Supporting Information

The First F-ring Modified Ciguatoxin Analogue Showing Significant Toxicity

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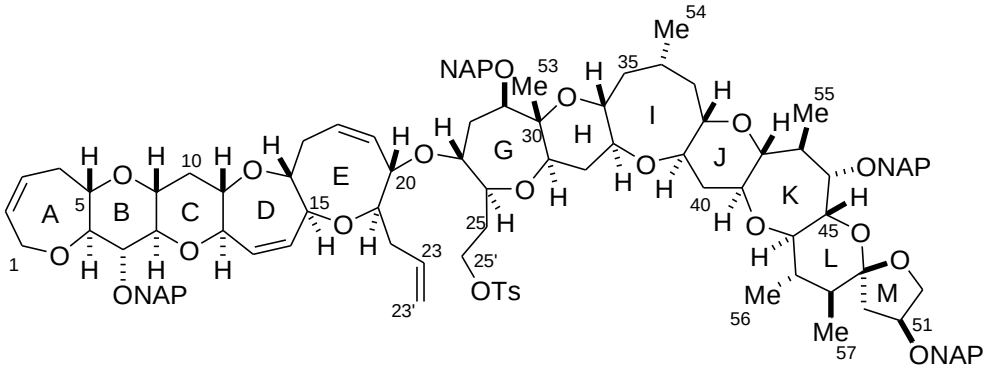
Experimental

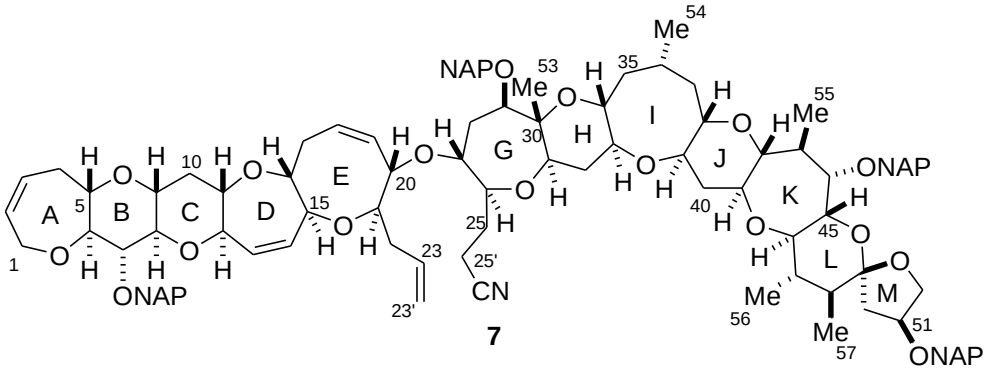
All reactions sensitive to air or moisture were carried out under argon or nitrogen atmosphere in dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed using E. Merck Silica gel 60 F254 pre-coated plates. Column chromatography was performed using 100-210 μm Silica Gel 60N (Kanto Chemical Co., Inc.), and for flash column chromatography 40-50 μm Silica Gel 60N (Kanto Chemical Co., Inc.) was used.

^{1}H- NMR spectra were recorded on a Varian INOVA 500 (500 MHz) spectrometer. Chemical shifts are reported in δ (ppm) down field from tetramethylsilane with reference to solvent signals [^{1}H NMR: $CHCl_3$ (7.26), C_5HD_4N (7.56)]. Signal patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak. MALDI-TOF MS spectra were measured on Applied Biosystems Voyager DE STR SI-3 instrument. High resolution mass spectra were measured on Themo Fisher Scientific Orbitrap Discovery (ESI LTQ Orbitrap).



Tosylate. To a suspension of alcohol **6** (3.2 mg, 1.9 μmol) and powdered 4Å MS in pyridine (500 μL) at room temperature was added TsCl (1.8 mg, 9.5 μmol). After being stirred for 5 h, the reaction mixture was quenched with aqueous $NaHCO_3$. The mixture was extracted with EtOAc (x3), and the organic layer was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc 5:1–3:1) gave the tosylate (3.0 mg, 1.7 μmol) in 87% yield: colorless amorphous; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85–7.14 (32H, m, NAPx4, Ts), 5.88 (1H, m, H2), 5.82 (1H, m, H23), 5.78 (1H, m, H3), 5.77 (1H, m, H13), 5.64 (1H, m, H18), 5.62 (1H, m, H14), 5.20 (1H, dd, *J* = 11.0, 5.5 Hz, H19), 5.11 (1H, m, H23'), 5.08 (1H, m, H23'), 5.06 (1H, d, *J* = 12.0 Hz, NAP), 5.00 (1H, d, *J* = 12.0 Hz, NAP), 4.96 (1H, d, *J* = 12.5 Hz, NAP), 4.82 (1H, d, *J* = 12.0 Hz, NAP), 4.78 (1H, d, *J* = 12.0 Hz, NAP), 4.75 (1H, d, *J* = 12.5 Hz, NAP), 4.62 (1H, d, *J* = 12.0 Hz, NAP), 4.58 (1H, d, *J* = 12.0 Hz, NAP), 4.32 (1H, dd, *J* = 16.0, 6.0 Hz, H1), 4.26 (1H, m, H51), 4.05 (1H, m, H26), 4.04 (1H, m, H1), 4.01 (1H, m, H25'), 3.97 (1H, dd, *J* = 7.5, 1.5 Hz, H52), 3.89 (1H, m, H41), 3.82 (1H, m, H12), 3.81 (1H, m, H52), 3.78 (1H, m, H15), 3.71 (1H, m, H20), 3.67 (1H, m, H29), 3.64 (1H, m, H45), 3.62 (1H, m, H25'), 3.54 (1H, m, H7), 3.53 (1H, m, H16), 3.42 (1H, m, H27), 3.41 (1H, m, H44), 3.39 (1H, m, H31), 3.38 (1H, m, H46), 3.33 (1H, m, H6), 3.31 (1H, m, H34), 3.29 (1H, m, H5), 3.22 (1H, m, H11), 3.14 (1H, m, H8), 3.13 (1H, m, H39), 3.12 (1H, m, H9), 3.09 (1H, m, H33), 3.00 (1H, m, H38), 2.91 (1H, m, H21), 2.88

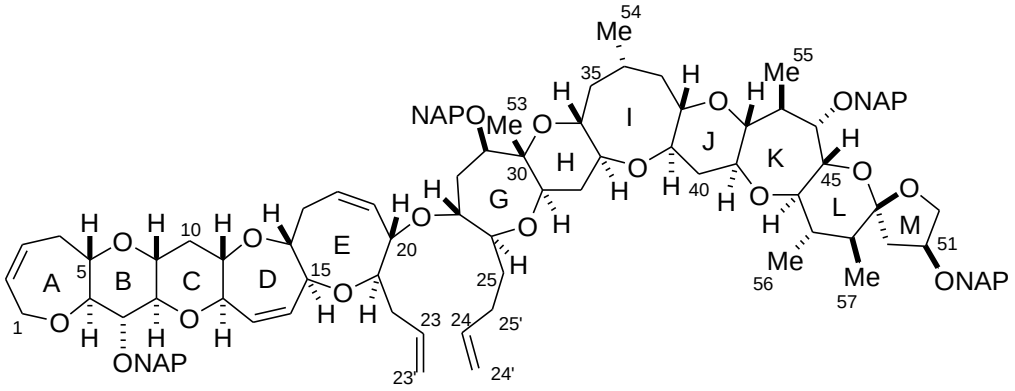
(1H, dd, *J* = 9.5, 4.5 Hz, H42), 2.66 (1H, ddd, *J* = 16.0, 8.0, 4.0 Hz, H4), 2.58 (1H, m, H17), 2.43 (1H, m, H22), 2.43 (3H, s, Ts), 2.36 (1H, m, H4), 2.34 (1H, m, H10), 2.31 (1H, m, H40), 2.28 (1H, m, H43), 2.19 (1H, m, H17), 2.15 (1H, m, H50), 2.14 (1H, m, H50), 2.04 (1H, m, H22), 2.01 (1H, m, H32), 1.93 (1H, m, H47), 1.92 (1H, m, H35), 1.89 (1H, m, H37), 1.88 (1H, m, H36), 1.79 (1H, m, H28), 1.68 (1H, m,H28), 1.64 (1H, m, H37), 1.62 (1H, m, H25), 1.62 (1H, m, H25), 1.60 (1H, m, H32), 1.55 (1H, m, H35), 1.53 (1H, m, H10), 1.41 (1H, m, H40), 1.29 (1H, m, H48), 1.27 (3H, s, Me53), 1.11 (3H, d, *J* = 7.0 Hz, Me55), 1.05 (3H, d, *J* = 6.5 Hz, Me54), 1.03 (3H, d, *J* = 6.5 Hz, Me56), 0.97 (3H, d, *J* = 7.0 Hz, Me57); MALDI-TOF MS, calcd. for $C_{109}H_{124}NaO_{20}S$ 1807.83 ($M+Na^+$), found 1807.65.



7

Nitrile 7. To a solution of tosylate (3.0 mg, 1.7 μmol) in DMSO (500 μL) at room temperature was added NaCN (1.0 mg, 17 mmol). The stirred mixture was allowed to warm up to 45 ˚C. After being stirred for 12 h, the reaction mixture was quenched with EtOAc and aqueous $NaHCO_3$. The mixture was extracted with EtOAc (x3), and the organic layer was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc 10:1–1:1) gave the nitrile **7** (2.8 mg, 1.7 μmol) in 99% yield: colorless amorphous; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85–7.14 (28H, m, NAPx4), 5.88 (1H, m, H2), 5.82 (1H, m, H23), 5.76 (1H, m, H3), 5.74 (1H, m, H13), 5.66 (1H, m, H18), 5.62 (1H, m, H14), 5.23 (1H, dd, *J* = 11.0, 5.0 Hz, H19), 5.12 (1H, m, H23'), 5.09 (1H, m, H23'), 5.06 (1H, d, *J* = 12.0 Hz, NAP), 5.00 (1H, d, *J* = 12.0 Hz, NAP), 4.96 (1H, d, *J* = 12.5 Hz, NAP), 4.82 (1H, d, *J* =

12.0 Hz, NAP), 4.78 (1H, d, *J* = 12.0 Hz, NAP), 4.75 (1H, d, *J* = 12.5 Hz, NAP), 4.62 (1H, d, *J* = 12.0 Hz, NAP), 4.58 (1H, d, *J* = 12.0 Hz, NAP), 4.33 (1H, dd, *J* = 15.5, 6.0 Hz, H1), 4.25 (1H, m, H51), 4.05 (1H, m, H26), 3.97 (1H, m, H1), 3.95 (1H, m, H52), 3.86 (1H, m, H41), 3.82 (1H, m, H12), 3.81 (1H, m, H52), 3.80 (1H, m, H15), 3.76 (1H, m, H20), 3.73 (1H, m, H29), 3.61 (1H, d, *J* = 9.0 Hz, H45), 3.54 (1H, m, H7), 3.53 (1H,m, H16), 3.43 (1H, m, H27), 3.41 (1H, m, H44), 3.40 (1H, m, H31), 3.39 (1H, m, H34), 3.38 (1H, m, H46), 3.29 (1H, m H5), 3.23 (1H, m, H6), 3.22 (1H, m, H11), 3.14 (1H, m, H39), 3.13 (1H, m, H9), 3.12 (1H, m, H8), 3.08 (1H, m, H33), 2.99 (1H, m, H38), 2.92 (1H, m, H42), 2.87 (1H, dd, *J* = 9.0, 5.0 Hz, H21), 2.66 (1H, ddd, *J* = 16.0, 8.0, 4.0 Hz, H4), 2.60 (1H, m, H17), 2.44 (1H, m, H22), 2.43 (1H, m, H25'), 2.41 (1H, m, H4), 2.36 (1H, m, H10), 2.35 (1H, m, H25'), 2.30 (1H, m, H40), 2.29 (1H, m, H43), 2.17 (1H, m, H17), 2.15 (1H, m, H50), 2.14 (1H, m, H50), 2.03 (1H, m, H22), 2.02 (1H, m, H32), 1.92 (1H, m, H35), 1.89 (1H, m, H37), 1.88 (1H, m, H36), 1.72 (1H, m, H28), 1.64 (1H, m, H37), 1.62 (1H, m, H28), 1.60 (1H, m, H32), 1.55 (1H, m, H35), 1.54 (1H, m, H47), 1.53 (1H, m, H10), 1.49 (1H, m, H25), 1.47 (1H, m, H25), 1.41 (1H, m, H40), 1.29 (1H, m, H48), 1.27 (3H, s, Me53), 1.11 (3H, d, *J* = 7.0 Hz, Me55), 1.05 (3H, d, *J* = 6.5 Hz, Me54), 1.03 (3H, d, *J* = 6.5 Hz, Me56), 0.97 (3H, d, *J* = 7.0 Hz, Me57); MALDI-TOF MS, calcd. for $C_{103}H_{117}NNaO_{17}$ 1662.82 ($M+Na^+$), found 1662.66.

