Supporting Information

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Synthesis and Biological Activity of Phospholipase C-Resistant Analogues of Phosphatidylinositol 4, 5-bisphosphate

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Experimental details for chemical synthesis.

General. Chemicals were purchased from Aldrich and Acros Chemical Corporation and used without prior purification. Solvents were reagent-grade and distilled before use: CH_2Cl_2 was distilled from CaH_2 and THF was distilled from sodium wire. TLC used precoated silica gel glass sheets (EM SCIENCE silica gel $60F_{254}$). Flash chromatography (FC) employed Whatman 230~400 mesh ASTM silica gel. NMR spectra were recorded on a Varian INOVA 400 at 400 MHz (^{1}H), 101 MHz (^{13}C), 162 MHz (^{31}P) and 376 MHz (^{19}F) at 25 ^{o}C. Chemical shifts are reported in ppm with TMS as internal standard (δ = 0.00); ^{31}P, 85% H_3PO_4 (δ=0.00); ^{19}F, $CFCl_3$ (δ=0.00). Low- and high-resolution mass spectra were obtained on HP5971A MSD and Finnigan MAT95 double focusing mass spectrometer (MS) instruments, respectively. DiC_8-PtdIns(4,5)P_2 was obtained from Echelon Biosciences (Salt Lake City, UT).

(2*S*)-1,2-*O*-cyclohexylidenebut-3-ene-4-bromo-4-fluoro-1,2-diol (7). To a solution of triphenylphosphine (6.29 g, 24 mmol), tribromofluoromethane (2.4 mL, 24 mmol,), and aldehyde **6** (3.4 g, 20 mmol) in anhydrous THF (150 mL), a solution of diethylzinc in hexane (24 mL, 24 mmol) was added dropwise over 1 h at room temperature under argon. The mixture was stirred at room temperature for another 1 h, and concentrated under reduced pressure, the residue was then dissolved in Et_2O and filtered through a short silica gel column, washed with Et_2O. the filtrate was concentrated, and the residue was chromatographied on silica gel (petroleum ether (30 - 50^{o}C)/Et_2O = 100 : 2) to afford bromofluoroolefin **7** (4 g, 76%) as a colorless liquid. Compound **7** is a inseparable *E/Z* mixture (1:1, as determined by ^{19}F NMR).

$[\alpha]^D_{20}$ = -4.2 (*c* 1.1, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$) δ 5.60 (dd, *J* = 12.0, 8.4 Hz, 0.5H),

5.19 (dd, *J* = 30.0, 8.4 Hz, 0.5H), 4.89 (q, *J* = 7.6 Hz, 0.5H), 4.62 (qd, *J* = 7.6, 2.0 Hz, 0.5H), 4.12 (dt, *J* = 10.0, 6.4 Hz, 1H), 3.62 (q, *J* = 8.4 Hz, 1H), 1.61 (m, 8H), 1.39 (m, 2H). ^{19}F NMR (376MHz, $CDCl_3$) δ -66.23 (d, *J* = 12.4 Hz, 0.5F), -71.25 (d, *J* = 32.4 Hz, 0.5F).

Benzyl hydrogen 1-[2,3,6-*O*-tris(methoxymethylene)-4,5-*O*-bis(dibenzoxylphosphoryl) -*myo*-inositol] phosphite (11). A mixture of inositol **9** (100 mg, 0.12 mmol), benzyl *N, N, N, N*-tetraisopropylaminophosphoramidite (84.6 mg, 0.25 mmol) and *N,N*-diisopropylethylammonium 1*H*-tetrazole (27 mg, 0.16 mmol) in dry CH_2Cl_2 was stirred at rt for 1 d, then a second portion of phosphoramidite (84.6 mg, 0.25 mmol) and *N,N*-diisopropylethylammonium 1*H*-tetrazole (27 mg, 0.16 mmol) was added and stirred for an additional day. TLC show the end of the reaction. Then 1*H*-tetrazole (31 mg, 0.3 mmol) was added and stirred for 10 min, followed by addition of water (0.1 mL) and the entire mixture was stirred for 1h. then the mixture was diluted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, concentrated in vacuo, and purified on silica gel (hexane/acetone, 1:1) to give *H*-phosphonate **11**(90 mg, 76%).

$[\alpha]^{D}_{20}$ = -27.4 (*c* 1.255, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 51.2 Hz, 0.5H), 7.36 - 7.22 (m, 25H), 6.10 (d, *J* = 40.8 Hz, 0.5H), 5.15 – 4.84 (m, 10H), 4.79 – 4.56 (m, 4H), 4.52 (dd, *J* = 7.2, 1.6 Hz, 1H), 4.44 - 4.30 (m, 3H), 4.17 (s, 1H), 4.09 – 4.02 (m, 2H), 3.52 (ddd, *J* = 25.6, 10.0, 1.6 Hz, 1H), 3.39 – 3.20 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.31, 136.25, 136.21, 136.13, 136.05, 135.97, 135.71, 135.66, 129.07, 129.05, 128.94, 128.74, 128.73, 128.66, 128.63, 128.57, 128.52, 128.36, 128.33, 128.29, 128.15, 128.09, 99.66, 99.14, 97.78, 97.68, 97.15, 97.08, 79.04, 78.85, 77.72, 77.37, 76.44, 75.26, 74.86, 74.79, 74.66, 74.60, 70.05, 70.00, 69.94, 69.81, 69.75, 69.72, 69.67, 69.53, 69.49, 69.46, 67.81, 67.75, 67.25, 67.19, 56.85, 56.82, 56.13, 56.12, 56.05, 55.97; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.44, 9.21, -0.14, -0.16, -0.20; MS (MALDI) 1009.50 $[M + Na]^+$; MALDI-HRMS

$[M + Na]^+$ calcd for $C_{47}H_{57}O_{17}P_3Na$ 1009.2706, found 1009.2684.

Benzyl 1-[2,3,6-*O*-tris(methoxymethylene)-4,5-*O*-bis(dibenzoxylphosphoryl) -*myo*-inositol] 4-(*sn*-3,4-*O*-cyclohexylidene-1-fluoro-1-buten-1-yl) phosphonate (12). To a 4 mL screw cap reaction vial charged with *H*-Phosphite **11** (300 mg, 0.304 mmol) was added $Pd(OAc)_2$ (12 mg, 0.083 mmol) , dppf (60 mg, 0.106 mmol), bromofluoroolefin **7** (0.24 mL) and propylene oxide (0.5 mL) under argon. Then added dry THF to full. The resulting yellow solution was heated in 70 ^oC oil bath for 10 h. After cooling to room temperature, the solvent was removed in vacuum and the crude residue was purified by column chromatography (SiO_2, hexane/acetone 7:3) to afford compound **12** (218 mg, 62%) as a colorless oil.
$[\alpha]^D_{20}$ = -18.8 (*c* 1.14, $CHCl_3$); ^1H NMR(400 MHz, $CDCl_3$) δ 7.38 – 7.24 (m, 25H), 6.17 – 5.95 (m, 1H), 5.19 – 4.84 (m, 12H), 4.77 (d, *J* = 2.1 Hz, 1H), 4.75 (s, 1H), 4.73 – 4.67 (m, 1H), 4.64 – 4.52 (m, 2H), 4.43 (t, *J* = 6.8 Hz, 1H), 4.40 – 4.32 (m, 1H), 4.31 – 4.27 (m, 1H), 4.23 (td, *J* = 9.6, 2.0 Hz, 1H), 4.13 (td, *J* = 10.0, 4.0 Hz, 1H), 4.06 (td, *J* = 6.4, 2.0 Hz, 1H), 3.61 (td, *J* = 8.4, 1.6 Hz, 0.3 H), 3.50 (td, *J* = 8.4, 1.6 Hz, 1.7H), 3.38 – 3.21 (m, 9H), 1.62 – 1.56 (m, 8H), 1.39 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.38, 136.33, 136.31, 136.25, 136.20, 136.12, 135.31, 129.15, 129.11, 128.98, 128.93, 128.69, 128.64, 128.62, 128.58, 128.50, 128.47, 128.40, 128.32, 128.28. 128.25, 128.16, 125.55, 125.26, 110.96, 110.88, 98.89, 97.83, 97.76, 97.07, 78.81, 77.42, 76.09, 76.02, 75.93, 75.50, 75.14, 75.06, 74.70, 69.95, 69.90, 69.79, 69.74, 69.68, 69.63, 69.59, 69.53, 69.47, 69.42, 69.30, 69.26, 69.18, 68.94, 68.88, 68.82, 68.74, 68.63, 57.54, 56.93, 56.55, 56.15, 56.11, 56.05, 36.34, 35.39, 25.25, 24.09, 24.05; ^{19}F NMR (376 MHz, $CDCl_3$) δ -126.80 (m, 1F); ^{31}P NMR (162 MHz, $CDCl_3$) δ 5.49 (1P), -0.16 (1P), -0.18 (1P); MS (MALDI) 1193.41 $[M + Na]^+$; MALDI-HRMS $[M + Na]^+$ calcd for $C_{57}H_{70}FO_{19}P_3Na$ 1193.3606, found 1193.3616.

Benzyl 1-[2,3,6-*O*-tris(methoxymethylene)-4,5-*O*-bis(dibenzoxylphosphoryl) -*myo*-inositol]-4-(*sn*-3,4-dihydroxy-1-fluoro-1-buten-1-yl) phosphonate (13). A solution of compound **12** (100 mg, 0.085 mmol) in THF (1 mL) was cooled to 0 ^{o}C, to this solution was added pre-cooled 0 ^{o}C 60% aqueous trifluoroacetic acid (4 mL), the resulting mixture was stirred at 0 ^{o}C for 1 h. After neutralized with saturated sodium hydrogen carbonate solution at 0 ^{o}C, the mixture was extracted with ethyl acetate. The extract was washed with water, dried over Na_2SO_4 and concentrated to leave a residue, the crude residue was purified by column chromatography (SiO_2, ethyl acetate/methanol 10:1) to afford compound **13** (80 mg, 86%) as a colorless oil.

$[\alpha]^{D}_{20}$ = -17.0 (*c* 1.27, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$) δ 7.29 – 7.17 (m, 25H), 6.14 – 5.96 (m, 1H), 5.15 – 4.82 (m, 10H), 4.76 (q, *J* = 9.6 Hz, 1H), 4.68 (d, *J* = 2.0 Hz, 2H), 4.63 (d, *J* = 6.4 Hz, 2H), 4.56 (q, *J* = 6.4 Hz, 1H), 4.47 (d, *J* = 7.6 Hz, 1H), 4.39 – 4.17 (m, 4H), 4.04 (qd, *J* = 9.2, 2.8 Hz, 1H), 3.56 – 3.45 (m, 3H), 3.31 – 3.16 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 136.25, 136.18, 136.04, 135.97, 135.91, 135.26, 129.11, 129.08, 129.06, 128.94, 128.72, 128.67, 128.64, 128.61, 128.58, 128.51, 128.47, 128.37, 128.34, 128.30, 128.28, 128.17, 128.15, 126.42, 126.14, 98.93, 98.82, 97.70, 97.53, 97.43, 97.14, 78.84, 77.59, 77.48, 77.28, 76.96, 75.97, 75.92, 75.63, 75.24, 75.11, 74.91, 74.79, 70.09, 70.03, 69.97, 69.80, 69.75, 69.51, 69.46, 69.38, 69.34, 69.08, 66.68, 66.08, 65.47, 65.34, 57.16, 57.07, 56.96, 56.24, 56.18, 56.15, 56.10, 56.04; ^{19}F NMR (376 MHz, $CDCl_3$) δ -126.00 (m, 1F); ^{31}P NMR (162 MHz, $CDCl_3$) δ 5.80 (1P), -0.20 (1P), -0.26 (1P); MS (MALDI) 1113.35 $[M + Na]^+$; MALDI-HRMS $[M + Na]^+$ calcd for $C_{51}H_{62}FO_{19}P_3Na$ 1113.3123, found 1113.2980.

