

One-pot synthesis of natural-product inspired spiroindolines with anti-cancer activities

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General Experimental

¹H and ¹³C NMR were recorded on a Bruker 400 spectrometer. ¹H NMR data are reported as follows: chemical shift in ppm (δ), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz), relative intensity. ¹³C NMR data are reported as follows: chemical shift in ppm (δ). LC/MS analyses were performed on a Shimadzu-2020 LC-MS instrument using the following conditions: Shim-pack VP-ODS C18 column (reverse phase, 150 x 4.6 mm); a linear gradient from 10% water and 90% acetonitrile to 75% acetonitrile and 25% water over 6.0 min; flow rate of 0.5 mL/min; UV photodiode array detection from 200 to 400 nm. High-resolution mass spectra (HRMS) were recorded on Thermo Scientific Exactive Plus System. The products were purified by Biotage Isolera™ Spektra Systems and hexane/EtOAc solvent systems. All reagents and solvents were obtained from commercial sources and used without further purification. All microwave irradiation experiments were carried out in a Biotage® Initiator Classic microwave apparatus with continuous irradiation power from 0 to 400W with utilization of the standard absorbance level of 250W maximum power. The reactions were carried out in 5, 10 or 20 mL glass tubes, sealed with microwave cavity. The reaction was irradiated at a required ceiling temperature using maximum power for the stipulated time. Then it was cooled to 50 °C with gas jet cooling.

General procedures for compound (±)-6.

A solution of propiolic acid compound (1.0 mmol), aldehyde (1.0 mmol), *tert*-butyl 3-amino-1*H*-indole-1-carboxylate (1.0 mmol) and isonitrile (1.0 mmol), was stirred overnight in MeOH (2.0 mL) at room temperature. The reaction mixture was monitored by the TLC. When the reaction was completed, the solvent was removed under nitrogen blowing and the crude residue was dissolved in 10% TFA/DCE (3.0 mL) and treated in microwave at 100 °C for 10 min. After the microwave vial was cooled to room temperature, the reaction mixture was concentrated and diluted with EtOAc (15.0 mL), washed with sat. Na₂CO₃ and brine. The organic layer was dried

over MgSO₄ and concentrated. The residue was purified by silica gel column chromatography using a gradient of ethyl acetate/hexane (0-100%) to afford the relative targeted product (\pm)-**6**.

Measurement of cell viability and proliferation

U87, PANC-1, PC3, Hep3B and MCF 7 were all purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). U87, Hep3B and MCF 7 cells were cultured in high-glucose DMEM (Hyclone, SH30022.01, USA) medium, PC3 cells were cultured in the F12K medium (Hyclone, SH30026.01B, USA) and PANC-1 cells were cultured in RPMI medium modified (Hyclone, SH30809.01, USA) medium with 10% bovine fetal serum (FBS, Gibco, 10100147, Australia origin) and 1% penicillin/streptomycin (Gibco, USA) at 37 °C in a humidified incubator containing 5% CO₂.

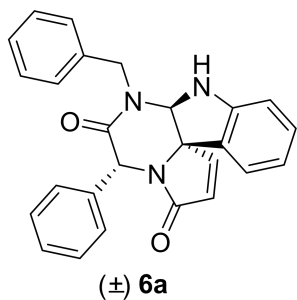
Cell viability measurement

The effects of compound (\pm)-**6d** on inhibition of cells proliferation and the IC₅₀ value were measured by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT, Beyotime, ST316, Shanghai, China) assay. Briefly, cancer cells were seeded on the 96 well-plates at a density of 3×10^3 - 6×10^3 cells per well with 200 μ L complete medium. Then, cells were treated with compounds at concentration of 0, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 μ M for 48h after incubating 24 h. After that, each well was added 20 μ L MTT (5mg/ml) and incubated for another 4 h. Then the medium was removed and insoluble formazan inside cells was dissolved in 200 μ L DMSO (Dimethylsulfoxide). The plate was placed into the table shaker and shaken about 10min at 140 rpm/min. The optical density (OD) of each well was measured by the microplate reader (Bio-Tek, Winooski, VT, USA) at 570 nm. The OD value of the control wells was used as 100%. The IC₅₀ values of all compounds were calculated by GraphPad Prism 6 and the experiments were repeated for three times.

Statistical analysis: Statistical analyses were performed by unpaired Student's t test for two group comparisons and one-way analysis of variance (ANOVA) for multi-group comparisons at a significance level of $p < 0.05$. Data were presented as

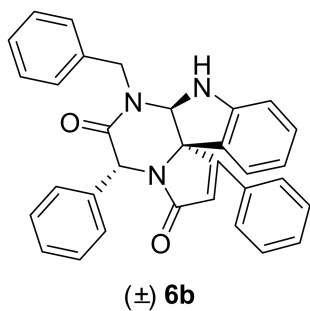
means \pm SD from three or more independent experiments.

(\pm)-7-benzyl-5-phenyl-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino[2,3-*b*]indole-3,6(7*H*)-dione, Compound (\pm)-**6a**, yellow solid, yield 64%. ¹H NMR (400 MHz,



CDCl₃) δ 7.42 – 7.34 (m, 3H, ArCH), 7.34 – 7.30 (m, 2H, ArCH), 7.07 (d, J = 6.9 Hz, 2H, ArCH), 7.06 – 6.94 (m, 4H, ArCH), 6.93 – 6.87 (m, 1H, ArCH), 6.39 (t, J = 7.3 Hz, 1H, ArCH), 6.29 (dd, J = 8.6, 6.9 Hz, 2H, ArCH), 6.25 – 6.18 (m, 2H, ArCH and COCHN), 5.46 – 5.28 (m, 1H, NCHN), 5.17 (s, 1H, NH), 4.89 (d, J = 15.1 Hz, 1H, PhCH), 4.81 (d, J = 15.1 Hz, 1H, PhCH). ¹³C NMR (100 MHz, CDCl₃) δ 170.9 (CO), 167.4 (CO), 149.3 (ArC), 146.7 (ArC), 136.5 (ArC), 135.6 (ArC), 130.1 (ArC), 129.2 (ArC), 128.1 (ArC), 127.7 (ArC), 127.4 (ArC), 125.7 (ArC), 124.3 (ArC), 122.7 (ArC), 120.2 (ArC), 110.4 (ArC), 74.2 (NCHN), 71.3 (NC₄), 56.5 (NCH), 48.6 (CH₂). HRMS (ESI) m/z calcd for C₂₆H₂₂N₃O₂⁺ (M+H)⁺ 408.1707, found 408.1709.

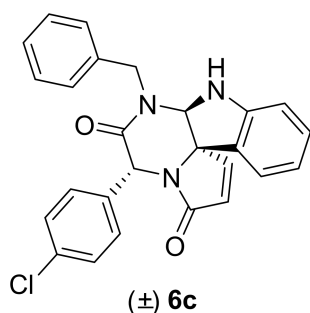
(\pm)-7-benzyl-1,5-diphenyl-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino[2,3-*b*]indole-3,6(7*H*)-dione, Compound (\pm)-**6b**, yellow solid, yield 75%. ¹H NMR (400 MHz,



CDCl₃) δ 7.33 – 7.25 (m, 7H, ArCH), 7.17 (t, J = 7.7 Hz, 2H, ArCH), 7.10 – 7.06 (m, 2H, ArCH), 6.96 (d, J = 5.5 Hz, 5H, ArCH), 6.87 – 6.81 (m, 1H, ArCH), 6.59 – 6.52 (m, 2H, ArCH), 6.42 (t, J = 7.4 Hz, 1H, NCHN), 6.34 (s, 1H, COCH), 6.15 (d, J = 7.9 Hz, 1H, CH), 5.30 (s, 1H, NH), 4.76 (q, J = 14.9 Hz, 2H, CH₂). ¹³C NMR (100 MHz, CDCl₃) δ 170.9 (CO), 168.8 (CO), 160.7 (ArC), 147.6 (ArC), 136.6 (ArC), 134.4 (ArC), 130.7 (ArC), 130.4 (ArC), 130.1 (ArC), 129.0 (ArC), 128.8 (ArC), 128.2 (ArC), 128.1 (ArC), 127.5 (ArC), 127.3 (ArC), 127.2 (ArC), 124.7 (ArC), 124.2 (ArC), 121.5 (ArC), 120.3 (ArC), 109.8 (ArC), 74.4 (NCHN), 72.8 (NC₄), 57.2 (NCH), 49.6 (CH₂). HRMS (ESI) m/z calcd for C₃₂H₂₆N₃O₂⁺ (M+H)⁺ 484.2020, found 484.2017.

(±)7-benzyl-5-(4-chlorophenyl)-7a,8-dihydro-3H,5H-pyrrolo[2',1':3,4]pyrazino

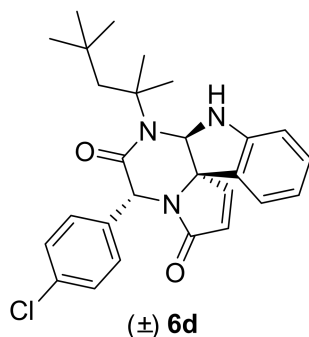
[2,3-b]indole-3,6(7H)-dione, Compound (±)-**6c**, yellow solid, yield 80%. ¹H NMR



(400 MHz, CDCl₃) δ 7.42 – 7.35 (m, 3H, ArCH), 7.31 – 7.29 (m, 2H, ArCH), 7.02 – 6.94 (m, 6H, ArCH), 6.48 (t, *J* = 7.5 Hz, 1H, ArCH), 6.33 – 6.25 (m, 3H, ArCH, CHCH), 6.14 (s, 1H, COCH), 5.19 (s, 1H, NCHN), 4.83 (q, *J* = 15.1 Hz, 2H, CH₂), 4.33 (s, 1H, NH). ¹³C NMR (100 MHz, CDCl₃) δ 171.0 (CO), 167.2 (CO), 149.4 (ArC), 146.8

(ArC), 136.3 (ArC), 134.0 (ArC), 133.3 (ArC), 130.3 (ArC), 129.2 (ArC), 128.2 (ArC), 128.1 (ArC), 127.8 (ArC), 125.9 (ArC), 124.2 (ArC), 122.7 (ArC), 120.3 (ArC), 110.5 (ArC), 74.1 (NCHN), 71.3 (NC₄), 56.1 (NCH), 48.8 (CH₂). HRMS (ESI) *m/z* calcd for C₂₆H₂₁ClN₃O₂⁺ (M+H)⁺ 442.1317, found 442.1316.

(±)5-(4-chlorophenyl)-7-(2,4,4-trimethylpentan-2-yl)-7a,8-dihydro-3H,5H-pyrrolo[2',1':3,4]pyrazino[2,3-b]indole-3,6(7H)-dione, Compound (±)-**6d**, white solid,

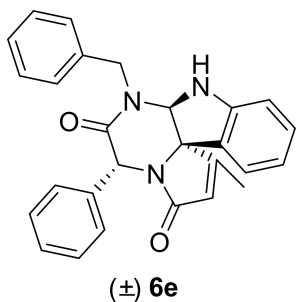


yield 83%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.02 (s, 1H, NH), 7.56 (s, 1H, ArCH), 7.16 (m, 5H, ArCH), 6.99 (m, 4H, ArCH), 6.79 (s, 1H, ArCH), 6.07 (s, 1H, COCH), 1.54 (s, 2H, CH₂), 1.27 (d, *J* = 13.1 Hz, 6H, 2CH₃), 0.85 (s, 9H, 3CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 170.2 (CO), 167.7 (CO), 154.1 (ArC), 133.9 (ArC), 132.1 (ArC), 131.8 (ArC), 127.2

(ArC), 120.9 (ArC), 118.9 (ArC), 114.5 (ArC), 111.4 (ArC), 81.4 (NCHN), 77.1 (NC₄), 54.2 (NCH), 50.5 (CH₂), 31.1 (C₄), 31.0 (C₄), 29.0 (CH₃), 28.3 (CH₃). HRMS (ESI) *m/z* calcd for C₂₇H₃₁ClN₃O₂⁺ (M+H)⁺ 464.2099, found 464.2111.

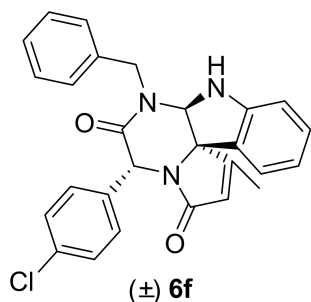
(±)7-benzyl-1-methyl-5-phenyl-7a,8-dihydro-3H,5H-pyrrolo[2',1':3,4]pyrazino

[2,3-b]indole-3,6(7H)-dione, Compound (±)-**6e**, yellow solid, yield 56%. ¹H NMR (400 MHz, CDCl₃) δ 7.39 – 7.30 (m, 5H, ArCH), 7.23 – 7.15 (m, 1H, ArCH), 7.07 – 6.96 (m, 5H, ArCH), 6.88 (t, *J* = 7.6 Hz, 1H, ArCH), 6.37 (t, *J* = 7.5 Hz, 1H, ArCH), 6.26 (d, *J* = 7.9 Hz, 1H, ArCH), 6.19 (d, *J* = 7.8 Hz, 2H, ArCH, COCH), 5.99 (d, *J* =



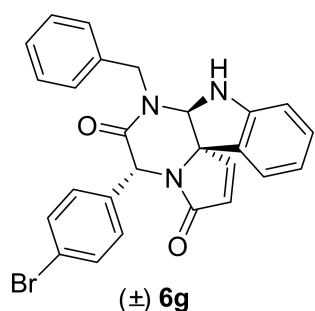
1.4 Hz, 1H, NCHN), 5.07 (s, 1H, NH), 4.93 (d, $J = 15.1$ Hz, 1H, 1CH₂), 4.78 (d, $J = 15.1$ Hz, 1H, 1CH₂), 1.65 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 171.1 (CO), 167.8 (CO), 160.9 (ArC), 147.2 (ArC), 136.6 (ArC), 136.1 (ArC), 130.0 (ArC), 129.1 (ArC), 128.1 (ArC), 127.7 (ArC), 127.2 (ArC), 123.9 (ArC), 123.7 (ArC), 121.5 (ArC), 120.2 (ArC), 110.1 (ArC), 74.2 (NCHN), 72.8 (NC₄), 56.4 (NCH), 48.8 (CH₂), 12.5 (CH₃). HRMS (ESI) m/z calcd for C₂₇H₂₄N₃O₂⁺ (M+H)⁺ 422.1863, found 422.1769.

(±)-7-benzyl-5-(4-chlorophenyl)-1-methyl-7a,8-dihydro-3H,5H-pyrrolo[2',1':3,4]pyrazino[2,3-b]indole-3,6(7H)-dione, Compound (±)-**6f**, yellow solid, yield 83%. ¹H



NMR (400 MHz, CDCl₃) δ 7.43 – 7.33 (m, 3H, ArCH), 7.32 – 7.27 (m, 2H, ArCH), 7.26 – 7.20 (m, 1H, ArCH), 7.04 – 6.89 (m, 5H, ArCH), 6.46 (t, $J = 7.5$ Hz, 1H, ArCH), 6.25 (t, $J = 6.7$ Hz, 2H, ArCH), 6.15 (s, 1H, COCH), 6.00 (d, $J = 1.4$ Hz, 1H, NCHN), 5.08 (s, 1H, NH), 4.90 (d, $J = 15.1$ Hz, 1H, 1CH₂), 4.75 (d, $J = 15.1$ Hz, 1H, 1CH₂), 1.66 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 171.2 (CO), 167.7 (CO), 161.0 (ArC), 147.2 (ArC), 136.4 (ArC), 134.5 (ArC), 133.2 (ArC), 130.3 (ArC), 129.2 (ArC), 129.0 (ArC), 128.2 (ArC), 128.1 (ArC), 127.7 (ArC), 123.9 (ArC), 123.6 (ArC), 121.7 (ArC), 120.3, 110.3 (ArC), 74.0 (NCHN), 72.8 (NC₄), 56.0 (NCH), 48.9 (CH₂), 12.6 (CH₃). HRMS (ESI) m/z calcd for C₂₇H₂₃ClN₃O₂⁺ (M+H)⁺ 456.1473, found 456.1536.

