SUPPORTING INFORMATION

Synthesis of the 2-methylene analogue of the HRV 3C protease inhibitor thysanone (2-carbathysanone)

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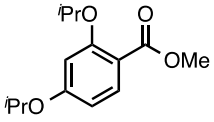
General information:

Unless otherwise stated, all non-aq. reactions and distillations were performed under a nitrogen or argon atmosphere in oven- or flame-dried glassware. Tetrahydrofuran was freshly distilled from sodium/benzophenone. Dichloromethane (CH_2Cl_2) and toluene were freshly distilled from calcium hydride. TMEDA was distilled from calcium hydride and stored under a nitrogen atmosphere over magnesium sulfate. Reactions performed at low temperature were either cooled in an acetone-dry ice bath for temperatures below 0 °C or using a water-ice bath for 0 °C. Flash chromatography was carried out using 0.063-0.1 mm silica gel (Davisil R LC60A 40-63 Micron) with the indicated solvent. Preparatory TLC was carried out on 500 μm UniplateTM (Analtech) silica gel (20 x 20 cm) thin layer chromatography plates. Infrared spectra were recorded using a Perkin Elmer R Spectrum 1000 Fourier Transform Infrared spectrometer. Values are expressed in wavenumbers (cm^{-1}) and recorded in a range of 4000 to 450 cm^{-1}. NMR spectra were recorded at 21 °C in $CDCl_3$ or C_6D_6 on either a Bruker® Avance 300 spectrometer operating at 300 MHz for 1H nuclei and 75 MHz for ^{13}C nuclei or on a Bruker® DRX400 or Bruker® 400 spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei. All chemical shifts are reported in parts per million (ppm) from tetramethylsilane (d = 0) and were measured relative to the solvent in which the sample was analysed ($CDCl_3$: TMS d = 0.00 for ^{1}H NMR and $CDCl_3$ d = 77.16 for ^{13}C NMR; acetone-d_6: d = 2.05 for ^{1}H NMR and d = 29.84±0.01 for ^{13}C NMR). Coupling constants (*J*) are reported in Hertz (Hz). ^{1}H-NMR data is reported as chemical shift in ppm, followed by relative integral, multiplicity ("s" singlet, "d" doublet, "dd" doublet of doublets, "ddd", douplet of douplets of douplets, "dt" doublet of triplets, "t" triplet, "q" quartet, "quint." quintuplet, "sept." septet, "m" multiplet, "b" broad), coupling constant where applicable and assignment. ^{13}C NMR spectra are reported as chemical shift in ppm followed by the assignment. High resolution mass spectra were recorded using a Bruker micrOTOF-QII mass spectrometer.

Methyl 2,4-diisopropoxybenzoate 13

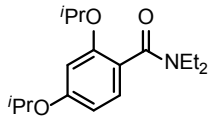


13

To a solution of methyl 2,4-dihydroxybenzoate **24** (200 mg, 1.19 mmol) in acetone-DMF (10:1, 4 mL) were added cesium carbonate (500 mg, 5.57 mmol), TBAI (2 mg) and 2-bromopropane (1.2 mL, 13 mmol). The suspension was heated at reflux for 16 h then quenched at r.t. with 1 M aq. HCl to pH 7. The aq. layer was extracted with CH_2Cl_2 (3 x 10 mL), the combined organic layers washed with brine (10 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue purified by flash chromatography using hexanes-EtOAc (9:1) as eluent to afford the *title compound* (297 mg, 1.19 mmol, quant.) as a colourless oil.

R_f (hexanes:EtOAc = 9:1): 0.31. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.79 (1H, d, *J* = 9.4 Hz), 6.46 (1H, dd, *J* = 6.8 Hz, 2.4 Hz), 6.46 (1H, s), 4,59, (1H, sept., *J* = 5.9 Hz), 4.53 (1H, sept., *J* = 5.9 Hz), 3.84 (3H, s), 1.37 (6H, d, *J* = 5.9 Hz), 1.35 (6H, d, *J* = 5.9 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 166.2, 162.5, 153.4, 133.8, 117.4, 106.8, 103.9, 72.1, 70.2, 51.6, 22.2, 22.1.
The spectroscopic data was in agreement with that reported in the literature.[1]

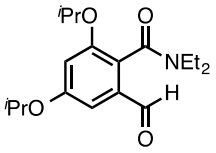
N,*N*-Diethyl-2,4-diisopropoxybenzamide



To a solution of methyl 2,4-diisopropoxybenzoate **13** (3 g, 11.9 mmol) in toluene (15 mL) were added trimethylaluminium (2 M in hexane, 5.9 mL, 11.9 mmol) and diethylamine (1.9 mL, 17.8 mmol) under an atmosphere of nitrogen. The reaction mixture was stirred overnight at r.t. then quenched with satd. aq. NH_4Cl solution (10 mL). The aq. layer was extracted with EtOAc (3 x 30 mL) and the combined organic layers were washed with brine (30 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue purified by flash chromatography using hexanes-EtOAc (1:1) as eluent to afford the *title compound* (3.48 g, 11.9 mmol, quant.) as a colourless oil.

R_f (hexanes:EtOAc = 1:1): 0.5. **ν_{max}** (film/cm^{-1}): 2976, 2934, 1627, 1606, 1277, 1127, 1109. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.11 (1H, d, *J* = 8.4 Hz), 6.47 (1H, dd, *J* = 8.4 Hz, 2.1 Hz), 6.41 (1H, d, *J* = 2.1 Hz), 4.54 (1H, sept., *J* = 6.0 Hz), 4.47 (1H, sept., *J* = 6.0 Hz), 3.83 (1H, bs), 3.25 (1H, bs), 3.20 (2H, bs), 1.33 (6H, d, *J* = 6.0 Hz), 1.29 (6H, d, *J* = 6.0 Hz), 1.21 (3H, t, *J* = 7.1 Hz), 1.02 (3H, t, *J* = 7.1 Hz). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 169.2, 159.4, 154.9, 128.6, 121.1, 107.0, 102.9, 70.9, 70.2, 42.7, 38.7, 22.2, 14.2, 12.8. **HRMS** (ESI): Calculated for $C_{17}H_{28}NO_3$ $[MH]^+$: 294.2069, found: 294.2070; calculated for $C_{17}H_{27}NaNO_3$ $[MNa]^+$: 316.1889, found: 316.1896; calculated for $C_{17}H_{27}KNO_3$ $[MK]^+$: 332.1628, found: 332.1629.

N,*N*-Diethyl-2-formyl-4,6-diisopropoxybenzamide 14

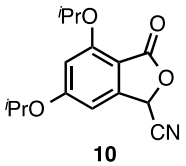


14

To a solution of *N*,*N*-diethyl-2,4-diisopropoxybenzamide (2.9 g, 9.9 mmol) in THF (15 mL) was added TMEDA (1.6 mL, 10.9 mmol) under an atmosphere of nitrogen. The reaction mixture was cooled under nitrogen to −78 °C. *t*-BuLi (1.6 M in hexane, 9.3 mL, 14.8 mmol) was added slowly. The mixture was stirred 15 min until a dark red colour was reached. DMF (1.5 mL, 19.8 mmol) was added at −78 °C and the solution was warmed to r.t. overnight. The reaction was quenched with 4 M aq. HCl (15 mL) and stirred for further 30 min. The aq. layer was extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with brine (30 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue purified by flash chromatography using hexanes-EtOAc (7:3) as eluent to afford the *title compound* (3.17 g, 9.9 mmol, quant.) as a colourless oil.

R_f (hexanes:EtOAc = 1:1): 0.57. **ν_{max}** (film/cm^{-1}): 2979, 2935, 2252, 1698, 1624, 1598, 1312, 1155, 1111, 907, 726. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 9.95 (1H, s), 6.70 (1H, d, *J* = 2.4 Hz), 6.64 (1H, d, *J* = 2.3 Hz), 4.63 (1H, sept, *J* = 6.1 Hz), 4.52 (1H, sept. *J* = 6.1 Hz) 3.80 (1H, sext., *J* = 5.9 Hz), 3.42 (1H, sext., *J* = 7.0 Hz), 3.19-3.11 (2H, m, *J* = 5.9 Hz), 1.37-1.32 (12H, m), 1.29 (3H, t, *J* = 0.8 Hz), 1.26 (3H, t, *J* = 0.8 HzH_3). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 198.5, 190.7, 166.1, 159.3, 155.5, 134.7, 108.4, 104.2, 71.3, 70.5, 42.8, 39.0, 22.2, 22.0, 14.3, 12.7. **HRMS** (ESI): Calculated for $C_{18}H_{28}NO_4$ $[MH]^+$: 244.1838, found: 322.2017; calculated for $C_{18}H_{27}NaNO_4$ $[MNa]^+$: 344.1838, found: 344.1840; calculated for $C_{18}H_{27}KNO_4$ $[MK]^+$: 360.1577, found: 360.1573.

4,6-Diisopropoxy-3-oxo-1,3-dihydroisobenzofuran-1-carbonitrile 10



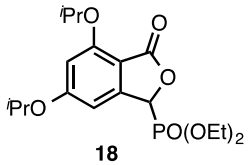
10

To a solution of *N*,*N*-diethyl-2-formyl-4,6-diisopropoxybenzamide **14** (0.93 g, 3.4 mmol) in CH_2Cl_2 (10 mL) were added TMSCN (0.84 mL, 6.7 mmol), 18-crown-6 (0.1 g, 0.34 mmol), and KCN (22 mg, 0.34 mmol) at 0 °C under an atmosphere of nitrogen. The reaction mixture was stirred 3 hours at 0 °C then warmed to r.t. and stirred for 30 min. The solvent was then removed. Glacial acetic acid (10 mL) was added and the mixture was stirred overnight at r.t. The mixture was quenched with 4 M aq. NaOH solution and the pH was adjusted to pH 10. The aq. layer was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (20 mL) and dried over $MgSO_4$.

The solvent was removed *in vacuo* and the residue purified by flash chromatography using hexanes-EtOAc (7:3) as eluent to afford the *title compound* (0.49 g, 1.8 mmol, 53 %) as a colourless solid.

mp 87-89 °C. $\mathbf{R_f}$ (hexanes:EtOAc = 1:1): 0.80. ν_{max} (film/cm^{-1}): 2981, 2937, 1772, 1614, 1596, 1480, 1319, 1186, 1107, 1014, 992. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 6.59 (1H, t, *J* = 1.4 Hz), 6.48 (1H, d, *J* = 1.4 Hz), 5.86 (1H, s), 4.68 (1H, sept., *J* = 6.1 Hz), 4.66 (1H, sept., *J* = 6.1 Hz), 1.43 (3H, d, *J* = 6.1 Hz), 1.43 (3H, d, *J* = 6.1 Hz), 1.41 (3H, d, *J* = 6.1 Hz), 1.40 (3H, d, *J* = 6.1 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 166.3, 165.1, 159.2, 146.7, 114.5, 105.2, 104.0, 99.5, 72.4, 71.6, 21.9, 21.9. **HRMS** (ESI): Calculated for $C_{15}H_{18}NO_4$ $[MH]^+$: 276.1236, found: 276.1235; calculated for $C_{15}H_{17}NaNO_4$ $[MNa]^+$: 298.1055, found: 298.1058; calculated for $C_{15}H_{17}KNO_4$ $[MK]^+$: 314.0795, found: 314.0794.

Diethyl (4′,6′-diisopropoxy-3′-oxo-1′,3′-dihydroisobenzofuran-1′-yl)phosphonate 18

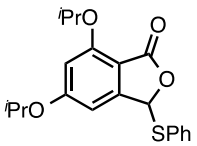


18

Triethylphosphonate (54 µL, 0.31 mmol) and oxalic acid (3 mg, 31 µmol) were added to *N*,*N*-diethyl-2-formyl-4,6-diisopropoxybenzamide **14** (100 mg, 0.31 mmol) under an atmosphere of nitrogen. The reaction mixture was heated at 50 °C overnight then at 100 °C for 7 h. The reaction mixture was cooled to r.t., water (1 mL) was added and the mixture extracted with CH_2Cl_2 (3 x 3 mL). The combined organic layers were washed with brine (2 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue purified by flash chromatography using hexanes-EtOAc (1:1) as eluent to afford the *title compound* (97 mg, 0.25 mmol, 81 %) as a yellow oil.

$\mathbf{R_f}$ (hexanes:EtOAc =1:1): 0.20. ν_{max} (film/cm^{-1}): 2979, 2935, 2874, 1767, 1608, 1475, 1317, 1109, 1018, 984. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 6.73 (1H, m), 6.42 (1H, m), 5.49 (1H, d, *J* = 5.49 Hz), 4.29 (1H, sept., *J* = 6.4 Hz), 4.29 (1H, sept., *J* = 6.0 Hz), 4.42-3.82 (4H, m), 1.40 (6H, dd, *J* = 16.0 Hz, 6.0 Hz), 1.39 (6H, dd, *J* = 16 Hz, 6.4 Hz), 1.35 (3H, t, *J* = 6.1 Hz), 1.13 (3H, t, *J* = 6.1 Hz). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 167.4, 165.2, 158.7, 148.7, 103.5, 100.2, 77.4, 72.1, 71.1, 64.5, 64.0, 22.0, 21.9, 16.6, 16.4. **HRMS** (ESI): Calculated for $C_{18}H_{28}O_7P$ $[MH]^+$: 387.1567, found: 387.1559; calculated for: $C_{18}H_{27}NaO_7P$ $[MNa]^+$: 409.1387, found: 409.1383.

5,7-Diisopropoxy-3-(phenylthio)isobenzofuran-1(3*H*)-one 19



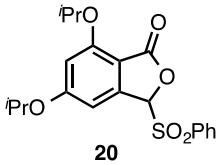
19

To a solution of *N*,*N*-diethyl-2-formyl-4,6-diisopropoxybenzamide **14** (500 mg, 1.56 mmol) in THF (2 mL) was added 2 M aq. KOH (5 mL). The reaction mixture was stirred overnight at r.t. then

acidified with concentrated HCl to pH 1. The aq. layer was extracted with EtOAc (3x10 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed at reduced pressure. Toluene (5 mL) was added followed by thiophenol (190 µL, 1.87 mmol) and *para*-toluenesulfonic acid (20 mg). The reaction was stirred for 3 days at r.t. The solvent was removed *in vacuo* and the residue was purified by flash chromatography using hexanes-EtOAc (1:1) as eluent to afford the *title compound* (200 mg, 0.56 mmol, 36 %) as a yellow oil.

