Electronic Supporting Information

Recognition of glycosaminoglycan chemical patterns using an unbiased sensor array

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Fluorescence titration curves

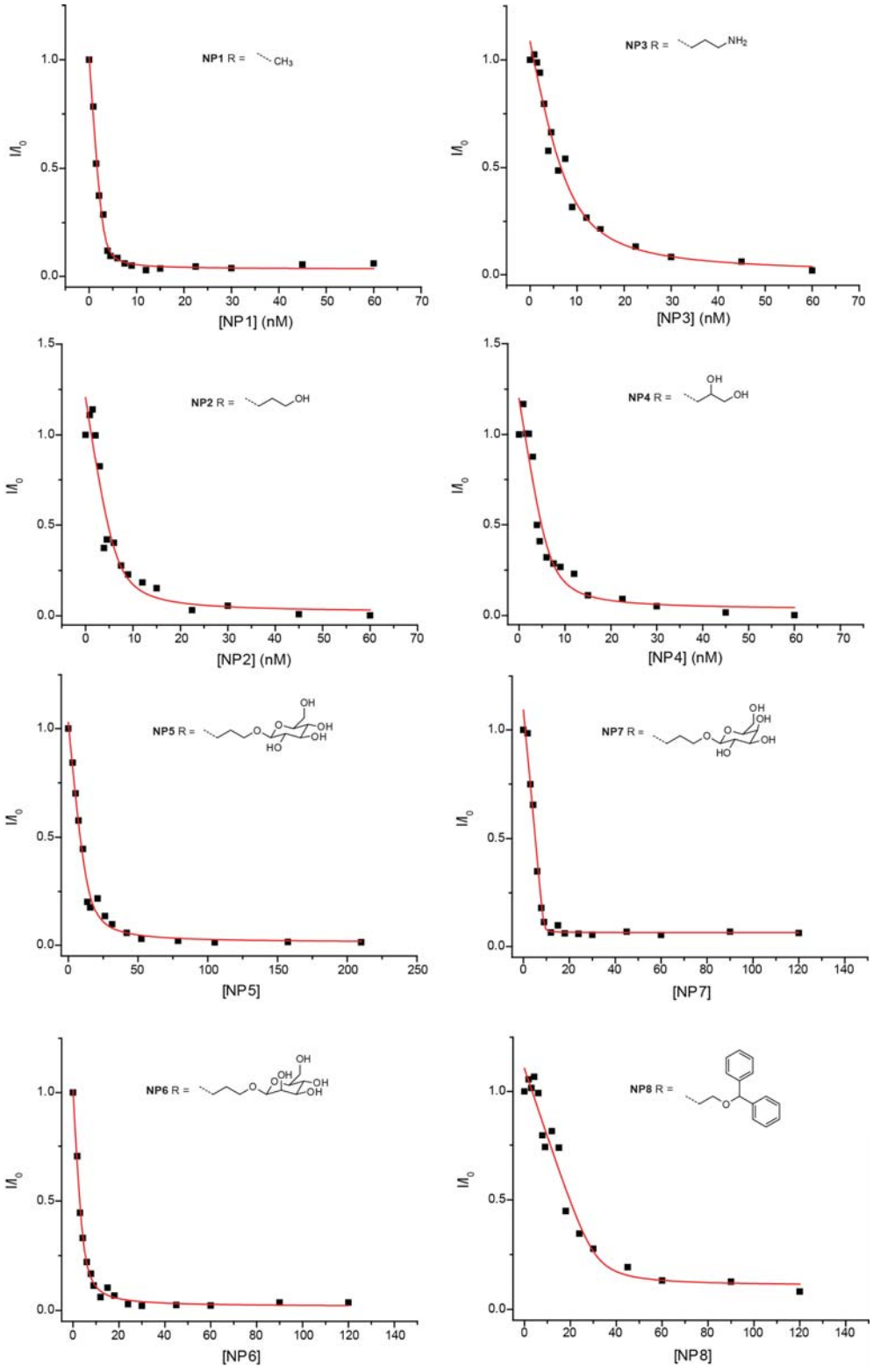


Figure S1. Fluorescence titration curves of the gold nanoparticles with the polymer PPE.

Fluorescence response patterns

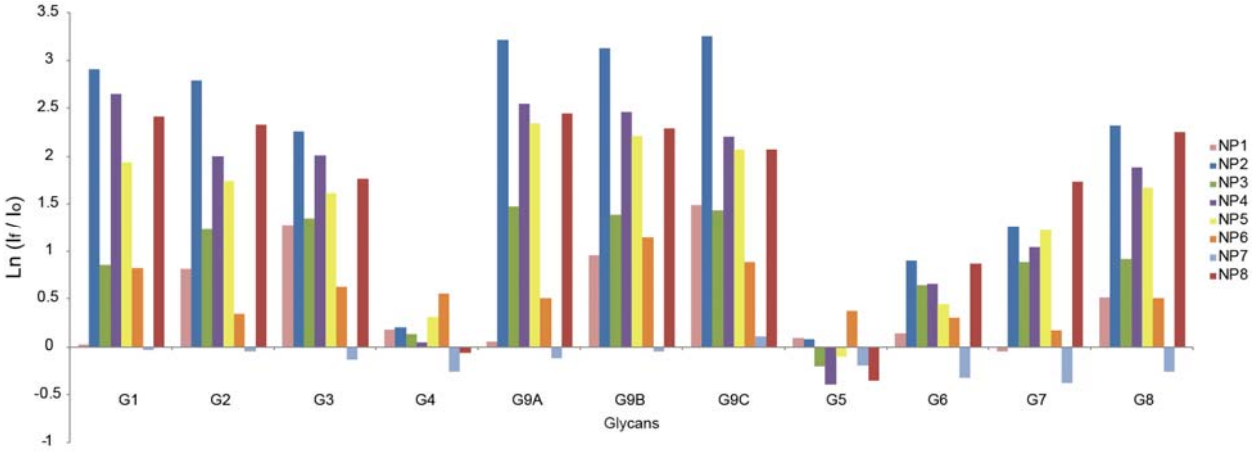


Figure S2. Fluorescence response patterns for all the glycosaminoglycans under study.

