

Supporting Information

Stereoelectronic effects impact glycan recognition

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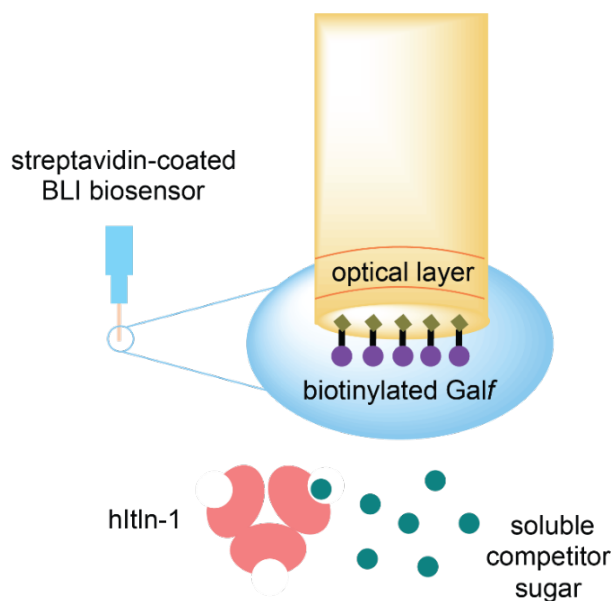


Figure S1. Schematic of biolayer interferometry (BLI) competition assay used to assess IC_{50} values of hItln-1 monosaccharide ligands. Biotinylated galactofuranose (Gal β) was immobilized on streptavidin-coated biosensors and incubated with hItln-1 in the presence of varying concentrations of soluble monosaccharides.

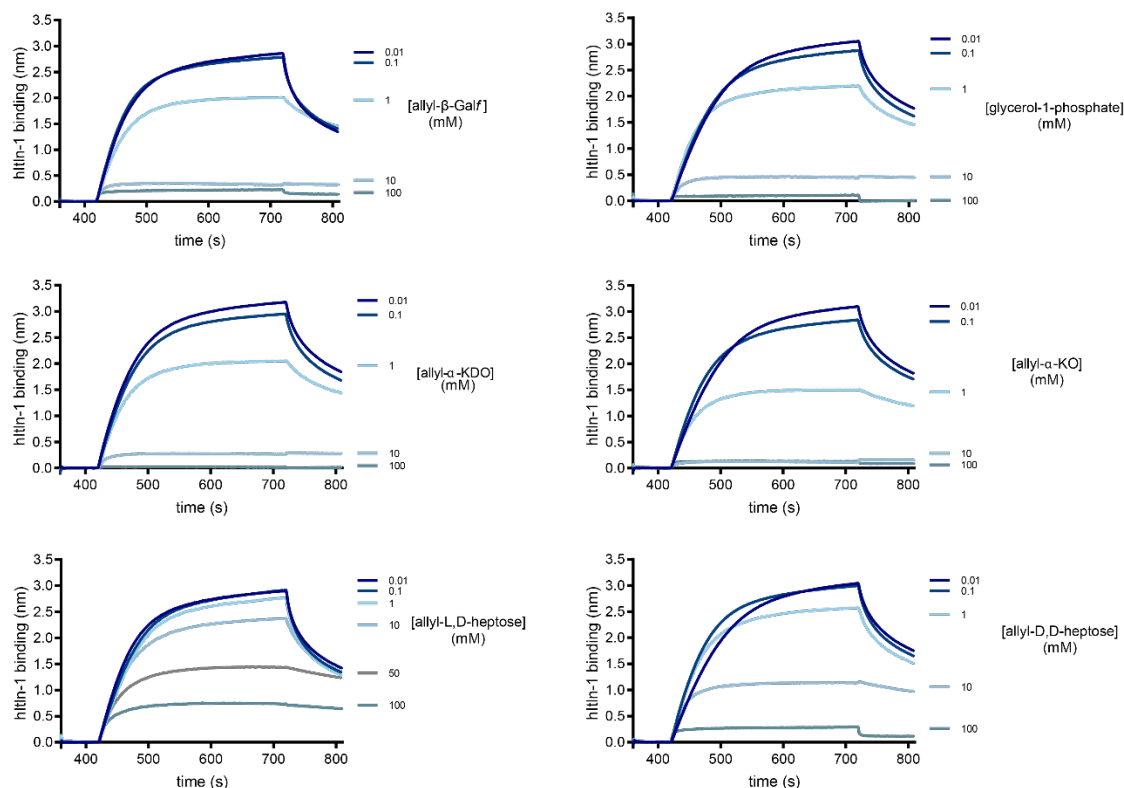


Figure S2. Biolayer interferometry (BLI) sensorgrams of soluble monosaccharides in competition with immobilized Galf. Real time sensorgrams are shown for competition with allyl- β -Galf, glycerol-1-phosphate, allyl- α -KDO, allyl- α -KO, allyl-L,D-heptose, and allyl-D,D-heptose over a range of concentrations. Data from 710–720 s of each binding curve were averaged and used for IC₅₀ determination (Figure 2).

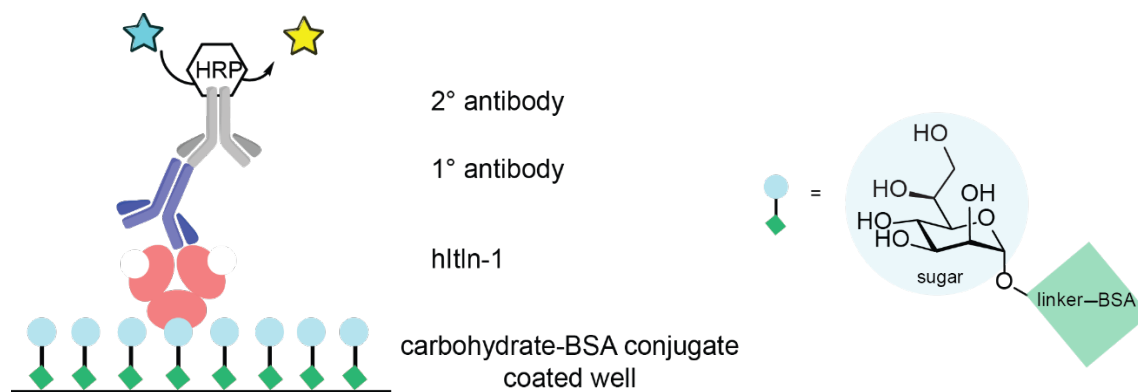


Figure S3. Schematic of ELISA-like assay used to test binding of hItln-1 to immobilized monosaccharide ligands. Bovine serum albumin (BSA)-conjugated sugars were coated onto a plate and incubated with various concentrations of hItln-1. Binding to hItln-1 is detected via the enzyme horseradish peroxidase (HRP) conjugated to an antibody (either a secondary as shown or a directly conjugated primary) reacting with a chromogenic HRP substrate.

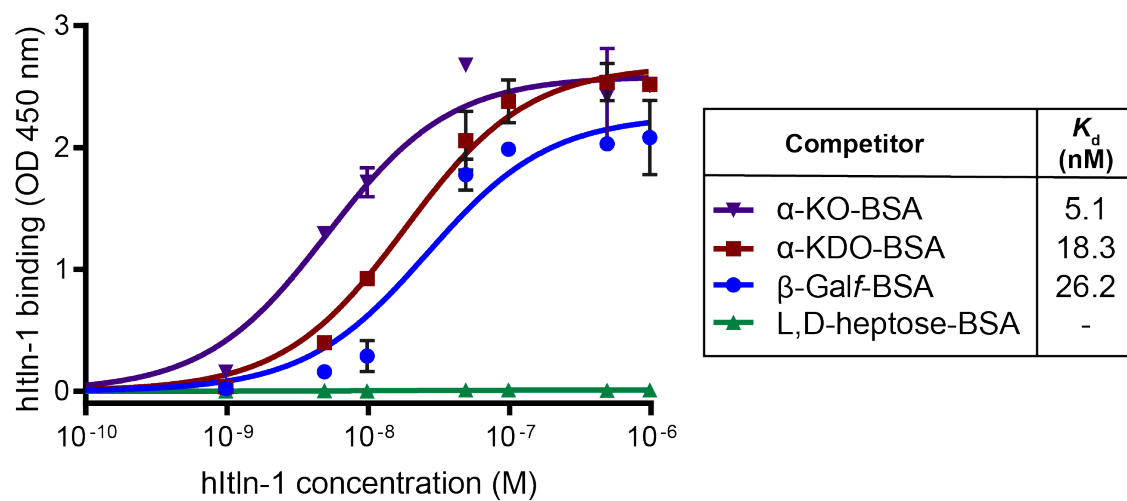


Figure S4. hItln-1 binding to monosaccharides in an ELISA-like assay using BSA-conjugated sugars. Data are shown as mean \pm SEM ($n = 2$ technical replicates). Data are representative of two independent experiments. Data were fitted to a single-site binding equation (solid lines). OD, optical density.

Table S1. Data collection and refinement statistics

	Allyl-α-KO bound hItln-1
PDB code	6USC
Data Collection	
X-ray source	23-ID-B
Detector	Eiger-16m
Wavelength, Å	1.033202
Resolution, Å	48.25–1.59 (1.79–1.59)
Space group	P 2 ₁ 3
<i>a</i> , <i>b</i> , <i>c</i> (Å)	118.14, 118.14, 118.14
α , β , γ (°)	90, 90, 90
No. of Reflections	2,622,179 (70,740)
No. Unique Reflections	73,131 (6,680)
Redundancy	35.8 (10.6)
Mean <i>I</i> / σ	16.9 (1.9)
Completeness	99.2 (92.0)
<i>R</i> _{meas}	0.1384 (0.8223)
<i>R</i> _{merge}	0.1403 (0.8629)
<i>R</i> _{pim}	0.02237 (0.2486)
CC _{1/2}	0.999 (0.514)
Wilson <i>B</i> -factor	18.3
Refinement	
Working set	73,130 (6680)
Test set	3,522 (338)
<i>R</i> _{work}	0.1582 (0.2666)
<i>R</i> _{free}	0.1822 (0.3098)
RMS deviation bond lengths (Å)	0.007
RMS deviation bond angles (°)	0.86
Protein residues	558
Total number of atoms	4811
Protein	4438
Allyl- α -KO	47
Solvent	326
Mean <i>B</i> -factor (Å ²)	21.94
Protein	21.35
Allyl- α -KO	29.38
Solvent	28.88
Ramachandran favored, allowed, outliers (%)	97, 3, 0
Values in parentheses are for highest-resolution shell.	

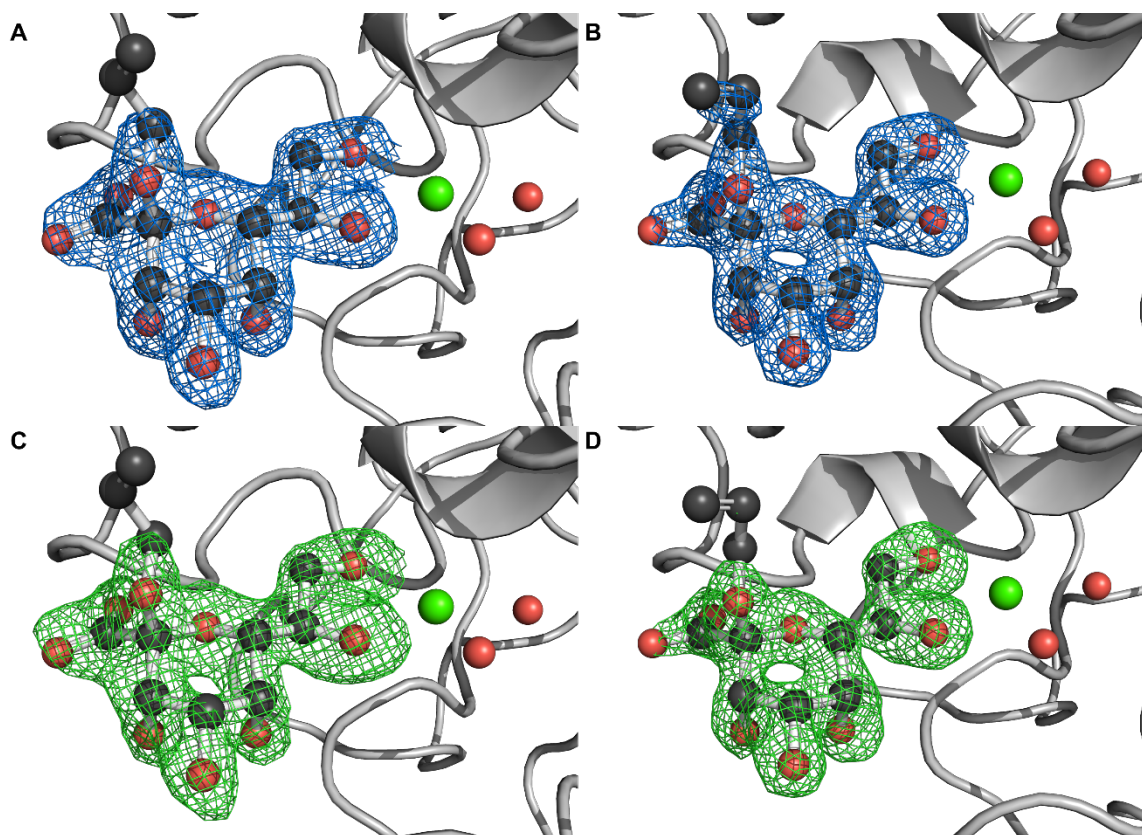


Figure S5. Electron density of allyl- α -KO bound to hItln-1. Final structures of allyl- α -KO bound to chain A (A) and chain B (B) shown with the $2F_o - F_c$ map contoured at 1.0σ . Both allyl- α -KO ligands were removed from the final model and simulated annealing refinement was performed. Final structures of allyl- α -KO bound to chain A (C) and chain B (D) are shown with the ensuing the $F_o - F_c$ map contoured at 3.0σ . Maps were prepared with phenix.refine and visualized with PyMOL.

