

Electronic Supporting Information (ESI)

Synthesis and cytotoxicity of (+/-)-7,9-dideoxy-pancratistatin analogues

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General Remarks

Solvents for moisture-sensitive reactions were distilled and dried according to standard procedures. Anhydrous Na₂SO₄ was used to dry organic solutions during workups. Reagents were purchased in the highest available commercial quality and used as supplied. Reactions were monitored by TLC with pre-coated silica gel 60 F254 aluminum plates (Merck KGaA, Darmstadt) using UV light as the visualizing agent and by dipping the plate into a solution of (NH₄)₆Mo₇O₂₄·4 H₂O (12.5 g) and Ce(SO₄)₂·4 H₂O (5 g) in 10% aqueous H₂SO₄ (500 mL), followed by heating. Flash column chromatography was performed with silica gel (0.035-0.070 mm, 60 Å) from Merck. Concentrations were carried out in a rotary evaporator. ¹H, ¹³C, DEPT and 2D NMR were recorded using either Bruker DPX-250, AMX-300 and WM-500 spectrometers, or Varian Mercury 300 and Inova 400 spectrometers, as indicated; chemical shifts are reported in ppm and coupling constants in Hz; multiplicities are given as follows: s (singlet), br s (broad singlet), br d (broad doublet), d (doublet), t (triplet), and m (multiplet). Mass spectra were recorded on Micromass Autospec, TRACE MS and HP-5988-A spectrometers using Electronic Impact (EI, 70 eV), Electrospray (ESI) or Chemical Ionization (CI). Melting points were determined on a Büchi apparatus (Dr. Tottoli, Flawil, Switzerland).

Methyl ((1S*,5R*,6R*,7R*,8S*)-6-(furan-2-yl)-8-((methoxycarbonyl)oxy)-3,3-dimethyl-9-oxo-2,4-dioxabicyclo[3.3.1]nonan-7-yl)carbamate (+/-)-19. A suspension of **15** (490 mg, 1.64 mmol), 10% Pd/C (590 mg) and ammonium formate (494 mg, 7.60 mmol) in dry MeOH (2.8 mL) was stirred at rt. After completion of the reduction (as monitored by TLC), the catalyst was filtered off and washed with methanol and EtOAc and the combined washings and the filtrate were evaporated in vacuo. The residue was dissolved in dry CH₂Cl₂ (13 mL) under argon and treated with methyl chloroformate (215 µL, 2.75 mmol) and DMAP (400 mg, 2.75 mmol). After stirring for 2 h at rt, the reaction mixture was treated with a saturated aqueous solution of NaHCO₃ (13 mL) and extracted with CH₂Cl₂ (3 x 10 mL). Chromatography (35% EtOAc/hexane) gave **19** (127 mg, 15%) as white foam: *R*_f = 0.38 (30% EtOAc/hexane); ¹H NMR (CDCl₃, 400 MHz) δ: δ 7.35 (d, *J* = 1.8 Hz, 1H), 6.43 (d, *J* = 2.9 Hz, 1H), 6.34 (dd, *J* = 2.9, 1.8 Hz, 1H), 5.33-5.14 (m, 2H), 4.78 (br d, *J* = 10.0 Hz, 1H), 4.50-4.34 (m, 2H), 3.82 (s, 3H),

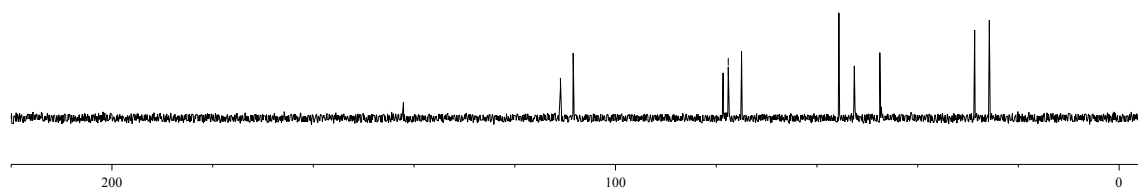
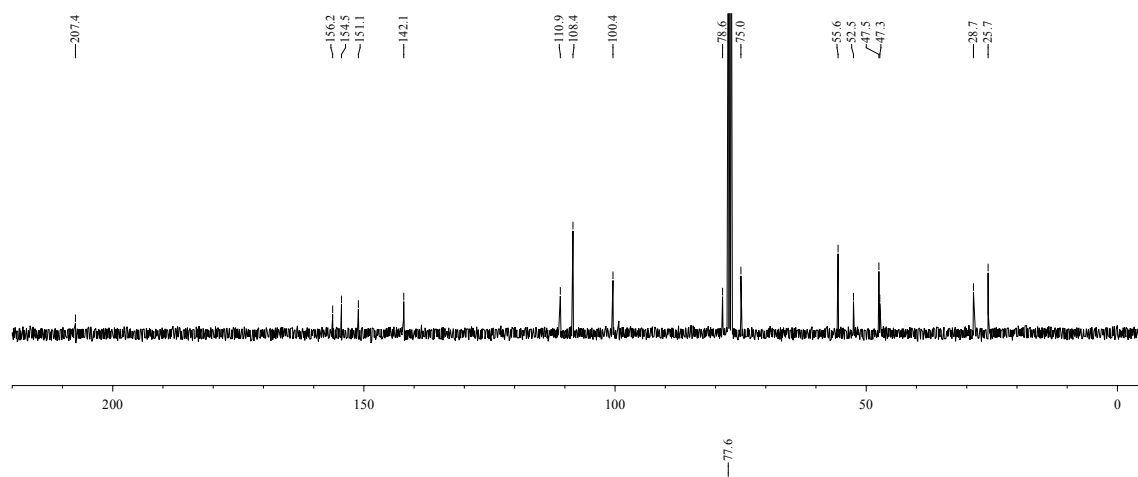
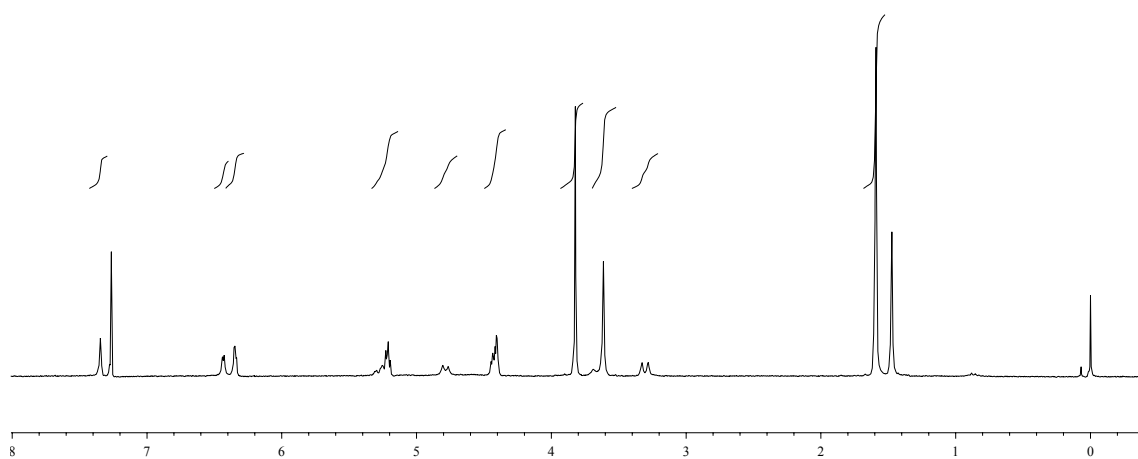
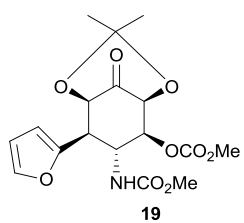
3.61 (s, 3H), 3.30 (br d, $J = 11.7$ Hz, 1H), 1.59 (s, 6H); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 207.4, 156.2, 154.5, 151.1, 142.1, 110.9, 108.4, 100.4, 78.6, 77.6, 75.0, 55.6, 52.5, 47.5, 47.3, 28.7, 25.7.

