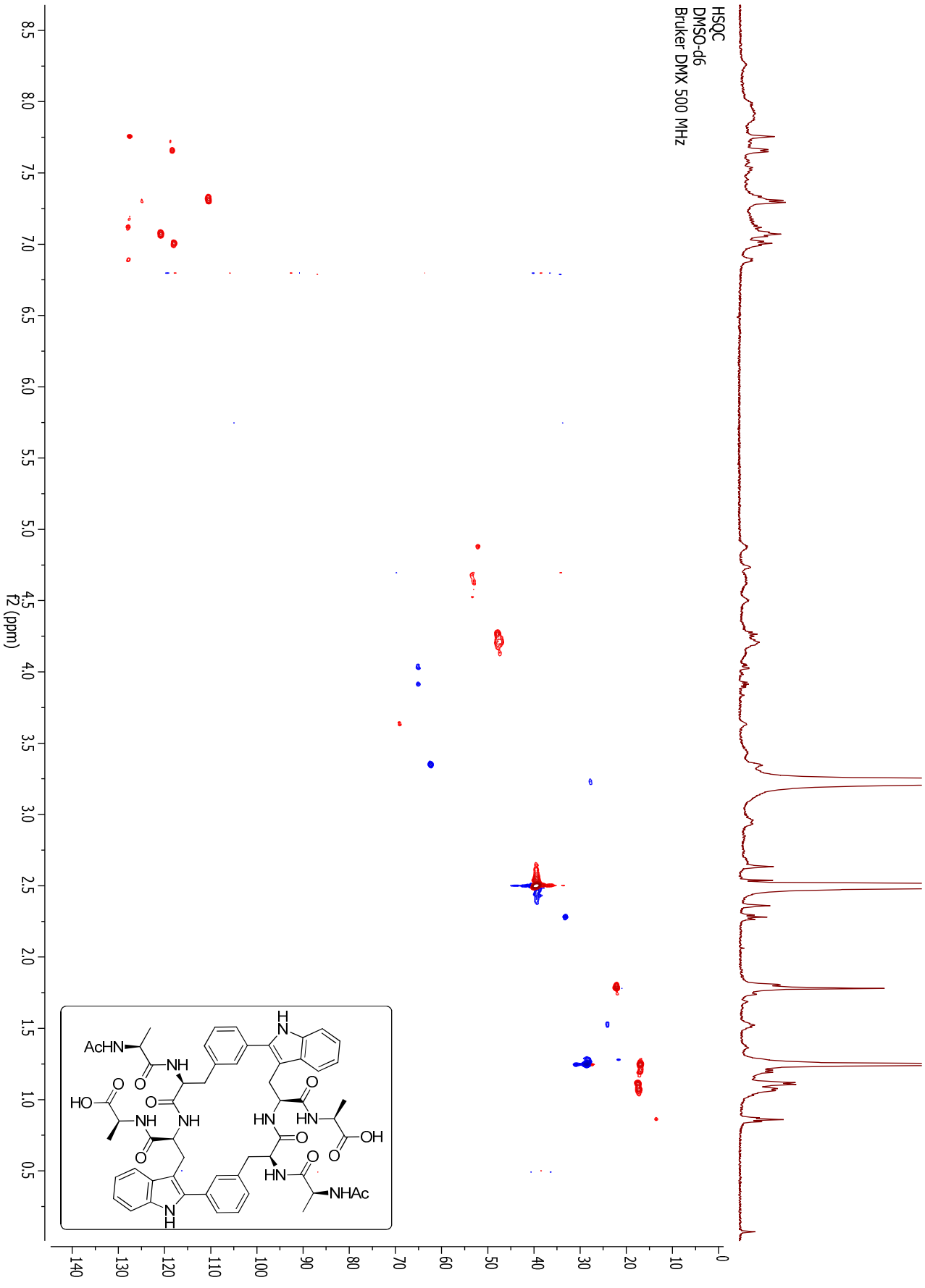
[Ac-*C2*-Trp-OH)—(Fmoc-*p*-Phe-OH)] adduct (1b) TOCSY NMR



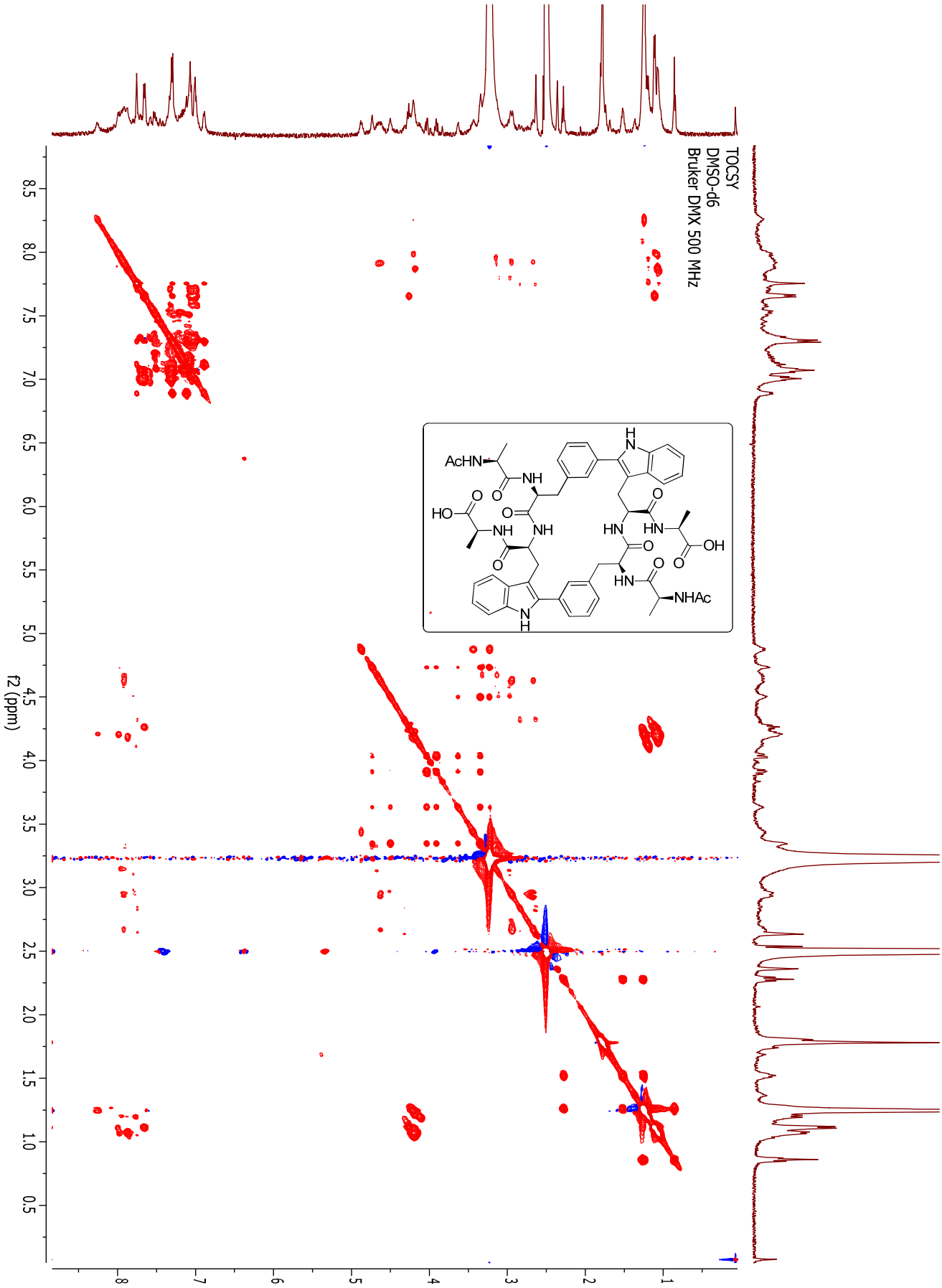
(Cyclo-*m,m*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (3) ^{1}H NMR T=318 K



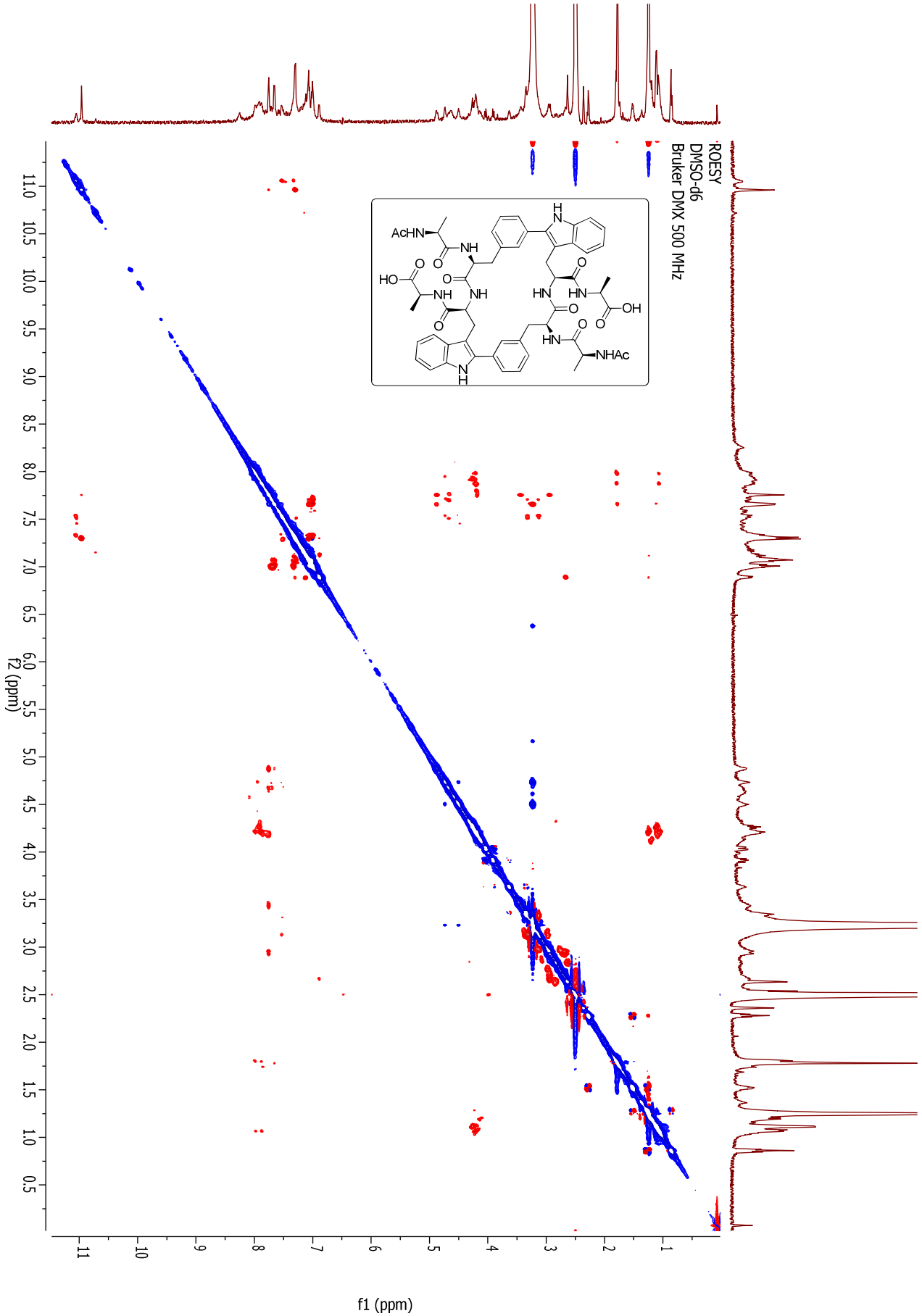
(Cyclo-*m,m)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (3) ^{1}H-^{13}C HSQC NMR T=318 K



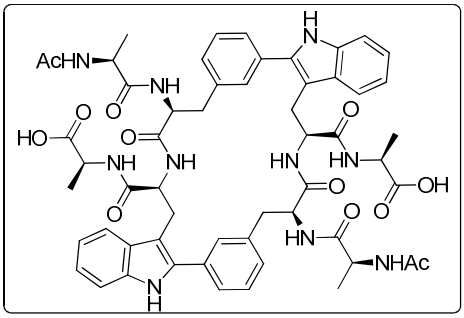
(Cyclo-*m,m*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (3) TOCSY NMR T=318 K

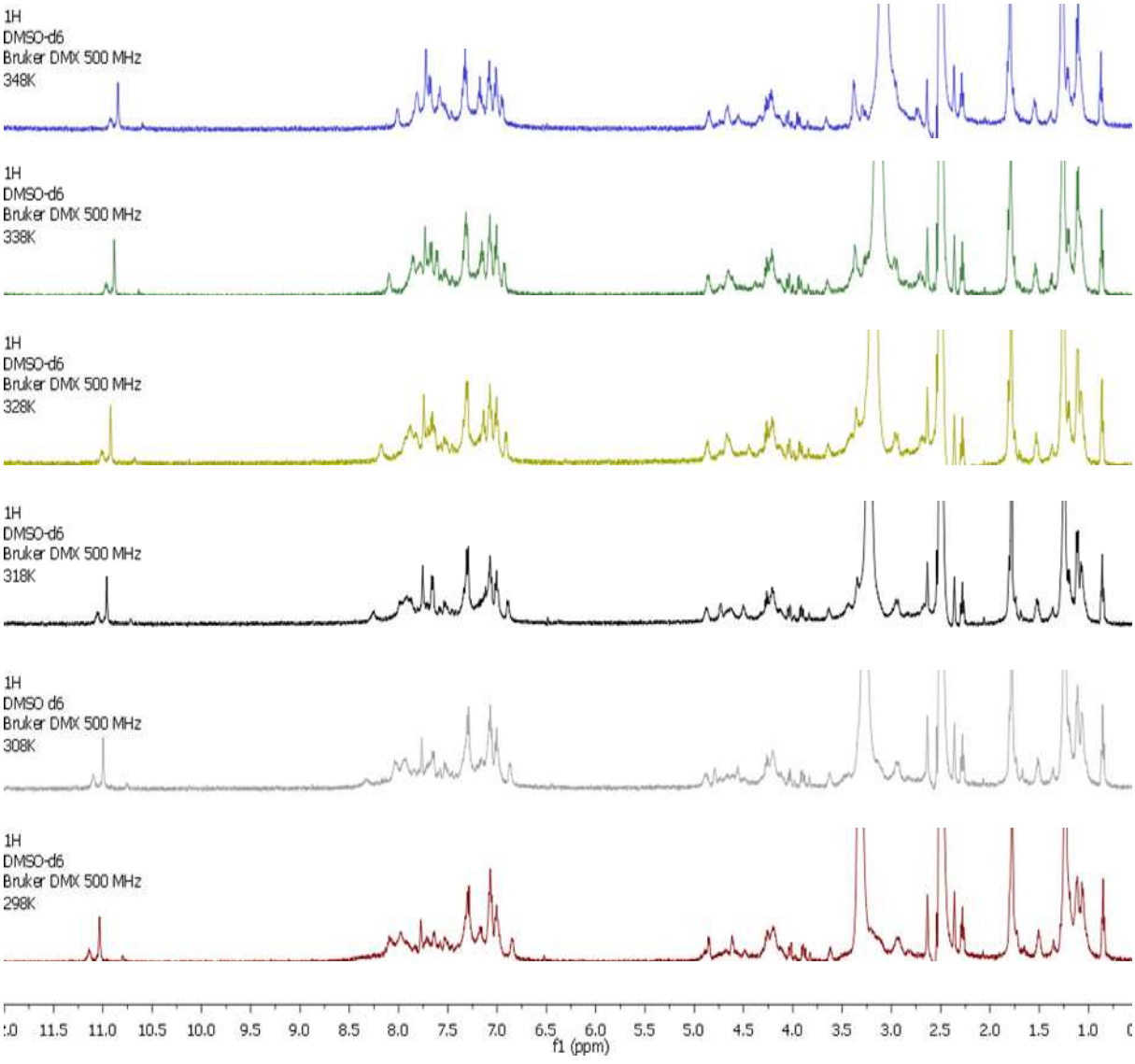


(Cyclo-*m,m)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (3) ROESY NMR T=318 K

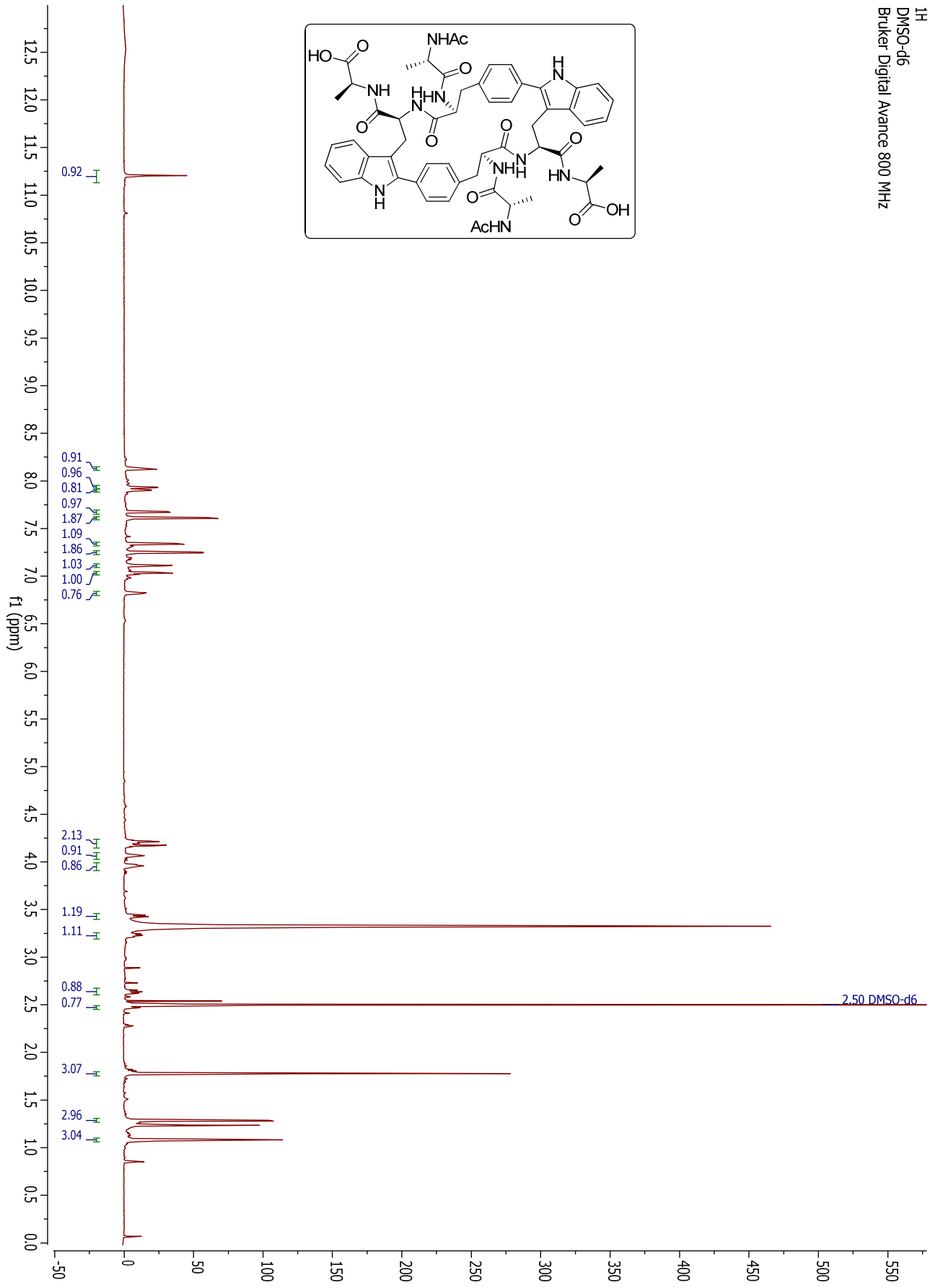


(Cyclo-*m,m)*bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (3) ^{1}H NMR T=298-348 K

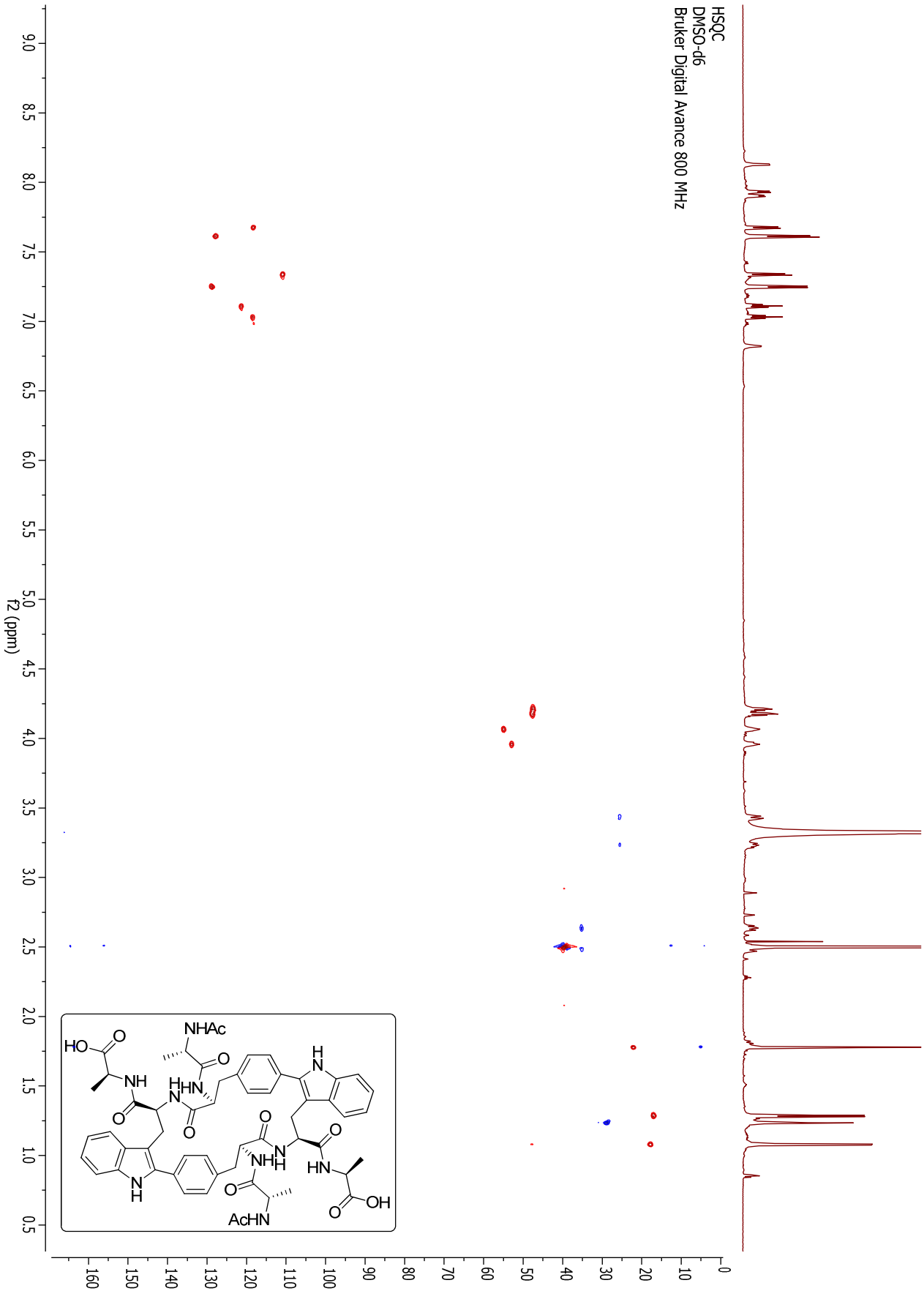




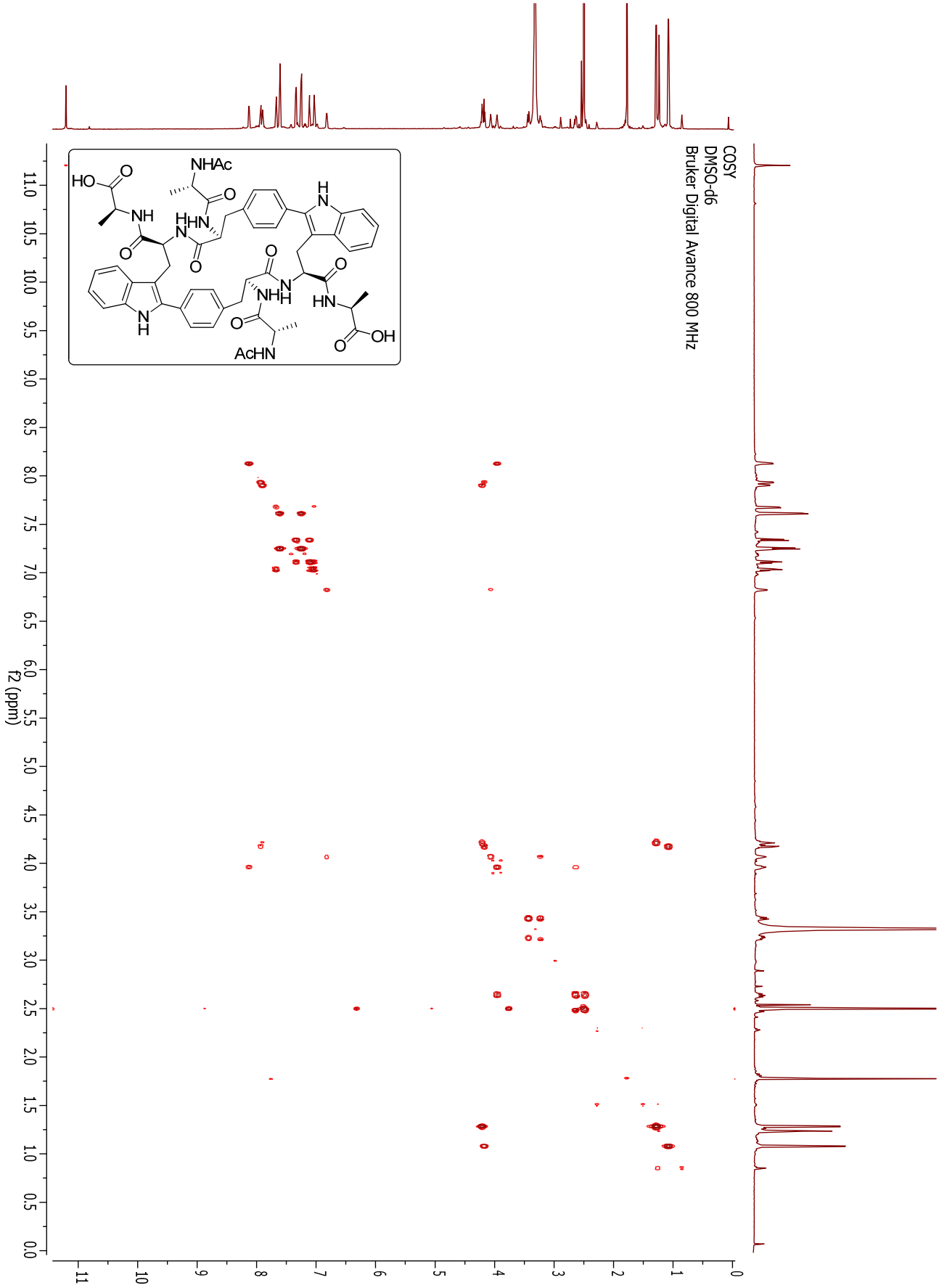
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (5a) ^{1}H NMR



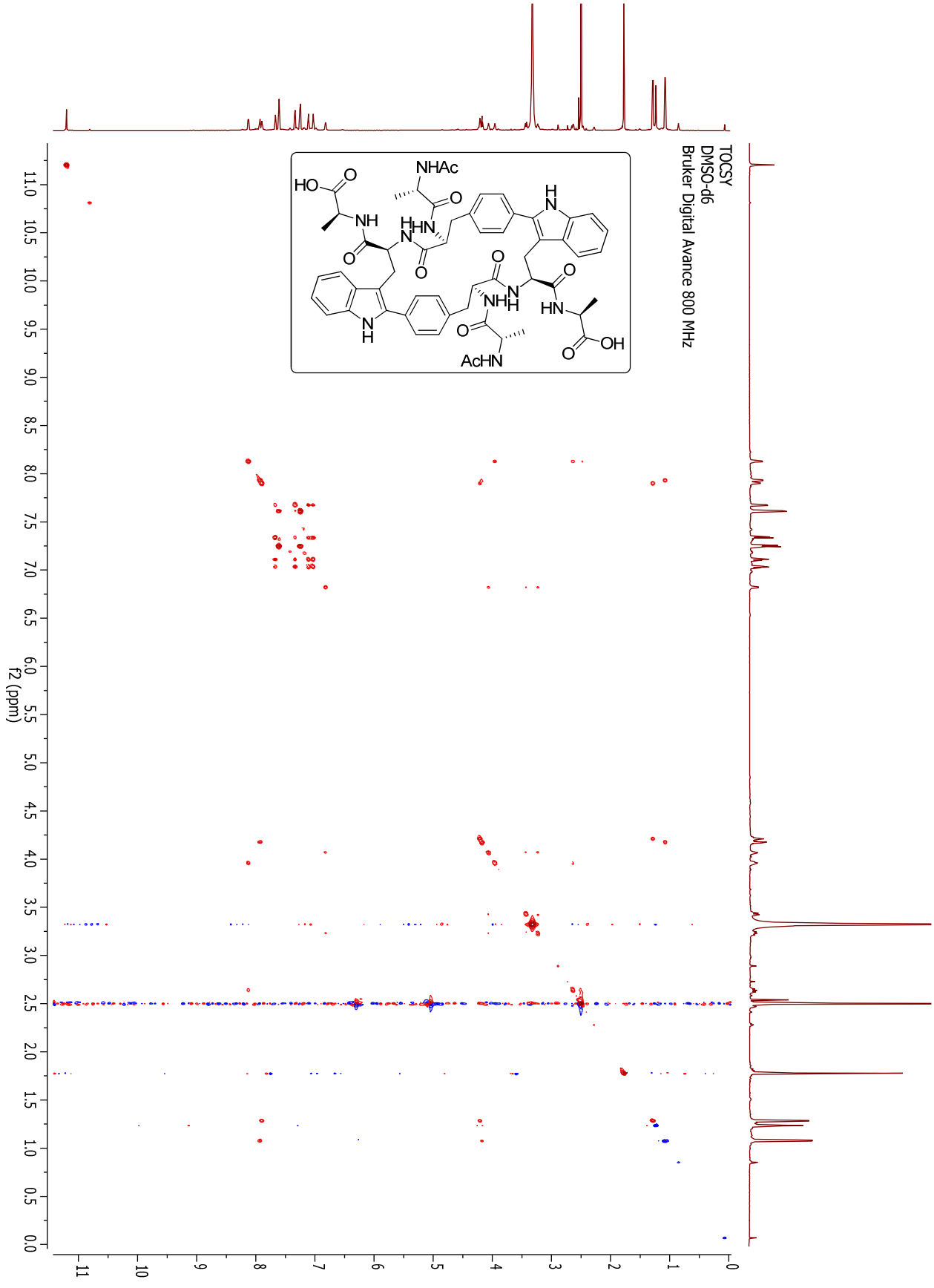
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (5a) ^{1}H-^{13}C HSQC NMR



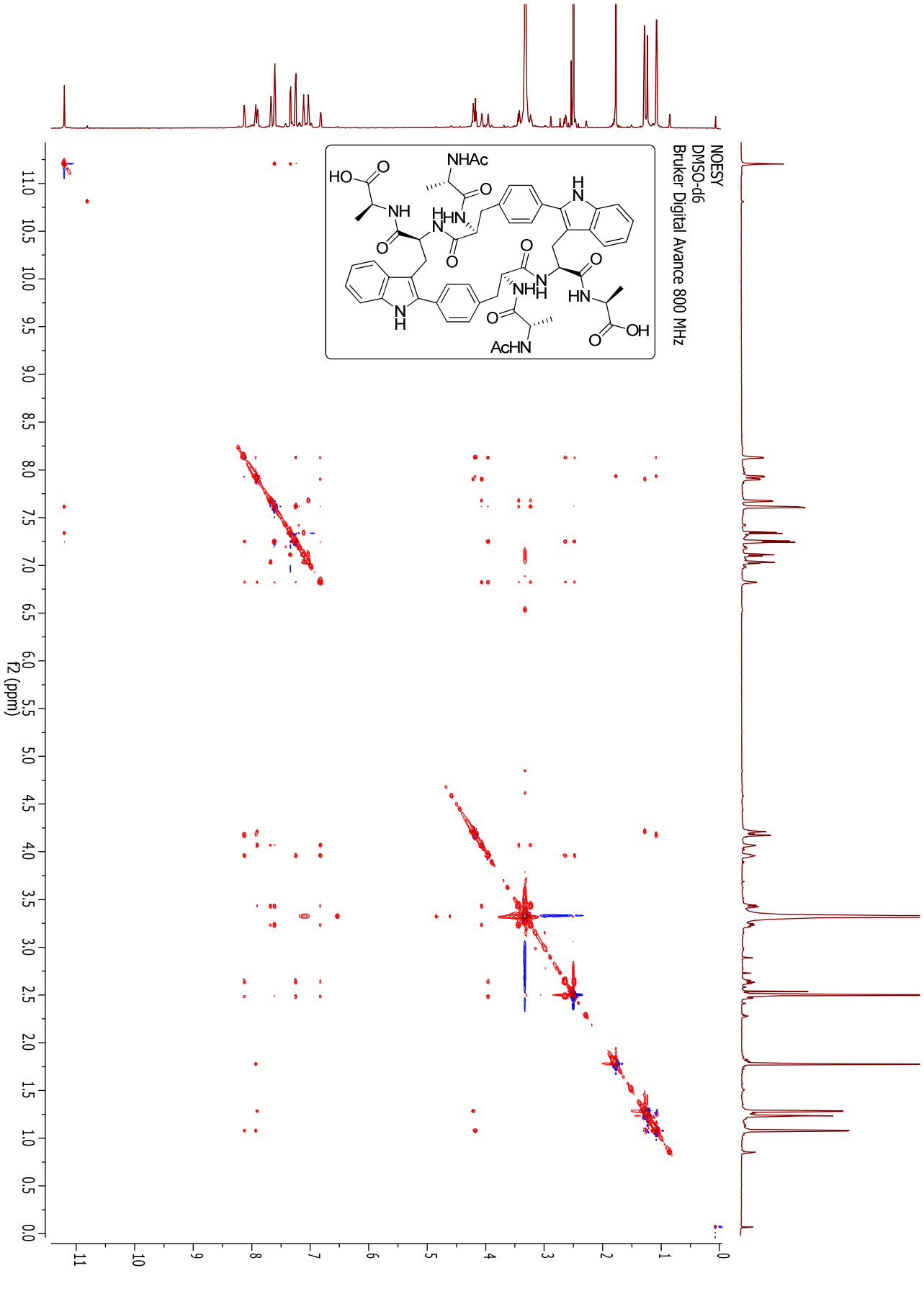
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (5a) COSY NMR



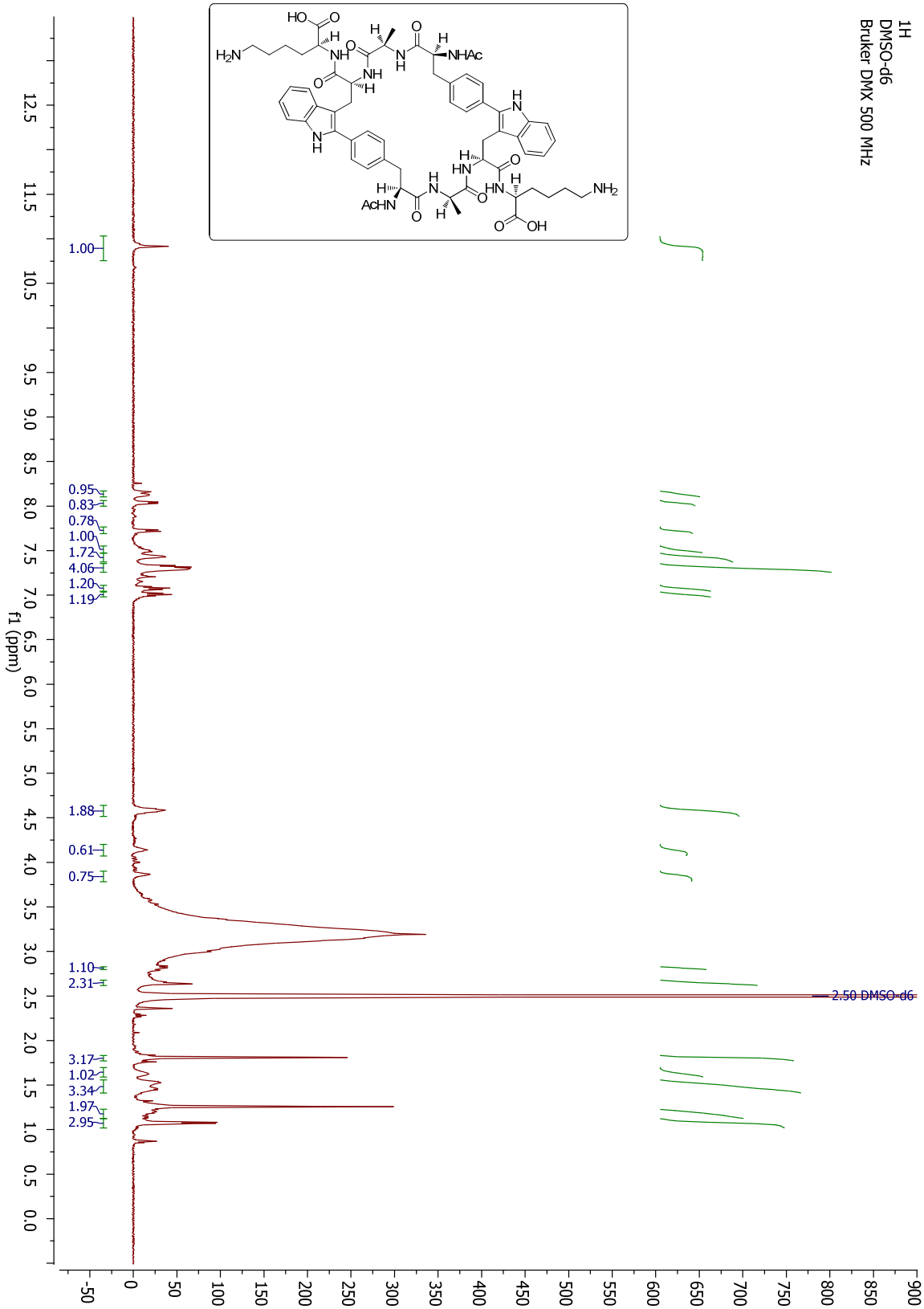
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (5a) TOCSY NMR



