SUPPORTING INFORMATION

Reversed Immunoglycomics Identifies α-Galactosyl-Bearing Glycotopes specific for *Leishmania major* infection

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GENERAL INFORMATION

All chemicals were purchased as reagent grade from Thermo Fisher Scientific, Sigma-Aldrich, or Acros Organic, and used without further purification. The ACS grade solvents used for reactions were obtained from Thermo Fisher Scientific and they were distilled from the appropriate drying agents. Molecular sieves (3 Å and 4 Å) were purchased from Alfa Aesar and Thermo Fisher Scientific, respectively, and activated under high vacuum and heat prior to use. Reactions were performed under an argon atmosphere, strictly anhydrous conditions and monitored by TLC on silica gel 60 F254 plates from EMD Millipore or Dynamic Adsorbents, Inc. Spots were detected under UV light (254 nm) and/or by charring with 4% sulfuric acid in ethanol. The purification of the compounds was performed by flash column chromatography on silica gel (40-60 μm) from Thermo Fisher Scientific, and the ratio between silica and crude product ranged from 50:1 to 120:1 (dry w/w). FPLC purifications were performed with an AKTA Purifier 100 FPLC system from Cytiva (former GE Healthcare) using a Resource RPC column with a stationary phase of 15 μm polystyrene/divinylbenzene beads, solvent A: 2% CH3CN/H_2O; solvent B: 85% CH3CN/H_2O. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III HD 400 MHz NMR spectrometer at 400 and 101 MHz or on a JEOL 600 MHz NMR spectrometer at 600 and 150 MHz, respectively. Chemical shifts (in ppm) were determined relative to tetramethylsilane (δ 0.00 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. In case of spectra measured in D_2O, a solution of tetramethylsilane in $CDCl_3$ in a sealed capillary was used as an external standard for calibration. Coupling constant(s) [Hz] were measured from one-dimensional ^{1}H-NMR spectra. Full or partial assignments were made by 1D spectra as well as standard COSY, HSQC, and TOCSY experiments. In disaccharides and trisaccharides, protons of galactopyranose are labeled with an italized “*p*“, protons of galactofuranose with an italized “*f*“, and protons of mannopyranose with an italized “*m*“. Protons in the allyl group are labeled as “a“ for the sp^3-hybridized CH_2, “b“ for the sp^2-hybridized CH, and “c“ for the terminal sp^2-hybridized CH_2. MS analyses of the carbohydrate derivatives were performed on a high-resolution JEOL AccuTOF mass spectrometer using an electrospray ionization (ESI) source. The thiol-ene reactions were performed in a Rayonet RPR200 photochemical reactor (Southern New England Ultraviolet Company, Branford, CT) equipped with 16 UV lamps (350 nm). Bovine serum albumin (BSA) and BSA derivatives (neoglycoproteins (NGPs) and 2-mercaptoethanol-BSA) were measured by matrix-assisted laser/desorption/ionization mass spectrometer (MALDI)-TOF-MS (MALDI-8020, Shimadzu) using 10 mg/mL sinapinic acid, 0.1% trifluoroacetic acid, in 50% acetonitrile as a matrix. Polystyrene Nunc MaxiSorp 96-well ELISA plates, and chemiluminescent ELISA reagents were purchased from Thermo Fisher Scientific or Jackson ImmunoResearch, and chemiluminescence was recorded on a Luminoskan Ascent, Thermo Fisher Scientific. Optical rotations were measured on an ATAGO AP-300 Automatic Polarimeter.

Safety Statement: No unexpected or unusually high safety hazards were encountered.

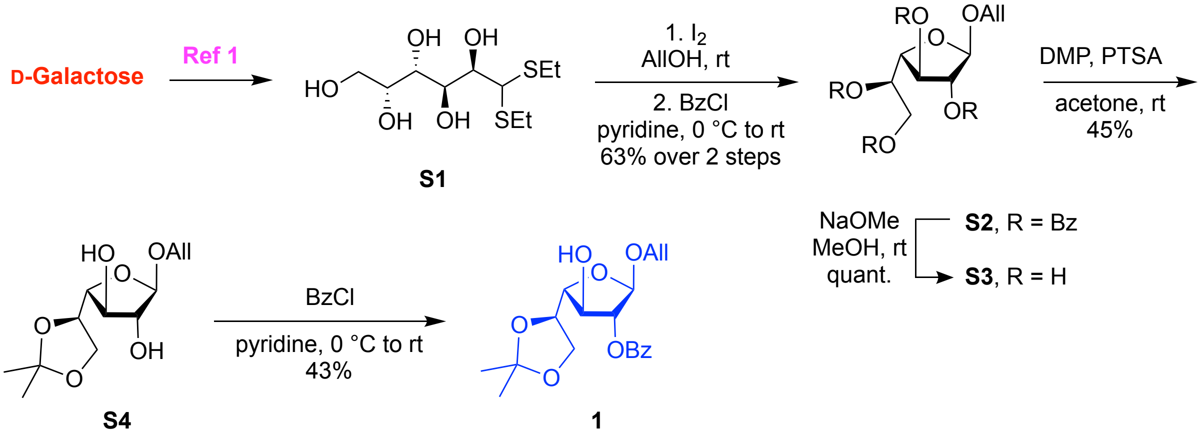
ABBREVIATIONS USED

Å	angstrom
Abs	antibodies
Ac	acetyl
Ac_2O	acetic anhydride
AcSH	thiolacetic acid
AgOTf	silver trifluoromethanesulfonate
AIBN	azobisisobutyronitrile
All	allyl
AllOH	allyl alcohol

Ar	argon
$BF_3 \cdot Et_2O$	boron trifluoride etherate
BMKs	biomarkers
BSA	bovine serum albumin
Bz	benzoyl
BzCl	benzoyl chloride
CCl_3CN	trichloroacetonitrile
ChD	Chagas disease
CL	cutaneous leishmaniasis
$Cu(OTf)_2$	cupric trifluoromethanesulfonate
Cys	cysteine
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	methylene dichloride
DMP	2,2-dimethoxypropane
DPAP	2,2-dimethoxy-2-phenylacetophenone
DTBS	di-*tert*-butylsilylene (protecting group)
$DTBS(OTf)_2$	di-*tert*-butylsilyl bis(trifluoromethanesulfonate)
ELISA	enzyme-linked immunosorbent assay
equiv.	equivalent
ESI-TOF HRMS	Electrospray ionization Time-of-Flight high resolution mass spectra
Et_3N	triethylamine
EtOAc	ethyl acetate
EtSH	ethanethiol
FPLC	Fast protein liquid chromatography
GIPL	glycoinositolphospholipid
h	hour(s)
L. major	*Leishmania major*
L. tropica	*Leishmania tropica*
MALDI-TOF	matrix-assisted laser desorption ionization time-of-flight
2-ME	2-mercaptoethanol
MeOH	methanol
min	minute(s)
MS	molecular sieves
m/z	mass-to-charge ratio
NBS	*N*-bromosuccinimide
NGP	neoglycoprotein
NHS	normal human serum
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
PTLC	preparative thin-layer chromatography
PTSA	*para*-toluenesulfonic acid
quant.	quantitative
RLU	relative luminescence unit(s)
rt	room temperature
*t*Bu	*tert*-butyl
TCEP	tris(2-carboxyethyl)phosphine hydrochloride
TFA	trifluoroacetic acid
TLC	thin-layer chromatography
TolSH	*para*-thiocresol
THF	tetrahydrofuran
TMS-OTf	trimethylsilyl trifluoromethanesulfonate

SYNTHETIC SCHEMES, PROCEDURES, AND CHARACTERIATIONS

Synthesis of the acceptor 1



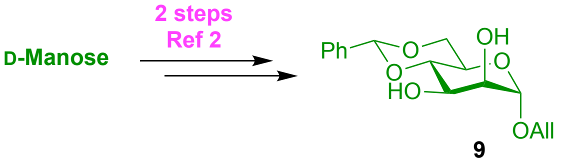
Scheme S1. Synthesis of allyl 2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (Gal*f*β) acceptor **1**.

Allyl β-D-galactofuranoside (S3). Dithioacetal galactose **S1**[1] (1.0 g, 3.50 mmol) was dissolved in 1.5% I_2 in anhydrous AllOH (w/v, 75 mL) under Ar and stirred overnight at rt. Excess of I_2 was quenched by the incremental addition of solid $Na_2S_2O_3$ until the solution turned colorless or slightly yellow. The reaction mixture was then neutralized by the addition of $NaHCO_3$. Filtration followed by evaporation of the solvent gave the crude product allyl β-D-galactofuranoside as an amorphous light-yellow solid **S3**. Without further purification, anhydrous pyridine (60 mL) was added to the crude mixture followed by the addition of BzCl (2.5 mL, 21.45 mmol) at 0°C. The reaction mixture was stirred under Ar overnight, poured into an ice-water mixture, and extracted with DCM (3 x 75 mL). The combined organic layers were washed with 1.0 M HCl (50 mL), brine (50 mL), dried over $MgSO_4$, filtered, concentrated and purified by flash column chromatography on silica gel (EtOAc/hexanes = 1:3) to furnish the allyl 2,3,5,6-*O*-benzoyl β-D-galactofuranoside **S2** as a white powder (β-configured compound; 1.4 g, 63% over two steps). R_f 0.4 (EtOAc/hexanes = 1:3). ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calculated for $C_{37}H_{32}NaO_{10}$ 659.1893, found 659.1892. Finally, the benzoylated compound **S2** (1.2 g, 1.89 mmol), was dissolved in 60 mL of 0.25 M NaOMe in MeOH under Ar and stirred at rt overnight. Amberlyst-15 ion-exchange resin was added and stirred until pH 7 was reached, followed by filtration through Celite and concentration to give **S3** as a white solid (415 mg, quant.). R_f 0.37 (DCM/MeOH = 6:1). ^1H NMR (400 MHz, CD_3OD, 300K) δ 0.75-1.65 (m, 3H, 3 × O*H*); 2.50-2.79 (m, 1H, O*H*); 3.56-3.76 (m, 4H, H-4, H-5, H-6a,b); 3.87-4.04 (m, 3H, Ha, H-2, H-3); 4.20 (dd, *J* = 13.1, 5.0 Hz, 1H, Ha'); 4.90 (s, 1H, H-1); 5.15 (d, *J* = 10.5 Hz, 1H, H-c); 5.29 (d, *J* = 17.2 Hz, 1H, H-c'); 5.94 (m, 1H, H-b) ppm. ^{13}C NMR (101 MHz, CD_3OD, 300K) δ 64.6 (*C*-6); 69.5 (*C*-a); 72.5; 78.9; 83.5; 84.4; 108.7 (*C*-1); 117.2 (C-c); 135.9 (C-b) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calculated for $C_9H_{16}NaO_6$ 243.0845, found 243.1199.

Allyl 5,6-*O*-isopropylidene-β-D-galactofuranoside (S4). To a solution of allyl β-D-galactofuranoside **S3** (333 mg, 1.51 mmol) in 24 mL of non-anhydrous acetone, DMP (1.7 mL, 13.8 mmol) and PTSA (50 mg, 0.30 mmol) were added and stirred 14 h at rt. Then, the solution was neutralized with Et_3N, concentrated and purified by flash column chromatography on silica gel (DCM/MeOH = 15:1) to yield **S4** as a colorless oil (180 mg, 45%). R_f 0.31 (DCM/MeOH = 15:1). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 1.40 (s, 3H, C*H*$_3$); 1.43 (s, 3H, C*H*$_3$); 3.00 (d, *J* = 11.7 Hz, 1H, O*H*); 3.95-4.16 (m, 7H, O*H*, H-a, H-6a,b); 4.24 (m, 1H, H-a'); 4.36 (m, 1H); 5.08 (s, 1H, H-1); 5.21 (m, 1H, H-c); 5.28 (m, 1H, H-c'); 5.89 (m, 1H, H-b) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 25.5 (*C*H$_3$); 25.6 (*C*H$_3$); 65.7 (C-6); 68.2 (C-a); 75.7; 78.2; 78.6; 85.6; 107.8 (*C*-1); 110.2 (*C*q-*isop.*); 117.7 (C-c); 133.6 (C-b) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{12}H_{20}NaO_6$ 283.1158, found 283.1130.

Allyl 2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (1). To a solution of allyl 5,6-*O*-isopropylidene-β-D-galactofuranoside **S4** (180 mg, 0.69 mmol) in pyridine/DCM (0.7 mL/7.0 mL) was added BzCl (105 μL, 0.90 mmol) dropwise at 0 °C, and the resulting mixture was allowed to warm up to room temperature gradually. The reaction was stirred for 3 h at rt, until completion as indicated by TLC. The reaction was quenched with MeOH followed by evaporation of the solvents. The remainder was diluted with DCM (50 mL), and then the mixture was washed with water (25 mL) and brine (25 mL). The organic layer was separated and dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (EtOAc/hexanes = 1:3) to afford the Gal*f*β acceptor **1** (108 mg, 43%) as a colorless syrup. R_f 0.33 (EtOAc/hexanes = 1:3). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 1.39 (s, 3H, C*H*$_3$); 1.46 (s, 3H, C*H*$_3$); 3.25 (d, *J* = 5.2 Hz, 1H, O*H*); 3.97 (dd, *J* = 8.4, 6.7 Hz, 1H, H-6a or b); 4.03 (m, 1H, H-3); 4.06-4.15 (m, 3H, H-6a or b, H-a, H-5); 4.24-4.36 (m, 2H, H-a', H-4); 5.10 (dd, $^3J_{2,3}$ = 2.7 Hz, $^3J_{1,2}$ = 1.1 Hz, 1H, H-2); 5.23 (m, 1H, H-c); 5.30-5.37 (m, 2H, H-1, H-c); 5.94 (m, 1H, H-b); 7.43-7.50 (m, 2H, arom.); 7.61 (m, 1H, arom.); 8.00-8.06 (m, 2H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 25.4 (*C*H$_3$); 26.5 (*C*H$_3$); 65.5 (C-6); 68.5 (C-a); 76.1 (C-4); 77.7 (C-3); 84.1 (C-5); 86.2 (C-2); 104.5 (*C*-1); 109.9 (*C*q-*isop.*); 117.7 (C-c); 128.6 (2 × *C*-arom.); 129.0 (*C*q, arom.); 129.8 (2 × *C*-arom.); 133.6 (C-b); 133.7 (*C*-arom.); 166.8 (C=O) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{19}H_{24}NaO_7$ 387.1420, found 387.1346.

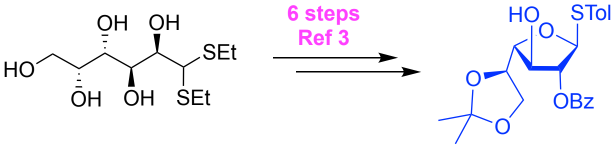
Synthesis of the acceptor 9



9

Scheme S2. The Manα acceptor **9** (allyl 4,6-*O*-benzylidene-α-D-mannopyranoside) was synthesized following a known procedure.[2,3]

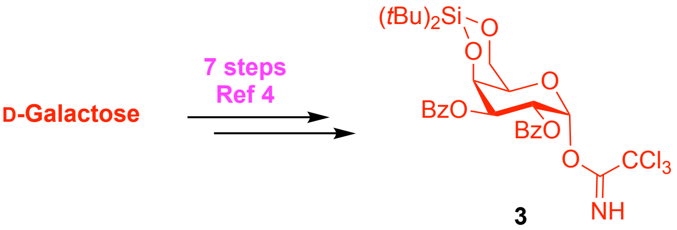
Synthesis of the donor 2



S1 **2**

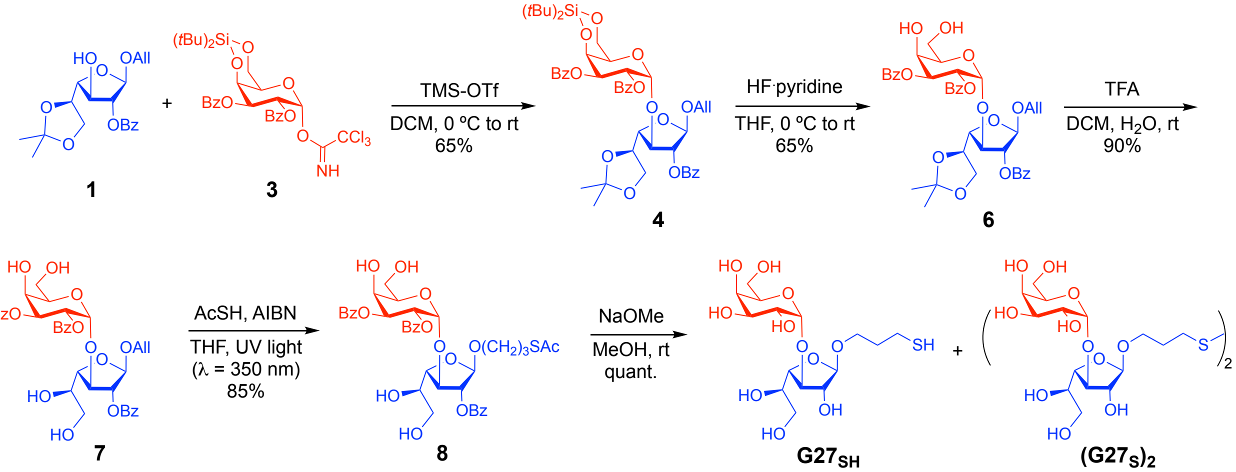
Scheme S3. The Gal*f*β donor **2** (allyl 2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranoside) was synthesized following known procedures.[4-6]

Synthesis of the Kiso donor 3



Scheme S4. Synthesis of 2,3-Di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranosyl trichloroacetimidate (Gal*p*α) donor **3** following known procedures.[7,8]

Synthesis of G27$_{SH}$



Scheme S5. Synthesis of the 3-thiopropyl disaccharide **G27$_{SH}$**.

Allyl 2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (4). To a solution of Gal*f* acceptor **1** (150 mg, 0.41 mmol) and Gal*p* donor **3**[7,8] (373 mg, 0.56 mmol) in anhydrous DCM (54 mL), freshly activated MS 4Å was added and stirred under Ar for 1 h at rt. Then, the solution was cooled down to 0°C and TMS-OTf (22 µL, 0.12 mmol) was added dropwise. The solution was gradually brought to rt and after 1 h, the reaction mixture was quenched by addition of Et_3N, filtered, and washed with water and brine. The organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography on silica gel (EtOAc/hexanes = 1:4) to give disaccharide **4** (234 mg, 65%), as a light-yellow solid. R_f 0.37 (EtOAc/hexanes = 1:4). $[\alpha]_D^{23}$ = +83.4° (c = 0.06, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 0.95 (s, 9H, *t*Bu); 1.08 (s, 9H, *t*Bu); 1.22 (s, 3H, C*H*$_3$); 1.35 (s, 3H, C*H*$_3$); 3.70-3.87 (m, 2H, H*f*-6a,b); 4.04 (m, 1H, H-a), 4.11-4.34 (m, 7H, H-a', H*p*-5, H*p*-6a,b, H*f*-3, H*f*-4, H*f*-5); 4.90 (d, $^3J_{3,4}$ = 2.9 Hz, 1H, H*p*-4); 5.15-5.23 (m, 2H, H*f*-1, H-c); 5.32 (dd, *J* = 17.2, 1.7 Hz, 1H, H-c'); 5.45 (d, $^3J_{1,2}$ = 1.0 Hz, 1H, H*f*-2); 5.48 (d, $^3J_{1,2}$ = 3.8 Hz, 1H, H*p*-1); 5.61 (dd, $^3J_{2,3}$ = 10.6 Hz, $^3J_{3,4}$ = 2.9 Hz, 1H, H*p*-3); 5.77 (dd, $^3J_{1,2}$ = 3.8 Hz, 1H, H*p*-2); 5.89 (m, 1H, H-b); 7.35-7.64 (m, 9H, arom.); 7.99-8.05 (m, 6H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 20.7 (*C*q-*t*Bu); 23.2 (*C*q-*t*Bu); 24.8 (*C*H$_3$); 26.0 (*C*H$_3$); 27.2 (*t*Bu); 27.5 (*t*Bu); 65.3 (C*f*-6); 66.9 (C*p*-6); 67.6; 67.9

(C-a); 68.4 (*Cp*-2); 71.0 (*Cp*-3); 71.1 (*Cp*-4); 74.4; 81.9 (*Cf*-2); 82.5; 83.3; 97.0 (*Cp*-1); 104.9 (*Cf*-1); 109.8 (*C*q-*isop.*); 117.4 (C-c); 128.3 (*C*-arom.); 128.4 (*C*-arom.); 128.5 (*C*-arom.); 129.2 (*C*q, arom.); 129.4 (*C*q, arom.); 129.7 (*C*-arom.); 129.8 (2 × *C*-arom.); 129.9 (*C*q, arom.); 133.1 (*C*-arom.); 133.3 (*C*-arom.); 133.5 (*C*-arom.); 133.8 (C-b); 165.3 (*C*=O); 166.0 (*C*=O); 166.2 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{47}H_{58}NaO_{14}Si$ 897.3494, found 897.3494.

Allyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (6). Fully protected disaccharide **4** (160 mg, 0.18 mmol) was dissolved in a mixture of HF-Pyr (70%)/dry THF (320 μL/32 mL) in a plastic conical tube and stirred for 30 min at 0°C and then 30 min at rt under Ar. The reaction mixture was cooled down again to 0°C and quenched with saturated $NaHCO_3$ solution. Then, the mixture was diluted and extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, concentrated and purified by flash column chromatography on silica gel to give the partially protected disaccharide **6** (87 mg, 65%) as a white powder. R_f 0.37 (EtOAc/hexanes = 1:1). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 1.20 (s, 3H, C*H*$_3$); 1.32 (s, 3H, C*H*$_3$); 2.84 (br. s, 1H, O*H*-6); 3.07 (br. s, 1H, O*H*-4); 3.75 (dd, 1H, H*f*-6a); 3.82 (dd, 1H, H*f*-6b); 3.93 (dd, 1H, H*p*-6a); 4.00 (dd, 1H, H*p*-6b); 4.06 (m, 1H, H-a); 4.09 - 4.20 (m, 3H, H*f*-3, H*f*-5, H*f*-4); 4.25 (m, 1H, H-a’); 4.38 (t, $^3J_{3,4}$ = 4.6, 1H, H*p*-5); 4.49 (s, 1H, H*p*-4); 5.19-5.25 (m, 2H, H*f*-1, H-c); 5.35 (m, 1H, H-c’); 5.54 (d, $^3J_{1,2}$ = 2.6 Hz, 1H, H*p*-1); 5.62 (d, *J* = 1.1 Hz, 1H, H*f*-2); 5.71 (m, 2H, H*p*-2, H*p*-3); 5.92 (m, 1H, H-b); 7.34-7.40 (m, 4H, arom.); 7.41-7.47 (m, 2H, arom.); 7.49-7.54 (m, 2H, arom.); 7.60 (m, 1H, arom.); 7.96-8.05 (m, 6H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 25.0 (*C*H$_3$); 26.1 (*C*H$_3$); 63.3 (C*p*-6); 65.3 (C*f*-6); 67.9 (C-a); 68.7 (*Cp*-2); 69.6 (*Cp*-4); 70.3 (*Cf*-3); 71.0 (*Cp*-3); 74.9; 81.5 (*Cf*-2); 83.4; 84.2; 97.7 (*Cp*-1); 104.9 (*Cf*-1); 109.8 (*C*q-*isop.*); 117.5 (C-c); 128.4 (2 × *C*-arom.); 128.5 (*C*-arom.); 129.0 (*C*q, arom.); 129.2 (*C*q, arom.); 129.3 (*C*q, arom.); 129.7 (*C*-arom.); 129.8 (*C*-arom.); 133.4 (*C*-arom.); 133.6 (*C*-arom.); 133.7 (C-b); 165.6 (*C*=O); 165.8 (*C*=O); 165.9 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{39}H_{42}NaO_{14}$ 757.2472, found 757.2470.

Allyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-β-D-galactofuranoside (7). To a solution of desilylated disaccharide **6** (110 mg, 0.15 mmol) in 15 mL DCM, water and TFA (1.5 mL each) were consecutively added and stirred vigorously at rt for 15 min. After consumption of the starting material, the resulting solution was co-evaporated with 10 mL ethanol twice under reduced pressure at <40 °C. The residue was then dried under vacuum and purified by column chromatography on silica gel (DCM/MeOH = 20:1) to afford disaccharide **7** (94 mg, 90%) as a yellow solid. R_f 0.27 (DCM/MeOH = 20:1). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 3.40-3.58 (m, 2H, H*f*-6a,b); 3.64 (m, 1H, H*f*-5); 3.88 (dd, 1H, H*p*-6a); 3.94 (dd, 1H, H*p*-6b); 4.02 (m, 1H, H-a); 4.09-4.20 (m, 2H, H-a’, H*p*-5); 4.33 (t, $^3J_{3,4}$ = 4.6, 1H, H*f*-3); 4.39 (d, 1H, H*f*-4); 4.47 (s, 1H, H*p*-4); 5.13-5.21 (m, 2H, H*f*-1, H-c); 5.31 (m, 1H, H-c’); 5.51 (d, $^3J_{1,2}$ = 1.3 Hz, 1H, H*f*-2); 5.58 (d, $^3J_{1,2}$ = 2.2 Hz, 1H, H*p*-1); 5.65-5.74 (m, 2H, H*p*-2, H*p*-3); 5.86 (m, 1H, H-b); 7.32-7.44 (m, 6H, arom.); 7.46-7.58 (m, 3H, arom.); 7.91-8.05 (m, 6H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 63.1 (C*p*-6); 63.9 (C*f*-6); 68.0 (C-a); 69.0; 69.5 (*Cp*-4); 70.4 (*Cf*-3); 70.6 (*Cf*-5); 70.9; 81.9 (*Cf*-2); 83.0 (*Cf*-4); 83.6 (*Cp*-5); 97.2 (*Cp*-1); 104.9 (*Cf*-1); 117.6 (C-c); 128.3 (*C*-arom.); 128.4 (*C*-arom.); 128.5 (*C*-arom.); 129.0 (*C*q, arom.); 129.1 (*C*q, arom.); 129.4 (*C*q, arom.); 129.7 (*C*-arom.); 129.8 (2 ×*C*-arom.); 133.3 (*C*-arom.); 133.5 (*C*-arom.); 133.6 (C-b, *C*-arom.); 165.8 (*C*=O); 165.9 (*C*=O); 166.2 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{36}H_{38}NaO_{14}$ 717.2159, found 717.2158.

***S*-acetyl-3-thiopropyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-β-D-galactofuranoside (8)**. To a solution of disaccharide **7** (37 mg, 0.053 mmol) and AIBN (9 mg, 0.055 mmol) in 8.5 mL dry THF, thioacetic acid (26 µL, 0.36 mmol) was added and stirred under Ar at rt for 6 h in a Rayonet UV reactor (350 nm). The reaction mixture was co-evaporated with toluene and concentrated. The crude product was purified by flash chromatography (DCM/MeOH

= 15:1) to give the thioester **8** (35 mg, 85%) as a white solid. R_f 0.37 (DCM/MeOH = 15:1). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 1.84 (m, 2H, $OCH_2C\underline{\mathit{H}_2}CH_2S$); 2.02 (br. s, 1H, O*H*); 2.30 (s, 3H, $C\mathit{H}_3$); 2.83 (br. s, 1H, O*H*); 2.94 (t, $^3J_{b,c}$ = 7.1 Hz, 2H, $OCH_2CH_2C\underline{\mathit{H}_2}S$); 3.26 (br. s, 1H, O*H*); 3.40-3.76 (m, 6H, O*H,* $OC\underline{\mathit{H}_2}CH_2CH_2S$, H*f*-5, H*f*-6a,b); 3.89 (dd, 1H, H*p*-6a); 3.96 dd, 1H, H*p*-6b); 4.13 (dd, *J* = 5.2, 3.7 Hz, 1H, H*p*-5); 4.29-4.40 (m, 2H, H*f*-3, H*f*-4); 4.49 (s, 1H, H*p*-4); 5.12 (s, 1H, H*f*-1); 5.48 (d, $^3J_{1,2}$ = 1.1 Hz, 1H, H*f*-2); 5.58 (d, $^3J_{1,2}$ = 3.06 Hz, 1H, H*p*-1); 5.63-5.75 (m, 2H, H*p*-2, H*p*-3); 7.31-7.45 (m, 6H, arom.); 7.46-7.60 (m, 3H, arom.); 7.91-8.06 (m, 6H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 25.9 (C-c); 29.4 (C-b); 30.6 ($\mathit{C}H_3$); 63.0 (C*p*-6); 63.9 (C*f*-6); 65.9 (C-a); 69.0; 69.4 (*Cp*-4); 70.4 (*Cf*-3); 70.6 (*Cf*-5); 70.9; 81.8 (*Cf*-2); 83.3 (*Cf*-4); 83.5 (*Cp*-5); 97.5 (*Cp*-1); 105.9 (*Cf*-1); 128.3 (*C*-arom.); 128.4 (*C*-arom.); 128.5 (*C*-arom.); 129.0 (*C*q, arom.); 129.1 (*C*q, arom.); 129.4 (*C*q, arom.); 129.7 (*C*-arom.); 129.8 (*C*-arom.); 133.3 (*C*-arom.); 133.5 (*C*-arom.); 133.6 (*C*-arom.); 165.7 (*C*=O); 165.8 (*C*=O); 166.1 (*C*=O); 196.1 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{38}H_{42}NaO_{15}S$ 793.2142, found 793.2147.

3-Thiopropyl α-D-galactopyranosyl-(1→3)-β-D-galactofuranoside ($G27_{SH}$). The acyl-protected disaccharide **8** (35 mg, 0.045 mmol) was dissolved in 3 mL of 0.25 M NaOMe in anhydrous MeOH, and stirred for 2 h under Ar. The removal of benzoyl and acetyl groups was monitored by mass spectrometry. The solution was then neutralized with Amberlyst-15, filtered through Celite, concentrated and dissolved in water and lyophilized. Initially, the unprotected 3-thiopropyl trisaccharide **$G27_{SH}$** is produced, which oxidizes by handling on air within hours to the disulfide **$(G27_S)_2$** (19 mg, quant.) as an off-white solid. R_f 0.37 [*i*PrOH/H_2O = 5:1 w/ 3 drops AcOH (27 μL per 12 mL of eluent)]. ^{1}H NMR (600 MHz, D_2O, 300K) δ 1.82 (m, 2H, $OCH_2C\underline{\mathit{H}_2}CH_2S$); 2.65 (t, ^3J = 6.0 Hz, 2H, $OCH_2CH_2C\underline{\mathit{H}_2}S$); 3.46-3.50 (m, 3H, $OC\underline{\mathit{H}_2}CH_2CH_2S$, H*f*-$6_a$); 3.53 (dd, 1H, H*f*-$6_b$); 3.55- 3.58 (m, 2H, H*p*-$6_{a,b}$); 3.61-3.69 (m, 3H, H*p*-5, H*p*-2, H*p*-3); 3.81 (m, 1H, H*p*-4); 3.85 (dd, 1H, H*f*-3); 3.87 (m, 1H, H*p*-5); 3.98 (dd, ^3J = 6.1, 4.0 Hz, 1H, H*f*-4); 4.09 (dd, $^3J_{2,3}$ = 2.9 Hz, $^3J_{1,2}$ = 1.5 Hz, 1H, H*f*-2); 4.84 (d, 1H, H*f*-1); 4.86 (d, $^3J_{1,2}$ = 3.8 Hz, 1H, H*p*-1) ppm. ^{13}C NMR (150 MHz, D_2O, 300K) δ 28.4 ($OCH_2\underline{C}H_2CH_2S$); 34.9 ($OCH_2CH_2\underline{C}H_2S$); 61.2 (C*p*-6); 62.9 (C*f*-6); 66.3 (C-$O\underline{C}H_2CH_2CH_2S$); 68.3 (*Cp*-2); 69.3 (*Cp*-3, *Cp*-4); 71.0 (*Cf*-5); 71.5 (*Cp*-5); 79.3 (*Cf*-2); 82.1 (*Cf*-4); 85.1 (*Cf*-3); 100.0 (*Cp*-1); 107.6 (*Cf*-1) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{15}H_{28}NaO_{11}S$ 439.1250, found 439.1198; for $C_{30}H_{54}NaO_{22}S_2$ 853.2446, found 853.2331.

Conjugation of $G27_{SH}$ to maleimide-derivatized BSA to produce NGP27b and MALDI-TOF MS analysis

The kit for the conjugation of the thiol-containing glycan **$G27_{SH}$** to BSA (Imject™ Maleimide-Activated BSA, 77116 cat. number), was purchased from Thermo Fisher Scientific, and the conjugation procedure followed was similar to the one described by the manufacturer and previously published.[9] Tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl, 0.8 mg, 2.8 μmol) was dissolved in 250 μL of conjugation buffer provided in the kit (83 mM sodium phosphate buffer, 0.1 M EDTA, 0.9 M sodium chloride, 0.02% sodium azide, pH 7.2). The TCEP·HCl solution was added to a 1.5 mL micro-centrifuge tube that contained sugar-disulfide **$(G27_S)_2$** (2.5 mg, 3.0 μmol), and the mixture was agitated in a shaker for 30 min to furnish sugar-thiol **$G27_{SH}$**. An aliquot of 10 μL was set aside for the colorimetric determination of the thiol concentration. The maleimide-activated BSA (2 mg, 15-25 moles of maleimide/mole BSA) was reconstituted with 200 μL of ultrapure water to produce a 10 mg/mL solution. The disaccharide solution was added to the reconstituted BSA and incubated at rt for 2-3 h in a shaker. Then, 18 μL were removed from the conjugation mixture to determined the concentration of unreacted thiol. This aliquot was diluted to 2.75 mL with reaction buffer (0.1 M sodium phosphate, pH 8.0, containing 1 mM EDTA), combined with 50 μL of Ellman's reagent [5,5'dithiobis-(2-nitrobenzoic acid) = DTNB] solution (4 mg DTNB in 1 mL of reaction buffer), and reacted for 15 min at rt. With a UV Vis spectrophotometer, the absorbance at 412 nm was measured. The thiol concentration was determined using the molar extinction coefficient of 2-nitro-5-thiobenzoic acid (TNB, ε = 14,150 M^{-1} cm^{-1}), and the amount of sugar conjugated, typically 2.0 μmol, was calculated. The conjugation mixture was diluted with ultrapure water to a volume of 1 mL and desalted using an Amicon Ultra 3K centrifugal filter and was centrifuged for 20 min at 4,000 × g, rt. The mixture was washed with 1 mL of ultrapure water three times following the same procedure. The tube with the filtrate was then removed, and 500 μL of ultrapure water was added to the **NGP27b** solution remaining in the filter. Since, a small amount of aggregation can occur, the solution/suspension was transferred onto a 2 mL ZebaTM spin desalting column (7K MWCO), provided in the kit, that was previously washed with 1 mL of ultrapure pure water 4 times and centrifuged at 1,000 × g for 2 min at rt. This procedure removed all salts and aggregated protein. The filtrate was lyophilized and can be stored at -50 ºC for at least 6 months. In our hands, this combination of filtration and size exclusion chromatography avoids or minimizes aggregation of the NGP. To determine the **NGP27b** quantity, a solution of 1-2 mg of it in 1-3 mL of ultrapure water was prepared, and the concentration was determined with a Pierce BCA Protein Assay Reagent kit using a spectrophotometer at a detection wavelength of 562 nm. To determine the mass of BSA and **NGP27b**, in a 1.5 mL microcentrifuge tube, 1 μL of a solution of ~ 0.1 mg BSA /100 μL H_2O, was combined with 1 μL of a solution of ~ 0.1 mg **NGP27b**/100 μL H_2O and 2 μL of matrix (10 mg/mL sinapinic acid, 50% acetonitrile, 0.1% TFA). Two μL of the combined sample:matrix was spotted onto a 48-well steel MALDI plate and allowed to crystallize at rt for approximately 20 minutes. The mass spectra were acquired using a SHIMADZU MALDI-8020 mass spectrometer set to linear mode with dithering at a scan range of 10,000 to 100,000 *m/z*. Data acquisition included a laser power of 110, laser rep. rate (Hz) 50, accumulated shots 5, blast shots 2, profiles at 200, pulse extraction set to 66,431, and a blanking mass of 15,000. Spectra were processed by Threshold Apex set at constant Threshold, Gaussian smoothing, smoothing filter width 200 and peak width 2. BSA standard was used for calibration and internal references set at $[BSA+H]^+$ = 66,120 with a 5ppm mass tolerance.

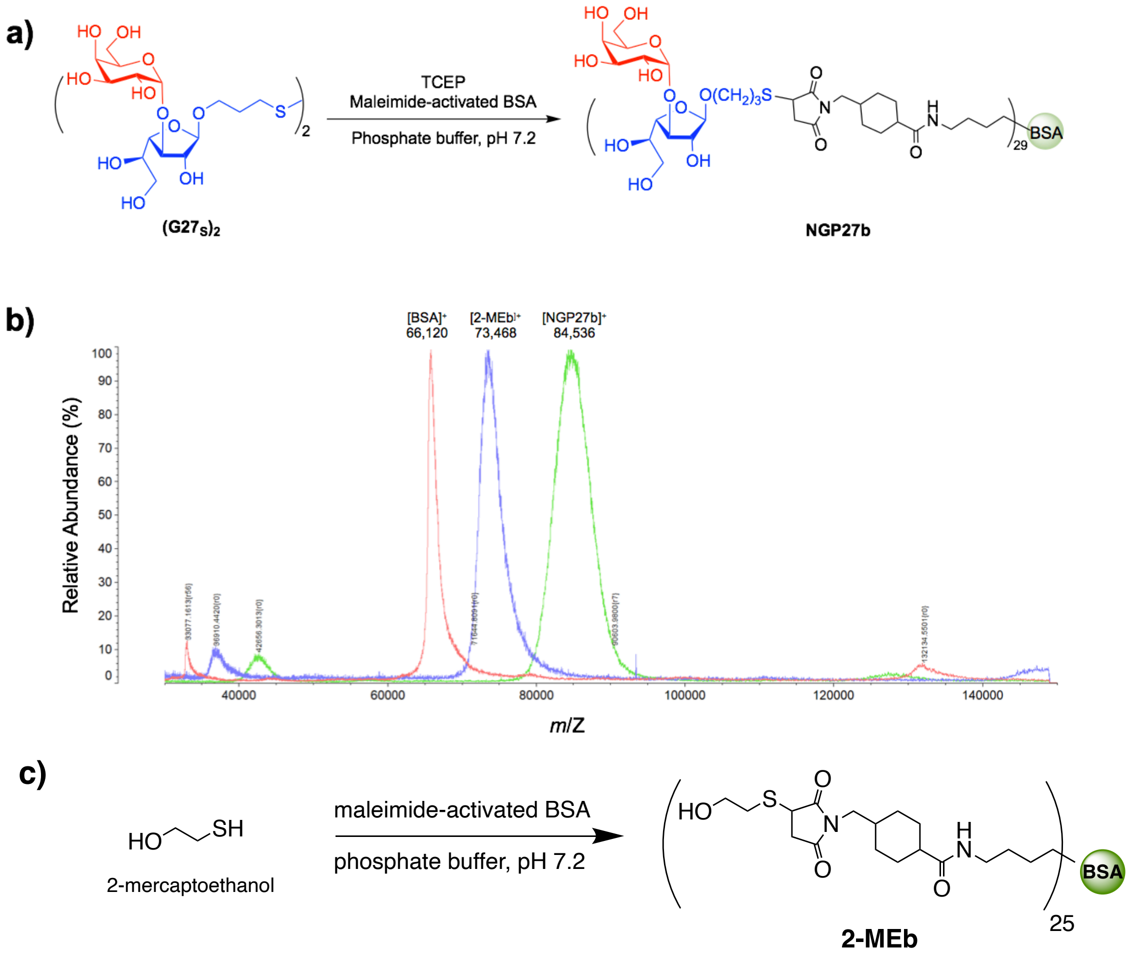


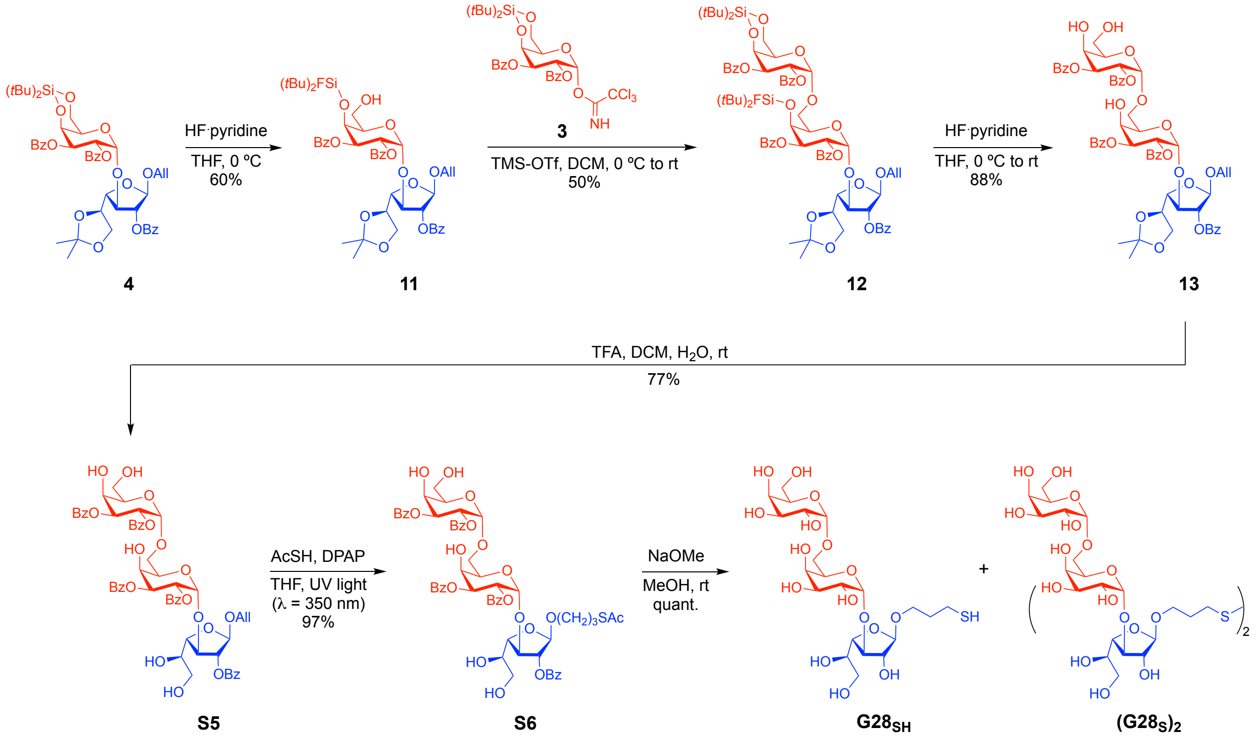
Figure S1. **a**) Conjugation of **$G27_{SH}$** to BSA. **b**) MALDI-TOF mass spectra of **NGP27b** overlaid with underivatized BSA and **2-MEb**. *m/z*, mass-to-charge ratio; **c**) Conjugation of 2-mercaptoethanol to BSA.

MALDI-TOF MS: *m/z* for BSA, $[M+H]^+$ = 66,120; for NGP27b, $[M+H]^+$ = 84,536; for 2-MEb, $[M+H]^+$ = 73,468. The average payload of **$G27_{SH}$** units per BSA molecule was 29, and the average payload of 2-mercaptoethanol units per BSA was 25.

Conjugation of 2-mercaptoethanol to maleimide-derivatized BSA to produce 2-MEb

2-MEb was used as a negative control antigen in the cross-titrations of NGP antigens and pooled sera by chemilumescent ELISA (see Figure S5). The conjugation of 2-mercaptoethanol to maleimide-derivatized BSA (2 mg) was performed following the same procedure as described for the conjugation of **$G27_{SH}$** to maleimide-derivatized BSA (p. S10), except for that 1.4 μmol of 2-mercaptoethanol was used (0.1 μL), and TCEP-HCl was omitted. A disulfide reduction was not necessary because pristine 2-mercaptoethanol from an ampoule was added to the reconstituted BSA immediately after opening the ampoule.

Synthesis of G28$_{SH}$



Scheme S6. Synthesis of the 3-thiopropyl trisaccharide **G28$_{SH}$**.

Allyl 2,3-di-*O*-benzoyl-4-*O*-di-*tert*-butylfluorosilyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (11). The fully protected disaccharide **4** (**Scheme S5**) (300 mg, 0.34 mmol) was dissolved in 50 mL of anhydrous THF in a plastic conical tube and cooled 0°C. Next, 100 μL of HF-Pyr (70%) was added and the reaction mixture was stirred for 2 h at 0°C under Ar. Then, it was quenched with saturated NaHCO$_3$ solution, extracted with EtOAc 5x, washed with water, brine and dried over MgSO$_4$. The residue was purified by flash chromatography on silica gel (EtOAc/hexanes = 1:4) to furnish the ring-opened silylated disaccharide **11** (180 mg, 60%) as a white powder. R_f 0.24 (EtOAc/hexanes = 1:4). ^{1}H NMR (400 MHz, CDCl$_3$, 300K) δ 1.00 (s, 9H, *t*Bu); 1.02 (s, 9H, *t*Bu); 1.21 (s, 3H, C*H*$_3$); 1.30 (s, 3H, C*H*$_3$); **2.74 (dd, *J* = 8.6, 4.6 Hz, 1H, O*H*)**; 3.68-3.87 (m, 3H, H*f*-6a,b, H*p*-6a or b); 3.96 (m, 1H, H*p*-6a or b); 4.04-4.22 (m, 4H, H-a, H*f*-3, H*f*-4, H*f*-5); 4.27 (m, 1H, H-a'); 4.41 (dd, *J* = 7.9, 4.5 Hz, 1H, H*p*-5); 4.89 (d, $^3J_{3,4}$ = 2.3 Hz, 1H, H*p*-4); 5.19-5.28 (m, 2H, H*f*-1, H-c); 5.37 (m, 1H, H-c'); 5.49 (d, $^3J_{1,2}$ = 3.8 Hz, 1H, H*p*-1); 5.64-5.79 (m, 3H, H*f*-2, H*p*-2, H*p*-3); 5.93 (m, 1H, H-b); 7.32-7.39 (m, 4H, arom.); 7.41-7.53 (m, 4H, arom.); 7.60 (m, 1H, arom.); 7.93-8.05 (m, 6H, arom.) ppm. ^{13}C NMR (101 MHz, CDCl$_3$, 300K) δ 20.4 (*C*q-*t*Bu); 20.6 (*C*q-*t*Bu); 25.0 (*C*H$_3$); 26.1 (*C*H$_3$); 27.1 (*t*Bu); 27.2 (*t*Bu); 62.4 (C*p*-6); 65.3 (C*f*-6); 67.8 (C-a); 68.4 (C*p*-2); 71.2 (C*p*-3); 71.4 (C*p*-4); 72.4 (C*p*-5); 75.0; 81.4 (C*f*-2); 83.8; 84.3; 97.9 (C*p*-1); 104.9 (C*f*-1); 109.8 (Cq-*isop.*); 117.6 (C-c); 128.2 (*C*-arom.); 128.4 (*C*-arom.); 128.5 (*C*-arom.); 129.1 (*C*q, arom.); 129.3 (*C*q, arom.); 129.5 (*C*q, arom.); 129.7 (*C*-arom.); 129.8 (*C*-arom.); 129.9 (2 × *C*-arom.); 133.2 (*C*-arom.); 133.3 (*C*-arom.); 133.6 (*C*-arom.); 133.7 (C-b); 165.7 (*C*=O), 165.9 (*C*=O), 166.5 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* [M+Na]$^+$ calcd for C$_{47}$H$_{59}$FNaO$_{14}$Si 917.3556, found: 917.3560. The fact that the only OH of compound **11** appears as a dd in the ^{1}H-NMR spectrum shows that the opening of the silylene ring was indeed regioselective and that the 6-OH group is unprotected.

Allyl 2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranosyl-(1→6)-2,3-di-*O*-benzoyl-4-*O*-di-*tert*-butylfluorosilyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (12). To a solution of disaccharide acceptor **11** (180 mg, 0.20 mmol) and 4,6-di-*O*-*tert*butylsilylene-2,3-di-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate donor **3**[7,8] (180 mg, 0.27 mmol) in anhydrous DCM (24 mL), crushed and freshly activated 4Å molecular sieves was added and stirred under Ar for 1 h at rt. Then, the solution was cooled down at 0°C, TMS-OTf (11 μL, 0.060 mmol) was added dropwise, and the mixture was gradually brought to rt and stirred for 1.5 h. To quench the reaction, Et_3N was added. The solution was diluted with DCM, extracted with water and brine solution, dried over $MgSO_4$, filtered, concentrated and purified by column chromatography on silica gel (EtOAc/hexanes = 1:4) to give trisaccharide **12** (132 mg, 50%), as a light-yellow solid. R_f 0.47 (EtOAc/hexanes = 1:3). $[\alpha]_D^{23}$ = +126.1° (c = 0.05, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 0.83 (s, 9H, *t*Bu); 0.89 (s, 9H, *t*Bu); 0.92 (s, 9H, *t*Bu); 1.13 (s, 9H, *t*Bu); 1.20 (s, 3H, CH_3); 1.28 (s, 3H, CH_3); 3.61-3.86 (m, 3H, H*f*-6a,b, H*p*-6a or b); 3.98 (m, 1H, H*p*-6a or b); 4.04 (m, 1H, H*f*-4); 4.08-4.39 (m, 8H, H-a,a', H*p'*-6a,b, H*f*-3, H*f*-5, H*p'*-4, H*p'*-5); 4.56 (m, 1H, H*p*-5); 4.82 (m, 1H, H*p*-4); 5.15 (m, 1H, H-c); 5.27 (d, $^{3}J_{1,2}$ = 3.4 Hz, 1H, H*p'*-1); 5.31-5.41 (m, 2H, H*f*-1, H-c'); 5.51 (s, 1H, H*p*-1); 5.54-5.61 (m, 2H, H*p*-3, H*f*-2); 5.71-5.86 (m, 3H, H*p*-2, H*p'*-2, H*p'*-3); 5.94 (m, 1H, H-b); 7.28-7.41 (m, 10H, arom.); 7.42-7.56 (m, 5H, arom.); 7.90-8.03 (m, 10H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 20.3 (*C*q-*t*Bu); 20.5 (*C*q-*t*Bu); 20.7 (*C*q-*t*Bu); 23.2 (*C*q-*t*Bu); 25.1 (CH_3); 26.2 (CH_3); 26.8 (*t*Bu); 27.0 (*t*Bu); 27.3 (*t*Bu); 27.5 (*t*Bu); 65.4 (C*p*-6); 65.5 (C*f*-6); 67.1; 67.1 (CH_2, C*p'*-6); 67.7 (C-a); 68.4 (*Cp*-2); 68.6; 70.2 (C*p*-5); 71.0 (*Cp*-3); 71.2 (*Cp*-4); 71.5; 75.4; 77.2; 81.0 (*Cf*-2); 83.9; 85.0 (C*f*-4); 96.5 (*Cp'*-1); 98.3 (*Cp*-1); 105.0 (*Cf*-1); 109.8 (Cq-*isop.*); 116.9 (C-c); 128.2 (2 × *C*-arom.); 128.3 (*C*-arom.); 128.4 (2 × *C*-arom.); 129.2 (*C*q, arom.); 129.3 (*C*q, arom.); 129.5 (*C*q, arom.); 129.6 (*C*-arom.); 129.7 (*C*q, arom.); 129.7 (*C*-arom.); 129.8 (*C*-arom.); 129.9 (2 × *C*-arom.); 130.0 (*C*q, arom.); 132.8 (*C*-arom.); 133.0 (*C*-arom.); 133.1 (*C*-arom.); 133.2 (*C*-arom.); 133.4 (*C*-arom.); 134.1 (C-b); 165.5 (*C*=O), 165.7 (*C*=O), 165.9 (*C*=O), 166.2 (*C*=O), 166.3 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* [M+Na]$^+$ calcd for $C_{75}H_{93}FNaO_{21}Si_2$ 1427.5630, found: 1427.5625.

Allyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→6)-2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (13). The fully protected trisaccharide **12** (39 mg, 0.028 mmol) was dissolved in 8.0 mL of anhydrous THF in a plastic conical tube and cooled to 0°C. Then, 80 μL of HF-Pyr (70%) was added and the mixture was stirred for 30 min. at 0°C and then 30 min. at rt under Ar. The reaction mixture was cooled again to 0°C and quenched with saturated $NaHCO_3$ solution. Then, the mixture was extracted with EtOAc 5×, washed with water and brine, dried over $MgSO_4$, concentrated, and purified by PTLC on silica gel (DCM/MeOH = 20:1) to furnish the desilylated trisaccharide **13** (27 mg, 88%) as a white powder. R_f 0.36 (DCM/MeOH = 15:1). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 1.18 (s, 3H, CH_3); 1.29 (s, 3H, CH_3); 3.67-3.89 (m, 3H, H*f*-6a,b, H*p*-6a or b); 3.92-4.21 (m, 8H, H-a, H*p*-6a or b, H*p'*-6a,b, H*p*-4, H*f*-3, H*f*-4, H*f*-5,); 4.28 (m, 1H, H-a'); 4.34 (m, 1H, H*p'*-4); 4.43 (m, 1H, H*p'*-5); 4.53 (m, 1H, H*p*-5); 5.19 (dd, *J* = 10.4, 1.5 Hz, 1H, H-c); 5.25 (s, 1H, H*f*-1); 5.34 (m, 1H, H-c'); 5.40 (d, *J* = 2.9 Hz, 1H, H*p'*-1); 5.43 (d, *J* = 3.8 Hz, 1H, H*p*-1); 5.49-5.56 (m, 2H, H*p*-3, H*f*-2); 5.60-5.73 (m, 3H, H*p*-2, H*p'*-2, H*p'*-3); 5.92 (m, 1H, H-b); 7.17-7.25 (m, 3H, arom.); 7.32-7.61 (m, 12H, arom.); 7.87-8.07 (m, 10H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 25.0 (CH_3); 26.2 (CH_3); 63.1 (C*p'*-6); 65.3 (C*f*-6); 66.8 (C*p*-6); 67.9 (C-a); 68.1; 68.7; 68.8 (C*p*-5); 68.8; 69.5; 69.6; 70.6; 71.0; 74.9; 81.5 (*Cf*-2); 83.0; 84.4; 97.5 (*Cp'*-1); 97.9 (*Cp*-1); 105.0 (*Cf*-1); 109.8 (Cq-*isop.*); 117.3 (C-c); 128.3 (*C*-arom.); 128.4 (*C*-arom.); 128.6 (*C*-arom.); 129.1 (*C*q, arom.); 129.2 (2 × *C*q, arom.); 129.3 (*C*q, arom.); 129.4 (*C*q, arom.); 129.6 (*C*-arom.); 129.7 (*C*-arom.); 129.8 (*C*-arom.); 133.1 (*C*-arom.); 133.2 (*C*-arom.); 133.3 (*C*-arom.); 133.6 (*C*-arom.); 133.9 (C-b); 165.4 (*C*=O);

165.6 (*C*=O); 165.7 (*C*=O); 165.8 (*C*=O); 165.9 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* [M+Na]$^+$ calcd for $C_{59}H_{60}NaO_{21}$ 1127.3525, found: 1127.3524.

Allyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→6)-2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-β-D-galactofuranoside (S5). To a solution of trisaccharide **13** (27 mg, 0.024 mmol) in DCM (3.0 mL), H_2O (300 μL) and TFA (300 μL) were consecutively added, and the mixture was vigorously stirred at rt for 15 min. After disappearance of starting material based on TLC, the resulting solution was twice co-evaporated with EtOH (10 mL). The residue was then further dried under vacuum and purified by PTLC on silica gel (DCM/MeOH = 15:1) to afford the partially deprotected trisaccharide **S5** (20 mg, 77%) as a yellow syrup. R_f 0.30 (DCM/MeOH = 15:1). ^{1}H NMR (400 MHz, CD_3OD, 300K) δ 3.46-3.61 (m, 2H, H*f*-6a,b); 3.66 (m, 1H); 3.72-3.90 (m, 3H, H*p*-6a,b, H*p'*-6a or b); 3.98-4.12 (m, 2H, H-a, H*p'*-6a or b); 4.20-4.33 (m, 5H, H-a'); 4.39 (m, 1H); 4.62 (m, 1H); 5.11 (m, 1H, H-c); 5.26-5.38 (m, 3H, H-c', H*f*-1, H*p'*-1); 5.55 (br. s, 1H, H*p*-1); 5.60-5.71 (m, 5H, H*f*-2, H*p*-2, H*p*-3, H*p'*-2, H*p'*-3); 5.91 (m, 1H, H-b); 7.25-7.43 (m, 11H, arom.); 7.48-7.57 (m, 4H, arom.); 7.87-7.98 (m, 8H, arom.); 8.04-8.09 (m, 2H, arom.) ppm. ^{13}C NMR (101 MHz, CD_3OD, 300K) δ 62.6 (C*p'*-6); 64.4 (C*f*-6); 67.7 (C*p*-6); 68.8; 69.0 (C-a); 69.3; 70.5; 70.7; 71.1; 72.2; 72.7; 72.9; 73.0; 83.0; 85.0; 85.4; 98.5 (*Cp'*-1); 99.4 (*Cp*-1); 106.6 (*Cf*-1); 117.4 (C-c); 129.5 (*C*H, arom.); 129.6 (*C*H, arom.); 129.71 (2 × *C*H, arom.); 129.8 (*C*H, arom.); 130.8 x 2 (*C*H, arom.); 130.9 (2 × *C*H, arom.); 131.0 (*C*q, arom.); 131.1 (*C*q, arom.); 131.2 (*C*q, arom.); 131.3 (*C*q, arom.); 135.7 134.4 (*C*-arom.); 134.5 (*C*-arom.); 134.6 (*C*-arom.); 134.7 x 2 (*C*-arom.); (C-b); 167.3 (*C*=O); 167.4 (*C*=O); 167.5 x 2 (*C*=O); 167.6 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* [M+Na]$^+$ calcd for $C_{56}H_{56}NaO_{21}$ 1087.3212, found: 1087.3202.

