

SUPPLEMENTARY MATERIALS

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

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GENERAL INFORMATION

^1H and ^{13}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 (δ 0.00 ppm) was used as a reference to determine the chemical shift δ [ppm] as an internal standard in CDCl_3 and CD_3OD or relative to the CDCl_3 signal (δ 77.0 ppm) in ^{13}C NMR spectra. When spectra were measured in D_2O , a solution of tetramethylsilane in CDCl_3 in a sealed capillary was used as an external standard for calibration. The coupling constants J [Hz] were measured from one-dimensional ^1H -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

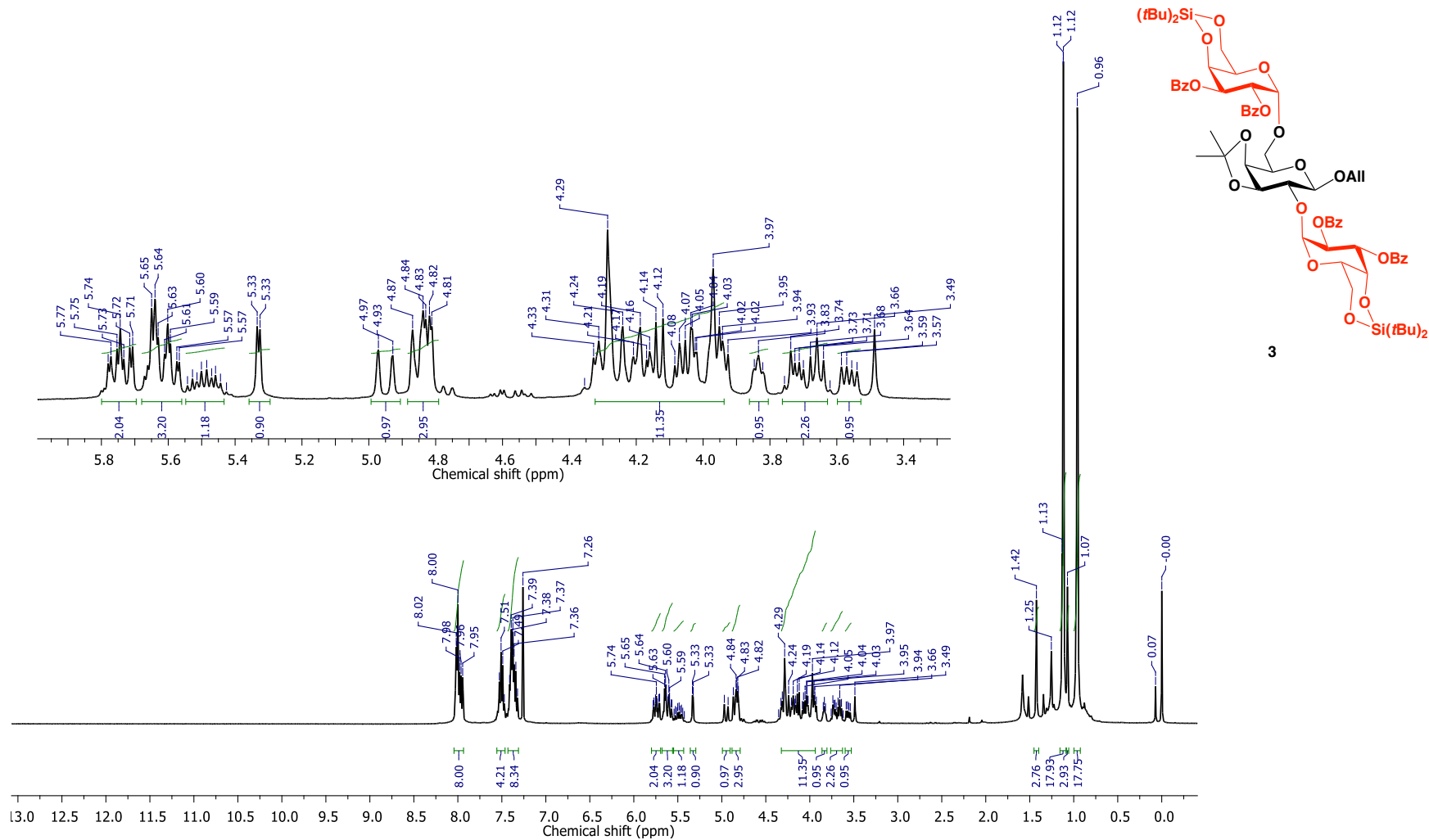


Figure S1: ^1H NMR spectrum of compound **3**, (400 MHz, in CDCl_3)

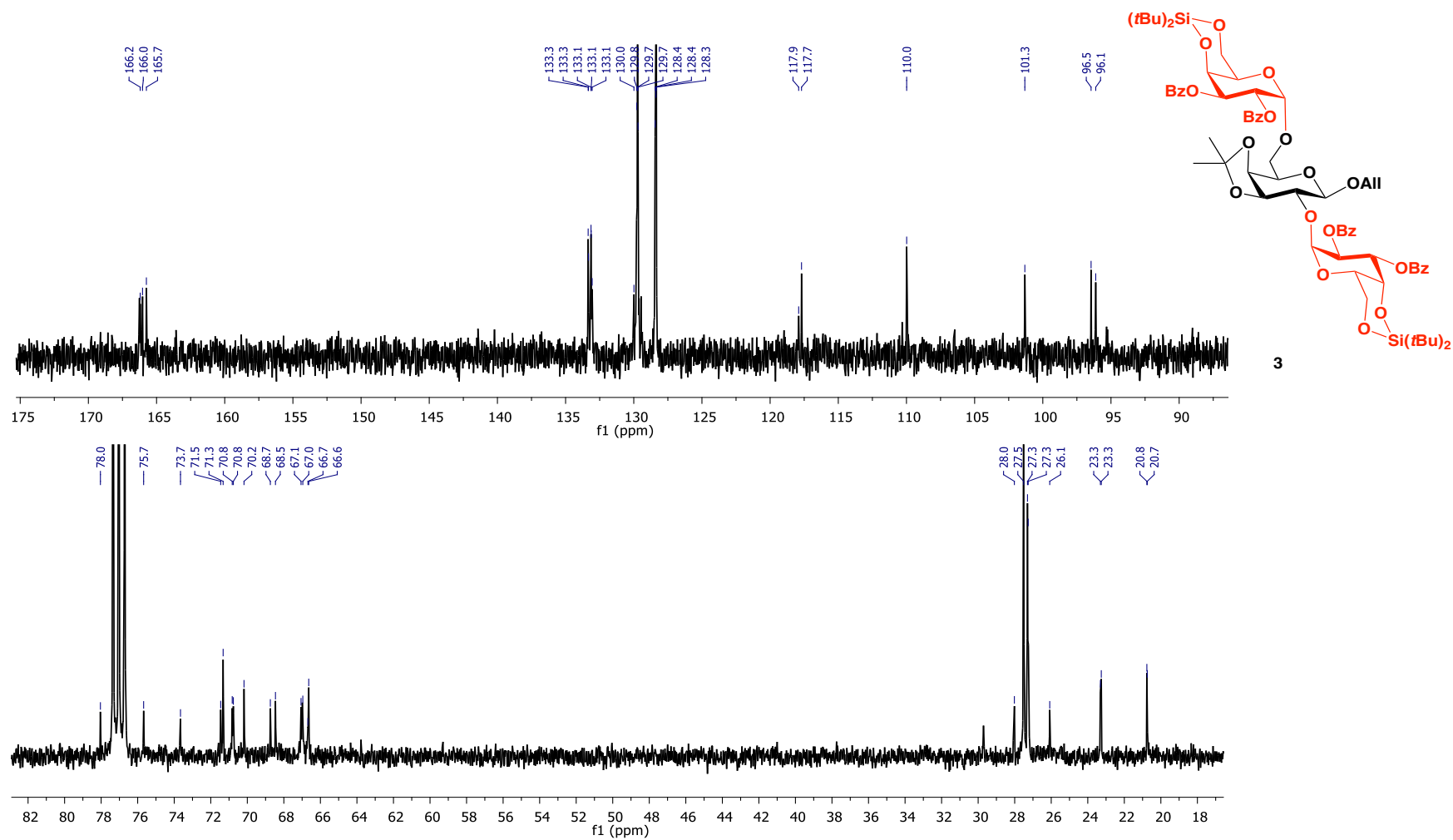


Figure S2: ^{13}C NMR spectrum of compound **3** (101 MHz, in CDCl_3)

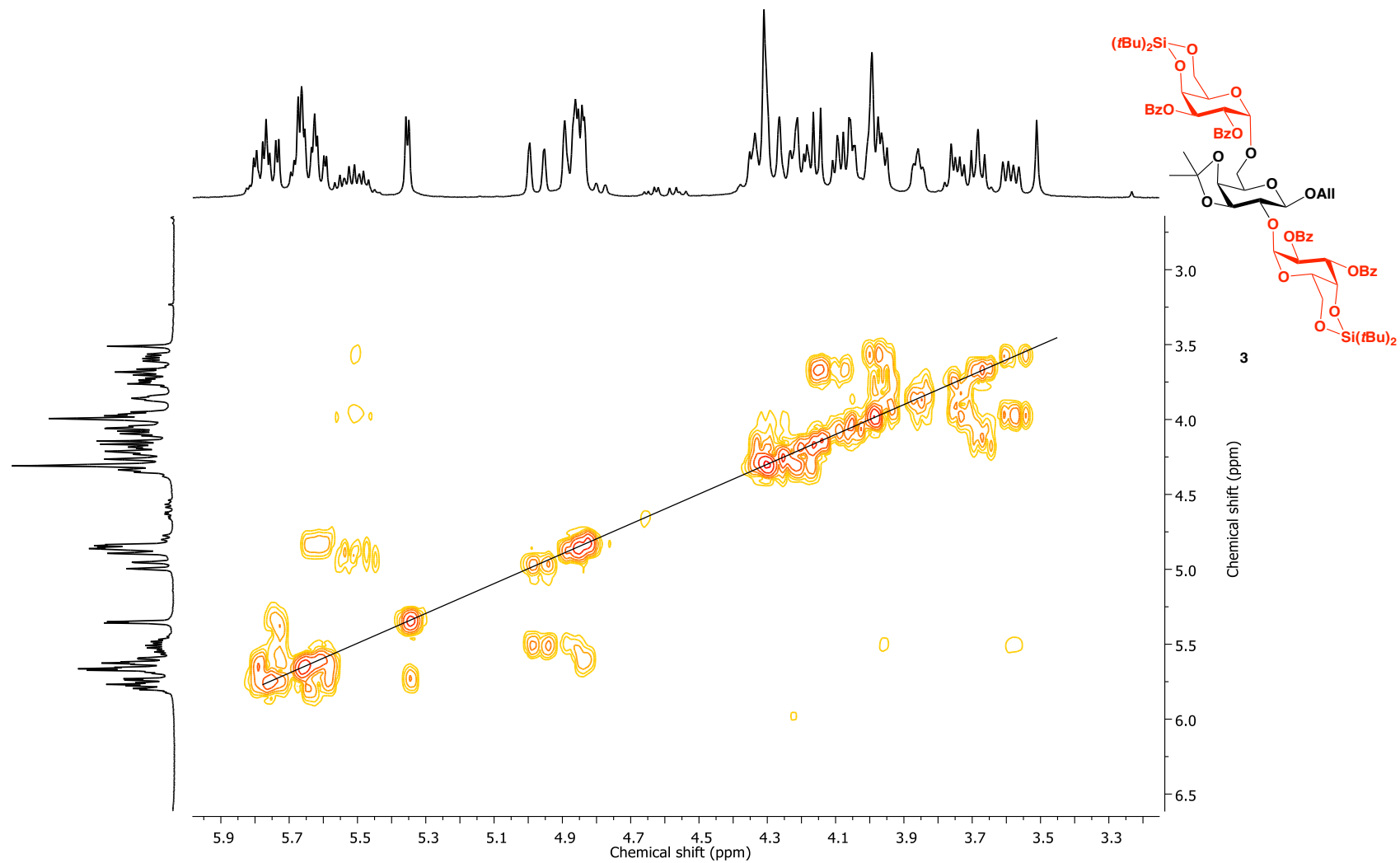


Figure S3: COSY NMR spectrum of compound **3** (400 MHz, in CDCl₃)