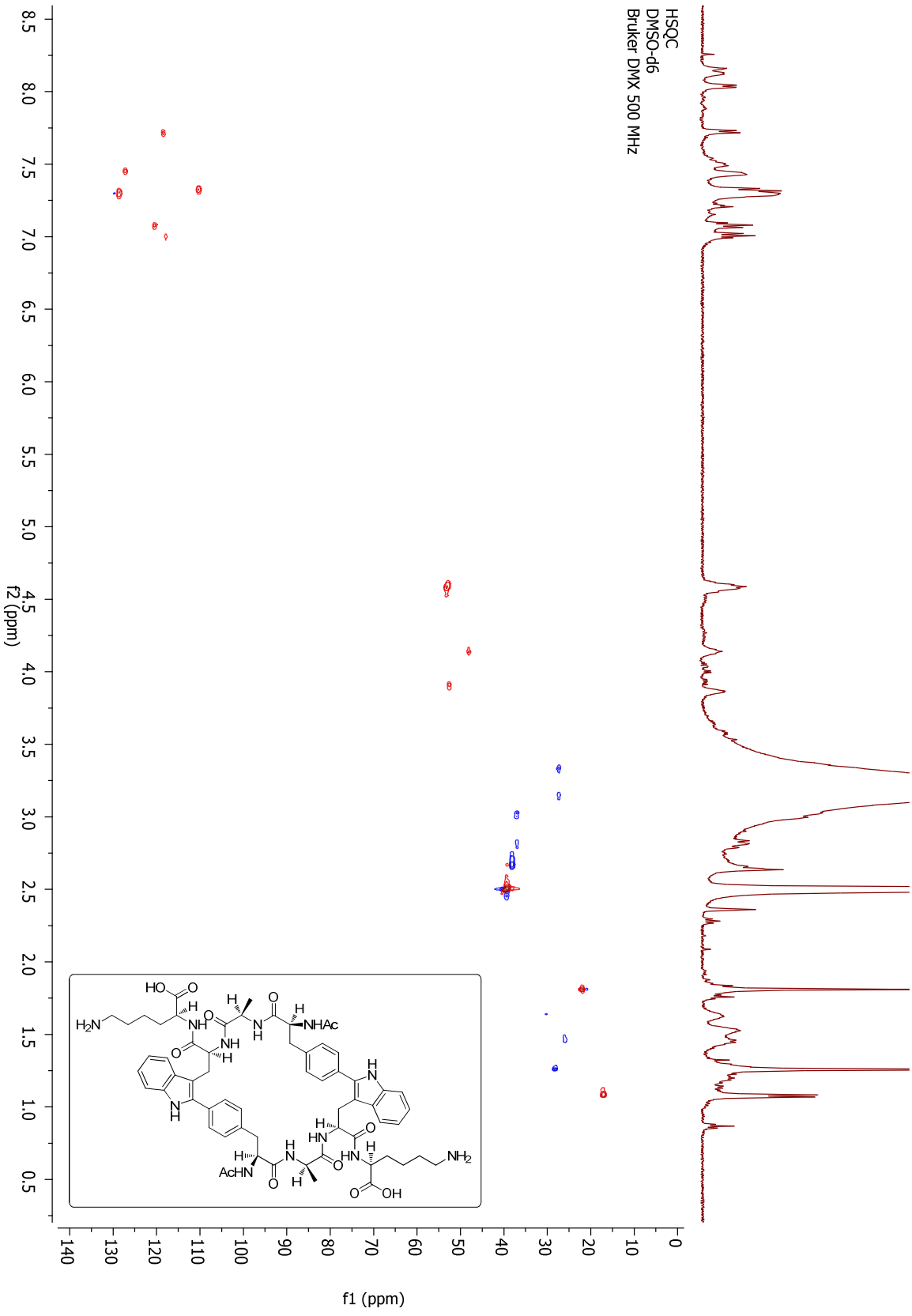
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Trp-Ala-OH) (5a) NOESY NMR



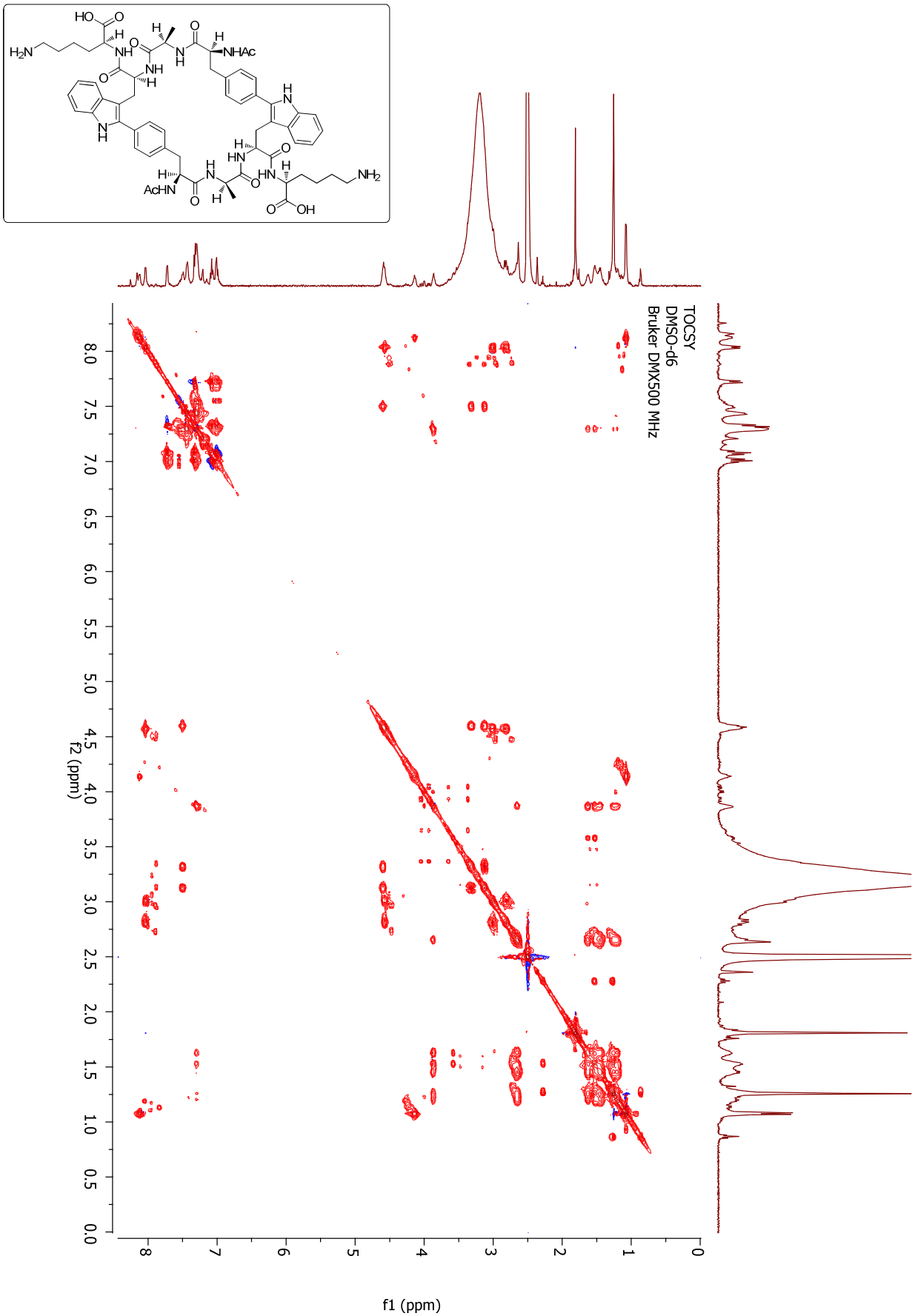
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Phe-Ala-Trp-Lys-OH) (5b) ^{1}H NMR T=338 K



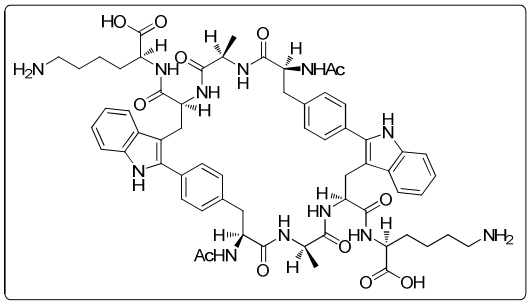
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Phe-Ala-Trp-Lys-OH) (5b) ^{1}H-^{13}C HSQC NMR T=338 K

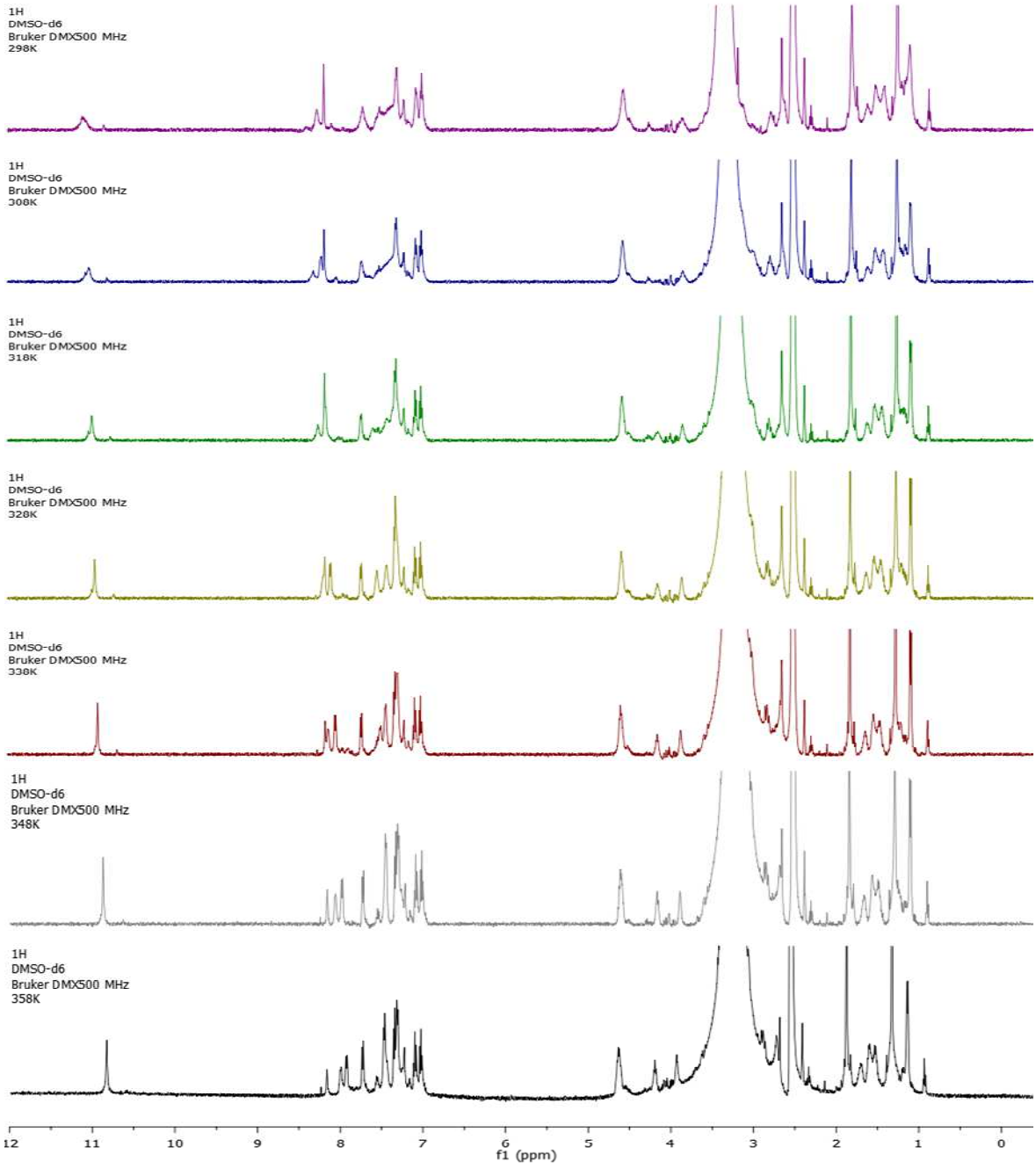


(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Phe-Ala-Trp-Lys-OH) (5b) TOCSY NMR T=338 K

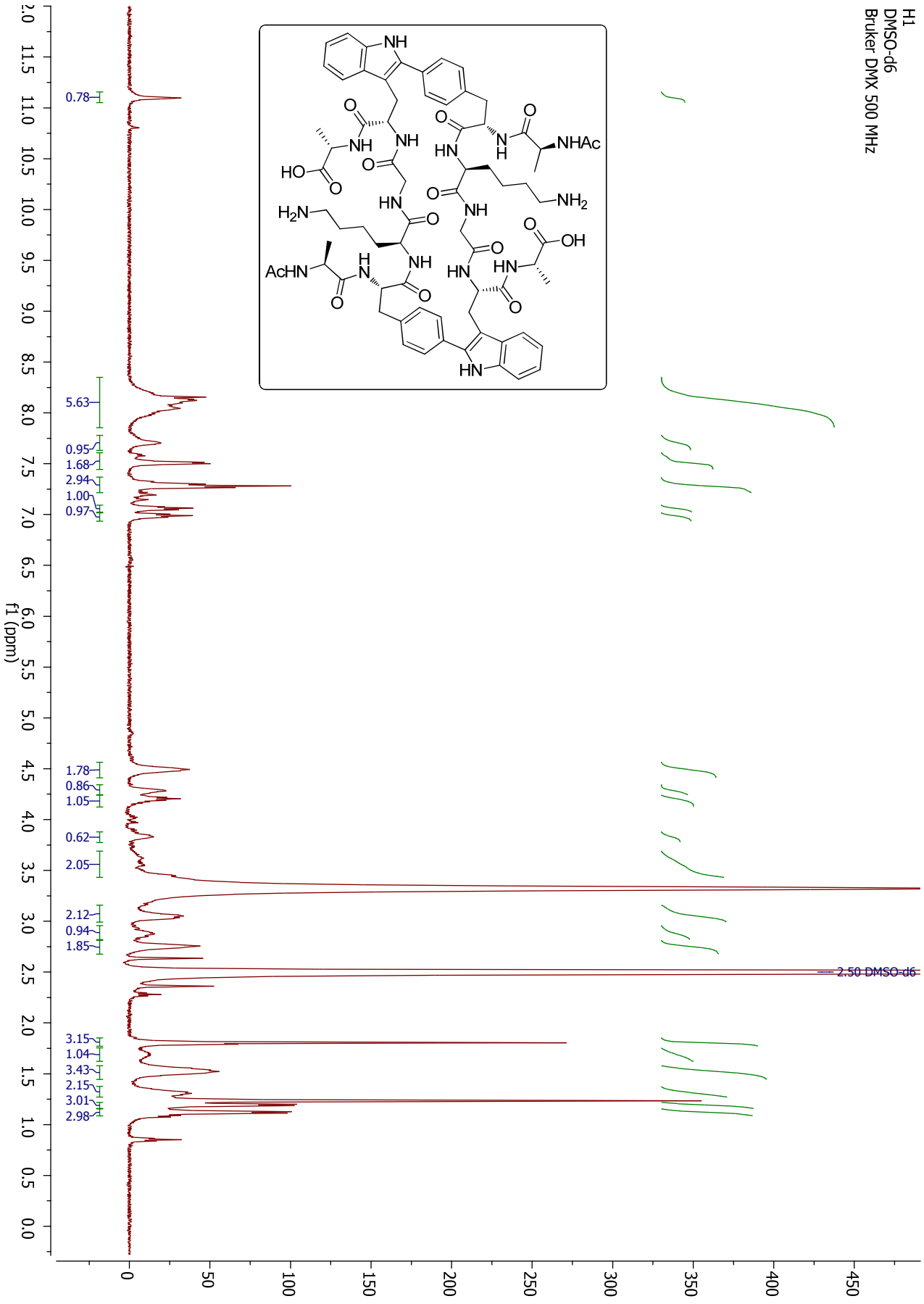


(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Phe-Ala-Trp-Lys-OH) (5b) ^{1}H NMR T=298-358 K

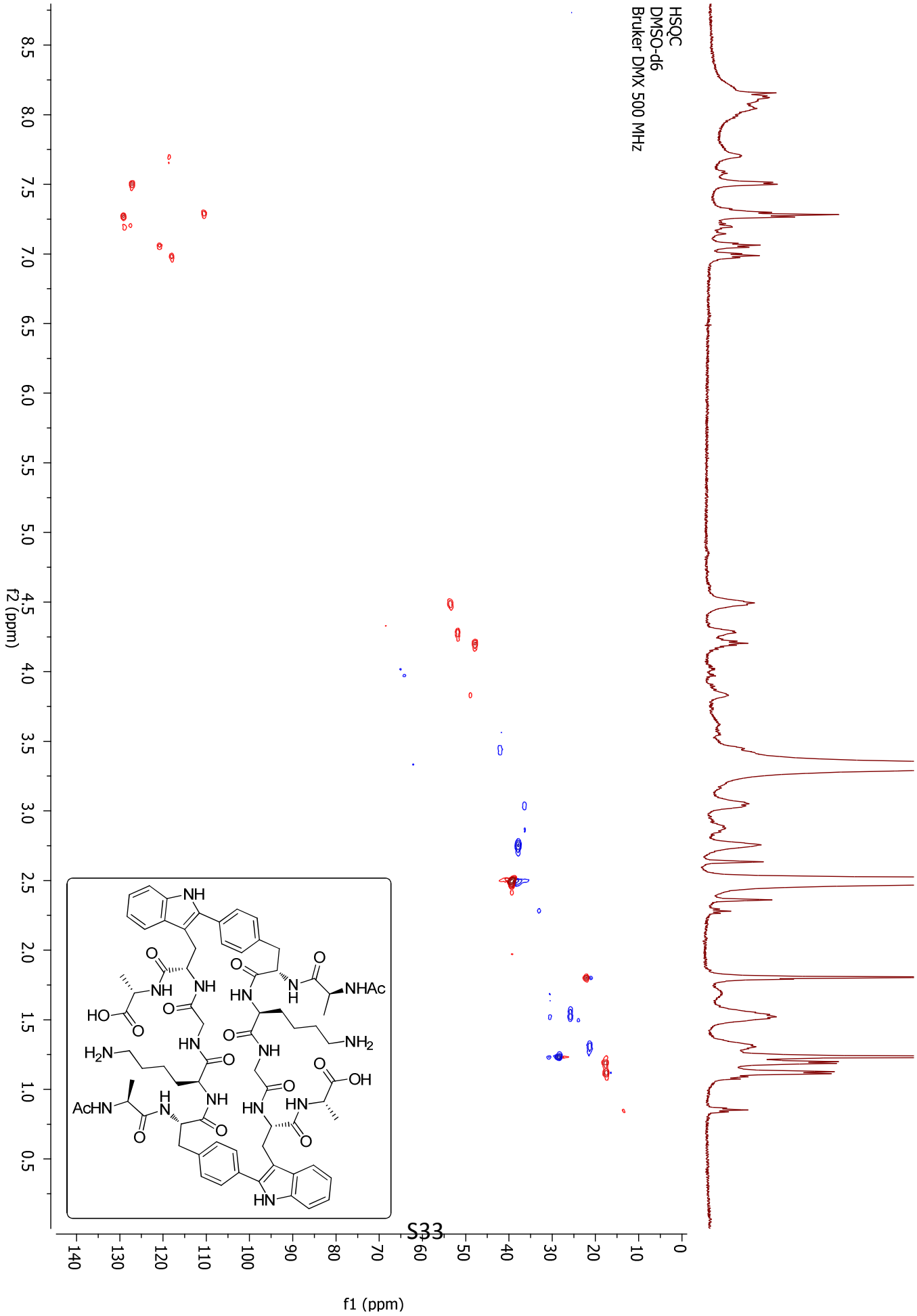




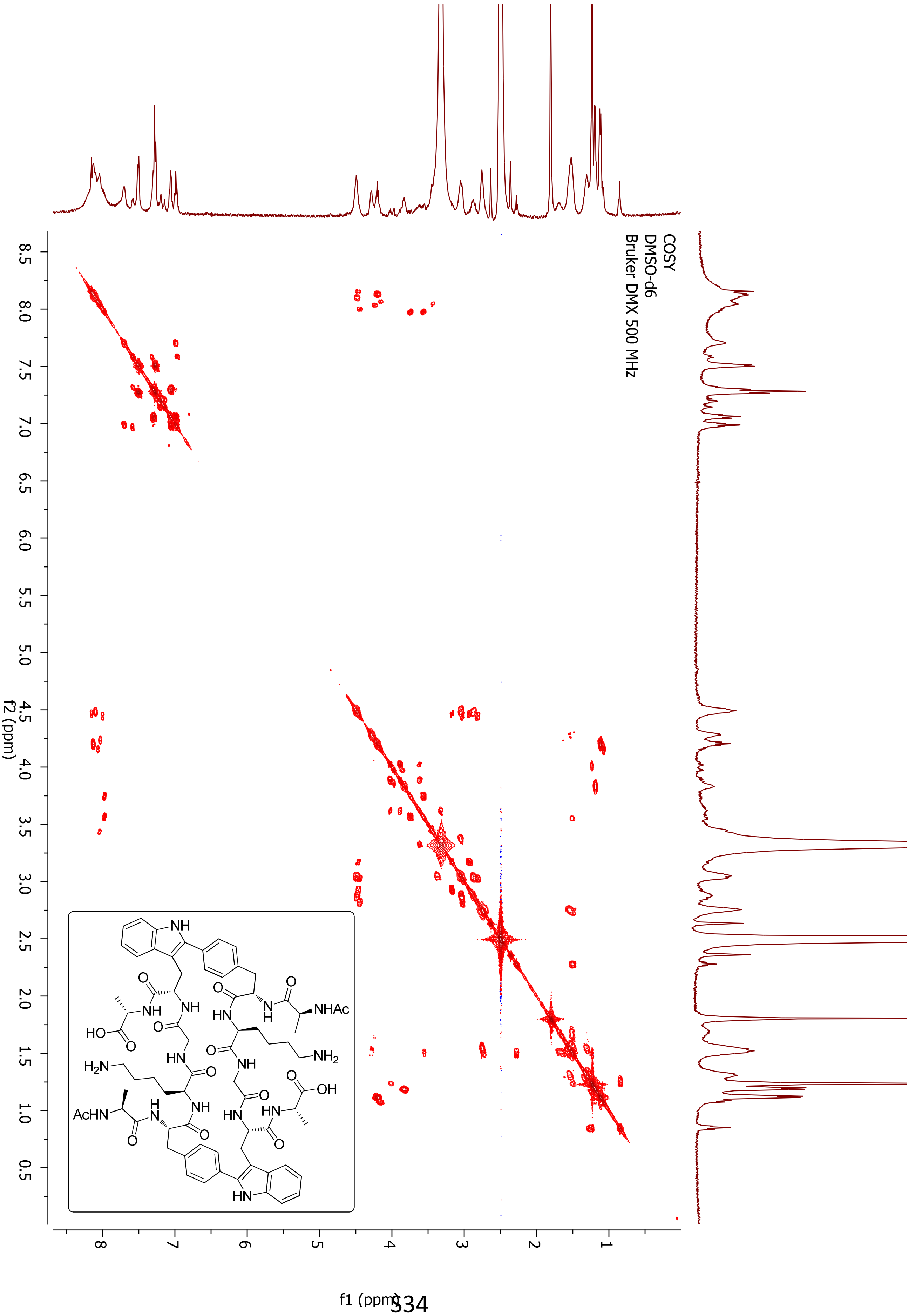
Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-Phe-Lys-Gly-Trp-Ala-OH) (5c) ^{1}H NMR T=338 K



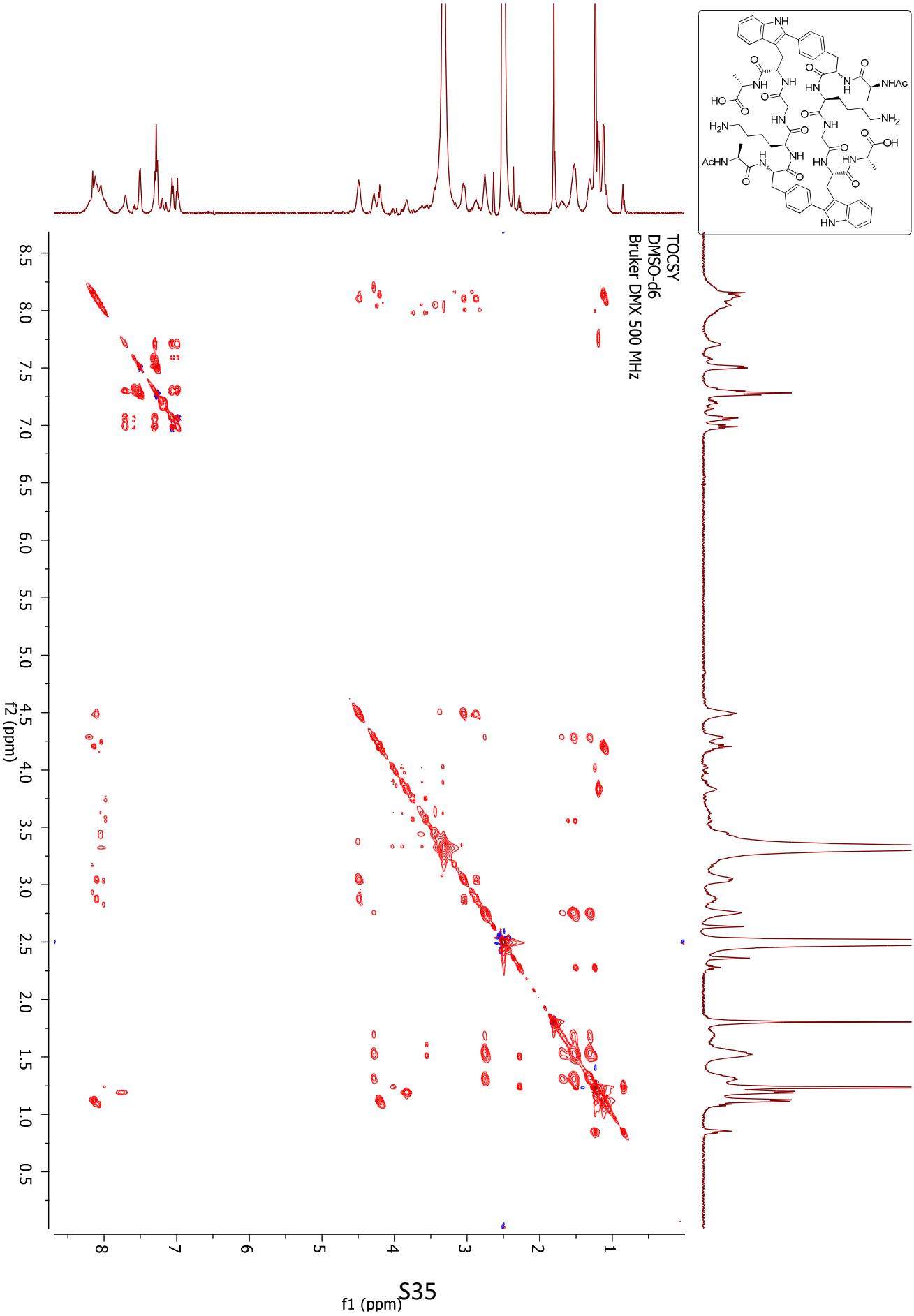
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Lys-Gly-Trp-Ala-OH) (5c) ^{1}H-^{13}C HSQC NMR T=338 K

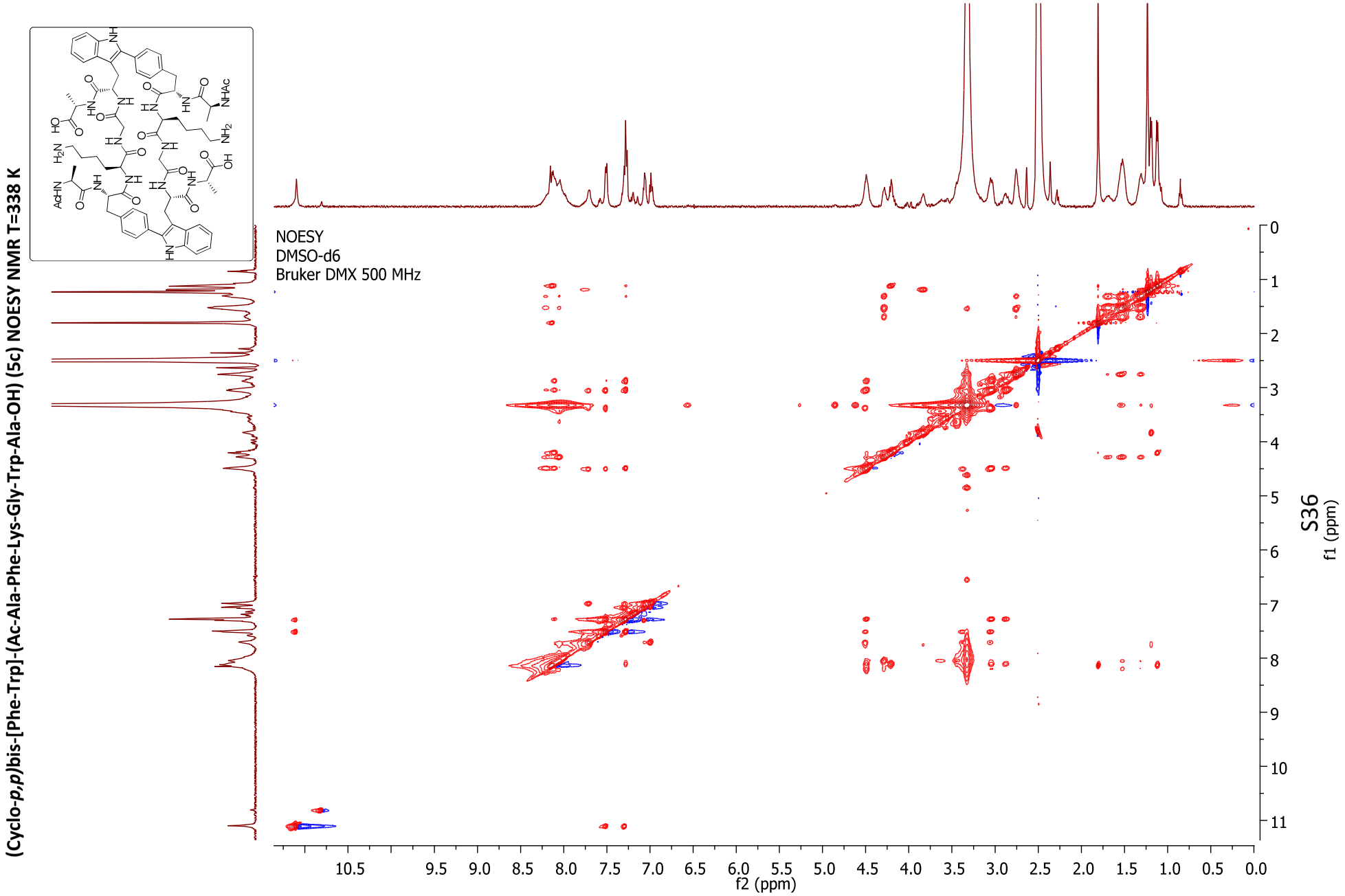


(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Lys-Gly-Trp-Ala-OH) (5c) COSY NMR T=338 K

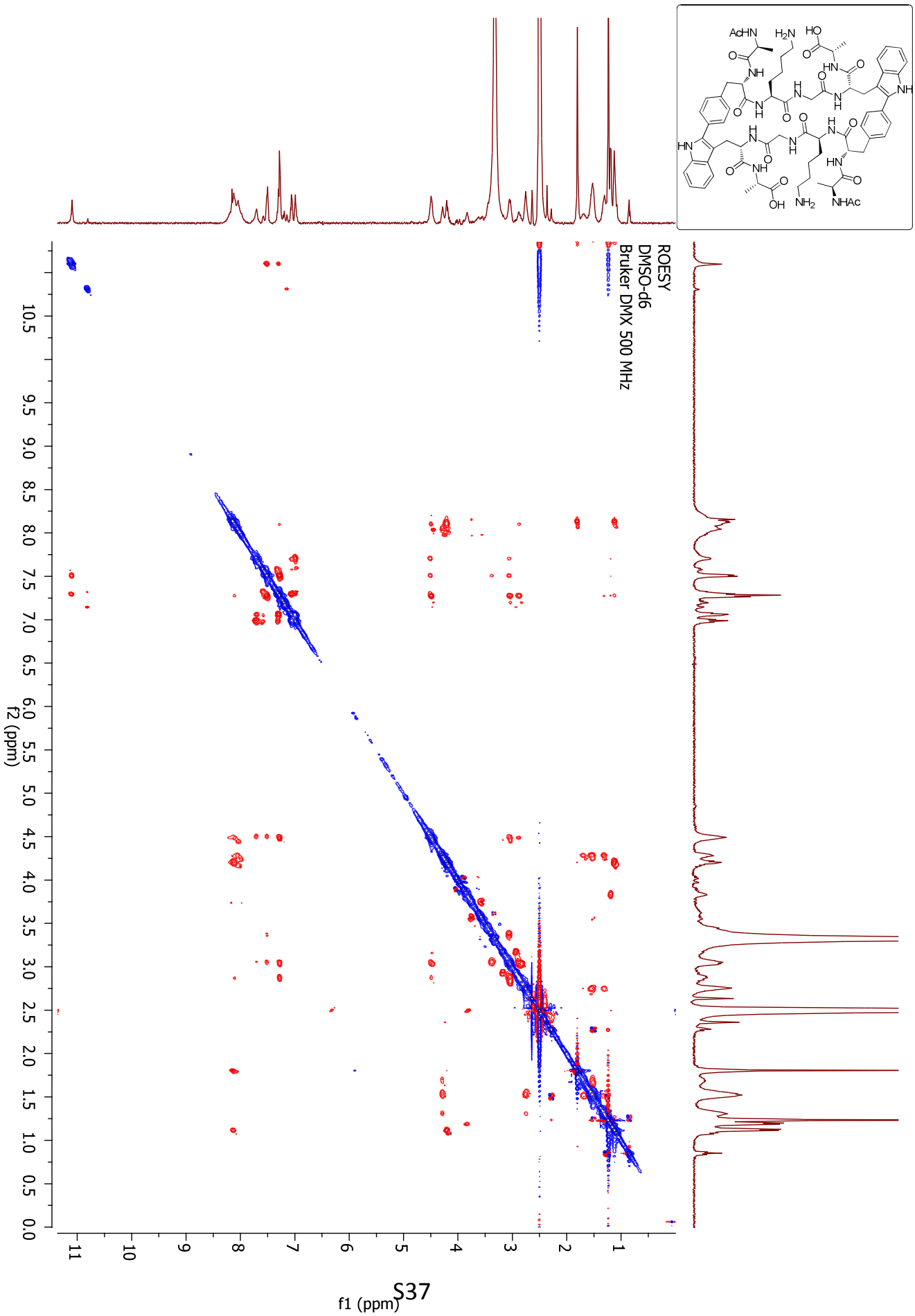


(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Lys-Gly-Trp-Ala-OH) (5c) TOCSY NMR T=338 K

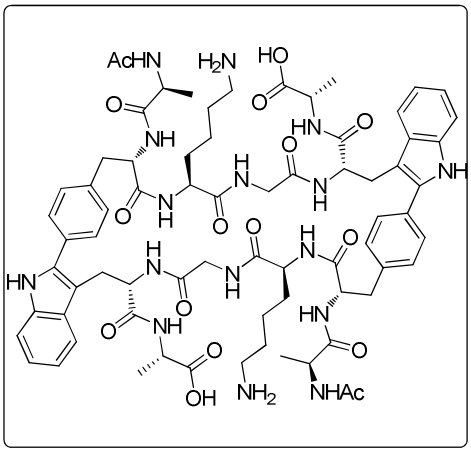


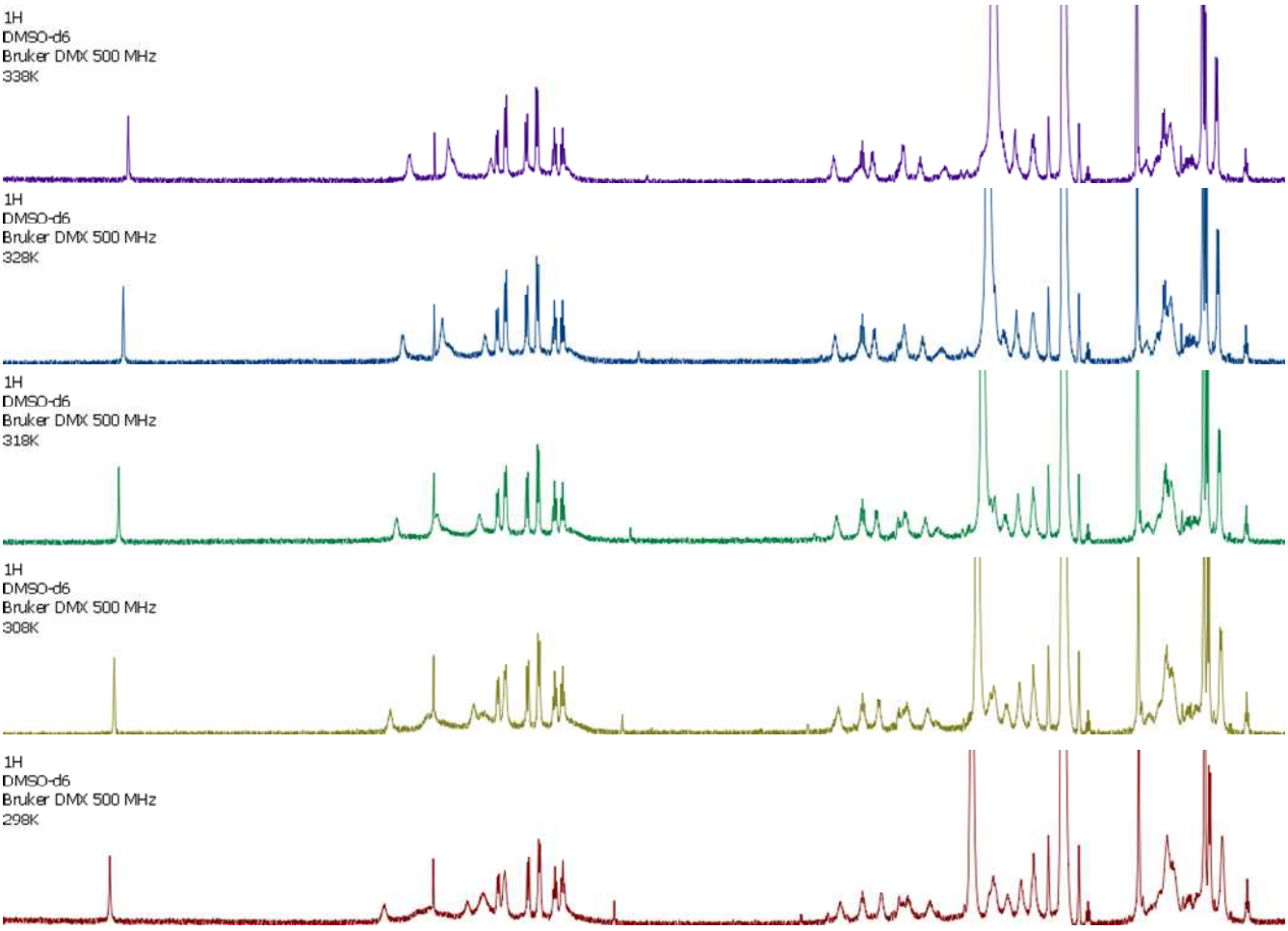

(Cyclo-p,p)bis-[Phe-Trp]-(Ac-Ala-Phe-Lys-Gly-Trp-Ala-OH) (5c) NOESY NMR T=338 K
NOESY
DMSO-d6
Bruker DMX 500 MHz
S36
f1 (ppm)
f2 (ppm)
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
6
7
8
9
10
11

