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Glycoclusters as lectin inhibitors: comparative analysis on two plant agglutinins with different folding as a step towards rules for selectivity

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ABSTRACT

The emerging physiological significance of carbohydrate (glycan)–protein (lectin) recognition engenders the interest to design synthetic inhibitors with a high level of selectivity among natural sugar receptors. Plant agglutinins are common models to determine structure–activity relationships. Focussing on the contribution of valency towards selectivity, copper-catalysed azide (sugar derivative)–alkyne (scaffold) cycloaddition yielded a panel of 10 bi- to tetravalent glycoclusters with *N*-acetylglucosamine as the bioactive headgroup. They were introduced into assays using (neo)glycoproteins and cell surfaces as platforms to study carbohydrate-dependent lectin binding. The ability of the bivalent compounds, which exhibit a distance profile of the sugar headgroups of about 16–21 Å, for intramolecular bridging of two contact sites from the eight hevein domains of wheat germ agglutinin led to comparatively high enhancements of inhibitory potency relative to a tetrameric leguminous lectin (distance profile of 50–70 Å between sugar-specific sites), especially for a β-S-glycoside. The extent of inhibition at fixed concentrations of the sugar depended on the type of matrix used for the assay. Increases to tri- and tetravalency played a less important role than the anomeric position to keep cross-reactivity low, these tested topologies enabling cross-linking for both lectins. The potential for cis-interactions (intramolecular interactions), with glycoclusters serving as molecular rulers, is suggested to help designing selective blocking reagents.

1. Introduction

Synthetic chemistry substantially contributes to the progress in unravelling structure–activity relationships of carbohydrate (glycan)–protein (lectin) interactions, also referred to as cracking the sugar code.[1] A major question to be answered concerns the origin of the apparent specificity and selectivity of carbohydrate-binding proteins (lectins) for distinct cellular glycans presented by specific glycoconjugates (counterreceptors), in structural and topological terms. Growing insights into the natural occurrence of diverse sugar receptors is facilitating to classify them into groups, either formed by homologous family members or by non-related proteins sharing at least monosaccharide specificity, for example, β-galactoside-specific receptors defined as C-type lectins or galectins.[1f,2] Due to their functional cooperation in vivo, versatile tools are needed to comparatively dissect recognition properties of each constituent of such a network. Towards this end, glycoclusters (glycosylated scaffolds with at least bivalency) with galactosides as headgroups have proven their applicability in the test case of galectins, revealing routes towards selectivity among the three types of structural design of these lectins and natural/engineered variants (for examples, please see Ref. 3). Such data strengthen the assumption that these synthetic products can be potent sensors of the topological and/or spatial aspects of lectin binding and that their design can achieve differential reactivity to various types of receptors for compounds presenting the same carbohydrate epitope. Herein, we test the concept on a further letter of the sugar alphabet and two folding patterns of lectins.

N-Acetylglucosamine (GlcNAc), like galactose, can be spatially accessible when positioned at branch termini of N- and mucin-type O-glycans as well as on glycolipids. Physiologically, N-glycans with terminal β-GlcNAc are abundant in basophils and eosinophils, and an increase in such GlcNAc-terminated structures in several types of carcinoma intimates a value as tumour marker, with potential functional significance.[4] In fact, a panel of

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endogenous lectins is known with specificity for GlcNAc, among them soluble host defence effectors (collectins, ficolins), transmembrane pattern recognition receptors such as the tandem-repeat-type macrophage mannose receptor, the trimeric langerin, chitinase 3-like-1/2 (YKL-40/39) and also the trefoil factor 2.[5] Truncated N-glycan structures are also presented by viral glycoproteins, turning them into docking sites for ensuing virus entry. With this route defined, the *l*iver/lymph node *s*inusoidal *e*ndothelial *c*ell C-type lec*tin* (LSECtin, CLEC4G) with its micromolar affinity to the GlcNAcβ1,2Man disaccharide becomes a prominent potential target for therapeutic lectin blocking, as the chitin-binding protein GbpA of *Vibrio cholerae* is, which acts as adherence factor to intestinal mucins.[6] These examples underscore the emerging interest in developing bioactive and selective GlcNAc-presenting glycoclusters. In this respect, previous research has proven the particular usefulness of the plant lectin wheat germ agglutinin (WGA) as a model.[7] This sugar receptor with a total of eight hevein domains (about 14 Å apart) binds terminal GlcNAc irrespective of its anomeric linkage and also interacts with the innermost core GlcNAc moiety of complex-type N-glycans.[8] Intriguingly, bivalent (dumb-bell-like) compounds can be accommodated by two sites of the same protein, if linker length (somehow) matches distance between contact sites, or cross-link two proteins, in both cases saturating GlcNAc-binding sites.[7]

As proof-of-principle for a sensor activity of topological aspects, we added a structurally different (tetrameric) leguminous lectin to our test panel, i.e., *Griffonia* (*Bandeiraea*) *simplicifolia* agglutinin-II (GSA-II). It prefers the α-anomeric linkage of GlcNAc to its β-isomeric form.[8b,9] The common distance profile between sugar-binding sites in leguminous lectins of 50–70 Å makes intramolecular bridging impossible for the glycoclusters presented herein. Integrating a specificity control with scaffold-presented *N*-acetylgalactosamine (GalNAc) to exclude carbohydrate-independent interactions to the linker and building on previous experience with WGA and two bivalent compounds,[7g] we prepared a panel of 10 bi- to tetravalent glycoclusters bearing GlcNAc (Fig. 1). To ensure comparability, the nature of the linker was strictly kept constant and only one parameter was altered. Structural variations were introduced by changing anomeric configuration and valency, and using either an O- or S-glycosidic linkage. The products were assayed under identical conditions (e.g., aliquots of the same lectin solution or cell suspensions per experimental series) to determine their capacity to block binding of these two structurally dissimilar model lectins to surface-immobilised (neo)glycoproteins and to cells. The obtained data in the inhibition studies reveal glycocluster- and lectin-type-dependent results, underscoring the suitability to use glycoclusters as molecular rulers and as source of selectivity.

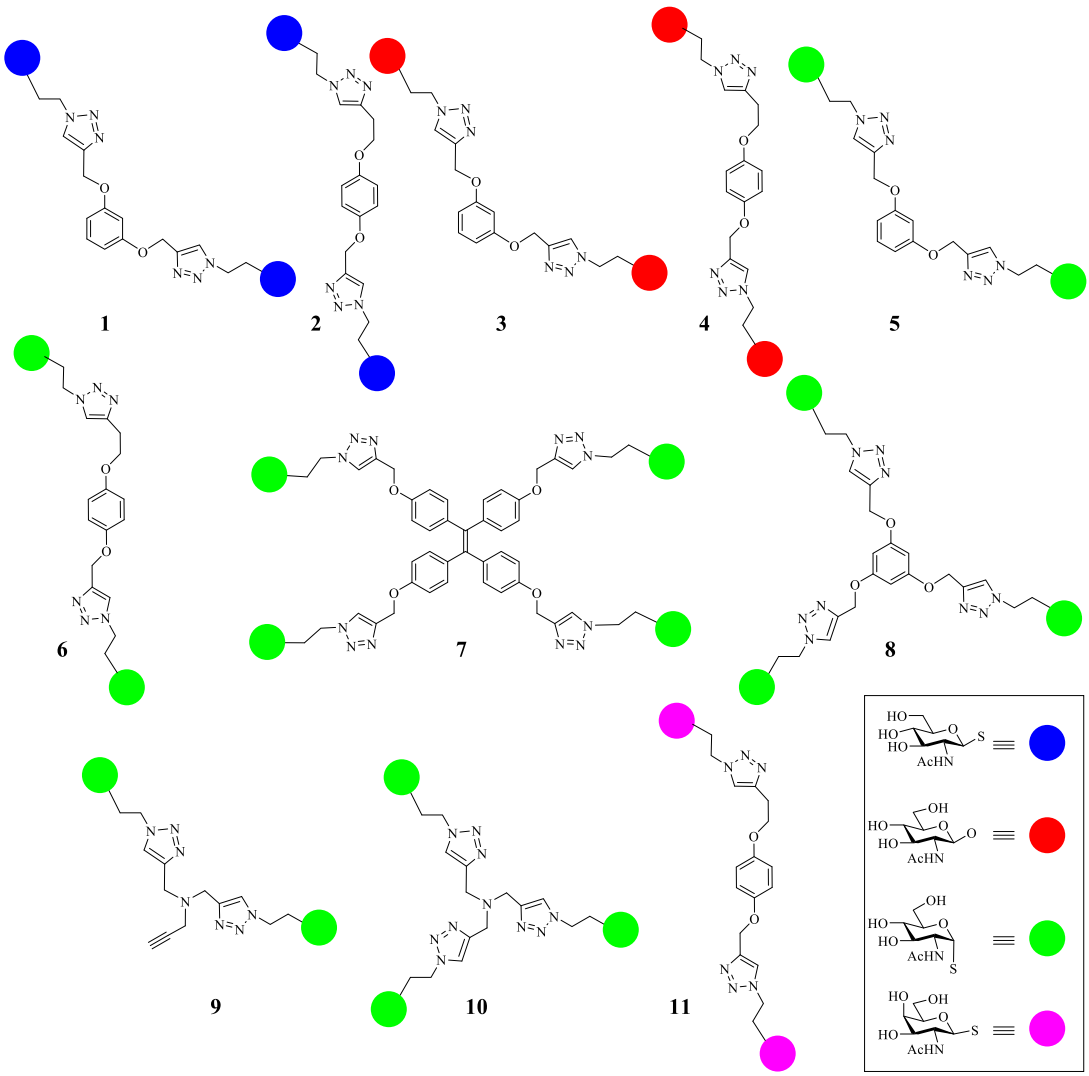

Fig. 1. Structure of glycoclusters **1**–**11**.

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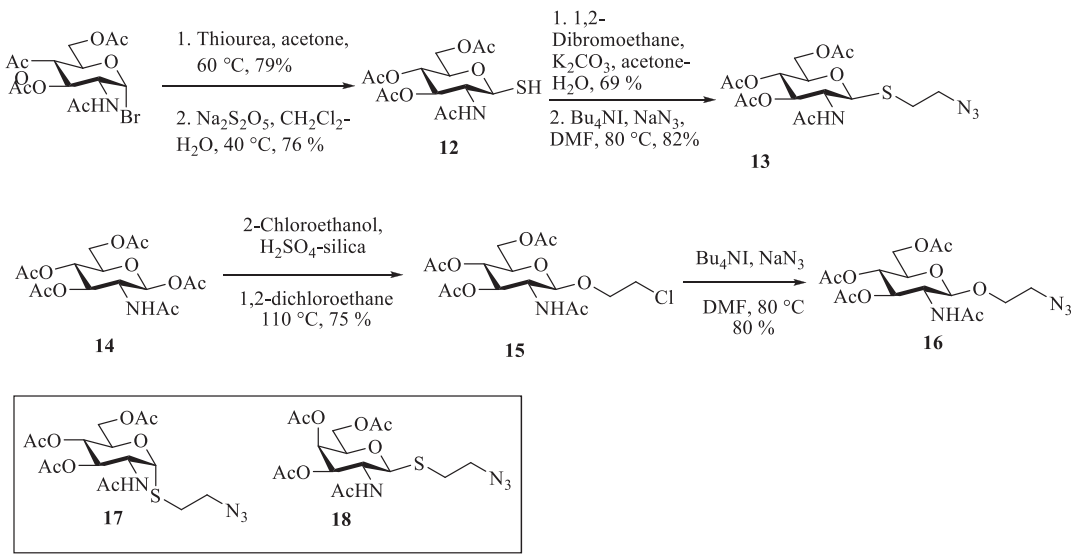
2. Results and discussion

2.1. Synthesis of the test panel

The spacered precursors were selected to ensure identical linker length and flexibility. Thus, thioglycoside **13** was obtained in two steps from the thiol **12**, prepared by a previously reported procedure (Scheme 1).[10] In detail, the thiol **12** was first reacted with 1,2-dibromoethane in the presence of potassium carbonate in acetone-water, and the resulting monobrominated product then converted to the azide **13** in the presence of sodium azide and tetra-*N*-butyl ammonium iodide in DMF. Next, the corresponding O-glycosidic analogue **16** was generated. Glycosidation of **14**[11] using 2-chloroethanol in 1,2-dichloroethane with sulfuric acid on silica as the promoter gave intermediate **15**. Reaction of **15** with sodium azide as for the preparation of **13** yielded **16**. The preparation of **17** and **18** was carried out as previously described.[6g] Herewith, β-O/S- and α-S-derivatives were made available for the cluster design and synthesis, **18** with GalNAc that will later provide a glycocluster serving as a specificity control.

2.2. Distance profiles of sugar headgroups by modelling

Extending our previous study on GalNAc-presenting clusters, in which we estimated average distances between the carbohydrate headgroups of 21–22 Å (for bi- and trivalent compounds with the aromatic core) and 14 Å/27 Å/30 Å (latter across the diagonal) for the tetravalent compound **7**,[7g] the respective parameter was herein calculated for both bi- and trivalent **9** and **10**. The Maestro interface (www.schrodinger.com) was used to build a conformer for **9**, implementing a distance constraint of 8.8 Å from the anomeric carbon of GlcNAc to the CH_2 group bonded directly to the central nitrogen atom; this constraint was chosen as it matches the corresponding distance in glycoclusters **1**–**8** and **11** (Fig. 2). Next, energy minimisations were carried out in Macromodel (OPLSAA force field, gas phase; www.schrodinger.com). Under these conditions, the two or three anomeric carbon atoms are separated by about 16 Å, in **10** adopting an equilateral triangular arrangement (Figs. 2 and 3). Owing to conformational flexibility this distance can be expected to be a dynamic average. As with the other bi- to trivalent glycoclusters, compounds **9** and **10** can apparently present their



Scheme 1.