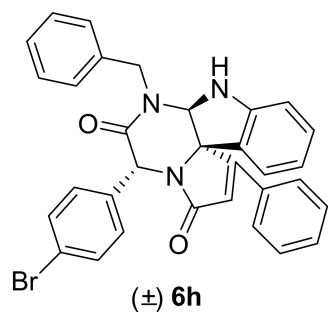
(±)-7-benzyl-5-(4-bromophenyl)-7a,8-dihydro-3H,5H-pyrrolo[2',1':3,4]pyrazino[2,3-b]indole-3,6(7H)-dione, Compound (±)-**6g**, yellow solid, yield 79%. ¹H NMR



(400 MHz, CDCl₃) δ 7.38 (td, $J = 8.9, 4.5$ Hz, 3H, ArCH), 7.32 – 7.28 (m, 2H, ArCH), 7.23 (s, 1H, ArCH), 7.12 (d, $J = 8.5$ Hz, 2H, ArCH), 6.96 (dd, $J = 15.0, 7.0$ Hz, 4H, ArCH), 6.48 (t, $J = 7.7$ Hz, 1H, ArCH), 6.33 – 6.24 (m, 3H, ArCH, COCH), 6.12 (s, 1H, NCHN), 5.18 (s, 1H, NH),

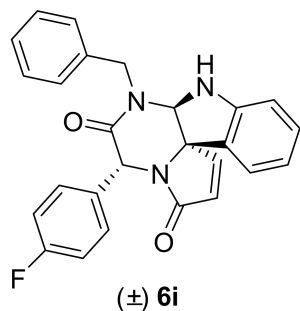
4.82 (q, $J = 15.1$ Hz, 2H, CH₂). ¹³C NMR (100 MHz, CDCl₃) δ 171.1 (CO), 167.2 (CO), 149.4 (ArC), 146.8 (ArC), 136.3 (ArC), 134.5 (ArC), 130.7 (ArC), 130.3 (ArC), 129.4 (ArC), 129.2 (ArC), 128.3 (ArC), 128.1 (ArC), 125.9 (ArC), 124.2 (ArC), 122.7 (ArC), 121.5 (ArC), 120.3 (ArC), 110.5 (ArC), 74.1 (NCHN), 71.4 (NC₄), 56.2 (NCH), 48.8 (CH₂). HRMS (ESI) m/z calcd for C₂₆H₂₁BrN₃O₂⁺ (M+H)⁺ 486.0812, found 486.0810; 488.0800.

(±)7-benzyl-5-(4-bromophenyl)-1-phenyl-7a,8-dihydro-3H,5H-pyrrolo[2',1':3,4]pyrazino[2,3-b]indole-3,6(7H)-dione, Compound **(±)-6h**, yellow solid, yield 68%.



¹H NMR (400 MHz, CDCl₃) δ 7.32 – 7.23 (m, 7H, ArCH), 7.18 (t, $J = 7.7$ Hz, 2H, ArCH), 7.06 (d, $J = 8.5$ Hz, 2H, ArCH), 6.99 – 6.91 (m, 5H, ArCH), 6.58 (t, $J = 3.4$ Hz, 2H, ArCH), 6.50 (t, $J = 7.4$ Hz, 1H, ArCH), 6.25 (s, 1H, COCH), 6.16 (d, $J = 7.9$ Hz, 1H, NCHN), 5.30 (s, 1H, NH), 4.73 (s, 2H, CH₂). ¹³C NMR (100 MHz, CDCl₃) δ 171.0 (CO), 168.6 (CO), 160.7 (ArC), 147.5 (ArC), 136.5 (ArC), 133.3 (ArC), 130.5 (ArC), 130.4 (ArC), 130.2 (ArC), 129.1 (ArC), 128.9 (ArC), 128.3 (ArC), 127.3 (ArC), 124.7 (ArC), 124.1 (ArC), 121.4 (ArC), 121.3 (ArC), 120.5 (ArC), 110.0 (ArC), 74.4 (NCHN), 72.8 (NC₄), 56.8 (NCH), 49.7 (CH₂). HRMS (ESI) m/z calcd for C₃₂H₂₅BrN₃O₂⁺ (M+H)⁺ 562.1125, found 562.1126; 564.1118.

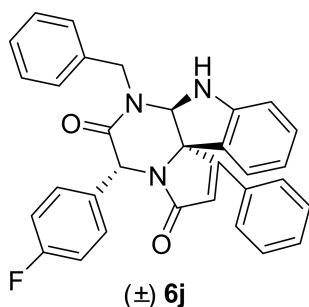
(±)7-benzyl-5-(4-fluorophenyl)-7a,8-dihydro-3H,5H-pyrrolo[2',1':3,4]pyrazino[2,3-b]indole-3,6(7H)-dione, Compound **(±)-6i**, yellow solid, yield 67%.



¹H NMR (400 MHz, CDCl₃) δ 7.41 – 7.34 (m, 3H, ArCH), 7.32 – 7.28 (m, 2H, ArCH), 7.04 (dd, $J = 8.6, 5.4$ Hz, 2H, ArCH), 6.95 (dd, $J = 13.3, 6.7$ Hz, 2H, ArCH), 6.68 (t, $J = 8.7$ Hz, 2H, ArCH), 6.46 (t, $J = 7.5$ Hz, 1H, ArCH), 6.31 – 6.25 (m, 3H, ArCH, COCH), 6.15 (s, 1H, NCHN), 5.18 (s, 1H, NH), 4.89 (d, $J = 15.1$ Hz, 1H, 1CH₂), 4.78 (d, $J = 15.1$ Hz, 1H, CH₂). ¹³C NMR (100 MHz, CDCl₃) δ 170.9 (CO), 167.4 (CO), 149.4 (ArC), 146.8

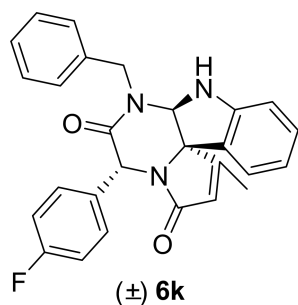
(ArC), 136.3 (ArC), 131.4 (ArC), 130.3 (ArC), 129.5 (ArC), 129.2 (ArC), 128.2 (ArC), 128.1 (ArC), 125.8 (ArC), 124.2 (ArC), 122.7 (ArC), 120.2 (ArC), 114.6 (ArC), 114.4 (ArC), 110.5 (ArC), 74.0 (NCHN), 71.3 (NC₄), 56.0 (NCH), 48.7 (CH₂). HRMS (ESI) *m/z* calcd for C₂₆H₂₁FN₃O₂⁺ (M+H)⁺ 426.1612, found 426.1618.

(±)7-benzyl-5-(4-fluorophenyl)-1-phenyl-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino [2,3-*b*]indole-3,6(7*H*)-dione, Compound (±)-**6j**, yellow solid, yield 73%.



¹H NMR (400 MHz, CDCl₃) δ 7.28 (dt, *J* = 10.9, 6.5 Hz, 7H, ArCH), 7.17 (t, *J* = 7.7 Hz, 2H, ArCH), 7.05 (dd, *J* = 8.3, 5.4 Hz, 2H, ArCH), 6.96 (d, *J* = 7.6 Hz, 2H, ArCH), 6.90 (t, *J* = 7.6 Hz, 1H, ArCH), 6.63 (t, *J* = 8.7 Hz, 2H, ArCH), 6.56 (d, *J* = 7.6 Hz, 2H, ArCH), 6.48 (t, *J* = 7.4 Hz, 1H, ArCH), 6.29 (s, 1H, COCH), 6.18 (d, *J* = 7.9 Hz, 1H, NCHN), 5.30 (s, 1H, NH), 4.79 – 4.69 (m, 2H, CH₂). ¹³C NMR (100 MHz, CDCl₃) δ 171.0 (CO), 168.8 (CO), 163.1 (ArC), 160.7 (ArC), 147.5 (ArC), 136.5 (ArC), 130.6 (ArC), 130.2 (ArC), 129.2 (ArC), 128.9 (ArC), 128.2 (ArC), 128.1 (ArC), 127.3 (ArC), 124.7 (ArC), 124.1 (ArC), 121.4 (ArC), 120.4 (ArC), 114.4 (ArC), 114.2 (ArC), 109.9 (ArC), 74.3 (NCHN), 72.7 (NC₄), 56.6 (NCH), 49.7 (CH₂). HRMS (ESI) *m/z* calcd for C₃₂H₂₅FN₃O₂⁺ (M+H)⁺ 502.1925, found 502.1931.

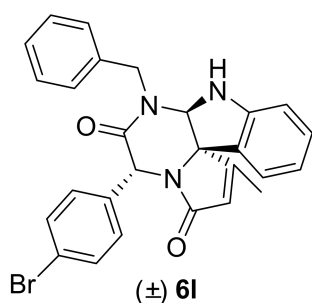
(±)7-benzyl-5-(4-fluorophenyl)-1-methyl-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino[2,3-*b*]indole-3,6(7*H*)-dione, Compound (±)-**6k**, yellow solid, yield 85%.



¹H NMR (400 MHz, CDCl₃) δ 7.37 (dt, *J* = 6.8, 5.9 Hz, 3H, ArCH), 7.32 – 7.28 (m, 2H, ArCH), 7.02 (dd, *J* = 8.4, 5.4 Hz, 2H, ArCH), 6.96 – 6.90 (m, 1H, ArCH), 6.67 (t, *J* = 8.7 Hz, 2H, ArCH), 6.44 (t, *J* = 7.4 Hz, 1H, ArCH), 6.28 (d, *J* = 7.9 Hz, 1H, ArCH), 6.23 (d, *J* = 7.5 Hz, 1H, ArCH), 6.16 (s, 1H, COCH), 6.00 (d, *J* = 1.4 Hz, 1H, NCHN), 5.08 (s, 1H, NH), 4.92 (d, *J* = 15.1 Hz, 1H, 1CH₂), 4.76 (d, *J* = 15.1 Hz, 1H, 1CH₂), 1.66 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 171.2 (CO), 167.8 (CO), 161.0 (ArC), 147.1 (ArC),

136.4 (ArC), 131.8 (ArC), 130.2 (ArC), 129.5 (ArC), 129.2 (ArC), 128.2 (ArC), 128.0 (ArC), 123.8 (ArC), 123.6 (ArC), 120.3 (ArC), 114.6 (ArC), 114.4 (ArC), 110.2 (ArC), 74.1 (NCHN), 72.8 (NC₄), 55.9 (NCH), 48.9 (CH₂), 12.6 (CH₃). HRMS (ESI) *m/z* calcd for C₂₇H₂₃FN₃O₂⁺ (M+H)⁺ 440.1769, found 440.1768.

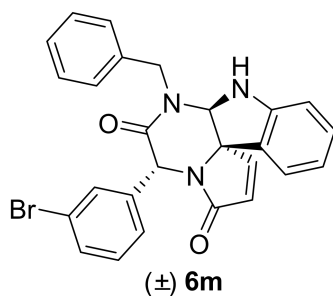
(±)7-benzyl-5-(4-bromophenyl)-1-methyl-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino[2,3-*b*]indole-3,6(7*H*)-dione, Compound (±)-**6l**, yellow solid, yield 83%. ¹H



NMR (400 MHz, CDCl₃) δ 7.40 – 7.34 (m, 3H, ArCH), 7.30 – 7.29 (m, 2H, ArCH), 7.10 (d, *J* = 8.4 Hz, 2H, ArCH), 6.94 (dd, *J* = 17.9, 8.0 Hz, 3H, ArCH), 6.47 (t, *J* = 7.5 Hz, 1H, ArCH), 6.27 – 6.24 (m, 2H, ArCH), 6.13 (s, 1H, COCH), 6.00 (s, 1H, NCHN), 5.08 (s, 1H, NH), 4.90 (d, *J* = 15.1 Hz, 1H, 1CH₂), 4.76 (d, *J* = 15.1 Hz, 1H,

1CH₂), 1.66 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 171.2 (CO), 167.6 (CO), 161.0 (ArC), 147.2 (ArC), 136.4 (ArC), 135.0 (ArC), 130.6 (ArC), 130.2 (ArC), 129.3 (ArC), 128.2 (ArC), 128.1 (ArC), 123.9 (ArC), 123.6 (ArC), 121.7 (ArC), 121.4 (ArC), 120.3 (ArC), 110.2 (ArC), 74.0 (NCHN), 72.8 (NC₄), 56.0 (NCH), 48.9 (CH₂), 12.6 (CH₃). HRMS (ESI) *m/z* calcd for C₂₇H₂₃BrN₃O₂⁺ (M+H)⁺ 500.0968, found 500.0950; 502.0952.

(±)7-benzyl-5-(3-bromophenyl)-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino[2,3-*b*]indole-3,6(7*H*)-dione, Compound (±)-**6m**, yellow solid, yield 75%. ¹H NMR

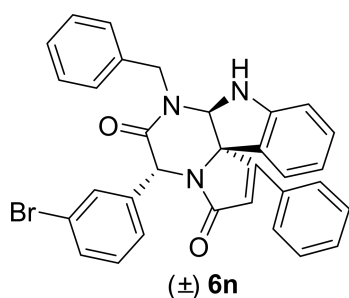


(400 MHz, CDCl₃) δ 7.42 – 7.34 (m, 3H, ArCH), 7.30 (d, *J* = 7.0 Hz, 2H, ArCH), 7.17 (d, *J* = 6.9 Hz, 2H, ArCH), 7.07 (d, *J* = 7.7 Hz, 1H, ArCH), 6.99 – 6.87 (m, 3H, ArCH), 6.48 (t, *J* = 7.5 Hz, 1H, ArCH), 6.31 (dd, *J* = 12.7, 7.4 Hz, 3H, ArCH), 6.14 (s, 1H, COCH), 5.20 (d, *J* = 2.0 Hz, 1H, NCHN), 4.87 (d, *J* = 15.1 Hz, 1H, 1CH₂), 4.78 (d,

J = 15.1 Hz, 1H, 1CH₂), 4.38 (s, 1H, NH). ¹³C NMR (100 MHz, CDCl₃) δ 171.0 (CO), 167.1 (CO), 149.5 (ArC), 146.8 (ArC), 137.5 (ArC), 136.3 (ArC), 130.8 (ArC), 130.6

(ArC), 129.2 (ArC), 128.2 (ArC), 128.1 (ArC), 126.5 (ArC), 125.9 (ArC), 124.1 (ArC), 122.6 (ArC), 121.8 (ArC), 120.3 (ArC), 110.4 (ArC), 74.0 (NCHN), 71.4 (NC₄), 56.2 (NCH), 48.8 (CH₂). HRMS (ESI) *m/z* calcd for C₂₆H₂₁BrN₃O₂⁺ (M+H)⁺ 486.0812, found 486.0800; 488.0798.

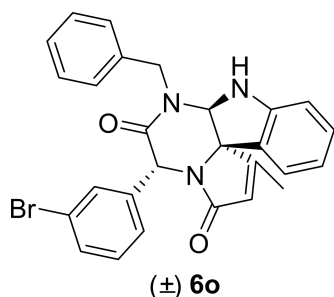
(±)7-benzyl-5-(3-bromophenyl)-1-phenyl-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino[2,3-*b*]indole-3,6(7*H*)-dione, Compound (±)-**6n**, yellow solid, yield 71%.



¹H NMR (400 MHz, CDCl₃) δ 7.34 – 7.25 (m, 7H, ArCH), 7.20 – 7.16 (m, 3H, ArCH), 7.11 – 7.06 (m, 2H, ArCH), 6.99 (d, *J* = 7.5 Hz, 2H, ArCH), 6.92-6.83 (m, 2H, ArCH), 6.63 – 6.58 (m, 2H, ArCH), 6.50 (t, *J* = 7.4 Hz, 1H, ArCH), 6.28 (s, 1H, COCH), 6.21 (d, *J* = 7.9 Hz, 1H, NCHN), 5.31 (s, 1H, NH), 4.74 (s, 2H, CH₂).

¹³C NMR (100 MHz, CDCl₃) δ 171.0 (CO), 168.5 (CO), 160.8 (ArC), 147.4 (ArC), 136.5 (ArC), 130.8 (ArC), 130.5 (ArC), 130.3 (ArC), 129.1 (ArC), 128.9 (ArC), 128.2 (ArC), 127.3 (ArC), 126.2 (ArC), 124.5 (ArC), 124.0 (ArC), 121.7 (ArC), 121.3 (ArC), 120.6 (ArC), 109.9 (ArC), 74.3 (NCHN), 72.8 (NC₄), 56.7 (NCH), 49.7 (CH₂). HRMS (ESI) *m/z* calcd for C₃₂H₂₅BrN₃O₂⁺ (M+H)⁺ 562.1125, found 562.1098; 564.1096.

(±)7-benzyl-5-(3-bromophenyl)-1-methyl-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino [2,3-*b*]indole-3,6(7*H*)-dione, Compound(±)-**6o**, yellow solid, yield 79%.

