Synthesis and preliminary biological evaluation of carba analogues from *Neisseria meningitidis* A capsular polysaccharide

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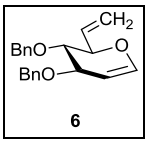
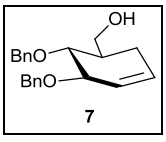


6

Synthesis of 1,5-Anhydro-di-*O*-benzyl-2,6,7-trideoxy-D-arabino-hept-1,6-dienitol (6). Freshly prepared 2-iodoxybenzoic acid[1] (IBX, 89 g, 319 mmol) was added to a solution of **5** (12.3 g, 38 mmol), prepared from glucal **4** according to the procedure reported in the literature,[2] in dry EtOAc (400 mL), and the suspension was stirred under nitrogen at 75 °C for 4 h. The mixture was then cooled, filtered over a Celite pad and concentrated. The crude was co-evaporated with toluene (3 × 50 mL) to obtain the aldehyde intermediate (12.3 g, 99%). NMR analysis showed the complete conversion of **5** into the aldehyde intermediate.

Freshly prepared PPh_3CH_3I (23.3 g, 57 mmol) was dissolved in dry THF (40 mL), the solution was cooled to -78 °C, then KHMDS (1M solution in THF, 57 mL, 57 mmol) was added under nitrogen. The mixture was stirred at -78 °C for 30 minutes, then another 1 h at 0 °C. A solution of the aldehyde (12.3 g, 38 mmol) in dry THF was added to the mixture at -78 °C. The reaction mixture was then stirred at room temperature. After 3 h, a saturated aqueous solution of NH_4Cl (200 mL) was added and the mixture was stirred for 10 min, then diluted with CH_2Cl_2 (300 mL) and the organic layer was washed with brine, dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (toluene:hexane 40:60) providing **6 (**9.4 g, 77 %) as a clear oil. The optical rotation and the spectroscopic characterization data of compound **6** were in agreement with those previously reported.[3]

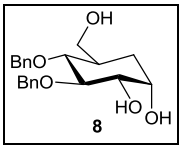


7

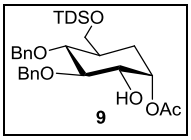
Synthesis of (3R,4R,5R)-3,4-dibenzyloxy-5-(hydroxymethyl)cyclohexene (7). Compound **6** (7.5 g, 23.3 mol) was dissolved in 1,6-dichlorobenzene (25 mL) in a sealed tube and heated at 240 °C for 2 h. After cooling down, the mixture was slowly poured into a suspension of $NaBH_4$ (500 mg, 13 mmol) in THF (100 mL) and EtOH (25 mL) and stirred for 15 min. Then water (200 mL) was added, and the mixture was extracted with CH_2Cl_2 (3×100 mL). The organic layer was washed with

[1] M. Frigerio, M. Santagostino and S. Sputore, *J. Org. Chem.*, 1999, **12**, 4537-4538.
[2] A. V. R. L. Sudha and M. Nagarajan, *Chem. Commun.*, 1998, 925-926.
[3] R. V. Stick and K. A. Stubbs, *J. Carbohydr. Chem.*, 2005, **24**, 529–547.

brine (200 mL), dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (EtOAc:hexane 20:80 → 50:50), providing **7** (6.5 g, 86 %) as a clear oil. The optical rotation and the spectroscopic characterization data of compound **7** were in agreement with those previously reported.[3]



Synthesis of 3,4-Di-*O*-benzyl-5a-carba-α-D-glucopyranose (8). Compound **7** (6.5 g, 20.8 mmol) was dissolved in a mixture of acetone (75 mL) and H_2O (25 mL), then a solution of OsO_4 (CAUTION!) (250 mg in 4.5 mL H_2O and 18 mL acetone) was added at room temperature, followed by Me_3NO (5.075 g, 46 mmol), and stirring was continued for 48 h at room temperature. A saturated aqueous solution of $Na_2S_2O_3$ (50 mL) was added and stirred for 30 min to reduce the OsO_4, then chloroform (300 mL) was added and the organic layer was washed with brine (200 mL), dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (MeOH:CH_2Cl_2 10:90), providing **8** (6.23 g, 86.3%) as a white solid. The optical rotation and the spectroscopic characterization data of triol **8** were in agreement with those previously reported.[4]

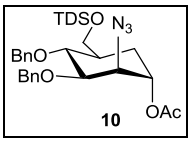


Synthesis of 1-*O*-Acetyl-3,4-di-*O*-benzyl-6-*O*-thexyldimethylsilyl-5a-carba-α-D-glucopyranose (9). To a solution of **8** (8.1 g, 22.6 mmol) and imidazole (4.6 g, 68 mmol) in THF (200 mL), thexyldimethylsilyl chloride (9.8 mL, 52 mmol) was added dropwise at 15 °C. The mixture was stirred at room temperature for 24 h, then a saturated aqueous solution of $NaHCO_3$ (100 mL) was added, followed by extraction with EtOAc (3 × 150 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated. The crude was purified by flash chromatography (EtOAc:hexane 30:70), yielding the 6-*O*-silylated intermediate (10.5 g, 93 %) as a colourless oil.

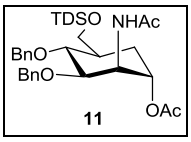
The *O*-silylated intermediate was dissolved in dry CH_3CN (150 mL) under nitrogen. Trimethyl orthoacetate (6.25 mL, 50 mmol) was added at room temperature, followed by a catalytic amount of

[4] L. Toma, L. Legnani, A. Rencurosi, L. Poletti, L. Lay and G. Russo, *Org. Biomol. Chem.*, 2009, **7**, 3734–3740.

p-toluenesulfonic acid. After 15 min, a 80% aqueous solution of acetic acid (150 mL) was added and stirring was continued for 15 min. CH_2Cl_2 (200 mL) was added and the organic layer was washed with H_2O (200 mL) and a saturated aqueous solution of $NaHCO_3$ (200 mL), dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash chromatography (EtOAc:hexane 25:75), providing **9** (10.45 g, 91%) as a clear oil. The optical rotation and the spectroscopic characterization data of alcohol **9** were in agreement with those previously reported.[4]



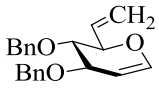
Synthesis of 1-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose (10). Alcohol **9** (10.3 g, 19 mmol) was dissolved in a 5:1 mixture of CH_2Cl_2-Pyridine (360 mL), then trifluoromethanesulfonic anhydride (17 mL, 104 mmol) was added dropwise at -10 °C. The mixture was stirred at 0 °C for 60 min, then a saturated aqueous solution of $NaHCO_3$ (150 mL) was added, and the organic layer was washed with brine (200 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was then dissolved in a 19:1 mixture of DMF-H_2O (100 mL), and NaN_3 (6.2 g, 95 mmol) was added. The reaction mixture was stirred overnight at 40 °C, then the solvent was evaporated and the crude residue was purified by flash chromatography (EtOAc:toluene 2:98), giving **10** (8.5 g, 79%) as an oil. The optical rotation and the spectroscopic characterization data of compound **10** were in agreement with those previously reported.[4]

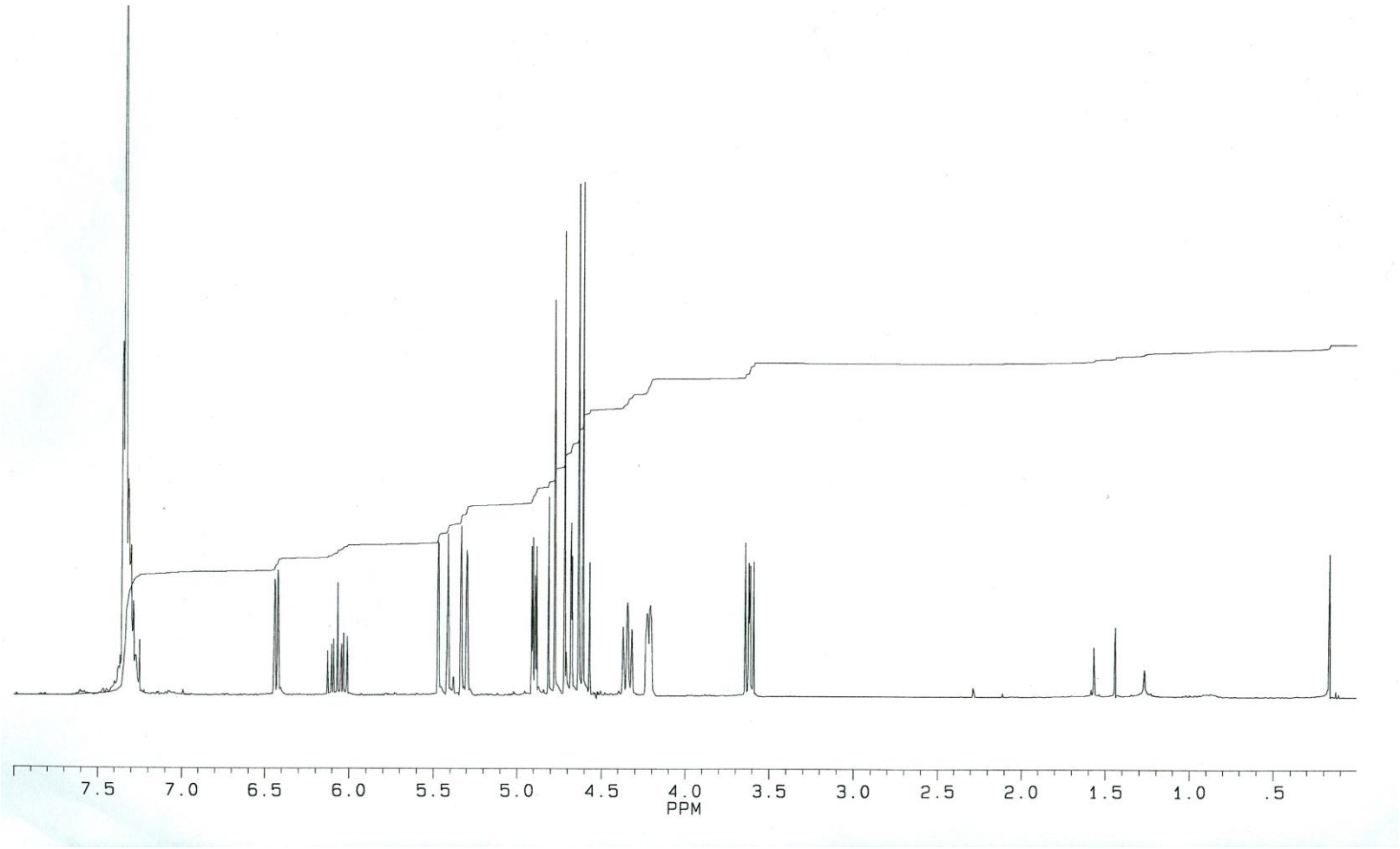


Synthesis of 2-Acetamido-1-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-6-*O*-thexyldimethylsilyl-5a-carba-α-D-mannopyranose (11). A mixture of **10** (9.1 g, 16 mmol) and freshly crystallized PPh_3 (12.6 g, 48 mmol) in dry THF (250 mL) was stirred overnight at 60 °C under nitrogen atmosphere. After addition of water (40 mL), the reaction was stirred for further 24 h at the same temperature, then the solvent was evaporated. The residue was dissolved in MeOH (200 mL) and acetic anhydride (30 mL, 320 mmol) was added. After 24 h the solvent was evaporated and the crude

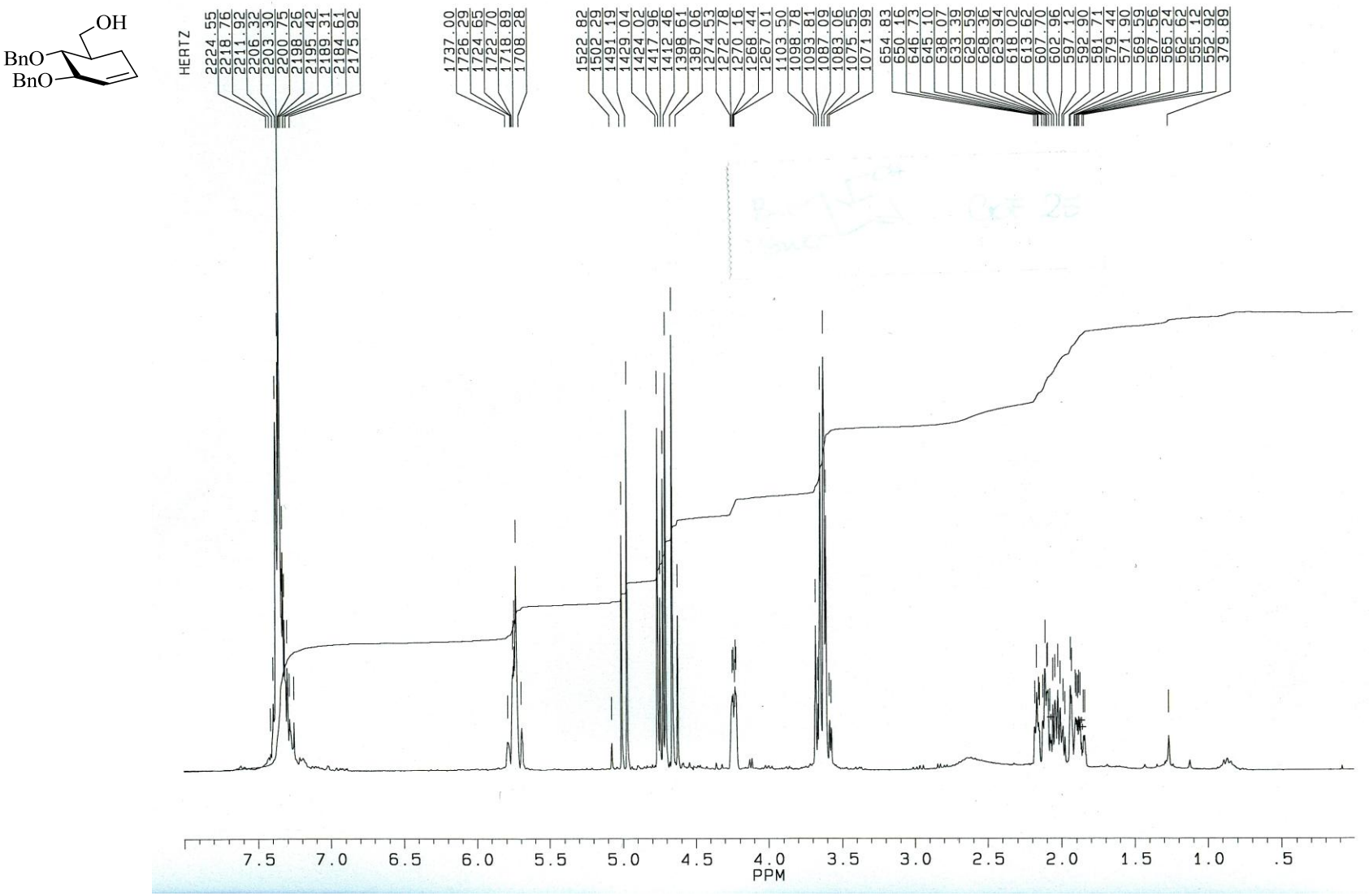
material was purified by flash chromatography (EtOAc:hexane 30:70), providing **11** (8.86 g, 95%) as an oil. The optical rotation and the spectroscopic characterization data of compound **11** were in agreement with those previously reported.[4]