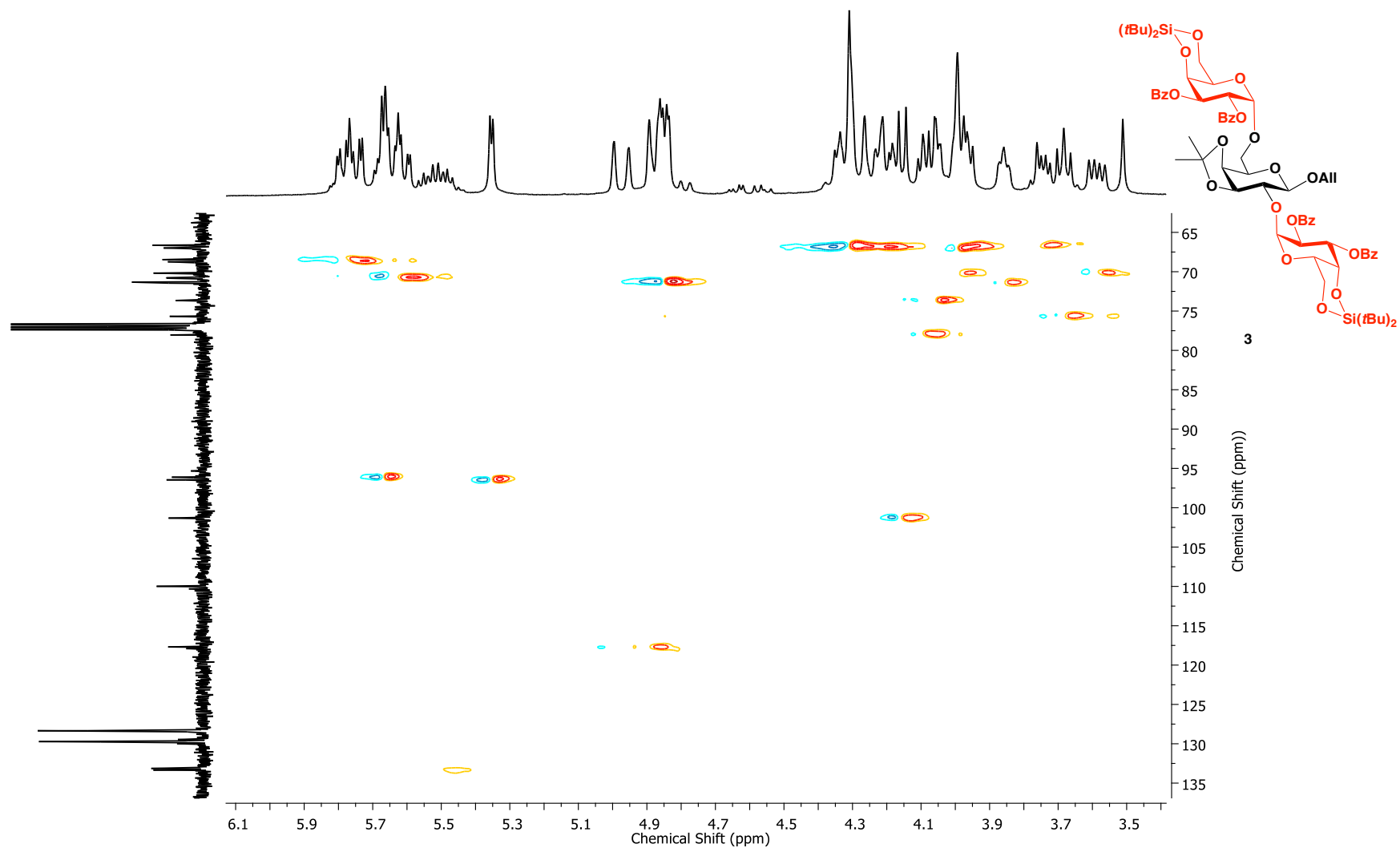


Figure S4: HSQC NMR spectrum of compound **3** (in CDCl_3)

Acq. Data Name: EGC_051319_ULTRAMARK
Creation Parameters: Average(MS[1] Time:0.5670..0.6310)
x10³ Relative Intensity