Table S2A. Conformational analysis of saccharides containing exocyclic diols in the PDB.

PDB ID	Ligand ID	Residue	Trans to ring C–O: ring, side-chain	Proximal rotamer (y)	Distal rotamer (z)	Glycan interactions	Protein interactions	Interacting residues
Pyranose								
1q9w	KDO	B301	H,O	tg	gt	no	no	
	KDO	B302	H,O	tg	gt	1-Oz	no	
	KDO	B303	H,O	tg	gt	no	1-Oy	Tyr92 mainchain
	KDO	C311	H,O	tg	gt	no	no	
	KDO	C312	H,O	tg	gt	no	no	
	KDO	C313	H,O	tg	gt	1-Oz	1-Oy	Tyr92 mainchain
2r1y	KDO	B212	H,O	tg	gg	1-Oz	no	
	KDR	B213	H,O	tg	tg	no	1-Oz	Asn52 sidechain
2r23	KO1	B214	H,O	tg	gg	no	no	
2r2b	KDO	B215	H,O	tg	gg	no	no	
2r2h	KO2	B212	H,O	tg	gg	no	1-Oy	Asn30 sidechain
2ria	289	A356	O,H	gt	gt	no	2-Oy	Arg343 sidechain
	289	B356	O,H	gt	gt	no	no	
	289	C356	O,H	gt	gt	no	no	
2rib	GMH	A356	O,H	gg	gt	no	Ca	coordinates calcium
	GMH	B356	O,H	gg	gt	no	Ca	
	GMH	C356	O,H	gg	gt	no	Ca	
2ric	GMH	A356	O,H	gg	gt	no	Ca	coordinates calcium
	GMH	B356	O,H	gg	gt	no	Ca	
	GMH	C356	O,H	gg	gt	no	Ca	
2rie	293	A356	O,H	gg	gt	no	Ca	coordinates calcium
	293	B356	O,H	gg	gt	no	Ca	
	293	C356	O,H	gg	gt	no	Ca	
3dur	KDO	A303	H,O	tg	gg	no	2-Oy, 1-Oz	Tyr92 mainchain, Lys30 sidechain (both)
	KDO	D303	H,O	tg	gg	no	2-Oy	Tyr92 mainchain, Lys30 sidechain
3dus	KDO	A107	H,O	tg	gg	no	2-Oy, 1-Oz	Tyr92 mainchain, Lys30 sidechain (both)
	KDO	C108	H,O	tg	gg	no	2-Oy, 2-Oz	Tyr92 mainchain, Lys30 sidechain (both), PEG
3duu	KDO	B114	H,O	tg	gg	no	2-Oy, 1-Oz	Tyr92 mainchain, Lys30 sidechain (both)
	KDO	D114	H,O	tg	gg	no	2-Oy	Tyr92 mainchain, Lys30 sidechain
3dv6	KDO	B114	H,O	tg	gg	no	2-Oy, 1-Oz	Tyr33, Arg52 (both)
3etn	CMK	B500	H,O	tg	gg	no	1-Oy,1-Oz	Asn96 both
	CMK	C500	H,O	tg	gg	no	1-Oy,1-Oz	Asn96 both
	CMK	D500	H,O	tg	gg	no	1-Oy,1-Oz	Asn96 both

3hzm	KDO	B303	H,O	tg	gt	no	no	
3hzv	KDA	A301	H,O	tg	gt	1-Oy	no	
	KDO	A303	H,O	tg	gt	no	Oz-1	Arg27 sidechain
3k2v	CMK	A1	H,O	tg	gt	no	1-Oy, 1-Oz	Asn242 mainchain, His307 sidechain
	CMK	B2	H,O	tg	gt	no	1-Oy	Asn242 mainchain
3k8d	KDO	A1244	H,O	tg	gg	no	2-Oy	His181 and Gln211 sidechains
	KDO	B1244	H,O	tg	gg	no	2-Oy	His181 and Gln211 sidechains
	KDO	C1244	H,O	tg	gg	no	2-Oy	His181 and Gln211 sidechains
	KDO	D1244	H,O	tg	gg	no	2-Oy	His181 and Gln211 sidechains
3okd	KDO	B214	H,O	tg	gg	no	no	
3okk	KDO	B225	H,O	tg	gg	1-Oz	2-Oy	Tyr92 mainchain, Asn27 sidechain
3okl	KDO	B216	H,O	tg	gg	no	no	
3sy0	KDA	B301	H,O	tg	gg	no	no	
	KDO	B303	H,O	tg	gg	no	no	
3t4y	KDO	B303	H,O	tg	gg	no	no	
3t65	KDO	B303	H,O	tg	gg	no	1-Oy	Asn30 sidechain
3t77	KDO	B303	H,O	tg	gt	no	1-Oy	Tyr92 mainchain
3v0w	GMH	H307	O,H	gg	gt	1-Oy	no	
	GM0	H308	O,H	gg	gt	no	no	
	KDO	H309	H,O	tg	gt	1-Oy	no	
4e52	GMH	B404	O,H	gg	gt	no	Ca	coordinates calcium
	GMH	C404	O,H	gg	gt	no	Ca	coordinates calcium
4hgw	KDO	B302	H,O	tg	gg	no	1-Oy	Asn93 sidechain
4m7j	KDA	H303	H,O	tg	gt	no	no	
	KDO	H305	H,O	tg	tg	2-Oz	1-Oy, 2-Oz	Thr99 sidechain, Asn28 and Tyr32 sidechain
4o9k	CMK	A401	H,O	tg	gg	no	2-Oy	Leu240 mainchain, Asn321 sidechain
	CMK	B401	H,O	tg	gt	no	2-Oy, 1-Oz	Leu240 mainchain, Asn321 sidechain and Arg304 sidechain
4pf6	KDO	A401	H,O	tg	gg	no	1-Oy, 1-Oz	Arg42 sidechain
5fvn	KDO	A417	H,O	tg	gt	no	1-Oz	Arg199 mainchain
	KDO	B409	H,O	tg	gt	no	no	
	GMH	B410	O,H	gg	gt	no	no	
	KDO	B411	H,O	tg	gt	no	1-Oz	Arg199 mainchain
	KDO	C415	H,O	tg	gt	1-Oy	no	
	GMH	C416	H,O	tg	gt	no	no	
	KDO	C417	H,C	gg	tg	no	Ca	coordinates calcium
	KDO	C427	H,O	tg	gt	no	no	
	KDO	C428	H,O	tg	gt	no	1-Oz	Arg199 mainchain
	KDO	D425	H,O	tg	gt	no	no	
	KDO	D426	H,O	tg	gt	no	1-Oz	Arg199 mainchain

	KDO	E407	H,O	tg	gt	no	no	
	KDO	E408	H,O	tg	gt	no	1-Oz	Arg199 mainchain
	KDO	F408	H,O	tg	gg	1-Oz	no	
	KDO	F410	H,C	gg	tg	no	Ca	coordinates calcium
	KDO	F420	H,O	tg	gt	no	no	
	KDO	F421	H,O	tg	gt	no	1-Oz	Arg199 mainchain
5oxr	GMH	B405	O,H	gg	gt	no	Ca	coordinates calcium
	GMH	B406	O,H	gg	tg	1-Oz	no	
	GMH	C405	O,H	gg	gt	no	Ca	coordinates calcium
5oxs	GMH	A405	O,H	gg	tg	2-Oz	no	
	GMH	B404	O,H	gg	gt	no	Ca	coordinates calcium
	GMH	B405	O,H	gg	gt	no	no	
	GMH	C404	O,H	gg	gt	no	Ca	coordinates calcium
6c5h	KDO	H409	H,O	tg	gt	no	no	
	KDO	H411	H,O	tg	gg	1-Oz	1-Oz	Asn28 sidechain
6c5k	KDO	A406	H,O	tg	gg	no	no	
	KDO	A408	H,O	tg	gg	1-Oz	1-Oz	Asn28 sidechain
	KDO	H406	H,O	tg	gg	no	no	
	KDO	H408	H,O	tg	gg	1-Oz	1-Oz	Asn28 sidechain
Furanose^a								
2vk2	GZL	A1298	O,C	gt	gt	3-Oy, 1-Oz		
2ydg	A5C	A1131	H,H	gg	tg		1-Oy	Asn65 sidechain
4xad	3ZW	A207	O,H	gg	tg		3-Oy	Ser50, Asn59, Arg61 sidechain
4wmy	3S6	A404	O,H	gg	gt		Ca	Both coordinated
5lsh	KTS	A205_1	O,H	gg	tg/gt		no	
	KTS	A205_2	O,H	gg	gg		2-Oy	Arg98 and Asp102 sidechain

^a GLZ, 3ZW, 3S6, and KTS are all β -D-galactofuranose-containing

Table S2B. Summary of conformational analysis results

Proximal rotamer	Number of instances	Distal rotamer	Number of instances
Pyranose, equatorial hydrogen (67 structures)			
gg	2	gg	0
		tg	2
		gt	0
tg	65	gg	34
		tg	2
		gt	29
gt	0		0
Pyranose, equatorial hydroxyl group (24 structures)			
gg	21	gg	0
		tg	2
		gt	19
tg	0		0
gt	3	gg	0
		tg	0
		gt	3
Furanose (6 structures)			
gg	5	gg	1
		tg	3*
		gt	2*
tg	0		0
gt	1	gg	0
		tg	0
		gt	1

*one structure showed alternate conformations with distal hydroxyl in tg and gt

Table S3. Cartesian coordinates of saccharides optimized at the M06-2X/6-311+G(d,p); IEFPCM:water level of theory

KO				DD_gtgt			
C	0.85349500	-1.87232600	-0.15394700	C	-1.25090400	0.99202400	0.67333800
O	-0.24235900	0.08517200	0.72447000	C	-0.64381300	-0.37806100	0.39774500
C	-0.14406900	-1.89765100	-1.31608400	C	1.03284000	0.53549900	-1.03003400
O	-2.14431300	-1.94466900	0.07189900	C	0.54444400	1.96160500	-0.78948200
C	-1.45575000	-1.20247600	-0.93245100	C	-0.15930200	2.05495000	0.56688600
O	-0.39478000	-3.23370300	-1.71959200	H	0.13424400	-0.57492900	1.14480100
C	-1.15197500	0.17934800	-0.36659900	H	1.39276100	2.64646400	-0.81411200
O	1.47707000	0.00528600	2.71277600	H	0.56792500	1.89111700	1.36626200
C	1.02000300	-0.44623300	0.38166200	O	-0.05407900	-0.36717400	-0.90727700
O	3.08177400	-0.90822200	1.43785400	O	2.04510000	0.27719900	-0.11265400
C	1.94182800	-0.44697900	1.65263600	C	2.66970200	-0.99738800	-0.29030300
O	1.60126300	0.29029000	-0.66791600	H	3.08602600	-1.06591400	-1.30151800
C	-2.40071200	0.89023900	0.15695500	H	1.91674300	-1.78447000	-0.16760900
O	-3.40310400	0.91705400	-0.84607100	C	5.00574100	-1.41607100	0.45746000
C	-2.06039500	2.28333100	0.65221800	H	5.74776900	-1.54021900	1.23720700
O	-3.22475400	2.84370500	1.23884900	H	5.33800400	-1.53569500	-0.56905800
C	1.93734400	1.63268800	-0.32144500	C	3.73970600	-1.13798900	0.74421500
O	0.42938100	-2.74924200	0.87834600	H	3.41857300	-1.01750200	1.77578400
C	2.26576800	2.36921900	-1.58118300	C	-1.61342200	-1.55653100	0.43038600
C	3.40234200	3.02832900	-1.77380500	H	-1.98569400	-1.66506100	1.45135300
H	4.17758300	3.04591000	-1.01398600	C	-2.80076300	-1.38127000	-0.50297400
H	1.82786400	-2.23070700	-0.48523100	H	-3.34138300	-0.46844100	-0.23306800
H	0.29048900	-1.36722600	-2.16620900	H	-2.44930600	-1.28877600	-1.53760800
H	-2.46975500	-2.75677800	-0.33110800	O	-0.89483100	-2.74460600	0.14260900
H	-2.08838000	-1.09694300	-1.81781300	H	-0.46896600	-2.61920000	-0.71429400
H	-0.28860100	-3.79061800	-0.93767400	O	-3.64181600	-2.51358000	-0.35071600
H	-0.70802400	0.78158800	-1.17197300	H	-4.40188300	-2.40602600	-0.92882700
H	-2.81687000	0.30764000	0.98125700	H	1.38349200	0.41213500	-2.05997800
H	-3.14591300	1.55067200	-1.52536900	H	-2.02128900	1.21196500	-0.07898100
H	-1.72053700	2.89524800	-0.19589300	O	-0.33446900	2.36506800	-1.82772900
H	-2.99852300	3.70354200	1.60360000	H	-0.91539700	1.62745400	-2.05197000
H	2.78757300	1.63830200	0.36949200	O	-0.71058000	3.34244600	0.76933200
H	-0.45980400	-2.47214400	1.14469600	H	-1.14634300	3.59922100	-0.05410900
H	1.49865300	2.35355500	-2.35205600	O	-1.82655000	0.97052100	1.96207600
H	3.59225300	3.57485200	-2.69004100	H	-2.06283600	1.87827600	2.18718300
H	-1.24619100	2.21237600	1.37811700				
H	1.07948500	2.10043100	0.17754300				