^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **6**

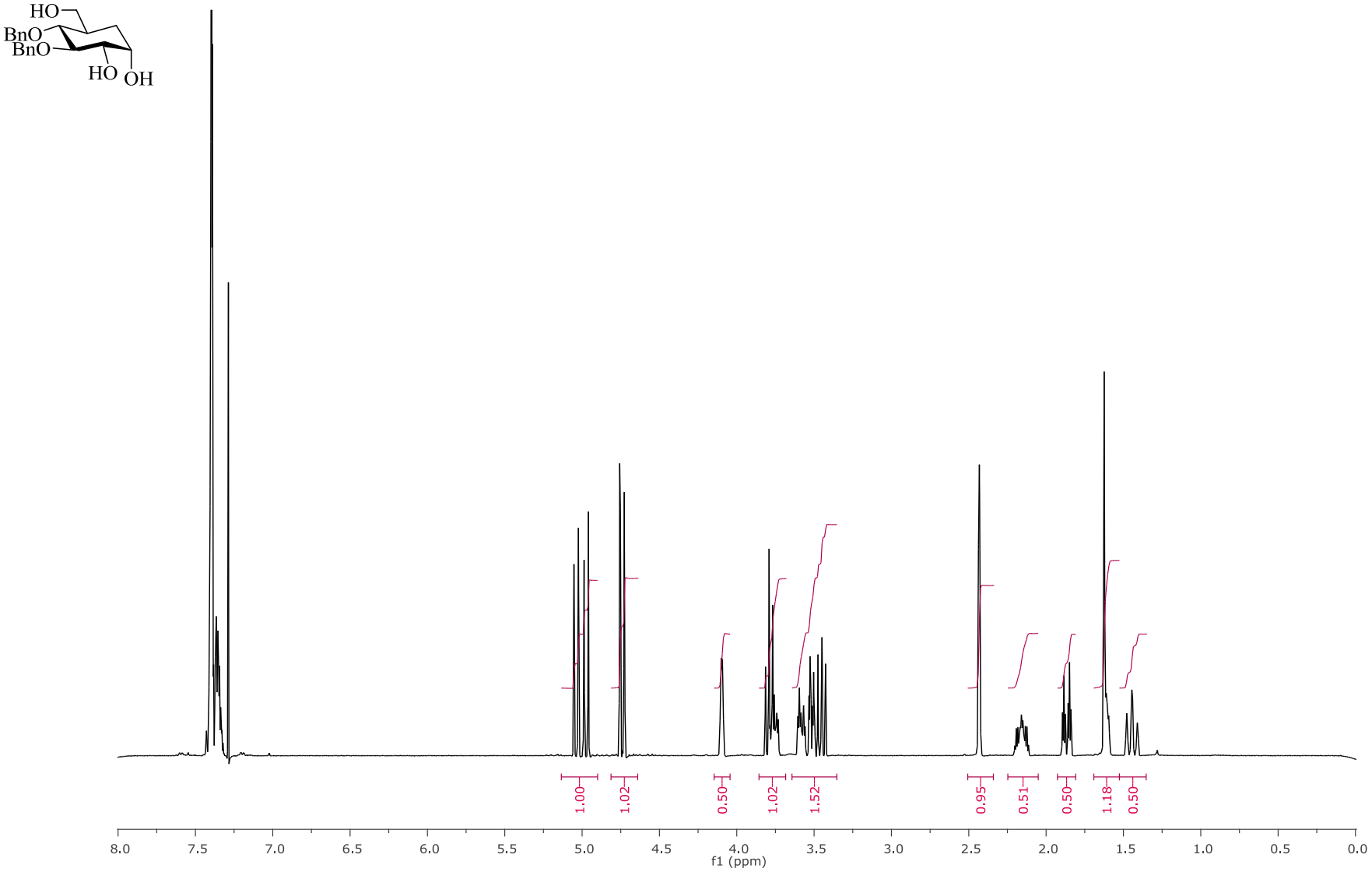




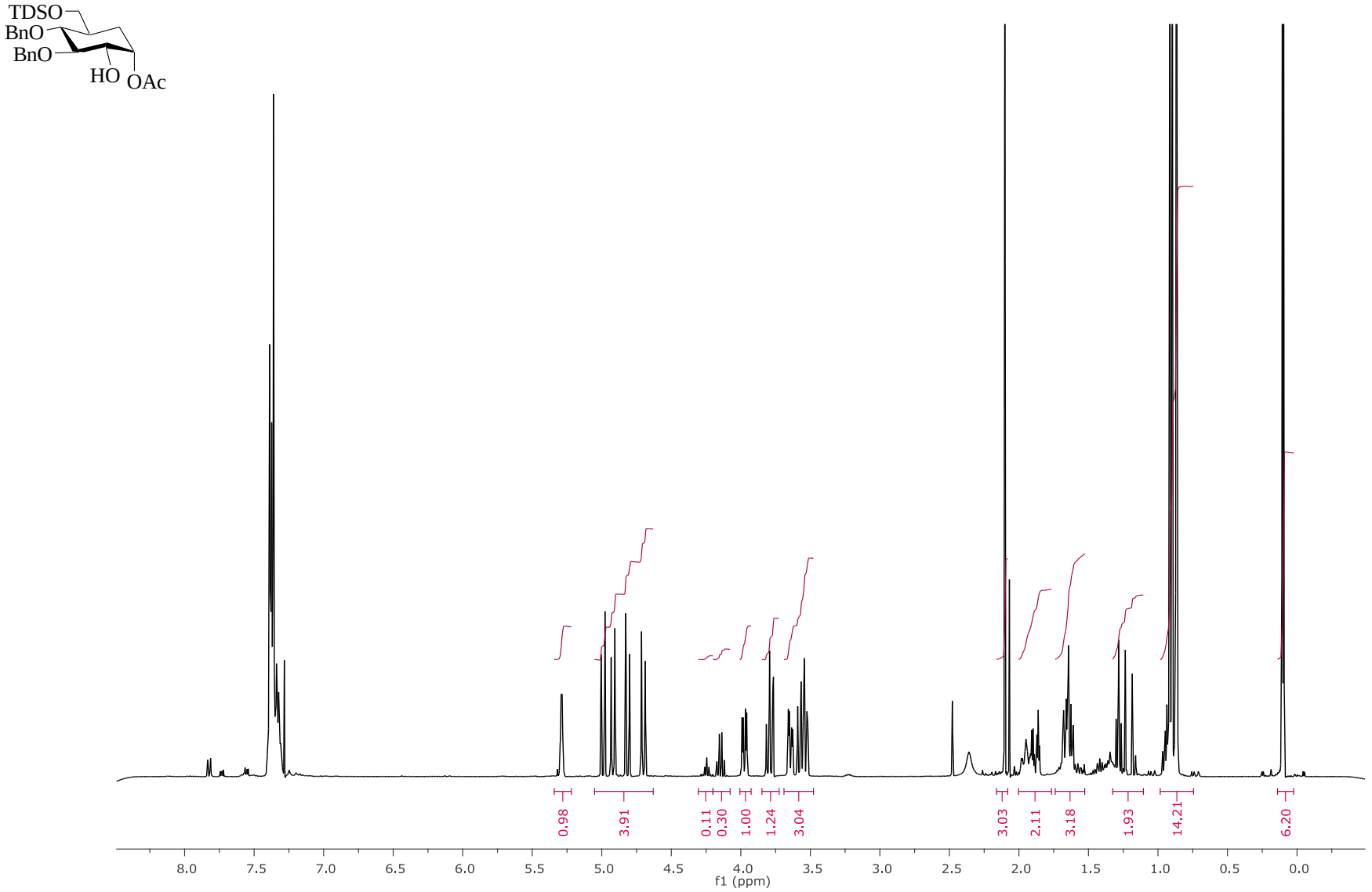
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **7**



^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **8**



^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **9**



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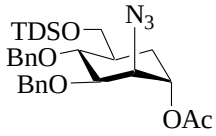

