Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Synthesis and immunochemical evaluation of a novel *Neisseria meningitidis* serogroup A tetrasaccharide and its conjugate

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Supporting Information

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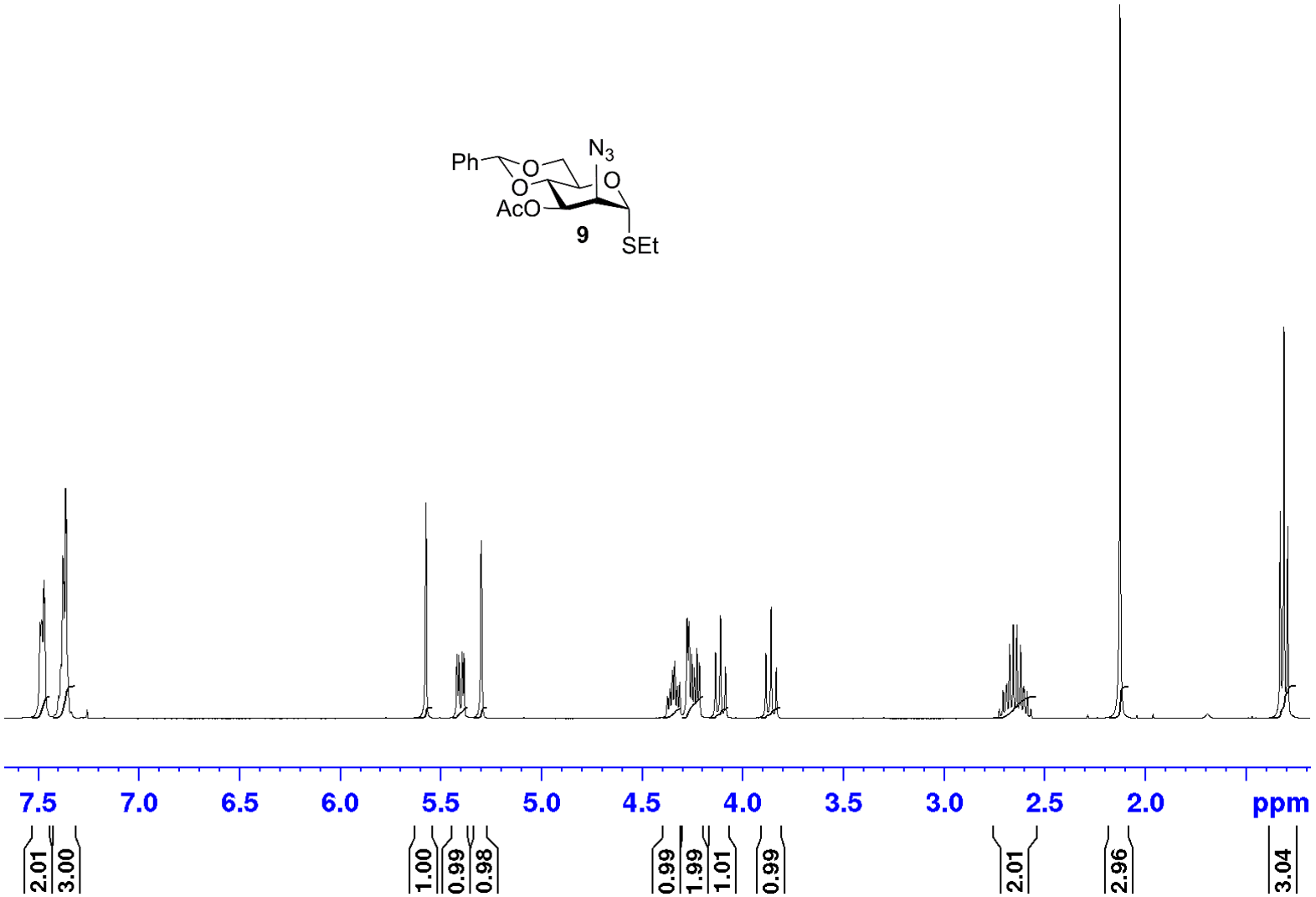
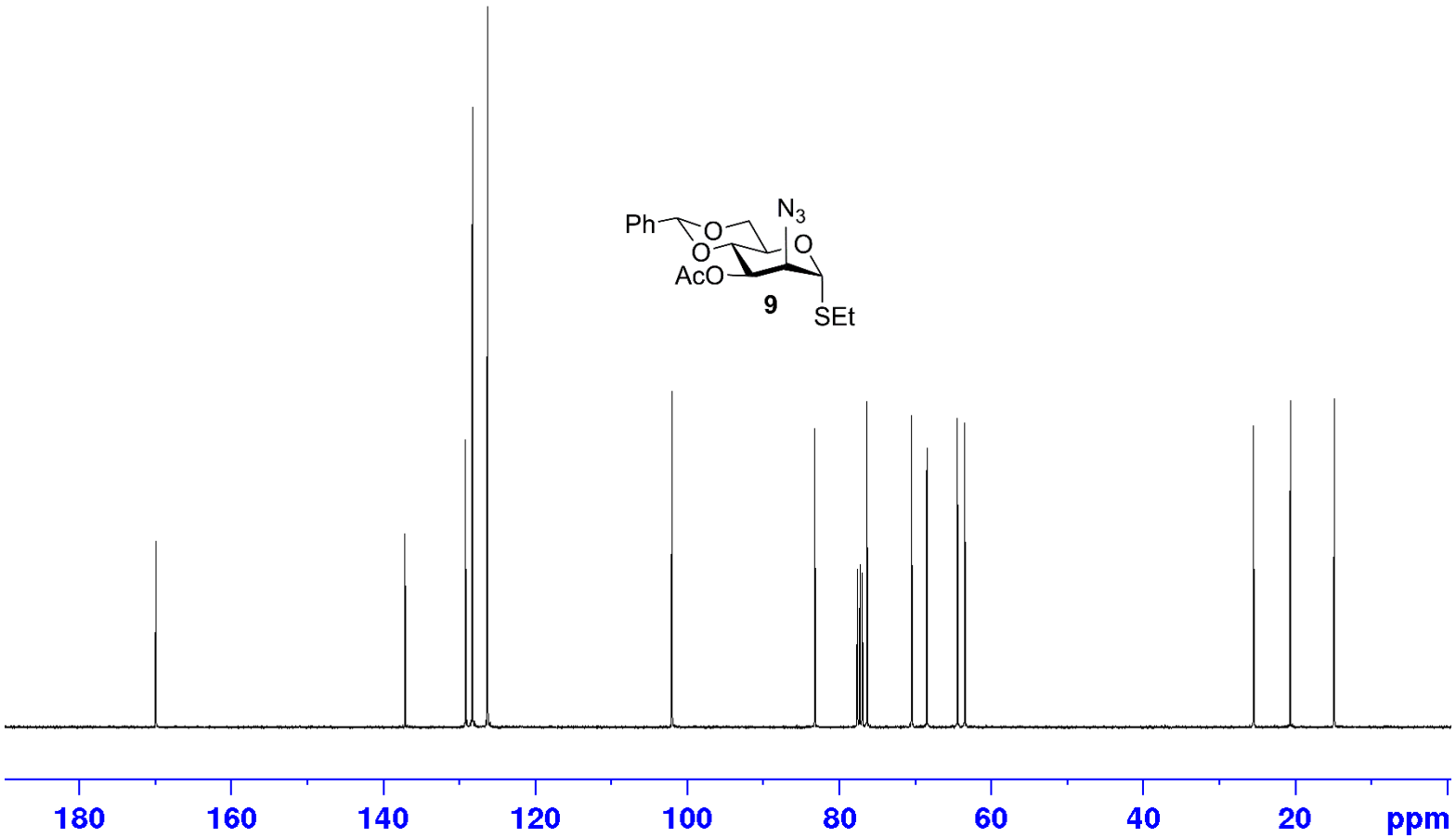
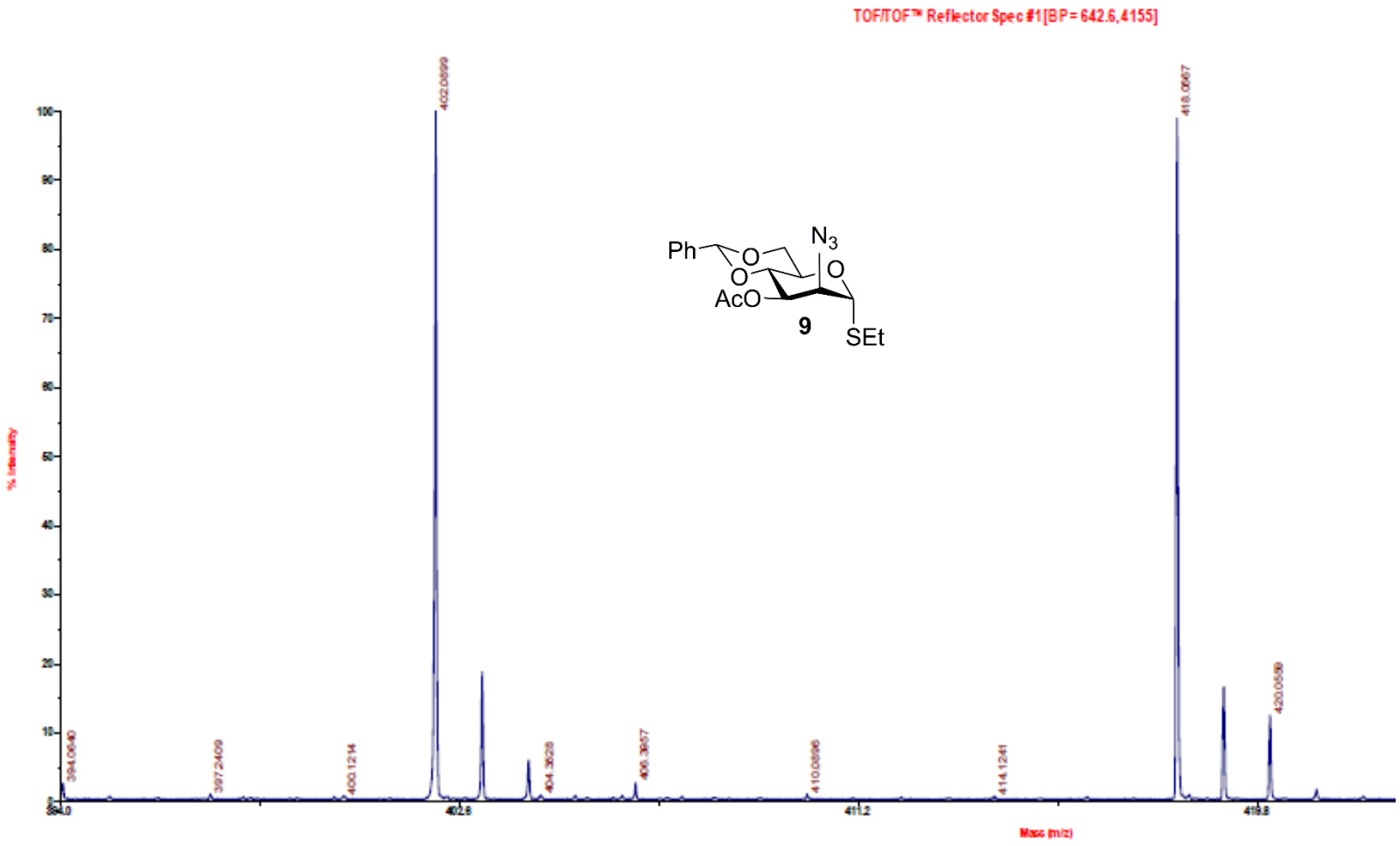
^{1}HNMR of compound **9** ($CDCl_3$, 500 MHz)



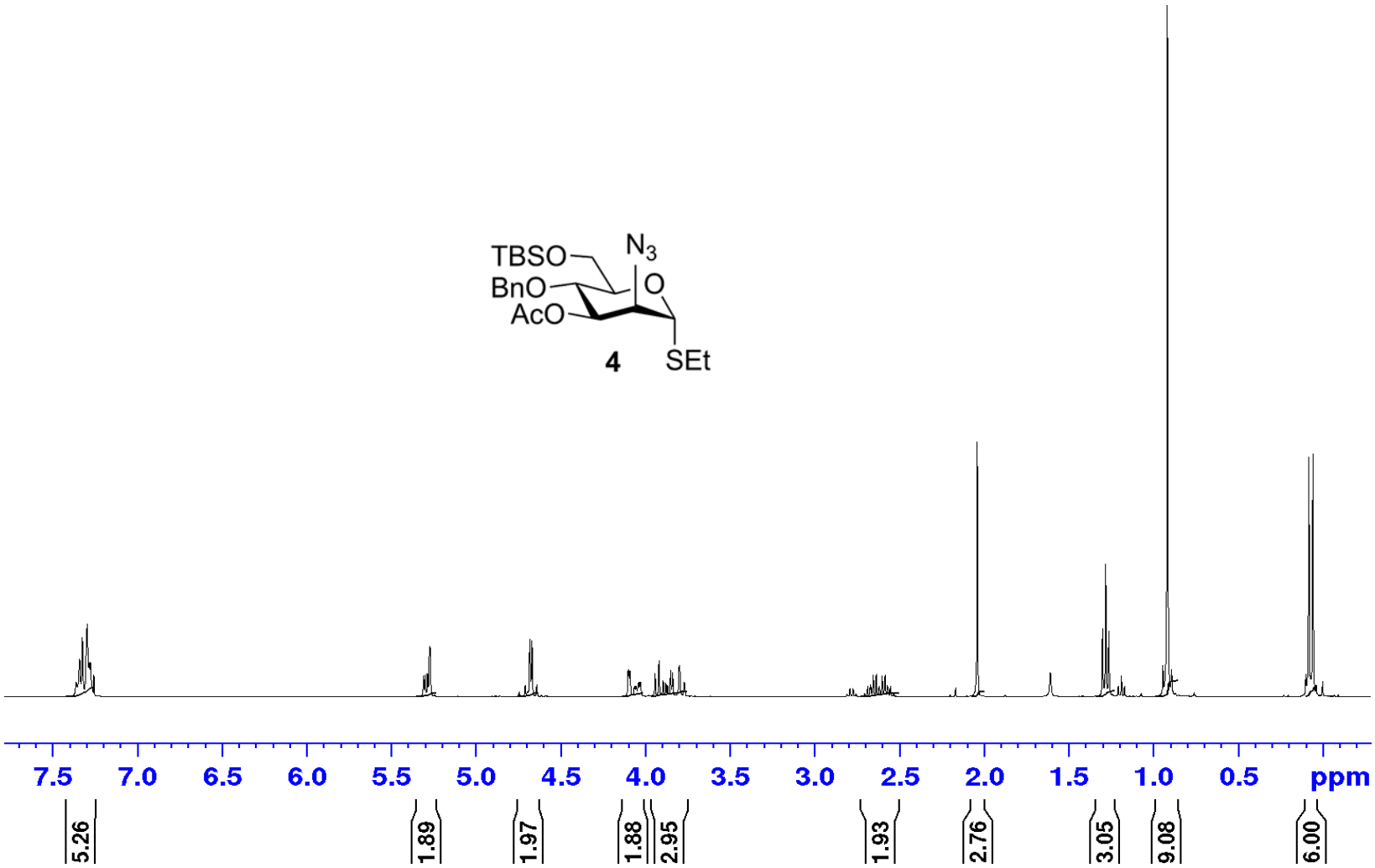
^{13}CNMR of compound **9** ($CDCl_3$, 125 MHz)



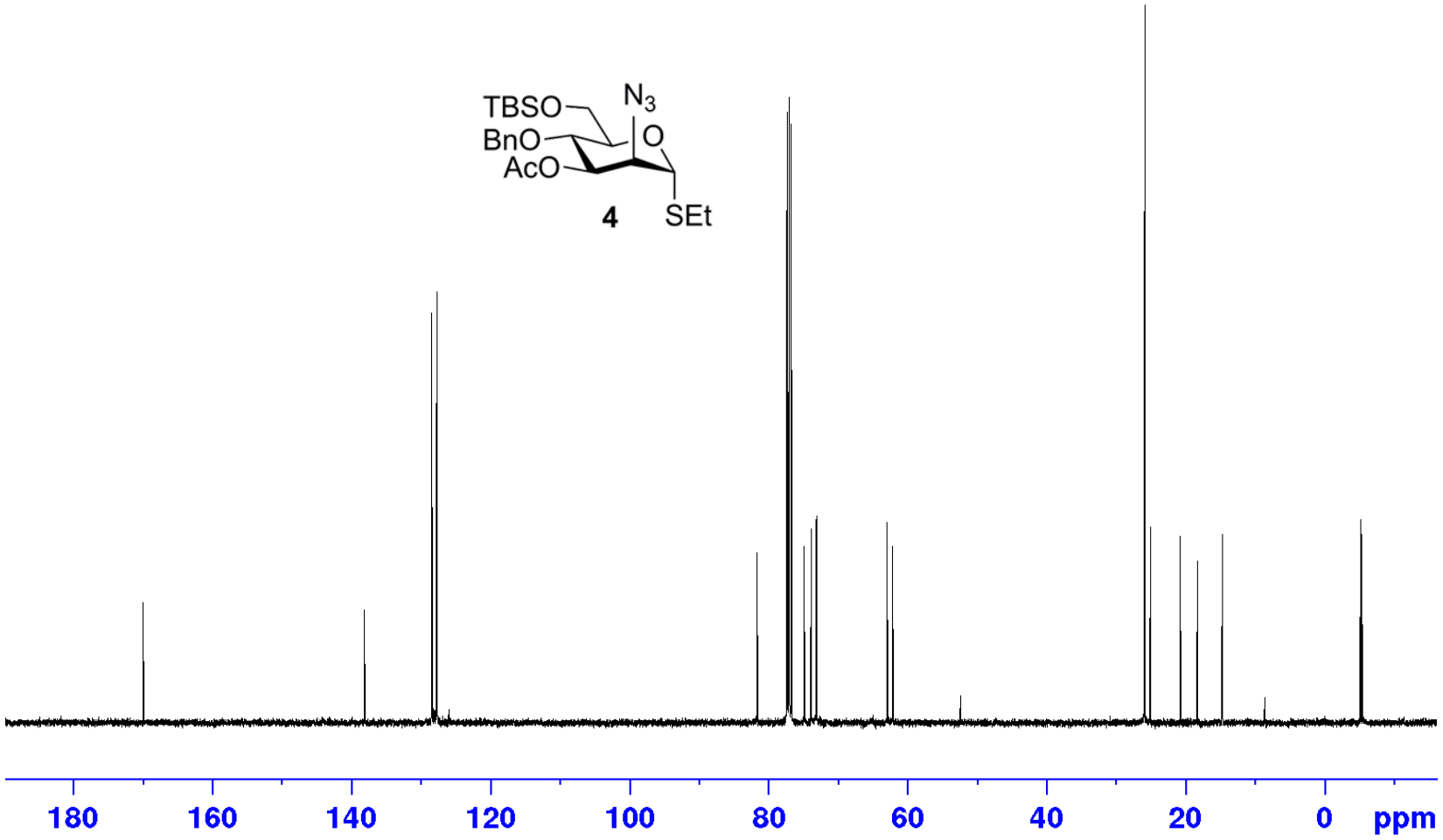
MALDI-TOF MS spectra of compound **9**



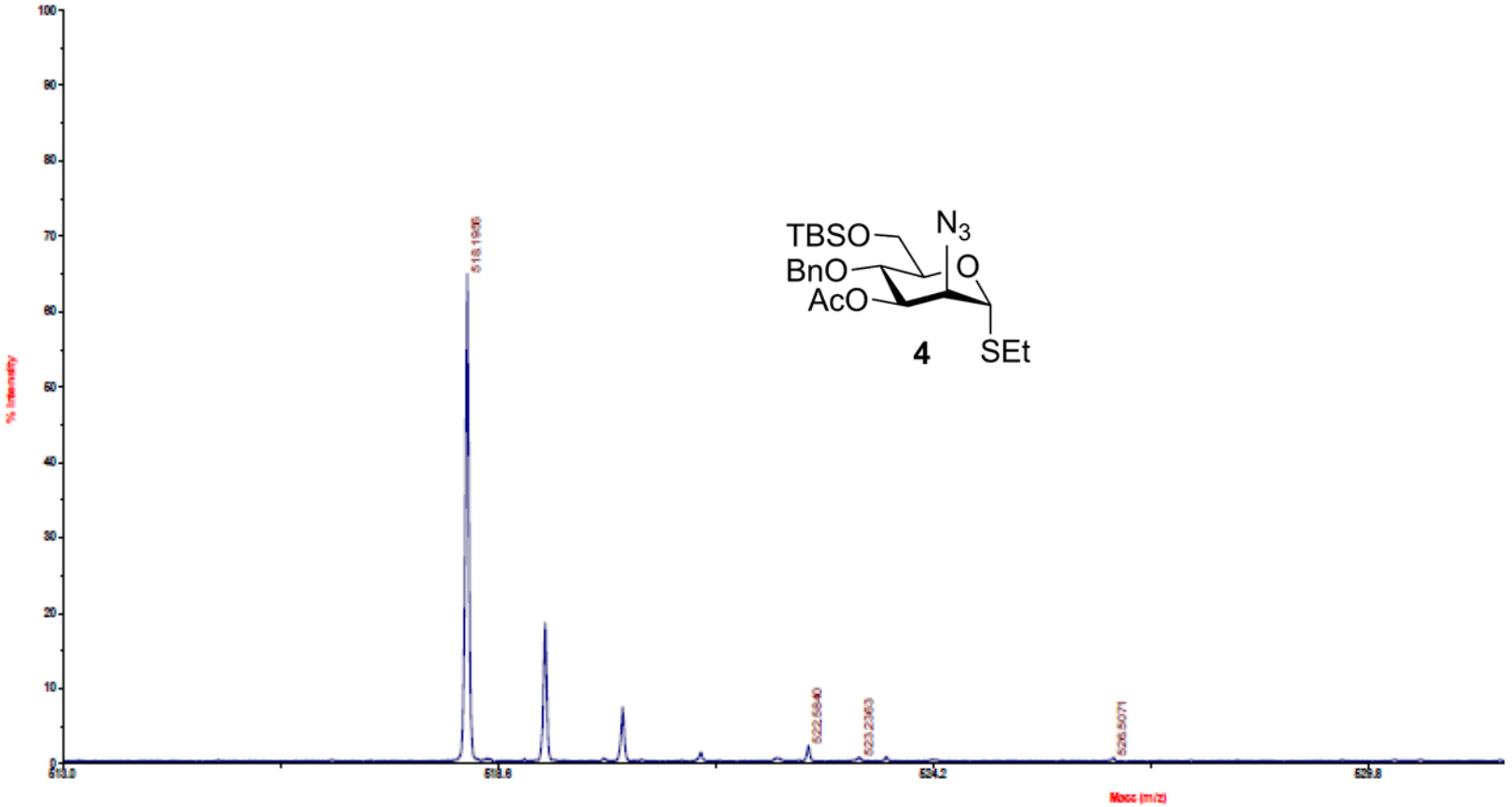
^{1}HNMR of compound **4** ($CDCl_3$, 500 MHz)



