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Design and Synthesis of Hydrolytically Stable Multivalent Ligands Bearing Thiodigalactoside Analogues for Peanut Lectin and Human Galectin-3 Binding

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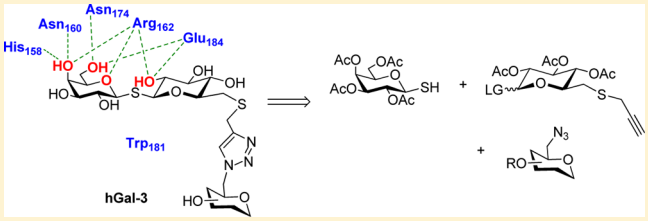
Supporting Information

ABSTRACT: Herein, we describe the design and synthesis of a novel family of hydrolytically stable glycoclusters bearing thiodigalactoside (TDG) analogues as recognition elements of β-galactoside binding lectins. The TDG analogue was synthesized by thioglycosylation of a 6-*S*-acetyl-α-D-glucosyl bromide with the isothiouronium salt of 2,3,4,6-tetra-*O*-acetyl-β-D-galactose. Further propargylation of the TDG analogue allowed the coupling to azido-functionalized oligosaccharide scaffolds through copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC) under microwave activation. The final mono-, di-, and tetravalent ligands were resistant to enzymatic hydrolisis by *Escherichia coli* β-galactosidase. Binding affinities to peanut agglutinin and human galectin-3 were measured by isothermal titration calorimetry which showed K_a constants in the micromolar range as well as a multivalent effect. Monovalent ligand exhibited a binding affinity higher than that of thiodigalactoside. Docking studies performed with a model ligand on both β-galactoside binding lectins showed additional interactions between the triazole ring and lectin amino acid residues, suggesting a positive effect of this aromatic residue on the biological activity.



INTRODUCTION

The design of multivalent glycosylated architectures that mimic the natural systems has been actively pursued over the last years for the synthesis of high affinity ligands of lectins, the carbohydrate recognition proteins.[1,2] Carbohydrate−protein interactions are involved in cellular recognition processes that include viral and bacterial infections, inflammation, and tumor metastasis.[3−5] In natural systems, the usually weak binding affinity of carbohydrates to their protein receptors is overcome by a multivalent display of sugar residues at the surface of cells, which leads to the so-called "glycoside clustering effect".[6] Hence, glycoclusters that interfere with carbohydrate−protein recognition processes are seen as promising chemotherapeutics owing to the relevance of these interactions in triggering many cellular recognition processes.[7,8]

However, most of the multivalent ligands synthesized present O-linked saccharides, and their preparation involves classic glycosylation methods.[2] Although some of these ligands have shown interesting binding activities, O-glycosides are expected to have limited half-life in biological media since they are sensitive to glycosidases. Thioglycosides, on the contrary, are more resistant toward enzymatic and acidic hydrolysis, and therefore a better bioavailability under physiological conditions is expected. Aryl thiogalactosides have been described as ligands for galectin-1, -3 and -7.[9,10]

Among the legume lectins that recognize β-galactoside residues, peanut (*Arachis hypogaea*) lectin (PNA) has been used as model lectin in several studies.[11,12] To our knowledge, in literature there are very few comparative studies between the β-galactoside recognition domains of this lectin and mammalian galectins.[13,14]

In fact, galectins have been actively studied because of their role in numerous processes of vital cell activity, such as cell cycle regulation, cell−cell and cell−intercellular matrix adhesion, and transmission of intercellular signals.[15,16] Human galectin-3, for instance, is implicated in a variety of biological processes such as cancer, inflammation, host−pathogen interaction, and nerve injury, among others.[17]

Thiodigalactoside (TDG, **1**) has been shown to interact with galectins with approximately the same affinity as the natural ligand *N*-acetyllactosamine (LacNAc, **2**).[18,19] Furthermore, it has been demonstrated by X-crystallographic studies that TDG binds to galectin-3 in a similar manner than LacNAc (Figure 1).[20] The effect of thiodigalactoside on anticancer immune responses and tumor growth in vivo has also been reported.[21] Thus, TDG offered an interesting initial structural framework for the synthesis of glycoclusters designed to interfere in galectin-mediated biological processes.

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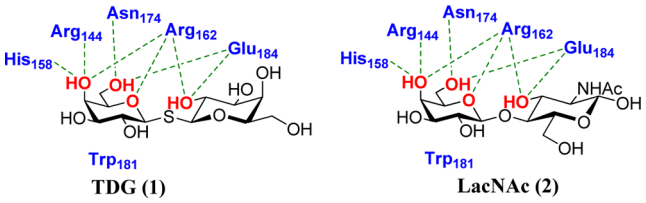


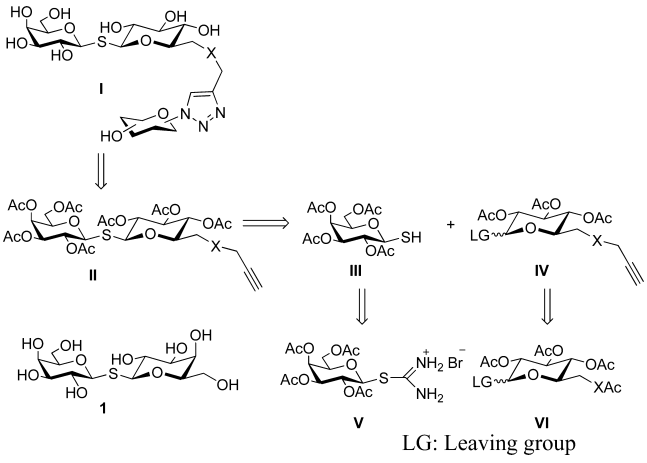
Figure 1. Representation of human galectin-3−TDG (**1**)[20] and human galectin-3−LacNAc (**2**)[22] complexes. Hydrogen-bond interactions are depicted with dotted lines.

In this work we report the rational design and synthesis of a new family of multivalent ligands bearing TDG analogues as the recognition element (RE). The resistance to hydrolysis of the synthesized ligands was evaluated toward *Escherichia coli* β-galactosidase. Also, their affinity to β-galactoside-binding lectins, namely peanut agglutinin (PNA) and human galectin-3 (hGal-3), was determined by isothermal titration calorimetry (ITC). Additionally, computational studies (*docking*) were realized in order to evaluate specific interactions between the different HO- and hydrophobic groups, and key amino acid residues in the carbohydrate recognition domain (CRD) of both lectins.

RESULTS AND DISCUSSION

For the synthesis of the hydrolytically stable multivalent ligands (generically described by **I**), we designed the precursor **II**, inspired by the structure of TDG (**1**). The retro-synthetic pathway is depicted in Scheme 1.

Scheme 1. Retrosynthetic Pathway for the Synthesis of Compound I



Compared to TDG (**1**), which possess two β-linked galactose residues, the proposed RE armed with an alkyne group (**II**) exposes one galactose residue thioglycosidically β-linked to the anomeric position of a conveniently C-6 functionalized glucose residue. This difference should not affect the binding properties, as only one galactose residue in TDG is involved in the recognition process (Figure 1).[20] We rationalized that, by functionalization of the primary HO-6 of the glucose moiety with a terminal alkyne group, this RE could be further linked to azido-functionalized scaffolds, to obtain multivalent products. We also took into account that a glucose residue can be selectively functionalized at C-6 easier than a galactose residue, as in the latter the axially disposed HO-4 hampers substitutions at C-6. On the other hand, we selected sulfur (X = S) in **II**, to ensure the chemoselective introduction of the alkyne group in this position, as is described below.

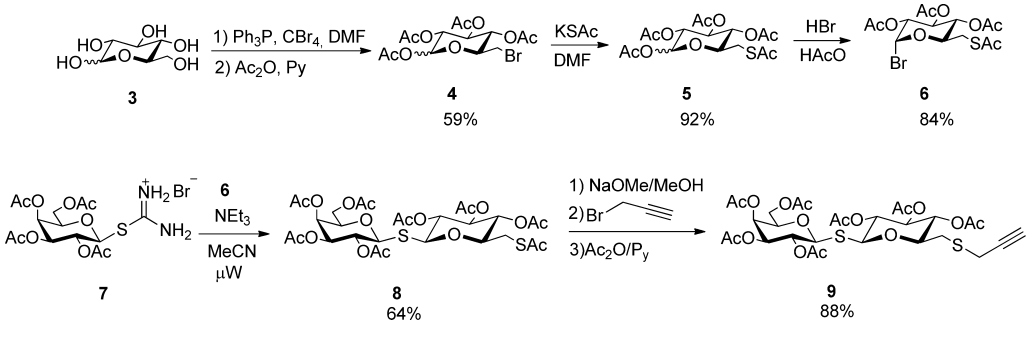
The key step in the synthesis is the thioglycosylation step, which could be achieved by treatment of 1-thio-β-D-galactose (**III**) with a glycosyl donor (**IV**). These precursors could be prepared from the peracetylated isothiouronium salt of galactose (**V**) and a *gluco* derivative such as **VI**, respectively.