***S*-acetyl-3-thiopropyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→6)-2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-β-D-galactofuranoside (S6)**. To a solution of allyl trisaccharide **S5** (40 mg, 0.038 mmol) and DPAP (96 µL of a solution of 5 mg DPAP in 500 µL DCM, 0.0038 mmol) in anhydrous DCM (1.4 mL) under Ar, thioacetic acid (14 µL, 0.18 mmol) was added, and the mixture was stirred under water cooling (~ 25 °C) for 30 min in a Rayonet UV reactor equipped with 350 nm lamps. The solution was then co-evaporated with toluene and concentrated to near dryness. The crude product was purified by PTLC on silica gel (DCM/MeOH = 20:1) to afford the acyl-protected trisaccharide **S6** (42 mg, 97%) as a white solid. R_f 0.30 (DCM/MeOH = 10:1).^{1}H NMR (400 MHz, CD_3OD, 300K) δ 1.72-1.90 (m, 2H, $OCH_2C\underline{H_2}CH_2S$); 2.20 (s, 3H, CH_3); 2.89-2.98 (m, 2H, $OCH_2CH_2C\underline{H_2}S$); 3.46-3.60 (m, 3H, H*f*-6a,b); 3.62-3.68 (m, 1H); 3.73-3.90 (m, 4H); 4.08 (dd, 1H); 4.25-4.35 (m, 4H); 4.36 (s, 1H); 4.69 (t, 1H); 5.31 (d, $^3J_{1,2}$ = 2.7 Hz, 1H, H*p'*-1); 5.36 (s, 1H, H*f*-1); 5.57 (d, $^3J_{1,2}$ = 2.2 Hz, 1H, H*p*-1); 5.61-5.69 (m, 4H, H*f*-2, H*p*-2, H*p*-3, H*p'*-2); 5.72 (s, 1H, H*p'*-3); 7.26-7.46 (m, 11H, arom.); 7.47-7.56 (m, 4H, arom.); 7.85-7.99 (m, 8H, arom.); 8.04-8.09 (m, 2H, arom.) ppm. ^{13}C NMR (400 MHz, CD_3OD, 300K) δ 27.2 (C-c); 30.5 (CH_3); 31.9 (C-b); 62.5 (C*p'*-6); 64.3 (C*f*-6); 66.5 (C*p*-6); 67.7(C-a); 68.9; 69.2; 70.4; 70.8; 71.1; 72.9; 72.5; 72.7; 73.0; 82.3; 85.2; 85.8; 98.1 (*Cp'*-1); 99.1 (*Cp*-1); 99.5; 106.9 (*Cf*-1); 129.5 (*C*-arom.); 129.6 (*C*-arom.); 129.7 (2 × *C*-arom.); 129.8 (*C*-arom.); 130.8 (*C*-arom.); 130.9 (*C*-arom.); 131.0 (*C*q, arom.); 131.1 (*C*q, arom.); 131.2 (*C*q, arom.); 131.3 (2 × *C*q, arom.); 134.4 (*C*-arom.); 134.5 (*C*-arom.); 134.6 (2 × *C*-arom.); 134.7 (*C*-arom.); 167.1 (*C*=O); 167.2 (*C*=O); 167.3 (*C*=O); 167.4 (*C*=O); 167.5 (*C*=O); 197.4 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* [M+Na]$^+$ calcd for $C_{58}H_{60}NaO_{22}S$ 1163.3195, found: 1163.3194.

3-Thiopropyl α-D-galactopyranosyl-(1→6)-α-D-galactopyranosyl-(1→3)-β-D-galactofuranoside (G28$_{SH}$). The acyl-protected trisaccharide **S6** (20 mg, 0.018) was dissolved in 3.0 mL of anhydrous 0.25 M NaOMe, and stirred for 3 h under Ar. The removal of benzoyl and acetyl groups was monitored by mass spectrometry. The solution was then neutralized with Amberlyst-15, filtered through Celite, concentrated and finally dissolved in water and lyophilized.

Initially, the unprotected 3-thiopropyl trisaccharide **G28$_{SH}$** is produced, which oxidizes by handling on air within hours to the disulfide **(G28$_{S}$)$_2$** (10 mg, quant.) as an off-white solid. The compound was purified by reversed phase FPLC on polystyrene/divinylbenzene beads using 2% CH_3CN/H_2O (solvent A) and 85% CH_3CN/H_2O (solvent B) in a gradient of 0-90. R_f 0.20 [*i*PrOH/H_2O = 5:1 w/ 3 drops AcOH (27 μL per 12 mL of eluent)]. ^1H NMR (600 MHz, D_2O, 300K) δ 1.83 (m, 2H, $OCH_2C\underline{H_2}CH_2S$); 2.66 (t, ^3J = 7.1 Hz, 2H, $OCH_2CH_2C\underline{H_2}S$); 3.45-3.51 (m, 3H); 3.53-3.58 (m, 3H); 3.61-3.66 (m, 3H); 3.67-3.74 (m, 4H); 3.78-3.82 (m, 2H); 3.85 (dd, 1H, ^3J = 7.7 Hz; 2.1 Hz); 3.87 (m, 1H); 3.99 (dd, 1H, ^3J = 5.5 Hz; 4.5 Hz); 4.07 (dd, 1H, ^3J = 6.9 Hz; 5.5 Hz); 4.17 (m, 1H); 4.80 (d, 1H, ^3J = 3.7 Hz, anomeric); 4.87 (s, 1H, anomeric); 4.88. (d, 1H, ^3J = 3.7 Hz, anomeric) ppm. ^{13}C NMR (150 MHz, D_2O, 300K) δ 28.4 ($OCH_2\underline{C}H_2CH_2S$); 35.0 ($OCH_2CH_2\underline{C}H_2S$); 61.2; 62.9; 65.9; 66.8; 68.3; 68.4; 3 × 69.3; 2 × 69.6; 71.1; 71.3; 79.0; 82.7; 85.5; 98.5 (anomeric); 100.0 (anomeric); 107.5 (anomeric) ppm. ESI-TOF HRMS: *m*/*z* $[M+H]^+$ calcd for $C_{42}H_{75}O_{32}S_2$ 1155.3683, found 1155.3538; *m*/*z* $[M+Na]^+$ calcd for $C_{42}H_{74}NaO_{32}S_2$ 1177.3502, found 1177.3554.

Conjugation of G28$_{SH}$ to maleimide-derivatized BSA to produce NGP28b and MALDI-TOF MS analysis

The conjugation of **G28$_{SH}$** to BSA and the analysis of **NGP28b** by MALDI-TOF MS were performed following the same procedures as described for the conjugation of **G27$_{SH}$** to BSA, and the MS analysis of **NGP27b**, see p. S10.

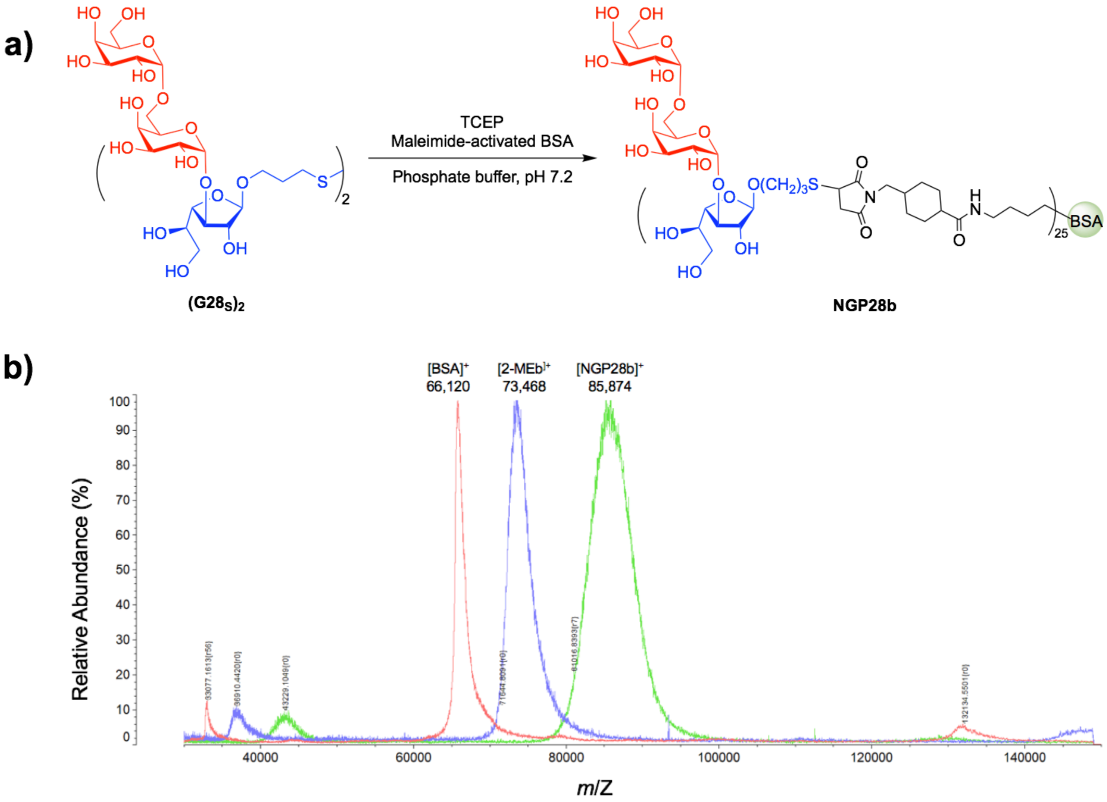
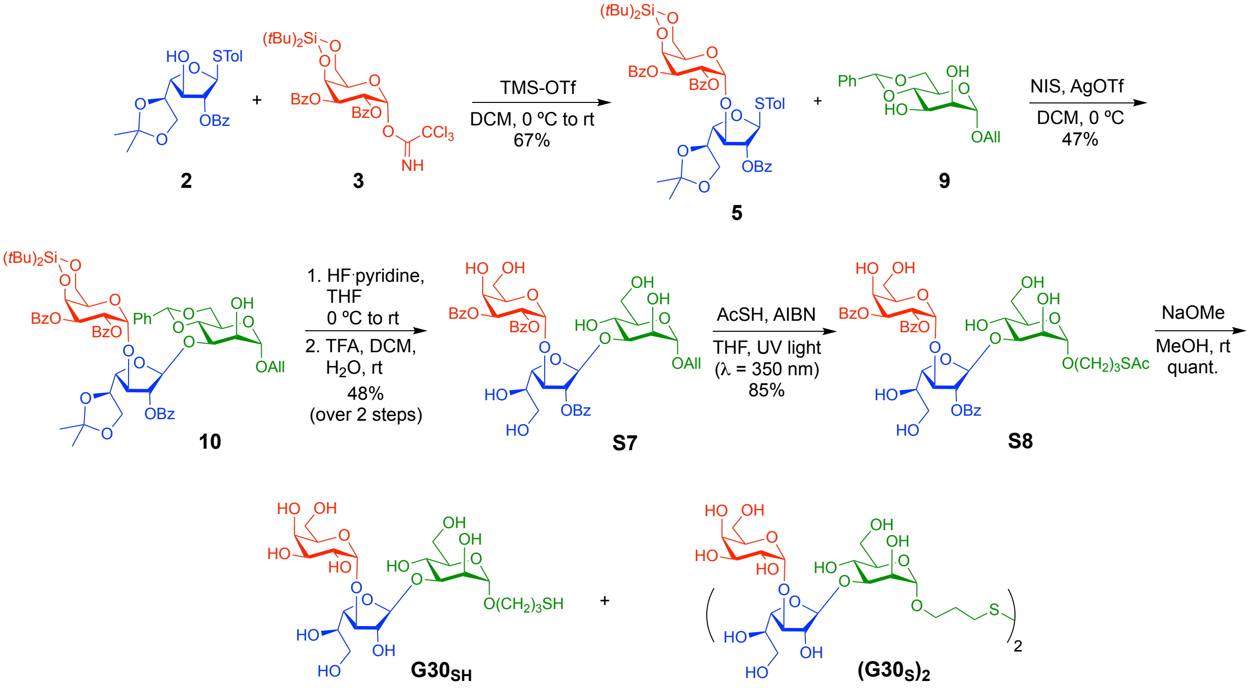


Figure S2. **a**) Conjugation of **G28$_{SH}$** to BSA. **b**) MALDI-TOF mass spectra of **NGP28b** overlaid with underivatized BSA and **2-MEb**. *m*/*z*, mass-to-charge ratio.

MALDI-TOF MS: *m/z* for BSA $[M+H]^+$ 66,120; for NGP2B $[M+H]^+$ 85,874. The average payload of **G28$_{SH}$** units per BSA molecule was 25.

Synthesis of the G30$_{SH}$



Scheme S7. Synthesis of the 3-thiopropyl trisaccharide **G30$_{SH}$**.

***p*-Tolyl 2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-5,6-*O*-isopropylidene-1-thio-β-D-galactofuranoside (5)**. To a solution of Gal*f* acceptor **2**$^{4\text{-}6}$ (520 mg, 1.21 mmol) and Gal*p* donor **3**^{7,8} (1.11 g, 1.65 mmol) in anhydrous DCM (186 mL), freshly activated MS 4Å was added and the mixture was stirred under Ar for 1 h at rt. Then, the solution was cool down to 0°C and TMS-OTf (77.0 μL, 0.43 mmol) was added dropwise. The solution was gradually brought to rt and after 1 h, the reaction mixture was quenched by addition of Et_3N, filtered, and washed with water and brine. The organic layers were dried over $MgSO_4$, concentrated, and purified by flash column chromatography on silica gel (EtOAc/Hexanes = 1:5) to give the disaccharide donor **5** as a yellow powder (756 mg, 67%). R_f = 0.30 (EtOAc/Hexanes = 1:5). $[\alpha]_D^{23.5}$ = +65.5° (c = 0.11, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (s, 9H, *t*Bu); 1.06 (s, 9H, *t*Bu); 1.19 (s, 3H, C*H*$_3$); 1.36 (s, 3H, C*H*$_3$); 2.32 (s, 3H, arom.C*H*$_3$); 3.81 (d, *J* = 6.6 Hz, 2H, H*f*-6a,b); 4.12-4.23 (m, 3H, H*p*-5, H*f*-5, H*p*-6a or b); 4.27-4.38 (m, 3H, H*f*-3, H*f*-4, H*p*-6a or b); 4.93 (d, $^3J_{3,4}$ = 3.0 Hz, 1H, H*p*-4); 5.49 (d, $^3J_{1,2}$ = 3.8 Hz, 1H, H*p*-1); 5.53-5.59 (m, 2H, H*f*-1, H*f*-2); 5.63 (dd, $^3J_{2,3}$ = 10.6 Hz, 1H, H*p*-3); 5.80 (dd, $^3J_{1,2}$ = 3.8 Hz, 1H, H*p*-2); 7.10 (d, *J* = 8.19 Hz, 2H, arom.); 7.31-7.64 (m, 11H, arom.); 7.99-8.14 (m, 6H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 20.7 (*C*q-*t*Bu); 21.1 (arom.*C*H$_3$); 23.2 (*C*q-*t*Bu); 24.7 (*C*H$_3$); 25.9 (*C*H$_3$); 27.2 (*t*Bu); 27.5 (*t*Bu); 65.2 (C*f*-6); 66.8 (C*p*-6); 67.8 (C*p*-5); 68.3 (C*p*-2); 71.0 (C*p*-3); 71.1 (C*p*-4); 73.7 (C*f*-5); 81.5; 82.2 (C*f*-2); 82.5; 91.0 (C*f*-1); 96.6 (C*p*-1); 109.8 (Cq-*isop.*); 128.3 (*C*-arom.); 128.4 (*C*-arom.); 128.6 (*C*-arom.); 129.1 (*C*q, arom.); 129.4 (*C*q, arom.); 129.7 (2 × *C*-arom.); 129.8 (*C*-arom.); 129.9 (2 ×

*C*q, arom.); 130.0 (*C*-arom.); 132.8 (*C*-arom.); 133.1 (*C*-arom.); 133.3 (*C*-arom.); 133.6 (*C*-arom.); 137.9 (Cq); 165.3 (*C*=O); 166.0 (*C*=O); 166.2 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* [M+Na]$^+$ calcd for $C_{51}H_{60}NaO_{13}SSi$ 963.3422, found 963.3489.

Allyl 2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilylene-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranosyl-(1→3)-4,6-*O*-benzylidene-α-D-mannopyranoside (10). To a solution of Man*p* acceptor **9**[3] (429 mg, 1.39 mmol) and disaccharide donor **5** (414 mg, 0.44 mmol) in anhydrous DCM (78 mL), NIS (248 mg, 1.10 mmol) and AgOTf (7.0 mg, 0.027 mmol) were added consecutively at 0 °C. After 45 min, the reaction mixture was quenched by addition of Et_3N, filtered, and washed with a saturated solution of $Na_2S_2O_3$ and brine. The organic layers were dried over $MgSO_4$, concentrated, and purified by column chromatography on silica gel (EtOAc/Hexanes = 1:3) to give the desired trisaccharide **10** (231 mg, 47%), as a light-yellow powder. R_f 0.27 (EtOAc/Hexanes = 1:3). $[\alpha]_D^{24}$ = +103.1° (c = 0.10, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 0.94 (s, 9H, *t*Bu); 1.06 (s, 12H, *t*Bu, C*H*$_3$); 1.30 (s, 3H, C*H*$_3$); 3.21 (s, 1H); 3.36 (m, 1H, H*f*-6a or b); 3.59 (dd, *J* = 8.4, 6.5 Hz, 1H, H*f*-6a or b); 3.71-3.90 (m, 3H, H*m*-6a or b); 3.92-4.26 (m, 10H, Ha,a', H*p*-6a,b, H*m*-6a or b); 4.32 (dd, *J* = 7.2, 3.4 Hz, 1H, H*f*-3); 4.84 (d, $^3J_{3,4}$ = 2.8 Hz, 1H, H*p*-4); 4.97 (d, *J* = 1.1 Hz, 1H, H*m*-1); 5.15-5.24 (m, 3H, H*f*-1, H-c, H*f*-2); 5.29 (m, 1H, H-c'); 5.38 (s, 1H, OC*H*Ph); 5.49 (d, $^3J_{1,2}$ = 3.7 Hz, 1H, H*p*-1); 5.61 (dd, $^3J_{2,3}$ = 10.6 Hz, $^3J_{3,4}$ = 2.8 Hz, 1H, H*p*-3); 5.76 (dd, 1H, H*p*-2); 5.88 (m, 1H, H-b); 7.23-7.30 (m, 3H, arom.); 7.32-7.64 (m, 11H, arom.); 7.95-8.07 (m, 6H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 20.7 (*C*q-*t*Bu); 23.2 (*C*q-*t*Bu); 24.8 (*C*H$_3$); 25.9 (*C*H$_3$); 27.2 (*t*Bu); 27.4 (*t*Bu); 63.7; 64.9 (C*f*-6); 66.7 (C*p*-6); 67.8; 68.2 (C-a); 68.5 (*Cp*-2); 68.7 (C*m*-6); 69.0; 70.6 (*Cp*-3); 71.2 (*Cp*-4); 73.0; 73.6; 76.6; 79.8; 81.2 (*Cf*-3); 83.9 (*Cf*-2); 97.2 (*Cp*-1); 99.3 (C*m*-1); 101.6 (OC*H*Ph); 103.1 (C*f*-1); 109.5 (Cq-*isop.*); 117.9 (C-c); 126.1 (*C*-arom.); 128.1 (*C*-arom.); 128.4 (*C*-arom.); 128.6 (*C*-arom.); 128.7 (*C*-arom.); 128.8 (*C*q, arom.); 129.3 (*C*q, arom.); 129.7 (*C*-arom.); 129.8 (*C*-arom.); 129.9 (*C*-arom.); 133.1 (*C*-arom.); 133.4 (*C*-arom.); 133.5 (C-b); 133.7 (*C*-arom.); 137.6 (Cq); 166.0 (*C*=O); 166.1 (*C*=O); 166.3 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* [M+Na]$^+$ calcd for $C_{60}H_{72}NaO_{19}Si$ 1147.4335, found 1147.4332.

Allyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-α-D-mannopyranoside (S7). The trisaccharide **10** (60 mg, 0.053 mmol) was dissolved in a mixture of HF-Pyr (70%)/dry THF (120 μL/12 mL) in a plastic conical tube and stirred for 30 min at 0°C and 30 min at rt under Ar. The reaction mixture was cooled down again to 0°C and quenched with saturated $NaHCO_3$ solution. Then, the resulting solution was extracted with EtOAc 5×, washed with water and brine, dried over $MgSO_4$, and concentrated to yield the desilylated trisaccharide as a light-yellow oil. R_f 0.16 (EtOAc/Hexanes 1:1). Without further purification, to a solution of the resulting trisaccharide (60 mg, 0.06 mmol) in DCM (9.0 mL), H_2O (900 μL) and TFA (900 μL) were added consecutively, and the mixture was vigorously stirred at rt for 20 min. After disappearance of starting material based on TLC, the resulting solution was co-evaporated with EtOH (10 mL) twice. The residue was then further dried under vacuum and purified by PTLC on silica gel (DCM/MeOH = 10:1) to afford the partially deprotected trisaccharide **S7** (22 mg, 48%) as a light-yellow syrup. R_f 0.33 (DCM/MeOH = 10:1). ^{1}H NMR (400 MHz, CD_3OD, 300K) δ 3.49-3.70 (m, 5H); 3.71-3.97 (m, 6H); 3.99-4.10 (m, 2H, H-a); 4.23 (dd, *J* = 13.0, 5.0 Hz, 1H, H-a'); 4.34-4.41 (m, 2H, H*p*-4); 4.44-4.50 (m, 2H, H*p*-5); 4.89 (1H, H*m*-1, overlapped with MeOH signal, based on HSQC spectrum); 5.17 (d, *J* = 10.4 Hz, 1H H-c); 5.26-5.38 (m, 2H, H*f*-1, H-c'); 5.55 (d, $^3J_{1,2}$ = 3.8 Hz, 1H, H*p*-1); 5.59-5.75 (m, 3H, H*p*-2, H*p*-3, H*f*-2); 5.93 (m, 1H, H-b); 7.35-7.44 (m, 4H, arom.); 7.46- 7.58 (m, 4H, arom.); 7.63 (m, 1H, arom.); 7.98 (t, *J* = 8.7 Hz, 4H, arom.); 8.08 (d, *J* = 7.7 Hz, 2H, arom.) ppm. ^{13}C NMR (101 MHz, CD_3OD, 300K) δ 62.2 (CH_2); 63.2 (CH_2); 64.5 (CH_2); 67.0; 68.9; 69.1; 69.0 (C-a); 70.5; 72.3; 72.7; 73.0; 75.1; 78.2; 83.8; 84.0 (*Cf*-2); 85.4

(C*p*-5); 99.6 (*Cp*-1); 100.6 (C*m*-1); 105.0 (*Cf*-1); 117.7 (C-c); 129.6 (*C*-arom.); 129.7 (*C*-arom.); 129.8 (*C*-arom.); 130.8 (*C*q, arom.); 130.9 (*C*-arom.); 131.0 (*C*q, arom.); 131.1 (*C*-arom.); 131.2 (*C*q, arom.); 134.6 (*C*-arom.); 134.7 (*C*-arom.); 134.8 (*C*-arom.); 135.5 (C-b); 167.6 (*C*=O); 167.7 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{42}H_{48}NaO_{19}$ 879.2687, found 879.2897.

***S*-acetyl-3-thiopropyl 2,3-di-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-α-D-mannopyranoside (S8)**. To a solution of trisaccharide **S7** (22 mg, 0.026 mmol) and AIBN (6.3 mg, 0.038 mmol) in anhydrous THF (6 mL), AcSH (18 μL, 0.25 mmol) was added and stirred under argon for 5 min. The solution was then placed in a Rayonet UV reactor (350 nm) and stirred for 12 h under water cooling (~rt). The solution was concentrated by two co-evaporations with toluene, and purified by PTLC on silica gel (DCM/MeOH = 10:1) to give the acyl-protected trisaccharide **S8** as a white powder (20.5 mg, 85%). R_f = 0.32 (DCM/MeOH = 10:1). ^{1}H NMR (400 MHz, CD_3OD, 300K) δ 1.81-1.91 (m, 2H, $OCH_2C\underline{H_2}CH_2S$); 2.29 (s, 3H, CH_3); 2.85-3.06 (m, 2H, $OCH_2CH_2C\underline{H_2}S$); 3.43-3.96 (m, 12H); 4.07 (br. s, 1H, H*m*-2); 4.34-4.41 (m, 2H, H*p*-4); 4.44-4.50 (m, 2H, H*p*-5); 4.59 (s, 1H); 4.81 (s, 1H, H*m*-1); 5.36 (s, 1H, H*f*-1); 5.49 (s, 2H); 5.55 (d, $^3J_{1,2}$ = 3.7 Hz, 1H, H*p*-1); 5.59-5.75 (m, 3H, H*p*-2, H*p*-3, H*f*-2); 7.35-7.45 (m, 4H, arom.); 7.46-7.58 (m, 4H, arom.); 7.64 (m, 1H, arom.); 7.97 (m, 4H, arom.); 8.08 (d, *J* = 7.6 Hz, 2H, arom.) ppm. ^{13}C NMR (101 MHz, CD_3OD, 300K) δ 27.0 (C-c); 30.6 (CH_3); 30.8 (C-b); 62.2 (CH_2); 63.2 (CH_2); 64.6 (CH_2); 67.0; 67.2 (C-a); 68.9; 69.1; 70.5; 72.3; 72.8; 73.0; 75.1; 78.4; 83.8; 84.0; 85.3; 99.6 (*Cp*-1); 101.5 (C*m*-1); 105.1 (*Cf*-1); 129.6 (*C*-arom.); 129.7 (*C*-arom.); 129.8 (*C*-arom.); 130.8 (*C*q, arom.); 130.9 (*C*-arom.); 131.0 (*C*q, arom.); 131.1 (*C*-arom.); 131.2 (*C*q, arom.); 134.6 (*C*-arom.); 134.7 (*C*-arom.); 134.8 (*C*-arom.); 167.6 (*C*=O); 167.7 (*C*=O); 197.6 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{44}H_{52}NaO_{20}S$ 955.2670, found 955.2994.

3-Thiopropyl α-D-galactopyranosyl-(1→3)-β-D-galactofuranosyl-(1→3)-α-D-mannopyranoside ($G30_{SH}$). The acyl-protected trisaccharide **S8** (20 mg, 0.021) was dissolved in 3.0 mL of 0.25 M NaOMe in anhydrous MeOH, and stirred for 2 h under Ar. The removal of benzoyl and acetyl groups was monitored by mass spectrometry. The solution was then neutralized with Amberlyst-15, filtered through Celite, concentrated and finally dissolved in water and lyophilized. Initially, the unprotected 3-thiopropyl trisaccharide **$G30_{SH}$** was produced, which oxidizes by handling on air within hours to the disulfide **$(G30_S)_2$** (12 mg, quant.) as an off-white solid. R_f 0.25 [*i*PrOH/H_2O = 5:1 w/ 3 drops AcOH (27 μL per 12 mL of eluent)]. ^{1}H NMR (400 MHz, D_2O, 300K) δ 1.81 (m, 2H, $OCH_2C\underline{H_2}CH_2S$); 2.63 (t, 2H, $OCH_2CH_2C\underline{H_2}S$); 3.50-3.69 (m, 13H); 3.77 (s, 1H); 3.82-3.92 (m, 4H); 4.04 (dd, 1H); 4.16 (s, 1H); 4.68 (s, 1H); 4.84 (d, $^3J_{1,2}$ = 4.0 Hz, 1H); 4.93 (s, 1H) ppm. ^{13}C NMR (101 MHz, D_2O, 300K) δ 28.1; 34.9; 61.0; 61.3; 62.9; 65.1; 66.1; 66.9; 68.3; 2 × 69.3; 70.8; 71.5; 72.9; 75.5; 79.6; 81.7; 84.8; 99.6; 99.7; 104.8. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{42}H_{74}NaO_{32}S_2$ 1177.3502, found 1177.3508.

Conjugation of $G30_{SH}$ to maleimide-derivatized BSA to produce NGP30b and MALDI-TOF MS analysis

The conjugation of **$G30_{SH}$** to BSA and the analysis of **NGP30b** by MALDI-TOF MS were performed following the same procedures as described for the conjugation of **$G27_{SH}$** to BSA, and the MS analysis of **NGP27b**, see p. S10.

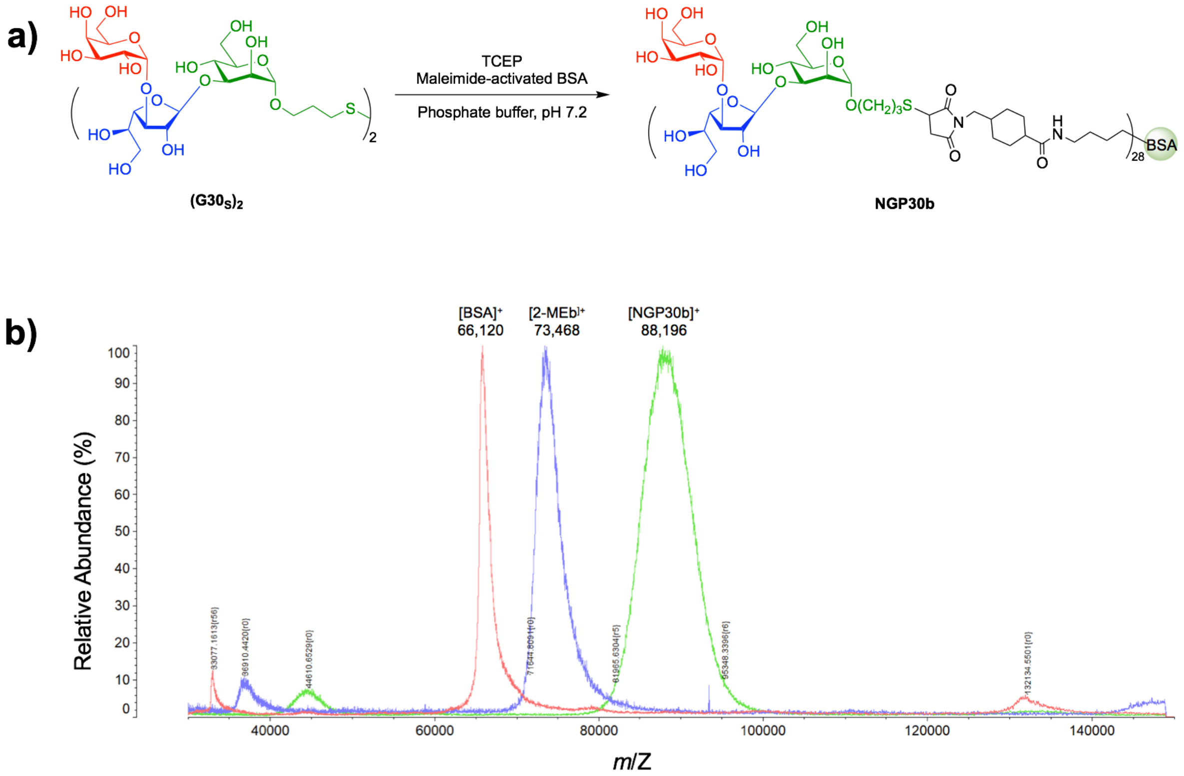
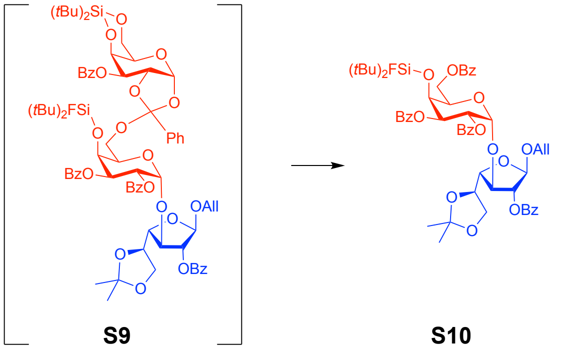


Figure S3. **a**) Conjugation of **G30$_{SH}$** to BSA. **b**) MALDI-TOF mass spectra of **NGP30b** overlaid with underivatized BSA and **2-MEb**. *m/z*, mass-to-charge ratio.

MALDI-TOF MS: *m/z* for BSA $[M+H]^+$ 66,120; for NGP30B $[M+H]^+$ 88,196. The average payload of **G30$_{SH}$** units per BSA molecule was 28.

ADDITIONAL RESULTS

As shown in Scheme **1** and described in detail in the experimental section above, the fully protected trisaccharide **12** was produced in only 50% yield. Therefore, we looked into the formation of any byproducts and identified the benzoylated disaccharide **S10**, most likely formed via orthoester **S9** (not identified or isolated), **Figure S4**.



S9 **S10**

Figure S4. Byproduct **S10** formed in the synthesis of trisaccharide **12**, presumably via orthoester **S9**.

Allyl 2,3-di-*O*-benzoyl-4-*O*-di-*tert*-butylfluorosilyl-6-*O*-benzoyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-5,6-*O*-isopropylidene-β-D-galactofuranoside (S10). The crude product obtained in the synthesis of compound **12** was purified by flash chromatography on silica gel (EtOAc/hexanes = 1:4), which, besides the desired trisaccharide **12**, also furnished the byproduct **S10** (30%) as a light-yellow powder. R_f 0.50 (EtOAc/hexanes = 1:3). ^{1}H NMR (400 MHz, $CDCl_3$, 300K) δ 1.00 (s, 9H, *t*Bu); 1.01 (s, 9H, *t*Bu); 1.22 (s, 3H, CH_3); 1.32 (s, 3H, CH_3); 3.71-3.77 (m, 1H); 3.80-3.86 (m, 1H); 3.93 (dd, *J* = 12.8, 6.1 Hz, 1H, H-a); 4.10-4.23 (m, 4H, H-a); 4.44-4.53 (m, 1H); 4.67-4.72 (m, 2H); 5.00-5.11 (m, 3H, H-c, H*f*-1); 5.25 (d, *J* = 17.0 Hz, 1H, H-c); 5.57-5.62 (m, 2H, H*p*-1); 5.74-5.85 (m, 3H, H-b); 7.27-7.57 (m, 12H, arom.); 7.91-8.05 (m, 8H, arom.) ppm. ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ 20.4 (*C*q-*t*Bu); 20.6 (*C*q-*t*Bu); 25.0 (CH_3); 26.1 (CH_3); 27.1 (*t*Bu); 27.2 (*t*Bu); 63.0 (C-6); 65.4 (C-6); 68.0 (C-a); 68.2; 69.4; 71.0; 71.5; 75.0; 81.2; 83.1; 84.1; 96.9 (*Cp*-1); 104.8 (*Cf*-1); 109.9 (*C*q-*isop.*); 117.5 (C-c); 128.2 (*C*-arom.); 128.3 (*C*-arom.); 128.4 (*C*-arom.); 128.5 (*C*-arom.); 129.2 (*C*q, arom.); 129.3 (*C*q, arom.); 129.4 (*C*q, arom.); 129.6 (*C*-arom.); 129.7 (*C*-arom.); 129.8 (*C*q, arom.); 130.0 (*C*-arom.); 132.9 (*C*-arom.); 133.2 (*C*-arom.); 133.3 (*C*-arom.); 133.4 (*C*-arom.); 133.8 (C-b); 165.2 (*C*=O), 165.9 (*C*=O), 166.0 (*C*=O), 166.5 (*C*=O) ppm. ESI-TOF HRMS: *m*/*z* $[M+Na]^+$ calcd for $C_{54}H_{63}FNaO_{15}Si$ 1021.3818, found: 1021.3804.

BIOLOGICAL DATA

Ethics Statement and Cohort Description

All human serum samples were collected chiefly by Dr. Waleed Al-Salem, Dr. Yasser Alraey, and the Saudi Ministry of Health, in collaboration with other colleagues at Liverpool School of Tropical Medicine (LSTM). All patient samples were obtained at either national leishmaniasis reference clinics or at field sites (construction sites) with the assistance of the Ministry of Health Leishmaniasis Control Team. Each study subject provided a serum sample and lesion aspirate for parasite identification. All patients signed an informed consent form prior to the participation and collection of samples. Moreover, archived samples collected in 2013 from individuals residing in CL endemic regions throughout Saudi Arabia were used for this study.[10] This study was approved by the Institutional Review Board (IRB) of the LSTM (LSTM ethics application 12.06R).

Molecular analysis of the samples was performed by Dr. Waleed Al-Salem and Dr. Yasser Alraey. The collection of samples was conducted at two sites in Saudi Arabia: Al Ahsa (endemic for *L. major* CL) and Asir (endemic for *L. tropica* CL) (**Table S1**). Individuals with suspected CL were referred to the clinic and diagnosed first by dermatological examination and microscopy analysis, and then by PCR. Briefly, lesion aspirates were taken for culture and microscopy confirmation of parasites, as were swabs for restriction fragment length polymorphism (*ITS*1-PCR RFLP) for identification of *Leishmania* species.[11] Serum samples were also collected at the same time. Patient samples were collected before any treatment had commenced. When secondary (fungal or bacterial) infection was present, antibiotics or antifungals were prescribed first, before administration of either intralesional or intramuscular sodium stibogluconate (Pentostam®). When patients returned to the clinic for subsequent treatment, additional samples were collected. Blood type, gender, number of lesions, nationality, and age were recorded. Samples from patients with heterologous diseases, which included eczema, or bacterial or fungal skin infection, were mostly collected at the Al Ahsa site (**Table S1**).

Table S1. Description of the cohort evaluated in this study.

Characteristics	***L. major* (n = 81)**	***L. tropica* (n = 15)**	**Heterologous diseases (n = 24)**
Gender	Male = 77 Female = 2 NA [b] = 2	Male = 7 Female = 8	Male = 19 Female = 2 NA = 3
Age [a]	34 (16-67)	20 (10-58)	36 (22-50)
Province	Al Ahsa = 76 Asir = 1 Central = 2 NA = 2	Asir = 15	Al Ahsa = 21 NA = 3
Lesion Number	1-5 = 59 6-11 = 14 12+ = 6 NA = 2	1 = 13 2-5 = 2	Not relevant
Lesion Appearance	Nodular = 18 Nodular/Ulcer = 23 Papular = 15 Scar = 3 Ulcerative = 14 NA = 5 Mixed = 3 [d]	Nodular = 2 Nodular/Ulcer = 5 Papular = 3 Scar = 4 Ulcerative =1	Not relevant
Diagnosis	Cutaneous leishmaniasis	Cutaneous leishmaniasis	Eczema = 14 Bacterial Infection = 2 Fungal Infection = 2 NA = 6
Nationality	Bangladeshi = 5 Egyptian = 21 Filipino = 3 Indian = 17 Nepalese = 6 Pakistani = 8 Saudi = 12 Indian = 3 Other = 5 [e] NA = 1	Bangladeshi = 1 Saudi = 14	Saudi = 23 Bangladeshi = 1
Blood Type	A = 17 B = 14 AB = 4 O = 14 NA = 32	A = 7 AB = 1 O = 7	NA = 24

[a] NA, Not available

[b] Median age with range in parenthesis

[c] Three or more lesion types

[d] Yemen/Lebanon/Bedouin-Saudi (n= 1), Sudan (n = 2)

Description of the chemiluminescent ELISA

Levels of *Leishmania*-specific anti-α-Gal IgG antibodies in human sera was determined by chemiluminescent ELISA essentially as previously described,[10,12] with modifications. The serum dilutions and concentrations of NGPs (**NGP27b**, **NGP28b**, and **NGP30b**) and control antigen (**2-MEb**) varied between assays, but the overall immunoassay steps remained the same. Briefly, MaxiSorp white 96-well microplates (Nunc, Thermo Fisher Scientific) were coated with the appropriate NGP in 0.2 M carbonate-bicarbonate buffer, pH 9.6 (CBB), at 4°C, 16 h, at concentrations determined through antigen and serum cross-titration experiments. The free sites on the microplate wells were blocked with 200 µL/well PBS-1% BSA (PBS-B), and incubated with sera from patients with *L. major* or *L. tropica* CL infection, heterologous diseases, or from healthy individuals, diluted in PBS-B as indicated in each experiment. Anti-human IgG (H+L) biotin conjugate (1:10,000 dilution, Cat # 31770, Thermo Fisher Scientific) was added (50 µL/well). The biotin complex was recognized by adding 50 µL/well of Pierce High Sensitivity NeutrAvidin-HRP (1:5,000 dilution, Cat# 31030, Thermo Fisher Scientific). In all incubation steps, plates were incubated for 1 h at 37°C. Between incubation steps, plates were washed three times with 200 µL/well PBS-0.05% Tween 20 (PBS-T). Following addition of SuperSignal™ ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific, 37070), diluted 1:8 (v/v) in CBB, the luminescence was immediately measured, as relative luminescence units (RLUs), using a FLUOstar Omega multi-mode microplate reader (BMG LabTech, Ortenberg, Germany). Positive and negative controls for each microplate were included in triplicate or duplicate. The negative control consisted of a serum pool of ten randomly selected healthy individuals from Saudi Arabia. The positive control consisted of a serum pool of ten randomly selected *L. major* CL patients. In preliminary immunoassays, to determine the nonspecific background reactivities, we also included negative control wells lacking the antigen, primary antibody (serum), secondary conjugate (biotinylated anti-human IgG antibody), or NeutrAvidin-HRP. The average RLU was taken for the negative and positive controls and subtracted from the average of the experimental sample, tested in duplicate or triplicate, to control for nonspecific/background signal from the reagents.

The cutoff of each immunoassay microplate was determined using the method described by Frey et al.[13] Briefly, we first defined the upper prediction limit, expressed as the standard deviation (SD) multiplied by a factor (*f*) (SD*f*), calculated based on the Student *t*-distribution, according to the number of negative control (NC) replicates in each plate and a confidence level ($1 - \alpha$) of 95%. Therefore, the cutoff value in each was calculated as the NC RLU mean + SD*f*. Since the titer of each ELISA was defined as the ratio of the experimental sample's average RLU value to the cutoff value. A serum sample was considered positive when its titer was equal to or higher than 1.000 and negative when the titer was lower than 1.000.

Cross-titrations of NGP antigens and pooled sera by chemilumescent ELISA

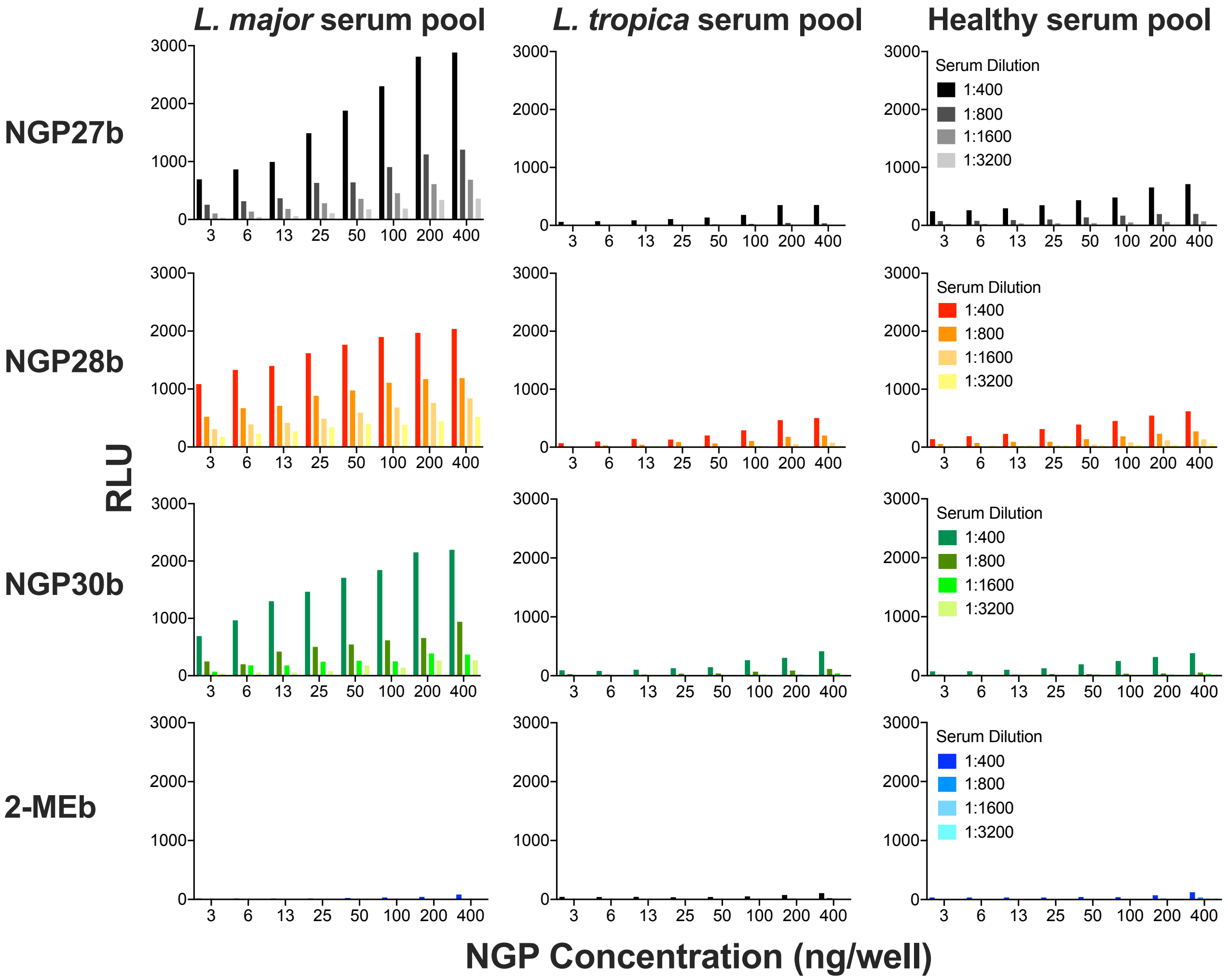


Figure S5. Antigen and serum cross-titration by chemiluminescent ELISA of **NGP27b**, **NGP28b**, **NGP30b**, and **2-MEb**. The three NGPs and their negative control antigen (**2-MEb**) were titrated at different concentrations (3, 6, 13, 25, 50, 100, 200, or 400 ng/well) with pools of sera (diluted at 1:400, 1:800, 1:1600, or 1:3,200) from patients with active *L. major* or *L. tropica* infection (each pool consisting of sera from ten individuals), or pool of sera from ten healthy individuals from Saudi Arabia. The chemiluminescent ELISA was performed as described above.

Chemiluminescent ELISA reactivity of NGPs, and 2-MEb with sera from OWCL patients, heterologous disease, or from healthy individuals

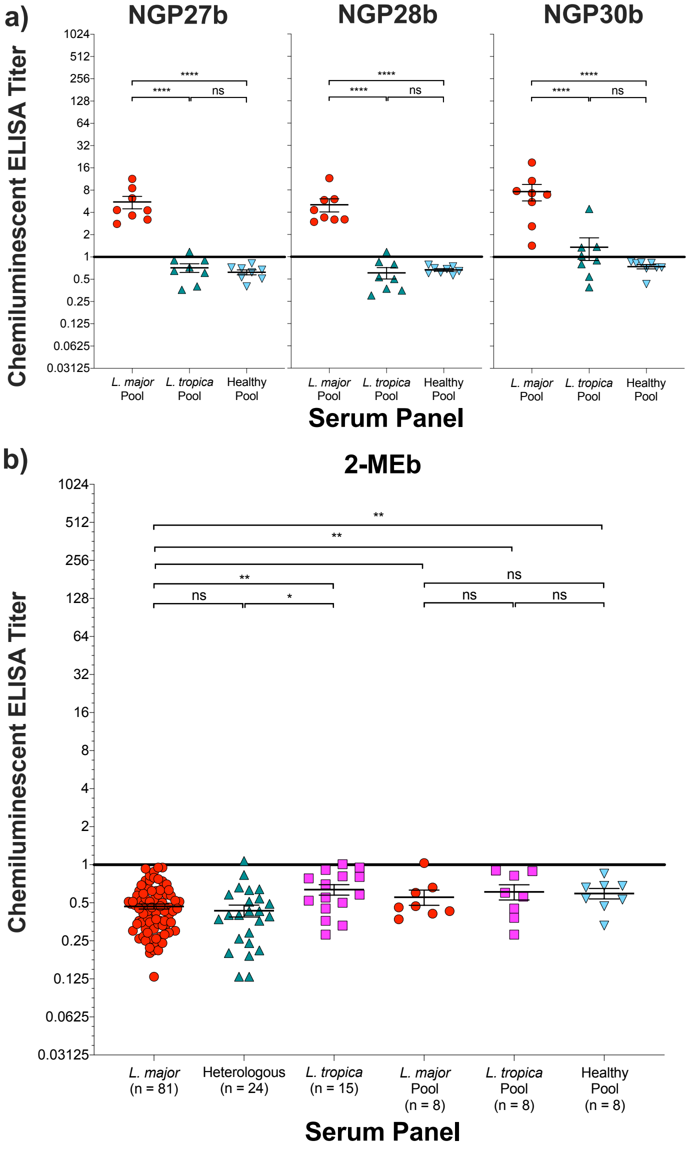


Figure S6. Chemiluminescent ELISA reactivity of **NGP27b**, **NGP28b**, **NGP30b**, and **2-Meb** with sera from patients with *L. major* or *L. tropica* infection, heterologous disease, or from healthy individuals. **a**) Pooled sera (at 1:800 dilution) from patients (n = 10) with active *L. major* or *L. tropica* infection, or from healthy individuals (n = 10) from Saudi Arabia were evaluated by chemiluminescent ELISA against **NGP27b**, **NGP28b**, and **NGP30b**, each at 25 ng/well. Each point represents the mean of triplicate or duplicate of each pool, tested in separate microplate reactions (total = 8), performed at the same or different days. The immunoassay was performed as described in the Supporting Information (Chemiluminescent ELISA). **b**) Grouped scatter plot analysis of individual sera from patients with *L. major* infection, heterologous disease, or *L. tropica* infection, and control serum pools (as in a) tested with **2-MEb** (negative control antigen). The horizontal line indicates the initial cutoff value (C_i, titer = 1.000), calculated as described in the Supporting Information (Chemiluminescent ELISA). Statistical analysis: nonparametric Mann-

Whitney test; significance level: $p < 0.05$; *, $p < 0.05$; **, $p < 0.001$; ****, $p < 0.0001$; ns, non-significant.

