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Synthetic Prodigiosenes and the Influence of C-Ring Substitution on DNA Cleavage, Transmembrane Chloride Transport and Compound Basicity

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Supporting Information

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1) Synthetic procedures and characterization data

General procedures

All chemicals were purchased and used as received unless otherwise indicated. Moisture sensitive reactions were performed in flame-dried glassware under a positive pressure of nitrogen or argon. Air- and moisture-sensitive compounds were introduced via syringe or cannula through a rubber septum. Flash chromatography was performed using Silicycle ultra pure silica (230-400 mm) or 150 mesh Brockmann III activated neutral alumina oxide as indicated. The NMR spectra were recorded using a 500 MHz spectrometer instrument using $CDCl_3$, DMSO-d_6 or MeOD as solvent and are reported in part per million (δ) using the solvent signals at 7.26 ppm for ^{1}H and at 71.16 ppm for ^{13}C while $CDCl_3$ was used, at 2.50 ppm for ^{1}H and at 39.52 ppm for ^{13}C while DMSO-d_6 was used and at 3.31 ppm for ^{1}H and at 49.00 ppm for ^{13}C while MeOD was used, as an internal reference with *J* values given in Hertz. Mass spectra were obtained using TOF and LCQ Duo ion trap instruments operating in ESI+ mode. The purity of all tested compounds was ≥95%, as determined by analytical HPLC. Melting points were determined using a Fisher-Johns melting point apparatus. Compounds **6**,[39] **7**,[39] **8c**,[39] **9**,[39] **3a-c**[39] and 4-methoxy-3-pyrrolin-2-one[49] were prepared using literature procedures.

General procedure 1 for the synthesis of esters 8d-i: To a stirred solution of 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylic acid **9** (1.5 g, 8.97 mmol) in dry dichloromethane (50 mL) was added DMAP (1.2 g, 9.82 mmol) and EDCI (1.9 g, 9.91 mmol) followed by the alcohol (desired amount, as indicated for each compound) and the resulting solution was heated at reflux temperature for 2 days. The reaction mixture was then cooled to room temperature, washed twice with water and then with brine, dried (Na_2SO_4) and concentrated *in vacuo*. Purification using chromatography over silica (ethyl acetate/hexanes, 20/80) gave the desired products.

General procedure 2 for the synthesis of dipyrrinone 10d-m: To a solution of 4-methoxy-3-pyrrolin-2-one (340 mg, 3.02 mmol, 2.2 eq.) in dry DCM (45 mL) was added triethylamine (1.14 mL, 8.22 mmol, 6.0 eq.) at 0 °C. Then TMSOTf (750 μL, 4.11 mmol, 3.0 eq.) was added drop-wise. After 20 min the aldehyde **8** (1 eq.) was added in dry DCM (45 mL). The reaction was stirred at this temperature for three hours, then TMSOTf (150 μL, 4.11 mmol, 0.6 eq.) was added. After one hour stirring at 0 °C the reaction was quenched by the addition of phosphate buffer (pH = 7, 100 mL). The solution was extracted with DCM (3 x 100 mL), washed with brine and then

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dried (Na_2SO_4). After evaporation of the solvent the resulting brown oil was dissolved in THF (90 mL) and concentrated aqueous HCl (300 μL) was added. After a few minutes the reaction was quenched via addition of saturated aqueous $NaHCO_3$ (200 mL), then extracted with DCM (3 x 100 mL) and washed with water (2 x 100 mL). After concentration of the combined organic fractions under vacuum, the resulting suspension was washed with water and hexane, using filtration, to give the product as a yellow solid.

General procedure 3 for the synthesis of bromodipyrrins 11d-g: To a stirred suspension of **10** (11.7 mmol) in dry CH_2Cl_2 (250 mL) was added $POBr_3$ (6.70 g, 23.37 mmol, 2.0 eq.). The resulting solution was heated at reflux temperature under nitrogen for 17 h. After the reaction mixture was cooled to room temperature, sat. aqueous $NaHCO_3$ (500 mL) was added at 0 ^oC and the organic layer was separated, washed with brine and water, then dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The crude product was purified by passing a solution in EtOAc through a pad of silica gel eluting with EtOAc/hexane 80/20 to give a solid.

General procedure 4 for the synthesis of triflyl dipyrrins 12d, 12h-m: To a suspension of the dipyrrinone **10** (0.85 mmol, 1 eq.), in dry DCM (60 mL) at 0 °C was slowly added Tf_2O (400 μL, 2.38 mmol, 2.8 eq.). After 4 h stirring at this temperature, the reaction was quenched with sat. aqueous $NaHCO_3$ (70 mL), then extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine, and then dried (Na_2SO_4). After evaporation of the solvents under reduced pressure, the crude material was purified using flash column chromatography (SiO_2, EtOAc/hexane 1/9).

General procedure 5 for the synthesis of prodigiosenes 3d-m: Compound **11** or **12** (0.48 mmol, 1 eq.) was dissolved in DME (9 mL) then LiCl (60 mg, 1.44 mmol, 3 eq) and boronic acid (121 mg, 0.57 mmol, 1.2 eq) were added. The solution was degassed by bubbling with N_2, and then palladium tetrakis (56 mg, 10 mol/%) was added. Then a degassed 2 M solution of Na_2CO_3 was added (1.0 mL, 1.92 mmol, 4 eq.) and the suspension was stirred at 85 °C for 18 h. After cooling the solution was poured into water (100 mL) and extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine (100 mL), and then dried (Na_2SO_4). Purification using chromatography (Al_2O_3 neutral type III, EtOAc/hexane 1/9 then 2/8) gave a red film. It was dissolved in a mixture of MeOH/$CHCl_3$ (20:1) then a 0.1 M solution of HCl in MeOH (1.5 eq.) was added. After 15 min stirring the solvents were removed under reduced pressure. The

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obtained solid was filtrated and washed with water and hexane or methanol, to give a dark brown-red solid.

Benzyl 2,4-dimethyl-1*H*-pyrrole-3-carboxylate 7: To a stirred solution of 4-benzyl 2-tert-butyl 3,5-dimethyl-1*H*-pyrrole-2,4-dicarboxylate **6** (165.0 g, 0.5 mol) in ethanol (500 mL) at 0 °C was slowly added 10 M hydrochloric acid (225 mL, 2.3 mol) and the resulting reaction mixture was stirred at 65 °C for 4 hours. After cooling to room temperature, the reaction mixture was poured into a mixture of ice and water (2 L) and extracted with dichloromethane (3 x 500 mL). The combined organic extracts were washed with brine and water, dried (Na_2SO_4), and concentrated in *vacuo* to give an off-white solid (114.0 g, 99%) which was used for the next step without further purification.

Benzyl 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylate 8c: To a stirred solution of DMF (33.3 mL, 0.43 mol) in dichloromethane (100 mL) at 0 °C was added drop-wise $POCl_3$ (39.5 mL, 0.43 mol) under nitrogen. The solution was stirred at room temperature for 15 minutes, and the resulting mixture was added drop-wise to a stirred solution of **7** (90.0 g, 0.39 mol) in dichloromethane (300 mL) at 0 °C over a period of 15 minutes. The reaction mixture was then heated at reflux temperature for 2 hours. After cooling to room temperature, 1 M $NaHCO_3$ (2 L, 2.0 mole) was added, slowly at first and then as rapidly as possible: the mixture was then heated at reflux temperature for 1 hour. After cooling to room temperature, the organic layer was separated and the aqueous layer was extracted with dichloromethane (3 x 500 mL). The combined organic fractions were washed with brine and water several times, dried (Na_2SO_4) and concentrated in *vacuo* to give a light brown solid which was crystallized from hexane/EtOAc (70:30) to give an off-white fluffy crystalline solid (98.0 g, 97%).

5-Formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid 9: To a mixture of benzyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate **8c** (5.1 g, 20.0 mmol) and a catalytic amount of palladium on activated carbon (10 mol%) in a 500 mL round-bottom flask was added ethanol/methanol (9:1) (250 mL) followed by few drops of triethylamine. Hydrogenolysis of the benzyl ester was achieved by stirring the mixture for 5 h under 1 atmosphere of hydrogen. The mixture was then filtered through a plug of Celite® to remove the catalyst and rinsed with methanol (3 x 50 mL). Removal of the solvent in *vacuo* gave a bright white solid (3.08 g, 93%).

Butyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8e: According to general procedure 1 and using *n*-butanol (15 eq.), this compound was obtained as an off-white solid (112 mg, 99%).

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^{1}H NMR ($CDCl_3$, 500 MHz) 0.97 (t, J = 7.5 Hz, 3 H), 1.42-1.50 (m, 2 H), 1.69-1.85 (m, 2 H), 2.55 (s, 3 H), 2.58 (s, 3 H), 4.25 (t, J = 6.5 Hz, 2 H), 9.60 (s, 1 H), 10.43 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.9, 13.9, 14.5, 19.6, 31.0, 63.8, 114.3, 128.4, 136.5, 144.0, 165.2, 177.5. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{12}H_{17}N_1O_3Na$: 246.1101; found 246.1088.

Octyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8f: According to general procedure 1 using octanol (1.1 eq), this compound was obtained as an off-white solid (3.0 g, 90%). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, J = 7.0 Hz, 3 H), 1.24-1.44 (m, 8 H), 1.73 (quint, J = 6.5 Hz, 2 H), 2.54 (s, 3 H), 2.56 (s, 3 H), 4.24 (t, J = 6.5 Hz, 2 H), 9.38 (s, 1 H), 9.62 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.9, 14.3, 14.7, 22.9, 26.4, 29.0, 29.4, 29.5, 32.0, 64.3, 114.5, 128.4, 135.7, 143.0, 165.3, 177.5. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{16}H_{25}N_1O_3Na$: 302.1727; found 302.1719.

Phenyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8g: According to general procedure 1 using phenol (1.1 eq), this compound was obtained as an off-white solid (1.8 g, 83%). ^{1}H NMR ($CDCl_3$, 500 MHz) 2.64 (s, 3 H), 2.67 (s, 3 H), 7.18-7.20 (m, 2 H), 7.25-7.28 (m, 1 H), 7.41-7.45 (m, 2 H), 9.67 (s, 1 H),10.41 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.0, 14.7, 113.4, 122.1, 125.9, 128.7, 129.6, 136.8, 144.8, 150.6, 163.4, 177.7. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{14}H_{13}N_1O_3Na$: 266.0788; found: 266.0788.

Pentyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8h: According to an amended general procedure 1 using pentanol (5.0 eq.) in DMF (10 mL), this compound was obtained as an off-white solid (348 mg, 38%). Mp = 166 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.92 (t, J = 6.8 Hz, 3 H), 1.34-1.45 (m, 4 H), 1.74 (qint, J = 6.8 Hz, 2 H), 2.55 (s, 3 H), 2.57 (s, 3 H), 4.24 (t, J = 6.8 Hz, 2 H), 9.60 (s, 1 H), 9.99 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.8, 10.9, 14.6, 22.5, 28.5, 28.6, 64.1, 114.4, 128.4, 136.2, 143.6, 165.2, 177.5. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{13}H_{19}NO_3$: 260.1263; found: 260.1257.

Hexyl 5-Formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8i: According to an amended general procedure 1 using hexanol (5.0 eq.) in DMF (10 mL), this compound was obtained as an off-white solid (192 mg, 31%). Mp = 167 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.90 (t, J = 7.0 Hz, 3 H), 1.30-1.33 (m, 4 H), 1.39-1.45 (m, 2 H), 1.73 (quint, J = 7.0 Hz, 2 H), 2.55 (s, 3 H), 2.57 (s, 3 H), 4.24 (t, J = 7.0 Hz, 2 H), 9.61 (s, 1 H), 10.79 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.8, 14.1, 14.4, 22.7, 26.0, 28.9, 31.6, 64.0, 114.3, 128.4, 136.8, 144.3, 165.2, 177.5. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{14}H_{21}NO_3$: 274.1419; found: 274.1414.

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Tridecyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8j: 5-Formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid **9** (700 mg, 4.2 mmol), tridecanol (2.5 g, 12.5 mmol), EDC (782 mg, 5.04 mmol) and DMAP (615 mg, 5.04 mmol) were dissolved in DMF (20 mL) under nitrogen. After stirring at 50 °C for two days, water (100 mL) was added and the crude mixture was extracted with diethyl ether (3 x 40 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), and then dried (Na_2SO_4). After evaporation of the solvent under vacuum the crude solid was quickly purified using flash column chromatography (SiO_2, EtOAc/hexane 3/7) to give a mixture of tridecanol and the expected compound. Crystallization from hot methanol (20 mL) gave the product as a white solid (760 mg, 52%). Mp = 70 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, J = 7.0 Hz, 3 H), 1.26-1.34 (m, 18 H), 1.39-1.44 (m, 2 H), 1.73 (quint., J = 7.0 Hz, 2 H), 2.54 (s, 3 H), 2.56 (s, 3 H), 4.24 (t, J = 7.0 Hz, 2 H), 9.44 (bs, 1 H), 9.62 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.8, 14.3, 14.6, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7 (2 C), 29.8 (3 C), 32.1, 64.1, 114.4, 128.3, 135.9, 143.3, 165.2, 177.4. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{21}H_{35}N_1NaO_3$: 372.509; found 372.2505.

Tetradecyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8k: 5-Formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid **9** (400 mg, 2.39 mmol), tetradecanol (614 mg, 2.87 mmol), EDC (445 mg, 2.87 mmol) and DMAP (350 mg, 2.87 mmol) were dissolved in DMF (10 mL) under nitrogen. After stirring at 50 °C for two days, water (100 mL) was added and the crude mixture was extracted with diethyl ether (3 x 40 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), and then dried (Na_2SO_4). After evaporation of the solvent under vacuum the crude solid was quickly purified using flash column chromatography (SiO_2, EtOAc/hexane 5/5) to give a mixture of tetradecanol and the expected compound. Crystallization from hot methanol (15 mL) gave a white solid (300 mg, 34%). Mp = 78 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.87 (t, J = 7.0 Hz, 3 H), 1.31-1.35 (m, 20 H), 1.40-1.45 (m, 2 H), 1.73 (qint., J = 7.0 Hz, 2 H), 2.54 (s, 3 H), 2.56 (s, 3 H), 4.24 (t, J = 7.0, 2 H), 9.59 (bs, 1 H), 9.62 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.8, 14.3, 14.6, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7 (2C), 29.8 (4C), 32.1, 64.1, 114.4, 128.3, 135.8, 143.2, 165.2, 177.4. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{22}H_{38}N_1O_3$: 364.2846; found 364.2854.

Pentadecyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8l: 5-Formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid **9** (620 mg, 3.7 mmol), pentadecanol (3.4 mg, 14.8 mmol), EDC (688 mg, 4.44 mmol) and DMAP (540 mg, 4.44 mmol) were dissolved in DMF (18 mL) under

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nitrogen. After stirring at 50 °C for 24 hours, water (100 mL) was added and the crude mixture was extracted with diethyl ether (3 x 40 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), and then dried (Na_2SO_4). After evaporation of the solvent under vacuum the crude solid was quickly purified using flash column chromatography (SiO_2, EtOAc/hexane 5/5) to give a mixture of pentadecanol and the expected compound. Crystallization from hot methanol (20 mL) gave a white solid (470 mg, 33%). Mp = 80 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, *J* = 7.0 Hz, 3 H), 1.25-1.35 (m, 22 H), 1.39-1.46 (m, 2 H), 1.74 (quint., *J* = 7.0 Hz, 2 H), 2.55 (s, 3 H), 2.57 (s, 3 H), 4.24 (t, *J* = 7.0 Hz, 2 H), 9.61 (bs, 1 H), 10.01 (s, 1 H).^{13}C NMR ($CDCl_3$, 125 MHz) 10.9, 14.3, 14.6, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7 (2 C), 29.8 (5 C), 32.1, 64.1, 114.4, 128.4, 136.2, 143.7, 165.2, 177.5. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{23}H_{39}N_1NaO_3$: 400.2822; found: 400.2812.

Hexadecyl 5-formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 8m: 5-Formyl-2,4-dimethyl-1*H*-pyrrole-3-carboxylic acid **9** (700 mg, 4.2 mmol), hexadecanol (3.0 mg, 12.5 mmol), EDC (782 mg, 5.04 mmol) and DMAP (615 mg, 5.04 mmol) were dissolved in DMF (20 mL) under nitrogen. After stirring at 50 °C for two days, water (100 mL) was added and the crude mixture was extracted with diethyl ether (3 x 40 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), and then dried (Na_2SO_4). After evaporation of the solvent under *vacuum* the crude solid was quickly purified using flash column chromatography (SiO_2, EtOAc/hexane 5/5) to give a mixture of hexadecanol and the expected compound. Crystallization from hot methanol (20 mL) gave a white solid (871 mg, 54%). Mp = 82 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, *J* = 7.0 Hz, 3 H), 1.25-1.35 (m, 24 H), 1.39-1.43 (m, 2 H), 1.73 (quint., *J* = 7.0 Hz, 2 H), 2.54 (s, 3 H), 2.55 (s, 3 H), 4.24 (t, *J* = 7.0 Hz, 2 H), 9.17 (bs, 1 H), 9.63 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.9, 14.3, 14.6, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7 (2 C), 29.8 (6 C), 32.1, 64.1, 114.4, 128.4, 136.2, 143.7, 165.2, 177.5. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{24}H_{41}N_1NaO_3$: 414.2979; found 414.2959.

((*Z*)-Butyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5*H*)-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10e: According to general procedure 2 using aldehyde **8e** (1.5 g, 6.7 mmol), this compound was obtained as a yellow solid (1.34 g, 63%). ^{1}H NMR (DMSO-d_6, 500 MHz) 0.91 (t, *J* = 7.5 Hz, 3 H), 1.35-1.43 (m, 2 H), 1.60-1.65 (m, 2 H), 2.20 (s, 3 H), 2.44 (s, 3 H), 3.84 (s, 3 H), 4.12 (t, *J* = 6.5 Hz, 2 H), 5.25 (s, 1 H), 6.02 (s, 1 H), 9.64 (s, 1 H), 10.90 (s, 1 H). ^{13}C NMR (DMSO-d_6, 125 MHz) 11.1, 13.8, 19.2, 30.6, 58.7, 62.9, 91.5, 95.0, 111.7, 122.2, 124.4, 125.4,

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139.5, 165.1, 167.2, 171.3. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{17}H_{22}N_2O_4Na$: 341.1472; found 341.1469.

(*Z*)-Octyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5*H*)-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10f: According to general procedure 2 using aldehyde **8f** (1.5 g, 5.37 mmol), this compound was obtained as a yellow solid (1.35 g, 67% of a 10/90 E/*Z* mixture (determined using NMR)). ^{1}H NMR (DMSO-d_6, 500 MHz) 0.85 (t, *J* = 6.5 Hz, 3 H). 1.25-1.39 (m, 10 H), 1.64 (quint, *J* = 6.5 Hz, 2 H), 2.21 (s, 3 H), 2.45 (s, 3 H), 3.85 (s, 3 H), 4.12 (t, *J* = 6.5 Hz, 2 H), 5.27 (s, 1 H), 6.02 (s, 1 H), 9.64 (s, 1 H), 10.91 (s, 1 H). ^{13}C NMR (DMSO-d_6, 125 MHz) 10.9, 13.6, 14.0, 22.1, 25.7, 28.3, 28.6 (2 C), 31.2, 58.4, 62.8, 91.4, 94.4, 111.4, 122.1, 124.0, 125.3, 139.1, 164.8, 166.9, 170.8. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{21}H_{30}N_2O_4Na$: 397.2098; found: 397.2097.

(*Z*)-Phenyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5*H*)-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10g: According to general procedure 2 using aldehyde **8g** (1.5 g, 7.4 mmol), this compound was obtained as a yellow solid (2.29 g, 92% of a 5/95 *E*/*Z* mixture (determined using NMR)). ^{1}H NMR (DMSO-d_6, 500 MHz) 2.28 (s, 3 H), 2.54 (s, 3 H), 3.87 (s, 3 H), 5.29 (s, 1 H), 6.06 (s, 1 H), 7.18-7.20 (m, 2 H), 7.25-7.28 (m, 1 H), 7.42-7.45 (m, 2 H), 9.69 (s, 1 H), 11.10 (s, 1 H). ^{13}C NMR (DMSO-d_6, 125 MHz) 11.0, 13.8, 58.5, 91.6, 94.3, 110.4, 122.2 (2 C), 122.5, 124.2, 125.4, 125.8, 129.4, 140.3, 150.5, 163.0, 166.9, 170.9. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{19}H_{18}N_2O_4Na$: 361.1159; found: 361.1171.

(Z)-Pentyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5*H*)-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10h: According to general procedure 2 using aldehyde **8h** (338 mg, 1.42 mmol), this compound was obtained as a bright yellow solid (422 mg, 89%). Mp = 160 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) 0.88 (t, *J* = 6.8 Hz, 3 H), 1.30-1.37 (m, 4 H), 1.65 (quint., *J* = 6.8 Hz, 2 H), 2.21 (s, 3 H), 2.45 (s, 3 H), 3.85 (s, 3 H), 4.12 (t, *J* = 6.8 Hz, 2 H), 5.26 (s, 1 H), 6.02 (s, 1 H), 9.64 (s, 1 H), 10.90 (s, 1 H). ^{13}C NMR (DMSO-d_6, 125 MHz) 10.9, 13.6, 13.9, 21.8, 27.9, 28.0, 58.4, 62.8, 91.4, 94.5, 111.5, 122.0, 124.0, 125.3, 139.1, 164.8, 166.9, 170.8. HR-MS (ESI): $[M+Na]^+$ calcd. for: $C_{18}H_{24}N_2O_4$: 355.1634; found: 355.1628.

(*Z*)-Hexyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5H)-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10i: According to general procedure 2 using aldehyde **8i** (178 mg, 0.70 mmol), this compound was obtained as a bright yellow solid (213 mg, 87% of a 20/80 *E*/*Z* mixture). Mp = 162 °C. ^{1}H NMR (MeOD, 500 MHz) 0.79 (t, *J* = 7.0 Hz, 3 H), 1.19-1.22 (m, 4 H), 1.28-1.31

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(m, 2 H), 1.56 (qint., J = 7.0 Hz, 2 H), 2.13 (s, 3 H), 2.37 (s, 3 H), 3.77 (s, 3 H), 4.04 (t, J = 7.0 Hz, 2 H), 5.18 (s, 1 H), 5.94 (s, 1 H). ^{13}C NMR (DMSO-d6, 125 MHz) 11.0, 13.7, 13.9, 22.1, 25.4, 28.3, 30.9, 58.5, 59.0, 62.9, 91.4, 94.6, 95.4, 100.8, 111.2, 111.5, 122.1, 123.6, 124.0, 125.3, 127.7, 137.3, 139.2, 163.8, 164.8, 166.9, 168.5, 170.9. HR-MS (ESI): $[M+Na]^+$ calcd. for: $C_{19}H_{26}N_2O_4$: 369.1790; found: 369.1785.

(*Z*)-tridecyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5*H*)-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10j: According to general procedure 2 using aldehyde **8j** (500 mg, 1.32 mmol), this compound was obtained as a yellow solid (530 mg, 88% of a 10/90 *E*/*Z* mixture (determined using NMR)). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, J = 7.0 Hz, 3 H), 1.26-1.35 (m, 18 H), 1.40-1.45 (m, 2 H), 1.73 (quint., J = 7.0 Hz, 2 H), 2.34 (s, 0.17 H, *E* isomer), 2.38 (s, 3 H), 2.53 (s, 0.14 H, *E* isomer), 2.66 (s, 3 H), 3.91 (s, 3 H), 4.07 (s, 0.23 H, *E* isomer), 4.23 (t, J = 7.0 Hz, 2 H), 5.12 (s, 1 H), 5.39 (s, *E* isomer), 6.28 (s, *E* isomer), 6.40 (s, 1 H), 10.58 (s, 1 H), 11.00 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.5, 14.3, 22.8, 26.4, 29.0, 29.4, 29.5, 29.7 (2 C), 29.8 (3 C), 32.1, 58.4, 63.7, 90.3, 99.9, 112.7, 122.5, 123.6, 128.4, 141.8, 166.0, 168.3, 173.6. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{26}H_{41}N_2O_4$: 445.3061; found 445.3084.

(*Z*)-tetradecyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5*H*)-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10k: According to general procedure 2 using aldehyde **8k** (500 mg, 1.37 mmol), this compound was obtained as a yellow solid (490 mg, 76% of a 5/95 *E*/*Z* mixture (determined using NMR)). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.87 (t, J = 7.0 Hz, 3 H), 1.26-1.35 (m, 20 H), 1.40-1.45, (m, 2 H), 1.70-1.76 (m, 2 H), 2.34 (s, 0.22 H, *E* isomer), 2.38 (s, 3 H), 2.53 (s, 0.22 H, *E* isomer), 2.65 (s, 3 H), 3.91 (s, 3 H), 4.06 (s, 0.22 H, *E* isomer), 4.23 (t, J = 7.0 Hz, 2 H), 5.12 (s, 1 H), 5.39 (s, 0.10 H, *E* isomer), 6.29 (s, 0.10 H, *E* isomer), 6.40 (s, 1 H), 10.58 (s, 1 H), 10.99 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.5, 14.3, 22.8, 26.4, 29.0, 29.4, 29.5, 29.7 (2 C), 29.8 (5 C), 32.1, 58.4, 63.7, 90.3, 99.8, 112.7, 122.5, 123.6, 128.3, 141.8, 166.0, 168.2, 173.6. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{27}H_{43}N_3O_4$: 459.3217; found 459.3202.

(*Z*)-pentadecyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5*H*)-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10l: According to general procedure 2 using aldehyde **8l** (400 mg, 1.06 mmol), this compound was obtained as a yellow solid (214 mg, 43% of a 5/95 *E*/*Z* mixture (determined using NMR)). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.87 (t, J = 7.0 Hz, 3 H), 1.25-1.35 (m, 22 H), 1.40-1.46 (m, 2 H), 1.73 (quint., J = 7.0 Hz, 2 H), 2.38 (s, 3 H), 2.66 (s, 3 H), 3.92 (s, 3 H), 4.23 (t, J = 7.0 Hz, 2 H), 5.14 (s, 1 H), 6.41 (s, 1 H), 10.55 (bs, 1 H), 10.97 (bs, 1 H). Because

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of the poor solubility of this compound no ^{13}C NMR is available. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{28}H_{45}N_2O_4$: 473.3374; found 473.3347.

(*Z*)-hexadecyl 5-((3-methoxy-5-oxo-1*H*-pyrrol-2(5*H*)-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 10m: According to general procedure 2 using aldehyde **8m** (500 mg, 1.27 mmol), this compound was obtained as a yellow solid (430 mg, 69% of a 5/95 *E*/*Z* mixture (determined using NMR)). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.87 (t, *J* = 7.0 Hz, 3 H), 1.25-1.35 (m, 24H), 1.41-1.46 (m, 2 H), 1.73 (quint., *J* = 7.0 Hz, 2 H), 2.34 (s, 0.13 H, *E* isomer), 2.38 (s, 3 H), 2.53 (s, 0.18 H, *E* isomer), 2.65 (s, 3 H), 3.75 (s, 0.15 H, *E* isomer), 3.90 (s, 3 H), 4.06 (s, 0.18 H, *E* isomer), 4.23 (t, *J* = 7.0 Hz, 2 H), 5.11 (s, 1 H), 5.39 (s, 0.08 H, *E* isomer), 6.30 (s, 0.07 H, *E* isomer), 6.39 (s, 1 H), 10.59 (bs, 1 H), 11.01 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.5, 14.3, 22.8, 26.4, 29.0, 29.4, 29.5, 29.7 (2 C), 29.8 (7 C), 32.1, 58.4, 63.7, 90.3, 99.8, 112.7, 122.5, 123.6, 128.3, 141.8, 166.0, 168.2, 173.6. HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{29}H_{46}N_2NaO_4$: 509.3350; found 509.3338.

(*Z*)-Butyl 5-((5-bromo-3-methoxy-2*H*-pyrrol-2-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 11e: According to general procedure 3 using dipyrrinone **10e** (1.34 g, 4.2 mmol), this compound was obtained as a green-yellow solid (1.50 g, 94%). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.97 (t, *J* = 7.1 Hz, 3 H), 1.46 (sext., J = 7.1 Hz, 2 H), 1.72 (quint., *J* = 7.1 Hz, 2 H), 2.40 (s, 3 H), 2.59 (s, 3 H), 3.85 (s, 3 H), 4.24 (t, *J* = 6.1 Hz, 2 H), 5.59 (s, 1 H), 6.93 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.7, 13.9, 15.2, 19.6, 31.0, 58.7, 63.6, 100.1, 114.1, 115.9, 126.4, 134.1, 139.2, 144.1, 147.1, 165.5, 167.4. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{17}H_{22}BrN_2O_3$: 381.0808; found 381.0798.

(*Z*)-Octyl 5-((5-bromo-3-methoxy-2*H*-pyrrol-2-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 11f: According to general procedure 3 using dipyrrinone **10f** (1.0 g, 2.67 mmol), this compound was obtained as a green-yellow solid (1.00 g, 87%). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, *J* = 6.5 Hz, 3 H), 1.28-1.36 (m, 8 H), 1.40-1.45 (m, 2 H), 1.67 (quint, *J* = 6.5 Hz, 2 H), 2.40 (s, 3 H), 2.59 (s, 3 H), 3.85 (s, 3 H), 4.23 (t, *J* = 6.5 Hz, 2 H), 5.59 (s, 1 H), 6.94 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.7, 14.3, 15.2, 22.8, 26.4, 29.0, 29.3, 29.4, 31.9, 58.7, 64.0, 100.2, 114.1, 115.9, 126.5, 134.1, 139.2, 144.2, 147.2, 165.5, 167.5. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{21}H_{30}N_2O_3$: 337.1434; found 437.1426.

(*Z*)-Phenyl 5-((5-bromo-3-methoxy-2*H*-pyrrol-2-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate 11g: According to general procedure 3 using dipyrrinone **10g** (1.0 g, 2.96 mmol),

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this compound was obtained as a green-yellow solid (1.10 g, 93%).^{1}H NMR ($CDCl_3$, 500 MHz) 2.48 (s, 3 H), 2.67 (s, 3 H), 3.87 (s, 3 H), 5.61 (s, 1 H), 6.98 (s, 1 H), 7.18-7.20 (m, 2 H), 7.25-7.26 (m, 1 H), 7.40-7.43 (m, 2 H), 11.35 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.8, 15.3, 58.8, 100.3, 113.0, 115.7, 122.2, 125.7, 126.7, 129.5, 134.2, 139.7, 145.0, 147.8, 150.8, 163.7, 167.6. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{19}H_{18}BrN_2O_3$: 401.0495; found 401.0485.

(*Z*)-Pentyl 2-((3-methoxy-5-(((trifluoromethyl)sulfonyl)oxy)-1*H*-pyrrol-2-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate 12h: According to general procedure 4 using dipyrrinone **10h** (194 mg, 0.64 mmol), this compound was obtained as a bright yellow solid (186 mg, 78%). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.92 (t, J = 6.9 Hz, 3 H), 1.34-1.43 (m, 4 H), 1.74 (quint., J = 6.9 Hz, 2 H), 2.42 (s, 3 H), 2.57 (s, 3 H), 3.90 (s, 3 H), 4.24 (t, J = 6.9 Hz, 2 H), 5.43 (s, 1 H), 7.12 (s, 1 H), 10.99 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.7, 14.2, 15.1, 22.5, 28.5, 28.6, 56.0, 64.0, 87.6, 114.3, 118.8 (q, J = 319 Hz), 119.1, 126.0, 133.3, 135.4, 144.8, 161.8, 165.3, 168.2. HR-MS (ESI): $[M+Na]^+$ calcd. for: $C_{19}H_{19}F_3N_2O_6S$: 487.1127; found: 487.1132.

(*Z*)-Hexyl 2-((3-methoxy-5-(((trifluoromethyl)sulfonyl)oxy)-*1H*-pyrrol-2-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate 12i: According to general procedure 4 using dipyrrinone **10i** (79 mg, 0.23 mmol), this compound was obtained as a bright yellow solid (100 mg, 92%). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.90 (t, J = 6.9 Hz, 3 H), 1.31-1.35 (m, 4 H), 1.40-1.46 (m, 2 H), 1.73 (quint., J = 6.9 Hz, 2 H), 2.42 (s, 3 H), 2.57 (s, 3 H), 3.90 (s, 3 H), 4.23 (t, J = 6.9 Hz, 2 H), 5.43 (s, 1 H), 7.13 (s, 1 H), 11.02 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.7, 14.2, 15.1, 22.7, 26.0, 28.9, 31.6, 59.0, 64.0, 87.6, 114.4, 119.1, 119.7 (q, J = 319 Hz), 126.1, 133.3, 135.4, 144.8, 161.8, 165.3, 168.2. HR-MS (ESI): $[M+H]^+$ calcd. for: $C_{20}H_{26}F_3N_2O_6S$: 479.1419; found: 479.1458.

***(Z)*-Tridecyl 2-((3-methoxy-5-(((trifluoromethyl)sulfonyl)oxy)-1*H*-pyrrol-2-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate 12j:** According to general procedure 4 using dipyrrinone **10j** (400 mg, 0.89 mmol), this compound was obtained as a yellow solid (296 mg, 59%). Mp = 68 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.87 (t, J = 7.0 Hz, 3 H), 1.26-1.35 (m, 18 H), 1.39-1.45 (m, 2 H), 1.73 (quint., J = 7.0 Hz, 2 H), 2.42 (s, 3 H), 2.57 (s, 3 H), 3.89 (s, 3 H), 4.23 (t, J = 7.0 Hz, 2 H), 5.43 (s, 1 H), 7.12 (s, 1 H), 10.99 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.7, 14.3, 15.1, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7 (2 C), 29.8 (3 C), 32.1, 59.0, 64.0, 87.6, 114.4, 118.8 (q., J = 319 Hz), 119.1, 126.0, 133.3, 135.4, 144.8, 161.8, 165.3, 168.2. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{27}H_{40}F_3N_2O_6S$: 577.2554; found 577.2531.

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(*Z*)-Tetradecyl 2-((3-methoxy-5-(((trifluoromethyl)sulfonyl)oxy)-1*H*-pyrrol-2-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate 12k: According to general procedure 4 using dipyrrinone **10k** (390 mg, 0.85 mmol), this compound was obtained as a yellow solid (500 mg, quant.). Mp = 64 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.87 (t, *J* = 6.7 Hz, 3 H), 1.25-1.35 (m, 20 H), 1.40-1.44 (m, 2 H), 1.73 (qint., *J* = 6.7 Hz, 2 H), 2.41 (s, 3 H), 2.56 (s, 3 H), 3.88 (s, 3 H), 4.23 (t, *J* = 6.7 Hz, 3 H), 5.42 (s, 1 H), 7.10 (s, 1 H), 10.98 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz,) 11.6, 14.2, 15.1, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7, (2 C), 29.8 (4 C), 32.1, 58.9, 64.0, 87.5, 114.3, 117.0 (q, *J* = 319 Hz), 117.5, 126.0, 133.3, 135.3, 144.7, 161.8, 165.3, 168.2. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{28}H_{42}F_3N_2O_6S$: 591.2710; found 591.2708.

(*Z*)-Pentadecyl 2-((3-methoxy-5-(((trifluoromethyl)sulfonyl)oxy)-1*H*-pyrrol-2-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate 12l: According to general procedure 4 using dipyrrinone **10l** (294 mg, 0.62 mmol), this compound was obtained as a yellow solid (115 mg, 31%). Mp = 78 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, *J* = 7.0 Hz, 3 H), 1.25-1.35 (m, 22 H), 1.39-1.45 (m, 2 H), 1,73 (quint., *J* = 7.0 Hz, 2 H), 2.42 (s, 3 H), 2.57 (s, 3 H), 3.90 (s, 3 H), 4.23 (t, *J* = 7.0 Hz, 2 H), 5.43 (s, 1 H), 7.12 (s, 1 H), 11.00 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.6, 14.2, 15.1, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7 (2 C), 29.8 (5 C), 32.1, 58.9, 64.0, 87.5, 114.3, 118.7 (q, *J* = 318 Hz), 119.0, 126.0, 133.3, 135.3, 144.7, 161.8, 165.3, 168.2. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{29}H_{44}F_3N_2O_6S$: 605.2867; found: 605.2877.

(*Z*)-Hexadecyl 2-((3-methoxy-5-(((trifluoromethyl)sulfonyl)oxy)-1*H*-pyrrol-2-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate 12m: According to general procedure 4 using dipyrrinone **10m** (350 mg, 0.78 mmol), this compound was obtained as a yellow solid (260 mg, 52%). Mp = 74 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, *J* = 7.0 Hz, 3 H), 1.25-1.34 (m, 24 H), 1.39-1.45 (m, 2 H), 1,73 (quint., *J* = 7.0 Hz, 2 H), 2.42 (s, 3 H), 2.57 (s, 3 H), 3.90 (s, 3 H), 4.23 (t, *J* = 7.0 Hz, 2 H), 5.43 (s, 1 H), 7.13 (s, 1 H), 11.00 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 11.7, 14.3, 15.1, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7, 29.8 (6 C), 32.1, 59.0, 64.1, 87.6, 114.4, 118.8 (q, *J* = 318 Hz), 119.1, 126.1, 133.3, 135.4, 144.8, 161.8, 165.3, 168.2. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{30}H_{46}F_3N_2O_6S$: 619.3023; found 619.3042.

(*Z*)-Butyl 2-((4-methoxy-1*H*,1'*H*-2,2'-bipyrrol-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate hydrochloride 3e: According to general procedure 5 using bromodipyrrinone **11e** (1.4 g, 3.67 mmol), this compound was obtained as a red HCl salt (730 mg, 54%). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.97 (t, *J* = 7.5 Hz, 3 H), 1.43-1.50 (m, 2 H), 1.70-1.76 (m, 2 H), 2.51 (s, 3

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H), 2.81 (s, 3 H,), 4.04 (s, 3 H), 4.26 (t, J = 6.5 Hz, 2 H), 6.10 (bs. 1 H), 6.37-6.39 (m, 1 H), 7.00 (bs, 1 H), 7.10 (s, 1 H), 7.29 (bs, 1 H), 12.66 (bs, 1 H), 12.72 (bs, 1 H), 12.93 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.0, 13.9, 15.0, 19.6, 31.0, 59.1, 64.0, 93.4, 112.5, 113.0, 116.0, 119.1, 122.2, 122.6, 123.5, 128.6, 140.6, 150.1, 150.5, 164.8, 166.8. HR-MS (ESI): $[M\text{-}HCl+H]^+$ calcd. for $C_{21}H_{26}N_3O_3$: 368.1969; found 368.1963. UV ($CHCl_3$) λ_{max} (ε): 500 (52 400), 528 (109 700). HPLC: Regis Pirkle Covalent Whelk 01 (250 × 4.60 mm), MeOH/(H_2O/NH_4OH, 500/1, v/v) 80/20, 0.75 mL/min, λ = 451 nm, ^{t}R = 95.7 min.

(*Z*)-Octyl 2-((4-methoxy-1*H*,1'*H*-2,2'-bipyrrol-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate hydrochloride 3f: According to general procedure 5 using bromodipyrrinone **11f** (1.0 g, 2.29 mmol), this compound was obtained as a red HCl salt (639 mg, 61%). ^{1}H NMR ($CDCl_3$, 500 MHz) 0.88 (t, J = 6.5 Hz, 3 H), 1.25-1.45 (m, 10 H), 1.73 (quint, J = 6.5 Hz, 2 H), 2.50 (s, 3 H), 2.80 (s, 3 H), 4.02 (s, 3 H), 4.23 (t, J = 6.5 Hz, 2 H), 6.07 (bs, 1 H), 6.36 (bs, 1 H), 6.97 (bs, 1 H), 7.06 (s, 1 H), 7.26 (bs, 1 H), 12.63 (bs, 1 H), 12.67 (bs, 1 H), 12.86 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.0, 14.2, 15.0, 22.8, 26.3, 28.9, 29.3, 29.4, 31.9, 59.2, 64.3, 93.5, 112.5, 112.9, 115.9, 119.1, 122.1, 122.5, 123.5, 128.5, 140.5, 150.0, 150.4, 164.7, 166.7. HR-MS (ESI): $[M\text{-}HCl+H]^+$ calcd. for $C_{25}H_{34}N_3O_3$: 424.2595; found 424.2569. λ_{max} (ε): 501 (59 100), 528 (121 000). HPLC: Regis Pirkle Covalent Whelk 01 (250 × 4.60 mm), MeOH 100%, 0.75 mL/min, λ = 451 nm, ^{t}R = 42.4 min.

(*Z*)-Phenyl 2-((4-methoxy-1*H*,1'*H*-2,2'-bipyrrol-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate hydrochloride 3g: According to general procedure 5 using bromodipyrrinone **11f** (1.1 g, 2.74 mmol), this compound was obtained as a red HCl salt (756 mg, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz) 2.57 (s, 3 H) 2.89 (s, 3 H), 4.04 (s, 3 H), 6.09 (bs, 1 H), 6.39 (bs, 1 H), 7.01 (bs, 1 H), 7.12 (bs, 1 H), 7.18-7.20 (m, 2 H), 7.24-7.27 (m, 1 H), 7.30 (s, 1 H), 7.40-7.43 (m, 1 H), 12.68 (bs, 1 H), 12.82 (bs, 1 H),12.95 (bs, 1 H), ^{13}C NMR ($CDCl_3$, 125 MHz) 12.1, 15.0, 59.3, 93.7, 112.7, 114.7, 119.6, 122.0, 122.1, 123.0, 123.6, 125.8, 128.9, 129.5, 140.5, 150.5, 150.6, 150.8, 162.9, 166.9 (1 quaternary carbon missing). HR-MS (ESI): $[M\text{-}HCl+H]^+$ calcd. for $C_{23}H_{22}N_3O_3$: 388.1656; found 388.1630. λ_{max} (ε): 501 (57 000), 526 (109 000). HPLC: Regis Pirkle Covalent Whelk 01 (250 × 4.60 mm), MeOH 100%, 0.75 mL/min, λ = 451 nm, ^{t}R = 29.4 min.