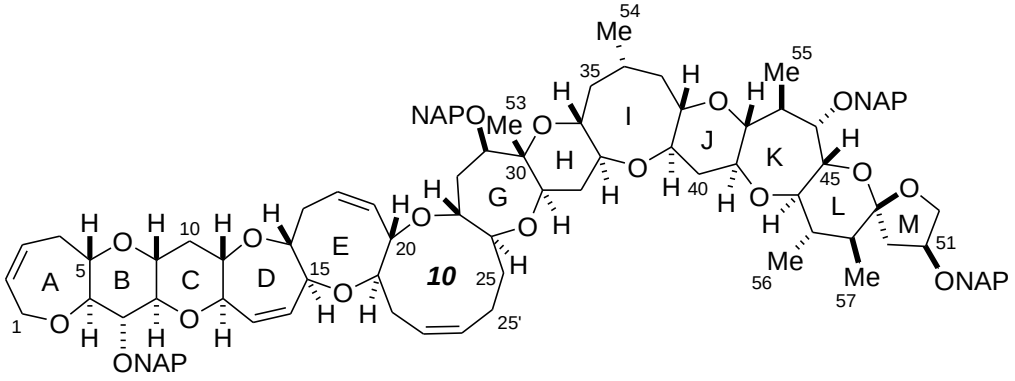


9

Pentaene 9. To a solution of nitrile **7** (2.8 mg, 1.7 μmol) in CH_2Cl_2 (500 μL) at –50 °C was added DIBAL (0.98 M solution of in hexane, 17 μL, 17 μmol). After being stirred for 1.5 h at –50 °C, the reaction mixture was quenched with EtOAc and 1N HCl. The mixture was extracted with EtOAc (x3), and the organic layer was washed with brine and dried over Na_2SO_4. Concentration and filtration through a pad of flash silica gel gave the aldehyde **8**, which was used in the next reaction without further purification.

To a suspension of Ph_3PCH_3Br (60.0 mg, 168 μmol) in THF (1.7 mL) at 0 °C was added *t*-BuOK (15.5 mg, 84.0 μmol). After 15 min, a solution of aldehyde **8** in THF (3.6 mL) at 0 °C was added dropwise to the reaction mixture. After being stirred for 30 min at 0 °C, the reaction mixture was quenched with aqueous NH_4Cl. The mixture was extracted with EtOAc (x3), and the organic layer was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc 10:1–3:1) gave the pentaene **9** (2.4 mg, 15 μmol) in 90% yield over 2 steps: colorless amorphous; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85–7.14 (28H, m, NAPx4), 5.89 (1H, m, H2), 5.81 (1H, m, H23), 5.79 (1H, m, H3), 5.75 (1H, m, H13), 5.67 (1H, m, H24), 5.65 (1H, m, H18), 5.62 (1H, m, H14), 5.25 (1H, dd, *J* = 10.5, 5.0 Hz, H19), 5.10 (1H, m, H23'), 5.09 (1H, m, H24'), 5.08 (1H, m, H23'), 5.07 (1H, m, H24'), 5.06 (1H, d, *J* = 12.0 Hz, NAP), 5.00 (1H, d, *J* = 12.0 Hz, NAP), 4.96 (1H, d, *J* = 12.5 Hz, NAP), 4.82 (1H, d,

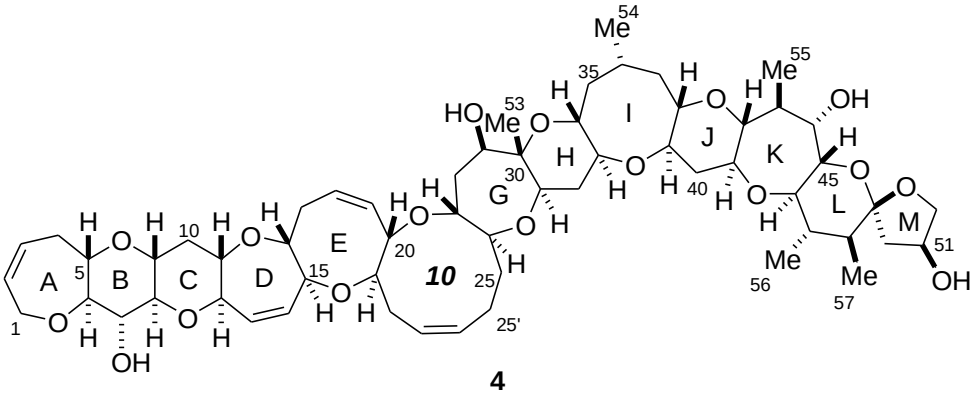
J = 12.0 Hz, NAP), 4.78 (1H, d, *J* = 12.0 Hz, NAP), 4.75 (1H, d, *J* = 12.5 Hz, NAP), 4.62 (1H, d, *J* = 12.0 Hz, NAP), 4.58 (1H, d, *J* = 12.0 Hz, NAP), 4.32 (1H, dd, *J* = 15.0, 5.5 Hz, H1), 4.26 (1H, m, H51), 4.05 (1H, m, H1), 3.97 (1H, dd, *J* = 9.5, 1.5 Hz, H52), 3.85 (1H, m, H41), 3.84 (1H, m, H12), 3.82 (1H, m, H52), 3.80 (1H, m, H15), 3.71 (1H, m, H20), 3.70 (1H, m, H29), 3.63 (1H, m, H45), 3.56 (1H, m, H26), 3.55 (1H, m, H16), 3.54 (1H, m, H7), 3.44 (1H, m, H44), 3.40 (1H, m, H6), 3.40 (1H, m, H34), 3.39 (1H, m, H46), 3.37 (1H, m, H27), 3.29 (1H, ddd, *J* = 9.0, 9.0, 1.5 Hz, H5), 3.22 (1H, m, H31), 3.21 (1H, m, H11), 3.14 (1H, m, H8), 3.13 (1H, m, H39), 3.12 (1H, m, H9), 3.10 (1H, m, H33), 3.09 (1H, m, H38), 3.04 (1H, m, H21), 2.87 (1H, m, H42), 2.66 (1H, m, H4), 2.57 (1H, m, H17), 2.48 (1H, m, H22), 2.41 (1H, m, H25'), 2.36 (1H, m, H4), 2.29 (1H, m, H10), 2.27 (1H, m, H40), 2.20 (1H, m, H43), 2.16 (1H, m, H17), 2.15 (1H, m, H50), 2.14 (1H, m, H50), 2.03 (1H, m, H22), 2.01 (1H, m, H32), 1.93 (1H, m, H47), 1.92 (1H, m, H35), 1.89 (1H, m, H37), 1.88 (1H, m, H36), 1.82 (1H, m, H28), 1.77 (1H, m, H28), 1.68 (1H, m, H25'), 1.64 (1H, m, H37), 1.60 (1H, m, H32), 1.55 (1H, m, H35), 1.53 (1H, m, H10), 1.48 (1H, m, H25), 1.41 (1H, m, H40), 1.31 (1H, m, H25), 1.29 (1H, m, H48), 1.27 (3H, s, Me53), 1.11 (3H, d, *J* = 7.0 Hz, Me55), 1.05 (3H, d, *J* = 6.5 Hz, Me54), 1.03 (3H, d, *J* = 6.5 Hz, Me56), 0.97 (3H, d, *J* = 7.0 Hz, Me57); MALDI-TOF MS, calcd. for $C_{109}H_{124}NaO_{20}S$ 1807.83 ($M+Na^+$), found 1807.65.



10

Tetrakis-NAP 10-membered F-ring 51-hydroxyCTX3C 10. To a degassed solution of pentaene **9** (2.4 mg, 15 μmol) in CH_2Cl_2 (3.0 mL) at room temperature was added $(PCy_3)_2Cl_2Ru$=CHPh (1.0 mg, 1.2 μmol). After being stirred for 12 h at 40 °C, two drops of Et_3N were added at room temperature, and the mixture was concentrated and subjected to flash column chromatography (hexane/EtOAc 8:1-1:1) gave tetrakis-NAP 10-membered F-ring 51-hydroxyCTX3C **10** (2.4 mg, 15 μmol) in 99% yield: colorless amorphous; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85–7.44 (28H, m, NAPx4), 5.87 (1H, m, H2), 5.82 (1H, m, H13), 5.80 (1H, m H19), 5.77 (1H, m, H3), 5.68 (1H, m, H18), 5.62 (1H, m, H23), 5.60 (1H, m, H14), 5.52 (1H, m, H24), 5.04 (1H, d, *J* = 12.0 Hz, NAP), 4.99 (1H, d, *J* = 12.0 Hz, NAP), 4.82 (1H, d, *J* = 12.5 Hz, NAP), 4.82 (1H, d, *J* = 12.5 Hz, NAP), 4.77 (1H, d, *J* = 12.5 Hz, NAP), 4.74 (1H, d, *J* = 12.5 Hz, NAP), 4.62 (1H, d, *J* = 12.5 Hz, NAP), 4.59 (1H, d, *J* = 12.5 Hz, NAP), 4.31 (1H, dd, *J* = 15.5, 6.0 Hz, H1), 4.27 (1H, m, H51), 4.05 (1H, m, H1), 3.99 (1H, m, H15), 3.99 (1H, m, H52), 3.84 (1H, m, H41), 3.83 (1H, m, H20), 3.82 (1H, m, H52), 3.81 (1H, m, H12), 3.64 (1H, m, H45), 3.70 (1H, m, H29), 3.57 (1H, m, H26), 3.55 (1H, m, H16), 3.52 (1H, m, H7), 3.51 (1H, m, H21), 3.45 (1H, m, H44), 3.40 (1H, m, H46), 3.38 (1H, m, H6), 3.36 (1H, m, H34), 3.29 (1H, m, H27), 3.28 (1H, m, H5), 3.22 (1H, m, H11), 3.14 (1H, m, H31), 3.12 (1H, m, H8), 3.11 (1H, m, H39), 3.12 (1H, m, H9), 3.09 (1H, m, H33), 3.00 (1H, m, H38), 2.99 (1H, m, H22), 2.88 (1H, dd, *J* = 9.5, 4.0 Hz, H42), 2.72 (1H, m, H25'), 2.65 (1H, ddd, *J* = 16.0, 8.0, 4.0 Hz, H4), 2.64 (1H, m, H17), 2.36 (1H, m, H4), 2.30 (1H, m, H10), 2.26 (1H, m, H40), 2.23 (1H, m, H28), 2.21 (1H, m, H43), 2.18 (1H, m, H17), 2.16 (1H, m, H50), 2.15 (1H, m, H50), 2.13 (1H, m, H22), 2.09 (1H, m, H32), 2.08 (1H, m, H28), 2.04 (1H, m, H25'), 1.87 (1H, m, H35), 1.87 (1H, m, H36), 1.87 (1H, m, H37), 1.82 (1H, m, H25), 1.69 (1H, m, H32), 1.60 (1H, m, H47), 1.56 (1H, m, H10), 1.53 (1H, m, H48), 1.52 (1H, m, H37), 1.46 (1H, m, H35), 1.43 (1H, m,