Treatment of D-glucose (**3**) with carbon tetrabromide and triphenylphosphine in DMF, gave 1,2,3,4-tetra-*O*-acetyl-6-bromo-6-deoxy-D-glucose (**4**) in 59% yield, after an acetylation step (Scheme 2),[23] which was employed directly for the next step without further purification.

The nucleophilic substitution of the bromide in compound **4** with potassium thioacetate readily took place and gave the expected thioacetate derivative **5** in 92% yield. Attempts to construct the thioglycosidic linkage by treatment of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose or its isothiouronium derivative (**7**) with derivatives **4** or **5** in the presence of $BF_3 \cdot Et_2O$ or $SnCl_4$, under the conditions described by Falconer,[24] were unsuccessful. These results suggest that the 6-bromide or the 6-thioacetate groups decrease the reactivity of the precursor in the thioglycosylation reaction, and thus, a better leaving group than the anomeric acetate was required. Thus, compound **5** was then treated with HBr (33% in AcOH solution) to afford glucosyl bromide **6** in 84% yield. By treatment of **6** with the isothiouronium salt **7** in the presence of triethylamine, thiodisaccharide **8** was obtained.[25] The reaction time and yield (64%) were optimized under microwave irradiation.[26]

^{1}H NMR spectrum of thiodisaccharide **8** confirmed that both residues were β-(1 → 1)-linked, as two doublets were observed at 4.74 ppm ($J_{1,2}$ = 9.2 Hz) and 4.81 ppm ($J_{1',2'}$ = 10.2 Hz), consistent with the anomeric protons of β-thioglucose and β-thiogalactose, respectively.

Scheme 2. Synthesis of Compound 9



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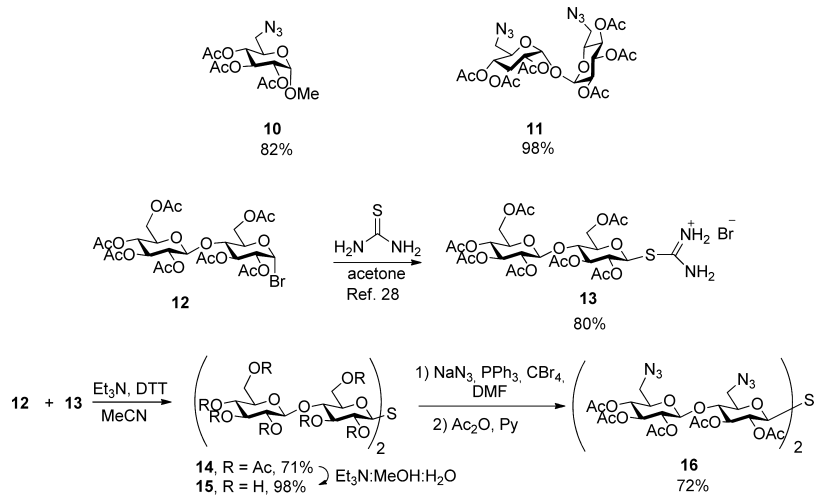
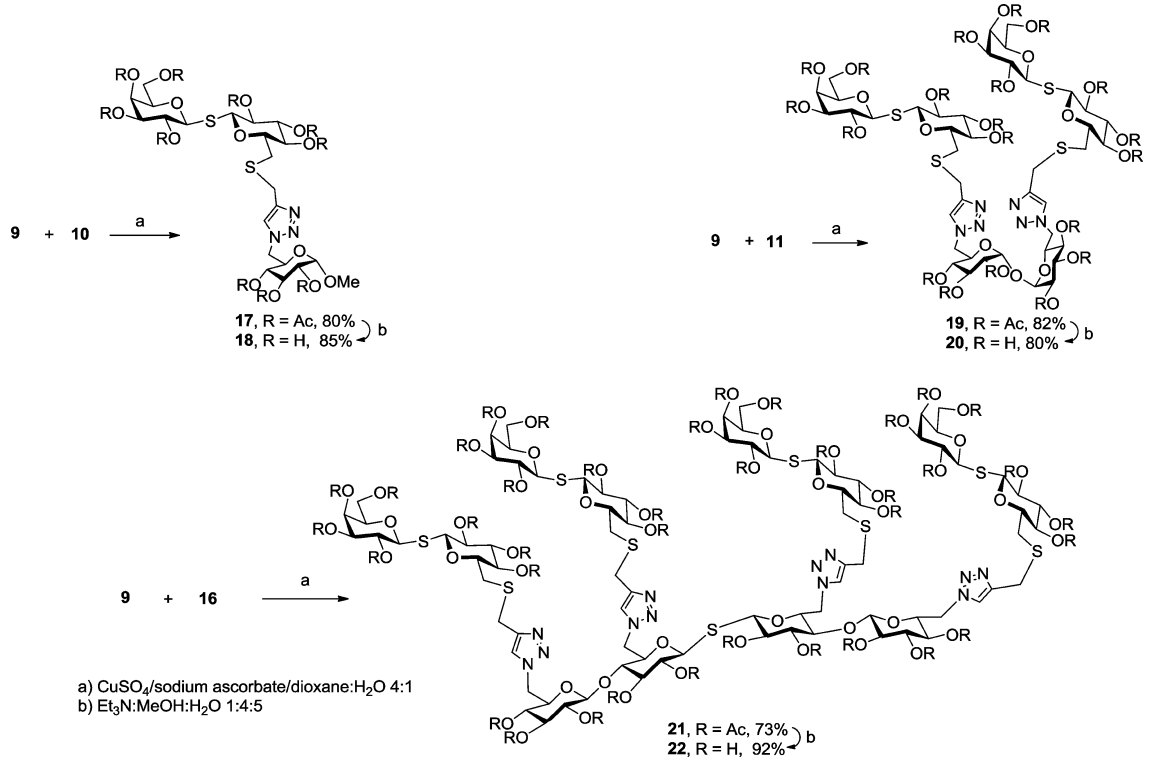
Scheme 3. Azide-Scaffolds 10, 11, and 16



Scheme 4. Synthesis of the Multivalent Ligands through Click Reaction



Thiodisaccharide **8** was then treated with 0.5 M NaOMe/MeOH, and the thiolate obtained reacted chemoselectively with propargyl bromide. Thus, after a reacetylation step, the desired precursor **9** was obtained (88%).

Azide-containing scaffolds **10** and **11** were prepared as previously reported,[27,28] starting from the corresponding free sugars, α-methyl D-glucoside and trehalose, respectively (Scheme 3).

Taking into account that humans lack β-(1 → 4) glucosidase activity, disaccharide cellobiose was chosen as a suitable starting material for a new sugar-derived scaffold, expected to be hydrolytically stable in human biological media. Indeed, we envisaged a suitable scaffold for a tetravalent ligand by coupling two cellobiose units through a thioglycosidic bond. In this way, the resistance to glycosidases would be reinforced.

Thus, cellobiose octaacetate was treated with HBr/AcOH to afford the corresponding glycosyl bromide **12**,[29] which was converted into the isothiouronium salt **13**, by treatment with thiourea in acetone (80% yield).[30] Reaction of **12** and **13** in the presence of Et_3N under the same conditions described above,[25,26] afforded derivative **14**, a symmetric dimer of cellobiose linked through a β-(1 → 1)-thioglycosidic bond. The reaction required the presence of dithiothreitol (DTT) to avoid the formation of disulfides. The ^{1}H NMR spectrum of **14** showed anomeric signals at 4.69 ppm ($J_{1,2}$ = 10.1 Hz) and at 4.47 ppm ($J_{1',2'}$ = 7.9 Hz) which were assigned to the H-1′ (β-O-linked glucose) and the H-1 (β-S-linked glucose), respectively.

Compound **14** was deacetylated with a solution of Et_3N:MeOH:H_2O 1:4:5 to give derivative **15** in 98% yield.

