

## SUPPLEMENTARY MATERIALS

### A Branched and Double Alpha-Gal-Bearing Synthetic Neoglycoprotein as a Biomarker for Chagas Disease

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## GENERAL INFORMATION

$^1\text{H}$  and  $^{13}\text{C}$  Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Advance III HD 400 MHz NMR spectrometer at 400 and 101 MHz, respectively. Tetramethylsilane ( $\delta$  0.00 ppm) was used as a reference to determine the chemical shift  $\delta$  [ppm] as an internal standard in  $\text{CDCl}_3$  and  $\text{CD}_3\text{OD}$  or relative to the  $\text{CDCl}_3$  signal ( $\delta$  77.0 ppm) in  $^{13}\text{C}$  NMR spectra. When spectra were measured in  $\text{D}_2\text{O}$ , a solution of tetramethylsilane in  $\text{CDCl}_3$  in a sealed capillary was used as an external standard for calibration. The coupling constants  $J$  [Hz] were measured from one-dimensional  $^1\text{H}$ -NMR spectra. Full or partial assignments were made by standard COSY, HSQC, and TOCSY experiments. High-resolution mass spectra were recorded using electrospray ionization mass spectrometry (ESI-MS) on a high-resolution JEOL AccuTOF mass spectrometer.

## ABBREVIATIONS USED

COSY	Correlated Spectroscopy
HR ESI-TOF-MS	High Resolution Electrospray Ionization Time-of-Flight Mass Spectrum
HSQC	Heteronuclear Single Quantum Coherence
m/z	Mass-to-Charge Ratio
NMR	Nuclear Magnetic Resonance

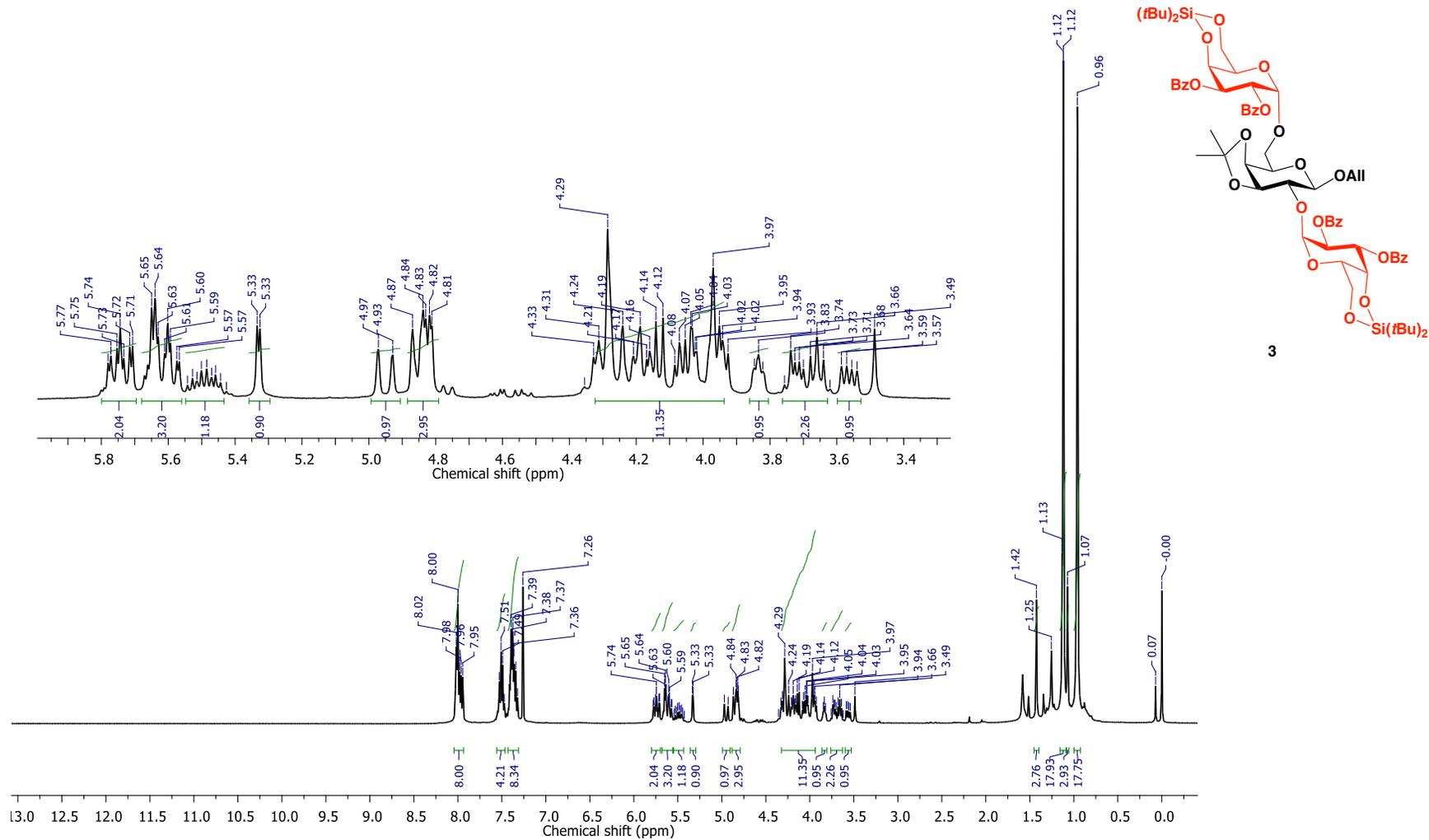


Figure S1:  $^1\text{H}$  NMR spectrum of compound **3**, (400 MHz, in  $\text{CDCl}_3$ )

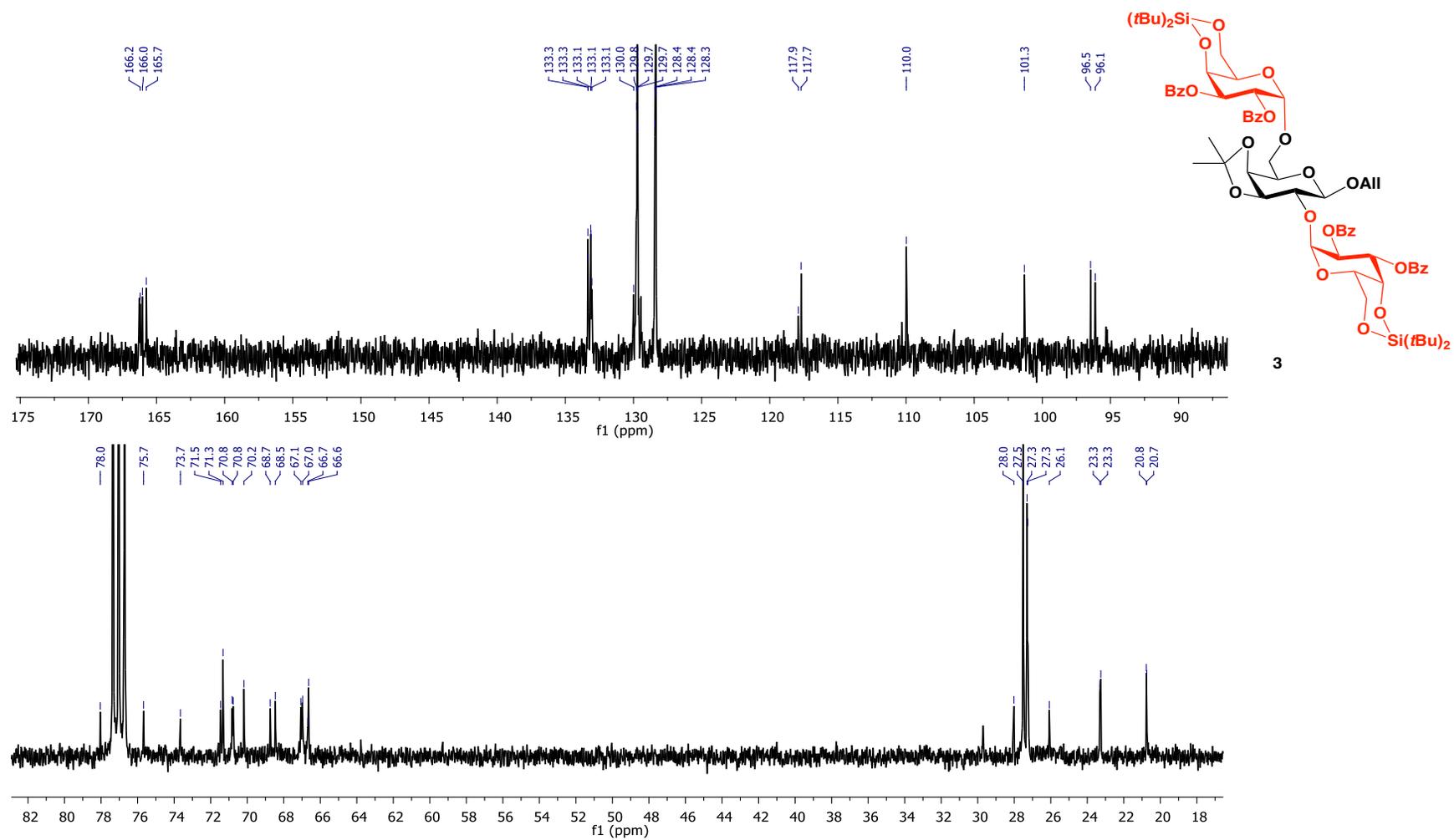


Figure S2:  $^{13}\text{C}$  NMR spectrum of compound **3** (101 MHz, in  $\text{CDCl}_3$ )

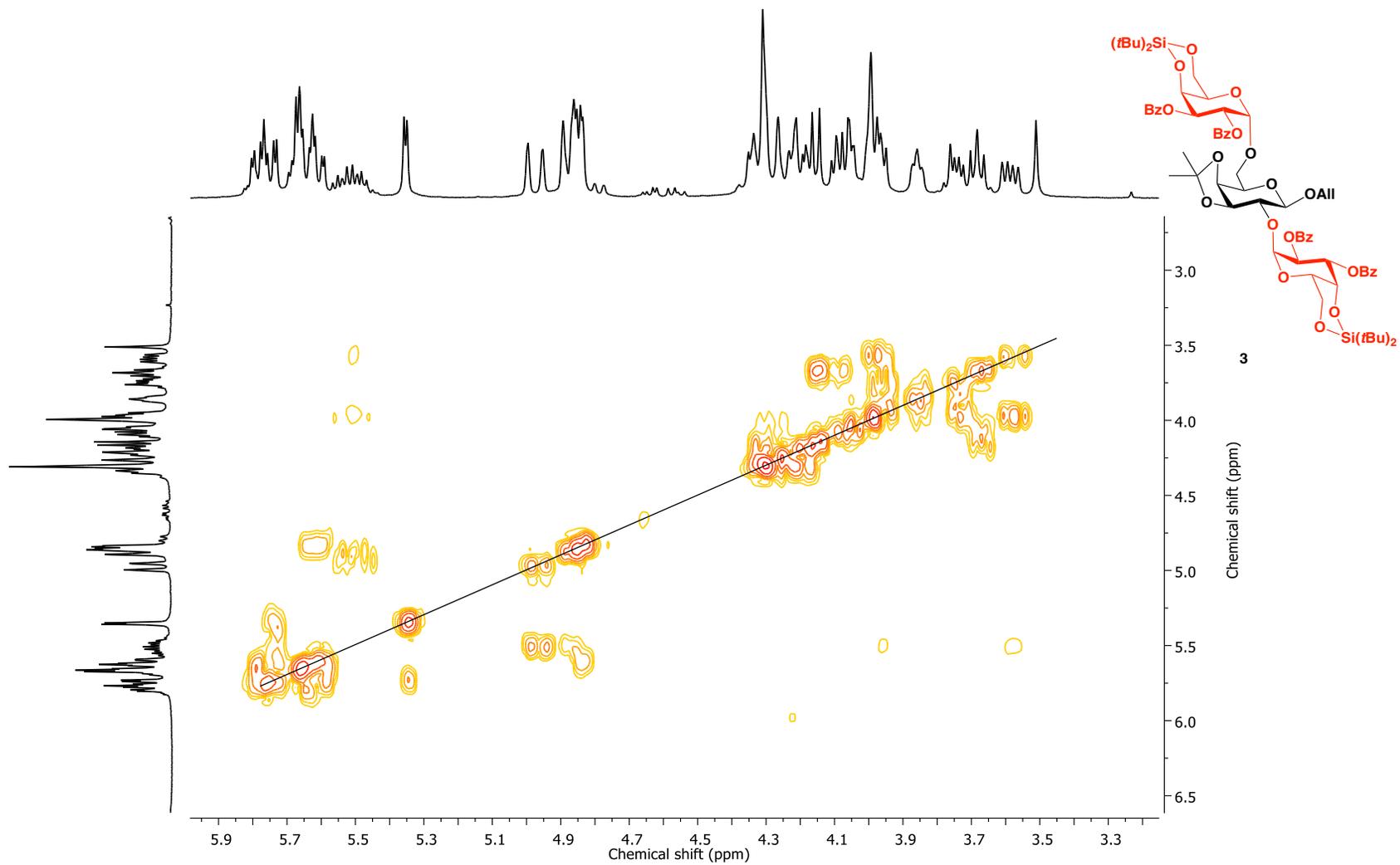


Figure S3: COSY NMR spectrum of compound **3** (400 MHz, in CDCl<sub>3</sub>)