The routine application of the copper-catalysed azide–alkyne cycloaddition (CuAAC) using pairs of azides (Scheme 1) and alkyne derivatives (Scheme 2), followed by deacetylation, resulted in the panel of the glycoclusters (Fig. 1). As listed, the propargyl derivatives obtained from hydroquinone, resorcinol, trihydroxybenzene, tetraphenylethene and ammonia (**19**–**23**)[12] were reacted with the appropriate azide (**12**, **13**, **15** or **18**) in the presence of Cu(II) $SO_4 \cdot 5H_2O$ and sodium ascorbate, in 1:1 THF/H_2O, to lead to the acetylated intermediates. The alkyne derivatives were generally used as limiting reagents. In the case of the reaction of tripropargylamine, the mono-, bi- and trivalent intermediates (**24**–**26**) were all obtained after chromatography. Each compound, which still had the sugar residue protected, was separated from the reagents by chromatography, and then the sugar residues de-O-acetylated using a catalytic amount of a freshly prepared 1 M sodium methoxide solution in methanol. Bi- to tetravalent products **1**–**11** (Fig. 1) were obtained by this approach and purified in all cases using reverse-phase column chromatography.

sugar headgroups in a special way to bridge adjacent binding sites in hevein domains of WGA. In contrast, with binding sites in leguminous lectins about 50–70 Å apart, such intramolecular bridging will not occur for GSA-II.

In order to determine lectin reactivity of the glycoclusters we performed inhibition assays, using incubation of a mixture of a synthetic compound (or free sugar) with labelled lectin with a (neo)glycoprotein matrix or with cells, and measuring the extent of binding to glycan ligands.

2.3. Glycoclusters as inhibitors of GSA-II binding to a neoglycoprotein and cells

The leguminous lectin GSA-II is specific for non-reducing-end GlcNAc residues. In the solid-phase assay, the labelled lectin did not bind to complex-type N-glycans of asialofetuin (ASF). Removal of the β1,3/4-linked galactose moieties from these N-glycans by treatment with β-galactosidase led to a signal in binding assays.

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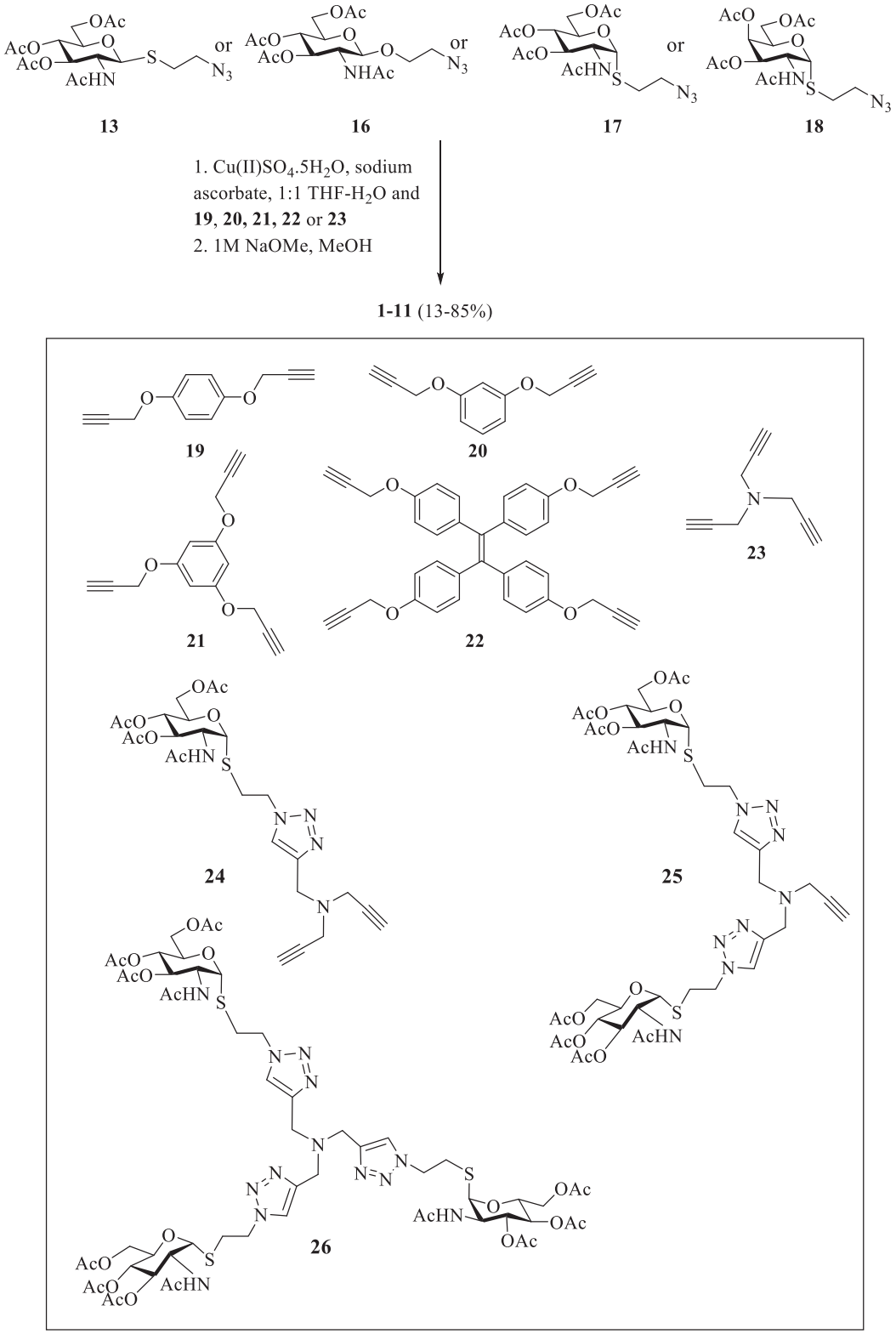

Scheme 2.

However, in relation, a neoglycoprotein presenting GlcNAc as a *p*-isothiocyanato derivative on bovine serum albumin as carrier was most reactive. Thus, this latter product was adsorbed to the plastic surface of microtiter plate wells, and lectin binding was ascertained to be saturable. It was dependent on the nature of the monosaccharide and of the matrix, GalNAc-containing neoglycoprotein serving as specificity control, and inhibitable by cognate sugar. The presence of the sugar diminished binding of GSA-II to the neoglycoprotein to background level. Optimal sensitivity to the presence of an inhibitor was achieved by using a lectin

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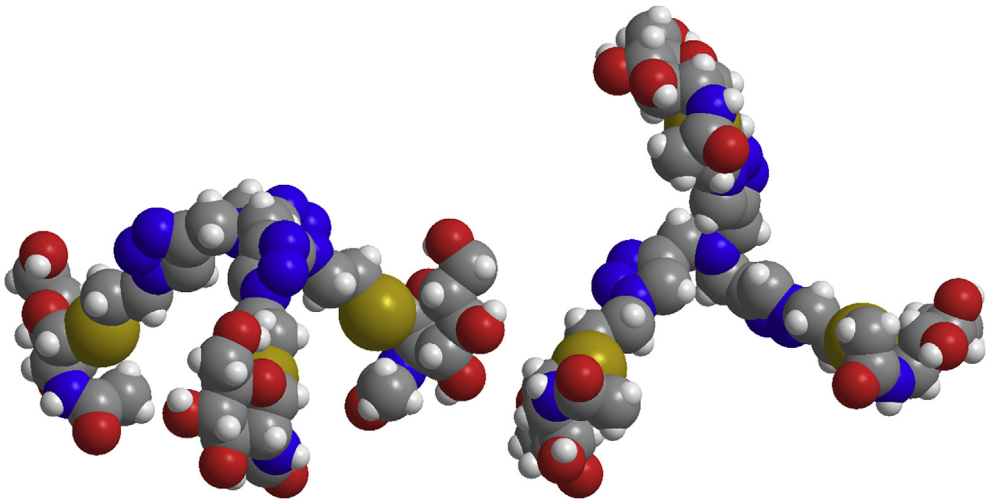

Fig. 2. Space filling models of **9** (two views).

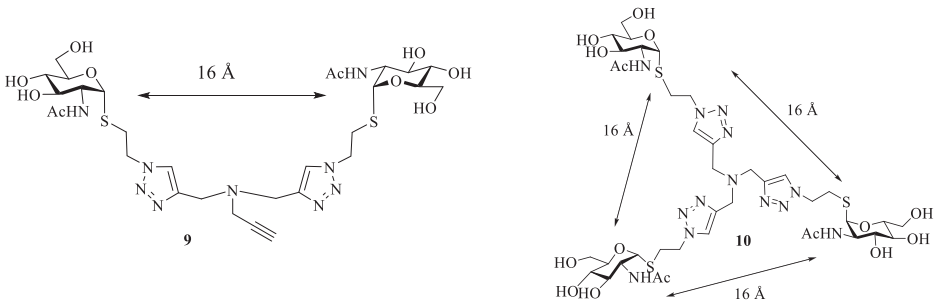

Fig. 3. Schematic illustration of inter-residue distances in compounds **9** and **10** based on molecular modelling using Macromodel.

concentration, which just yielded a plateau level of optical density. Titrations with increasing quantities of free GlcNAc and GlcNAc conjugated to various scaffolds (thus all concentrations are always expressed as concentration of sugar) enabled to calculate the sugar concentrations, at which the signal intensity of lectin binding was reduced by 50% (IC_{50}-value).

Reflecting the known preference for the α-anomer, the four β-GlcNAc-containing divalent glycoclusters **1**–**4** were relatively weak inhibitors of the interaction at the surface; this was irrespective of the nature of the atom (S or O) at the anomeric centre (Table 1). Respective 5–8-fold enhancements compared to compounds **1**–**4** were seen with the three bivalent glycoclusters with the α-S-glycosidic linkage (compounds **5**, **6** and **9**), with further increases in valency leading to further stepwise improvements (Table 1). The tetravalent **7** reached an approximately 200-fold enhancement of relative inhibitory capacity. Considering the distance profiles in the glycoclusters and in GSA-II, each glycocluster can be expected to bind to a lectin molecule only via a single sugar, then with the possibility for functional bi- to tetravalency, which will lead to lectin cross-linking. As documented in detail for binding to up to nine chains of the three complex-type N-glycans in ASF by galectins, successive binding steps to the nonavalent ligand apparently

Table 1
IC_{50}-values of the di-to tetravalent compounds[a] and the free monosaccharide in binding assays with biotinylated lectins and surface-immobilised (neo)glycoproteins (given in μM for the concentration of sugar)

Test compound	GSA-II (15 μg/mL)/GlcNAc-BSA (100 ng)	WGA (20 ng/mL)/GlcNAc-BSA (20 ng)	WGA (20 μg/mL)/ASF (500 ng)
1	145 (4.1)	11 (2273)	1.6 (1000)
2	130 (4.6)	8.5 (2941)	1.0 (1600)
3	140 (4.3)	19 (1316)	2.1 (762)
4	115 (5.2)	15 (1667)	1.6 (1000)
5	24 (25)	26 (962)	2.6[b] (615)
6	17 (35)	15 (1667)	1.3[b] (1231)
7	3 (200)	2.8 (8929)	12 (133)
8	11 (55)	22 (1136)	44 (36)
9	28 (21)	46 (543)	13 (123)
10	22 (27)	39 (641)	78 (21)
11	n.i.[c]	n.i.[c]	n.i.[c]
GlcNAc	600	25,000	1600

Numbers in parentheses denote relative inhibitory potency set in relation to the free monosaccharide.
[a] For structures, please see Fig. 1.
[b] Tested in a previous study.[7g]
[c] n.i.: not inhibitory (at least 1 mM).

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exhibit a gradient of decreasing binding constants.[13] Kinetically, the local vicinity of sugar headgroups in glycoclusters can have a positive impact on interfering with lectin binding to the ligand-exposing surface in the solid-phase assay.

In order to address the question whether presentation of the ligand on a cell surface may affect the inhibitory profile we selected a cell system with known expression of terminal β-GlcNAc moieties, i.e., the Lec8 line from the panel of Chinese hamster ovary (CHO) glycosylation mutants.[14] In contrast to the wild-type (WT) cells and the Lec2 mutant (reduced sialylation), these cells with impaired galactosylation can be stained with labelled GSA-II in a concentration-dependent and sugar-inhibitable manner. GlcNAc (shown at 2 mM) drastically reduced lectin binding, and the impact of the anomeric position in glycoclusters was as evident as in the solid-phase assay (Fig. 4A). Also, increase in valency had a similar impact, leading to strong reductions of cell staining with the trivalent compound **8** (Fig. 4B). Respective measurements with WGA were next carried out to answer the question on relative potency of the glycoclusters.

