Supplementary information for:

Synthesis of the *O*-linked hexasaccharide containing β-D-Gal*f*-(1→2)-β-D-Gal*f* in *Trypanosoma cruzi* mucins

*Gustavo A. Kashiwagi, Verónica M. Mendoza, Rosa M. de Lederkremer and Carola Gallo-Rodriguez**

CIHIDECAR, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 1428 Buenos Aires, Argentina

cgallo@qo.fcen.uba.ar.

Table of Contents

Additional Experimental Procedures

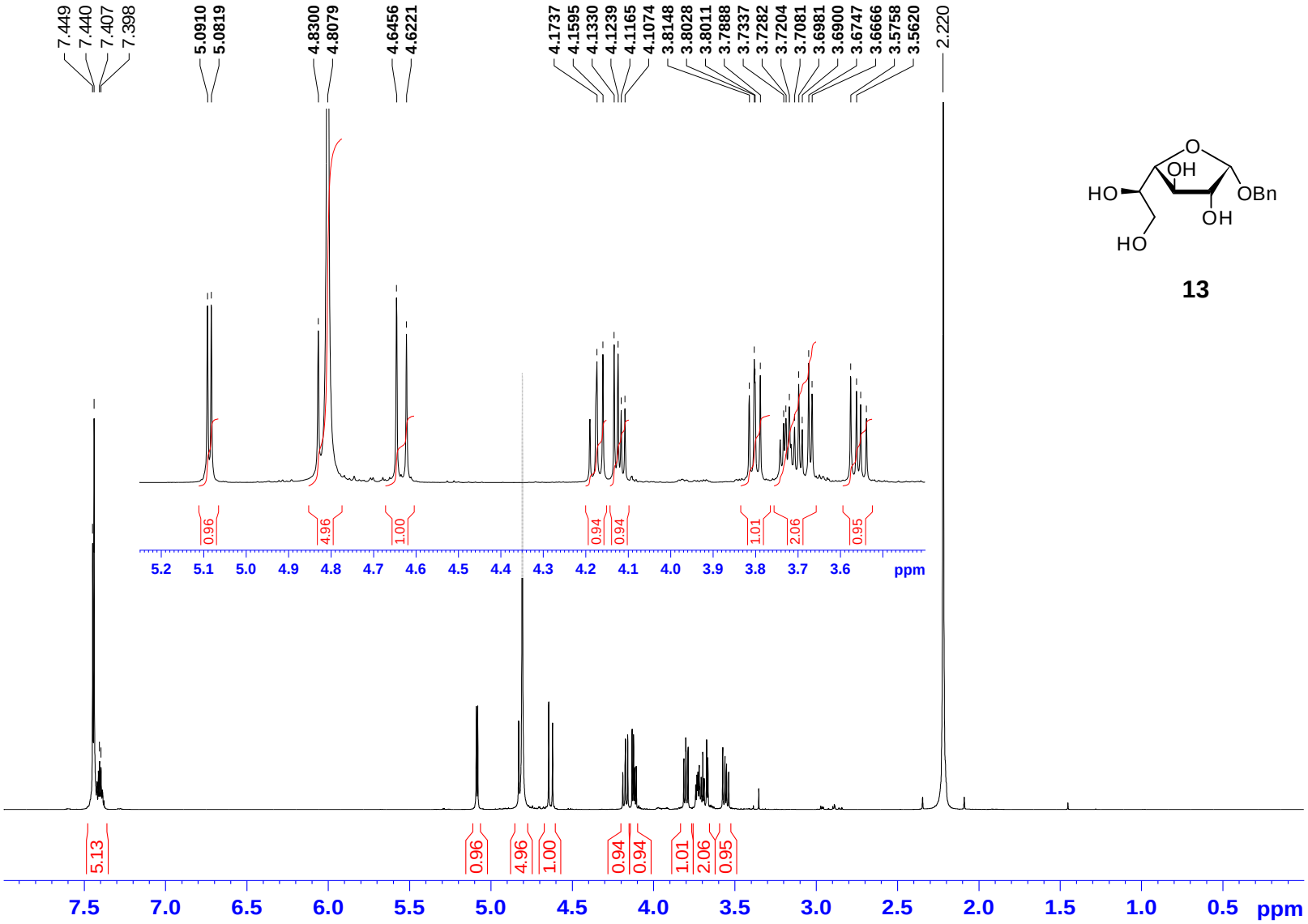
Benzyl 2-*O*-levulinoyl-5,6-*O*-isopropylidene-α-D-galactofuranoside (15). To a cooled solution (-18 ºC) of **14** (2.23 g, 6.85 mmol) and 4-(dimethylamino)pyridine (262 mg, 2.14 mmol) in anh CH_2Cl_2 (24 mL), levulinic acid (562 μL, 5.49 mmol, 0.80 equiv) was added with vigorous stirring under argon atmosphere, followed by dicyclohexylcarbodiimide (1.13 g, 5.48 mmol, 0.80 equiv). After 20 h at -20 ºC, TLC monitoring of the mixture showed that the reaction reached to a standstill. The mixture was filtered off and the filtrate was diluted with CH_2Cl_2 (150 mL), washed with 10 % $NaHCO_3$ (2 x 100 mL), water (2 x 100 mL), dried and concentrated. Purification of the residue by silica gel column chromatography (120:1 CH_2Cl_2-CH_3OH) gave a first fraction of 2,3-di-*O*-levulinoyl-5,6-*O*-isopropylidene-α-D-galactofuranoside (**16**, 208 mg, 6%): R_f 0.75 (20:1 CH_2Cl_2-CH_3OH), $[\alpha]_D$ +50.7º (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.45 - 7.30 (m, 5H, arom.), 5.47 (dd, 1H, *J* = 6.8, 5.7 Hz, H-3). 5.32 (d, 1H, *J* = 4.4 Hz, H-1), 5.07 (dd, 1H, *J* = 6.8, 4.4 Hz, H-2), 4.85, 4.50 (2d, 2H, *J* = 11.7 Hz, C*H*$_2$Ph), 4.26 (dt, 1H, *J* = 8.1, 6.7 Hz, H-5), 4.01 (dd, 1H, *J* = 8.5, 6.6 Hz, H-6a), 3.97 (dd, 1H, *J* = 8.1, 5.7 Hz, H-4), 3.67 (dd, 1H, *J* = 8.5, 7.1 Hz, H-6b), 2.82-2.54 (m, 8H, (C*H*$_2$)$_2$), 2.18-2.14 (2s, 6H, C*H*$_3$CO), 1.44, 1.37 (2s, 6H, (C*H*$_3$)$_2$C)). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 206.0, 204.4 (CH_3*C*O), 172.0, 171.8 (*C*OO), 137.1, 128.3, 127.8 (C-arom.), 109.8 ((CH$_3$)$_2$*C*), 99.4 (C-1), 81.1 (C-4), 77.5, 77.3, 74.9 (C-2, C-5, C-3), 69.8 (*C*H$_2$Ph), 65.5 (C-6), 37.8, 37.7, 27.9, 27.7 ((*C*H$_2$)$_2$), 29.72, 29.67 (*C*H$_3$CO), 26.6, 25.4 ((*C*H$_3$)$_2$C); HRMS (ESI/APCI) m/z calcd for $C_{26}H_{34}NaO_{10}$ $(M+Na)^+$: 529.2044. Found: 529.2051.

A second fraction from the column gave 1.06 g of **15** (38 %): mp 136 ºC (1:1 hexane-EtOAc); R_f 0.40 (20:1 CH_2Cl_2-CH_3OH); $[\alpha]_D$ +70.2º (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.34 - 7.28 (m, 5H, arom.), 5.24 (d, 1H, *J* = 4.6 Hz, H-1), 4.87 (dd, 1H, *J* = 8.0, 4.6 Hz, H-2), 4.81, 4.51 (2d, 2H, *J* = 12.0 Hz,

C*H*$_2$Ph), 4.33 (dt , 1H, *J* = 7.5, 3.5 Hz, H-3), 4.25 (apparent q, 1H, *J* = 7.0 Hz, H-5), 4.03 (dd, 1H, *J* = 8.5, 6.5 Hz, H-6a), 3.93 (t, 1H, *J* = 7.0 Hz, H-4), 3.92 (dd, 1H, *J* = 8.5, 7.0 Hz, H-6b), 3.22 (d, 1H, *J* = 3.5 Hz , O*H*), 2.77-2.57 (m, 4H, (C*H*$_2$)$_2$), 2.15 (s, 3H, C*H*$_3$CO), 1.46, 1.38 (2s, 6H, (C*H*$_3$)$_2$C)). ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 206.5 (CH$_3$*C*O), 173.2 (*C*OO), 137.6, 128.4, 127.8 (C-arom.), 109.8 ((CH$_3$)$_2$*C*), 99.4 (C-1), 82.2 (C-4), 80.1 (C-2), 77.7 (C-5), 73.8 (C-3), 69.6 (*C*H$_2$Ph), 65.0 (C-6), 37.8, 27.7 ((*C*H$_2$)$_2$), 29.8 (*C*H$_3$CO), 26.6, 25.2 ((*C*H$_3$)$_2$C). Anal. Calcd for $C_{21}H_{28}O_8$: C 61.75, H 6.91. Found: C 61.87, H 6.68.

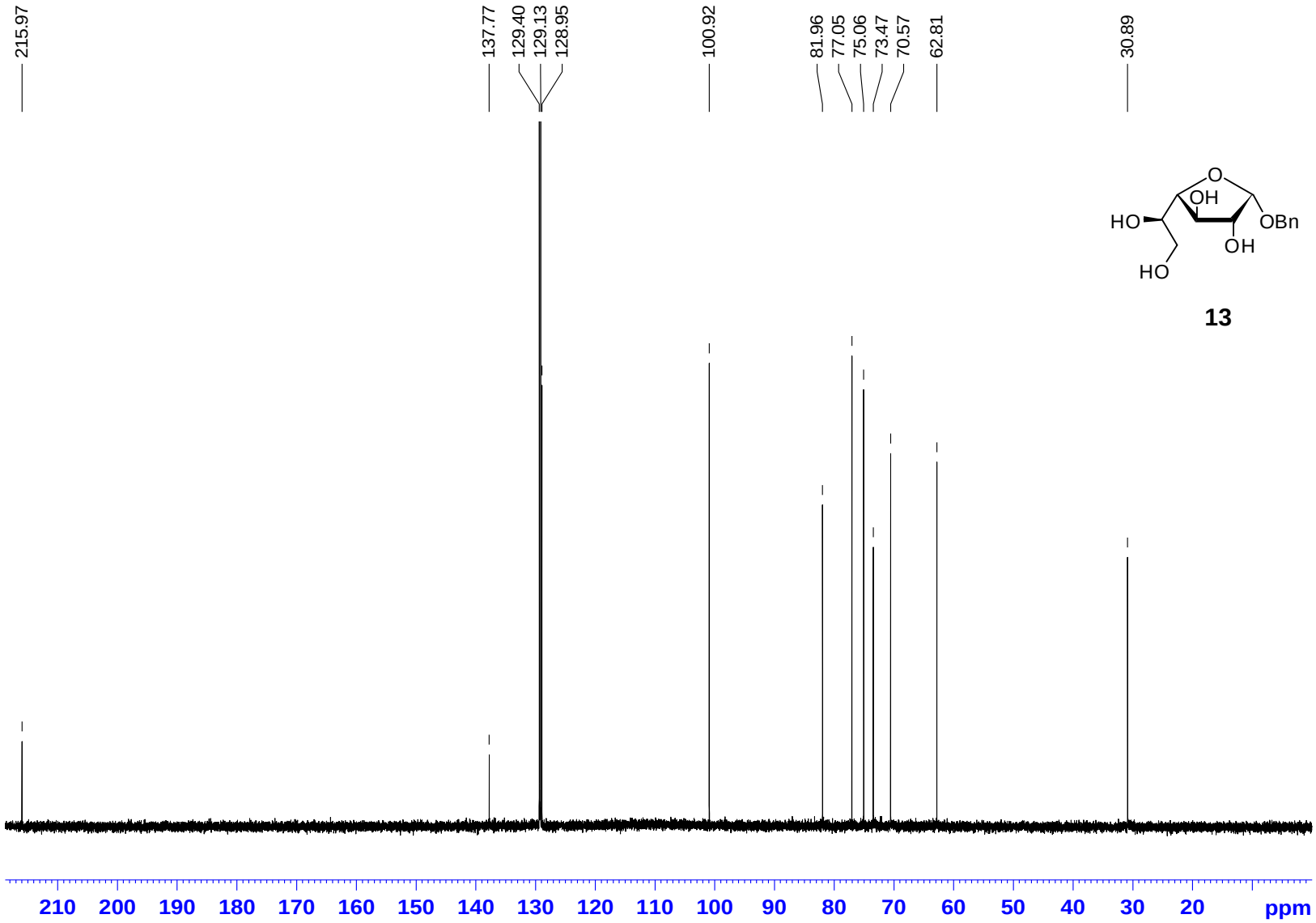
Unreacted **14** was also recovered (0.65g, 30.6%).

Benzyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl-(1→2)-3,5,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→4)-2-acetamido-3-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (8). A solution of 5% HF (48 wt % in H_2O) in acetonitrile (24 mL) was added to **24** (764 mg, 0.45 mmol) and the solution was stirred at 25 ºC for 18 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with H_2O (2 x 70 mL), 10% $NaHCO_3$ (2 x 70 mL) and H_2O (3 x 70 mL) until pH 7, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification by column chromatography of the residue (5:6 hexane–EtOAc) yielded **24** (576 mg, 88%). Physical properties, ^{1}H and ^{13}C-NMR spectra matched lit.[26]

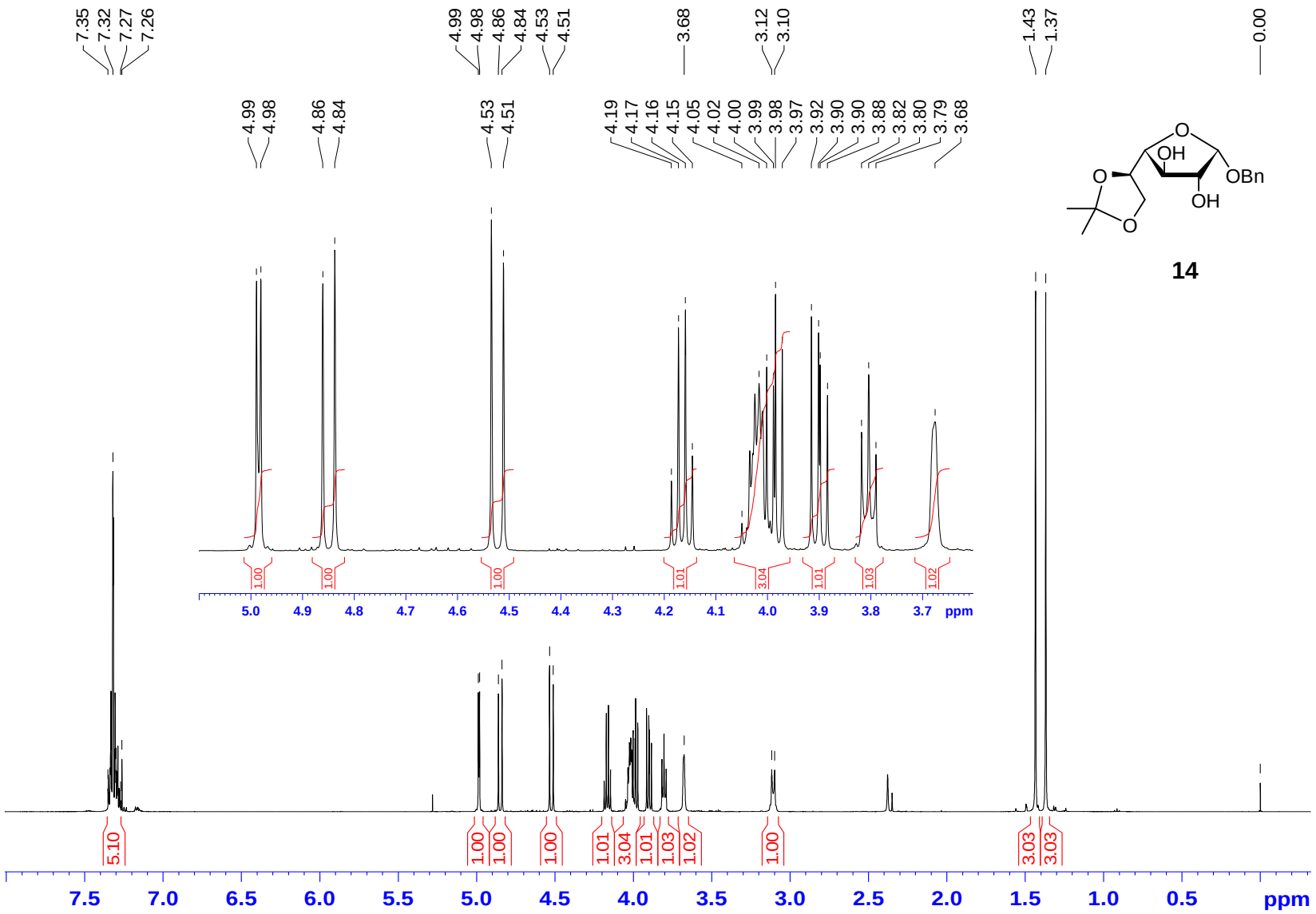


13

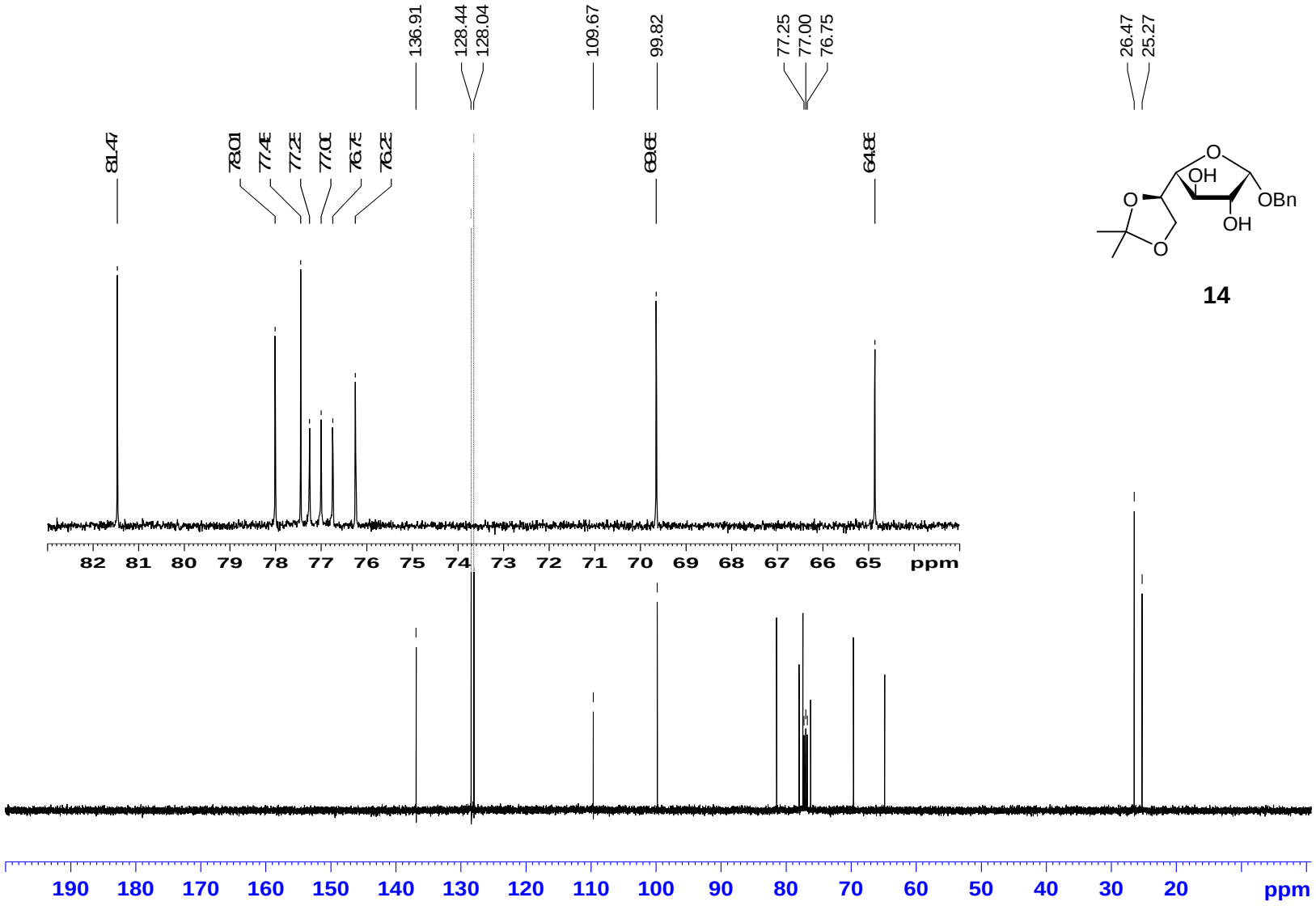
^{1}H NMR spectrum of compound **13** (D_2O, 500 MHz).