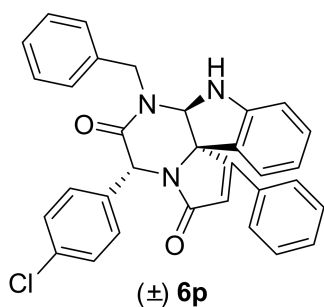


¹H NMR (400 MHz, CDCl₃) δ 7.41 – 7.35 (m, 3H, ArCH), 7.30 (d, *J* = 6.6 Hz, 2H, ArCH), 7.15 (d, *J* = 6.7 Hz, 2H, ArCH), 7.05 (d, *J* = 8.0 Hz, 1H, ArCH), 6.90 (dt, *J* = 16.4, 7.7 Hz, 2H, ArCH), 6.46 (t, *J* = 7.5 Hz, 1H, ArCH), 6.32 – 6.27 (m, 2H, ArCH), 6.14 (s, 1H, COCH), 6.00 (d, *J* = 1.4 Hz, 1H, NCHN), 5.09 (s, 1H, NH), 4.92

(d, *J* = 15.1 Hz, 1H, 1CH₂), 4.74 (d, *J* = 15.1 Hz, 1H, 1CH₂), 1.66 (s, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 171.2 (CO), 167.5 (CO), 161.0 (ArC), 147.2 (ArC), 138.0

(ArC), 136.4 (ArC), 130.7 (ArC), 130.5 (ArC), 129.2 (ArC), 128.2 (ArC), 128.1 (ArC), 126.4 (ArC), 123.8 (ArC), 123.5 (ArC), 121.8 (ArC), 120.4 (ArC), 110.2 (ArC), 73.9 (NCHN), 72.9 (NC₄), 56.1 (NCH), 48.9 (CH₂), 12.64 (CH₃). HRMS (ESI) *m/z* calcd for C₂₇H₂₃BrN₃O₂⁺ (M+H)⁺ 500.0968, found 500.0952; 502.0950.

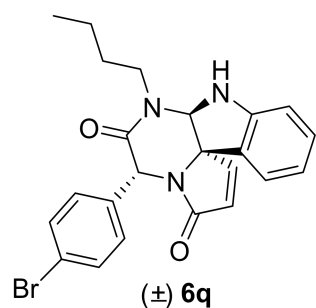
(±)7-benzyl-5-(4-chlorophenyl)-1-phenyl-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino[2,3-*b*]indole-3,6(7*H*)-dione, Compound (±)-**6p**, yellow solid, yield 78%.



¹H NMR (400 MHz, CDCl₃) δ 7.30 – 7.27 (m, 3H, ArCH), 7.26 – 7.22 (m, 3H, ArCH), 7.19 – 7.15 (m, 3H, ArCH), 7.02– 6.97 (m, 4H, ArCH), 6.91 (t, *J* = 8.1 Hz, 3H, ArCH), 6.59 – 6.55 (m, 2H, ArCH), 6.49 (t, *J* = 7.4 Hz, 1H, ArCH), 6.27 (s, 1H, COCH), 6.16 (d, *J* = 7.9 Hz, 1H, NCHN), 5.30 (s, 1H, NH), 4.73 (q, *J* = 14.9 Hz, 2H, CH₂).

¹³C NMR (100 MHz, CDCl₃) δ 171.1 (CO), 168.7 (CO), 160.8 (ArC), 147.5 (ArC), 136.4 (ArC), 133.1 (ArC), 132.8 (ArC), 131.2 (ArC), 130.5 (ArC), 130.2 (ArC), 129.1 (ArC), 128.9 (ArC), 128.2 (ArC), 128.1 (ArC), 127.5 (ArC), 127.3 (ArC), 124.7 (ArC), 124.1 (ArC), 121.3 (ArC), 120.4 (ArC), 109.9 (ArC), 74.3 (NCHN), 72.8 (NC₄), 56.7 (NCH), 49.7 (CH₂). HRMS (ESI) *m/z* calcd for C₃₂H₂₅ClN₃O₂⁺ (M+H)⁺ 518.1630, found 518.1487.

(±)5-(4-bromophenyl)-7-butyl-7*a*,8-dihydro-3*H*,5*H*-pyrrolo[2',1':3,4]pyrazino[2,3-*b*]indole-3,6(7*H*)-dione, Compound (±)-**6q**, yellow solid, yield 61%. ¹H NMR

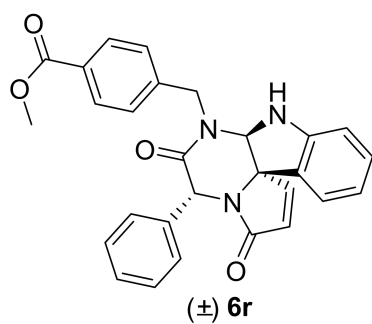


(400 MHz, CDCl₃) δ 7.10 – 7.07 (m, 3H, ArCH), 7.01 (t, *J* = 7.6 Hz, 1H, ArCH), 6.89 (d, *J* = 8.3 Hz, 2H, ArCH), 6.52 – 6.44 (m, 2H, ArCH), 6.34 (d, *J* = 5.7 Hz, 1H, ArCH), 6.29 (d, *J* = 7.5 Hz, 1H, ArCH), 6.00 (s, 1H, COCH), 5.25 (s, 1H, NCHN), 4.71 (s, 1H, NH), 3.75 (ddd, *J* = 13.9, 9.7, 6.0 Hz, 1H, 1CH₂), 3.32 – 3.24 (m, 1H, 1CH₂), 1.62 – 1.47

(m, 2H, CH₂), 1.39 – 1.33 (m, 2H, CH₂), 0.96 (t, *J* = 7.3 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃) δ 171.0 (CO), 166.6 (CO), 149.4 (ArC), 146.8 (ArC), 134.7 (ArC),

130.6 (ArC), 130.3 (ArC), 129.4 (ArC), 125.9 (ArC), 124.2 (ArC), 122.9 (ArC), 121.4 (ArC), 120.3 (ArC), 110.5 (ArC), 74.2 (NCHN), 71.3 (NC₄), 56.0 (NCH), 45.47 (CH₂), 30.0 (CH₂), 20.2 (CH₂), 13.8 (CH₃). HRMS (ESI) *m/z* calcd for C₂₃H₂₃BrN₃O₂⁺ (M+H)⁺ 452.0968, found 452.0925; 452.0919.

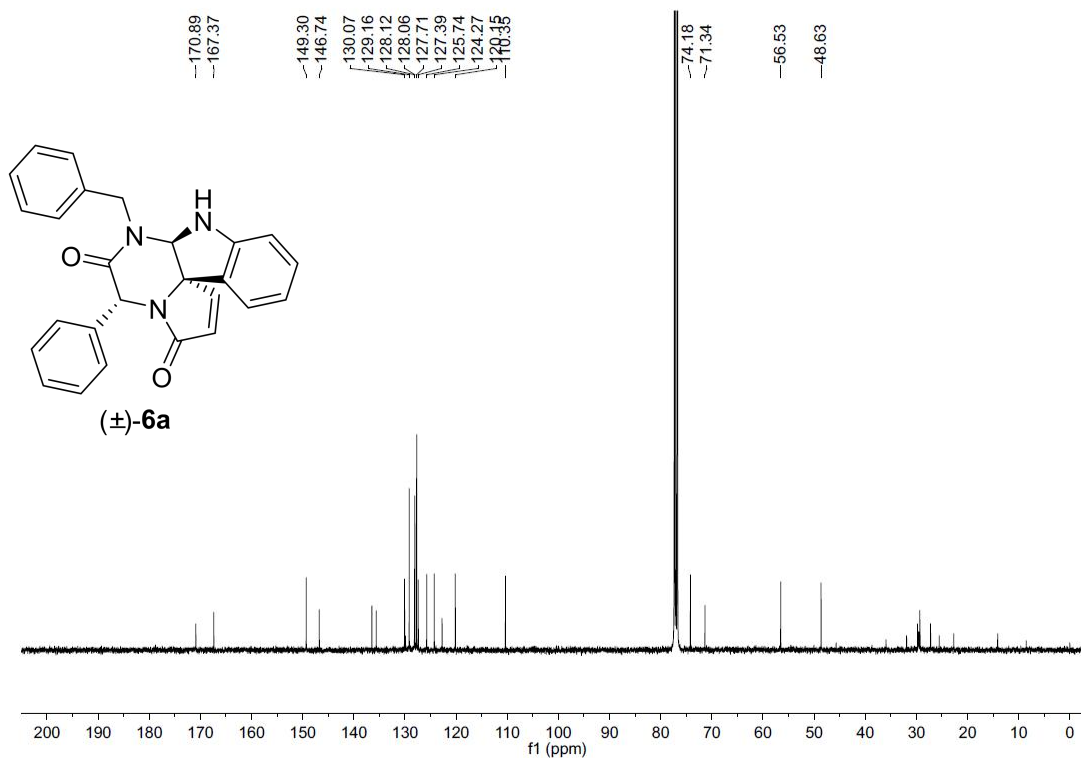
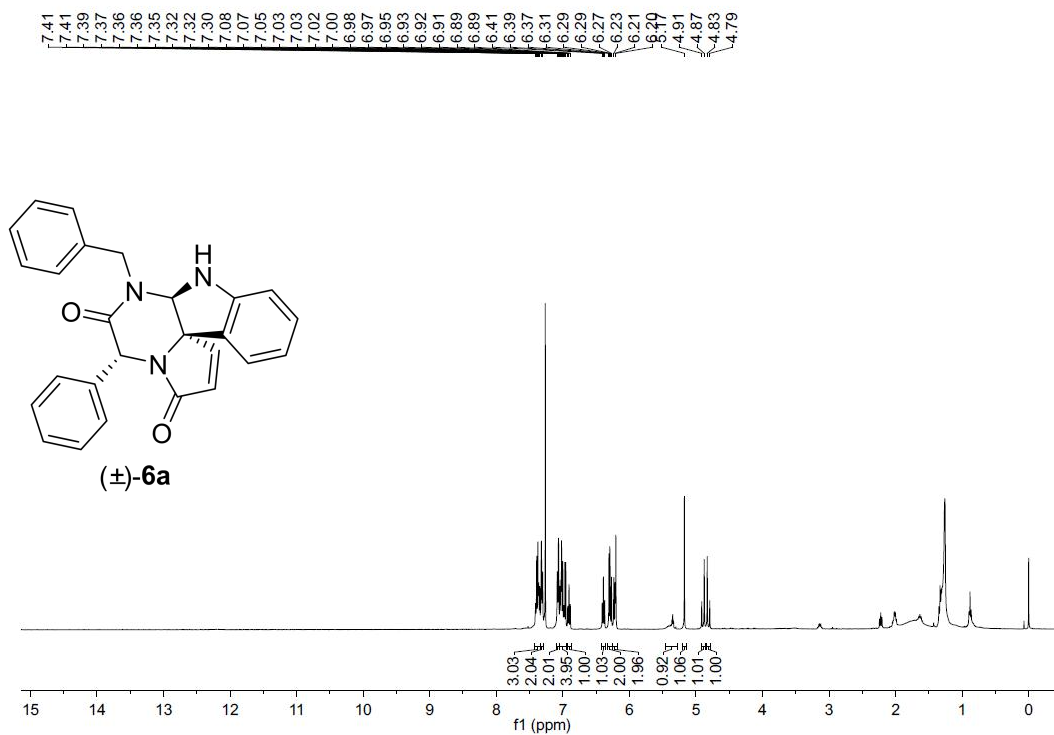
(±)methyl 4-((3,6-dioxo-5-phenyl-5,6,7*a*,8-tetrahydro-3*H*,7*H*-pyrrolo[2',1':3,4]pyrazino[2,3-*b*]indol-7-yl)methyl)benzoate, Compound (±)-6r, yellow solid, yield



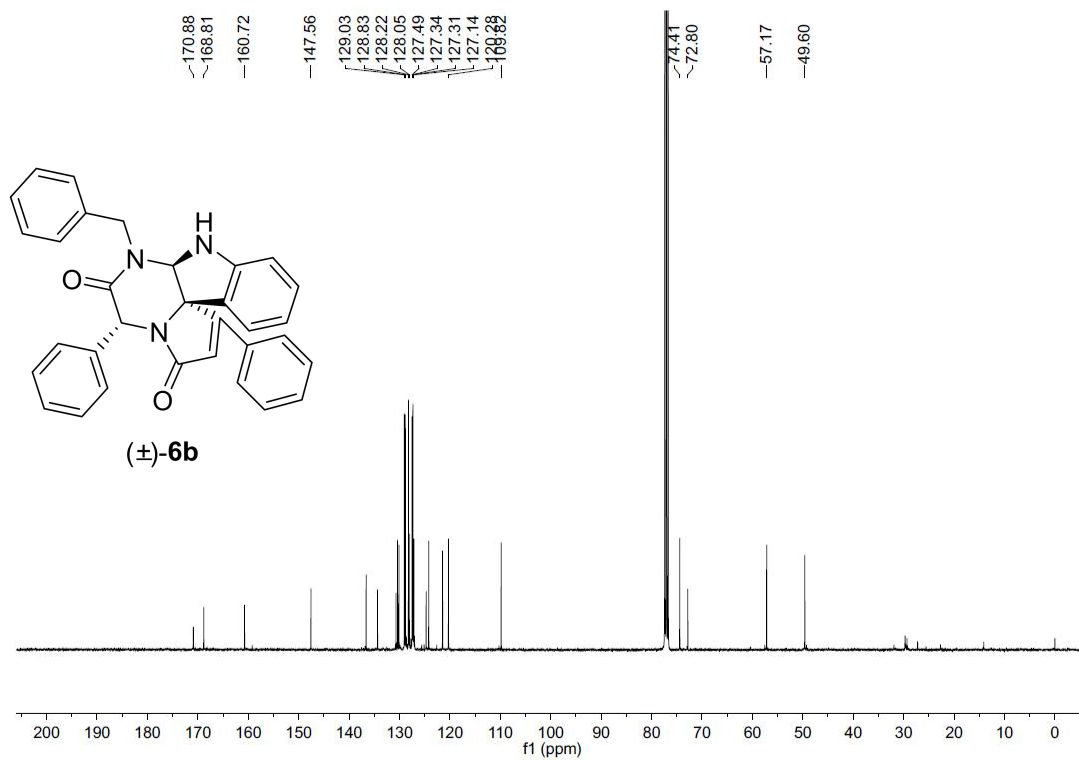
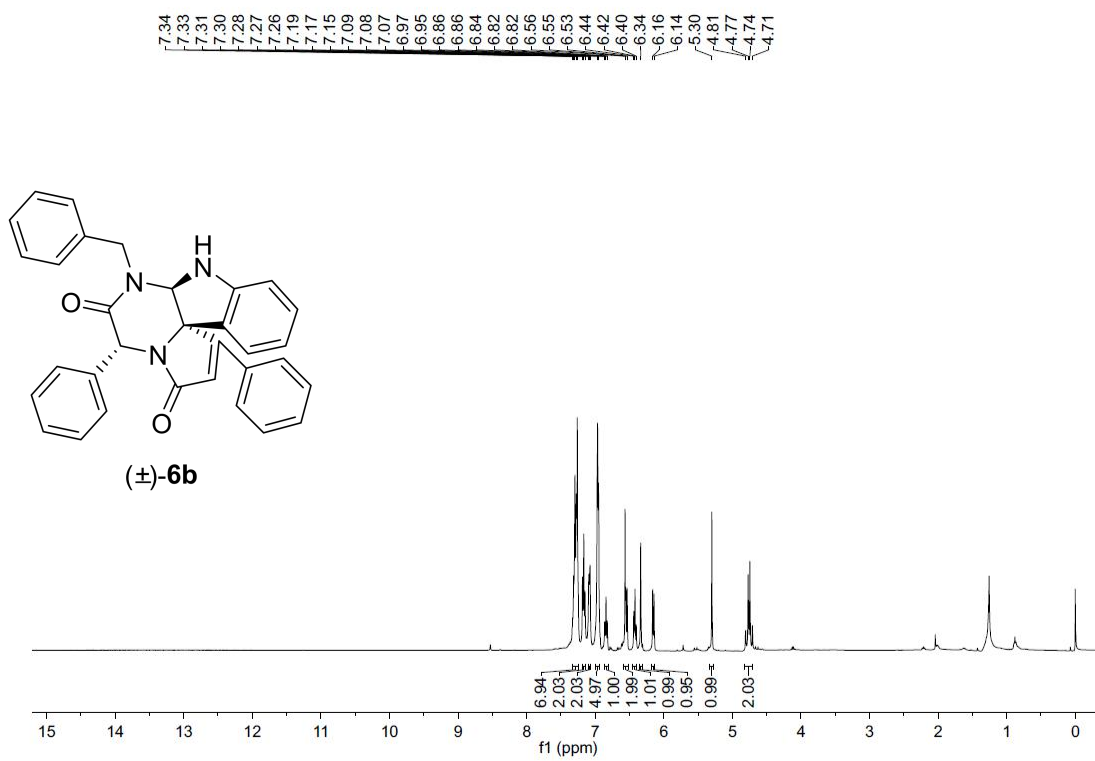
83%. ¹H NMR (400 MHz, *d*₆-DMSO) δ 7.92 (d, *J* = 8.2 Hz, 2H, ArCH), 7.36 – 7.32 (m, 3H, ArCH), 7.23 (s, 1H, ArCH), 7.07 – 6.98 (m, 5H, ArCH), 6.93 – 6.87 (m, 1H, ArCH), 6.39 (t, *J* = 6.9 Hz, 2H, ArCH), 6.33 (t, *J* = 7.4 Hz, 1H, ArCH), 6.26 (d, *J* = 7.2 Hz, 1H, ArCH), 5.82 (s, 1H, COCH), 5.30 (d, *J* = 2.9 Hz, 1H, NCHN),

5.16 (d, *J* = 15.8 Hz, 1H, 1CH₂), 4.46 (d, *J* = 15.8 Hz, 1H, 1CH₂), 3.85 (s, 3H, CH₃). ¹³C NMR (100 MHz, *d*₆-DMSO) δ 171.7 (CO), 167.4 (CO), 166.5 (ArC), 151.9 (ArC), 148.6 (ArC), 143.3 (ArC), 135.9 (ArC), 130.3 (ArC), 129.8 (ArC), 129.1 (ArC), 128.1 (ArC), 128.0 (ArC), 127.7 (ArC), 124.9 (ArC), 124.1 (ArC), 123.4 (ArC), 118.7 (ArC), 109.9 (ArC), 73.7 (NCHN), 71.4 (NC₄), 56.5 (NCH), 52.6 (OCH₃), 47.5 (CH₂). HRMS (ESI) *m/z* calcd for C₂₈H₂₄N₃O₄⁺ (M+H)⁺ 466.1761, found 466.1799.