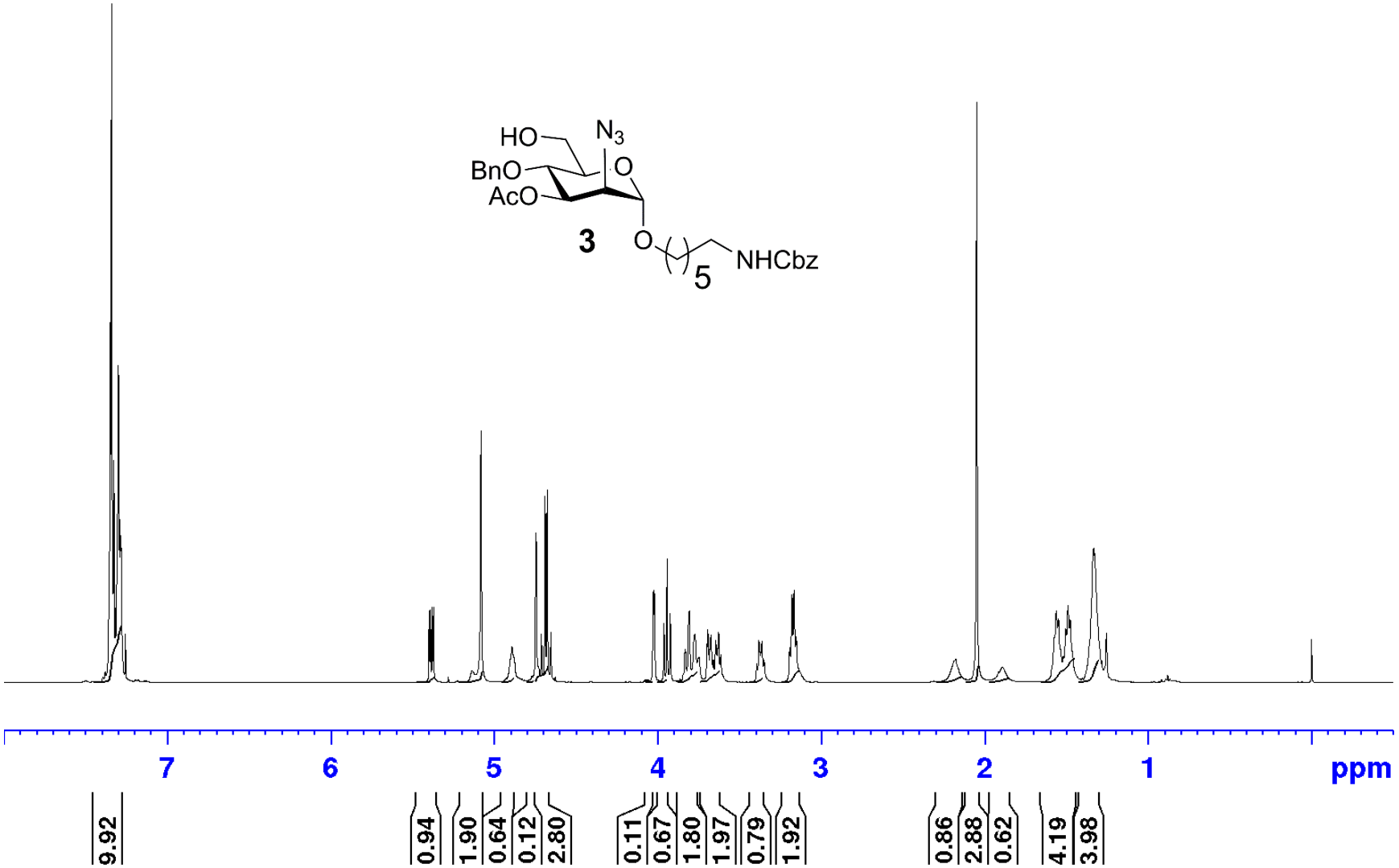
^{13}C NMR of compound **4** ($CDCl_3$, 125 MHz)



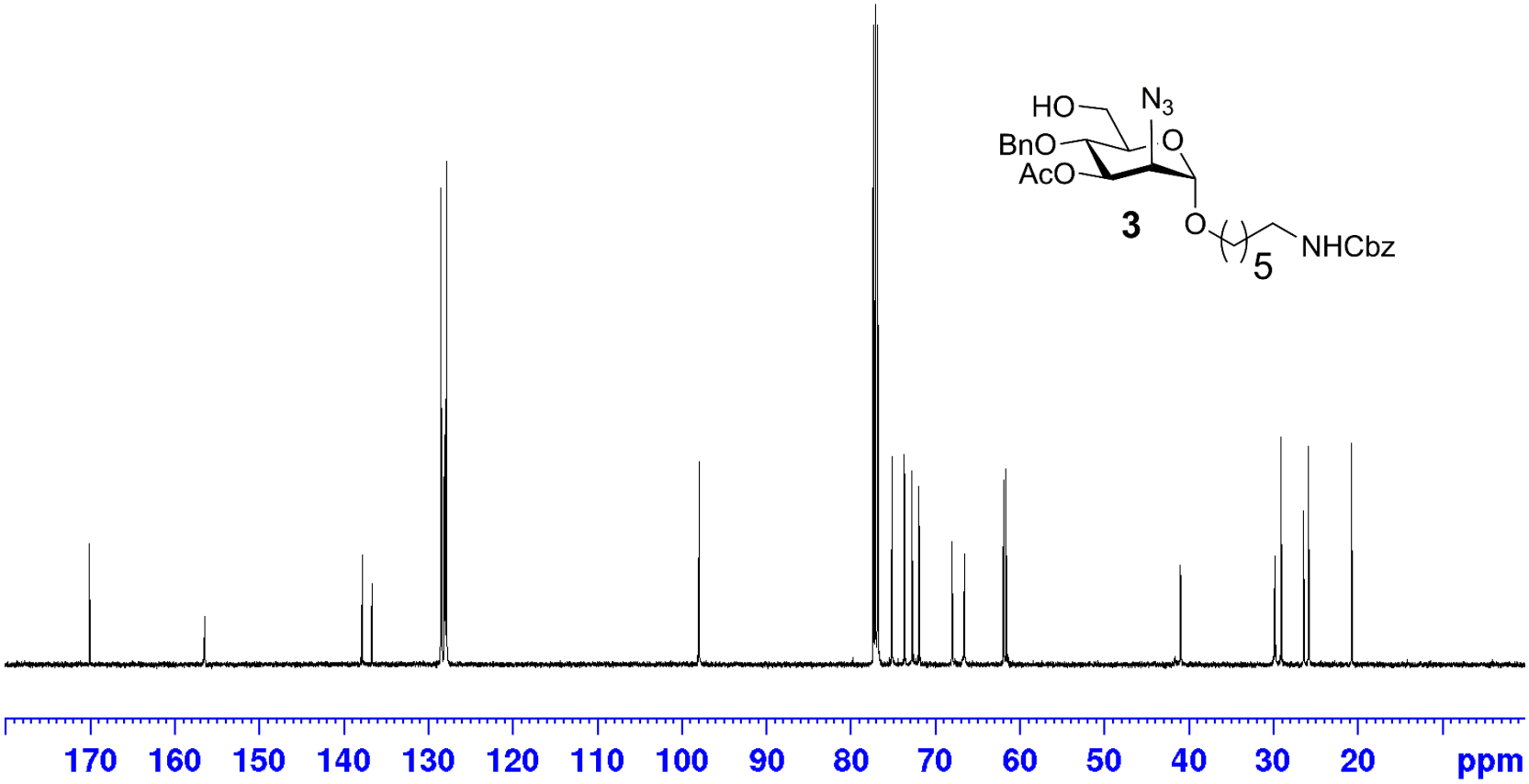
MALDI-TOF MS spectra of compound **4**



^{1}HNMR of compound **3** ($CDCl_3$, 500 MHz)



^{13}C NMR of compound **3** ($CDCl_3$, 125 MHz)



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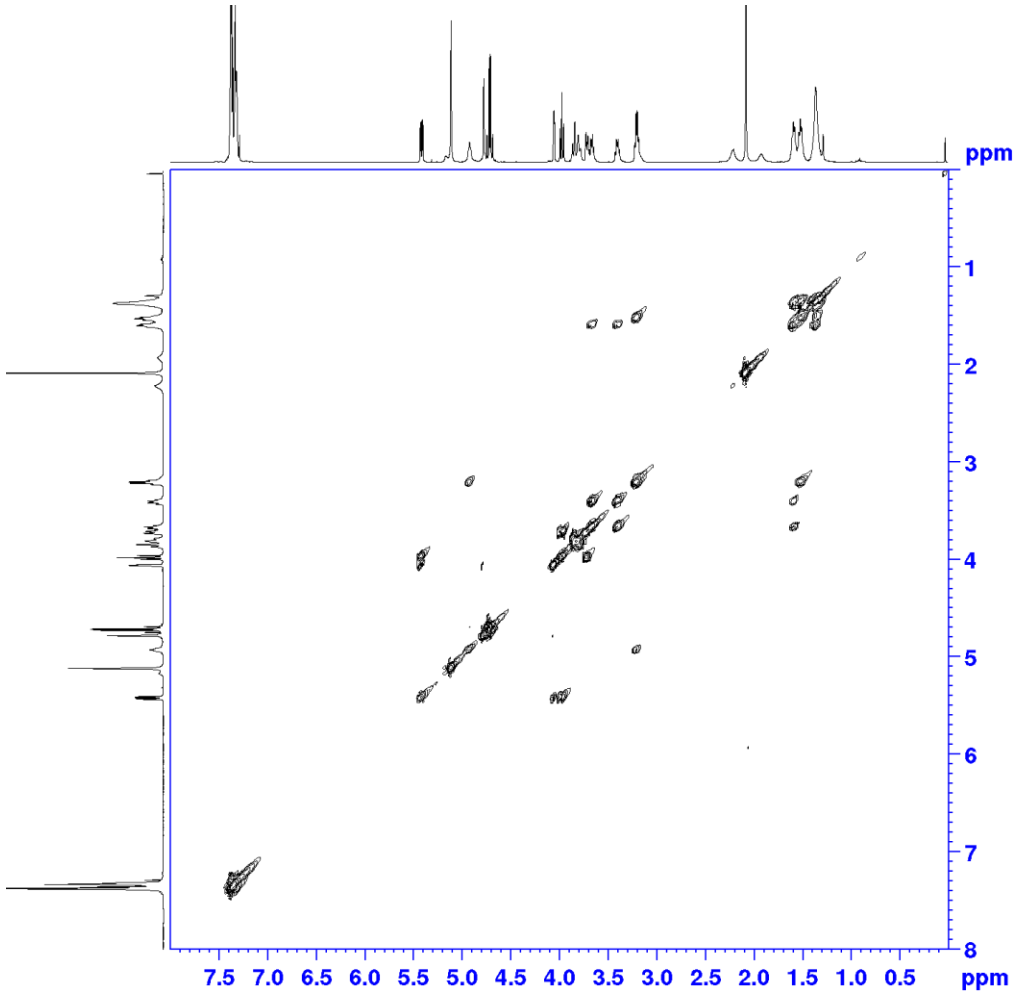
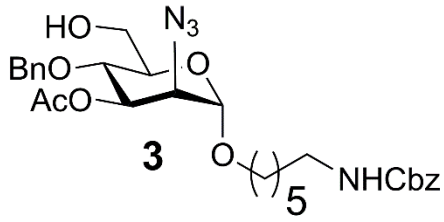
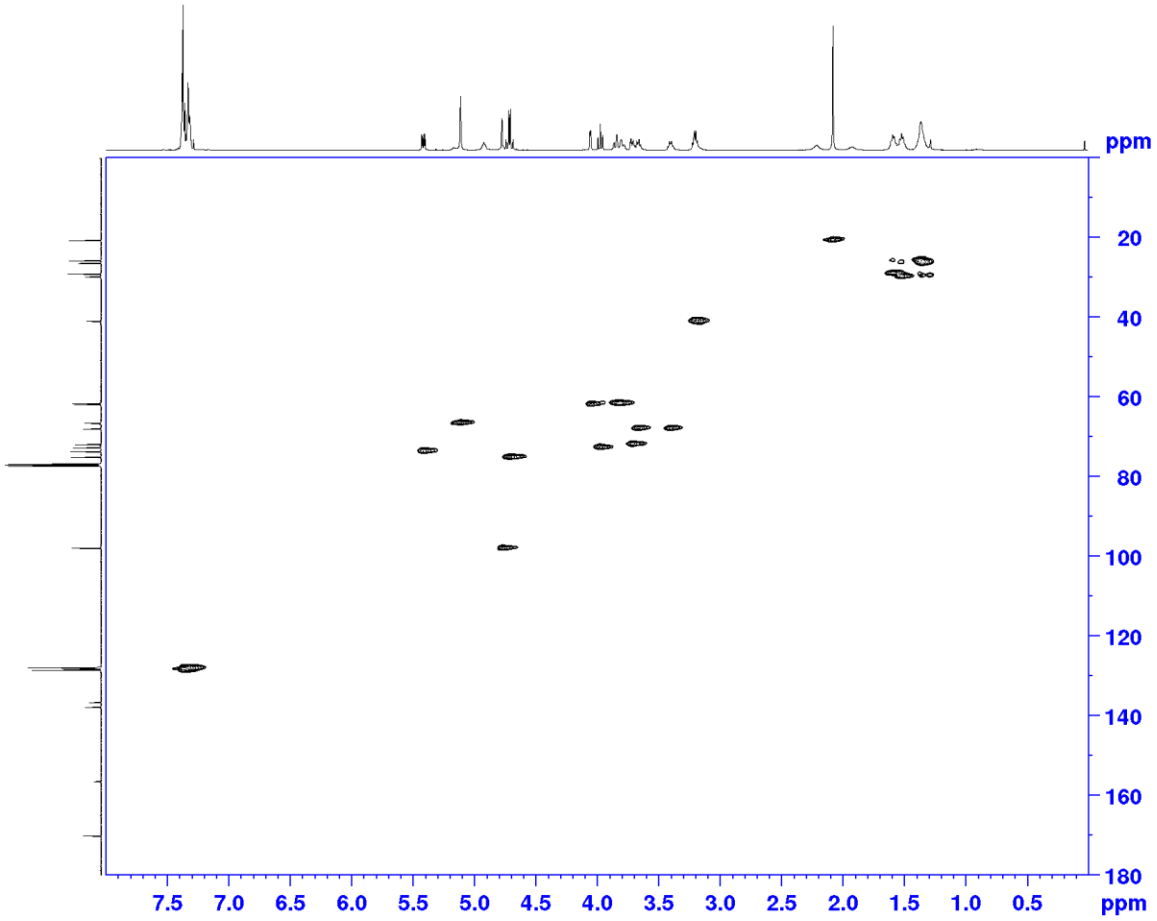
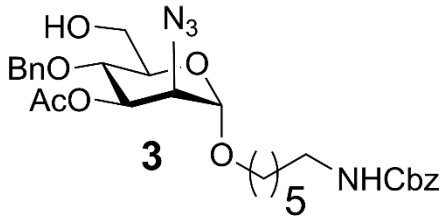


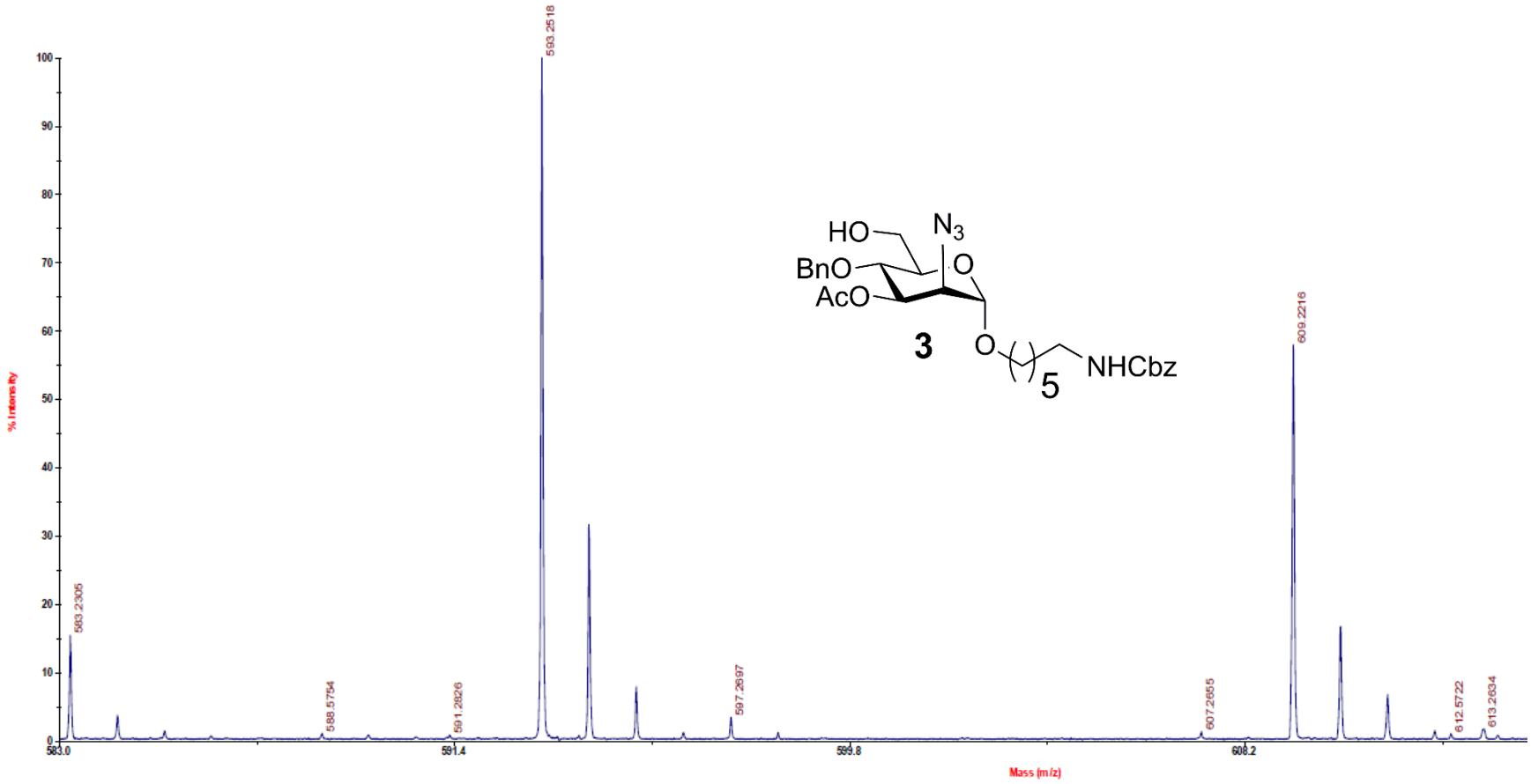


2D HSQC of compound **3** ($CDCl_3$, 500 MHz)