(Z)-Pentyl 2-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2H-pyrrole-

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4-carboxylate hydrochloride 3h: According to general procedure 5 using triflate **12h**, this compound was obtained as a purple solid (82 mg, 46%). Mp = 164 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.93 (t, *J* = 6.9 Hz, 3 H), 1.36-1.44 (m, 5 H), 1.75 (quint., *J* = 6.9 Hz, 2 H), 2.52 (s, 3 H), 2.82 (s, 3 H), 4.05 (s, 3 H), 4.25 (t, *J* = 6.9 Hz, 2 H), 6.10 (s, 1 H), 6.39 (t, *J* = 1.7 Hz, 1 H), 7.00 (s, 1 H), 7.11 (s, 1 H), 7.29 (s, 1 H), 12.66 (s, 1 H), 12.72 (s, 1 H), 12.95 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.0, 14.1, 14.9, 22.44, 28.4, 28.5, 59.1, 64.2, 76.9, 77.2, 77.4, 93.4, 112.5, 112.8, 115.8, 119.0, 122.0, 122.4, 123.43, 128.3, 140.4, 149.9, 150.2, 164.6, 166.7. HR-MS (ESI): $[M+H]^+$ calcd. for: $C_{22}H_{28}N_3O_3$: 354.2131; found: 382.2125. UV (CH_2Cl_2) λ_{max} (ε): 525 (19 000).HPLC: Regis Pirkle Covalent Whelk 01 (250 × 4.60 mm), MeOH/(H_2O/HCl, 250/1, v/v) 80/20, 1.0 mL/min, λ = 520 nm, ^{t}R = 36.5.7 min.

(*Z*)-Hexyl 5-((4'-methoxy-1*H*,5'*H*-[2,2'-bipyrrol]-5'-ylidene)methyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxylate hydrochloride 3i: According to general procedure 5 using triflate **12i**, this compound was obtained as a purple-red solid (22 mg, 30%). Mp = 165 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.90 (t, *J* = 6.9 Hz, 3 H), 1.31-1.35 (m, 4 H), 1.40-1.45 (m, 2 H), 1.73 (quint., *J* = 6.9, 2 H), 2.49 (s, 3 H), 2.79 (s, 3 H), 4.02 (s, 3 H), 4.23 (t, *J* = 6.9 Hz, 2 H), 6.06 (s, 1 H), 6.36 (s, 1 H), 6.96 (s, 1 H), 7.07 (s, 1 H), 12.64 (bs, 1 H), 12.68 (s, 1 H), 12.87 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.0, 14.1, 15.0, 22.7, 26.0, 28.9, 31.6, 59.1, 64.3, 93.4, 112.5, 113.0, 115.9, 119.0, 122.1, 122.5, 123.5, 128.5, 140.6, 150.1, 150.4, 164.7, 166.7. HR-MS (ESI): $[M+H]^+$ calcd. for: $C_{23}H_{30}N_3O_3$: 396.2287; found: 396.2282. UV (CH_2Cl_2) λ_{max} (ε): 525 (19 000). HPLC: Regis Pirkle Covalent Whelk 01 (250 × 4.60 mm), MeOH/(H_2O/HCl, 250/1, v/v) 80/20, 1.0 mL/min, λ = 520 nm, ^{t}R = 42.1 min.

(*Z*)-Tridecyl 2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate hydrochloride 3j: According to general procedure 5 using triflate **12j** (280 mg, 0.48 mmol), this compound was obtained as a brown-red HCl salt (127 mg, 50%). Mp = 160 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.87 (t, *J* = 7.0 Hz, 3 H), 1.25-1.35 (m, 18 H), 1.39-1.44 (m, 2 H), 1.73 (quint., *J* = 7.0 Hz, 2 H), 2.49 (s, 3 H), 2.80 (s, 3 H), 4.02 (s, 3 H), 4.23 (t, *J* = 7.0 Hz, 2 H), 6.06 (d, *J* = 1.5 Hz, 1 H), 6.35-6.37 (m, 1 H), 6.96-6.96 (m, 1 H), 7.07 (s, 1 H), 7.26 (s, 1 H), 12.64 (bs, 1 H), 12.68 (bs, 1 H), 12.87 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.0, 14.3, 15.0, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7 (4 C), 29.8, 32.0, 59.1, 64.3, 93.4, 112.5, 112.9, 115.9, 119.0, 122.1, 122.5, 123.5, 128.5, 140.5, 150.0, 150.4, 164.7, 166.7. HR-MS (ESI): $[M+H-HCl]^+$

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calcd. for $C_{30}H_{44}N_3O_3$: 494.3377; found 494.3371. UV (DMSO) λ_{max} (ε): 460 (36 500), 528 (17 400). HPLC: Polaris Si A 5 μ (150 × 4.60 mm), IPA/(H_2O/HCl, 250/1, v/v) 80/20, 0.3 mL/min, λ = 520 nm, ^tR = 6.4 min.

(*Z*)-Tetradecyl 2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate hydrochloride 3k: According to general procedure 5 using triflate **12k** (500 mg, 0.84 mmol), this compound was obtained as a brown-red solid (210 mg, 46%). Rf = 0.15 (EtOAc/hexane, 1/9). Mp = 160 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.87 (t, *J* = 7.0 Hz, 3 H), 1.25-1.35 (m, 20 H), 1.39-1.45 (m, 2 H), 1.73 (quint., *J* = 7.0 Hz, 2 H), 2.51 (s, 3 H), 2.81 (s, 3 H), 4.04 (s, 3 H), 4.24 (t, *J* = 7.0 Hz, 2 H), 6.09 (d, *J* = 1.5 Hz, 1 H), 6.37-6.38 (m, 1 H), 6.98-6.99 (m, 1 H), 7.10 (s, 1 H), 7.27-7.28 (m, 1 H), 12.66 (bs, 1 H), 12.71 (bs, 1 H), 12.92 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.0, 14.3, 15.0, 22.8, 26.3, 28.9, 29.4, 29.5 (2 C), 29.7 (3 C), 29.8, 32.1, 59.1, 64.3, 93.4, 112.5, 113.0, 115.9, 119.0, 122.1, 122.5, 123.5, 128.5, 140.6, 150.1, 150.5, 164.8, 166.7. HR-MS (ESI): $[M+H- HCl]^+$ calcd. for $C_{31}H_{46}N_3O_3$: 508.3534; found 508.3514. UV (DMSO) λ_{max} (ε): 457 (36 400). HPLC: Polaris Si A 5 μ (150 × 4.60 mm), IPA/(H_2O/HCl, 250/1, v/v) 80/20, 0.3 mL/min, λ = 520 nm, ^tR = 6.0 min.

(*Z*)-Pentadecyl 2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate hydrochloride 3l: According to general procedure 5 using triflate **12l** (115 mg, 0.19 mmol), this compound was obtained as a brown-red solid (36 mg, 33%). Mp = 160 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.86 (t, *J* = 7.0 Hz, 3 H), 1.24-1.33 (m, 22 H), 1.38-1.42 (m, 2 H), 1.72 (quint., *J* = 7.0 Hz, 2 H), 2.47 (s, 3 H), 2.78 (s, 3 H), 3.99 (s, 3 H), 4.22 (t, *J* = 7.0 Hz, 2 H), 6.03 (s, 1 H), 6.33 (s, 1 H), 6.93 (s, 1 H), 7.03 (s, 1 H), 7.24 (s, 1 H), 12.61 (bs, 1 H), 12.64 (bs, 1 H), 12.81 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.0, 14.2, 14.9, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7 (2 C), 29.8 (5 C), 32.0, 59.1, 64.3, 93.4, 112.5, 112.8, 115.8, 119.0, 122.1, 122.4, 123.4, 128.3, 140.5, 150.0, 150.3, 164.7, 166.7. HR-MS (ESI): $[M+H-HCl]^+$ calcd. for $C_{32}H_{48}N_3O_3$: 522.3690; found 522.3675. λ_{max} (ε): 529 (33 000), 463 (44 000). HPLC: Polaris Si A 5 μ (150 × 4.60 mm), IPA/(H_2O/HCl, 250/1, v/v) 80/20, 0.3 mL/min, λ = 520 nm, ^tR = 6.2 min.

(*Z*)-Hexadecyl 2-((4-methoxy-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dimethyl-2*H*-pyrrole-4-carboxylate hydrochloride 3m: According to general procedure 5 using triflate **12m** (240 mg, 0.38 mmol), this compound was obtained as a brown-red solid (106 mg, 49%). Rf =

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0.15 (EtOAc/hexane, 1/9). Mp = 160 °C. ^{1}H NMR ($CDCl_3$, 500 MHz) 0.87 (t, J = 7.0 Hz, 3 H), 1.25-1.35 (m, 24 H), 1.39-1.43 (m, 2 H), 1.73 (quint., J = 7.0 Hz, 2 H), 2.50 (s, 3 H), 2.80 (s, 3 H), 4.02 (s, 3 H), 4.23 (t, J = 7.0 Hz, 2 H), 6.07 (d, J = 1.5 Hz, 1 H), 6.35-6.37 (m, 1 H), 6.96-6.97 (m, 1 H), 7.08 (s, 1 H), 7.26-7.27 (m, 1 H), 12.64 (bs, 1 H), 12.69 (bs, 1 H), 12.89 (bs, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.0, 14.3, 15.0, 22.8, 26.3, 28.9, 29.4, 29.5, 29.7 (2 C), 29.8 (6 C), 32.1, 59.1, 64.3, 93.4, 112.5, 112.9, 115.9, 119.0, 122.1, 122.5, 123.5, 128.5, 140.6, 150.0, 150.4, 164.7, 166.7. HR-MS (ESI): $[M+H-HCl]^+$ calcd. for $C_{33}H_{50}N_3O_3$: 536.3847; found 536.3855. UV (DMSO) λ_{max} (ε): 462 (37 900), 528 (24 500). HPLC: Polaris Si A 5 μ (150 × 4.60 mm), IPA/(H_2O/HCl, 250/1, v/v) 80/20, 0.3 mL/min, λ = 520 nm, ^tR = 6.2 min.

Zn(II)[(*Z*)-5-(4-Methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrole-3-carboxylic acid benzyl ester]$_2$ 4: To a solution of benzyl-ester prodigiosene **3c** free-base (201 mg, 0.50 mmol) in chloroform (10 mL) was added a solution of zinc acetate dihydrate (263 mg, 1.20 mmol) and sodium acetate trihydrate (163 mg, 1.20 mmol) in methanol (10 mL). The resulting solution was stirred at 40°C overnight. The resulting dark red solution was poured onto water (50 mL) and extracted with dichloromethane (3 x 20 mL). The organic fractions were combined, dried (Na_2SO_4) and concentrated in *vacuo*. Purification over silica-gel (EtOAc/hexane 20/80 to 50/50) gave the desired Zn-complex as a bright red crystalline powder (115 mg, 54%). ^{1}H NMR ($CDCl_3$, 500 MHz) 2.16 (s, 6 H,), 2.54 (s, 6 H), 3.97 (s, 6 H), 5.22 (s, 4 H), 6.04 (s, 2 H), 6.09-6.11 (m, 2 H), 6.48-6.50 (m, 2 H), 6.59-6.60 (m, 2 H), 7.28-7.30 (m, 2 H), 7.31-7.35 (m, 6 H), 7.36-7.39 (m, 4 H), 9.21 (bs, 2 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 12.3 (2C), 17.3 (2C), 58.5 (2C), 65.4 (2C), 96.0 (2C), 110.7 (2C), 114.2 (2C), 116.7 (2C), 117.9 (2C), 123.0 (2C), 126.5 (2C), 127.9 (2C), 128.3 (4C), 128.6 (4C), 131.7 (2C), 133.1 (2C), 136.9 (2C), 141.4 (2C), 155.4 (2C), 156.6 (2C), 165.5 (2c), 167.0 (2C). HR-MS (ESI): $[M+Na]^+$ calcd. for $C_{48}H_{44}N_6O_6ZnNa$: 887.2506; found 887.2464.

(*Z*)-5-(4-Methoxy-1*H*,1'*H*-[2,2']bipyrrolyl-5-ylmethylene)-2,4-dimethyl-5*H*-pyrrole-3-carboxylic acid isopropylamide 3n: To a mixture of **4** (132 mg, 0.15 mmol) and a catalytic amount of palladium on activated carbon (10 mol%) in 50 mL round-bottom flask was added dry THF (10 mL) followed by 1 drop of triethylamine. Hydrogenolysis of the benzyl ester was achieved via stirring the reaction mixture for 15 h under 1 atm hydrogen. The mixture was then filtered through a plug of Celite® to remove the catalyst and rinsed with dry THF (5 mL) and the

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mixture was quickly used in the next step. To this solution of carboxylic acid in dry THF (15 mL) was added DMAP (42 mg, 0.34 mmol) and HBTU (129 mg, 0.34 mmol) followed by *i*-propyl amine (10 mL) and the resulting solution was stirred at room temperature for 18 h. The reaction mixture was then passed through neutral (type III) alumina (**3n** is soluble in water) to remove reagents and concentrated in *vacuo*. The crude-material was then dissolved in dry THF and conc. aqueous HCl (42 mL, 1.5 equiv.) was added to the solution. The mixture was stirred for 30 minutes, and then 2 M NaOH (20 mL) was added and stirring was continued for another 30 minutes. The solution was then diluted with EtOAc and the organic layer was separated, dried (Na_2SO_4) and concentrated in *vacuo*. Purification using chromatography over neutral alumina (type III) (EtOAc/hexane 0/10 to 40/60) gave the desired product as a deep brown solid (14 mg, 12%) ^{1}H NMR ($CDCl_3$, 500 MHz) 1.18 (d, J = 6.5 Hz, 6 H) 1.98 (s, 3 H), 2.29 (s, 3 H), 3.98 (s, 3 H), 4.16-4.23 (m, 1 H), 5.29 (d, J = 8.0 Hz, 1 H), 6.07 (s, 1 H), 6.16-6.17 (m, 1 H), 6.68-6.70 (m, 2 H), 6.88 (s, 1 H). ^{13}C NMR ($CDCl_3$, 125 MHz) 10.7 11.4, 23.1, 41.4, 58.7, 96.1, 110.5, 112.5, 113.7, 119.7, 123.5, 125.8, 128.1, 128.2, 138.8, 139.1, 160.8, 165.3, 169.2. HR-MS (ESI): $[M+H]^+$ calcd. for $C_{20}H_{25}N_4O_2$: 353.1972; found 353.1956. HPLC: Regis Pirkle Covalent Whelk 01 (250 × 4.60 mm), MeOH/(H_2O/NH_4OH, 500/1, v/v) 90/10, 0.75 mL/min, λ = 451 nm, ^{t}R = 15.2 min.

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2) Growth inhibition and cytotoxity assays

Human cell-line screening and *in vivo* toxicity evaluations were performed by the Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute (http://dtp.cancer.gov).

3) Anion exchange transport assays

Materials. Egg-yolk phosphatidylcholine (EYPC) lipid was purchased from Avanti Polar Lipids. Polycarbonate membranes and the extrusion apparatus used for making the liposomes was also from Avanti Polar Lipids. Salts (> 99% purity) were purchased from Sigma-Aldrich and used as received. The fluorescent dye lucigenin was purchased from Sigma-Aldrich. Prodigiosin **1** was provided by the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, NCI. Buffer solutions were made using ultra-pure water (distilled and then passed through a Millipore filtering system). The pH adjustments were made using concentrated NaOH solution. Fluorescence experiments were completed using a Hitachi model F-4500 fluorescence spectrophotometer.

Preparation of EYPC liposomes. EYPC lipid solution (~60 mg of lipid) was evaporated under reduced pressure to produce a thin film that was then dried *in vacuo* overnight. The resulting lipid film was hydrated with 1 mL of a solution containing 20 mM HEPES (pH = 7.4), 100 mM NaCl and 1 mM of the lucigenin dye.[1] After 9 freeze/thaw cycles (thawing and then warming to 45°C) the liposomes were extruded through a 200 nm polycarbonate membrane 31 times at room temperature to convert any large multi-lamellar vesicles (LMVs) into small unilamellar vesicles (SUV). The liposome solution was then passed through a Sephadex (G-25) column to remove any excess lucigenin dye. The isolated liposomes were diluted in 20 mM HEPES (pH 7.4, 75 mM Na_2SO_4) to give a final concentration of 13 mM in EYPC lipid, assuming complete retention of lipid during the gel filtration process. The size of the liposomes was confirmed using dynamic light scattering experiments.

Chloride-Nitrate Anion Exchange Transport Assay in EYPC Liposomes. In a typical experiment, 0.04 mL of the stock EYPC liposome solution was diluted into 2 mL of a solution of 20 mM HEPES (pH 7.4, 100 mM $NaNO_3$) to give a solution that was 0.2 mM in lipid. The lucigenin's fluorescence was monitored for 720 s with an excitation wavelength of 372 nm and

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an emission at 504 nm. At t = 30 s we added 1 μL of a 0.2 mM DMSO solution of the prodigiosene transporter to the cuvette containing the EYPC solution, giving a 1:2,500 ligand to lipid ratio (or 0.04 mol %). The efflux of Cl^- from within the liposomes due to the transporter's catalysis of chloride-nitrate exchange was observed by an increase in the lucigenin's fluorescence. At t=660 s, we added 0.05 mL of a solution of 10 % Triton-X detergent in order to destroy the liposomes and allow for determination of the maximal fluorescence quenching of lucigenin by Cl^-. Experiments were repeated in triplicate and all traces reported in Figures 2-4 of the paper are the average of the three trials.

Determination of EC_{50} values for anion exchange by prodigiosin 1 and prodigiosenes 2 and 3h. The Hill equation describes the relationship between the concentration of a substrate and an observed effect.[2] It is often used in pharmacodynamics to describe the relationship between drug concentration (X) and observed effect (Y). A form of the Hill equation that is useful in the context of the supramolecular function of ion transport is shown below (equation 1), where K (EC_{50}) is the transporter's concentration for which 50% of maximum transport is obtained and n is the Hill coefficient of sigmoidality.

$$Y = V_{max}.X^n / (K^n + X^n) \quad \text{(equation 1)}$$

The Hill equation can be applied to ion transport by examining the effect of varying the concentration of transporter [X] on ion flux (Y). In this way, a value of K (EC_{50}) can be calculated and used to quantify transport activity; the lower the value of EC_{50}, the more potent the transporter.

To calculate the values of K and n for each compound, the transport assay was repeated for different concentrations of transporters with the same batch of EYPC liposomes. The transport experiment was initiated by addition of an aliquot of transporter solution at 25 ºC. The fluorescence of intravesicular lucigenin was monitored by excitation at 372 nm, and the emission was recorded at 503 nm with a 10 nm slit width. At the end of the experiment, 10 % aqueous Triton-X was injected to lyze the liposomes. This procedure describes how ion transport assays depicted in Figures **Figure S2-4** were performed.

Lucigenin fluorescence was converted to chloride concentration using the Stern-Volmer constant determined under the assay conditions. To measure the Stern-Volmer constant,

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liposomes were prepared as above, except that the liposomes were lysed with Triton-X at t = 30s. Then, 10 μL of 2.0 M NaCl was titrated every 30 s via the injection port. The titration was completed thrice. A plot of f_0/f vs. chloride concentration was generated (**Figure S1**), the slope of which is taken to be the Stern-Volmer constant. Using the Stern-Volmer constant and the lucigenin fluorescence data in **Figure S2-4**, the percentage of chloride anion that had entered into the vesicle at each transporter concentration was calculated at t = 150 s. The percentage of external chloride vs. natural log of transporter's concentration was plotted and fitted to Hill equation 1. This enabled us to calculate values for K (EC_{50}), 150s.

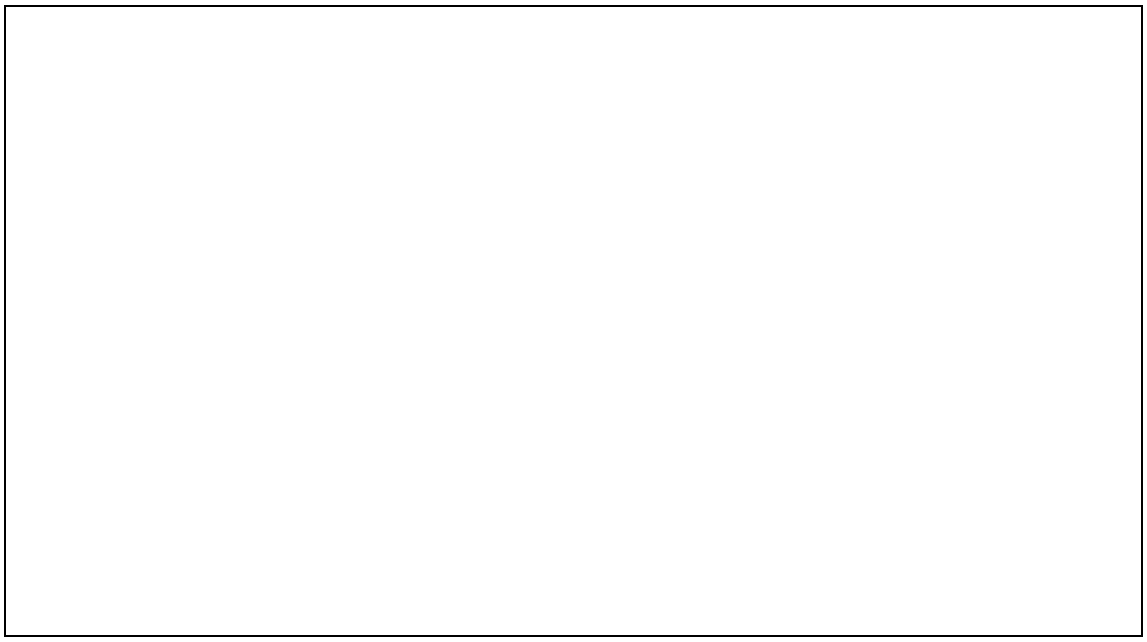

Figure S1: Determination of the Stern-Volmer constant for lucigenin quenching by Cl^-.

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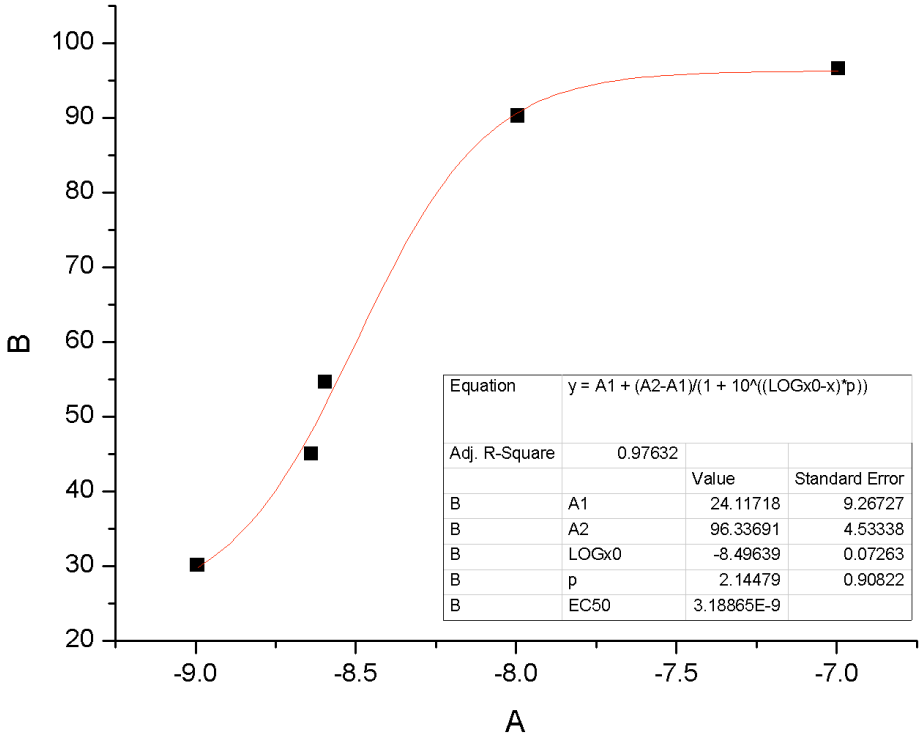

Figure S2: EC_{50} dose response curve for prodigiosin **1** at 150 s. Using the Stern-Volmer constant and lucigenin fluorescence measurements, the percentage of chloride that had exited the liposome after 150 vs. the natural log of concentration of prodigiosin **1** was plotted and fitted to Hill equation 1. This enabled us to calculate values for K (EC_{50}), 150s.

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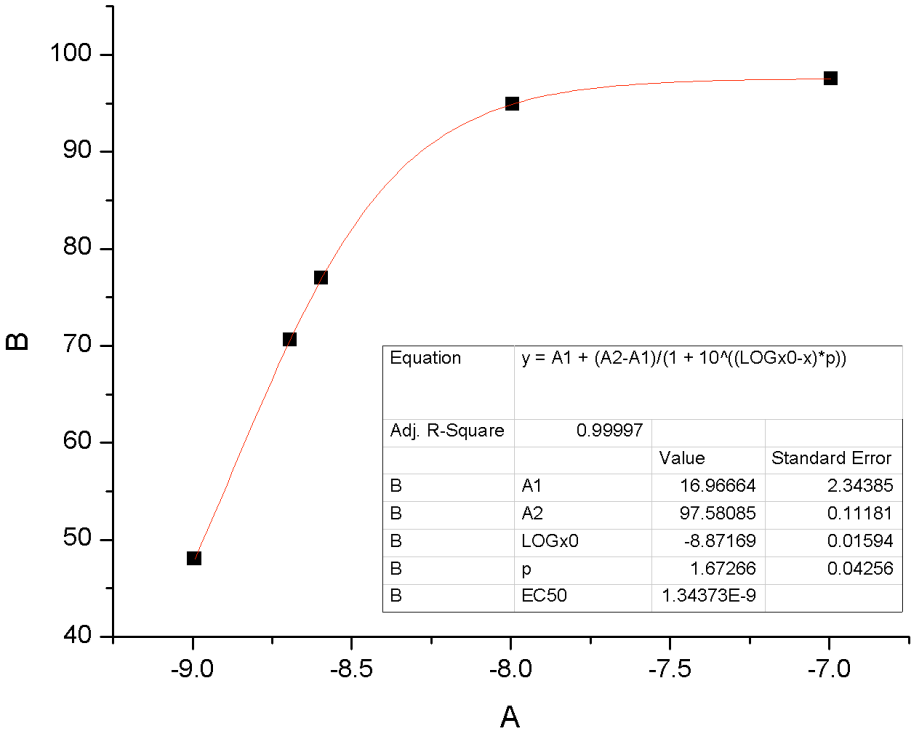

Figure S3: EC_{50} dose response curve for prodigiosene **2** at 150 s. Using the Stern-Volmer constant and lucigenin fluorescence measurements, the percentage of chloride that had exited the liposome after 150 vs. the natural log of concentration of prodigiosine **2** was plotted and fitted to Hill equation 1. This enabled us to calculate values for K (EC_{50}), 150s.

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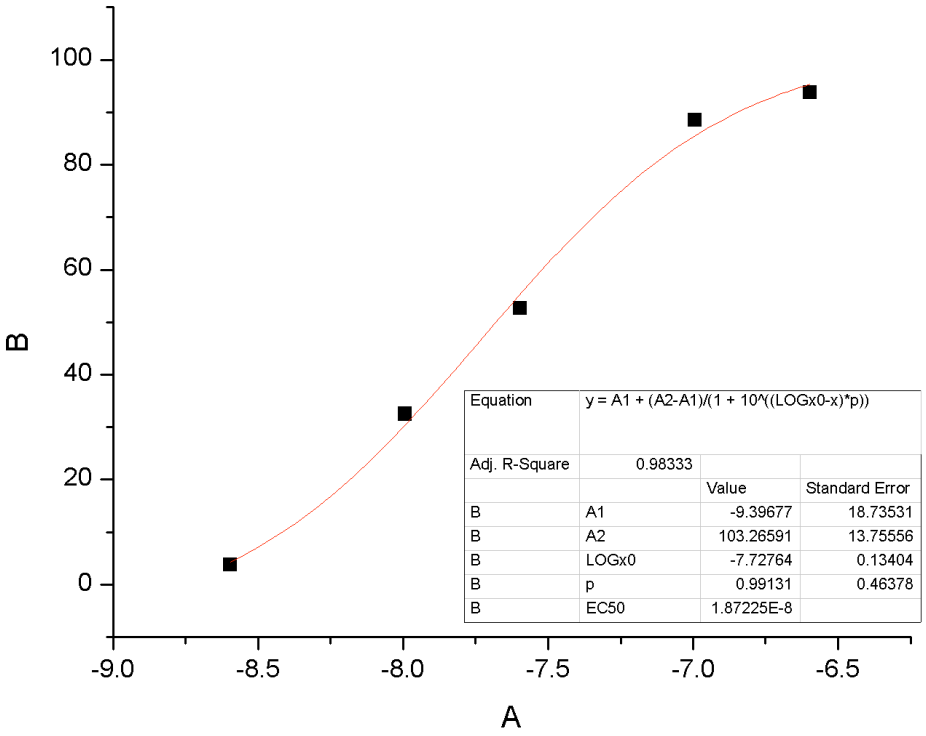

Figure S4: EC_{50} dose response curve for prodigiosene **3h** at 150 s. Using the Stern-Volmer constant and lucigenin fluorescence measurements, the percentage of chloride that had exited the liposome after 150 vs. the natural log of concentration of prodigiosine **3h** was plotted and fitted to Hill equation 1. This enabled us to calculate values for K (EC_{50}), 150s.

Preparation of EYPC liposomes for pH Variation Assay. EYPC lipid solution (~60 mg of lipid) was evaporated under reduced pressure to produce a thin film that was then dried *in vacuo* overnight. The resulting lipid film was hydrated with 1 mL of a solution containing 10 mM phosphate (pH = 7.5), 100 mM $NaNO_3$ and 1 mM of the lucigenin dye. After 9 freeze/thaw cycles (thawing and then warming to 45°C) the liposomes were extruded through a 200 nm polycarbonate membrane 31 times at room temperature to convert any large multi-lamellar vesicles (LMVs) into small unilamellar vesicles (SUV). The liposome solution was then passed through a Sephadex (G-25) column to remove any excess lucigenin dye. The isolated liposomes were diluted in 10 mM phosphate (pH 7.5, 75 mM Na_2SO_4) to give a final concentration of 12 mM in EYPC lipid, assuming complete retention of lipid during the gel filtration process. The size of the liposomes was confirmed by dynamic light scattering experiments.

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pH Variation Assay in EYPC Liposomes. In a typical experiment, 0.04 mL of the stock EYPC liposome solution was diluted into 2 mL of a solution of 10 mM phosphate (pH 6.5, 7.5 or 8.5) containing 100 mM $NaNO_3$ to give a solution that was 0.2 mM in lipid. The lucigenin's fluorescence was monitored for 720 s with an excitation wavelength of 372 nm and an emission at 504 nm. At t = 30 s, we added 1 μL of a 0.2 mM DMSO solution of the prodigiosene transporter to the cuvette containing the EYPC solution, giving a 1:2,500 ligand to lipid ratio (or 0.04 mol %). A pulse of 10 mM phosphate (pH 6.5, 7.5 or 8.5), 1 M NaCl and 100 mM $NaNO_3$ was added at 60 s to get a final concentration of 100 mM NaCl in the cuvette. The influx of Cl^- to the inside of liposomes, due to the transporter's catalysis of chloride-nitrate exchange, was observed by a decrease in the lucigenin's fluorescence. At t = 660 s, we added 0.07 mL of a solution of 10 % Triton-X detergent in order to destroy the liposomes and allow for determination of the maximal fluorescence quenching of lucigenin by Cl^-. Experiments were repeated in triplicate and all traces reported in Figure 8 of the paper are the average of the 3 trials.

4) NMR titrations

As shown in Figure 5 of the paper, ^{1}H-NMR experiments were performed at 3 °C wherein a mixture of two compounds in CD_3CN was titrated with acid. Methanesulfonic acid (MSA) was used as a source of the protons. In a typical NMR experiment involving a mixture of two compounds, solutions were prepared containing 1 mM of each compound in a total volume of 0.8 mL. If the compound mixture was in the form of an HCl salt, then 2 μL of 0.4 M solution of tetraethylammonium bicarbonate (TEAB) was first added to the solution to create the free-base form of the compounds. Then, 2 μL of a 0.08 M solution of MSA in CD_3CN was consecutively added to obtain 0.2, 0.4, 0.6, 0.8 and 1.0 eq in the NMR tube. Then, 5 μL of MSA was added twice to obtain 1.5 and 2.0 eq. Finally, 10 μL of MSA solution was added to obtain 3 eq of MSA in the NMR tube. After each addition of MSA, a ^{1}H-NMR spectrum was recorded using a Bruker AVIII-600MHz instrument at 3°C. Chemical shifts were referenced to the non-deuterated methyl group at δ 1.93 in the CD_3CN solvent.

Determination of pK_a Values for Prodigiosin 1 and Prodigiosene Ester 3i

We used a spectrophotometric procedure described by Manderville and colleagues to determine the apparent pK_a values for prodigiosin **1** and prodigiosene Ester **3i**. [33] The UV-vis spectra were recorded on a Cary 100 UV-Visible spectrophotometer. Standard 10 mm quartz glass cells from Starna Cells Inc. were used. All UV-Vis spectra were recorded at 25 °C with baseline correction.

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The pH measurements were made using an Accumet AR25A pH/ion meter equipped with a Accumet pH microelectrode. Calibration was achieved using commercial buffers (Thermo Scientific, pH 4.00, 7.00 and 10.00, all ±0.01). Stock solutions (2 mM) of prodigiosin **1** and prodigiosene-ester **3i** were prepared in CH_3CN. Then, 15 μL of the stock solution was added to a quartz cuvette containing 2 mL total volume of 1:1 CH_3CN/H2O (v/v). The ionic strength was kept constant by using 0.1 M NaCl. Acidity constants were determined spectrophotometrically by monitoring absorbance changes in the UV-Vis spectra after additions of dilute HCl or NaOH solutions under constant temperature conditions (25 °C). The λ_{max} for the protonated species of prodigiosin **1•H$^+$** was observed at 533 nm and the λ_{max} for the free-base **1** occurred at 460 nm (**Figure S5**). For the hexyl ester derivative **3i**, the λ_{max} for the protonated form **3i•H$^+$** was at 517 nm and λ_{max} for the free-base **3i** was around 450 nm (**Figure S6**). The pKa values were determined from a plot of log (ionization ratio) vs. pH.[3] Based on these UV-vis spectra, pKa values were calculated to be 8.2 for prodigiosin **1•H$^+$** and 6.5 for ester **3i•H$^+$**.

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Brandon Groves,[†] Julie Colpitts,[§] Sherri A. McFarland[§*]
Jeffery T. Davis,[‡*] and Alison Thompson[†*]

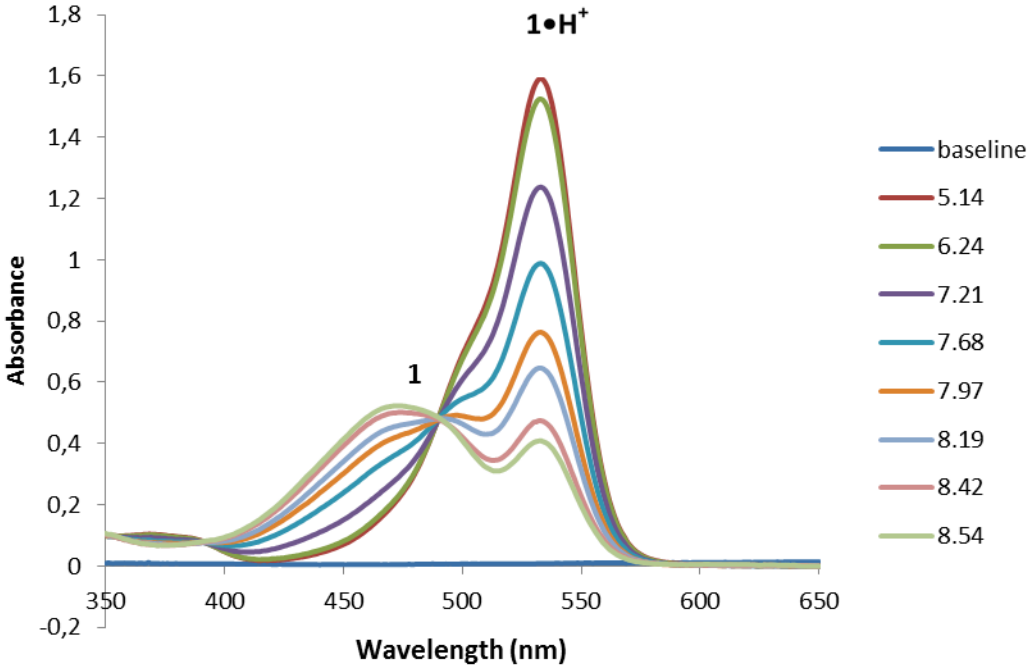

Figure S5: UV-vis absorbance spectra for prodigiosin **1** as a function of pH in 1:1 CH_3CN/H_2O (v/v) at 25 °C (0.1 M NaCl).

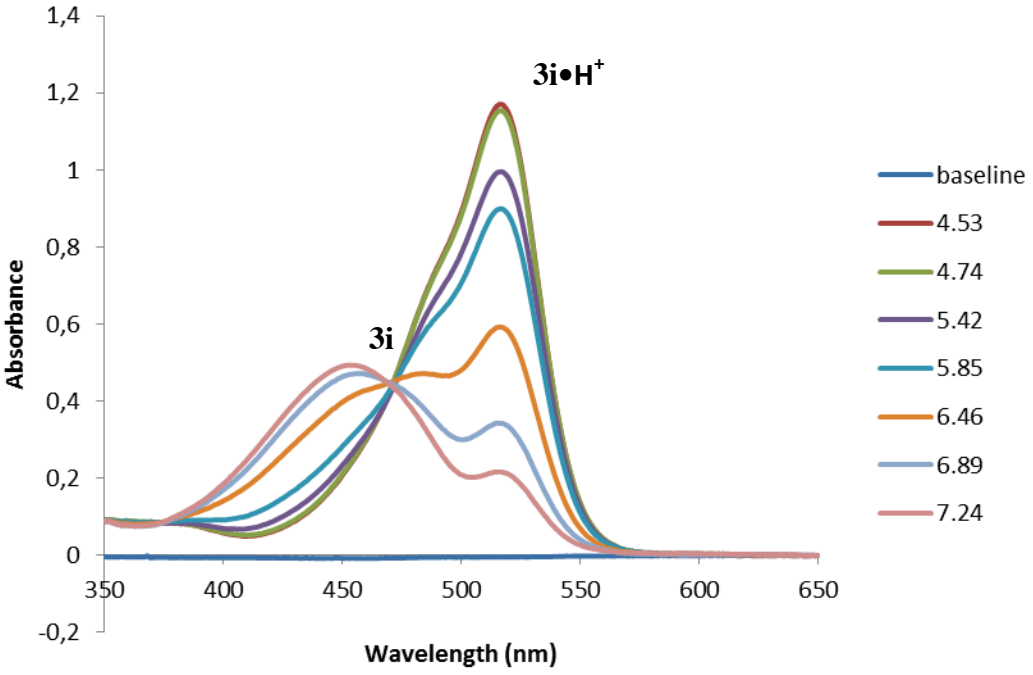

Figure S6: UV-vis absorbance spectra for ester **3i** as a function of pH in 1:1 CH_3CN/H_2O (v/v) at 25 °C (0.1 M NaCl).

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5) DNA cleavage assays

Materials and methods

Supercoiled pUC19 DNA plasmid (Form I) was transformed in NovaBlue cells (Novagen) followed by purification using the QIAprep Spin Miniprep Kit (Qiagen), yielding approximately 30 μg of plasmid DNA per 20-mL culture. All prodigiosenes were dissolved initially in DMSO and subsequent dilutions were made with water (distilled, deionized from a Milli-Q system) so that the final assay tubes contained <5% DMSO. Reaction mixtures (20 μL total volume) were prepared in 0.5 mL sterile microfuge tubes. Transformed pUC19 plasmid (130 ng, >95% Form I) was pipetted into the assay tubes as a solution in 10 mM Tris-Cl (pH 8.5) along with 5 mM (final concentration) Tris 50 mM (final concentration) NaCl buffer (pH 7.4) to give a final concentration of 20 μM DNA bases. When prodigiosene-copper mixtures where desired, prodigiosene dilutions were pre-mixed with equimolar cupric acetate and added to the assay tubes to achieve the desired final concentrations. All reaction mixtures were diluted to a final volume of 20 μL with ultra-pure water and incubated at 37°C for 2 hours. Samples were then quenched by the addition of 4 μL gel loading buffer, loaded onto 1% agarose gels containing ethidium bromide (0.75 μg mL^{-1}) and electrophoresced for 30 min at 8 V cm^{-1} in 1X TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.2). The bands were visualized with UV-transillumination (UVP transilluminator).

Densitometric analysis of the gels was performed using VisionWorks®LS image software. Non-linear sigmoidal dose-response curves were generated by least-squares fits using KaleidaGraph 4.03. EC_{50} values show the effective concentration where 50% DNA nicking occurs. All experiments were run in duplicate or triplicate to give an EC50 value with +/- 2% error.

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Jeffery T. Davis,[‡*] and Alison Thompson[†*]

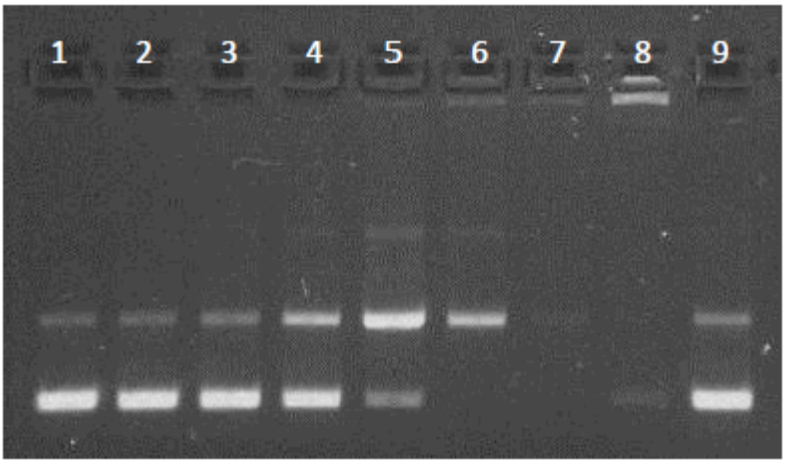

Figure S7: Copper-mediated DNA cleavage by **2**. Reaction mixtures (20 μL total volume) contained pUC19 DNA plasmid (20 μM bases) in 10 mM Tris, 100 mM NaCl buffer, pH 7.4 and were incubated at 37 ˚C for 2 hours: lane 1, DNA only; lane 2, 1 μM **2**/Cu(II); lane 3, 5 μM **2**/Cu(II); lane 4, 10 μM **2**/Cu(II); lane 5, 25 μM **2**/Cu(II); lane 6, 50 μM **2**/Cu(II); lane 7, 75 μM **2**/Cu(II); lane 8, 75 μM **2**; lane 9, 75 μM Cu(II).