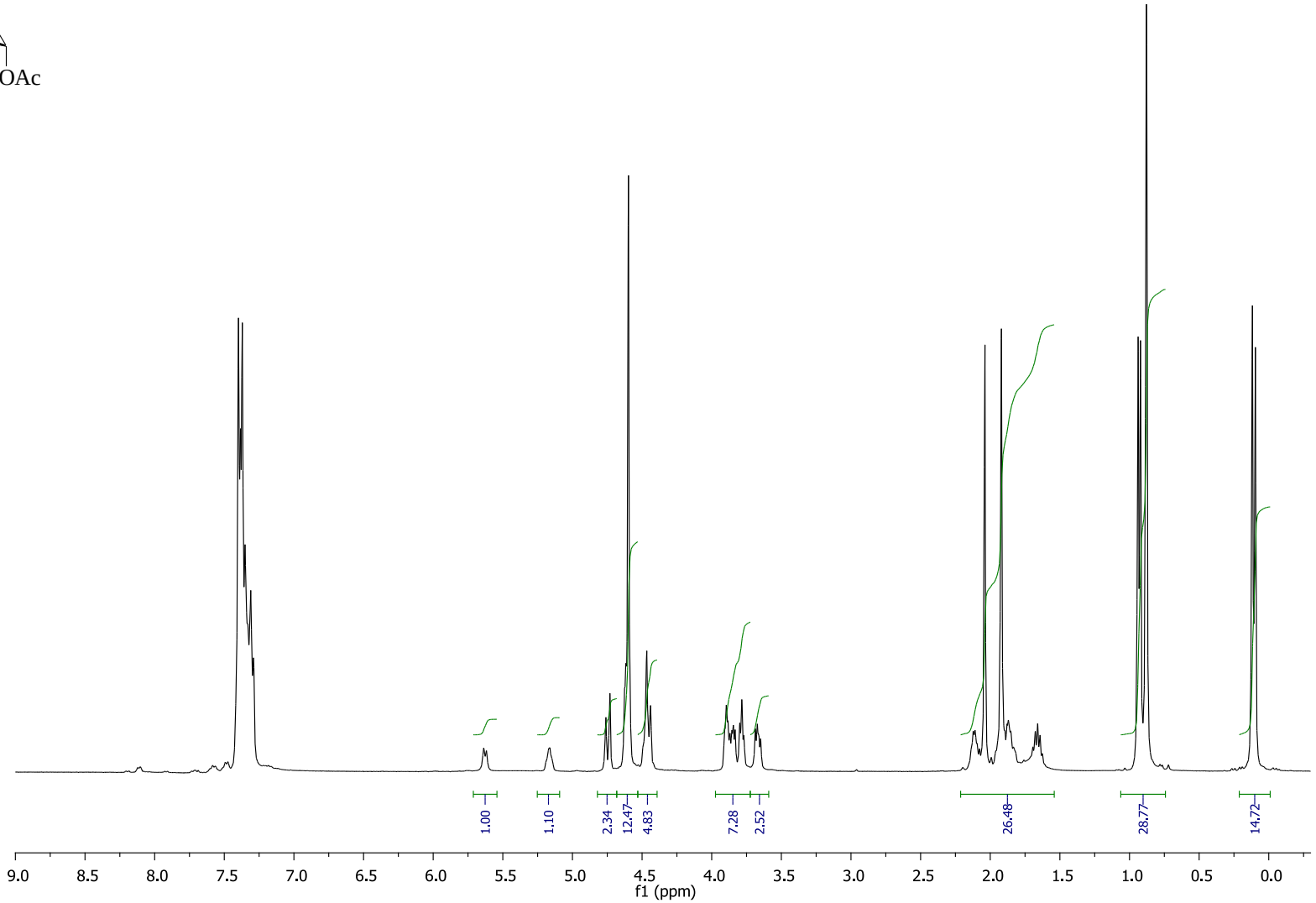

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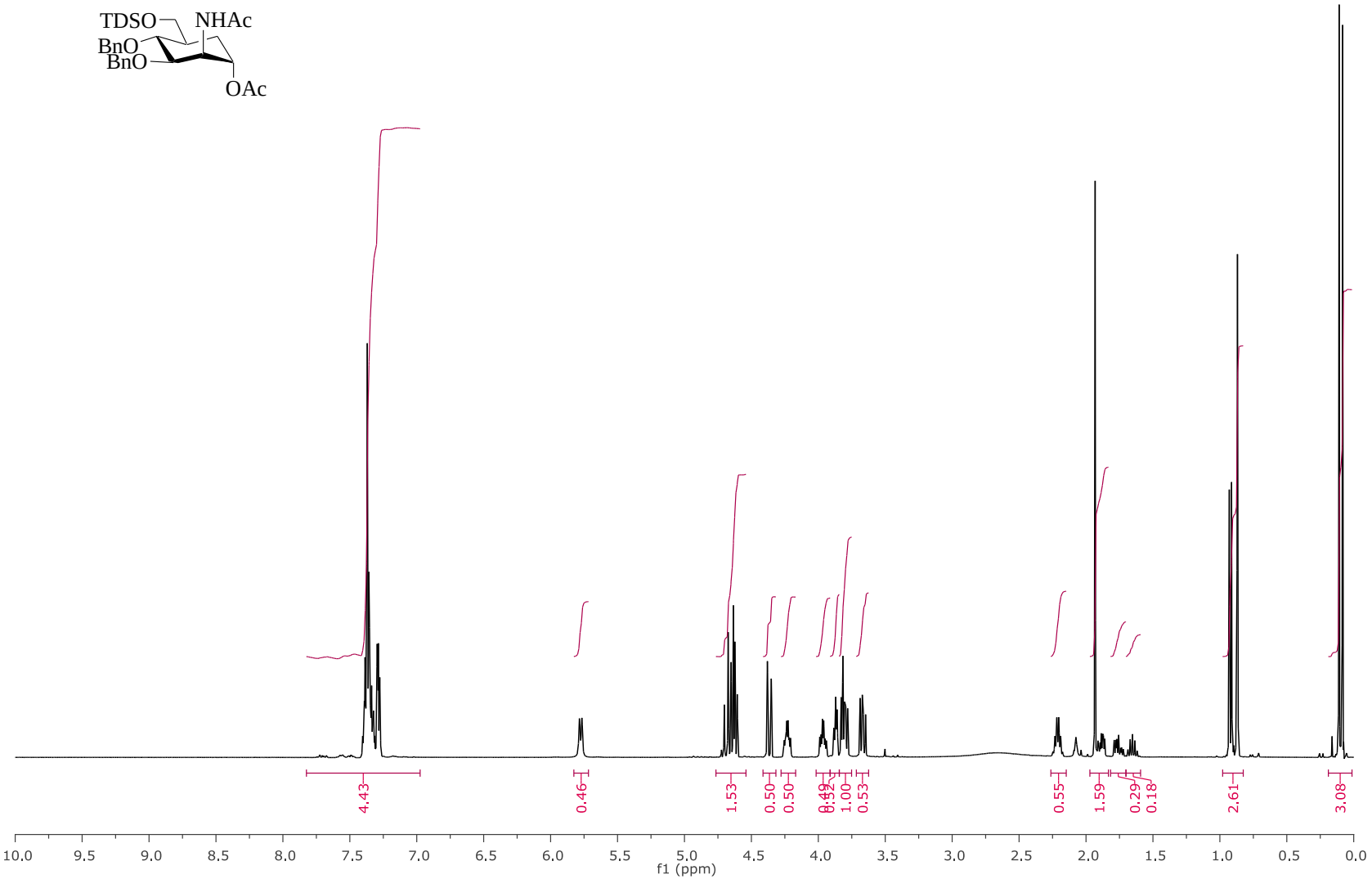
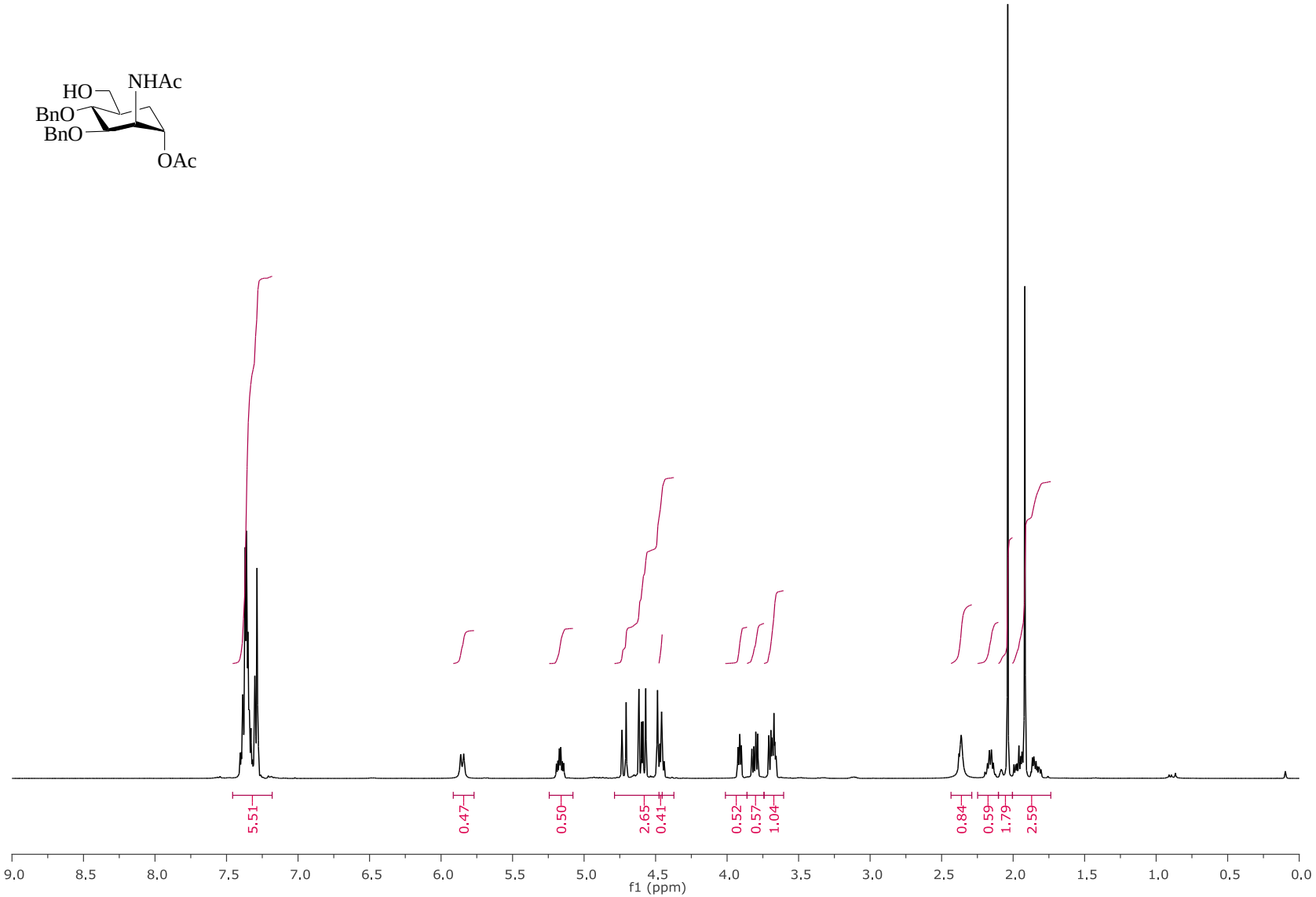
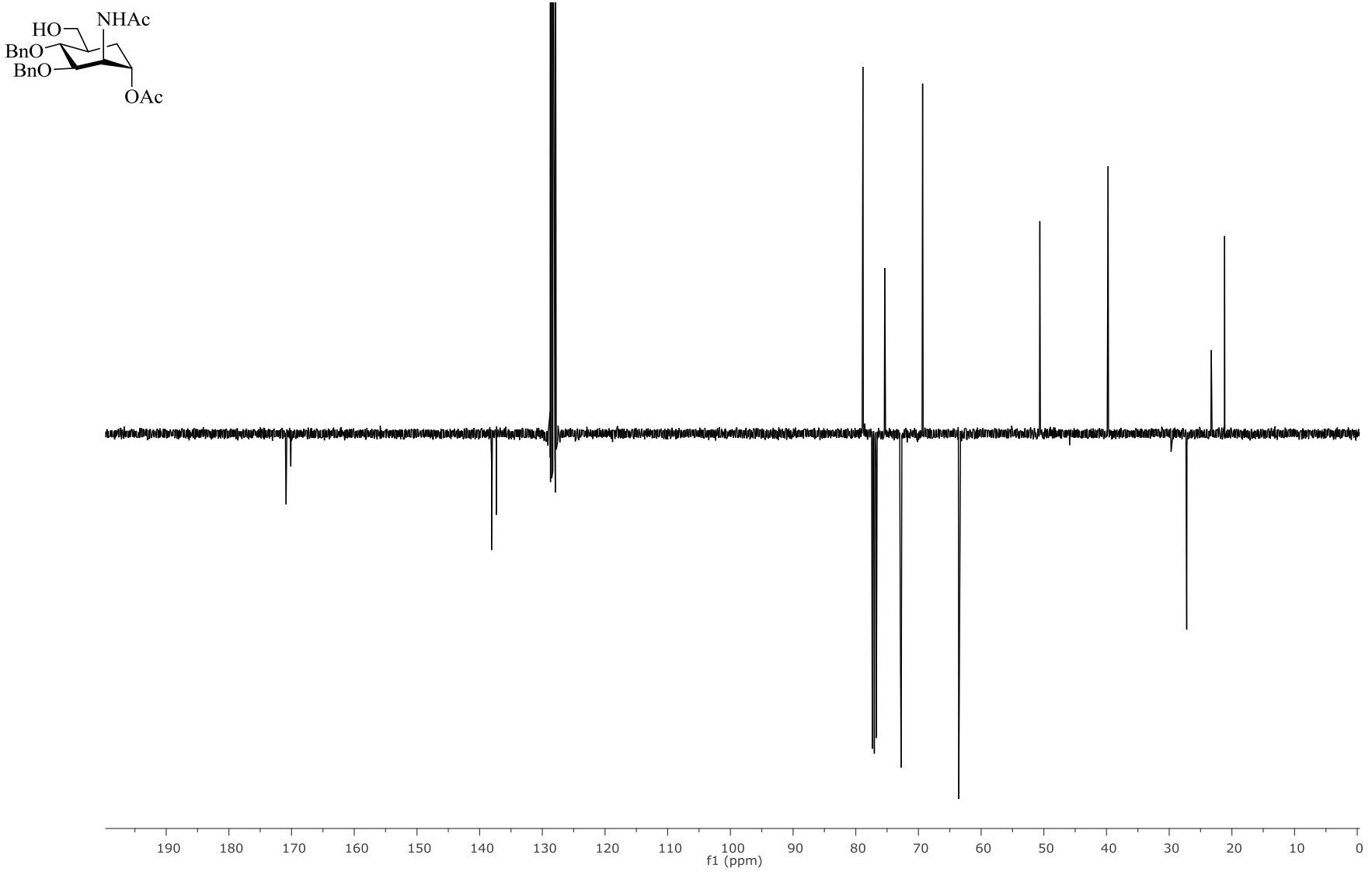


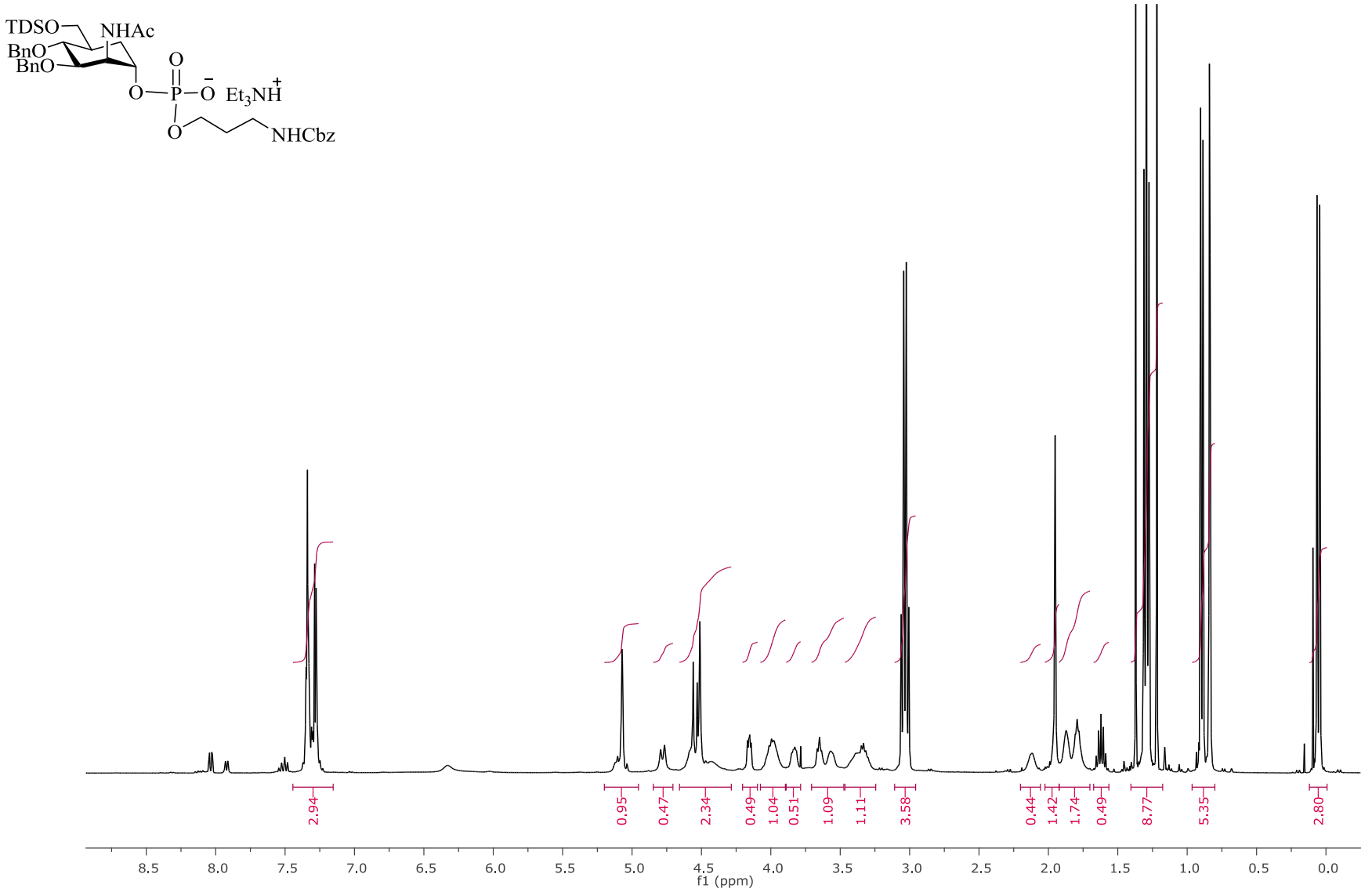
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **12**



