Design, Synthesis, and Biological Evaluation of Conormationally Constrained *cis*-Amide Hsp90 Inhibitors

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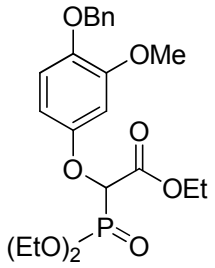
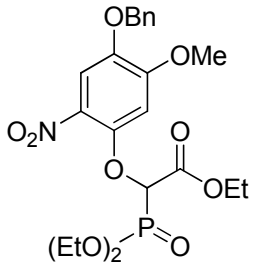
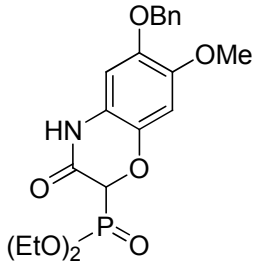


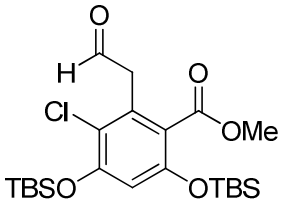
Ethyl 2-(4-(benzyloxy)-3-methoxyphenoxy)-2-(diethoxyphosphoryl)acetate (10): Rhodium (II) acetate (1 mol%) was added to a solution of **8** (2.90 g, 12.61 mmol) and **9** (1.58 g, 6.32 mmol) dissolved in anhydrous toluene (50 mL) at rt. The suspension was warmed to 90 °C for 18 h under argon atmosphere. The cooled solution was poured over a plug of celite and concentrated in vacuo. Flash chromatography (SiO_2, 20% EtOAc in Et_2O) gave **10** (2.00 g, 70%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45 – 7.31 (m, 5H), 6.77 (d, *J* = 8.8, 1H), 6.67 (d, *J* = 2.9, 1H), 6.31 (dd, *J* = 2.9, 8.8, 1H), 5.09 (s, 2H), 4.97 (d, *J* = 18.8, 1H), 4.36 – 4.26 (m, 6H), 3.88 (s, 3H), 1.39 (t, *J* = 7.1, 6H), 1.30 (t, *J* = 7.1, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.6, 152.8 (d, *J* = 13.6), 150.8, 143.7, 137.2, 128.5 (2C), 127.8, 127.4 (2C), 114.9, 104.5, 102.1, 75.2 (d, *J* = 157.7), 71.8, 64.1 (d, *J* = 7.5), 64.0 (d, *J* = 6.3), 62.2, 55.6, 16.4 (d, *J* = 6.3), 16.4 (d, *J* = 5.0), 14.1; IR (film) ν_{max} 2982, 2935, 2908, 1751, 1601, 1508, 1450, 1391, 1369 cm^{-1}; ESI-HRMS *m/z* 453.1667 (M + H$^+$, $C_{22}H_{29}O_8P$ requires 453.1678).



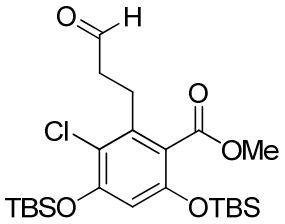
Ethyl 2-(4-(benzyloxy)-5-methoxy-2-nitrophenoxy)-2-(diethoxyphosphoryl)acetate (4): Compound **10** (0.96 g, 2.12 mmol) was dissolved in anhydrous THF (22 mL) and cooled to 0 °C under an argon atmosphere. Ammonium nitrate (0.25 g, 3.12 mmol) was added at once and trifluoroacetic anhydride (1.76 g, 8.38 mmol) was added dropwise to the stirred suspension. The resulting solution was warmed to 25 °C and stirred 1 h, before saturated aqueous $NaHCO_3$ (10 mL) was added. The resulting biphasic solution was poured into EtOAc (25 mL) and the aqueous layer washed with EtOAc (2 x 20 mL). The combined organic layers were combined, dried with Na_2SO_4, and concentrated. Flash chromatography (SiO_2, 20% EtOAc in Et_2O) gave **5** (0.95 g, 91%) as a yellow amorphous solid: ^{1}H NMR (400 MHz, CDCl3) δ 7.63 (s, 1H), 7.47 – 7.33 (m, 5H), 6.75 (s, 1H), 5.15 (s, 2H), 5.10 (d, *J* = 17.3, 1H), 4.42 – 4.28 (m, 6H), 3.93 (s, 3H), 1.44 – 1.36 (m, 6H), 1.31 (t, *J* = 7.1, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8, 154.9, 147.7 (d, *J* = 12.2), 143.3, 135.7 (2C), 132.5, 128.7 (2C), 128.4, 127.6, 110.9, 102.1, 77.2 (d, *J* = 155.4), 71.6, 64.7 (d, *J* = 6.3), 64.4 (d, *J* = 7.5), 62.5, 56.5, 16.4 (d, *J* = 5.0), 16.4 (d, *J* = 6.3), 14.1; IR (film) ν_{max} 2984, 1749, 1614, 1589, 1521, 1445, 1389, 1367, 1337 cm^{-1}; ESI-HRMS *m/z* 496.1380 (M – H$^+$, $C_{22}H_{28}NO_{10}P$ requires 496.1373).



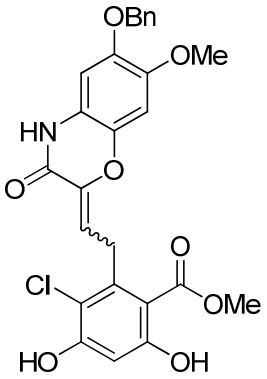
Diethyl 6-(benzyloxy)-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ylphosphonate (1): Tin (II) chloride (2.50 g, 13.16 mmol) was added to a solution of **4** (0.97 g, 1.95 mmol) in absolute ethanol (2.5 mL) and the suspension was refluxed for 1 h before pouring into saturated aqueous $NaHCO_3$ (25 mL). Precipitate was filtered and washed with EtOAc (25 mL) and H_2O (25 mL). The filtrate was rinsed with EtOAc (3 x 15 mL) and the organic layers were combined, dried with Na_2SO_4, and concentrated. Recrystallization with EtOAc and hexanes gave **6** (0.59 g, 72%) as a white amorphous solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.46 – 7.30 (m, 5H), 6.65 (s, 1H), 6.43 (s, 1H), 5.02 (s, 2H), 5.00 (d, *J* = 16.2, 1H), 4.25 – 4.03 (m, 4H), 3.82 (s, 3H), 1.34 (t, *J* = 7.1, 3H), 1.24 (t, *J* = 7.1, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.7 (d, *J* = 3.1), 146.5, 143.7, 136.8, 136.4 (d, *J* = 1.8), 128.6 (2C), 128.0, 127.5 (2C), 117.9, 103.8, 102.0, 74.1 (d, *J* = 151.5), 72.0, 63.8 (d, *J* = 6.3), 63.7 (d, *J* = 7.5), 56.4, 16.4 (d, *J* = 7.5), 16.3 (d, *J* = 5.0); IR (film) ν_{max} 1697, 1628, 1520, 1445, 1389 cm^{-1}; ESI-HRMS *m/z* 420.1207 (M – H$^+$, $C_{20}H_{24}NO_7P$ requires 420.1212).



Methyl 4,6-bis(tert-butyldimethylsilyloxy)-3-chloro-2-(2-oxoethyl)benzoate (2): A 1.0 M solution of lithium diisopropylamide (3.7 mL, 3.71 mmol) was added dropwise to a solution of **5** (1.50 g, 3.37 mmol) dissolved in anhydrous THF (35 mL) at -78°C. After stirring 5 min under argon atmosphere, DMF (4.93 g, 67.45 mmol) was added at once under the solution level and the reaction stirred at -78°C for 10 min. The resulting solution was poured into saturated aqueous NH_4Cl (100 mL) previously cooled to 0°C. This mixture stirred at 0°C for 30 minutes. The product was extracted with EtOAc (50 mL) and the aqueous layer was washed with EtOAc (3 x 30 mL). The combined organic layers were washed with saturated aqueous NaCl, dried with Na_2SO_4, and concentrated. Flash chromatography (SiO_2, 5% EtOAc in hexanes) gave **2** as a yellow oil (0.53 g, 34%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.65 (t, *J* = 1.5, 1H), 6.42 (s, 1H), 3.84 (s, 3H), 3.83 (d, *J* = 1.5, 2H), 1.05 (s, 9H), 0.98 (s, 9H), 0.27 (s, 6H), 0.24 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 195.3, 165.1, 151.1, 149.9, 128.7, 119.0, 117.3, 108.3, 49.9, 43.7, 23.2 (3C), 23.1 (3C), 16.0, 15.7, -6.7 (2C), -6.8 (2C); IR (film) ν_{max} 2953, 2932, 2887, 2858, 2716, 2361, 1732, 1585, 1466, 1433, 1412, 1391, 1362 cm^{-1}; ESI-HRMS *m/z* 473.1940 (M + H$^+$, $C_{22}H_{37}ClO_5Si_2$ requires 473.1946).



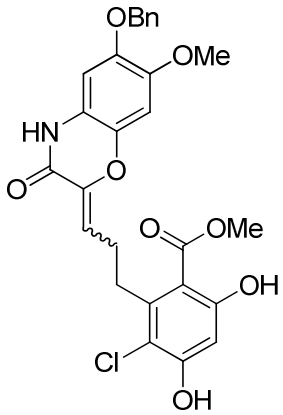
Methyl 4,6-bis(tert-butyldimethylsilyloxy)-3-chloro-2-(3-oxopropyl)benzoate (3): Osmium tetraoxide (96 µL) and sodium periodate (0.42 g, 1.96 mmol) were added consecutively to a solution of **6** (0.32 g, 0.66 mmol) in dioxane : H_2O (3 : 1) (10 mL) and the resulting solution stirred for 8 h. The precipitate was filtered and washed with EtOAc (20 mL). The filtrate was washed with H_2O (2 x 20 mL) and saturated aqueous NaCl (20 mL). The organic layer was collected, dried with Na_2SO_4, and concentrated in vacuo. Flash Chromatography (SiO_2, 5% EtOAc in hexanes) gave **3** as a colorless oil (0.27g, 84%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.59 (s, 1H), 6.11 (s, 1H), 3.63 (s, 3H), 2.73 (dd, *J* = 6.6, 9.3, 2H), 2.56 (dd, *J* = 6.5, 9.3), 0.81 (s, 9H), 0.74 (s, 9H), 0.02 (s, 6H), 0.00 (s, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 201.0, 168.0, 153.1, 151.6, 137.7, 121.1, 118.4, 109.6, 52.3, 43.4, 25.6 (3C), 25.4 (3C), 24.7, 18.3, 18.0, -4.4 (2C), -4.4 (2C); IR (film) ν_{max} 2897, 2858, 2046, 1728, 1630, 1587, 1470, 1433, 1410, 1364 cm^{-1}; ESI-HRMS *m/z* 487.2104 (M + H$^+$, $C_{23}H_{39}ClO_5Si_2$ requires 487.2103).



(*E*)-Methyl 2-(2-(6-(benzyloxy)-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ylidene)ethyl)-3-chloro-4,6-dihydroxybenzoate (11): Sodium hydride (30.0 mg) was added to a suspension of **1** (0.26 g, 0.62 mmol) in anhydrous THF (6 mL) at 0 °C. The solution was warmed to 25 °C and stirred for 30 min under argon atmosphere before cooling to 0 °C. A 0.1M solution of **2** (0.35 g, 0.74 mmol) in anhydrous THF was canulated into the reaction mixture. The reaction was stirred at 0 °C for 30 min then warmed to 25 °C for 12 h. Tetrabutylammonium fluoride (2.5 mL, 2.47 mmol, 0.1 M solution in THF) was added dropwise and the reaction mixture stirred for 1 h. The reaction was quenched with saturated aqueous NH_4Cl and extracted with EtOAc (20 mL). The aqueous layer was rinsed with EtOAc (2 x 20 mL). The organic layers were combined, washed with saturated aqueous NaCl, dried with Na_2SO_4, and concentrated. Repeated flash chromatography (SiO_2, 30% EtOAc in hexanes) afforded the *trans/cis* (3:1) isomers giving **11** (0.16 g) and **12** (0.05g) in 67% overall yield: ^{1}H NMR (Acetone, 400 MHz) δ 9.58 (s, 1H), 7.48–7.32 (m, 5H), 6.72 (s, 1H), 6.66 (s, 1H), 6.53 (s, 1H), 5.55 (t, *J* = 6.8, 1H), 5.04 (s, 2H), 4.49 (d, *J* = 6.8, 2H), 3.89 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.3, 162.1, 158.6, 158.3, 147.1, 144.7, 142.2, 142.1, 138.4, 137.4,

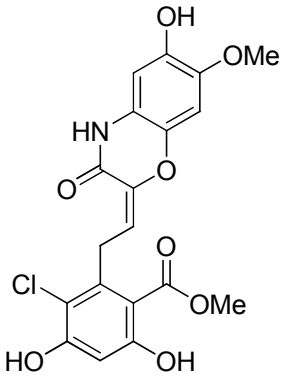
129.2 (2C), 128.7, 128.6 (2C), 119.1, 118.9, 115.2, 108.6, 104.1, 103.3, 102.1, 72.5, 56.8, 52.8, 31.1; IR (film) ν_{max} 1647, 1609, 1576, 1520, 1437, 1398, 1317 cm^{-1}; ESI-HRMS 510.0938 ($M - H^+$, $C_{26}H_{22}ClNO_8$ requires 510.0956).

(*Z*)-Methyl 2-(2-(6-(benzyloxy)-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ylidene)ethyl)-3-chloro-4,6-dihydroxybenzoate (12): ^{1}H NMR (Acetone, 400 MHz) δ 9.40 (s, 1H), 7.37–7.17 (m, 5H), 6.73 (s, 1H), 6.61 (s, 1H), 6.42 (s, 1H), 5.78 (t, *J* = 6.9, 1H), 4.93 (s, 2H), 3.97 (d, *J* = 6.9, 2H), 3.78 (s, 3H), 3.73 (s, 3H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.3, 162.3, 158.8, 156.8, 147.1, 144.9, 143.4, 141.4, 138.4, 136.5, 129.2 (2C), 128.7, 128.6 (2C), 115.2, 112.4, 108.3, 104.2, 104.1, 103.3, 102.2, 72.4, 56.8, 53.0, 28.9; IR (film) ν_{max} 1647, 1518, 1441, 1389, 1321 cm^{-1}; ESI-HRMS 510.0959 ($M - H^+$, $C_{26}H_{22}ClNO_8$ requires 510.0956).

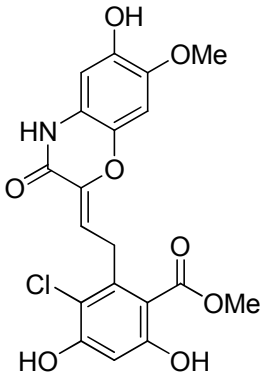


(*E*)-Methyl 2-(3-(6-(benzyloxy)-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ylidene)propyl)-3-chloro-4,6-dihydroxybenzoate (13): Following the procedure to generate **11** and **12**, cyclophosphonate **1** (0.26 g, 0.62 mmol) was reacted with **3** (0.32 g, 0.66 mmol) in anhydrous THF (6 mL). Repeated flash chromatography (SiO_2, 30% EtOAc in hexanes) afforded the *trans/cis* (3:1) isomers giving **13** (0.15 g) and **14** (0.05 g) in 70% overall yield: ^{1}H NMR (Acetone, 400 MHz) δ 9.44 (s, 1H), 7.48–7.29(m, 5H), 6.69 (s, 1H), 6.69 (s, 1H), 6.49 (s, 1H), 5.72 (t, *J* = 8.0, 1H), 5.03 (s, 2H), 3.95 (s, 3H), 3.82 (s, 3H), 3.25–3.20 (m, 2H), 3.03–2.96 (m, 2H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.6, 162.4, 158.6, 158.1, 147.1, 144.6, 143.5, 142.2, 138.4, 137.4, 129.2 (2C), 128.7, 128.6 (2C), 120.4, 119.2, 114.8, 108.1, 104.1, 103.0, 102.1, 72.5, 56.8, 52.8, 32.9, 27.4; IR (film) ν_{max} 1678, 1647, 1609, 1578, 1520, 1435, 1402, 1319 cm^{-1}; ESI-HRMS 524.1115 ($M - H^+$, $C_{27}H_{24}ClNO_8$ requires 511.1112).

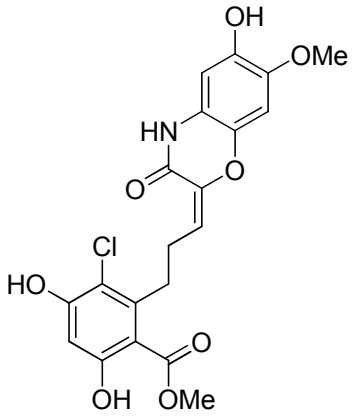
(*Z*)-Methyl 2-(3-(6-(benzyloxy)-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ylidene)propyl)-3-chloro-4,6-dihydroxybenzoate (14): ^{1}H NMR (Acetone, 400 MHz) δ 9.48 (s, 1H), 7.50–7.29(m, 5H), 6.76 (s, 1H), 6.72 (s, 1H), 6.50 (s, 1H), 6.03 (t, *J* = 7.8, 1H), 5.06 (s, 2H), 3.98 (s, 3H), 3.83 (s, 3H), 3.30–3.20 (m, 2H), 2.63–2.57 (m, 2H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.5, 158.7, 156.9, 147.0, 144.7, 143.8, 143.3, 138.4, 136.5, 129.2 (2C), 128.7 (2C), 128.6 (2C), 128.5, 118.7, 114.8, 113.9, 108.1, 104.2, 103.1, 102.1, 72.4, 56.8, 52.9, 32.0, 25.3; IR (film) ν_{max} 1641, 1620, 1574, 1518, 1439, 1416, 1396, 1371, 1319 cm^{-1}; ESI-HRMS 524.1109 ($M - H^+$, $C_{27}H_{24}ClNO_8$ requires 511.1112).