Benzyl 1-[2,3,6-*O*-tris(methoxymethylene)-4,5-*O*-bis(dibenzoxylphosphoryl) -*myo*-inositol]-4-(*sn*-3,4-*O*-dipalmitoyl-1-fluoro-1-buten-1-yl) phosphonate (14a). A solution of

diol **13** (30 mg, 0.0275 mmol) and palmitic acid (29 mg, 0.11 mmol), EDCI (42 mg, 0.22 mmol) and DMAP (14 mg, 0.11mmol) in 3 mL of dry CH_2Cl_2 was stirred overnight at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with water and dried with Na_2SO_4. concentration of the solvent gave a crude residue, which was purified by column chromatography (SiO_2, ethyl acetate/hexane 3:2 to 2:1) to afford the phosphoinositide **14a** (36 mg, 84%) as a colorless oil. $[\alpha]^D_{20}$ = -12.7 (*c* 1.13, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$) δ 7.31 - 7.16 (m, 25H), 6.00 - 5.82 (m, 1H), 5.80 – 5.70 (m, 1H), 5.13 – 4.84 (m, 10H), 4.80 (q, *J* = 9.2 Hz, 1H), 4.71 – 4.60 (m, 3H), 4.54 (dd, *J* = 10.8, 6.8 Hz, 1H), 4.43 (dd, *J* = 21.0, 6.8 Hz, 1 H), 4.38 – 4.24 (m, 2H), 4.22 – 4.15 (m, 2H), 4.13 – 3.95 (m, 3H), 3.45 (t, *J* = 9.6 Hz, 1H), 3.30 – 3.14 (m, 9H), 2.22 (q, *J* = 6.8 Hz, 4H), 1.52 (m, 4H), 1.18 (brs, 48H), 0.81, t = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.42, 172.69, 172.67, 172.63, 172.58, 136.39, 136.31, 136.21, 136.12, 136.19, 136.12, 129.21, 129.16, 129.14, 128.96, 128.68, 128.64, 128.63, 128.61, 128.57, 128.54, 128.50, 128.45, 128.40, 128.37, 128.27, 128.15, 121.76, 121.76, 121.42, 121.12, 98.92, 97.89, 97.79, 97.72, 97.15, 97.06, 96.83, 78.81, 77.47, 76.14, 75.52, 75.09, 75.04, 69.94, 69.89, 69.79, 69.73, 69.69, 69.64, 69.56, 69.45, 69.40, 69.34, 65.47, 63.71, 56.89, 56.13, 56.09, 56.02, 34.30, 34.27, 34.20, 32.15, 29.93, 29.89, 29.72, 29.60, 29.52, 29.34, 25.04, 25.00, 22.92, 14.36; ^{19}F NMR (376 MHz, $CDCl_3$) δ -121.50 (m, 1F); ^{31}P NMR (162 MHz, $CDCl_3$) δ 4.95 (1P), -0.17 (2P); MS (MALDI) 1589.79 $[M + Na]^+$; MALDI-HRMS $[M + Na]^+$ calcd for $C_{83}H_{122}FO_{21}P_3Na$ 1589.7573, found 1589.7508.

Benzyl 1-[2,3,6-*O*-tris(methoxymethylene)-4,5-*O*-bis(dibenzoxylphosphoryl) -*myo*-inositol]-4-(*sn*-3,4-*O*-dioctanoyl-1-fluoro-1-buten-1-yl) phosphonate (14b). Was obtained from **13** in 68% yield analogously as described for compound **14a.**

$[\alpha]^D_{20}$ = -14.8 (*c* 0.85, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$) δ 7.29 -7.18 (m, 25H), 6.04 -

5.82 (m, 1H), 5.80 – 5.70 (m, 1H), 5.15 – 4.89 (m, 10H), 4.80 (q, *J* = 9.2 Hz, 1H), 4.71 – 4.52 (m, 3H), 4.54 (dd, *J* = 10.8, 6.8 Hz, 1H), 4.43 (dd, *J* = 21.0, 6.8 Hz, 1 H), 4.38 – 4.24 (m, 2H), 4.22 – 4.14 (m, 2H), 4.13 – 3.95 (m, 3H), 3.45 (t, *J* = 9.6 Hz, 1H), 3.29 – 3.14 (m, 9H), 2.22 (q, *J* = 6.8 Hz, 4H), 1.52 (m, 4H), 1.18 (br, 16H), 0.80, t = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) 173.41, 172.68, 172.63, 172.58, 136.39, 136.31, 136.28, 136.20, 136.12, 135.25, 135.18, 135.15, 135.12, 129.21, 129.17, 129.14, 128.97, 128.76, 128.69, 128.61, 128.55, 128.50, 128.45, 128.41, 128.36, 128.26, 128.15, 121.77, 121.48, 121.11, 98.93, 97.89, 97.78, 97.72, 97.15, 97.06, 96.82, 78.81, 77.51, 77.39, 76.20, 76.14, 75.53, 75.09, 75.04, 74.78, 74.71, 74.51, 69.94, 69.88, 69.78, 69.73, 69.68, 69.63, 69.60, 69.55, 69.45, 69.39, 69.33, 65.52, 65.47, 65.39, 65.35, 63.72, 56.89, 56.13, 56.09, 56.02, 34.29, 34.25, 34.18, 31.86, 29.92, 29.27, 29.24, 29.13, 25.03, 25.00, 24.97, 22.82, 14.29; ^{19}F NMR 376MHz, $CDCl_3$) -121.57 (m, 1F); ^{31}P NMR (162 MHz, $CDCl_3$) 4.95 (1P), -0.18 (2P); MS (MALDI) 1365.44 $[M + Na]^+$; MALDI-HRMS $[M + Na]^+$ calcd for $C_{67}H_{90}FO_{21}P_3Na$ 1365.5069, found 1365.5064.

Benzyl 1-[2,3,6-*O*-tris(methoxymethylene)-4,5-*O*-bis(dibenzoxylphosphoryl) -*myo*-inositol]-4-(*sn*-3,4-*O*-dioleoyl-1-fluoro-1-buten-1-yl) phosphonate (14c). Was obtained from **13** in 82% yield analogously as described for compound **14a.** $[]^D_{20}$ = -13.7 (*c* 0.50, $CHCl_3$); ^{1}H NMR(400 MHz, $CDCl_3$) 7.20 (m, 25H), 6.00 – 5.80 (m, 1H), 5.80 – 5.68 (m, 1H), 5.27 (s, 4H), 5.11 – 4.85 (m, 10H), 4.80 (q, 1H), 4.71 – 4.60 (m, 3H), 4.54 (q, *J* = 6.8 Hz, 1H), 4.45 (dd, *J* = 20.4, 6.8 Hz, 1H), 4.38 -4.24 (m, 2H), 4.20 (br, 2H), 4.12 – 3.95 (m, 3H), 3.46 (t, *J* = 9.6 Hz, 1H), 3.29 – 3.14 (m, 9H), 2.21 (t, *J* = 7.2 Hz, 4H), 1.93 (brd, *J* = 6.0 Hz, 8H), 1.52 (br, 4H), 1.20 (br, 40 H), 0.80 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$) 176.58, 173.38, 172.65, 172.60, 172.55, 136.38, 136.31, 136.20, 136.12, 135.25, 135.19, 135.12, 130.24, 129.98, 129.92, 129.21, 129.14, 128.96, 128.68, 128.63, 128.55, 128.50, 128.45,

128.41, 128.36, 128.27, 128.16, 121.77, 121.47, 121.11, 98.92, 97.89, 97.79, 97.72, 97.15, 97.06, 96.84, 78.80, 77.50, 77.42, 76.14, 75.53, 75.11, 75.05, 74.79, 74.72, 74.52, 69.96, 69.90, 69.80, 69.74, 69.70, 69.65, 69.62, 69.57, 69.47, 69.43, 69.35, 65.49, 63.73, 56.89, 56.13, 56.08, 56.01, 34.29, 34.24, 34.17, 34.12, 32.13, 29.99, 29.75, 29.66, 29.54, 29.42, 29.35, 27.44, 27.41, 25.10, 25.01, 22.91, 14.35; ^{19}F NMR (376 MHz, $CDCl_3$) δ -121.67 (m, 1F); ^{31}P NMR (162 MHz, $CDCl_3$) δ 4.95 (1P), -0.19 (1P), -0.21 (1P); MS (MALDI) 1641.81 $[M + Na]^+$; MALDI-HRMS $[M + Na]^+$ calcd for $C_{87}H_{126}FO_{21}P_3Na$ 1641.7886, found 1641.7881.

1-[2,3,6-*O*-tris(hydroxyl)-4,5-*O*-bis(diphosphoryl)-*myo*-inositol]-4-(*sn*-3,4-*O*-dipalmitoyl-1-fluoro-1-butyl) phosphonic acid (1). A solution of fully protected phosphoinositide **14a** (50 mg, 0.032 mmol) and Pd/C (10%, 10 mg) in MeOH (10 mL) was stirred at 1 atm H_2 atmosphere for 6 h, the catalyst was filtered off through a celite pad. The filtrate was concentrated to an almost pure product which was completely dried in vacuum. The residue was subject to ethanethiol (5 mL) and stirred for 1.5 h to afford the benzyl and MOM removed product **1**, which was washed with cold methanol to afford a pure white solid **1** (18 mg, 57%).

$[\alpha]^D_{20}$ = -5.6 (*c* 0.65, $CHCl_3$/MeOH 1:1); ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 5.25 (br, 1H), 4.95 (br, 0.45H), 4.83 (br, 0.55H), 4.43 (q, *J* = 8.8 Hz, 1H), 4.30 (dd, *J* = 25.6, 2.4 Hz, 1H), 4.21-4.00 (m, 3H), 3.97 – 3.88 (m, 2H), 3.56 (br, 1H), 2.26 -2.14 (m, 6H), 1.53 (m, 4H), 1.18 (m, 48H), 0.80(t, *J* = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 175.28, 174.15, 173.96, 34.47, 34.34, 34.21, 32.05, 29.84, 29.79, 29.68, 29.54, 29.49, 29.34, 29.28, 29.21, 25.04, 24.50, 24.96, 22.79, 14.05, 14.03; ^{19}F NMR (376 MHz, $CDCl_3/CD_3OD$ = 1:1) δ -207.26, -208.20, -210.27, -210.93; ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 17.09 (1P), 1.69 (1P), 1.43 (1P); MS (MALDI) 985.52 $[M - H]^+$; MALDI-HRMS $[M + Na]^+$

calcd for $C_{42}H_{82}FO_{18}P_3Na$ 1009.4596, found 1009.4464.

1-[2,3,6-*O*-tris(hydroxyl)-4,5-*O*-bis(diphosphoryl)-*myo*-inositol]-4-(*sn*-3,4-*O*-dioctanoyl-1-fluoro-1-butyl) phosphonic acid (2).Was obtained from **14b** in 53% yield analogously as described for compound **1.**

$[\alpha]^{D}_{20}$ = -6.4 (*c* 1.10, $CHCl_3$/MeOH 1:1); ^{1}H NMR(400 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 5.27 (br,1H), 4.69 (br,1H), 4.41- 4.00 (m, 5H), 3.93 (m, 2H), 3.59 (m, 1H), 2.24 (m, 6H), 1.52 (brs, 4H), 1.20 (brs, 16H), 0.80 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 174.09, 174.06, 34.32, 34.20, 34.06, 34.02, 31.77, 31.75, 29.68, 29.70, 29.11, 29.04, 29.02, 24.97, 24.92, 24.88, 22.63, 13.74.; ^{19}F NMR (376 MHz, $CDCl_3/CD_3OD$ = 1:1) δ -206.82, -207.89, -209.89, -210.56; ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 16.50 (1P), 1.64 (1P), 1.05 (1P); MS (MALDI) 785.22 $[M + Na]^+$; MALDI-HRMS $[M + Na]^+$ calcd for $C_{26}H_{50}FO_{18}P_3Na$ 785.2092, found 785.2086.