Dose response curve

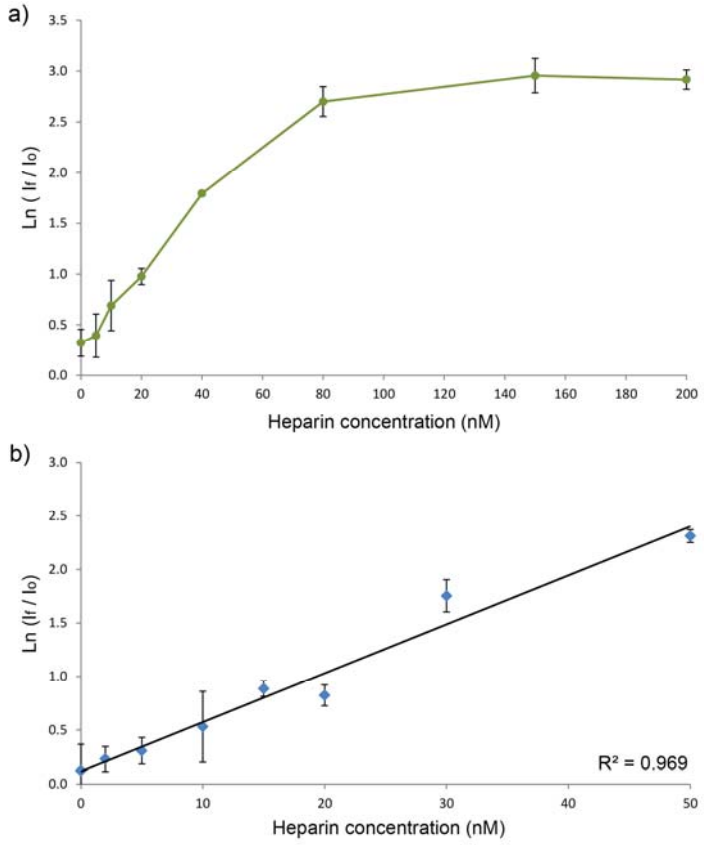


Figure S3. (a) Response curve from 0 to 200 nM of heparin.
(b) Dynamic range of the response curve from 0 to 50 nM of heparin.

Classifications analysis

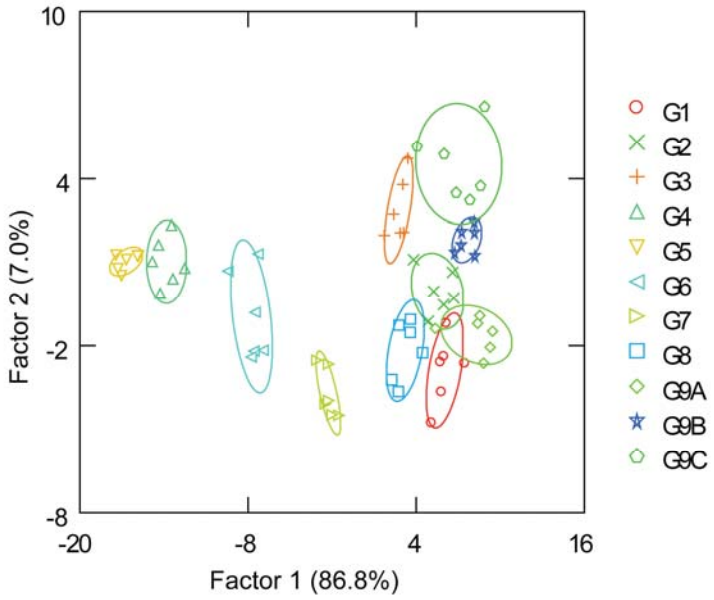


Figure S4. LDA canonical score plots for all the glycosaminoglycans under study.

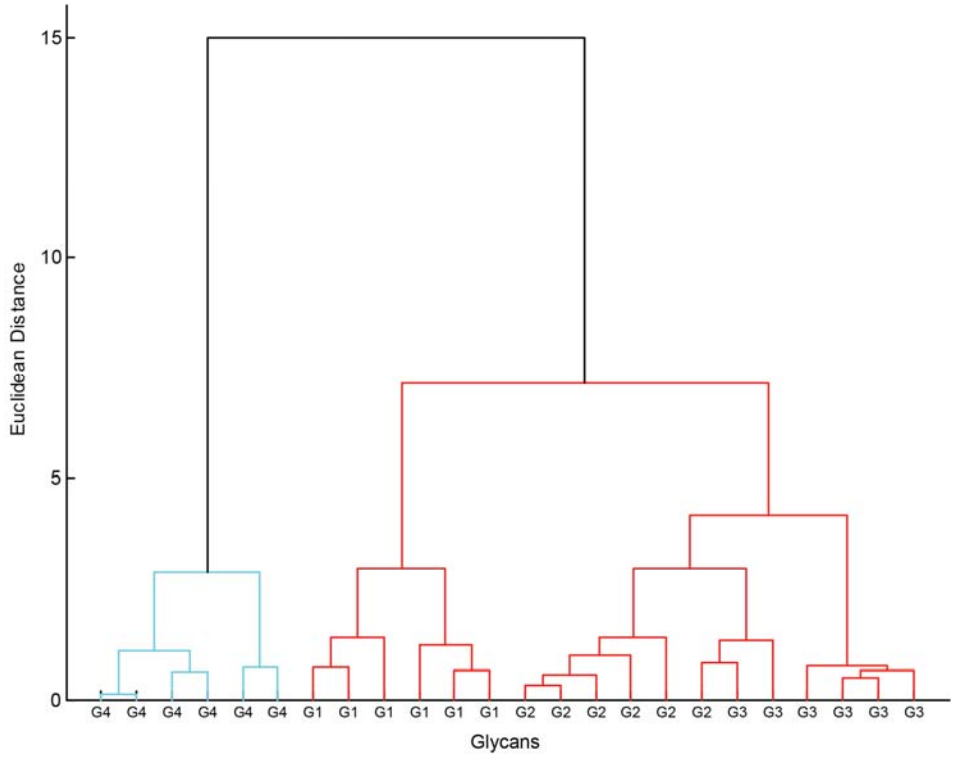


Figure S5. HCA of structurally different heparin molecules (G1:Heparin, G2:N-acetyl Heparin, G3: De-N-sulfated Heparin and G4: N-acetyl-de-O-sulfated Heparin).

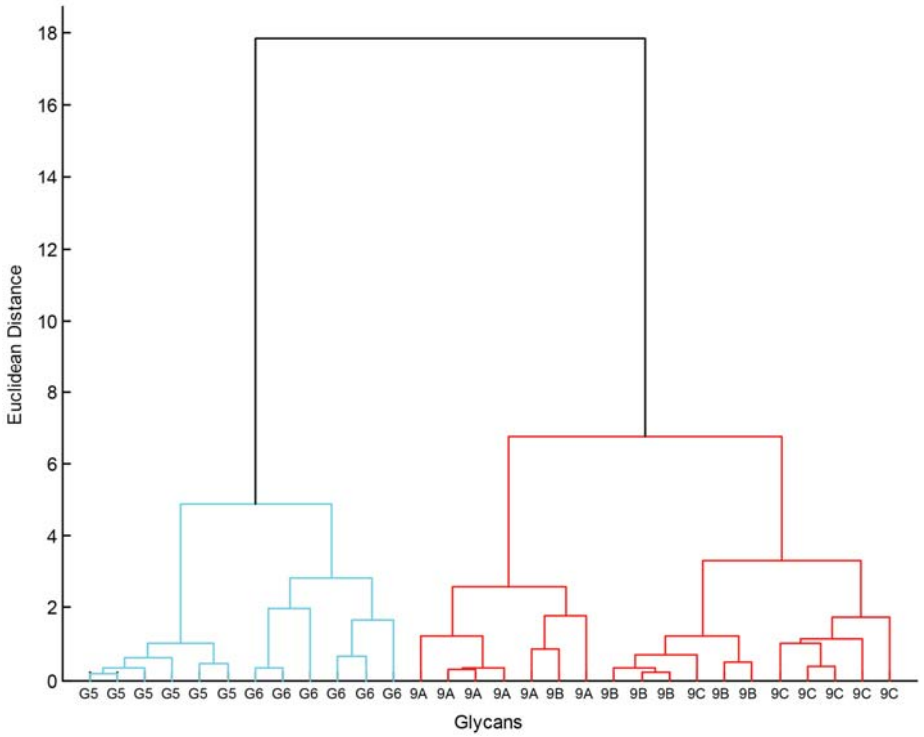


Figure S6. HCA of glycans with different size and charge. (G5:Chitosan, G6: Hyaluronic Acid, 9A: Dextran Small (8kDa), 9B: Dextran Medium (15kDa) and 9C: Dextran Large (500kDa)).