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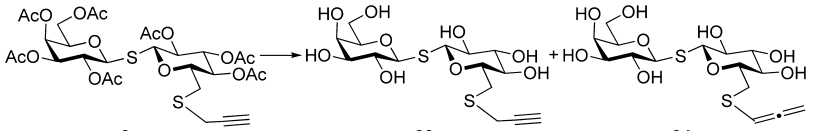
Scheme 5. Deacetylation of 9



Conditions	23	24
Et_3N, MeOH, H_2O, 18 h	20%	80%
NaOMe 1 M, MeOH, 18 h	25%	75%
NaOMe 0.5 M, MeOH, 2 h	100%	0%

Table 1. Thermodynamic Binding Parameters of the Synthetic Ligands Towards Peanut Agglutinin Related to TDG (1), Used As Reference[a]

cmpd	val	n	$K_a \times 10^{-3}(M^{-1})$	ΔH(kcal mol^{-1})	$T\Delta S$(kcal mol^{-1})	ΔG(kcal mol^{-1})	rel Pot	rel pot per RE
TDG (1)	1	1	1.57	−7.54	−3.91	−3.63	1	1
23	1	0.97	1.33	−6.67	−3.12	−3.55	0.85	0.85
18	1	1.20	2.22	−9.06	−5.44	−3.62	1.41	1.41
20	2	0.60	6.44	−16.60	−12.37	−4.23	4.10	2.05
22	4	0.24	13.15	−29.65	−24.99	−4.66	8.37	2.09

[a]Val refers to the structural valency of the ligand; n is the stoichiometry of the binding; rel pot is the relative potency of the ligand referred to reference **1**, and rel pot per RE is the corrected potency on a recognition element (RE) molar basis.

Finally, direct azidation of **15**, followed by acetylation,[27] afforded the tetra-azide scaffold **16** in 72% yield. Alternative strategies to obtain **16** from diazido cellobiose precursors were unsuccessful because direct azidation of cellobiose was not selective in favor of the desired 6,6′-diazido derivative, and thus, an unresolved mixture of products was obtained. One major advantage of the use of scaffold **16**, in terms of the characterization of the derived tetravalent ligand, was the simplicity of the NMR spectra of as a result of the symmetry (see Supporting Information [SI]).

Coupling of **9** to the azide-containing scaffolds, **10**, **11**, and **16**, was conducted by click reaction in the presence of copper sulfate and sodium ascorbate in dioxane/H_2O, under microwave irradiation (Scheme 4).[28,31,32] In all cases, the reaction mixtures were followed by TLC and ESI-MS to evaluate the consumption of the starting materials. The latter technique was particularly useful to analyze reaction mixtures that involved polar partners of high molecular weight, difficult to detect by TLC.

The cycloaddition of **9** and **10** afforded monovalent product **17** (80% yield). Then, di- and tetravalent compounds **19** (82%) and **21** (73%) were obtained by click reaction of **9** and scaffolds **11** and **16**, respectively. All the obtained glycoclusters were *O*-deacetylated with Et_3N in MeOH/H_2O to afford the free products **18**, **20**, and **22** in very good yields (Scheme 4).

Additionally to monovalent ligand **18**, containing a triazole ring and a monosaccharide-derived scaffold, a monovalent reference was necessary to evaluate the effect of these two structural moieties in the biological activity. Thus, compound **9** was also deacetylated under the standard conditions previously used (Et_3N/MeOH/H_2O 1:4:5 overnight at room temperature) to obtain reference thiodisaccharide **23**. Nevertheless, the rearranged allene **24** was obtained as the major product, whose structure was confirmed by the diagnostic signal at 210 ppm in the ^{13}C NMR spectrum, corresponding to the *sp* allene carbon atom (See SI). Propargyl-allene rearrangements under basic conditions have been previously described in literature.[33] Deacetylation of **9** to obtain **23** as a single product was achieved by using mild basic conditions and short reaction times (Scheme 5).

Evaluation of the Hydrolytic Stability. The resistance of the new products toward *Escherichia coli* β-galactosidase was determined to evaluate their stability in biological systems. This enzyme has been extensively studied and was used as a model in the study of a variety of β-thiogalactosides.[28,34−38] Glycoclusters **18**, **20**, **22**, and **23** were incubated with the enzyme at 37 °C (pH = 7.3), and the reaction mixture was analyzed by NMR and TLC. No hydrolysis products were observed, and all the starting glycoclusters were recovered intact even after 24 h incubation. 2-Nitro-β-D-galactopyranose was used as the reference for the control experiment. Release of 2-nitrophenol was spectrophotometrically quantified.

Isothermal Titration Calorimetry. Peanut agglutinin (PNA) is a 110-kDa, homotetrameric, nonglycosylated legume lectin, specific to β-galactose at the monosaccharide level, although it displays a higher affinity toward lactose derivatives. PNA shows high specificity for the T-antigenic disaccharide, Galβ(1 → 3)GalNAc (Thomsen−Friedenreich antigen), which is found as *O*-linked glycans on poorly differentiated and tumor cells, but not on normal cells.[39] The single chain in each subunit is 236 amino acid residues long and is homologous to the subunits in other legume lectins.[40,41] The cluster effect has been previously observed by interaction of this lectin with a variety of multivalent *O*-glycosides.[11,12,42]

It is well-known that isothermal titration calorimetry (ITC) analysis is a useful tool for the determination of the

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Table 2. Thermodynamic Binding Parameters of the Synthetic Ligands Towards Human Galectin-3 Related to TDG (1), Used As Reference[a]

cmpd	val	n	$K_a \times 10^{-3}(M^{-1})$	ΔH (kcal mol^{-1})	$T\Delta S$(kcal mol^{-1})	ΔG (kcal mol^{-1})	rel pot	rel pot per RE
TDG (1)	1	0.95	21.5	−10.0	−4.1	−5.9	1	1
23	1	0.92	17.5	−10.1	−4.4	−5.7	0.81	0.81
18	1	1.08	68.2	−11.3	−4.7	−6.6	3.17	3.17
20	2	0.48	446.0	−25.0	−18.0	−7.0	20.7	10.4
22	4	0.24	566.1	−41.1	−31.9	−9.2	26.3	6.58

[a]Val refers to the structural valency of the ligand; n is the stoichiometry of the binding; rel pot is the relative potency of the ligand referred to the TDG reference, and rel pot per RE is the corrected potency on a recognition element (RE) molar basis.

thermodynamic parameters of binding (K_a, ΔG, ΔH, ΔS, and n). Glycoclusters **18**, **20**, **22**, and **23** were evaluated as ligands for peanut agglutinin, using TDG as reference compound (Table 1).

Recognition element **23** exhibited a binding affinity toward PNA lower than that of the TDG (**1**) reference. On the other hand, monovalent compound **18** displayed a binding affinity higher than that of **1**, which is in agreement with a favorable effect of the triazole aromatic ring in the recognition process, as we have previously observed.[28,36] Di- and tetravalent compounds **20** and **22** showed a moderate cluster effect, exhibiting a 4.10-fold higher and a 8.37-fold higher binding affinity (2.05-fold and 2.09-fold on a RE basis), respectively.

Glycoclusters **18**, **20**, **22**, as well as **23**, were also evaluated as ligands for human galectin-3 (Table 2), in order to compare the binding affinities to both β-galactoside-binding lectins. Human galectin-3 (hGal-3) is a member of the galectin family of lectins defined by a conserved ∼14 kDa carbohydrate recognition domain (CRD) showing affinity for β-galactosides. This chimera-type galectin is monomeric in solution, but it can precipitate as a pentamer.[43]

The thiodigalactoside reference and the free glycoclusters showed in general a higher binding affinity with hGal-3 than with PNA. Compound **23** exhibited a somewhat lower binding affinity than the reference **1**, as observed with PNA. This is consistent with a statistic effect due to the symmetry of **1**. Monovalent derivative **18** showed an important improvement in the binding affinity compared to those of **1** and to **23**, ascribed to a favorable effect of the triazole ring in the binding process. Divalent glycoconjugate **20** showed a clear cluster effect since it binds to galectin-3 with a relative potency per RE of 10.4, compared to that of the reference compound **1**. In fact, **20** displayed the highest relative potency per recognition element. Finally, tetravalent glycocluster **22** exhibited the highest binding affinity (56.61×10^4 M^{-1}) and a relative potency per RE of 6.58, compared to those of the reference **1**.

The binding enthalpies of the glycoconjugates to the lectin increase almost linearly with the number of sugar residues attached to the scaffold. As shown in Table 2, the entropic terms increase concomitantly. The relatively narrow range of binding free energies provided binding curves well fit by a single-site model, and indicating a compensation of enthalpy and entropy factors for these glycoclusters.

Docking Studies. To further study the structural aspects of the ligand−lectin interactions we proceeded to investigate the binding process by molecular docking. Although PNA and galectin-3 have the same binding specificity for lactose, the amino acid residues in the carbohydrate recognition domain (CRD) of both lectins are rather different, as depicted in Figure 2 for the lactose−lectin complexes.

