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Supporting Information

Label-Free Detection of Glycan–Protein Interactions for Array Development by Surface-Enhanced Raman Spectroscopy (SERS)

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Supporting Information

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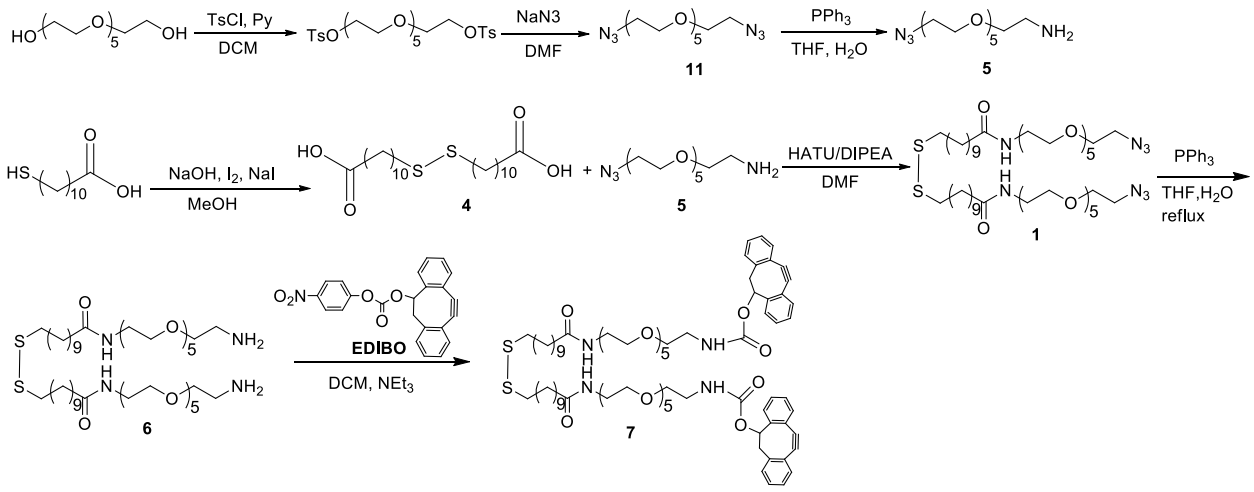
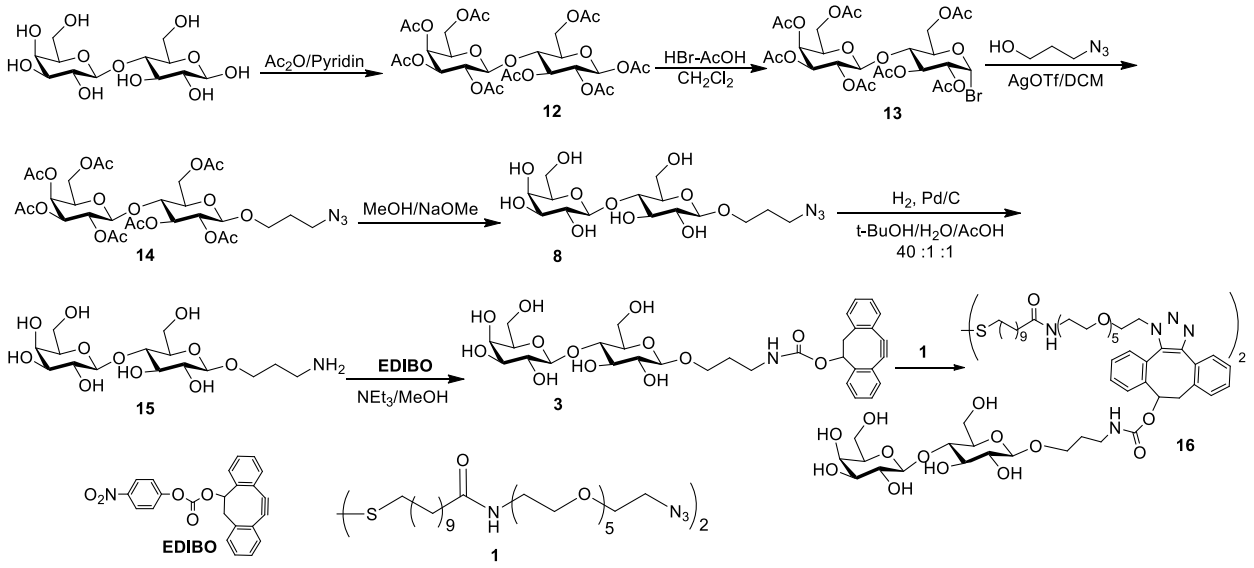
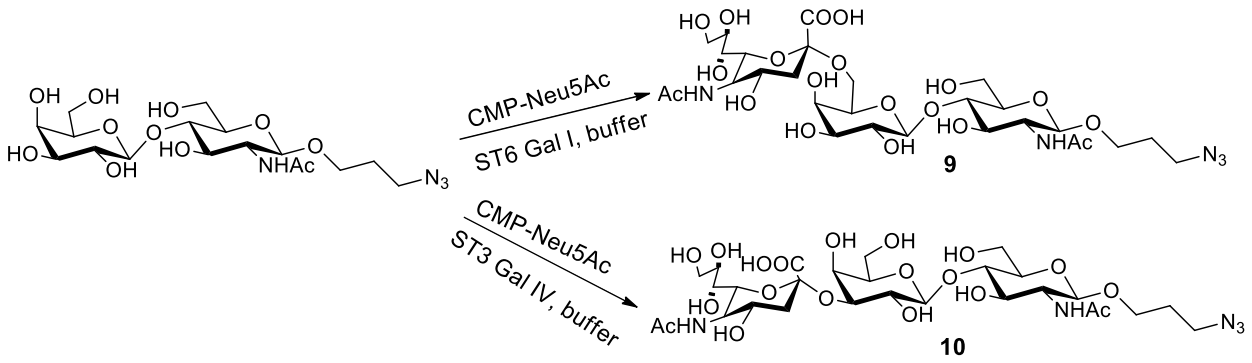


Scheme S1. The synthesis of ligands for immobilization of the carbohydrates.



Scheme S2. The synthesis of DIBO-lactose and the model compound.



Scheme S3. The synthesis of sialylated LacNAc. Buffer: sodium cacodylate buffer (50mM, pH 7.3), BSA (0.1%), CIAP (0.1%).

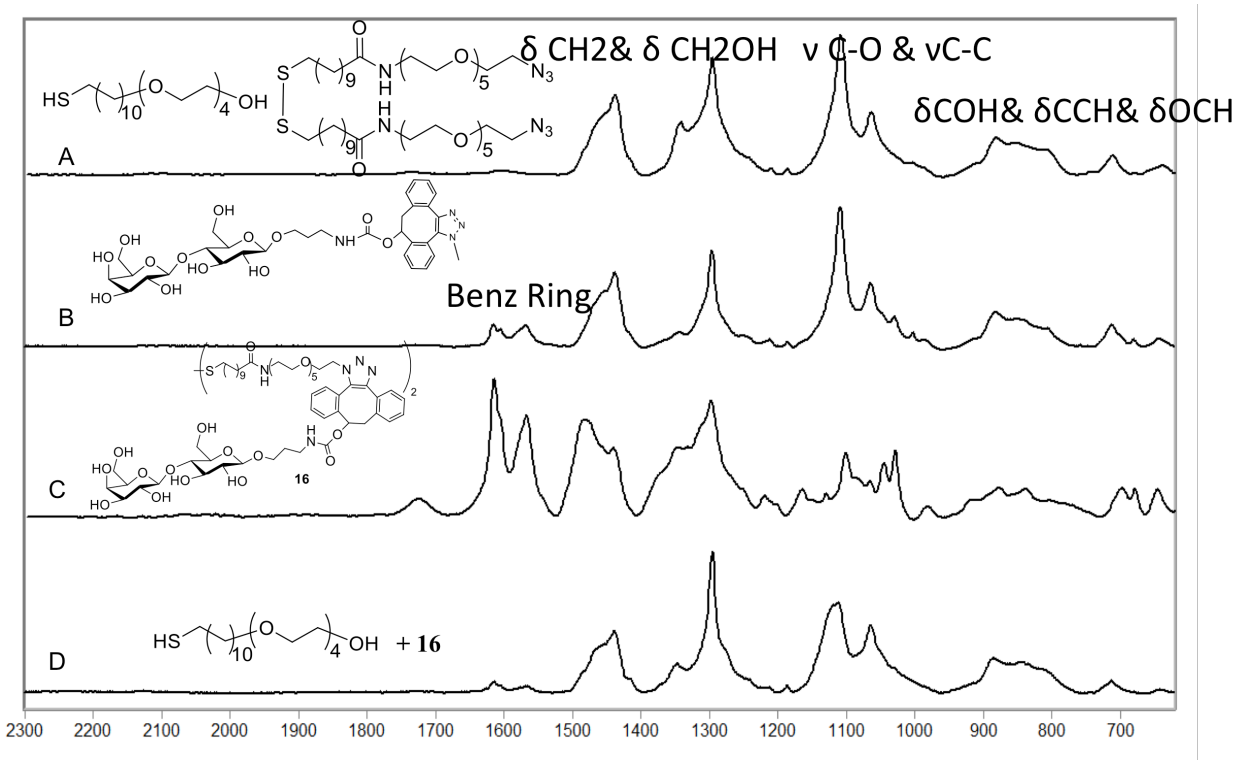
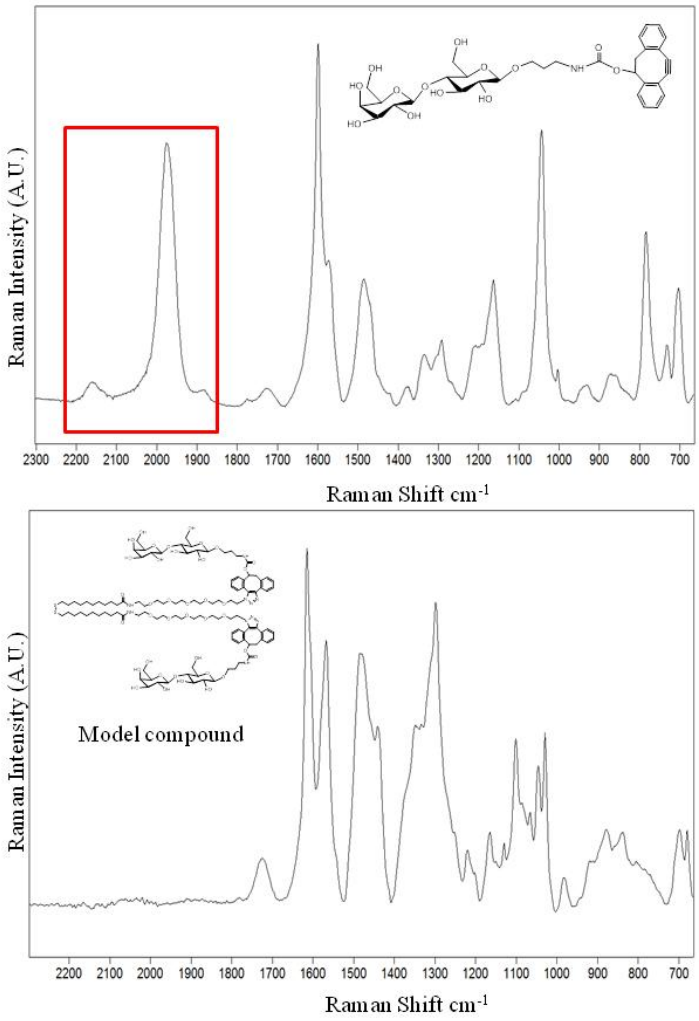


Figure S1. SERS spectra of **1** + **2** (A), **1** + **2** + **3** (B), model compound **16** (C) and **2** + **16** (D). Each spectrum shown is an average of 10 individual spectra for each sample. More detail assignments for the corresponding spectrum are listed in Table S1 and Figure S2.

Table S1. The assignment of major peaks for the spectrum in Figure S1 (A).

Wave number (cm^{-1})	Assignment
638	v(C-S)G
712	v(C-S)T
803	CH_2 rocking
843	CH_2 rocking
885	CH_2 rocking
1000	v(C-C)
1062	v(C-O) stretching
1114	CH_2 twisting
1297	v(C-O) stretching
1438	v(C-H) stretching
1457	v(C-H) stretching



DIBO-Lactose wavenumber (cm^{-1})	Assignment
857	δ(COH), δ(CCH),δ (OCH) side group deformations
1004	ν(C-O) and ν(C-C)
1039	ν(C-O) and ν(C-C)
1161	ν(C-O) and ν(C-C)
1264	$\delta(CH_2)$ AND $\delta(CH_2OH)$
1465	$\delta(CH_2)$ AND $\delta(CH_2OH)$
1571	Ring stretches
1599	Ring stretches
1964	C≡C stretch
2133	C≡C stretch

Model compound Wavenumber (cm-1)	Assignment
1722	C=O Stretch
1614	Benzene Ring Bands
1606	Benzene Ring Bands
1570	Ring stretches
1029	ν(C-O) and ν(C-C)
1001	ν(C-O) and ν(C-C)
678	Ring Breathing

Figure S2. SERS spectra and the corresponding assignments of DIBO-lactose and model compound directly deposited in the SERS substrate.

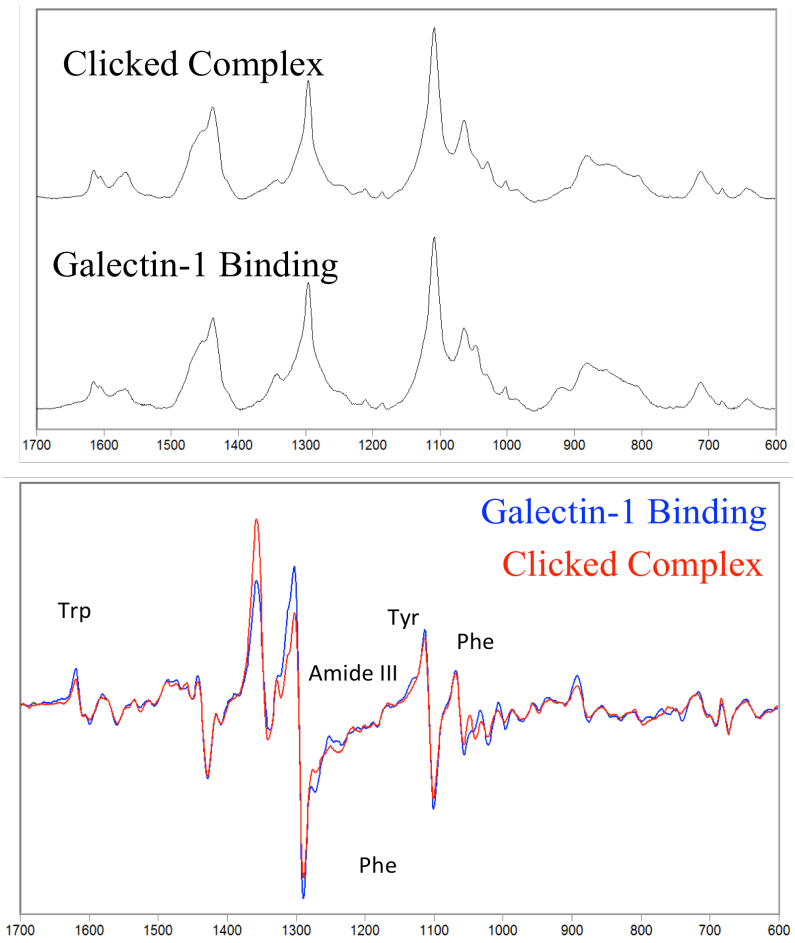


Figure S3. Overlap of normalized Savitzky-Golay SERS spectra of clicked complex containing DIBO-lactose and the capture of Gal1.