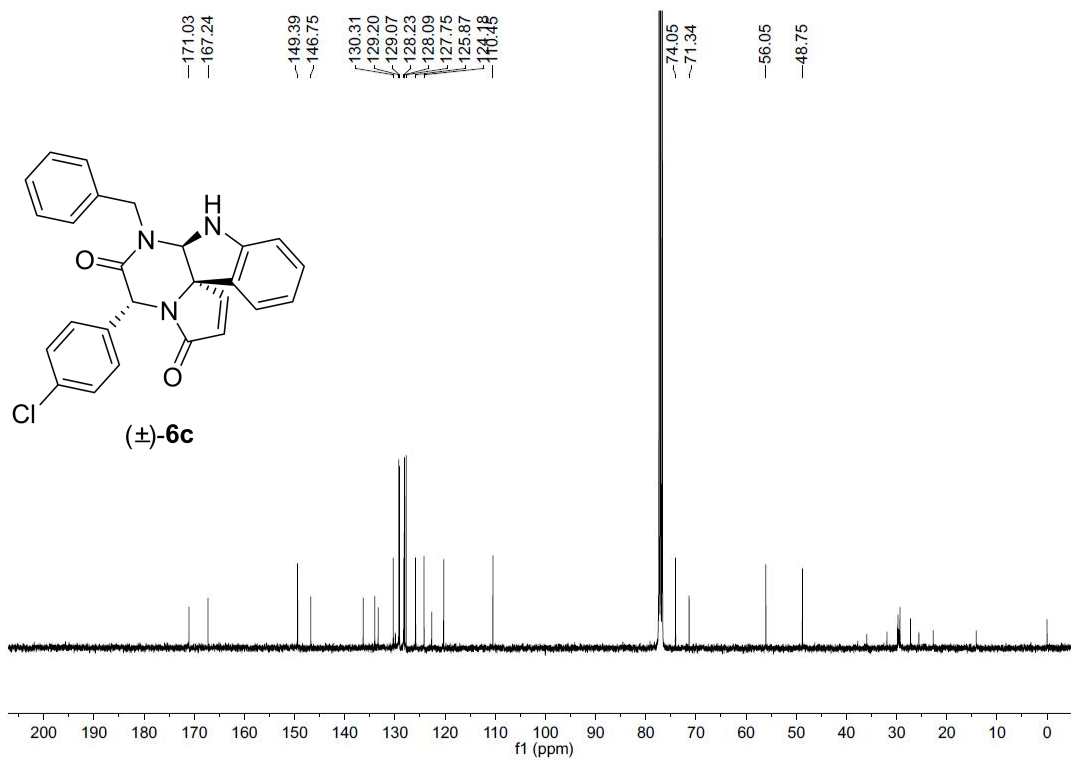
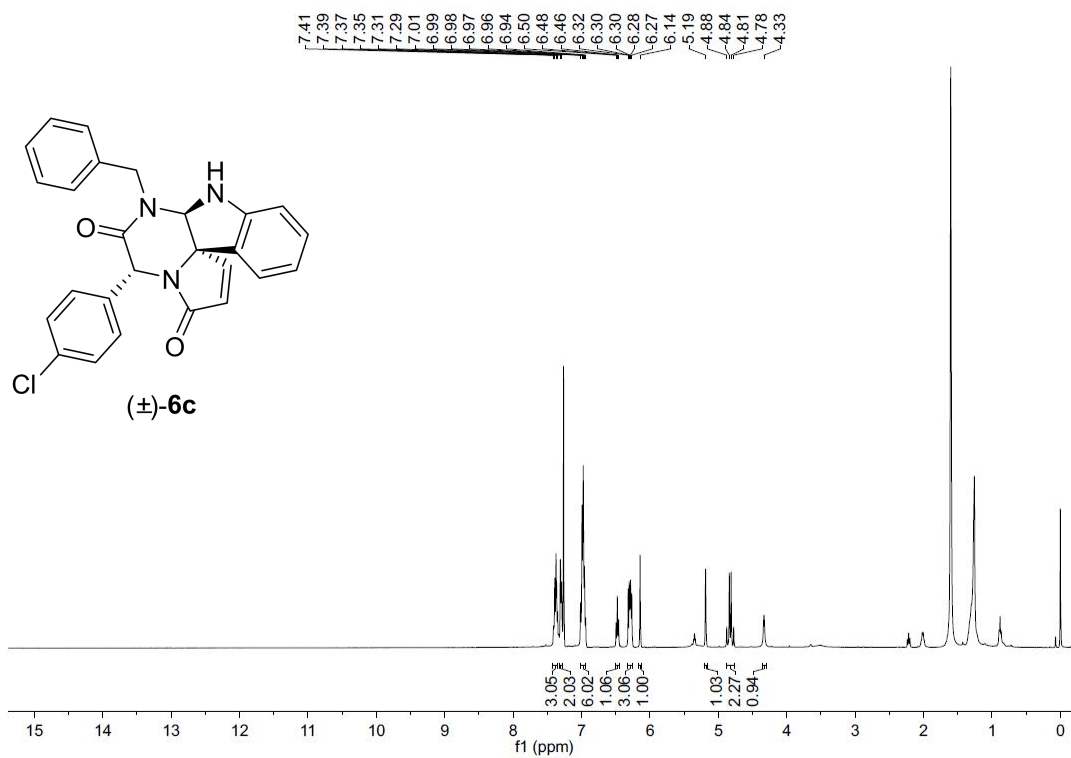
Compound (±)-6a



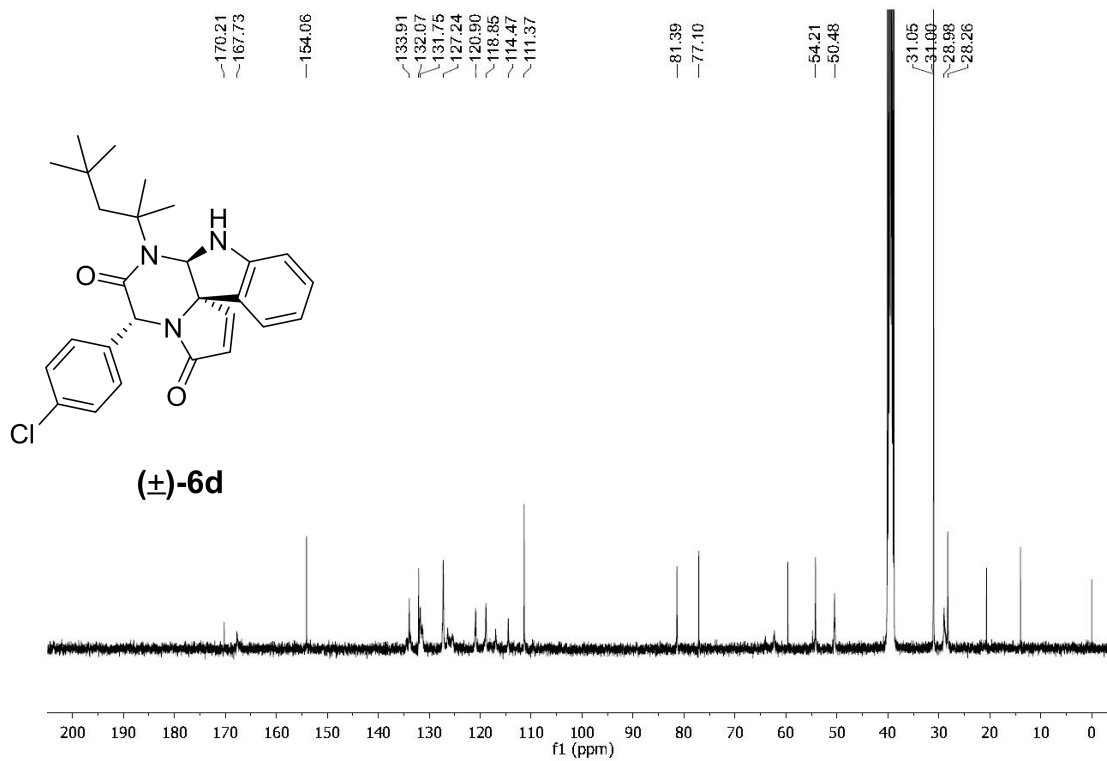
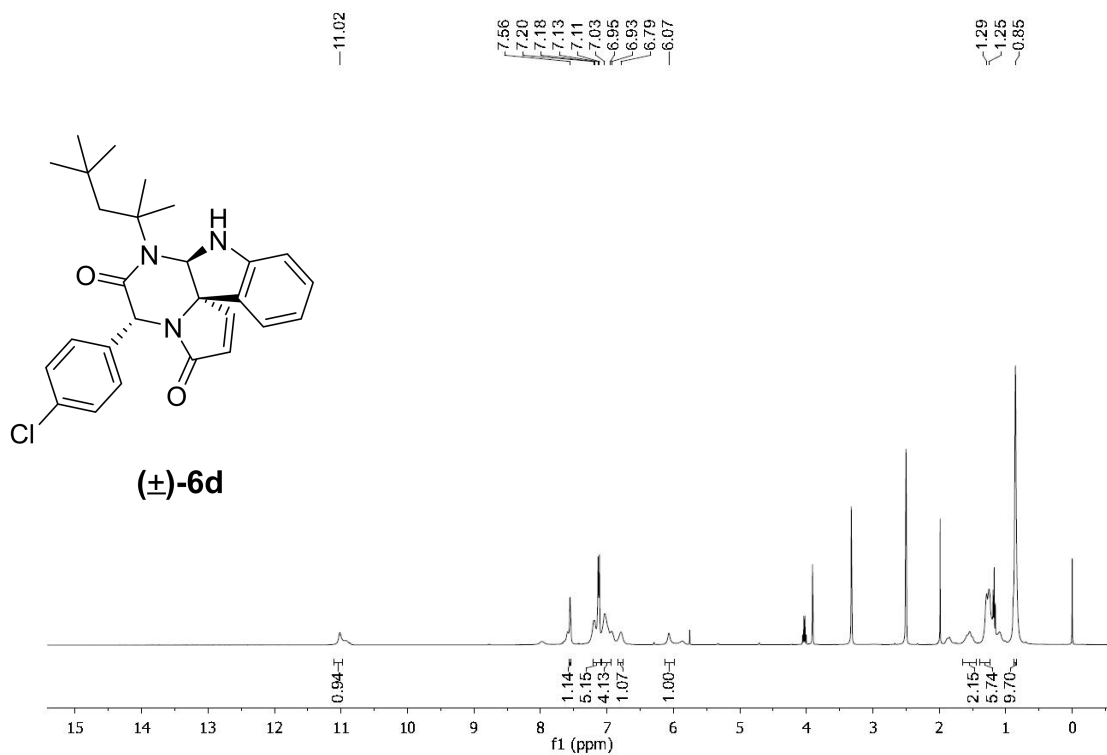
Compound (\pm)-6b



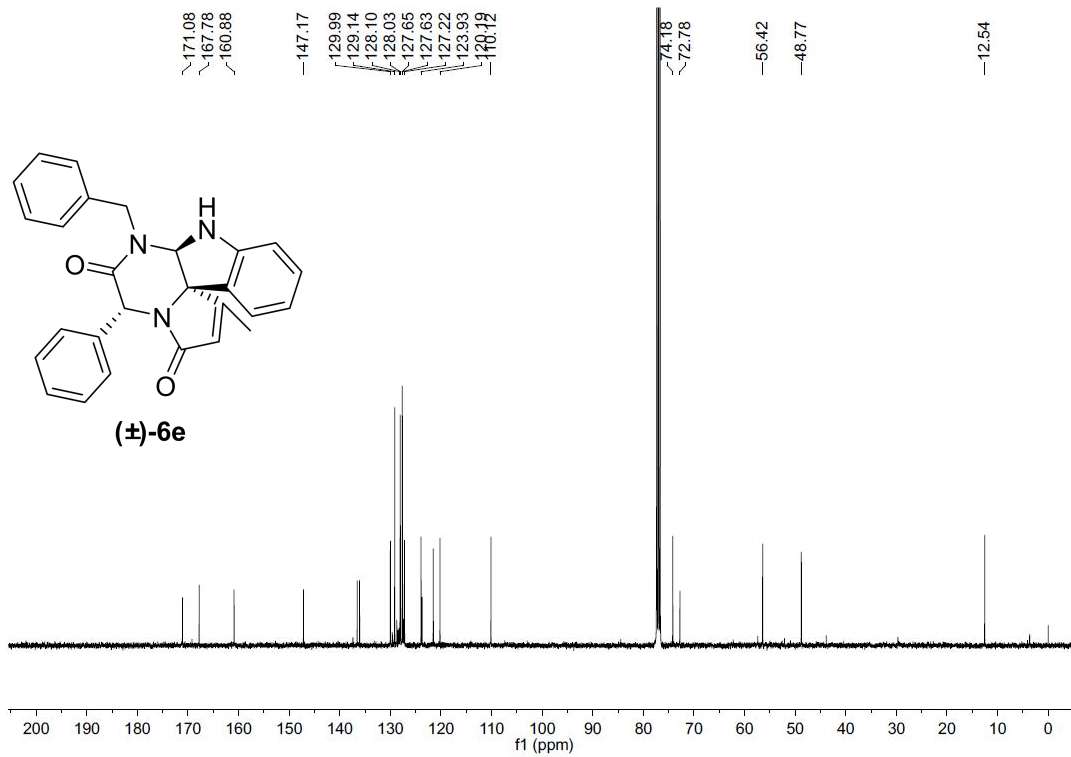
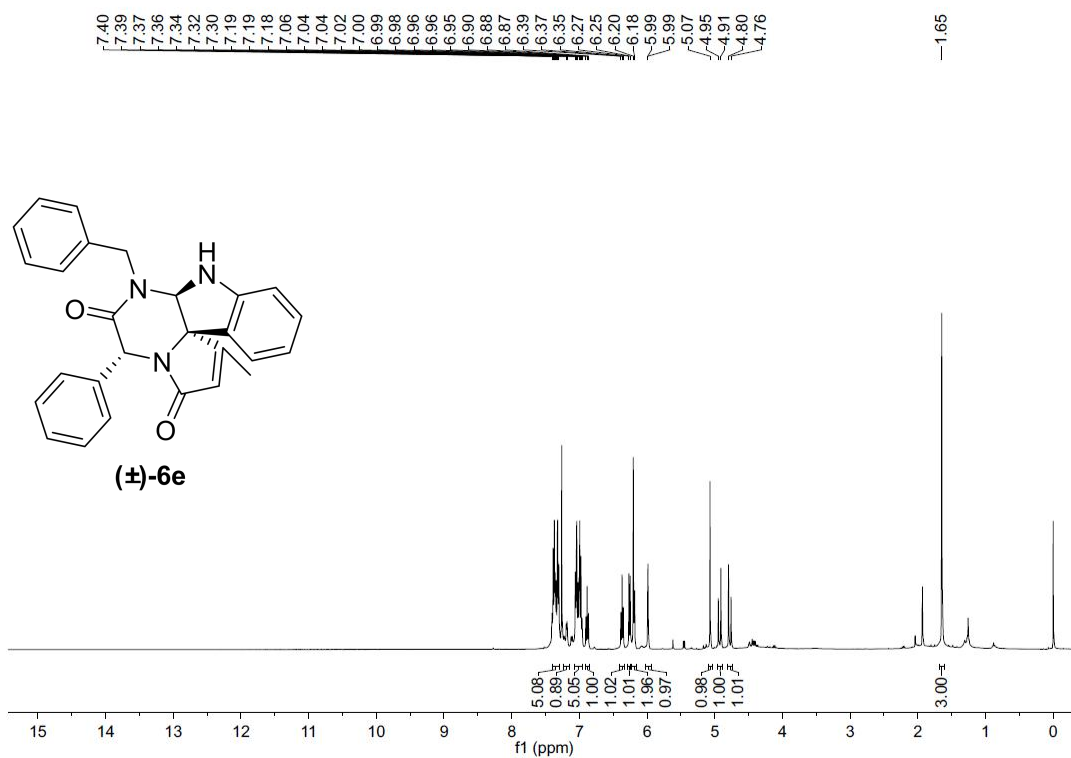
Compound (±)-6c