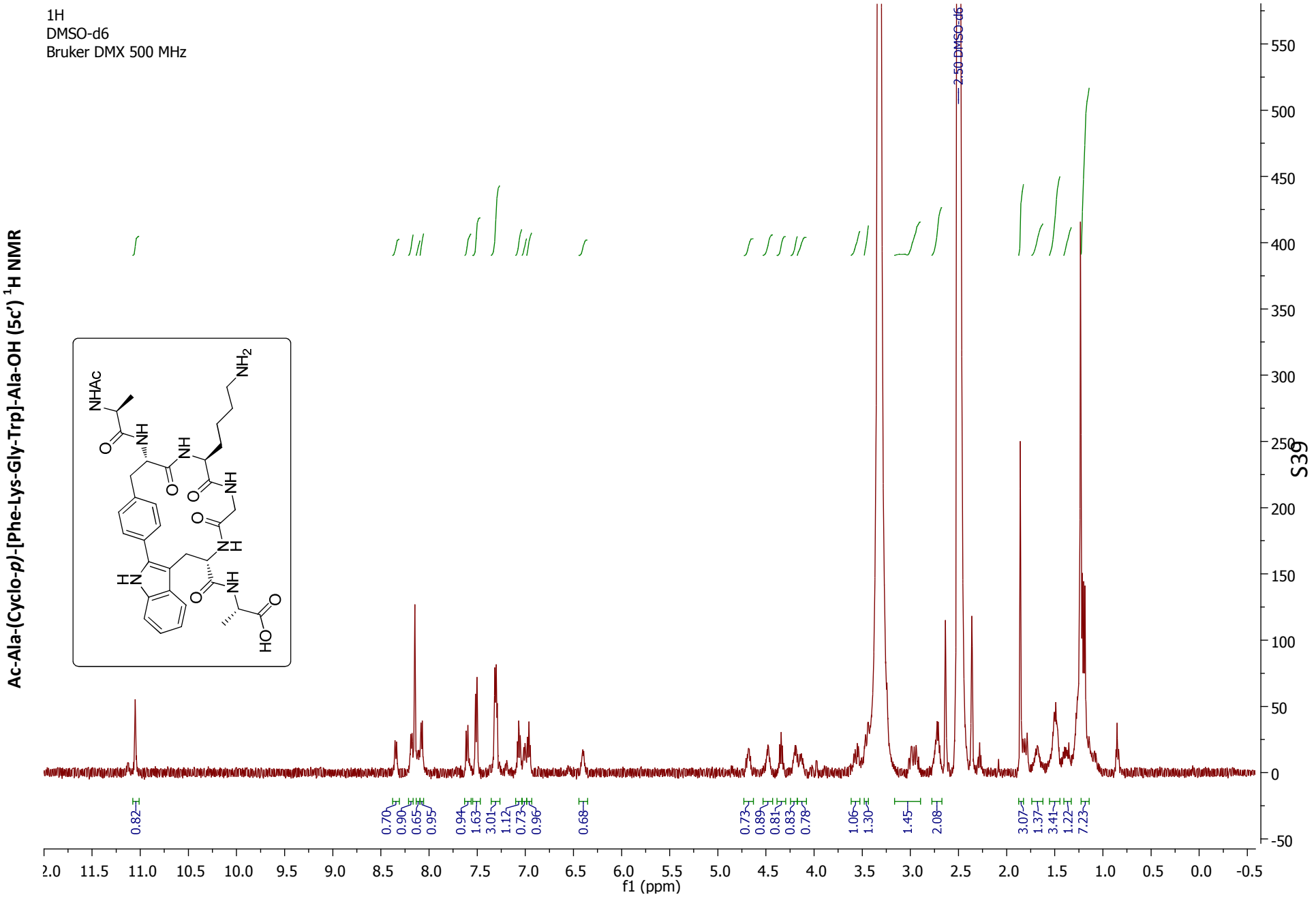
(Cyclo-*p,p*)bis-[Phe-Trp]-(Ac-Ala-Phe-Lys-Gly-Trp-Ala-OH) (5c) ROESY NMR T=338 K



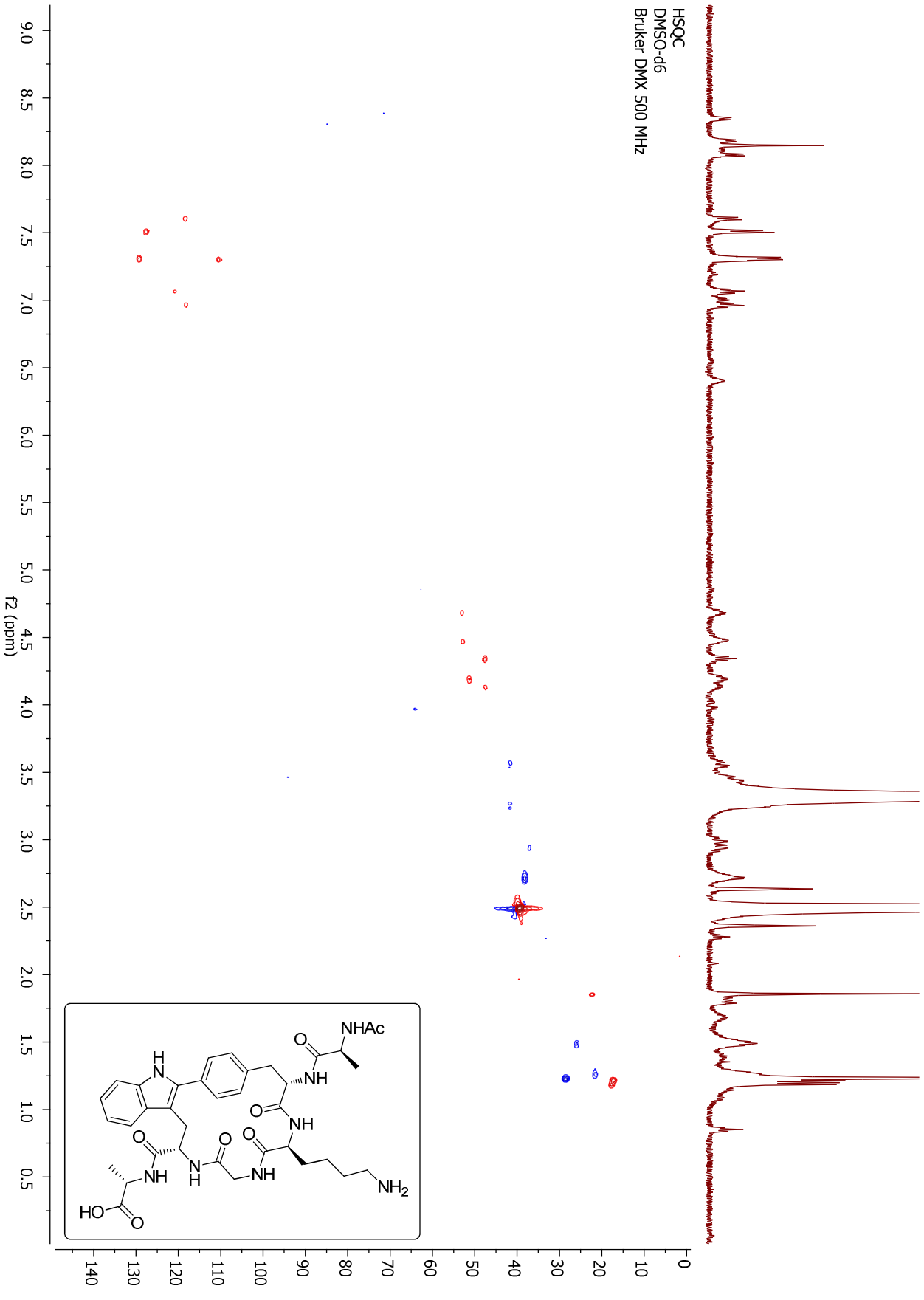
(Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-Phe-Lys-Gly-Trp-Ala-OH) (5c) ^{1}H NMR T=298-338 K



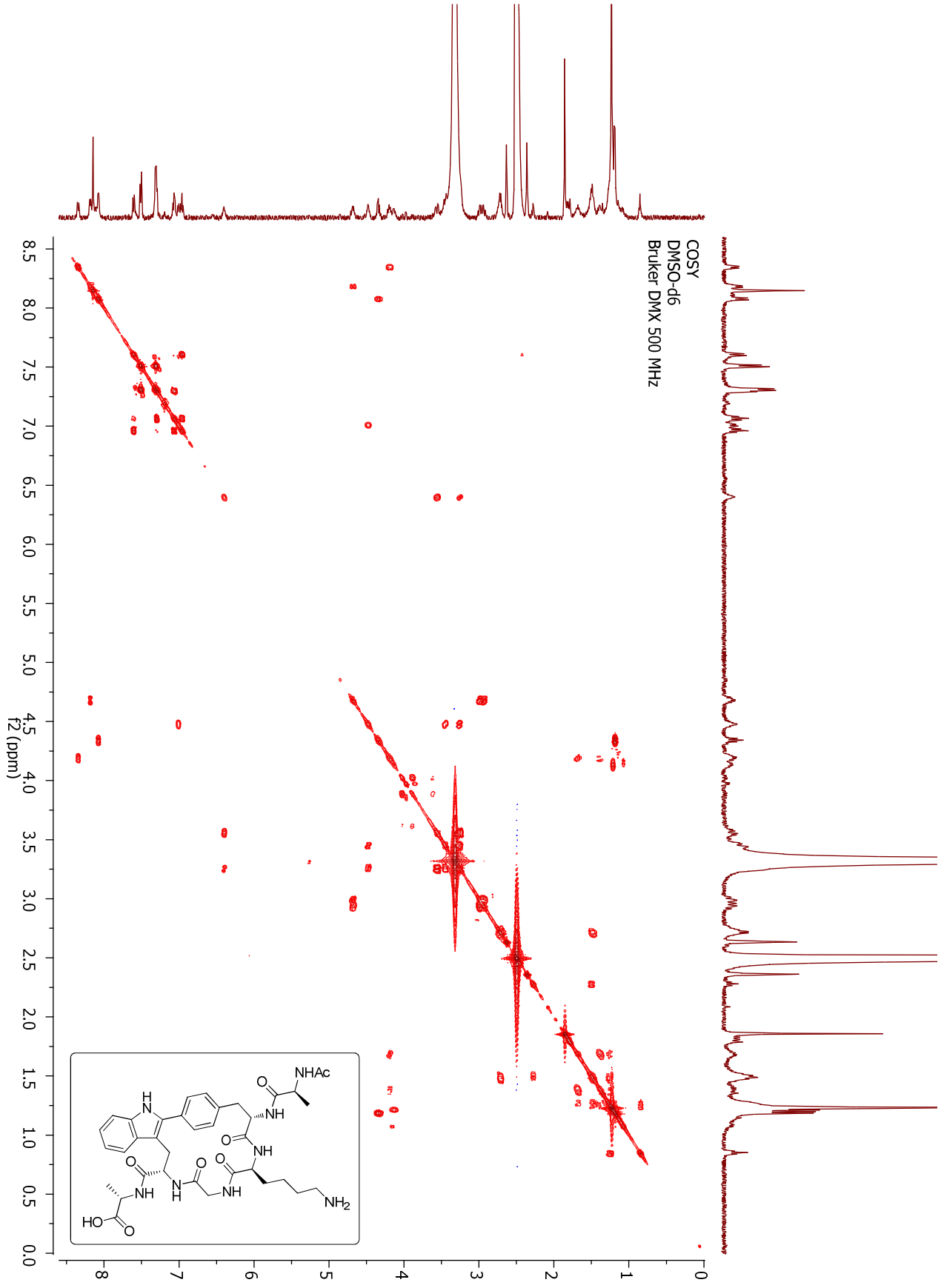



1H
DMSO-d6
Bruker DMX 500 MHz
Ac-Ala-(Cyclo-p)-[Phe-Lys-Gly-Trp]-Ala-OH (5c′) 1H NMR
2.50 DMSO-d6
0.82
0.70
0.90
0.65
0.95
0.94
1.63
3.01
1.12
0.73
0.96
0.68
0.73
0.89
0.81
0.83
0.78
1.06
1.30
1.45
2.08
3.07
1.37
3.41
1.22
7.23
f1 (ppm)
S39

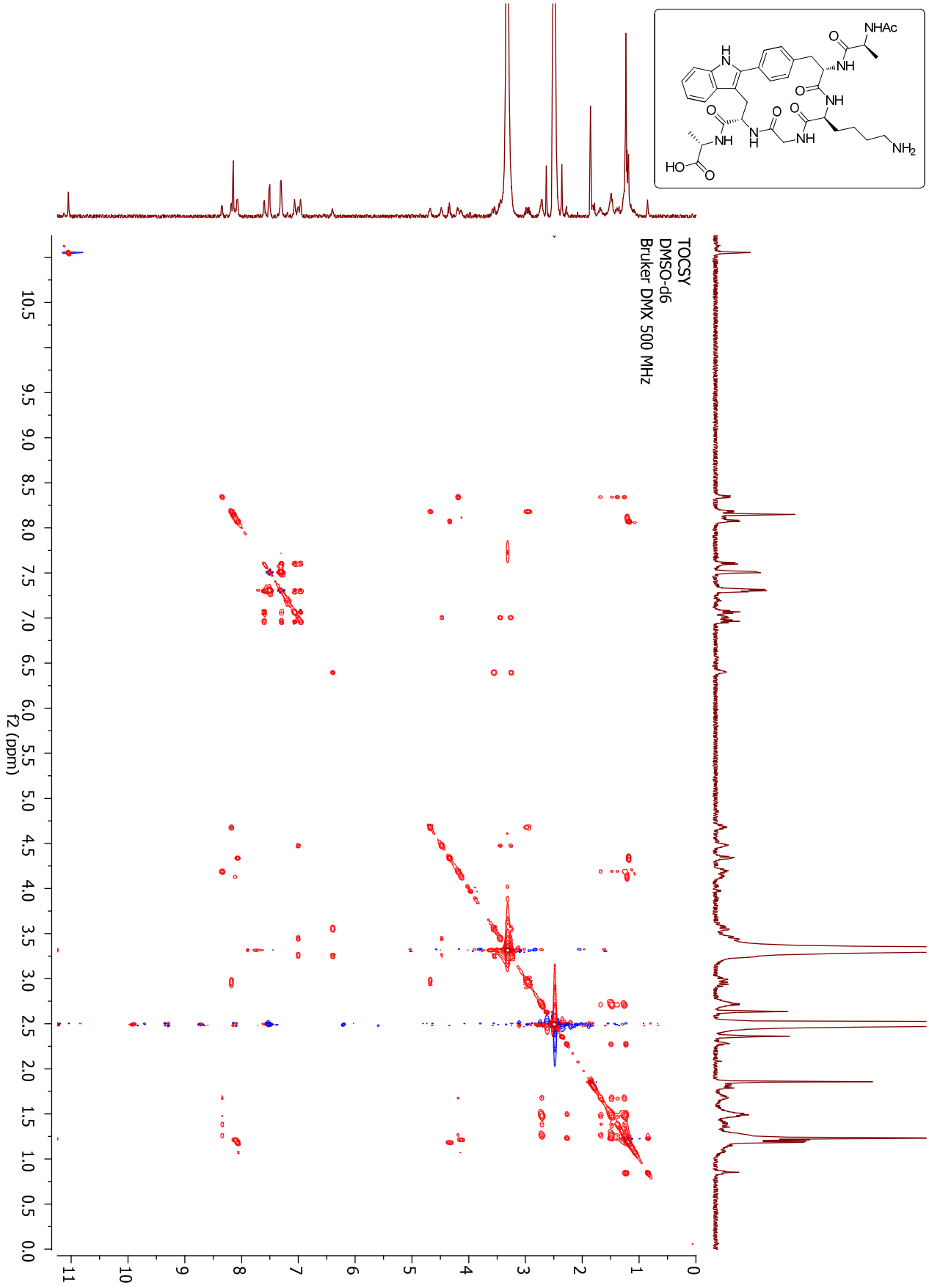
Ac-Ala-(Cyclo-*p*)-[Phe-Lys-Gly-Trp]-Ala-OH (5*c'*) ^{1}H-^{13}C HSQC NMR



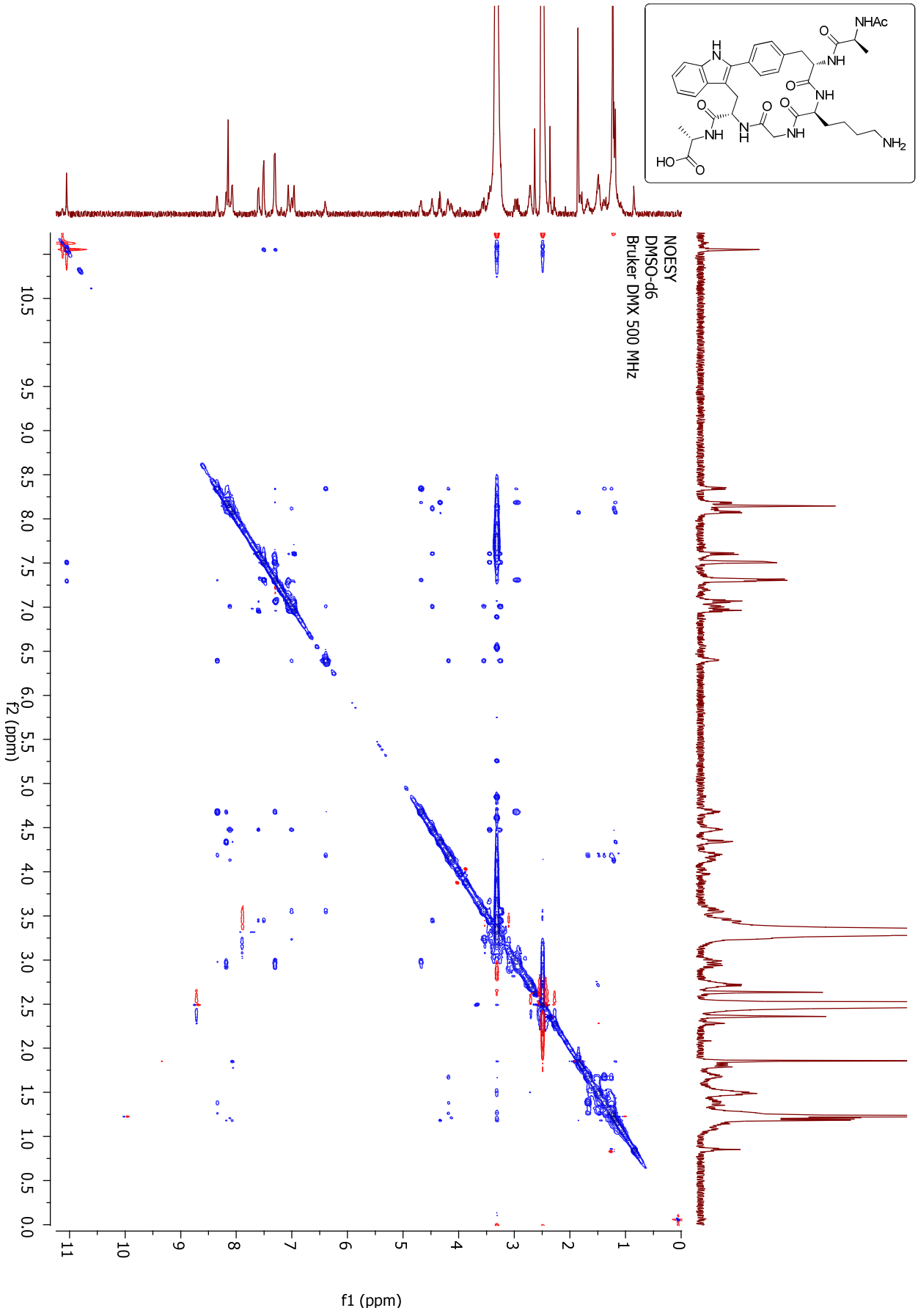
Ac-Ala-(Cyclo-*p*)-[Phe-Lys-Gly-Trp]-Ala-OH (5c') COSY NMR



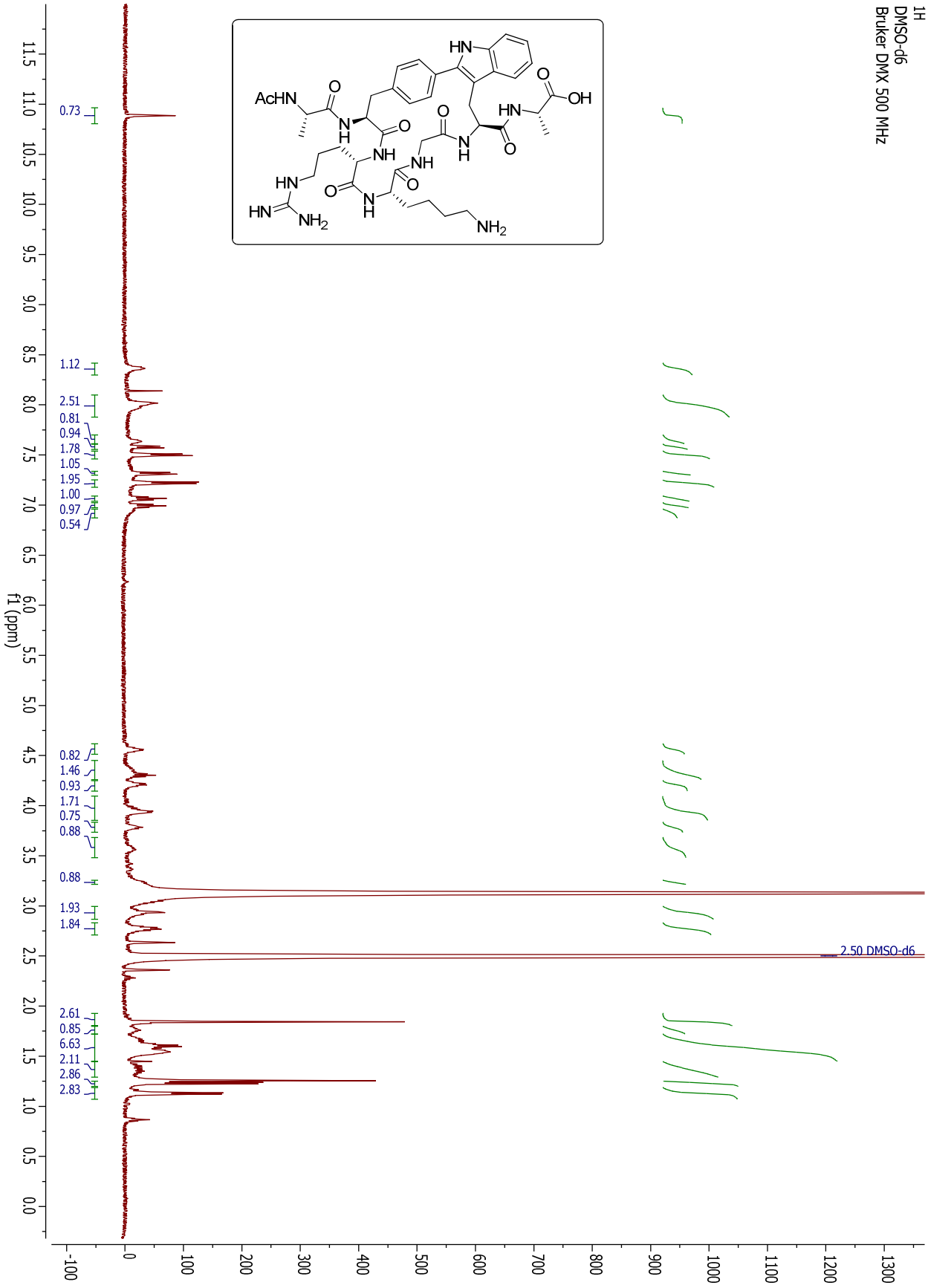
Ac-Ala-(Cyclo-*p*)-[Phe-Lys-Gly-Trp]-Ala-OH (5c') TOCSY NMR



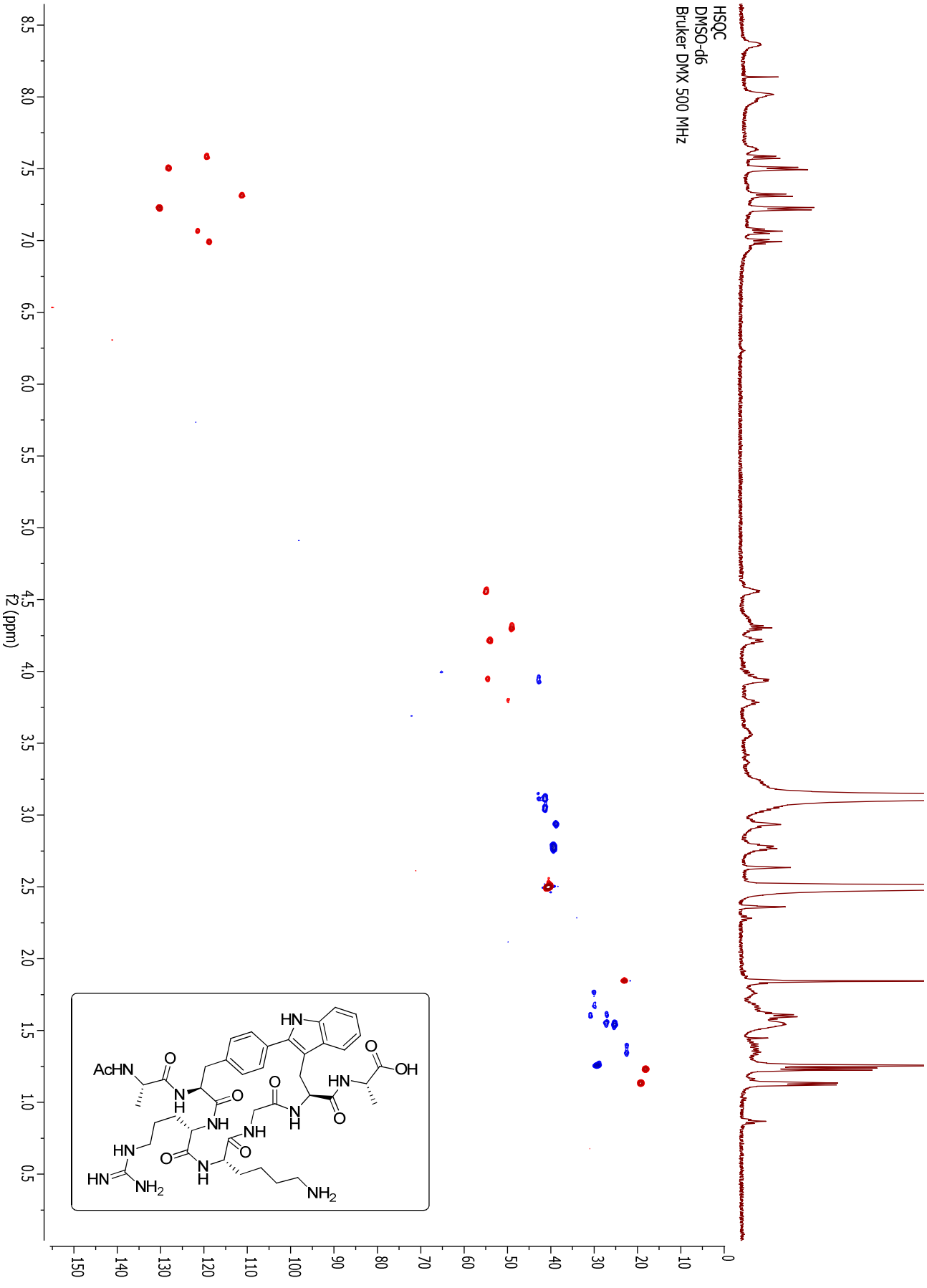
Ac-Ala-(Cyclo-*p*)-[Phe-Lys-Gly-Trp]-Ala-OH (5c') NOESY NMR



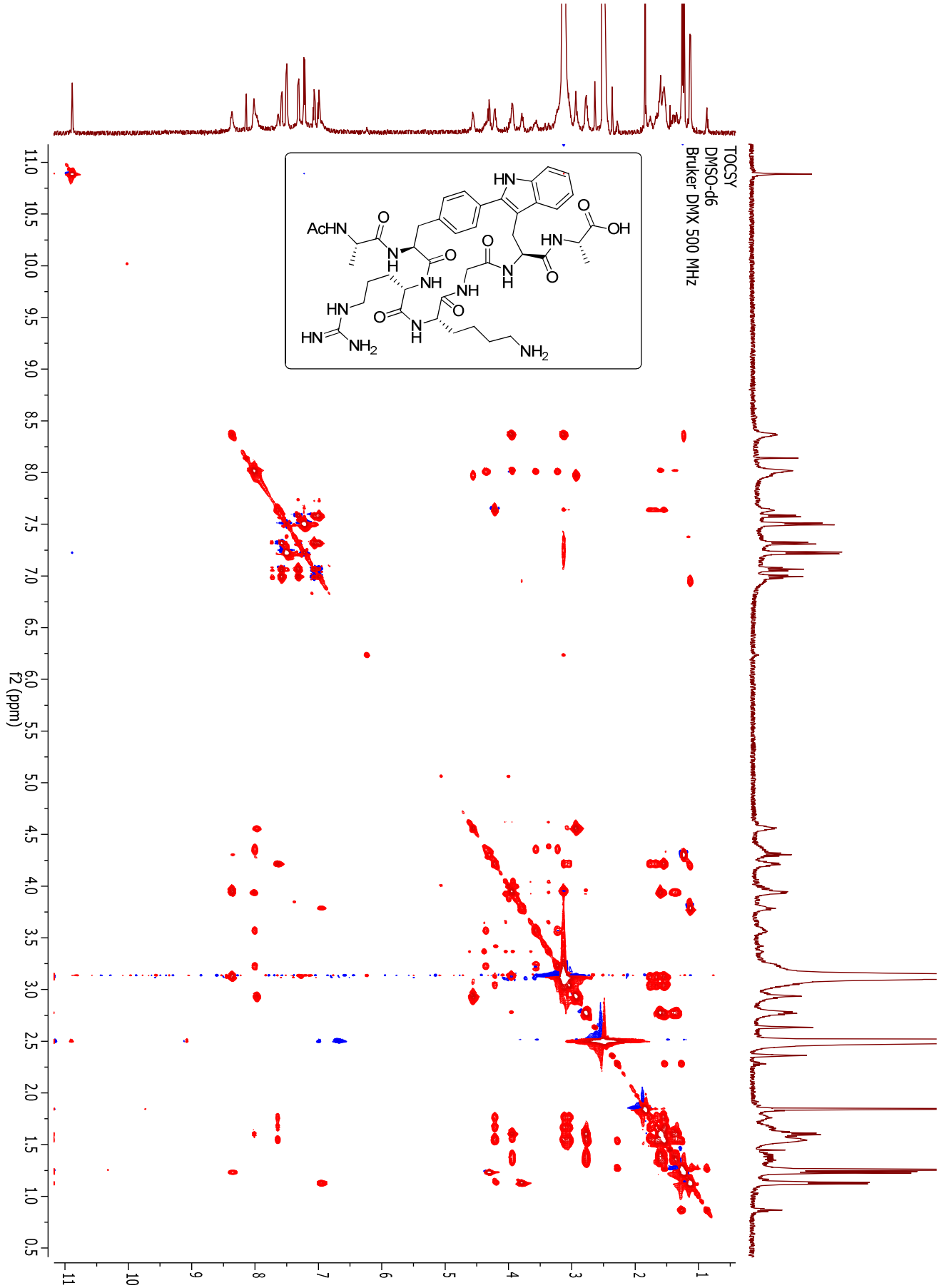
Ac-Ala-(Cyclo-*p*)-[Phe-Arg-Lys-Gly-Trp]-Ala-OH (5d') ^{1}H NMR



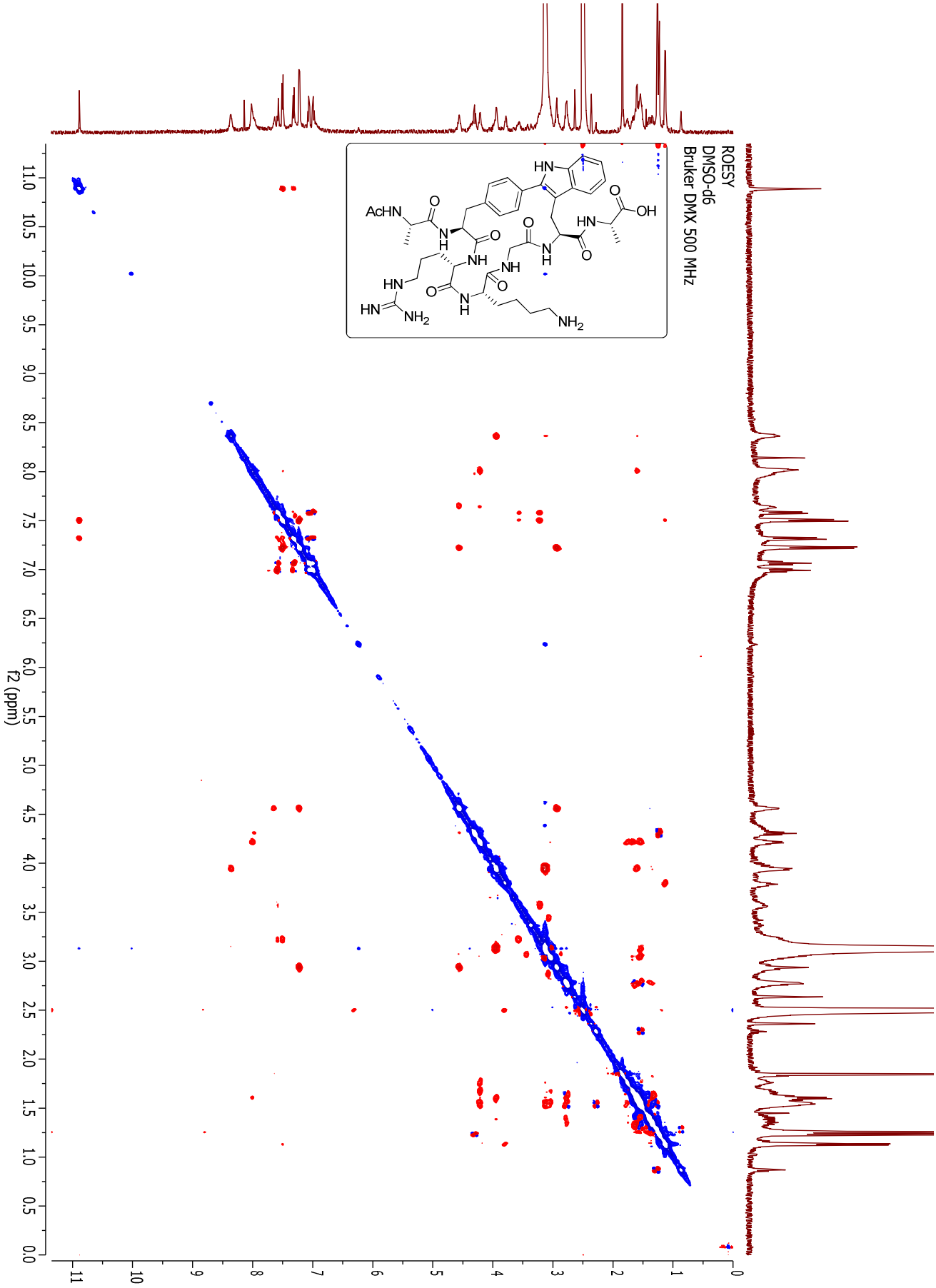
Ac-Ala-(Cyclo-*p*)-[Phe-Arg-Lys-Gly-Trp]-Ala-OH (5d') ^{1}H-^{13}C HSQC NMR