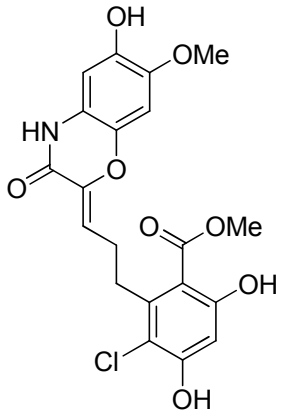
(*E*)-methyl 3-chloro-4,6-dihydroxy-2-(2-(6-hydroxy-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ylidene)ethyl)benzoate (15): Aluminum (III) chloride (42.0 mg, 0.31 mmol) was added to a solution of **11** (40.0 mg, 0.08 mmol) in anhydrous anisole (10 mL) at 0 °C. This mixture was stirred at 0 °C and monitored closely by TLC. Upon complete conversion of **11**, MeOH (10 mL) was added and stirring continued while warming to 25°C. Concentration of the resulting solution followed by flash chromatography (SiO_2, 50% EtOAc in hexanes) gave **15** as a white amorphous solid (48.0 mg, 83%): ^{1}H NMR (Acetone, 400 MHz) δ 9.58 (s, 1H), 6.63 (s, 1H), 6.57 (s, 1H), 6.52 (s, 1H), 5.52 (t, J = 6.8, 1H), 4.48 (d, J = 6.8, 2H), 3.89 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.2, 162.0, 158.7, 158.5, 144.0, 142.9, 142.3, 142.1, 136.0, 119.7, 118.6, 115.2, 108.6, 103.3, 103.1, 101.3, 56.8, 52.8, 31.1; IR (film) ν_{max} 1647, 1609, 1582, 1520, 1400, 1317 cm^{-1}; ESI-HRMS 420.0486 (M – H$^+$, $C_{19}H_{16}ClNO_8$ requires 420.0486).



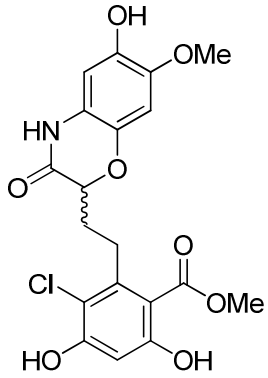
(Z)-methyl 3-chloro-4,6-dihydroxy-2-(2-(6-hydroxy-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ylidene)ethyl)benzoate (16): Compound **12** (22.0 mg, 0.04 mmol) was subjected to the conditions used to generate **15**. Flash chromatography (SiO_2, 50% EtOAc in hexanes) gave **16** as a white amorphous solid (4.0 mg, 24%): ^{1}H NMR (Acetone, 400 MHz) δ 9.53 (s, 1H), 6.83 (s, 1H), 6.58 (s, 1H), 6.53 (s, 1H), 5.90 (t, J = 6.9, 1H), 4.10 (d, J = 6.9, 2H), 3.90 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.5, 162.6, 159.9, 157.0, 144.1, 143.5, 143.1, 141.3, 135.1, 119.3, 115.7, 112.4, 107.4, 103.4, 103.3, 101.4, 56.9, 52.8, 29.0; IR (film) ν_{max} 1715, 1693, 1568, 1520, 1445, 1393, 1385, 1321 cm^{-1}; ESI-HRMS 420.0496 (M – H$^+$, $C_{19}H_{16}ClNO_8$ requires 420.0486).



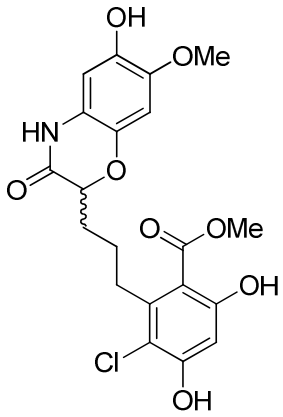
(*E*)-methyl 3-chloro-4,6-dihydroxy-2-(3-(6-hydroxy-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ylidene)propyl)benzoate (17): Compound **13** (70.0 mg, 0.13 mmol) was subjected to the conditions used to generate **15**. Flash chromatography (SiO_2, 50% EtOAc in hexanes) gave **17** as a white amorphous solid (36.0 mg, 62%): ^{1}H NMR (Acetone, 400 MHz) δ 9.46 (s, 1H), 6.66 (s, 1H), 6.54 (s, 1H), 6.50 (s, 1H), 5.70 (t, J = 8.0, 1H), 3.95 (s, 3H), 3.82 (s, 3H), 3.24 – 3.21 (m, 2H), 3.00 (dd, J = 7.9, 15.9, 2H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.6, 162.4, 158.9, 158.3, 144.0, 143.4, 142.8, 142.4, 136.0, 120.1, 119.7, 115.0, 107.9, 103.0, 103.0, 101.3, 56.9, 52.8, 33.0, 27.4; IR (film) ν_{max} 1676, 1653, 1605, 1518, 1319 cm^{-1}; ESI-HRMS 434.0645 (M – H$^+$, $C_{20}H_{18}ClNO_8$ requires 434.0643).



(Z)-methyl 3-chloro-4,6-dihydroxy-2-(3-(6-hydroxy-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-ylidene)propyl)benzoate (18): Compound **14** (7.0 mg, 0.013 mmol) was subjected to the conditions used to generate **15**. Flash chromatography (SiO_2, 50% EtOAc in hexanes) gave **18** as a white amorphous solid (3.2 mg, 57%): ^{1}H NMR (Acetone, 400 MHz) δ 9.37 (s, 1H), 6.59 (s, 1H), 6.43 (s, 1H), 6.37 (s, 1H), 5.88 (t, J = 7.8, 1H), 3.85 (s, 3H), 3.69 (s, 3H), 3.16 – 3.06 (m, 2H), 2.49 – 2.42 (m, 2H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.5, 162.4, 158.7, 157.1, 144.0, 144.0, 143.4, 143.0, 135.1, 119.2, 114.8, 113.6, 108.1, 103.2, 103.1, 101.4, 56.8, 52.9, 32.1, 25.3; IR (film) ν_{max} 1718, 1651, 1518, 1435, 1377, 1319 cm^{-1}; ESI-HRMS 434.0635 (M – H$^+$, $C_{20}H_{18}ClNO_8$ requires 434.0643).



(±) Methyl 3-chloro-4,6-dihydroxy-2-(2-(6-hydroxy-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-yl)ethyl)benzoate (19): Palladium on carbon (10 mol%) was added to a stirred solution of mixed isomers **11** and **12** (60.0 mg, 0.12 mmol) in EtOAc (10 mL). Hydrogen was bubbled through the reaction mixture and then the reaction stirred under H_2 atmosphere for 12 h. The reaction was filtered through a plug of celite and the resulting filtrate was concentrated and purified by flash chromatography (SiO_2, 30% hexanes in EtOAc) to give **19** as an amorphous white solid (42.4 mg, 85%): ^{1}H NMR (Acetone, 400 MHz) δ 9.38 (s, 1H), 6.70 (s, 1H), 6.55 (s, 1H), 6.49 (s, 1H), 4.59–4.50 (m, 1H), 3.93 (s, 3H), 3.82 (s, 3H), 3.42–3.18 (m, 2H), 2.18–2.08 (m, 2H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.4, 166.9, 162.3, 158.6, 144.0, 143.3, 142.8, 136.3, 121.5, 114.8, 108.2, 103.6, 103.4, 103.0, 78.0, 56.9, 52.8, 30.7, 29.1; IR (film) ν_{max} 1651, 1607, 1377, 1321 cm^{-1}; ESI-HRMS 424.0783 (M + H$^+$, $C_{19}H_{18}ClNO_8$ requires 424.0799). Chiral HPLC with 15% EtOAc in hexanes provided the pure enantiomers: **(+)-21**: $[\alpha]_D$ +137.1 (*c* = 0.24, $CHCl_3$); **(–)-22**: $[\alpha]_D$ -164.9 (*c* = 0.24, $CHCl_3$).



(±) Methyl 3-chloro-4,6-dihydroxy-2-(3-(6-hydroxy-7-methoxy-3-oxo-3,4-dihydro-2H-benzo[b][1,4]oxazin-2-yl)propyl)benzoate (20): Following the same procedure used to generate **19**, an E/Z mixture of **13** and **14** (106.9 mg, 0.20 mmol) were introduced to the conditions mentioned above. The resulting filtrate was concentrated and purified by flash chromatography (SiO_2, 30% hexanes in EtOAc) to give **20** as an amorphous white solid (49.0 mg, 85%): ^{1}H NMR (Acetone, 400 MHz) δ 9.32 (s, 1H), 6.68 (s, 1H), 6.54 (s, 1H), 6.49 (s, 1H), 4.47 (dd, *J* = 4.4, 8.2, 1H), 3.94 (s, 3H), 3.80 (s, 3H), 3.17–3.05 (m, 2H), 2.01–1.91 (m, 2H), 1.90–1.78 (m, 2H); ^{13}C NMR (Acetone, 126 MHZ) δ 171.6, 167.3, 162.4, 158.7, 144.2, 144.0,

142.8, 136.3, 121.6, 114.7, 107.9, 103.6, 102.9, 102.9, 77.6, 56.8, 52.8, 32.8, 31.0, 25.9; IR (film) ν_{max} 1655, 1649, 1602, 1510, 1433, 1369, 1311 cm^{-1}; ESI-HRMS 438.0969 ($M + H^+$, $C_{20}H_{20}ClNO_8$ requires 438.0956). Chiral HPLC with 15% EtOAc in hexanes provided the pure enantiomers: **(+)** $[\alpha]_D$ +11.7 (*c* = 0.115, $CHCl_3$); **(−)** $[\alpha]_D$ -14.7 (*c* = 0.115, $CHCl_3$).

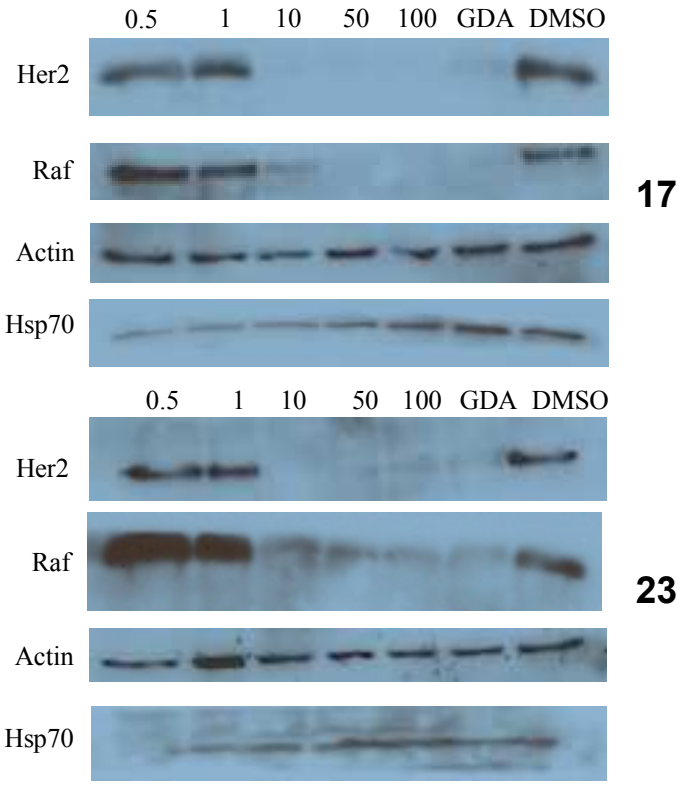
Anti-proliferation Assay: MCF-7 and SKBr3 cells were maintained in a 1:1 mixture of Advanced DMEM/F12 (Gibco) supplemented with non-essential amino acids, L-glutamine (2 mM), streptomycin (500 µg/mL), penicillin (100 units/mL), and 10% FBS. Cells were grown to confluence in a humidified atmosphere (37 °C, 5% CO_2), seeded (2000/well, 100 µL) in 96-well plates, and allowed to attach overnight. Compound or geldanamycin at varying concentrations in DMSO (1% DMSO final concentration) was added, and cells were returned to the incubator for 72 h. At 72 h, the number of viable cells was determined using an MTS/PMS cell proliferation kit (Promega) per the manufacturer's instructions. Cells incubated in 1% DMSO were used as 100% proliferation, and values were adjusted accordingly. IC_{50} values were calculated from separate experiments performed in triplicate using GraphPad Prism.

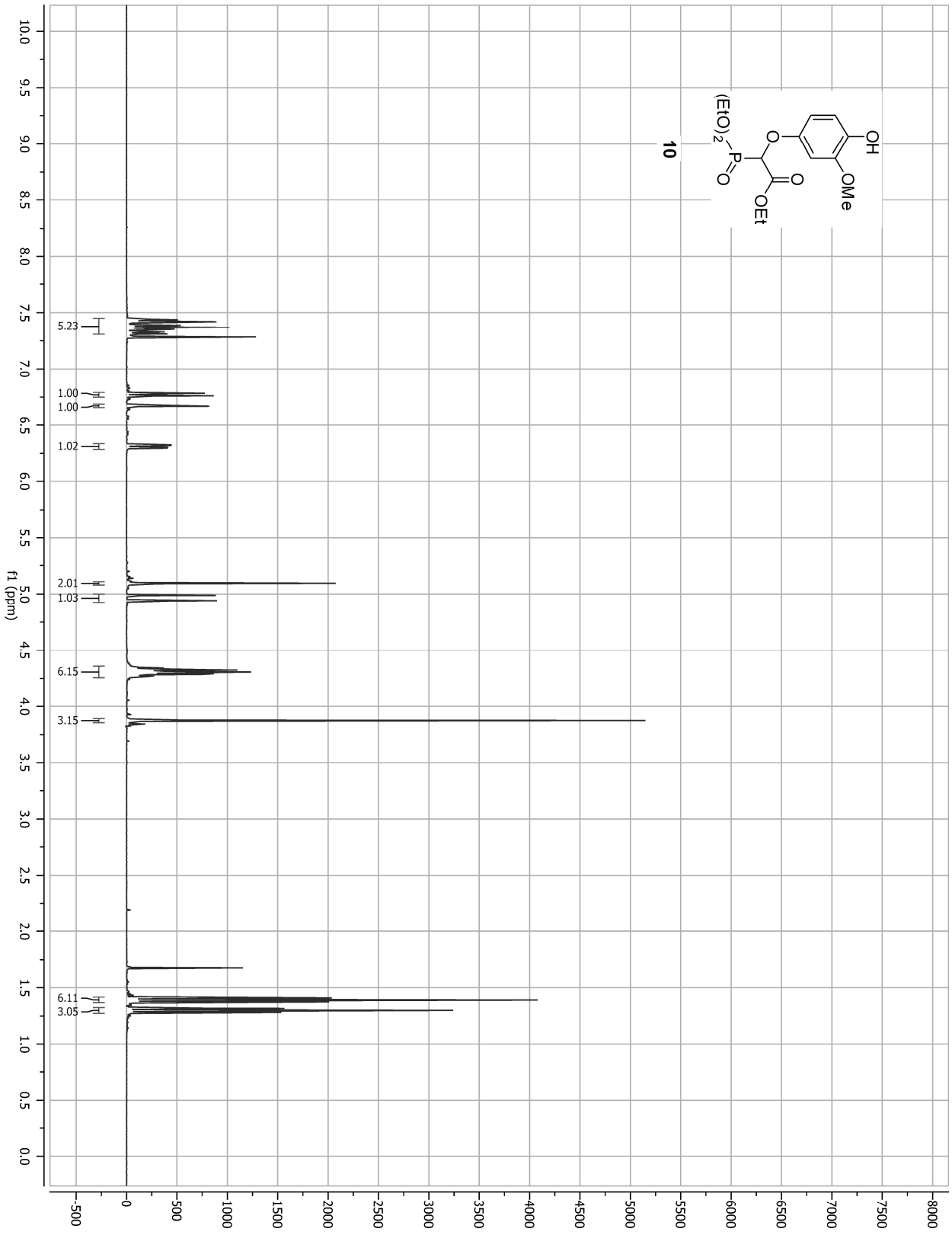
ATPase Assay: Recombinant yeast Hsp90 was overexpressed and purified[1,2]. The assay was run using optimized conditions previously reported and the P_iPer™ Phosphate Assay Kit (Molecular Probes #P-22061). Proper dilutions were made using the provided manufacturer's instructions. Assay solutions and conditions were taken directly from the optimized conditions previously published[2]. Each well contained a final volume of 100 µL. Wells were mixed by pipette and then shaken for approximately 30 s. Plates were then covered and incubated at 42 ºC while shaken for 2 h. Absorbance was measured at 563 nm and IC_{50} values were calculated using GraphPad Prism. Each compound was tested in triplicate on three separate occasions.

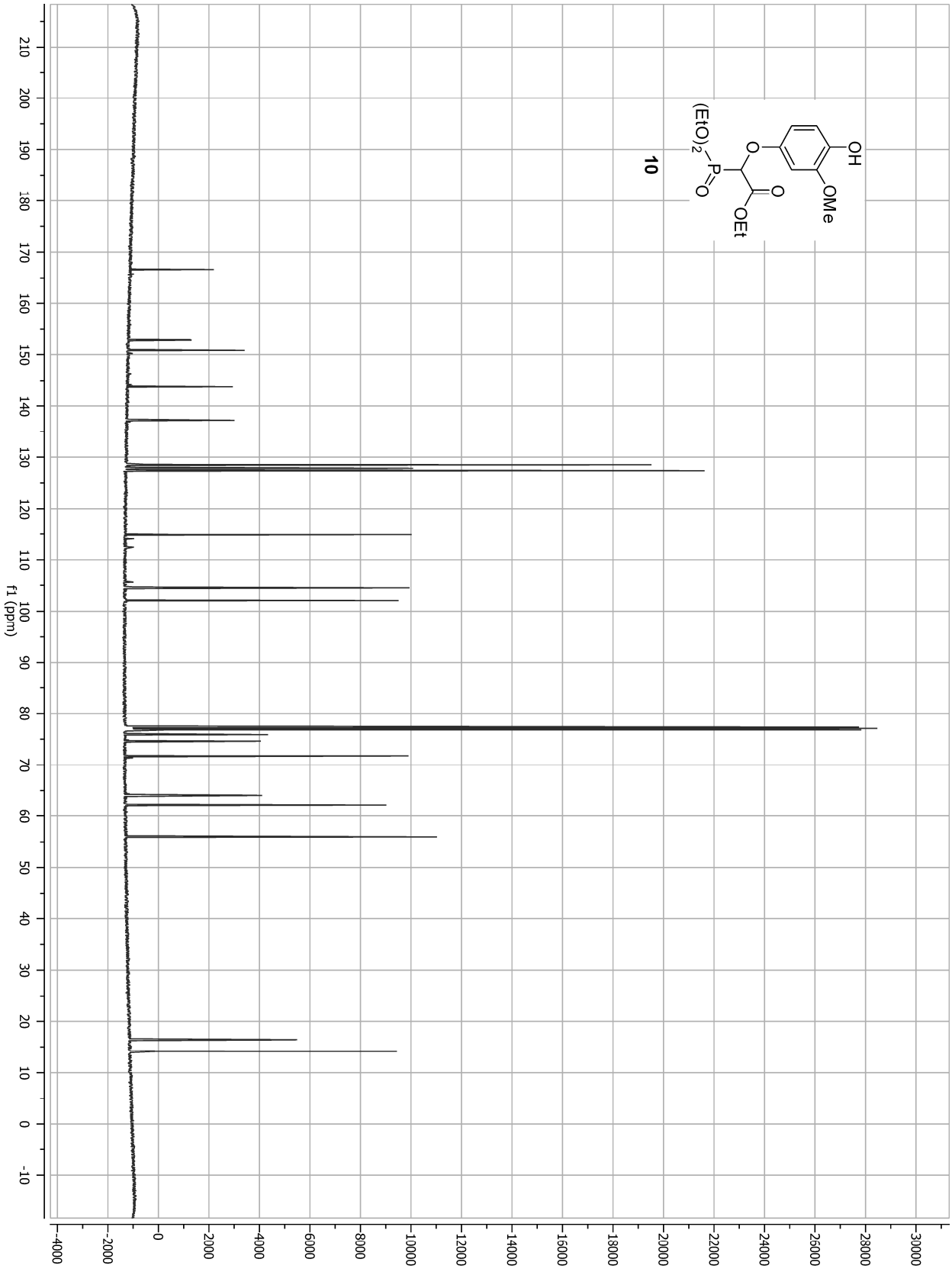
Western Blot Analysis: MCF-7 cells were cultured as described previously and treated with various concentrations of drug, GDA in DMSO (1% DMSO final concentration), or vehicle (DMSO) for 24 h. Cells were harvested in cold PBS and lysed in RIPA lysis buffer containing 1 mM PMSF, 2 mM sodium orthovanadate, and protease inhibitors on ice for 1 h. Lysates were clarified at 1400 g for 10 min at 4 ºC. Protein concentrations were determined by using the Pierce BCA assay kit per the manufacturer's instructions. Equal amounts of protein (20 µg) were electrophoresed under reducing conditions, transferred to a nitrocellulose membrane, and immunoblotted with the corresponding specific antibodies. Membranes were incubated with an appropriate horseradish peroxidase-labeled secondary anti-body, developed with chemiluminescent substrate, and visualized. The western blots from compounds **17** and **23** are shown below.