Table S2. VIP scores for differentiation of non protein (lactose) and proteins (Gal1 and Gal3). Spectral bands that recorded a value greater than one are listed along with their spectral assignments.

Wavenumber (cm^{-1})	VIP Value	Assignment
1498	1.08	CH_2 sciss
1424	2.87	Trp
1413	16.67	Phe, Tyr, Trp
1352	34.90	Tyr
1235	9.07	Trp
1100	7.72	C-N str.
1090	15.11	Tyr
1038	3.75	Phe
1023	1.61	Phe
994	2.19	Phe
721	6.78	Phe, Trp, Tyr

Table S3. Cross validation results for the PLS-DA Y-CV predicted score plots shown in Figure 2.

Protein Binding Classification		
	No protein	Protein
Sensitivity	1.000	1.000
Specificity	1.000	1.000
Class Error (CV)	0.000	0.000
RMSECV	0.109	0.109

Table S4. PLS-DA cross validation results of samples from binary models.

Protein Binding Classification							
		Gal1 (μg/mL)			Gal3 (μg/mL)		
	lactose	1	5	100	1	5	100
Sensitivity	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Specificity	1.000	0.950	1.000	1.000	1.000	1.000	1.000

Table S5. SERS signal assignments for Gal1 and Gal3 on nanoparticles.

Gal1 Wave number (cm^{-1})	**Gal3 Wave number (cm^{-1})**	**Assignment**
	1608	Amide I
	1558	Indole ring Trp
1459	1449	Trp
1352	1352	Tyr
	1325	Trp, C-H
1237	1236	Amide III
	1128	C-N str.
1086	1087	Tyr
1036	1035	Phe
1003		Phe
990	991	Phe
877	883	Trp

Table S6. Summary of the PLS-DA cross validated results for classification of Gal1 and Gal3 at various concentrations shown in Figure 3 (B-D).

	Protein Classification					
	Gal1 (μg/mL)			Gal3 (μg/mL)		
	1	5	100	1	5	100
Sensitivity	0.769	1.000	0.900	1.000	1.000	0.900
Specificity	1.000	1.000	0.900	0.769	1.000	0.900

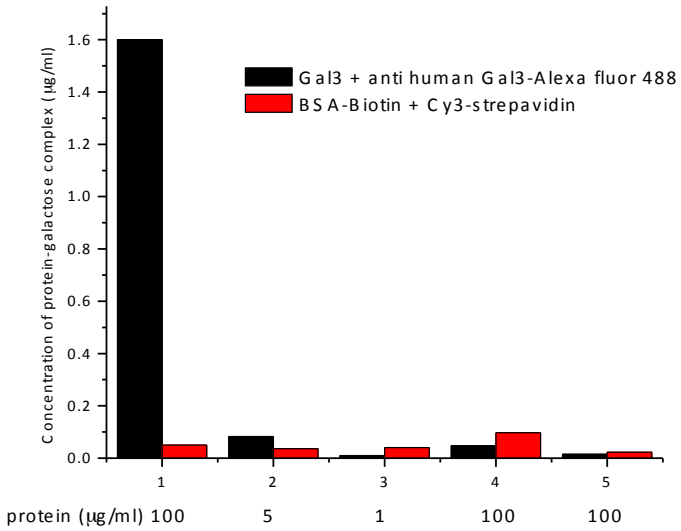


Figure S4. Fluorescence detection of the bound Gal3 and BSA on the AuNPs immobilized with galectose (1-3), AuNPs with **2** (4) and bare AuNPs (5). The initial concentration of the proteins (Gal3 and BSA-biotin) added to 1, 2, 3 (Au nanoparticles attached with **1**-DIBO-lactose), 4 (AuNPs + **2**) and 5 (bare AuNPs) are 100, 5, 1, 100 and 100 μg/mL, respectively.

(B)

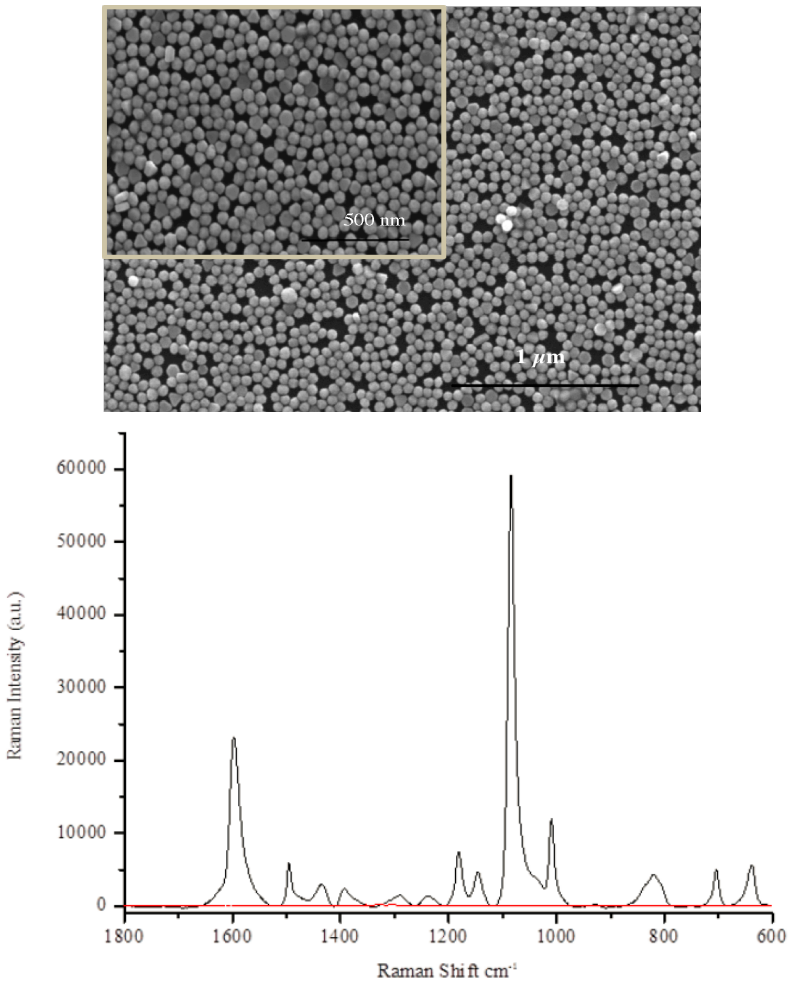


Figure S5. (A) SEM image of the Au nanoparticle monolayer captured by amine anchored on a gold thin film coating on a glass slide by Au-sulfide bonds; (B) Typical spectra of 4-ATP on the substrate with (black) and without (red) Au nanoparticle monolayer.

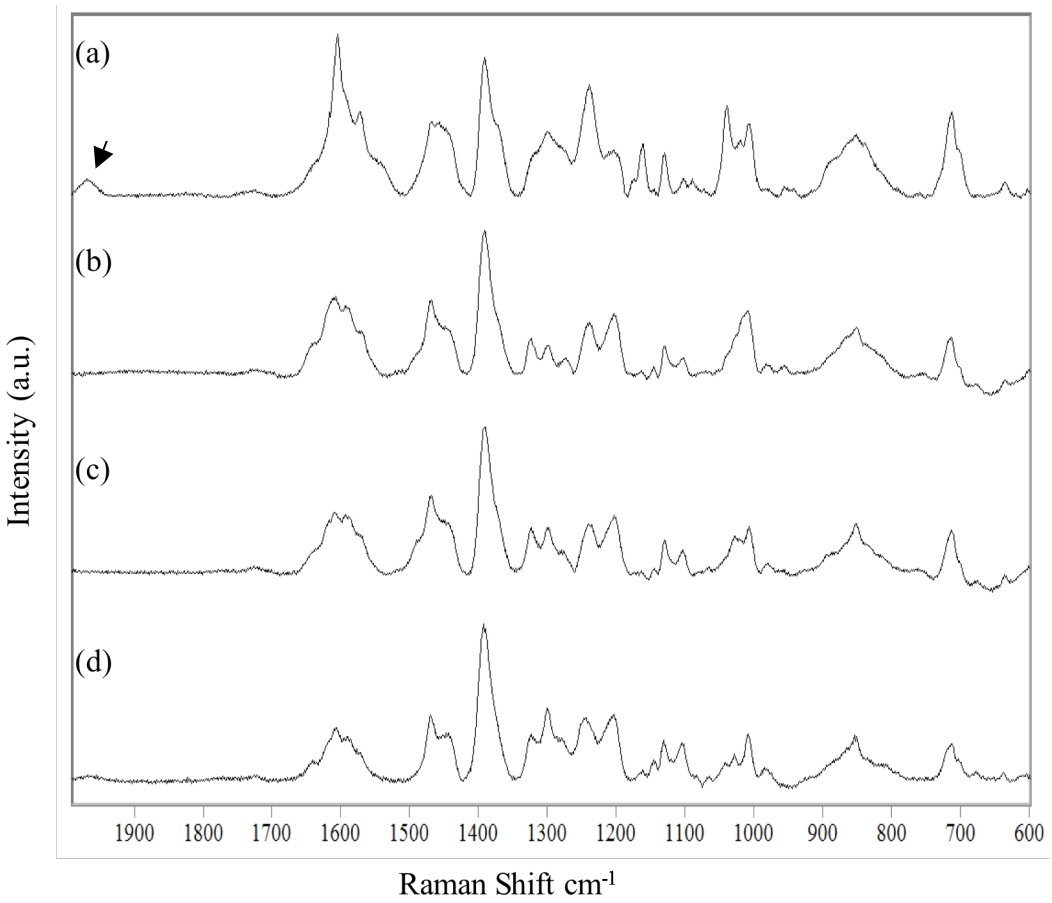


Figure S6. Representative SERS spectra of **7** (a), **7** + **8** (b), **7** + **9** (c) and **7** + **10** (d). Each spectrum shown is an average of 10 individual spectra for each sample.

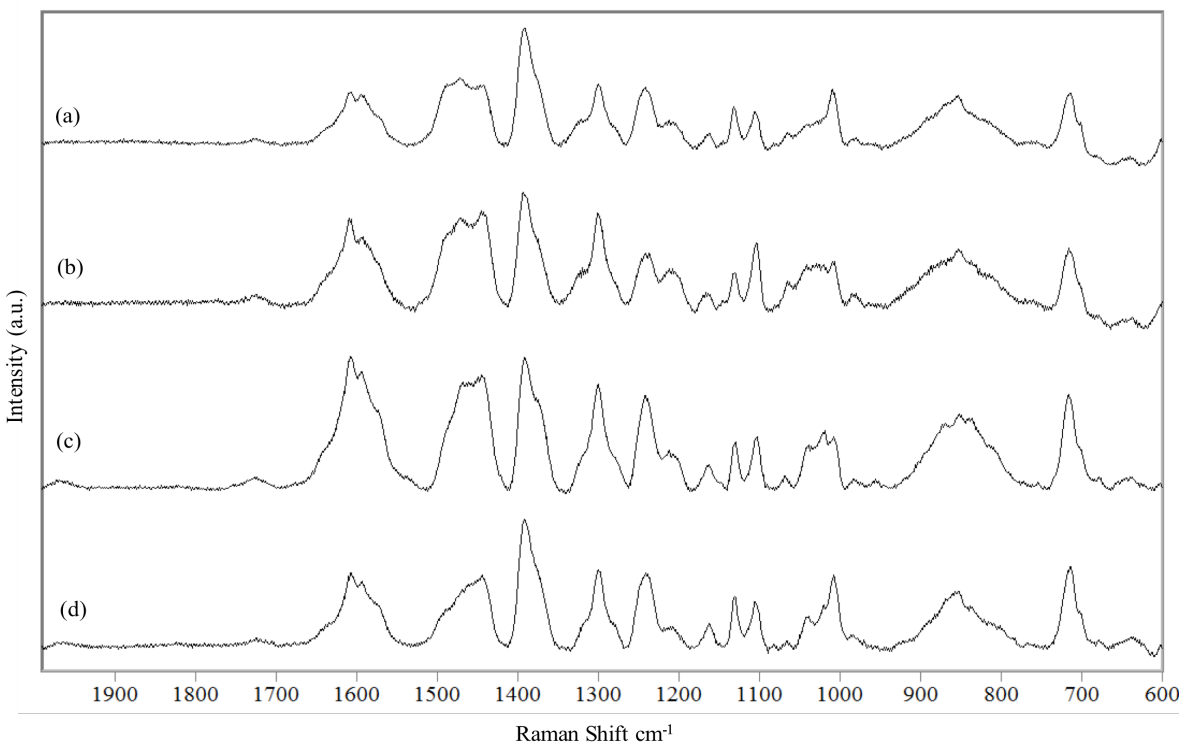
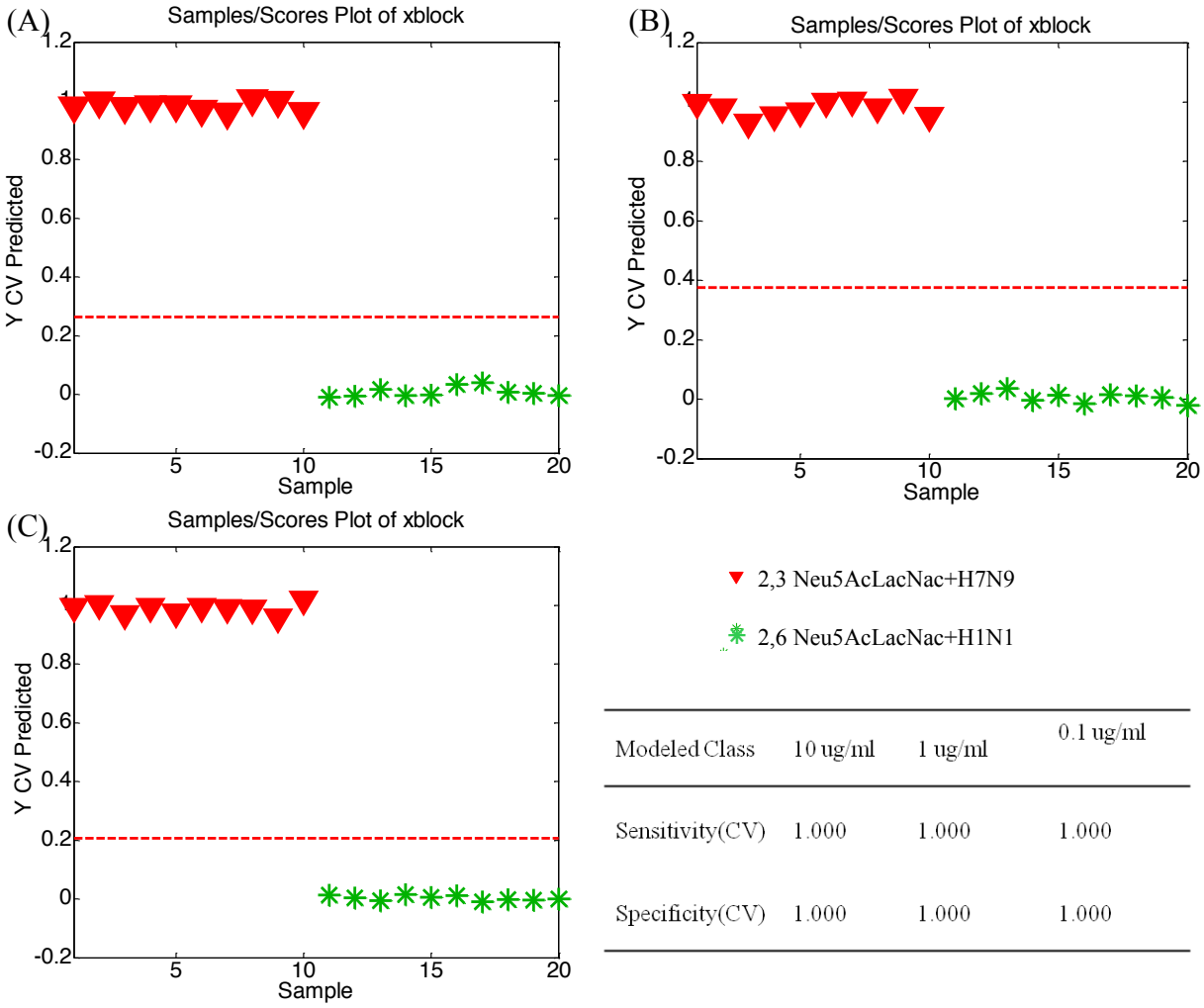


