Supporting information

Synthesis of phenanthiridine spiropyrans and studies of their effects on G-quadruplex DNA

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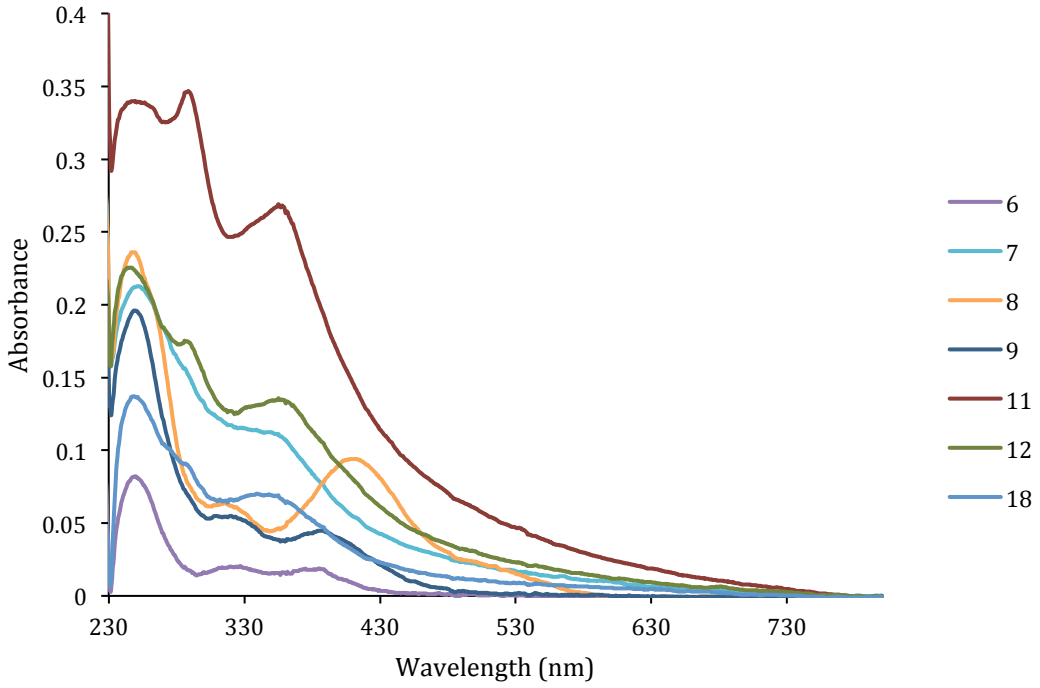
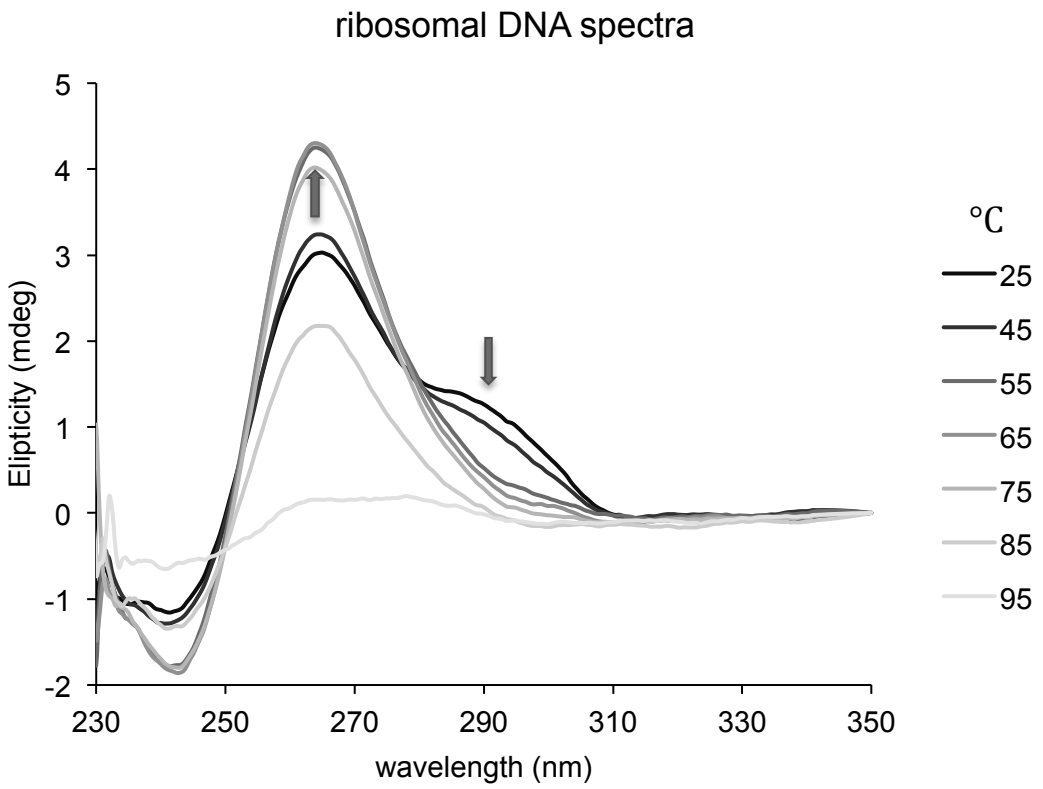
Supporting Information Figure S1



UV Vis absorption spectra of the selected molecules used in the CD experiments. Absorbance of 16 µM compounds was measured in 10 mM Tris (pH 7.5) and 100 mM KCl. Concentration of DMSO was 1.25% (v/v) in all samples. Absorption spectra were recorded on a CARY 50 UV-vis spectrometer (Varian, USA). The optical path length was 1.0 cm.

Supporting Information Figure S2



CD spectra of 5 uM ribosomal DNA treated with 80 uM compound **8** recorded at different temperatures (25, 45, 55, 65, 75, 85 and 95 °C). The peak at 295 nm (represents antiparallel folding) is quickly decreasing with increasing temperature while the peak at 264 nm (represents parallel folding) initially is increasing and has it's maximum at 75 °C (arrows).

Folding of the G4 structures

The oligonucleotides used in this study were purchased from Eurofins MWG Operon (Germany). To fold the oligonucleotides, 50 μM oligonucleotide solution was prepared in 10 mM Tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl; pH 7.5) and 100 mM KCl, heated to 95 °C for 5 min, and slowly cooled to room temperature overnight.

Thioflavin T (ThT) displacement assay

1 μM folded oligonucleotides was incubated in 0.5 μM ThT, 10 mM Tris pH=7.5, 100 mM KCl treated with 0.0002, 0.0008, 0.003, 0.01, 0.25, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.13, 6.25, 12.5, 25 or 50 μM concentration of the compounds in a final volume of 40 μl. 2.5% v/v DMSO was used as a reference sample. All compounds were dissolved in 100% v/v DMSO. The final concentration of DMSO in each sample was 2.5%. 10 μL from the mixture was transferred into three wells in 384-well Corning black flat-bottom microplates to obtain technical triplicates. BioTek Synergy H4 Microtiter plate reader was used for readout at λ = 435/480 nm (ex/em). Background fluorescence of the buffer containing appropriate compound was subtracted from all the measurements. The relative fluorescence was calculated as a fold change (increase or decrease) to reference sample treated with DMSO. DC_{50} values of ThT displacement were calculated using Origin 8.5 software by fitting a dose response function with fixed bottom asymptote to 0.

CD measurements

For CD measurements, 50 μM folded oligonucleotide were diluted to 5 μM concentration in a buffer containing 10 mm Tris–HCl (pH 7.5) and 100 mm KCl and in the presence or absence of 80 μM of the tested compound. The blank sample contained the same buffer without the compound but with 1.25% (v/v) DMSO. A JASCO-720 spectropolarimeter with Peltier temperature control was used for the measurements. Four accumulations of CD spectra of 5 μM oligonucleotide were recorded at 25 °C over λ = 230–350 nm. The measurements were performed in a quartz cuvette with a path length of 0.1 cm. For melting curves, the wavelength was held constant at λ =264 nm and melting curves were recorded in a range of 25-90 °C. The T_m value is defined as the temperature at which 50% of the G4 structures are

unfolded. T_m values were estimated by fitting the melting curves into a dose response function in Origin 8.5 Software.

Taq-polymerase stop assay.

1 μM TET-labeled primer was annealed to 1.25 μM template DNA in 100 mM KCl by heating at 95 °C for 5 min and was slowly cooled to room temperature, overnight. Annealed DNA (40 nm) was incubated with 25 μM compound or 5% (v/v) DMSO for 30 min in 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 1.5 mM $MgCl_2$, and 200 mM dNTPs (dATP, dTTP, dCTP, dGTP) at room temperature. Primer extension assay was accomplished by the addition of 1 U of Taq DNA polymerase (Thermo Scientific) diluted in 10 mM Tris-HCl (pH 7.5) and 50 mM KCl into the reaction mixture at 50 °C. The final volume of the reaction was 45 μL. Reaction was stopped after 0.5, 1, 5 or 10 minutes by transferring 10 uL of the reaction mixture into a stop solution (containing 95% formamide, 20 mM ethylenediaminetetraacetic acid (EDTA), and 0.1% bromophenol blue). 5 uL of the final mixture was loaded onto a 10% polyacrylamide gel containing 8 M urea, 25% formamide and 1×Tris/borate/ethylenediaminetetraacetic acid (TBE). The gel was visualized with a Typhoon Scanner 9400 (GE Healthcare) and quantified with the Image Quant 5.2 software (GE Healthcare).

NMR Analysis

The reference sample of Pu24T was prepared by adding 20mL D_2O to 200mL stock solution of Pu24T (100 mM Pu24T in PBS buffer 10mM phosphate, 25mM KCl, pH 7) and transferring the solution to a 3mm NMR tube.

Samples containing compound 7 or 8 were prepared in the same way as the reference sample but with addition of 2mL of stock solution of 7 or 8 (10mM in DMSO) to get a 1:1 ratio between Pu24T and the added compound.

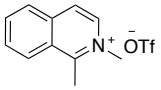
All spectra were acquired on a Bruker 850MHz Avance III HD spectrometer equipped with a 5mm TCI cryo-probe. 256 scans were added with a relaxation delay of 1s and with excitation sculpting for water suppression. 1Hz line-broadening was applied before fourier transform. All processing was done in Topspin 3.2 (www.bruker.com).

General Synthesis

Unless stated otherwise, all reagents and solvents were used as received from commercial suppliers. 3-Ethyl-2-hydroxybenzaldehyde was prepared according to previously published procedure.[1] TLC was performed on aluminum backed silica gel plates (median pore size 60 Å, fluorescent indicator 254 nm) and detected with UV light. Flash column chromatography was performed using silica gel with an average particle diameter 50 μm (range 40−65 μm, pore diameter 53 Å), and eluents are given in brackets. DMF, THF and DCM were dried in a solvent drying system (THF and DCM drying agent: neutral alumina ; DMF drying agent: activated molecular sieves, also equipped with an isocyanate scrubber) and were collected fresh prior to every reaction. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer at 298 K or on a Bruker 600 MHz spectrometer at 298 K, and calibrated by using the residual peak of the solvents as the internal standard ($CDCl_3$: δ H= 7.26 ppm; δ C = 77.16 ppm. DMSO-d_6: δ H = 2.50 ppm; δ C = 39.50 ppm). LC-MS was conducted on an Agilent 6100 Series Quadropole LC/MSD system . HRMS was performed by using a Agilent 1290 binary LC System connected to a Agilent 6230 Accurate-Mass TOF LC/MS (ESI+); calibrated with Agilent G1969-85001 ES-TOF Reference Mix containing ammonium trifluoroacetate, purine and hexakis(1H, 1H, 3H-tetrafluoropropoxy)phosphazine in 90:10 acetonitrile:water. Preparatory HPLC was performed on a C18 reversed-phase column (25 cm x 21.2 mm, 5 mm) with H_2O/MeCN mixtures as the eluent.

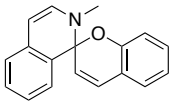
Abbreviations

DMF – dimethylformamide, MeOH – methanol, THF - tetrahydrofuran, EtOAc – ethyl acetate, TFA – trifluoro acetic acid, DCE – dichloroethane, DCM – dichloromethane, MeCN – acetonitrile, DMAP – dimethylaminopyridine, TLC – thin layer chromatography, HPLC – high pressure liquid chromatography, LCMS – liquid chromatography mass spectrometry, rt – room temperature

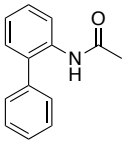


1,2-dimethylisoquinolin-2-ium trifluoromethanesulfonate (**1**): Methyltrifluoromethansulfonate (307 μl, 2.71 mmol) was added to a solution of 1-methylisoquinoline (360 μl, 2.71 mmol) in 1,2-dichloroethene (5 ml). The reaction

mixture was stirred at rt for 5 h. A white solid precipitated from the solution. After filtering off the solid, it was washed with cold diethyl ether (0 °C) and dried under reduced pressure. The product was isolated as a white solid in 87 % yield (722 mg, 2.35 mmol) ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 8.77 (d, J = 8.7 Hz, 1H), 8.68 (d, J = 6.9 Hz, 1H), 8.38 (d, J = 6.9 Hz, 1H), 8.29 (d, J = 8.1 Hz, 1H), 8.21 (t, J = 7.1 Hz, 1H), 8.04 (ddd, J = 8.4, 6.9, 1.3 Hz, 1H), 4.38 (s, 3H), 3.21 (s, 3H).^{13}C-NMR (100 MHz, DMSO-d_6): δ = 160.6, 136.6, 136.5, 135.8, 130.9, 128.4, 127.9, 127.1, 123.4, 121.1 ($J_{C\text{-}F}$ = 321 Hz), 46.4, 16.8. LCMS: Calculated M^+ (cationic part only) (158); Found (M^+) 158 m/z, Rt = 1.29 min.

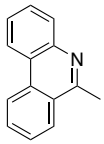


2'-Methyl-3,8a-dihydro-2'H-spiro[chromene-2,1'-isoquinoline] (**2**) (ECH23): Made using the general procedure (*vide infra*) from 1 (100 mg, 0.33 mmol) and salicylaldehyde (34 µl, 0.33 mmol).The crude mixture was concentrated and purified by preparative HPLC using MeCN:water with 0.75% formic acid as eluent, giving the open zwitterionic intermediate after condensation with the aldehyde. The isolated intermediate was then dissolved in DMSO and sodium carbonate (35 mg, 0.33 mmol) was added to the solution. The suspension was stirred at rt for 12 h. The reaction mixture was diluted with dichloromethane and washed with water three times. The organic layer was dried with sodium sulfate and the solvent was removed under reduced pressure. The product was a red solid. Yield: 77 % (65 mg, 0.25 mmol).1H-NMR (400 MHz, DMSO-d6): δ = 7.27 (t, J = 7.2 Hz, 1H), 7.24 (d, J = 7.0 Hz, 1H), 7.23 (t, J = 7.3 Hz, 1H), 7.16 (d, J = 7.0 Hz, 1H), 7.11 (t, J = 6.8 Hz, 1H), 7.11 (t, J = 6.8 Hz, 1H), 7.06 (d, J = 10.1 Hz, 1H), 6.84 (t, J = 7.4 Hz, 1H), 6.54 (t, J = 8.2 Hz, 2H), 5.93 (d, J = 10.0 Hz, 1H), 5.70 (d, J = 7.5 Hz 1H), 3.01 (s, 3H).13C-NMR (151 MHz, DMSO-d6): δ = 152.2, 134.4, 130.4, 130.1, 128.5, 127.4, 127.1, 127.1, 126.7, 124.8, 123.5, 121.6, 120.1, 118.4, 113.6, 40.4. HRMS ESI-TOF+: m/z calcd for $C18H16NO^+$ [$M+H^+$]: 262.1226; found 262.1235.

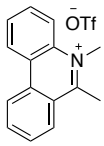


N-([1,1'-biphenyl]-2-yl)acetamide (**3**): Lithium hydroxide (727 mg, 17.3 mmol) was added to a solution of 2-aminobiphenyl (1.00 g, 5.91 mmol) in DCM (88 mL). The suspension was cooled to 0 °C before a solution of acetyl bromide (1.31 ml, 17.7 mmol) in DCM (8 mL) was added dropwise. The reaction mixture was

stirred for 4 h at rt, filtered and concentrated under reduced pressure. The residue was dissolved in DCM and washed with water. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by crystallization at 0 °C from a DCM:*n*-heptane solution giving the product as colourless needles in 93 % yield (1.16 g, 5.48 mmol). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.27 (d, *J* = 8.1 Hz, 1H), 7.35 – 7.51 (m, 6H), 7.25 (d, *J* = 7.1 Hz, 1H), 7.18 (t, *J* = 7.3 Hz, 1H), 7.11 (s, 1H), 2.02 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 168.4, 138.3, 134.8, 132.3, 130.2, 129.4, 129.2, 128.6, 128.1, 124.5, 121.8, 24.8. LCMS: Calculated MH^+ (212); Found (MH^+) 212 m/z, Rt = 4.68 min.



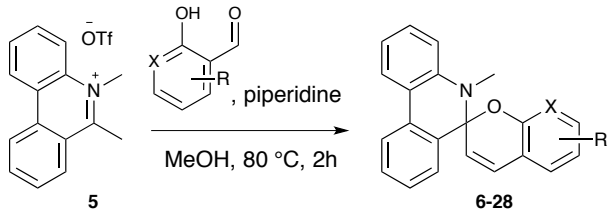
6-methylphenanthridine (**4**): **3** (208 mg, 985 µmol) and polyphosphoric acid (3 ml, 115 % H_3PO_4 equiv.) were mixed and stirred for 2.5 h at 140 °C. The reaction was then cooled down to 0 °C and saturated aqueous sodium hydroxide solution was added until pH 14 was reached. The aqueous solution was then extracted with DCM and the organic phase was dried with sodium sulfate. Evaporation of the solvent under reduced pressure gave the product as a white solid in 87 % yield (165 mg, 854 mmol). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 8.64 (d, *J* = 8.3 Hz, 1H), 8.55 (d, *J* = 8.1 Hz, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 8.10 (d, *J* = 8.1 Hz, 1H), 7.85 (ddd, *J* = 8.3 Hz, 7.1, 1.3 Hz, 1H), 7.71 (m, 2H), 7.63 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 3.06 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 159.0, 143.8, 132.7, 130.6, 129.5, 128.8, 127.5, 126.7, 126.5, 126.1, 123.9, 122.5, 122.1, 23.6. LCMS: Calculated MH^+ (194); Found (MH^+) 194 m/z, Rt = 3.91 min.



5,6-dimethylphenanthridin-5-ium trifluoromethanesulfonate (**5**): **4** (719 mg, 3.72 mmol) was dissolved in DCM (40 mL) at rt. Methyl trifluoromethanesulfonate (505 µL, 4.47 mmol) was then added dropwise and the reaction was left stirring at rt over night. The formed precipitate was filtered off, washed with Et_2O and dried under vacuum. The product was isolated in 89% yield (1.18 g, 3.30 mmol) as white needles. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (d, *J* = 8.0 Hz, 2H), 8.91 (d, *J* = 8.5 Hz, 1H), 8.63 (d, *J* = 8.3 Hz, 1H), 8.35 (t, *J* = 7.6 Hz, 1H), 8.16 – 8.01 (m, 3H), 4.57 (s, 3H),

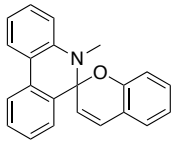
3.43 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 165.9, 136.8, 135.0, 133.1, 131.7, 130.6, 130.2, 129.6, 124.5, 124.4, 124.4, 123.3, 120.7 ($J_{C\text{-}F}$ = 321 Hz), 120.0, 41.3, 19.6. LCMS: Calculated M^+ (cationic part only) (208); Found (M^+) 208 m/z, Rt = 0.95 min.

General procedure for preparation of spiropyrans

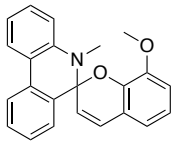


5 **6-28**

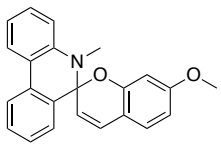
2-hydroxybenzaldehyde (1 equiv.) was dissolved in methanol (0.2 M) in a capped microwave vial at rt and piperidine (1 equiv.) was added. The mixture was then heated to reflux before removed from the oilbath and 0.25 equiv. of triflate salt of 5,6-dimethylphenanthridin-5-ium (**5**) in methanol (0.2 M) was added. The reaction was then refluxed for 20 min and then again removed from the oilbath and an additional 0.25 equiv. of **5** was added. This sequence was repeated until a total of 1 equiv. of **5** had been added. The reaction was then left to reflux an additional hour and then allowed to cool down under stirring over night. Unless otherwise noted, the formed precipitate was filtered off over a small glass-frit funnel and then dried under vacuum to give the phenanthridine spiropyrans as racemates. No additional purification was needed.



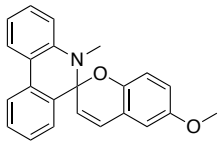
5'-Methyl-5'H-spiro[chromene-2,6'-phenanthridine] (**6**) (ECH24): Made using the general procedure from **5** (53 mg, 0.15 mmol) and salicyl aldehyde (15 μL, 0.15 mmol), giving **6** as a white solid in 74% yield (34 mg, 0.11 mmol). ^{1}H NMR (600 MHz, DMSO-d6) δ 8.14 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 7.4 Hz, 1H), 7.41 – 7.34 (m, 3H), 7.27 (d, J = 7.5, 1H), 7.17 (d, J = 10.0 Hz, 1H), 7.10 – 7.01 (m, 3H), 6.86 (t, J = 7.4, 1H), 6.46 (d, J = 8.0 Hz, 1H), 6.02 (d, J = 10.0 Hz, 1H), 3.10 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 151.9, 140.8, 131.4, 130.1, 129.6, 129.0, 128.2, 127.8, 127.5, 127.2, 127.0, 123.1, 123.0, 122.1, 120.4, 119.6, 119. 2, 118.4, 114.9, 114.0, 91.2, 33.0. HRMS ESI-TOF+: m/z calcd for $C_{22}H_{18}NO^+$ [$M+H^+$]: 312.1383; found 312.1393.



8-Methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**7**) (ECH25): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-3-methoxybenzaldehyde (18 μL, 0.14 mmol), giving **7** as a white solid in 69% yield (33 mg, 0.10 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.15 (d, J = 7.9, 1H), 8.11 (d, J = 7.9, 1H), 7.49 (td, J = 7.3, 1.9 Hz, 1H), 7.41 – 7.34 (m, 3H), 7.13 (d, J = 10.0 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.02 (t, J = 7.3 Hz, 2H), 6.88 (dd, J = 7.0, 2.2 Hz, 1H), 6.84 – 6.78 (m, 2H), 5.98 (d, J = 10.0 Hz, 1H), 3.46 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 146.6, 141.0, 140.6, 131.2, 129.6, 129.0, 128.1, 127.5, 126.8, 123.1, 123.0, 122.0, 119.9, 119.4, 119.3, 119.1, 118.7, 113.8, 113.7, 91.2, 55.4, 32.9. HRMS ESI-TOF+: *m/z* calcd for C23H20NO2^{+} [M+H^{+}]: 342.1489; found 342.1498.

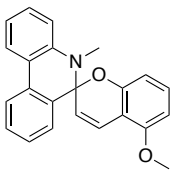


7-Methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**8**) (ECH26): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-4-methoxybenzaldehyde (14 μL, 0.14 mmol), giving **8** as a white solid in 71% yield (34 mg, 0.10 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.17 – 8.13 (d, J = 8.1 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 7.50 (t, J = 7.3 Hz, 1H), 7.44 – 7.34 (m, 3H), 7.17 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 10.0 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 7.06 – 7.02 (t, J = 7.4 Hz, 1H), 6.43 (dd, J = 8.4, 2.4 Hz, 1H), 6.08 (d, J = 2.4 Hz, 1H), 5.84 (d, J = 10.0 Hz, 1H), 3.61 (s, 3H), 3.11 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 161.1, 153.3, 140.8, 131.4, 129.5, 129.0, 128.2, 128.0, 127.9, 127.5, 126.7, 123.0, 122.0, 120.1, 119.6, 119.2, 114.0, 111.5, 107.0, 100.1, 91.5, 55.1, 33.0. HRMS ESI-TOF+: *m/z* calcd for C23H20NO2^{+} [M+H^{+}]: 342.1489; found 342.1499.

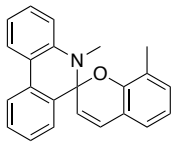


6-Methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**9**) (ECH27): Made using the general procedure from **5** (20 mg, 0.06 mmol) and 2-hydroxy-5-methoxybenzaldehyde (7 μL, 0.06 mmol), giving **9** as a white solid in 52% yield (10 mg, 0.03 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (d, J = 7.9 Hz, 1H),

8.08 (d, *J* = 8.0 Hz, 1H), 7.48 (t, *J* = 6.7 Hz, 1H), 7.38 – 7.34 (m, *J* = 2.0 Hz, 2H), 7.14 (d, *J* = 10.0 Hz, 1H), 7.07 – 7.00 (m, 2H), 6.89 (d, *J* = 3.0 Hz, 1H), 6.65 (dd, *J* = 8.7, 3.0 Hz, 1H), 6.40 (d, *J* = 8.7 Hz, 1H), 6.04 (d, *J* = 10.0 Hz, 1H), 3.70 (s, 3H), 3.08 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 153.0, 145.8, 140.9, 131.4, 129.5, 129.0, 128.2, 127.7, 127.4, 127.1, 123.9, 123.0, 122.1, 119.6, 119.1, 118.9, 115.7, 115.6, 113.9, 111.7, 90.8, 55.4, 33.1. HRMS ESI-TOF+: *m/z* calcd for $C23H20NO2^+$ [$M+H^+$]: 342.1489; found 342.1496.

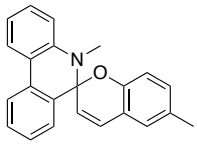


5-Methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**10**) (ECH28): Made using the general procedure from **5** (30 mg, 0.08 mmol) and methyl 2-hydroxy-6-methoxybenzaldehyde (13 mg, 0.08 mmol), giving **10** as a white solid in 52% yield (15 mg, 0.04 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (dd, *J* = 8.0, 7.7 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.44 – 7.29 (m, 4H), 7.06 – 7.01 (m, 3H), 6.52 (d, *J* = 8.3 Hz, 1H), 6.09 (d, *J* = 8.2 Hz, 1H), 5.93 (d, *J* = 10.2 Hz, 1H), 3.86 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 155.2, 152.5, 140.7, 131.4, 130.3, 129.6, 129.0, 128.2, 127.9, 127.5, 123.0, 122.1, 121.4, 121.0, 119.6, 119.2, 114.0, 107.9, 107.2, 102.9, 90.9, 55.7, 32.9. HRMS ESI-TOF+: *m/z* calcd for $C23H20NO2^+$ [$M+H^+$]: 342.1489; found 342.1497.