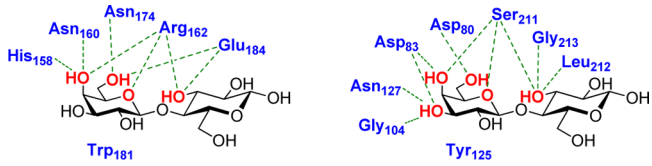

Figure 2. Representation of human galectin-3−lactose (left)[44] and PNA−lactose (right)[41] complexes. Hydrogen-bond interactions are depicted with dotted lines.

The sugar binding region in PNA is made up of four loops which are 75−83 (loop A), 91−106 (loop B), 125−135 (loop C), and 211−216 (loop D).[39,41,45] Concerning the galactose residue, Gal O3 interacts with Asp[83], Gly[104], and Asn[127], Gal O4 with Asp[83], and Gal O6 with Asp[80]. These five protein−sugar hydrogen bonds involve the first three loops. In addition, Gal O4 and Gal O5 interact with Ser[211], which involves the D-loop, often described as the specificity loop. Additionally, the side chain of an aromatic residue, Tyr[125], stacks against the galactose ring at the primary binding site (CH/π bonds).[46] Thus, the interactions at the primary site with Gal involve seven hydrogen bonds and the stacking interaction referred to earlier. In contrast, direct interactions of the glucose residue with the protein are fewer and weaker. Glc O3 interacts with Ser[211] and the backbone of Gly[213] and Leu[212] (loop D).

Regarding hGal-3 the carbohydrate binding site is formed by β-strands S4−S6a/S6b.[22] The amino acids making direct interaction with the bound carbohydrate are highly conserved among all galectins sequenced to date. The galactose moiety of lactose is most deeply buried in the binding site, and Gal O4 plays a central role in the binding, forming strong hydrogen-bond interactions with His[158], Arg[162], and Asn[160]. Gal O6 also interacts with Glu[184] and Asn[174]. Also, the lower α face of the galactose moiety interacts through CH/π bonds with the aromatic Trp[181], in a fashion similar to that described above for PNA. The glucose moiety of lactose is more exposed to the solvent, and the only hydrogen bonds occur between Glc O3 and Glu[184] and Arg[162]. These interactions, involving the glucose residue with an acidic (Glu[184]) and a basic (Arg[162]) amino acid residues, are expected to be stronger than those discussed above for PNA, established between Glc O3 and the nonpolar amino acid residues Gly[213] and Leu[212].

The major conformer of the recognition element **23** and model ligand **25** (Figure 3) were docked into the binding sites of peanut agglutinin and human galectin-3 using the program AUTODOCK 4.2.[47] Compound **25** is a model of the synthesized glycoclusters where the scaffold has been replaced by a methyl group, in order to analyze the effect of the triazole ring in the recognition process.

Regarding peanut agglutinin, to our knowledge the recognition of TDG (**1**) by PNA has not been previously

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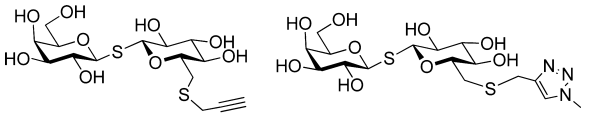

23 25

Figure 3. Representation of the structures used in the docking studies: compound **23** and model structure **25**.

described in literature. When the structure of TDG was docked into PNA, it adopted a similar conformation than lactose (See Figure S3 in the SI). Indeed, almost the same H-bond and hydrophobic interactions were observed with TDG (**1**) than with lactose (shown in Figure 2), where Gly^{213}, Asp^{80}, Tyr^{125}, Asn^{127}, Asp^{83}, Gly^{104}, and Ser^{211} were the amino acid residues involved. However, the distal galactose residue from TDG is twisted when compared to the glucose residue in lactose. The position of Glc O3 in lactose is occupied by the distal Gal O2 in TDG, which interacts with Ser^{211} and Gly^{213}. Glucose H1, H3, and H5 are disposed toward nonpolar amino acid residues Leu^{212} and Ile^{101}. On the contrary, the analogous hydrogen atoms in TDG are arranged in the opposite direction. That means that the hydrophobic faces of the distal residues of both disaccharides are displayed in opposite directions.

In opposition to lactose, no interaction with Leu^{212} was observed, probably due to the longer C−S thioglycosidic bond in TDG, compared to the C−O bond in lactose. These differences would account for the lower affinity found for TDG with PNA, compared to lactose.

Compound **23** was also docked into the binding site of PNA. The lectin−ligand interactions found were similar to those described above for TDG (**1**). The central Tyr^{125} residue provided CH/π stacking interactions with the Gal residue. Asp^{80}, Asn^{127}, and Asp^{83} residues provided hydrogen-bonding interactions with the Gal moiety, while Ser^{211} lateral chain provided simultaneous contacts to both the Gal and Glc units. Gly^{213} only interacts with HO-3 of the glucose moiety. As well as with TDG, no interactions were observed with Leu^{212}. No major contacts between the alkyne moiety of **23** and PNA were observed (Figure 4a).

Model compound **25** was also docked into the binding site of PNA using AUTODOCK. The thiodisaccharide moiety adopted a similar conformation than compound **23**, exhibiting the same interactions with the key amino acid residues of the binding site of the lectin (See Figure 4b). Regarding the aromatic triazole ring, it was disposed into a hydrophobic pocket formed by the nonpolar amino acid residues Val^{40}, Ile^{101}, and Leu^{212} (see Figure 4, c and d).

For hGal-3, rigid-body docking could be performed with remarkable fit, and the formation of the lectin−ligand complexes proceeded without any notable structural deviation of the carbohydrate ligands from their low-energy conformation in the free-state. When TDG was docked into de CRD of hGal-3 we observed interactions the same as those found by X-ray crystallographic studies.[20] The thioglycosidic bond does not seem to interfere with the tight binding of TDG with the lectin.

RE **23** adopted the same conformation as TDG (**1**),[20] and the same H-bond and hydrophobic interactions were observed. As illustrated for lectin−ligand contacts formed, a model of the

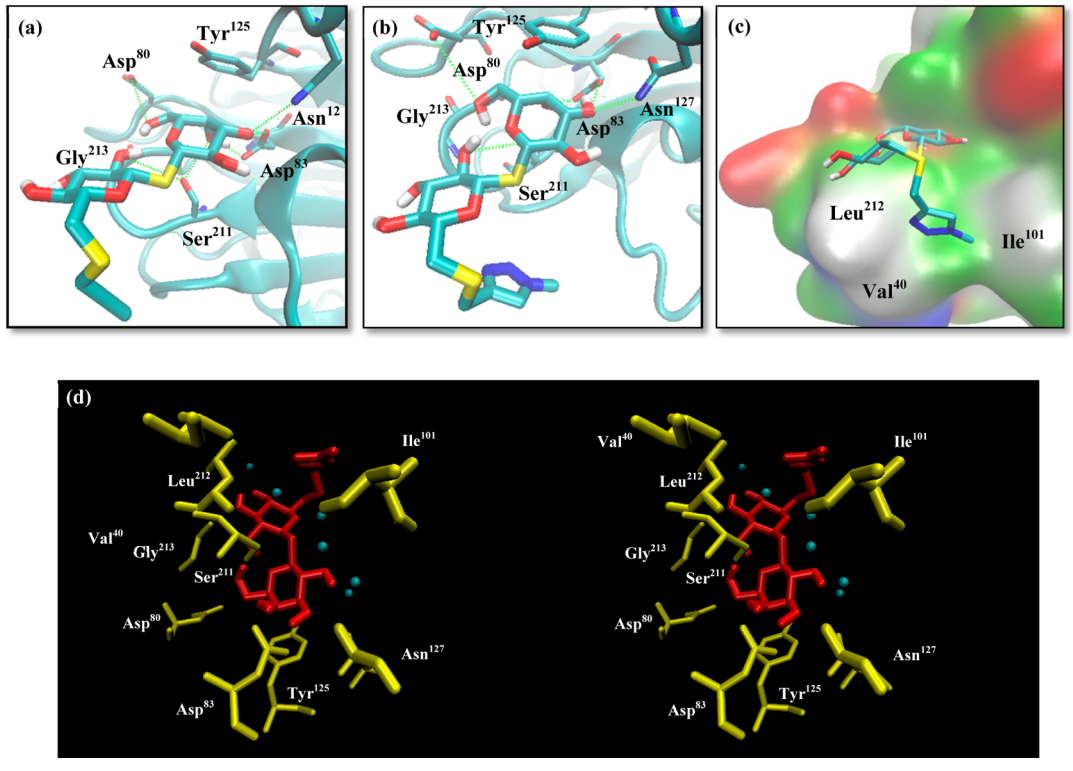

Figure 4. Model of the interaction of PNA with (a) compound **23** and (b) compound **25**. The lectin site of PNA is shown in NewCartoon representation with key side chains in licorice. Hydrogen bonds are indicated in green. (c) PNA−compound **25** complex. The accessible protein surface is shown and colored according to the residue type (blue is basic, red is acidic, green is polar, and white is nonpolar). (d) Stereo view of the protein−carbohydrate interactions in PNA−compound **25** complex. The ligand is shown in red, whereas water molecules are represented by cyan-colored spheres. This figure was prepared by using VMD.[48]