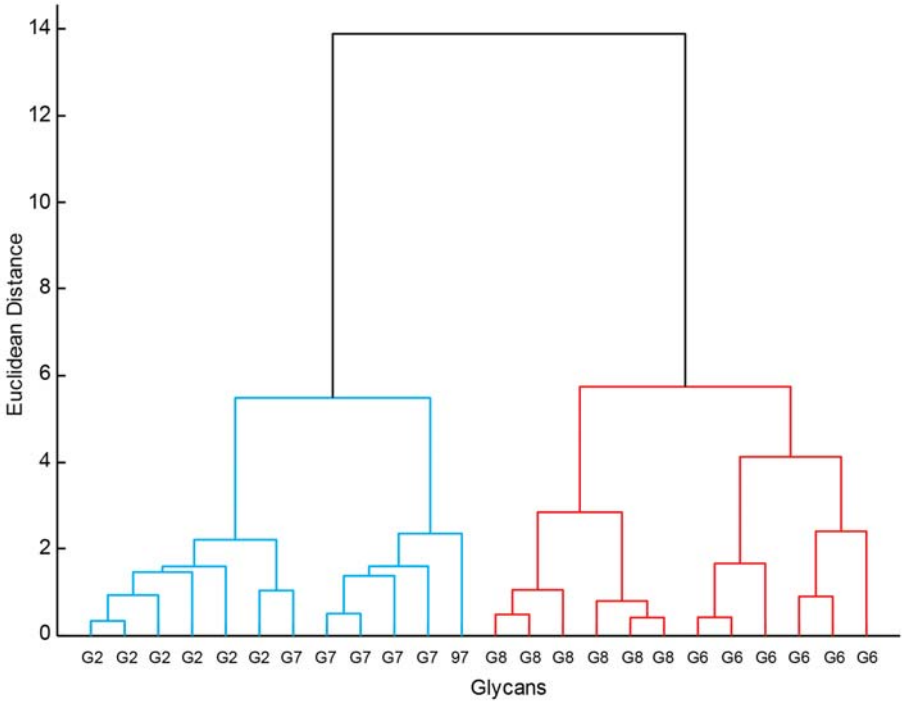


Figure S7. HCA of glycans with different chirality. (G7: Chondroitin sulfate B, G8: Chondroitin sulfate A, G2: N-acetyl Heparin and G6: Hyaluronic acid).

Unknown Classifications

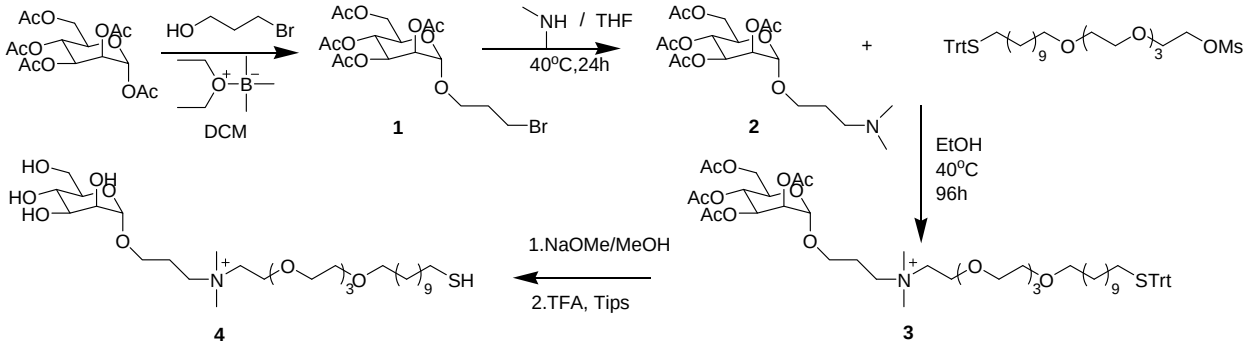
Table S1. Classification of unknown samples

