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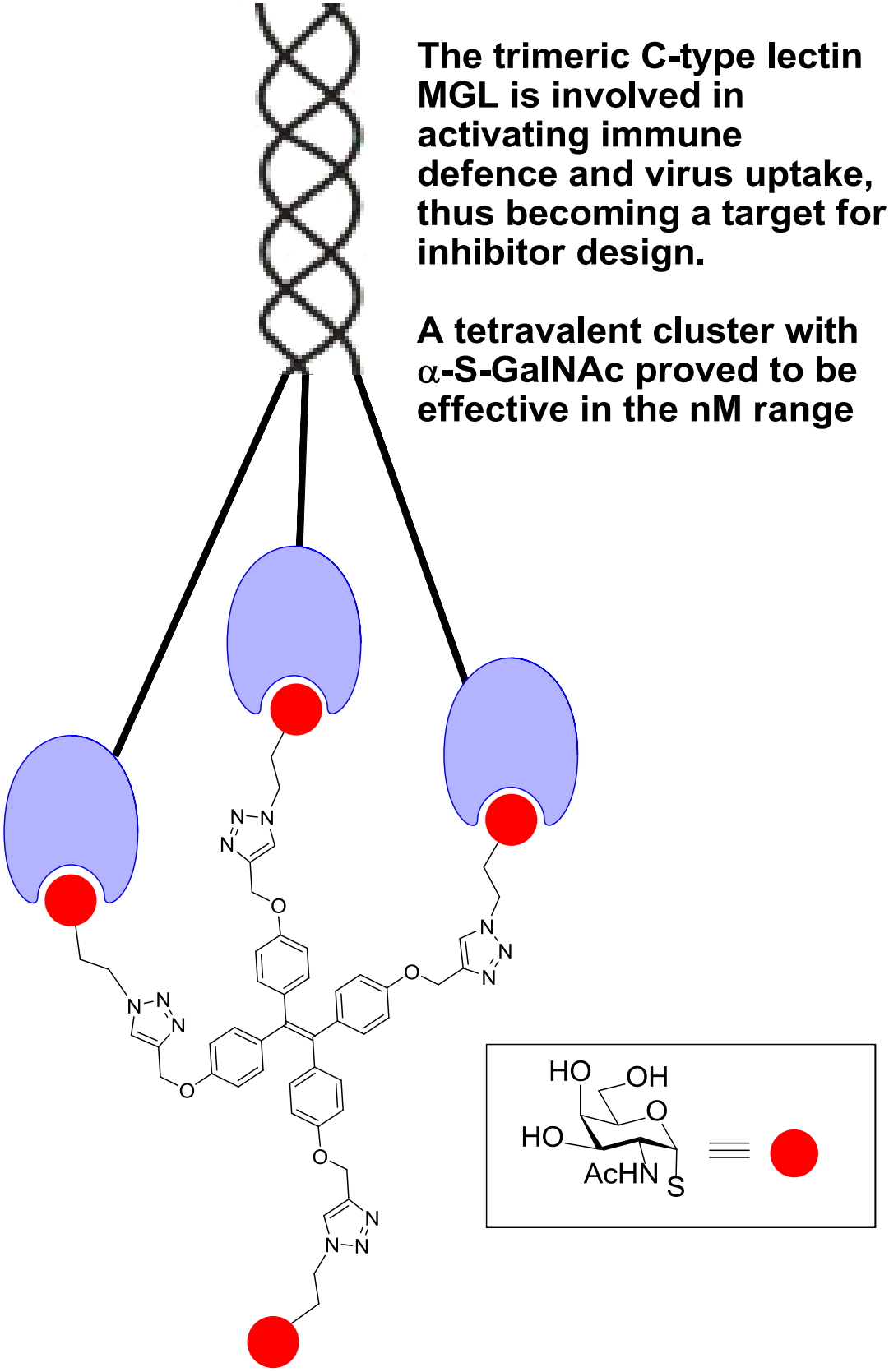
Publication Date	2015-02-17
Publication Information	Andre, S,O'Sullivan, S,Koller, C,Murphy, PV,Gabius, HJ (2015) 'Bi- to tetravalent glycoclusters presenting GlcNAc/GalNAc as inhibitors: from plant agglutinins to human macrophage galactose-type lectin (CD301) and galectins'. Organic & Biomolecular Chemistry, 13 :4190-4203.
Publisher	Royal Society of Chemistry
Link to publisher's version	http://dx.doi.org/10.1039/C5OB00048C
Item record	http://hdl.handle.net/10379/5375
DOI	http://dx.doi.org/10.1039/c5ob00048c

Graphical Abstract



Bi- to tetravalent glycoclusters presenting GlcNAc/GalNAc as inhibitors: from plant agglutinins to human macrophage galactose-type lectin (CD301) and galectins

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Dedication: This paper is dedicated to Professor Richard J. K. Taylor on the occasion of his 65th Birthday

Running title

Glycoclusters as lectin inhibitors

Abstract: Emerging insights into the functional spectrum of tissue lectins leads to identification of new targets for the custom-made design of potent inhibitors, providing a challenge for synthetic chemistry. The affinity and selectivity of a carbohydrate ligand for a lectin may immensely be increased by a number of approaches, which includes varying geometrical or topological features. This perspective leads to the design and synthesis of glycoclusters and their testing using assays of physiological relevance. Herein, hydroquinone, resorcinol, benzene-1,3,5-triol and tetra(4-hydroxyphenyl)ethene have been employed as scaffolds and propargyl derivatives obtained. The triazole-containing linker to the α/β-O/S-glycosides of GlcNAc/GalNAc presented on these scaffolds was generated by copper-catalysed azide-alkyne cycloaddition. This strategy was used to give a panel of nine glycoclusters with bi-, tri- and tetravalency. Maintained activity for lectin binding after conjugation was ascertained for both sugars in solid-phase assays with the plant agglutinins WGA (GlcNAc) and DBA (GalNAc). Absence of cross-reactivity excluded any carbohydrate-independent reactivity of the bivalent compounds, allowing us to proceed to further testing with a biomedically relevant lectin specific for GalNAc. Macrophage galactose(-binding C)-type lectin, involved in immune defence by dendritic cells and in virus uptake, was produced as a soluble protein without/with its α-helical coiled-coil stalk region. Binding to ligands presented on a matrix and on cell surfaces was highly susceptible to the presence of the tetravalent inhibitor derived from the tetraphenylethene-containing scaffold, and presentation of GalNAc with an α-thioglycosidic linkage proved favorable. Cross-reactivity of this glycocluster to human galectins-3 and -4, which interact with T_n-antigen-presenting mucins, was rather small. Evidently, the valency and spatial display of α-GalNAc residues is a key factor to design potent and selective inhibitors for this lectin.

Keywords: agglutinin - C-type lectin - dendritic cells – glycocluster - immune defence - neoglycoconjugate - virus uptake

Introduction

The ability of the glycans of cellular glycoconjugates to present docking sites for tissue lectins pervades diverse aspects of cell physiology.[1] Used as tools, carrier-immobilized carbohydrates had been instrumental to detect and map presence of such endogenous sugar receptors, revealing an exquisite specificity, which, for example, accurately distinguishes the letters of the sugar alphabet such as N-acetylgalactosamine (GalNAc) and N-acetylglucosamine (GlcNAc).[2] In addition to the structure of the sugar the geometry, spatial properties or topology of its presentation are factors that influence the level of affinity of the recognition by a receptor. Local clustering of ligands on a scaffold is a means to reach optimal levels of avidity and selectivity. As the classical work on the hepatic C-type lectins from mammals (Gal/GalNAc-specific) and chicken (GlcNAc-specific) attests, systematic application of neoglycoconjugates and glycoclusters was crucial to measure distinct spatial arrangements in the aggregates of carbohydrate recognition domains (CRDs).[4] The merit of these chemical products as molecular rulers for mammalian lectins is further exemplified for the oligomeric serum collectin mannose (mannan)-binding protein and for galectins (a network of adhesion/growth-regulatory lectins sharing specificity for β-galactosides and the β-sandwich folding[1b, 5]), which form non-covalent di- or oligomers or display two CRDs connected by a linker.[6] Their synthetic origin endows neoglycoconjugates with the potential to let the influence of valency and other structural factors, e. g. relative positioning of the ligands, chemical properties and length of linker as well as changes in the anomeric position including the nature of the atom at the glycosidic linkage, on lectin reactivity become thoroughly examined. If the target lectin has pathophysiological significance, this project line can build the basis for biomedical applications.

Mucin-type O-glycosylation undergoes dynamic alterations, with shifts between elaborated branched structures and the short T/T_n-antigens.[7] The human macrophage galactose(-binding

C)-type lectin (MGL, also referred to as CD301 or CLEC10A), an oligomeric cell surface glycoprotein as the hepatic asialoglycoprotein receptor and other endocytic C-type lectins, is specific for Ser/Thr-linked α-GalNAc residues (T_n antigen and 6'-substituted derivatives thereof such as sialyl T_n) and also β-GalNAc, as presented by bacterial GalNAc-terminated chains (especially those from *Proteus mirabilis*) and the LacdiNAc (GalNAcβ1,4GlcNAc) epitope, e.g. present on helminth parasites.[8] Giving reasons to consider applications, MGL engagement in immature dendritic cells enhances their performance as antigen-presenting cells,[9] while its role as entry site for viral uptake prompts efforts toward targeted blocking.[10] Obviously, MGL can become a target for delivery or blocking.

To initiate glycocluster testing for human MGL we first prepared a panel of nine compounds (for overview on structures, please see Chart 1). Conceptually, we kept the triazole-containing linker between sugar headgroup and the core, i.e. hydroquinone, resorcinol, benzene-1,3,5-triol and tetra(4-hydroxyphenyl)ethene, constant. The two bivalent α-GlcNAc-presenting compounds **1** and **2** served as specificity controls, their equivalents **3** and **4** contained α-GalNAc also with an S-glycosidic linkage, then increasing the number of ligands to tri- and tetravalency in compounds **5** and **6**. Finally, bivalency with β-GalNAc was established, with both S- (**7**) and O-glycosidic linkages (**8**, **9**). The S-glycosidic linkage offers the advantage of its resistance to hydrolysis. Bioactivity and selectivity of these synthetic products were first ascertained by using the agglutinins from *Triticum vulgare* (wheat germ agglutinin, WGA) and *Dolichos biflorus* (DBA) as probes. WGA is specific for GlcNAc irrespective of type of anomeric linkage with only minor affinity for GalNAc. In complex-type N-glycans WGA homes in on the innermost core GlcNAc moiety.[11] DBA is GalNAc specific with some preference for binding to the α-anomer.[11b, 12] The compounds were then tested as inhibitors of human MGL in solid-phase and cell assays. Its physiological presentation and the possibility

of shedding required to test two forms of MGL: the CRD which occurs physiologically after proteolytic cleavage, and also as the complete extracellular section with the CRD and the α-helical coiled-coil stalk section (CRD + stalk). In addition, the issue on occurrence of cross-reactivity of the synthetic compounds to human galectins-3 and -4, known to interact with GalNAc and mucins with T_n-antigen, albeit comparatively weakly,[13] is examined.

Results and discussion

Synthesis of GlcNAc derivatives

The synthetic routes to the bivalent compounds **1** and **2** started from the thiol **10**, which was obtained in line with a reported procedure (Scheme 1).[14] As shown, in the top part of the Scheme, the peracetylated α-GlcNAc derivative was first reacted with 1,2-dibromoethane in the presence of potassium carbonate in acetone-water, and the resulting monobrominated product then converted by sodium azide in the presence of tetrabutylammonium iodide in DMF to the corresponding azide **11**.

Scheme 1

Using the azide, copper-catalysed azide-alkyne cycloaddition (CuAAC) with the two alkynes **19** and **20** yielded the protected form of the test compound (Scheme 2).

Scheme 2

In detail, both bispropargyloxybenzenes, prepared from hydroquinone or resorcinol as previously described,[15] were reacted with **11**, in the presence of $Cu(II)SO_4x5H_2O$ and sodium ascorbate, in a 1:1 THF-H_2O mixture.[16] The acceptors were the limiting reagent to ensure that exclusively a di-triazole was obtained. Each divalent product was separated from unreacted

reagents by chromatography and then the O-acetyl groups were removed from the sugar using a catalytic amount of a freshly prepared 1M sodium methoxide solution in methanol. Products **1** and **2** from this type of reaction were obtained after reverse phase column chromatography in good yield (Scheme 2).

The α-thioglycosidic linkage in the two GlcNAc derivatives leading to **1** and **2** was deliberately prepared to obtain appropriate material for the specificity controls with MGL, because the α-anomer of GalNAc had best reactivity with MGL in solid-phase testing of monosaccharide derivatives.[8b, c] In the case of WGA, α-linked GlcNAc in bivalent scaffolds binders was superior to the β-anomer, and four such molecules gained access to all eight binding sites of WGA.[17] Interestingly, only a slight difference in K_d-values between O- and S-glycosidic linkages had been found when performing titration calorimetry on WGA and tetravalent glycocyclopeptides (0.307 ± 0.003 μM for the O-glycosidic compound *vs* 0.54 ± 0.02 μM for the thioether).[18] No such data are available for MGL. In order to predict the reactivity level of the bivalent compounds for WGA we next performed molecular modeling and calculated the distance profiles of these bivalent compounds. In each case, the anomeric center was the reference point.