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

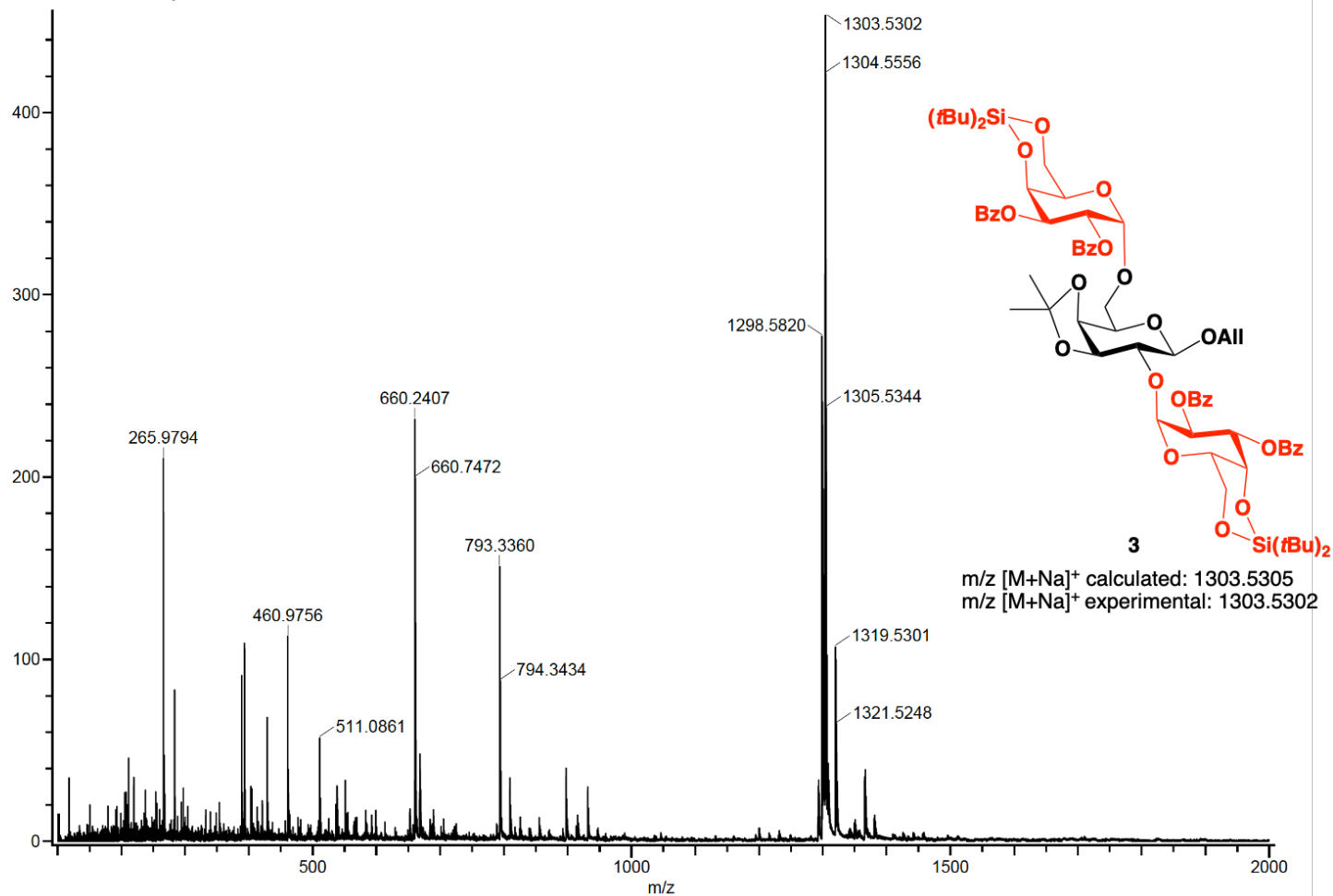


Figure S5: HR ESI-TOF mass spectrum of compound **3**

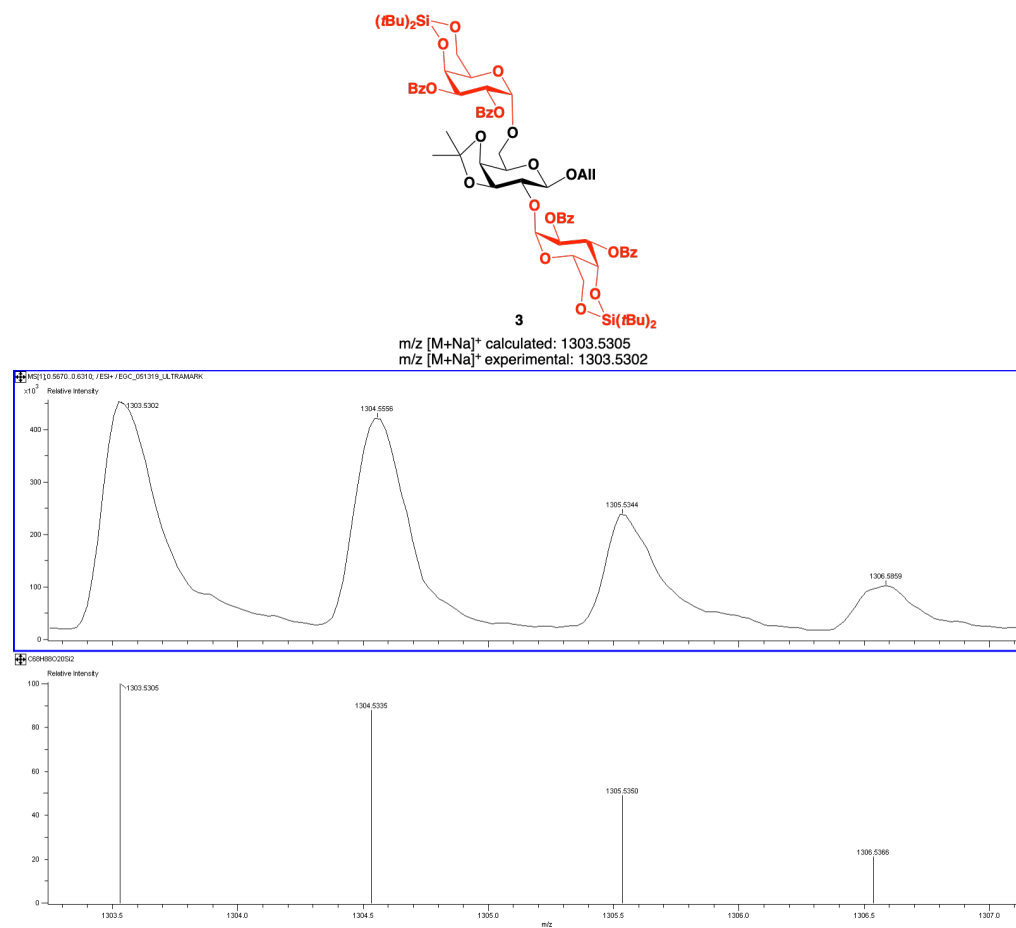


Figure S6: Actual and simulated HR ESI-TOF mass spectrum of compound **3**

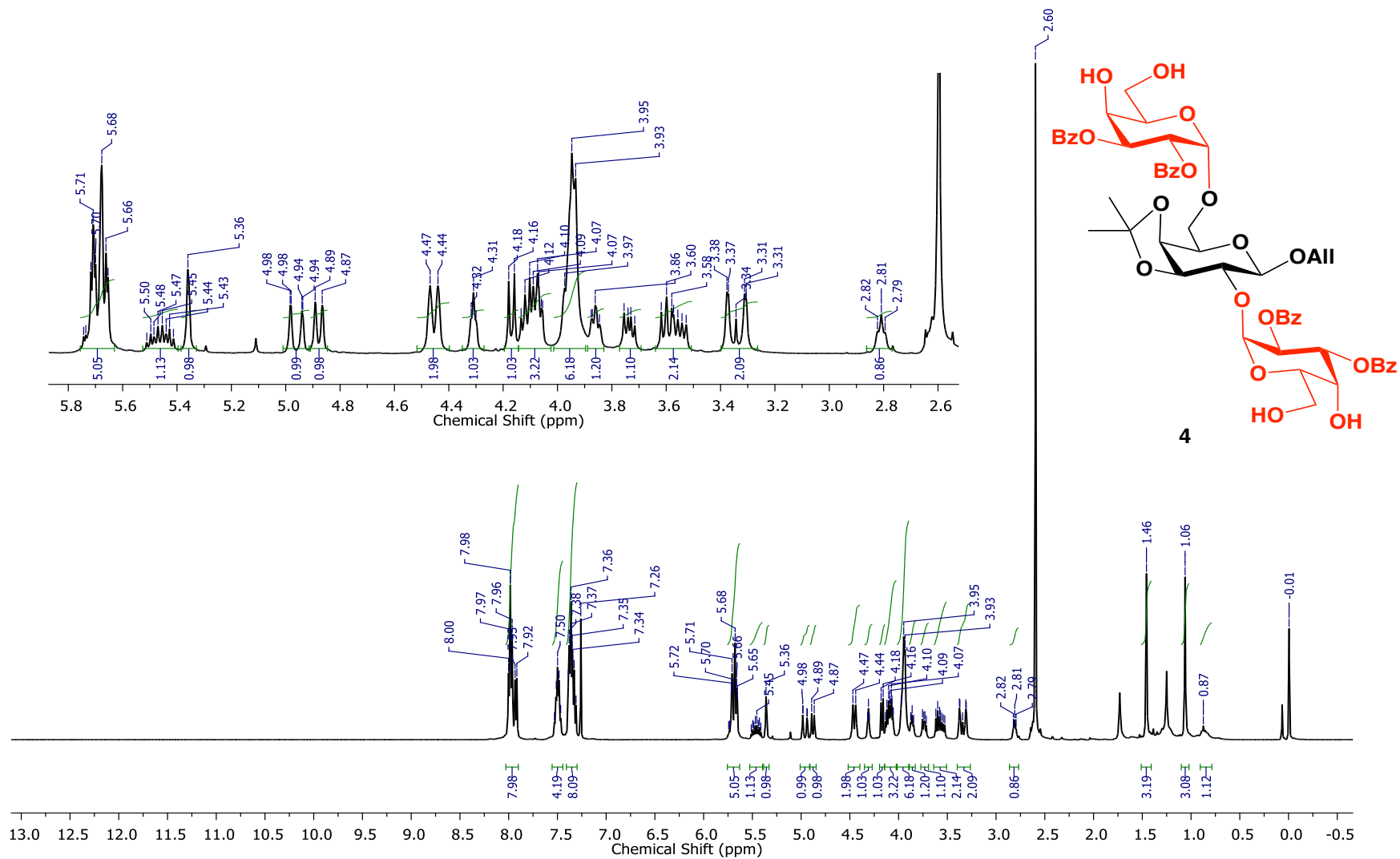


Figure S7: ^1H NMR spectrum of compound **4** (400 MHz, in CDCl_3)

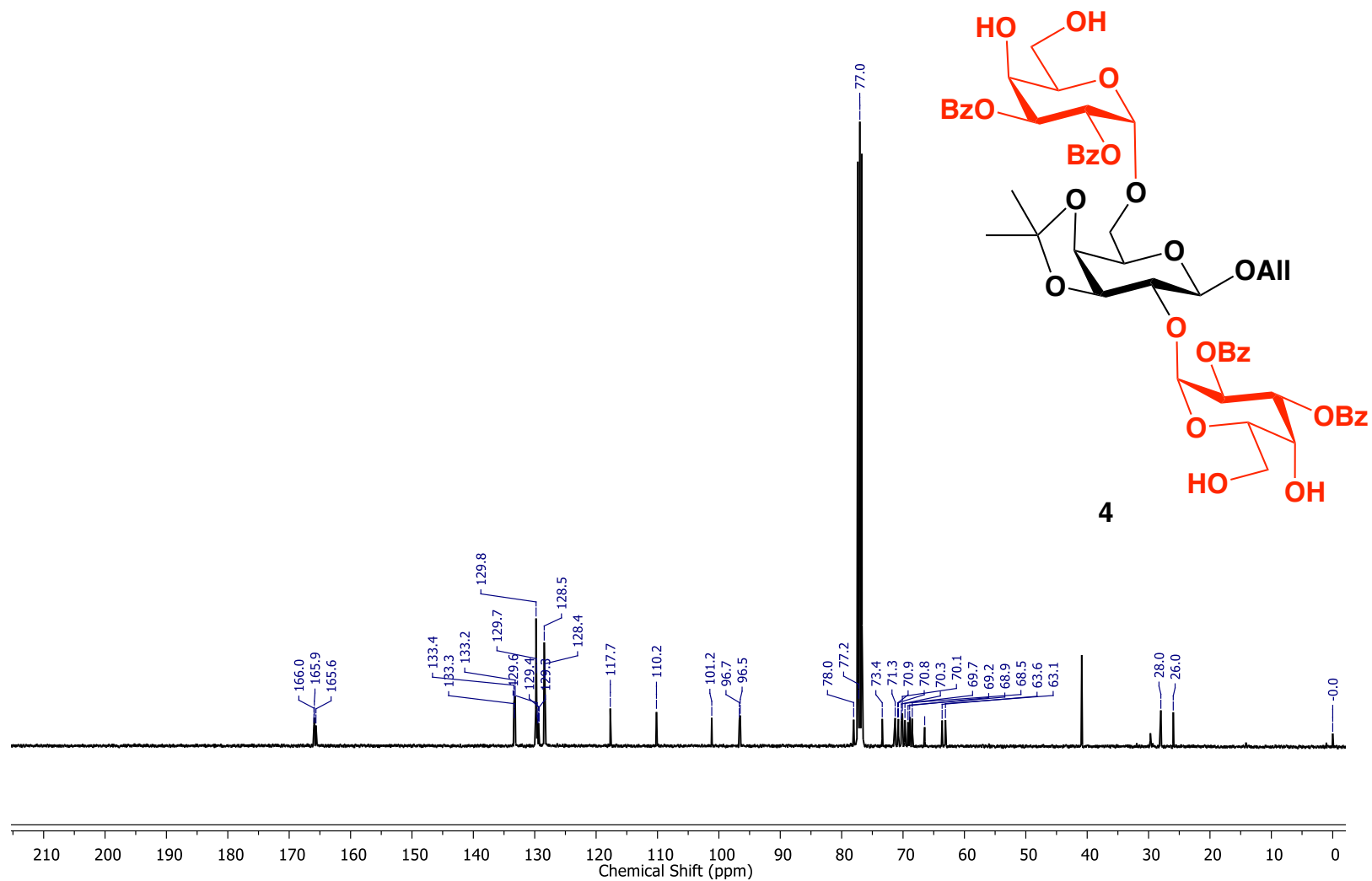


Figure S8: ^{13}C NMR spectrum of compound **4** (101 MHz, in CDCl_3)

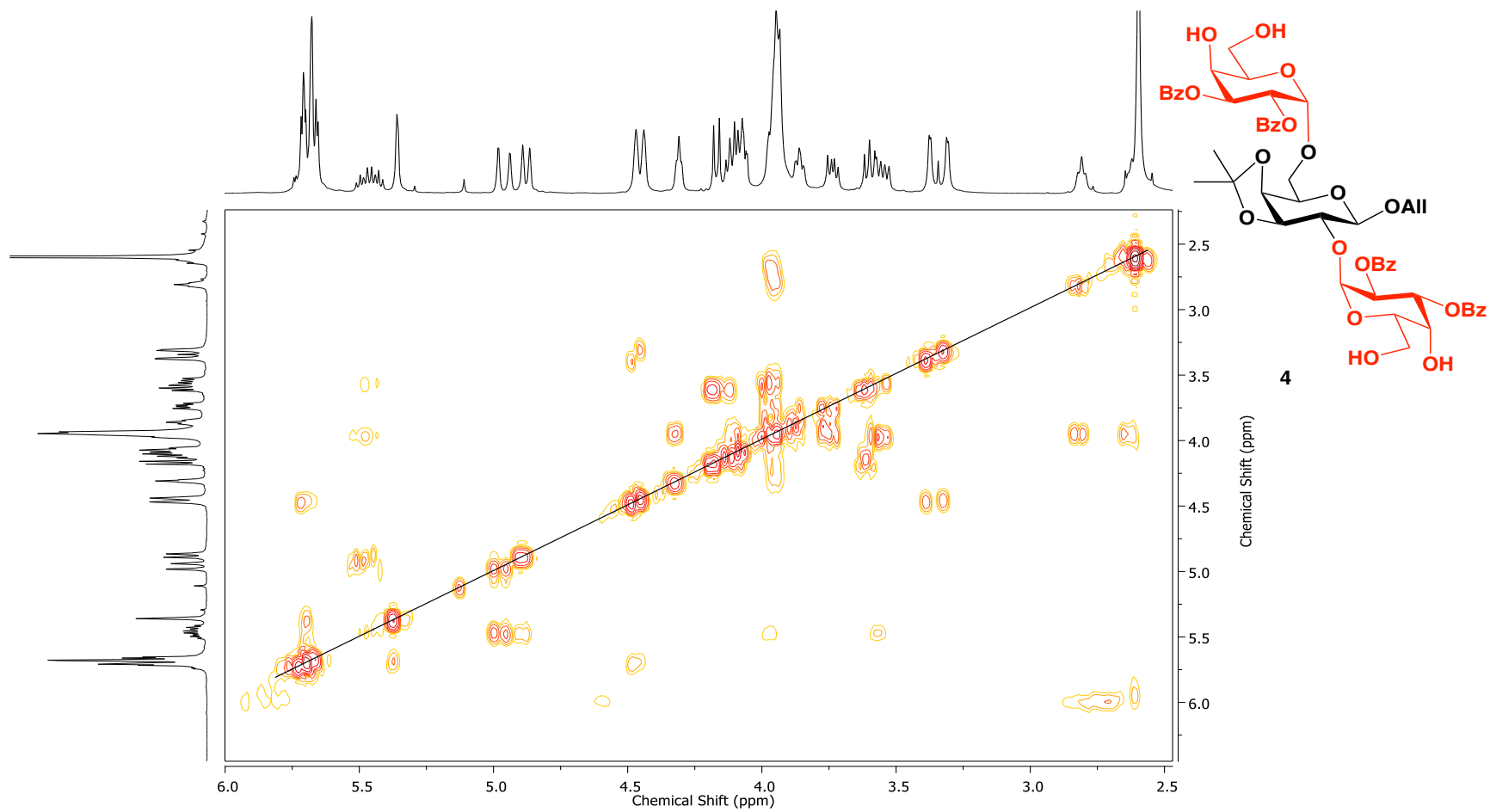


Figure S9: COSY NMR spectrum of compound **4** (in CDCl₃)