NP1	NP2	NP3	NP4	NP5	NP6	NP7	NP8	Identity	Verification
0.23011	2.62852	1.09913	2.53989	2.47026	0.87087	0.29267	2.16106	**G1**	**G1**
0.25107	3.48374	0.75197	2.77665	2.53028	0.69824	0.00756	2.33915	**G1**	**G1**
-0.03274	3.22708	0.64278	2.80231	2.99879	0.38854	-0.07536	2.29070	**G1**	**G1**
-0.41372	3.89802	0.65861	3.09519	2.48115	0.49710	0.04105	2.24210	**G1**	**G1**
-0.30274	3.62769	1.08294	3.03728	3.15689	0.64159	0.07582	2.41414	**G1**	**G9A**
-0.51377	3.99601	0.69047	3.79785	3.07282	0.86019	0.17706	2.25901	**G1**	**G1**
1.19403	2.98342	1.04351	2.41802	2.26564	0.57310	0.03280	1.92123	**G2**	**G9C**
0.96326	2.41867	1.32892	2.47930	2.03123	0.10774	-0.06216	1.85271	**G2**	**G2**
0.80858	2.72752	1.30081	1.84413	1.85378	0.86395	-0.20727	1.99599	**G2**	**G2**
0.90981	3.64351	1.18991	2.95465	2.24096	0.44287	-0.19508	2.23863	**G2**	**G9B**
0.75032	2.20783	1.37880	1.98889	1.98987	0.38108	-0.00944	2.17829	**G2**	**G2**
0.34252	2.96816	1.20866	2.48504	2.02603	0.57819	-0.18098	2.28784	**G2**	**G9A**
1.38839	2.56889	1.34190	2.61267	1.78112	0.69434	0.04135	1.84547	**G3**	**G3**
1.12704	2.80075	1.29280	2.78135	1.67620	0.04955	-0.01319	1.73316	**G3**	**G3**
1.34682	2.55157	1.32105	2.51677	2.00926	0.53871	-0.24401	1.76503	**G3**	**G3**
0.75665	2.67539	1.36139	2.43381	1.85696	0.78778	-0.19739	1.85088	**G3**	**G3**
1.15531	2.90624	1.47080	2.30376	1.62455	0.60865	0.14062	1.81926	**G3**	**G9C**
0.80165	2.34517	1.47734	2.05645	1.66468	0.89462	-0.28500	1.57764	**G3**	**G3**
0.07517	0.31512	0.41995	0.07213	0.69686	0.93734	0.05114	0.86589	**G4**	**G4**
-0.18408	-0.08558	0.45803	0.86907	0.54860	0.81149	-0.12640	-0.05869	**G4**	**G4**
-0.09391	0.41540	0.50290	0.53594	0.37220	0.95021	-0.06983	-0.04082	**G4**	**G4**
0.05011	0.31229	0.50476	0.60955	0.55410	0.90811	-0.05904	-0.25090	**G4**	**G4**
0.13052	-0.14066	0.37709	0.91067	0.66896	0.80707	-0.10919	-0.27102	**G4**	**G4**
0.17996	0.14409	0.30453	0.67674	-0.29017	0.90048	-0.24001	-0.01586	**G4**	**G4**
0.06960	0.05808	-0.18884	-0.27833	-0.06847	0.44814	-0.03194	-0.29521	**G5**	**G5**
0.20819	0.38862	-0.25583	-0.44989	0.03195	0.53868	-0.09715	-0.17140	**G5**	**G5**
0.02033	0.37536	-0.33322	-0.20054	-0.21263	0.51808	-0.09686	-0.34210	**G5**	**G5**
0.30665	0.40405	-0.30497	-0.41332	-0.03376	0.53195	-0.10049	-0.23033	**G5**	**G5**
0.14392	0.51757	-0.09722	0.06636	-0.00550	0.52348	-0.14841	-0.33608	**G5**	**G5**
-0.04047	0.21946	-0.23483	-0.01116	-0.16191	0.55656	-0.13725	-0.42552	**G5**	**G5**
-0.13044	3.18652	1.17021	2.85139	2.59960	0.31517	0.01121	2.29432	**G9A**	**G9A**
-0.13488	3.30074	1.53148	3.04935	2.11365	0.49502	-0.15795	2.46254	**G9A**	**G9A**
0.00593	3.28824	1.50241	2.66430	2.31823	0.21380	-0.16387	2.23977	**G9A**	**G9A**
-0.01861	3.45415	1.22386	2.79929	2.35131	0.46562	-0.08792	2.45958	**G9A**	**G9A**
-0.10853	3.23574	1.30938	2.36958	2.30540	0.45124	-0.21848	2.34982	**G9A**	**G9A**
0.00533	3.04582	1.45738	2.65629	2.09070	0.73955	-0.16931	2.38488	**G9A**	**G9A**
0.97160	3.57468	1.49609	2.75990	2.21658	0.94380	0.01842	2.35802	**G9B**	**G9B**
0.66322	3.65963	1.53707	2.41619	2.37431	1.22669	-0.06181	2.34943	**G9B**	**G9B**
0.99203	3.56847	1.29789	2.73654	2.15387	1.02119	0.09023	2.43687	**G9B**	**G9B**
1.03706	3.31087	1.26132	2.45976	2.08616	0.97584	0.04746	2.32174	**G9B**	**G9B**
0.90538	3.22469	1.04229	2.66065	2.03515	0.88033	-0.21470	2.27063	**G9B**	**G9B**
0.99593	3.16857	1.24966	2.49138	1.86287	0.87693	0.07089	2.28286	**G9B**	**G9B**
1.42387	2.91926	1.67605	2.78131	2.19236	1.47929	0.34968	1.76280	**G9C**	**G9C**
1.38532	3.69330	1.49673	2.88577	2.26150	1.39922	0.35919	2.26004	**G9C**	**G9C**
1.81367	3.42884	1.69517	3.09034	2.29715	1.48856	0.07676	2.28262	**G9C**	**G9C**
1.46829	3.48516	1.57043	2.62526	2.35092	1.31037	0.17902	2.33217	**G9C**	**G9C**
1.29573	3.05096	1.69222	2.47550	2.32763	1.27824	0.01848	2.36528	**G9C**	**G9B**
1.24138	2.87239	1.49679	1.96622	2.09587	1.34090	0.06974	2.44995	**G9C**	**G9B**
-0.10953	1.39081	0.58553	0.64442	0.35278	-0.08481	-0.31127	0.68192	**G6**	**G6**
0.15431	0.57439	0.35794	0.51783	0.23120	-0.12781	-0.30993	1.03146	**G6**	**G6**
0.08293	0.98861	0.35812	0.85742	1.10295	0.12396	0.00357	0.78693	**G6**	**G6**
-0.07003	1.13310	0.57626	1.45765	0.44327	0.43118	-0.24583	0.57027	**G6**	**G6**
0.20931	1.73824	0.63645	0.81044	1.39682	0.55509	-0.17418	0.77215	**G6**	**G6**
0.13895	1.55698	0.29907	1.06327	1.00069	0.19921	-0.37279	0.72490	**G6**	**G6**

0.10133	1.68468	0.97581	1.50914	1.54605	0.08962	-0.31229	1.67396	**G7**	**G7**
-0.23823	2.56585	1.03282	2.03615	1.40145	0.19754	-0.52068	1.74576	**G7**	**G8**
0.08374	2.00329	0.86365	1.28285	1.40387	0.27361	-0.40131	1.75252	**G7**	**G7**
-0.03948	1.61530	0.75870	1.46178	1.07193	0.07004	-0.36582	1.56702	**G7**	**G7**
0.15232	1.71858	0.78308	1.05655	1.14035	0.45188	-0.55975	1.60236	**G7**	**G7**
0.07774	1.37924	0.51821	0.80086	0.98634	0.14253	-0.45347	1.86134	**G7**	**G7**
0.65332	2.32017	1.06489	2.25471	1.86292	0.56746	-0.24862	2.14344	**G8**	**G8**
0.47452	2.18629	0.56756	2.06045	1.88787	1.08667	-0.31297	2.42429	**G8**	**G8**
-0.31914	2.40421	0.94703	2.08987	1.77177	0.02417	-0.19685	2.03064	**G8**	**G8**
-0.25451	2.34494	0.86217	2.16386	1.53885	0.22671	-0.21762	2.06711	**G8**	**G8**
0.10043	1.89011	0.93449	1.98109	1.42675	-0.29270	-0.33047	2.02211	**G8**	**G8**
0.24980	2.35363	0.63455	1.75312	1.52394	0.03464	-0.46502	2.25375	**G8**	**G8**

Nanoparticle synthesis

Gold nanoparticles were synthesized according to methods reported by You, et. al.[1] In brief, 1-pentanethiol coated gold nanoparticles (d = ~2 nm) were prepared according to the protocol developed by Schiffrin, et. al.[2] A Murray place-exchange reaction[3] was performed by dissolving the thiolated ligand (for ligand synthesis check below) in dry DCM with the pentanethiol-coated gold cores and stirring for 3 days at room temperature. Then, DCM was evaporated under reduced pressure and the oily residue was dissolved in a small amount of distilled water. Dialysis was performed during 5 days (membrane MWCO = 10,000) to remove excess of ligand and salts remaining in the nanoparticle solution. After dialysis, the particles were lyophilized and redissolved in deionized water to concentrate the solution.
The ligands for NP1-NP4 and NP8 were synthesized according to the reported procedure.[4] The ligands for NP5, NP6 and NP7 were synthesized as follows:



Scheme S1. Synthetic route of glycan-functionalized ligands.

Compound 1: Boron trifluoride etherate (8 mL, 65 mmol) was added to a solution of 1,2,3,4,6-penta-O-acetyl-alpha-D-glucopyranose (6.5 g, 16.65 mmol) and 3-bromo-1-propanol (1.8 mL, 20 mmol) in dry CH_2Cl_2 (100ml). The reaction mixture was stirred under a nitrogen atmosphere overnight. The reaction mixture was neutralized by adding 100 mL of a saturated solution of sodium bicarbonate and then washed with distilled water (2x100 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting oil was then purified using column chromatography (ethyl acetate/hexane (1:1, v/v)).