R_f (hexanes:EtOAc =1:1): 0.79. **ν_{max}** (film/cm^{-1}): 2978, 1764, 1611, 1476, 1366, 1318, 1037, 841. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.54-7.53 (2H, m), 7.31-7.27 (3H, m), 6.59 (1H, d, *J* = 1.6 Hz), 6.51 (1H, s), 6.37 (1H, d, *J* = 1.6 Hz), 4.65 (1H, sept., *J* = 6.0 Hz), 4.60 (1H, sept., *J* = 6.2 Hz), 1.41-1.36 (12H, m). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 165.1, 158.4, 151.0, 133.4, 131.4, 129.2, 128.7, 103.9, 103.8, 100.5, 85.0, 72.3, 71.1, 22.1, 22.0, 21.9. **HRMS** (ESI): Calculated for $C_{20}H_{23}O_4S$ $[MH]^+$: 359.1312, found: 359.1310; calculated for $C_{20}H_{22}NaO_4S$ $[MNa]^+$: 381.1131, found: 381.1128.

5,7-Diisopropoxy-3-(phenylsulfonyl)isobenzofuran-1(3*H*)-one 20

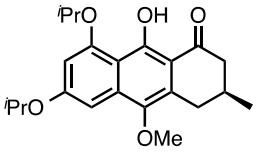


20

To a solution of *N,N*-diethyl-2-formyl-4,6-diisopropoxybenzamide **14** (80 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) was added *m*-CPBA (92 mg, 0.54 mmol) at 0 °C. The reaction mixture was stirred for 20 min then warmed to r.t. and stirred for a further 30 min. *m*-CPBA (16 mg, 0.09 mmol) was added and the mixture was stirred for 1 h. The reaction was quenched with satd. aq. $NaHCO_3$ solution (2 mL). The layers were separated and the organic layer was dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was purified by flash chromatography using hexanes-EtOAc (4:1) as eluent to afford the *title compound* (83 mg, 0.21 mmol, 97 %) as a colourless solid.

R_f (hexanes:EtOAc = 4:1): 0.49. ν_{max} (film/cm^{-1}): 2978, 1764, 1611, 1476, 1366, 1318, 1037, 841. **m.p.**: 162.4-162.6 °C. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.87-7.85 (2H, m), 7.68-7.64 (1H, m), 7.54-7.50 (2H, m), 6.92 (1H, d, *J* = 1.7 Hz), 6.45 (1H, d, *J* = 1.7 Hz), 5.96 (1H, s), 4.72 (1H, sept., *J* = 6.5 Hz), 4.58 (1H, sept., *J* = 6.0 Hz), 1.42 (6H, dd, *J* = 12.6 Hz, 6.5 Hz), 1.36 (6H, d, *J* = 6.0 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 153.0, 151.8, 134.9, 134.6, 130.4, 129.9, 129.3, 127.5, 104.9, 101.7, 89.7, 72.3, 71.4, 22.1, 22.0, 21.9. **HRMS** (ESI): Calculated for $C_{20}H_{23}O_6S$ $[MH]^+$: 391.1210, found: 391.1209; calculated for $C_{20}H_{22}O_6NaS$ [MNa]+: 413.1035, found: 413.1024.

9-Hydroxy-6,8-diisopropoxy-10-methoxy-3-methyl-3,4-dihydroanthracen-1(2*H*)-one (±)-17

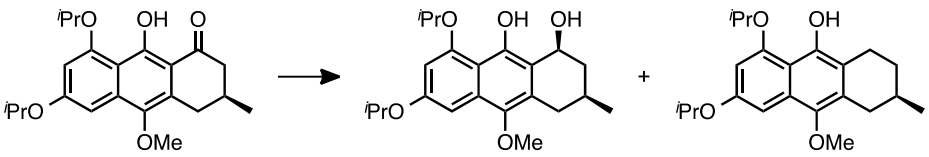


(±)-**17**

To a solution of 4,6-diisopropoxy-3-oxo-1,3-dihydroisobenzofuran-1-carbonitrile **10** (100 mg, 0.36 mmol) and cyclohexenone (±)-**11** (79 mg, 0.72 mmol) in THF (1 mL) was added ^{t}BuOK (45 mg, 0.4 mmol) at r.t. under an atmosphere of nitrogen. The reaction mixture was stirred for 60 min. Dimethyl sulfate (0.17 mL, 1.8 mmol), KOH (40 mg) and water (3 drops) were added. The reaction was heated to 60 °C and stirred for further 17 h. Water (5 mL) was added and the aq. layer extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue purified by flash chromatography using hexanes-EtOAc (7:3) as eluent to afford the *title compound* (56 mg, 0.15 mmol, 42 %) as a yellow fluorescent oil.

R_f (hexanes:EtOAc =7:3): 0.83. **ν_{max}** (film/cm^{-1}): 2977, 2934, 2873, 1612, 1599, 1410, 1382, 1057, 899. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 13.25 (1H, s), 6.91 (1H, d, *J* = 2.3 Hz), 6.47 (1H, d, *J* = 2.3 Hz), 4.76 (1H, sept., *J* = 6.0 Hz), 4.64 (1H, sept., *J* = 6.0 Hz), 3.77 (3H, s), 3.26 (1H, dq, *J* = 2.2 Hz, 0.3 Hz), 2.73 (1H, dq, *J* = 2.2 Hz, 0.3 Hz), 2.52-2.34 (2H, m), 2.29-2.17 (1H, m), 1.46 (6H, d, *J* = 6.0 Hz), 1.42 (6H, d, *J* = 6.0 Hz), 1.95 (3H, d, *J* = 6.4 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 203.6, 163.4, 160.8, 160.5, 142.5, 137.0, 128.5, 112.0, 109.1, 102.8, 95.8, 72.5, 70.1, 60.6, 46.6, 31.8, 29.3, 22.2, 21.4. **HRMS** (ESI): Calculated for $C_{22}H_{29}O_5$ $[MH]^+$: 373.2010, found: 373.2000; calculated for $C_{22}H_{28}NaO_5$ $[MNa]^+$: 395.1834, found: 395.1815; calculated for $C_{22}H_{28}KO_5$ $[MK]^+$: 411.1568, found: 411.1557.

***cis*-6,8-Diisopropoxy-10-methoxy-3-methyl-1,2,3,4-tetrahydroanthracene-1,9-diol (±)-*cis*-21**

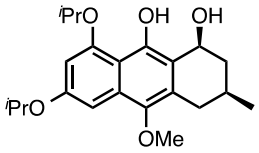


(±)-**17** (±)-*cis*-**21** (±)-**22**

To a solution of 9-hydroxy-6,8-diisopropoxy-10-methoxy-3-methyl-3,4-dihydroanthracen-1(2*H*)-one (±)-**17** (40 mg, 0.10 mmol) in THF (1 mL) was added a solution of cyclohexylborane (1 M, 0.1 mL, 0.1 mmol) at 0 °C under an atmosphere of nitrogen. The reaction mixture was warmed to r.t. stirred for 1 hour then quenched with satd. aq. NH_4Cl solution (3 mL). The aq. layer was extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with brine (10 mL) and dried

over $MgSO_4$. The solvent was removed *in vacuo* and the residue purified by flash chromatography using hexanes-EtOAc (4:1) as eluent to afford the *title compound* (28 mg, 75 µmol, 72%) as a yellow oil and 6,8-diisopropoxy-10-methoxy-3-methyl-1,2,3,4-tetrahydroanthracen-9-ol (±)-**22** (10 mg, 28 µmol, 25%) as a yellow oil.

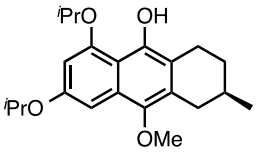
***cis*-6,8-Diisopropoxy-10-methoxy-3-methyl-1,2,3,4-tetrahydroanthracene-1,9-diol** (±)-*cis*-**21**



(±)-*cis*-**21**

R_f (hexanes:EtOAc =4:1): 0.45. **ν_{max}** (film/cm^{-1}): 3394, 2974, 2927, 2874, 1627, 1612, 1449, 1365, 1227, 1034. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 10.02 (1H, s), 6.91 (1H, d, *J* = 2.1 Hz), 6.42 (1H, d, *J* = 2.1 Hz), 5.23 (1H, dd, *J* = 9.6 Hz, 7.4 Hz), 4.81 (1H, sept., *J* = 6.0 Hz), 4.72 (1H, sept., *J* = 6.0 Hz), 4.51 (1H, s), 3.77 (3H, s), 3.10 (1H, dq, *J* = 16.5 Hz, 2.1 Hz), 2.36-2.26 (2H, m), 1.87-1.70 (1H, m), 1.5 (6H, d, *J* = 6.0 Hz), 1.40 (6H, dd, *J* = 6.0 Hz, 2.1 Hz), 1.15 (3H, d, *J* = 6.5 Hz). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 156.0, 155.5, 149.21, 144.2, 130.2, 129.6, 119.3, 110.2, 100.8, 95.5, 73.1, 69.9, 67.3, 60.5, 40.0, 33.2, 27.9, 22.3, 22.2, 22.1. **HRMS** (ESI): Calculated for $C_{22}H_{31}O_5$ $[MH]^+$: 359.2217, found: 359.2206; calculated for $C_{22}H_{30}NaO_5$ $[MNa]^+$: 381.2036, found: 381.2019.

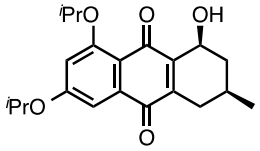
6,8-Diisopropoxy-10-methoxy-3-methyl-1,2,3,4-tetrahydroanthracen-9-ol (±)-**22**



(±)-**22**

R_f (hexanes:EtOAc =4:1): 0.60. **ν_{max}** (film/cm^{-1}): 3566, 3338, 2977, 2930, 2877, 2834, 1656, 1626, 1611, 1450, 1364, 1225, 1108, 874, 613. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 9.62 (1H, s), 6.90 (1H, d, *J* = 2.2 Hz), 6.39 (1H, d, *J* = 2.2 Hz), 4.79 (1H, sept., *J* = 6.0 Hz), 4.71 (1H, sept., *J* = 6.0 Hz), 3.78 (3H, s), 3.12 (1H, ddd, *J* = 17.3 Hz, 4.6 Hz, 1.8 Hz), 2.99 (1H, ddd, *J* = 17.3 Hz, 5.8 Hz, 3.1 Hz), 2.69-2.57 (1H, m), 2.31 (1H, dd, *J* = 16.9 Hz, 10.7 Hz), 2.02-1.90 (1H, m), 1.86-1.74 (1H, m), 1.49 (6H, d, *J* = 6.0 Hz), 1.40 (6H, d, *J* = 6.0 Hz), 1.38-1.30 (1H, m), 1.11 (3H, d, *J* = 6.5 Hz). **^{13}C NMR** (75 MHz, $CDCl_3$): δ = 155.4, 155.2, 147.6, 138.2, 129.6, 128.9, 116.8, 100.3, 95.5, 77.4, 72.7, 69.9, 60.5, 32.8, 31.2, 28.7, 23.3, 22.2, 22.2, 22.1. **HRMS** (ESI): Calculated for $C_{22}H_{30}NaO_4$ $[MNa]^+$: 397.1985, found: 397.1971.

cis-1-Hydroxy-6,8-diisopropoxy-3-methyl-1,2,3,4-tetrahydroanthracene-9,10-dione (±)-*cis*-23

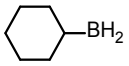


(±)-*cis*-**23**

To a solution of *cis*-6,8-diisopropoxy-10-methoxy-3-methyl-1,2,3,4-tetrahydroanthracene-1,9-diol (±)-*cis*-**21** (10 mg, 27 µmol) in acetonitrile (1 mL) was slowly added a solution of CAN (29 mg, 53 µmol) in water (1 mL) at 0 ^{0}C. The reaction mixture was stirred at 0 ^{0}C for 5 min then warmed to r.t., stirred for further 5 min. then poured into ice cold water (10 mL). The aq. layer was extracted with EtOAc (3 x 10 mL) and the combined organic extracts were dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue purified by preparative TLC using hexanes-EtOAc (7:1) as eluent to afford the *title compound* (7 mg, 20 µmol, 74%) as a yellow oil.

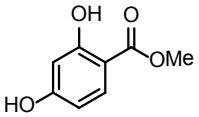
R_f (hexanes:EtOAc =7:3): 0.52. **ν_{max}** (film/cm^{-1}): 3499, 2980, 2940, 2928, 2863, 1647, 1557, 1592, 1370, 1312, 1110. **^{1}H NMR** (300 MHz, acetone-d_6): δ = 7.14 (1H, d, *J* = 2.5 Hz), 6.87 (1H, d, *J* = 2.5 Hz), 4.93-4.86 (1H, m), 4.87 (1H, sept., *J* = 6.0 Hz), 4.79 (1H, sept., *J* = 6.0 Hz), 4.23 (1H, d, *J* = 2.4 Hz, H-OH), 2.79-2.75 (1H, m), 2.16-2.09 (1H, m), 1.95 (1H, ddd, *J* = 18.8 Hz, 10.6 Hz, 3.1 Hz), 1.81-1.71 (1H, m), 1.38 (6H, d, *J* = 6.0 Hz), 1.37 (6H, d, *J* = 6.0 Hz), 1.21-1.31 (1H, m), 1.09 (3H, d, *J* = 6.6 Hz). **^{13}C NMR** (75 MHz, acetone-d_6): δ = 185.8, 184.9, 163.9, 161.7, 146.7, 142.6, 136.9, 108.3, 106.1, 72.7, 71.4, 61.2, 39.6, 32.2, 27.2, 22.3, 22.1, 21.9. **HRMS** (ESI): Calculated for $C_{21}H_{27}O_5$ $[MH]^+$: 359.1853, found: 359.1858; calculated for $C_{21}H_{26}NaO_5$ $[MNa]^+$: 381.1672, found: 381.1677.

Cyclohexylborane



A 1 M cyclohexylborane solution in THF was prepared by adding cyclohexene (107 µL, 1.0 mmol) to a solution of borane-dimethyl sulfide (100 µL, 1.0 mmol) in THF (1 mL) at 0 °C. The cloudy reaction mixture was allowed to warm to r.t. over 1 h and used directly without further purification.

Methyl 2,4-dihydroxybenzoate 24



24

To a solution of 2,4-dihydroxybenzoic acid **12** (5.0 g, 32.4 mmol) in methanol (100 mL) was added concentrated H_2SO_4 (5 mL) and the solution stirred at reflux for 5 days. The reaction mixture was quenched with water (50 mL) and the aq. layer extracted with EtOAc (5 x 30 mL). The combined organic layers were washed with brine (50 mL) and dried over $MgSO_4$ and the solvent was removed *in vacuo*. Purification by flash chromatography using hexanes-EtOAc (7:3) as eluent afforded the *title compound* (5.4 g, 32.4 mmol, quant.) as a colourless solid. $\mathbf{R_f}$ (hexanes:EtOAc = 4:1): 0.1. ^{1}**H NMR** (300 MHz, $CDCl_3$): δ = 10.95 (1H, s), 7.73 (1H, d, *J* = 8.9 Hz), 6.40-6.35 (2H, m), 3.19 (3H, s). ^{13}**C NMR** (75 MHz, $CDCl_3$): δ = 169.7, 163.8, 161.9, 132.0, 107.9, 105.2, 103.3, 52.2.