Molecular modeling of GlcNAc-presenting scaffolds

Using the Maestro interface (www.schrodinger.com), conformers were first built in an iterative manner, implementing the distance constraint from the reference point to its nearest aromatic C atom (bonded to an O atom) at 10.7 Å, then minimalisations were carried out in Macromodel (OPLSAA force field, gas phase).[3d] Under these conditions, the two anomeric centers are separated by about 21 – 22 Å (Fig. 1, top part). Owing to conformational flexibility this distance can be shortened. It is likely that the two compounds **1** and **2** can thus

present their sugar moieties in a way that is able to bridge adjacent binding sites in WGA. They are about 14 Å apart,[17] nourishing the assumption of strong inhibitory capacity.

Assaying inhibitory potency of the bivalent glycoclusters with GlcNAc on WGA

This hypothesis was tested in an assay, in which WGA interacts with the core of the complex-type N-glycans of asialofetuin. The glycoprotein was adsorbed to the surface of microtiter plate wells, presenting its three bi- and triantennary N-glycans. Extent of binding of the labeled lectin is visualized by colorimetric detection so that any reduction in signal intensity by compound-dependent blocking of lectin binding was readily detected. As shown in Table 1, both compounds are active as inhibitors, with about a twofold difference. Bivalency led to a large increase of inhibitory potency when compared to free GlcNAc, although the triazole as constituent of the linker is known to be much less favorable than a carbamate in the case of WGA.[17-19] Examined for other classes of lectins, e.g. galectins, such a difference between linkers is not a general phenomenon.[6e, 20] Extrapolation of inhibitory activity from the solid-phase assay to cell binding was shown to be possible. WGA association to cell surfaces was susceptible to the presence of GlcNAc (Fig. 2A). As in the solid-phase system, both bivalent compounds were active, with the considerable enhancement relative to the free sugar (Fig. 2A, B). To exclude a carbohydrate-independent effect by the core/linker parts an exchange of the sugar headgroup was performed, by incorporation of GalNAc in α-thioglycosidic linkage. The synthetic procedure now started with the respective α-GalNAc derivative (Scheme 1), with following processing performed as described above (Scheme 2) to produce compounds **3** and **4** from **12**. These two pairs, i.e. **1**/**2** and **3**/**4**, are thus sensors for carbohydrate-independent binding, an essential control for synthetic products. In order to characterize responses to a valency increase, to changes in anomeric linkage and the switch to an O-glycosidic bond we completed preparation of the panel of probes shown in Chart 1.

Synthesis and molecular modeling of the glycoclusters with GalNAc

The α- and β-anomer derivatives of the thioglycoside **13** and **15** were prepared as described in Scheme 1 for GlcNAc (**11**). The β-O-glycoside-containing azide **18** was obtained from the peracetylated **16** by glycosidation promoted by microwave irradiation with dichloroethane (to give **17**) and then heating with reagents as given in Scheme 1 (bottom). These azide precursors **13**, **15** and **18**, together with the four alkyne reactants **19**-**22** (**21** and **22** prepared as described[21]), generated the compounds **5** and **6** (Scheme 2) and **7**-**9** (Scheme 3). Molecular modeling was performed as above to assess the distance profiles for sugar headgroups in the tri- and tetravalent products **5** and **6**. The triangular presentation of **5** is characterized by a distance of about 21 Å (Fig. 1, bottom part). Put in a rectangular conformation, distances of 14 Å and 27 Å were apparent for tetravalent **6** (Fig. 1, bottom part). The length of the line across the diagonal was about 30 Å. Rotations around linkages in the linker portion will dynamically broaden the range of distances, making smaller spacings possible. Of note, inter-CRD distances of 15 Å, 22 Å and 25 Å are known from the C-type lectin of mammalian hepatocytes.[4d, e] If the sugars in the glycocluster panel were to maintain their binding activity to lectins, then use of the panel should be able to answer questions on effect of valency, anomeric linkage and atom at the anomeric center, for a plant lectin and then for MGL. Of course, an influence of the aglycone parts needed to be excluded first by testing compounds **3** and **4** on WGA.

Assaying the GalNAc-containing compounds **3** and **4** in concentrations up to 1 mM in the inhibition assays yielded no significant reduction of extent of signal development, thus no evidence for any carbohydrate-independent binding. As consequence, the panel could further be examined, first by assaying DBA.

Assaying inhibitory potency of the glycoclusters with GalNAc on DBA

Using a matrix with neoglycoprotein (β-GalNAc as ligand presented by bovine serum albumin), DBA binding was found to be saturable and carbohydrate dependent. The binding of DBA to the sugar part of the neoglycoprotein was effectively reduced with the two GalNAc-containing compounds **3** and **4**, but not by compounds **1** and **2** (Table 1). This reactivity prompted us to proceed to determine activity levels of the other compounds with tri- and tetravalency (**5**, **6**), the β-linked thioglycoside (**7**) and the pair of bivalent β-linked O-glycosides (**8**, **9**). The outcome of the measurements was in line with the known preference to the α-anomer, and rather small enhancements were recorded for the tri- and tetravalent compounds (Table 1). Because the distance between the lectin's binding sites are outside of the range of distances in the synthetic clusters, with 55 Å and 68 Å for the two types of sides in the DBA tetramer,[22] no intramolecular bridging (chelate effect) as in WGA is possible. This limits the inhibitory capacity, nonetheless documenting bioactivity of the sugar. As intended, these binding data set the stage for testing the glycoclusters against MGL.

Assaying inhibitory potency of glycoclusters on MGL in the solid-phase assay

Binding of the human C-type lectin to the matrix in both forms, i.e. as CRD and as the CRD + stalk, was saturable and completely dependent on the presence of Ca^{2+}. When Ca^{2+} was removed from the lectin by treatment with EDTA, abolishing the involvement of the cation in GalNAc binding by coordination bonds to the 3- and 4-OH groups,[8, 23] no significant signal in the assay was obtained. The same holds true if the 4-OH group is in the equatorial position as is the case for the GlcNAc derivatives; these products are not suited for coordination bonding. Altering parameters in the five bivalent compounds, which present GalNAc, disclosed a preference for the α-anomer (Table 1). The reactivity was increased considerably for the MGL version containing the neck region. This was apparent for the α-anomers at all degrees

of valency (Table 1). The tetravalent compound reached the highest level of inhibitory potency in the solid-phase assay. Like in related C-type lectins and lectin-like proteins,[24] the α-helical coiled-coil stalk acts as a mediator for trimerization,[8d] and the effect of the tetravalent **6** on the CRD indicates the possibility of a glycocluster-induced aggregation contributing to the measured potency. Some flexibility between CRD and the α-helical region will allow to adapt to a high-affinity interaction with clustered arrangements of α-GalNAc residues. Physiologically, this will be the case for the T_n-antigen and its sialylated version.

Assaying inhibitory potency of glycoclusters on MGL in cell assays

For the cell assays, a test system with large expression of the T_n-antigen and its sialylated version would be ideal. The Lec8 mutant of the CHO system affords such a desired surface platform, because impairment in Gal transport into the Golgi attests mucin-type O-glycosylation at the stage of the sialyl T_n-antigen.[25] This system was thus used, and dependence of MGL binding on the concentration for the CRD (Fig. 3A) and the (CRD + stalk) construct (Fig. 4A), as well as the effect of free sugar (Fig. 3B, 4B) corresponded to the results seen in the solid-phase assay. Increased efficiency for blocking (CRD + stalk) was apparent for the bi- and trivalent compounds **4** and **5** (Fig. 3C, Fig. 4C) and especially for the tetravalent **6**. Panels D in Fig. 3 and Fig. 4 underscore the strong response to presence of **6**. Glycocluster **6**, along with the trivalent compound **5**, also proved reactive with the two human galectins. In both cases, inhibition in the solid-phase assay reached an extent of about 25-30 % of the signal at concentrations of 1.25-2.5 mM, that is at least about 1000fold less than that measured for MGL. Cross-reactivity of these compounds to both galectins will thus be minimal, fully in line with previous observations on lysine-/lysyllysine-based glycoclusters with Gal as headgroup, reactive with the C-type lectin of hepatocytes, and galectins-1 and -3.[26]

Conclusions

Our aim was to answer the question on favorable properties to turn synthetic glycoclusters into potent inhibitors of human MGL. Keeping linker structure and length constant, we varied the anomeric position, the atom in the anomeric centre and valency. The bioactivity of the compounds, together with a specificity control using a non-cognate headgroup, was first ascertained with two plant lectins. For the human lectin in solution, carbohydrate conjugation in the α-anomeric position as the thioglycoside gave the best results for bivalent compounds. Increase in valency enhanced inhibitory activity. The tetravalent compound **6** was highly potent, blocking binding to a matrix and to cells at nM concentration. Because association of a respective conjugate with an influenza virus led to a marked increase in fluorescence emission at 460 nm from this scaffold, already detectable by visual inspection under UV illumination,[21b] a corresponding application may detect MGL shedding and resulting presence in serum. Cross-reactivity to mucin-binding galectins is already at a low level. If required, it could even be further diminished by using sialyl T_n as a headgroup, a disaccharide which is unreactive with galectins.[13] However, the lectin of hepatocytes could recognize glycoclusters presenting T_n and sialyl T_n epitopes. Despite its original name "asialoglycoprotein receptor", this mammalian C-type lectin on the hepatic parenchymal cells binds N-glycans with α2,6-sialylated LacdiNAc along with unsubstituted β-GalNAc termini (but not the 4'-sulfated LacdiNAc), with interspecies variations in relative affinity, and involvement of this lectin in mucin clearance from serum in mice suggests a respective reactivity confirmed for the rat receptor by glycan array data.[27] Of further note when considering extrapolations of data for MGL of different species, mice express MGL proteins with distinct carbohydrate specificities and expression profiles from two different genes.[28]

Experimental section

General experimental

Unless otherwise noted, all commercially available compounds were used as provided without further purification. Petroleum ether 40-60 °C was used for column chromatography and thin layer chromatography (TLC). NMR spectra were recorded (25 °C) at 500 MHz for ^{1}H NMR and 126 MHz for ^{13}C NMR. Data are reported in the following order: chemical shift (δ) in ppm; multiplicities indicated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet); coupling constants (J) given in Hertz (Hz). Chemical shifts are reported relative to internal standard Me_4Si in $CDCl_3$ (δ 0.0) or HOD for D_2O (δ 4.72, 25 °C) for ^{1}H and Me_4Si in $CDCl_3$ (δ 0.0) or $CDCl_3$ (δ 77.0) for ^{13}C. ^{1}H NMR signals were assigned with the aid of COSY, ^{13}C NMR signals using DEPT, gHSQCAD and/or gHMBCAD. Low- and high-resolution mass spectra were in positive and/or negative mode as indicated in each case. TLC was performed on aluminium sheets precoated with silica gel and spots visualized by UV and charring with H_2SO_4-EtOH (1:20) or cerium molybdate, unless otherwise stated. Chromatography was carried out with silica gel 60 (0.040-0.630 mm) and using a stepwise solvent polarity gradient correlated with TLC mobility, unless otherwise stated. Reverse phase silica used was a C18 - reverse phase silica gel (100 Å pore size) available from Sigma-Aldrich (60756). CH_2Cl_2, MeOH, toluene and THF reaction solvents were used as obtained from a Pure Solv™ Solvent Purification System.