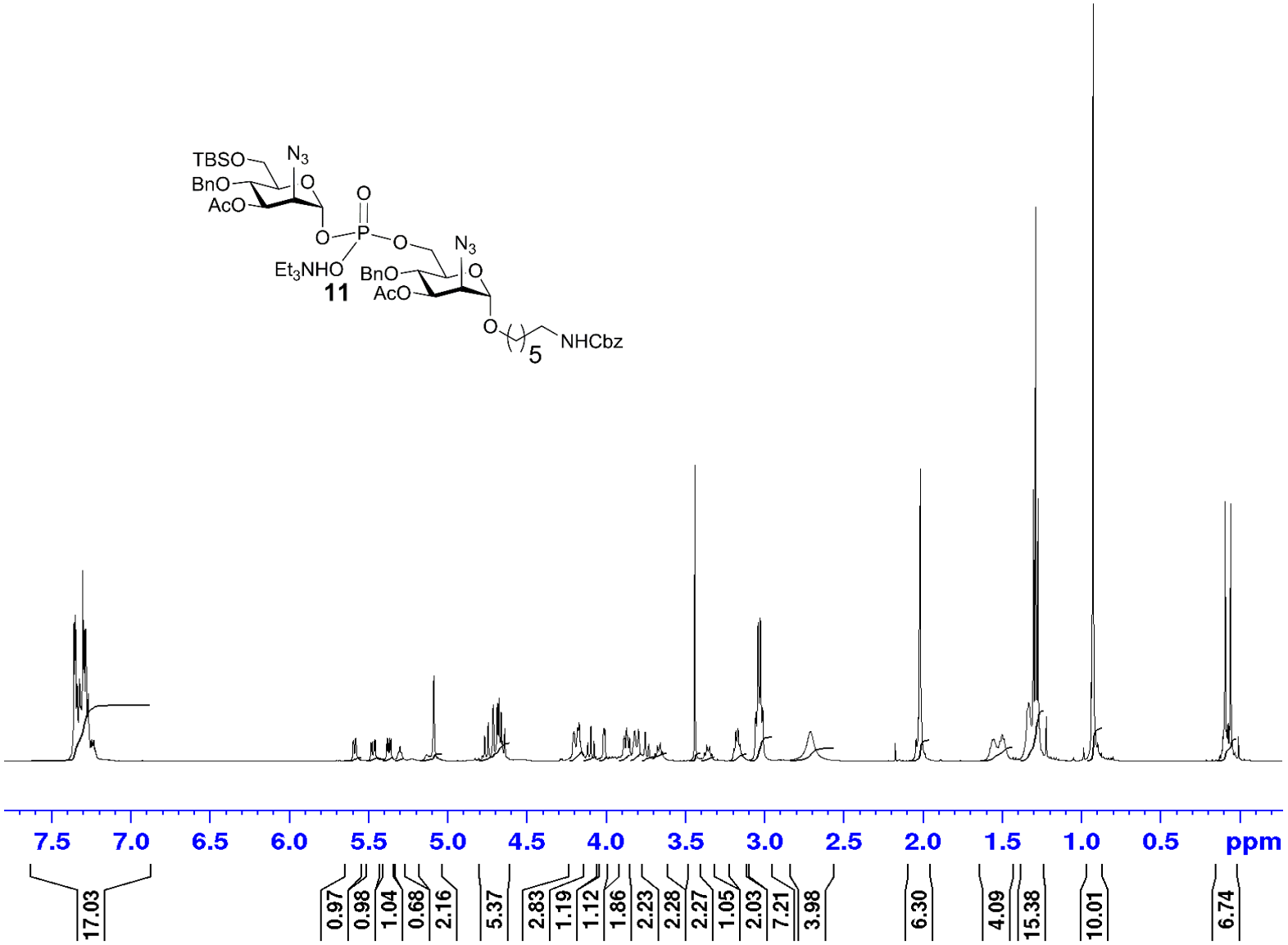




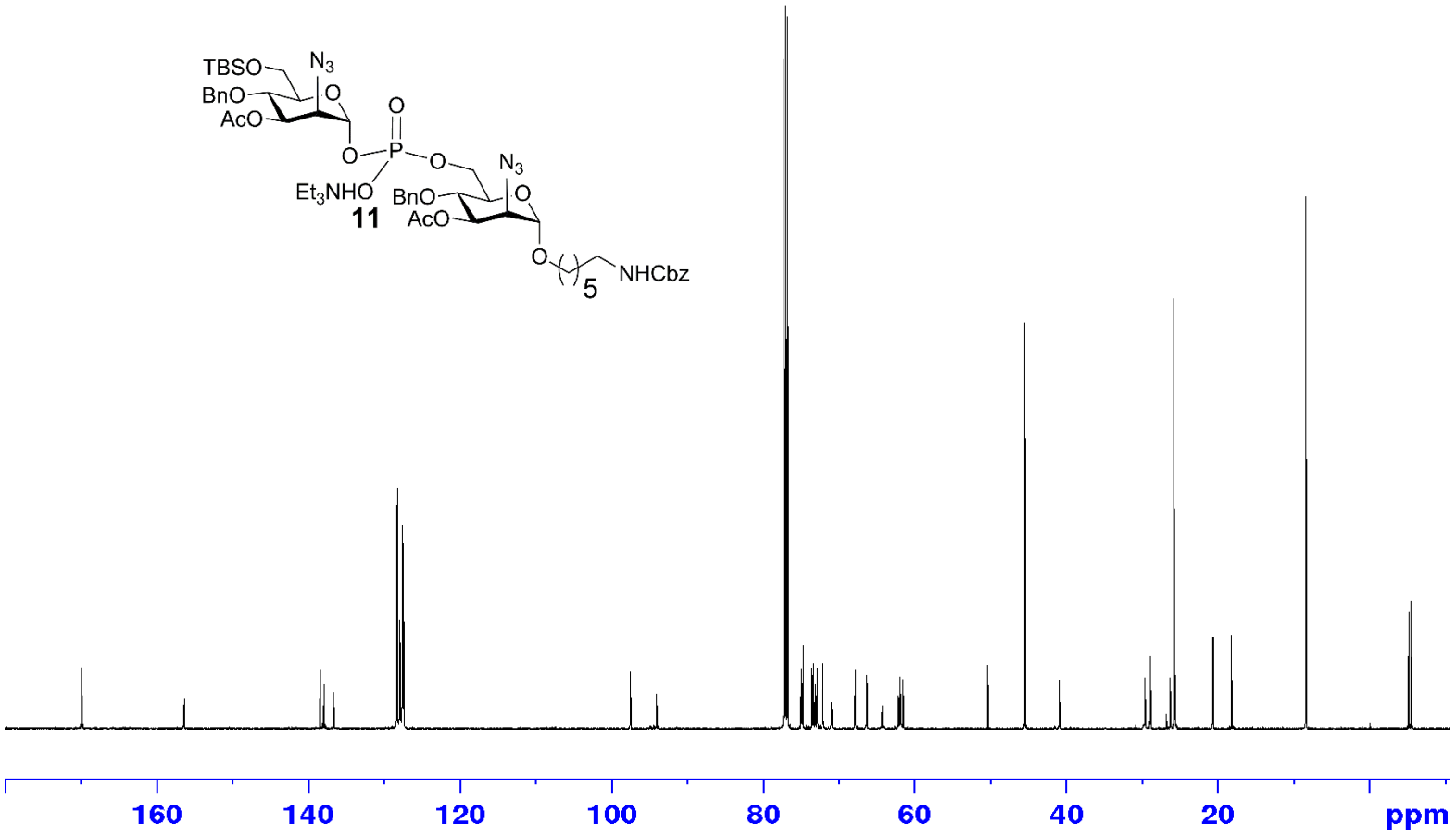
MALDI-TOF MS spectra of compound **3**



[1]HNMR of compound **11** ($CDCl_3$, 500 MHz)



^{13}C NMR of compound **11** ($CDCl_3$, 125 MHz)



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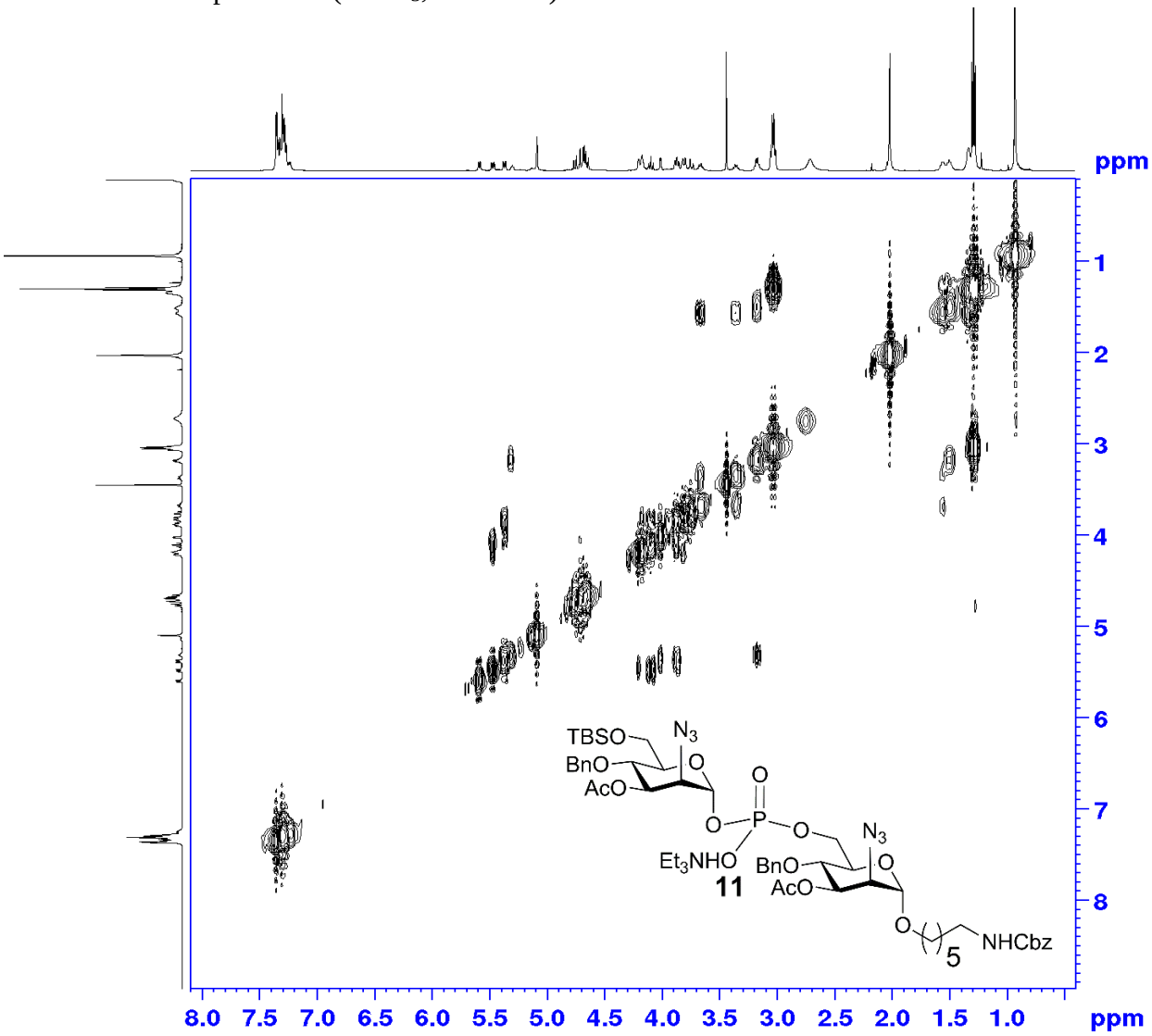
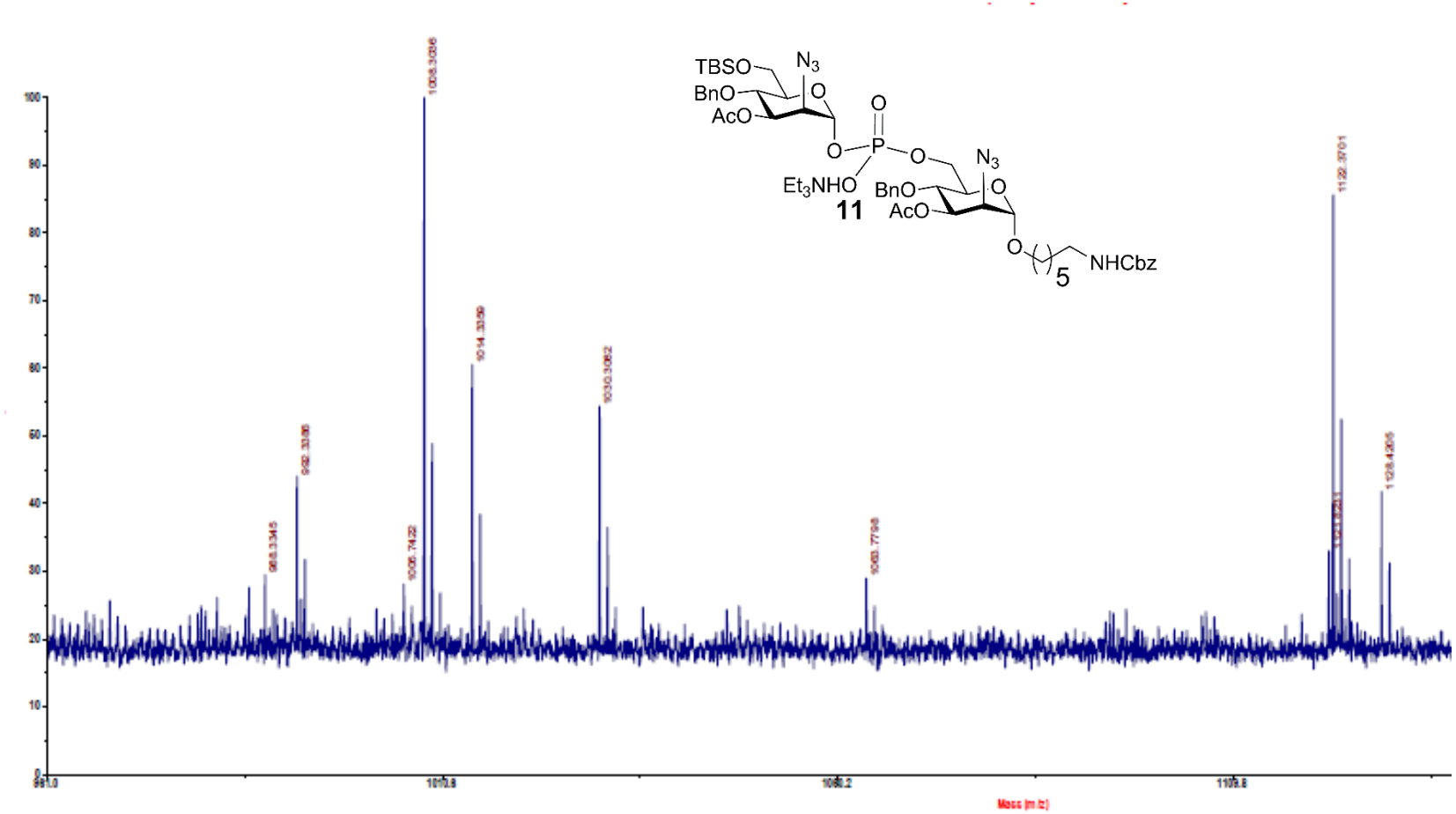
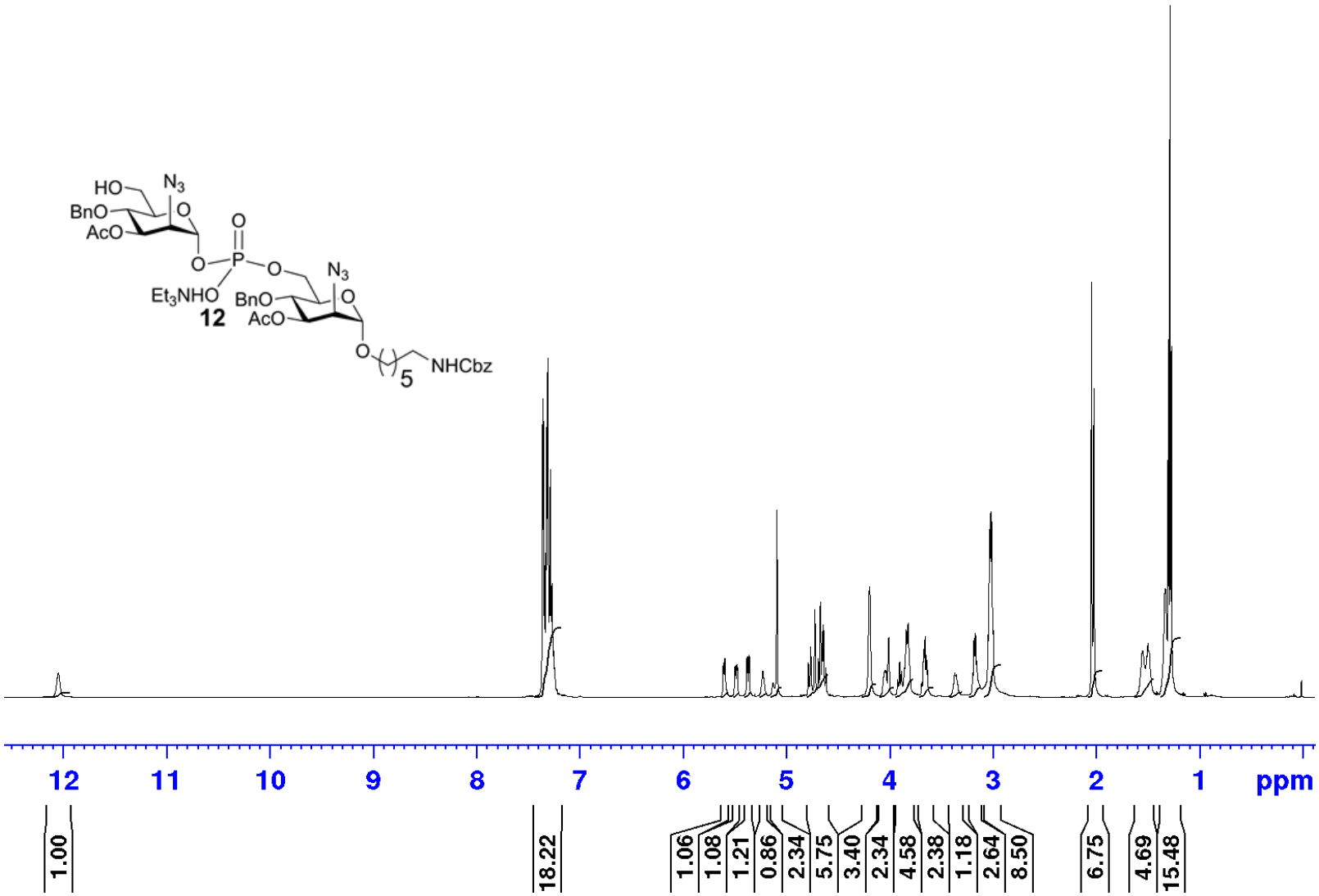


MALDI-TOF MS spectra of compound **11**



[1]HNMR of compound **12** ($CDCl_3$, 500 MHz)