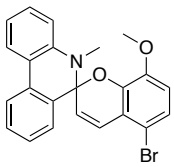


5',8-Dimethyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**11**) (ECH29): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-3-methylbenzaldehyde (17 μL, 0.14 mmol), giving **11** as a white solid in 70% yield (32 mg, 0.10 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (dd, *J* = 8.0, 1.2 Hz, 1H), 8.09 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.49 (ddd, *J* = 7.9, 7.1, 1.5 Hz, 1H), 7.41 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.36 (dddd, *J* = 8.0, 7.0, 4.2, 1.4 Hz, 2H), 7.14 (d, *J* = 10.1 Hz, 1H), 7.10 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.06 – 7.00 (m, 2H), 6.96 (ddd, *J* = 7.5, 1.8, 0.9 Hz, 1H), 6.77 (t, *J* = 7.5 Hz, 1H), 6.04 (d, *J* = 10.0 Hz, 1H), 3.03 (s, 3H), 1.66 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 149.8, 140.9, 132.1, 131.2, 129.5, 128.9, 128.5, 127.6, 127.3, 127.3, 124.8, 123.5, 123.0, 122.7, 122.1, 119.9, 119.2, 118.2, 113.9, 90.6, 32.7,

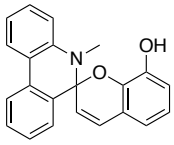
14.7. HRMS ESI-TOF+: *m/z* calcd for C23H20NO$^+$ [M+H$^+$]: 326.1539; found 326.1548.



5',6-Dimethyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**12**) (ECH30): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-5-methylbenzaldehyde (19 mg, 0.14 mmol), giving **12** as a white solid in 77% yield (35 mg, 0.11 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (d, J = 7.9 Hz, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.49 (t, J = 7.4 Hz, 1H), 7.41 – 7.34 (m, 3H), 7.12 (d, J = 10.0 Hz, 1H), 7.08 – 7.02 (m, 3H), 6.89 – 6.86 (d, J = 8.4 Hz, 1H), 6.36 (d, J = 8.2 Hz, 1H), 6.00 (d, J = 10.0 Hz, 1H), 3.09 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 149.8, 140.8, 131.5, 130.5, 129.6, 129.1, 129.0, 128.2, 127.8, 127.5, 127.1, 123.2, 123.0, 122.1, 119.6, 119.2, 118.2, 114.7, 113.9, 91.0, 33.0, 20.1. HRMS ESI-TOF+: *m/z* calcd for C23H20NO$^+$ [M+H$^+$]: 326.1539; found 326.1548.

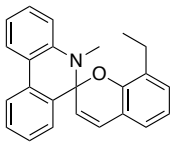


5-Bromo-8-methoxy-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**13**) (ECH31): Made using the general procedure from **5** (30 mg, 0.08 mmol) and **o** (19 mg, 0.08 mmol), giving **13** as a white solid in 74% yield (26 mg, 0.06 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.17 (d, J = 8.1 Hz, 1H), 8.12 (d, J = 7.9 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.43 – 7.36 (m, 2H), 7.35 (d, J = 8.0 Hz, 1H), 7.29 (d, J = 10.2 Hz, 1H), 7.11 – 7.03 (m, 3H), 6.80 (d, J = 8.8 Hz, 1H), 6.17 (d, J = 10.2 Hz, 1H), 3.47 (s, 3H), 3.08 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 146.6, 142.4, 140.3, 130.5, 129.7, 129.3, 128.1, 128.1, 127.7, 125.2, 125.0, 123.2, 123.1, 122.1, 119.4, 119.2, 117.6, 114.6, 113.9, 111.7, 91.8, 55.6, 33.0. HRMS ESI-TOF+: *m/z* calcd for C23H19BrNO2$^+$ [M+H$^+$]: 420.0594; found 420.0598.

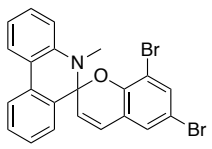


5'-Methyl-5'*H*-spiro[chromene-2,6'-phenanthridin]-8-ol (**14**) (ECH32): Made using the general procedure from **5** (20 mg, 0.06 mmol) and 2,3-dihydroxybenzaldehyde (8.0 mg, 0.06 mmol). The crude mixture was concentrated and purified by preparative HPLC using MeCN:water with 0.75% formic acid as

eluent, giving the open zwitterionic intermediate after condensation with the aldehyde. The isolated intermediate was then dissolved in a small amount of methanol and poured into 5% KOH in water. The closed spirocyclic compound was then extracted by EtOAc and dried under vacuum giving **14** in 66% yield (12 mg, 0.04 mmol). Trace amount of the ring-opened merocyanine form still present. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, J = 7.9 Hz, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.48 (t, J = 7.4 Hz, 1H), 7.41 – 7.32 (m, 3H), 7.10 – 6.98 (m, 3H), 6.74 – 6.61 (m, 3H), 5.92 (d, J = 10.0 Hz, 1H), 3.07 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 144.3, 140.8, 139.9, 131.5, 129.5, 128.9, 128.2, 127.4, 126.9, 123.0, 122.9, 121.8, 119.9, 119.2, 119.0, 118.9, 117.7, 117.1, 113.7, 90.7, 32.9. HRMS ESI-TOF+: *m/z* calcd for $C22H18NO2^{+}$ [$M+H^{+}$]: 328.1332; found 328.1338.

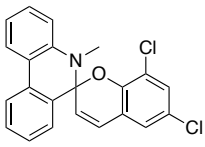


8-Ethyl-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**15**) (ECH33): Made using the general procedure from **5** (20 mg, 0.06 mmol) and 3-ethyl-2-hydroxybenzaldehyde (8.0 mg, 0.06 mmol), giving **15** as a white solid in 87% yield (17 mg, 0.05 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.13 (d, J = 8.0 Hz, 1H), 8.09 (d, J = 7.8 Hz, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.43 – 7.33 (m, 3H), 7.15 (d, J = 10.0 Hz, 1H), 7.10 (dd, J = 7.6, 1.7 Hz, 1H), 7.06 – 7.00 (m, 2H), 6.95 (dd, J = 7.5 Hz, 1H), 6.78 (t, J = 7.5 Hz, 1H), 6.04 (d, J = 10.0 Hz, 1H), 3.06 (s, 3H), 2.10 – 2.02 (m, 2H), 0.65 (t, J = 7.5 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 149.5, 140.9, 131.9, 129.9, 129.7, 129.4, 128.9, 128.5, 127.6, 127.4, 127.3, 124.9, 122.9, 122.7, 122.1, 120.1, 119.9, 119.2, 118.3, 113.9, 90.6, 32.9, 22.3, 13.7. HRMS ESI-TOF+: *m/z* calcd for $C24H22NO^{+}$ [$M+H^{+}$]: 340.1696; found 340.1703.

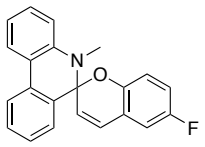


6,8-Dibromo-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**16**) (ECH34): Made using the general procedure from **5** (30 mg, 0.08 mmol) and 3,5-dibromosalicylaldehyde (23 mg, 0.08 mmol), giving **16** as a white solid in 61% yield (24 mg, 0.05 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.19 (d, J = 7.9 Hz, 1H), 8.13 (d, J = 7.9 Hz, 1H), 7.57 (dd, J = 8.5, 2.3 Hz, 2H), 7.53 (t, J = 7.7 Hz, 1H), 7.43 – 7.34 (m, 3H), 7.19 (d, J = 10.1 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.06 (t, J = 7.5 Hz,

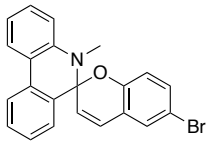
1H), 6.21 (d, *J* = 10.1 Hz, 1H), 3.07 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 148.0, 140.2, 134.4, 130.5, 129.6, 129.4, 129.0, 128.4, 127.8, 127.6, 125.8, 124.9, 123.1, 122.2, 121.8, 119.6, 119.5, 114.0, 111.4, 109.6, 93.3, 32.9. HRMS ESI-TOF+: *m/z* calcd for C22H16Br2NO$^+$ [M+H$^+$]: 467.9593; found 467.9597.



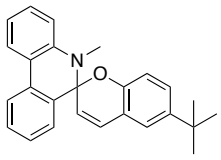
6,8-Dichloro-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**17**) (ECH35): Made using the general procedure from **5** (30 mg, 0.08 mmol) and 3,5-dichloro-2-hydroxybenzaldehyde (16 mg, 0.08 mmol), giving **17** as a white solid in 85% yield (27 mg, 0.07 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.20 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 7.54 (t, *J* = 7.3 Hz, 1H), 7.44 (d, *J* = 2.5 Hz, 1H), 7.43 – 7.34 (m, 4H), 7.22 (d, *J* = 10.1 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.24 (d, *J* = 10.1 Hz, 1H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 148.0, 140.2, 134.4, 130.5, 129.7, 129.4, 129.0, 128.4, 127.8, 127.6, 125.8, 124.9, 123.1, 122.2, 121.8, 119.6, 119.5, 114.0, 111.4, 109.6, 93.3, 32.9. HRMS ESI-TOF+: *m/z* calcd for C22H16Cl2NO$^+$ [M+H$^+$]: 380.0603; found 380.0611.



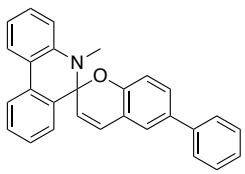
6-Fluoro-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**18**) (ECH36): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 5-fluorosalicylaldehyde (20 mg, 0.14 mmol), giving **18** as a white solid in 76% yield (35 mg, 0.11 mmol).^{1}H NMR (600 MHz, DMSO-d_6) δ 8.14 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 7.9 Hz, 1H), 7.50 (m, 1H), 7.41 – 7.34 (m, 2H), 7.21 – 7.14 (m, 2H), 7.07 (d, *J* = 8.4 Hz 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 6.89 (td, *J* = 8.4, 3.0 Hz, 1H), 6.48 (dd, *J* = 8.7, 4.5 Hz, 1H), 6.12 (d, *J* = 10.0 Hz, 1H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 156.8, 155.3, 148.1, 140.7, 131.1, 129.6, 129.1, 128.2, 127.7, 127.6, 126.4, 124.5, 123.1, 122.2, 119.6, 119.5, 119.4, 119.3, 116.3, 116.1, 116.1, 114.0, 113.2, 113.0, 91.4, 33.1. HRMS ESI-TOF+: *m/z* calcd for C22H17FNO$^+$ [M+H$^+$]: 330.1289; found 330.1294.



6-Bromo-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**19**) (ECH37): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 5-bromosalicylaldehyde (28 mg, 0.14 mmol), giving **19** as a white solid in 71% yield (39 mg, 0.10 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, J = 8.0 Hz, 1H), 8.10 (dd, J = 7.9, 1.5 Hz, 1H), 7.55 – 7.47 (m, 2H), 7.41 – 7.34 (m, 3H), 7.23 – 7.15 (m, 2H), 7.11 – 7.02 (m, 2H), 6.45 (d, J = 8.6 Hz, 1H), 6.11 (d, J = 10.1 Hz, 1H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 151.1, 140.5, 132.3, 130.9, 129.7, 129.4, 129.2, 128.2, 127.8, 127.6, 126.0, 124.3, 123.1, 122.2, 120.7, 119.5, 119.4, 117.2, 114.0, 111.4, 91.8, 33.1. HRMS ESI-TOF+: *m/z* calcd for $C_{22}H_{17}BrNO^{+}$ [M+H^{+}]: 390.0488; found 390.0497.

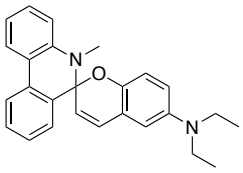


6-(*tert*-Butyl)-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**20**) (ECH38): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 5-*tert*-butyl-2-hydroxybenzaldehyde (24 μL, 0.14 mmol), giving **20** as a white solid in 72% yield (37 mg, 0.10 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, J = 7.9 Hz, 1H), 8.09 (dd, J = 7.9, 1.5 Hz, 1H), 7.48 (dt, J = 7.4, 2.3 Hz, 1H), 7.41 – 7.33 (m, 3H), 7.28 (d, J = 2.4 Hz, 1H), 7.17 (d, J = 10.0 Hz, 1H), 7.12 – 6.99 (m, 3H), 6.39 (d, J = 8.5 Hz, 1H), 5.98 (d, J = 10.0 Hz, 1H), 3.08 (s, 3H), 1.25 (s, 9H). ^{13}C NMR (151 MHz, DMSO) δ 149.7, 142.5, 140.8, 131.6, 129.6, 129.0, 128.2, 127.8, 127.5, 127.4, 127.0, 123.9, 123.0, 122.8, 122.1, 119.6, 119.2, 117.5, 114.2, 113.9, 91.0, 33.8, 33.0, 31.3. HRMS ESI-TOF+: *m/z* calcd for $C_{26}H_{26}NO^{+}$ [M+H^{+}]: 368.2009; found 368.2018.

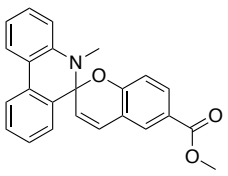


5'-Methyl-6-phenyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**21**) (ECH39): Made using the general procedure from **5** (20 mg, 0.06 mmol) and **29** (11 mg, 0.06 mmol), giving **21** as a white solid in 78% yield (17 mg, 0.04 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.17 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 7.8 Hz, 1H), 7.66 – 7.59 (m, 4H), 7.53 (t, J = 7.5 Hz, 1H), 7.47 – 7.35 (m, 5H), 7.32 (t, J = 7.3 Hz, 1H),

7.27 (d, *J* = 10.1 Hz, 1H), 7.10 (d, *J* = 8.2 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.57 (d, *J* = 8.2 Hz, 1H), 6.09 (d, *J* = 10.1 Hz, 1H), 3.14 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 151.6, 140.7, 139.6, 132.4, 131.3, 129.6, 129.1, 128.9, 128.3, 128.2, 127.8, 127.6, 127.1, 126.8, 126.1, 125.5, 123.5, 123.1, 122.1, 119.6, 119.3, 118.7, 115.4, 114.0, 91.6, 33.0. HRMS ESI-TOF+: *m/z* calcd for $C_{28}H_{22}NO^+$ [M+H$^+$]: 388.1696; found 388.1708.

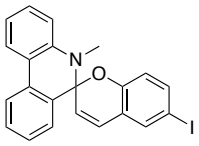


N,*N*-diethyl-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridin]-6-amine (**22**) (ECH40): Made using the general procedure from **5** (30 mg, 0.08 mmol) and 4-diethylaminosalicylaldehyde (16 mg, 0.08 mmol). The crude mixture was concentrated and purified by preparative HPLC using MeCN:water with 0.75% formic acid as eluent, giving the open zwitterionic intermediate after condensation with the aldehyde, as a deep purple solid. The isolated solid was then dissolved in a small amount of methanol and poured into 5% KOH in water. The closed spirocyclic compound was then extracted by EtOAc and dried under vacuum giving **22** in 11% yield (3.5 mg, 0.0092 mmol). Trace amount of the ring-opened merocyanine form still present. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 – 8.06 (dd, *J* = 8.2, 7.9 Hz, 2H), 7.50 – 7.40 (m, 2H), 7.38 – 7.33 (m, 2H), 7.08 – 6.96 (m, 4H), 6.16 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.68 (d, *J* = 2.5 Hz, 1H), 5.62 (d, *J* = 9.9 Hz, 1H), 3.19 (q, *J* = 7.0 Hz, 4H), 3.10 (s, 3H), 0.96 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, DMSO) δ 153.5, 149.4, 141.0, 131.9, 129.5, 128.8, 128.2, 128.1, 128.0, 127.4, 126.8, 123.0, 121.9, 119.5, 118.9, 117.5, 113.9, 106.6, 103.9, 96.8, 91.1, 43.5, 32.9, 12.5. HRMS ESI-TOF+: *m/z* calcd for $C_{26}H_{27}N_2O^+$ [M+H$^+$]: 383.2118; found 383.2128.

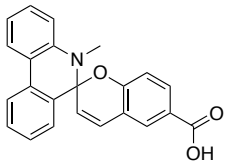


Methyl 5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine]-6-carboxylate (**23**) (ECH41): Made using the general procedure from **5** (50 mg, 0.14 mmol) and methyl 3-formyl-4-hydroxybenzoate (21 μL, 0.14 mmol), giving **23** as a white solid in 77% yield (40 mg, 0.11 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, *J* = 8.0 Hz, 1H), 8.12 (d, *J* = 7.6 Hz, 1H), 7.95 (d, *J* = 2.2 Hz, 1H), 7.67 (dd, *J* =

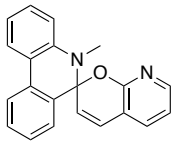
8.5, 2.2 Hz, 1H), 7.54 – 7.50 (m, 1H), 7.41 – 7.36 (m, 3H), 7.32 (d, *J* = 10.2 Hz, 1H), 7.12 – 7.03 (m, 2H), 6.57 (d, *J* = 8.5 Hz, 1H), 6.14 (d, *J* = 10.2 Hz, 1H), 3.81 (s, 3H), 3.11 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 165.7, 155.9, 140.4, 131.5, 130.8, 129.7, 129.3, 128.8, 128.2, 127.8, 127.7, 126.6, 123.8, 123.1, 122.2, 121.6, 119.6, 119.5, 118.4, 115.3, 114.1, 92.7, 51.9, 33.0. HRMS ESI-TOF+: *m/z* calcd for $C_{24}H_{20}NO_3^+$ [M+H$^+$]: 370.1438; found 370.1447.



6-Iodo-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine] (**24**) (ECH42): Made using the general procedure from **5** (20 mg, 0.06 mmol) and 5-iodosalicylaldehyde (14 mg, 0.06 mmol), giving **24** as a white solid in 70% yield (17 mg, 0.04 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 7.9, 1H), 7.65 (d, *J* = 2.2 Hz, 1H), 7.52 – 7.48 (m, 1H), 7.43 – 7.31 (m, 4H), 7.16 (d, *J* = 10.1 Hz, 1H), 7.10 – 7.01 (m, 2H), 6.32 (d, *J* = 8.5 Hz, 1H), 6.08 (d, *J* = 10.1 Hz, 1H), 3.09 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 151.8, 140.5, 138.2, 135.2, 131.0, 129.6, 129.2, 128.2, 127.8, 127.6, 125.9, 124.0, 123.1, 122.1, 121.2, 119.5, 119.4, 117.6, 114.0, 91.7, 82.5, 33.0. HRMS ESI-TOF+: *m/z* calcd for $C_{22}H_{17}INO^+$ [M+H$^+$]: 438.0349; found 438.0350.

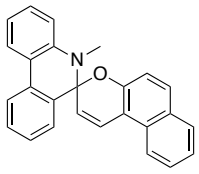


5'-Methyl-5'*H*-spiro[chromene-2,6'-phenanthridine]-6-carboxylic acid (**25**) (ECH43): Made using the general procedure from **5** (50 mg, 0.14 mmol) and **30** (23 mg, 0.14 mmol). Removal of unknown byproduct was accomplished by several recrystalisations using DCM and MeOH, finally giving **25** as a beige solid in 30% yield (15 mg, 0.04 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (d, *J* = 7.9 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 7.91 (d, *J* = 2.2 Hz, 1H), 7.65 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.54 – 7.50 (m, 1H), 7.42 – 7.38 (m, 3H), 7.30 (d, *J* = 10.1 Hz, 1H), 7.11 – 7.05 (m, 2H), 6.54 (d, *J* = 8.5 Hz, 1H), 6.12 (d, *J* = 10.1 Hz, 1H), 3.12 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 166.8, 155.6, 140.5, 131.6, 130.9, 129.7, 129.3, 129.0, 128.2, 127.9, 127.7, 126.7, 123.6, 123.1, 122.2, 119.5, 118.1, 115.0, 114.1, 92.5, 33.0. HRMS ESI-TOF+: *m/z* calcd for $C_{23}H_{18}NO_3^+$ [M+H$^+$]: 356.1281; found 356.1294.

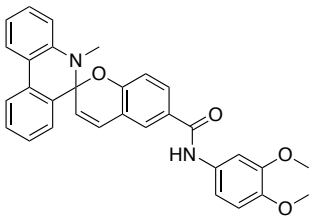


5-Methyl-5*H*-spiro[phenanthridine-6,2'-pyrano[2,3-*b*]pyridine] (**26**) (ECH44): Made using the general procedure from **5** (35 mg, 0.10 mmol) and 2-oxo-1,2-dihydro-3-pyridinecarbaldehyde (12 mg, 0.10 mmol), giving the open cationic intermediate after condensation with the aldehyde, as a beige solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.30 (bs, 1H), 9.17 (d, J = 7.8 Hz, 2H), 8.72 – 8.51 (m, 3H), 8.36 (t, J = 7.7 Hz, 1H), 8.23 – 7.90 (m, 3H), 7.66 (d, J = 5.6 Hz, 1H), 7.26 (d, J = 16.2 Hz, 1H), 6.45 (t, J = 6.5 Hz, 1H), 4.57 (s, 3H).

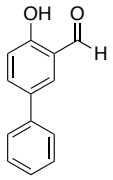
The isolated solid was then dissolved in a small amount of methanol and poured into 5% KOH in water. The closed spirocyclic compound was then extracted by EtOAc and dried under vacuum giving **26** in 52% yield (16 mg, 0.05 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, J = 7.9 Hz, 1H), 8.13 (d, J = 7.7 Hz, 1H), 7.91 (dd, J = 5.0, 2.0 Hz, 1H), 7.71 (dd, J = 7.3, 2.0 Hz, 1H), 7.55 – 7.51 (m, 1H), 7.44 – 7.34 (m, 3H), 7.22 (d, J = 10.0 Hz, 1H), 7.14 – 7.07 (m, 2H), 6.93 (dd, J = 7.3, 5.0 Hz, 1H), 6.16 (d, J = 10.0 Hz, 1H), 3.11 (s, 3H). HRMS ESI-TOF+: *m/z* calcd for C21H17N2O [M+H$^+$]: 313.1335; found 313.1339.



5'-Methyl-5'*H*-spiro[benzo[*f*]chromene-3,6'-phenanthridine] (**27**) (ECH45): Made using the general procedure from **5** (50 mg, 0.14 mmol) and 2-hydroxy-1-naphtaldehyde (24 mg, 0.14 mmol), giving **27** as a gray solid in 81% yield (41 mg, 0.11 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (d, J = 8.5 Hz, 1H), 8.17 (d, J = 8.1 Hz, 1H), 8.13 (d, J = 7.5 Hz, 1H), 7.98 (d, J = 10.4 Hz, 1H), 7.81 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.58 (t, J = 7.6 Hz, 1H), 7.54 – 7.43 (m, 2H), 7.42 – 7.33 (m, 3H), 7.11 – 7.03 (m, 2H), 6.77 (d, J = 8.9 Hz, 1H), 6.13 (d, J = 10.4 Hz, 1H), 3.13 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 150.0, 140.6, 131.4, 130.4, 129.6, 129.6, 129.1, 128.4, 128.4, 128.2, 127.6, 127.0, 123.5, 123.1, 122.7, 122.0, 121.6, 121.2, 119.6, 119.3, 117.3, 114.0, 110.1, 91.3, 32.9. HRMS ESI-TOF+: *m/z* calcd for C26H20NO$^+$ [M+H$^+$]: 362.1539; found 362.1550.

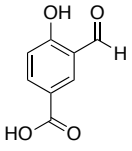


N-(3,4-dimethoxyphenyl)-5'-methyl-5'*H*-spiro[chromene-2,6'-phenanthridine]-6-carboxamide (**28**) (ECH46): Made using the general procedure from **5** (35 mg, 0.10 mmol) and **31** (42 mg, 0.10 mmol), giving **28** as a gray solid in 56% yield (27 mg, 0.06 mmol). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (s, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.13 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 2.3 Hz, 1H), 7.71 (dd, J = 8.5, 2.3 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.46 – 7.37 (m, 4H), 7.34 – 7.26 (m, 2H), 7.11 (d, J = 8.3 Hz, 1H), 7.09 – 7.05 (t, J = 7.4 Hz, 1H), 6.92 (d, J = 8.7 Hz, 1H), 6.59 (d, J = 8.4 Hz, 1H), 6.15 (d, J = 10.1 Hz, 1H), 3.75 (s, 3H), 3.73 (s, 3H), 3.13 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 164.3, 154.5, 148.4, 145.0, 140.5, 132.9, 131.0, 129.7, 129.3, 128.2, 127.8, 127.6, 127.1, 127.1, 126.8, 123.7, 123.1, 122.2, 119.6, 119.5, 118.0, 114.7, 114.1, 112.1, 111.9, 105.4, 92.3, 55.7, 55.4, 33.1. HRMS ESI-TOF+: *m/z* calcd for C31H26N2O4^{+} [M+H^{+}]: 491.1965; found 491.1968.

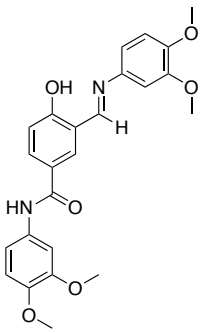


4-Hydroxy-[1,1'-biphenyl]-3-carbaldehyde (**29**): $Pd(PPh_3)_4$ (115 mg, 0.10 mmol) was added to a stirred solution of 5-bromosalicylaldehyde (200 mg, 1.00 mmol), phenylboronic acid (122 mg, 1.00 mmol), K_2CO_3 (344 mg, 2.50 mmol) in dry toluene (12.5 ml) and ethanol (2.5 ml) at room temperature under N_2 atmosphere and the contents were stirred at the same temperature for 20 min. The reaction mixture slowly heated to 75-80 ^{0}C for 2 hr. After completion of the reaction (TLC), the solvents were removed under reduced pressure, the reaction mixture was extracted with ethylacetate and washed with water, the organic layer was separated, dried over sodium sulfate, solvent removed under reduced pressure and the obtained crude product was purified by flash chromatography using silica gel with heptane: ethyl acetate as eluent to give 2-hydroxy-5-phenylbenzaldehyde (**29**) in 47% yield as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.01 (s, 1H), 9.99 (s, 1H), 7.84 – 7.73 (m, 2H), 7.59 – 7.53 (m, 2H), 7.49 – 7.43 (m, 2H), 7.39 – 7.33 (m, 1H), 7.08 (d, J = 7.9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.8, 161.1, 139.5, 135.9, 133.4,

132.0, 129.1, 127.5, 126.7, 120.8, 118.3. LCMS: Calculated M^+ (199); no ionization found m/z, Rt = 4.65 min.