2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucopyranoside 11

Thiol **10** (3.12 g, 8.59 mmol) was dissolved in acetone-H_2O (2:1, 36 mL) mixture and potassium carbonate (1.4 g, 10.1 mmol) and 1,2 dibromoethane (6 ml, 69.3 mmol) was added to this solution. This mixture was stirred at room temperature for 3 h after which point it was diluted with dichloromethane. The layers were separated, the aqueous layer then re-extracted with a further portion of dichloromethane. The combined organic layers were dried over

Na_2SO_4, and the solvent was removed under reduced pressure. Chromatography (EtOAc-petroleum ether, 1:3) gave the intermediate 2-bromoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucopyranose (2.61 g, 65 %) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.74 (d, *J* = 8.4 Hz, 1H, NH), 5.52 (d, *J* = 5.4 Hz, 1H, H-1), 5.10 (t, *J* = 9.5 Hz, 1H, H-4), 5.05 (dd, *J* = 10.8, 9.3 Hz, 1H, H-3), 4.48 (ddd, *J* = 10.8, 8.4, 5.4 Hz, 1H, H-2), 4.37 (ddd, *J* = 9.8, 5.4, 2.2 Hz, 1H H-5), 4.24 (dd, *J* = 12.3, 5.4 Hz, 1H, H-6a), 4.10 (dd, *J* = 12.3, 2.2 Hz, 1H, H-6b), 3.58 (td, *J* = 9.8, 5.8 Hz, 1H, SCH_2CH_2Br), 3.49 (td, *J* = 9.9, 6.3 Hz, 1H, SCH_2CH_2Br), 3.10 (ddd, *J* = 14.0, 9.7, 6.3 Hz, 1H, SCH_2CH_2Br), 3.03 (ddd, *J* = 13.9, 9.6, 5.8 Hz, 1H, SCH_2CH_2Br), 2.11 (s, 3H), 2.05 & 2.04 (each s, each 3H, each OAc), 1.96 (s, 3H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.69, 170.56, 169.95, 169.23 (each C=O), 85.09 (C-1), 71.02 (C-3), 68.71 (C-5), 68.06 (C-4), 62.10 (C-6), 52.62 (C-2), 33.92 (SCH_2CH_2Br), 30.28 (SCH_2CH_2Br), 23.20 (NHAc), 20.72, 20.70, 20.58 (each OAc); ES-HRMS calcd for $C_{16}H_{24}N_1O_8Na_1S_1Br_1$ 492.0304, found *m/z* 492.0296 $[M+Na]^+$; IR cm^{-1}: 1744, 1665, 1535, 1367, 1229, 1088, 912; R_f: 0.60 (MeOH-dichloromethane, 3:47); $[\alpha]_D^{20}$ +139 (*c* 0.63, $CHCl_3$). This intermediate (2.52 g, 5.36 mmol) was dissolved in DMF (40 ml), tetrabutylammonium iodide (1.99 g, 5.39 mmol) and sodium azide (1.40 g, 21.54 mmol) were added to the solution. The reaction was heated to 80 °C and stirred overnight at this temperature. It was then allowed to cool, after which it was diluted with dichloromethane and washed a number of times with H_2O, to remove the DMF. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Chromatography (EtOAc-petroleum ether, 1:3-1:1 gradient elution) led to the title compound (2.05 g, 88 %) as a white solid.; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.74 (d, *J* = 8.5 Hz, 1H, NH), 5.52 (d, *J* = 5.4 Hz, 1H, H-1), 5.12 (t, *J* = 9.6 Hz, 1H, H-4), 5.06 (dd, *J* = 11.0, 9.3 Hz, 1H, H-3), 4.50 (ddd, *J* = 11.0, 8.5, 5.4 Hz, 1H, H-2), 4.35 (ddd, *J* = 9.9, 4.7, 2.3 Hz, 1H, H-5), 4.26 (dd, *J* = 12.4, 4.8 Hz, 1H, H-6a), 4.10 (dd, *J* = 12.4, 2.3 Hz, 1H, H-6b), 3.53 (dt, *J* = 11.3, 5.7 Hz, 1H, $SCH_2CH_2N_3$), 3.50 – 3.45 (m, 1H, $SCH_2CH_2N_3$), 2.87 (dt, *J* = 13.6, 6.8 Hz, 1H, $SCH_2CH_2N_3$), 2.81 – 2.75 (m, 1H, $SCH_2CH_2N_3$),

2.10 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H) (each OAc), 1.96 (s, 3H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.68, 170.56, 169.98, 169.23 (each C=O), 84.86 (C-1), 71.08 (C-3), 68.65 (C-5), 67.99 (C-4), 61.98 (C-6), 52.56 (C2), 50.80 ($SCH_2\mathit{C}H_2N_3$), 30.99 ($S\mathit{C}H_2CH_2N_3$), 23.19 (NHAc), 20.69, 20.69, 20.58 (each OAc); ES-HRMS calcd for $C_{16}H_{24}N_4O_8Na_1S_1$ 455.1213, found *m/z* 455.1228 $[M+Na]^+$; IR cm^{-1}: 2962, 2104, 1741, 1666, 1535, 1367, 1225, 1088, 1034, 734; R_f*:* 0.64 (MeOH/dichloromethane, 3:47)

$[\alpha]_D^{20}$ 131 (*c* 0.44, $CHCl_3$).

2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-galactopyranoside 13 Thiol **12** (2.50 g, 6.88 mmol) was treated as described for **10** and gave the title compound (1.76 g, 87 %) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$): 5.66 (d, *J* = 8.5 Hz, 1H, NH), 5.60 (d, *J* = 5.3 Hz, 1H, H-1), 5.39 (dd, *J* = 3.5, 1.2 Hz, 1H, H-4), 5.05 (dd, *J* = 11.7, 3.2 Hz, 1H, H-3), 4.76 (ddd, *J* = 11.7, 8.5, 5.3 Hz, 1H, H-2), 4.57 – 4.52 (m, 1H, H-5), 4.14 (dd, *J* = 11.4, 5.8 Hz, 1H, H-6a), 4.08 (dd, *J* = 11.4, 7.0 Hz, 1H, H-6b), 3.53 (dt, *J* = 13.4, 6.8 Hz, 1H, $SCH_2C\mathit{H}_2N_3$), 3.50 – 3.44 (m, 1H, $SCH_2C\mathit{H}_2N_3$), 2.87 (dt, *J* = 13.7, 6.8 Hz, 1H $S\mathit{C}H_2CH_2N_3$), 2.77 (dt, *J* = 13.6, 6.6 Hz, 1H $S\mathit{C}H_2CH_2N_3$), 2.15 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H) (each OAc), 1.97 (s, 3H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ ^{13}C NMR (126 MHz, $cdcl_3$) δ 171.02, 170.32, 170.23, 170.16 (each C=O), 85.29 (C-1), 68.23 (C-3), 67.56 (C-5), 67.25 (C-4), 61.95 (C-6), 50.86 ($SCH_2\mathit{C}H_2N_3$), 48.44 (C-2), 30.64 ($S\mathit{C}H_2CH_2N_3$), 23.27 (NHAc), 20.72, 20.66, 20.65 (each OAc); ES-HRMS calcd for $C_{16}H_{24}N_4O_8Na_1S_1$ 455.1213, found *m/z* 455.1222 $[M+Na]^+$; IR cm^{-1}: 2103, 1744, 1661, 1537, 1369, 1217, 1081, 1046, 734; R_f*:* 0.57 (3:47 MeOH-dichloromethane); $[\alpha]_D^{20}$ +157 (*c* 0.35, $CHCl_3$)

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-galactopyranose 14 Freshly prepared 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (1.3 g, 3.17 mmol) was dissolved in acetone (20 ml), thiourea (415 mg, 5.45 mmol) was added. The

reaction mixture was then heated to reflux (60 °C) and stirred for 2 h. The solid obtained was then filtered off, with the filtrate returned to the reaction vessel and heated for an additional 2 h. The solid obtained was dried under reduced pressure to give the intermediate thiourea derivative (1.22 g, 79 %) as a white solid; ^{1}H NMR (500 MHz, DMSO) δ 9.15 (s, 2H, NH_2), 8.99 (s, 2H, NH_2), 8.27 (d, *J* = 9.0 Hz, 1H, NH), 5.45 (d, *J* = 10.5 Hz, 1H, H-1), 5.34 (d, *J* = 3.1 Hz, 1H, H-4), 5.00 (dd, *J* = 10.9, 3.2 Hz, 1H, H-3), 4.33 (t, *J* = 6.2 Hz, 1H, H-5), 4.13 (q, *J* = 10.2 Hz, 1H, H-2), 4.04 (t, *J* = 5.9 Hz, 2H, H-6a, H-6b), 2.11 (s, 3H), 1.99 (s, 3H), 1.92 (s, 3H) (each OAc), 1.81 (s, 3H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.55, 170.36, 170.32, 169.91 (each C=O), 167.81 (C=NH_2Br), 82.22 (C-1), 74.43 (C-5), 70.76 (C-3), 66.78 (C-4), 61.86 (C-6), 47.49 (C-2), 23.04 (NHAc), 21.02, 20.87, 20.86 (each OAc); ES-HRMS calcd for $C_{15}H_{23}N_3O_8S_1Br_1$ 484.0389 found *m/z* 484.0399 $[M-H]^-$; IR cm^{-1}: 3056, 1747, 1640, 1551, 1374, 1224, 1213, 1080, 1035, 913. This intermediate (1.12 g, 2.30 mmol) and sodium metabisulphite (485 mg, 2.55 mmol) were added to dichloromethane and water (3:2, 15 ml). The mixture was heated to reflux and stirred at reflux for 3 h, then allowed to cool, and the layers were separated. The aqueous layer was re-extracted with dichloromethane. The combined organic layers were then washed with H_2O, separated and dried over $MgSO_4$. The solvent was removed under reduced pressure to give the title compound (640 mg, 76 %) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.47 (d, *J* = 9.5 Hz, 1H, NH), 5.38 (dd, *J* = 3.5, 1.2 Hz, 1H, H-4), 5.05 (dd, *J* = 10.9, 3.3 Hz, 1H, H-3), 4.59 (dd, *J* = 10.0, 9.3 Hz, 1H, H-1), 4.28 (dt, *J* = 10.8, 9.7 Hz, 1H, H-2), 4.13 (dd, *J* = 6.6, 1.1 Hz, 2H, H-6a, H-6b), 3.91 (td, *J* = 6.6, 1.2 Hz, 1H, H-5), 2.61 (d, *J* = 9.2 Hz, 1H, SH), 2.17 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H) (each OAc), 1.99 (s, 3H, HNAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.77, 170.52, 170.41, 170.19 (each C=O), 80.78 (C-1), 74.93 (C-5), 71.01 (C-3), 66.76 (C-4), 61.63 (C-6), 53.22 (C-2), 23.40 (NHAc), 20.71, 20.69, 20.66 (each OAc); ES-HRMS calcd for $C_{14}H_{21}N_1O_8Na_1S_1$ 386.0886, found *m/z* 386.0889 $[M+Na]^+$; IR cm^{-1}: 3286, 1743, 1661, 1542, 1370, 1227, 1083, 1049, 732; R_f: 0.33 (1:19 MeOH-dichloromethane); $[\alpha]_D^{20}$ -1.2 (*c* 0.68, $CHCl_3$)

2-Azidoethyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-galactopyranoside 15

Thiol **14** (802 mg, 2.21 mmol) was reacted as described for **10** and gave the title compound (62 %) as a white solid; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.40 (dd, J = 3.2, 1.0 Hz, 1H, H-4), 5.38 (d, J = 9.5 Hz, 1H, NH), 5.14 (dd, J = 10.8, 3.3 Hz, 1H, H-3), 4.68 (d, J = 10.3 Hz, 1H, H-1), 4.26 (q, J = 10.4 Hz, 1H, H-2), 4.19 – 4.05 (m, 2H, H-6a, H-6b), 3.93 (td, J = 6.5, 1.2 Hz, 1H, H-5), 3.56 (dt, J = 13.5, 6.8 Hz, 1H, $SCH_2CH_2N_3$), 3.50 (dt, J = 12.6, 7.0 Hz, 1H, $SCH_2CH_2N_3$), 3.02 (dt, J = 14.0, 7.0 Hz, 1H, $SCH_2CH_2N_3$), 2.81 (ddd, J = 13.8, 7.2, 6.3 Hz, 1H, $SCH_2CH_2N_3$), 2.17 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H) (each OAc), 1.98 (s, 3H, NHAc); ^{13}C-NMR (126 MHz, $CDCl_3$): δ 170.64, 170.40, 170.27, 170.15 (each C=O), 85.14 (C-1), 74.76 (C-5), 71.07 (C-3), 66.88 (C-4), 61.71 (C-6), 51.62 ($SCH_2CH_2N_3$), 49.62 (C-2), 29.66 ($SCH_2CH_2N_3$), 23.39 (NHAc), 20.72, 20.66, 20.66 (each OAc); ES-HRMS calcd for $C_{16}H_{24}N_4O_8S_1Na_1$ 455.1213, found m/z 455.1201 $[M+Na]^+$; IR cm^{-1}: 2102, 1743, 1659, 1542, 1370, 1300, 1225, 1081, 1034, 919; Rf: 0.42 (1:19 MeOH-dichloromethane); $[\alpha]_D^{20}$ -49 (c 0.17, $CHCl_3$).