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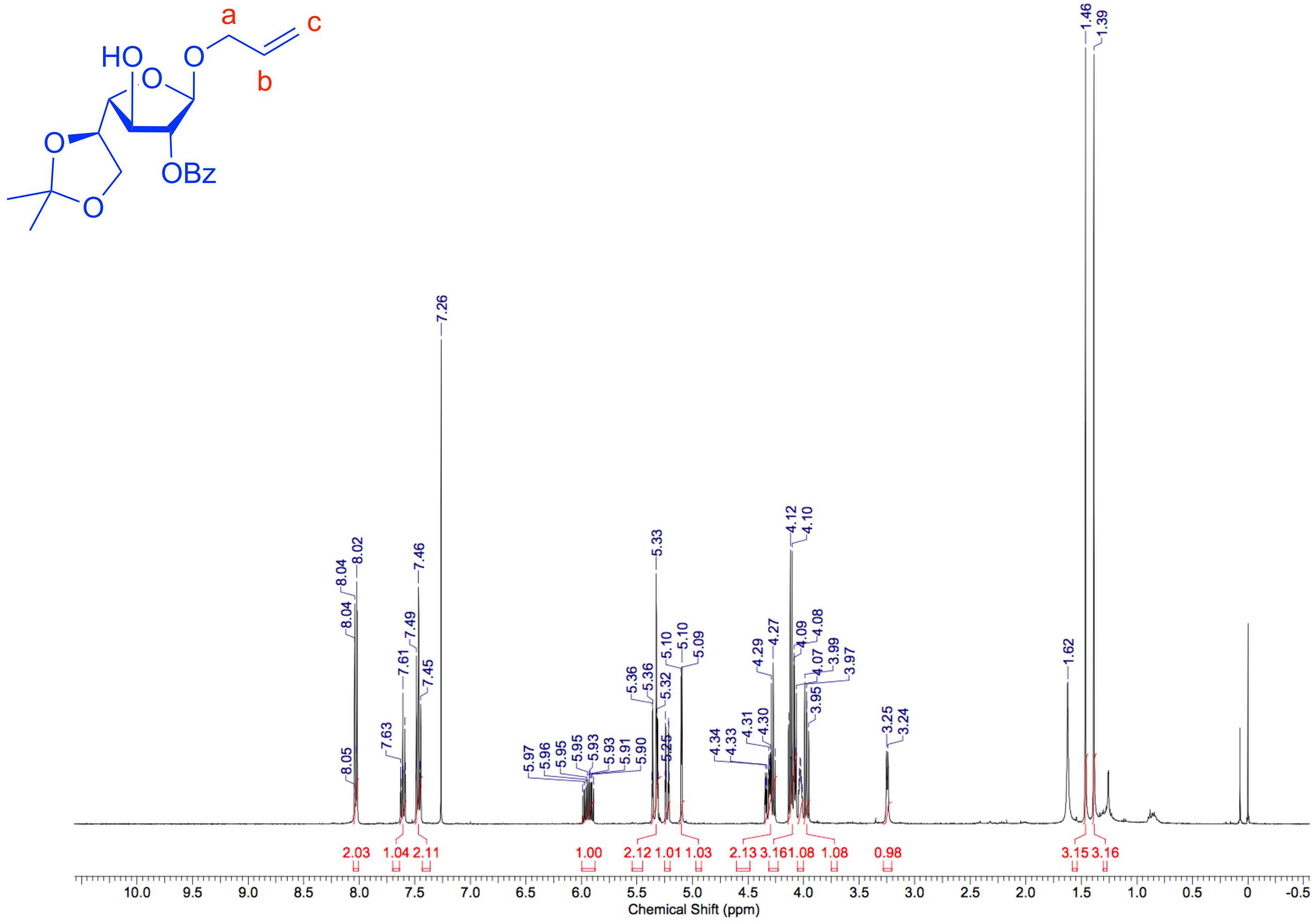
APPENDIX

^{1}H and ^{13}C NMR Spectra, and Mass Spectra

^{1}H NMR, 400 MHz, $CDCl_3$, compound 1



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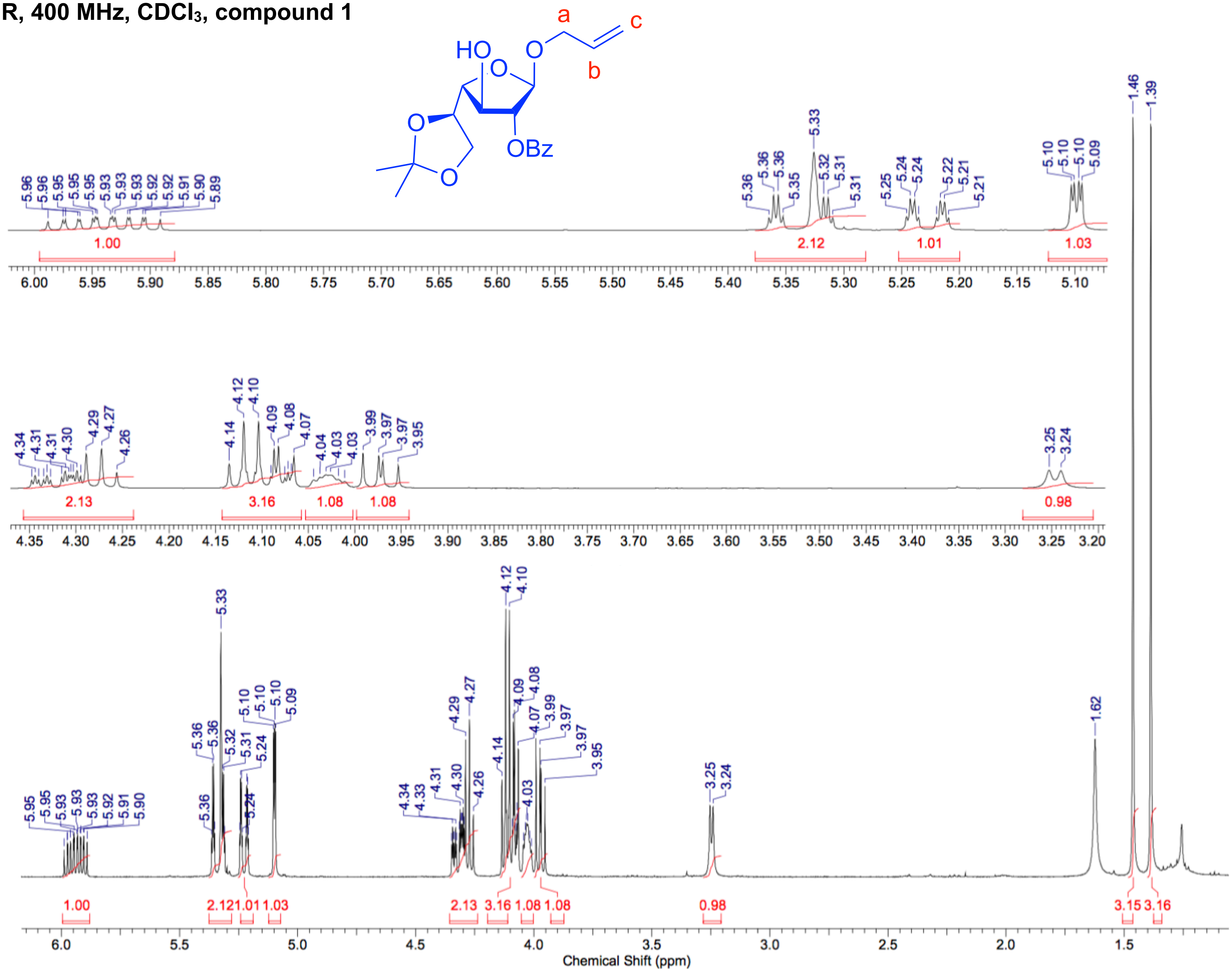

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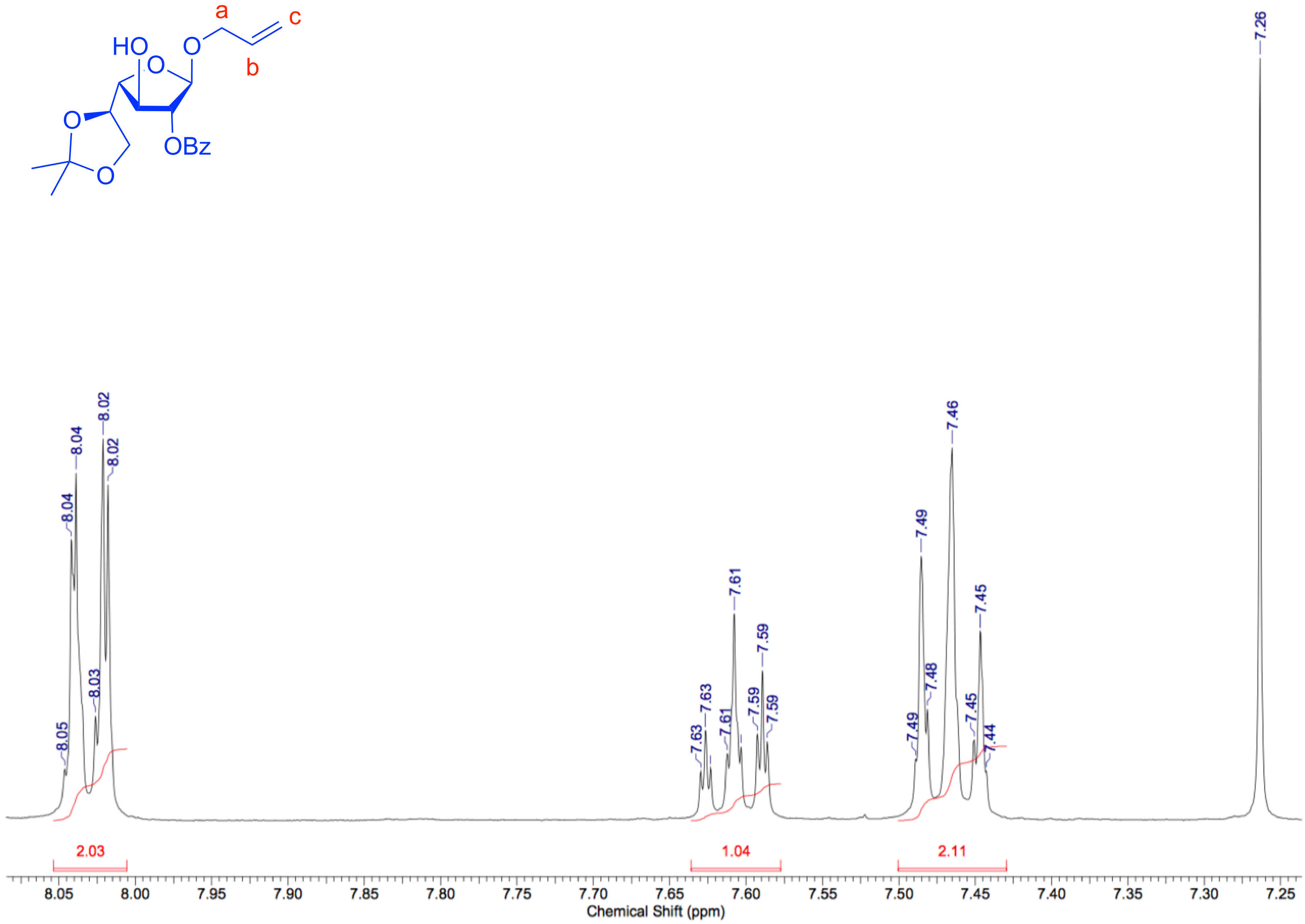
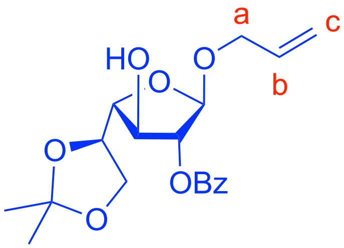
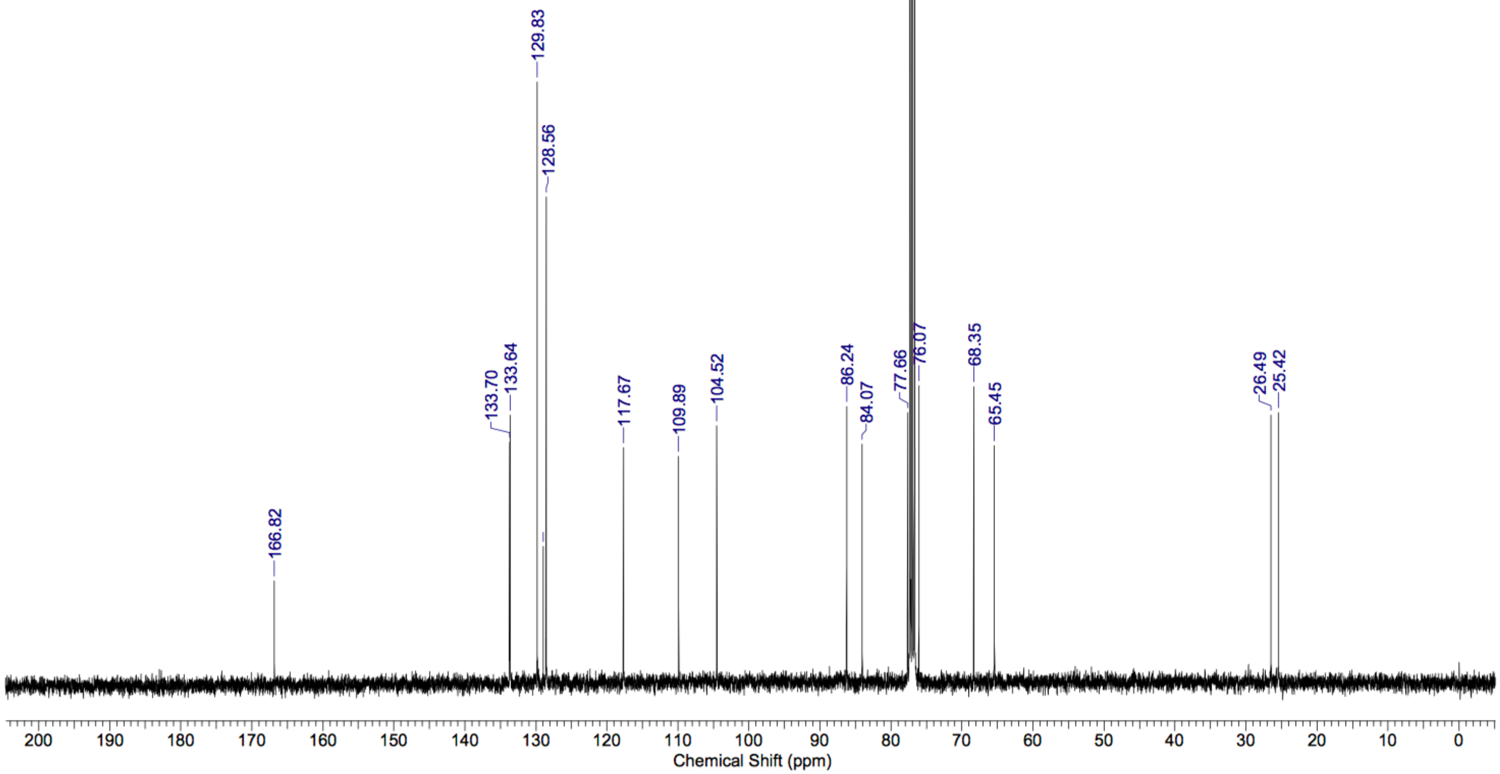
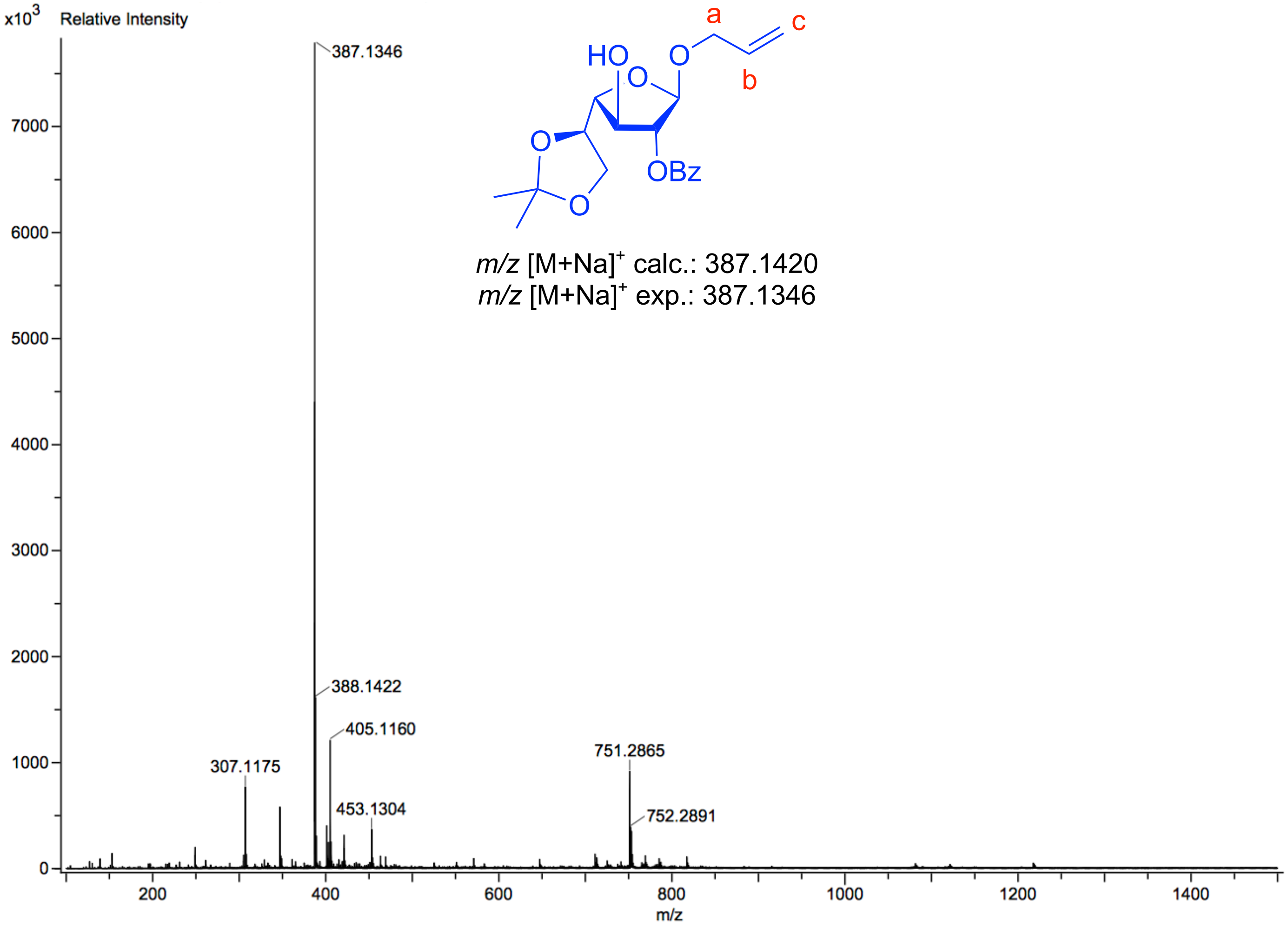


^{13}C NMR, 100 MHz, CDCl$_3$, compound 1



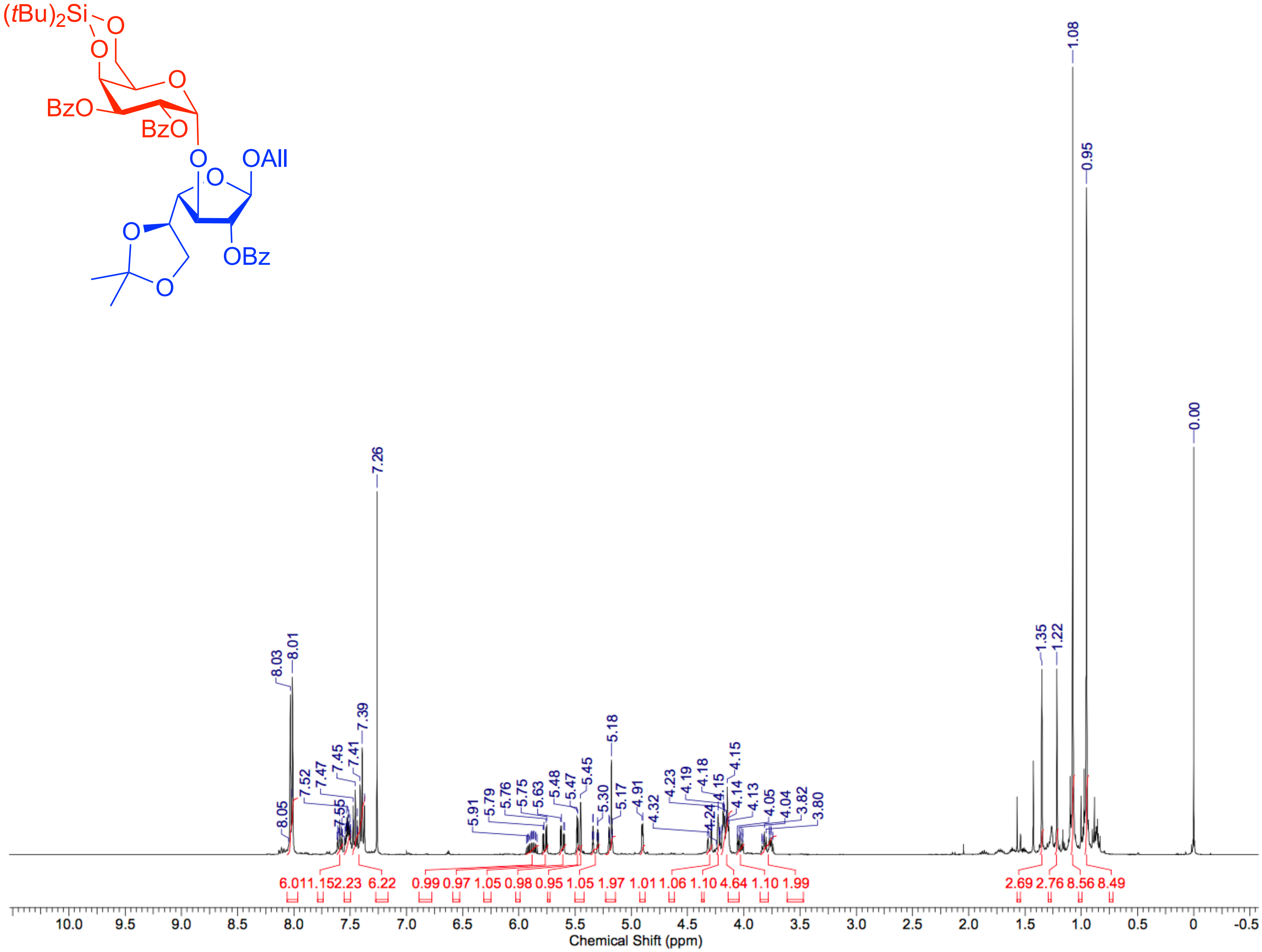


ESI-TOF HR mass spectrum of compound 1

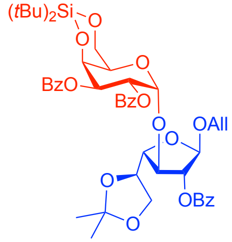


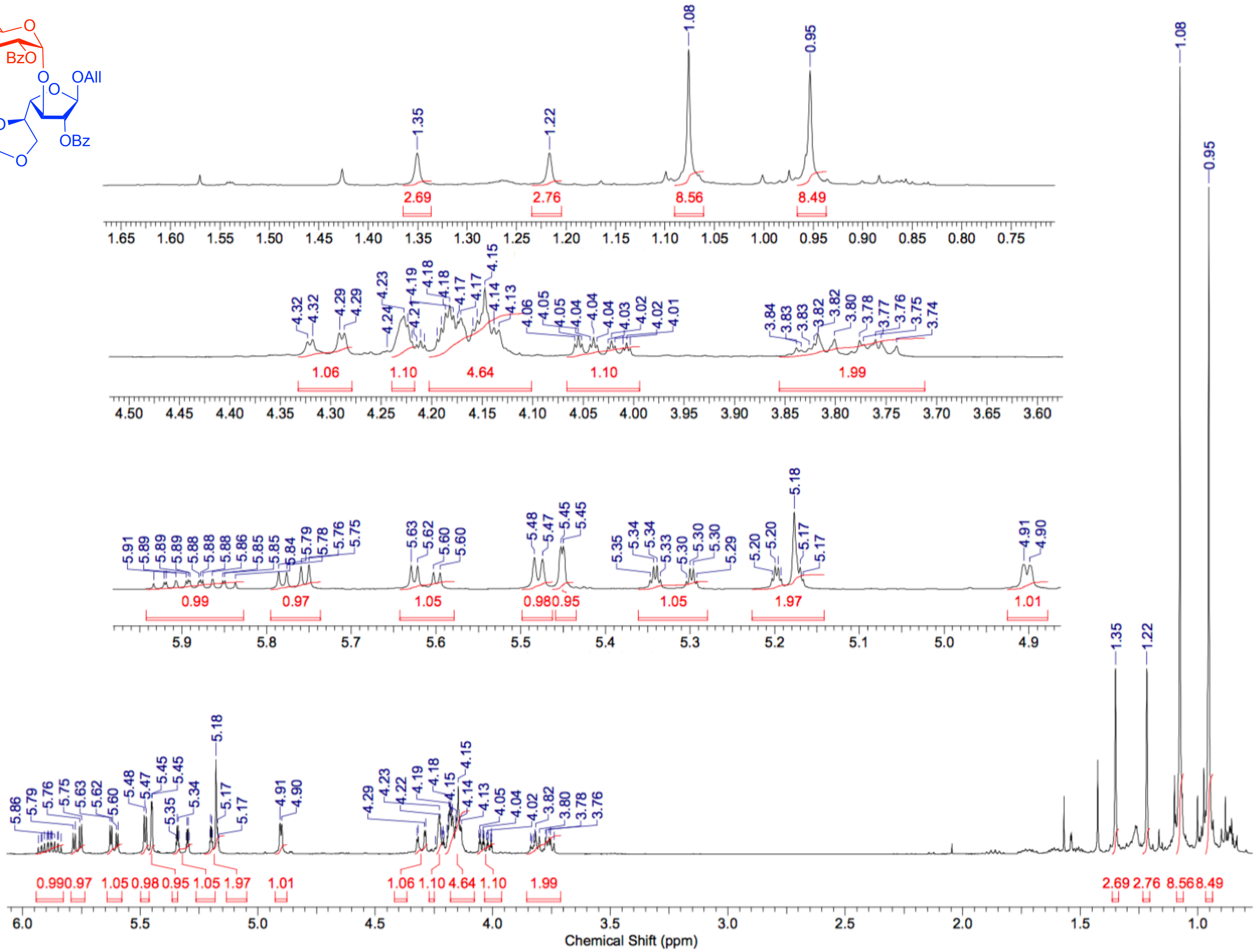
m/z [M+Na]$^+$ calc.: 387.1420
m/z [M+Na]$^+$ exp.: 387.1346

^{1}H NMR, 400 MHz, CDCl$_3$, compound 4

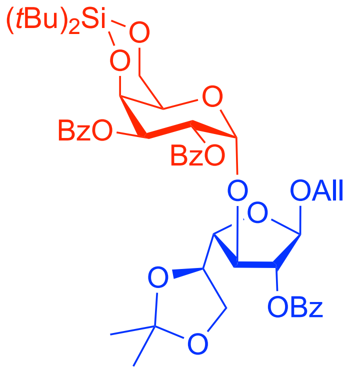


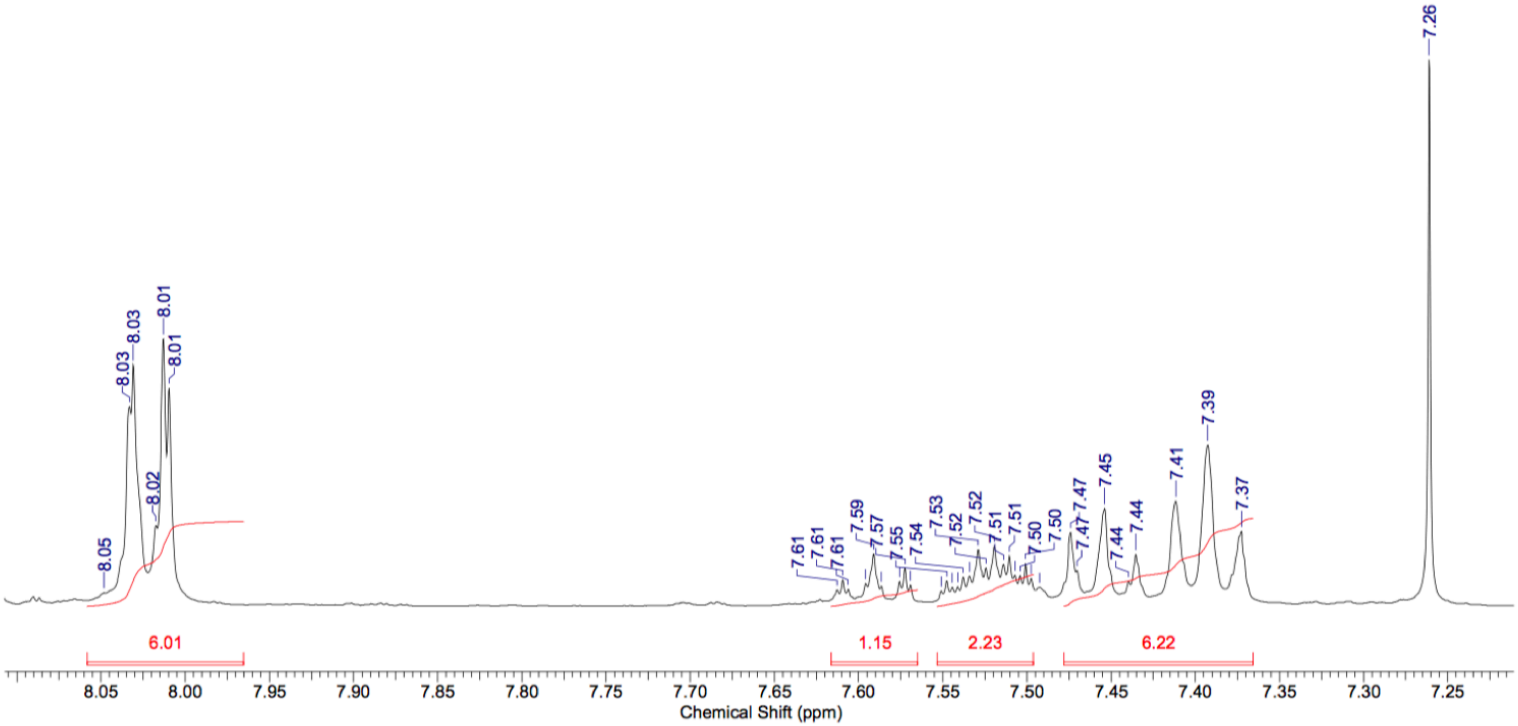
^{1}H NMR, 400 MHz, CDCl$_3$, compound 4



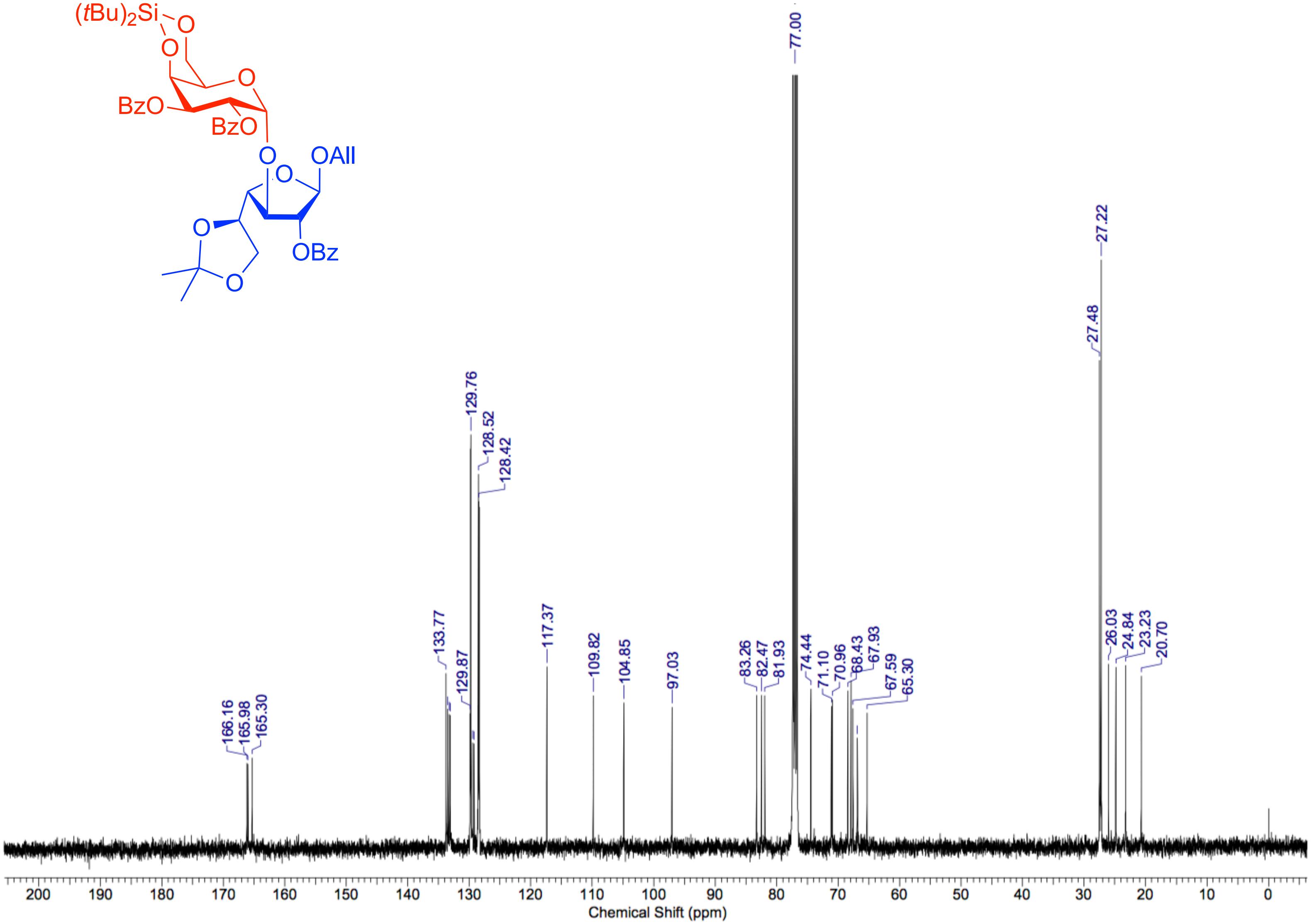


^{1}H NMR, 400 MHz, CDCl$_3$, compound 4

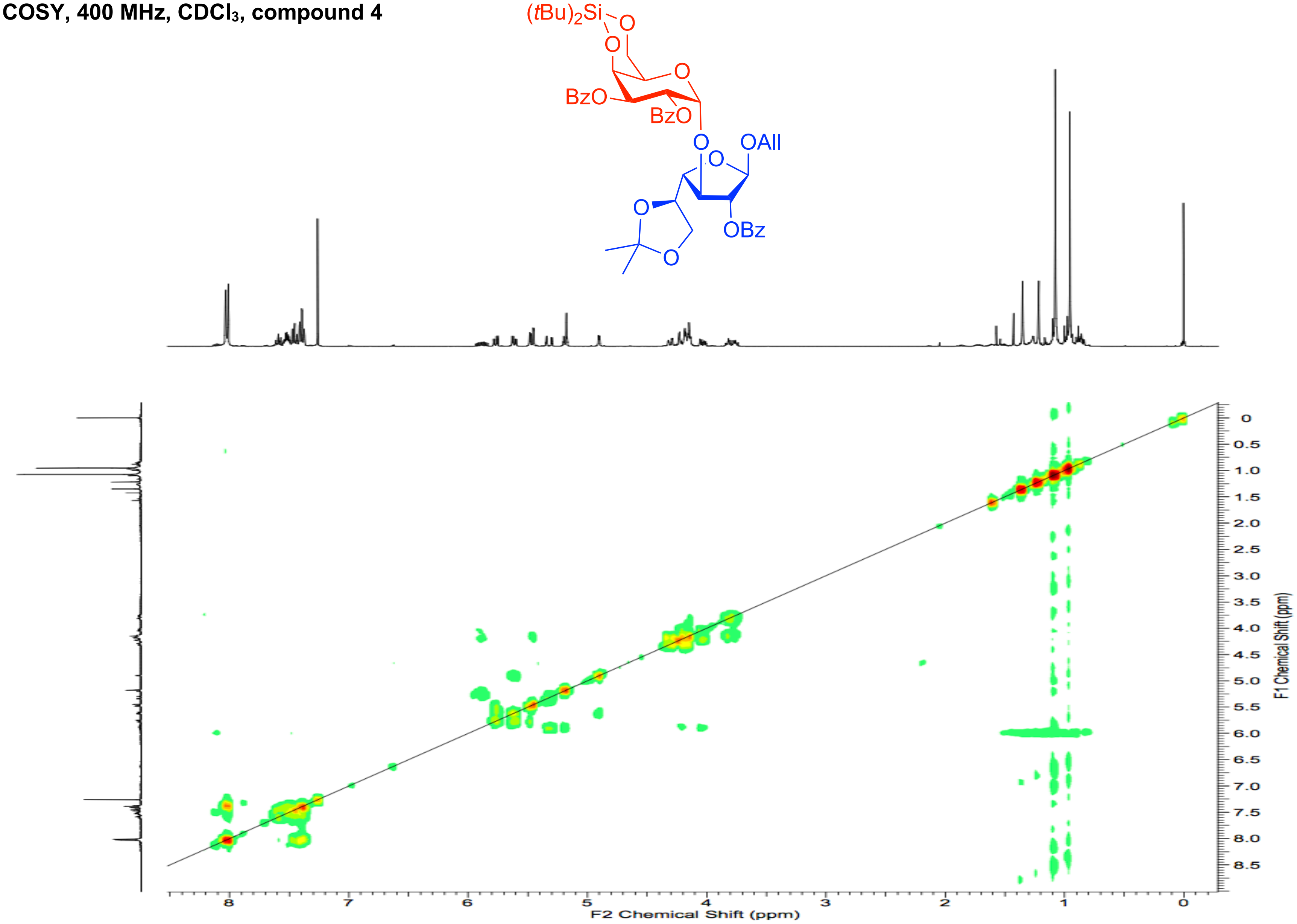




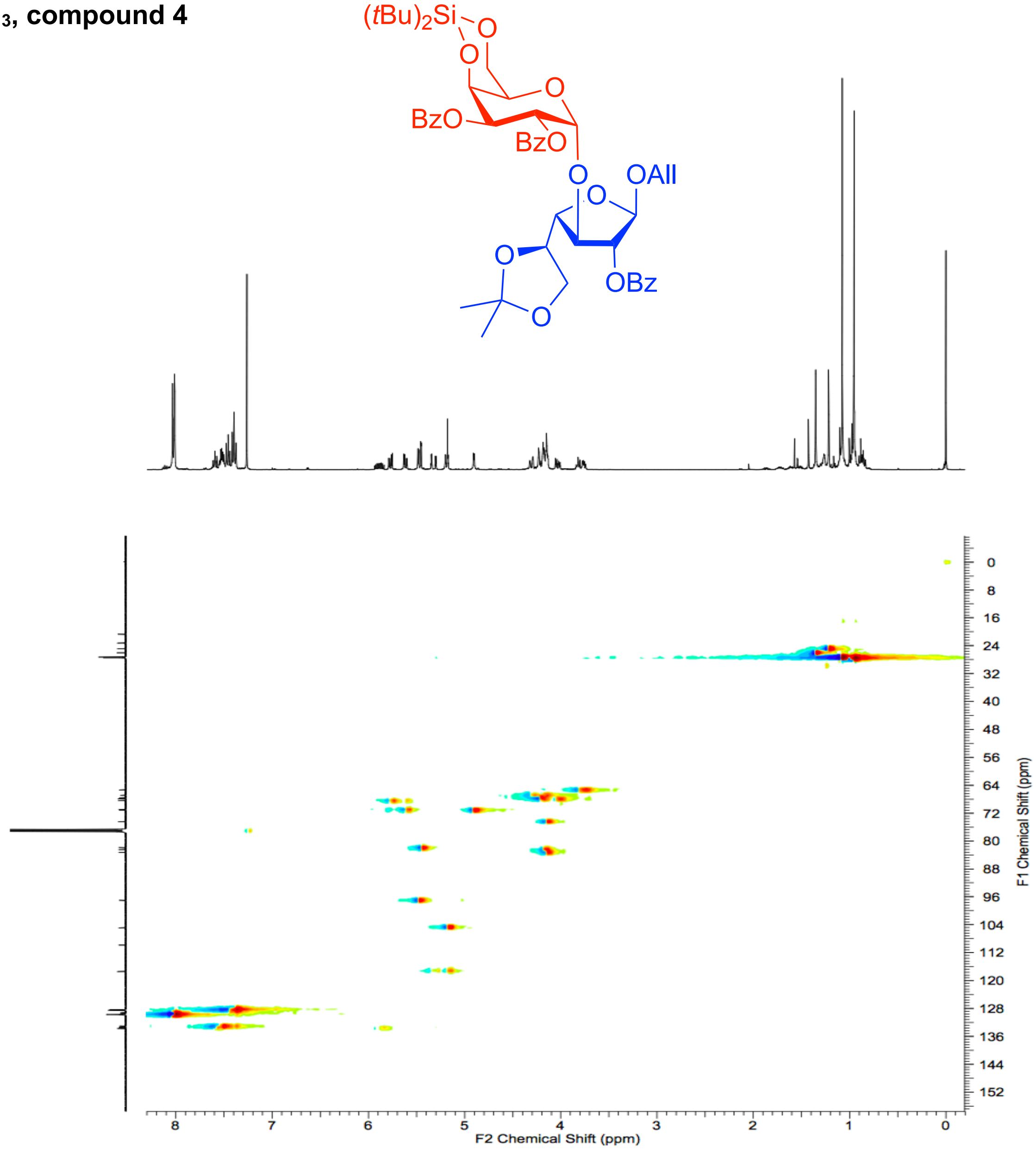
^{13}C NMR, 100 MHz, $CDCl_3$, compound 4



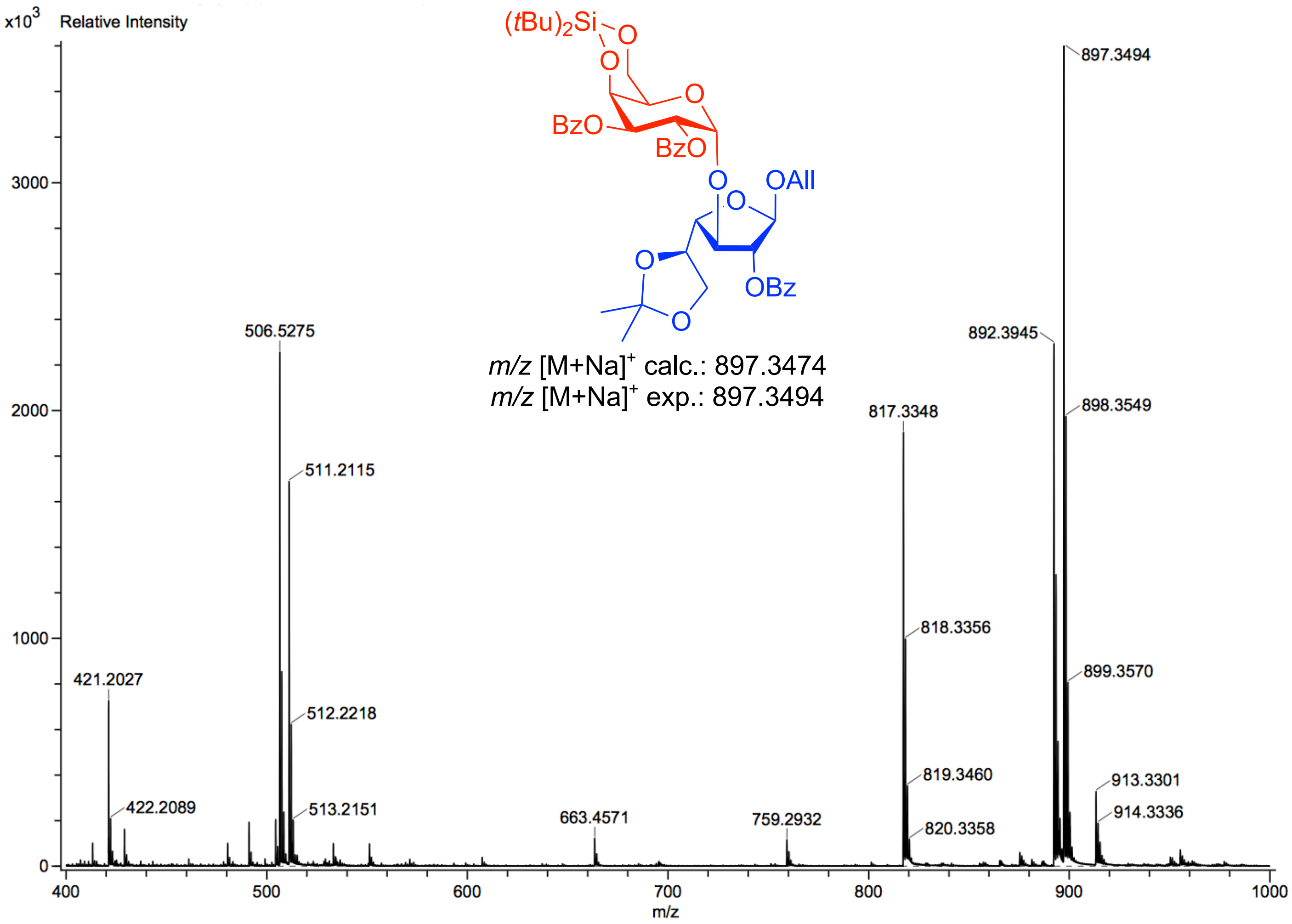
COSY, 400 MHz, $CDCl_3$, compound 4



HSQC, 400 MHz, $CDCl_3$, compound 4

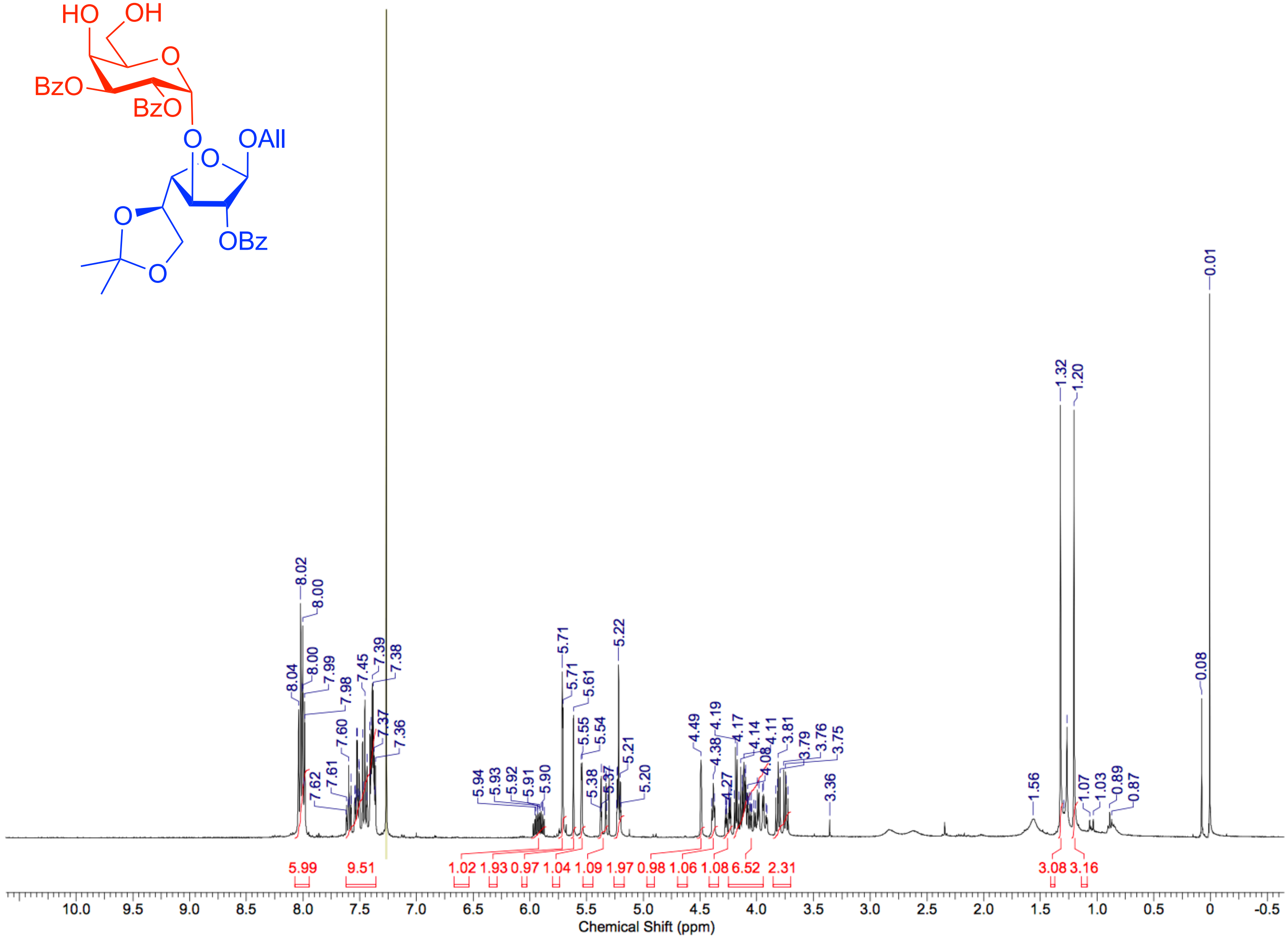


ESI-TOF HR mass spectrum of compound 4

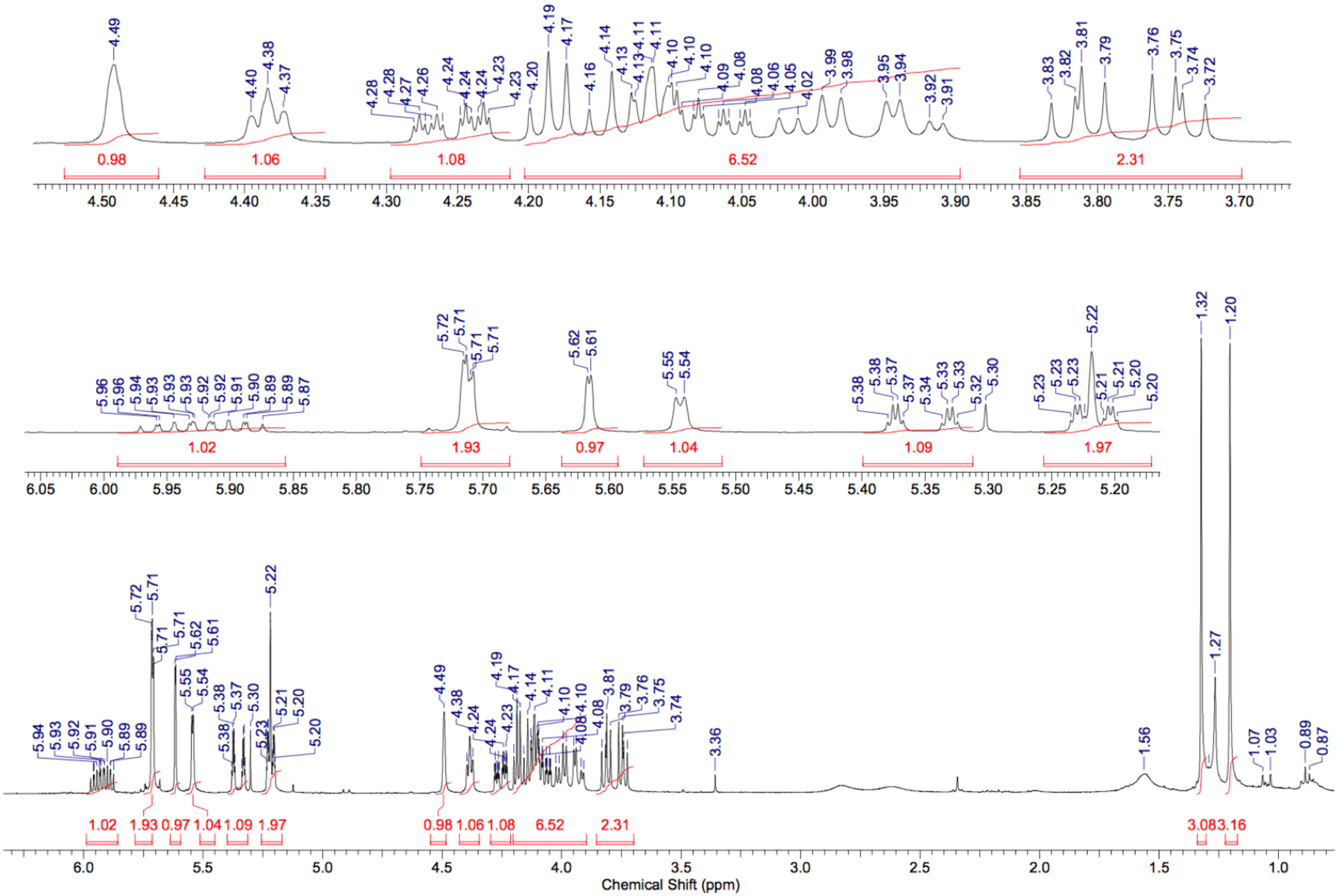


m/z $[M+Na]^+$ calc.: 897.3474
m/z $[M+Na]^+$ exp.: 897.3494

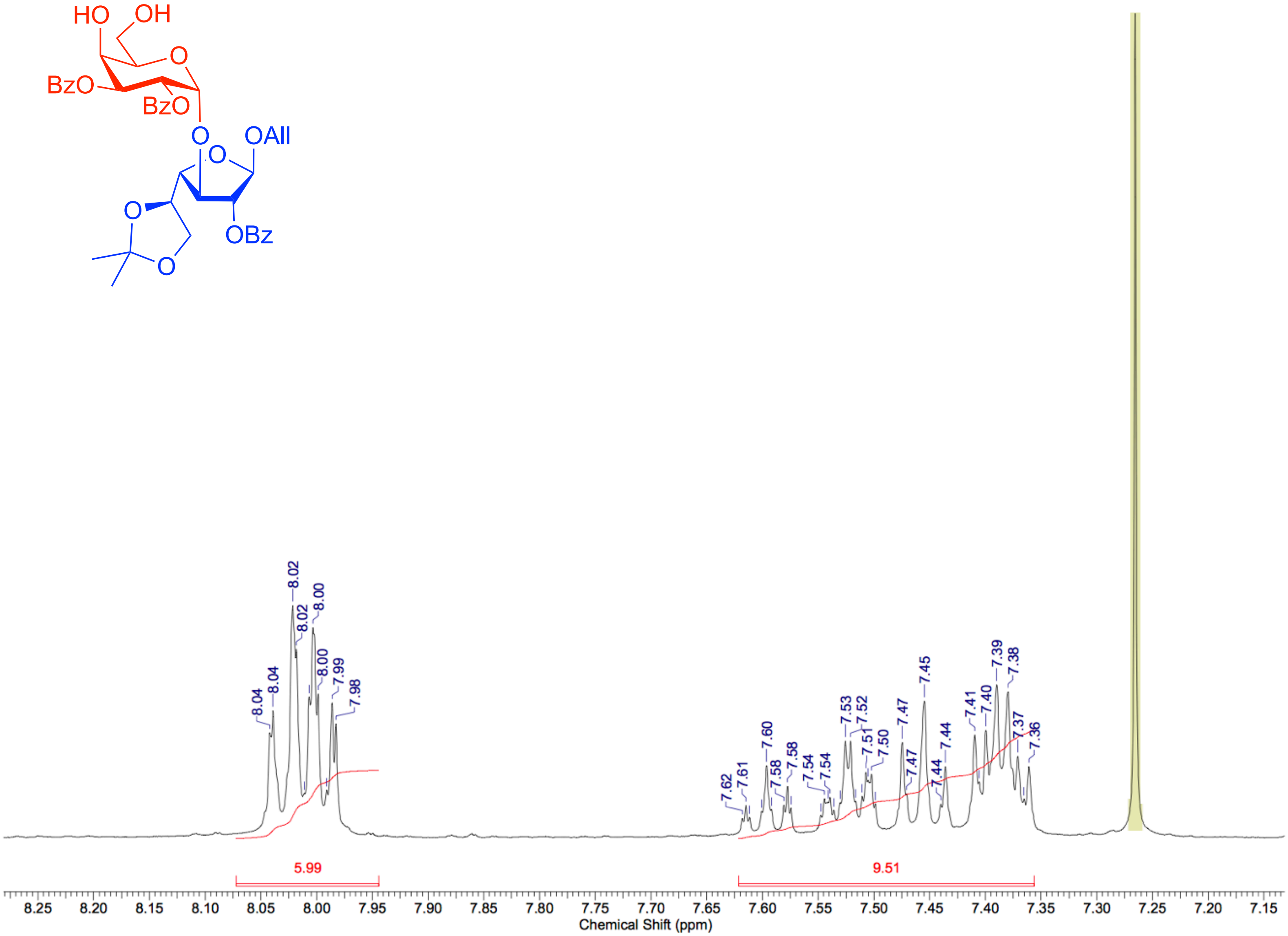
^{1}H NMR, 400 MHz, CDCl$_3$, compound 6



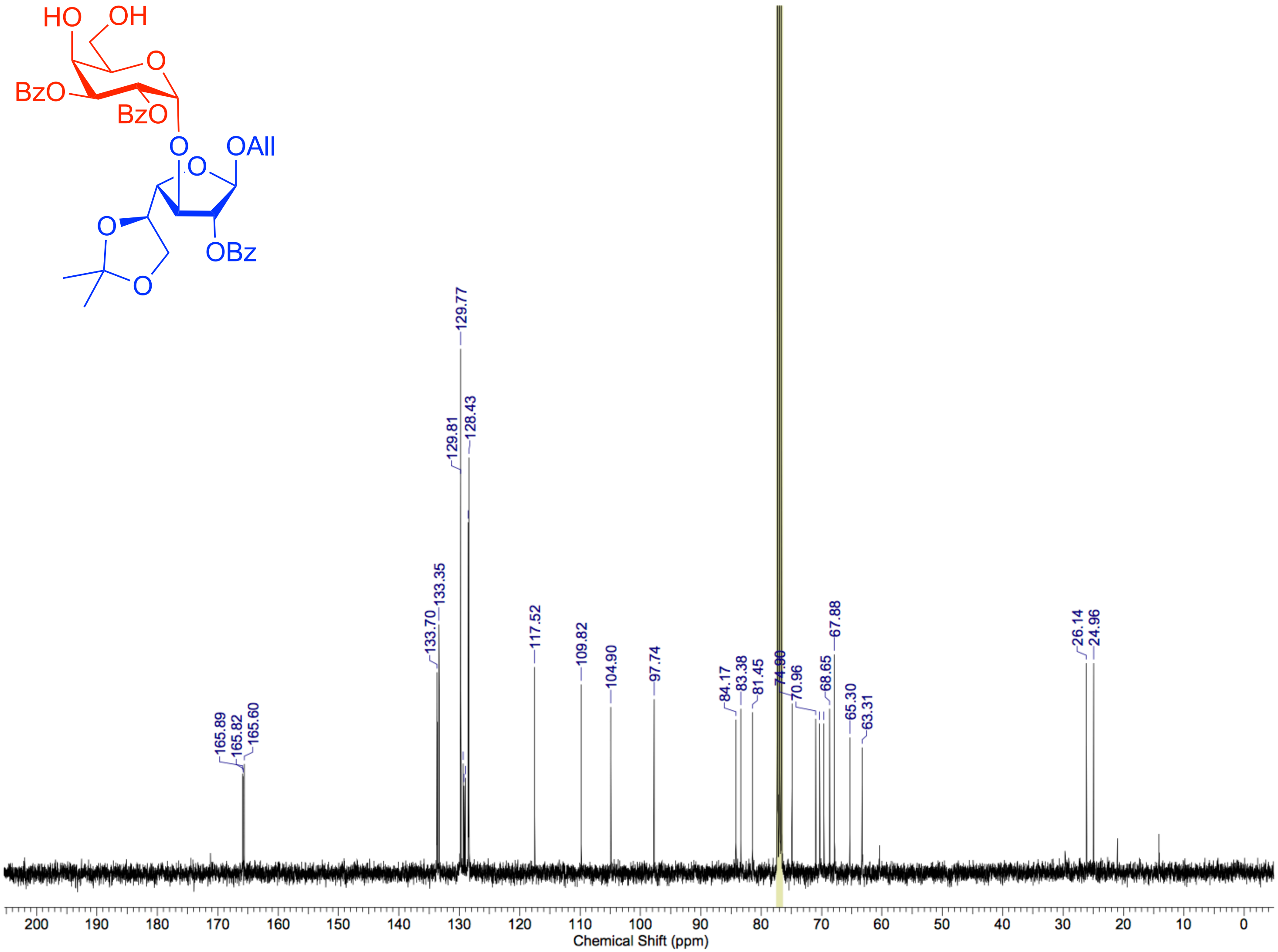
^{1}H NMR, 400 MHz, $CDCl_3$, compound 6