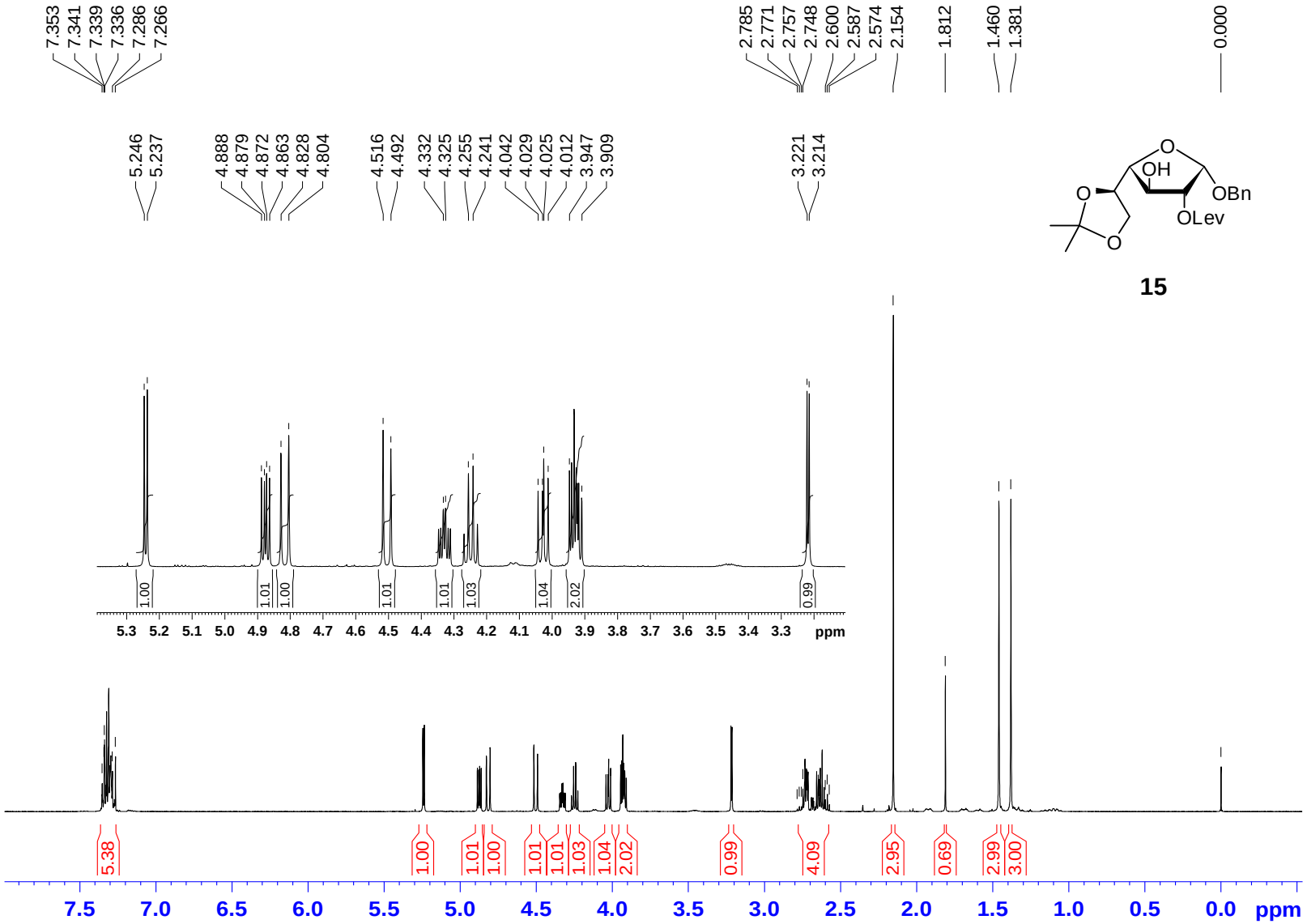
^{13}C NMR spectrum of compound **13** (D_2O, 125.8 MHz).



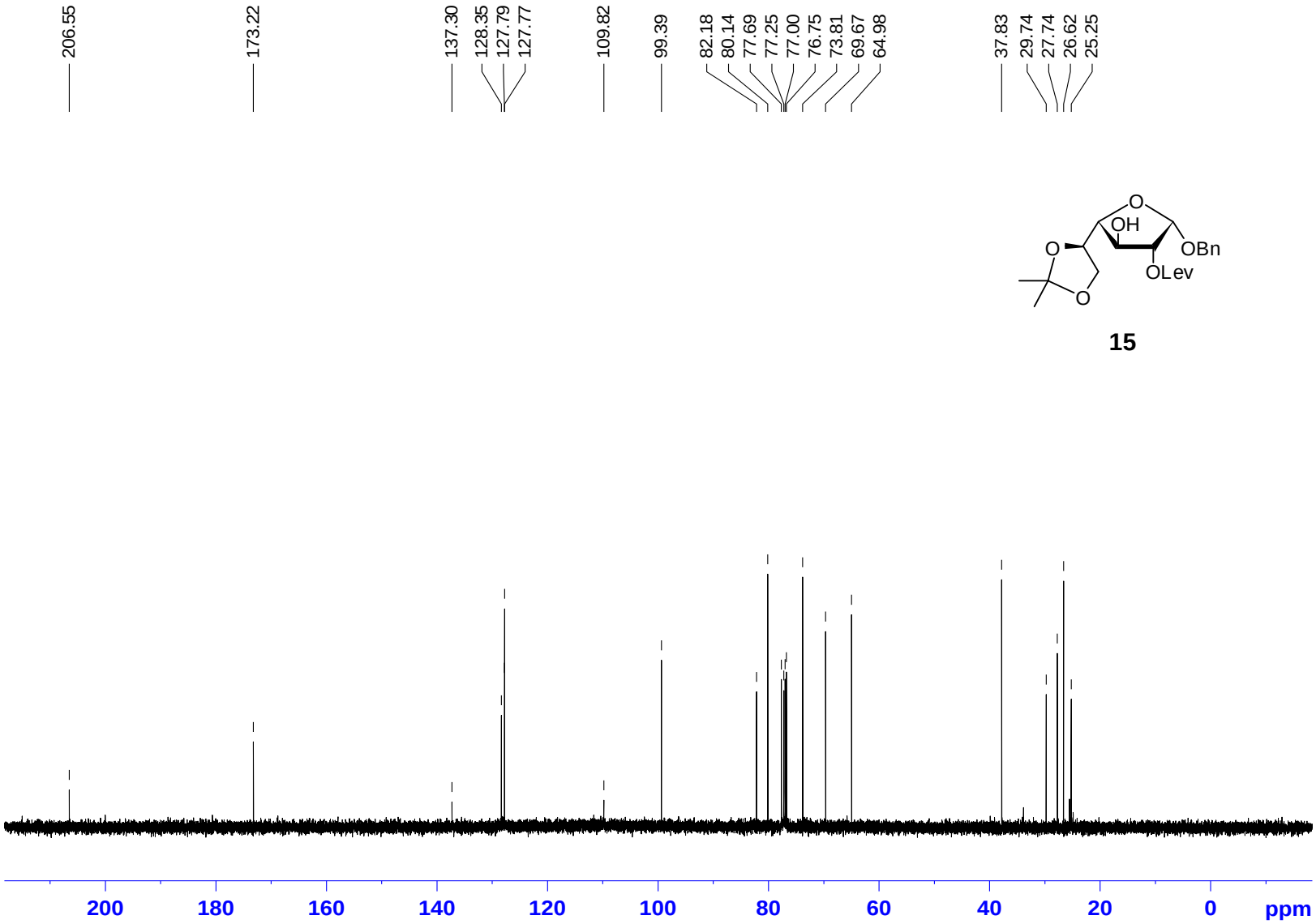
^{1}H NMR spectrum of compound **14** ($CDCl_3$, 500 MHz).



^{13}C NMR spectrum of compound **14** ($CDCl_3$, 125.8 MHz).

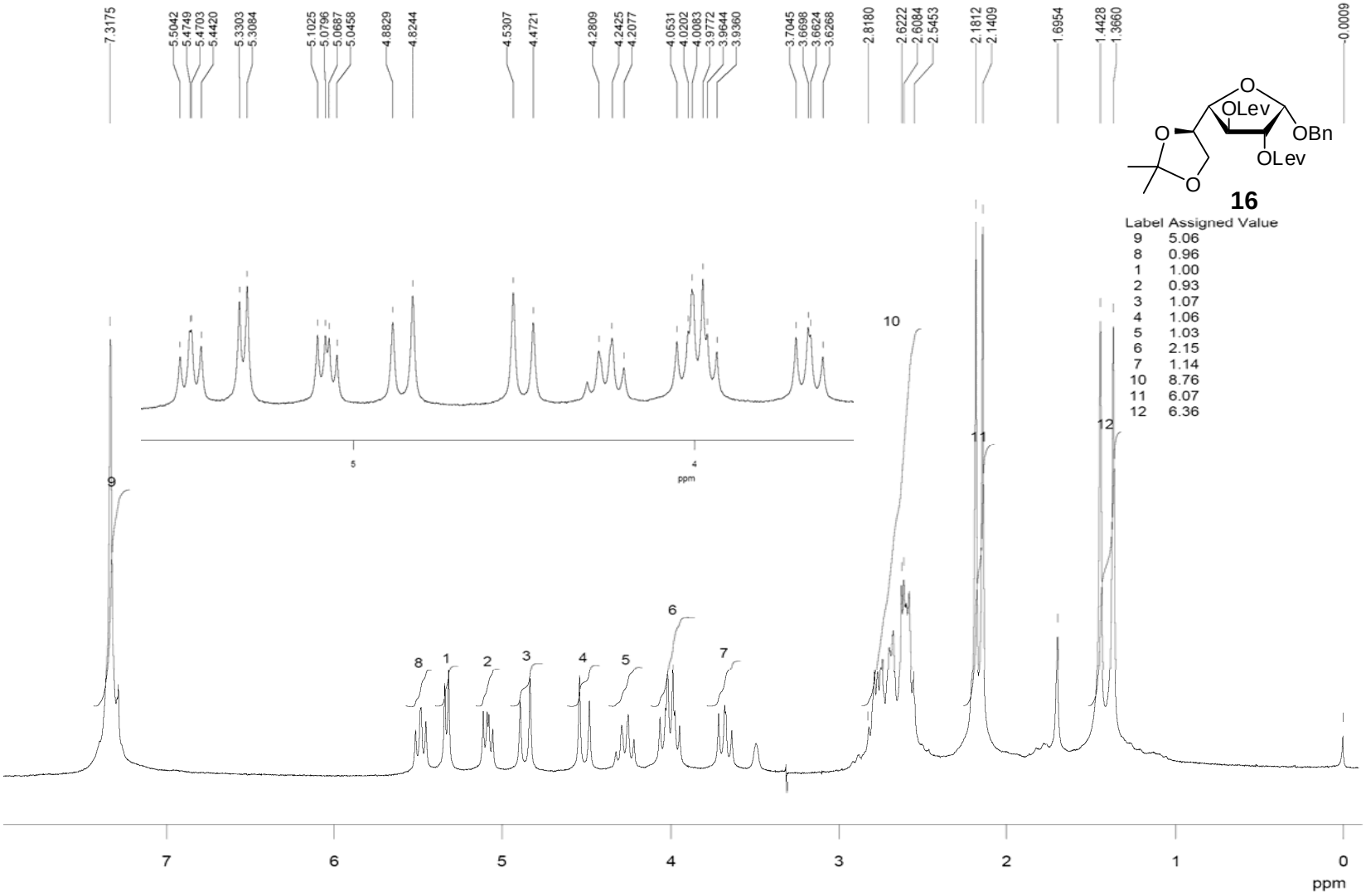


^{1}H NMR spectrum of compound **15** ($CDCl_3$, 500 MHz).

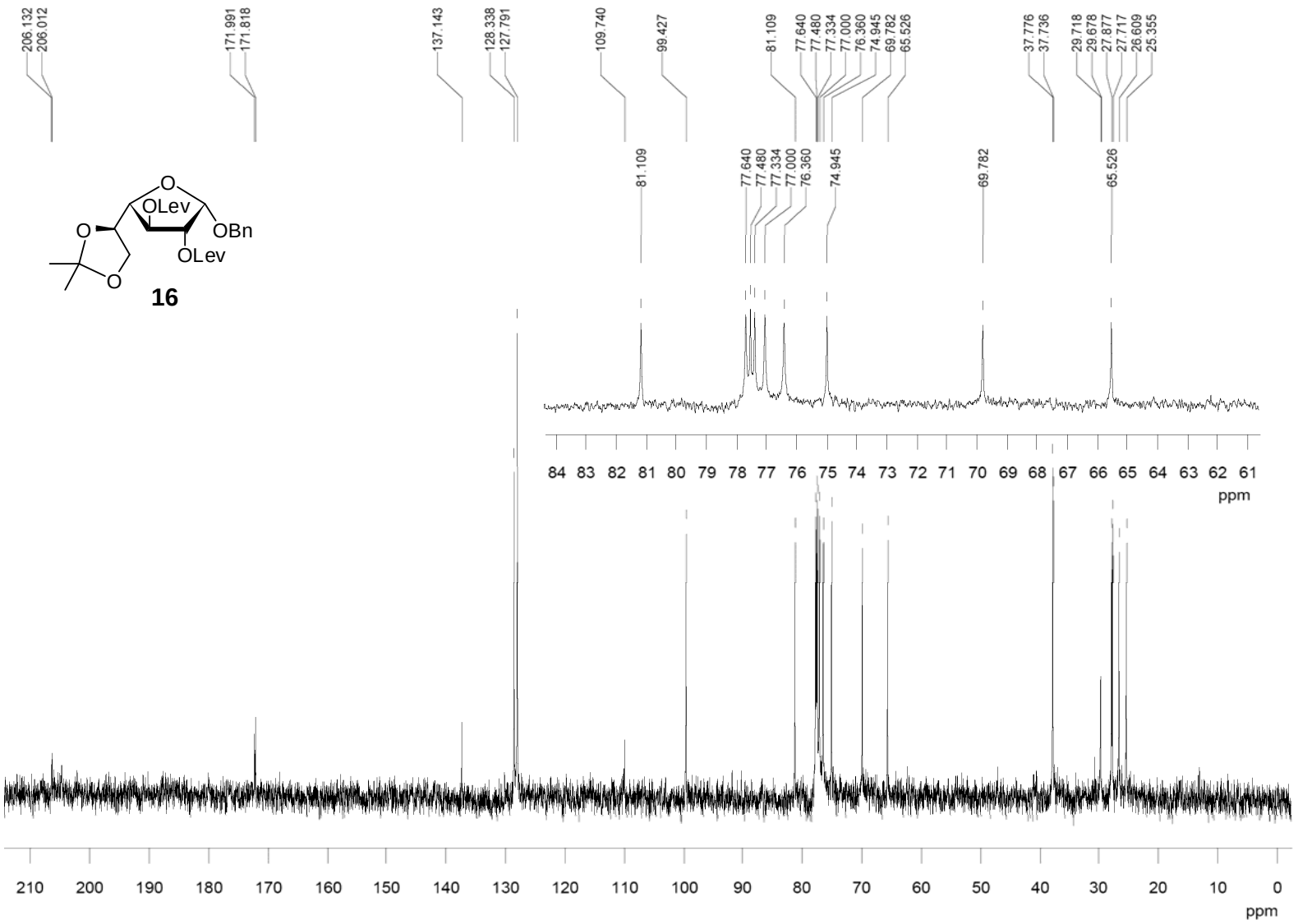


15

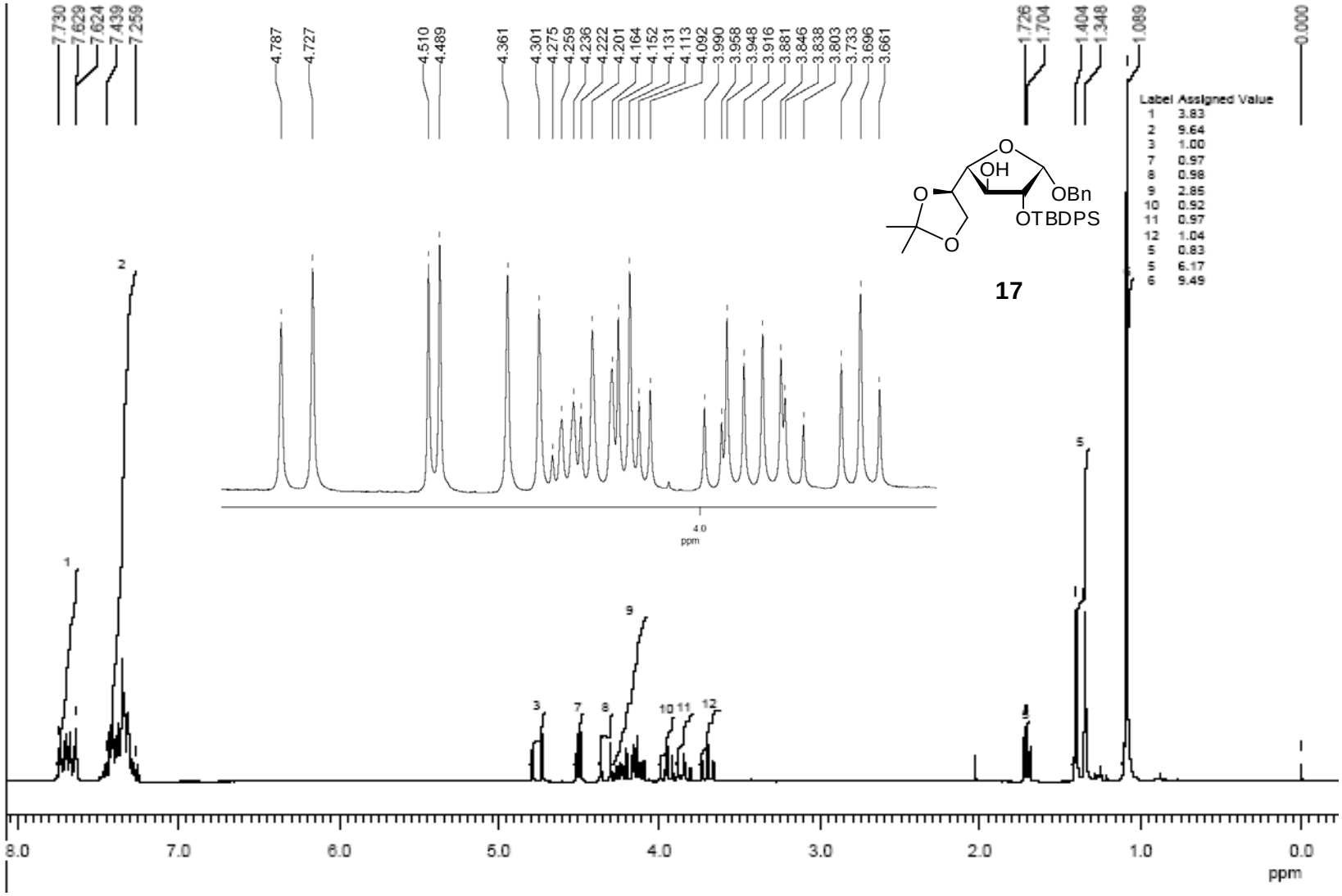
^{13}C NMR spectrum of compound **15** ($CDCl_3$, 125.8 MHz).