After collecting the relevant fractions, the solvent was evaporated and 3'-bromopropyl 2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranose was obtained as a light yellow oil (4.4 g, 56%).

Compound 2: Compound 1 (730 mg, 1.556 mmol) was dissolved into 2M dimethylamine solution in THF (15 ml). The reaction mixture was stirred at ~40^{o}C overnight. The crude product was checked by TLC and THF was evaporated at reduced pressure. The light yellowish residue was washed with hexanes using sonication and centrifugation, and it was further dried at high vacuum. The product formation was quantitative (>95%). NMRs in Figures S8, S9 and S10.

Compound 3: Compound 2 (550 mg, 1.27 mmol) was added to a solution in ethanol of 1,1,1-triphenyl-14,17,20,23-tetraoxa-2-thiapentacosan-25-yl methanesulphonate (2.67 g, 3.81 mmol). The reaction mixture was stirred at ~40^{o}C for 96 h. When the reaction was finally completed (according to TLC), the ethanol was evaporated. The crude product was washed three times with hexane and six times with diethylether to afford compound 3 (yellowish oil, 1.55 g, 79 %).

Compound 4: To a solution of compound 3 (300mg, 0.29 mmol) in dry and deoxygenated MeOH (5ml), NaOMe was added. After 10 minutes the reaction was neutralized with H^{+} Dowex resin, filtered and the solvent was removed, giving the product without any other purification. The product was then dissolved in dry dichloromethane (5 mL) and an excess of trifluoroacetic acid (TFA, ~20 equivalents) was added. The color of the solution turned yellow immediately. Subsequently, triisopropylsilane (TIPS, ~1.5 equivalents) was added to the reaction mixture. The reaction mixture was stirred overnight under N_2 at room temperature. The solvent, TFA and TIPS were distilled off under reduced pressure. The pale yellow residue was purified washing with hexanes (3 times) and ether (6 times) combining successive sonication and centrifugation. The product formation was quantitative (>95%). NMRs in Figures S11, S12 and S13. MALDI-MS spectrum in Figure S14.

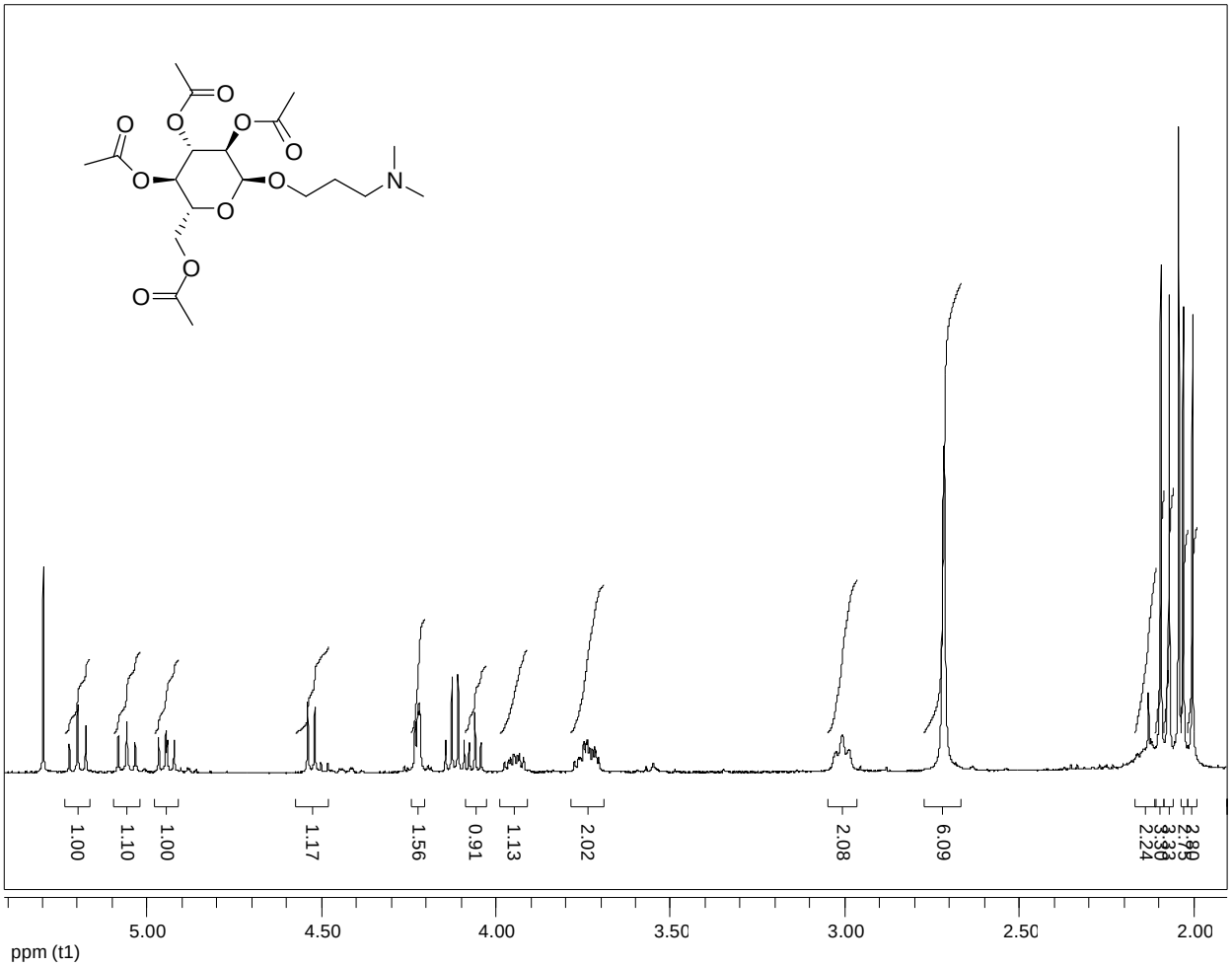


Figure S8. 400 MHz ^{1}H NMR spectra of 3`-dimethylaminopropyl 2,3,4,6-tetra-O-acetyl-alpha-D-glucopyranose in chloroform-D (D, 99.8%).