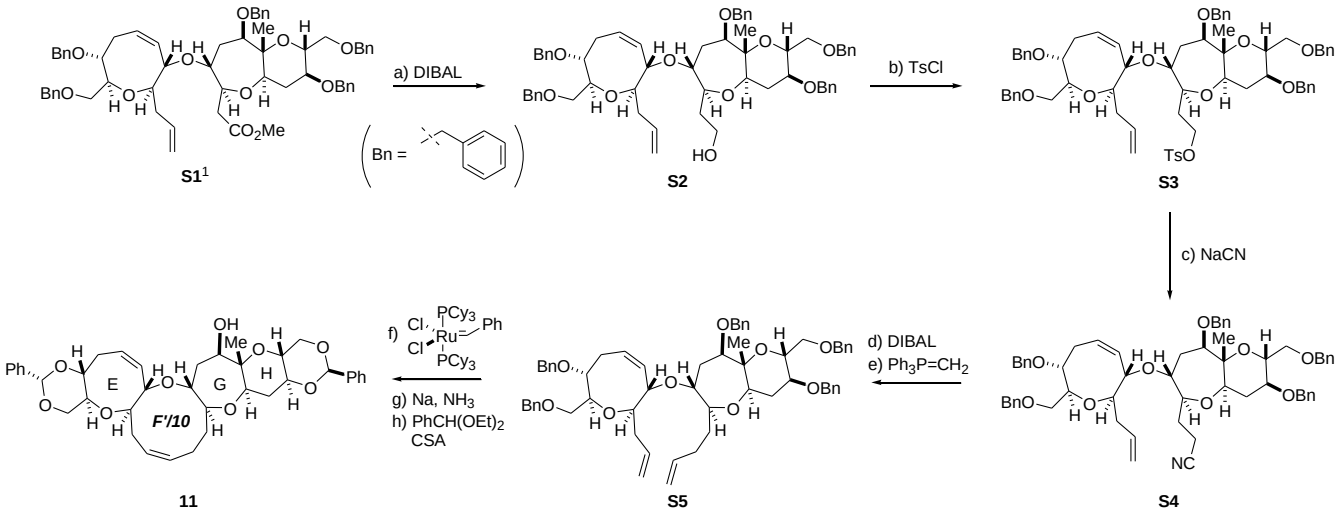
H25), 1.42 (1H, m H40), 1.24 (3H, s, Me53), 1.11 (3H, d, *J* = 7.5 Hz, Me55), 1.06 (3H, d, *J* = 6.5 Hz, Me54), 1.05 (3H, d, *J* = 6.5 Hz, Me56), 1.04 (3H, d, *J* = 6.5 Hz, Me57); MALDI-TOF MS, calcd. for $C_{102}H_{116}NaO_{17}$ 1635.81 ($M+Na^+$), found 1635.64.



4

10-membered F-ring 51-hydroxyCTX3C 4. To a solution of tetrakis-NAP 10-membered F-ring 51-hydroxyCTX3C **10** (1.8 mg, 1.1 μmol) in CH_2Cl_2 (310 μL) and H_2O (310 μL) at room temperature was added DDQ (3.0 mg, 11 μmol). After being stirred for 30 min at room temperature, the mixture was quenched with saturated aqueous $Na_2S_2O_3$ at room temperature, diluted with EtOAc and saturated aqueous $NaHCO_3$, and extracted with EtOAc (x3). The organic layer was concentrated, and the residue were separated by reversed-phase HPLC (Shodex Asahipak ODP 50-6D, 6.0x150 mm, UV 210 nm, CH_3CN/H_2O 55:45, 1.0 mL/min) to give pure 10-membered F-ring 51-hydroxyCTX3C (**4**, t_R = 22.5 min, 496 μg, 0.47 μmol) in 43% yield: coorless amorphous; ^{1}H NMR (500 MHz, C_6D_5N, 25 °C) δ7.28 (1H, d, *J* = 3.5 Hz, OH7), 6.72 (1H, d, *J* = 3.5 Hz, OH44), 6.52 (1H, d, *J* = 4.0 Hz, OH51), 6.07 (1H, m, H19), 5.89 (1H, m, H13), 5.85 (1H, m, H23), 5.83 (1H, m, H18), 5.81 (1H, m, H2), 5.72 (1H, m, H3), 5.69 (1H, m, H14), 5.57 (1H, m, H24), 4.98 (1H, m, OH29), 4.84 (1H, m, H51), 4.46 (1H, ddd, *J* = 10.5, 10.5, 4.5 Hz, H41), 4.30 (1H, dd, *J* = 10.5, 5.5 Hz, H1), 4.22 (1H, m, H29), 4.21 (1H, m, H44), 4.19 (1H, m, H12), 4.16 (2H, m, H52x2), 4.16 (1H, m, H15), 4.05 (1H, m, H20), 4.05 (1H, m, H45), 4.02 (1H, m,

H1), 3.99 (1H, m, H7), 3.95 (1H, dd, *J* = 10.5, 10.5 Hz, H46), 3.89 (1H, m, H27), 3.71 (1H, m, H16), 3.68 (1H, m, H26), 3.62 (1H, m, H6), 3.55 (1H, m, H5), 3.49 (1H, dd, *J* = 8.5, 8.5 Hz, H11), 3.44 (1H, ddd, *J* = 9.5, 9.5, 3.5 Hz, H21), 3.40 (1H, m, H34), 3.38 (1H, m, H9), 3.37 (1H, m, H8), 3.32 (1H, m, H39), 3.26 (1H, ddd, *J* = 12.5, 10.5, 4.5 Hz, H33), 3.20 (1H, m, H31), 3.19 (1H, m, H38), 3.17 (1H, m, H22), 3.15 (1H, m, H42), 2.87 (1H, m, H17), 2.66 (1H, m, H4), 2.62 (1H, m, H28), 2.60 (1H, m. H47), 2.58 (1H, m, H40), 2.57 (1H, m, H22), 2.50 (1H, ddd, *J* = 11.0, 4.5, 4.5 Hz, H10), 2.43 (1H, m, H4), 2.32 (1H, dd, *J* = 13.5, 3.5 Hz, H50), 2.29 (1H, m, H25'), 2.28 (1H, m, H28), 2.27 (1H, m, H17), 2.25 (1H, m, H32), 2.04 (1H, m, H25), 2.02 (1H, m, H25'), 2.01 (1H, m, H37), 1.99 (1H, m, H43), 1.91 (1H, m, H32), 1.90 (1H, m, H36), 1.83 (1H, m, H35), 1.78 (1H, m, H40), 1.74 (1H, m, H10), 1.71 (1H, m, H37), 1.66 (1H, m, H48), 1.61 (1H, m, H25), 1.60 (1H, m, H35), 1.46 (3H, s, Me53), 1.29 (3H, d, *J* = 7.5 Hz, Me56), 1.27 (3H, d, *J* = 6.5 Hz, Me55), 1.21 (3H, d, *J* = 7.0 Hz, Me57) 0.92 (3H, d, *J* = 7.5 Hz, Me54); HRMS (ESI), calcd. for $C_{58}H_{84}NaO_{17}$ 1075.5601 ($M+Na^+$), found 1075.5601.



[1] Inoue, M.; Wang, G.-X.; Wang, J.; Hirama, M. *Org. Lett.* **2002**, *4*, 3439.

Scheme S1. Synthesis of EF'(10)GH model **11**

Alcohol S2. To a solution of methylester **S1** (9.1 mg, 9.4 μmol) in CH_2Cl_2 (1.0 mL) at –90 °C was added DIBAL (0.9 M solution of in hexane, 105 μL, 94 μmol). After being stirred for 1 hour at –90 °C to –40 °C, the reaction mixture was quenched with EtOAc and aqueous NH_4Cl. The mixture was extracted with EtOAc (x3), and the organic layer was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc 10:1) gave primary alcohol **S2** (8.5 mg, 9.1 μmol) in 97% yield: colorless oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.20 (25H, m, Bn), 5.95 (1H, ddt, *J* = 17.0, 10.0, 6.5 Hz, H23), 5.75 (1H, dddd, *J* = 11.5, 11.5, 6.5, 2.0 Hz, H18), 5.61 (1H, dd, *J* = 11.5, 5.0 Hz, H19), 5.04 (1H, dd, *J* = 17.0, 2.0 Hz, H23'), 4.99 (1H, dd, *J* = 10.5, 2.0 Hz, H23'), 4.89 (1H, d, *J* = 12.0 Hz, Bn), 4.63 (1H, d, *J* = 12.0 Hz, Bn), 4.62 (1H, d, *J* = 12.0 Hz, Bn), 4.61 (1H, d, *J* = 12.0 Hz, Bn), 4.60 (1H, d, *J* = 12.0 Hz, Bn), 4.55 (1H, d, *J* = 12.0 Hz, Bn), 4.52 (1H, d, *J* = 12.0 Hz, Bn), 4.48 (1H, d, *J* = 12.0 Hz, Bn), 4.43 (1H, d, *J* = 12.0 Hz, Bn), 4.36 (1H, d, *J* = 12.0 Hz, Bn), 3.92 (1H, m, H20), 3.91 (1H, m, H26), 3.77 (1H, dd, *J* = 11.5, 3.0 Hz, H29), 3.77 (1H, m, H16), 3.75 (1H, m, H35), 3.75 (1H, m, H14), 3.72 (1H, m, H25'), 3.67 (1H, m, H14), 3.67 (1H, m, H35), 3.64 (1H, m, H25'), 3.61 (1H, m, H34), 3.51 (1H, m, H15), 3.49 (1H, m, H27), 3.46 (1H, dd, *J* = 12.5, 5.0 Hz, H31), 3.39 (1H, ddd, *J* = 12.5, 10.0, 5.0 Hz, H33), 3.36 (1H, dd, *J* = 9.0, 9.0, 3.5 Hz, H21), 2.56 (1H, m, H22), 2.51 (1H, m, H17), 2.45 (1H, ddd, *J* = 13.0, 6.5, 3.0 Hz, H17), 2.25 (1H, ddd, *J* = 12.5, 5.0, 5.0 Hz, H32), 2.21 (1H, m, H22), 1.96 (1H, ddd, *J* = 14.0, 7.0, 3.0 Hz, H28), 1.83 (1H, ddd, *J* = 14.0, 11.5 Hz, H28), 1.65 (1H, m, H25), 1.60 (1H, m, H25), 1.51 (1H, m, H32), 1.28 (3H, s, Me53); MALDI-TOF MS, calcd for $C_{59}H_{70}O_{10}Na$ 961.4867 ($M+Na^+$), found 961.4402.

Tosylate S3. To a suspension of alcohol **S2** (9.0 mg, 9.6 μmol) and powdered 4Å MS in pyridine (1

mL) at room temperature was added TsCl (3.7 mg, 19 μmol). After being stirred for 4 h, the reaction mixture was quenched with aqueous $NaHCO_3$. The mixture was extracted with EtOAc (x3), and the organic layer was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc 5:1–3:1) gave the tosylate **S3** (10.5 mg, 9.6 μmol) in 99% yield: colorless amorphous; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89–7.78 (4H, m, Ts), 7.34–7.21 (25H, m, Bn, Ts), 5.95 (1H, ddt, *J* = 17.0, 10.0, 7.0 Hz, H23), 5.76 (1H, m, H18), 5.59 (1H, dd, *J* = 11.5, 5.5 Hz, H19), 5.04 (1H, dd, *J* = 17.0, 2.0 Hz, H23'), 4.99 (1H, dd, *J* = 10.5, 2.0 Hz, H23'), 4.89 (1H, d, *J* = 12.0 Hz, Bn), 4.63 (1H, d, *J* = 12.0 Hz, Bn), 4.62 (1H, d, *J* = 12.0 Hz, Bn), 4.61 (1H, d, *J* = 12.0 Hz, Bn), 4.60 (1H, d, *J* = 12.0 Hz, Bn), 4.55 (1H, d, *J* = 12.0 Hz, Bn), 4.52 (1H, d, *J* = 12.0 Hz, Bn), 4.48 (1H, d, *J* = 12.0 Hz, Bn), 4.43 (1H, d, *J* = 12.0 Hz, Bn), 4.36 (1H, d, *J* = 12.0 Hz, Bn), 4.15 (1H, m, H25'), 4.05 (1H, m, H25'), 3.88 (1H, m, H20), 3.78 (1H, m, H26), 3.77 (1H, m, H29), 3.76 (1H, m, H16), 3.75 (1H, m, H14), 3.64 (1H, m, H14), 3.62 (1H, m, H35), 3.60 (1H, m, H34), 3.51 (1H, m, H15), 3.48 (1H, dd, *J* = 10.0, 5.5 Hz, H35), 3.44 (1H, m, H27), 3.38 (1H, m, H31), 3.37 (1H, m, H33), 3.34 (1H, m, H21), 2.53 (1H, m, H17), 2.50 (1H, m, H22), 2.45 (1H, m, H17), 2.42 (3H, s, Ts), 2.20 (1H, m, H22), 2.19 (1H, m, H32), 1.91 (1H, ddd, *J* = 14.0, 6.5, 2.5 Hz, H28), 1.67 (1H, m, H28), 1.61 (1H, m, H25), 1.58 (1H, m, H25), 1.50 (1H, ddd, *J* = 12.0, 12.0, 12.0 Hz, H32), 1.26 (3H, s, Me53); MALDI-TOF MS, calcd for $C_{60}H_{76}O_{12}NaS$ 1115.4950 ($M+Na^+$), found 1115.4076.