1-[2,3,6-*O*-tris(hydroxyl)-4,5-*O*-bis(diphosphoryl)-*myo*-inositol]-4-(*sn*-3,4-*O*-dipalmitoyl-1-fluoro-1-buten-1-yl) phosphonic acid (3). To completely dried compound **14a** (40 mg, 0.0255 mmol) in a 1.5 mL reaction vial under argon atmosphere was added TMSBr (1 mL) and TMSI (0.2 mL) at room temperature. The reaction mixture was stirred at room temperature for 1.5 h. The mixture was concentrated under vacuum to remove excess TMSBr, TMSI, methyl and benzyl bromide or iodide product. The colored residue was shown no MOM and benzyl groups existing through ^{1}H NMR. The residue was treated with methanol (1 mL) and stirred for 1 h, white precipitate was produced. The solvent was removed under vacuum, The colored solid was dried under vacuum and washed with a 0.5 mL of cold methanol to afford a white solid **3** (22 mg, 87.5%) as a pure product.

$[\alpha]^{D}_{20}$ = 5.0 (*c* 0.2, $CHCl_3$/MeOH 1:1); ^{1}H NMR(400 MHz, $CDCl_3/CD_3OD$ = 1:1) δ 5.70 (br,

2H), 4.37 (d, *J* = 9.2 Hz, 1H), 4.18 (d, *J* = 11.6 Hz, 1H), 4.04 (m, 4H), 3.89 (s, 1H), 5.52 (d, *J* = 8.8 Hz, 1H), 2.20 (t, *J* = 7.2 Hz, 4H), 1.48 (t, *J* = 6.8 Hz, 4H), 1.20 (br, 48H), 0.75 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 1:1) 175.31, 174.02, 173.41, 117.00, 79.98, 79.69, 78.72, 78.43, 78.00, 71.32, 70.30, 65.67, 63.85, 34.22, 34.13, 34.11, 32.02, 29.79, 29.75, 29.65, 29.61, 29.56, 29.51, 29.45, 29.44, 29.40, 29.31, 29.21, 29.18, 25.00, 24.94, 22.74, 18.86, 13.91; ^{19}F NMR (376 MHz, $CDCl_3/CD_3OD$ = 1:1) -121.25 (m, 1F); ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ = 1:1) 2.86, 2.21, 1.60, 1.21; MS (MALDI) 1007.44 $[M + Na]^+$; MALDI-HRMS $[M + Na]^+$ calcd for $C_{42}H_{80}FO_{18}P_3Na$ 1007.4439, found 1007.4433.

1-[2,3,6-*O*-tris(hydroxyl)-4,5-*O*-bis(diphosphoryl)-*myo*-inositol]-4-(*sn*-3,4-*O*-dioctanoyl-1-fluoro-1-buten-1-yl) phosphonic acid (4). Was obtained from **14b** in 91% yield analogously as described for compound **3.** $[\alpha]^D_{20}$ = 7.3 (*c* 0.3, $CHCl_3$/MeOH 1:1); ^{1}H NMR(400 MHz, $CDCl_3/CD_3OD$ = 1:1) 5.72 (br, 2H), 4.37 (q, *J* = 8.8 Hz, 1H), 4.18 (d, *J* = 11.6 Hz, 1H), 4.03 (m, 4H), 3.90 (d, *J* = 8.4 Hz, 1H), 3.53 (d, *J* = 8.4 Hz, 1H), 2.21 (t, *J* = 7.2 Hz, 4H), 1.48 (t, *J* = 6.8 Hz, 4H), 1.17 (br, 16H), 0.75 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3/CD_3OD$ = 1:1) 174.51, 173.28, 173.22, 172.61, 117.00, 79.16, 78.90, 77.89, 70.52, 69.50, 64.98, 63.05, 33.37, 33.30, 33.26, 30.95, 28.91, 28.30, 28.28, 28.19, 24.19, 24.09, 21.83, 13.02; ^{19}F NMR (376 MHz, $CDCl_3/CD_3OD$ = 1:1) -121.30 (m, 1F); ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ = 1:1) 2.80 (0.5P), 2.15 (0.5P); 1.58 (1P), 1.17 (1P); MS (MALDI) 783.21 $[M + Na]^+$; MALDI-HRMS $[M + Na]^+$ calcd for $C_{26}H_{48}FO_{18}P_3Na$ 783.1935, found 783.1927.

1-[2,3,6-*O*-tris(hydroxyl)-4,5-*O*-bis(diphosphoryl)-*myo*-inositol]-4-(*sn*-3,4-*O*-dioleoyl-1-fluoro-1-buten-1-yl) phosphonic acid (5). Was obtained from **14c** in 96% yield analogously

as described for compound **3.**

$[\alpha]^{D}_{20}$ = 2.3 (*c* 1.02, $CHCl_3$/MeOH 1:1); ^{1}H NMR(400 MHz, $CD_3OD/CDCl_3$ = 5:1) δ 5.82 - 5.75 (br, 2H), 5.24 (t, *J* = 4.8 Hz, 4H), 4.72 – 4.54 (m, 1 H), 4.42 (s, 1H), 4.25 (d, *J* = 10.8 Hz, 1H), 4.07 – 4.01 (m, 4H), 3.57 (s, OH, 3H), 3.48 (s, 1H), 2.22 (2t, *J* = 7.6 Hz, 4H), 1.93 (d, *J* = 5.6 Hz, 4H), 1.78 -1.70 (m, 2H), 1.64 - 1.56 (m, 2H), 1.52 (m, 4H), 1.22 (br, 40H), 0.80 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, $CD_3OD/CDCl_3$ = 5:1) δ 174.97, 173.73, 173.14, 173.00, 129.83, 129.79, 129.66, 129.58, 117.00, 80.12, 79.85, 79.48, 79.32, 79.03, 78.78, 77.16, 71.91, 71.54, 70.29, 69.80, 66.57, 65.76, 65.13, 63.82, 63.08, 40.76, 40.72, 40.69, 39.94, 39.86, 39.82, 34.00, 33.89, 33.84, 31.94, 31.89, 29.76, 29.74, 29.66, 29.54, 29.51, 29.46, 29.42, 29.37, 29.34, 29.28, 29.26, 29.14, 29.11, 29.08, 29.04, 28.98, 28.84, 28.81, 28.70, 28.59, 27.12, 27.09, 27.04, 24.91, 24.87, 24.83, 22.64, 13.59, 13.56; ^{19}F NMR (376 MHz, $CDCl_3/CD_3OD$ = 1:5) δ -120.94, -123.13, -125.31, -126.60; ^{31}P NMR (162 MHz, $CDCl_3/CD_3OD$ = 1:5) δ 2.21 (br); MS (MALDI) 1059.56 $[M + Na]^+$; MALDI-HRMS $[M + Na]^+$ calcd for $C_{46}H_{84}FO_{18}P_3Na$ 1059.4752, found 1059.4747.

General information for NMR spectra

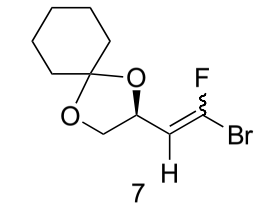
There is a racemic phosphorus stereocenter in compounds **12-14** and **1-5**. In addition, compounds **12-14** and **3-5** are approximately equimolar mixures of E and Z geometries. None of the individual diastereomers can be separated chromatographically. Similarly, compounds **1** and **2** are inseparable mixtures of the two diastereomers at the C-F center. As a result, there are four stereoisomers present in each compound, which leads to the complexity of their NMR spectra.

General experimental protocols for biological studies

Recording of TRPM4 currents. TRPM4b fused to the C terminus of CFP under a CMV promoter was expressed in ChoK1 cells as previously described.[1] Cells were transfected with Fugene (Roche,) according to the manufacturer's instructions and recordings were performed 48-72 hours after transfection. Patch clamp recording was performed from CFP-mTRPM4 expressing cells that were identified under epifluorescence. After formation of a gigaohm seal, the patch was excised into zero Ca^{2+} containing solution and placed in front of a linear array of micro perfusion pipes from which test solutions were delivered (Warner Instruments, Hamden, CT). For all experiments, the membrane potential was held at –80 mV. All recording were made with an Axopatch 200B amplifier, digitized with a digidata 1322a, acquired with pClamp 8.2 and analyzed with Clampfit 8.2 (Axon Instruments, Union City, CA). Patch pipets (1.5-3 MOhm) were fabricated from borosilicate glass. The pipette solution contained (in mM): NaCl, 145; HEPES, 10; $CaCl_2$, 2 (pH 7.4 with NaOH). Bath (cytosolic) solutions were as follows. Zero Ca^{2+} solution was (in mM): NaCl, 145; HEPES, 10; HEDTA, 2 (pH 7.4 with NaOH). 100 mM Ca^{2+} solutions contained (in mM) NaCl, 145; HEPES, 10; 0.1 mM $CaCl_2$ (pH 7.4 with NaOH). Dioctanooyl-PtdIns(4,5)P_2 was obtained from Echelon Biosciences, Inc. (Salt Lake City, UT).

[1]Zhang, Z.; Okawa, H.; Wang, Y.; Liman, E. R. *J. Biol. Chem.* **2005**, *280*, 39185-39192.

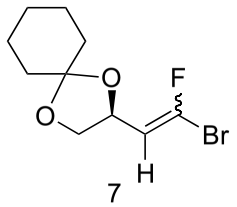
zh12751



7

^{1}HNMR (400 MHz, $CDCl_3$)

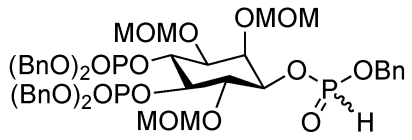
/51F



7

^{19}FNMR (376 MHz, $CDCl_3$)