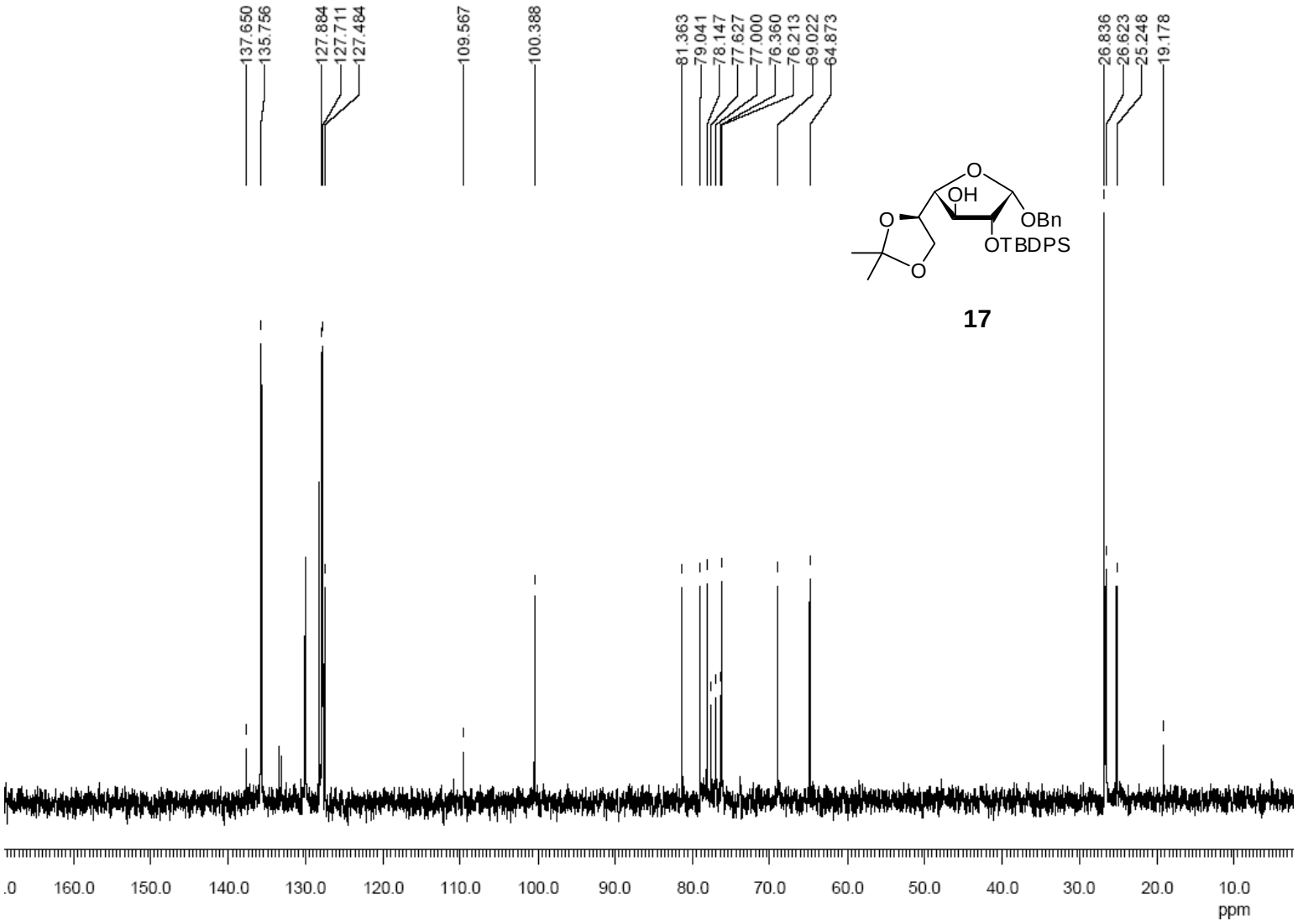
^{1}H NMR spectrum of compound **16** ($CDCl_3$, 200 MHz).



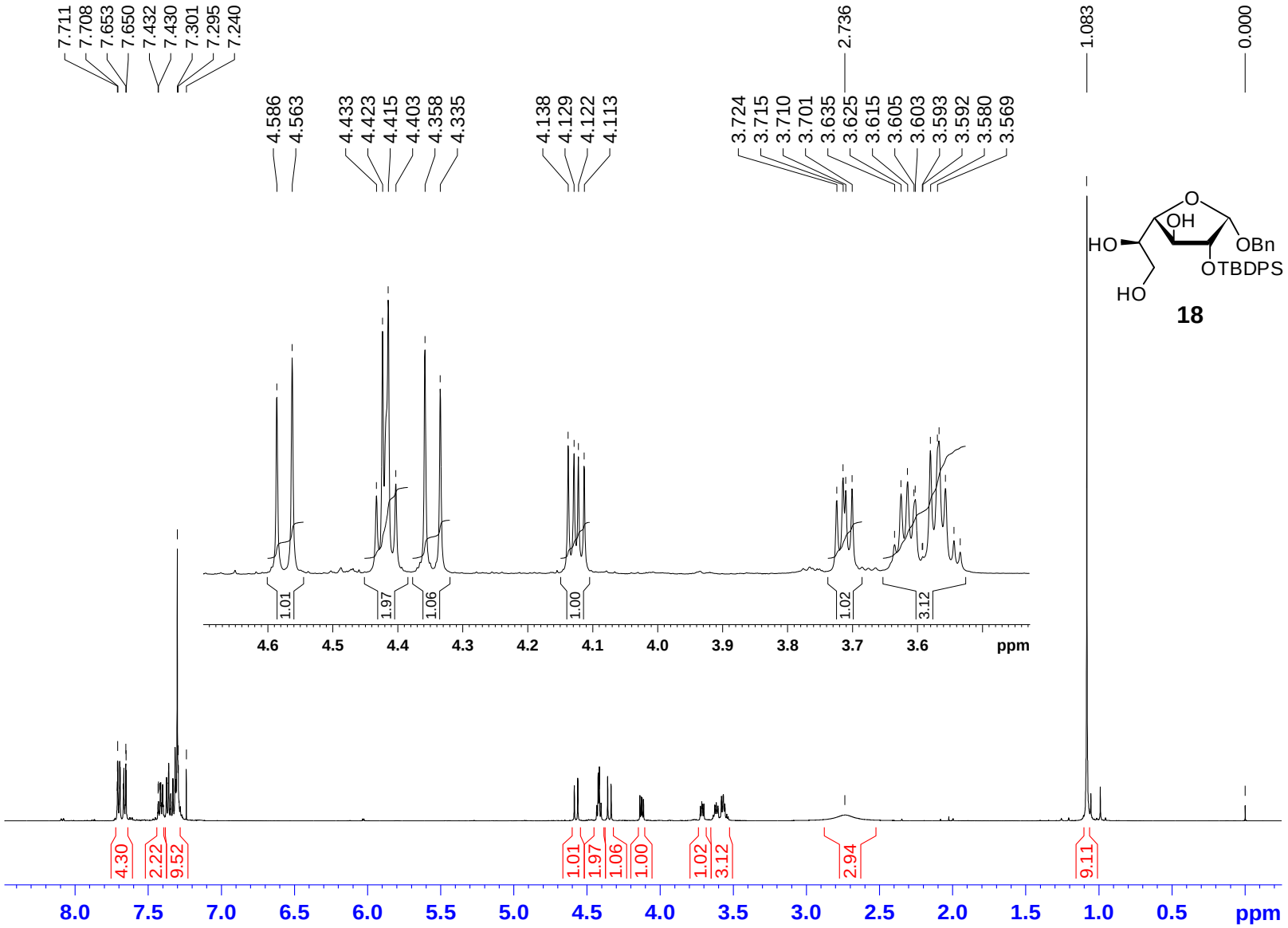
^{13}C NMR spectrum of compound **16** ($CDCl_3$, 50.3 MHz).



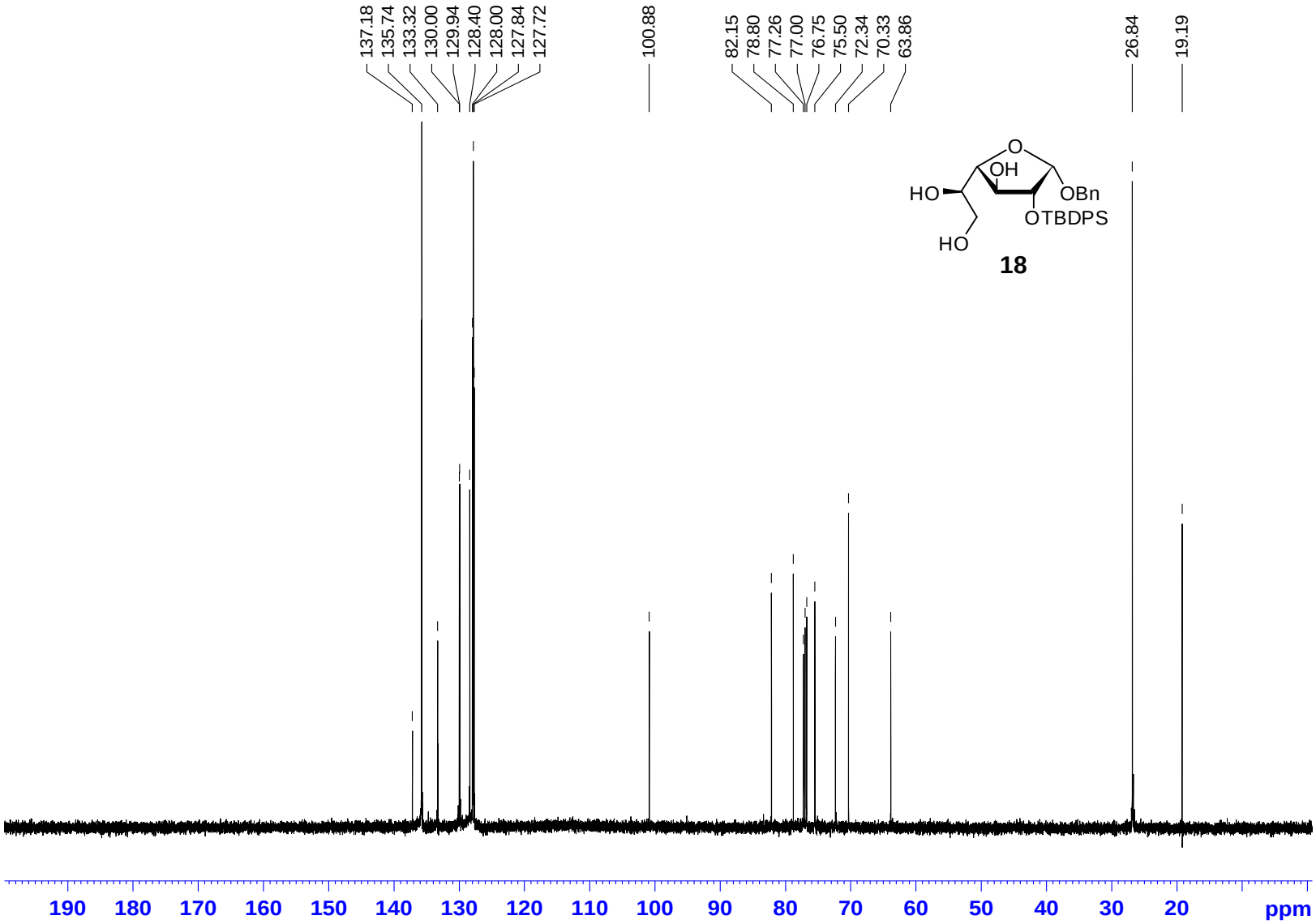
^{1}H NMR spectrum of compound **17** ($CDCl_3$, 200 MHz).



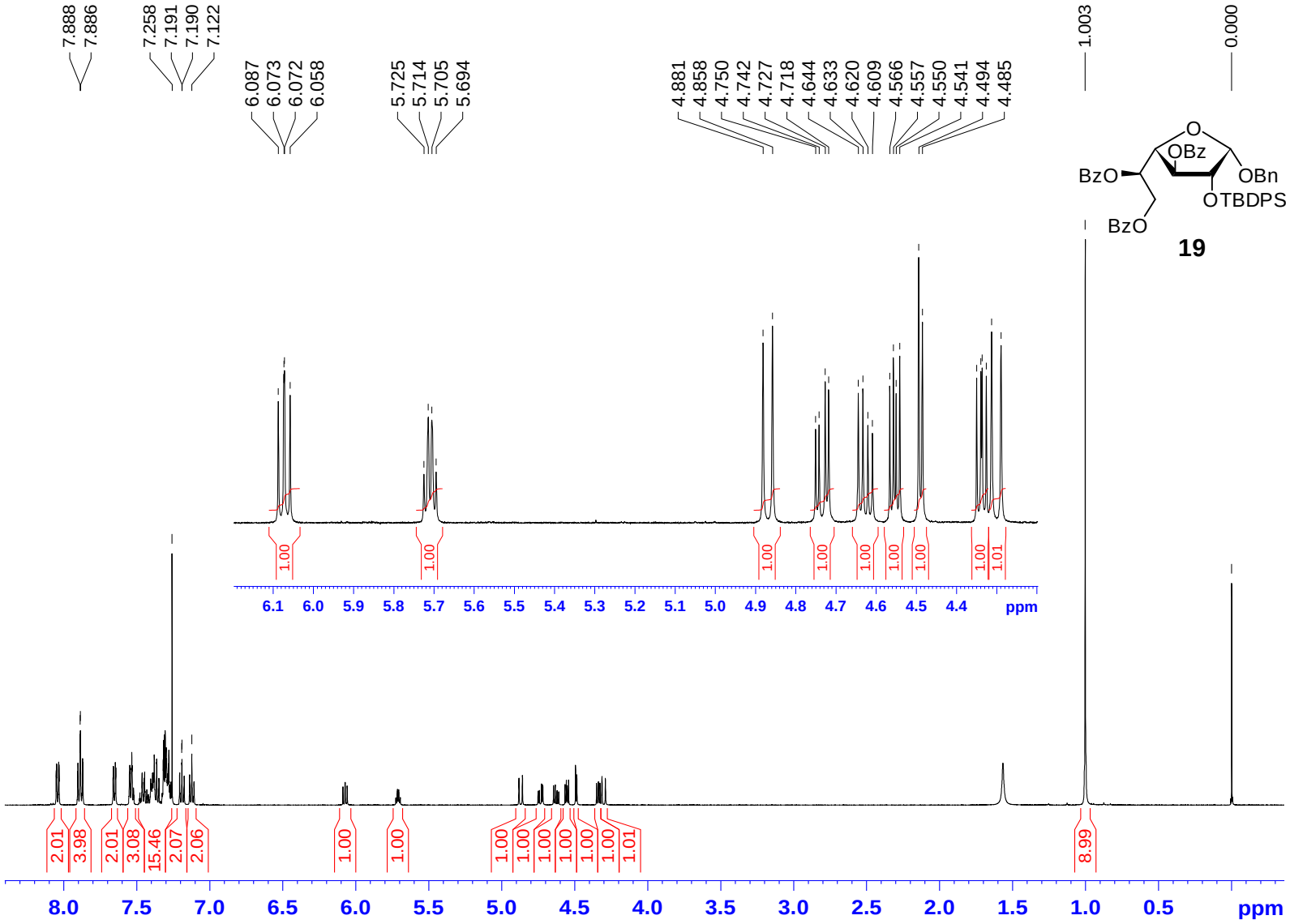
^{13}C NMR spectrum of compound **17** ($CDCl_3$, 50.3 MHz).



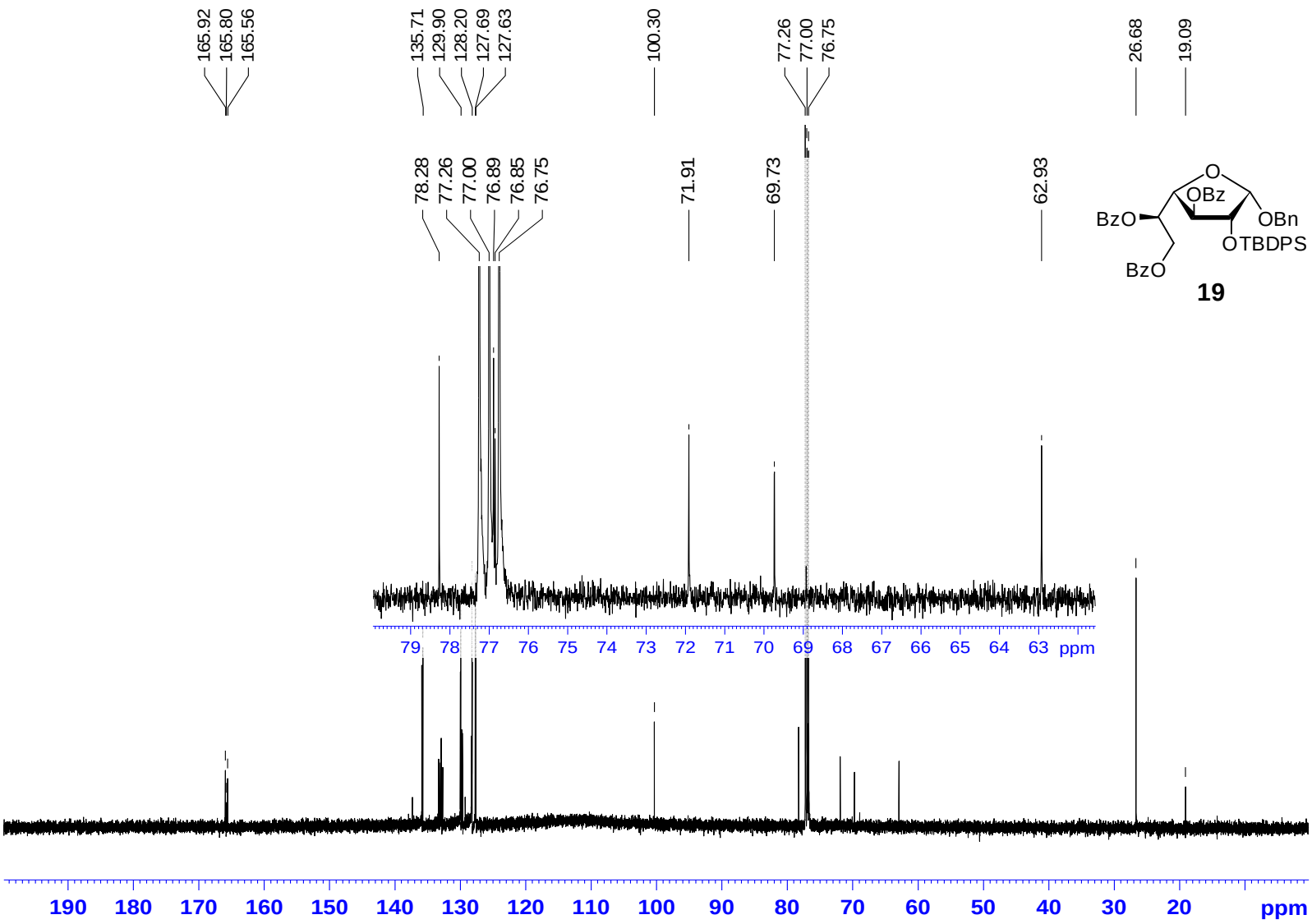
^{1}H NMR spectrum of compound **18** (D_2O, 500 MHz).