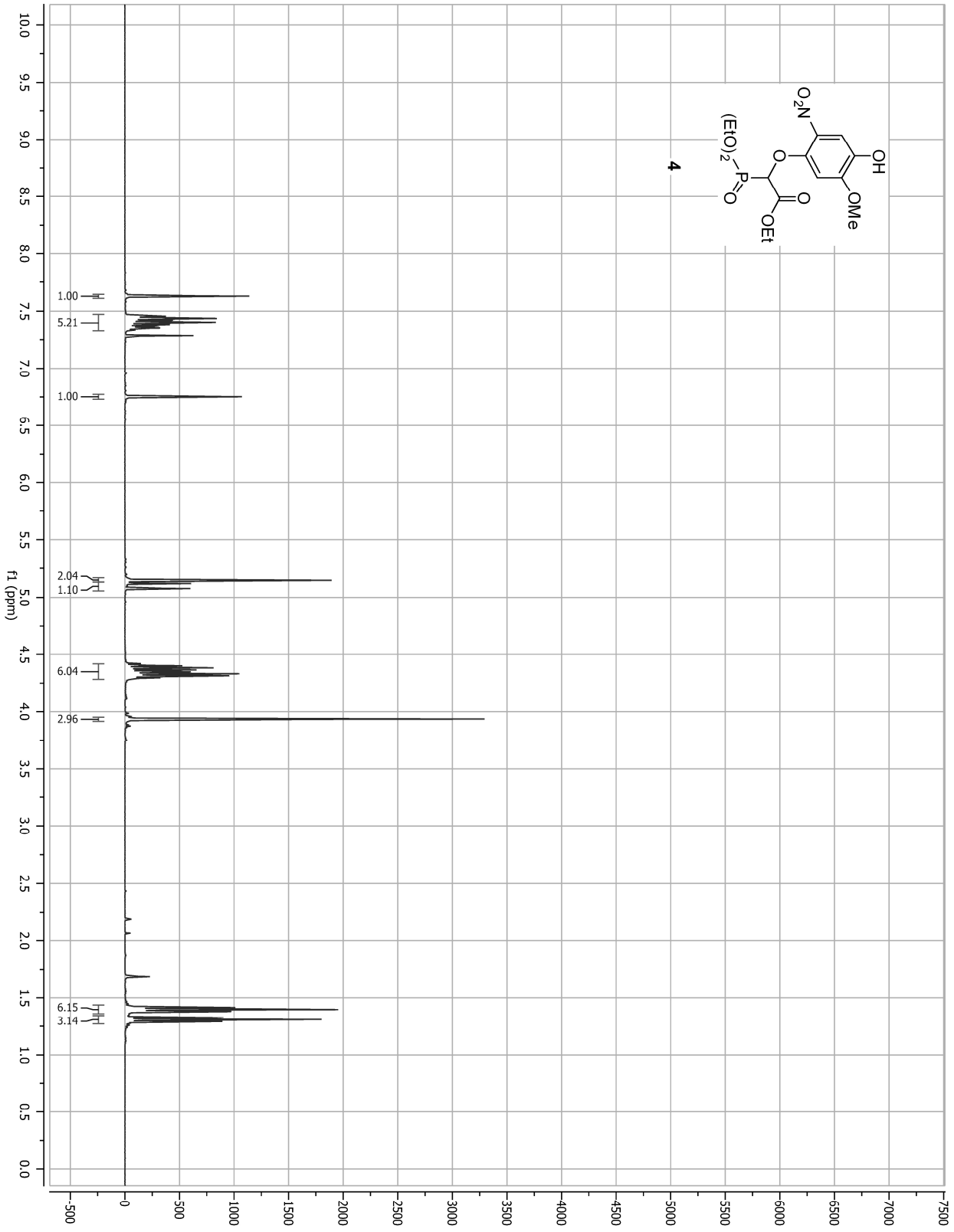
1.) Richter, K., Muschler, P., Hainzl, O., Buchner, J., *J. Biol. Chem.* **2001**, *276*, 33689–33696.

2.) Avila, C., Kornilayev, B. A., Blagg, B. S. J., *Bioorg. & Med. Chem.* **2006**, *14*, 1134–1142.


0.5 1 10 50 100 GDA DMSO
Her2
Raf
Actin
Hsp70
17
0.5 1 10 50 100 GDA DMSO
Her2
Raf
Actin
Hsp70
23


10
(EtO)2P
O
O
OEt
OMe
OH
f1 (ppm)
5.23
1.00
1.00
1.02
2.01
1.03
6.15
3.15
6.11
3.05


OH
OMe
O
O
OEt
(EtO)2
P
O
10
f1 (ppm)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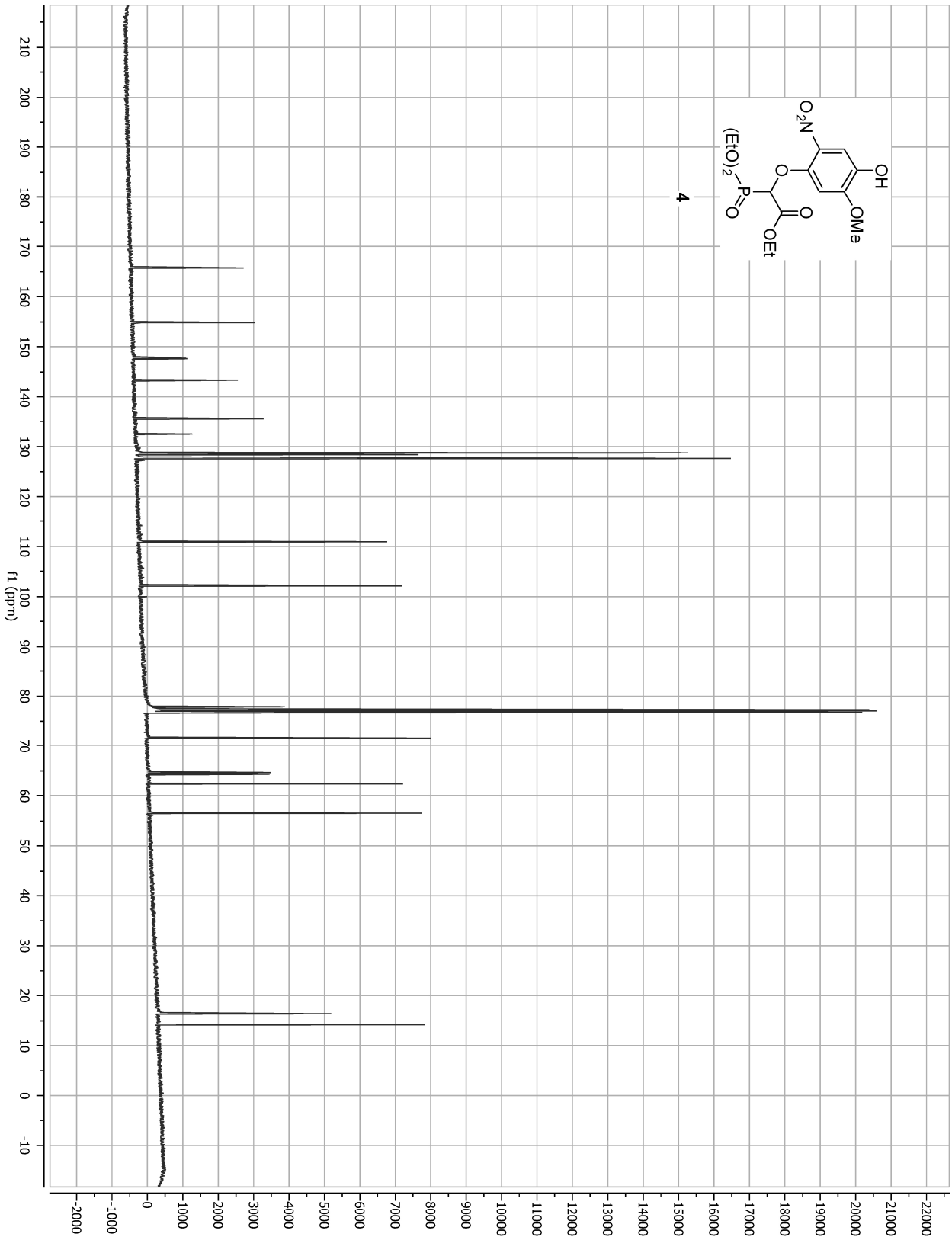
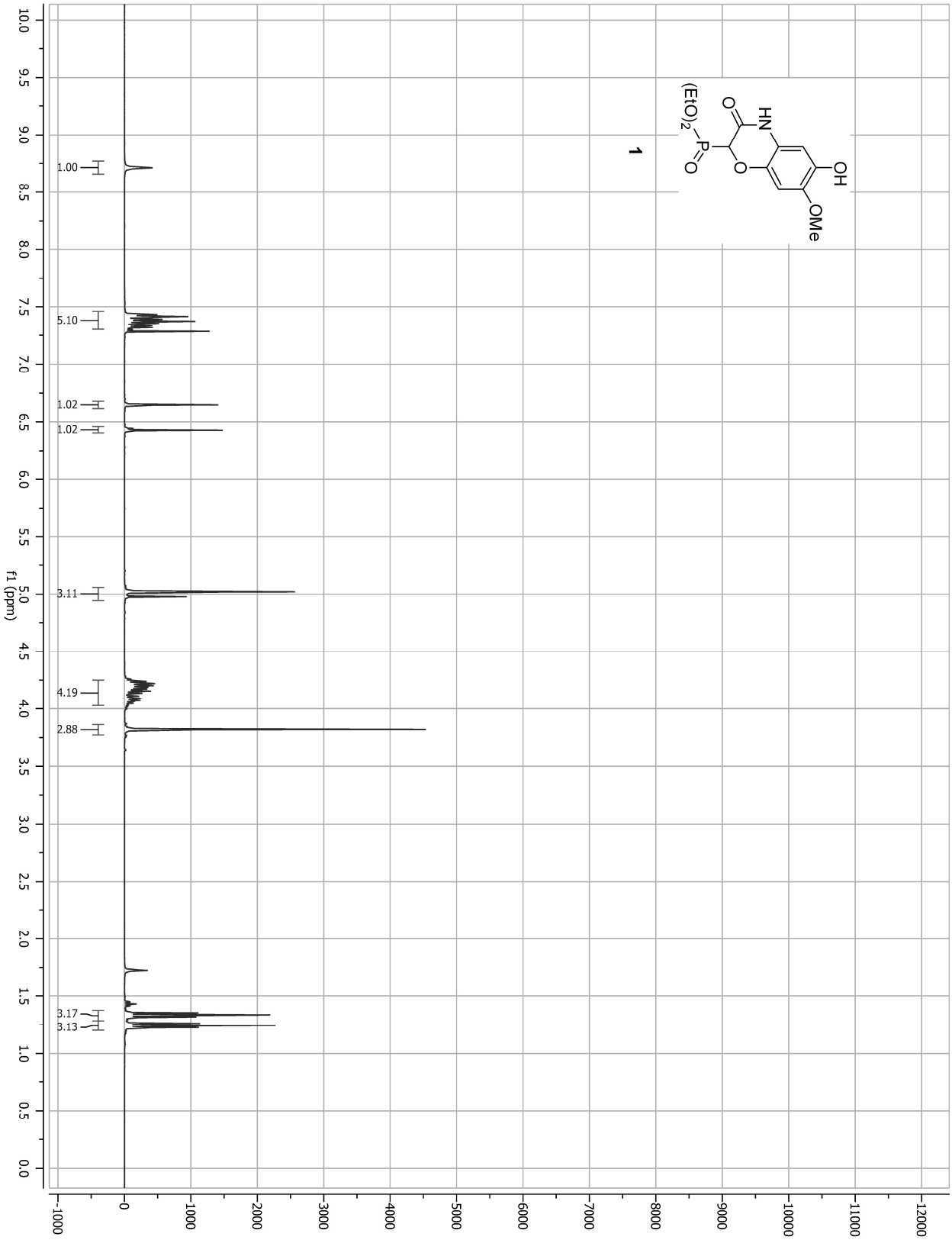
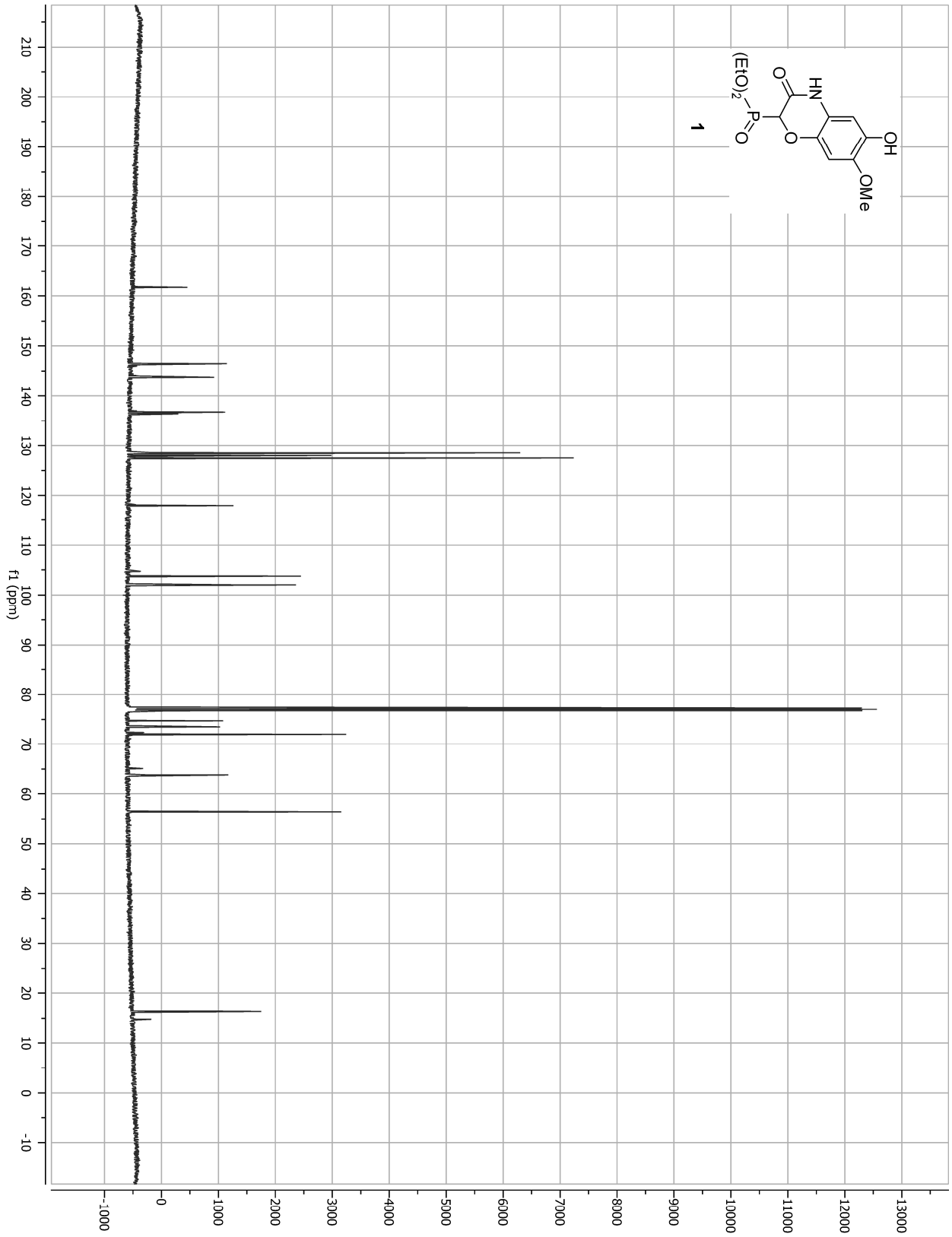
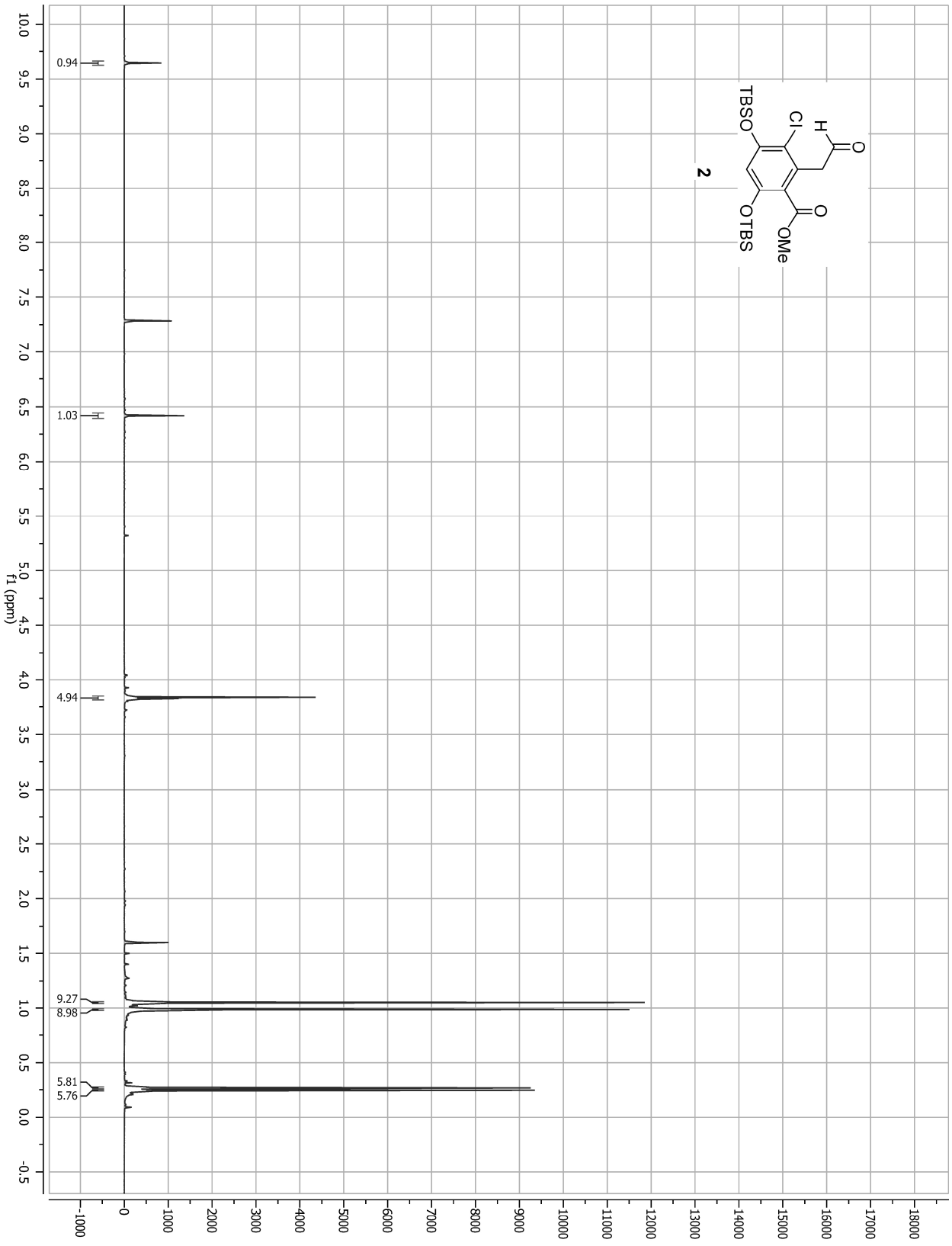
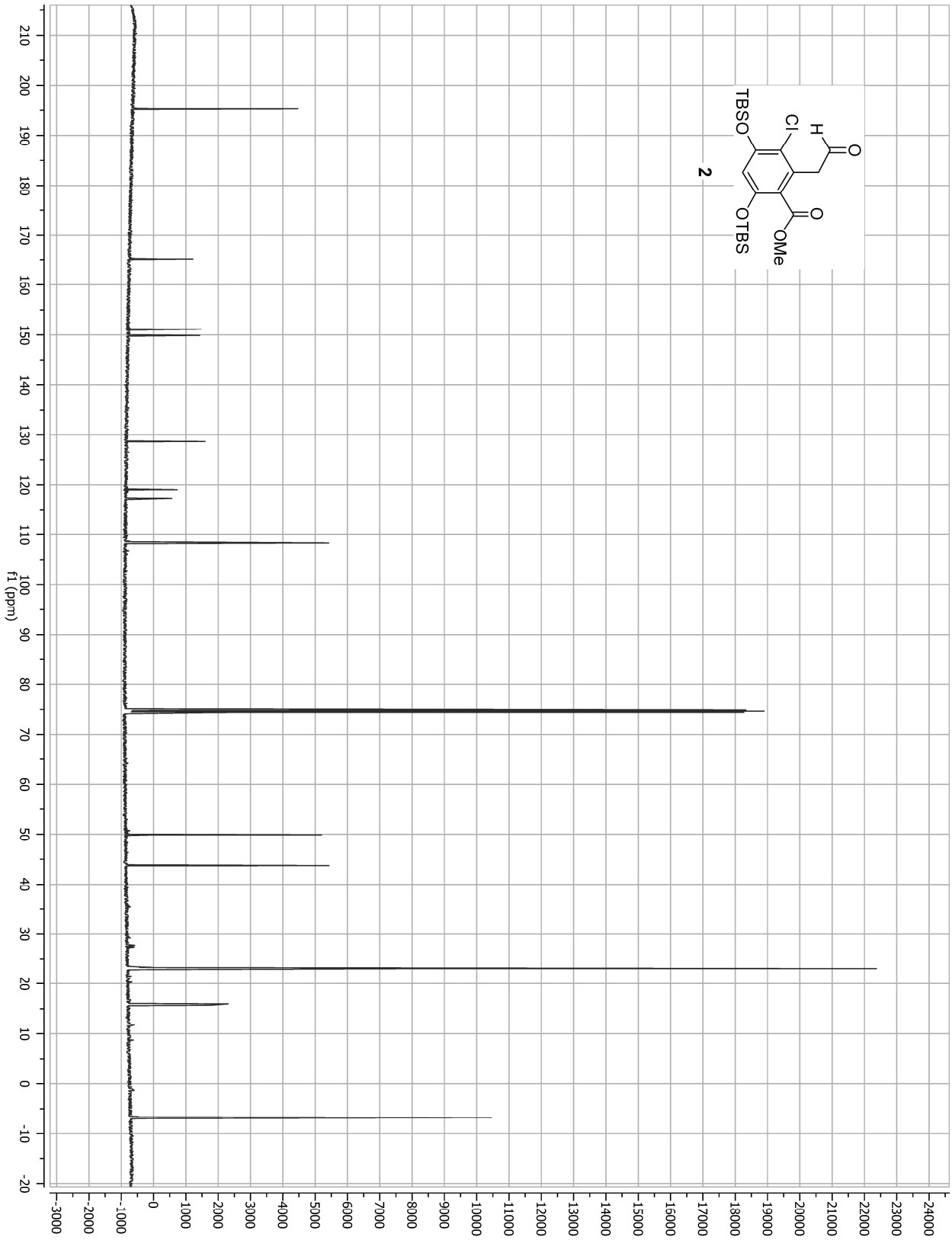
1.00
5.21
1.00
2.04
1.10
6.04
2.96
6.15
3.14
f1 (ppm)