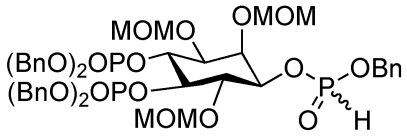
zh10211
CDCl3



11

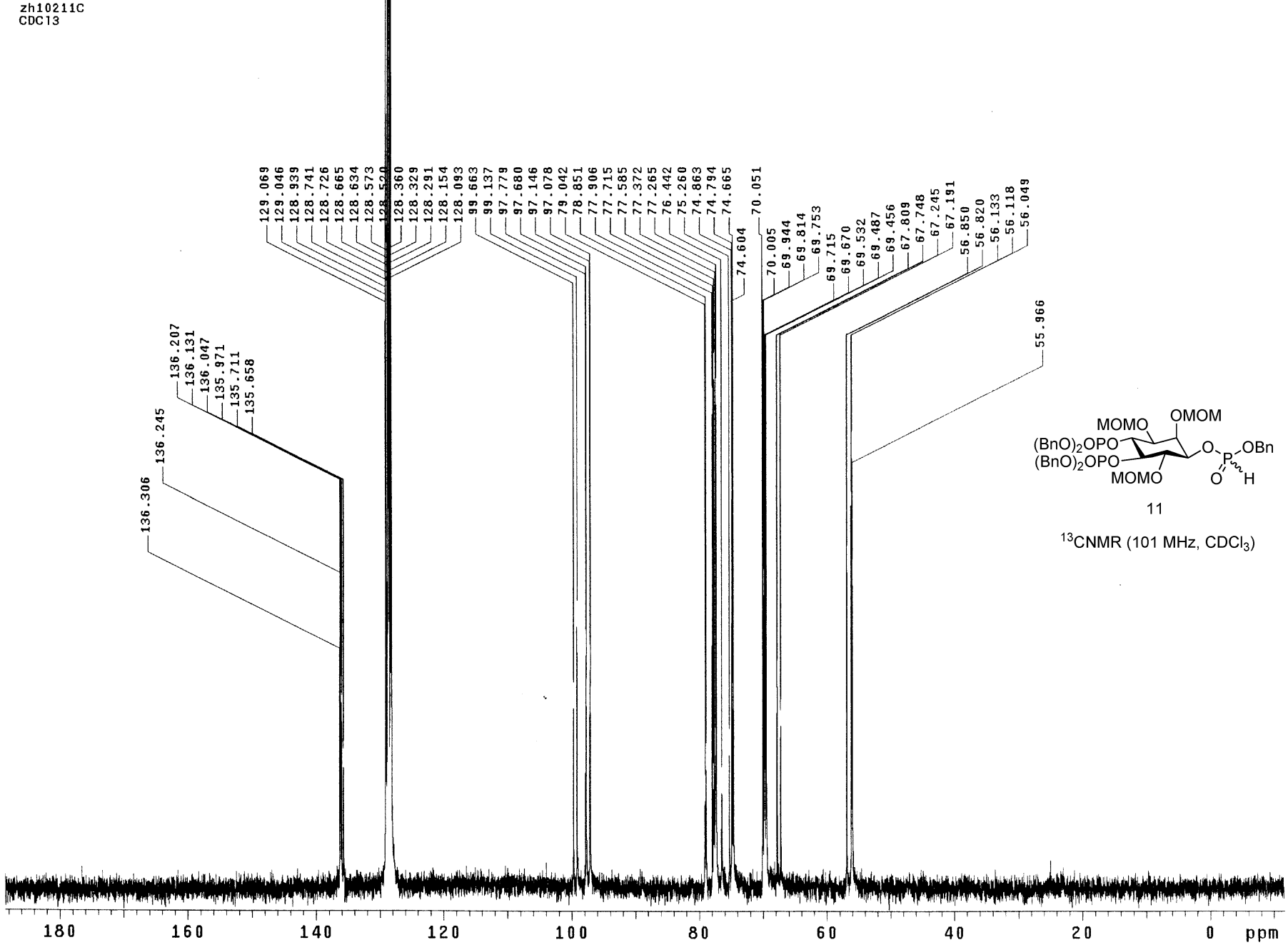
^{1}HNMR (400 MHz, $CDCl_3$)

zh10211P
CDCl3



11

^{31}PNMR (162 MHz, $CDCl_3$)


zh10211C
CDCl3
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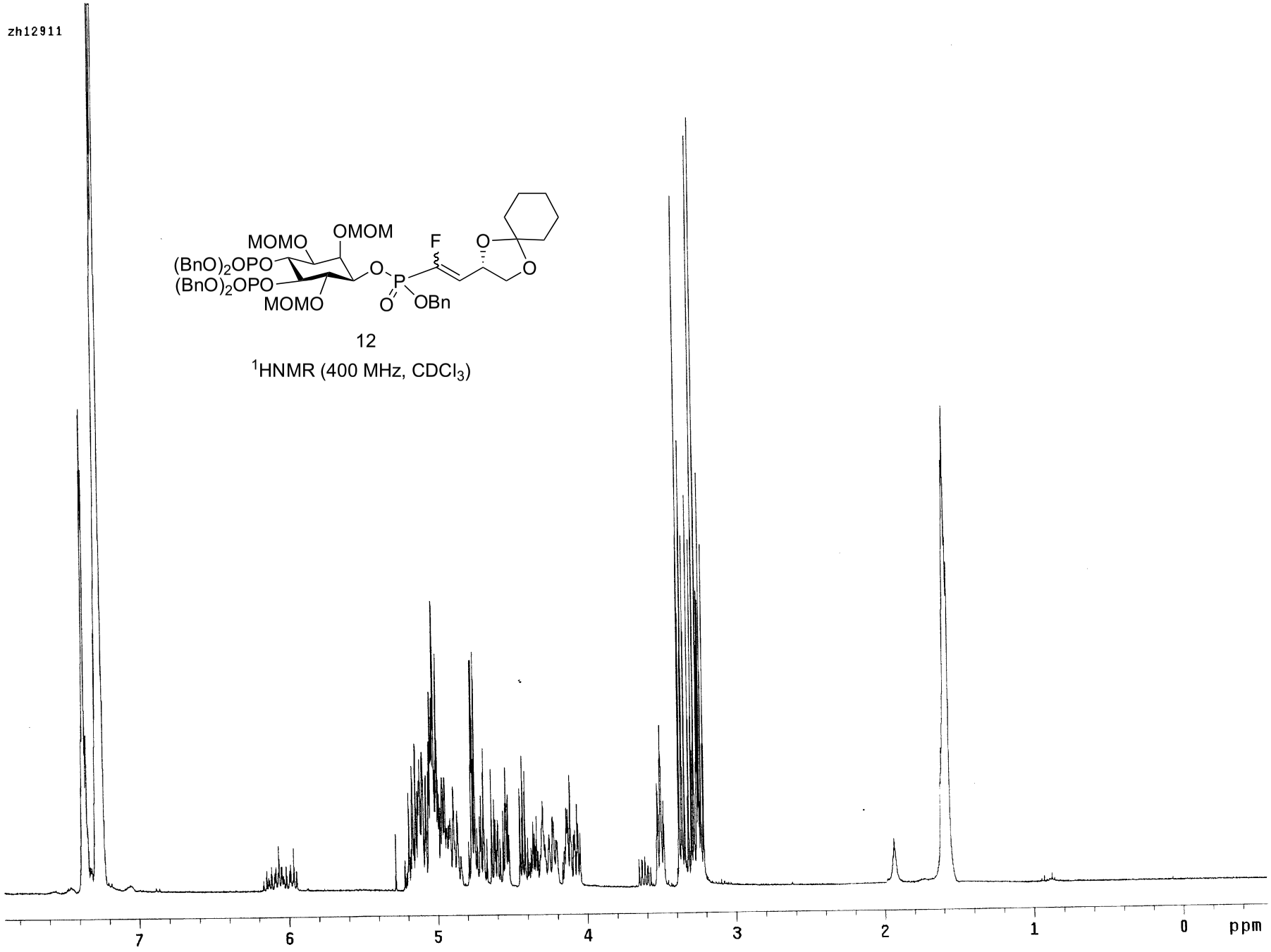
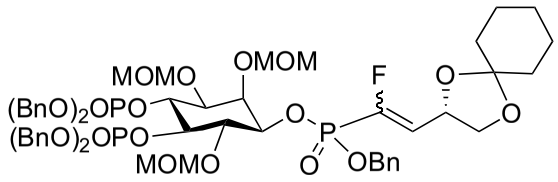
129.046
128.939
128.741
128.726
128.665
128.634
128.573
128.520
128.360
128.329
128.291
128.154
128.093
99.663
99.137
97.779
97.680
97.146
97.078
79.042
78.851
77.906
77.715
77.585
77.372
77.265
76.442
75.260
74.863
74.794
74.665
74.604
70.051
70.005
69.944
69.814
69.753
69.715
69.670
69.532
69.487
69.456
67.809
67.748
67.245
67.191
56.850
56.820
56.133
56.118
56.049
55.966
136.207
136.131
136.047
135.971
135.711
135.658
136.245
136.306
MOMO
OMOM
(BnO)2OPO
(BnO)2OPO
MOMO
O
OBn
P
O
H
11
13CNMR (101 MHz, CDCl3)
180
160
140
120
100
80
60
40
20
0
ppm



12

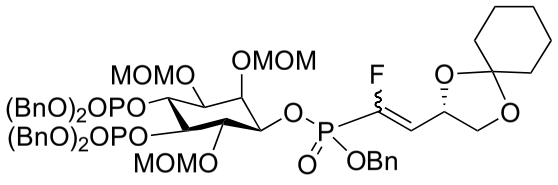
^{1}HNMR (400 MHz, $CDCl_3$)



12

^{19}FNMR (376 MHz, $CDCl_3$)

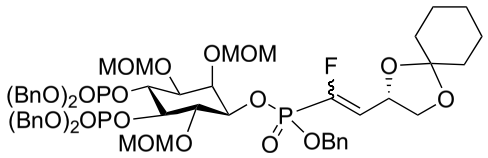
zh12911P



12

^{31}PNMR (162 MHz, $CDCl_3$)