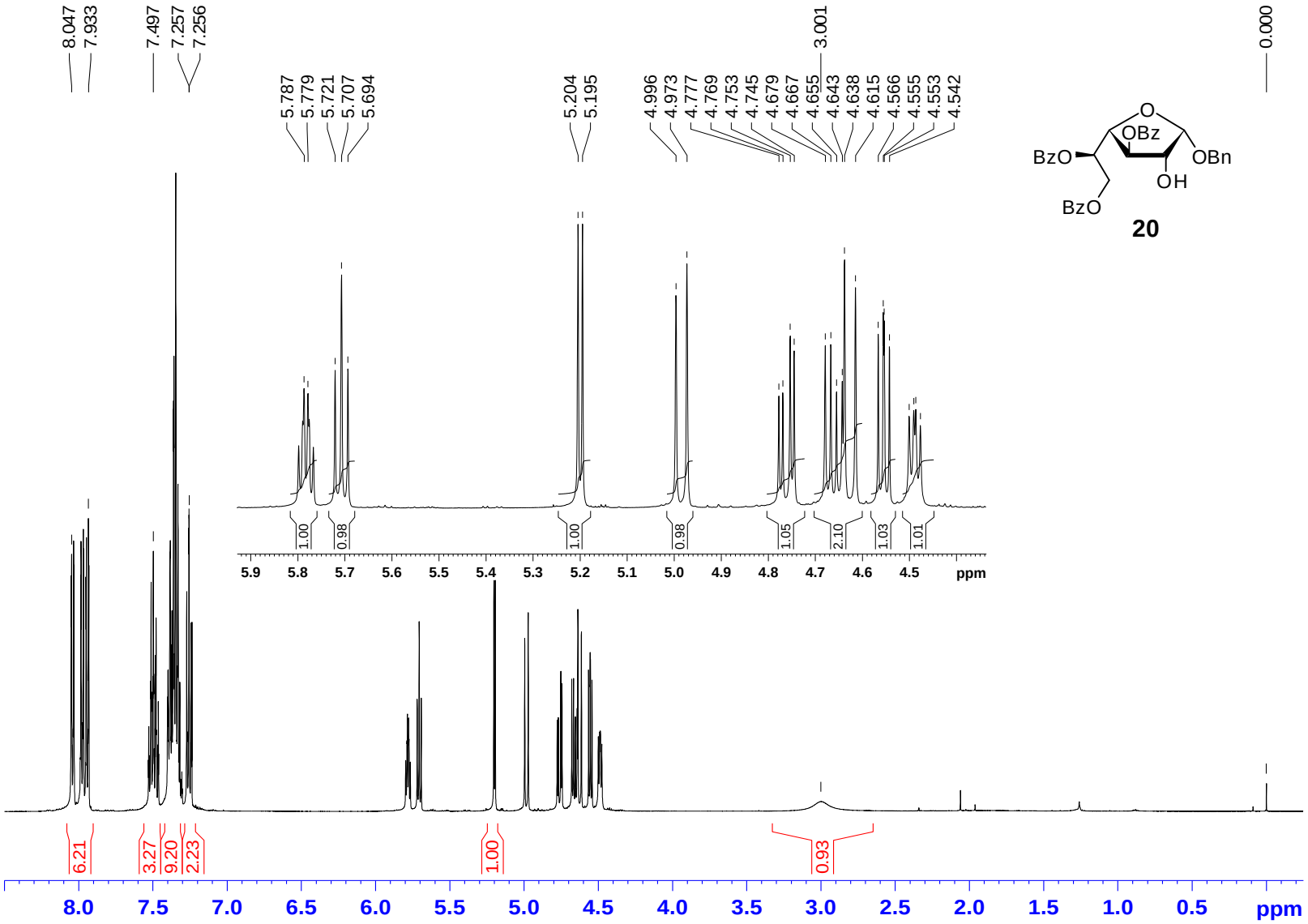
^{13}C NMR spectrum of compound **18** (D_2O, 125.8 MHz).



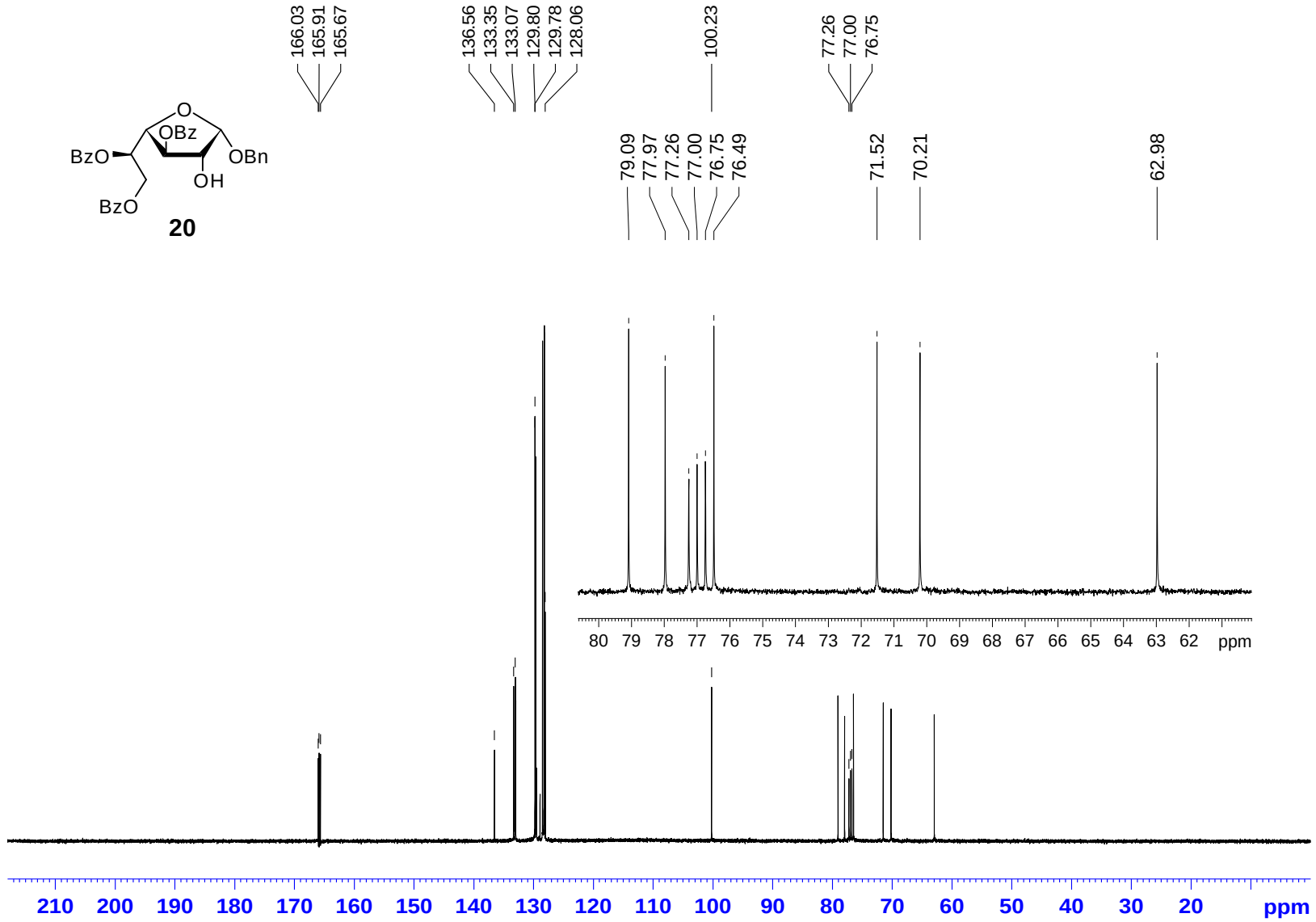
^{1}H NMR spectrum of compound **19** ($CDCl_3$, 500 MHz).



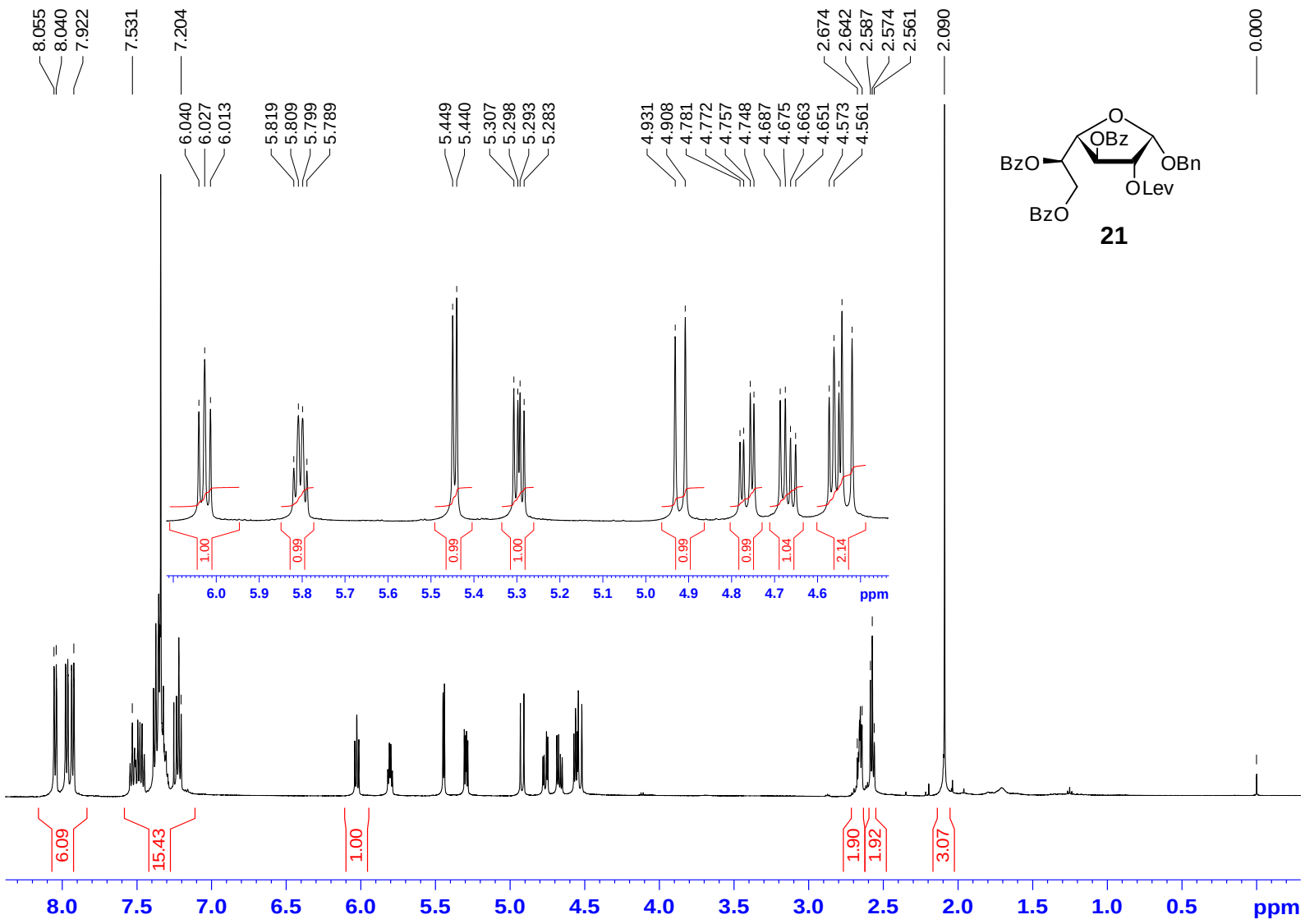
^{13}C NMR spectrum of compound **19** ($CDCl_3$, 125.8 MHz).



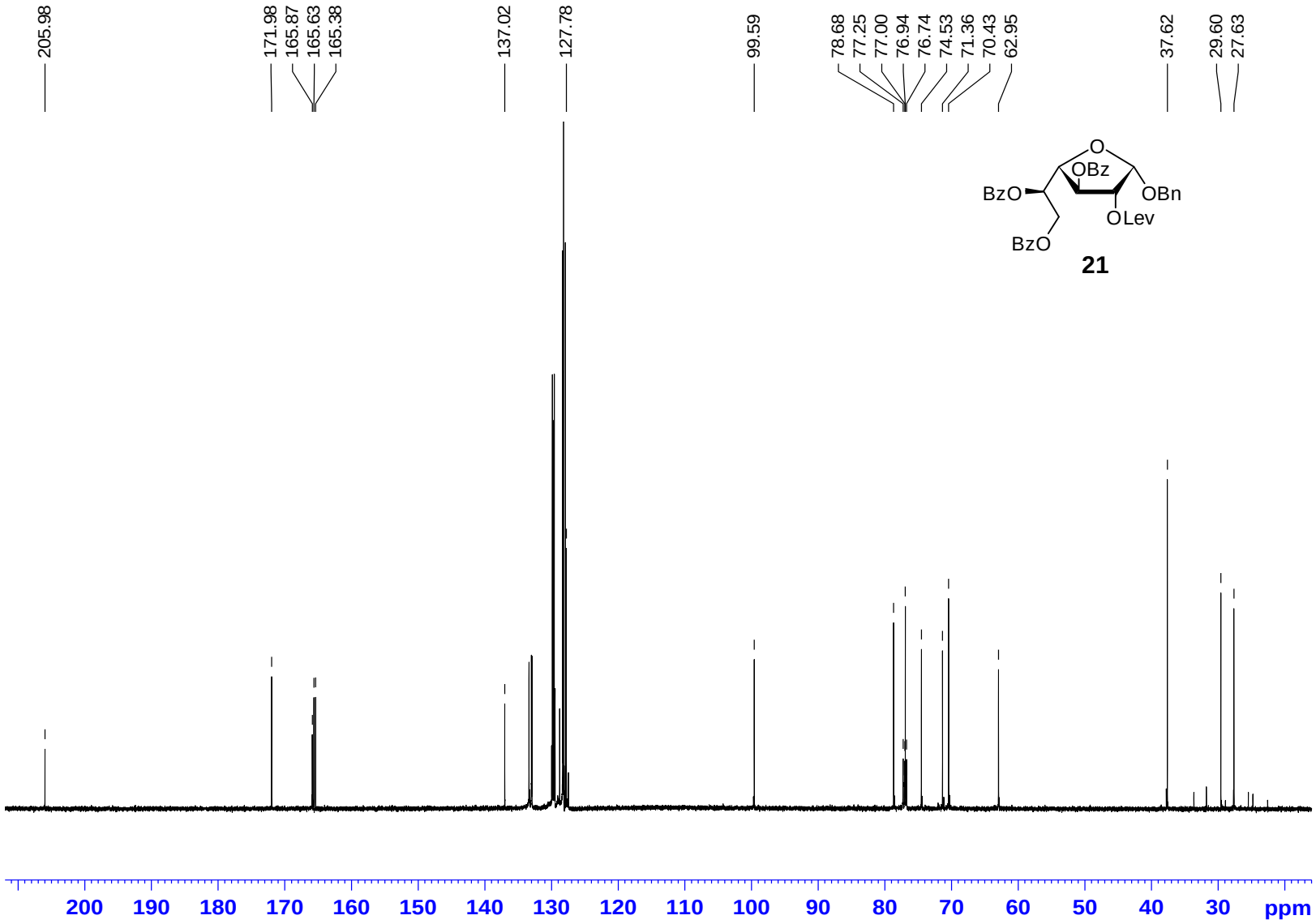
^{1}H NMR spectrum of compound **20** ($CDCl_3$, 500 MHz).



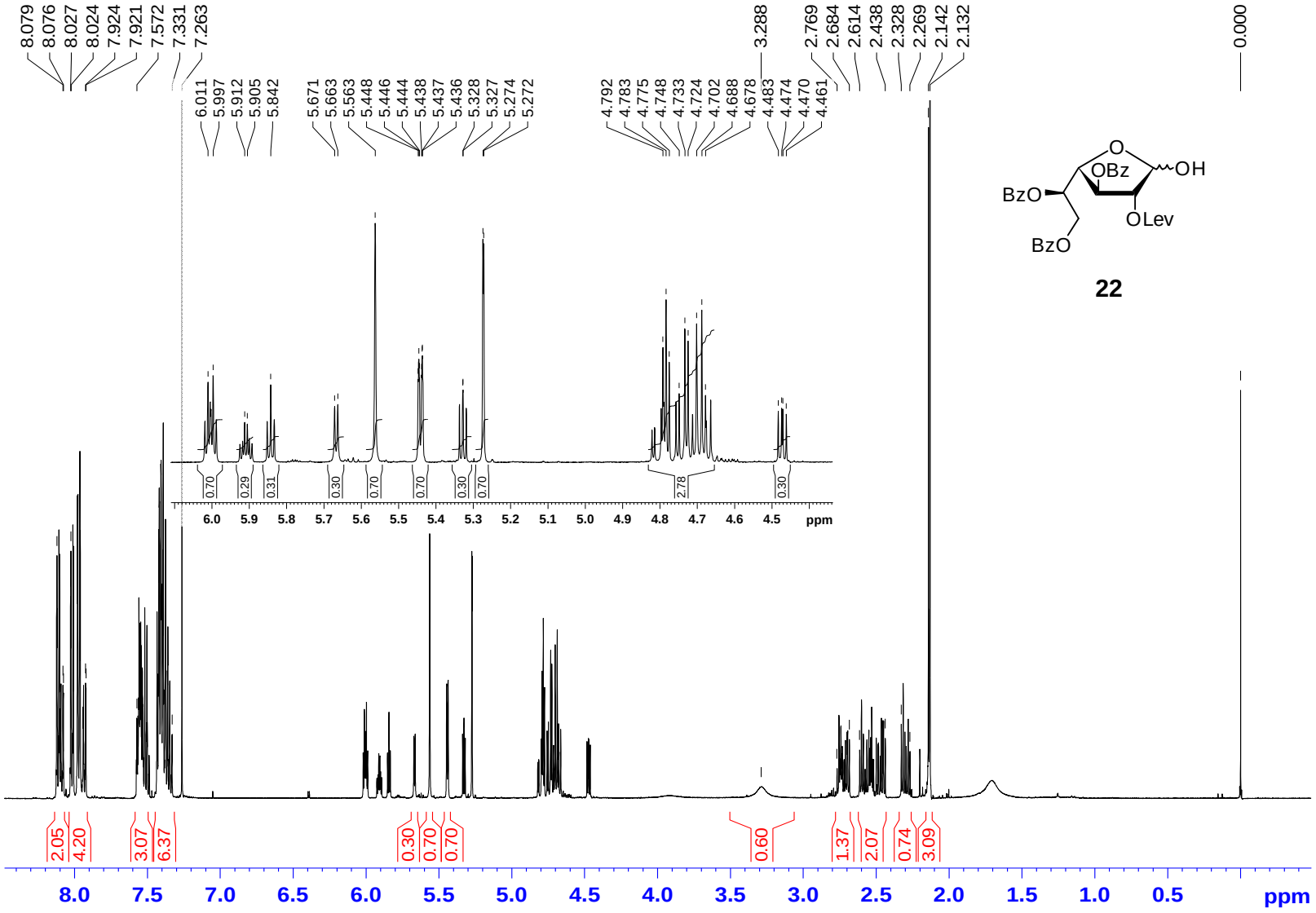
^{13}C NMR spectrum of compound **20** ($CDCl_3$, 125.8 MHz).



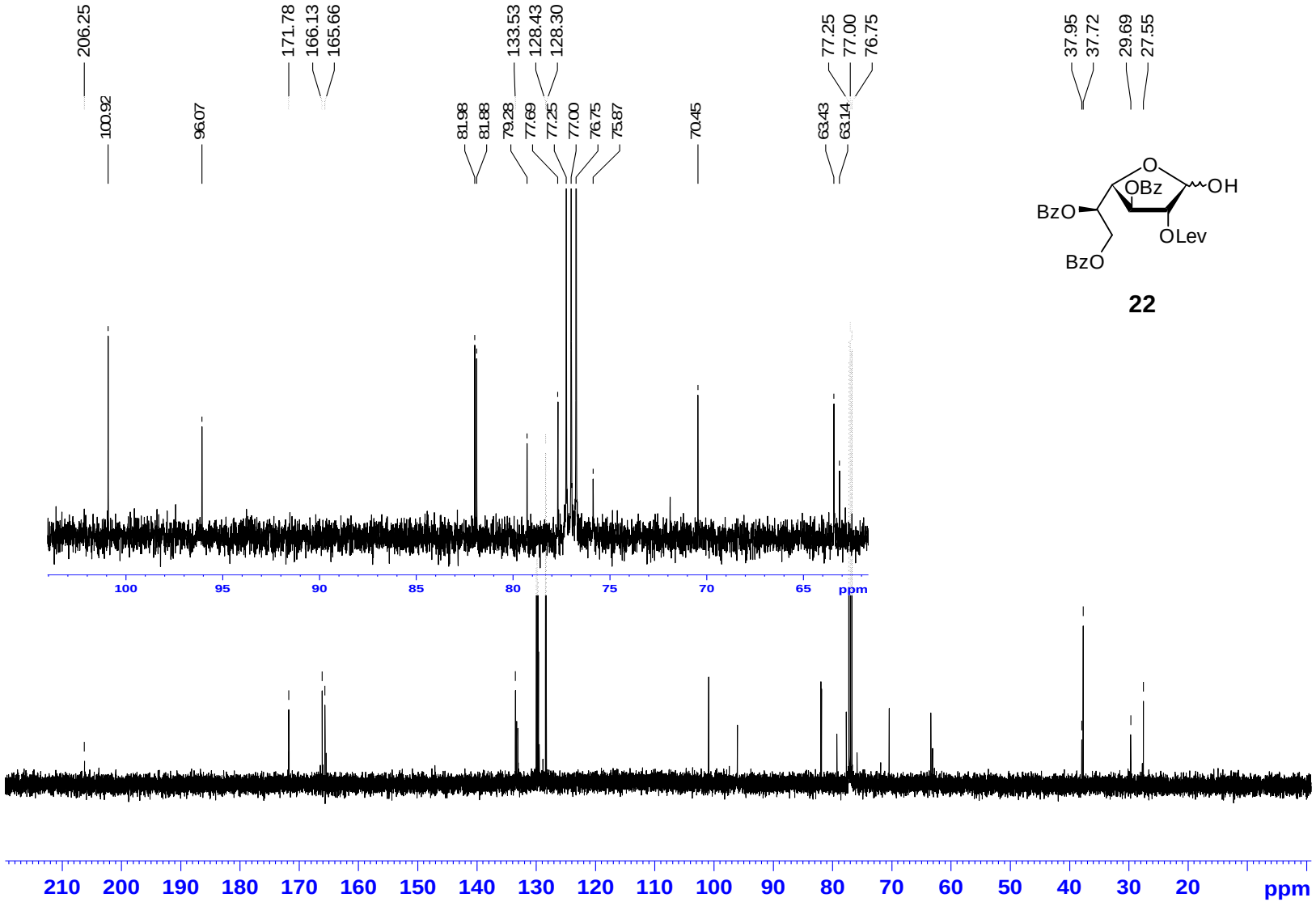
^{1}H NMR spectrum of compound **21** ($CDCl_3$, 500 MHz).