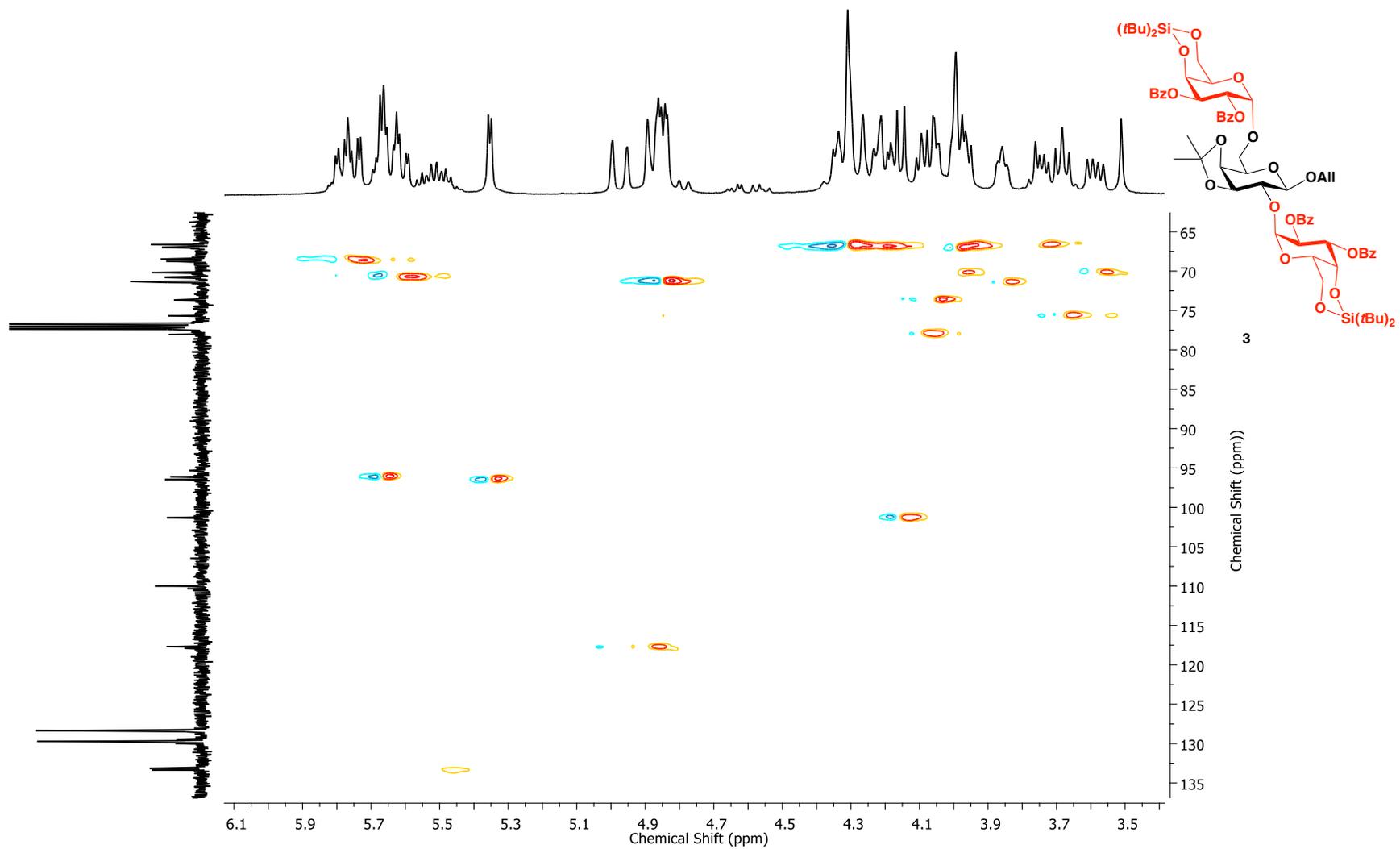


Figure S4: HSQC NMR spectrum of compound **3** (in CDCl<sub>3</sub>)

Acq. Data Name: EGC\_051319\_ULTRAMARK  
Creation Parameters: Average(MS[1] Time:0.5670..0.6310)  
x10<sup>3</sup> Relative Intensity

Experiment Date/Time: 5/20/2019 11:36:55 AM  
Ionization Mode: ESI+

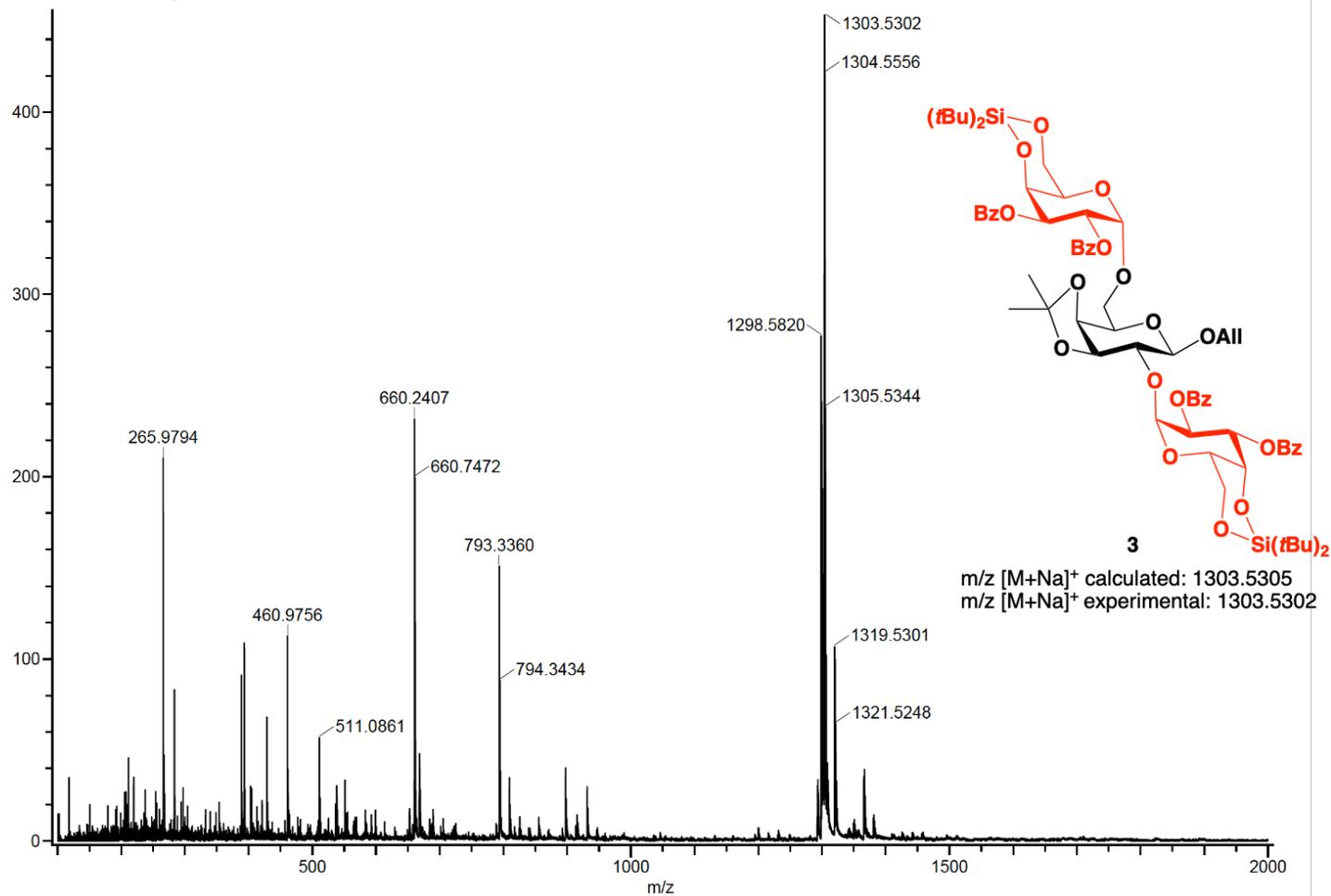
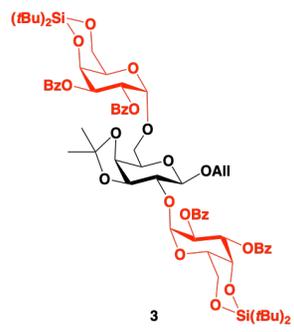


Figure S5: HR ESI-TOF mass spectrum of compound **3**



m/z [M+Na]<sup>+</sup> calculated: 1303.5305  
 m/z [M+Na]<sup>+</sup> experimental: 1303.5302

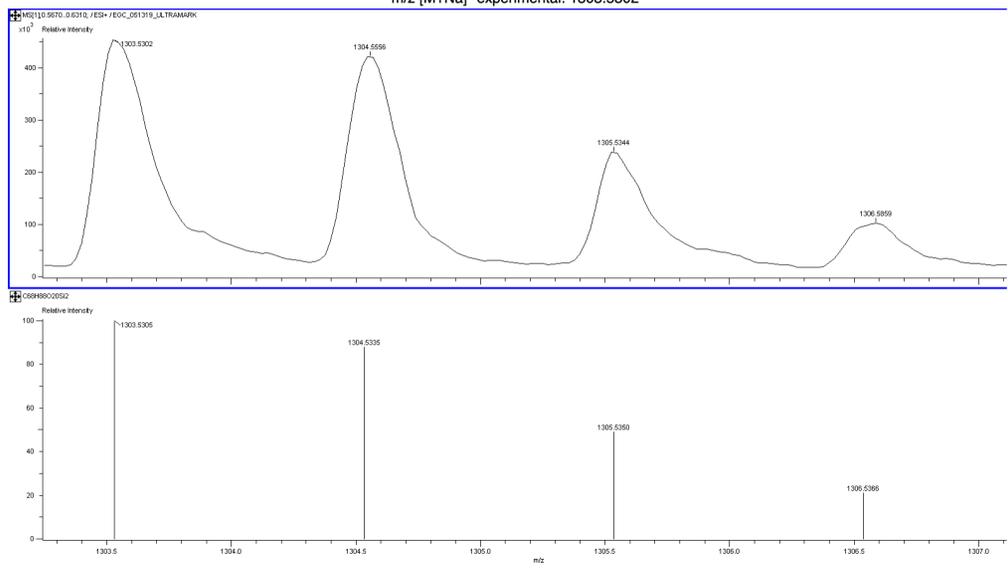


Figure S6: Actual and simulated HR ESI-TOF mass spectrum of compound **3**

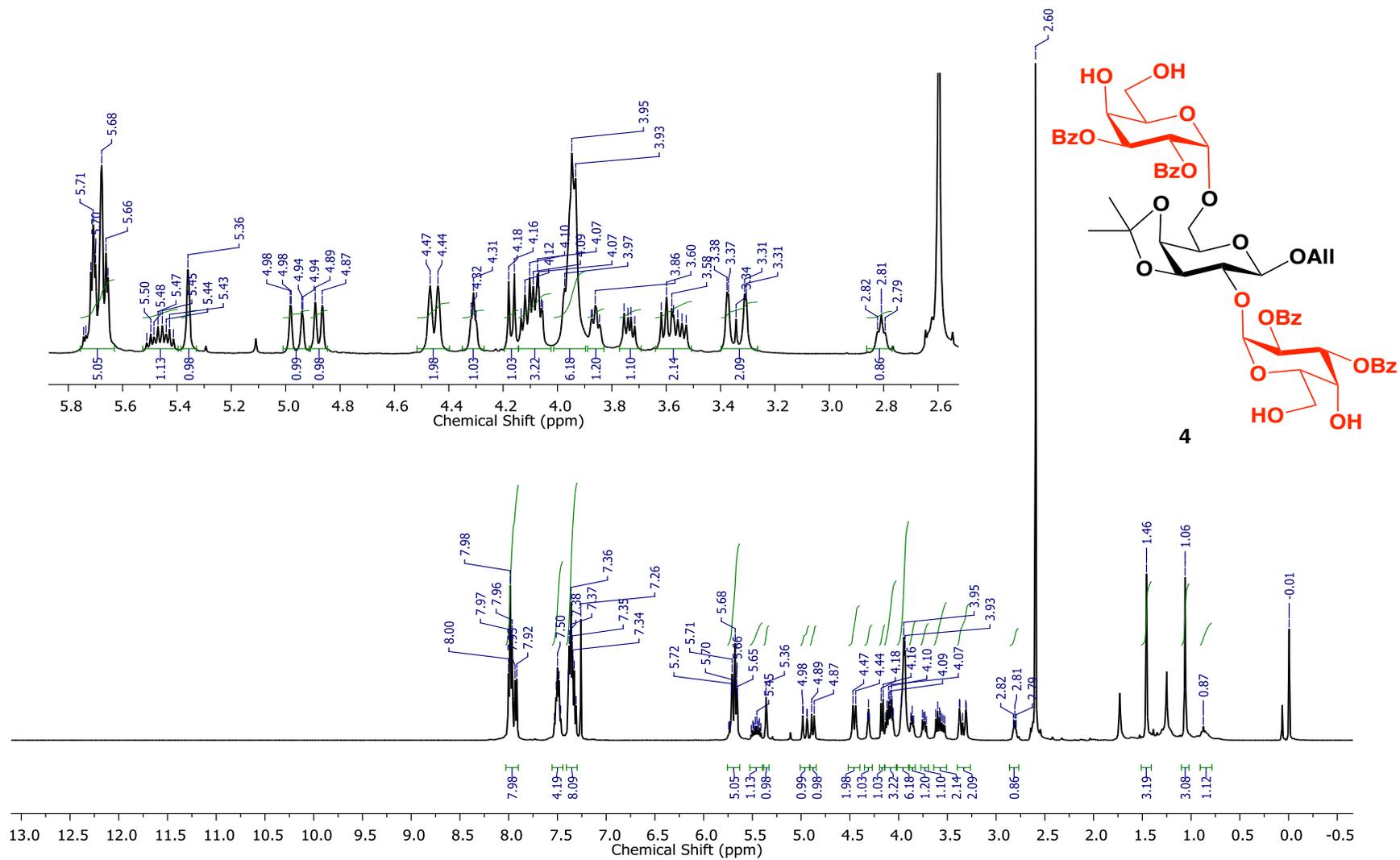


Figure S7: <sup>1</sup>H NMR spectrum of compound 4 (400 MHz, in CDCl<sub>3</sub>)