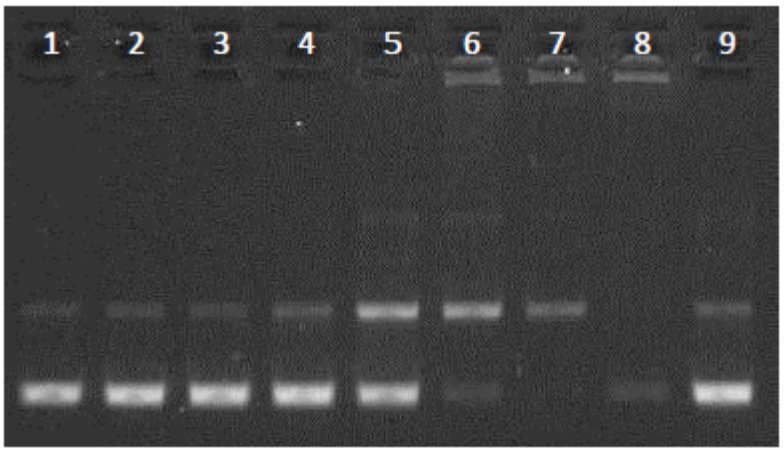

Figure S8: Copper-mediated DNA cleavage by **3b**. Reaction mixtures (20 μL total volume) contained pUC19 DNA plasmid (20 μM bases) in 10 mM Tris, 100 mM NaCl buffer, pH 7.4 and were incubated at 37 ˚C for 2 hours: lane 1, DNA only; lane 2, 1 μM **3b**/Cu(II); lane 3, 5 μM **3b**/Cu(II); lane 4, 10 μM **3b**/Cu(II); lane 5, 25 μM **3b**/Cu(II); lane 6, 50 μM **3b**/Cu(II); lane 7, 75 μM **3b**/Cu(II); lane 8, 75 μM **3b**; lane 9, 75 μM Cu(II).

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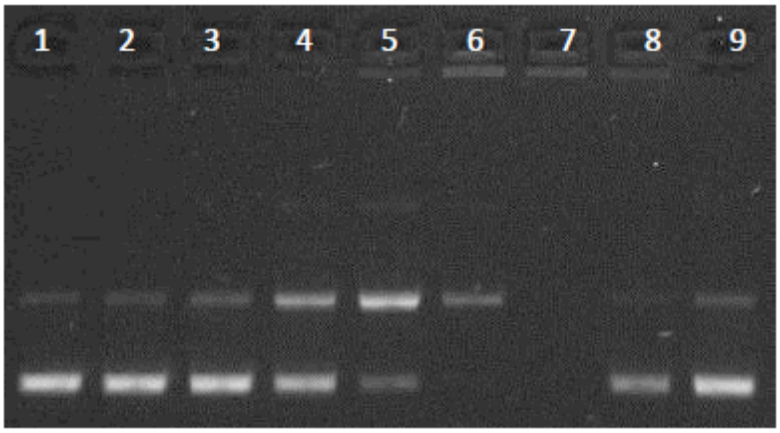

Figure S9: Copper-mediated DNA cleavage by **3c**. Reaction mixtures (20 μL total volume) contained pUC19 DNA plasmid (20 μM bases) in 10 mM Tris, 100 mM NaCl buffer, pH 7.4 and were incubated at 37 ˚C for 2 hours: lane 1, DNA only; lane 2, 1 μM **3c**/Cu(II); lane 3, 5 μM **3c**/Cu(II); lane 4, 10 μM **3c**/Cu(II); lane 5, 25 μM **3c**/Cu(II); lane 6, 50 μM **3c**/Cu(II); lane 7, 75 μM **3c**/Cu(II); lane 8, 75 μM **3c**; lane 9, 75 μM Cu(II).

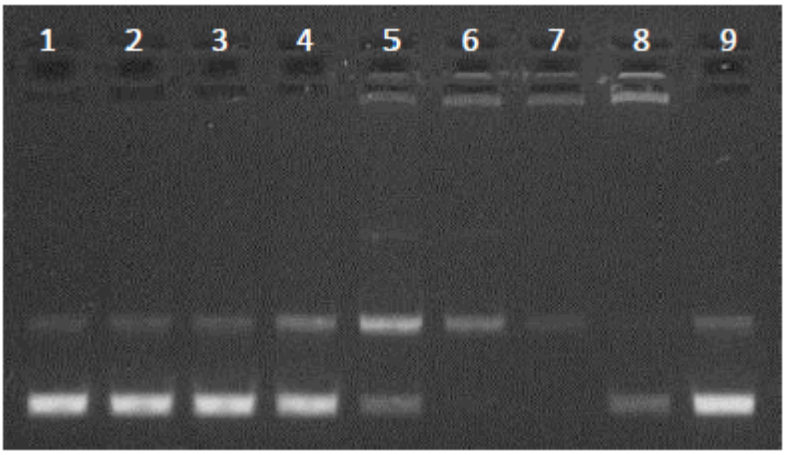

Figure S10: Copper-mediated DNA cleavage by **3d**. Reaction mixtures (20 μL total volume) contained pUC19 DNA plasmid (20 μM bases) in 10 mM Tris, 100 mM NaCl buffer, pH 7.4 and were incubated at 37 ˚C for 2 hours: lane 1, DNA only; lane 2, 1 μM **3d**/Cu(II); lane 3, 5 μM **3d**/Cu(II); lane 4, 10 μM **3d**/Cu(II); lane 5, 25 μM **3d**/Cu(II); lane 6, 50 μM **3d**/Cu(II); lane 7, 75 μM **3d**/Cu(II); lane 8, 75 μM **3d**; lane 9, 75 μM Cu(II).

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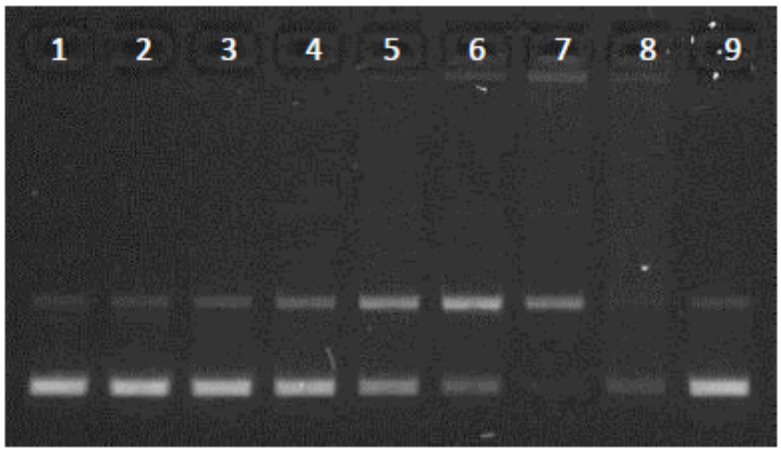

Figure S11: Copper-mediated DNA cleavage by **3f**. Reaction mixtures (20 μL total volume) contained pUC19 DNA plasmid (20 μM bases) in 10 mM Tris, 100 mM NaCl buffer, pH 7.4 and were incubated at 37 ˚C for 2 hours: lane 1, DNA only; lane 2, 1 μM **3f**/Cu(II); lane 3, 5 μM **3f**/Cu(II); lane 4, 10 μM **3f**/Cu(II); lane 5, 25 μM **3f**/Cu(II); lane 6, 50 μM **3f**/Cu(II); lane 7, 75 μM **3f**/Cu(II); lane 8, 75 μM **3f**; lane 9, 75 μM Cu(II).

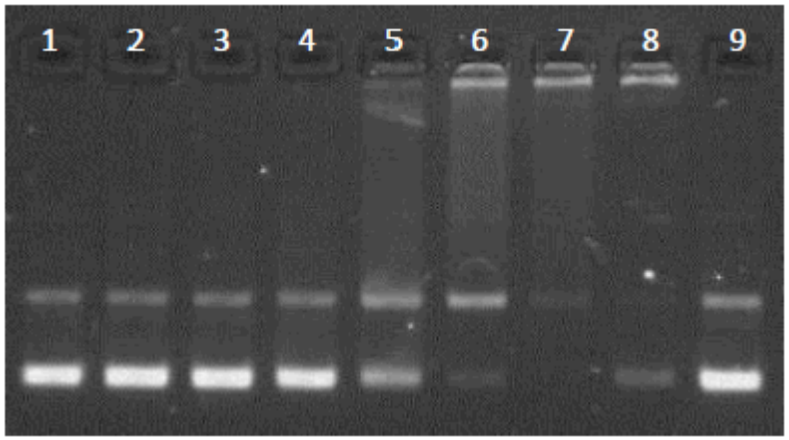

Figure S12: Copper-mediated DNA cleavage by **3g**. Reaction mixtures (20 μL total volume) contained pUC19 DNA plasmid (20 μM bases) in 10 mM Tris, 100 mM NaCl buffer, pH 7.4 and were incubated at 37 ˚C for 2 hours: lane 1, DNA only; lane 2, 1 μM **3g**/Cu(II); lane 3, 5 μM **3g**/Cu(II); lane 4, 10 μM **3g**/Cu(II); lane 5, 25 μM **3g**/Cu(II); lane 6, 50 μM **3g**/Cu(II); lane 7, 75 μM **3g**/Cu(II); lane 8, 75 μM **3g**; lane 9, 75 μM Cu(II).

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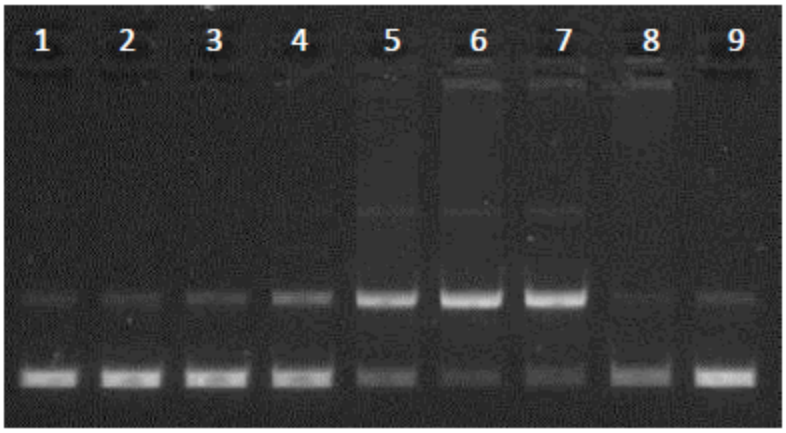

Figure S13: Copper-mediated DNA cleavage by **3j**. Reaction mixtures (20 μL total volume) contained pUC19 DNA plasmid (20 μM bases) in 10 mM Tris, 100 mM NaCl buffer, pH 7.4 and were incubated at 37 ˚C for 2 hours: lane 1, DNA only; lane 2, 1 μM **3j**/Cu(II); lane 3, 5 μM **3j**/Cu(II); lane 4, 10 μM **3j**/Cu(II); lane 5, 25 μM **3j**/Cu(II); lane 6, 50 μM **3j**/Cu(II); lane 7, 75 μM **3j**/Cu(II); lane 8, 75 μM **3j**; lane 9, 75 μM Cu(II).

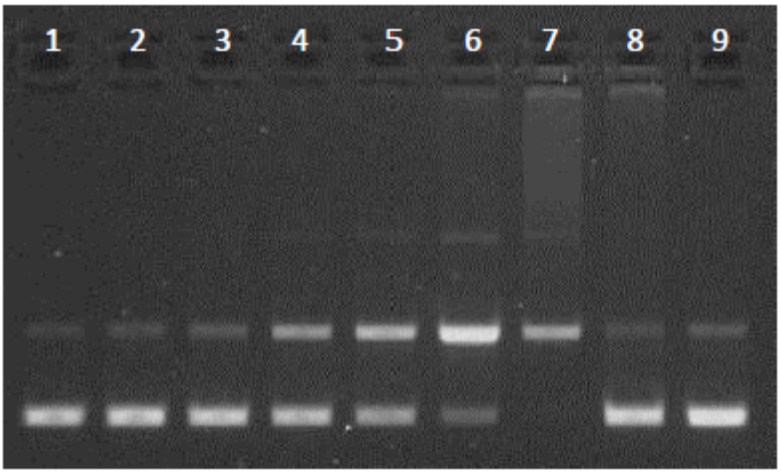

Figure S14: Copper-mediated DNA cleavage by **3k**. Reaction mixtures (20 μL total volume) contained pUC19 DNA plasmid (20 μM bases) in 10 mM Tris, 100 mM NaCl buffer, pH 7.4 and were incubated at 37 ˚C for 2 hours: lane 1, DNA only; lane 2, 1 μM **3k**/Cu(II); lane 3, 5 μM **3k**/Cu(II); lane 4, 10 μM **3k**/Cu(II); lane 5, 25 μM **3k**/Cu(II); lane 6, 50 μM **3k**/Cu(II); lane 7, 75 μM **3k**/Cu(II); lane 8, 75 μM **3k**; lane 9, 75 μM Cu(II).

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Jeffery T. Davis,[‡*] and Alison Thompson[†*]

6) Total growth inhibition and half maximal lethal concentrations

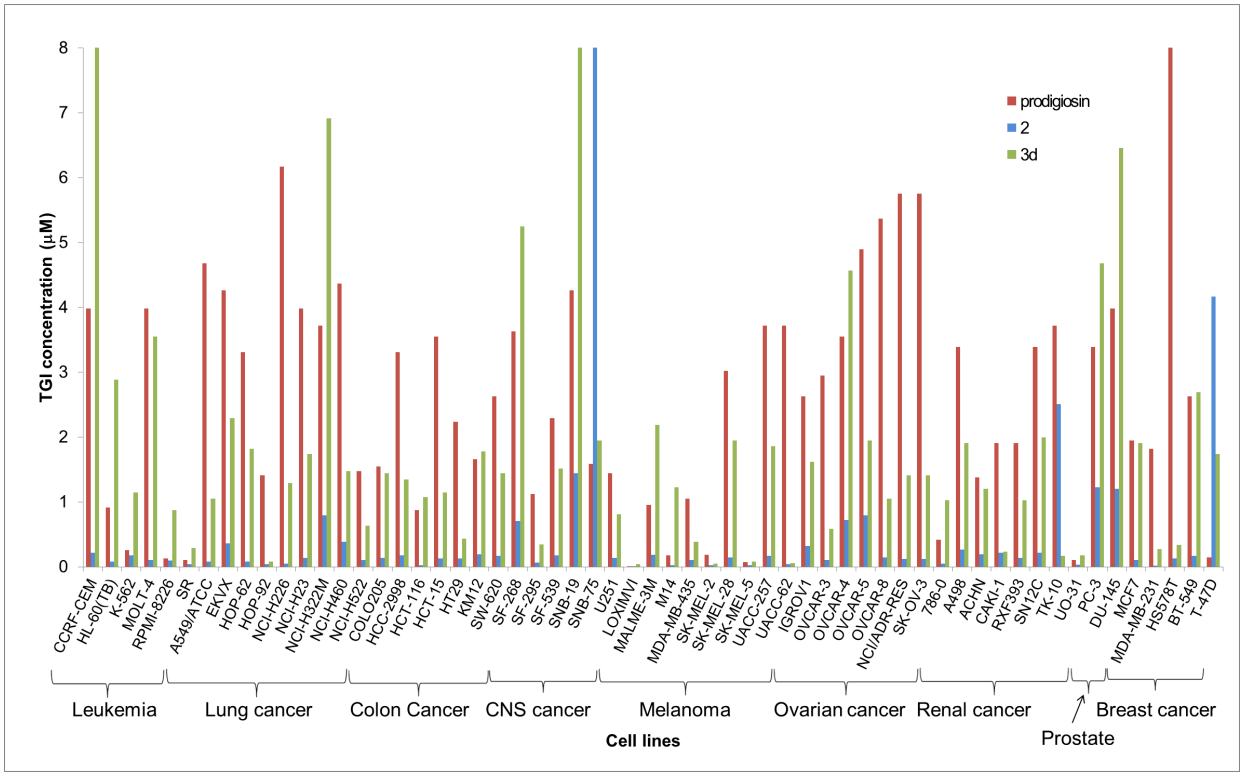

Figure S15: TGI concentrations (total growth inhibition concentrations) of prodigiosin, **2** and **3d** against 59 human cancer cell lines representing 9 different cancer types; http://dtp.cancer.gov.

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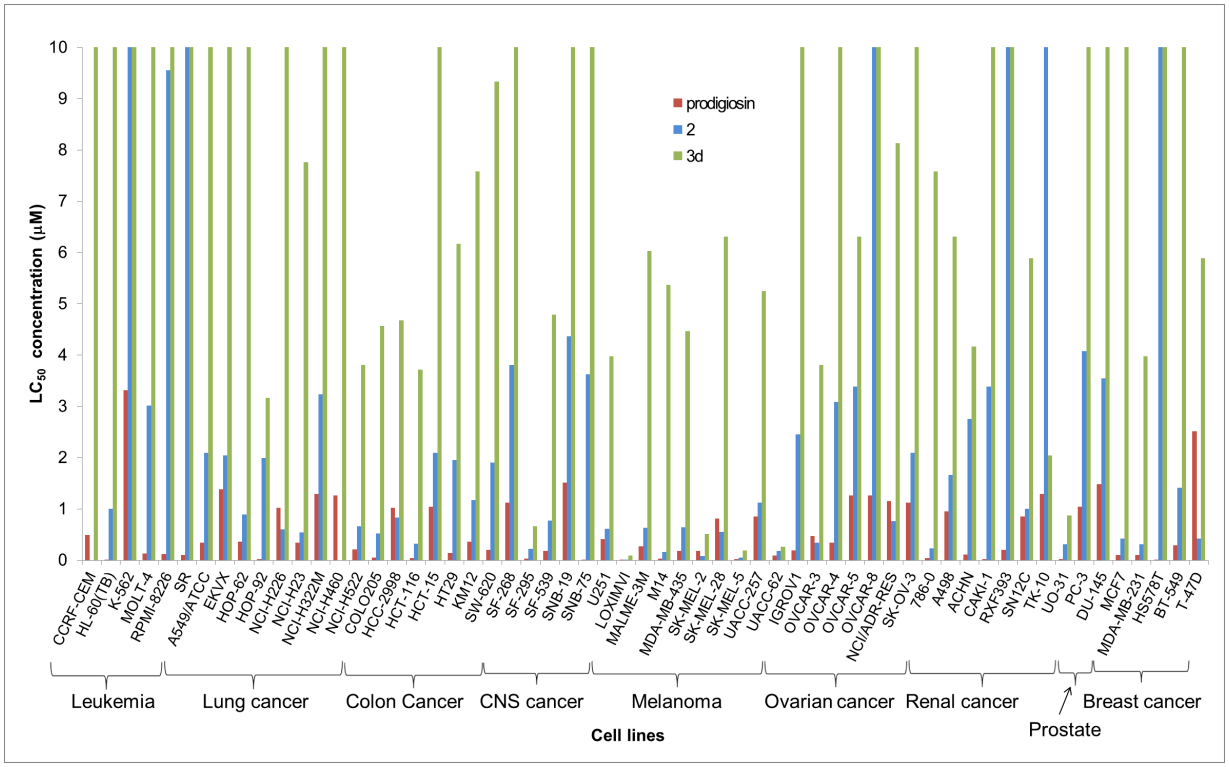

Figure S16: LC_{50} concentrations (half maximal lethal concentrations) of prodigiosin, **2** and **3d** against 59 human cancer cell lines representing 9 different cancer types; http://dtp.cancer.gov.

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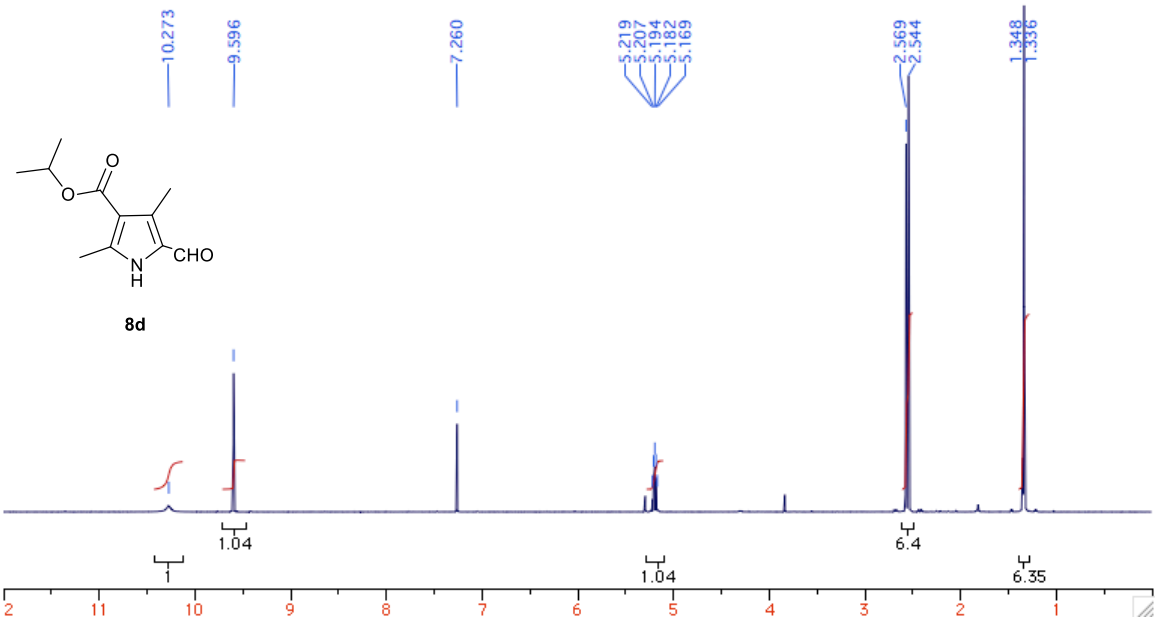
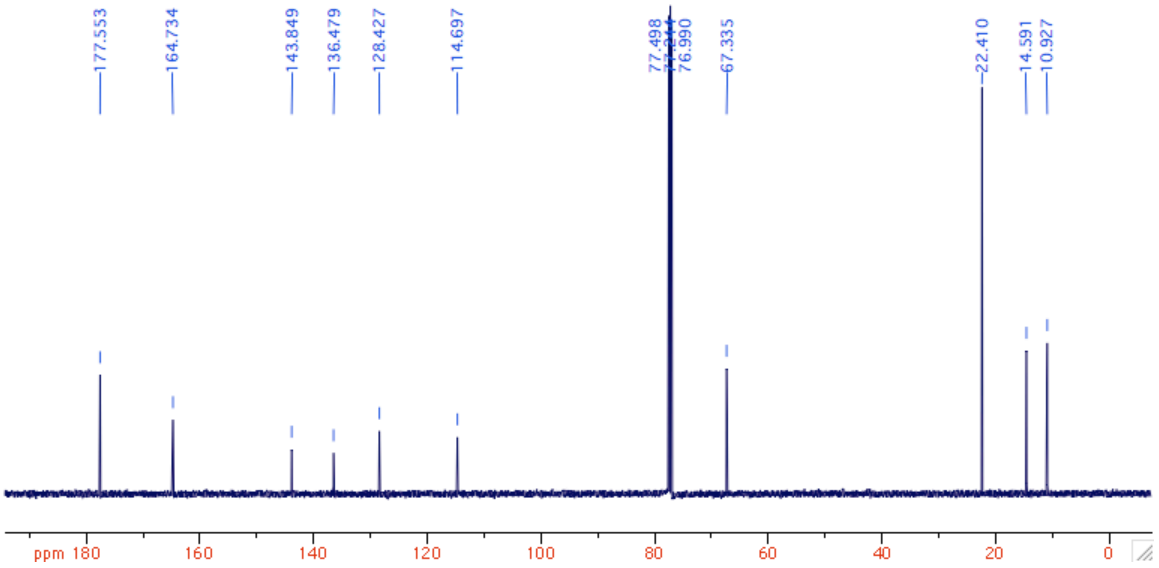
Brandon Groves,[†] Julie Colpitts,[§] Sherri A. McFarland[§*]
Jeffery T. Davis,[‡*] and Alison Thompson[†*]

7) ^{1}H and ^{13}C NMR spectra

^{1}H NMR, $CDCl_3$, 500 MHz



^{13}C NMR, $CDCl_3$, 125 MHz



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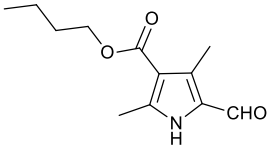
^{1}H NMR, $CDCl_3$, 500 MHz



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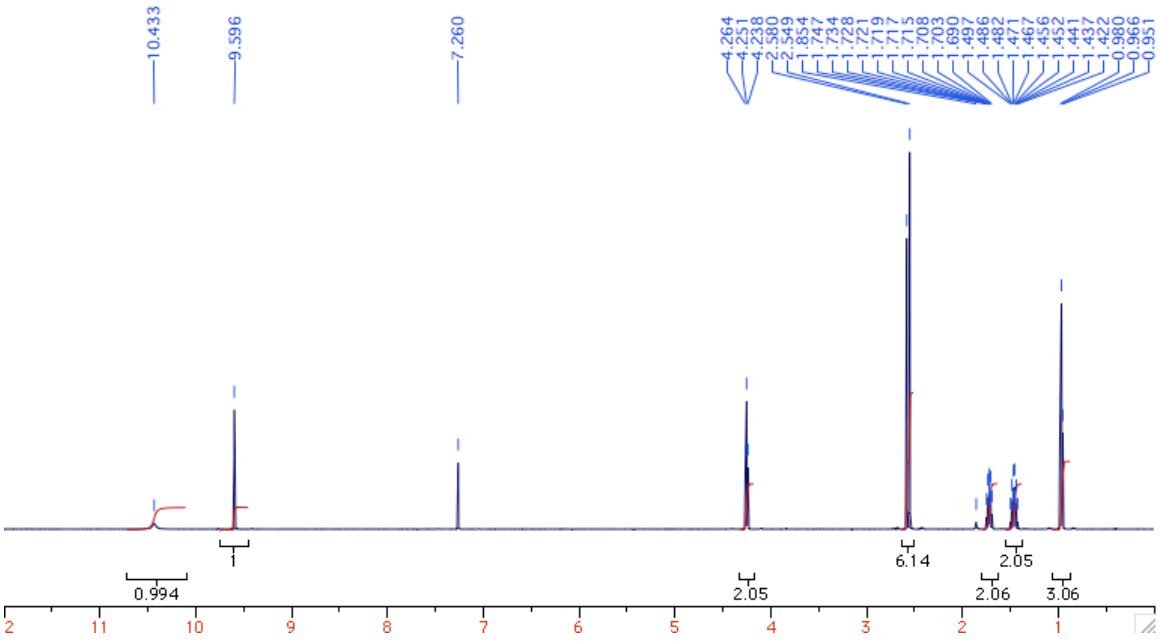

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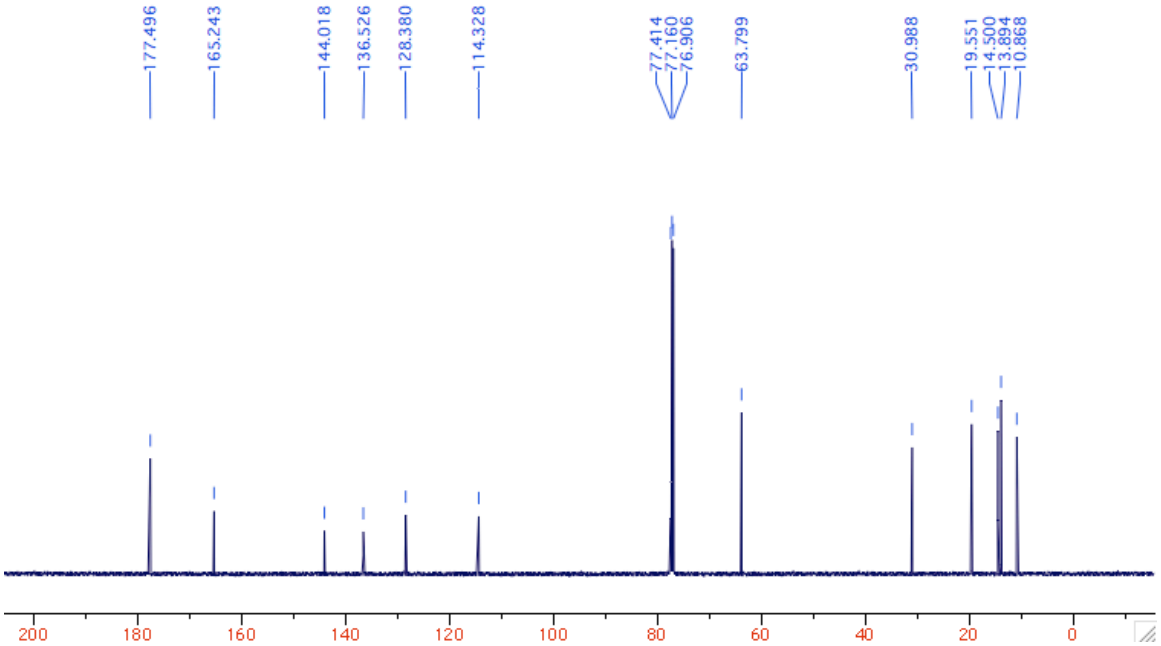

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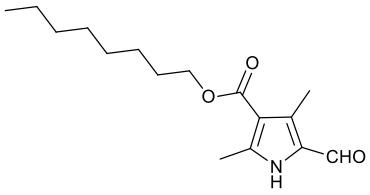
^{1}H NMR, $CDCl_3$, 500 MHz



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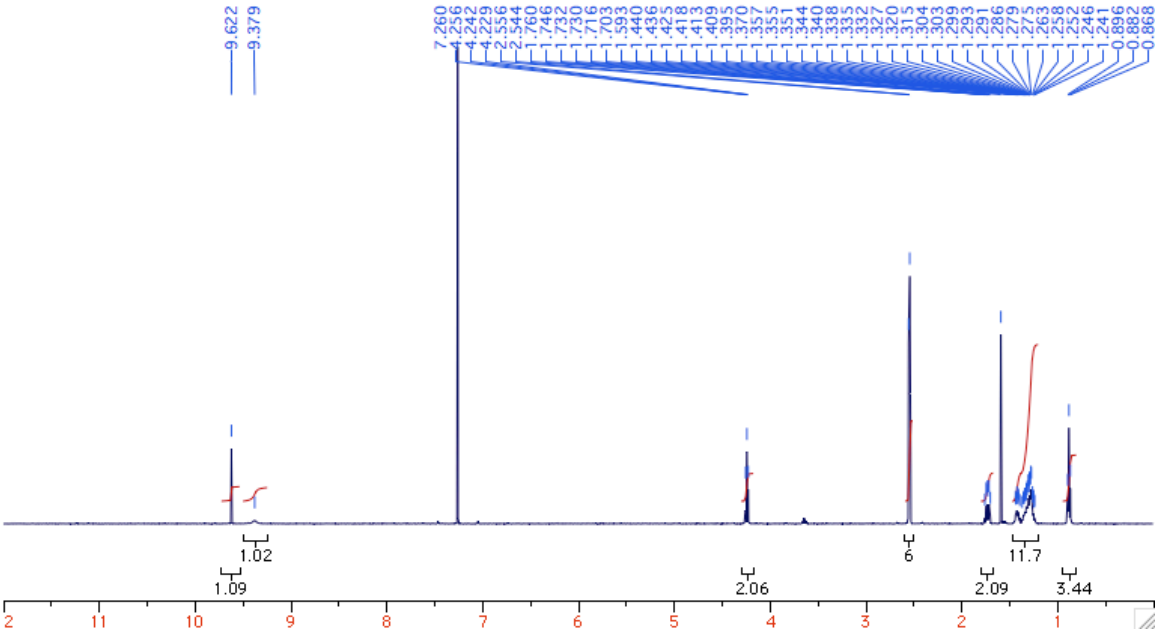

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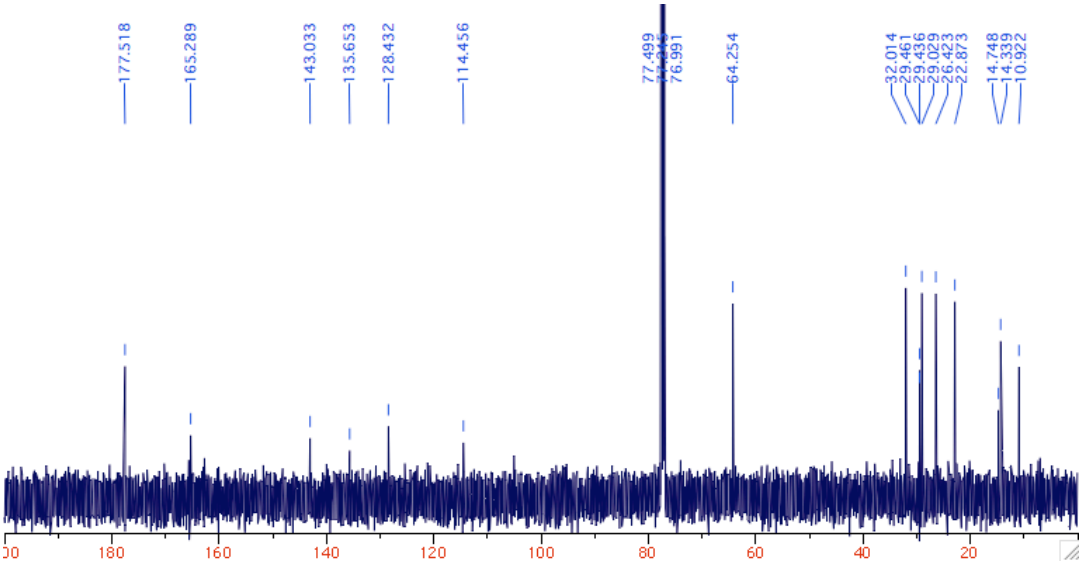

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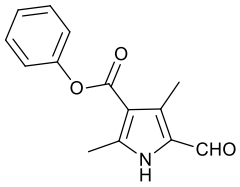

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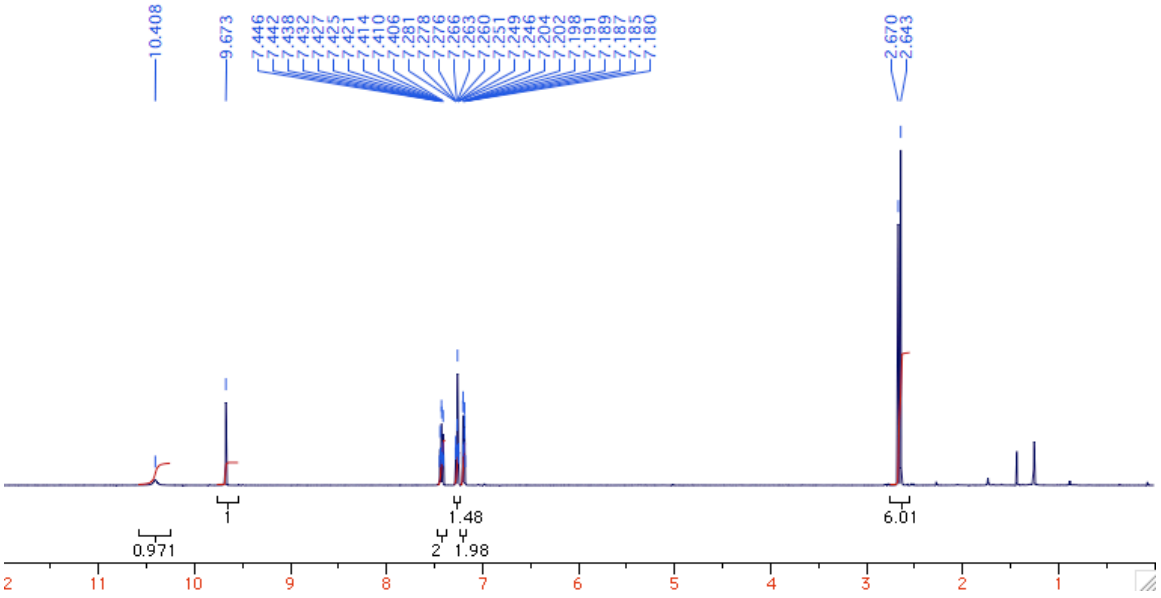
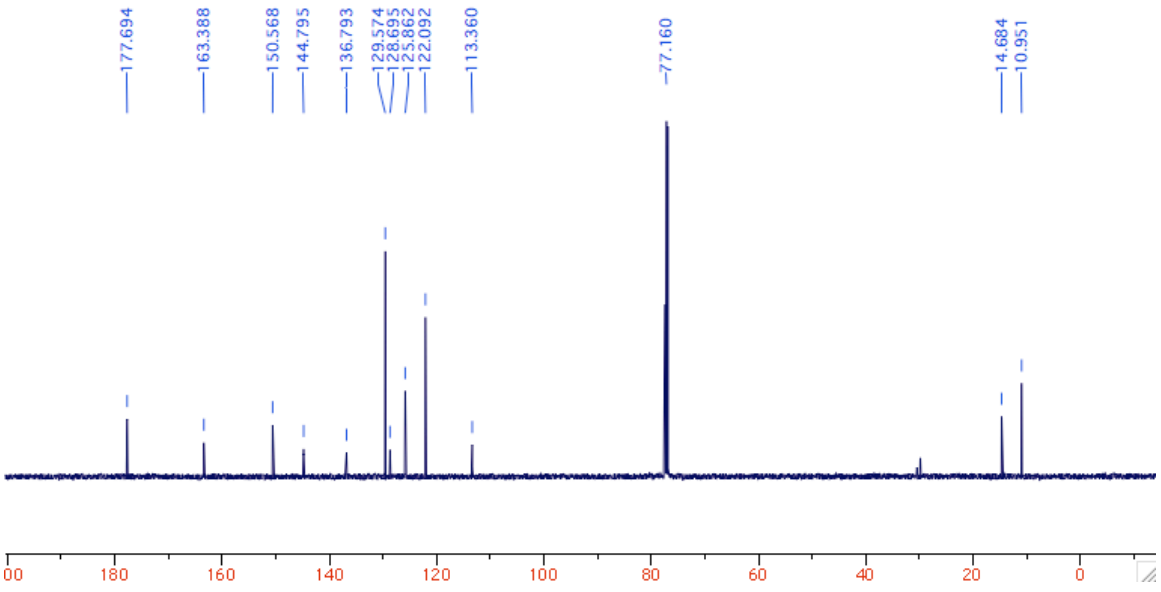


^{13}C NMR, $CDCl_3$, 125 MHz



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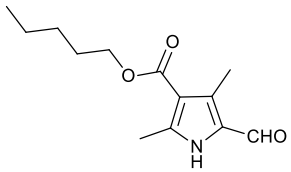

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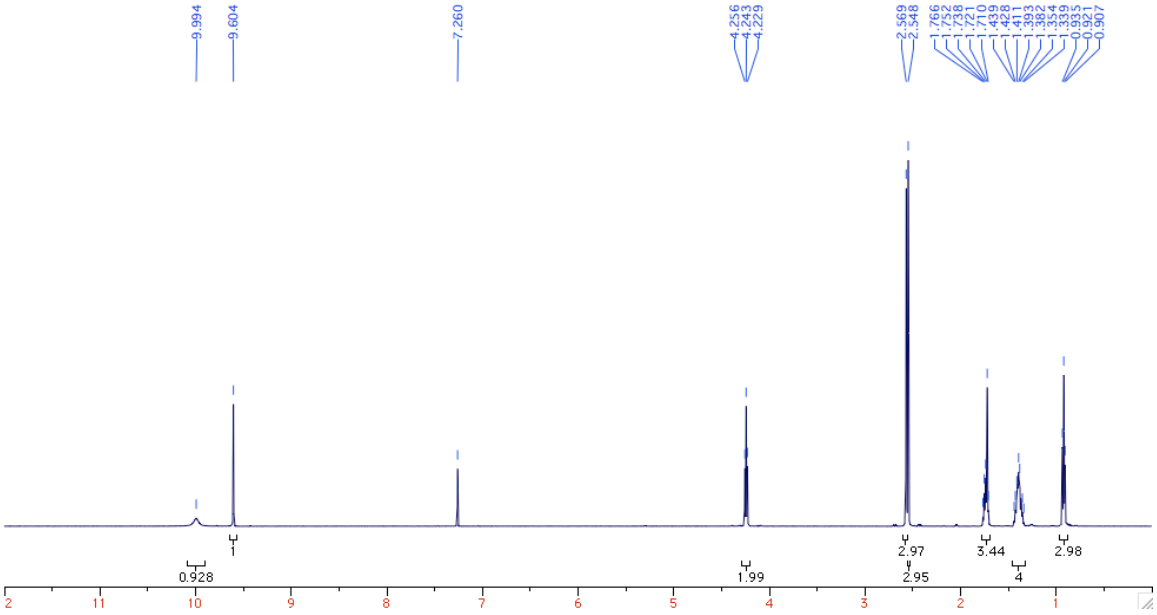