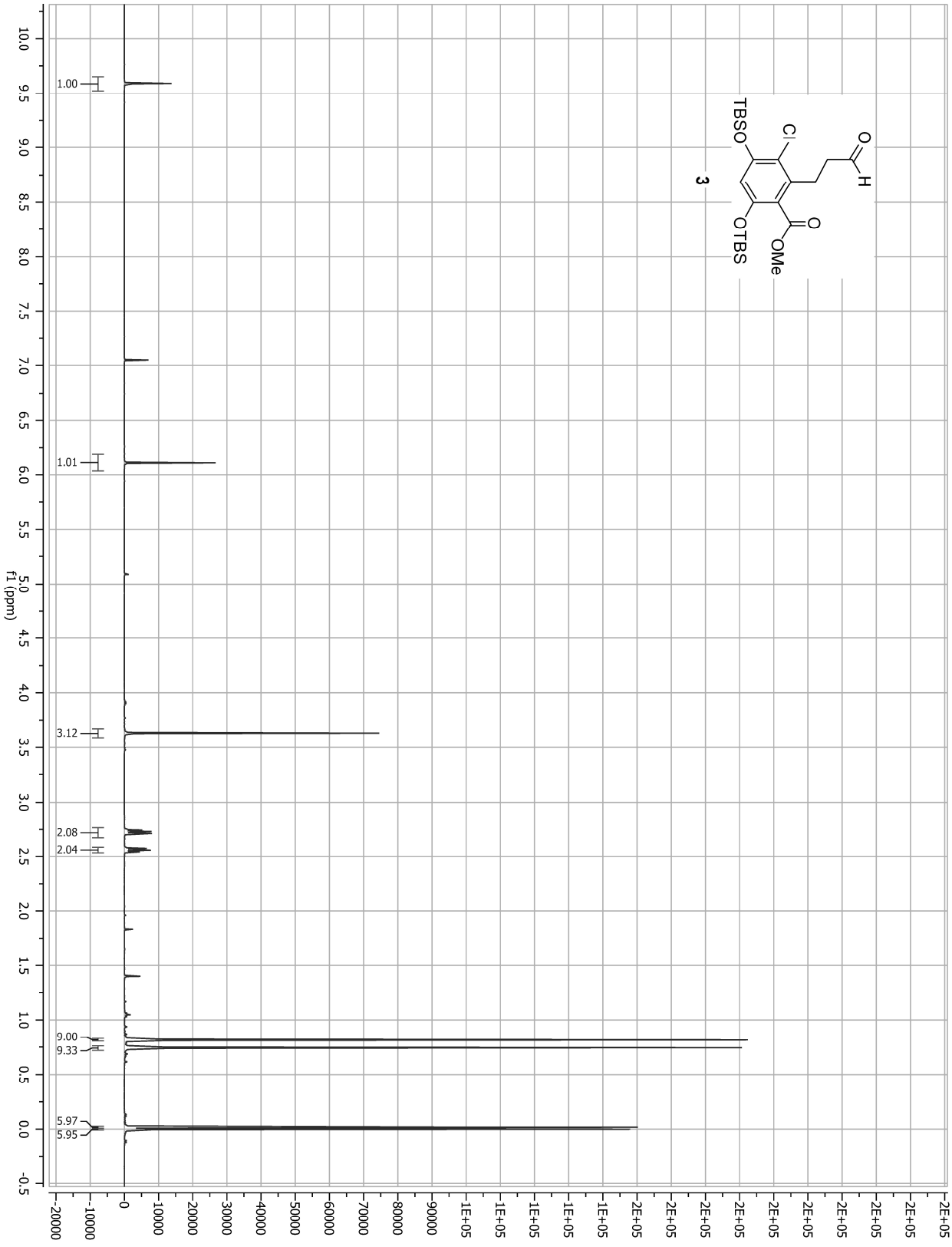

4
f1 (ppm)

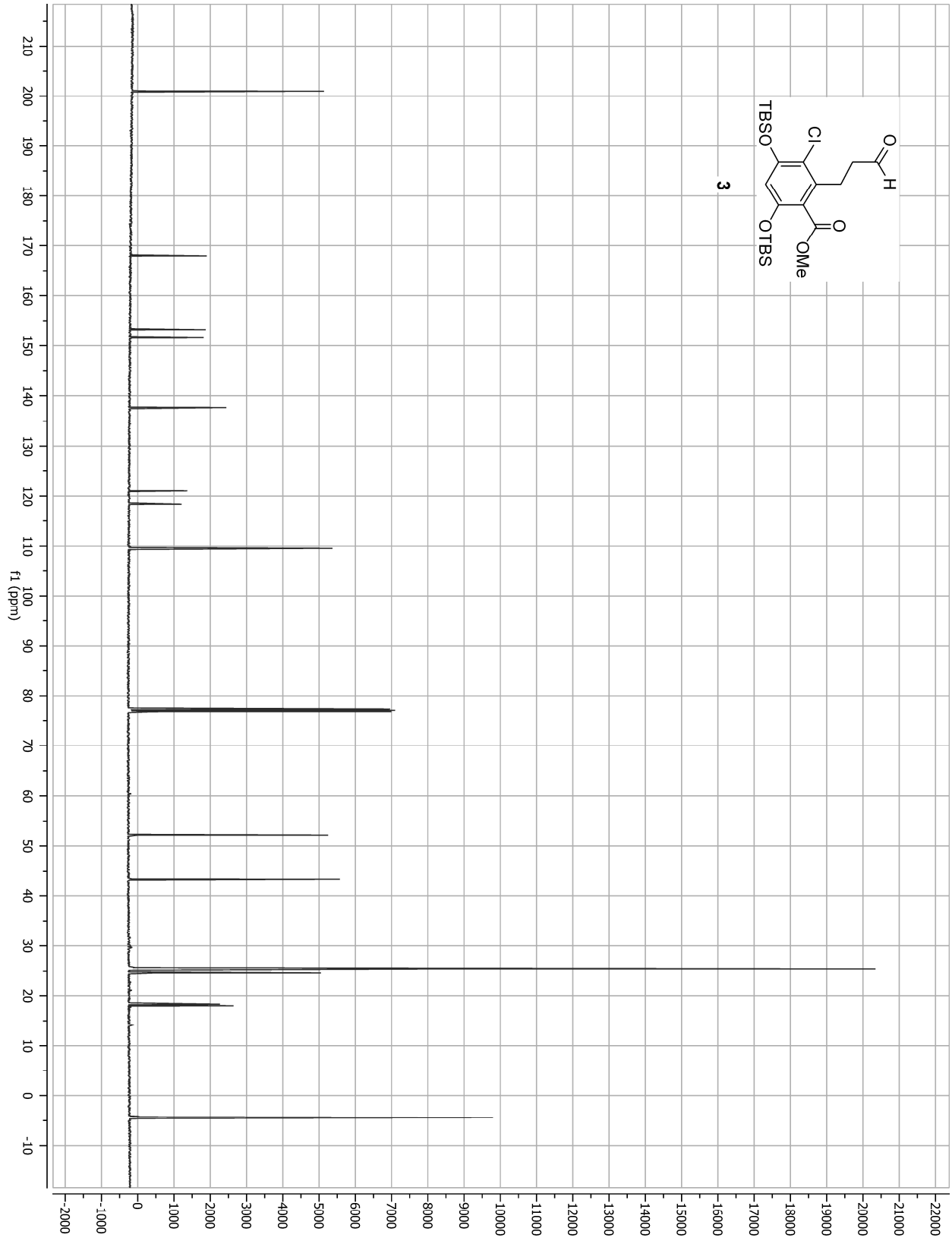

1
HN
O
O
$(EtO)_2$—P=O
OH
OMe
1.00
5.10
1.02
1.02
3.11
4.19
2.88
3.17
3.13
f1 (ppm)

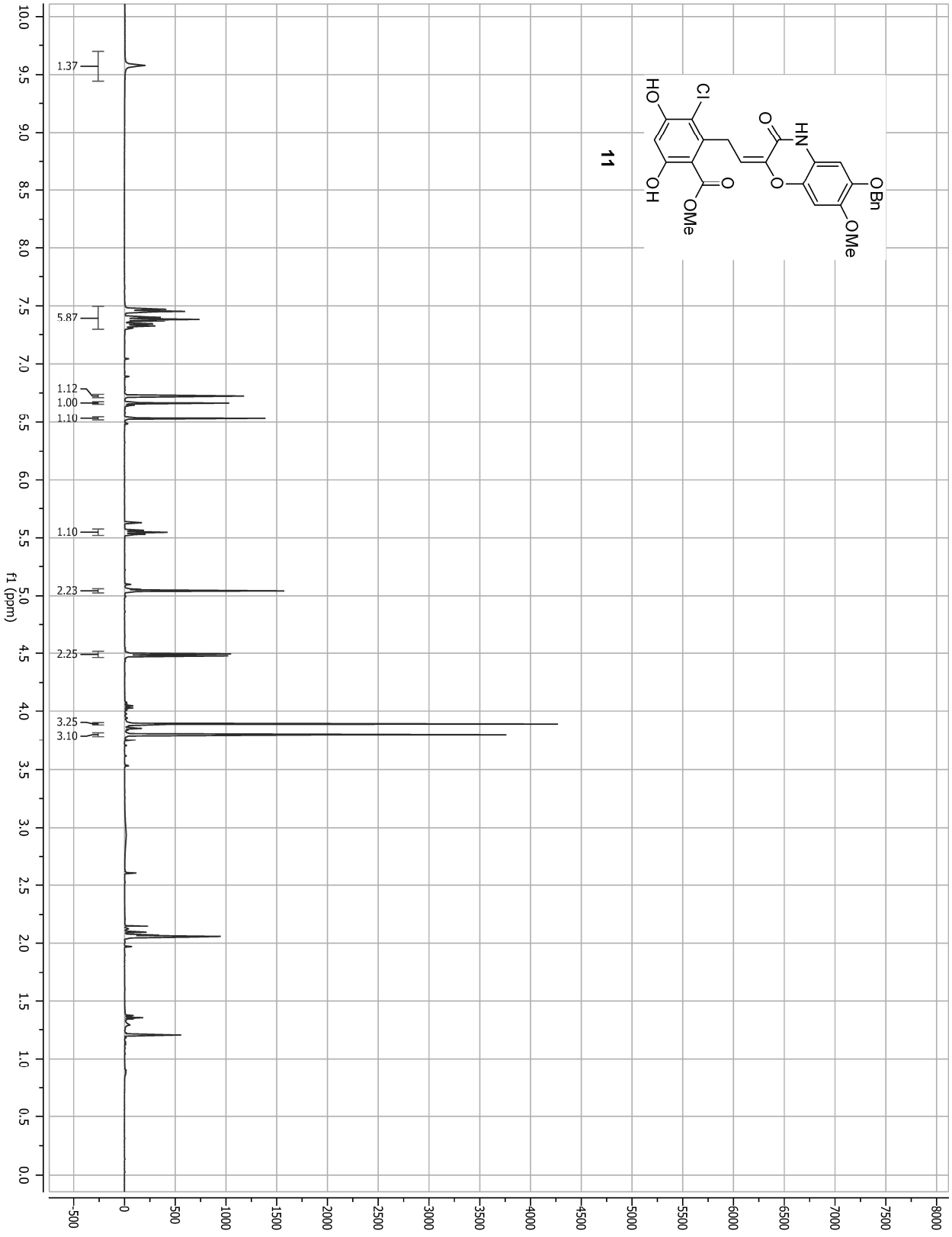

HN
O
O
OH
OMe
(EtO)2
P
O
1
f1 (ppm)