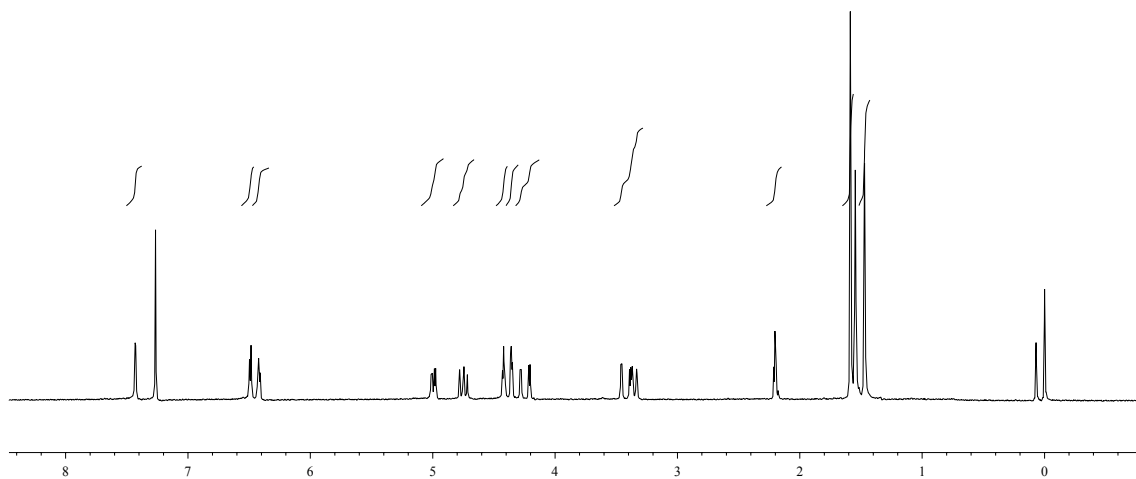
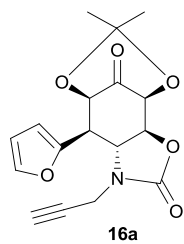
(3aS*,4S*,8R*,9R*,9aR*)-9-(Furan-2-yl)-6,6-dimethyl-1-(prop-2-yn-1-yl)tetrahydro-1H-4,8-methano[1,3]dioxocino[6,5-d]oxazole-2,10(8H)-dione (+/-)-16a. NaH (7.8 mg, 0.20 mmol) was added to a solution of **19** (50 mg, 0.14 mmol) in dry THF (1.3 mL) under argon. After stirring for 15 min at rt, propargyl bromide (17 μL , 0.16 mmol) was added, the mixture stirred for 4.5 h at rt and then heated for 3.5 h at 60 $^\circ\text{C}$. After removing the volatiles, chromatography (25% EtOAc/hexane) gave **16a** (27 mg, 63%) as a yellow solid: $R_f = 0.54$ (30% EtOAc/hexane); ^1H NMR (CDCl_3 , 300 MHz) δ : δ 7.43 (d, $J = 1.8$ Hz, 1H), 6.49 (d, $J = 3.3$ Hz, 1H), 6.42 (dd, $J = 3.3, 1.8$ Hz, 1H), 4.99 (dd, $J = 6.8, 2.5$ Hz, 1H), 4.75 (dd, $J = 8.8, 6.8$ Hz, 1H), 4.42 (dd, $J = 2.5, 2.5$ Hz, 1H), 4.35 (dd, $J = 2.5, 1.4$ Hz, 1H), 4.25 (dd, $J = 17.8, 2.6$ Hz, 1H), 3.42 (dd, $J = 17.8, 2.6$ Hz, 1H), 3.37 (br d, $J = 8.8$ Hz, 1H), 2.22 (t, $J = 2.6$ Hz, 1H), 1.59 (s, 3H), 1.47 (s, 3H); ^{13}C NMR (CDCl_3 , 75 MHz) δ : 207.8, 155.3, 151.1, 142.7, 111.2, 108.6, 100.8, 81.0, 77.6, 73.4, 72.9, 54.3, 50.3, 49.5, 33.2, 28.9, 25.1.

N-((1R*,5S*,6S*,7R*,8R*)-6-((tert-Butyldimethylsilyl)oxy)-8-(furan-2-yl)-3,3-dimethyl-9-oxo-2,4-dioxabicyclo[3.3.1]nonan-7-yl)-4-methylbenzenesulfonamide (+/-)-20. Imidazole (1.15 g, 16.82 mmol) and TBSCl (1.6 mL, 8.41 mmol) were added to a solution of **15** (500 mg, 1.68 mmol) in dry CH_2Cl_2 (3.5 mL) under argon. After stirring for 1.5 h at rt, the mixture was diluted with a saturated aqueous solution of NH_4Cl (3.5 mL) and extracted with CH_2Cl_2 (3 x 2mL). Chromatography (5% EtOAc/hexane) afforded the corresponding silyl ether of **15** (479 mg, 69%). The product obtained from two reactions (1 g, 2.43 mmol) was dissolved in MeOH/THF (1:1, 12 mL), treated with Raney®-Nickel (≈ 1.2 mL) and stirred for 24 h at rt under $\text{H}_2(\text{g})$. The catalyst was filtered off and washed with MeOH (80 mL) and EtOAc (80 mL). The combined washings and the filtrate were evaporated in vacuo and the residue dissolved in dry CH_2Cl_2 (8 mL), treated with Et_3N (322 μL , 2.19 mmol), TsCl (386 mg, 2.02 mmol) and DMAP (258 mg, 1.77 mmol) and stirred for 4 h at rt. Removal of the volatiles and chromatography (30% EtOAc/hexane) gave **20** (515 mg, 40%) as a white solid: $R_f = 0.37$ (30% EtOAc/hexane); ^1H NMR (CDCl_3 , 400 MHz) δ : 7.43-7.38 (m, 2H), 7.11-7.01 (m, 2H), 6.97 (dd, $J = 1.8, 0.8$ Hz, 1H), 6.04 (dd, $J = 3.3, 1.8$ Hz, 1H),

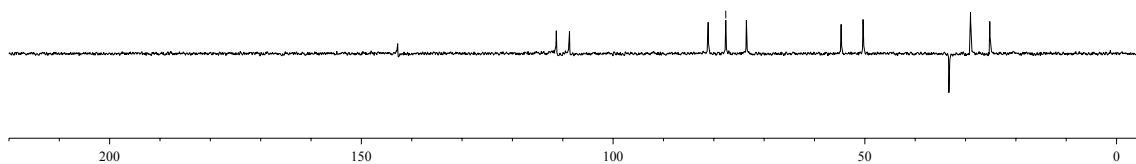
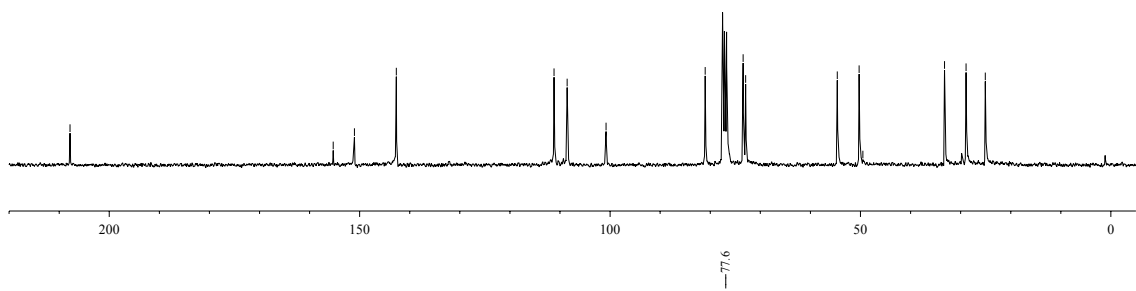
5.87 (d, $J = 3.3$ Hz, 1H), 4.76 (d, $J = 6.2$ Hz, 1H), 4.24 (dd, $J = 3.8, 2.6$ Hz, 1H), 4.17 (dd, $J = 3.8, 2.0$ Hz, 1H), 4.14 (dd, $J = 9.2, 2.6$ Hz, 1H), 3.92 (ddd, $J = 12.3, 9.2, 6.2$ Hz, 1H), 3.81-3.76 (m, 1H), 3.09 (dd, $J = 12.3, 2.0$ Hz, 1H), 2.35 (s, 3H), 1.65 (s, 3H), 1.63 (s, 3H), 0.97 (s, 9H), 0.23 (s, 3H), 0.18 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 152.6, 143.3, 141.2, 136.1, 129.5, 129.3, 127.1, 126.6, 110.2, 109.4, 107.1, 74.7, 72.6, 71.6, 68.2, 57.1, 44.9, 26.0, 25.8 (2C), 25.7, 23.9, 21.5, 18.0, -4.4, -5.0; HRMS [CI, $(\text{M}+3\text{H})^+$] m/z : calcd. for $(\text{C}_{26}\text{H}_{40}\text{NO}_7\text{SSi})$: 538.2295, found: 538.2295.