2.4. Glycoclusters as inhibitors of WGA binding to (neo)glycoproteins and cells

In the case of WGA, binding of glycoclusters is more complex than for GSA-II: bi- and tetravalent compounds can engage in trans(intermolecular)- and cis(intramolecular)-interactions.[7b–e] Also, the target selection of WGA is different from that of GSA-II. WGA interacts with its cognate sugar when presented by the natural glycoprotein (ASF or fetuin), here invariably in contact with both GlcNAc units of the N-glycan core (not the branch-end sialic acid if N-glycans are sialylated as is the case for fetuin),[8c] and by the neoglycoprotein (GlcNAc-BSA). This dual specificity by WGA to core or terminal sugar units has facilitated study into the occurrence of an effect of the matrix composition on the inhibition profile. In fact, an influence of the nature of the scaffold for GlcNAc presentation (porcine stomach mucin or covalently attached GlcNAc) had been reported, with the relative inhibitory potency being larger when testing the synthetic surface than the mucin.[7b] In our experiments with the neoglycoprotein, the high-density of sugar per carrier protein required a high concentration of cognate sugar to achieve inhibition. The S-glycosidic linkage was more suited for inhibition than its O-version for both surfaces (compare **1** with **3** and **2** with **4** in Table 1). Interestingly, this concurs with the behaviour of correspondingly compared glycocyclopeptides in a solid-phase assay, although measurements of K_D-values by isothermal titration calorimetry were in favour of the O-linkage.[8f] Comparing relative potencies of the bivalent compounds determined for GSA-II and WGA, listed in Table 1, indicates an intramolecular binding of the glycoclusters to WGA, as is also likely for two sites of the tri- and tetravalent compounds. In fact, conformational flexibility to allow finding an optimal fit to two hevein domains in WGA has been implied to explain the pronounced activity of a tris-(2-aminoethyl) amine-based glycocluster.[15] The low concentration of lectin used argues against a trans-interaction. In contrast, precipitation analysis and detection of bridging between WGA molecules in a surface plasmon resonance (SPR) assay documented the inherent capacity of bi- and tetravalent glycoclusters also to engage in intermolecular contacts.[7c,d] Of note, the details on assay condition and type must thus always be considered when interpreting data on glycoclusters as inhibitors, to avoid invalid extrapolations.

When WGA and the core region of complex-type N-glycans of ASF are in contact and the WGA concentration needed to be higher for adequate signal generation, the bivalent compounds remained fairly active (Table 1). Cross-linking phenomena under these conditions, as seen in SPR assays,[7c,d] may counterbalance lectin blocking, hereby diminishing the direct inhibitory capacity. Also, spatial constraints around the stem region of the glycan may reduce probe accessibility especially for the bulky compounds. The CHO cell panel enables to take these two constellations of glycan presentation for WGA binding to the level of cells.

The Lec8 mutant cells present β-GlcNAc at branch ends of N-glycans, in addition to GlcNAc in the N-glycans' core region. WGA binding to the cells is efficiently blocked by the free monosaccharide, and the tetravalent **7** is highly potent (Fig. 5A). Grading in solid-phase and cell assays appeared comparable, as shown for **1**, **5** and **9** (Fig. 5B). The same holds true, when WT cells (GlcNAc exclusively in the core of N-glycans) were processed (Fig. 6). In relative terms, the results in both assays are in qualitative accordance. However, grading can become different when comparing inhibition patterns for homologous lectins with disparate display of carbohydrate-binding sites to glycoproteins with three natural forms of glycan branching.[16] Using the same matrix (porcine stomach mucin), two plant lectins with specificity either to GlcNAc (WGA) or to *N*-acetyllactosamine (ECA, a leguminous lectin) and

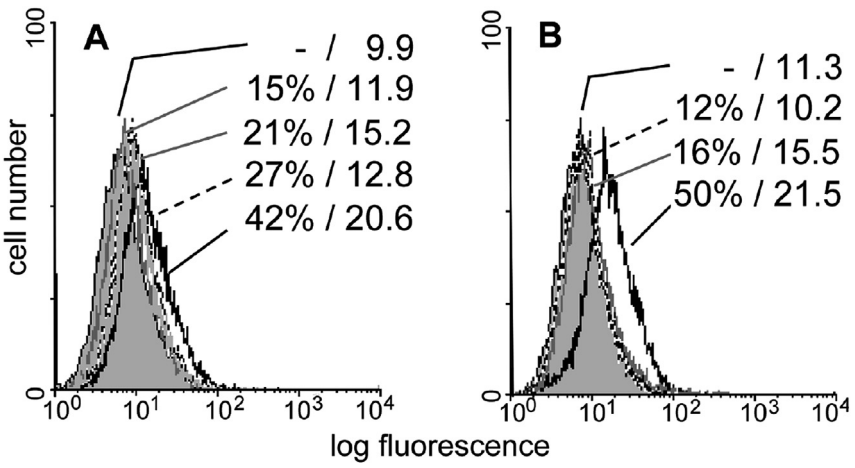

Fig. 4. Fluorescent cell (CHO Lec8 glycosylation mutant) surface staining by labelled GSA-II (20 μg/mL). The control value (lectin-independent background) obtained by cell processing without applying lectin is given as grey-shaded area, the 100%-value (lectin-dependent staining in the absence of a test compound) as thick black line. Numbers for staining parameters (percentage of positive cells/mean fluorescence intensity) are presented for each scan, presented in semilogarithmic scaling. A: inhibition of lectin binding by **3** (at 200 μM), **5** (at 50 μM) and GlcNAc (at 2 mM). The list of numbers (from bottom to top) present the 100%-value and the results of the test compounds as given (100%-value, staining in the presence of **3**, **5** and GlcNAc). B: Inhibition of lectin binding (100%-value at bottom) by **10** and **8** (at 100 μM).

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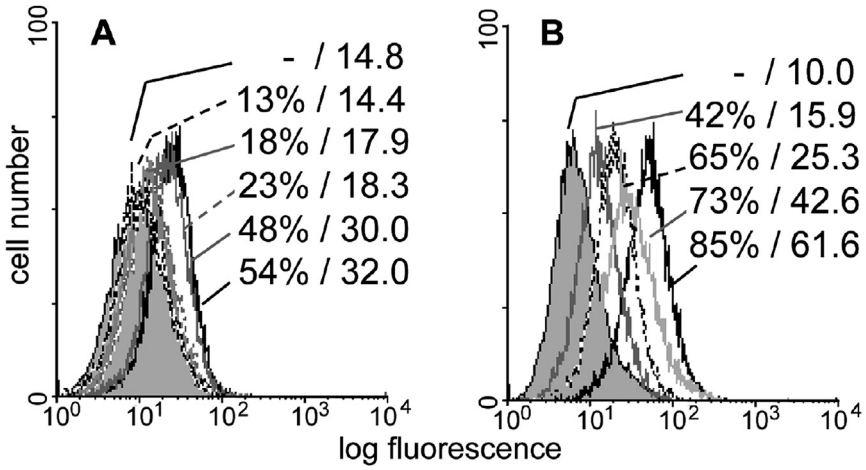

Fig. 5. Fluorescent cell (CHO Lec8 glycosylation mutant) surface staining by labelled WGA (2 μg/mL) (for details on representation, please see legend to Fig. 4). A: Inhibition of lectin binding by increasing concentrations of GlcNAc (2 mM, 10 mM, 20 mM) and by **7** (at 20 μM). B: Inhibition of lectin binding by **9** (at 20 μM), **5** (at 20 μM) and **1** (at 25 μM).

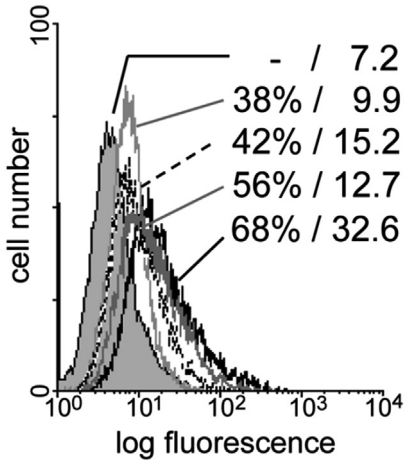

Fig. 6. Fluorescent cell (CHO WT line) surface staining by labelled WGA (8 μg/mL) (for details on representation, please see legend to Fig. 4). Inhibition of lectin binding by **9** (at 6 μM), **5** (at 2 μM) and **1** (2 μM).

lysine-based glycoclusters, the final IC_{50}-values were similar for the octavalent compound, with relative potency considerably higher in the case of WGA.[17] As a consequence, although the enhancement factor may look impressive, tuning of selectivity would remain a task in such a case. A similar phenomenon occurred in inhibition of haemagglutination using persubstituted cyclodextrin, whereas use of an octasilsesquioxane scaffold accounted for a more than 1000-fold difference in IC_{50}-values between WGA and concanavalin A in an inhibition setting.[18] Evidently, increasing the probability for cis-interactions, also referred to as 'cooperative interactions',[19a] as in calix[4]arenes or carbosilane dendrimers, can enhance selectivity, for WGA and also shown among human galectins.[19] In our case, allowing reactivity of the same glycoclusters to two lectins, selectivity for WGA can be attained at sites of low ligand density (N-glycan core) with **5** and **6**, combined with anomeric preference then at any sites with bivalent **1**–**4**.

3. Conclusions

Our model study characterises the impact of glycocluster valency, along with anomeric presentation of GlcNAc and the nature of the atom of the anomeric centre, on inhibitory potency for two plant agglutinins with different folding. In contrast to the four β-sandwich monomers (with a 50–70 Å distance profile; GSA-II), molecular design by eight hevein domains (with about 14 Å distance between contact sites for GlcNAc; WGA) makes intramolecular bridging possible for WGA. Intermolecular cross-linking, however, can occur in both cases. Relative inhibitory potency of the glycoclusters depended on the type of matrix: marked differences in impairing WGA binding were obtained, when either the N-glycan core of a glycoprotein or a high-density GlcNAc presentation of a neoglycoprotein was tested as ligand. Also considering the disparate sensitivity to inhibition by GlcNAc, selective reactivity of a test compound between the two lectins (with up to more than 100-fold difference in IC_{50}-values) was achieved by teaming up bivalency with an α-S-glycosidic linkage. Of note, the tested tetravalent glycocluster was relatively more potent as an inhibitor of WGA but the measured IC_{50}-values were rather similar for both lectins in the inhibition assays, due to the higher susceptibility of GSA-II binding to the presence of GlcNAc. In aggregate, these results attest the merit of the concept to determine the impact of each structural parameter of a glycocluster in a comparative setting. After all, the type of linker can also deliver contributions to avidity, for WGA[7f,15] or human lectins,[3b,20] and possibly selectivity, deserving a thorough systematic study. Equally important, the realisation of the impact of the matrix gives direction to take the inhibition assay to the level of tissues, the actual site of bioactivity. Monitoring reduction of signal intensity in lectin histochemistry when the sugar receptor is in contact with in situ ligands (counterreceptors) is technically as feasible as a direct binding assay using labelled glycoclusters.[2c,21] The data on cell assays documented herein encourage to extend assessment of inhibitory glycocluster activity to tissue sections, giving our work direction.

4. Experimental

4.1. General experimental

Unless otherwise noted, all commercially available compounds were used as provided without further purification. Petroleum ether with boiling point 40–60 °C was used for column chromatography and thin-layer chromatography (TLC). The 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 parts per million (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

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internal standard Me_4Si in $CDCl_3$ (δ 0.0) or HOD for D_2O (δ 4.72, 25 °C) for ^1H and Me_4Si in $CDCl_3$ (δ 0.0) or $CDCl_3$ (δ 77.0) for ^{13}C. ^1H 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 visualised 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.