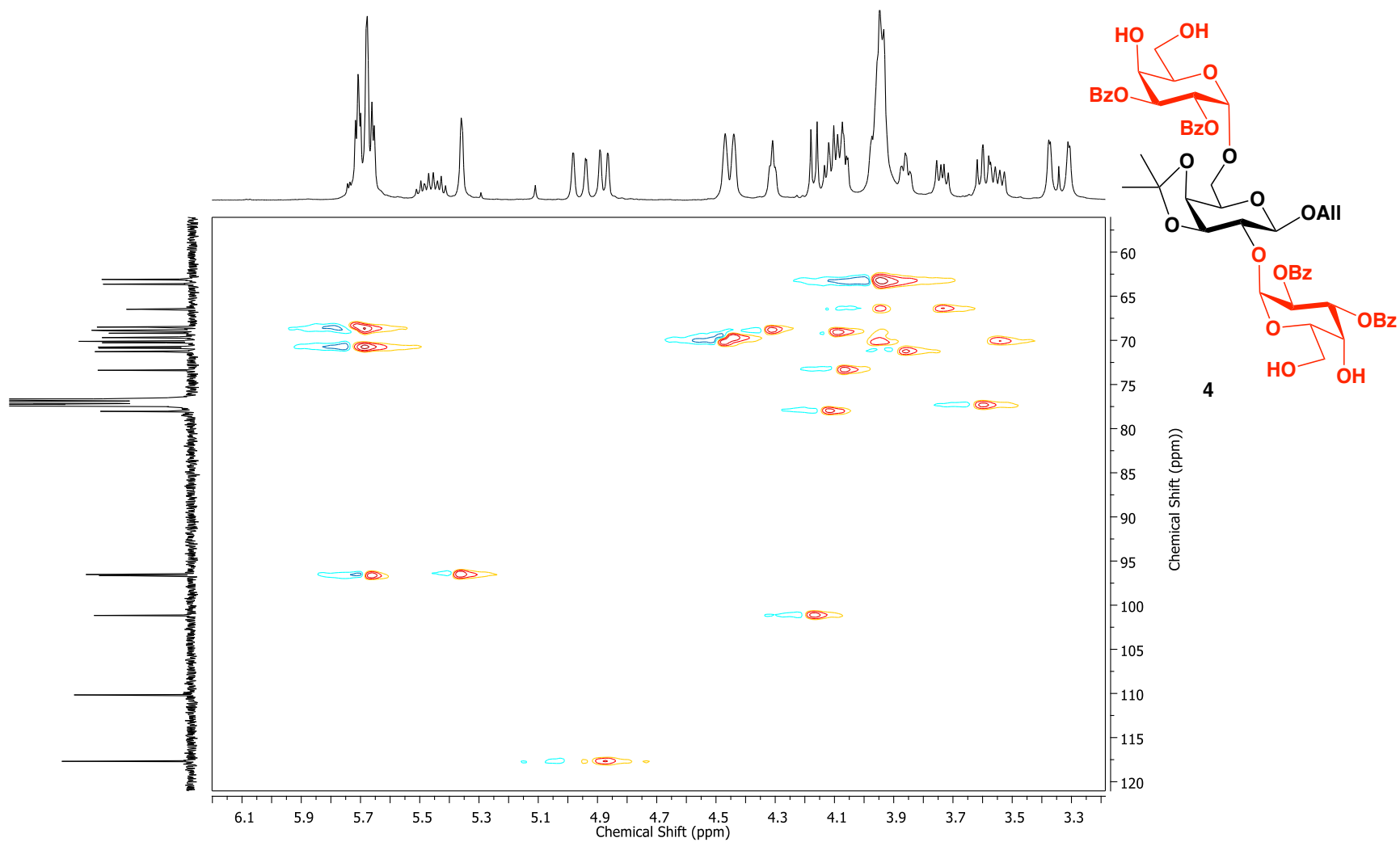


Figure S10: HSQC NMR spectrum of compound **4** (in CDCl_3)

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

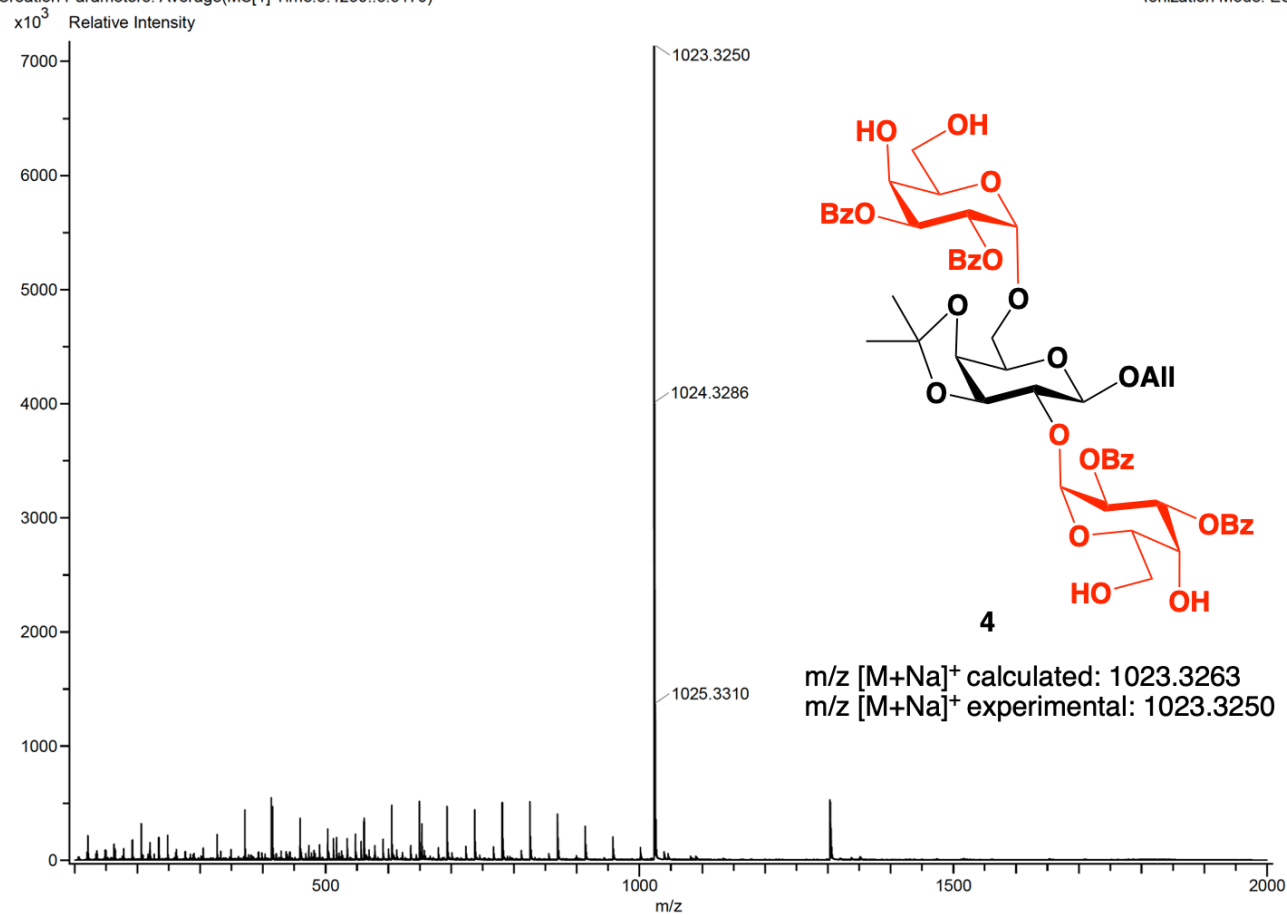


Figure S11: HR ESI-TOF mass spectrum of compound **4**

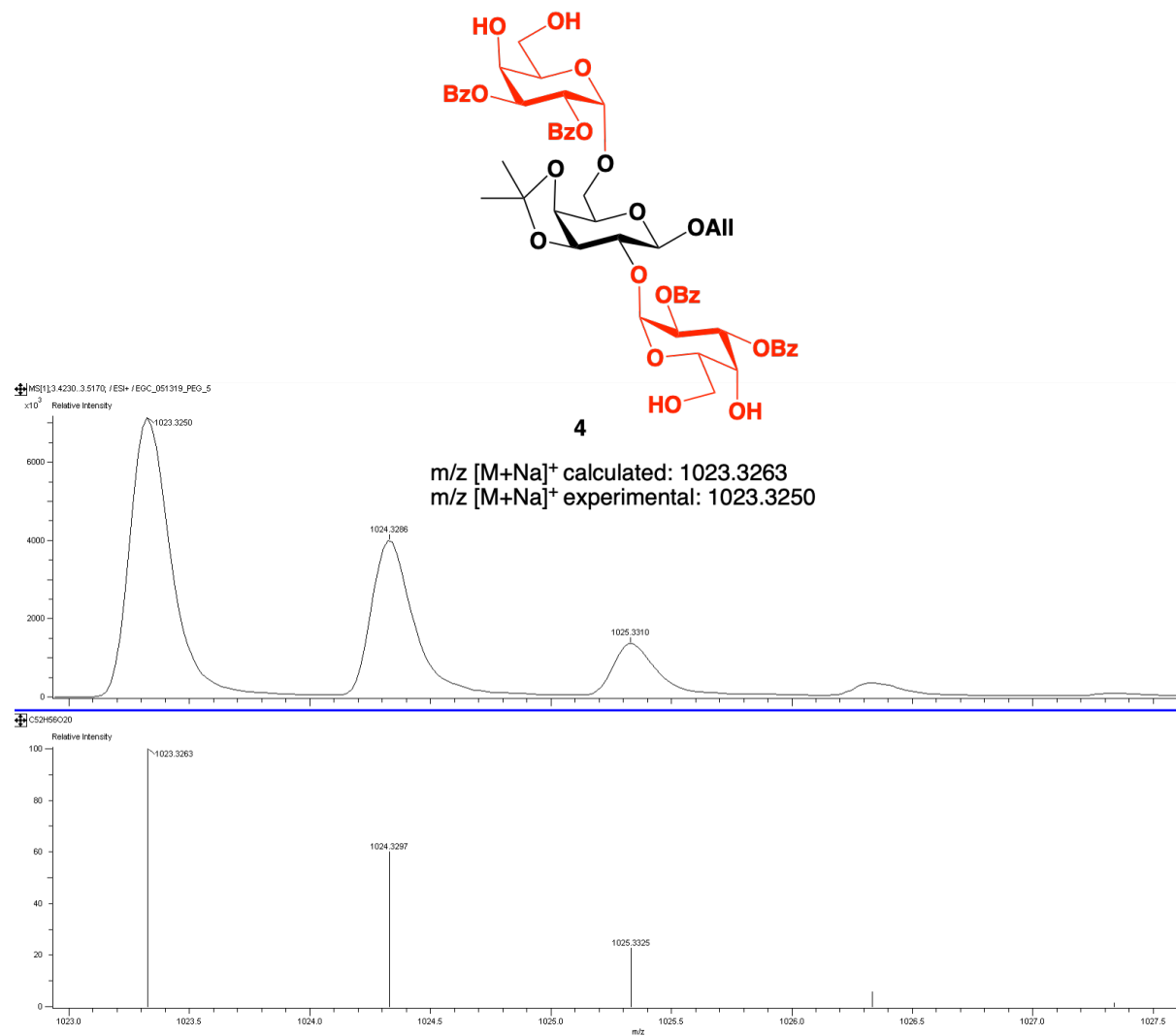


Figure S12: Actual (top) and simulated (bottom) HR ESI-TOF mass spectrum of compound **4**

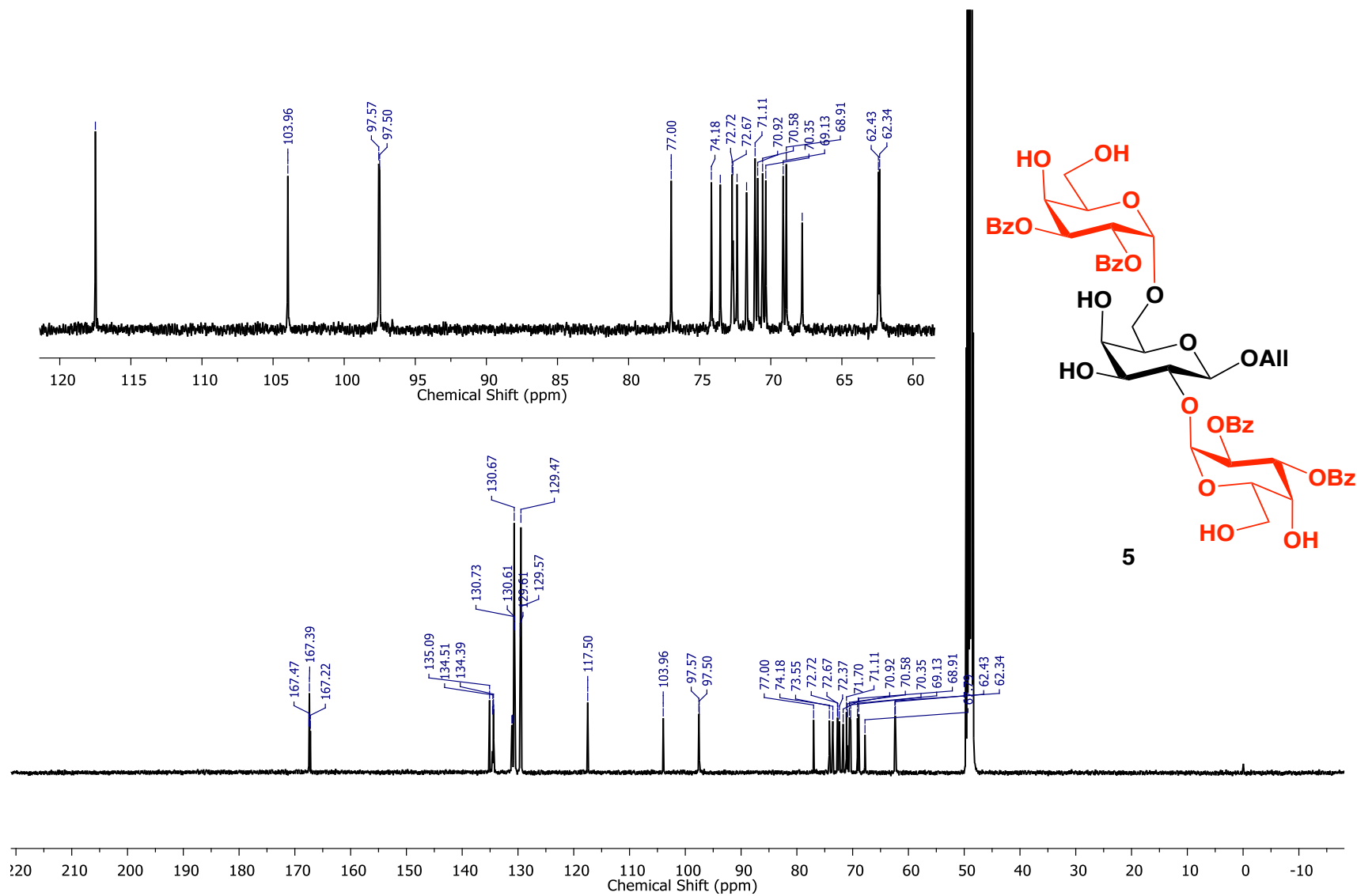


Figure S14: ^{13}C NMR spectrum of compound **5** (101 MHz, in methanol- d_4)