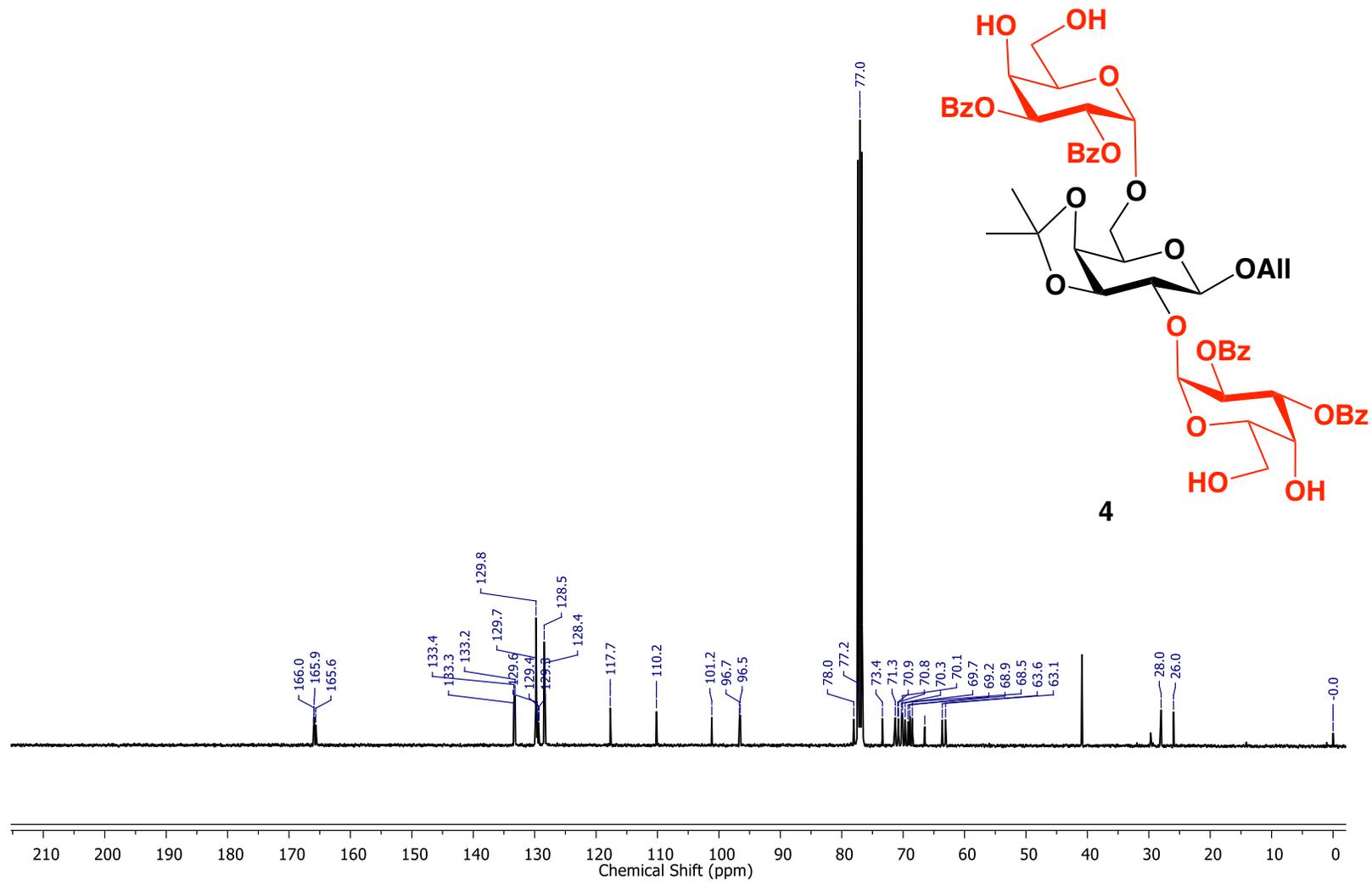


Figure S8: <sup>13</sup>C NMR spectrum of compound **4** (101 MHz, in CDCl<sub>3</sub>)

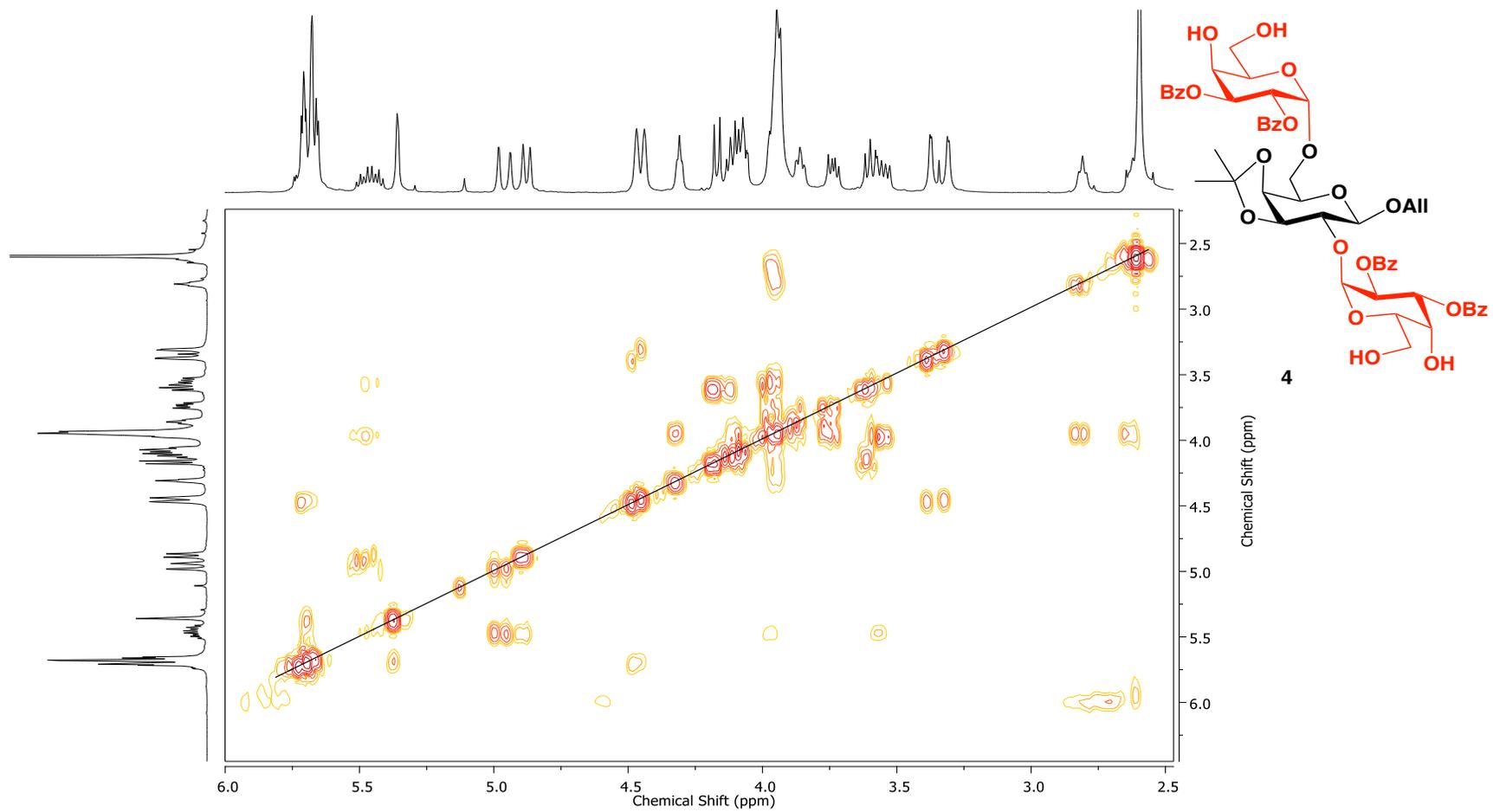


Figure S9: COSY NMR spectrum of compound **4** (in CDCl<sub>3</sub>)

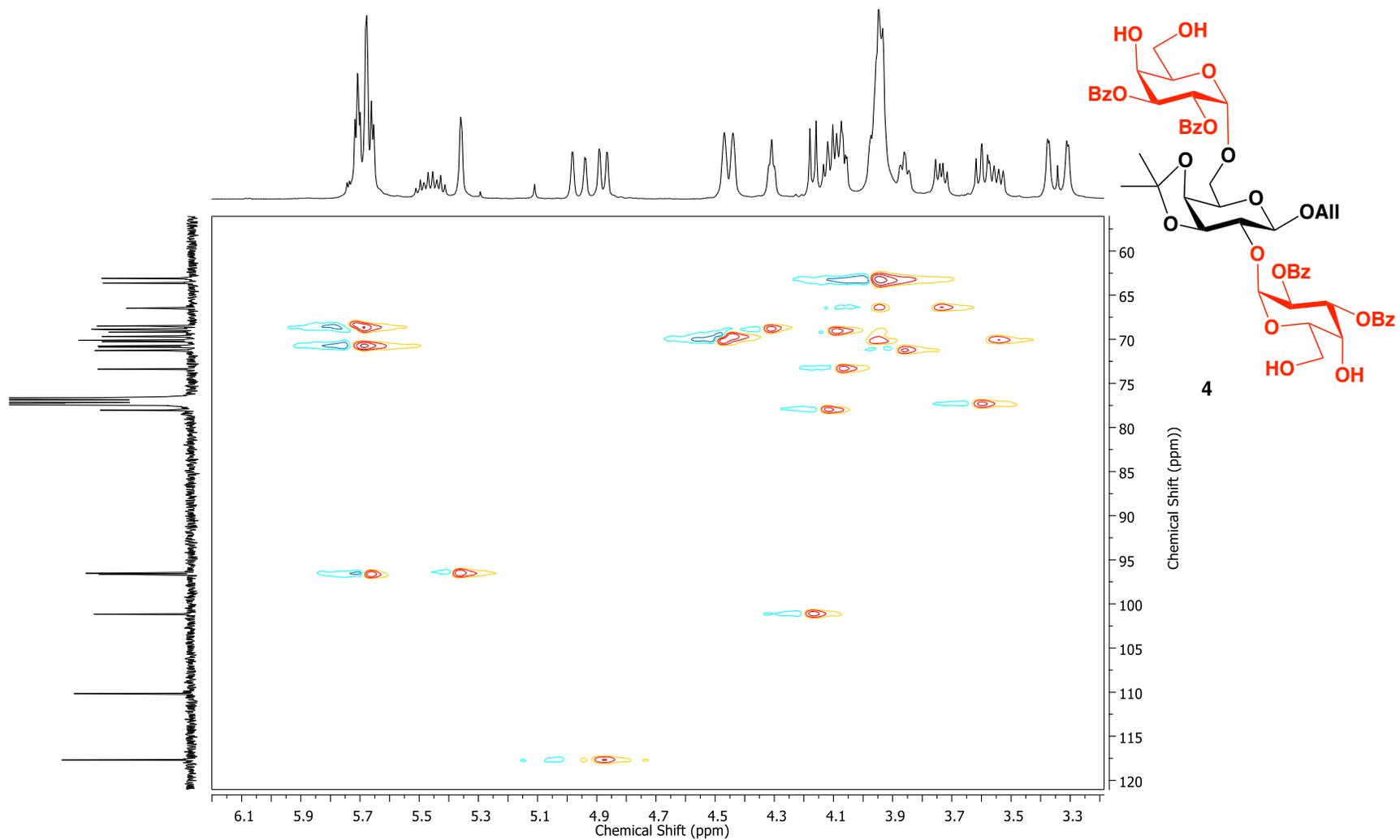


Figure S10: HSQC NMR spectrum of compound **4** (in CDCl<sub>3</sub>)

Acq. Data Name: EGC\_051319\_PEG\_5  
Creation Parameters: Average(MS[1] Time:3.4230..3.5170)