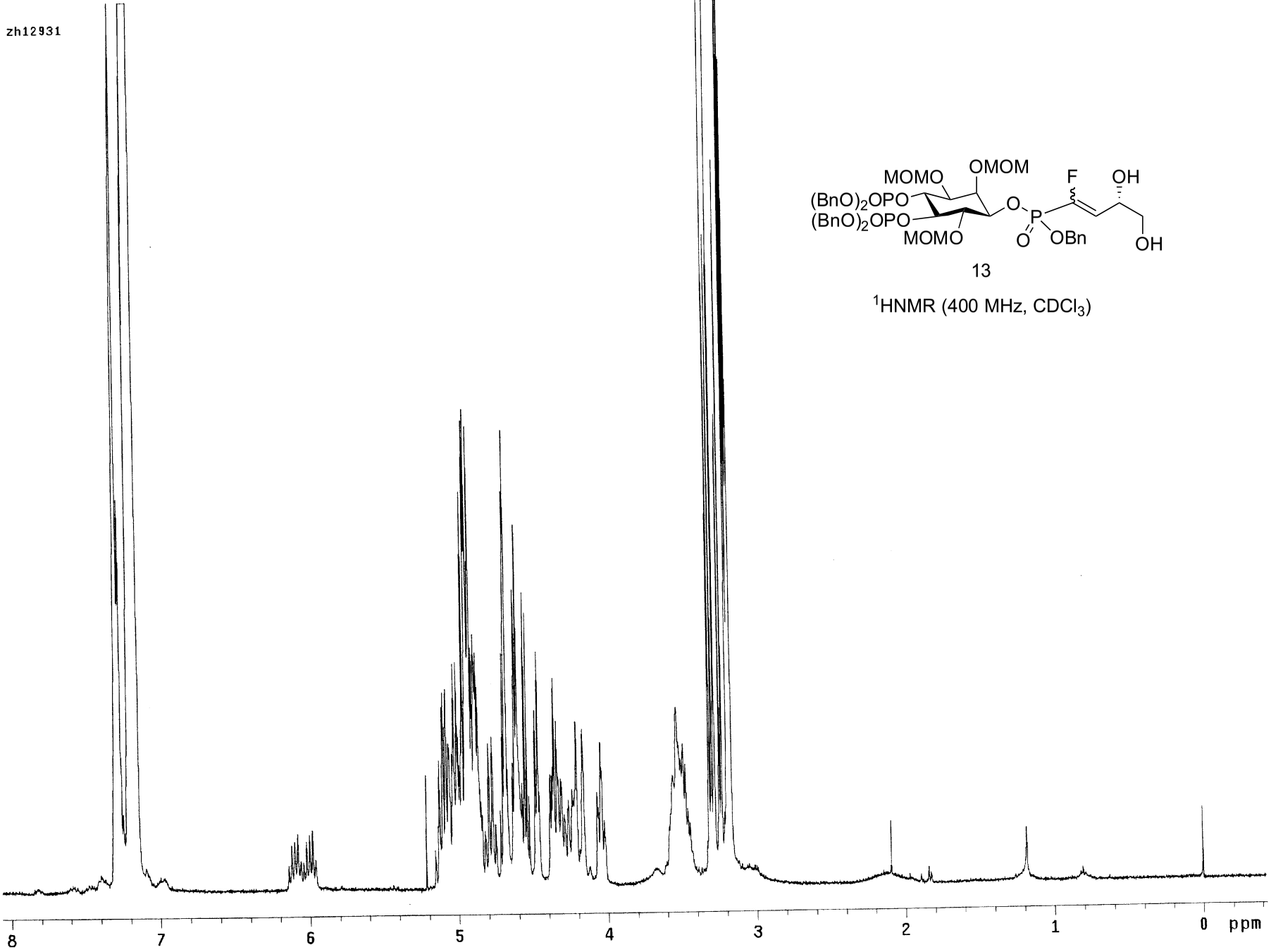
zh12911C



12

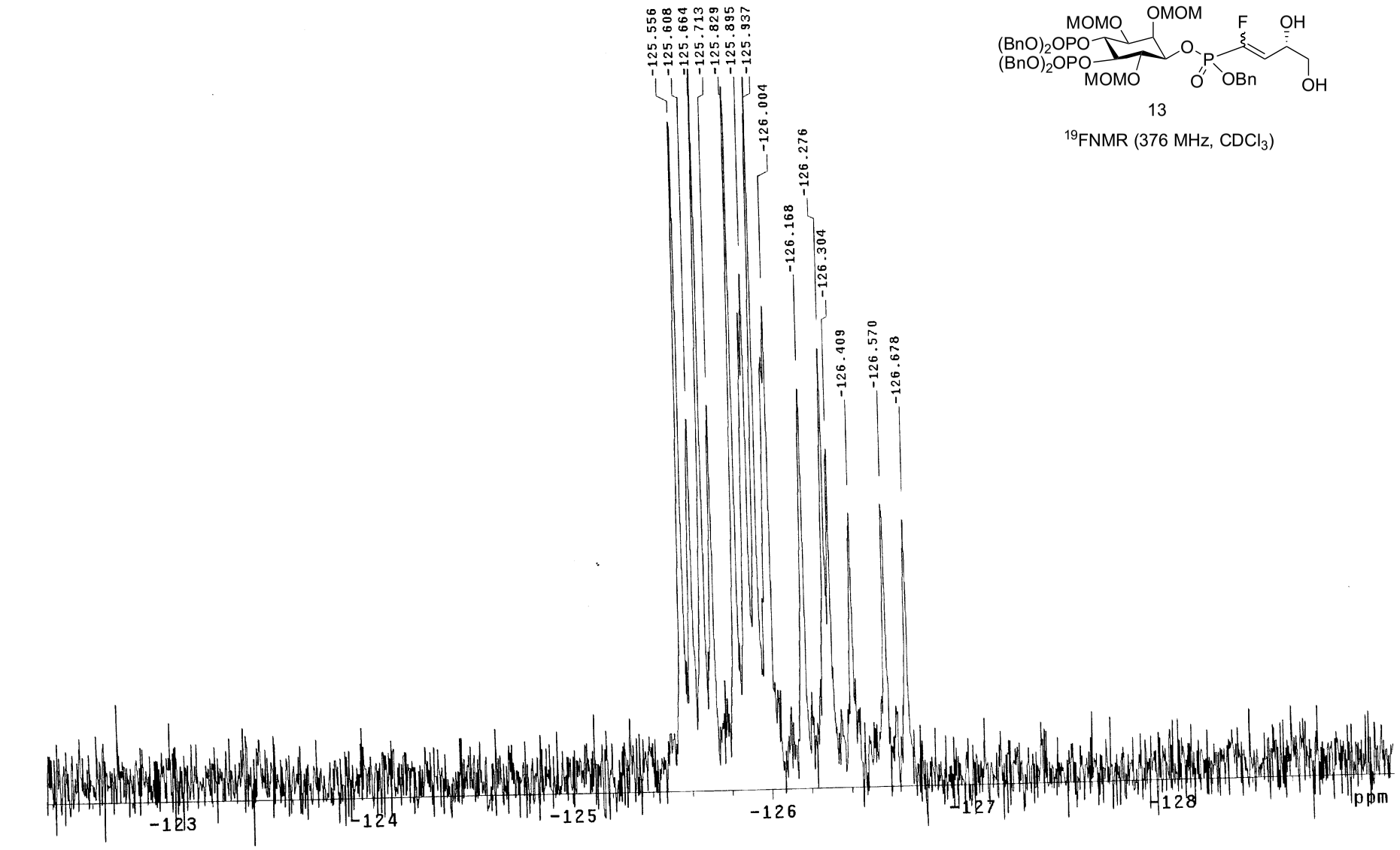
^{13}CNMR (101 MHz, $CDCl_3$)

160 140 120 100 80 60 40 20 0 ppm



13

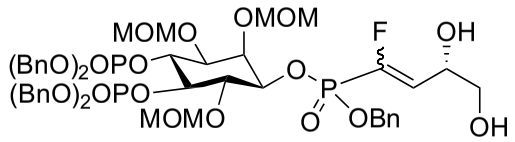
^{1}HNMR (400 MHz, $CDCl_3$)



13

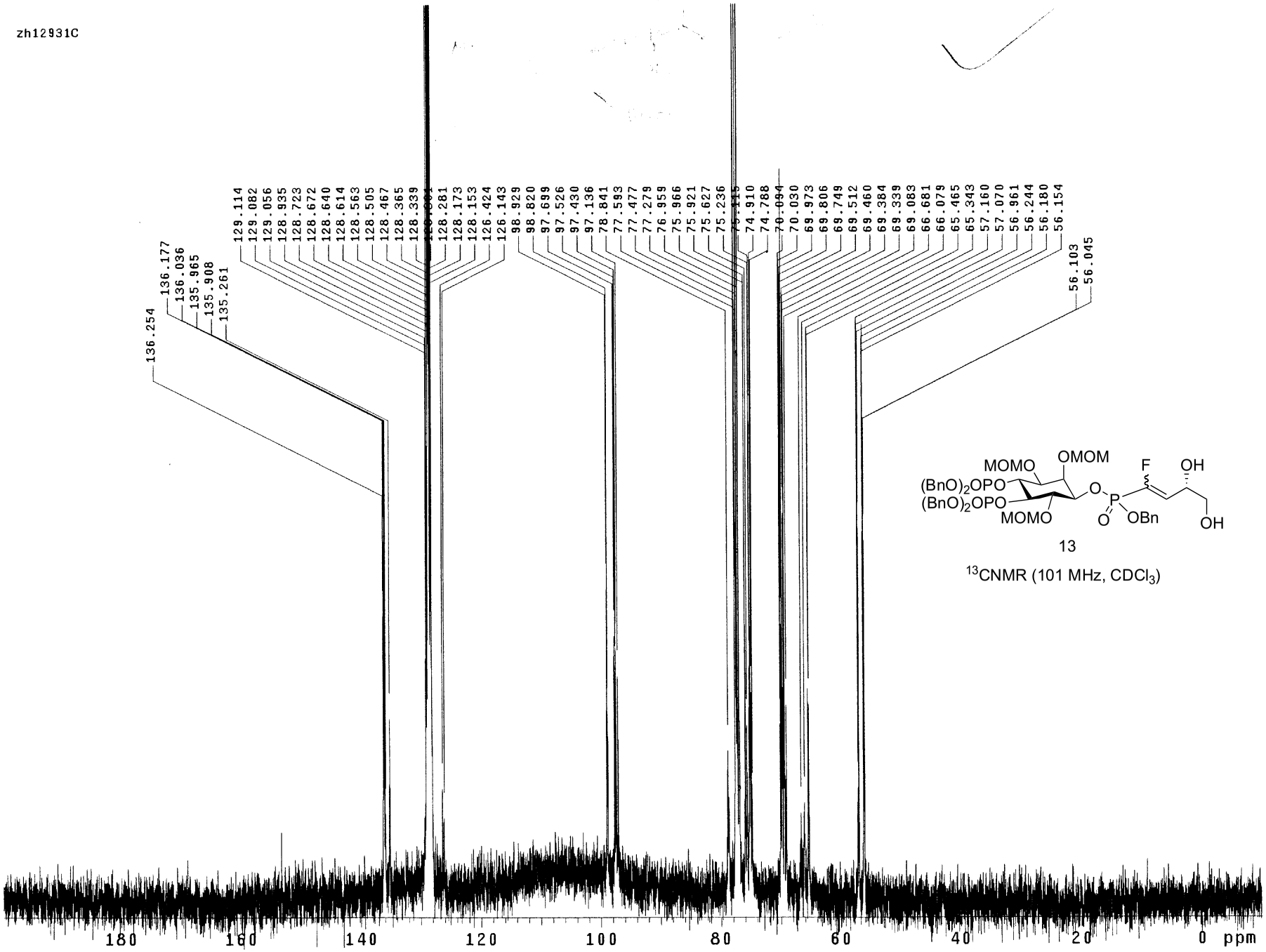
^{19}FNMR (376 MHz, $CDCl_3$)

zh12931P



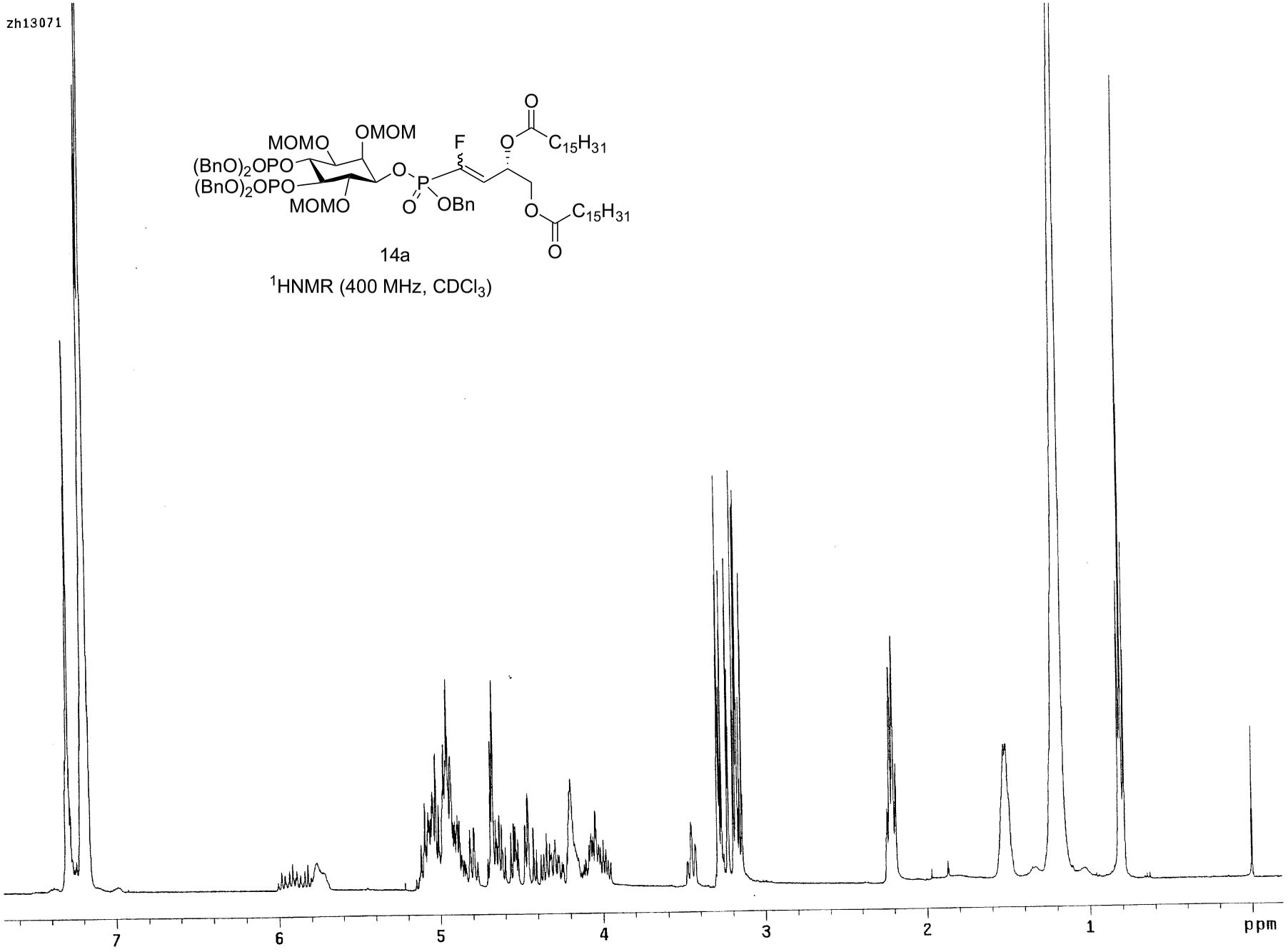
13

^{31}PNMR (162 MHz, $CDCl_3$)