TBSO
Cl
H
O
O
OMe
OTBS
2
0.94
1.03
4.94
9.27
8.98
5.81
5.76
f1 (ppm)


TBSO
Cl
H
O
O
OMe
OTBS
2
f1 (ppm)


TBSO
Cl
O
H
OTBS
O
OMe
3
1.00
1.01
3.12
2.08
2.04
9.00
9.33
5.97
5.95
f1 (ppm)


TBSO
Cl
O
H
O
OMe
OTBS
3
f1 (ppm)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HO
Cl
O
HN
OBn
OH
O
O
OMe
OMe
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1.12
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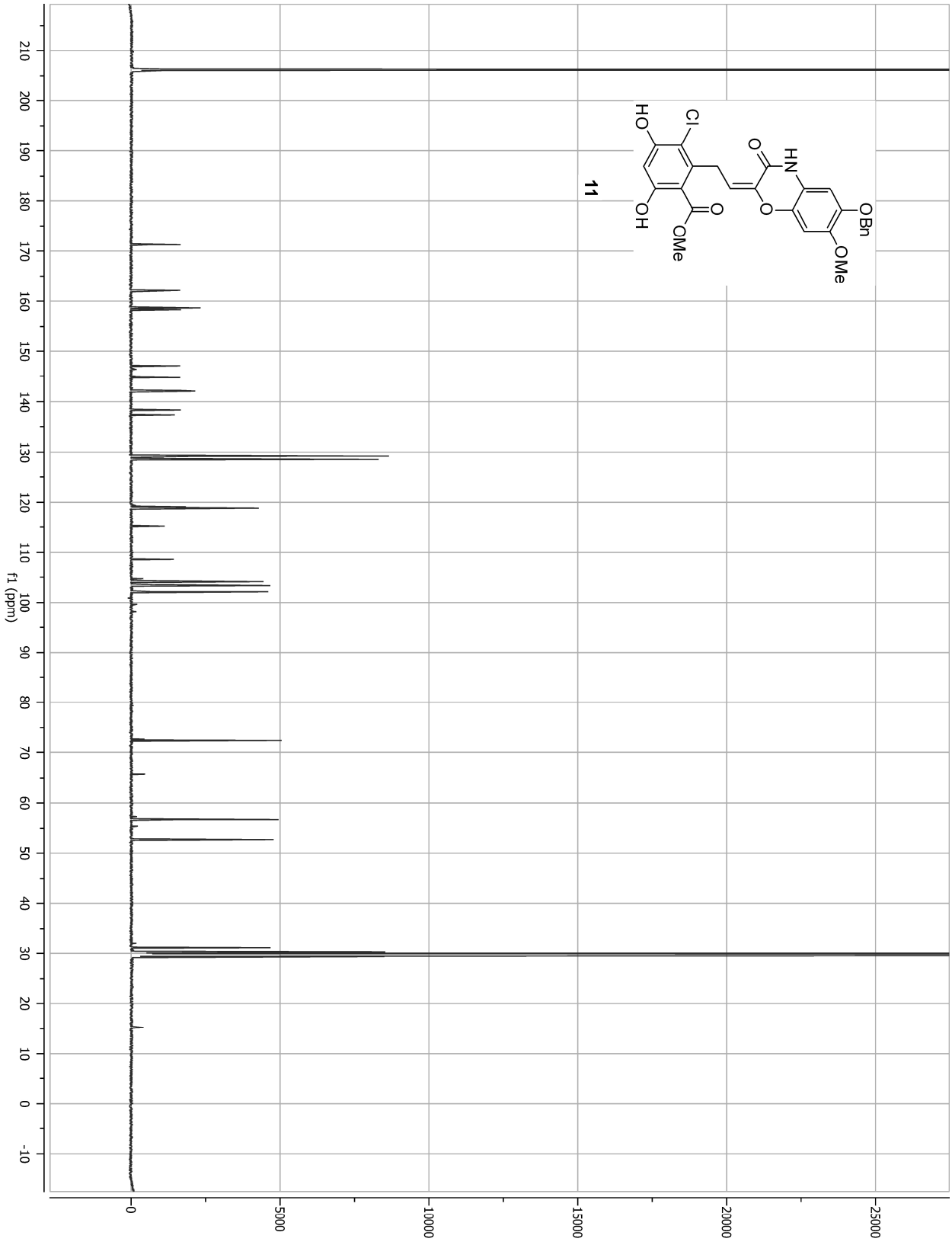
1.10
1.10
2.23
2.25
3.25
3.10
f1 (ppm)


11
HO
Cl
OH
O
OMe
HN
O
O
OBn
OMe
f1 (ppm)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
0
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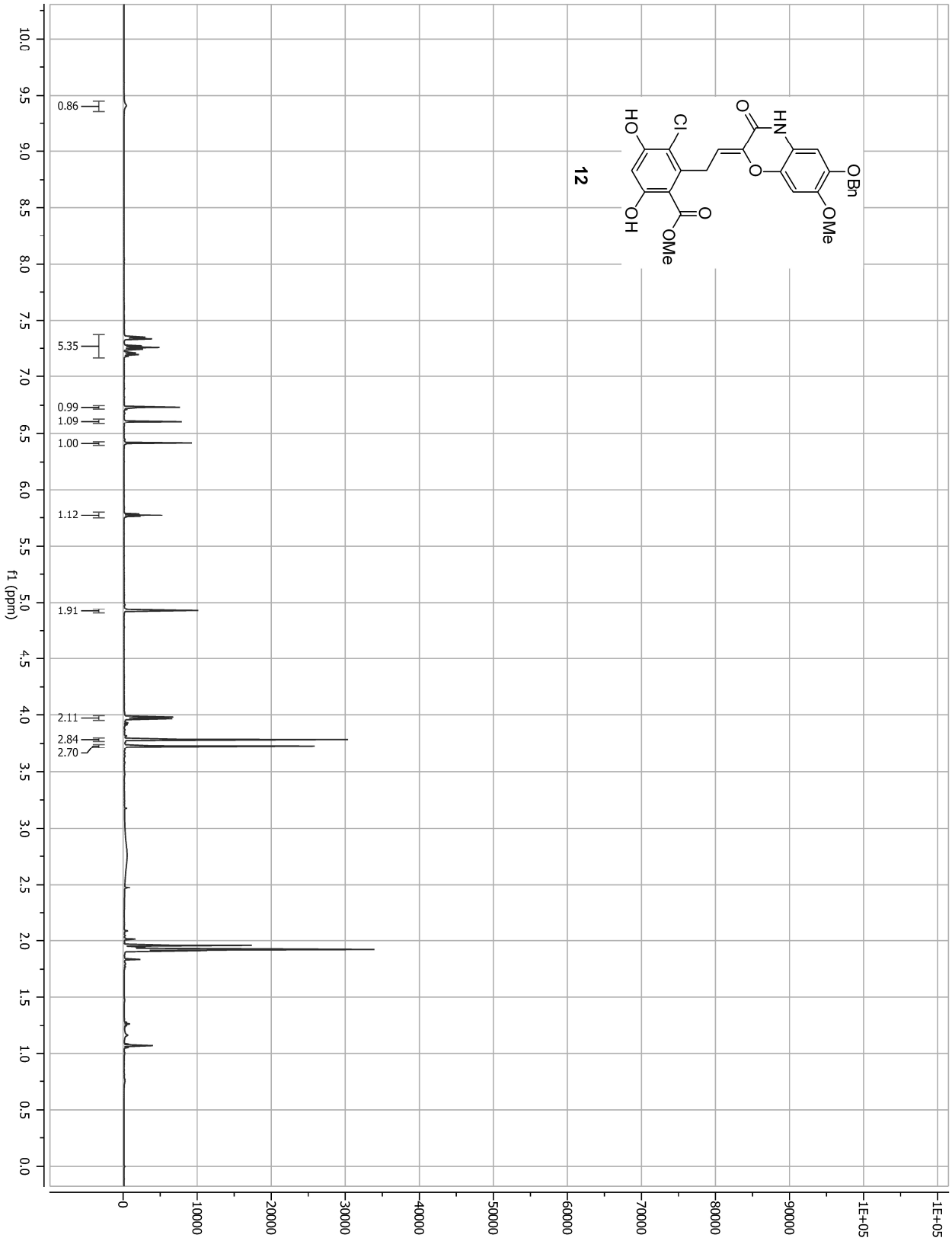
10000
15000
20000
25000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Cl
HN
O
OBn
OMe
OH
O
OMe
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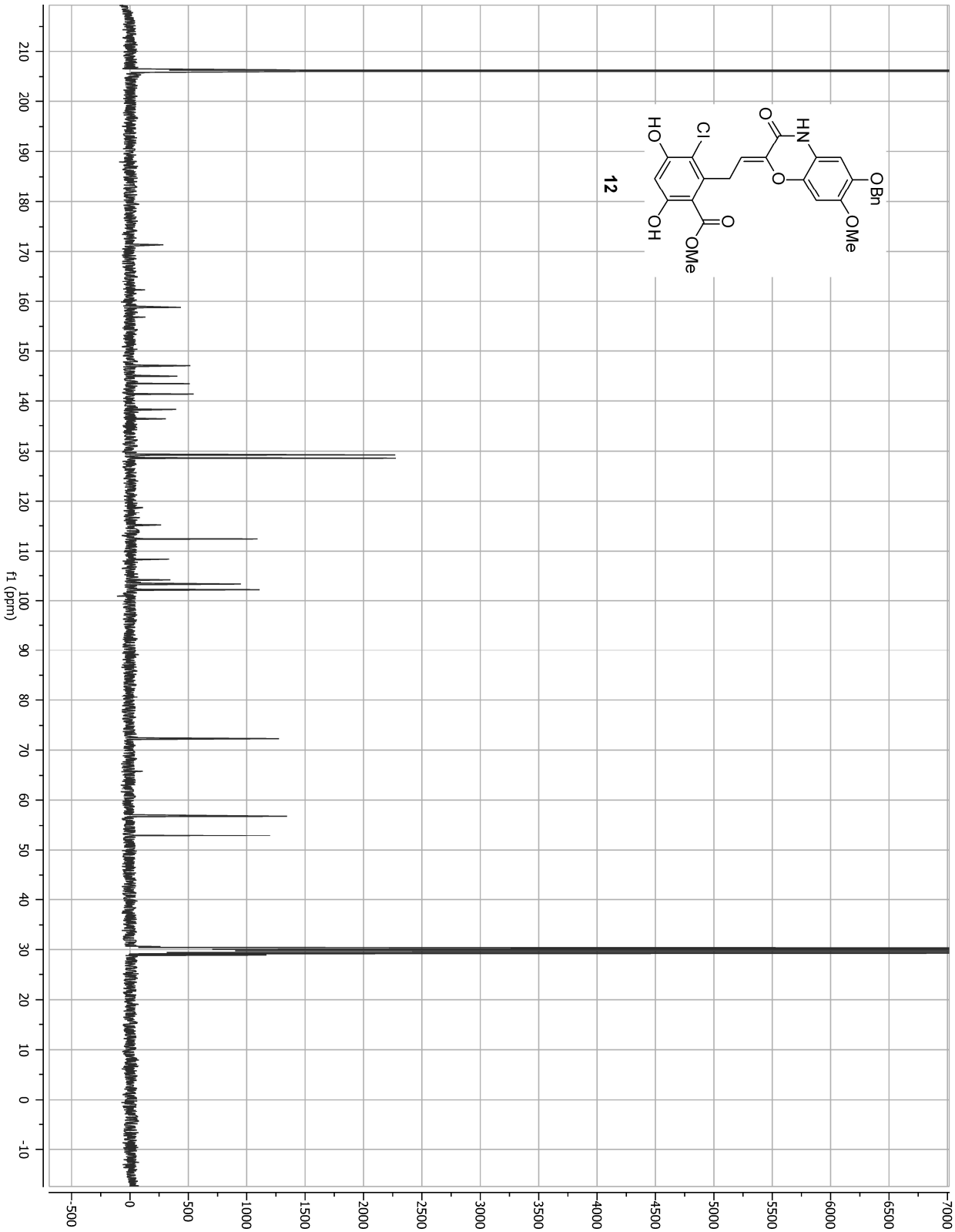
0.99
1.09
1.00
1.12
1.91
2.11
2.84
2.70
f1 (ppm)


12
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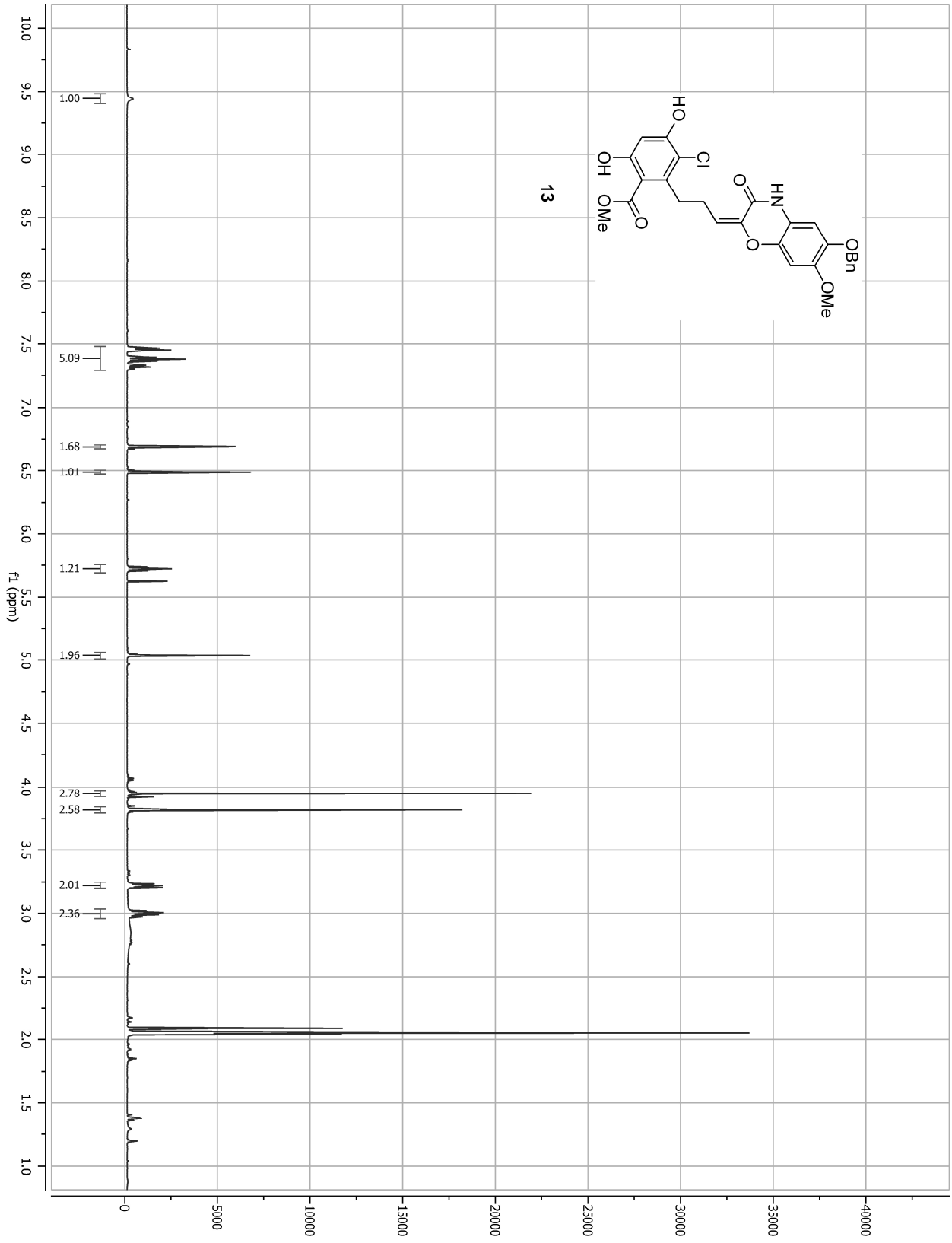
Cl
HN
O
O
OBn
OMe
OH
O
OMe
f1 (ppm)