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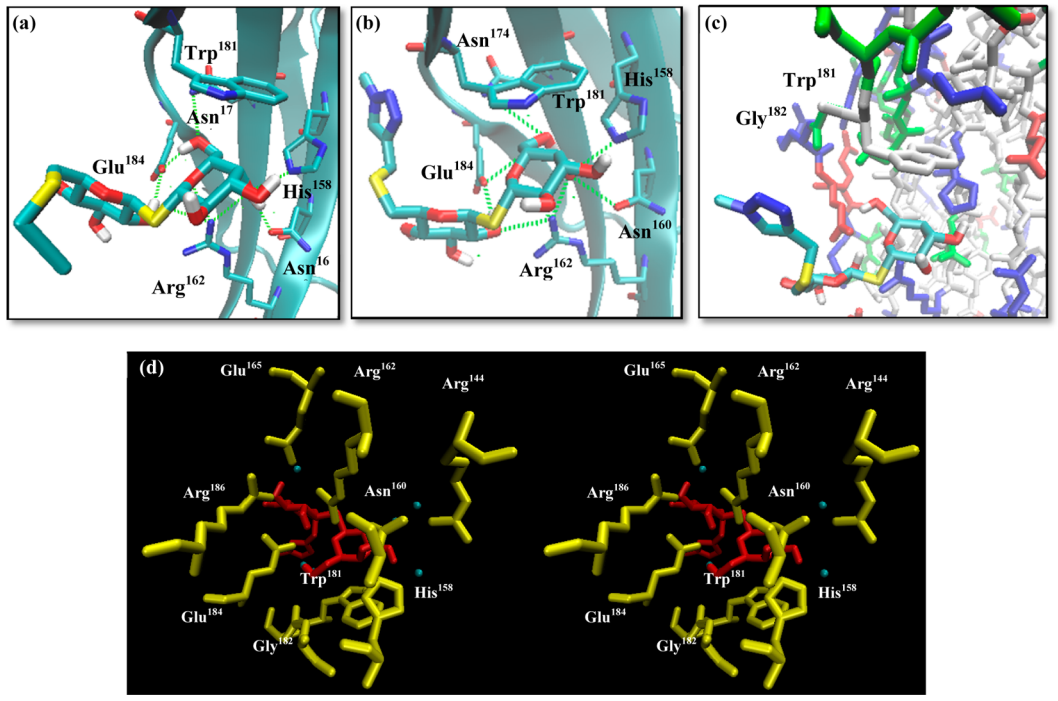

Figure 5. Model of the interaction of hGal-3 with (a) compound **23** and (b) model compound **25**. The lectin site of hGal-3 is shown in NewCartoon representation with key side chains in licorice. Hydrogen bonds are indicated in green. (c) hGal-3–compound **25** complex. The protein is shown in licorice representation and colored according to the residue type (blue is basic, red is acidic, green is polar and white is nonpolar). (d) Stereo view of the protein–carbohydrate interactions in hGal3–compound **25** complex. The ligand is shown in red, whereas water molecules are represented by cyan-colored spheres. This figure was prepared by using VMD.[48]

complex of hGal-3 with compound **23** is shown in Figure 5a. As well as with PNA, no major interactions between the alkyne moiety and the protein were observed.

Model compound **25** also adopted a similar conformation in the binding site of the lectin. As seen in Figure 5b, the galactose moiety of **25** exhibited H-bond interactions with His^{158}, Asn^{160}, and Asn^{174} as well as CH/π interactions with Trp^{181}. Arg^{162} and Glu^{184} lateral chains provided simultaneous contacts to both the Gal and Glc units. The triazole ring turns toward the loop between S5 and S6 strands, and hydrophobic interactions with Gly^{182} and Trp^{181} were observed (see Figure 5c and d).

The change in binding affinity from **23** to **18** is higher for hGal-3 than for PNA, suggesting that the π-stacking interaction triazol-Trp observed on the docking into the binding site of the galectin would be stronger than the hydrophobic interaction triazole-Val, -Ile, -Leu, found for the PNA.

CONCLUSION

In conclusion, we have developed an efficient strategy for the synthesis of multivalent ligands with a TDG analogue as recognition element. Precursor **9** was designed to be resistant to hydrolysis and, at the same time, to be recognized by β-galactoside-binding lectins. It was successfully prepared in five steps with a 26% overall yield, starting from D-glucose and the isothiouronium salt of galactose. This fragment was coupled to azide-containing oligosaccharide scaffolds to afford glycoclusters **18**, **20**, and **22**. These ligands proved to be resistant to the enzymatic activity of *E. coli* β-galactosidase, and so, a better bioavailability and a higher lifetime in biological fluids may be expected, compared to that of *O*-glycosides.

The affinity of the multivalent ligands toward two β-galactoside binding lectins, peanut agglutinin and human galectin-3, was evaluated by isothermal titration calorimetry. The glycoclusters were in all cases recognized by the lectins, demonstrating that the replacement of the oxygen of the glycosidic bond by a sulfur atom, and the distal galactose residue by a glucose one in the RE, does not affect the recognition process. For PNA, the multivalent ligands exhibited a moderate cluster effect, where the tetravalent compound showed the highest relative potency per RE. These glycoclusters exhibited a higher binding affinity with hGal-3, as expected, and a higher multivalent effect was observed. In this case, the divalent ligand showed the highest relative potency per RE. For both lectins, a positive effect of the triazole ring in the biological activity can be deduced when comparing the K_a values of the monovalent ligand (**18**) with respect to those of the simple disaccharide-like structure **23** and TDG (**1**).

Molecular docking studies were therefore performed with RE **23** and the model ligand **25** and both lectins in order to study and compare the protein–ligand complexes and the interactions implicated in the recognition process. A detailed study on the role of the different amino acid residues present on both recognition sites provided a rational explanation for the different affinities observed by ITC. For example, the interactions between the CRD of hGal-3 and the distal sugar residue (mainly Glc O2) of compound **23**, involve polar amino acid such as Glu^{184} and Arg^{162}. On the contrary, weak interactions were observed in compound **23**–PNA complex between Glc O2 and nonpolar amino acids Gly^{213} and Leu^{212}. Additionally, for compound **23**, no interactions were observed between the alkyne group and the lectins. However, for model compound **25**, bearing a triazole ring coupled to the RE, additional interactions between this moiety and certain amino acid residues of both lectins were observed. These interactions seem to be stronger for hGal-3 than for PNA. Docking calculations provided for the first time a strong indication that

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these interactions could account at least partially for the higher binding affinity of triazole-containing glycoclusters.

EXPERIMENTAL SECTION

General Methods. Analytical thin layer chromatography (TLC) was performed on Silica Gel 60 F254 aluminum supported plates (layer thickness 0.2 mm) with solvent systems given in the text. Visualization of the spots was effected by exposure to UV light and charring with a solution of 5% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography was carried out with Silica Gel 60 (230−400 mesh). Optical rotations were measured at 20 °C in a 1 cm cell in the stated solvent; $[\alpha]_D$ values are given in 10^{-1} deg·cm^2·g^{-1} (concentration c given as g/100 mL). Microwave irradiation was carried out at 70 °C in a CEM Discover MW instrument with a System Internal IR probe type (power max 300 W). High-resolution mass spectra HRMS where obtained by electrospray ionization (ESI) and Q-TOF detection. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 25 °C at 500 and 125 MHz, respectively, or at 600 and 150 MHz, respectively. For ^{1}H, ^{13}C NMR spectra, chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak ($CHCl_3$: ^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.2 ppm). Assignments of ^{1}H and ^{13}C were assisted by two-dimensional (2D) ^{1}H COSY and 2D ^{1}H−^{13}C CORR experiments. Peak multiplicity is reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). In the description of the spectra, the signals corresponding to the glucose, trehalose, or cellobiose moieties were labeled as "G", "T". or "C", respectively.