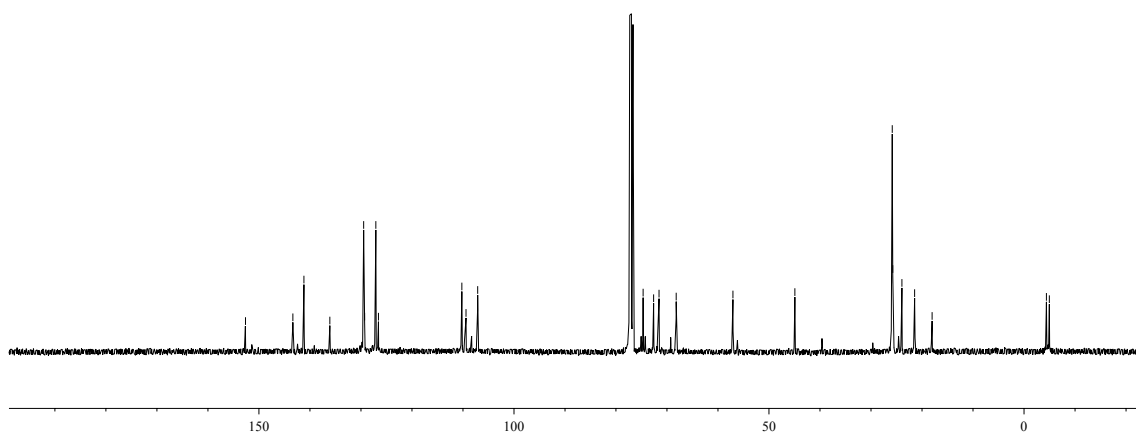
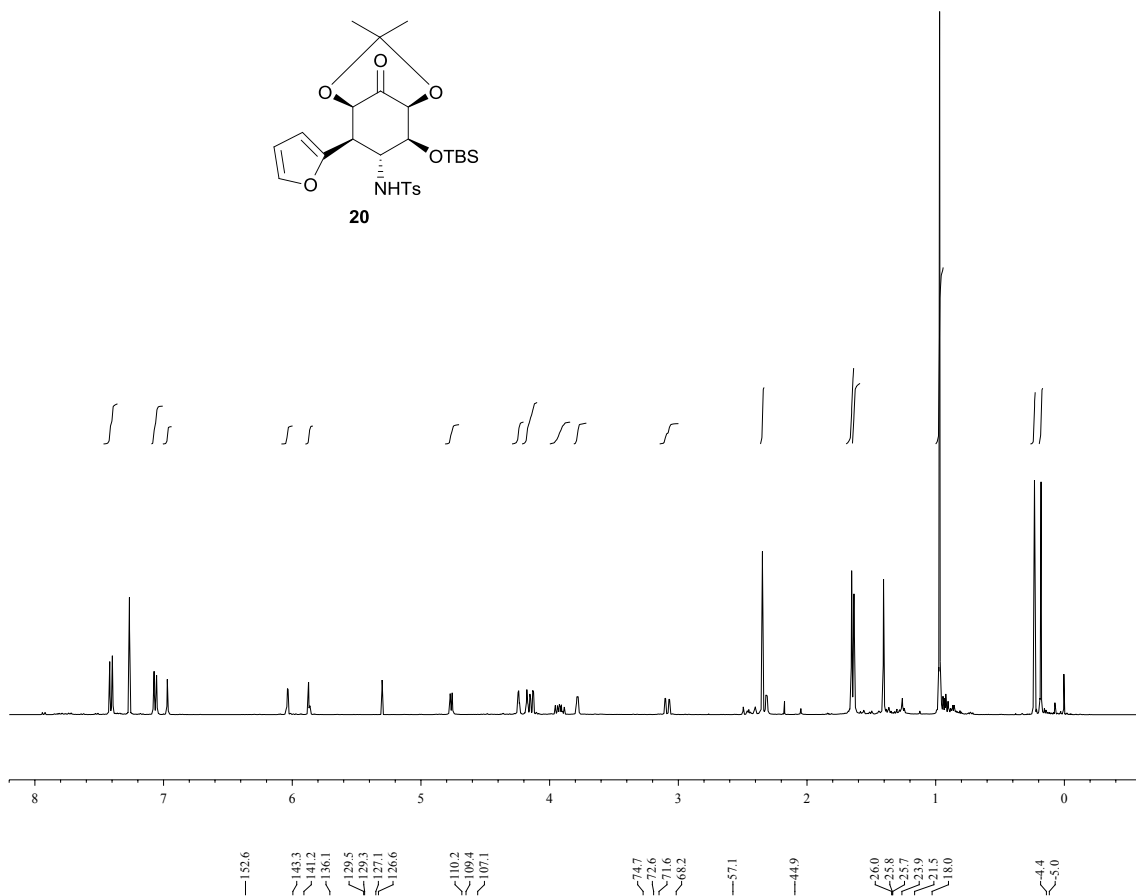
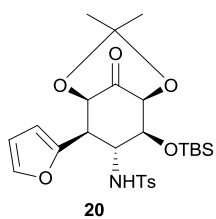
***N*-((1*R**,5*S**,6*S**,7*R**,8*R**)-6-((*tert*-Butyldimethylsilyl)oxy)-8-(furan-2-yl)-3,3-dimethyl-9-oxo-2,4-dioxabicyclo[3.3.1]nonan-7-yl)-4-methyl-*N*-(prop-2-yn-1-yl)benzenesulfonamide (+/-)-16b.** NaH (50 mg, 2.12 mmol) was added to a solution of **20** (760 mg, 1.42 mmol) in dry THF (7 mL) under argon. After stirring for 15 min at rt, propargyl bromide (200 μL , 2.84 mmol) was added and the mixture stirred for 4.5 h at rt. The solution was diluted with a saturated aqueous solution of NH_4Cl (7 mL) and extracted with CH_2Cl_2 (3 x 5 mL). Chromatography (25% EtOAc/hexane) afforded **16b** (376 mg, 46%) as a white solid: $R_f = 0.56$ (30% EtOAc/hexane); ^1H NMR (CDCl_3 , 400 MHz) δ : 7.46 (d, $J = 8.2$ Hz, 2H), 7.17-7.05 (m, 3H), 6.30-6.21 (m, 1H), 6.19 (s, 1H), 4.58 (dd, $J = 9.3, 2.4$ Hz, 1H), 4.36-4.19 (m, 3H), 4.14-4.04 (m, 1H), 3.88 (d, $J = 3.7$ Hz, 1H), 3.80 (br d, $J = 12.2$ Hz, 1H), 2.36 (s, 1H), 1.69 (s, 3H), 1.51 (s, 3H), 0.95 (s, 9H), 0.22 (s, 3H), 0.20 (s, 3H); ^{13}C NMR (CDCl_3 , 100 MHz) δ : 152.7, 143.0, 140.5, 137.8, 129.2 (2C), 127.2, 110.2, 107.8, 97.9, 78.44, 76.5, 75.0, 75.0, 74.0, 66.0, 64.1, 43.1, 42.1, 31.8 (2C), 28.9, 25.8 (2C), 21.4, 18.2, 14.2, -4.2, -4.6; LRMS (CI) m/z (%): 574.2 [($\text{M}+\text{H}$) $^+$, 25]; HRMS [CI, ($\text{M}+\text{H}$) $^+$] m/z : calcd. for $(\text{C}_{29}\text{H}_{40}\text{NO}_7\text{SSi})$: 574.2295, found: 574.2294.

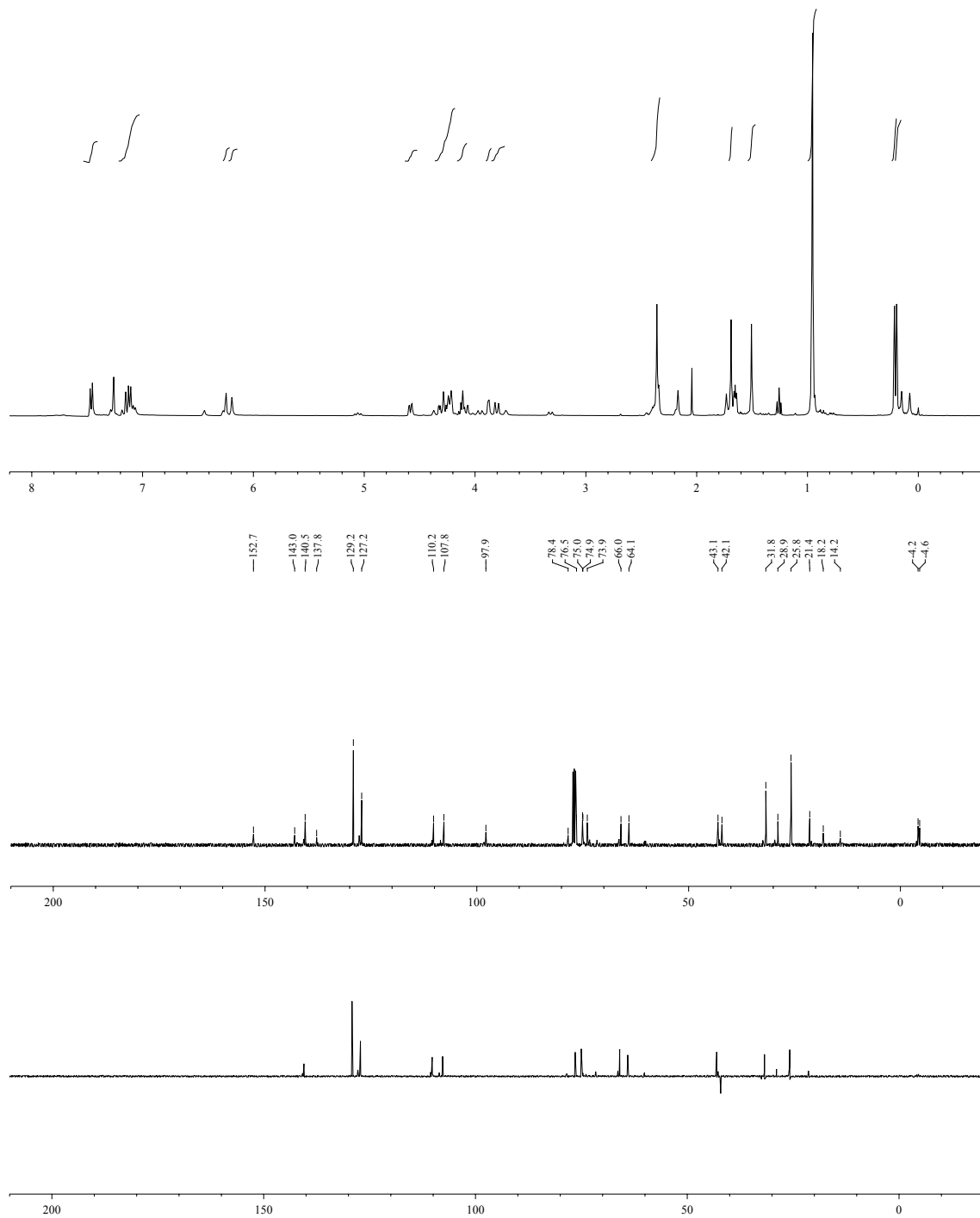
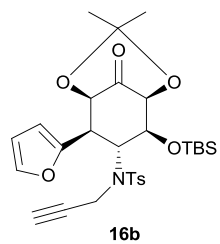