Figure S7. Representative SERS spectra of **7** + **9** (a), **7** + **9** + H7N9 (b), **7** + **10** (c) and **7** + **10** + H1N1 (d). The HA concentration of each sample is 10 μg/mL and the spectra shown are averages of 10 individual spectra for each sample.

Table S7. Summary of the PLS-DA cross validated results for classification of spacer (**2**) + DIBO (**7**), **2** + **7** + α2,3SA-LacNAc (**8**) and **2** + **7** + α2,3SA-LacNAc +Avian HA (H7N9) at various concentrations or spacer + DIBO, spacer + DIBO + α2,6SA-LacNAc and spacer + DIBO + α2,6SA-LacNAc + human HA (H1N1).

Modeled Class	Sensitivity (CV)	Specificity (CV)	Modeled Class	Sensitivity (CV)	Specificity (CV)
DIBO-spacer	1.000	1.000	DIBO-spacer	1.000	1.000
DIBO-α2,3 Neu5AcLacNAc (avg)	0.967	0.942	DIBO-α2,6 Neu5AcLacNAc (avg)	0.967	0.921
H7N9 (50 μg/mL)	1.000	1.000	H1N1 (50 μg/mL)	1.000	0.950
H7N9 (25 μg/mL)	1.000	1.000	H1N1 (25 μg/mL)	1.000	0.900
H7N9 (10 μg/mL)	0.800	0.850	H1N1 (10 μg/mL)	1.000	0.895
H7N9 (1 μg/mL)	1.000	0.947	H1N1 (1 μg/mL)	1.000	0.950
7N9 (0.5 μg/mL)	1.000	0.950	H1N1 (0.5 μg/mL)	1.000	1.000
7N9 (0.1 μg/mL)	1.000	0.950	H1N1 (0.1 μg/mL)	1.000	1.000



Modeled Class	10 ug/ml	1 ug/ml	0.1 ug/ml
Sensitivity(CV)	1.000	1.000	1.000
Specificity(CV)	1.000	1.000	1.000

Figure S8. Cross-validated PLS-DA plots for H7N9 vs H1N1. Concentration of HA: 10 µg/mL (A); 1 µg/mL (B) and 0.1 µg/mL (C).

Experimental

Reagents and general procedures

Gold nanoparticles with a diameter of 60 nm (4.58×10^{11}) were purchased from Ted Pella. Indium tin oxide (ITO) glass slides with square resistivity of 70-100 Ω/sq and (11-mercaptoundecyl)tetra(ethylene glycol) (95%), recombinant human Gal1, recombinant human Gal3, streptavidin-cy3, albumin, biotin labeled bovine (BSA) were purchased from Sigma Aldrich. Human Gal3 alexa fluor 488 was purchased from R&D systems. Alkaline phosphatase from calf intestine (CIAP) was purchased from Calbiochem EMD Millipore. Cytidine 5'-monophospho-*N*-acetylneuraminic (CMP-Neu5Ac) acid was purchased from Carbosynth Limited**.** The recombinant enzymes ST3Gal-IV (α2-3Sialyltransferase) and ST6Gal-I (α2-6Sialyltransferase) were provided by Prof. Kelley Moremen (Complex Carbohydrate Research Center, University of Georgia, Athens, GA). Avian influenza A hemagglutinin (Avian HA) full length protein (ab182675) from H7N9 (Shanghai/017/2013) and influenza A virus hemagglutinin H1 (Human HA) full length protein H1N1 (ab69741, New Caledonia/20/1999) were purchased from Abcam®. Gold (99.999%) and titanium (99.999%) evaporation pellets were purchased from Kurt J. Lesker. Other reagents were obtained from commercial sources and used as purchased. Dichloromethane (DCM) was freshly distilled using standard procedures. Other organic solvents were purchased anhydrous and used without further purification. Unless otherwise noted, all reactions were carried out at room temperature in oven-dried glassware with magnetic stirring. Molecular sieves were flame dried under high vacuum prior to use. Organic solutions were concentrated under diminished pressure with bath temperatures < 40 ^{o}C. Flash column chromatography was carried out on silica gel G60 (Silicycle, 60-200 μm, 60 Å). Thin-layer chromatography (TLC) was carried out on Silica gel 60 F_{254} (EMD Chemicals Inc.) with detection by UV absorption (254 nm) were applicable and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 ^{o}C or by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ~150 ^{o}C. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-300 (300/75 MHz), a Varian Inova-500 (500 MHz) and a Varian Inova-600 (600/150 MHz) spectrometer equipped with sun workstations. Multiplicities are quoted as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), or multiplet (m). All NMR signals were assigned on the basis of ^{1}H NMR, ^{13}C NMR, gCOSY and gHSQC experiments. All chemical shifts are quoted on the δ-scale in parts per million (ppm). Signals

marked with a superscript Roman numeral I were the reducing end, whereas II and III were the second and the third sugar from the reducing end, IV is the branching sugar. Residual solvent signals were used as an internal reference. Mass spectra were recorded on an Applied Biosystems 5800 MALDI-TOF mass spectrometer. The matrix used was 2,5-dihydroxy-benzoic acid (DHB). Reverse-Phase HPLC was performed on an Aglient 1200 series system equipped with an auto-sampler, fraction-collector, UV-detector and eclipse XDB-C18 column (5 μm, 4.6 × 250 mm or 9.4 × 250 mm).

Synthesis

Diazido hexaethylene glycol (11). To a solution of hexaethylene glycol (25.0 g, 88.5 mmol) in 300 mL of DCM was added *p*-toluenesulfonyl chloride (37.1 g, 194.8 mmol) and pyridine (35.0 g, 442.7 mmol). The reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated and dried under vacuum. The residue was dissolved in 300 mL of DMF and sodium azide (28.8 g, 442.7 mmol) was added. The suspension was stirred at 80 °C for 16 h, then the mixture was filtered and the filtrate was evaporated. The residue was purified by flash chromatography on silica gel (1:1 to 1:0 DCM/EtOAc) to give **11** as a colorless oil (21.0 g, 63 mmol) in a 71.4% yield. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 3.63-3.60 (m, 20H, CH2O), 3.34-3.31(t, 4H, CH2N3). ^{13}C-NMR ($CDCl_3$, 300 MHz): δ 70.86, 70.84, 70.80, 70.76, 70.20, 50.85. MALDI-MS ($C_{12}H_{24}N_6O_5$): Calculated ($[M+Na]^+$): 335.1706, found: 335.1696.

Azido hexaethylene glycol amine (5). To a solution of **11** (11.60 g, 34.90 mmol) in 150 mL of THF and 5 mL of H_2O was added triphenyl phosphine (10.98 g, 41.88 mmol) and stirred at room temperature for 16 h. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (5-50% MeOH/DCM) to afford compound **5** as a yellowish oil (5.0 g, 16.3 mmol) in 46.8% yield. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 5.29 (s, 2H), 3.62-3.56 (m, 16H), 3.54-3.51 (m, 2H), 3.36 (m, 2H), 2.88 (m, 4H). ^{13}C-NMR ($CDCl_3$, 300 MHz): δ 72.73, 72.59, 72.55, 70.84, 70.82, 70.76, 70.69, 70.56, 70.45, 70.19, 53.67, 50.88, 49.23, 41.80. MALDI-MS ($C_{12}H_{26}N_4O_5$): Calculated ($[M+H]^+$): 307.1981, found: 307.1988.

11-(10'-carboxy-decyldisulfanyl)undecanoic acid (4). This compound was synthesized as described.[1] Sodium hydroxide (732 mg, 18.32 mmol), potassium iodide (50.0 mg, 0.30 mmol)

and iodine (2.32 g, 9.16 mmol) were added to a solution of 11-mercaptoundecanoic acid (4.00 g, 18.32 mmol) in methanol (100 mL) and the solution was stirred for 30 min. The brown reaction mixture was decolored with a saturated sodium sulfite solution, the solvents concentrated under reduced pressure, the precipitate dissolved in CH_2Cl_2 (100 mL) and the resulting solution washed with a HCl solution (1 M, 100 mL) and water (100 mL). The organic phase was dried over MgSO4, filtered and concentrated under reduced pressure. Purification of the crude material by crystallization (hexanes/AcOEt) gave **3** (2.5 g, 63%) as a white solid. The NMR of the material matched reported data.

Diazido hexaethylene glycol 11-(10'-carboxy-decyldisulfanyl)undecanoic amide (**1**)**.** HATU (1.36 g, 3.59 mmol) was added to a solution of **4** (1.10 g, 3.59 mmol), **5** (0.71 g, 1.63 mmol) and DIPEA (0.63 g, 4.90 mmol) in 40 mL of dry DMF. The reaction mixture was stirred at room temperature for 2 h. Then the solvent was removed under vacuum and the residue was separated by silica gel chromatography (5-50% MeOH/DCM) to achieve **1** as a yellowish solid (3.52 g, 3.45 mmol) in 98.0% yield. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 6.13 (s, 2H), 3.66-3.58 (m, 36H), 3.52-3.50 (m, 4H), 3.44-3.34 (m, 8H), 2.65 (t, 4H,), 2.14 (t, 4H), 1.66-1.60 (m, 8H), 1.59-1.24 (m, 24H). ^{13}C-NMR ($CDCl_3$, 300 MHz): δ 173.46, 70.88, 70.80, 70.77, 70.73, 70.71, 70.42, 70.23, 70.18, 53.67, 50.88, 39.35, 36.91, 29.42-29.67, 28.72, 25.95. MALDI-MS ($C_{46}H_{90}N_8O_{12}S_2$): Calculated ($[M+Na]^+$): 1033.6018, found: 1033.6010.

Diamine hexaethylene glycol 11-(10'-carboxy-decyldisulfanyl)undecanoic amide (**6**)**.** To a solution of **1** (0.8 g, 0.791 mmol) in THF (40 mL) and H2O (2 mL) was added triphenylphosphine (0.436 g, 1.661 mmol). The reaction mixture was stirred under refluxing for 18 h. Then the solvent was removed under vacuum and the residue was separated by flash chromatography on Iatrobeads (10-40% MeOH/DCM) to give the product as white solid (0.628 g) in 83% yield. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 6.41 (s, 2H), 3.64-3.61 (m, 32H), 3.54-3.43 (m, 12H), 2.85-2.82 (t, 4H), 2.82-2.65 (t, 4H), 2.17-2.15 (t, 4H), 2.12 (s, 4H), 1.66-1.62 (m, 8H), 1.59-1.25 (m, 24H). ^{13}C-NMR ($CDCl_3$, 300 MHz): δ 173.50, 73.59, 70.81, 70.78, 70.72, 70.47, 70.40, 70.26, 41.99, 39.36, 39.33, 36.88, 29.68, 29.66, 29.60, 29.57, 29.43, 28.73, 25.97. MALDI-MS ($C_{46}H_{94}N_4O_{12}S_2$): Calculated ($[M+Na]^+$): 981.6208, found: 981.5950.