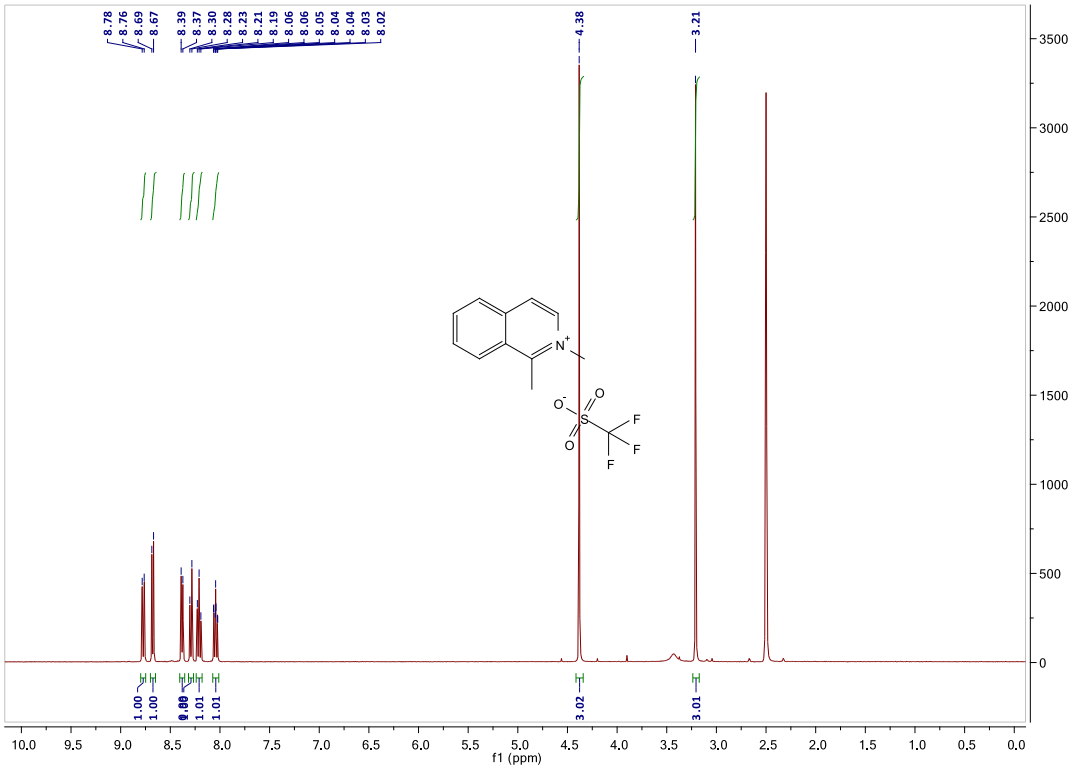
3-Formyl-4-hydroxybenzoic acid (**30**): Methyl 3-formyl-4-hydroxybenzoate (50 mg, 0.28 mmol) was dissolved in MeOH (2.5 mL) and LiOH (70 μL, 0.28 mmol, 4M aqueous sol.) was added. The reaction was heated to reflux for 2h and then allowed to cool down. The solvent was evaporated and the resulting crude was dissolved in a small amount of water. Acidic pH was reached by dropwise addition of 1M HCl which led to a precipitation. The product was then isolated by filtration then eluted into a new flask using MeOH and dried under Na_2SO_4. The resulting solution was concentrated giving the product as a white solid in 95% yield (44 mg, 0.27 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (s, 1H), 10.29 (s, 1H), 8.23 (d, J = 2.3 Hz, 1H), 8.04 (dd, J = 8.7, 2.3 Hz, 1H), 7.08 (d, J = 8.7 Hz, 1H). ^{13}C NMR (151 MHz, DMSO) δ 190.5, 166.4, 164.1, 136.8, 130.7, 122.1, 121.9, 117.5. LCMS: Calculated M^- (165); Found (M^-) 165 m/z, Rt = 3.19 min.



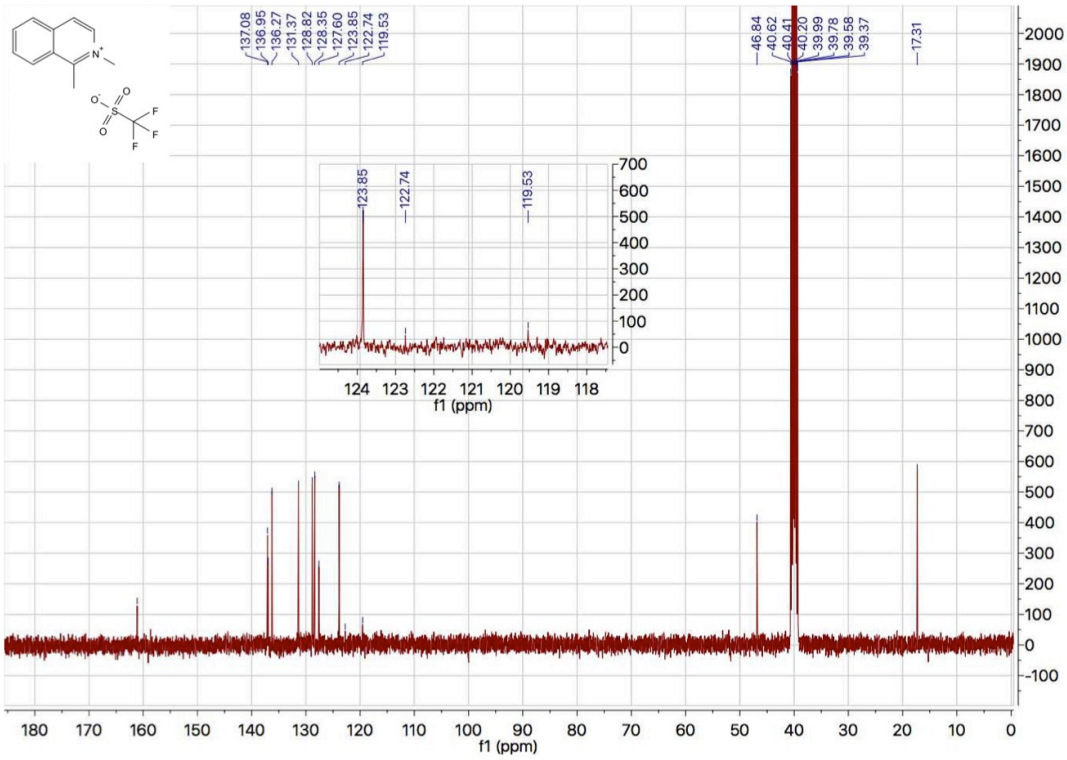
(*E*)-*N*-(3,4-dimethoxyphenyl)-3-(((3,4-dimethoxyphenyl)imino) methyl)-4-hydroxybenzamide (**31**): Acid **30** (30 mg, 0.18 mmol) and 3,4-dimethoxyaniline (55 mg, 0.36 mmol) were dissolved in DMF (2 mL). 1-Hydroxybenzotriazole hydrate (5.5 mg, 0.04 mmol) was added followed by 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (69 mg, 0.36 mmol). The reaction was left to stir over night and then diluted with EtOAc and washed with saturated NaHCO3 solution. The resulting organic phase was dried under Na_2SO_4 and concentrated. The crude mixture was purified by column chromatography using EtOAc:heptane as eluent. Upon isolation of the product it was found that it was the imine that had formed and not the desired aldehyde. The imine was however successfully used for the following step as such. The imine **31** was isolated as a yellow solid in 46% yield

(36 mg, 0.08 mmol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.87 (s, 1H), 10.04 (s, 1H), 9.11 (s, 1H), 8.30 (d, J = 2.3 Hz, 1H), 8.02 (dd, J = 8.7, 2.4 Hz, 1H), 7.44 (d, J = 2.4 Hz, 1H), 7.35 (dd, J = 8.7, 2.4 Hz, 1H), 7.21 (d, J = 1.8 Hz, 1H), 7.13 – 7.00 (m, 3H), 6.93 (d, J = 8.8 Hz, 1H), 3.85 (s, 3H), 3.80 (s, 3H), 3.76 (s, 3H), 3.74 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 164.1, 162.8, 160.6, 149.4, 148.5, 148.4, 145.0, 140.3, 132.8, 132.4, 131.8, 125.8, 118.8, 116.5, 114.4, 112.3, 112.0, 112.0, 111.9, 105.5, 104.8, 55.7, 55.6, 55.4. LCMS: Calculated M^+ (aldehyde) (302); Found (M^+) 302 m/z, Rt = 4.97 min.