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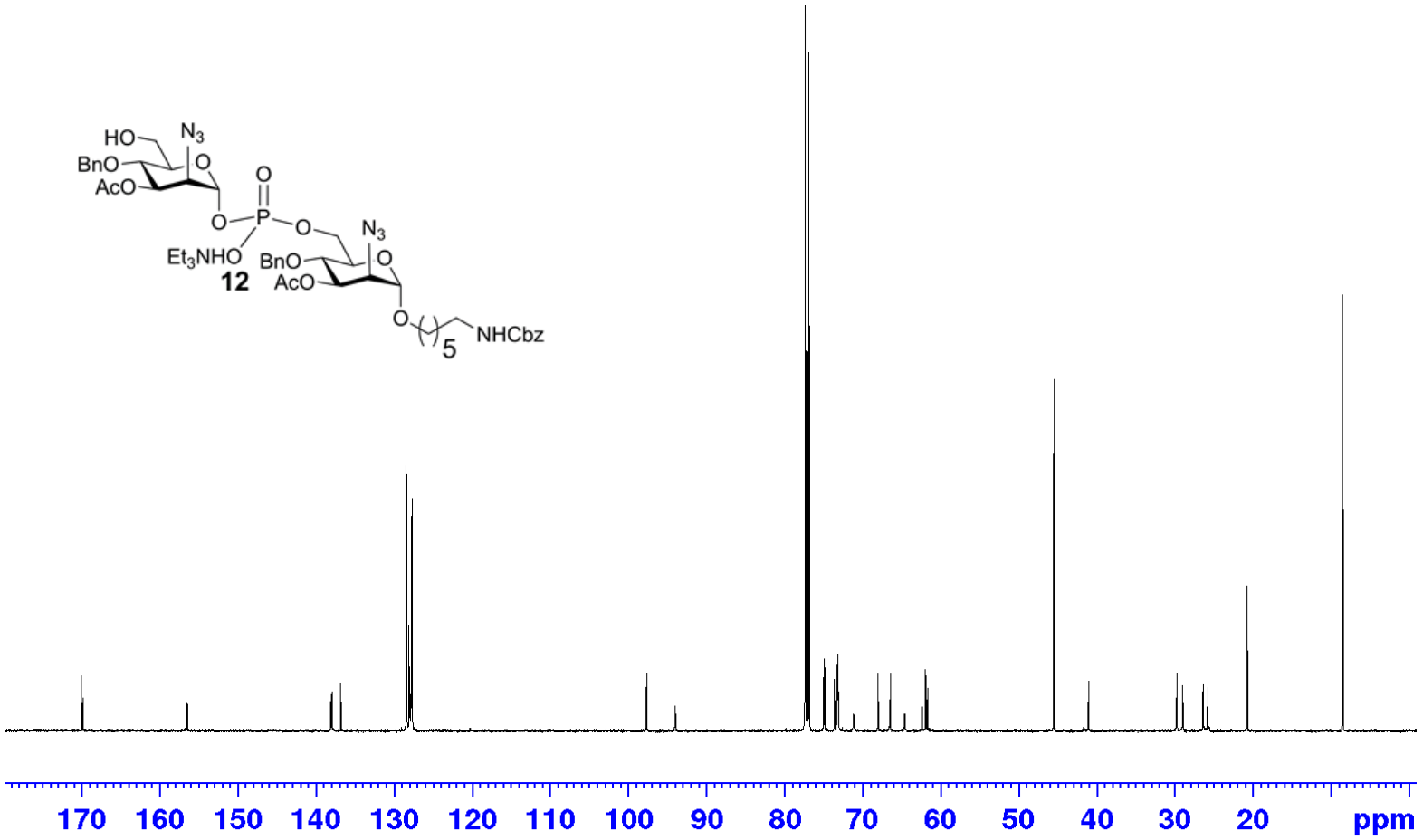

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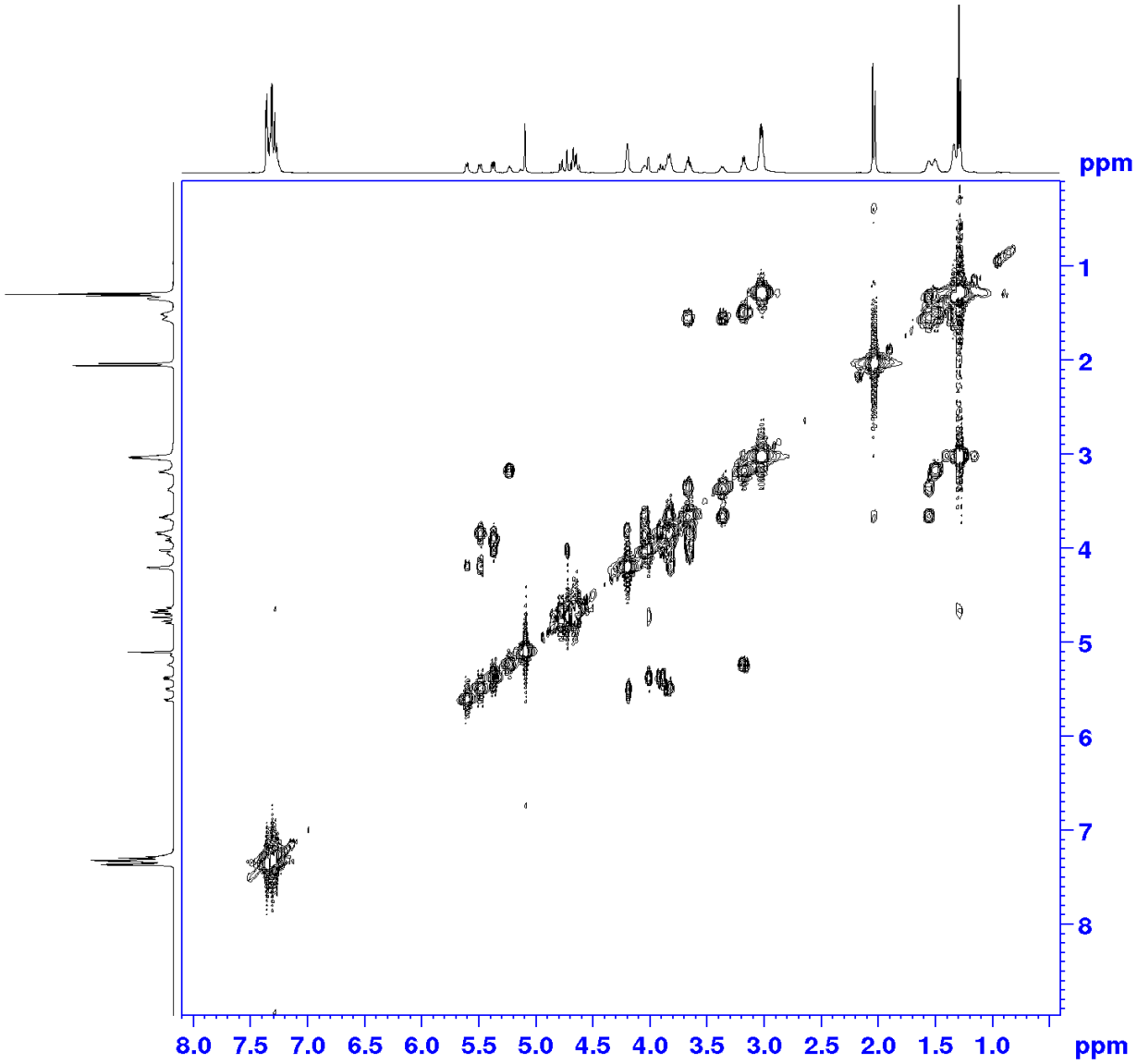
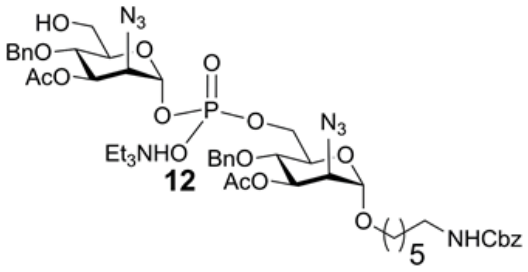
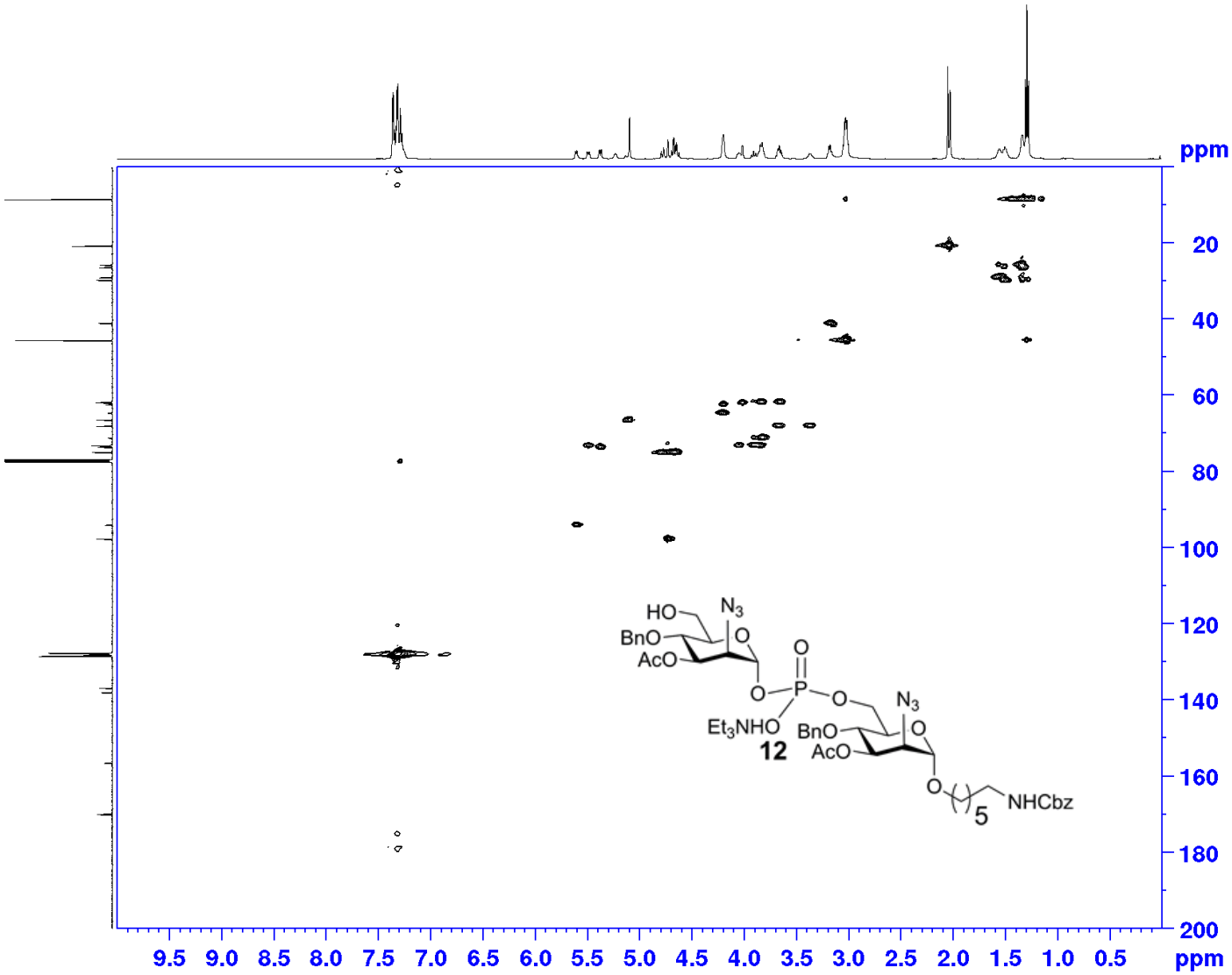
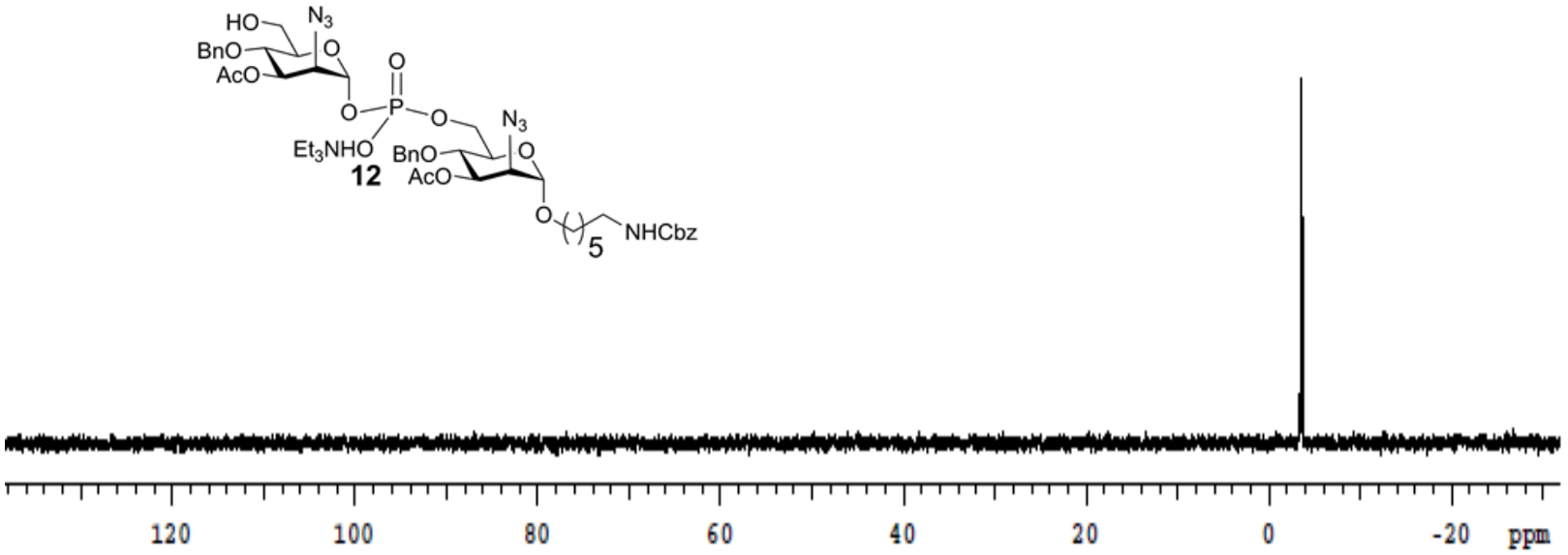




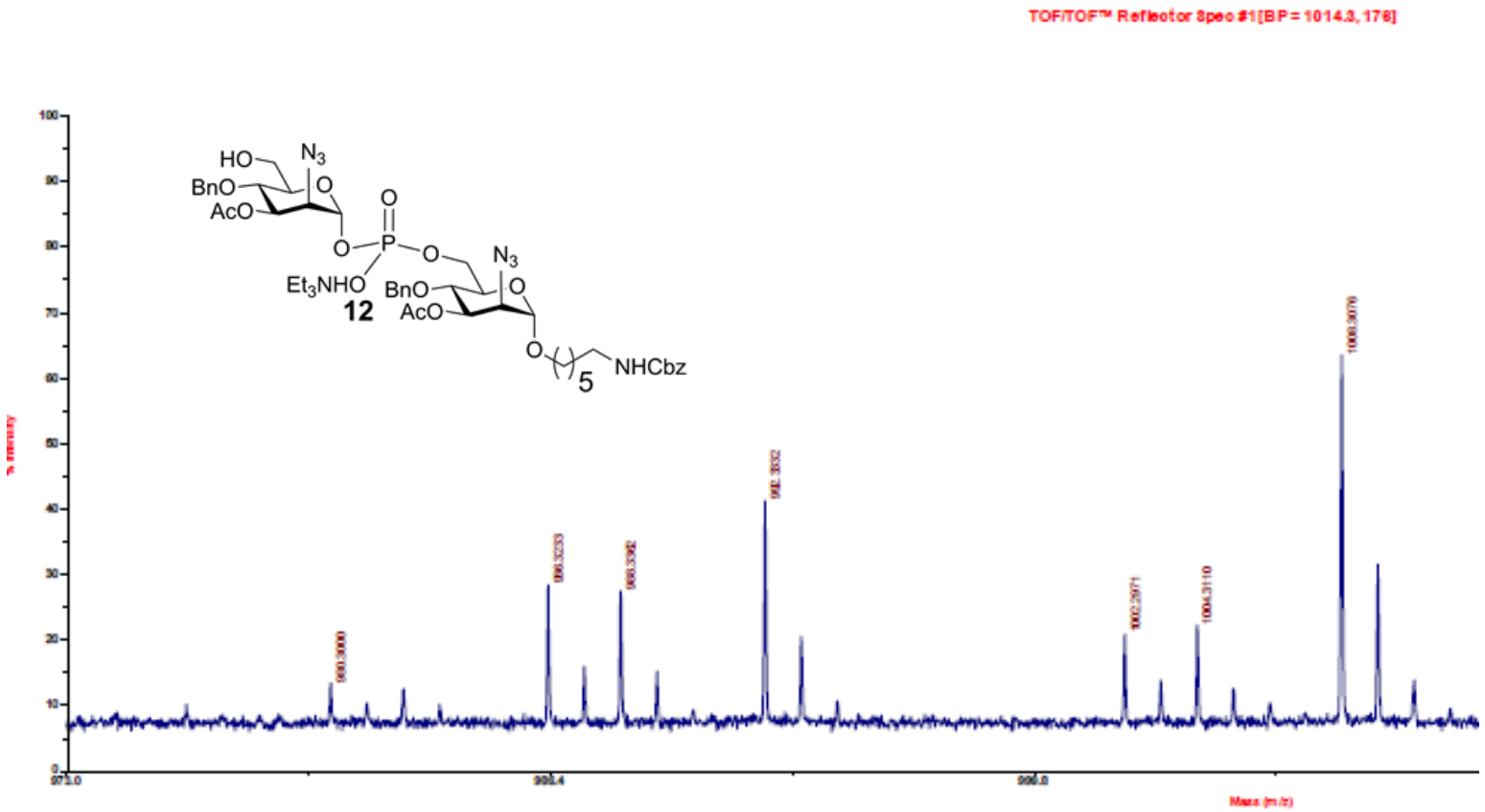
2D HSQC of compound **12** ($CDCl_3$, 500 MHz)



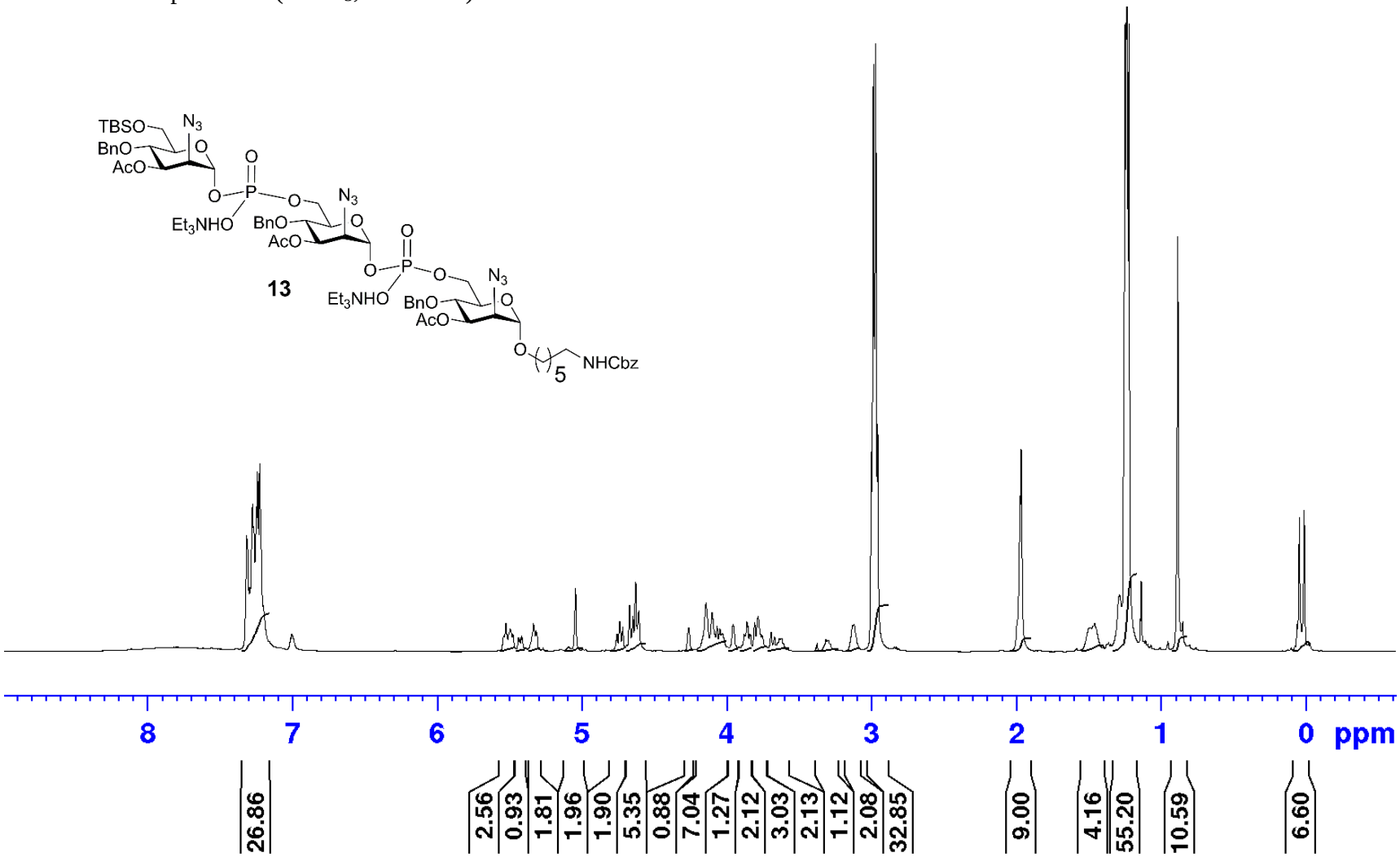
^{31}P NMR of compound **12** (CDCl3, 202 MHz)