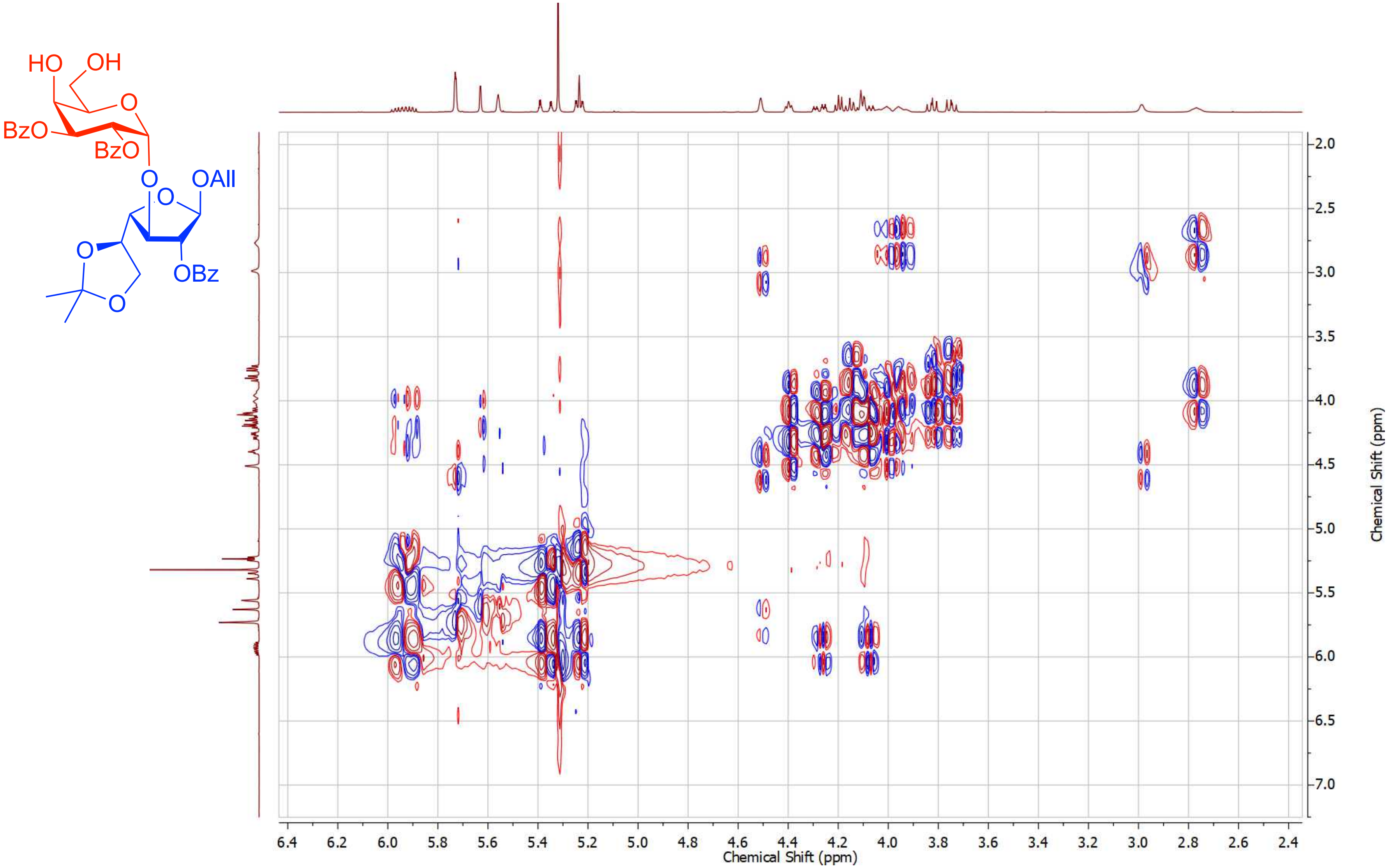
^{1}H NMR, 400 MHz, CDCl$_3$, compound 6



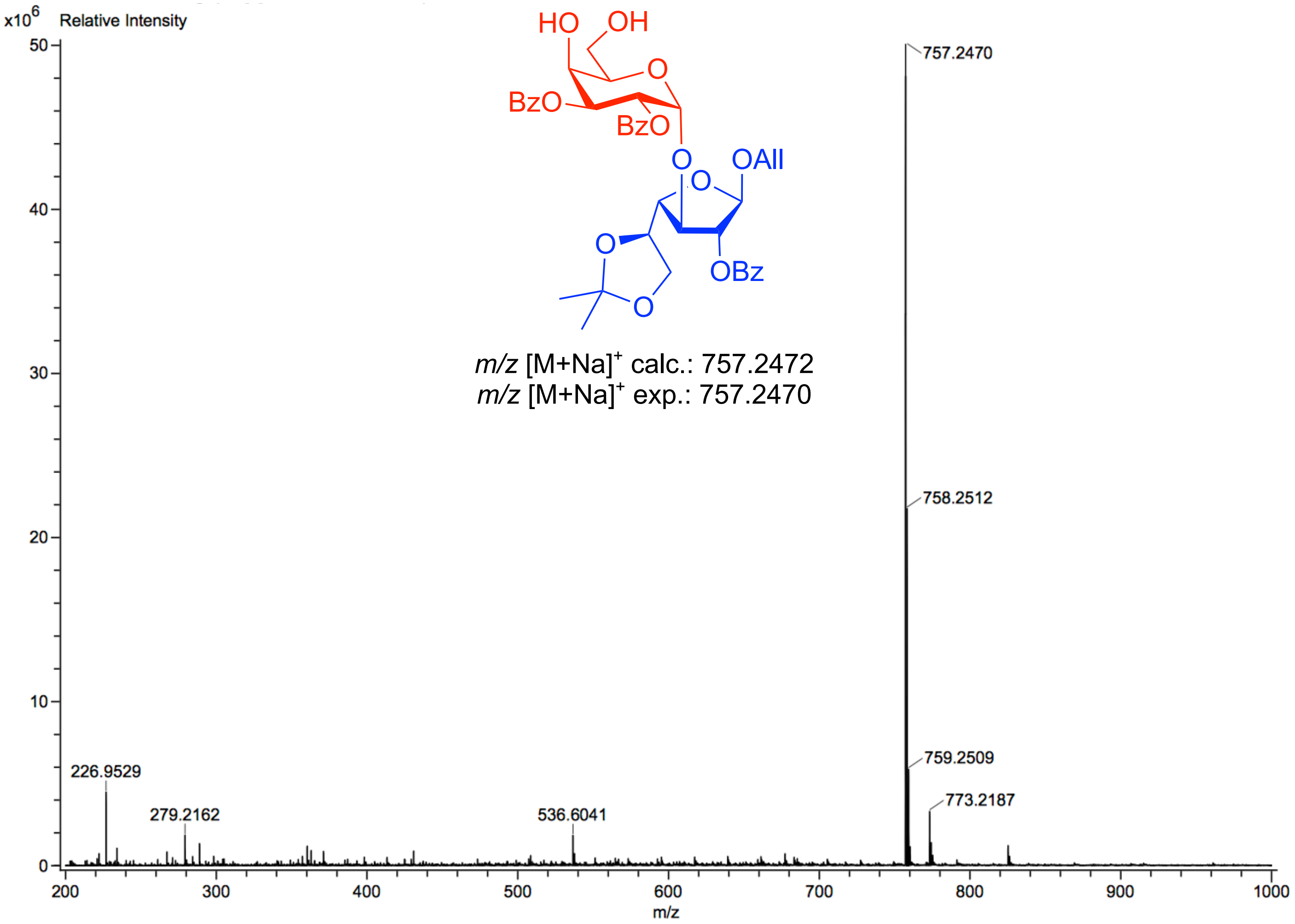
^{13}C NMR, 100 MHz, CDCl$_3$, compound 6



COSY NMR, 400 MHz, $CDCl_3$, compound 6

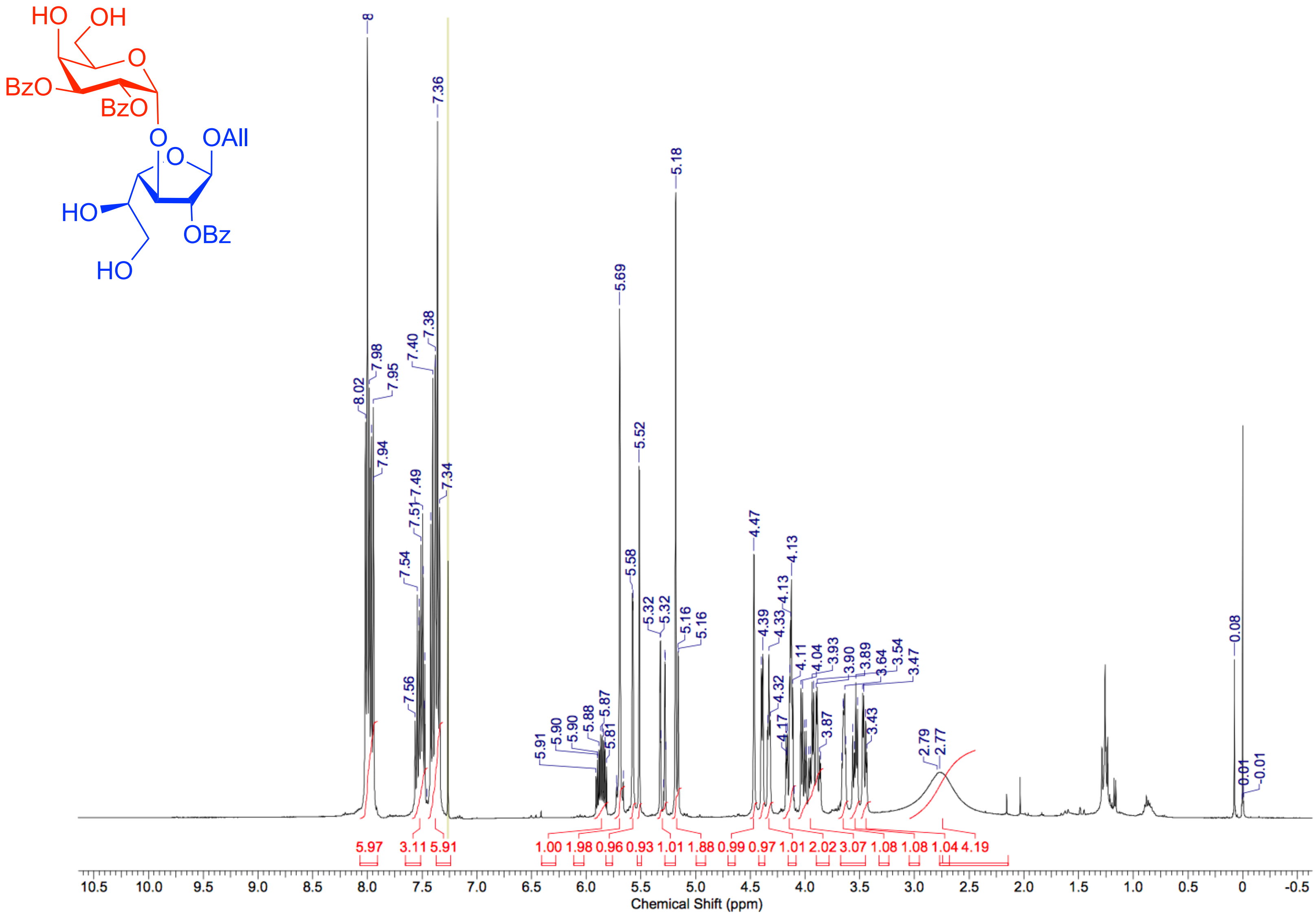


ESI-TOF HR mass spectrum of compound 6

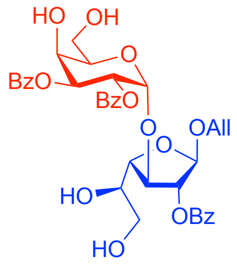


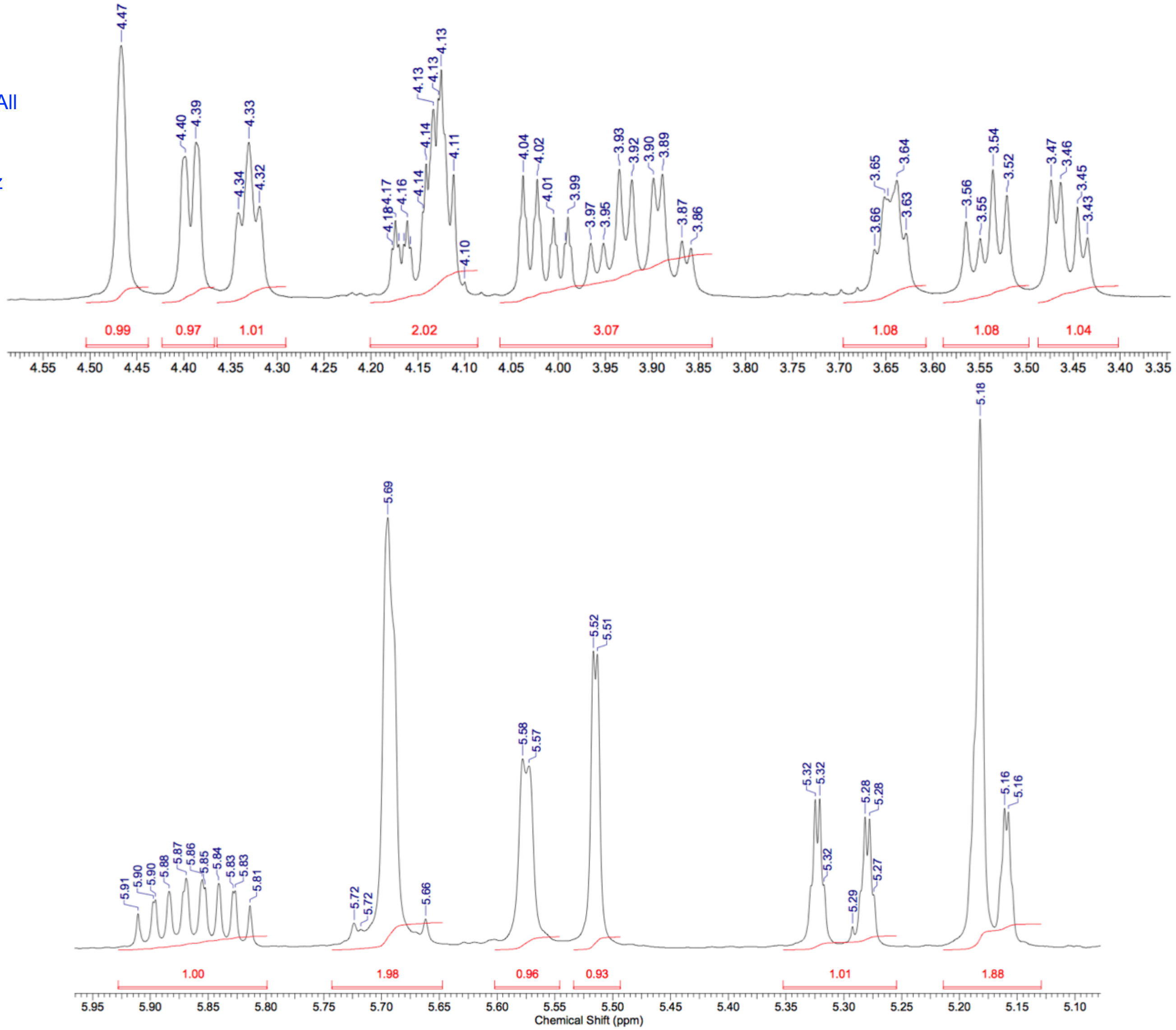
m/z [M+Na]$^+$ calc.: 757.2472
m/z [M+Na]$^+$ exp.: 757.2470

^{1}H NMR, 400 MHz, CDCl$_3$, compound 7

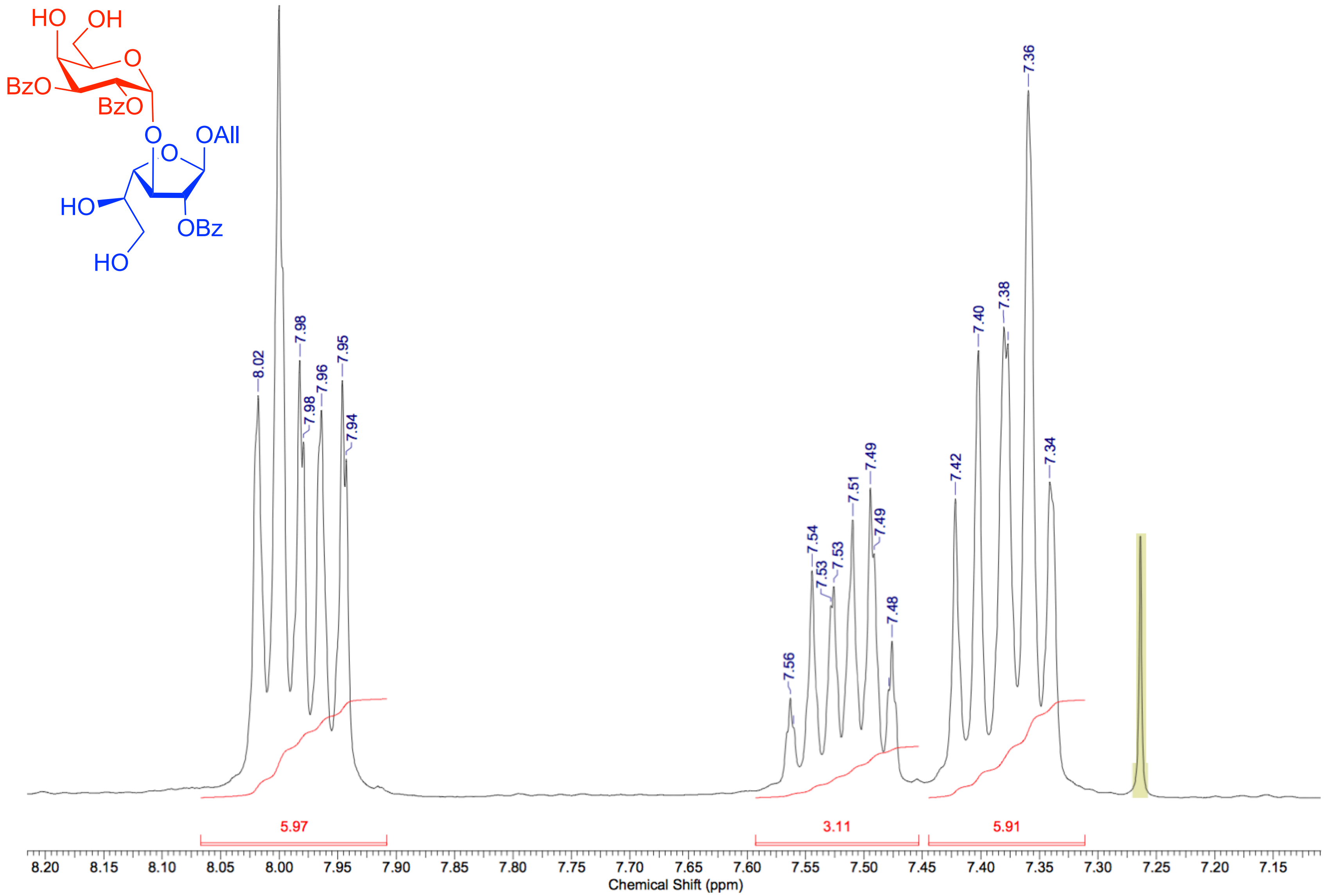


^{1}H NMR, 400 MHz, CDCl$_3$, compound 7

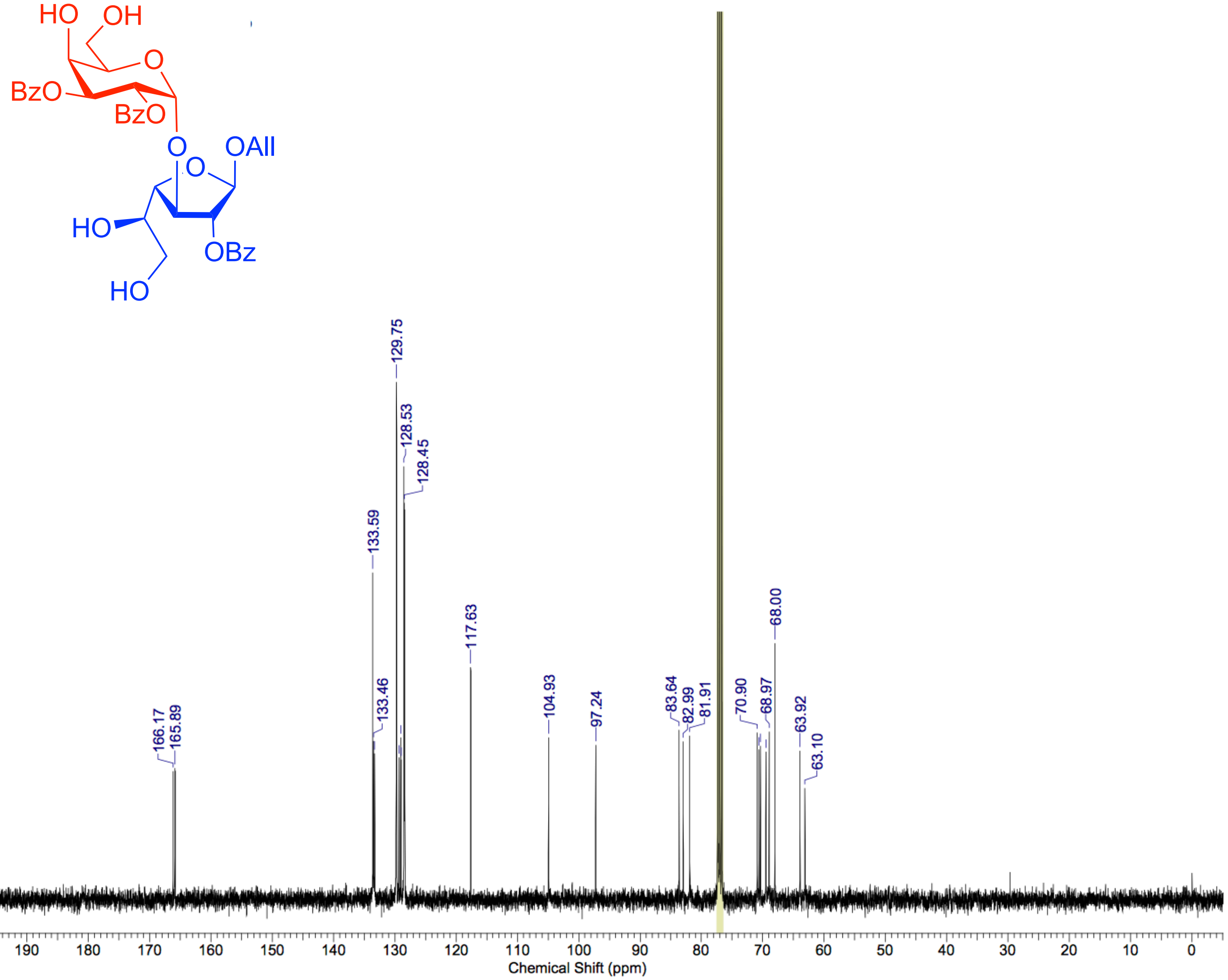




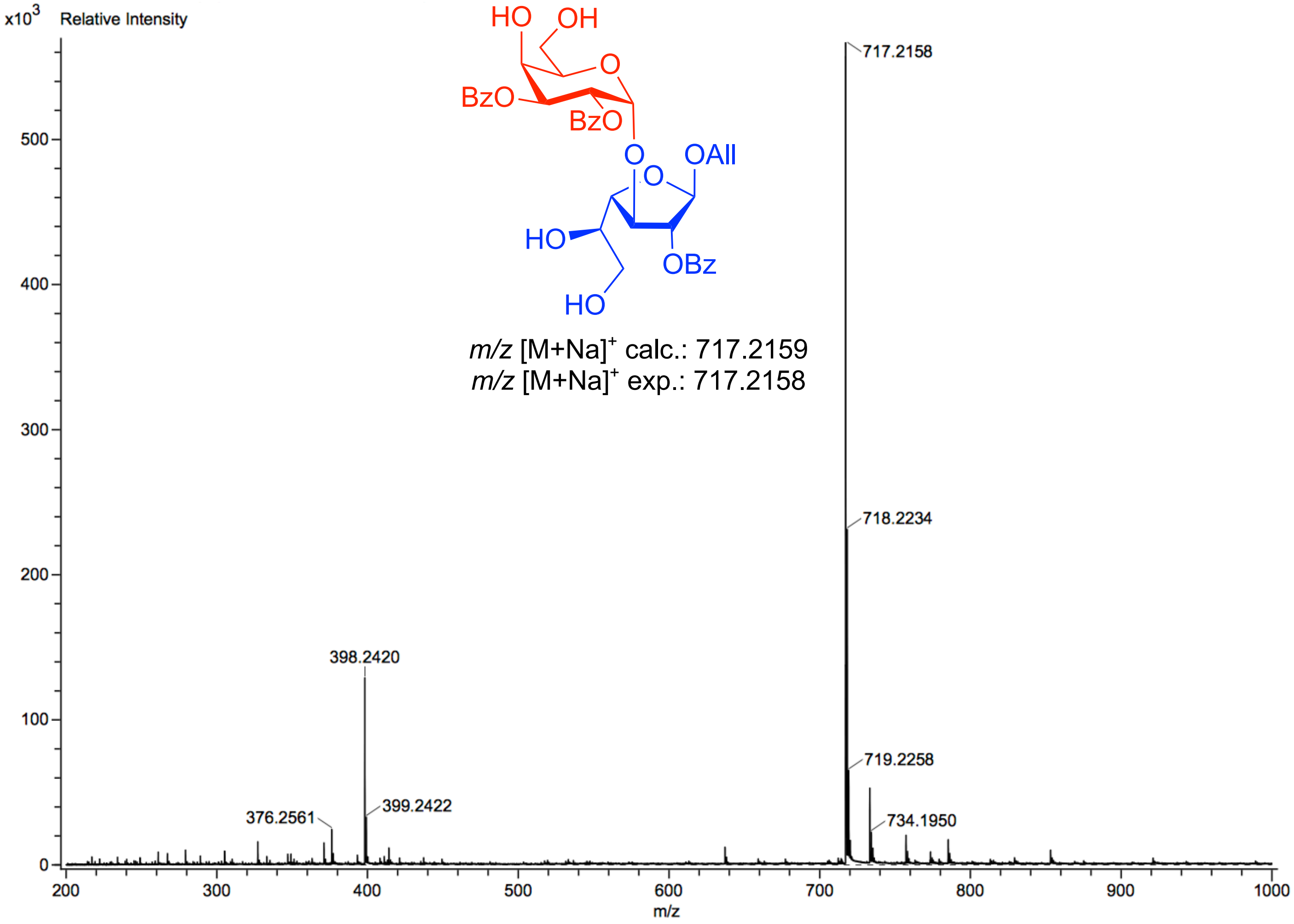
^{1}H NMR, 400 MHz, CDCl$_3$, compound 7



^{13}C NMR, 100 MHz, CDCl$_3$, compound 7

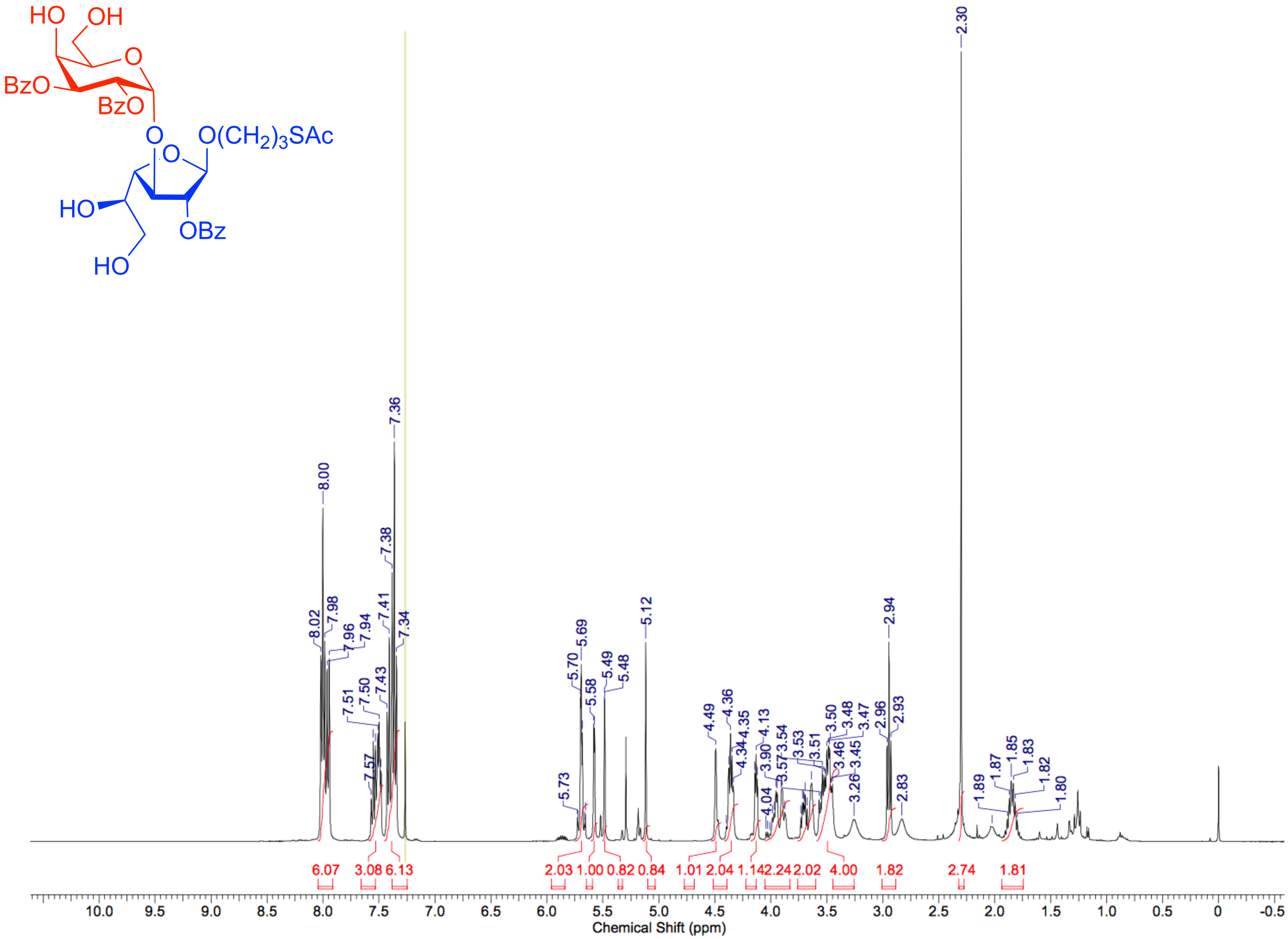


ESI-TOF HR mass spectrum of compound 7

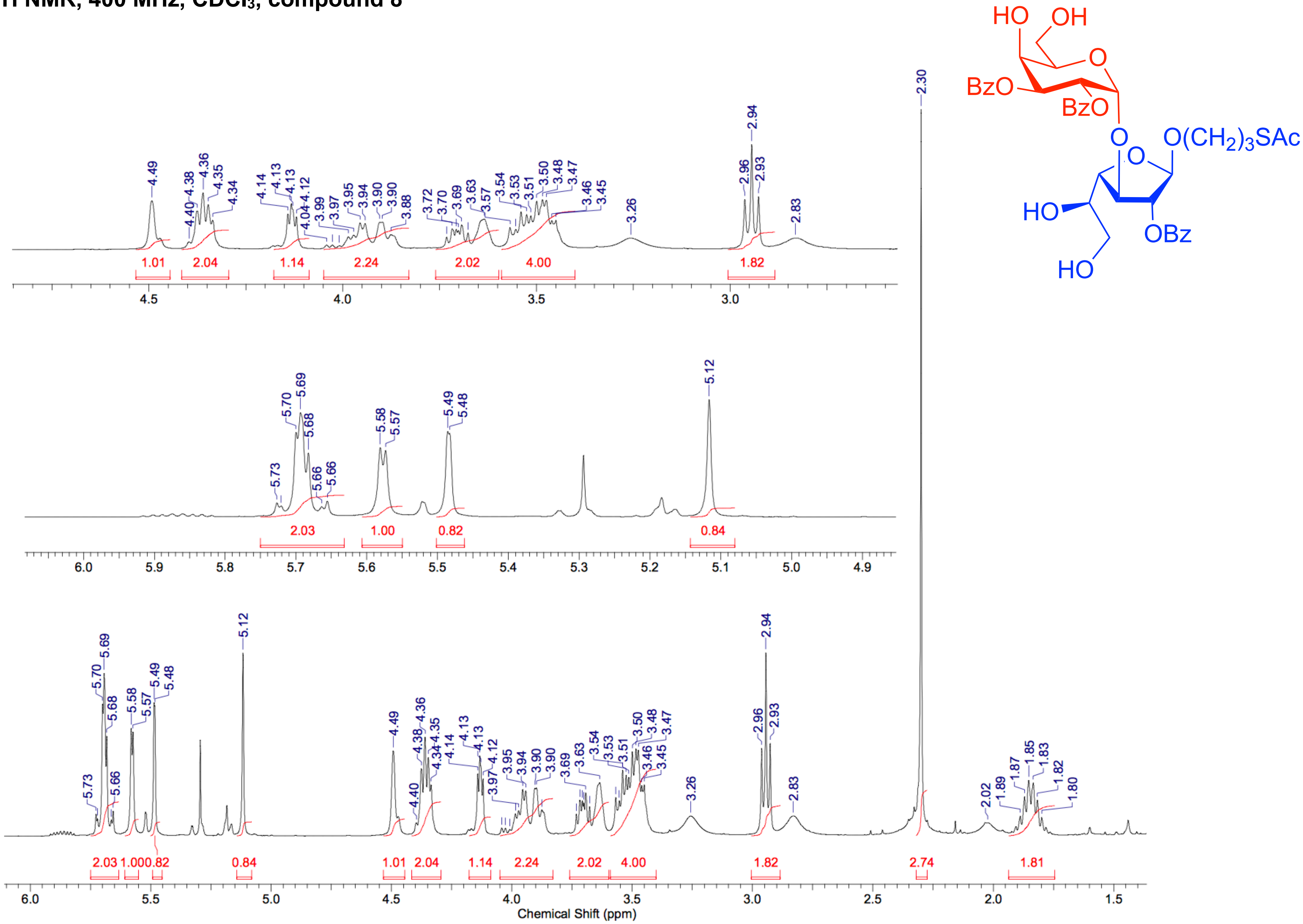


m/z $[M+Na]^+$ calc.: 717.2159
m/z $[M+Na]^+$ exp.: 717.2158

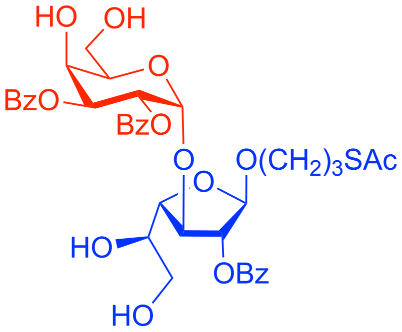
^{1}H NMR, 400 MHz, $CDCl_3$, compound 8

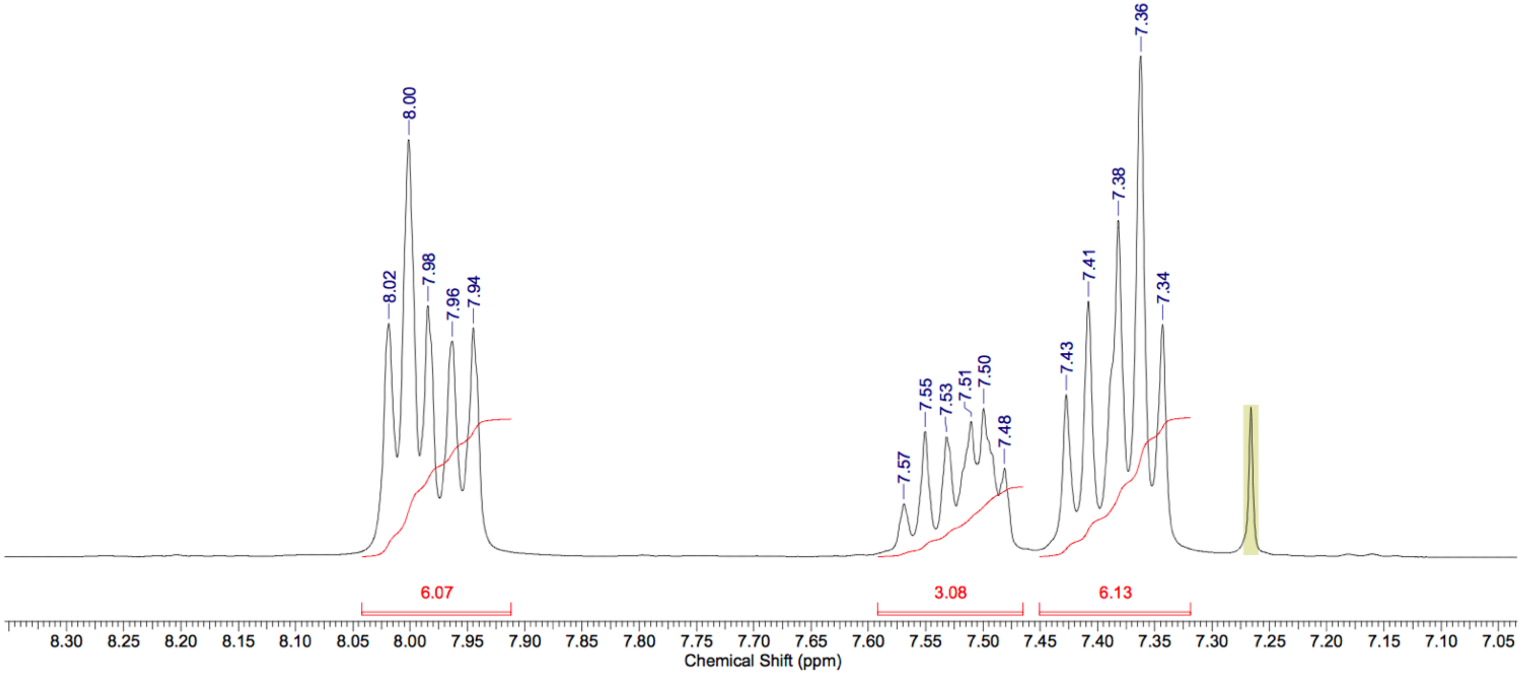


^{1}H NMR, 400 MHz, CDCl$_3$, compound 8

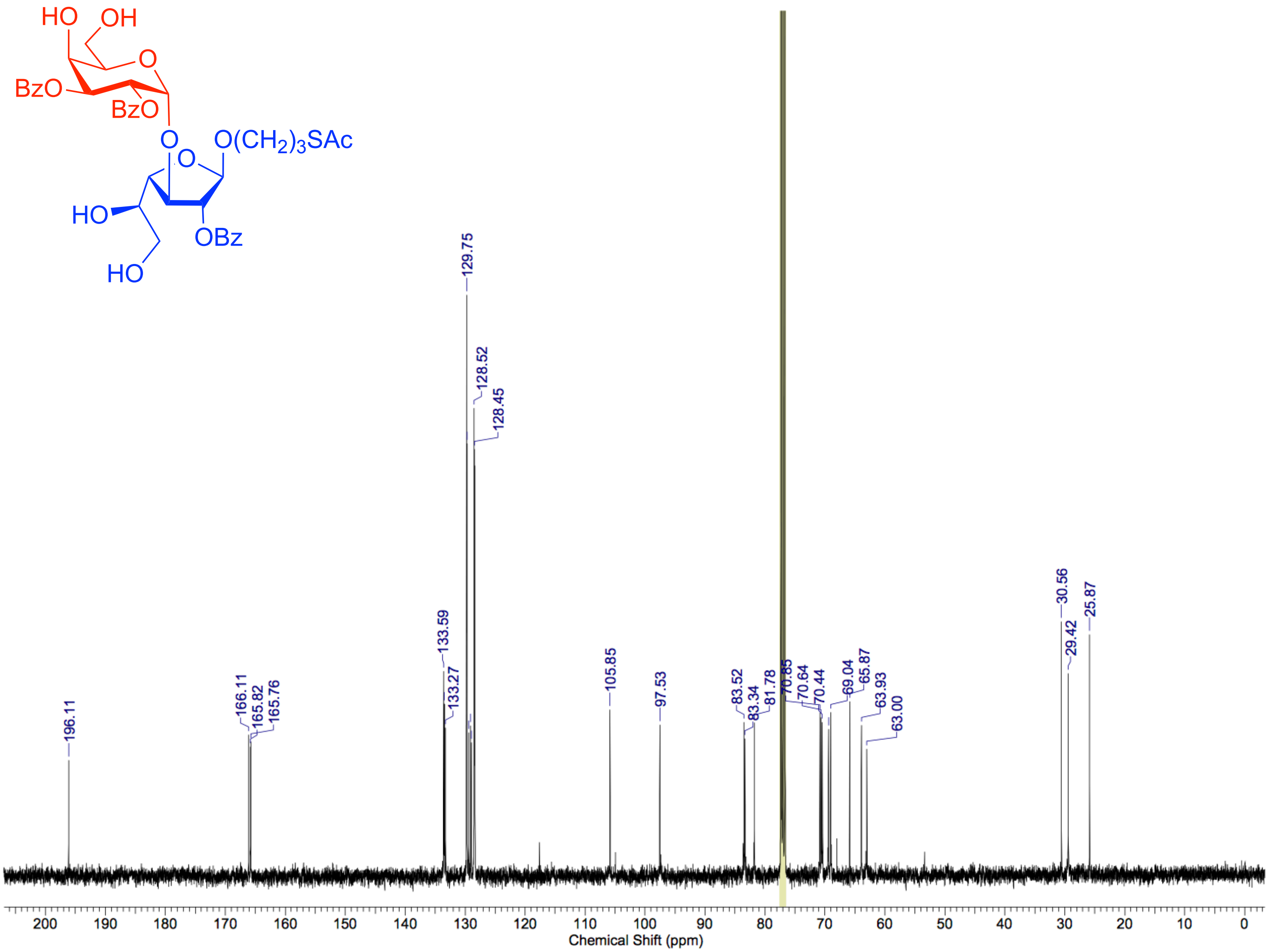


^{1}H NMR, 400 MHz, $CDCl_3$, compound 8

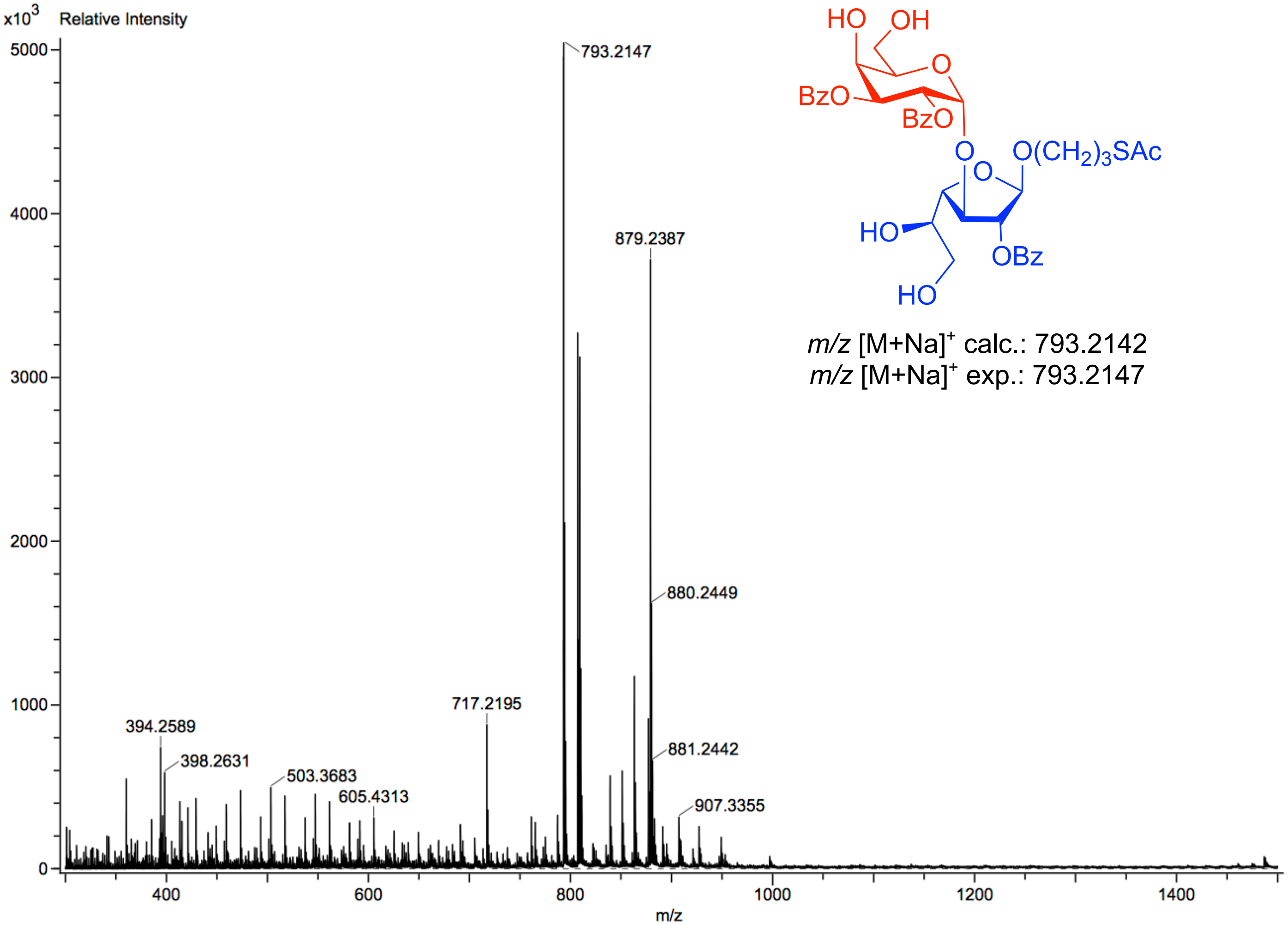




^{13}C NMR, 100 MHz, CDCl$_3$, compound 8

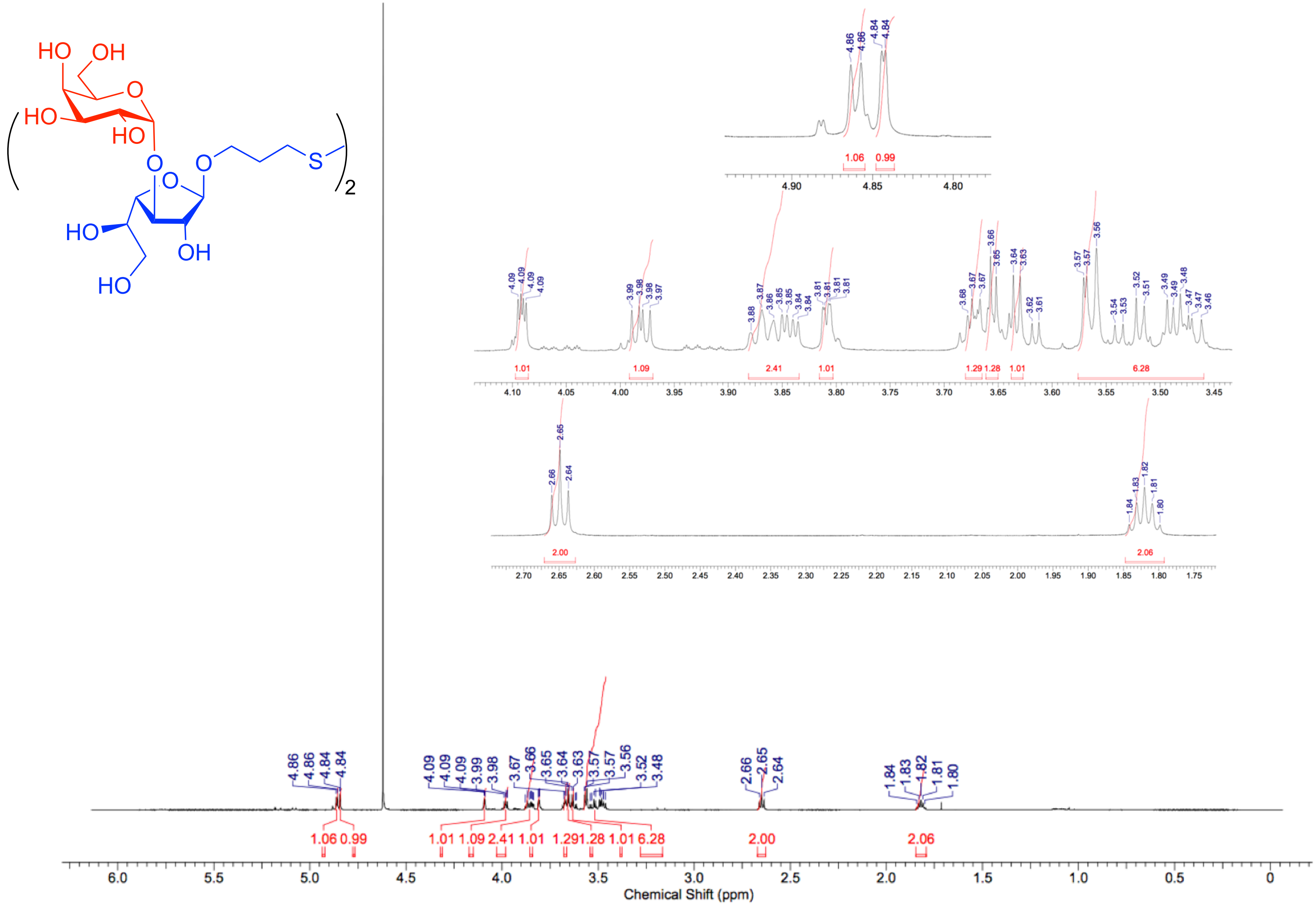


ESI-TOF HR mass spectrum of compound 8

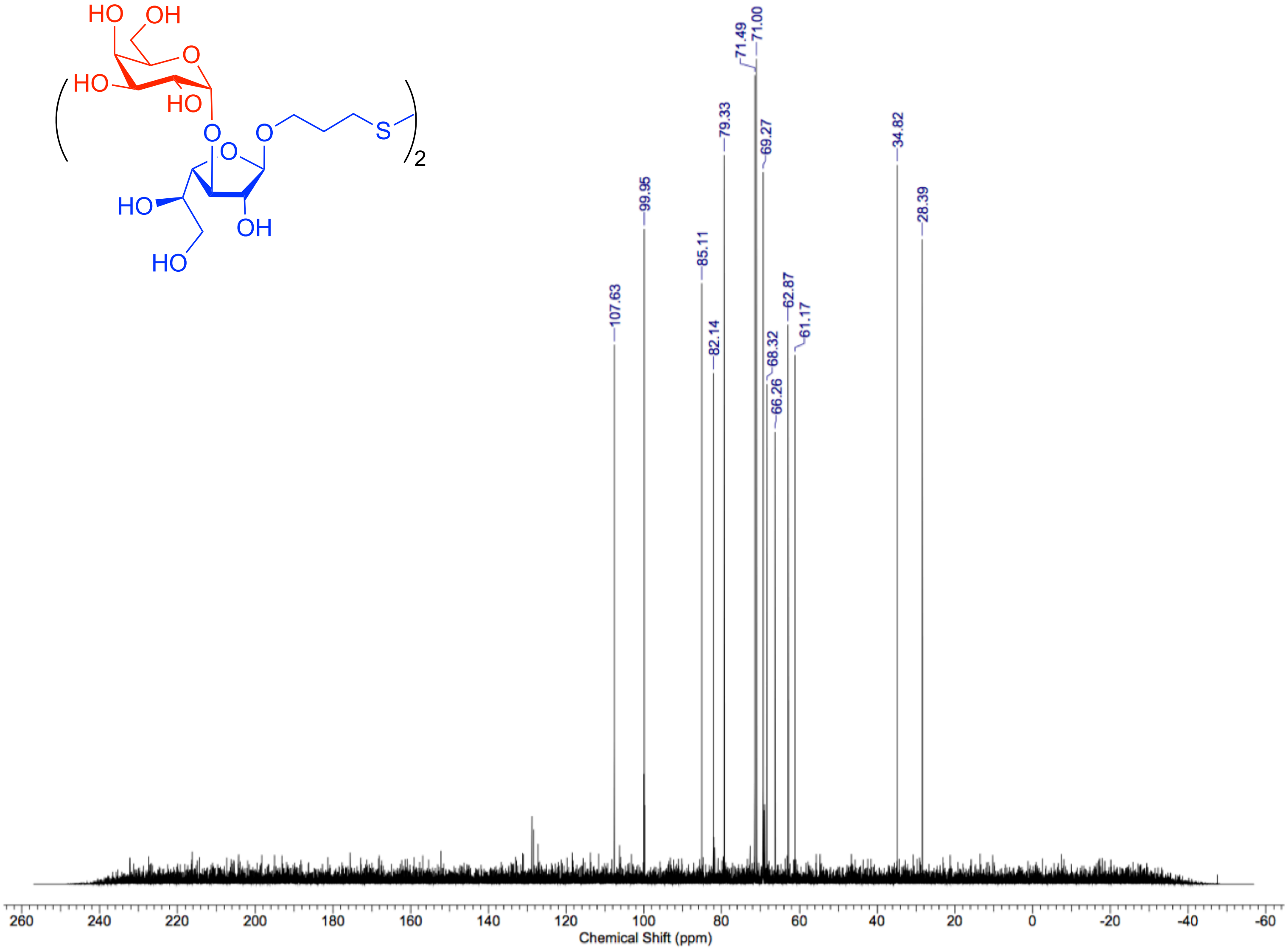


m/z $[M+Na]^+$ calc.: 793.2142
m/z $[M+Na]^+$ exp.: 793.2147

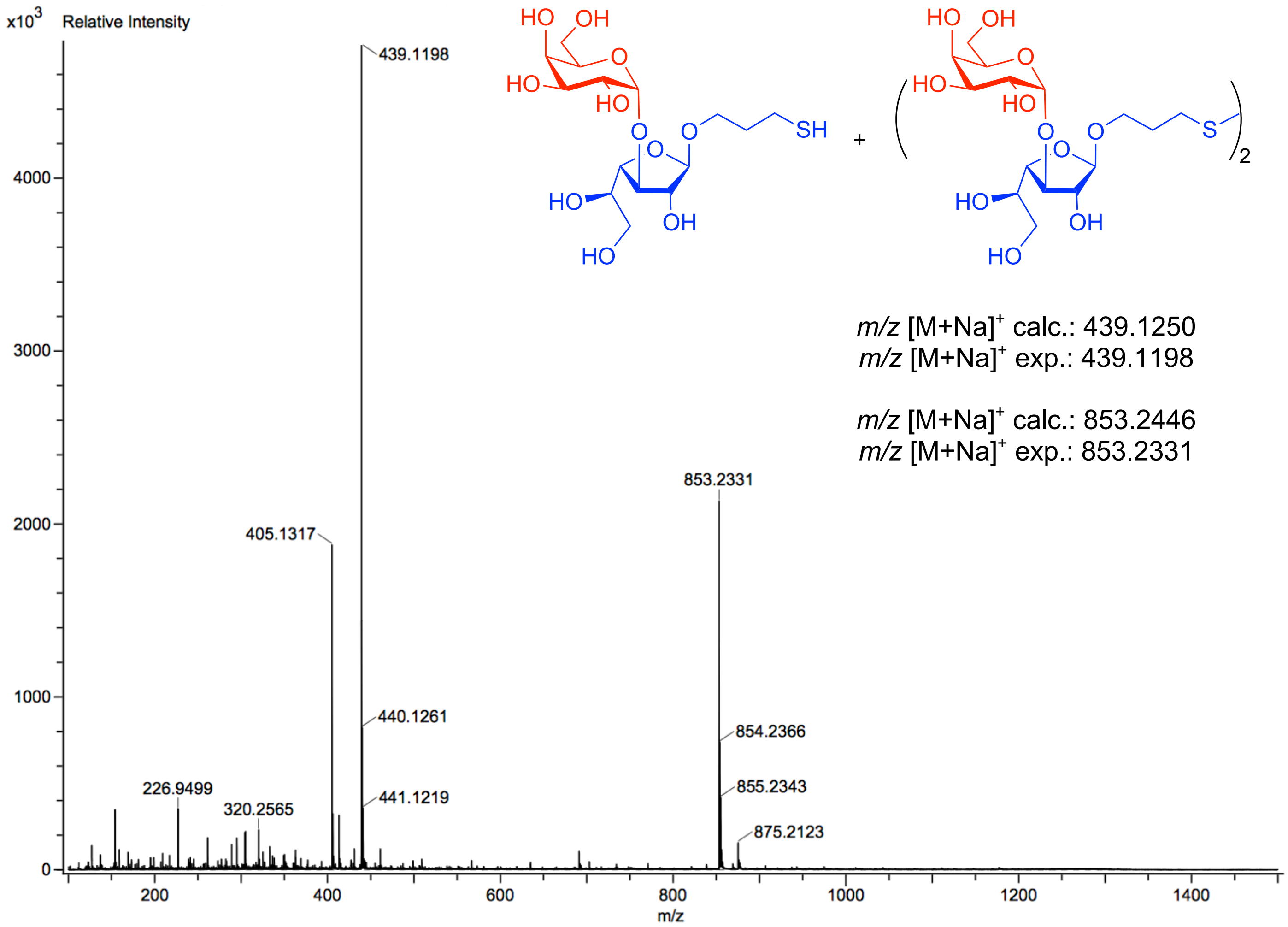
^{1}H NMR, 600 MHz, D_2O, compound $(G27_S)_2$