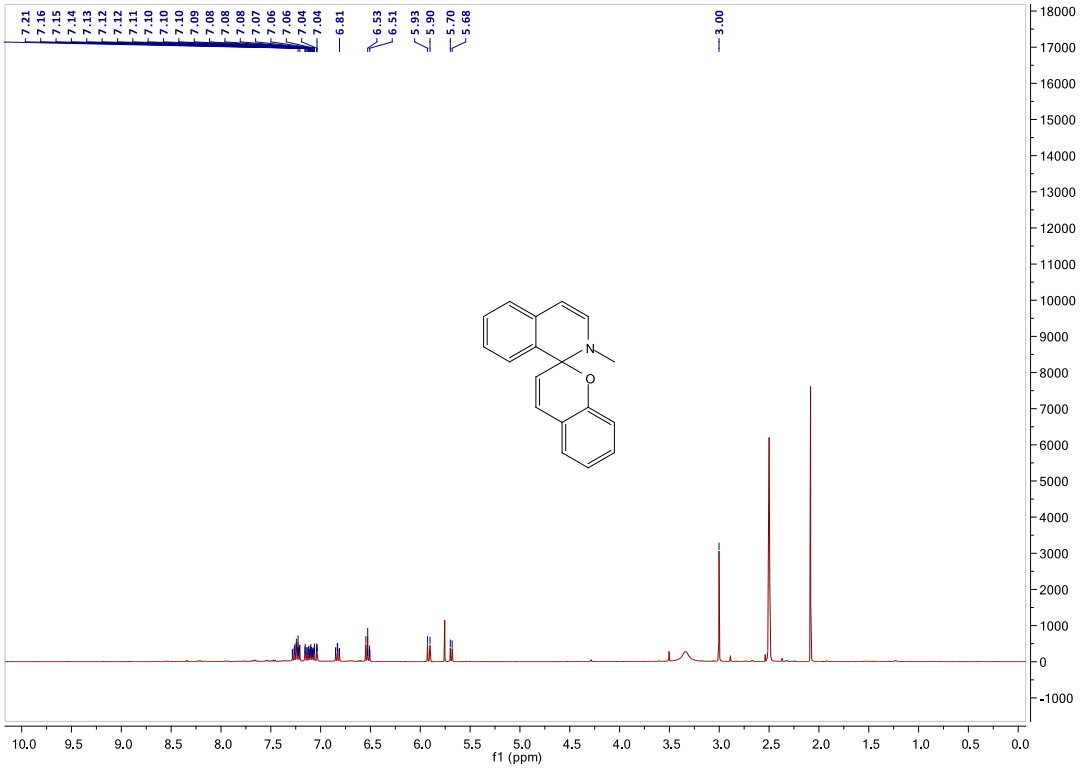
NMR spectra for compounds 1-31



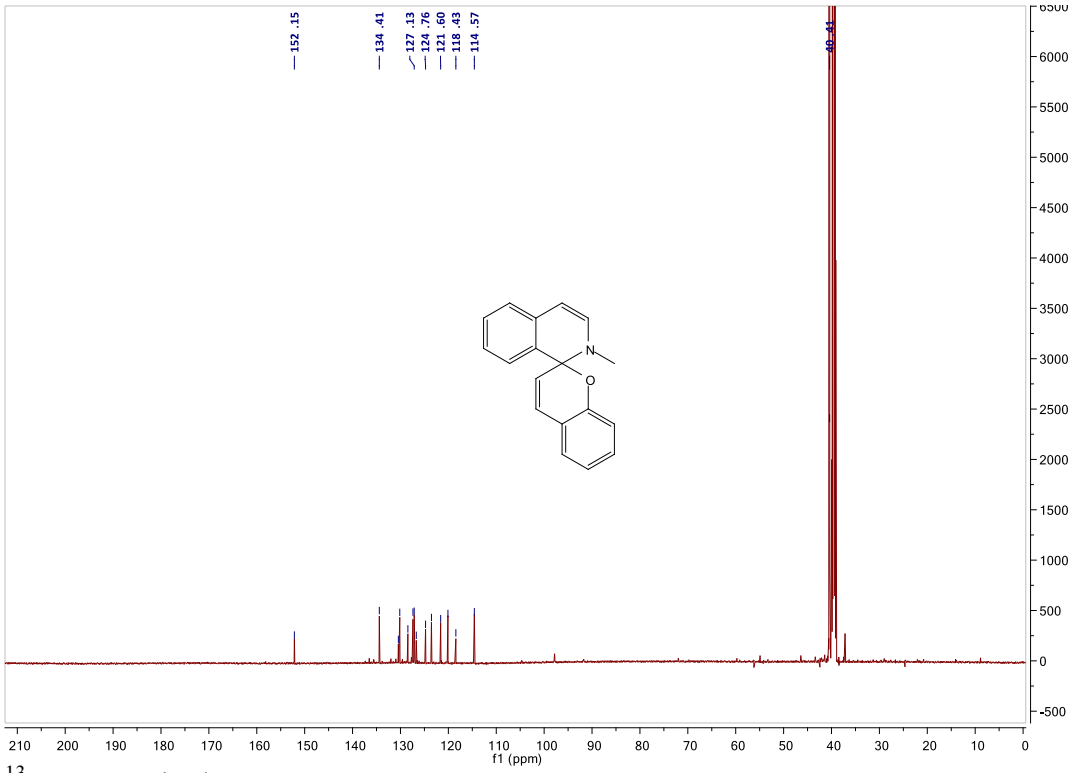
^{1}H NMR **1** in d6-DMSO



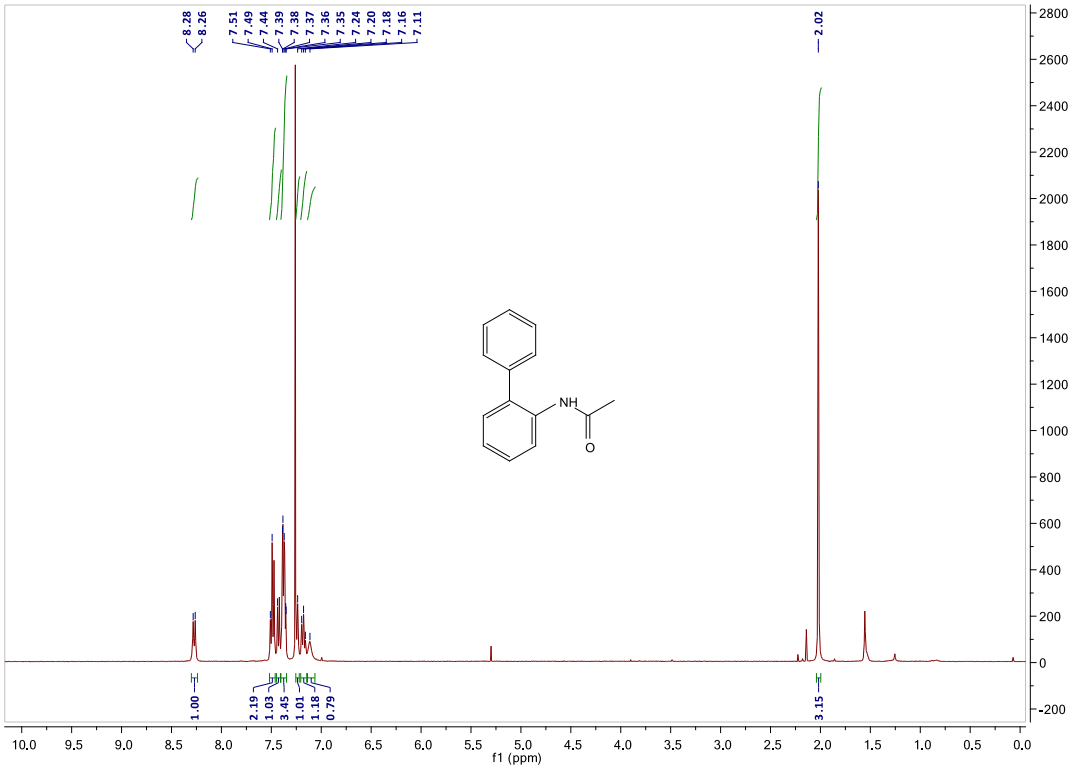
^{13}C NMR **1** in d6-DMSO



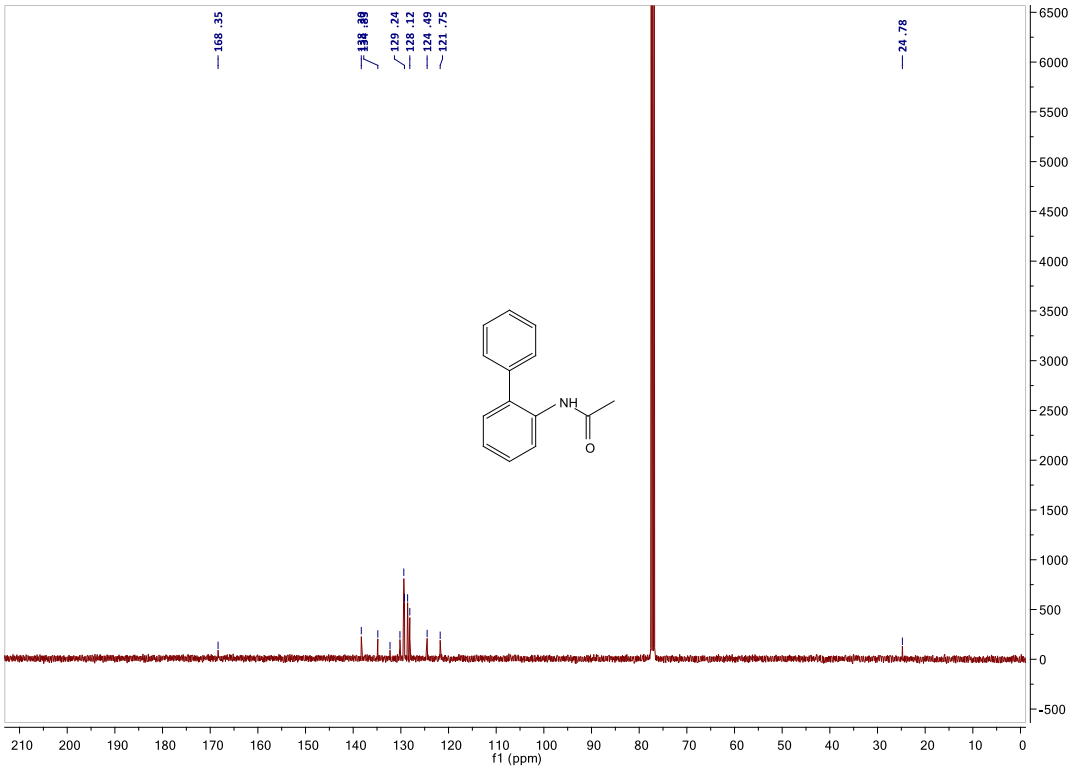
^{1}H NMR **2** in d6-DMSO



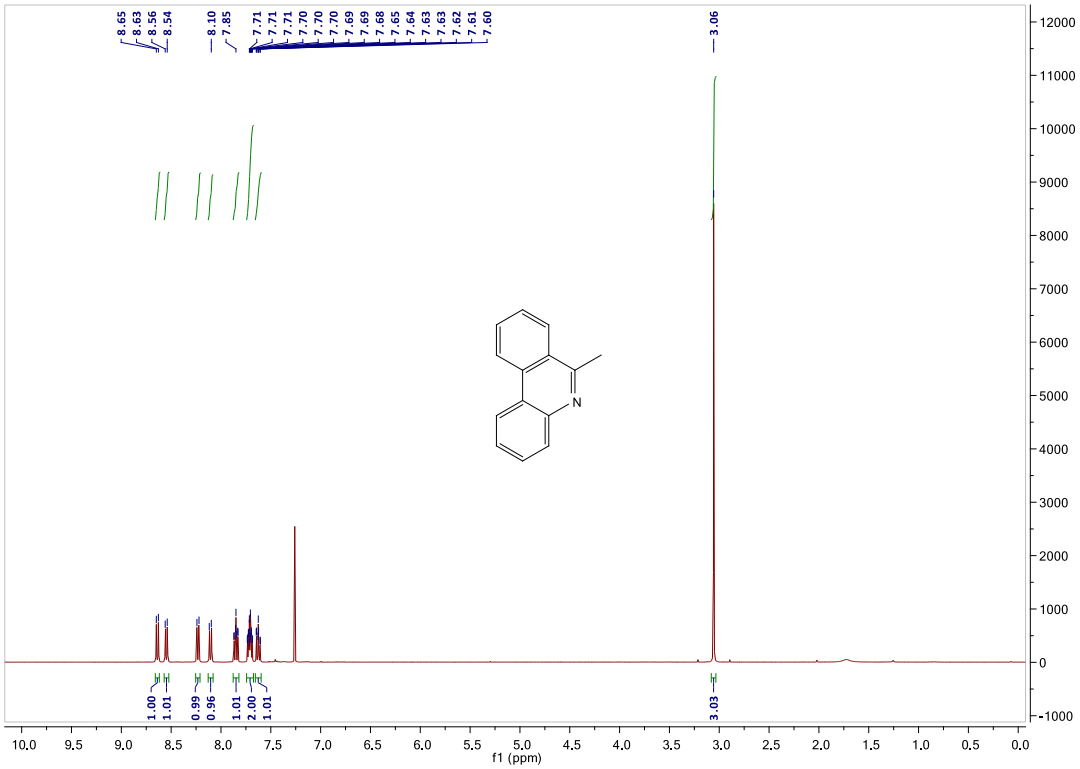
^{13}C NMR **2** in d6-DMSO



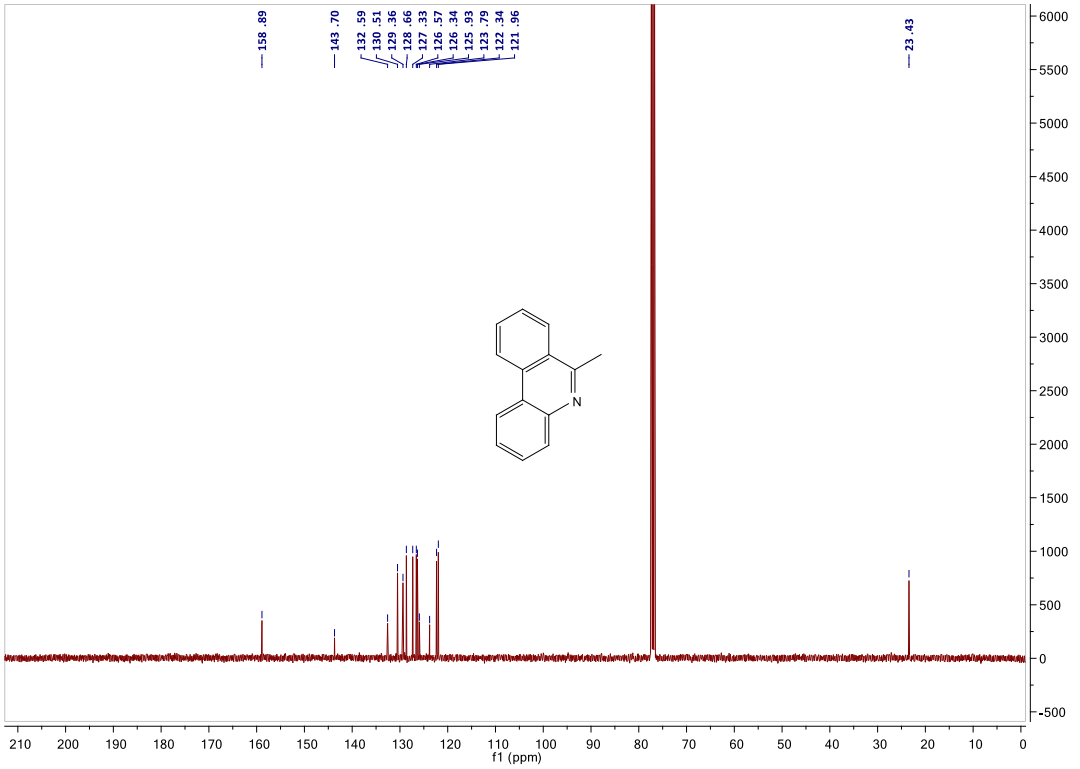
^{1}H NMR **3** in d6-DMSO



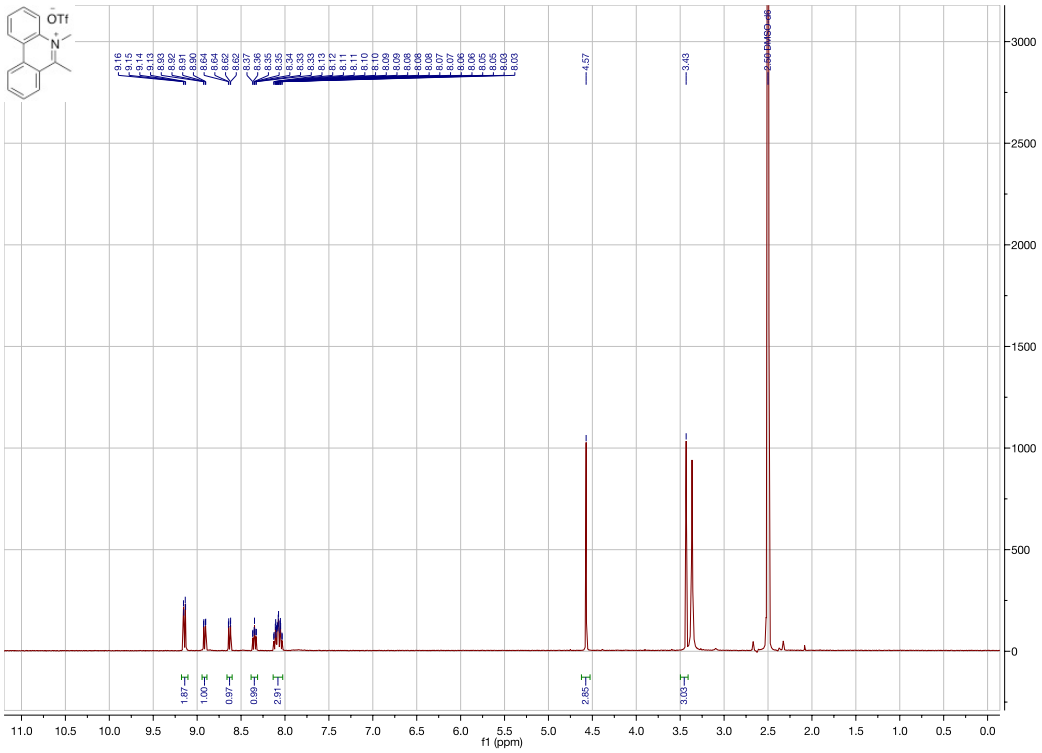
^{13}C NMR **3** in d6-DMSO



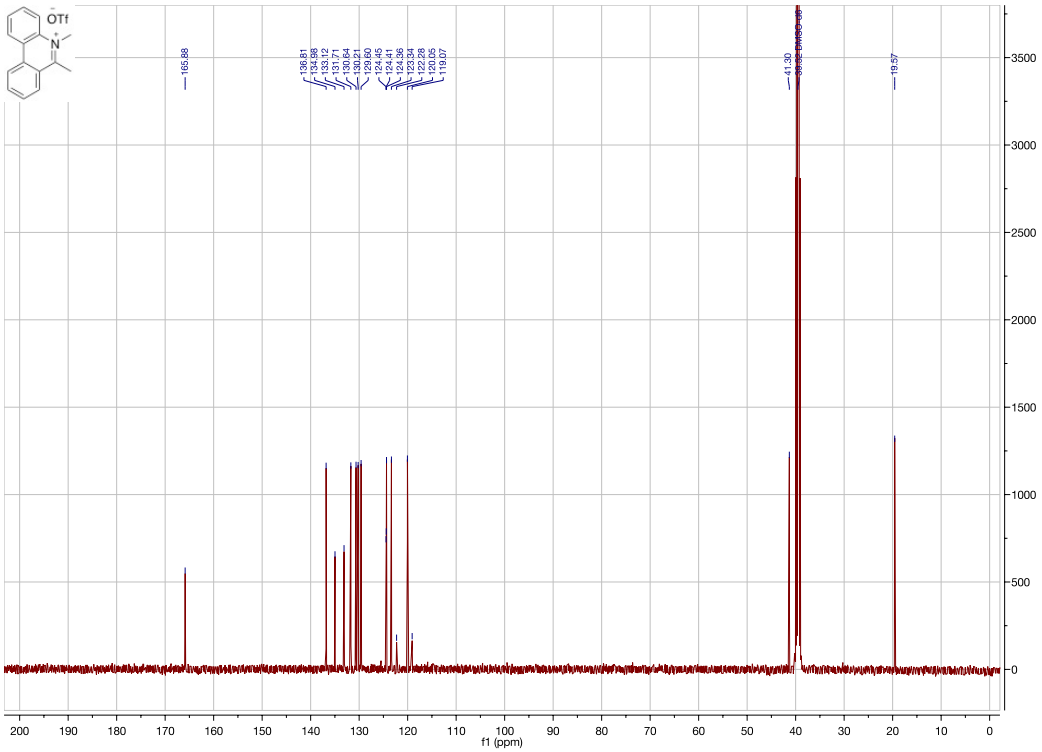
^{1}H NMR **4** in d6-DMSO



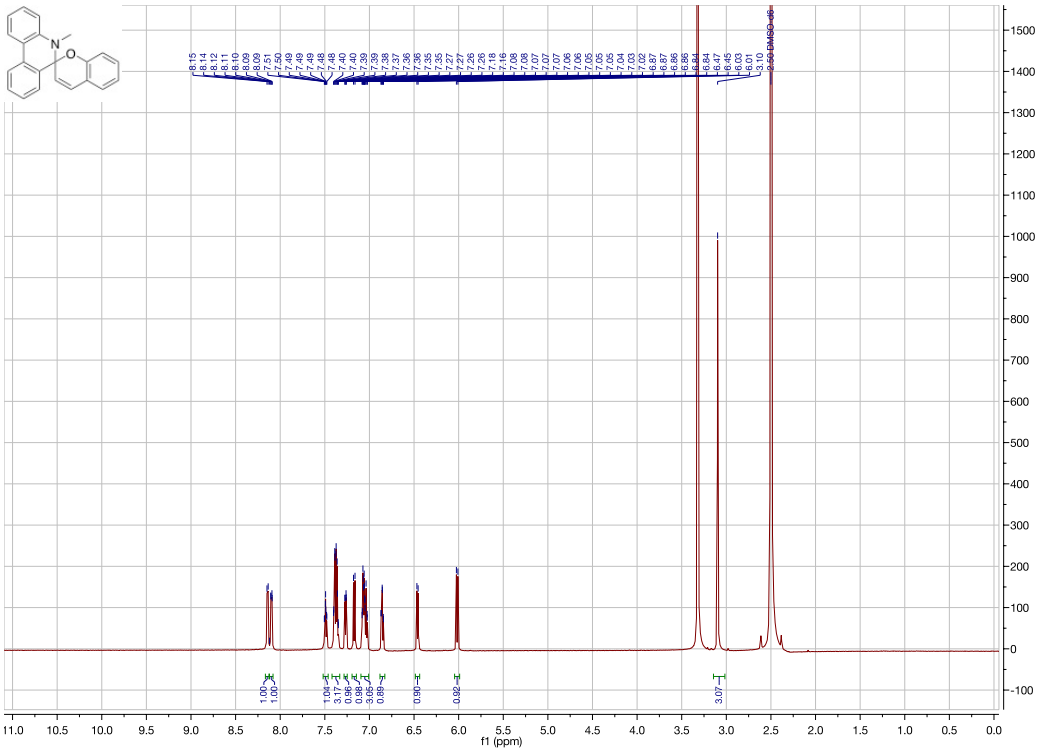
^{13}C NMR **4** in d6-DMSO



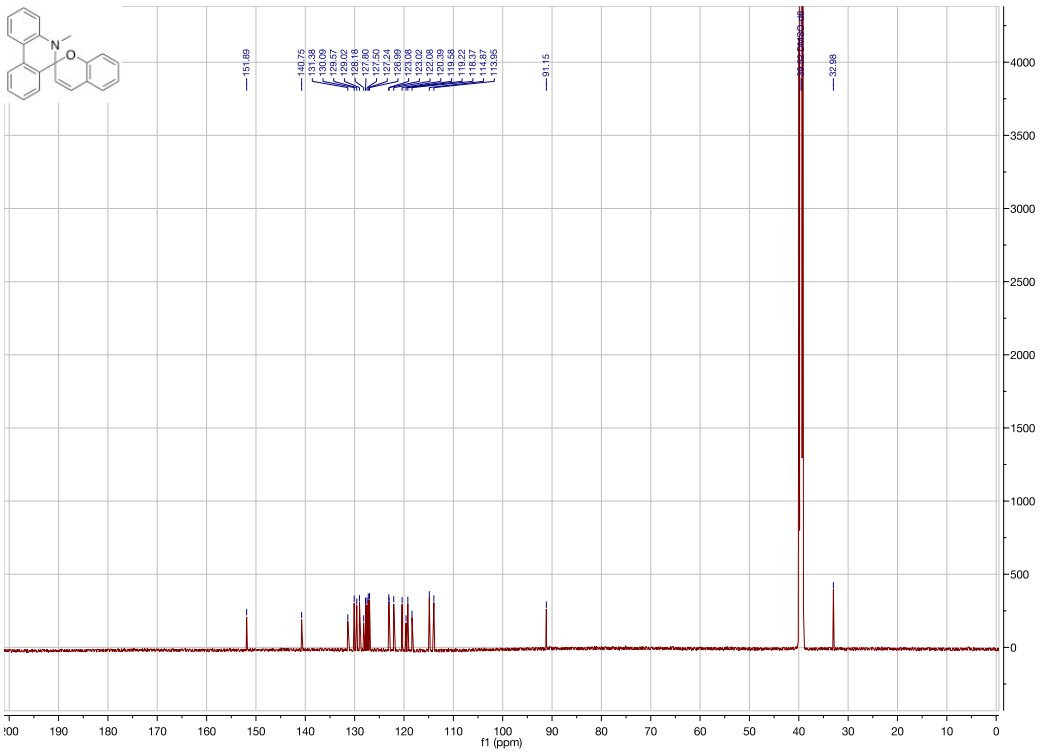