Experiment Date/Time: 5/20/2019 11:08:32 AI  
Ionization Mode: ESI

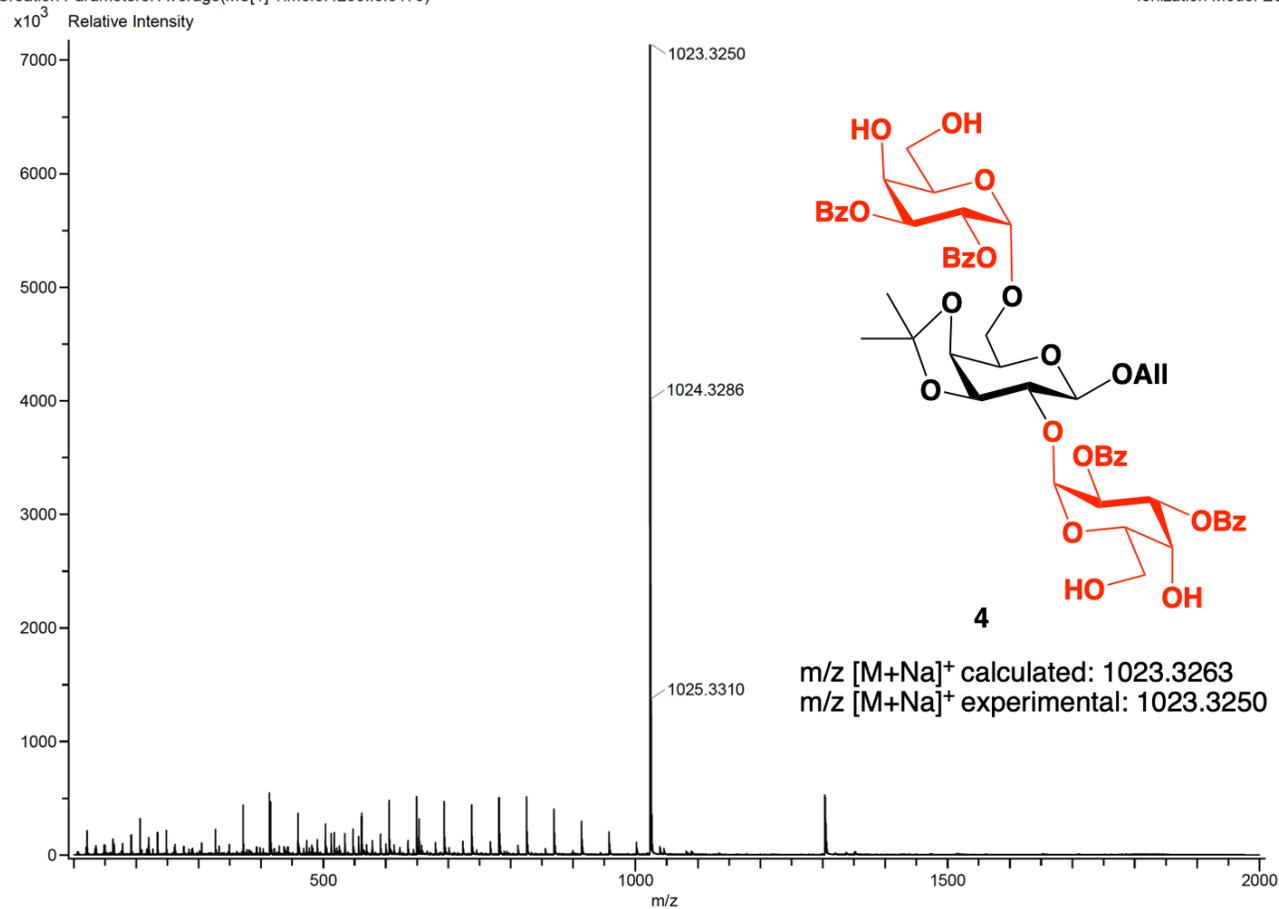
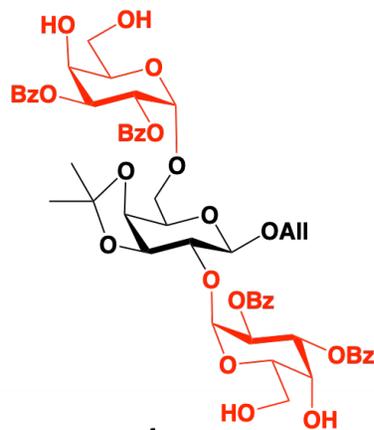


Figure S11: HR ESI-TOF mass spectrum of compound 4



$m/z$   $[M+Na]^+$  calculated: 1023.3263  
 $m/z$   $[M+Na]^+$  experimental: 1023.3250

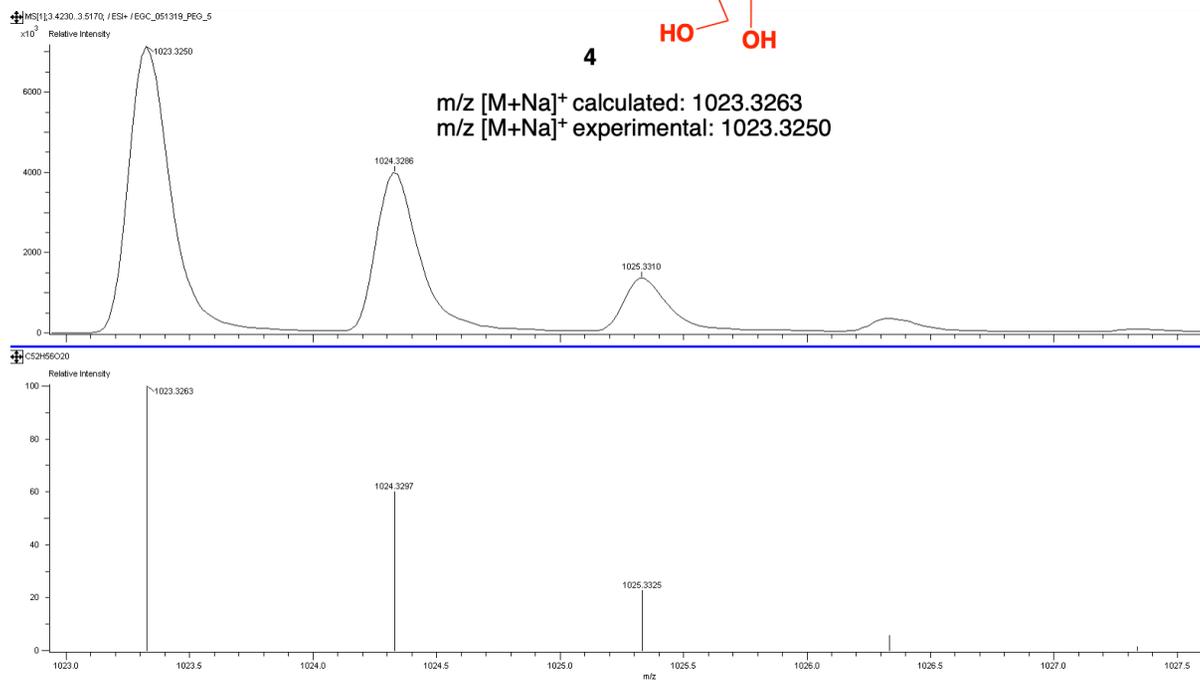


Figure S12: Actual (top) and simulated (bottom) HR ESI-TOF mass spectrum of compound 4

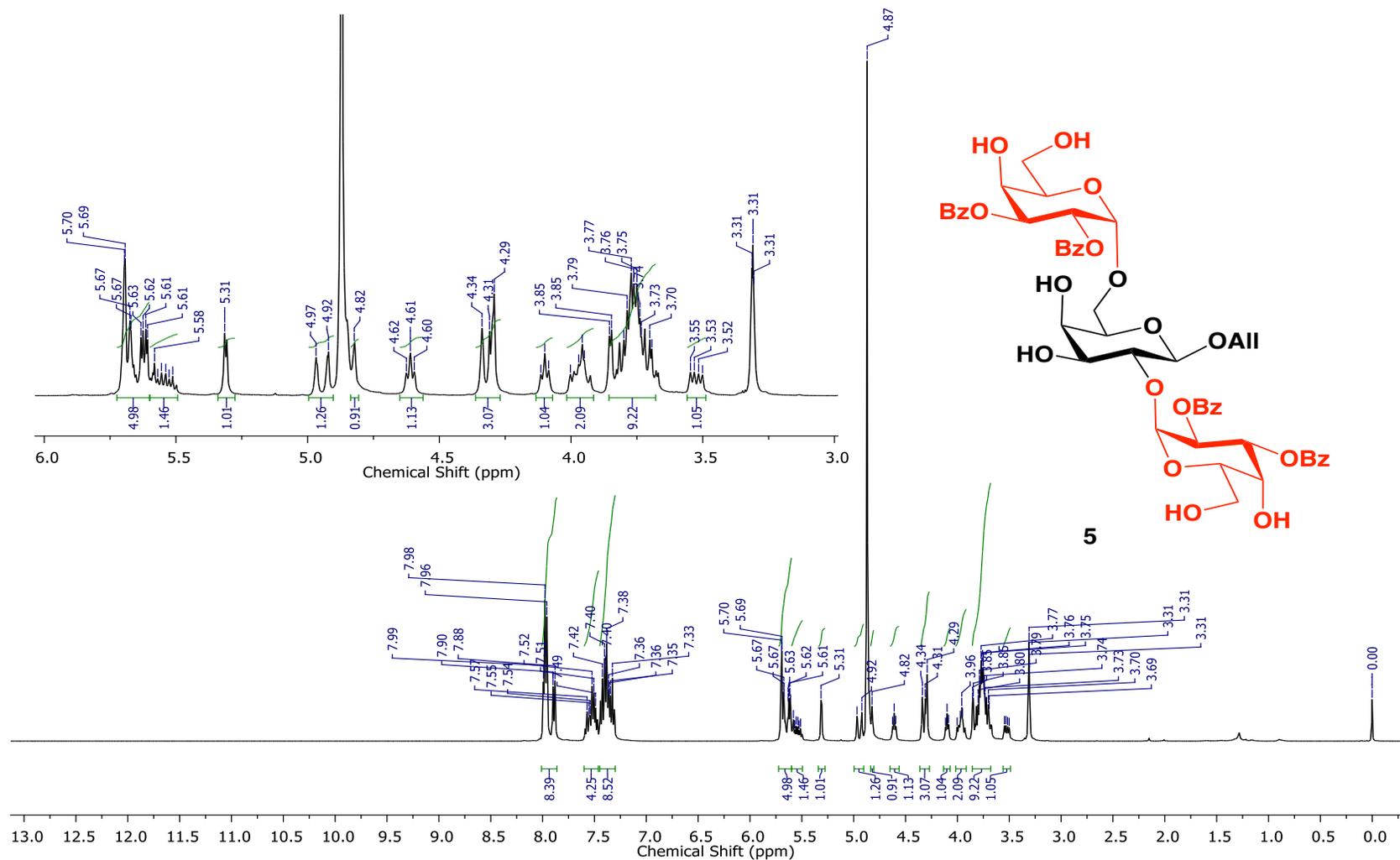


Figure S13:  $^1\text{H}$  NMR spectrum of compound 5 (400 MHz, in methanol- $d_4$ )