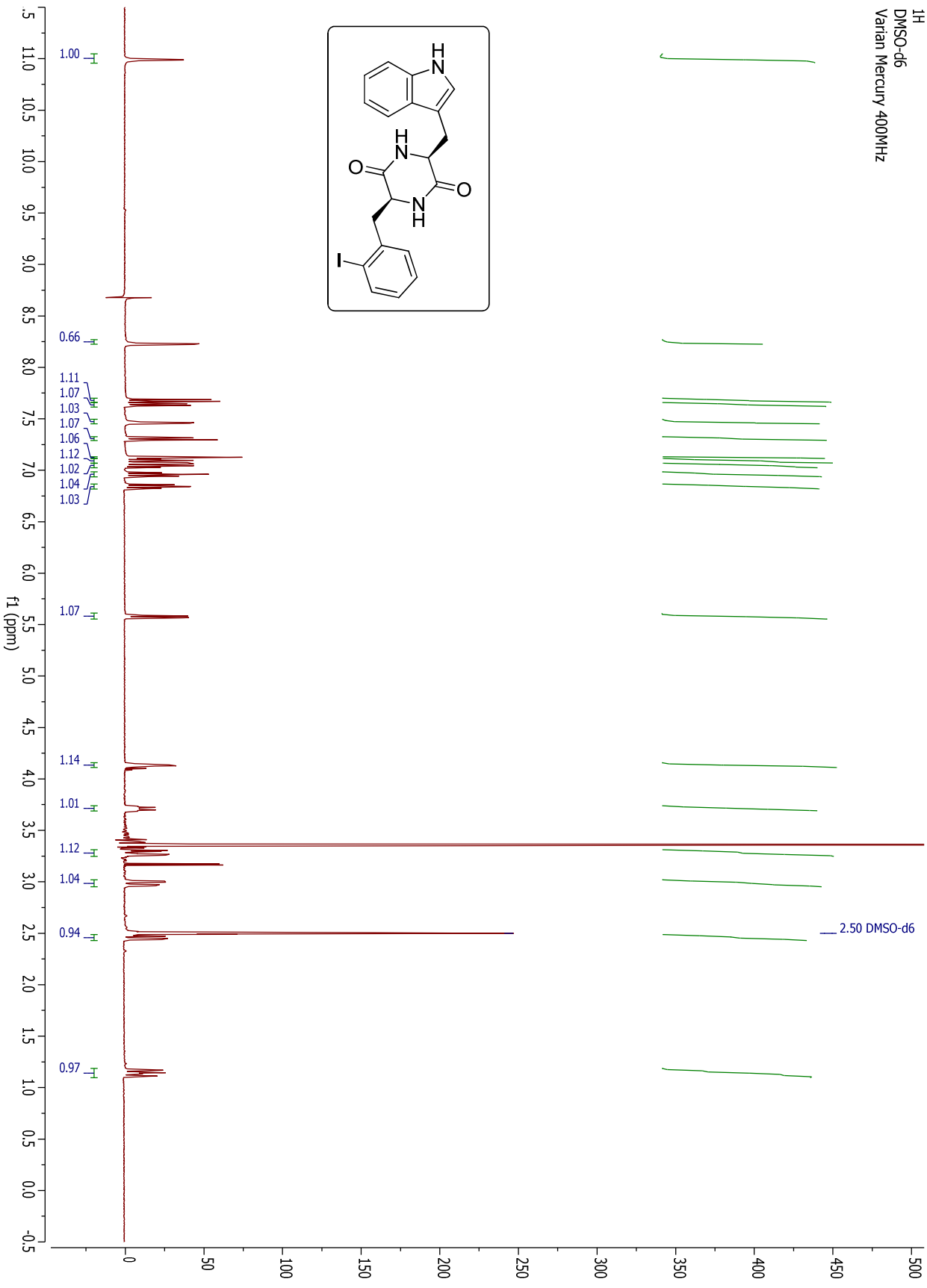
Ac-Ala-(Cyclo-*p*)-[Phe-Arg-Lys-Gly-Trp]-Ala-OH (5d') TOCSY NMR



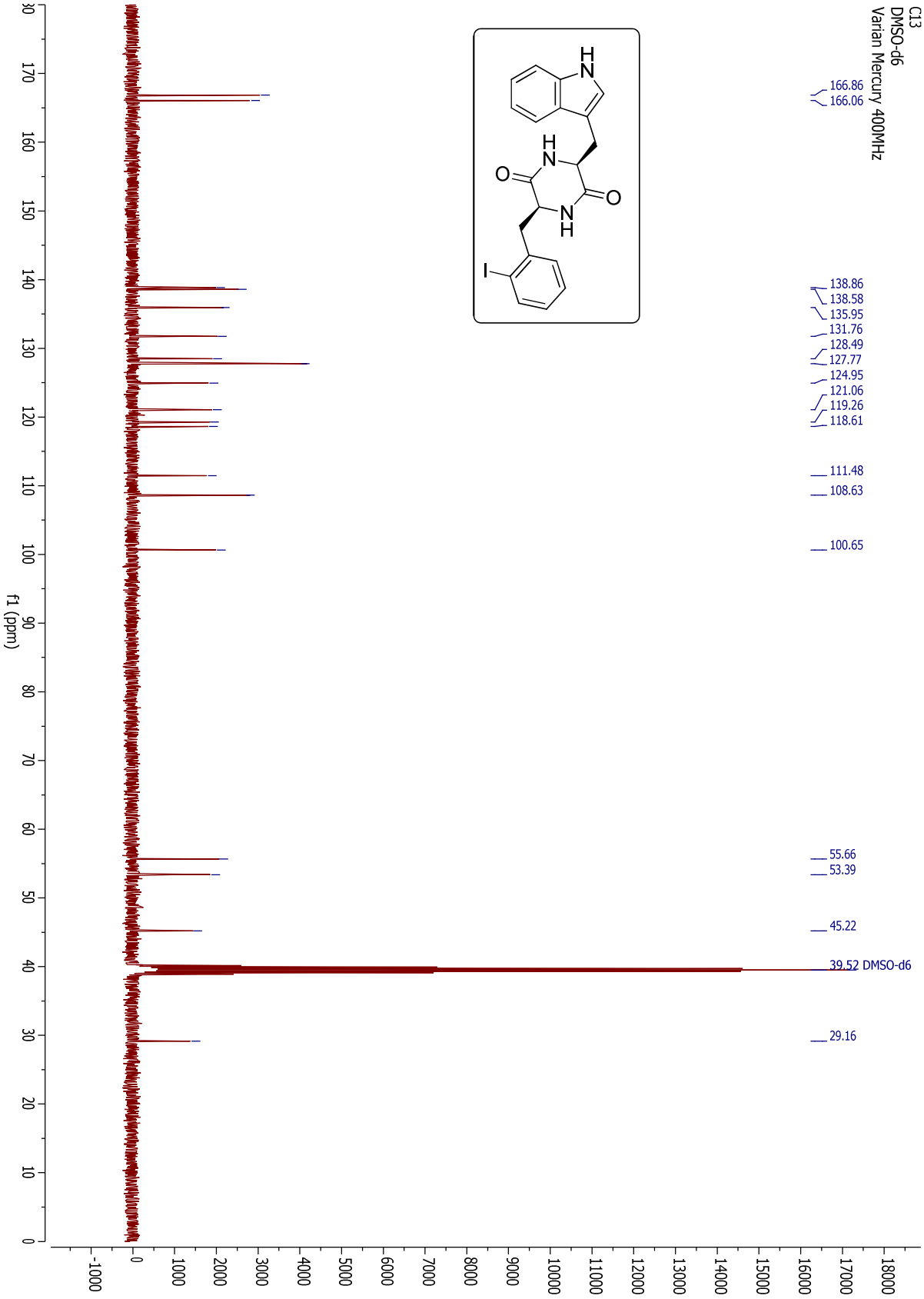
Ac-Ala-(Cyclo-*p*)-[Phe-Arg-Lys-Gly-Trp]-Ala-OH (5d') ROESY NMR



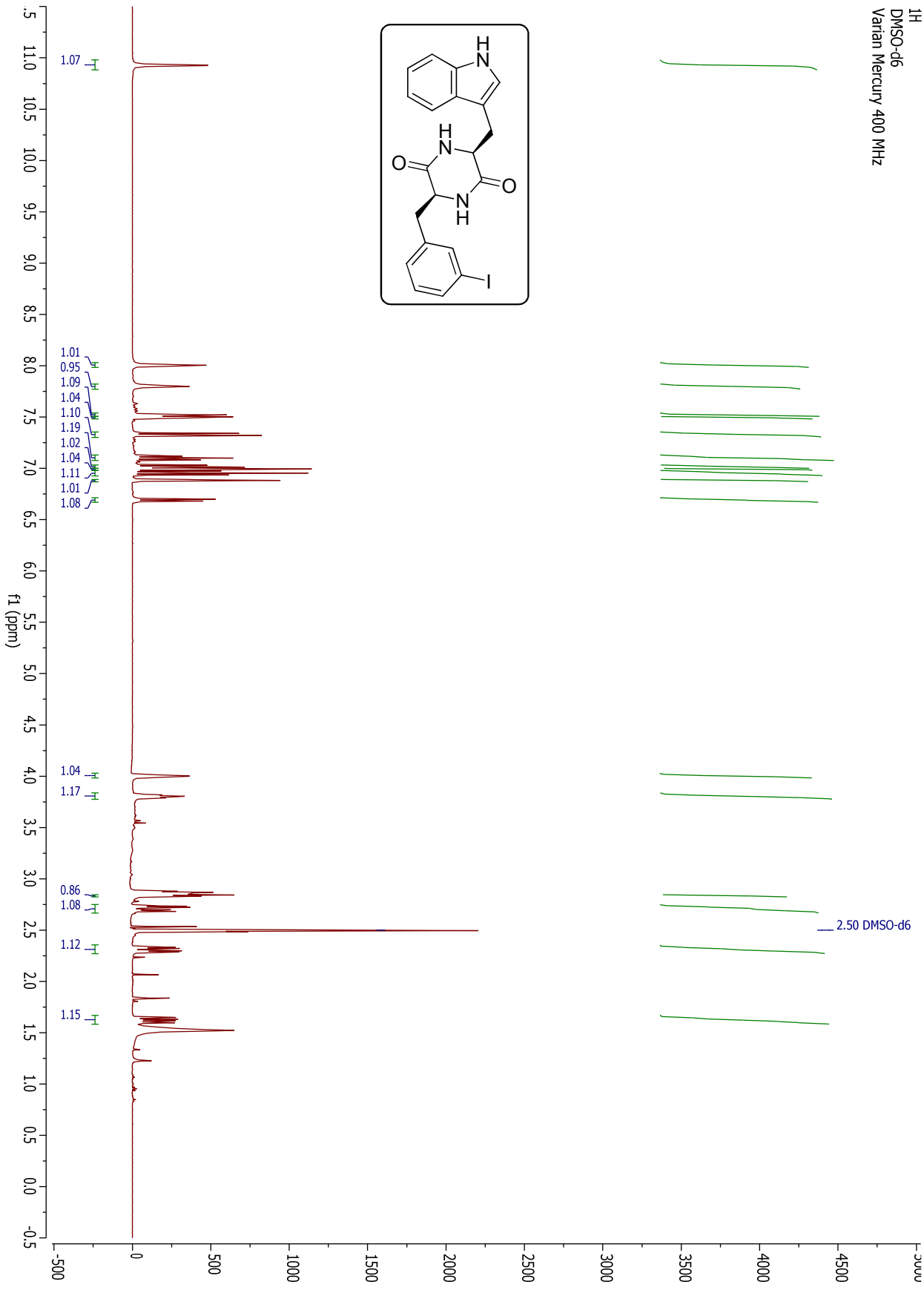
Cyclo(2-I-Phe-Trp) (6a) ^{1}H NMR



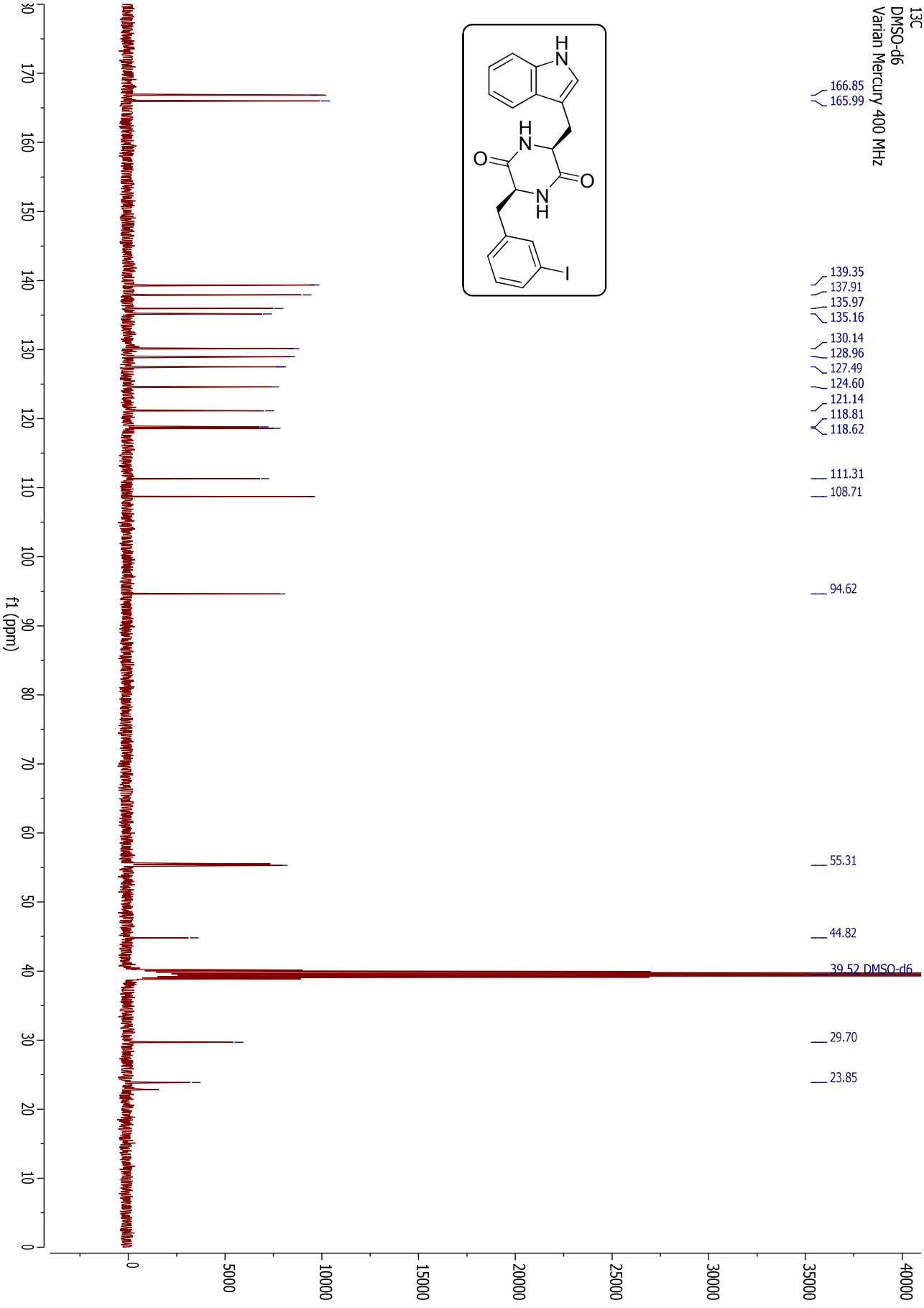
Cyclo(2-I-Phe-Trp) (6a) ^{13}C NMR



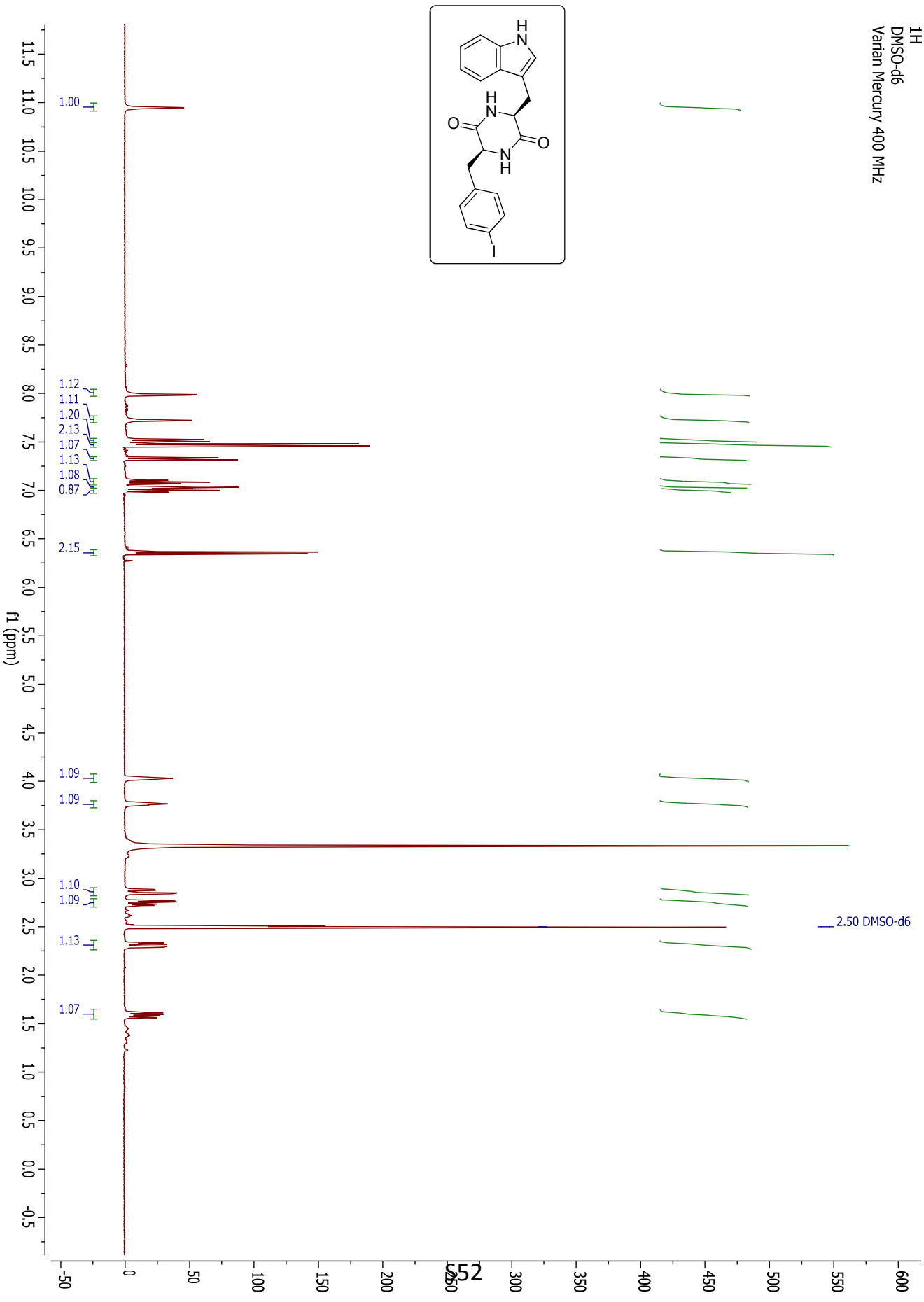
Cyclo(3-I-Phe-Trp) (6b) ^{1}H NMR



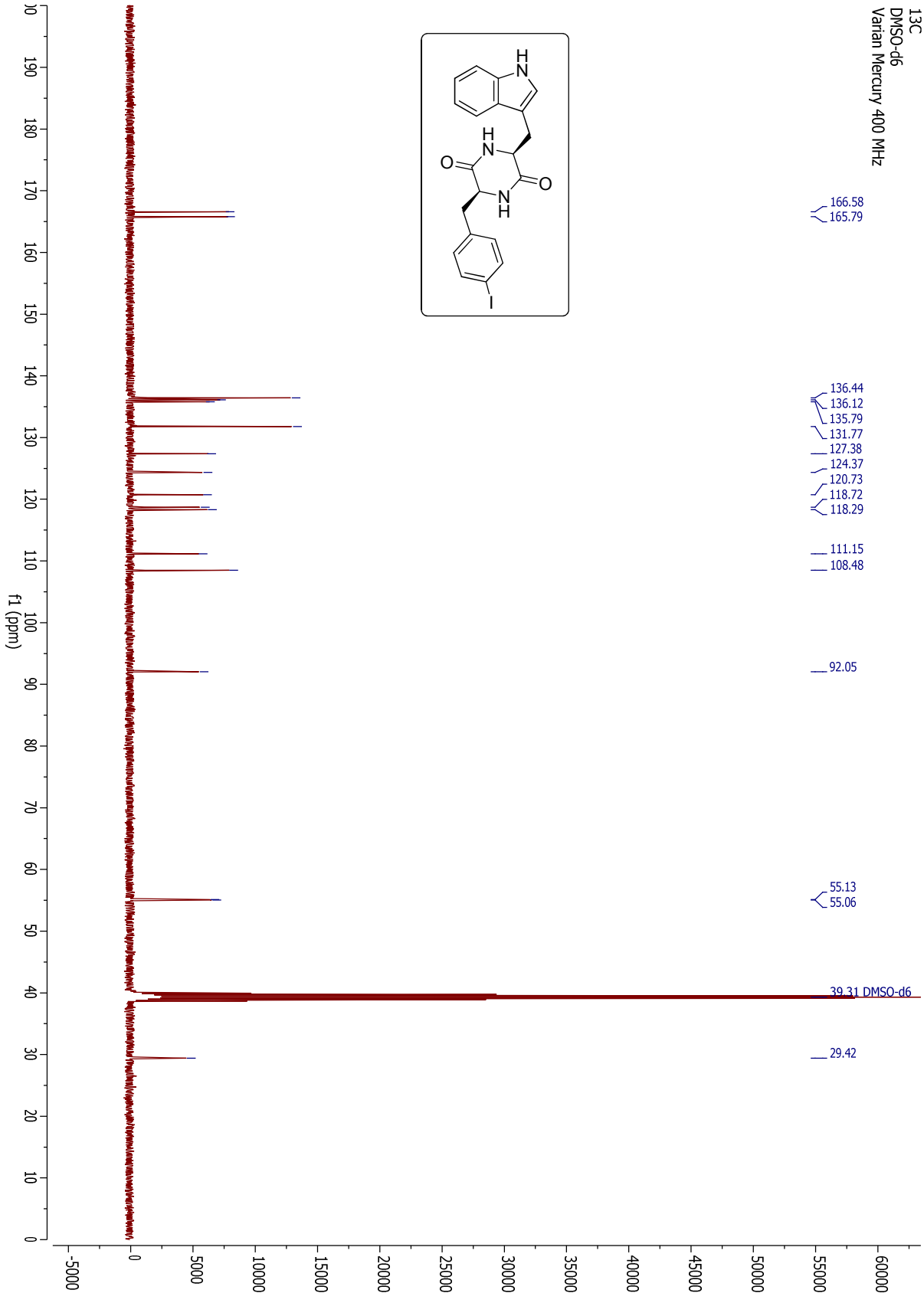
Cyclo(3-I-Phe-Trp) (6b) ^{13}C NMR



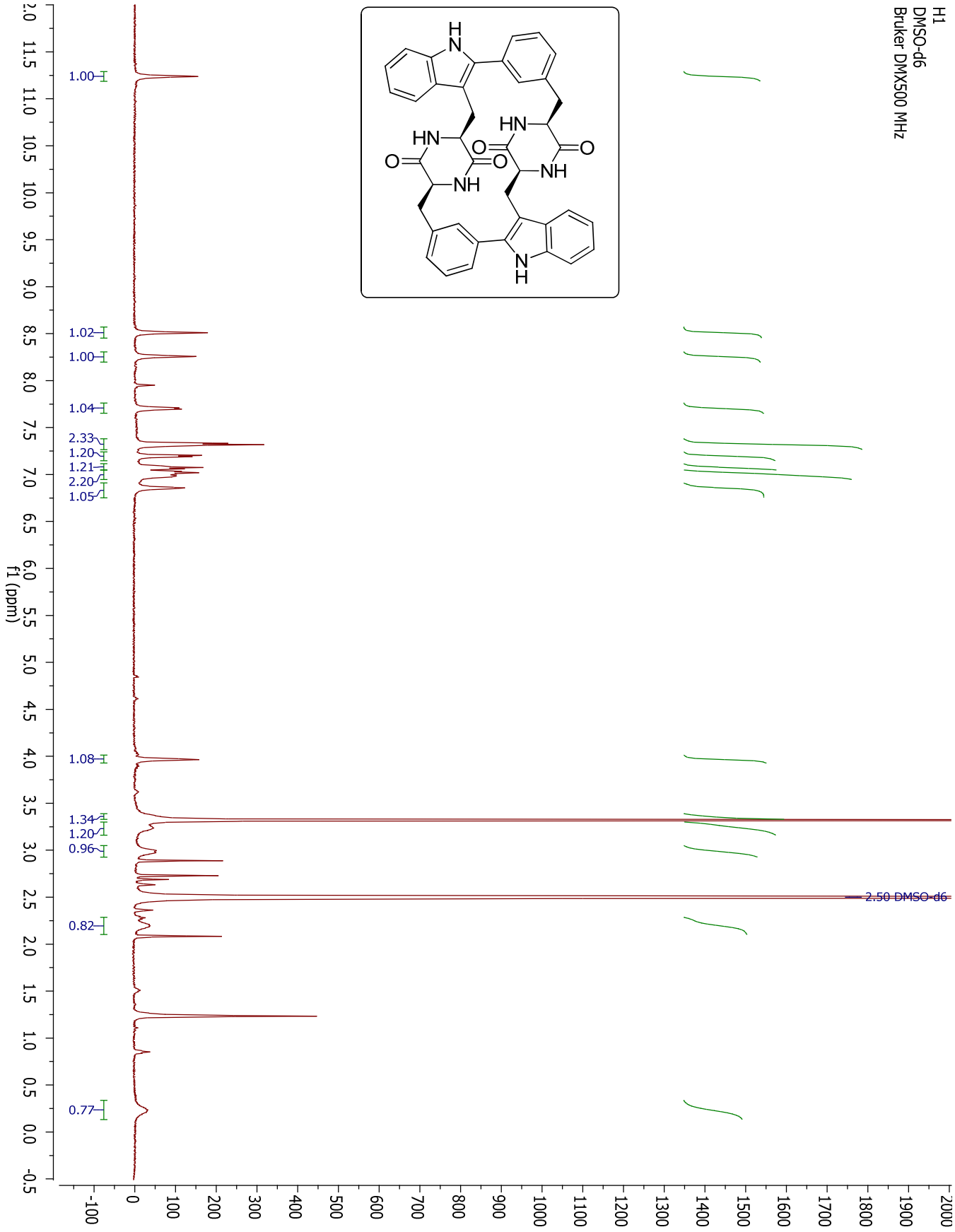
Cyclo(4-I-Phe-Trp) (6c) ^{1}H NMR



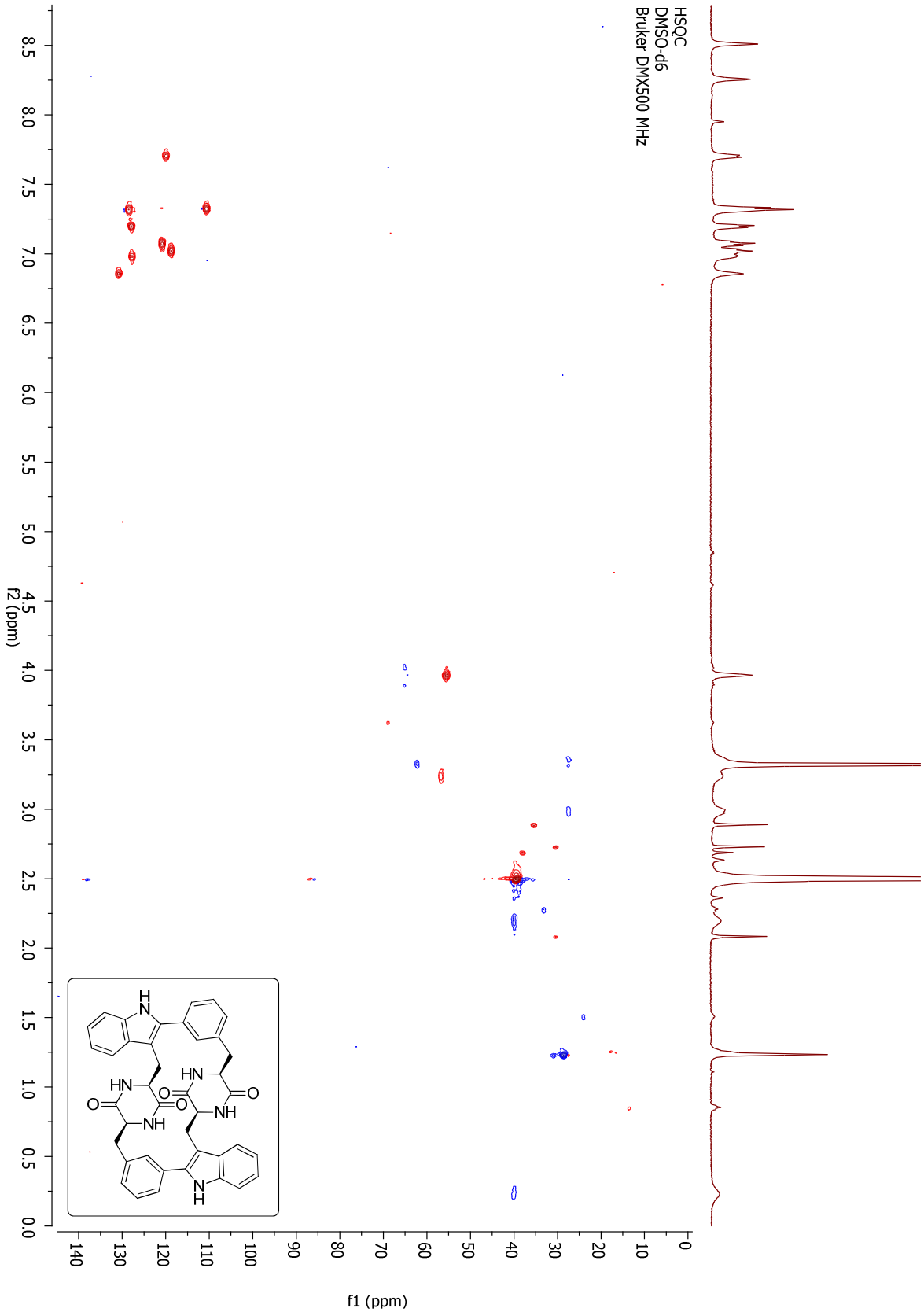
Cyclo(4-I-Phe-Trp) (6c) ^{13}C NMR



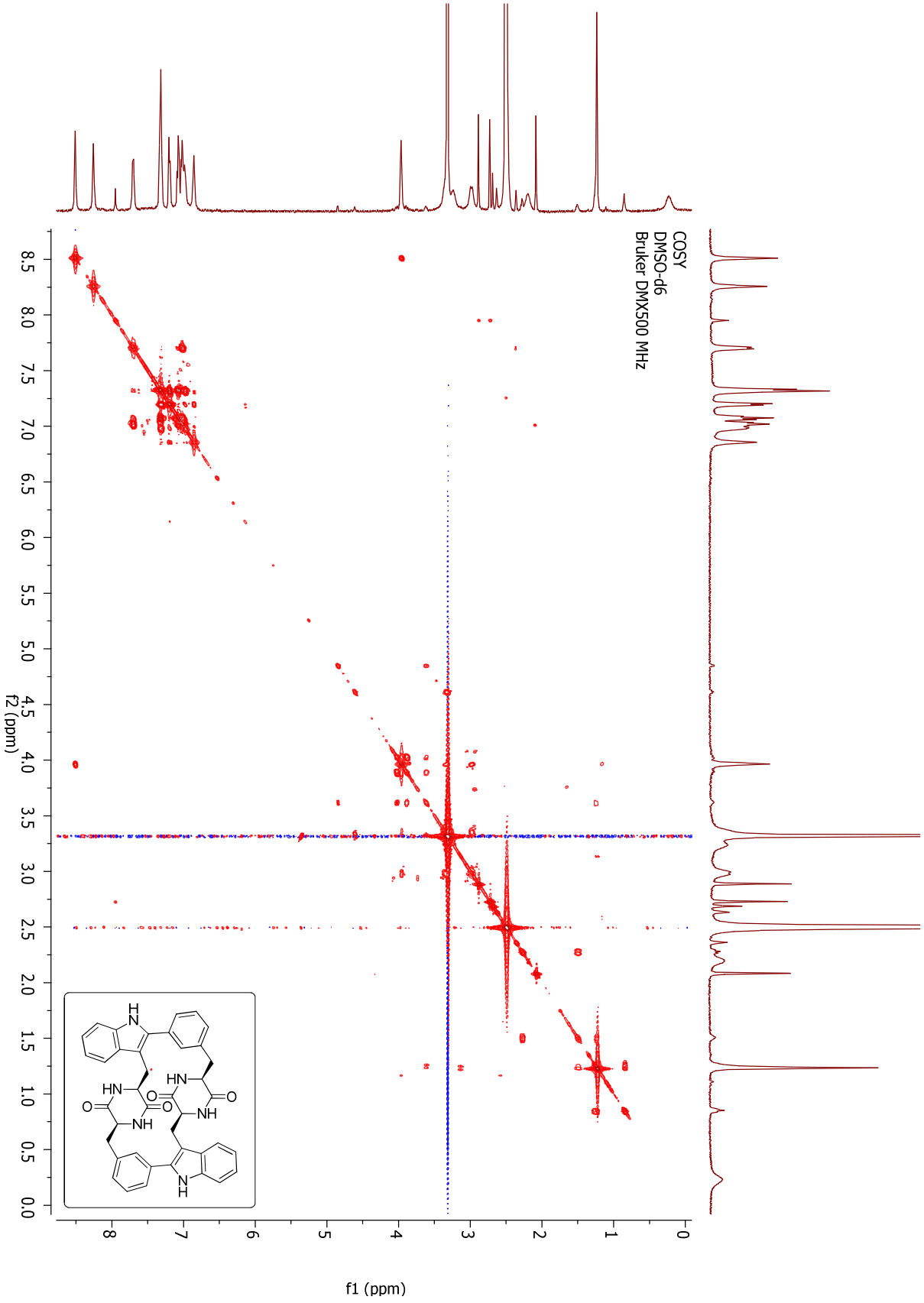
(Cyclo-*m,m*)bis-[Phe-Trp]-cyclo(Phe-Trp) (7b) ^{1}H NMR



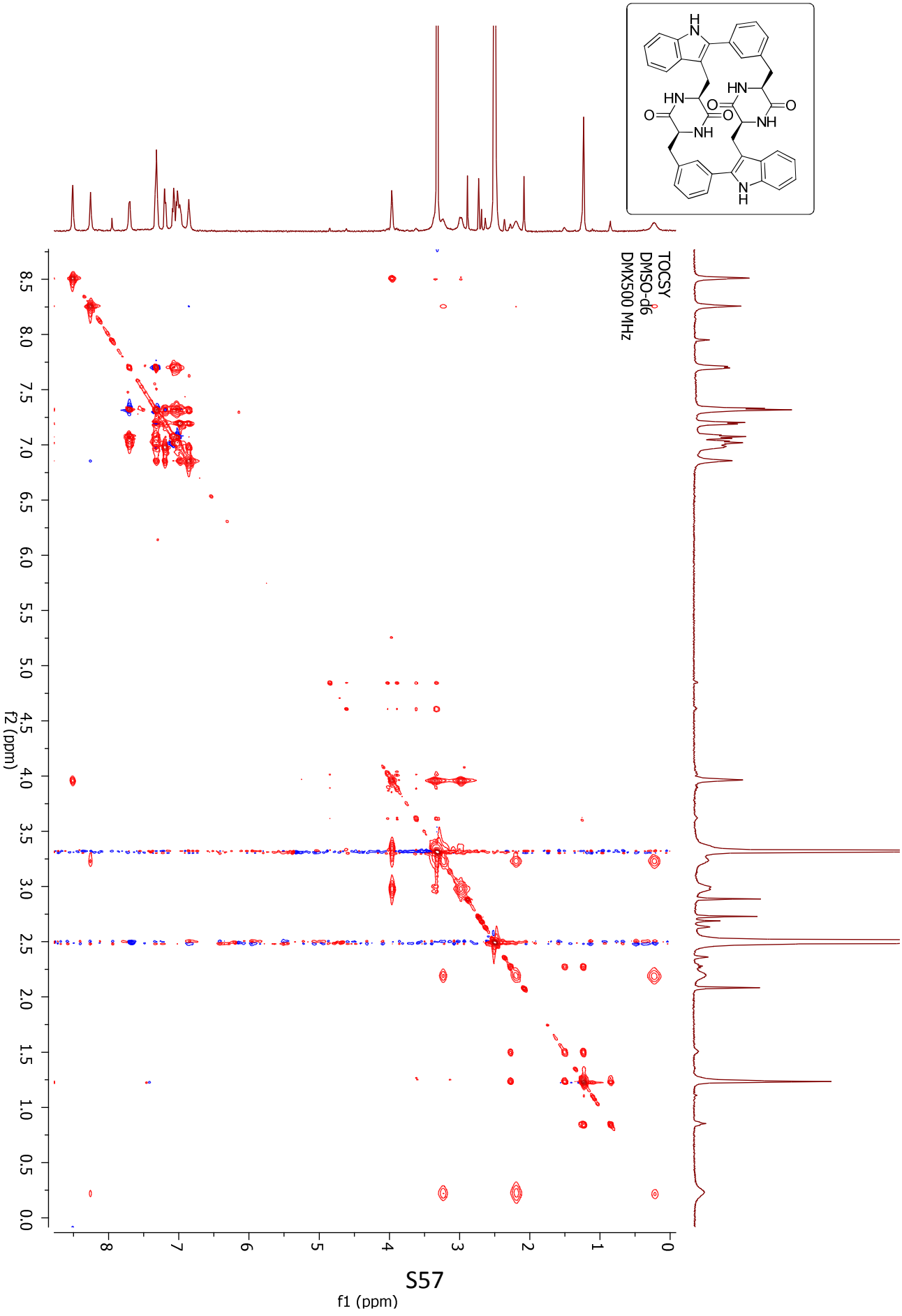
(Cyclo-*m,m)*bis-[Phe-Trp]-cyclo(Phe-Trp) (7b) ^{1}H-^{13}C HSQC NMR