The spectroscopic data was in agreement with that reported in the literature.[1]

HRV 3C protease assay

A typical protease assay[2] was performed at room temperature for 1 h in a 50 μL reaction containing 50 μg/mL rHRV 3C protease, 52 μM of substrate and 5 μM TCEP in PBS buffer pH 6.8 under continuous rotation. The reaction mixture was centrifuged and 10 μL of the supernatant was added to 200 μL PBS buffer pH 6.8 in a 96-well opti plate. The fluorescence intensity (FI) was measured on a Perkin Elmer EnSpire Multimode Plate Reader with an excitation wavelength of 495 nm and an emission wavelength of 516 nm using 100 excitation flashes. A blank was measured containing enzyme only in PBS at pH 6.8 and an assay using (−)-thysanone (**1**) was run as a control (detected IC_{50} for (−)-thysanone (**1**): 53±0 μM).

Cytotoxicity assays

Materials:

Complete RPMI 1640. Roswell Park Memorial Institute media 1640 pH 7.4 (Invitrogen, USA) supplemented with 1.5 mg/mL sodium bicarbonate, 50 U/mL penicillin (Gibco, Invitrogen, USA), 50 μg/mL streptomycin (Gibco, Invitrogen USA), 2 mM L-glutamine (Gibco, Invitrogen, USA), 110 μg/mL sodium pyruvate (Gibco, Invitrogen, USA) and 10% FCS.

Fetal calf serum. Heat inactivated for 30 min at 56 °C and filtered (Invitrogen, USA).

Cells. NCI-H441 (Human lung adenocarcinoma epithelial cell line).

Thymidine solution. [methyl-^{3}H] Thymidine, 12.5 μCi/mL, GE Healthcare TRK 120.

Assays:

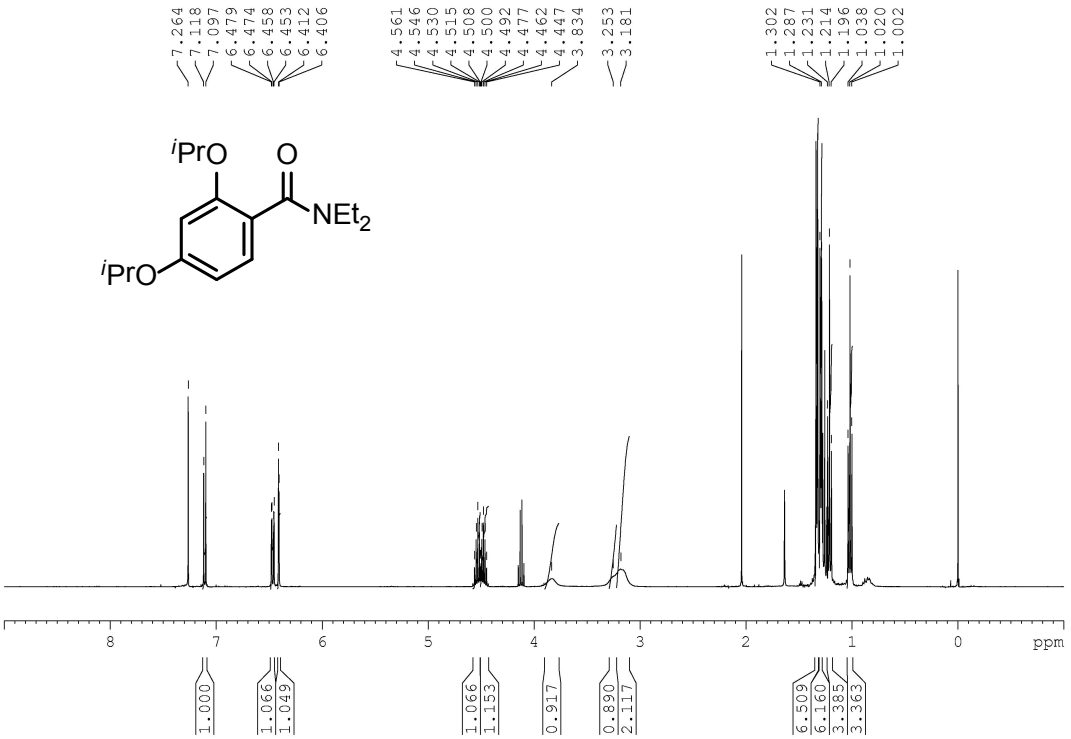
The NCI-H441 human lung adenocarcinoma epithelial cell line was used for cytotoxicity assays. The cell line was maintained in complete RPMI 1640 supplemented with 1.5 mg/mL sodium bicarbonate, 50 U/mL penicillin, 50 μg/mL streptomycin, 2 mM L-glutamine, 110 μg/mL sodium pyruvate and 10% heat-inactivated FCS (cRPMI; all from Gibco, Invitrogen, USA). Cells were harvested with 0.5% v/v Trypsin-EDTA, washed in cRPMI and incubated at 37 °C, 5% CO_2 with serial two-fold dilutions of inhibitor (80 μg/mL → 0.63 μg/mL) in cRPMI. For measurement of cytotoxicity 40,000 cells/well were added to flat-bottom 96 well plates for 36 h and cytotoxicity was quantified by adding 0.25 μCi/well methyl-[^{3}H] thymidine (GE Healthcare, TRK 120) to the cells for an additional 6 h. Plates were harvested onto glass filter mats using a Tomtec Harvester 96, dried, and Betaplate scint added prior to measurement of radioactivity with a Microbeta TriLux counter (all materials from Perkin Elmer).

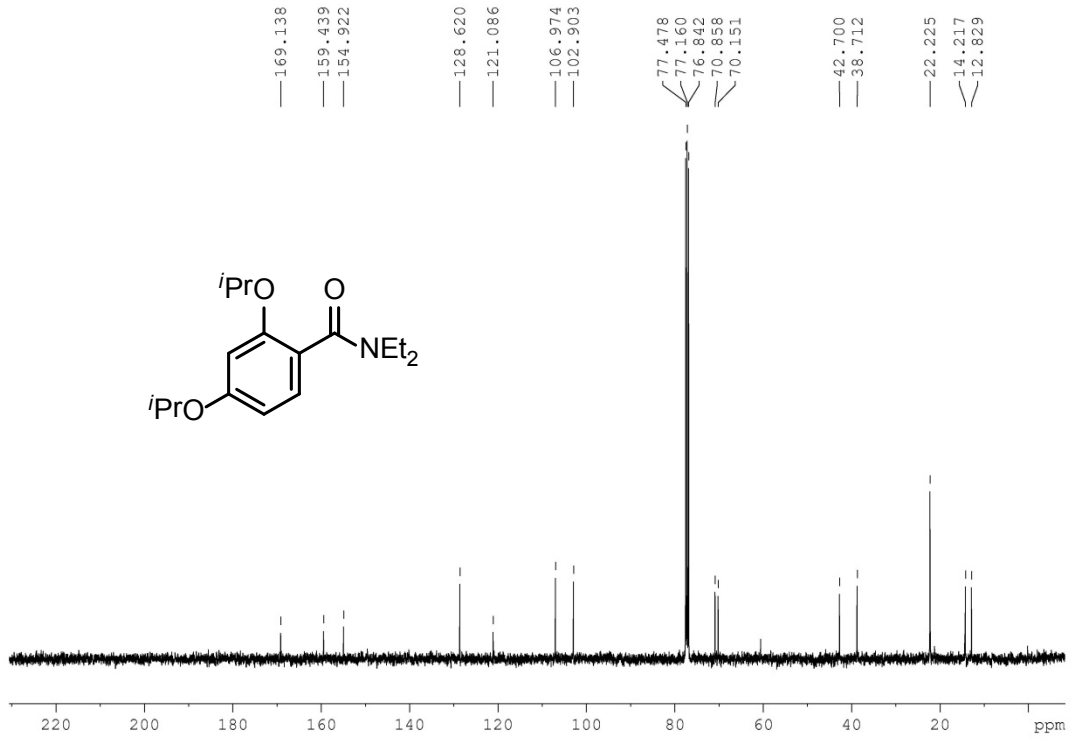
TCEP = tris(2-carboxyethyl)phosphine; PBS = phosphate buffered saline; RPMI = Roswell Park Memorial Institute; TRK = **t**ropomyosin-**r**eceptor-**k**inase

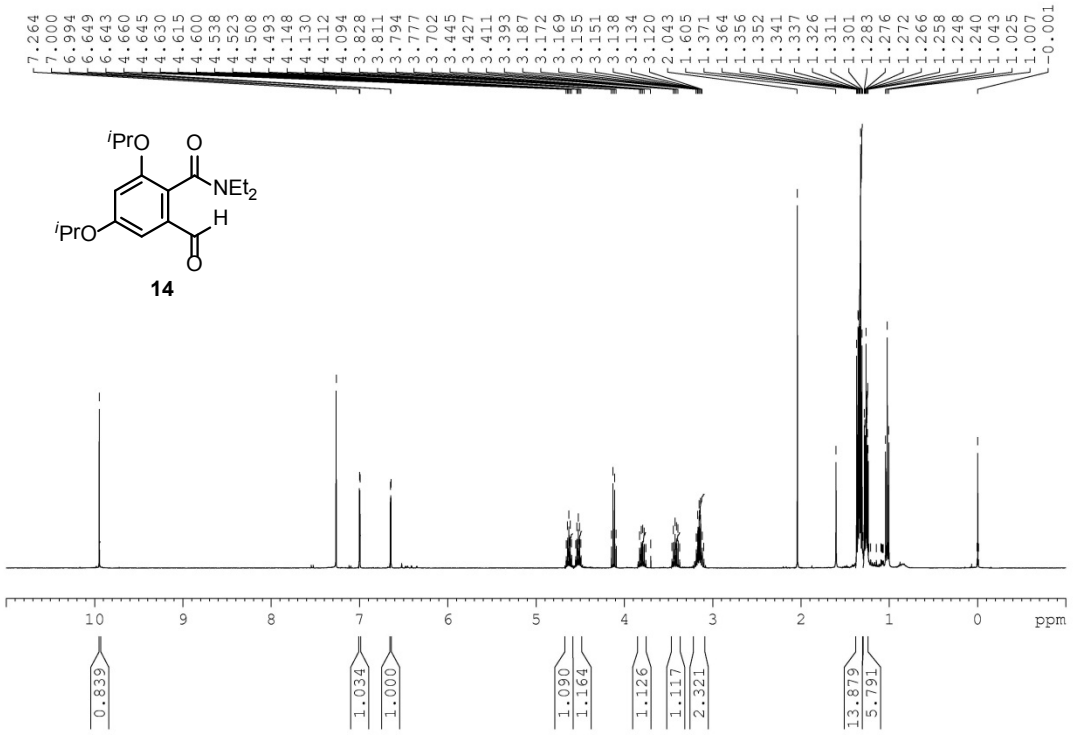
References

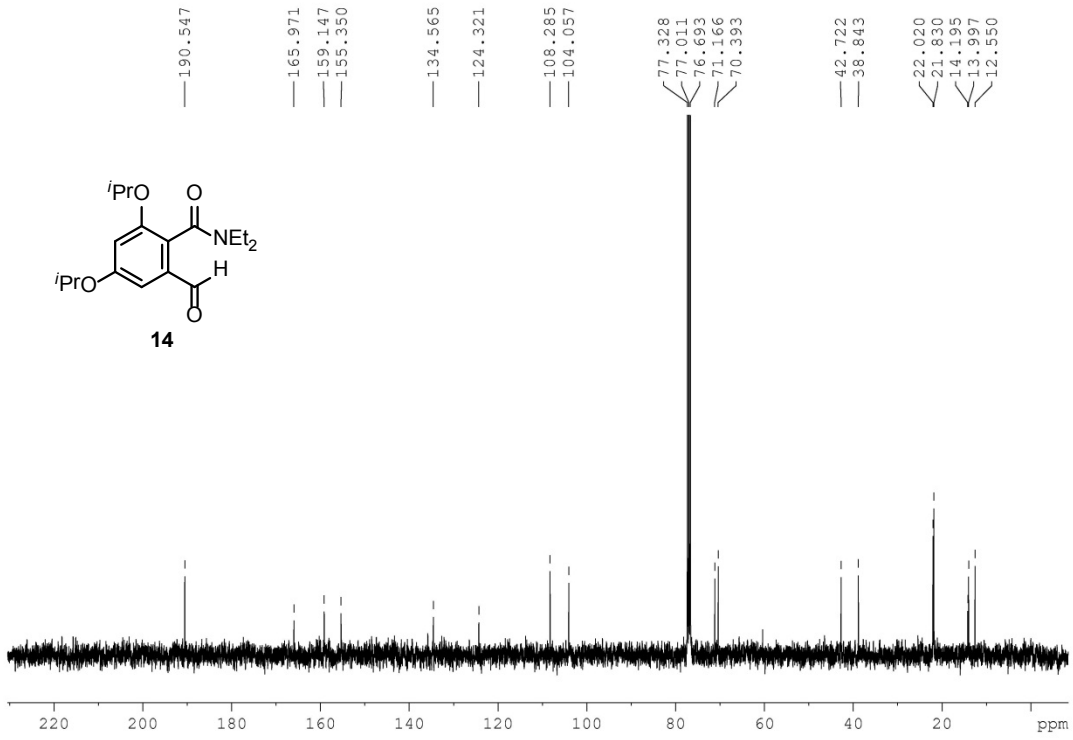
1. T. Ishiyama, Y. Nobuta, J. F. Hartwig and N. Miyaura, *Chem. Commun.*, 2003, 2924-2925.
2. K. Schünemann, S. Connelly, R. Kowalczyk, J. Sperry, I. A. Wilson, J. D. Fraser and M. A. Brimble, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 5018-5024.