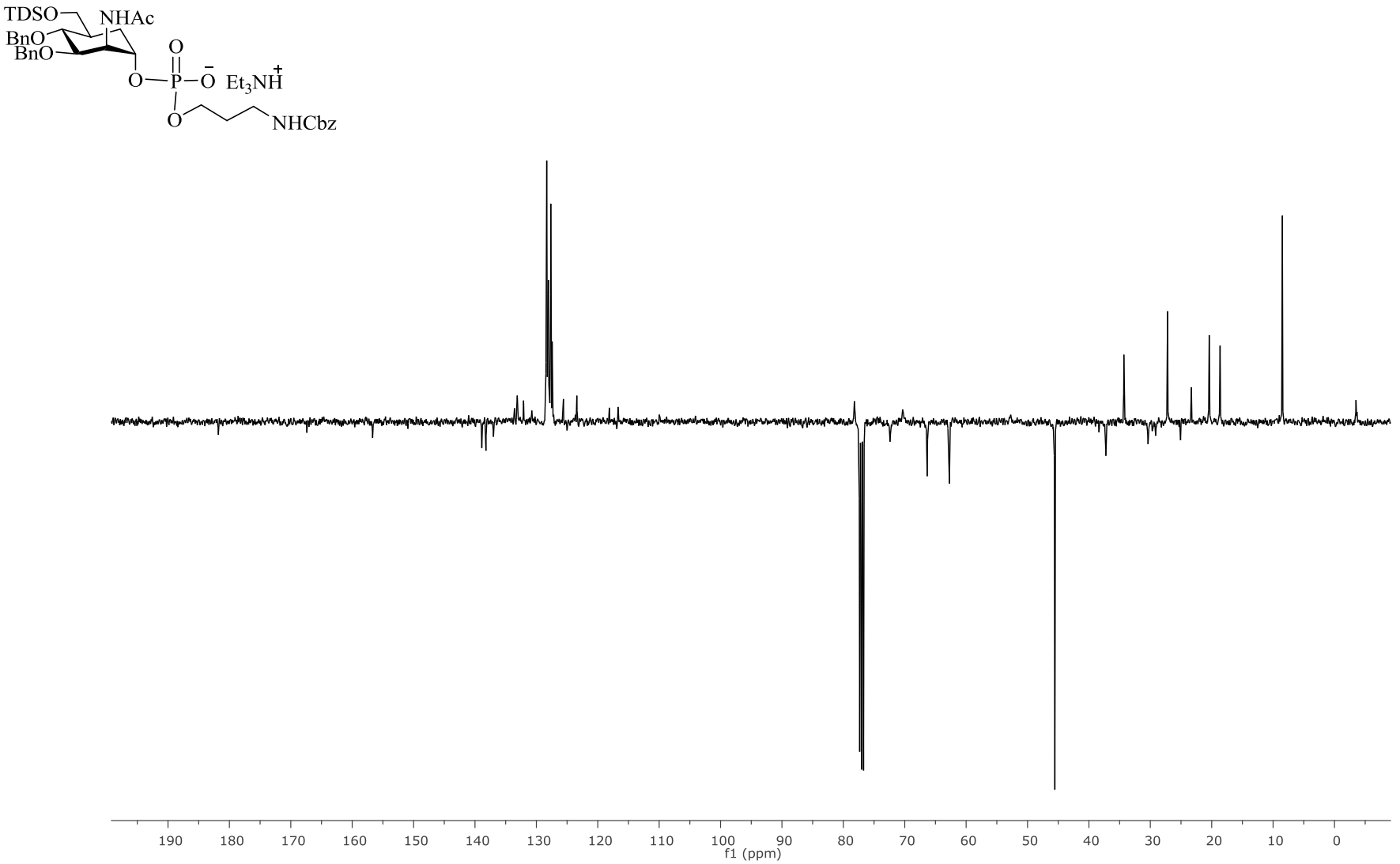
^{13}C NMR (100.6 MHz, $CDCl_3$) spectrum of compound **12**



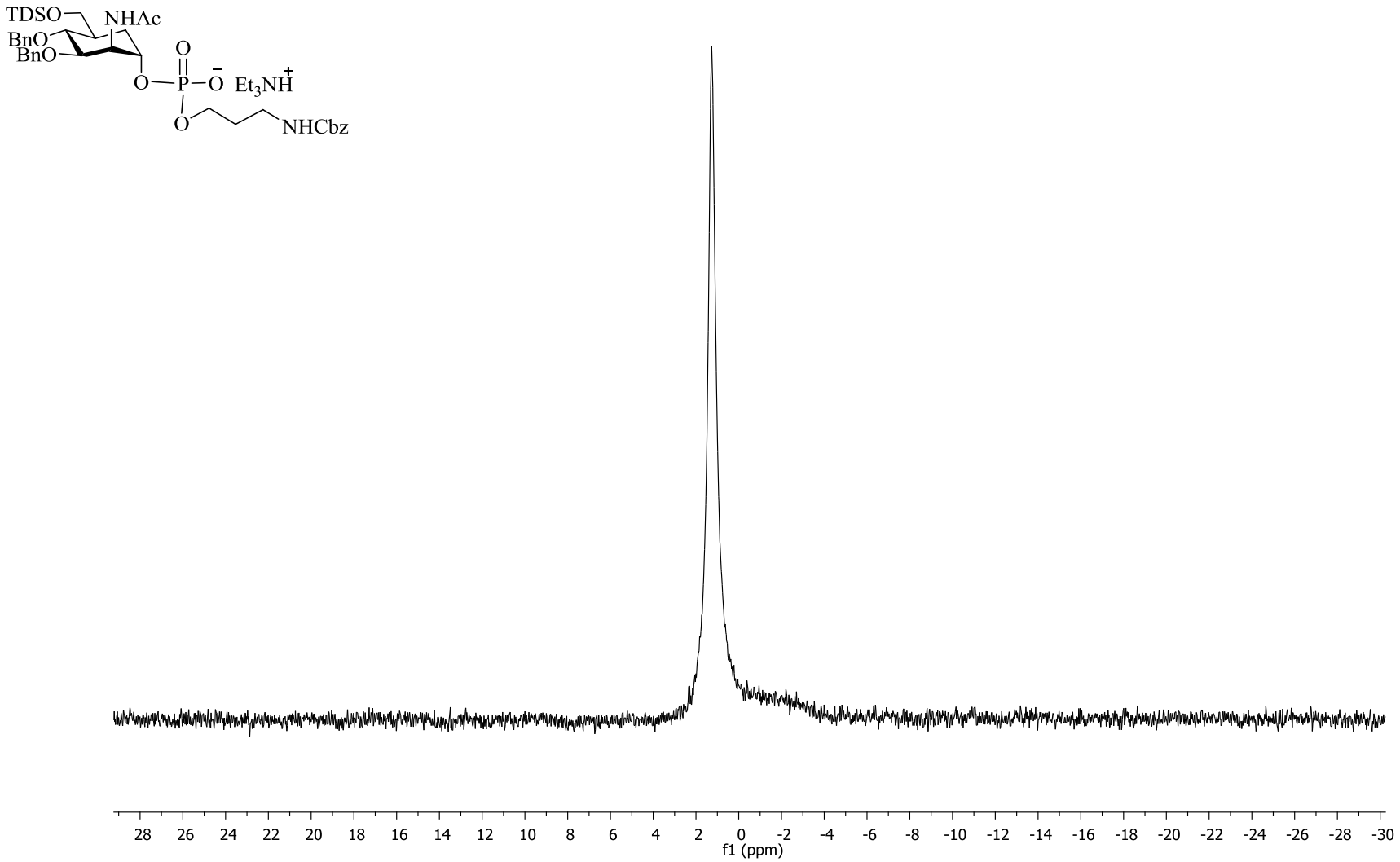
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **17**



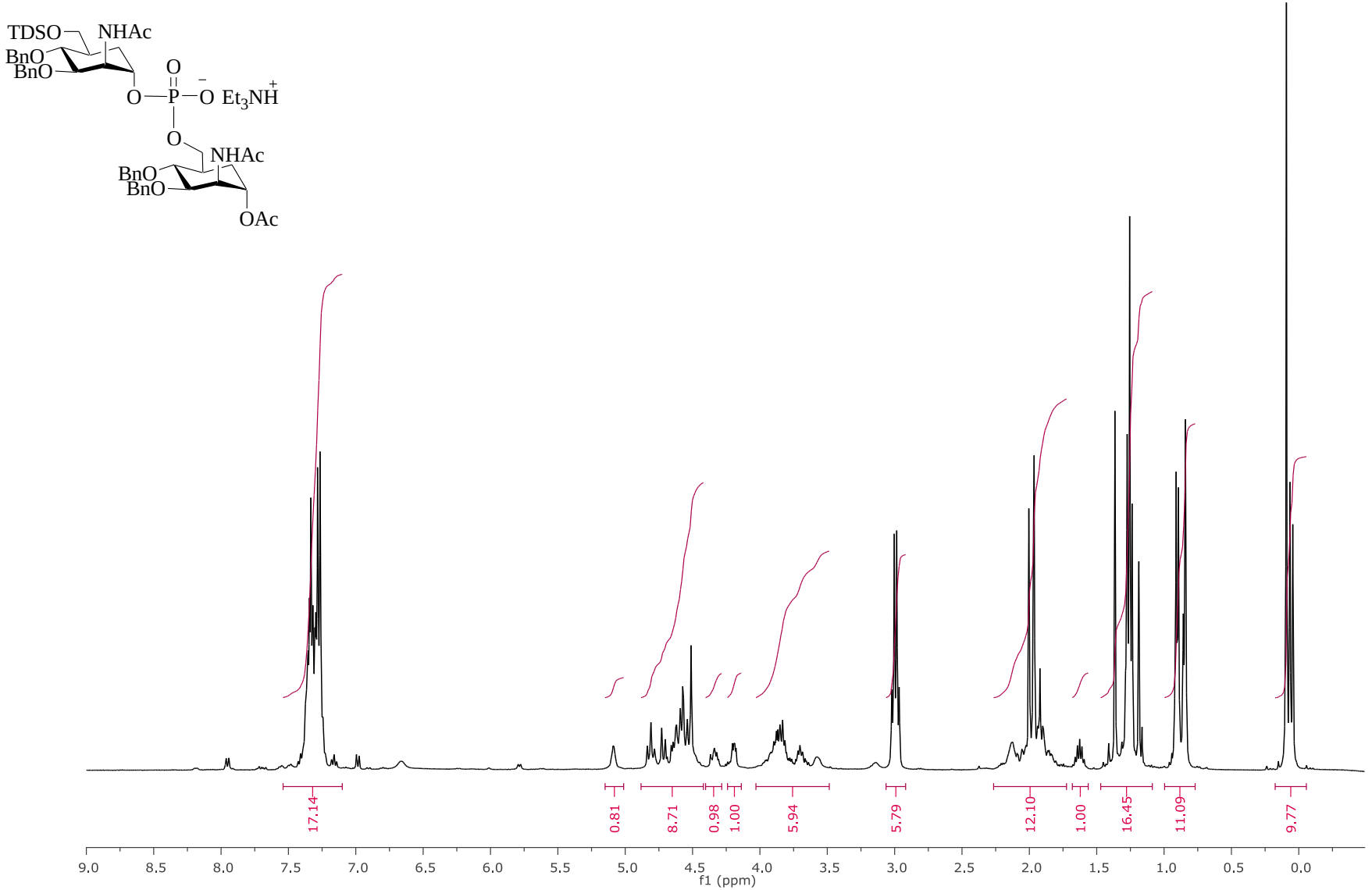
^{13}C NMR (100.6 MHz, $CDCl_3$) spectrum of compound **17**