Synthetic Methods. *Compound 4.* Compound **4** was prepared following the methodology reported by Beaupère et al.[1] A solution of D-glucose (**3**, 1 g, 5.55 mM) in DMF (50 mL) was treated with triphenylphosphine (2.91 g, 11.1 mmol) and CBr_4 (3.68 g, 11.1 mmol). The reaction mixture was stirred for 90 min under argon atmosphere at 50 °C. This mixture was then concentrated under reduced pressure, diluted with water, and washed with CH_2Cl_2. The aqueous phases were concentrated, and the residue reacetylated with Ac_2O (25 mL) and pyridine (25 mL). The isolated product (1.35g, 59%) showed physical properties identical to those reported in literature.[2]

Compound 5. Compound **4** (500 mg, 1.217 mmol) was disolved in anhydrous DMF (25 mL). KSAc (152 mg, 1.345 mmol) was added, and the mixture was stirred for 2 h at 50 °C. The solution was concentrated, and the residue was purified by flash chromatography (hexane/ethyl acetate 7:3) to afford compound **5** (455 mg, 92%), which showed spectroscopic properties the same as those reported in literature.[3] ^{1}H NMR (200 MHz, $CDCl_3$) of **5*β*** δ = 5.65 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 5.40 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 5.25−4.90 (m, 2 H, H-2, H-4), 3.77 (m, 1 H, H-5), 3.15 (m, 2 H, H-6a, H-6b), 2.32 (s, 3 H, C*H*$_3$C(O)S-), 2.10, 2.03, 2.00, 1.98 (4 s, 12 H, C*H*$_3$C(O)O-); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 194.5 (-S*C*(O)CH$_3$), 170.3, 170.2, 170.1, 170.0 (-OC(O)CH$_3$), 88.9 (C-1), 73.7 (C-5), 73.8 (C-3), 70.7 (C-4), 70.4 (C-2), 30.4, 29.7 (C-6, *C*H$_3$C(O)S-), 20.8, 20.7 (2 ×), 20.6(2 ×), 20.5 (2 ×) (*C*H$_3$C(O)O-).

Compound 6. Compound **6** was prepared following the methodology reported by Driguez et al.[4] To a solution of **5** (208 mg, 0.512 mmol) in anhydrous CH_2Cl_2 (2.55 mL) was added a solution of HBr in AcOH dropwise (30−32%, 1 mL). The reaction mixture was stirred in the dark at 0 °C until complete conversion of the starting materials, analyzed by TLC (hexane/EtOAc 1:1). The mixture was diluted with CH_2Cl_2 and washed with water and ice (3 × 50 mL), saturated solution of $NaHCO_3$ (50 mL) and water again (50 mL). The organic layers were collected together and concentrated under reduced pressure. Compound **6** (184 mg, 84%) was isolated as a white-yellow syrup, which was pure enough to be used in the fruther reactions. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 6.50 (d, 1 H, $J_{1,2}$ = 4.0 Hz, H-1), 5.43 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 10.5 Hz, H-3), 4.99 (dd, 1 H, $J_{3,4}$ = 10.2, $J_{4,5}$ = 9.4 Hz, H-4), 4.73 (dd, 1 H, $J_{1,2}$ = 4.0, $J_{2,3}$ = 10.0 Hz, H-2), 4.23 (m, 1 H, H-5), 3.16 (m, 2 H, H-6a, H-6b), 2.28 (s, 3 H, C*H*$_3$C(O)S-), 2.03, 2.02, 1.95 (3 s, 9 H, C*H*$_3$C(O)O-); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 193.5 (-SC(O)CH$_3$), 169.2, 169.1 (-OC(O)CH$_3$), 85.9 (C-1), 72.5 (C-5), 70.0, 69.6, 68.6 (C-3, C-4, C-2), 29.8, 28.6 (C-6, *C*H$_3$C(O)S-), 20.0 (3 ×) (*C*H$_3$C(O)O-).

Compound 8. The conditions for the thioglycosylation reaction reported by Tiwari et al.[25] and El Ashry et al. were followed.[26] Isothiuronium salt of galactose (**7**, 490 mg, 1.006 mmol) and 6-thioglucosyl bromide derivative **6** (431 mg, 1.009 mmol) were dissolved in dry acetonitrile (1 mL). Et_3N (0.492 mL, 3.525 mmol) was added, and the reaction mixture was stirred for 3 h at room temperature. The mixture was then concentrated under reduced pressure, and the residue was purified by flash chromatography (hexane/EtoAc 3:2) to give thiodisaccharide **8** (458 mg, 64%). $[\alpha]^{20}_D$ −11.8 (c = 0.3, $CHCl_3$); R_f = 0.41 (Hexane/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.39 (dd, 1 H, $J_{4',5'}$ < 1, $J_{3',4'}$ = 3.2 Hz, H-4′), 5.16 (t, 1 H, $J_{1',2'}$ = $J_{2',3'}$ = 10.0 Hz, H-2′), 5.13 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3), 5.02 (dd, 1 H, $J_{3',4'}$ = 3.3, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.94 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 9.2 Hz, H-2), 4.92 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 4.81 (d, 1 H, $J_{1',2'}$ = 10.2 Hz, H-1′), 4.74 (d, 1 H, $J_{1,2}$ = 9.2 Hz, H-1), 4.10 (m, 2 H, H-6′a, H-6′b), 3.90 (t, 1 H, $J_{4',5'}$ < 1, $J_{5',6'a}$ = $J_{5',6'b}$ = 6.5 Hz, H-5′), 3.47 (m, 1 H, $J_{5,6a}$ = 2.5, $J_{5,6b}$ = 7.8, $J_{4,5}$ = 9.5 Hz, H-5), 3.25 (dd, 1 H, $J_{5,6a}$ = 2.5, $J_{6a,6b}$ = 14.3 Hz, H-6a), 2.92 (dd, 1 H, $J_{5,6b}$ = 7.8, $J_{6a,6b}$ = 14.3 Hz, H-6b), 2.32 (s, 3 H, C*H*$_3$C(O)S-), 2.10, 2.03, 2.00, 1.98, 1.97, 1.93 (2 ×) (7 s, 21 H, C*H*$_3$C(O)O-); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 194.4 (-S*C*(O)CH$_3$), 170.3, 170.2, 170.1, 170.0, 169.8, 169.5, 169.3 (-OC(O)CH$_3$), 80.6 (C-1′), 79.8 (C-1), 77.6 (C-5), 74.7 (C-5′), 73.8 (C-3), 71.9 (C-3′), 70.8 (C-4), 70.4 (C-2), 67.2 (2 ×) (C-2′, C-4′), 61.5 (C-6′), 30.5, 30.4 (C-6, *C*H$_3$C(O)S-), 20.8, 20.7(2 ×), 20.6(2 ×), 20.5 (2 ×) (*C*H$_3$C(O)O-). Anal. Calcd for $C_{28}H_{38}O_{17}S_2$: C, 47.32; H, 5.39; S, 9.02. Found: C, 47.19; H, 5.58; S, 9.14. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{28}H_{38}NaO_{17}S_2$: 733.1448, found: 733.1418.