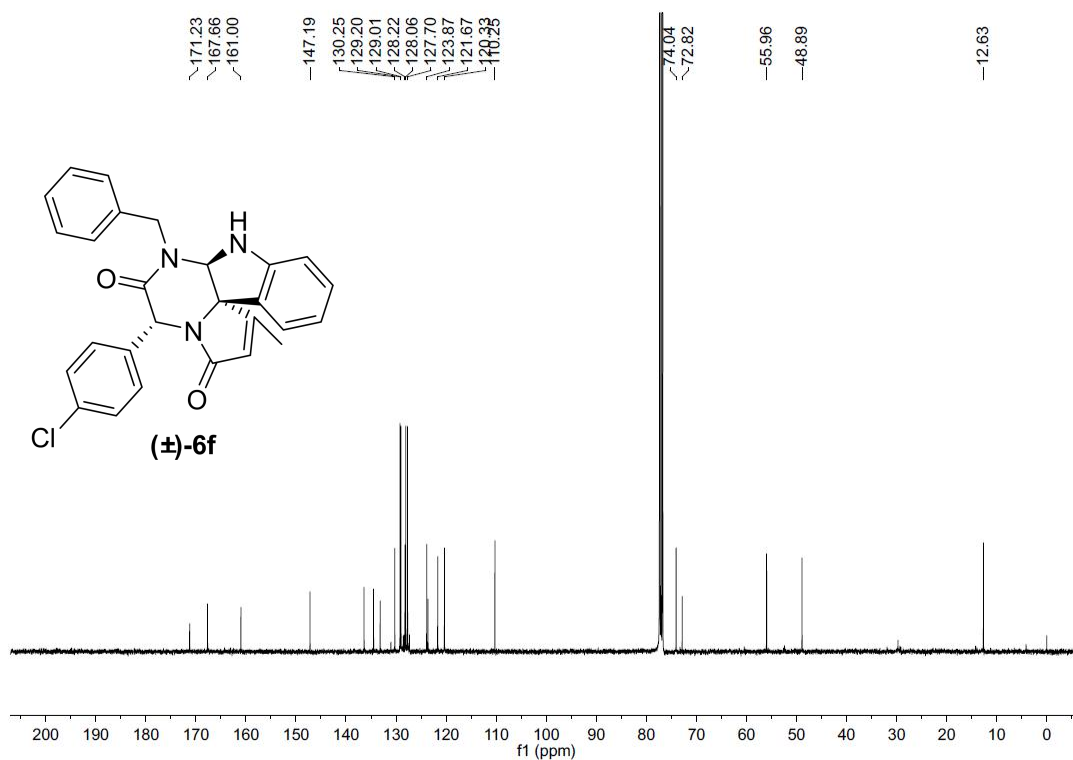
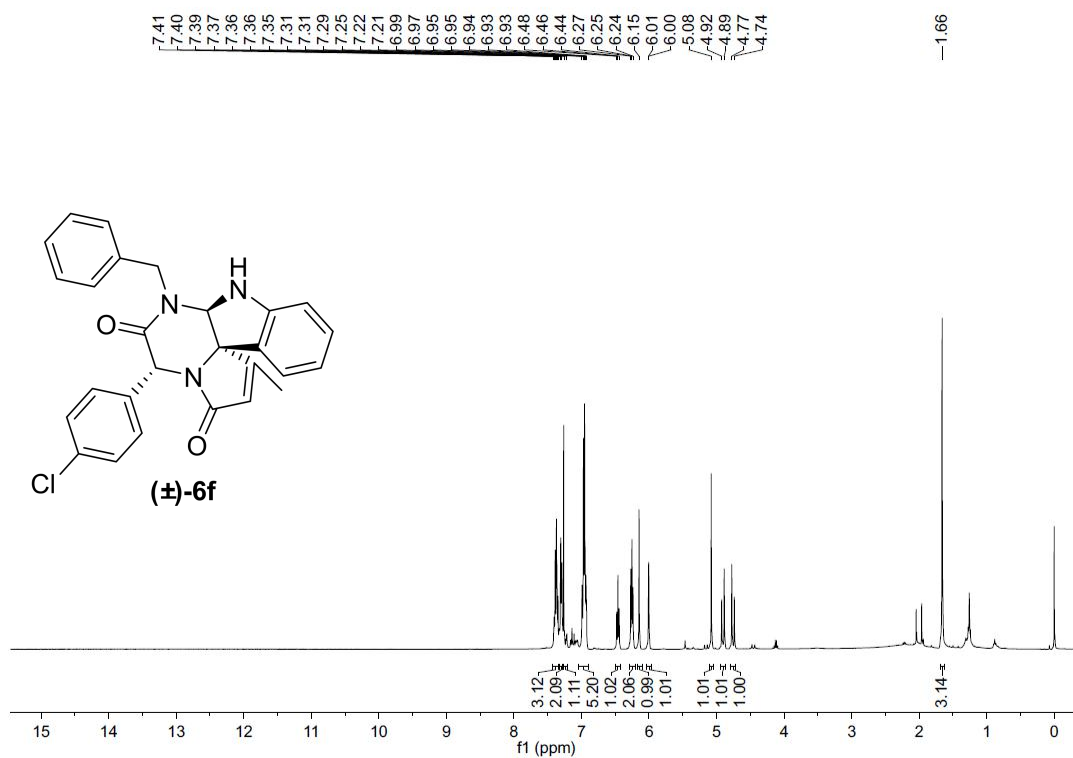
Compound (±)-6d



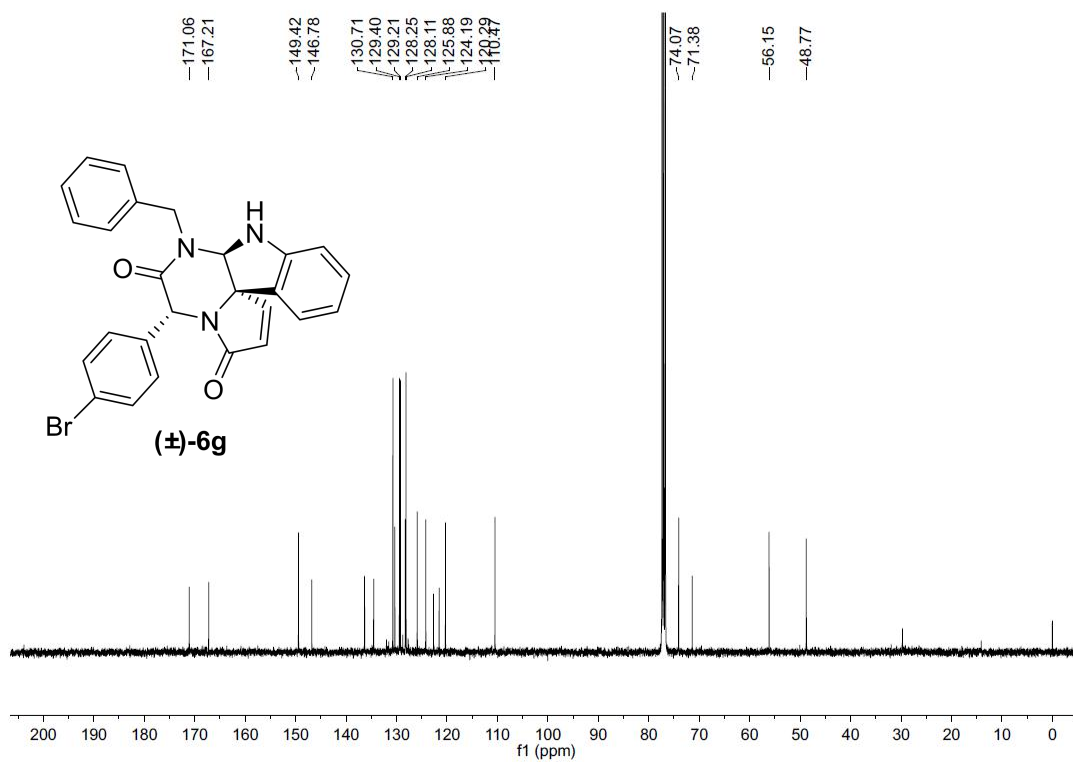
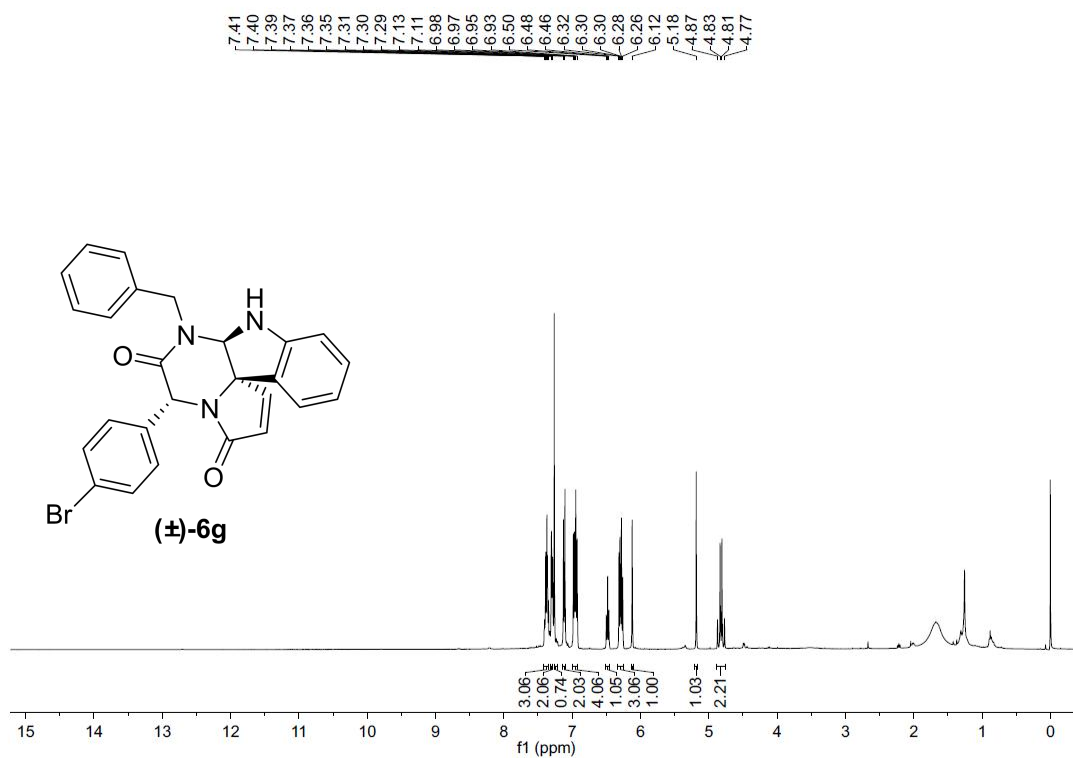
Compound (\pm)-6e



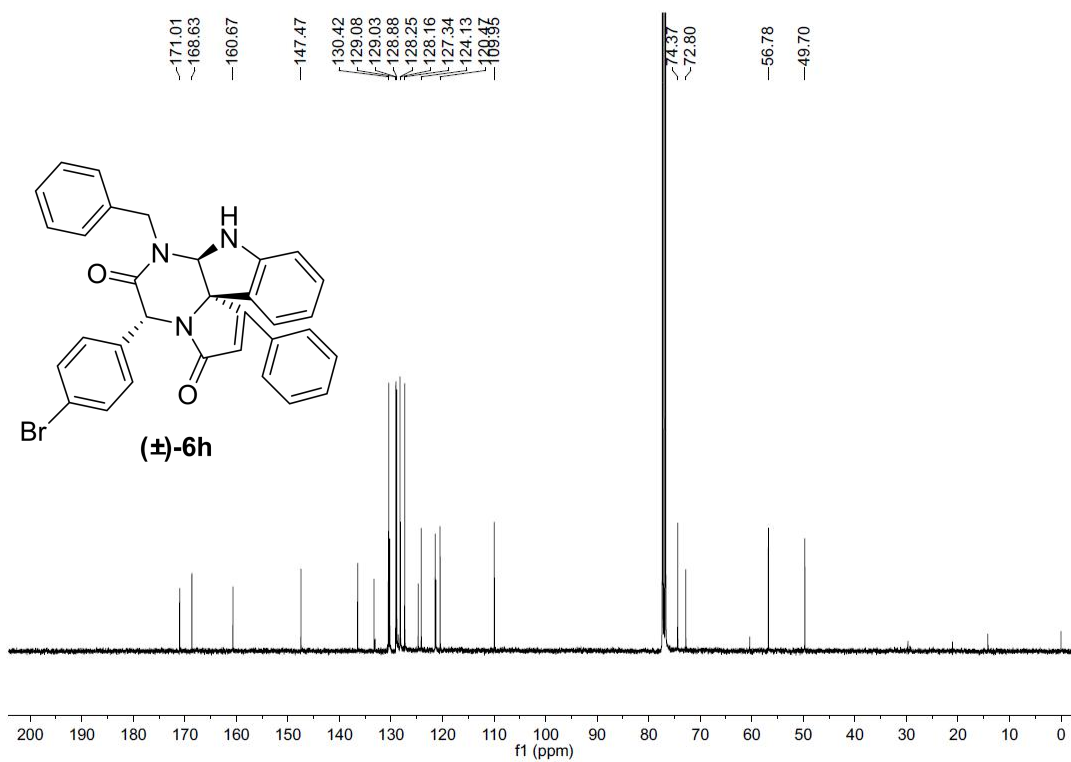
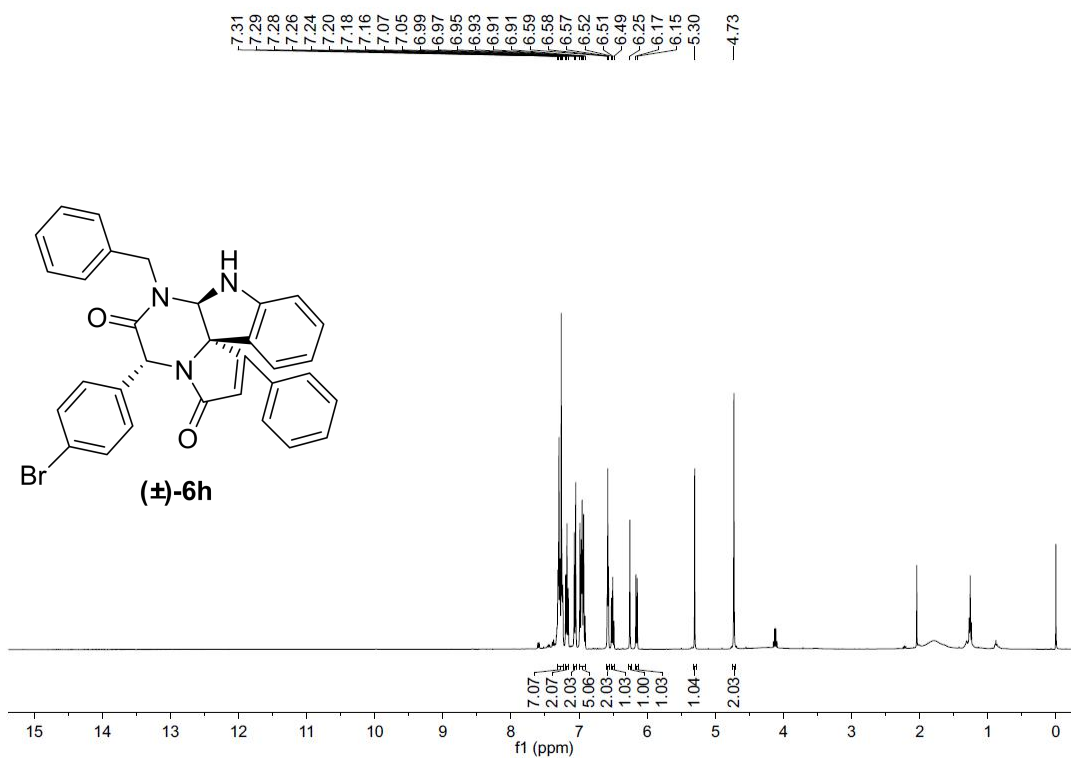
Compound (\pm)-6f