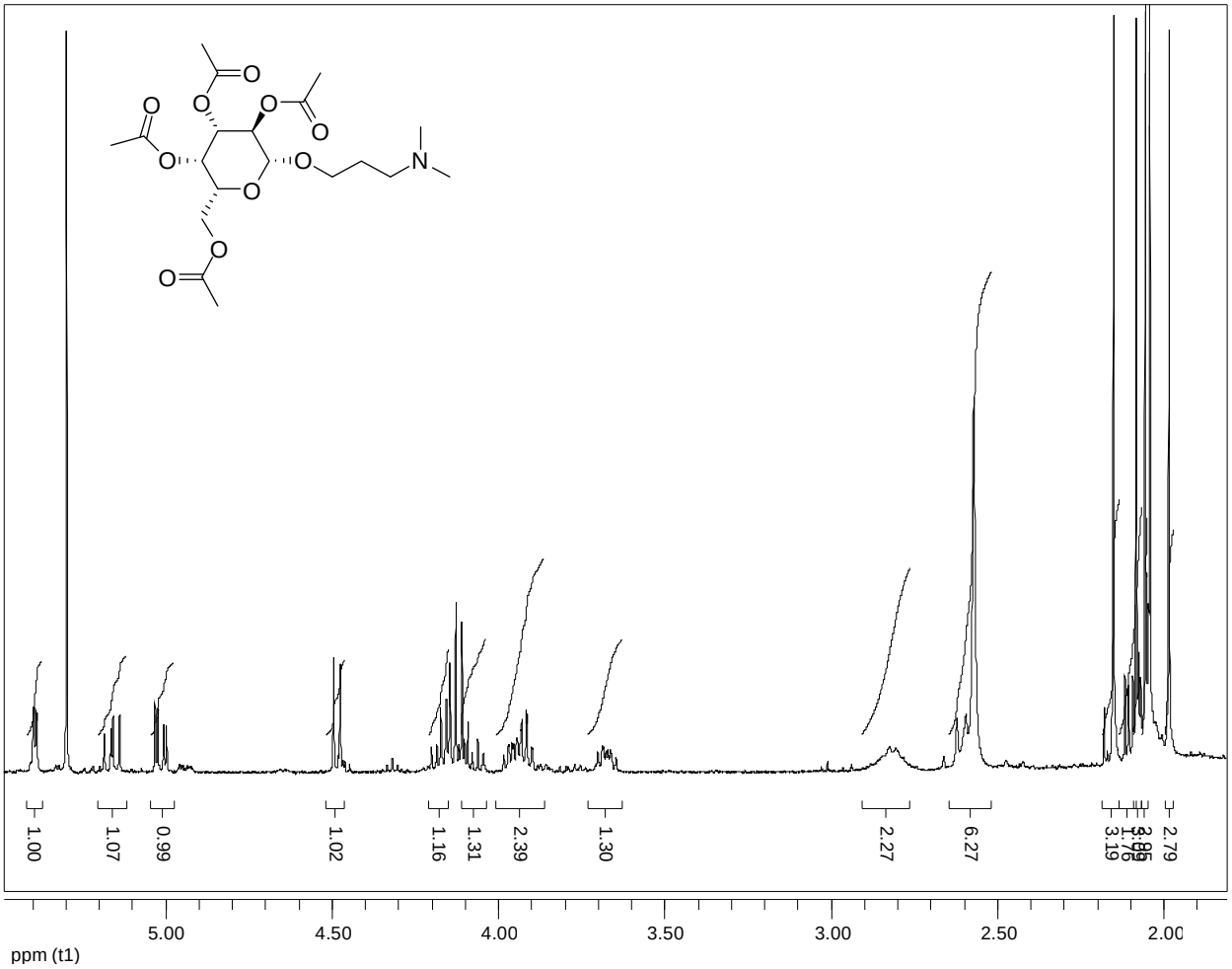


Figure S9. 400 MHz ^{1}H NMR spectra of 3`-dimethylamoinopropyl 2,3,4,6-tetra-O-acetyl-alpha-D-galactopyranose in chloroform-D (D, 99.8%).

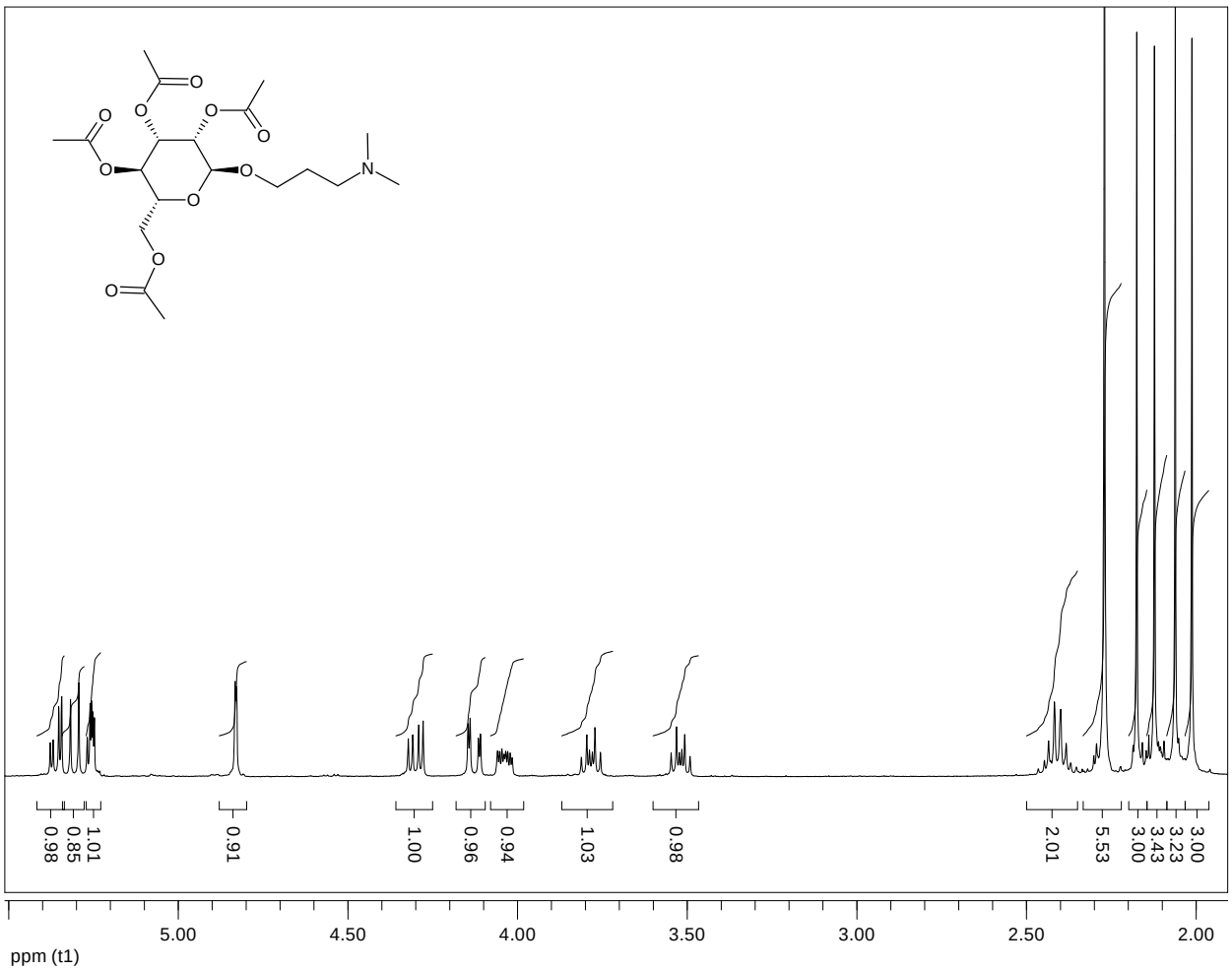


Figure S10. 400 MHz ^{1}H NMR spectra of 3`-dimethylaminopropyl 2,3,4,6-tetra-O-acetyl-alpha-D-mannopyranoside in chloroform-D (D, 99.8%).