^{13}C NMR, 150 MHz, D$_2$O, compound (G27$_S$)$_2$



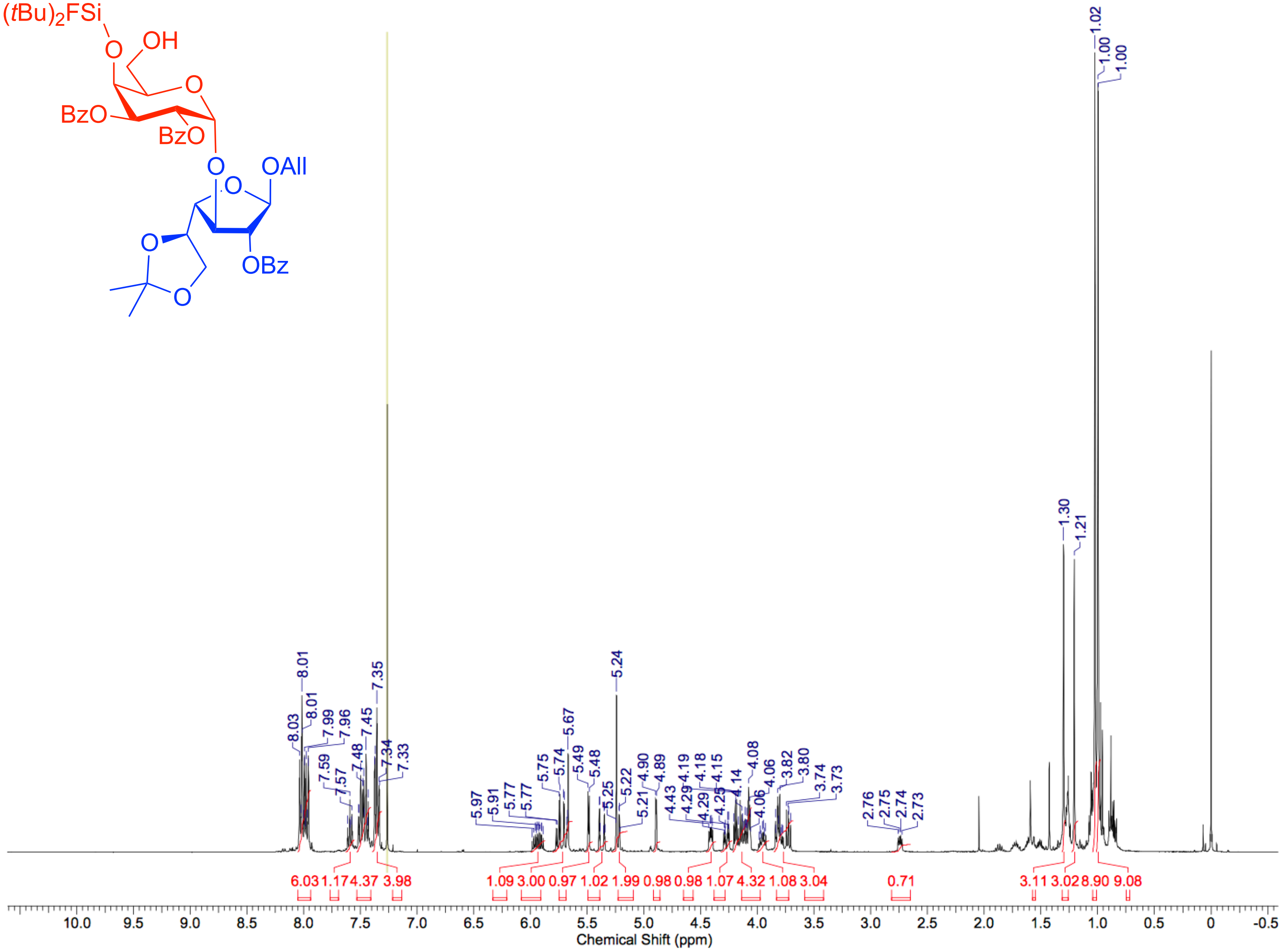
ESI-TOF HR mass spectrum of compound (G27$_S$)$_2$



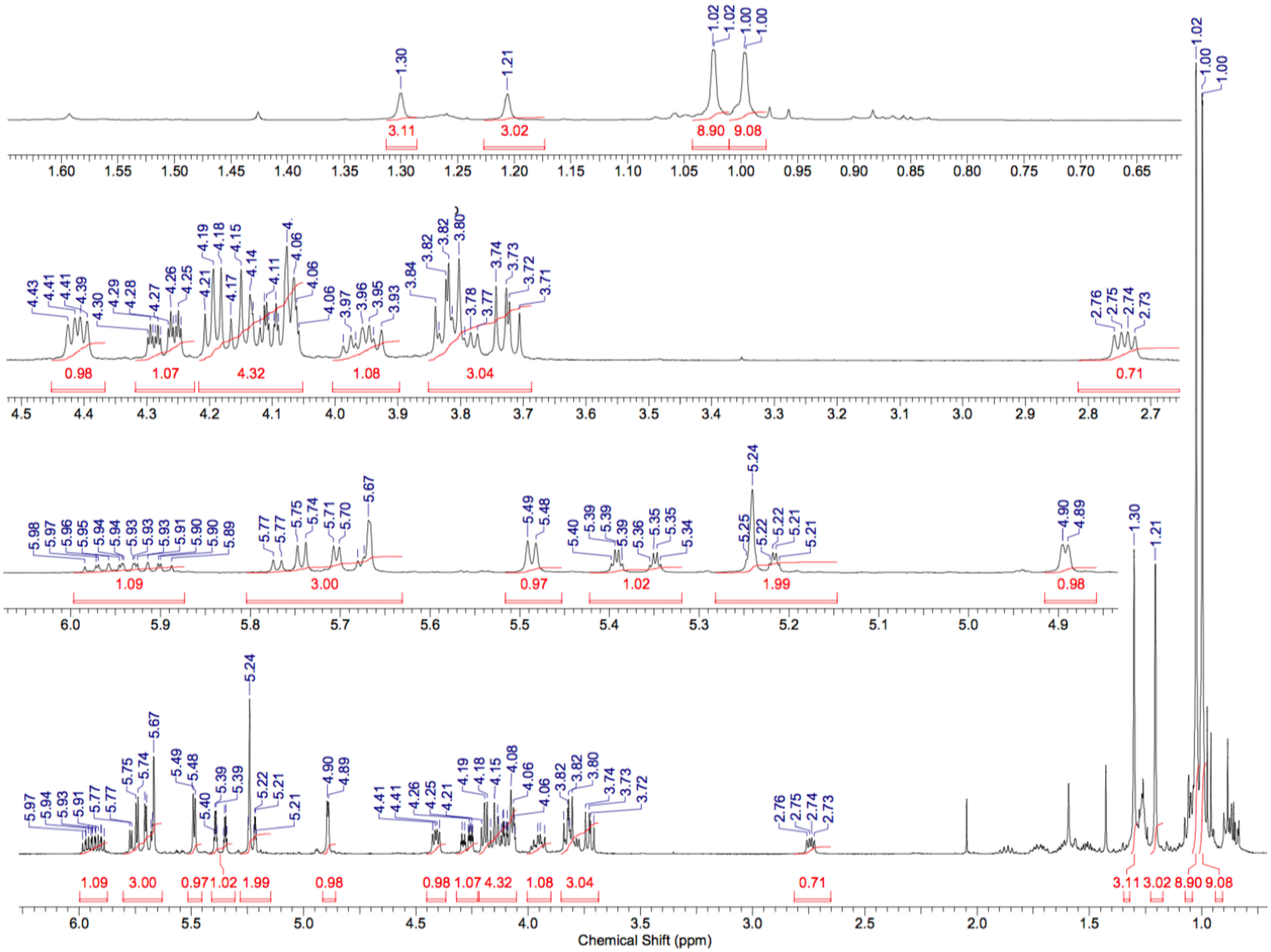
m/z [M+Na]$^+$ calc.: 439.1250
m/z [M+Na]$^+$ exp.: 439.1198

m/z [M+Na]$^+$ calc.: 853.2446
m/z [M+Na]$^+$ exp.: 853.2331

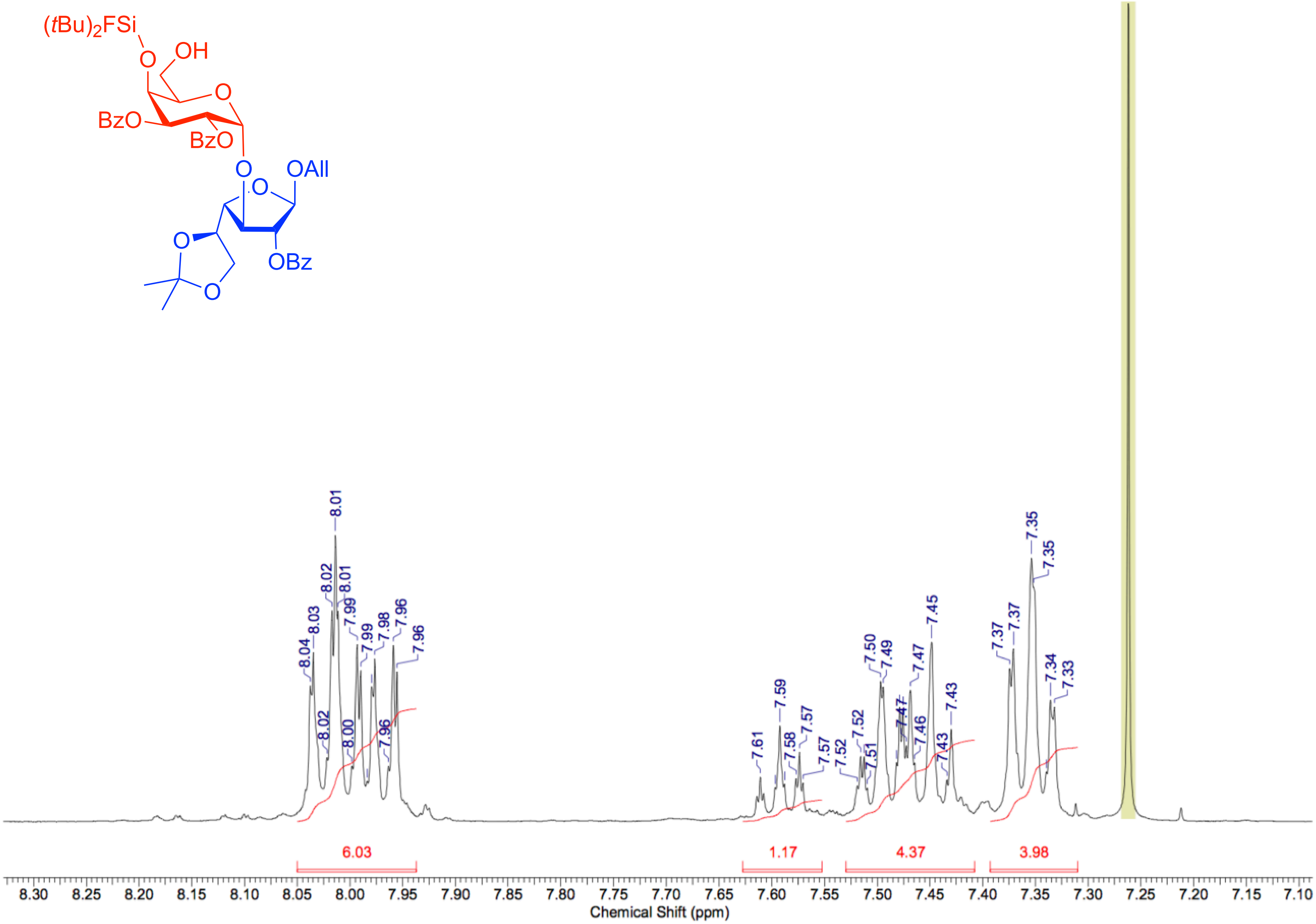
^{1}H NMR, 400 MHz, CDCl$_3$, compound 11



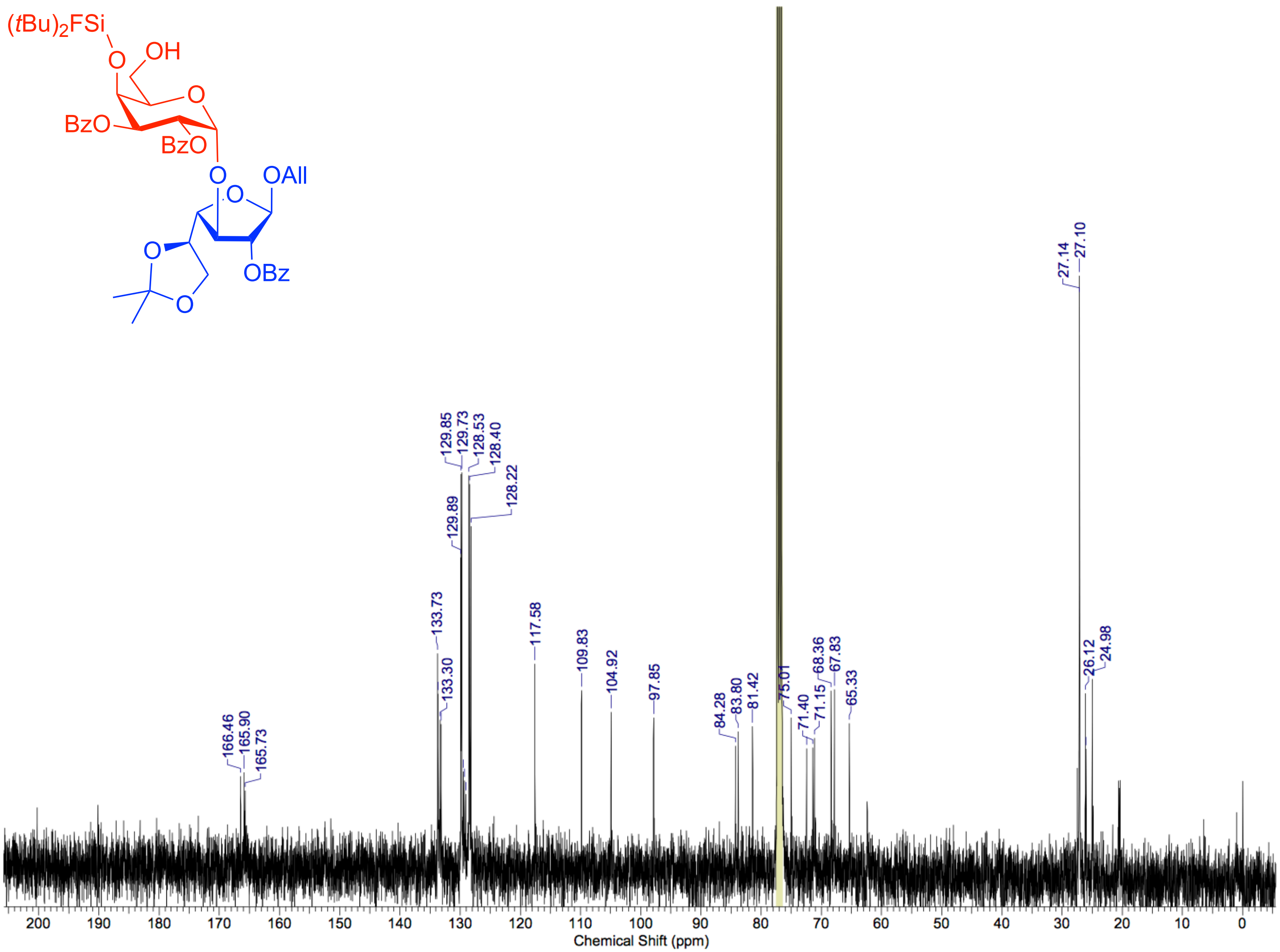
^{1}H NMR, 400 MHz, CDCl$_3$, compound 11



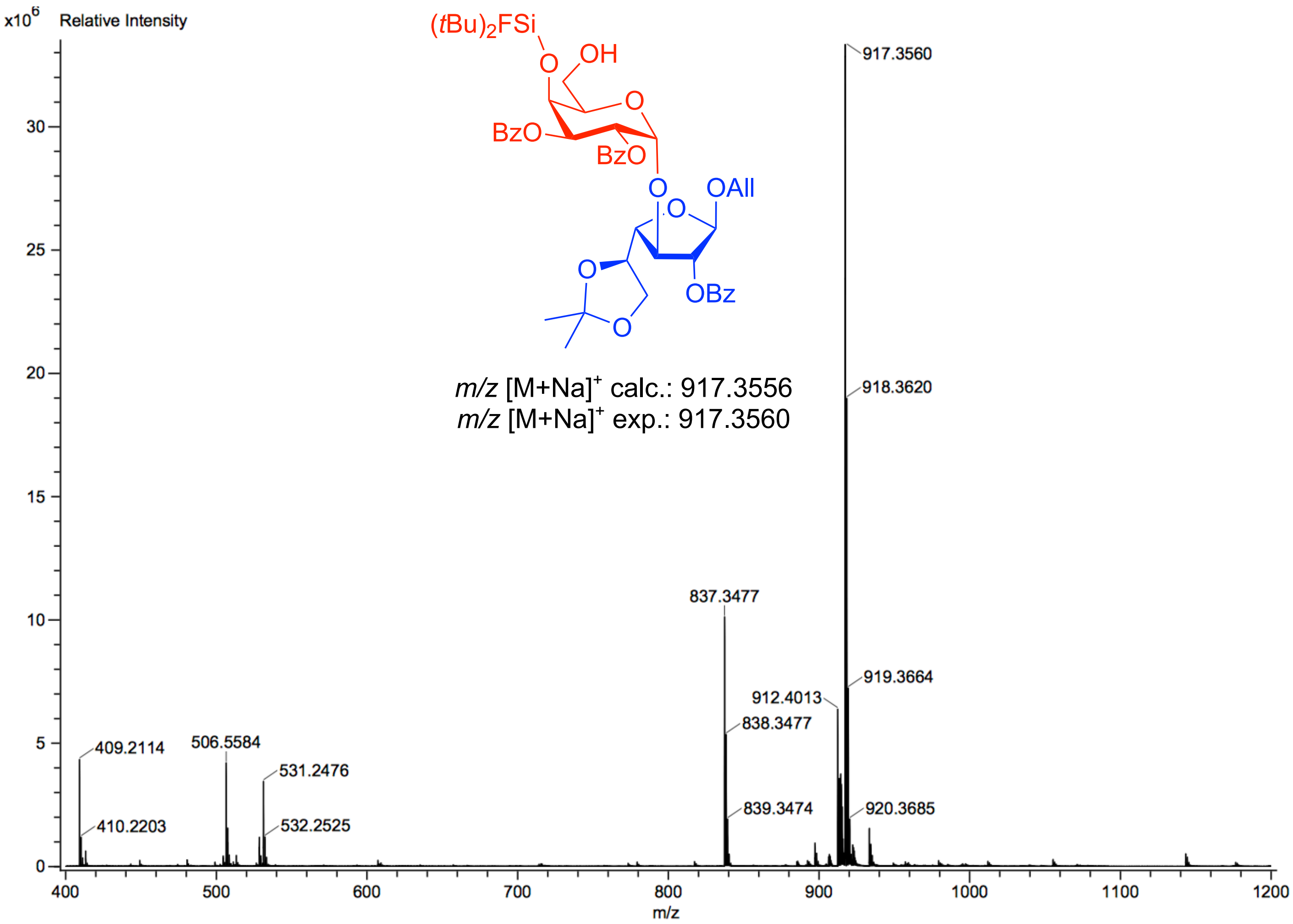
^{1}H NMR, 400 MHz, CDCl$_3$, compound 11



^{13}C NMR, 100 MHz, CDCl$_3$, compound 11

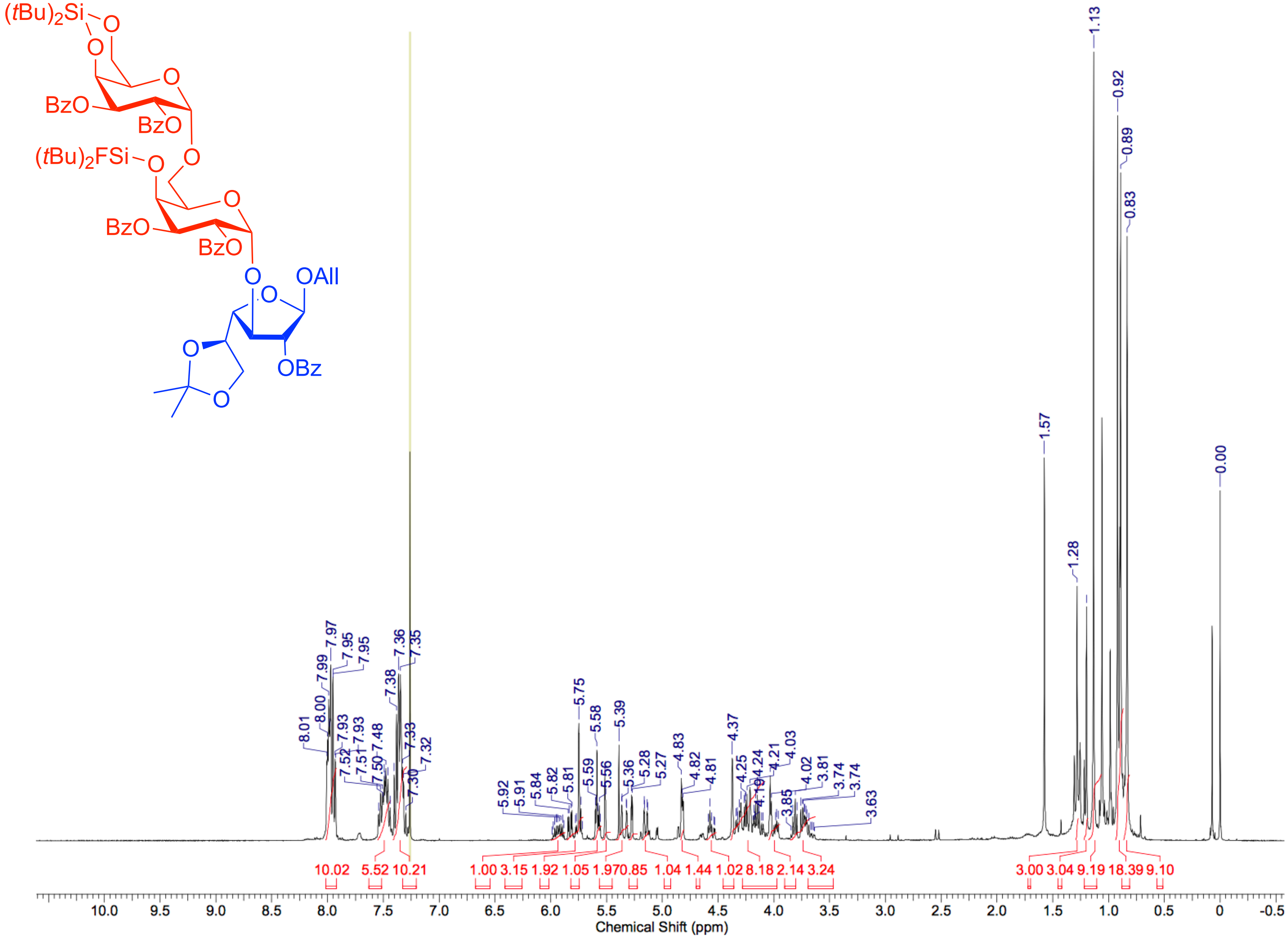


ESI-TOF HR mass spectrum of compound 11

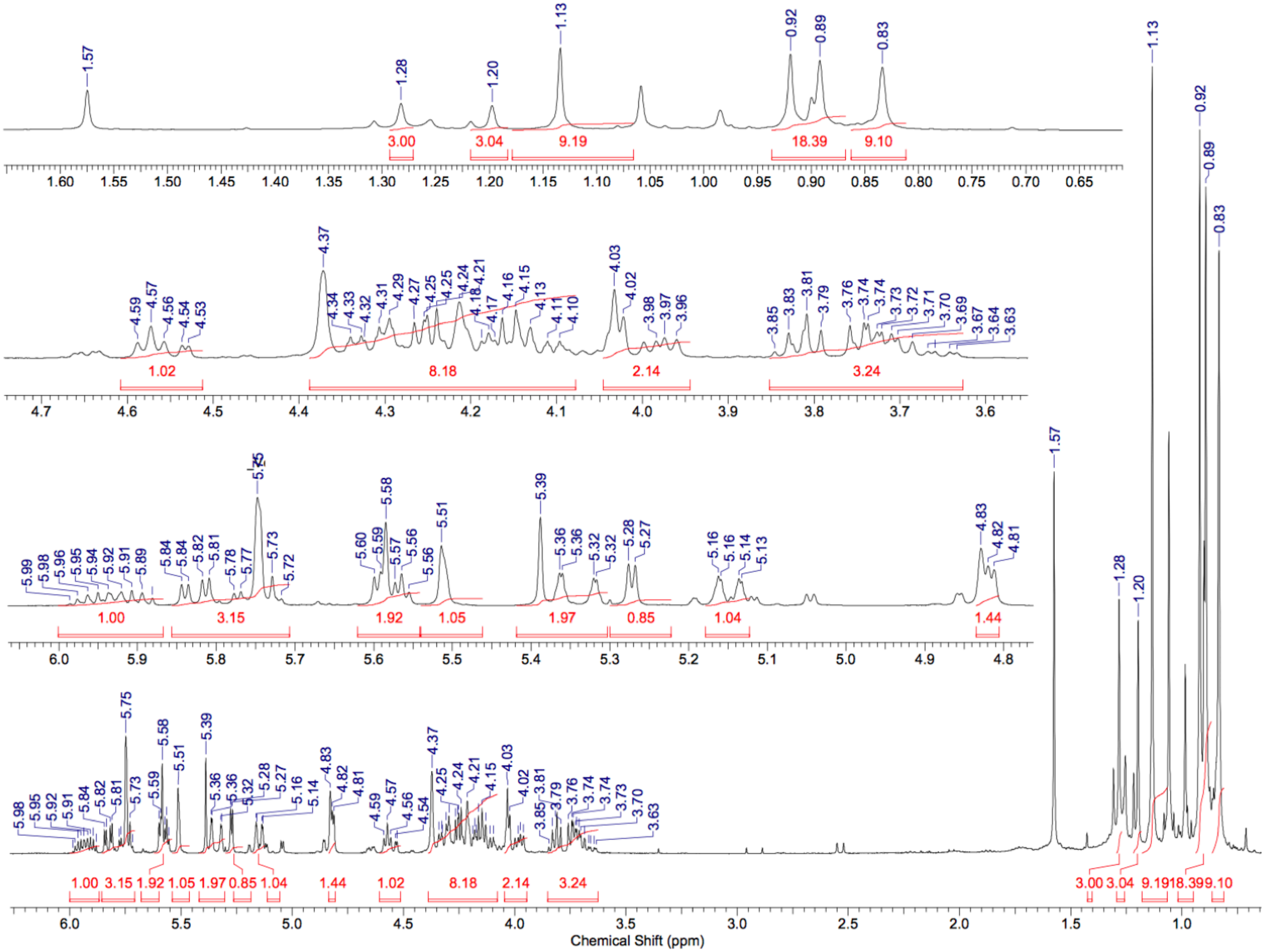


m/z $[M+Na]^+$ calc.: 917.3556
m/z $[M+Na]^+$ exp.: 917.3560

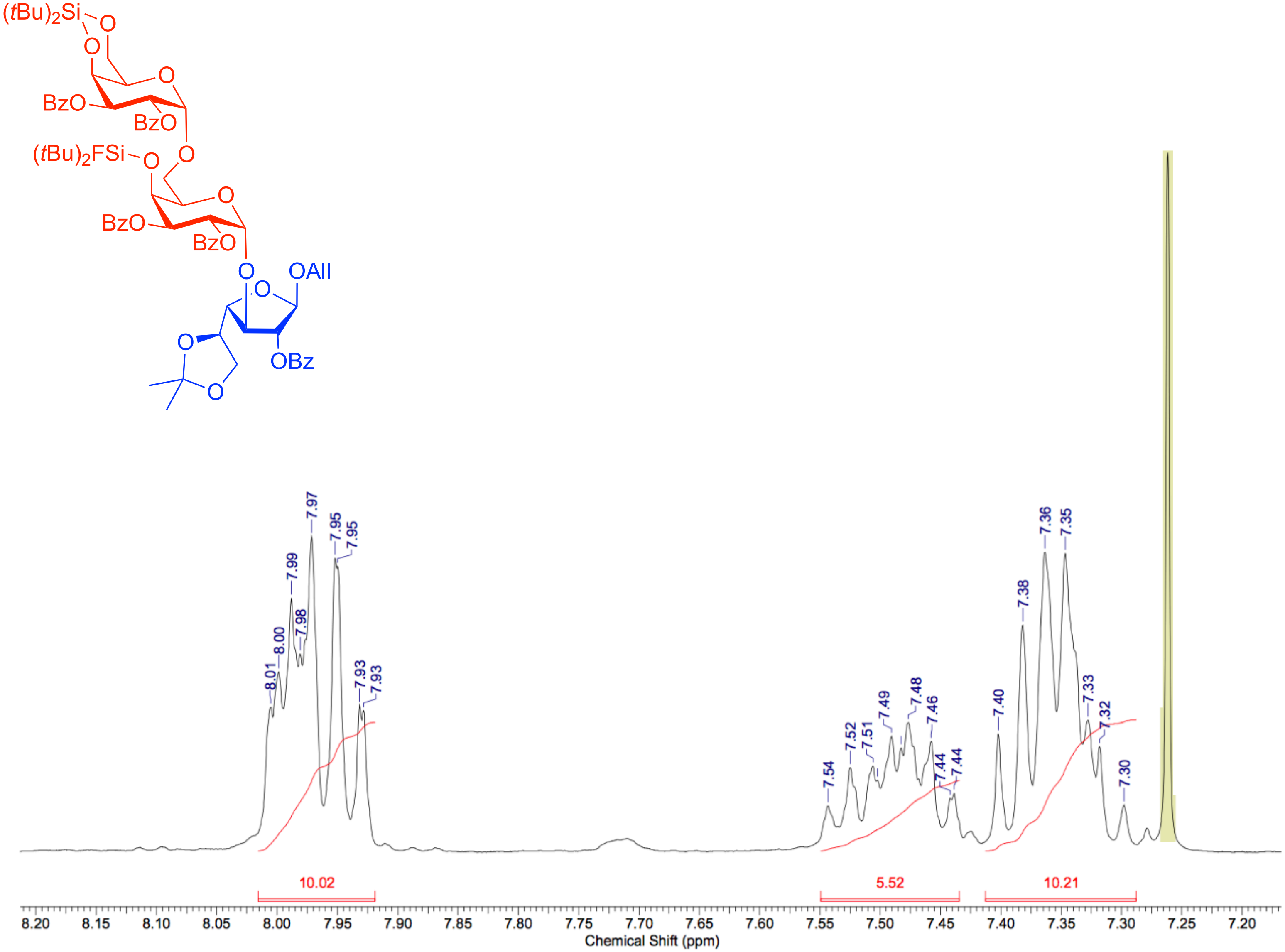
^{1}H NMR, 400 MHz, CDCl$_3$, compound 12



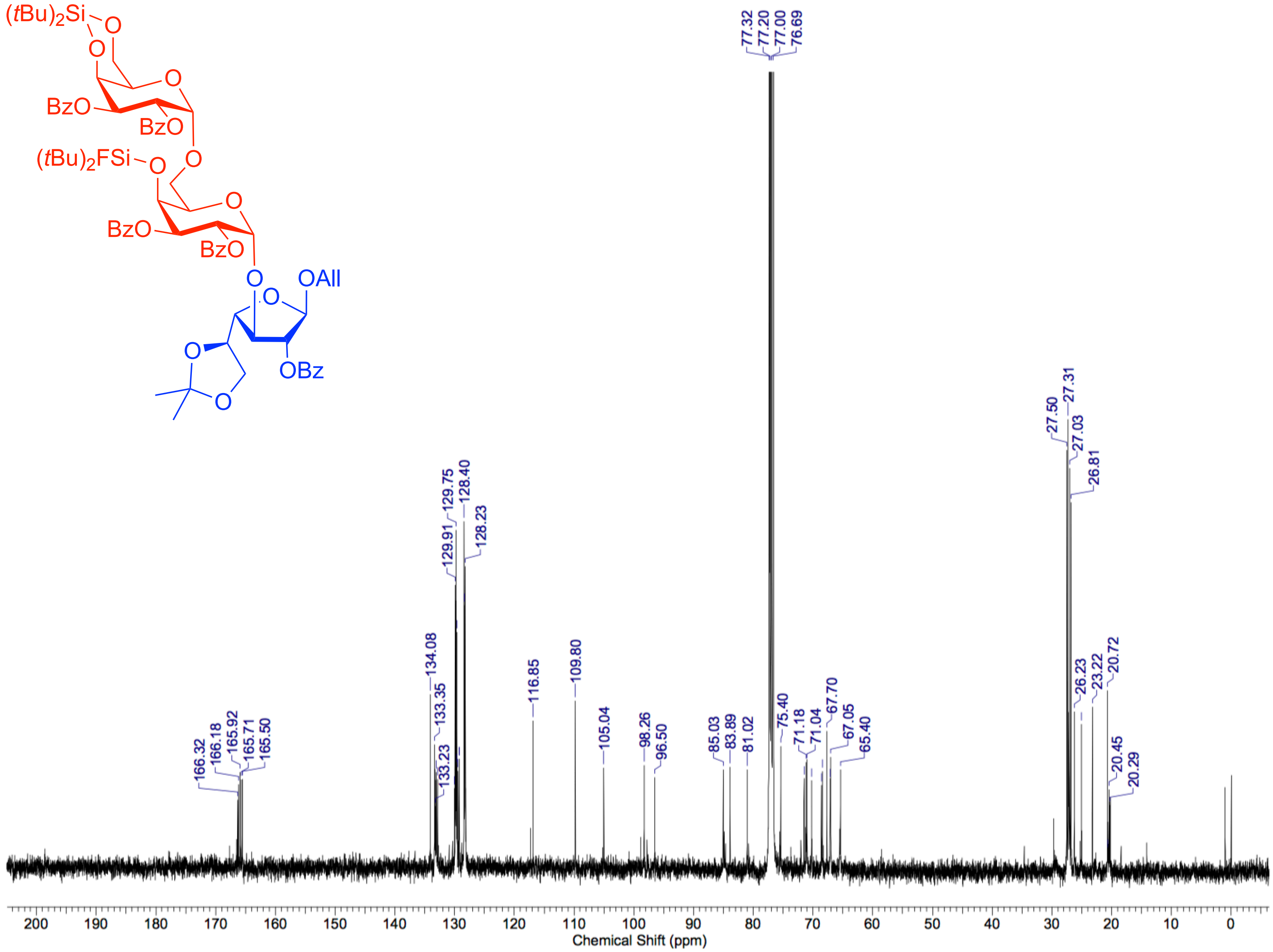
^{1}H NMR, 400 MHz, CDCl$_3$, compound 12



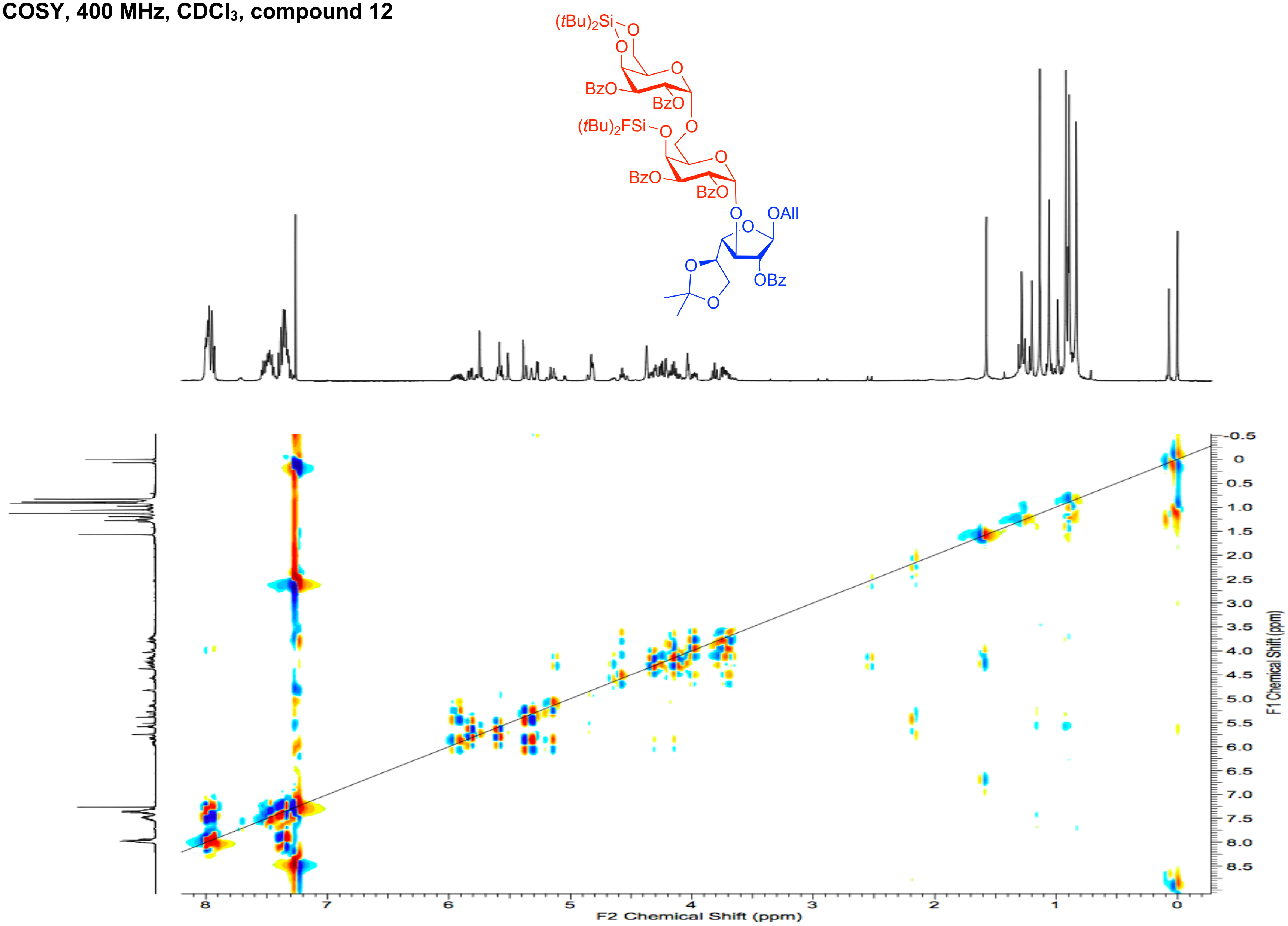
^{1}H NMR, 400 MHz, $CDCl_3$, compound 12



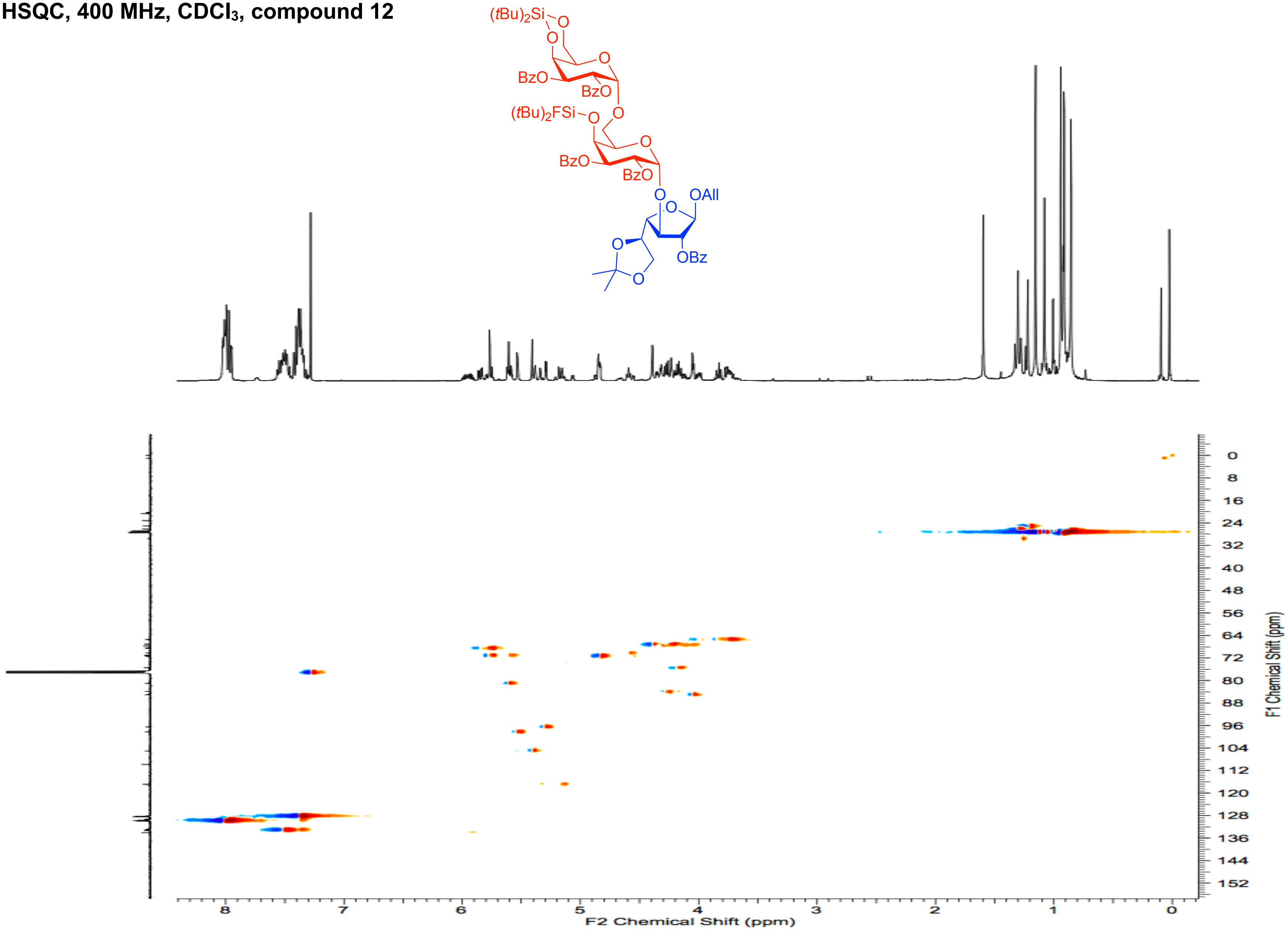
^{13}C NMR, 100 MHz, CDCl$_3$, compound 12



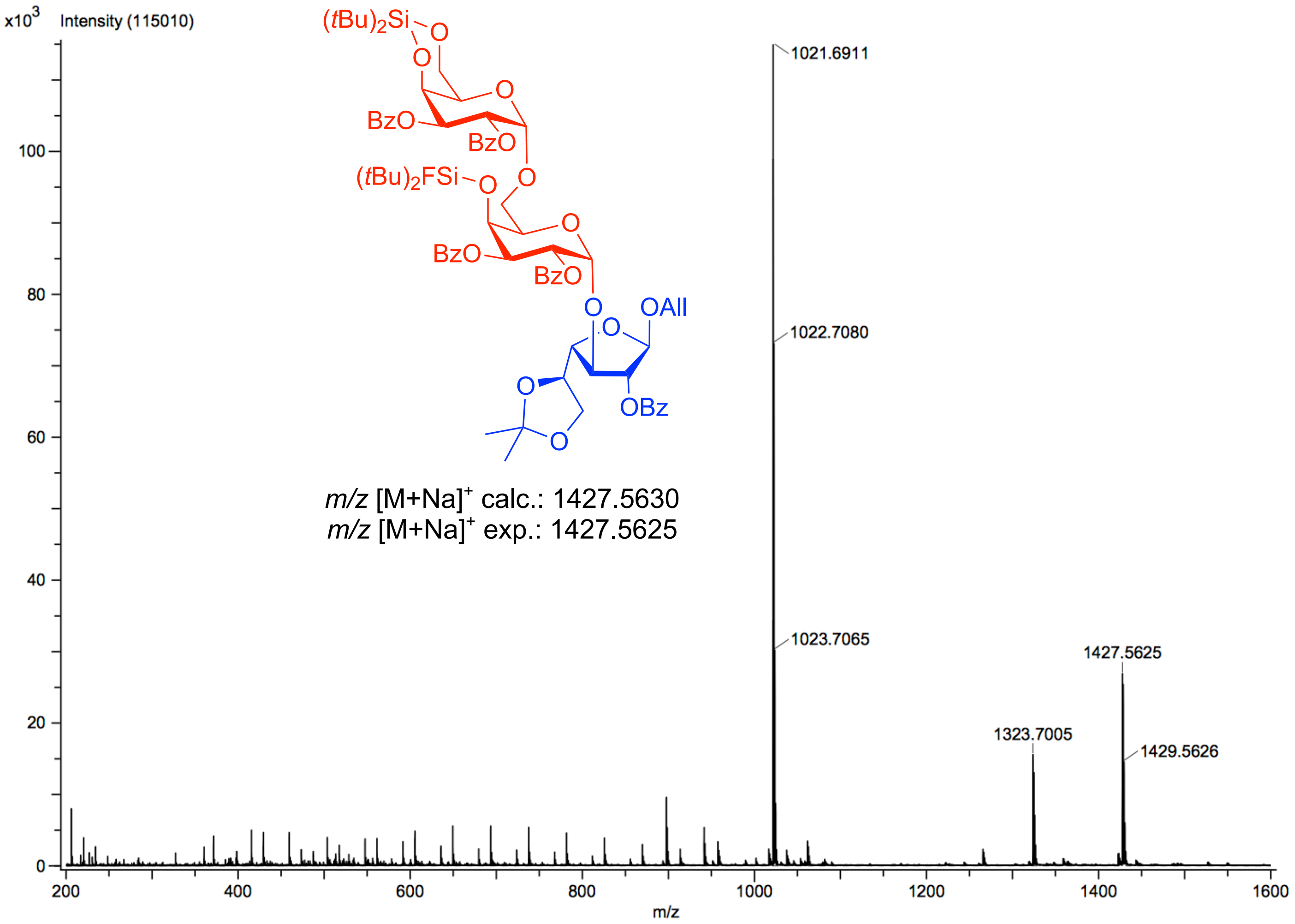
COSY, 400 MHz, $CDCl_3$, compound 12



HSQC, 400 MHz, $CDCl_3$, compound 12

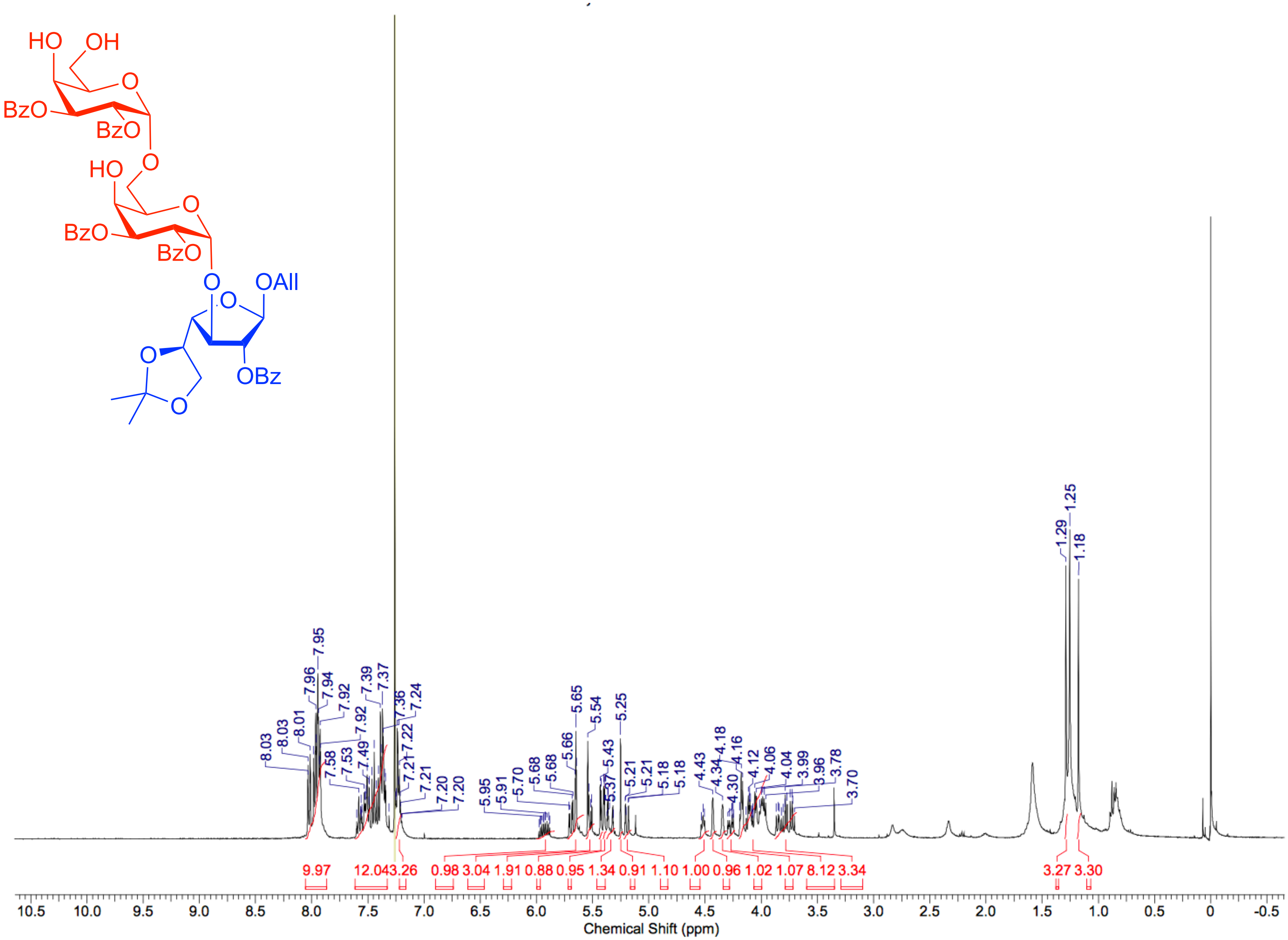


ESI-TOF HR mass spectrum of compound 12

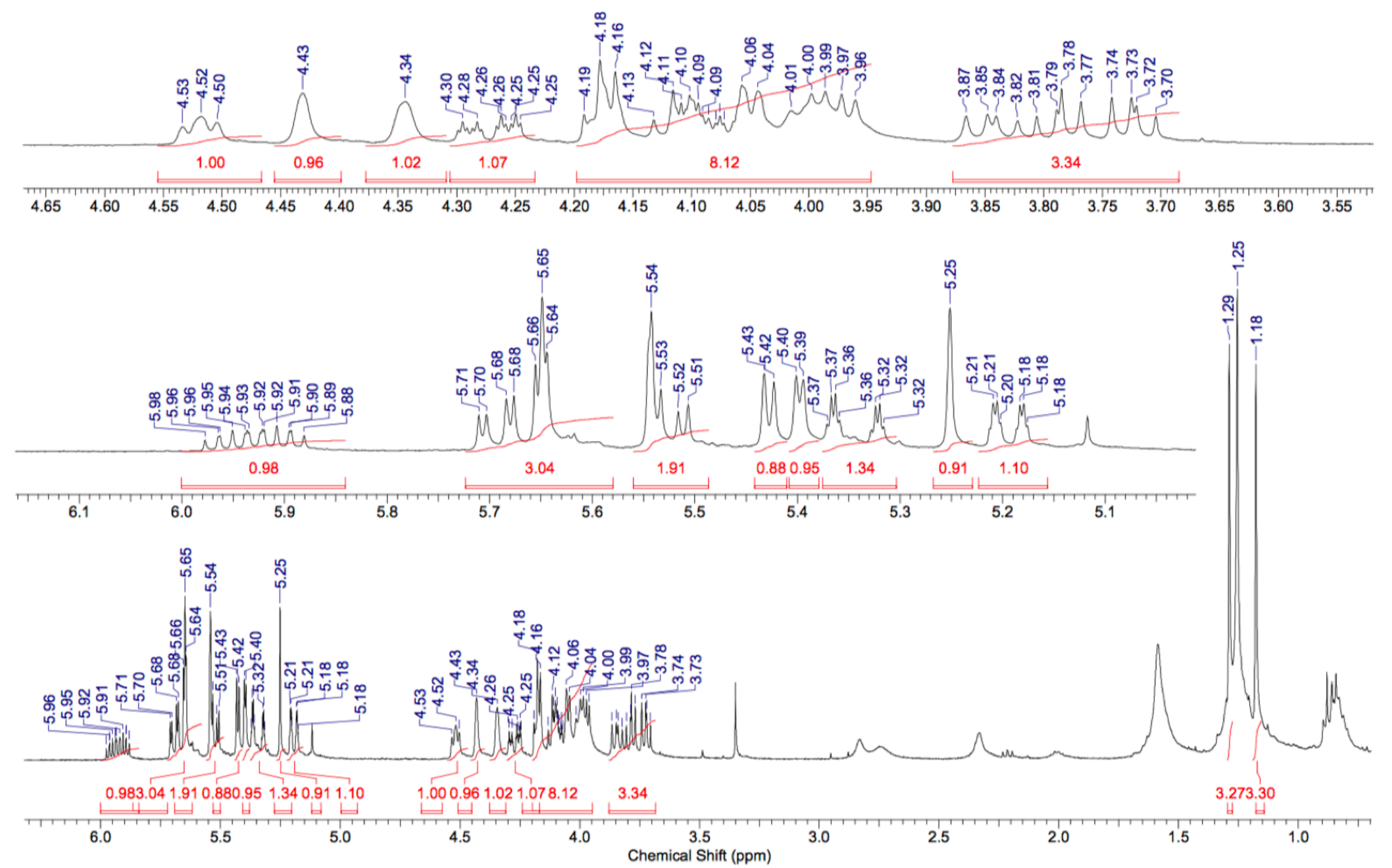


m/z $[M+Na]^+$ calc.: 1427.5630
m/z $[M+Na]^+$ exp.: 1427.5625

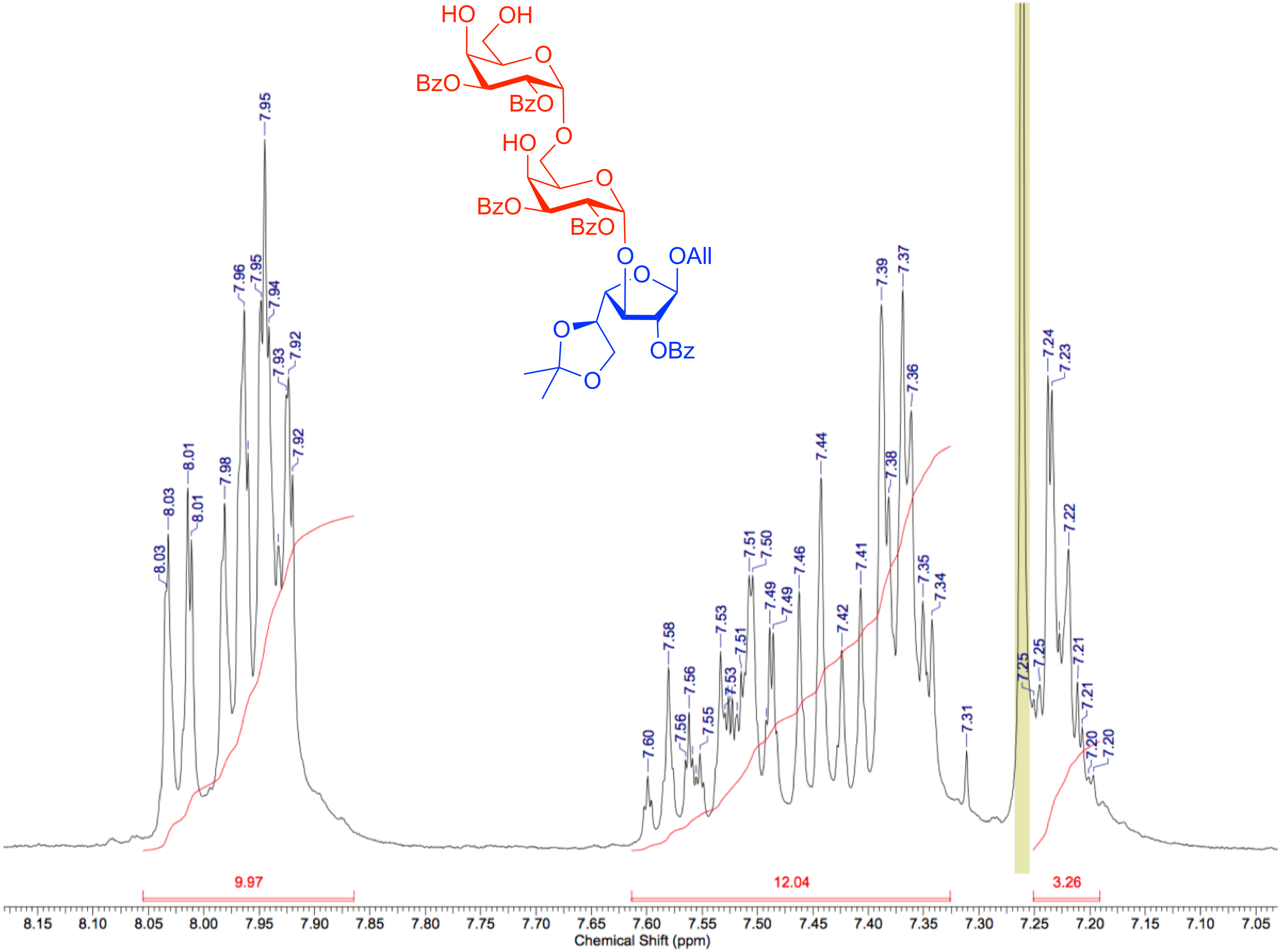
^{1}H NMR, 400 MHz, CDCl$_3$, compound 13