2-Chloroethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside 17

Compound **16** (2.0 g, 5.14 mmol) was dissolved in 1,2-dichloroethane (10 ml) in a microwave vial equipped with a stir bar. H_2SO_4-silica (37 mg) and 2-chloroethanol (0.43 ml, 6.41 mmol) were added to this mixture. It was then heated, stirred under microwave conditions at 110 °C for 15 min then filtered through Celite, followed by washing with dichloromethane. The filtrate washed with satd $NaHCO_3$, brine, dried and the solvent was removed under reduced pressure. Column chromatography (EtOAc-petroleum ether, 1:3-1:1-2:1-1:0) gave the title compound (1.69 g, 80 %) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.41 (d, *J* = 8.7 Hz, 1H, NH), 5.37 (dd, *J* = 3.5, 1.1 Hz, 1H, H-4), 5.31 (dd, *J* = 11.2, 3.4 Hz, 1H, H-3), 4.81 (d, *J* = 8.4 Hz, 1H H-1), 4.24 – 4.06 (m, 3H, H-6a, H-6b, O*CH*$_2$CH$_2$Cl), 3.97 (dt, *J* = 11.2, 8.5 Hz, 1H, H-2), 3.92 (dd, *J* = 6.5, 1.3 Hz, 1H, H-5), 3.79 (dt, *J* = 11.4, 6.3 Hz, 1H, O*CH*$_2$CH$_2$Cl), 3.65 (dd, *J* = 6.3, 4.9 Hz, 2H, OCH$_2$C*H*$_2$Cl), 2.15 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H) (each

OAc), 1.98 (s, 3H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.54, 170.38, 170.38, 170.16 (each C=O), 101.26 (C-1), 70.84 (C-5), 69.70 (C-3), 69.65 (O*C*$H_2$$CH_2$Cl), 66.70 (C-4), 61.40 (C-6), 51.63 (C-2), 43.01 (OCH$_2$*C*H_2Cl), 23.50 (NHAc), 20.68, 20.67, 20.65 (each OAc); ES-HRMS calcd for $C_{16}H_{24}Cl_1N_1O_9Na_1$ 432.1037, found *m/z* 432.1024 $[M+Na]^+$; IR cm^{-1}: 1744, 1662, 1556, 1370, 1227, 1168, 1136, 1080, 1046, 923; R_f: 0.48 (1:19 MeOH-dichloromethane); $[\alpha]_D^{20}$ -17 (*c* 0.13, $CHCl_3$).

2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside 18 To **17** (1.68 g, 4.10 mmol) dissolved in DMF (25 ml), tetrabutylammonium iodide (1.59 g, 4.30 mmol) and sodium azide (1.07 g, 16.46 mmol) were added. The mixture was heated to 80 °C and stirred at this temperature overnight, then cooled, diluted with dichloromethane and washed with water. The aqueous layer was re-extracted with dichloromethane and the combined organic layers then washed with successive portions of water and brine, dried over Na_2SO_4, finally solvent was removed under reduced pressure. Chromatography (EtOAc-petroleum ether, 1:3-1:1-2:1-100% EtOAc) gave the title compound (1.35 g, 79 %) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ 5.59 (d, *J* = 8.5 Hz, 1H, NH), 5.41 – 5.33 (m, 2H, H-3, H-4), 4.86 (d, *J* = 8.4 Hz, 1H, H-1), 4.16 (dd, *J* = 11.2, 6.6 Hz, 1H, H-6a), 4.12 (dd, *J* = 11.3, 6.7 Hz, 1H, H-6b), 4.07 (ddd, *J* = 10.8, 4.7, 3.4 Hz, 1H, OC*H*$_2$$CH_2N_3$), 3.95 (t, *J* = 6.4 Hz, 2H, H-5), 3.94 – 3.88 (m, 1H, H-2), 3.71 (ddd, *J* = 11.2, 8.6, 3.1 Hz, 1H, OC*H*$_2$$CH_2N_3$), 3.52 (ddd, *J* = 13.5, 8.6, 3.3 Hz, 1H, OCH$_2$C*H*$_2$$N_3$), 3.27 (ddd, *J* = 13.5, 4.7, 3.1 Hz, 1H, OCH$_2$C*H*$_2$$N_3$), 2.14 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H) (each OAc), 1.96 (s, 3H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.63, 170.41, 170.33, 170.19 (each C=O), 100.61 (C-1), 70.81 (C-5), 69.54 (C-3), 68.36 (O*C*$H_2$$CH_2N_3$), 66.80 (C-4), 61.44 (C-6), 51.76 (C-2), 50.62 (OCH$_2$*C*H_2N_3), 23.47 (NHAc); ES-HRMS calcd for $C_{16}H_{24}N_4O_9Na_1$ 439.1441, found *m/z* 439.1431 $[M+Na]^+$; IR cm^{-1}: 2105, 1742, 1661, 1548, 1433, 1369, 1223, 1167, 1136, 1046, 932*; R_f:* 0.38 (1:19 MeOH-dichloromethane); $[\alpha]_D^{20}$ -28 (*c* 0.36, $CHCl_3$)

1,4-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 1 To a mixture of **11** (335 mg, 0.775 mmol) and **19** (65 mg, 0.349 mmol) in THF-H_2O (1:1, 12 ml) sodium ascorbate (42 mg, 0.212 mmol) and copper sulphate pentahydrate (52 mg, 0.208 mmol) were added. The resulting mixture was stirred at room temperature for 18 h. The THF was then removed under reduced pressure. This was followed by the dilution of the concentrate with dichloromethane, and subsequent washing with water. The aqueous layer was re-extracted with a further portion of dichloromethane. The combined organic layers were then washed with water, dried over Na_2SO_4 and solvent was removed under reduced pressure. Chromatography of the solution (95:5 dichloromethane-MeOH) gave the acetylated intermediate (353 mg, 96 %) as a white solid. The acetylated intermediate (327 mg, 0.311 mmol) was stirred in methanol (30 ml). A catalytic amount of a freshly prepared 1M NaOMe solution in MeOH (0.16 ml, 0.16 mmol) was added. The resulting mixture was stirred for 1.5 h at room temperature at which point Amberlite IR-120 H^+ was added, then stirred gently for 10 min, to neutralize (pH = 7) the solution. The resin was removed by filtration and washed with water and acetonitrile. The solvents were removed, and reverse phase column chromatography was carried out. For the chromatography, the compound is dissolved in a minimal volume of water, using heating if necessary before loading to the column. It was first run with 3 column volumes of water. This was then followed by elution with a 1:1 MeCN-H_2O mixture to give the title compound (196 mg, 71 %) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.17 (s, 2H, CC*H*, triazole), 7.83 (d, *J* = 7.0 Hz, 2H, NH), 6.95 (s, 4H, Ar-H), 5.44 (d, *J* = 5.2 Hz, 2H, H-1), 5.12 (d, *J* = 5.6 Hz, 2H, OH-4), 5.04 (s, 4H, C*H*$_2$OAr), 4.82 (d, *J* = 5.7 Hz, 2H, OH-3), 4.64 (t, *J* = 5.7 Hz, 2H, OH-6), 4.58 (dt, *J* = 13.7, 6.8 Hz, 2H, SCH$_2$C*H*$_2$triazole), 4.51 (dt, *J* = 13.9, 7.0 Hz, 2H, SCH$_2$C*H*$_2$triazole), 3.79 (ddd, *J* = 11.0, 7.0, 5.2 Hz, 2H, H-2), 3.74 – 3.67 (m, 4H, H-5, H-6a), 3.48 (dt, *J* = 12.0, 6.2 Hz, 2H, H-6b), 3.34 (ddd, *J* = 11.1, 8.7, 5.8 Hz, 2H, H-3), 3.10 (ddd, *J* = 9.9, 8.6, 5.6 Hz, 2H, H-4), 3.04 (dt, *J* = 13.9, 7.0 Hz, 2H, SC*H*$_2$CH$_2$triazole),

2.96 (dt, *J* = 13.8, 6.8 Hz, 2H, SC*H*$_2$CH$_2$triazole), 1.81 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.02 (C=O), 152.80 (Ar-C), 143.15 (*C*CH, triazole), 125.03 (C*C*H, triazole), 116.04 (Ar-*C*H), 84.48 (C-1), 74.19 (C-5), 71.42 (C-4), 71.08 (C-3), 62.09 (*C*H$_2$OAr), 61.32 (C-6), 54.61 (C-2), 49.72 (SCH$_2$*C*H$_2$triazole), 30.57 (S*C*H$_2$CH$_2$triazole), 23.03 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{12}S_2Na_1$ 821.2574, found m/z 821.2578 $[M+Na]^+$; IR (ATR) cm^{-1}: 3283, 1644, 1543, 1508, 1215, 1064, 1039, 1007, 823; R_f: 0.61 (1:1 MeCN-H$_2$O, reverse phase silica gel); $[\alpha]_D^{20}$ +146 (*c* 0.39, DMSO)

1,3-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 2 Treatment of **11** (335 mg, 0.775 mmol) and **20** (65 mg, 0.349 mmol) as described in preparation of **1** gave the intermediate acetylated compound (327 mg, 89 %) as a white solid. De-*O*-acetylation of this intermediate (314 mg, 0.299 mmol) generated the title compound (226 mg, 95 %) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.20 (s, 2H, CC*H*, triazole), 7.83 (d, *J* = 7.0 Hz, 2H, NH), 7.19 (t, *J* = 8.2 Hz, 1H, Ar-H), 6.70 (t, *J* = 2.4 Hz, 1H, Ar-H), 6.62 (dd, *J* = 8.3, 2.3 Hz, 2H, Ar-H), 5.44 (d, *J* = 5.3 Hz, 2H, H-1), 5.11 (d, *J* = 5.6 Hz, 2H, OH-4), 5.09 (s, 4H, C*H*$_2$OAr), 4.82 (d, *J* = 5.7 Hz, 2H, OH-3), 4.64 (t, *J* = 5.8 Hz, 2H, OH-6), 4.59 (dt, *J* = 13.7, 6.8 Hz, 2H, SCH$_2$C*H*$_2$triazole), 4.52 (dt, *J* = 13.9, 7.0 Hz, 2H, SCH$_2$C*H*$_2$triazole), 3.79 (ddd, *J* = 11.0, 7.0, 5.3 Hz, 2H, H-2), 3.75 – 3.67 (m, 4H, H-5, H-6a), 3.48 (dt, *J* = 11.9, 6.2 Hz, 2H, H-6b), 3.34 (ddd, *J* = 10.4, 8.4, 5.4 Hz, 2H, H-3), 3.11 (td, *J* = 9.3, 5.6 Hz, 2H, H-4), 3.05 (dt, *J* = 13.9, 7.0 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.96 (dt, *J* = 13.8, 6.8 Hz, 2H, SC*H*$_2$CH$_2$triazole), 1.81 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.03 (C=O), 159.74 (Ar-C), 142.87 (*C*CH, triazole), 130.47 (Ar-*C*H), 125.15 (C*C*H, triazole), 107.68 (Ar-*C*H), 102.03 (Ar-*C*H), 84.47 (C-1), 74.19 (C-5), 71.42 (C-4), 71.08 (C-3), 61.61 (*C*H$_2$OAr), 61.31 (C-6), 54.60 (C-2), 49.74 (SCH$_2$*C*H$_2$triazole), 30.54 (S*C*H$_2$CH$_2$triazole), 23.03 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{12}S_2Na_1$ 821.2574, found m/z 821.2585 $[M+Na]^+$; IR (ATR) cm^{-1}: 3278, 1644,

1594, 1547, 1283, 1150, 1041, 1028, 763; R_f: 0.57 (1:1 MeCN-H_2O, reverse phase silica gel); $[\alpha]_D^{20}$ +115 (*c* 0.42, DMSO).

1,3-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-galactopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 3 Treatment of **13** (335 mg, 0.775 mmol) and **19** (65 mg, 0.349 mmol) as described in preparation of **1** gave the intermediate acetylated compound (363 mg, >95%) as a white solid. De-*O*-acetylation of this intermediate (314 mg, 0.299 mmol) produced the title compound (186 mg, 69 %) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.17 (s, 2H, CC*H*, triazole), 7.74 (d, *J* = 7.0 Hz, 2H, NH), 6.95 (s, 4H, Ar-H), 5.50 (d, *J* = 5.3 Hz, 2H, H-1), 5.05 (s, 4H, CH_2OAr), 4.65 (br s, 4H, OH), 4.57 (dt, *J* = 13.7, 6.8 Hz, 2H, SCH$_2$CH_2triazole), 4.51 (dt, *J* = 13.9, 7.0 Hz, 2H, SCH$_2$CH_2triazole), 4.17 (ddd, *J* = 11.9, 7.0, 5.2 Hz, 2H, H-2), 3.90 (t, *J* = 6.0 Hz, 2H, H-5), 3.71 (d, *J* = 3.1 Hz, 2H, H-4), 3.54 (d, *J* = 6.0 Hz, 4H, H-6a, H-6b), 3.48 (dd, *J* = 11.2, 3.1 Hz, 2H, H-3), 3.31 (br s, 2H, OH), 3.02 (dt, *J* = 13.9, 6.9 Hz, 2H, SCH_2CH$_2$triazole), 2.93 (dt, *J* = 13.8, 6.8 Hz, 2H, SCH_2CH$_2$triazole), 1.79 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.20 (C=O), 152.79 (Ar-C), 143.12 (*C*CH, triazole), 125.09 (C*C*H, triazole), 116.04 (Ar-*C*H), 84.81 (C-1), 72.69 (C-5), 68.55 (C-4), 67.94 (C-3), 62.07 (*C*H$_2$OAr), 61.36 (C-6), 50.46 (C-2), 49.74 (SCH$_2$*C*H$_2$triazole), 30.34 (S*C*H$_2$CH$_2$triazole), 23.09 NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{12}S_2Na_1$ 821.2574, found m/z 821.2562 $[M+Na]^+$; IR (ATR) cm^{-1}: 3279, 1635, 1546, 1508, 1212, 1115, 1045, 1011, 827, 802; R_f: 0.67 (1:1 MeCN-H_2O, reverse phase silica gel); $[\alpha]_D^{20}$ +223.8° (*c* 0.27, DMSO)