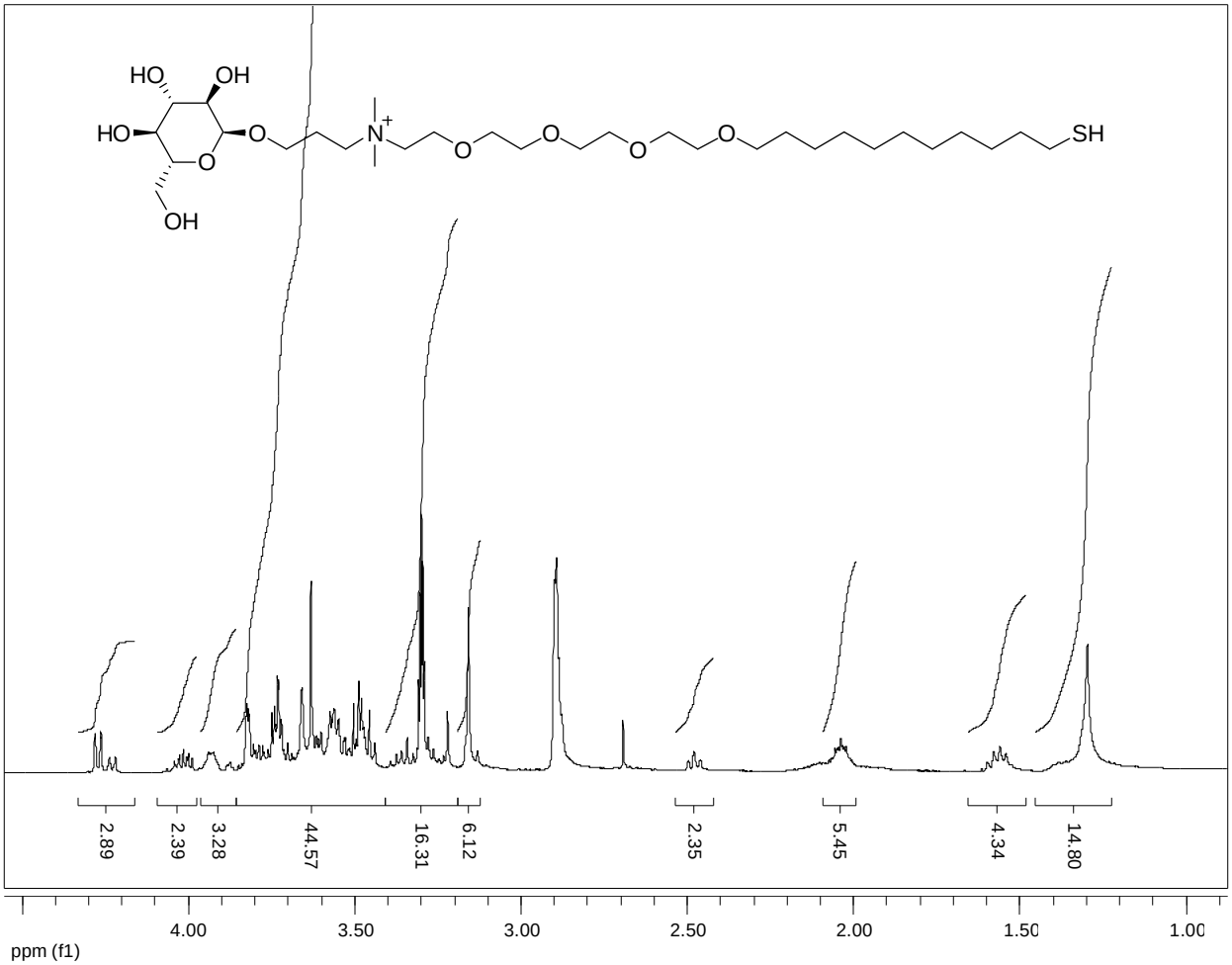


Figure S11. 400 MHz ^{1}H NMR spectra of **glucose ligand** in MeOD(D, 99.8%).

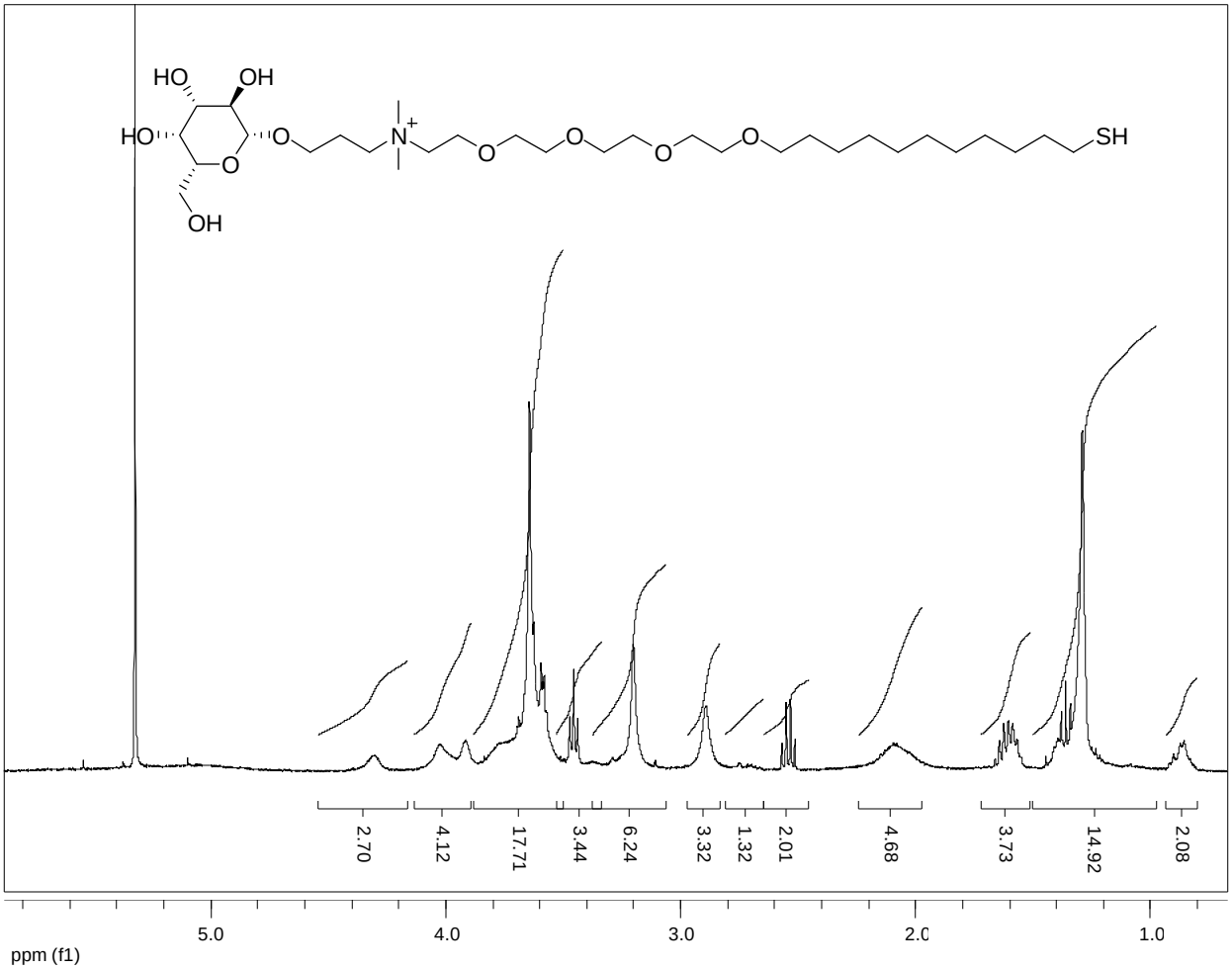


Figure S12. 400 MHz ^{1}H NMR spectra of the **galactose ligand** in MeOD(D, 99.8%).