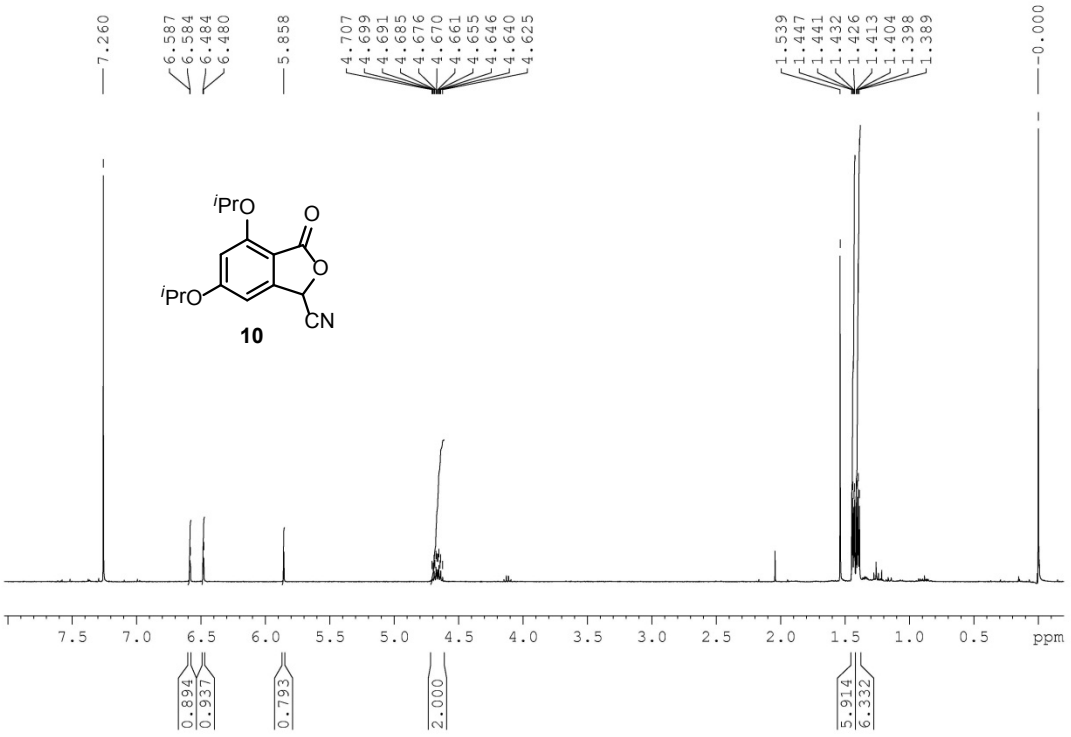
NMR data

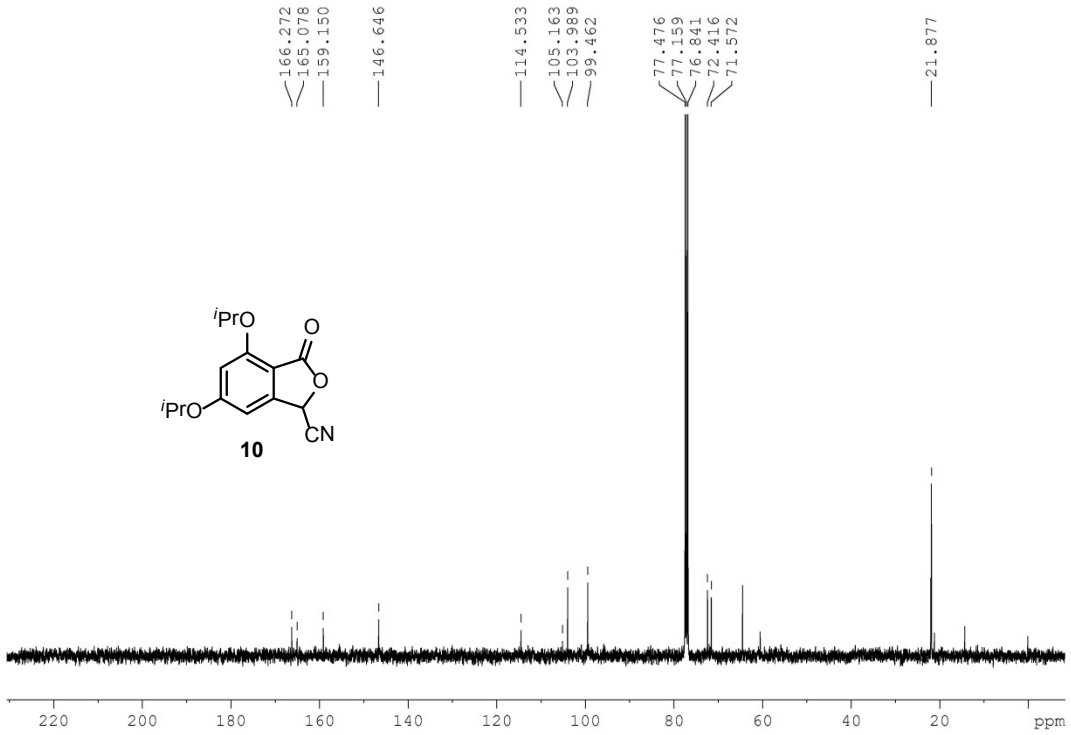


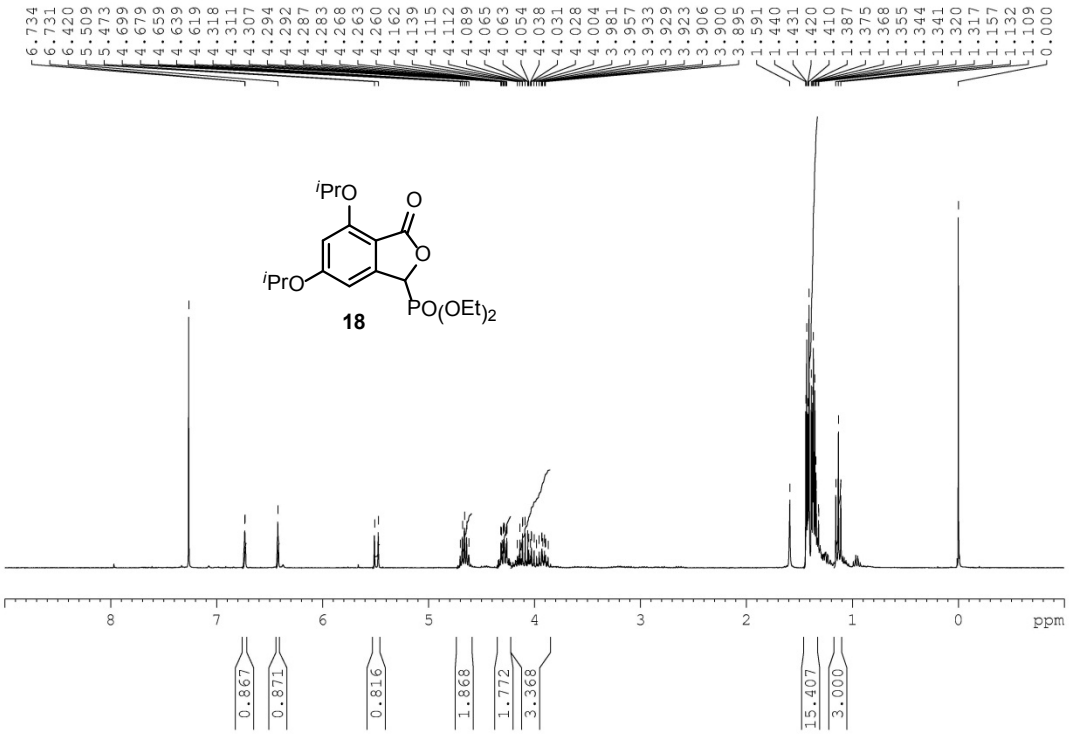


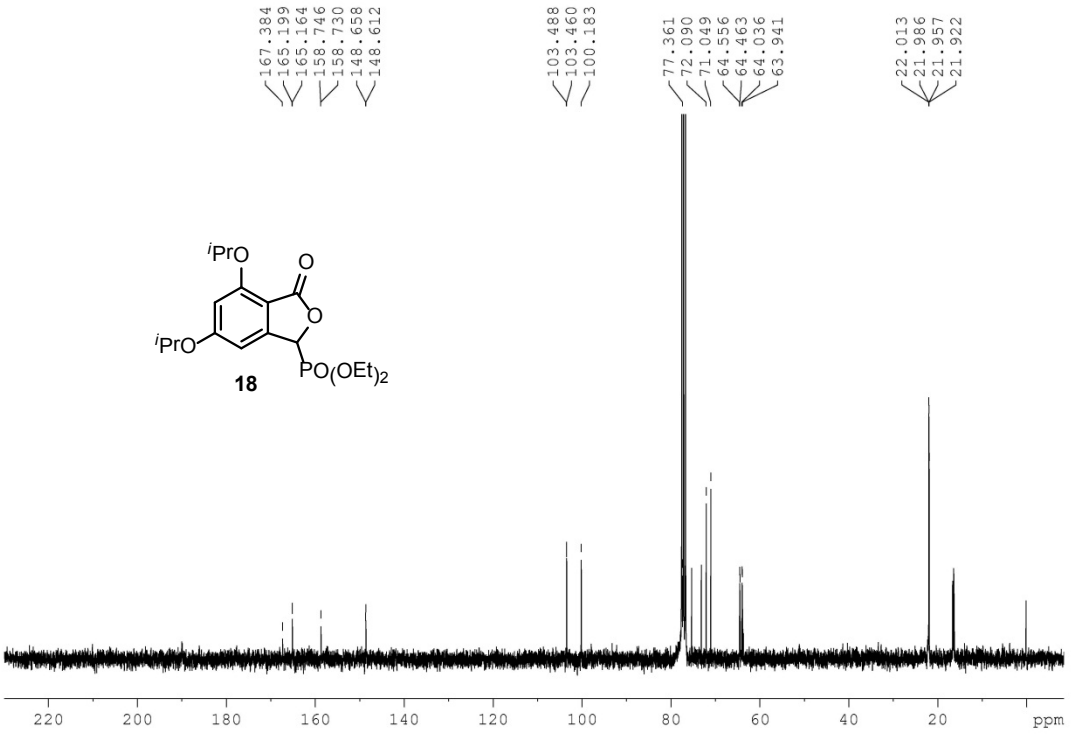


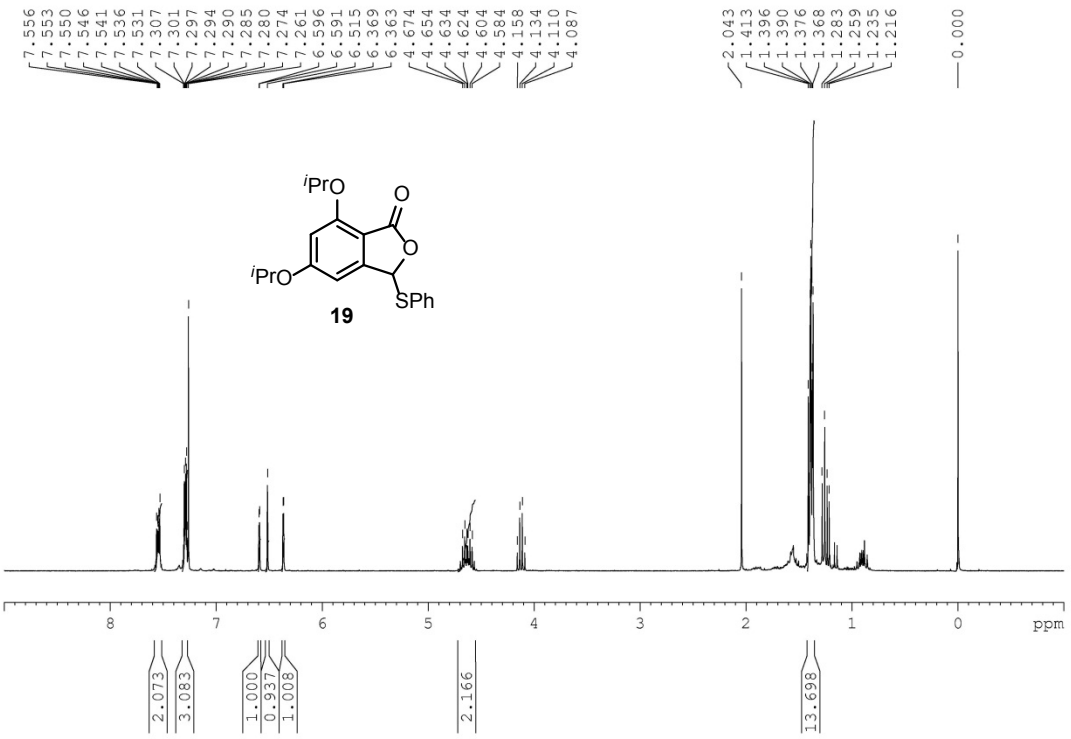


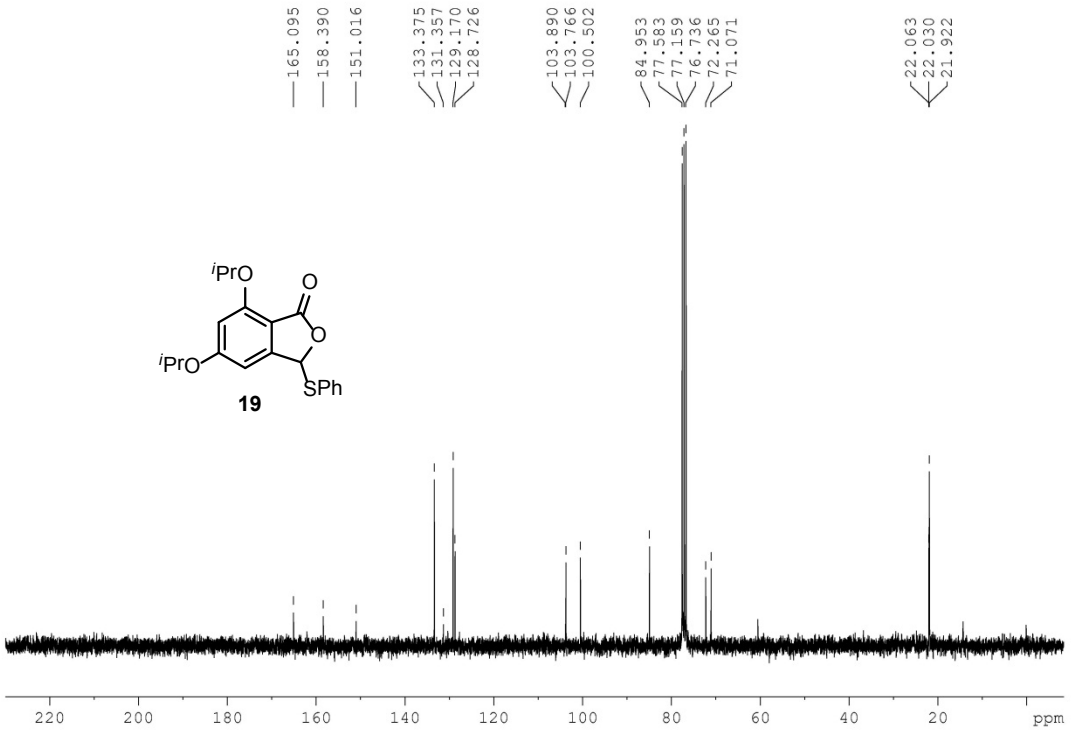