DD_gggt				LD_gggt			
C	1.12682100	0.95322000	-0.57972400	C	1.43296500	-0.90169400	0.68699200
C	0.66899800	-0.38602800	-0.00557100	C	0.71924500	0.39063800	0.30483600
C	-1.29525900	0.61193900	0.94914600	C	-0.87532300	-0.78204200	-1.03696800
C	-0.91562500	2.00477900	0.45926500	C	-0.24021100	-2.13365900	-0.72515800
C	-0.06694500	1.88099000	-0.80306600	C	0.45907900	-2.07551600	0.63288800
H	0.07632700	-0.91821000	-0.75942400	H	-0.04509200	0.60949500	1.06021000
H	-1.81823200	2.57888000	0.24692900	H	-1.01072600	-2.90537100	-0.71376900
H	-0.67928000	1.46429000	-1.60662100	H	-0.28937800	-1.94338600	1.41840700
O	-0.12331800	-0.15107200	1.16578500	O	0.10965700	0.23472100	-0.98096500
O	-2.12797500	0.03955600	-0.00671600	O	-1.89693400	-0.57193400	-0.11662700
C	-2.66524300	-1.22648600	0.38694100	C	-2.68202400	0.59270700	-0.38952400
H	-3.27462200	-1.10320700	1.28913300	H	-3.22712800	0.45639800	-1.33003200
H	-1.83911500	-1.90981200	0.61445800	H	-2.01519700	1.45619700	-0.49464800
C	-4.75334300	-2.12200900	-0.62758100	C	-4.93809100	0.89453400	0.61696500
H	-5.30543800	-2.53039800	-1.46569300	H	-5.58422800	1.07342100	1.46815500
H	-5.27960500	-2.02993300	0.31742600	H	-5.41086100	0.80363800	-0.35599000
C	-3.48255800	-1.75679000	-0.74685700	C	-3.62119400	0.79886800	0.75500600
H	-2.96597900	-1.85001800	-1.69887800	H	-3.15855900	0.88741100	1.73489500
C	1.79248500	-1.29623000	0.48939900	C	1.66956500	1.57627700	0.18063900
H	1.31031400	-2.20904300	0.86318000	C	0.89954500	2.86679700	-0.04660600
C	2.77803100	-1.70187100	-0.58567600	H	0.41779700	2.83654000	-1.03069500
H	2.23687700	-1.95504200	-1.50275200	H	0.12085800	2.95851800	0.71970200
H	3.44916900	-0.86642100	-0.79872700	O	1.81636000	3.94565100	0.03684200
O	2.51490100	-0.67268900	1.53841900	H	1.35698500	4.75683200	-0.19633600
H	1.89320100	-0.49552300	2.25312000	H	-1.24809400	-0.75460600	-2.06607600
O	3.49900100	-2.82546100	-0.09535600	H	2.25102900	-1.07303700	-0.02613200
H	4.20664200	-3.02482200	-0.71403100	O	0.68141400	-2.49463800	-1.74165100
H	-1.78161700	0.66006200	1.92903300	H	1.19283100	-1.71524300	-1.99211700
H	1.82301900	1.41845800	0.13225300	O	1.13710700	-3.28666400	0.91341400
O	-0.21084200	2.71221200	1.46666400	H	1.61459900	-3.53862900	0.11210700
H	0.41637000	2.11256900	1.88946900	O	1.95580900	-0.74143400	1.99075200
O	0.38397800	3.14766000	-1.24851600	H	2.28721300	-1.60140100	2.27452400
H	0.70680800	3.62503200	-0.47297300	H	2.23263500	1.66106800	1.11308100
O	1.78241400	0.71147000	-1.81035900	O	2.62556200	1.34811300	-0.84021400
H	1.95572100	1.57064100	-2.21293100	H	2.14217500	1.20016800	-1.66212000

LD_gtgt				H	2.76963000	-1.74561900	1.89698200
C	-1.18963400	1.13901600	0.44739600	C	-1.87898400	-1.16513100	-0.52237300
C	-0.75794000	-0.24775600	-0.02717100	C	-2.83048400	-1.60355700	0.57038300
C	1.28557400	0.61883600	-0.95100800	H	-2.27519200	-2.19307400	1.30979700
C	0.94929200	2.05042400	-0.55057900	H	-3.25209500	-0.72602400	1.06138400
C	0.03720500	2.02710400	0.67227500	O	-3.84994500	-2.38694100	-0.03681400
H	-0.19884800	-0.75119500	0.77185000	H	-4.48753300	-2.62665600	0.64082900
H	1.86516800	2.59624600	-0.32137400	H	1.81147800	0.59195600	-1.91106300
H	0.59215800	1.62817500	1.52510700	H	-1.82785200	1.59224900	-0.32610500
O	0.08221100	-0.09222800	-1.18012500	O	0.32430800	2.73289600	-1.62508000
O	2.04829300	0.05850100	0.06662000	H	-0.30321800	2.13539800	-2.05074800
C	2.52423200	-1.25741200	-0.23168500	O	-0.37985700	3.33175600	1.03078900
H	3.14916400	-1.22818600	-1.13117100	H	-0.64208300	3.78238000	0.21718200
H	1.66652100	-1.91300400	-0.42269000	O	-1.90839300	1.02150800	1.65955700
C	4.54951800	-2.19022700	0.87189600	H	-2.00469200	1.91252100	2.01747500
H	5.06993800	-2.56731800	1.74422100	H	-2.44608900	-0.62759800	-1.29526000
H	5.08899400	-2.19483400	-0.07009600	O	-1.29932600	-2.33501600	-1.07681800
C	3.29992900	-1.74859700	0.94799100	H	-0.64602000	-2.04543500	-1.72409500

Table S4. NBO Donor-acceptor interaction energies and calculated ΔE_{NBO} of bond rotation.

Glycan conformation	Interaction	Energy (kcal/mol)
DD-heptose-gtgt	$\sigma_{\text{C19-H20}} \rightarrow \sigma^*_{\text{C2-O9}}$	5.34
	$\sigma_{\text{C1-C2}} \rightarrow \sigma^*_{\text{C19-O24}}$	2.42
	$\sigma_{\text{C19-C21}} \rightarrow \sigma^*_{\text{C2-H6}}$	1.95
	$\sigma_{\text{C2-H6}} \rightarrow \sigma^*_{\text{C19-C21}}$	3.54
	Sum of all interactions	13.25
DD-heptose-gggt	$\sigma_{\text{C19-C21}} \rightarrow \sigma^*_{\text{C2-O9}}$	2.72
	$\sigma_{\text{C2-H6}} \rightarrow \sigma^*_{\text{C19-O24}}$	4.93
	$\sigma_{\text{C19-H20}} \rightarrow \sigma^*_{\text{C1-C2}}$	3.93
	$\sigma_{\text{C1-C2}} \rightarrow \sigma^*_{\text{C19-H20}}$	1.48
	Sum of all interactions	13.06
DD-heptose ΔE_{NBO}		0.19
LD-heptose-gggt	$\sigma_{\text{C19-H33}} \rightarrow \sigma^*_{\text{C2-O9}}$	4.73
	$\sigma_{\text{C19-H20}} \rightarrow \sigma^*_{\text{C1-C2}}$	2.06
	$\sigma_{\text{C2-H6}} \rightarrow \sigma^*_{\text{C19-O34}}$	4.52
	$\sigma_{\text{C1-C2}} \rightarrow \sigma^*_{\text{C19-C20}}$	1.64
	Sum of all interactions	13.25
LD-heptose-gtgt	$\sigma_{\text{C19-H33}} \rightarrow \sigma^*_{\text{C2-H6}}$	2.86
	$\sigma_{\text{C19-C20}} \rightarrow \sigma^*_{\text{C2-O9}}$	2.15
	$\sigma_{\text{C2-H6}} \rightarrow \sigma^*_{\text{C19-H33}}$	2.57
	$\sigma_{\text{C1-C2}} \rightarrow \sigma^*_{\text{C19-O34}}$	2.12
	Sum of all interactions	9.7
LD-heptose ΔE_{NBO}		3.25

II. Synthetic Procedures

a. General Procedures and Materials

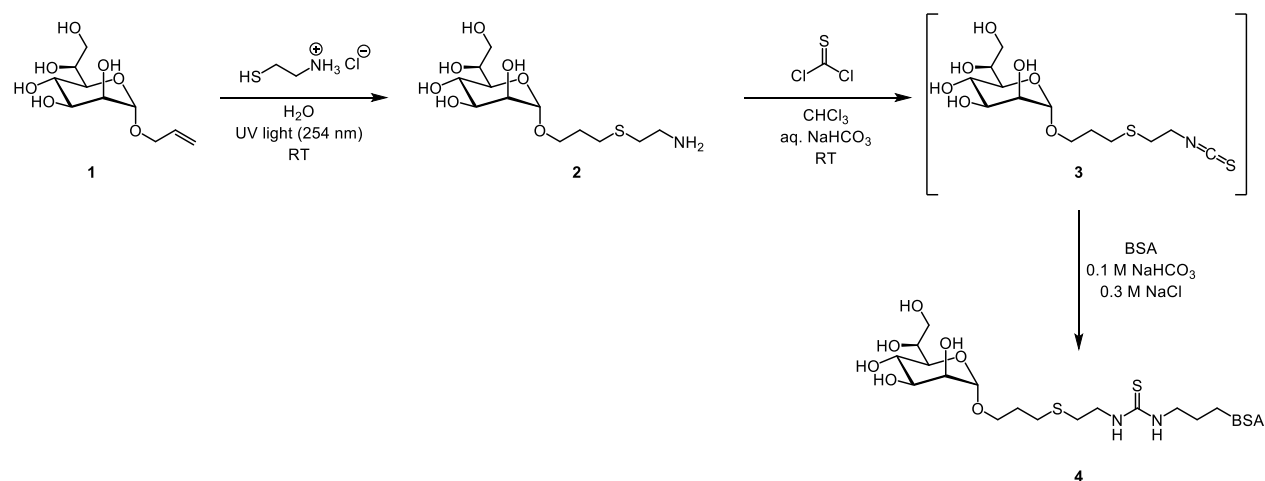
All reagents and solvents were purchased from Sigma Aldrich and used without further purification unless otherwise noted. Methanol was dried over 4Å molecular sieves or neutral alumina. Dichloromethane was dried over 4Å molecular sieves. Aqueous solutions of salts were saturated unless stated otherwise. All reactions were run under an inert N₂ atmosphere unless otherwise noted. All glassware and stir bars were dried prior to use. Analytical thin layer chromatography (TLC) was carried out on E. Merck (Darnstadt) or Silicycle TLC plates pre-coated with silica gel 60 F254 (250-µm layer thickness) or on HPTLC plates with a 2.5-cm concentration zone (Merck). Analyte visualization was accomplished using a UV lamp and by charring with *p*-anisaldehyde or potassium permanganate solution. For column chromatography, silica gel (0.040–0.063 mm) was used. Optical rotations were measured with an Anton Paar MCP100 Polarimeter. Proton and carbon magnetic resonance spectra (¹H NMR and ¹³C NMR) were recorded on a Bruker Avance III 600 instrument or a Bruker Avance DRX 600 instrument (¹H NMR at 600 MHz and ¹³C NMR at 151 MHz) using standard Bruker NMR software. Solvent resonance was used as the internal standard - ¹H spectra were referenced to 7.26 ppm (CDCl₃) and 0.00 ppm (D₂O, external calibration to 2,2-dimethyl-2-silapentane-5-sulfonic acid) or 0.00 ppm (D₂O, external calibration to 3-(trimethylsilyl)propionic acid-*d*₄ sodium salt). ¹³C spectra were referenced to 77.00 ppm (CDCl₃) and 67.40 ppm (D₂O, external calibration to 1,4-dioxane) or to 0.00 ppm (D₂O, external calibration to 3-(trimethylsilyl)propionic acid-*d*₄ sodium salt). Some assignments were based on COSY and HSQC data. ¹H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = doublet of doublets, ddd = doublet of doublet of doublets, td = triplet of doublets, qd = quartet of doublets, m = multiplet, br. s. = broad singlet), coupling constants (Hz), and integration. ESI-MS data were obtained on a Micromass Q-TOF Ultima Global instrument. DART-TOF MS data were obtained on a JEOL AccuTOF DART instrument at 300 °C. Matrix-assisted laser desorption ionization time-of-flight mass spectra (MALDI-TOF-MS) were obtained on Bruker Autoflex MALDI TOF/TOF or Bruker microflex LRF™ instrument using 2,5-dihydroxyacetophenone or sinapic acid as matrix.