Nitrile S4. To a solution of tosylate **S3** (10.5 mg, 9.6 μmol) in DMSO (1 mL) at room temperature was added NaCN (4.7 mg, 96 mmol). The stirred mixture was allowed to warm up to 45 ˚C. After being stirred for 20 h, the reaction mixture was quenched with EtOAc and aqueous $NaHCO_3$. The mixture

was extracted with EtOAc (x3), and the organic layer was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc 10:1–1:1) gave the nitrile **S4** (5.8 mg, 6.2 μmol) in 64% yield: colorless amorphous; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.24 (25H, m, Bn), 5.95 (1H, ddt, *J* = 17.0, 10.0, 7.0 Hz, H23), 5.76 (1H, m, H18), 5.61 (1H, dd, *J* = 11.5, 5.5 Hz, H19), 5.06 (1H, dd, *J* = 17.0, 2.0 Hz, H23'), 5.01 (1H, dd, *J* = 10.5, 2.0 Hz, H23'), 4.88 (1H, d, *J* = 12.0 Hz, Bn), 4.63 (1H, d, *J* = 12.0 Hz, Bn), 4.62 (1H, d, *J* = 12.0 Hz, Bn), 4.61 (1H, d, *J* = 12.0 Hz, Bn), 4.60 (1H, d, *J* = 12.0 Hz, Bn), 4.55 (1H, d, *J* = 12.0 Hz, Bn), 4.52 (1H, d, *J* = 12.0 Hz, Bn), 4.48 (1H, d, *J* = 12.0 Hz, Bn), 4.43 (1H, d, *J* = 12.0 Hz, Bn), 4.36 (1H, d, *J* = 12.0 Hz, Bn), 3.91 (1H, m, H20), 3.79 (1H, m, H26), 3.76 (1H, m, H29), 3.75 (1H, m, H16), 3.74 (1H, m, H35), 3.66 (1H, m, H35), 3.64 (1H, m, H14), 3.60 (1H, m, H34), 3.53 (1H, m, H15), 3.47 (1H, m, H14), 3.45 (1H, m, H27), 3.42 (1H, m, H31), 3.39 (1H, m, H33), 3.36 (1H, m, H21), 2.56 (1H, m, H22), 2.52 (1H, m, H17), 2.46 (1H, ddd, *J* = 13.0, 6.5, 3.0 Hz, H17), 2.42 (1H, m, H25'), 2.40 (1H, m, H25'), 2.26 (1H, ddd, *J* = 12.5, 5.0, 5.0 Hz, H32), 2.22 (1H, m, H22), 1.95 (1H, ddd, *J* = 14.0, 7.0, 3.0 Hz, H28), 1.74 (1H, ddd, *J* = 14.0, 12.0 Hz, H28), 1.65 (1H, m, H25), 1.62 (1H, m, H32), 1.61 (1H, m, H25), 1.28 (3H, s, Me53); MALDI-TOF MS, calcd for $C_{59}H_{70}O_{10}Na$ 961.4867 ($M+Na^+$), found 961.4402.

Pentaene S5. To a solution of nitrile **S4** (6.2 mg, 6.5 μmol) in CH_2Cl_2 (1 mL) at –80 °C was added DIBAL (0.98 M solution of in hexane, 70 μL, 65 μmol). After being stirred for 1 h at –50 °C, the reaction mixture was quenched with EtOAc and 1N HCl. The mixture was extracted with EtOAc (x3), and the organic layer was washed with brine and dried over Na_2SO_4. Concentration and filtration through a pad of flash silica gel gave the aldehyde, which was used in the next reaction without further

purification.

To a suspension of Ph_3PCH_3Br (234 mg, 654 μmol) in THF (1 mL) at 0 °C was added *t*-BuOK (60.3 mg, 327.0 μmol). After 15 min, a solution of aldehyde in THF (1 mL) at 0 °C was added dropwise to the reaction mixture. After being stirred for 30 min at 0 °C, the reaction mixture was quenched with aqueous NH_4Cl. The mixture was extracted with EtOAc (x3), and the organic layer was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc 10:1–3:1) gave the pentaene **S5** (3.0 mg, 3.2 μmol) in 48% yield over 2 steps: colorless amorphous; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34–7.20 (25H, m, Bn), 5.94 (1H, ddt, *J* = 17.0, 10.0, 6.5 Hz, H23), 5.78 (1H, m, H24), 5.75 (1H, m, H18), 5.62 (1H, dd, *J* = 11.5, 5.5 Hz, H19), 5.04 (1H, dd, *J* = 18.0, 2.0 Hz, H23'), 5.01 (1H, dd, *J* = 10.5, 2.0 Hz, H23'), 4.99 (1H, dd, *J* = 17.5, 1.0 Hz, H24'), 4.96 (1H, dd, *J* = 10.5, 1.0 Hz, H24'), 4.89 (1H, d, *J* = 12.0 Hz, Bn), 4.63 (1H, d, *J* = 12.0 Hz, Bn), 4.62 (1H, d, *J* = 12.0 Hz, Bn), 4.61 (1H, d, *J* = 12.0 Hz, Bn), 4.60 (1H, d, *J* = 12.0 Hz, Bn), 4.55 (1H, d, *J* = 12.0 Hz, Bn), 4.52 (1H, d, *J* = 12.0 Hz, Bn), 4.48 (1H, d, *J* = 12.0 Hz, Bn), 4.43 (1H, d, *J* = 12.0 Hz, Bn), 4.36 (1H, d, *J* = 12.0 Hz, Bn), 3.89 (1H, m, H20), 3.80 (1H, m, H29), 3.77 (1H, m, H35), 3.75 (1H, m, H16), 3.67 (1H, m, H34), 3.65 (1H, m, H35), 3.63 (1H, m, H26), 3.59 (1H, m, H14), 3.52 (1H, m, H15), 3.49 (1H, m, H27), 3.48 (1H, m, H14), 3.39 (1H, m, H31), 3.36 (1H, m, H33), 3.35 (1H, m, H21), 2.55 (1H, m, H22), 2.51 (1H, m, H17), 2.47 (1H, m, H25'), 2.23 (1H, m, H22), 2.21 (1H, m, H17), 2.17 (1H, m, H25'), 2.06 (1H, m, H32), 1.92 (1H, ddd, *J* = 14.0, 7.0, 3.0 Hz, H28), 1.91 (1H, m, H25), 1.80 (1H, ddd, *J* = 14.0, 12.0 Hz, H28), 1.63 (1H, m, H25), 1.62 (1H, m, H32), 1.26 (3H, s, Me53); MALDI-TOF MS, calcd for $C_{61}H_{72}O_9Na$ 971.3883 ($M+Na^+$), found 971.5069.

EF'(10)GH model 11. To a degassed solution of pentaene **S5** (3.0 mg, 3.2 μmol) in CH_2Cl_2 (4.0 mL) at room temperature was added $(PCy_3)_2Cl_2Ru$=CHPh (0.4 mg, 0.42 μmol). After being stirred for 12 h at 40 °C, two drops of Et_3N were added at room temperature. Concentration and filtration through a pad of flash silica gel gave tetrakis-Bn EF'(10)GH model, which was used in the next reaction without further purification.

To a solution of tetrakis-Bn EF'(10)GH model in THF (310 μL) and EtOH (310 μL) at -90 °C was added liquid ammonia (ca. 400 μL). Then, Sodium (ca. 1 mg) was added to this solution, and the mixture was stirrered at 3 h. The reaction mixture was quenched with solid NH_4Cl, diluted with EtOAc, and extracted with EtOAc (x3). The organic layer was concentrated, and the residue was used for next reaction without further purification.

To a solution of tetraol in THF (300 μL) at room temperature were added 10-camphorsulfonic acid (1.0 mg, 3.3 μmol) $PhCH(OEt)_2$ (4.6 μL, 31 μmol). After stirring for 4 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$. The mixture was extracted with EtOAc (x3), and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc 10:1–3:1) gave the pentaene **S5** (1.0 mg, 1.5 μmol) in 47% yield over 3 steps.: colorless solid; ^{1}H NMR (500 MHz, C_6D_5N, 25 °C) δ 7.75–7.70 (3H, m, Ph), 7.42–7.37 (7H, m, Ph), 6.14 (1H, m, H19), 5.87 (1H, m,H23), 5.85 (1H, m, H18), 5.79 (1H, s, Ph acetal), 5.66 (1H, s, Ph acetal), 5.57 (1H, m, H24), 4.36 (1H, m, H35), 4.34 (1H, m, H14), 4.27 (1H, m, H29), 4.07 (1H, m, H20), 3.99 (1H, m, H27), 3.95 (1H, m, H16), 3.88 (1H, m, H35), 3.86 (1H, m, H14), 3.83 (1H, m, H34), 3.77 (1H, m, H21), 3.67 (1H, m, H15), 3.64 (1H, m, H33), 3.61 (1H, m, H26), 3.47 (1H, dd, *J* = 12.0, 4.5 Hz, H31), 3.16 (1H, m, H22), 2.98 (1H, m, H17), 2.51 (1H, m, H17'), 2.48 (1H, m, H32), 2.43 (1H, m, H25'), 2.28 (1H, m, H22), 2.19 (1H, m, H28, 2.15 (1H, ddd, *J* =

11.5, 11.5, 11.5 Hz, H32), 2.06 (1H, m, H25'), 2.05 (1H, m, H28), 1.91 (1H, m, H25), 1.55 (1H, m, H25), 1.57 (3H, s, Me53); MALDI-TOF MS, calcd for $C_{38}H_{46}O_9Na$ 669.3034 ($M+Na^+$), found 669.3158.

Mouse neuroblastoma cell assays. Neuro-2a cells (ATCC, CCL131), obtained from Institute of Development Aging and Cancer (Tohoku University, Sendai, Japan), were grown and maintained in 75 cm^2 tissue culture flasks (Falcon) at 37 °C in a humidified 5% CO_2 atmosphere using RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (Gibco), 2 mM L-glutamine, non-essential amino acid, and 1% of antibiotic antifungal solution (50 unit/ ml penicillin G, 50 μg/ ml streptomycin). Cells were harvested by trypsin-EDTA solution (0.5%-0.2%, 5 min at room temperature) and transferred into 96 well microplates (Falcon). For the assay, each well was inoculated with 100 μL of a suspension of 4 x 10^5 cell/ ml in the assay medium. Small aliquots of 100 μL containing the synthetic polyether (51-hydroxyCTX3C or 10-membered F-ring analogue) in the growth medium to give a range of concentrations between 10^{-6} and 10^{-16} M were added to replicate wells (n=3) in the presence of veratridine (20 μM), a site 2-specific sodium channel activator and ouabain (45 μM), a blocker of the Na^+/K^+ ATPase. After incubation for 20 hours, cells were treated with 50 μL of PMS/XTT solution[1] in the growth medium, followed by further incubation for 4 hours. Absorbance at 490 nm was measured on the microplate reader (Bio Rad Model 550). The EC_{50} values of the synthetic polyethers were calculated using GraphPad Prism (v. 4.0) (**Figure S1**, **Table S1**).