4.2. 2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranoside 13

The thiol **14** (1.00 g, 2.75 mmol) was dissolved in acetone/H_2O (2:1, 15 mL), and potassium carbonate (450 mg, 3.26 mmol) and 1,2-dibromoethane (2 mL, 23.2 mmol) were then added. This mixture was stirred at room temperature for 15 h, after which point it was diluted with CH_2Cl_2. The layers were separated, with the aqueous layer being re-extracted with further CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, and the solvent was removed. Chromatography of the residue (CH_2Cl_2/MeOH, 95:5 to 93:7) gave the mono-bromide precursor (898 mg, 69%) as a colourless solid; ^1H NMR (500 MHz, $CDCl_3$): δ 5.67 (d, J=9.2 Hz, 1H, NH), 5.18 (dd, J=10.3, 9.3 Hz, 1H, H-3), 5.06 (t, J=9.7 Hz, 1H, H-4), 4.69 (d, J=10.4 Hz, 1H, H-1), 4.16 (d, J=4.0 Hz, 2H, H-6a, H-6b), 4.06 (td, J=10.3, 9.1 Hz, 1H, H-2), 3.72 (dt, J=10.0, 4.0 Hz, 1H, H-5), 3.60 (ddd, J=10.9, 9.8, 5.6 Hz, 1H, SCH_2CH_2Br), 3.51 (ddd, J=10.9, 9.7, 5.6 Hz, 1H, SCH_2CH_2Br), 3.19 (ddd, J=14.0, 11.0, 5.6 Hz, 1H, SCH_2CH_2Br), 2.99 (ddd, J=14.0, 11.0, 5.6 Hz, 1H, SCH_2CH_2Br), 2.11, 2.03, 2.03 (each s, each 3H, each OAc), 1.95 (s, 3H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.06, 170.65, 170.14, 169.26 (each C=O), 85.11 (C-1), 76.02 (C-5), 73.40 (C-3), 68.30 (C-4), 62.29 (C-6), 53.40 (C-2), 32.90 (SCH_2CH_2Br), 30.95 (SCH_2CH_2Br), 23.24 (NHAc), 20.76, 20.65, 20.58 (each OAc); ES-HRMS calcd for $C_{16}H_{25}NO_8SBr$ 470.0484, found m/z 470.0471 $[M+H]^+$; IR (cm^{-1}): 3288, 1746, 1661, 1543, 1373, 1233, 1047, 946, 735; R_f: 0.46 (2:23 MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ −42.5 (c 0.48, $CHCl_3$). This bromide intermediate (1.24 g, 2.64 mmol) was dissolved in DMF (20 mL), and to this were added tetrabutylammonium iodide (1.12 g, 3.03 mmol) and sodium azide (705 mg, 10.8 mmol). The mixture was heated to 80 °C and stirred for 15 h at this temperature and then allowed to cool to room temperature, after which it was diluted with CH_2Cl_2 and washed a number of times with H_2O, in an effort to remove DMF. The organic layer was then dried over Na_2SO_4 and the solvent was removed. Chromatography of the residue (EtOAc/petroleum ether, 1:3 to 1:1) gave the title compound (934 mg, 82%) as a white solid; ^1H NMR (500 MHz, $CDCl_3$): δ 5.56 (d, J=9.3 Hz, 1H, NH), 5.16 (dd, J=10.3, 9.3 Hz, 1H, H-3), 5.09 (t, J=9.7 Hz, 1H, H-4), 4.65 (d, J=10.3 Hz, 1H, H-1), 4.22 (dd, J=12.4, 5.1 Hz, 1H, H-6a), 4.15 (dd, J=12.4, 2.4 Hz, 1H, H-6b), 4.10 (td, J=10.3, 9.2 Hz, 1H, H-2), 3.70 (ddd, J=9.9, 5.0, 2.4 Hz, 1H, H-5), 3.56 (dt, J=13.2, 6.7 Hz, 1H, $SCH_2CH_2N_3$), 3.48 (dt, J=12.6, 6.9 Hz, 1H, $SCH_2CH_2N_3$), 2.99 (dt, J=13.9, 6.9 Hz, 1H, $SCH_2CH_2N_3$), 2.79 (dt, J=13.8, 6.7 Hz, 1H, $SCH_2CH_2N_3$), 2.09, 2.04, 2.03 (each s, each 3H, each OAc), 1.96 (s, 3H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 171.08, 170.62, 170.12, 169.24 (each C=O), 84.69 (C-1), 76.09 (C-5), 73.55 (C-3), 68.19 (C-4), 62.18 (C-6), 53.21 (C-2), 51.60 ($SCH_2CH_2N_3$), 29.64 ($SCH_2CH_2N_3$), 23.26 (NHAc), 20.71, 20.65, 20.58 (each OAc); ES-HRMS calcd for $C_{16}H_{24}N_4O_8NaS$ 455.1213, found m/z 455.1210 $[M+Na]^+$; IR (cm^{-1}): 2099, 1742, 1659, 1543, 1371, 1223, 1036, 916; R_f: 0.57 (3:47 MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ −60.5 (c 0.44, $CHCl_3$).

4.3. 2-Chloroethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside 15

2-Acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (1.00 g, 2.57 mmol) was dissolved in 1,2-dichloroethane (5 mL) in a microwave vial equipped with a stirring bar. To this mixture were added H_2SO_4-silica (18 mg) and 2-chloroethanol (0.21 mL, 3.13 mmol). This mixture was then heated and stirred under microwave conditions at 110 °C for 15 min. The reaction mixture was then filtered through Celite and washed thoroughly with CH_2Cl_2. The filtrate was then washed with saturated aqueous $NaHCO_3$, brine, then dried over $NaSO_4$ and the solvent was removed. Column chromatography (EtOAc/petroleum ether, 1:3 to 1:1 to 2:1 to 100% EtOAc) gave the title compound (784 mg, 75%) as a white solid; ^1H NMR (500 MHz, $CDCl_3$-d) δ 5.52 (d, J=8.7 Hz, 1H, NH), 5.30 (dd, J=10.7, 9.3 Hz, 1H, H-3), 5.07 (t, J=9.7 Hz, 1H, H-4), 4.77 (d, J=8.4 Hz, 1H, H-1), 4.26 (dd, J=12.3, 4.8 Hz, 1H, H-6a), 4.14 (dd, J=12.3, 2.5 Hz, 1H, H-6b), 4.10 (dd, J=10.7, 5.6 Hz, 1H, OCH_2CH_2Cl), 3.86 (dt, J=10.6, 8.5 Hz, 1H, H-2), 3.78 (dt, J=11.4, 6.4 Hz, 1H, OCH_2CH_2Cl), 3.71 (ddd, J=10.0, 4.8, 2.4 Hz, 1H, H-5), 3.64 (dd, J=6.3, 5.0 Hz, 2H, OCH_2CH_2Cl), 2.09, 2.03, 2.03 (each 3H, each s, each OAc), 1.96 (s, 3H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.81, 170.66, 170.40, 169.36 (each C=O), 101.06 (C-1), 72.09 (C-3), 71.97 (C-5), 69.67 (OCH_2CH_2Cl), 68.48 (C-4), 62.00 (C-6), 54.70 (C-2), 42.98 (OCH_2CH_2Cl), 23.35 (NHAc), 20.73, 20.67, 20.61 (each OAc); ES-HRMS calcd for $C_{16}H_{24}ClNO_9Na$ 432.1037, found m/z 432.1023 $[M+Na]^+$; IR (cm^{-1}): 1742, 1659, 1556, 1432, 1368, 1223, 1120, 1039, 907; R_f: 0.51 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ −4.0 (c 0.10, $CHCl_3$).

4.4. 2-Azidoethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside 16

Chloride **15** (1.36 g, 3.32 mmol) was dissolved in DMF (22 mL), and to this tetra-*N*-butylammonium iodide (1.29 g, 3.49 mmol) and sodium azide (863 mg, 13.3 mmol) were added. The reaction mixture was then heated to 80 °C and was then stirred at this temperature for 15 h. The reaction mixture was cooled, diluted with CH_2Cl_2 and washed with water. The aqueous layer was re-extracted with a portion of CH_2Cl_2. The organic portions were combined and were then washed with water. The organic layer was then washed with brine, dried over $NaSO_4$ and the solvent then removed. Chromatography (EtOAc/petroleum ether, 1:3 to 1:1 to 2:1 to 1:0) gave the title compound (1.10 g, 80%) as a white solid; ^1H NMR (500 MHz, $CDCl_3$-d) δ 5.50 (d, J=8.6 Hz, 1H, NH), 5.36 (dd, J=10.6, 9.3 Hz, 1H, H-3), 5.08 (dd, J=10.0, 9.3 Hz, 1H, H-4), 4.83 (d, J=8.3 Hz, 1H, H-1), 4.26 (dd, J=12.3, 4.7 Hz, 1H, H-6a), 4.15 (dd, J=12.3, 2.4 Hz, 1H, H-6b), 4.05 (ddd, J=10.8, 4.7, 3.2 Hz, 1H, $OCH_2CH_2N_3$), 3.80 (dt, J=10.7, 8.5 Hz, 1H, H-2), 3.74–3.66 (m, 2H, H-5, $OCH_2CH_2N_3$), 3.51 (ddd, J=13.5, 8.6, 3.3 Hz, 1H, $OCH_2CH_2N_3$), 3.26 (ddd, J=13.4, 4.7, 3.1 Hz, 1H, $OCH_2CH_2N_3$), 2.09 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H) (each OAc), 1.96 (s, 3H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.74, 170.65, 170.40, 169.38 (each C=O), 100.46 (C-1), 72.04 (C-4), 71.97 (C-5), 68.55 (C-3), 68.41 ($OCH_2CH_2N_3$), 61.97 (C-6), 54.93 (C-2), 50.60 ($OCH_2CH_2N_3$), 23.37 (NHAc), 20.74, 20.66, 20.62 (each OAc); ES-HRMS calcd for $C_{16}H_{24}N_4O_9Na$ 439.1441, found m/z 439.1429 $[M+Na]^+$; IR (cm^{-1}): 2106, 1744, 1656, 1566, 1436, 1368, 1225, 1170, 1125, 1033, 910; R_f: 0.41 (1:19 MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ −22.8 (c 0.36, $CHCl_3$).

4.5. 1,3-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 1

To a mixture of azide **13** (350 mg, 0.809 mmol) and *meta*-bispropargyloxybenzene **20** (63 mg, 0.338 mmol), in THF/H_2O (1:1, 12 mL), were added sodium ascorbate (41 mg, 0.207 mmol) and copper sulfate pentahydrate (51 mg, 0.204 mmol). The mixture was

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stirred at room temperature for 18 h. Tetrahydrofuran was then removed and CH_2Cl_2 was added and the mixture was then washed with water. The aqueous layer was re-extracted further with CH_2Cl_2. The combined organic layers were then washed with water, dried over $NaSO_4$ and the solvent removed. Chromatography of the residue (95:5 CH_2Cl_2/MeOH) gave the acetylated intermediate (350 mg, ~95%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 2H, CC*H*, triazole), 7.20 (t, *J*=8.3 Hz, 1H, aromatic H), 6.65–6.61 (m, 3H, aromatic H), 6.01 (d, *J*=9.3 Hz, 2H, NH), 5.20 (s, 4H, ArOC*H*$_2$), 5.17 (dd, *J*=10.3, 9.4 Hz, 2H, H-3), 5.05 (dd, *J*=10.1, 9.3 Hz, 2H, H-4), 4.67 (dt, *J*=13.1, 6.4 Hz, 2H, SCH$_2$C*H*$_2$triazole), 4.61–4.55 (m, 2H, SCH$_2$C*H*$_2$triazole), 4.53 (d, *J*=10.4 Hz, 2H, H-1), 4.19–4.13 (m, 4H, H-6a, H-6b), 4.05 (td, *J*=10.3, 9.2 Hz, 2H, H-2), 3.67 (ddd, *J*=10.1, 4.6, 3.0 Hz, 2H, H-5), 3.30 (dt, *J*=14.6, 6.7 Hz, 2H, SC*H*$_2$CH$_2$triazole), 3.08 (dt, *J*=14.6, 6.4 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.05, 2.03, 2.03 (each s, each 6H, each OAc), 1.93 (s, 6H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.88, 170.56, 170.40, 169.31 (each C=O), 159.30 (aromatic C), 143.60 (*C*CH, triazole), 130.40 (aromatic CH), 124.22 (C*C*H, triazole), 108.26 (aromatic CH), 102.45 (aromatic CH), 84.48 (C-1), 76.08 (C-5), 73.34 (C-3), 68.25 (C-4), 62.04 (C-6), 62.04 (*C*H$_2$OAr), 53.15 (C-2), 50.38 (SCH$_2$CH$_2$triazole), 30.44 (SCH$_2$CH$_2$triazole), 23.19 (NHAc), 20.72, 20.65, 20.59 (each OAc); ES-HRMS calcd for $C_{44}H_{58}N_8O_{18}S_2Na$ 1073.3208, found *m*/*z* 1073.3201 [M+Na]$^+$; IR (ATR, cm^{-1}): 1743, 1660, 1525, 1371, 1221, 1035, 733, 703; R_f: 0.44 (2:23 MeOH/CH_2Cl_2).

The acetylated intermediate (350 mg, 0.333 mmol) was stirred in methanol (30 mL) and a catalytic amount of a freshly prepared 1 M NaOMe solution in MeOH (0.17 mL, 0.170 mmol) was added. The resulting mixture was stirred for 2 h at room temperature, and Amberlite IR-120H$^+$ was then added and the mixture stirred for a further 10 min. The resin was removed by filtration and washed with water and acetonitrile. The solvents were removed until a minimal amount of water remained and the concentrate was subjected to reverse-phase column chromatography. The solution of the compound, in a minimal amount of water, which was heated or made acidic if necessary to dissolve, was loaded onto the reverse-phase silica gel. Three volumes of water were first passed through the column and the title compound (207 mg, 78%, white solid) was then eluted with 1:1 MeCN/H_2O; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.20 (s, 2H, CC*H*, triazole), 7.72 (d, *J*=9.3 Hz, 2H, NH), 7.18 (t, *J*=8.2 Hz, 1H, aromatic H), 6.71 (t, *J*=2.3 Hz, 1H, aromatic H), 6.62 (dd, *J*=8.3, 2.3 Hz, 2H, aromatic H), 5.09 (s, 4H, ArOC*H*$_2$), 5.04 (d, *J*=5.3 Hz, 2H, OH-4), 4.99 (d, *J*=5.4 Hz, 2H, OH-3), 4.67–4.52 (m, 6H, SCH$_2$C*H*$_2$triazole, OH-6), 4.40 (d, *J*=10.3 Hz, 2H, H-1), 3.71 (ddd, *J*=11.8, 5.4, 2.0 Hz, 2H, H-6a), 3.53 (q, *J*=9.8 Hz, 2H, H-2), 3.44 (dt, *J*=11.9, 6.1 Hz, 2H, H-6b), 3.29–3.23 (m, 2H, H-3), 3.20–3.13 (m, 4H, H-5, SC*H*$_2$CH$_2$triazole), 3.09 (td, *J*=9.1, 5.2 Hz, 2H, H-4), 2.97 (dt, *J*=13.9, 6.9 Hz, 2H, SC*H*$_2$CH$_2$triazole), 1.78 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 169.52 (C=O), 159.75 (aromatic C), 142.62 (*C*CH, triazole), 130.45 (aromatic CH), 125.60 (C*C*H, triazole), 107.67 (aromatic CH), 102.01 (aromatic CH), 85.03 (C-1), 81.60 (C-5), 75.81 (C-3), 70.90 (C-4), 61.68 (C-6), 61.60 (*C*H$_2$OAr), 54.70 (C-2), 50.14 (SCH$_2$C*H*$_2$triazole), 30.50 (SC*H*$_2$CH$_2$triazole), 23.46 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{12}S_2Na$ 821.2574, found *m*/*z* 821.2573 [M+Na]$^+$; IR (ATR, cm^{-1}): 3269, 1645, 1551, 1373, 1264, 1153, 1048, 1027, 820. R_f: 0.61 (1:1 MeCN/H_2O, reverse-phase silica gel); $[\alpha]_D^{20}$ −26.9 (*c* 0.26, DMSO).