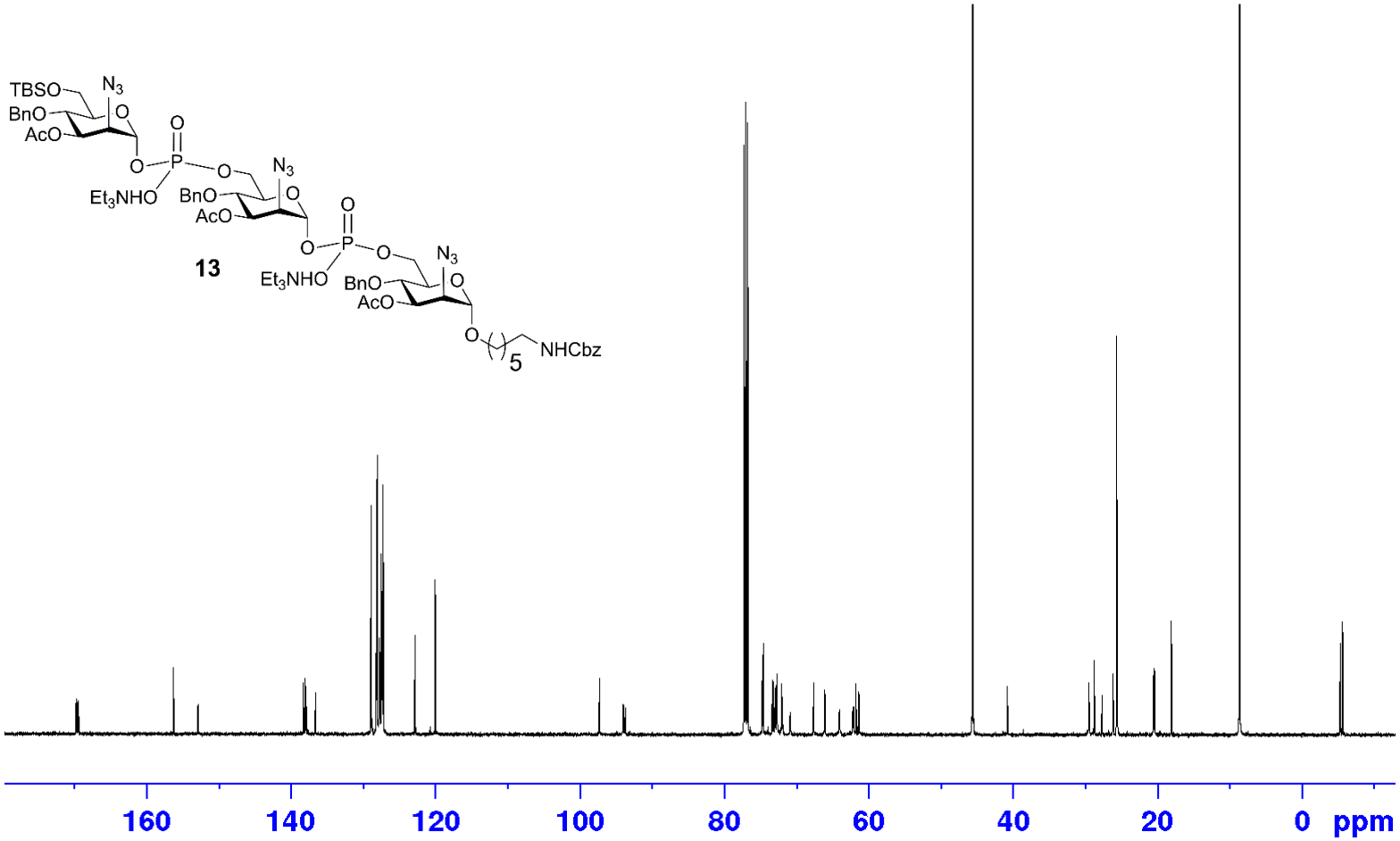
MALDI-TOF MS spectra of compound **12**



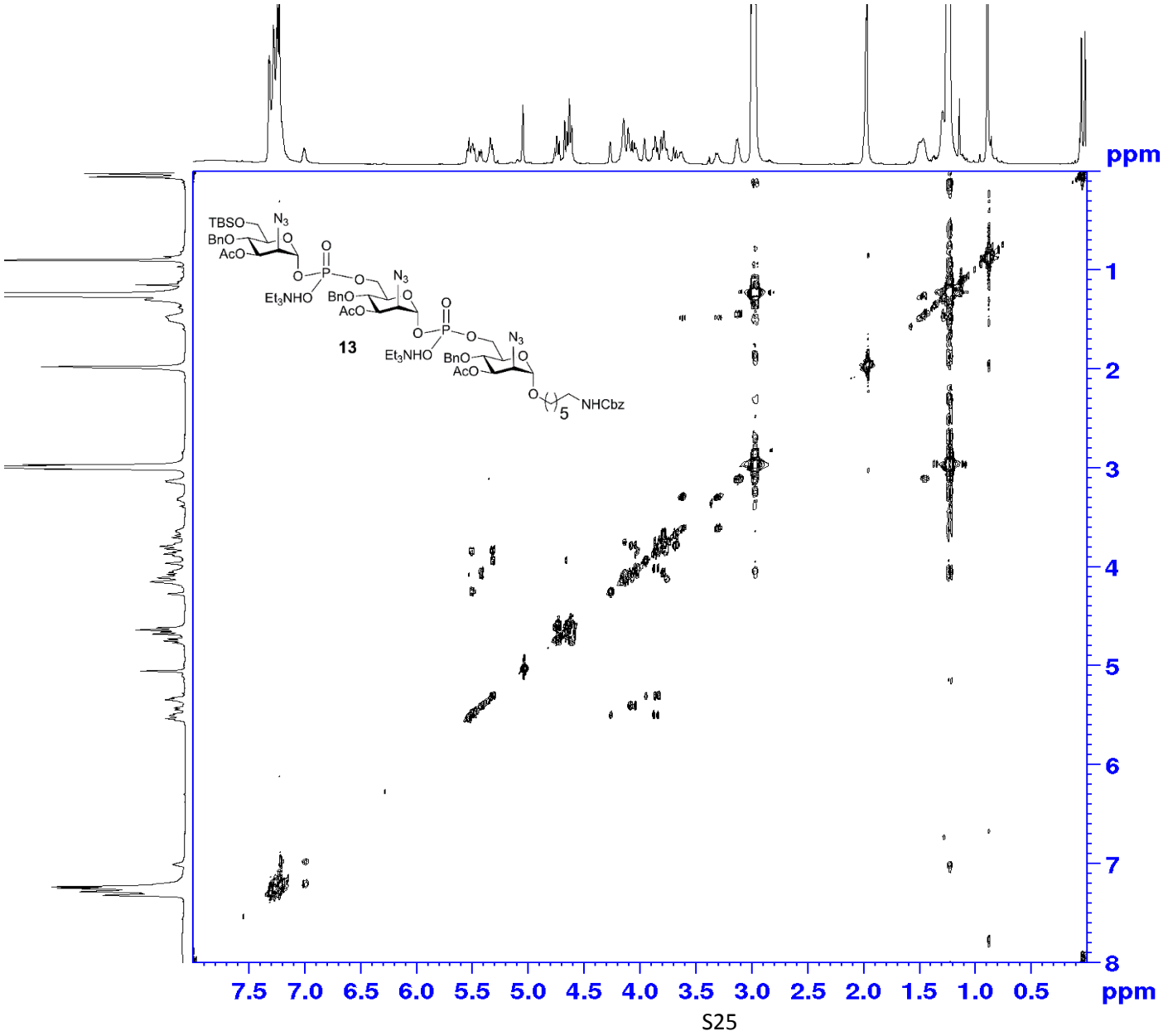
^{1}HNMR of compound **13** ($CDCl_3$, 500 MHz)



^{13}C NMR of compound **13** ($CDCl_3$, 125 MHz)



2D COSY of compound **13** ($CDCl_3$, 500 MHz)



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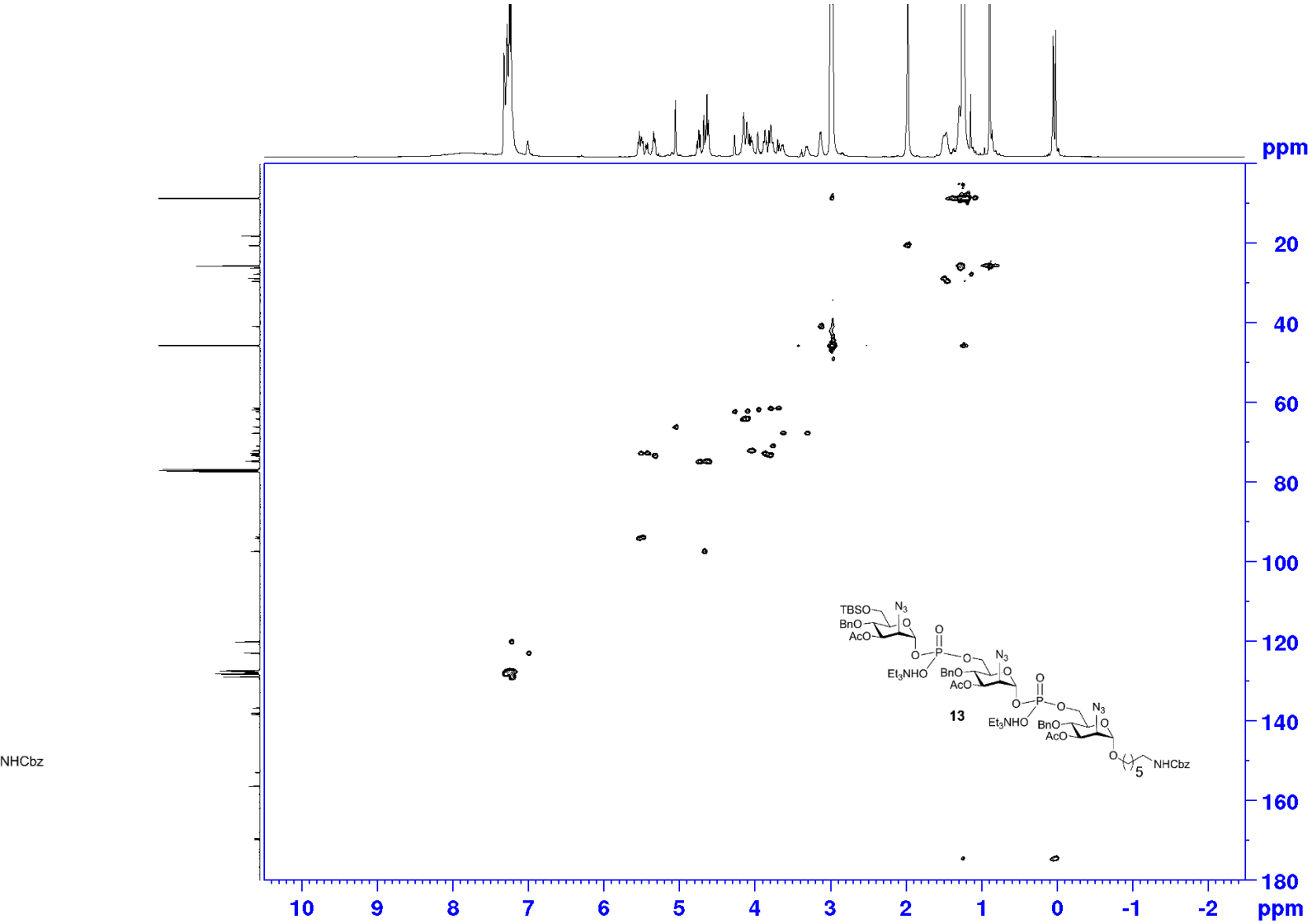
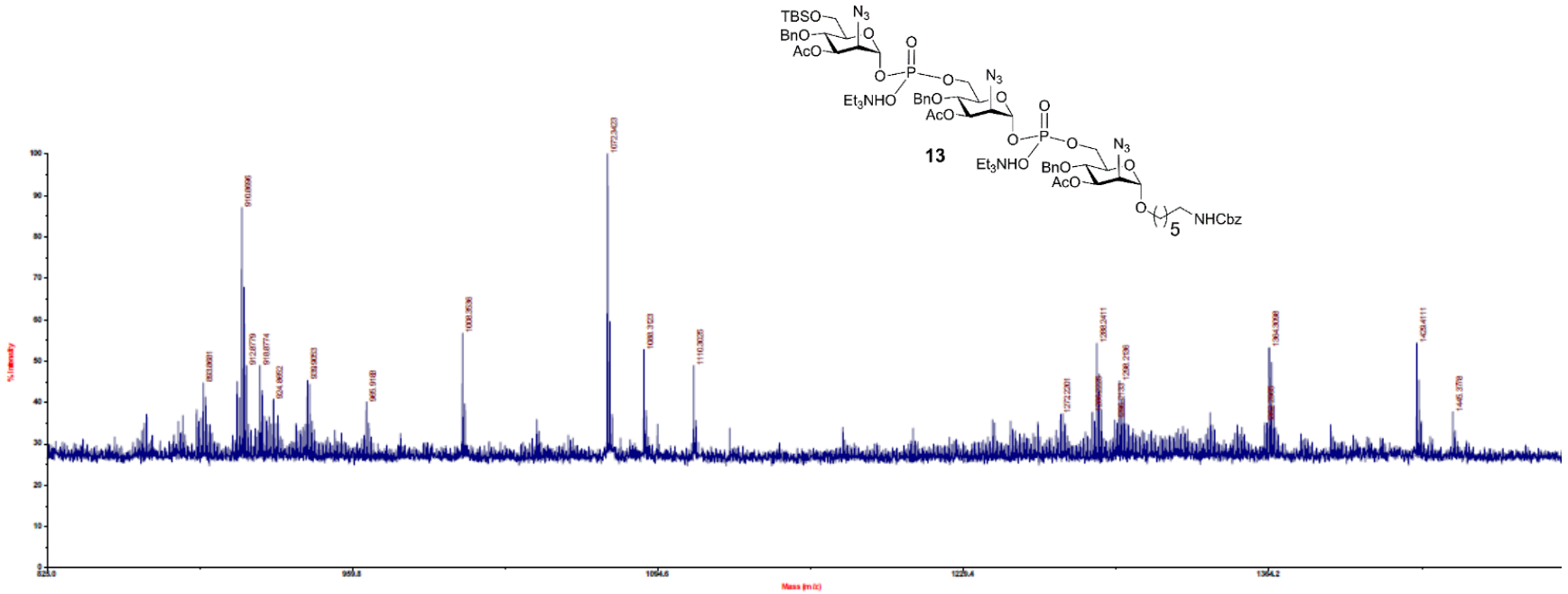


MALDI-TOF MS spectra of compound **13**



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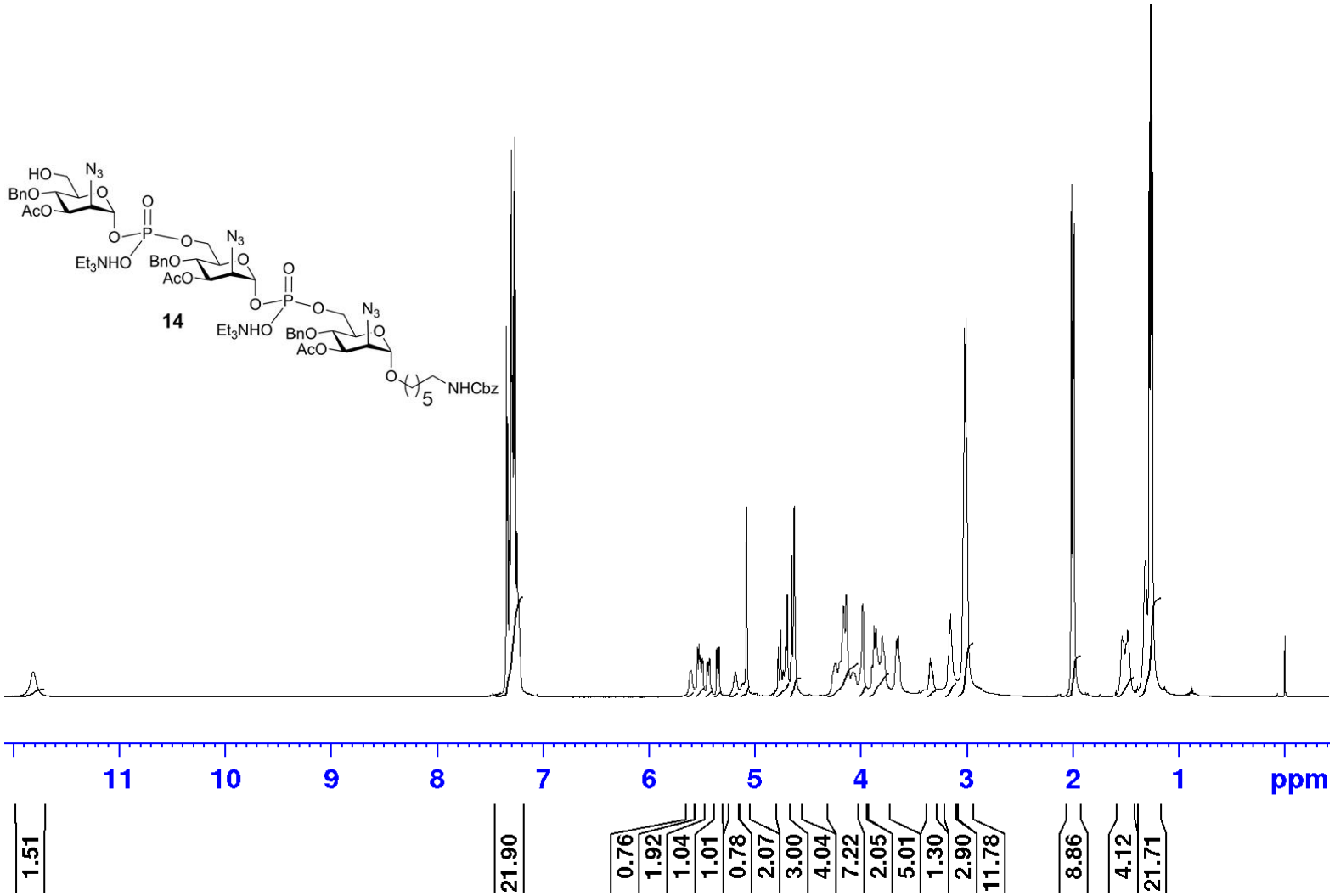
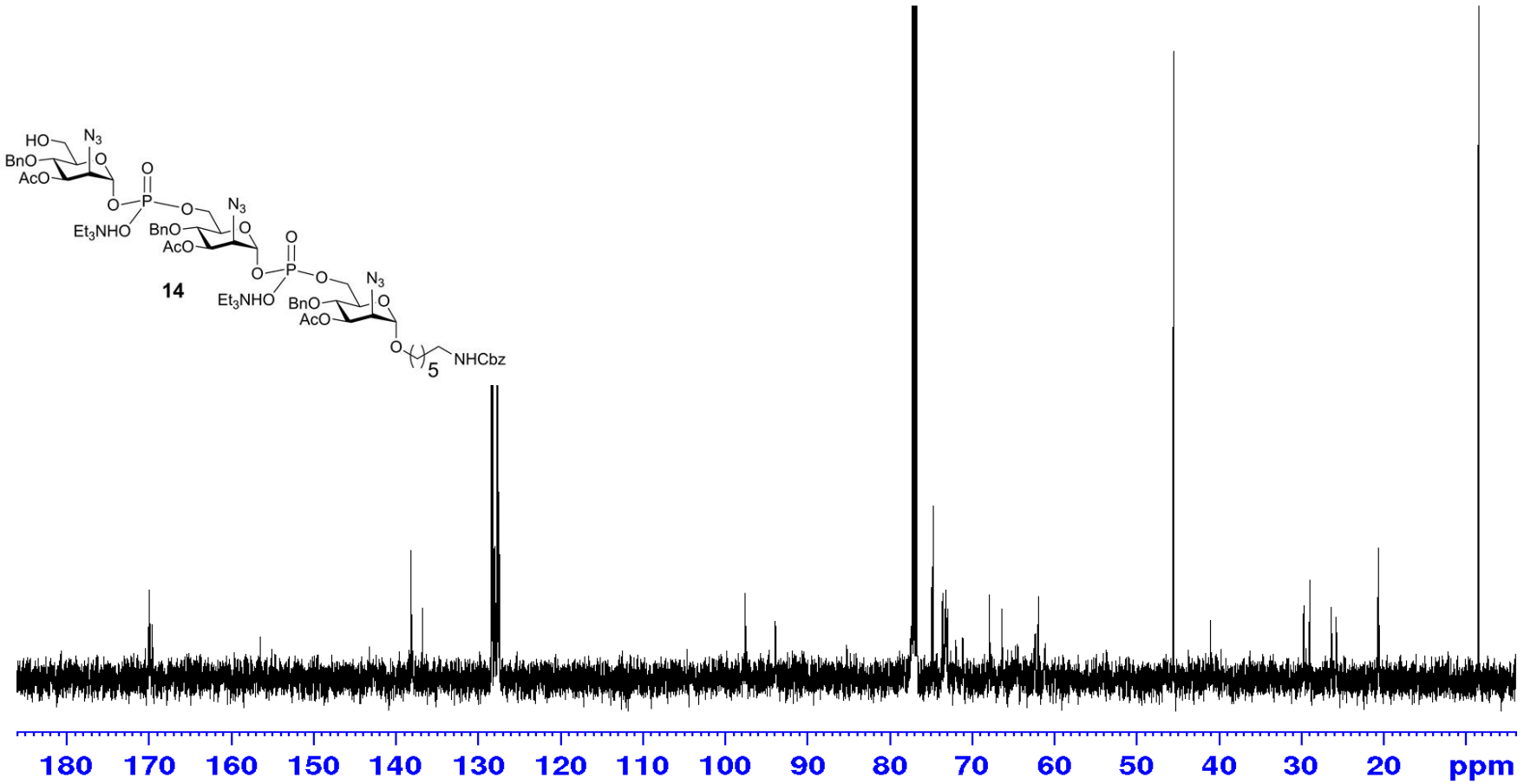
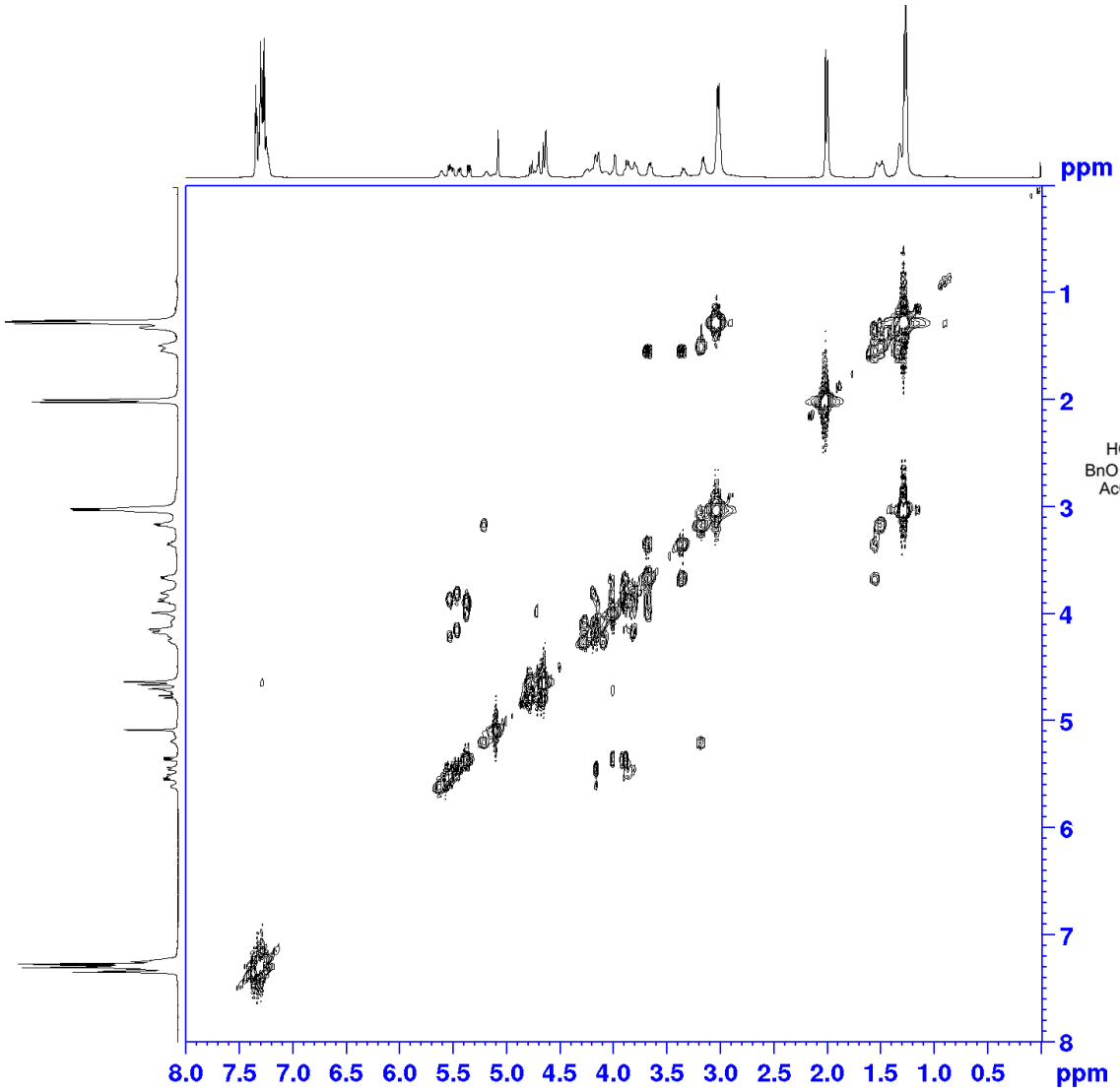
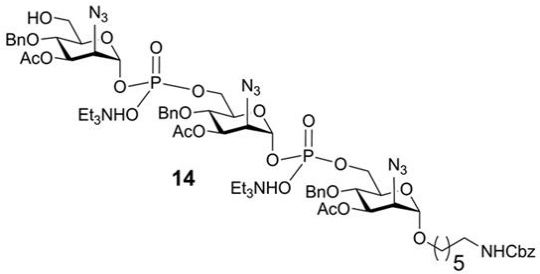