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[1] **Scudiero, D. A.; Shoemaker, R. H.; Paull, K. D.; Monks, A.; Tierney, S.; Nofziger, T. H.; Currens, M. J.; Seniff, D.; Boyd, M. R.** ***Cancer Res.*** 1998, ***48***, **4827.**

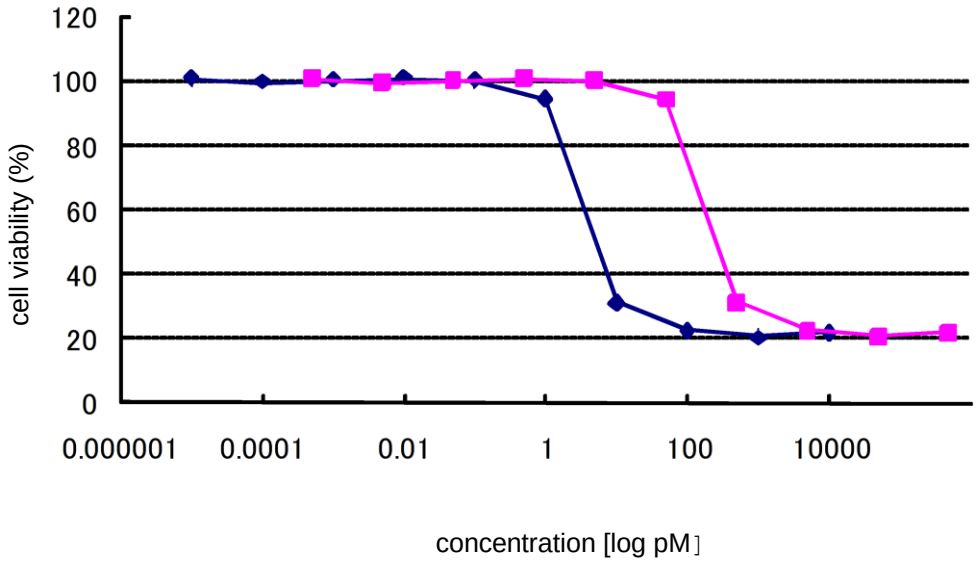


Figure S1. Cytotoxicity of 51-hydroxyCTX3C analogues on Neuro2a (blue diamonds: 51-hydroxyCTX3C (**1**), magenta squares: 10-membered F-ring analogue (**4**)).

	51-hydroxyCTX3C (**1**)	10-membered F-ring analogue (**4**)
EC_{50} (pM)	4.9	240

Table S1. 50% efficient concentration (EC_{50}) of 51-hydroxyCTX3C analogues

Mouse acute toxicity assays. Male ddY mice weight 14-15 g, were obtained from Kumagai Shouten Co., LTD. (Sendai, Japan). Mouse acute toxicity of 10-membered F-ring analogue was evaluated by i.p. injection of the toxin of 14-15 g ddY mice (male). Three mice were administered with different increase dosage of **4** suspended in 0.5 mL of a PBS containing 0.1% Tween 60. Mice were monitored for 24 hours. Signs, death-times and percentage of dead animals were recorded, and LD_{50} was calculated to give 600 μg/kg for **4**.

Molecular Modeling of Synthetic Polyethers. It should be noted that the purpose of this study was not to obtain a statistical distribution of the various possible structures, but to arrive at the conformation that generally satisfied the spectral data in solutions. The molecular modeling was performed using MacroModel Ver. 8.1. Monte Carlo method was used to search the conformational space in which each conformer was minimized with the MM2* force field using a Polak-Ribier conjugate gradient. A ring closure distance range of 0.5-2.5 Å was used. The obtained conformations were subjected to a multiconformer minimization, where a truncated Newton conjugate gradient was applied. The final structure was selected so that the nine- and ten-membered rings adopted a conformation that correlated well with NOE and coupling constant data (**Figures S2** and **S3**).

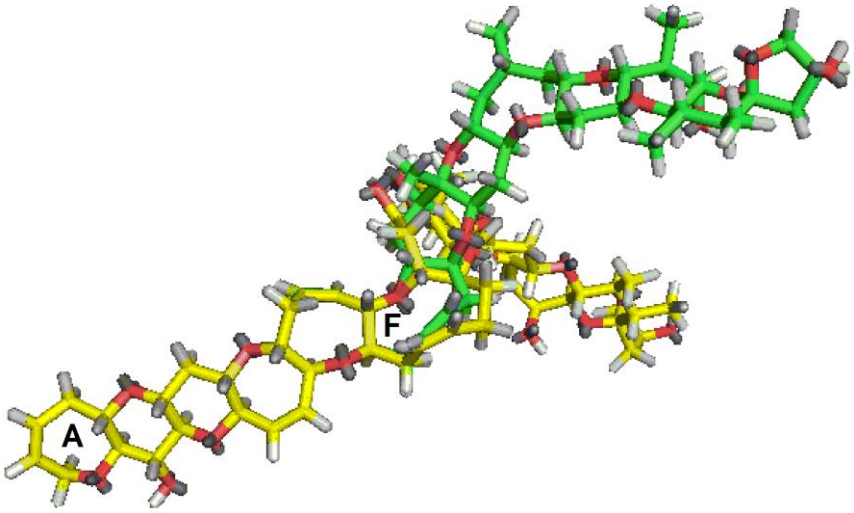


Figure S2. The most stable conformations of 51-hydroxyCTX3C analogues (green: 51-hydroxyCTX3C (**1**), yellow: 10-membered F ring analogue (**4**)). Two molecules were superimposed at ABCDE rings (MM2*, MacroModel ver. 8.1).

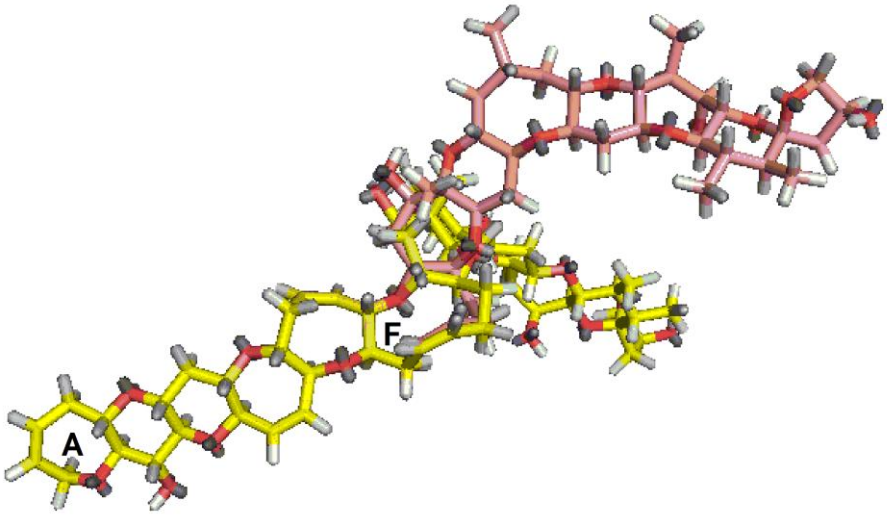
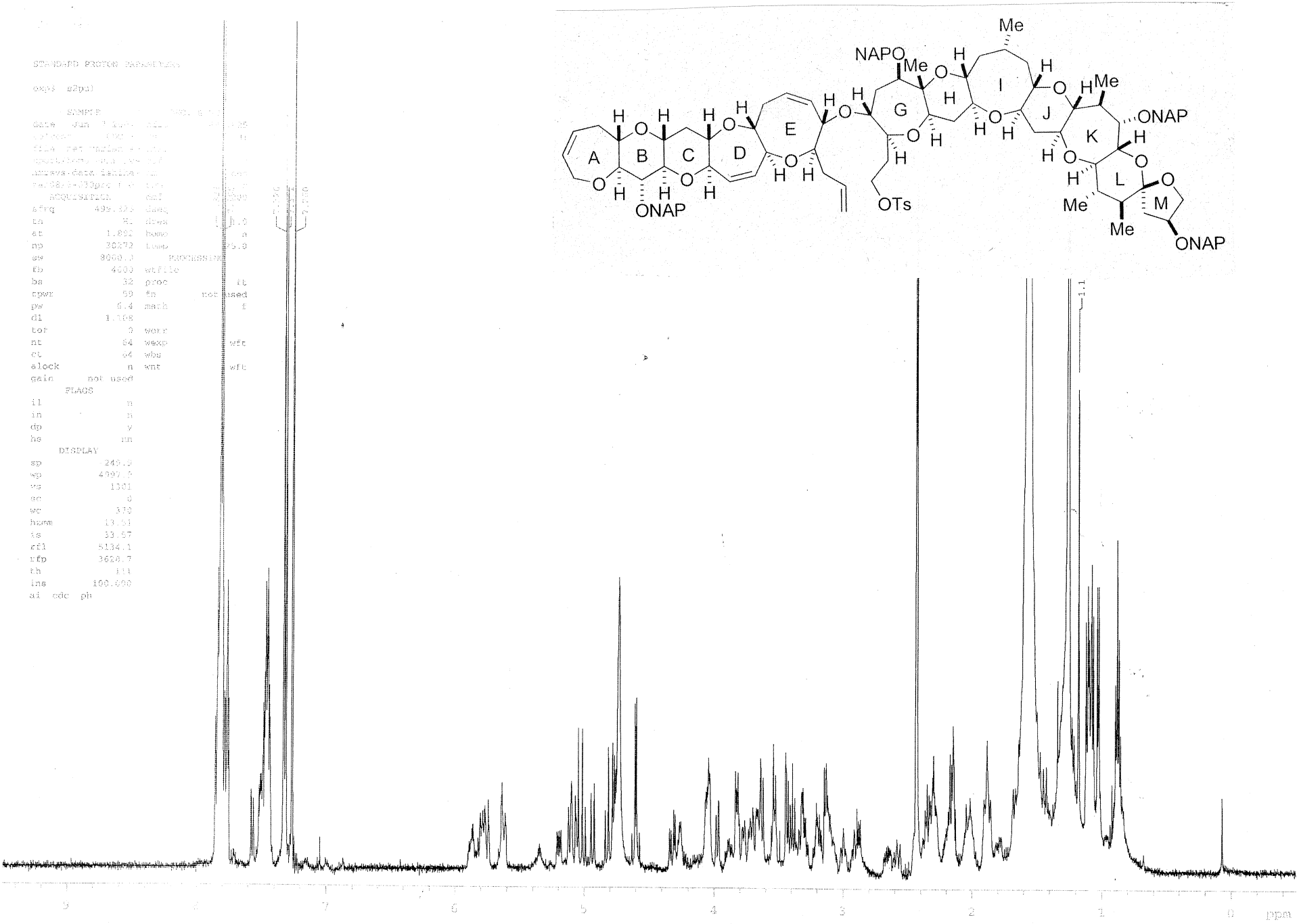
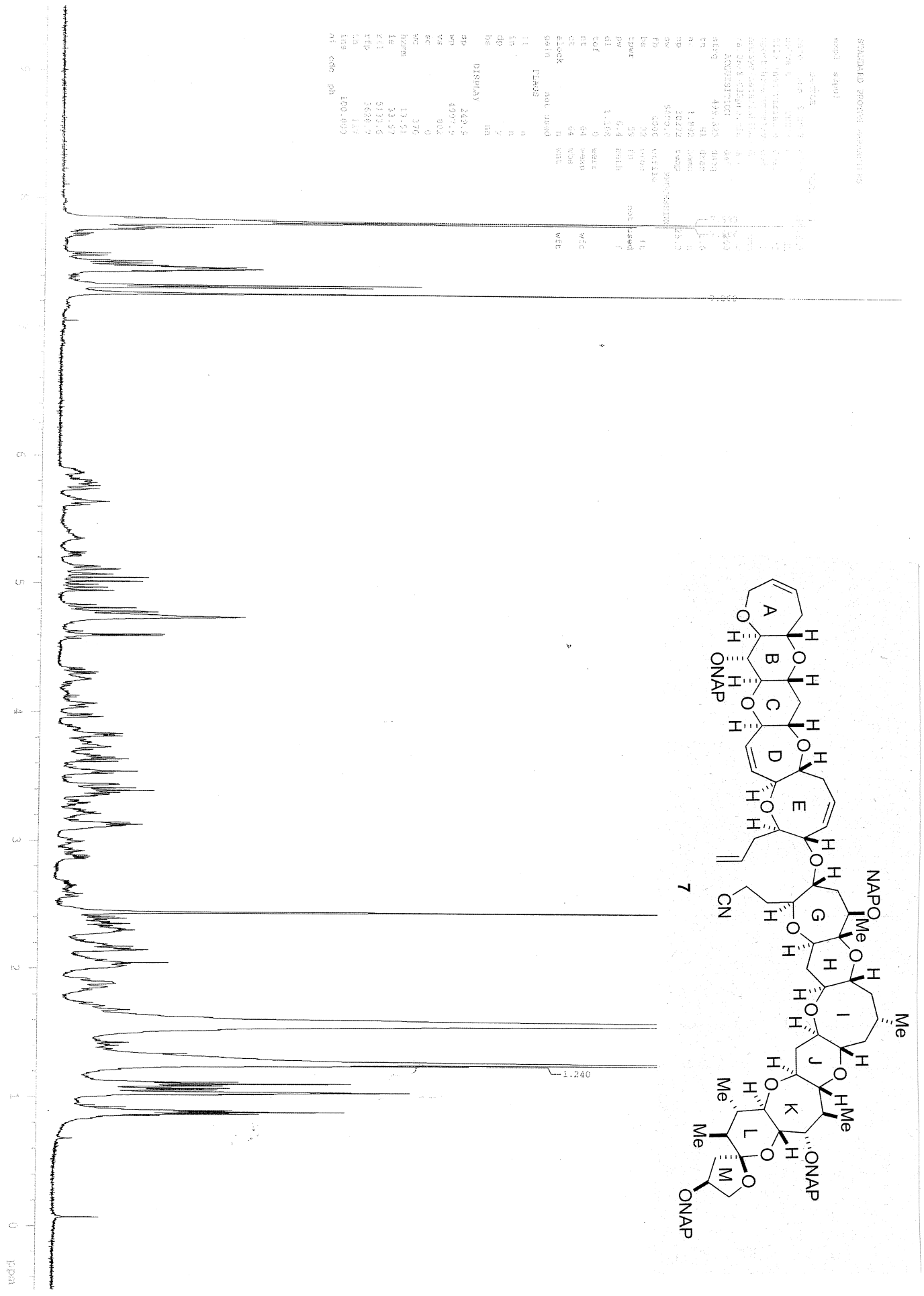
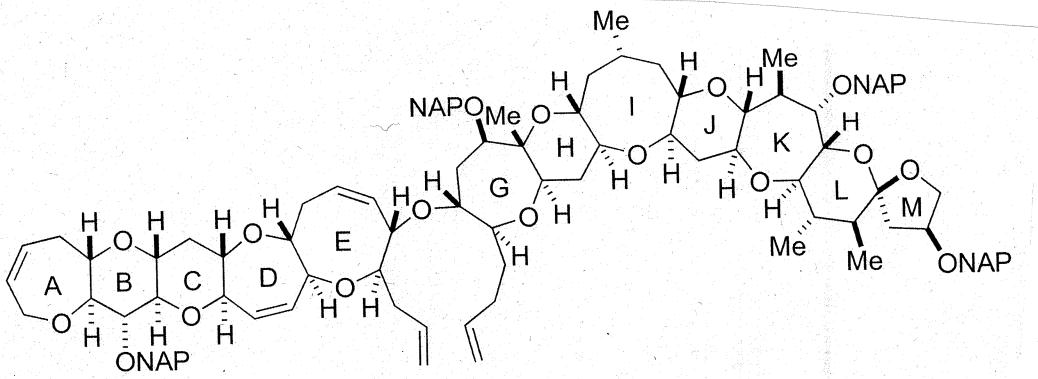