^{31}P NMR (162 MHz, $CDCl_3$) spectrum of compound **17**



^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **18**



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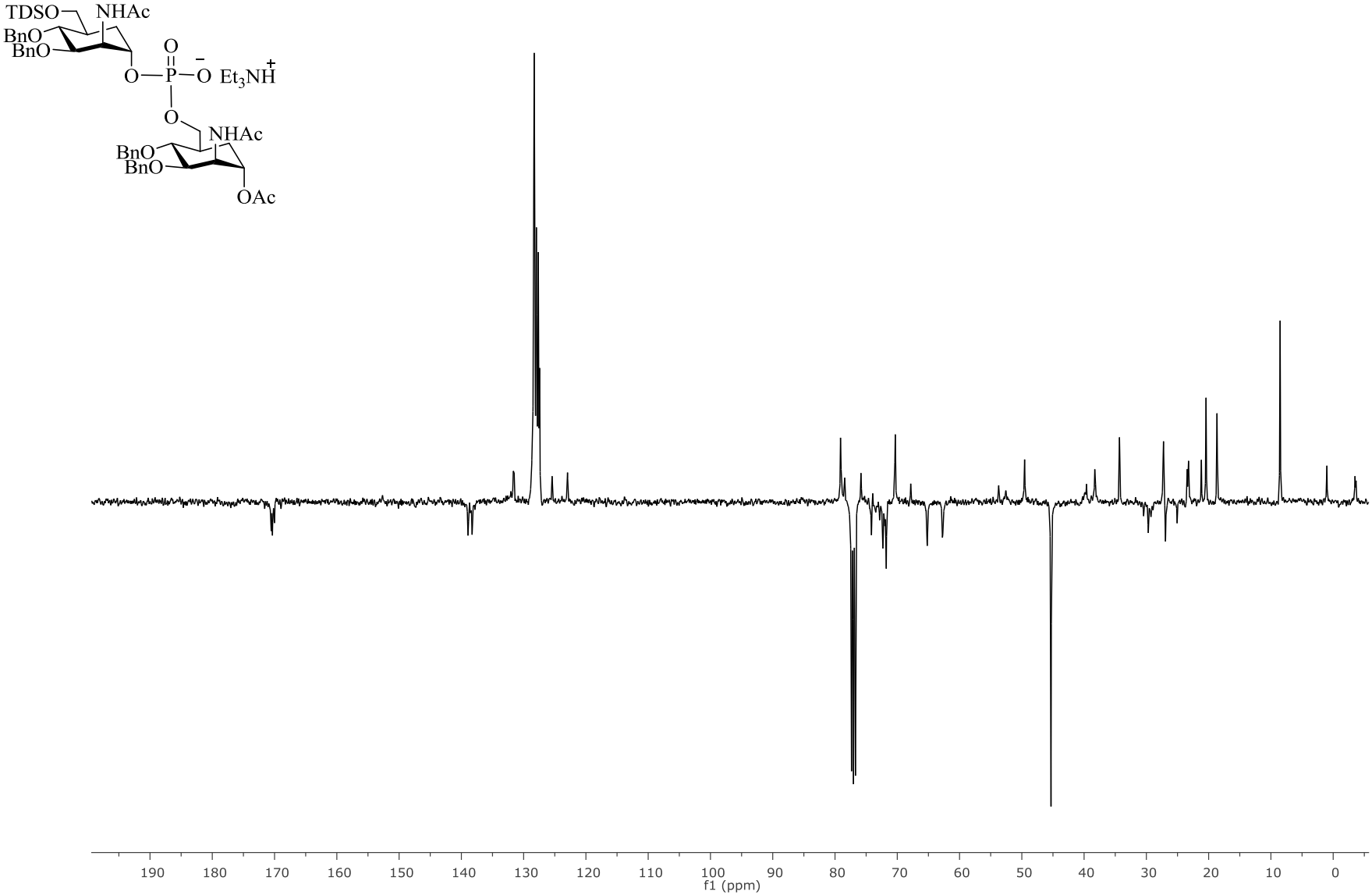
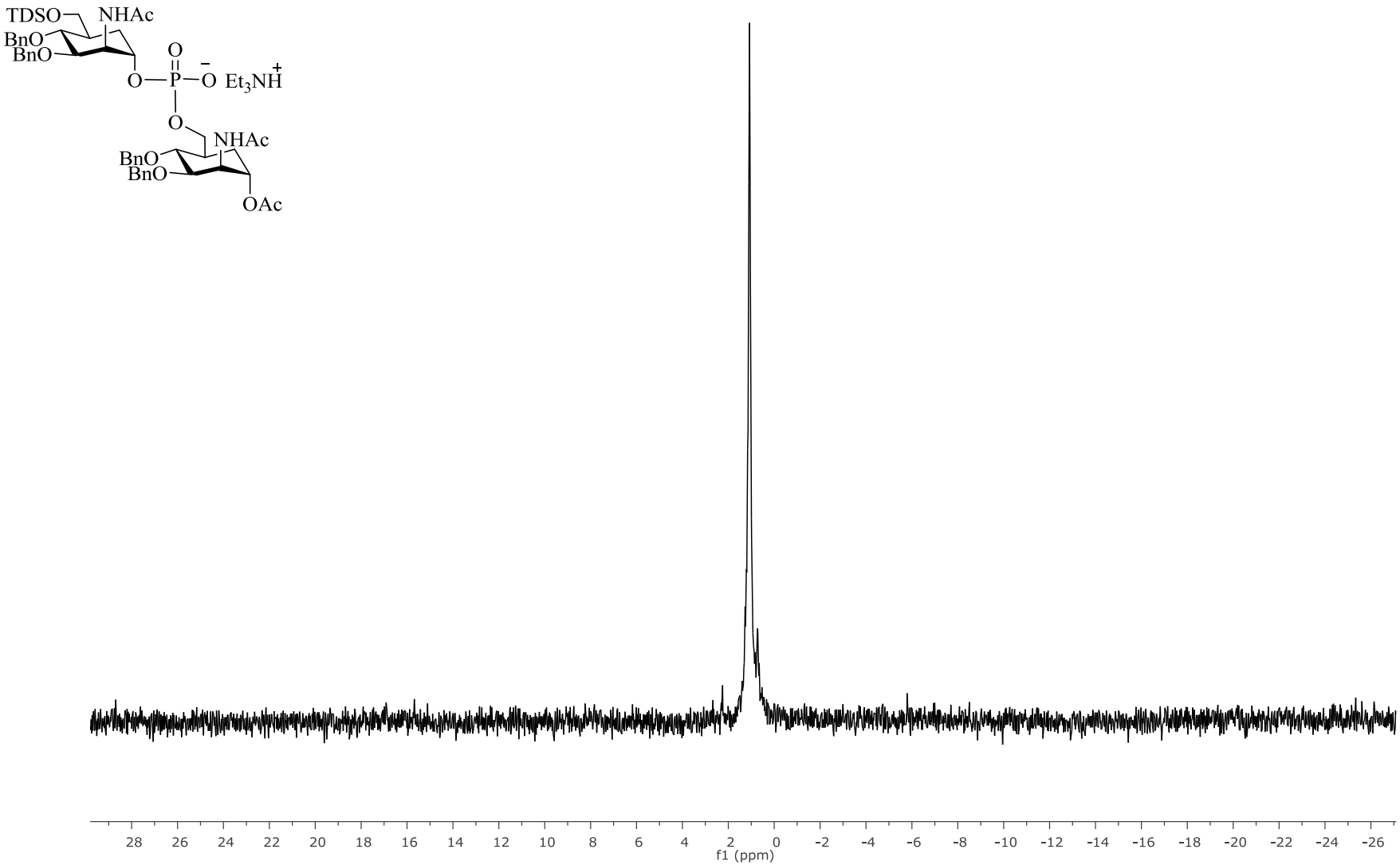
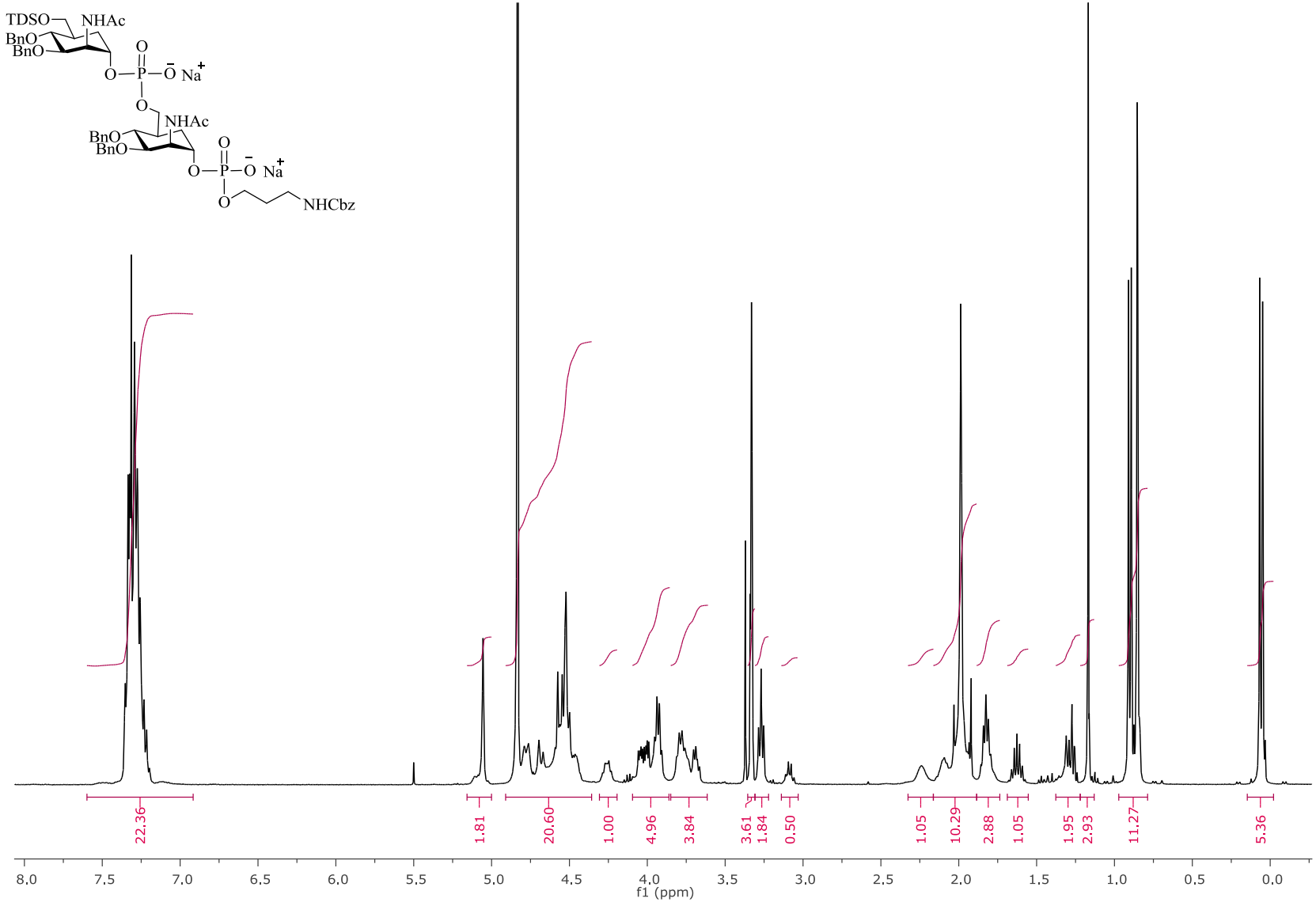


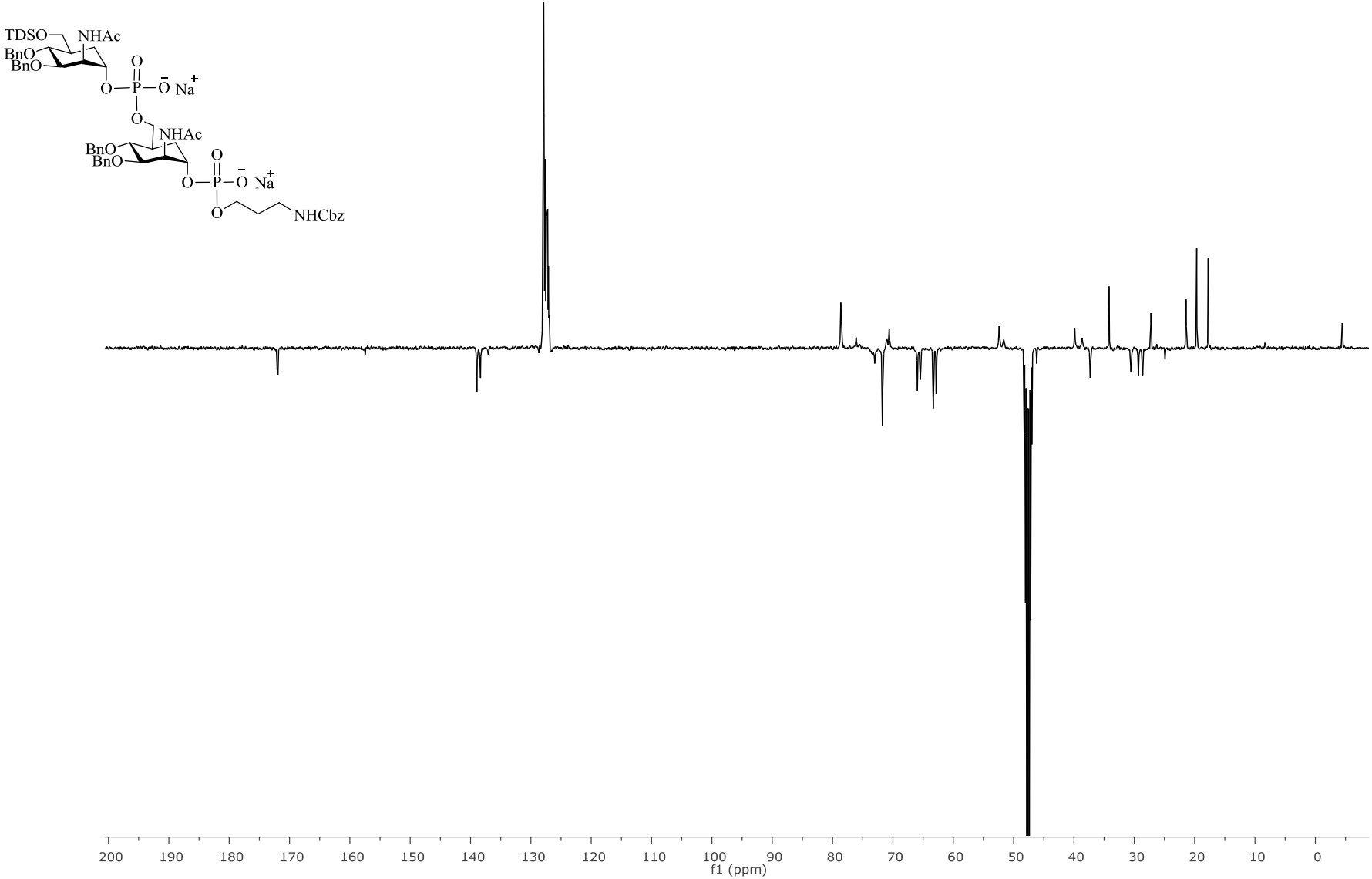
^{31}P NMR (162 MHz, $CDCl_3$) spectrum of compound **18**



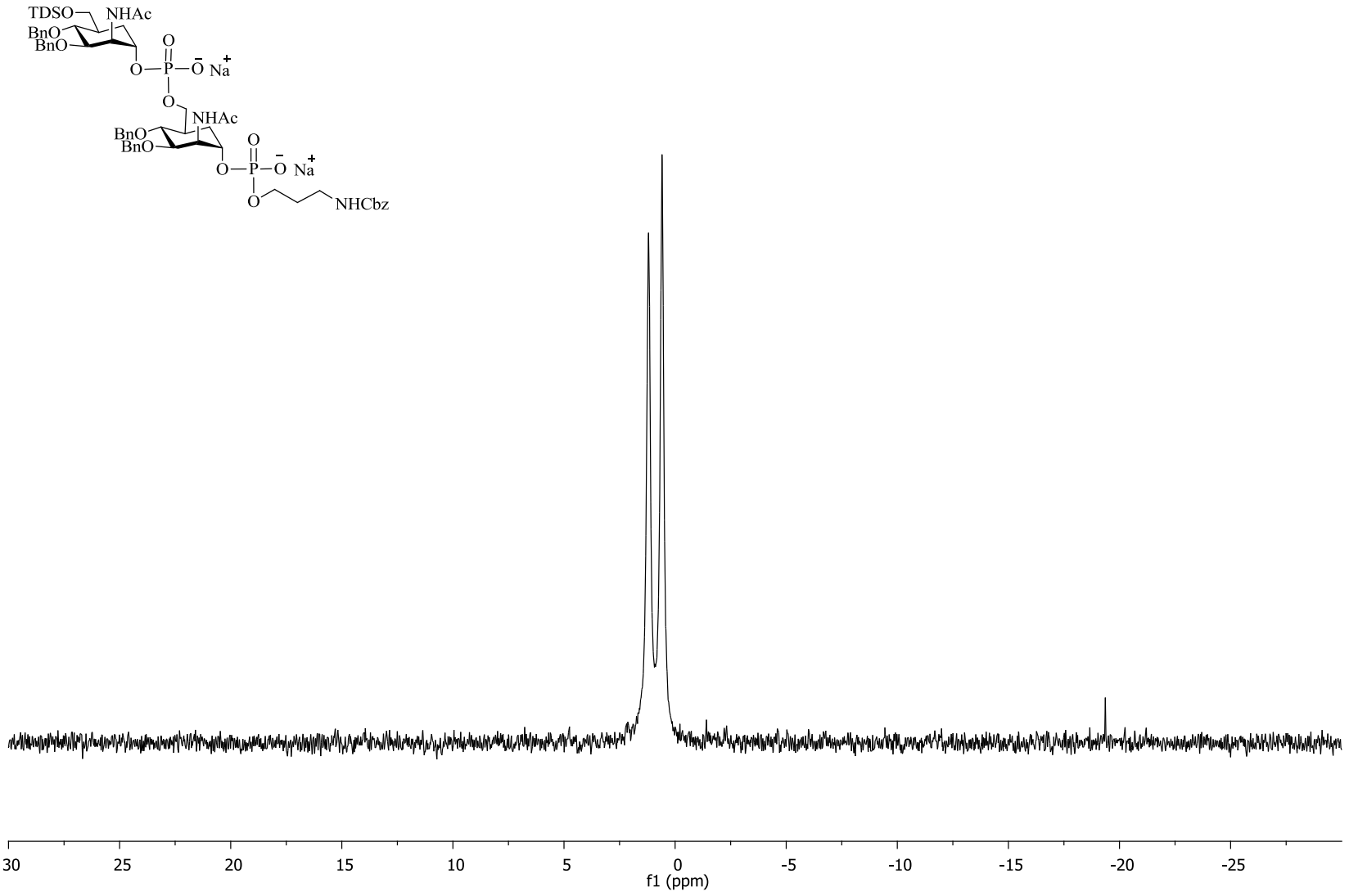
^{1}H NMR (400 MHz, CD_3OD, T = 313 K) spectrum of compound **19**