^{13}C NMR of compound **14** ($CDCl_3$, 125 MHz)



2D COSY of compound **14** ($CDCl_3$, 500 MHz)





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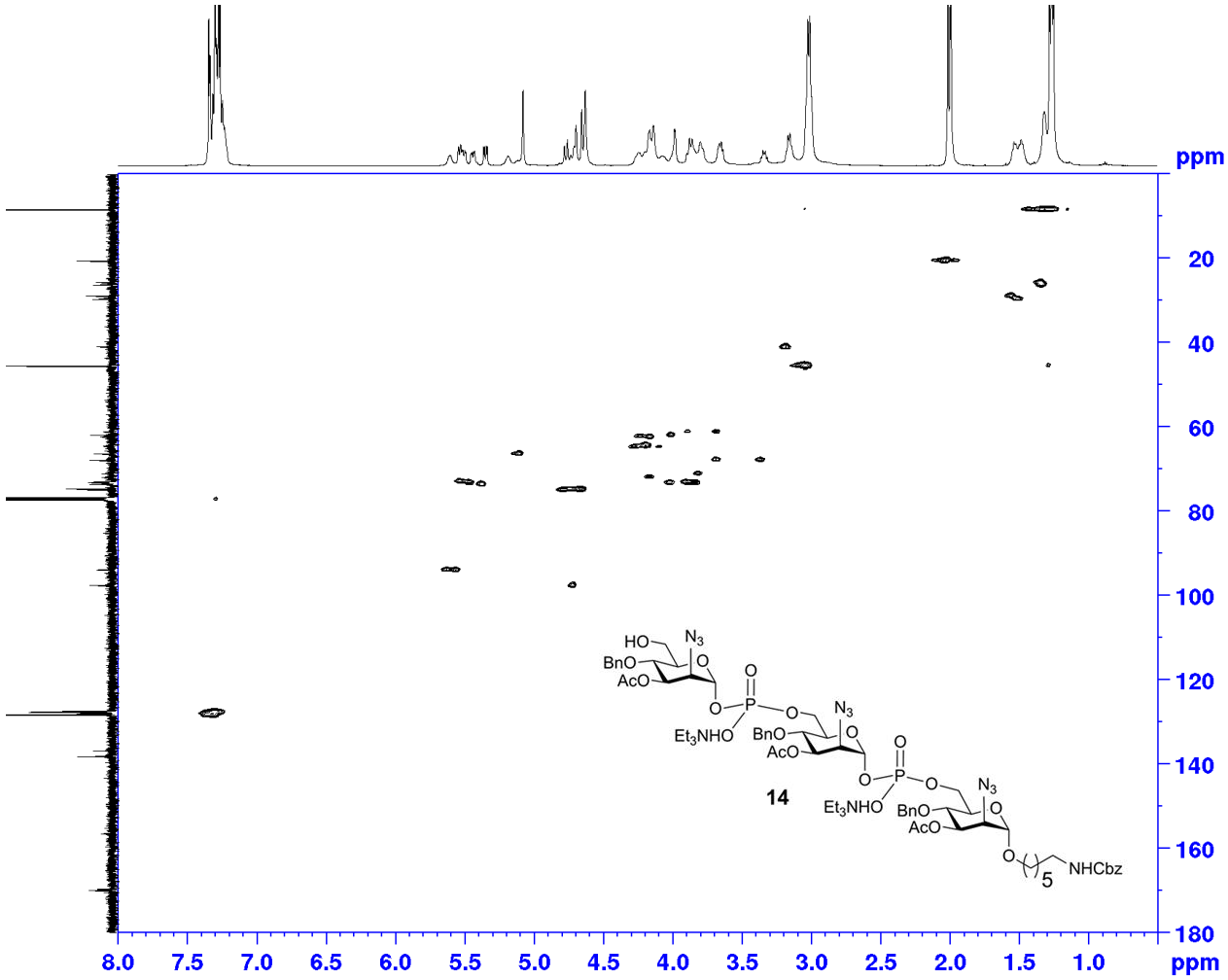
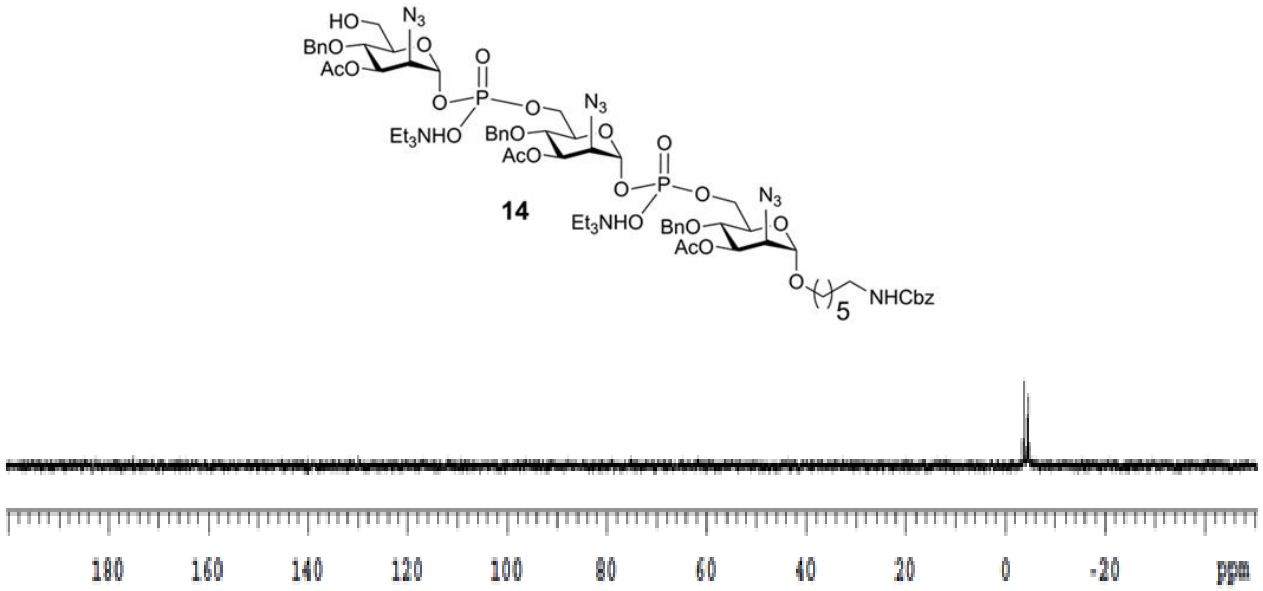
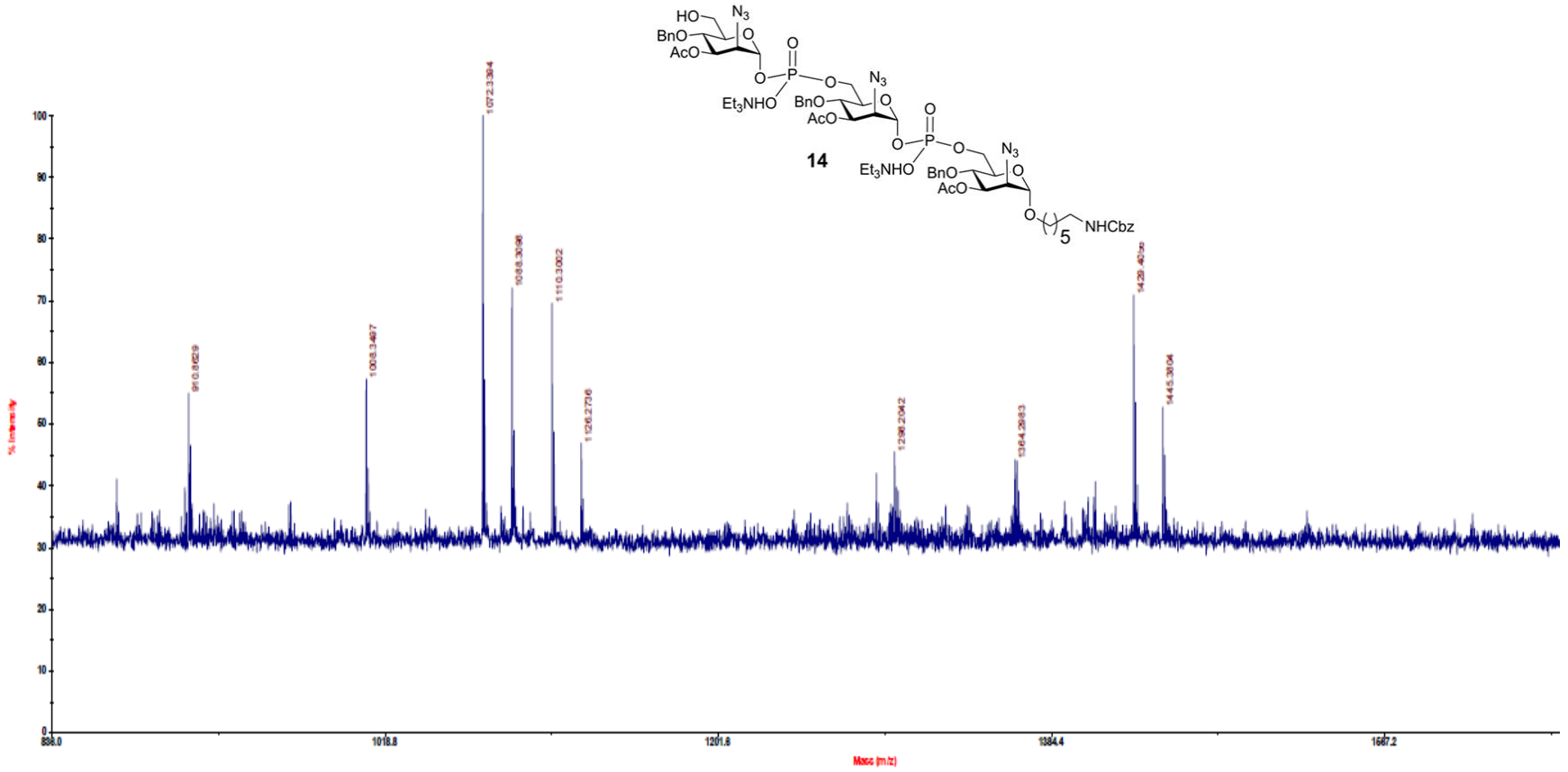


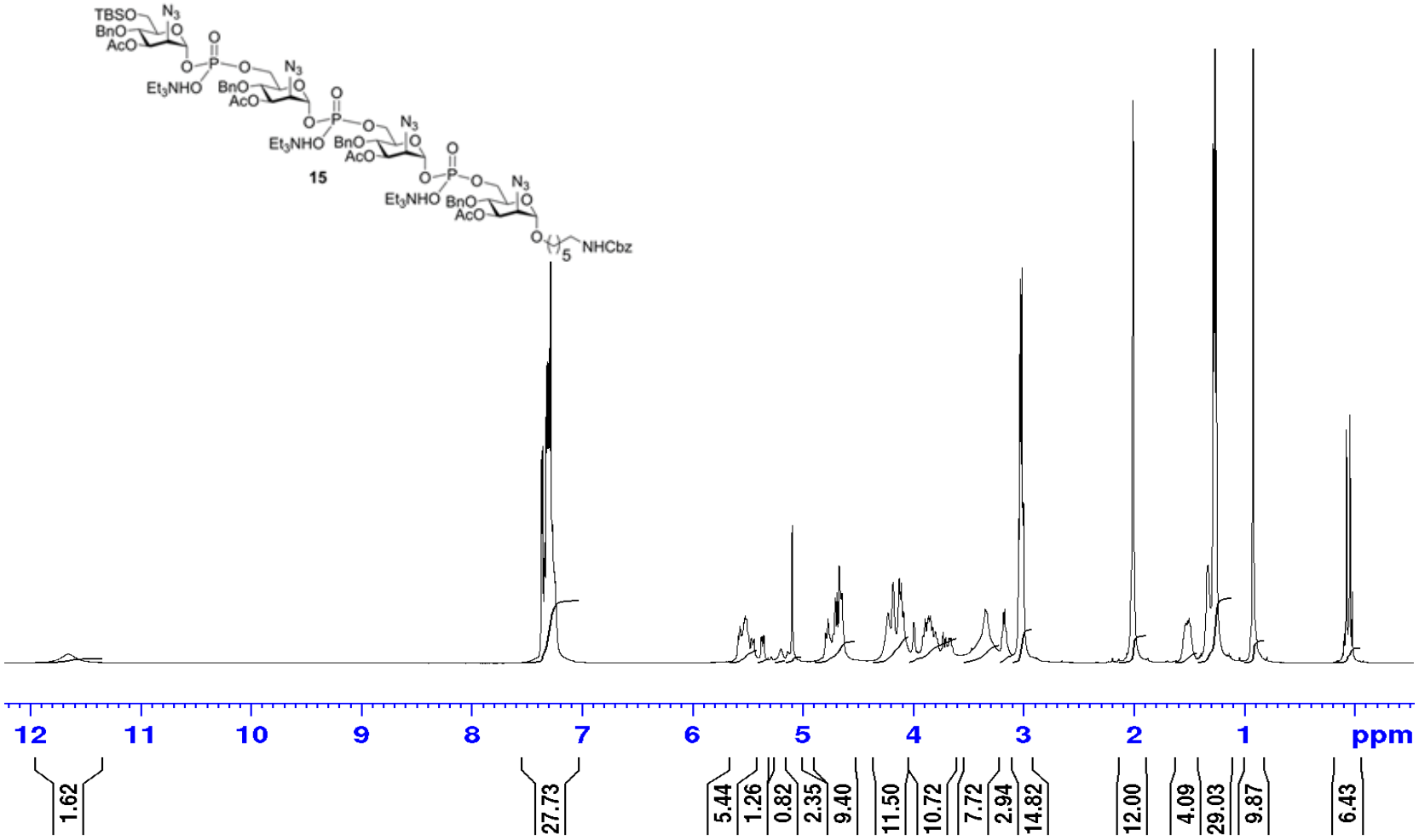
^{31}P NMR of compound **14** ($CDCl_3$, 202 MHz)



MALDI-TOF MS spectra of compound **14**



^{1}HNMR of compound **15** ($CDCl_3$, 500 MHz)



