Supporting Information

Physicochemical and Biological Characterization of Synthetic Phosphatidylinositol Dimannosides and Analogues

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Experimental section

2-*O*-(Benzyl-1,2-di-*O*-hexadecanoyl-*sn*-glycero-3-phosphoryl)-1,3-bis-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)glycerol (6b).

A solution of **3b** (105 mg, 0.101 mmol) and **4** (127 mg, 0.158 mmol) in CH_2Cl_2 was concentrated and dried under high vacuum for 1 h. The mixture was redissolved in dry CH_2Cl_2 (2 mL) and cooled in an ice/water bath before adding 4,5-dicyanoimidazole (19 mg, 0.161 mmol). The cooling bath was removed and the initial suspension became homogenous over 10 min. The reaction was monitored for loss of **3b** by TLC (2:3 EA/petroleum ether) and after 1 h *m*CPBA (60%, 75 mg as a solution in CH_2Cl_2 and dried over $MgSO_4$, 0.26 mmol) was added over a period of 1 min. After stirring for 45 min the mixture was diluted with diethyl ether (50 mL) and washed with $Na_2S_2O_3$ (30 mL). The aqueous phase was back extracted with diethyl ether (20 mL) and the combined organic portions were washed with $NaHCO_3$ (3 x 30 mL), brine (1 x 30 mL), dried ($MgSO_4$) and concentrated. Purification using flash silica gel chromatography (1:2-2:3 EA/petroleum ether) afforded **6b** as a pair of diastereoisomers (149 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (dd, *J* = 6.7, 7.1 Hz, 6H, 2 x CH_3), 1.19-1.34 (m, 48H), 1.48-1.61 (m, 4H), 2.11 (s, 3H, C(O)CH_3), 2.12 (s, 3H, C(O)CH_3), 2.18-2.25 (m, 4H), 3.57-3.71 (m, 4H), 3.71-3.85 (m, 6H), 3.85-3.97 (m, 4H), 3.99-4.16 (m, 3H), 4.16-4.26 (m, 1H), 4.38-4.53 (m, 6H), 4.55-4.70 (m, 5H), 4.78-4.91 (m, 4H), 5.01-5.08 (m, 1H), 5.09-5.18 (m, 1H), 5.31-5.39 (m, 2H, 2-H' and 2-H''), 7.11-7.17 (m, 4H, Ar), 7.20-7.36 (m, 31H, Ar); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.1, 21.0, 22.7, 24.81, 24.83, 29.1, 29.2, 29.31, 29.33, 29.37, 29.45, 29.5, 29.67, 29.72, 31.9, 34.0, 34.08, 34.09, 61.7, 65.58, 65.60, 65.62, 65.64, 66.42, 66.45, 66.47, 66.51, 66.71, 66.75, 66.8, 68.4, 68.68, 68.73, 68.8, 69.3, 69.39, 69.43, 69.5, 69.57, 69.59, 69.62, 71.89, 71.95, 73.42, 73.44, 74.0,

74.1, 75.13, 75.14, 75.2, 75.5, 75.60, 75.64, 78.2, 98.16, 98.18, 98.37, 98.38, 127.6, 127.7, 127.83, 127.84, 127.86, 127.89, 128.06, 128.07, 128.11, 128.12, 128.25, 128.26, 128.30, 128.31, 128.35, 128.38, 128.58, 128.64, 128.7, 135.57, 135.61, 135.62, 135.7, 137.92, 137.95, 138.0, 138.26, 138.28, 138.46, 138.47, 138.48, 170.22, 170.25, 172.69, 172.70, 173.1; ^{31}P NMR (202 MHz, $CDCl_3$) δ -1.57 (s), -1.46 (s); *m/z* (ESI) 1783.9554 $[MNa]^+$; $C_{103}H_{141}NaO_{22}P$ (1783.9550).

2-*O*-(Benzyl-1,2-di-*O*-decanoyl-*sn*-glycero-3-phosphoryl)-1,3-*bis*-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)glycerol (6c).

Compound **3c** (0.033 g, 0.029 mmol) and phosphoramidite **5** (0.116 g, 0.181 mmol) were stripped down together from dry CH_2Cl_2 and evaporated under high vacuum for 90 min. The mixture was dissolved in dry CH_2Cl_2 stored over 4Å molecular sieves (5 mL) at ambient temperature and then cooled down in a water/ice bath. After addition of 4,5-dicyanoimidazole (0.011 g, 0.097 mmol) the cooling bath was immediately removed. The disappearance of **3c** and **5** was monitored by TLC (3:7 EA/petroleum ether) and after 3 h a dried ($MgSO_4$) solution of *m*CPBA (50%, 0.053 g, 0.154 mmol) in dry CH_2Cl_2 (approx. 10 mL) was added. After stirring for 30 min the reaction was diluted with ether (25 mL) and washed with 10 % $Na_2S_2O_3$ (25 mL). The aqueous phase was back extracted with diethyl ether (25 mL). The combined organic phases were washed with saturated $NaHCO_3$ (3 x 25 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified using flash silica gel chromatography (1:3-1:2-2:3 EA/petroleum ether) to afford the title compound **6c** (0.048 g, 56 %) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83-0.92 (m, 6 H, 2 x CH_3), 1.15-1.38 (m, 24 H), 1.45-1.60 (m, 4 H), 2.15-2.27 (m, 4 H), 3.48-3.92 (m, 12 H), 3.92-4.38 (m, 8 H), 4.39-4.80 (m, 12 H), 4.82-5.05 (m, 6 H), 5.05-5.19 (m, 2 H), 6.92-7.67 (m, 45 H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$)

δ 14.16, 22.72, 24.85, 29.12, 29.17, 29.34, 29.49, 29.76, 31.92, 34.00, 34.11, 61.71, 61.75, 69.17, 69.41, 72.06, 72.13, 72.24, 72.27, 72.30, 72.36, 72.41, 72.65, 72.71, 72.73, 72.75, 73.37, 73.42, 74.40, 74.76, 74.88, 75.12, 80.16, 98.63, 127.50, 127.50, 127.57, 127.61, 127.64, 127.65, 127.66, 127.71, 127.74, 127.76, 127.78, 127.82, 128.00, 128.03, 128.07, 128.29, 128.30, 128.32, 128.34, 128.36, 128.37, 128.38,128.67, 128.69, 138.40, 138.41, 138.50, 138.53, 138.56, 172.95; ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.65 (s), -1.39 (s); *m/z* (HRMS-ESI) 1711.8414 $[MNa]^+$; $C_{101}H_{125}NaO_{20}P^+$ (1711.8394).

2-*O*-(1,2-di-*O*-hexadecanoyl-*sn*-glycero-3-phosphoryl)-1,3-bis-*O*-(2-*O*-acetyl-α-D-mannopyranosyl)glycerol (7b).

A mixture of **6b** (921 mg, 0.523 mmol) and $Pd(OH)_2$ (20% on C, 125 mg) in 2:3 THF/MeOH (30 mL) was stirred under an atmosphere of H_2 at ambient temperature for 18 h, filtered and concentrated. Flash chromatography on silica gel, using 1:4-0.8:1 MeOH/$CHCl_3$ gave **7b** as a colorless powder (515 mg, 87%). $[\alpha]^{20}_D$ +24 (c = 0.74, 2:1 $CHCl_3$/MeOH); ^{1}H NMR (500 MHz, 2:1 $CDCl_3$/CD_3OD) δ 0.89 (dd, *J* = 6.9, 7.1 Hz, 6H, 2 x CH_3), 1.21-1.36 (m, 48H), 1.55-1.66 (m, 4H), 2.12 (s, 6H, 2 x C(O)CH_3), 2.32 (dd, *J* = 5.4, 7.7 Hz, 2H, OCOC$\underline{H}_2$), 2.34 (dd, *J* = 7.5, 5.5 Hz, 2H, OCOC$\underline{H}_2$), 3.53-3.80 (m, 8H), 3.86-4.00 (m, 6H), 4.00-4.09 (m, 2H), 4.20 (dd, *J* = 6.9, 12.0 Hz, 1 H), 4.40-4.49 (m, 2H), 4.81 (s, 1H, 1-H'). 4.83 (s, 1H, 1-H''), 5.02-5.08 (m, 2H, 2-H' and 2-H''), 5.22-5.29 (m, 1H, 2-H). ^{13}C NMR δ (126 MHz, 2:1 $CDCl_3$/CD_3OD) δ 14.2, 20.9, 23.0, 25.20, 25.24, 29.46, 29.49, 29.67, 29.70, 29.85, 29.88, 29.97, 30.02, 32.2, 34.4, 34.6, 62.4, 62.5, 63.0, 64.3, 67.06, 67.13, 68.5, 68.7, 69.7, 70.7 (d, $^3J(^{31}P\text{-}^{13}C)$ = 8.0 Hz, 2-C), 72.66, 72.69, 73.5, 73.6, 73.9, 98.0, 171.59, 171.65, 174.1, 174.4. *m/z* (ESI) 1129.6278 $[M-H]^-$;

$C_{54}H_{98}O_{22}P^-$ (1129.6287). For ^{31}P NMR analysis a small portion was treated with DowexWX8-100 (Na^+) ion-exchange resin. ^{31}P NMR (202 MHz, 2:1 $CDCl_3/CD_3OD$) δ -0.7 (s).

2-*O*-(1,2-di-*O*-hexadecanoyl-*sn*-glycero-3-phosphoryl)-1,3-bis-*O*-(α-D-mannopyranosyl)glycerol (2b).

To a solution of **7b** (35 mg, 30.9 μmol) in MeOH (5 mL) was added sodium methoxide (0.5 M, 50 μL, 25 μmol) and the reaction mixture was stirred at ambient temperature. The reaction was monitored by TLC (2:1:0.17 $CHCl_3$/MeOH/H_2O) and at 20 min quenched by the addition of DowexWX8-100 (H^+) resin (approx. 0.5 g), filtered and subjected to two sequential treatments with DowexWX8-100 (Na^+) resin (2 x approx. 0.5 g). Flash chromatography on silica gel, using 2:1:0-2:1:0.05-2:1:0.17 $CHCl_3$/MeOH/H_2O gave **2b** as a colorless powder (21 mg, 64%). $[\alpha]^{20}_D$ +45 (c = 0.2, 2:1:0.17 $CHCl_3$/MeOH/H_2O); ^1H NMR (500 MHz, 2:1:0.17 $CDCl_3/CD_3OD/D_2O$) δ 0.89 (dd, *J* = 6.7. 7.2 Hz, 6H, 2 x CH_3), 1.20-1.43 (m, 48H), 1.54-1.67 (m, 4H), 2.28-2.32 (m, 4H), 3.57-3.71 (m, 6H), 3.71-3.79 (m, 4H), 3.80-3.92 (m, 6H), 3.93-4.04 (m, 2H), 4.34-4.45 (m, 2H), 4.83 (s, 1H, 1-H'), 4.86 (s, 1H, 1-H''), 5.20-5.28 (m, 1H, 2-H); ^{13}C NMR (126 MHz, 2:1:0.17 $CDCl_3/CD_3OD/D_2O$) δ 13.4, 22.2, 24.4, 24.5, 28.7, 28.86, 28.92, 29.2, 31.4, 33.7, 33.8, 60.9, 62.5, 63.0, 66.1, 66.4, 66.8, 66.9, 69.97, 70.02, 70.1 (d, $^3J(^{31}P\text{-}^{13}C)$ = 8.0 Hz, 2-C), 70.6, 72.5, 72.56, 72.65, 99.77, 99.84, 173.6, 173.9; ^{31}P NMR (202 MHz, 2:1:0.17 $CDCl_3/CD_3OD/D_2O$) δ -0.7 (s); *m/z* (ESI) 1045.6090 $[M-H]^-$; $C_{50}H_{94}O_{20}P$ (1045.6076). Compound **2b** was eluted using a gradient for HPLC analysis: solvent A H_2O; solvent B 100 mM NH_4OAc; solvent C Methanol; Program 0-10 min 5-0% A, 5% B, 85-95% C, 10-28 min hold (0% A, 5% B, 95% C). The HPLC purity was found to be 94.7%.