4.6. 1,4-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-β-D-gluco-pyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 2

Reaction of **13** (350 mg, 0.809 mmol) and *para*-bispropargyloxybenzene **19** (63 mg, 0.338 mmol) as described in preparation of **1** gave the acetylated intermediate (319 mg, 90%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (s, 2H, CC*H*, triazole), 6.91 (s, 4H, aromatic H), 5.92 (d, *J*=9.3 Hz, 2H, H-1), 5.16 (s, 4H, ArOC*H*$_2$), 5.14 (t, *J*=10.0 Hz, 2H, H-3), 5.06 (t, *J*=9.7 Hz, 2H, H-4), 4.67 (dt, *J*=12.9, 6.2 Hz, 2H, SCH$_2$C*H*$_2$triazole), 4.56 (dt, *J*=13.7, 6.6 Hz, 2H, SCH$_2$C*H*$_2$triazole), 4.43 (d, *J*=10.4 Hz, 2H, NH), 4.25–4.14 (m, 4H, H-6a, H-6b), 4.06 (q, *J*=9.9 Hz, 2H, H-2), 3.67 (ddd, *J*=10.1, 4.8, 2.6 Hz, 2H, H-5), 3.30 (dt, *J*=13.8, 6.7 Hz, 2H, SC*H*$_2$CH$_2$triazole), 3.06 (dt, *J*=14.6, 6.3 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.06, 2.03, 2.02 (each s, each 6H, each OAc), 1.93 (s, 6H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.91, 170.56, 170.34, 169.30 (each C=O), 152.70 (aromatic C), 143.86 (*C*CH, triazole), 124.18 (C*C*H, triazole), 116.20 (aromatic CH), 84.54 (C-1), 76.08 (C5), 73.39 (C-3), 68.21 (C-4), 62.68 (*C*H$_2$OAr), 62.06 (C-6), 53.02 (C-2), 50.45 (SCH$_2$*C*H$_2$triazole), 30.37 (SCH$_2$CH$_2$triazole), 23.17 (NHAc), 20.72, 20.64, 20.58 (each OAc); ES-HRMS calcd for $C_{44}H_{58}N_8O_{18}S_2Na$ 1073.3208, found *m*/*z* 1073.3193 [M+Na]$^+$; IR (ATR, cm^{-1}): 1743, 1661, 1536, 1508, 1372, 1221, 1043, 915, 731; R_f: 0.34 (2:23 MeOH/CH_2Cl_2). Deacetylation of this intermediate (315 mg, 0.300 mmol) as described in the preparation of **1** gave the title compound **2** (196 mg, 82%) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.18 (s, 2H, CC*H*, triazole), 7.71 (d, *J*=9.3 Hz, 2H, NH), 6.95 (s, 4H, aromatic H), 5.05 (d, *J*=1.4 Hz, 4H, ArOC*H*$_2$), 5.04 (d, *J*=3.9 Hz, 2H, OH-4), 4.99 (d, *J*=5.5 Hz, 2H, OH-3), 4.63–4.54 (m, 6H, SCH$_2$C*H*$_2$triazole, OH-6), 4.40 (d, *J*=10.3 Hz, 2H, H-1), 3.71 (ddd, *J*=11.9, 5.6, 2.0 Hz, 2H, H-6), 3.53 (q, *J*=9.8 Hz, 2H, H-2), 3.44 (dt, *J*=11.9, 6.1 Hz, 2H, H-6b), 3.30–3.21 (m, 2H, H-3), 3.20–3.13 (m, 4H, H-5, SC*H*$_2$CH$_2$triazole), 3.09 (td, *J*=9.1, 5.3 Hz, 2H, H-4), 2.97 (dt, *J*=13.9, 6.9 Hz, 2H, SC*H*$_2$CH$_2$triazole), 1.78 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 169.51 (C=O), 152.81 (aromatic C), 142.91 (*C*CH, triazole), 125.48 (C*C*H, triazole), 116.03 (aromatic CH), 85.03 (C-1), 81.61 (C-5), 75.81 (C-3), 70.90 (C-4), 62.07 (*C*H$_2$OAr), 61.68 (C-6), 54.70 (C-2), 50.11 (SCH$_2$*C*H$_2$triazole), 30.51 (SCH$_2$CH$_2$triazole), 23.46 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{12}S_2Na$ 821.2574, found *m*/*z* 821.2584 [M+Na]$^+$; IR (ATR, cm^{-1}): 3267, 1644, 1552, 1508, 1374, 1216, 1052, 1027, 1007, 822; R_f: 0.62 (1:1 MeCN/H_2O) reverse-phase silica gel; $[\alpha]_D^{20}$ −24.0 (*c* 0.27, DMSO).

4.7. 1,3-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-gluco-pyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 5

Reaction of azide **17** (335 mg, 0.775 mmol) and **20** as described in preparation of **1** gave the acetylated intermediate (327 mg, 89%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (s, 2H, CC*H*, triazole), 7.23–7.17 (m, 1H, aromatic H), 6.65–6.59 (m, 3H, aromatic H), 5.88 (d, *J*=8.0 Hz, 2H, NH), 5.58 (d, *J*=5.4 Hz, 2H, H-1), 5.20 (s, 4H, C*H*$_2$OAr), 5.11 (t, *J*=9.6 Hz, 2H, H-4), 5.02 (dd, *J*=11.1, 9.3 Hz, 2H, H-3), 4.59 (t, *J*=6.7 Hz, 4H, SCH$_2$C*H*$_2$triazole), 4.46 (ddd, *J*=11.1, 8.0, 5.4 Hz, 2H, H-2), 4.30 (ddd, *J*=9.9, 4.9, 2.2 Hz, 2H, H-5), 4.25 (dd, *J*=12.3, 4.8 Hz, 2H, H-6a), 4.12 (dd, *J*=12.3, 2.2 Hz, 2H, H-6b), 3.18 (dt, *J*=13.6, 6.7 Hz, 2H, SC*H*$_2$CH$_2$triazole), 3.09 (dt, *J*=14.0, 6.8 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.06, 2.04, 2.04 (each s, each 6H, each OAc), 1.97 (s, 6H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.72, 170.52, 170.20, 169.24 (each C=O), 159.37 (aromatic C), 144.26 (*C*CH, triazole), 130.13 (aromatic CH), 123.05 (C*C*H, triazole), 107.78 (aromatic CH), 102.13 (aromatic CH), 84.63 (C-1), 70.96 (C-3), 68.75 (C-5), 67.98 (C-4), 62.07 (*C*H$_2$OAr), 62.01 (C-6), 52.75 (C-2), 49.52 (SCH$_2$*C*H$_2$triazole), 31.44 (SC*H*$_2$CH$_2$triazole), 23.18 (NHAc), 20.70, 20.68, 20.58 (each OAc); ES-HRMS calcd for $C_{44}H_{58}N_8O_{18}S_2Na$ 1073.3208, found *m*/*z* 1073.3237 [M+Na]$^+$; IR (ATR, cm^{-1}): 3286, 1742, 1540, 1369, 1227, 1150, 1086, 1039, 734; R_f: 0.47 (2:23 MeOH/CH_2Cl_2).

Deacetylation of this intermediate (314 mg, 0.299 mmol) as described in the preparation of **1** gave the title compound **3** (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, aromatic H), 6.70 (t, *J*=2.4 Hz, 1H, aromatic H), 6.62 (dd, *J*=8.3, 2.3 Hz, 2H, aromatic 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,

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J=5.8 Hz, 2H, OH-6), 4.59 (dt, J=13.7, 6.8 Hz, 2H, SCH_2CH_2triazole), 4.52 (dt, J=13.9, 7.0 Hz, 2H, SCH_2CH_2triazole), 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, SCH_2CH_2triazole), 2.96 (dt, J=13.8, 6.8 Hz, 2H, SCH_2CH_2triazole), 1.81 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.03 (C=O), 159.74 (aromatic C), 142.87 (*C*CH, triazole), 130.47 (aromatic CH), 125.15 (C*C*H, triazole), 107.68 (aromatic CH), 102.03 (aromatic CH), 84.47 (C-1), 74.19 (C-5), 71.42 (C-4), 71.08 (C-3), 61.61 (CH_2OAr), 61.31 (C-6), 54.60 (C-2), 49.74 (SCH_2CH_2triazole), 30.54 (SCH_2CH_2triazole), 23.03 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{12}S_2Na$ 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); $[\alpha]_D^{20}$ +114.6 (c 0.42, DMSO).

4.8. 1,4-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-gluco-pyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 6

Reaction of azide **17** (335 mg, 0.775 mmol) and **19** (65 mg, 0.349 mmol) as described in the preparation of **1** gave the acetylated intermediate (353 mg, 96%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (s, 2H, C*H*, triazole), 6.92 (s, 4H, aromatic H), 5.83 (d, J=8.0 Hz, 2H, NH), 5.57 (d, J=5.4 Hz, 2H, H-1), 5.17 (s, 4H, CH_2OAr), 5.12 (t, J=9.6 Hz, 2H, H-4), 5.02 (dd, J=11.1, 9.3 Hz, 2H, H-3), 4.59 (t, J=6.7 Hz, 4H, SCH_2CH_2triazole), 4.46 (ddd, J=11.1, 7.9, 5.5 Hz, 2H, H-2), 4.30 (ddd, J=10.0, 4.9, 2.1 Hz, 2H, H-5), 4.26 (dd, J=12.3, 4.8 Hz, 2H, H-6a), 4.12 (dd, J=12.3, 2.1 Hz, 2H, H-6b), 3.18 (dt, J=13.6, 6.7 Hz, 2H, SCH_2CH_2triazole), 3.09 (dt, J=13.9, 6.8 Hz, 2H, SCH_2CH_2triazole), 2.06, 2.04, 2.04 (each s, each 6H, each OAc), 1.97 (s, 6H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.87, 171.77, 170.52, 170.18 (each C=O), 152.75 (aromatic C), 144.55 (*C*CH, triazole), 122.96 (C*C*H, triazole), 115.88 (aromatic CH), 84.64 (C-1), 70.99 (C-3), 68.76 (C-5), 67.93 (C-4), 62.66 (CH_2OAr), 62.01 (C-6), 52.77 (C-2), 49.50 (SCH_2CH_2triazole), 31.46 (SCH_2CH_2triazole), 23.18 (NHAc), 20.70, 20.69, 20.58 (each OAc); ES-HRMS calcd for $C_{44}H_{58}N_8O_{18}S_2Na$ 1073.3208, found m/z 1073.3203 $[M+Na]^+$; IR (ATR, cm^{-1}): 3278, 1744, 1635, 1547, 1376, 1228, 1085, 1043, 1027, 800; R_f: 0.47 (2:23 MeOH/CH_2Cl_2). Deacetylation of this intermediate (349 mg, 0.332 mmol) as described in the preparation of **1** gave the title compound (196 mg, 74%) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.17 (s, 2H, C*H*, triazole), 7.83 (d, J=7.0 Hz, 2H, NH), 6.95 (s, 4H, aromatic 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, CH_2OAr), 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_2CH_2triazole), 4.51 (dt, J=13.9, 7.0 Hz, 2H, SCH_2CH_2triazole), 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, SCH_2CH_2triazole), 2.96 (dt, J=13.8, 6.8 Hz, 2H, SCH_2CH_2triazole), 1.81 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.02 (C=O), 152.80 (aromatic C), 143.15 (*C*CH, triazole), 125.03 (C*C*H, triazole), 116.04 (aromatic CH), 84.48 (C-1), 74.19 (C-5), 71.42 (C-4), 71.08 (C-3), 62.09 (CH_2OAr), 61.32 (C-6), 54.61 (C-2), 49.72 (SCH_2CH_2triazole), 30.57 (SCH_2CH_2triazole), 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_2O, reverse-phase silica gel); $[\alpha]_D^{20}$ +146.4 (c 0.39, DMSO).