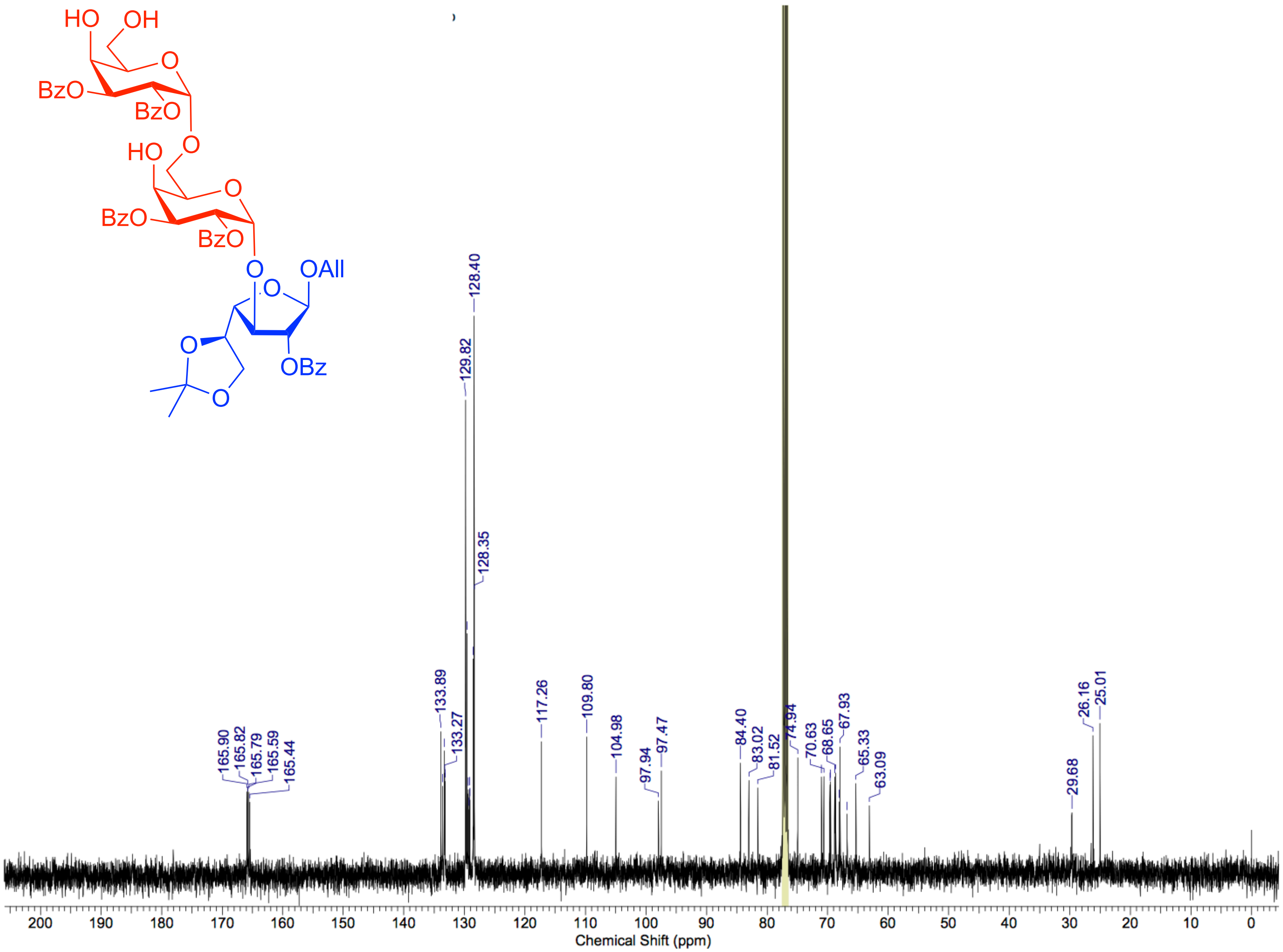
^{1}H NMR, 400 MHz, $CDCl_3$, compound 13



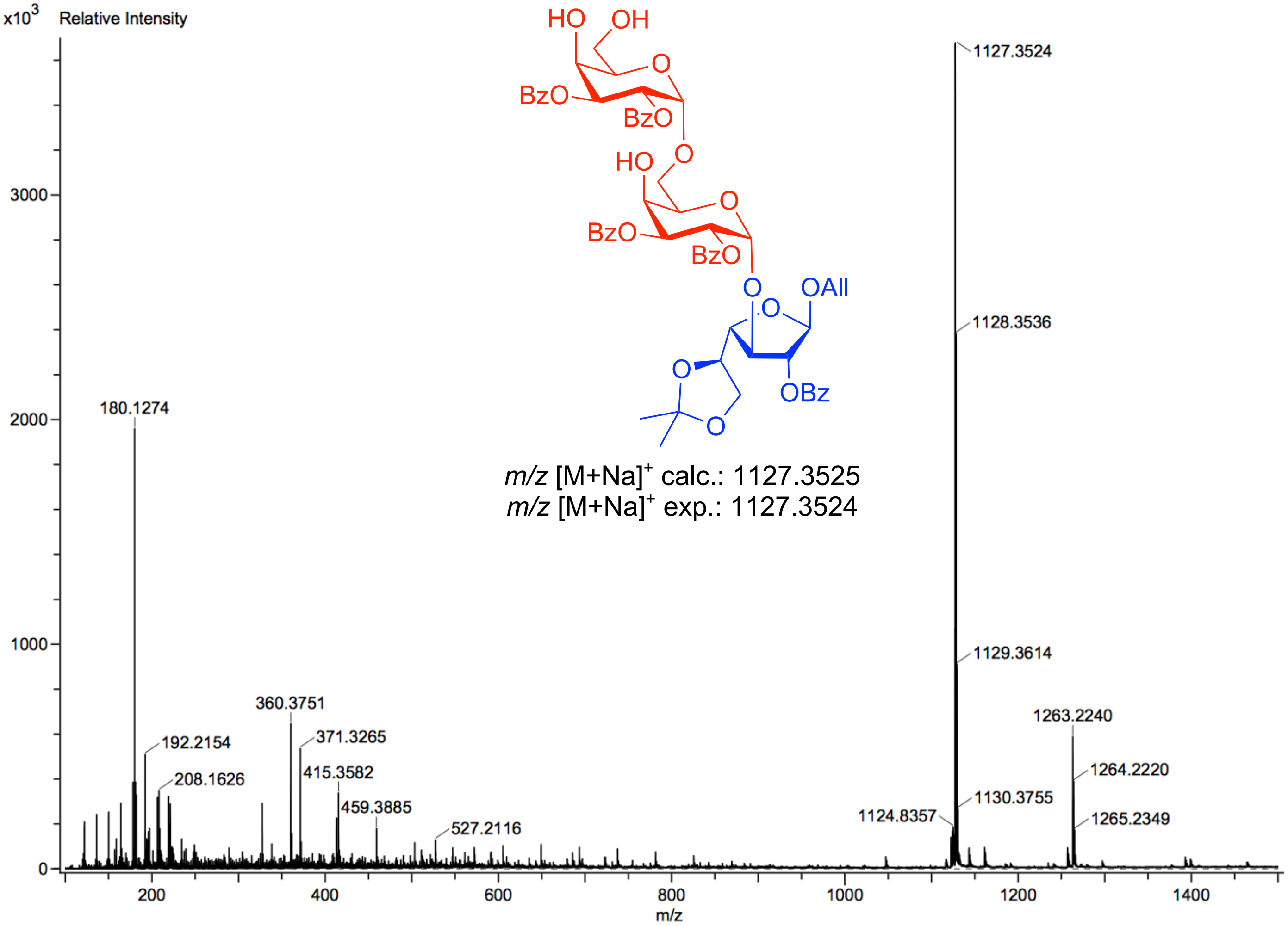
^{1}H NMR, 400 MHz, CDCl$_3$, compound 13



^{13}C NMR, 100 MHz, CDCl$_3$, compound 13

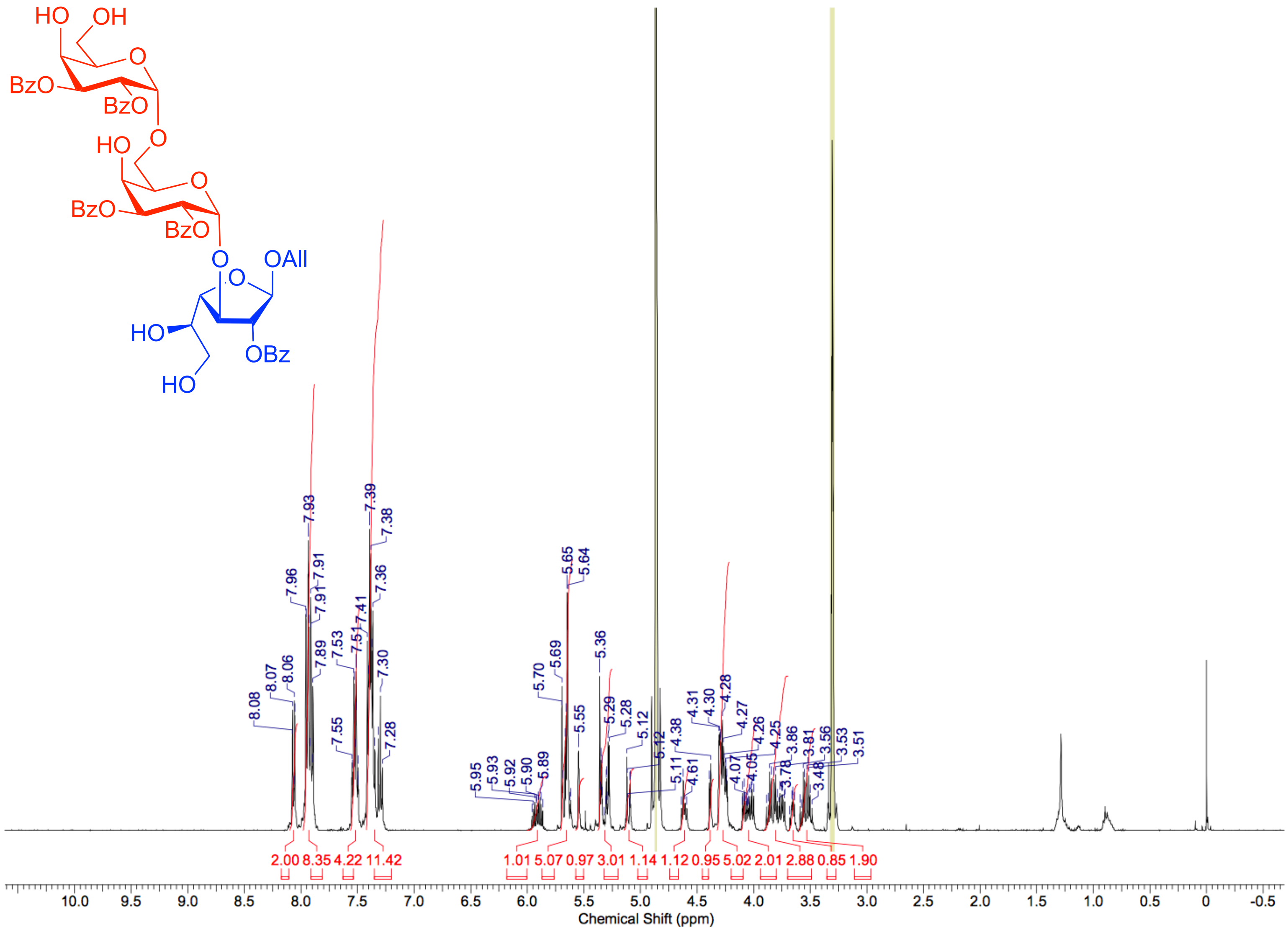


ESI-TOF HR mass spectrum of compound 13

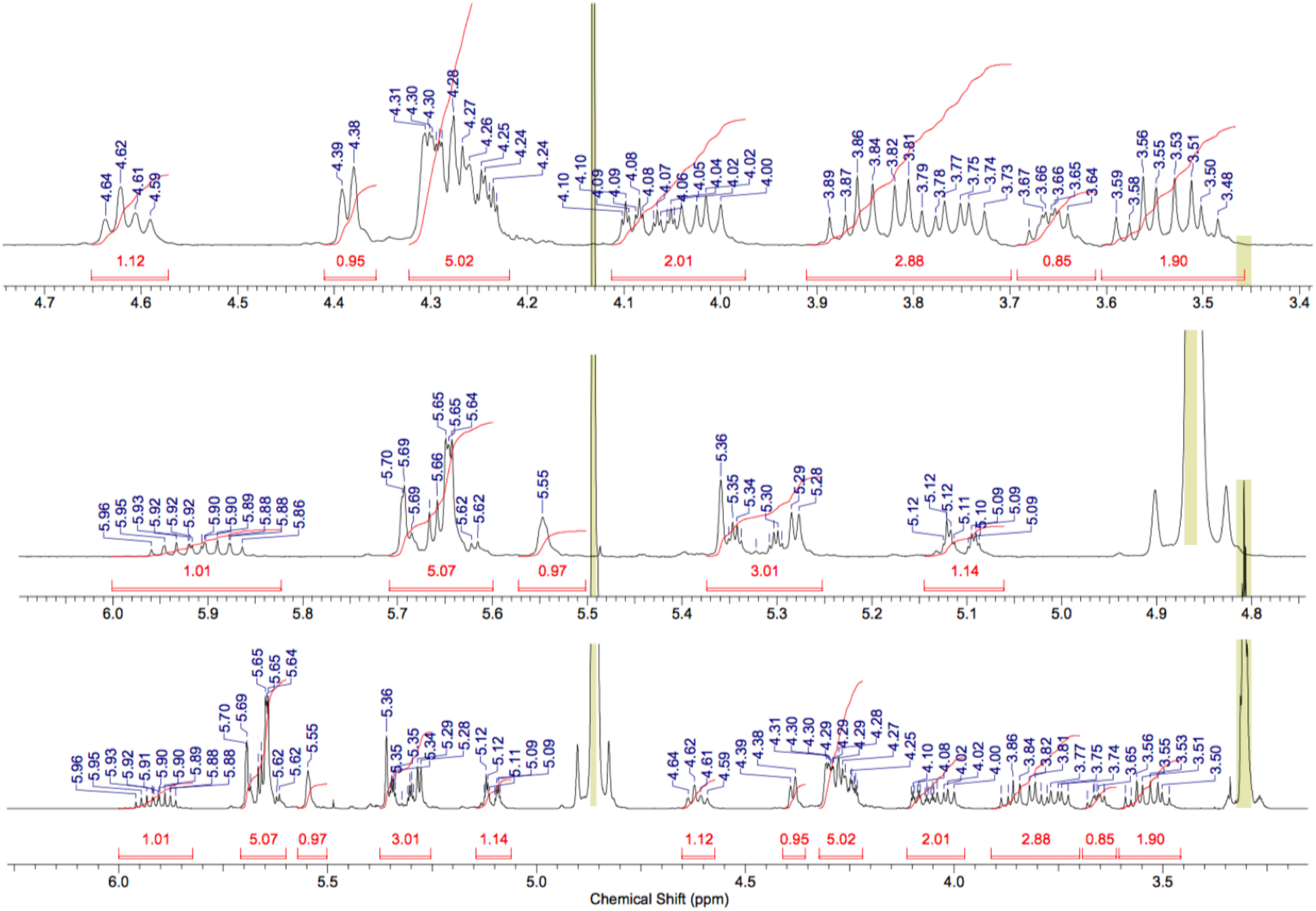


m/z [M+Na]$^+$ calc.: 1127.3525
m/z [M+Na]$^+$ exp.: 1127.3524

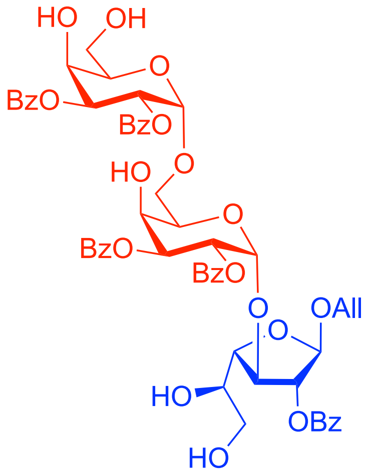
^{1}H NMR, 400 MHz, CD$_3$OD, compound S5

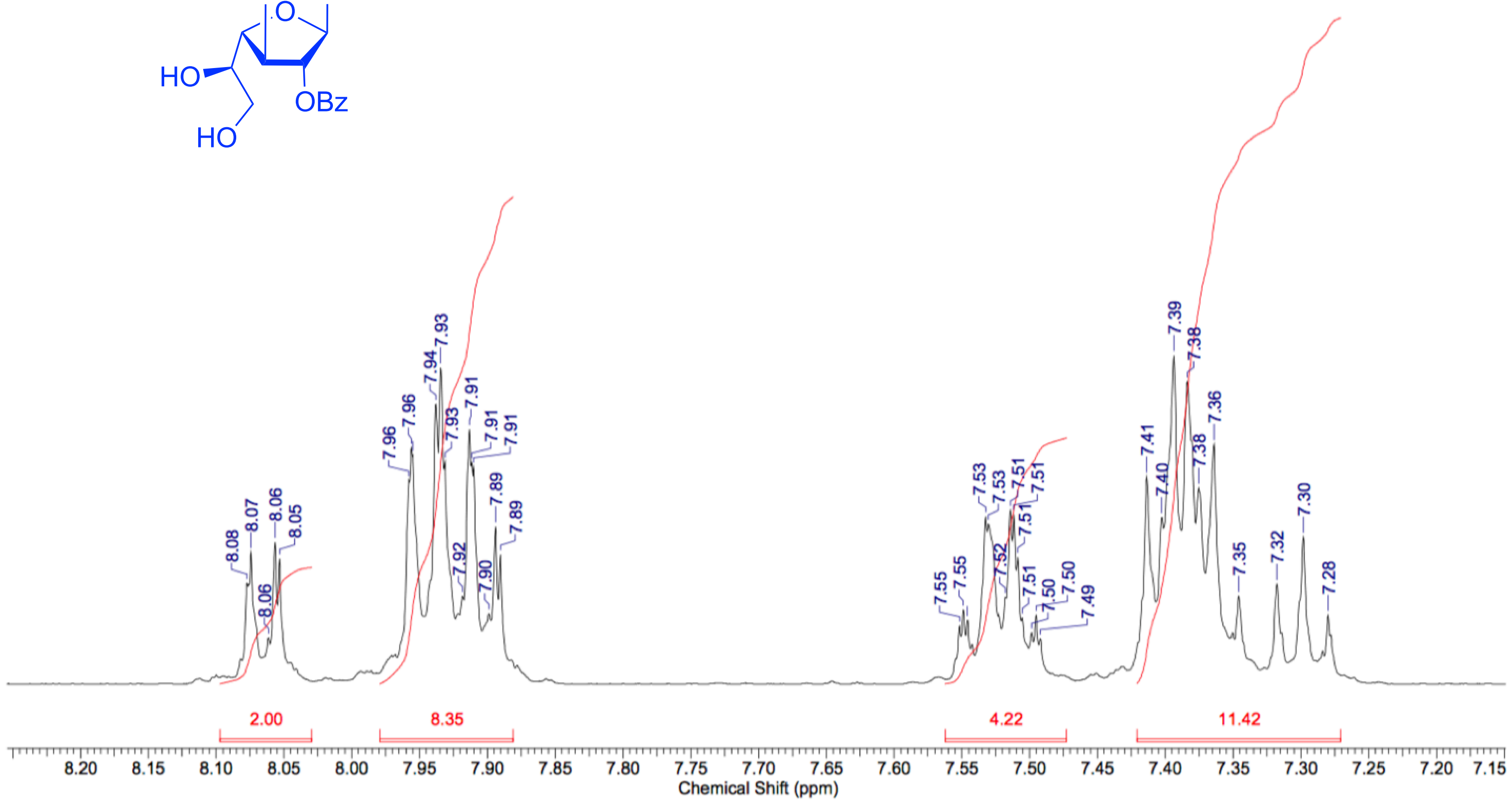


^{1}H NMR, 400 MHz, CD$_3$OD, compound S5

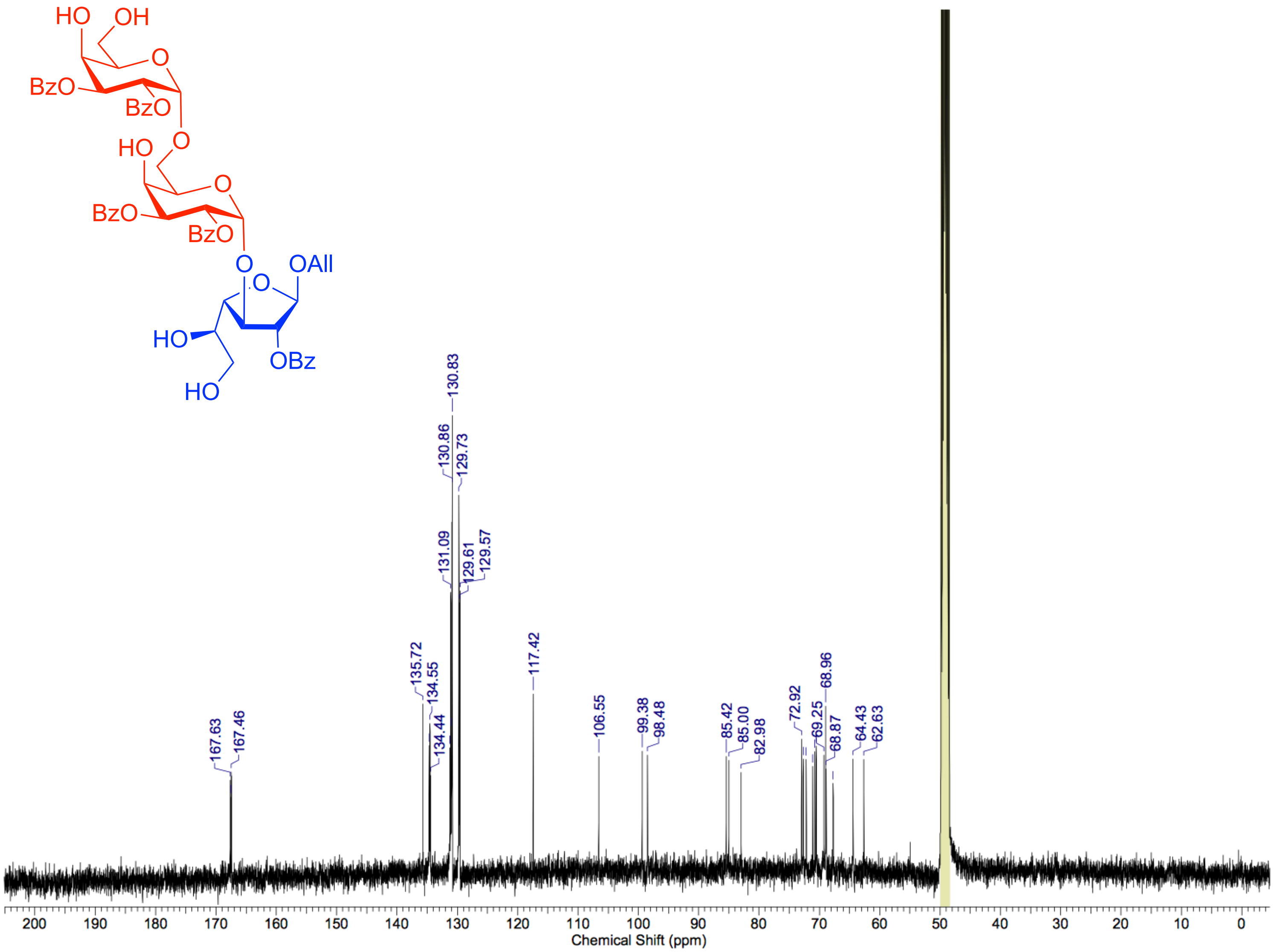


^{1}H NMR, 400 MHz, CD_3OD, compound S5

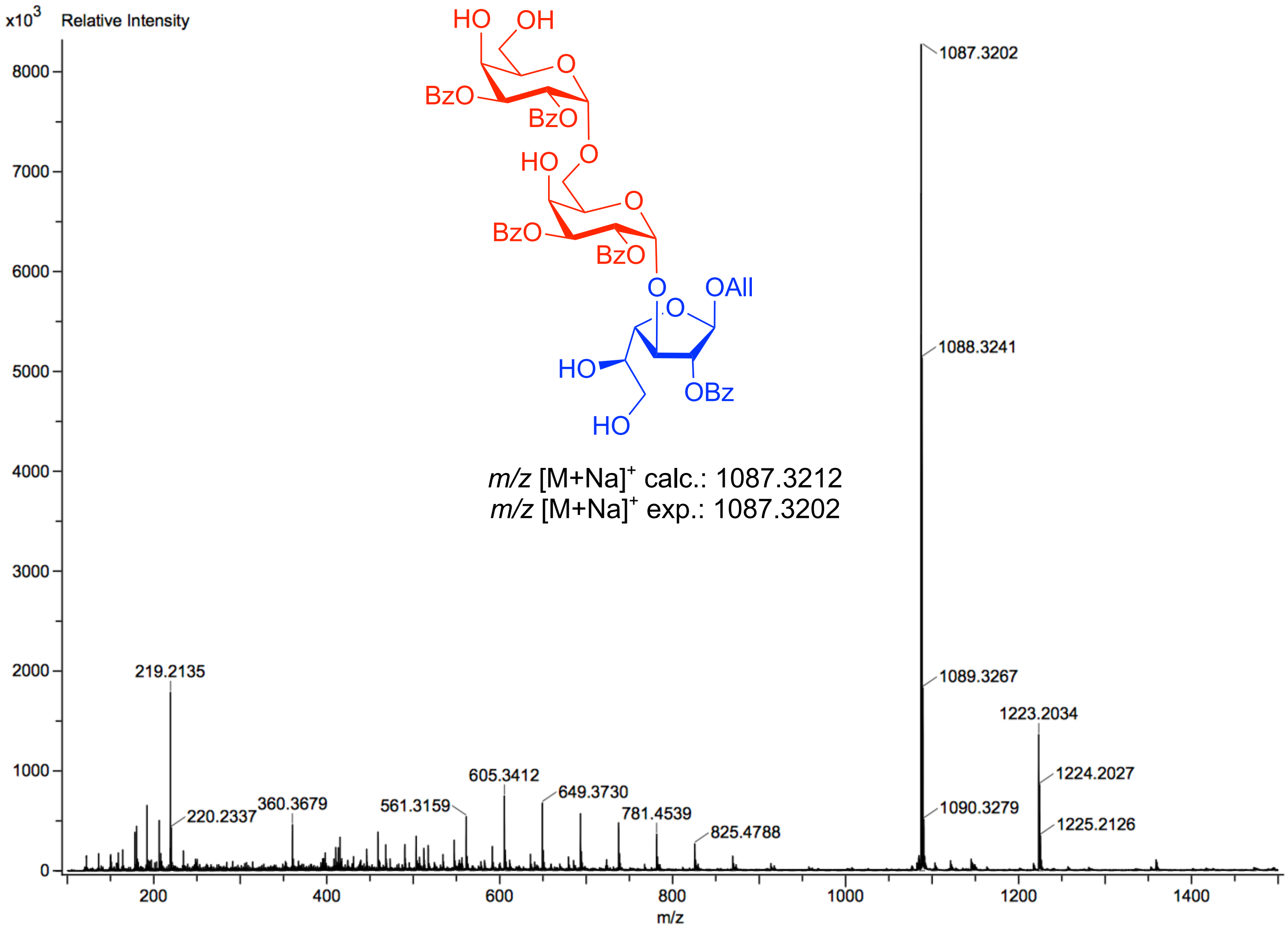




^{13}C NMR, 100 MHz, CD_3OD, compound S5

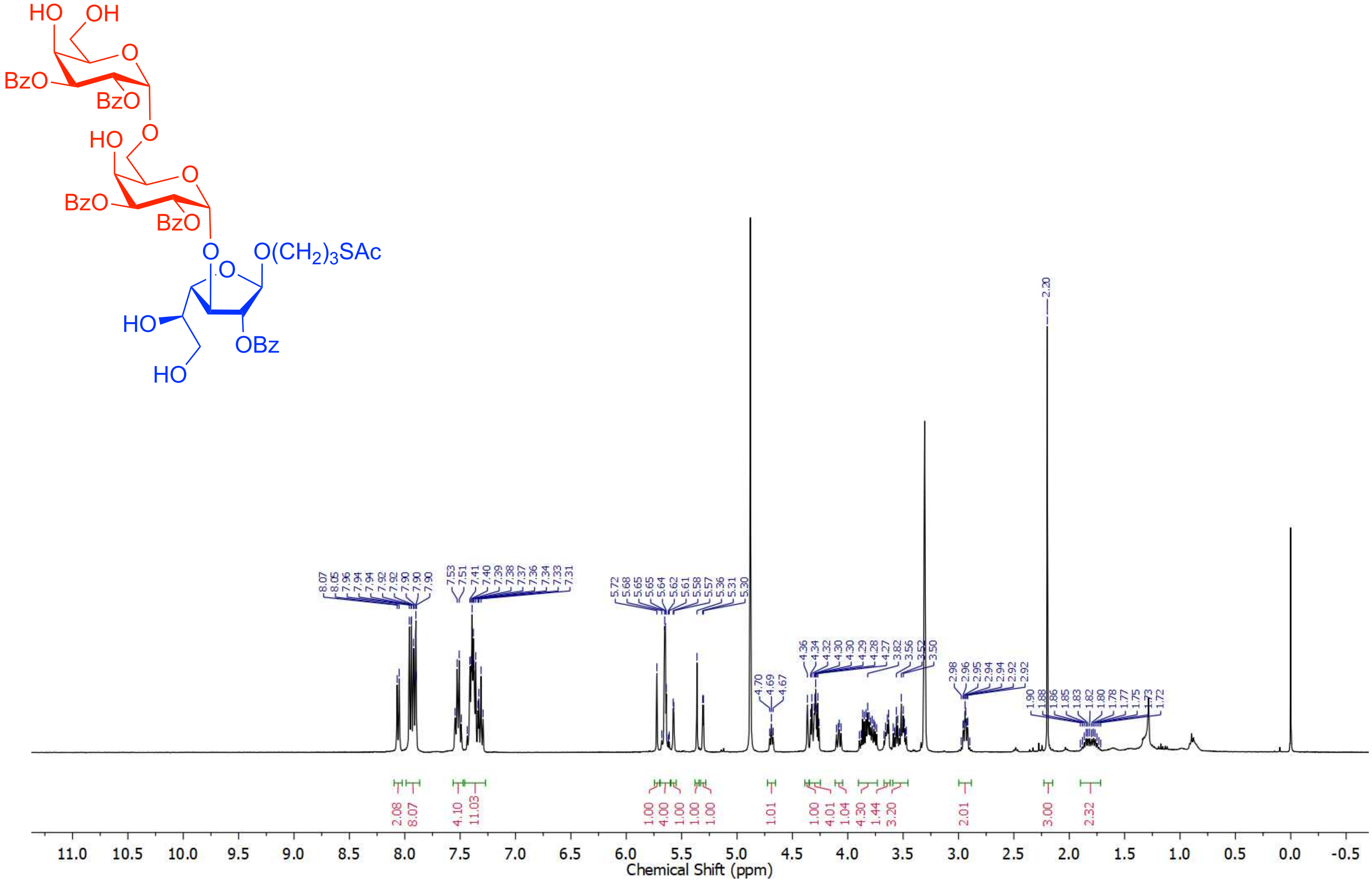


ESI-TOF HR mass spectrum of compound S5

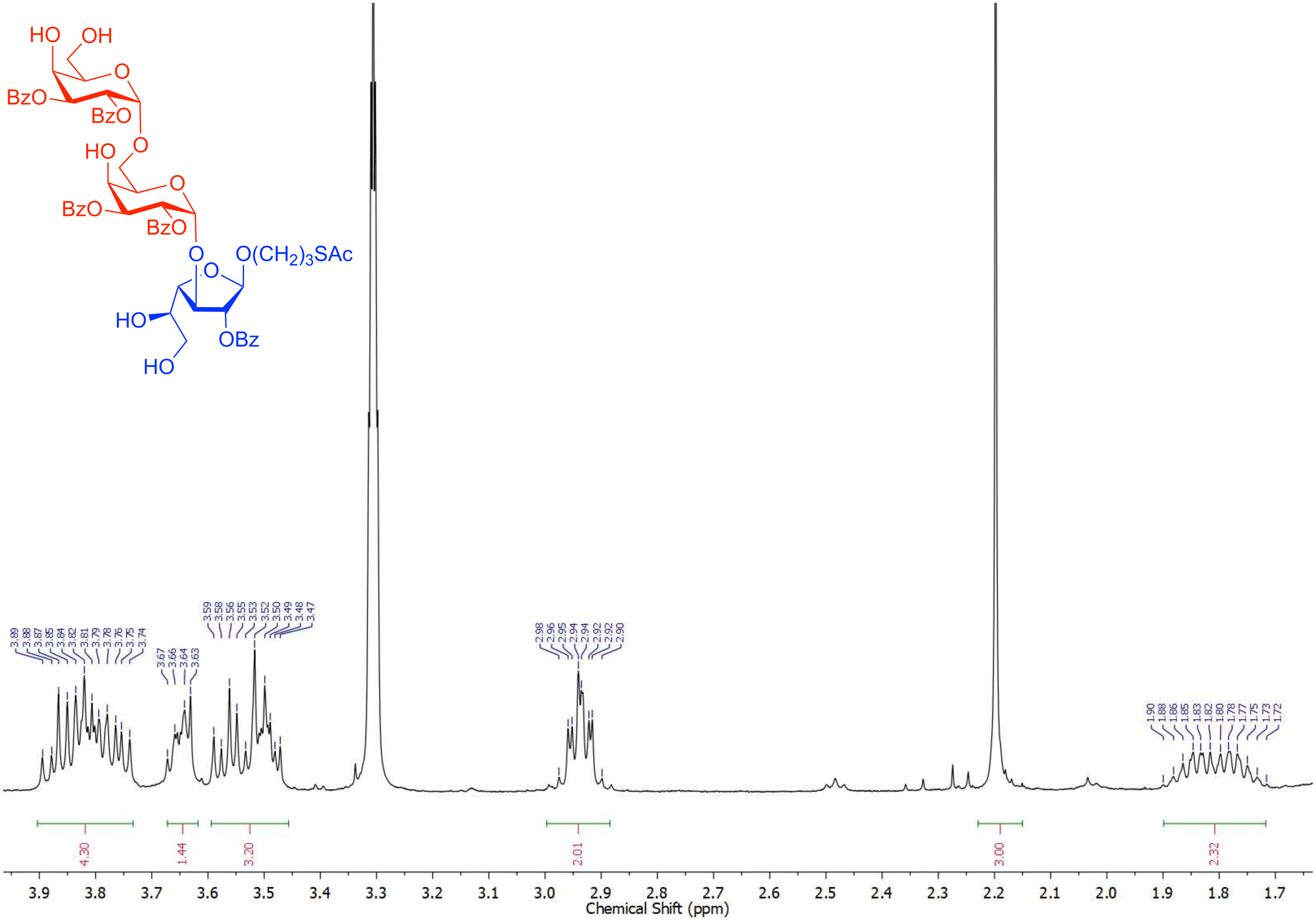


m/z [M+Na]$^+$ calc.: 1087.3212
m/z [M+Na]$^+$ exp.: 1087.3202

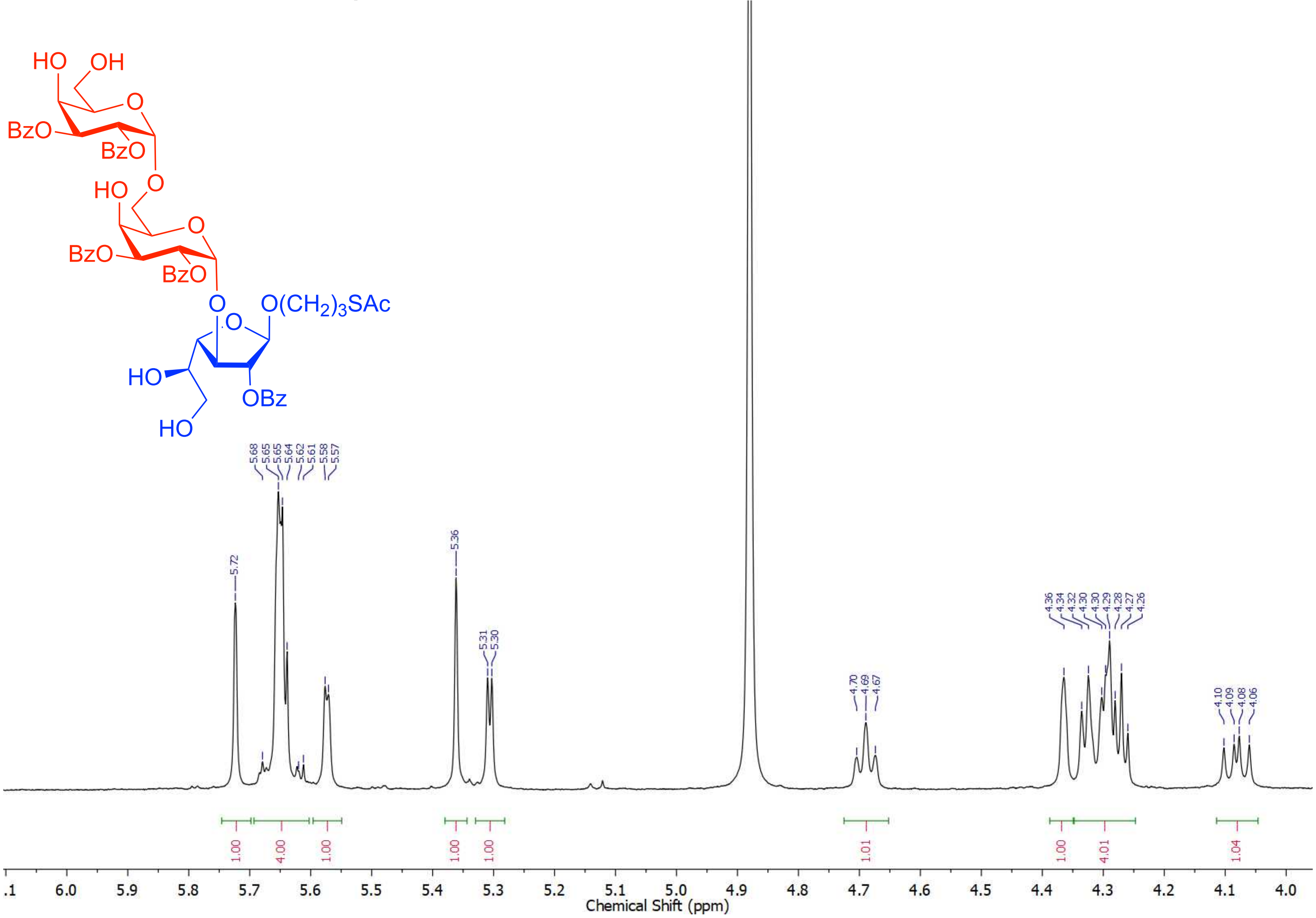
^{1}H NMR, 400 MHz, CD_3OD, compound S6



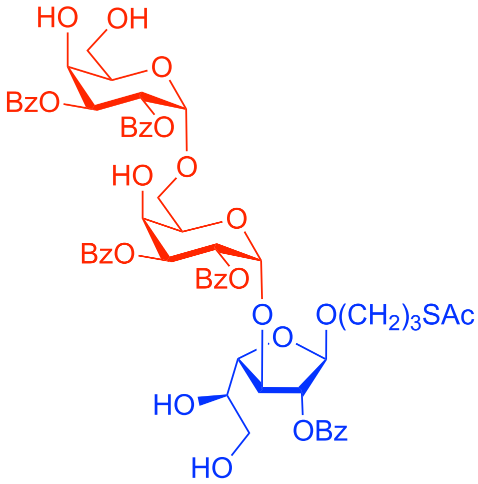
^{1}H NMR, 400 MHz, CD$_3$OD, compound S6

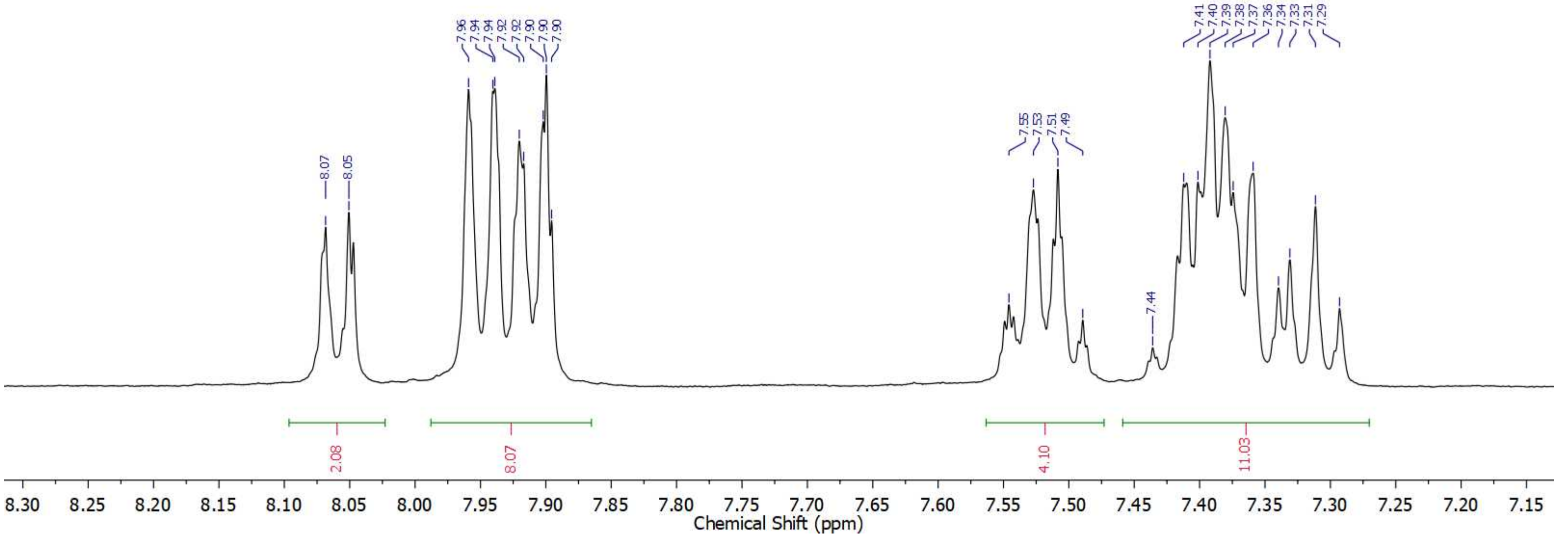


^{1}H NMR, 400 MHz, CD_3OD, compound S6

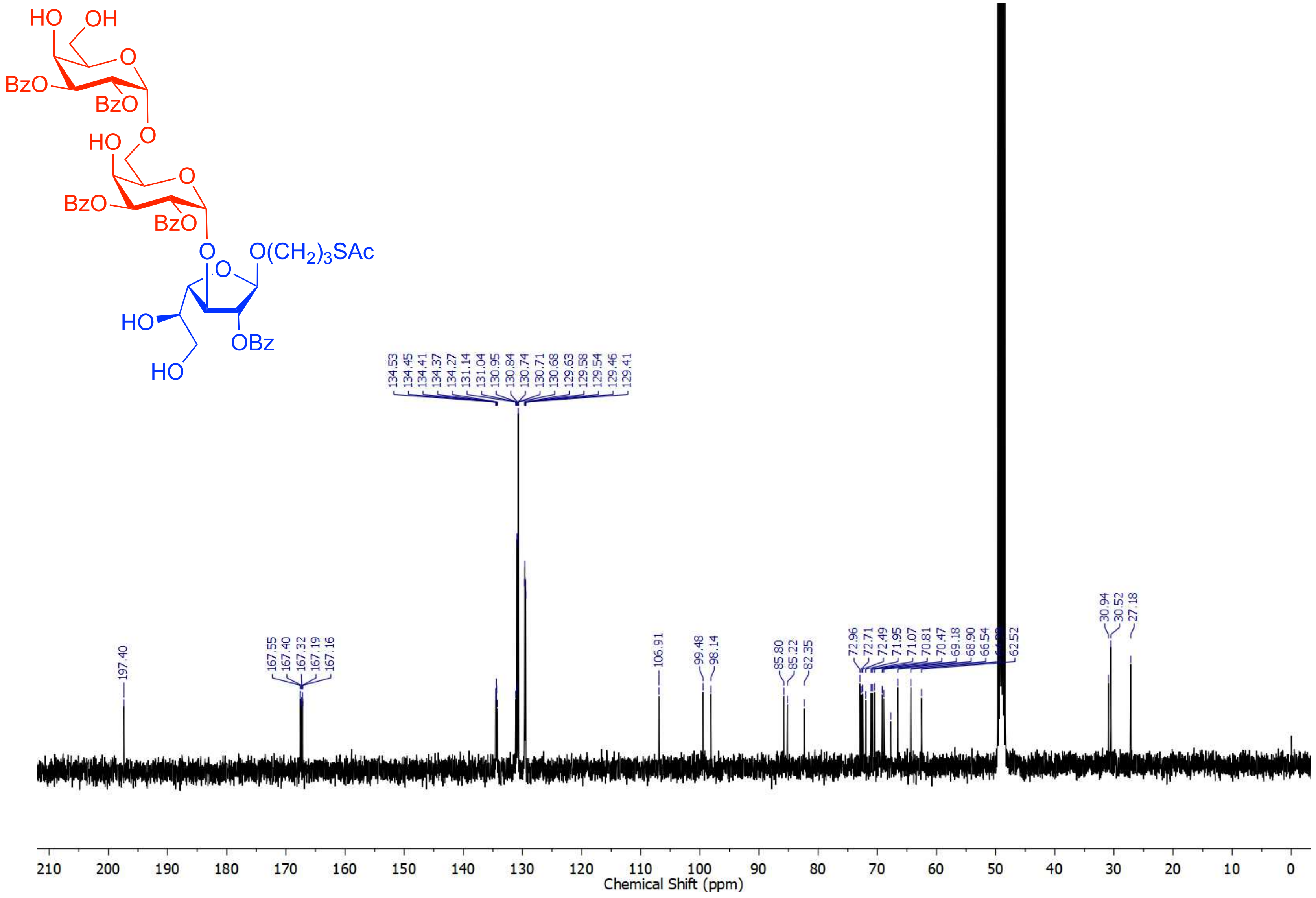


^{1}H NMR, 400 MHz, CD$_3$OD, compound S6

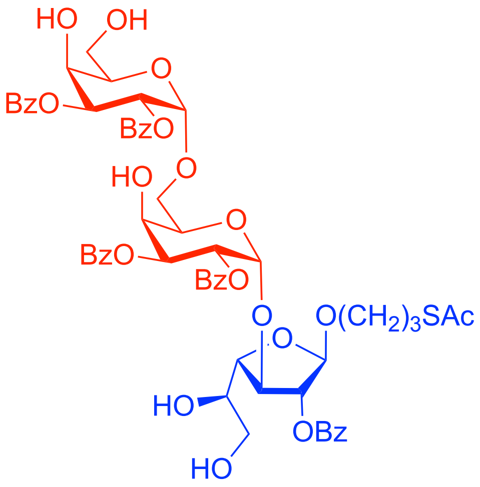


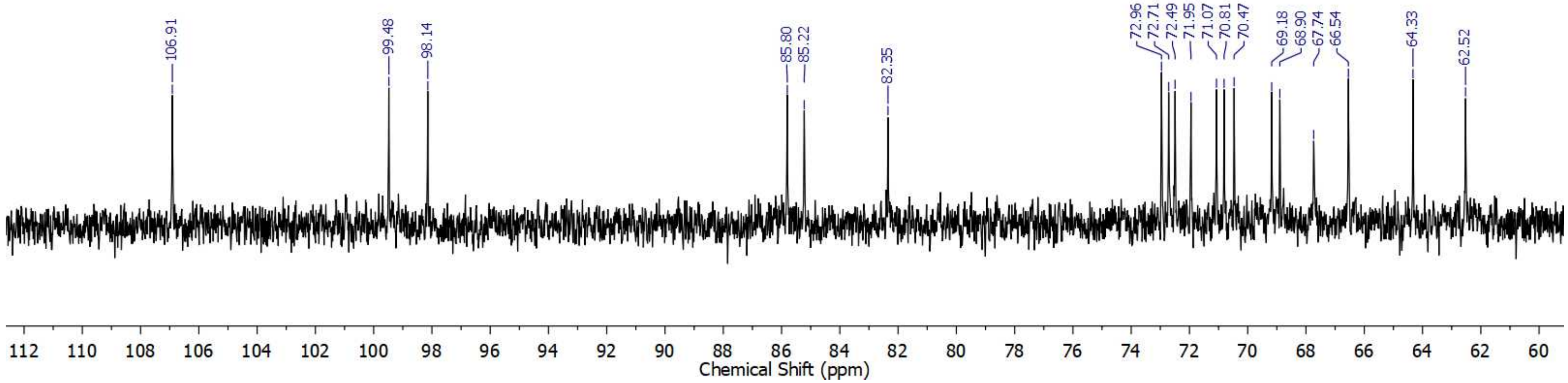


^{13}C NMR, 100 MHz, CD_3OD, compound S6

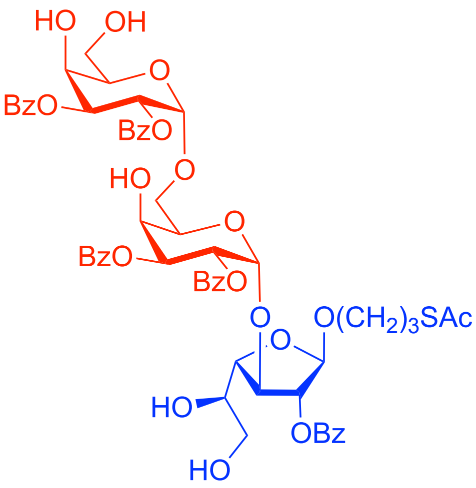


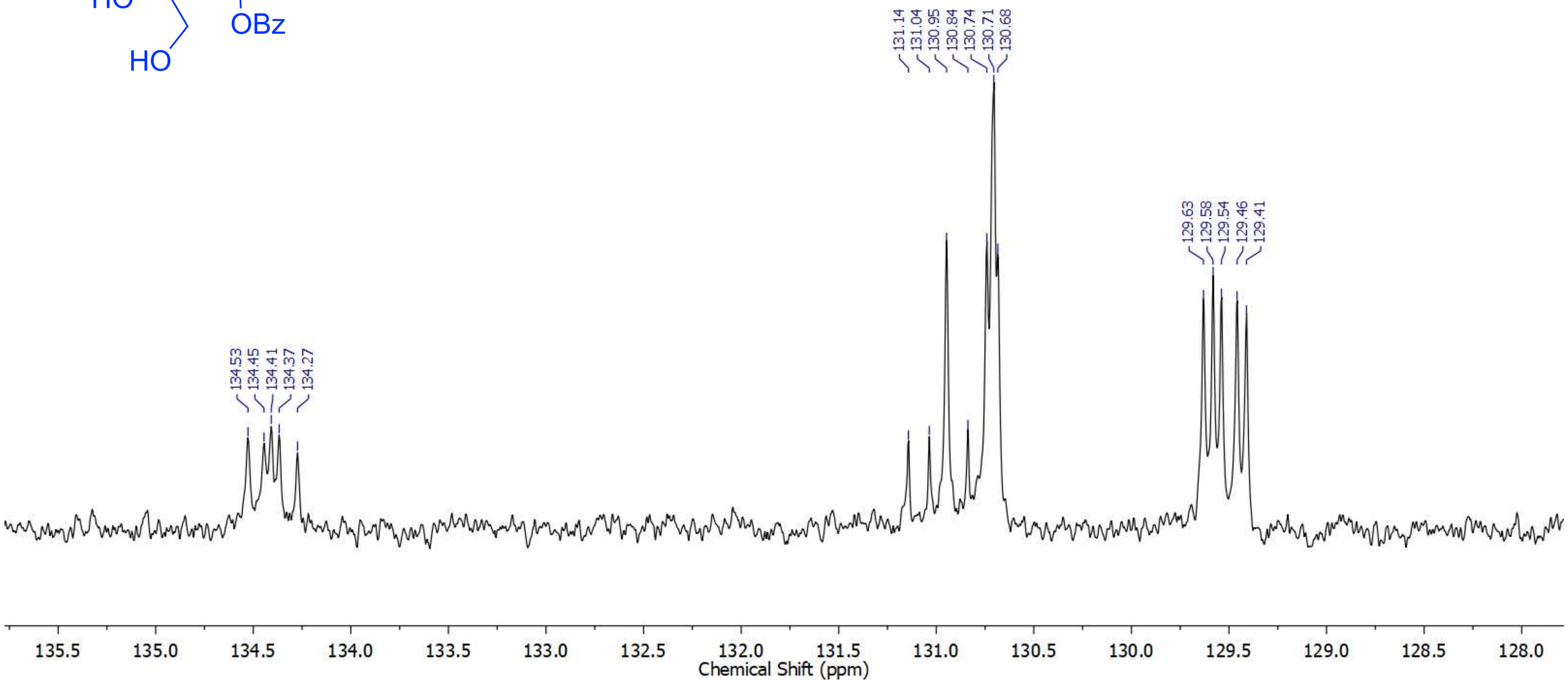
^{13}**C NMR, 100 MHz, CD$_3$OD, compound S6**



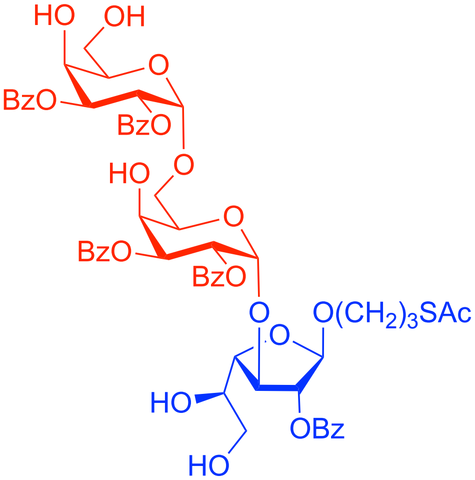


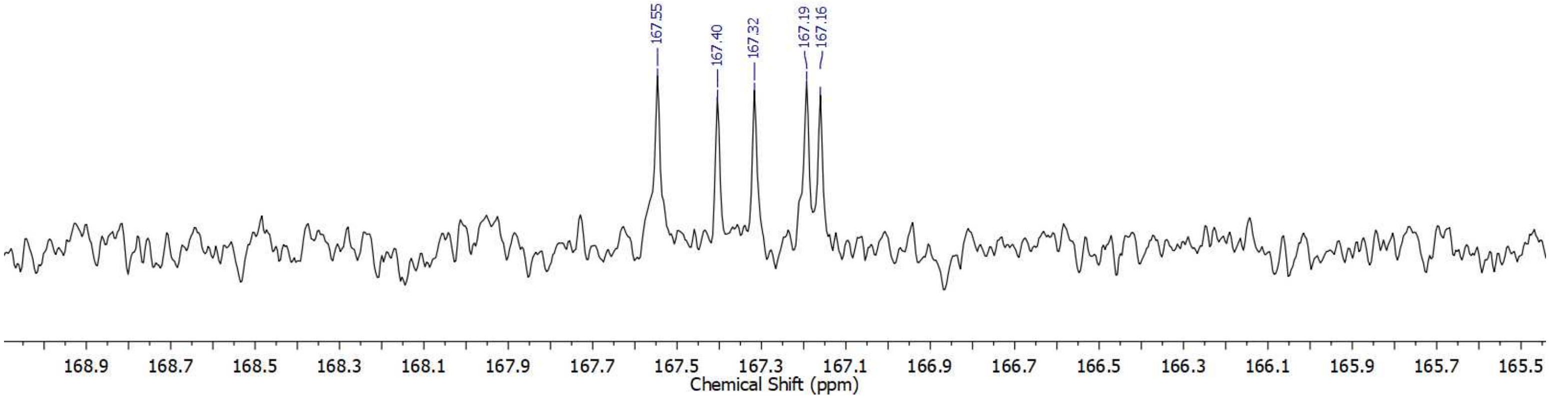
^{13}C NMR, 100 MHz, CD$_3$OD, compound S6