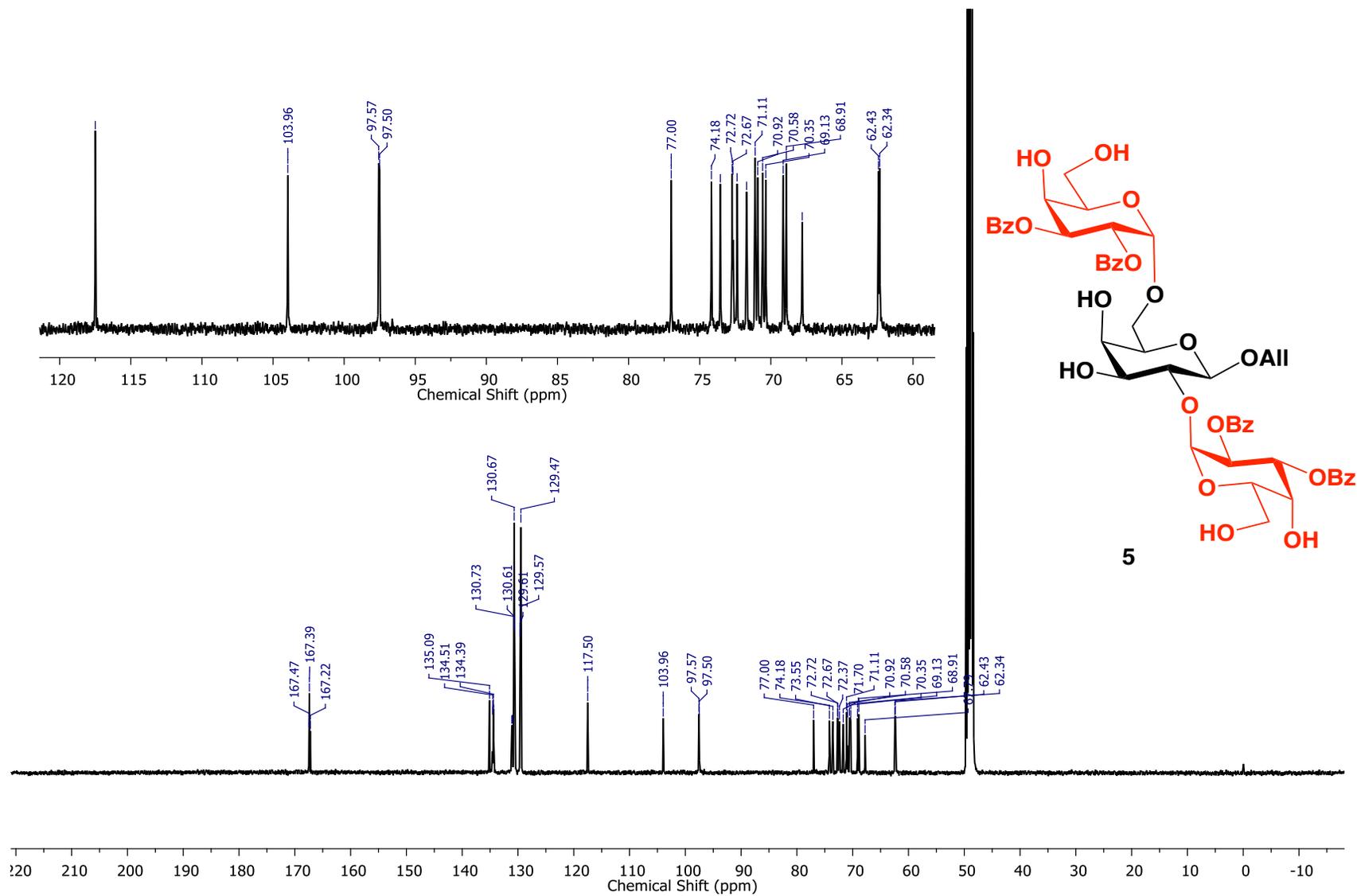
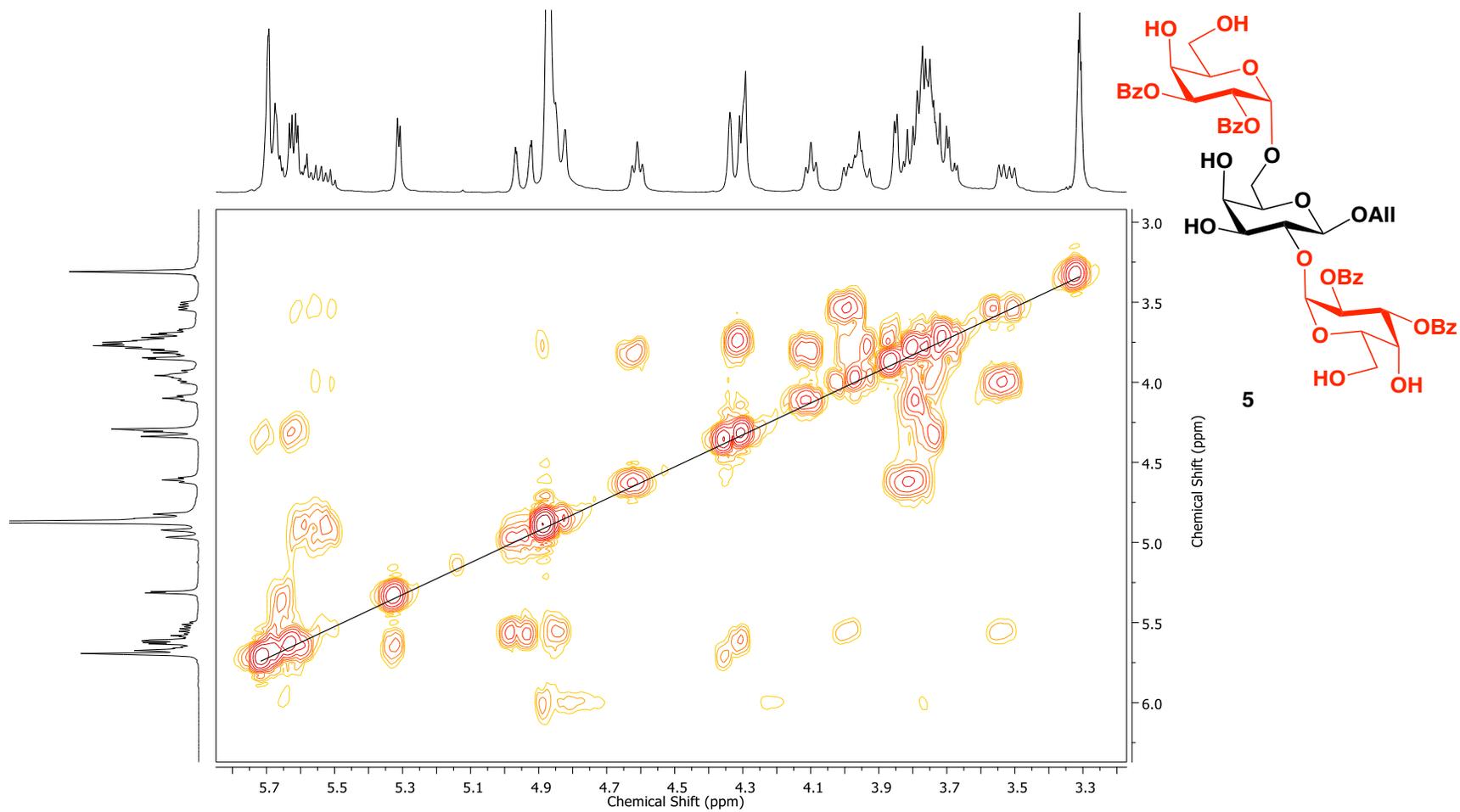


Figure S14:  $^{13}\text{C}$  NMR spectrum of compound **5** (101 MHz, in methanol- $\text{d}_4$ )



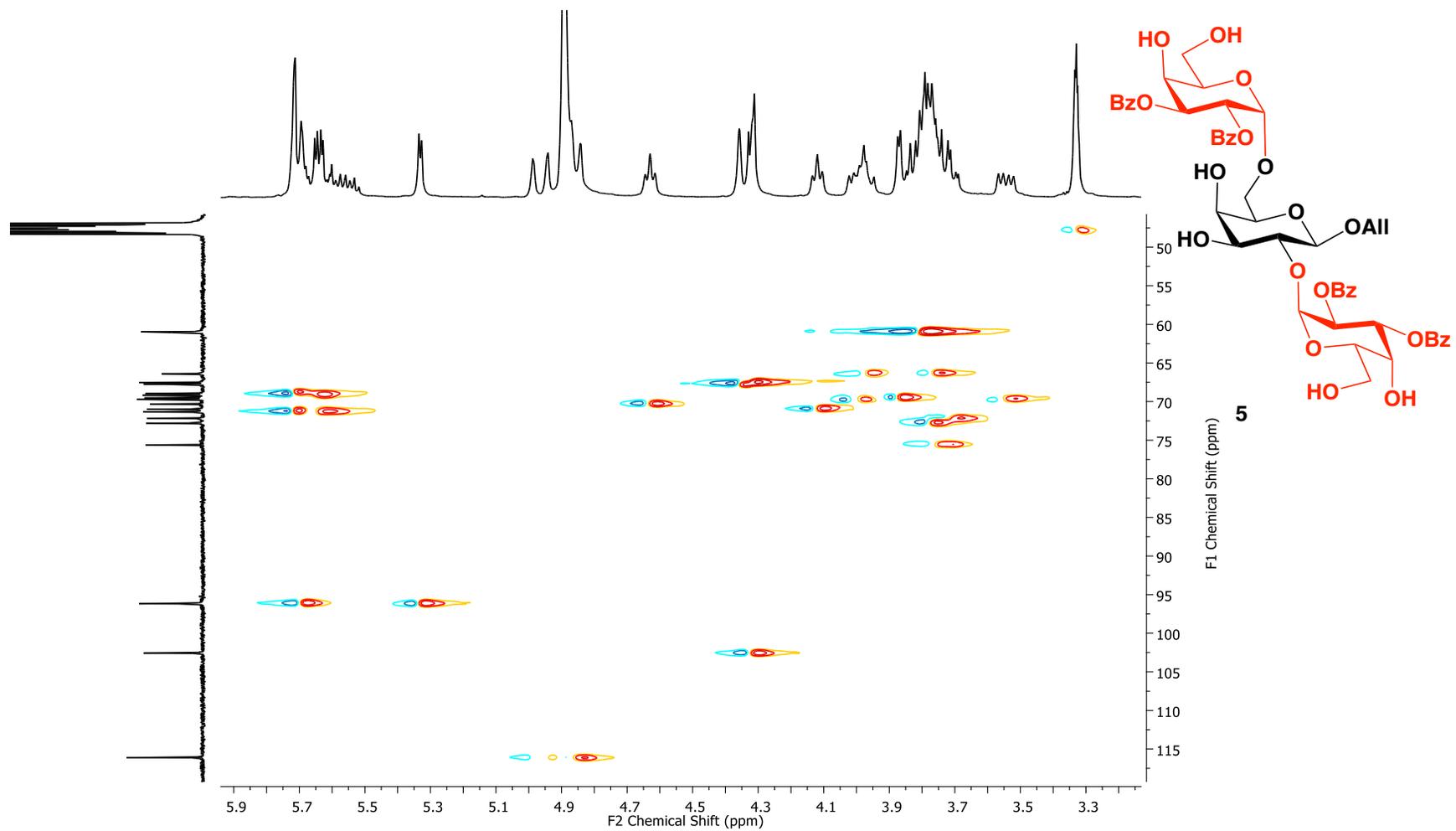


Figure S16: HSQC NMR spectrum of compound **5** (in methanol-d<sub>4</sub>)

Acq. Data Name: EGC\_052119\_ULTRAMARK\_2  
Creation Parameters: Average(MS[1] Time:0.2960..0.3560)