DIBO- hexaethylene glycol 11-(10'-carboxy-decyldisulfanyl)undecanoic amide (**7**)**.** To a solution of **6** (0.115 g, 0.12 mmol) and carbonic acid 7, 8- didehydro-1,2:5,6-dibenzocyclooctene-3-yl ester 4-nitrophenyl ester (EDIBO, 0.111 g, 0.288 mmol) in anhydrous DCM (30 ml) was added triethylamine (0.06 g, 0.6 mmol). The reaction mixture was stirred at room temperature for 30 h. The solvent was removed under reduce pressure and the residue was separated by flash chromatography on iatrobeads (1-5% MeOH/DCM) to give the product as a colorless oil (0.14 g, 80%). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.50 (d, 2H), 7.33-7.24 (m, 14H), 6.20 (s, 2H), 5.71 (s, 1H), 5.48 (s, 1H), 3.67-3.50 (m, 40H), 3.43-3.36 (m, 8H), 3.20 (dd, 2H), 2.90 (dd, 2H), 2.68 (t, 4H), 2.15 (t, 4H), 1.66-1.59 (m, 8H), 1.34-1.25 (m, 24H). ^{13}C-NMR ($CDCl_3$, 300 MHz): δ 173.49, 155.79, 152.44, 151.26, 130.16, 128.26, 128.14, 127.27, 126.44, 126.16, 124.05, 121.52, 113.13, 110.21, 70.80, 70.74, 70.56, 70.49, 70.39, 70.29, 70.20, 46.42, 41.19, 39.35, 36.87, 29.69, 29.66, 29.60, 29.57, 29.44, 29.43, 28.73, 25.96. MALDI-MS ($C_{80}H_{114}N_4O_{16}S_2$): Calculated ($[M+Na]^+$): 1473.7569, found: 1473.6699.

Per-*O*-acetyl lactose (**12**)**.** Lactose monohydrate (5.05 g, 15 mmol) was dissolved in pyridine (40 mL) at ambient temperature. Acetic anhydride (20 mL, 212 mmol) was added dropwise to this mixture and allowed to stir for 15 h with the exclusion of moisture. The reaction was cooled to 0 °C and methanol was added dropwise *carefully* (exothermic) and the mixture then left to warm up to ambient temperature. The solvents were then concentrated *in vacuo* and the resulting residue was dissolved in EtOAc. The organic phase was washed sequentially with saturated sodium bicarbonate solution and water. The organic layers were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo* to yield the β-glycoside **5** as a white solid (9.80 g, 99%): R*f* = 0.2 (hexane/EtOAc, 1:1, v/v); ^{1}H NMR (300 MHz, CDCl3) δ 6.29-6.28 (d, 1H, *J* = 3.0 Hz), 5.52-5.48 (t, 1H, *J* = 9.0 Hz), 5.39-5.38 (d, 1H, *J* = 3.0 Hz), 5.18-5.12 (dd, 1H, *J* = 3.0 Hz, *J* = 3.0 Hz), 5.06-4.97 (m, 2H), 4.53-4.46 (m, 2H), 4.18-4.12 (m, 3H), 3.94-3.82 (m, 2H), 2.21 (s, 3H), 2.20 (s, 3H), 2.19 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (300 MHz, CDCl3) δ 170.3-168.9 (C=O), 101.2, 88.9, 75.8, 71.0, 69.6, 69.4, 69.2, 66.6, 61.5, 60.8, 20.9-20.5; MALDI-MS ($C_{28}H_{38}O_{19}$): Calculated ($[M+Na]^+$): 701.1905, found: 701.1895.

Hepta-*O*-acetyl-β-D-lactosyl bromide (13). To a solution of compound **12** (2.5 g, 3.6 mmol) in dry CH2Cl2 (10 mL) was added dropwise 4 mL of HBr-AcOH (33%) and this mixture was allowed to stir for 2 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with water (120 mL). The organic layer was co-evaporated with toluene and dried in *vacuo* to give **13** as a white solid (2.16 g, 95%). This compound was used in the next step without further purification: R*f* = 0.5 (Hexane/EtOAc, 1:1, v/v); MALDI-MS ($C_{26}H_{35}BrO_{17}$): Calculated ($[M+Na]^+$): 721.0956, found: 721.0945.

Per-*O*-acetyl-(3-azidopropyl)-β-D-lactoside (14). To a solution of **13** (3.06 g, 4.29 mmol) in 50 mL of dry DCM with 4Å molecular sieves was added 3-azido-1-propanol[2] (2.2 g, 21.45 mmol) and AgOTf (3.37 g, 12.87 mmol) in 25 mL of toluene. The resulting mixture was stirred at ambient temperature for 18 h in the dark, filtered and evaporated. The crud solid thus obtained was purified by silica gel column chromatography (hexanes/EtOAc, from 2:1 to 1:1, v/v) to give **14** as a white solid (1.6 g, 52%).R*f* = 0.2 (hexane/EtOAc, 1:1, v/v); 1H NMR (300 MHz, CDCl3) δ 5.35-5.34 (d, 1H, *J* = 3.0 Hz), 5.23-5.14 (t, 1H, *J* = 9.0 Hz), 5.11-5.10 (d, 1H, *J* = 3.0 Hz), 4.98-4.93 (dd, 1H, *J* = 3.0 Hz, *J* = 3.0 Hz), 4.92-4.86 (m, 1H), 4.52-4.46 (m, 3H), 4.05-4.18 (m, 3H), 3.94-3.76 (m, 3H),3.63-3.55 (m, 2H), 3.37-3.33 (t, 2H), 2.15 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.10-2.06 (s, 9H), 2.05 (s, 3H), 1.98-1.79 (m, 2H). ^{13}C NMR (300 MHz, CDCl3) δ 170.32-168.91, 101.22, , 75.83, 73.65, 73.02, 71.03, 69.66, 69.45, 69.22, 66.66, 65.67, 61.52, 60.80, 20.92-20.55. MALDI-MS ($C_{29}H_{41}N_3O_{18}$): Calculated ($[M+Na]^+$): 742.2283, found: 742.2218.

3-azidopropyl-β-D-lactoside (8). This compound was synthesized as described previously.[3] To compound **14** (0.5 g, 0.6 mmol) dissolved in dry methanol (10 mL) and DCM (4 mL) was added a freshly made sodium methoxide solution (2 mL, 1 mol/L). The resulting mixture was stirred at ambient temperature for 1 h. The reaction was then neutralized with Dowex 50×2 (H^+) resin, filtered and concentrated to give the deacetylated target azido compound **8** as a white solid (280 mg, 95%). ^{1}H NMR (500 MHz, D_2O) δ 4.29 (d, 1H, *J* = 8.0 Hz, H-1), 4.25 (d, 1H, *J* = 7.0 Hz, H-1'), 3.81-3.77 (m, 2H), 3.73 (d, 1H, *J* = 3.0 Hz), 3.63-3.33 (m, 12H), 3.29 (t, 2H, *J* = 6.0 Hz), 3.12 (m, 1H), 1.75 (m, 2H). ^{13}C NMR (500MHz, D_2O) δ 103.45, 102.25, 78.49, 75.47, 74.89, 74.49, 72.92, 72.64, 71.07, 68.67, 67.47, 61.16, 60.20, 47.99, 28.37. MALDI-MS ($C_{15}H_{27}N_3O_{11}$): Calculated ($[M+Na]^+$): 448.1544, found: 448.1513.

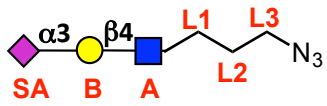
3-aminopropyl-β-D-lactoside (15). To a stirred solution of **8** (250 mg, 0.58 mmol) in 40 mL of a mixture of t-BuOH/AcOH/H2O (40/1/1, v/v/v) was added $Pd(OH)_2/C$ (500 mg, 20 wt.%, Degussa type). The resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 24 h, the catalyst was filtered off and washed thoroughly with MeOH. The combined filtrates were concentrated under reduced pressure to give **15** (230 mg, 98%) as a white solid. ^{1}H NMR (500MHz, CD_3OD) δ 4.38 (t, 1H), 4.32 (dd, 1H), 4.05 (m, 1H), 3.80 (m, 1H), 3.85 (m, 2H), 3.74 - 3.79 (m, 4H), 3.53 - 3.65 (m, 4H), 3.32 (t, 2H), 3.13 (t, 2H), 1.98 (m, 2H). ^{13}C NMR (500MHz, CD_3OD) δ 104.02, 103.22, 79.78, 76.95, 75.98, 75.25, 73.45, 71.53, 70.52, 69.06, 67.85, 61.56, 60.78, 37.98, 26.58, 23.22. MALDI-MS ($C_{15}H_{29}NO_{11}$): Calculated ($[M+Na]^+$): 422.1639, found: 422.1643.

Carbonic acid 7,8-didehydro-1,2:5,6-dibenzocyclooctene-3-yl ester 3-amide-β-D-lactoside (3). To a solution of **15** (50 mg, 0.12 mmol) and NEt_3 (30 mg, 0.30 mmol) in dry MeOH (5 mL) was added carbonic acid 7, 8- didehydro-1,2:5,6-dibenzocyclooctene-3-yl ester 4-nitrophenyl ester[4] (58 mg, 0.15 mmol) under an atmosphere of Argon. The resulting mixture was stirred at ambient temperature for 12 h and the solvent was removed under reduce pressure and the residue was purified by Tatrobeads 6Rs-8060 silica gel column chromatography (DCM/MeOH, ¼, v/v) to afford **3** (60 mg, 75%) as a white solid: R*f* = 0.2 (DCM/MeOH, ¼, v/v). ^{1}H NMR (500 MHz, CD_3OD) δ 7.61 – 7.53 (m, 1H), 7.44 – 7.25 (m, 7H), 5.43 (s, 1H), 4.37 (dd, *J* = 7.6, 2.3 Hz, 1H), 4.31 (dd, *J* = 7.8, 4.8 Hz, 1H), 3.99 – 3.87 (m, 2H), 3.87 – 3.74 (m, 4H), 3.71 (dd, *J* = 11.4, 4.6 Hz, 1H), 3.65 (dd, *J* = 6.7, 3.1 Hz, 1H), 3.61 – 3.46 (m, 4H), 3.33 – 3.23 (m, 4H), 2.83 (dd, *J* = 14.9, 4.0 Hz, 2H), 1.82 (t, *J* = 6.4 Hz, 2H). ^{1}C NMR (500 MHz, CD_3OD): δ 151.28, 129.88, 128.16, 128.08, 127.14, 127.08, 125.99, 125.71, 123.79, 121.42, 112.60, 109. 89, 103.92, 103.02, 79.48, 76.72, 75.90, 75.27, 73.64, 73.57, 71.38, 69.12, 67.08, 61.32, 60.74, 46.02, 37.77, 29.92. MALDI-MS ($C_{32}H_{39}NO_{13}$): Calculated ($[M+Na]^+$): 668.2319, found: 668.2382.

Model compound 16. To a solution of **1** (5 mg, 0.005 mmol) in 0.6 mL of methanol was added **3** (4.78 mg, 0.007 mmol). The mixture was stirred at ambient temperature for 3 h. The resulting solution was purified by LH20 Bio-gel column chromatography and after lyophilization of the appropriate fractions **16** (4.5 mg, 41%) was afforded as an amorphous white solid. ^{1}H NMR (600

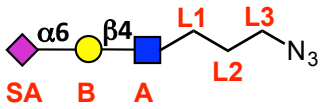
MHz, CD_3OD): δ 7.61 – 7.53 (m, 2H, ArH), 7.50 (d, *J* = 7.7 Hz, 2H, ArH), 7.48 – 7.39 (m, 2H, ArH), 7.39 – 7.34 (m, 2H, ArH), 7.34 – 7.17 (m, 6H, ArH), 7.11 (d, *J* = 18.6 Hz, 2H, ArH), 6.08 – 5.99 (m, 1H, C*H*OC(O)NH), 5.85 – 5.77 (m, 1H, C*H*OC(O)NH), 4.57 – 4.43 (m, 4H), 4.28 – 4.24 (m, 2H, 2xH-1), 4.21 – 4.12 (m, 2H, 2xH-1), 4.07 (d, *J* = 7.8 Hz, 2H), 4.02 – 3.88 (m, 2H), 3.88 – 3.81 (m, 2H), 3.81 – 3.75 (m, 4H), 3.75 – 3.70 (m, 2H), 3.70 – 3.64 (m, 2H), 3.60 (dd, *J* = 11.5, 4.1 Hz, 2H), 3.58 – 3.55 (m, 4H), 3.55 – 3.35 (m, 40H), 3.35 – 3.26 (m, 4H), 3.26 – 3.18 (m, 16H), 3.18 – 3.00 (m, 6H), 2.98 – 2.83 (m, 2H), 2.56 (t, *J* = 6.3 Hz, 8H, 4xCH_2), 2.08 (d, *J* = 8.1 Hz, 4H, 2x CH_2), 1.71 – 1.61 (m, 4H, 2xCH_2), 1.61 – 1.52 (m, 4H, 2xCH_2), 1.52 – 1.44 (m, 4H, 2xCH_2), 1.35 – 1.24 (m, 4H, 2xCH_2), 1.24 – 1.13 (m, 20H, 10xCH_2). MALDI-MS ($C_{110}H_{168}N_{10}O_{38}S_2$): Calculated ([M+Na]$^+$): 2324.1010, found: 2323.9386.

5-(acetamido)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonyl-(2→3)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-D-glucopyranosyl-β-1-azido propane (9).



Azido-linkered LacNAc[5] (3.2 mg, 6.86 µmol) and CMP-Neu5Ac (9 mg, 13.72 µmol) were dissolved in sodium cacodylate buffer (802 µL, 50 mM, pH 7.3) containing BSA (0.1%). CIAP (10 mU) and ST3Gal-IV (9.4 mU/µmol substrate) were added to achieve a final glycan concentration of 8 mM and the resulting reaction mixture was incubated at 37 °C for 36 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Biogel P-2 (eluent 5% aq. *n*-butanol). Fractions containing product were combined and lyophilized to give the compound (4 mg, 77%) as an amorphous white solid. ^{1}H NMR (500 MHz; D_2O): 1H-NMR (500 MHz; D2O): δ 1.74-1.65 (m, 3H, SA H-3b, L2-CH_2), 1.91 (d, *J* = 6.9 Hz, 6H, 2x CH_3CO), 2.63 (dd, *J* = 12.3, 4.2 Hz, 1H, SA H-3a), 3.25 (td, *J* = 6.5, 2.5 Hz, 2H, L3-CH_2), 3.63-3.42 (m, 13H, B H-2, SA H-7, A H-5, SA H-9b, SA H-6, L1-CH*H*, SA H-4, A H-3, B H-5, A H-4, A H-2, B H-6a,b), 3.78-3.70 (m, 4H, SA H-5, A H-6b, SA H-9a, SA H-8), 3.89-3.83 (m, 3H, A H-6a, L1- C*H*H, B H-4), 3.99 (dd, *J* = 9.7, 2.3 Hz, 1H, B H-3), 4.40 (d, *J* = 7.9 Hz, 1H, A H-1), 4.42 (d, *J* = 7.9 Hz, 1H, B H-1). ^{13}C NMR assigned from HSQC (125 MHz, D_2O): δ 22.22 (x2), 28.08, 39.41 (x2), 47.61, 51.52, 55.03, 59.72 (x2), 62.45 (x2), 66.75 (x2), 67.14, 67.53, 67.92, 69.09, 69.81 (x2), 71.44, 72.22, 72.61, 74.56, 74.95, 75.34, 78.08, 100.73, 102.29. MALDI-MS: [M+Na]$^+$ $C_{28}H_{47}N_5NaO_{19}$, calcd 780.2763, found 780.1310.

