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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Supporting Information

General experimental information.

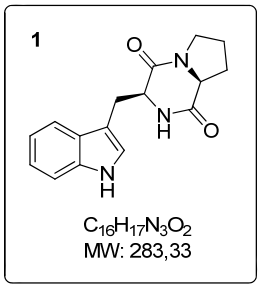
Unless stated otherwise, all reactions were carried out under argon atmosphere in dried glassware. Commercially available reactants were used without further purification. Thin-layer chromatography was performed on pre-coated Merk silica gel 60 F254 plates and visualized under a UV lamp. ^{1}H and ^{13}C 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

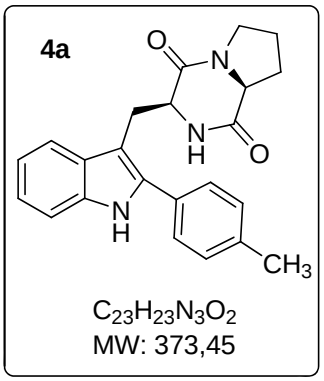
allowed to stand for 1 h at rt with occasional manual stirring. The resin was then washed with DCM (5×30s). Fmoc-Trp-OAl (1.3 g, 2.79 mmol) and PPTS (1.1 g, 4.38 mmol) in DCE were then added to the handle-resin and the suspension was shaken at 80 °C for 16 h in an Advanced Chemtech PLS 4x4 organic synthesizer. 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)

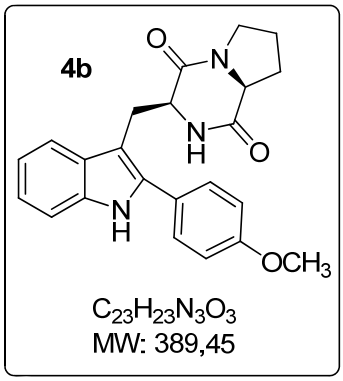


$C_{23}H_{23}N_3O_2$
MW: 373,45

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)

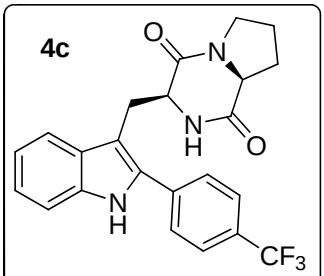


$C_{23}H_{23}N_3O_3$
MW: 389,45

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)

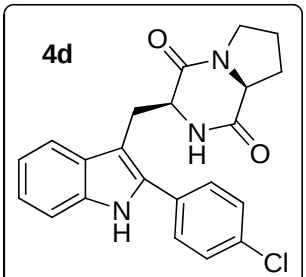


$C_{23}H_{20}F_3N_3O_2$
MW: 427,42

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)

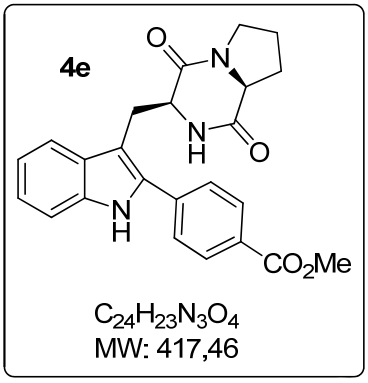


$C_{22}H_{20}ClN_3O_2$
MW: 393,87

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 $(M+H)^+$.

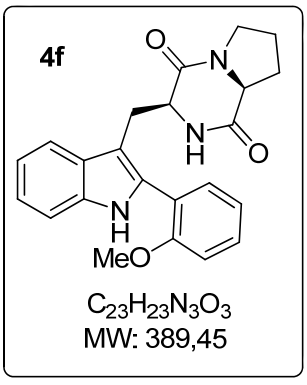
Methyl 4-(3-(((3S,8aS)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indol-2-yl)benzoate (4e)



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 $(M+H)^+$.

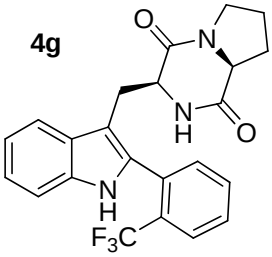
(3S,8aS)-3-((2-(2-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4f)



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)

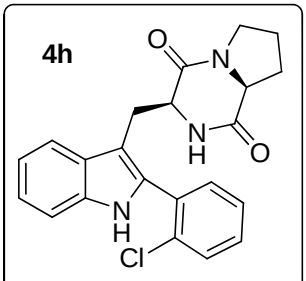


$C_{23}H_{20}F_3N_3O_2$
MW: 427,42

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)

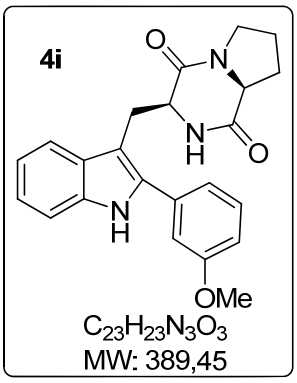


$C_{22}H_{20}ClN_3O_2$
MW: 393,87

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 $(M+H)^+$.

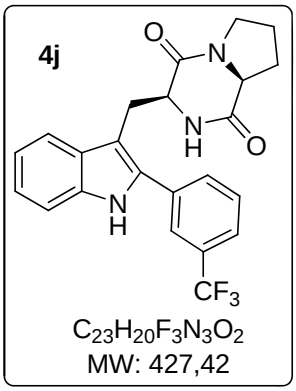
(3S,8aS)-3-((2-(2-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4i)



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 $(M+H)^+$.

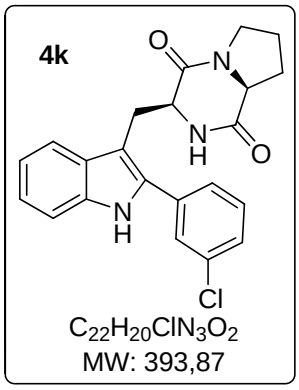
(3S,8aS)-3-((2-(3-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4j)



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 $(M+H)^+$.

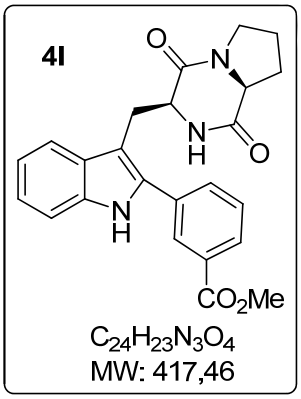
(3S,8aS)-3-((2-(3-Chlorophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4k)



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 $(M+H)^+$.

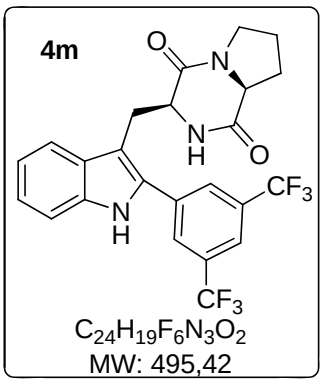
Methyl 3-(3-(((3S,8aS)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indol-2-yl)benzoate (4l)



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)

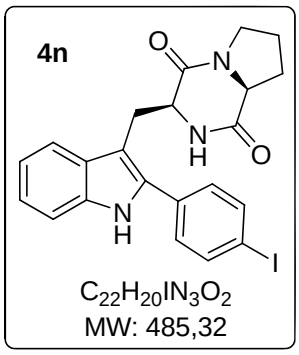


$C_{24}H_{19}F_6N_3O_2$
MW: 495,42

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 %).

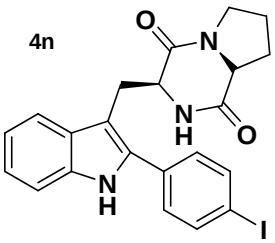
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.92 (s, 1H), 8.04 – 8.00 (m, 2H), 7.87 (s, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.32 – 7.15 (m, 2H), 5.50 (s, 1H), 4.50 – 4.37 (m, 1H), 4.03 (t, *J* = 7.5 Hz, 1H), 3.90 (dd, *J* = 15.3, 4.1 Hz, 1H), 3.68 – 3.48 (m, 2H), 3.15 (dd, *J* = 15.3, 11.2 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 $(M+H)^+$.

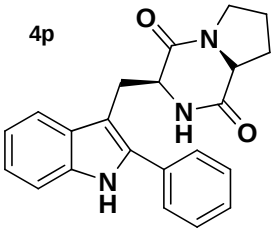
(3S,8aS)-3-((2-(4-Iodophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4n)

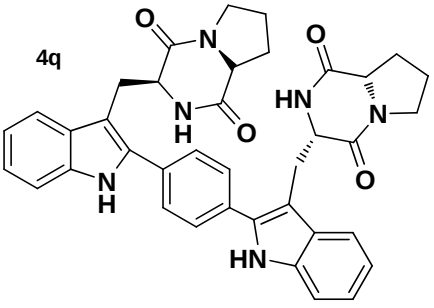


$C_{22}H_{20}IN_3O_2$
MW: 485,32

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).



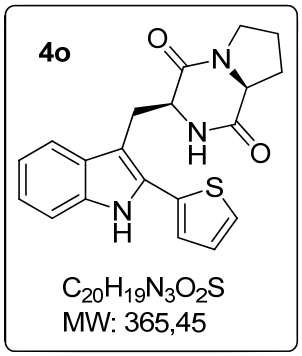




The crude product was purified by flash chromatography on silica using 80-90 % ethyl acetate to obtain **4n** as a pale solid (53.1 mg, 7.2%).

^{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 $(M+H)^+$.

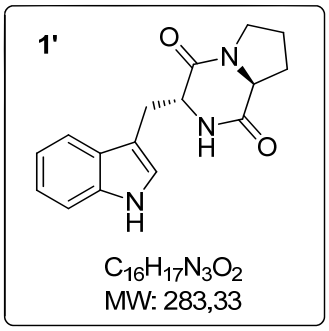
Synthesis of arylated diketopiperazines 4c'-4c'''

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.

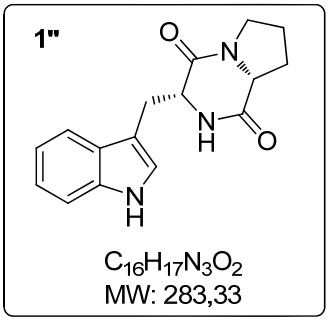
(3*R*,8a*S*)-3-((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (1')



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'')

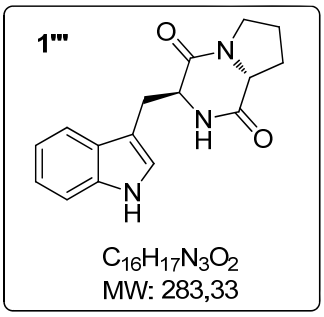


Compound **1'** was prepared according to the general procedure using Z-D-Trp-OH (1.74 g, 5.14 mmol) and H-D-Pro-OMe·HCl (0.85 g, 5.14 mmol). After filtration through Celite the solvent was removed affording a foamy white solid (**1'**) (1.20 g, 83 %).

^{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''')

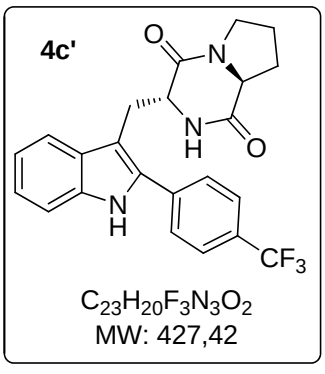


$C_{16}H_{17}N_3O_2$
MW: 283,33

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')



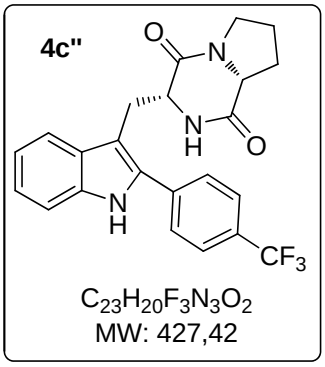
$C_{23}H_{20}F_3N_3O_2$
MW: 427,42

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 $(M+H)^+$.

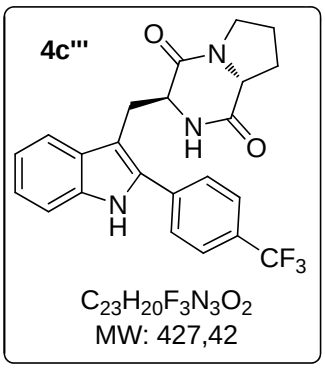
(3*R*,8a*R*)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (4c'')



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''')



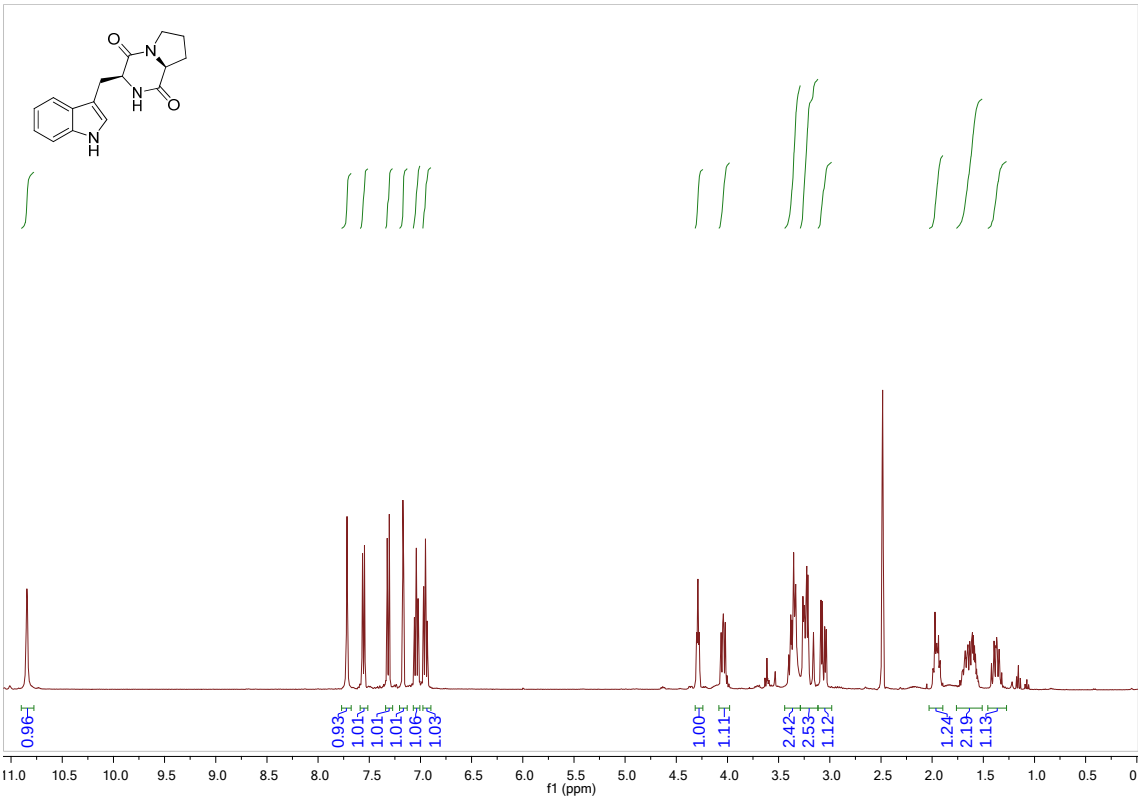
Compound **4c'''** was prepared according to the general procedure using 1-iodo-4-(trifluoromethyl)benzene (170 µL, 1.13 mmol). The crude product was purified by flash chromatography on silica using 97 % ethyl acetate to obtain **4c'''** as a pale solid (89.8 mg, 56 %).

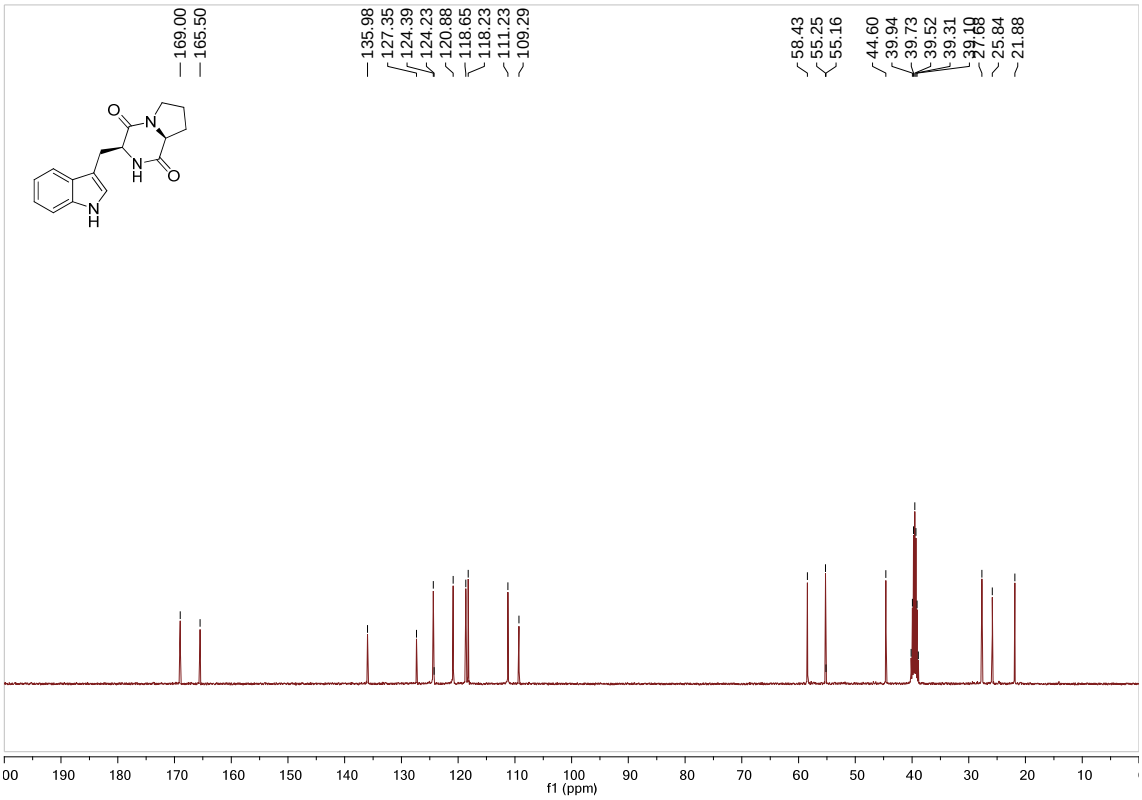
^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.38 (s, 1H), 7.64 – 7.60 (m, 2H), 7.59 – 7.52 (m, 3H), 7.34 – 7.30 (m, 1H), 7.21 – 7.16 (m, 1H), 7.11 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 5.46 – 5.32 (m, 1H), 4.19 – 4.09 (m, 1H), 3.48 – 3.36 (m, 2H), 3.32 (dd, *J* = 14.7, 4.9 Hz, 1H), 3.08 (ddd, *J* = 12.1, 9.4, 2.7 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 $(M+H)^+$.