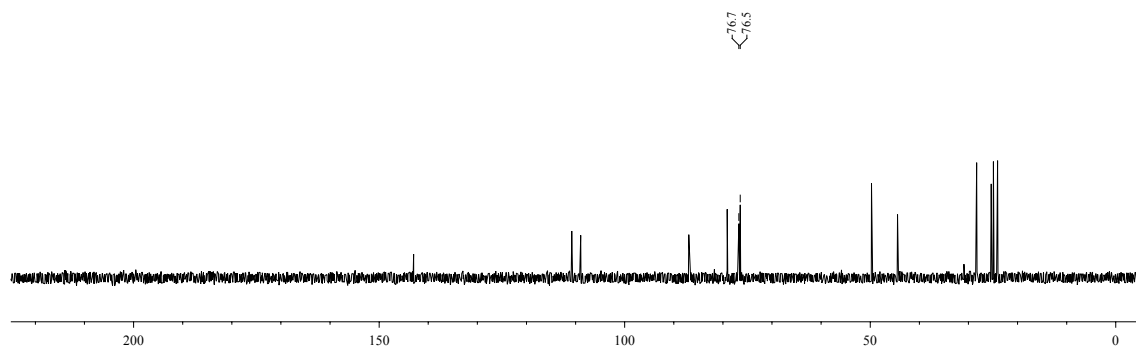
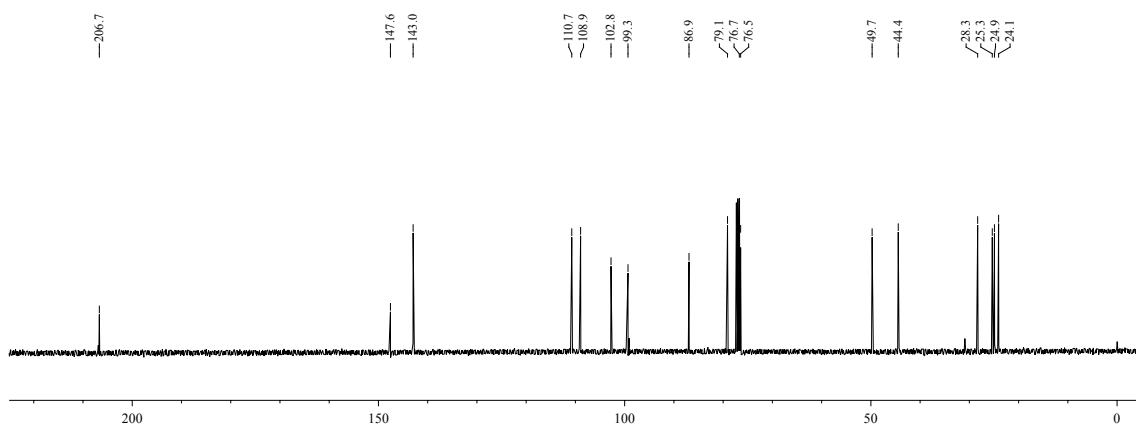
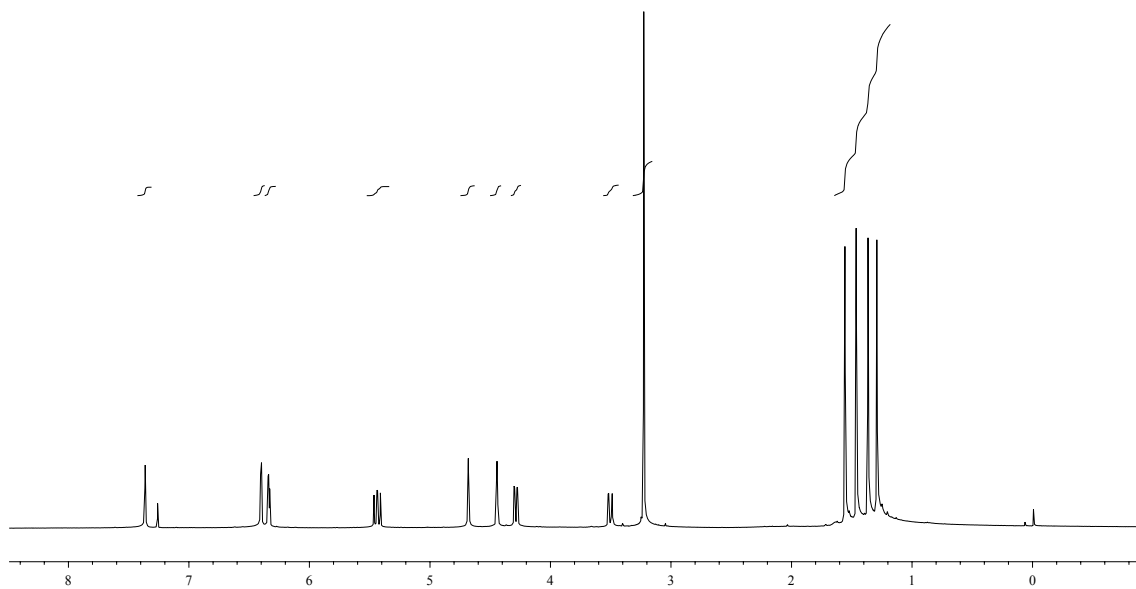
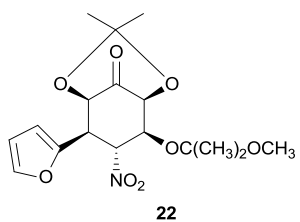


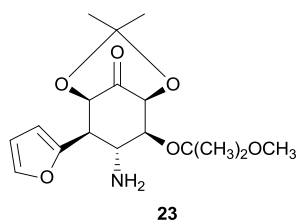
—207.8
—155.3
—151.0
—142.7
—111.2
—108.6
—100.8
—81.0
73.4
72.9
—54.6
—50.3
—49.5
—33.2
—28.9
—25.1



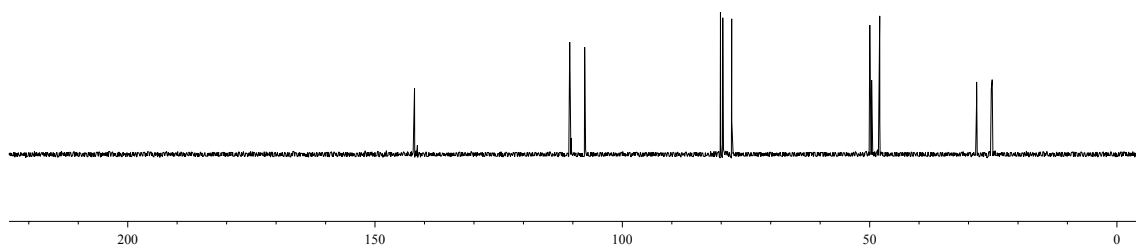
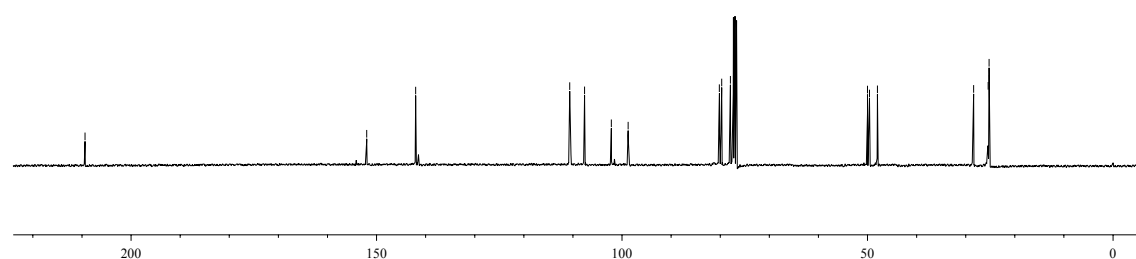
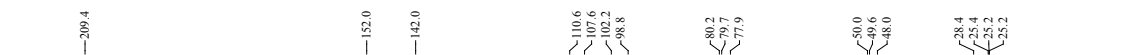
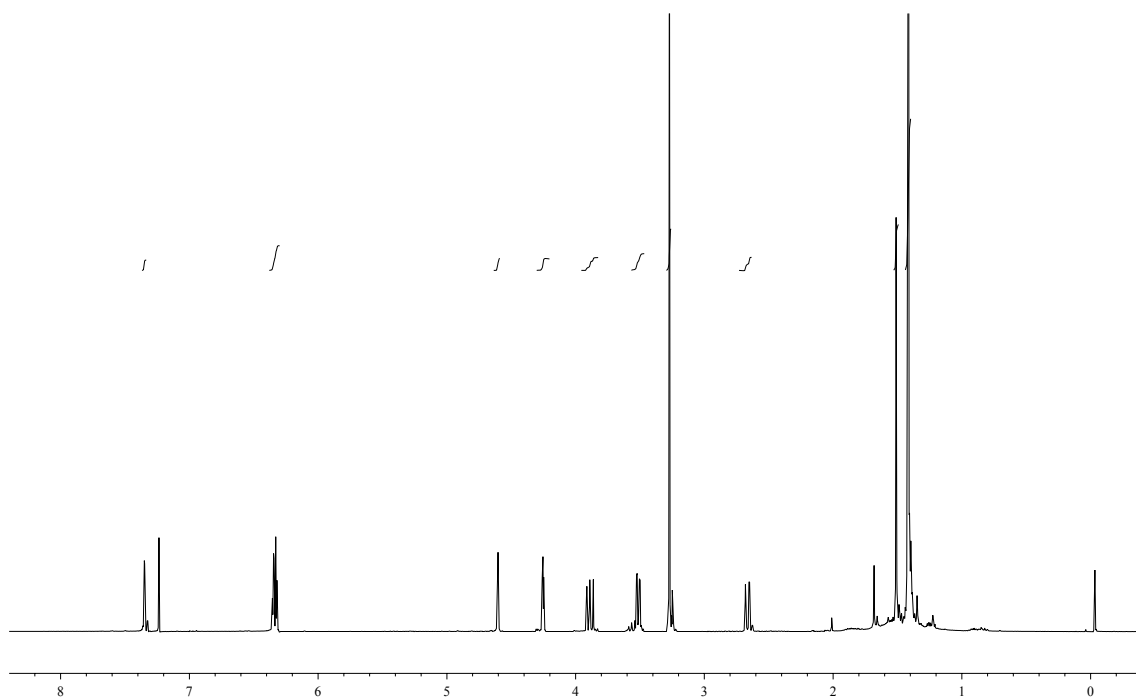


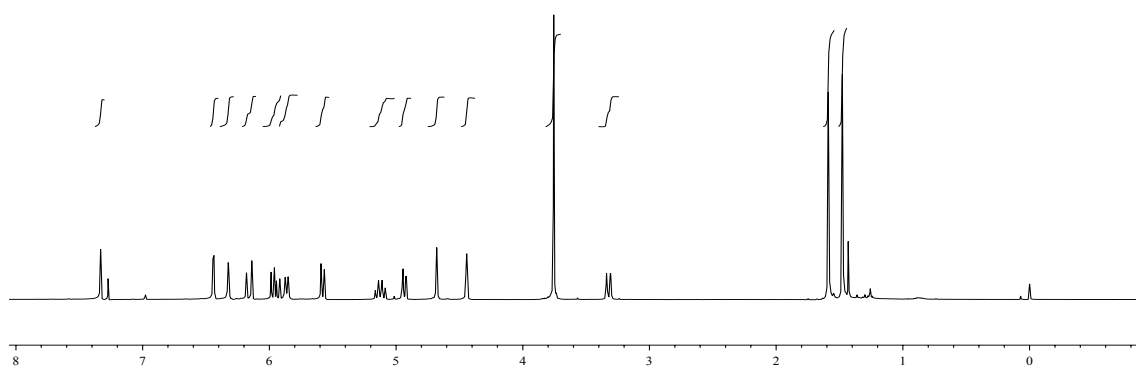
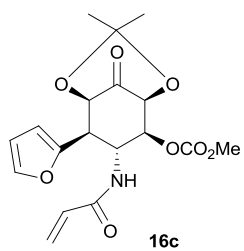