5-(acetamido)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-D-glucopyranosyl-β-1-azido propane (10).



Azido-linkered LacNAc (2.4 mg, 5.145 μmol) and CMP-Neu5Ac (6.8 mg, 10.29 μmol) were dissolved in sodium cacodylate buffer (612 μL, 50 mM, pH 7.3) containing BSA (0.1%). CIAP (10 mU) and ST6Gal-I (3.4 mU/μmol substrate) were added to achieve a final glycan concentration of 8 mM and the resulting reaction mixture was incubated at 37 ^{o}C for 18 h. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over Biogel P-2 (eluent 5% aq. *n*-butanol). Fractions containing product were combined and lyophilized to give compound **10** (2.8 mg, 72%) as an amorphous white solid. ^{1}H NMR (500 MHz; D_2O): δ 1.62 (t, *J* = 12.2 Hz, 1H, SA H-3b), 1.76 (quintet, *J* = 6.3 Hz, 2H, L2- CH_2), 1.94 (s, 3H, C*H*$_3$CO), 1.98 (s, 3H, C*H*$_3$CO), 2.58 (dd, *J* = 12.4, 4.6 Hz, 1H, SA H-3a), 3.30 (td, *J* = 6.6, 1.7 Hz, 2H, L3-C*H*$_3$), 3.48-3.43 (m, 3H, B H-2, B H-6a, SA H-7), 3.82-3.53 (m, 14H, A H-5, A H-4, SA H-9a, SA H-4, B H-3, SA H-6, A H-3, L1-CH*H*, A H-2, SA H-5, B H-5, A H-6a, SA H-9b, SA H-8), 3.84 (d, *J* = 3.3 Hz, 1H, B H-4), 3.92-3.87 (m, 3H, L1-C*H*H, B H-6b, A H-6b), 4.36 (d, *J* = 7.9 Hz, 1H, B H-1), 4.47 (d, *J* = 8.1 Hz, 1H, A H-1). ^{13}C NMR assigned from HSQC (125 MHz, D_2O): δ 21.83 (x2), 28.08, 39.79 (x2), 47.61, 51.91, 54.64, 60.11 (x2), 62.45 (x2), 63.23 (x2), 67.14 (x2), 67.92, 68.31, 68.31, 70.66, 71.44, 72.22 (x2), 72.61, 73.39, 74.17, 80.42, 100.74, 103.47. MALDI-MS: $[M+Na]^+$ $C_{28}H_{47}N_5NaO_{19}$, calcd 780.2763, found 780.1866.

Methods

SERS substrate fabrication. ITO glass slides and glass microscope slides were cleaned for 20 min using heated piranha solution containing a mixture of 4:1 H_2SO_4:H_2O_2, followed by rinsing with MilliQ 18.2 megaohm water and drying with a gentle nitrogen N_2 gas stream. Titanium (10 nm) and gold (300 nm) were deposited at a rate of 0.5-1 Å/s onto the ITO glass slides and 20 nm of Cr and 200 nm of gold were deposited onto glass microscope slides using the PVD75 electron beam deposition system where background pressure was maintained at less than 5.5 x 10^{-6} Torr.

The substrate for galectins detection: a multi-well array was assembled using a molding patterning on the ITO glass slides deposited with titanium and gold thin film. In this process a stainless-steel mold was pre-heated in an oven at 55 ºC for 30 min. After pre-heating, a mixture of polydimethylsiloxane (PDMS, Slygard 184 Elastomer Kit), curing agent and accelerator

(10:1:0.5, w/w) were poured through the mold opening. This mixture was cured at 70 ºC for 45 min. After the curing process, the mold was removed from the oven and cooled down to room temperature. The SERS substrate was detached from the stainless steel mold and excess PDMS was removed with a razor blade. This process generated a uniform 4 x 10 multi-well array substrate as shown in Scheme 1.

The substrate for influenza HA detection: the glass microscope slides deposited with gold were treated with disulfide amine (**6**) in methanol or DMF solution to form a monolayer of peg-amine to catch the monolayer of gold nanoparticles which was fabricated by ionic surfactant-mediated Langmuir-Blodgett method modified from a reported method.[6] Briefly, 3.5 mL of hexane was added to a solution of 7 mL of gold nanoparticles collid (60 nm, 1e11 particles/mL), 0.01% CTAB (0.35 mL) in a 5 cm petri dish to develop the water/hexane surface. Ethanol (3.5 mL) was slowly added to the solution in a dropwise fashion (0.2 mL/min). Upon the addition of the methanol, gold nanoparticles moved out of the aqueous phase to the water/hexane interface and a gold nanoparticles film was formed and packed with the slow evaporation of hexane. After the Au film was made, the slides treated with compound **6** were dipped into the solution and pulled out to transfer the Au monolayer film onto the slides. The assembled Au monolayer film was characterized by scanning electron microscopy (SEM). Then, a multi-well array was assembled using the molding patterning process described above on the substrate covered with the Au monolayer film.

To access the reproducibility of the SERS substrate fabrication method used in this paper, SERS spectra collected from substrates fabricated from the same batch and different batches were compared. SERS collection parameters used for measurements were 785 nm laser excitation source, 0.1% laser power, 10 sec acquisition time, 1 accumulation. A spectral range from 1800 to 600 cm^{-1} was collected. Ten microliters of a 4-aminothiphenol (4-ATP; 1 mM) solution in ethanol was dropped onto the AuNP substrate and allowed to evaporate. Three substrates were made for each batch and SERS spectra were collected from eight different spots on each substrate. Ten microliters of the 4-ATP solution were also placed onto a bare silicon wafer that had been functionalized with (3- mercaptopropyl)-trimethoxy-silane (MPTMS) without AuNP for control experiments.

SERS spectra collected are shown in Fig S5b. The spectrum of 4-ATP taken on the CTAB AuNp substrate agrees well with what has been reported in the literature. The spectrum shows

great enhancements over the SERS spectrum of 4-ATP taken on substrates that did not contain AuNPs. The SERS spectra were averaged and the relative standard deviation (RSD) was calculated and used to characterize the substrates reproducibility. The SERS band at 1084 cm^{-1} represents the (C-C) bond in 4-ATP.

Immobilization of DIBO-lactose to gold nanoparticles. The lactose was covalently linked to Au nanoparticles in two steps. First, the azide containing nanoparticle was prepared by adding 0.25 mL of **1** (2 mM) and **2** (8 mM) in DMF to 4.5 mL of 60 nm Au colloid stabilized with citrate acid in a centrifuge tube. The mixture was incubated at room temperature following by centrifuging at 1050 rpm for 10 min and washing with distilled water for three cycles after 8-12 h incubation. After the third time centrifugation, the nanoparticles were resuspended into 1.0 mL of water and sonicated for 20 min to ensure particles were free from aggregation. Next, 50 μL of DIBO-lactose (**3,** 4 mM) in water was added to 450 μL of the prepared azide containing Au nanoparticles. The solution was incubated for another 8-12 h and purified using the same centrifugation and washing steps to remove the excess of DIBO-lactose and sonication procedure mentioned above to reconstitute the final Au nanoparticles into sterile water.

A model compound (**16**) was synthesized to simulate the clicked product. Compound **16** was dissolved in DMF and attached to gold nanoparticles though the gold-sulfur bond by the same mixing and centrifuge procedure. After all samples were prepared for SERS analysis 10 μL of each sample was dropped into the multi-wells of the patterned gold film substrate and allowed to dry overnight.

Gal1, Gal3 and BSA binding studies by SERS. Gal1, Gal3 or BSA was added to the lactose attached Au nanoparticles at various final concentrations (100, 50, 25, 5 and 1 μg/ml) in PBS buffer and the mixture was incubated for 2 h at 37 ºC. The nanopaticles were purified by three cycles of centrifugation and washing with PBS and sterile water in the third cycle. Then the samples were sonicated to resuspend the particles into water. For SERS analysis 10 μL of the gold nanoparticle solution was placed onto the gold film multiwall substrate and allowed to dry overnight.

Raman spectroscopy. All samples were prepared in duplicates. SERS spectra were measured using a confocal Raman microscope (InVia, Renishaw, Inc. Gloucestershire, United Kingdom). Laser excitation was provided by a 785 nm near-IR diode laser. The sample was illuminated through a 20x (N.A. = 0.40) objective, resulting in a spot size of approximately 4.8 x 27.8 μm. The laser power measured at the sample was ~3.62 mW. SERS spectra were acquired from five different spots within a given micro-well using a 10 s acquisition time with three accumulations. The spectral range recorded was between 2300 and 350 cm^{-1}.

Gal3 and BSA binding study by fluorescence. Gal3 or BSA-biotin was added to the lactose attached Au nanoparticles and Au nanoparticles with citrate (negative control) at different final concentrations (100, 5, and 1 μg/mL) in PBS buffer and the mixture was incubated for 2 h at 37 ºC. The nanoparticles were purified by three cycles of centrifugation and washing with PBS and reconstituted into PBS. Anti-human Gal3-Alexa Fluor 488 (200 μL, 20 μg/mL) and Cy3-streptavidin (100 μL, 100 μg/mL) was added to the 100 μL Au nanopaticles with BSA-biotin and Gal3 treatment, respectively or without protein treatment as negative control and the solutions were incubated for 1 h at 37 ºC followed by three cycles of centrifugations and washes. The nanoparticles were resuspended into 200 μL PBS and their fluorescence (excitation 485 nm, emission 520 nm for anti-human Gal3-Alexa Fluor 488 and excitation 550 nm, emission 570 nm for Cy3-streptavidin) were measured in black 96-well plates using a fluorescent microplate reader (BMG Labtech). The bound proteins were calculated based on the corresponding standard curve of anti-human Gal3-Alexa Fluor 488 and Cy3-streptavidin.

Immobilization of azido-lactose, α2,3 and α2,6 Neu5AclacNAc to gold nanoparticles monolayer hooked on the substrate. The mixture of **7** (50 μL, 2 mM in DMF) and azido-lactose, or α2,3 or α2,6 Neu5AclacNAc (50 μL, 4 mM in sterile water) was stirred for 8 h at room temperature. Ten μL of **2** (40 μM) and the pre-mixture of **7** and azido-lactose, or α2,3 or α2,6 Neu5AclacNAc were added to the fabricated SERS substrate. The substrates were placed into a petri dish and sealed for incubation for 8 h in a humidity environment. Next, the wet substrates were washed three times with 50 μL sterile water, dried with a gentle N_2 stream and analyzed by SERS.

Binding of influenza hemagglutinin. H7N9 HA (avian HA) and H1N1 HA (human HA) were dissolved at 100 μg/mL and further diluted to concentrations of 50, 25, 10, 5, 1 and 0.1 μg/mL in a sterile buffer (Tris (0.6%), glycine (0.7%) in PBS, pH 7.5). Binding of the carbohydrates SERS sensing surface to HA was accomplished by adding 10 μL of the HA at the desired concentration to the carbohydrates immobilized substrates. The SERS substrates were incubated for at least 8 h at 37 ºC inside a humidity chamber. After incubation, HA solutions were removed by washing twice with the respective binding buffers and a final wash with 18.2 megaohm MilliQ water. The substrates were then dried by a gentle N2 stream and analyzed by SERS.

Data analysis. SERS spectra were imported into GRAMS 32/AI spectral software package (Galactic industries, Nashua, NH) for spectral averaging and baseline correction. Multivariate analysis for classification was performed using partial least-squares discriminate analysis (PLS-DA)[7] and carried out in PLS toolbox 4.0 (Eigenvector Research Inc., Wenatchee, WA) operated in a MATLAB environment (R2013b, The Mathworks Inc., Natick, MA). Prior to analysis, raw SERS spectra were preprocessed using a first order Savitsky-Golay derivative filter (15 point, 2^{nd} order polynomial algorithm), normalized to unit vector length and mean centered. These preprocessing methods eliminate non-analyte spectral variances such as baseline and substrate variations caused by instrumental drift, nonuniformity between different microwells on the substrate and environmental changes. Unless otherwise specified, all PLS-DA models were cross-validated using a Venetian blinds algorithm with 10 data splits.

Partial least squares discriminant analysis. PLS-DA is a supervised statistical classification method, where prior knowledge of the classes is used for classification.[7c] This method minimizes the contribution of spectral features which vary within a particular class and maximizes the contribution of spectral features among the different classes.[7b] PLS-DA incorporates both the xblock and yblock. The yblock contains variables of 0 or 1 which serve as a reference for each sample. This number indicates whether a sample belongs to a particular class. The models generated by PLS-DA are tested by cross-validation using the Venetian Blinds method. PLSDA was to differentiate different proteins and to access nonspecific protein binding.