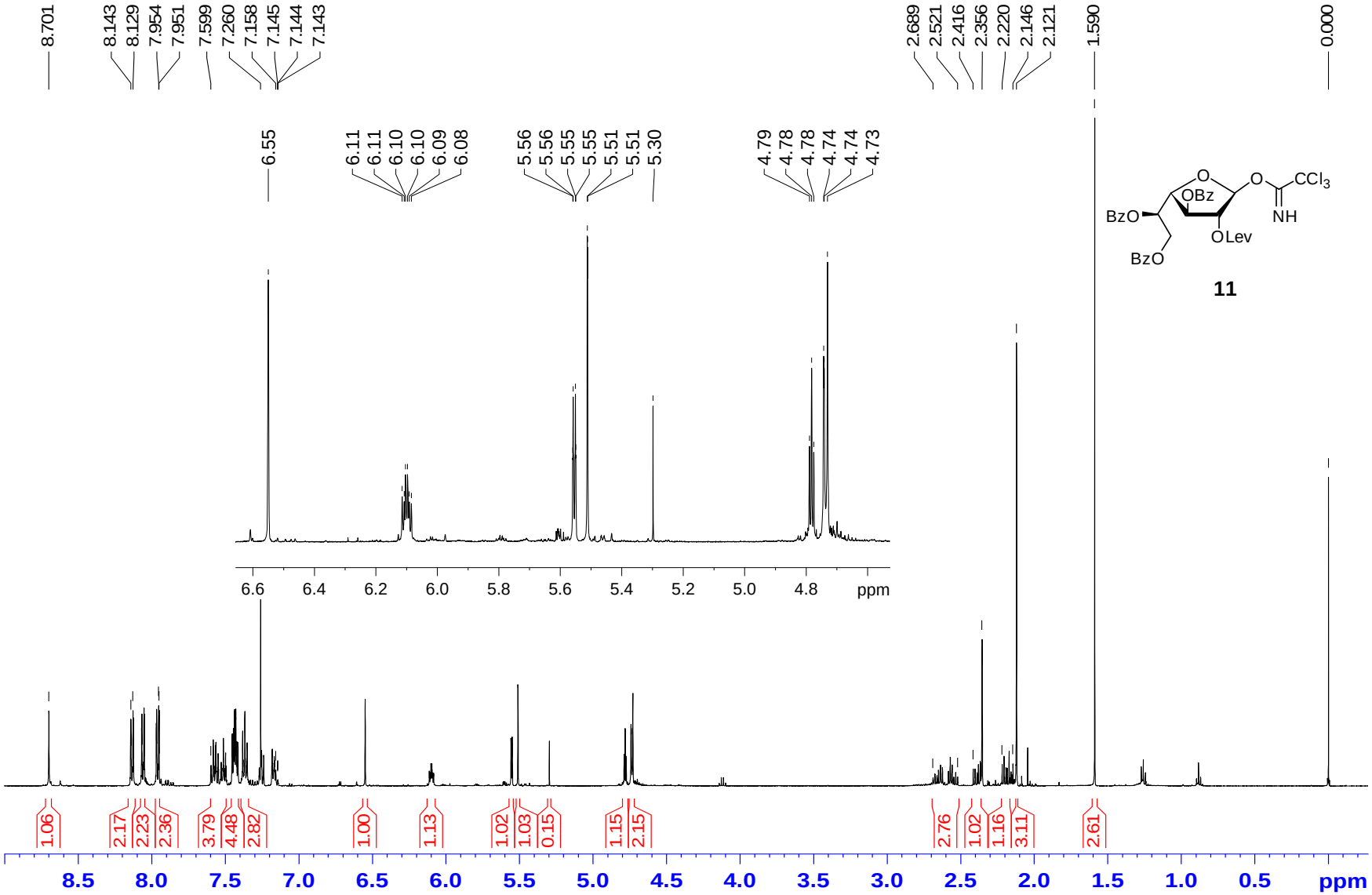
^{13}C NMR spectrum of compound **21** ($CDCl_3$, 125.8 MHz).



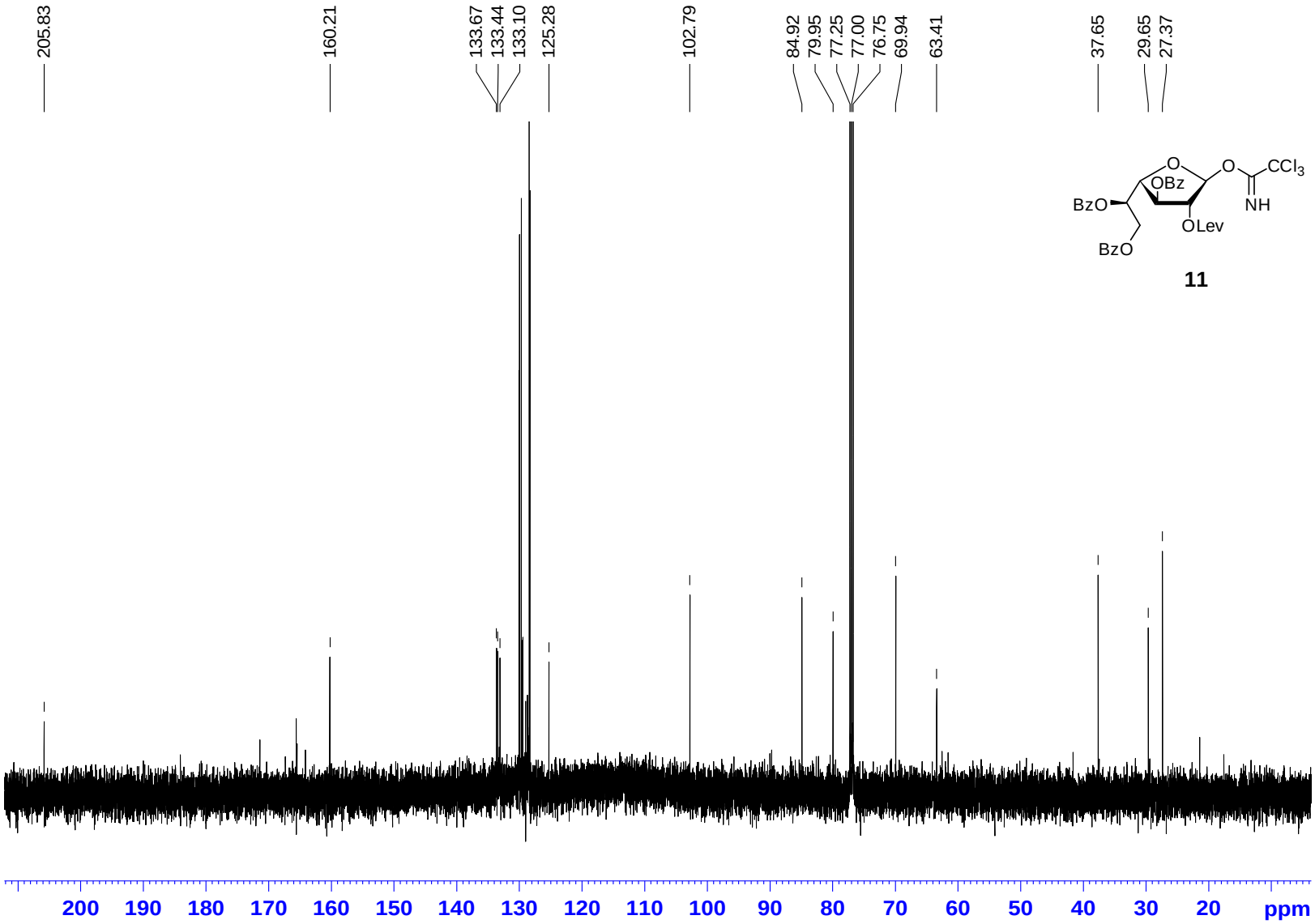
^{1}H NMR spectrum of compound **22** ($CDCl_3$, 500 MHz).



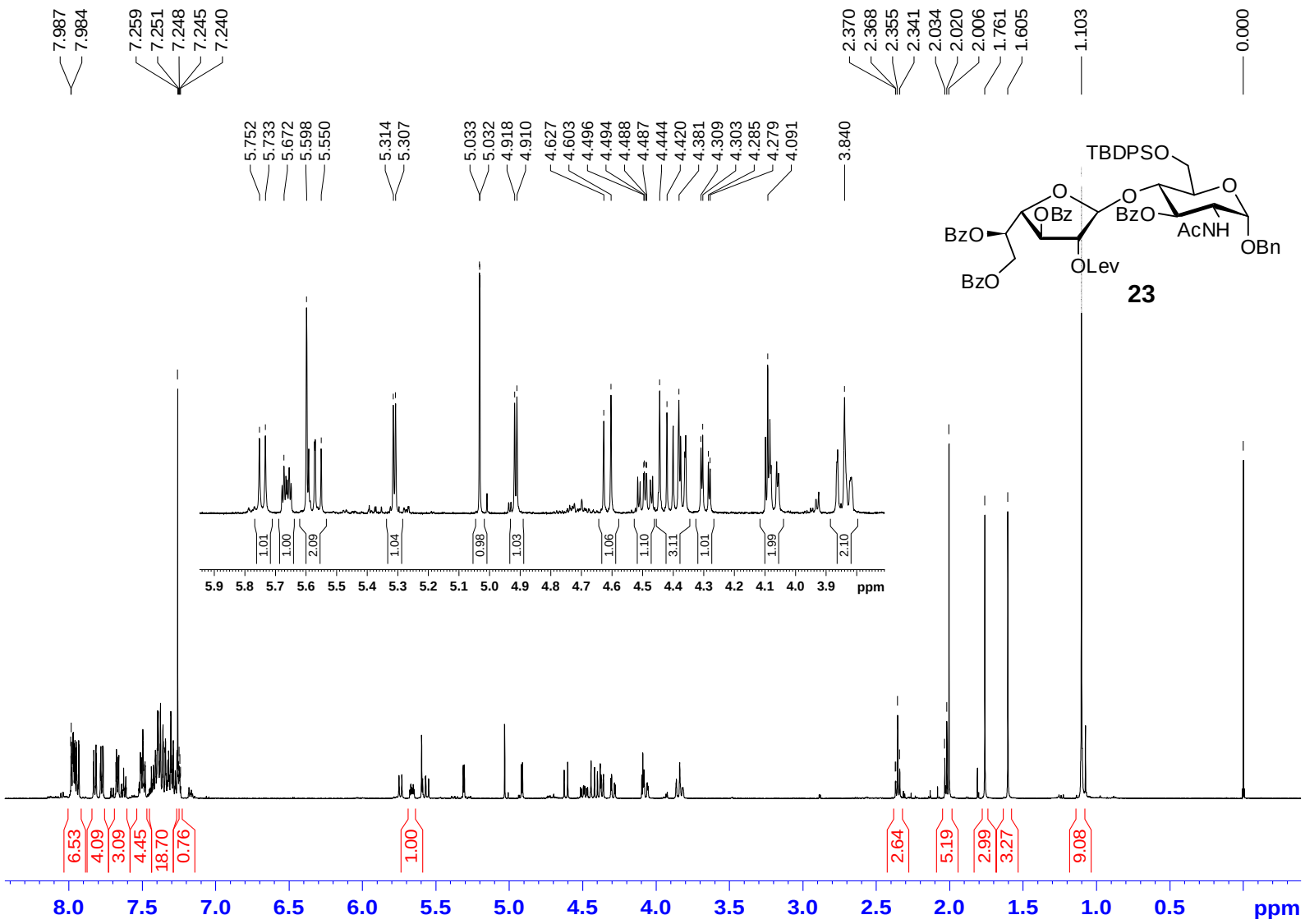
^{13}C NMR spectrum of compound **22** ($CDCl_3$, 125.8 MHz).



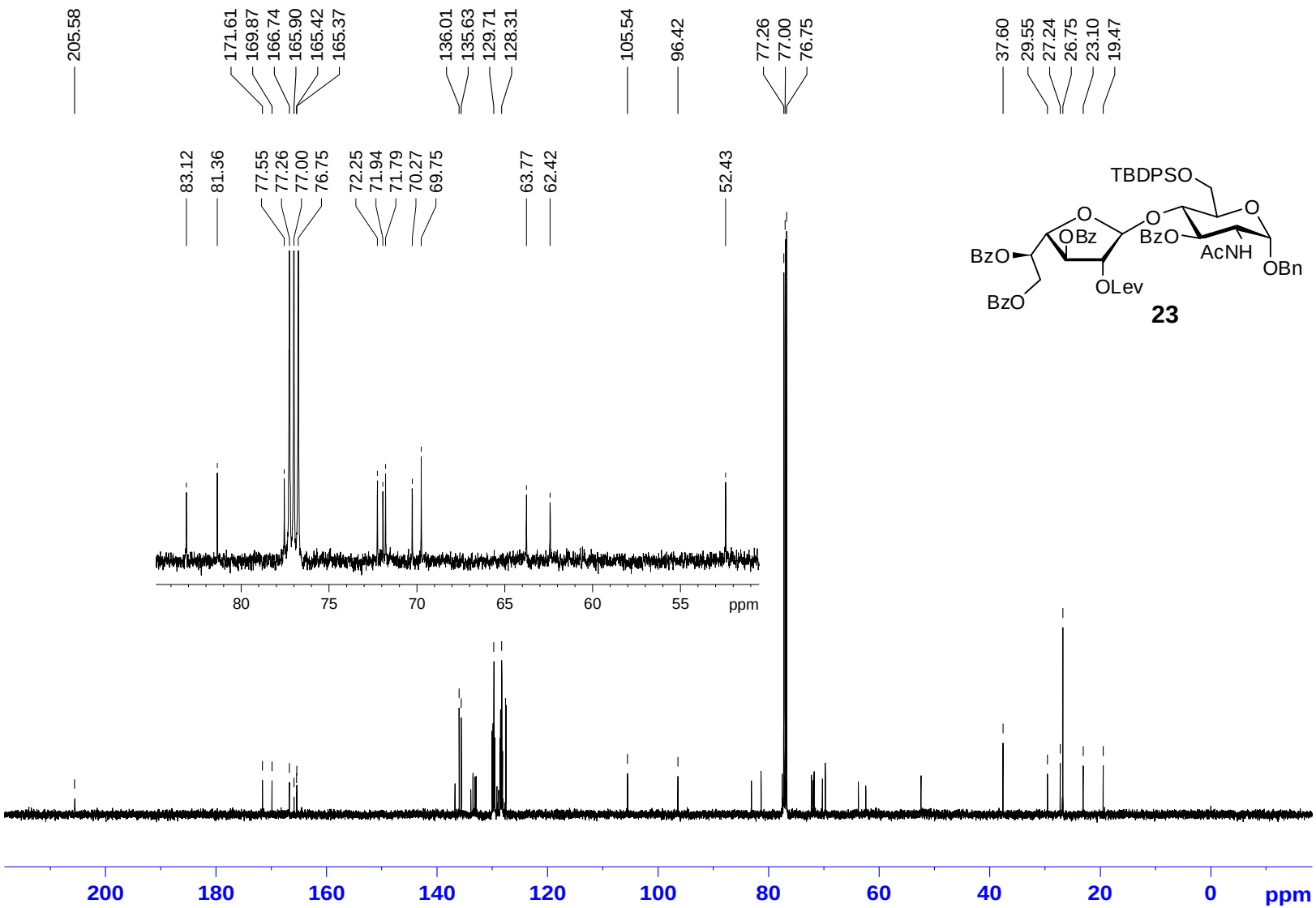
^{1}H NMR spectrum of compound **11** ($CDCl_3$, 500 MHz).



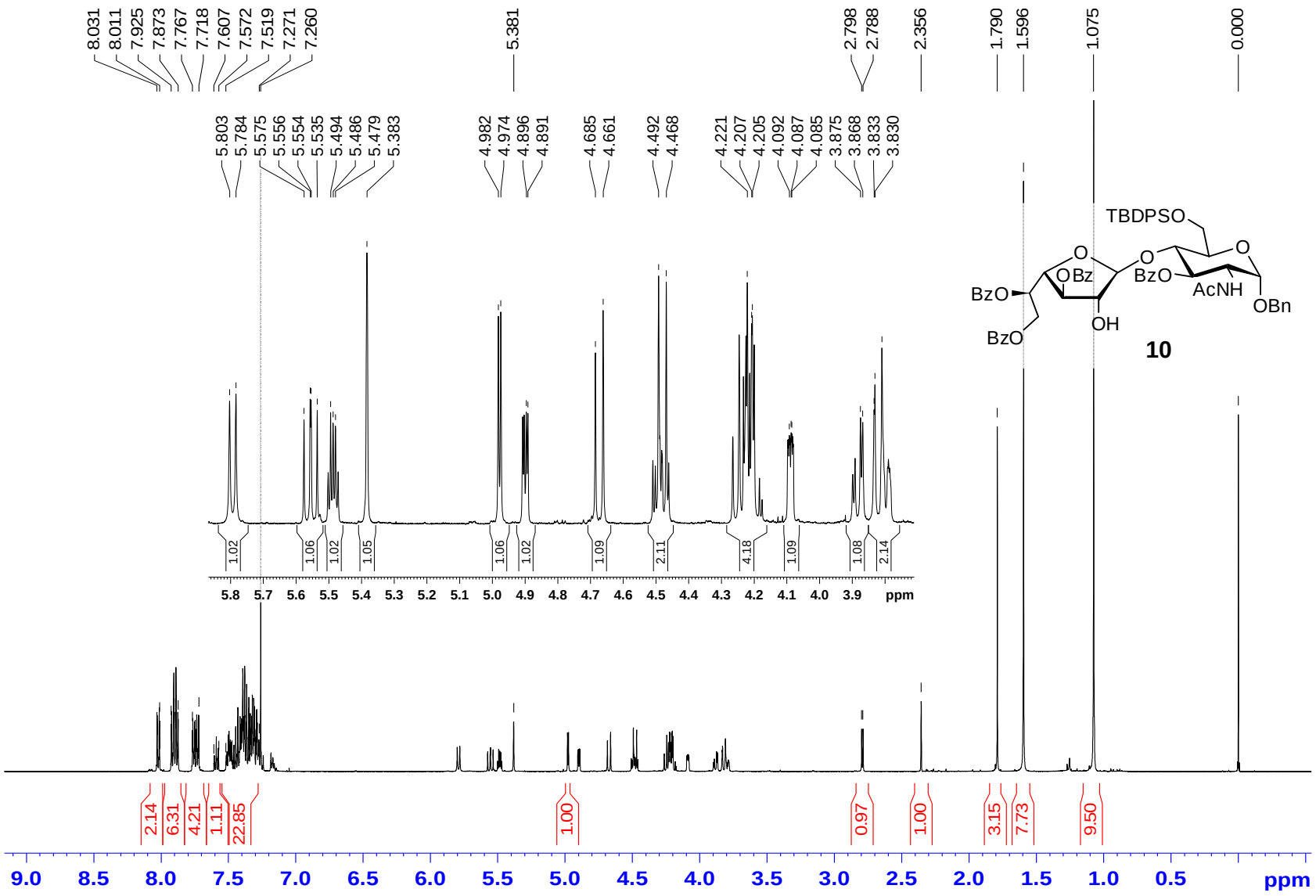
^{13}C NMR spectrum of compound **11** ($CDCl_3$, 125.8 MHz).