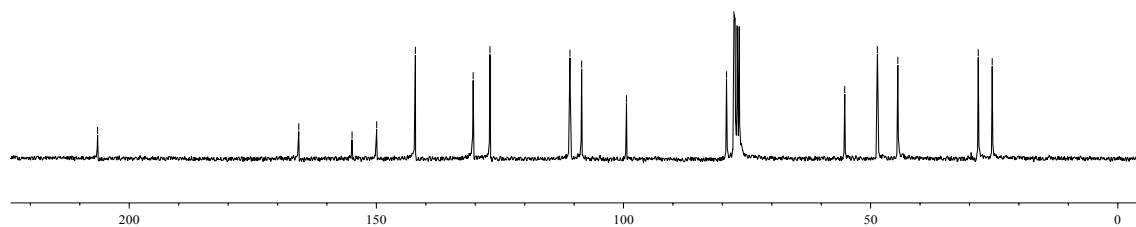


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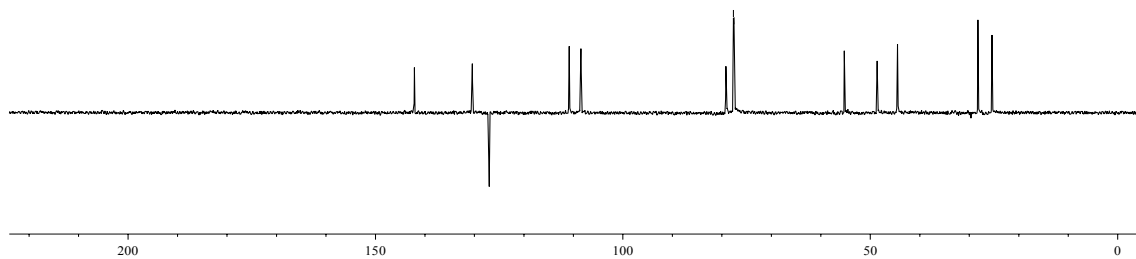


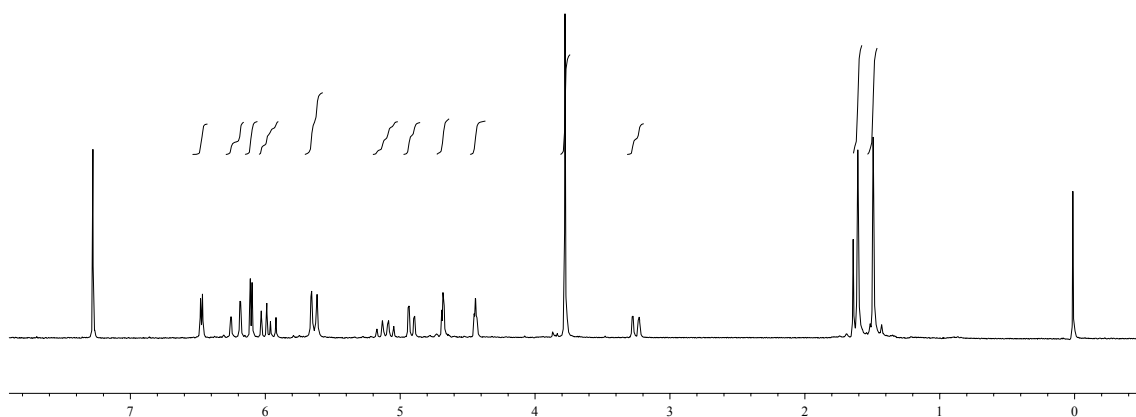
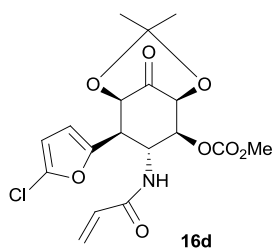


Chemical shift values (ppm): 206.4, 165.7, 154.9, 150.0, 142.1, 130.4, 127.0, 110.8, 108.5, 99.4, 79.1, 77.7, 77.5, 55.3, 48.6, 44.5, 28.2, 25.4.

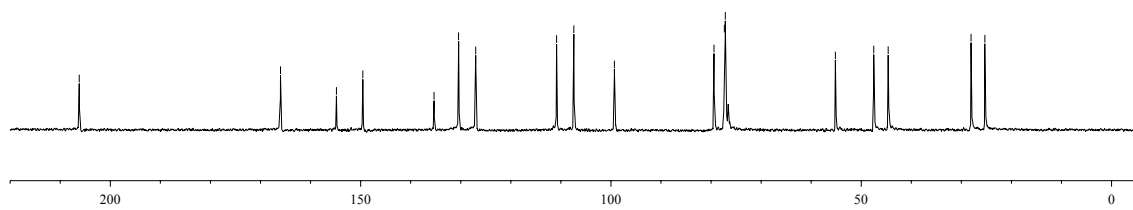


Chemical shift values (ppm): 77.7, 77.5.

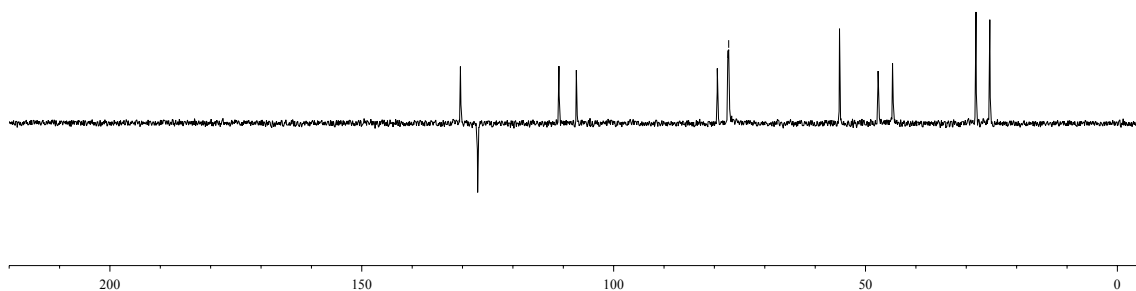


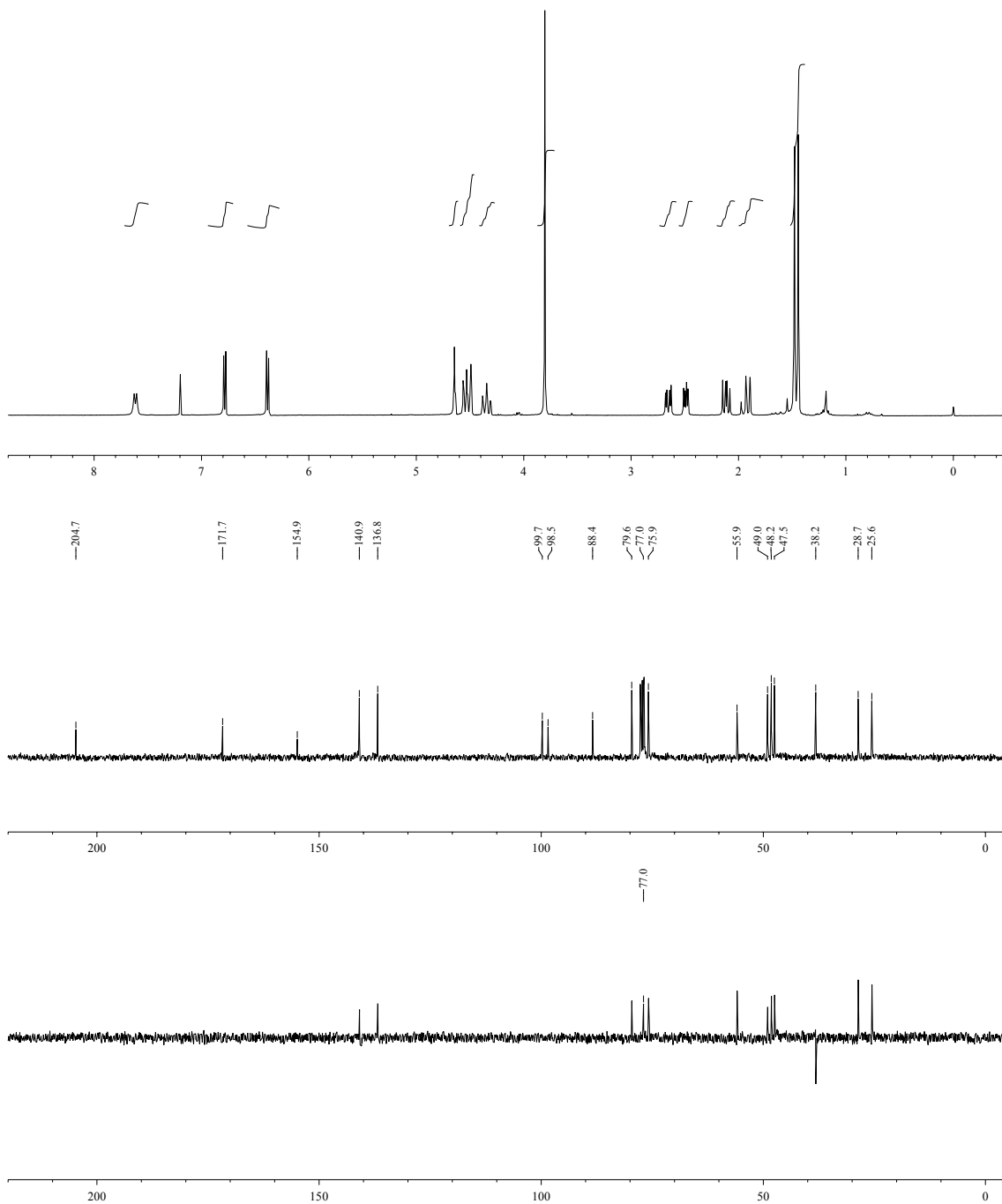
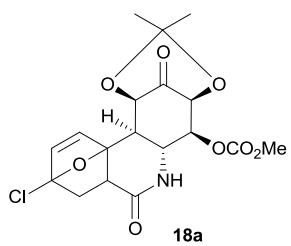