1,3-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-galactopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 4 To **13** (335 mg, 0.775 mmol) and **20** (65 mg, 0.349 mmol) as described in the preparation of **1** gave the acetylated intermediate (344 mg, 94 %) as a white solid. De-O-acetylation of this intermediate (327 mg, 0.311 mmol) as described in preparation of **1** resulted in the title compound (230 mg, 92 %) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.20 (s, 2H, CC*H*, triazole), 7.74 (d, *J* = 7.1 Hz, 2H, NH), 7.18 (t, *J* = 8.2

Hz, 1H, Ar-H), 6.70 (t, *J* = 2.4 Hz, 1H, Ar-H), 6.62 (dd, *J* = 8.3, 2.3 Hz, 2H, Ar-H), 5.50 (d, *J* = 5.3 Hz, 2H, H-1), 5.09 (s, 4H, C*H*$_2$OAr), 4.69 (d, *J* = 5.6 Hz, 2H, OH-6), 4.67 (d, *J* = 4.7 Hz, 2H, OH-4), 4.61 – 4.56 (m, 4H, OH-3, SCH$_2$C*H*$_2$triazole), 4.52 (td, *J* = 13.9, 13.5, 6.5 Hz, 2H, SCH$_2$C*H*$_2$triazole), 4.17 (ddd, *J* = 11.9, 6.9, 5.5 Hz, 2H, H-2), 3.90 (t, *J* = 6.0 Hz, 2H, H-5), 3.71 (br d, *J* = 3.0 Hz, 2H, H-4), 3.53 (td, *J* = 5.7, 1.3 Hz, 4H, H-6a, H-6b), 3.48 (ddd, *J* = 10.8, 6.8, 3.0 Hz, 2H, H-3), 3.03 (dt, *J* = 13.9, 7.0 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.93 (dt, *J* = 13.8, 6.9 Hz, 2H, SC*H*$_2$CH$_2$triazole), 1.79 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.21 (C=O), 159.73 (Ar-C), 142.83 (*C*CH, triazole), 130.45 (Ar-*C*H), 125.22 (C*C*H, triazole), 107.69 (Ar-*C*H), 102.04 (Ar-*C*H), 84.82 (C-1), 72.69 (C-5), 68.55 (C-4), 67.93 (C-3), 61.61 (*C*H$_2$OAr), 61.37 (C-6), 50.47 (C-2), 49.77(SCH$_2$*C*H$_2$triazole) , 30.34 (S*C*H$_2$CH$_2$triazole), 23.09 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{12}S_2Na_1$ 821.2574, found m/z 821.2555 $[M+Na]^+$; IR (ATR) cm^{-1}: 3279, 1637, 1596, 1548, 1491, 1283, 1150, 1116, 1044, 1023, 801; R_f: 0.60 (1:1 MeCN-H$_2$O, reverse phase silica gel); $[\alpha]_D^{20}$ +176 (*c* 0.25, DMSO)

1,3,5-Tri[1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-galactopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 5 To a mixture of **13** (345 mg, 0.798 mmol) and **21** (62 mg, 0.258 mmol) in THF-H$_2$O (1:1, 14 ml) sodium ascorbate (31 mg, 0.156 mmol) and copper sulphate pentahydrate (39 mg, 0.156 mmol) were added. The resulting mixture was stirred at room temperature for 20 h. The THF was then removed under diminished pressure and the concentrate was diluted with dichloromethane which was then washed with water. The aqueous layer was re-extracted with a further portion of dichloromethane. The combined organic layers were washed with water, dried over Na$_2$SO$_4$, and solvent removed under reduced pressure. Chromatography of the residue (dichloromethane-MeOH, 95:5-93:7) gave the acetylated intermediate (349 mg, 88 %) as a white solid; ^{1}H NMR (500 MHz, CDCl$_3$-*d*) δ 7.74 (s, 3H, CC*H*, triazole), 6.25 (s, 3H, Ar-H), 6.22 (d, *J* = 7.8 Hz, 3H, NH), 5.71 (d, *J* = 5.4

Hz, 3H, H-1), 5.39 (dd, *J* = 3.4, 1.3 Hz, 3H, H-4), 5.17 (s, 6H, C*H*$_2$OAr), 4.99 (dd, *J* = 11.8, 3.2 Hz, 3H, H-3), 4.73 (ddd, *J* = 12.5, 7.8, 5.3 Hz, 3H, H-2), 4.62 (dt, *J* = 13.4, 6.5 Hz, 3H, SCH$_2$C*H*$_2$triazole), 4.57 (dt, *J* = 13.8, 6.6 Hz, 3H, SCH$_2$C*H*$_2$triazole) 4.45 (t, *J* = 6.3 Hz, 3H, H-5), 4.10 (dd, *J* = 6.4, 2.2 Hz, 6H, H-6a, H-6b), 3.18 (dt, *J* = 13.5, 6.6 Hz, 3H, SC*H*$_2$CH$_2$triazole), 3.06 (dt, *J* = 13.8, 6.6 Hz, 3H, SC*H*$_2$CH$_2$triazole), 2.16 (s, 9H), 2.00 (s, 9H), 1.99 (s, 9H) (each OAc), 1.97 (s, 9H, NHAc); ^{13}C NMR (126 MHz, , $CDCl_3$) δ 170.89, 170.69, 170.40, 170.18 (each C=O), 159.88 (Ar-C), 143.88 (*C*CH, triazole), 123.52 (C*C*H, triazole), 95.83 (Ar-*C*H), 85.20 (C-1), 68.16 (C-3), 67.55 (C-5), 67.11 (C-4), 62.18 (C-6), 62.04 (*C*H$_2$OAr), 49.68 (SCH$_2$*C*H$_2$triazole), 48.46 (C-2), 31.38 (S*C*H$_2$CH$_2$triazole), 23.17 (NHAc), 20.75, 20.67, 20.62 (each OAc); ES-HRMS calcd for $C_{63}H_{84}N_{12}O_{27}S_3Na_1$ 1559.4629, found m/z 1559.4656 $[M+Na]^+$; IR (ATR) cm^{-1}: 1745, 1664, 1601, 1542, 1452, 1231, 1156, 1083, 1052, 712; 0.40 (2:23 MeOH-dichloromethane). This intermediate (343 mg, 0.223 mmol) was de-*O*-acetylated as described in the preparation of **1** in methanol (22 ml) to give the title compound (211 mg, 81 %) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.21 (s, 3H, CC*H*, triazole), 7.74 (d, *J* = 7.1 Hz, 3H, NH), 6.33 (s, 3H, Ar-H), 5.51 (d, *J* = 5.2 Hz, 3H, H-1), 5.07 (s, 6H, C*H*$_2$OAr), 4.58 (dt, *J* = 13.7, 6.8 Hz, 3H, SCH$_2$C*H*$_2$triazole), 4.52 (dt, *J* = 13.9, 7.0 Hz, 3H, SCH$_2$C*H*$_2$triazole), 4.17 (ddd, *J* = 11.9, 7.0, 5.3 Hz, 3H, H-2), 3.90 (t, *J* = 6.1 Hz, 3H, H-5), 3.71 (dd, *J* = 3.0, 1.5 Hz, 3H, H-4), 3.54 (d, *J* = 6.0 Hz, 6H, H-6a, H-6b), 3.48 (dd, *J* = 11.3, 3.0 Hz, 3H, H-3), 3.03 (dt, *J* = 13.9, 7.0 Hz, 3H, SC*H*$_2$CH$_2$triazole), 2.93 (dt, *J* = 13.8, 6.9 Hz, 3H, SC*H*$_2$CH$_2$triazole), 1.80 (s, 9H, NHAc); ^{13}C NMR (126 MHz, DMSO): δ 170.23 (C=O), 160.34 (OAr-C), 142.73 (*C*CH, triazole), 125.25 (C*C*H, triazole), 94.92 (Ar-*C*H), 84.83 (C-1), 72.69 (C-5), 68.55 (C-4), 67.94 (C-3), 61.68 (ArO*C*H$_2$) 61.37 (C-6), 50.47 (C-2), 49.78 (SCH$_2$*C*H$_2$triazole), 30.34 (S*C*H$_2$CH$_2$triazole), 23.10 (NHAc); ES-HRMS calcd for $C_{45}H_{65}N_{12}O_{18}S_3$ 1157.3702, found m/z 1157.3733 $[M-H]^-$; IR (ATR) cm^{-1}: 3282, 1634, 1546, 1374, 1151, 1116, 1052, 881, 802; R_f: 0.70 (1:1 MeCN-H$_2$O, reverse phase silica gel); $[\alpha]_D^{20}$ +169 (*c* 0.46, DMSO).

1,1,2,2-Tetrakis[4-(1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-galactopyranosyl)-1,2,3-triazol-4-ylmethyloxy-phenyl)]-ethene 6 To a mixture of **13** (149 mg, 0.345 mmol) and **22** (46 mg, 0.084 mmol) in THF-H_2O (1:1, 210 ml) sodium ascorbate (10 mg, 0.050 mmol) and copper sulphate pentahydrate (12 mg, 0.048 mmol) were added. Work-up and chromatography as for other compounds gave the acetylated intermediate compound (175 mg, 92 %) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$-*d*) δ 7.66 (s, 4H, CC*H*, triazole), 6.92 (d, *J* = 8.5 Hz, 8H, Ar-H), 6.71 (d, *J* = 8.7 Hz, 8H, Ar-H), 5.91 (d, *J* = 8.2 Hz, 4H, NH), 5.67 (d, *J* = 5.4 Hz, 4H, H-1), 5.41 – 5.39 (m, 4H, H-4), 5.15 (s, 8H, C*H*$_2$OAr), 5.01 (dd, *J* = 11.8, 3.2 Hz, 4H, H-3), 4.74 (ddd, *J* = 12.6, 8.0, 5.3 Hz, 4H, H-2), 4.59 (hept, *J* = 6.9 Hz, 8H, SCH$_2$C*H*$_2$triazole), 4.52 (t, *J* = 6.4 Hz, 4H, H-5), 4.15 (dd, *J* = 11.5, 5.9 Hz, 4H, H-6a), 4.11 (dd, *J* = 11.5, 7.0 Hz, 4H, H-6b), 3.18 (dt, *J* = 13.8, 6.8 Hz, 4H, SC*H*$_2$CH$_2$triazole), 3.08 (dt, *J* = 14.0, 6.9 Hz, 4H, SC*H*$_2$CH$_2$triazole), 2.17 (s, 12H), 2.01 (s, 12H), 2.00 (s, 12H) (each OAc), 1.97 (s, 12H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.99, 170.53, 170.39, 170.15(each C=O), 156.52 (OAr-C), 144.28 (*C*CH, triazole), 138.60 (Ar$_2$-*C*=*C*-Ar$_2$), 137.18 (Ar-C), 132.53 (Ar-*C*H), 123.12 (C*C*H, triazole), 113.93 (Ar-*C*H), 85.11 (C-1), 68.17 (C-3), 67.65 (C-5), 67.11 (C-4), 61.98 (C-6), 61.88 (*C*H$_2$OAr), 49.64 (SCH$_2$*C*H$_2$triazole), 48.52 (C-2), 31.14 (S*C*H$_2$CH$_2$triazole), 23.21 (NHAc), 20.73, 20.66, 20.66 (each OAc); ES-HRMS calcd for $C_{51}H_{62}N_8O_{18}S_2Na_1$ 1161.3521, found m/z 1161.3503 $[M/2+Na]^+$; IR (ATR) cm^{-1}: 2923, 1747, 1667, 1455, 1372, 1235, 1085, 1032, 1054, 710; R_f*:* 0.39 (2:23 MeOH/dichloromethane). De-*O*-acetylation of this intermediate (220 mg, 0.0966 mmol) as described for other compounds gave the title compound (159 mg, 93 %) as a yellow solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.18 (s, 4H, CC*H*, triazole), 7.74 (d, *J* = 7.1 Hz, 4H, NH), 6.87 (d, *J* = 8.4 Hz, 8H, Ar-H), 6.80 (d, *J* = 8.8 Hz, 8H, Ar-H), 5.50 (d, *J* = 5.3 Hz, 4H, H-1), 5.03 (s, 8H, C*H*$_2$OAr), 4.67 (br s, OH), 4.58 (dt, *J* = 13.8, 6.8 Hz, 4H, SCH$_2$C*H*$_2$triazole), 4.51 (dt, *J* = 13.9, 7.0 Hz, 4H, SCH$_2$C*H*$_2$triazole), 4.17 (ddd, *J* = 11.8, 7.0, 5.2 Hz, 4H, H-2), 3.91 (t, $J_{5,6a} = J_{5,6b}$ = 6.0 Hz, 4H, H-5), 3.75 – 3.67 (br d, J = 3.0Hz, 4H, H-4), 3.54 (d, $J_{5,6a} = J_{5,6b}$ =