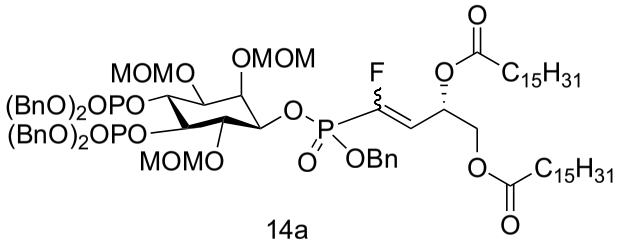
13

^{13}CNMR (101 MHz, $CDCl_3$)



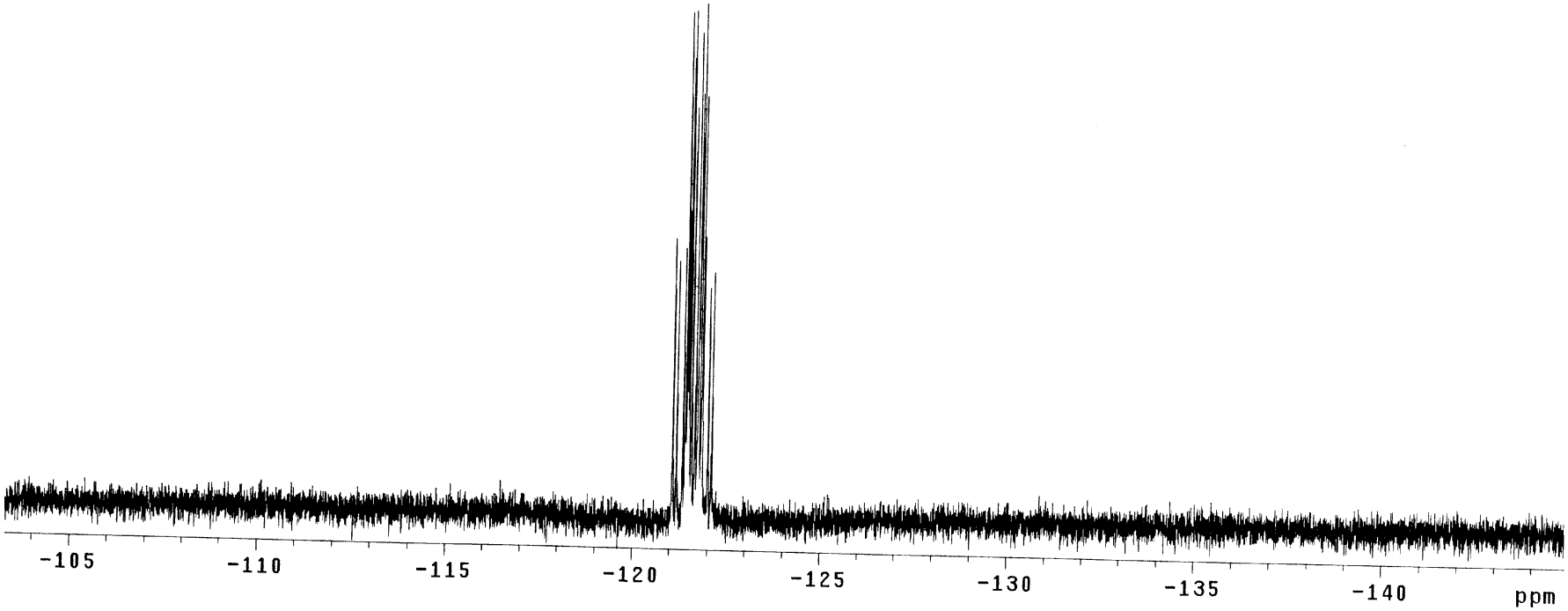
14a

^{1}HNMR (400 MHz, $CDCl_3$)

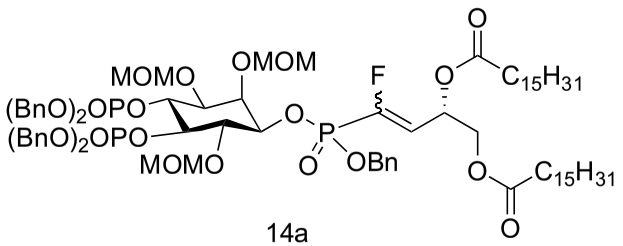


14a

^{19}FNMR (376 MHz, $CDCl_3$)



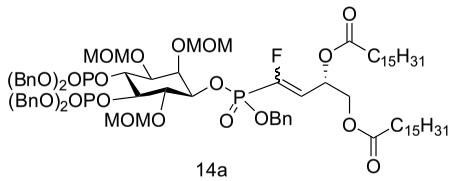
zh13071P



14a

^{31}PNMR (162 MHz, $CDCl_3$)

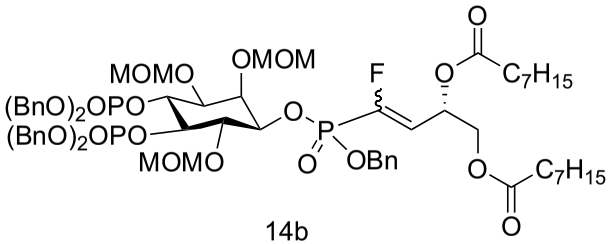
zh13071C



14a

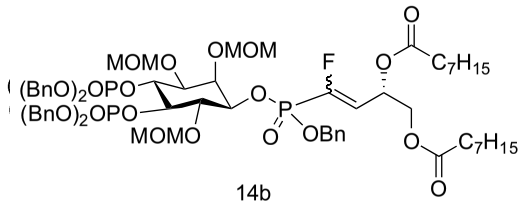
^{13}CNMR (101 MHz, $CDCl_3$)

zh20151



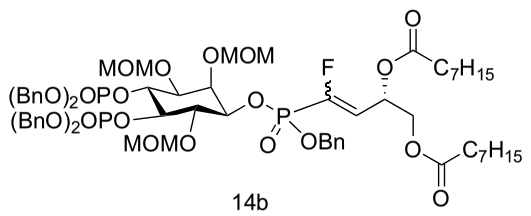
14b

^{1}HNMR (400 MHz, $CDCl_3$)



14b

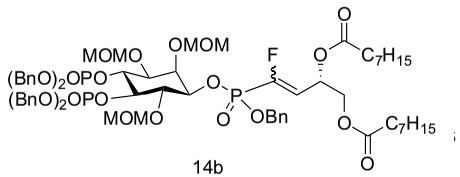
^{19}FNMR (376 MHz, $CDCl_3$)



14b

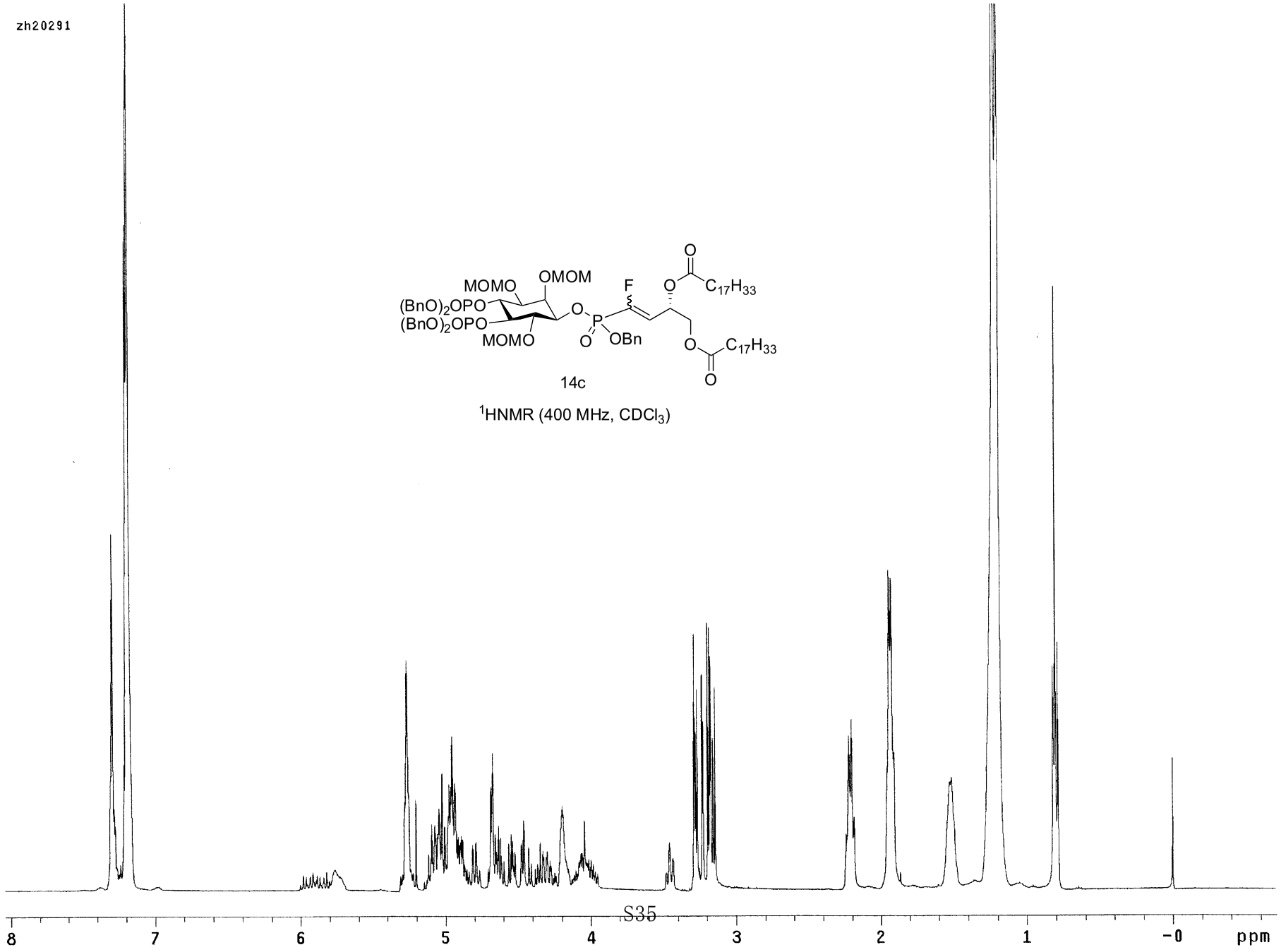
^{31}PNMR (162 MHz, $CDCl_3$)

zh20151C



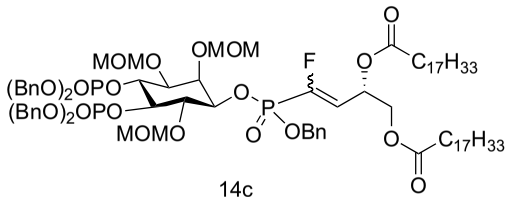
14b

^{13}CNMR (101 MHz, $CDCl_3$)