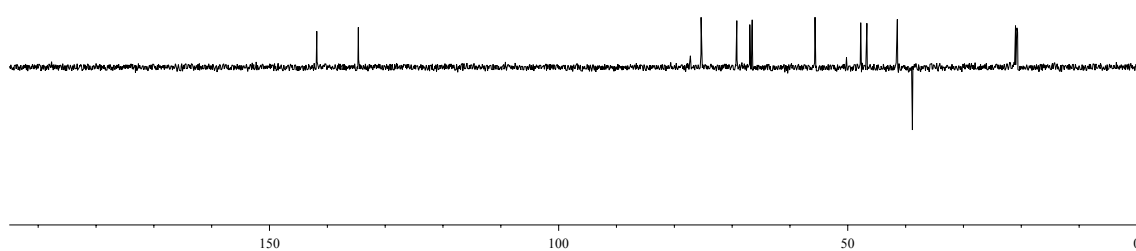
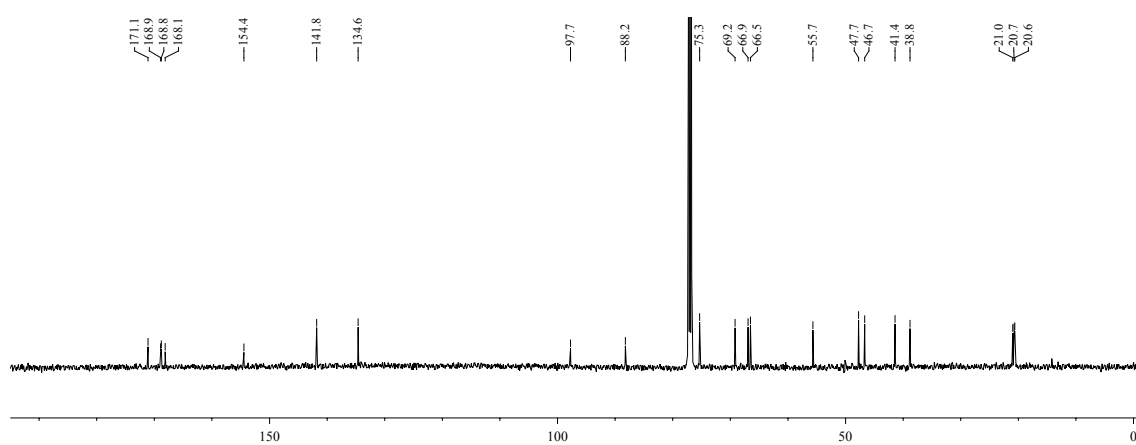
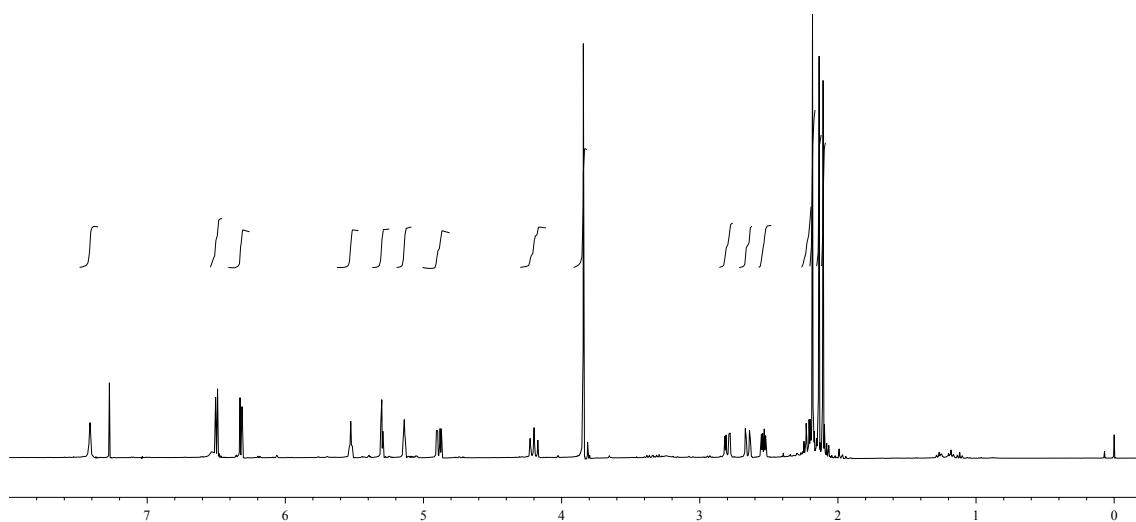
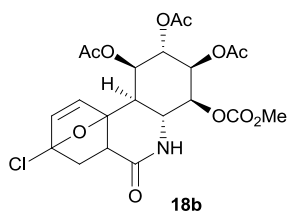
Chemical shift values (ppm): 7.062, 6.660, 6.548, 6.496, 6.353, 6.304, 6.270, 4.109, 4.074, 3.993, 1.794, 1.773, 1.772, 1.552, 1.475, 1.446, 0.281, 0.253.

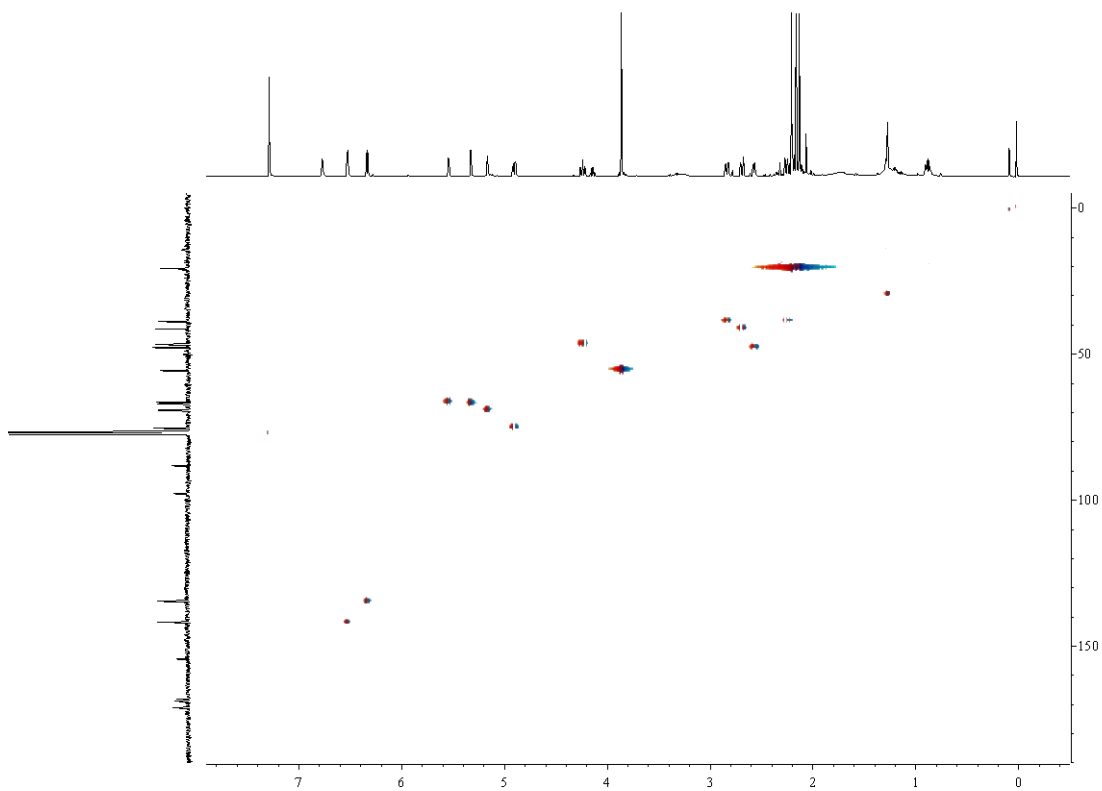
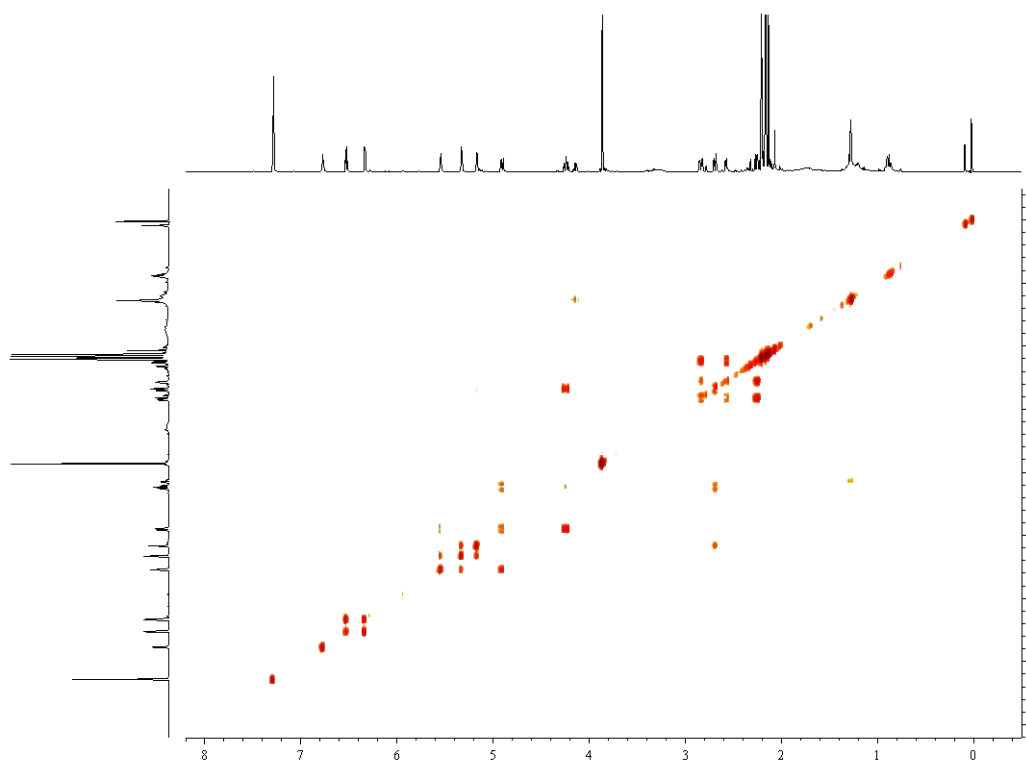
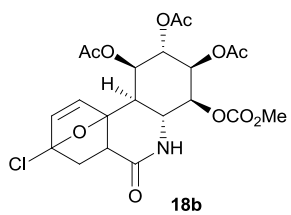