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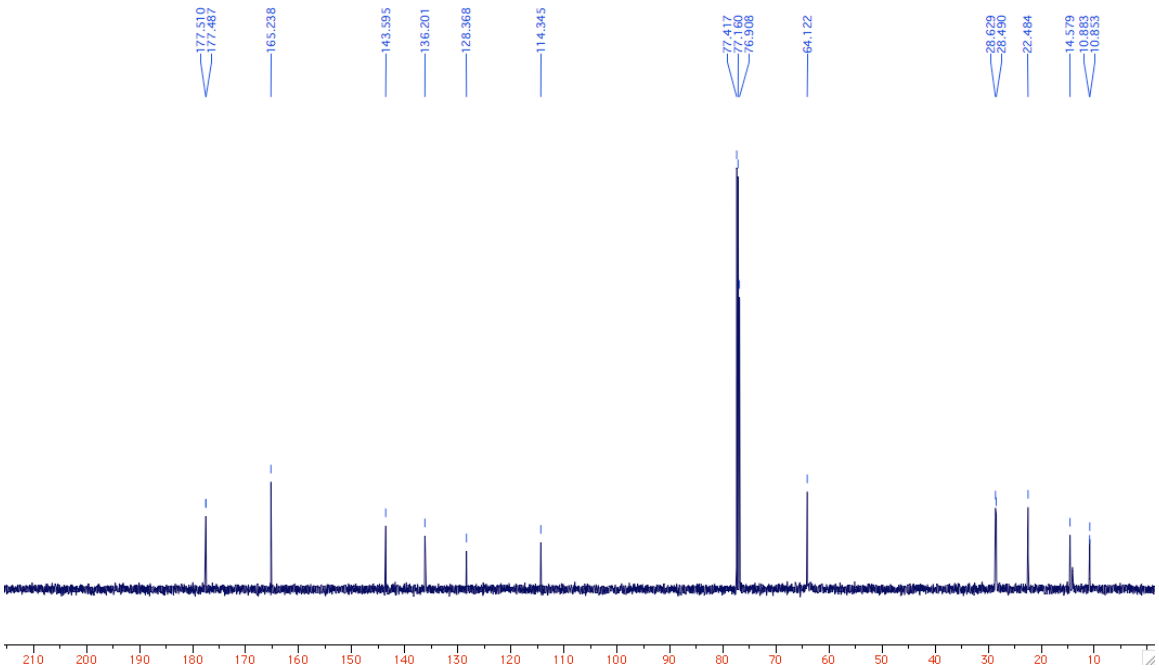

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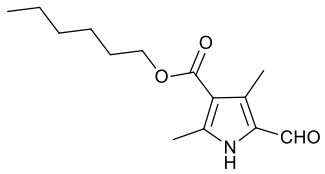

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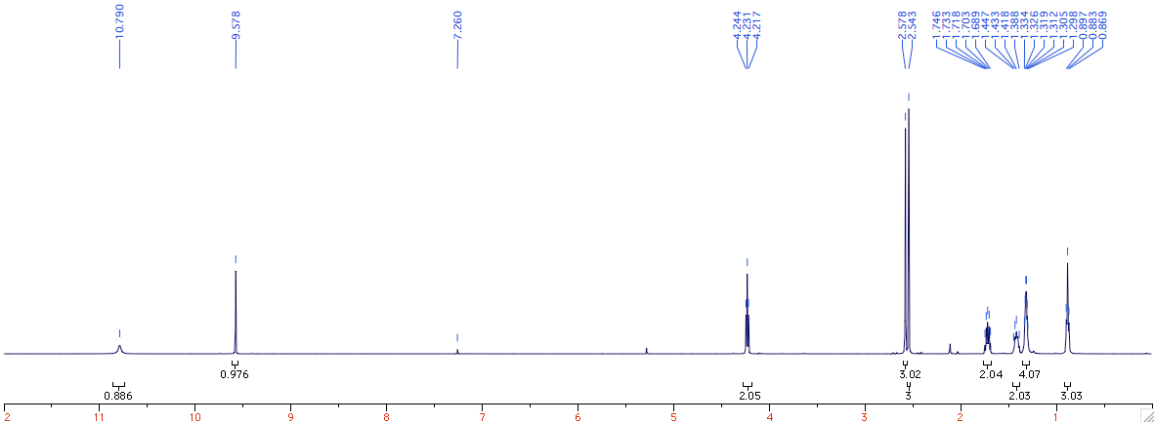

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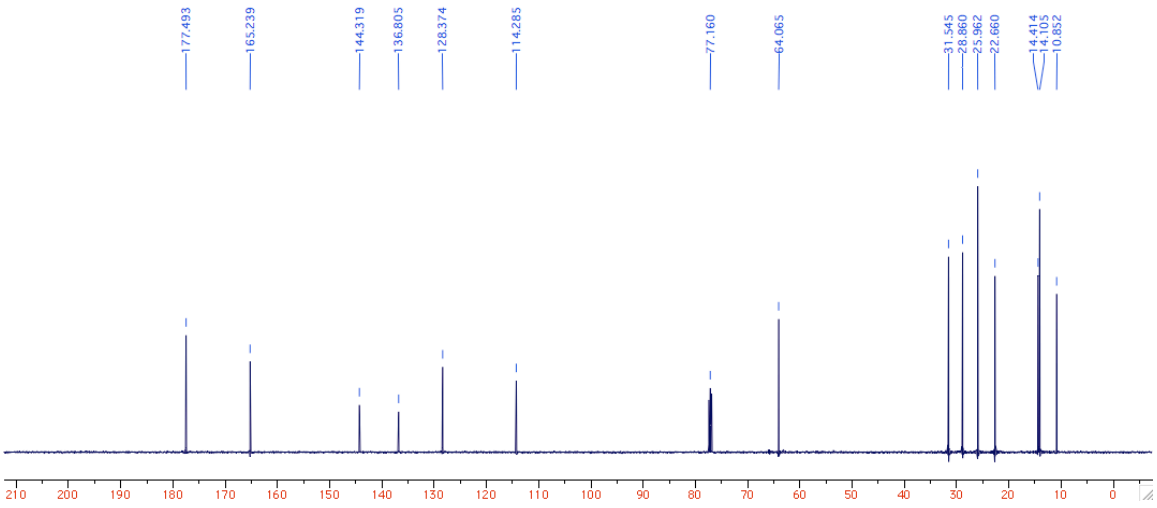

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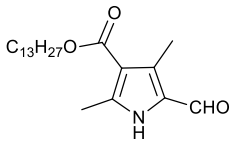
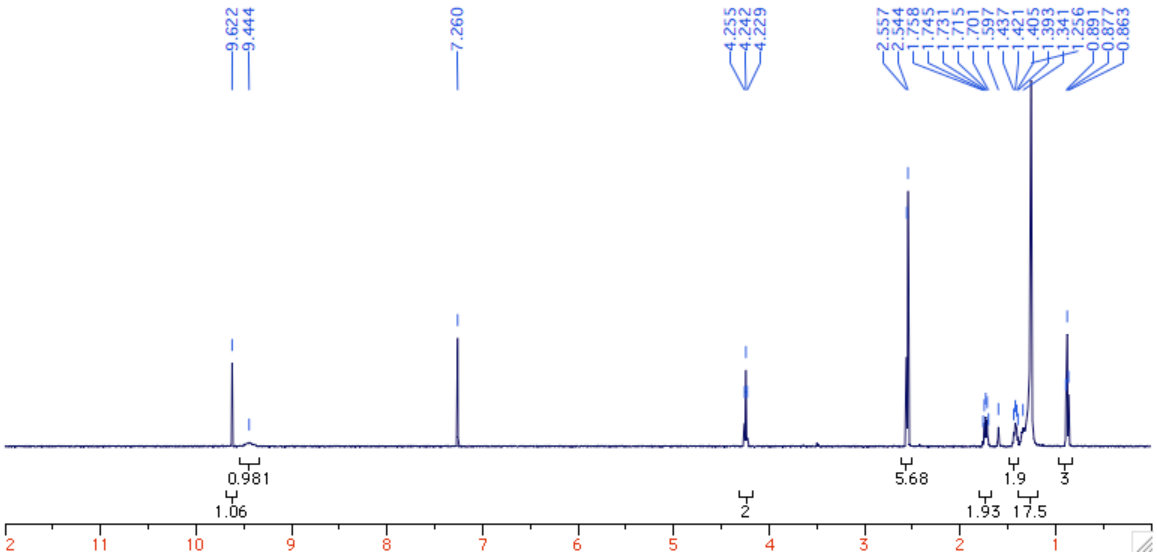
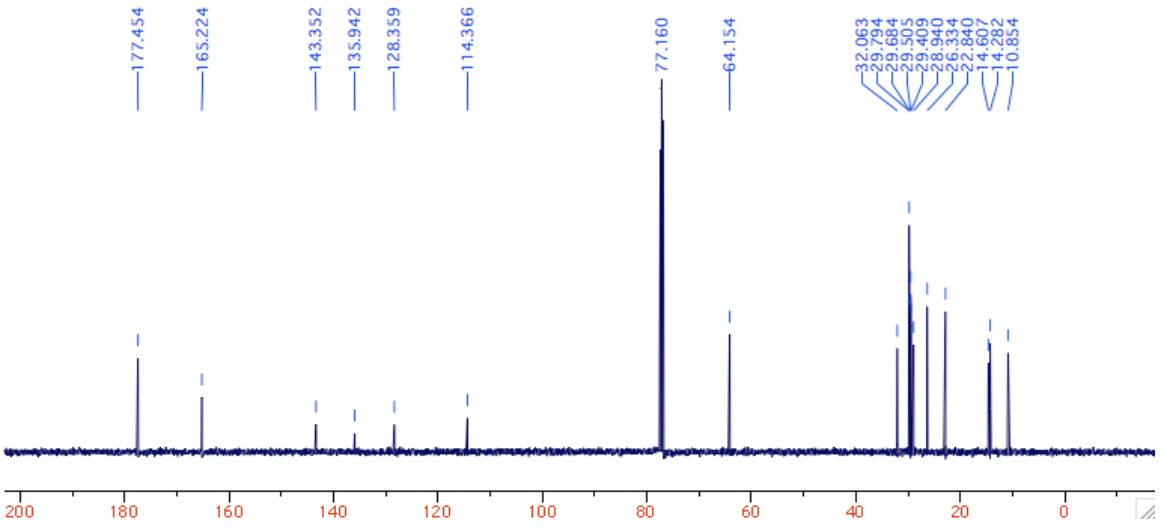


8j

^{1}H NMR, $CDCl_3$, 500 MHz



^{13}C NMR, $CDCl_3$, 125 MHz



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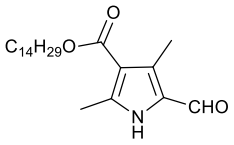

8k

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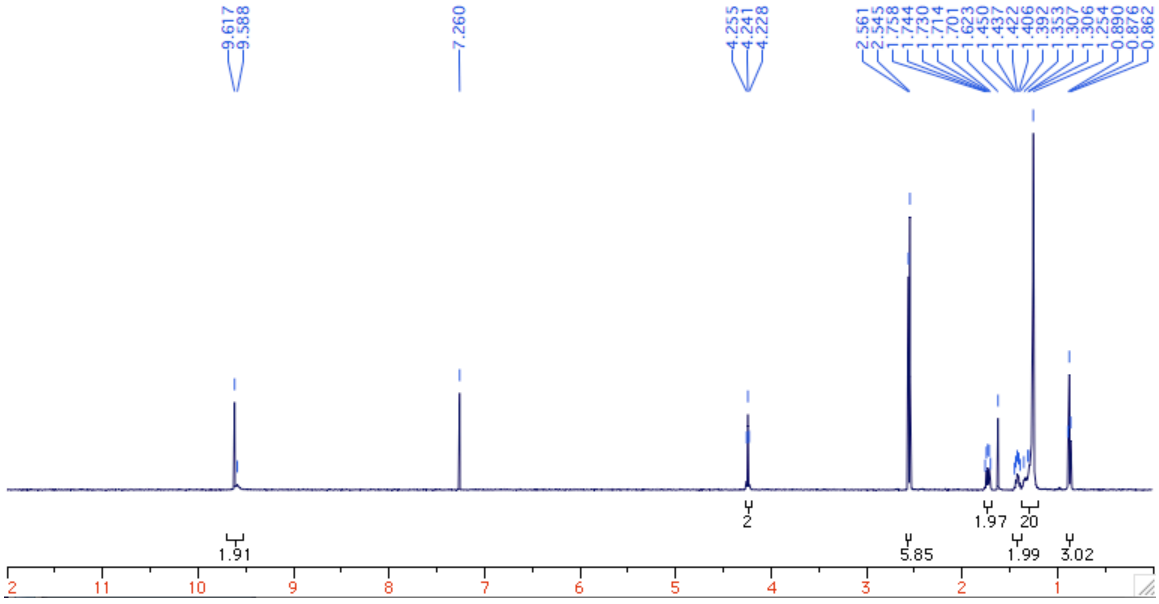
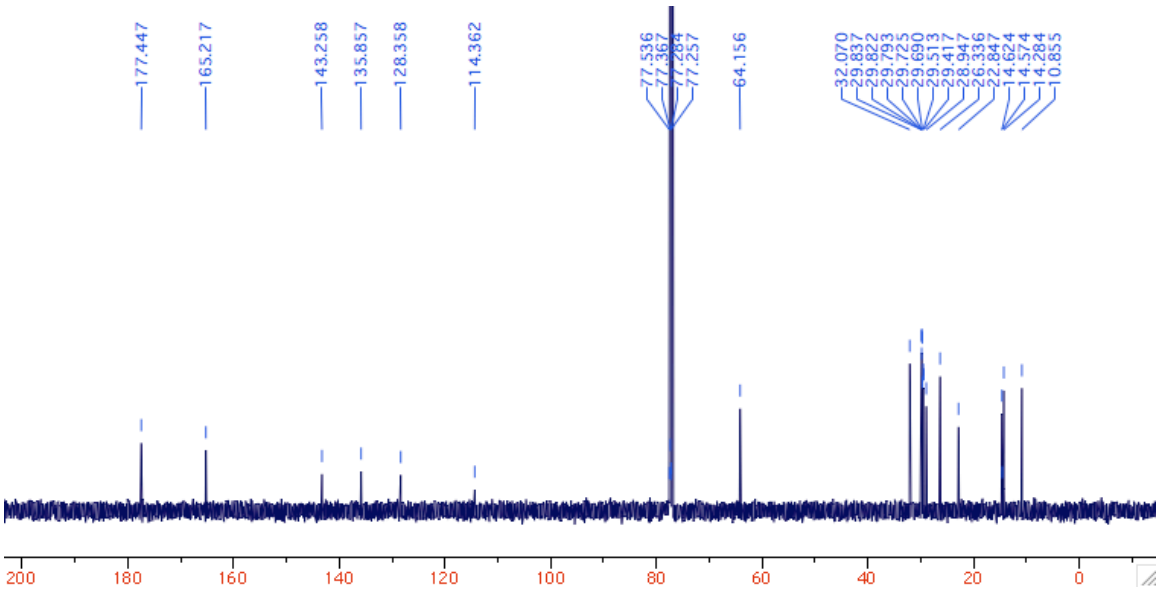


^{13}C NMR, $CDCl_3$, 125 MHz



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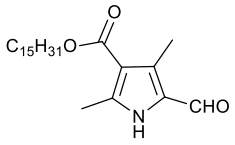

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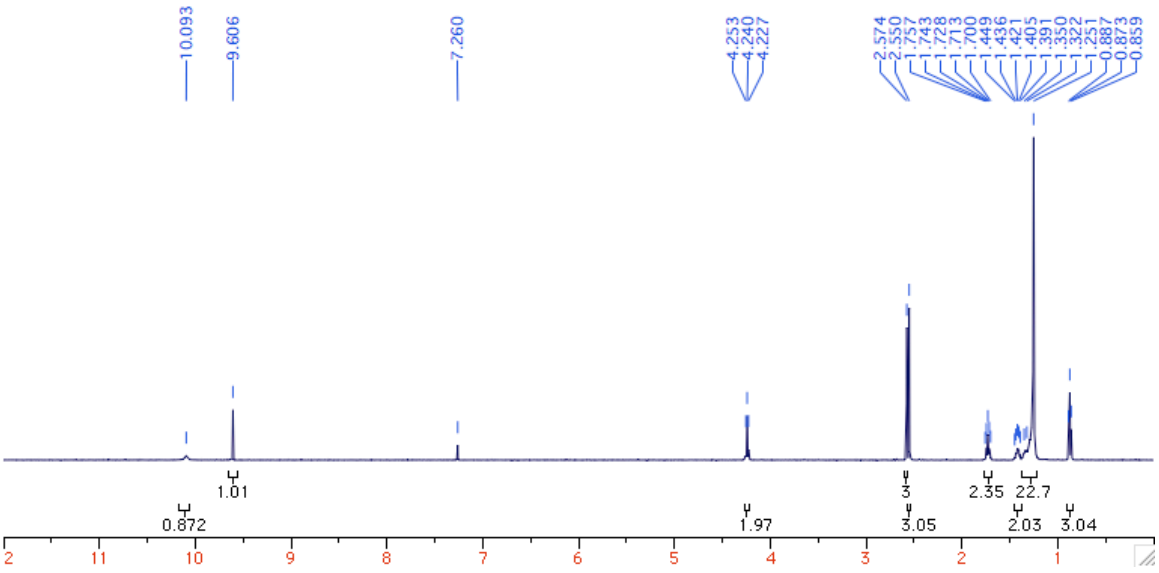

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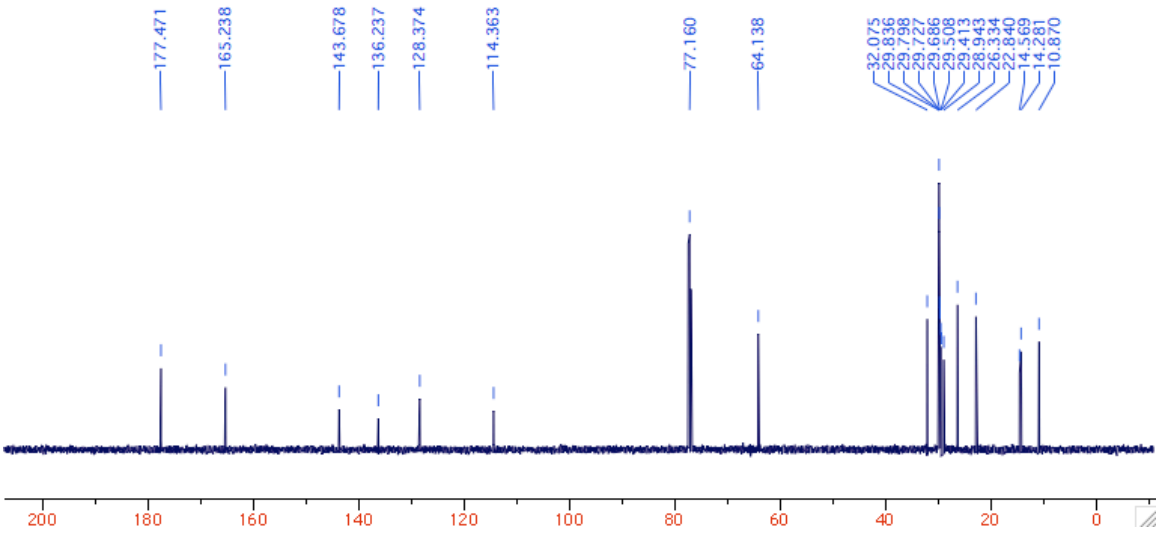

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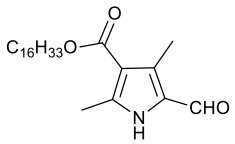
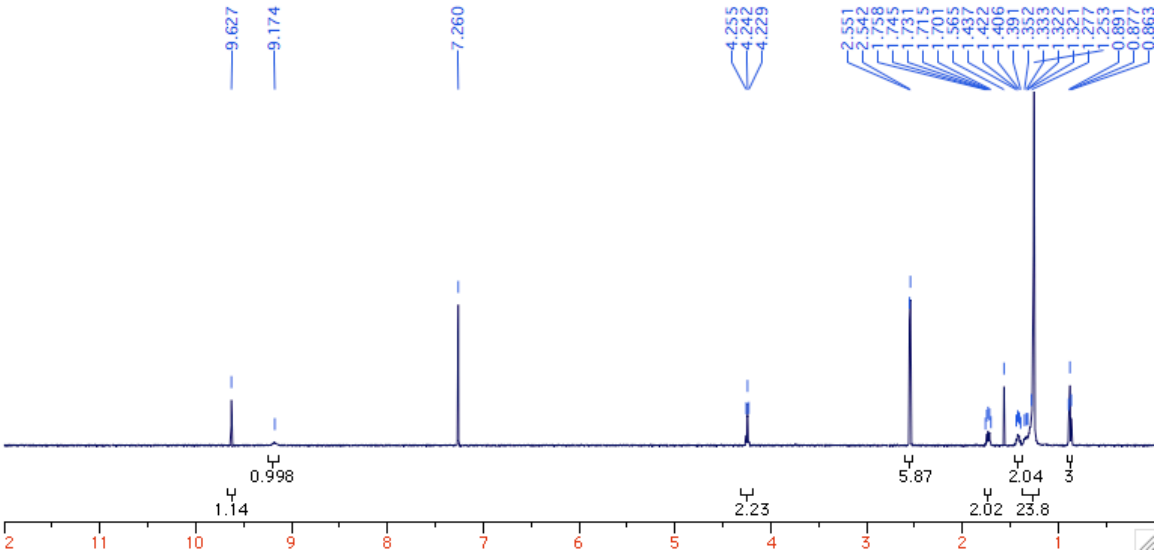
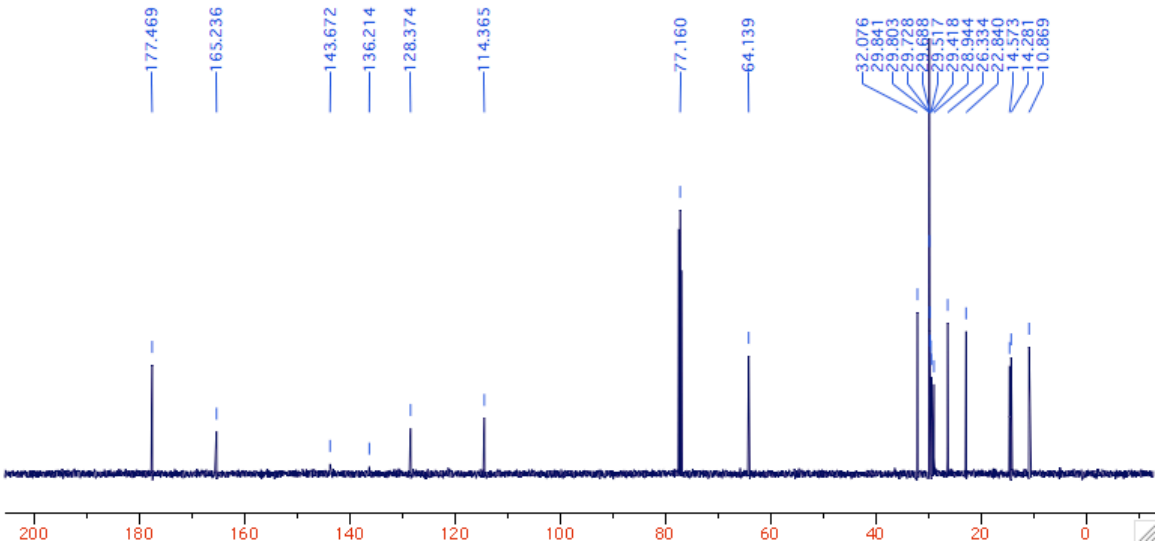


8m

^{1}H NMR, $CDCl_3$, 500 MHz



^{13}C NMR, $CDCl_3$, 125 MHz



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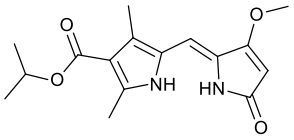
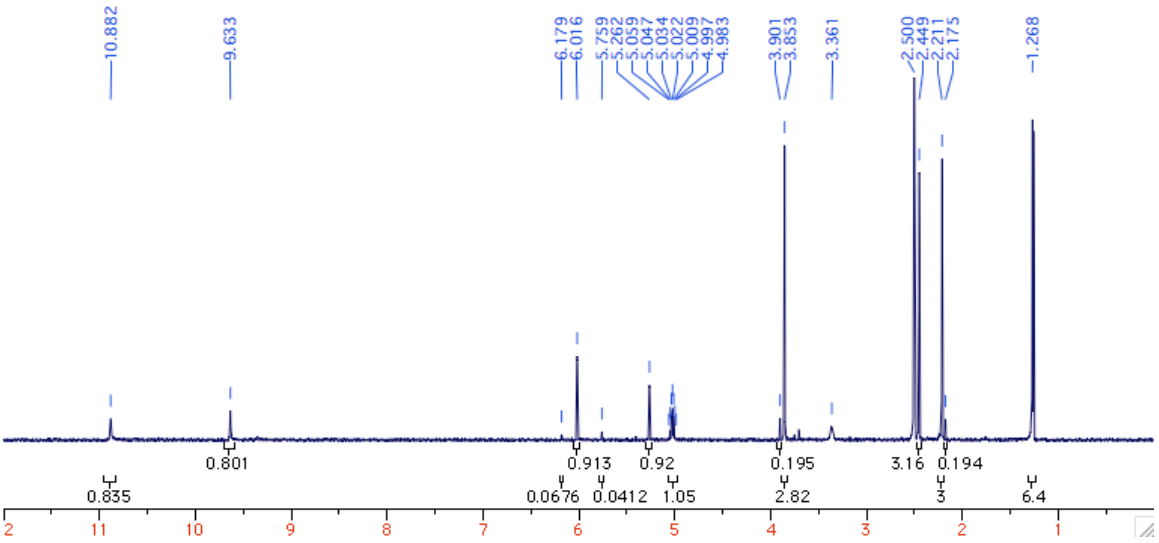
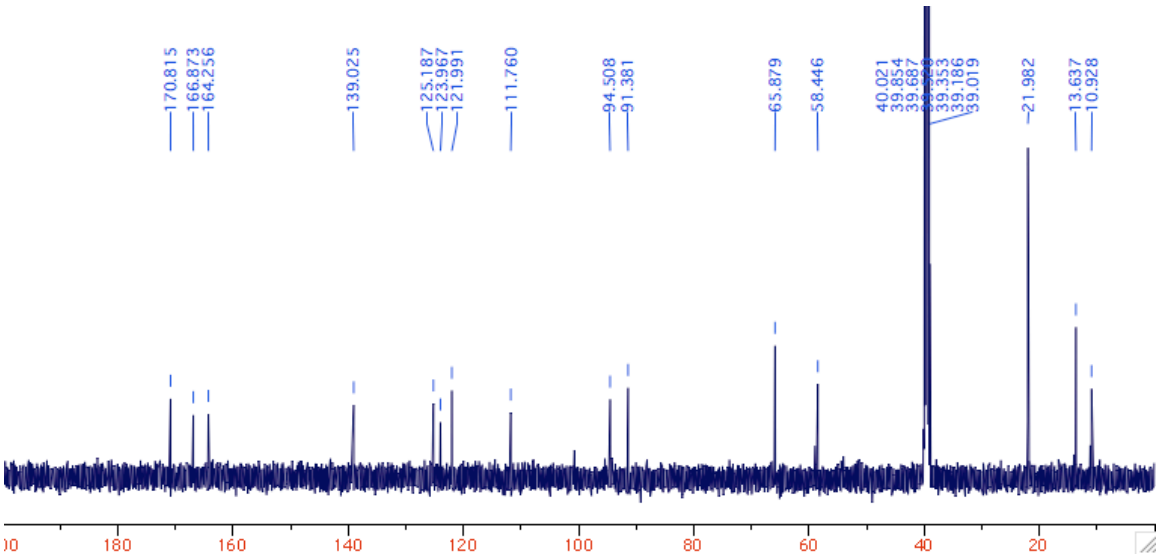


10d

^{1}H NMR, DMSO-d_6, 500 MHz



^{13}C NMR, DMSO-d_6, 125 MHz



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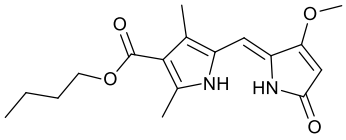
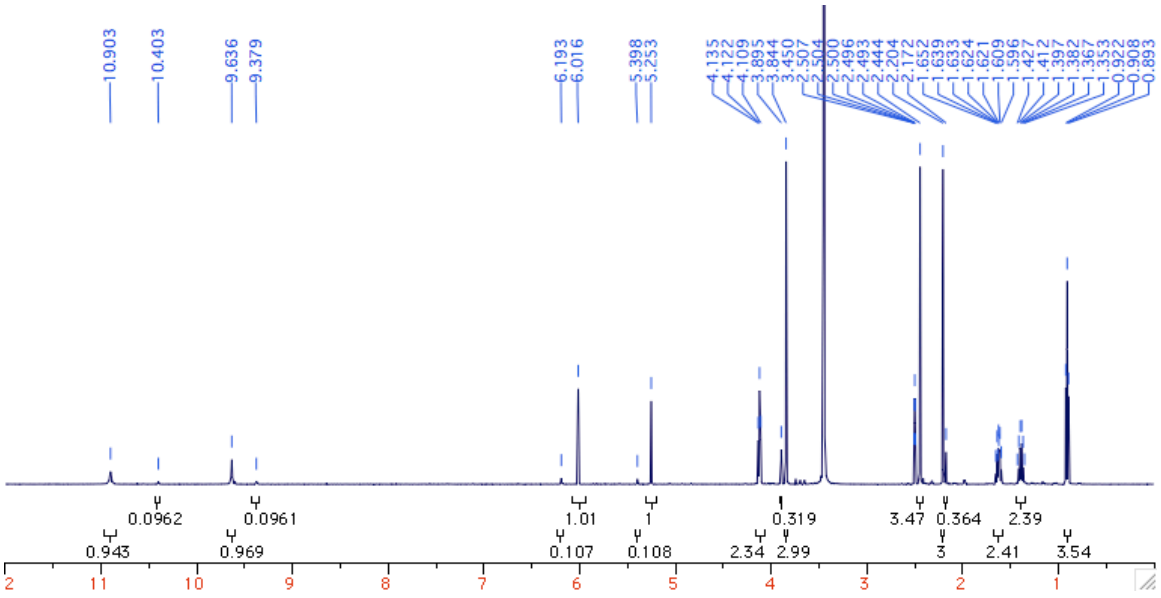


10e

^{1}H NMR, DMSO-d_6, 500 MHz



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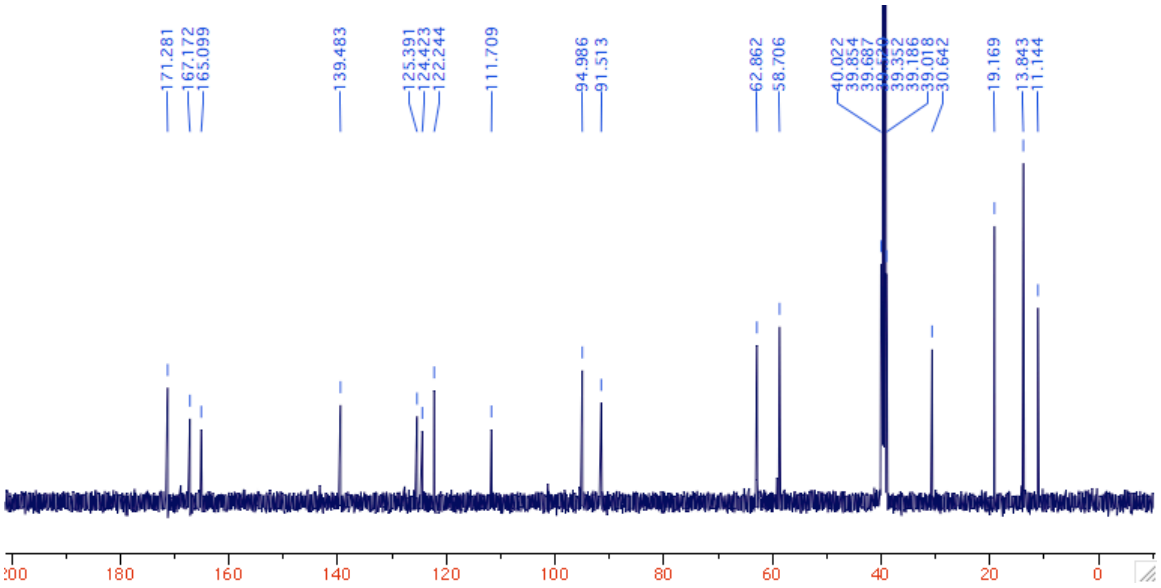

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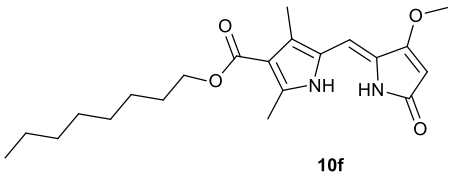

10f

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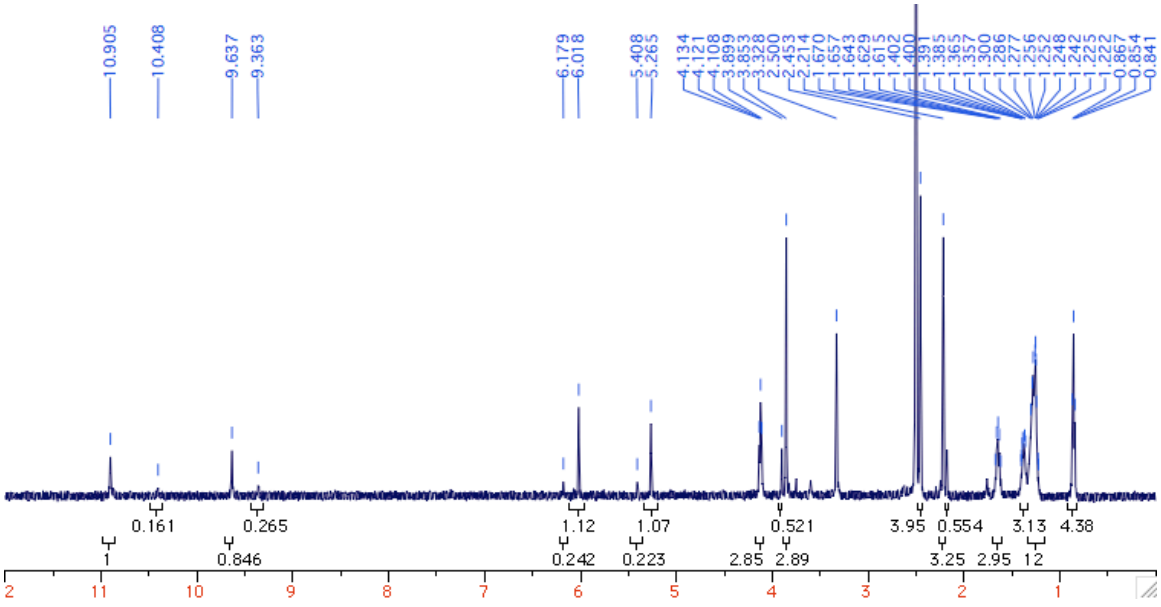
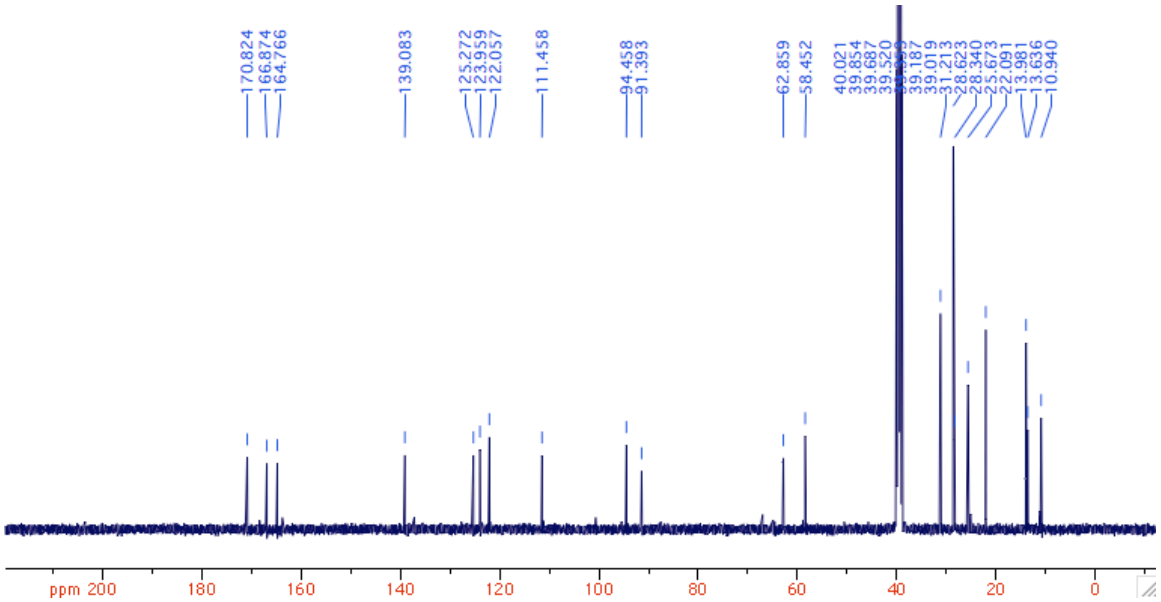


^{13}C NMR, DMSO-d_6, 125 MHz



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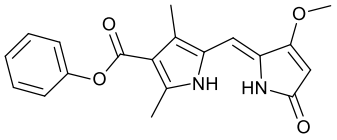

10g

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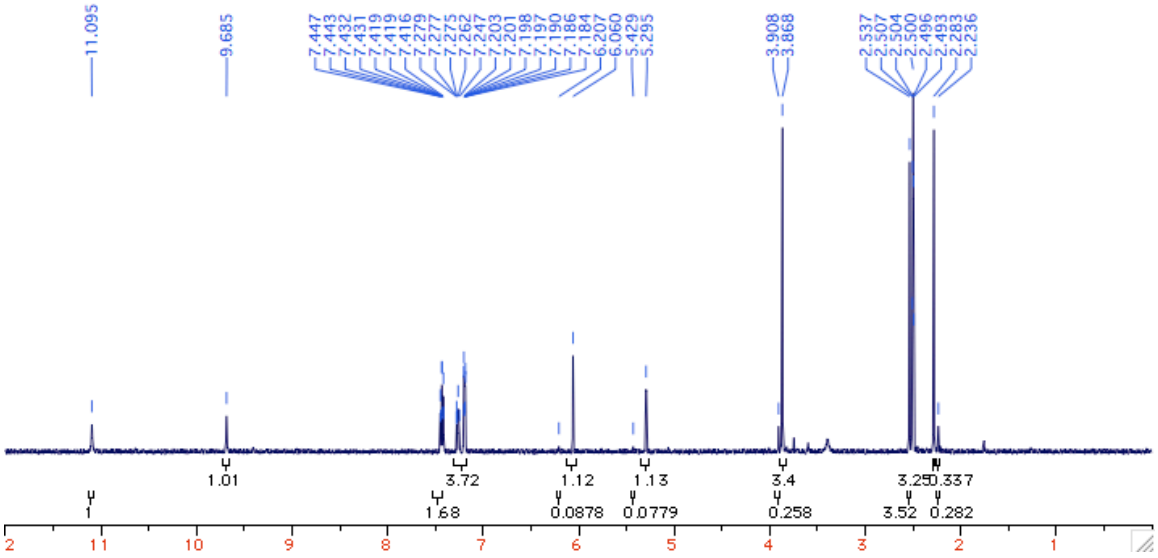
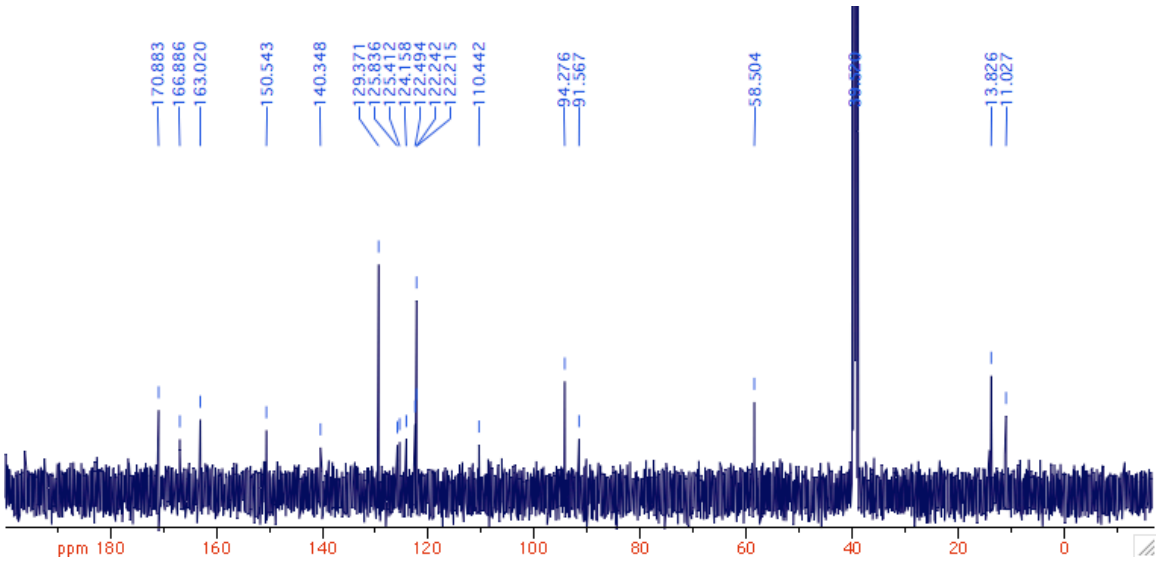


^{13}C NMR, DMSO-d_6, 125 MHz



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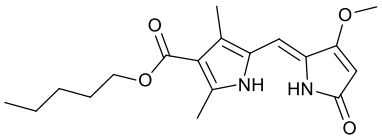