2-*O*-(1,2-di-*O*-decanoyl-*sn*-glycero-3-phosphoryl)-1,3-bis-*O*-(α-D-mannopyranosyl)glycerol (2c)

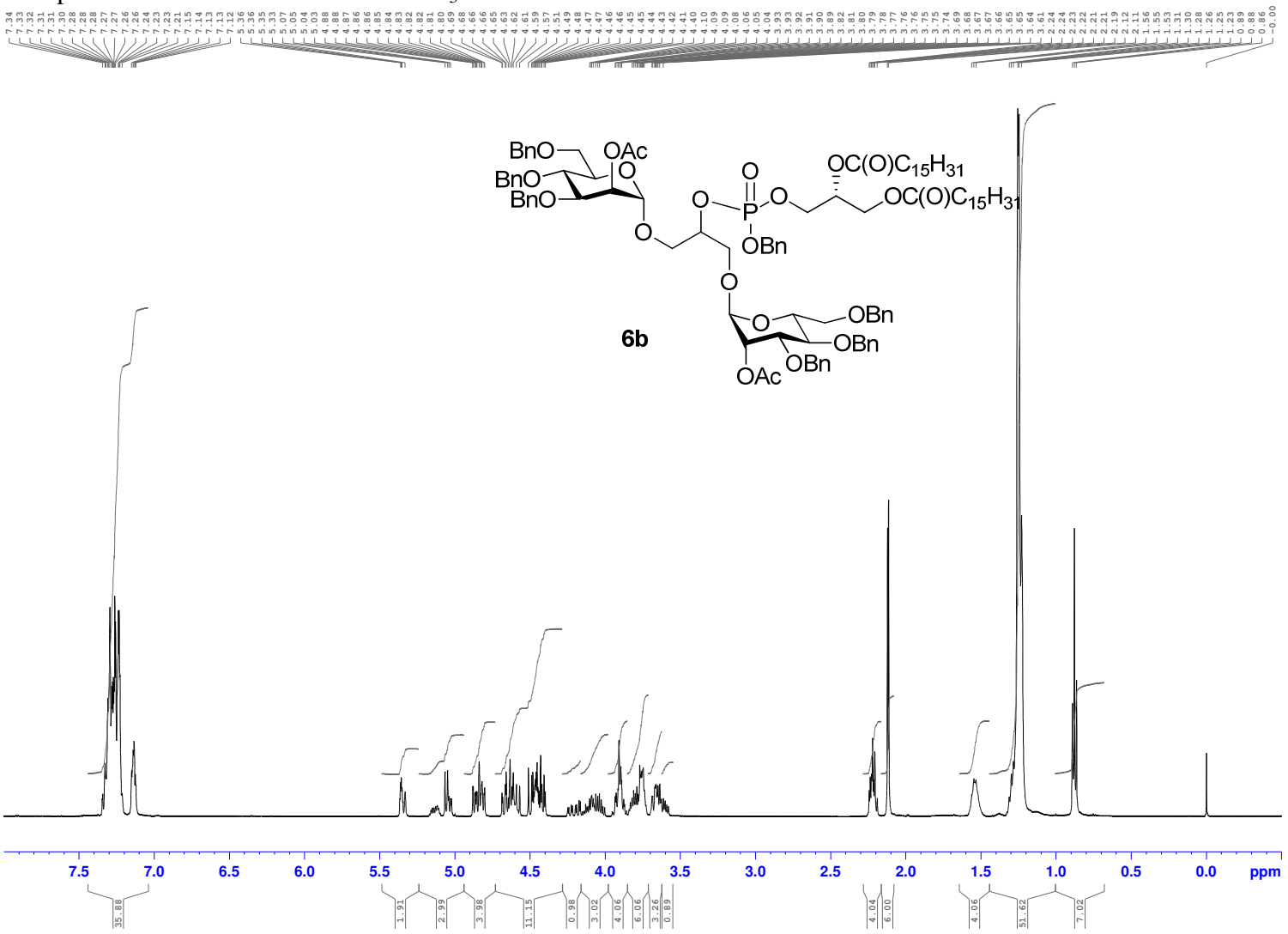
$Pd(OH)_2$/C (20%, 86 mg) was added to a solution of compound **6c** (120 mg, 0.071 mmol) in dry THF/MeOH (2:3, 10 mL). The mixture was stirred under hydrogen atmosphere for 4 h at ambient temperature. After removing the hydrogen atmosphere the mixture was filtered through Celite, the Celite was prewashed with dry THF/MeOH (2:3, 100 mL) and the solvent removed. The residue was purified using flash silica gel chromatography (9:1:0-8:2:0-2:1:0.05-2:1:0.1 $CHCl_3$/MeOH/H_2O) to afford the title compound **2c** (45.0 mg, 72%) as a white solid; $[\alpha]^{20}_D$ +38.4 (70:40:6 $CHCl_3$/MeOH/H_2O); ^{1}H NMR (500 MHz, 2:1 $CDCl_3$/CD_3OD) δ 0.84 (t, *J* = 6.8 Hz, 6 H, 2 x CH_3), 1.28 (m, 24 H), 1.55 (s, 4 H), 2.28 (dt, *J* = 15.1, 4 H, 7.5 Hz), 3.50-3.99 (m, 18 H), 4.06-4.20 (m, 3 H), 4.32-4.46 (m, 3 H), 4.85 (d, *J* = 15.7, 2 H, 1-H' and 1-H''), 5.20 (s, 1 H, 2-H); ^{13}C NMR (126 MHz, 2:1 $CDCl_3$/CD_3OD) δ 14.34, 23.06, 25.28, 25.37, 29.57, 29.60, 29.72, 29.75, 29.81, 29.90, 29.93, 30.07, 32.30, 34.51, 34.66, 61.86, 63.23, 64.14, 66.99, 67.80, 70.84, 71.57, 73.16, 73.70, 100.47, 174.3; *m/z* (HRMS-ESI) 877.4142 $[M-H]^-$; $C_{38}H_{70}O_{20}P^-$ (877.4204). The product was stirred with DOWEX 50X8-100 (Na^+) resin to afford the product as the sodium salt; *m/z* (HRMS-ESI) 877.4187 $[M-H]^-$; $C_{38}H_{70}O_{20}P^-$ (877.4204). Compound **2c** was eluted using a gradient for HPLC analysis: solvent A water; solvent B 1:1 MeOH/water containing 50 mM NH_4OAc (pH 5); solvent C MeOH; Program 0-20 min 0-10% B, 70 to 90% C; 20-28 min hold; 30-32 min return to 100% A; 32-40 min equilibrate at 100% A. A HPLC purity of 95% was determined.

2-*O*-(*sn*-glycero-3-phosphoryl)-1,3-bis-*O*-(α-D-mannopyranosyl)glycerol (2d)

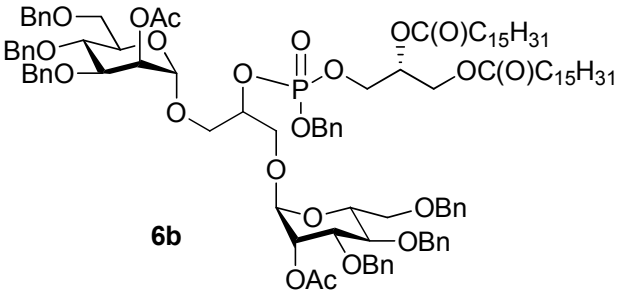
Sodium methoxide in MeOH (0.5 M solution, 200 μL) was added to a stirred solution of **2a** (11 mg, 0.010 mmol) in MeOH (10 mL). After 2 h the mixture was quenched with Dowex 50W8-100 (H^+) resin, filtered and subsequently made neutral by treatment with Dowex 50W8-100 (Na^+) resin then filtered and concentrated. The residue was dissolved in water and washed twice with $CHCl_3$ and freeze dried to give **2d** (5 mg, 90%) as a colorless foam. $[\alpha]^{20}_D$ +35 (c = 0.2, H_2O); ^1H NMR (500 MHz, D_2O) δ 3.57-3.78 (m, 10H), 3.79-3.96 (m, 9H), 3.97-4.01 (m, 2H), 4.42-4.49 (m, 1H), 4.87 (s, 1H), 4.88 (s, 1H); ^{13}C NMR (126 MHz, D_2O) δ 61.0, 62.2, 66.48, 66.51, 66.76, 66.80, 66.86, 66.90, 70.0, 70.6, 70.75, 70.81, 72.84, 72.92, 73.60 (d, $J(^{31}P\text{-}^{13}C)$ = 5.3 Hz, 2-C), 100.0, 100.2, ^{31}P NMR (202 MHz, D_2O) δ -0.3; *m/z* (HRMS-ESI) 569.1492 $[M\text{-}H]^-$; $C_{18}H_{34}O_{18}P^-$ (569.1483).

NMR spectra

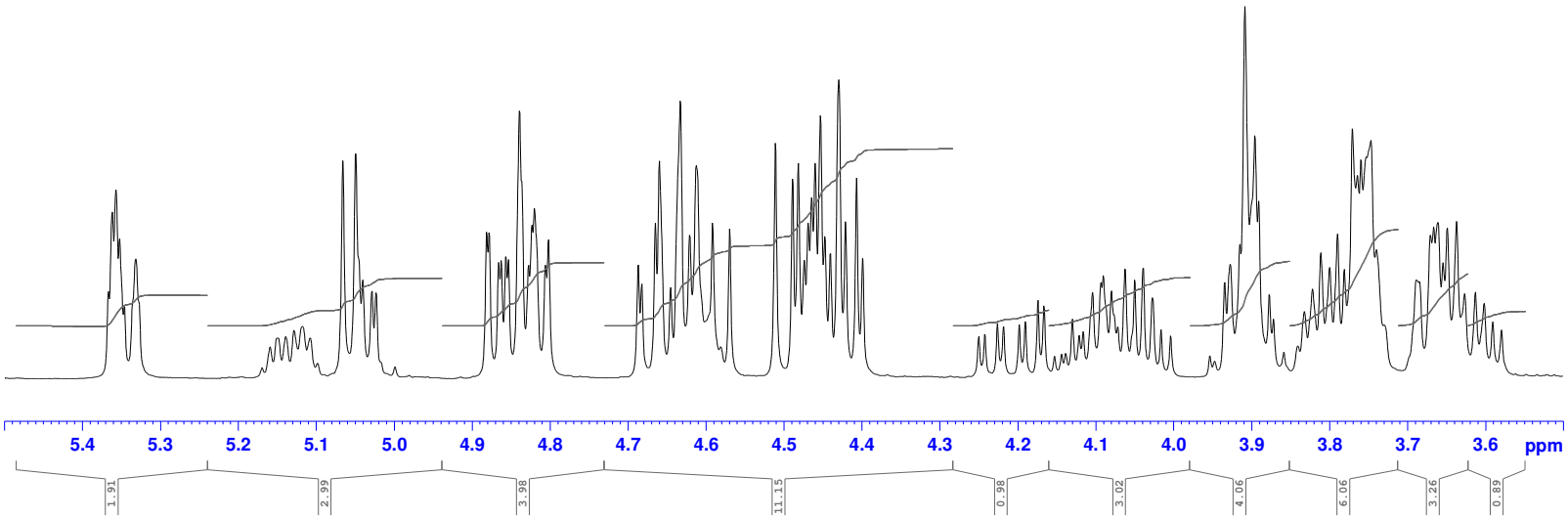
Compound **6b** 500 MHz ^{1}H NMR $CDCl_3$



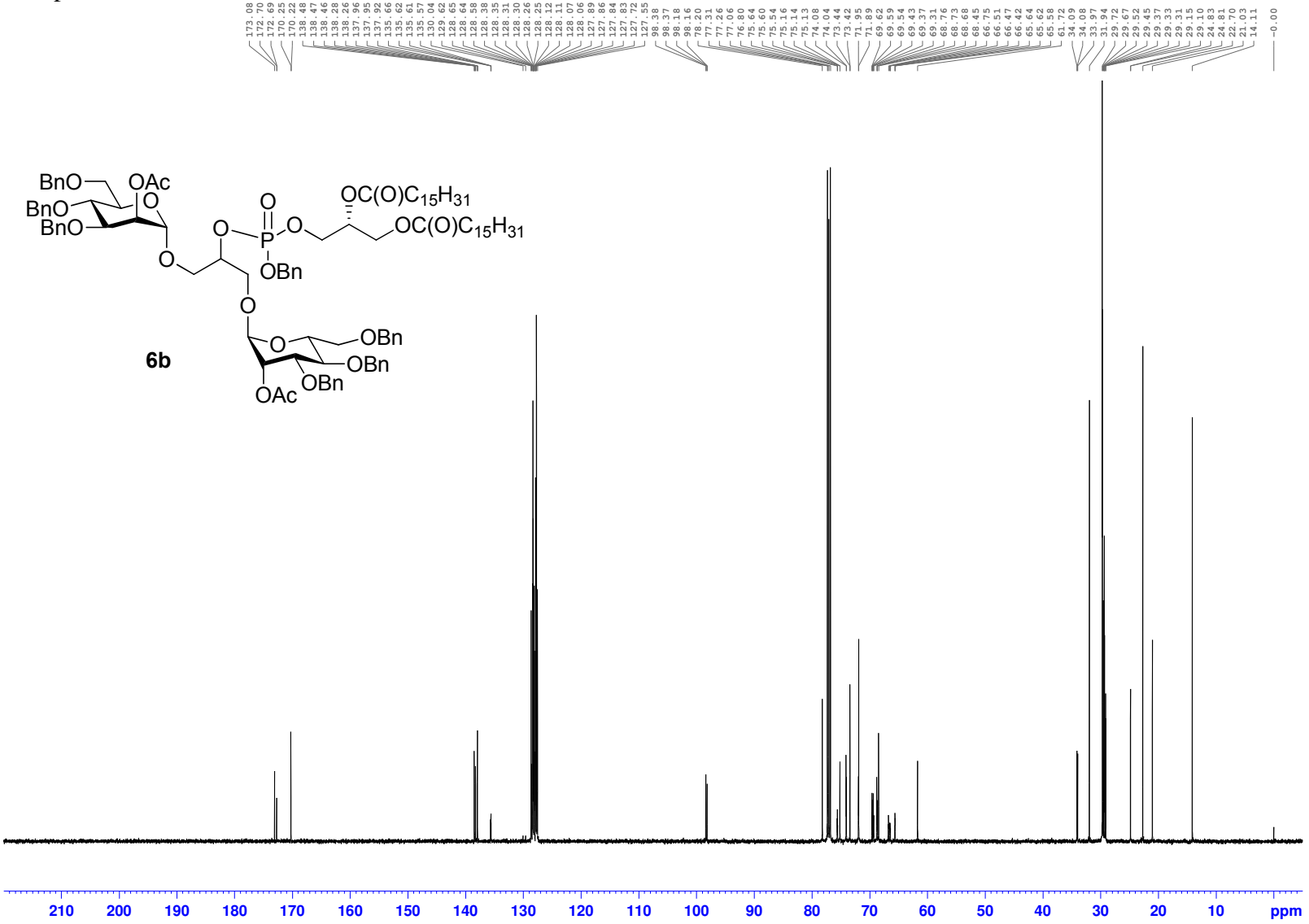
Compound **6b** 500 MHz ^{1}H NMR $CDCl_3$