6.0 Hz, 8H, H-6a, H-6b), 3.48 (dd, *J* = 11.3, 3.0 Hz, 4H, H-3), 3.02 (dt, *J* = 13.9, 7.0 Hz, 4H, SC*H*$_2$CH$_2$triazole), 2.93 (dt, *J* = 13.8, 6.9 Hz, 4H, SC*H*$_2$CH$_2$triazole), 1.79 (s, 12H, NHAc); ^{13}C NMR (126 MHz, dmso) δ 170.22 (C=O), 156.89 (OAr-C), 142.83 (*C*CH, triazole), 138.51 (Ar$_2$-*C*=*C*-Ar$_2$), 136.94 (Ar-C), 132.46 (Ar-*C*H), 125.21 (C*C*H, triazole), 114.33 (Ar-*C*H), 84.82 (C-1), 72.70 (C-5), 68.55 (C-4), 67.94 (C-3), 61.46 (*C*H$_2$OAr), 61.36 (C-6), 50.47 (C-2), 49.78 (SCH$_2$*C*H$_2$triazole), 30.33 (S*C*H$_2$CH$_2$triazole), 23.09 (NHAc); ES-HRMS calcd for $C_{78}H_{100}N_{16}O_{24}S_4Na_1$ 1795.5877, found m/z 1795.5940 $[M+Na]^+$; IR (ATR) cm^{-1}: 3277, 1638, 1545, 507, 1224, 1175, 1114, 1048, 1009, 830, 802; R_f*:* 0.48 (1:1 MeCN-H$_2$O, reverse phase silica gel); $[\alpha]_D^{20}$ +150 (*c* 0.28, DMSO)

1,3-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-β-D-galactopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 7 Reaction of **15** (96 mg, 0.222 mmol) and **19** (20 mg, 0.107 mmol) as described in preparation of **1** gave the intermediate acetylated compound (111 mg, 98 %) as a white solid. De-O-acetylation of this intermediate (107 mg, 0.102 mmol) as described for the other compounds gave the title compound (67 mg, 83 %) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (s, 2H, CC*H*, triazole), 7.64 (d, *J* = 9.5 Hz, 2H, NH), 7.19 (t, *J* = 8.2 Hz, 1H, Ar-H), 6.71 (t, *J* = 2.4 Hz, 1H, Ar-H), 6.62 (dd, *J* = 8.2, 2.3 Hz, 2H, Ar-H), 5.09 (s, 4H, ArOC*H*$_2$), 4.71 (d, *J* = 6.2 Hz, 2H, OH-3), 4.63 (d, *J* = 5.2 Hz, 2H, OH-6), 4.62 – 4.58 (m, 6H, OH-4, SCH$_2$C*H*$_2$triazole), 4.34 (d, *J* = 10.3 Hz, 2H, H-1), 3.89 (q, *J* = 10.0 Hz, 2H H-2), 3.71 – 3.67 (m, 2H, H-4), 3.57 – 3.48 (m, 4H, H-6a, H-6b), 3.44 – 3.36 (m, 4H, H-3, H-5), 3.20 (dt, *J* = 13.7, 6.8 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.95 (dt, *J* = 14.1, 7.1 Hz, 2H, SC*H*$_2$CH$_2$triazole), 1.78 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 169.77 (C=O), 159.75 (Ar-C), 142.66 (*C*CH, triazole), 130.45 (Ar-*C*H), 125.49 (C*C*H, triazole), 116.03 (Ar-*C*H), 107.67 (Ar-*C*H), 102.61 (Ar-*C*H), 85.15 (C-1), 79.90 (C-5), 72.77 (C-3), 68.16 (C-4), 61.60 (*C*H$_2$OAr), 61.25 (C-6), 50.79 (C-2), 50.14 (SCH$_2$*C*H$_2$triazole), 30.24 (SCH$_2$CH$_2$triazole), 23.51 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{12}S_2Na_1$ 821.2574, found m/z 821.2571 $[M+Na]^+$; IR (ATR) cm^{-1}: 3266, 1642, 1596, 1553, 1373, 1286, 1180, 1153, 1119, 1078, 1037, 1023, 864, 759; R_f: 0.64 (1:1 MeCN-H_2O, reverse phase silica gel); $[\alpha]_D^{20}$ -18.5 (*c* 0.28, DMSO)

1,4-Di[1-(ethyl 2-acetamido-2-deoxy-β-D-galactopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 8 Reaction of **18** (351 mg, 0.843 mmol) and **20** (74 mg, 0.397 mmol) as described for other compounds gave the acetylated intermediate (378 mg, 93 %) as a white solid. This intermediate (374 mg, 0.367 mmol) was de-O-acetylated as described for other compounds to give the title compound (224 mg, 80 %) as a white solid; ^{1}H NMR (500

MHz, DMSO-d_6) δ 8.06 (s, 2H, CC*H*, triazole), 7.56 (d, *J* = 9.2 Hz, 2H, NH), 6.97 (s, 4H, Ar-H), 5.03 (s, 4H, ArOCH_2), 4.59 (d, *J* = 6.4 Hz, 2H, OH-3), 4.58 (d, *J* = 6.0 Hz, 2H, OH-6), 4.53 (dd, *J* = 5.8, 3.6 Hz, 2H, OCH_2CH_2triazole), 4.51 (d, *J* = 4.4 Hz, 2H, OH-4), 4.48 (dd, *J* = 7.1, 3.8 Hz, 2H, OCH_2CH_2triazole), 4.26 (d, *J* = 8.4 Hz, 2H, H-1), 4.05 (ddd, *J* = 11.0, 5.9, 3.7 Hz, 2H, OCH_2CH_2triazole), 3.83 – 3.70 (m, 4H, H-2, OCH_2CH_2triazole), 3.65 – 3.62 (m, 2H, H-4), 3.51 (tt, *J* = 11.1, 5.6 Hz, 4H, H-6a, H-6b), 3.40 (ddd, *J* = 9.9, 6.4, 3.2 Hz, 2H, H-3), 3.34 – 3.32 (m, 2H, H-5), 1.74 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.00 (C=O), 152.81 (Ar-C), 143.08 (*C*CH, triazole), 125.35 (C*C*H, triazole), 115.98 (Ar-*C*H), 101.53 (C-1), 75.92 (C-5), 71.78 (C-3), 67.93 (C-4), 66.70 (OCH_2CH_2triazole), 62.03 (CH_2OAr), 60.94 (C-6), 52.12 (C-2), 50.01 (OCH_2CH_2triazole), 23.48 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{14}Na_1$ 789.3031, found m/z 789.3026 $[M+Na]^+$; IR (ATR) cm^{-1}: 3276, 1637, 1556, 1511, 1372, 1310, 1224, 1109, 1046, 1033, 980, 822, 782; R_f: 0.72 (1:1 MeCN-H_2O, reverse phase silica gel); $[\alpha]_D^{20}$ -7.8° (*c* 0.48, DMSO)

1,3-Di[1-(ethyl 2-acetamido-2-deoxy-β-D-galactopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 9 Reaction of **18** (350 mg, 0.841 mmol) and **19** (74 mg, 0.397 mmol) as described in the preparation of **1** gave the acetylated intermediate (355 mg, 88 %) as a white solid. De-O-acetylation of this intermediate (330 mg, 0.324 mmol) gave the title compound (208 mg, 84 %) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (s, 2H, CC*H*, triazole), 7.56 (d, *J* = 9.1 Hz, 2H, NH), 7.20 (t, *J* = 8.2 Hz, 1H, Ar-H), 6.71 (t, *J* = 2.4 Hz, 1H, Ar-H), 6.63 (dd, *J* = 8.2, 2.3 Hz, 2H, Ar-H), 5.07 (s, 4H, ArOCH_2), 4.59 (d, *J* = 6.5 Hz, 2H, OH-3), 4.58 (d, *J* = 4.5 Hz, 2H, OH-6), 4.55 (td, *J* = 6.0, 5.6, 2.6 Hz, 2H, OCH_2CH_2triazole), 4.50 (d, *J* = 4.4 Hz, 2H, OH-4), 4.49 – 4.45 (m, 2H, OCH_2CH_2triazole), 4.27 (d, *J* = 8.4 Hz, 2H, H-1), 4.05 (ddd, *J* = 11.1, 5.8, 3.7 Hz, 2H, OCH_2CH_2triazole), 3.81 – 3.72 (m, 4H, H-2, OCH_2CH_2triazole), 3.63 (t, *J* = 4.1 Hz, 2H, H-4), 3.51 (tt, *J* = 11.0, 5.4 Hz, 4H, H-6a, H-6b), 3.40 (ddd, J = 10.2, 6.4, 3.2 Hz, 2H H-3), 3.34 – 3.32 (m, 2H, H-5), 1.74 (s,

6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.01 (C=O), 159.75 (Ar-C), 142.80 (*C*CH, triazole), 130.47 (Ar-*C*H), 125.46 (C*C*H, triazole), 107.62 (Ar-*C*H), 102.00 (Ar-*C*H), 101.52 (C-1), 75.92 (C-5), 71.77 (C-3), 67.93 (C-4), 66.68 (O*C*H$_2$CH$_2$triazole), 61.60 (*C*H$_2$OAr), 60.94 (C-6), 52.11 (C-2), 50.03 (O*C*H$_2$CH$_2$triazole), 23.47 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{14}Na_1$ 789.3031, found m/z 789.3011 $[M+Na]^+$; IR (ATR) cm^{-1}: 3272, 1637, 1598, 1554, 1494, 1375, 1278, 1264, 1181, 1152, 1117, 1047, 1032, 1005, 891, 786, 759; R_f: 0.70 (1:1 MeCN-H_2O, reverse phase silica gel); $[\alpha]_D^{20}$ -5.5 (*c* 0.48, DMSO).

Lectin purification and quality controls Cloning of cDNA for the two MGL constructs and their recombinant production, further processing of inclusion bodies, protein solubilization, dialysis and affinity chromatography followed established protocols.[8f] Purity was controlled by one- and two-dimensional gel electrophoresis. WGA was similarly purified by affinity chromatography as crucial step using fetuin as ligand immobilized to the resin after its activation with divinyl sulfone.[29] Biotinylation was performed under activity-preserving conditions with the N-hydroxysuccinimide ester derivative (Sigma, Munich, Germany).[30] Biotinylated DBA was purchased from Enzo Life Sciences, (Lörrach, Germany).

Inhibition assays The surface of microtiter plate wells was coated with asialofetuin (for WGA and galectins) or an albumin-based neoglycoprotein presenting β-GalNAc moieties as p-isothiocyanatophenyl derivative (for DBA and MGL)[31] using 0.5 µg/per well in 50 µl phosphate-buffered saline overnight at 4 °C. Residual surface area capable to adsorb protein was saturated using 100 µl buffer containing 1 % (w/v) carbohydrate-free bovine serum albumin for 1 h at 37 °C. The assay was then carried out by a series of incubation steps comprising use of the lectin without/with test substances, a streptavidin-peroxidase conjugate (0.5 µg/ml; Sigma) and reagents for signal development (1 µg/ml *o*-phenylenediamine, 1 µl/ml hydrogen peroxide), separated by thorough washing steps.[20] Binding buffer was

phosphate-bufferd saline (pH 7.2) for the two plant lectins and 5 mM Hepes (pH 7.2) with 2 mM $CaCl_2$ and 150 mM NaCl for MGL. Assays were routinely performed in duplicates with up to six independent series.

Cell binding using the Lec8 mutant of the panel of glycosylation mutants of Chinese hamster ovary (CHO) cells (kindly provided by P. Stanley, Albert Einstein College of Medicine, Bronx, USA) was monitored by FACScan analysis using streptavidin-R-phycoerythrin (1:40; Sigma) as indicator as described.[6e, 32] Measurements with the same lectin were routinely run with aliquots of the cell suspensions of the same passage, at least in duplicates, with at least four independent series. Controls included omission of the incubation step with the labeled lectin to determine lectin-independent background staining (0 %-value).

Acknowledgements

This work was generously supported by EC funding (GLYCOPHARM), the Verein zur Förderung des biologisch-technologischen Fortschritts in der Medizin e. V. (Heidelberg, Germany) and Science Foundation Ireland (grant numbers 08/SRC/B1393 and 12/IA/1398). We are indebted to Drs. J. Domingo-Ekark, B. Friday, S. Namirha and G. Notelecs for inspiring discussions.