10h

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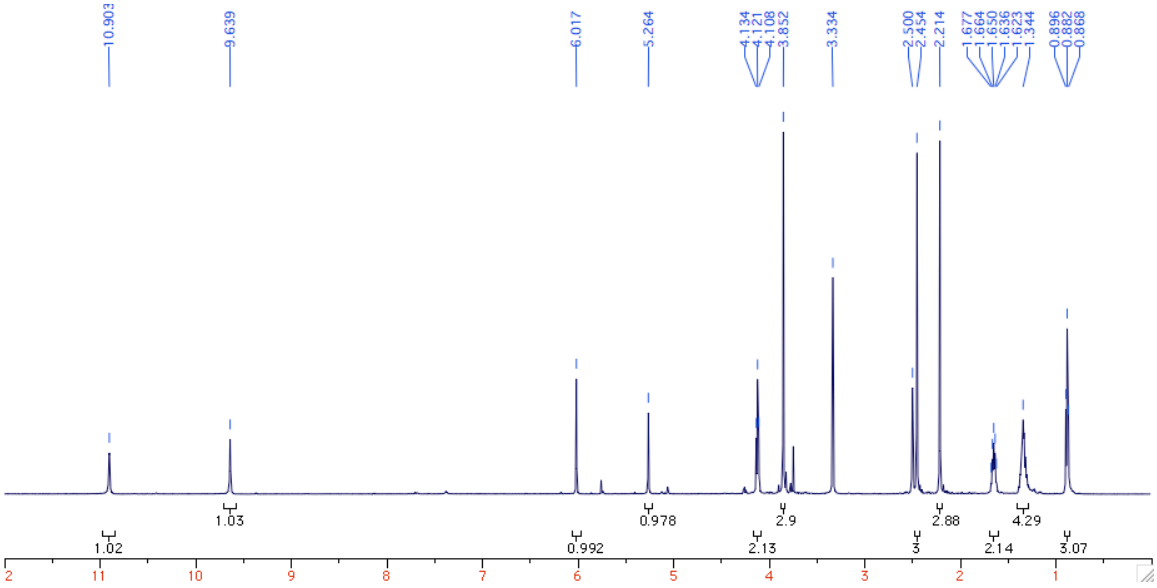
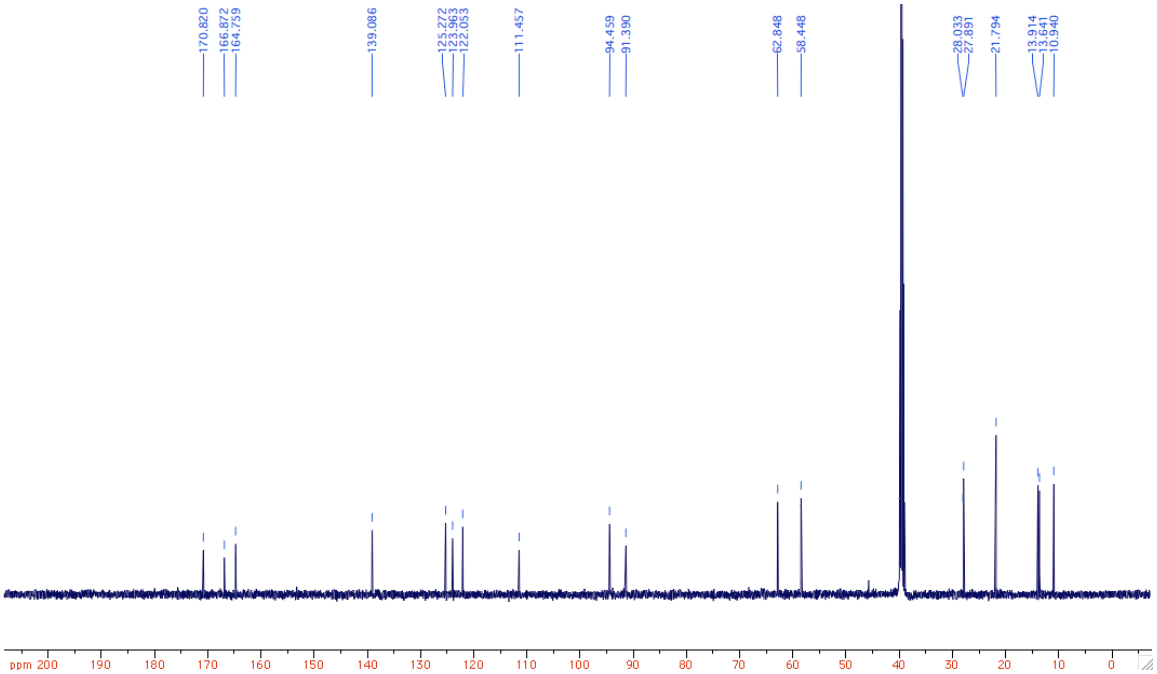


^{13}C NMR, DMSO-d_6, 125 MHz



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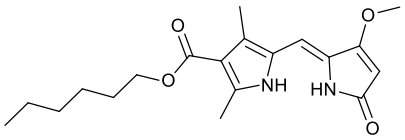
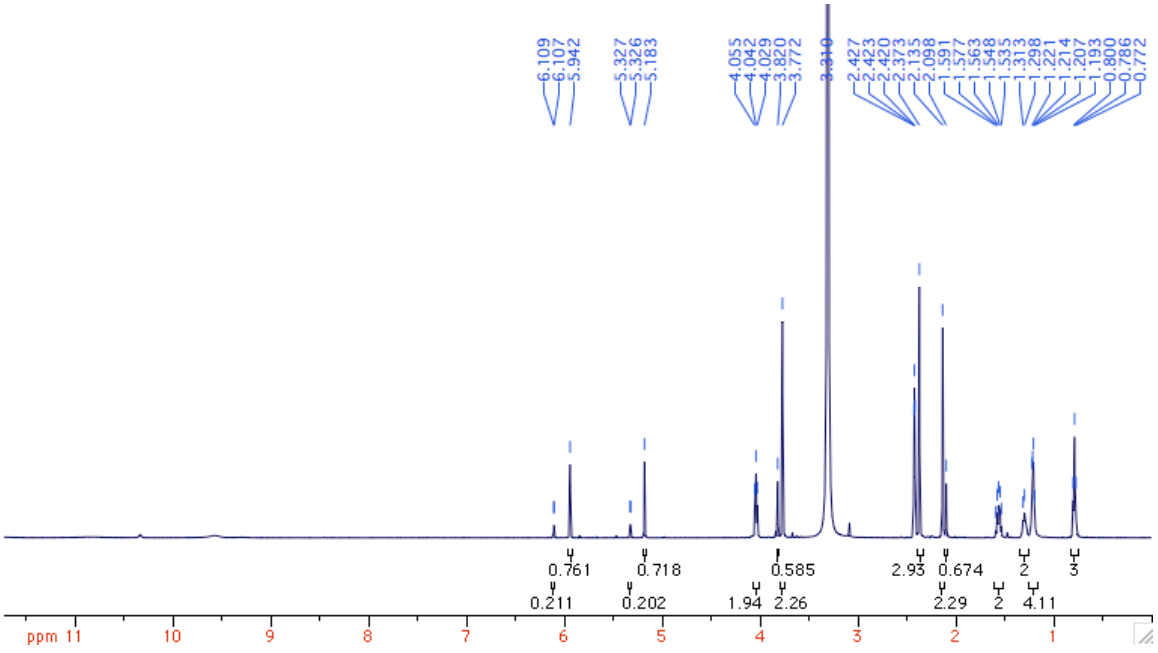
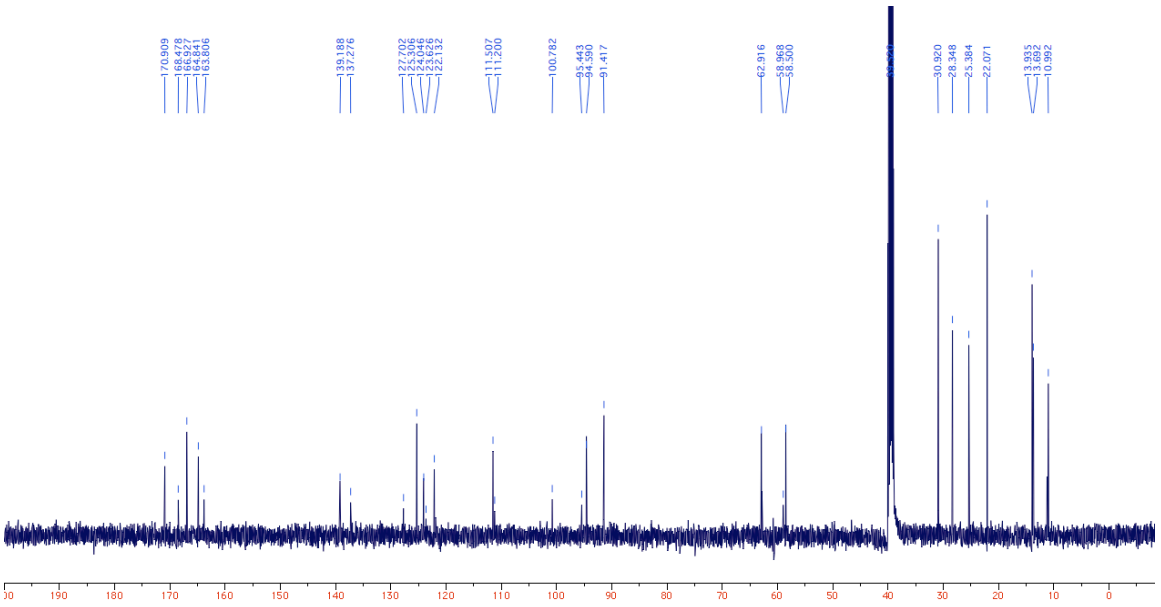


10i

^{1}H NMR, MeOD, 500 MHz



^{13}C NMR, DMSO-d_6, 125 MHz



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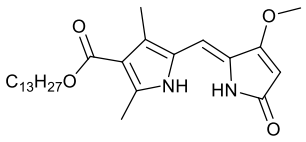

10j

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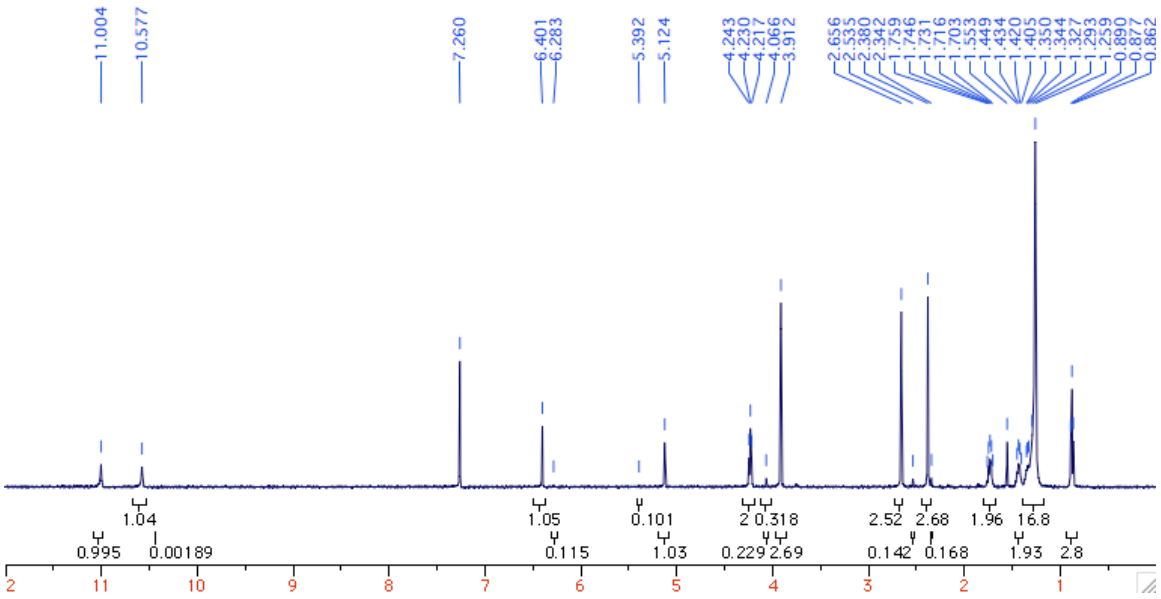
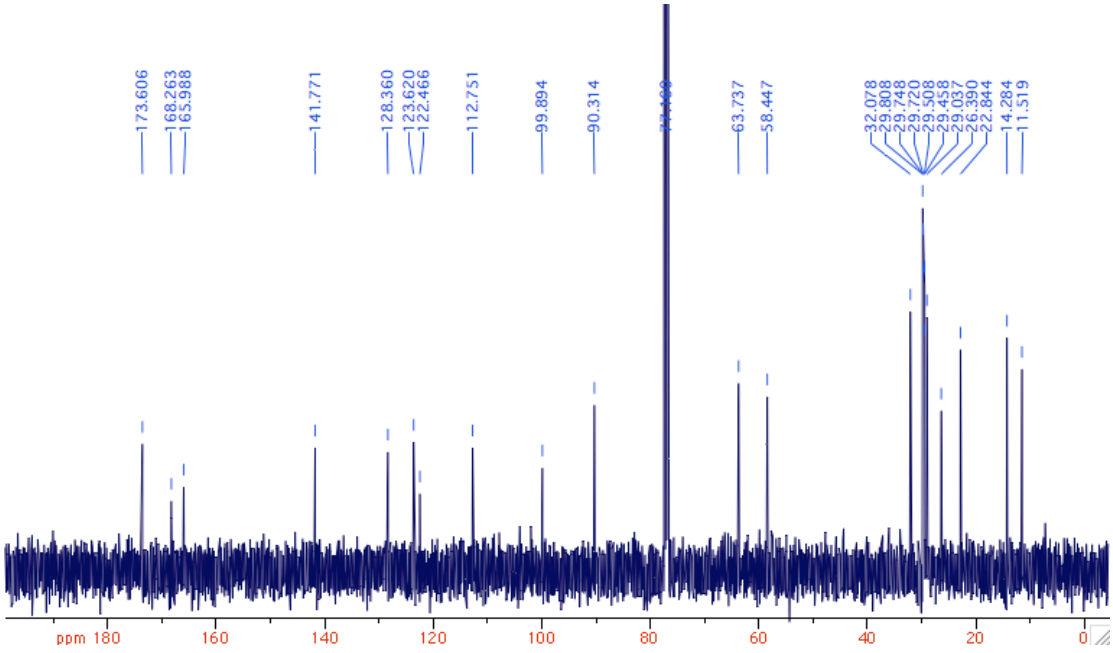


^{13}C NMR, $CDCl_3$, 125 MHz



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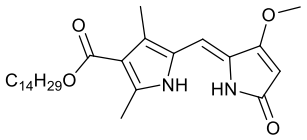

10k

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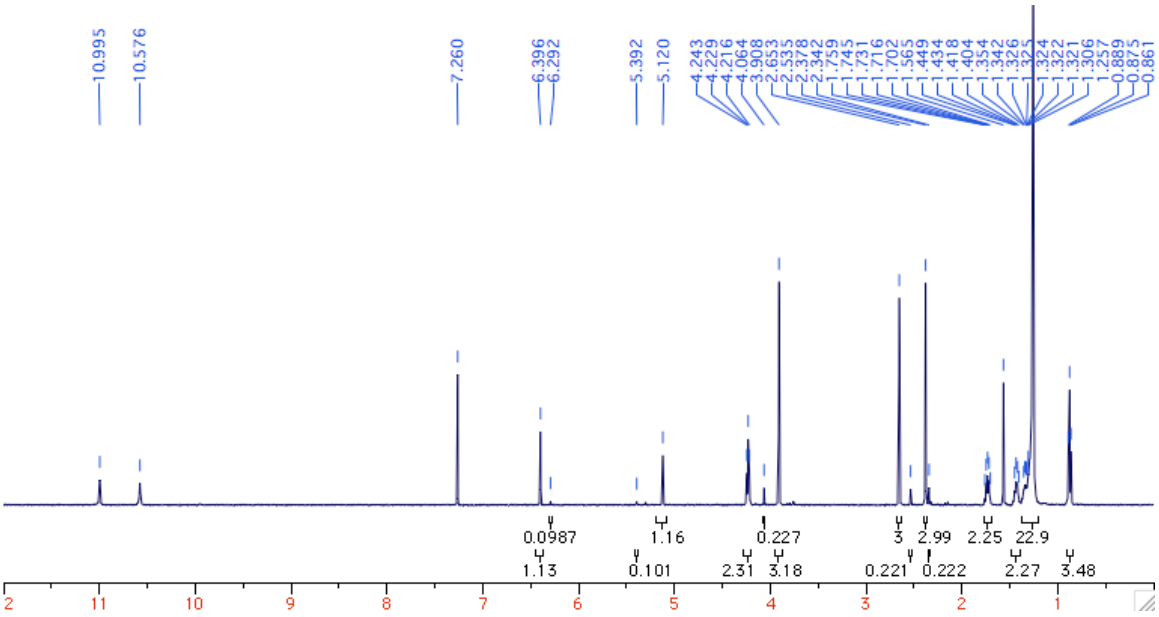
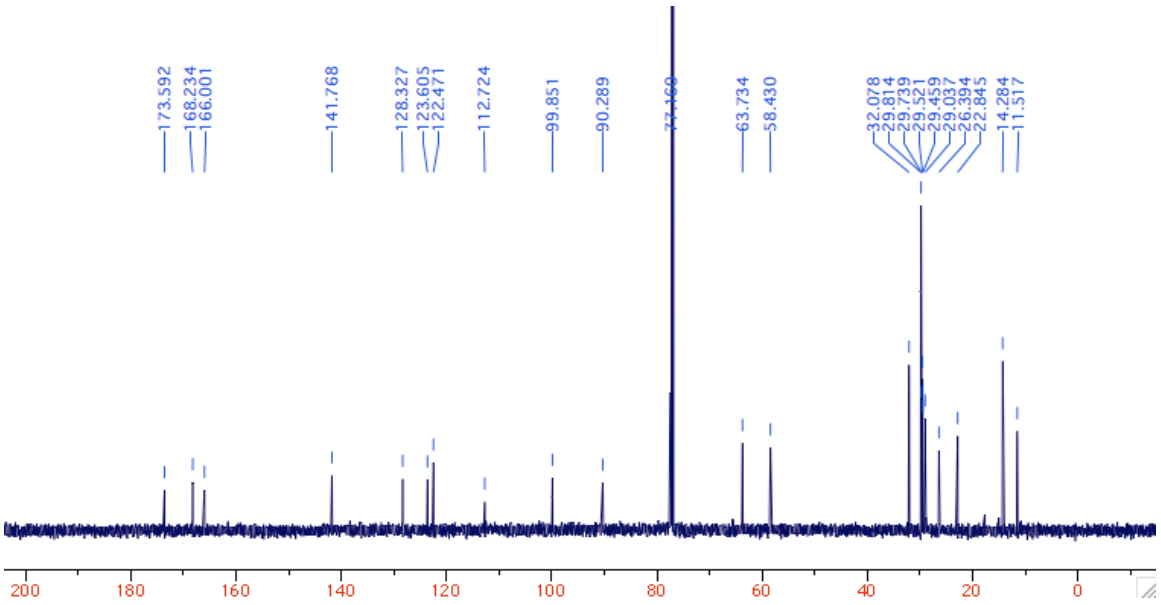


^{13}C NMR, $CDCl_3$, 125 MHz



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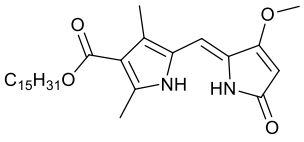
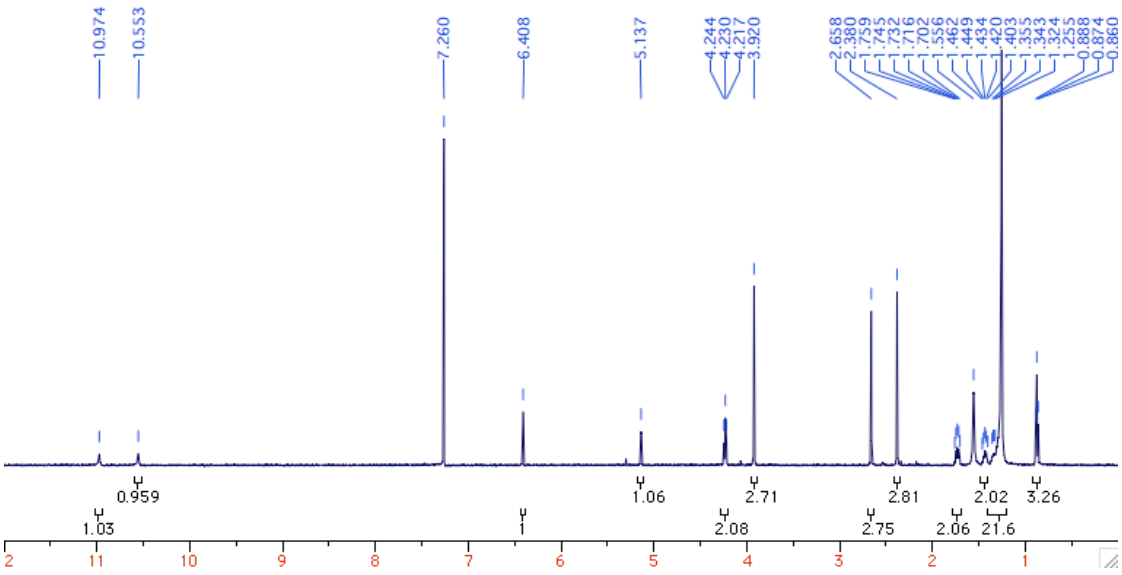


10l

^{1}H NMR, $CDCl_3$, 500 MHz



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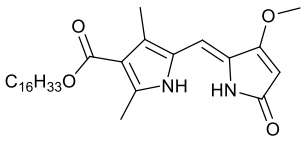

10m

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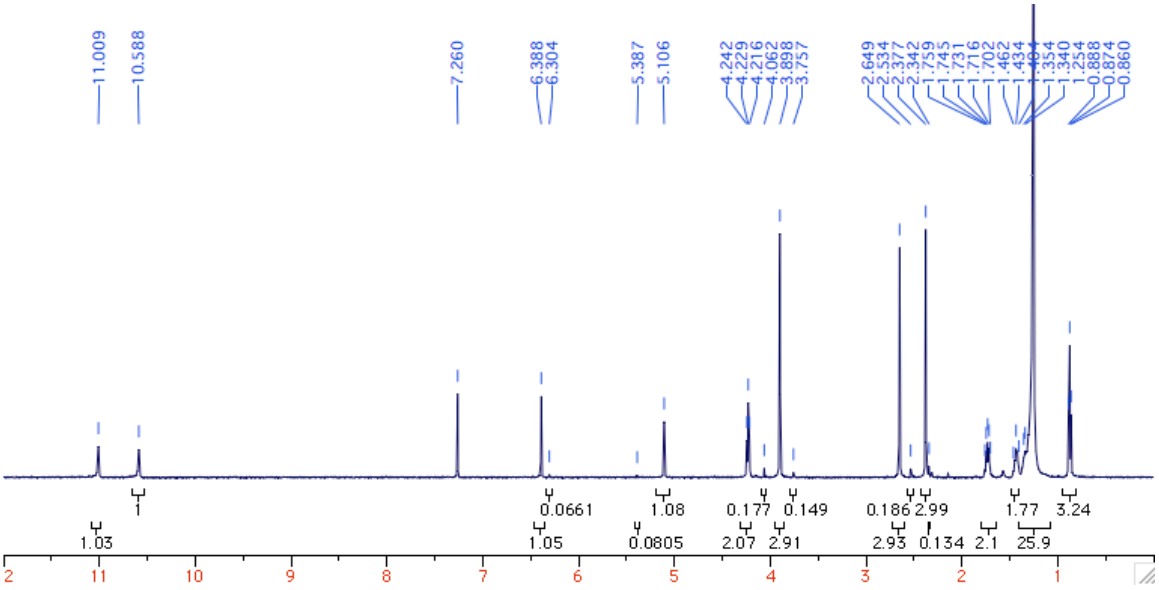

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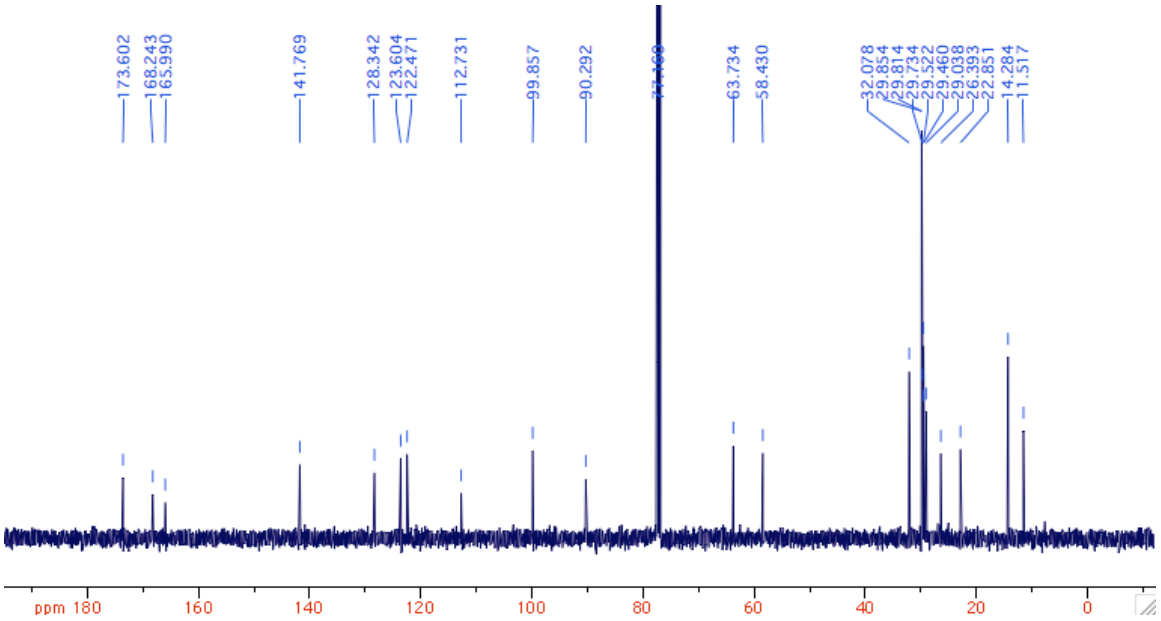

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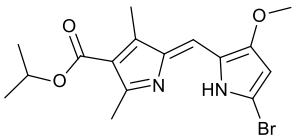

11d

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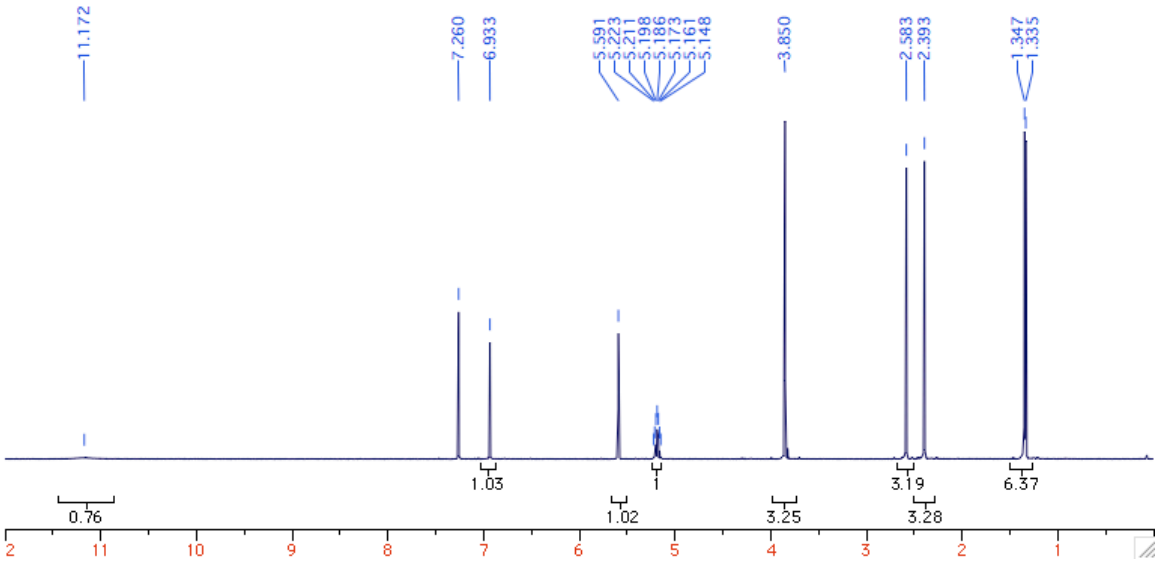
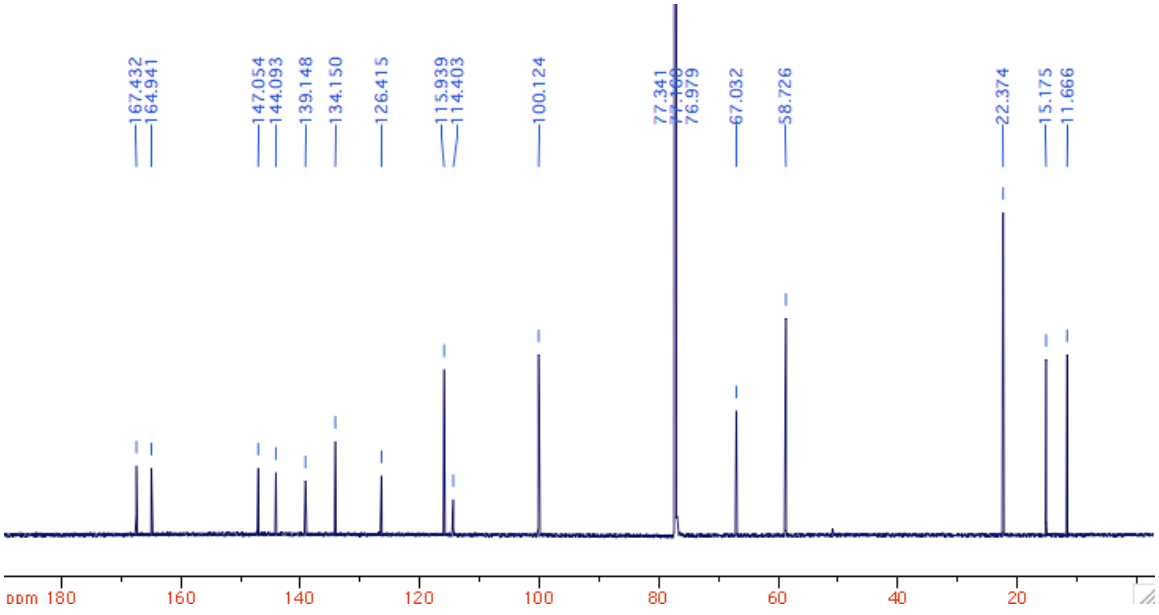


^{13}C NMR, $CDCl_3$, 125 MHz



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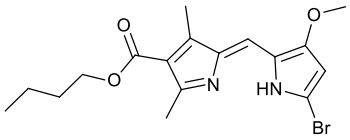

11e

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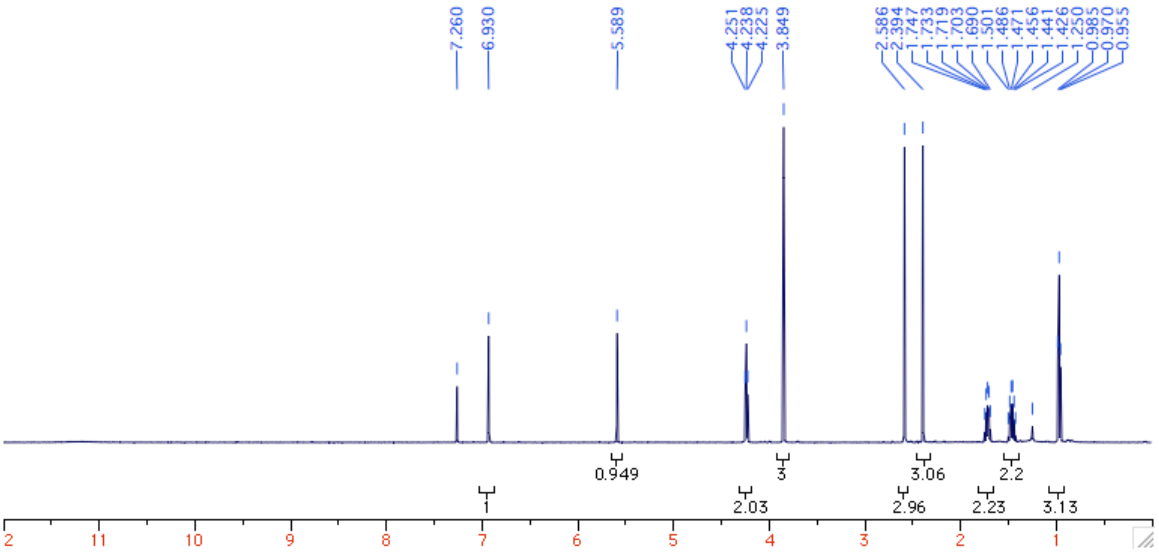

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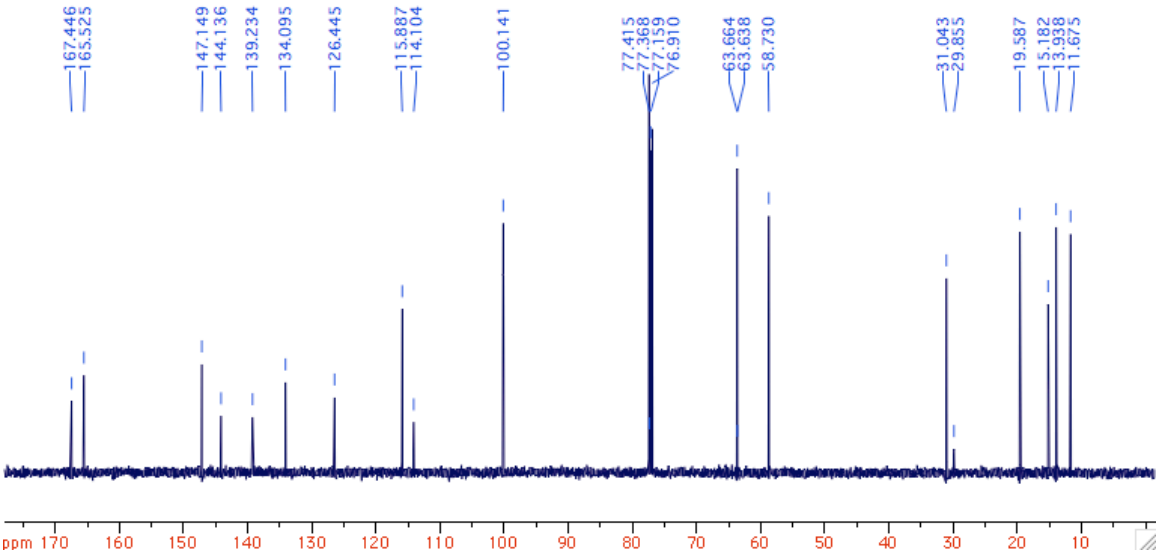

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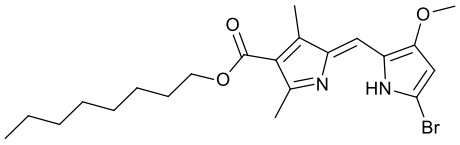
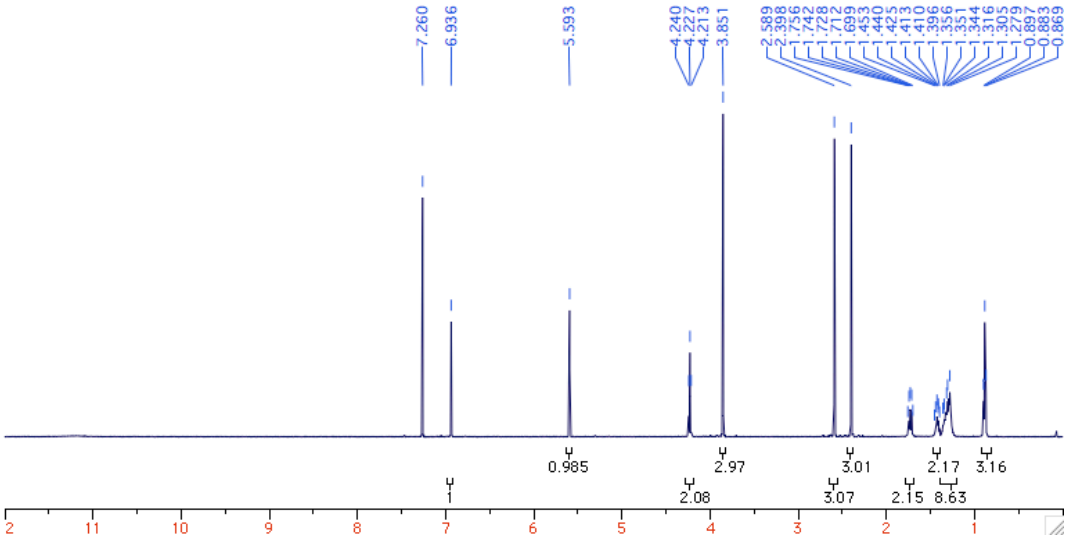
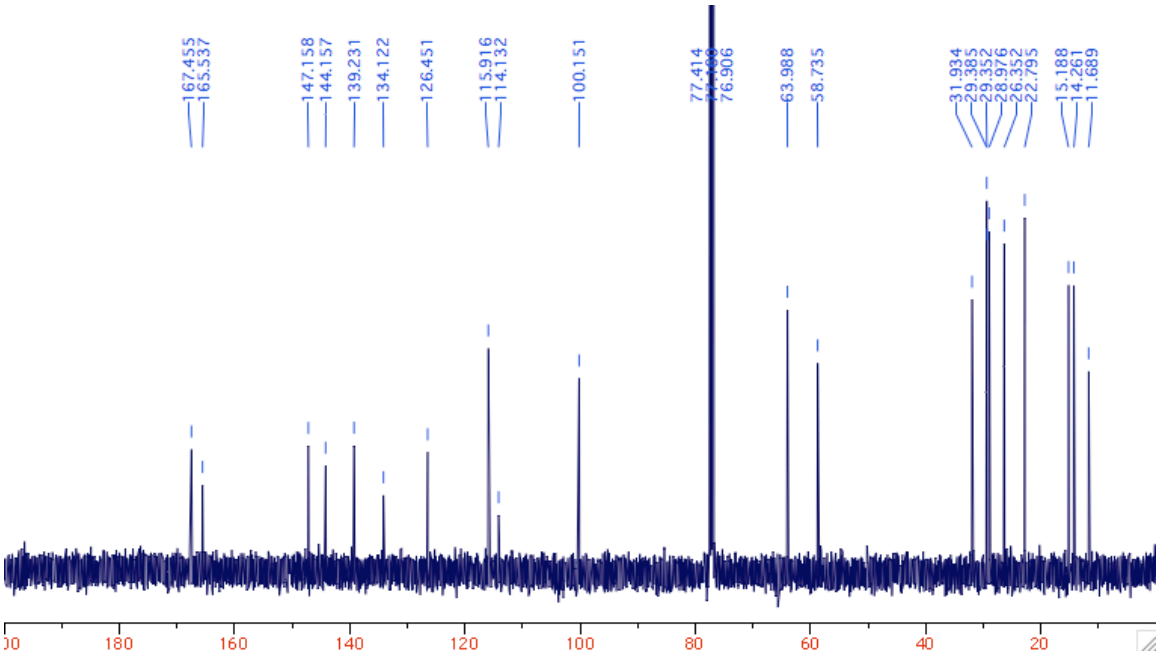


11f

^{1}H NMR, $CDCl_3$, 500 MHz



^{13}C NMR, $CDCl_3$, 125 MHz



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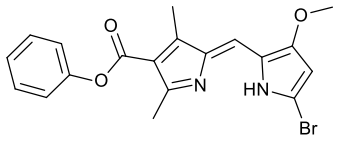

11g

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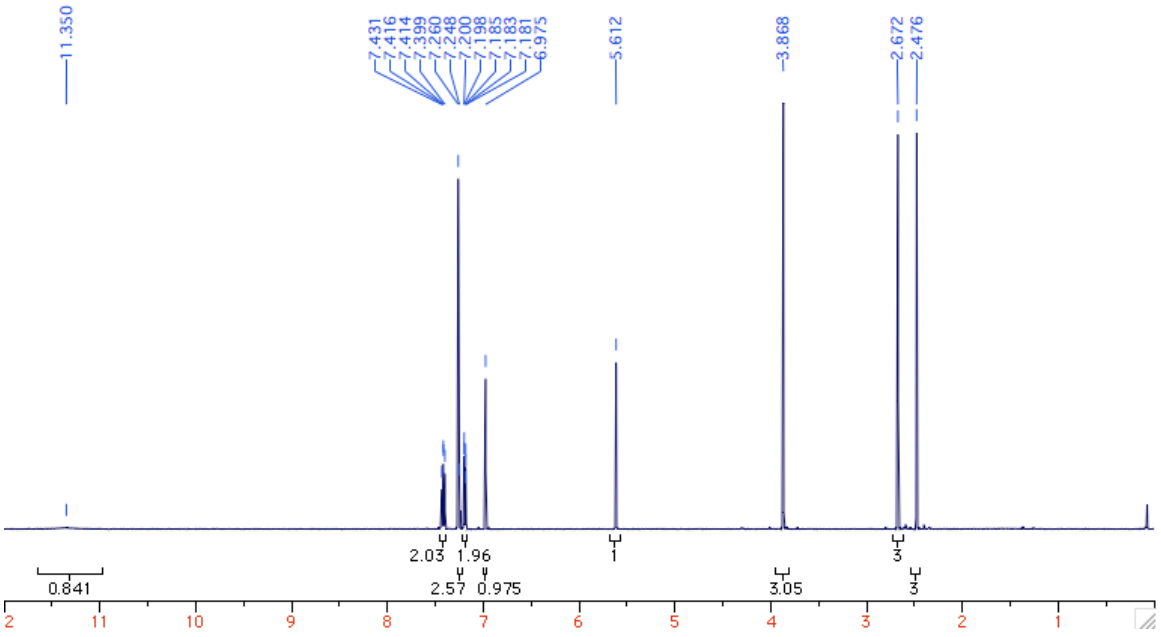
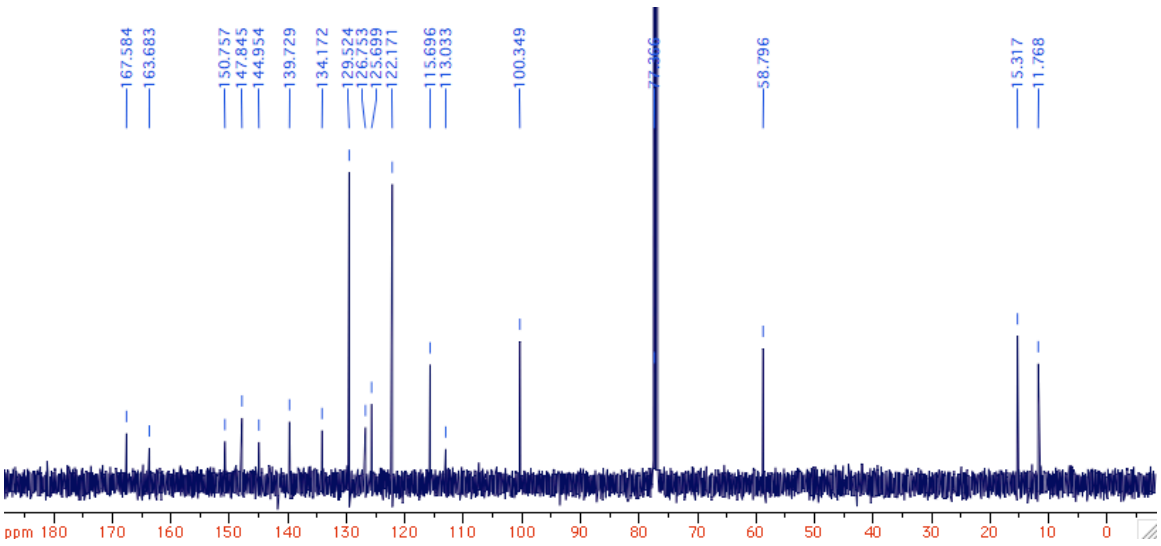