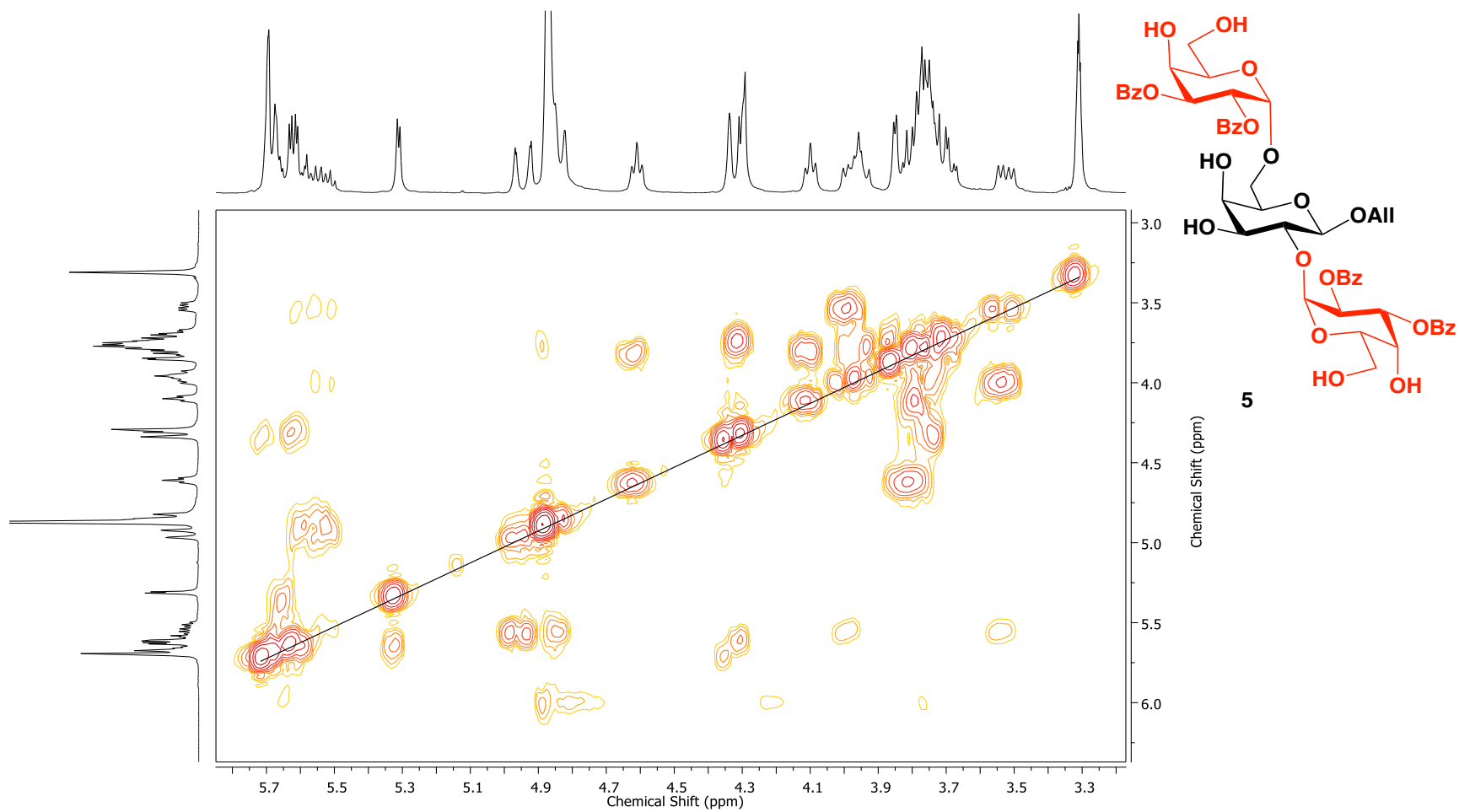


Figure S15: COSY NMR spectrum of compound **5** (in methanol-d₄)

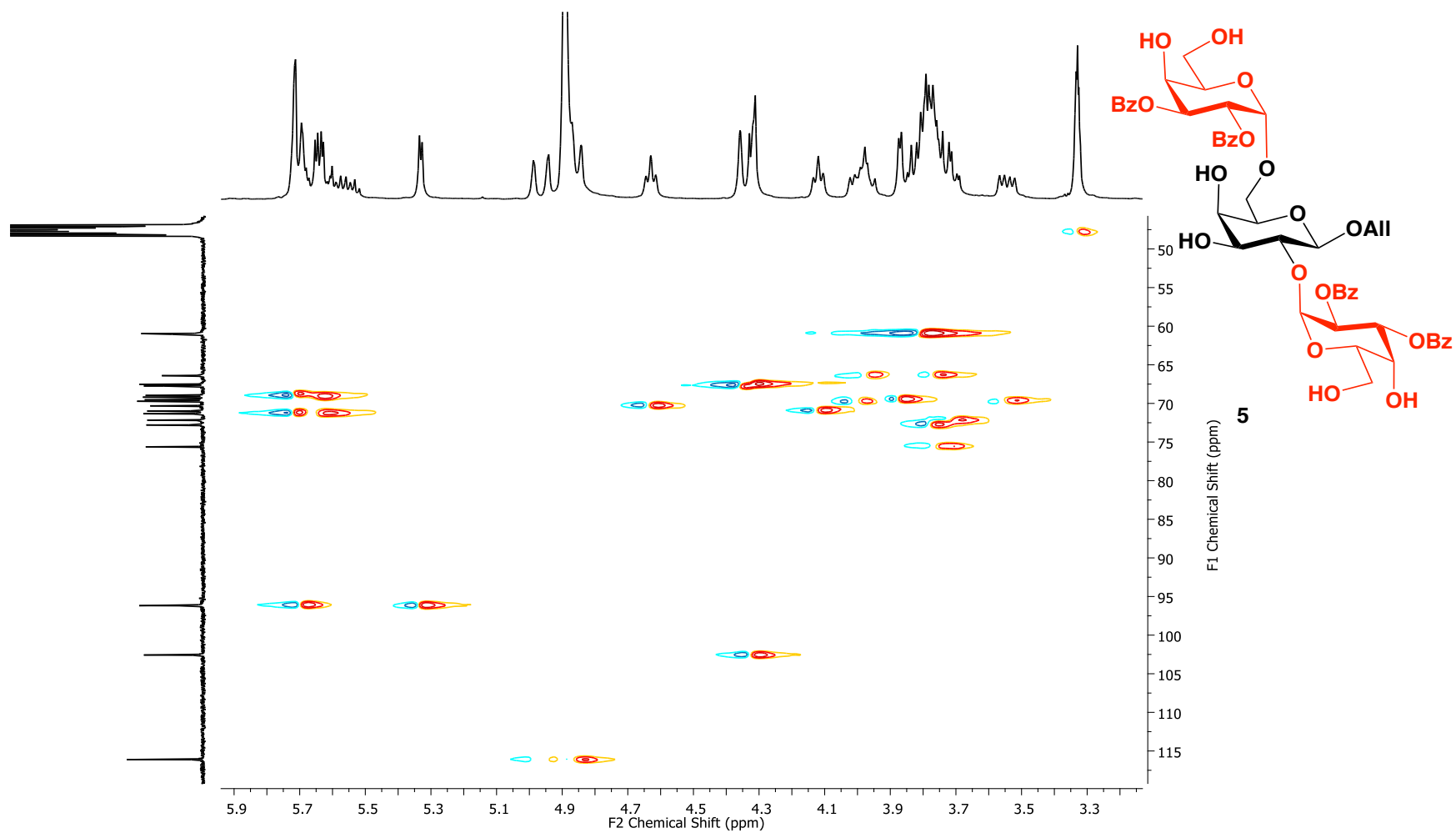


Figure S16: HSQC NMR spectrum of compound **5** (in methanol- d_4)

Acq. Data Name: EGC_052119_ULTRAMARK_2
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Experiment Date/Time: 5/22/2019 9:30:03 PM
Ionization Mode: ESI