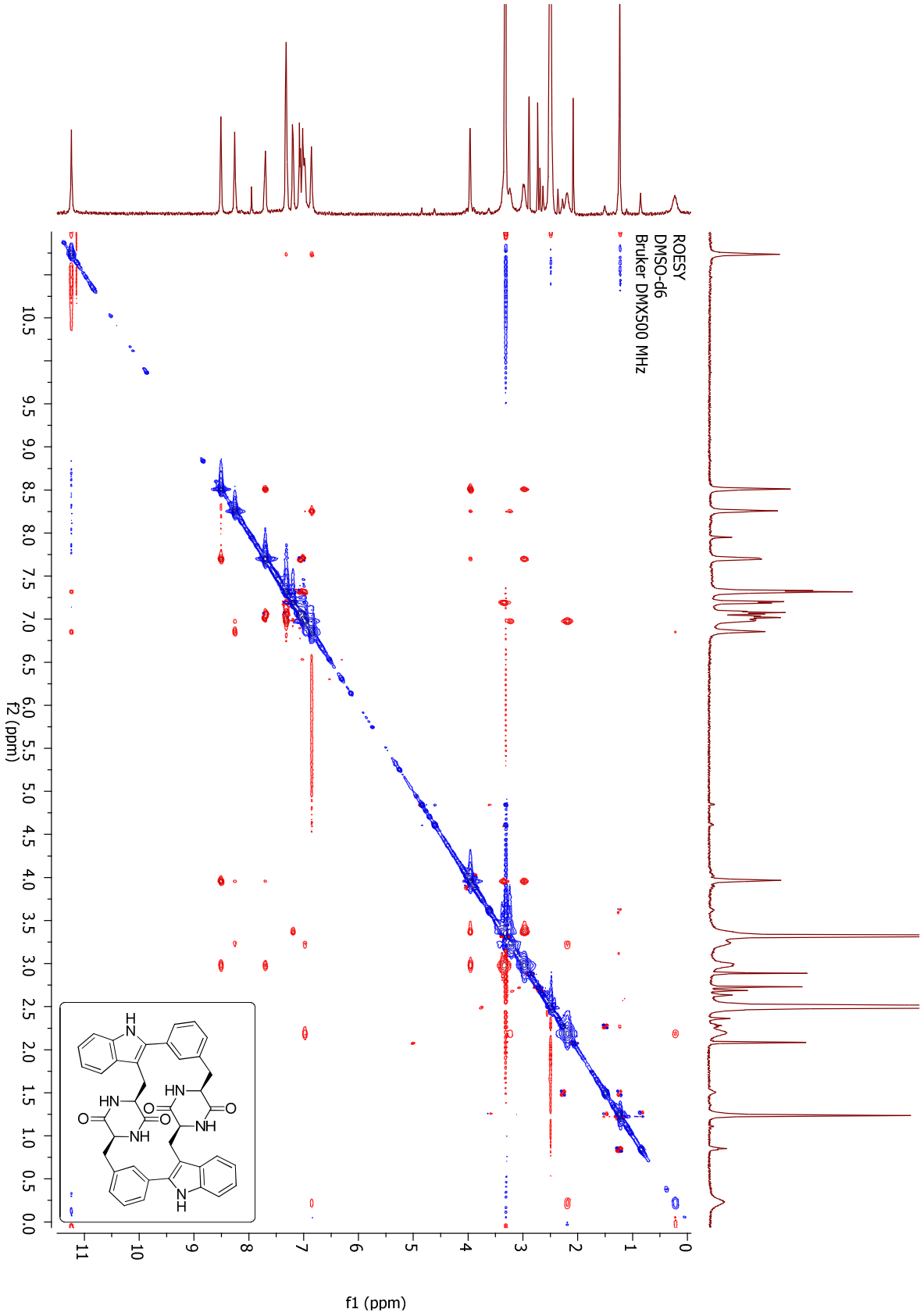
(Cyclo-*m,m)*bis-[Phe-Trp]-cyclo(Phe-Trp) (7b) COSY NMR



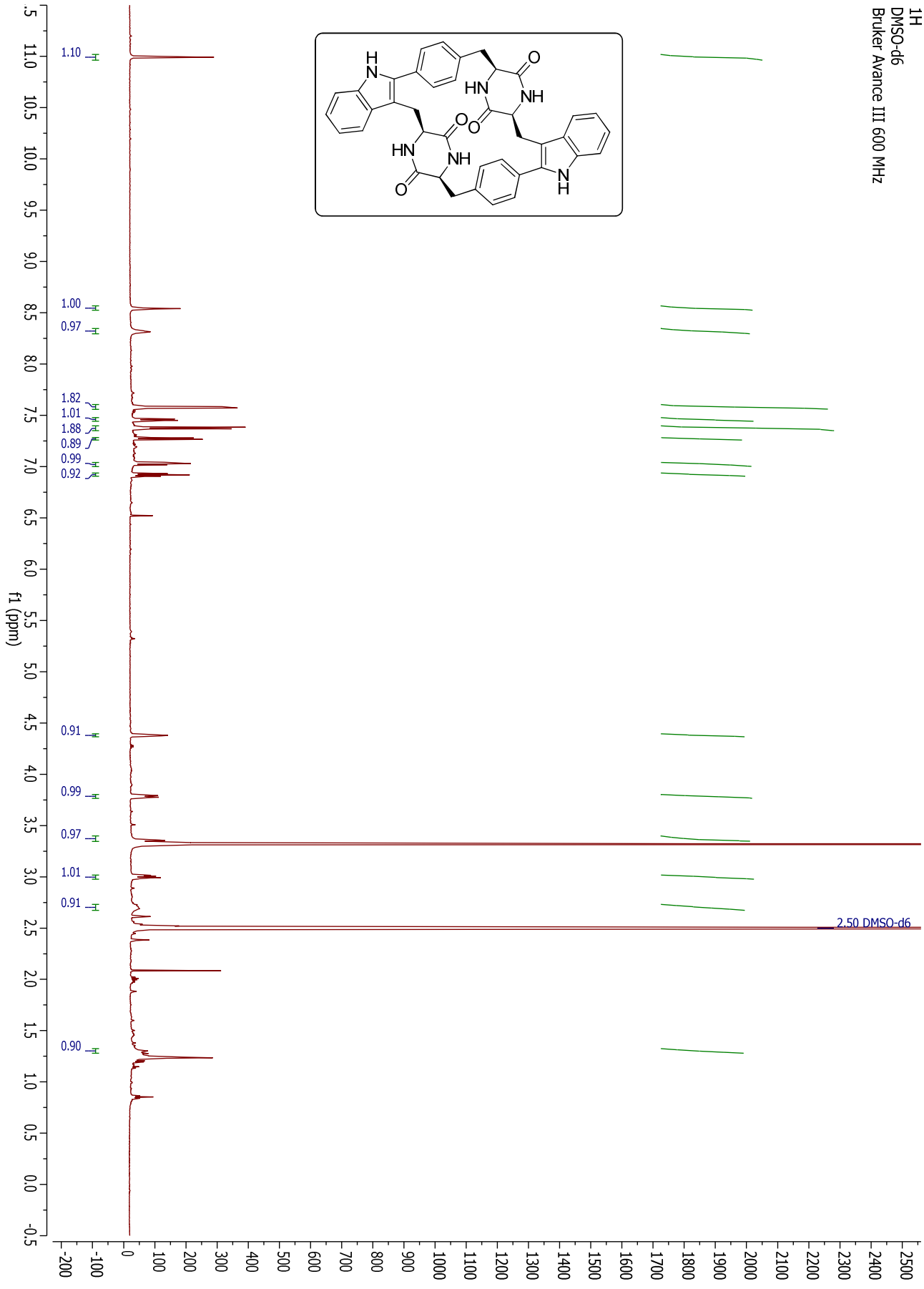
(Cyclo-*m,m)*bis-[Phe-Trp]-cyclo(Phe-Trp) (7b) TOCSY NMR



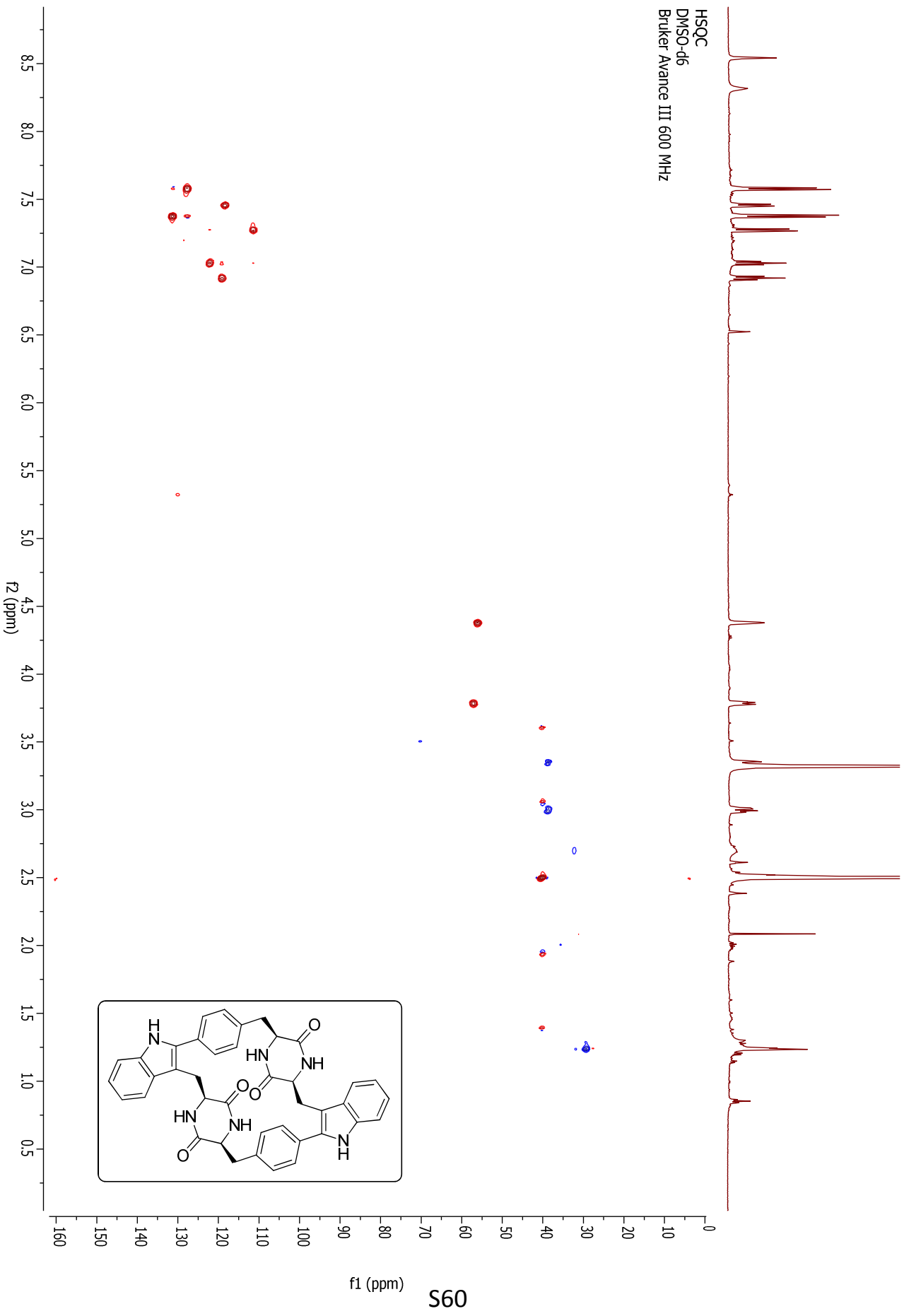
(Cyclo-*m,m)*bis-[Phe-Trp]-cyclo(Phe-Trp) (7b) ROESY NMR



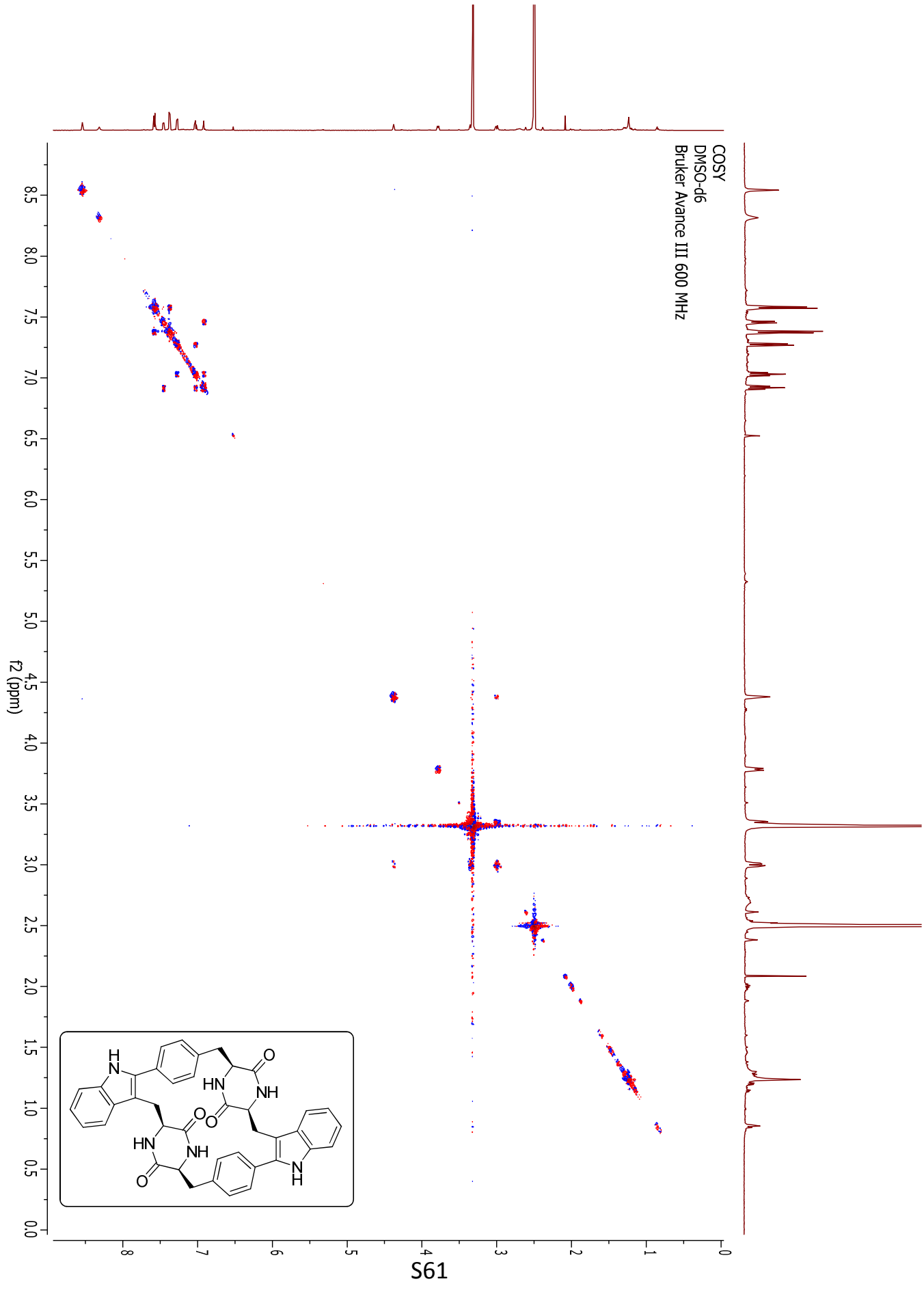
(Cyclo-*p,p*)bis-[Phe-Trp]-cyclo(Phe-Trp) (7c) ^{1}H NMR



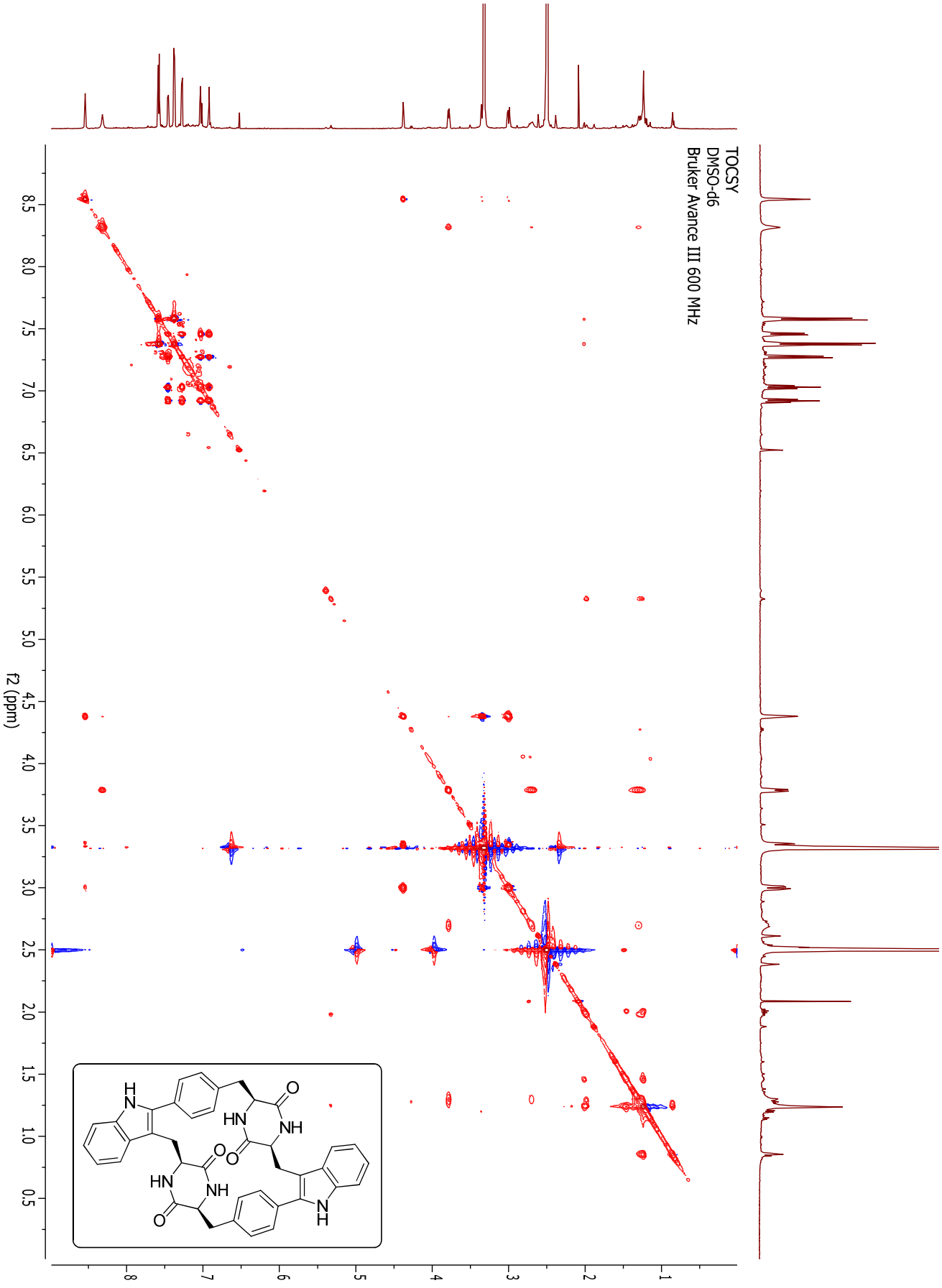
(Cyclo-*p,p*)bis-[Phe-Trp]-cyclo(Phe-Trp) (7c) ^{1}H-^{13}C HSQC NMR



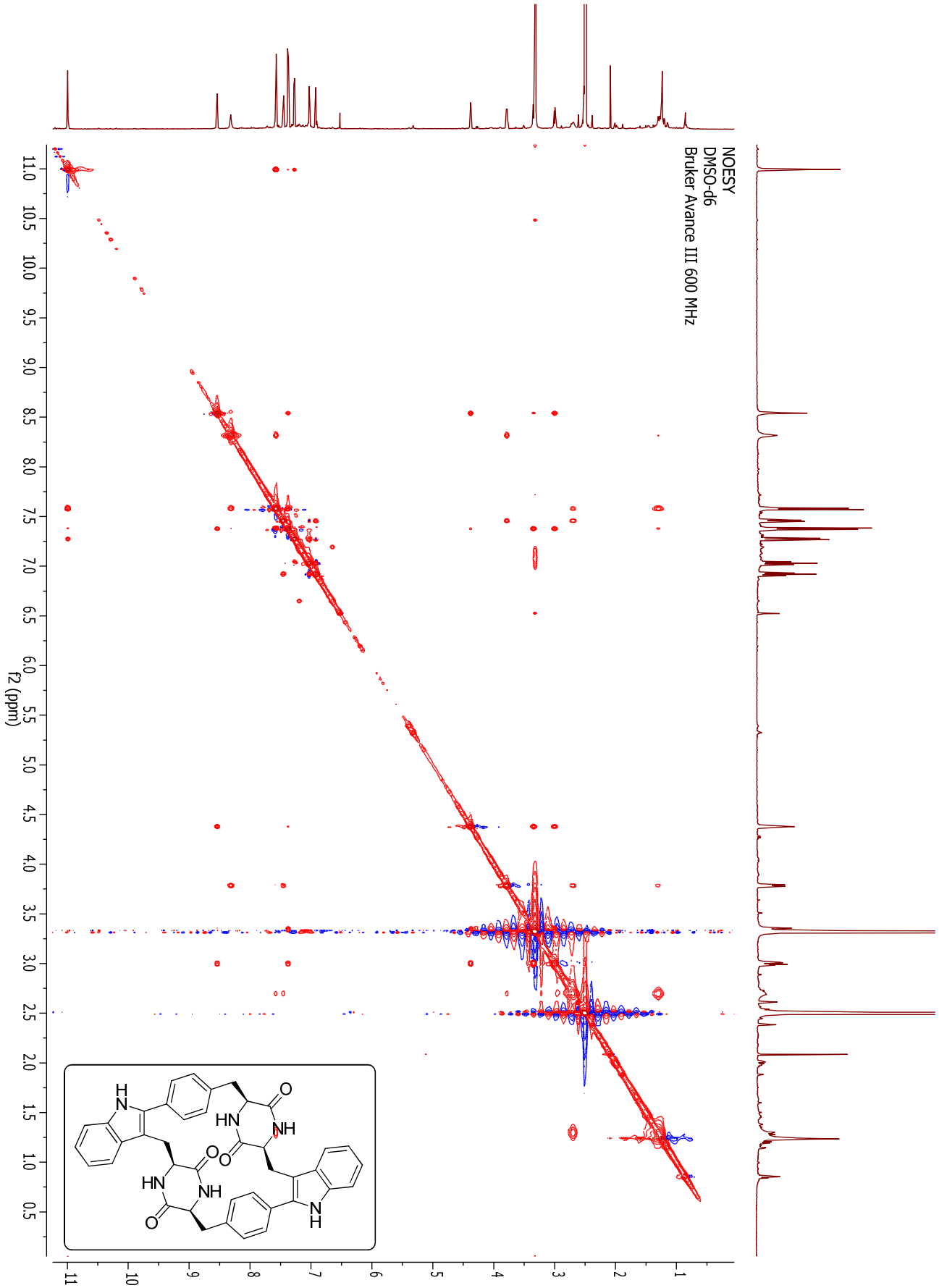
(Cyclo-*p,p*)bis-[Phe-Trp]-cyclo(Phe-Trp) (7c) COSY NMR



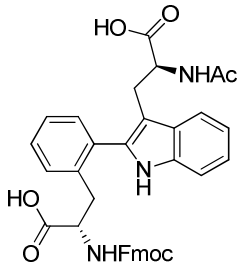
(Cyclo-*p,p)*bis-[Phe-Trp]-cyclo(Phe-Trp) (7c) TOCSY NMR



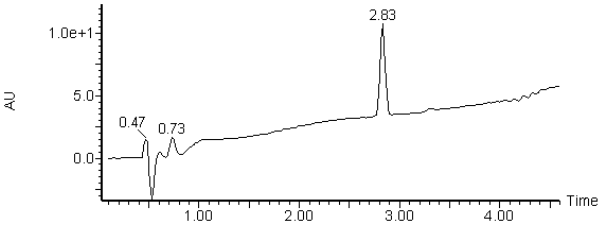
(Cyclo-*p,p)*bis-[Phe-Trp]-cyclo(Phe-Trp) (7c) NOESY NMR

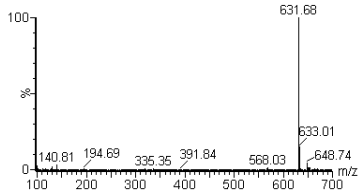


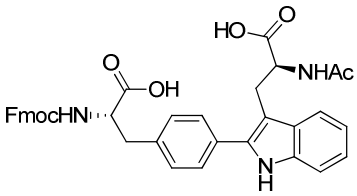
HPLC-MS chromatograms of compounds 1-7



1a







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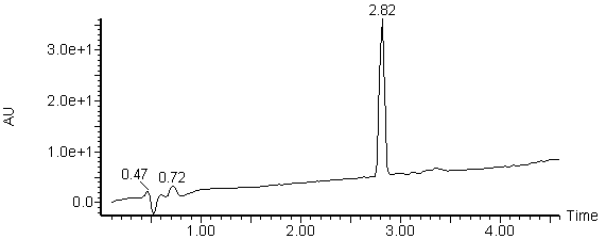
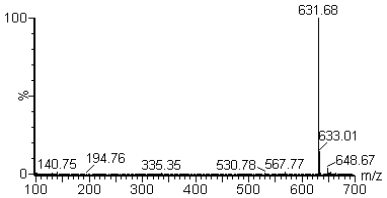
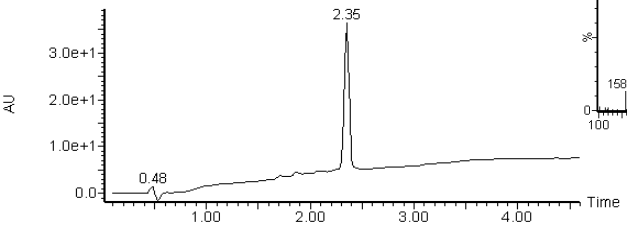
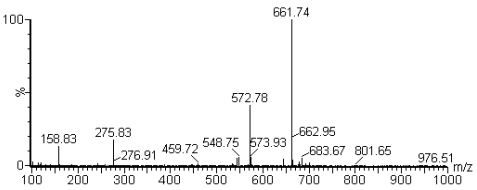


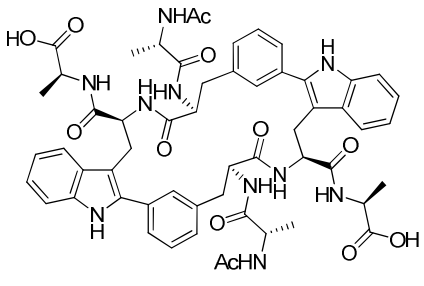


Ac-Ala-***m*-l-Phe-Trp**-Ala-OH

2







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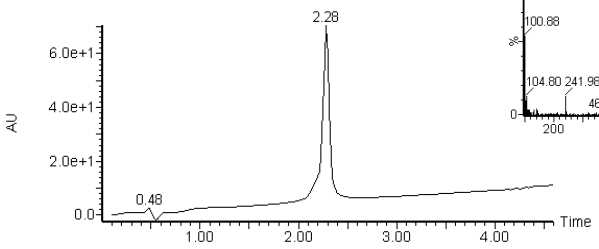
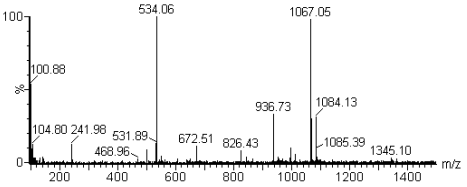
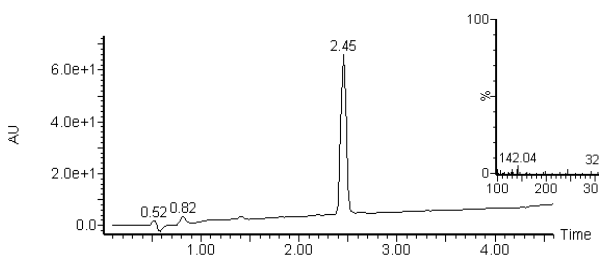
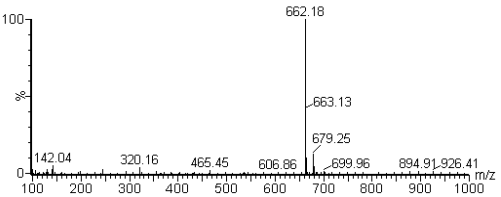




Ac-***p*-l-Phe-Trp**-Ala-OH

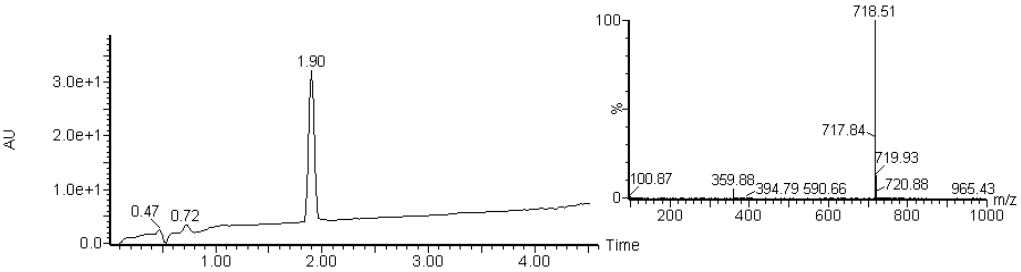
4a





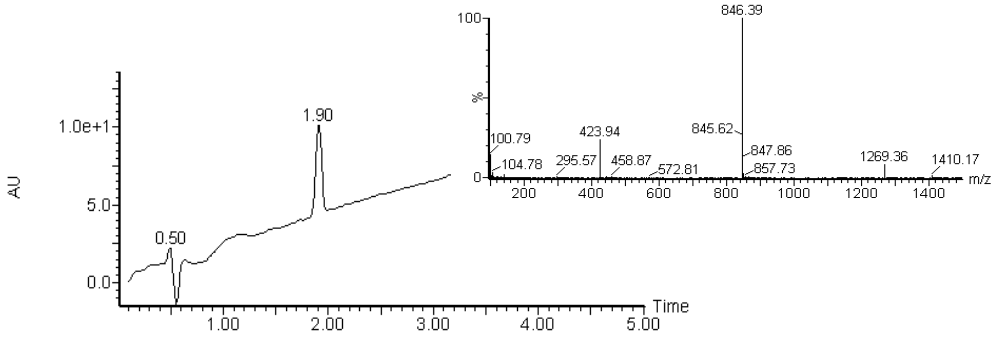
Ac-***p*-I-Phe**-Ala-**Trp**-Lys-OH

4b



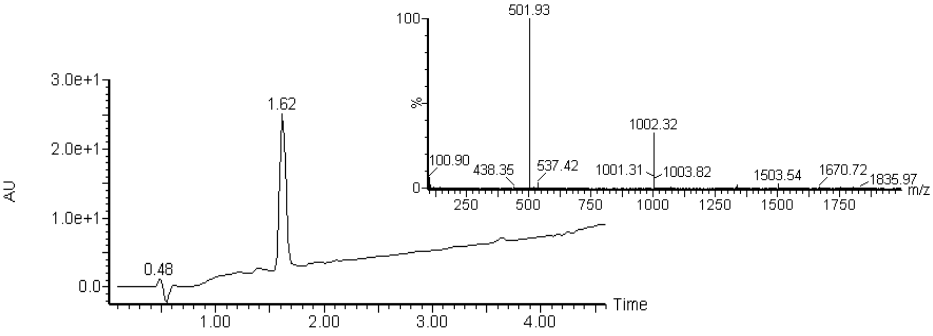
Ac-Ala-***p*-I-Phe**-Lys-Gly-**Trp**-Ala-OH

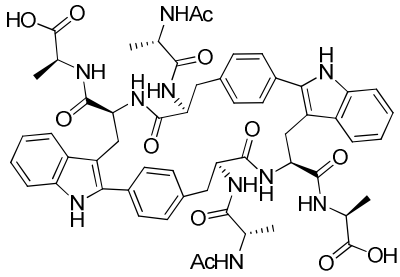
4c



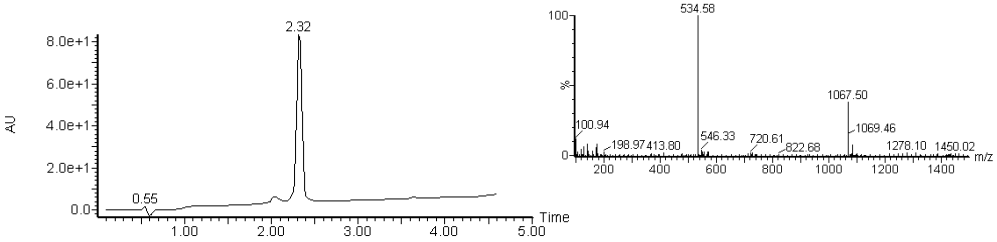
Ac-***p*-I-Phe**-Arg-Lys-Gly-**Trp**-Ala-OH

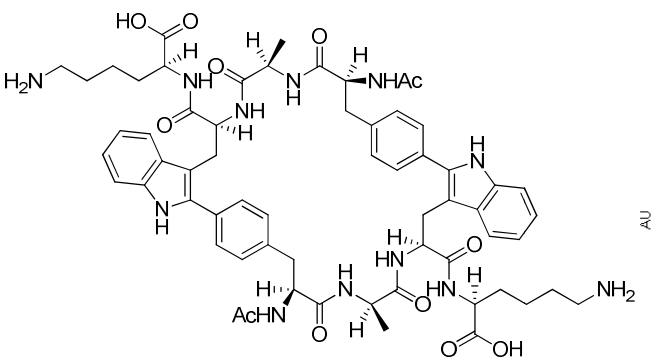
4d



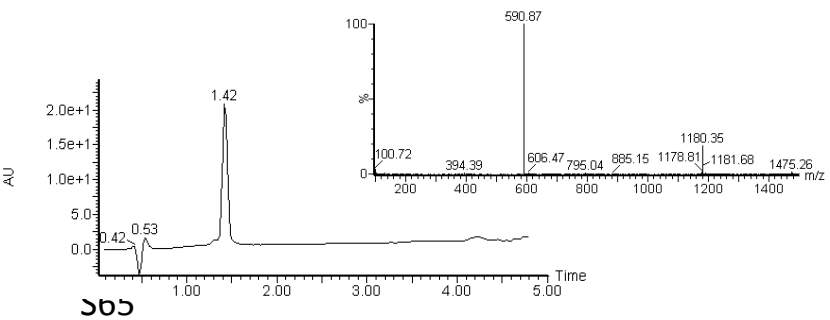


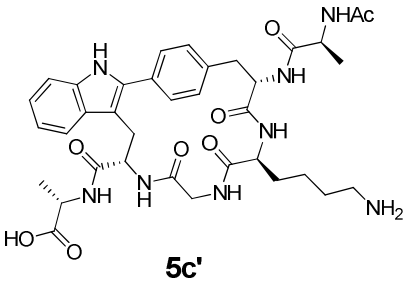
5a





5b





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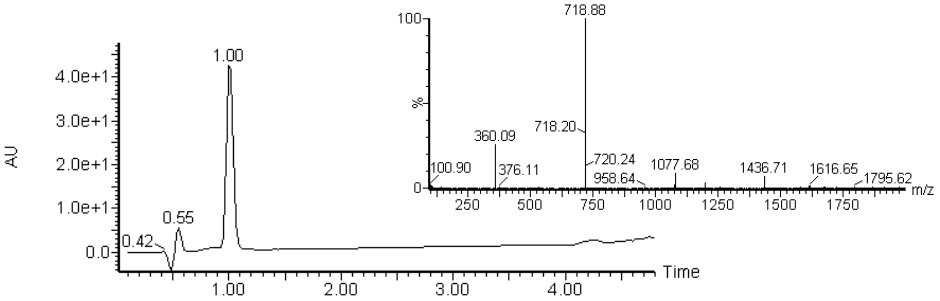
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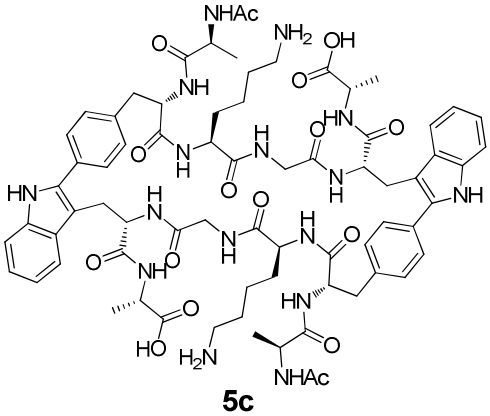
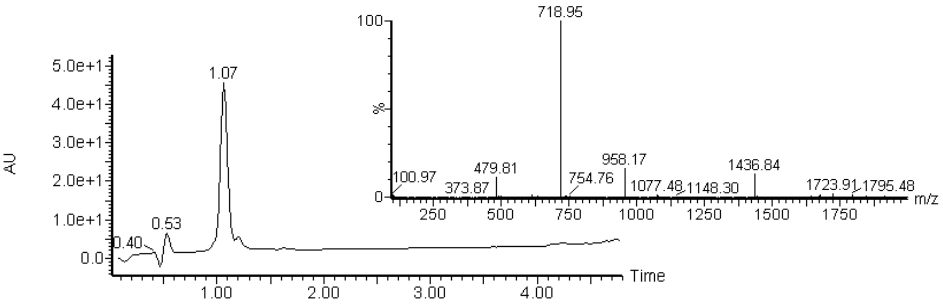
958.64



5c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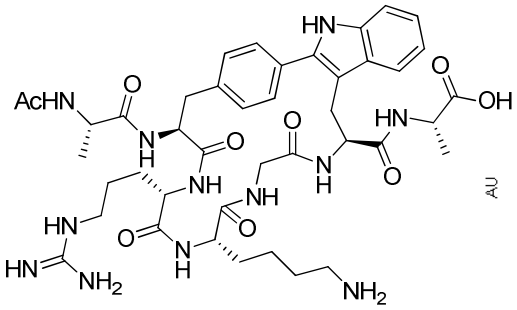
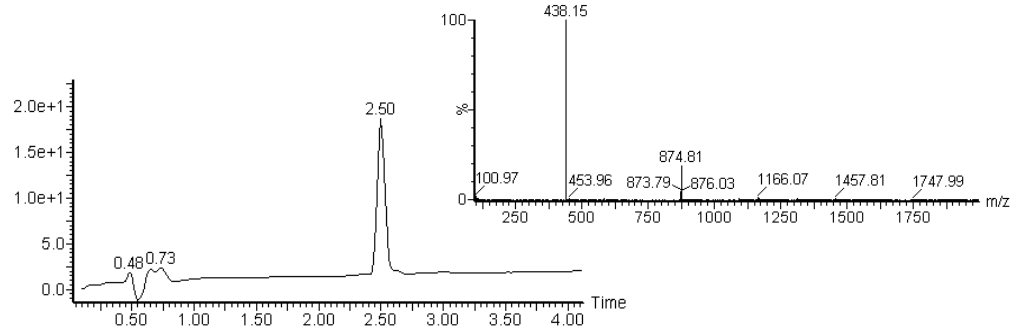


5d'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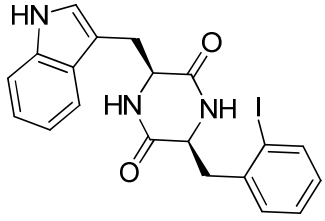
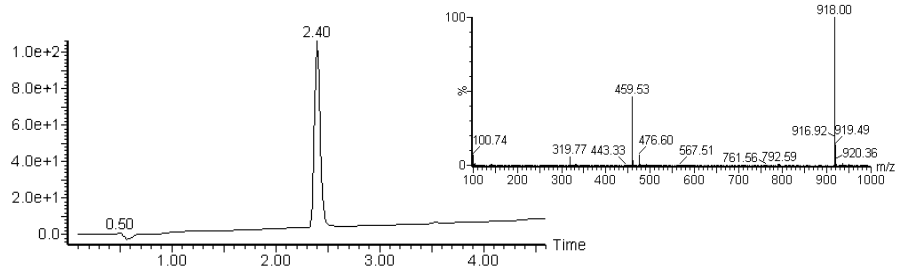