Compound 9. Thiodisaccharide **8** (450 mg, 0.634) was dissolved in 0.5 M NaOMe in MeOH (5 mL). The mixture was stirred for 30 min at −18 °C under argon atmosphere. Propargyl bomide (106 μL, 80% sol in toluene, 0.951 mmol) was added dropwise, and the reaction mixture was stirred for 4 h. The solution was neutralized with acetic acid and concentrated, and the residue was dissolved in pyridine (5 mL) and acetic anhydride (5 mL) and stirred at room temperature for 18 h. The residue obtained upon concentration was purified by flash chromatography to afford compound **9** (393 mg, 88%). $[\alpha]^{20}_D$ +13.0 (c = 0.3, $CHCl_3$); R_f = 0.40 (Hexane/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.38 (dd, 1 H, $J_{4',5'}$ < 1, $J_{3',4'}$ = 3.0 Hz, H-4′), 5.16 (t, 1 H, $J_{1',2'}$ = $J_{2',3'}$ = 10.1 Hz, H-2′), 5.13 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), 5.02 (dd, 1 H, $J_{3',4'}$ = 3.4, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.97 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 9.4 Hz, H-2), 4.95 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 4.89 (d, 1 H, $J_{1',2'}$ = 10.2 Hz, H-1′), 4.79 (d, 1 H, $J_{1,2}$ = 10.2 Hz, H-1), 4.12 (m, 2 H, H-6′a, H-6′b), 3.92 (t, 1 H, $J_{4',5'}$ < 1, $J_{5',6'a}$ = $J_{5',6'b}$ = 6.5 Hz, H-5′), 3.62 (m, 1 H, $J_{5,6a}$ = 2.5, $J_{5,6b}$ = 7.8, $J_{4,5}$ = 9.5 Hz, H-5), 3.30 (m, 2 H, J = 2.5 Hz, C*H*$_2$S), 2.85 (dd, 1 H, $J_{5,6a}$ = 2.6, $J_{6a,6b}$ = 14.4 Hz, H-6a), 2.72 (dd, 1 H, $J_{5,6b}$ = 8.5, $J_{6a,6b}$ = 14.4 Hz, H-6b), 2.22 (t, J = 2.5 Hz, C≡CH), 2.09, 2.01, 1.99, 1.98, 1.97, 1.94, 1.91 (7 s, 21H, C*H*$_3$CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.3, 170.2, 170.1, 170.0, 169.8, 169.5, 169.3 (-OC(O)CH$_3$), 80.8 (C-1′), 79.9 (C-1), 79.7 (*C*≡CH), 78.5 (C-5), 74.6 (C-5′), 73.8 (C-3), 71.9 (C-3′), 71.3 (C-4), 70.4 (C-2), 67.2, 67.1 (C-2′, C-4′), 61.3 (C-6′), 32.7 (C-6), 20.9, 20.8, 20.7(2 ×), 20.6(2 ×), 20.5 (2 ×) (*C*H$_2$S, *C*H$_3$C(O)O-). Anal. Calcd for $C_{29}H_{38}O_{16}S_2$: C, 49.28; H, 5.42; S, 9.07. Found: C, 48.99; H, 5.48; S, 9.14. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{29}H_{38}NaO_{16}S_2$: 729.1499, found: 729.1530.

Synthesis of the Cellobiose-Derived Scaffold. *Compound 12.* Compound **12** was prepared following the synthetic methodology reported by Watson et al.[5] To a solution of cellobiose peracetate (2.5 g, 3.69 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise a solution of HBr in AcOH (30−32%, 3.83 mL). The solution was stirred in the dark at 0 °C until complete conversion of the starting materials, observed by TLC (hexane/EtOAc 1:4). The reaction mixture was diluted in CH_2Cl_2 and washed with a H_2O−ice solution (3 × 50 mL), saturated solution of $NaHCO_3$ (50 mL), and H_2O again (50 mL). The organic layers were put together and concentrated under reduced pressure. Compound **12** (2.217 g, 86%) was isolated as a white-yellow syrup, which was pure enough to be used in further reactions.

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Compound ***13***. The isothiouronium salt of cellobiose (**13**) was prepared following the reported methodology.[6] To a solution of **12** (1.13 g, 1.617 mmol) in acetone (1.89 mL) was added thiourea (129 mg, 1.617 mmol), and the mixture was heated under reflux for 30 min. The isothiouronium salt **13** precipitated from the reaction mixture and was separated by filtration. The crude product was pure enough to be used in further reactions (1.00 g, 80%) and showed spectroscopic and physical properties identical to those reported in literature.[6]

Compound ***14***. Isothiouronium salt of cellobiose (**13**, 500 mg, 0.645 mmol), glycosyl bromide derivative **12** (451 mg, 0.645 mmol), and dithiothreitol (100 mg, 0.645 mmol) were dissolved in dry acetonitrile (2.5 mL). Et_3N (0.315 mL, 2.258 mmol) was added, and the reaction mixture was stirred for 3 h at room temperature. The mixture was then concentrated under reduced pressure, and the residue was purified by flash chromatography (hexane/EtOAc 3:2) to give compound **14** (606 mg, 74%). $[\alpha]^{20}_D$ −35.0 (c = 0.3, $CHCl_3$); R_f = 0.53 (Hexane/EtOAc 1:3); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.11 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.1 Hz, H-3), 5.09 (t, 1 H, $J_{2',3'} = J_{3',4'}$ = 9.1 Hz, H-3′), 5.00 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, H-4′), 4.85 (t, 2 H, $J_{1,2} = J_{2,3} = J_{1',2'} = J_{2',3'}$ = 9.2 Hz, H-2, H-2′), 4.69 (d, 1 H, $J_{1,2}$ = 10.1 Hz, H-1), 4.47 (d, 1 H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.43 (dd, 1 H, $J_{5,6a}$ = 1.8, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.30 (dd, 1 H, $J_{5',6'a}$ = 4.4, $J_{6'a,6'b}$ = 12.5 Hz, H-6′a), 4.05 (dd, 1 H, $J_{5,6b}$ = 4.1, $J_{6a,6b}$ = 12.6 Hz, H-6b), 3.98 (dd, 1 H, $J_{5',6'b}$ = 2.1, $J_{6'a,6'b}$ = 12.5 Hz, H-6′b), 3.71 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.60 (ddd, 1 H, $J_{5',6'b}$ = 2.2, $J_{5',6'a}$ = 4.1, $J_{4',5'}$ = 9.7 Hz, H-5′), 3.50 (ddd, 1 H, $J_{5,6a}$ = 1.6, $J_{5,6b}$ = 5.2, $J_{4,5}$ = 9.9 Hz, H-5), 2.07, 2.01, 1.96, 1.95, 1.94 (2 ×), 1.91 (7 s, 21H, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.4, 170.2, 169.7, 169.5, 169.3, 169.0 (-$COCH_3$), 100.8 (C-1′), 80.5 (C-1), 77.0 (C-5), 76.1 (C-4), 73.5, 72.9 (C-3, C-3′), 72.0 (C-5′), 71.6, 70.4 (C-2, C-2′), 67.7 (C-4′), 62.0 (C-6), 61.5 (C-6′), 20.9, 20.7, 20.6(2 ×), 20.5 (2 ×), 20.4 (CH_3CO-). Anal. Calcd for $C_{52}H_{70}O_{34}S$: C, 49.13; H, 5.55, S, 2.52. Found: C, 48.91; H, 5.21; S, 2.68. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{52}H_{70}NaO_{34}S$: 1293.3367, found: 1293.3328.

Compound ***15***. Compound **24** (600 mg, 0.472 mmol) was treated with a 1 M NaOMe solution in MeOH (0.512 mL) and stirred at room temperature. The solid was progressively dissolving and after 4−6 h TLC (EtOAc or EtOAc:MeOH, 9:1) showed complete consumption of the starting material. The reaction mixture was neutralized with Dowex 50W (H^+) ion-exchange resin, filtrated, and concentrated under reduced pressure. The residue was further purified by filtration through an Octadecyl C18 minicolumn. Evaporation of the solvent afforded the free product **15** (316 mg, 98%). $[\alpha]^{20}_D$ −44.4 (c = 0.2, H_2O); R_f = 0.13 (BuOH/EtOH/H_2O, 2.5:1:1); ^{1}H NMR (500 MHz, D_2O) δ = 4.77 (d, 1 H, $J_{1,2}$ = 10.0 Hz, H-1), 4.44 (d, 1 H, $J_{1',2'}$ = 7.9 Hz, H-1′), 3.90 (dd, 1 H, $J_{5,6a}$ = 1.8, $J_{6a,6b}$ = 12.4 Hz, H-6a), 43.84 (dd, 1 H, $J_{5',6'a}$ = 2.0, $J_{6'a,6'b}$ = 12.4 Hz, H-6′a), 3.72 (dd, 1 H, $J_{5,6b}$ = 5.2, $J_{6a,6b}$ = 12.4 Hz, H-6b), 3.66 (dd, 1 H, $J_{5',6'b}$ = 5.8, $J_{6'a,6'b}$ = 12.4 Hz, H-6′b), 3.761 (t, 1 H, $J_{3,4} = J_{4,5}$ = 8.9 Hz, H-4), 3.59 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.9 Hz, H-3), 3.53 (ddd, 1 H, $J_{5,6a}$ = 2.1, $J_{5,6b}$ = 5.2, $J_{4,5}$ = 8.9 Hz, H-5), 3.43 (t, 1 H, $J_{2',3'} = J_{3',4'}$ = 9.2 Hz, H-3′), 3.41 (ddd, 1 H, $J_{5',6'a}$ = 2.2, $J_{5',6'b}$ = 5.9, $J_{4',5'}$ = 9.5 Hz, H-5′), 3.37 (dd, 1 H, $J_{2,3}$ = 8.7, $J_{1,2}$ = 9.9 Hz, H-2), 3.34 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.5 Hz, H-4′), 3.24 (dd, 1 H, $J_{1',2'}$ = 8.1, $J_{2',3'}$ = 9.2 Hz, H-2′); ^{13}C NMR (125 MHz, D_2O) δ = 102.5 (C-1′), 82.7 (C-1), 78.7 (C-5), 78.3 (C-3), 76.0, 75.6 (C-5′, C-3′), 75.5 (C-4), 73.1 (C-2′), 72.0 (C-2), 69.4 (C-4′), 60.6 (C-6′), 60.1 (C-6). Anal. Calcd for $C_{24}H_{42}O_{20}S$: C, 42.23; H, 6.20; S, 4.70. Found: C, 42.11; H, 6.48; S, 4.87. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{24}H_{42}NaO_{20}S$: 705.1888, found: 705.1906.