^{1}H NMR **5** in d6-DMSO



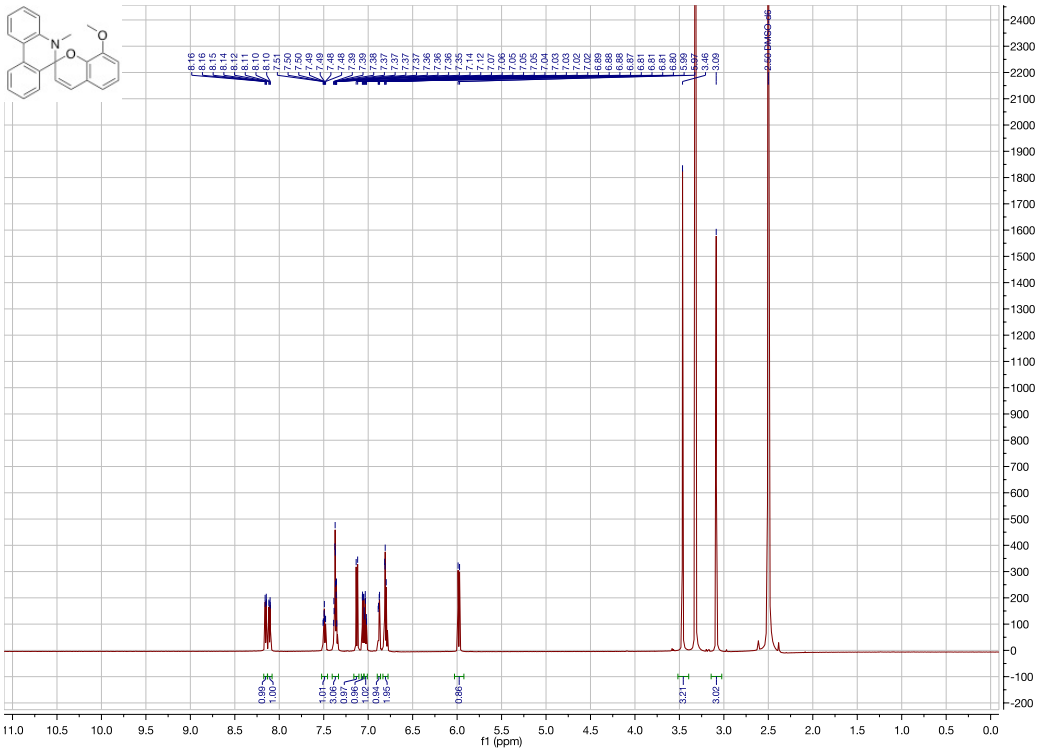
^{13}C NMR **5** in d6-DMSO



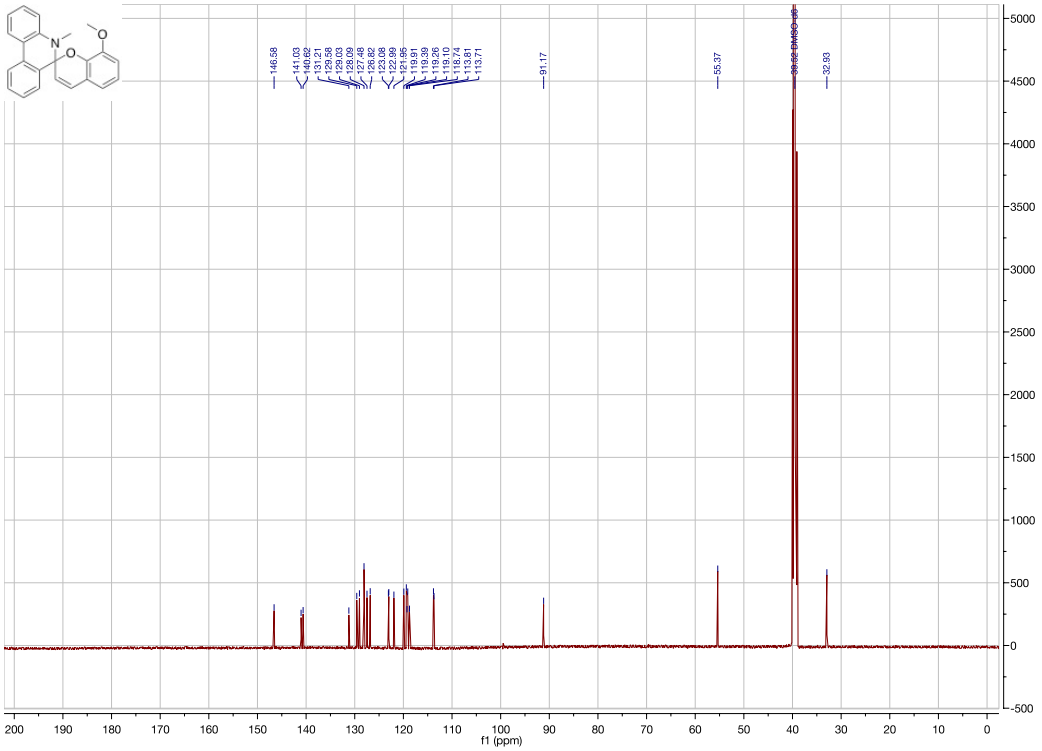
^{1}H NMR **6** in d6-DMSO



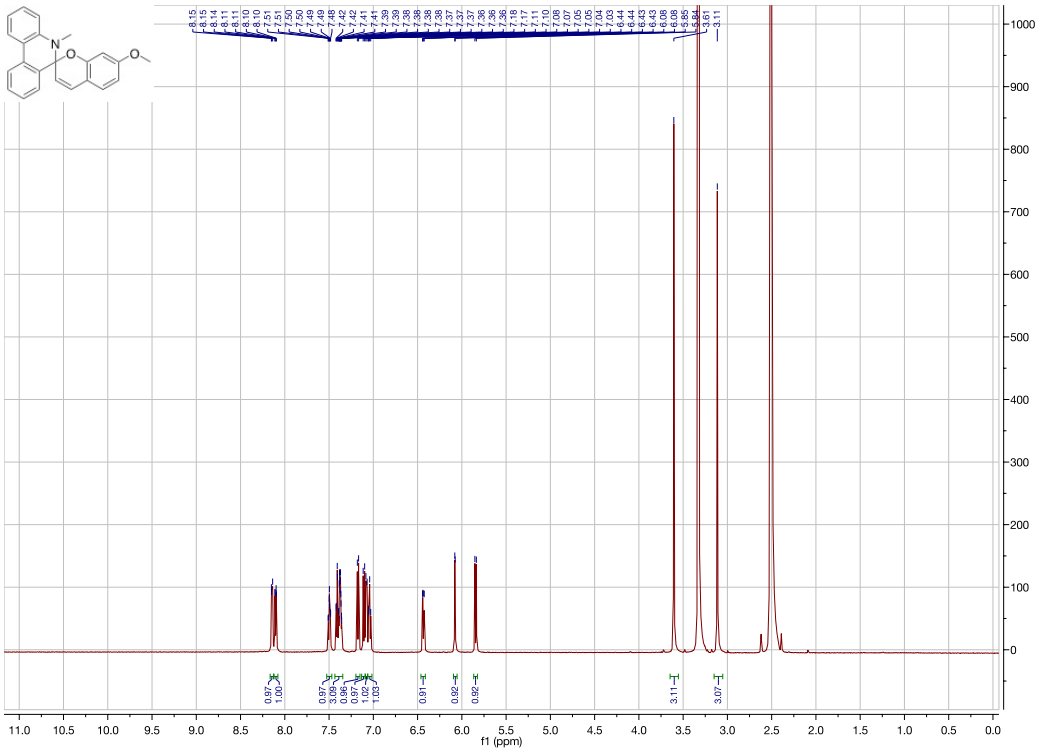
^{13}C NMR **6** in d6-DMSO



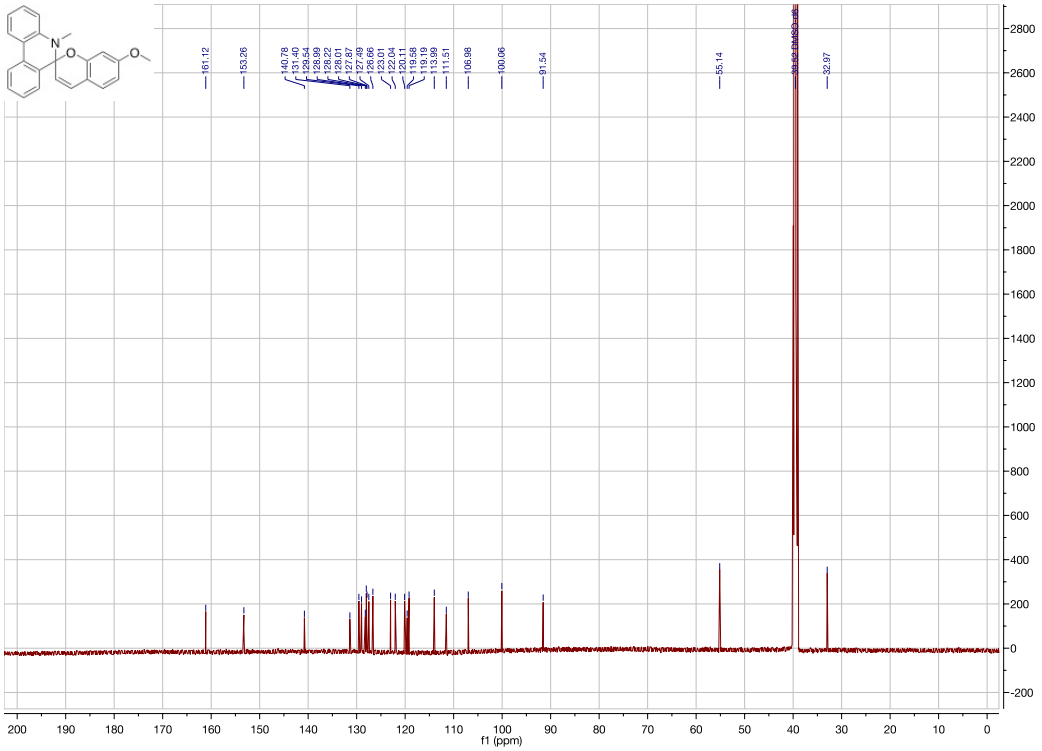
^{1}H NMR **7** in d6-DMSO



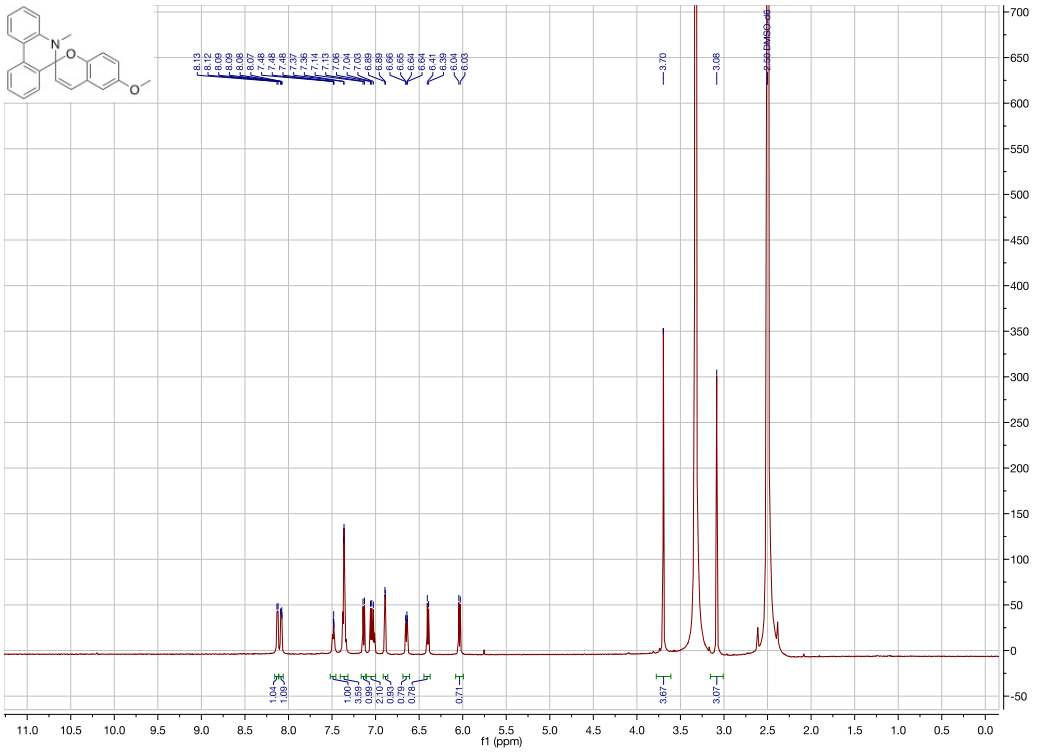
^{13}C NMR **7** in d6-DMSO



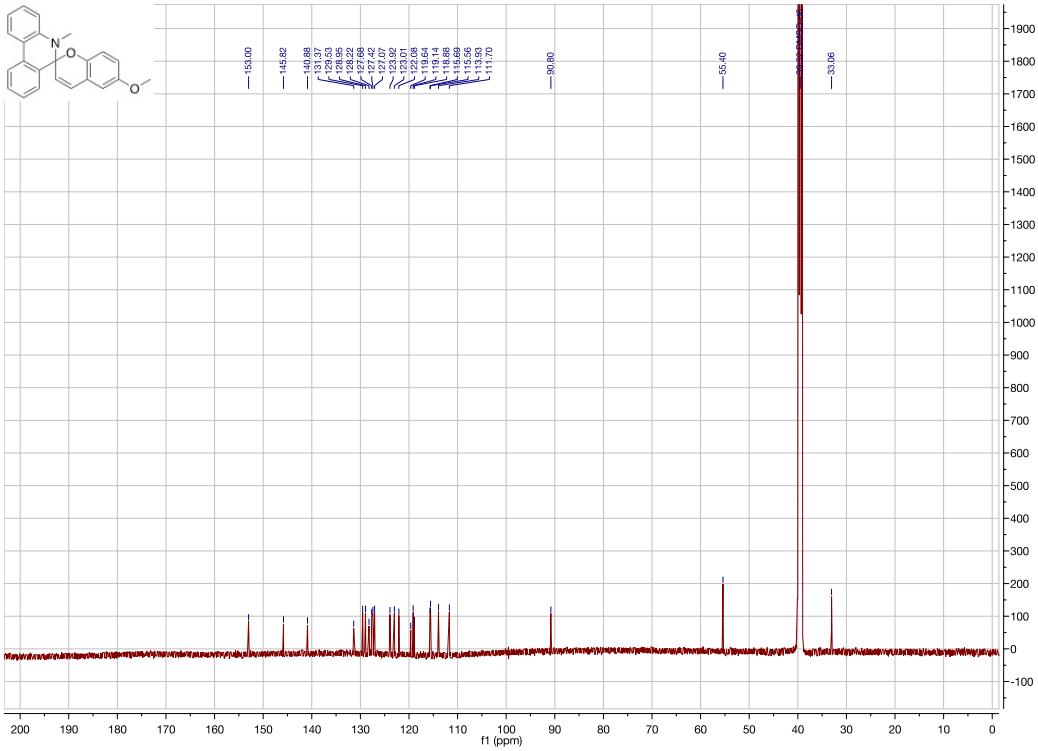
^{1}H NMR **8** in d6-DMSO



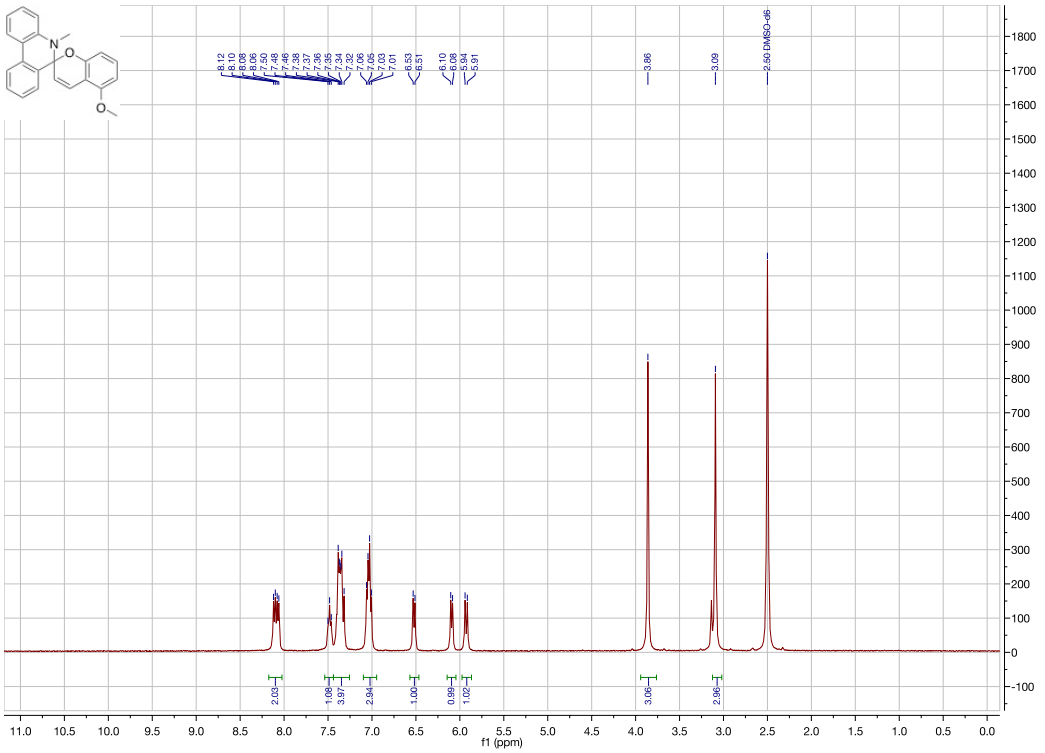
^{13}C NMR **8** in d6-DMSO



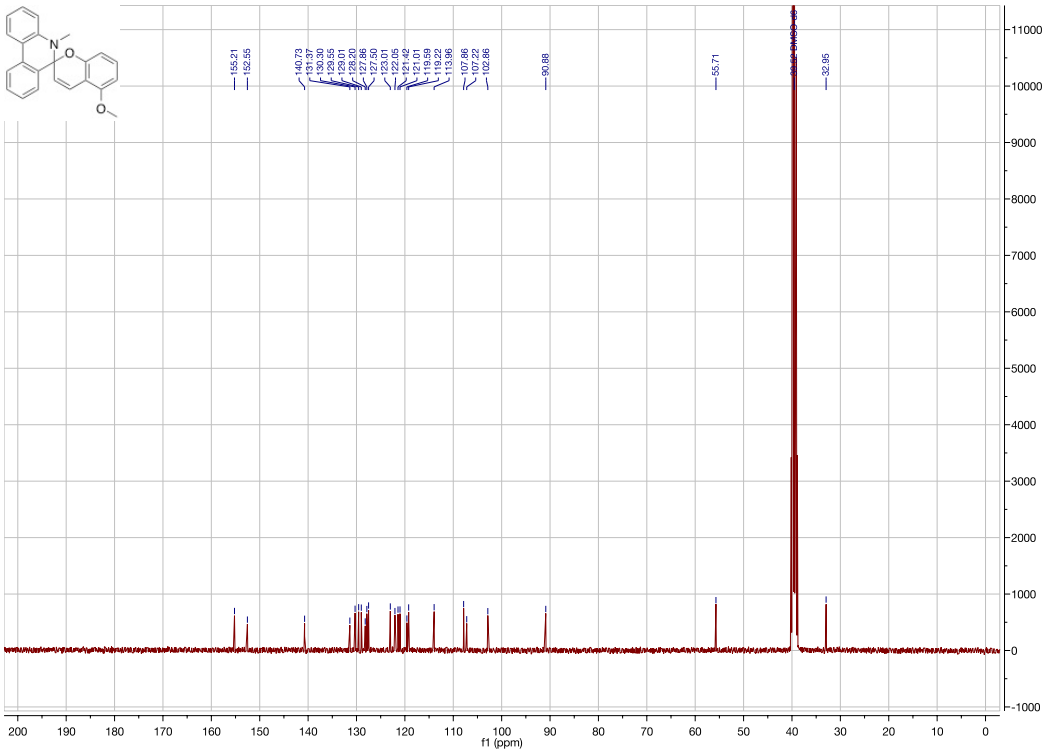
^{1}H NMR **9** in d6-DMSO



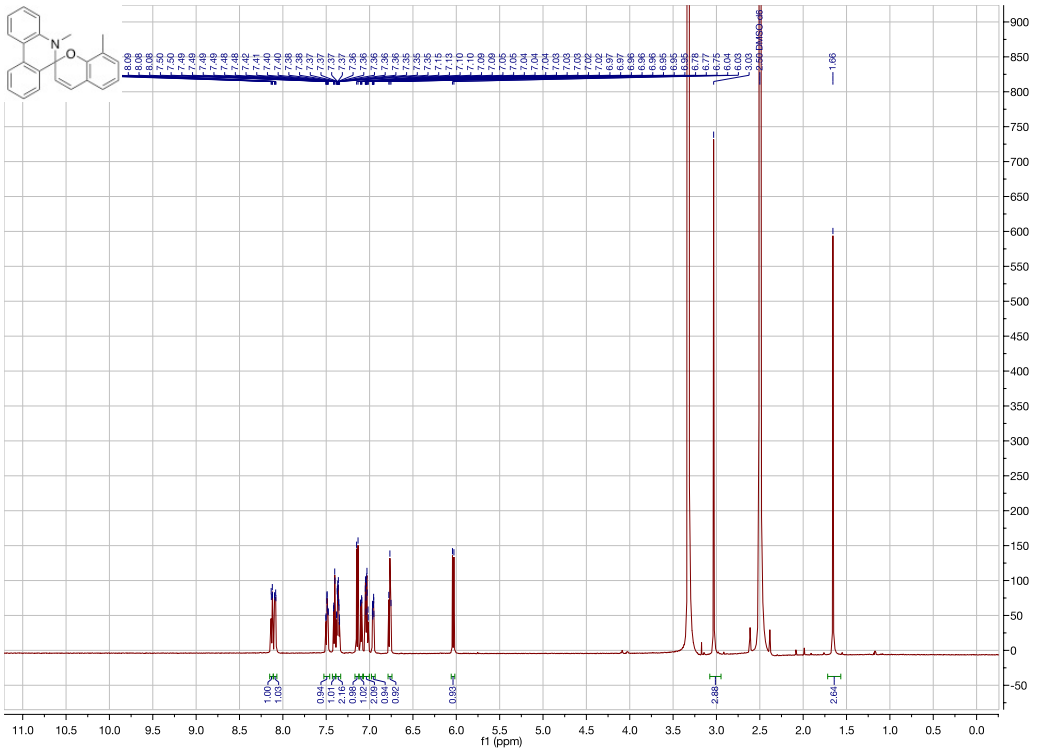
^{13}C NMR **9** in d6-DMSO