6a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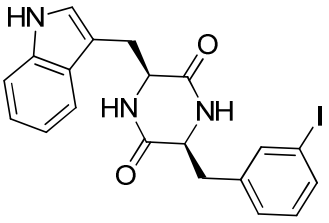
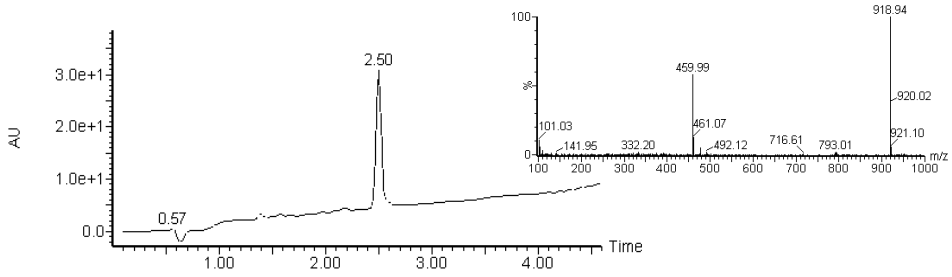
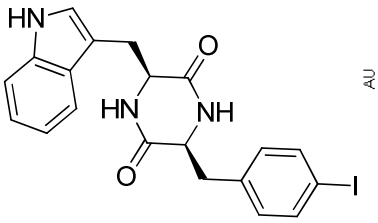


6b





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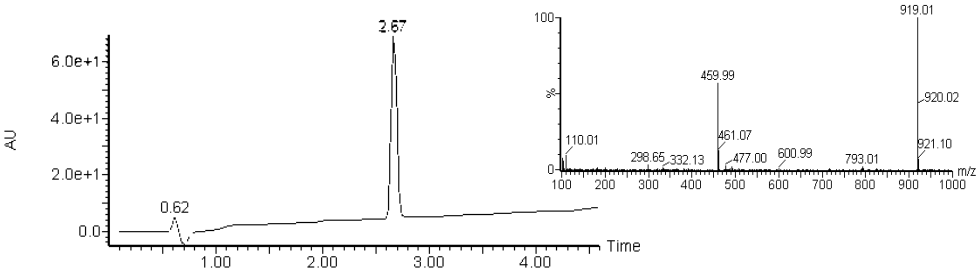

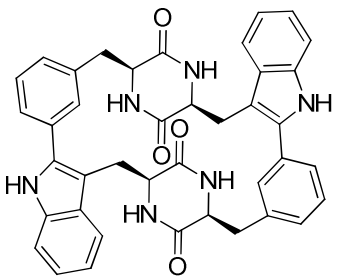

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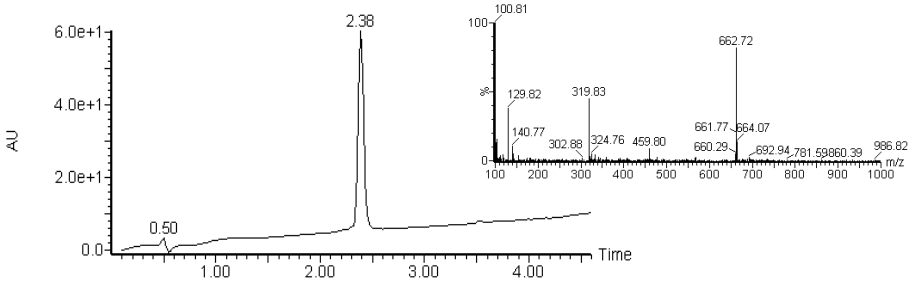
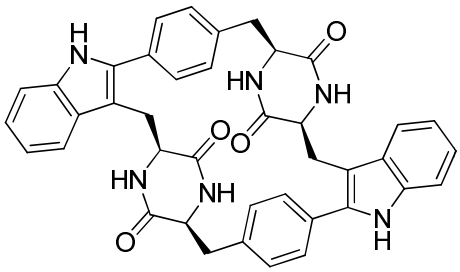
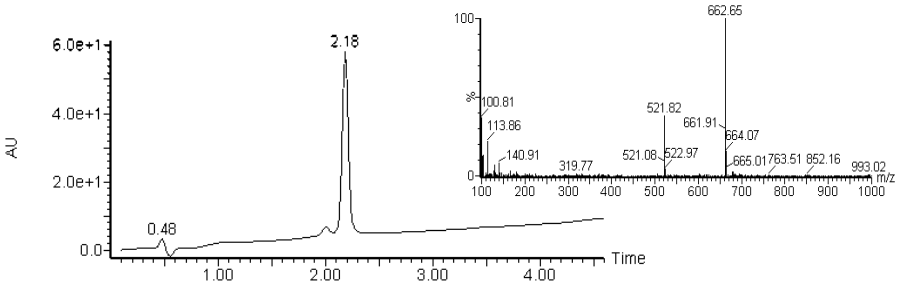




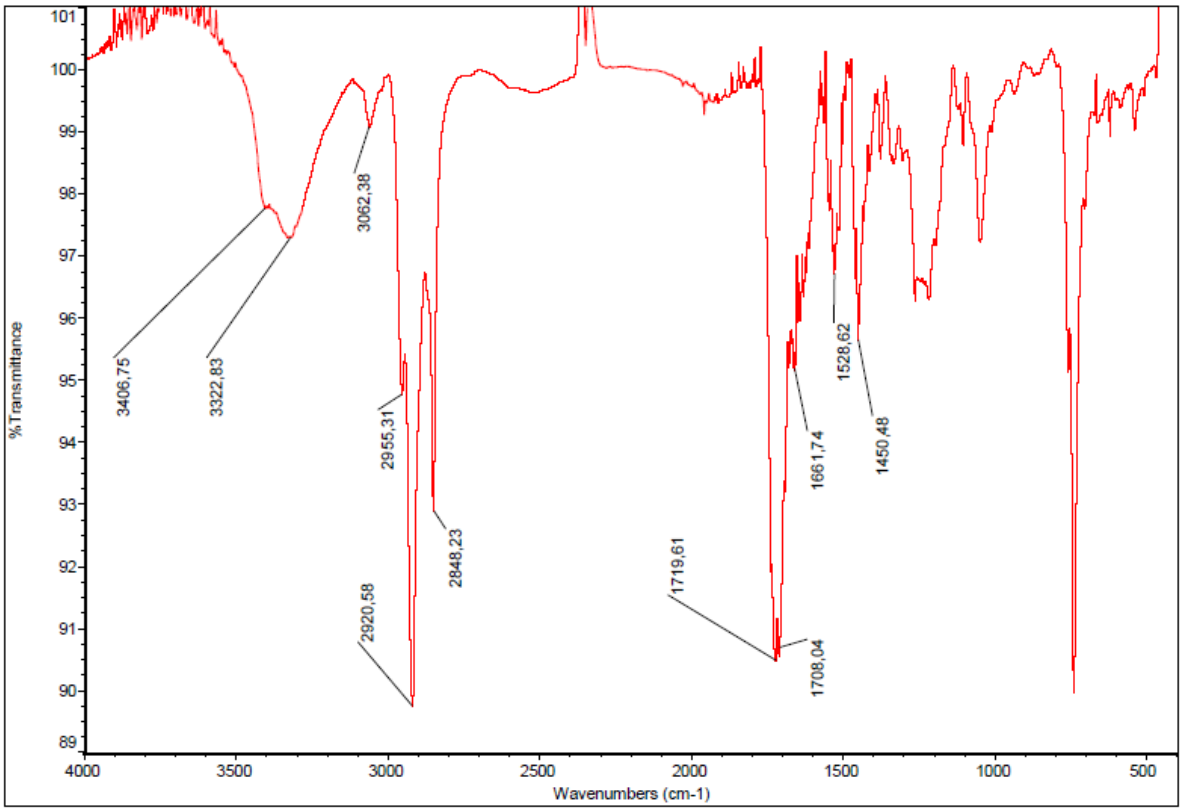
7c



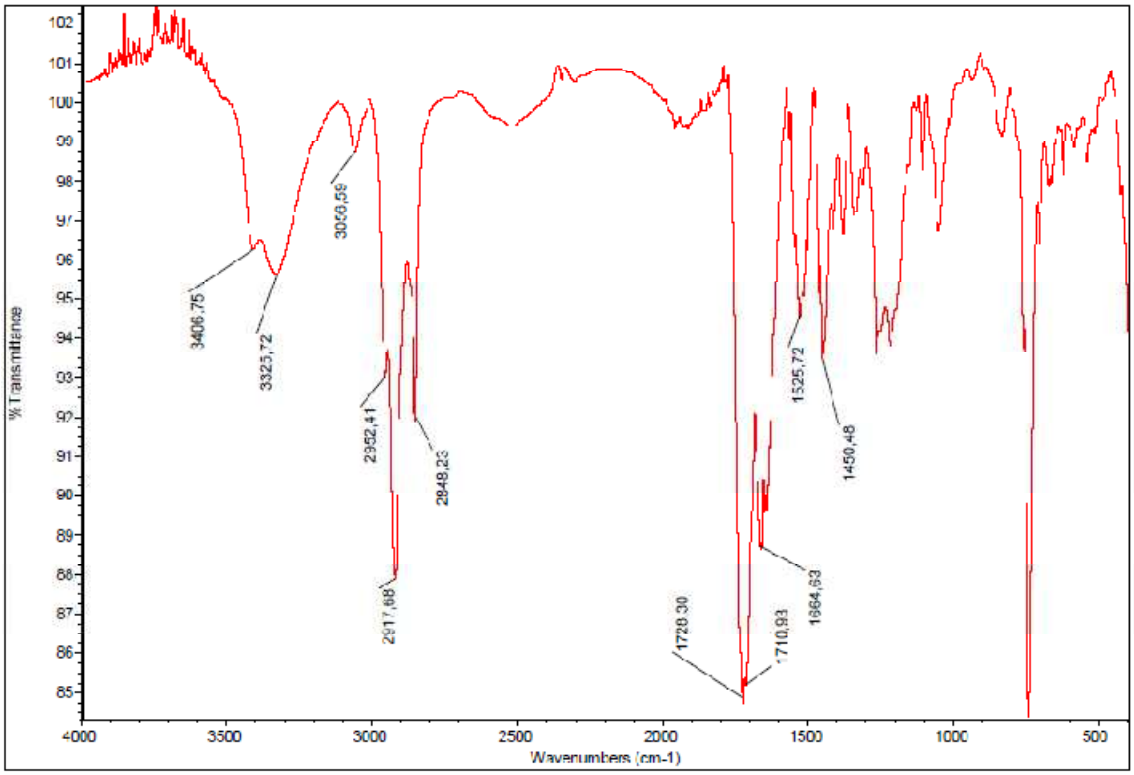
Linear gradients of ACN (+0.05% formic acid) into H_2O (+0.1% formic acid) were run at a flow rate of 1.6 mL·min^{-1} over 3.5 min from 5-100% ACN for compounds **1**-**4**, **5a** and **6**-**7**, 20-60% ACN for compounds **5b**-**c** and 5-30% for compound **5d**.

IR spectra of compounds 1 and 6

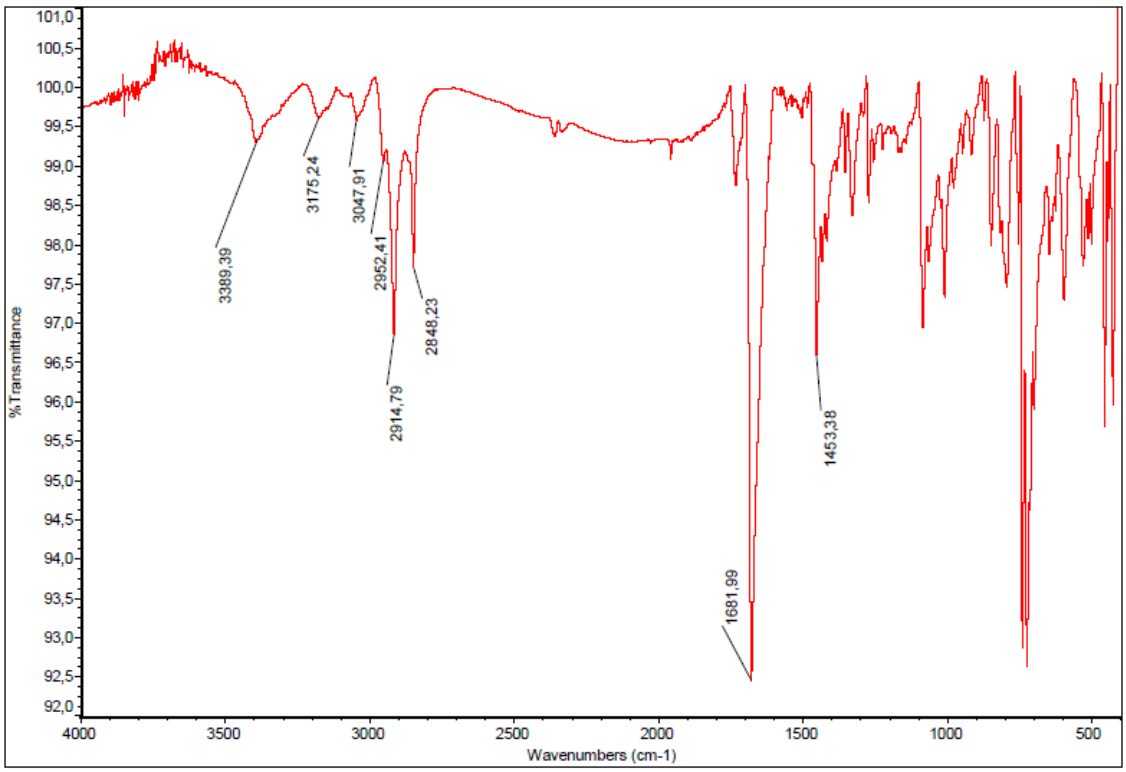
[Ac-*C2*-Trp-OH)—(Fmoc-*o*-Phe-OH)] adduct (1a)



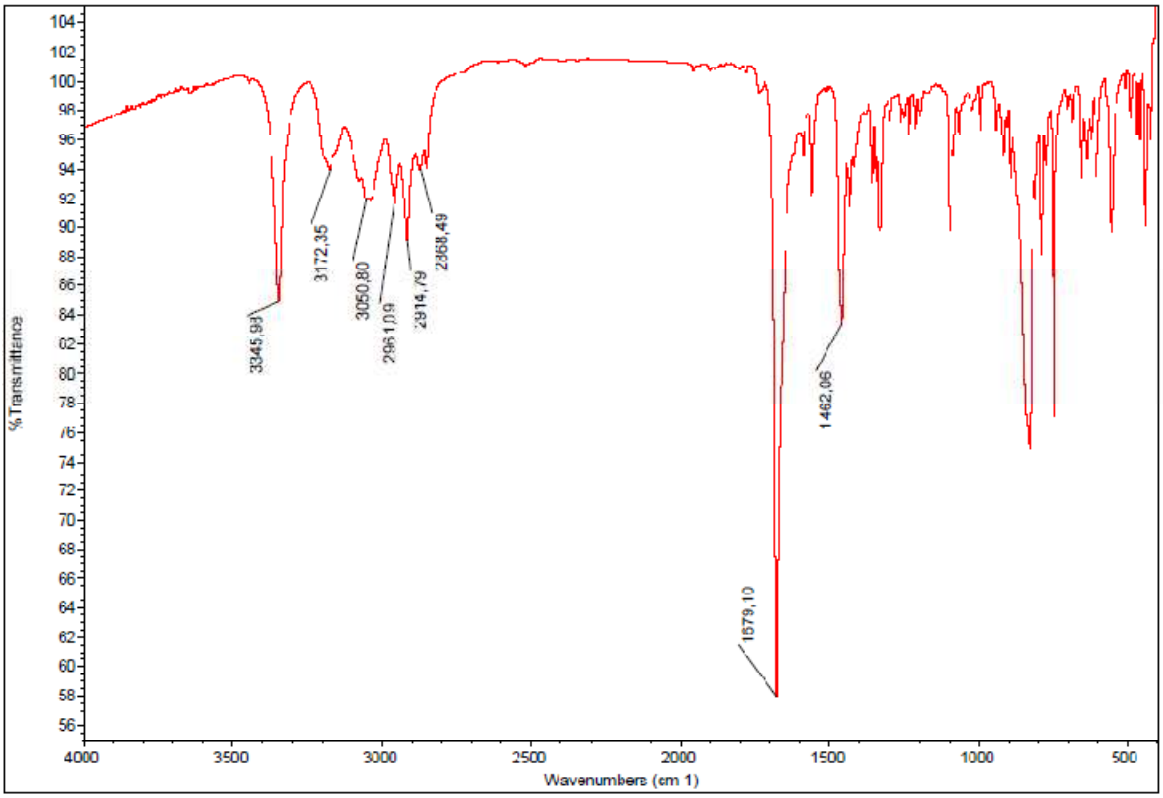
[Ac-*C2*-Trp-OH)—(Fmoc-*p*-Phe-OH)] adduct (1b)



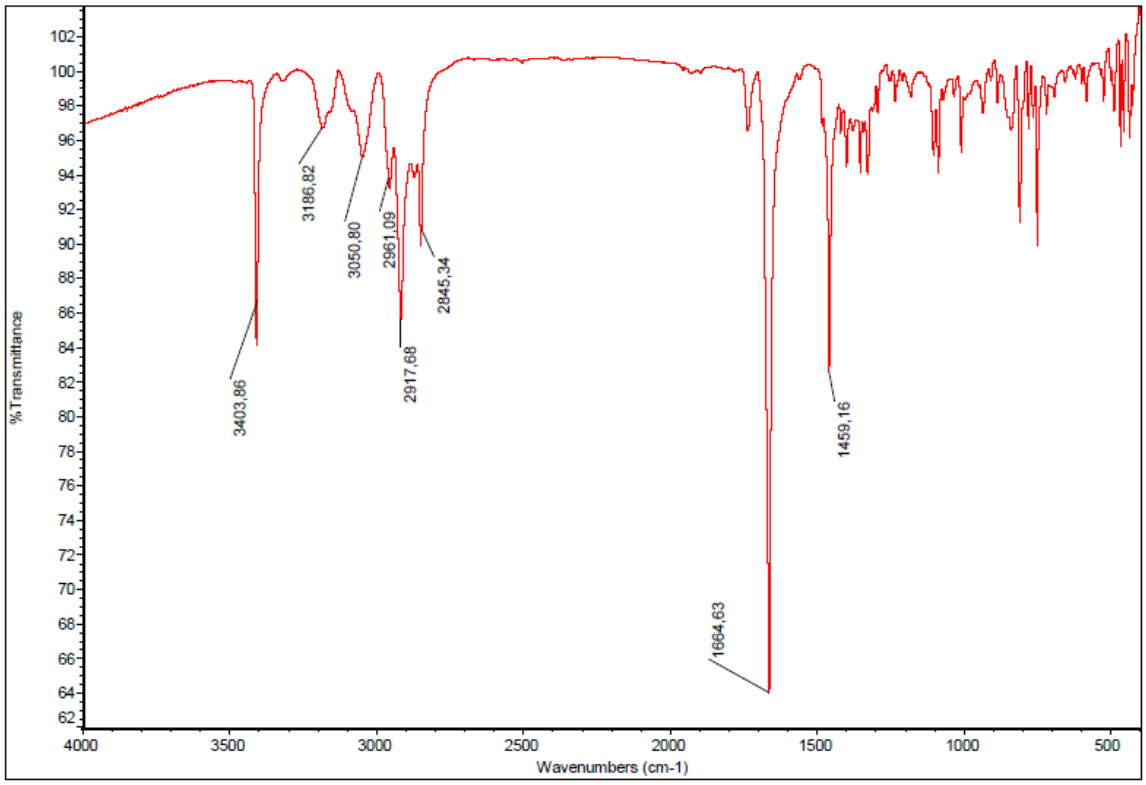
Cyclo(2-I-Phe-Trp) (6a)



Cyclo(3-I-Phe-Trp) (6b)

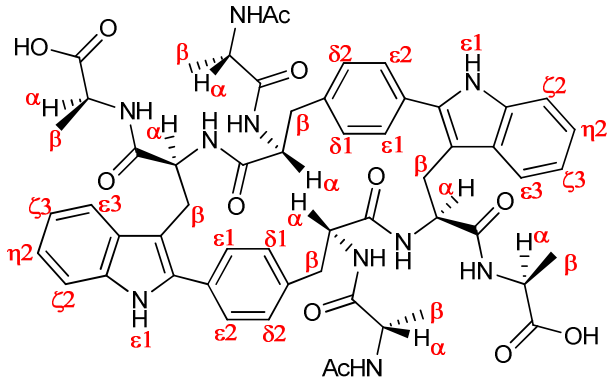


Cyclo(4-I-Phe-Trp) (6c)



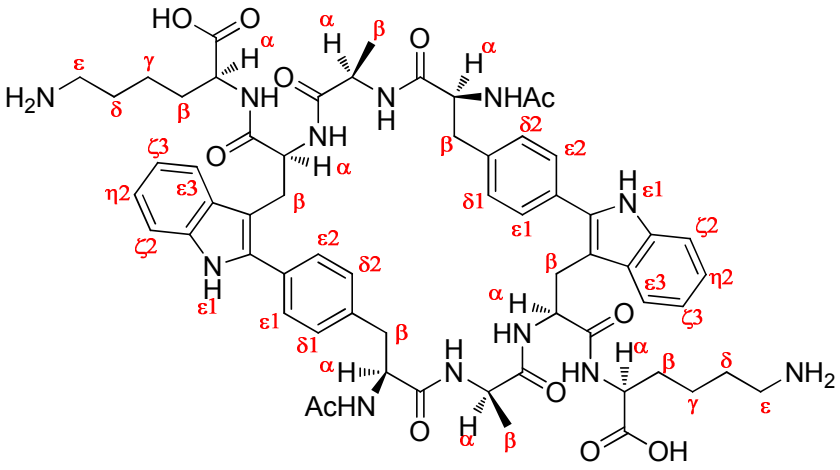
^{1}H and ^{13}C chemical shifts assignments of compounds 5 and 7

Due to the symmetric nature of dimeric peptides, both peptide moieties are chemically equivalent and have identical NMR signals.



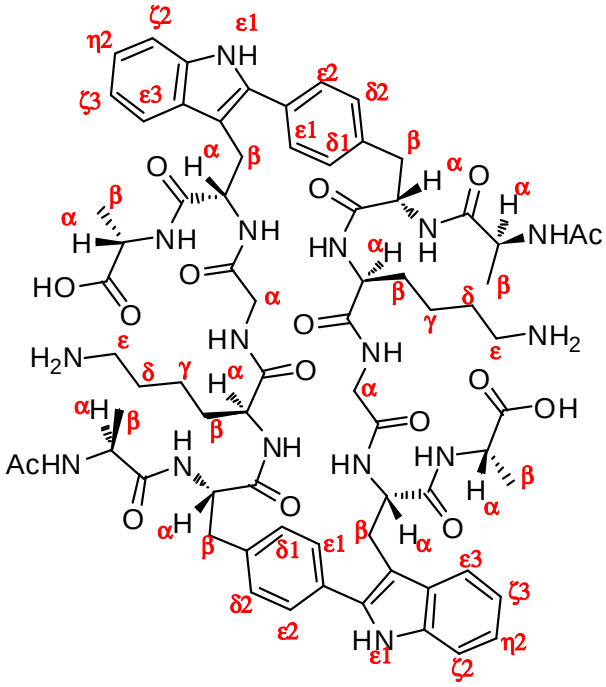
5a (^{1}H)						*δ* (ppm)				
AA	**NH**	**α**	**β**	**δ 1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	7.93	4.18	1.08	-	-	-	-	-	-	-	-
***p-I*-Phe**	8.13	3.96	2.64/2.48	7.25	7.25	7.61	7.61	-	-	-	-
Trp	6.82	4.07	3.43/3.43	-	-	-	11.20	7.34	7.11	7.03	7.67
Ala2	7.90	4.21	1.28	-	-	-	-	-	-	-	-

5a (^{13}C)					*δ* (ppm)					
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ε1**	**ζ2**	**η2**	**ζ3**	**ε3**
Ala1	47.7	17.8	-	-	-	-	-	-	-	-
***p-I*-Phe**	52.9	35.2	128.8	128.8	127.9	127.9	-	-	-	-
Trp	54.9	25.6	-	-	-	-	110.9	121.4	118.5	118.3
Ala2	47.5	17.0	-	-	-	-	-	-	-	-



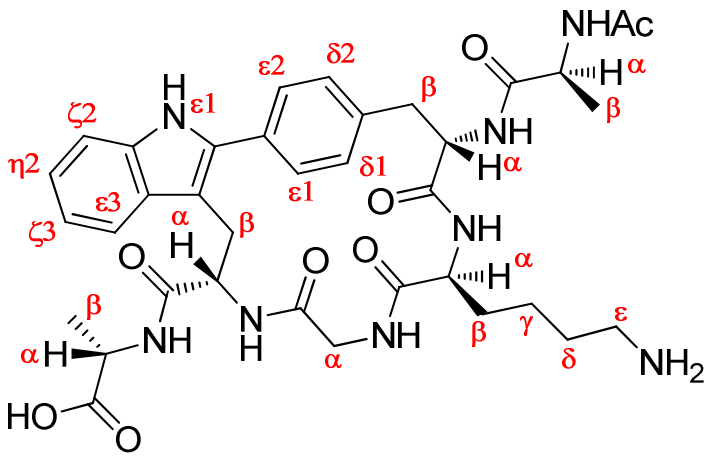
5b (^{1}H)						*δ* (ppm) (T:338 K)						
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Phe	8.04	4.59	3.01/2.81	7.30	7.30	7.43	7.43	-	-	-	-	-
Ala	8.14	4.14	1.08	-	-	-	-	-	-	-	-	-
Trp	7.49	4.59	3.32/3.13	-	-	10.92	-	7.32	7.08	7.01	7.72	-
Lys	7.29	3.86	1.63-1.16	1.63-1.16	-	2.66	-	-	-	-	-	1.63-1.16

5b (^{13}C)					*δ* (ppm) (T:338 K)						
AA	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Phe	53.2	37.2	128.9	128.9	127.4	127.4	-	-	-	-	-
Ala	48.3	17.2	-	-	-	-	-	-	-	-	-
Trp	53.2	27.5	-	-	-	-	110.6	120.6	118.1	119.0	-
Lys	52.8	30.6-26.1	30.6-26.1	-	38.3	-	-	-	-	-	30.6-26.1



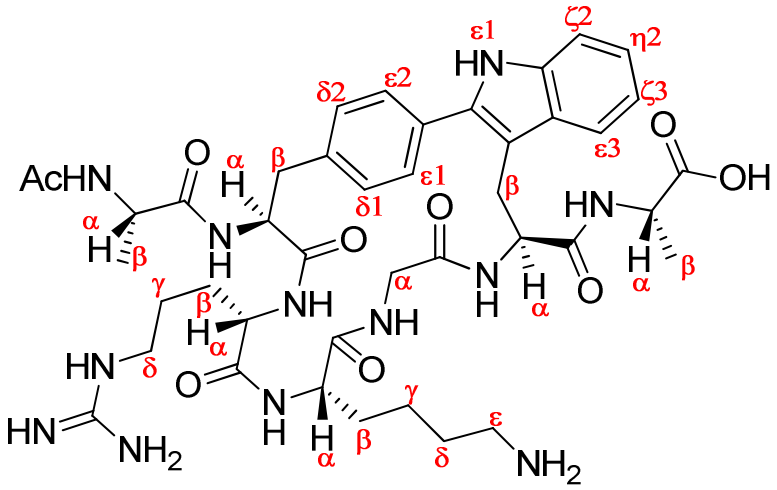
5c (^{1}H)												
						δ (ppm) (T:338 K)						
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Ala	8.35 – 7.86	4.20-3.83	1.20-1.11	-	-	-	-	-	-	-	-	-
Phe	8.35 – 7.86	4.50	3.05/2.88	7.27	7.27	7.51	7.51	-	-	-	-	-
Lys	8.35 – 7.86	4.29	1.68/1.53	1.55	-	2.76	-	-	-	-	-	1.30
Gly	8.35 – 7.86	3.76/3.56	-	-	-	-	-	-	-	-	-	-
Trp	8.35 – 7.86	4.50	3.43/3.05	-	-	11.10	-	7.29	7.06	6.99	7.70	-
Ala	8.35 – 7.86	4.20-3.83	1.20-1.11	-	-	-	-	-	-	-	-	-

5c (^{13}C)											
						δ (ppm) (T:338 K)					
AA	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Ala	47.9-48-8	17.6-17.7	-	-	-	-	-	-	-	-	-
Phe	53.7	36.5	129.2	129.2	127.3	127.3	-	-	-	-	-
Lys	51.9	30.9	25.7	-	37.4	-	-	-	-	-	21.6
Gly	42.0	-	-	-	-	-	-	-	-	-	-
Trp	53.7	42.2	-	-	-	-	110.5	120.8	118.2	118.7	-
Ala	47.9-48-8	17.6-17.7	-	-	-	-	-	-	-	-	-



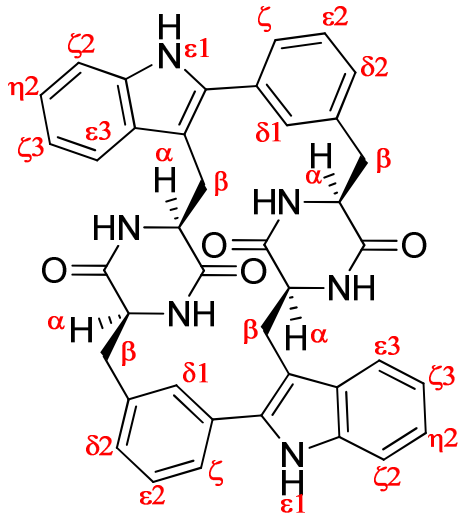
5c' (^{1}H)						δ (ppm)						
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Ala	8.08	4.34	1.19	-	-	-	-	-	-	-	-	-
Phe	8.18	4.68	2.96	7.31	7.31	7.51	7.51	-	-	-	-	-
Lys	8.35	4.19	1.68/1.36	1.49	-	2.72	-	-	-	-	-	1.39/1.26
Gly	6.40	3.56/3.26	-	-	-	-	-	-	-	-	-	-
Trp	7.00	4.48	3.45/3.27	-	-	11.05	-	7.30	7.07	6.96	7.61	-
Ala	8.11	4.14	1.22	-	-	-	-	-	-	-	-	-

5c' (^{13}C)						δ (ppm)					
AA	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Ala	47.7	17.8	-	-	-	-	-	-	-	-	-
Phe	53.1	37.2	129.3	129.3	127.7	127.7	-	-	-	-	-
Lys	51.4	unknown	26.1	-	38.4	-	-	-	-	-	21.9
Gly	41.8	-	-	-	-	-	-	-	-	-	-
Trp	52.9	unknown	-	-	-	-	110.5	120.8	118.2	118.4	-
Ala	47.6	17.4	-	-	-	-	-	-	-	-	-



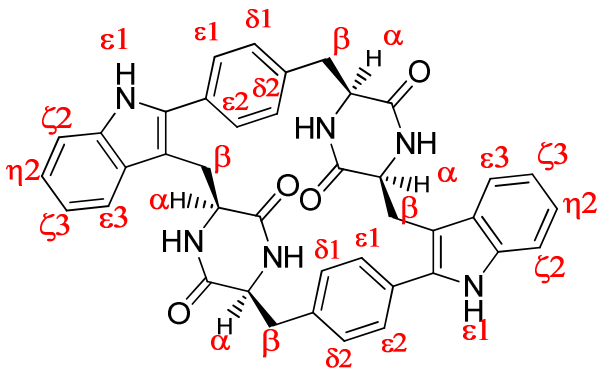
5d' (^{1}H)						*δ* (ppm)						
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Ala	6.95	3.79	1.13	-	-	-	-	-	-	-	-	-
Phe	7.97	4.56	2.94	7.23	7.23	7.50	7.50	-	-	-	-	-
Arg	7.64	4.22	1.77/1.67	3.12/3.05	-							1.54
Lys	8.02	3.95	1.60	1.61/1.55	-	2.78	-	-	-	-	-	1.40
Gly	8.36	3.95/3.13	-	-	-	-	-	-	-	-	-	-
Trp	8.10	4.30	3.56/3.24	-	-	10.89	-	7.32	7.06	6.99	7.58	-
Ala	8.36	4.30	1.23	-	-	-	-	-	-	-	-	-

5d' (^{13}C)					*δ* (ppm)						
AA	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**	**γ1**
Ala	49.9	19.0	-	-	-	-	-	-	-	-	-
Phe	54.9	38.7	130.3	130.3	128.2	128.2	-	-	-	-	-
Arg	54.1	30.1	41.2	-	-	-	-	-	-	-	25.2
Lys	54.6	30.8	27.1	-	39.2	-	-	-	-	-	22.4
Gly	42.8	-	-	-	-	-	-	-	-	-	-
Trp	49.0	unknown	-	-	-	-	111.3	121.4	118.8	119.3	-
Ala	49.0	18.1	-	-	-	-	-	-	-	-	-



7b (^{1}H)						*δ* (ppm)					
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ/ζ2**	**η2**	**ζ3**	**ε3**
Phe	8.26	3.24	2.20/0.24	6.86	6.98	-	7.32	7.20	-	-	-
Trp	8.51	3.97	3.38/2.99	-	-	11.24	-	7.33	7.08	7.02	7.71

7b (^{13}C)	*δ* (ppm)								
AA	**α**	**β**	**δ1**	**δ2**	**ε2**	**ζ/ζ2**	**η2**	**ζ3**	**ε3**
Phe	56.6	40.0	130.8	127.7	128.5	127.9	-	-	-
Trp	55.4	27.6	-	-	-	110.7	120.8	118.7	120.0



7c (^{1}H)						*δ* (ppm)					
AA	**NH**	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	8.54	4.38	3.36/3.00	7.38	7.38	7.58	7.58	-	-	-	-
Trp	8.32	3.78	2.70/1.33	-	-	10.99	-	7.27	7.04	6.92	7.46

7c (^{13}C)					*δ* (ppm)					
AA	**α**	**β**	**δ1**	**δ2**	**ε1**	**ε2**	**ζ2**	**η2**	**ζ3**	**ε3**
Phe	55.5	38.2	130.6	130.6	127.0	127.0		-	-	-
Trp	56.2	31.7	-		-	-	110.7	121.5	118.4	117.6

Additional comments on the NMR spectroscopy of cyclopeptides 3 and 5

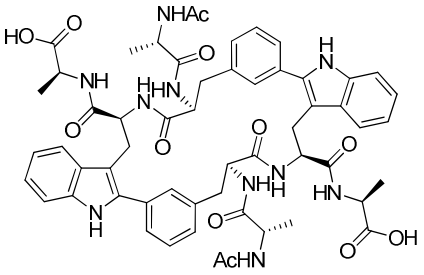
Cyclodimer peptides **3** and **5a**-**c** were characterized by NMR and indicated a C2-symmetric structure. Aromatic ring flipping was observed for Phe residues in all peptides. Rapid reorientation about the Cβ-Cγ bond renders the Hδ1/Hδ2 protons on one side and the Hε1/Hε2 protons on the other side, equivalent on the NMR timescale. In agreement with this observation, **5a** was the peptide that showed the largest chemical shift dispersion on the amide NH region at 298 K in all the series. Peptide **5b** showed moderate chemical shift dispersion on the amide region. In contrast, the six amide NH signals of **5c** overlapped at 7.9-8.3 ppm, indicating a significant flexibility.

With respect to cyclopeptides, the Gly residue of peptide **5c'** exhibited significant upfield shifted resonances with chemical shifts of 6.40 (NH) and 3.56/3.26 ppm (Hα). Some amide NH resonances of **5b** appear as broad signals at room temperature and sharpen as the temperature was increased.

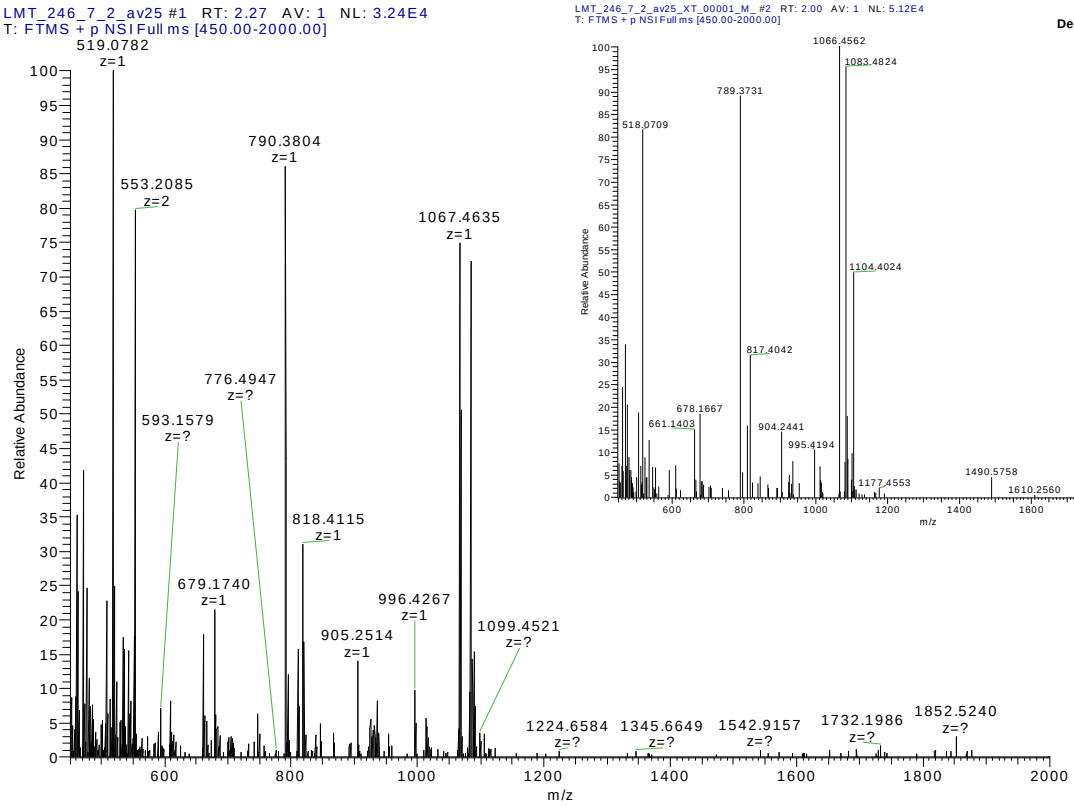
Regarding the DKP structure, cyclodimer formation was characterized by a large diastereotopic splitting of geminal Hβ protons. This effect was especially pronounced in the Phe Hβ protons of **7b**, where one Phe Hβ resonated at 2.20 ppm and the second Hβ was upfield shifted and appeared at 0.24 ppm.