4.9. 1,3,5-Tri[1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-gluco-pyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 8

Reaction of azide **17** (348 mg, 0.805 mmol) and tripropargyl ether **21** (62 mg, 0.258 mmol) as described in preparation of **1**, gave, after chromatography (95:5 to 93:7 CH_2Cl_2/MeOH), the acetylated intermediate (343 mg, 86%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$-d) δ 7.71 (s, 3H, C*H*, triazole), 6.28 (s, 3H, aromatic H), 5.99 (d, J=8.0 Hz, 3H, NH), 5.59 (d, J=5.4 Hz, 3H, H-1), 5.17 (s, 6H, CH_2OAr), 5.11 (t, J=9.6 Hz, 3H, H-4), 5.02 (dd, J=11.1, 9.3 Hz, 3H, H-3), 4.60 (t, J=6.7 Hz, 6H, SCH_2CH_2triazole), 4.47 (ddd, J=11.2, 8.0, 5.4 Hz, 3H, H-2), 4.31 (ddd, J=10.0, 4.8, 2.2 Hz, 3H, H-5), 4.26 (dd, J=12.3, 4.9 Hz, 3H, H-6a), 4.12 (dd, J=12.3, 2.2 Hz, 3H, H-6b), 3.18 (dt, J=13.6, 6.7 Hz, 3H, SCH_2CH_2triazole), 3.10 (dt, J=13.9, 6.8 Hz, 3H, SCH_2CH_2triazole), 2.05, 2.04, 2.04 (each s, each 9H, each OAc), 1.97 (s, 9H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.65, 170.53, 170.25, 169.28 (each C=O), 160.02 (aromatic C), 143.99 (*C*CH, triazole), 123.21 (C*C*H, triazole), 95.38 (aromatic CH), 84.64 (C-1), 70.92 (C-3), 68.73 (C-5), 68.05 (C-4), 62.03 (CH_2OAr), 62.03 (C-6), 52.71 (C-2), 49.53 (SCH_2CH_2triazole), 31.43 (SCH_2CH_2triazole), 23.17 (NHAc), 20.70, 20.69, 20.59 (each OAc); ES-HRMS calcd for $C_{63}H_{84}N_{12}O_{27}S_3Na$ 1559.4629, found m/z 1559.4636 $[M+Na]^+$; IR (ATR, cm^{-1}): 3276, 1744, 1635, 1541, 1509, 1376, 1230, 1152, 1118, 1044, 827, 803; R_f: 0.40 (2:23 MeOH/CH_2Cl_2). Deacetylation of this intermediate (330 mg, 0.215 mmol) as described in preparation of **1** gave the title compound **8** (224 mg, 90%) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.20 (s, 3H, C*H*, triazole), 7.83 (d, J=7.0 Hz, 3H, NH), 6.33 (s, 3H, aromatic H), 5.45 (d, J=5.3 Hz, 3H, H-1), 5.11 (d, J=5.7 Hz, 3H, OH-4), 5.07 (s, 6H, CH_2OAr), 4.81 (d, J=5.3 Hz, 3H, OH-3), 4.66–4.63 (m, 3H, OH-6), 4.59 (dt, J=13.7, 6.9 Hz, 3H, SCH_2CH_2triazole), 4.52 (dt, J=14.0, 7.0 Hz, 3H, SCH_2CH_2triazole), 3.79 (ddd, J=10.9, 7.0, 5.2 Hz, 3H, H-2), 3.71 (td, J=12.4, 11.1, 5.2 Hz, 6H, H-5. H-6a), 3.48 (dt, J=11.4, 5.3 Hz, 3H, H-6b), 3.38–3.33 (m, 3H, H-3), 3.11 (dt, J=9.6, 4.7 Hz, 3H, H-4), 3.05 (dt, J=14.0, 7.2 Hz, 3H, SCH_2CH_2triazole), 2.96 (dt, J=13.9, 6.9 Hz, 3H, SCH_2CH_2triazole), 1.81 (s, 9H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.04 (C=O), 160.36 (Oaromatic C), 142.77 (*C*CH, triazole), 125.18 (C*C*H, triazole), 94.91 (aromatic CH), 84.47 (C-1), 74.18 (C-5), 71.42 (C-4), 71.08 (C-3), 61.68 ($ArOCH_2$), 61.31 (C-6), 54.60 (C-2), 49.75 (SCH_2CH_2triazole), 30.53 (SCH_2CH_2triazole), 23.03 (NHAc); ES-HRMS calcd for $C_{45}H_{66}N_{12}O_{18}S_3Na$ 1181.3678, found m/z 1181.3660 $[M+Na]^+$; IR (ATR, cm^{-1}): 3286, 1641, 1543, 1376, 1168, 1099, 1066, 1004, 943, 810, 757; R_f: 0.71 (1:1 MeCN/H_2O, reverse-phase silica gel); $[\alpha]_D^{20}$ +142.2 (c 0.45, DMSO).

4.10. 1,1,2,2-Tetrakis[4-(1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranosyl)-1,2,3-triazol-4-ylmethyloxy-phenyl)]ethane 7

Reaction of azide **17** (333 mg, 0.770 mmol) and **22** (104 mg, 0.190 mmol) as described in the preparation of **1** gave, after chromatography (95:5 to 93:7 CH_2Cl_2/MeOH), the acetylated intermediate (357 mg, 83%) as a yellow solid; ^{1}H NMR (500 MHz, $CDCl_3$-d) δ 7.66 (s, 4H, C*H*, triazole), 6.93 (d, J=8.7 Hz, 8H, aromatic H), 6.72 (d, J=8.8 Hz, 8H, aromatic H), 5.89 (d, J=8.0 Hz, 4H, NH), 5.58 (d, J=5.4 Hz, 4H, H-1), 5.14 (s, 8H, CH_2OAr), 5.11 (t, J=9.8 Hz, 4H, H-4), 5.02 (dd, J=11.1, 9.3 Hz, 4H, H-3), 4.60 (t, J=6.8 Hz, 8H, SCH_2CH_2triazole), 4.47 (ddd, J=11.1, 8.0, 5.5 Hz, 4H, H-2), 4.31 (ddd, J=10.1, 4.8, 2.0 Hz, 4H, H-5), 4.26 (dd, J=12.3, 4.8 Hz, 4H, H-6a), 4.13 (dd, J=12.4, 2.2 Hz, 4H, H6b), 3.18 (dt, J=13.7, 6.7 Hz, 4H, SCH_2CH_2triazole), 3.10 (dt, J=14.0, 6.9 Hz, 4H, SCH_2CH_2triazole), 2.06 (s, 12H), 2.04 (s, 12H), 2.04 (s, 12H) (each OAc), 1.96 (s, 12H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.71, 170.54, 170.23, 169.25 (each C=O), 156.56 (aromatic C), 144.35 (*C*CH, triazole), 138.73 (Ar_2–*C*=*C*–Ar_2), 137.20 (aromatic C), 132.56 (aromatic CH), 123.01 (C*C*H, triazole), 113.88 (aromatic CH), 84.60 (C-1), 70.97 (C-3), 68.76 (C-5), 68.00 (C4), 62.01 (CH_2OAr), 62.01 (C-6), 52.71 (C-2), 49.52 (SCH_2CH_2triazole), 31.38 (SCH_2CH_2triazole), 23.17 (NHAc), 20.70, 20.70, 20.58 (each OAc); ES-HRMS calcd for $C_{102}H_{124}N_{16}O_{36}S_4Cl$ 2311.6935, found m/z 2311.6877 $[M+Cl]^-$; IR (ATR, cm^{-1}): 1741, 1669, 1508, 1367, 1225, 1039, 734; R_f: 0.39 (2:23 MeOH/CH_2Cl_2). Deacetylation of this intermediate (355 mg, 0.311 mmol) as

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described in the preparation of **1** gave the title compound **7** (263 mg, 95%) as a yellow solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.18 (s, 4H, CC*H*, triazole), 7.83 (d, *J*=7.0 Hz, 4H, NH), 6.87 (d, *J*=8.7 Hz, 8H, aromatic H), 6.80 (d, *J*=8.8 Hz, 8H, aromatic H), 5.44 (d, *J*=5.3 Hz, 4H, H-1), 5.11 (d, *J*=5.6 Hz, 4H, OH-4), 5.03 (s, 8H, CH_2OAr), 4.81 (d, *J*=5.8 Hz, 4H, OH-3), 4.64 (t, *J*=5.8 Hz, 4H, OH-6), 4.58 (dt, *J*=13.7, 6.8 Hz, 4H, SCH$_2$CH_2triazole), 4.51 (dt, *J*=14.0, 7.0 Hz, 4H, SCH$_2$CH_2triazole), 3.79 (ddd, *J*=10.9, 7.0, 5.2 Hz, 4H, H-2), 3.75–3.67 (m, 8H, H5, H-6a), 3.48 (dt, *J*=12.0, 6.2 Hz, 4H, H-6b), 3.37–3.31 (m, 4H, H-3), 3.11 (td, *J*=9.1, 5.9 Hz, 4H, H-4), 3.04 (dt, *J*=13.9, 7.0 Hz, 4H, SCH_2CH$_2$triazole), 2.96 (dt, *J*=13.8, 6.8 Hz, 4H, SCH_2CH$_2$triazole), 1.80 (s, 12H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.03 (C=O), 156.89 (Oaromatic C), 142.86 (*C*CH, triazole), 138.51 (Ar$_2$–*C*=*C*–Ar$_2$), 136.95 (aromatic C), 132.46 (aromatic CH), 125.15 (C*C*H, triazole), 114.33 (aromatic CH), 84.49 (C-1), 74.19 (C-5), 71.42 (C-4), 71.08 (C-3), 61.46 (*C*H$_2$OAr), 61.31 (C-6), 54.60 (C-2), 49.76 (SCH$_2$*C*H$_2$triazole), 30.56 (SC*H*$_2$CH$_2$triazole), 23.03 (NHAc); ES-HRMS calcd for $C_{78}H_{100}N_{16}O_{24}S_4Na_1$ 1795.5877, found *m*/*z* 1795.5903 [M+Na]$^+$; IR (ATR, cm^{-1}): 3279, 1644, 1506, 1235, 1175, 1051, 1004, 830, 761; *R*$_f$: 0.62 (1:1 MeCN/H$_2$O, reverse-phase silica gel); $[\alpha]_D^{20}$ +132.6 (*c* 0.33, DMSO).

4.11. 1,4-Di[1-(ethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 4

Reaction of azide **16** (320 mg, 0.769 mmol) and **19** (68 mg, 0.365 mmol) as described in the preparation of **1** gave, after chromatography (95:5 CH_2Cl_2/MeOH), the acetylated intermediate (315 mg, 85%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (s, 2H, CC*H*, triazole), 6.92 (s, 4H, aromatic H), 5.68 (d, *J*=8.9 Hz, 2H, NH), 5.16 (dd, *J*=10.7, 9.3 Hz, 2H, H-3), 5.13 (d, *J*=2.5 Hz, 4H, ArOCH_2), 5.06 (t, *J*=9.6 Hz, 2H, H-4), 4.62 (ddd, *J*=14.5, 4.5, 2.9 Hz, 2H, OCH$_2$CH_2triazole), 4.55 (d, *J*=8.4 Hz, 2H, H-1), 4.50 (ddd, *J*=14.5, 9.0, 3.2 Hz, 2H, OCH$_2$CH_2triazole), 4.27–4.23 (m, 2H, OCH_2CH$_2$triazole), 4.23 (dd, *J*=12.3, 4.7 Hz, 2H, H-6a), 4.13 (dd, *J*=12.3, 2.4 Hz, 2H, H-6b), 3.94 (dt, *J*=10.6, 8.6 Hz, 2H, H-2), 3.92–3.87 (m, 2H, OCH_2CH$_2$triazole), 3.67 (ddd, *J*=10.0, 4.7, 2.4 Hz, 2H, H-5), 2.08, 2.02, 2.01 (each s, each 6H, each OAc), 1.84 (s, 6H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.93, 170.62, 170.42, 169.30 (each C=O), 152.72 (aromatic C), 143.91 (*C*CH, triazole), 124.43 (C*C*H, triazole), 115.87 (aromatic CH), 100.73 (C-1), 72.17 (C-3), 71.98 (C-5), 68.28 (C-4), 67.32 (OCH$_2$CH$_2$triazole), 62.40 (*C*H$_2$OAr), 61.87 (C-6), 54.21 (C-2), 50.10 (OCH$_2$*C*H$_2$triazole), 23.18 (NHAc), 20.74, 20.63, 20.59 (each OAc); ES-HRMS calcd for $C_{44}H_{58}N_8O_{20}Na$ 1041.3665, found *m*/*z* 1041.3674 [M+Na]$^+$; IR (ATR, cm^{-1}): 1742, 1662, 1592, 1508, 1490, 1374, 1231, 1219, 1039, 1003, 734; *R*$_f$: 0.43 (2:23 MeOH/CH_2Cl_2). Deacetylation of this intermediate (310 mg, 0.304 mmol) as described in preparation of **1** gave the title compound **4** (207 mg, 89%) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.05 (s, 2H, CC*H*, triazole), 7.62 (d, *J*=9.0 Hz, 2H, NH), 6.97 (s, 4H, aromatic H), 5.03 (s, 4H, ArOCH_2), 4.55 (ddd, *J*=14.5, 5.8, 3.8 Hz, 2H, OCH$_2$CH_2triazole), 4.49 (ddd, *J*=14.5, 7.4, 3.8 Hz, 2H, OCH$_2$CH_2triazole), 4.31 (d, *J*=8.5 Hz, 2H, H-1), 4.06 (ddd, *J*=11.2, 5.8, 3.8 Hz, 2H, OCH_2CH$_2$triazole), 3.79 (ddd, *J*=11.1, 7.4, 3.7 Hz, 2H, OCH_2CH$_2$triazole), 3.68 (dd, *J*=11.9, 2.0 Hz, 2H, H-6a), 3.46–3.40 (m, 4H, H-2, H-6b), 3.25 (dd, *J*=10.2, 8.3 Hz, 2H, H-3), 3.10 (ddd, *J*=10.0, 5.9, 2.0 Hz, 2H, H-5), 3.05 (dd, *J*=9.7, 8.3 Hz, 2H, H-4), 1.74 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 169.67 (C=O), 152.80 (aromatic C), 143.07 (*C*CH, triazole), 125.40 (C*C*H, triazole), 115.97 (aromatic CH), 101.15 (C-1), 77.53 (C-5), 74.56 (C-3), 71.00 (C-4), 66.97 (OCH$_2$CH$_2$triazole), 62.01 (*C*H$_2$OAr), 61.48 (C-6), 55.55 (C-2), 49.96 (OCH$_2$*C*H$_2$triazole), 23.45 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{14}Na$ 789.3031, found *m*/*z* 789.3014 [M+Na]$^+$; IR (ATR, cm^{-1}): 3283, 644, 1549, 1512, 1375, 1236, 1226, 1101, 1055, 1030, 822, 806, 708; *R*$_f$: 0.70 (1:1 MeCN/H$_2$O, reverse-phase silica gel); $[\alpha]_D^{20}$ −32.1 (*c* 0.41, DMSO).