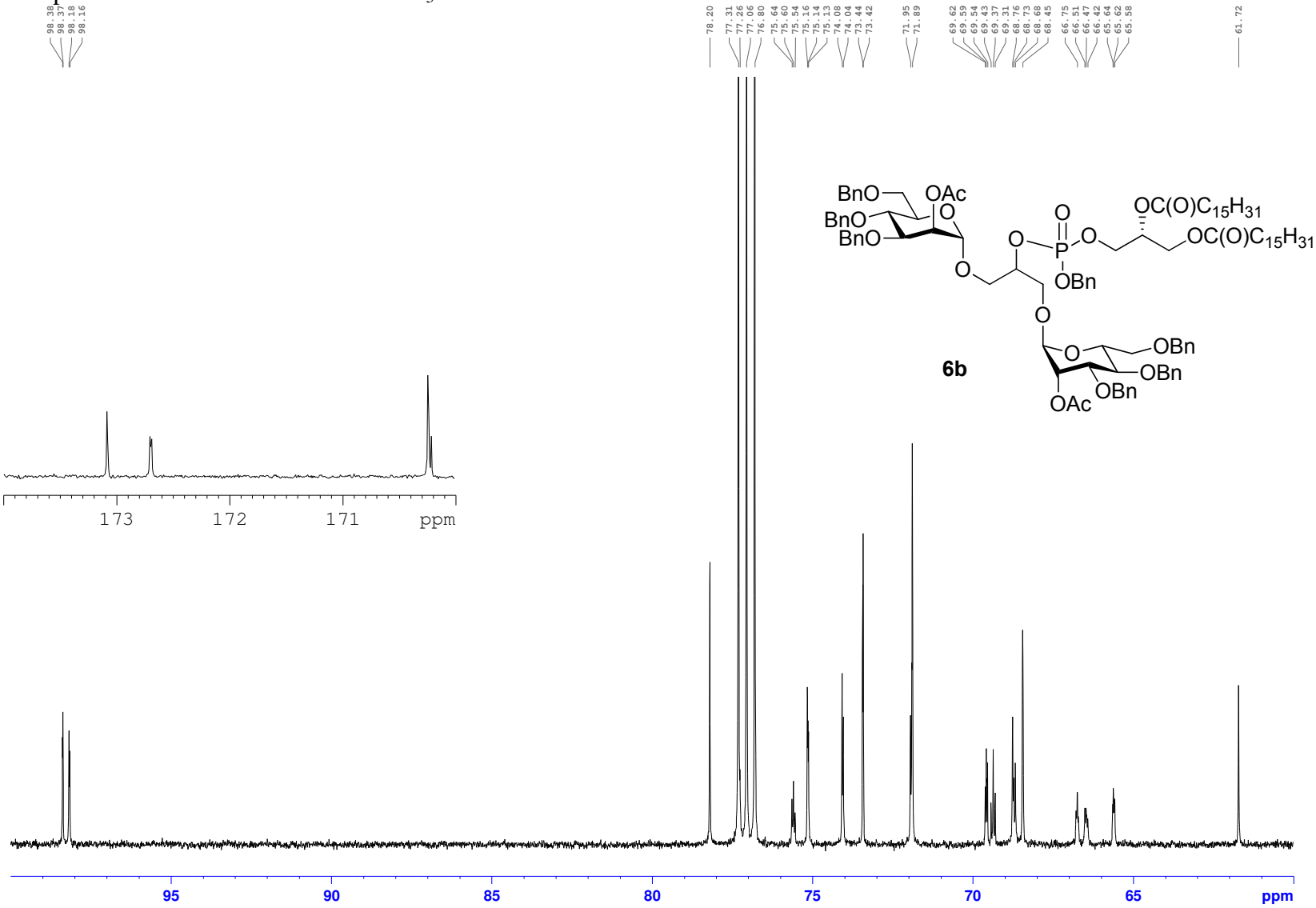
6b



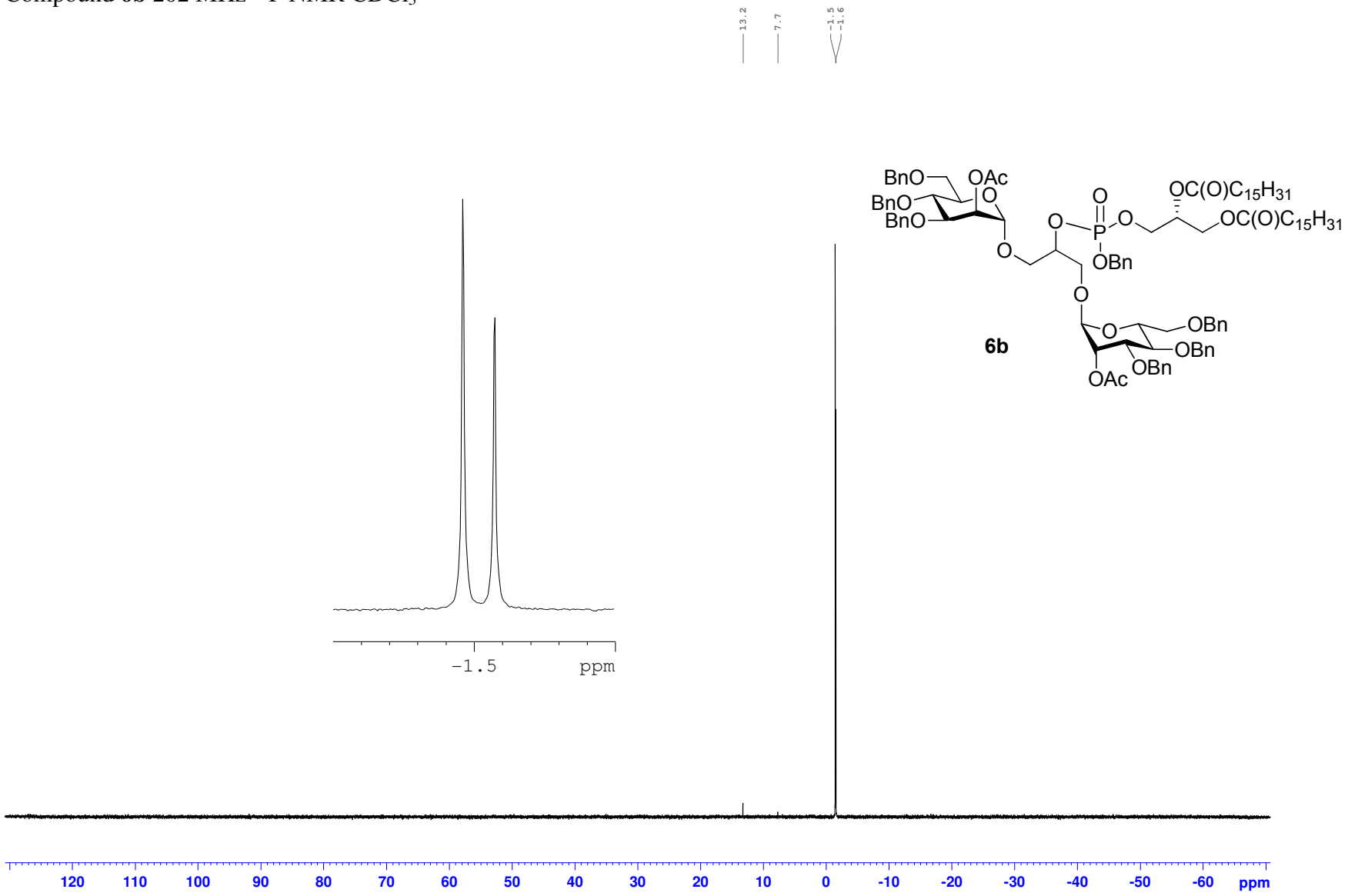
Compound **6b** 126 MHz ^{13}C NMR $CDCl_3$



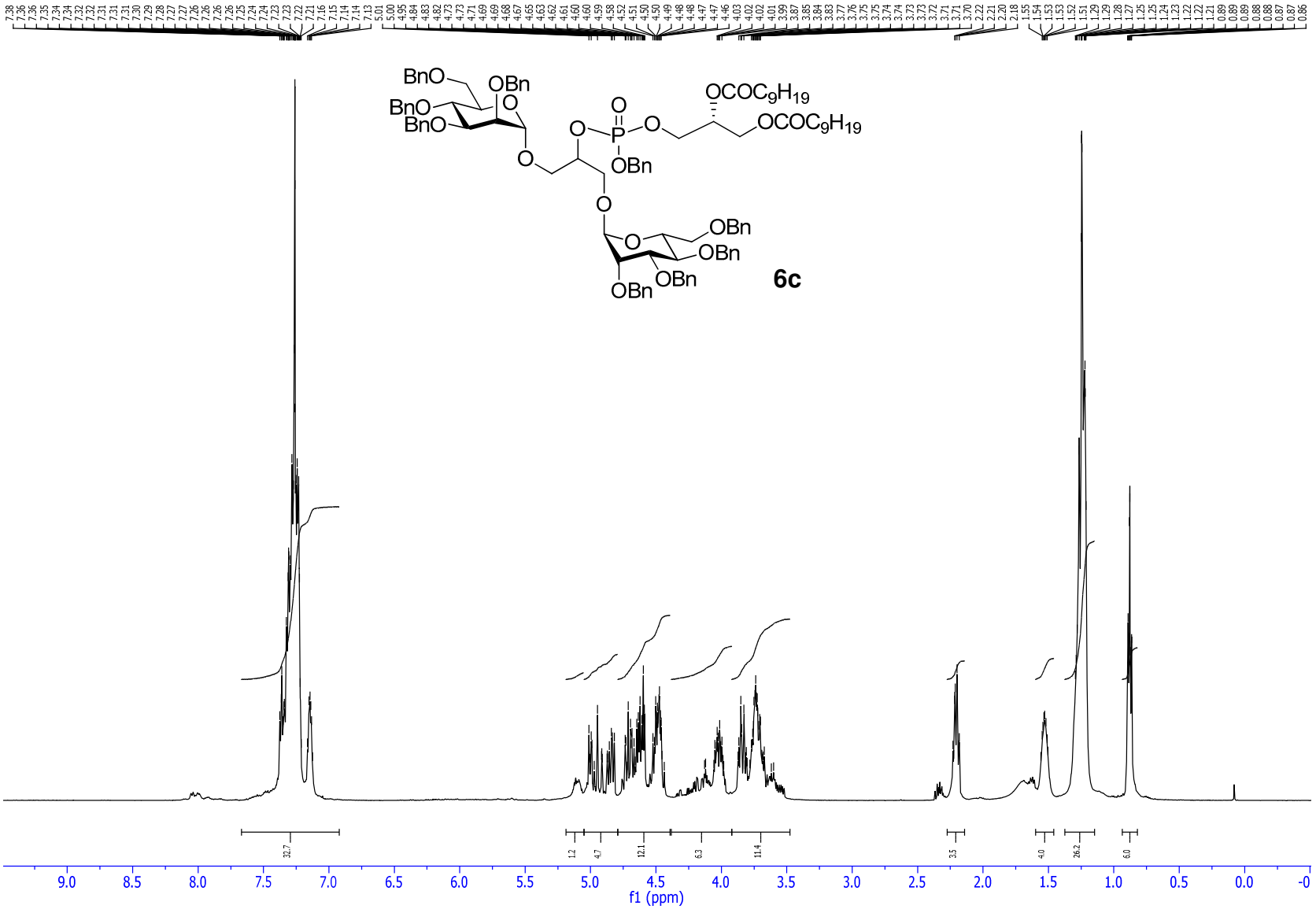
Compound **6b** 126 MHz ^{13}C NMR $CDCl_3$