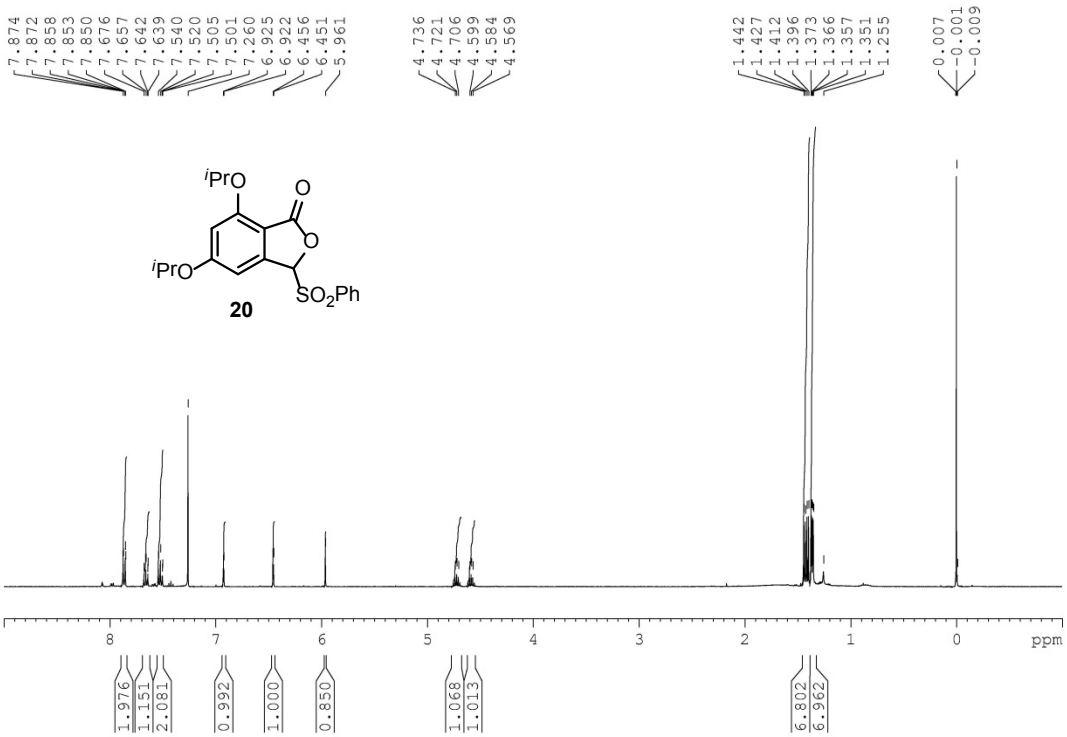


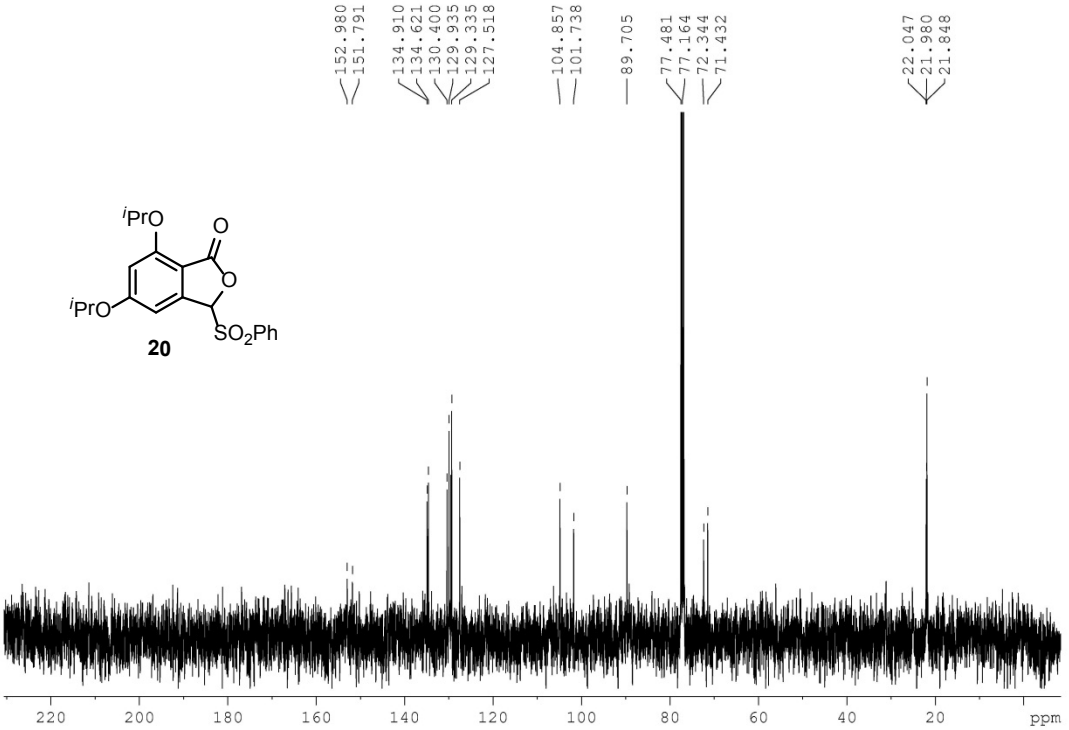


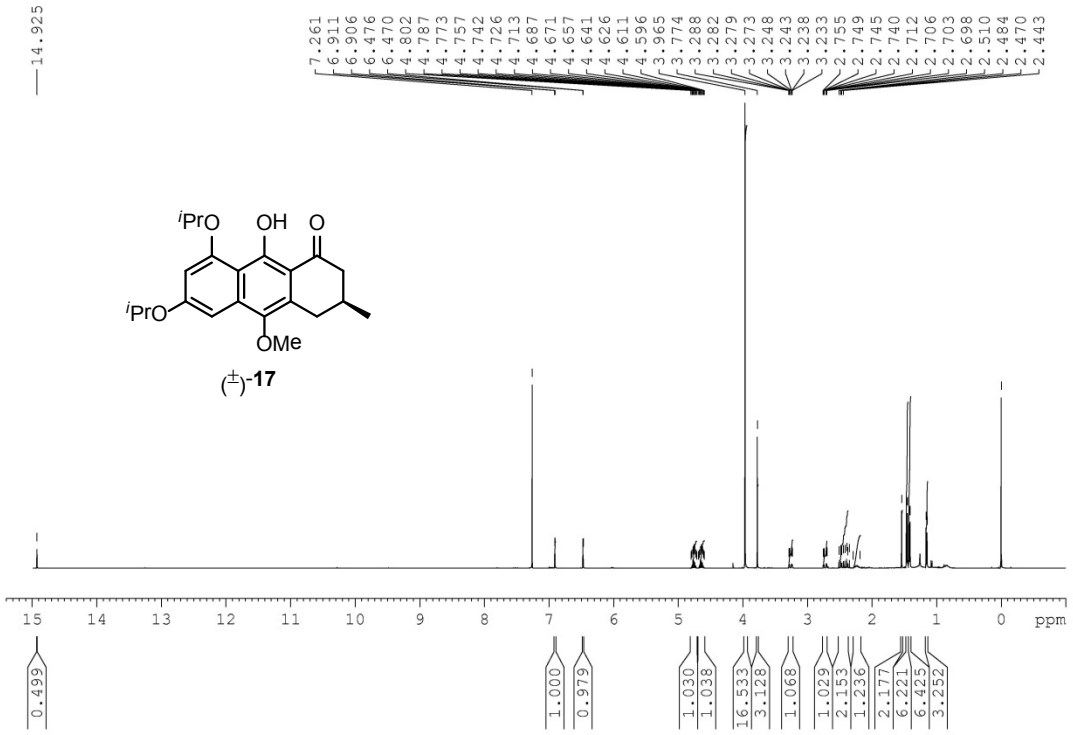


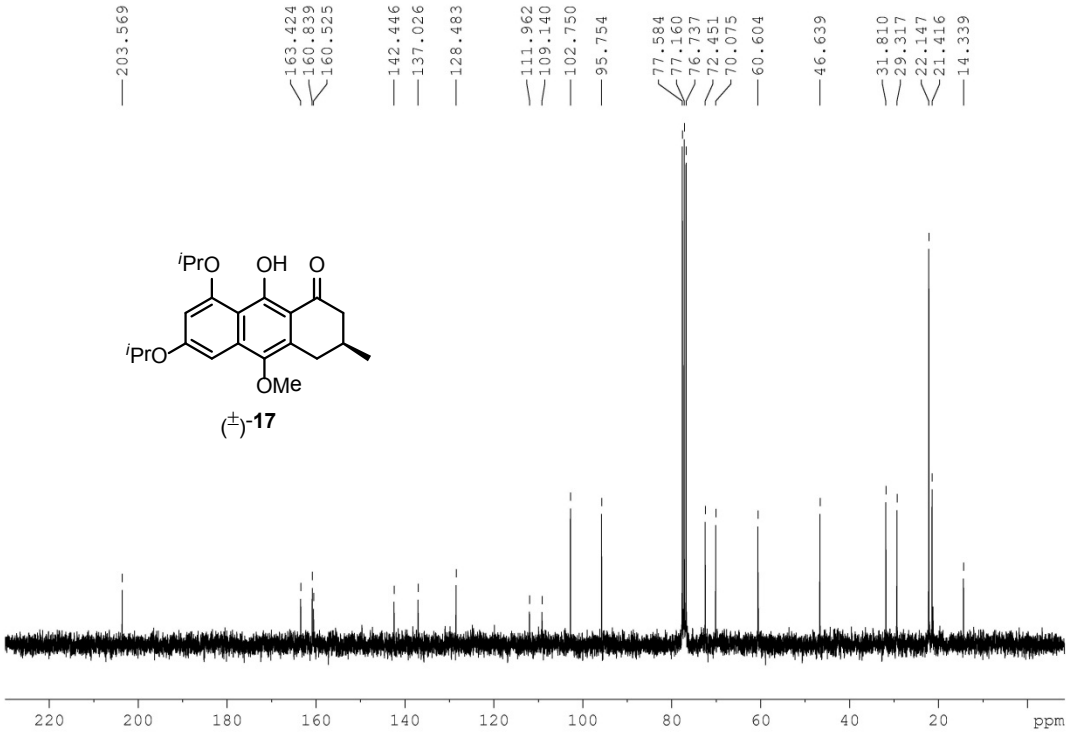


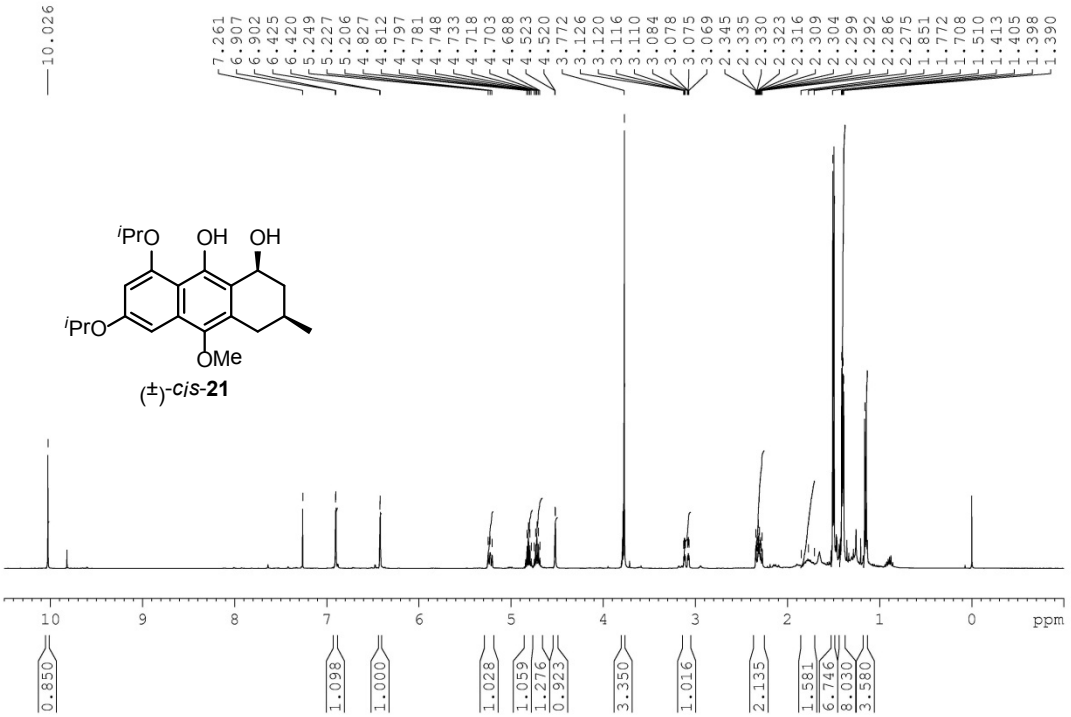


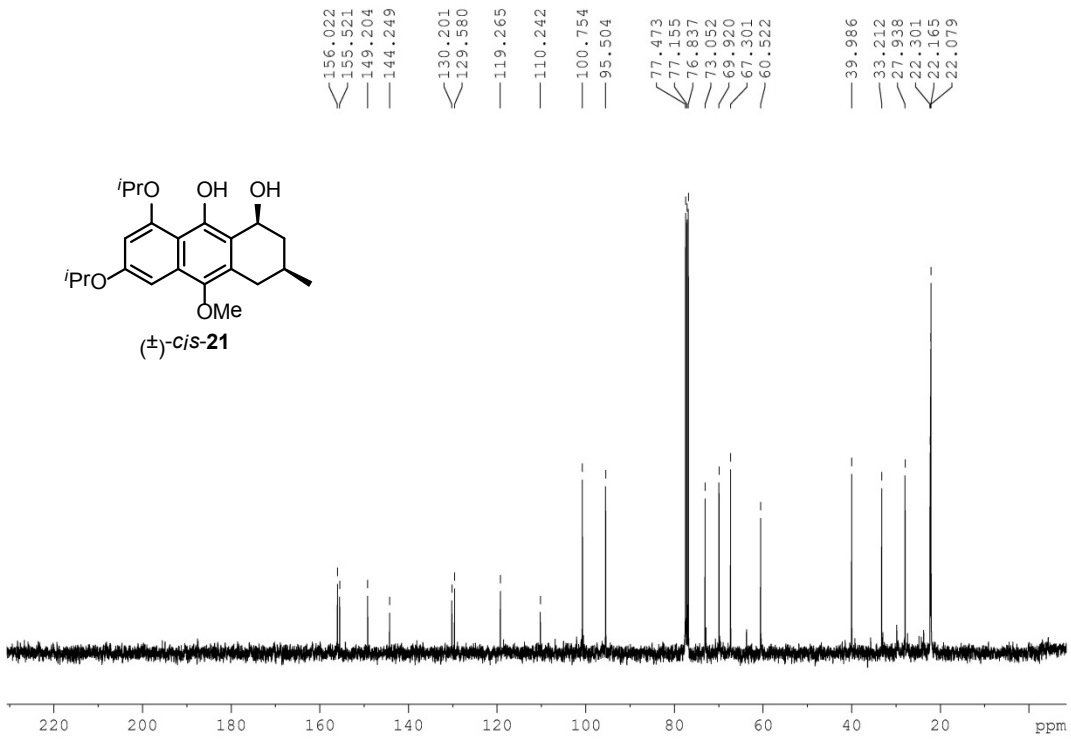


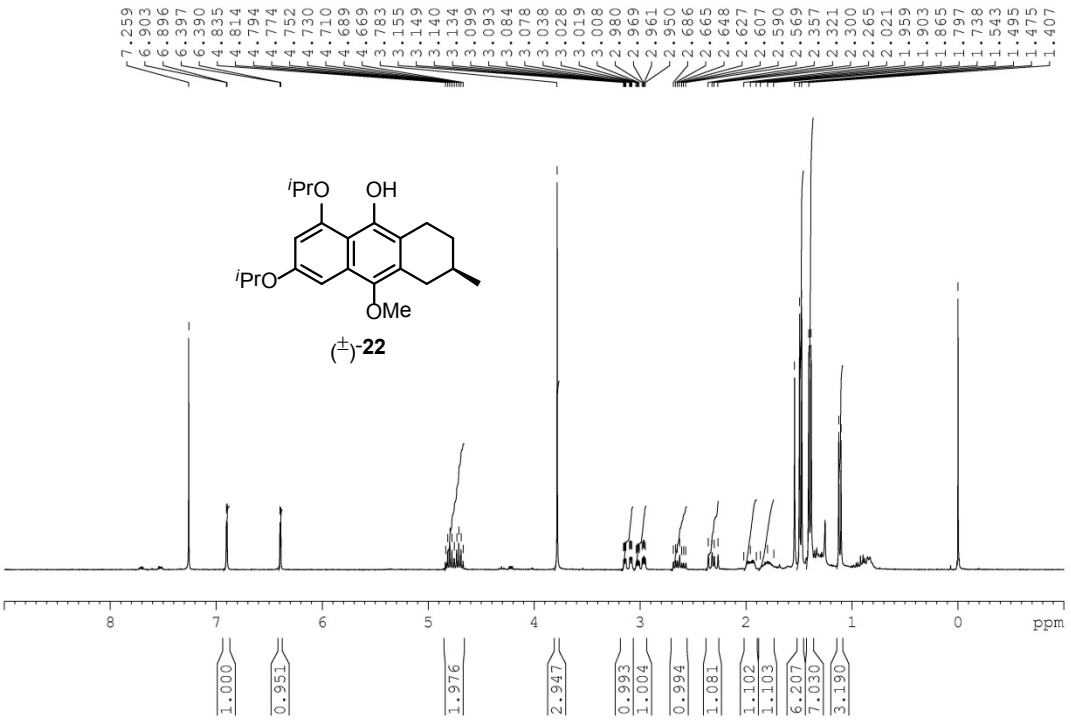