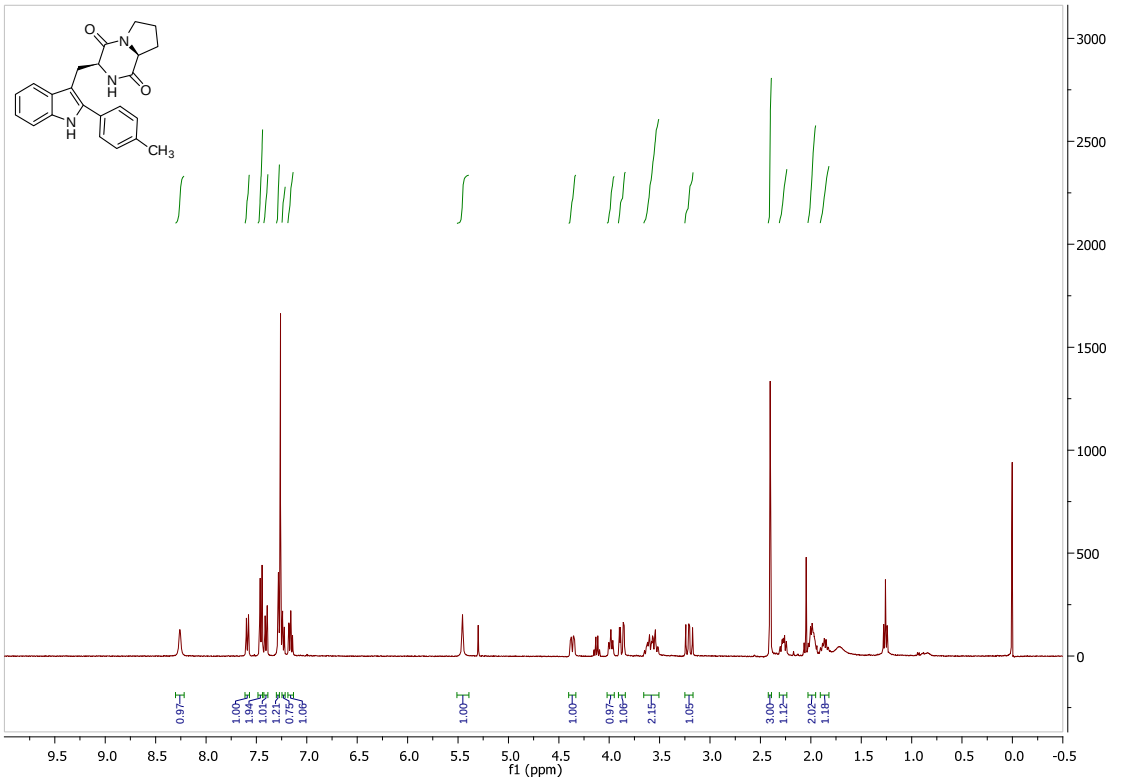
Spectroscopic data

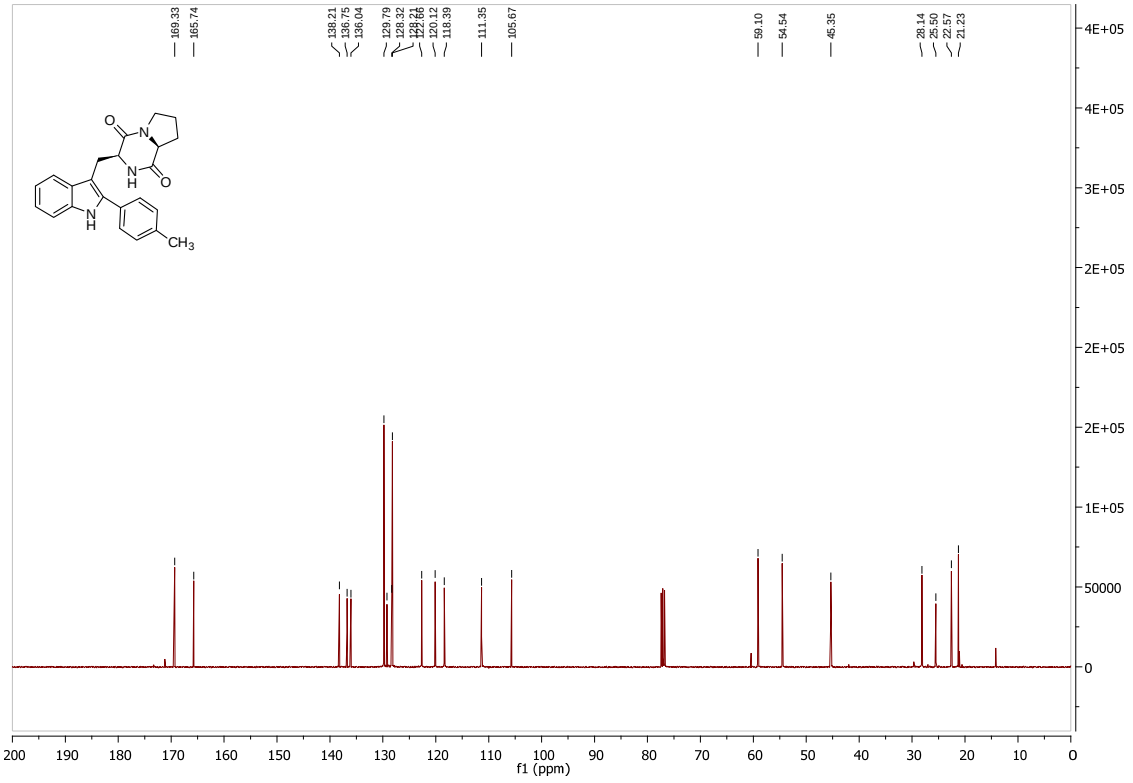
Brevianamide F (1)



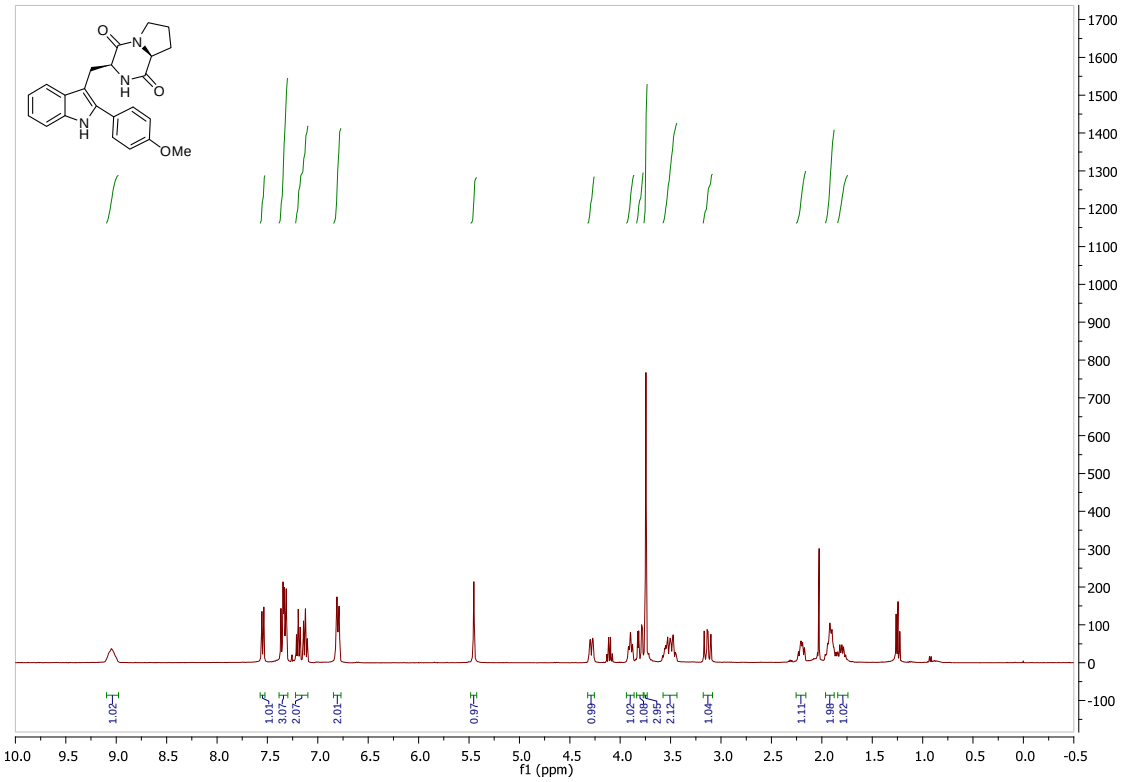


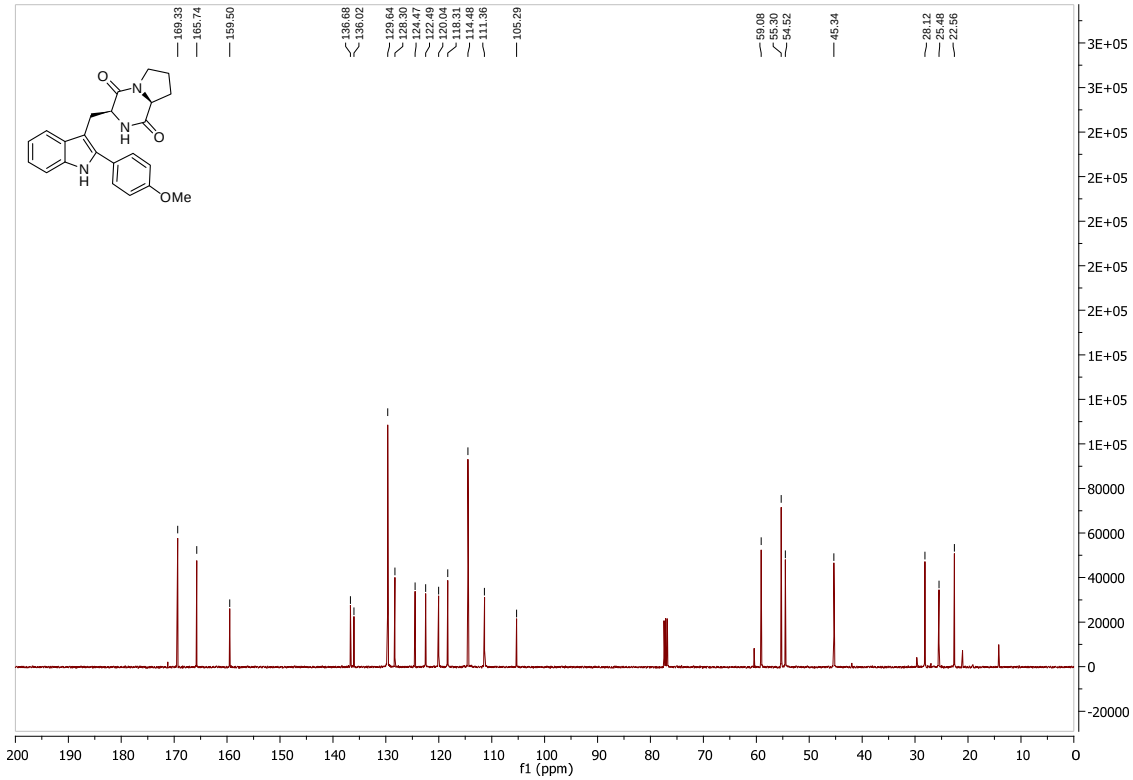
(3S,8aS)-3-((2-(*p*-Tolyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4a)



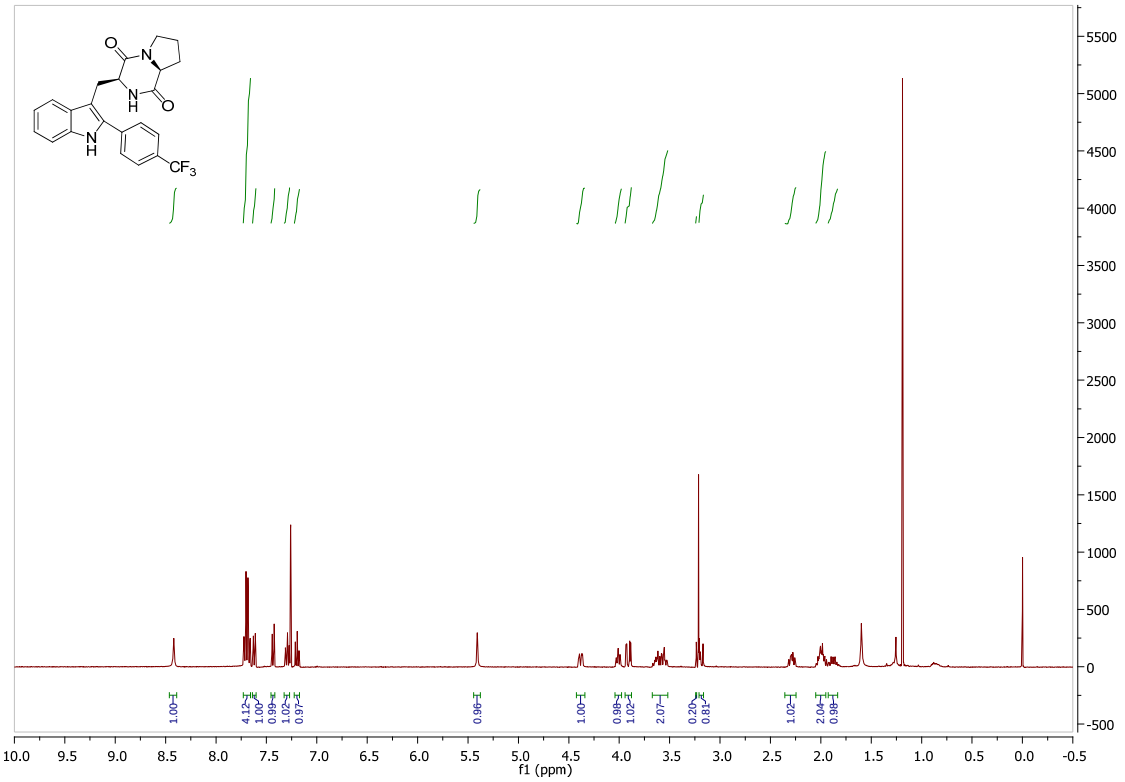


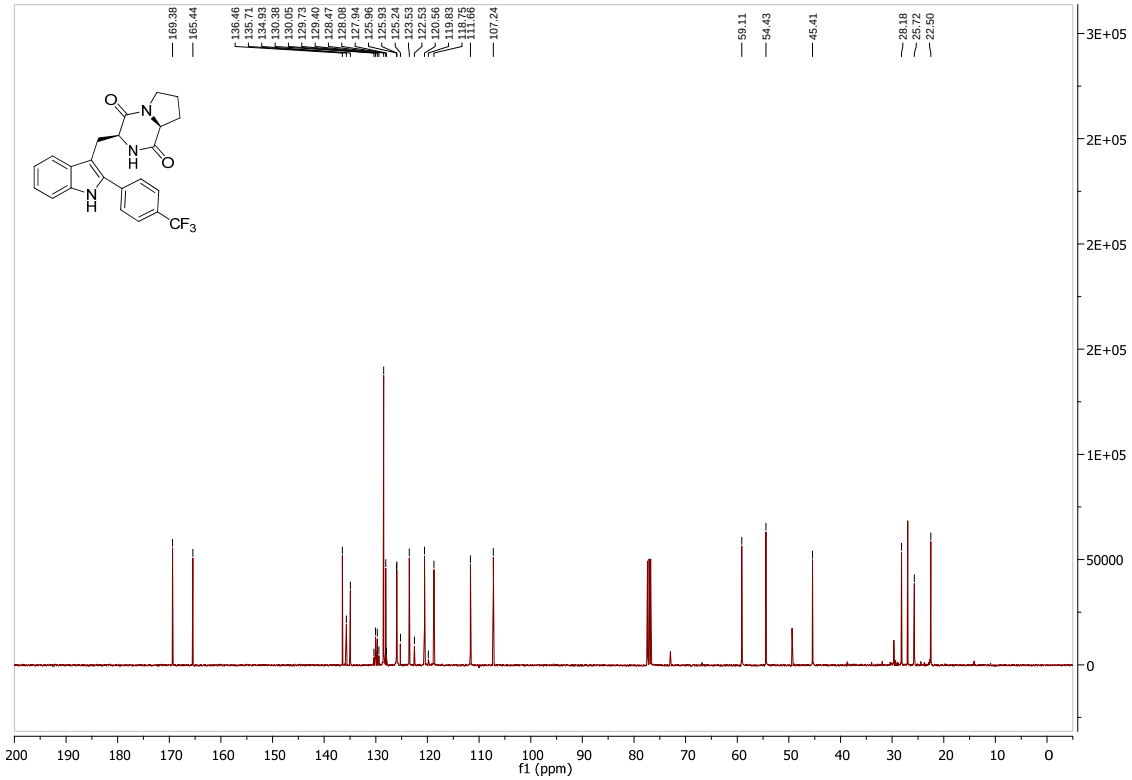
(3S,8aS)-3-((2-(4-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4b)