High resolution mass spectrometry analysis of compounds 3, 5 and 7

Samples were reconstituted in CH_3CN or H_2O/CH_3CN (1:1) 1% Formic acid and diluted in H_2O/CH_3CN (1:1) 1% Formic acid for MS analysis. Ion deconvolution to zero charged monoisotopic masses was performed using Xtract algorithm in Xcalibur software.



3

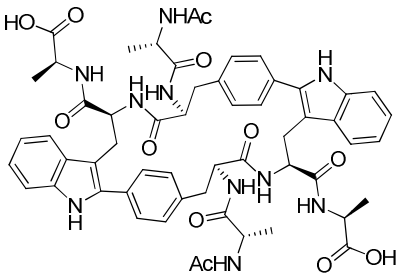


Elemental composition search on mass 1066.46

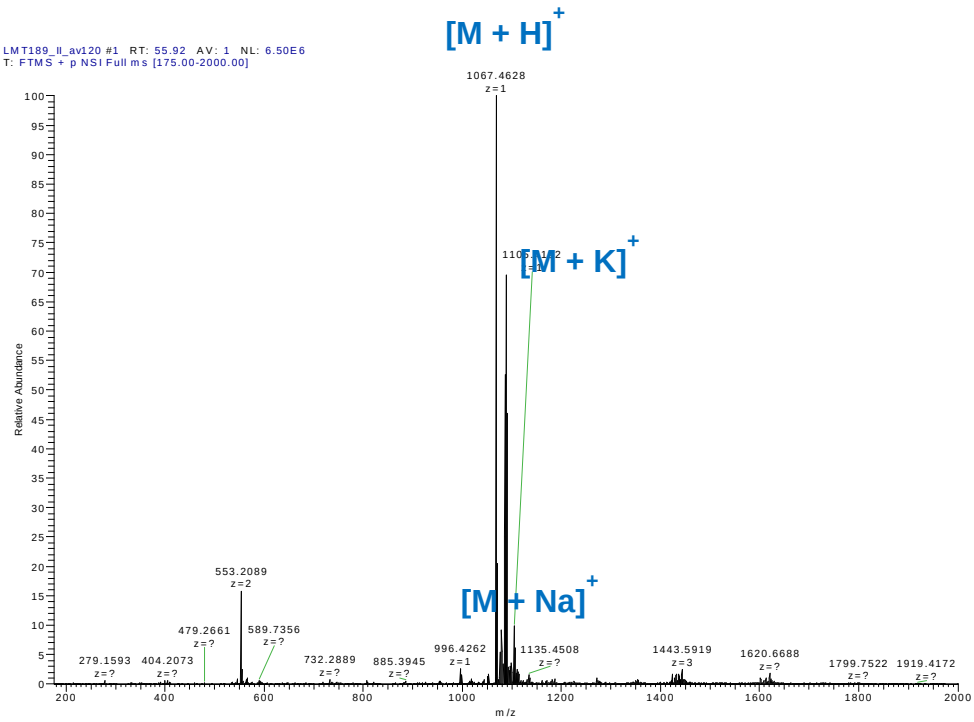
m/z= 1061.46-1071.46

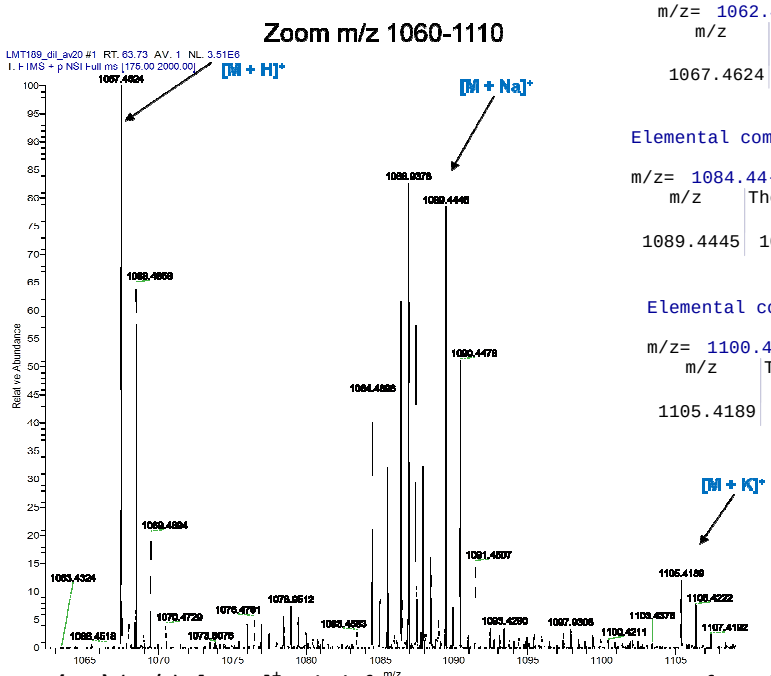
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition	
1066.45620	1066.45621	-0.01	30.5	$C_{58}H_{64}O_{13}N_7$	
	1066.45487	1.25	31.0	$C_{56}H_{62}O_{12}N_{10}$	← [M]
	1066.45755	-1.26	35.5	$C_{59}H_{60}O_9N_{11}$	
	1066.45755	-1.27	30.0	$C_{60}H_{66}O_{14}N_4$	
	1066.45084	5.02	27.0	$C_{51}H_{62}O_{14}N_{12}$	
	1066.46342	-6.77	26.5	$C_{52}H_{64}O_{14}N_{11}$	
	1066.44631	9.27	35.5	$C_{60}H_{60}O_{10}N_9$	
	1066.46610	-9.28	31.0	$C_{55}H_{62}O_{11}N_{12}$	

HRMS (ESI) (m/z): [M] calcd. for $C_{56}H_{62}N_{10}O_{12}$, 1066.45487; found, 1067.45620.



5a





Elemental composition search on mass 1067.46

m/z= 1062.46-1072.46

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1067.4624	1067.4621	0.22	30.5	$^{12}C_{56}H_{63}O_{12}N_{10}$

[M + H]+

Elemental composition search on mass 1089.44

m/z= 1084.44-1094.44

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1089.4445	1089.4441	0.42	30.5	$^{12}C_{56}H_{62}O_{12}N_{10}{}^{23}Na_1$

[M + Na]+

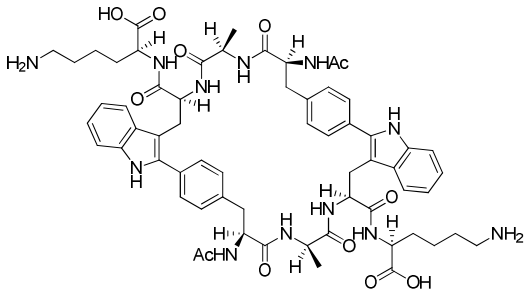
Elemental composition search on mass 1105.42

m/z= 1100.42-1110.42

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1105.4189	1105.4180	0.76	30.5	$^{12}C_{56}H_{62}O_{12}N_{10}{}^{39}K_1$

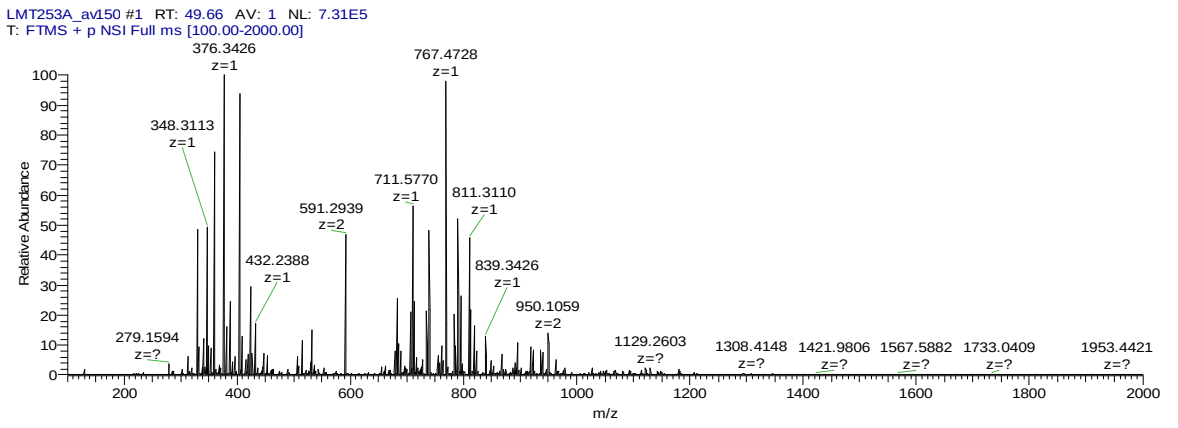
[M + K]+

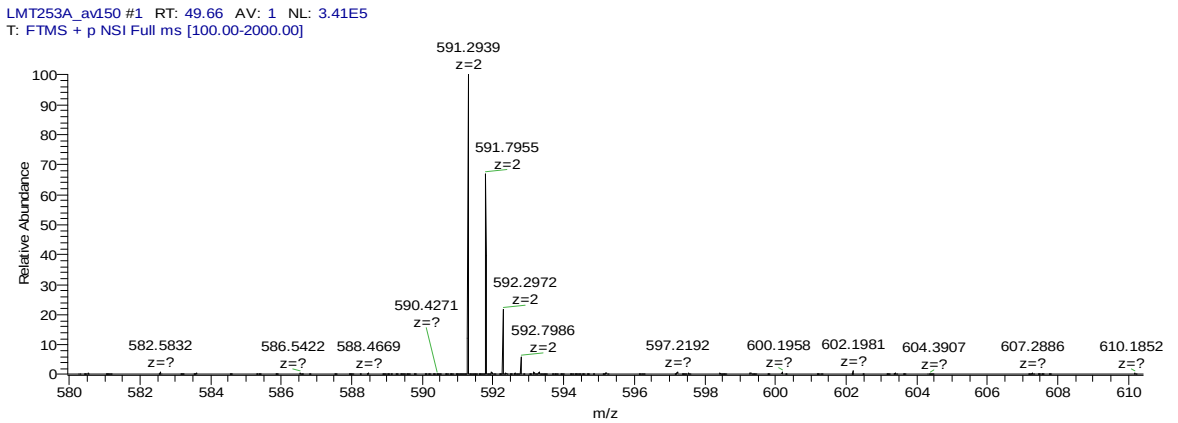
HRMS (ESI) (m/z): $[M+H]^+$ calcd. for $C_{56}H_{62}N_{10}O_{12}$, 1067.4621; found, 1067.4624.



5b

H2O/ACN 1%FA



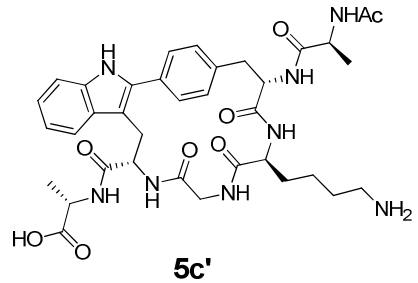


Elemental composition search on mass 591.29

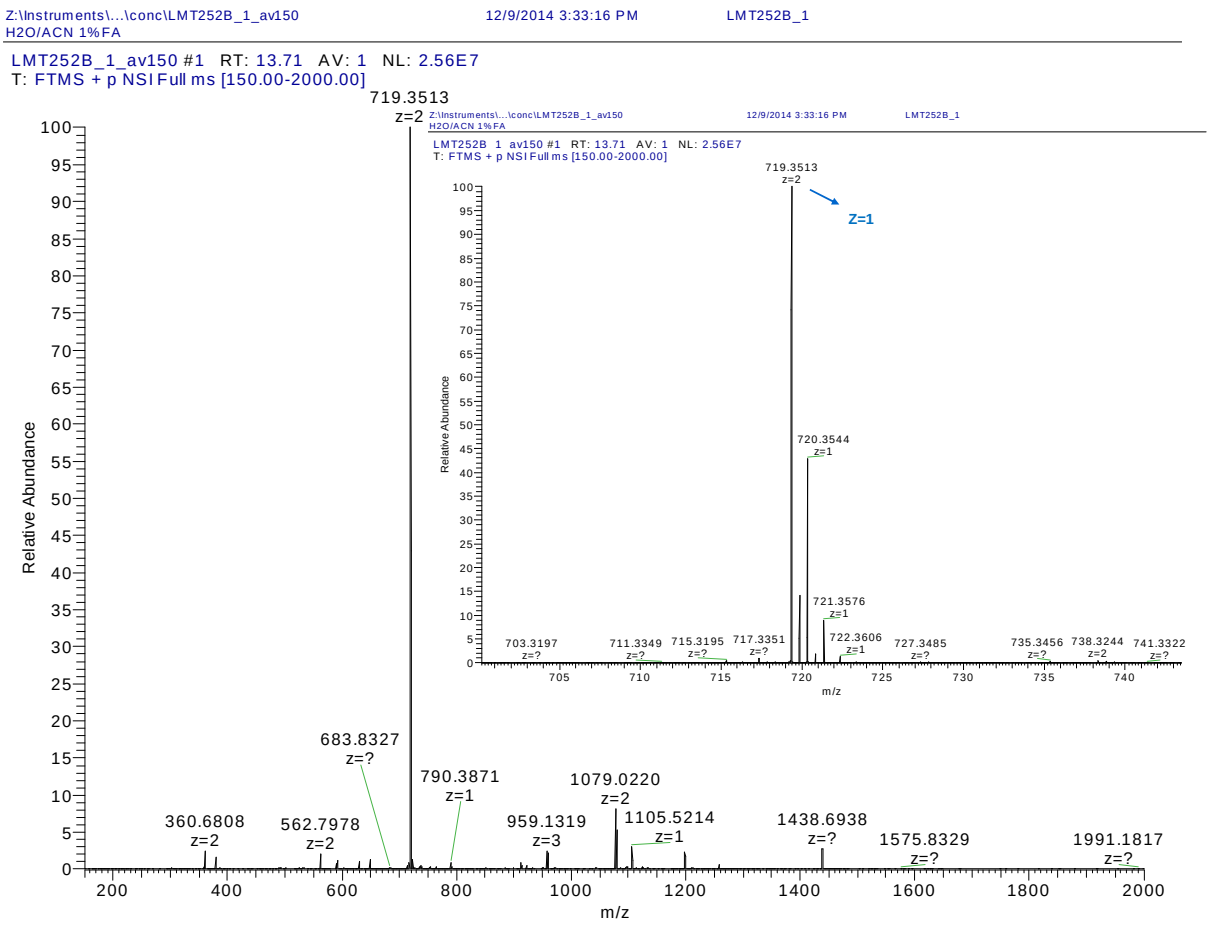
m/z= 586.29-596.29

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition	
591.29388	591.29390	-0.03	34.5	$C_{65}H_{76}O_9N_{13}$	
	591.29323	1.10	29.5	$C_{64}H_{80}O_{13}N_9$	
	591.29256	2.23	30.0	$C_{62}H_{78}O_{12}N_{12}$	← $[M+2H]^{2+}$
	591.29684	-5.00	25.5	$C_{58}H_{80}O_{14}N_{13}$	
	591.29055	5.64	26.0	$C_{57}H_{78}O_{14}N_{14}$	
	591.29818	-7.27	30.0	$C_{61}H_{78}O_{11}N_{14}$	
	591.29885	-8.40	29.5	$C_{63}H_{80}O_{12}N_{11}$	
	591.29952	-9.54	29.0	$C_{65}H_{82}O_{13}N_8$	

HRMS (ESI): (M: $C_{62}H_{76}O_{12}N_{12}$) m/z calcd 591.29390, found 591.29388 $[(M+2H)/2]^{2+}$.



5c'

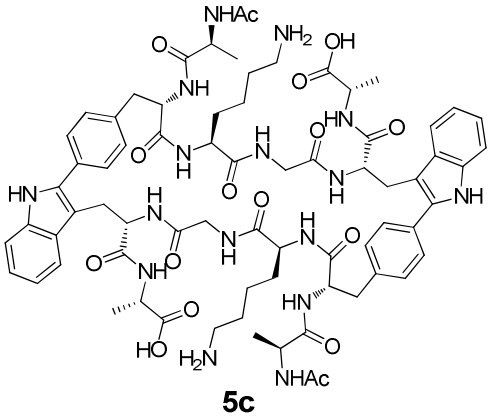


Elemental composition search on mass 719.35

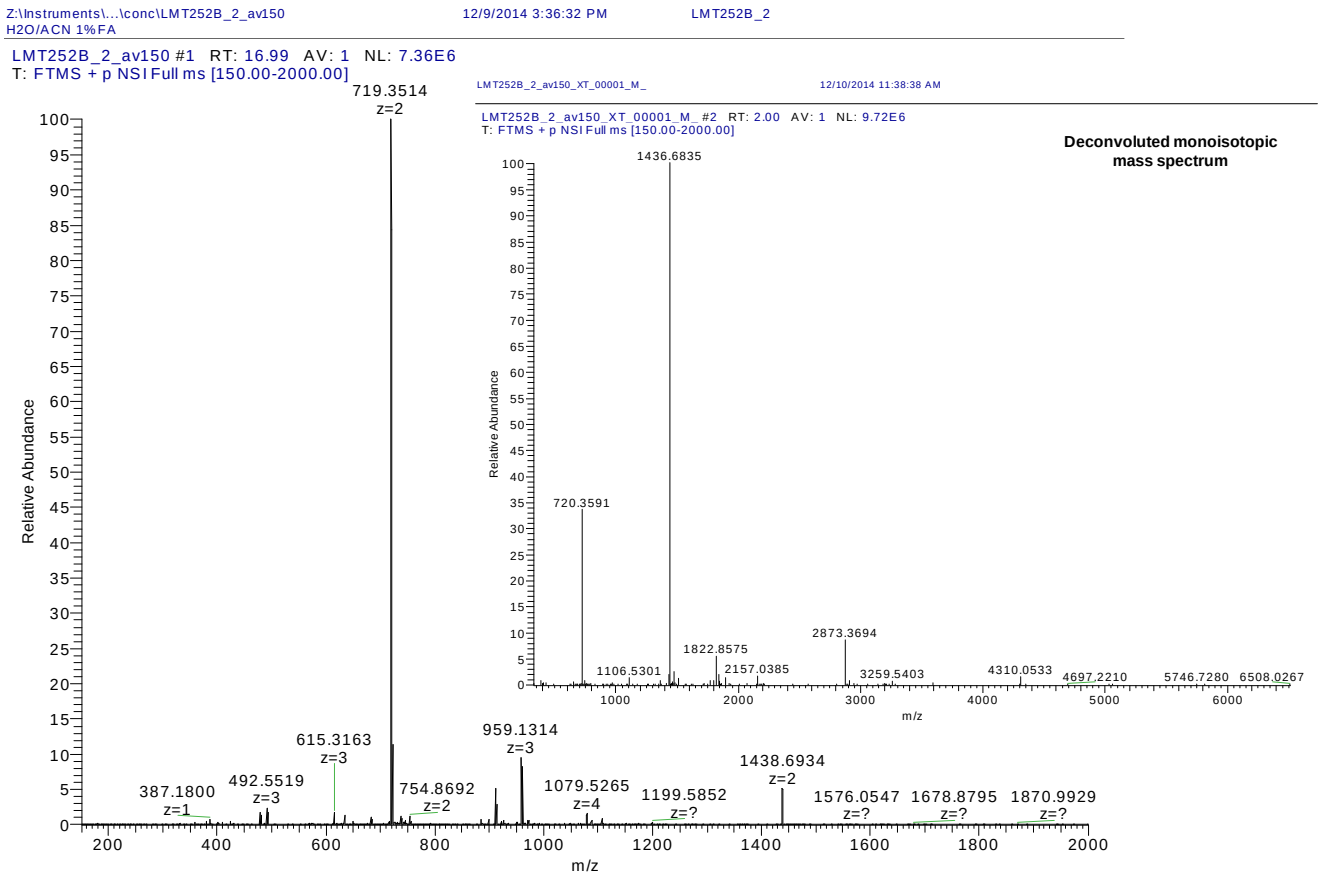
m/z= 714.35-724.35

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition	
719.35134	719.35114	0.28	17.5	$C_{36}H_{47}O_8N_8$	← [M+H]+
	719.35248	-1.58	17.0	$C_{38}H_{49}O_9N_5$	
	719.35382	-3.44	22.0	$C_{39}H_{45}O_5N_9$	

HRMS (ESI): (M: $C_{36}H_{46}N_8O_8$) m/z calcd. 719.35114, found 719.35134 $(M+H)^+$.



5c

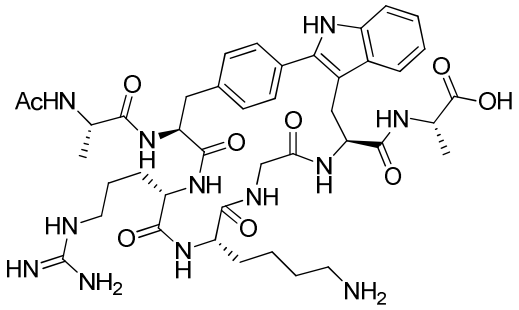


Elemental composition search on mass 1436.68

m/z= 1431.68-1441.68

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition	
1436.68351	1436.68772	-2.93	35.0	$C_{72}H_{92}O_{16}N_{16}$	← [M]
	1436.68906	-3.87	34.5	$C_{74}H_{94}O_{17}N_{13}$	
	1436.67649	4.89	35.0	$C_{73}H_{92}O_{17}N_{14}$	
	1436.67514	5.82	35.5	$C_{71}H_{90}O_{16}N_{17}$	

HRMS (ESI): (M: $C_{72}H_{92}N_{16}O_{16}$) m/z calcd. 1436.68772, found 1436.68351 (M).



5d'

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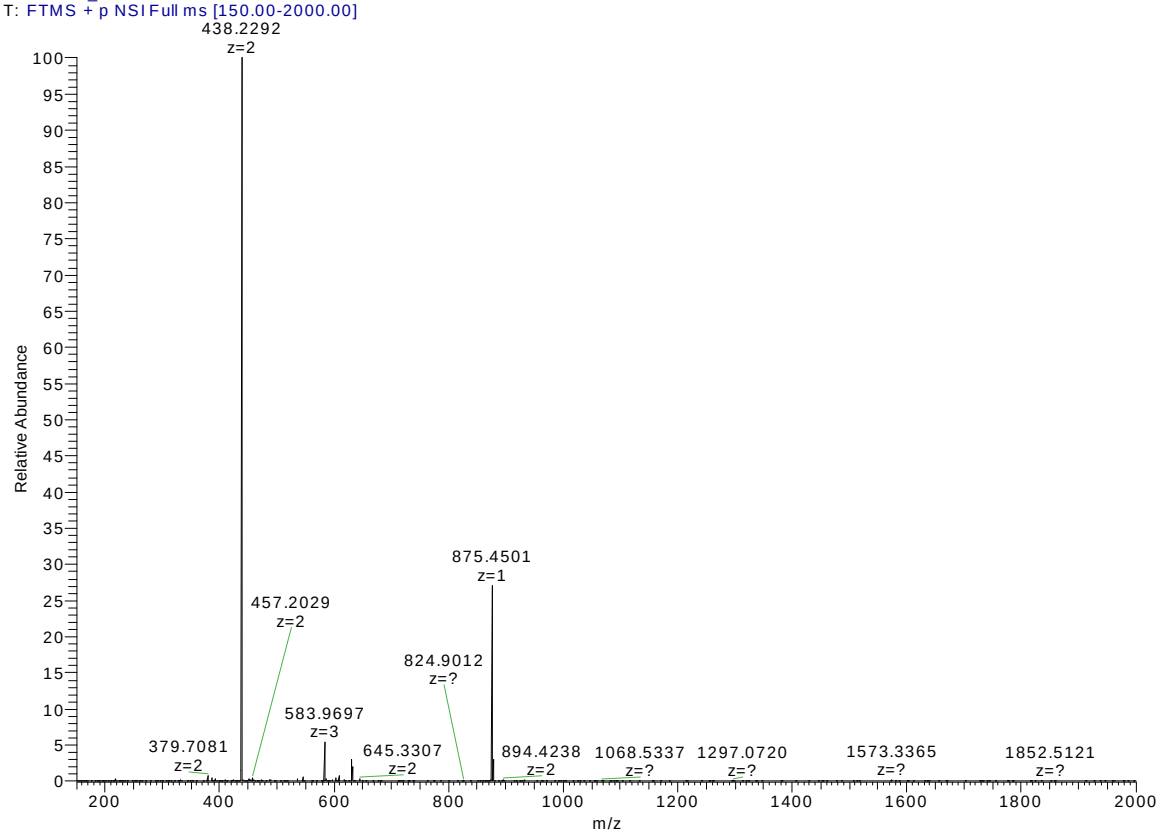
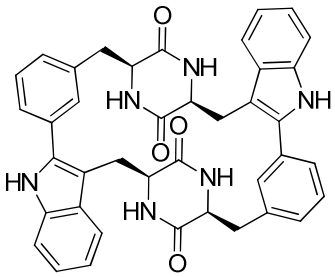


Elemental composition search on mass 875.45

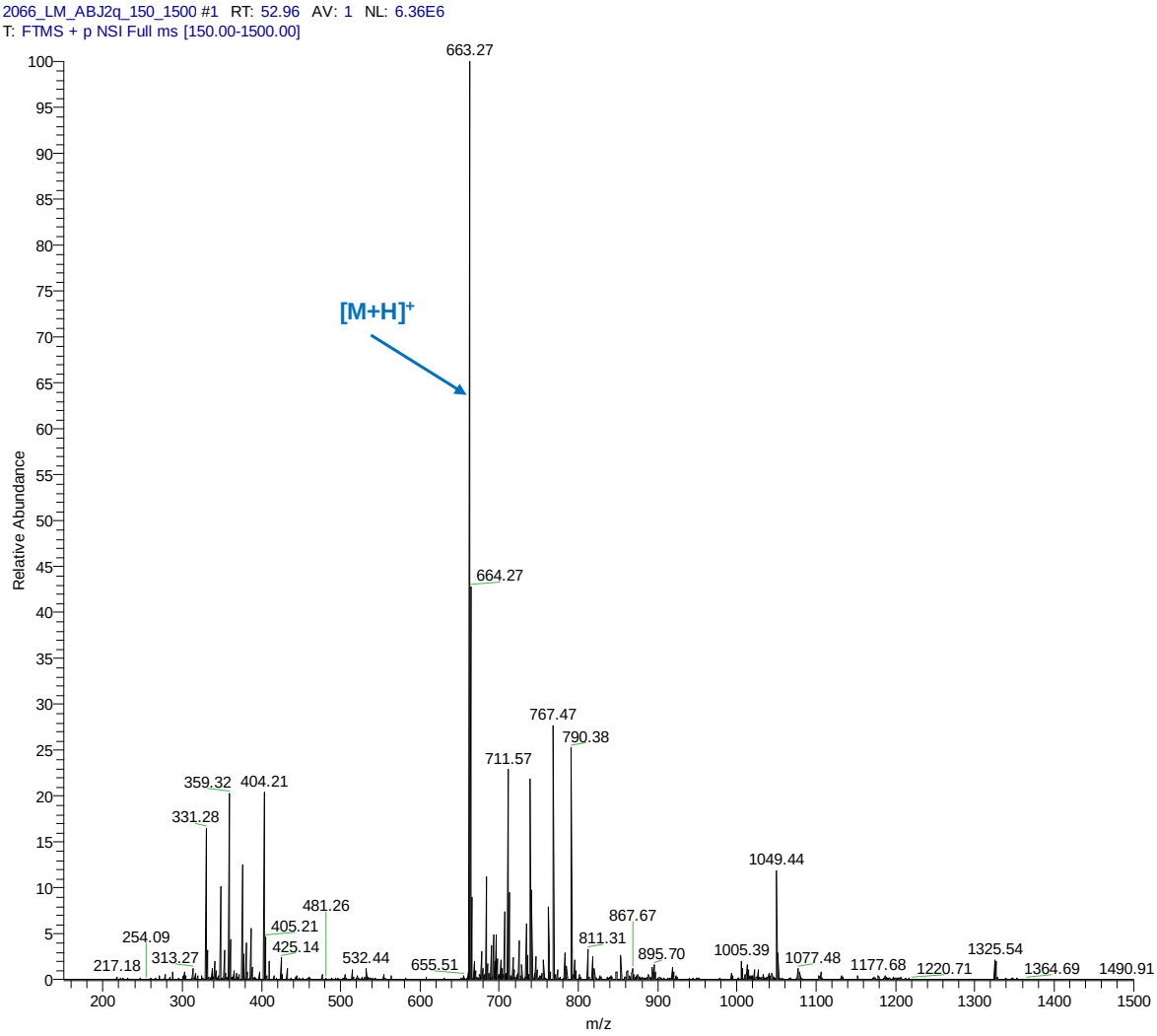
m/z= 870.45-880.45

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition	
875.45014	875.44822	2.19	15.5	$C_{37}H_{59}O_{11}N_{14}$	
	875.45225	-2.41	19.5	$C_{42}H_{59}O_{9}N_{12}$	← [M]
	875.45359	-3.94	19.0	$C_{44}H_{61}O_{10}N_{9}$	
	875.44235	8.90	24.5	$C_{44}H_{55}O_{6}N_{14}$	

HRMS (ESI): (M: $C_{42}H_{58}N_{12}O_9$) m/z calcd. 875.44822, found 875.45014 $(M+H)^+$.



7b

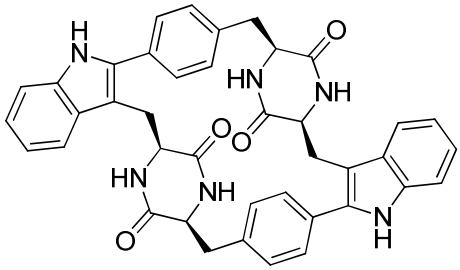


Elemental composition search on mass 663.27

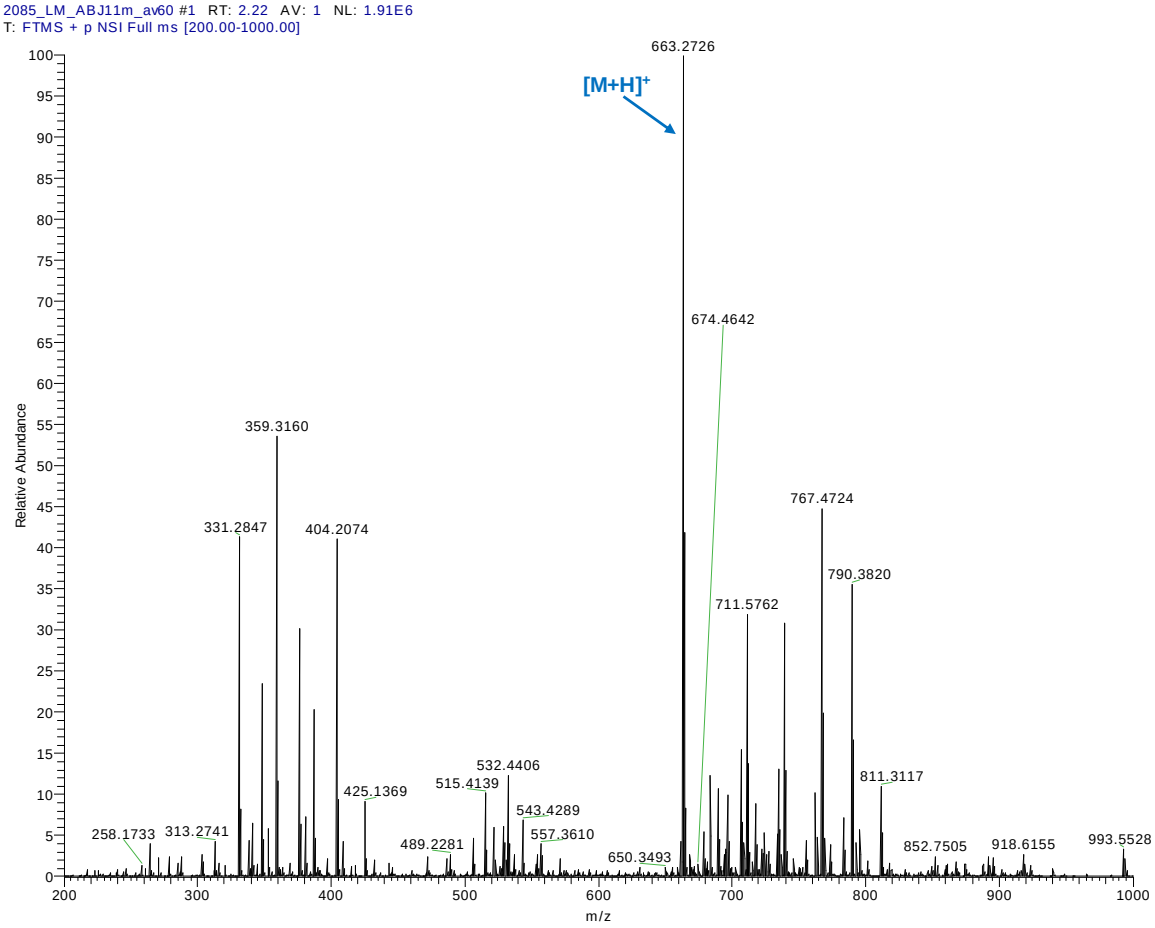
m/z= 658.27-668.27

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition	
663.2707	663.2714	-1.12	26.5	$^{12}C_{40}H_{35}O_4N_6$	$[M+H]^+$

HRMS (ESI): (M: $C_{40}H_{34}O_4N_6$) m/z calcd 663.2707, found 663.2714 $(M+H)^+$.



7c



Elemental composition search on mass 663.27

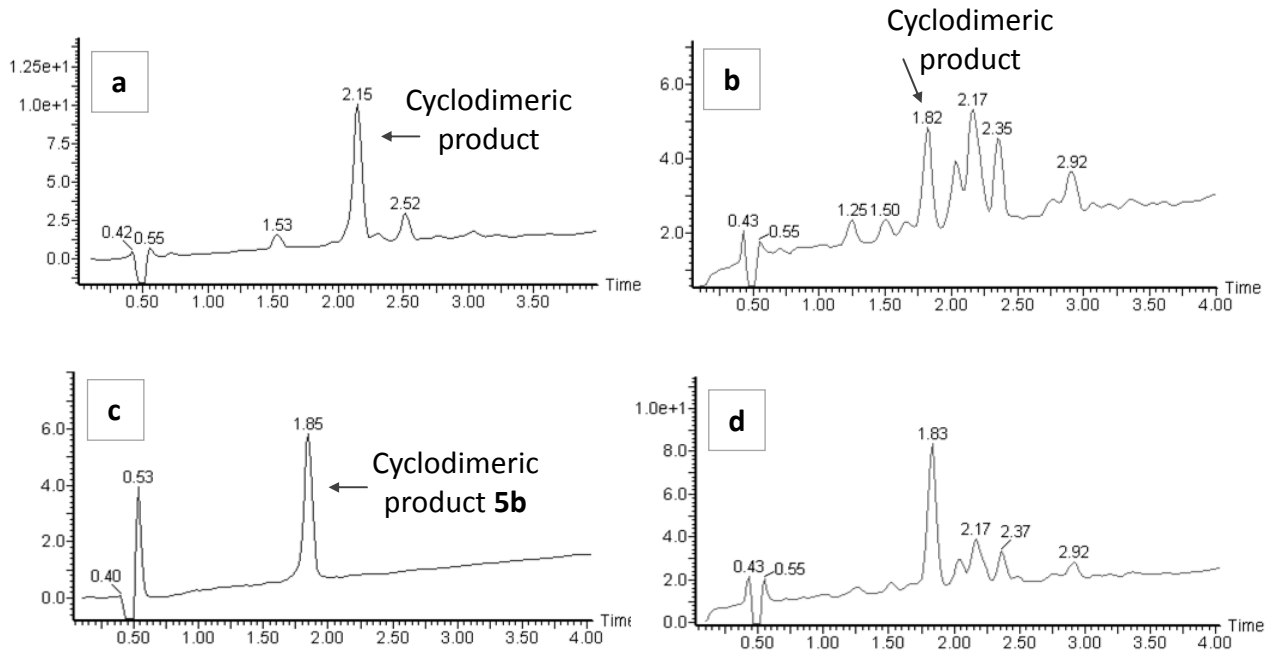
m/z= 658.27-668.27

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition	
663.2726	663.2714	1.69	26.5	$^{12}C_{40}H_{35}O_4N_6$	[M+H]+

HRMS (ESI): (M: $C_{40}H_{34}O_4N_6$) m/z calcd 663.2726, found 663.2714 $(M+H)^+$.

HPLC-MS chromatograms relative to C-H activation on resin

In a preliminary procedure, N-terminal acetylated Ala-m/p-I-Phe-Trp-Ala sequences anchored to a low functionalized TentaGel resin (f: 0.9 mmol/g resin) provided with (3-(4-hidroxymethylphenoxy)-propionic acid linker were subjected to a Pd-catalyzed coupling on-resin. The same trends were obtained compared to solution C-H arylation procedure and the corresponding cyclodimers were detected.



[a] Intermolecular C-H activation of Ala-*m*-I-Phe-Trp-Ala sequence on-resin to yield presumably the corresponding cyclodimeric product. [b] C-H activation of Ala-*p*-I-Phe-Trp-Ala sequence on-resin to yield the corresponding cyclodimeric product. [c] Isolated cyclodimeric product **5a**. [d] Co-elution of the crude relative to Ala-*p*-I-Phe-Trp-Ala C-H activation reaction with the isolated cyclodimeric product **5a**. Reaction conditions: 5 mol % $Pd(OAc)_2$, $AgBF_4$ (1.0 eq.), 2-nitrobenzoic acid (1.5 eq.) in DMF, MW 90 ºC, 20 min. All the peptides were cleaved from the resin with TFA-DCM (95:5), r.t, 1h to be analyzed by HPLC-MS. Gradient from 30 to 50% ACN (0,1% FA).