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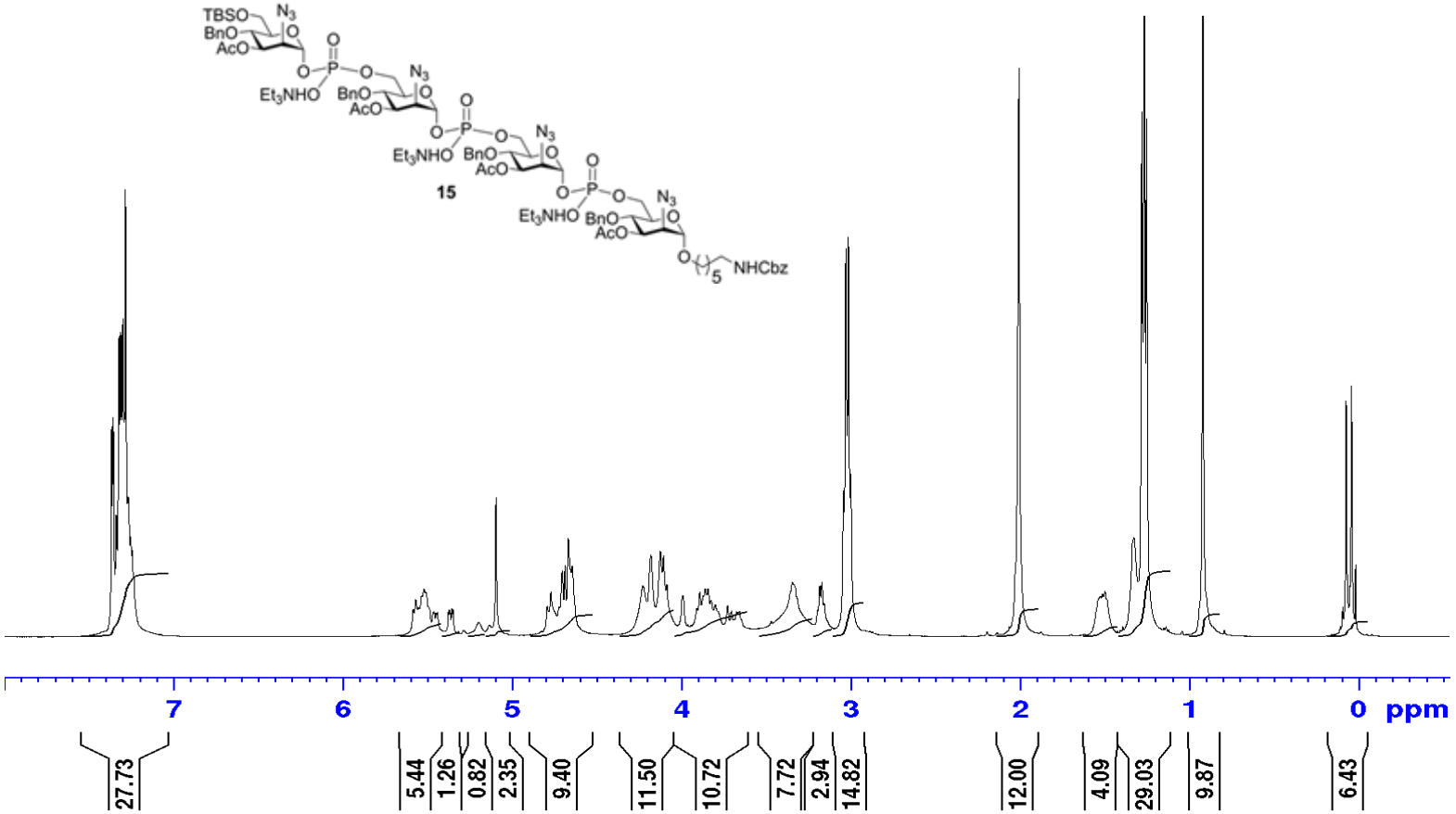
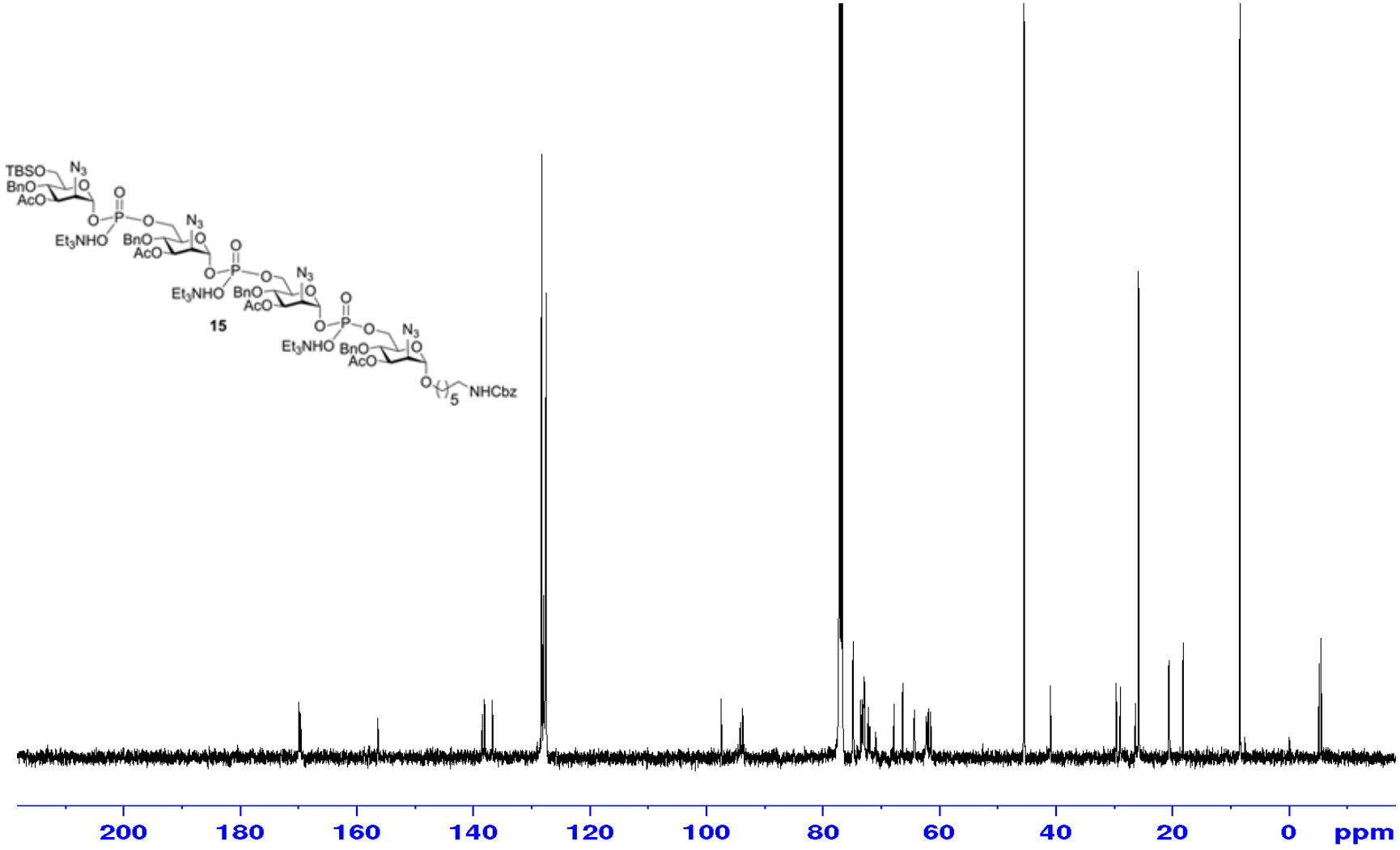
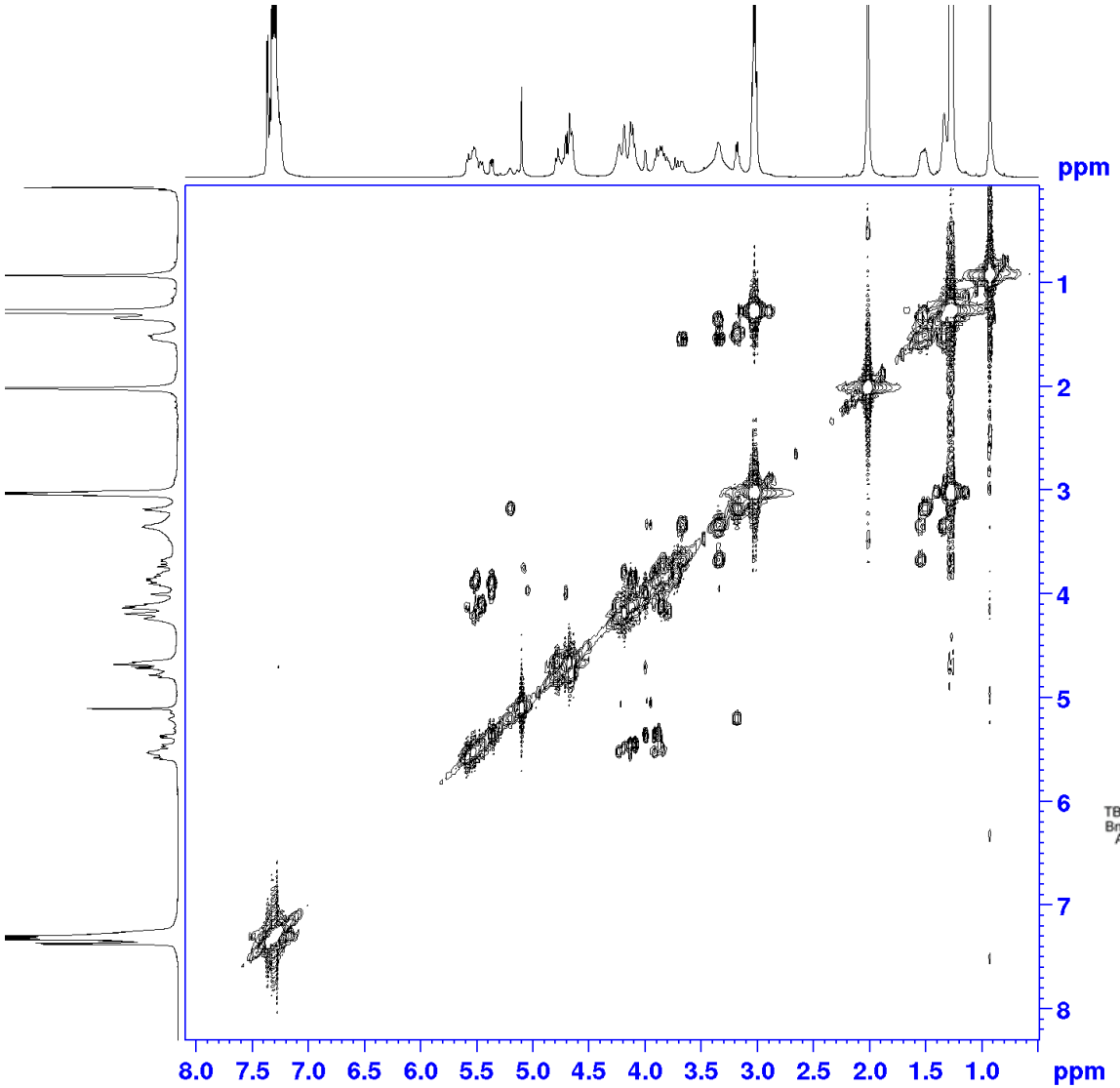
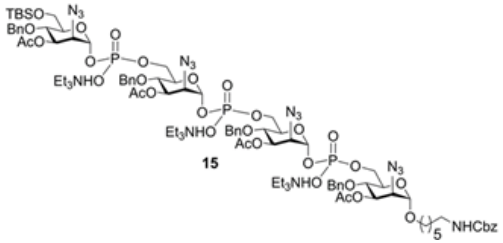


^{13}C NMR of compound **15** ($CDCl_3$, 125 MHz)



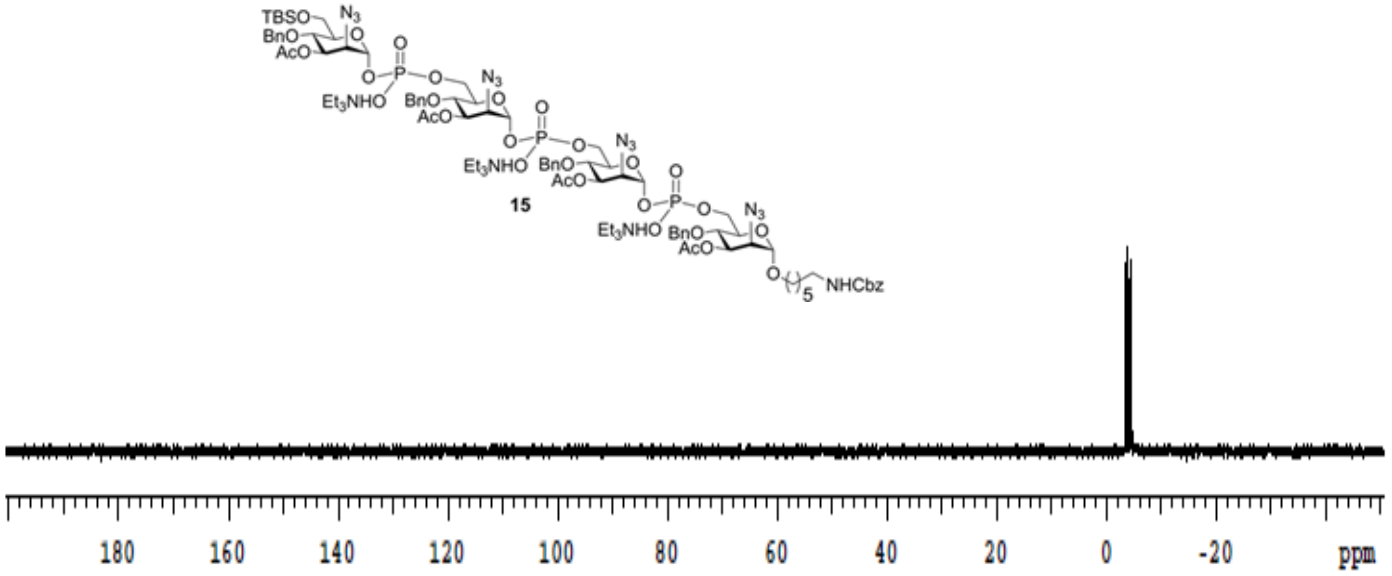
2D COSY of compound **15** ($CDCl_3$, 500 MHz)



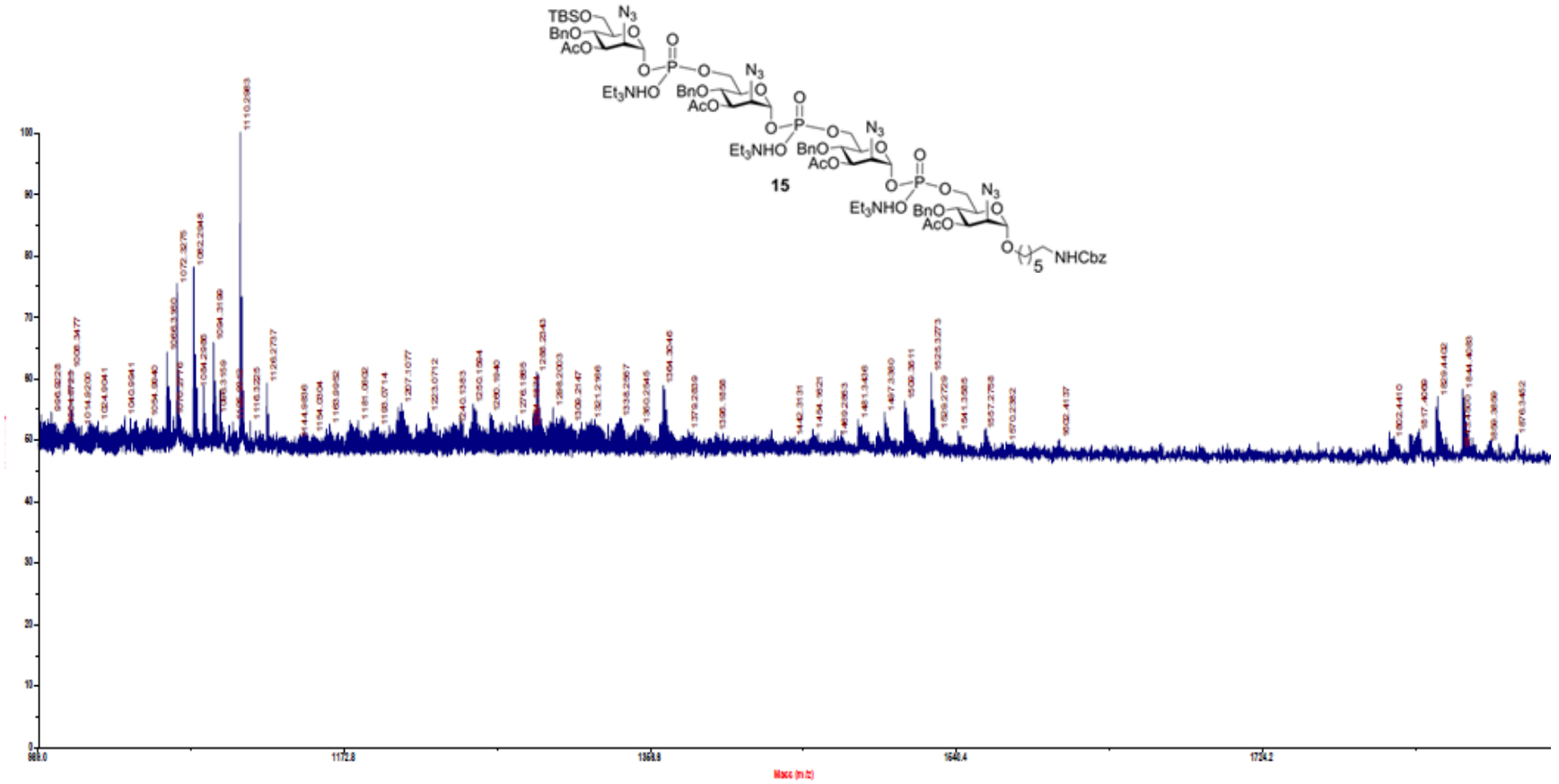


15

^{31}P NMR of compound **15** ($CDCl_3$, 202 MHz)