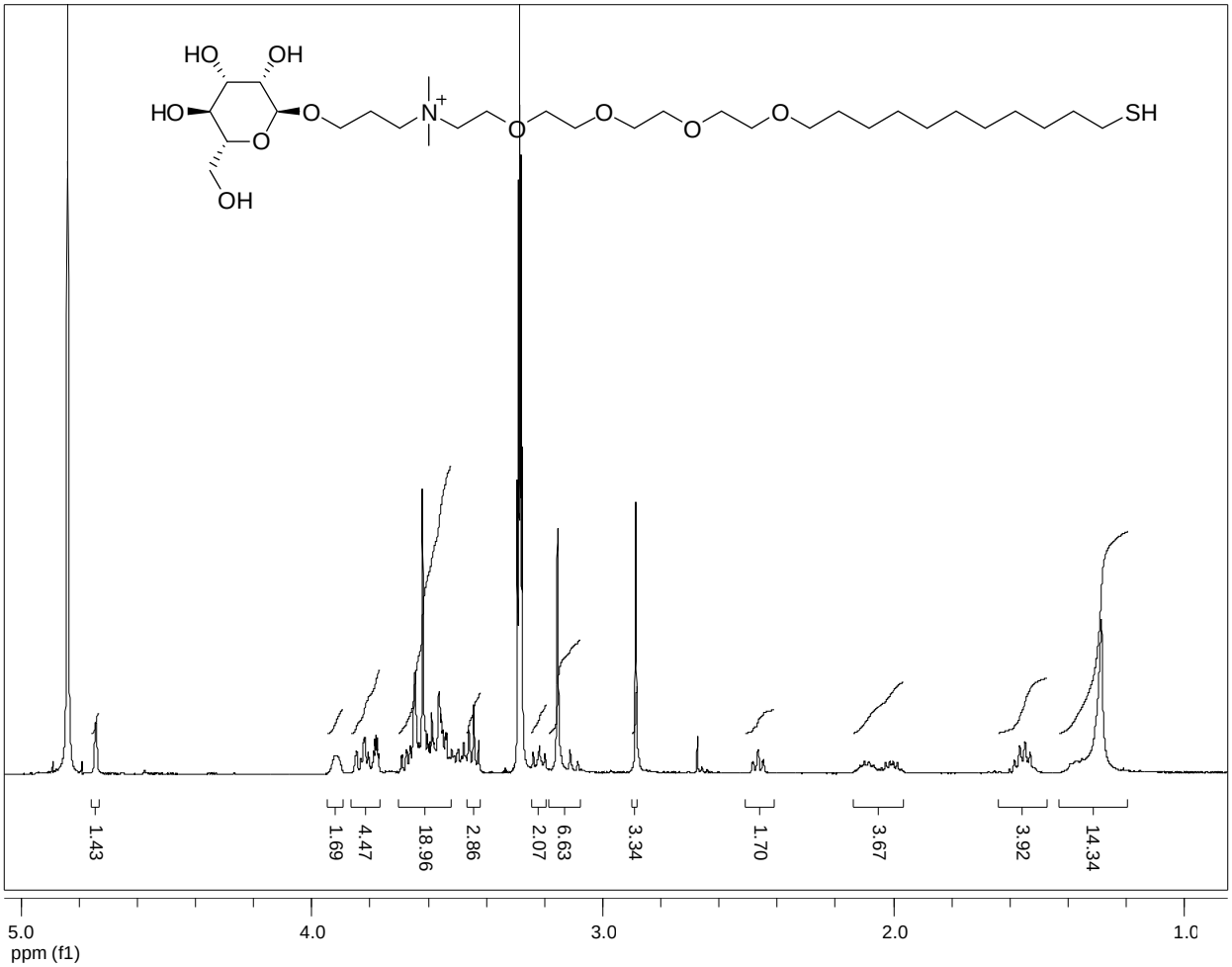


Figure S13. 400 MHz ^{1}H NMR spectra of the **mannose ligand** in MeOD(D, 99.8%).

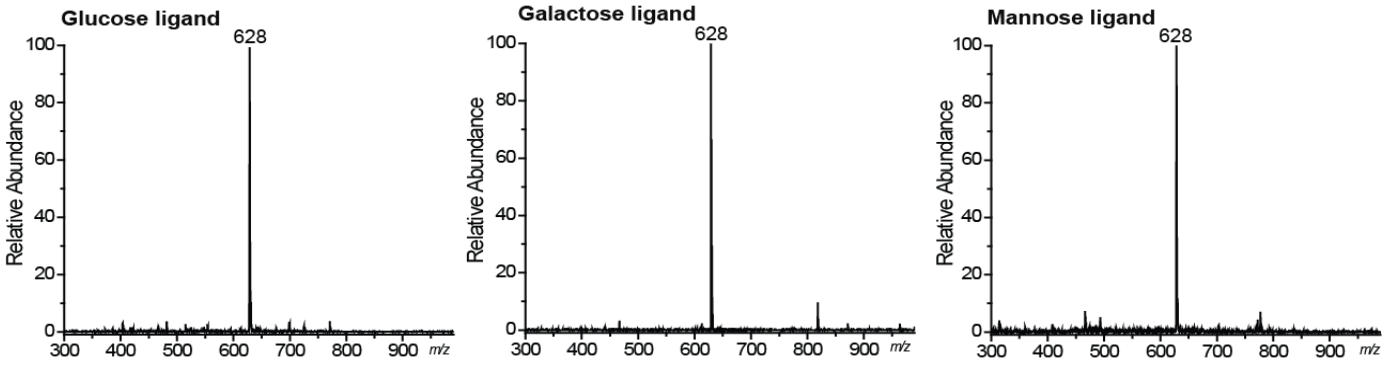


Figure S14. Matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) spectrum of three glycan ligands.

References

1) C. C. You, M. De, O. R. Miranda, B. Gider, S. P. Ghosh, I. Kim, B. Erdogan, S. A. Krovi, U. H. F. Bunz and V. M. Rotello, *Nature Nano.*, 2007, **2**, 318-323.
2) M. Brust, M. Walker, D. Bethell, D. J. Schiffrin and R. Whyman, *J. Chem. Soc. Chem. Commun.*, 1994, **7**, 801-802.
3) M. J. Hostetler, S. J. Green, J. J. Stokes and R. W. Murray, *J. Am. Chem. Soc.*, 1996, **118**, 4212-4213.
4) O. R. Miranda, H.-T. Chen, C.-C. You, D. E. Mortenson, X.-C. Yang, U. H. F. Bunz and V. M. Rotello, *J. Am. Chem. Soc.*, 2010, **132**, 5285-5289.