Emac values of compounds 3 and 5

Recently, James group reported a quantitative index for the macrocyclization efficiency (Emac) which is proportional to the concentration and the yield of the reaction:[5]

$$Emac = \log_{10}[Y^3 \times C]$$

where, Y = yield in %; C = concentration in mM

We calculated the Emac values for the following peptides:

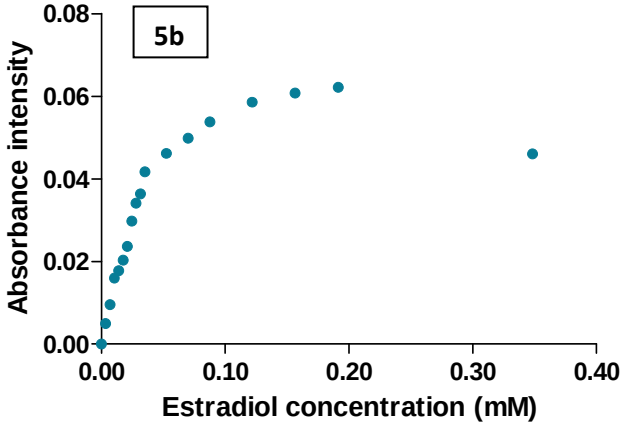
Compound	[C] (mM)	Yield (%)[a]	Emac
Ac-(Cyclo-*m*)-[**Phe**-Asn-Gly-Arg-**Trp**]-OH[3]	110	77	7.70
Ac-(Cyclo-*m*)-[**Phe**-Arg-Gly-Asp-**Trp**]-NH_2[3]	112	70	7.58
H-Ala-(Cyclo-*m)*-[**Phe**-Ser-Ala-**Trp**]-Ala-OH[3]	78	39	6.66
Ac-Ala-(Cyclo-*m)*-[**Phe**-Val-**Trp**]-Ala-OH[3]	187	71	7.83
(Cyclo-*m,m)*bis-[Phe-Trp]-(Ac-Ala-**Phe**-**Trp**-Ala-OH) (**3**)	151	48	7.22
(Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-**Phe**-**Trp**-Ala-OH) (**5a**)	151	41	7.02
(Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-**Phe**-Ala-**Trp**-Lys-OH) (**5b**)	240	54	7.58
(Cyclo-*p,p)*bis-[Phe-Trp]-(Ac-Ala-**Phe**-Lys-Gly-**Trp**-Ala-OH) (**5c**)	236	23	6.46
Ac-Ala-(Cyclo-*p)*-[**Phe**-Lys-Gly-**Trp**]-Ala-OH (**5c'**)	236	51	7.50
Ac-Ala-(Cyclo-*p)*-[**Phe**-Arg-Lys-Gly-**Trp**]-Ala-OH (**5d'**)	100	81	7.72

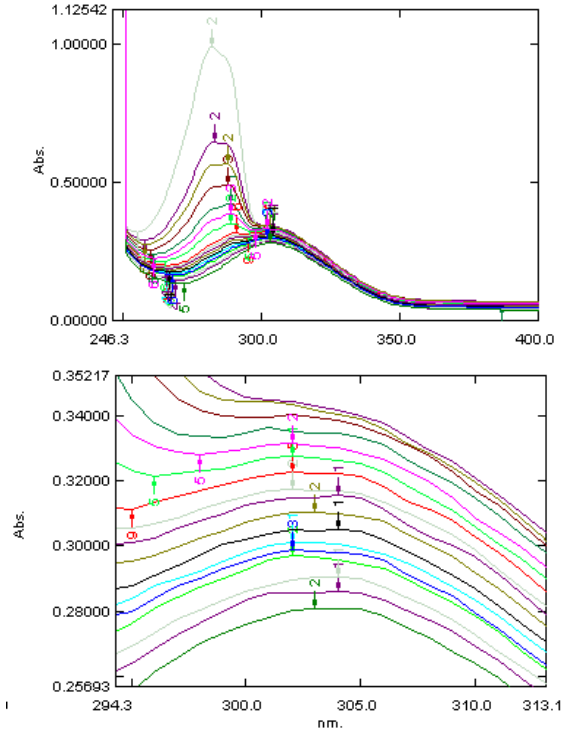
[a] Yields are from conversions estimated by HPLC-MS.

Preliminary absorbance host-guest complexation experiments of compounds 5b and 5c

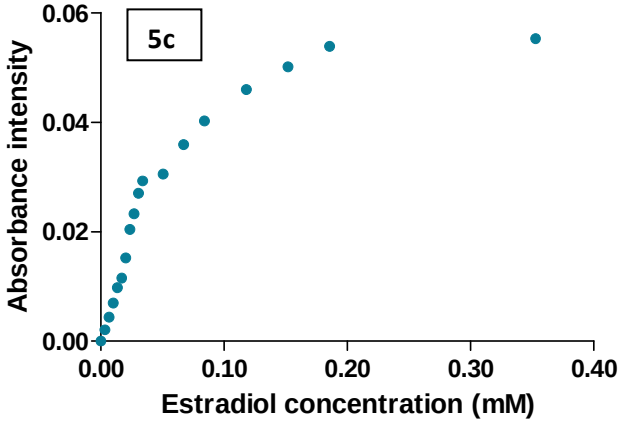
Increasing amounts of estradiol were added to **5b** or **5c** 0.01 mM solutions in MeOH and the corresponding absorbance intensity changes were measured with a UV-VIS.

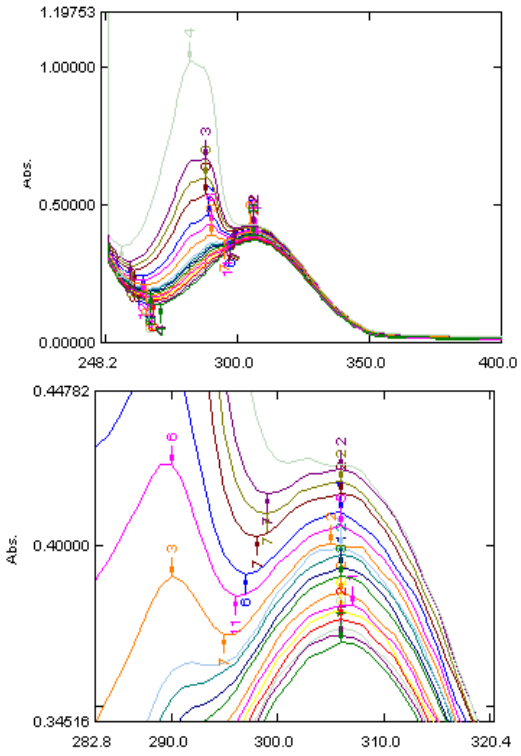
a)





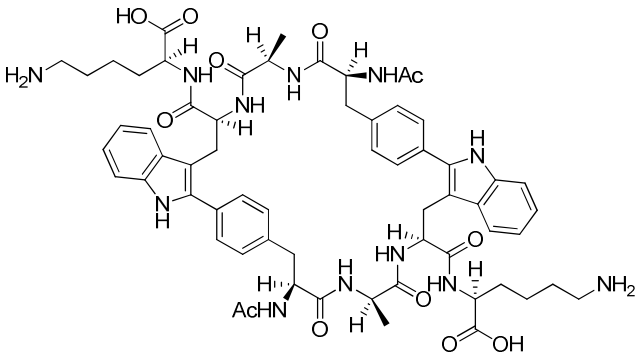
b)



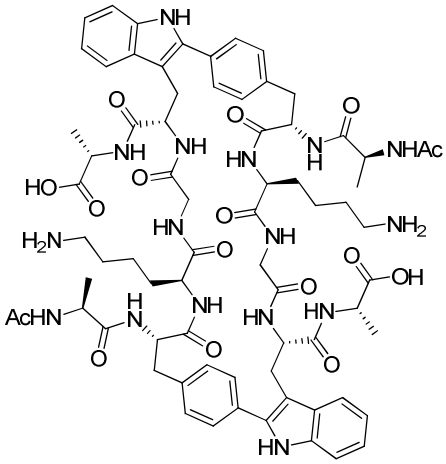


Absorbance intensity changes in MeOH of (a) **5b** (0.015 mM) at λ = 303 nm upon the addition of estradiol (from 0 to 0.35 mM) and (b) **5c** (0.014 mM) at λ = 306 nm upon the addition of estradiol (from 0 to 0.35 mM).

Taking into account that the presence of charged or hydrophilic amino acids (i.e. Lys) in the hosts may improve the binding with anionic guests,[5] we have prepared the cyclodimers **5b** and **5c**. However, the study of the interaction with cholic acid did not show strong evidences of binding.

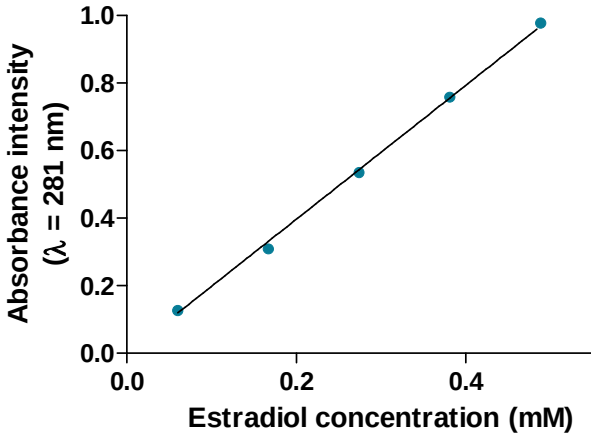


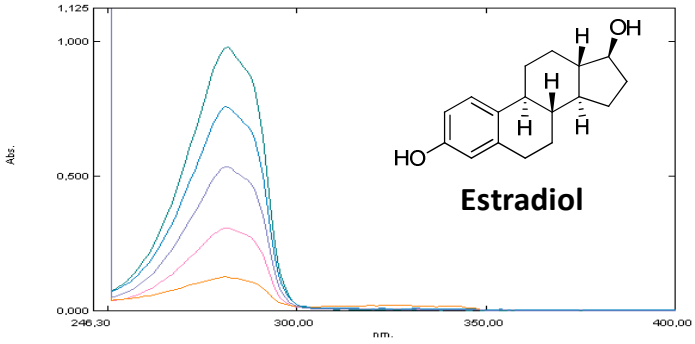
5b



5c

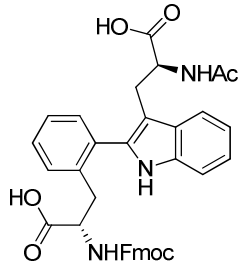
As a negative control, the absorbance intensity of Increasing estradiol solutions was measured at λ = 281, 303 and 306 nm.



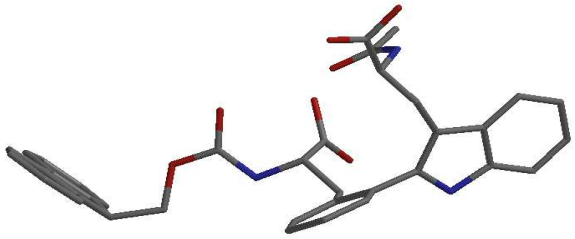


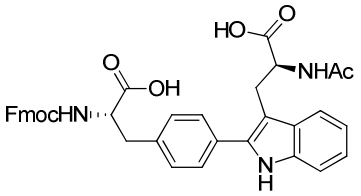
[estradiol] mM	A (λ: 281 nm)	A (λ: 303 nm)	A (λ: 306 nm)
0.488	0.977	0.005	0.014
0.381	0.758	0.009	0.016
0.274	0.535	0.006	0.011
0.167	0.309	0.007	0.010
0.060	0.126	0.015	0.015

Minimized geometries of compounds 1, 3, 5 and 7 generated by the Spartan '14 suite (Semi-Empirical, AM1)[4] Hydrogens omitted for clarity.

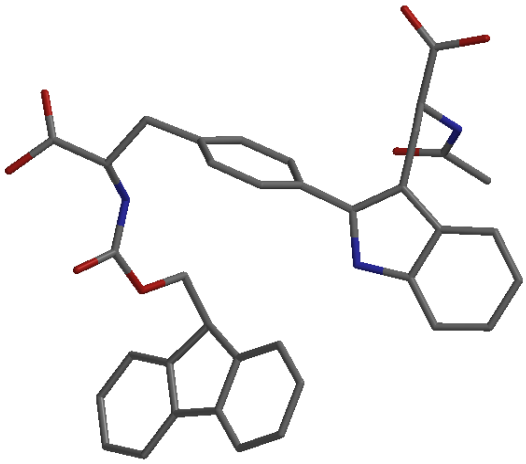


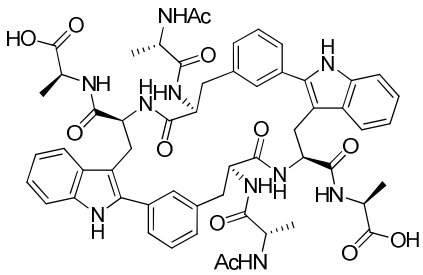
1a



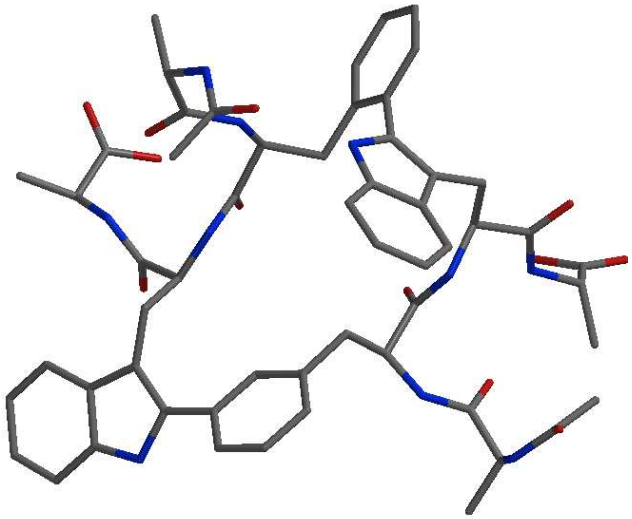


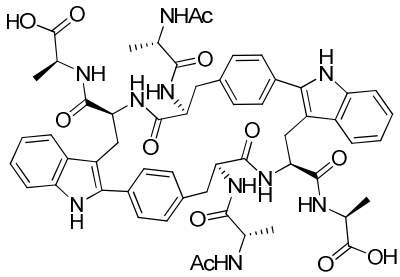
1b



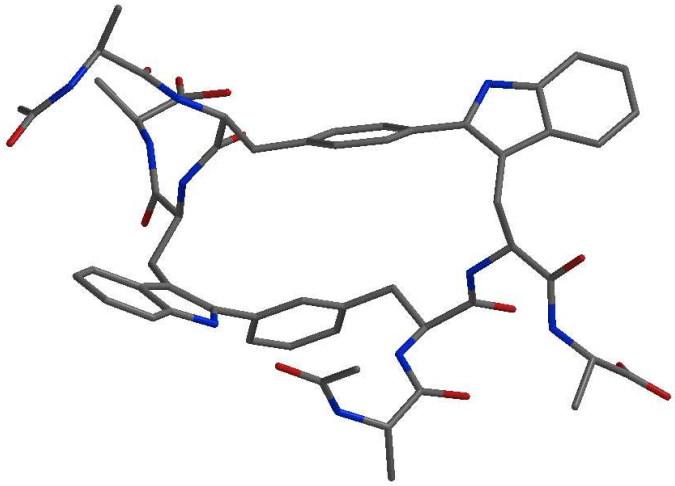


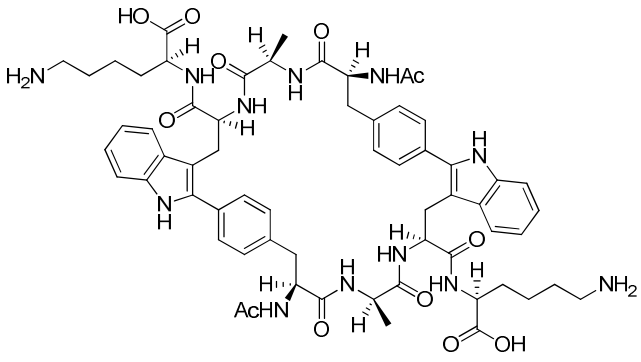
3



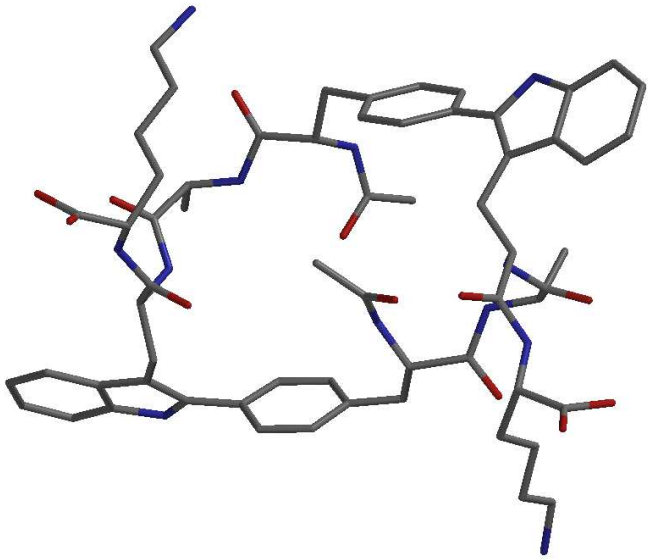


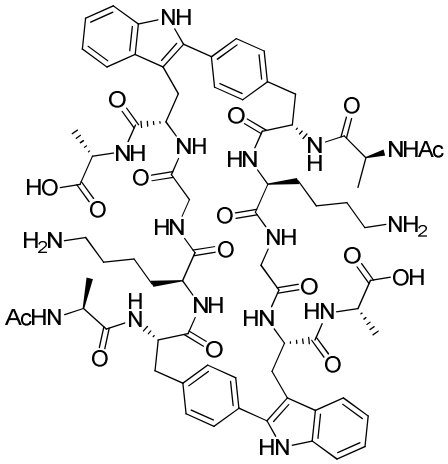
5a



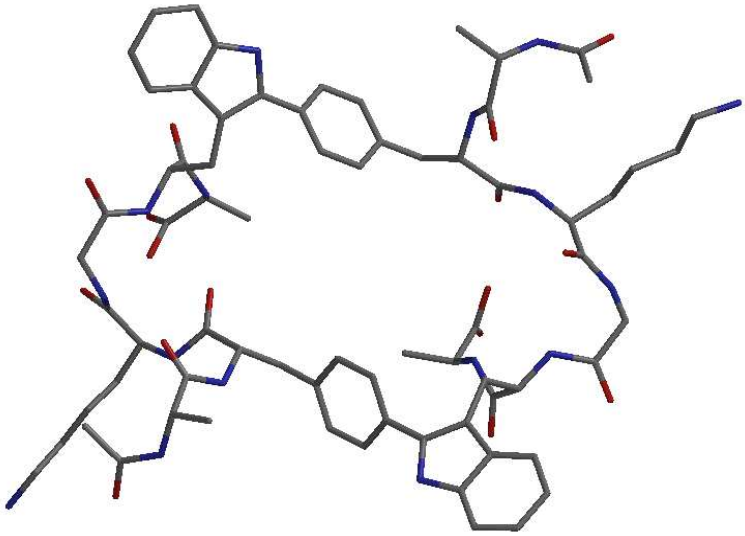


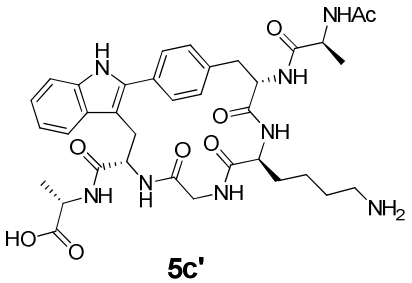
5b



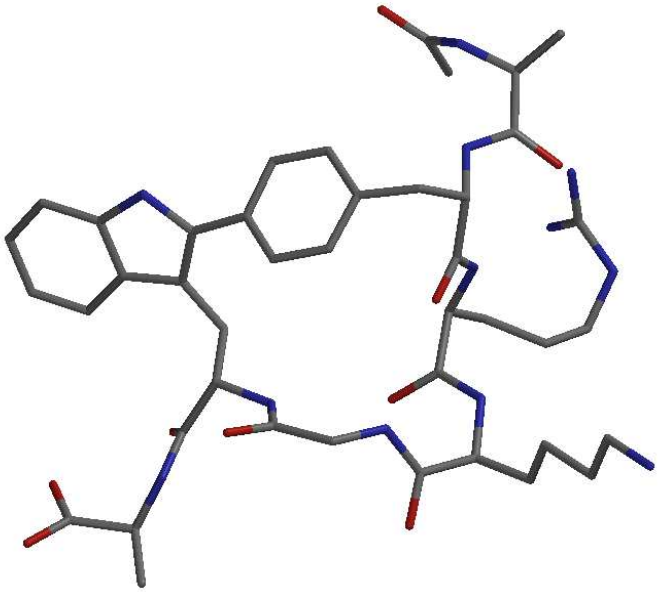


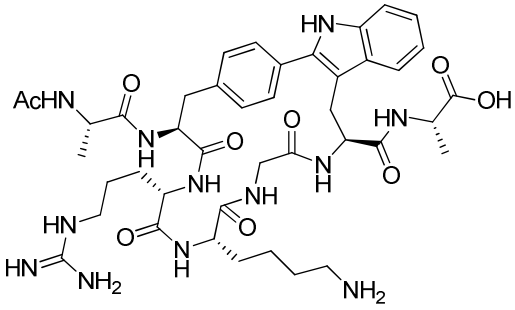
5c



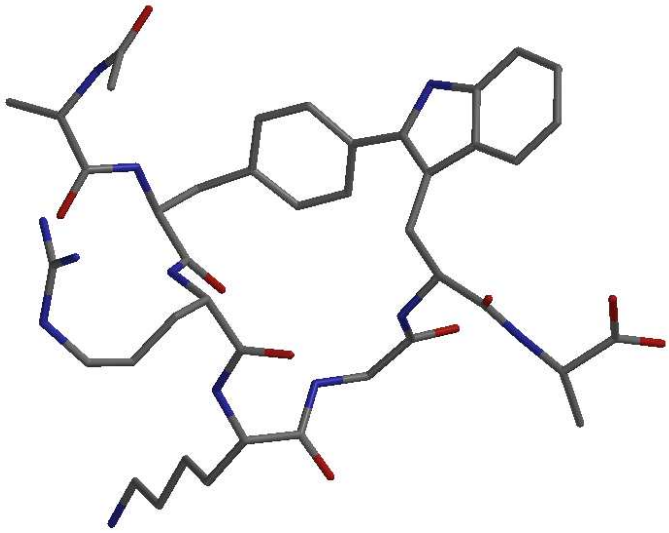


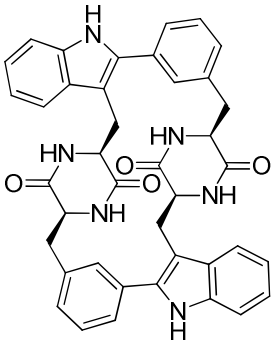
5c'



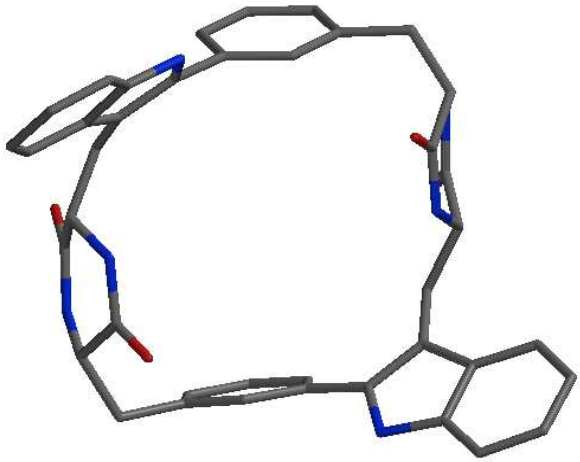


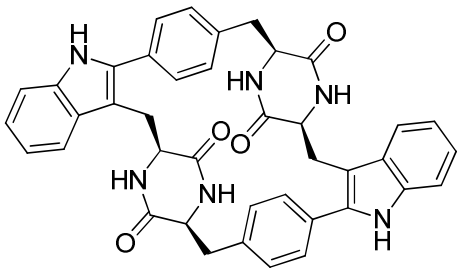
5d'





7b





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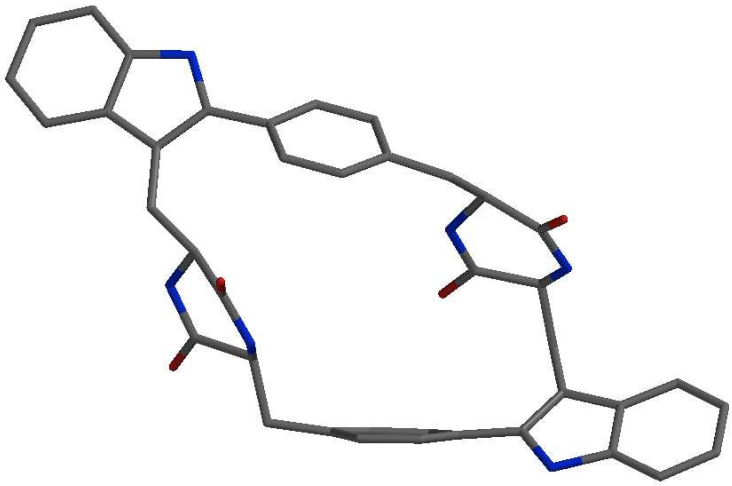
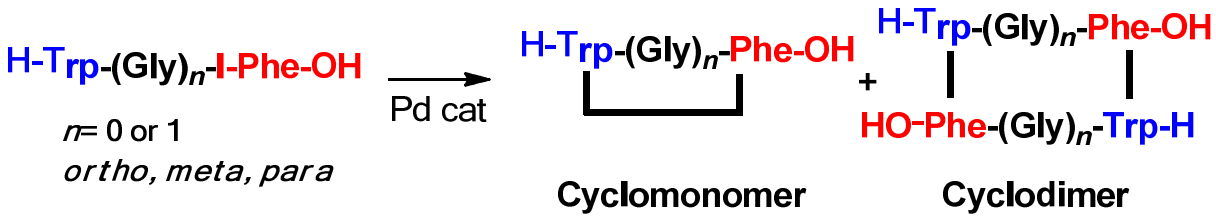


Preliminary calculations on the relative stabilities of the cyclic/cyclodimeric peptides after the Pd-catalyzed CH activation reaction

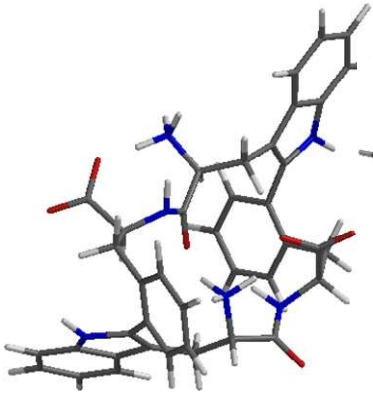
Estimation of the stabilities of the putative cyclic and cyclodimeric peptides arising from the Pd-catalyzed C-H activation upon the parent Trp-(Gly)$_n$-(I)Phe peptides, displaying different spacer lengths (*n*= 0 or 1) and regiochemistries (*ortho*, *meta* and *para*).



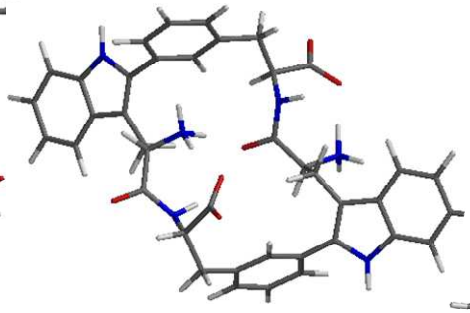
Data referring to the equilibrium geometries of the products, optimized through molecular mechanics (MMFF) and semiempirical methods (PM3), implemented in a Spartan suite.[4] For the sake of comparison, and as a rule of thumb, the cyclodimers may account for twice the enthalpy barrier to formation of their cyclic counterparts.

SIZE/Regio	CYCLOMONOMER calc. Enthaply barrier to fomation (kJ/mol)	CYCLOMONOMER exp. Detection	CYCLODIMER calc. Enthalpy barrier to formation (kJ/mol)	CYCLODIMER exp. Detection
0-*ortho*	-58.451	×	-222.670	×
0-*meta*	-74.395	×	-283.809	**ISOLATED**
0-*para*	-26.863	×	-286.967	**ISOLATED**
1-*ortho*	-237.066	×	-709.951	×
1-*meta*	-273.213	**ISOLATED**	-708.951	×
1-*para*	-251.280	×	-737.138	**ISOLATED**

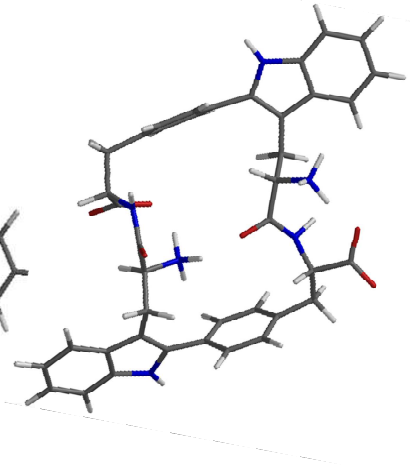
0-Trp-Phe *ortho*-Cyclodimer



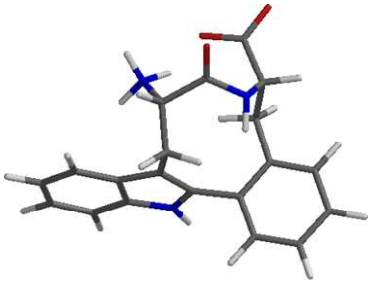
0-Trp-Phe *meta*-Cyclodimer



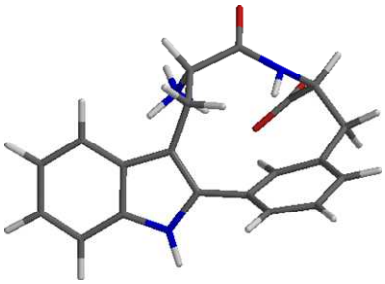
0-Trp-Phe *para*-Cyclodimer



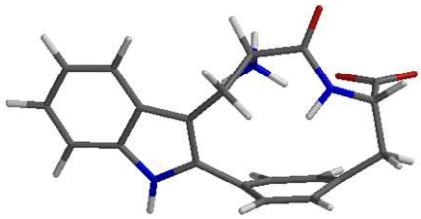
0-Trp-Phe *ortho*-Cycle



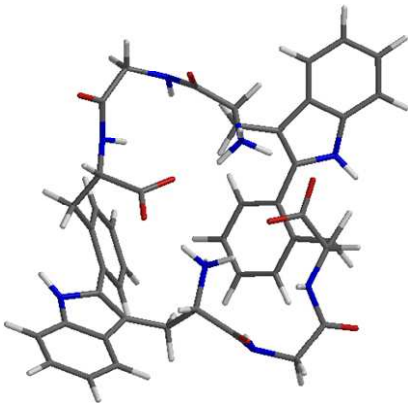
0-Trp-Phe *meta*-Cycle



0-Trp-Phe *para*-Cycle



1-Trp-Phe *ortho*-Cyclodimer



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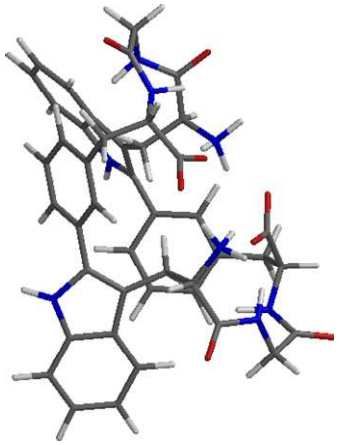

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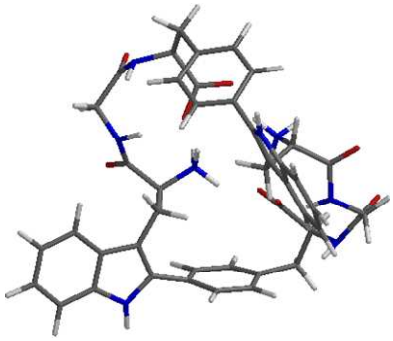

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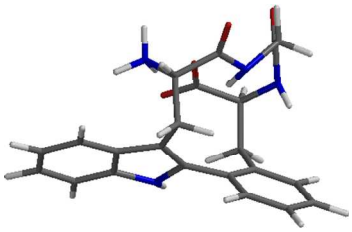

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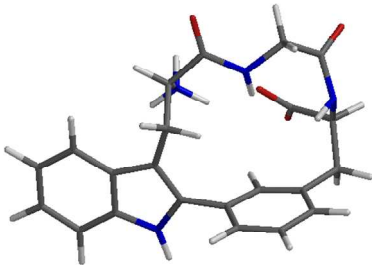

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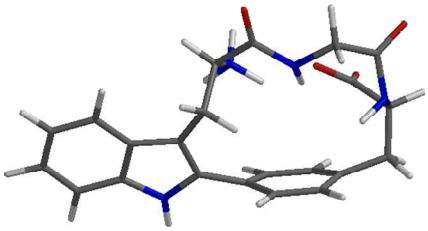
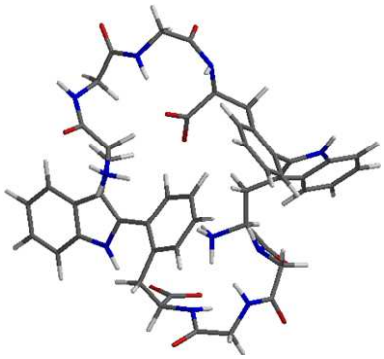
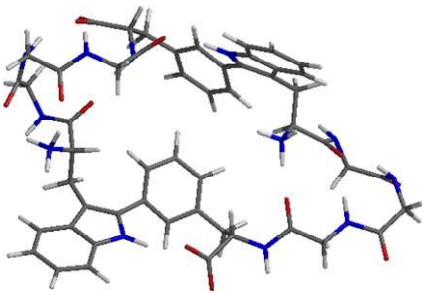


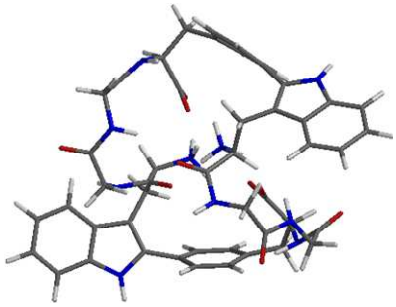
2-Trp-Phe *ortho*-Cyclodimer



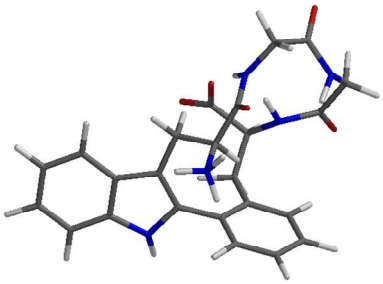
2-Trp-Phe *meta*-Cyclodimer



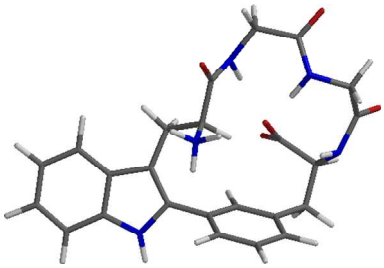
2-Trp-Phe *para*-Cyclodimer



2-Trp-Phe *ortho*-pCycle



2-Trp-Phe *meta*-Cycle



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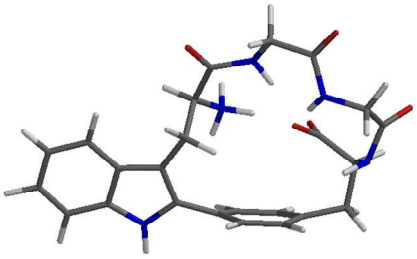

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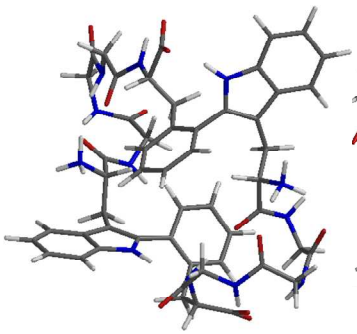

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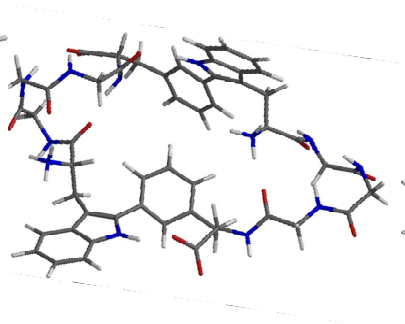

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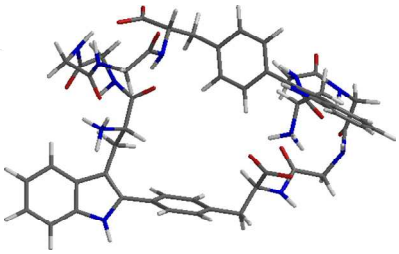

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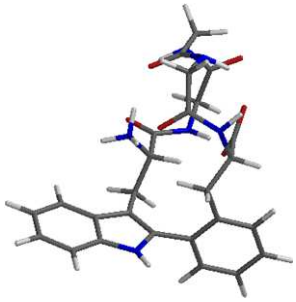

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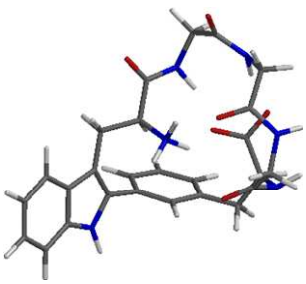

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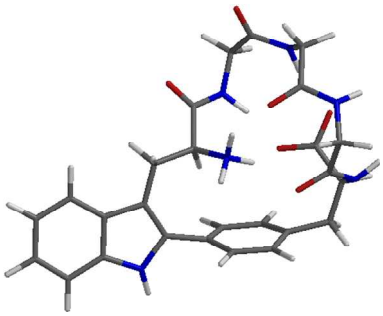

Although qualitatively, the preliminary estimations fit quite reasonably with the observed results for the models with smaller size (*n*= 0, 1).

Additional calculations on the remaining series (*n*=2, 3) are not conclusive at this point due to the fact that the conformational space relative to these compounds is much bigger and the possibility of dealing with local minima may bias the calculations. This makes difficult an appraisal of the chemical evolution regarding cyclization or cyclodimerization. Even so, it has to be said, that for these species, if the cyclization is energetically accessible, although it may not be as stable as the cyclodimer, likely it may be generated at faster rates because of entropic factors.

Ortho-regiochemistries seem to lead to strained geometries, which for the cases analyzed (*n*= 0, 1), no adducts have been detected (neither cyclomonomers nor cyclodimers). On the other hand, *meta*-arrangements, lead to very strained unstable structures on the peptide featuring adjacent amino acids (*n*=0), then leading to cyclodimer; whereas for the spacer of 1 Gly, the corresponding cyclic peptide has been isolated. The *para* isomers lead to the more stable cyclodimers for both *n*=0 and 1, likely because the putative cyclic peptides should be very unstable, featuring clearly non-planar benzene rings.

It has to be said that these processes are formally irreversible, and arise from a complex palladium-catalytic cycle, therefore the estimation of the activation barriers for the key steps, although relevant for the physicochemical interpretation of the process, should be extremely challenging. Furthermore, these barriers probably may display similar trends than the relative stabilities of the compounds.

The evidence that compounds **5b** and **5c** show broad ^{1}H NMR spectrum in contrast with **5a** which displayed sharp peaks, which suggests that backbone flexibility increases with the size of the polypeptide chain, reinforces the computational problems seen in this section.

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