Commercially available and previously characterized compounds:

Cysteamine hydrochloride was purchased from Fluka. Bovine serum albumin was purchased from US Biological. Glycerol phosphate was purchased from Sigma Aldrich (Milwaukee, WI: cat. no. G7886). The synthesis of the Gal^f-biotin ligand was described previously¹. Allyl-Gal^f, allyl-KO², allyl-KDO³, allyl-L,D-heptose⁴⁻⁶, and allyl-Neu5Ac⁷ have all been previously synthesized and characterized. KO-BSA conjugate⁸ and KDO-BSA conjugate⁹ have also been previously synthesized and characterized.

b. Experimental Procedures

i. Synthesis of L,D-heptose BSA conjugate (4)



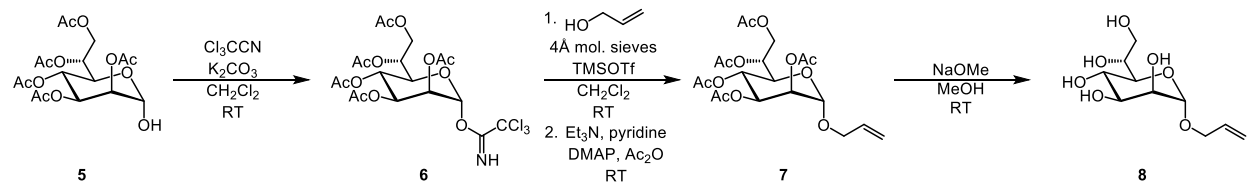
3-(2-Aminoethylthio)propyl L-glycero- α -D-manno-heptopyranoside (2). A solution of alkyl-L,D-heptose **1**⁴ (6.0 mg, 24 μ mol) and cysteamine hydrochloride (2.7 mg, 24 μ mol) in H₂O (0.1 mL) was irradiated at 254 nm for 3 h at RT. The reaction mixture was diluted with water and applied onto a column of Dowex 50 H⁺ cation exchange resin (10 cm x 1 cm diameter). Elution with 0.1 M aq. NH₃ afforded first a pool containing unreacted educt and cysteamine followed by fractions containing **2**. Lyophilization of the latter fractions afforded **2** (4.5 mg, 52%) as an amorphous solid. ¹H NMR (600 MHz, D₂O): δ 4.83 (d, J = 1.71 Hz, 1 H, H-1), 4.01 (ddd, J = 1.1, 5.3, 7.1 Hz, 1 H, H-6), 3.90 (dd, J = 1.7, 3.5 Hz, 1 H, H-2), 3.83 (t, J = 9.7 Hz, 1 H, H-4), 3.78-3.74 (m, 1 H, OCH₂), 3.76 (dd, J = 3.4, 9.7 Hz, 1 H, H-3), 3.71 4.42 (dd, J = 7.6, 11.3 Hz, 1 H, H-7a), 3.67 (dd, J = 5.4, 11.3 Hz, 1 H, H-7b), 3.58-3.53 (m, 2, H-5, OCH₂), 3.18 (t, J = 6.8 Hz, 0.6 H, NCH₂), 2.98 (t, J = 6.7 Hz, 1.4 H, CH₂N), 2.73 (t, J = 6.7 Hz, 1.4 H, SCH₂), 2.66-2.61 (m, 2.4 H, SCH₂), 1.92-1.84 (m, 2 H, CH₂CH₂S), 1.63-1.60 (m, 0.4 H, CH₂CH₂S); ¹³C NMR (D₂O): δ 100.6 (C-1), 72.1 (C-5), 71.7 (C-3), 70.9 (C-2), 69.6 (C-6), 66.9 (OCH₂), 66.8 (C-4), 63.8 (C-7), 41.4 and 39.9 (NCH₂), 31.8 and 28.4 (SCH₂), 29.2 (CH₂CH₂S). ESI TOF HRMS: m/z calc'd for C₁₂H₂₅NO₇S [M + H]⁺ 328.1424; found 328.1429.

BSA conjugate 4

A solution of **2** (4.5 mg, 12 μ mol) in 0.1 M NaHCO₃ (1.3 mL) was vigorously stirred with a solution of thiophosgene (2 μ L, 26 μ mol) in CHCl₃ (2 mL) for 1 h at RT to produce isocyanate **3**. The organic phase was removed using a pipette and the aqueous phase was extracted with three portions of CHCl₃. Traces of organic solvent were then removed by using a stream of air until a clear solution was obtained. The water phase was then combined with a solution of BSA (2 mg) in 0.1 M NaHCO₃/0.3 M NaCl (2 mL) and stirred

for 60 h at RT. The solution was twice dialyzed against water (2 L) and freeze-dried to give **4** as colorless powder (1.8 mg). MALDI-TOF-MS: M^+ 70096 (determined for BSA: 66488); 9.7 mol ligand/mol BSA.

ii. Synthesis of allyl D,D-heptose (**8**)



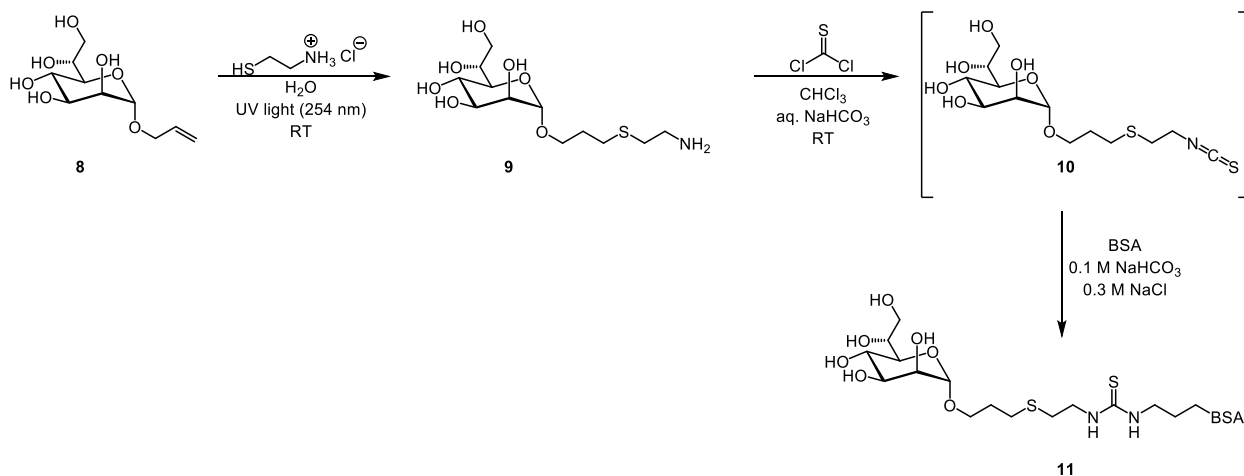
2,3,4,6,7-Penta-O-acetyl-D-glycero- α -D-manno-heptopyranosyl trichloroacetimidate (6**).** A suspension of 2,3,4,6,7-penta-O-acetyl-D,D-heptose **5**¹⁰ (33.8 mg, 80 μ mol), K_2CO_3 (140 mg, 1 mmol), and trichloroacetonitrile (65 μ L, 0.64 mmol) in dry CH_2Cl_2 (5 mL) was stirred for 18 h at RT. Additional portions of K_2CO_3 (40 mg) and trichloroacetonitrile (20 μ L) were added and stirring was continued for 3 h at RT. The suspension was diluted with toluene and concentrated. The residue was subjected to column chromatography on silica gel (toluene-EtOAc 3:1, containing 0.1% triethylamine) to give **6** (38 mg, 84%) as colorless prisms, m.p. 121-123° (from EtOAc-hexane), followed by the β -anomer (2 mg, 4%) as a syrup. 1H NMR (600 MHz, $CDCl_3$) for **6**: δ 8.79 (NH), 6.26 (d, J = 2.1 Hz, 1 H, H-1), 5.44 (br t, 1 H, H-2), 5.40-5.36 (m, 2 H, H-3, H-4), 5.20 (dt, J = 2 x 3.5, 7.1 Hz, 1 H, H-6), 4.42 (dd, J = 3.8, 12.0 Hz, 1 H, H-7a), 4.26 (dd, J = 7.3, 12.0 Hz, 1 H, H-7b), 4.22-4.20 (m, H-5), 2.19 (s, 3 H), 2.11 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H) and 2.00 (s, 3 H, 5 x CH_3CO). Compound **6** was directly used for the glycosylation reaction.

Allyl 2,3,4,6,7-penta-O-acetyl-D-glycero- α -D-manno-heptopyranoside (7**).** A suspension of allyl alcohol (25 μ L, 0.37 mmol) and molecular sieves 4Å (200 mg) in dry CH_2Cl_2 (5 mL) was stirred for 1 h at RT under Ar followed by addition of **6** (82 mg, 125 μ mol) in dry CH_2Cl_2 (5 mL). Stirring was continued for 30 min and TMSO-triflate (6 μ L, 33 μ mol) was added. The suspension was stirred for 4 h at RT. Additional portions of allyl alcohol (25 μ L) and TMSO-triflate (4 μ L) were added and stirring was continued for 2 h. The reaction was quenched by addition of triethylamine (0.1 mL) at ice-bath temperature followed by addition of dry pyridine (2 mL), a catalytic amount of *N,N*-dimethylaminopyridine, and acetic anhydride (0.25 mL). The suspension was stirred overnight at RT. Methanol (0.2 mL) was added, the suspension was filtered over Celite®, concentrated, and coevaporated three times with toluene. The residue was purified by silica gel chromatography (toluene-EtOAc 2:1), which gave **7** (31.8 mg, 47%) as a syrup followed by a fraction of **7** containing 10% of the β -anomer (28.5 mg, 43%); $[\alpha]_D^{20}$ +42 (*c* 0.3 $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$): δ 5.89 (m, 1 H, =CH-), 5.34 (dd, J = 3.4, 9.8 Hz, 1 H, H-3), 5.30 (m, 1 H, =CH_{2trans}), 5.27 (t, J = 9.9 Hz, 1 H, H-4), 5.24 (m, 1 H, =CH_{2cis}), 5.22 (dd, J = 1.9, 3.4 Hz, 1 H, H-2), 5.19 (dt, J = 2 x 3.3, 6.9

Hz, 1 H, H-6), 4.83 (d, $J = 1.8$ Hz, 1 H, H-1), 4.43 (dd, $J = 3.6, 12.1$ Hz, 1 H, H-7a), 4.26 (dd, $J = 7.4, 12.1$ Hz, 1 H, H-7b), 4.18 (m, 1 H, OCH₂), 4.02 (m, 1 H, OCH₂), 4.02 (dd, $J = 3.4, 9.7$ Hz, 1 H, H-5), 2.15 (s, 3 H), 2.08 (2s, 9 H) and 1.99 (s, 3 H, 5 x CH₃CO); ¹³C NMR (150 MHz, CDCl₃): δ 170.6, 169.6, 169.8 (2 C), and 169.7 (C=O), 132.9 (=CH-), 118.3 (=CH₂), 96.4 (C-1), 70.3 (C-6), 69.9 (C-5), 69.5 (C-2), 69.2 (C-3), 68.4 (OCH₂), 66.8 (C-4), 61.7 (C-7); ESI TOF HRMS: m/z calcd for C₂₀H₂₈O₁₂ [M + Na]⁺ 483.1473; found 483.1483.