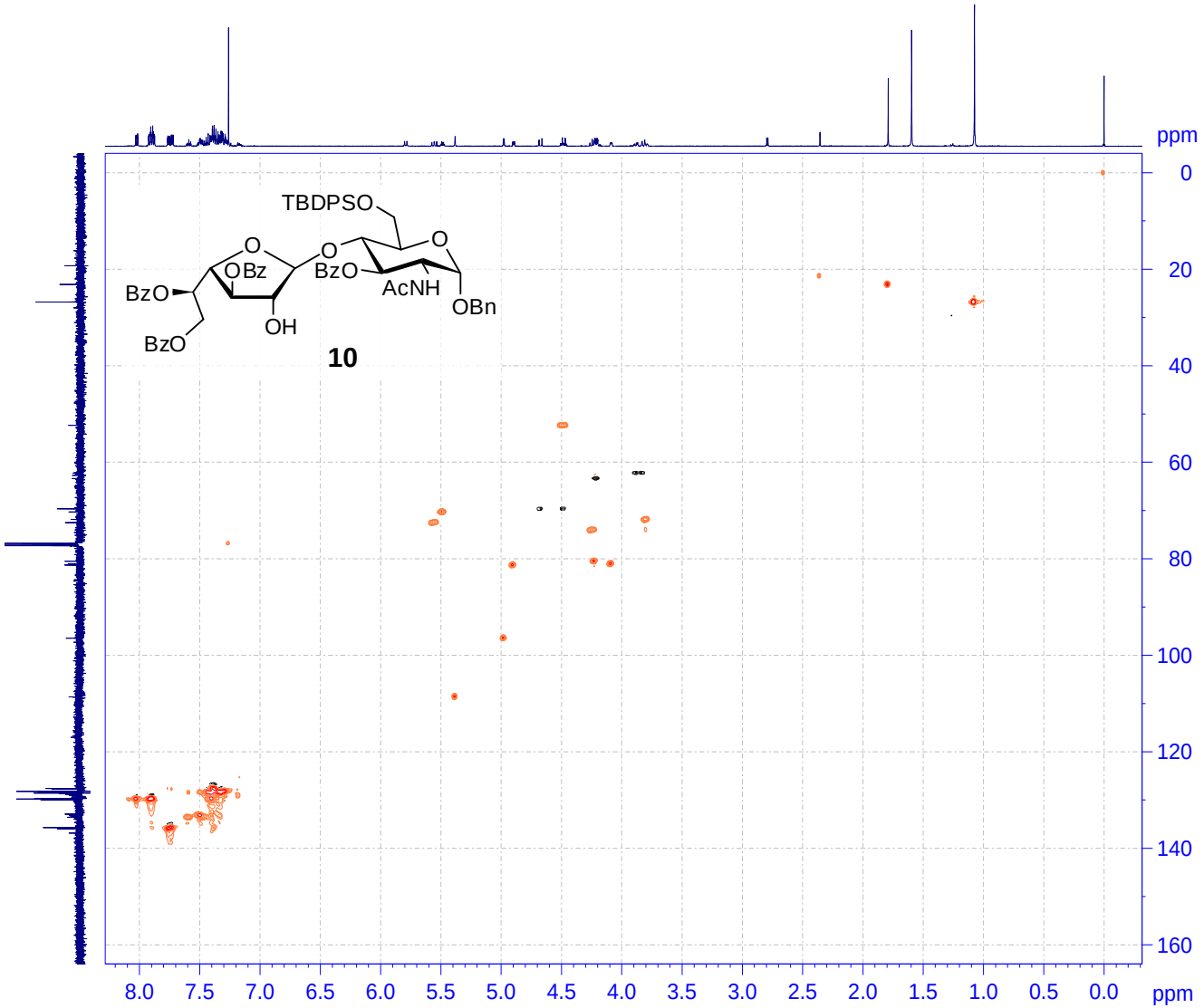
^{1}H NMR spectrum of compound **23** ($CDCl_3$, 500 MHz).



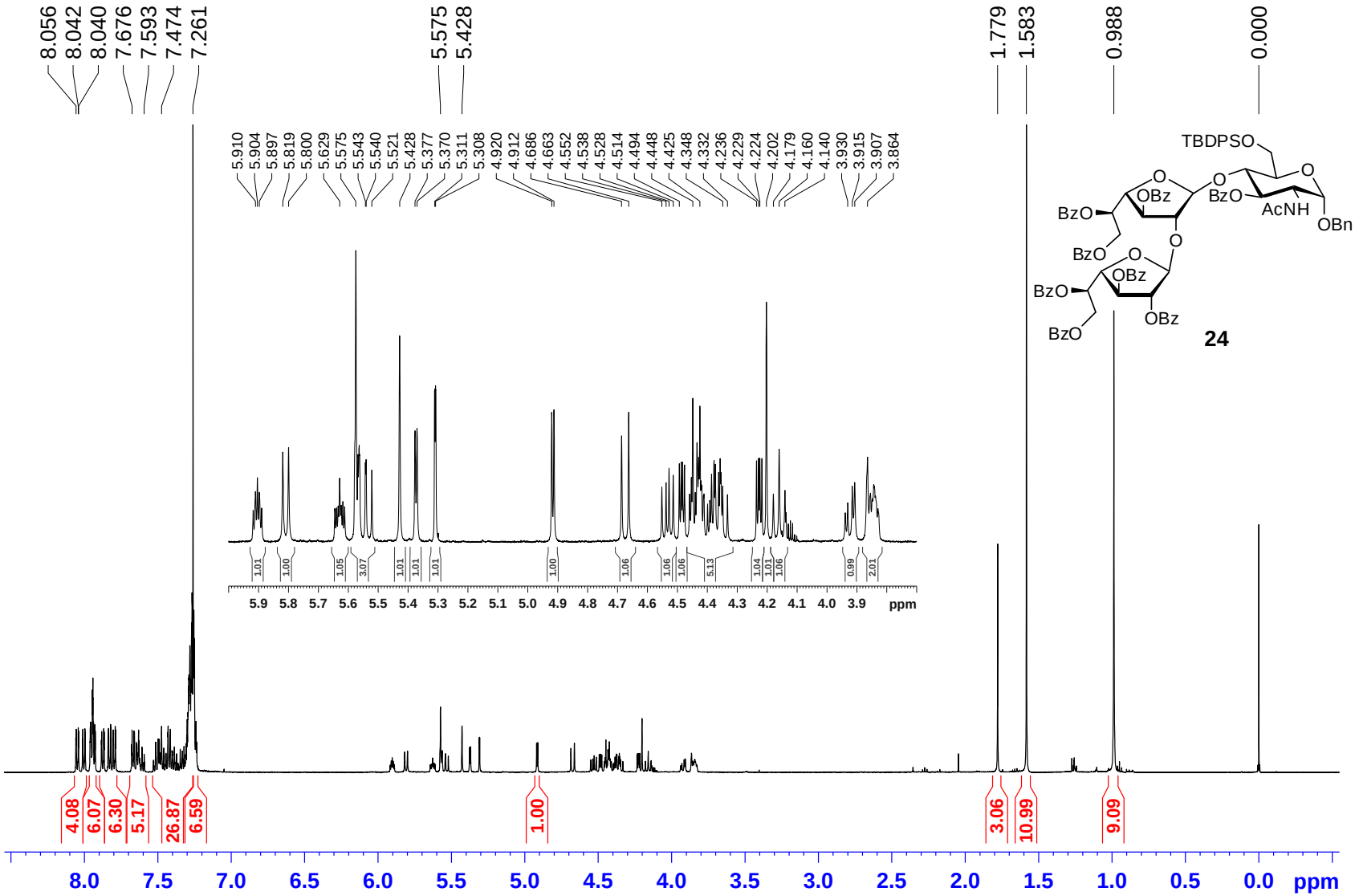
^{13}C NMR spectrum of compound **23** ($CDCl_3$, 125.8 MHz).



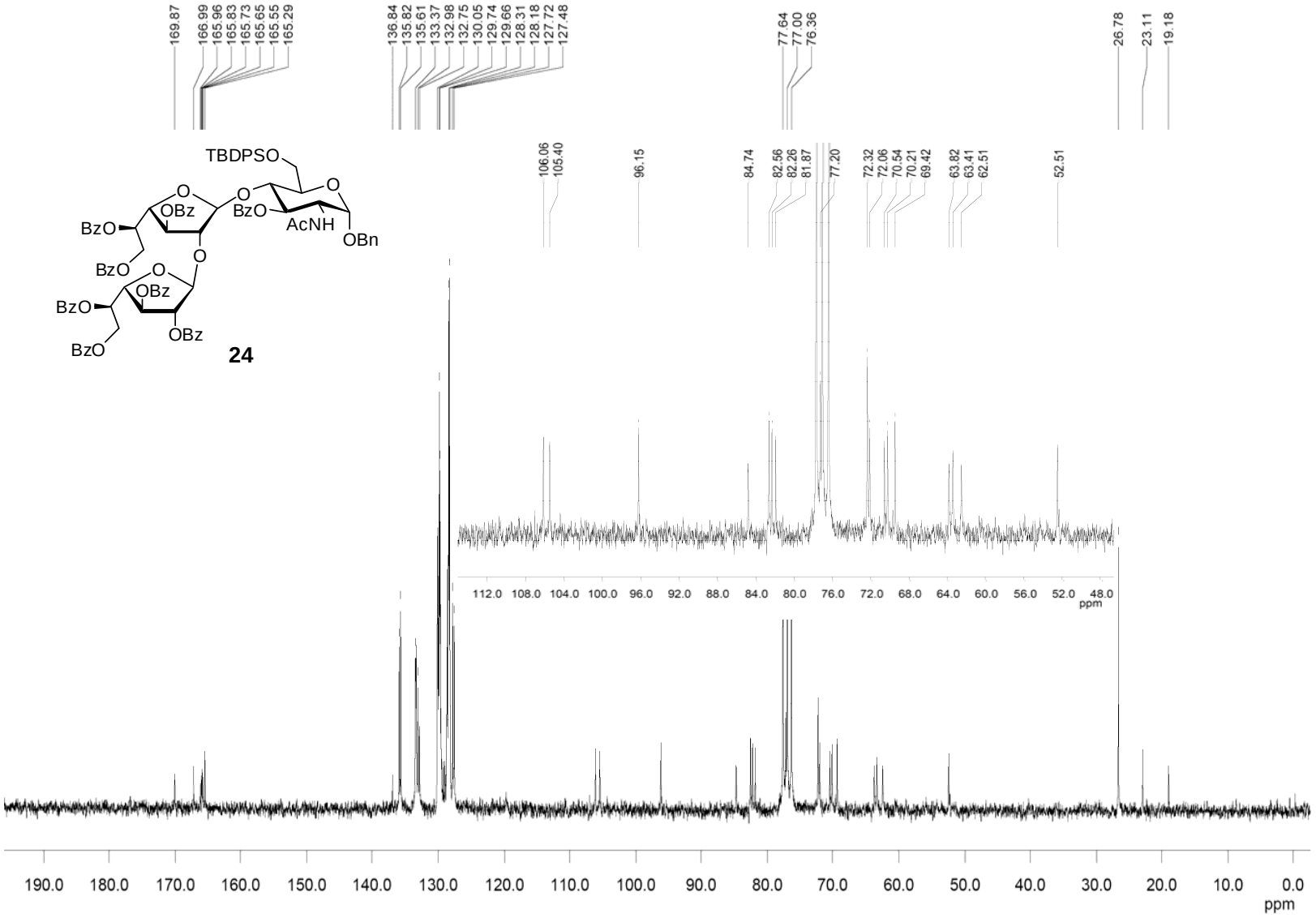
^{1}H NMR spectrum of compound **10** ($CDCl_3$, 500 MHz).



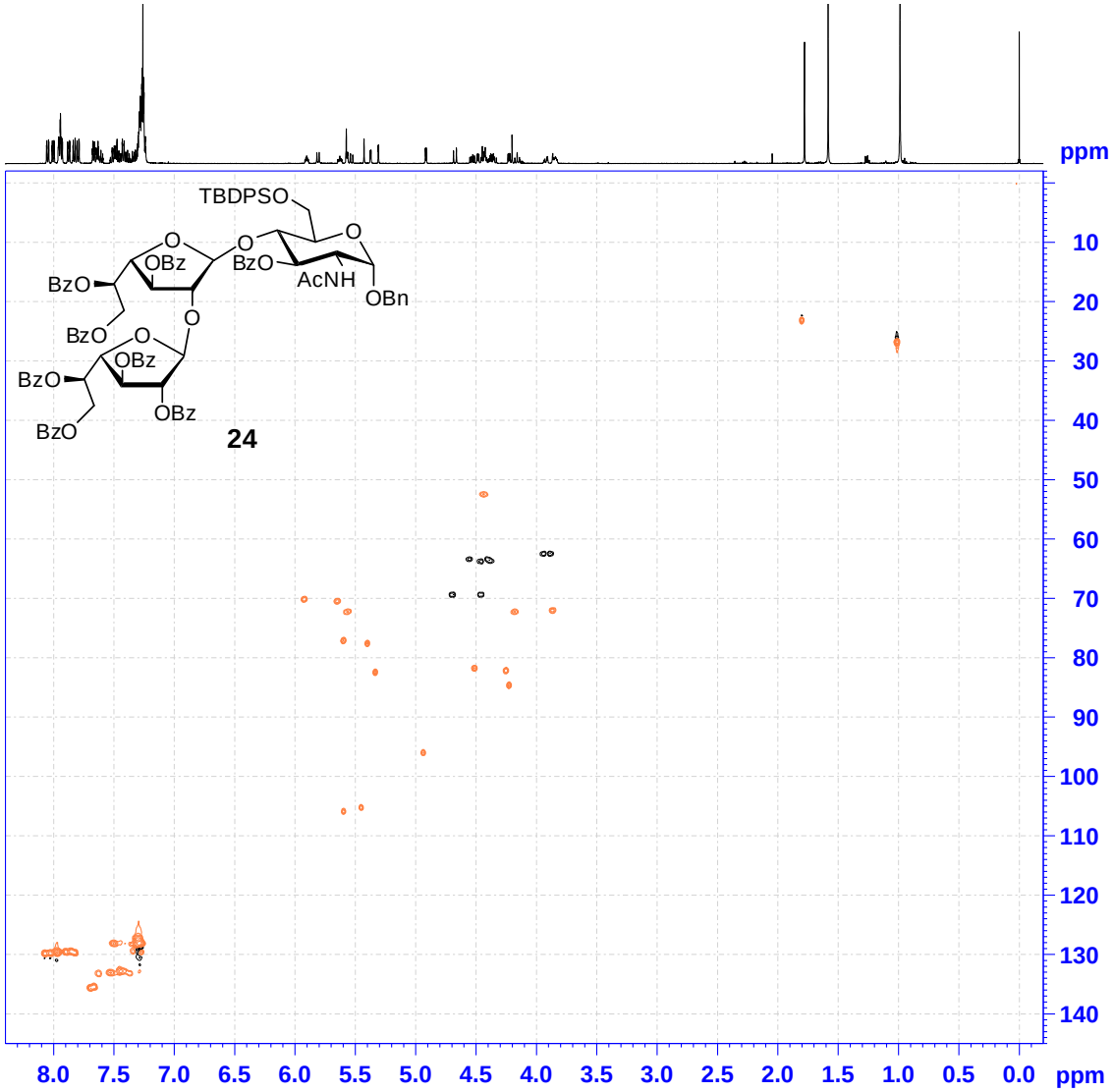
HSQC spectrum of compound **10** ($CDCl_3$, 500 MHz).



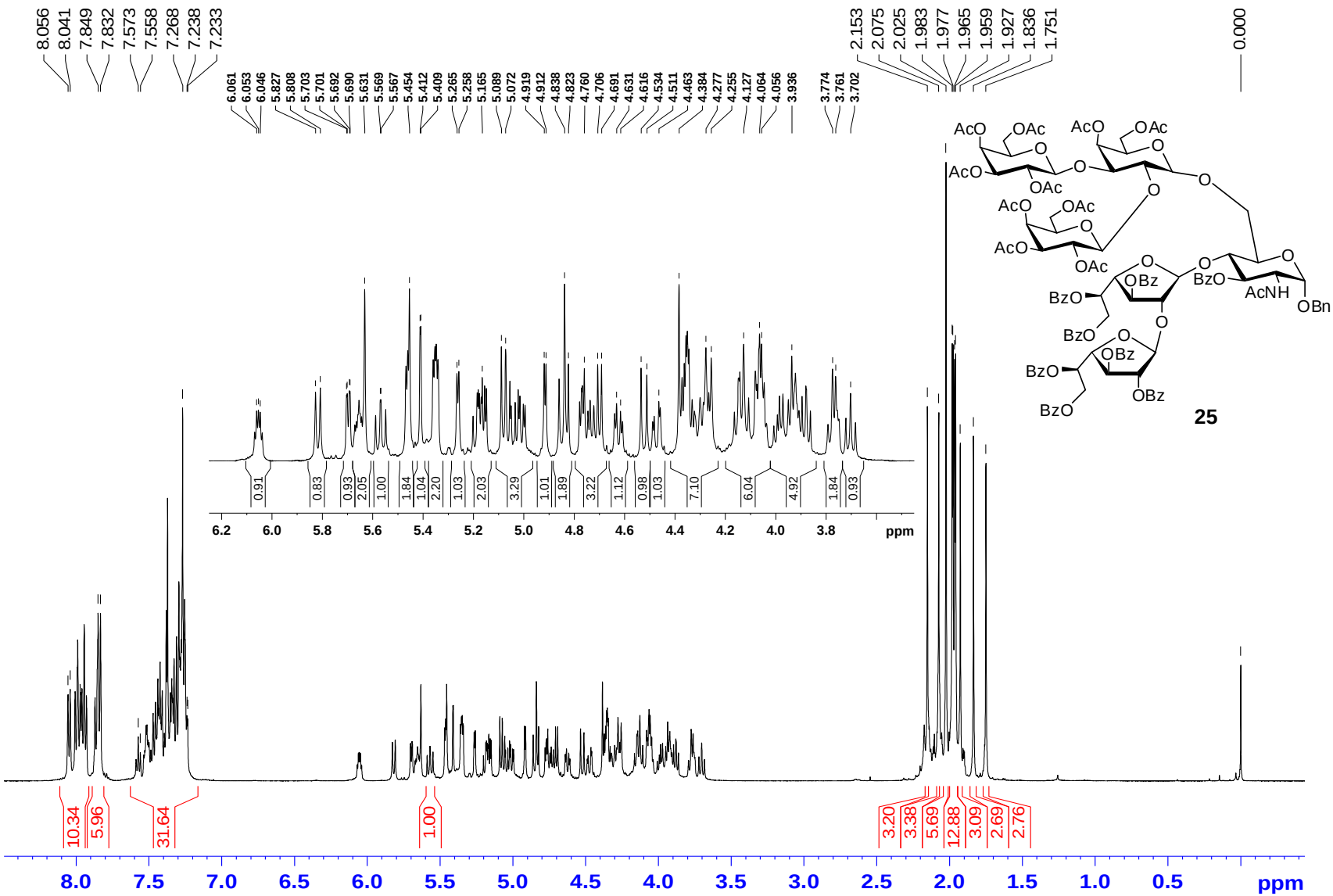
^{1}H NMR spectrum of compound **24** ($CDCl_3$, 500 MHz).