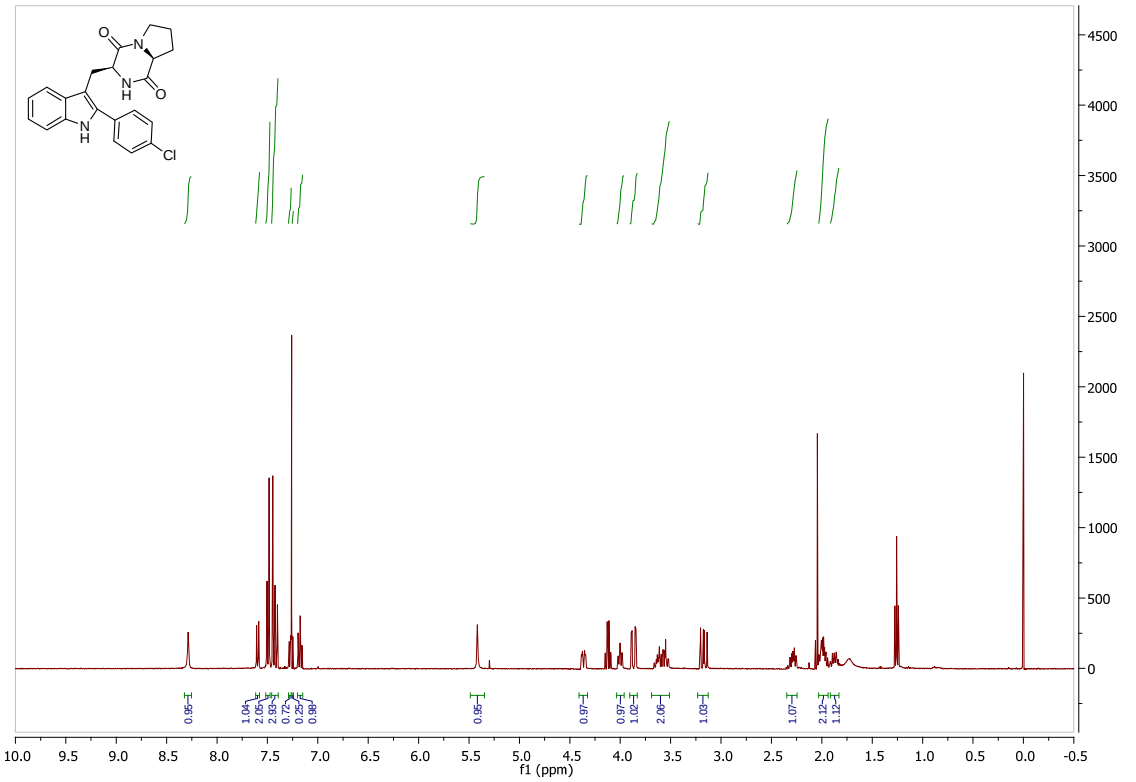


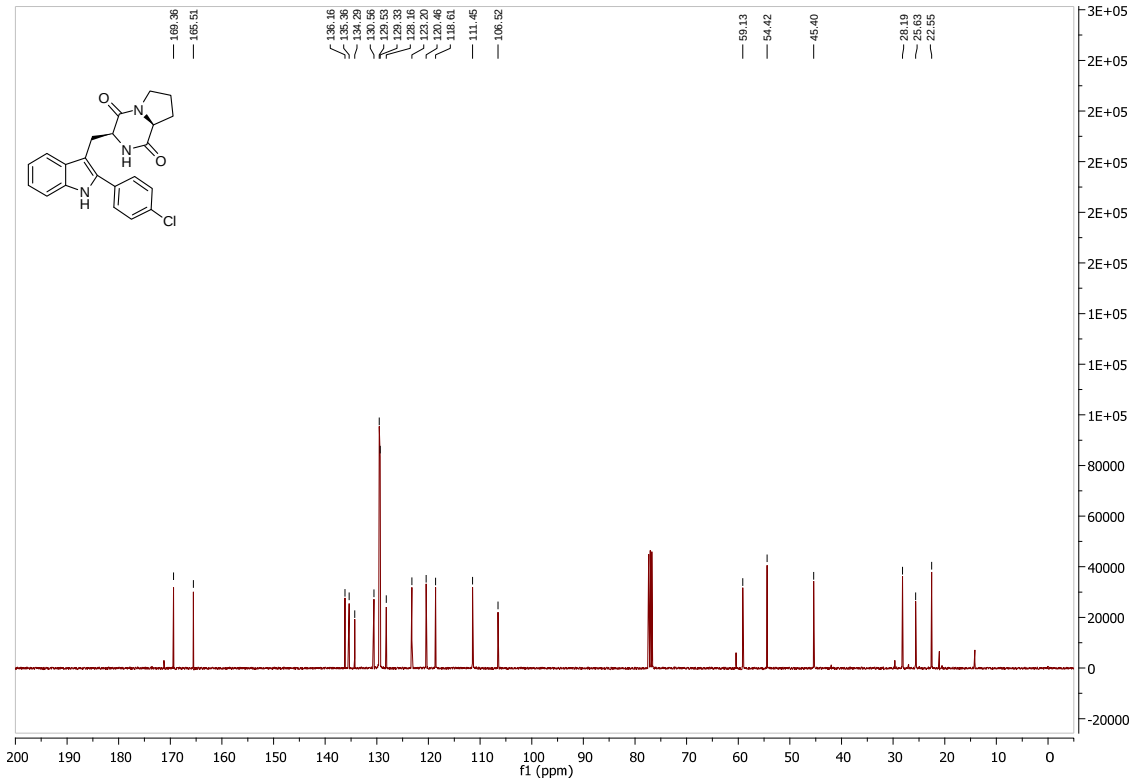
(3S,8aS)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4c)



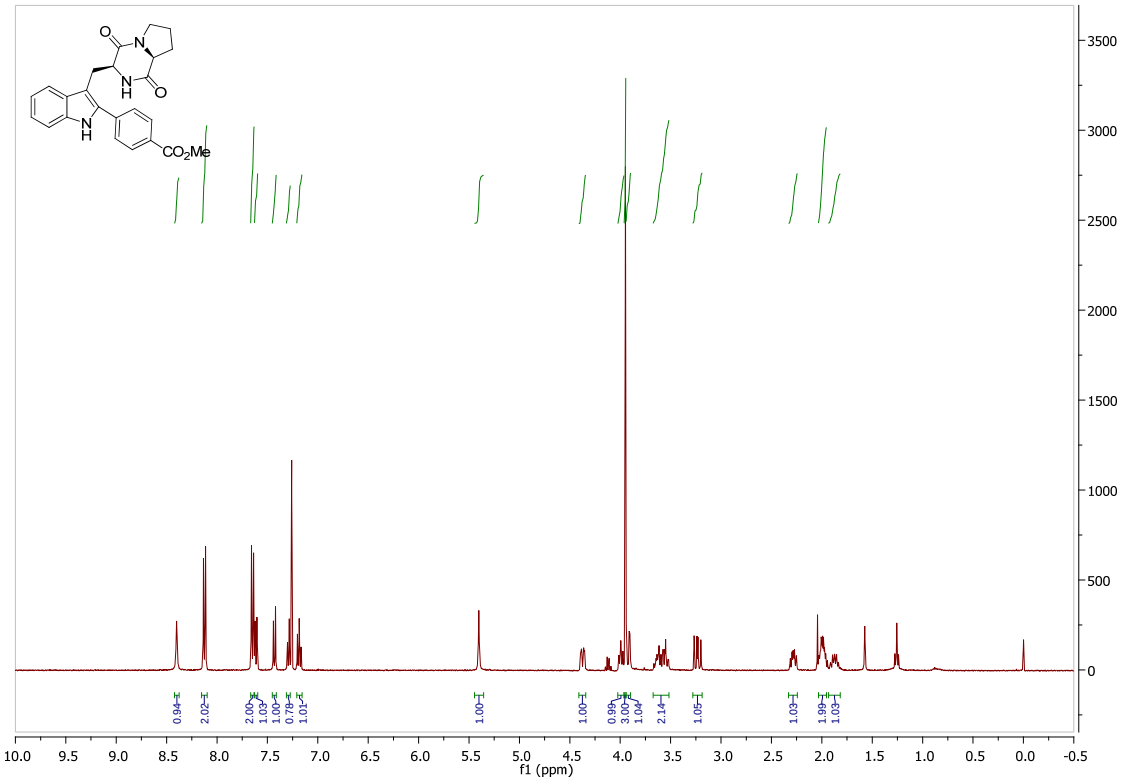


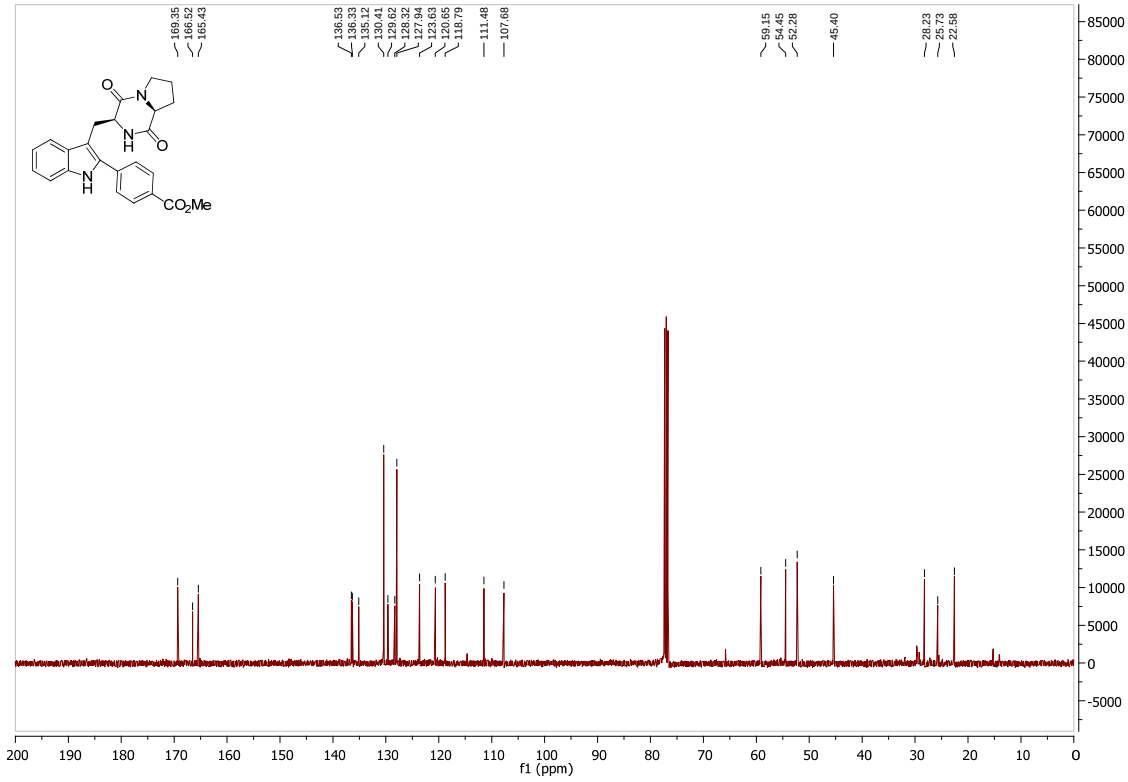
(3S,8aS)-3-((2-(4-Chlorophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4d)



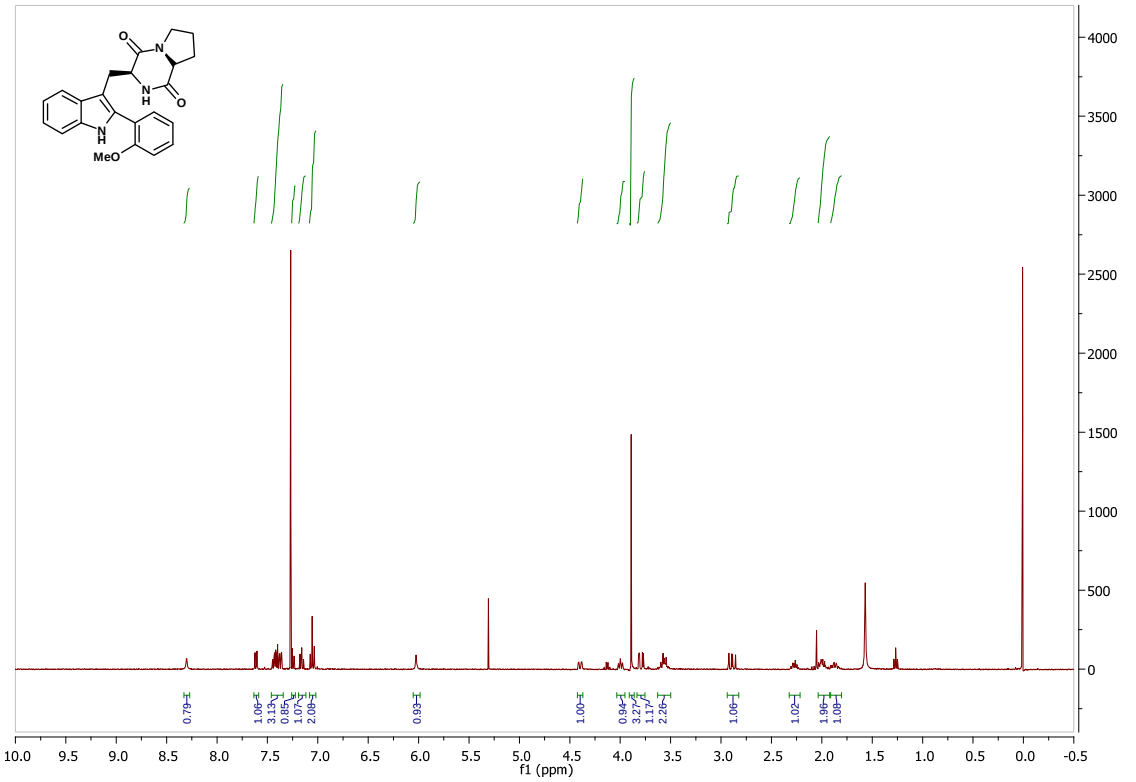


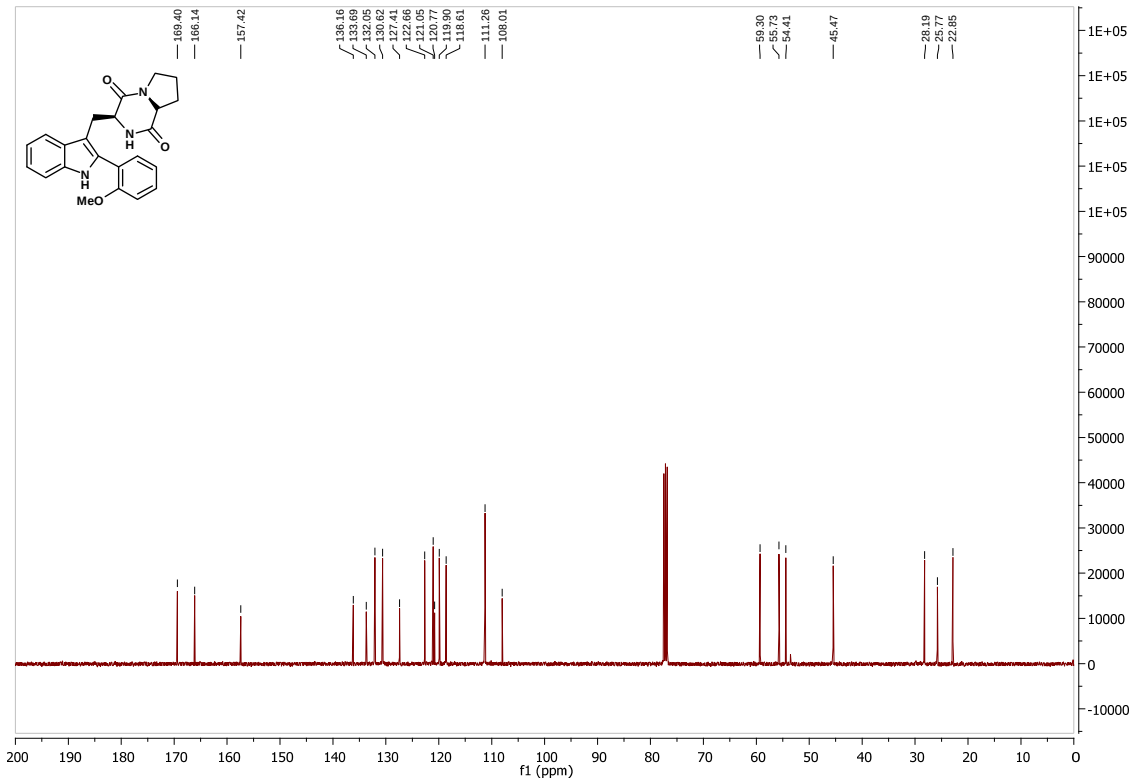
Methyl 4-(3-(((3S,8aS)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indol-2-yl)benzoate (4e)