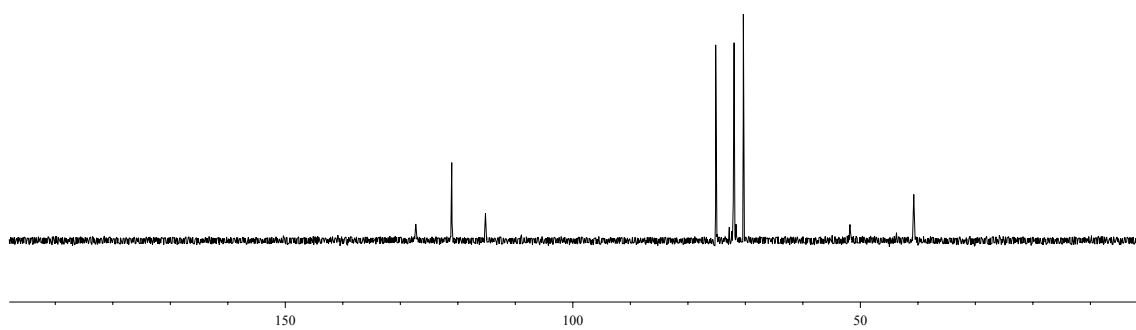
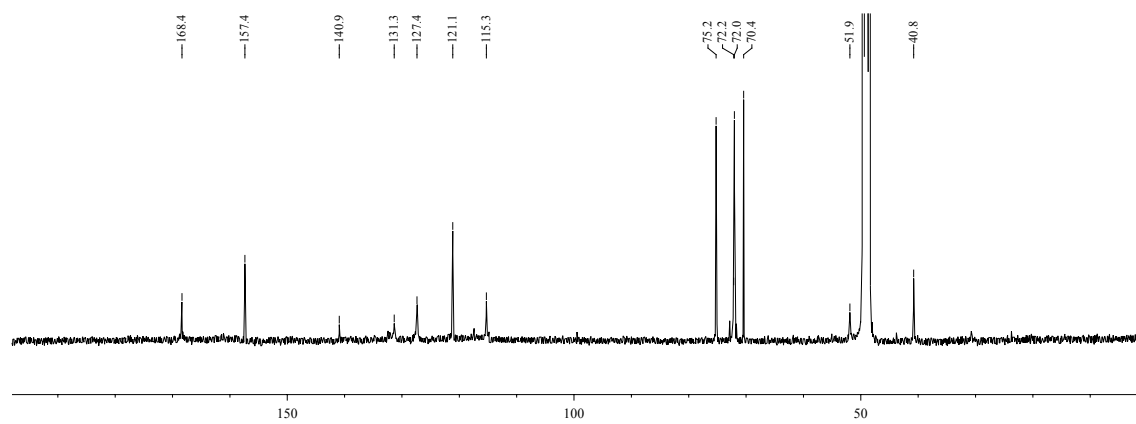
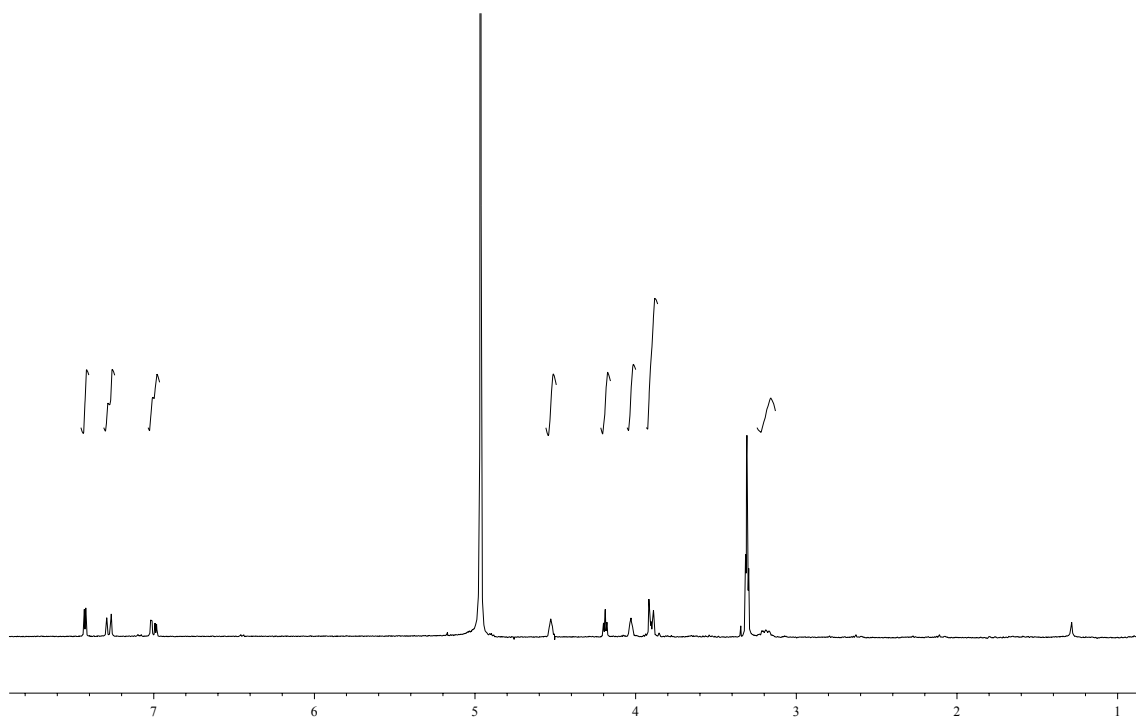
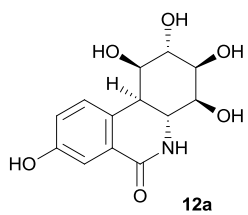
Chemical shift values (ppm): 77.3, 77.2.

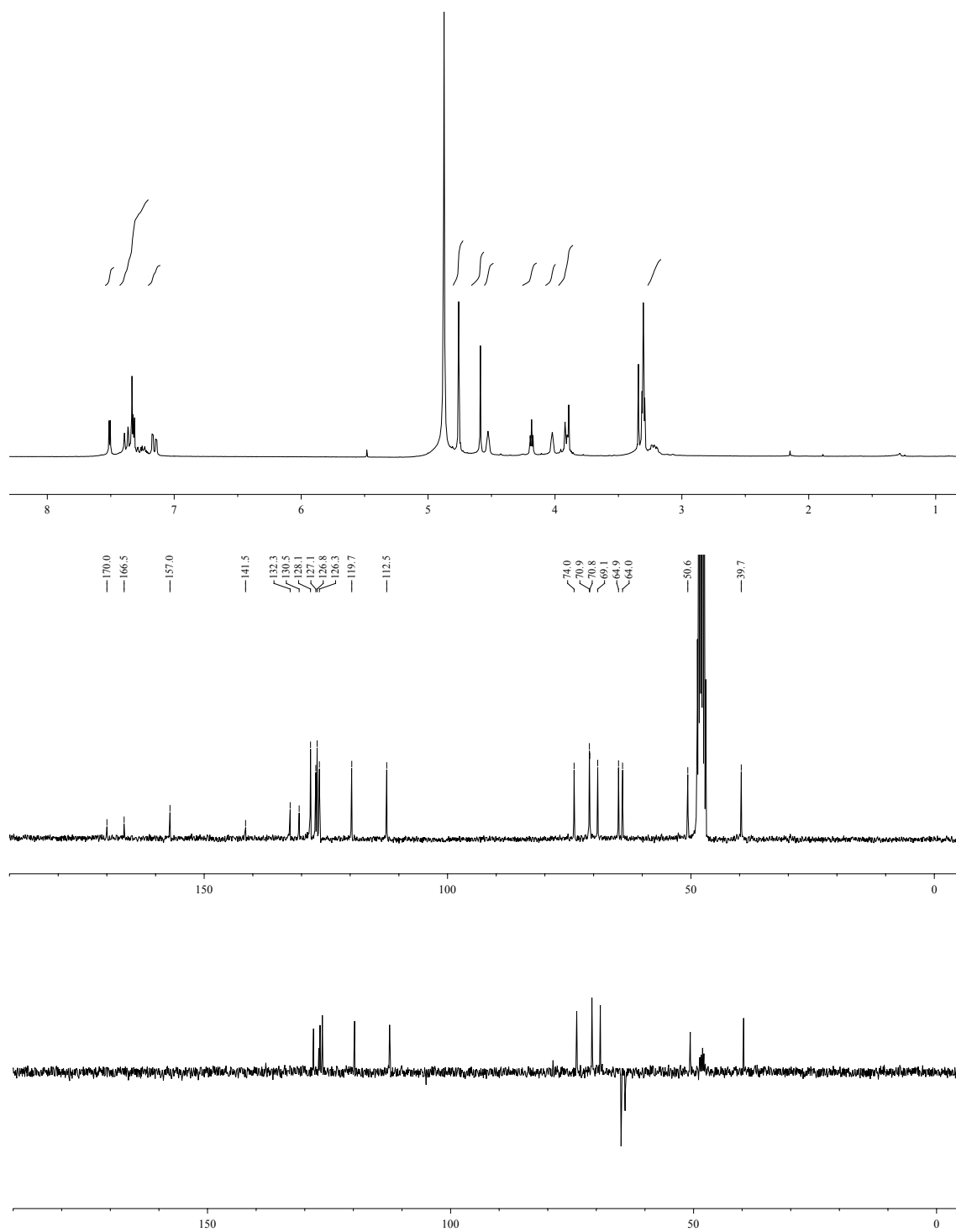
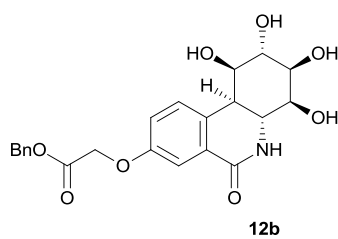