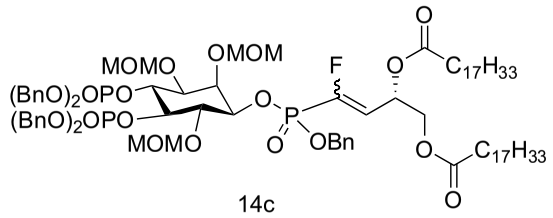
14c

^{1}HNMR (400 MHz, $CDCl_3$)



14c

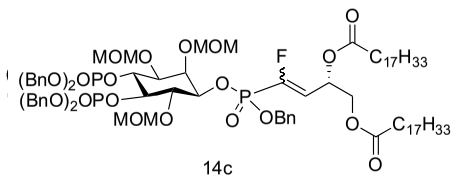
^{19}FNMR (376 MHz, $CDCl_3$)



14c

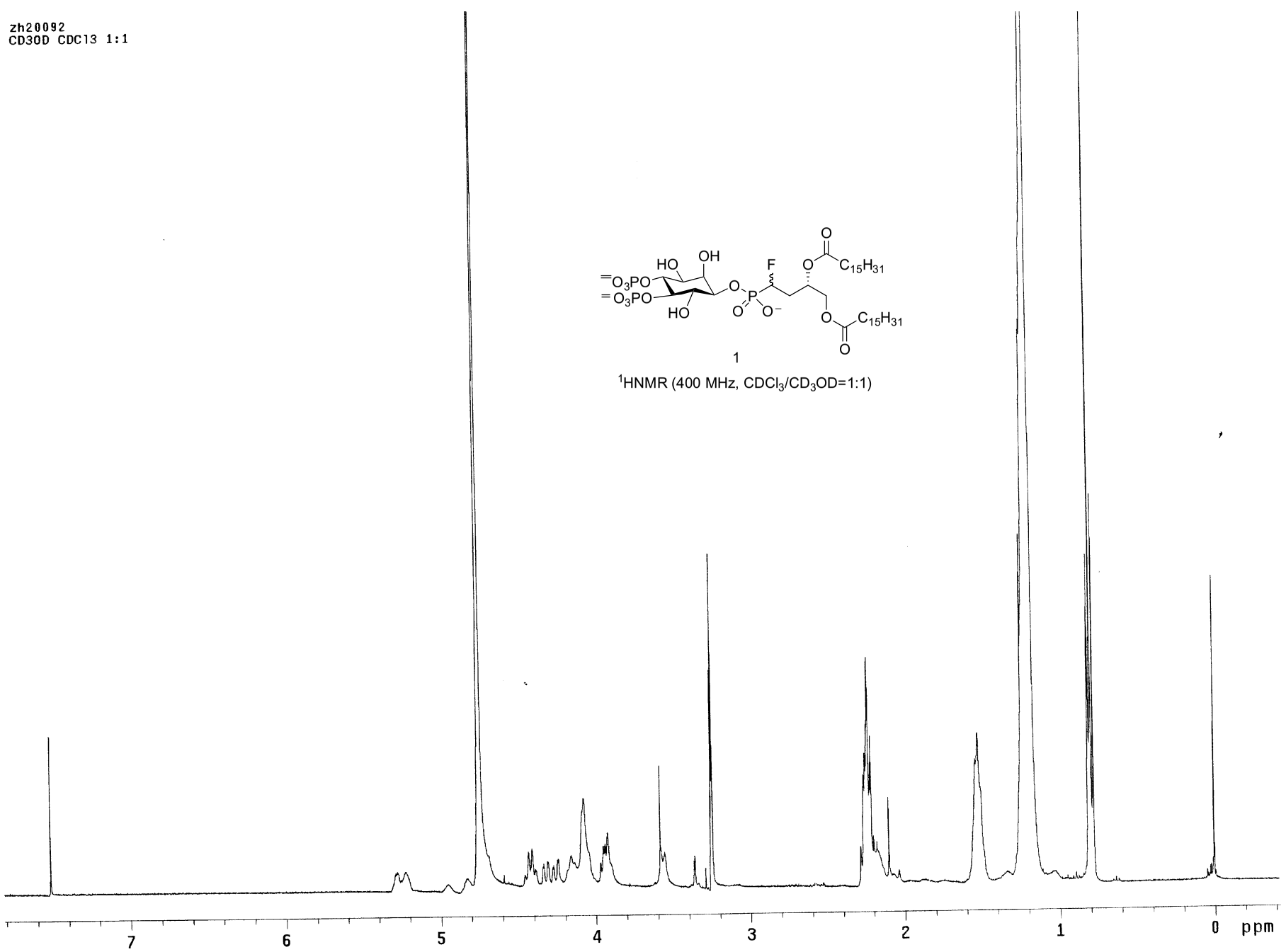
^{31}PNMR (162 MHz, $CDCl_3$)

zh20291C
CDCl3



14c

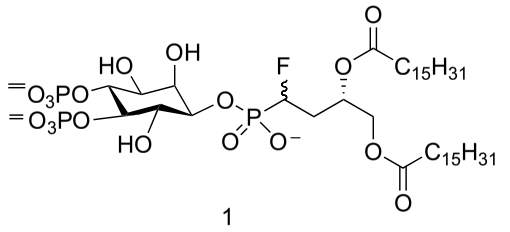
^{13}CNMR (101 MHz, $CDCl_3$)



1

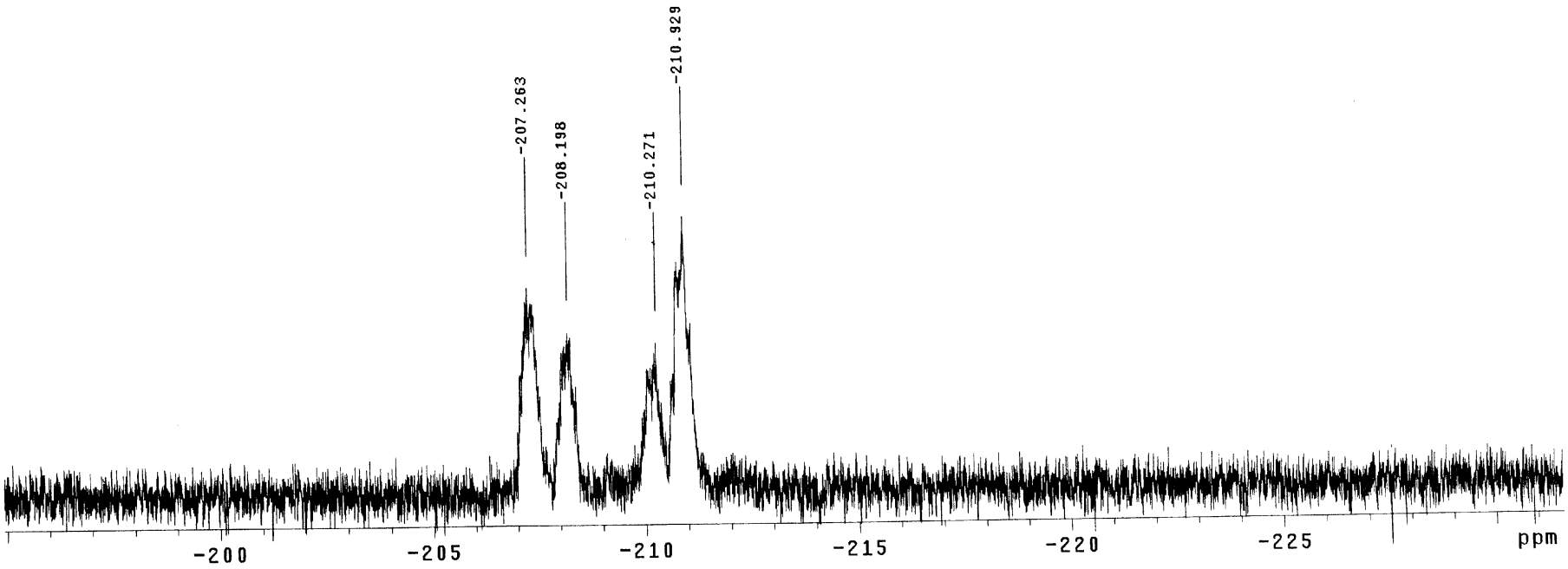
^{1}HNMR (400 MHz, $CDCl_3/CD_3OD$=1:1)

zh20092F
CD3OD CDC13 1:1

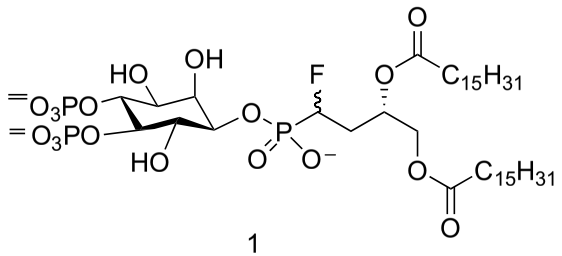


1

^{19}FNMR (376 MHz, $CDCl_3/CD_3OD$=1:1)



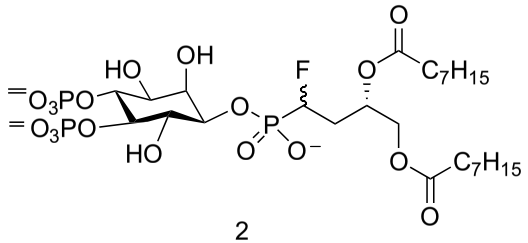
zh20092P
CD3OD CDC13 1:1



1

^{31}PNMR (162 MHz, $CDCl_3/CD_3OD$=1:1)

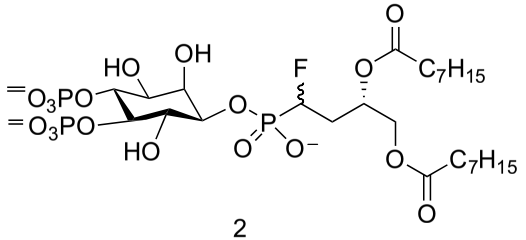
zh20191H
CD3OD CDCl3 1:1



2

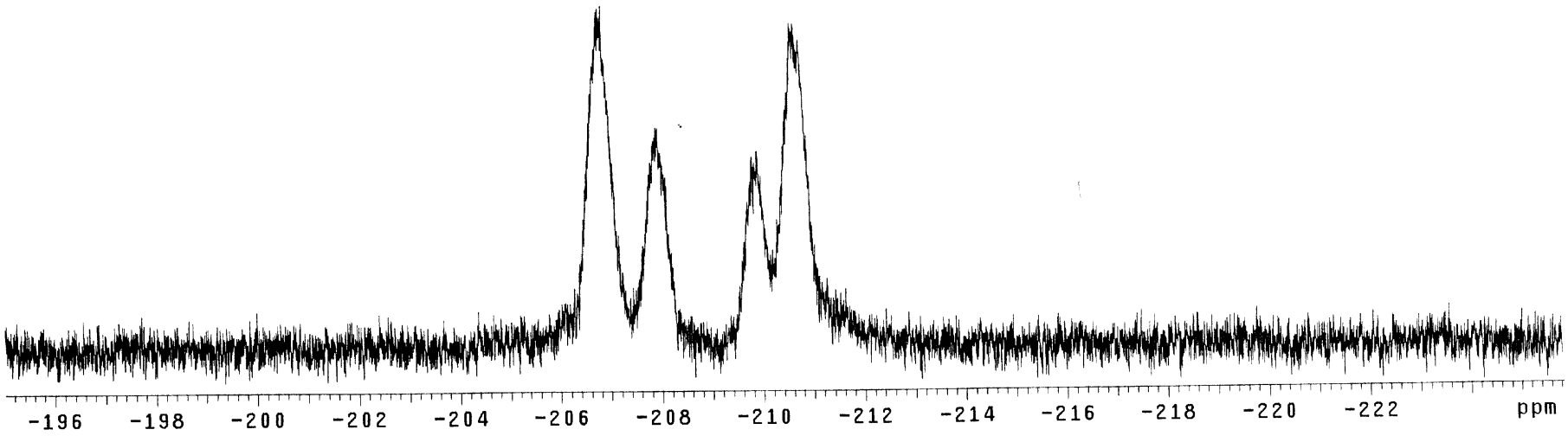
^{1}HNMR (400 MHz, $CDCl_3/CD_3OD$=1:1)