Experiment Date/Time: 5/22/2019 9:30:03 PT  
Ionization Mode: ESI

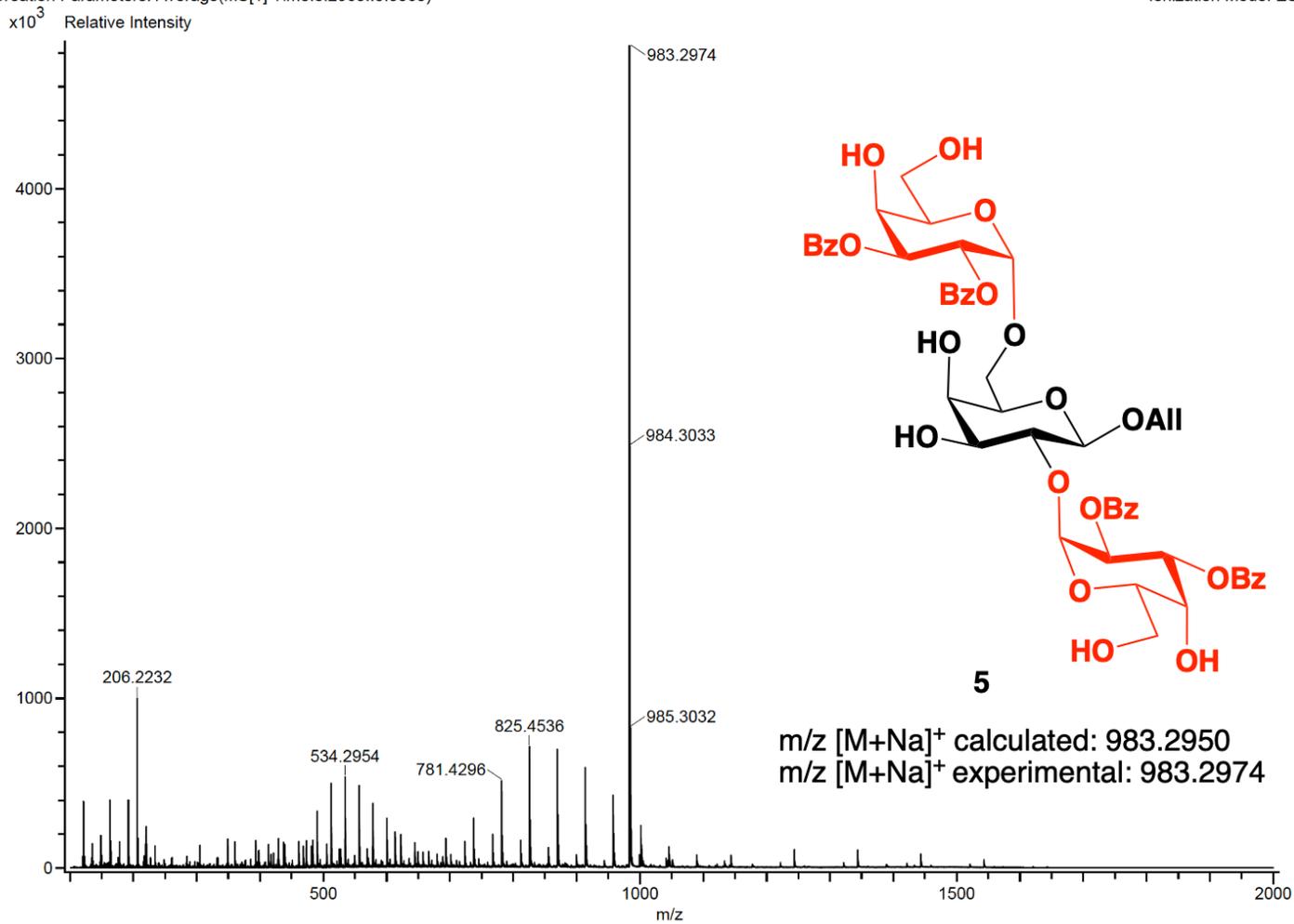


Figure S17: HR ESI-TOF mass spectrum of compound 5

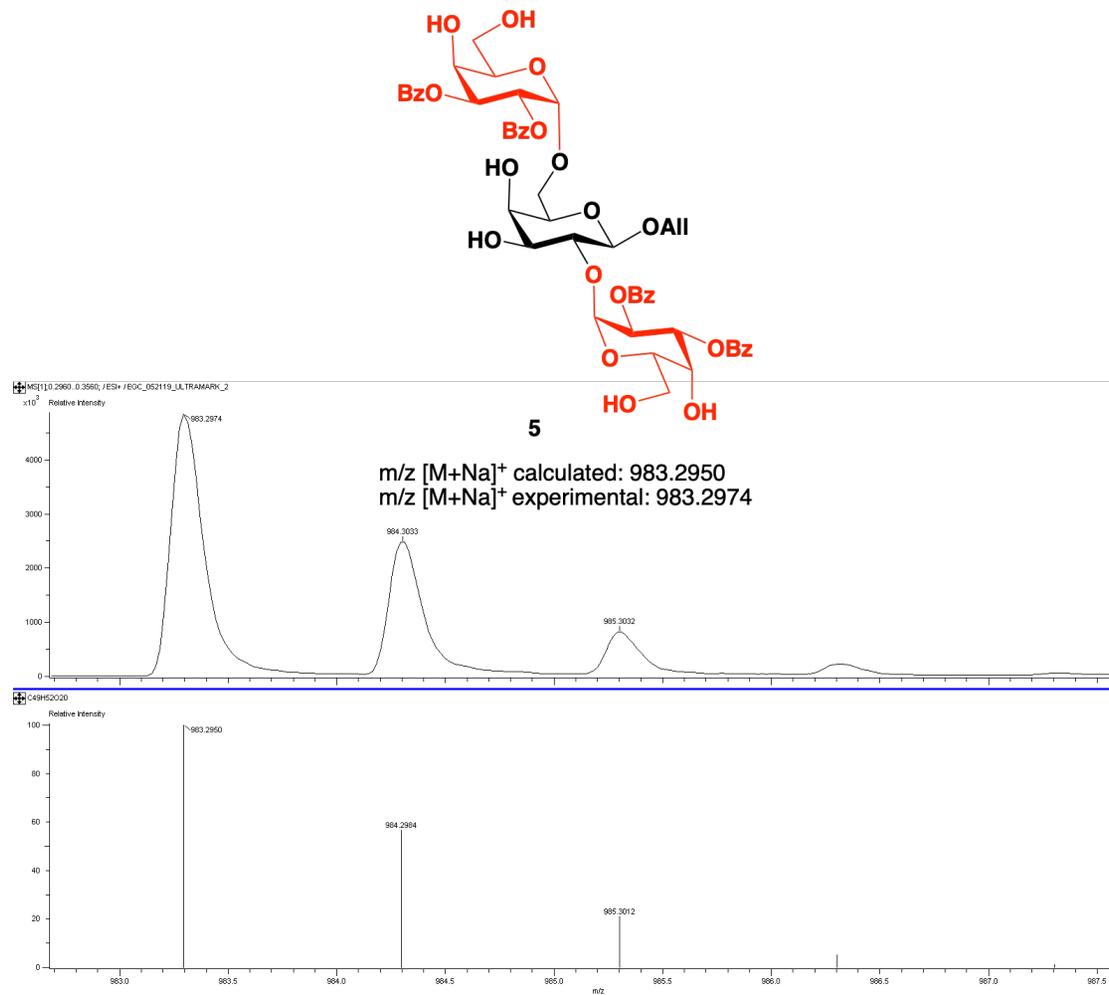


Figure S18: Actual (top) and simulated (bottom) HR ESI-TOF mass spectrum of compound **5**

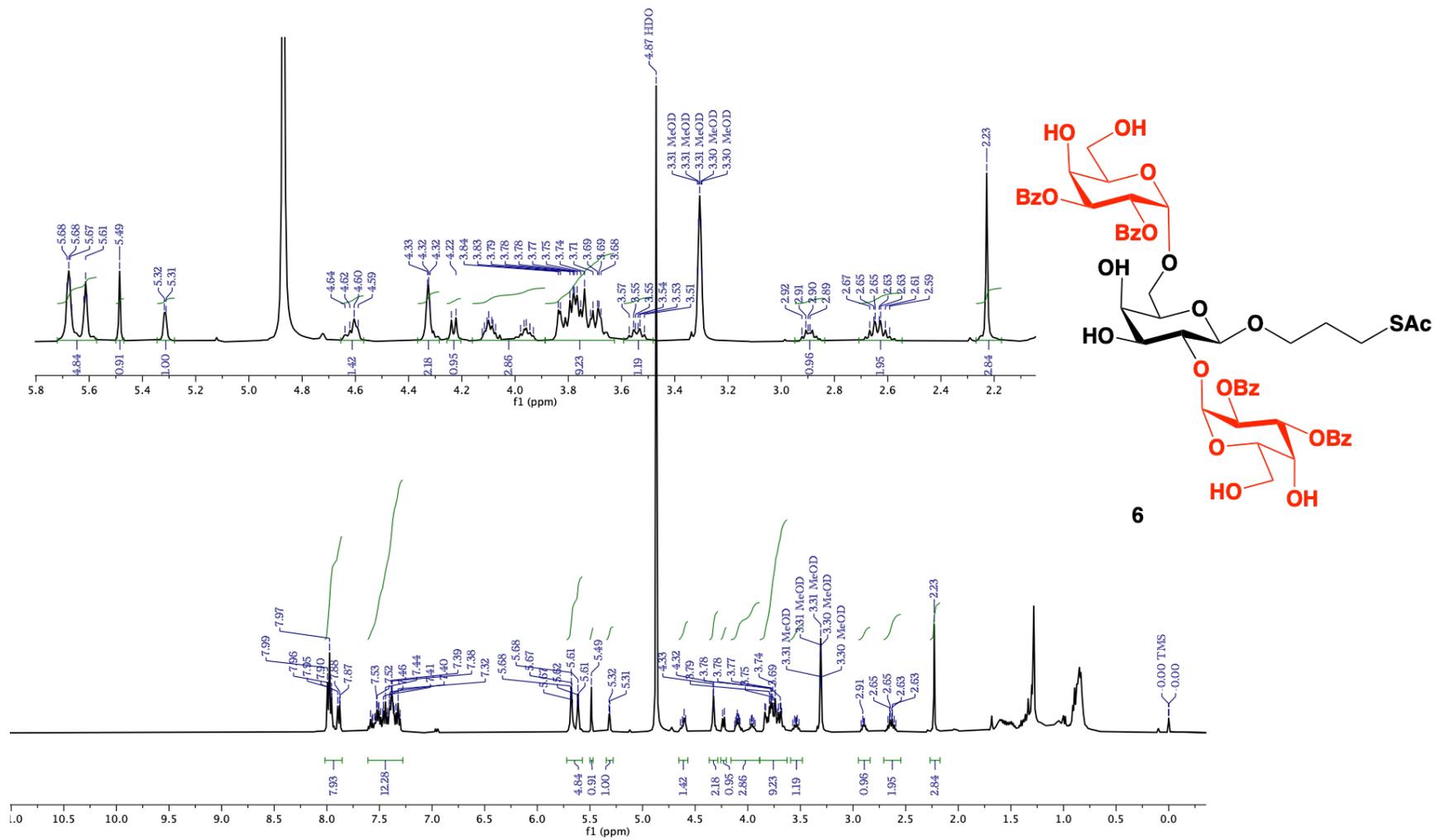


Figure S19:  $^1\text{H}$  NMR spectrum of compound **6** (in methanol- $d_4$ )

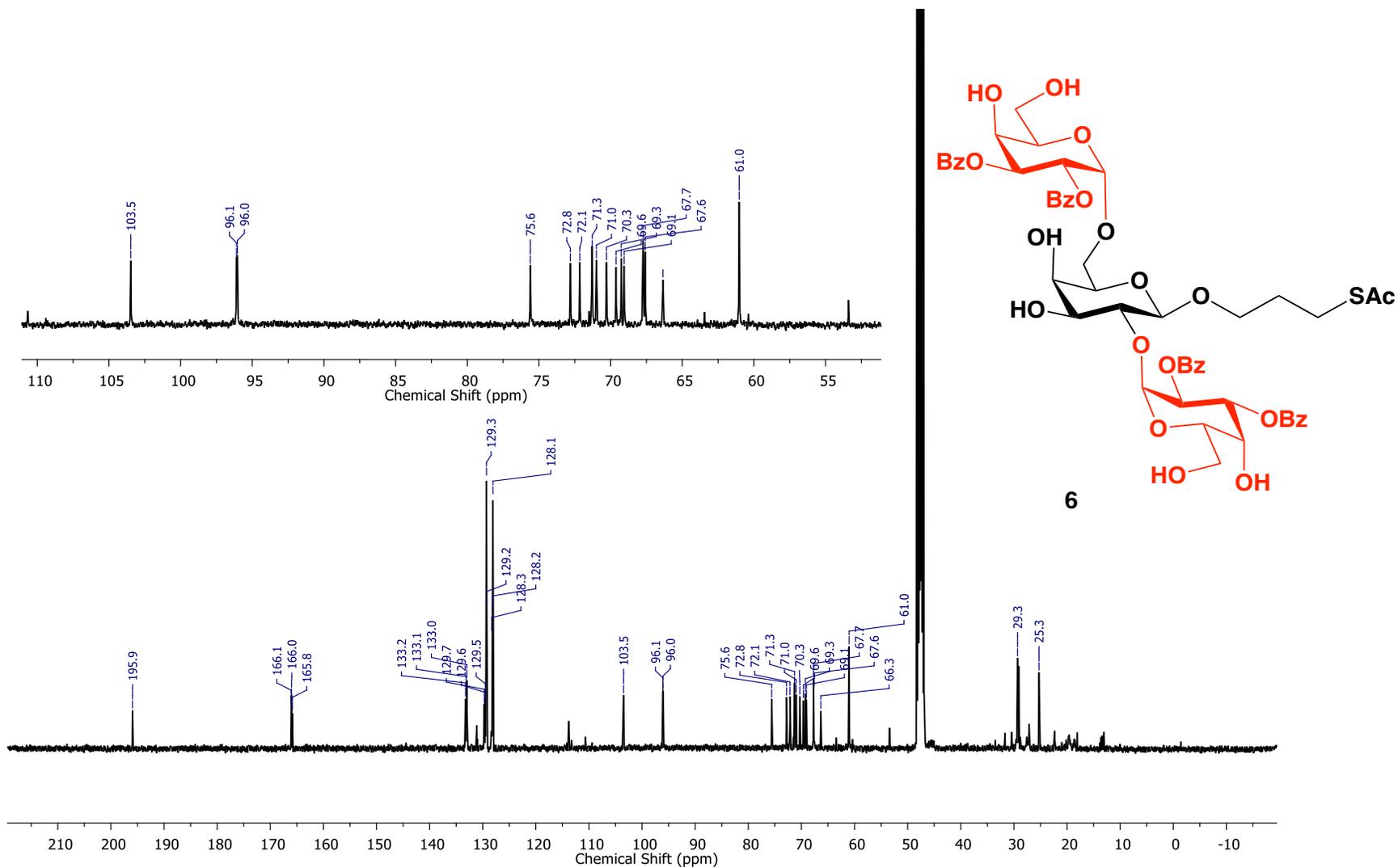


Figure S20:  $^{13}\text{C}$  NMR spectrum of compound **6** (in methanol- $\text{d}_4$ )

Acq. Data Name: EGC\_060619\_CRUDE  
Creation Parameters: Average(MS[1] Time:1.0770..1.2320)

Experiment Date/Time: 6/7/2019 11:50:12 AM  
Ionization Mode: ESI+

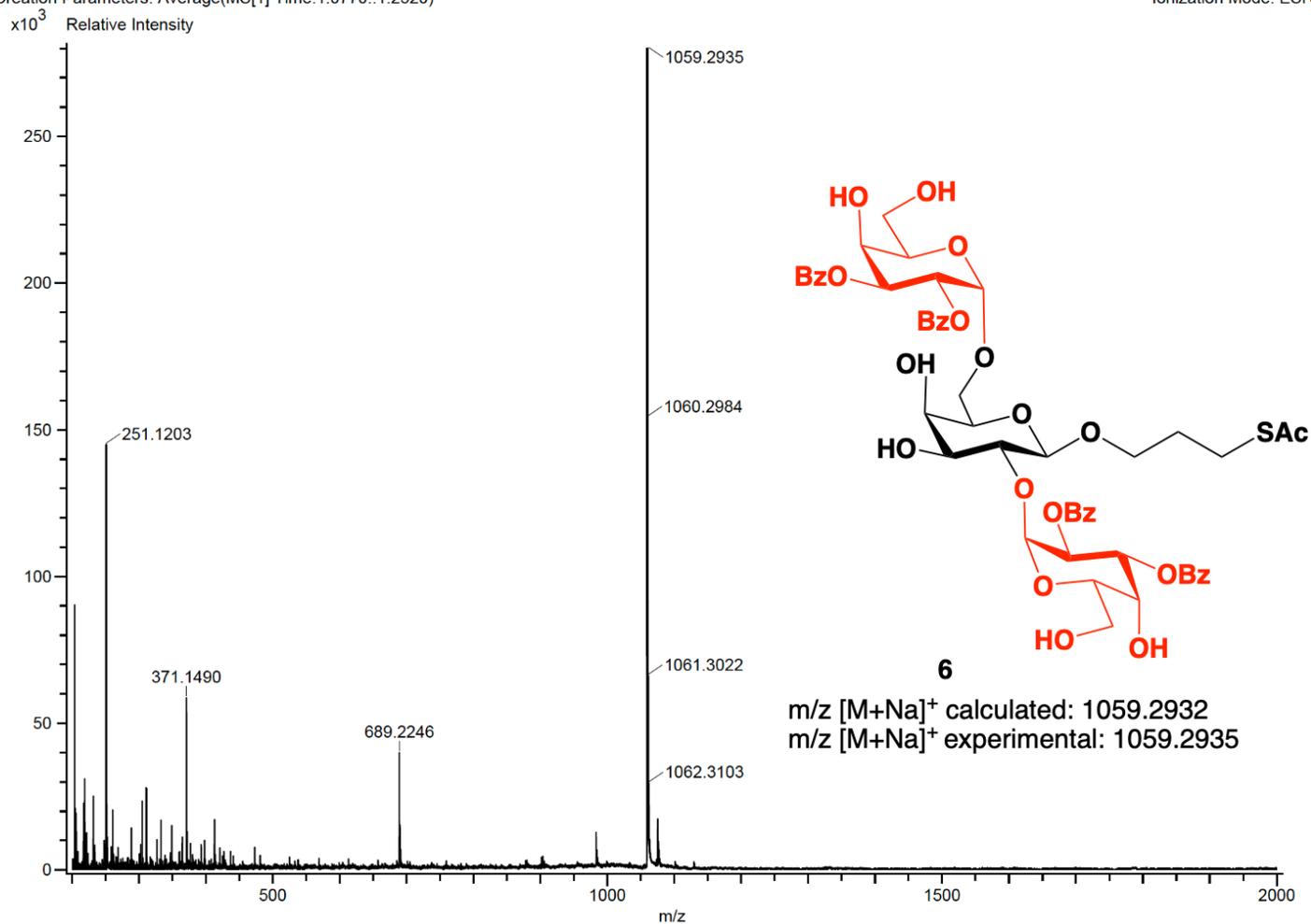
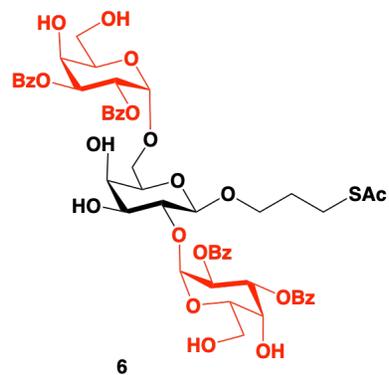