^{13}C NMR (100.6 MHz, CD_3OD, T = 313 K) spectrum of compound **19**



^{31}P NMR (162 MHz, CD_3OD) spectrum of compound **19**



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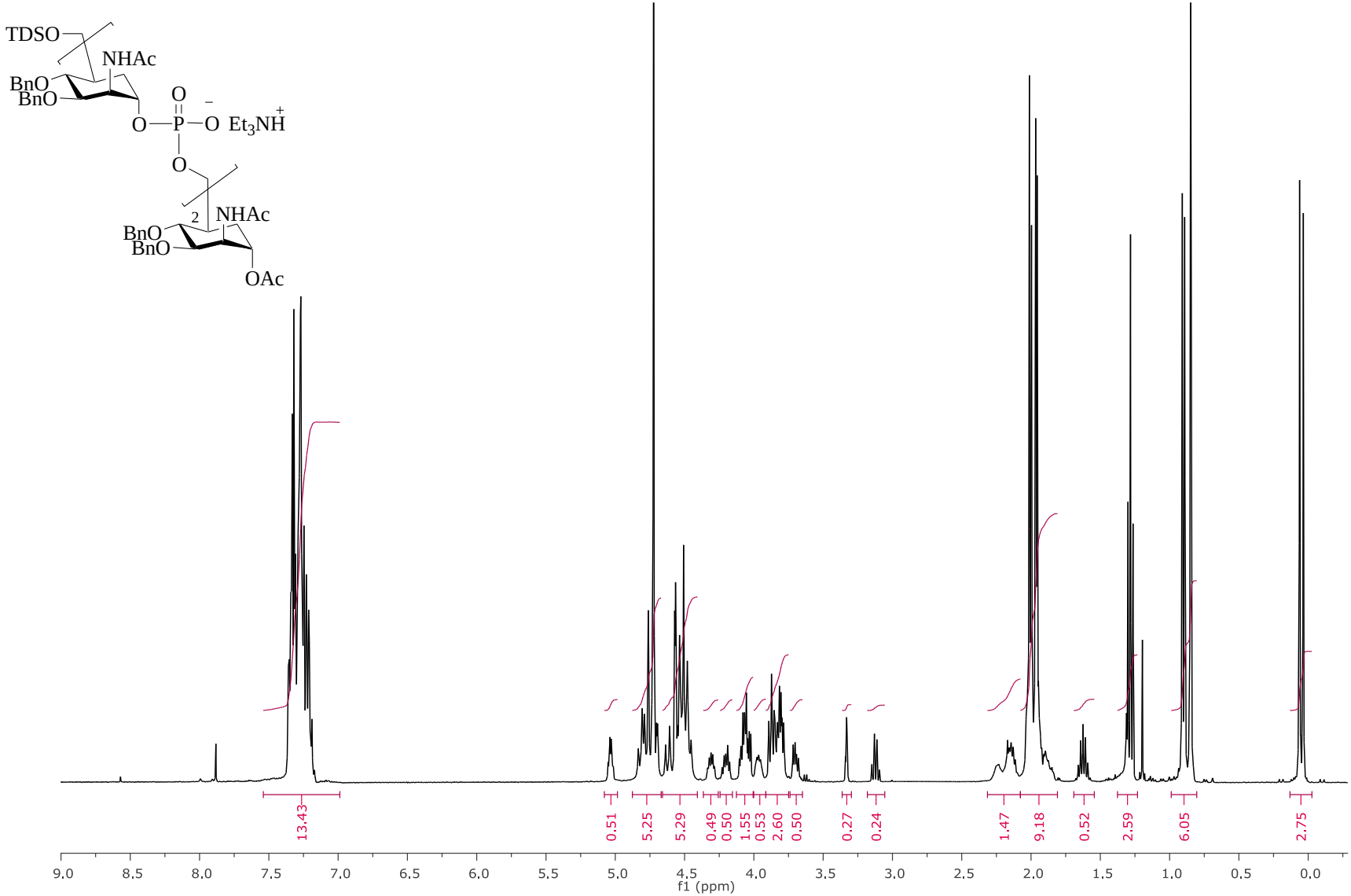
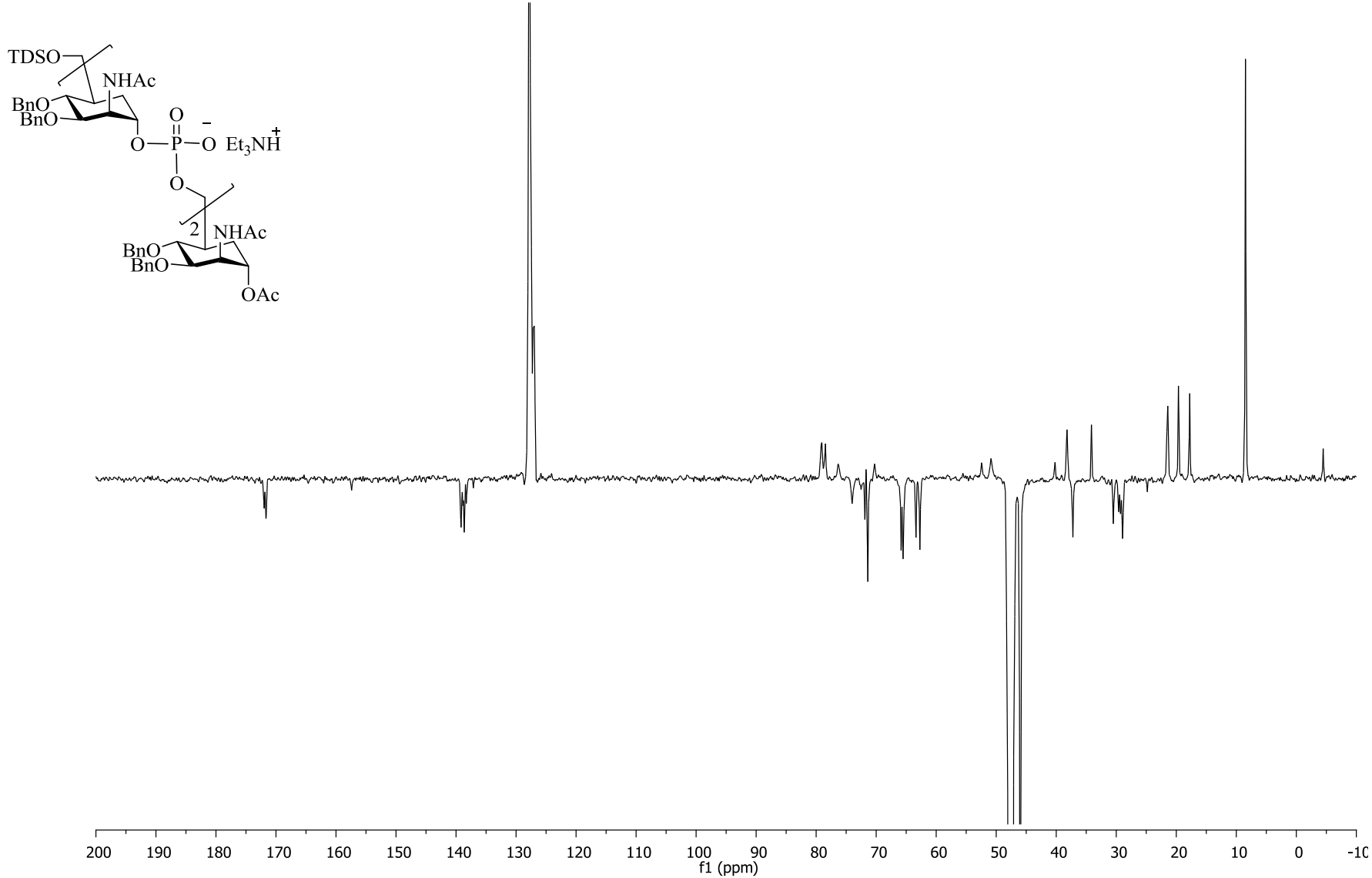
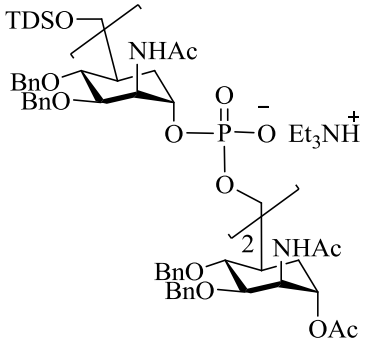


^{13}C NMR (100.6 MHz, CD_3OD, T = 313 K) spectrum of compound **20**



^{31}P NMR (162 MHz, CD_3OD, T = 313 K) spectrum of compound **20**



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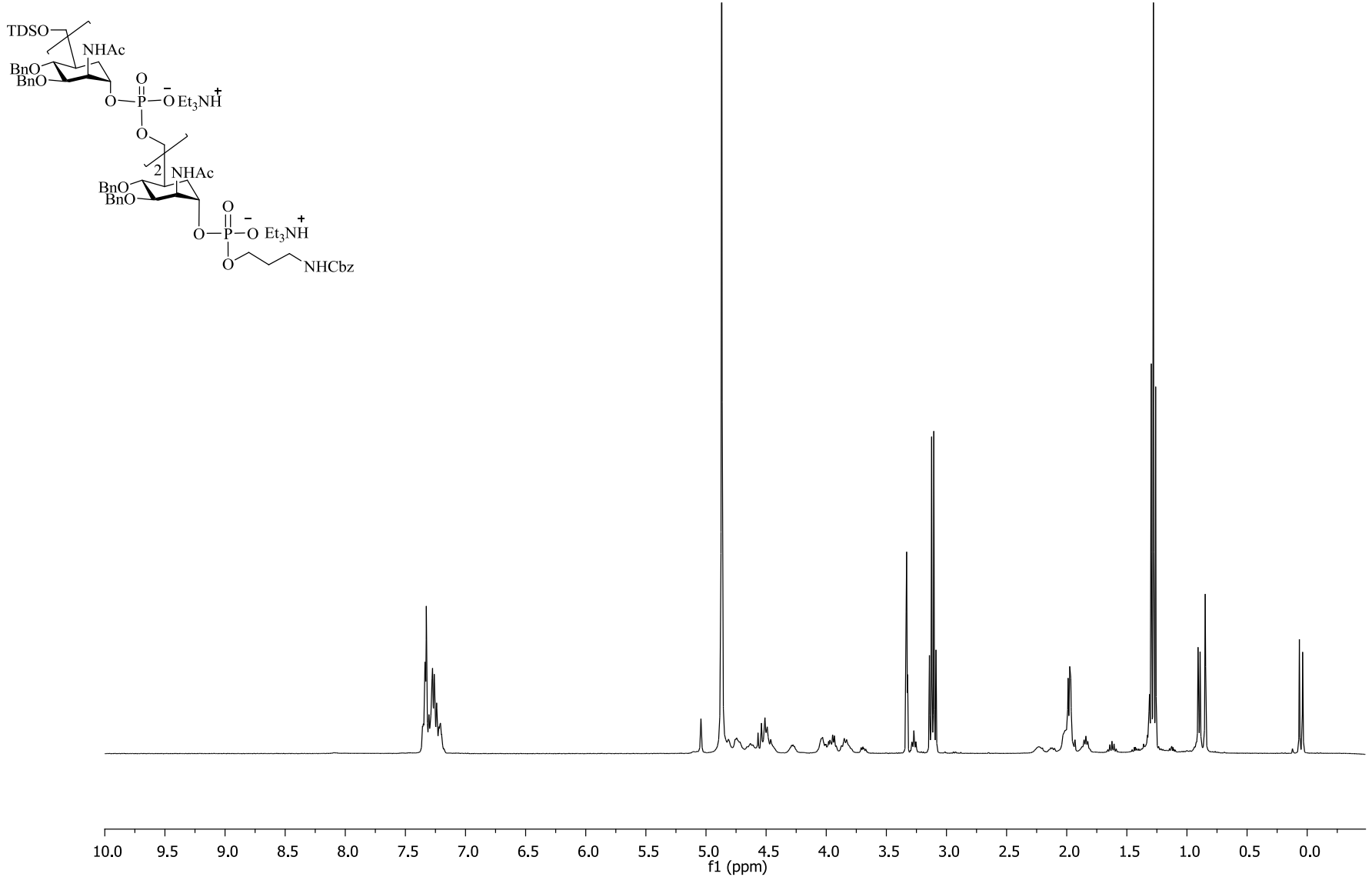
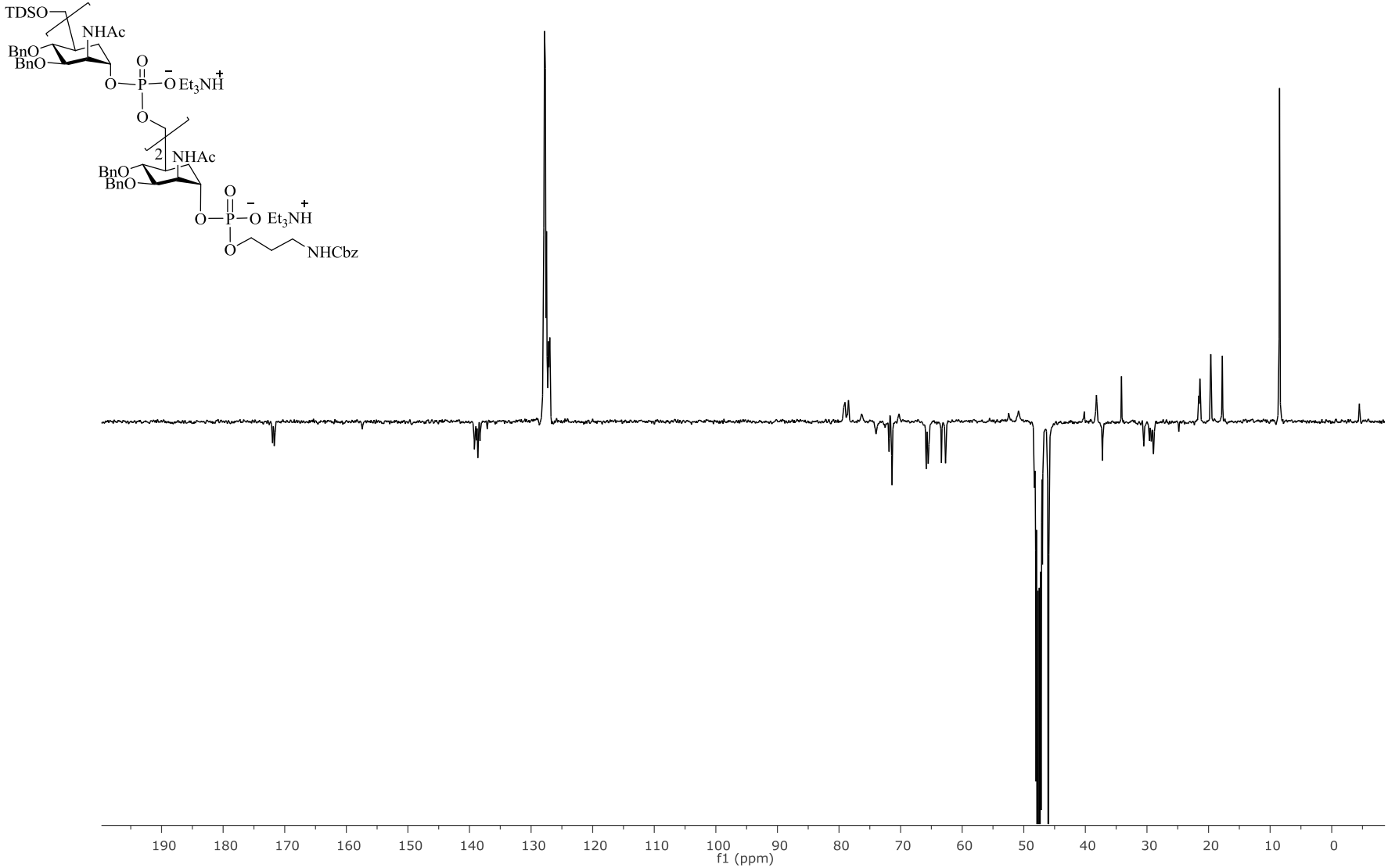