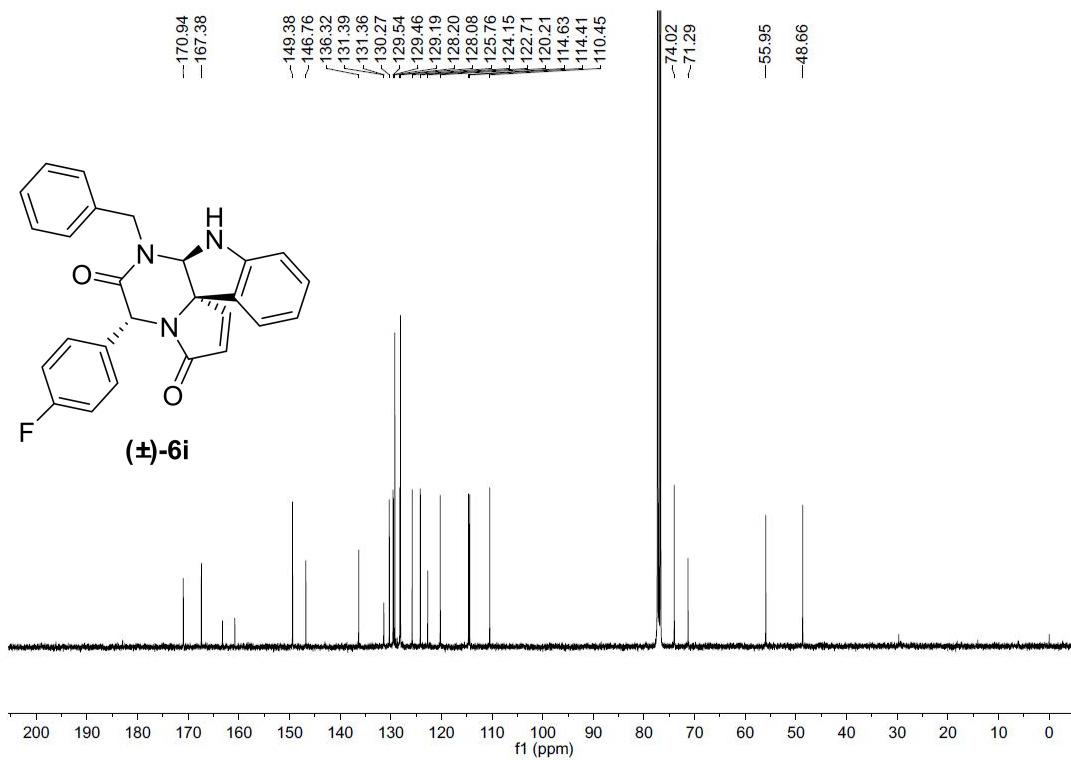
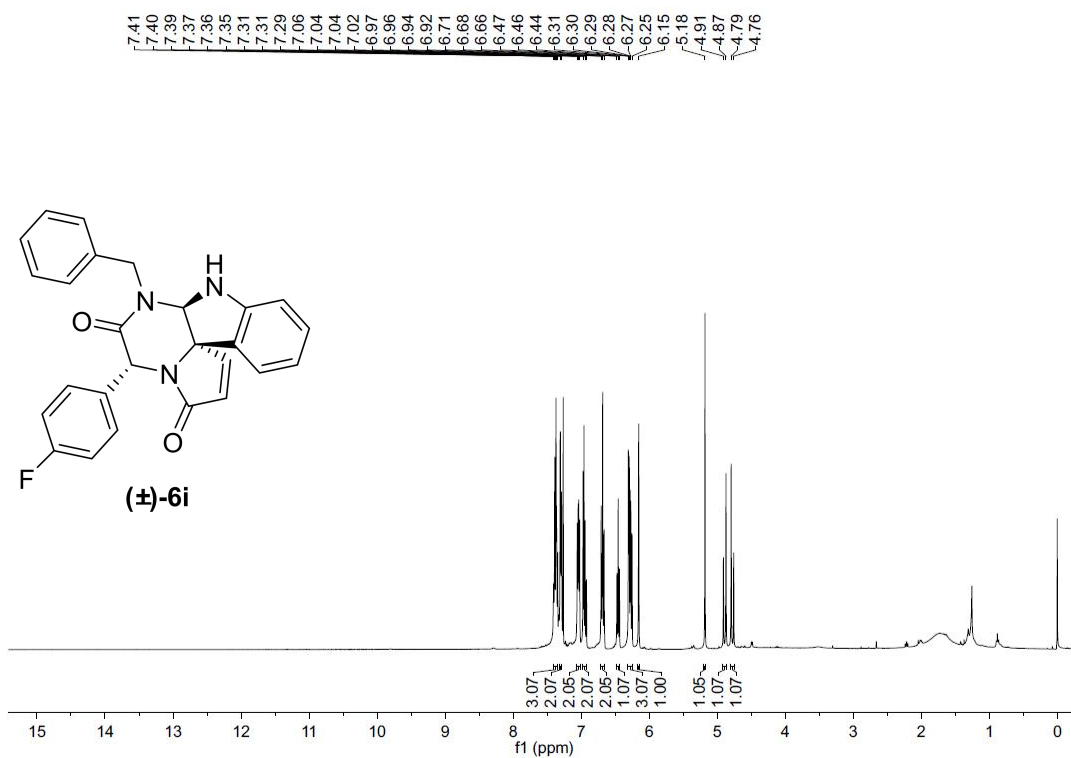
Compound (\pm)-6g



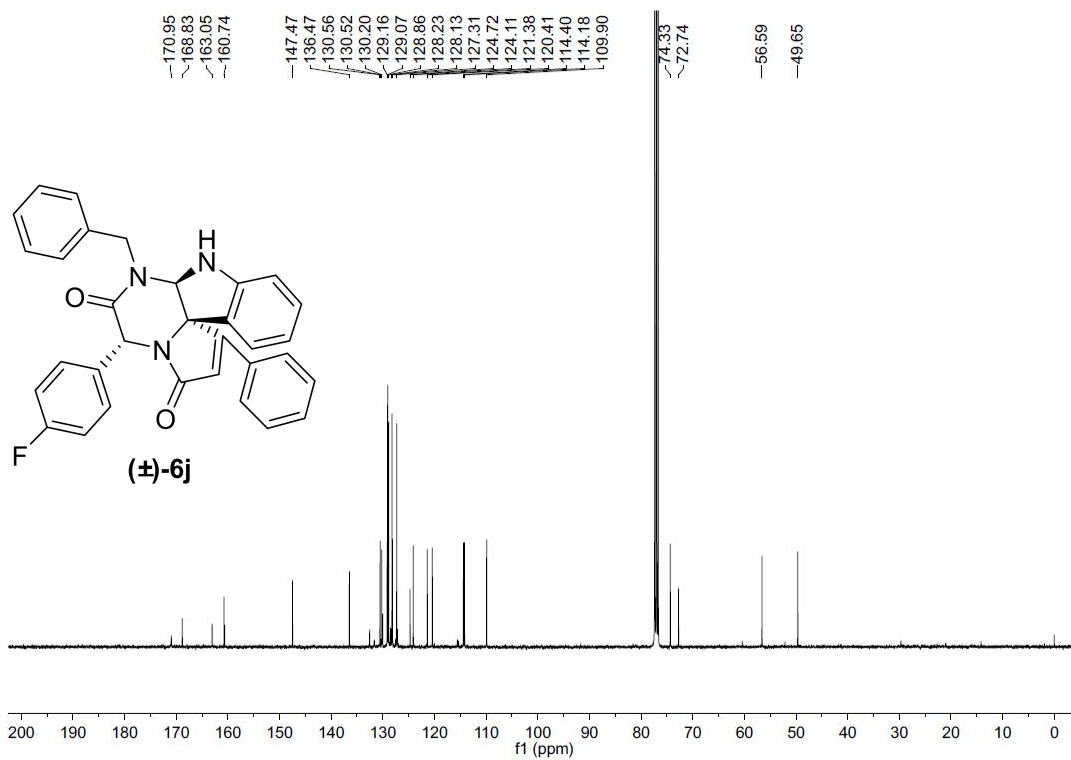
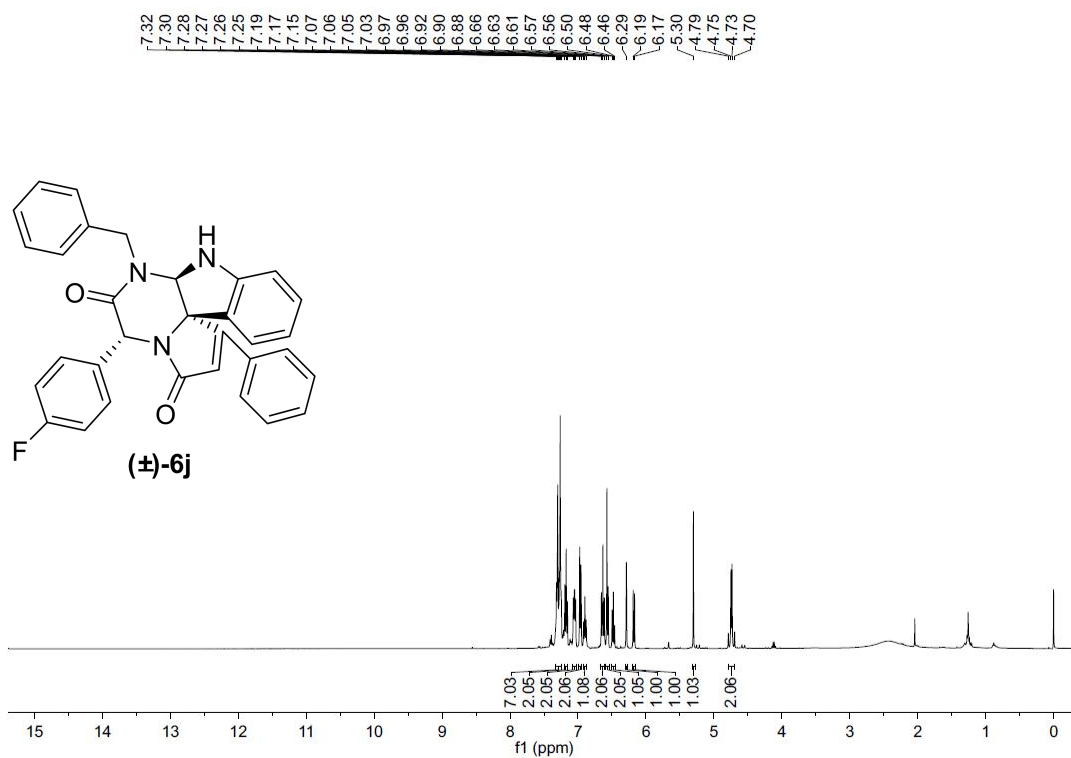
Compound (\pm)-6h



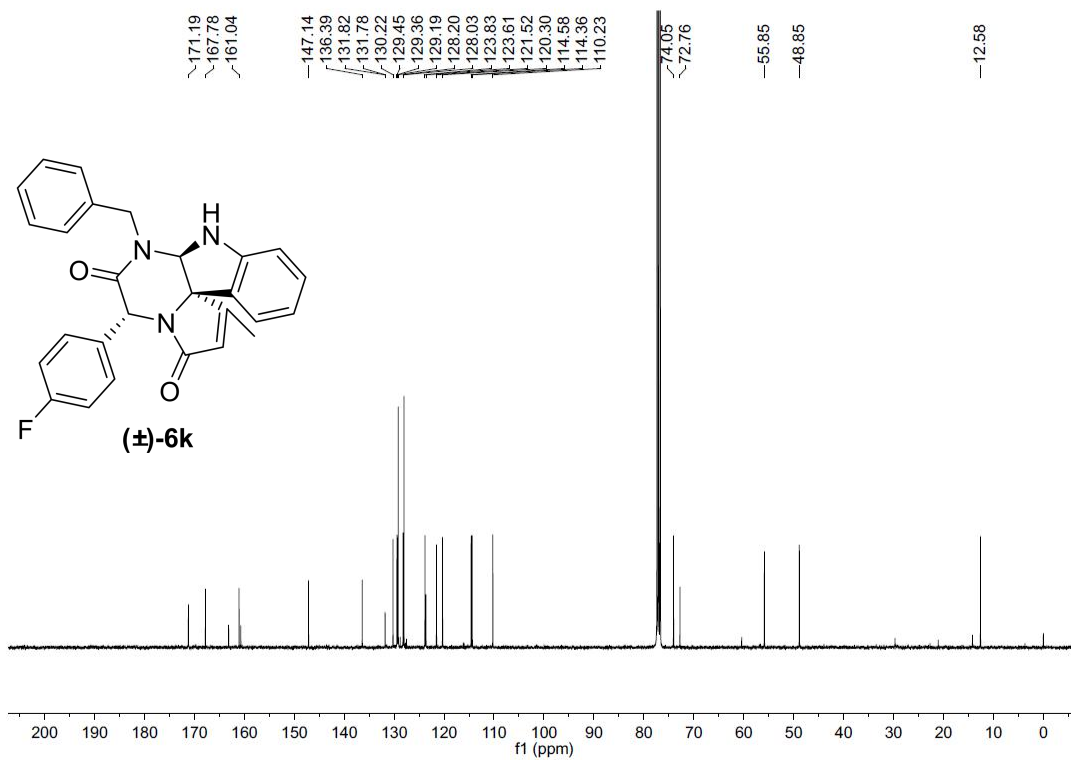
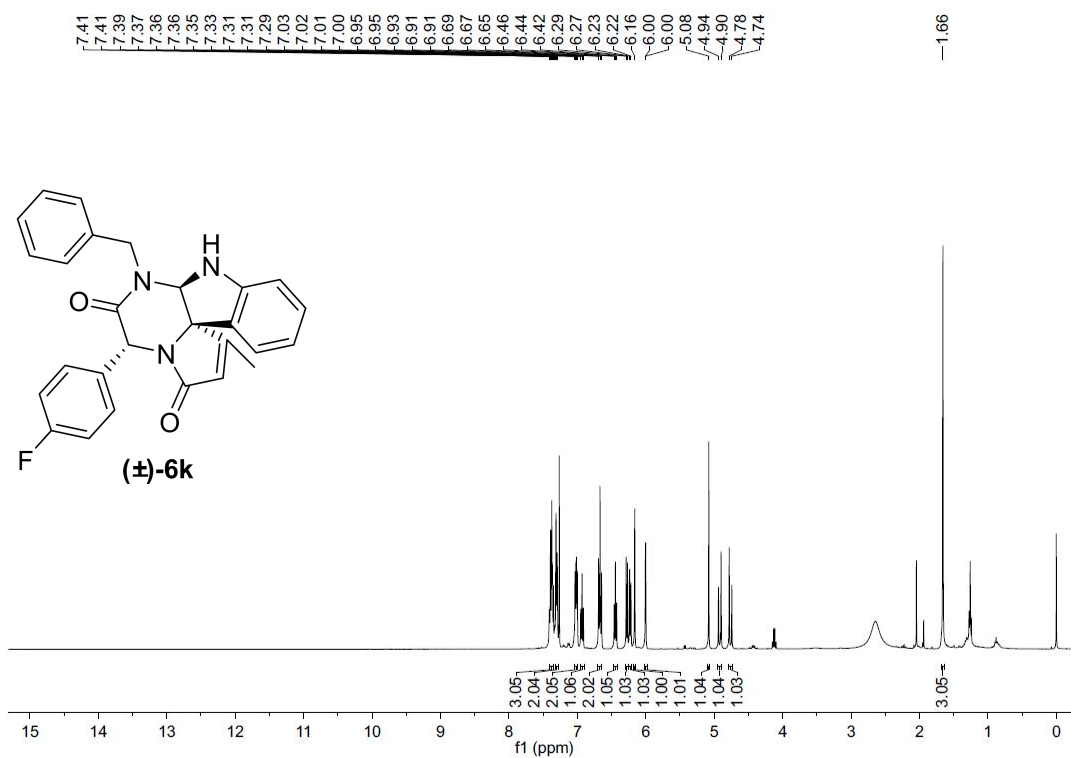
Compound (\pm)-6i