^{13}C NMR, $CDCl_3$, 125 MHz



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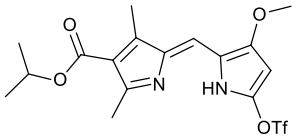

12d

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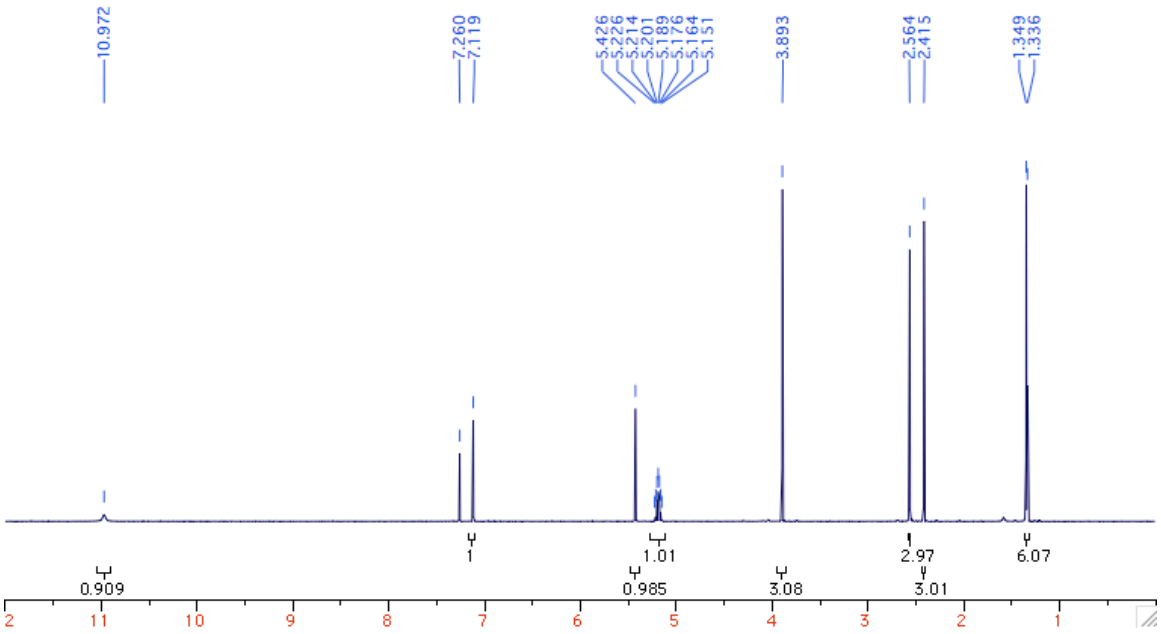
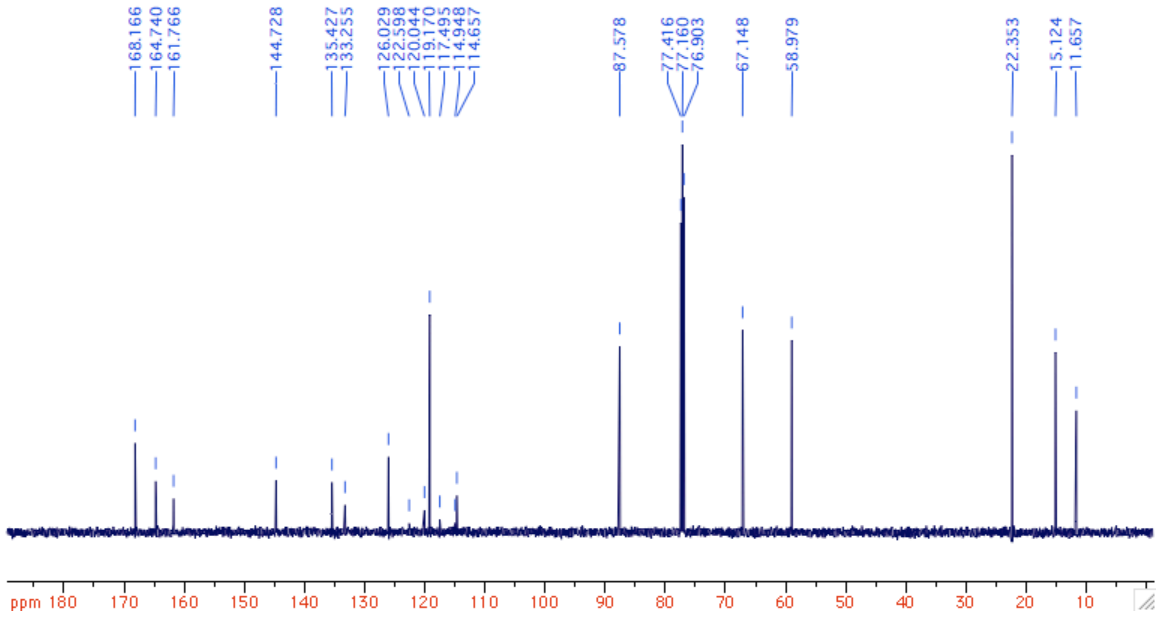


^{13}C NMR, $CDCl_3$, 125 MHz



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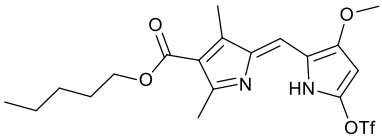

12h

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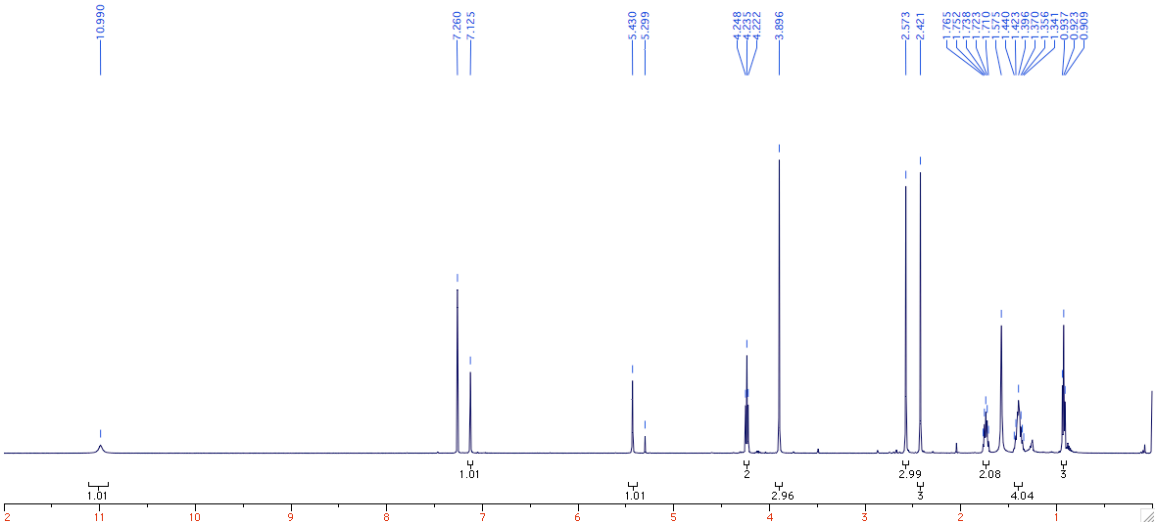
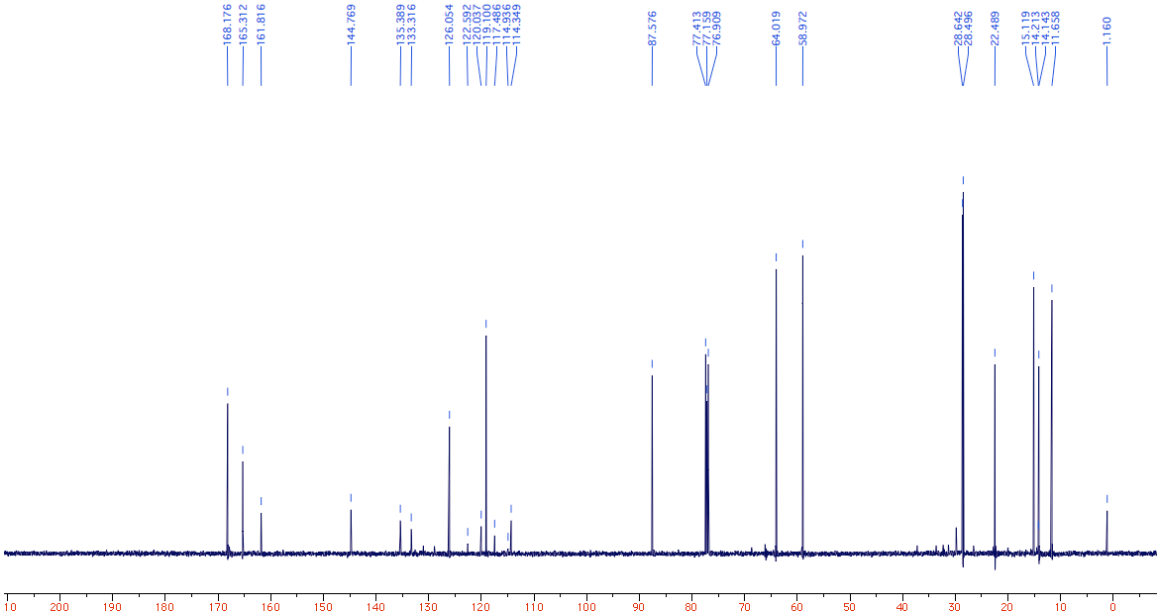


^{13}C NMR, $CDCl_3$, 125 MHz



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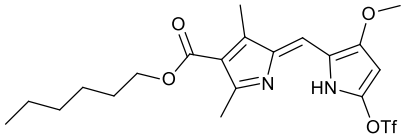

12i

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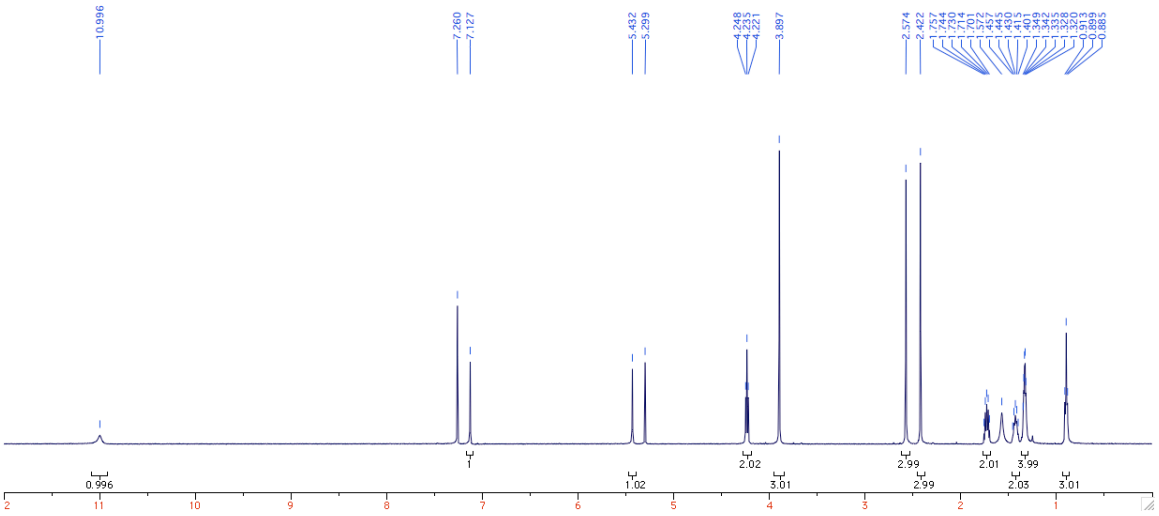
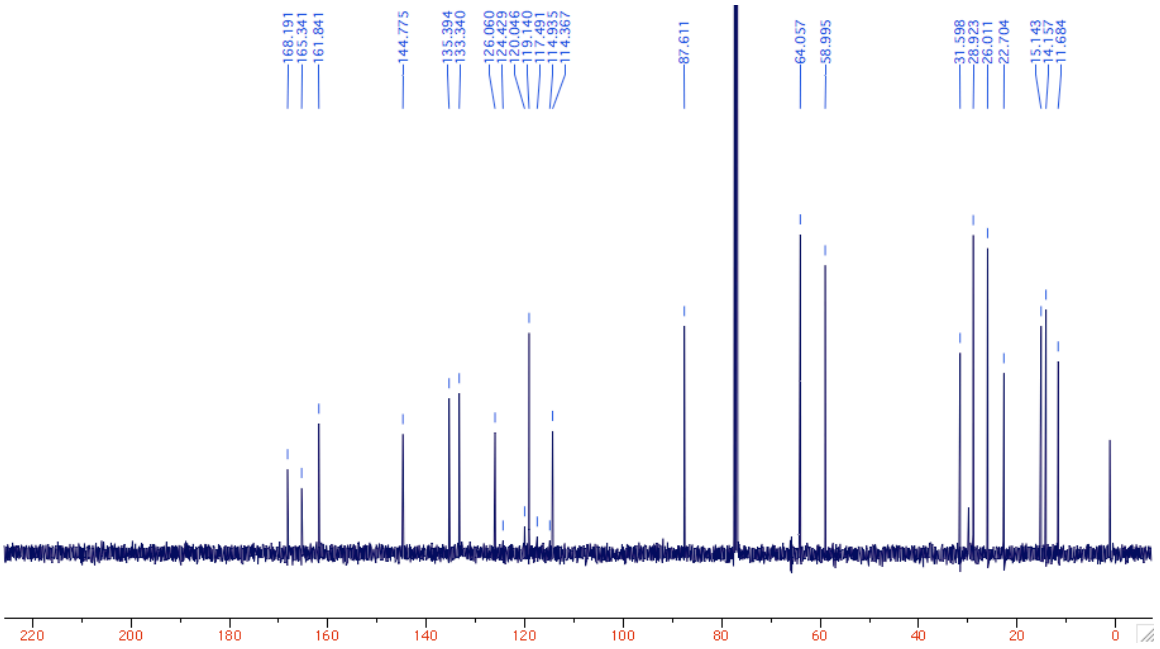


^{13}C NMR, $CDCl_3$, 125 MHz



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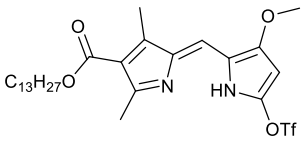

12j

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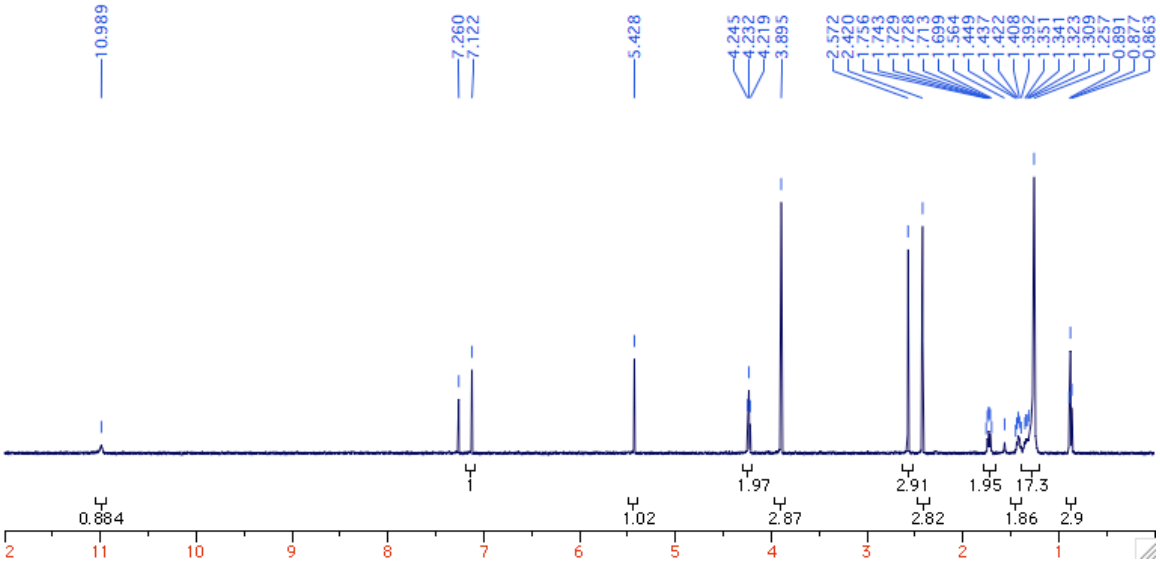
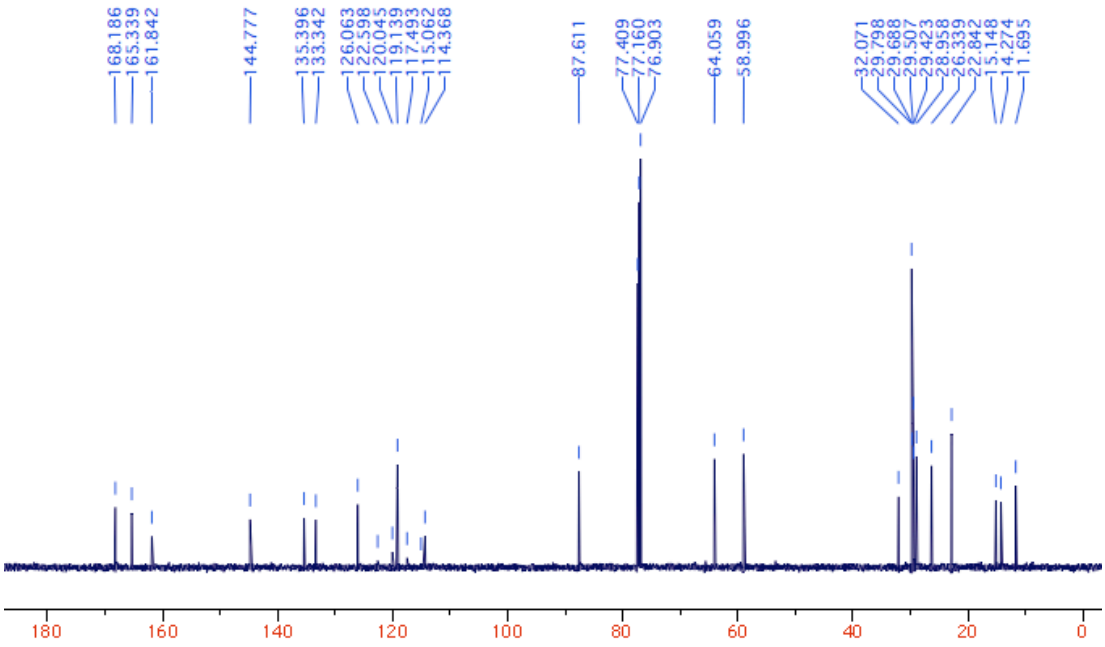


^{13}C NMR, $CDCl_3$, 125 MHz



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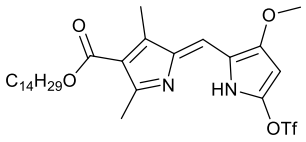

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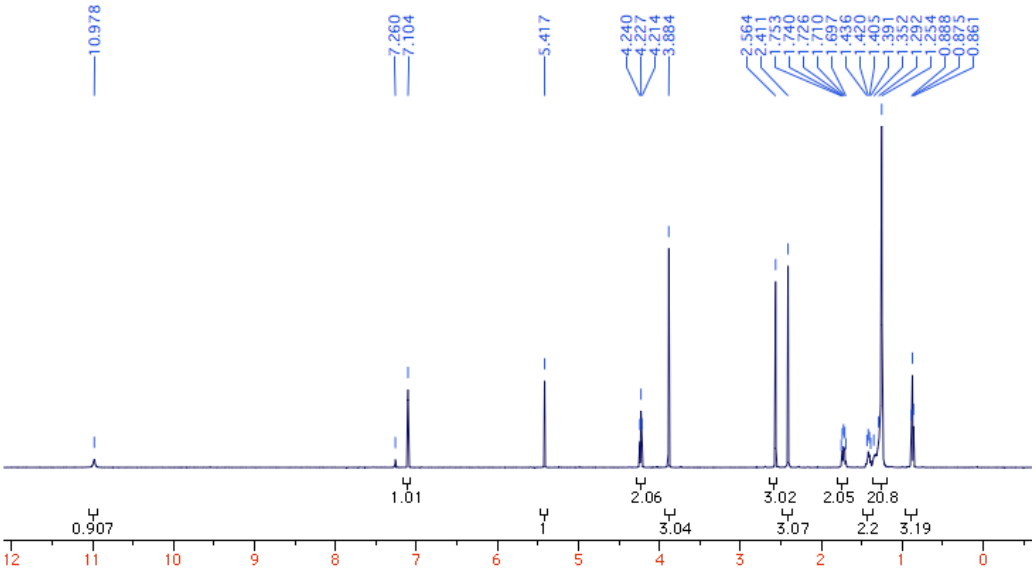

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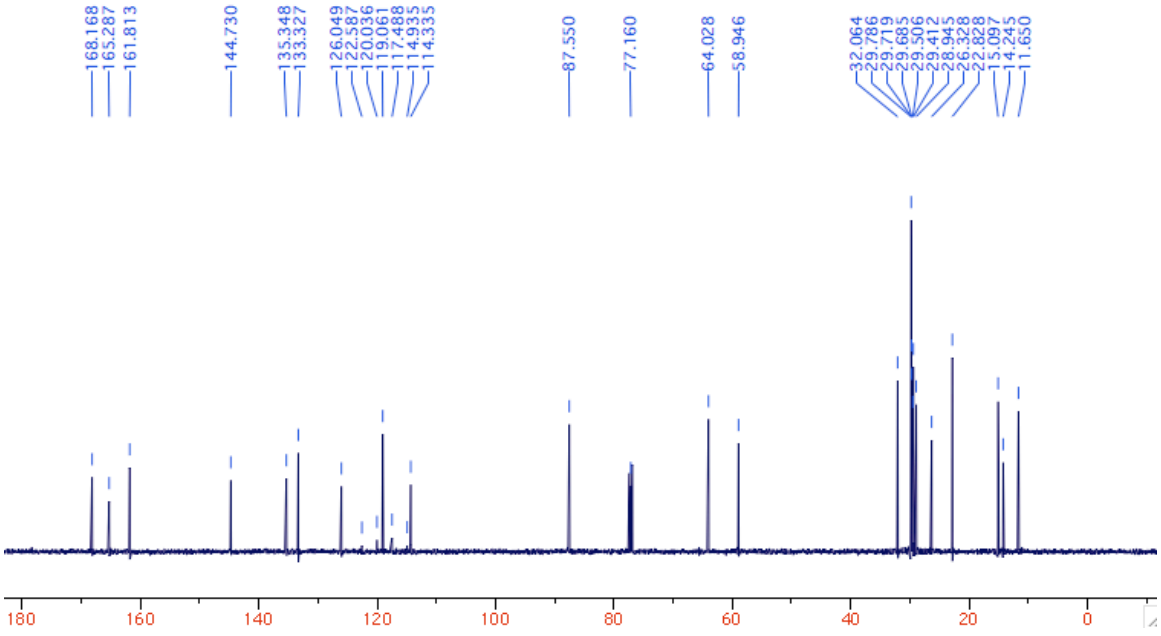

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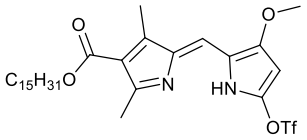

12l

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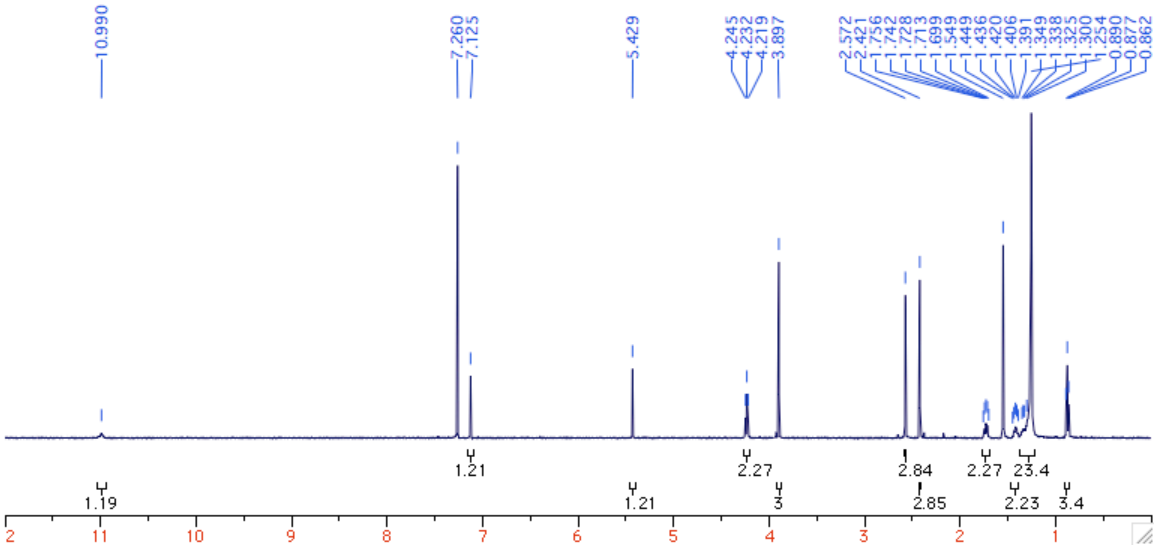

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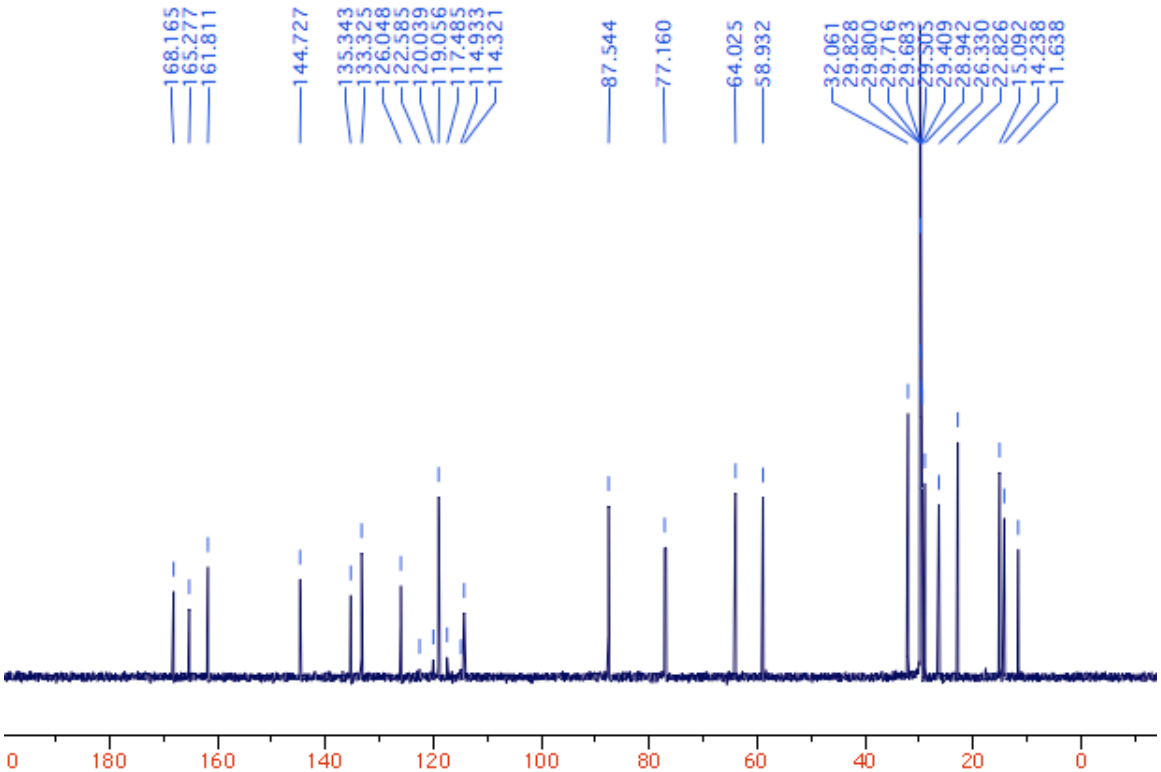

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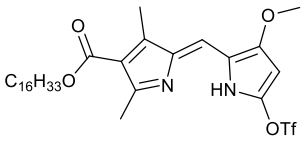
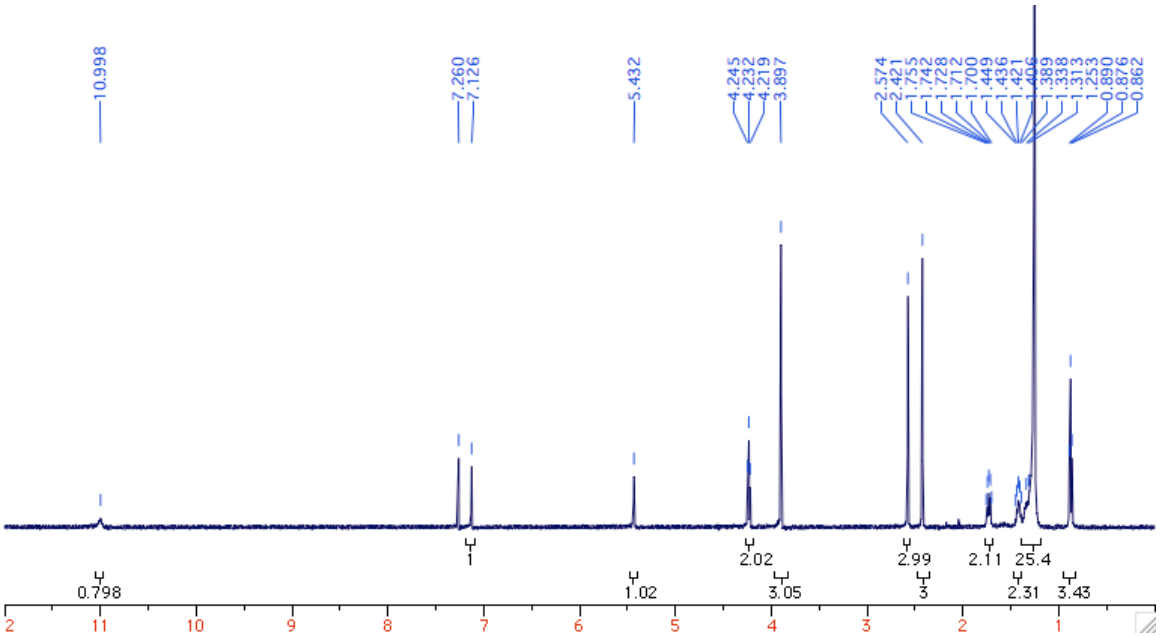
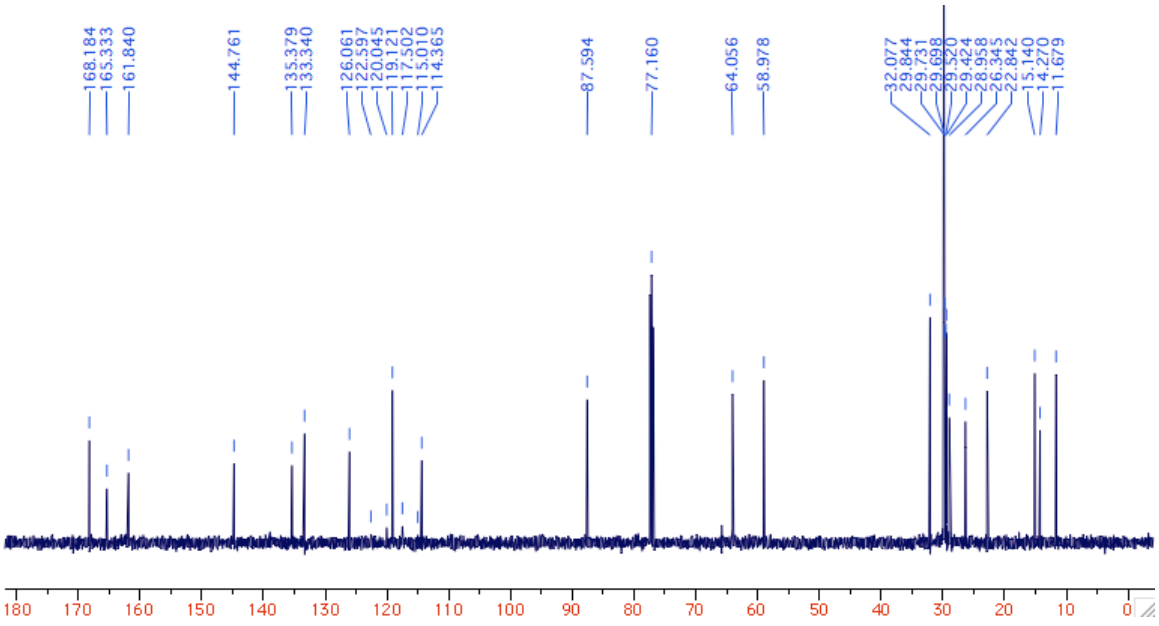


12m

^{1}H NMR, $CDCl_3$, 500 MHz



^{13}C NMR, $CDCl_3$, 125 MHz



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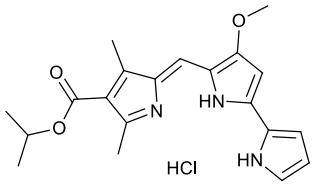

3d

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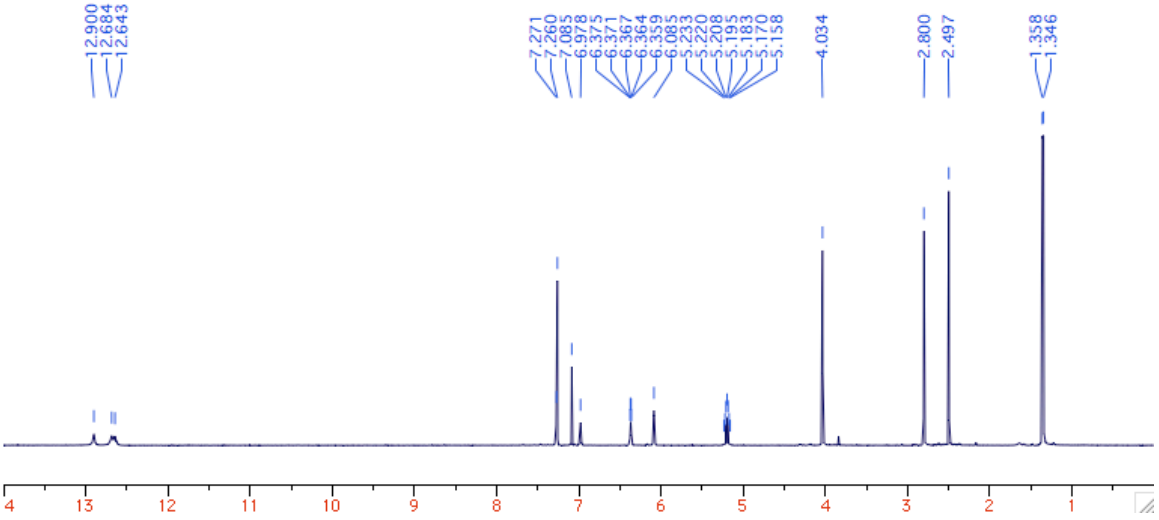

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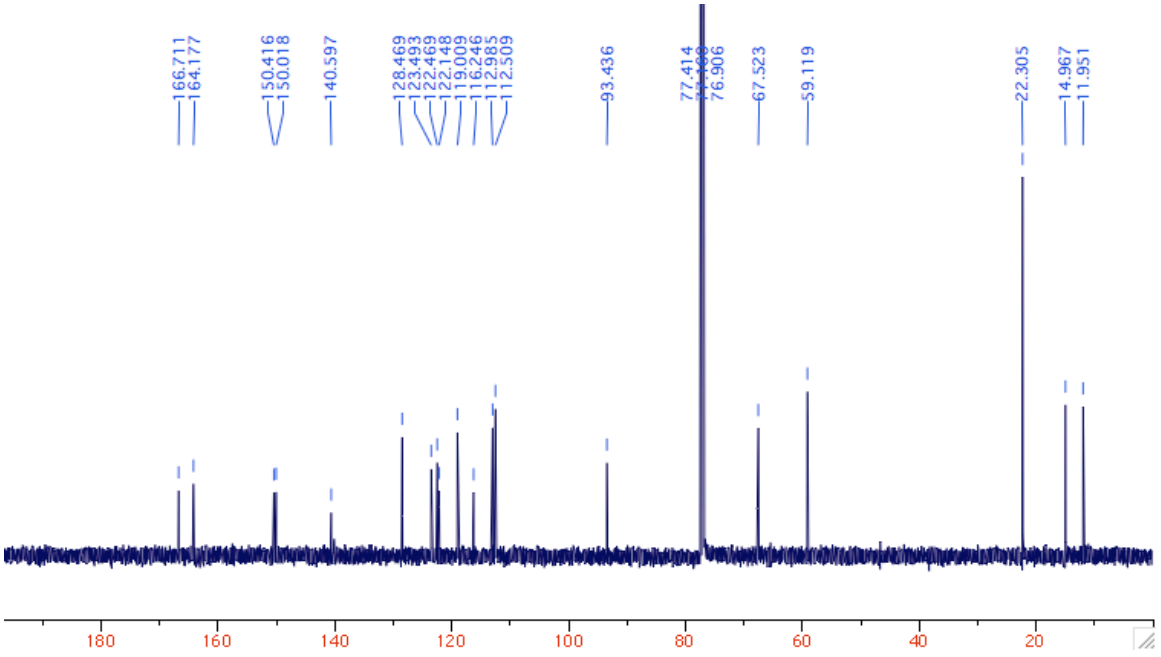

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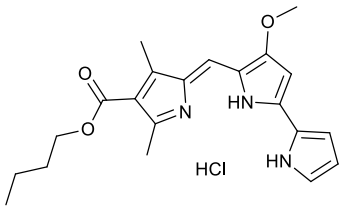

3e

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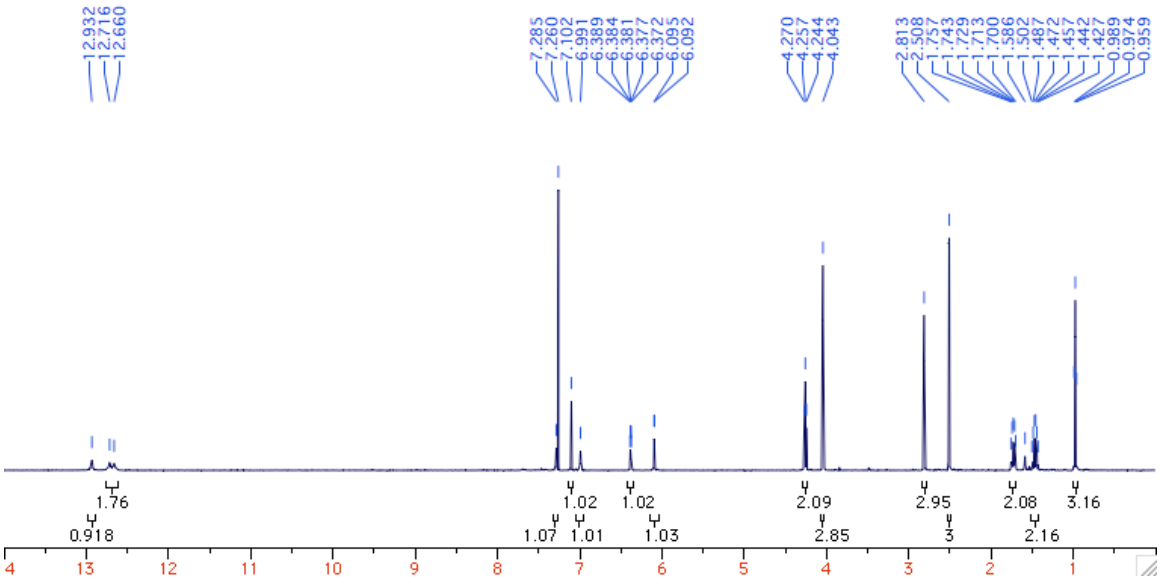