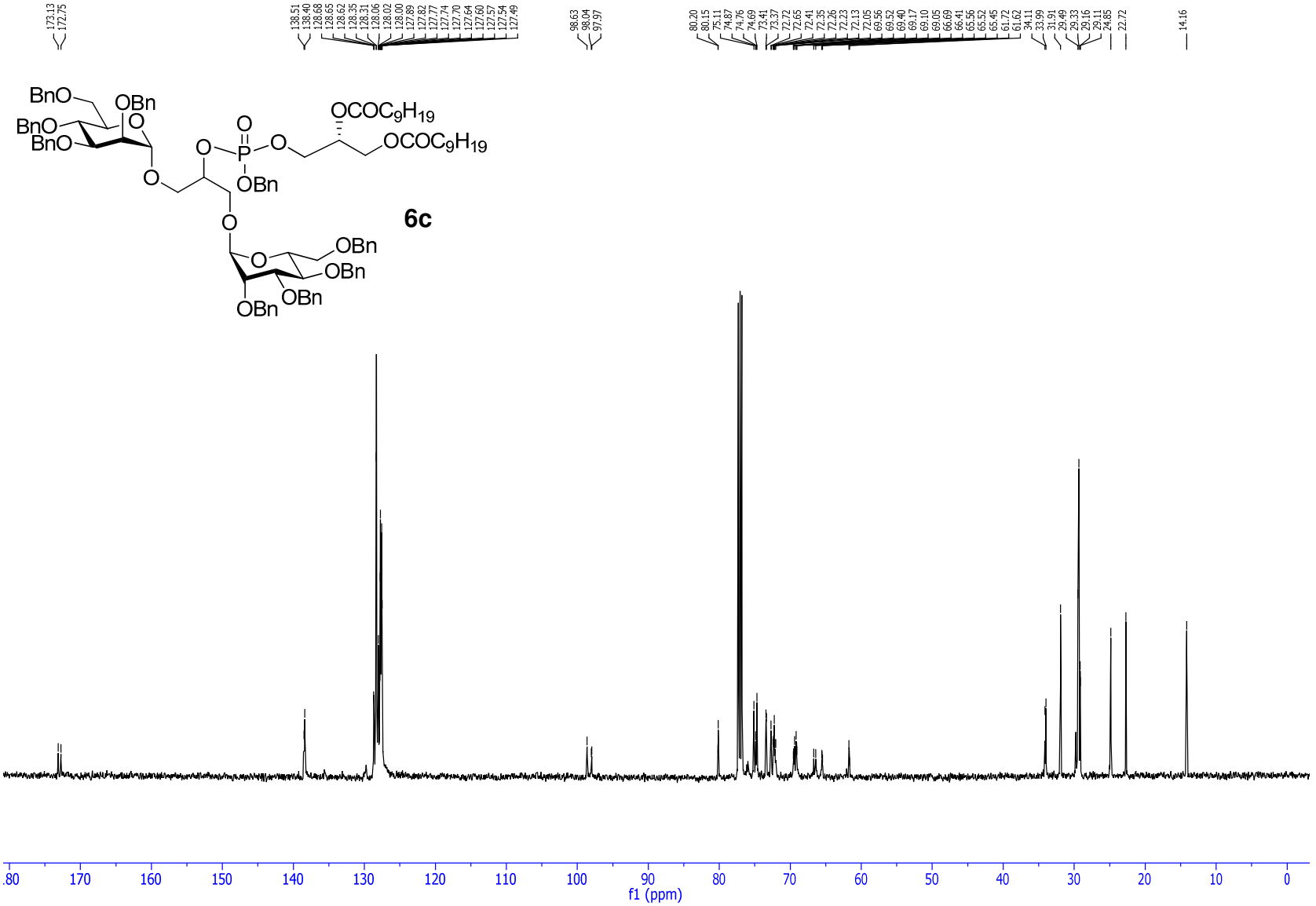
Compound **6b** 202 MHz ^{31}P NMR $CDCl_3$



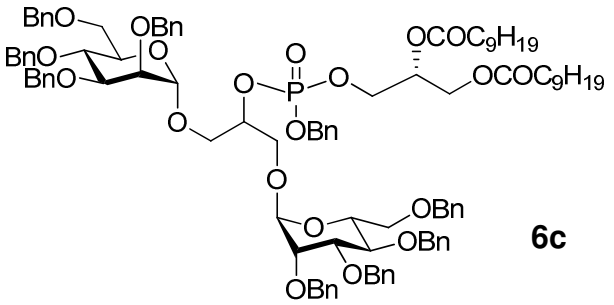
Compound **6c** 500 MHz ^{1}H NMR $CDCl_3$