Figure S3. Energy minimized structures of two characteristic conformations of 10-membered F ring analogue (**4**) of 51-hydroxyCTX3C (yellow: the most stable bent form, pink: the straight form found at 2.74 kcal/mol higher level from the most stable conformation). Two conformations were superimposed at ABCDE rings (MM2*, MacroModel ver. 8.1).


Me
NAPO
Me
H
O
I
H
Me
ONAP
H
G
H
J
K
H
H
H


1.240
ppm
7
NAPO
Me
CN
ONAP
Me
Me
Me
ONAP
ONAP
A
B
C
D
E
G
I
J
K
L
M



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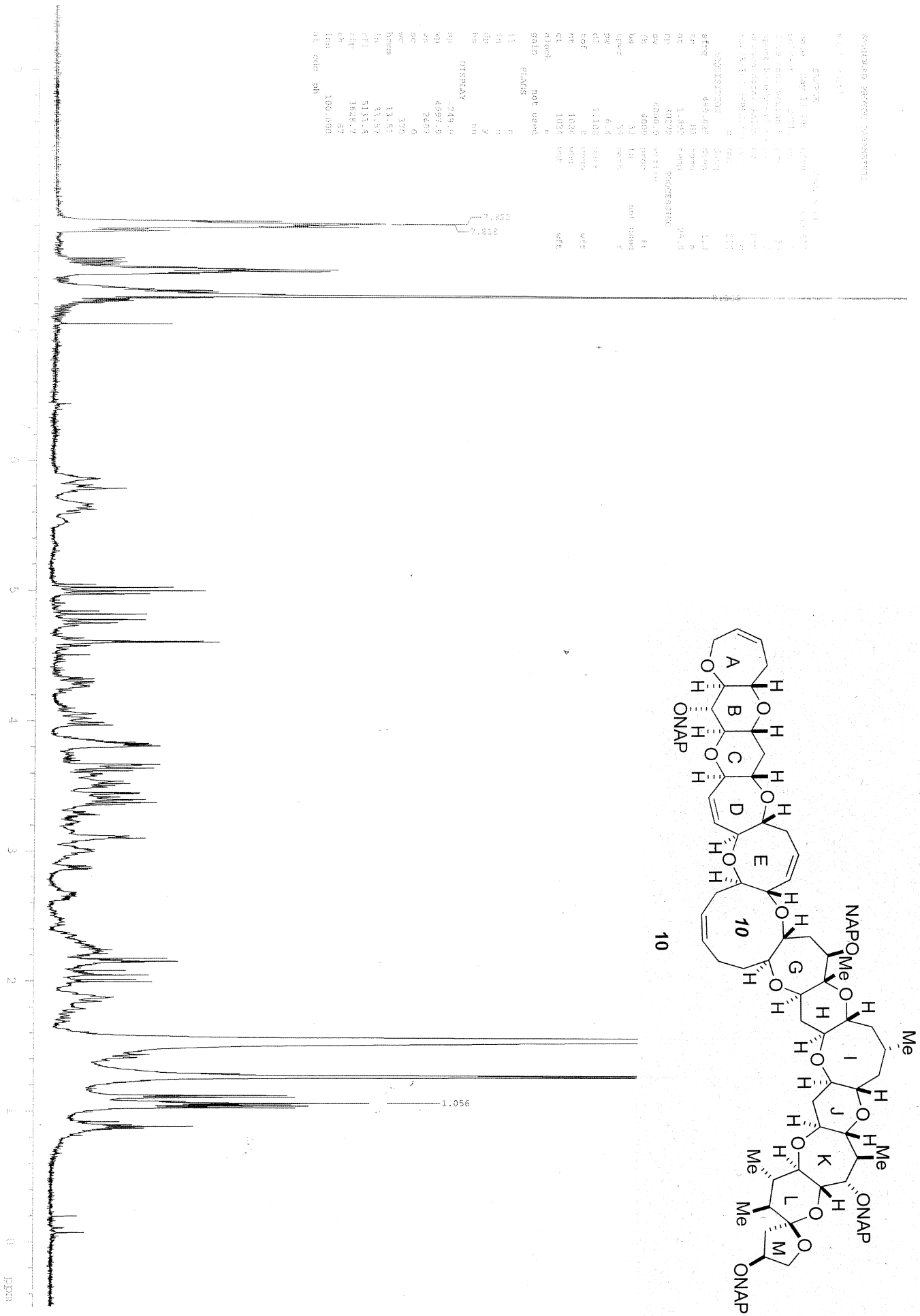
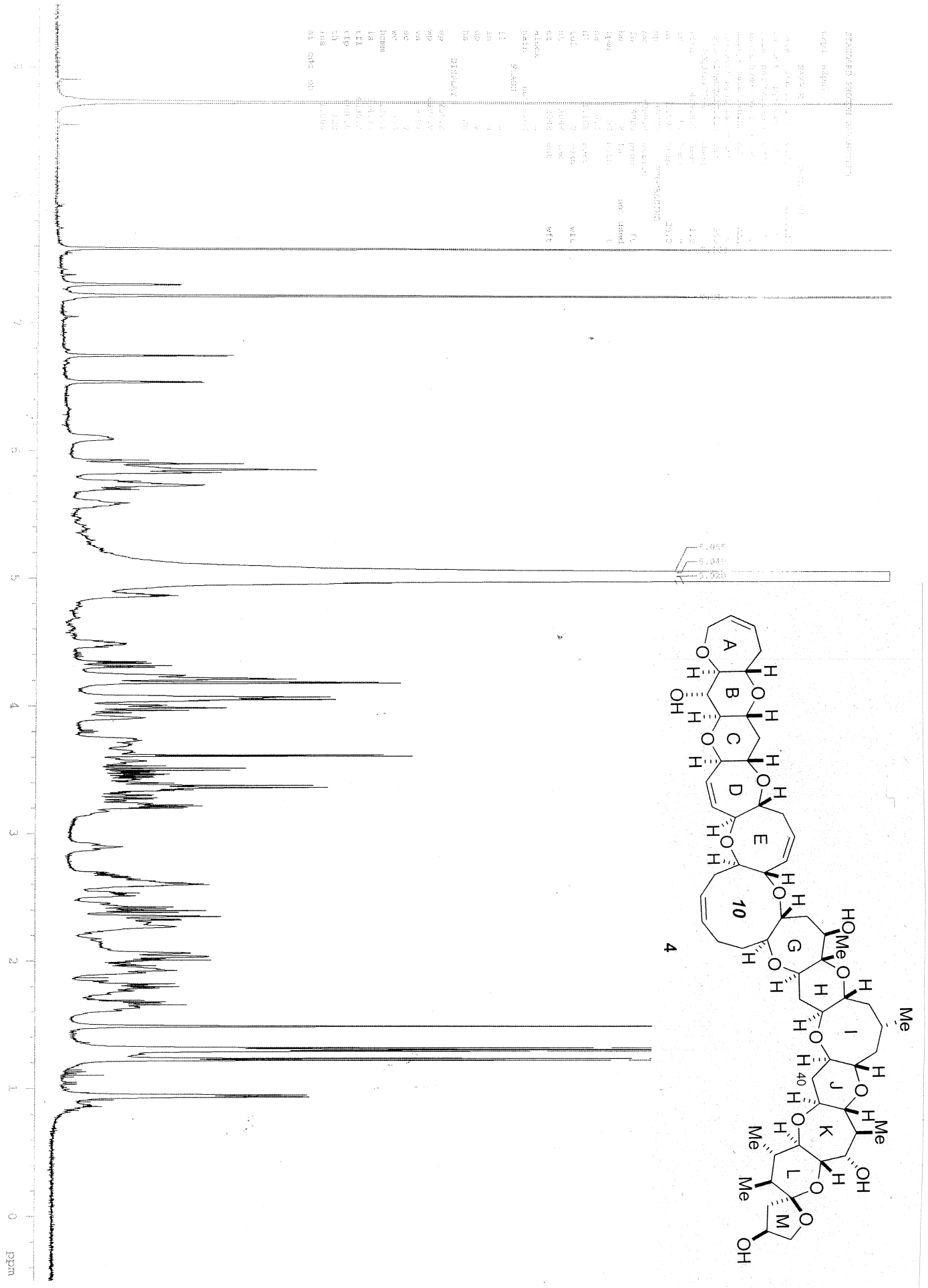