^{13}C NMR (100.6 MHz, CD_3OD) spectrum of compound **21**



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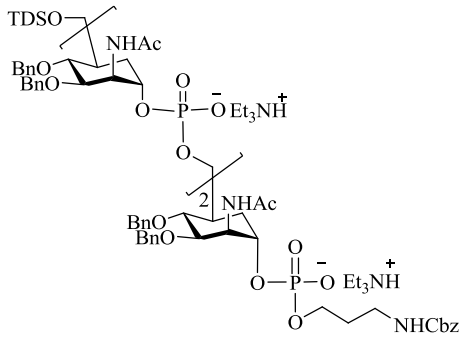
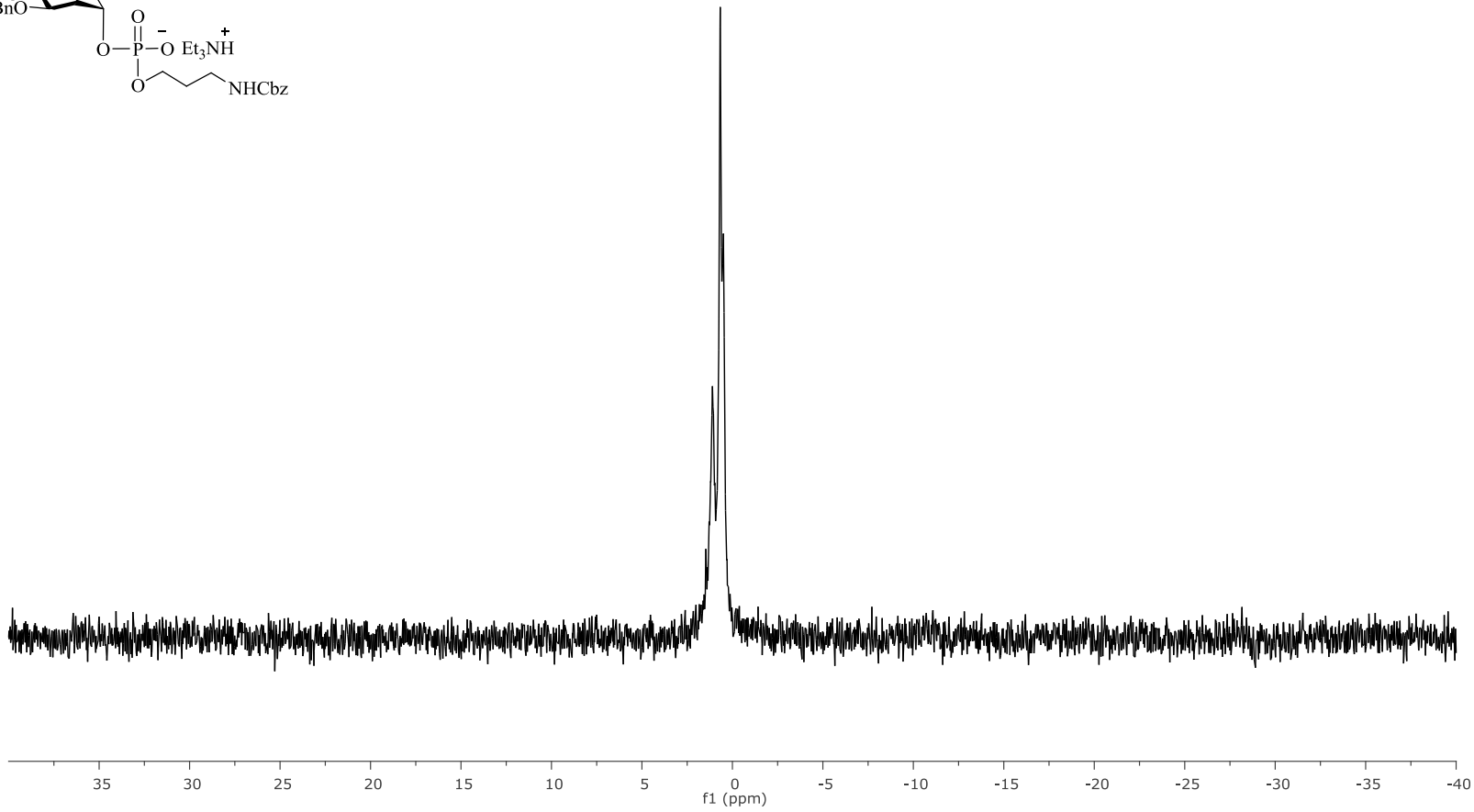
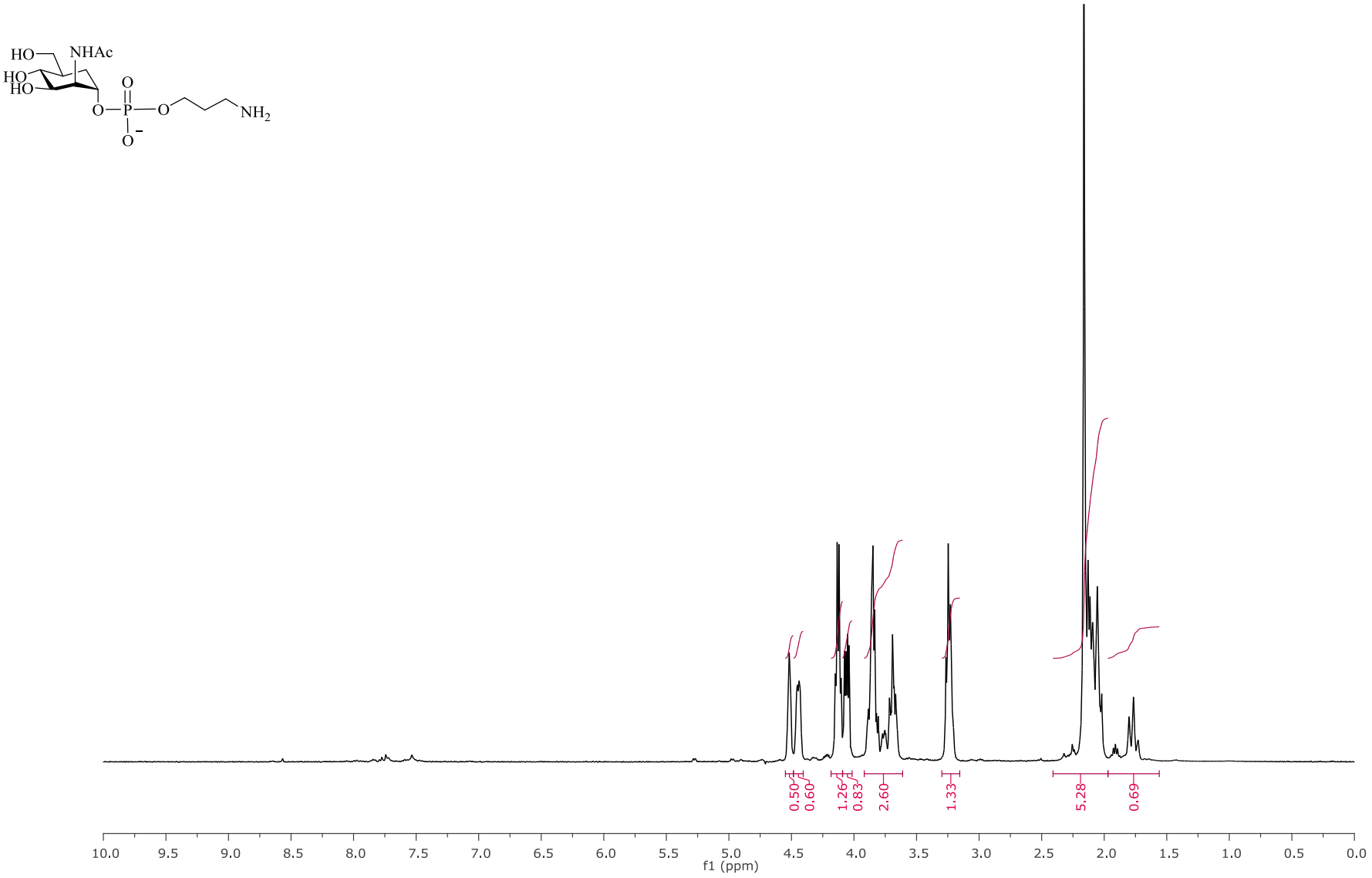




^{1}H NMR (400 MHz, D_2O, T = 313 K) spectrum of compound **1**



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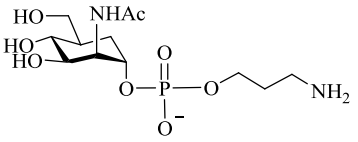

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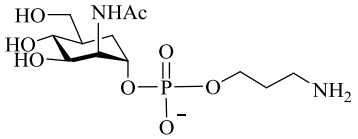