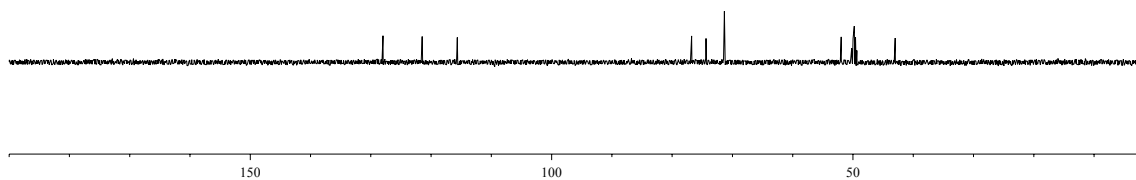
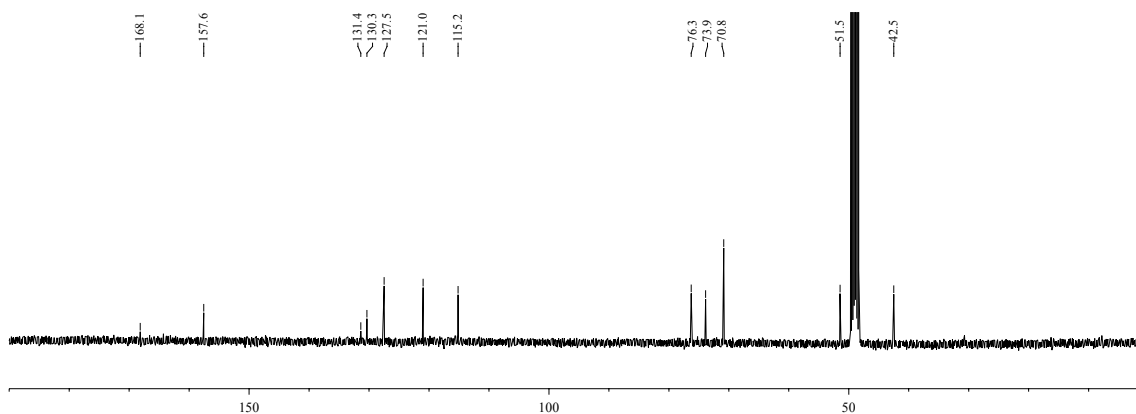
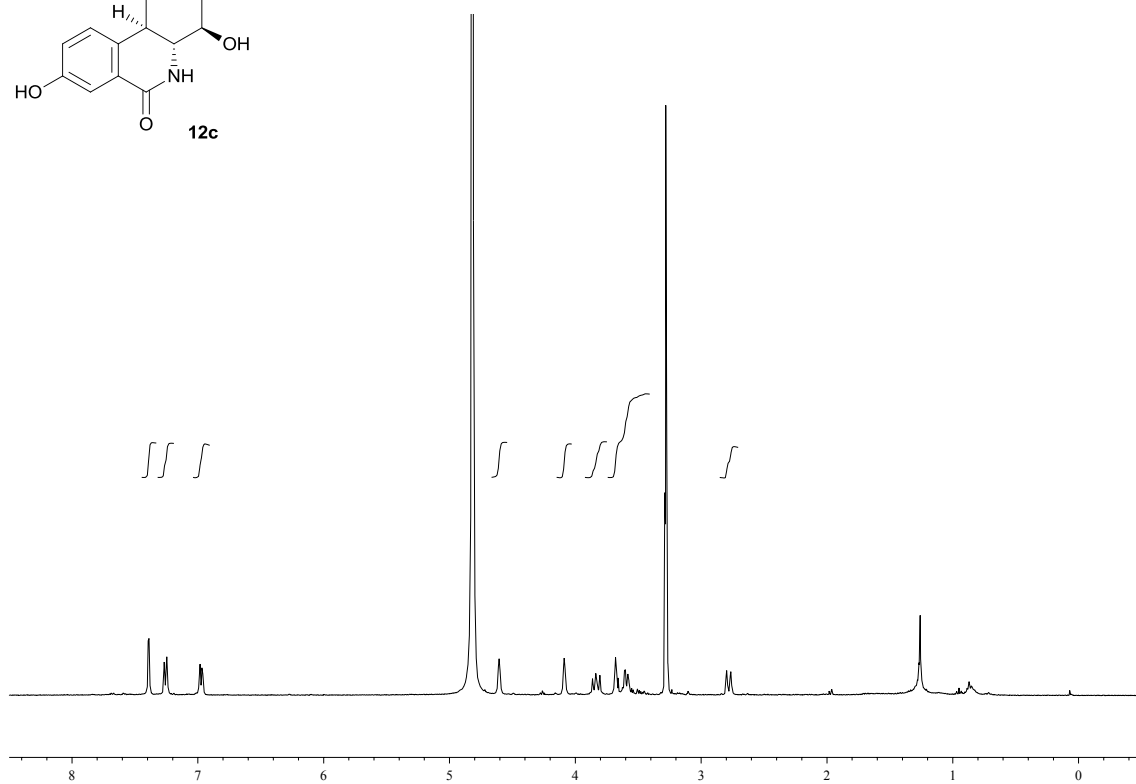
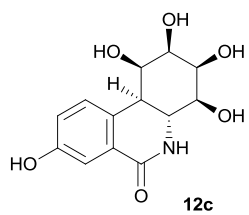












CYTOTOXIC STUDIES

a) GENERAL DATA for both NCI-H460 and MCF-7 cell lines

Culture conditions: RPMI 1640 supplemented with 10% FBS (Fetal Bovine Serum) in air/CO₂ (95:5) at 37°C.

Method: Sulforhodamine B assay at the USEF Drug Screening Platform at the University of Santiago de Compostela.

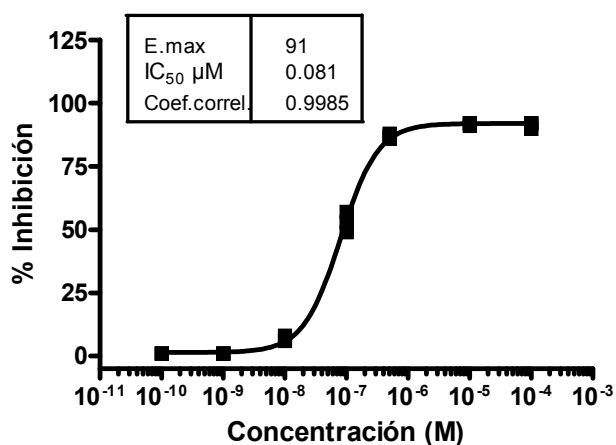
Positive control: natural narciclasine.

b) DATA AGAINST NCI-H460

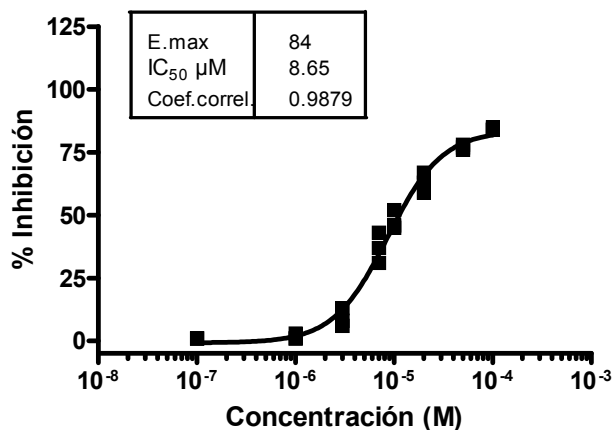
Compound	MW	IC ₅₀ (μ M and μ g/mL)	Standard error (μ M)	% Growth inhibition at 100 μ M
Narciclasine (3)	307.26	0.12 μ M = 0.037 μ g/mL	0.002	88 +/- 1
(+/-)-7-Deoxy-PST (<i>rac-2</i>)	309.27	5.08 μ M = 1.57 μ g/mL	0.09	85 +/- 1
(+/-)- 12a	281.09	> 28 μ g/mL		3.8 +/- 1
(+/-)- 12b	429.14	> 43 μ g/mL		7 +/- 2
(+/-)- 12c	281.09	> 28 μ g/mL		3.5 +/- 1

c) DATA AGAINST MCF-7

Compound	MW	IC ₅₀ (μ M and μ g/mL)	Standard error (μ M)	% Growth inhibition at 100 μ M
Narciclasine (3)	307.26	0.081 μ M = 0.024 μ g/mL	0.002	91 +/-1
(+/-)-7-Deoxy-PST (<i>rac-2</i>)	309.27	8.65 μ M = 2.67 μ g/mL	0.3	84 +/- 1
(+/-)- 12a	281.09	> 28 μ g/mL		4.6 +/- 1
(+/-)- 12b	429.14	> 43 μ g/mL		16 +/- 5
(+/-)- 12c	281.09	> 28 μ g/mL		1 +/- 1



Narciclasine (**3**)



(+/-)-7-Deoxy-PST (*rac-2*)