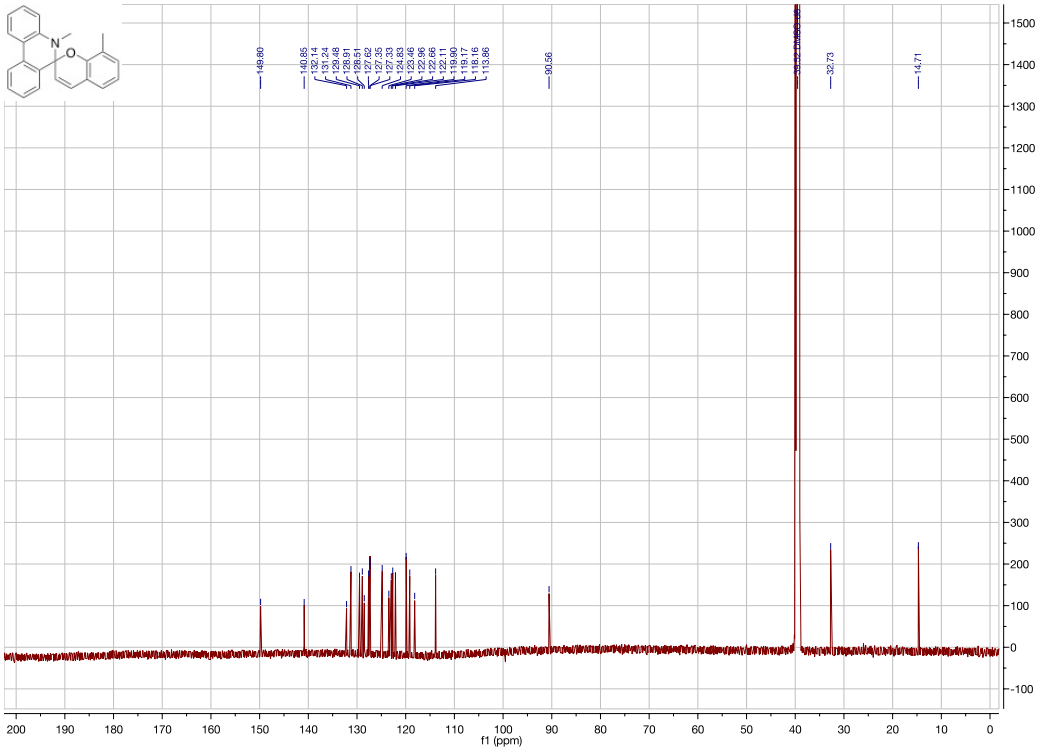
^{1}H NMR **10** in d6-DMSO



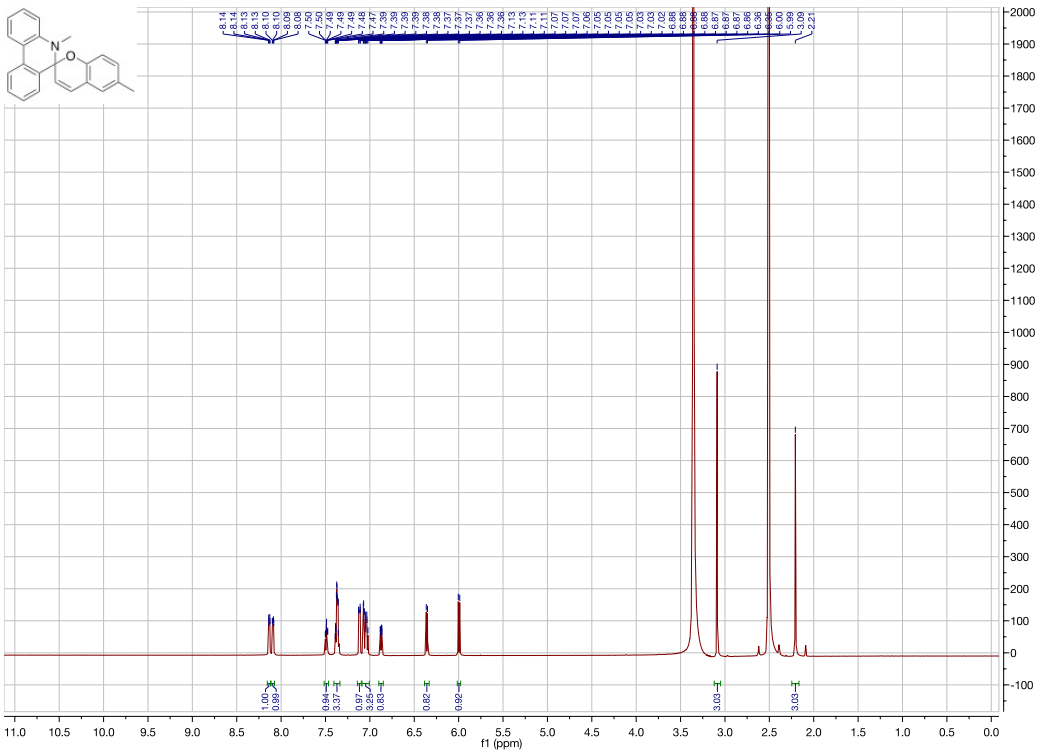
^{13}C NMR **10** in d6-DMSO



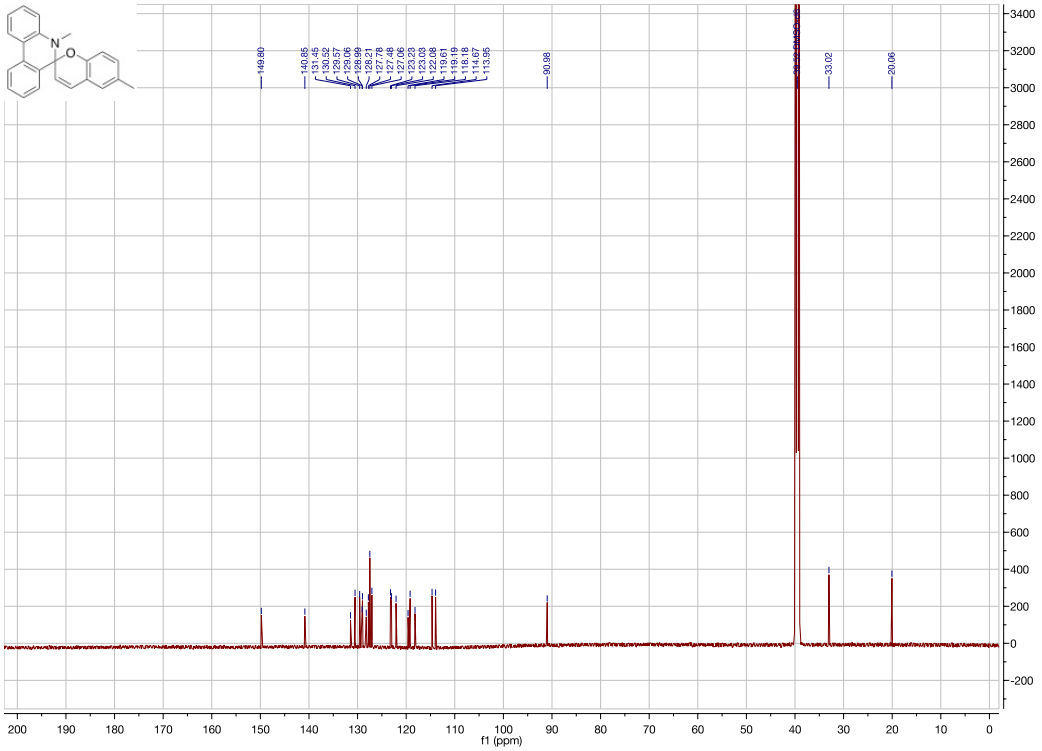
^{1}H NMR **11** in d6-DMSO



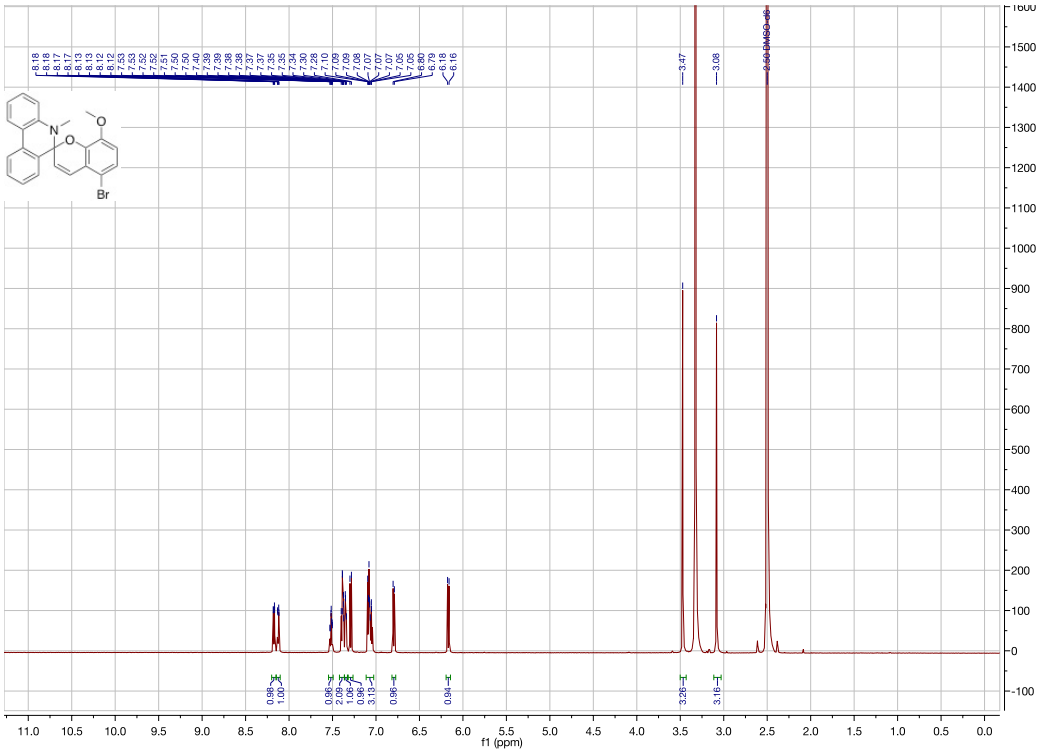
^{13}C NMR **11** in d6-DMSO



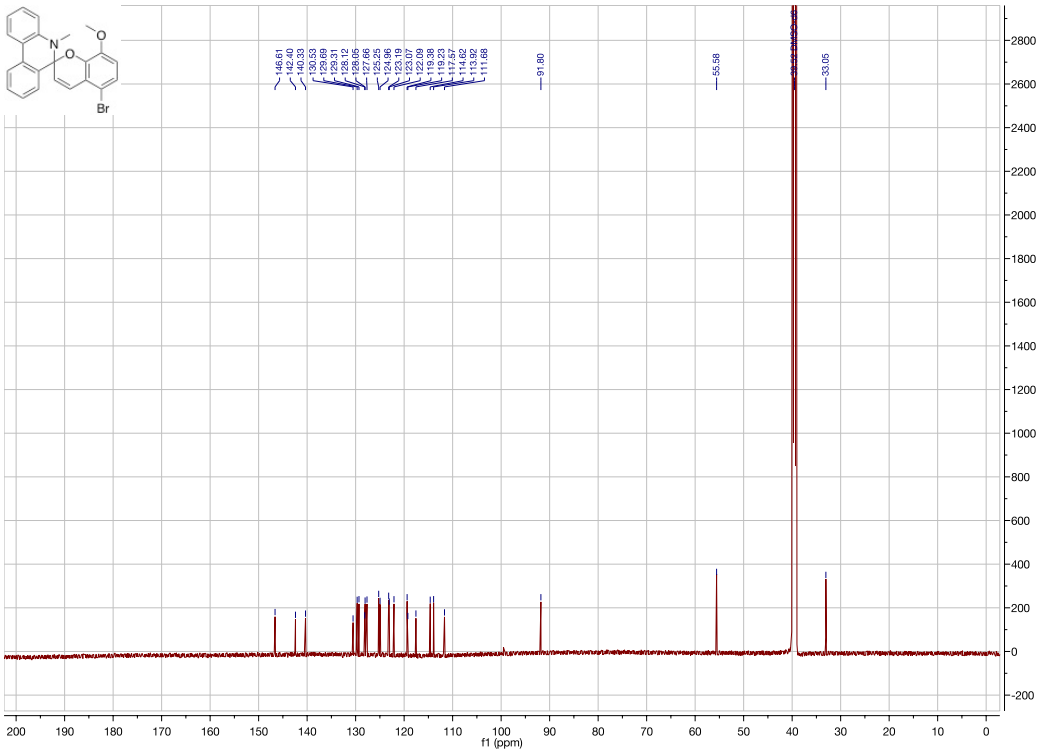
^{1}H NMR **12** in d6-DMSO



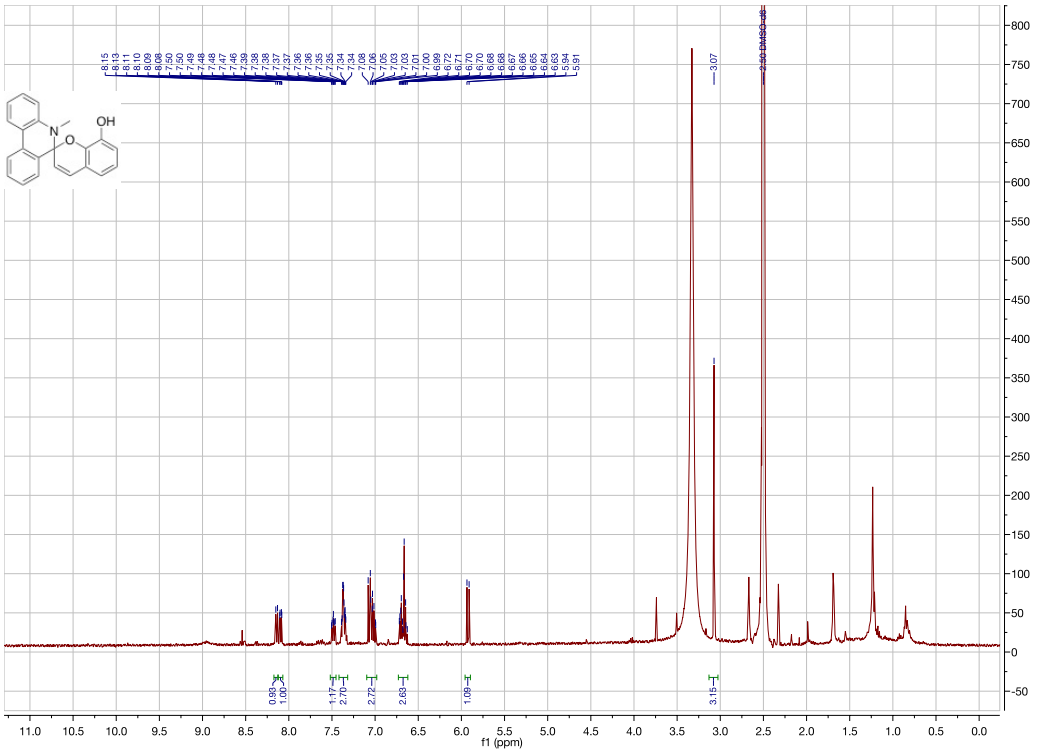
^{13}C NMR **12** in d6-DMSO



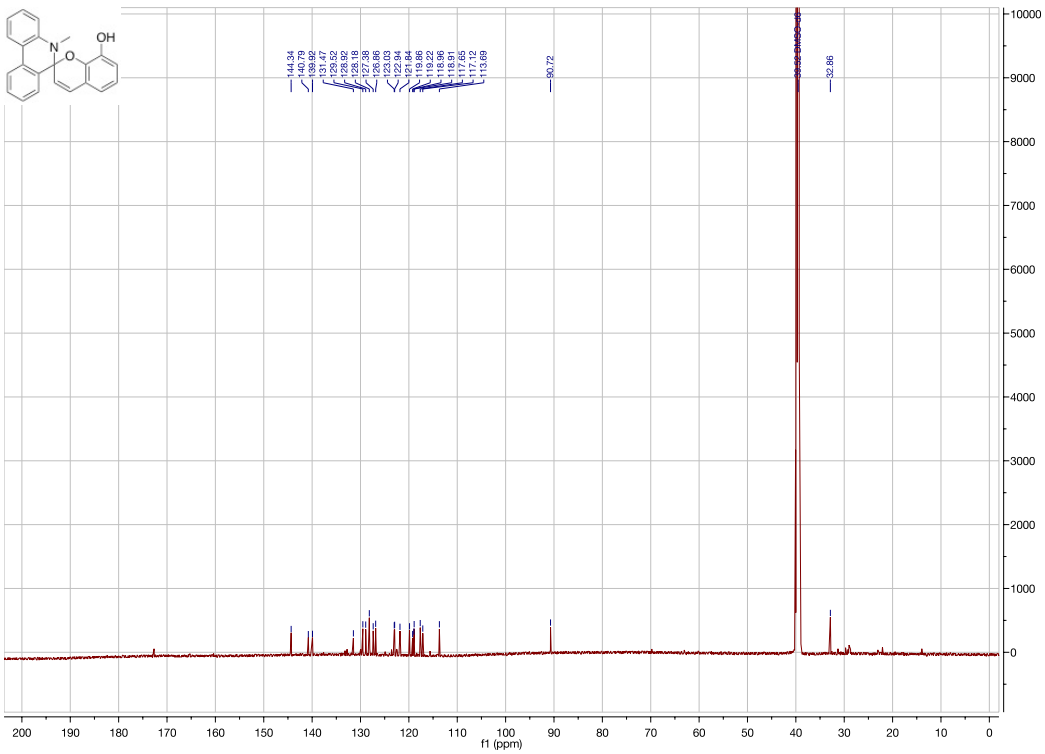
^{1}H NMR **13** in d6-DMSO



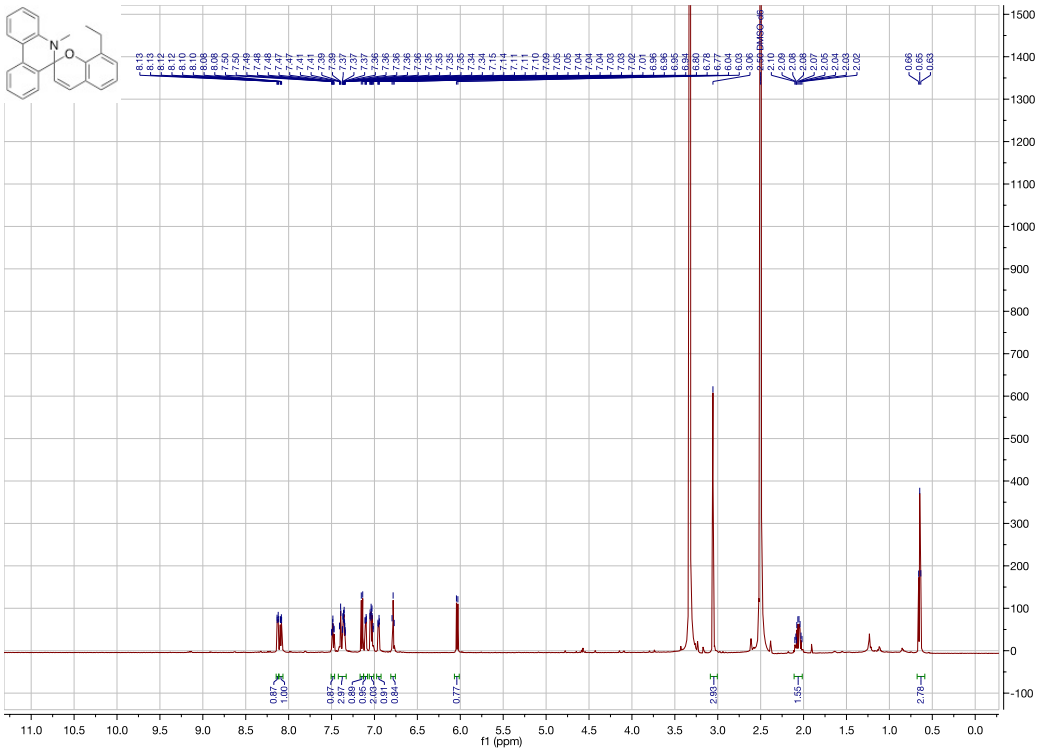
^{13}C NMR **13** in d6-DMSO



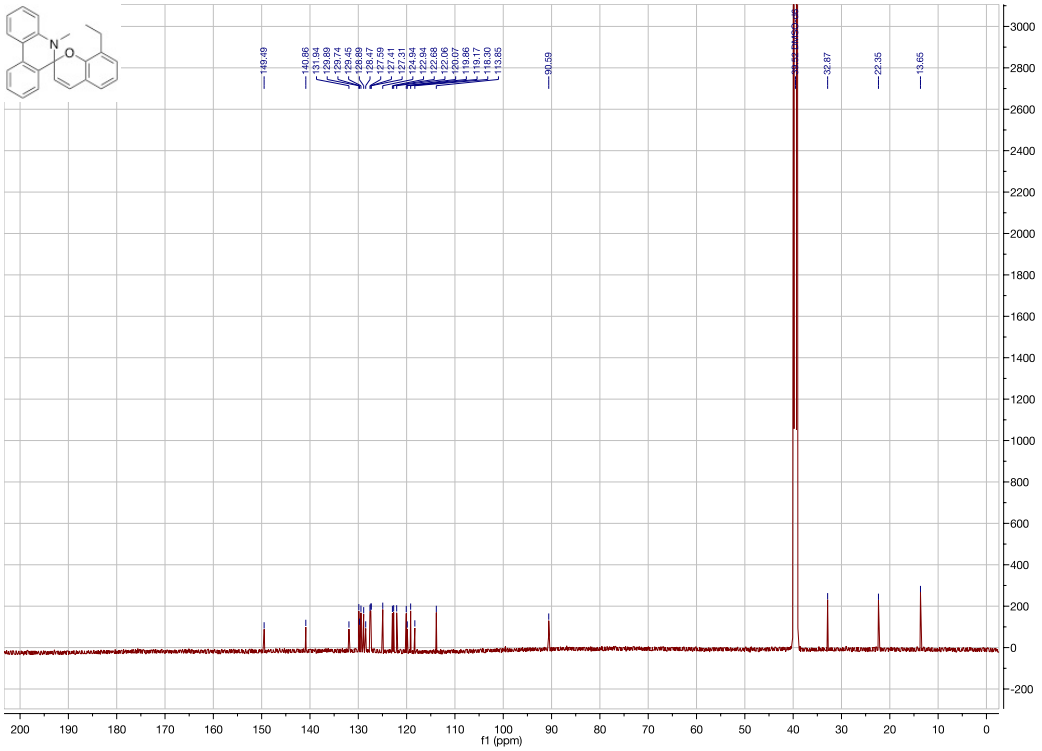
^{1}H NMR **14** in d6-DMSO. Trace amounts of ring-opened merocyanine form present.