MALDI-TOF MS spectra of compound **15**



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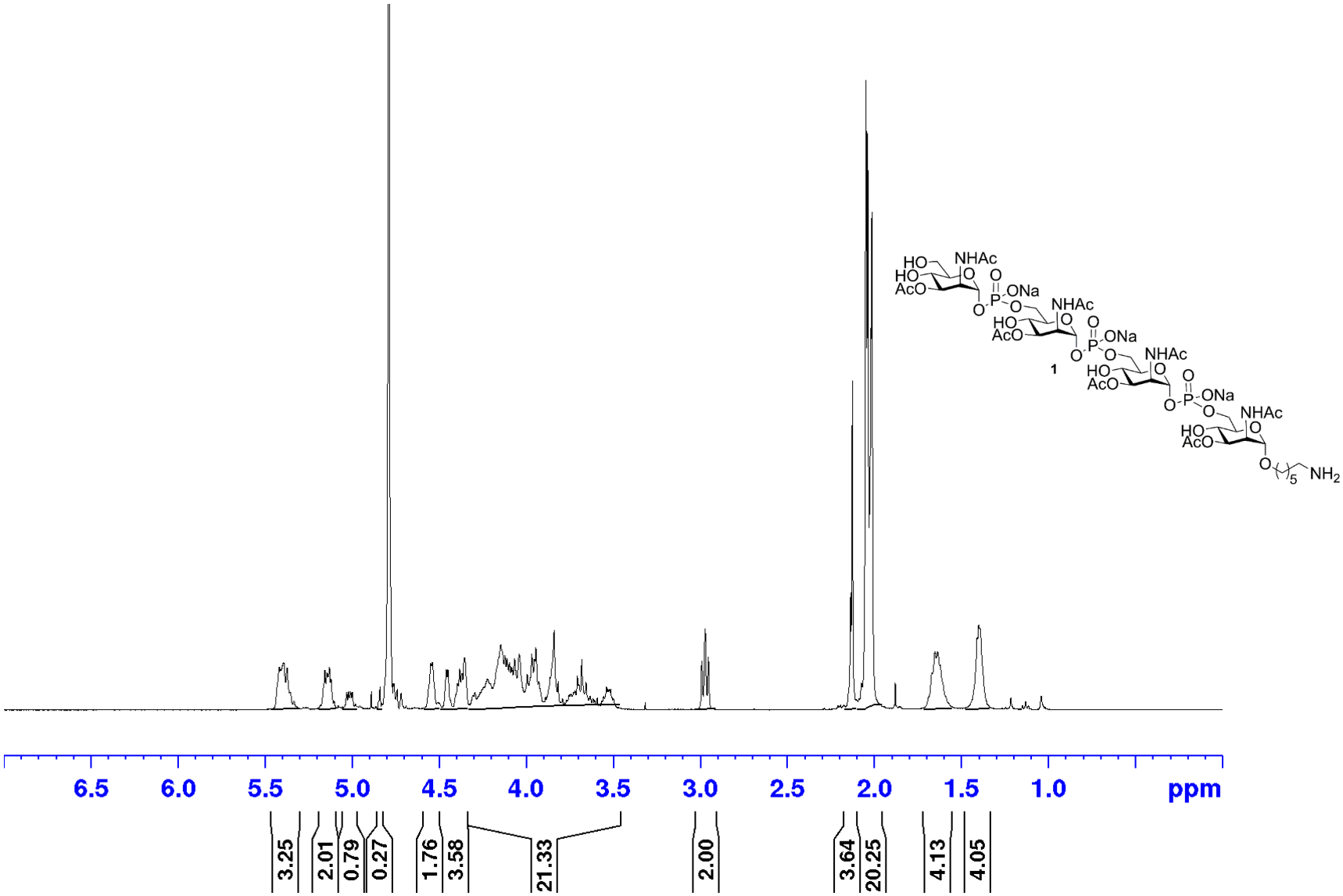
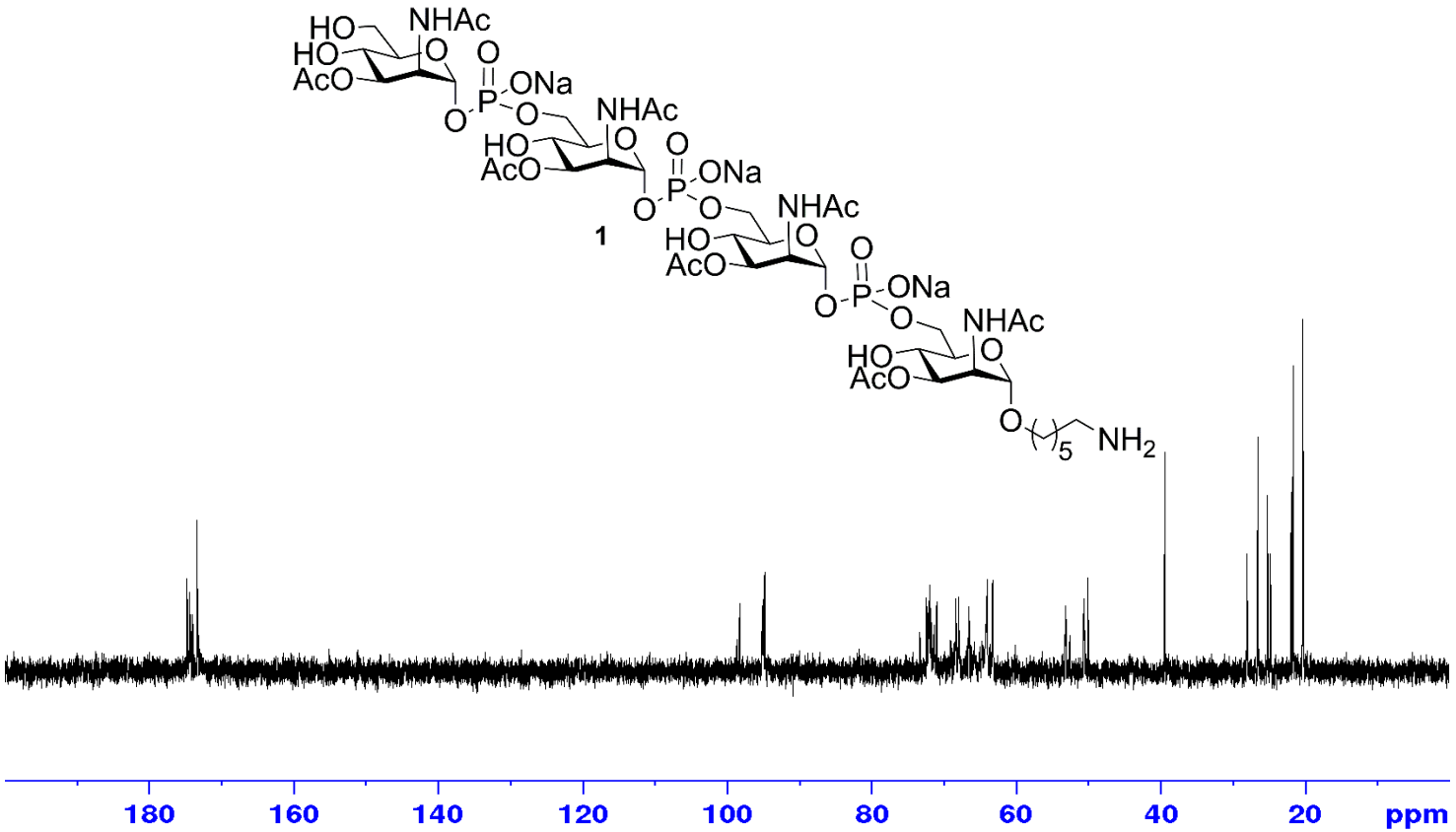
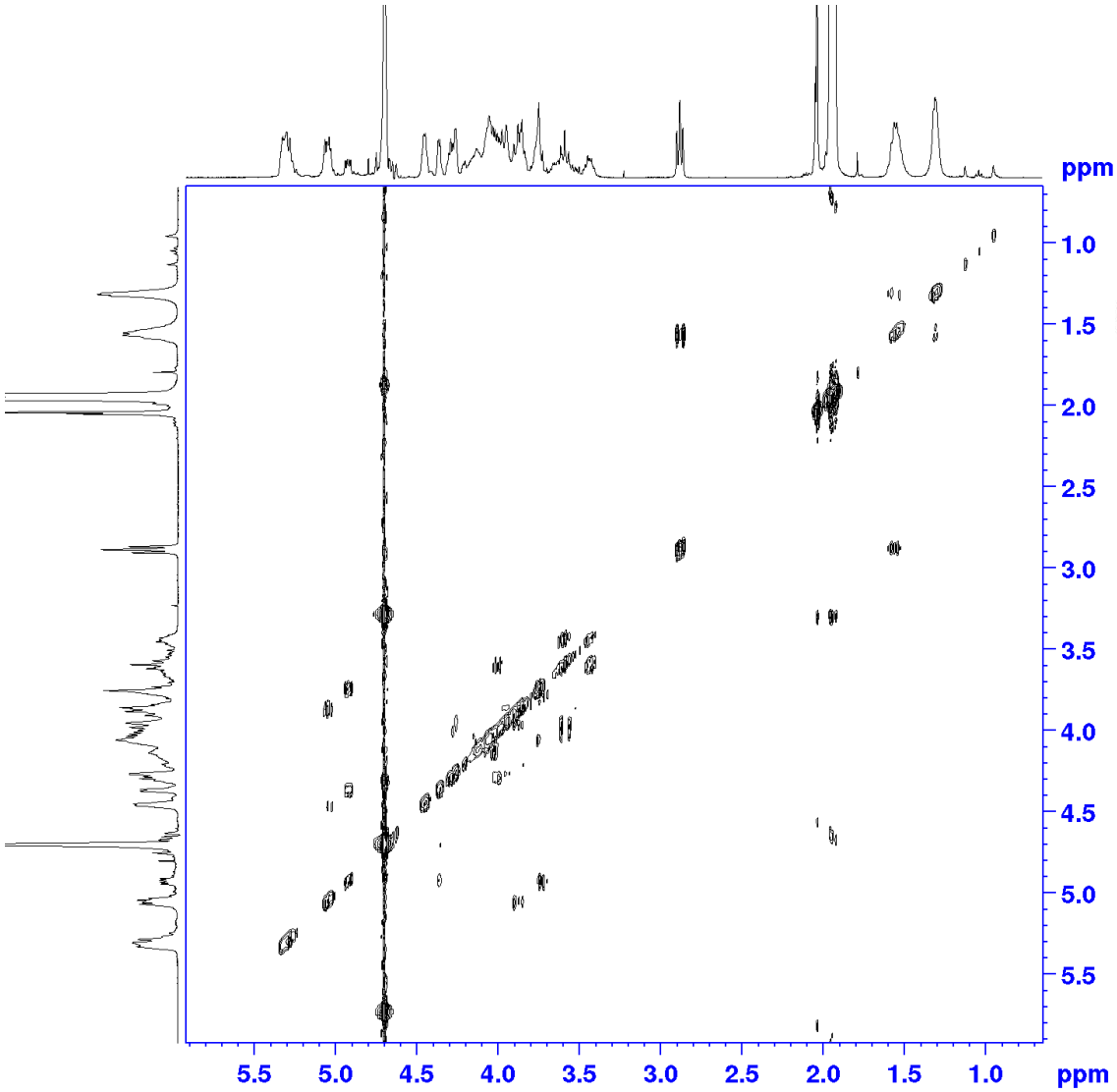
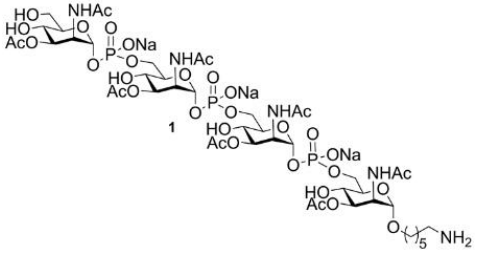


^{13}C NMR of compound **1** (D_2O, 125 MHz)

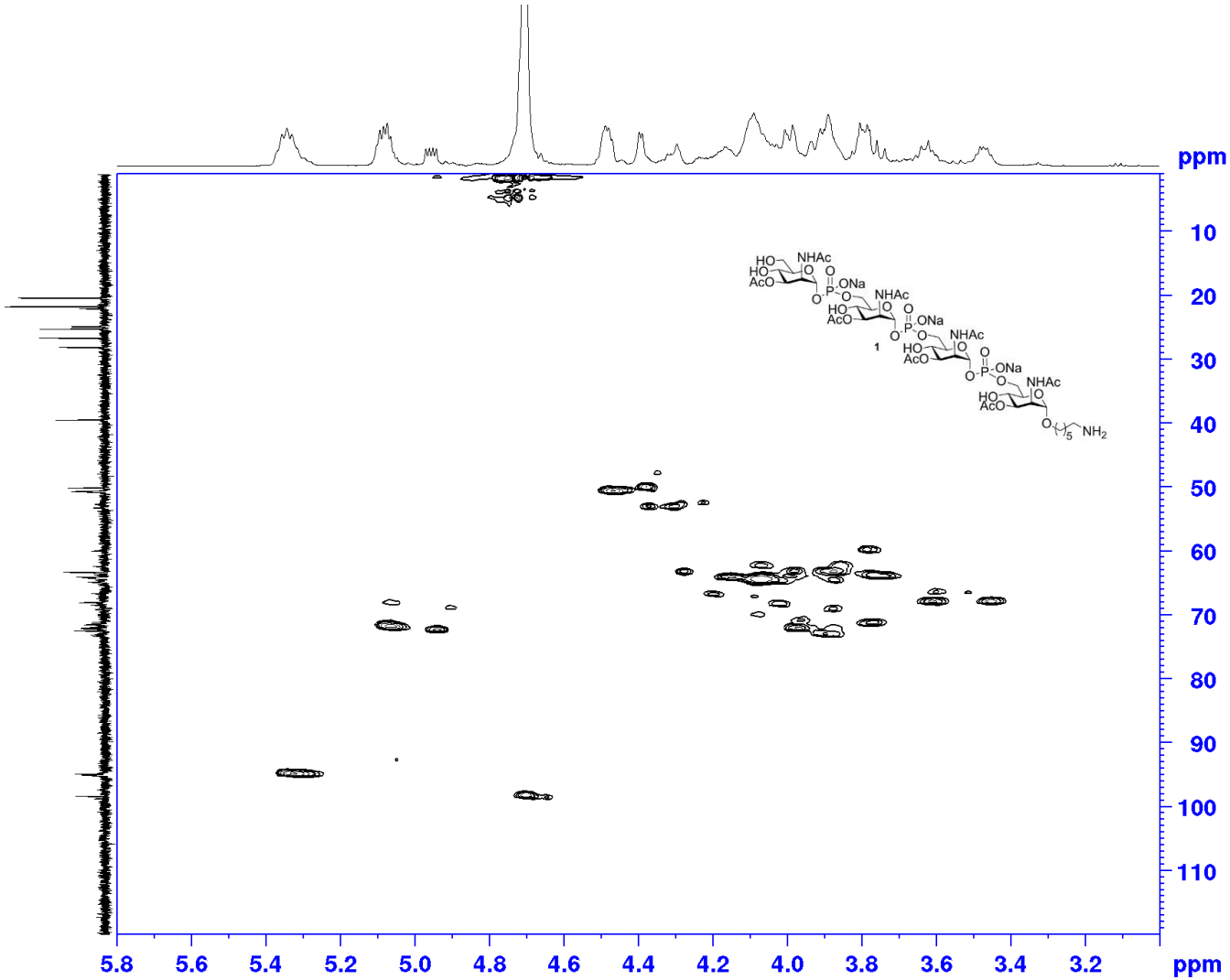


2D COSY NMR of compound **1** (D_2O, 500 MHz)

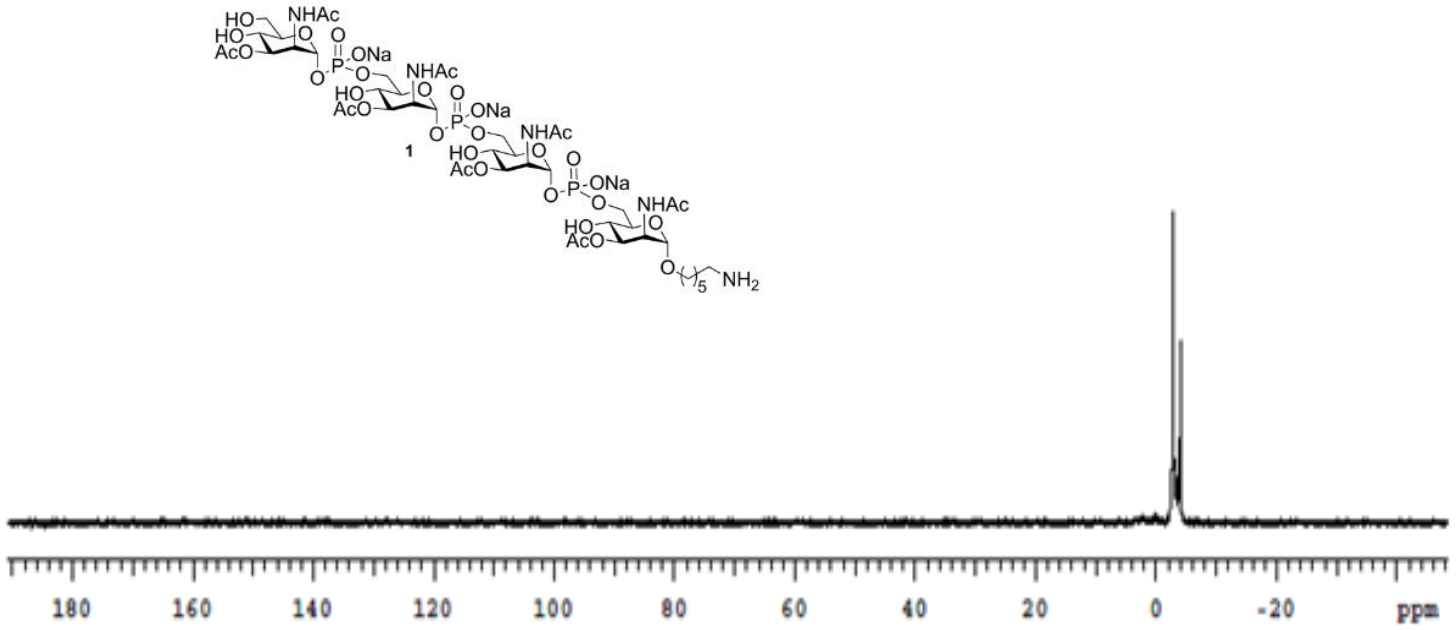




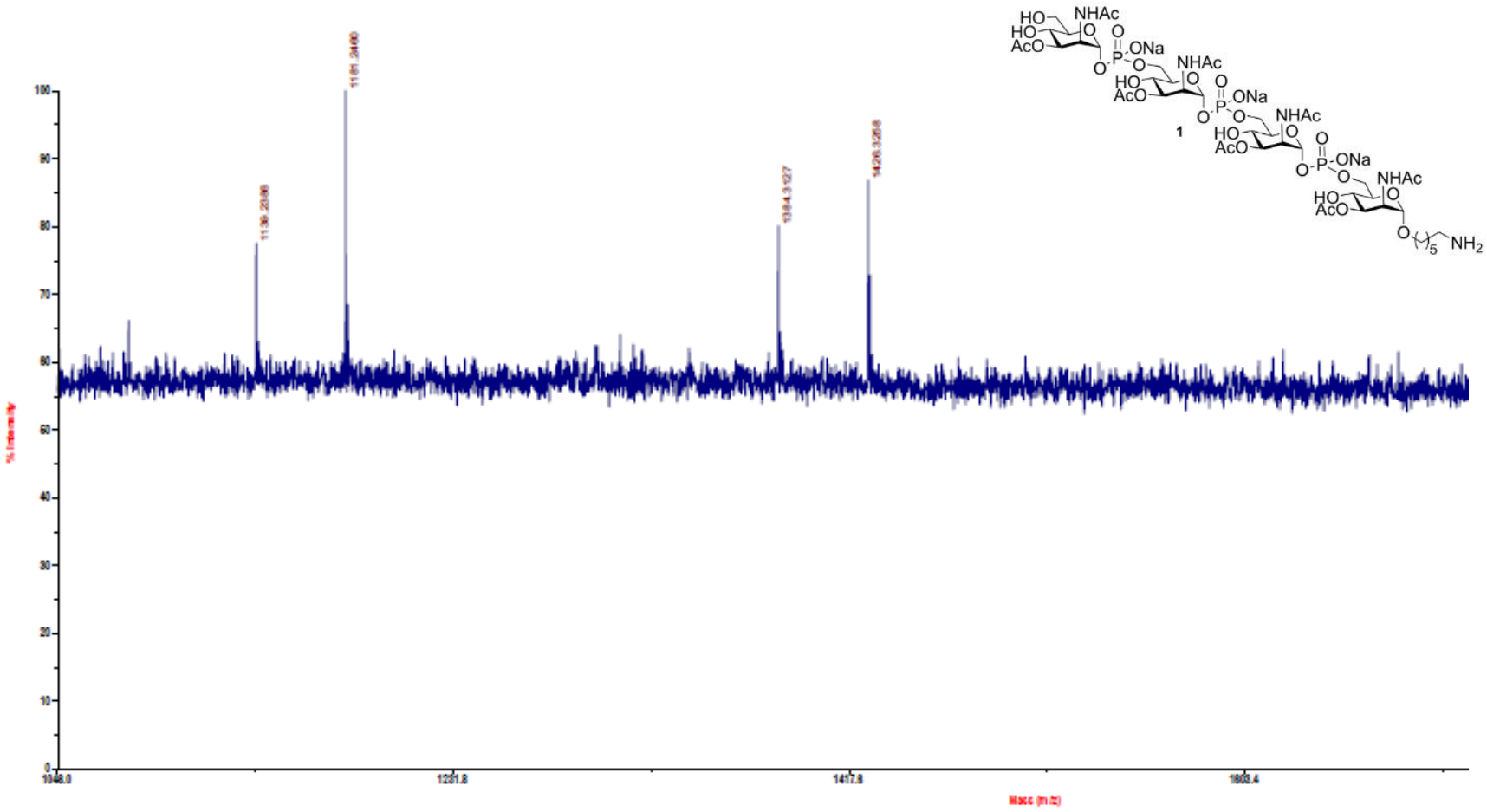
2D HSQC NMR of compound **1** (D_2O, 500 MHz)



^{31}P NMR of compound **1** (D_2O, 202 MHz)



MALDI-TOF MS spectra of compound **1**



Preparation of the compound 1 (MenA tetramer) and Compound 1-Tetanus toxoid (MenA tetramer-TT) conjugate: To a solution of compound **1** (18 mg, 14.56 mmol) in 0.1 M HEPES buffer containing 0.15 M NaCl, 10 mM EDTA, pH 7.5, was added a solution of *S*-acetylthioglycolic acid *N*-hydroxysuccinimide ester (SATA) (10 mg, 43.7 mmol) in dimethyl sulfoxide (218 µl) and the reaction mixture was stirred for 1 hour at room temperature. The resulting solution was purified by Sephadex G-10 chromatography to remove excess SATA. The resulting solution was reacted with hydroxylamine hydrochloride (35 mg, 35 equiv.) and solution was stirred at room temperature for 2 hours and stored at – 20 °C (13 mg, 70%) and used within 48 hours.

A solution of Tetanus toxoid (20 mg/mL) in 0.1 M HEPES, pH 7.6 was added to a solution of 3-(maleimido) propionic acid *N*-hydroxysuccinimide ester (7.2 mg) in 1-Methyl-2-pyrrolidinone (135 µl) and the reaction mixture was stirred for 2 hour at room temperature. The resulting solution was diafiltered against 0.1 M PBS containing 0.15 M NaCl, 5 mM EDTA, pH 6.8 through 50 kD cutoff membrane leading to a recovery of 16 mg (80%) modified TT.

A solution of thiolated oligosaccharide (13 mg) in buffer containing 0.1 M HEPES, 0.15 M NaCl, 10 mM EDTA, pH 7.5, was mixed with a solution of maleimide linked tetanus toxoid (10 mg) in 0.1 M PBS, 0.15 M NaCl, 5 mM EDTA, pH 6.8. The resulting solution was gently stirred overnight at 4 °C. At the end of the reaction, solution was diafiltered against buffer containing 0.1 M MES, 0.2 M NaCl, pH 6.5 through 50 kD cutoff membrane to achieve purified compound **1**-TT conjugate (5.5 mg conjugated compound **1** in the compound **1**-TT conjugate).