Allyl D-glycero- α -D-manno-heptopyranoside (8). A solution of **7** (15.5 mg, 33.7 μ mol) and 0.1 M methanolic NaOMe (0.2 mL) in dry MeOH (5 mL) was stirred for 4 h at RT. The pH of the solution was adjusted to 7 by addition of Dowex 50 H⁺ cation-exchange resin and the resin was filtered off. The filtrate was concentrated to afford **8** (8.4 mg, ~99%) as a colorless syrup; $[\alpha]_D^{20} +73.5$ (c 0.4, H₂O); ¹H NMR (600 MHz, D₂O): δ 5.95 (m, 1 H, =CH-), 5.34 (m, 1 H, =CH_{2trans}), 5.26 (m, 1 H, =CH_{2cis}), 4.86 (d, $J = 1.3$ Hz, 1 H, H-1), 4.21 (m, 1 H, OCH₂), 4.04 (m, 1 H, OCH₂), (dt, $J = 2 \times 3.2, 7.6$ Hz, 1 H, H-6), 3.90 (dd, 1 H, H-2), 3.79 (dd, $J = 3.4, 11.9$ Hz, 1 H, H-7a), 3.75 (dd, $J = 3.2, 9.1$ Hz, 1 H, H-3), 3.73 (t, $J = 9.2$ Hz, 1 H, H-4), 3.69 (dd, $J = 7.7$ Hz, 1 H, H-7b); ¹³C NMR (150 MHz, D₂O): δ 134.1 (=CH-), 119.2 (=CH₂), 99.8 (C-1), 73.8 (C-5), 72.6 (C-6), 71.7 (C-3), 70.7 (C-2), 68.9 (C-4), 68.2 (OCH₂), 62.6 (C-7); ESI TOF HRMS: m/z calcd for C₁₀H₁₈O₇ [M+H]⁺: 251.1125; found: 251.1133.

iii. Synthesis of D,D-heptose BSA conjugate (11)

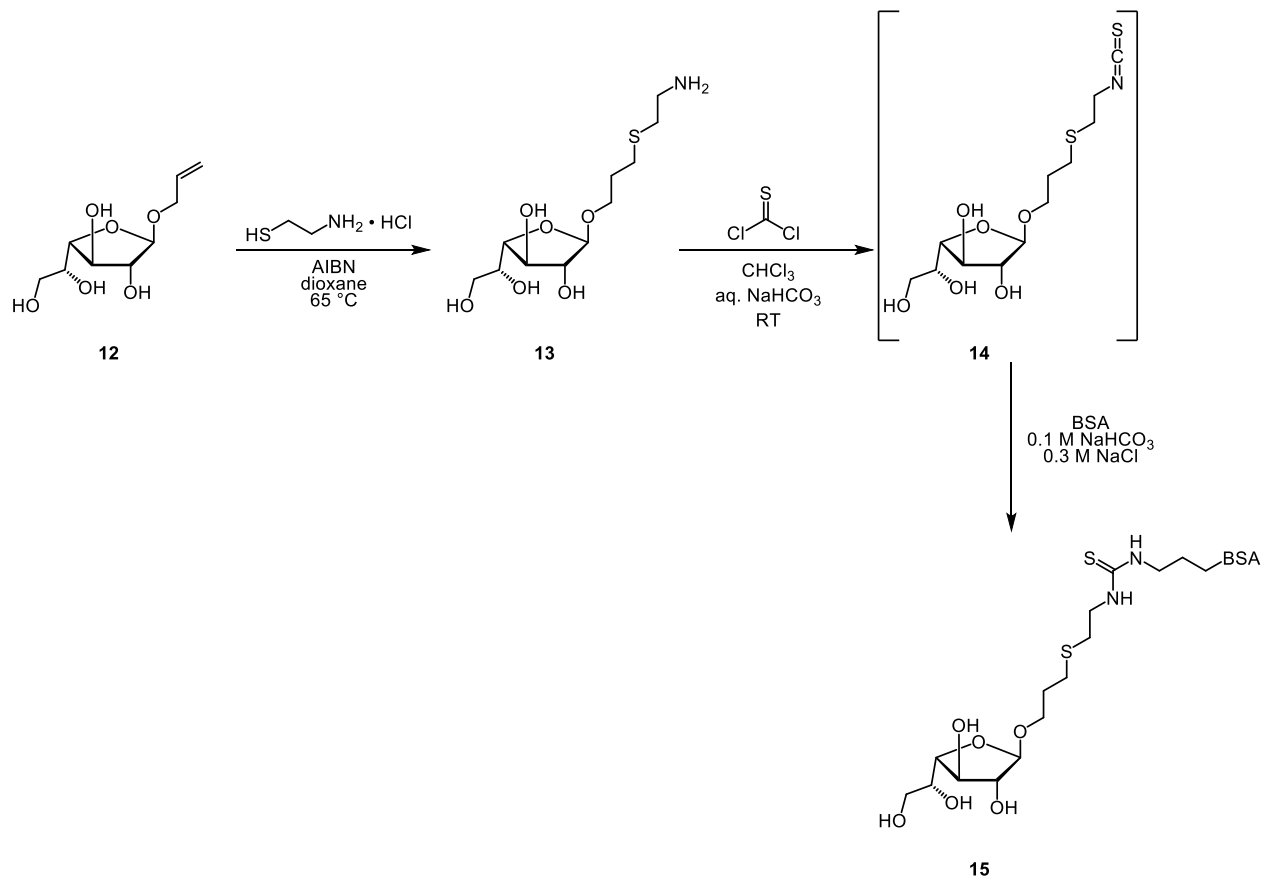


3-(2-Aminoethylthio)propyl D-glycero- α -D-manno-heptopyranoside (9). A solution of **8** (5.1 mg, 26 μ mol) and cysteamine hydrochloride (2.6 mg, 23 μ mol) in H₂O (50 μ L) was irradiated at 254 nm for 3 h at RT. A second portion of cysteamine hydrochloride (2 mg, 17.6 μ mol) was added and irradiation was continued for 1.5 h. The reaction mixture was diluted with water and applied onto a column of Dowex 50 H⁺ cation exchange resin (10 cm \times 1 cm diameter). Elution with 0.1 M aq. NH₃ afforded first a pool

containing unreacted educt and cysteamine (3.9 mg) followed by fractions containing **9** and byproducts. Final elution of the column with copious volumes of 0.1 M NH₃ (100 mL) afforded **9**. In order to remove aromatic residuals released from the resin, the crude was dissolved in water and passed over anion-exchange resin Dowex AG 1-X8 (HCO₃⁻ form). The resin was rinsed with water and the filtrate was lyophilized to give **9** (2.9 mg, 30%) as an amorphous solid. ¹H NMR (600 MHz, D₂O): δ 4.81 (d, *J* = 1.7 Hz, 1 H, H-1), 4.00 (dt, *J* = 3.3 (2 x) and 7.6 Hz, 1 H, H-6), 3.88 (dd, *J* = 1.9, 2.6 Hz, 1 H, H-2), 3.79 (m, 1 H, OCH₂), 3.78 (dd, *J* = 3.3, 11.9 Hz, 1 H, H-7a), 3.74-3.72 (m, 2 H, H-3, H-4), 3.69 (dd, *J* = 7.7, 12.0 Hz, 1H, H-7b), 3.67 (m, 1 H, H-5), 3.56 (dt, *J* = 5.8 Hz, 1 H, OCH₂), 3.16 (t, *J* = 6.7, 10.1 Hz, 2 H, NCH₂), 2.82 (t, *J* = 6.7 Hz, 2 H, SCH₂CH₂N), 2.68-2.63 (m, 2 H, CH₂CH₂CH₂S), 1.93-1.84 (m, 2H, CH₂CH₂CH₂); ¹³C NMR (150 MHz, D₂O): δ 100.3 (C-1), 73.7 (C-5), 72.3 (C-6), 71.6 (C-3), 70.3 (C-2), 68.1 (C-4), 66.7 (OCH₂), 62.5 (C-7), 39.4 (CH₂N), 29.3 (SCH₂CH₂N), 28.7 (CH₂CH₂CH₂), 28.5 (CH₂CH₂CH₂S); ESI TOF HRMS: *m/z* calcd for C₁₂H₂₅NO₇S [M + H]⁺ 328.1424; found 328.1429.

BSA conjugate 11. A solution of **9** (2.2 mg, 6 μmol) in 0.1 M NaHCO₃ (1.5 mL) was vigorously stirred with a solution of thiophosgene (1 μL, 13 μmol) in CHCl₃ (1 mL) for 2 h at RT to provide isocyanate **10**. The organic phase was removed using a pipette and the aqueous phase was extracted with four portions of CHCl₃. Traces of organic solvent were then removed by using a stream of N₂ until a clear solution was obtained. The water phase was then added to a solution of BSA (1.7 mg) in 0.1 M NaHCO₃/0.3 M NaCl (1.5 mL) and stirred for 48 h at RT. The solution was twice dialyzed against water (2 L) and freeze-dried to give **11** as an off-white powder (2.2 mg). MALDI-TOF-MS: M+ 69021 (determined for BSA: 66488); 6.8 mol ligand/mol BSA.

iv. Synthesis of Gal^f-BSA conjugate (15)

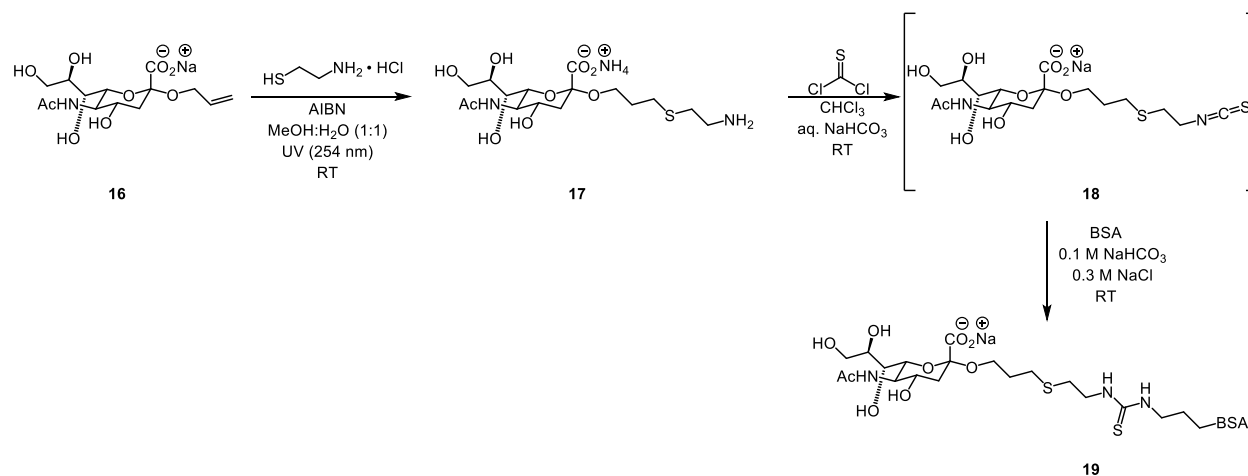


3-(2-Aminoethylthio)propyl-β-D-galactofuranoside (13). Allyl β-D-galactofuranose¹ (**12**) (12.6 mg, 0.057 mmol) was dissolved in anhydrous dioxane (Sigma-Aldrich, SureSeal) (219 μL, 0.26 M), along with cysteamine hydrochloride (32.4 mg, 0.285 mmol) and azobisisobutyronitrile (AIBN) (2.8 mg, 0.017 mmol), and heated to 65 °C. The reaction mixture was stirred for 3 h, diluted with methanol, and concentrated by rotary evaporation. The resulting product was purified on Dowex 50Wx8 H⁺ resin (100-200 mesh). The resin was pretreated with two cycles of 3N HCl, H₂O until neutral, 1.5 M NaOH, H₂O until neutral. After these cycles, the resin was again treated with 3N HCl and rinsed with water. The reaction mixture was added to the resin and eluted with 0.1 M aq. NH₃. Unreacted cysteamine began eluting off the column when the color change (orange-brown) reached the bottom of the column. Fractions were monitored using TLC staining with p-anisaldehyde. After cysteamine finished eluting, product was collected and fractions were evaporated, yielding 20 mg of **13** (quant.) as a solid. Analytical data for **13**: ¹H NMR (600 MHz, D₂O): δ 5.01 (d, *J* = 2.1 Hz, 1H), 4.10 – 4.04 (m, 2H), 3.97 (dd, *J* = 6.4, 4.0 Hz, 1H), 3.87 – 3.81 (m, 2H), 3.74 – 3.63 (m, 3H), 2.93 (t, *J* = 6.6 Hz, 2H), 2.73 (t, *J* = 6.6 Hz, 2H), 2.67 (t, *J* = 7.3 Hz, 3H), 1.91 (quint, *J* = 6.7 Hz, 2H); ¹³C NMR (151 MHz, D₂O): δ 110.0, 85.5, 83.8, 79.3, 73.6, 69.6, 65.6, 41.9, 34.3, 31.6, 30.2;

DART-SVP HRMS (positive ion mode) calculated for $C_{11}H_{23}NO_6S$ $[M+H]^+ = 298.13243$, found = 298.13242.