4.12. 1,3-Di[1-(ethyl 2-acetamido-2-deoxy-β-D-glucopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 3

Reaction of azide **16** (320 mg, 0.769 mmol) and **20** (68 mg, 0.365 mmol) in THF/H$_2$O (1:1, 12 mL) as described in preparation of **1** gave, after chromatography (95:5 CH_2Cl_2/MeOH), the acetylated intermediate (311 mg, 84%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 2H, CC*H*, triazole), 7.21 (t, *J*=8.2 Hz, 1H, aromatic H), 6.63 (dd, *J*=8.2, 2.3 Hz, 2H, aromatic H), 6.58 (t, *J*=2.4 Hz, 1H, aromatic H), 6.01 (d, *J*=8.8 Hz, 2H, NH), 5.21 (dd, *J*=10.6, 9.3 Hz, 2H, H-3), 5.20–5.12 (m, 4H, ArOCH_2), 5.03 (dd, *J*=10.0, 9.3 Hz, 2H, H-4), 4.65 (d, *J*=8.4 Hz, 2H, H-1), 4.64–4.59 (m, 2H, OCH$_2$CH_2triazole), 4.50 (ddd, *J*=14.5, 9.0, 3.2 Hz, 2H, OCH$_2$CH_2triazole), 4.26–4.23 (m, 2H, OCH_2CH$_2$triazole), 4.21 (dd, *J*=12.4, 4.8 Hz, 2H, H-6a), 4.12 (dd, *J*=12.3, 2.4 Hz, 2H, H-6b), 3.91 (ddd, *J*=11.2, 9.1, 2.9 Hz, 2H, OCH_2CH$_2$triazole), 3.87 (dt, *J*=10.7, 8.6 Hz, 2H, H-2), 3.68 (ddd, *J*=10.0, 4.8, 2.4 Hz, 2H, H-5), 2.08, 2.01, 2.01 (each 6H, each s, each OAc), 1.84 (s, 6H, NHAc); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.82, 170.61, 170.61, 169.33 (each C=O), 159.28 (aromatic C), 143.57 (*C*CH, triazole), 130.24 (aromatic CH), 124.54 (C*C*H, triazole), 107.88 (aromatic CH), 102.20 (aromatic CH), 100.59 (C-1), 72.09 (C-3), 71.92 (C-5), 68.43 (C-4), 67.30 (OCH$_2$CH$_2$triazole), 61.92 (*C*H$_2$OAr), 61.76 (C-6), 54.34 (C-2), 50.14 (OCH$_2$*C*H$_2$triazole), 23.16 (NHAc), 20.74, 20.64, 20.60 (each OAc); ES-HRMS calcd for $C_{44}H_{58}N_8O_{20}Na_1$ 1041.3665, found *m*/*z* 1041.3691 [M+Na]$^+$; IR (ATR, cm^{-1}): 1740, 1659, 1369, 1256, 1153, 1038, 733; *R*$_f$: 0.43 (2:23 MeOH/CH_2Cl_2). Deacetylation of this intermediate (303 mg, 0.297 mmol) as described in the preparation of **1** gave the title compound **3** (146 mg, 64%) as a white solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.07 (s, 2H, CC*H*, triazole), 7.62 (d, *J*=9.0 Hz, 2H, NH), 7.20 (t, *J*=8.2 Hz, 1H, aromatic H), 6.71 (t, *J*=2.4 Hz, 1H, aromatic H), 6.63 (dd, *J*=8.2, 2.3 Hz, 2H, aromatic H), 5.08 (s, 4H, ArOCH_2), 4.56 (ddd, *J*=14.5, 5.8, 3.8 Hz, 2H, OCH$_2$CH_2-triazole), 4.50 (ddd, *J*=14.4, 7.4, 3.8 Hz, 2H, OCH$_2$CH_2triazole), 4.31 (d, *J*=8.5 Hz, 2H, H-1), 4.06 (ddd, *J*=11.2, 5.8, 3.8 Hz, 2H, OCH_2CH$_2$triazole), 3.80 (ddd, *J*=11.1, 7.4, 3.8 Hz, 2H, OCH_2CH$_2$-triazole), 3.68 (dd, *J*=11.9, 2.0 Hz, 2H, H-6a), 3.46–3.39 (m, 4H, H-2, H-6b), 3.24 (dd, *J*=10.3, 8.4 Hz, 2H, H-3), 3.10 (ddd, *J*=9.8, 5.9, 2.0 Hz, 2H, H-5), 3.08–3.01 (m, 2H, H-4), 1.74 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 169.68 (C=O), 159.74 (aromatic C), 142.79 (*C*CH, triazole), 130.47 (aromatic CH), 125.51 (C*C*H, triazole), 107.62 (aromatic CH), 102.00 (aromatic CH), 101.14 (C-1), 77.53 (C-5), 74.56 (C-3), 71.00 (C-4), 66.96 (OCH$_2$CH$_2$triazole), 61.59 (*C*H$_2$OAr), 61.49 (C-6), 55.55 (C-2), 49.98 (OCH$_2$*C*H$_2$triazole), 23.45 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{14}Na$ 789.3031, found *m*/*z* 789.3037 [M+Na]$^+$; IR (ATR, cm^{-1}): 3269, 1644, 1605, 1547, 1294, 1185, 1161, 1054, 1028, 825, 765; *R*$_f$: 0.61 (1:1 MeCN/H$_2$O, reverse-phase silica gel); $[\alpha]_D^{20}$ −30.0 (*c* 0.34, DMSO).

4.13. Reaction of azide 17 with tripropargylamine 23[22]

To azide **17** (251 mg, 0.580 mmol) in THF/H$_2$O (1:1, 10 mL) were added sodium ascorbate (22 mg, 0.111 mmol) and copper sulfate pentahydrate (27 mg, 0.108 mmol) and tripropargylamine (38 μl, 0.25 mmol). The mixture was stirred at room temperature for 18 h. Tetrahydrofuran was then removed under diminished pressure followed by the dilution of the solution with CH_2Cl_2. This mixture was then washed with water. The aqueous layer was re-extracted with a further portion of CH_2Cl_2. The combined organic layers were then washed with water, dried over Na_2SO_4 and the solvent removed. Chromatography of the residue (95:5 CH_2Cl_2/MeOH) gave the monomeric product **24** (49 mg, 36%) as a white solid, the dimeric intermediate (131 mg, 51%) as a white solid, and the trimeric intermediate (46vmg, 13%) as a white solid. Analytical data for **24**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (s, 1H, CC*H*, triazole), 5.85 (d, *J*=8.1 Hz, 1H, NH), 5.55 (d, *J*=5.4 Hz, 1H, H-1), 5.11 (t, *J*=9.6 Hz, 1H, H-4), 5.01 (dd, *J*=11.2, 9.3 Hz, 1H, H-3), 4.57 (td, *J*=6.8, 1.5 Hz, 2H,

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SCH$_2$C*H*$_2$triazole), 4.46 (ddd, *J*=11.1, 8.1, 5.4 Hz, 1H, H-2), 4.30 (ddd, *J*=9.9, 4.7, 2.1 Hz, 1H, H-5), 4.26 (dd, *J*=12.2, 4.7 Hz, 1H, H-6a), 4.12 (dd, *J*=12.3, 2.1 Hz, 1H, H-6b), 3.86 (s, 2H, NC*H*$_2$triazole), 3.46 (d, *J*=2.4 Hz, 4H, NC*H*$_2$CCH), 3.16 (dt, *J*=13.6, 6.7 Hz, 1H, SC*H*$_2$CH$_2$triazole), 3.07 (dt, *J*=14.0, 6.9 Hz, 1H, SC*H*$_2$CH$_2$triazole), 2.27 (t, *J*=2.4 Hz, 2H, NCH$_2$CC*H*), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H) (each OAc), 1.97 (s, 3H, NHAc); ^{13}C NMR (126 MHz, CDCl$_3$) δ 171.74, 170.54, 170.23, 169.22 (each C=O), 144.76 (*C*CH, triazole), 123.18 (C*C*H, triazole), 84.62 (C-1), 78.47 (NCH$_2$*C*CH), 73.49 (NCH$_2$C*C*H), 70.99 (C-3), 68.74 (C-5), 67.92 (C-4), 61.98 (C-6), 52.69 (C-2), 49.44 (SCH$_2$*C*H$_2$triazole), 47.99 (N*C*H$_2$triazole), 41.97 (N*C*H$_2$CCH), 31.48 (S*C*H$_2$CH$_2$triazole), 23.19 (NHAc), 20.70, 20.69, 20.57 (each OAc); ES-HRMS calcd for $C_{25}H_{34}N_5O_8S$ 564.2128, found *m*/*z* 564.2116 [M+H]$^+$; IR (ATR, cm^{-1}): 3280, 1742, 1665, 1367, 1227, 1090, 1040, 910, 729; R_f:0.35 (1:19 MeOH/CH$_2$Cl$_2$). Analytical data for dimeric intermediate **25**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.66 (s, 2H, CC*H*, triazole), 6.13 (d, *J*=8.1 Hz, 2H, NH), 5.55 (d, *J*=5.4 Hz, 2H, H-1), 5.10 (t, *J*=9.6 Hz, 2H, H-4), 5.00 (dd, *J*=11.2, 9.3 Hz, 2H, H-3), 4.64–4.51 (m, 4H, SCH$_2$C*H*$_2$triazole), 4.47 (ddd, *J*=11.2, 8.1, 5.4 Hz, 2H, H-2), 4.30 (ddd, *J*=9.9, 4.7, 2.2 Hz, 2H, H-5), 4.26 (dd, *J*=12.2, 4.6 Hz, 2H, H-6a), 4.12 (dd, *J*=12.2, 2.1 Hz, 2H, H-6b), 3.85 (d, *J*=2.1 Hz, 4H, NC*H*$_2$triazole), 3.35 (dd, *J*=6.7, 2.4 Hz, 2H, NC*H*$_2$CCH), 3.16 (dt, *J*=14.2, 6.4 Hz, 2H, SC*H*$_2$CH$_2$triazole), 3.07 (dt, *J*=14.0, 6.9 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.31 (t, *J*=2.2 Hz, 1H, NCH$_2$CC*H*), 2.07 (s, 6H), 2.03 (s, 6H), 2.02 (s, 6H) (each OAc), 1.97 (s, 6H, NHAc); ^{13}C NMR (126 MHz, CDCl$_3$) δ 171.59, 170.57, 170.34, 169.26 (each C=O), 144.45 (*C*CH, triazole), 123.67 (C*C*H, triazole), 84.51 (C-1), 78.25 (NCH$_2$*C*CH), 73.96 (NCH$_2$C*C*H), 70.90 (C-3), 68.70 (C-5), 68.09 (C-4), 61.96 (C-6), 52.57 (C-2), 49.37 (SCH$_2$*C*H$_2$triazole), 47.76 (N*C*H$_2$triazole), 42.28 (N*C*H$_2$CCH), 31.40 (S*C*H$_2$CH$_2$triazole), 23.13 (NHAc), 20.71, 20.71, 20.58 (each OAc); ES-HRMS calcd for $C_{41}H_{57}N_9O_{16}S_2Na$ 1018.3262, found *m*/*z* 1018.3289 [M+Na]$^+$; IR (ATR, cm^{-1}): 1742, 1665, 1535, 1367, 1227, 1090, 1040, 910, 729; R_f: 0.25 (1:19 MeOH/CH$_2$Cl$_2$). Analytical data for trimeric intermediate **26**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.85 (s, 3H, CC*H*, triazole), 6.29 (d, *J*=8.2 Hz, 3H, NH), 5.54 (d, *J*=5.4 Hz, 3H, H-1), 5.09 (t, *J*=9.6 Hz, 3H, H-4), 5.01 (dd, *J*=11.1, 9.2 Hz, 3H, H-3), 4.60 (ddq, *J*=20.8, 13.9, 6.5 Hz, 6H, SCH$_2$C*H*$_2$triazole), 4.48 (ddd, *J*=11.1, 8.2, 5.4 Hz, 3H, H-2), 4.30 (ddd, *J*=9.9, 4.6, 2.2 Hz, 3H, H-5), 4.26 (dd, *J*=12.3, 4.6 Hz, 3H, H-6a), 4.12 (dd, *J*=12.4, 2.2 Hz, 3H, H-6b), 3.74 (q, *J*=14.2 Hz, 6H, NC*H*$_2$triazole), 3.18 (dt, *J*=14.2, 6.3 Hz, 3H, SC*H*$_2$CH$_2$triazole), 3.09 (dt, *J*=13.9, 6.8 Hz, 3H, SC*H*$_2$CH$_2$triazole), 2.07 (s, 9H), 2.02 (s, 9H), 2.00 (s, 9H) (each OAc), 1.97 (s, 9H, NHAc); ^{13}C NMR (126 MHz, CDCl$_3$) δ 171.48, 170.60, 170.42, 169.28 (each C=O), 143.65 (*C*CH, triazole), 124.44 (C*C*H, triazole), 84.55 (C-1), 70.84 (C-3), 68.67 (C-5), 68.20 (C-4), 61.97 (C-6), 52.47 (C-2), 49.47 (SCH$_2$*C*H$_2$triazole), 47.12 (N*C*H$_2$triazole), 31.40 (S*C*H$_2$CH$_2$triazole), 23.11 (NHAc), 20.72, 20.71 20.57 (each OAc); ES-HRMS calcd for $C_{57}H_{82}N_{13}O_{24}S_3$ 1428.4758, found *m*/*z* 1428.4789 [M+H]$^+$; IR (ATR, cm^{-1}): 1742, 1665, 1535, 1367, 1227, 1090, 1042, 912, 730; R_f: 0.18 (1:19 MeOH/CH$_2$Cl$_2$).