13
HO
Cl
OH
OMe
O
HN
O
O
OBn
OMe
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1.68
1.01
1.21
1.96
2.78
2.58
2.01
2.36
f1 (ppm)
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9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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3.0
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1.0
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20000
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30000
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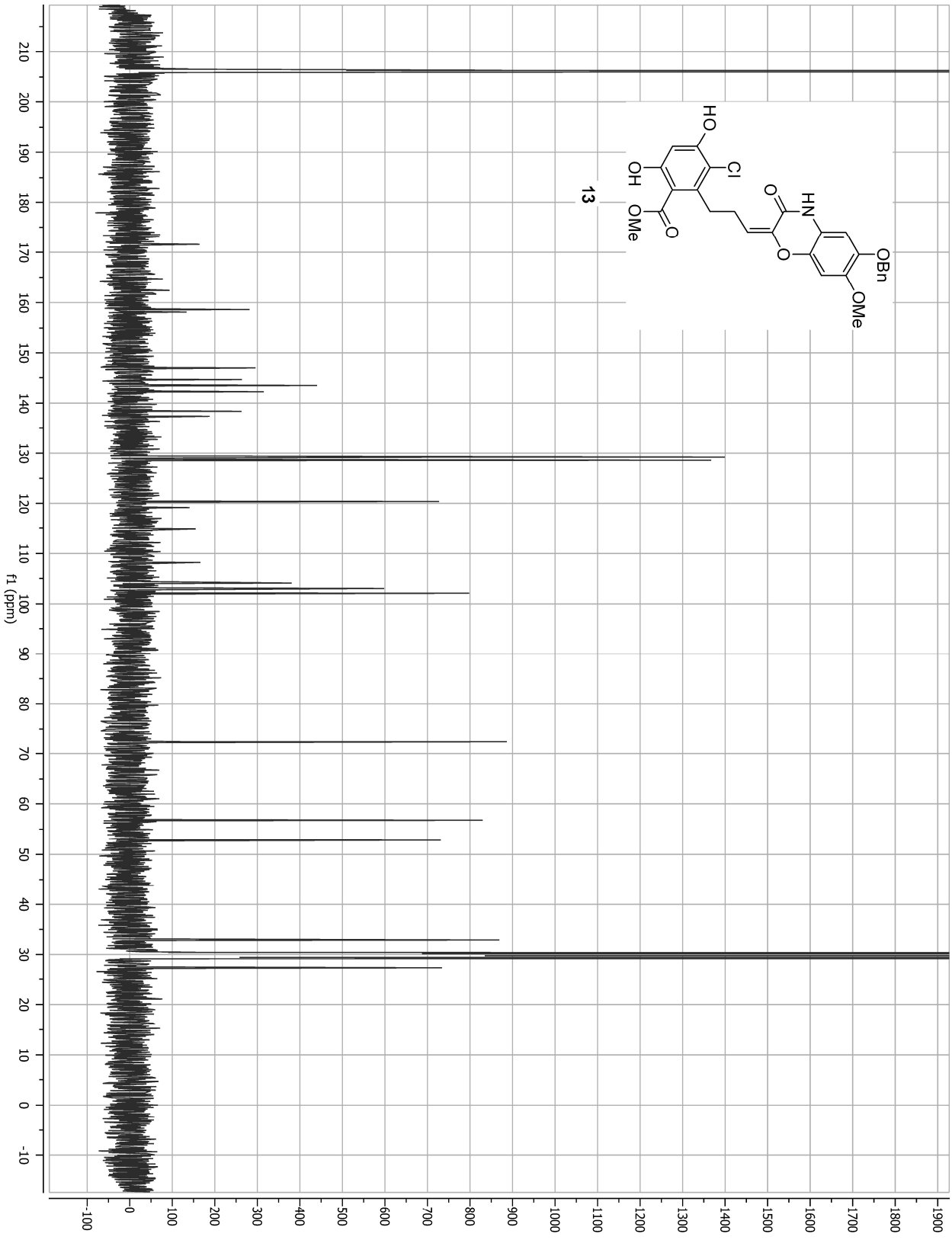
40000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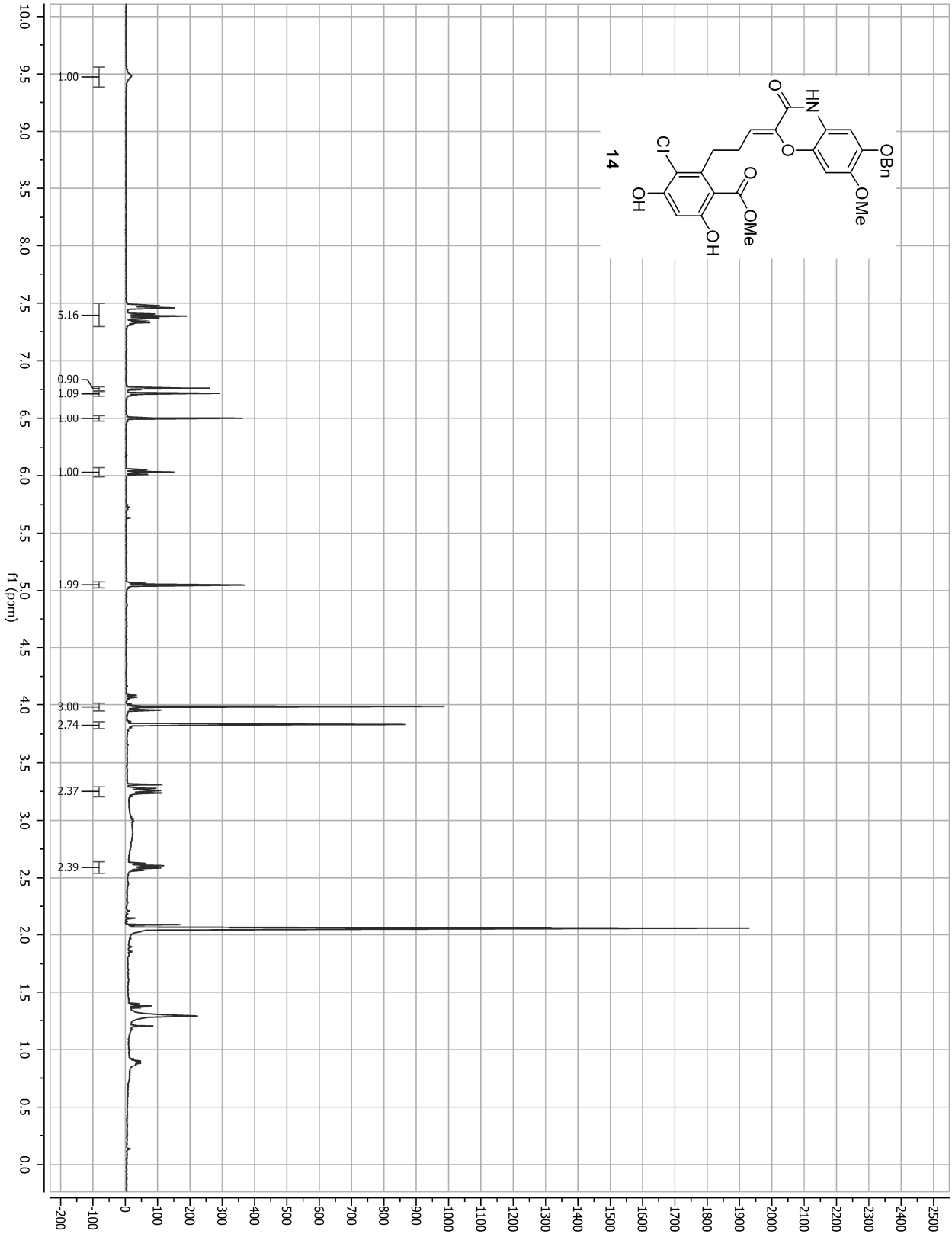
OH
OMe
O
HN
O
O
OBn
OMe
13
f1 (ppm)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O
O
OBn
OMe
Cl
OH
O
OMe
OH
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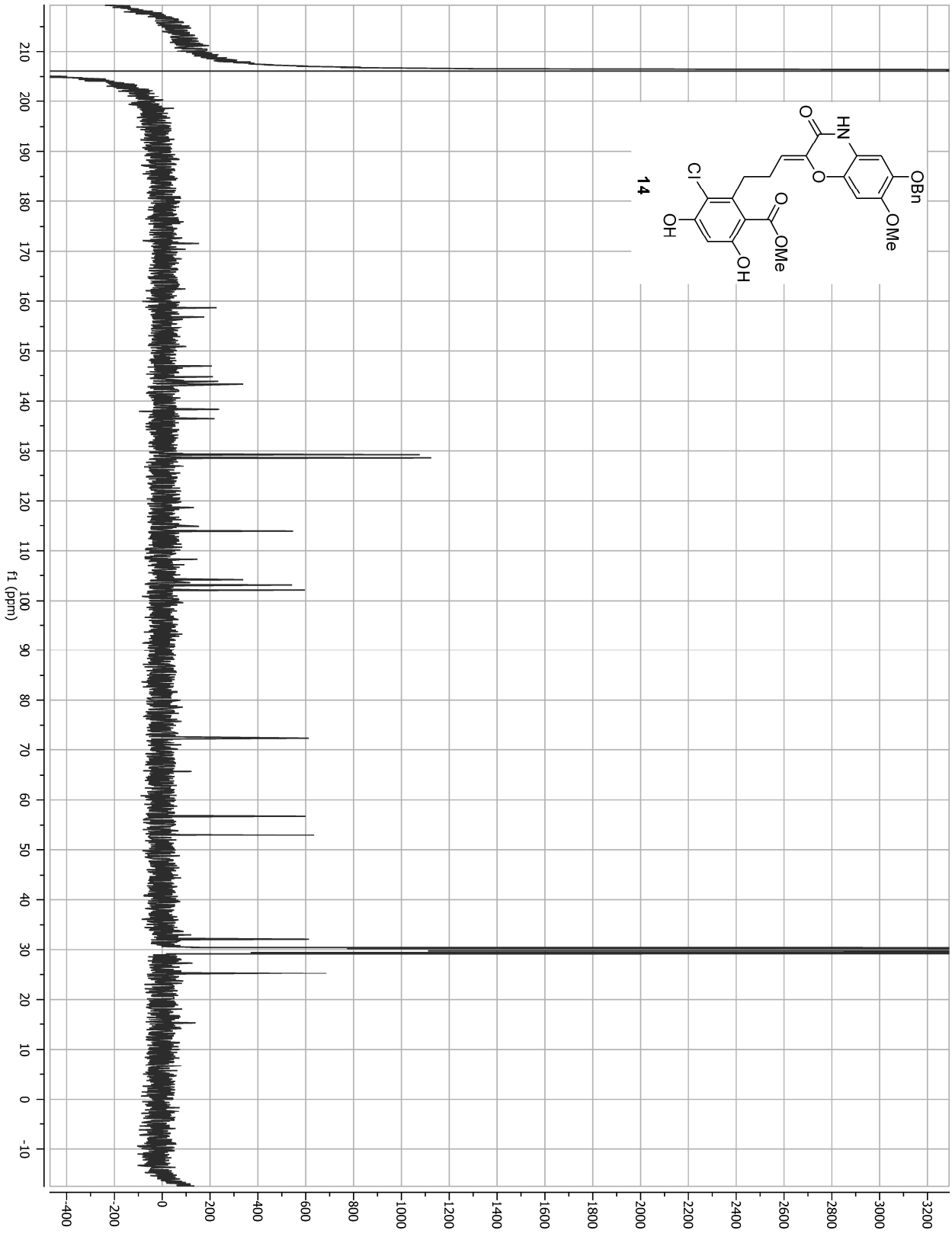
1.00
1.99
3.00
2.74
2.37
2.39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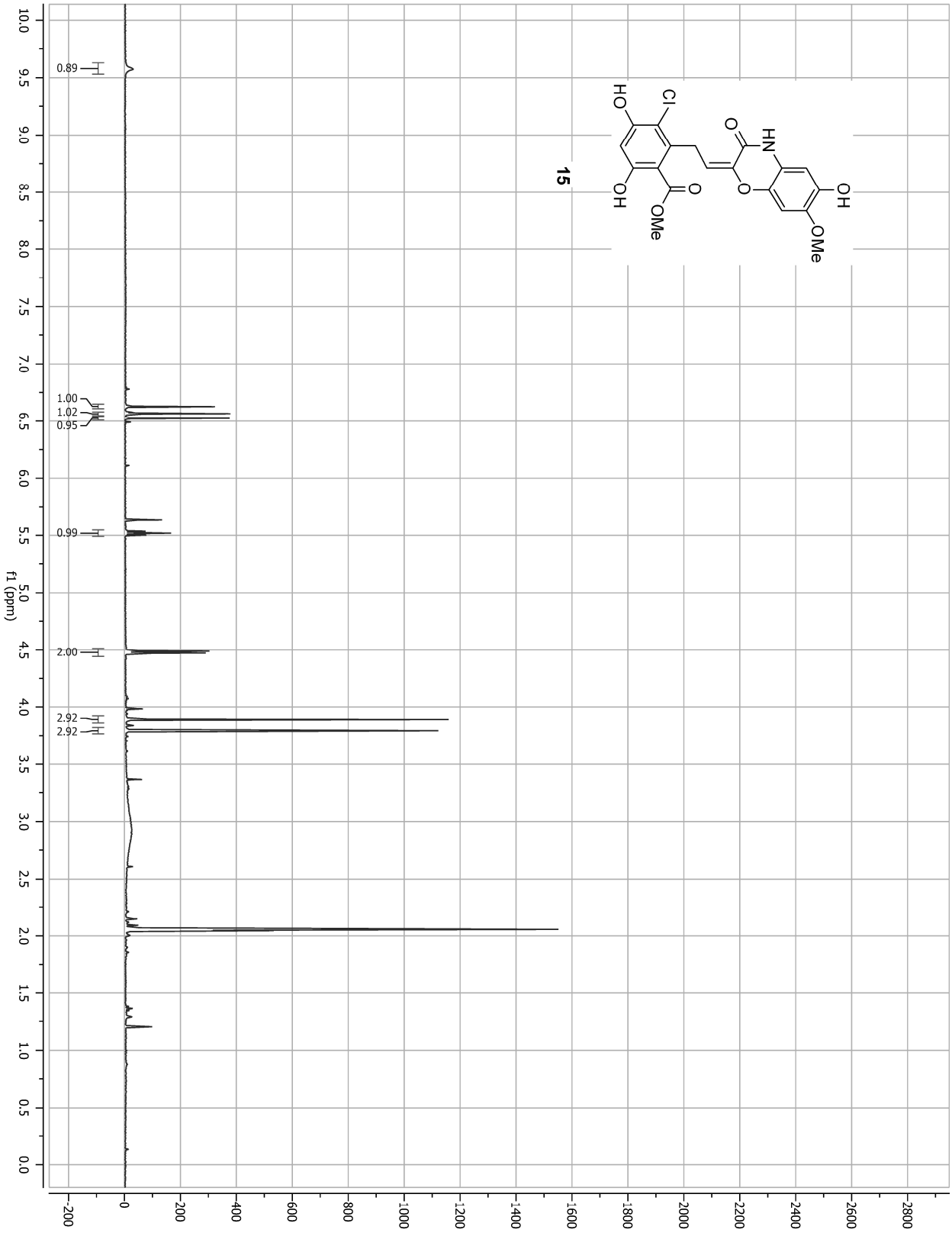
O
O
OBn
OMe
Cl
OH
O
OMe
OH
f1 (ppm)


15
HO
Cl
O
HN
OH
OMe
OH
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O
OMe
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1.02
0.95
0.99
2.00
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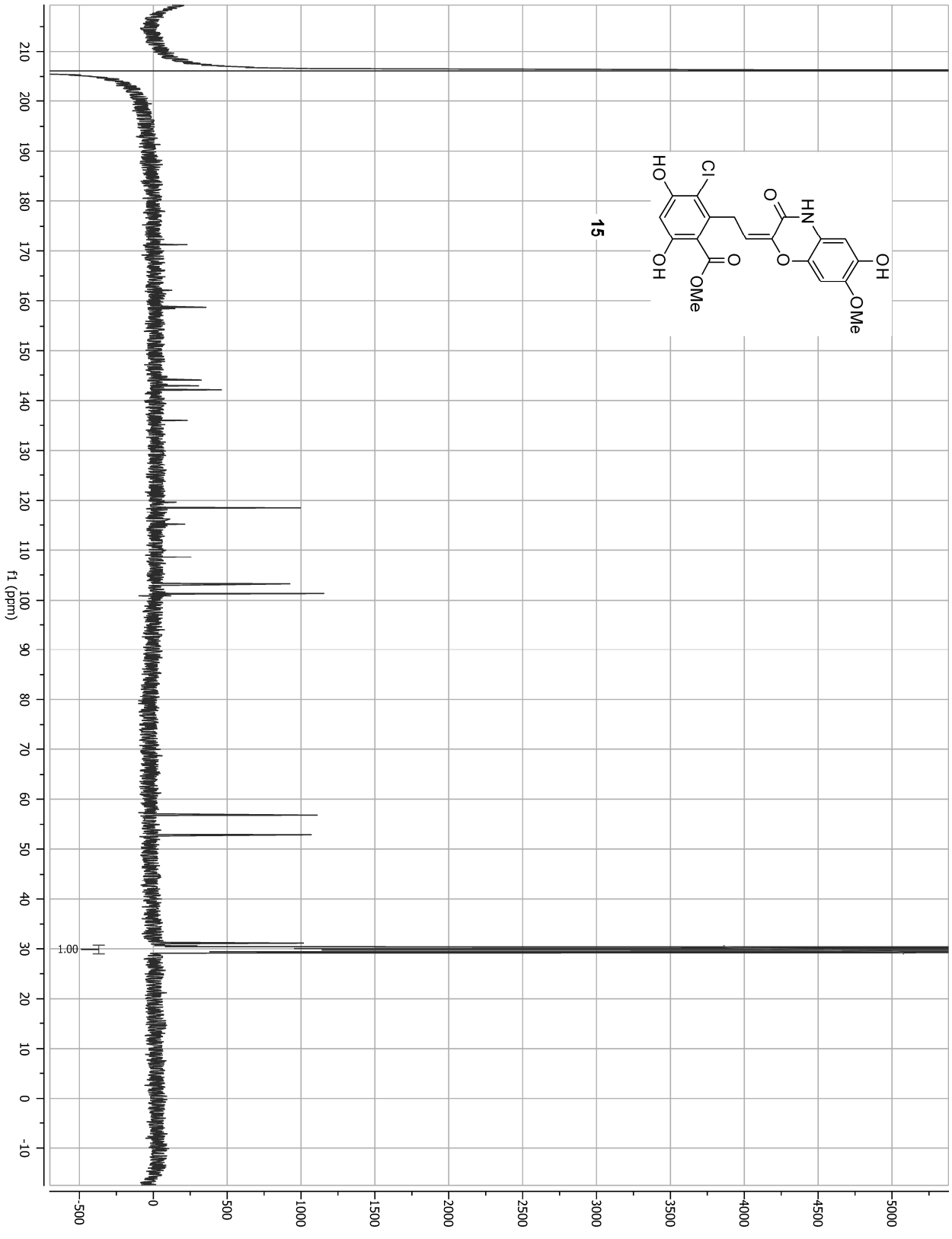
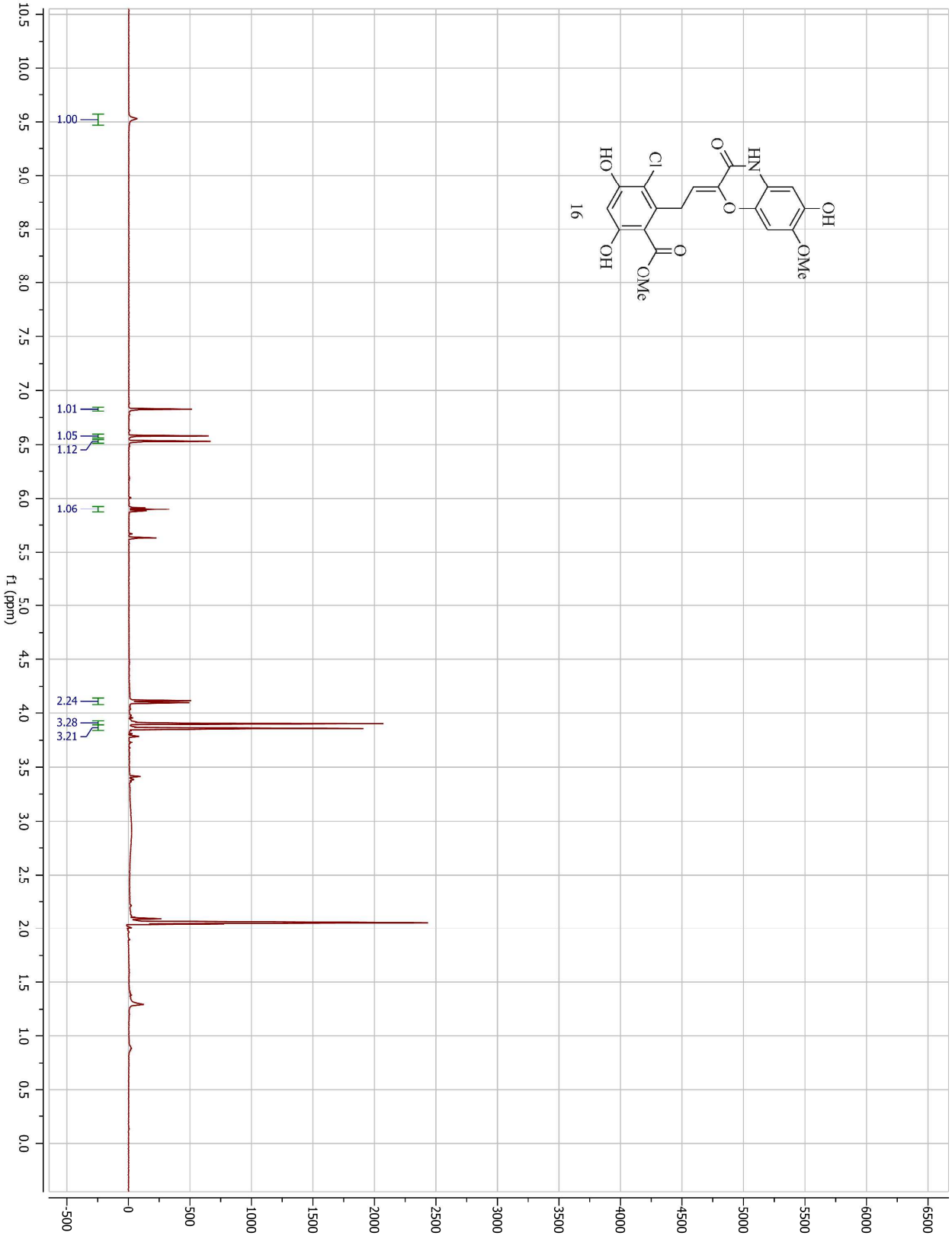
2.92
f1 (ppm)