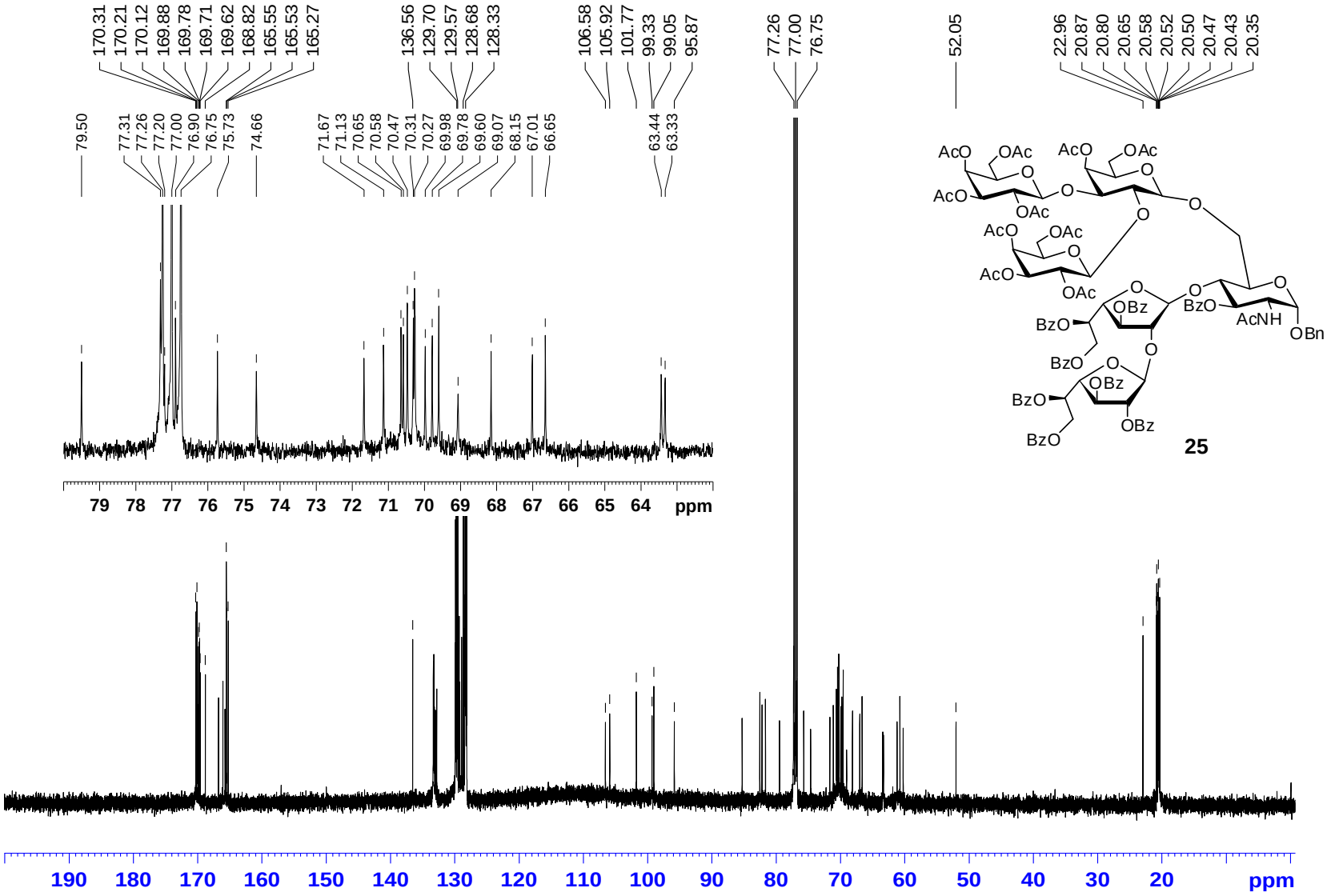
^{13}C NMR spectrum of compound **24** ($CDCl_3$, 50.3 MHz).



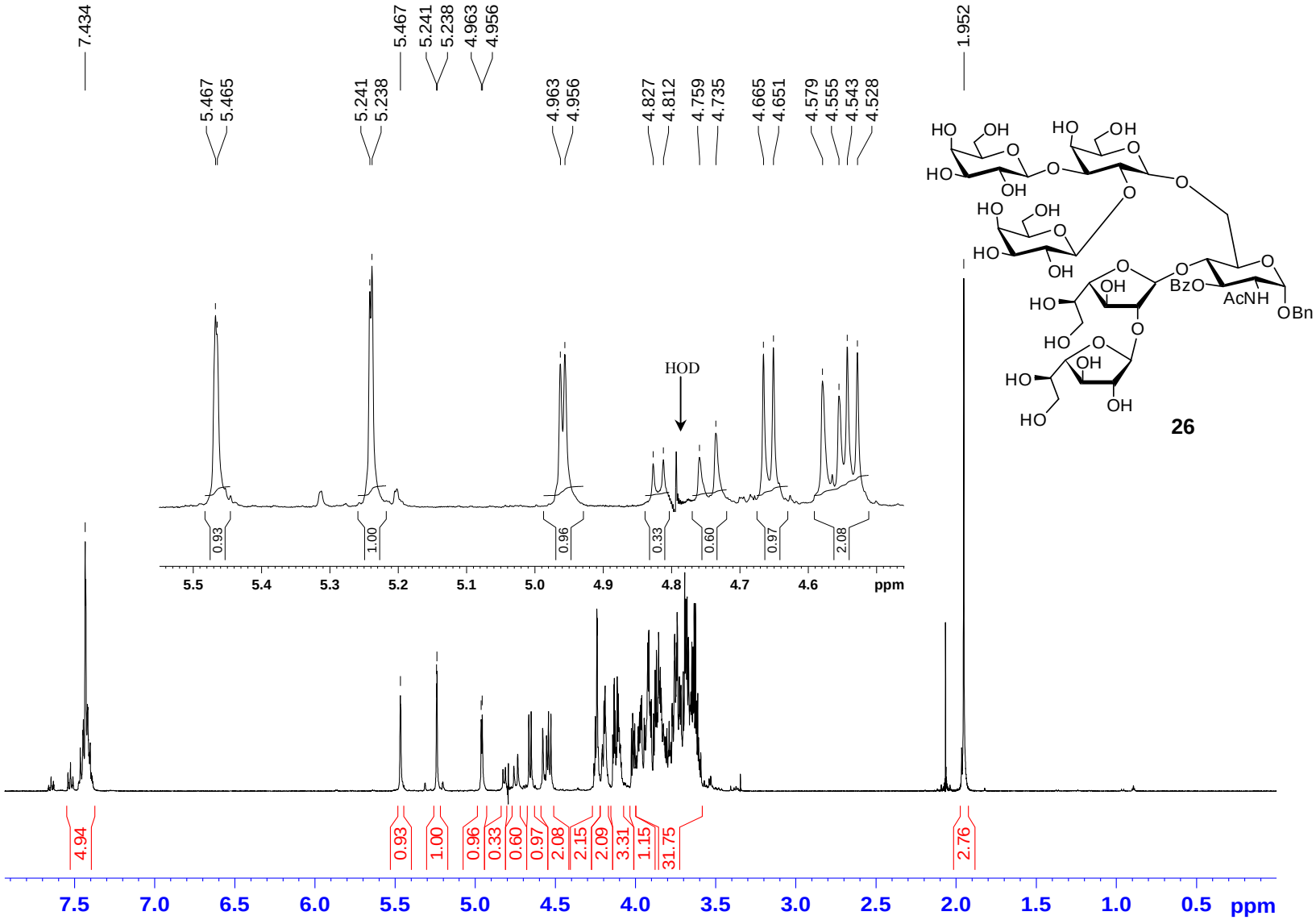
HSQC spectrum of compound **24** ($CDCl_3$, 500 MHz).



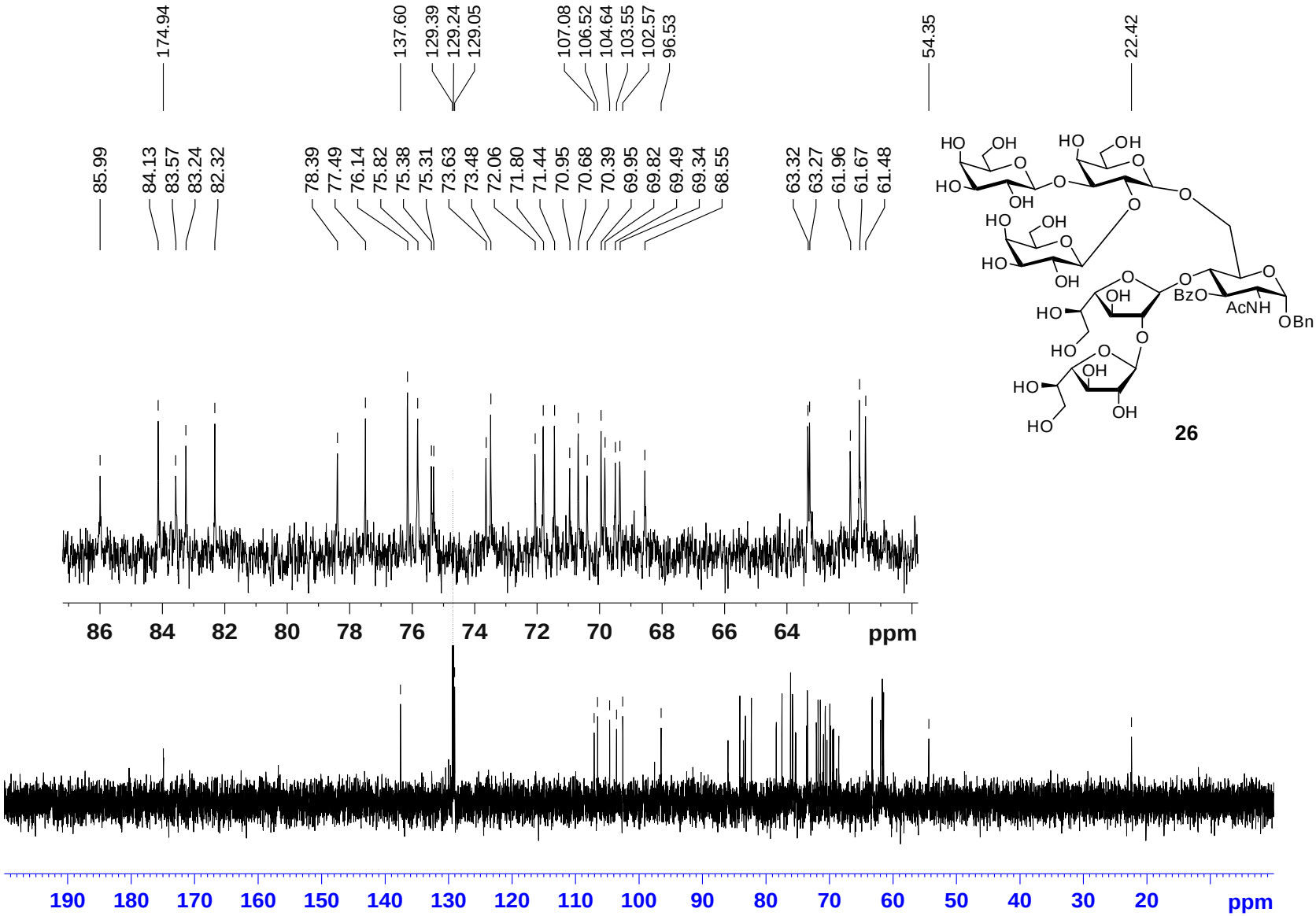
^{1}H NMR spectrum of compound **25** ($CDCl_3$, 500 MHz).



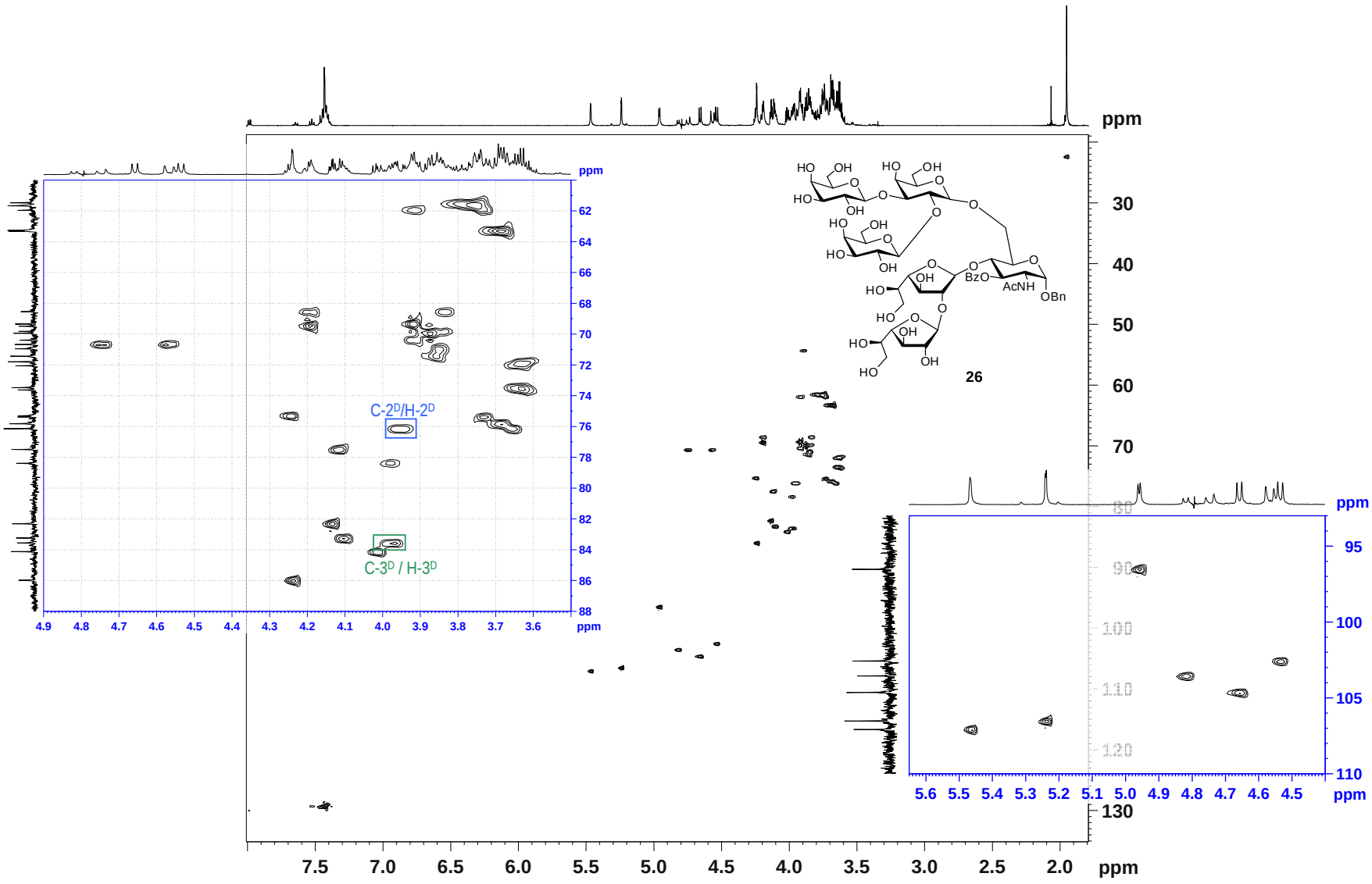
^{13}C NMR spectrum of compound **25** ($CDCl_3$, 125.8 MHz).



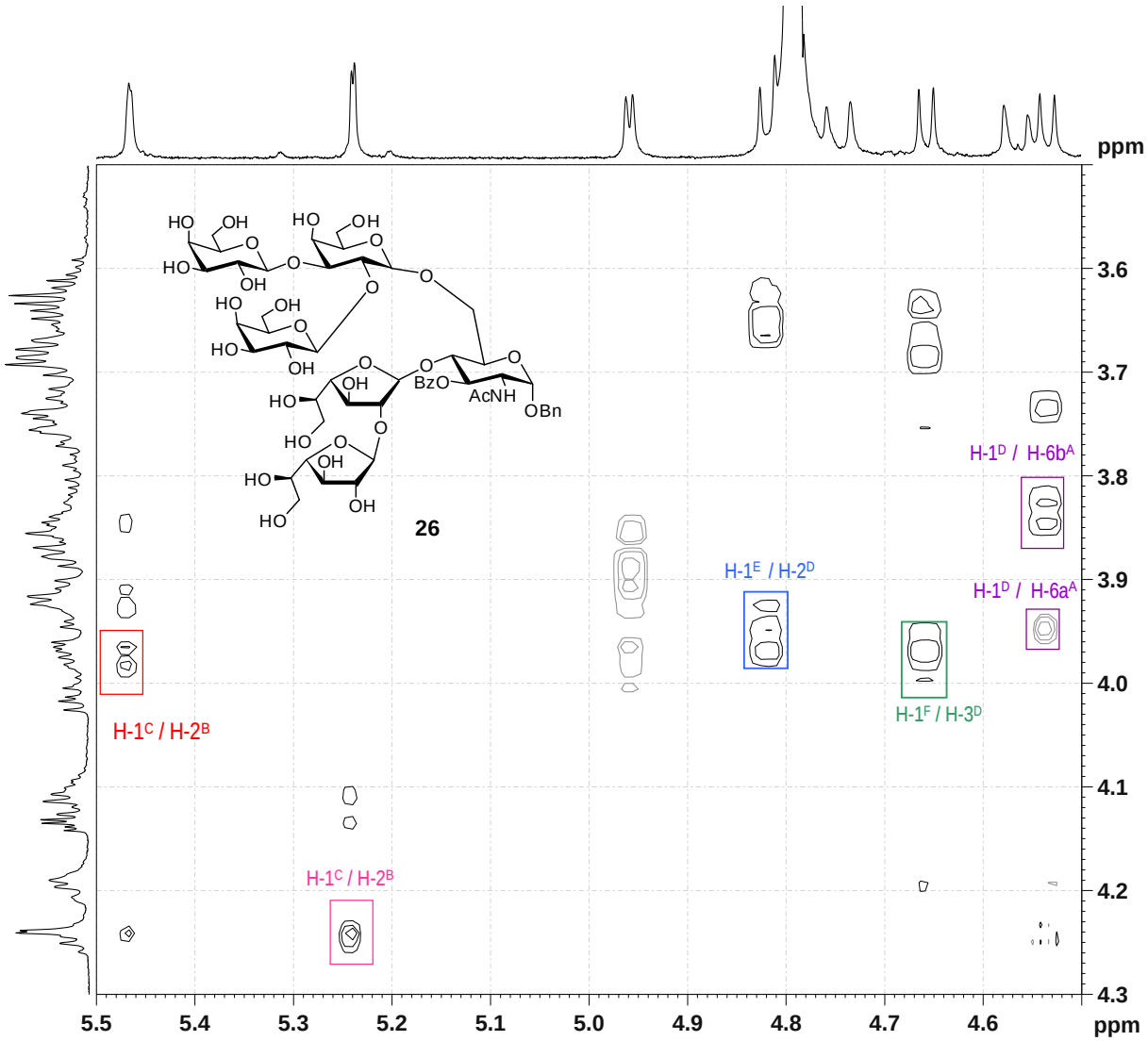
^{1}H NMR spectrum of compound **26** (D_2O, 500 MHz).



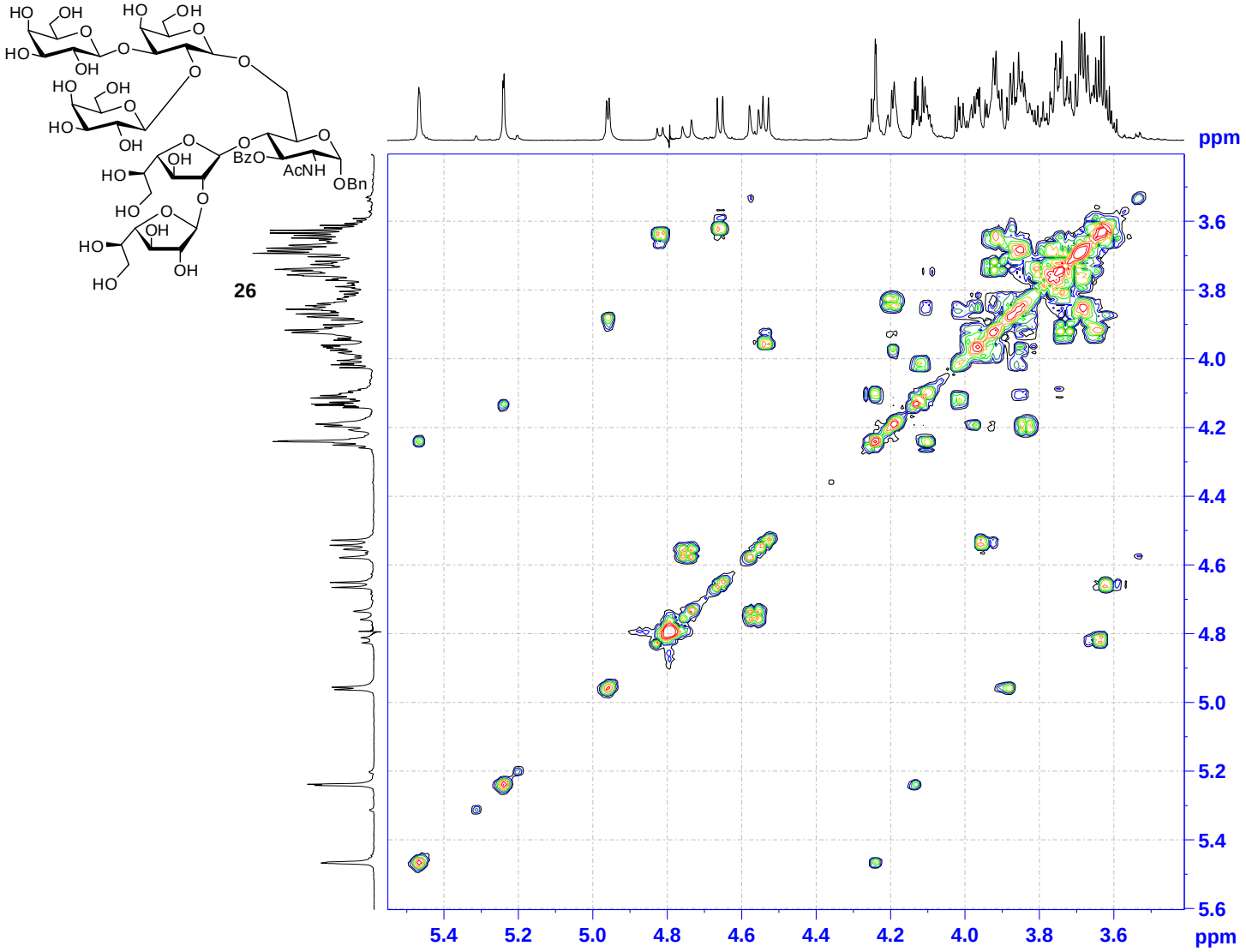
^{13}C NMR spectrum of compound **26** (D_2O, 125.8 MHz).