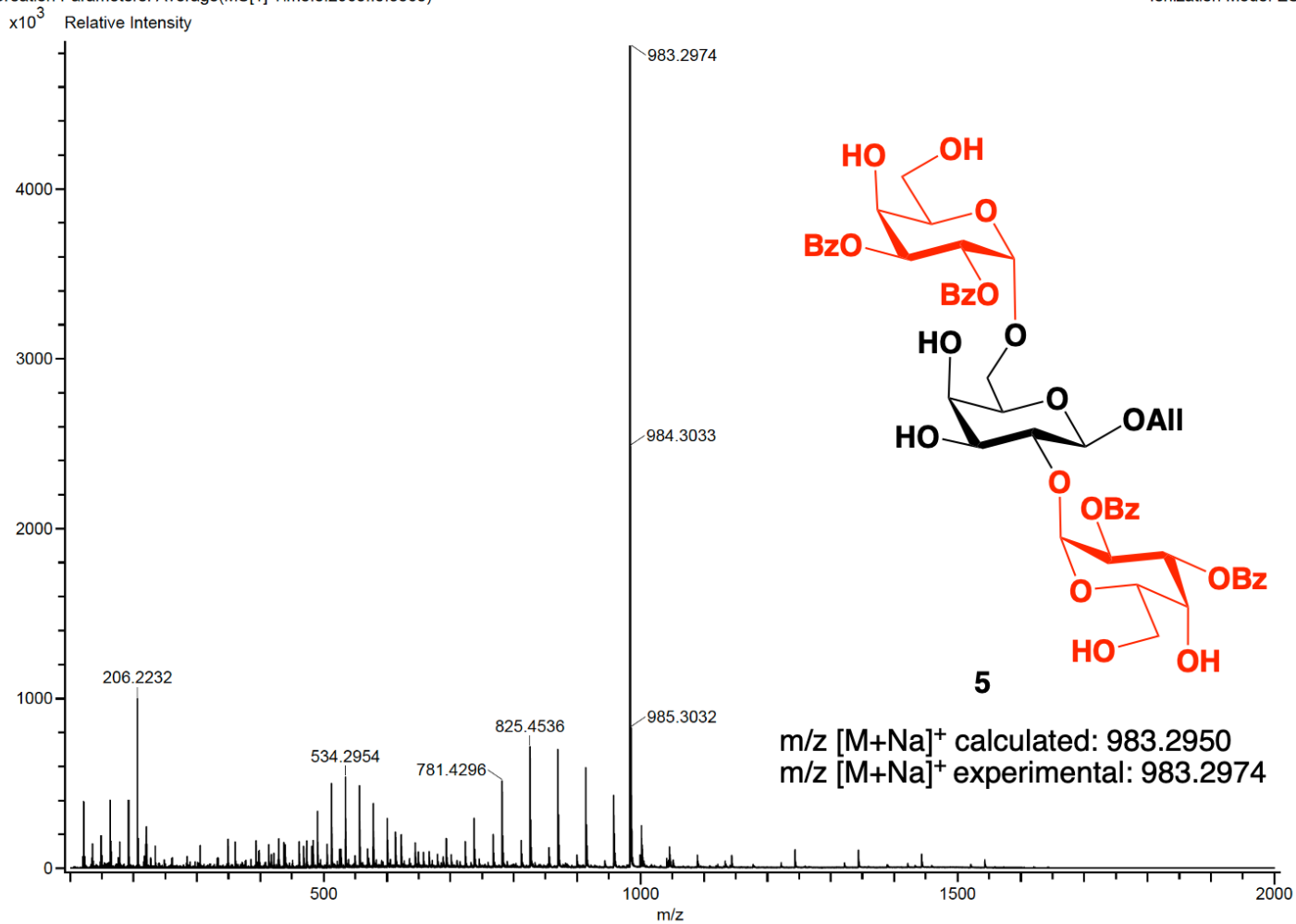


Figure S17: HR ESI-TOF mass spectrum of compound 5

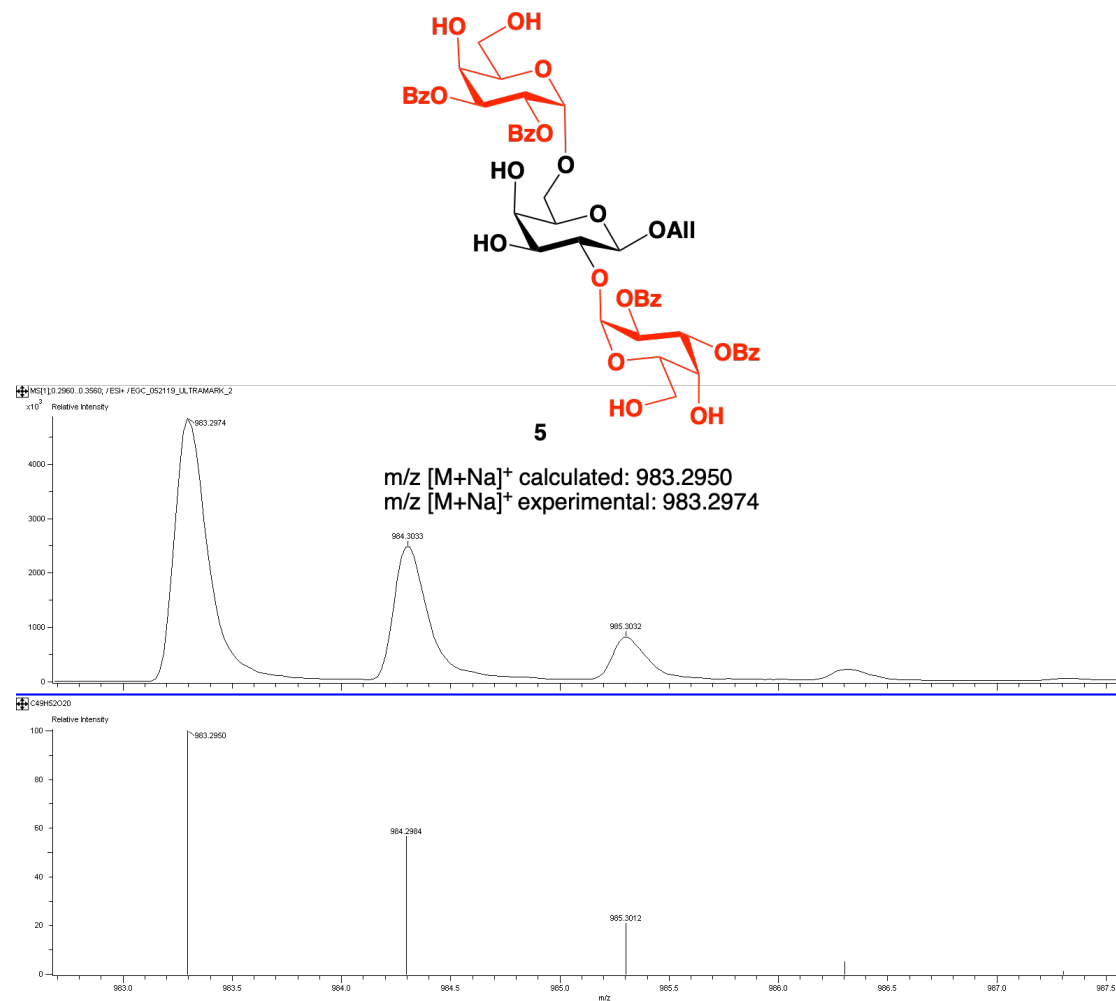
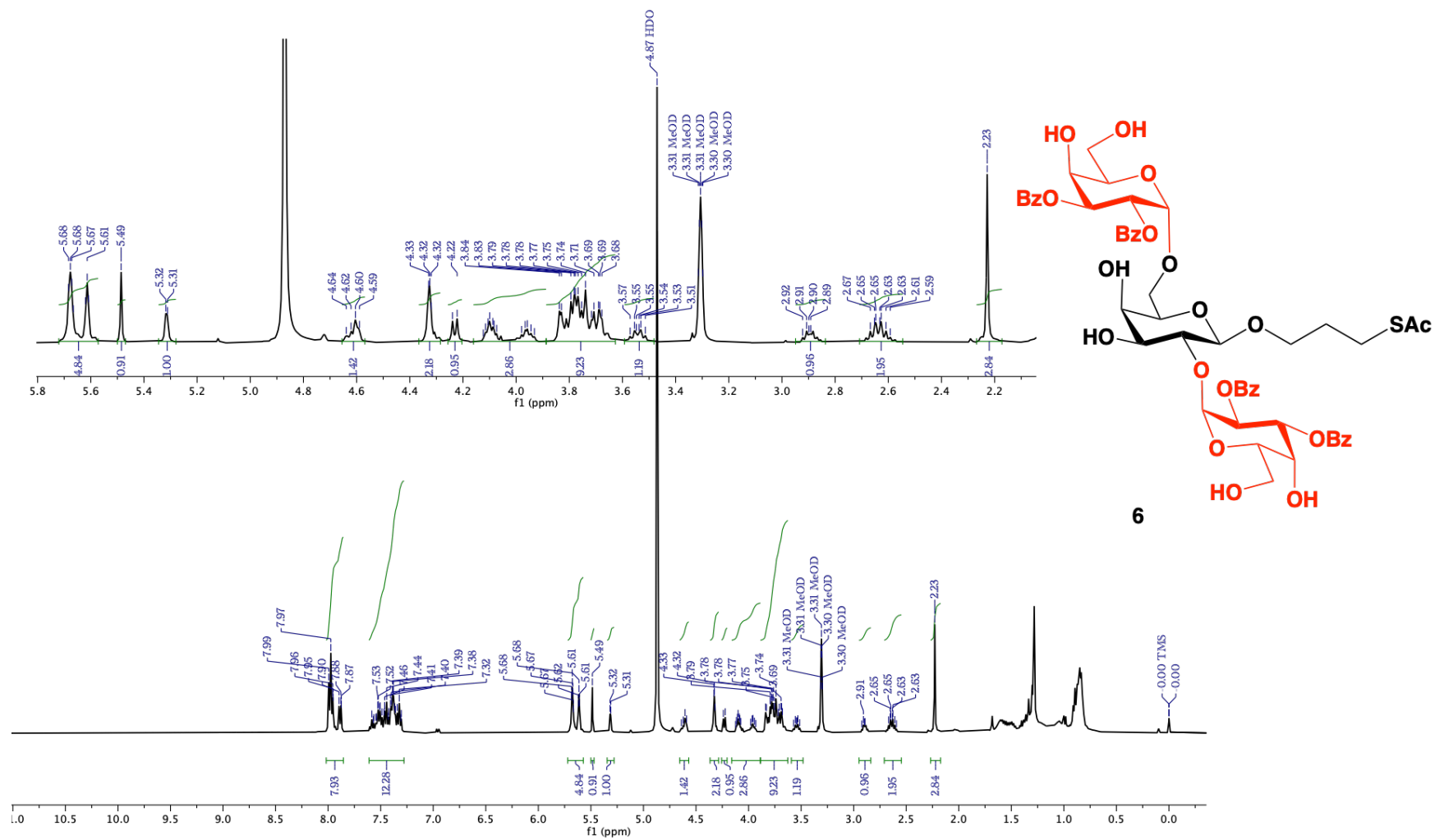


Figure S18: Actual (top) and simulated (bottom) HR ESI-TOF mass spectrum of compound **5**



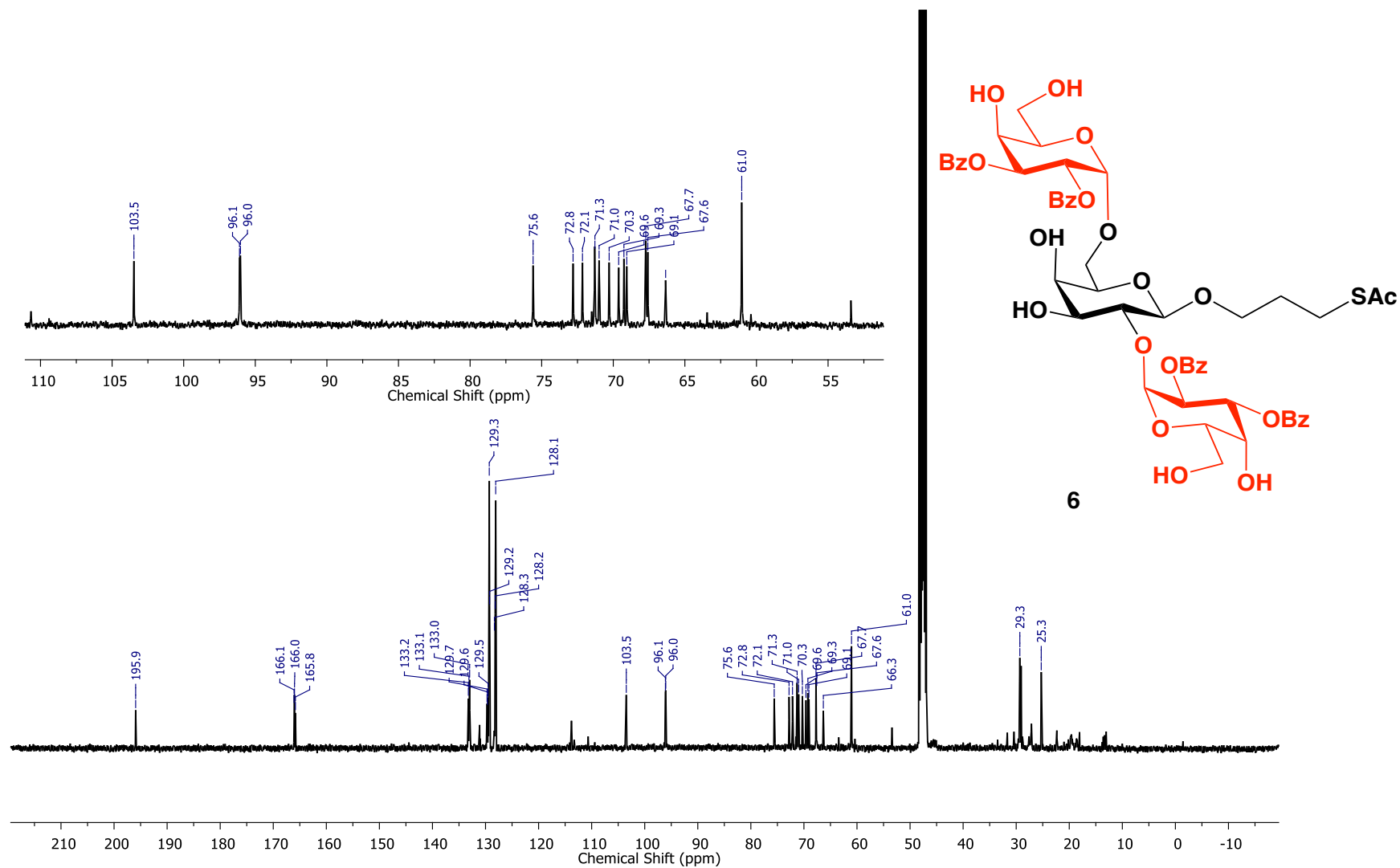


Figure S20: ^{13}C NMR spectrum of compound **6** (in methanol- d_4)