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Table 1. IC_{50}-values of the di- to tetravalent compounds[a] and free GlcNAc/GalNAc for blocking binding of biotinylated lectins to surface-immobilized (neo)glycoprotein (in µM)

Lectin inhibitor	WGA (20 µg/ml)	DBA (15 µg/ml)	CRD	CRD + stalk
1	1.2 (1250)	n. i.	n. i.	n. i.
2	2.7 (556)	n. i.	n. i.	n. i.
3	n. i.	120 (250)	5.8 (431)	2.1 (2000)
4	n. i.	250 (120)	5.7 (439)	3.5 (1200)
5	n. i.	100 (300)	4.3 (581)	1.4 (3000)
6	n. i.	80 (375)	0.02 (125000)	0.01 (420000)
7	n. i.	>1000 (<30)	18 (139)	19 (221)
8	n. i.	400 (75)	12 (208)	18 (233)
9	n. i.	600 (50)	14 (179)	18 (233)
GlcNAc	1500	n. i.	n. i.	n. i.
GalNAc	n. i.	25-40x10^3	2500	4200

[a] For structures, please see Scheme 1; titrations were performed using a fixed glycoprotein quantity for coating (0.5 µg ASF/neoglycoprotein per well) with eight concentrations of sugar in duplicates and up to five independent series, reaching an upper limit of 13.3 % for the standard deviation; the concentration is always given as sugar concentration, free in solution or conjugated to the scaffold (number in brackets denote relative potency compared to free sugar; for DBA set to 30 mM GalNAc); n. i.: not inhibitory

Fig. 1 Space filling models of **3-6** (left) and the estimated distances between anomeric carbon of the GalNAc residues in extended conformations of glycoclusters **3-6** (right) are specifically labelled. In compound **6**, the distance along the diagonal between the anomeric carbons is ~30 Å (not shown). To obtain these confomatins, models were built using Maestro and energy minimisations were carried out using Macromodel (www.schrodinger.com). In generating the extended conformations shown, the distances between the anomeric carbon and nearest aromatic carbon (bonded to O) were constrained at 10.7 Å during the modeling run to reach the low-energy conformer in each case, and all anomeric carbons are assumed to be coplanar.

Fig. 2 Semilogarithmic presentation of fluorescent surface staining of CHO cells of the Lec8 glycosylation mutant (reduced glycan galactosylation) by labeled WGA (1 µg/ml). The control value (background) obtained by cell processing without the incubation step using labeled lectin is given as grey-shaded area, the 100 %-value (lectin-dependent staining in the absence of a test compound) as thick black line. Numbers for staining (percentage of positive cells/mean fluorescence intensity) are presented for each scan. A: inhibition by increasing concentrations of GlcNAc (0 mM, 2 mM, 10 mM, 100 mM). The numbers (from bottom to top) refer to the 100 %-value (0 mM GlcNAc), presence of 2 mM GlcNAc, 10 mM GlcNAc, 100 mM GlcNac and the background value. B: inhibition by 10 µM of compounds **2** and **1**, numbers given (from bottom to top) referring to the 100 %- value, effects of **2**, effect of **1** and the background value.

Fig. 3 Fluorescent cell (CHO Lec8 mutant) surface staining by the labeled C-type CRD (for further details, please see legend to Fig. 2). A: staining parameters with increasing lectin concentrations (from top to bottom) in the listing of numbers of 1 µg/ml, 2 µg/ml and 5 µg/ml. B: staining parameters at a lectin concentration of 10 µg/ml in the absence of inhibitor (100 %-value) and in the presence of 10 mM GalNAc and 50 mM GalNAc (from bottom to top). C: staining parameters at a lectin concentration of 10 µg/ml in the absence of inhibitor (100 %-value) and in the presence of 10 µM of compound **7**, compound **4** and compound **5** (from bottom to top). D: staining parameters at a lectin concentration of 10 µg/ml in the absence of inhibitor (100 %-value) and in the presence of 25 nM/50 nM of compound **6**.

Fig. 4 Fluorescent cell (CHO Lec8 mutant) surface staining by the labeled C-type CRD + stalk construct (for further details, please see legend to Fig. 2). A: staining parameters with increasing lectin concentrations (from top to bottom) of 2 µg/ml, 10 µg/ml and 20 µg/ml. B: staining parameters at a lectin concentration of 20 µg/ml in the absence of inhibitor (100 %-value) and in the presence of 50 mM and 100 mM GalNAc (from bottom to top). C: staining parameters at a lectin concentration of 20 µg/ml in the absence of inhibitor (100 %-value) and in the presence of 500 µM of compound **2**, 0.5 µM of compound **5** and 1 µM of compound **4** (from bottom to top). D: staining parameters at a lectin concentration of 20 µg/ml in the absence of inhibitor (100 %-value) and in the presence of 10 µM compounds **9**, **7**, and **8** as well as of 20 nM of compound **6** (from bottom to top).

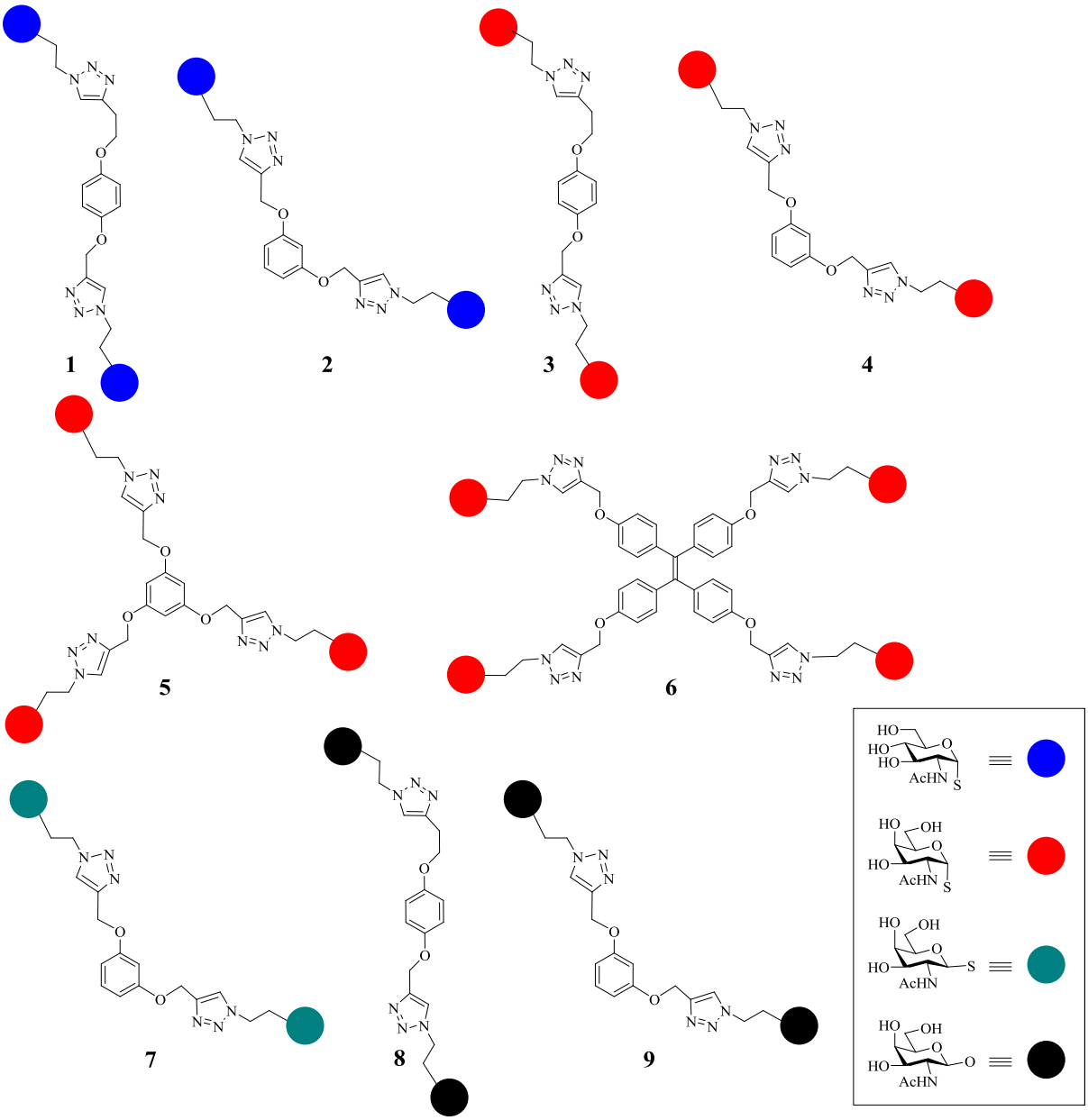
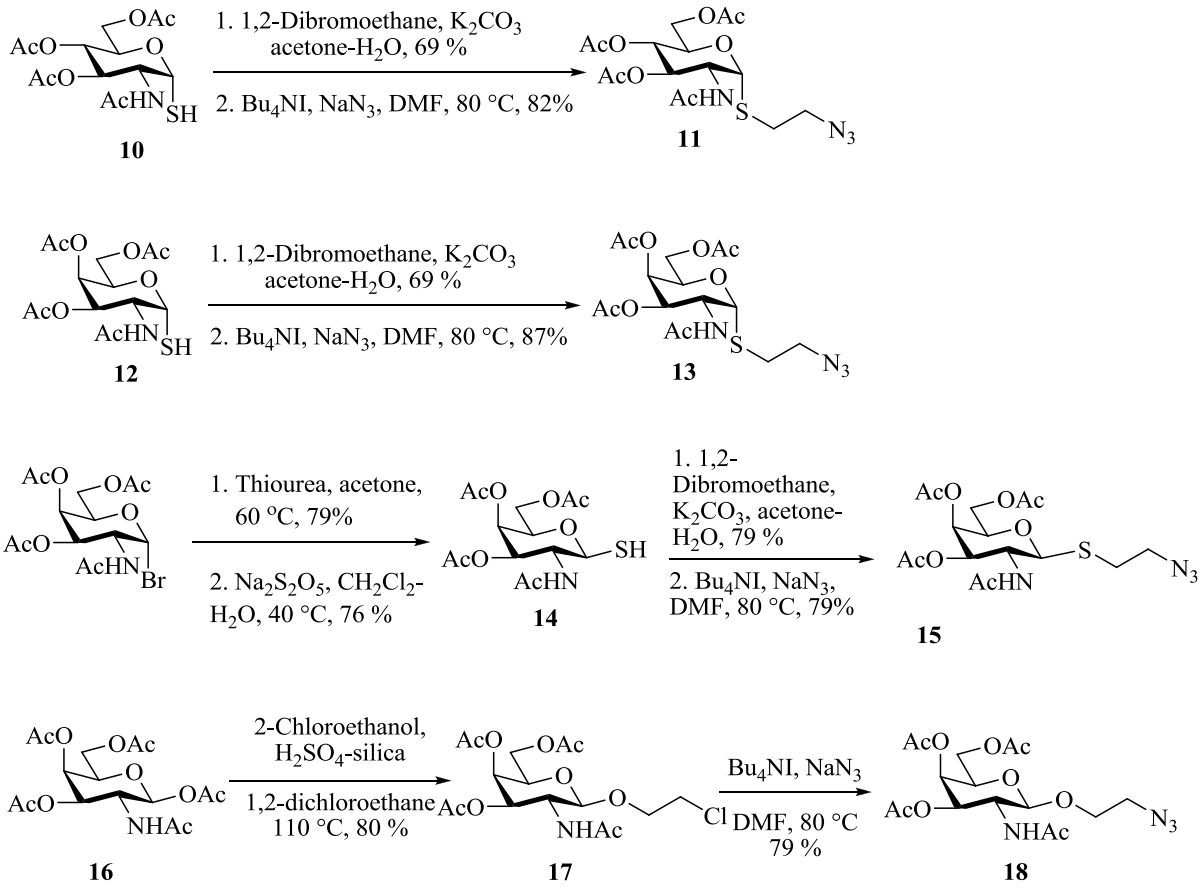
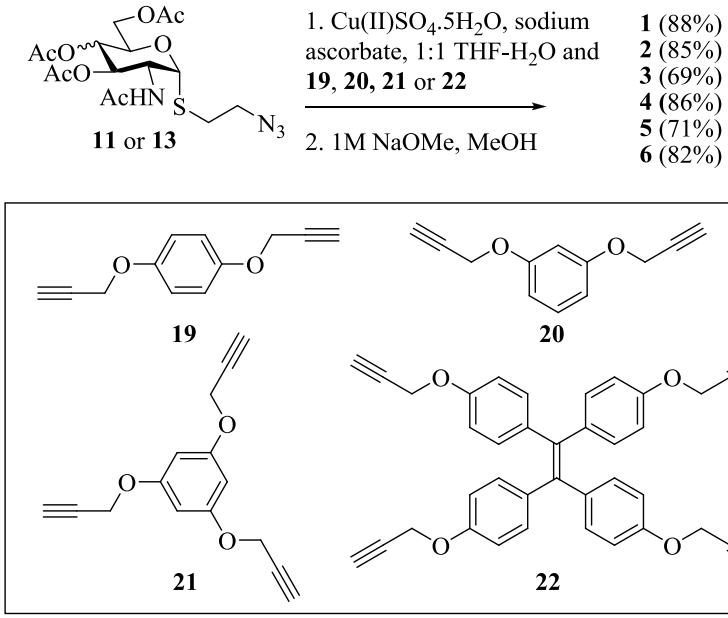