BSA conjugate 15. A solution of **13** (20 mg, 0.06 mmol) in 0.1 M $NaHCO_3$ (7.5 mL) was stirred at RT. A solution of thiophosgene (12.4 μ L, 0.162 mmol) in $CHCl_3$ (4.6 mL) was added. The reaction mixture was stirred vigorously at RT overnight to produce isocyanate **14**. The reaction mixture was extracted with $CHCl_3$ three times, and the aqueous layer was sparged with nitrogen to remove any remaining $CHCl_3$. The resulting aqueous solution was added to a solution of bovine serum albumin (BSA) (30 mg) in 4.7 mL buffer (0.1 M $NaHCO_3$, 0.3 M NaCl). The reaction mixture was stirred slowly at RT for 48 h. The reaction mixture was then dialyzed into water (20,000 MW cassette) for 2 h, then 24 h, then 24 h. The resulting solution was lyophilized to produce 27.8 mg of **15** as a white solid. A 20 μ M sample of **15** was made in water and mixed with a saturated sinapic acid matrix solution (30:70 acetonitrile:water) in a 1:1 ratio for MALDI characterization. MALDI-TOF-MS: M^+ 70443 (determined for BSA: 66933); 10 mol ligand/mol BSA.

v. Synthesis of Neu5Ac-BSA conjugate (19)



2-O-[3-(2-aminoethylthio)propyl]-3,5-dideoxy-5-acetamino-D-glycero- α -D-galacto-2-nonulopyranosidonic acid (2-O-[3-(2-aminoethylthio)propyl] sialic acid) (17**).** Allyl α -Neu5Ac⁷ (**16**) (10 mg, 26 μ mol) was dissolved in a 1:1 methanol:water mixture (400 μ L) in a quartz reaction tube, along with cysteamine hydrochloride (14.8 mg, 130 μ mol) and azobisisobutyronitrile (AIBN) (1.6 mg, 10 μ mol). The tube was stirred at RT overnight in a UV reactor, exposed to 254 nm light. The mixture was then diluted with methanol and concentrated by rotary evaporation. The product was purified as described for **13** (Dowex resin, elution with aq. NH_3) to provide 3.3 mg of **17** as a solid. Analytical data for **17** matched that of literature data.¹¹

BSA conjugate 19. A solution of **17** (3.3 mg, 7 μ mol) in 0.1 M NaHCO₃ (875 μ L) was stirred at RT. A solution of thiophosgene (2 μ L, 19 μ mol) in CHCl₃ (540 μ L) was added. The reaction mixture was stirred vigorously at RT overnight to produce isocyanate **18**. The reaction mixture was extracted with CHCl₃ three times, and the aqueous layer was sparged with nitrogen to remove any remaining CHCl₃. The resulting aqueous solution was added to a solution of bovine serum albumin (BSA) (3.5 mg) in 550 μ L of buffer (0.1 M NaHCO₃, 0.3 M NaCl). The reaction mixture was stirred slowly at RT for 48 h. The reaction mixture was then dialyzed into water (20,000 MW cassette) for 2 h, then 24 h, then 24 h. The final product was lyophilized to produce 3.95 mg of **19** as a white solid. A 20 μ M sample of **19** was made in water and mixed with a saturated sinapic acid matrix solution (30:70 acetonitrile:water) in a 1:1 ratio for MALDI characterization. MALDI-TOF-MS: M+ 72023 (determined for BSA: 66943); 10.3 mol ligand/mol BSA

III. References

1. Wesener, D. A.; Wangkanont, K.; McBride, R.; Song, X.; Kraft, M. B.; Hodges, H. L.; Zarling, L. C.; Splain, R. A.; Smith, D. F.; Cummings, R. D.; Paulson, J. C.; Forest, K. T.; Kiessling, L. L. Recognition of microbial glycans by human intelectin-1. *Nat. Struct. Mol. Biol.* **2015**, *22* (8), 603-610.
2. Kosma, P.; Strobl, M.; März, L.; Kusumoto, S.; Fukase, K.; Brade, L.; Brade, H. Synthesis and immunoreactivity of poly(acrylamide) copolymers containing C-3- and C-7-modified, carboxyl-reduced, 4-O- and 5-O-phosphorylated K. *Carbohydr. Res.* **1993**, *238*, 93-107.
3. Kosma, P.; Gass, J.; Schulz, G.; Christian, R.; Unger, F. M. Artificial antigens. Synthesis of polyacrylamide copolymers containing 3-deoxy-D-manno-2-octulopyranosylonic acid (KDO) residues. *Carbohydr. Res.* **1987**, *167*, 39-54.
4. Paulsen, H.; Wulff, A.; Brenken, M. Bausteine von Oligosacchariden, IC. Darstellung von synthetischen Antigenen der inneren Core-Region von Lipopolysacchariden durch Copolymerisation mit Acrylamid. **1991**, *11*, 1127-1145.
5. Reiter, A.; Zamyatina, A.; Schindl, H.; Hofinger, A.; Kosma, P. Synthesis of *Pseudomonas aeruginosa* lipopolysaccharide core antigens containing 7-O-carbamoyl-L-glycero- α -D-manno-heptopyranosyl residues. *Carbohydr. Res.* **1999**, *317* (1), 39-52.
6. Marchetti, R.; Malinowska, L.; Lameignère, E.; Adamova, L.; de Castro, C.; Cioci, G.; Stanetty, C.; Kosma, P.; Molinaro, A.; Wimmerova, M.; Imberty, A.; Silipo, A. Burkholderia cenocepacia lectin A binding to heptoses from the bacterial lipopolysaccharide. *Glycobiology* **2012**, *22* (10), 1387-1398.
7. Roy, R.; Laferrière, C. A. Synthesis of protein conjugates and analogues of N-acetylneuraminic acid. *Can. J. Chem.* **1990**, *68* (11), 2045-2054.
8. Wimmer, N.; Brade, H.; Kosma, P. Synthesis of neoglycoproteins containing D-glycero-D-talo-oct-2-ulopyranosylonic acid (KO) ligands corresponding to core units from Burkholderia and Acinetobacter lipopolysaccharide. *Carbohydr. Res.* **2000**, *329* (3), 549-560.
9. Holst, O.; Brade, L.; Kosma, P.; Brade, H. Structure, serological specificity, and synthesis of artificial glycoconjugates representing the genus-specific lipopolysaccharide epitope of Chlamydia spp. *J. Bacteriol.* **1991**, *173* (6), 1862-1866.
10. Zamyatina, A.; Gronow, S.; Puchberger, M.; Graziani, A.; Hofinger, A.; Kosma, P. Efficient chemical synthesis of both anomers of ADP L-glycero- and D-glycero-D-manno-heptopyranose. *Carbohydr. Res.* **2003**, *338* (23), 2571-2589.
11. Roy, R.; Laferrière, C. A. Synthesis of antigenic copolymers of N-acetylneuraminic acid binding to wheat germ agglutinin and antibodies. *Carbohydr. Res.* **1988**, *177*, c1-c4.

IV. ^1H and ^{13}C NMR Spectra

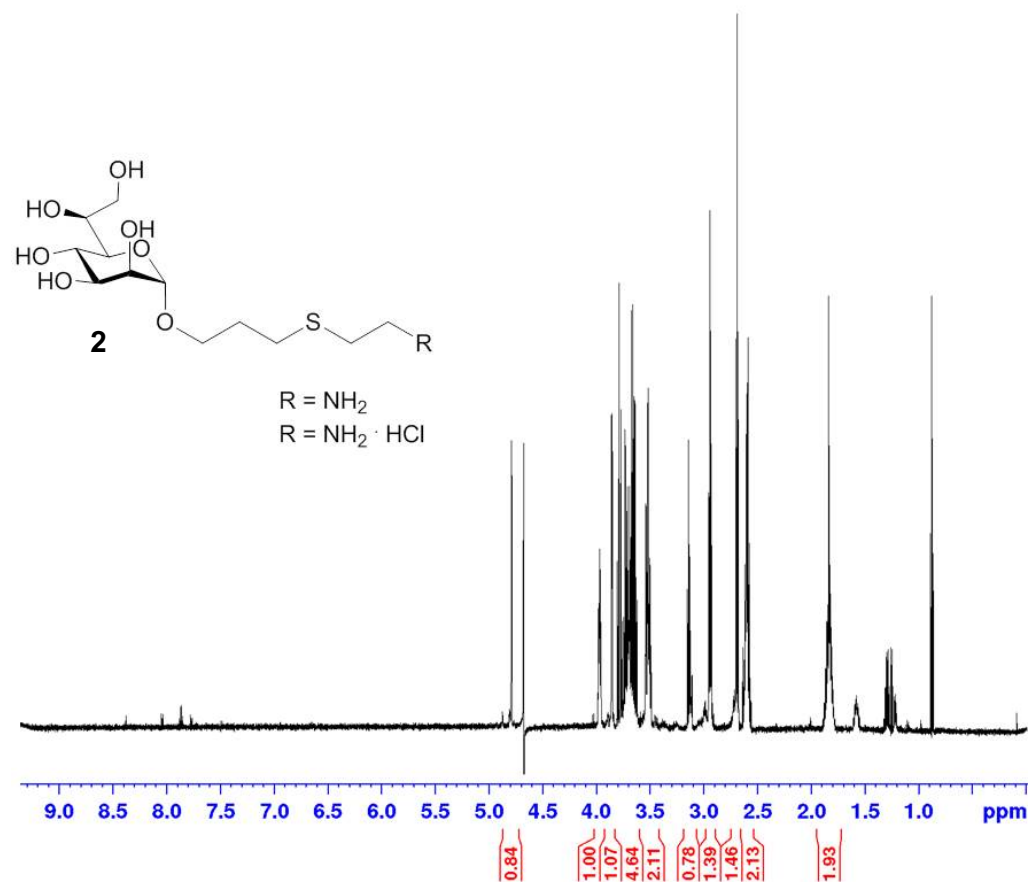


Fig. S6. 600 MHz ^1H NMR spectrum of **2**.

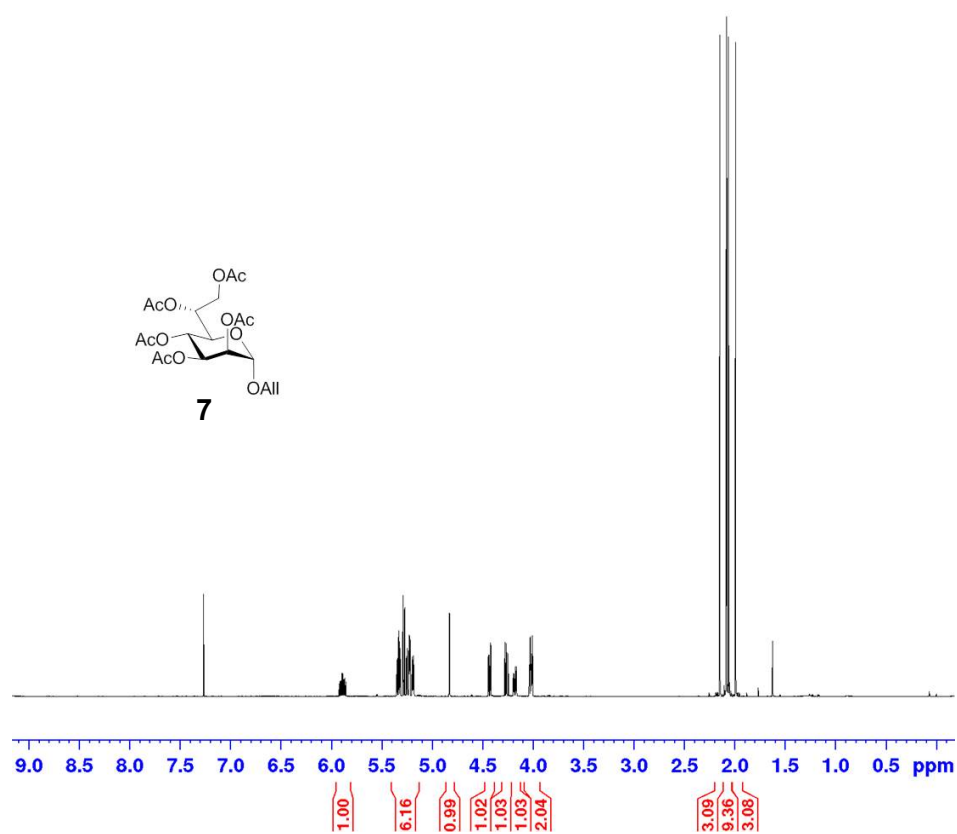


Fig. S7. 600 MHz ¹H NMR spectrum of **7**.