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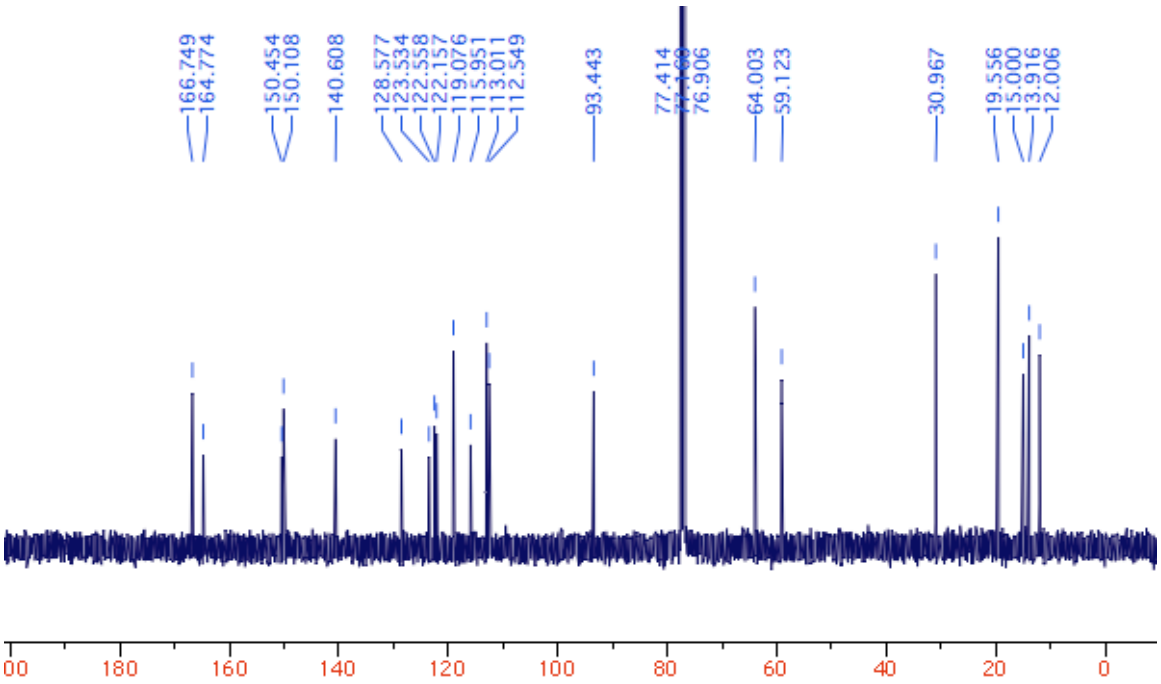

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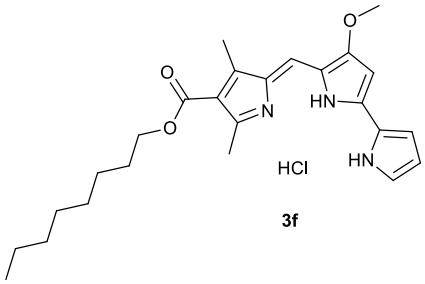

3f

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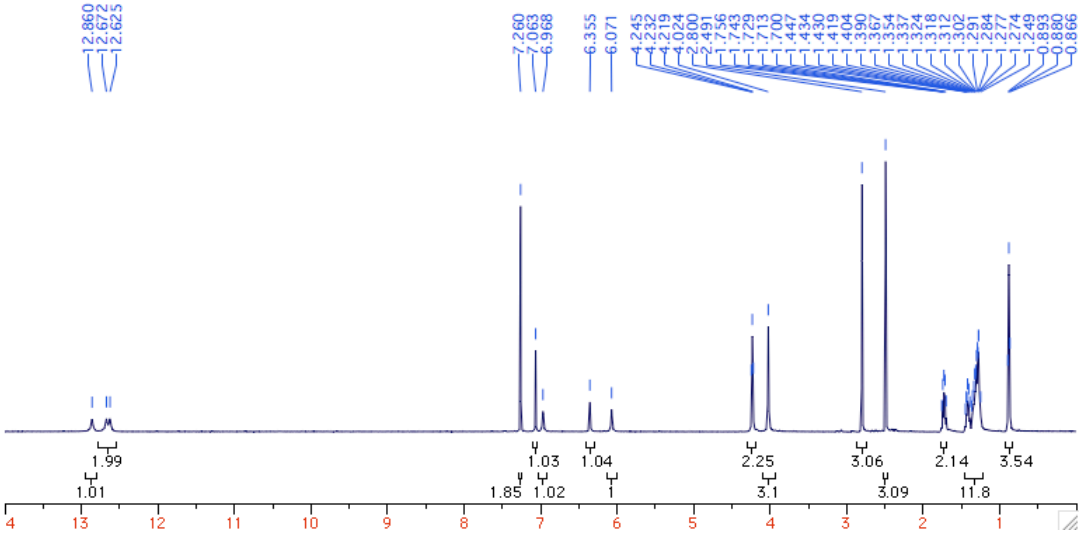

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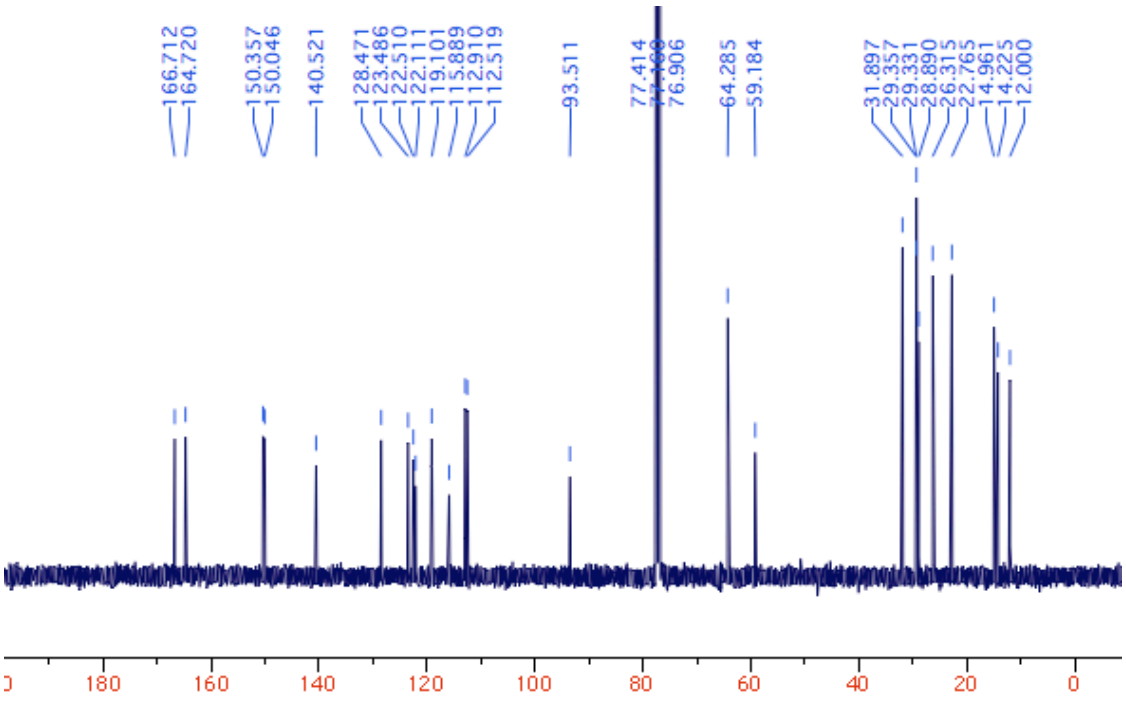

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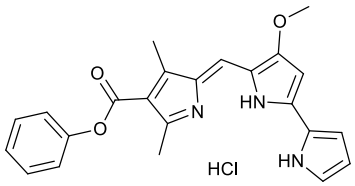

3g

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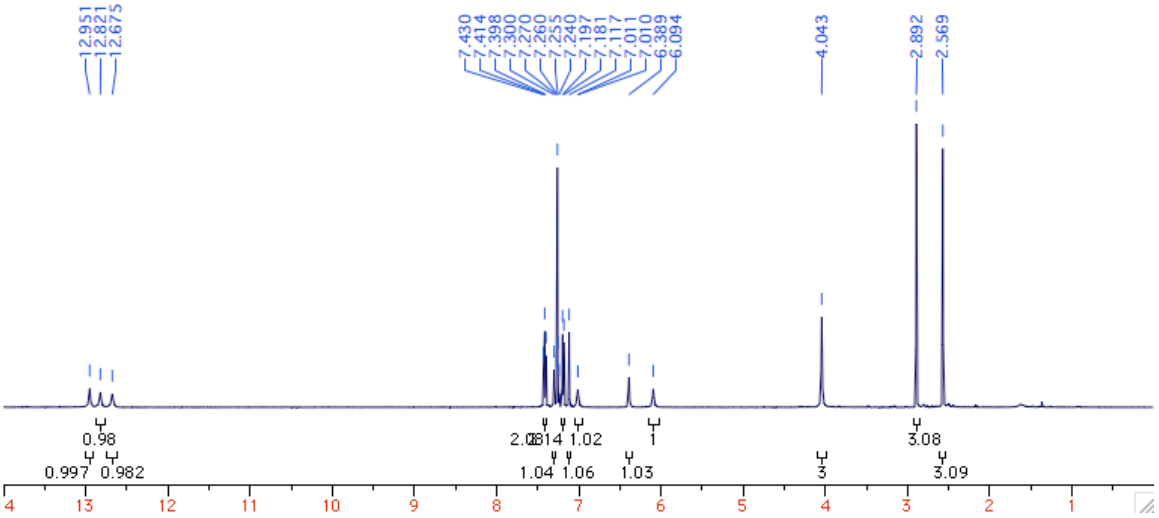

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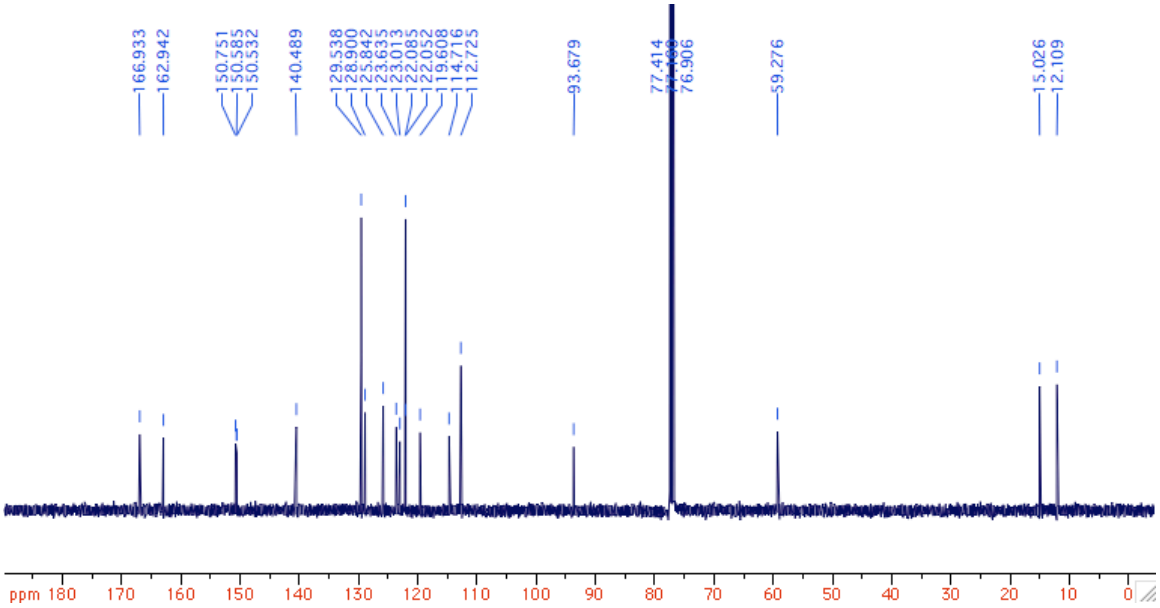

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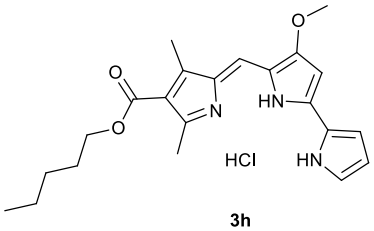

3h

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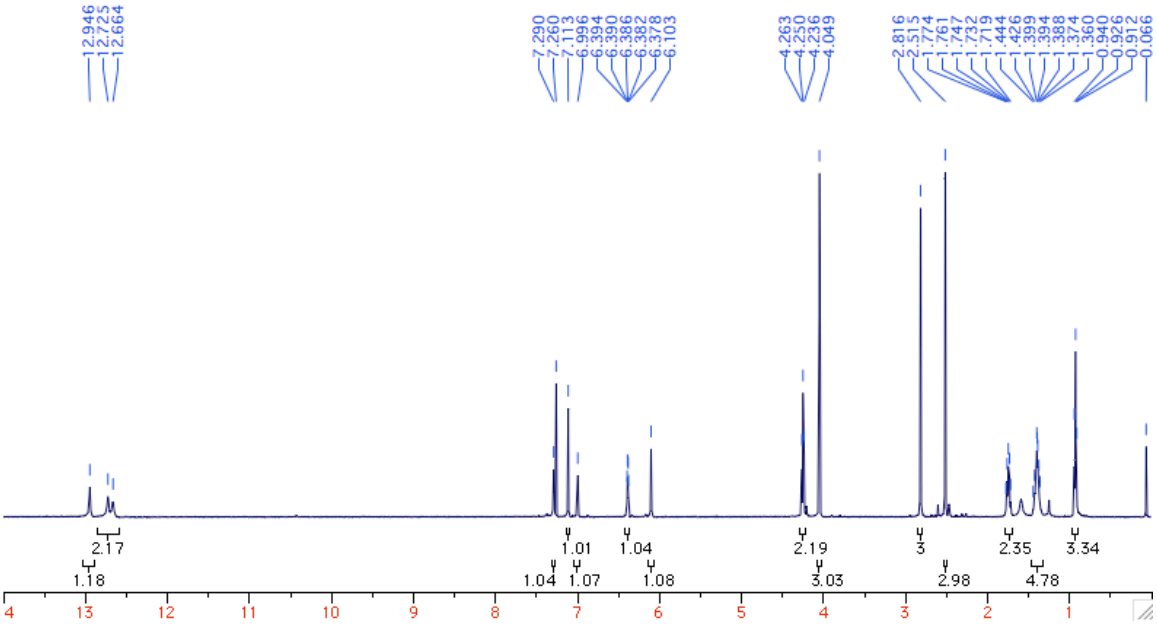
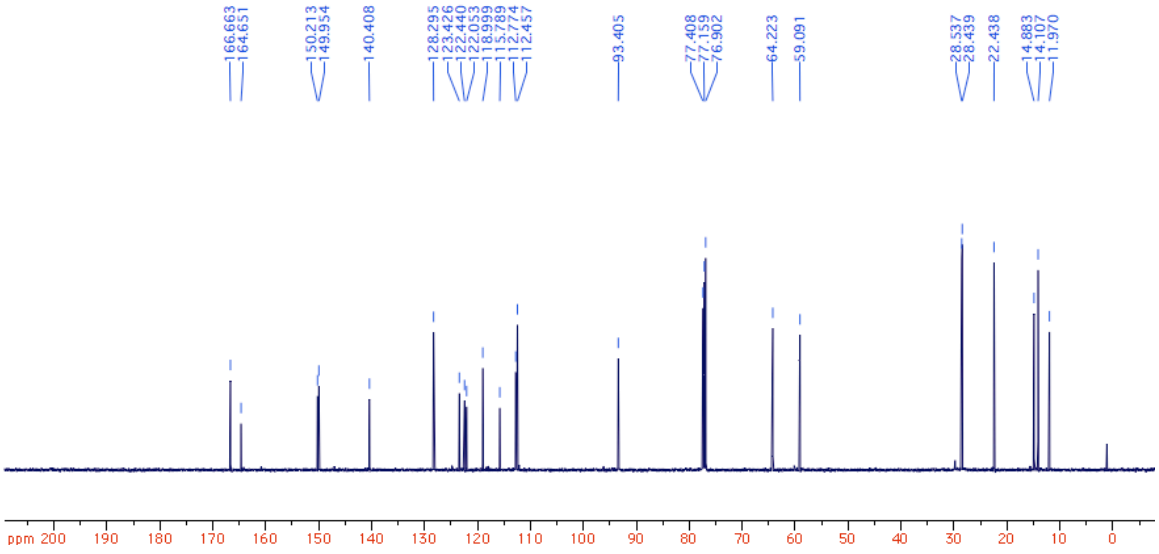


^{13}C NMR, $CDCl_3$, 125 MHz



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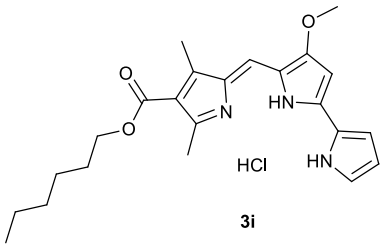

3i

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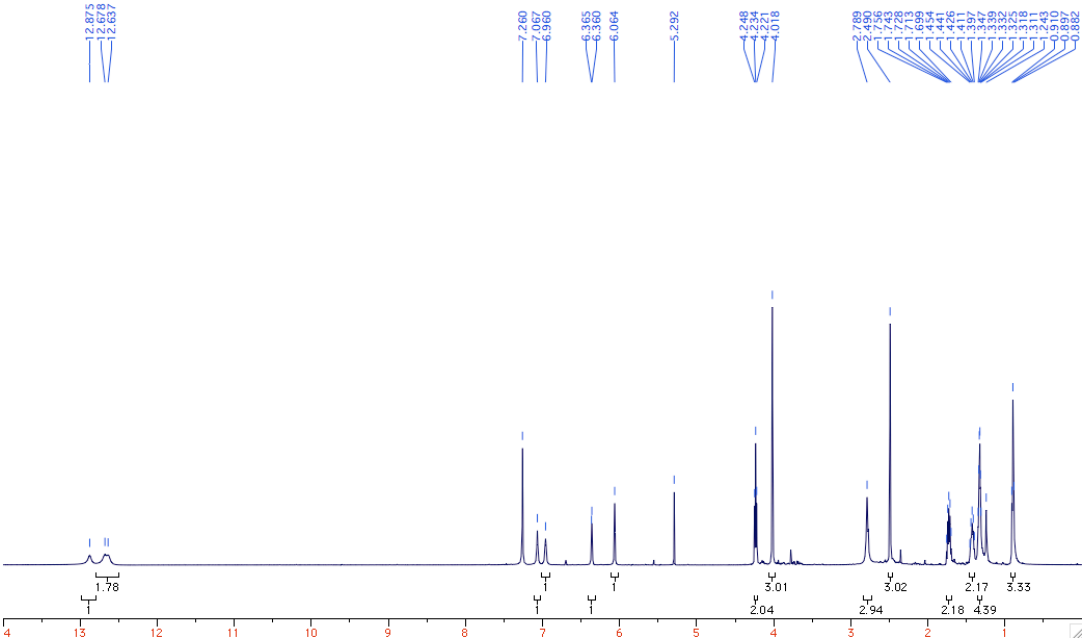

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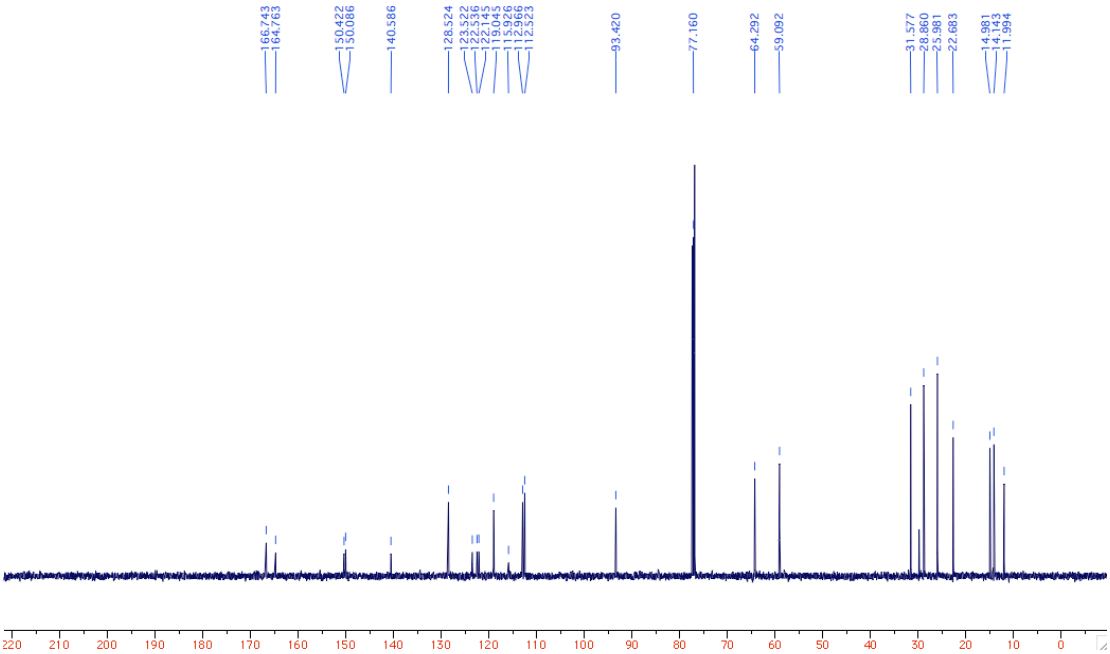

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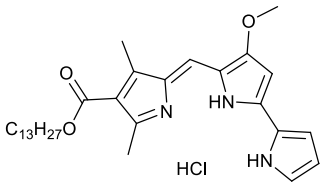

3j

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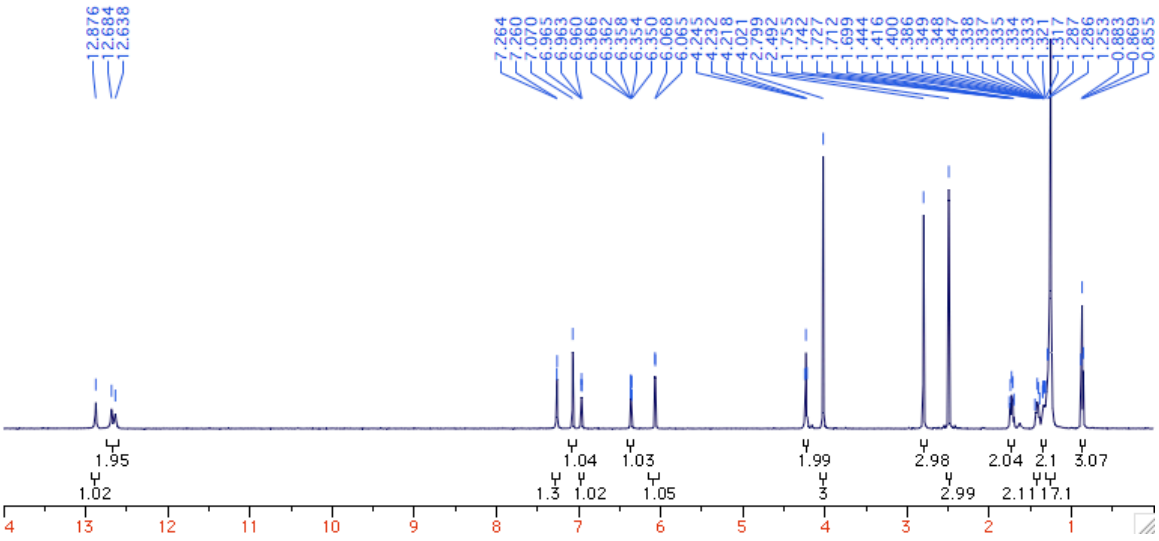

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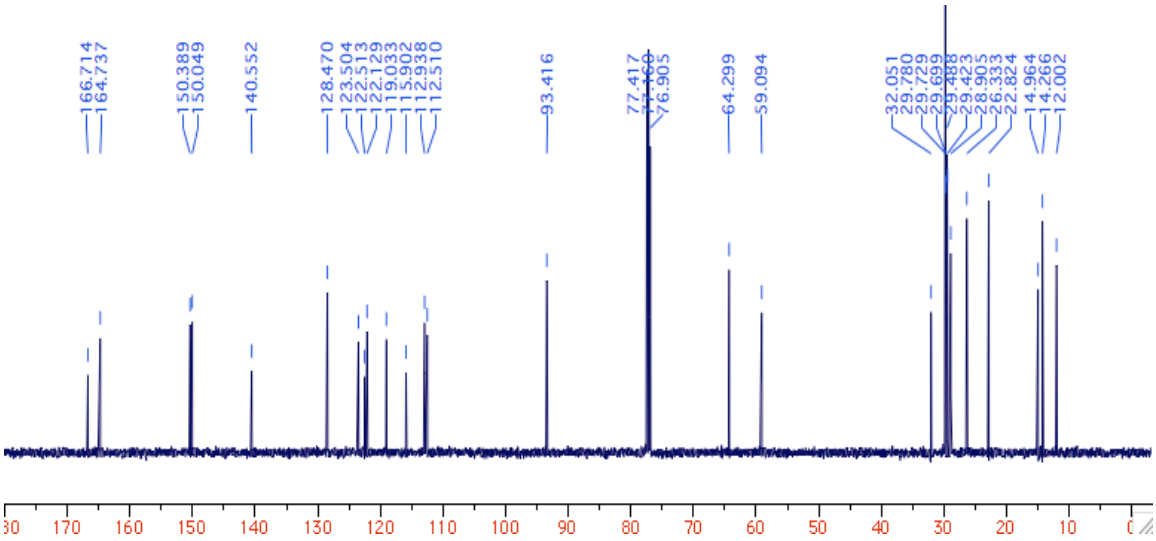

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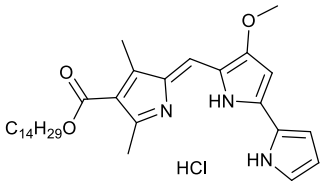
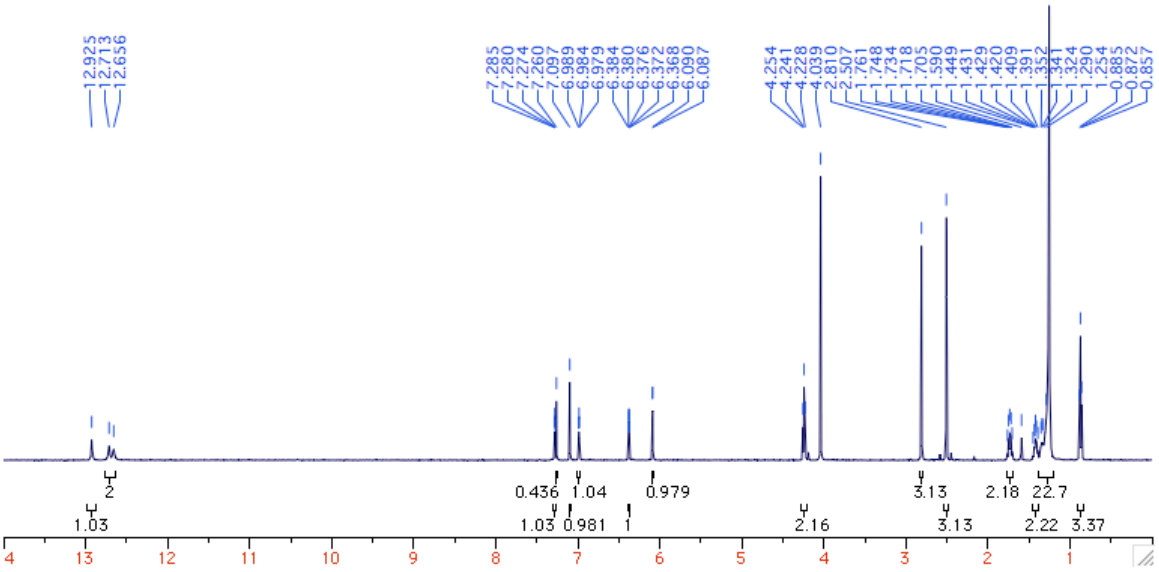


3k

^{1}H NMR, $CDCl_3$, 500 MHz



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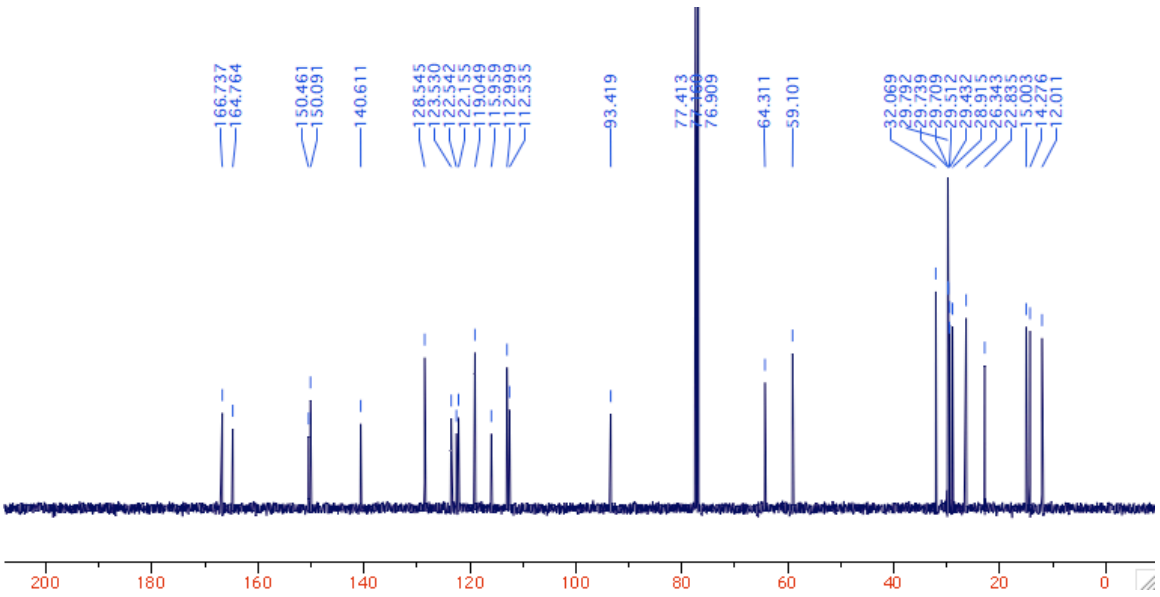

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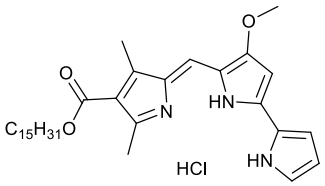

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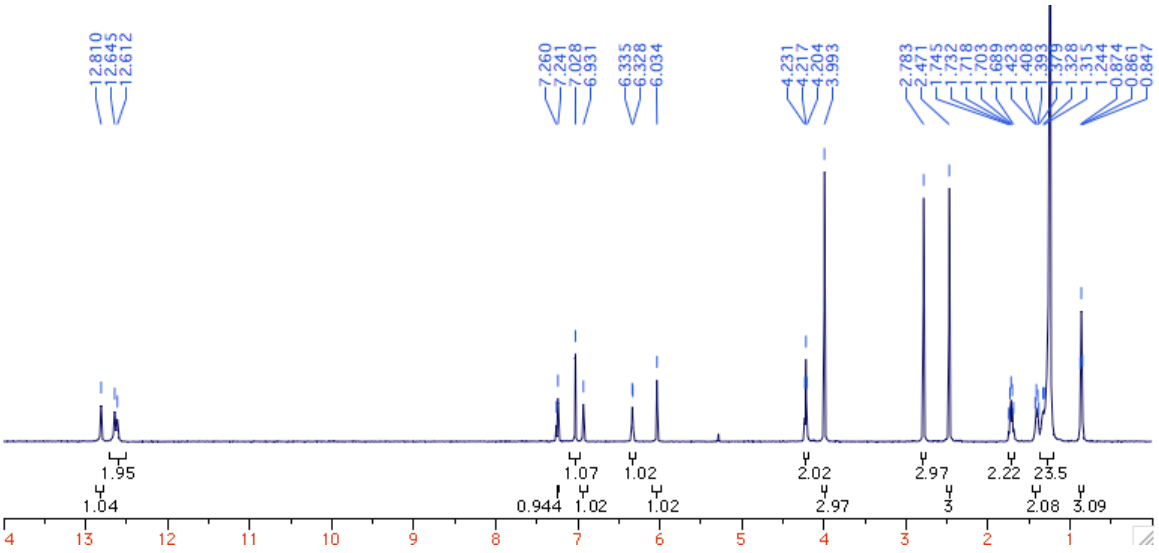

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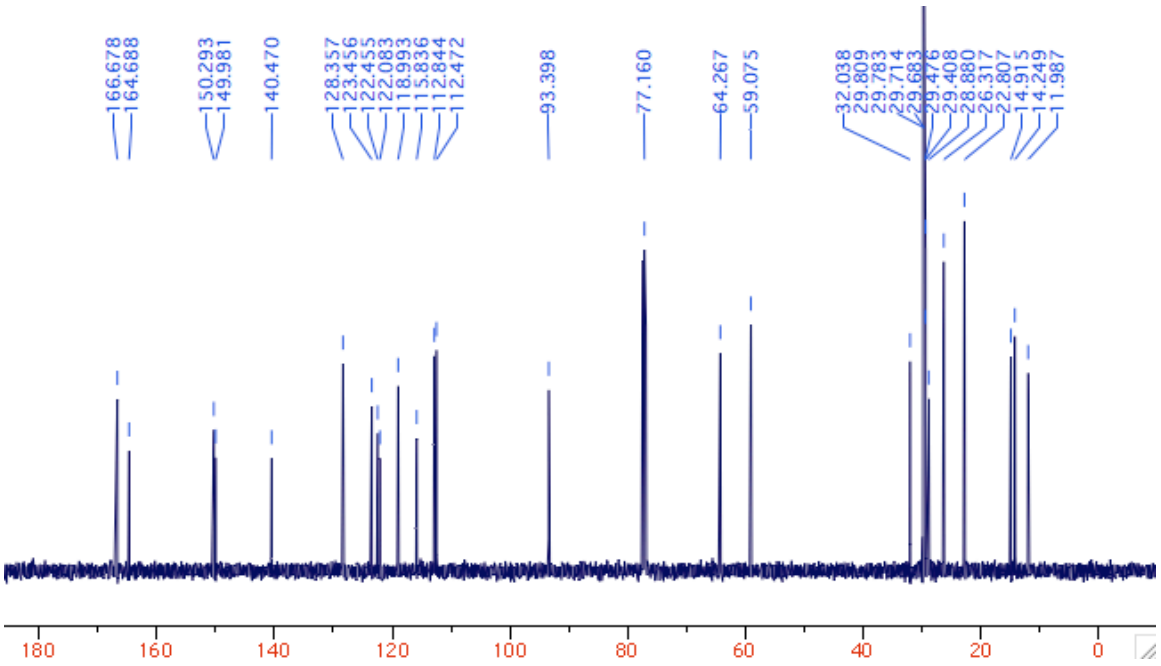

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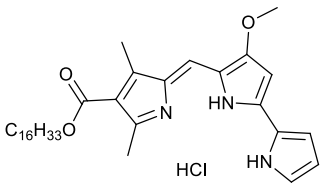

3m

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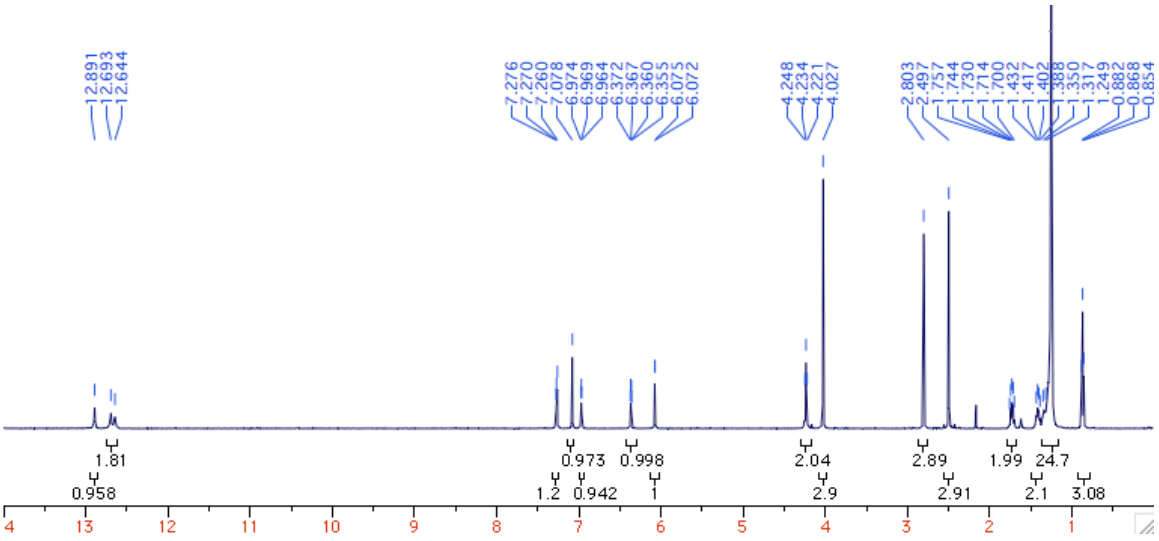
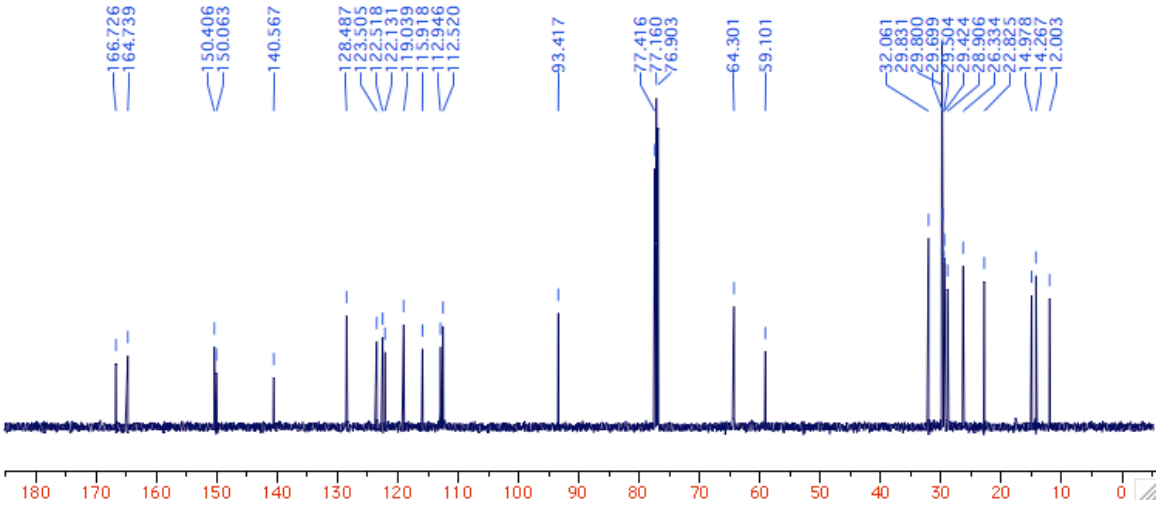


^{13}C NMR, $CDCl_3$, 125 MHz



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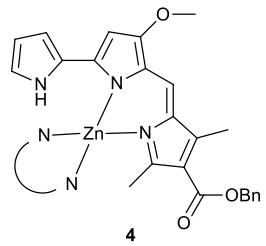

4

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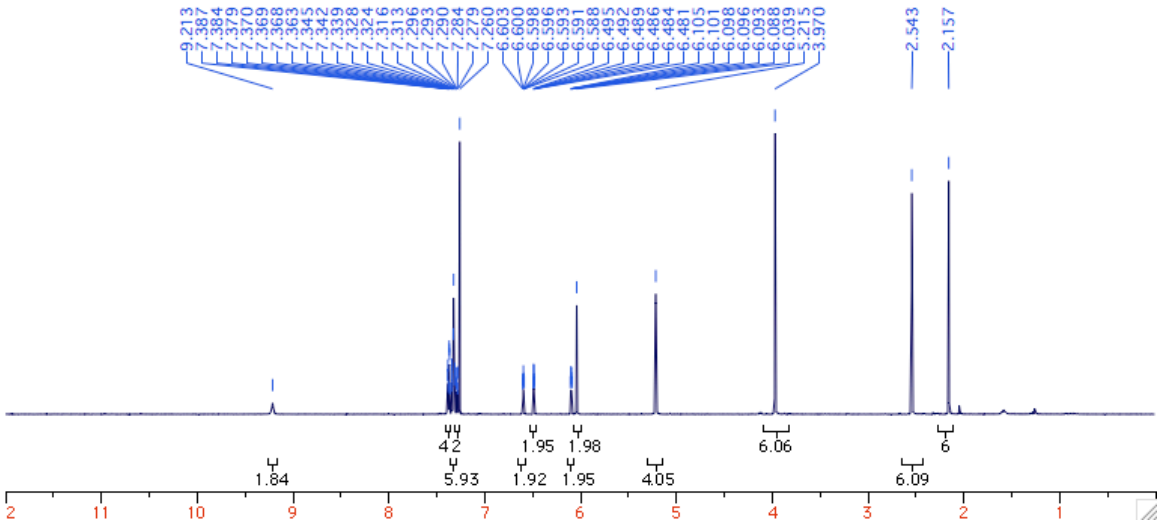

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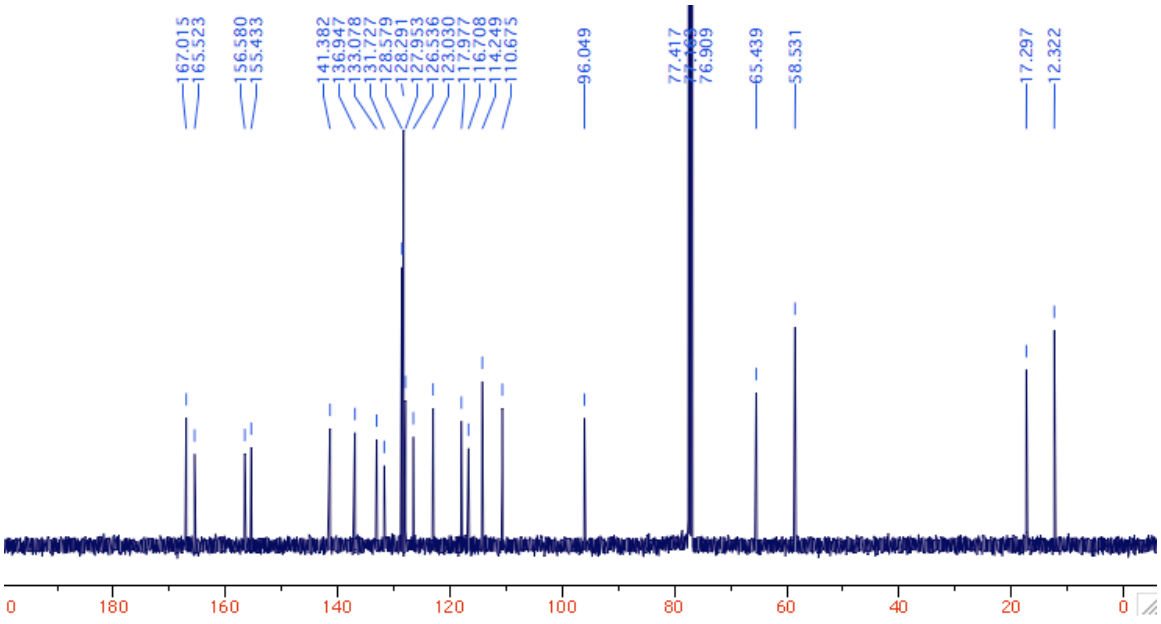