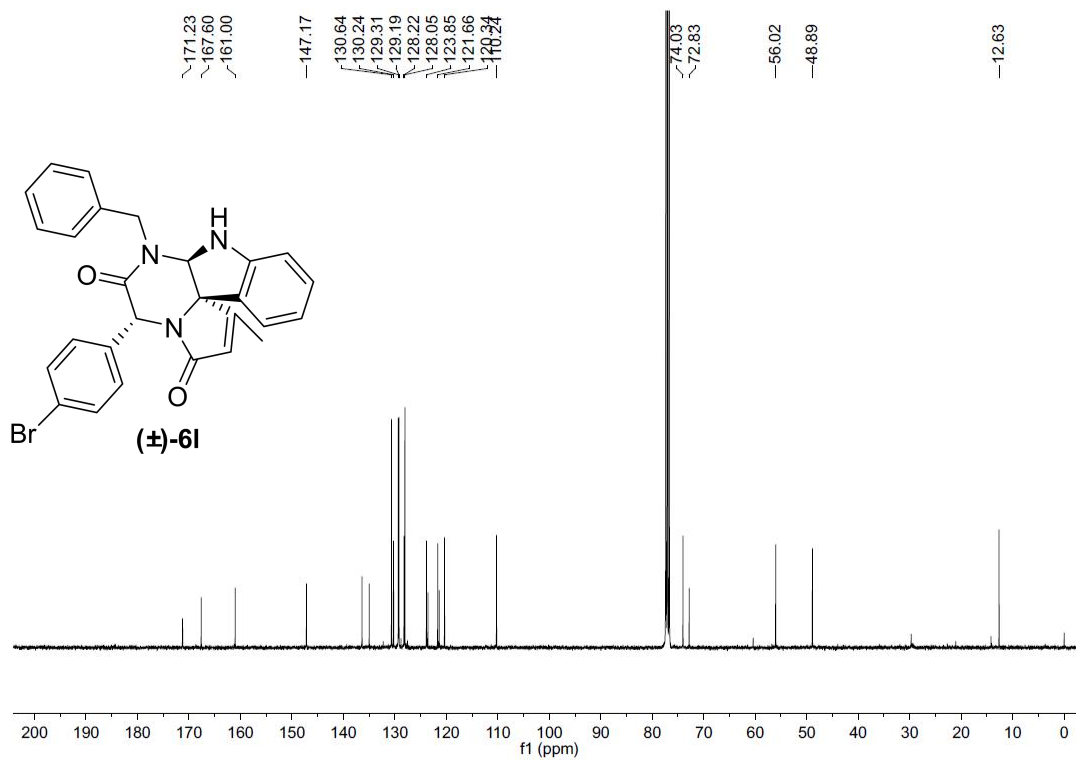
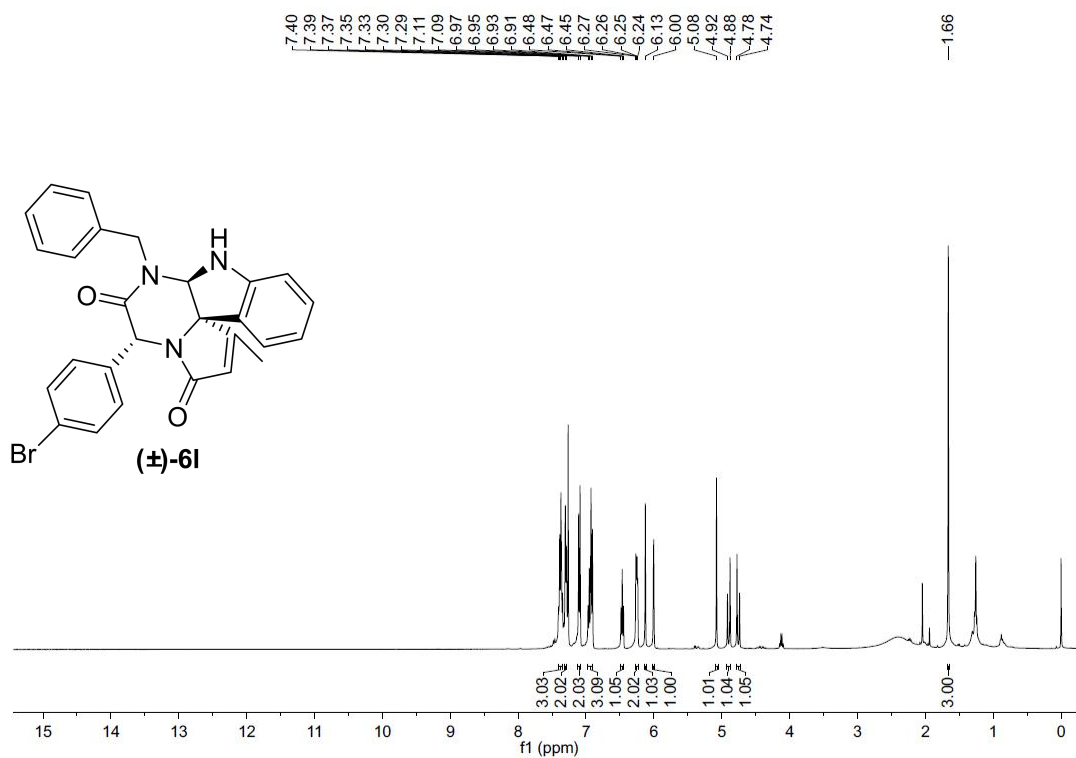
Compound (\pm)-6j



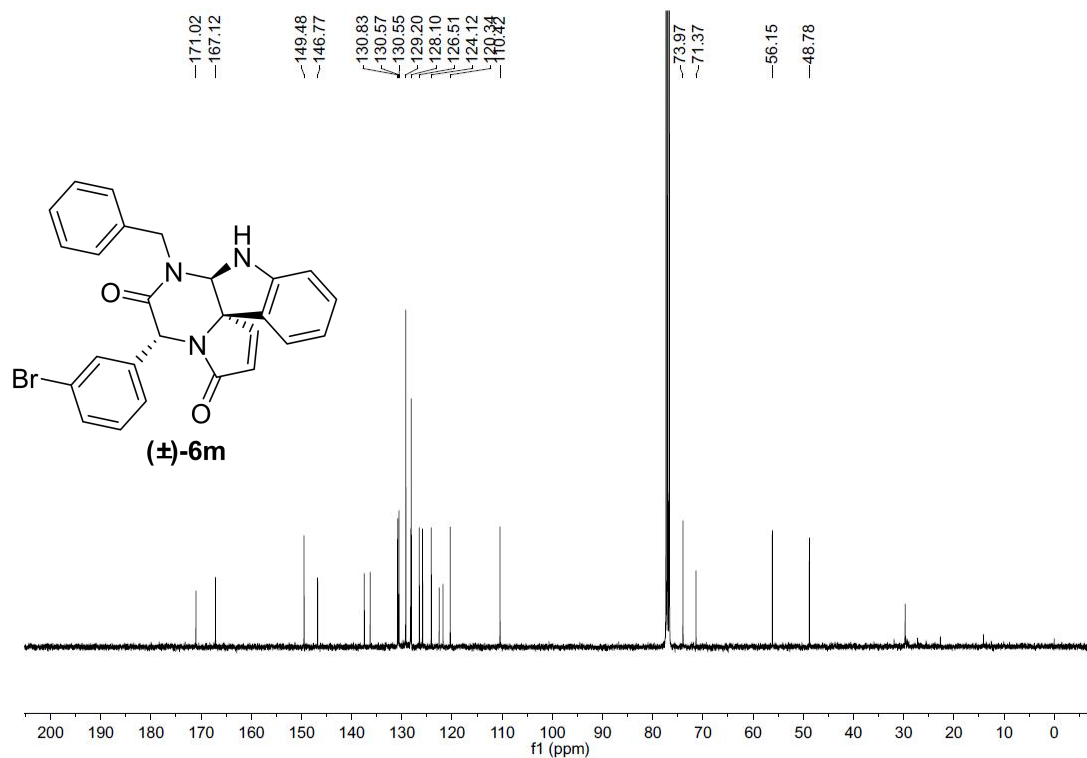
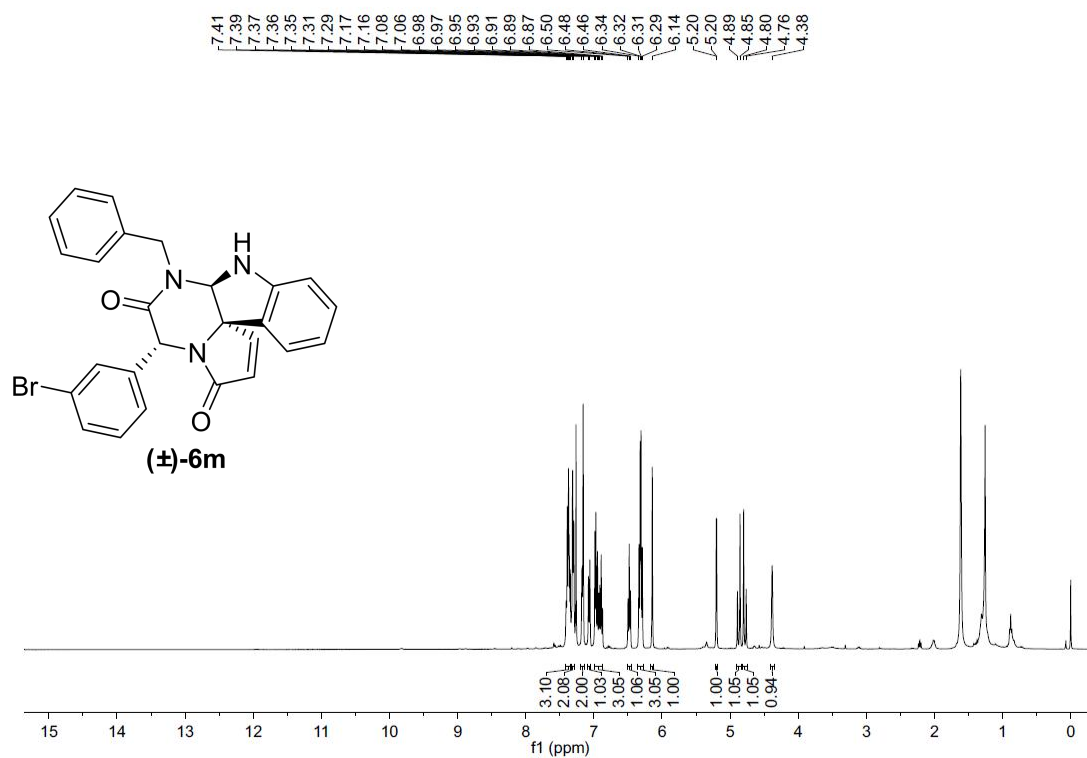
Compound (\pm)-6k



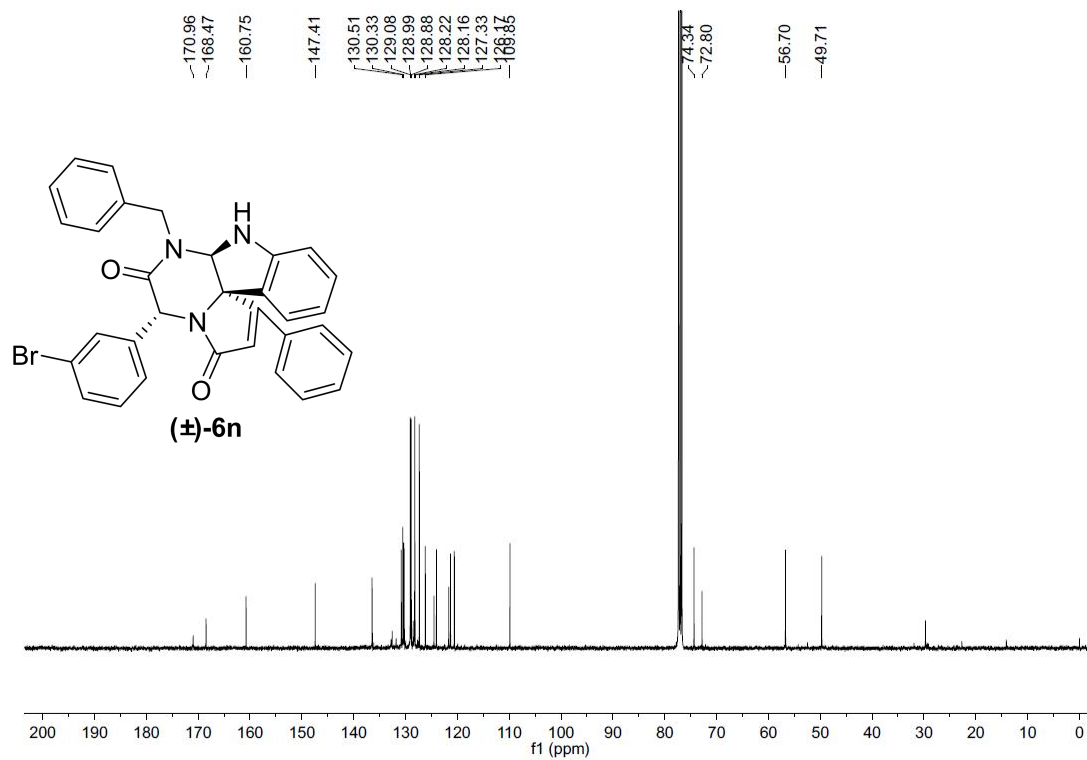
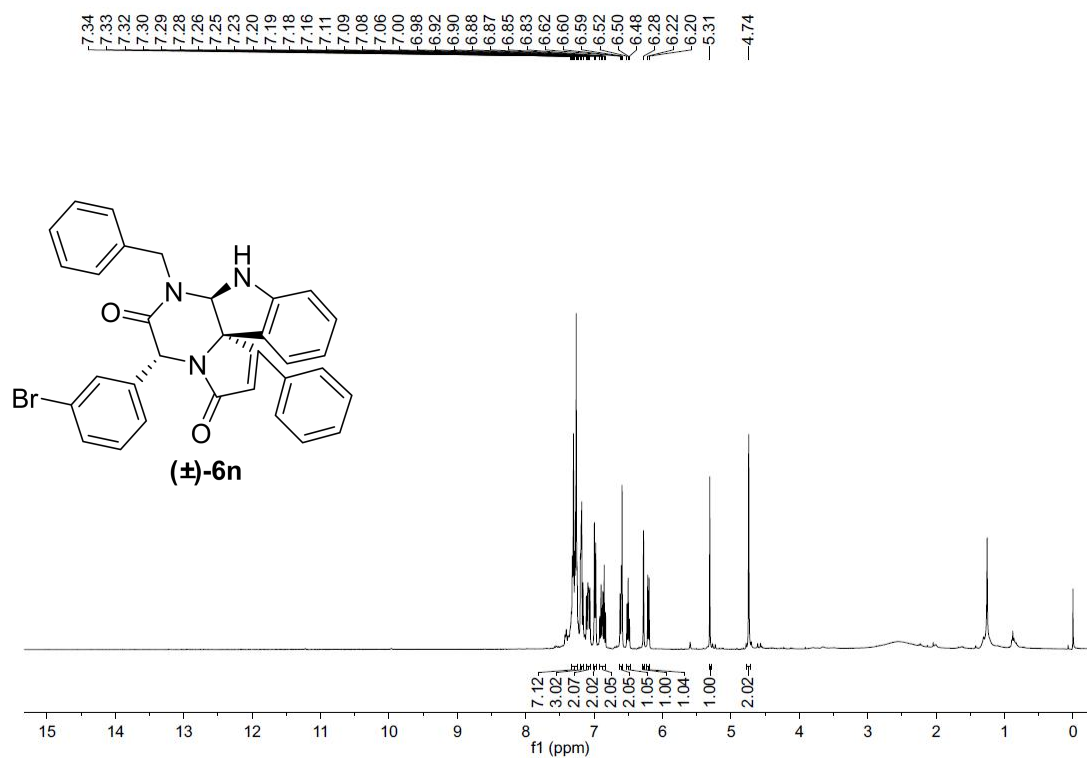
Compound (\pm)-61