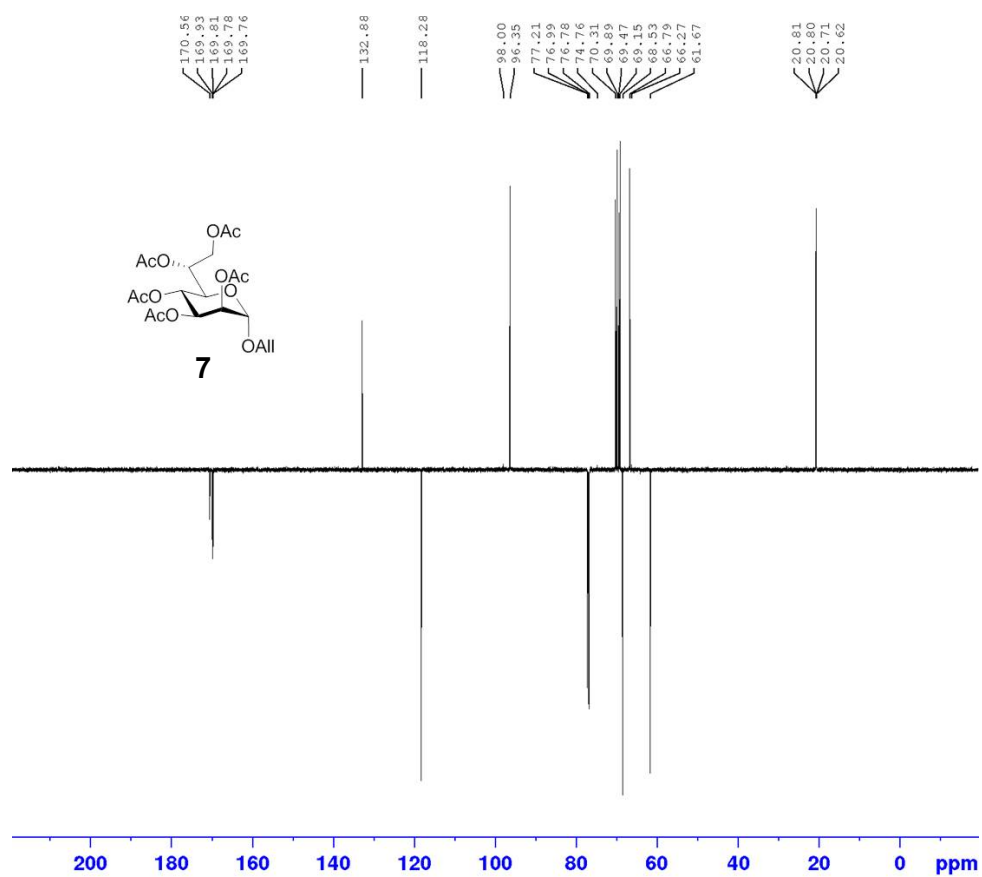


Fig. S8. 150 MHz ^{13}C NMR spectrum of 7.

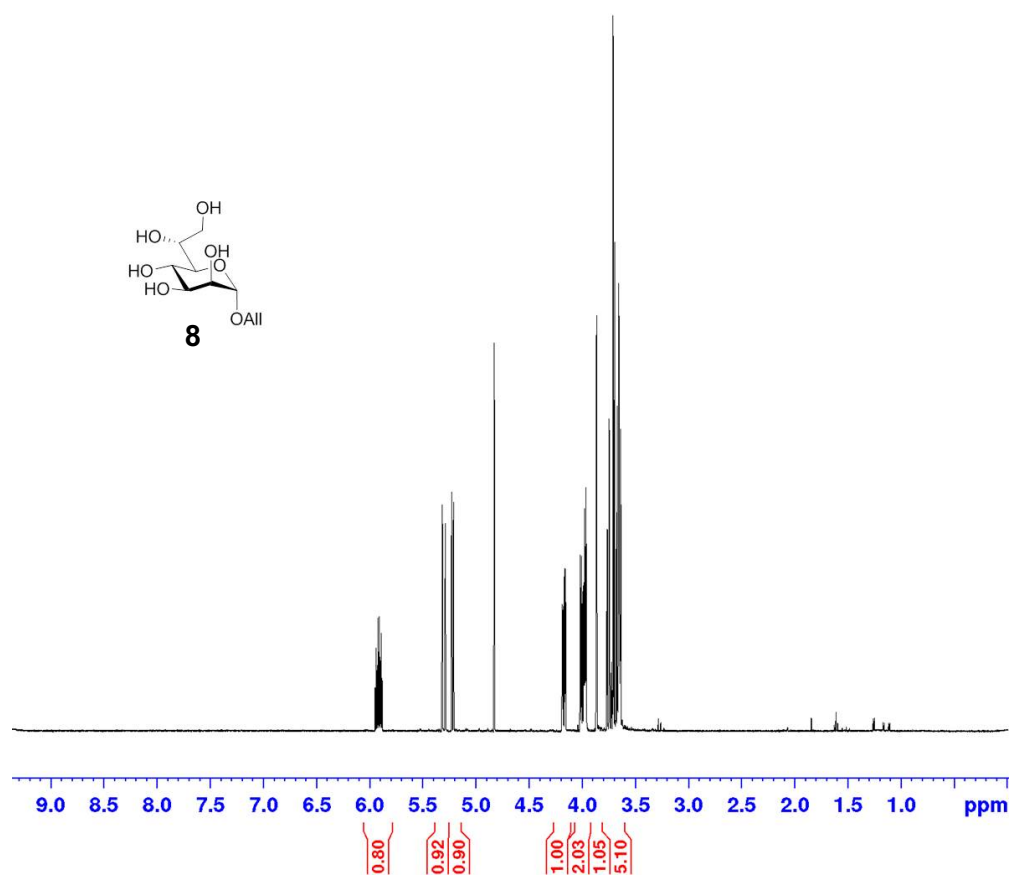


Fig. S9. 600 MHz ^1H NMR spectrum of **8**.

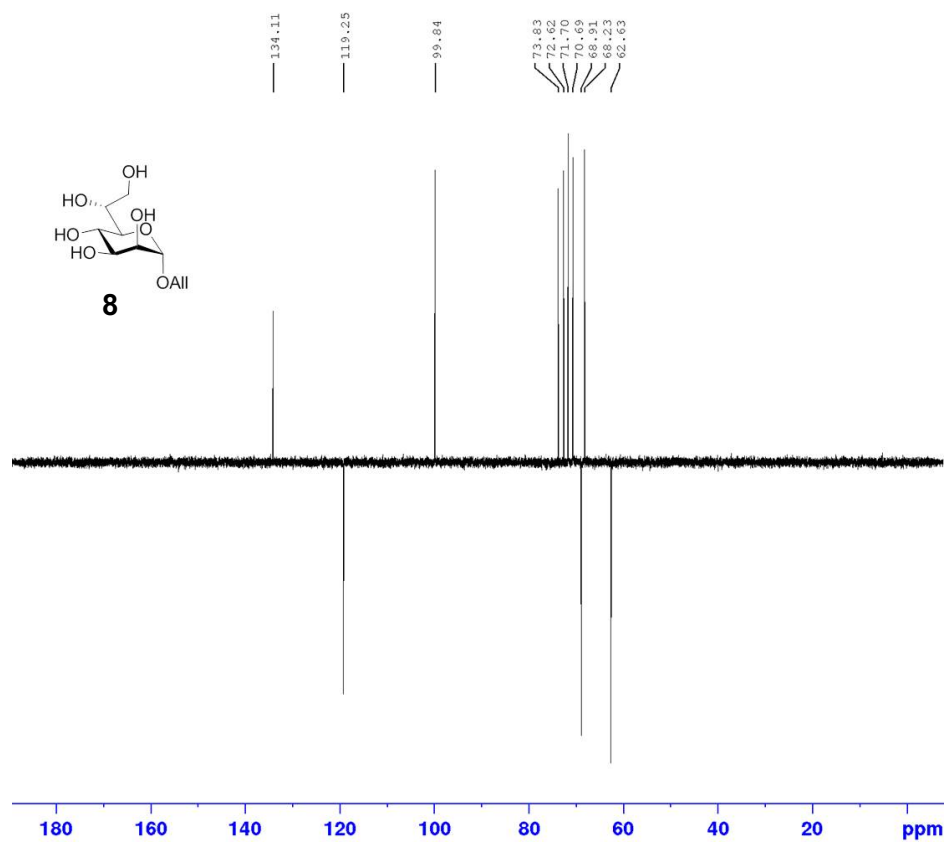


Fig. S10. 150 MHz ¹³C NMR spectrum of **8**.

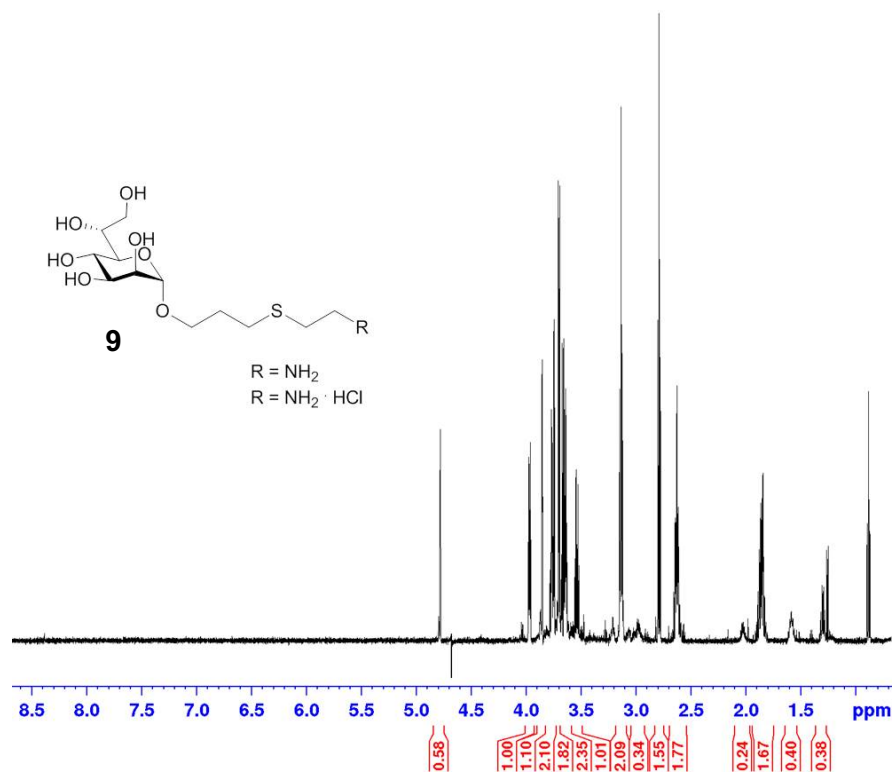


Fig. S11. 600 MHz ¹H NMR spectrum of **9**.

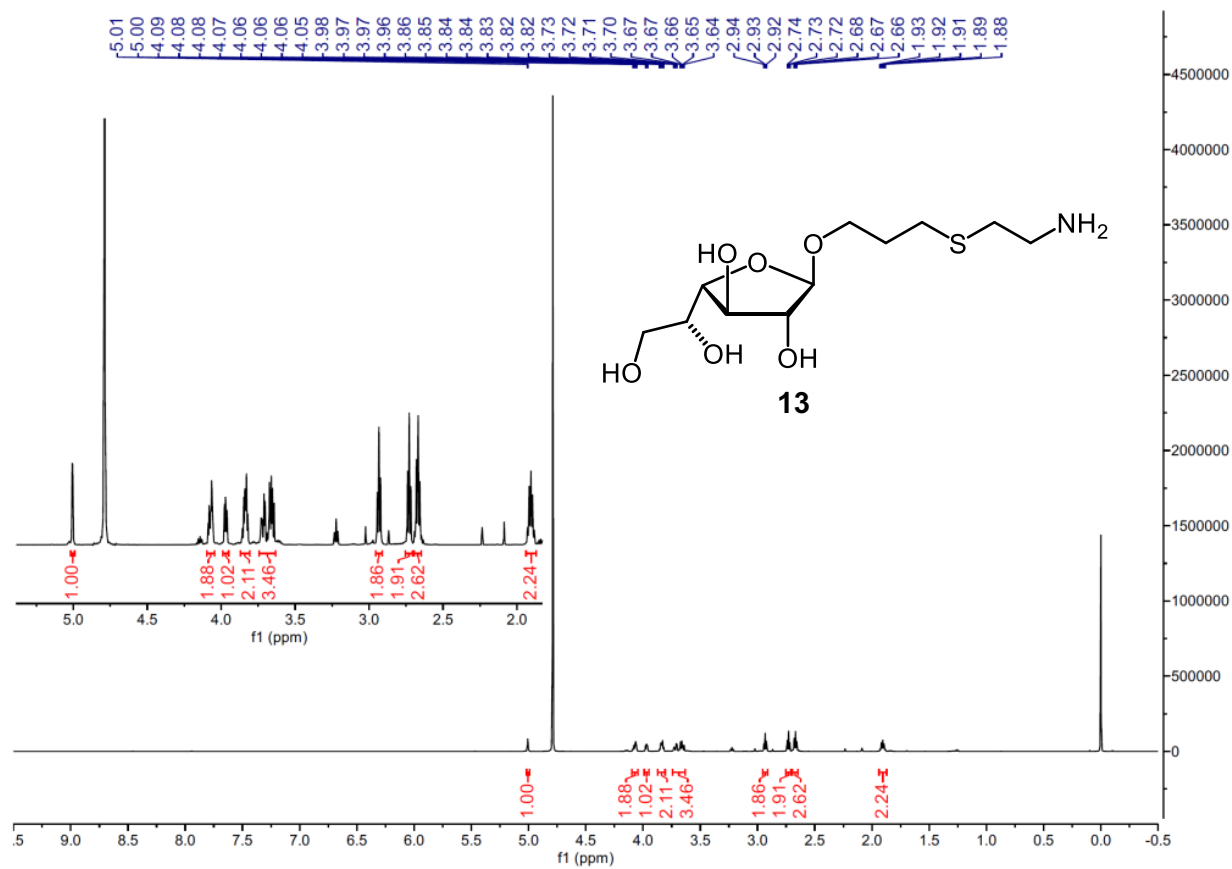


Fig. S12. 600 MHz ^1H NMR spectrum of **13**.