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+

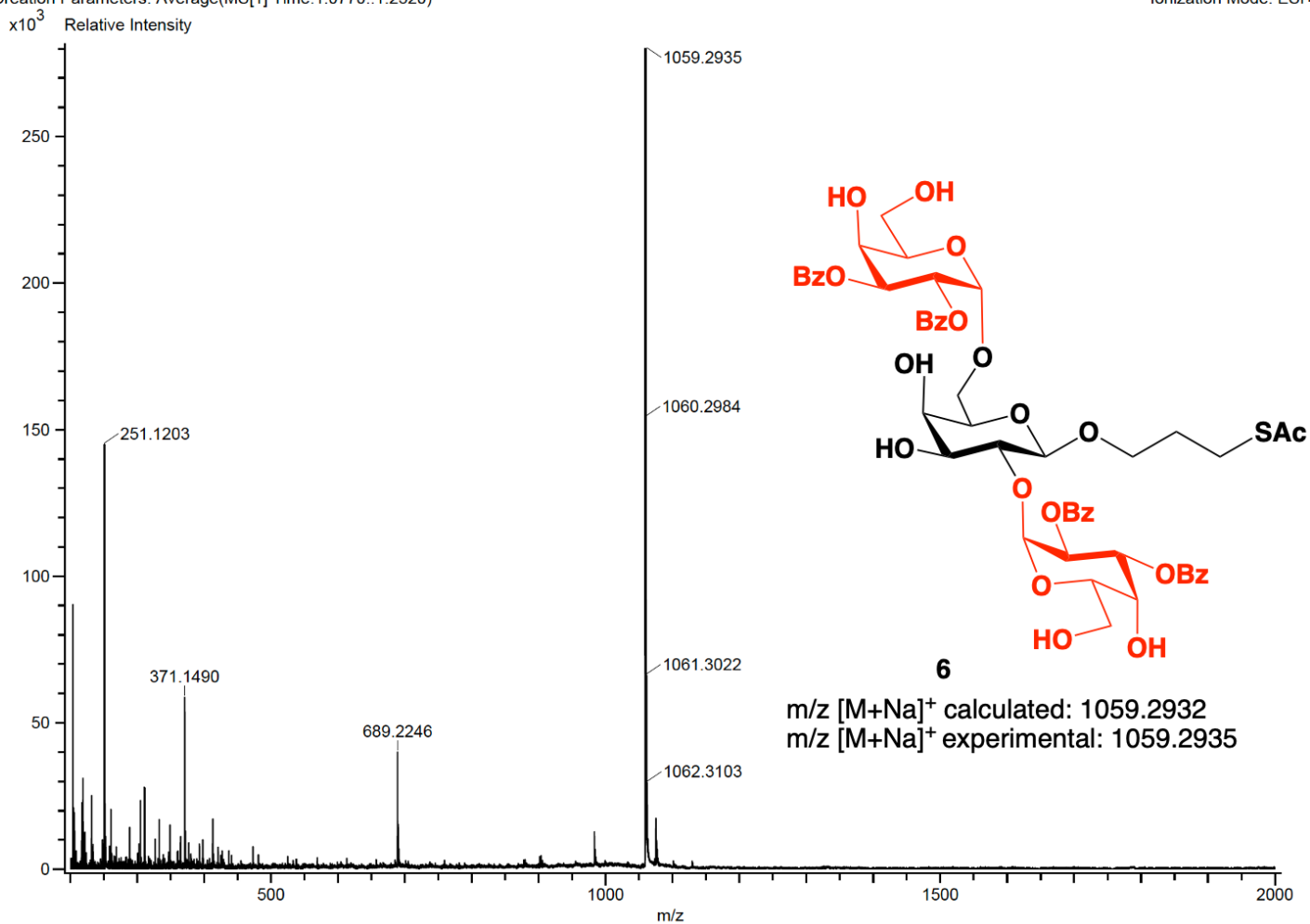
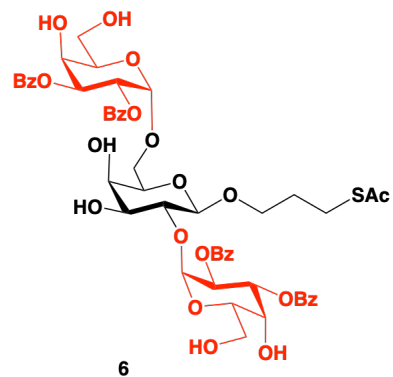


Figure S21: HR ESI-TOF mass spectrum of compound 6



m/z $[M+Na]^+$ calculated: 1059.2932
 m/z $[M+Na]^+$ experimental: 1059.2935

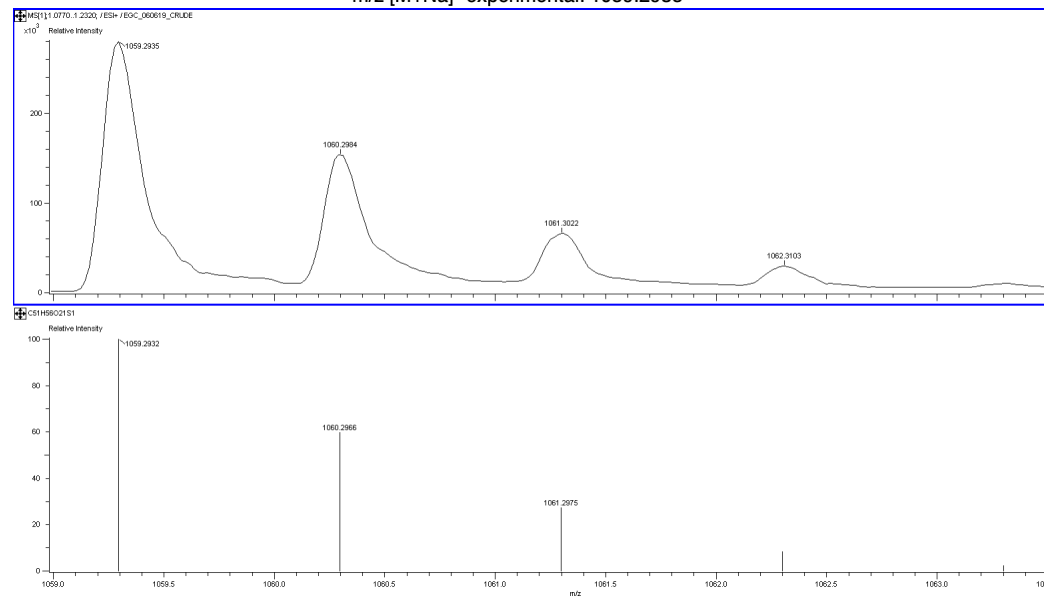


Figure S22: Actual (top) and simulated (bottom) HR ESI-TOF mass spectrum of compound **6**

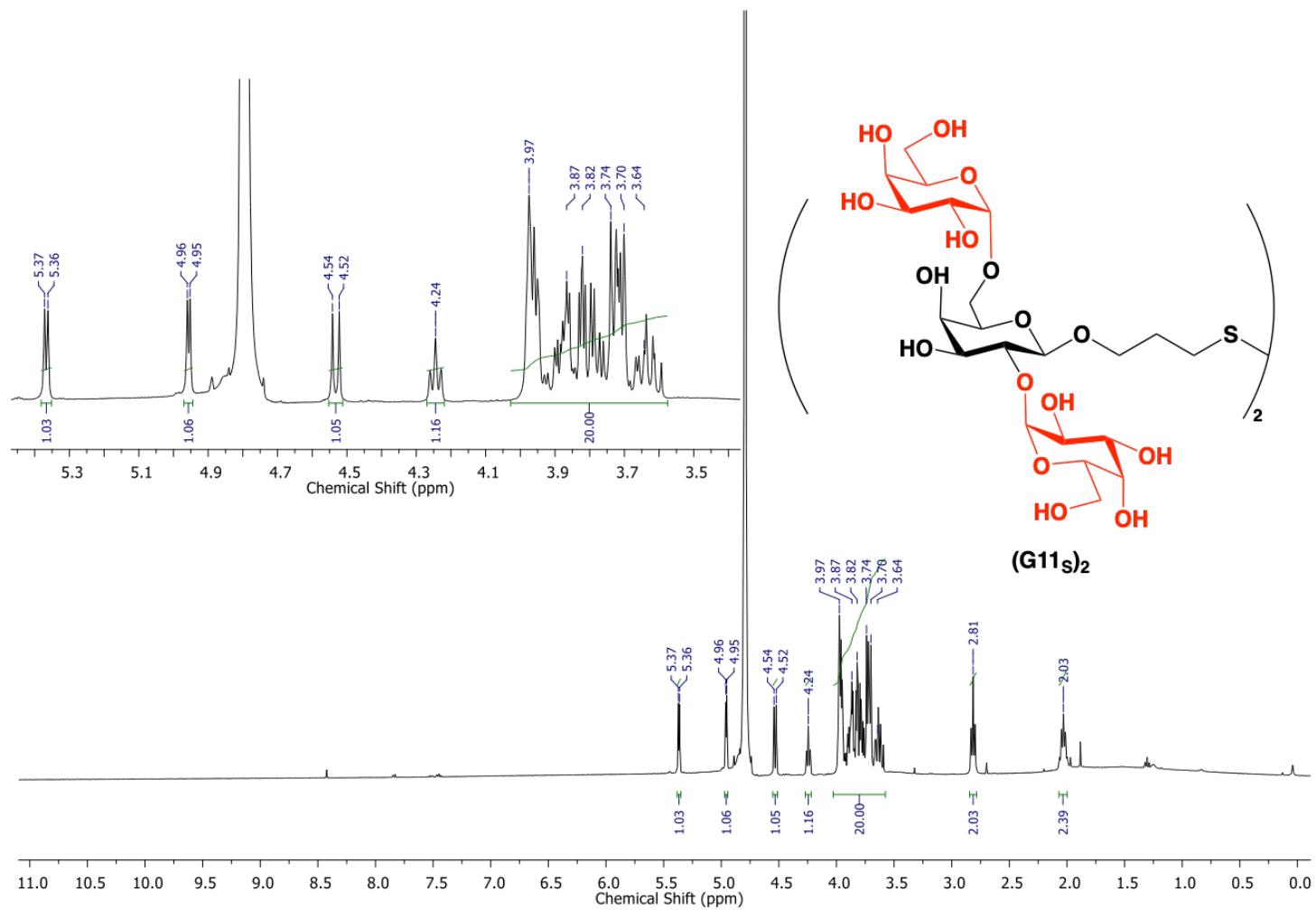


Figure S23: ^1H NMR spectrum of compound **(G11s)₂** (400 MHz, in D_2O)

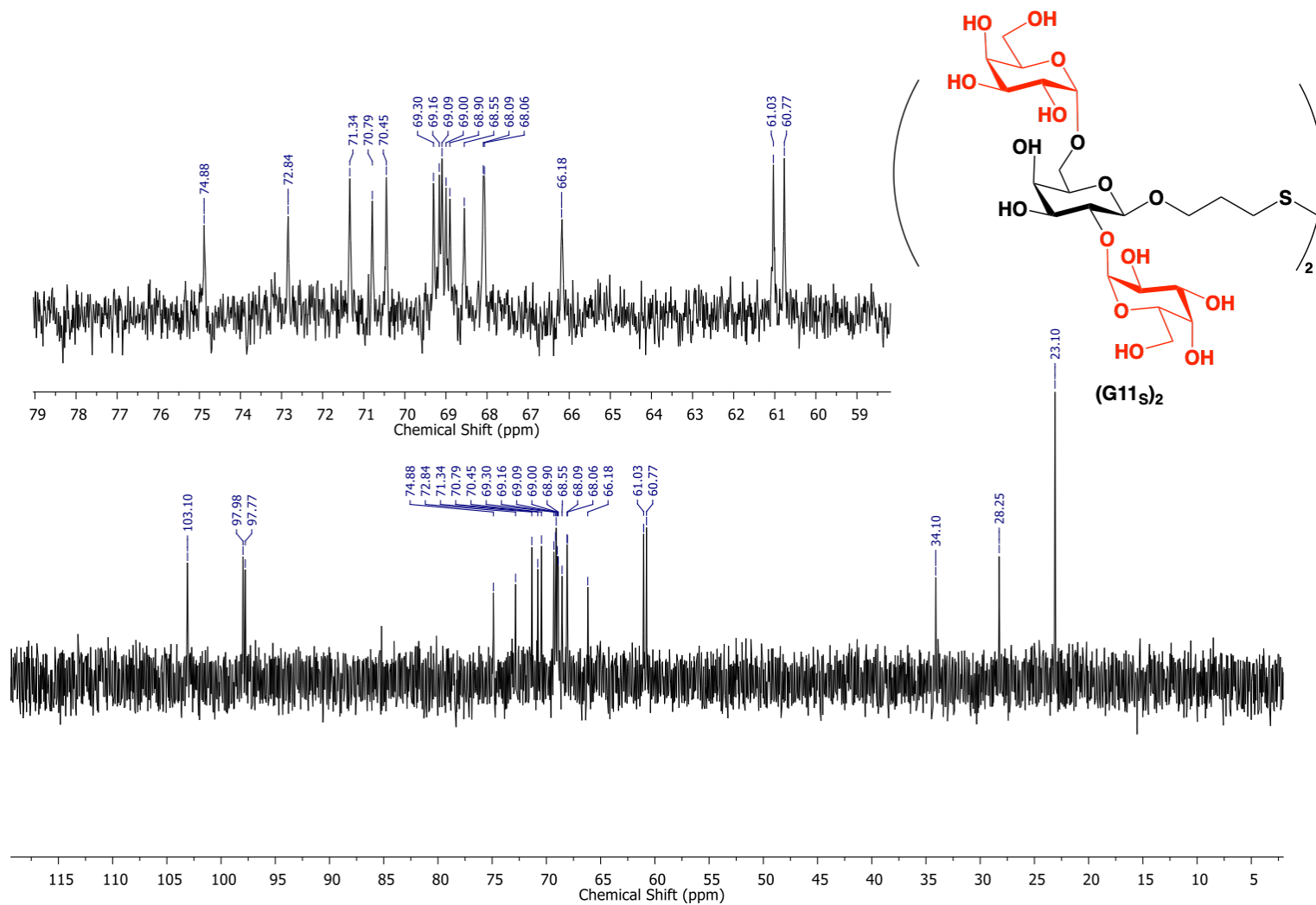


Figure S24: ^{13}C NMR spectrum of compound **(G11s)₂** (101 MHz, in D_2O)