4.14. *N,N*-Bis[1-(1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucopyranosyl)-1,2,3-triazol-4-ylmethyl)]-*N*-prop-2-yn-1-amine 9

Deacetylation of **25** (117 mg, 0.117 mmol) as described in the preparation of **1** gave the title compound (52 mg, 60%) as an off-white solid; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 7.98 (s, 2H, CC*H*, triazole), 7.82 (d, *J*=7.0 Hz, 2H, NH), 5.43 (d, *J*=5.3 Hz, 2H, H-1), 5.11 (br s, 2H, OH), 4.80 (br s, 2H, OH), 4.63 (br s, 2H, OH), 4.56 (dt, *J*=13.8, 6.9 Hz, 2H, SCH$_2$C*H*$_2$triazole), 4.48 (dt, *J*=14.0, 7.1 Hz, 2H, SCH$_2$C*H*$_2$triazole), 3.79 (ddd, *J*=11.7, 7.0, 5.2 Hz, 2H, H-2), 3.74–3.66 (m, 8H, H-5, H-6a, NC*H*$_2$triazole), 3.48 (dd, *J*=12.0, 6.3 Hz, 2H, H-6b), 3.31 (m, 4H, H-3, NC*H*$_2$CCH), 3.11 (t, *J*=9.3 Hz, 2H, H-4), 3.03 (dt, *J*=13.9, 7.0 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.94 (dt, *J*=13.8, 6.9 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.48 (m, 1H, NCH$_2$CC*H*), 1.80 (s, 6H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.04 (C=O), 143.53 (*C*CH, triazole), 124.58 (C*C*H, triazole), 84.36 (C-1), 74.13 (C-5), 71.38 (C-4), 71.06 (C-3), 61.28 (C-6), 54.57 (C-2), 49.64 (SCH$_2$*C*H$_2$triazole), 47.78 (NCH$_2$triazole), 41.38 (NCH$_2$CCH), 40.49 (NCH$_2$C*C*H), 30.50 (SCH$_2$CH$_2$triazole), 23.01 (NHAc); ES-HRMS calcd for $C_{29}H_{46}N_9O_{10}S_2$ 744.2809, found *m*/*z* 744.2811 [M+H]$^+$; IR (ATR, cm^{-1}): 3278, 1645, 1544, 1431, 1373, 1299, 1097, 1051, 1030, 1005, 848, 761; $[\alpha]_D^{20}$ 165.9 (*c* 0.14, DMSO).

4.15. *N,N,N*-Tris[1-(1-(ethyl 2-acetamido-2-deoxy-1-thio-α-D-glucoopyranosyl)-1,2,3-triazol-4-ylmethyl)]amine 10

Deacetylation of **26** (100 mg, 0.070 mmol) as described in the preparation of **1** gave the title compound **10** (44 mg, 59%) as a white solid; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.02 (s, 3H, CC*H*, triazole), 7.83 (d, *J*=7.0 Hz, 3H, NH), 5.44 (d, *J*=5.2 Hz, 3H, H-1), 5.11 (d, *J*=5.6 Hz, 3H, OH-4), 4.81 (d, *J*=5.7 Hz, 3H, OH-3), 4.65 (t, *J*=5.8 Hz, 3H, OH-6), 4.58 (dt, *J*=13.8, 6.9 Hz, 3H, SCH$_2$C*H*$_2$triazole), 4.50 (dt, *J*=14.0, 7.1 Hz, 3H, SCH$_2$C*H*$_2$triazole), 3.79 (ddd, *J*=11.8, 7.0, 5.3 Hz, 3H, H-2), 3.75–3.66 (m, 6H, H-5, H-6a), 3.61 (s, 6H, NC*H*$_2$triazole), 3.49 (dt, *J*=11.9, 6.2 Hz, 3H, H-6b), 3.34 (ddd, *J*=10.8, 8.7, 5.8 Hz, 3H, H-3), 3.11 (ddd, *J*=9.9, 8.4, 5.6 Hz, 3H, H-4), 3.04 (dt, *J*=14.0, 7.1 Hz, 3H, SC*H*$_2$CH$_2$triazole), 2.96 (dt, *J*=13.8, 7.0 Hz, 3H, SC*H*$_2$CH$_2$triazole), 1.78 (s, 9H, NHAc); ^{13}C NMR (126 MHz, DMSO) δ 170.04 (C=O), 143.67 (*C*CH, triazole), 124.58 (C*C*H, triazole), 84.41 (C-1), 74.15 (C-5), 71.39 (C-4), 71.08 (C-3), 61.29 (C-6), 54.59 (C-2), 49.68 (SCH$_2$*C*H$_2$triazole), 47.39 (NCH$_2$triazole), 30.55 (SCH$_2$CH$_2$triazole), 23.00 (NHAc); ES-HRMS calcd for $C_{39}H_{64}N_{13}O_{17}S_3$ 1094.3705, found *m*/*z* 1094.3727 [M+FA−H]$^-$; IR (ATR, cm^{-1}): 3277, 1645, 1544, 1432, 1373, 1300, 1221, 1097, 1051, 1028, 1005, 848, 762; $[\alpha]_D^{20}$ 191.5 (*c* 0.14, DMSO).

4.16. 1,4-Di[1-(ethyl 2-acetamido-2-deoxy-1-thio-β-D-galactopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 11

Reaction of azide **18** (215 mg, 0.497 mmol) and **19** (44 mg, 0.236 mmol) as described in the preparation of **1** gave, after chromatography (95:5 CH$_2$Cl$_2$/MeOH), the acetylated intermediate (232 mg, 94%) as a white solid; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.72 (s, 2H, CC*H*, triazole), 6.91 (s, 4H, Ar–H), 5.81 (d, *J*=8.7 Hz, 2H, NH), 5.37 (dd, *J*=3.4, 1.2 Hz, 2H, H-4), 5.17 (s, 4H, ArOC*H*$_2$), 5.04 (dd, *J*=10.2, 3.2 Hz, 2H, H-3), 4.70 (dt, *J*=13.0, 6.2 Hz, 2H, SCH$_2$C*H*$_2$triazole), 4.57 (dt, *J*=13.8, 6.7 Hz, 2H, SCH$_2$C*H*$_2$triazole), 4.30–4.24 (m, 4H, H-1, H-2), 4.11 (dd, *J*=6.5, 2.5 Hz, 4H, H-6a, H-6b), 3.87 (td, *J*=6.2, 1.1 Hz, 2H, H-5), 3.33 (dt, *J*=14.6, 6.4 Hz, 2H, SC*H*$_2$CH$_2$triazole), 3.07 (dt, *J*=14.5, 6.3 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.16 (s, 6H), 2.03 (s, 6H), 1.99 (s, 6H) (each OAc), 1.94 (s, 6H, NHAc); ^{13}C NMR (126 MHz, CDCl$_3$) δ 170.52, 170.45, 170.40, 170.15 (each C=O), 152.71 (Ar–C), 143.83 (*C*CH, triazole), 124.14 (C*C*H, triazole), 116.16 (Ar–*C*H), 84.98 (C-1), 74.77 (C-5), 71.11 (C-3), 66.82 (C-4), 62.70 (*C*H$_2$OAr), 61.74 (C-6), 50.54 (SCH$_2$*C*H$_2$triazole), 49.10 (C-2), 30.28 (S*C*H$_2$CH$_2$triazole), 23.26 (NHAc), 20.72, 20.66, 20.65 (each OAc); ES-HRMS calcd for $C_{44}H_{58}N_8O_{18}S_2Na_1$ 1073.3208, found *m*/*z* 1073.3197 [M+Na]$^+$; IR (ATR, cm^{-1}): 1746, 1663, 1508, 1371, 1234, 1054, 1032, 1016, 749; R_f: 0.38 (2:23 MeOH/CH$_2$Cl$_2$). Deacetylation of this intermediate (25 mg, 0.024 mmol) as described in the preparation of **1** gave the title compound **11** (16 mg, 84%) as a white solid; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.17 (s, 2H, CC*H*, triazole), 7.64 (d, *J*=9.5 Hz, 2H, NH), 6.96 (s, 4H, Ar–H), 5.05 (s, 4H, ArOC*H*$_2$), 4.71 (d, *J*=6.2 Hz, 2H, OH-3), 4.63 (d, *J*=5.5 Hz, 1H, OH-6), 4.61 (d, *J*=4.5 Hz, 1H, OH-4), 4.61–4.56 (m, 4H, SCH$_2$C*H*$_2$triazole), 4.35 (d, *J*=10.3 Hz, 2H, H-1), 3.89 (q, *J*=10.0 Hz, 2H, H-2), 3.69 (t, *J*=3.6 Hz, 2H, H-4), 3.52 (t, *J*=5.4 Hz, 4H, H-6a, H-6b), 3.47–3.36 (m, 4H, H-3, H-5), 3.20 (dt, *J*=13.7, 6.7 Hz, 2H, SC*H*$_2$CH$_2$triazole), 2.94 (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 169.74 (C=O), 152.80 (Ar–C), 142.94 (*C*CH, triazole),

125.37 (CCH, triazole), 116.03 (Ar–CH), 85.16 (C-1), 79.91 (C-5), 72.77 (C-3), 68.16 (C-4), 62.07 (CH_2OAr), 61.25 (C-6), 50.80 (C-2), 50.13 (SCH_2CH_2triazole), 30.24 (SCH_2CH_2triazole), 23.51 (NHAc); ES-HRMS calcd for $C_{32}H_{46}N_8O_{12}S_2Na$ 821.2574, found *m*/*z* 821.2551 $[M+Na]^+$; IR (ATR, cm^{-1}): 3271, 1635, 1549, 1507, 1372, 1312, 1225, 1115, 1041, 1016, 978, 864, 820, 705; R_f: 0.68 (1:1 MeCN/H_2O, reverse-phase silica gel); $[\alpha]_D^{20}$ 8.3 (*c* 0.17, DMSO).

4.17. Lectin purification and biotinylation

WGA was purified by affinity chromatography, after gel electrophoretic controls for purity biotinylated under activity-preserving conditions and checked for bioactivity by haemagglutination.[23] Labelled GSA-II was purchased from Vector Laboratories.

4.18. Inhibition assays

As lectin ligands, the glycoproteins fetuin, ASF or the agalacto version of ASF obtained by treatment with β-galactosidase as well as the neoglycoproteins based on bovine serum albumin presenting 22–26 β-GlcNAc (or β-GalNAc as control) moieties as *p*-isothiocyanatophenyl derivative were adsorbed to the surface of plastic microtiter plate wells, using a solution of 50 μl phosphate-buffered saline overnight at 4 °C. Remaining sites for protein binding were saturated with a solution of carbohydrate-free albumin (1% (w/v)) at 37 °C for 1 h. The binding assay was carried out in successive steps, starting with an incubation for 1 h at 37 °C using the biotinylated lectin without/with test compounds, continued with washing, and the application of the indicator conjugate streptavidin-peroxidase (0.5 μg/mL; Sigma). Finally, the signal for spectrophotometric quantitation at 490 nm was developed enzymatically using the substrate mixture of *o*-phenylenediamine (1 mg/mL) and hydrogen peroxide (1 μL/mL).[3b–d] Titrations were run in duplicates with at least four independent experimental series.

As platform for cell surface binding, the CHO WT line and the glycosylation mutants Lec2/Lec8, kindly provided by P. Stanley, Albert Einstein College of Medicine, Bronx (USA), were used. Cells were first carefully washed to remove all serum components, then sites for non-specific binding for proteins were saturated as above, and a suspension of 5×10^4 cells was incubated with biotinylated lectin in Dulbecco's phosphate-buffered saline for 30 min at 4 °C to minimise endocytic uptake. Following washing, cytofluorimetric analysis was based on detection of the signal of the R-phycoerythrin-streptavidin conjugate (1:40; Sigma) as described.[3c,d] Experimental series were routinely done with aliquots of the same cell suspension, with at least four independent titrations per compound and cell line.

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