Compound **6c** 126 MHz ^{13}C NMR $CDCl_3$

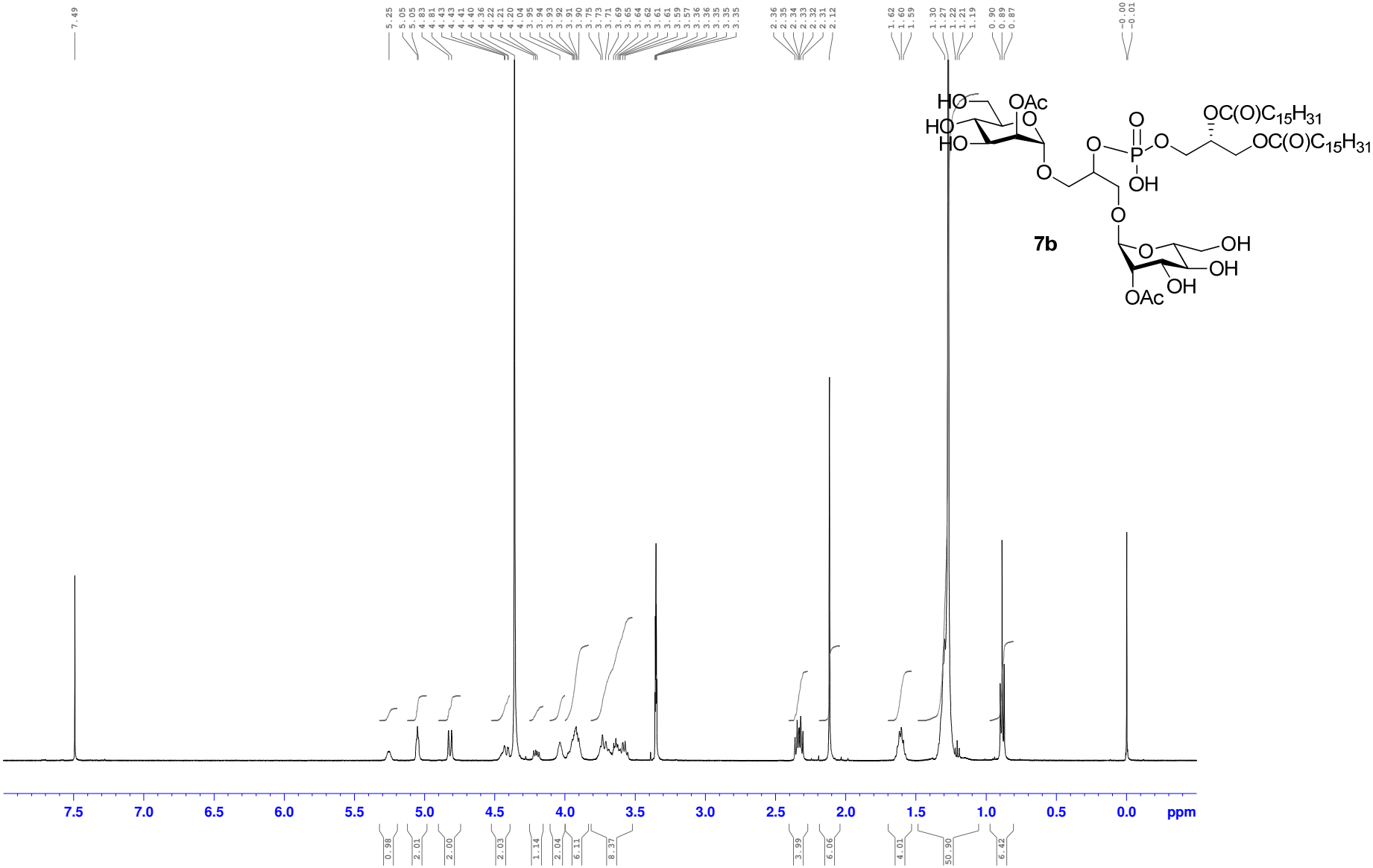


Compound **6c** 202 MHz ^{31}P NMR $CDCl_3$

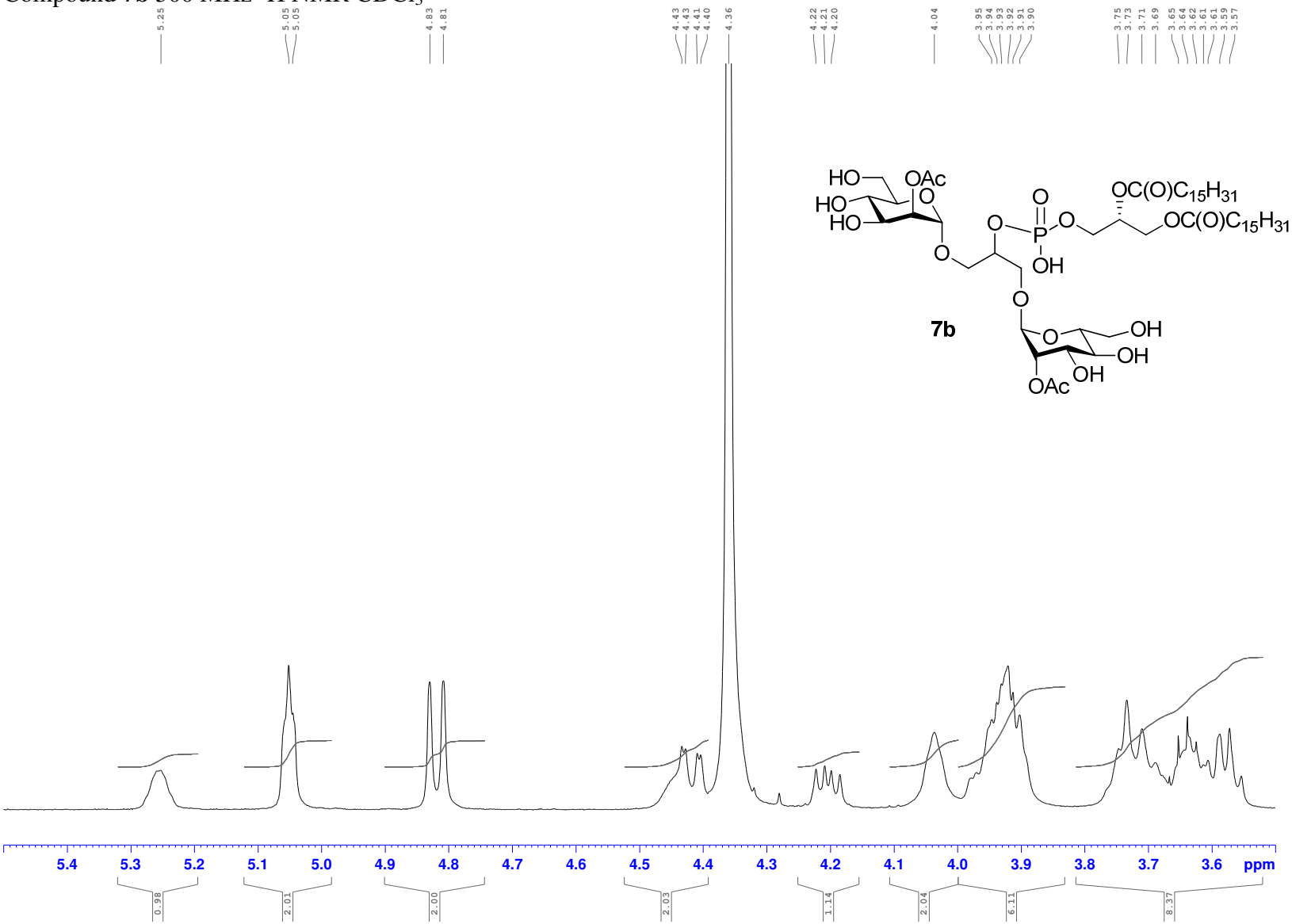


6c

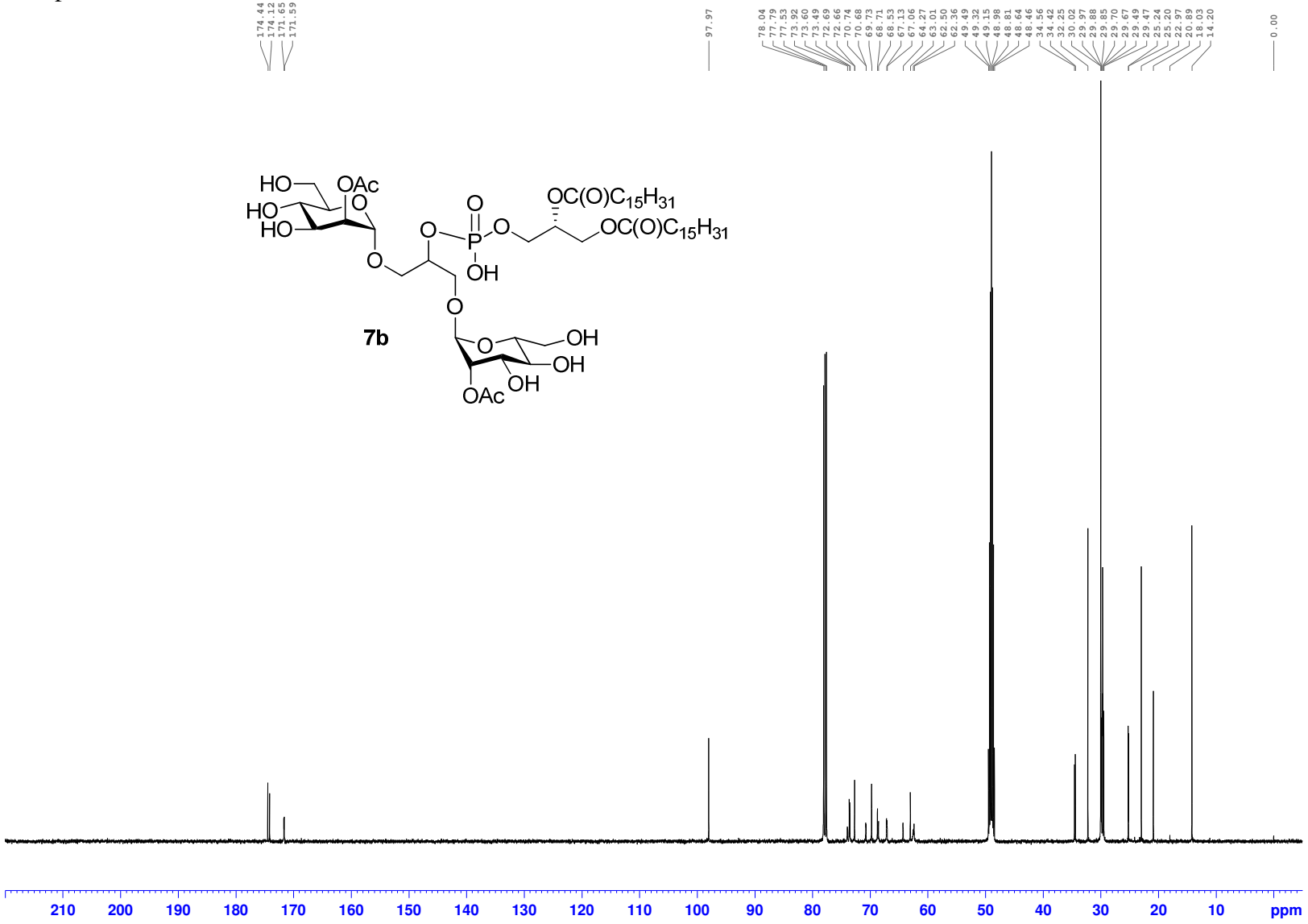
Compound **7b** 500 MHz ^{1}H NMR 2:1 $CDCl_3/CD_3OD$



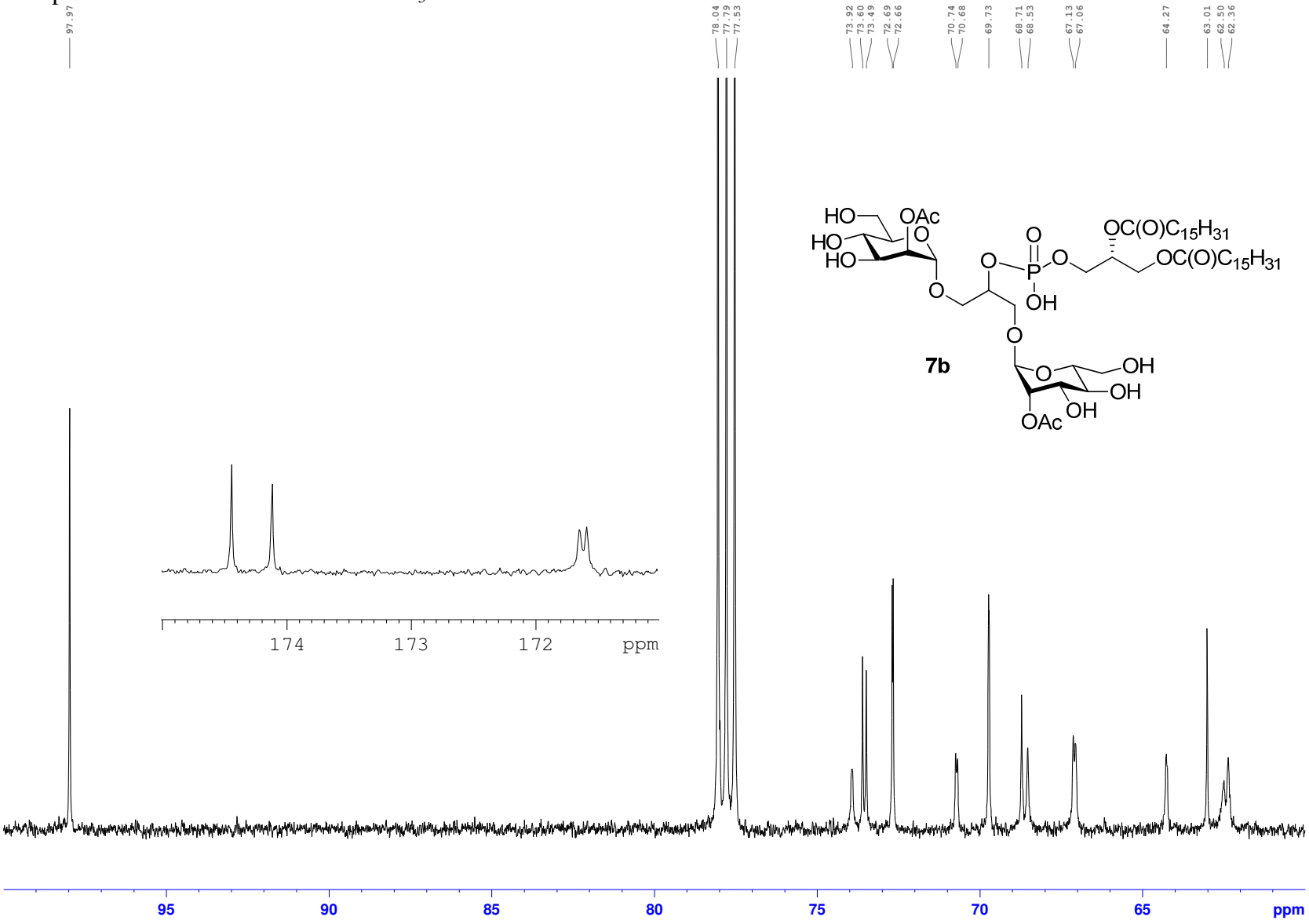
Compound **7b** 500 MHz ^{1}H NMR $CDCl_3$



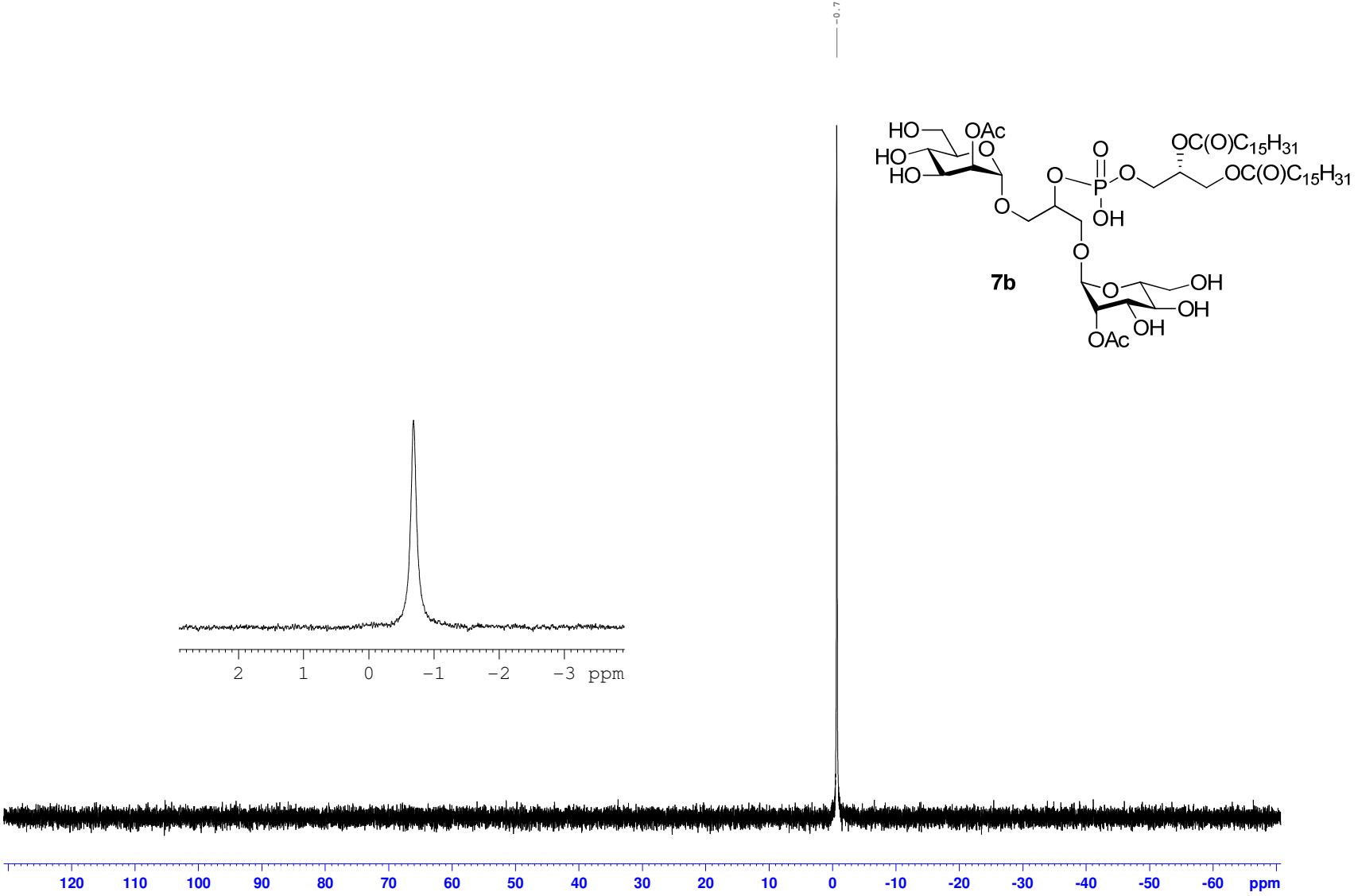
Compound **7b** 126 MHz ^{13}C NMR $CDCl_3$