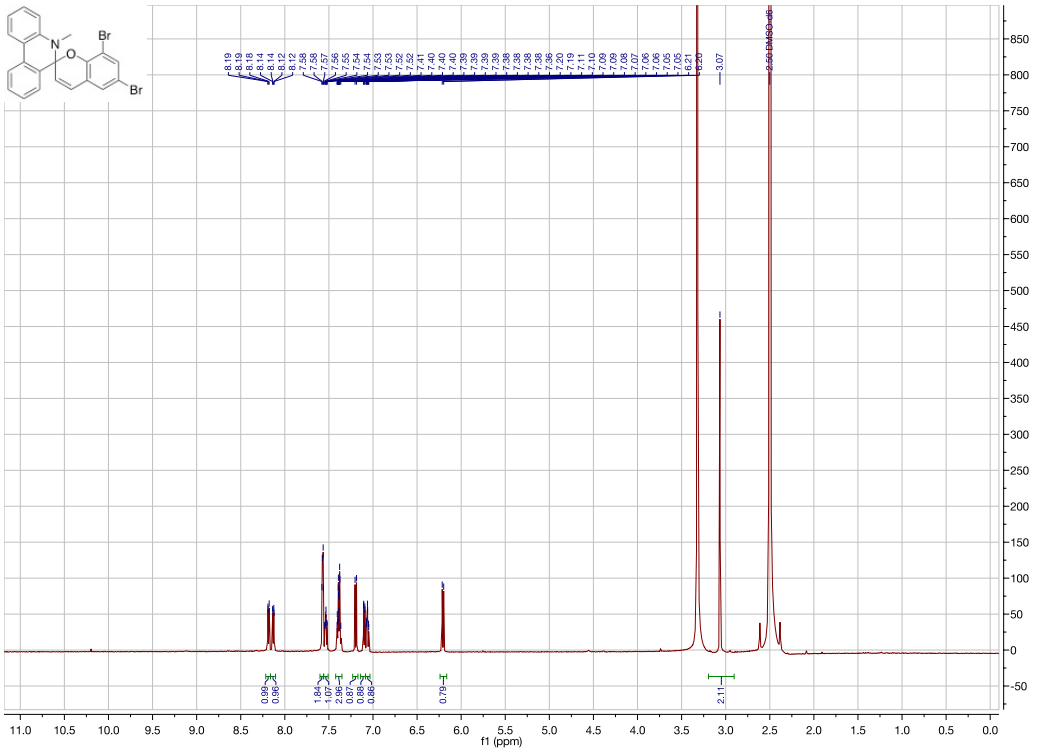
^{13}C NMR **14** in d6-DMSO



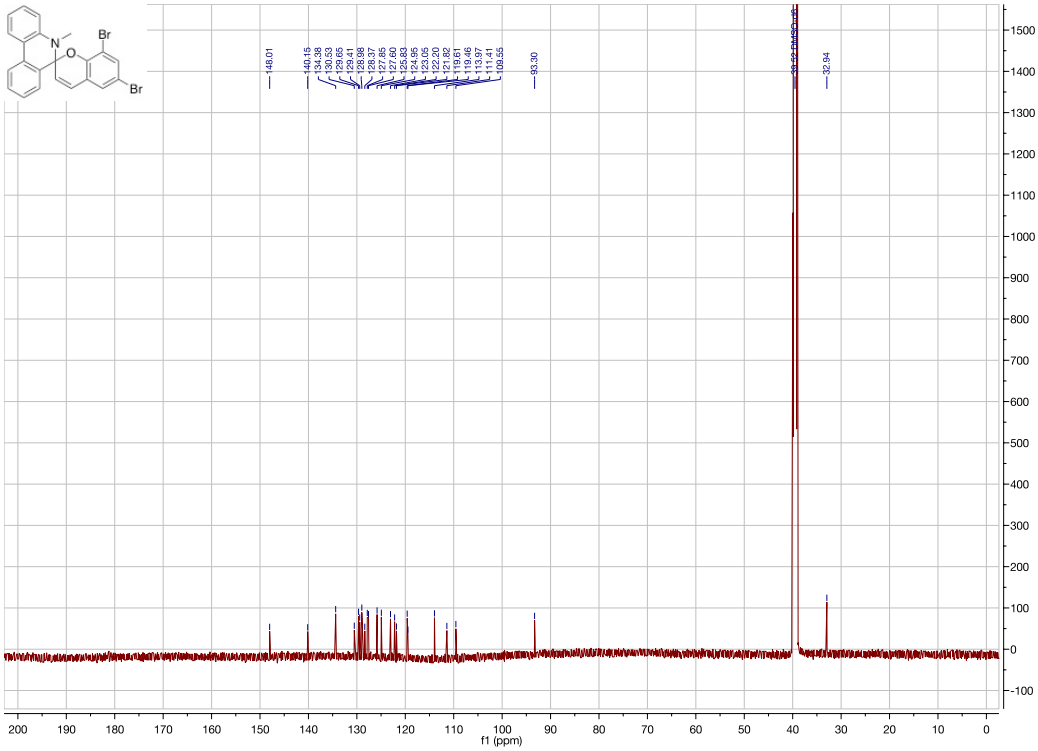
^{1}H NMR **15** in d6-DMSO



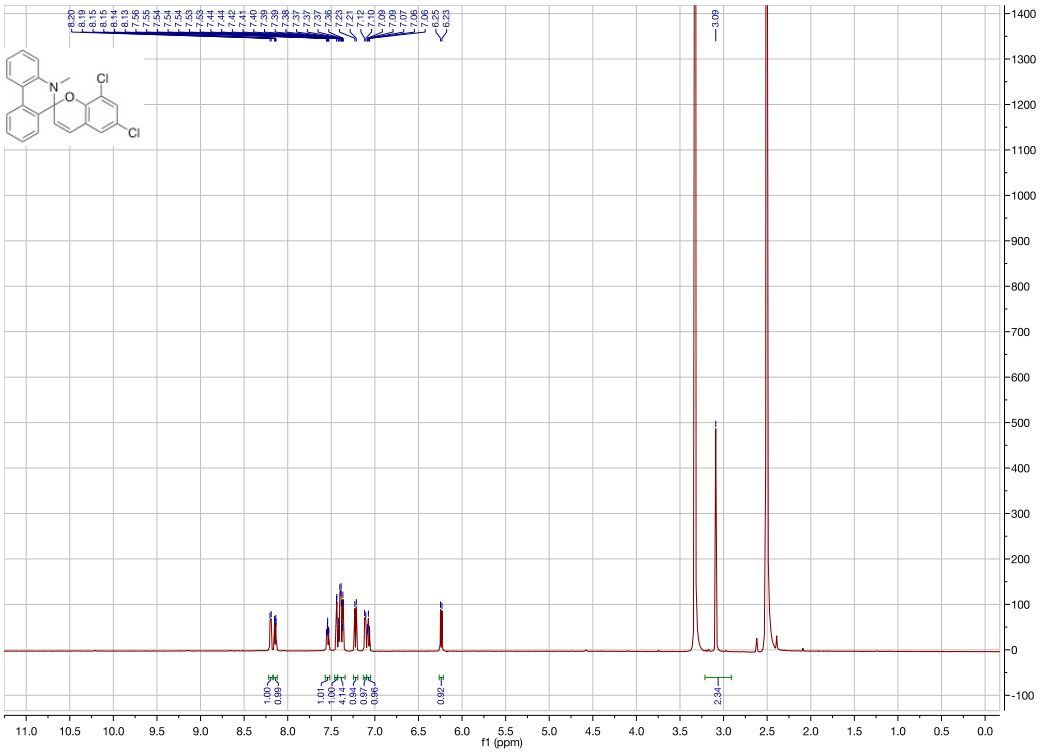
^{13}C NMR **15** in d6-DMSO



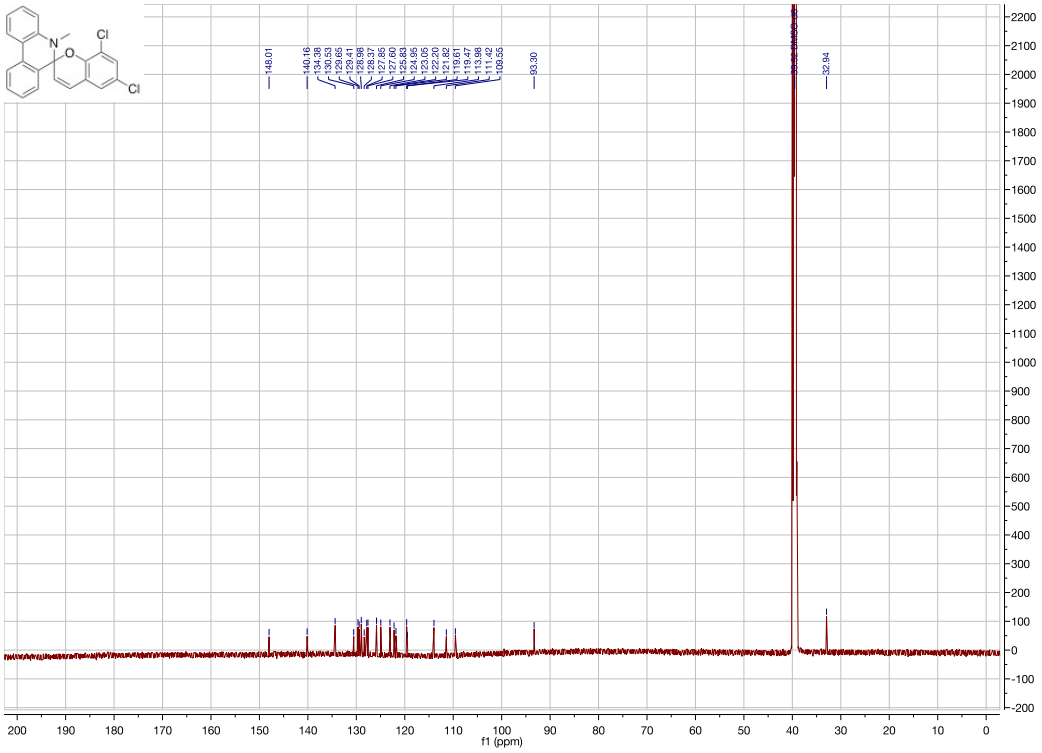
^{1}H NMR **16** in d6-DMSO



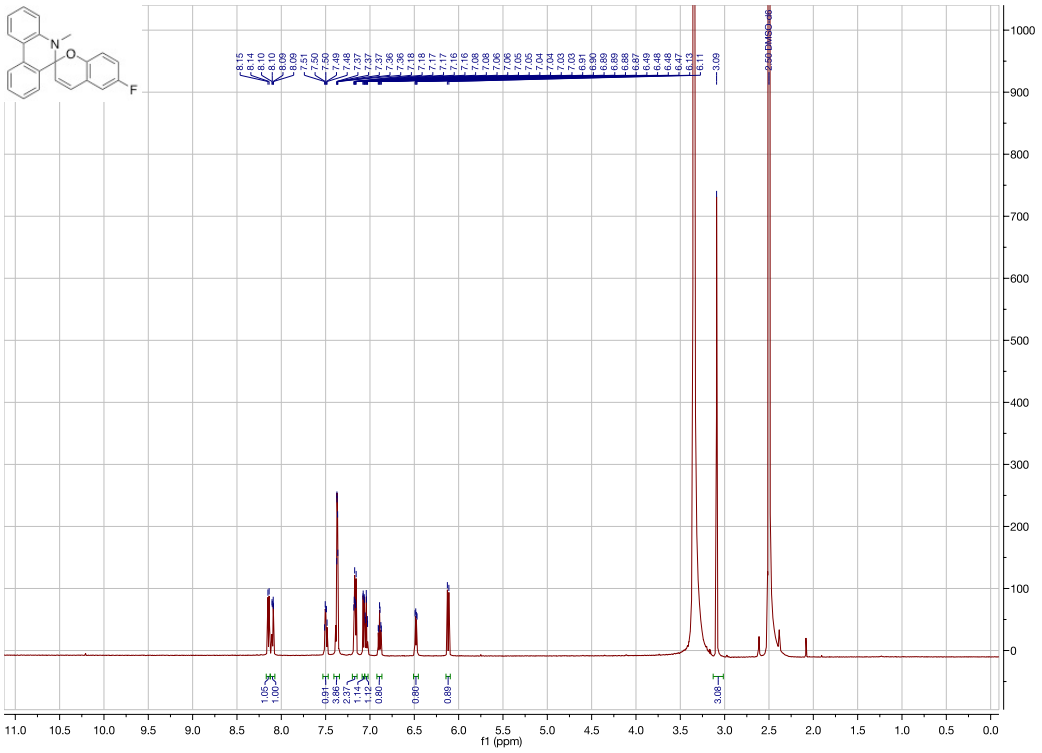
^{13}C NMR **16** in d6-DMSO



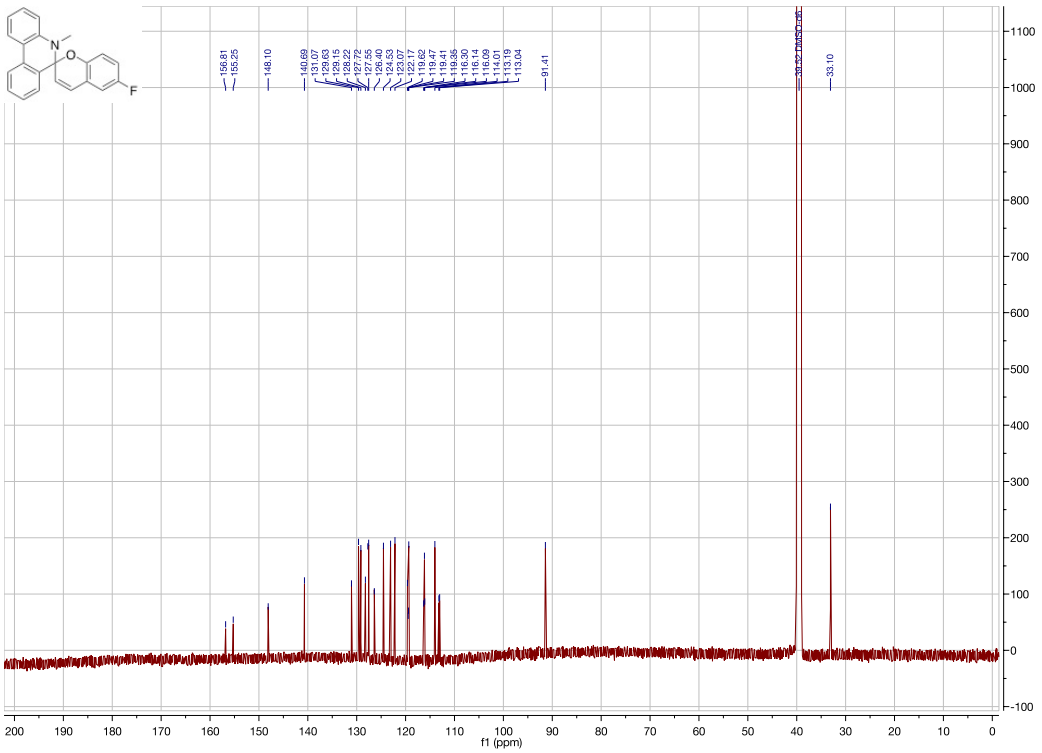
^{1}H NMR **17** in d6-DMSO



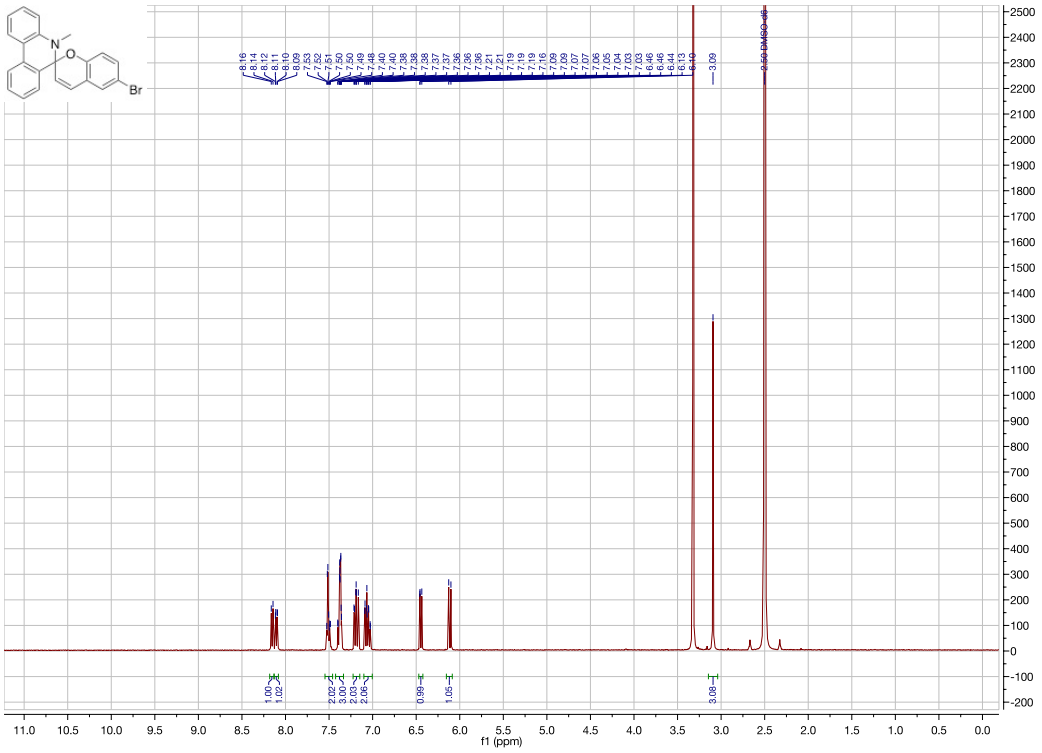
^{13}C NMR **17** in d6-DMSO



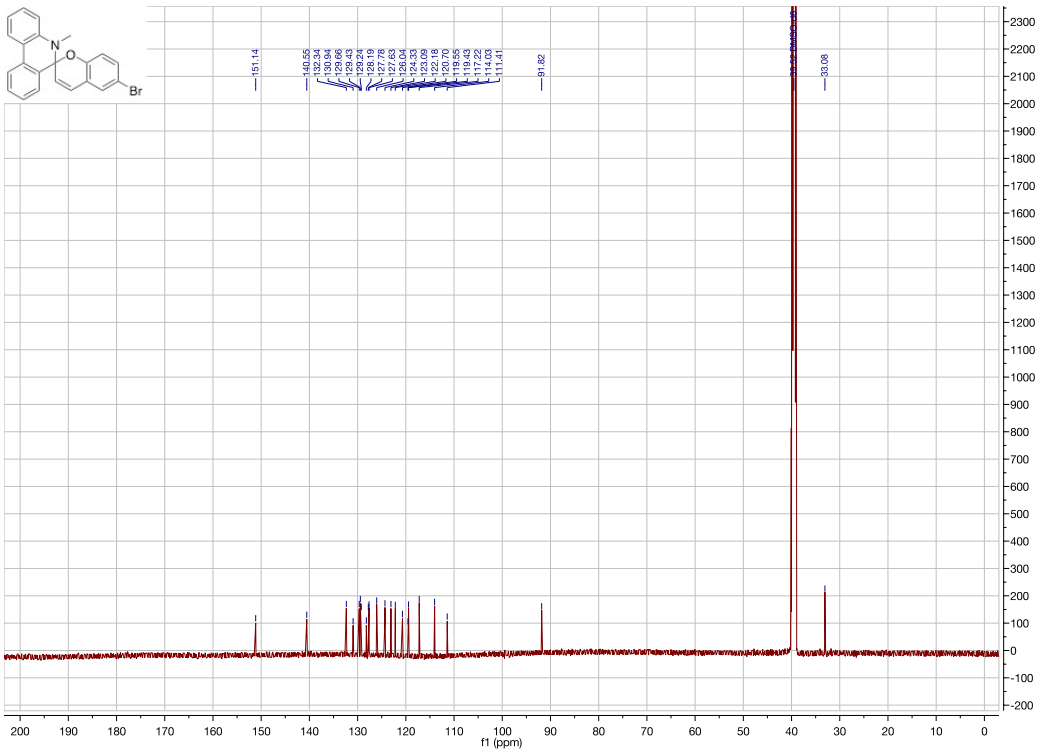
^{1}H NMR **18** in d6-DMSO



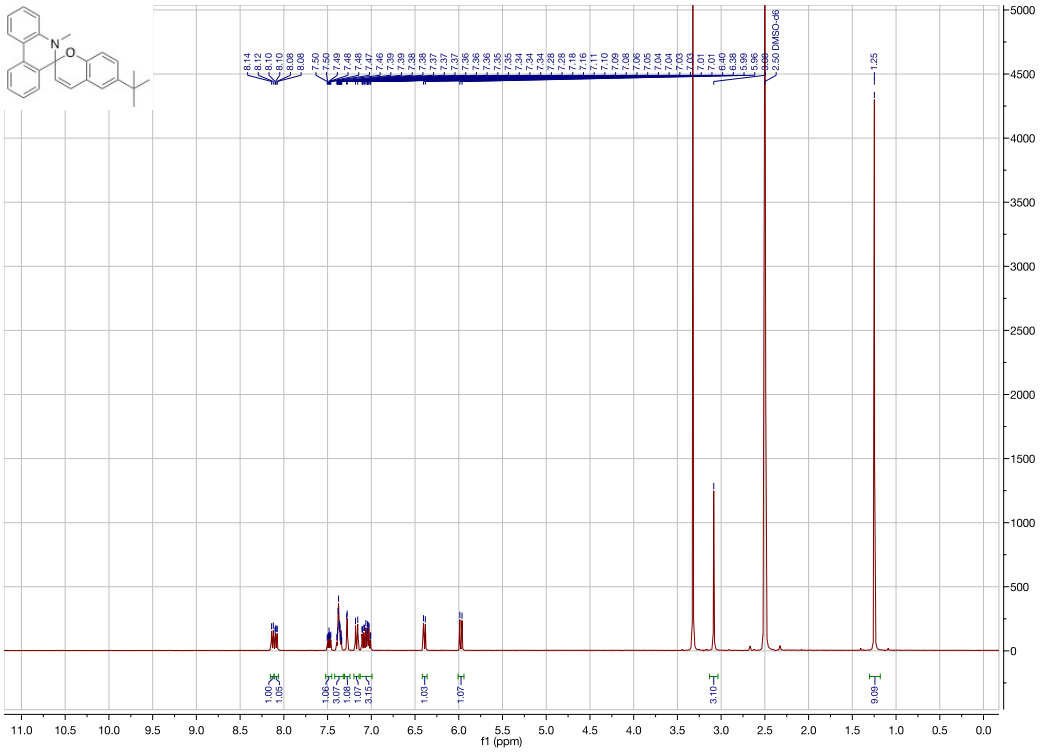
^{13}C NMR **18** in d6-DMSO



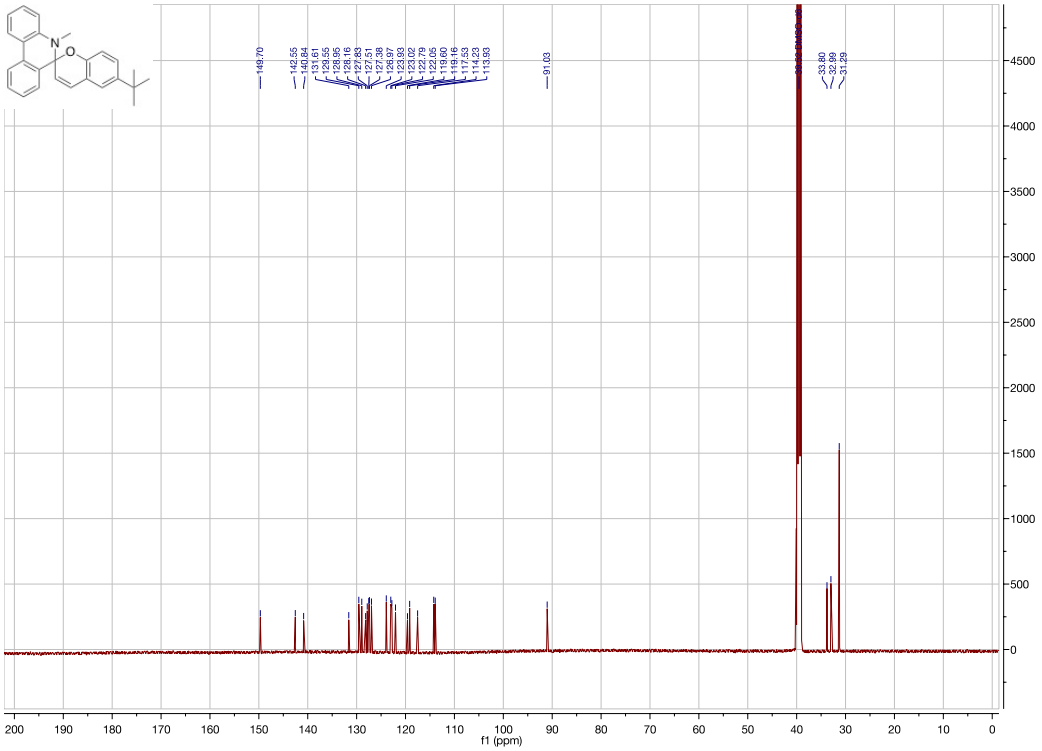
^{1}H NMR **19** in d6-DMSO



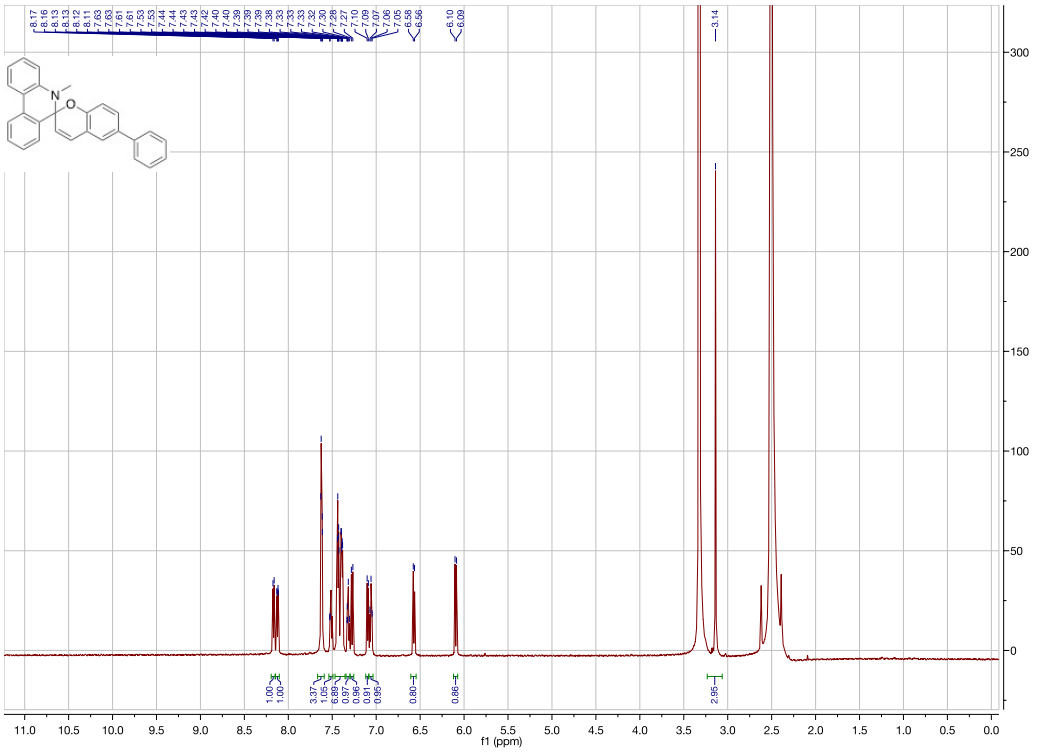
^{13}C NMR **19** in d6-DMSO



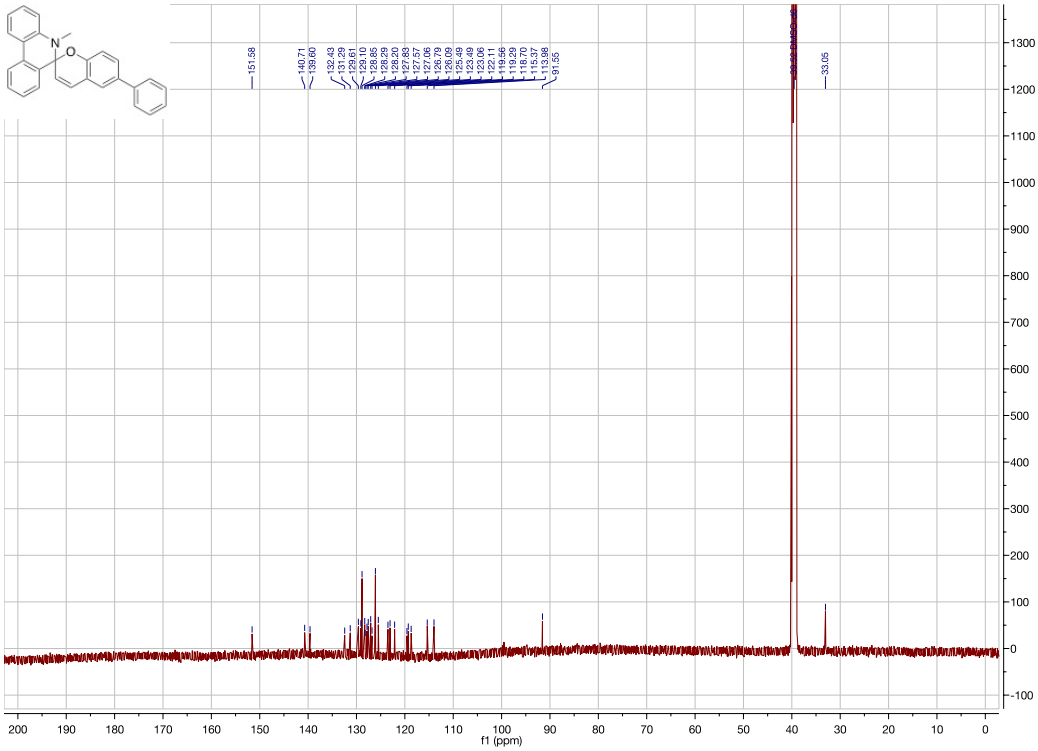
^{1}H NMR **20** in d6-DMSO



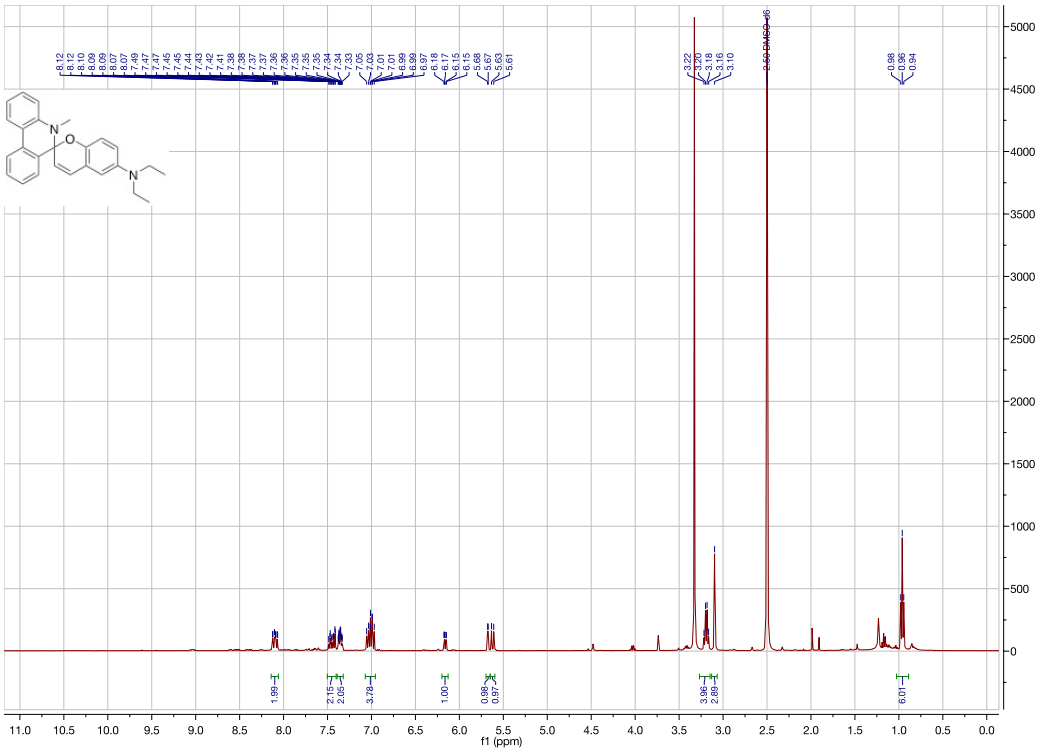
^{13}C NMR **20** in d6-DMSO



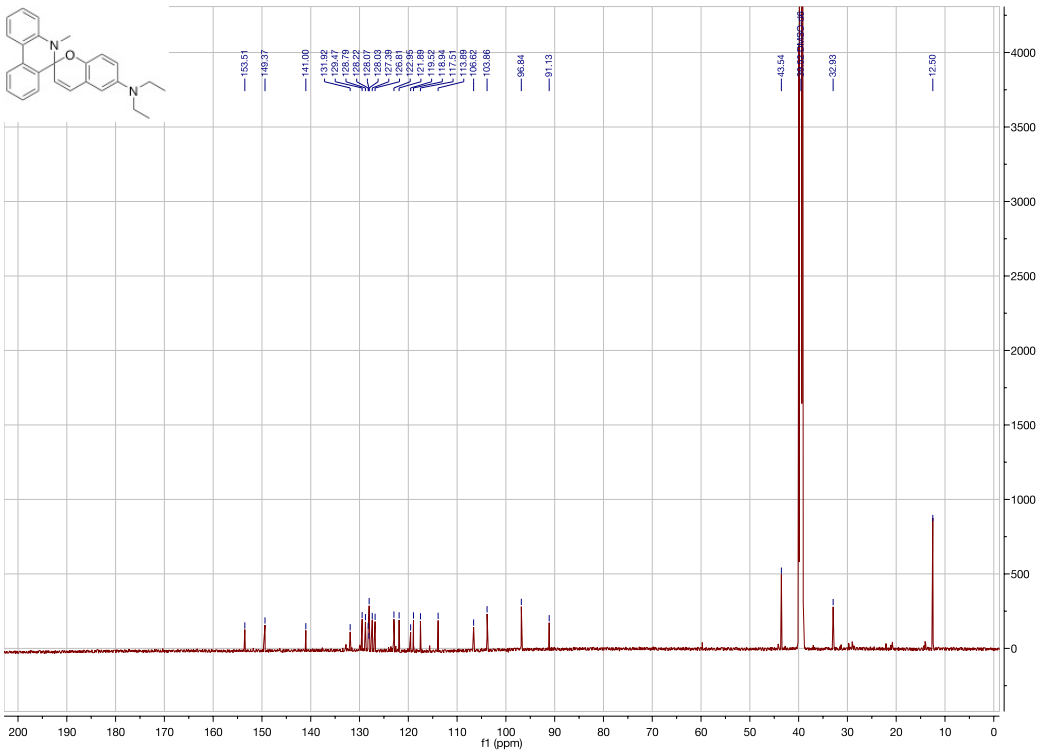
^{1}H NMR **21** in d6-DMSO



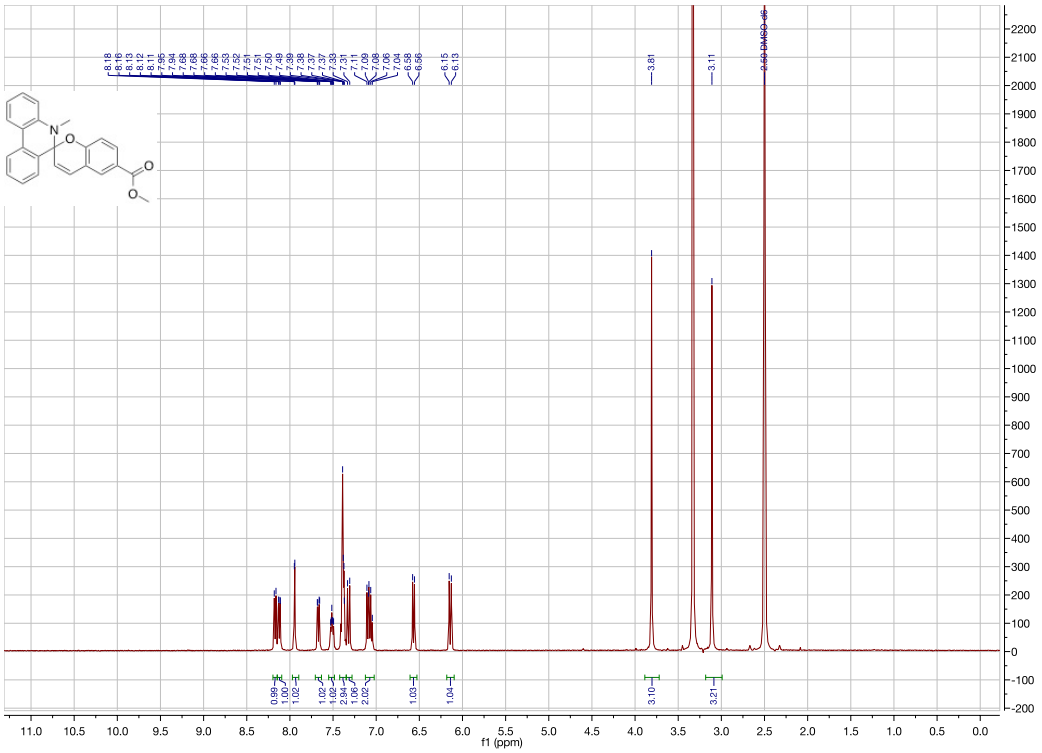
^{13}C NMR **21** in d6-DMSO



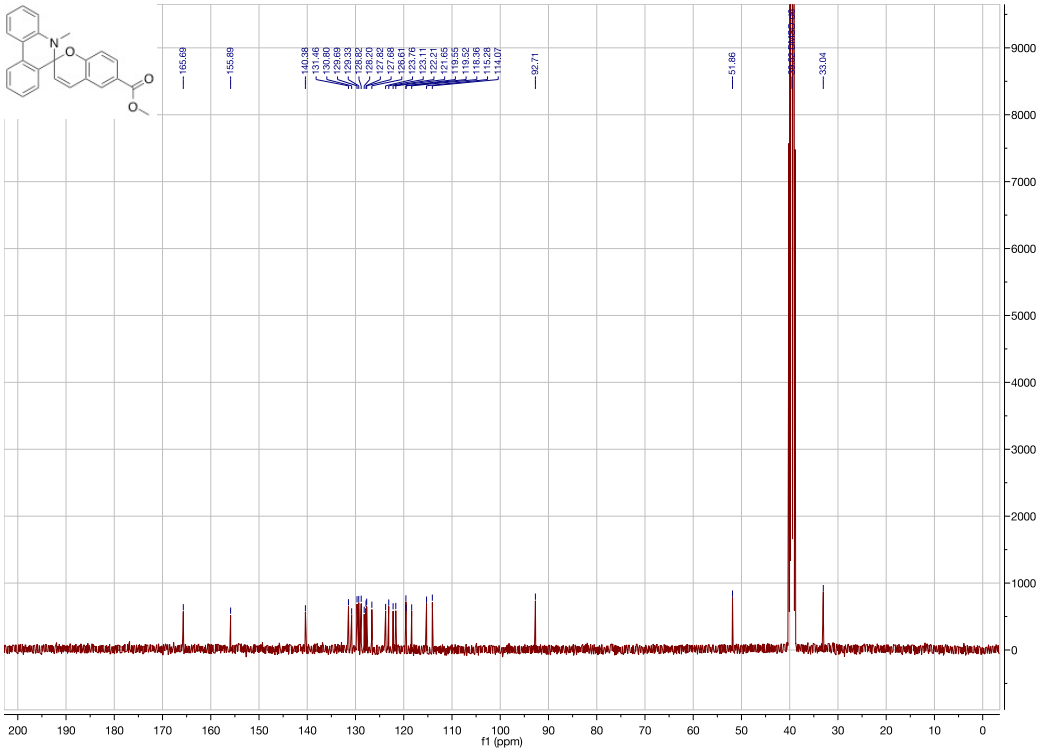
^{1}H NMR **22** in d6-DMSO. Trace amounts of ring-opened merocyanine form present.