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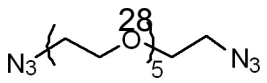
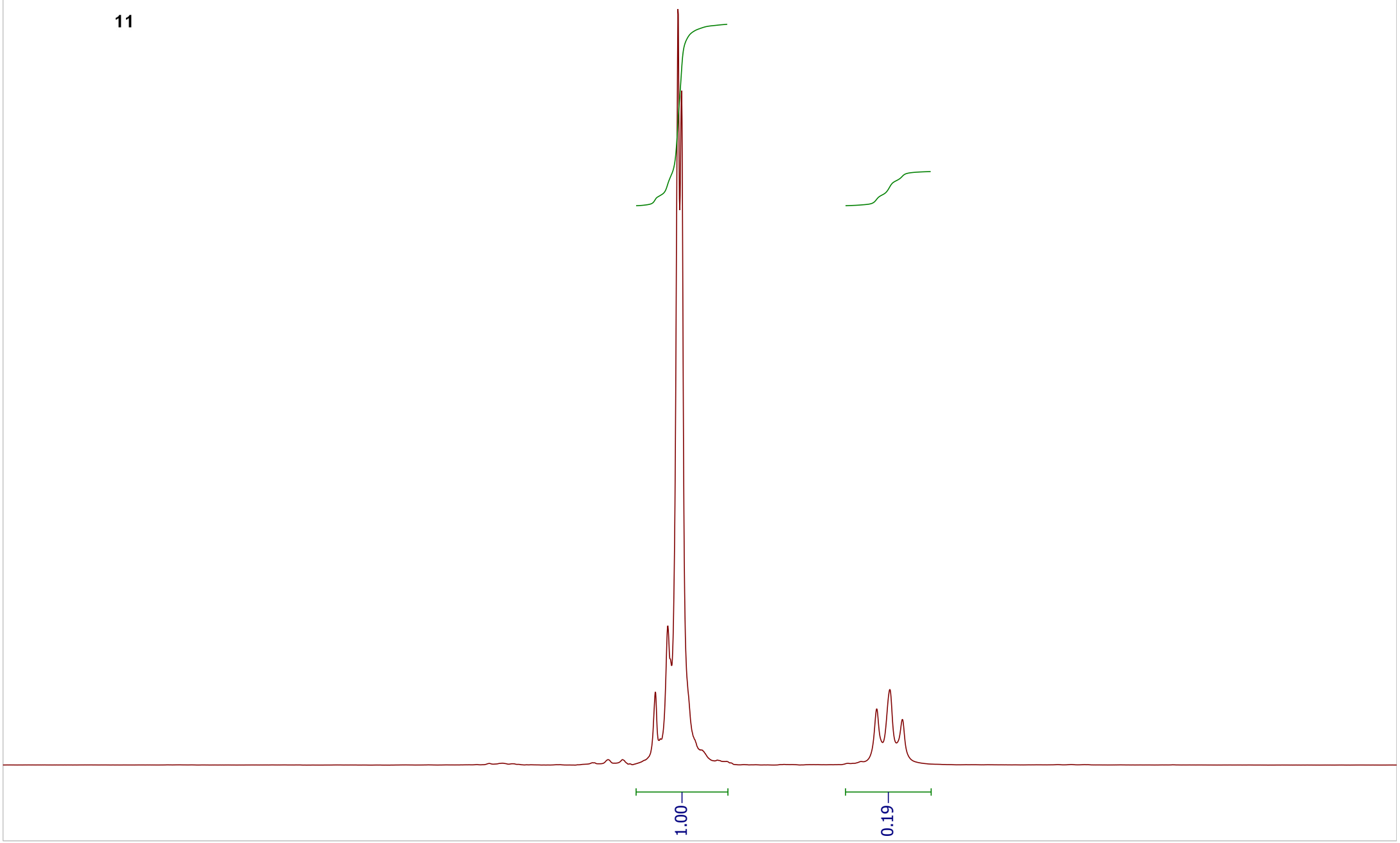
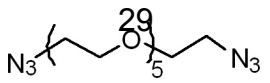

28
O
5
N3
N3

11

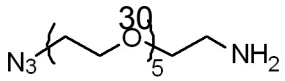

1.00
0.19
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
f1 (ppm)



11

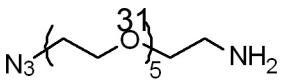
92 90 88 86 84 82 80 78 76 74 72 70 68 66 64 62 60 58 56 54 52 50 48 46 44 42 40 38

f1 (ppm)


30
O
5
N3
NH2

5

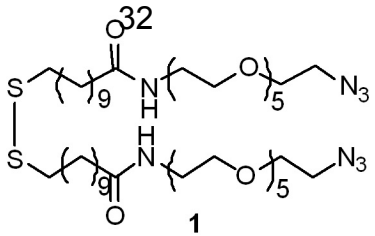
1.00
9.89
0.88
2.02
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6
f1 (ppm)



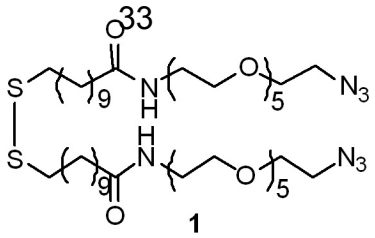
5

92 90 88 86 84 82 80 78 76 74 72 70 68 66 64 62 60 58 56 54 52 50 48 46 44 42 40 38 36

f1 (ppm)

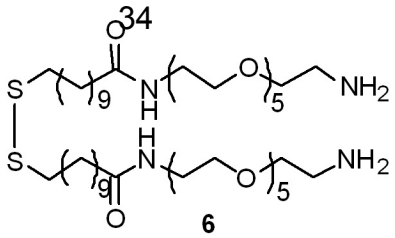

32
S
9
N
H
O
5
N3
1

1.00
17.44
1.89
3.51
1.75
1.70
3.52
11.69
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


O
33
S
9
N
H
O
5
N3
S
H
N
9
O
5
N3
O

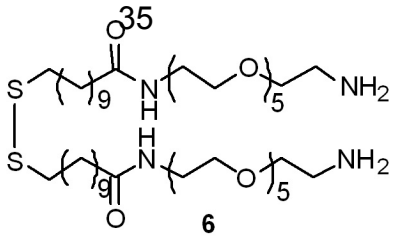
1

220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


O
34
S
9
N
H
O
5
NH2
S
9
N
H
O
5
NH2
O

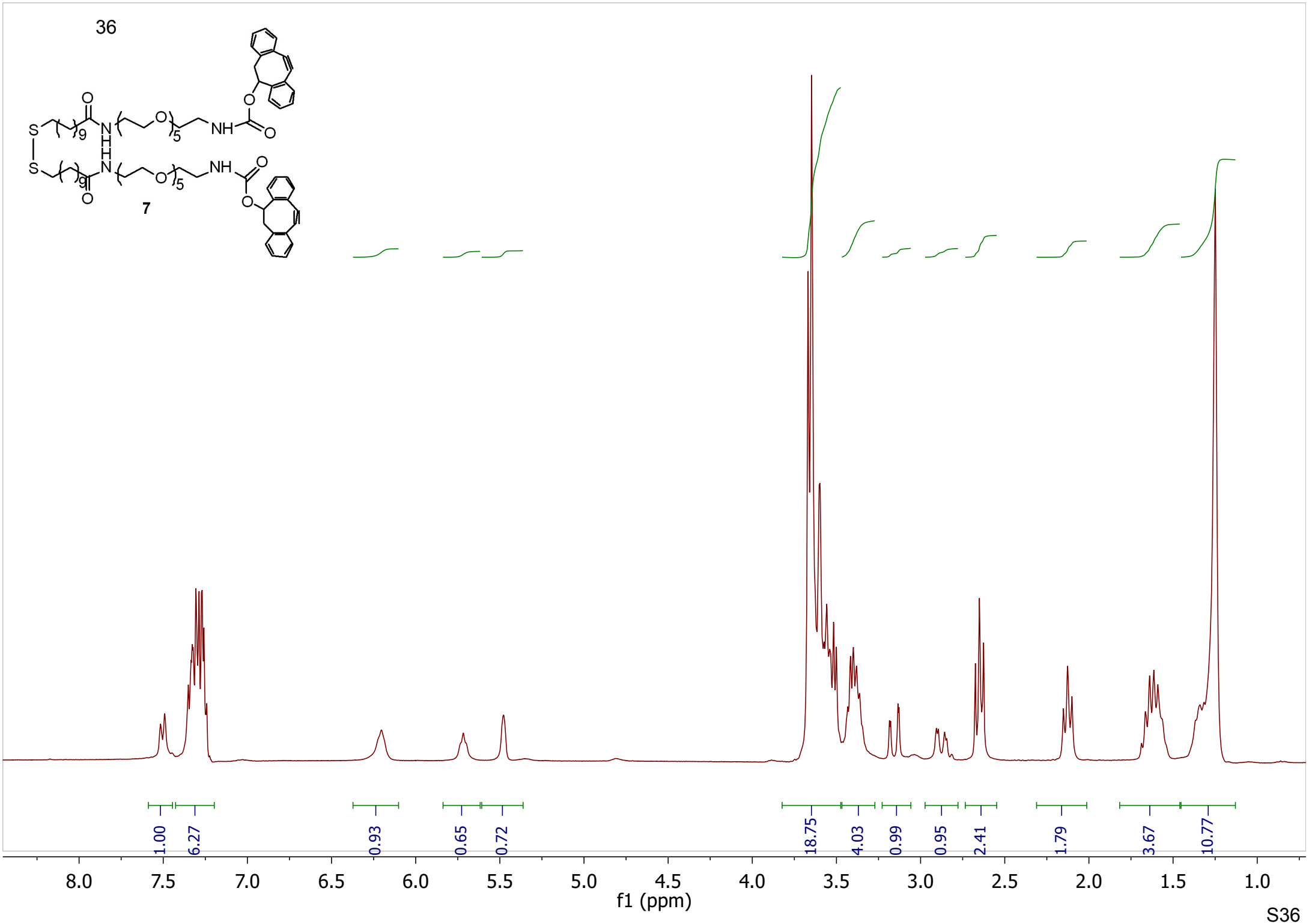
6

1.00
23.94
2.47
2.16
2.34
2.31
3.19
4.78
13.74
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

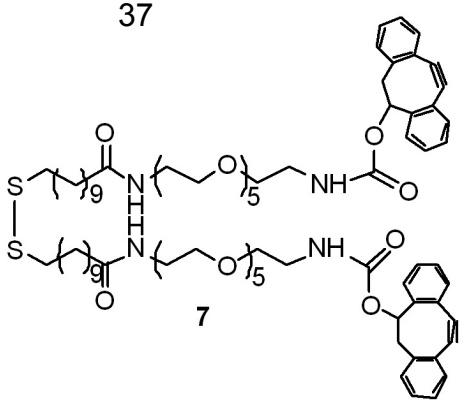

35
S
9
N
H
O
5
NH2
S
9
N
H
O
5
NH2
O

6

220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

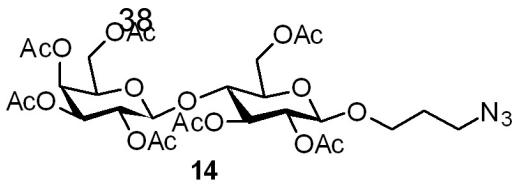

36
7
1.00
6.27
0.93
0.65
0.72
18.75
4.03
0.99
0.95
2.41
1.79
3.67
10.77
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

37

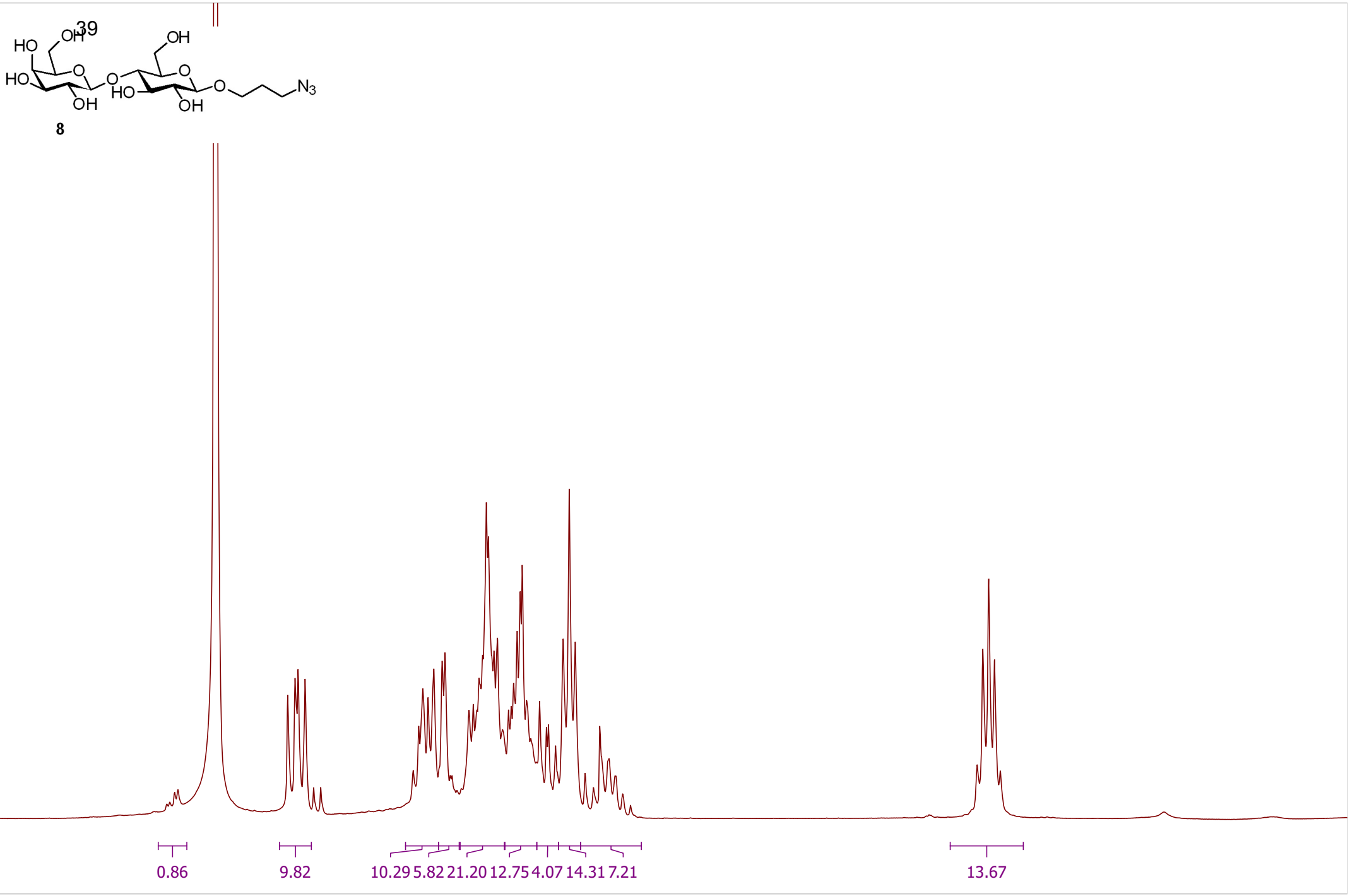


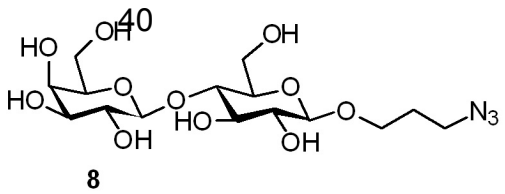
7

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


38
AcO
OAc
AcO
O
O
AcO
O
O
N3
OAc
OAc
OAc
OAc
14

0.03
1.00
1.16
1.13
1.18
1.09
3.13
3.76
3.58
2.27
2.05
3.35
4.21
14.36
3.14
2.02
0.95
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1
f1 (ppm)