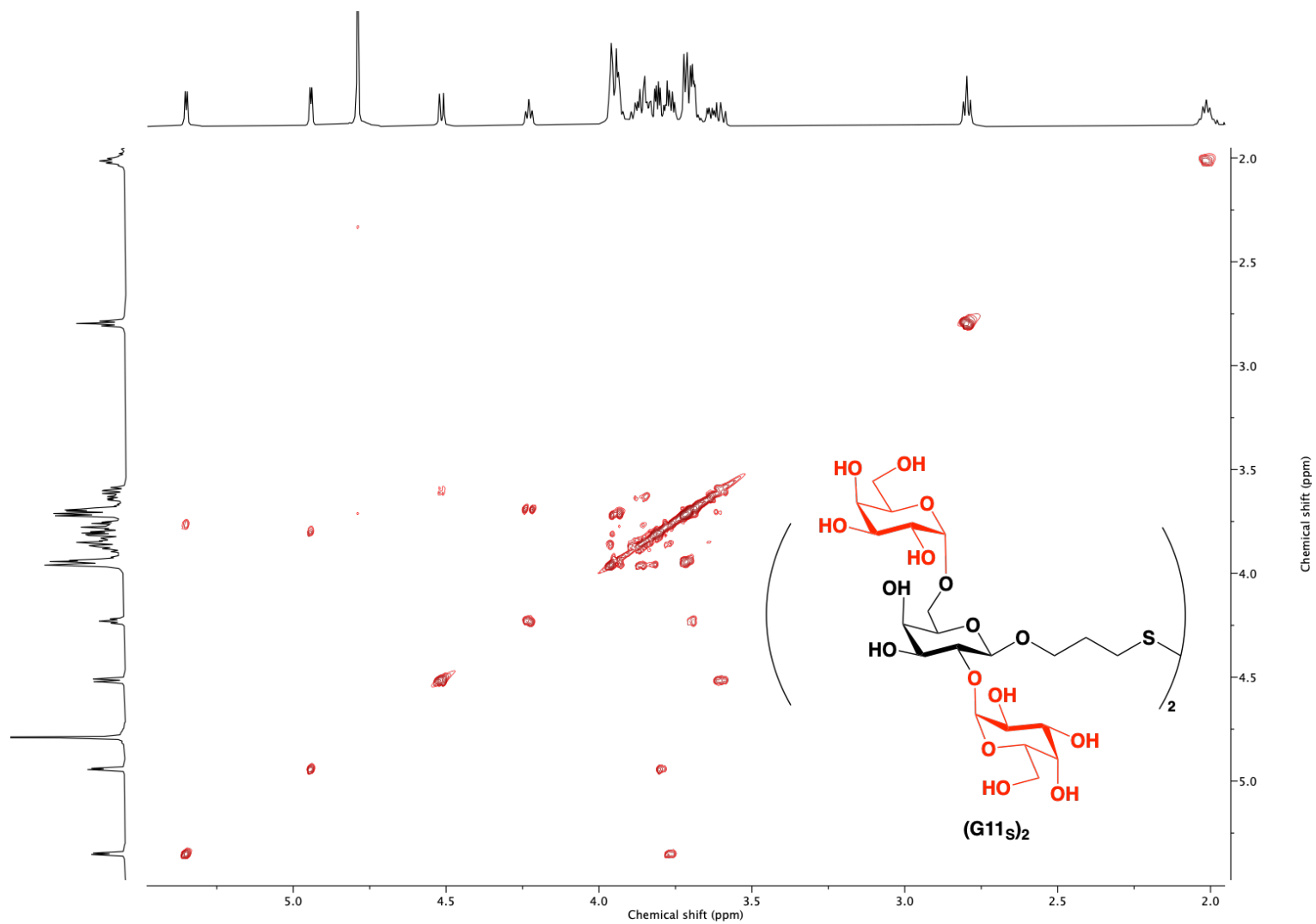


Figure S25: COSY NMR spectrum of compound **(G11s)₂** (400 MHz, in D₂O)

Acq. Data Name: EGC_KM11_FPLC_062421_2min
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Experiment Date/Time: 6/24/2021 9:04:58 AM
Ionization Mode: ESI+

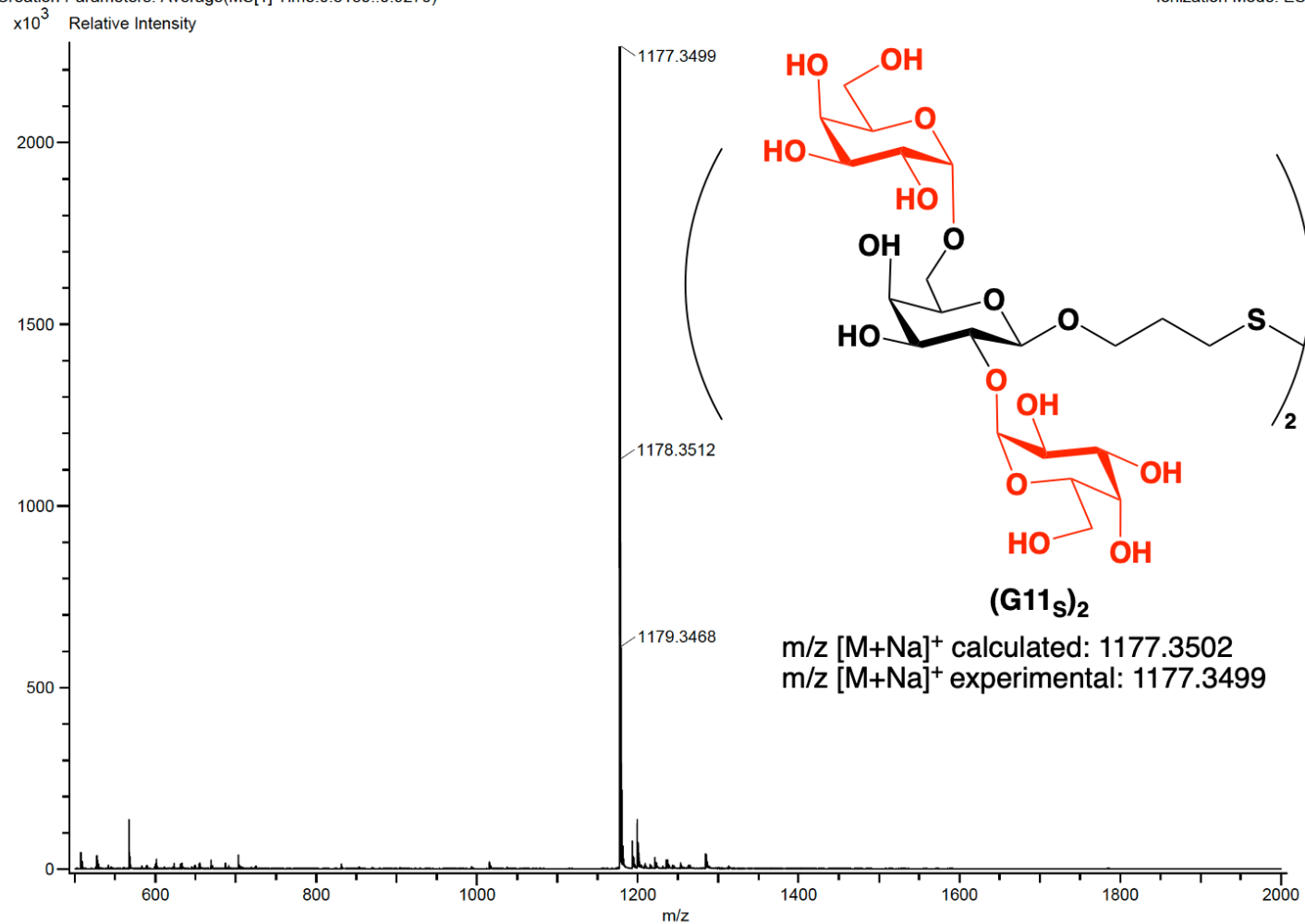
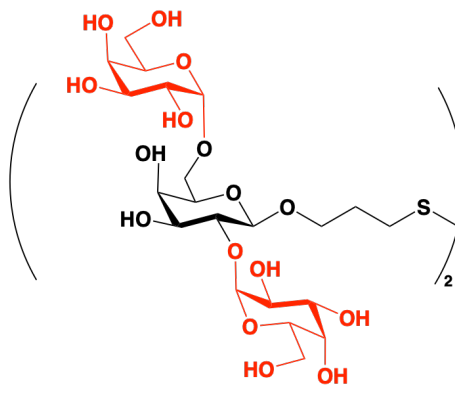


Figure S27: HR ESI-TOF mass spectrum of compound (G11s)₂



(G11s)₂

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

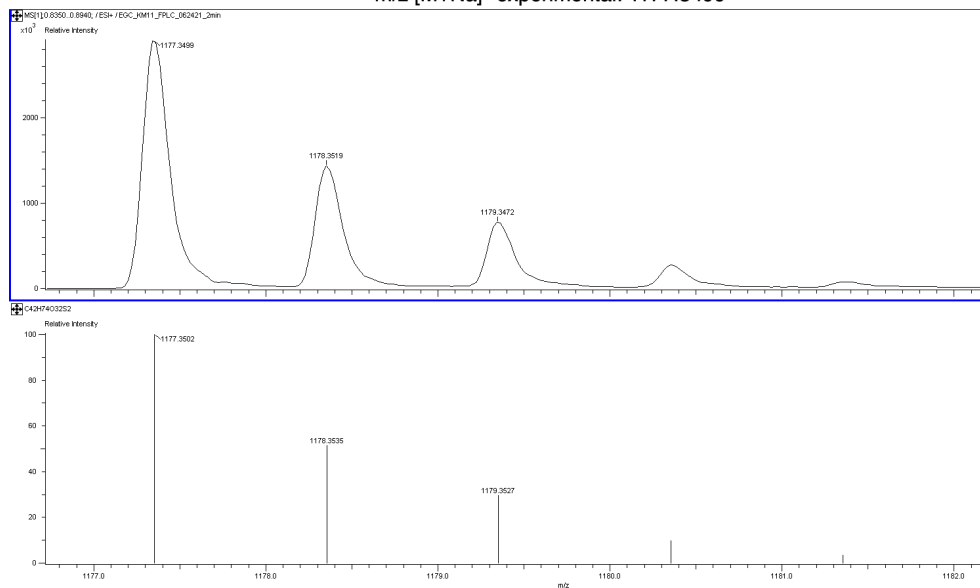


Figure S28: Actual (top) and simulated (bottom) HR ESI-TOF mass spectrum of compound (G11s)₂