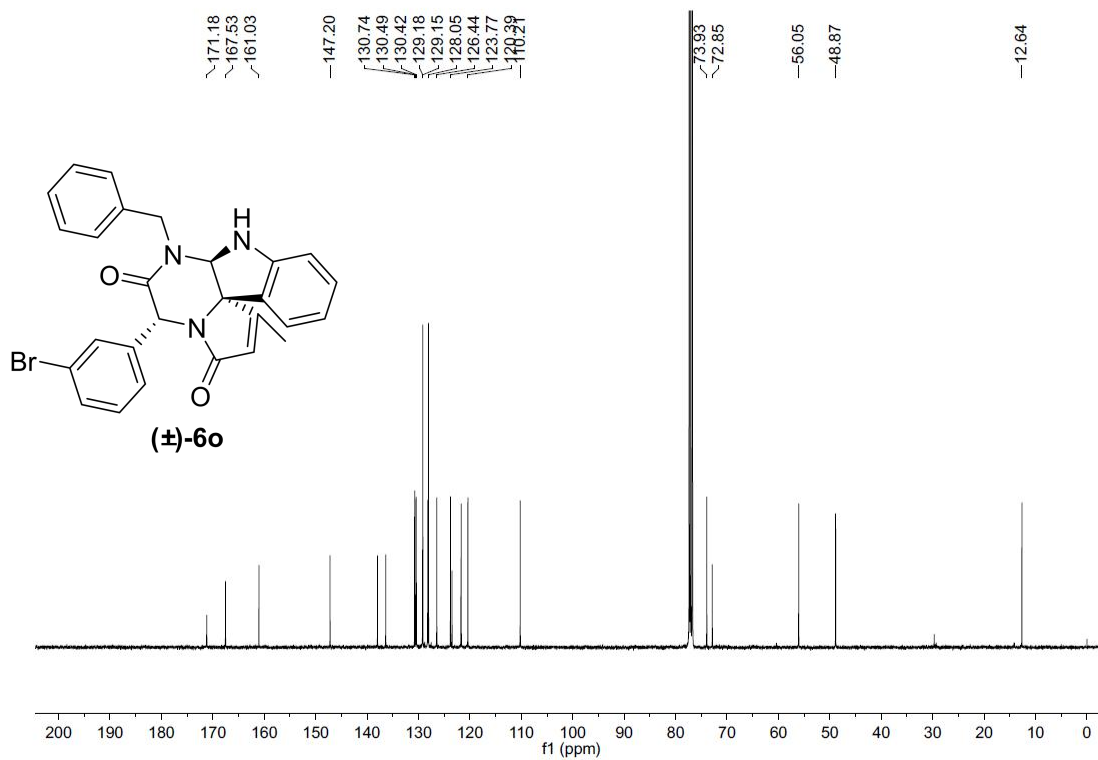
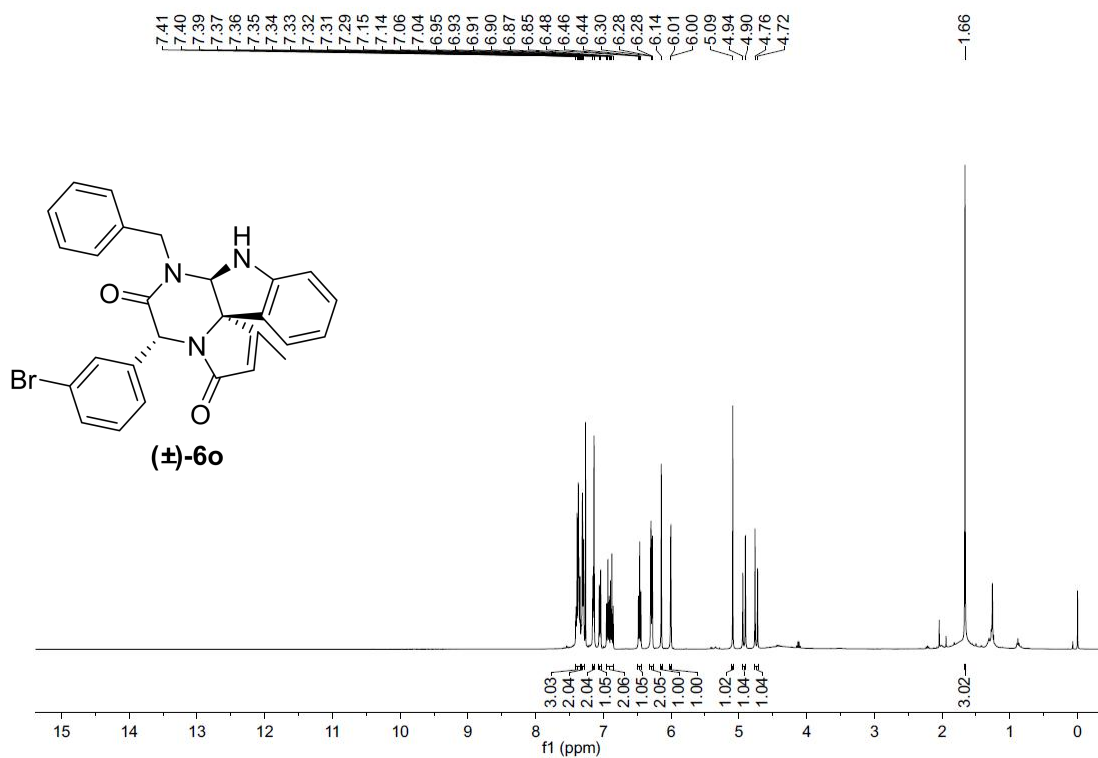
Compound (\pm)-6m



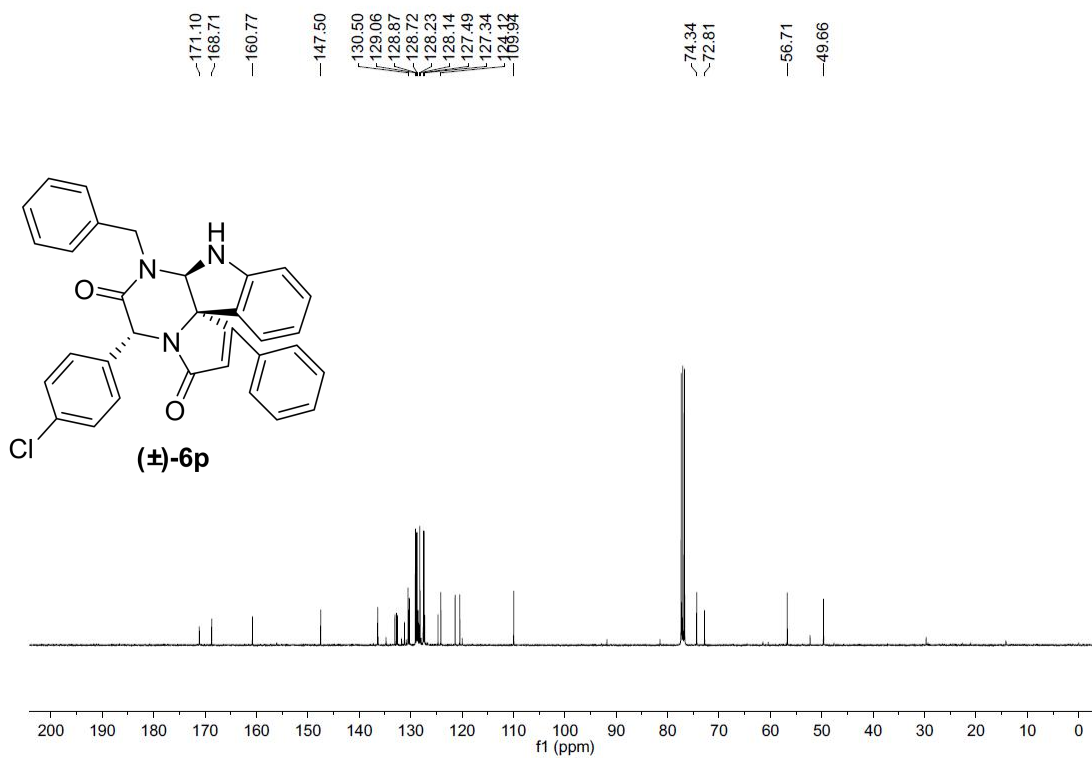
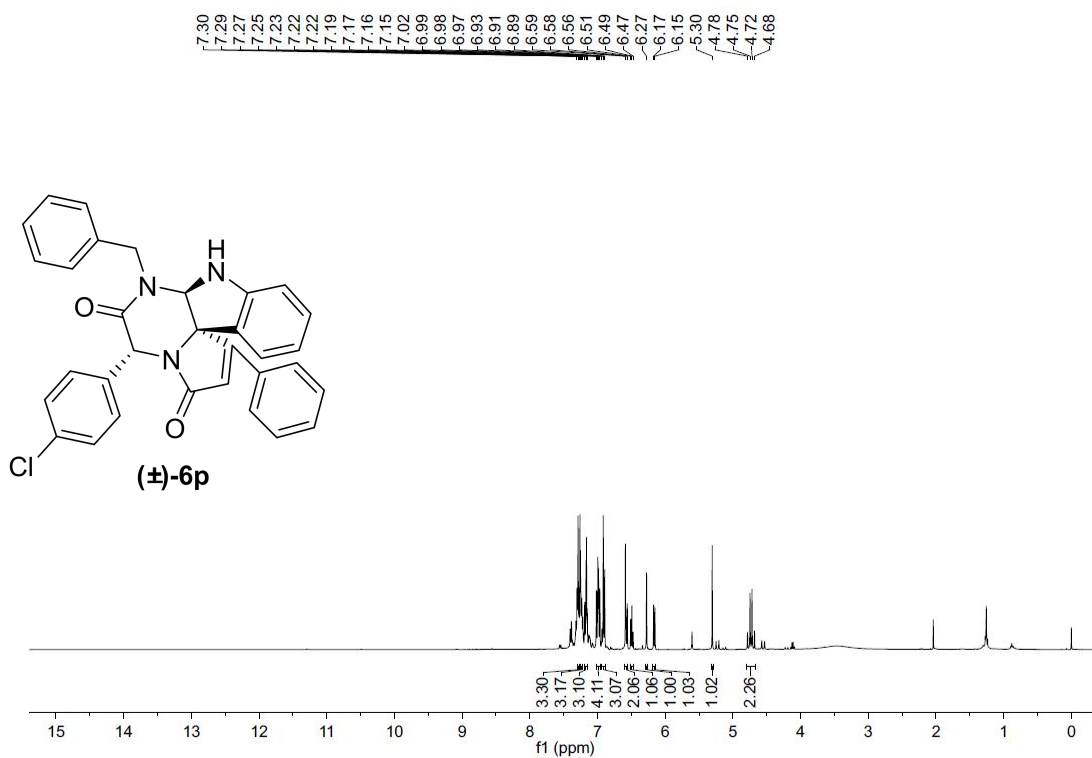
Compound (\pm)-6n



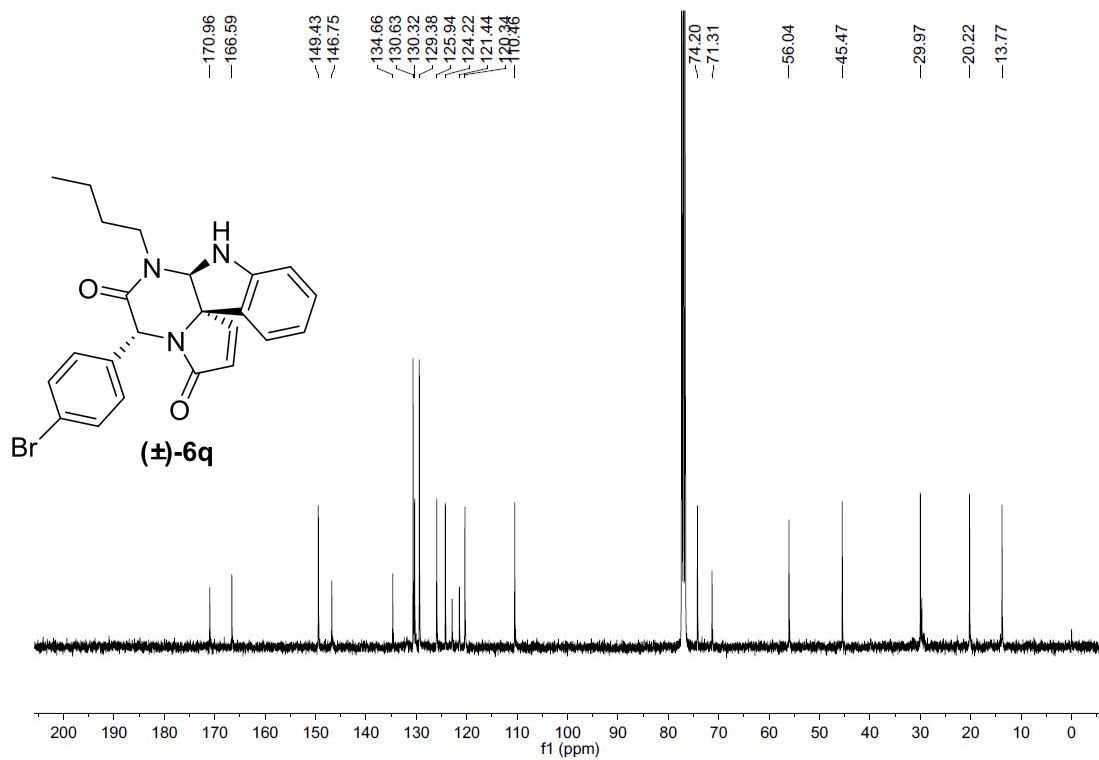
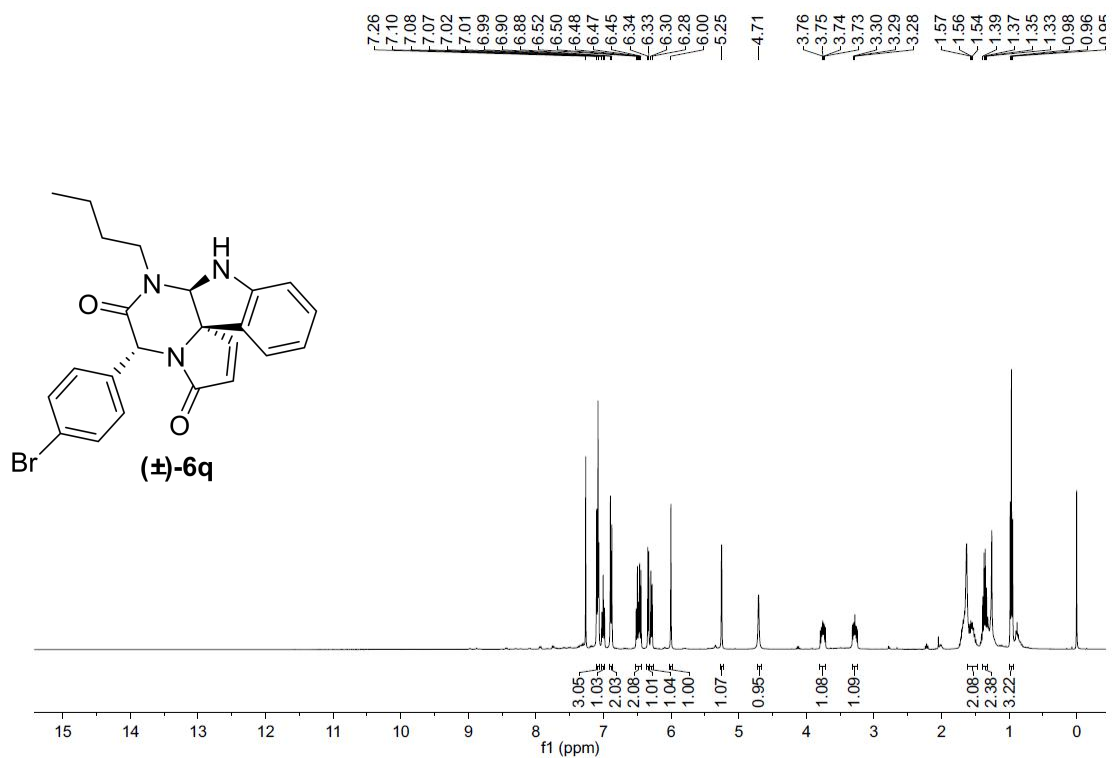
Compound (\pm)-60



Compound (\pm)-6p



Compound (\pm)-6q



Compound (\pm)-6r