15
HO
Cl
OH
O
OMe
O
HN
O
OH
OMe
1.00
f1 (ppm)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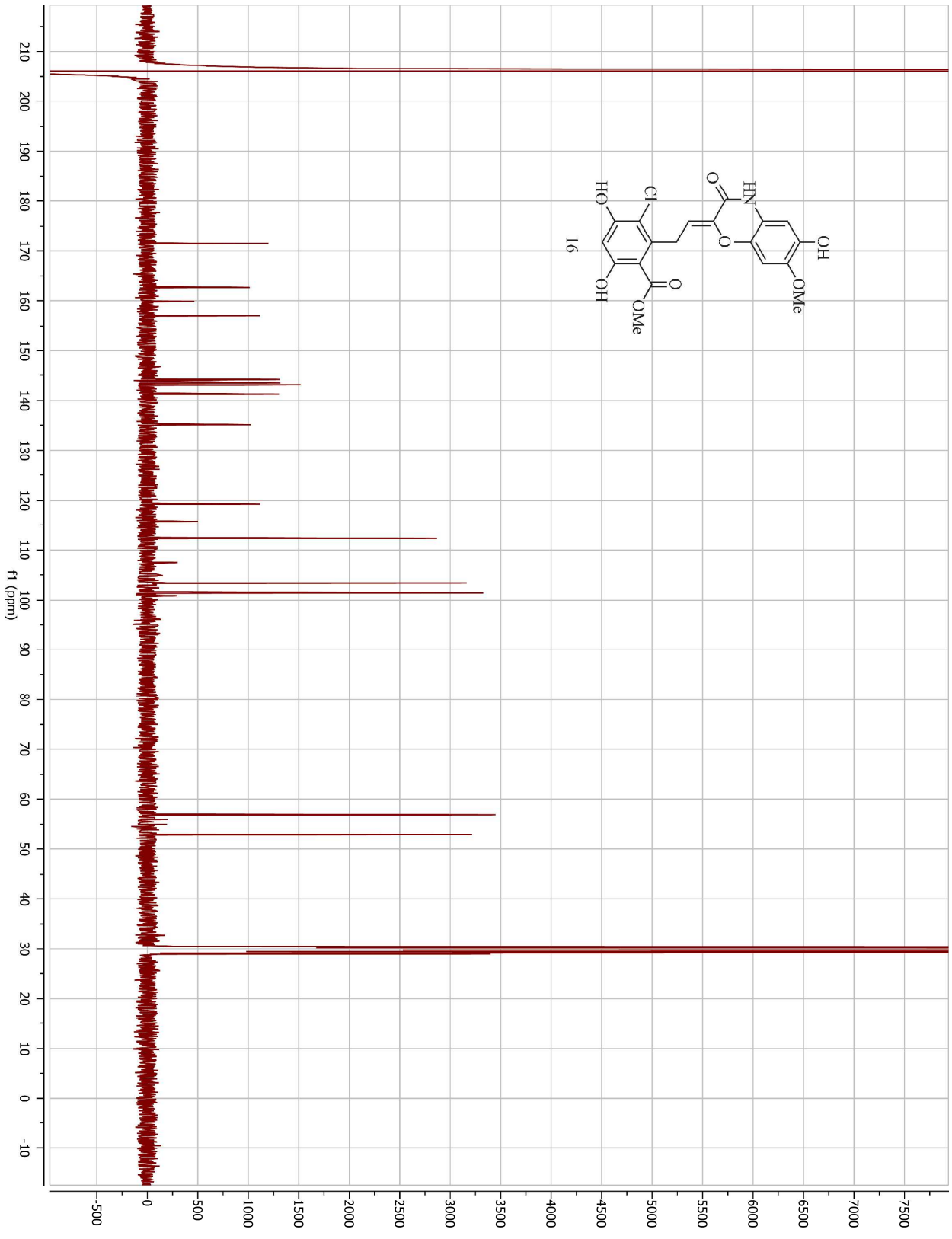
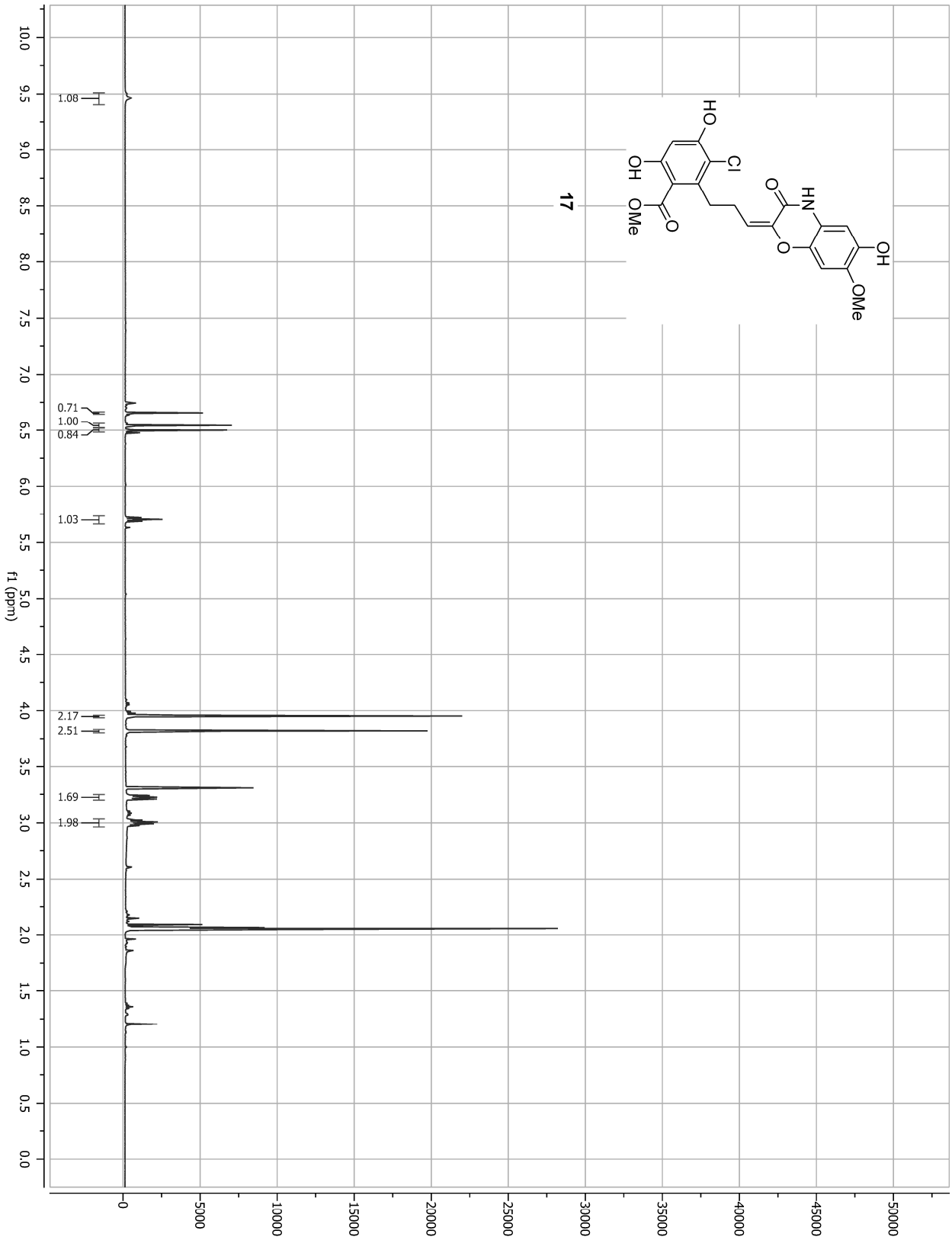
1.06
2.24
3.28
3.21
f1 (ppm)
16
HO
Cl
OH
O
OMe
O
HN
O
OH
OMe


16
HO
Cl
OH
O
OMe
HN
O
O
OH
OMe
f1 (ppm)


17
HO
Cl
OH
OMe
O
HN
O
O
OH
OMe
1.08
0.71
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0.84
1.03
2.17
2.51
1.69
1.98
f1 (ppm)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
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0
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10000
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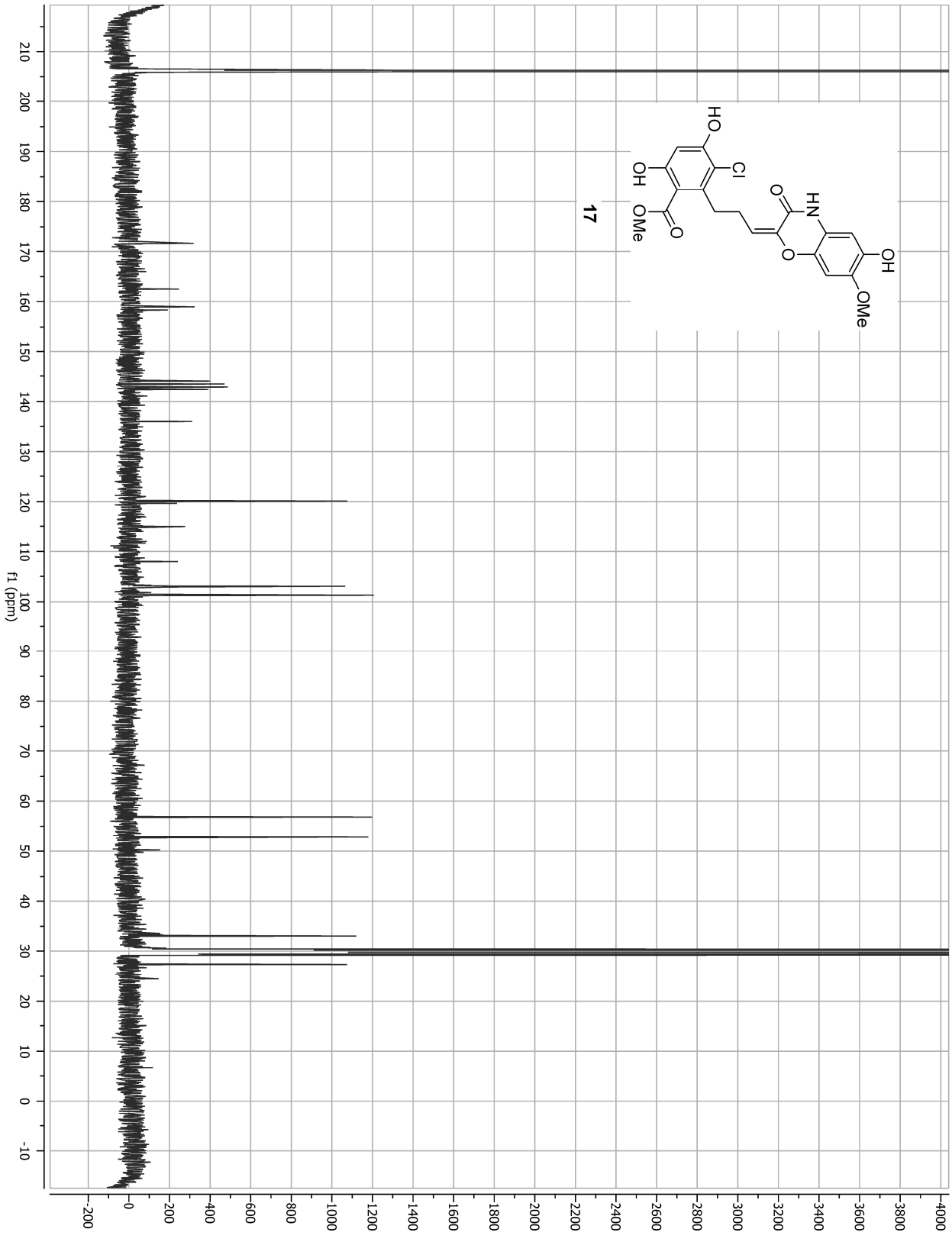
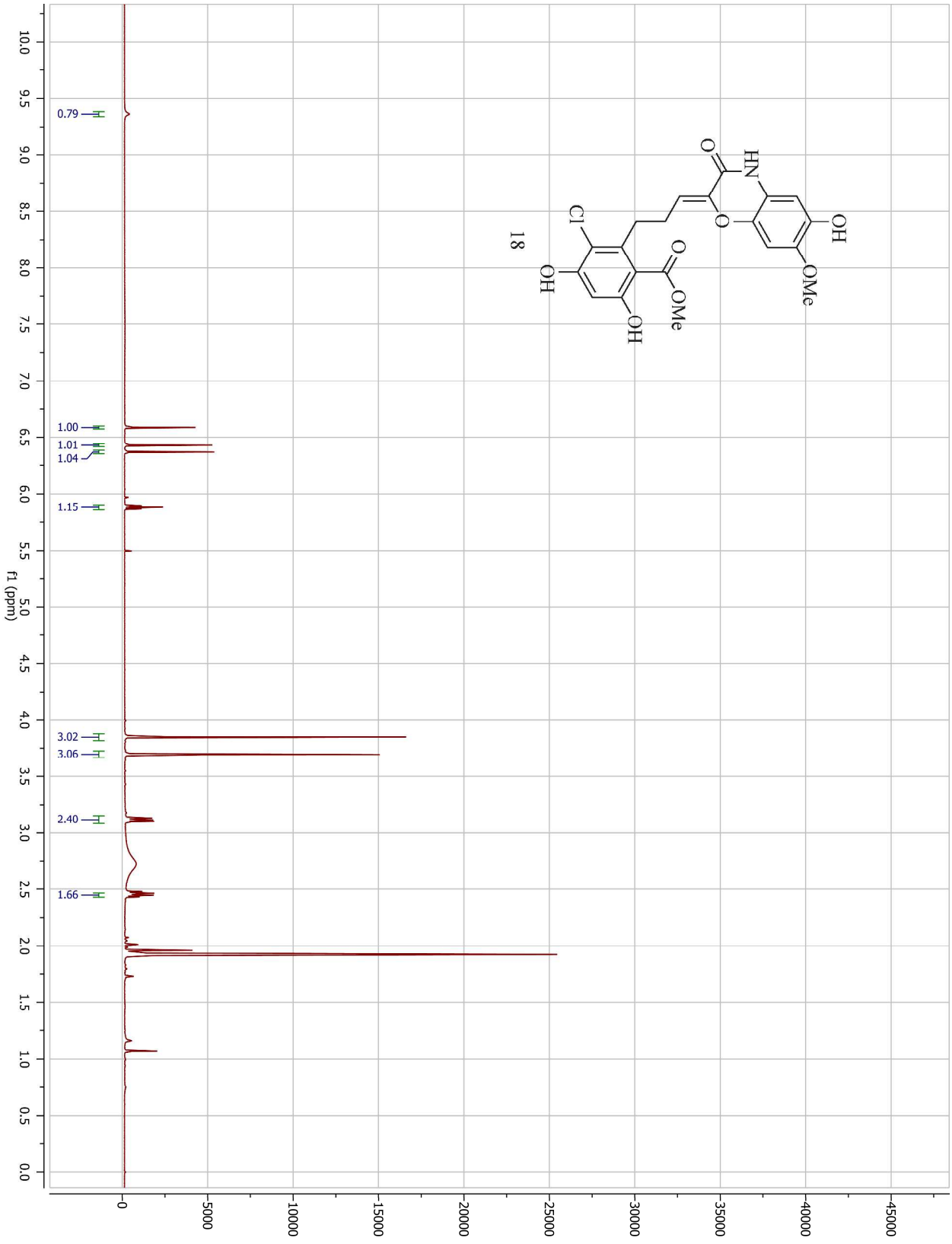
20000
25000
30000
35000
40000
45000
50000


HO
Cl
OH
OMe
O
HN
O
O
OH
OMe
17
f1 (ppm)