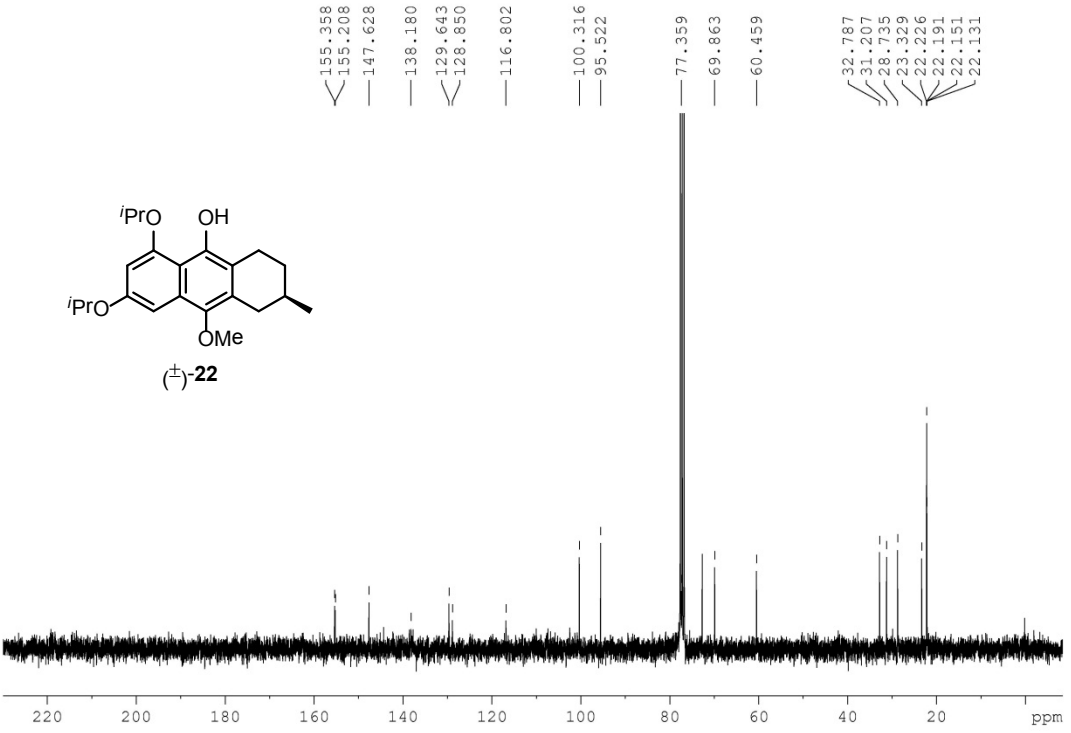


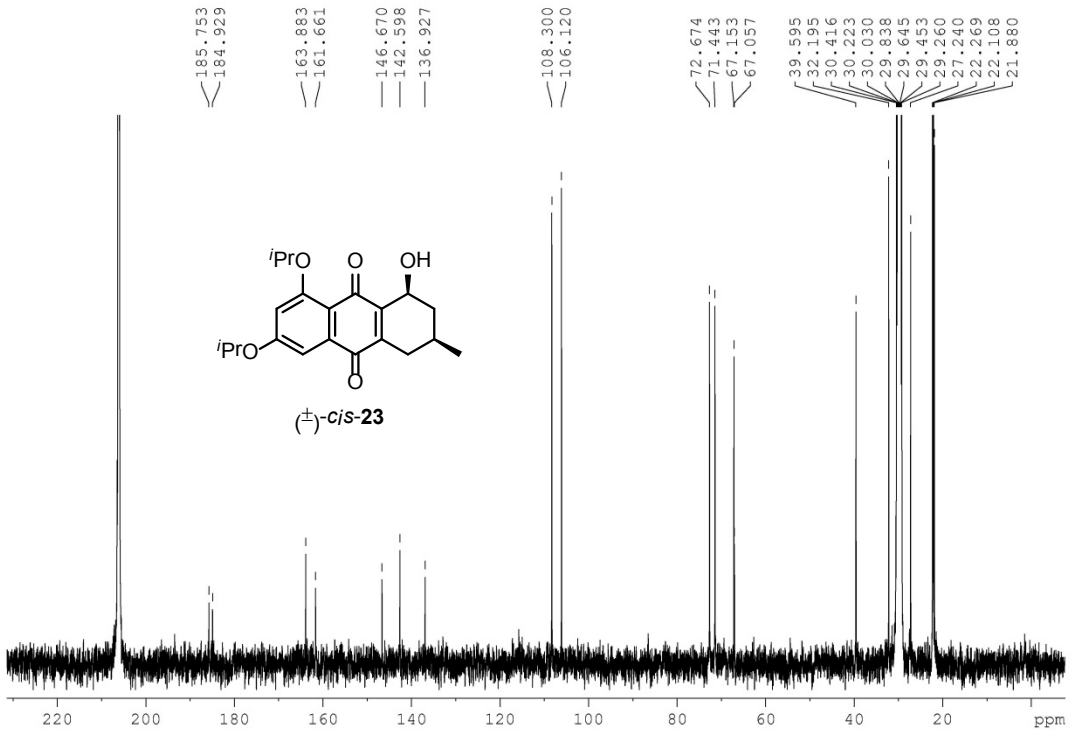


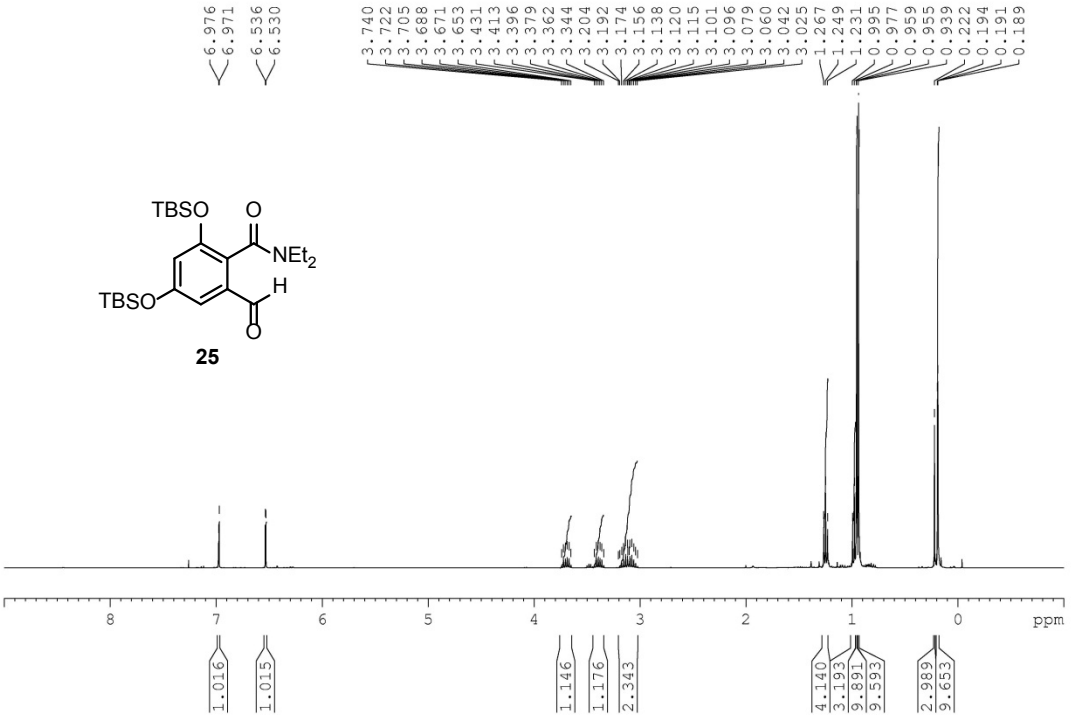


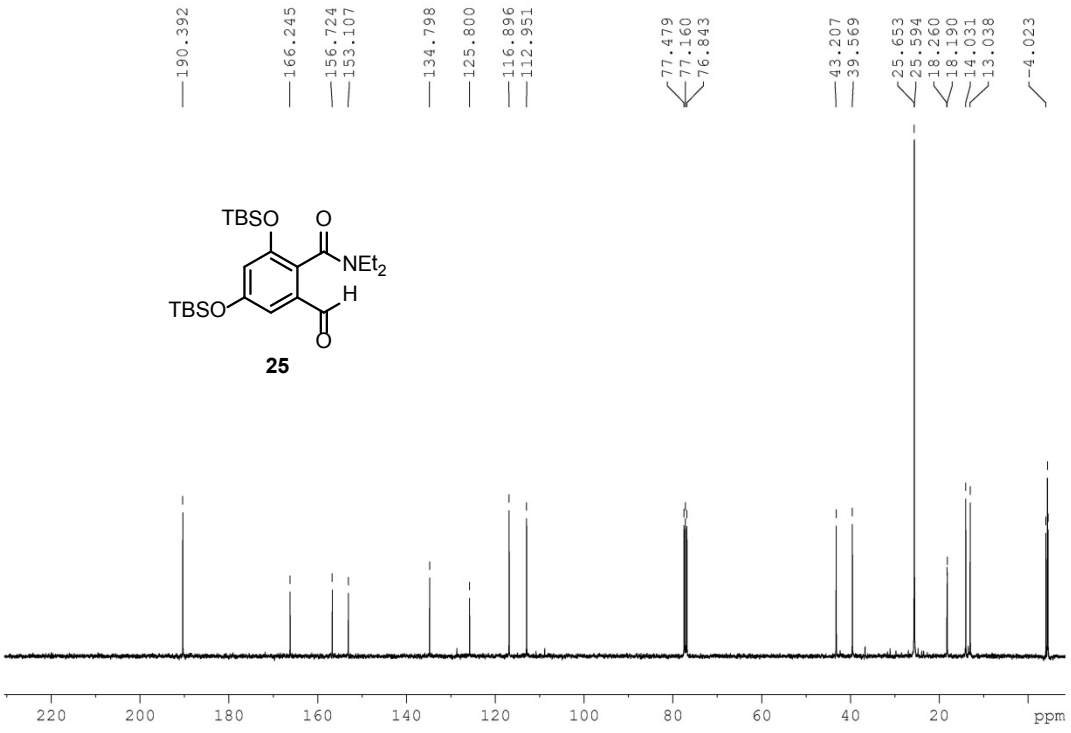


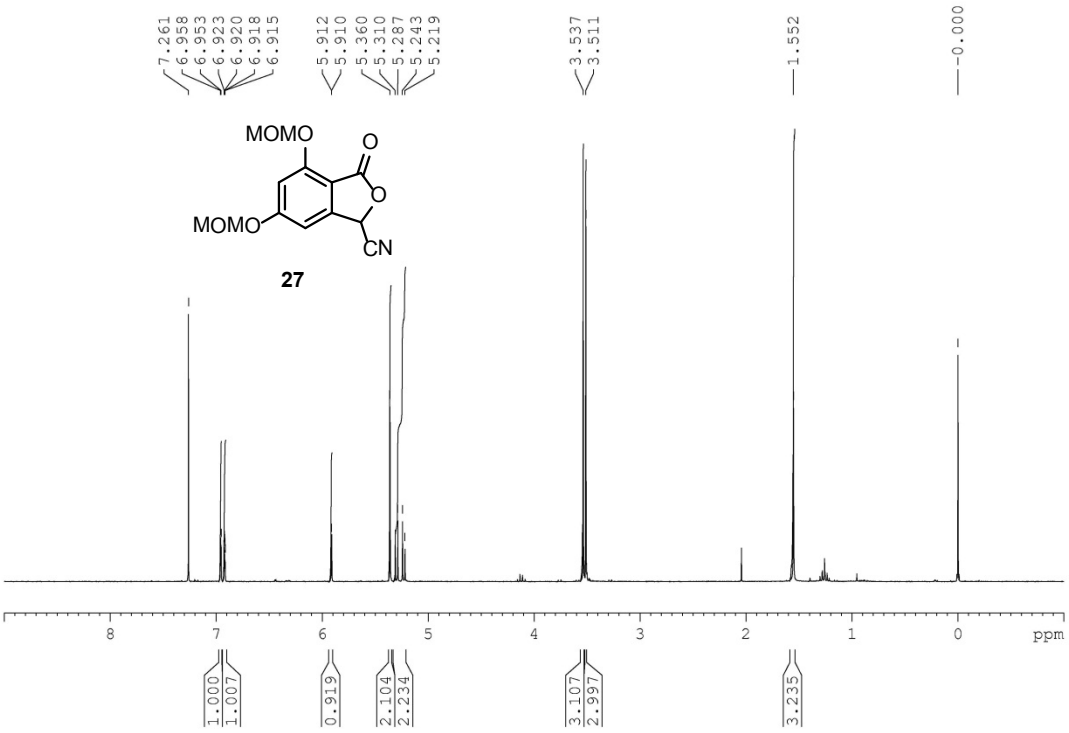


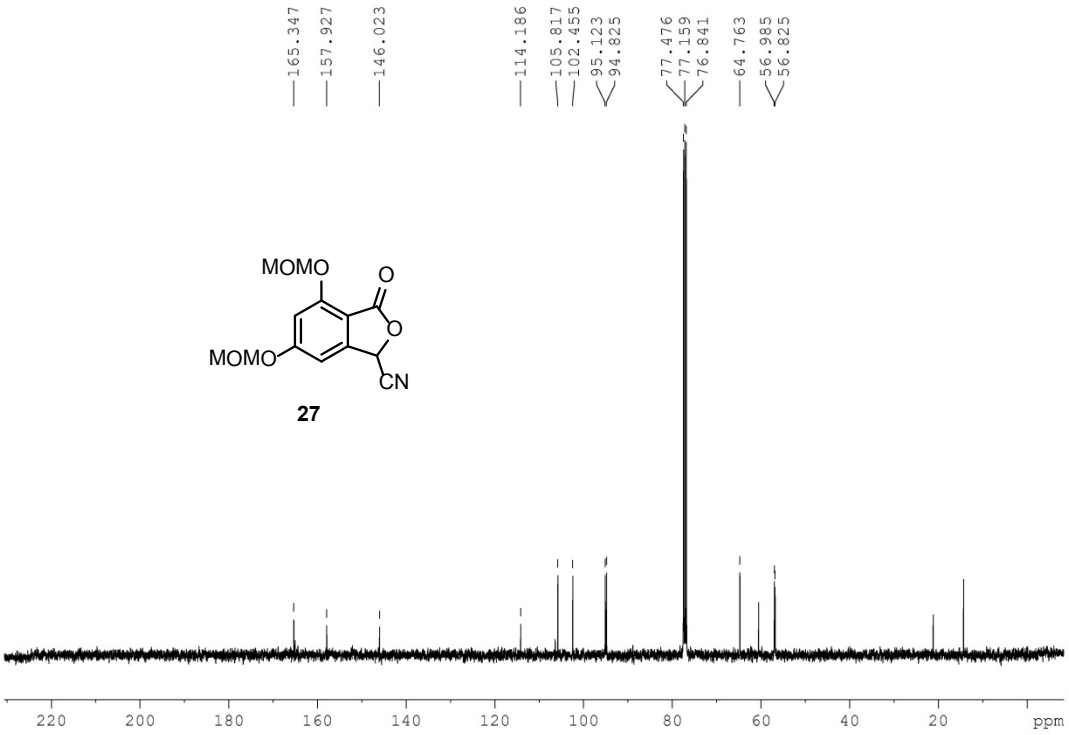


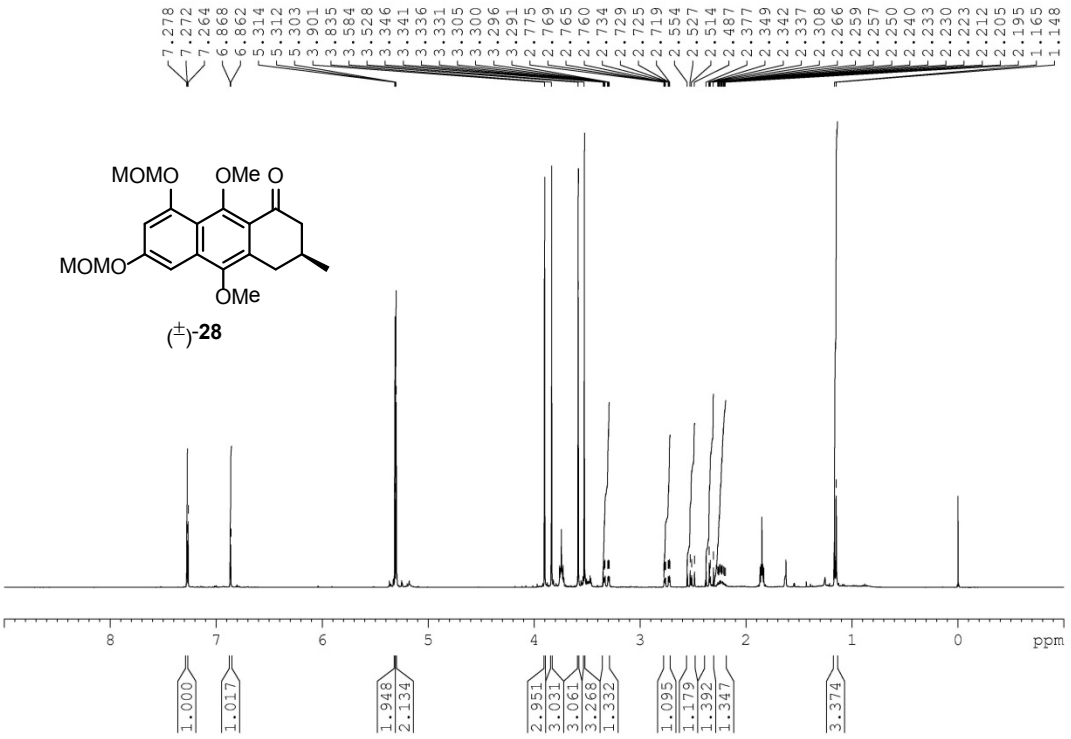