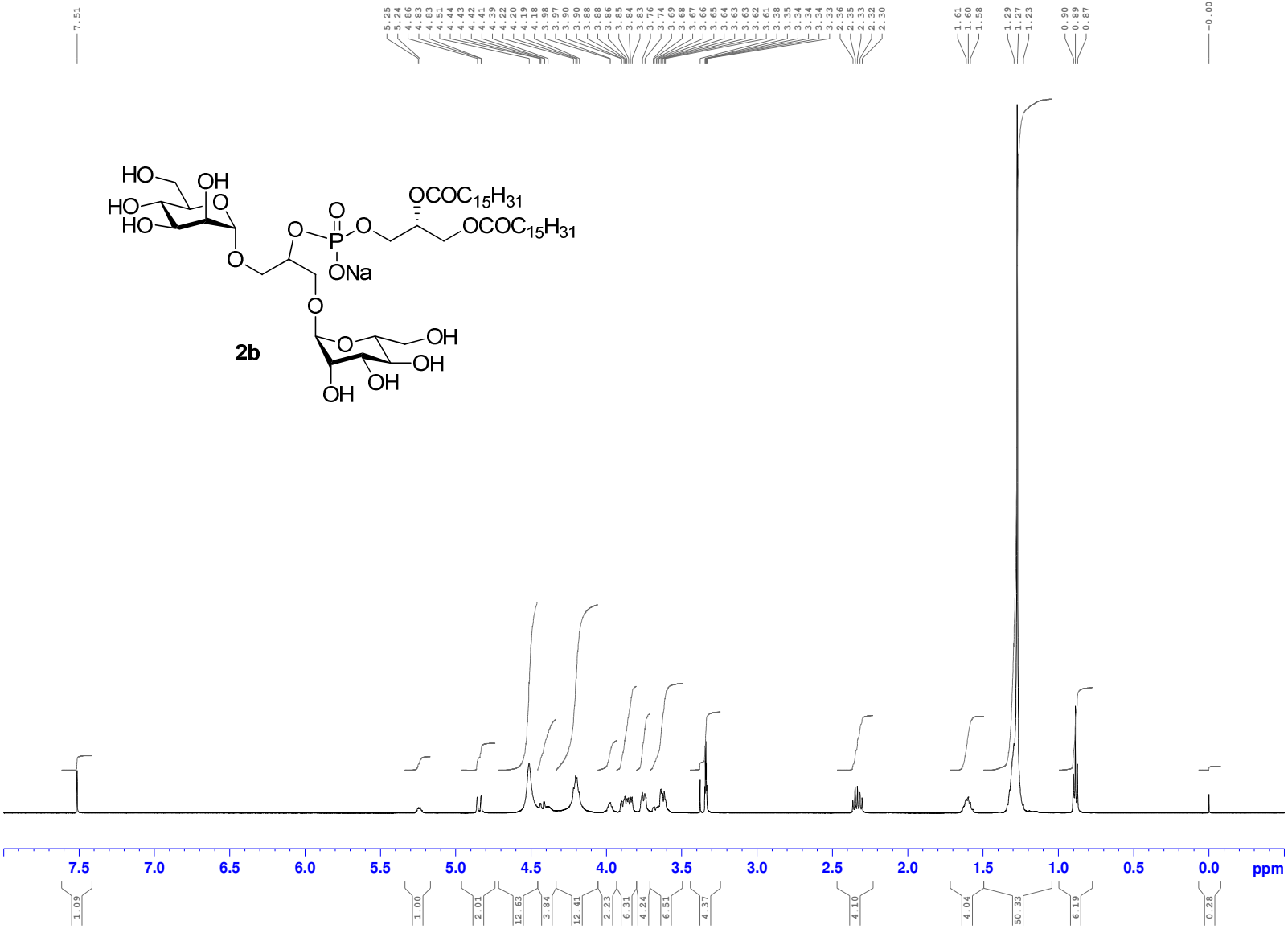
Compound **7b** 126 MHz ^{13}C NMR $CDCl_3$



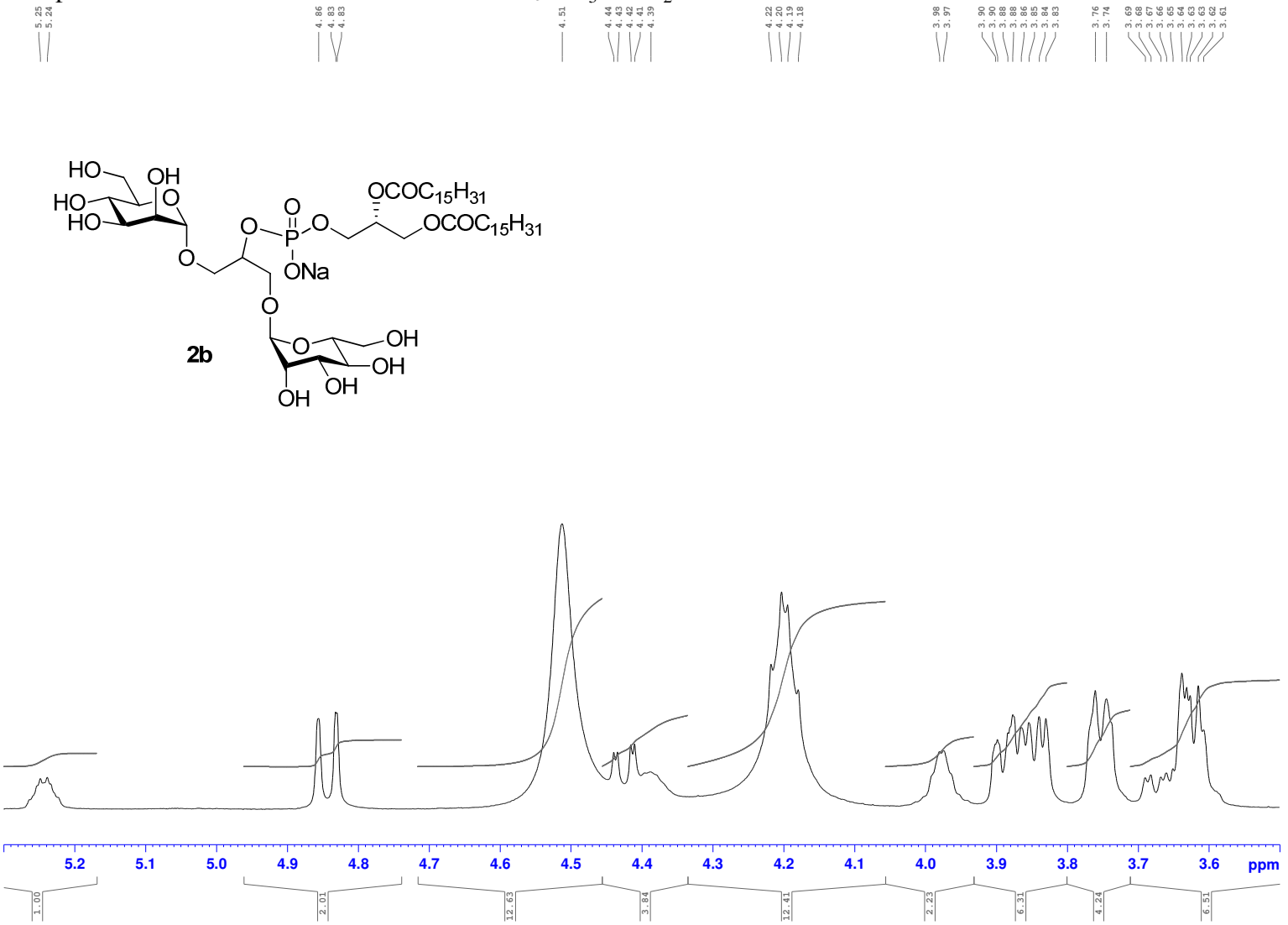
Compound **7b** 202 MHz ^{31}P NMR 2:1 $CDCl_3/CD_3OD$



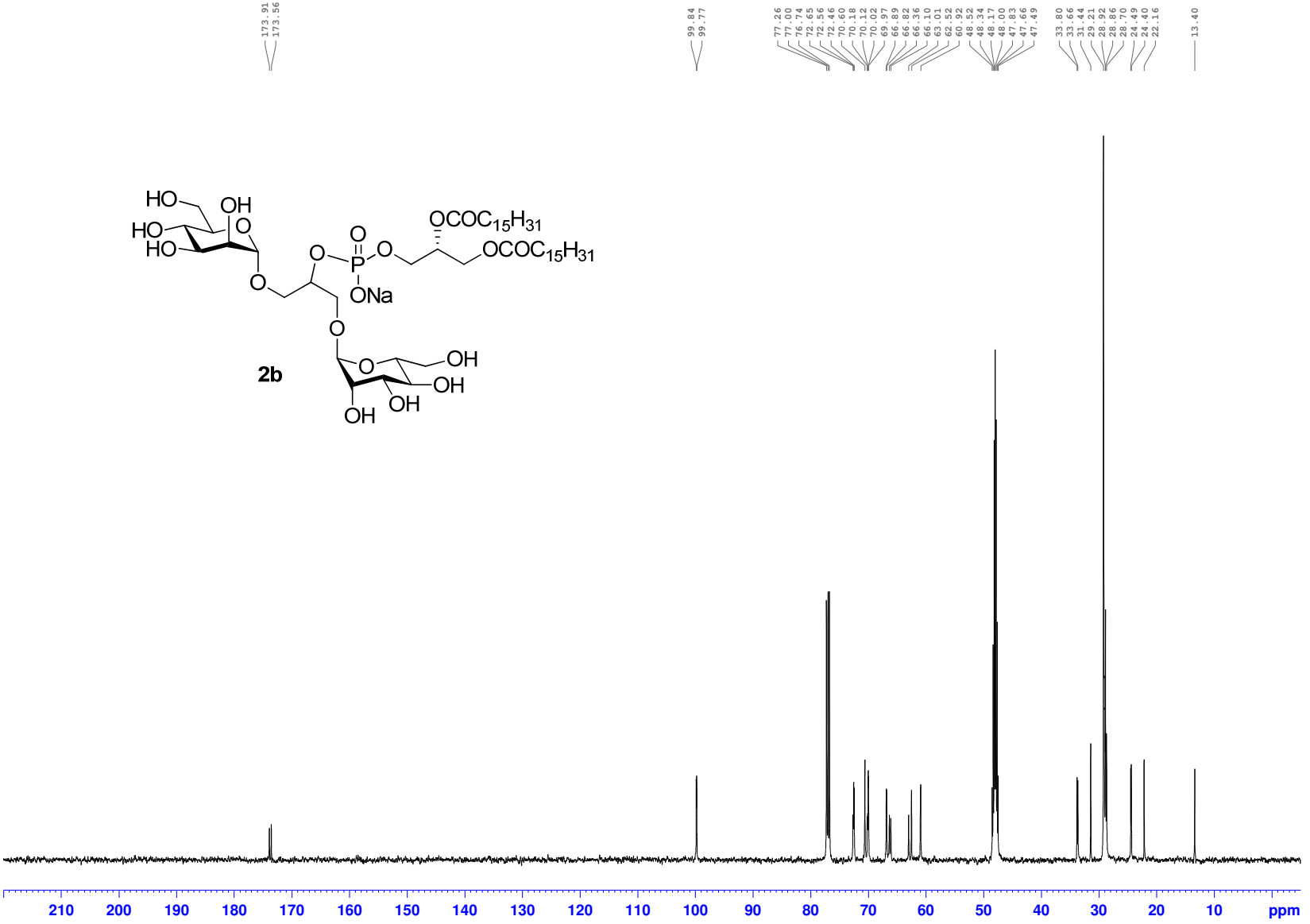
Compound **2b** 500 MHz ^{1}H NMR 2:1:0.17 $CDCl_3/CD_3OD/D_2O$



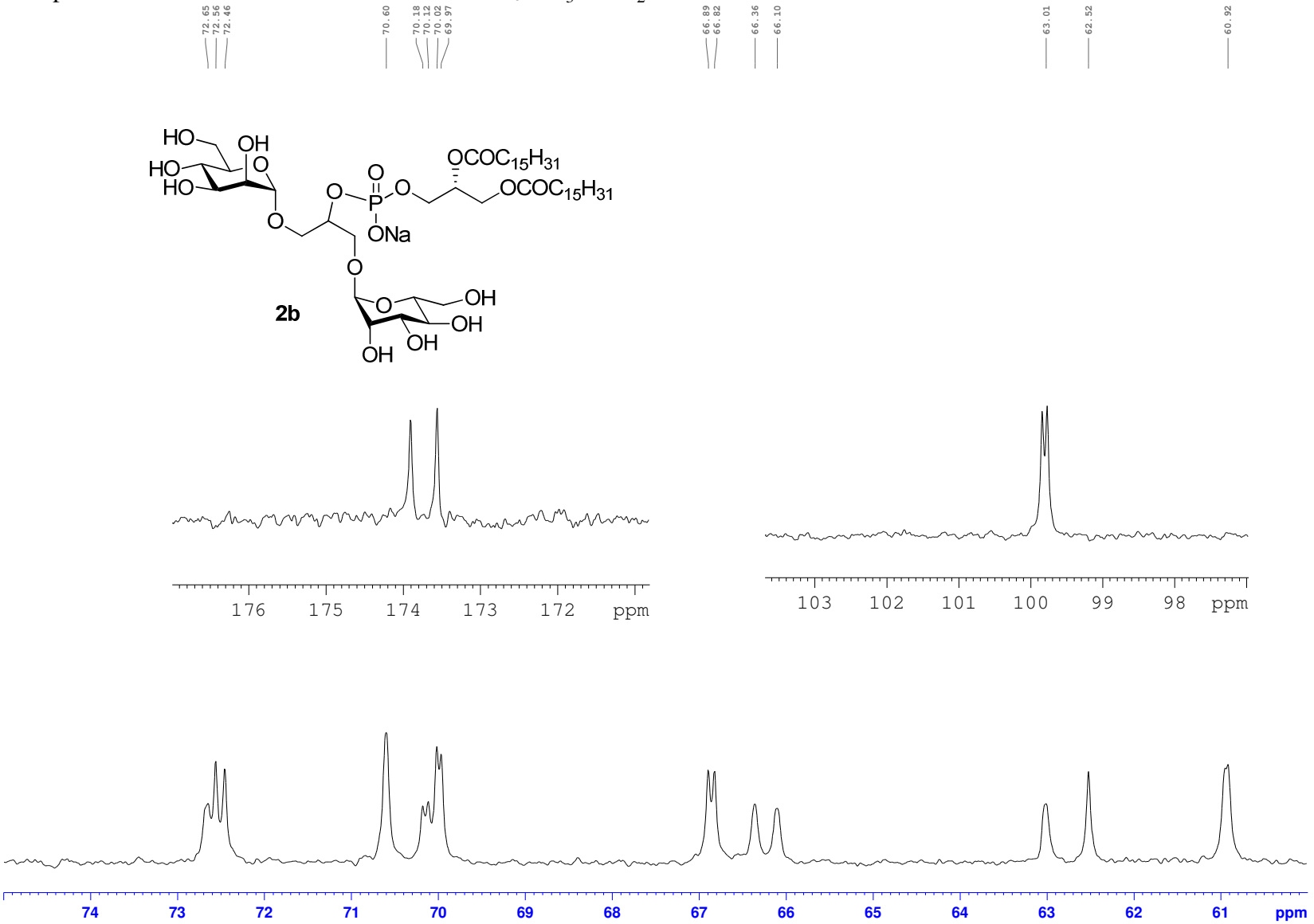
Compound **2b** 500 MHz ^{1}H NMR 2:1:0.17 $CDCl_3/CD_3OD/D_2O$