7.833
7.818
1.056
10
NAPO
Me
ONAP
A
B
C
D
E
G
H
I
J
K
L
M



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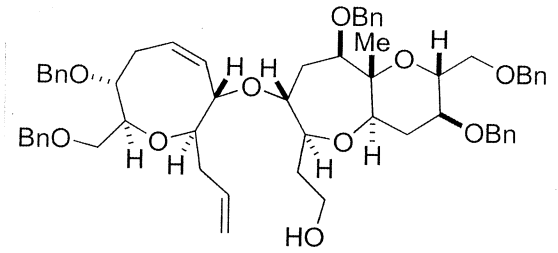

S2

STANDARD PROTON PARAMETERS

exp2 s2pul

SAMPLE		DEC. & VT	
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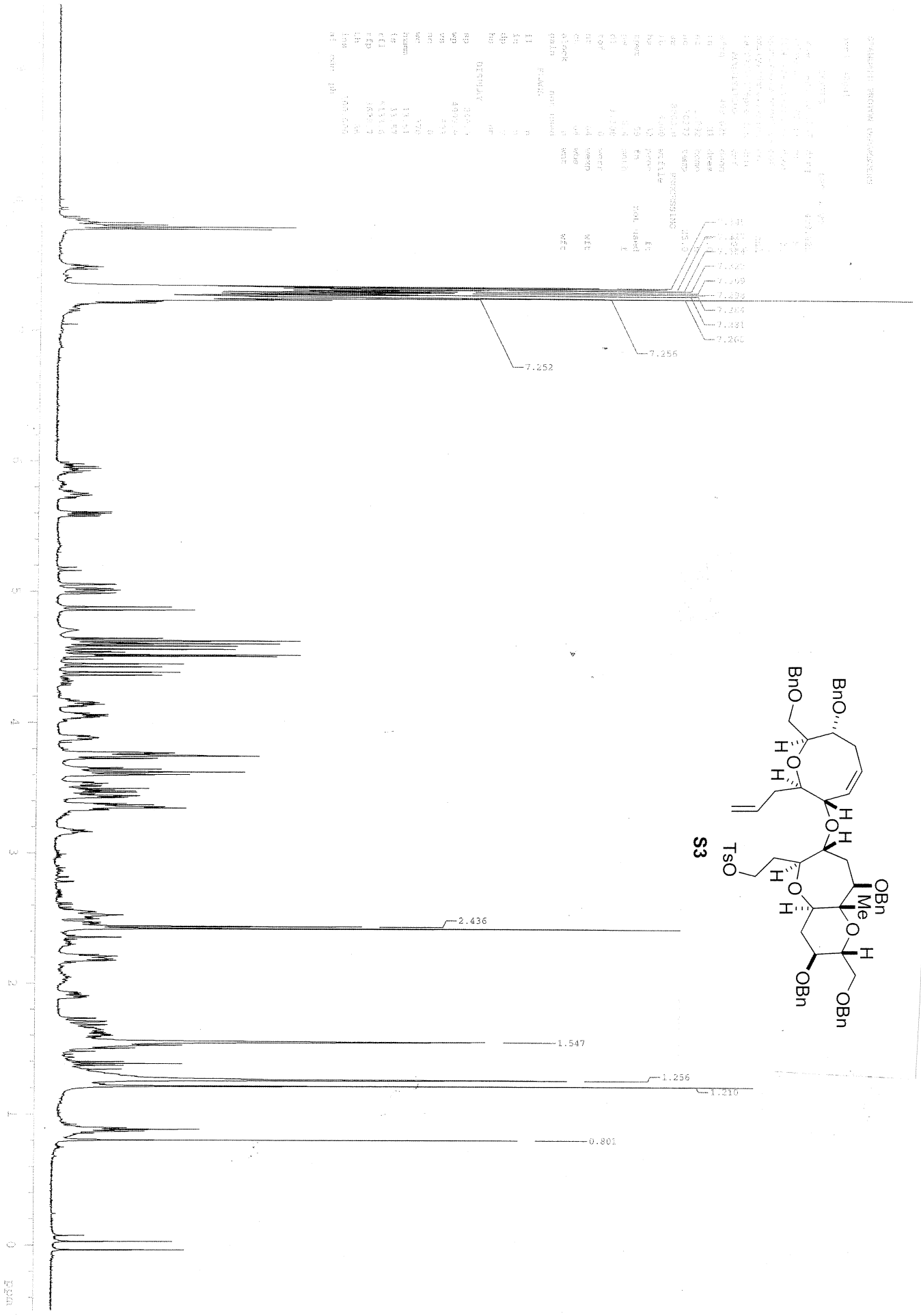
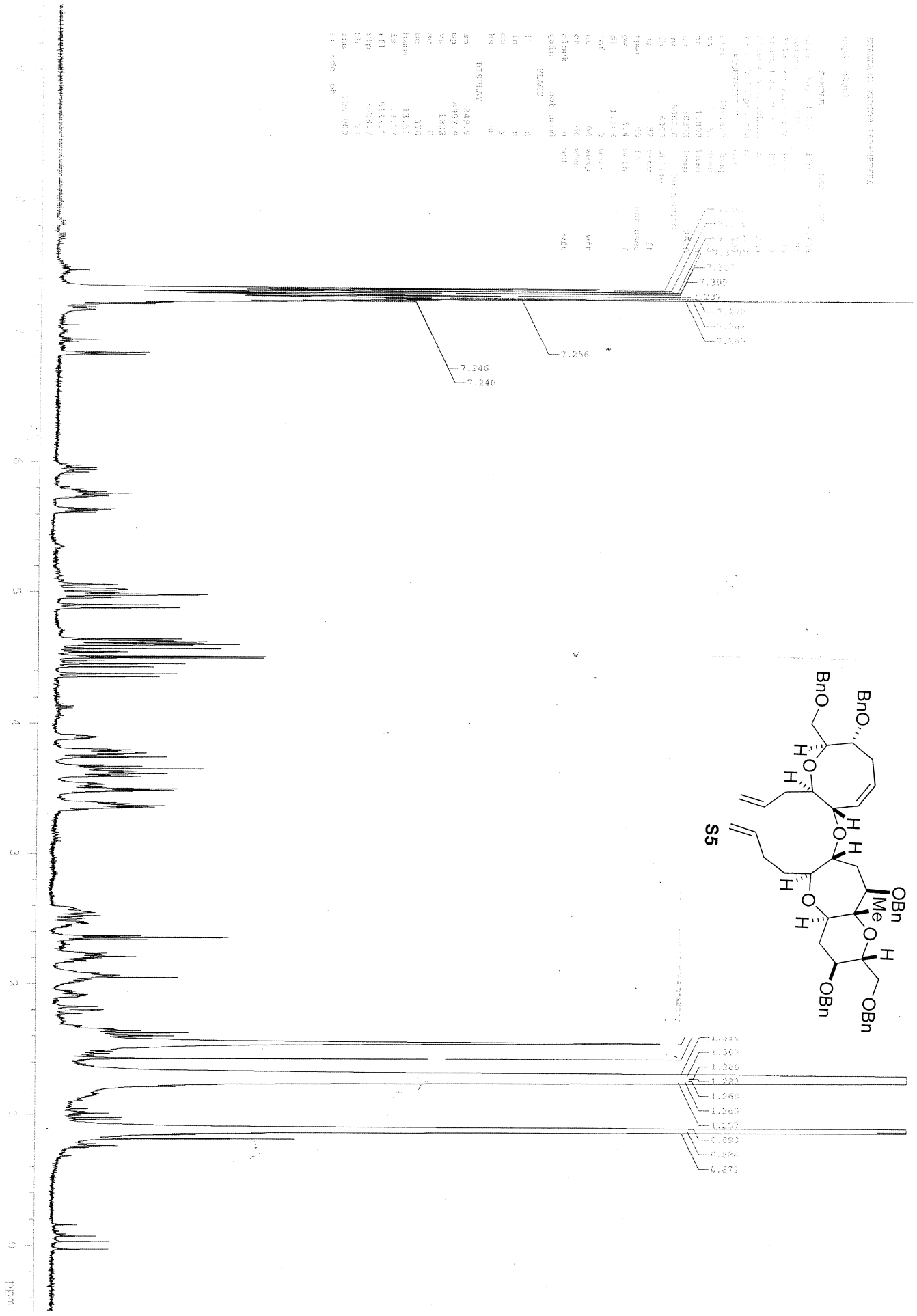
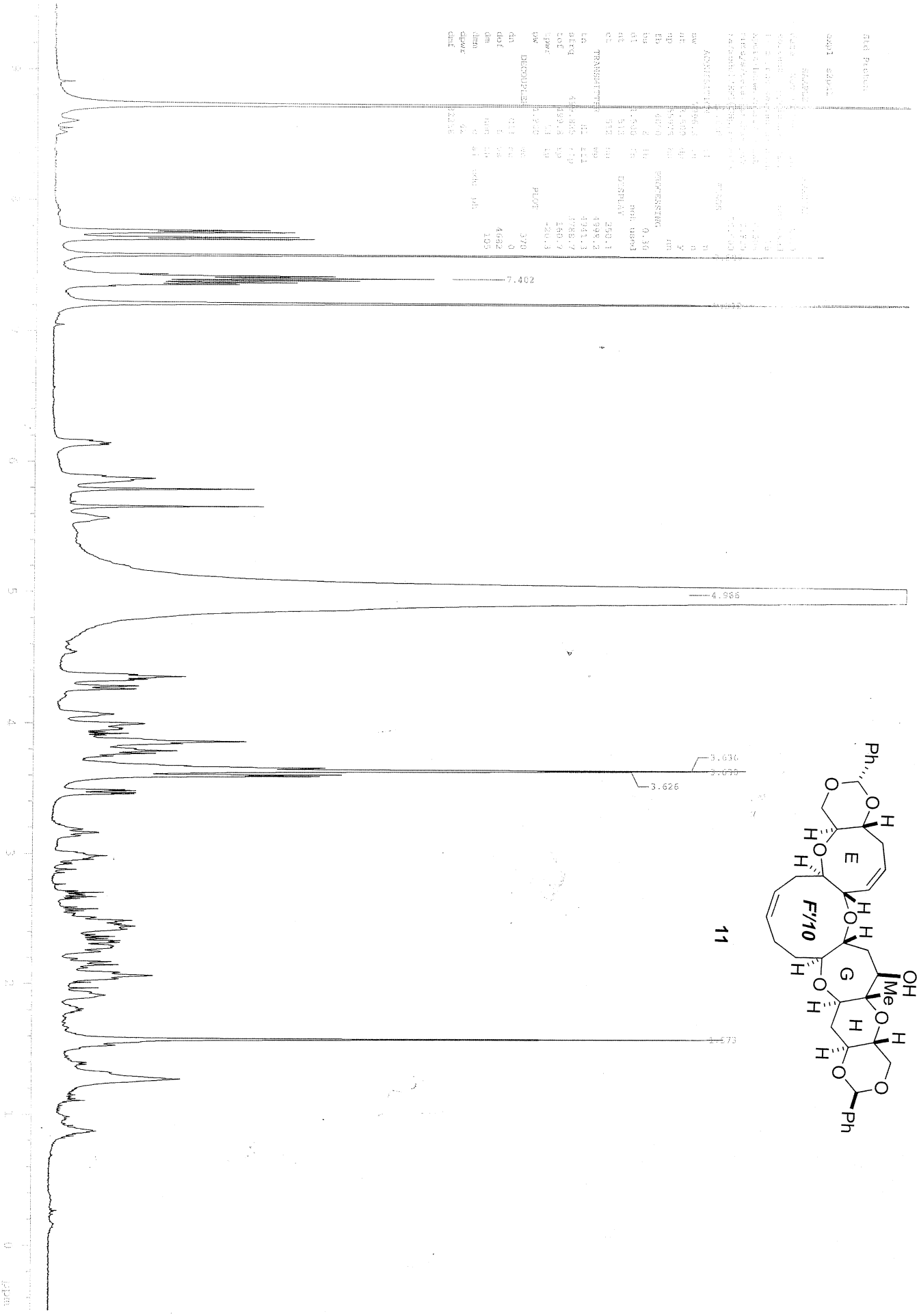
solvent	CDCl3	dn	H1
file	/net/varian/e~	dpwr	30
xport/home/vnmr1/v~		dof	0
nmrsys/data/Miyaza~		dm	nnn
ki/06/06-157pro.fi~		dmm	c
	d	dmf	200
ACQUISITION		dseq	
sfrq	499.825	dres	1.0
tn	H1	homo	n
at	1.892	temp	25.0
np	30272	PROCESSING	
sw	8000.0	wtfile	
fb	4000	proc	ft
bs	32	fn	not used
tpwr	59	math	f
pw	6.1		
d1	1.108	werr	
tof	0	wexp	wft
nt	8	wbs	
ct	8	wnt	wft
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
hs	nn		
DISPLAY			
sp	-249.9		
wp	4997.9		
vs	269		
sc	0		
wc	250		
hzmm	19.99		
is	33.57		
rfl	5133.6		
rfp	3628.7		
th	71		
ins	100.000		
ai cdc ph			