Figure S21: HR ESI-TOF mass spectrum of compound 6



m/z [M+Na]<sup>+</sup> calculated: 1059.2932  
m/z [M+Na]<sup>+</sup> experimental: 1059.2935

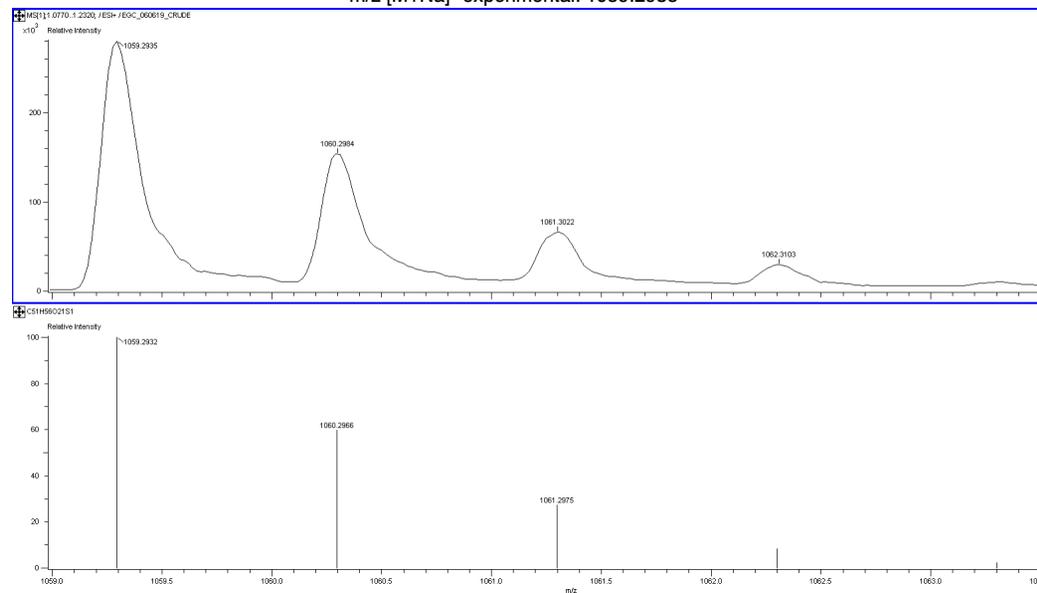


Figure S22: Actual (top) and simulated (bottom) HR ESI-TOF mass spectrum of compound **6**

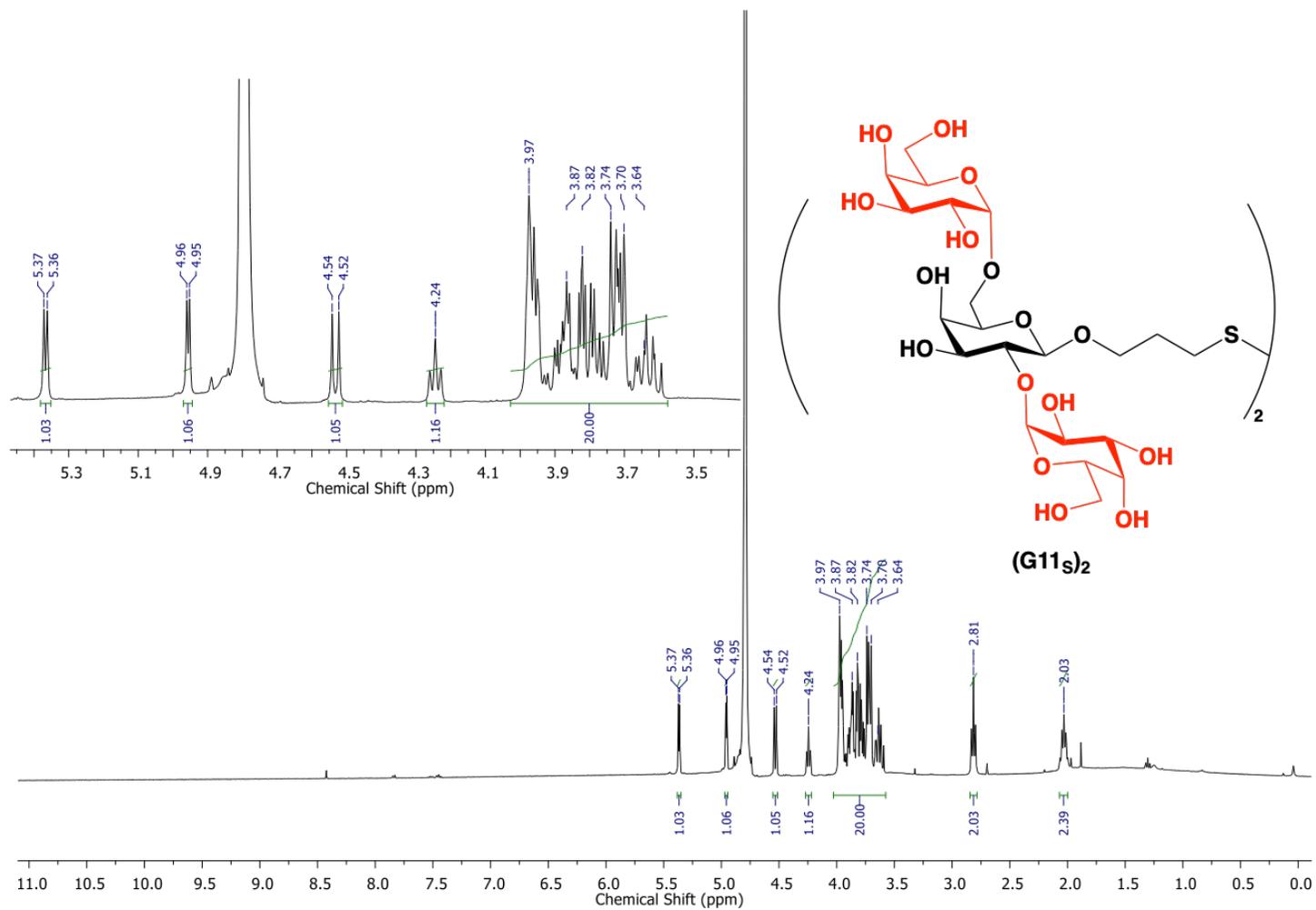


Figure S23:  $^1\text{H}$  NMR spectrum of compound  $(\text{G11s})_2$  (400 MHz, in  $\text{D}_2\text{O}$ )

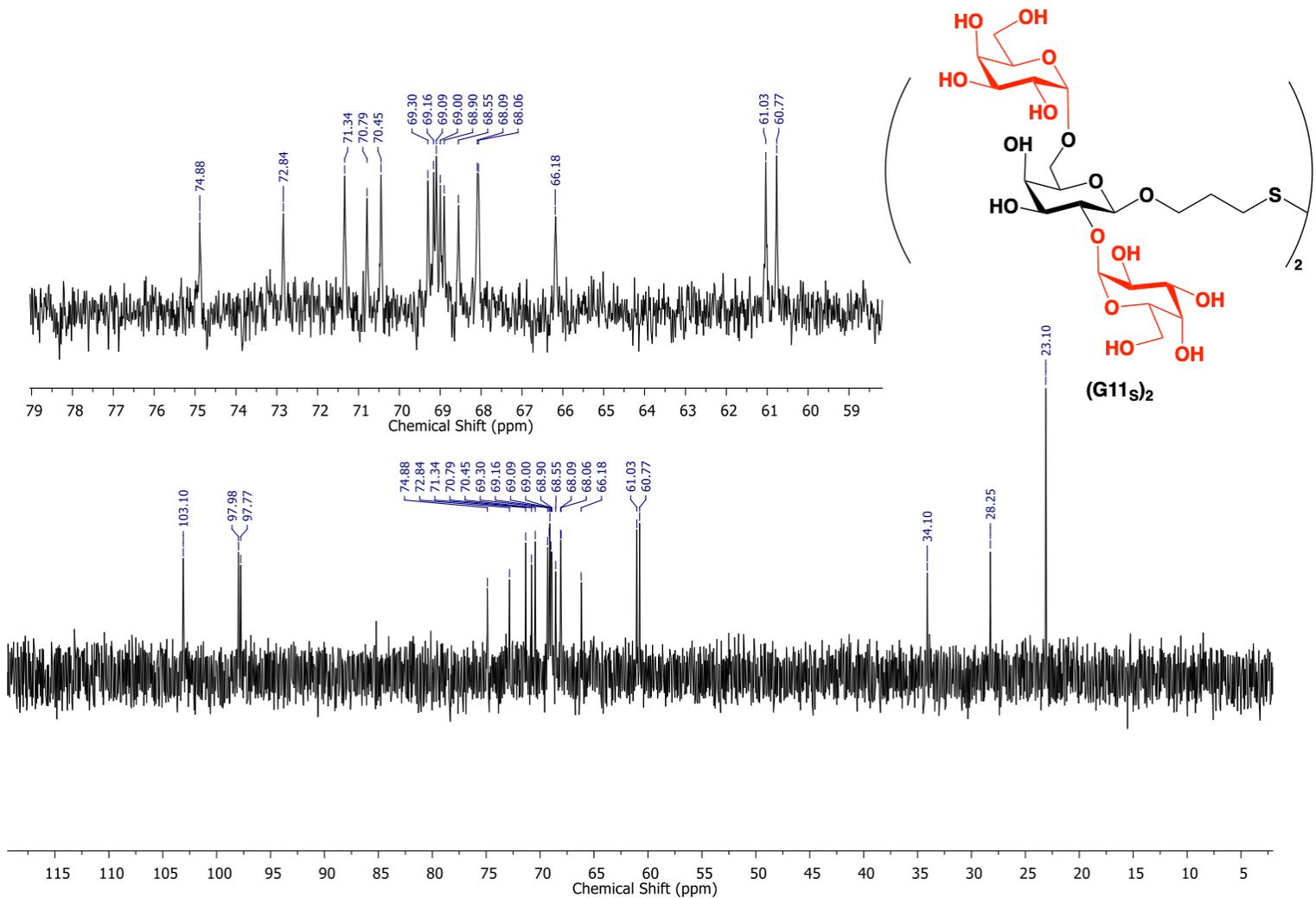


Figure S24: <sup>13</sup>C NMR spectrum of compound (G11s)<sub>2</sub> (101 MHz, in D<sub>2</sub>O)