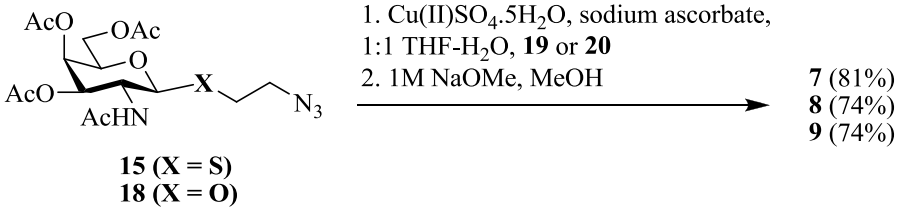
Chart 1. Structure of glycoclusters 1-9


1. 1,2-Dibromoethane, K_2CO_3
acetone-H_2O, 69 %
2. Bu_4NI, NaN_3, DMF, 80 °C, 82%
10
11
1. 1,2-Dibromoethane, K_2CO_3
acetone-H_2O, 69 %
2. Bu_4NI, NaN_3, DMF, 80 °C, 87%
12
13
1. Thiourea, acetone, 60 °C, 79%
2. $Na_2S_2O_5$, CH_2Cl_2-H_2O, 40 °C, 76 %
14
1. 1,2-Dibromoethane, K_2CO_3, acetone-H_2O, 79 %
2. Bu_4NI, NaN_3, DMF, 80 °C, 79%
15
2-Chloroethanol, H_2SO_4-silica
1,2-dichloroethane 110 °C, 80 %
16
17
Bu_4NI, NaN_3
DMF, 80 °C
79 %
18

Scheme 1



Scheme 2



Scheme 3

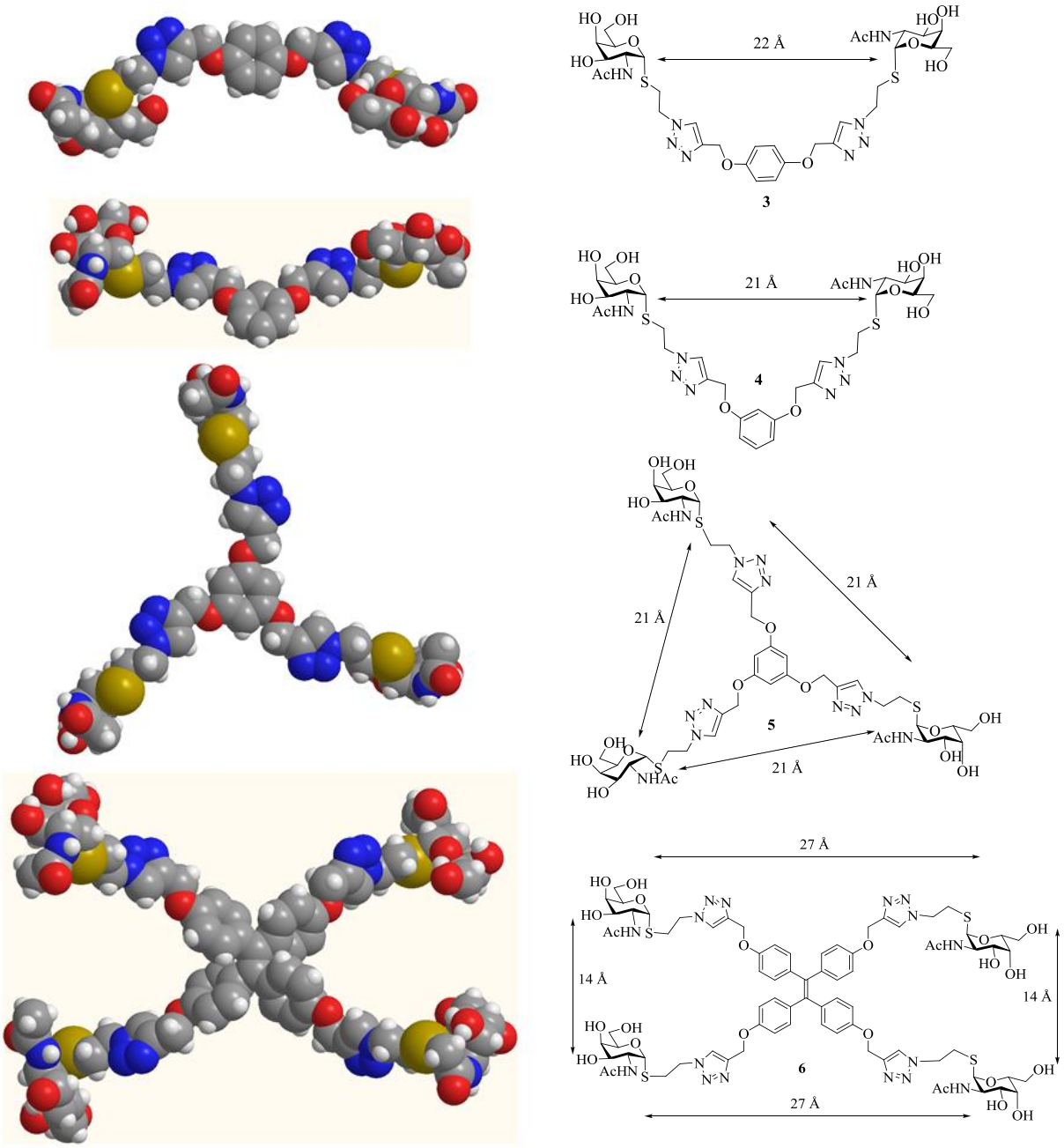


Figure 1

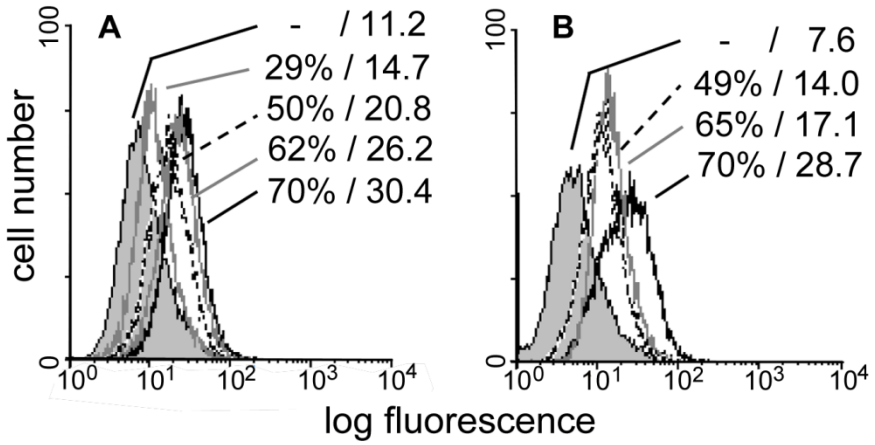

A
- / 11.2
29% / 14.7
50% / 20.8
62% / 26.2
70% / 30.4
B
- / 7.6
49% / 14.0
65% / 17.1
70% / 28.7
cell number
100
0
10^0 10^1 10^2 10^3 10^4
log fluorescence

Figure 2

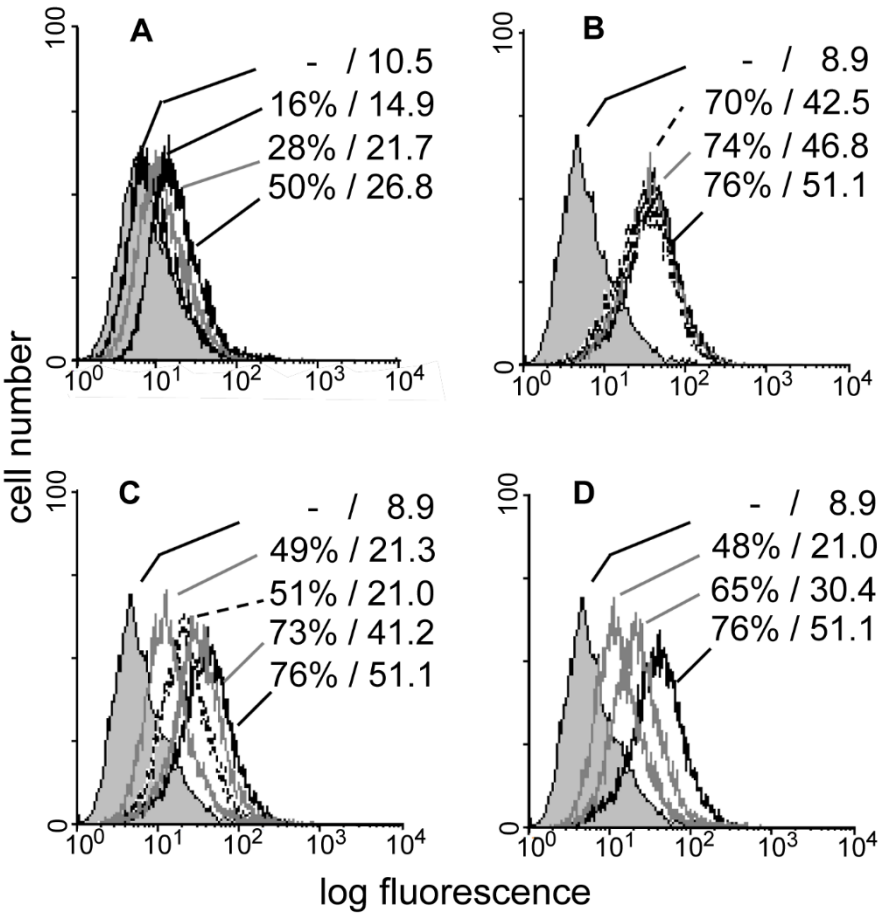

A
- / 10.5
16% / 14.9
28% / 21.7
50% / 26.8
B
- / 8.9
70% / 42.5
74% / 46.8
76% / 51.1
C
- / 8.9
49% / 21.3
51% / 21.0
73% / 41.2
76% / 51.1
D
- / 8.9
48% / 21.0
65% / 30.4
76% / 51.1
cell number
log fluorescence
100
0
10^0 10^1 10^2 10^3 10^4

Figure 3

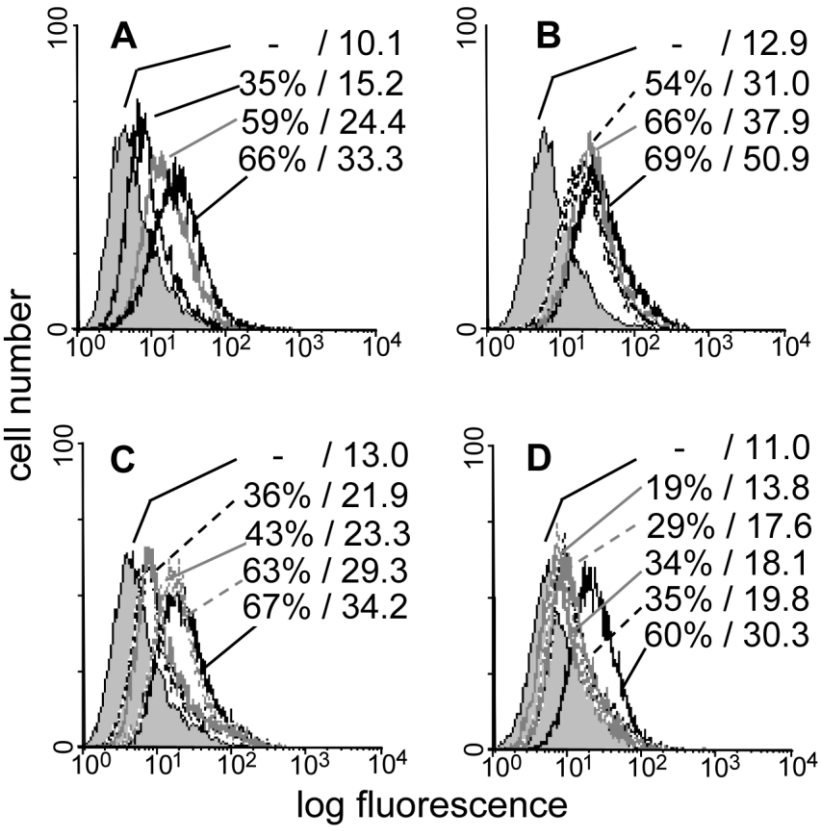

A
- / 10.1
35% / 15.2
59% / 24.4
66% / 33.3
B
- / 12.9
54% / 31.0
66% / 37.9
69% / 50.9
C
- / 13.0
36% / 21.9
43% / 23.3
63% / 29.3
67% / 34.2
D
- / 11.0
19% / 13.8
29% / 17.6
34% / 18.1
35% / 19.8
60% / 30.3
cell number
log fluorescence
100
0
10^0 10^1 10^2 10^3 10^4

Figure 4