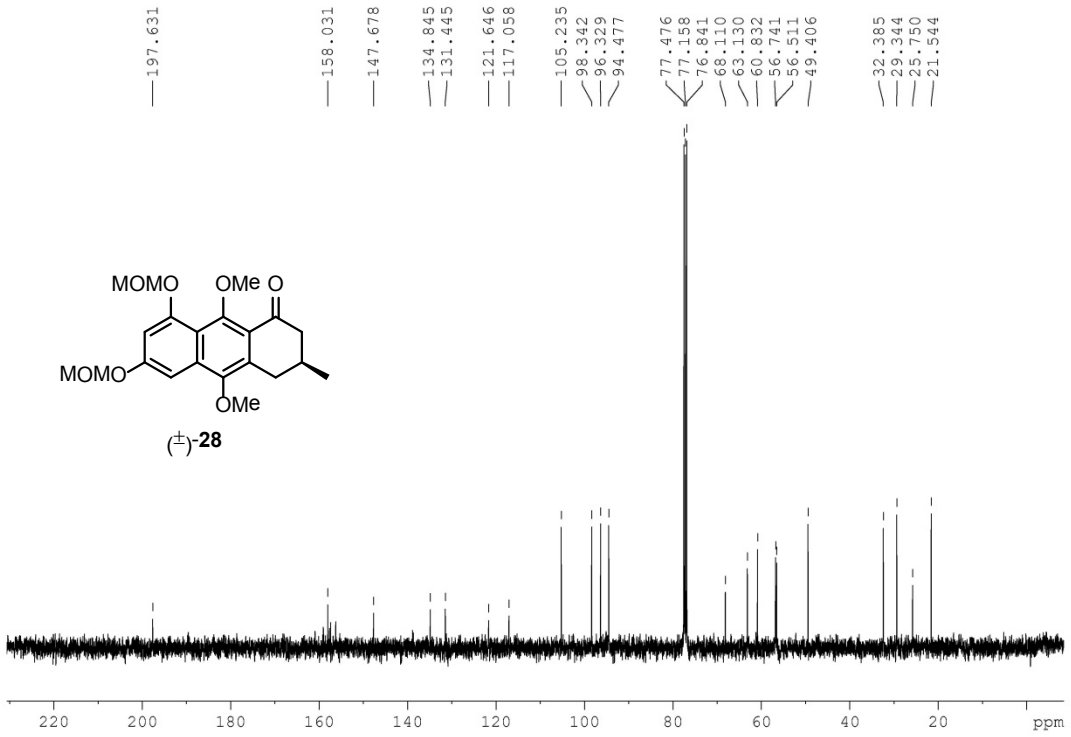


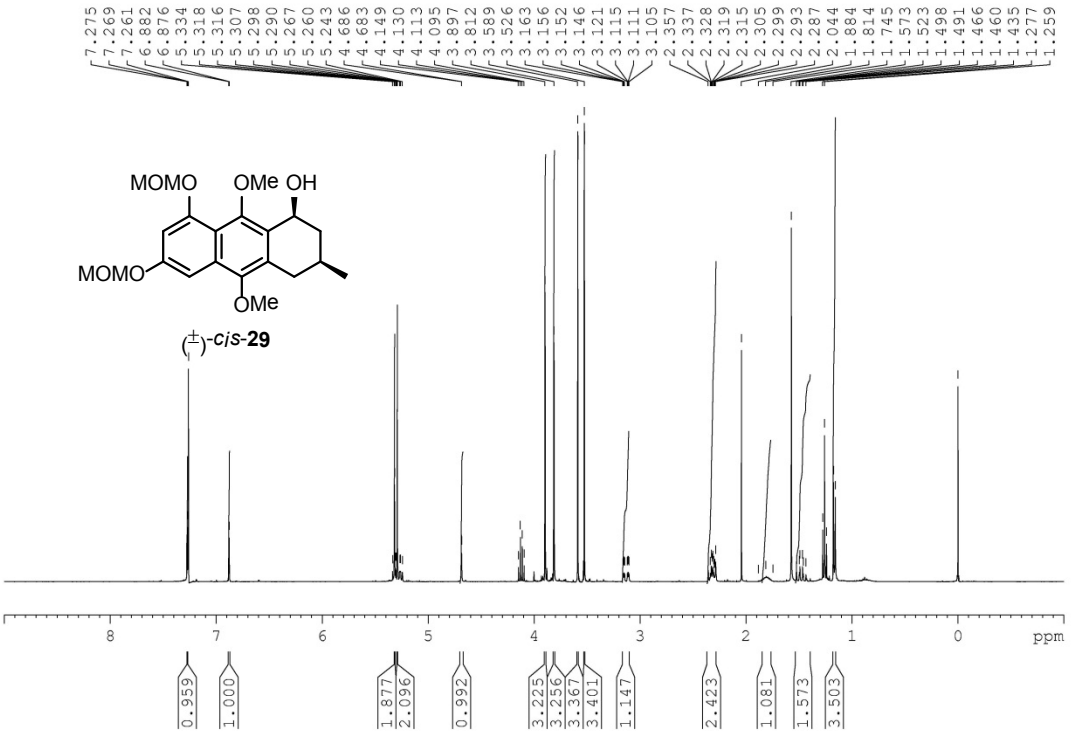








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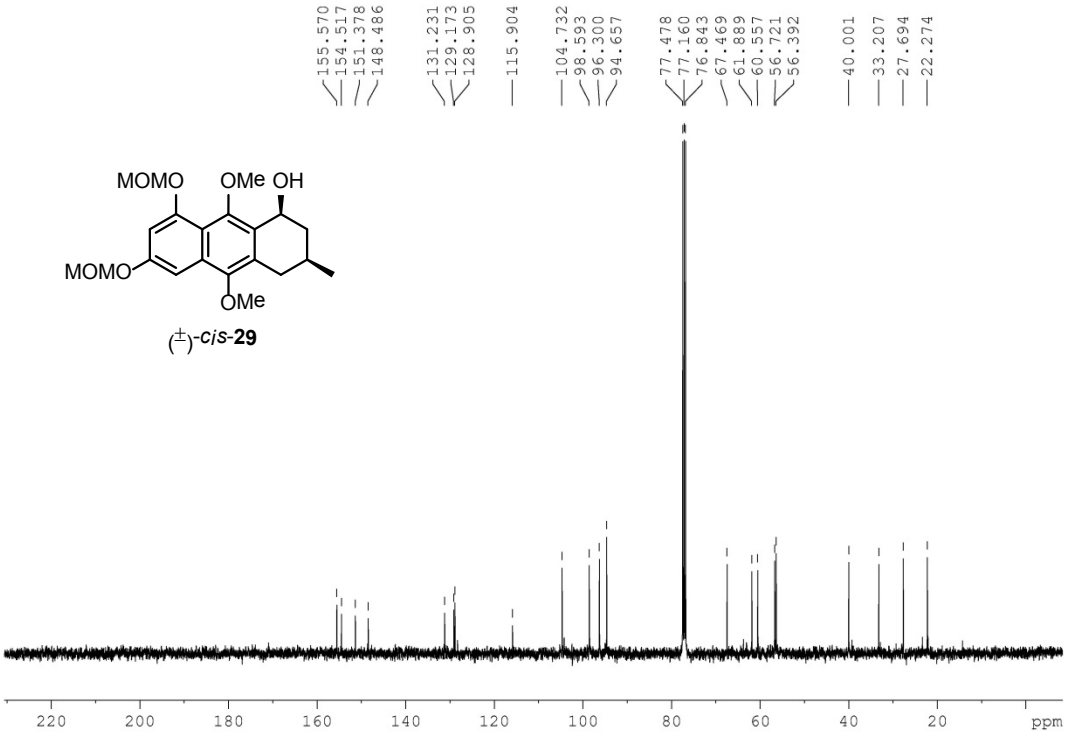
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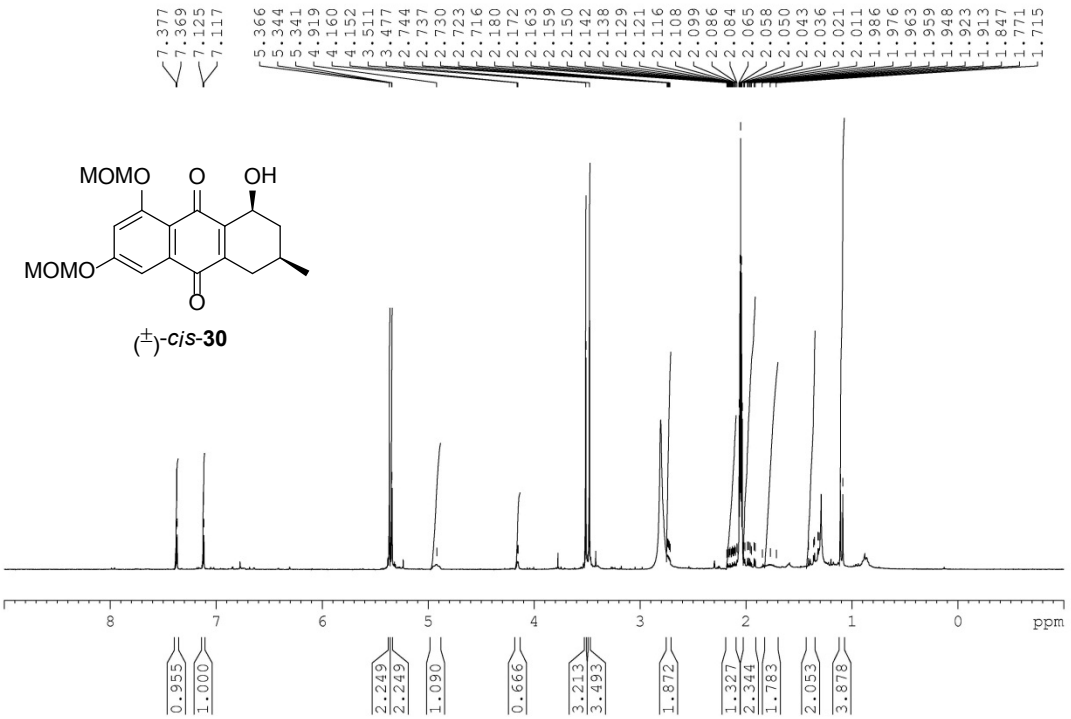