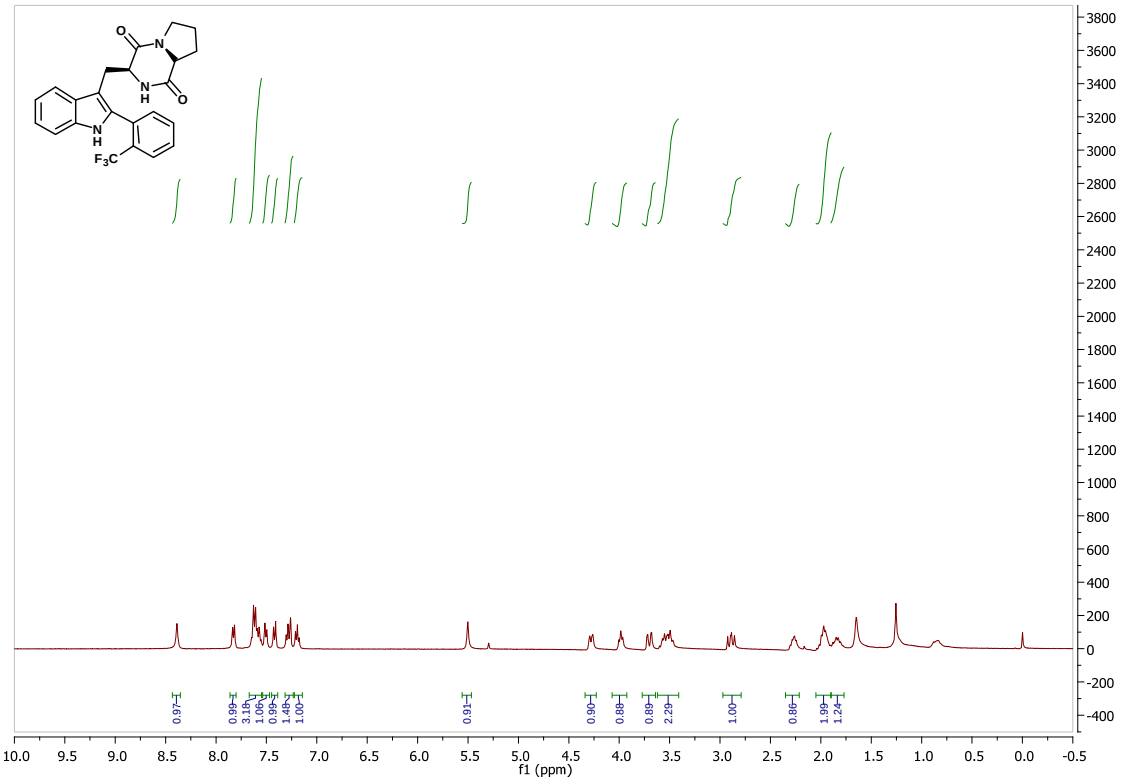


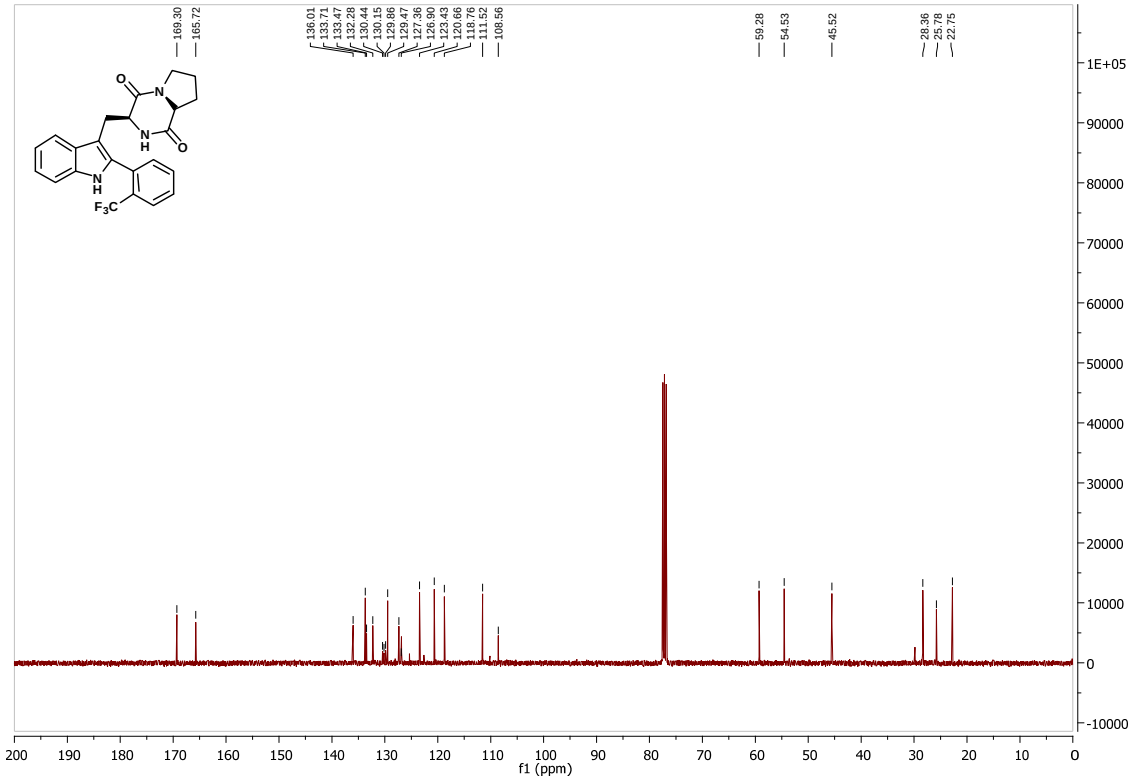
(3S,8aS)-3-((2-(2-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4f)



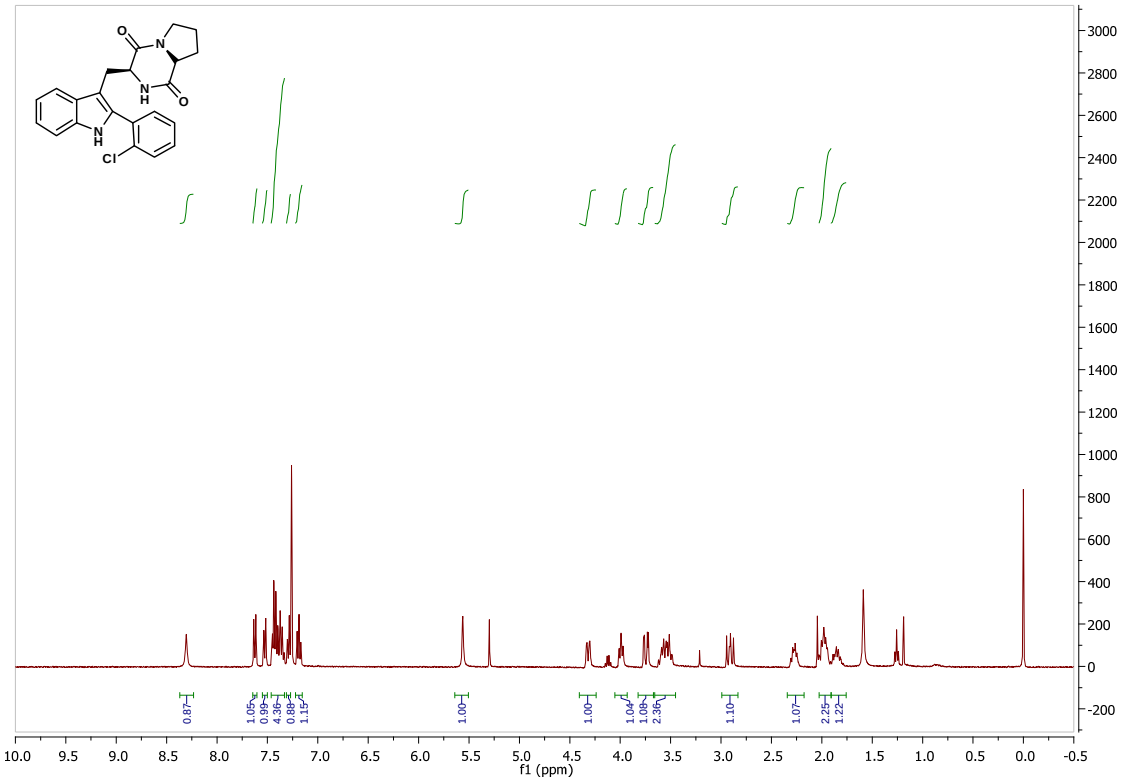


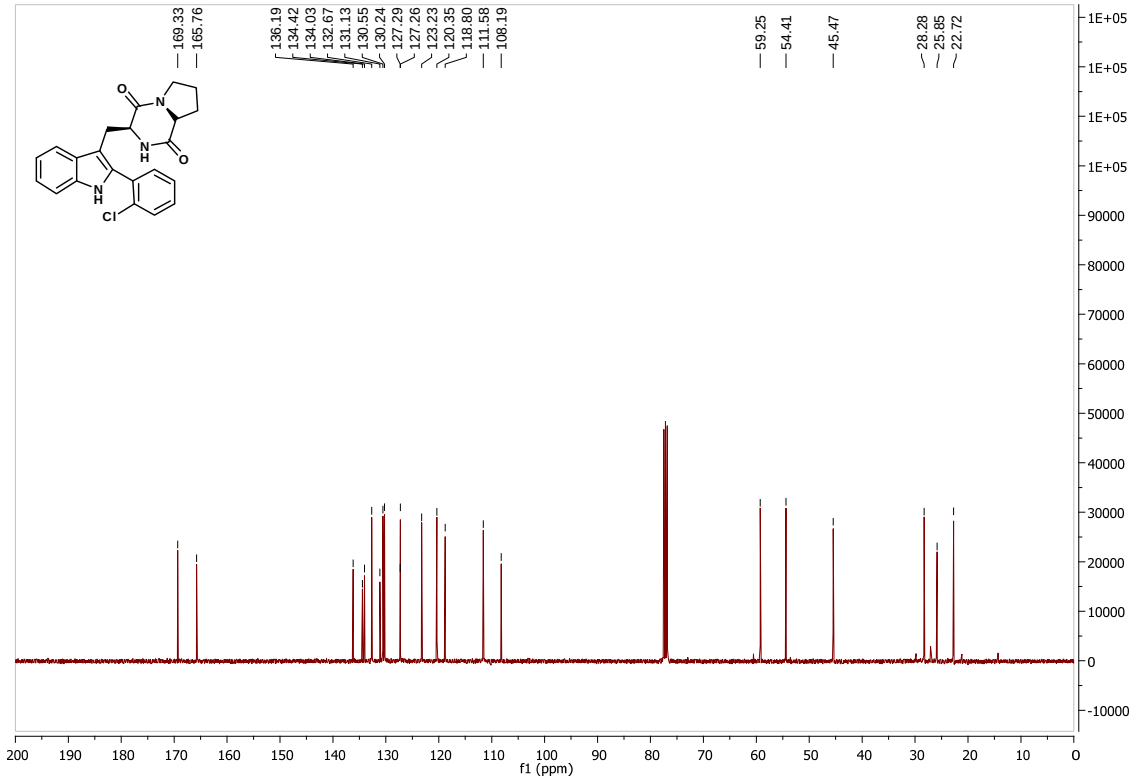
(3S,8aS)-3-((2-(2-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4g)



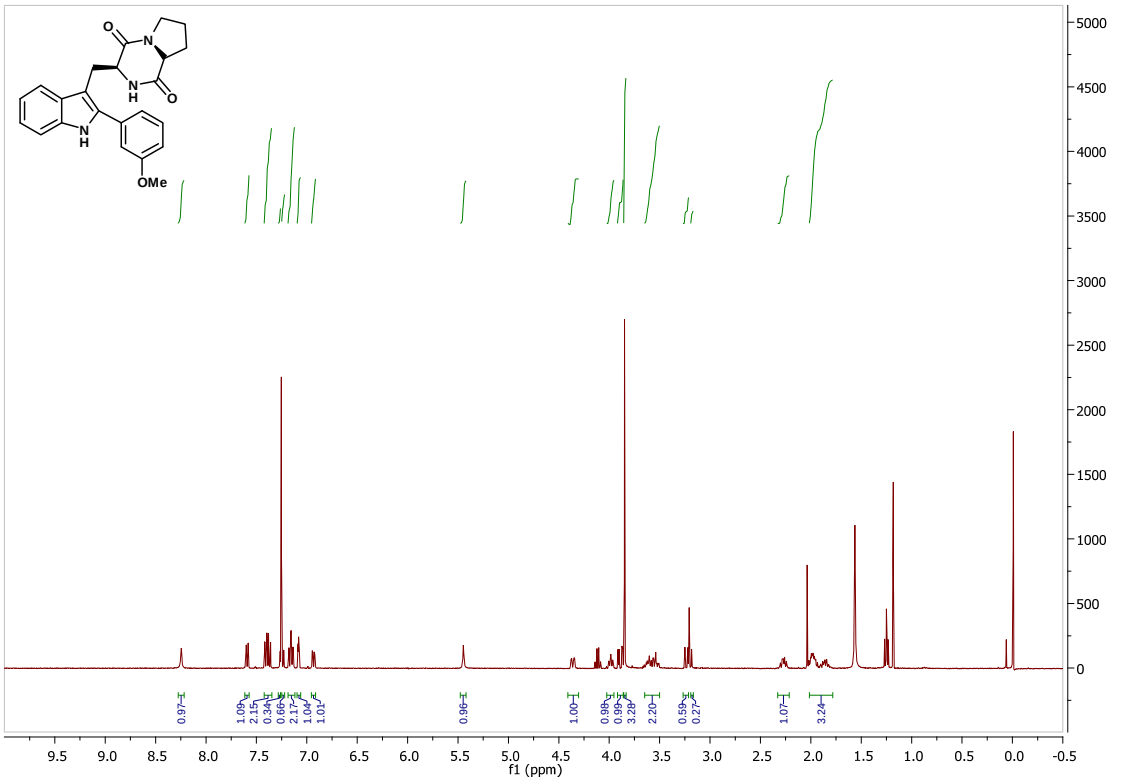


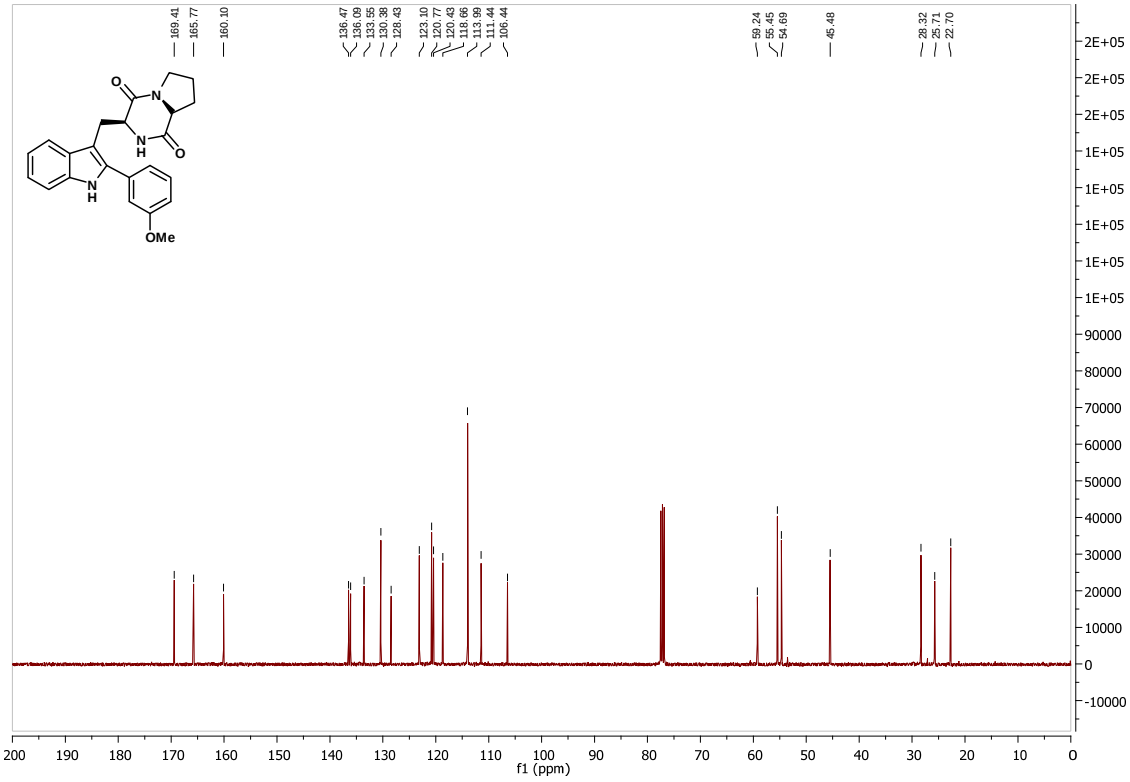
(3S,8aS)-3-((2-(2-Chlorophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4h)