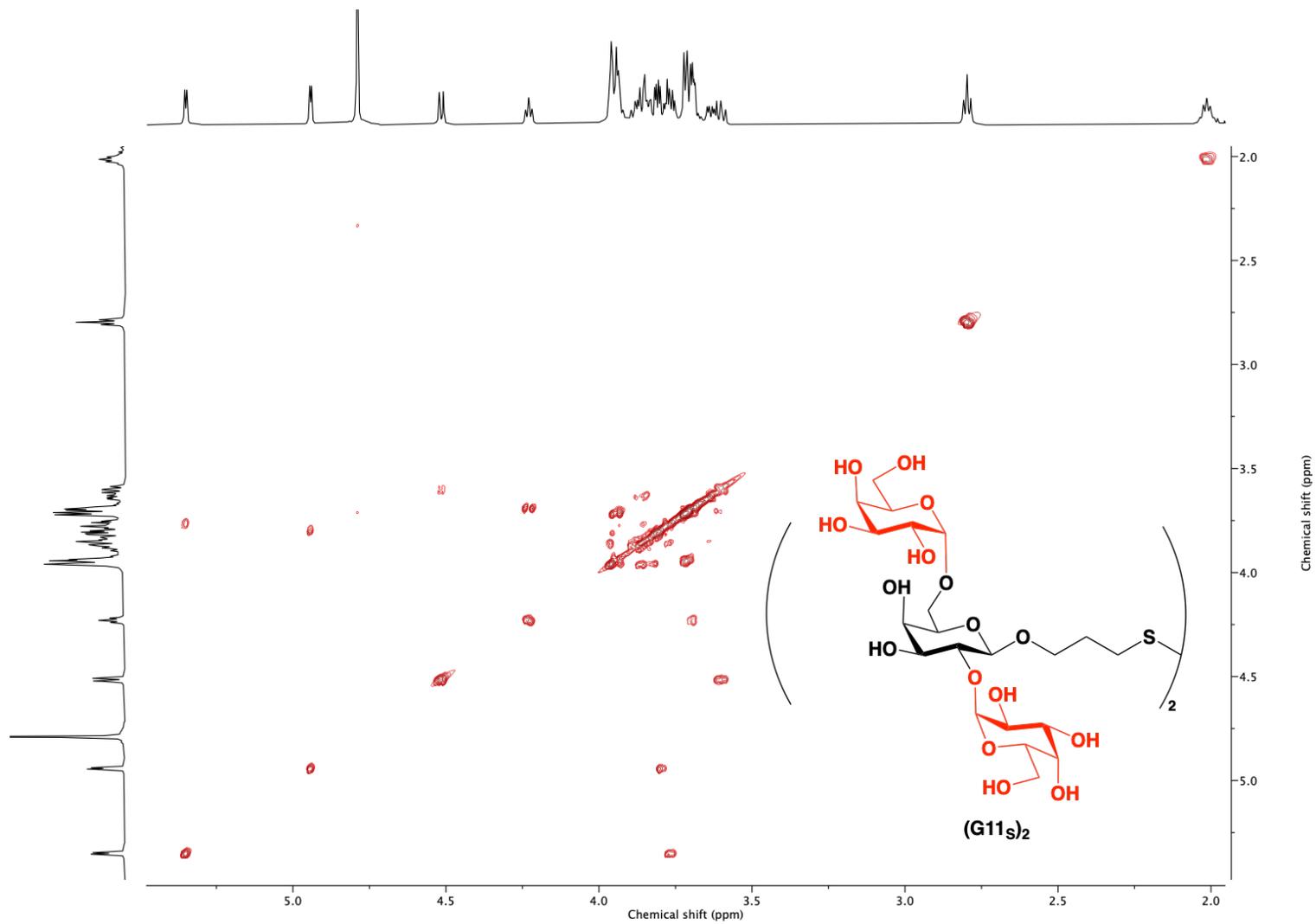


Figure S25: COSY NMR spectrum of compound  $(\text{G11s})_2$  (400 MHz, in  $\text{D}_2\text{O}$ )

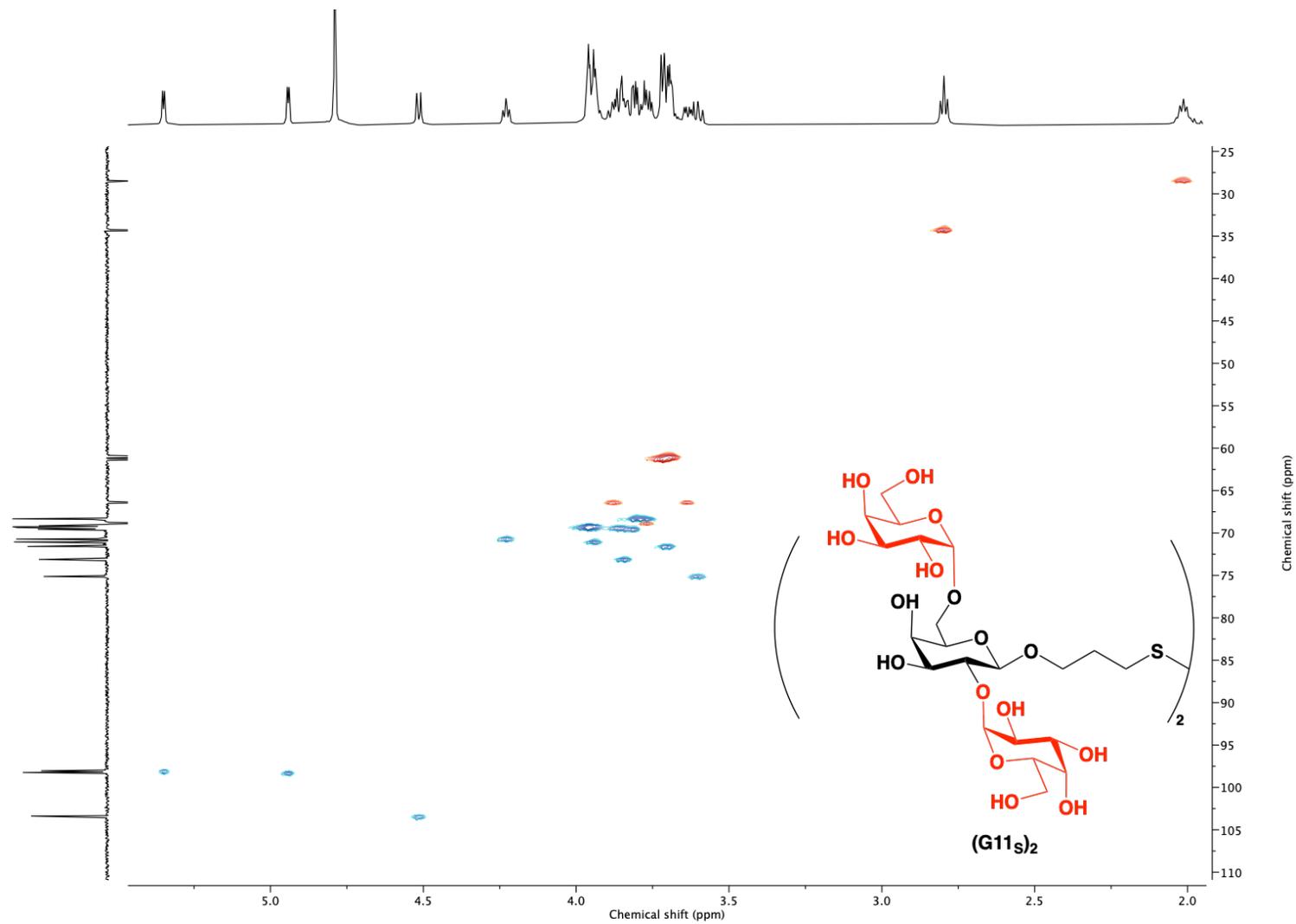


Figure S26: HSQC NMR spectrum of compound **(G11s)<sub>2</sub>** (in D<sub>2</sub>O)

Acq. Data Name: EGC\_KM11\_FPLC\_062421\_2min  
Creation Parameters: Average(MS[1] Time:0.8180..0.9270)

Experiment Date/Time: 6/24/2021 9:04:58 AM  
Ionization Mode: ESI+

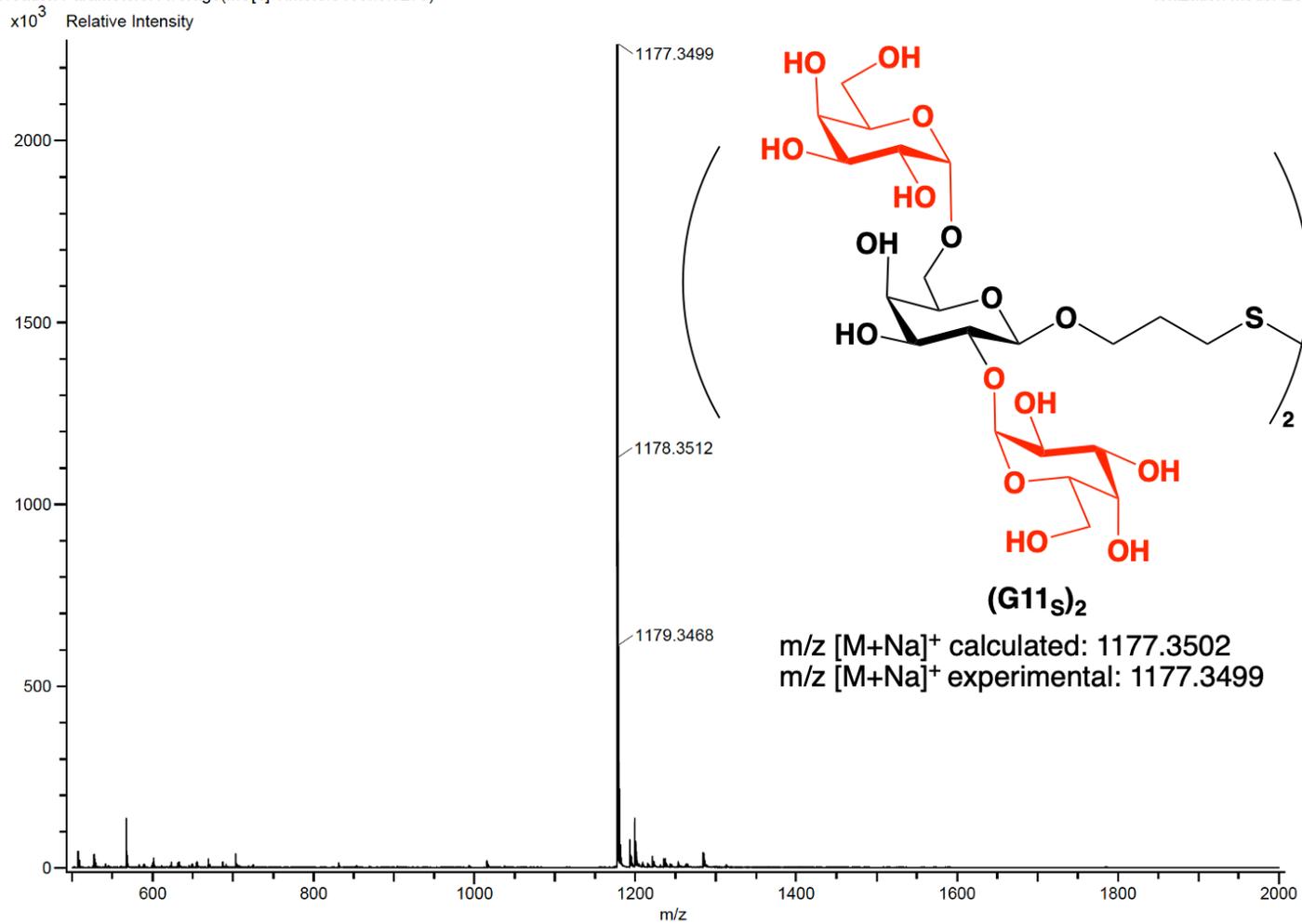
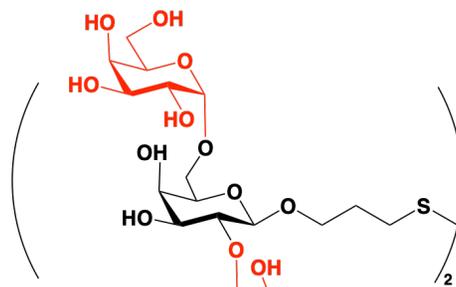


Figure S27: HR ESI-TOF mass spectrum of compound **(G11<sub>s</sub>)<sub>2</sub>**



(G11s)<sub>2</sub>

m/z [M+Na]<sup>+</sup> calculated: 1177.3502  
 m/z [M+Na]<sup>+</sup> experimental: 1177.3499

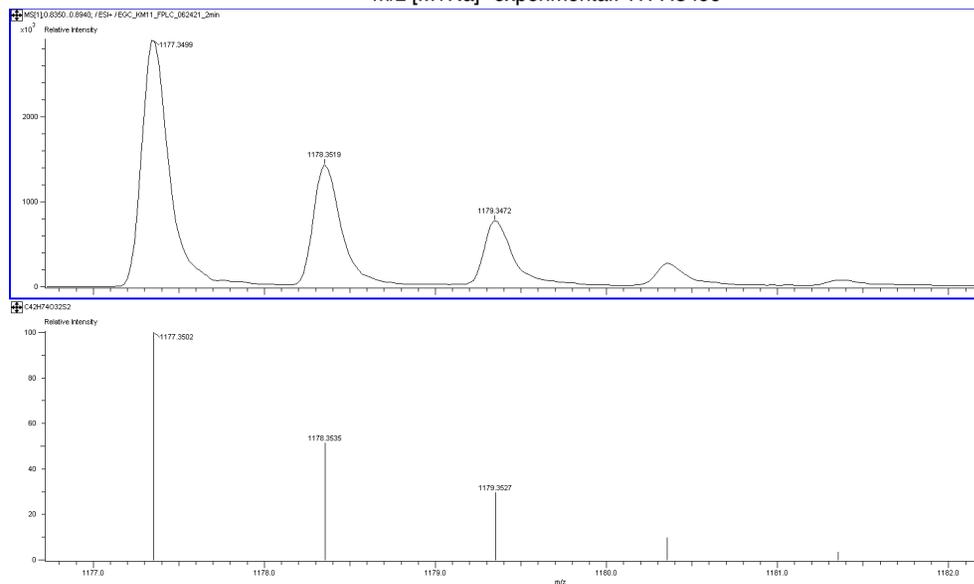


Figure S28: Actual (top) and simulated (bottom) HR ESI-TOF mass spectrum of compound (G11s)<sub>2</sub>