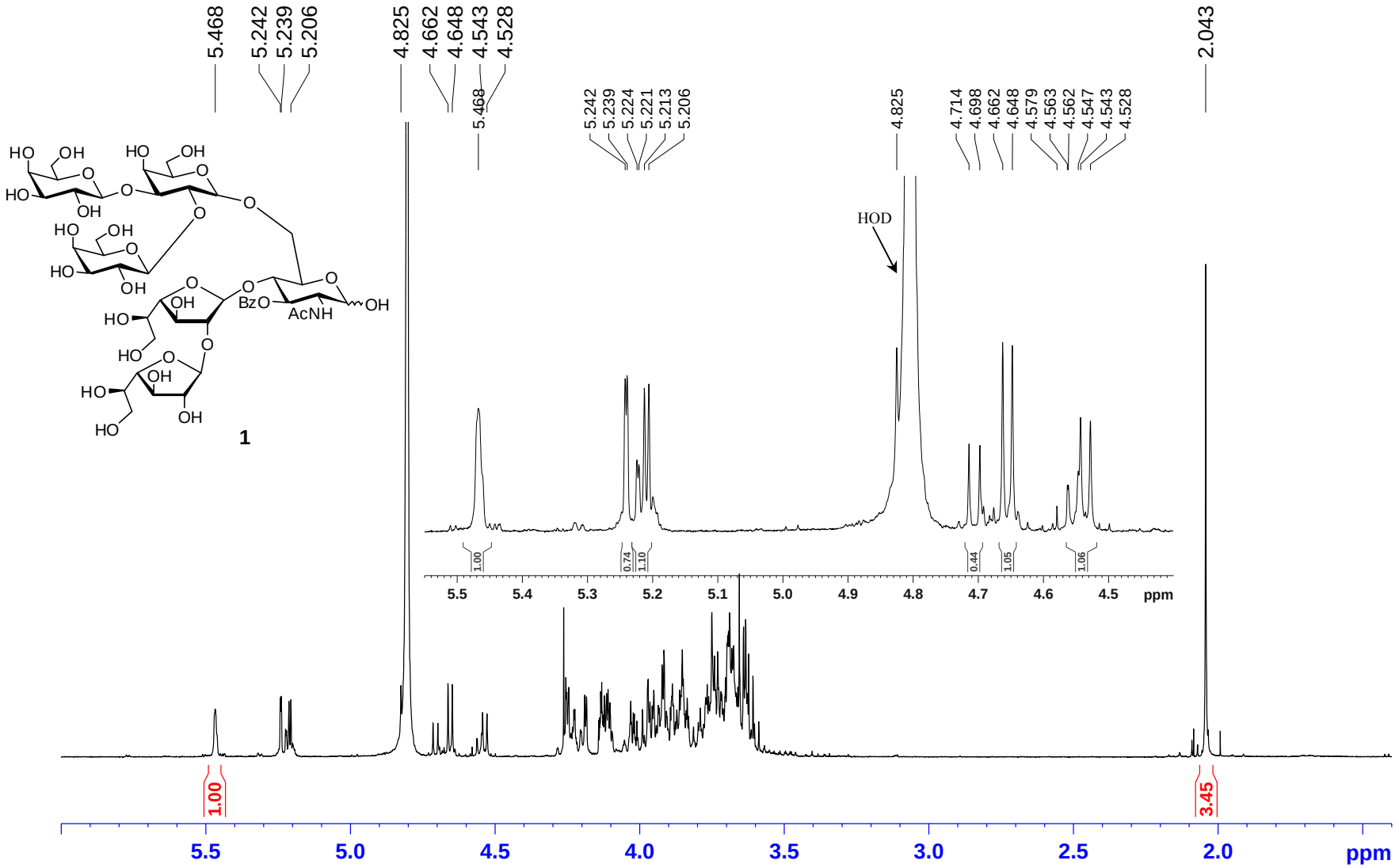
HMQC spectrum of compound **26** (D_2O, 125.8 MHz). Correlations of interest are highlighted.



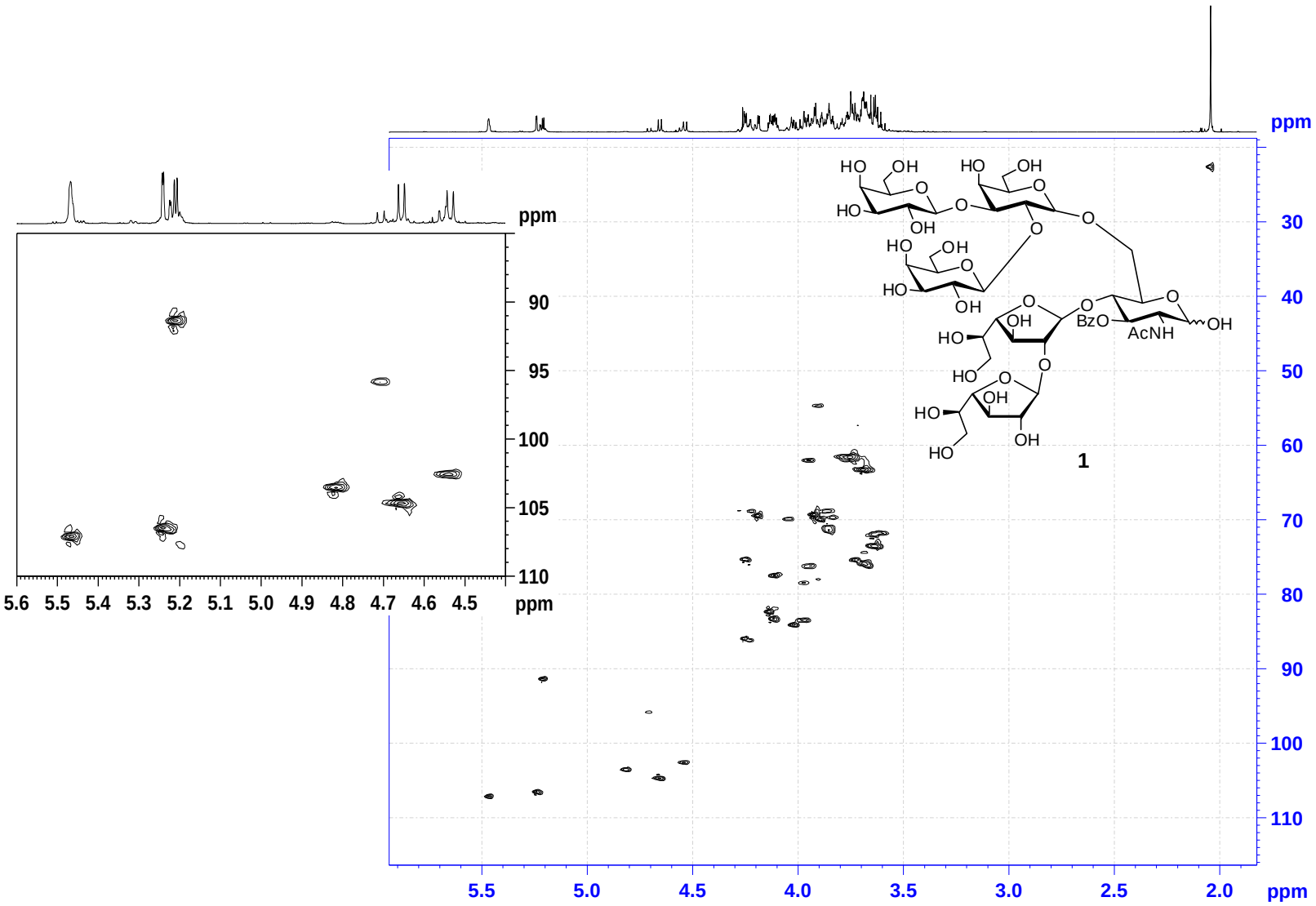
Anomeric correlation region from ROESY of compound **26** (D_2O, 500 MHz). Interresidue ROE correlations are indicated.



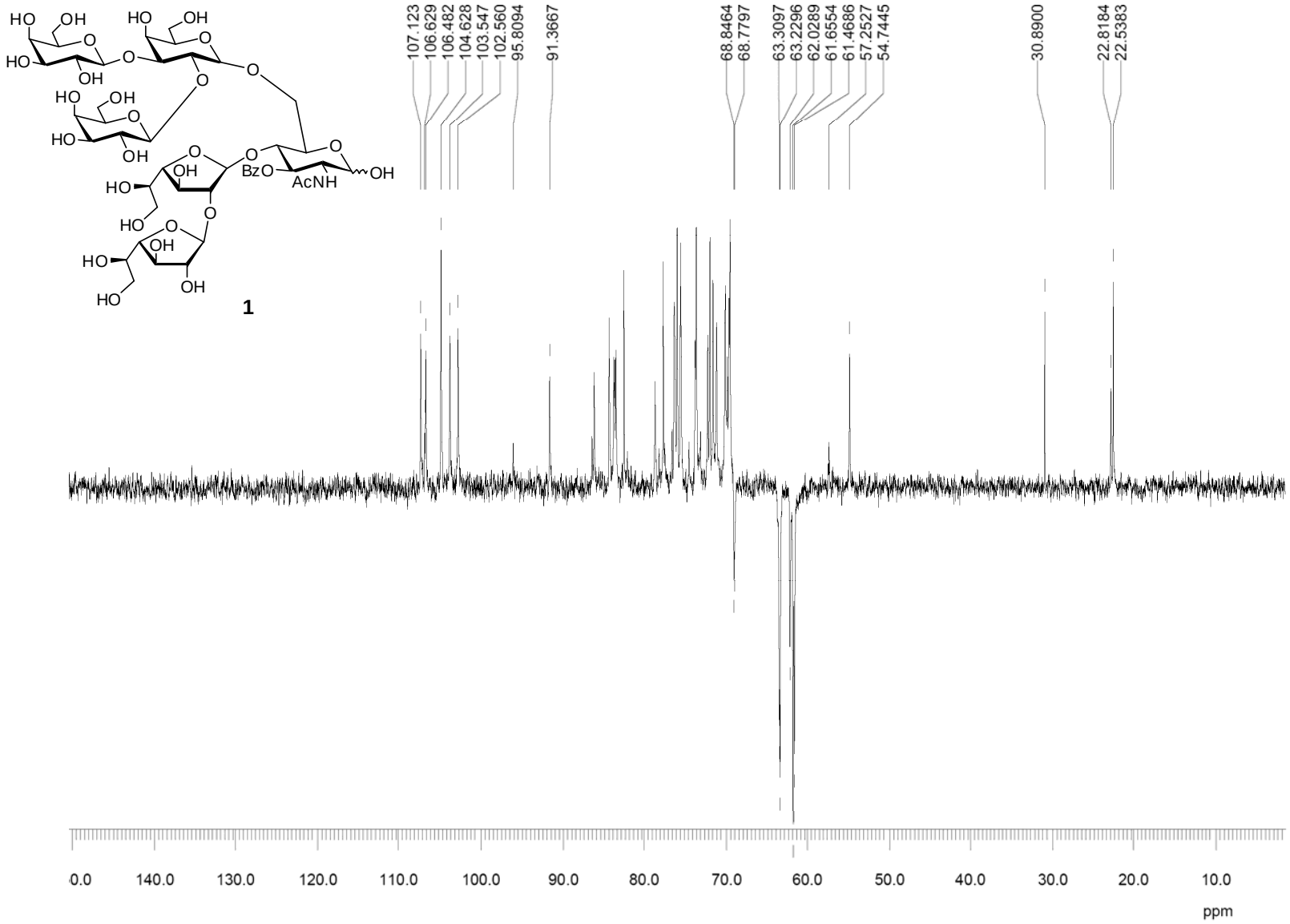
COSY spectrum of compound **26** (D_2O, 125.8 MHz).



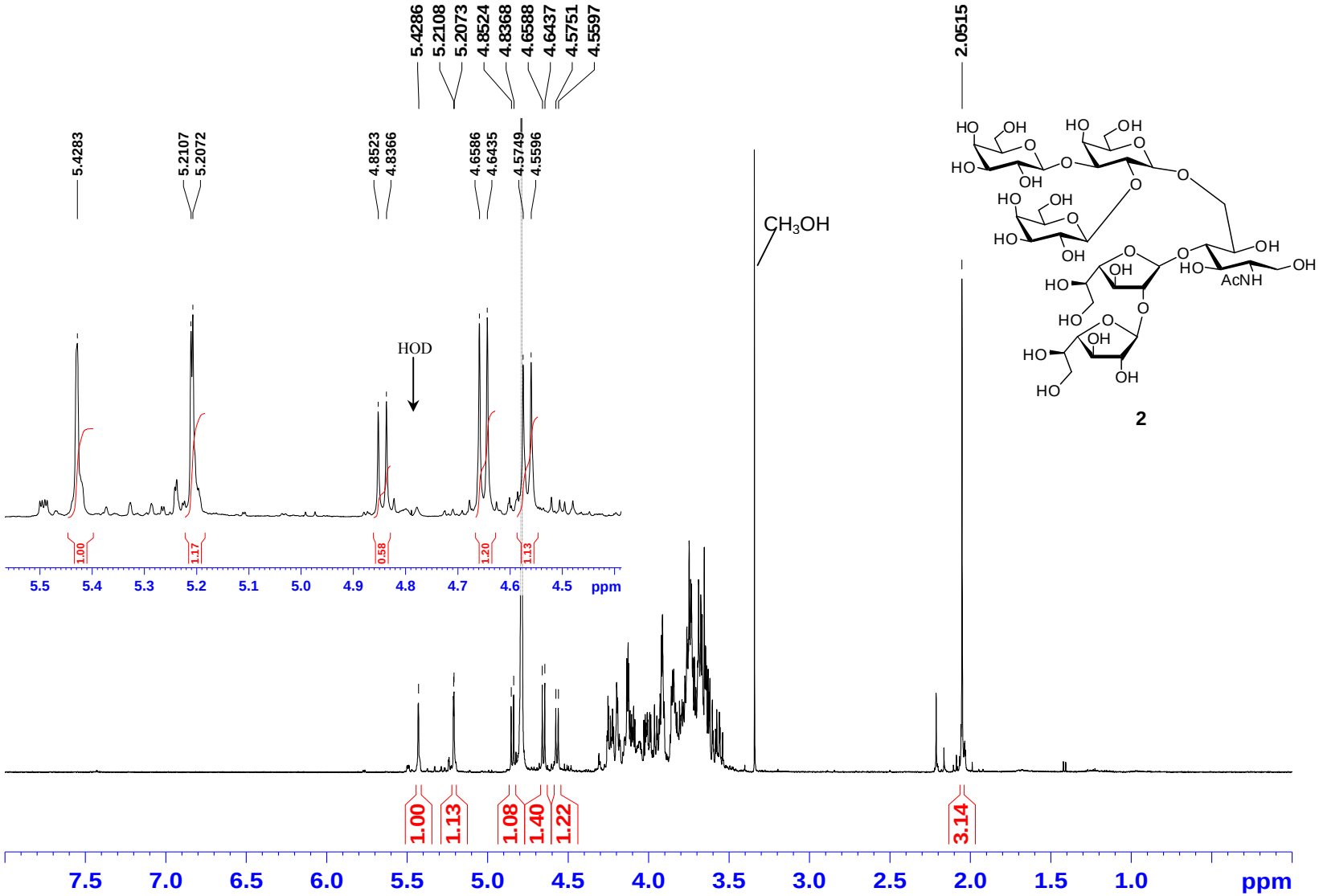
^{1}H NMR spectrum of compound **1** (D_2O, 500 MHz).



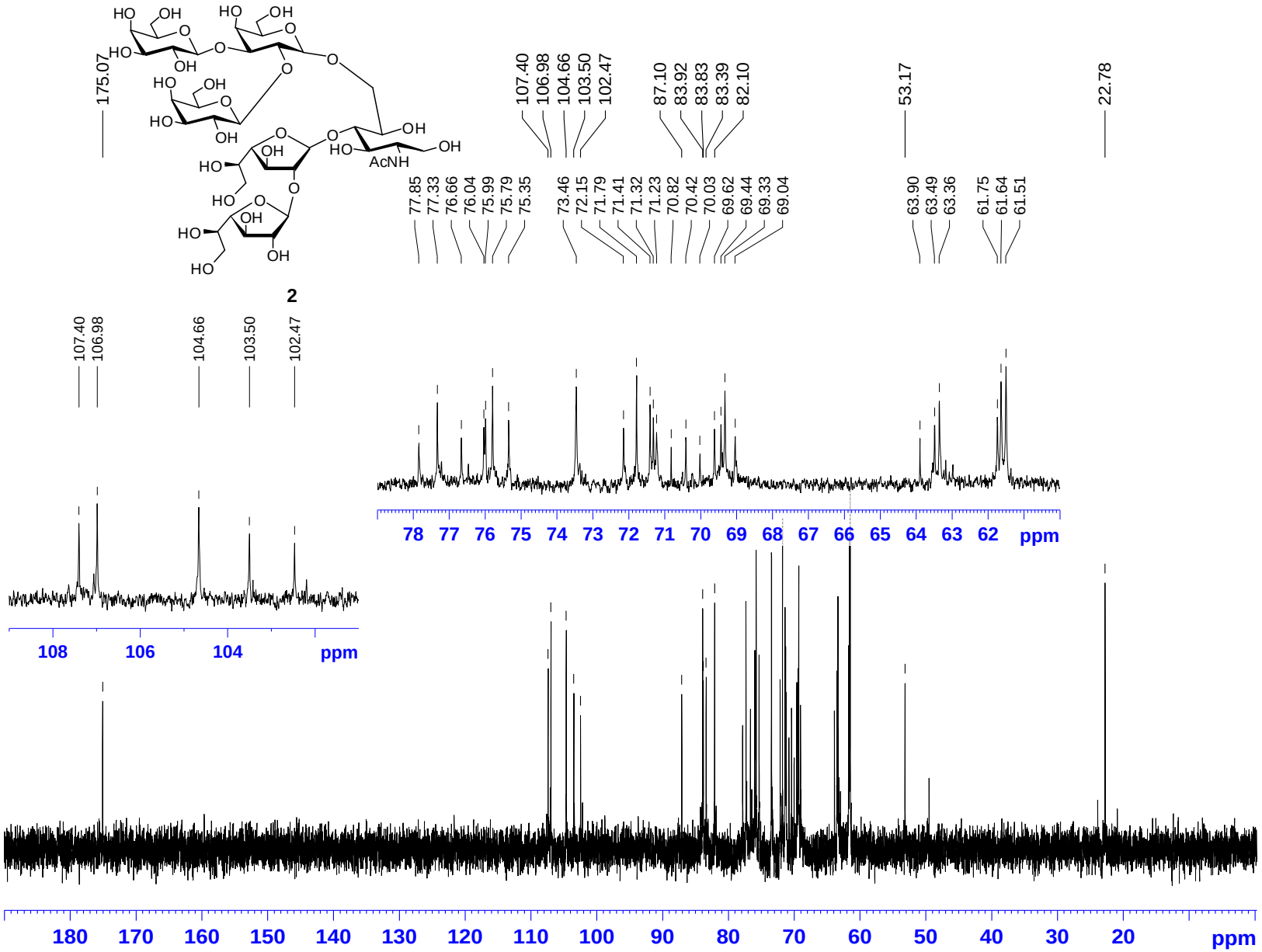
HSQC spectrum of compound **1** (D_2O, 500 MHz).



DEPT 135 spectrum of compound **1** (D_2O, 50.3 MHz).



^{1}H NMR spectrum of compound **2** (D_2O, 500 MHz). Anomeric region for the suppressed HDO signal spectrum is showed.



^{13}C NMR spectrum of compound **2** (D_2O, 125.8 MHz).