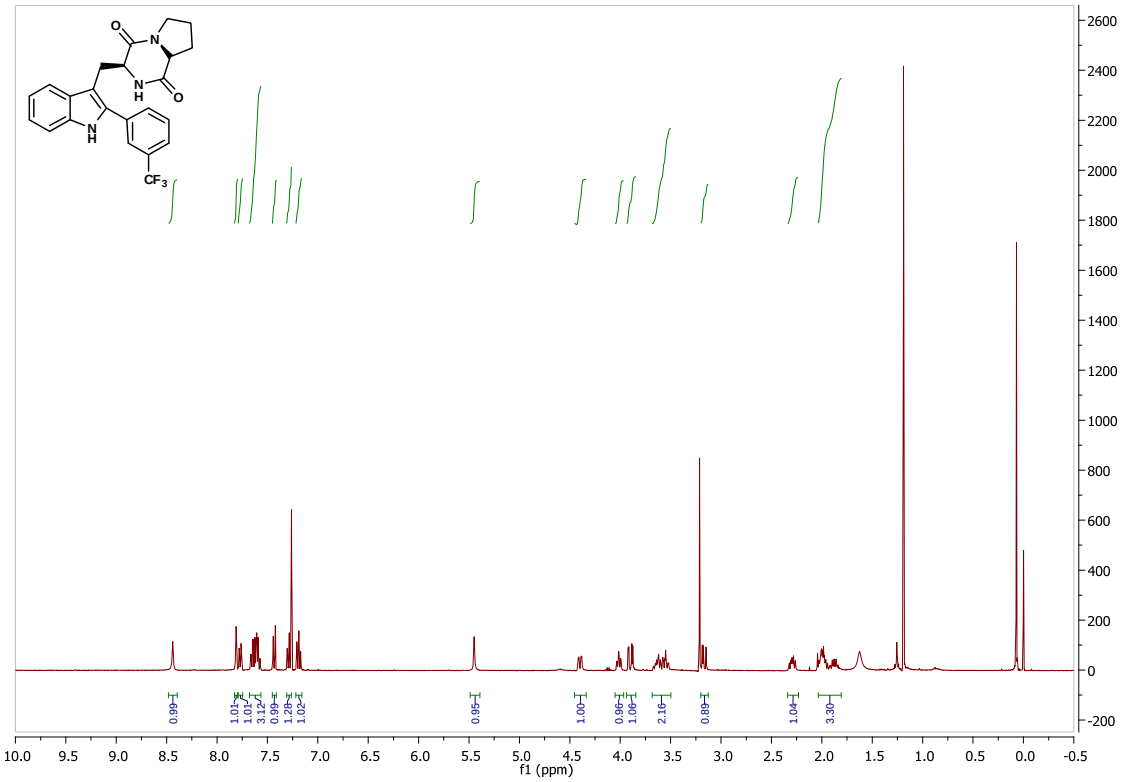


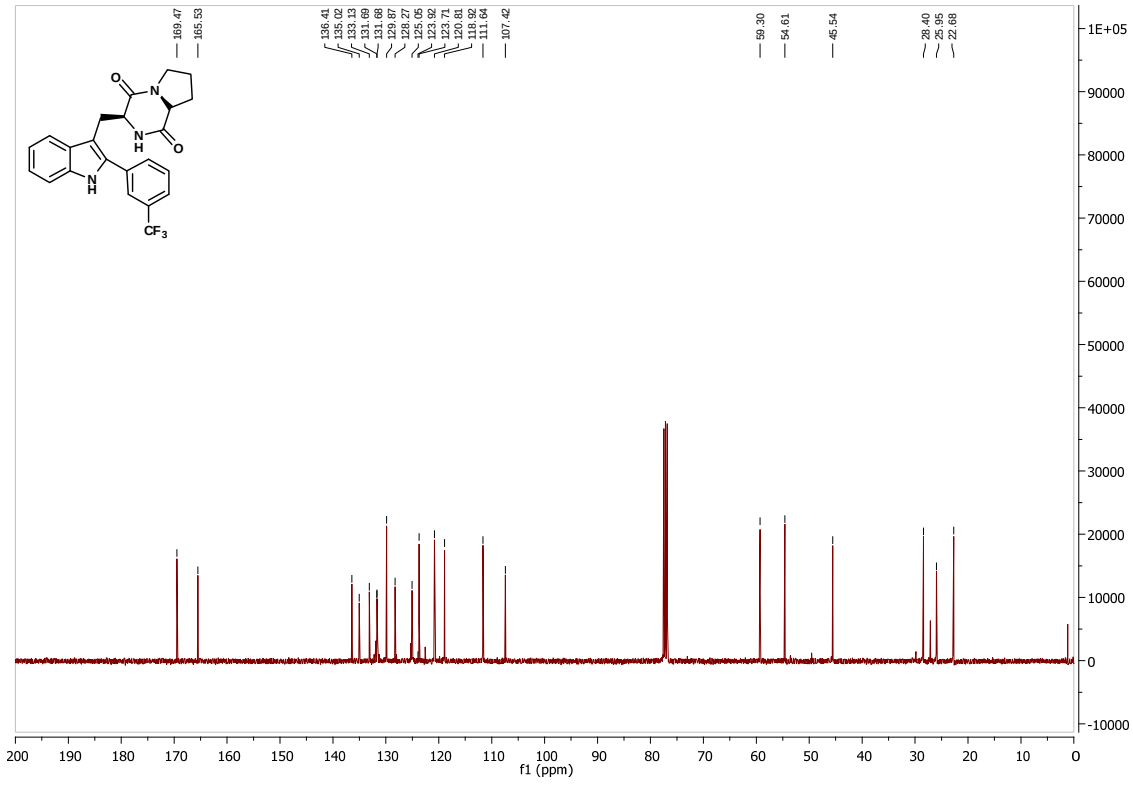
(3S,8aS)-3-((2-(3-Methoxyphenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4i)



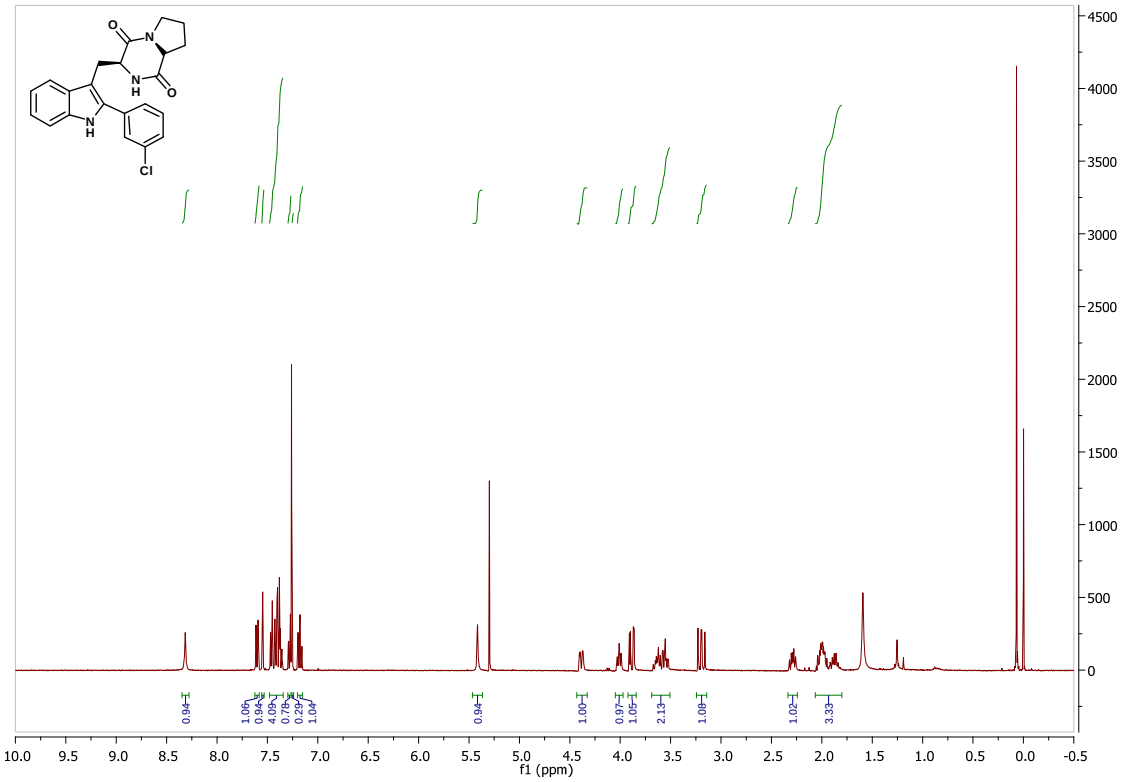


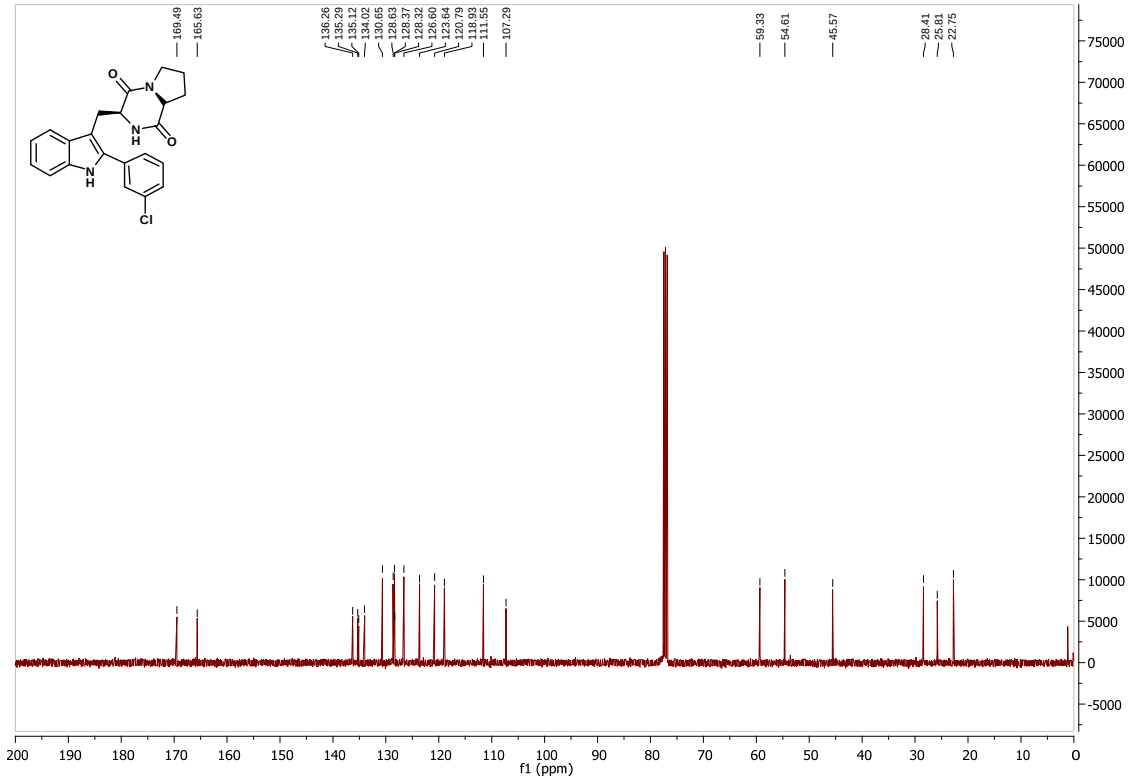
(3S,8aS)-3-((2-(3-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4j)



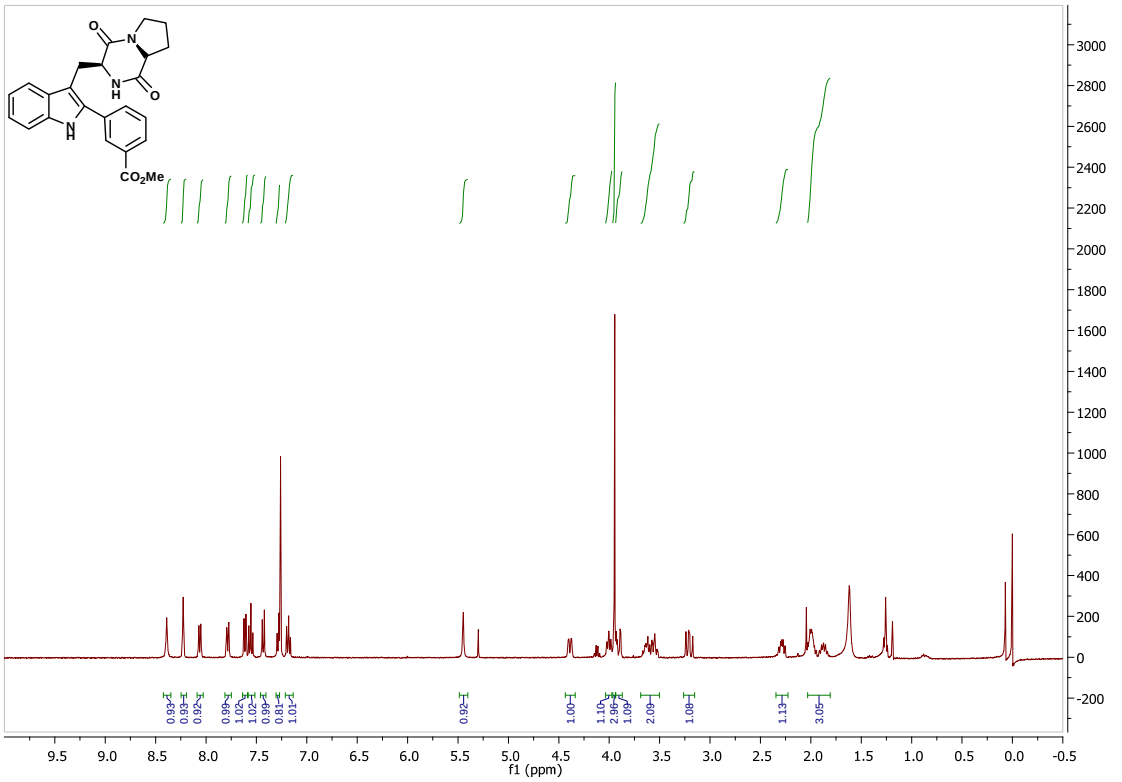


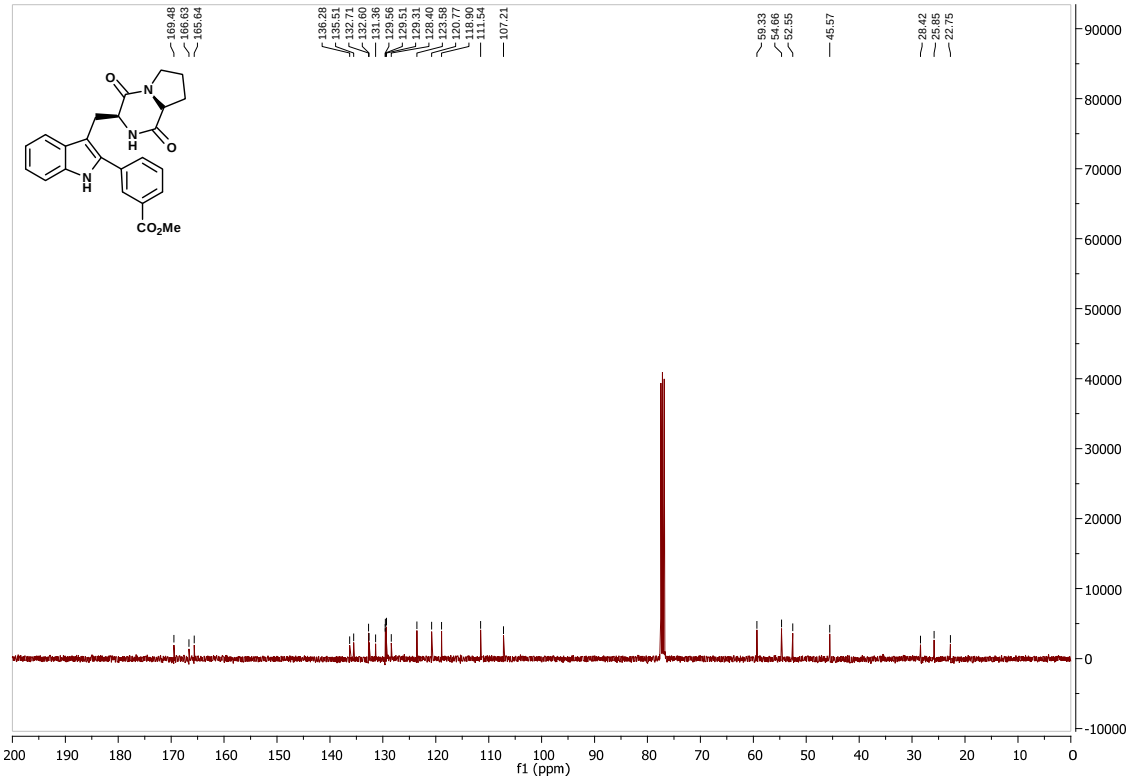
(3S,8aS)-3-((2-(3-Chlorophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4k)