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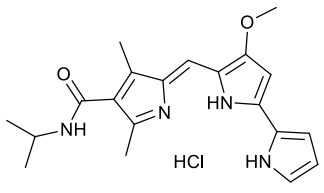

3n

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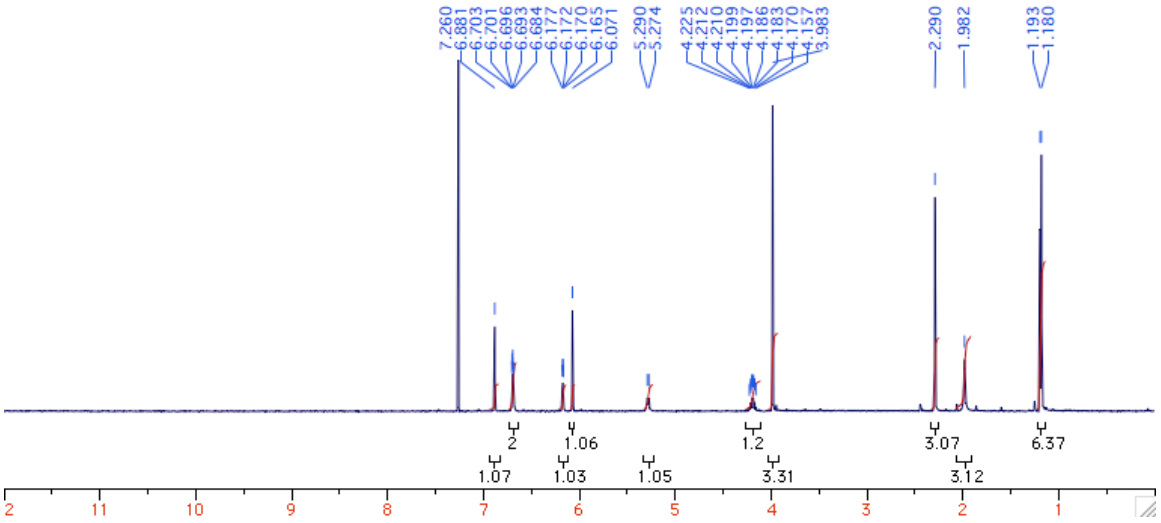
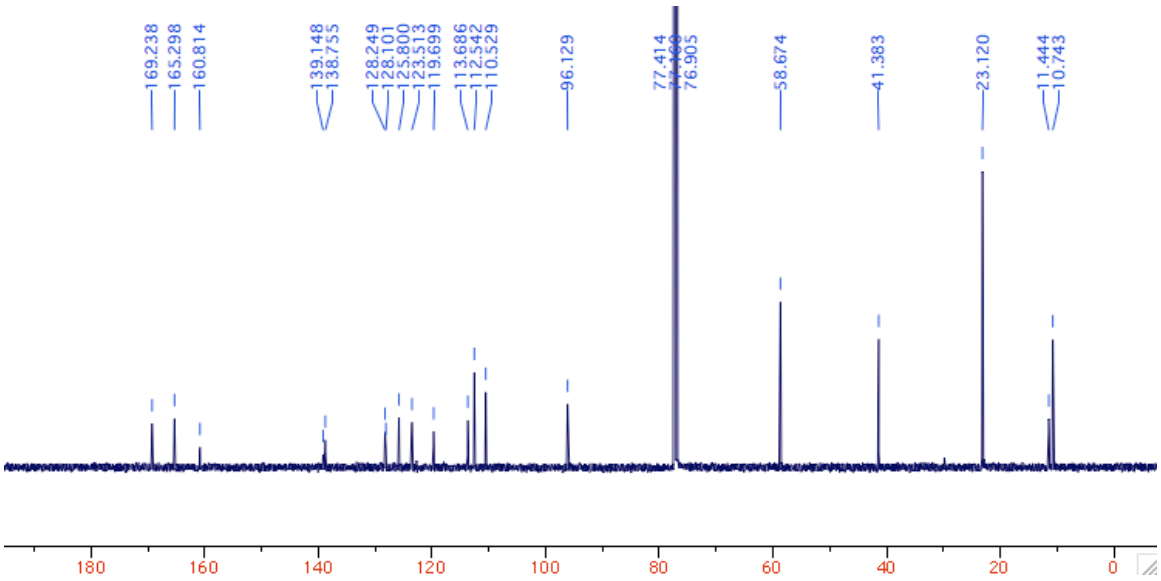


^{13}C NMR, $CDCl_3$, 125 MHz



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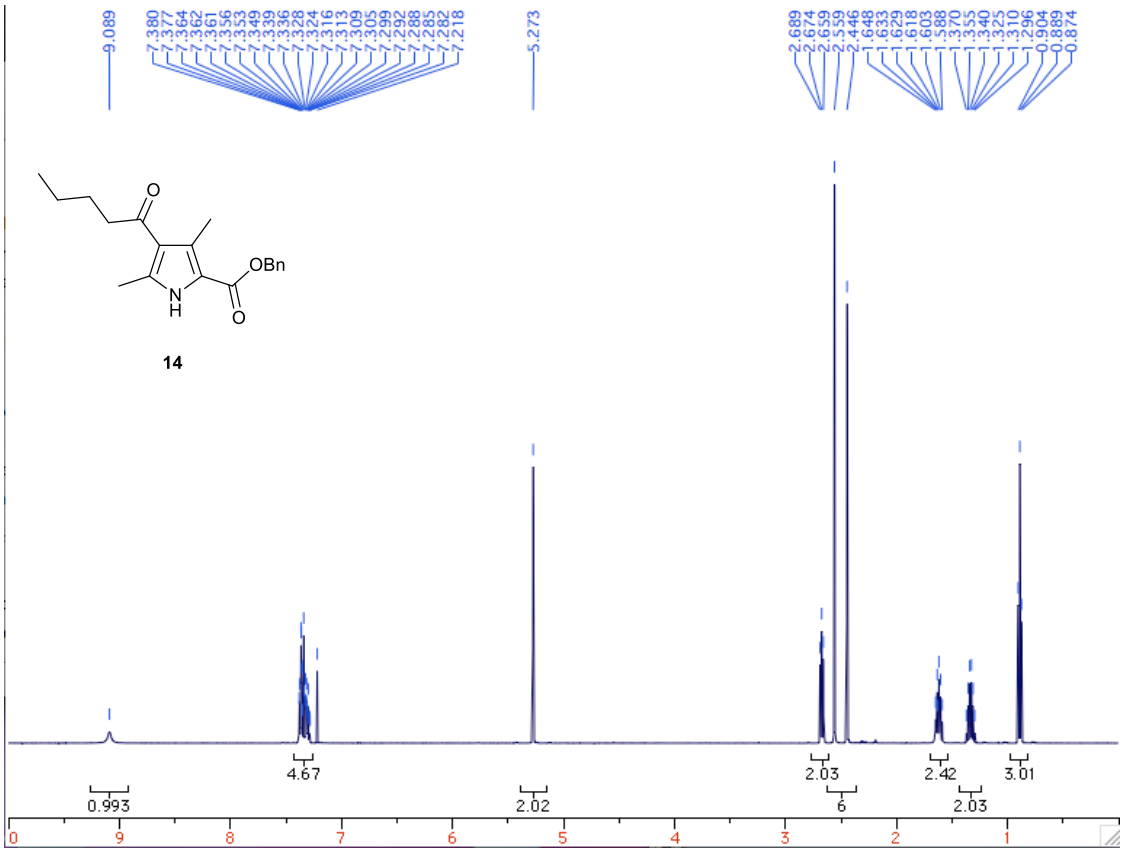
^{1}H NMR, $CDCl_3$, 500 MHz



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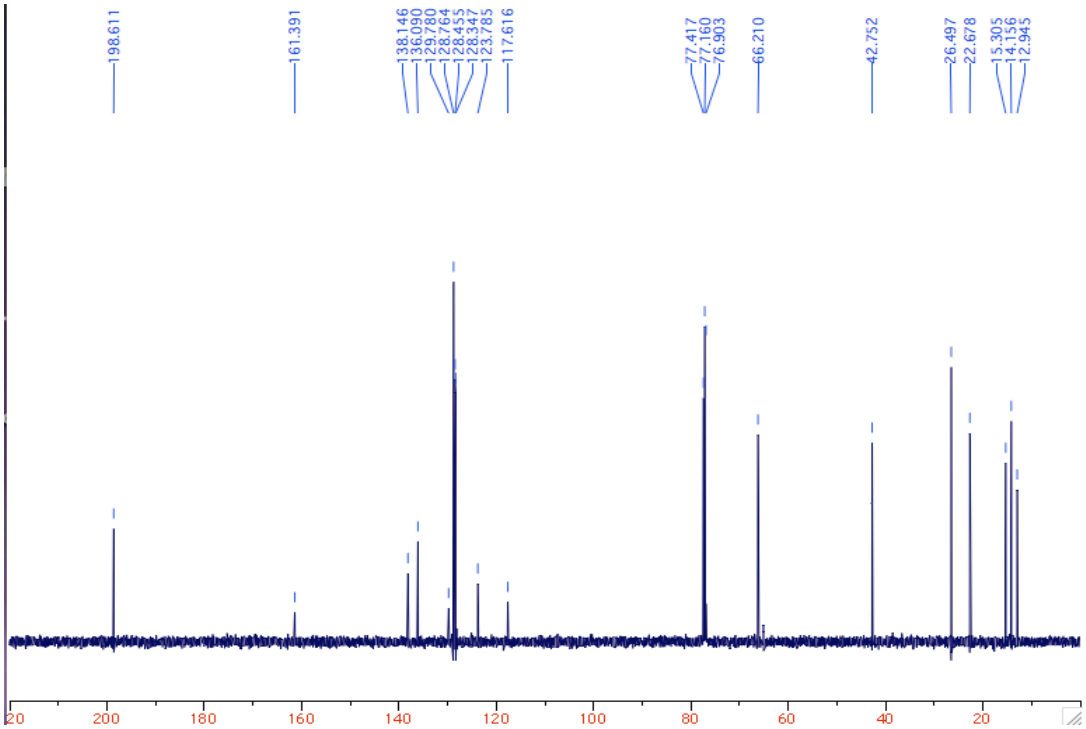

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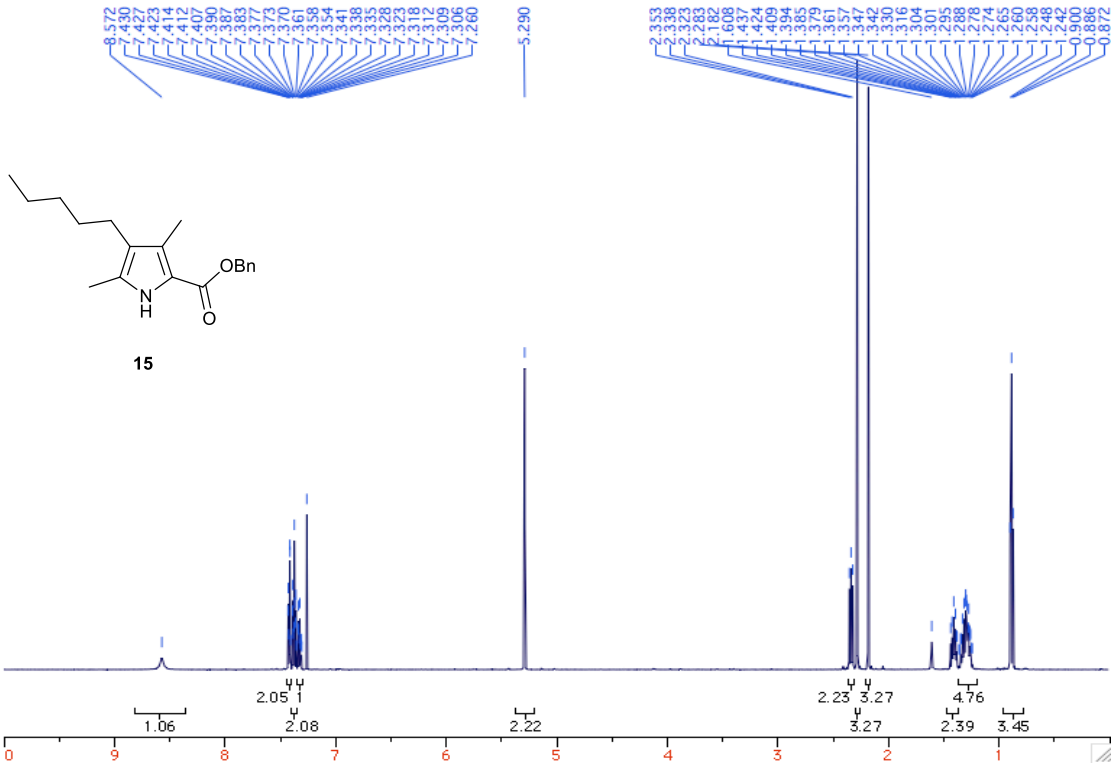
^{1}H NMR, $CDCl_3$, 500 MHz



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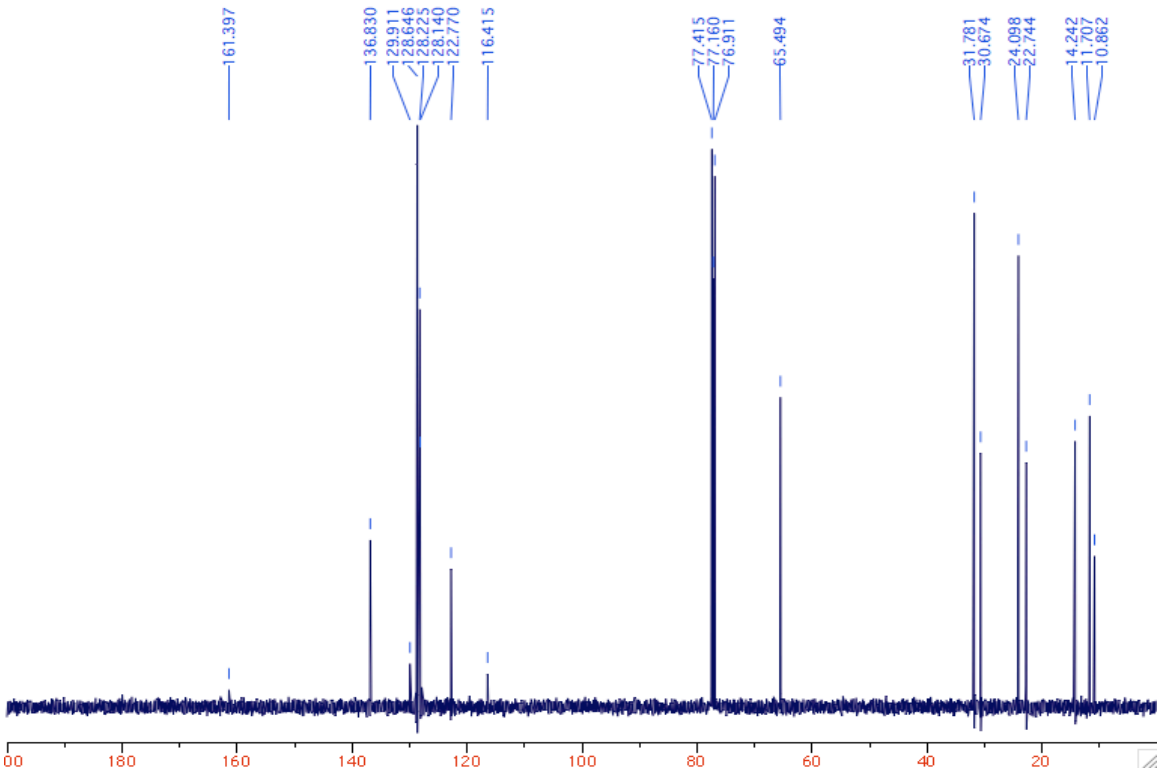

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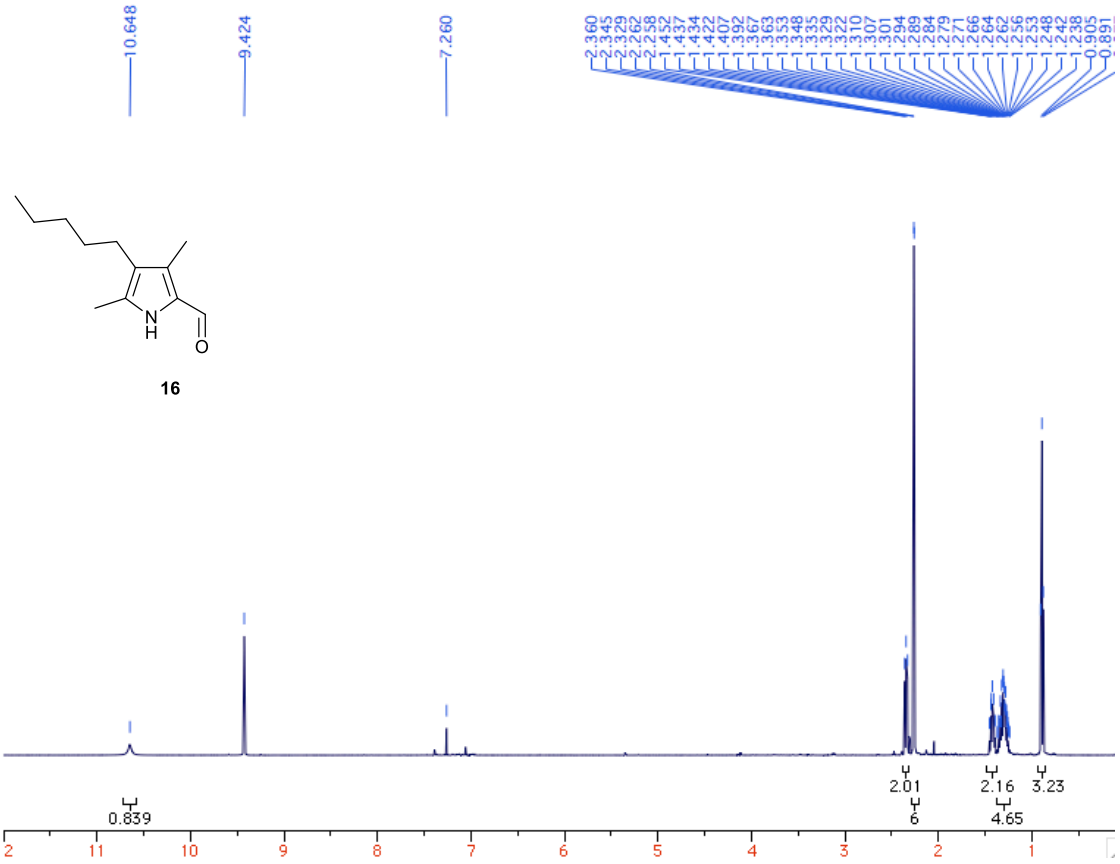
^{1}H NMR, $CDCl_3$, 500 MHz



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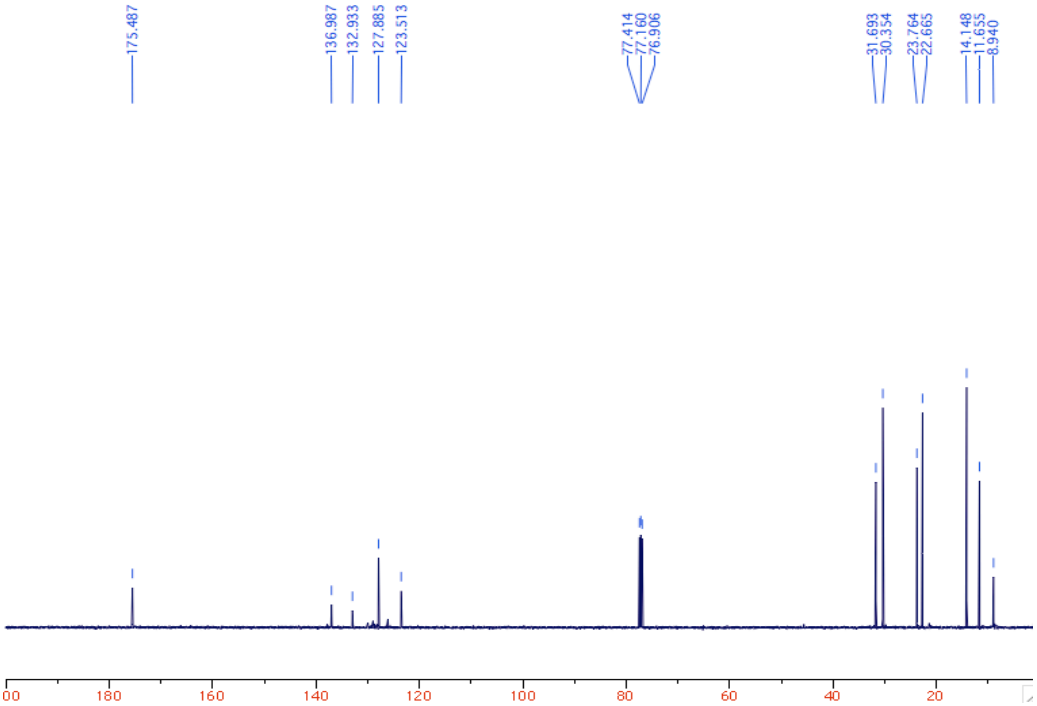

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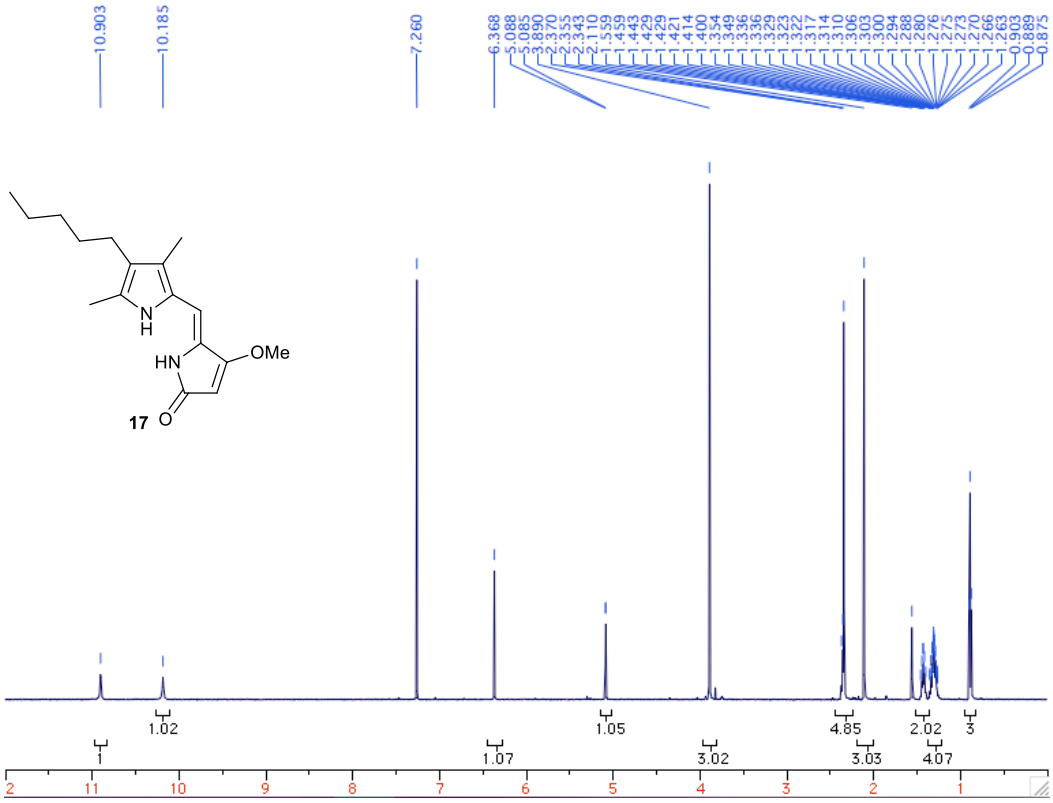
^{1}H NMR, $CDCl_3$, 500 MHz



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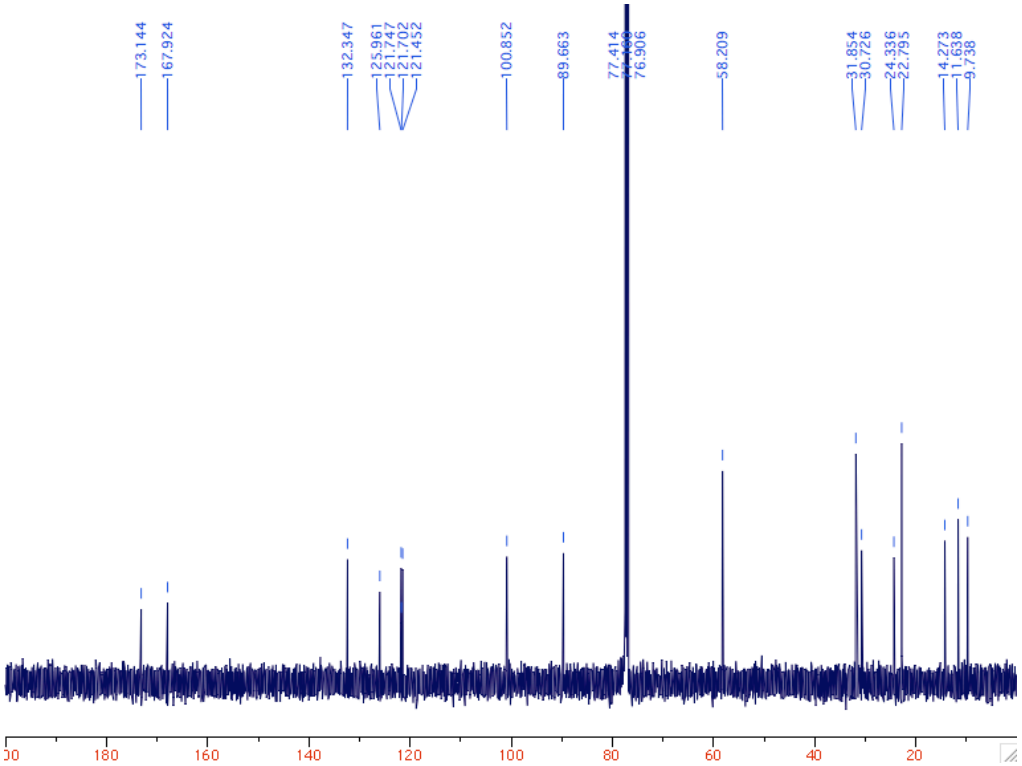

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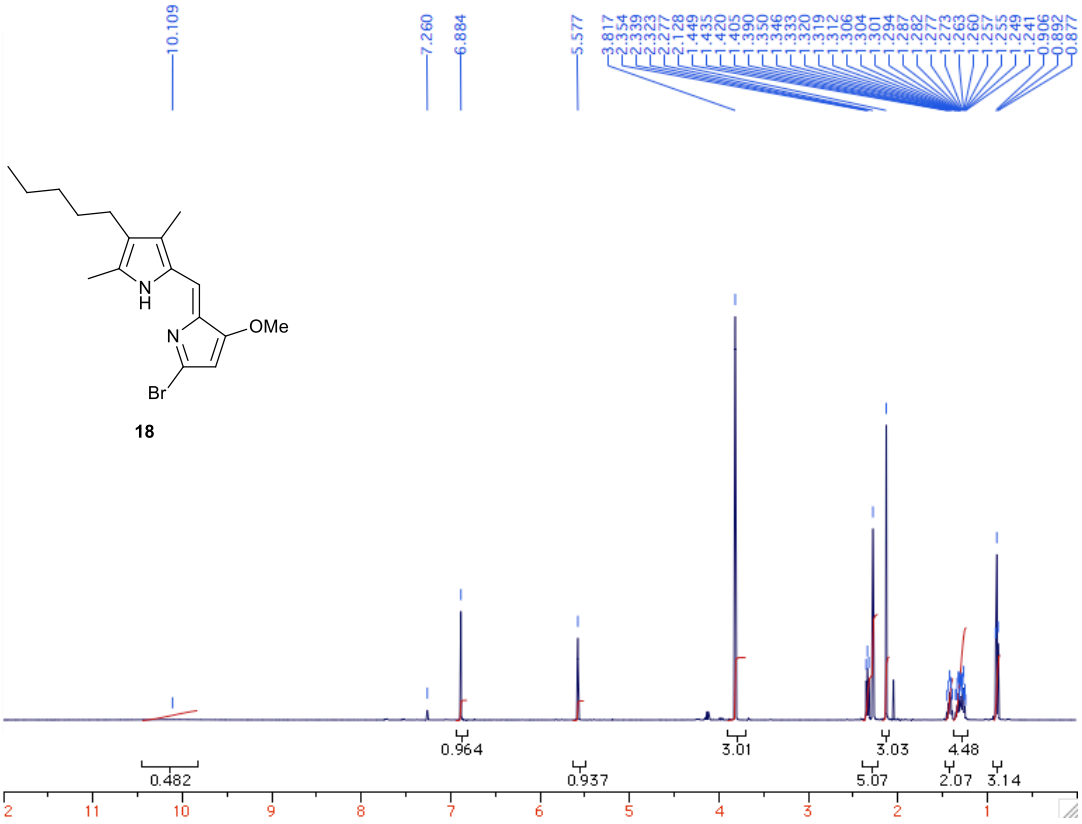

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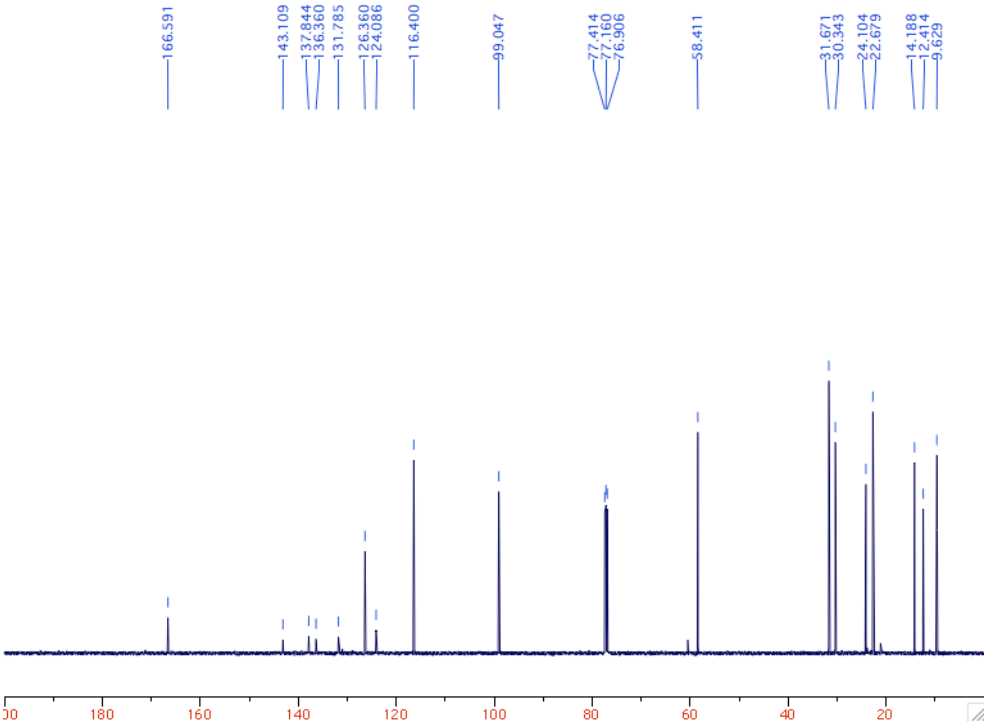

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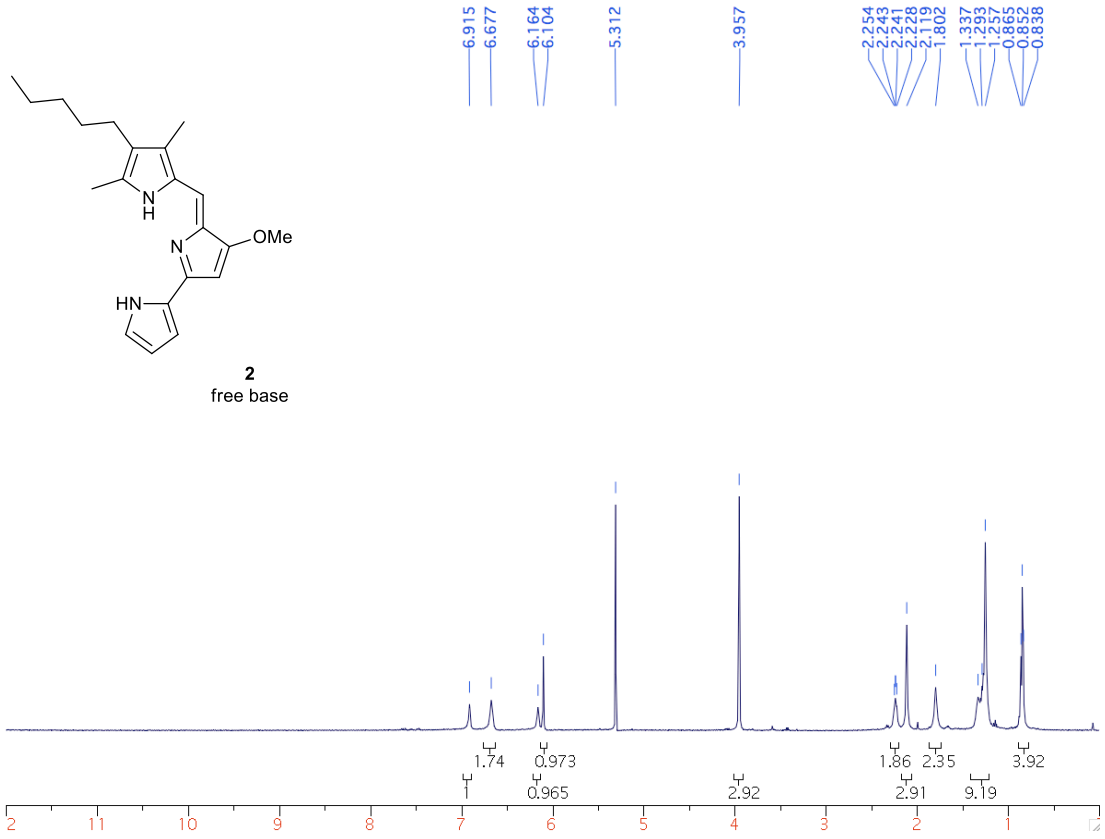
^{1}H NMR, CD_2Cl_2, 500 MHz



2
free base

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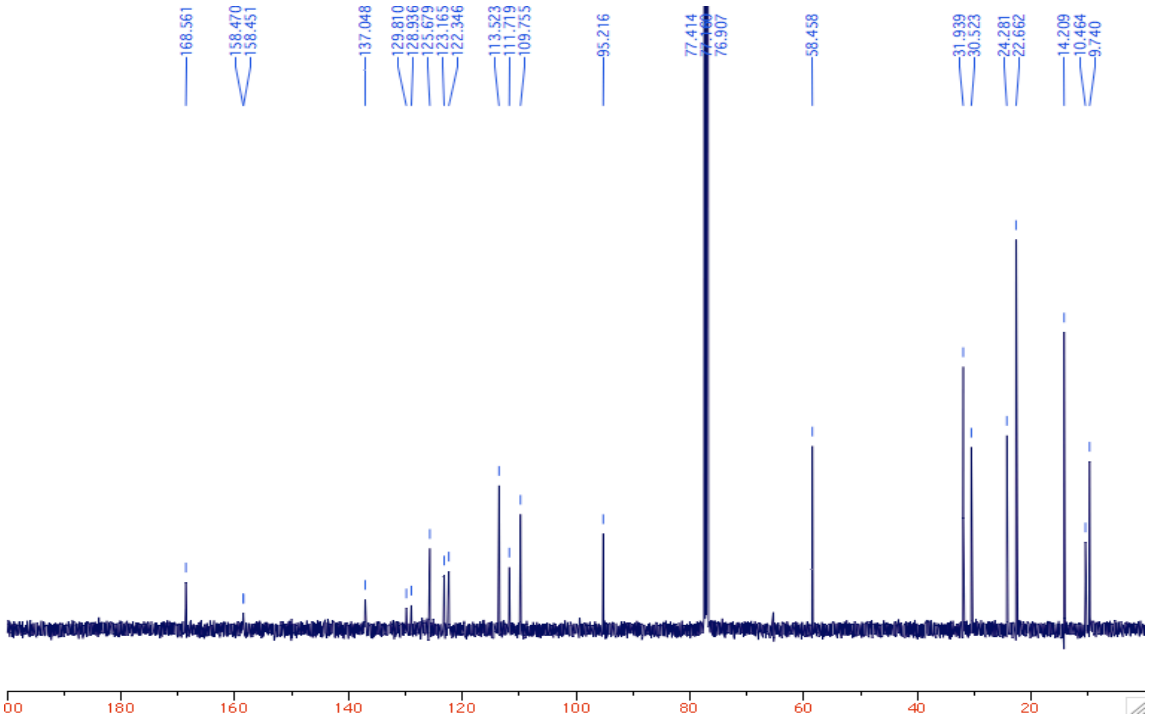

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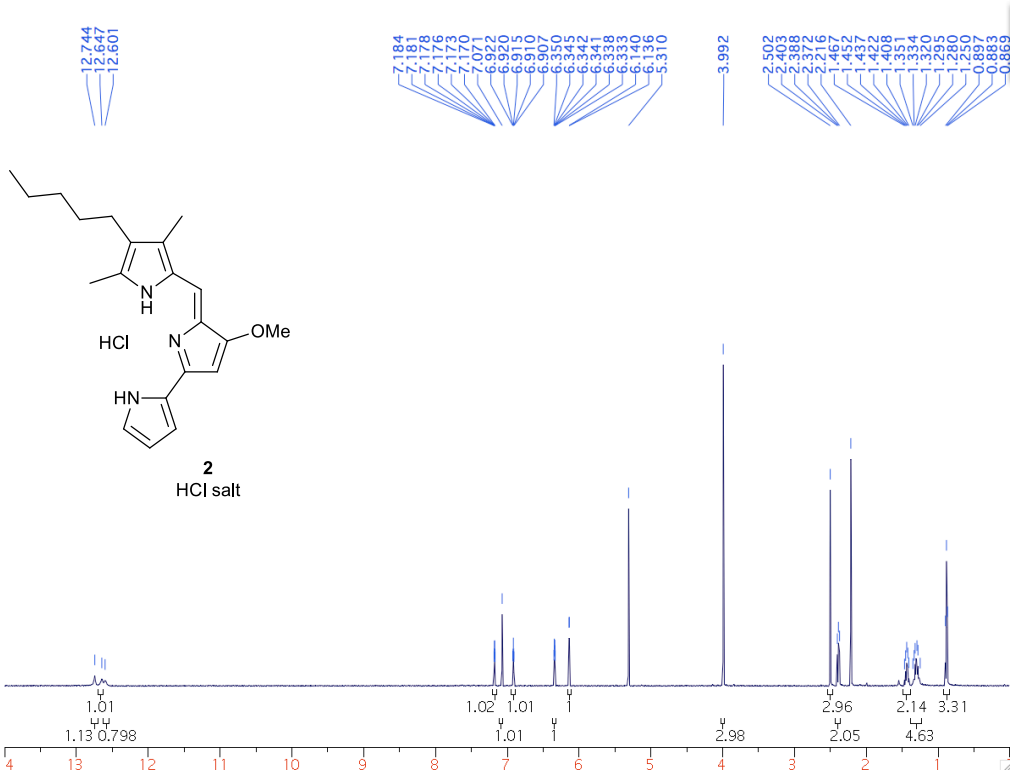
^{1}H NMR, CD_2Cl_2, 500 MHz



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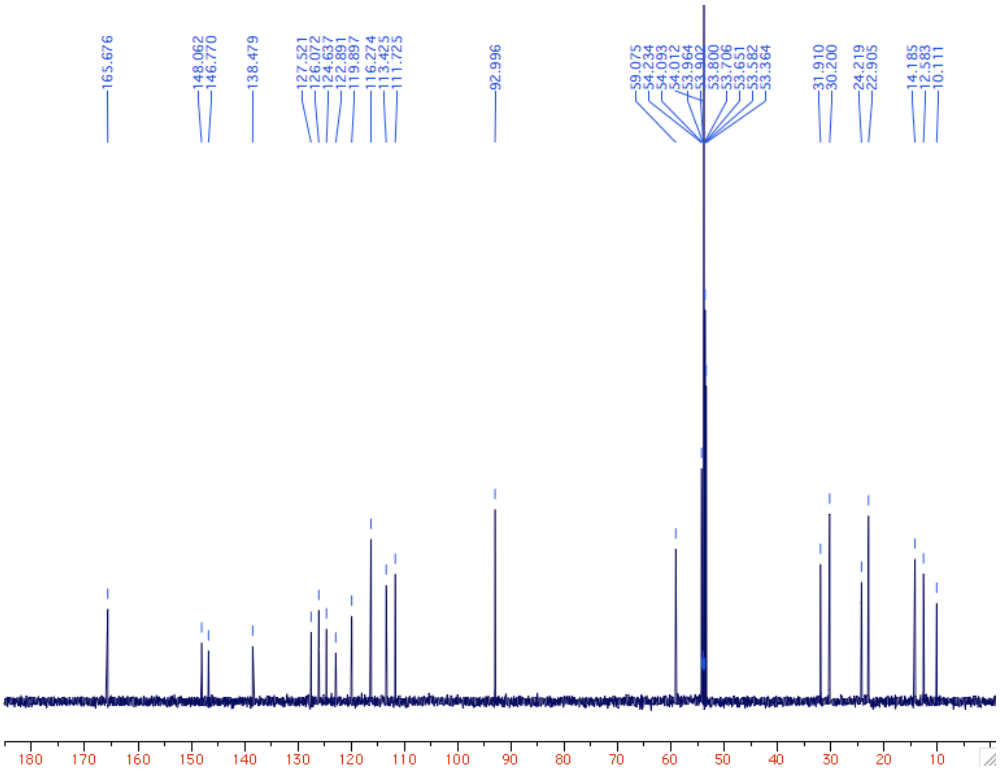

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8) References

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