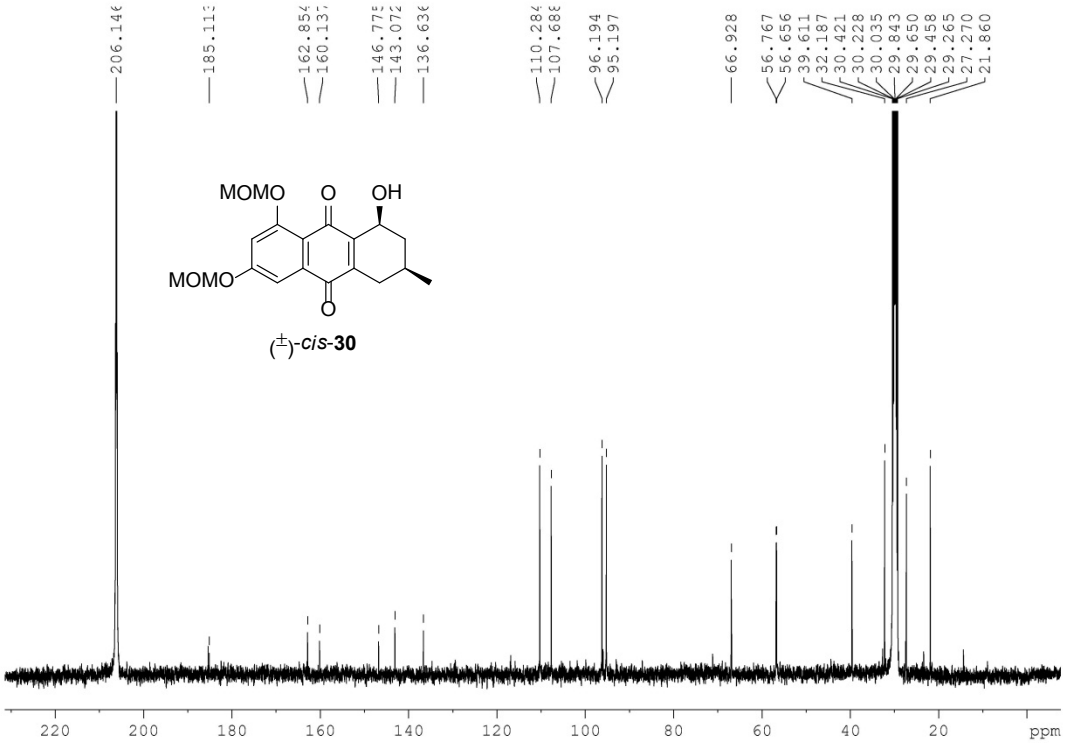

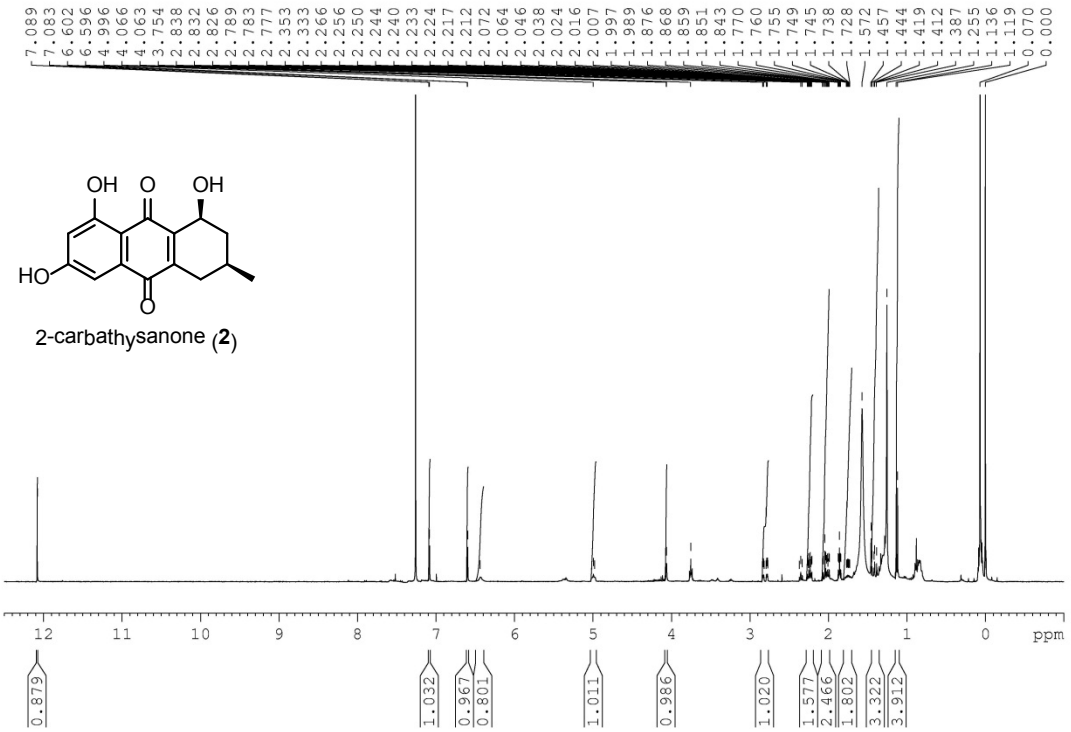

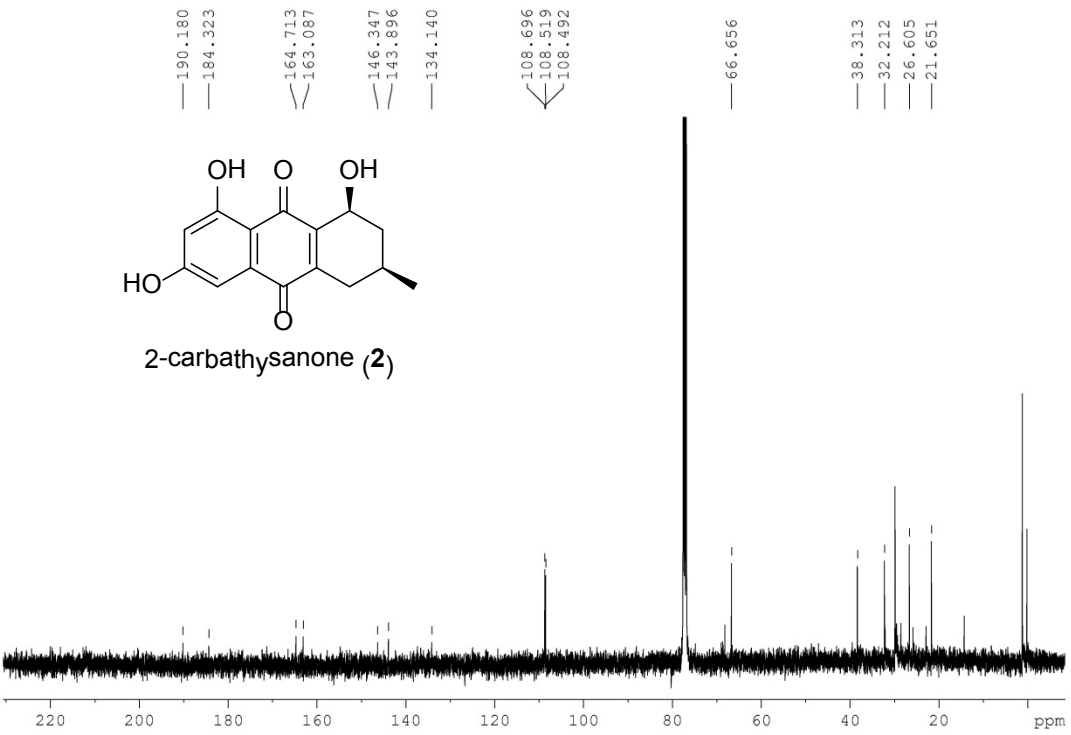

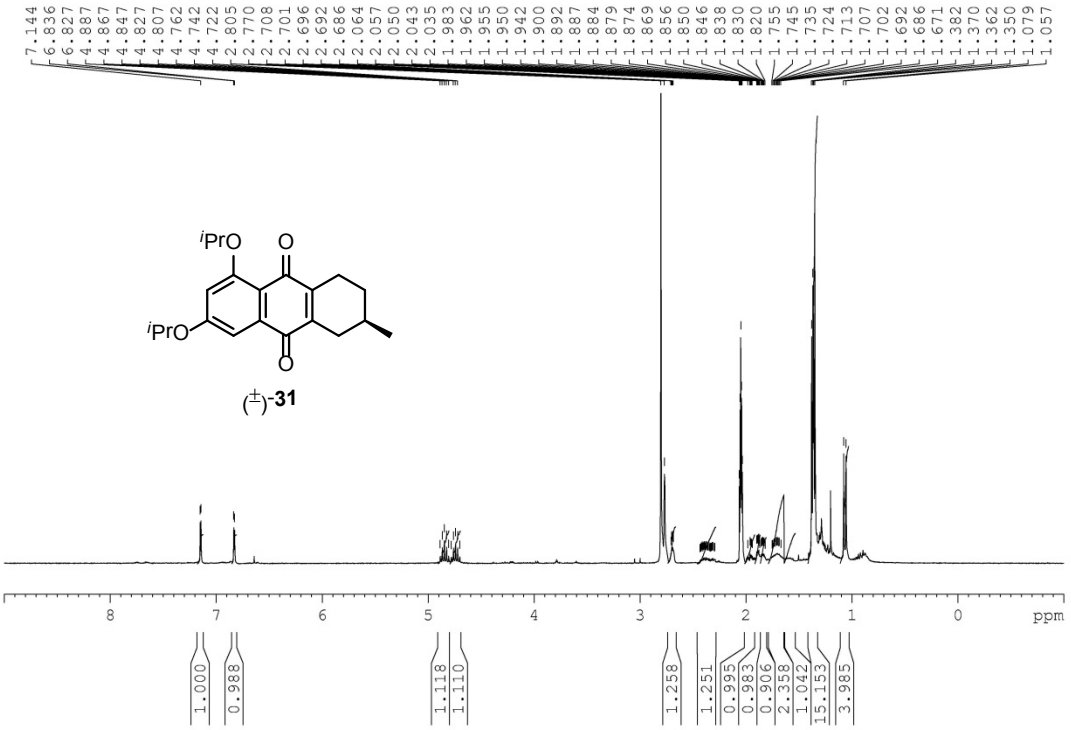
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2.805
2.770
2.708
2.701
2.696
2.692
2.686
2.064
2.057
2.050
2.043
2.035
1.983
1.962
1.955
1.950
1.942
1.900
1.892
1.887
1.884
1.879
1.874
1.869
1.856
1.850
1.846
1.838
1.830
1.820
1.755
1.745
1.735
1.724
1.713
1.707
1.702
1.692
1.686
1.671
1.382
1.370
1.362
1.350
1.079
1.057
iPrO
O
iPrO
O
(±)-31
8
7
6
5
4
3
2
1
0
ppm
1.000
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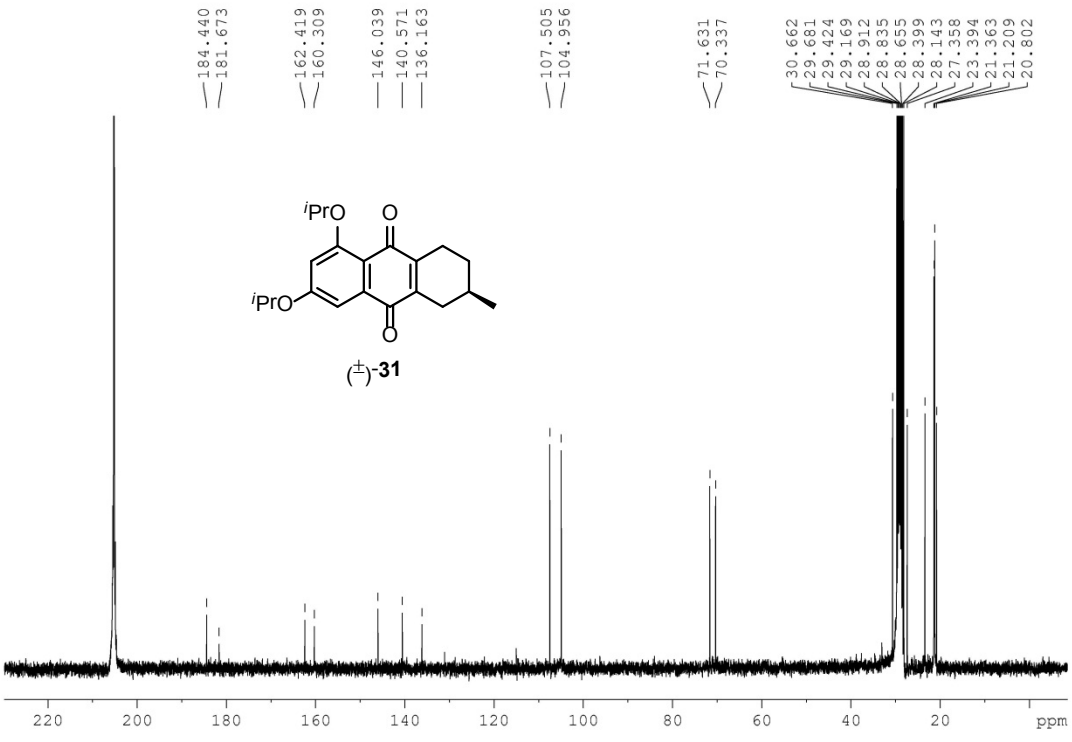
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iPrO
O
iPrO
O
(±)-31
220
200
180
160
140
120
100
80
60
40
20
ppm