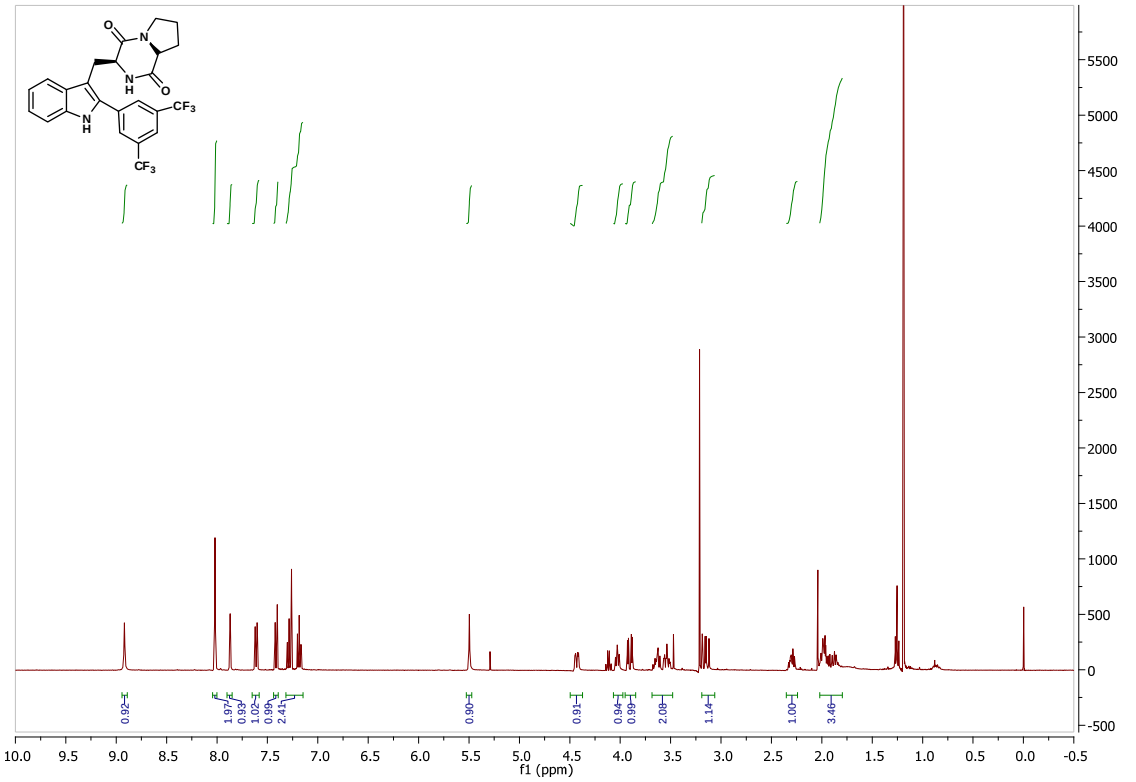


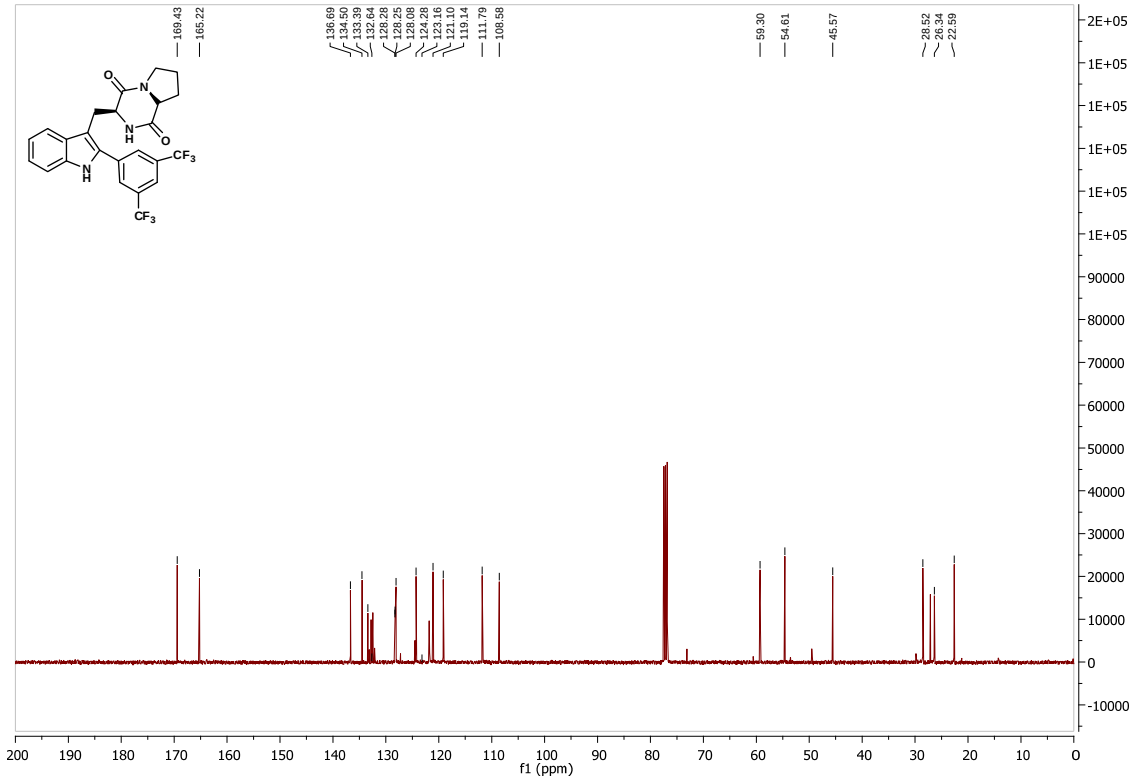
Methyl 3-(3-(((3S,8aS)-1,4-dioxooctahydropyrrolo[1,2-a]pyrazin-3-yl)methyl)-1*H*-indol-2-yl)benzoate (4l)



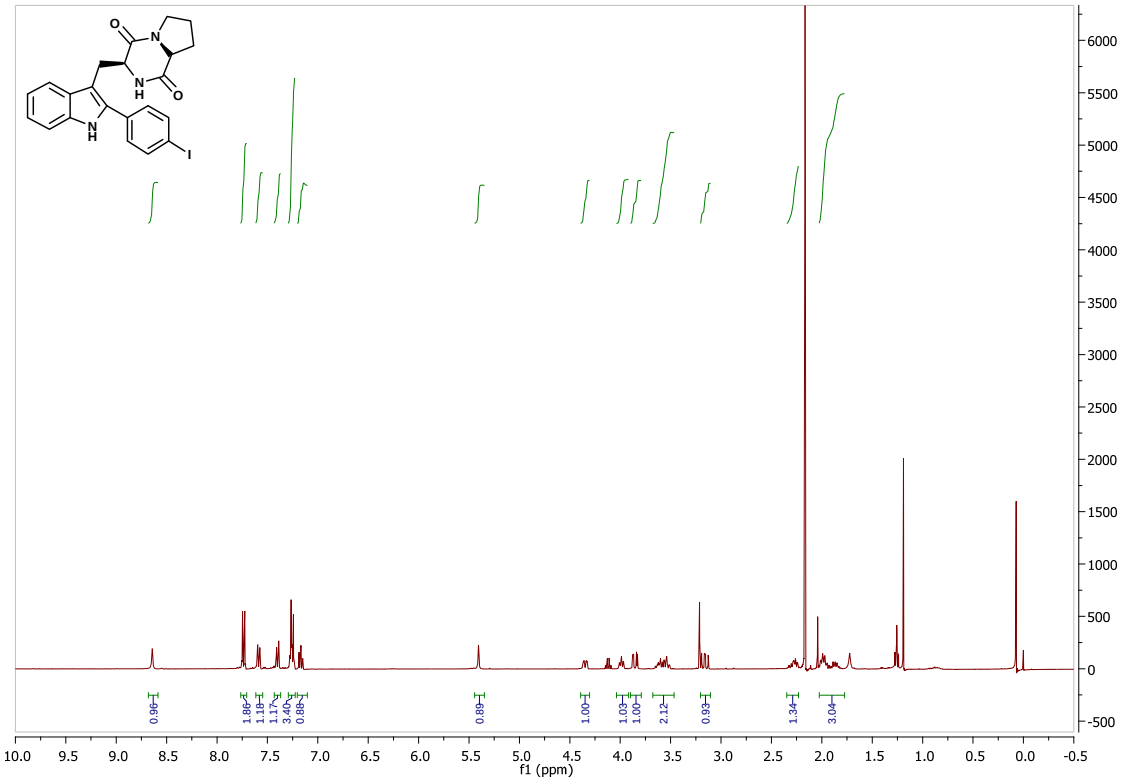


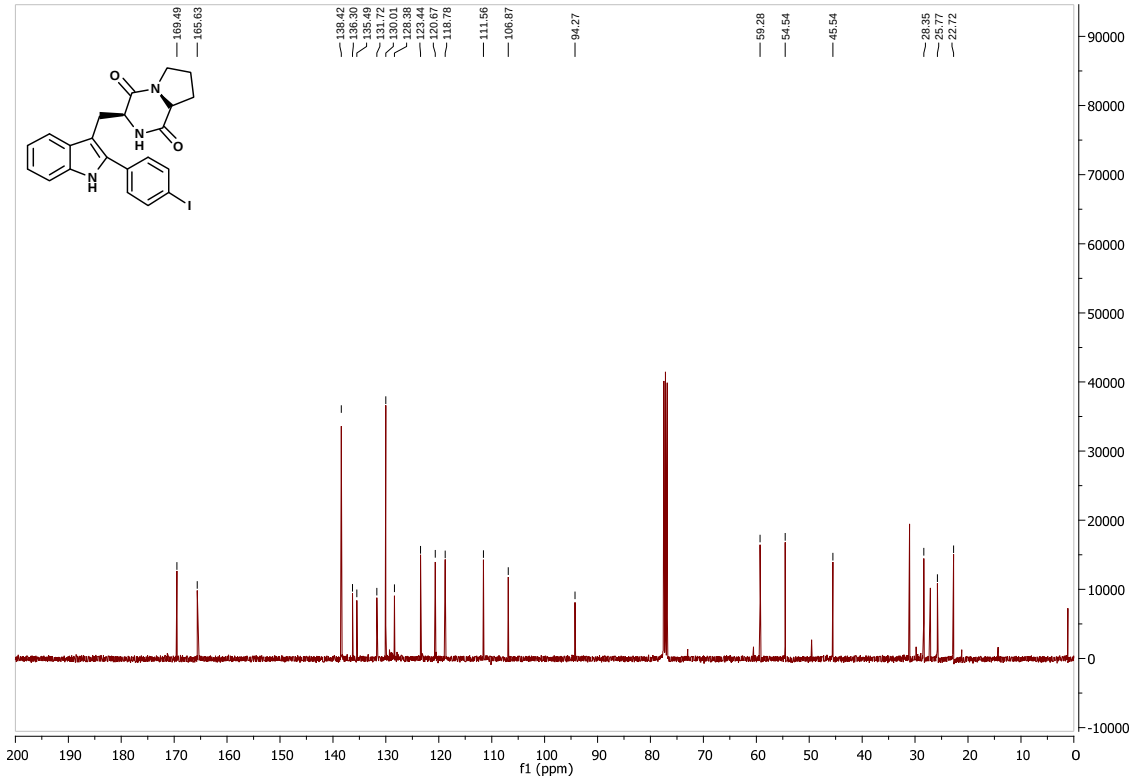
(3S,8aS)-3-((2-(3,5-bis(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4m)



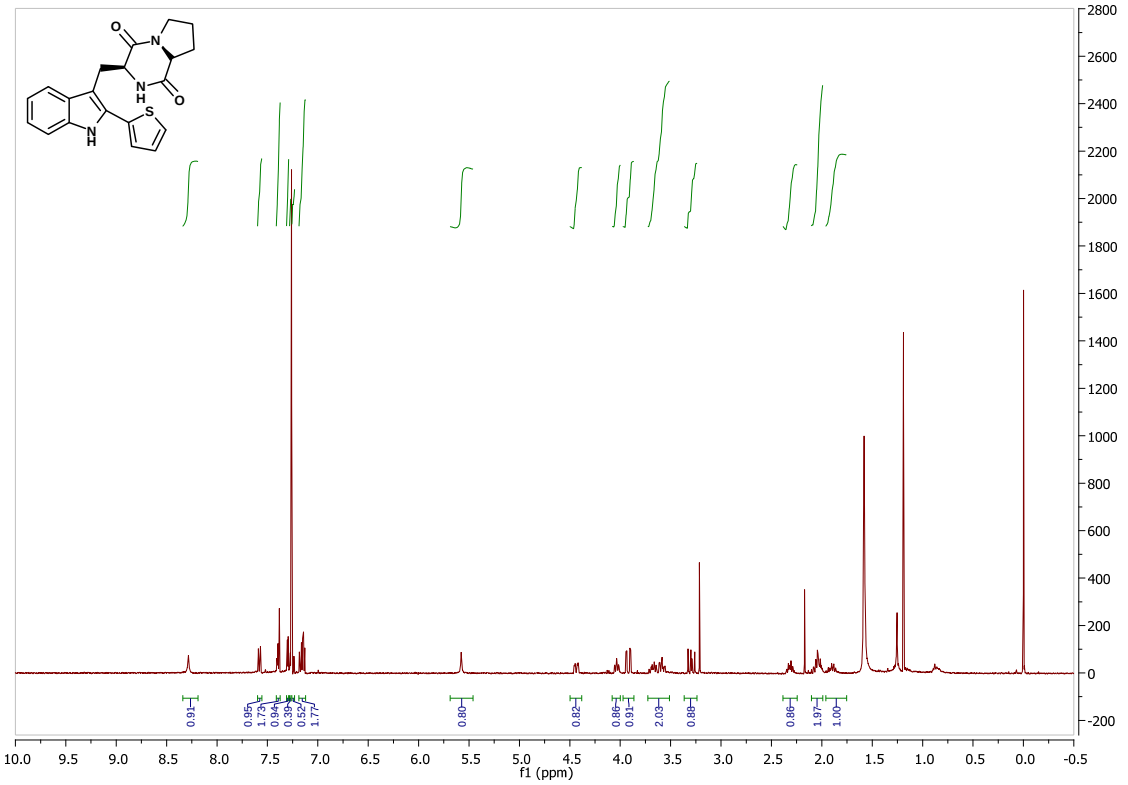


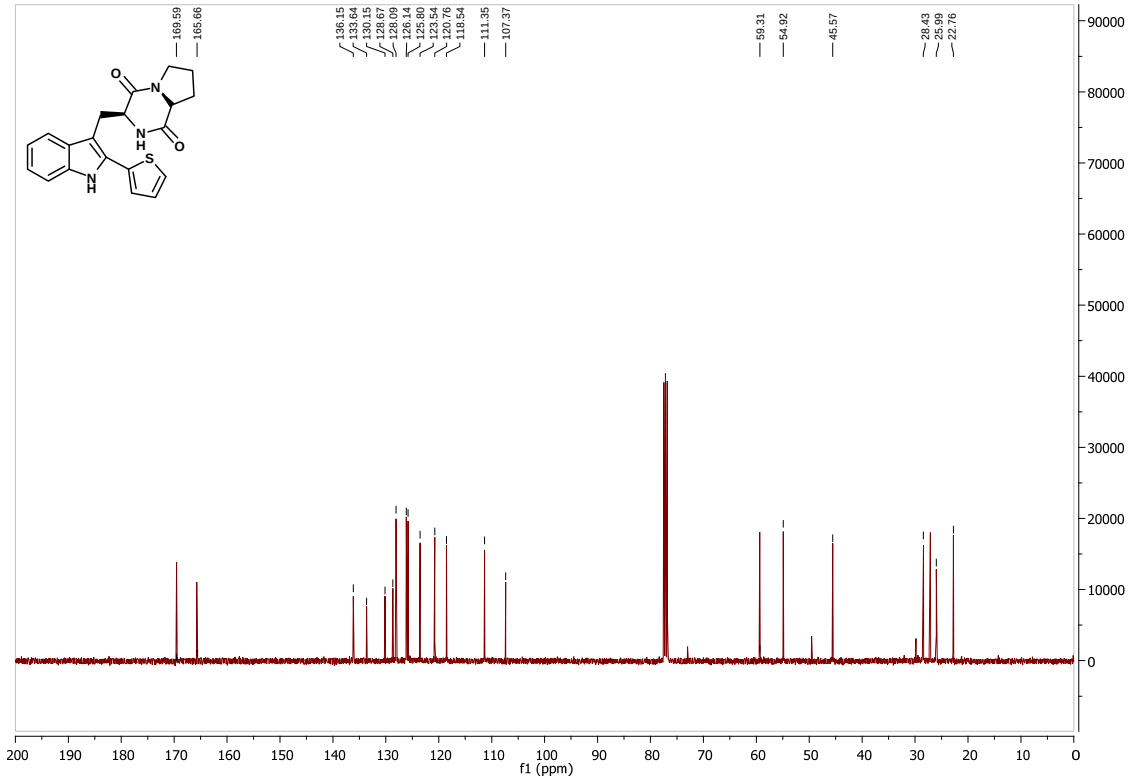
(3S,8aS)-3-((2-(4-Iodophenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4n)