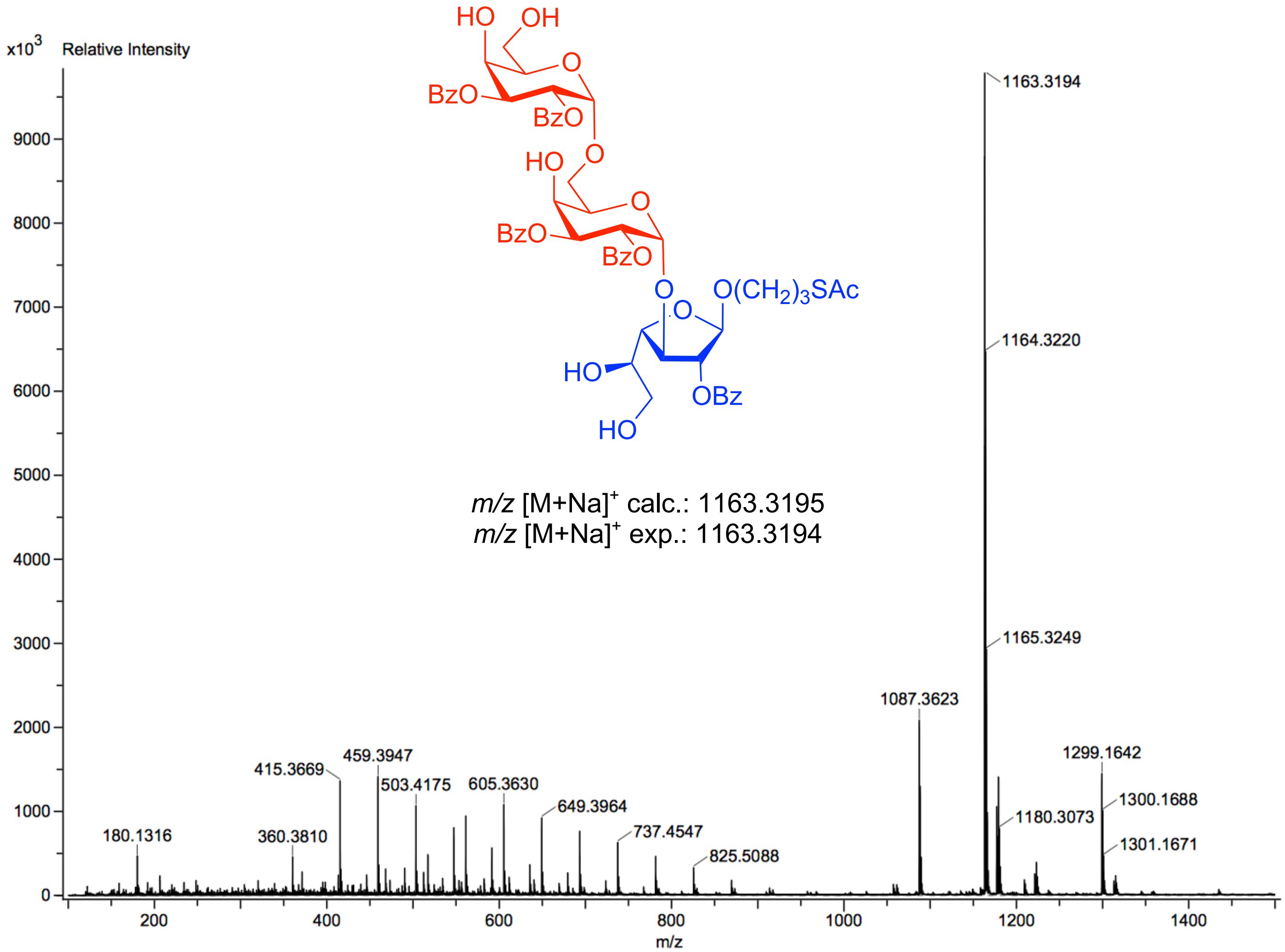


^{13}C NMR, 100 MHz, CD_3OD, compound S6



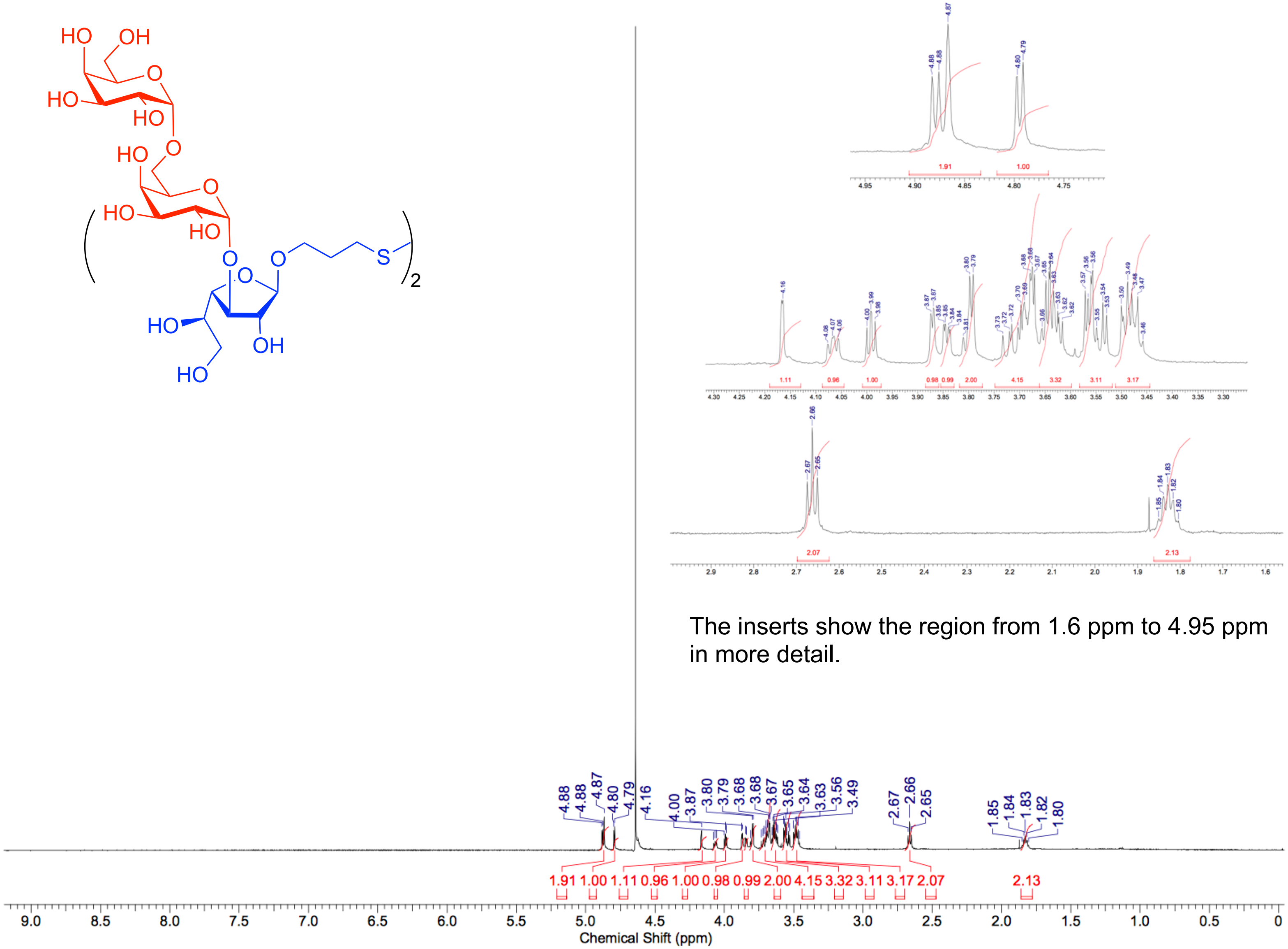


ESI-TOF HR mass spectrum of compound S6



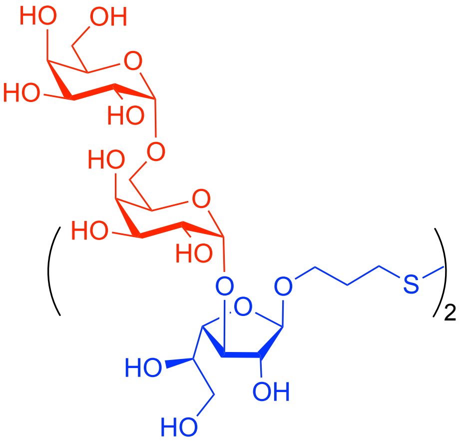
m/z [M+Na]$^+$ calc.: 1163.3195
m/z [M+Na]$^+$ exp.: 1163.3194

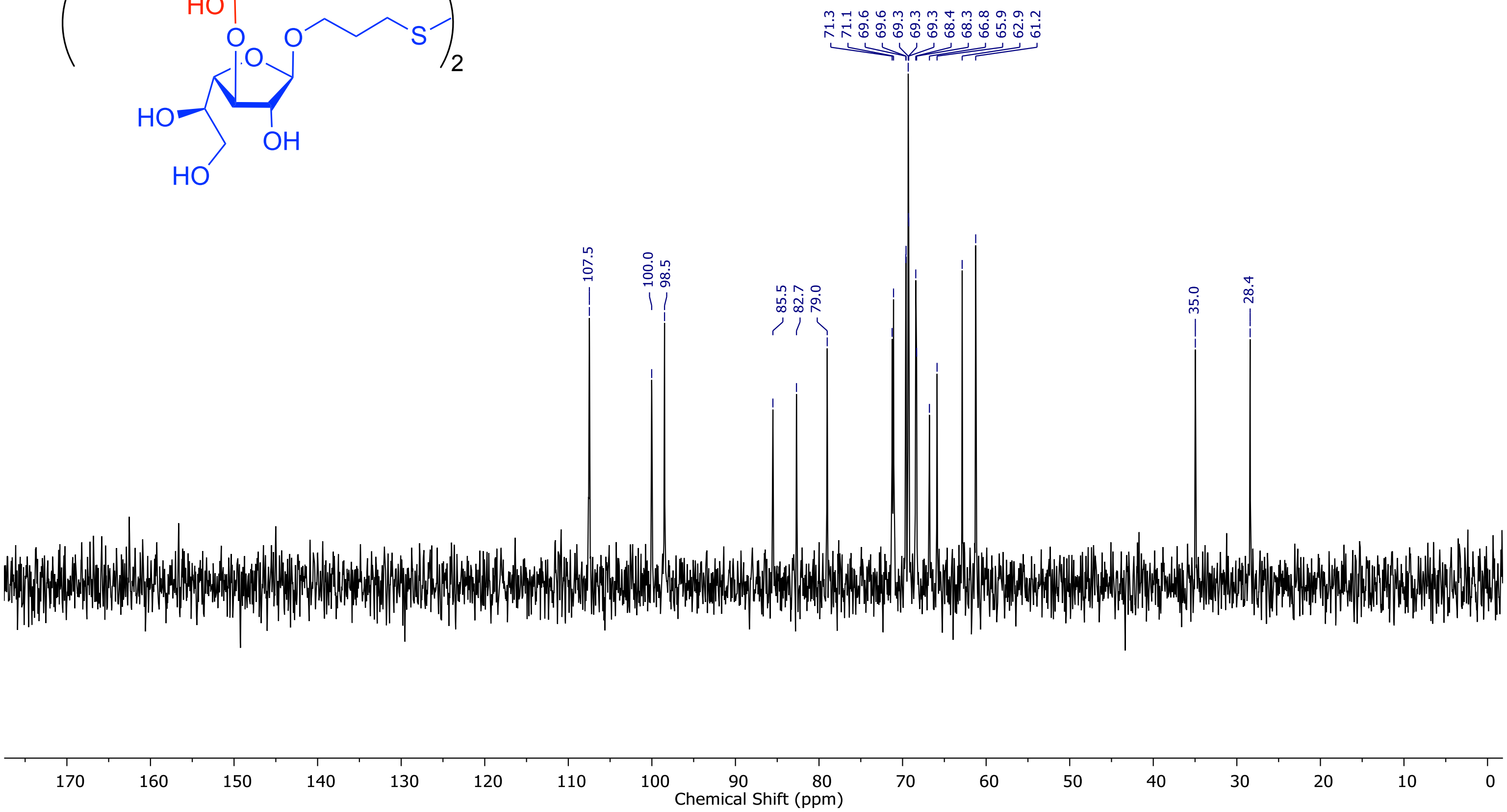
^{1}H NMR, 600 MHz, D_2O, compound $(G28_S)_2$



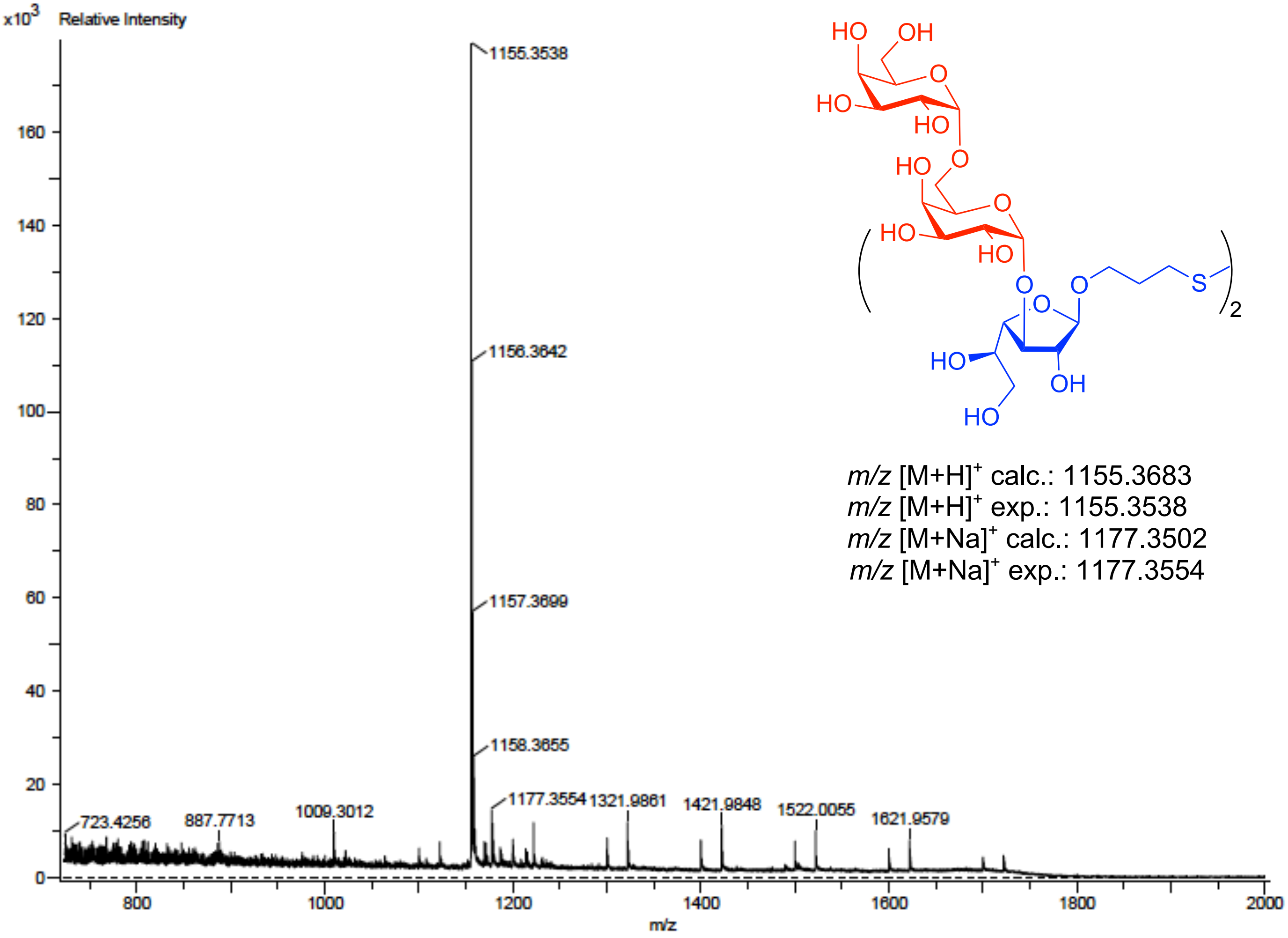
The inserts show the region from 1.6 ppm to 4.95 ppm in more detail.

^{13}C NMR, 150 MHz, D_2O, compound $(G28_S)_2$



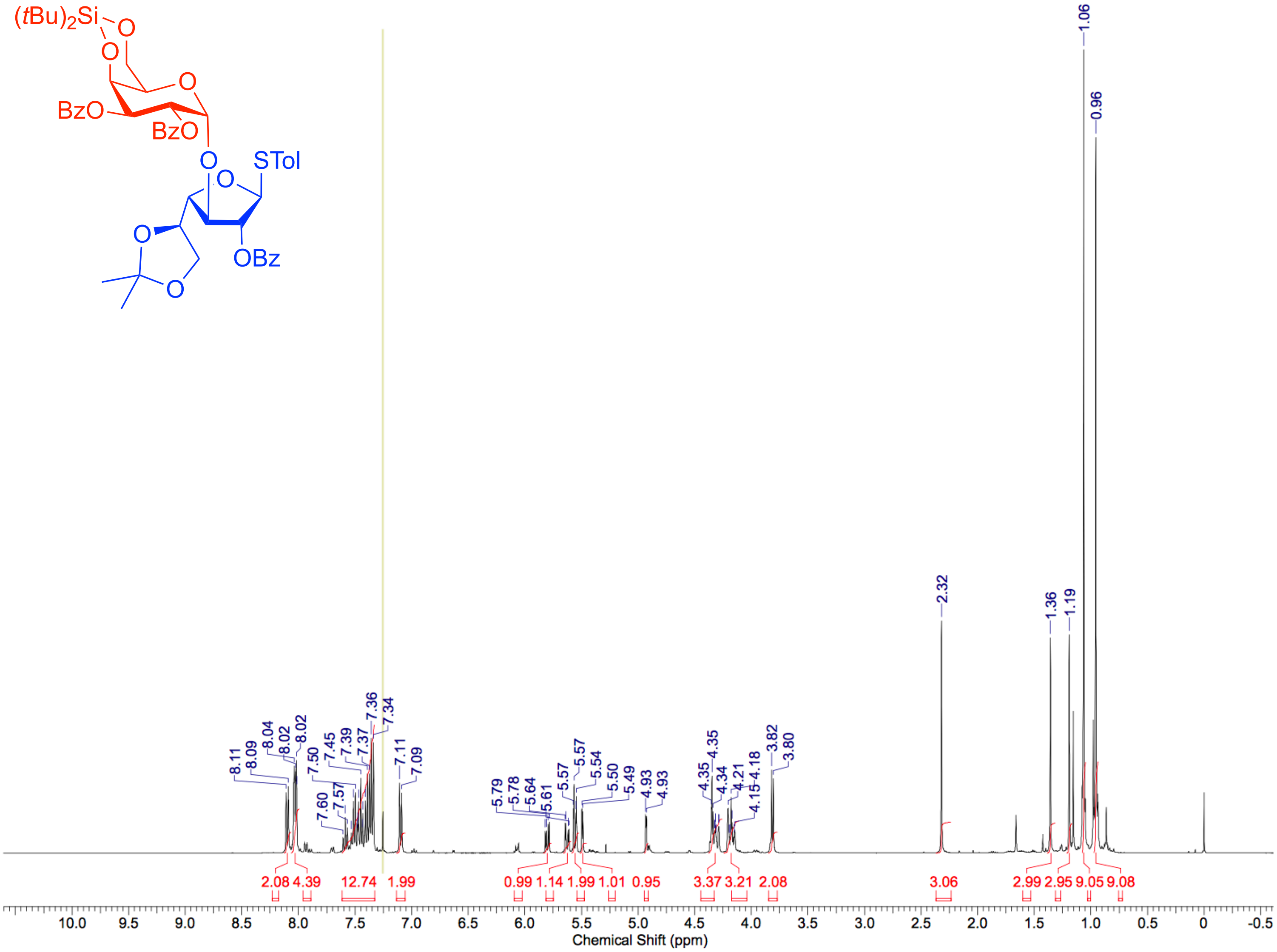


ESI-TOF HR mass spectrum of compound $(G28_S)_2$

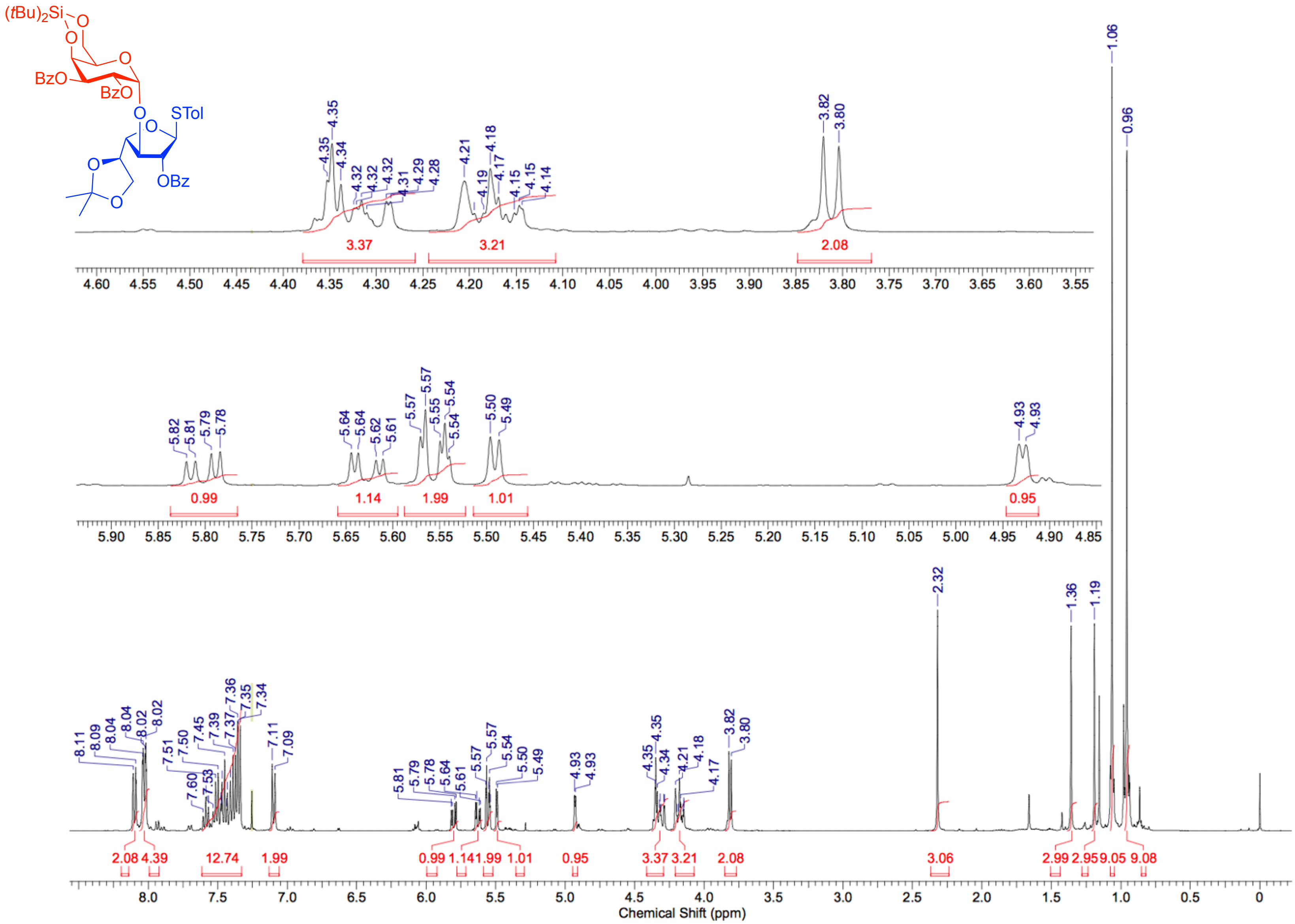


m/z $[M+H]^+$ calc.: 1155.3683
m/z $[M+H]^+$ exp.: 1155.3538
m/z $[M+Na]^+$ calc.: 1177.3502
m/z $[M+Na]^+$ exp.: 1177.3554

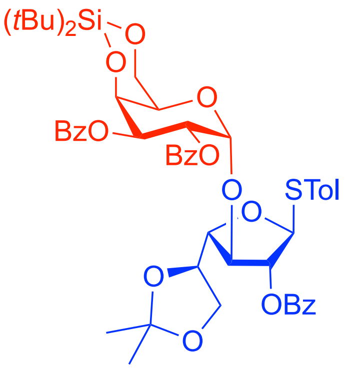
^{1}H NMR, 400 MHz, CDCl$_3$, compound 5

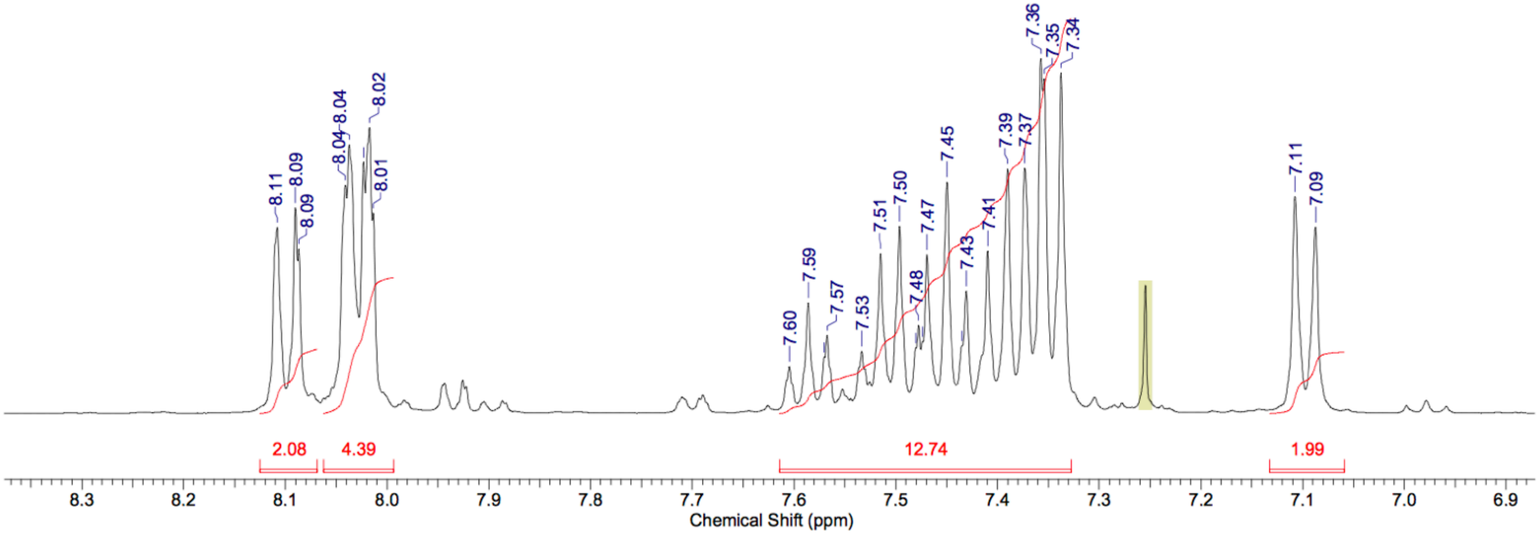


^{1}H NMR, 400 MHz, CDCl$_3$, compound 5

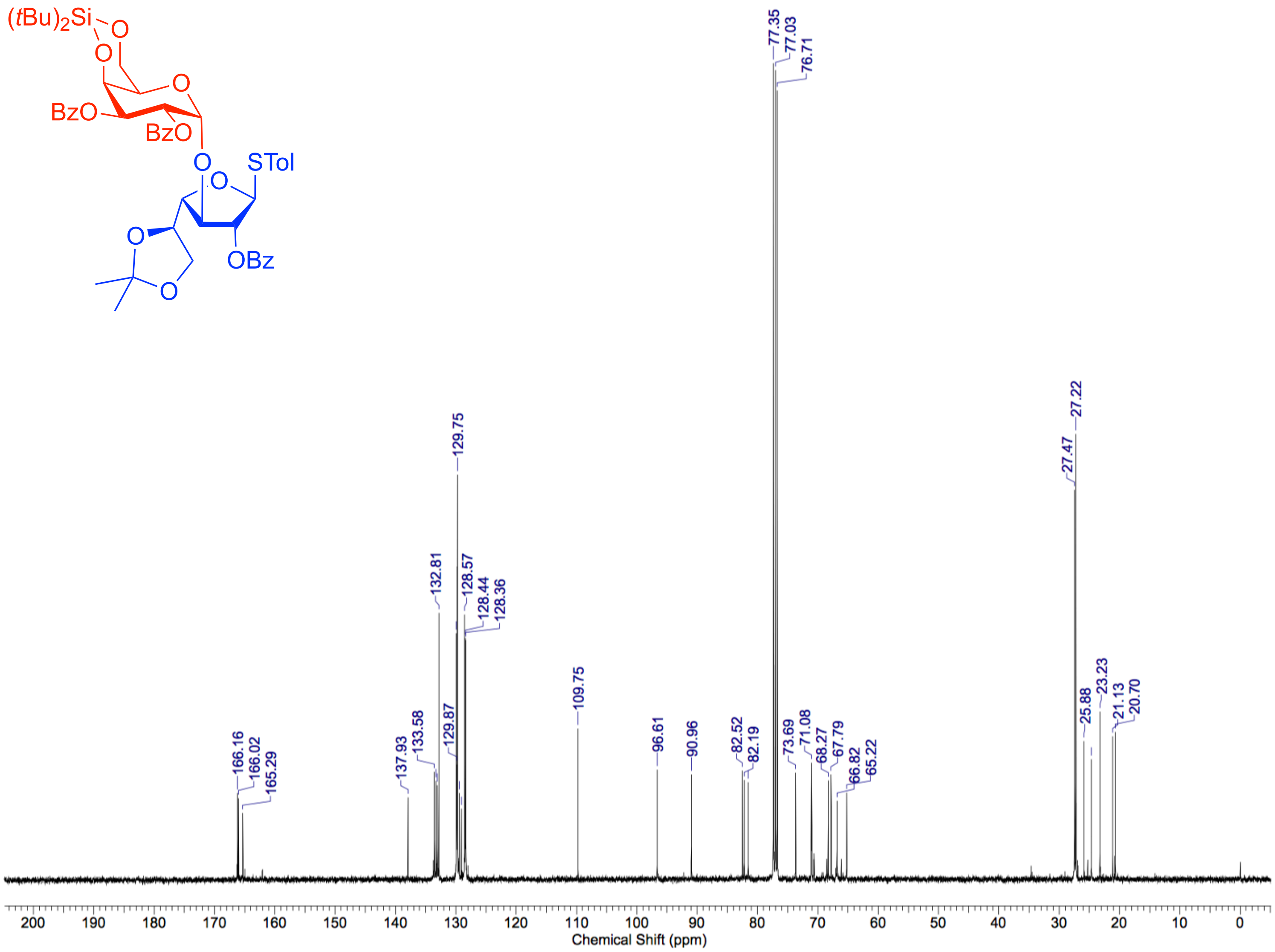


^{1}H NMR, 400 MHz, CDCl$_3$, compound 5

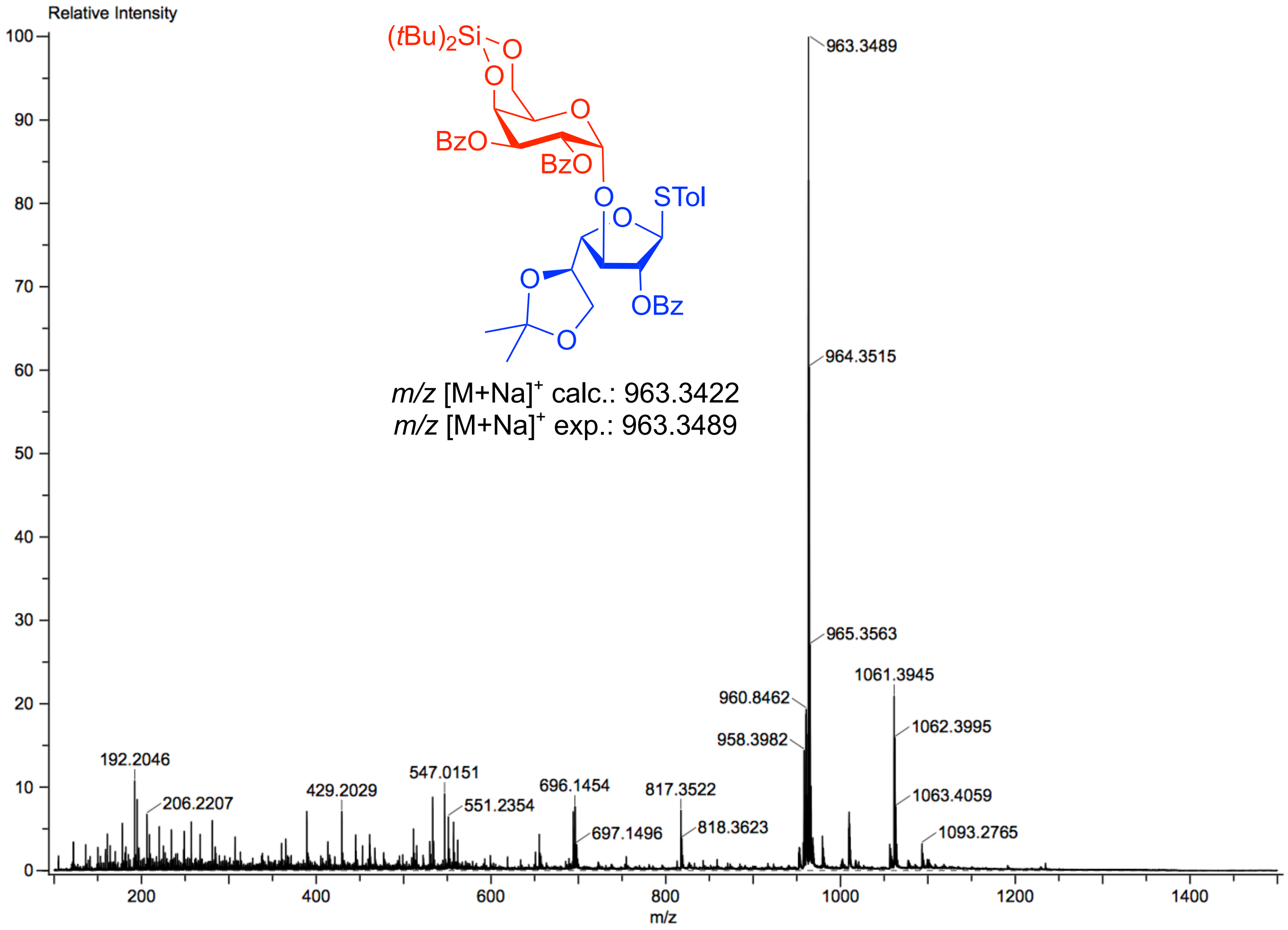




^{13}C NMR, 100 MHz, CDCl$_3$, compound 5

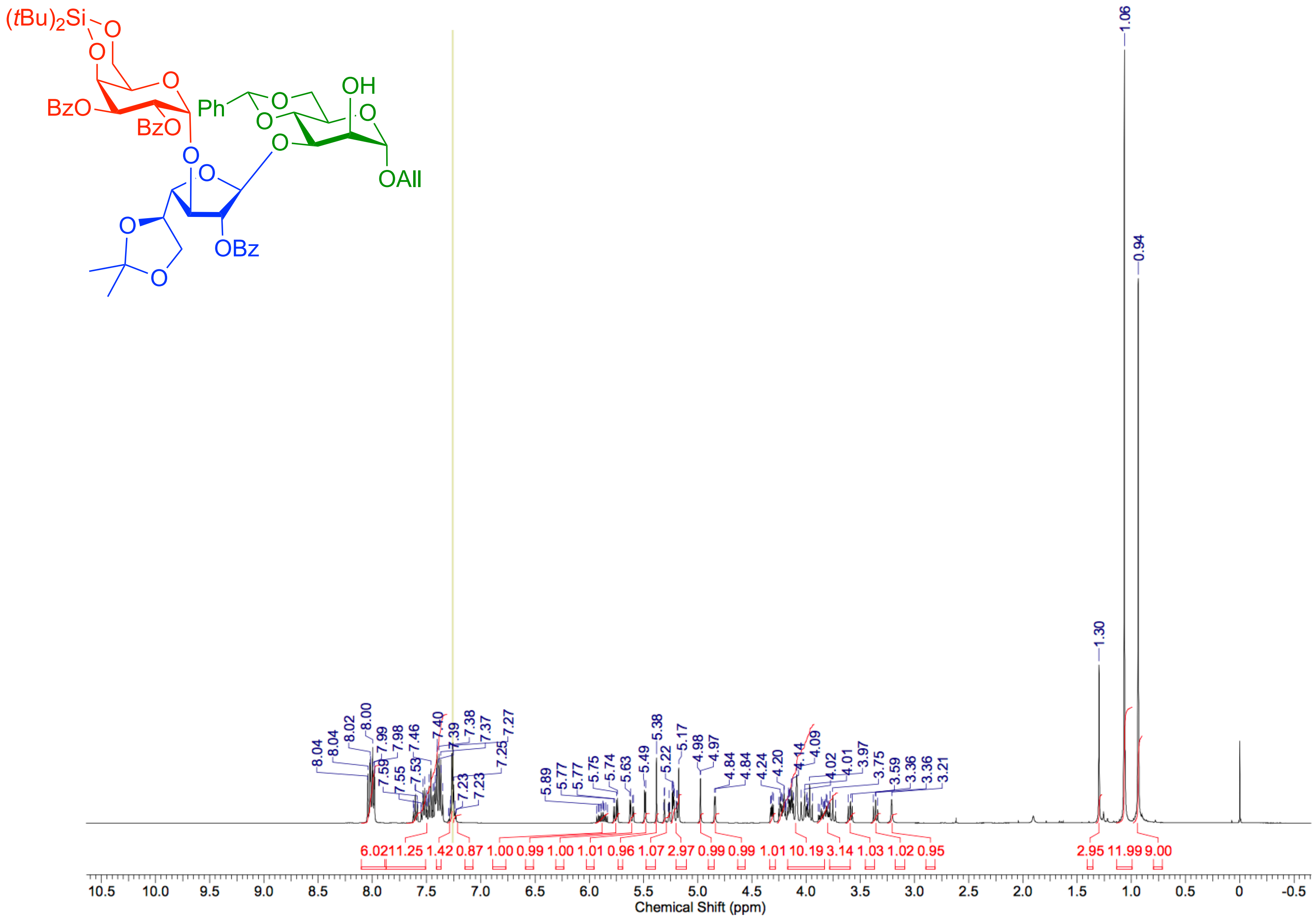


ESI-TOF HR mass spectrum of compound 5

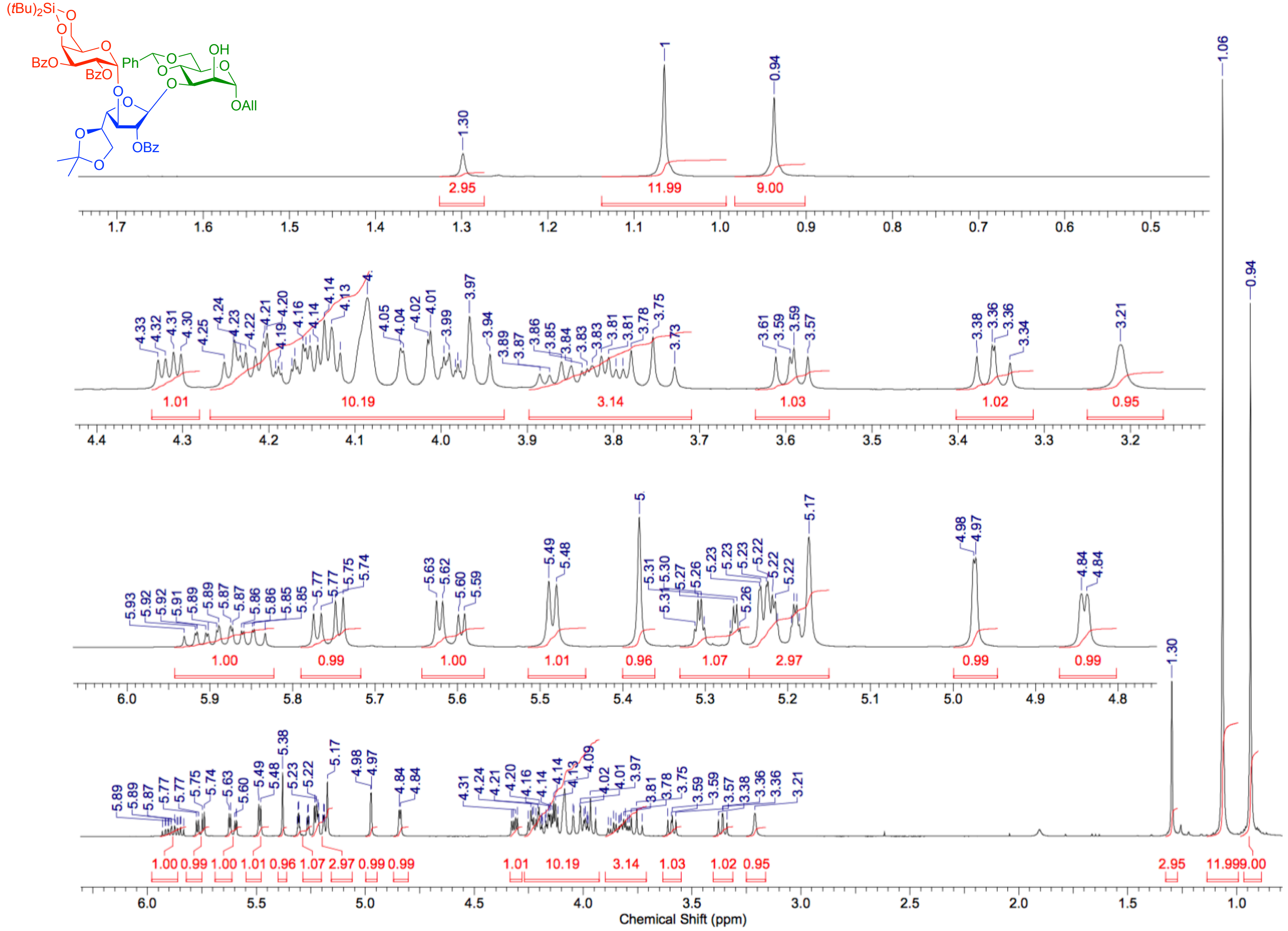


m/z $[M+Na]^+$ calc.: 963.3422
m/z $[M+Na]^+$ exp.: 963.3489

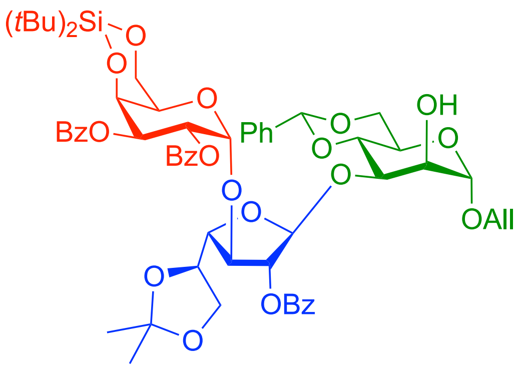
^{1}H NMR, 400 MHz, CDCl$_3$, compound 10

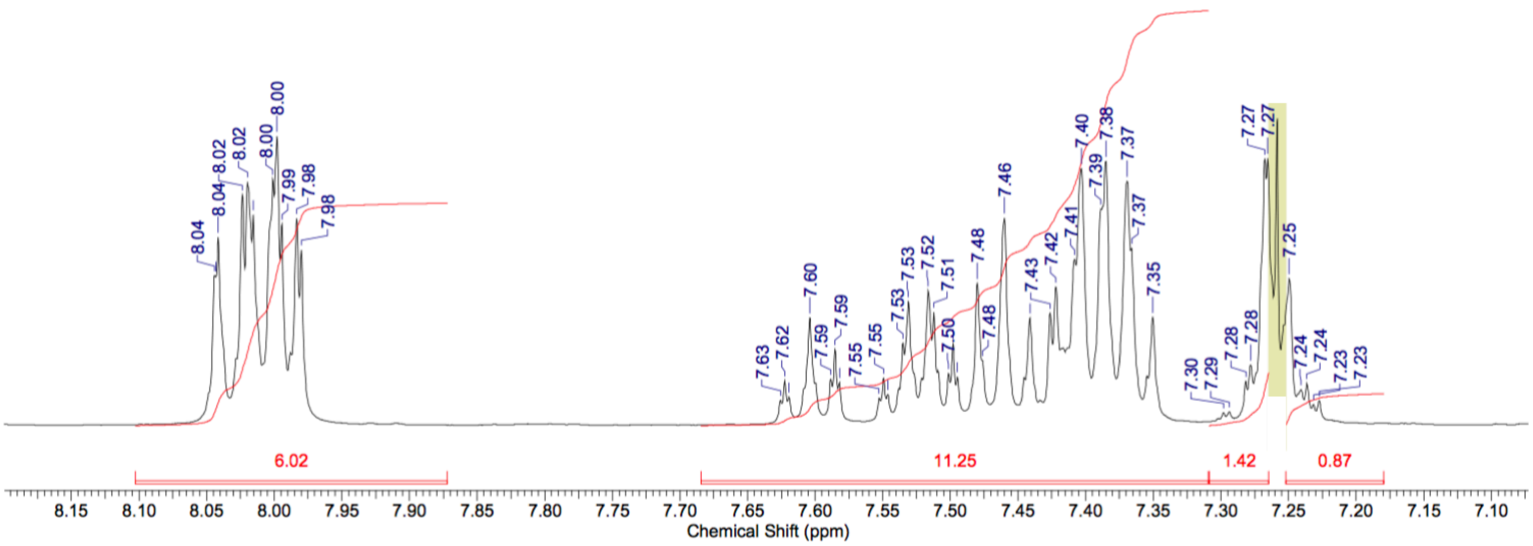


^{1}H NMR, 400 MHz, CDCl$_3$, compound 10

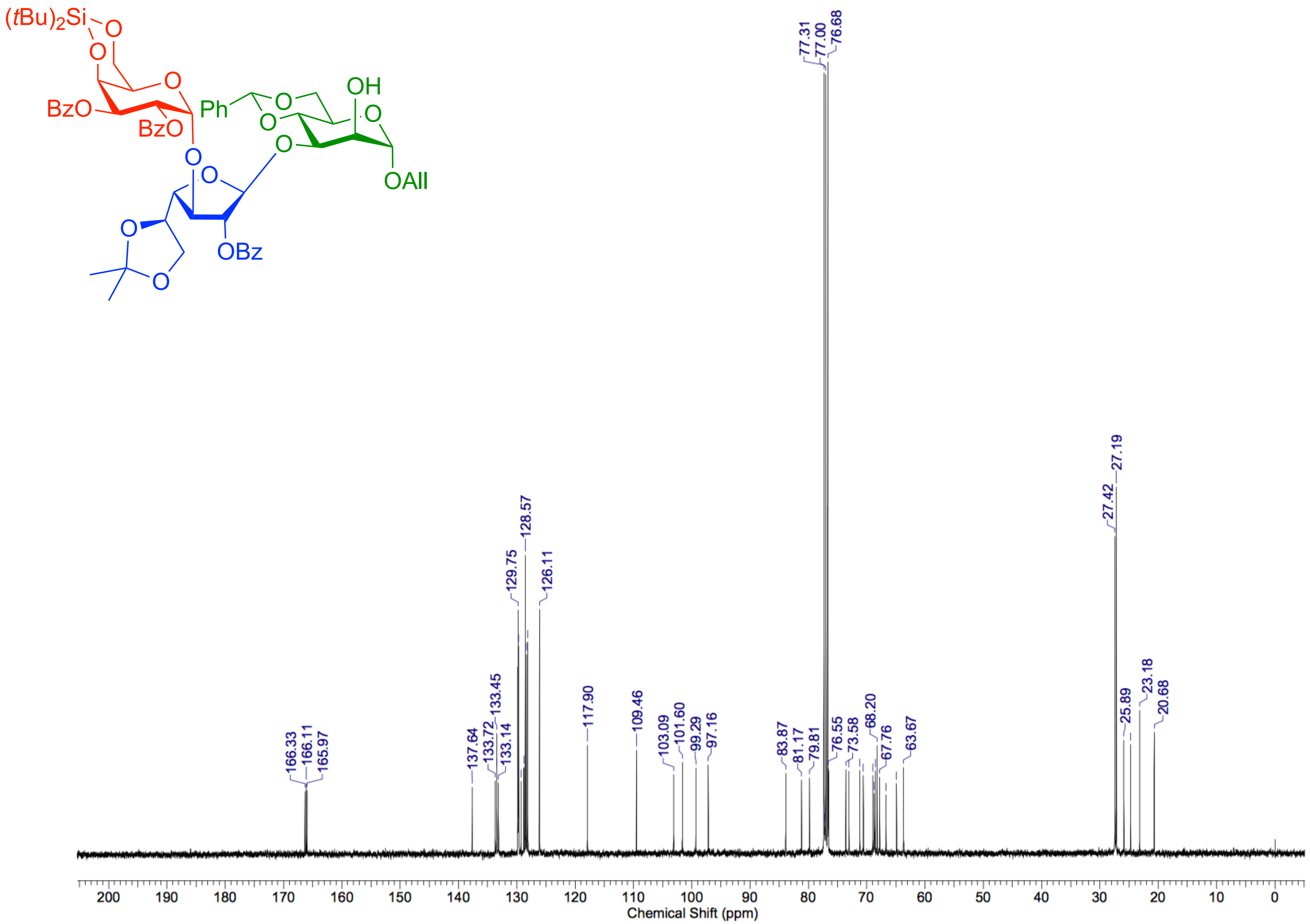


^{1}H NMR, 400 MHz, CDCl$_3$, compound 10

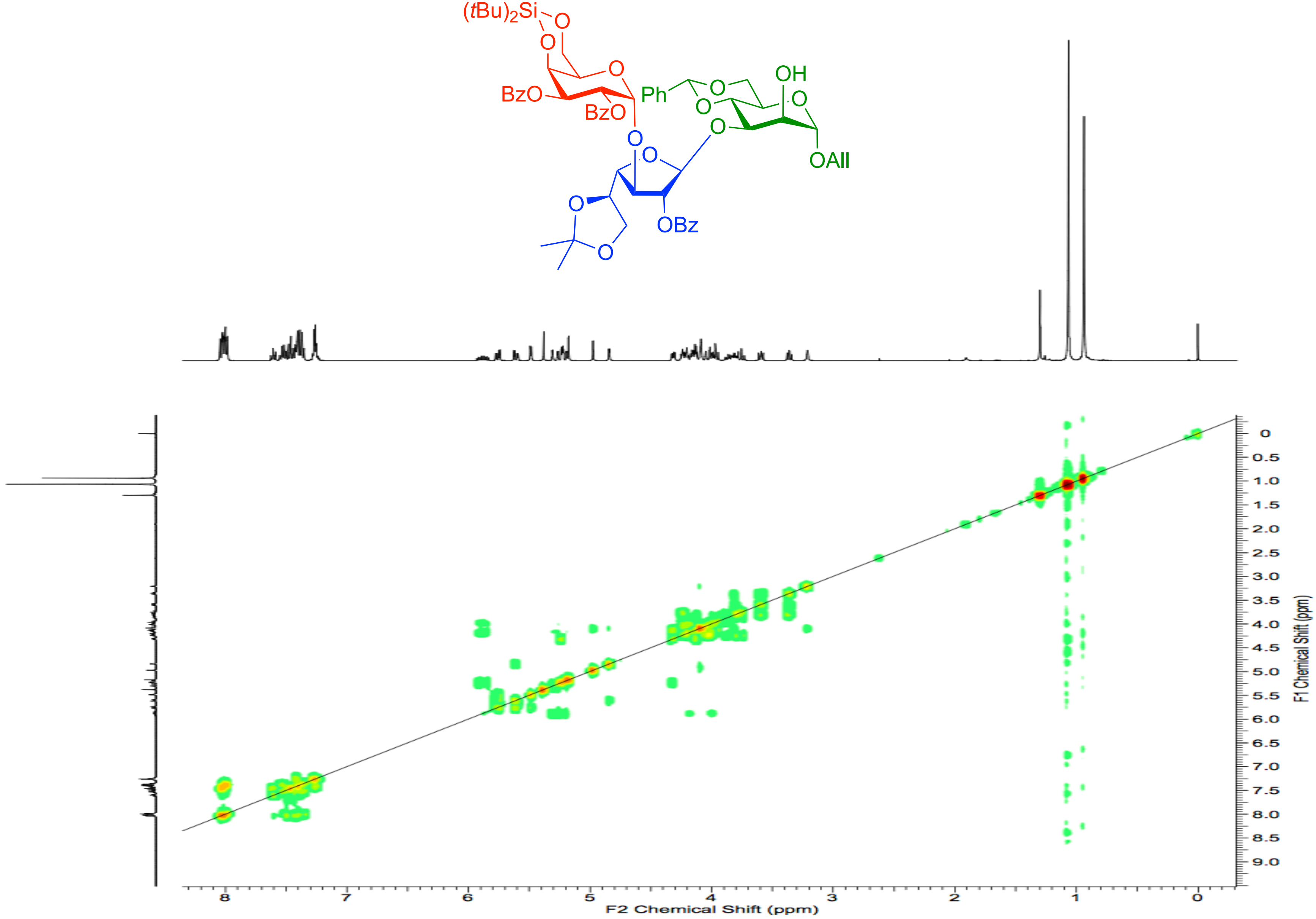




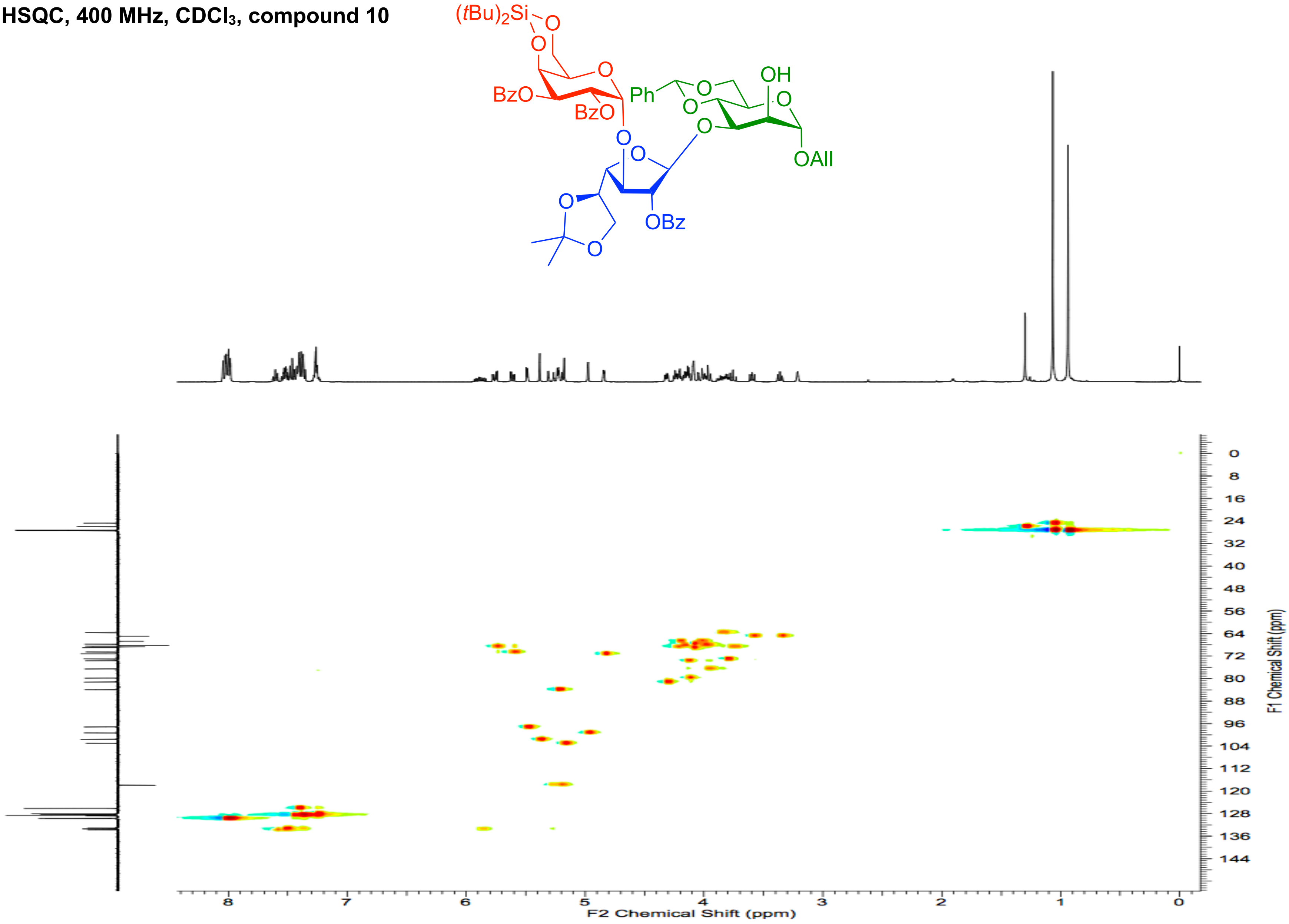
^{13}C NMR, 100 MHz, CDCl$_3$, compound 10