18
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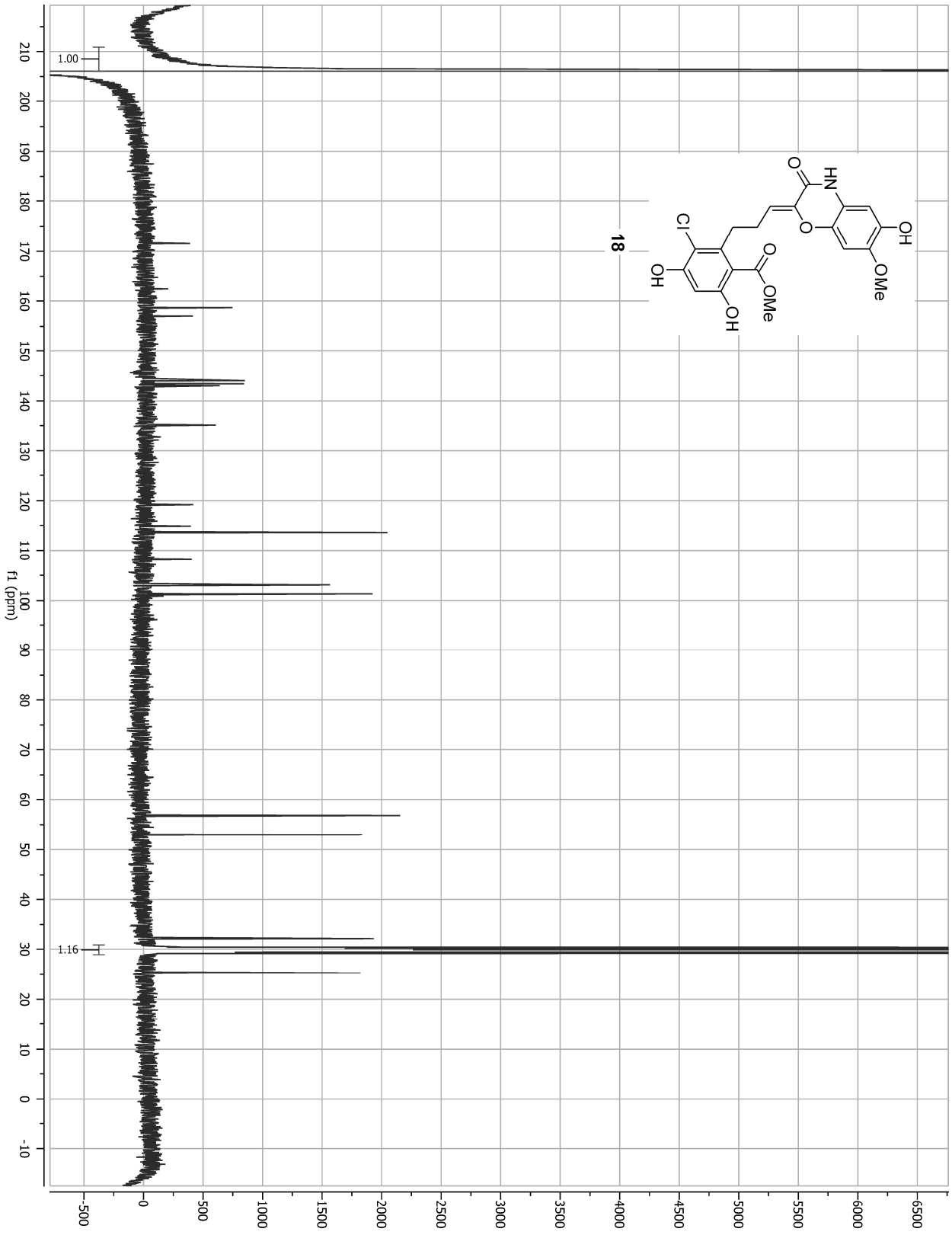
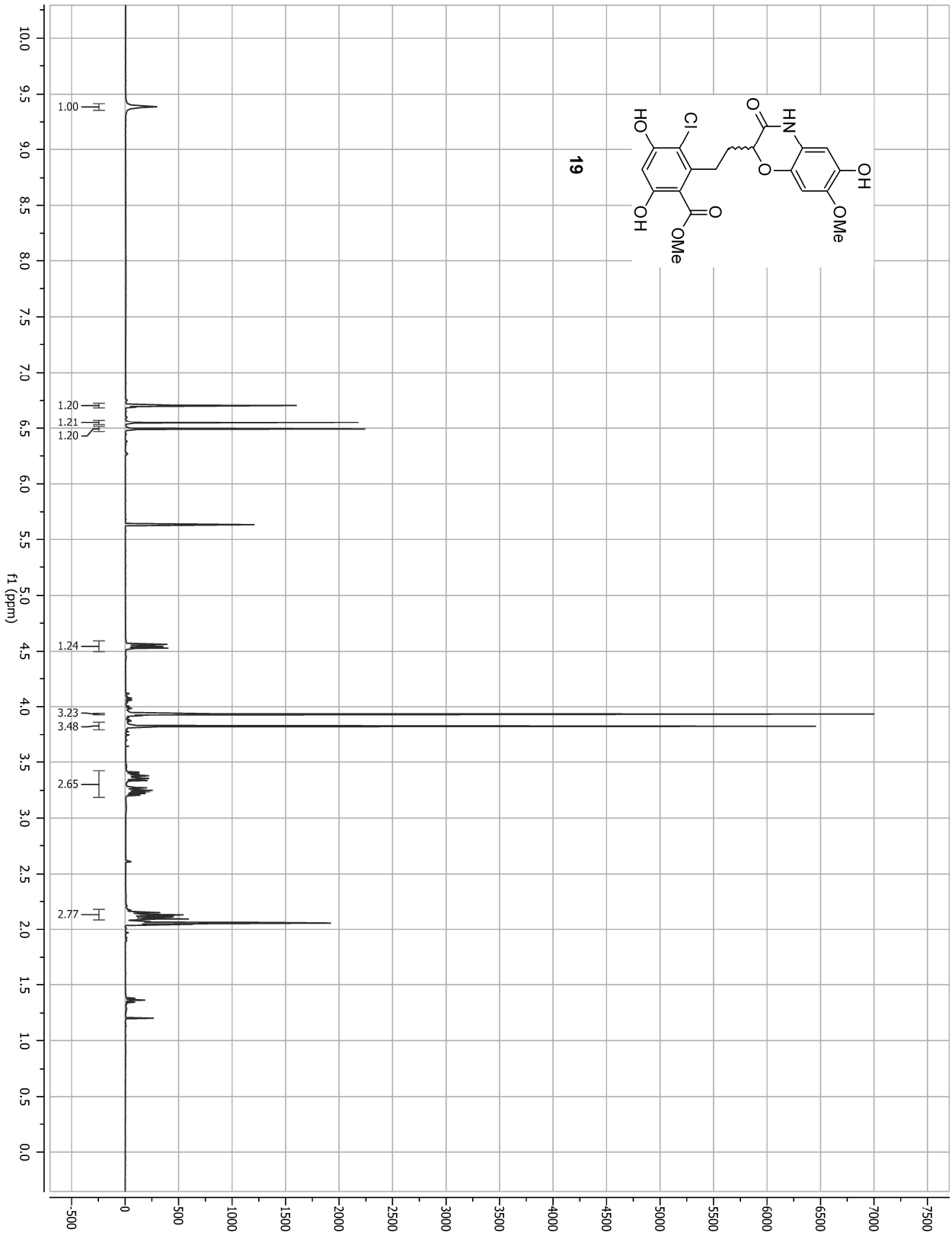
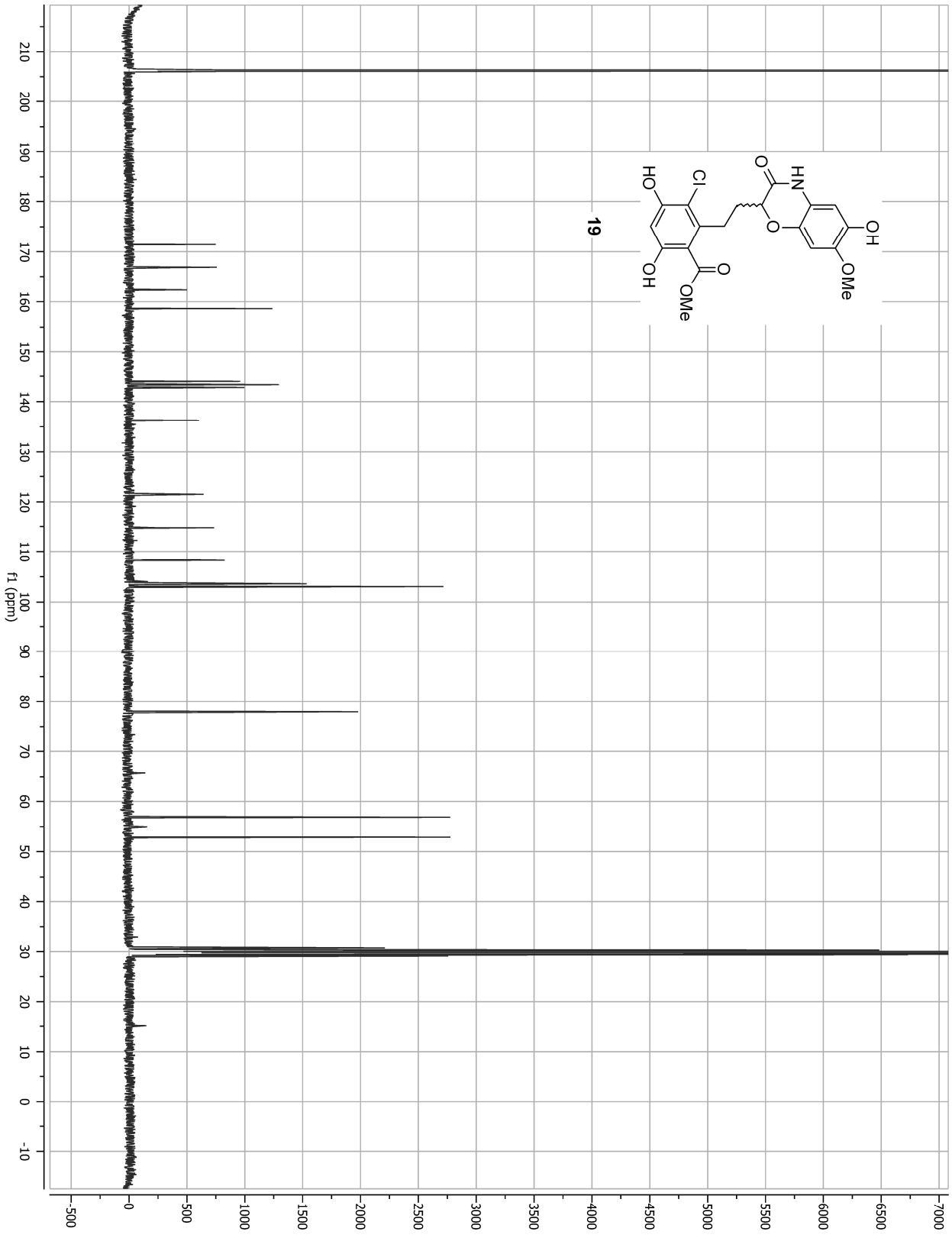
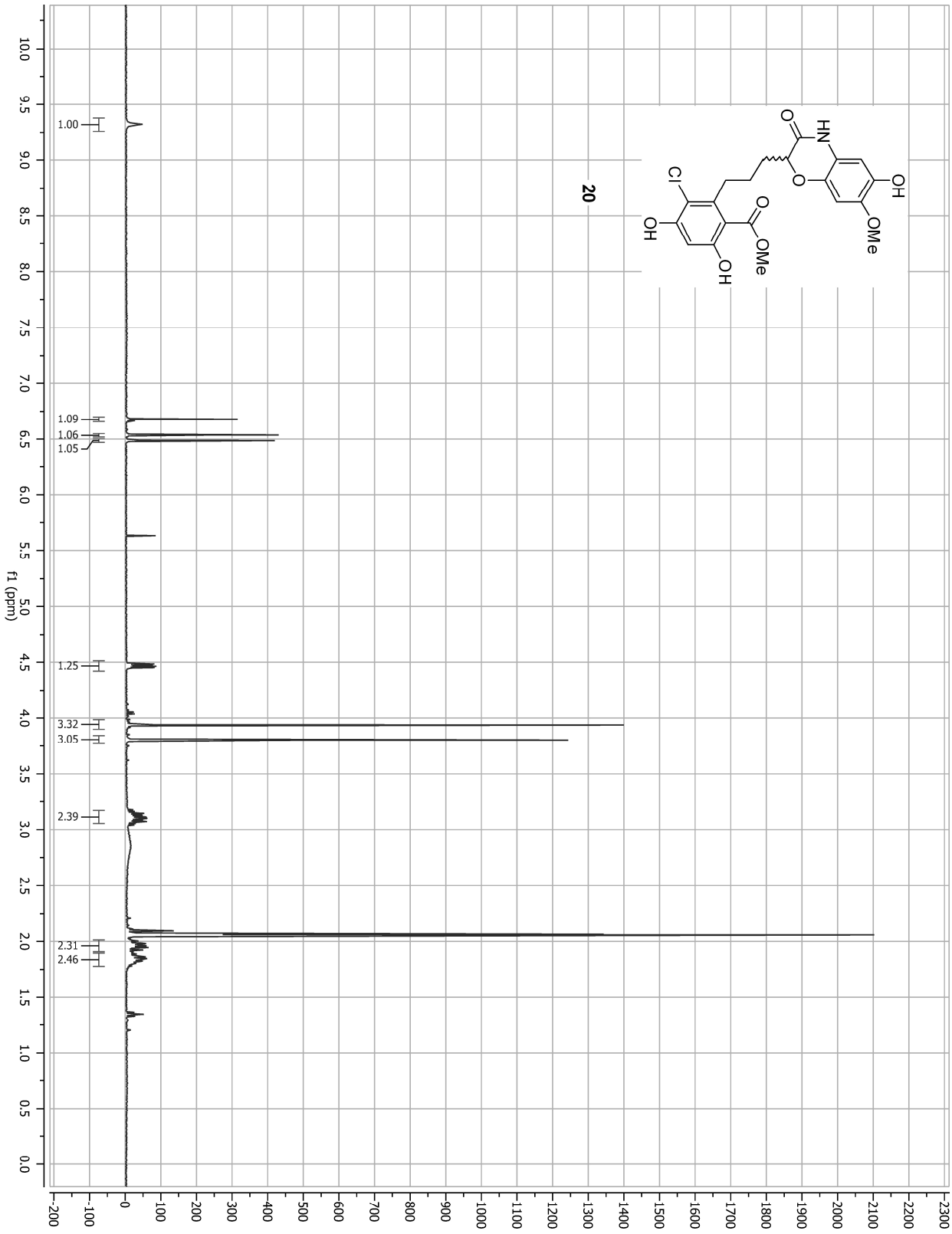
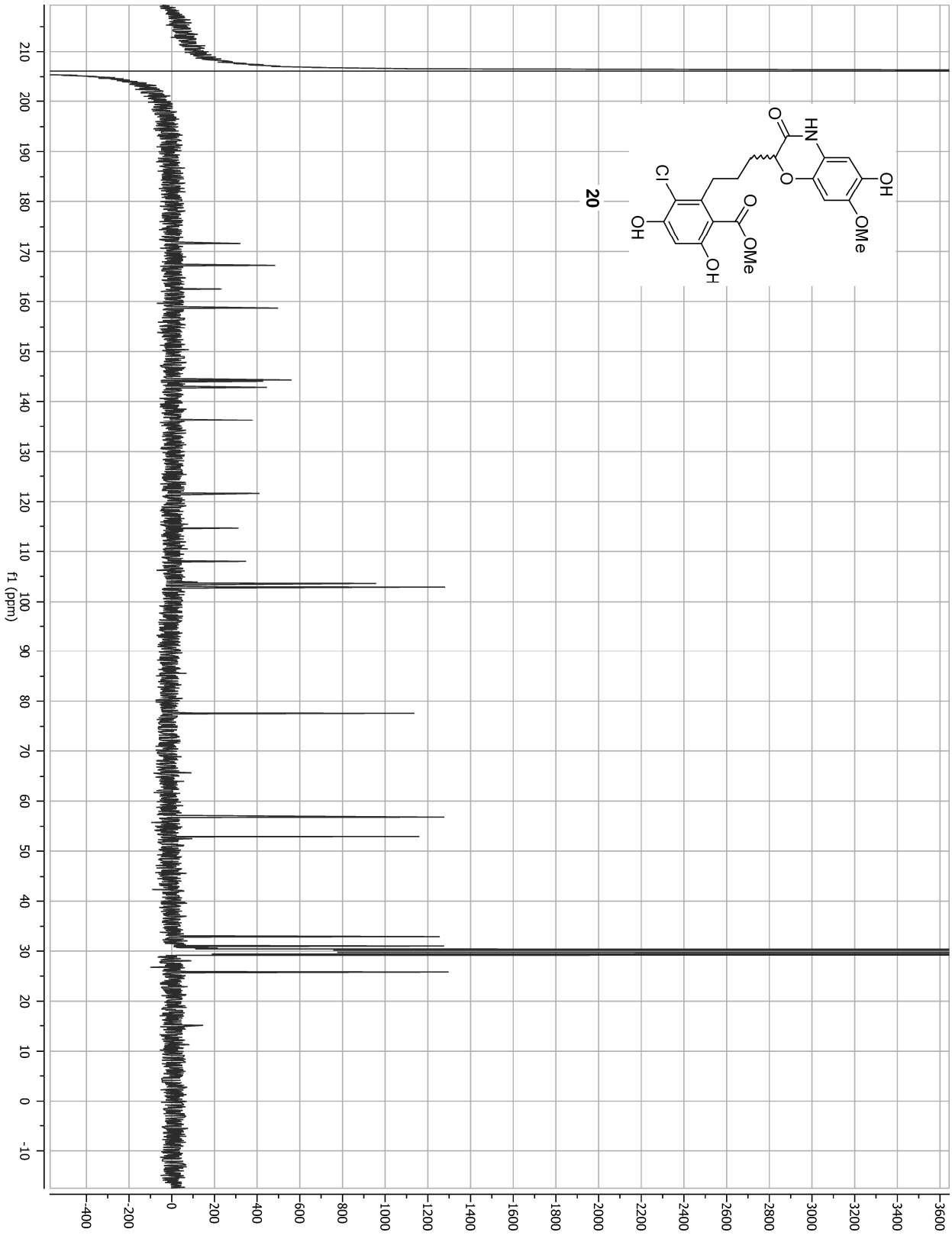
1.01
1.04
1.15
3.02
3.06
2.40
1.66
f1 (ppm)


1.00
1.16
f1 (ppm)
18
HN
O
O
Cl
OH
OMe
OH
OMe
OH


19
HO
Cl
O
HN
OH
OMe
OH
O
OMe
1.00
1.20
1.21
1.20
1.24
3.23
3.48
2.65
2.77
f1 (ppm)


19
HO
Cl
O
HN
OH
OMe
OH
O
OMe
f1 (ppm)


20
HN
O
O
OH
OMe
Cl
OH
O
OMe
OH
1.00
1.09
1.06
1.05
1.25
3.32
3.05
2.39
2.31
2.46
f1 (ppm)


20
HN
O
O
OH
OMe
Cl
OH
O
OMe
OH
f1 (ppm)