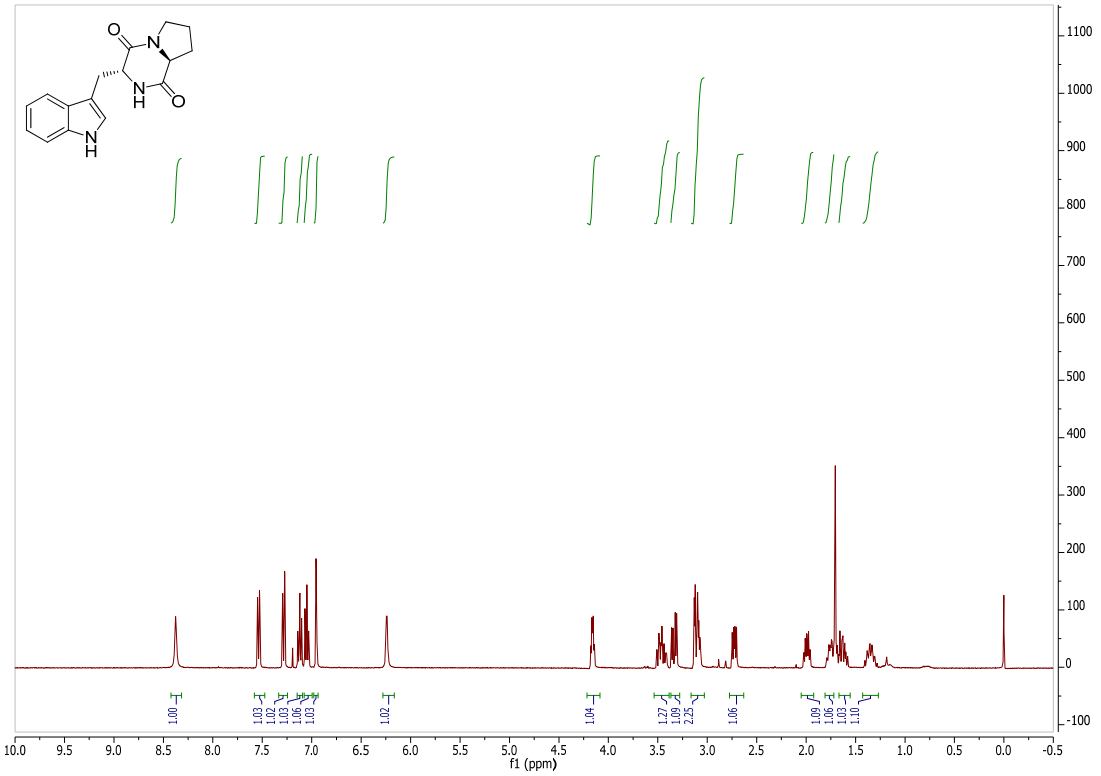


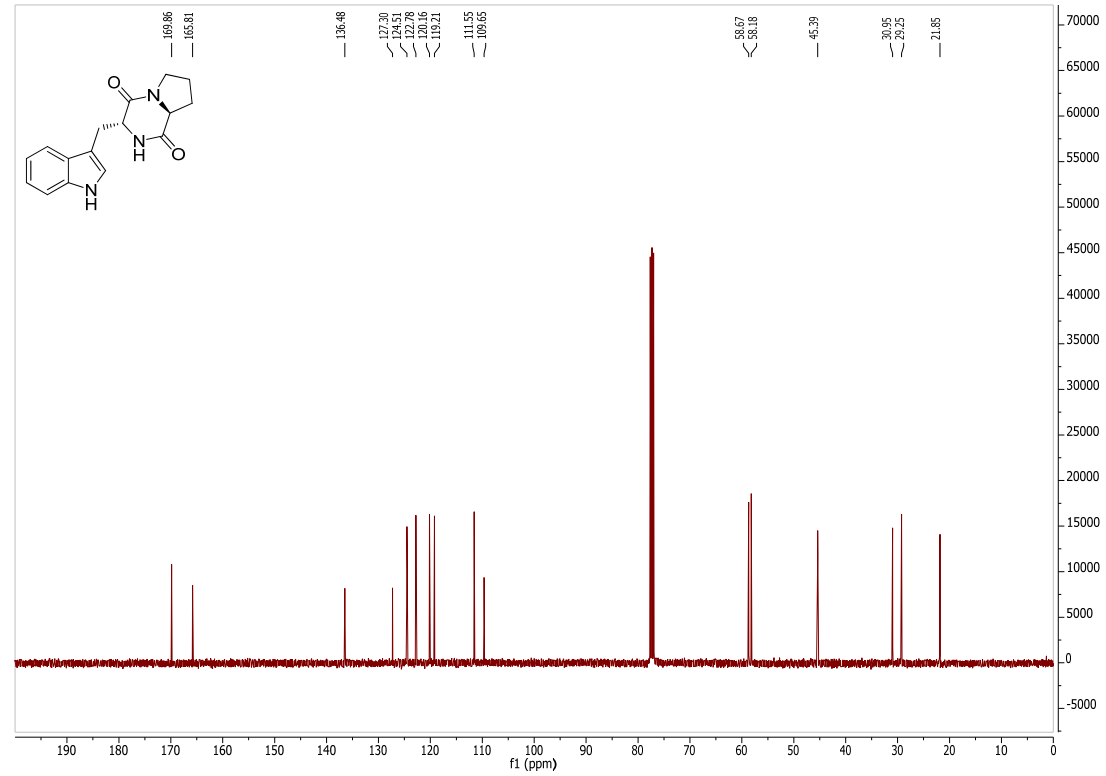
(3S,8aS)-3-((2-(2-Iodothiophene)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (4o)



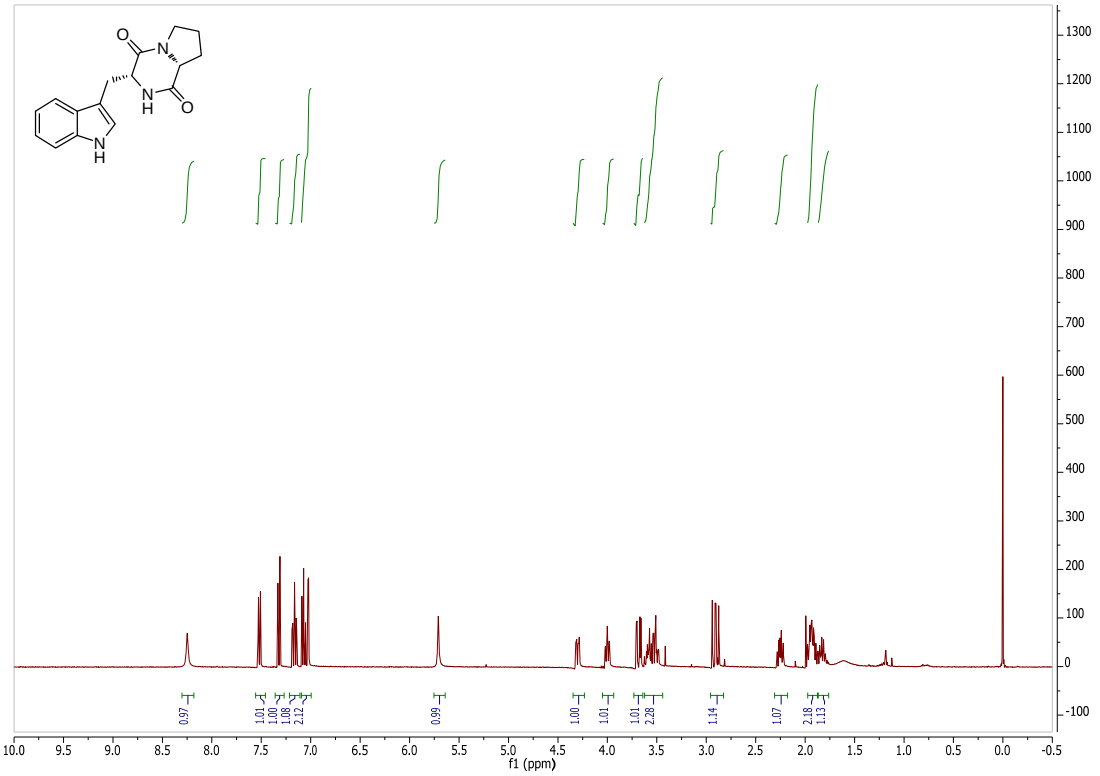


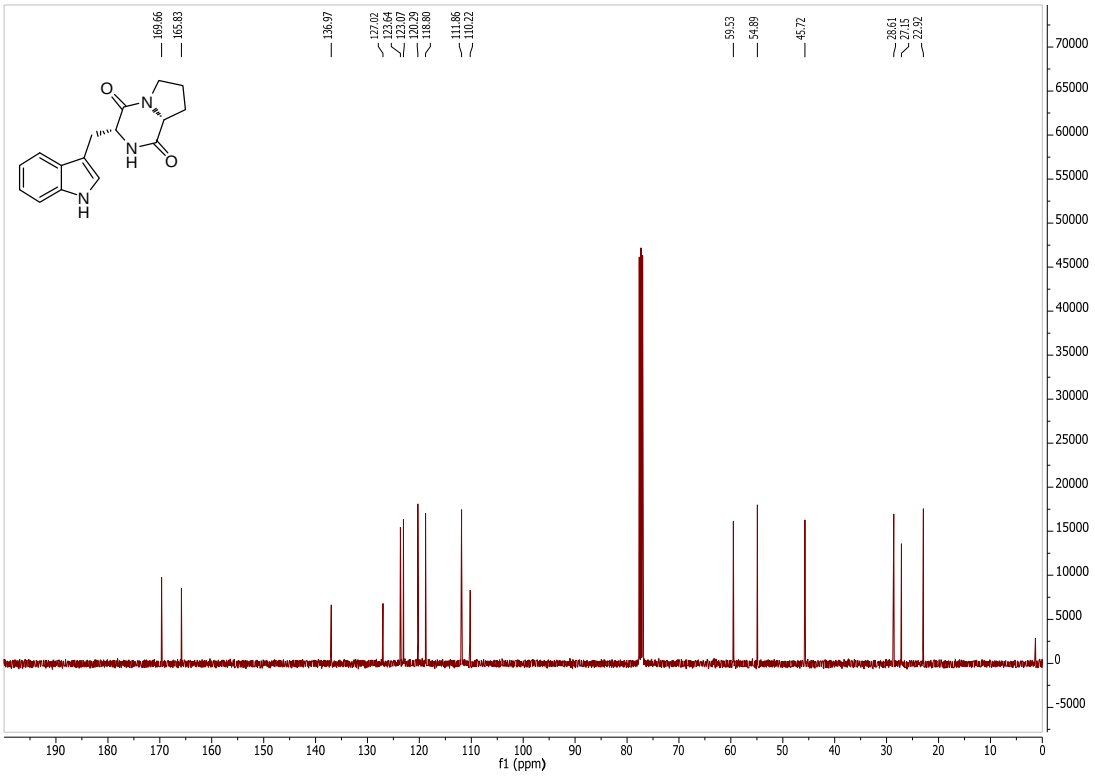
(3*R*,8a*S*)-3-((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (1')



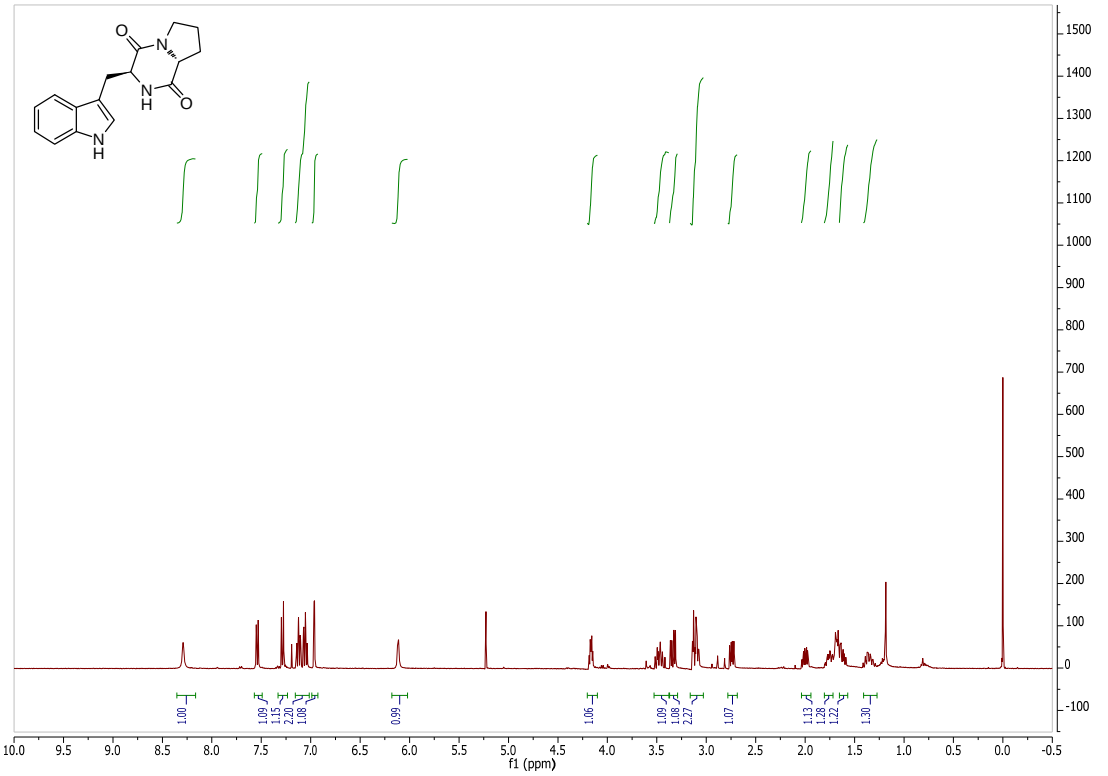


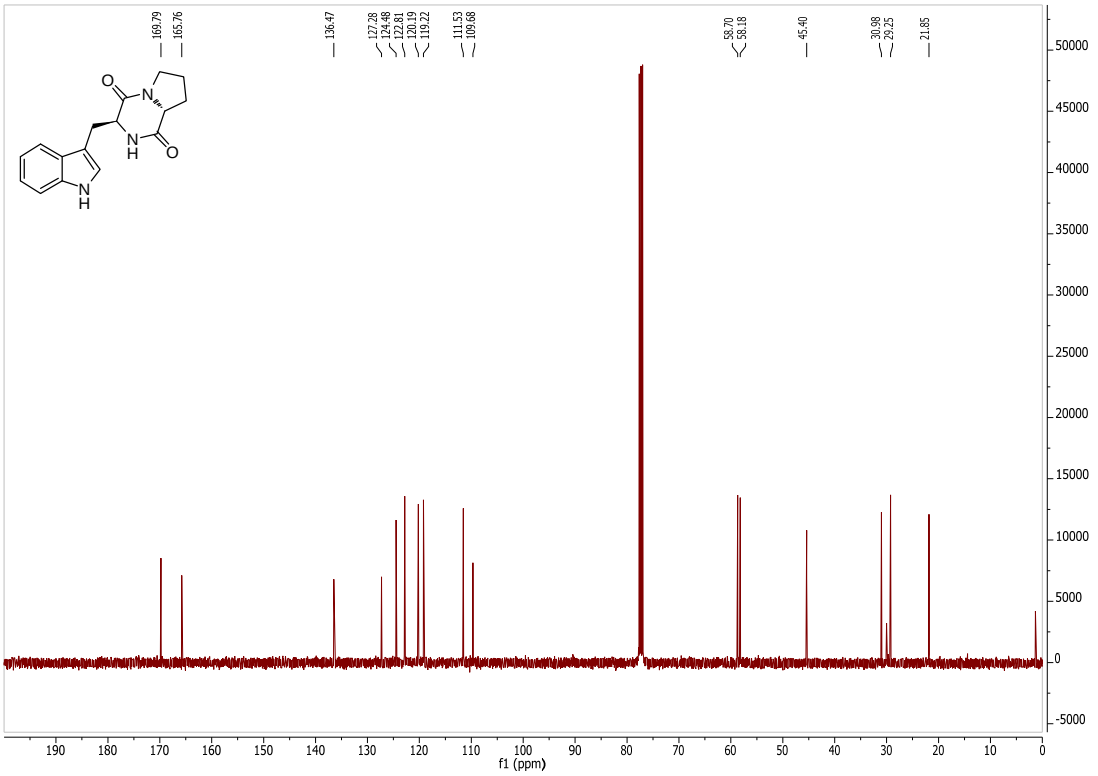
(3*R*,8a*R*)-3-((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (1'')