CD3OD CDC13 1:1

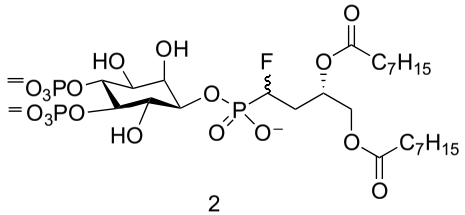


2

^{19}FNMR (376 MHz, $CDCl_3/CD_3OD$=1:1)



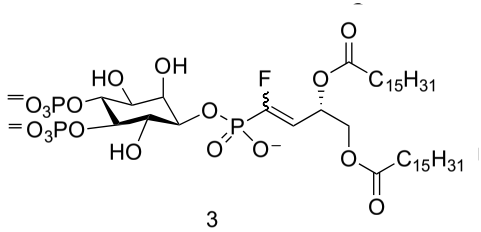
zh20191P
CD3OD CDC13 1:1



2

^{31}PNMR (162 MHz, $CDCl_3/CD_3OD$=1:1)

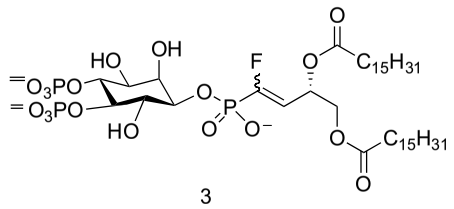
zh20251
CD3OD CDCl3 1:1



3

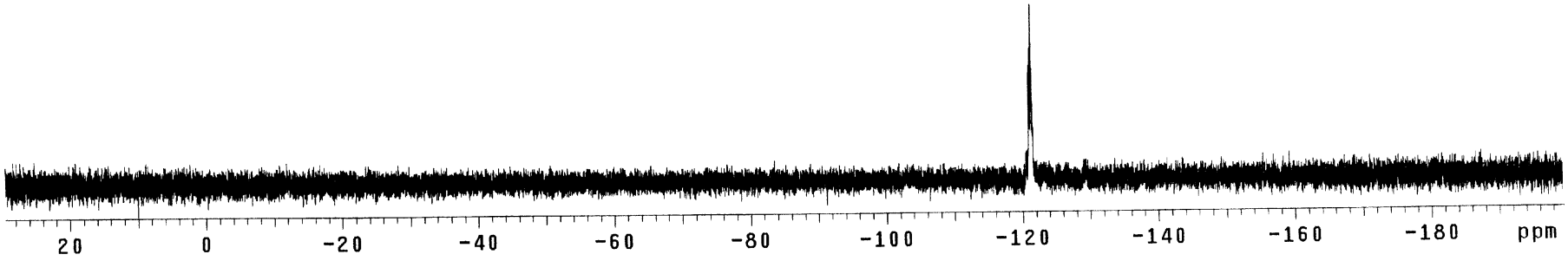
^{1}HNMR (400 MHz, $CDCl_3/CD_3OD$=1:1)

CDC13 1:1

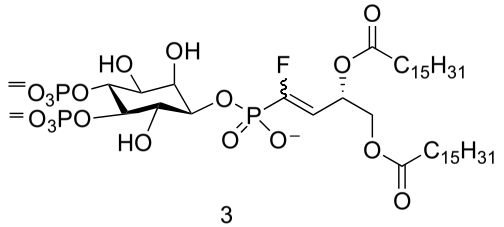


3

^{19}FNMR (376 MHz, $CDCl_3/CD_3OD$=1:1)

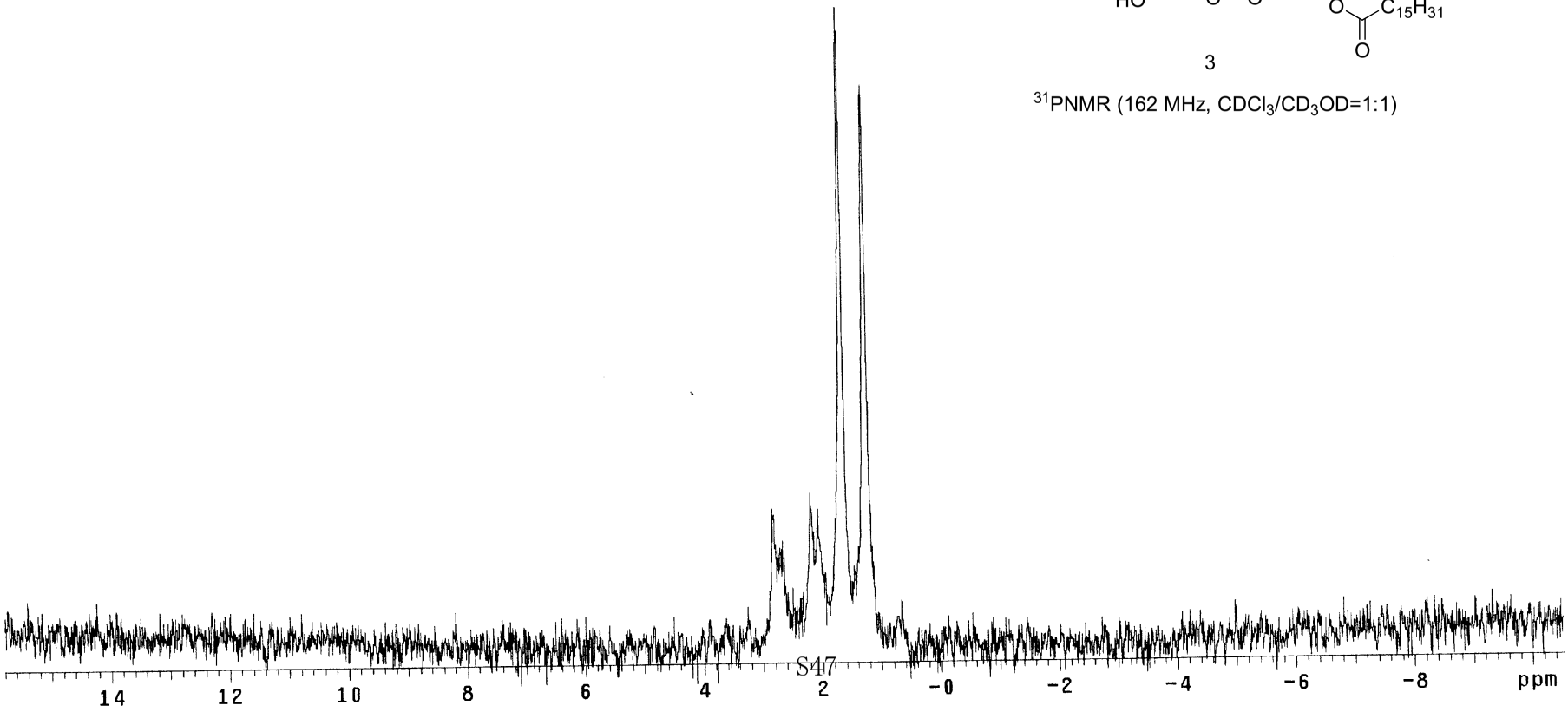


zh20241P
CD3OD CDC13
1:1

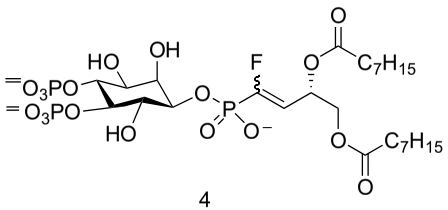


3

^{31}PNMR (162 MHz, $CDCl_3/CD_3OD$=1:1)



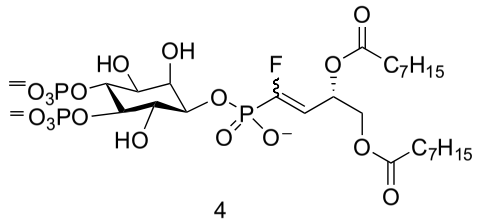
zh20271
CD3OD CDC13
1:1



4

^{1}HNMR (400 MHz, $CDCl_3/CD_3OD$=1:1)

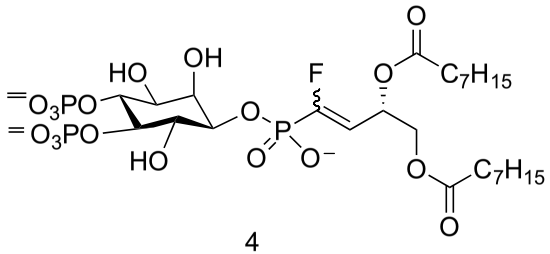
zh20271F
CD3OD CDC13
1:1



4

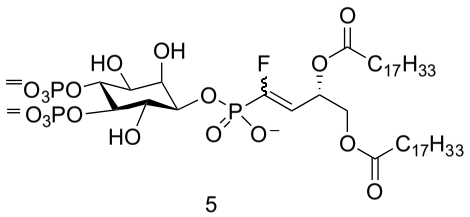
^{19}FNMR (376 MHz, $CDCl_3/CD_3OD$=1:1)

zh20271P
CD30D CDC13
1:1



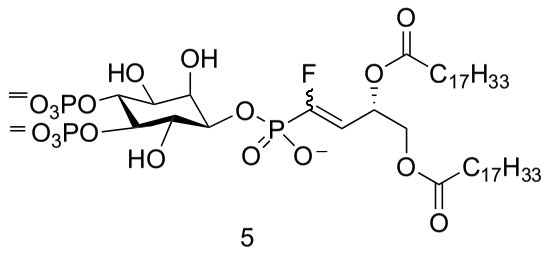
4

^{31}PNMR (162 MHz, $CDCl_3/CD_3OD$=1:1)



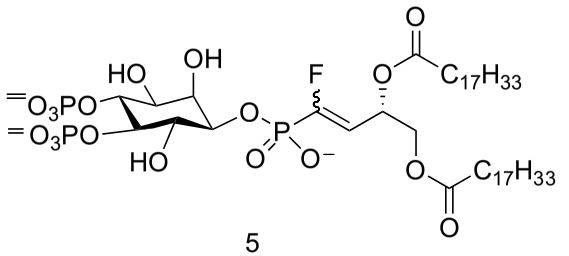
5

^{1}HNMR (400 MHz, $CDCl_3/CD_3OD$=1:5)



5

^{19}FNMR (376 MHz, $CDCl_3/CD_3OD$=1:5)



5

^{31}PNMR (162 MHz, $CDCl_3/CD_3OD$=1:5)