39
HO
OH
HO
O
O
OH
HO
OH
OH
O
O
HO
OH
O
N3
8
0.86
9.82
10.29
5.82
21.20
12.75
4.07
14.31
7.21
13.67
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
f1 (ppm)

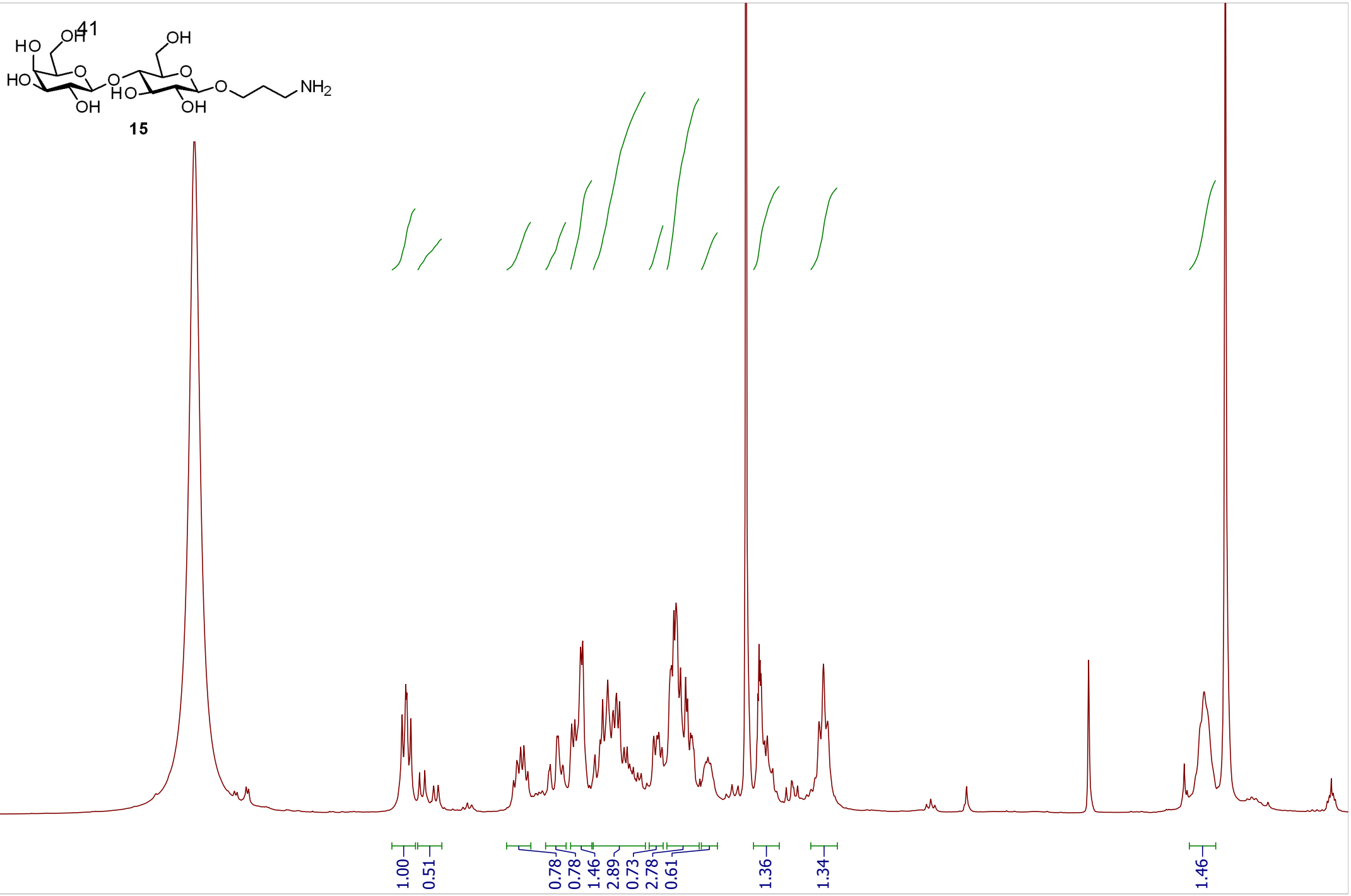


8

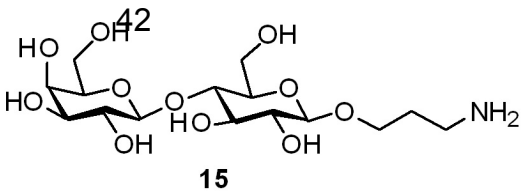
130.02
103.10
103.04
102.45
102.30
98.53
98.35
98.10
78.58
75.52
74.95
74.55
72.99
72.70
71.13
68.72
67.54
61.19
49.05
48.36
48.32
48.29
48.06
28.42
28.14

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

f1 (ppm)

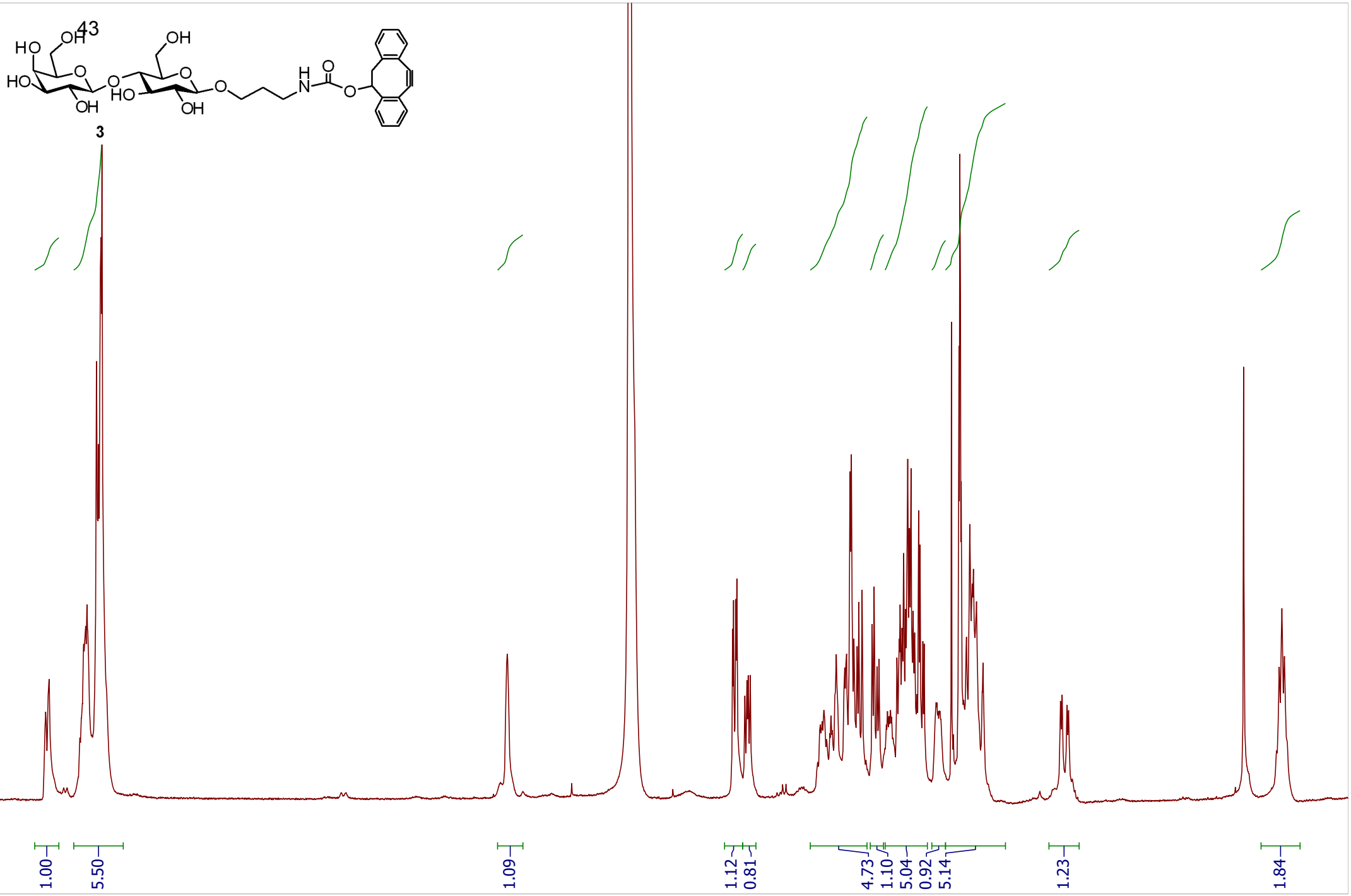

41
HO
OH
HO
O
O
HO
OH
OH
O
O
HO
OH
O
NH2
15
1.00
0.51
0.78
0.78
1.46
2.89
0.73
2.78
0.61
1.36
1.34
1.46
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
f1 (ppm)

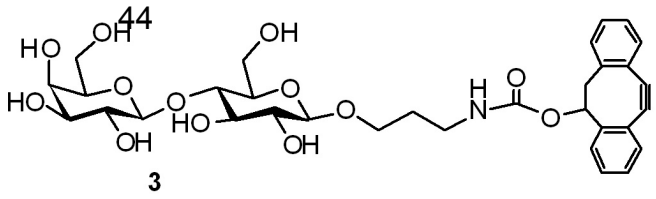
42


HO
OH
HO
O
O
HO
OH
OH
O
O
NH2
OH
OH

15

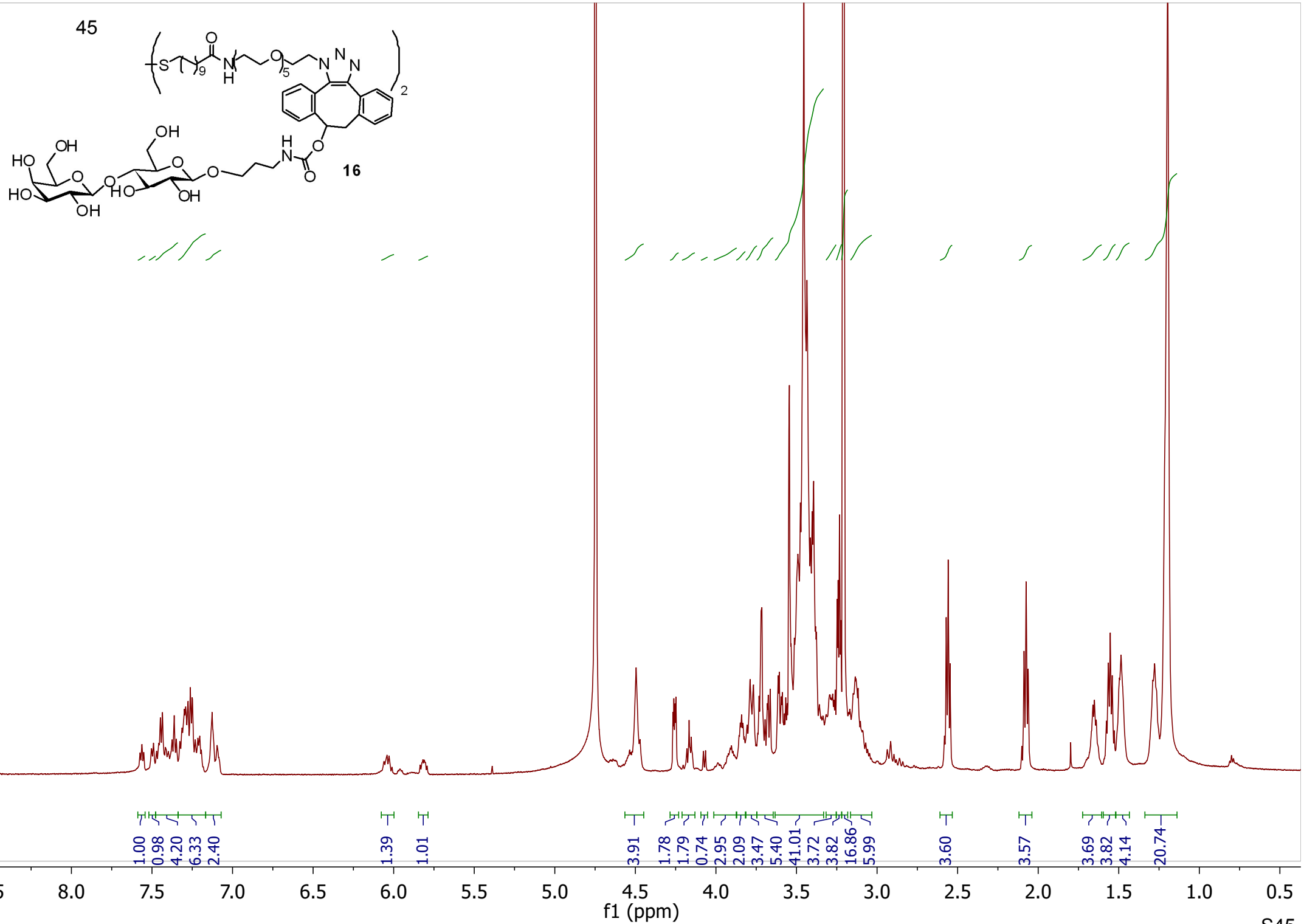
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
f1 (ppm)