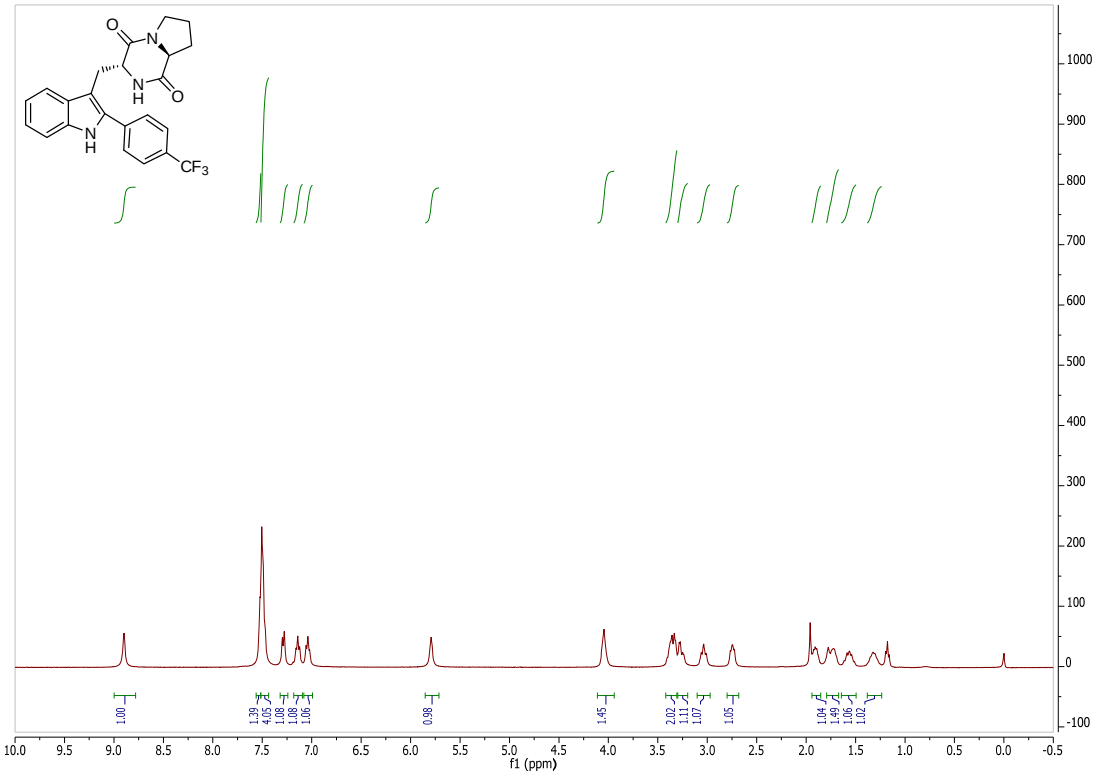


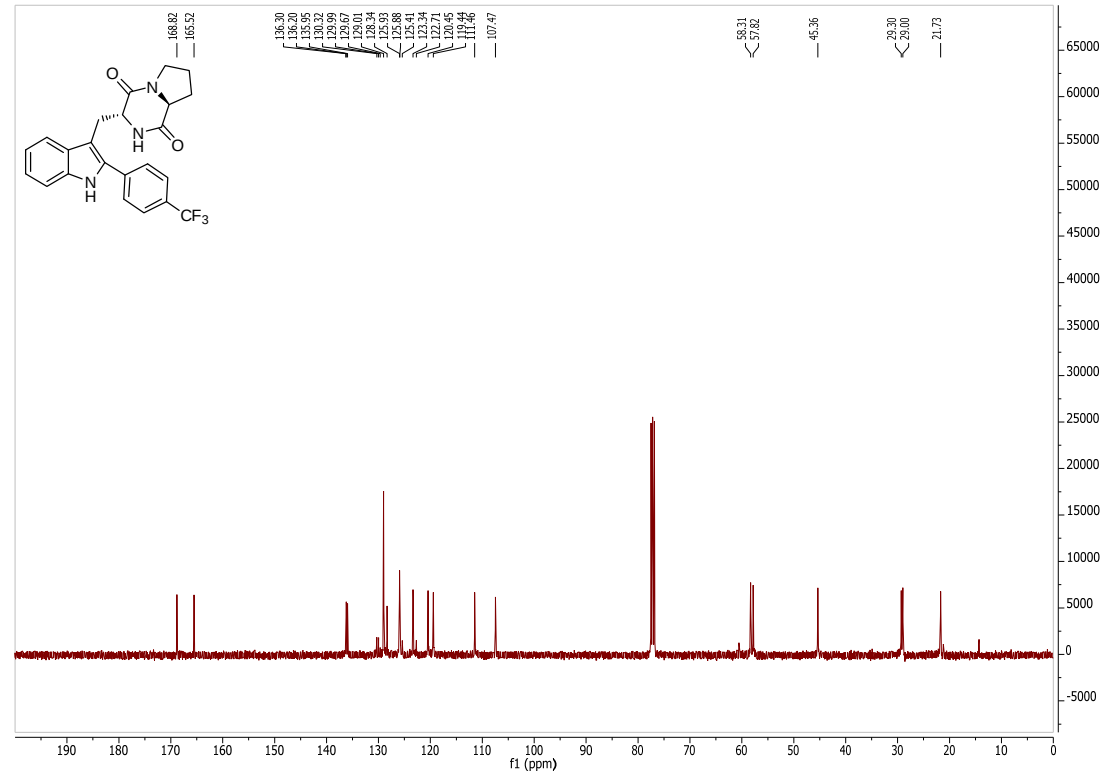
(3*S*,8a*R*)-3-((1H-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (1‘’’)



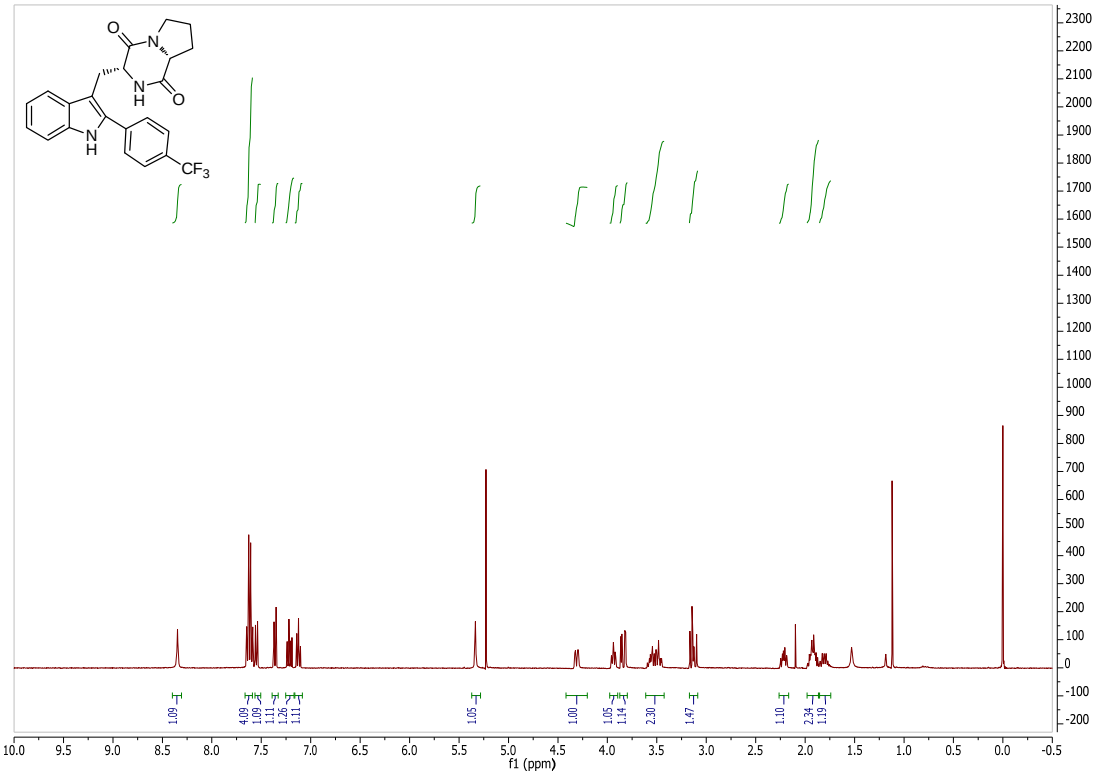


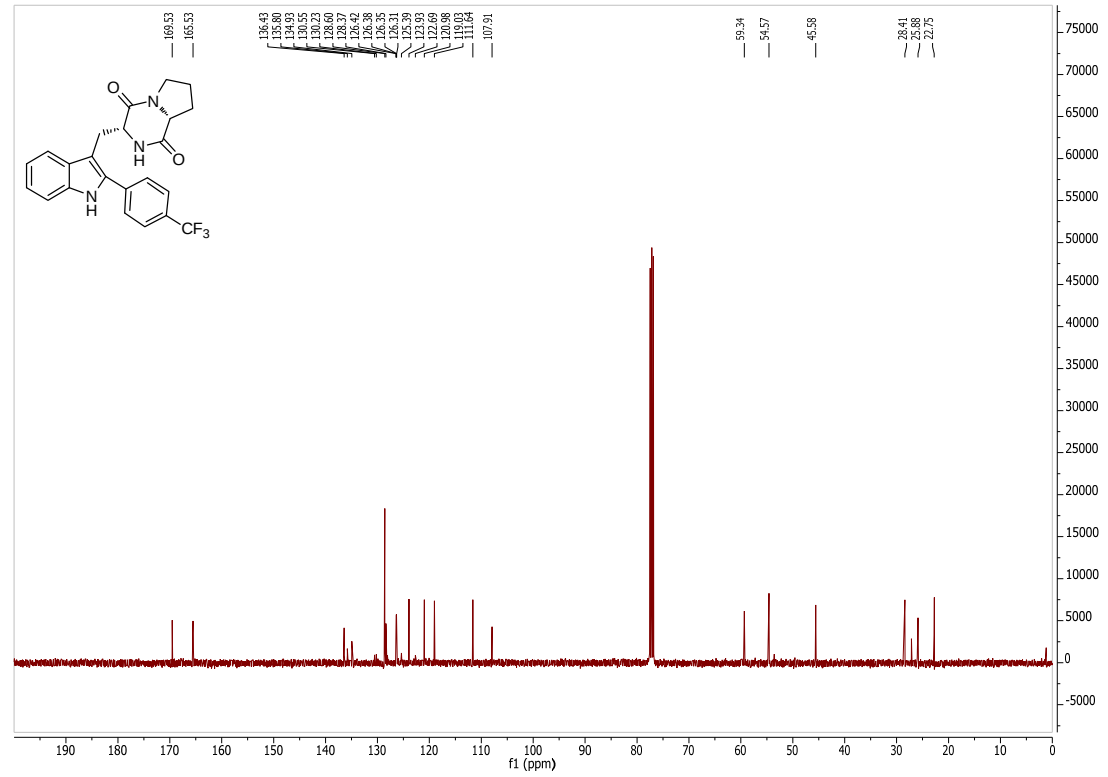
(3*R*,8a*S*)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (4c')



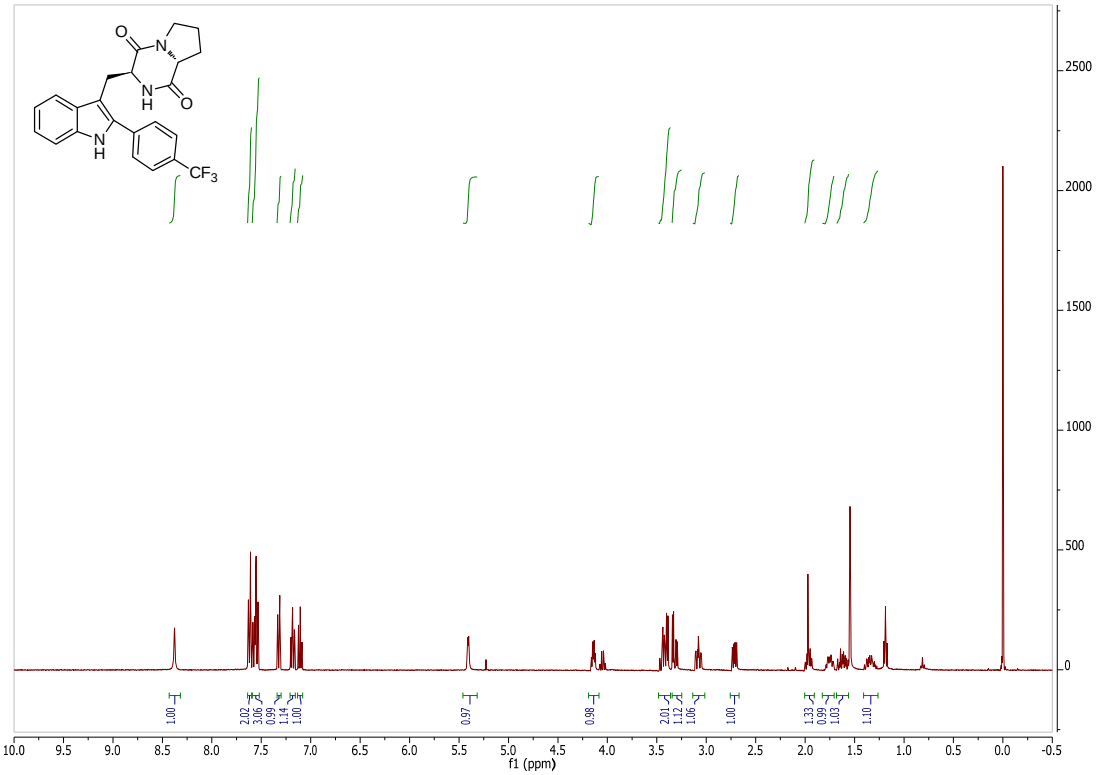


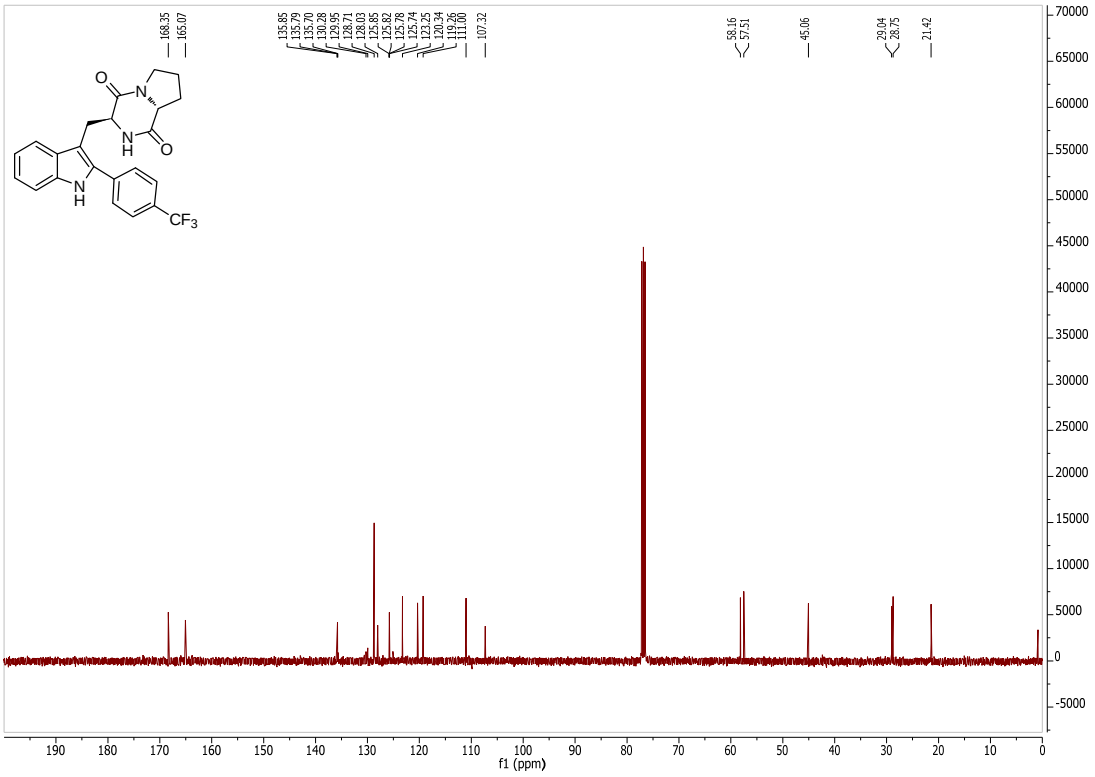
(3*R*,8a*R*)-3-((2-(4-(Trifluoromethyl)phenyl)-1*H*-indol-3-yl)methyl)hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (4c'')





(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

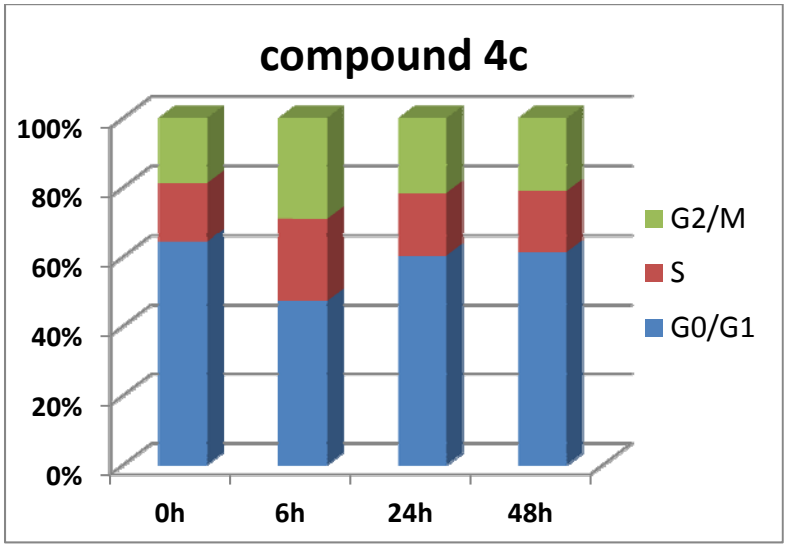


Table 5. Cell cycle analysis of compound **4c''**.

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