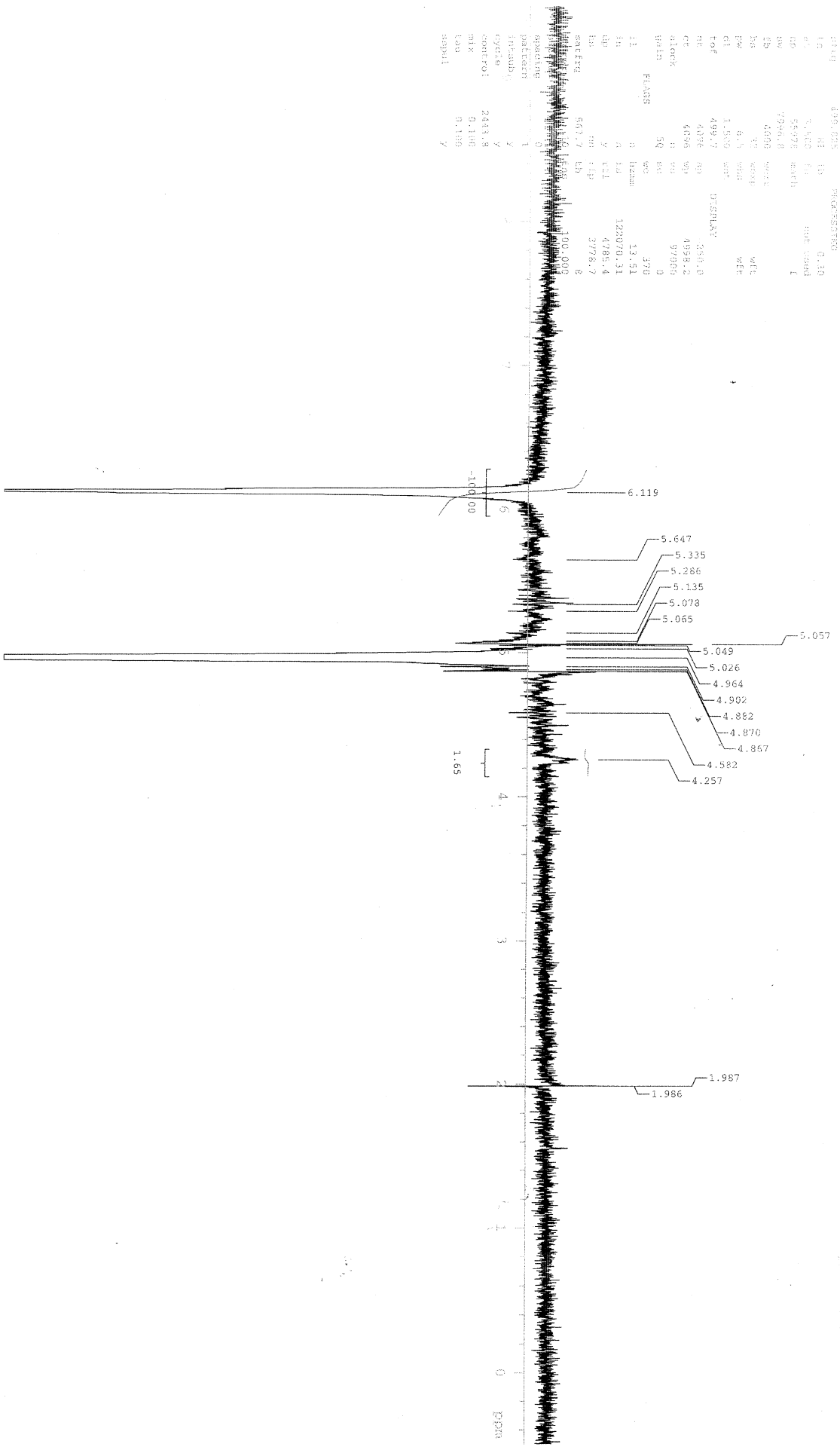

7.252
7.256
2.436
1.547
1.256
1.210
0.801
BnO
BnO
TsO
OBn
Me
OBn
OBn
S3

S4


7.246
7.240
7.256
1.302
1.288
1.282
1.268
1.263
1.257
0.899
0.884
0.871
BnO
BnO
OBn
Me
OBn
OBn
H
O

S5


7.402
4.986
3.630
3.626
3.573
11
Ph
O
H
E
F''10
G
OH
Me
Ph


6.119
5.647
5.335
5.286
5.135
5.078
5.065
5.057
5.049
5.026
4.964
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4.882
4.870
4.867
4.582
4.257
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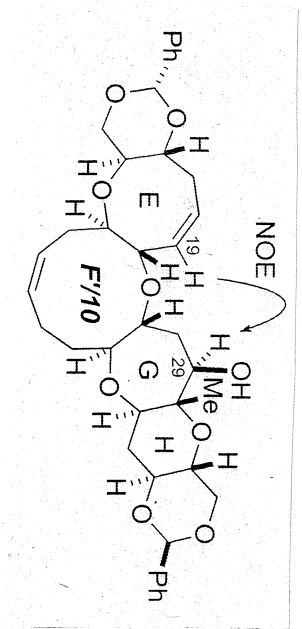
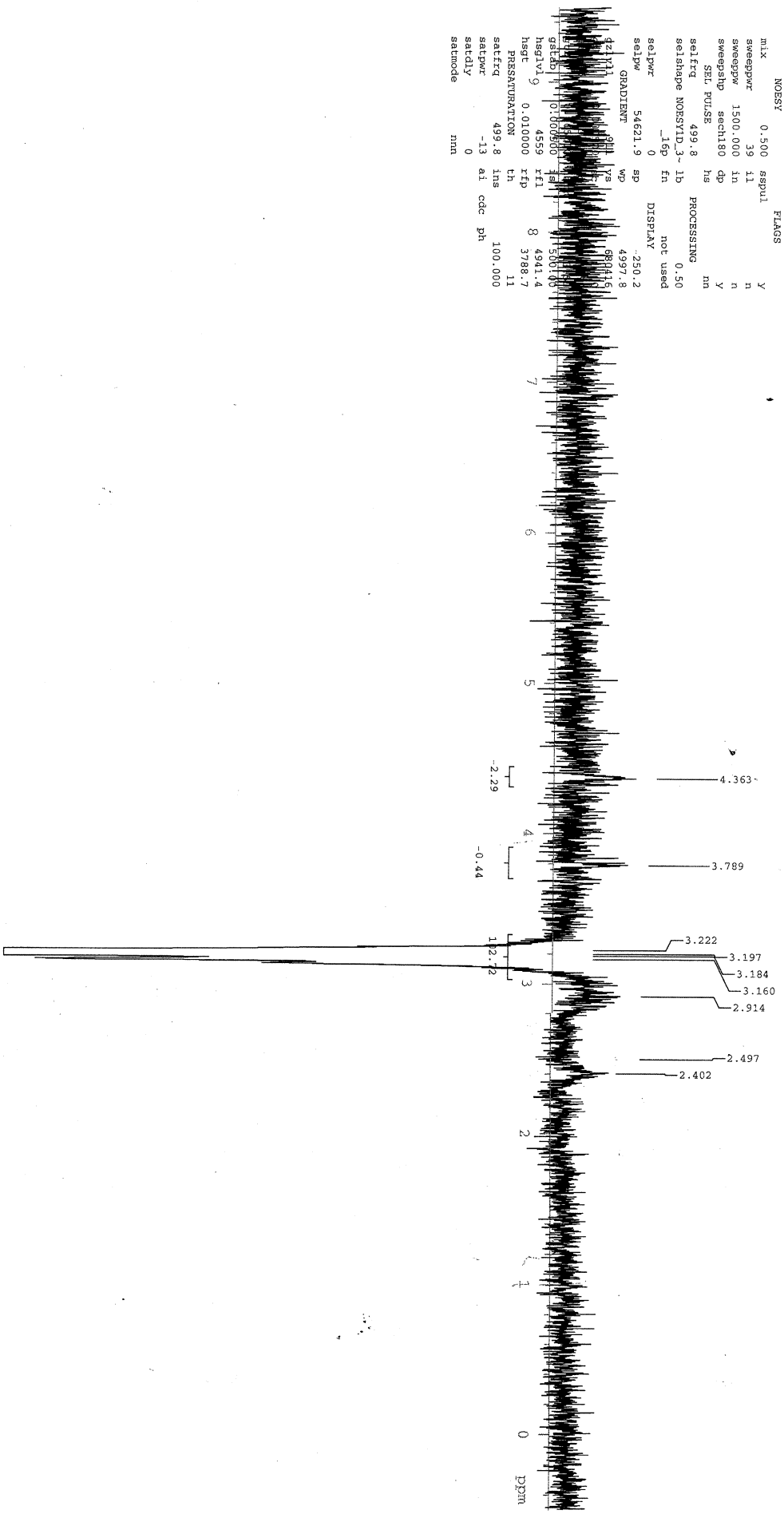
1.986
1.65
ppm


NOE
Ph
O
H
E
F'/10
G
Me
OH
19
29
Ph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at 1.998
np 31954
fb 4400
bs 8
ss 2
dl 2.000
nt 10000
ct 10000
TRANSMITTER
tn H1
sfrq 499.825
tof 499.8
tpwr 53
pw 9.800
NOESY
mix 0.500
sweeppwr 39
sweeppw 1500.000
sweepstp sech180
SEL PULSE
selfrq 499.8
selshape NOESY1D_3~
_16p
0
selpwr 54621.9
selpw 4997.8
GRADIENT
wp 250.2
ws 68041.6
gstab 1.0000000
hsglvl 9 4559
hsgt 0.010000
PRESATURATION
satfrq 499.8
satpwr -13
satdly 0
satmode nnn
DECOUPLER
dn C13
dm nnn
SAMPLE
date Nov 15 2009
solvent pyridine
file /net/varien/e~
xport/home/vnmrl/v~
nmrsys/data/ishiha~
ra/model/BF10GHpyr~
noe22.fid
PROCESSING
hs nn
lb 0.50
fn not used
DISPLAY
sp -250.2
wp 4997.8
vs 68041.6
sc 50010.0
wc 50010.0
rfl 4941.4
rfp 3788.7
th 11
ins 100.000
ai cdc ph
-2.29
-0.44
112.72
4.363
3.789
3.222
3.197
3.184
3.160
2.914
2.497
2.402
ppm
NOE
F'/10
E
G
OH
Me
Ph
Ph
22
25'