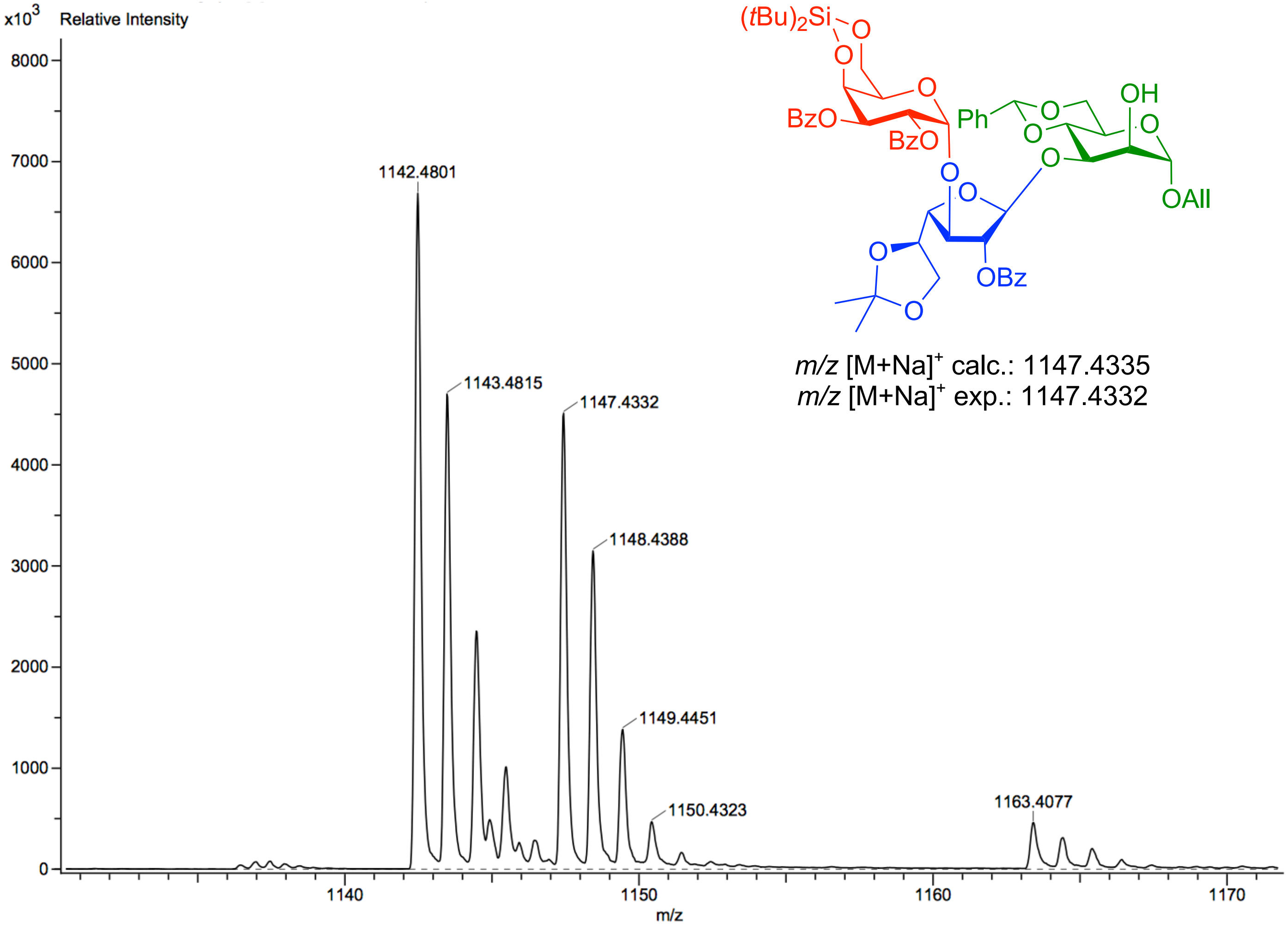
COSY, 400 MHz, $CDCl_3$, compound 10



HSQC, 400 MHz, CDCl$_3$, compound 10

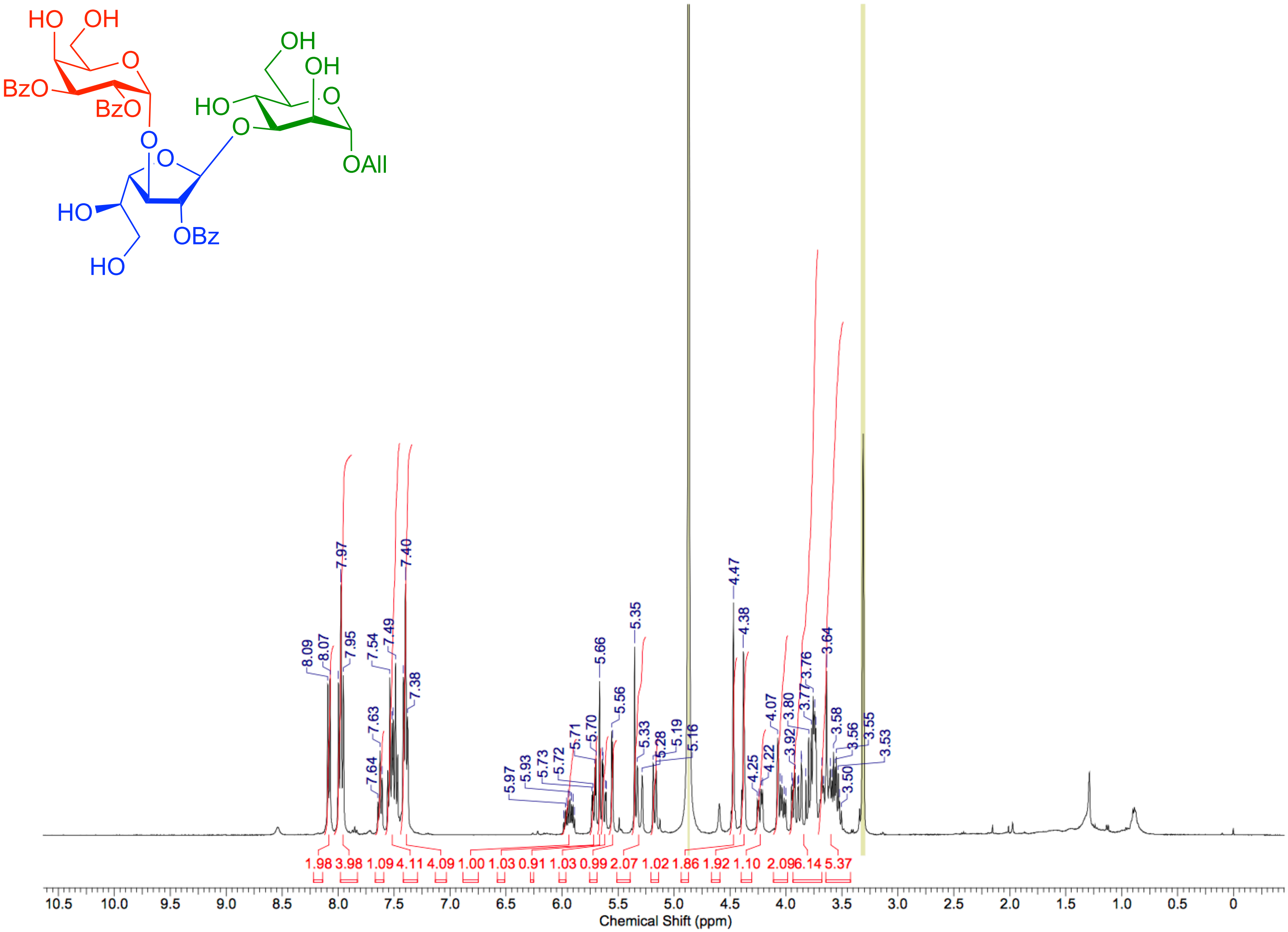


ESI-TOF HR mass spectrum of compound 10

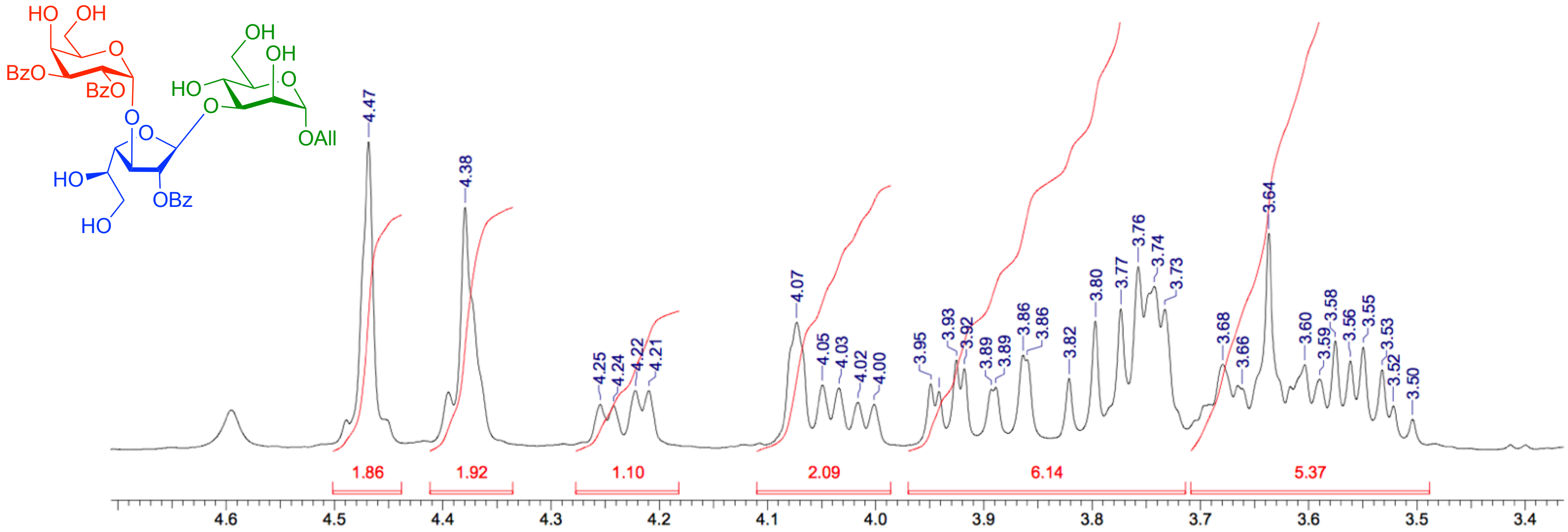


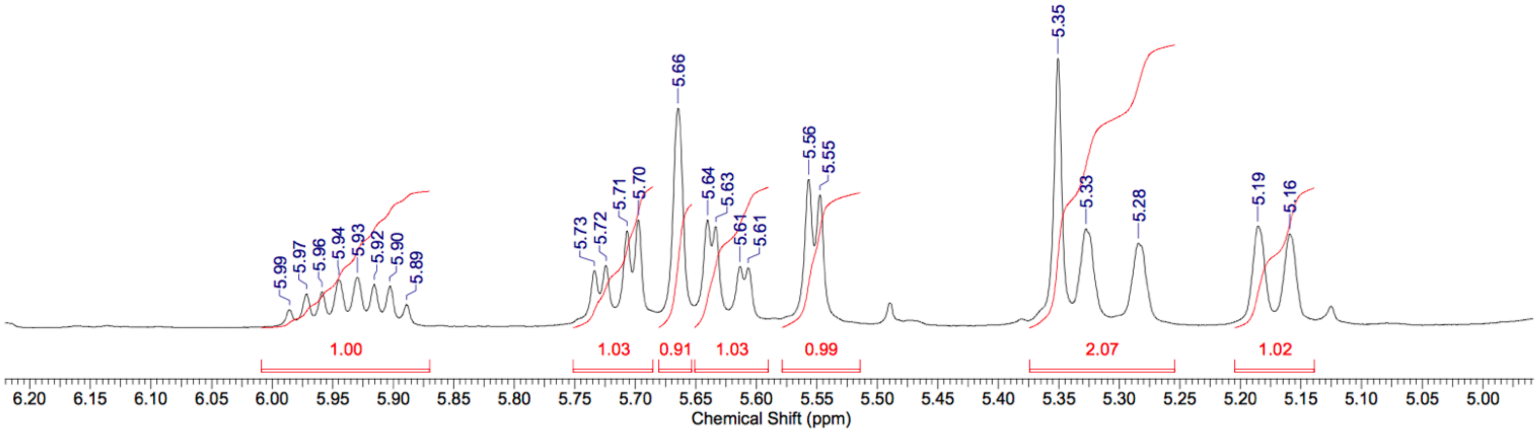
m/z [M+Na]$^+$ calc.: 1147.4335
m/z [M+Na]$^+$ exp.: 1147.4332

^{1}H NMR, 400 MHz, CD$_3$OD, compound S7

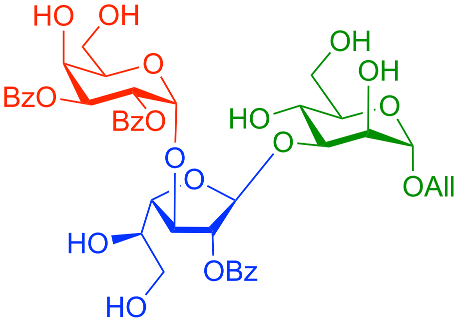


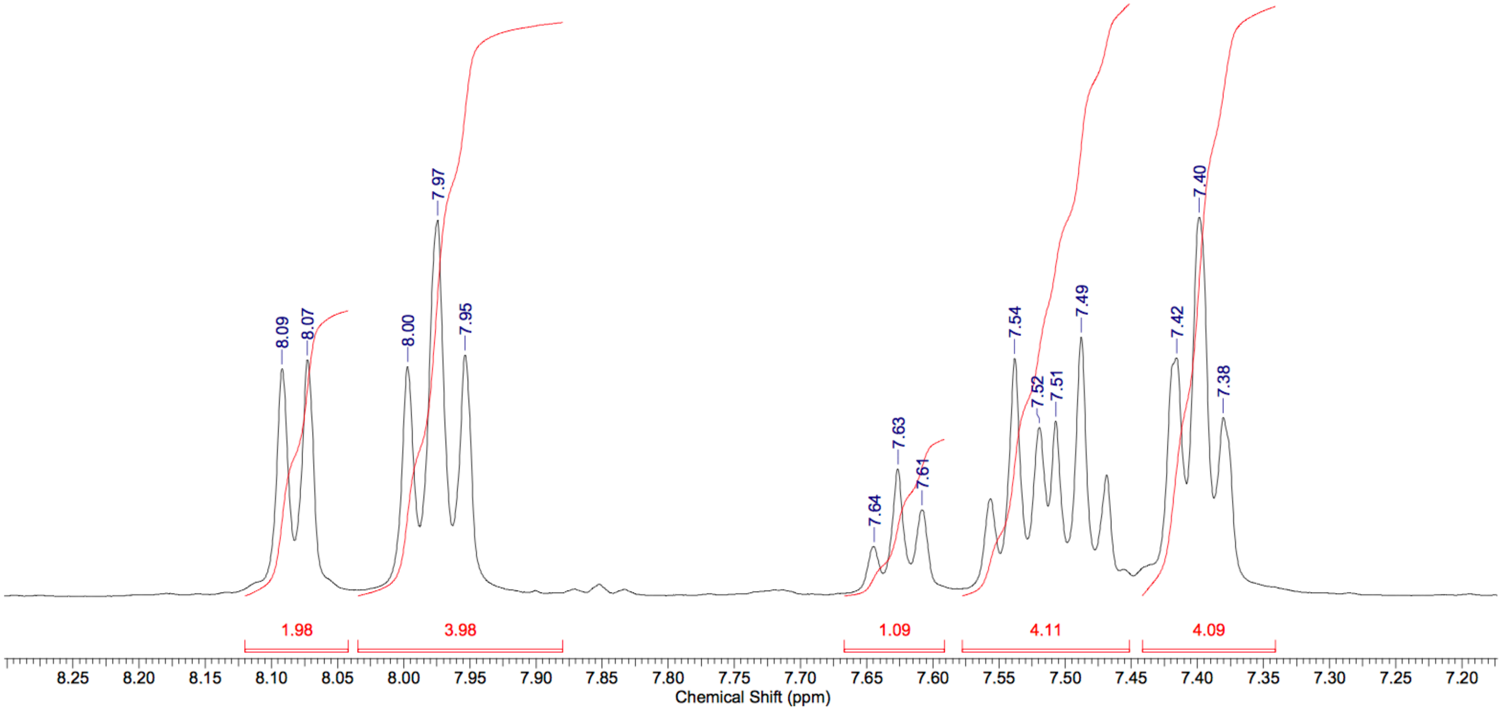
^{1}H NMR, 400 MHz, CD$_3$OD, compound S7



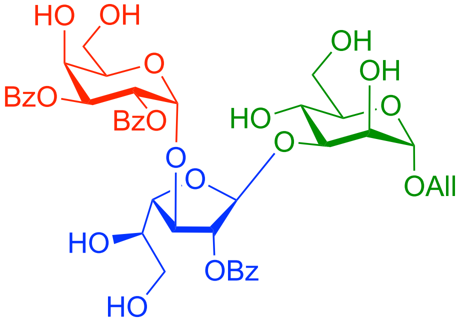


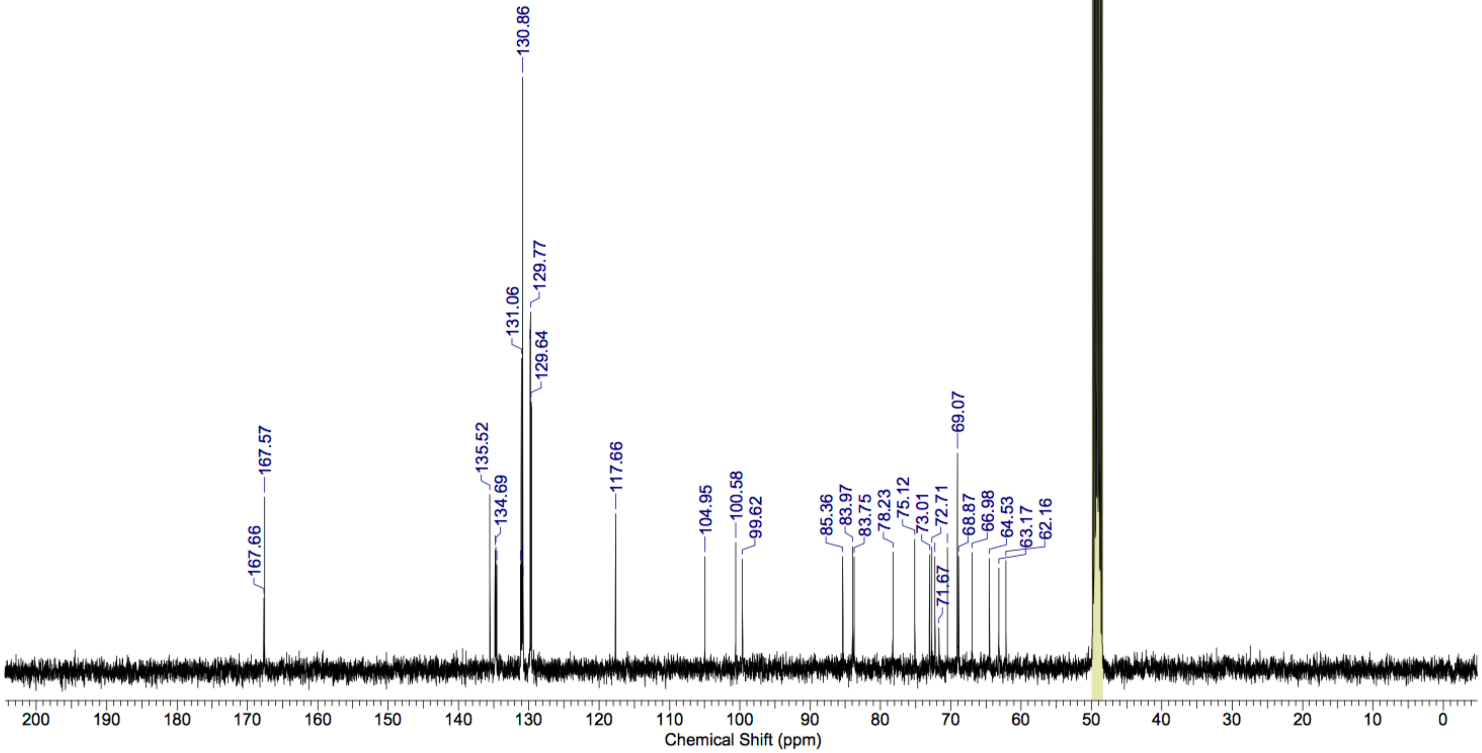
^{1}H NMR, 400 MHz, CD$_3$OD, compound S7



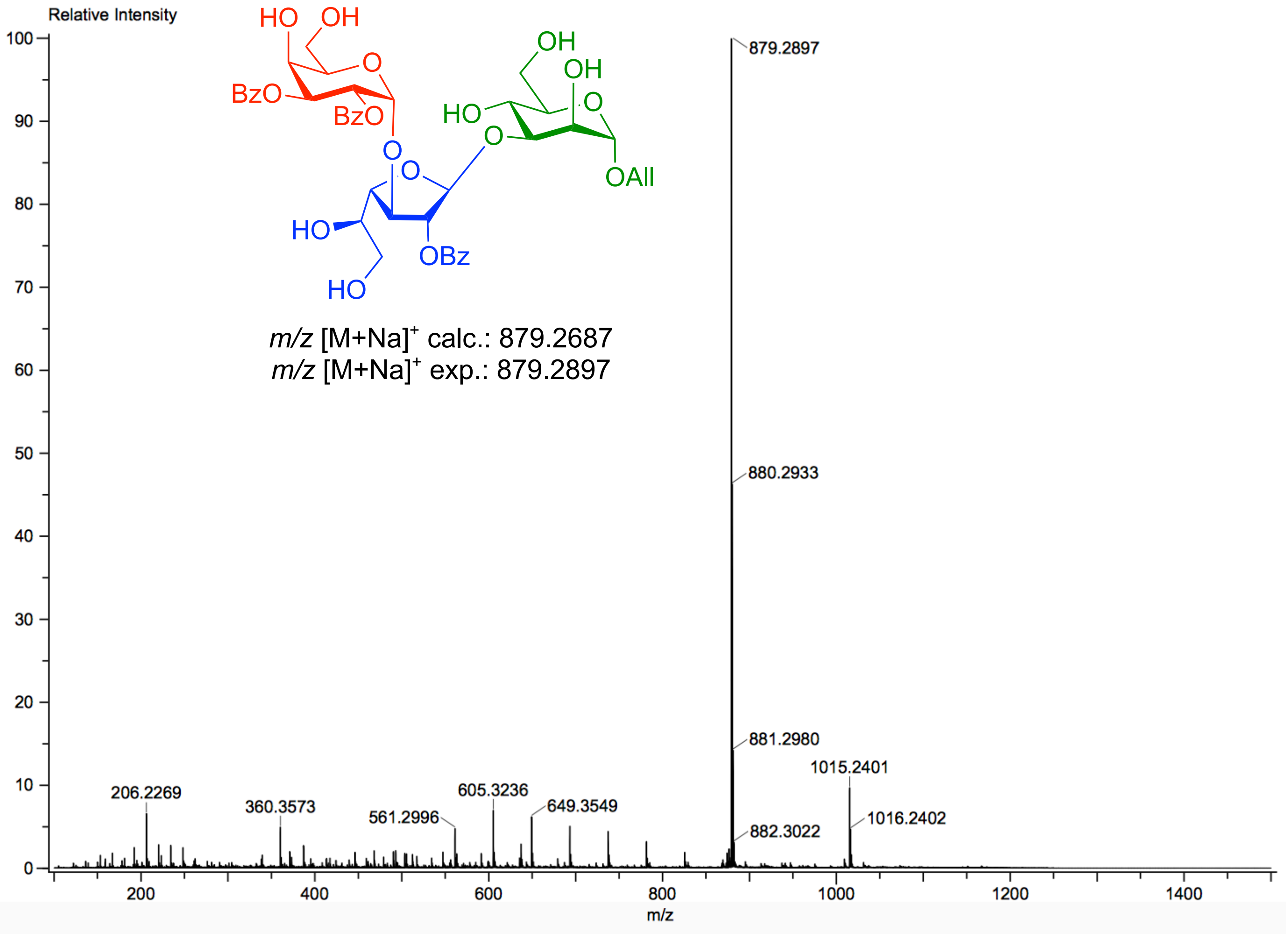


^{13}C NMR, 100 MHz, CD$_3$OD, compound S7



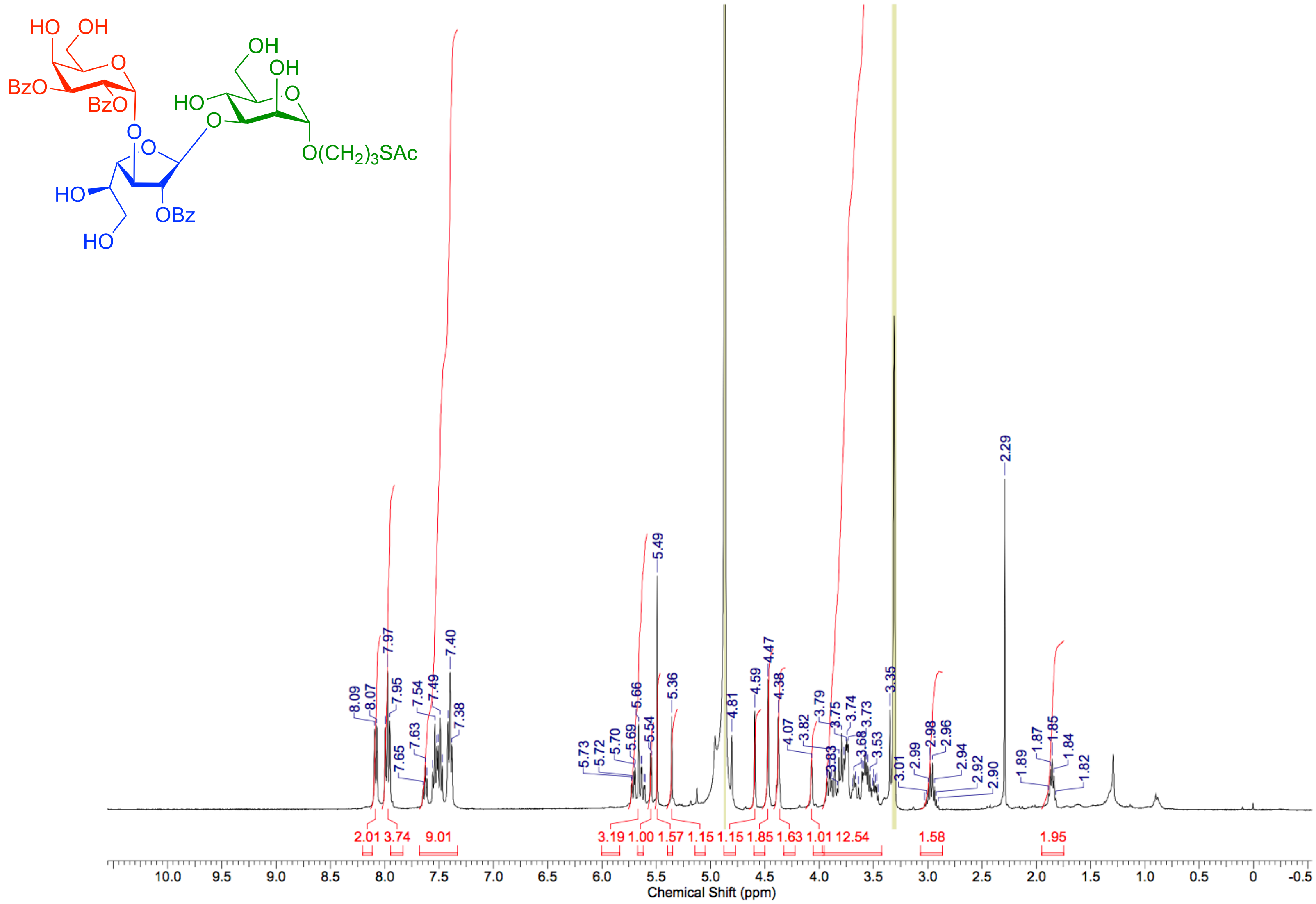


ESI-TOF HR mass spectrum of compound S7

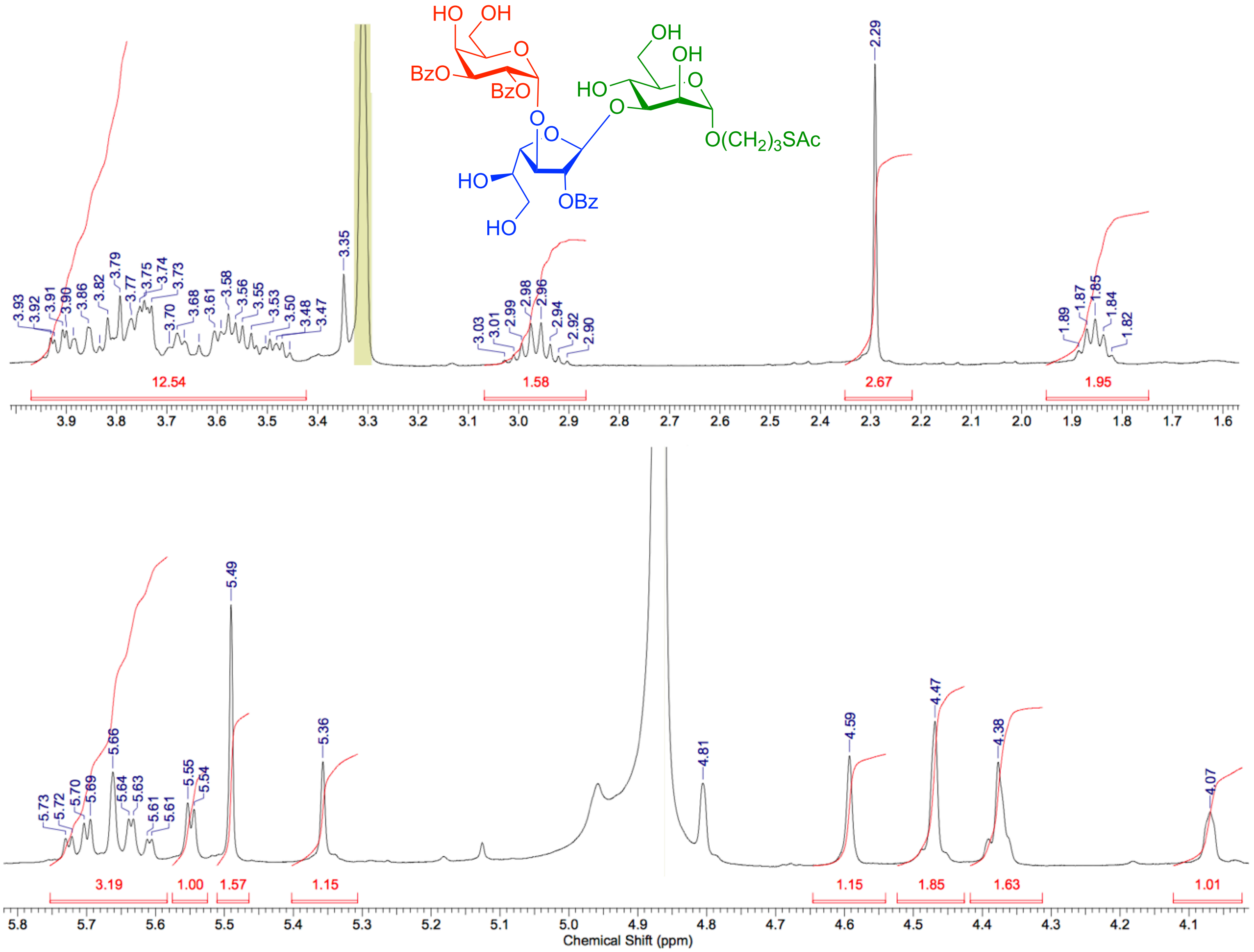


m/z [M+Na]$^{+}$ calc.: 879.2687
m/z [M+Na]$^{+}$ exp.: 879.2897

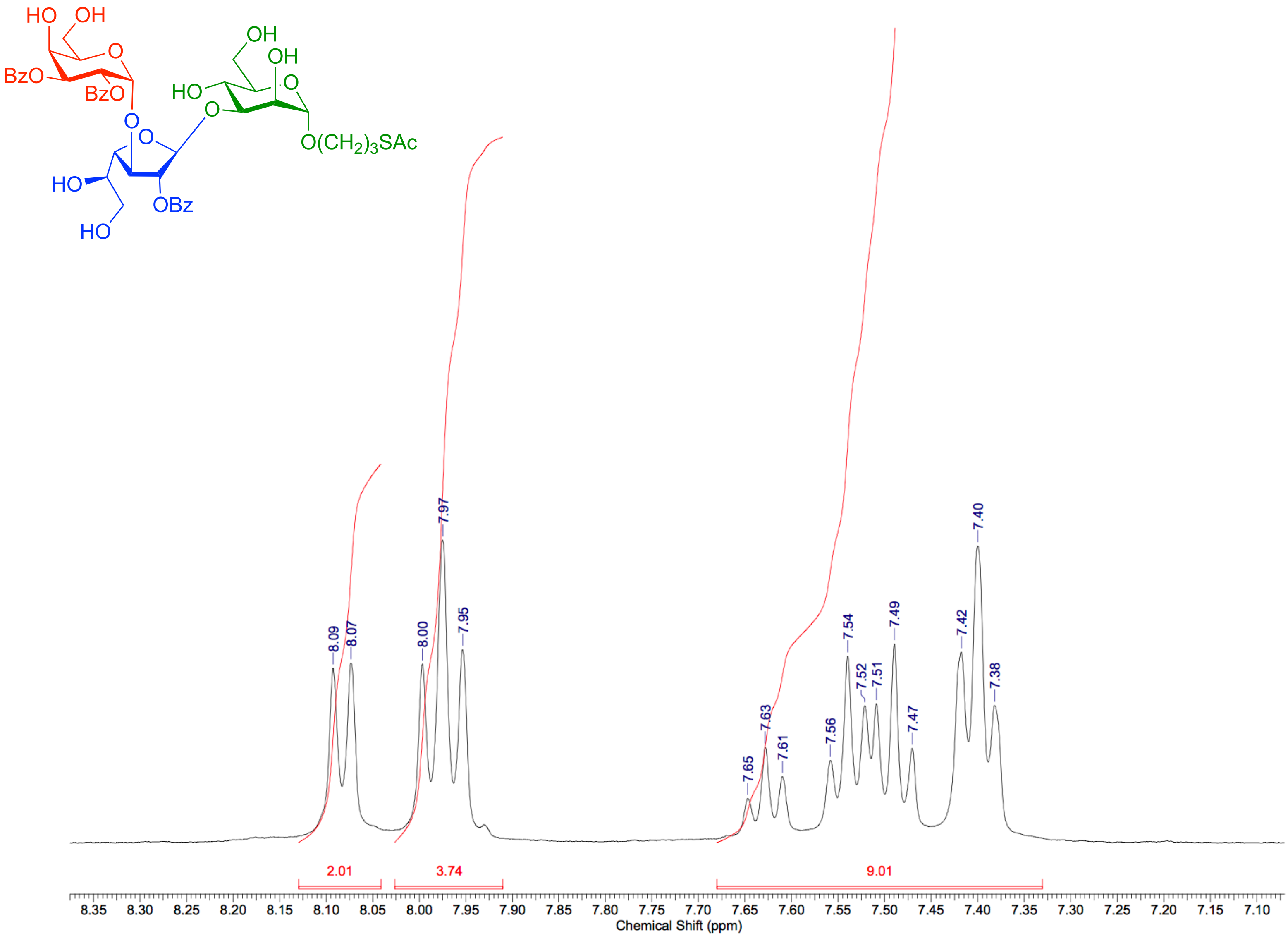
^{1}H NMR, 400 MHz, CD$_3$OD, compound S8



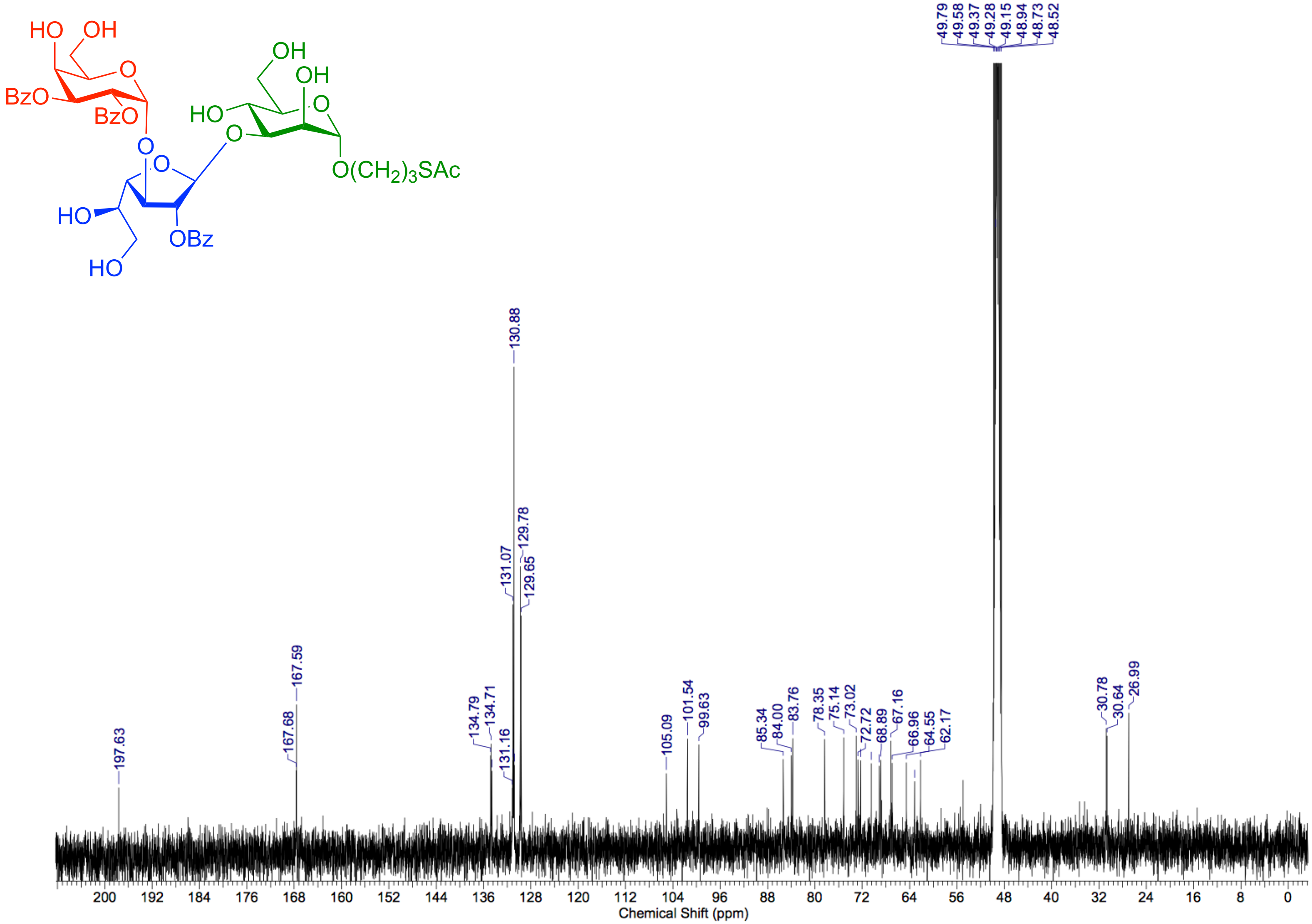
^{1}H NMR, 400 MHz, CD$_3$OD, compound S8



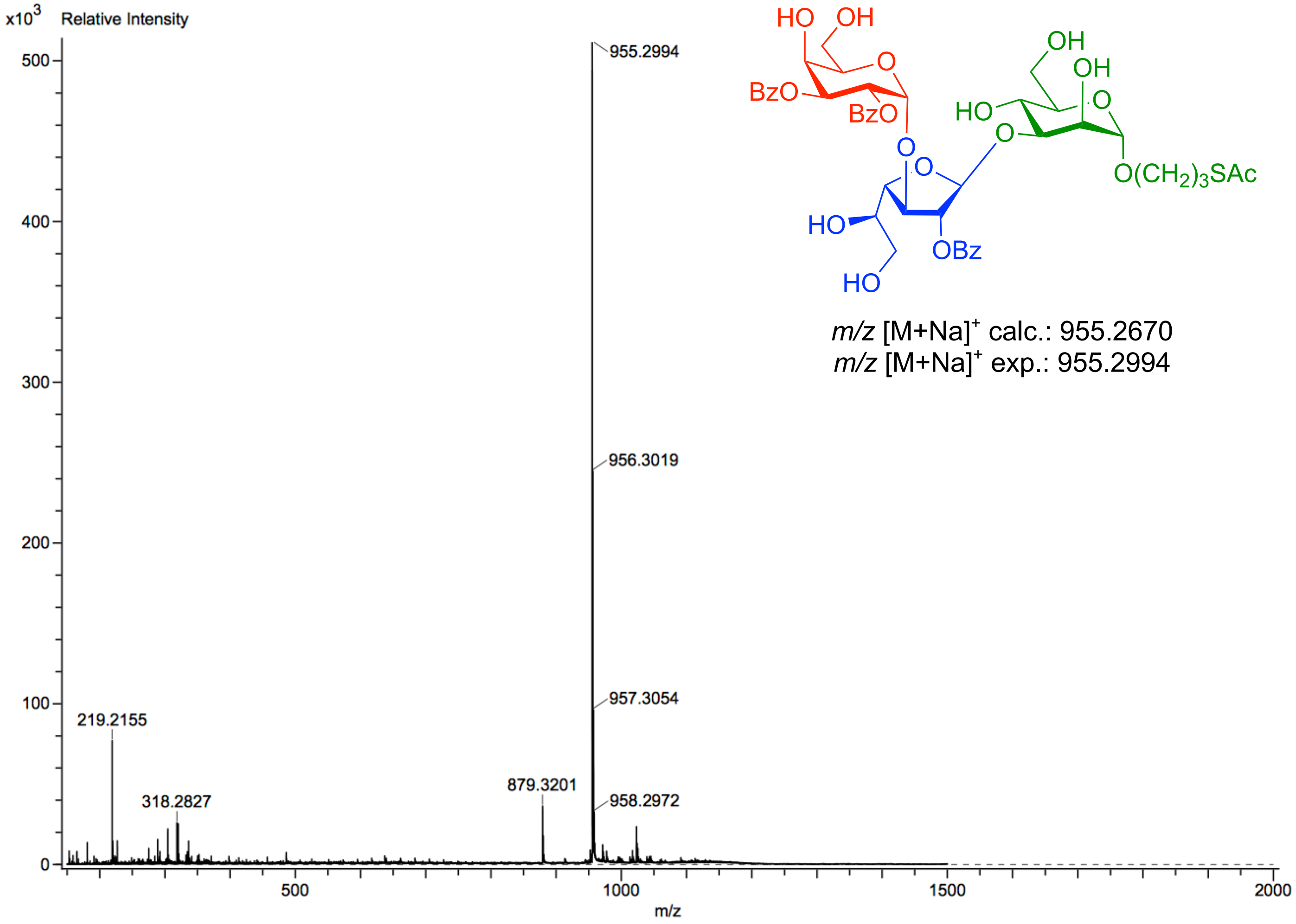
^{1}H NMR, 400 MHz, CD_3OD, compound S8



^{13}C NMR, 100 MHz, CD$_3$OD, compound S8

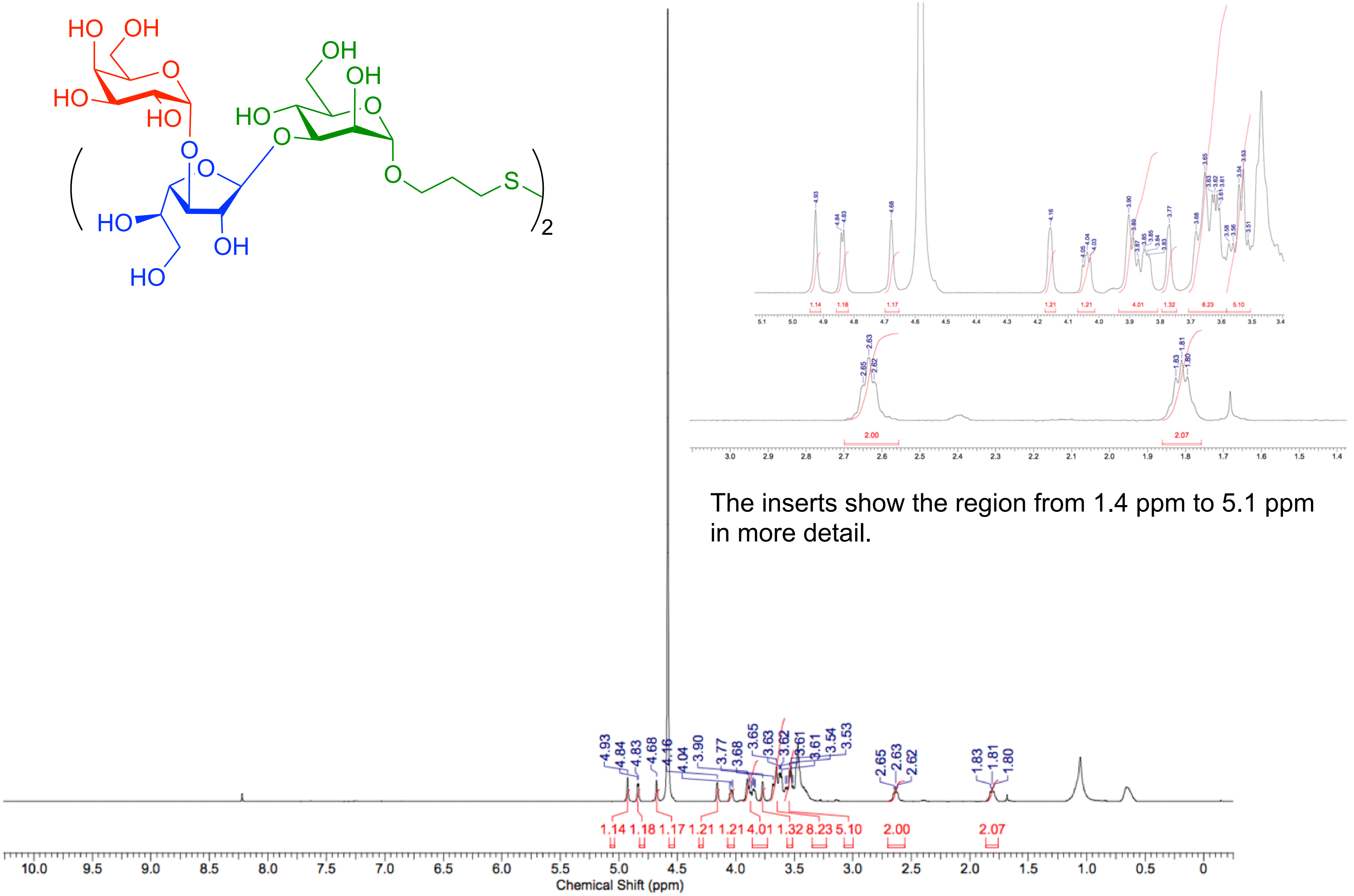


ESI-TOF HR mass spectrum of compound S8



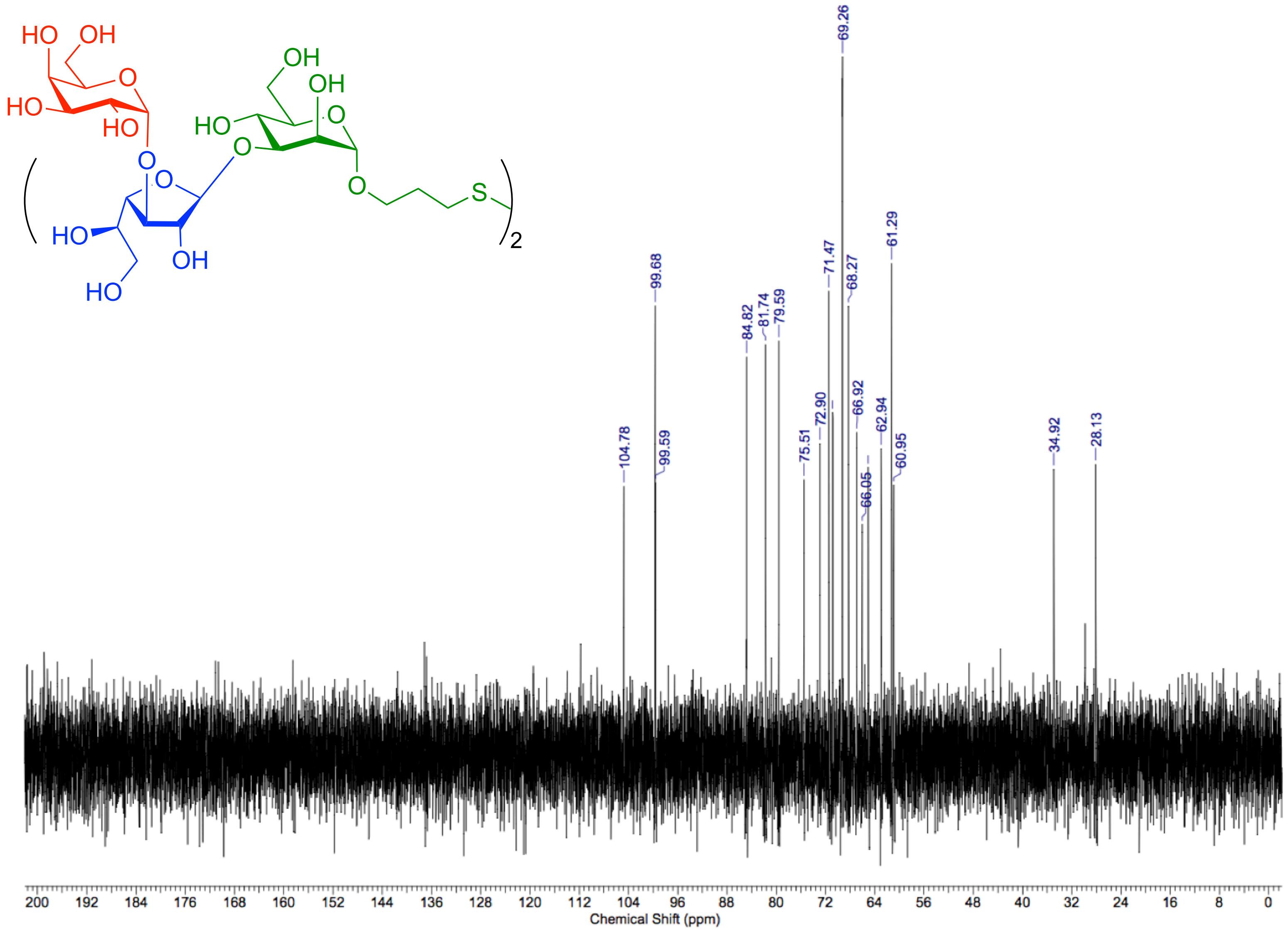
m/z $[M+Na]^+$ calc.: 955.2670
m/z $[M+Na]^+$ exp.: 955.2994

^{1}H NMR, 600 MHz, D_2O, compound $(G30_S)_2$

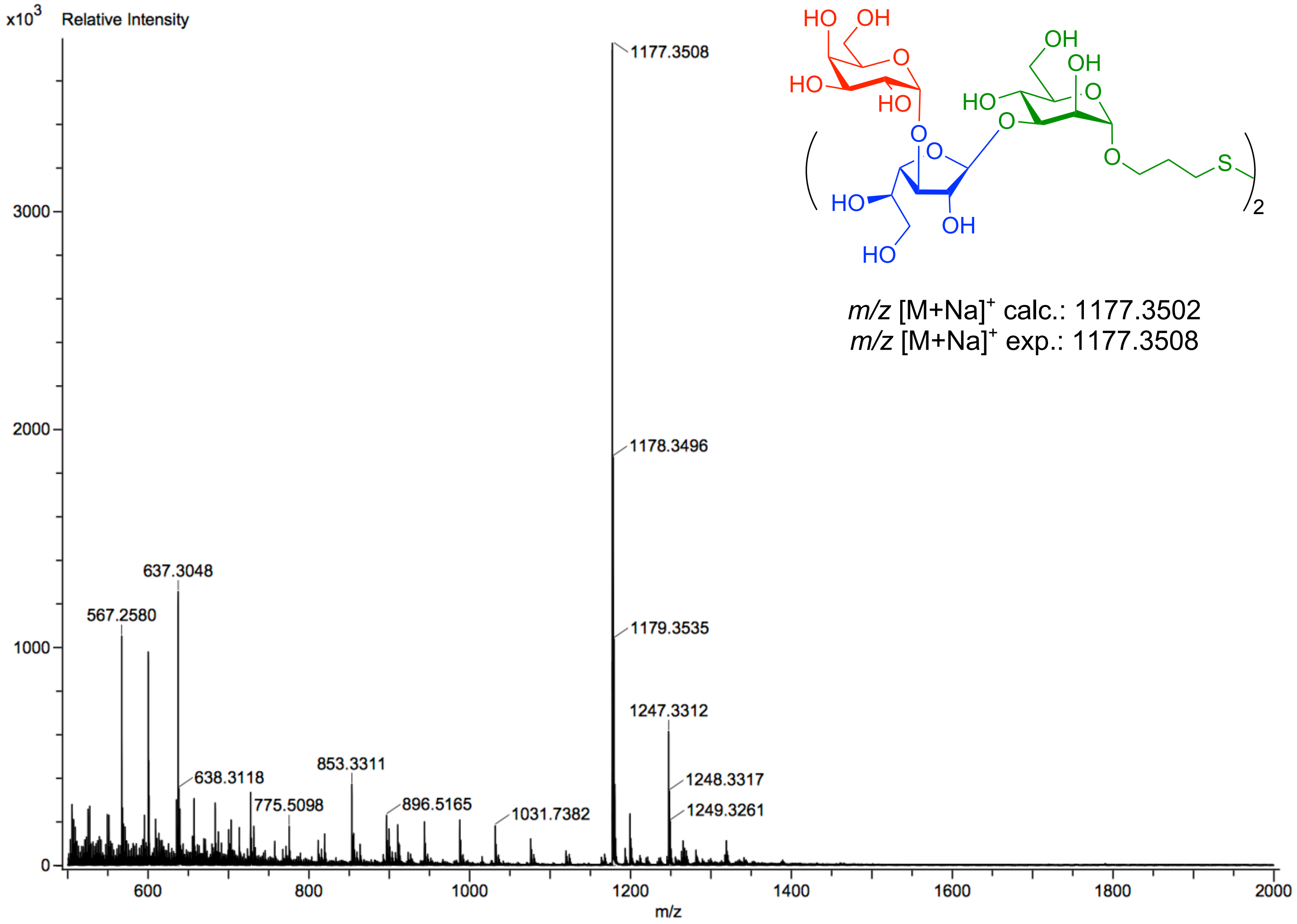


The inserts show the region from 1.4 ppm to 5.1 ppm in more detail.

^{13}C NMR, 100 MHz, D$_2$O, compound (G30$_S$)$_2$



ESI-TOF HR mass spectrum of compound $(G30_S)_2$



m/z $[M+Na]^+$ calc.: 1177.3502
m/z $[M+Na]^+$ exp.: 1177.3508