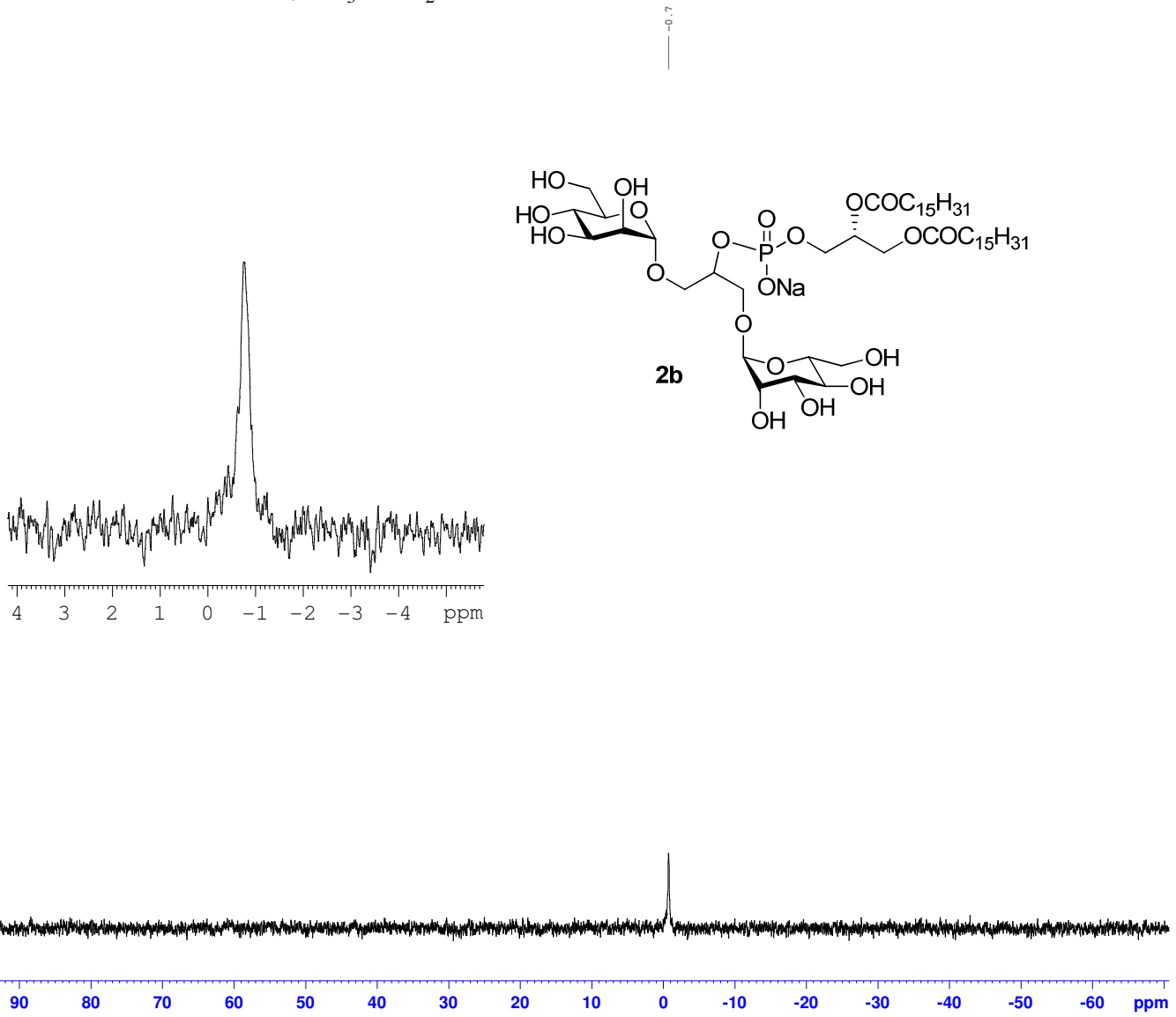
Compound **2b** 126 MHz ^{13}C NMR 2:1:0.17 $CDCl_3/CD_3OD/D_2O$



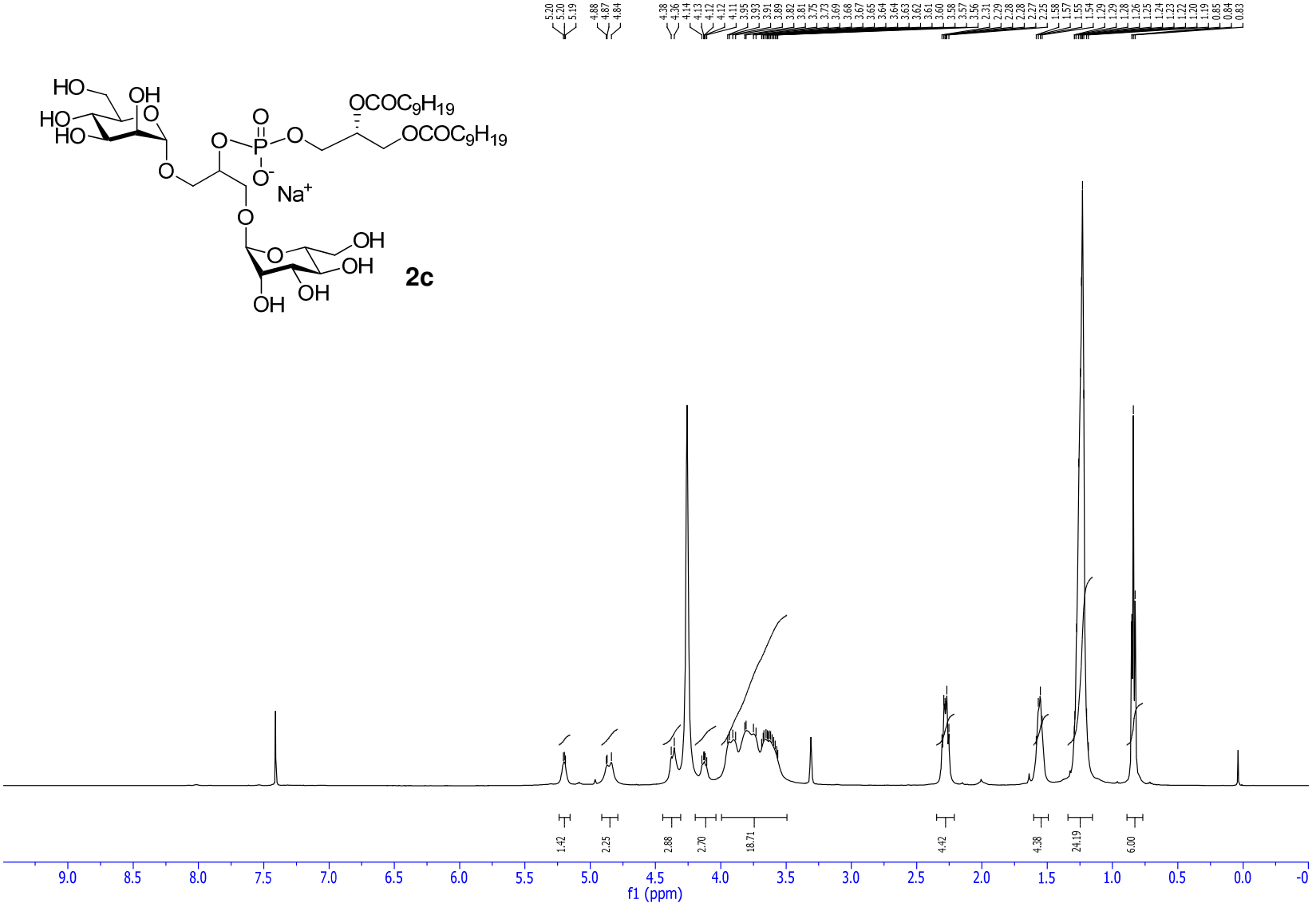
Compound **2b** 126 MHz ^{13}C NMR 2:1:0.17 $CDCl_3/CD_3OD/D_2O$



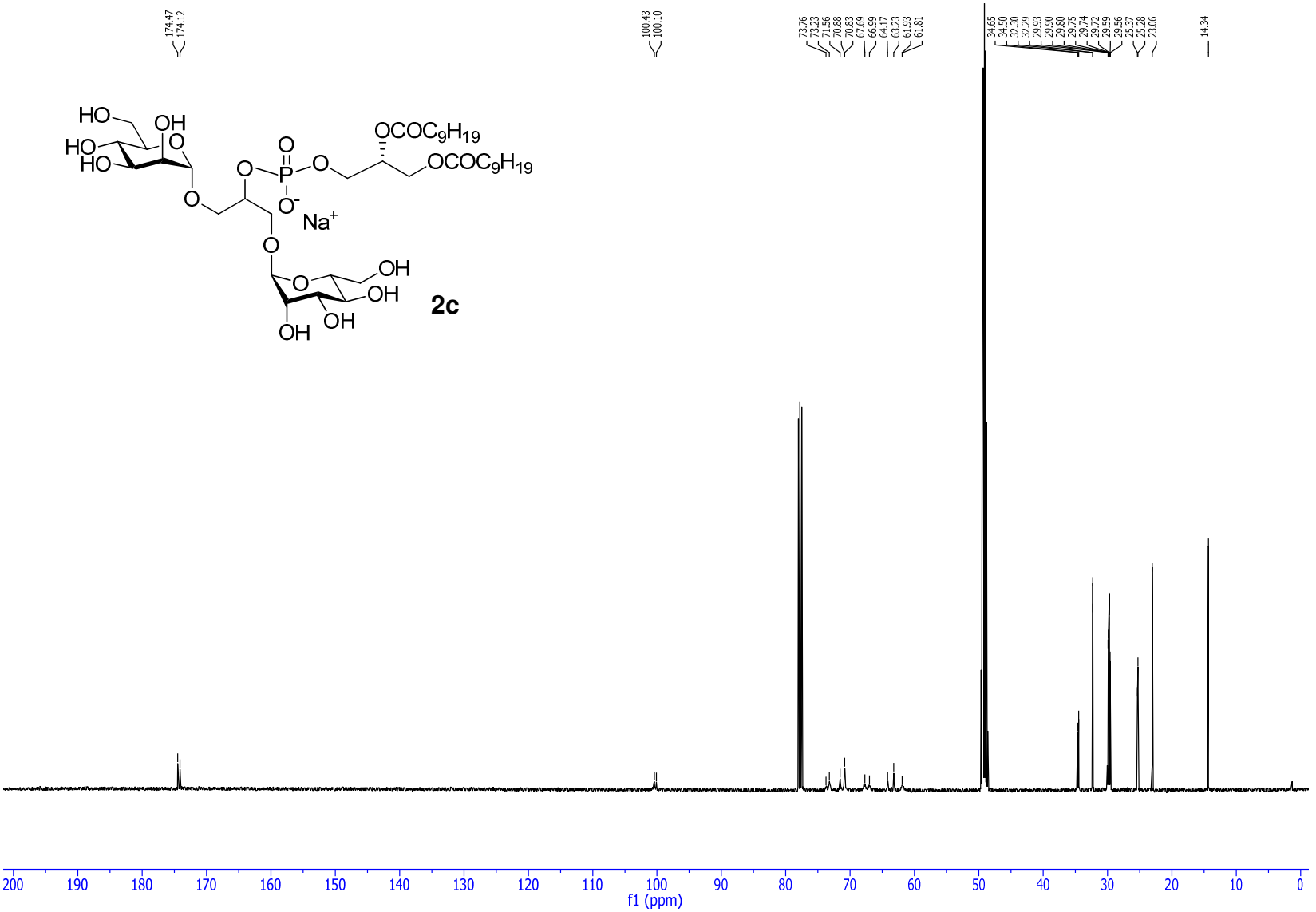
Compound **2b** 202 MHz ^{31}P NMR 2:1:0.17 $CDCl_3/CD_3OD/D_2O$