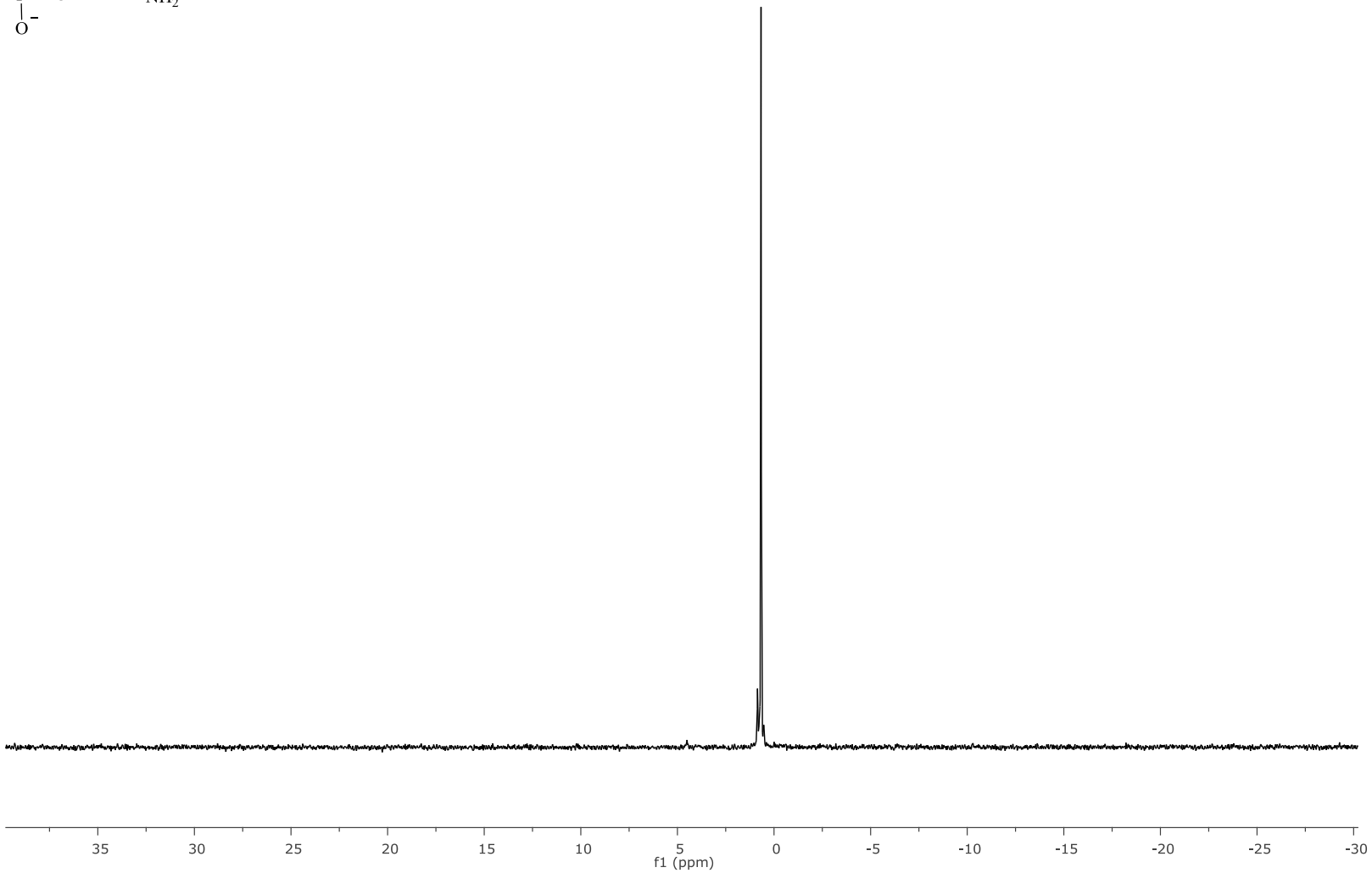

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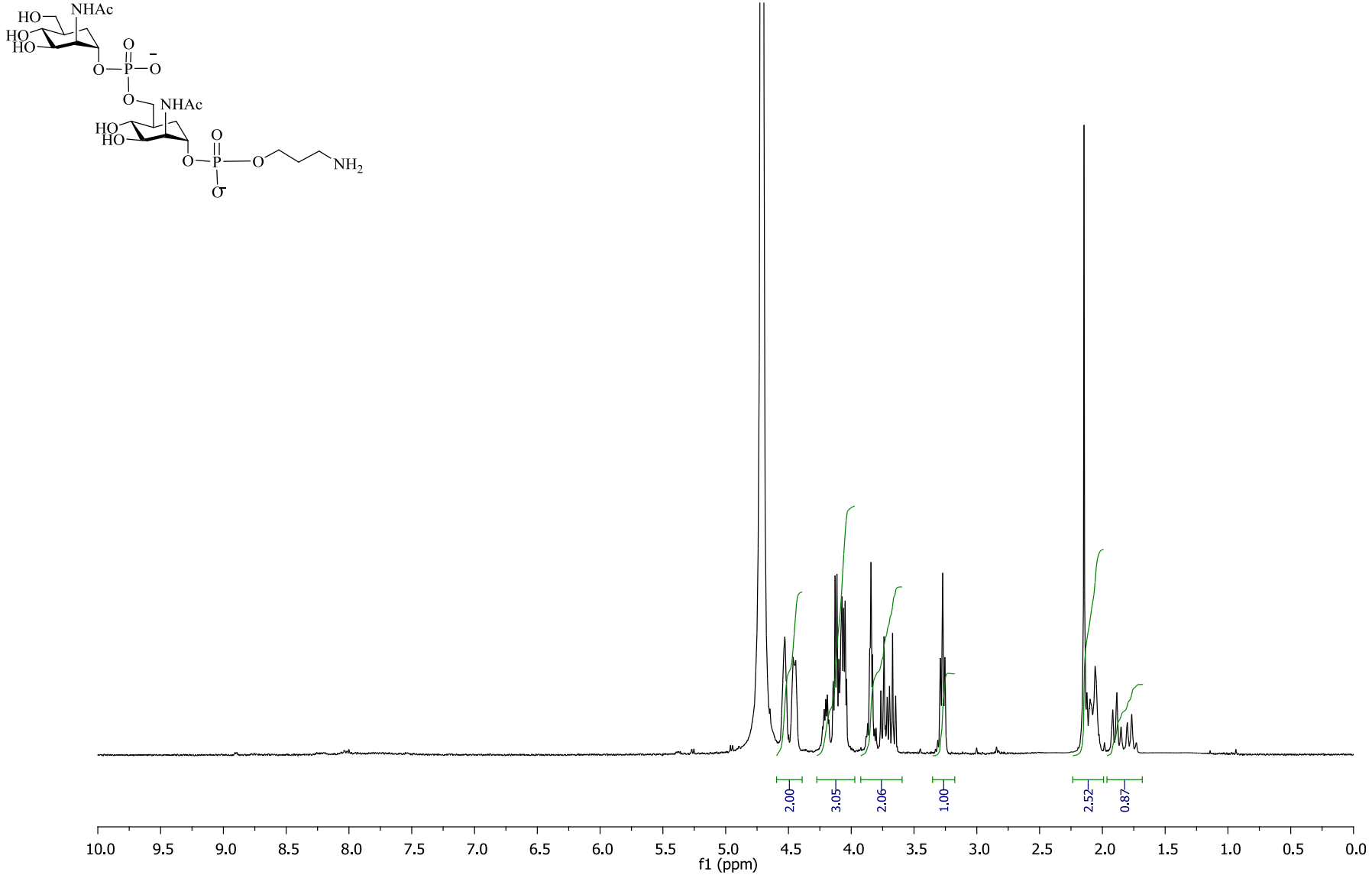
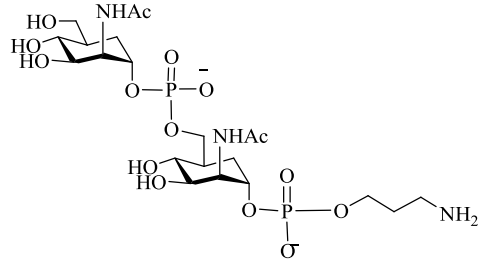


^{13}C NMR (100.6 MHz, D_2O, T = 313 K) spectrum of compound **2**



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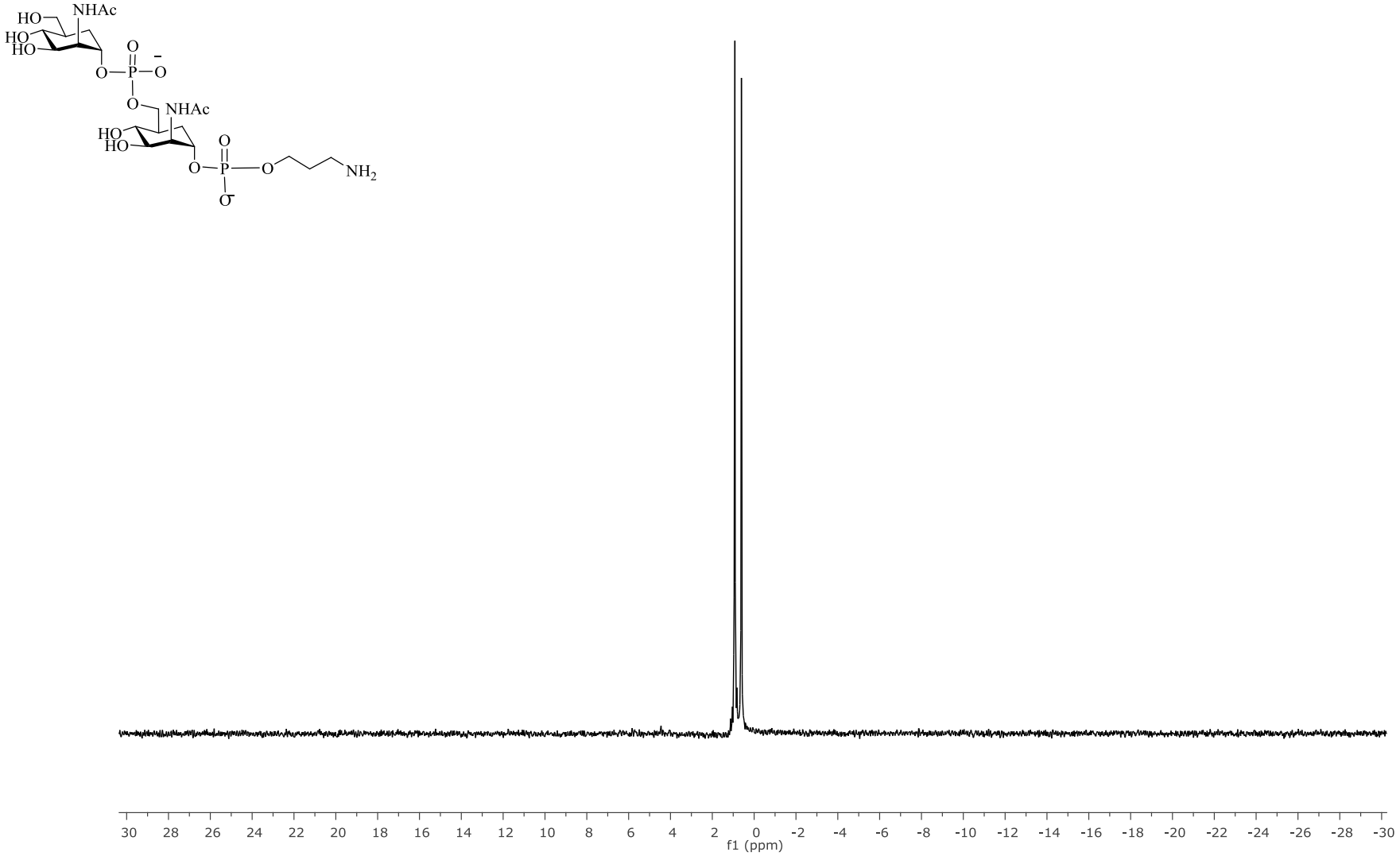
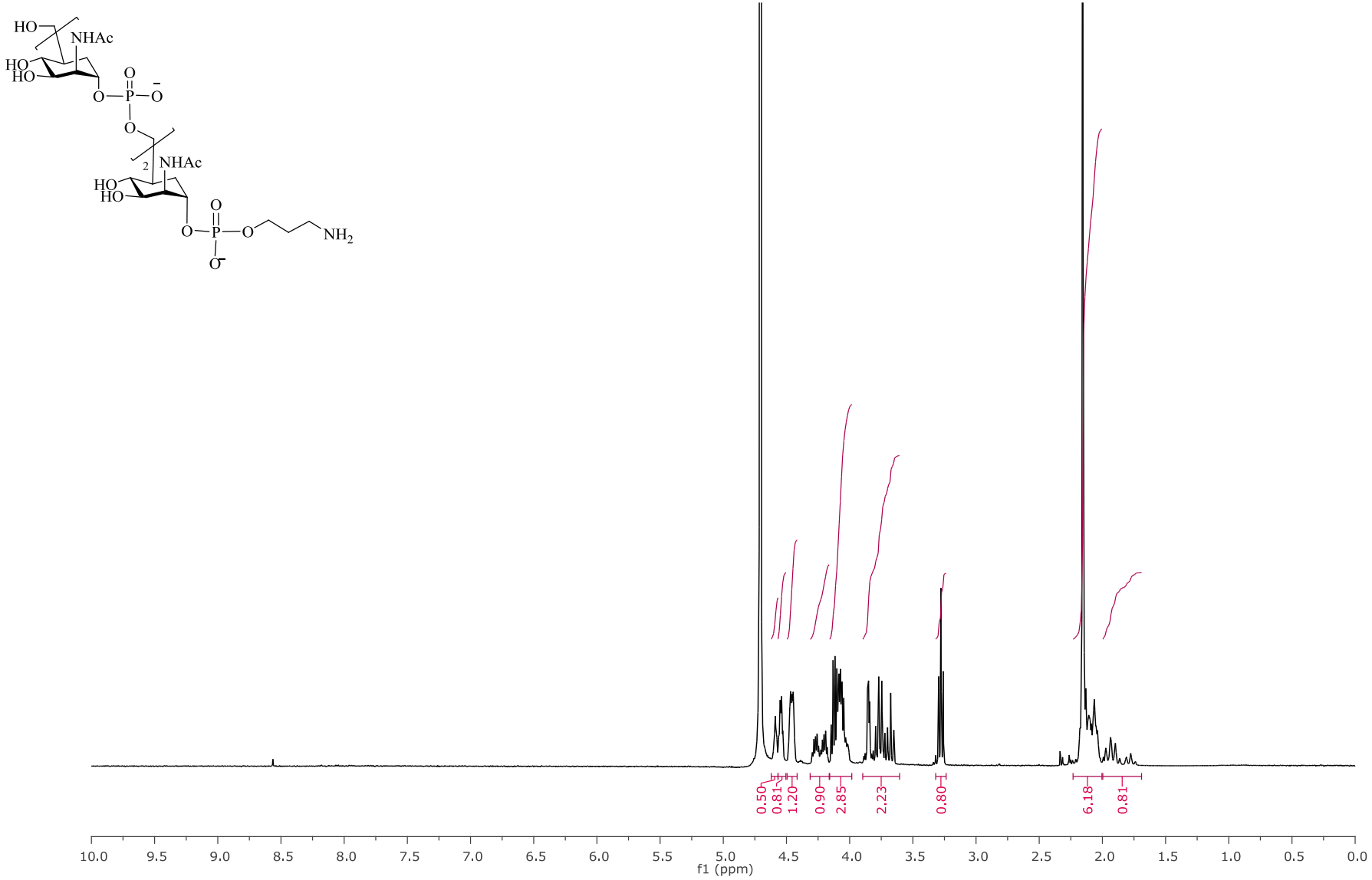
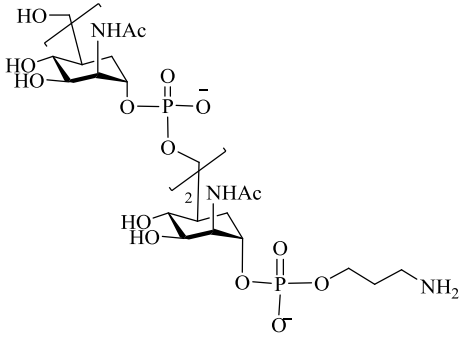


^{1}H NMR (400 MHz, D_2O, T = 313 K) spectrum of compound **3**



^{13}C NMR (100.6 MHz, D_2O, T = 308 K) spectrum of compound **3**



^{31}P NMR (162 MHz, D_2O, T = 308 K) spectrum of compound **3**