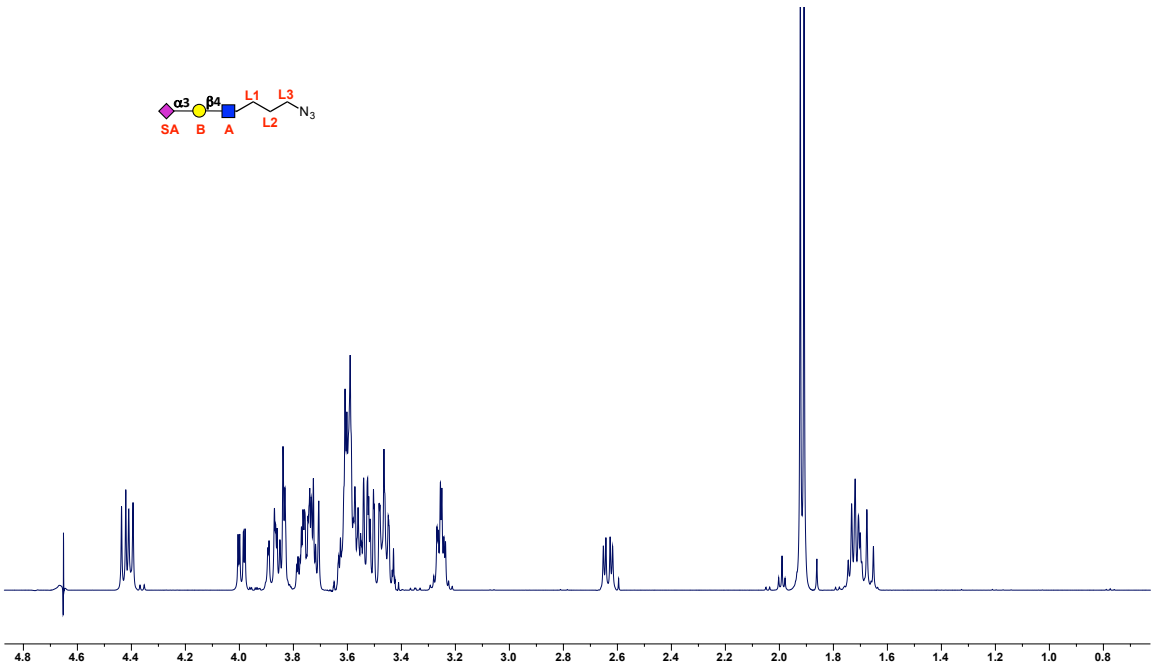

43
3
1.00
5.50
1.09
1.12
0.81
4.73
1.10
5.04
0.92
5.14
1.23
1.84
7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6
f1 (ppm)

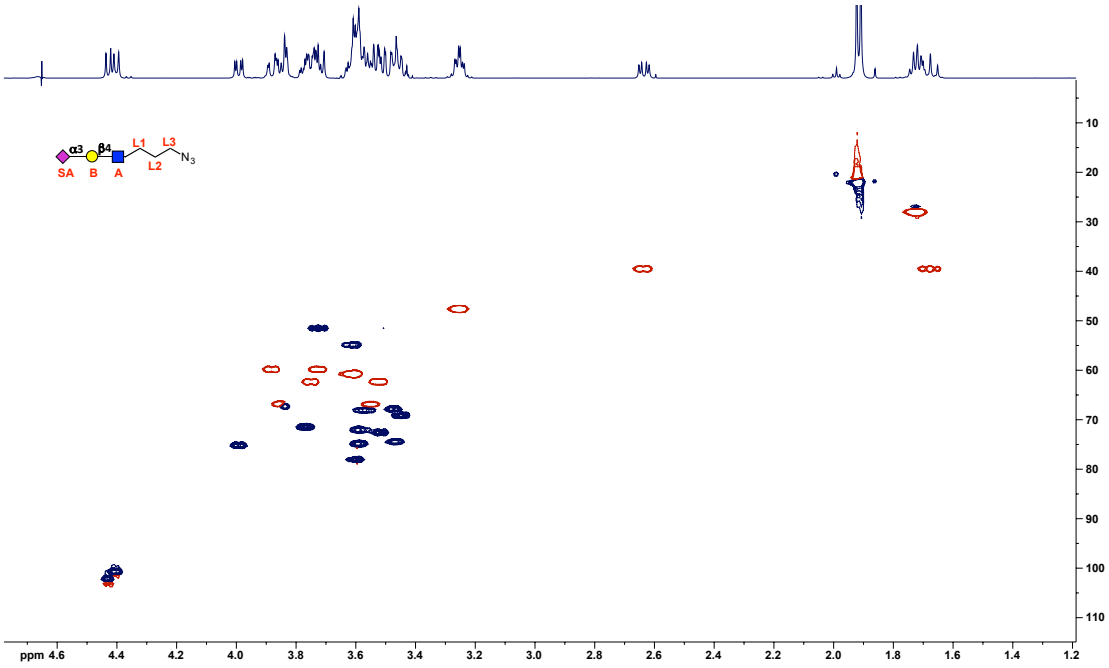

44
HO
OH
HO
O
O
OH
OH
O
HO
OH
O
H
N
O
O

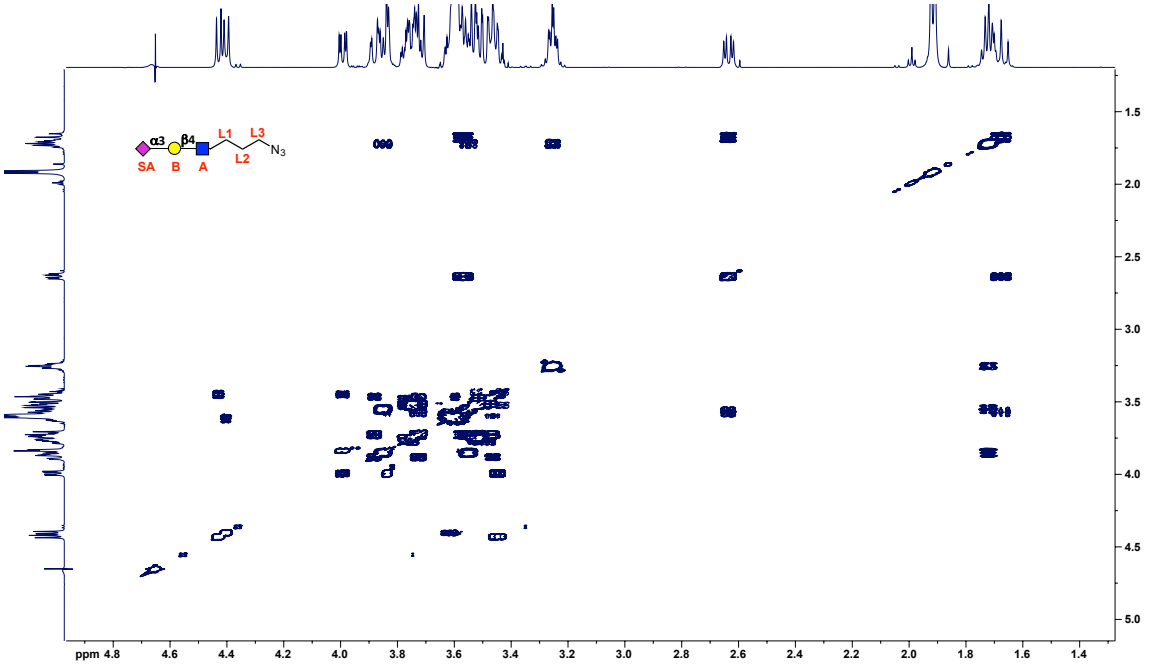
3

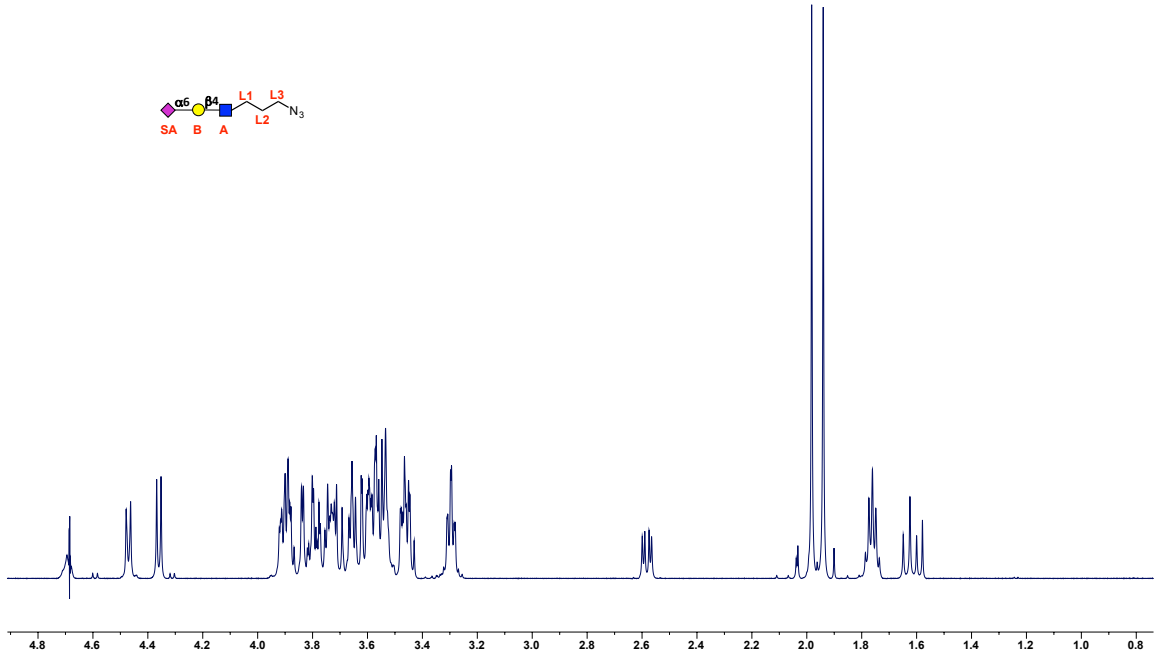
165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25
f1 (ppm)

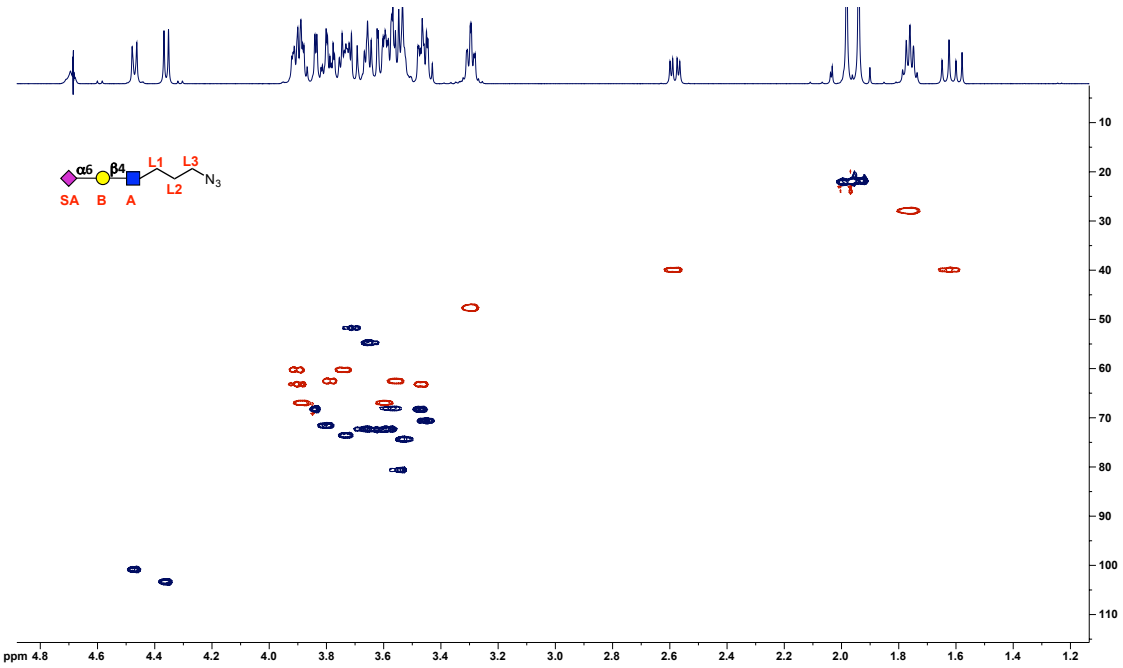

45
16
1.00
0.98
4.20
6.33
2.40
1.39
1.01
3.91
1.78
1.79
0.74
2.95
2.09
3.47
5.40
41.01
3.72
3.82
16.86
5.99
3.60
3.57
3.69
3.82
4.14
20.74
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

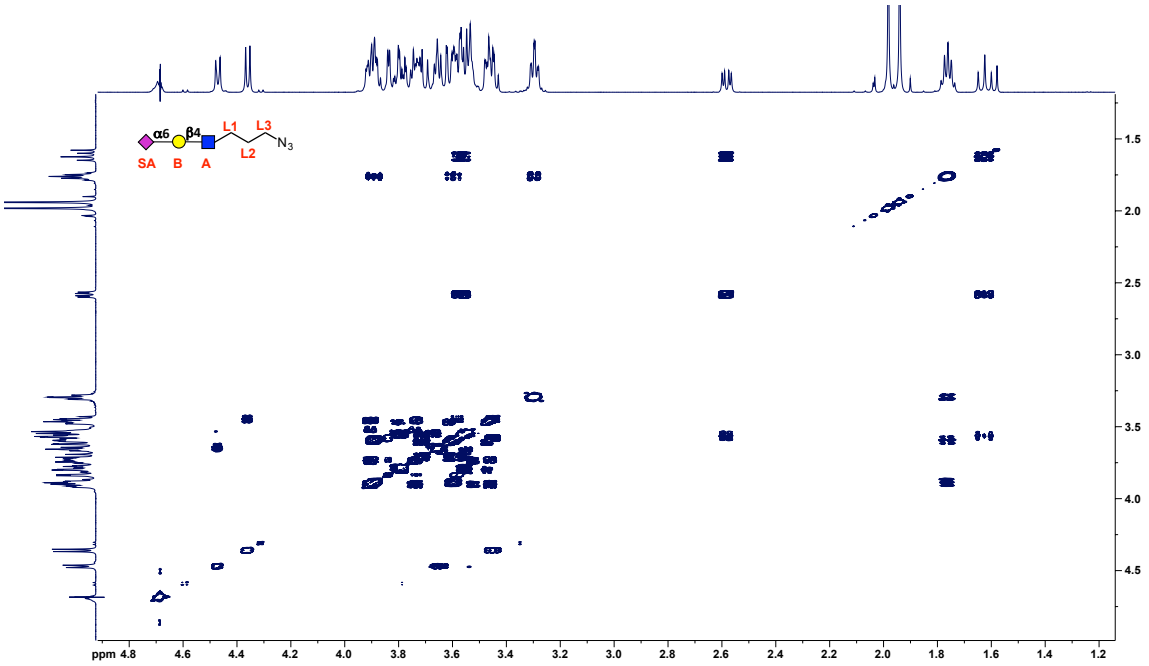

α3
β4
L1
L3
L2
SA
B
A
N_3
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8


α3
β4
L1
L3
L2
SA
B
A
N_3
10
20
30
40
50
60
70
80
90
100
110
ppm 4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2


α3 β4 L1 L3
SA B A L2
N3
ppm 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4
1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0


α6 β4 L1 L3
SA B A L2
N3
4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8


α6
β4
L1
L3
L2
N3
SA
B
A
ppm 4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
10
20
30
40
50
60
70
80
90
100
110


α6
β4
L1
L3
L2
N3
SA
B
A
ppm 4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.5
2.0
2.5
3.0
3.5
4.0
4.5