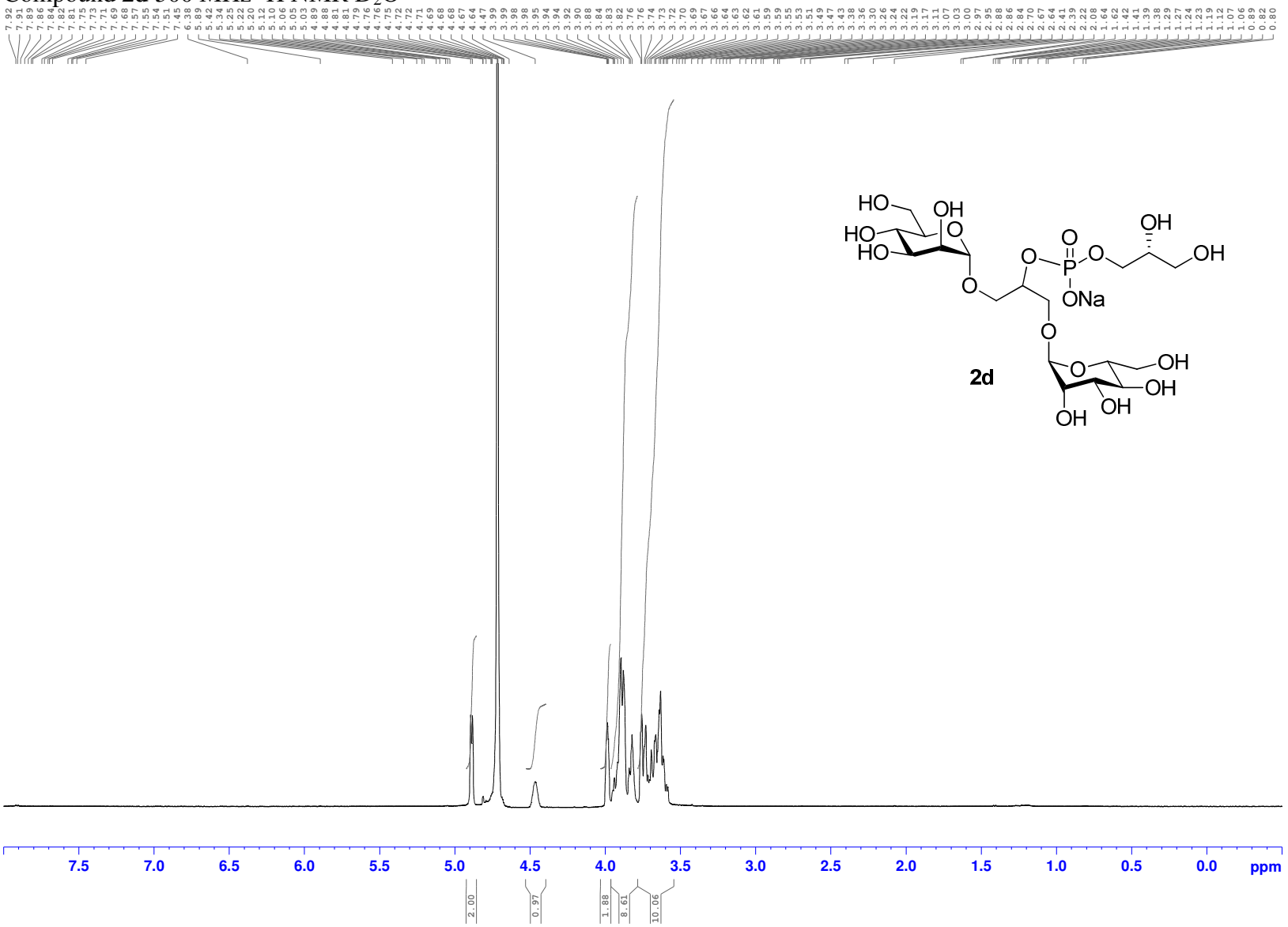
Compound **2c** 500 MHz ^{1}H NMR 2:1 $CDCl_3/CD_3OD$



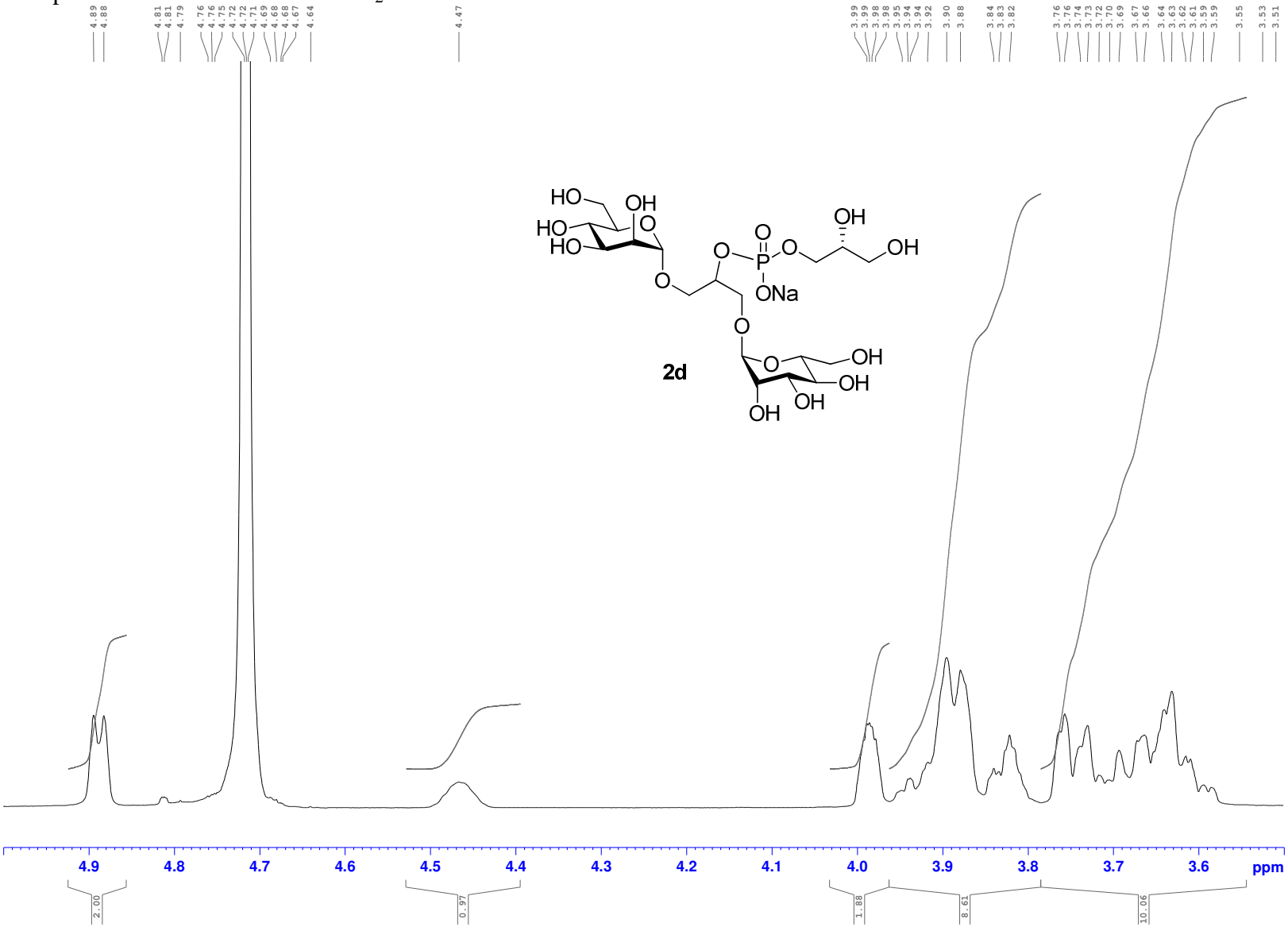
Compound **2c** 126 MHz ^{13}C NMR 2:1 $CDCl_3/CD_3OD$



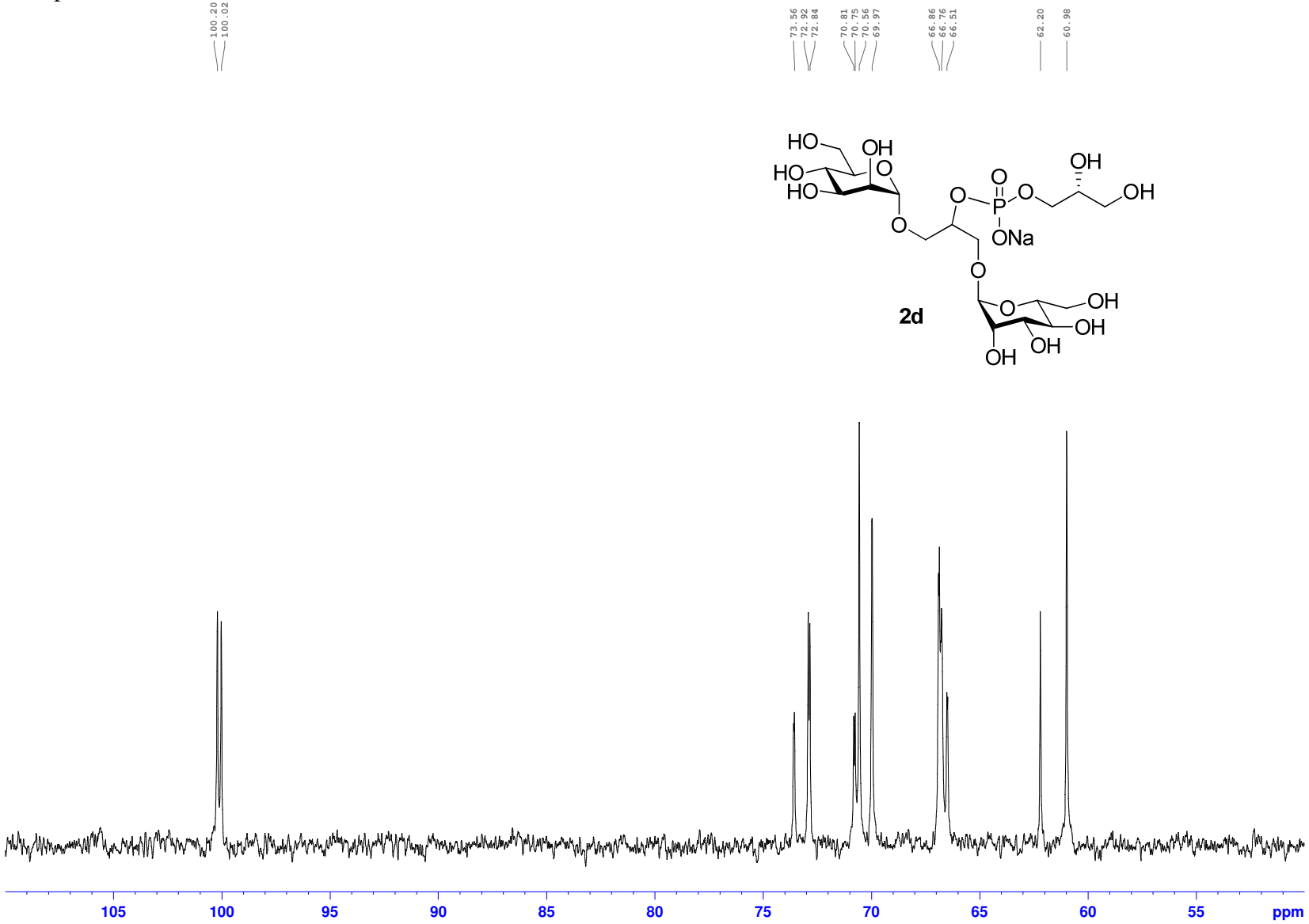
Compound **2d** 500 MHz ^{1}H NMR D_2O



Compound **2d** 500 MHz ^{1}H NMR D_2O



Compound **2d** 126 MHz ^{13}C NMR D_2O



Compound **2d** 202 MHz ^{31}P NMR D_2O