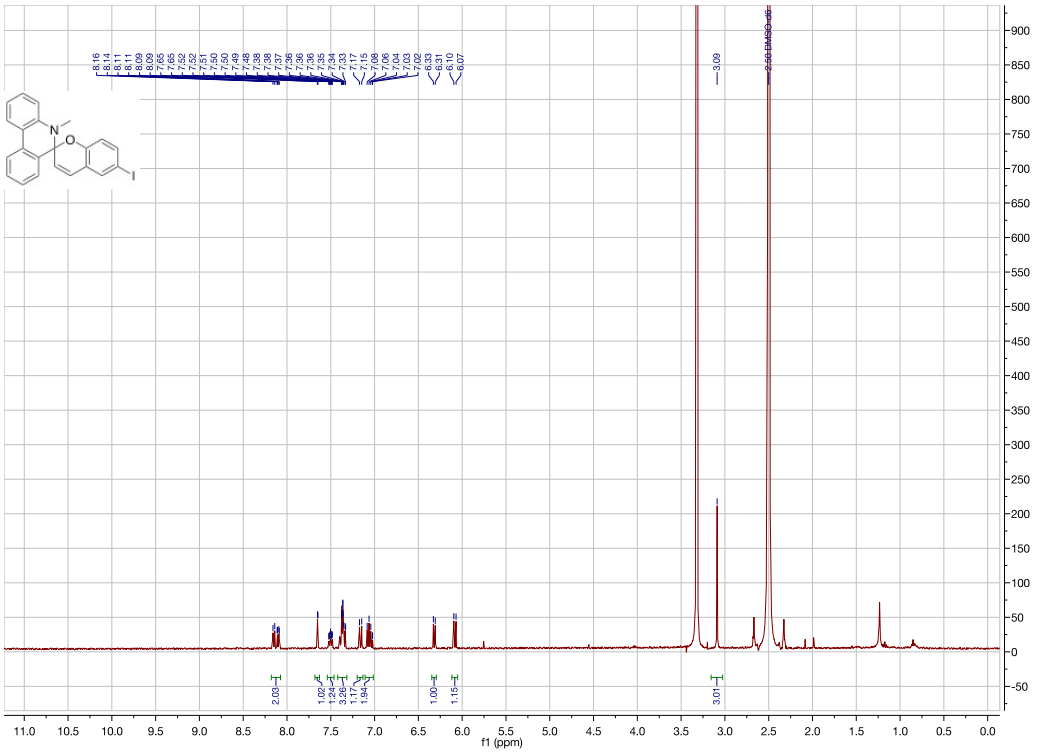
^{13}C NMR **22** in d6-DMSO



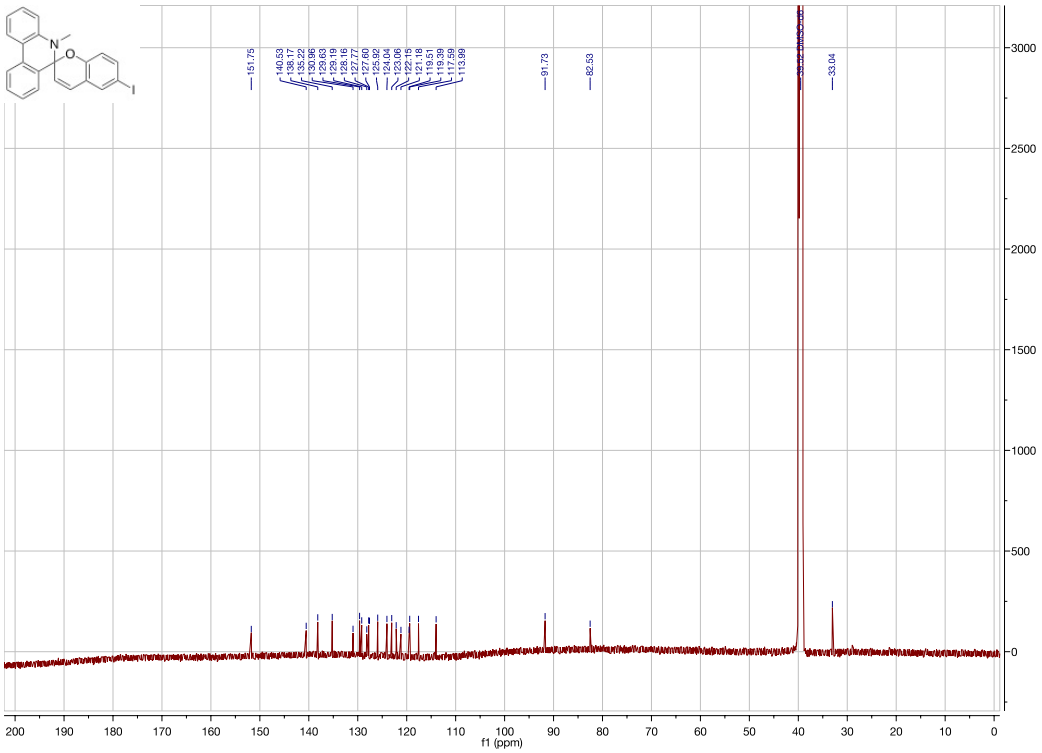
^{1}H NMR **23** in d6-DMSO



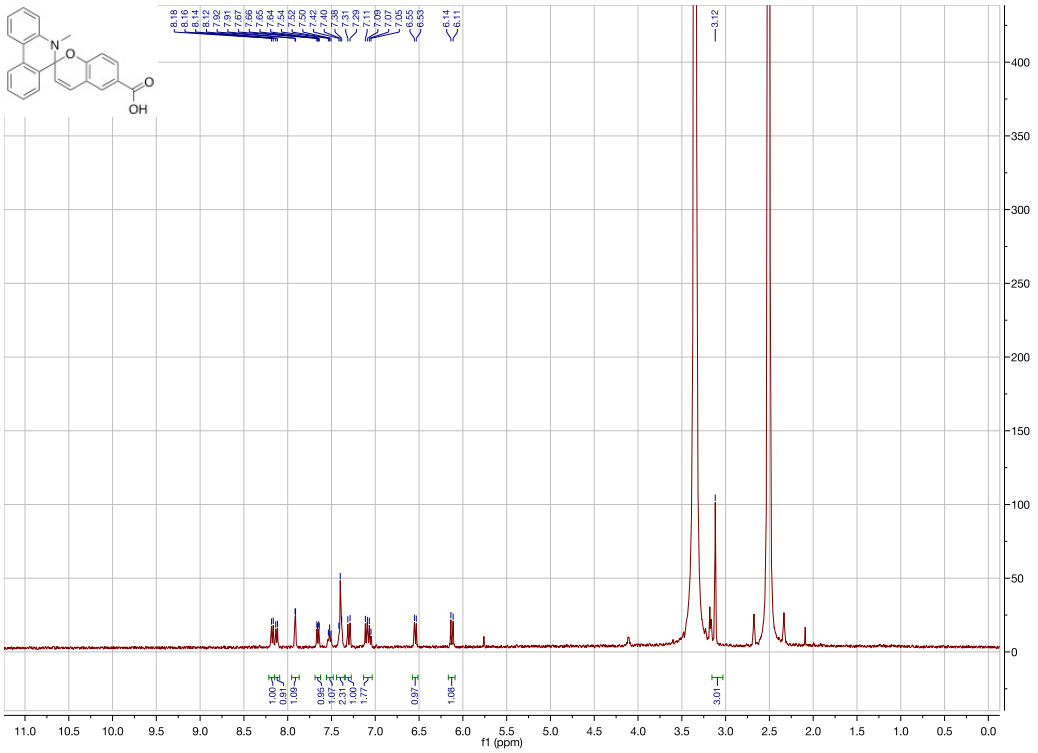
^{13}C NMR **23** in d6-DMSO



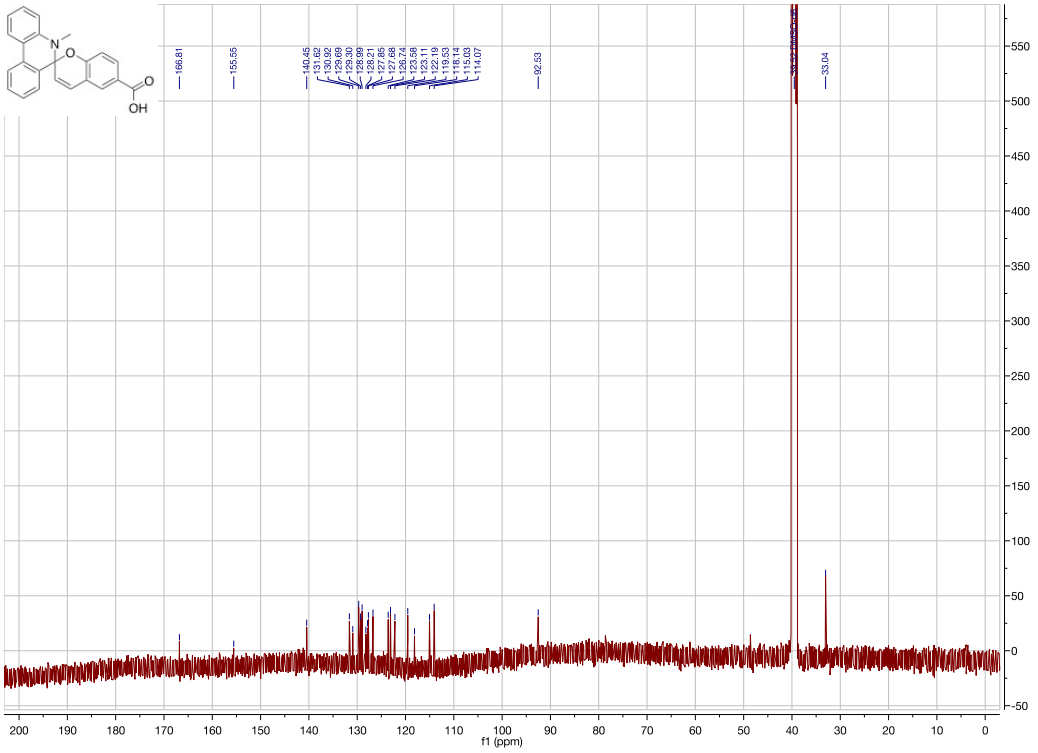
^{1}H NMR **24** in d6-DMSO



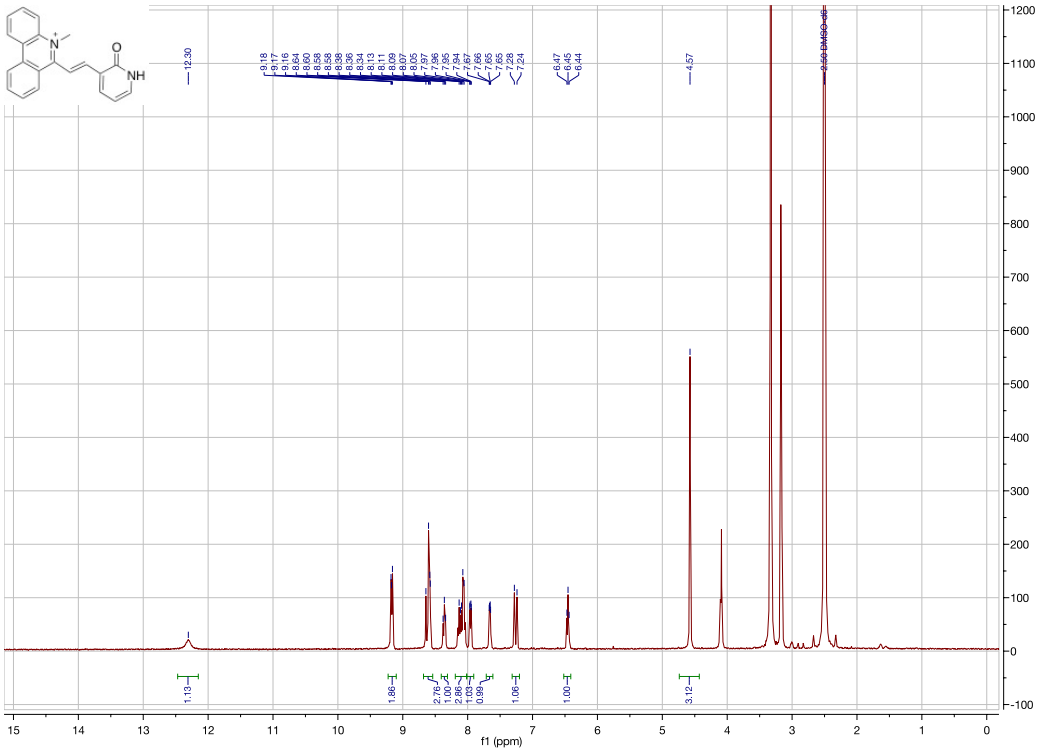
^{13}C NMR **24** in d6-DMSO



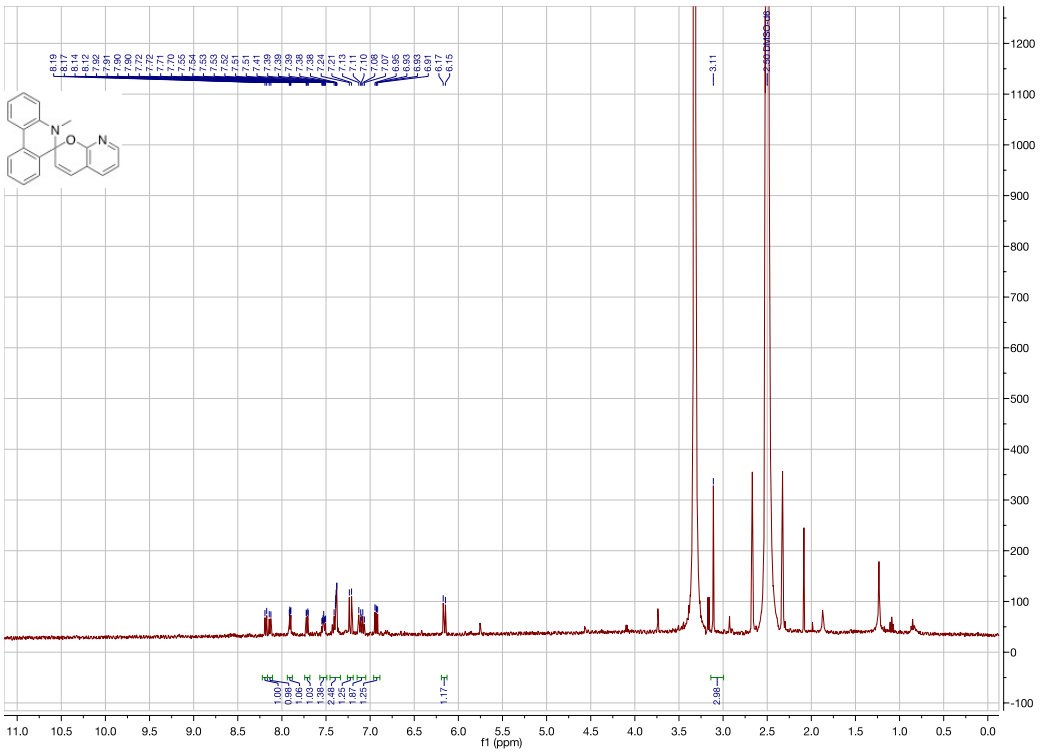
^{1}H NMR **25** in d6-DMSO



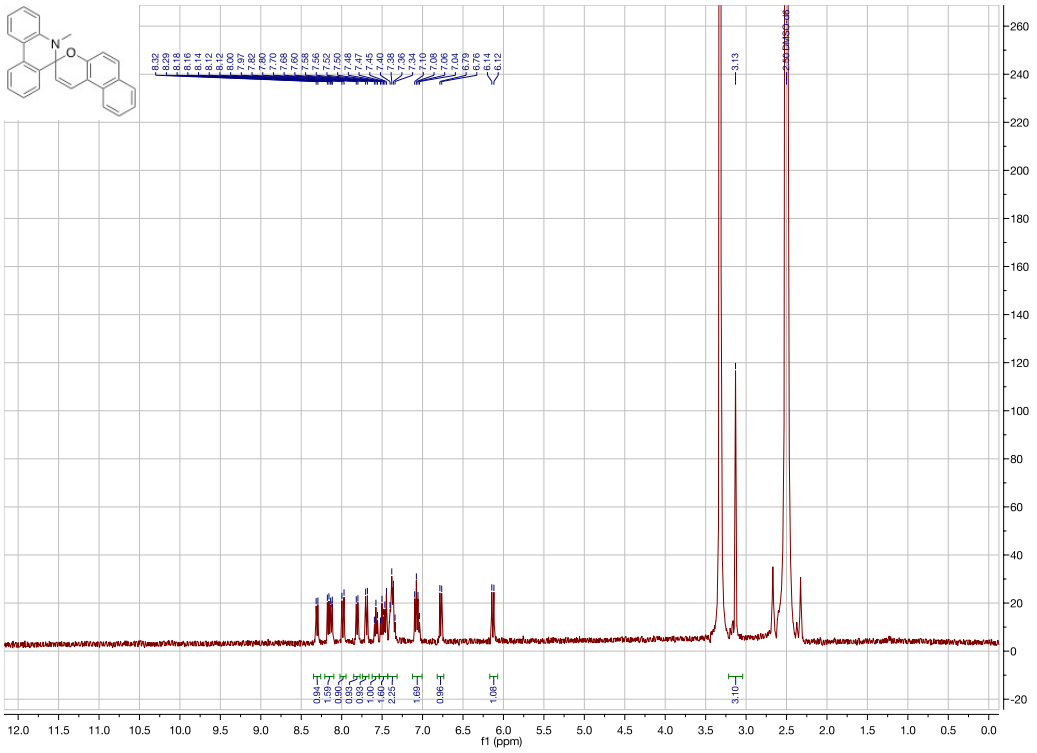
^{13}C NMR **25** in d6-DMSO



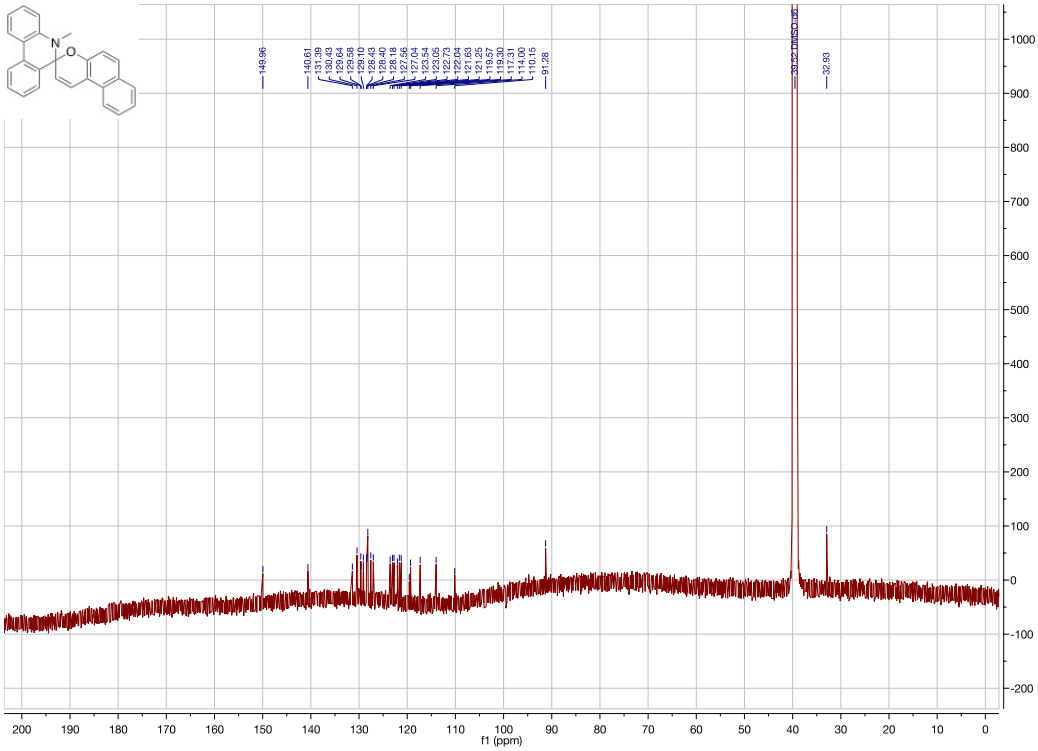
^{1}H NMR **26-merocyanine** in d6-DMSO



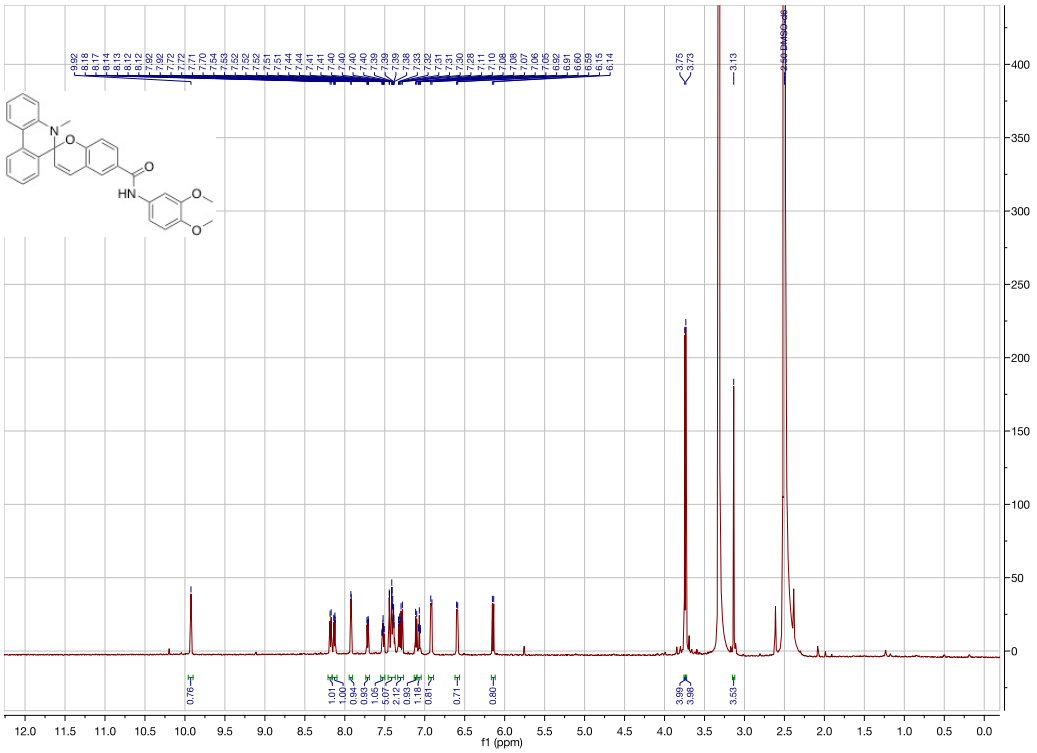
^{1}H NMR **26** in d6-DMSO



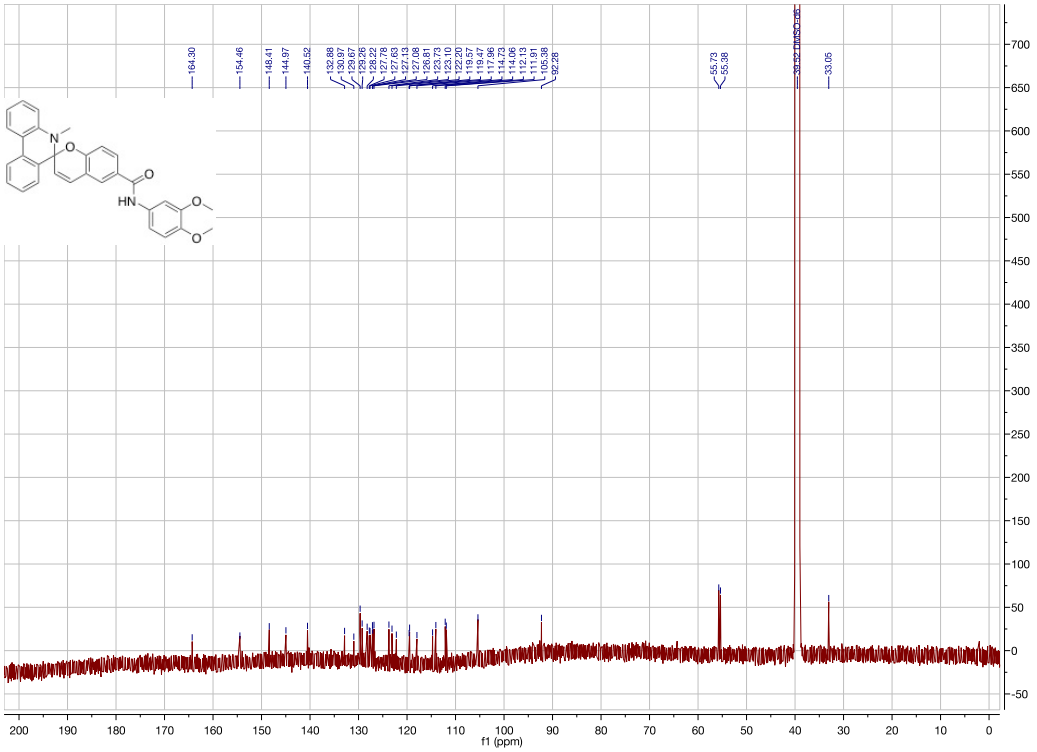
^{1}H NMR **27** in d6-DMSO



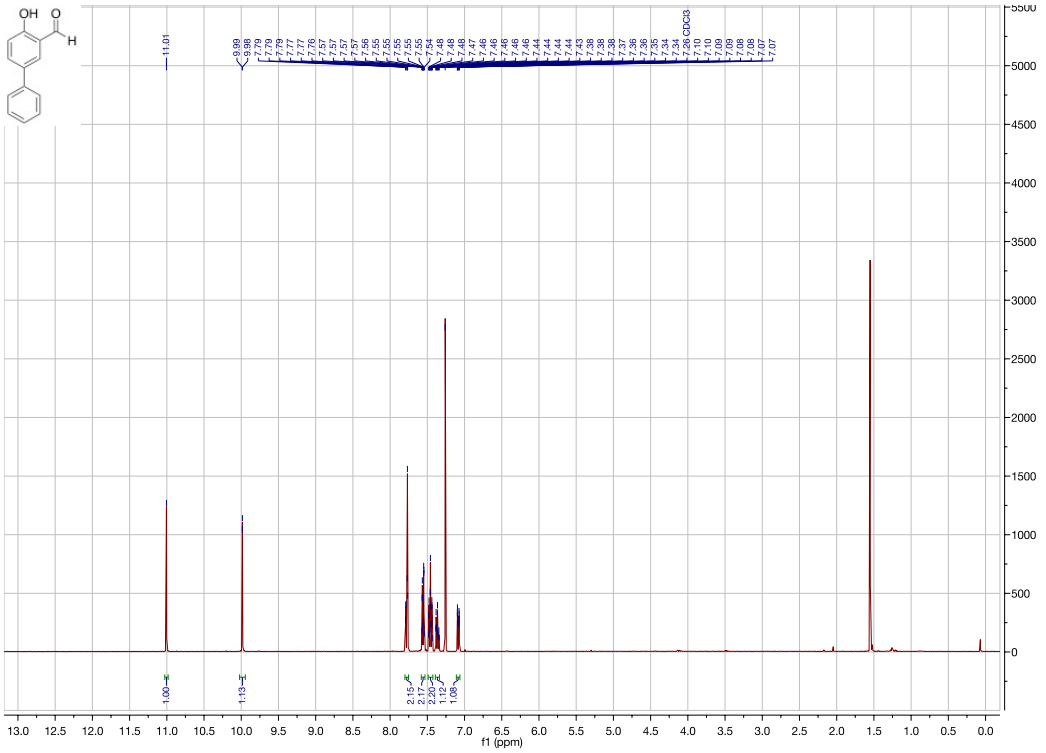
^{13}C NMR **27** in d6-DMSO



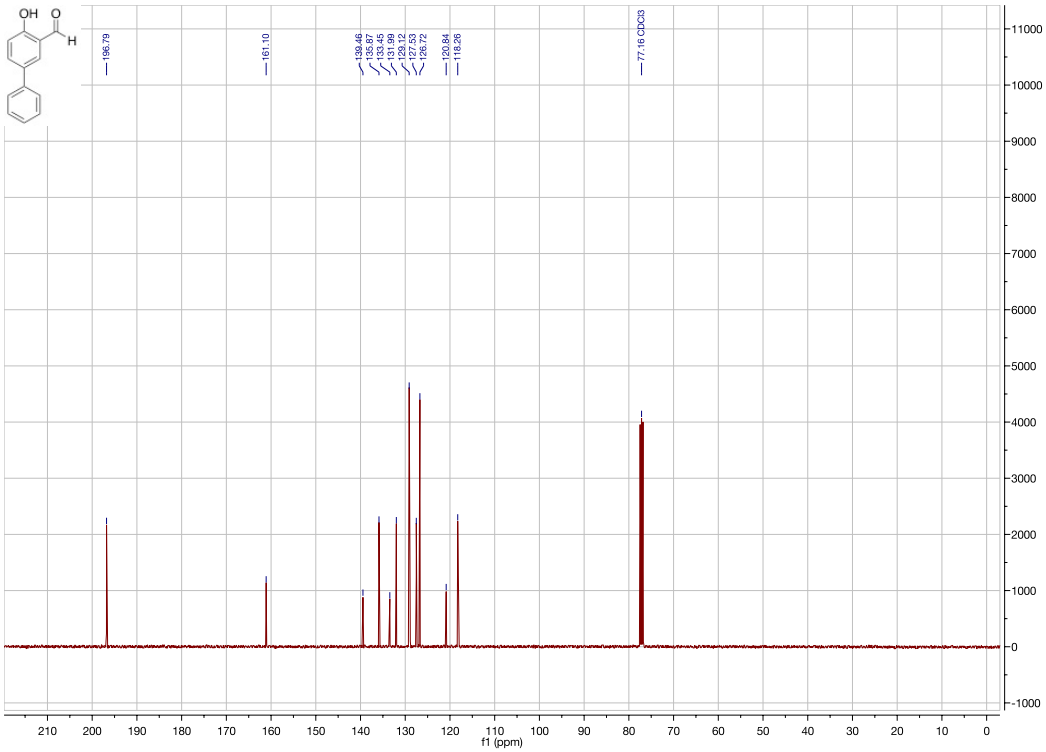
^{1}H NMR **28** in d6-DMSO



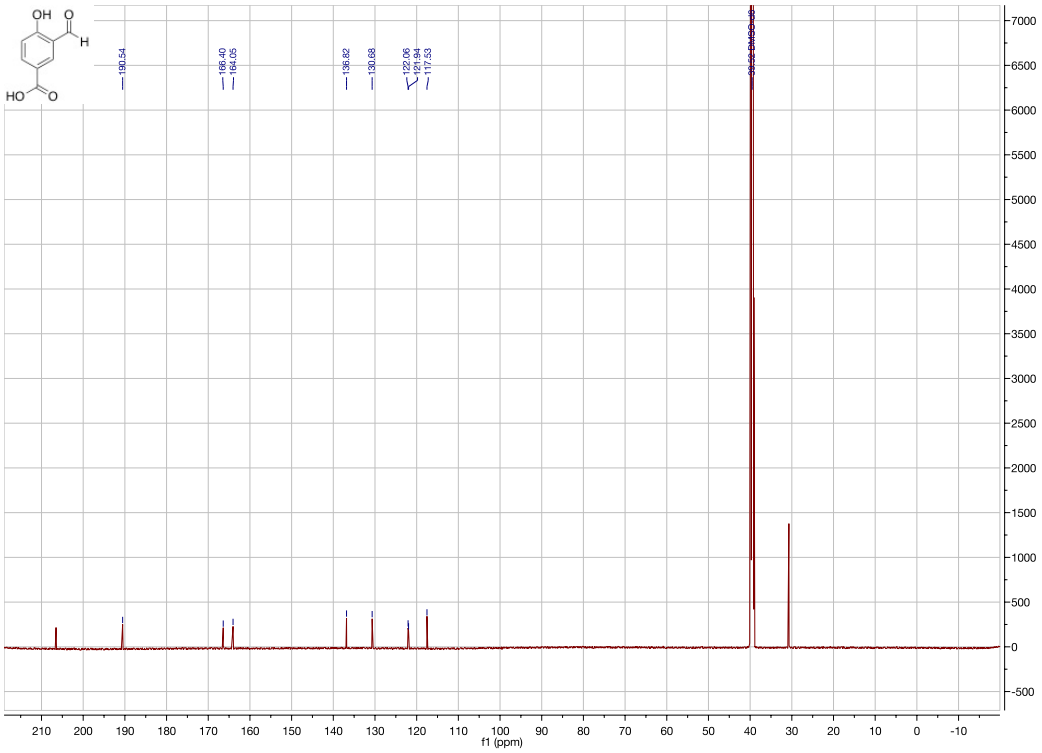
^{13}C NMR **28** in d6-DMSO



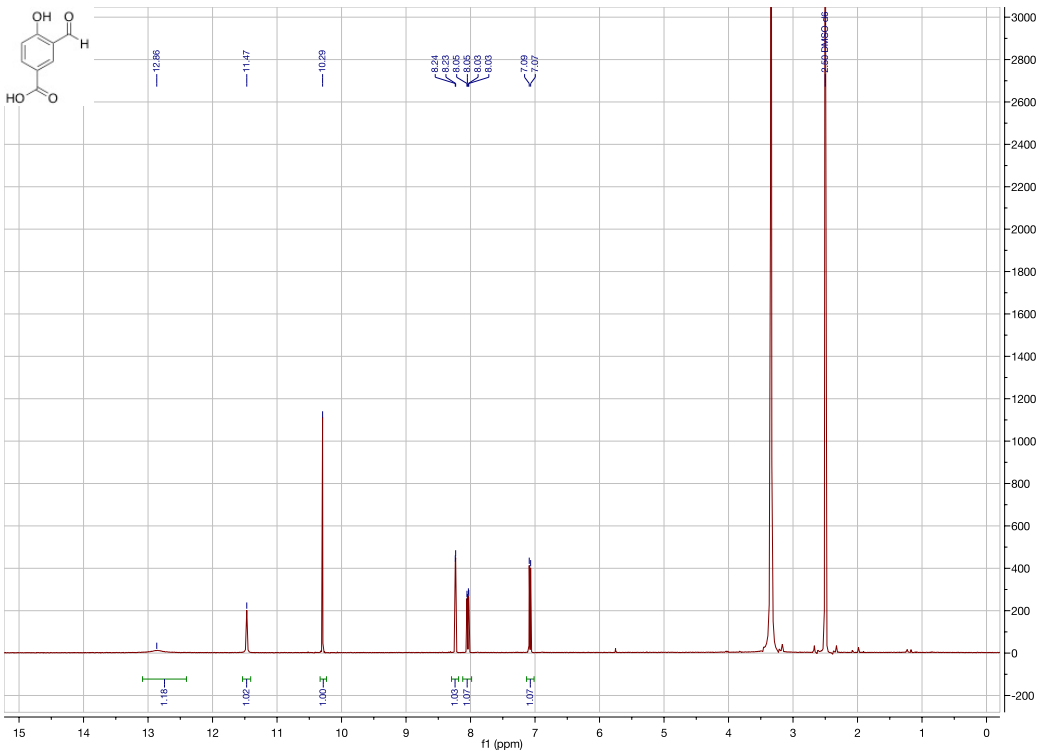
^{1}H NMR **29** in CDCl3



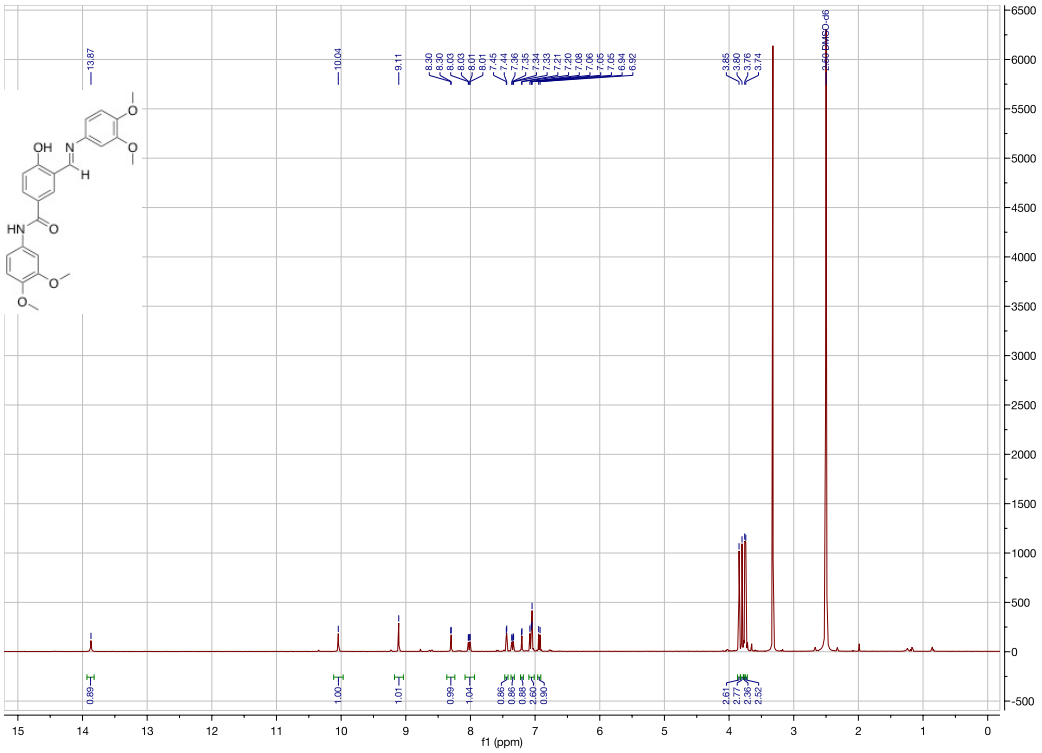
^{13}C NMR **29** in CDCl3



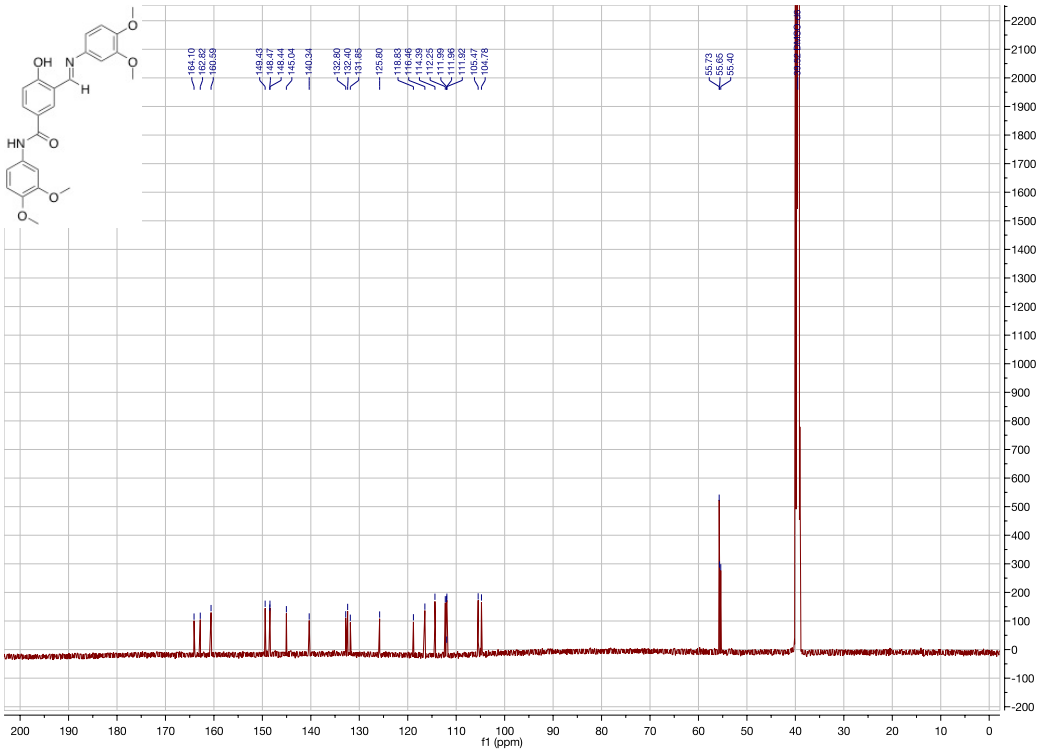
^{1}H NMR **30** in d6-DMSO



^{13}C NMR **30** in d6-DMSO



^{1}H NMR **31** in d6-DMSO



^{13}C NMR **31** in d6-DMSO

[1] M. Zaidlewicz, A. Tafelska-Kaczmarek, A. Prewysz-Kwinto, A. Chechlowska, *Tetrahedron: Assymmetry* **2003**, *14*, 1659-1664.