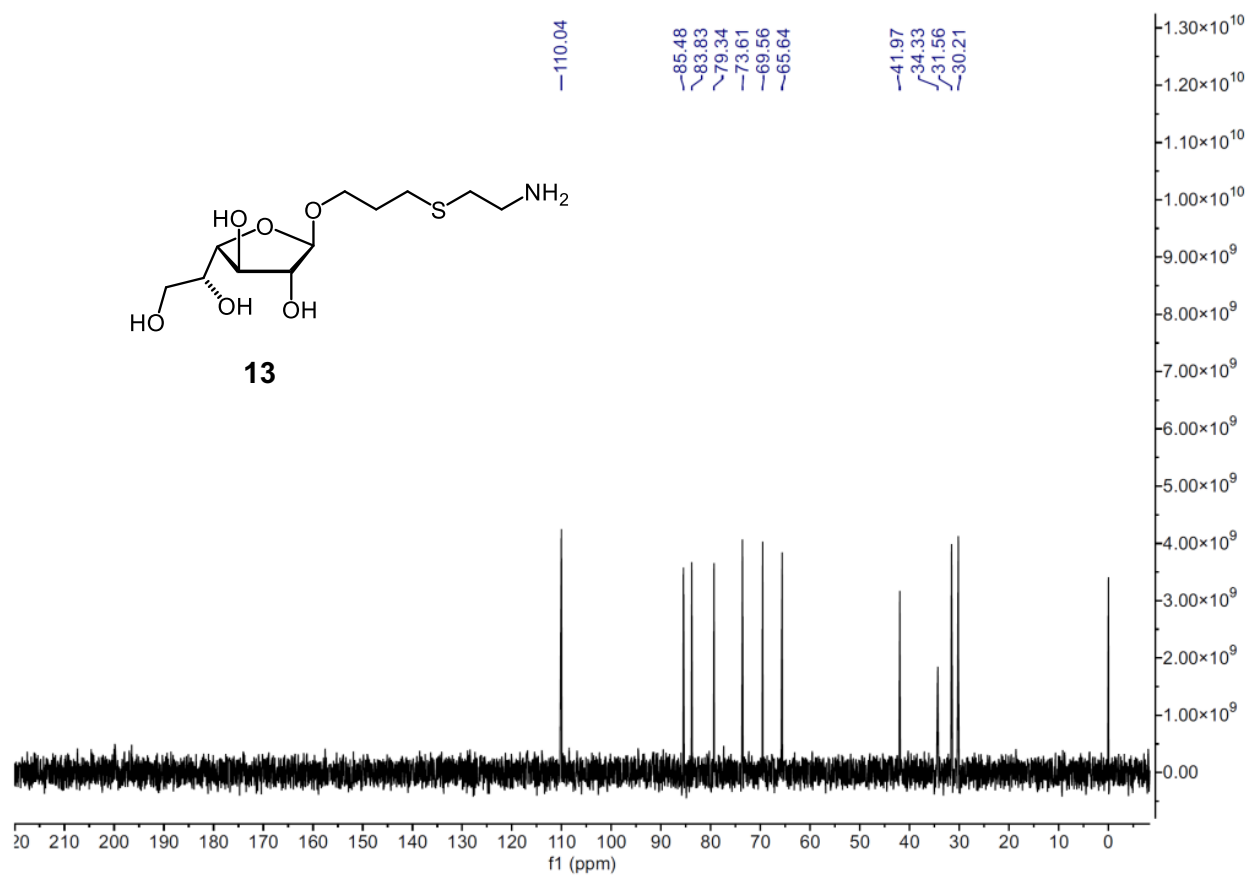


Fig. S13. 151 MHz ¹³C NMR spectrum of **13**.

V. MALDI Spectra of BSA Conjugates

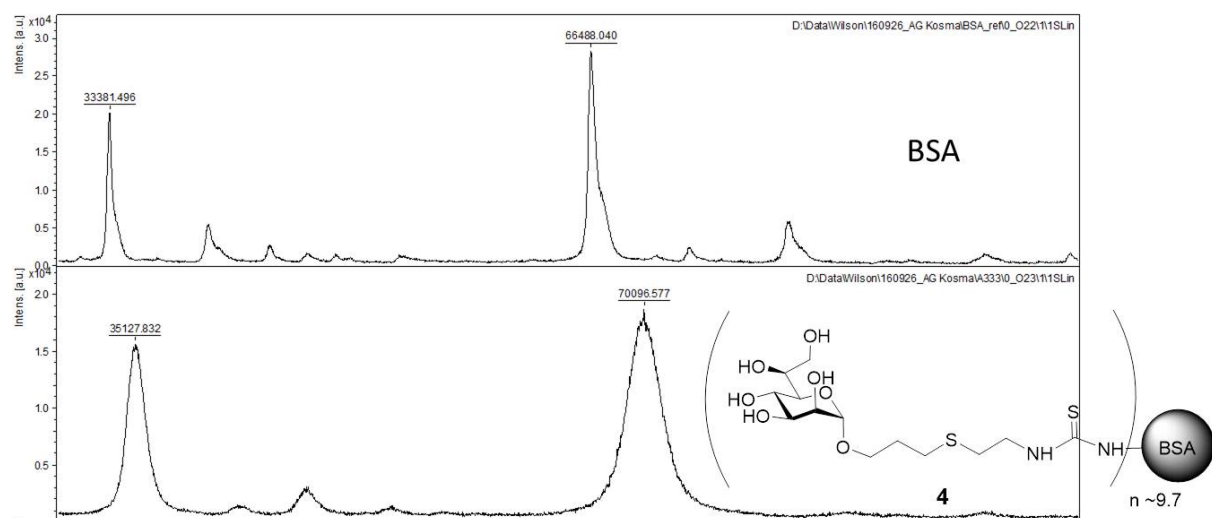


Fig. S14 MALDI-TOF spectra of BSA (top) and BSA conjugate **4**.

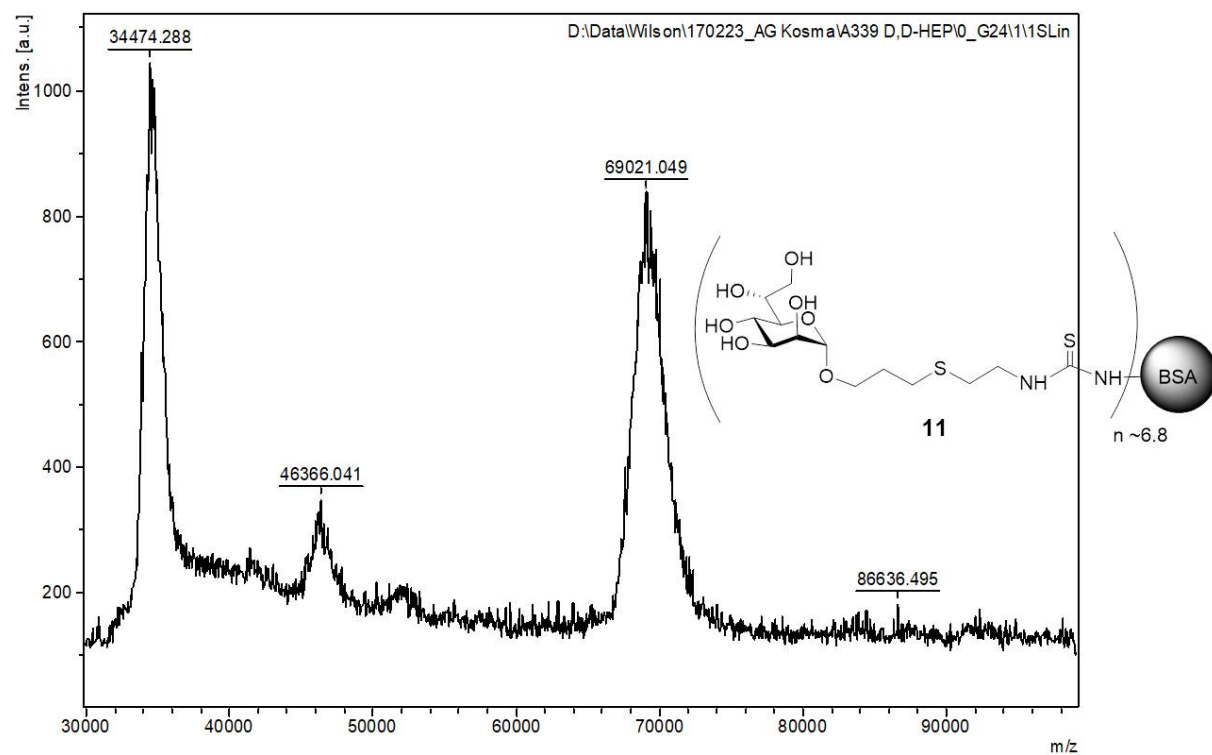


Fig. S15. MALDI-TOF spectrum of BSA conjugate **11**.

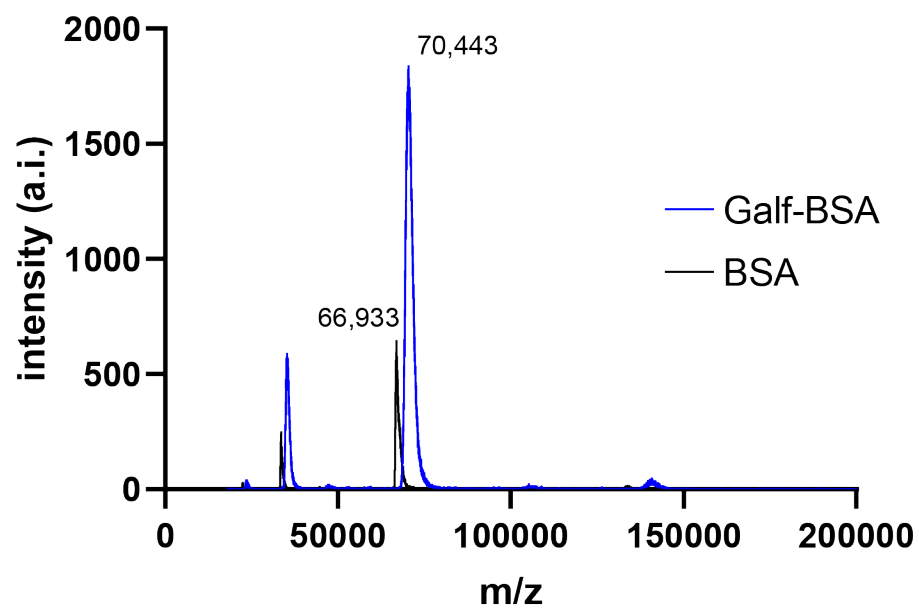


Fig. S16. MALDI-TOF spectra of BSA and BSA conjugate **15**.

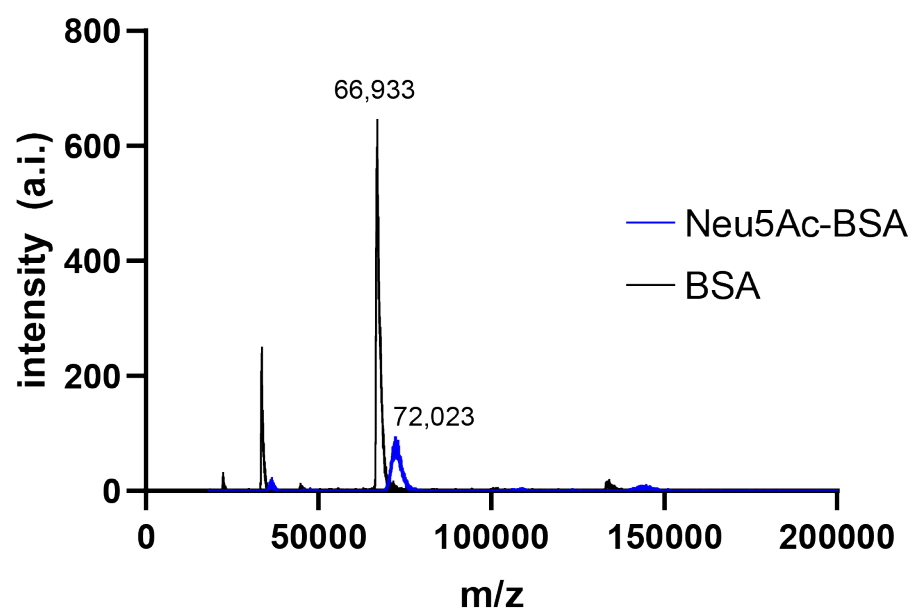


Fig. S17. MALDI-TOF spectra of BSA and BSA conjugate **19**.