General Procedures for the Synthesis of Azide-Containing Scaffolds. Compounds **10**, **11**, and **16** were synthesized from the corresponding free sugar (methyl α-D-glucopyranoside, trehalose, and **15**, respectively), sodium azide, PPh_3, and CBr_4 in anhydrous DMF as previously described.[7,8] Compounds **10** and **11** products showed properties identical to those previously reported.[7,8] Synthesis of cellobiose-derived scaffold **16** from **15** was achieved in 60% yield, following the same methodology.

Compound ***16***. Starting from compound **15** (100 mg, 0.146 mmol), NaN_3 (381 mg, 5.860 mmol), PPh_3 (316 mg, 1.172 mmol), and CBr_4 (389 mg, 1.172 mmol) in DMF (1 mL) was obtained compound **16** (105 mg) in 60% yield, after an overnight acetylation step with Ac_2O/Py (5 mL, 1:1); $[\alpha]^{20}_D$ −37.6 (c = 0.3, $CHCl_3$); R_f = 0.38 (Hexane/EtOAc 1:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.23 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3), 5.16 (t, 1 H, $J_{2',3'} = J_{3',4'}$ = 9.4 Hz, H-3′), 5.00 (t, 1 H, $J_{3',4'} = J_{4',5'}$ = 9.6 Hz, H-4′), 4.95 (t, 1 H, $J_{1,2} = J_{2,3}$ = 10.0 Hz, H-2), 4.93 (dd, 1 H, $J_{1',2'}$ = 8.0, $J_{2',3'}$ = 9.5 Hz, H-2′), 4.86 (d, 1 H, $J_{1,2}$ = 10.2 Hz, H-1), 4.60 (d, 1 H, $J_{1',2'}$ = 7.9 Hz, H-1′), 3.90 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.4 Hz, H-4), 3.64 (m, 3 H, H-5, H-5′, H-6a), 3.40 (m, 3 H, H-6b, H-6′a, H-6′b), 2.08, 2.06, 2.05, 2.03, 2.01 (5 s, 15H, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.2, 169.7, 169.5, 169.4, 168.8 (-$COCH_3$), 99.9 (C-1′), 80.6 (C-1), 78.2 (C-5), 75.6 (C-4), 73.2 (C-3), 72.8 (C-3′), 72.7 (C-5′), 71.7 (C-2′), 70.3 (C-2), 69.2 (C-4′), 51.0 (C-6′), 50.2 (C-6), 20.8, 20.7, 20.6, 20.5, 20.4 (CH_3CO-). Anal. Calcd for $C_{44}H_{58}N_{12}O_{26}S$: C, 43.93; H, 4.86; N, 13.97; S, 2.67. Found: C, 43.79; H, 4.68; N, 14,23; S, 2.89. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{44}H_{58}N_{12}NaO_{26}S$: 1225.3204, found: 1225.3219.

General Procedure for the Click Reaction. *Synthesis of Compounds* ***17***, ***19***, *and* ***21***. The corresponding azido-saccharide **10**, **11**, or **16** (0.20 mmol) and thiodisaccharide **9** (0.20 mmol per mole of reacting azide) were dissolved in a dioxane/H_2O mixture (8:2 mL, 2.5 mL). Copper sulfate (0.05 mmol per mole of reacting azide) and sodium ascorbate (0.10 mmol per mole of azide reacting group) were added, and the mixture was stirred at 70 °C under microwave irradiation during 40 min. The mixture was then poured into a 1:1 H_2O/NH_4Cl solution (20 mL) and extracted with EtOAc (4 × 15 mL). The organic layer was dried (Na_2SO_4) and filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography, using the solvent systems indicated in each case.

Compound ***17***. Yield: 168 mg, 80%; $[\alpha]^{20}_D$ +10.1 (c = 0.2, $CHCl_3$); R_f = 0.29 (Hexane/EtOAc 1:1.5); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.70 (H-triazole), 5.47 (m, 2 H, H-3G, H-4′), 5.21 (m, 2 H, H-3, H-2′), 5.13 (d, 1 H, $J_{1',2'}$ = 10.2 Hz, H-1′), 5.09 (dd, 1 H, $J_{3',4'}$ = 3.4, $J_{2',3'}$ = 9.8 Hz, H-3′), 5.01 (m, 2 H, H-2, H-4), 4.93−4.82 (m, 4 H, H-1G, H-2G, H-4G, H-1), 4.62 (dd, 1 H, $J_{5G,6aG}$ = 2.0, $J_{6aG,6bG}$ = 14.1 Hz, H-6aG), 4.40 (dd, 1 H, $J_{5G,6bG}$ = 8.2, $J_{6aG,6bG}$ = 14.1 Hz, H-6bG), 4.22−4.09 (m, 4 H, H-5′, H-6′a, H-6′b, H-5G), 3.92 (m, 2 H, CH_2-triazole), 3.68 (m, 1 H, $J_{5,6a}$ = 2.5, $J_{5,6b}$ = 7.8, $J_{4,5}$ = 9.5 Hz, H-5), 3.15 (s, 3 H, -OCH_3), 2.85 (dd, 1 H, $J_{5,6a}$ = 2.6, $J_{6a,6b}$ = 14.4 Hz, H-6a), 2.62 (dd, 1 H, $J_{5,6b}$ = 7.0, $J_{6a,6b}$ = 13.4 Hz, H-6b), 2.15, 2.11, 2.07, 2.06, 2.05, 2.04, 2.01, 2.00, 1.99, 1.95 (10 s, 30H, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.3, 170.2, 170.1, 170.0, 169.9, 169.8, 169.7, 169.5, 169.4, 169.3 (-$OCOCH_3$), 145.1 (C-4 triazole), 123.8 (C-5 triazole), 96.7 (C-1G), 80.5 (C-1′), 79.9 (C-1), 78.3 (C-5), 74.4 (C-5′), 73.8 (C-3), 71.8 (C-3′), 71.1 (C-2G), 70.6, 70.5 (C-4, C-2), 69.9 (C-4G), 69.7 (C-3G), 67.7 (C-5G), 67.7, 67.4 (C-2′, C-4′), 61.4 (C-6′), 55.5 (-OCH_3), 50.9 (C-6G), 32.5 (C-6), 26.3 (CH_2-triazole), 20.8, 20.7, 20.6(2 ×), 20.5 (2 ×), 20.4 (CH_3CO-). Anal. Calcd for $C_{42}H_{57}N_3O_{24}S_2$: C, 47.95; H, 5.46; N, 3.99; S, 6.10. Found: C, 48.19; H, 5.49; N, 4.25; S, 5.88. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{42}H_{57}N_3NaO_{24}S_2$: 1074.2666, found: 1074.2687.

Compound ***19***. Yield: 338 mg, 82%; $[\alpha]^{20}_D$ +34.3 (c = 0.4, $CHCl_3$); R_f = 0.22 (Hexane/EtOAc 1:4); ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6) δ = 7.77 (H-triazole), 5.33 (dd, 1 H, $J_{4',5'}$ < 1, $J_{3',4'}$ = 3.4 Hz, H-4′), 5.28 (t, 1 H, $J_{2T,3T} = J_{3T,4T}$ = 9.7 Hz, H-3T), 5.25 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.3 Hz, H-3), 5.12 (dd, 1 H, $J_{3',4'}$ = 3.5, $J_{2',3'}$ = 9.9 Hz, H-3′), 5.08 (d, 1 H, $J_{1',2'}$ = 10.3 Hz, H-1′), 5.02−4.90 (m, 5 H, H-1, H-4, H-2′, H-2T, H-4T), 4.86 (t, 1 H, $J_{1,2} = J_{2,3}$ = 9.7 Hz, H-2), 4.82 (d, 1 H, $J_{1T,2T}$ = 3.5 Hz, H-1T), 4.54 (dd, 1 H, $J_{5T,6aT}$ = 1.8, $J_{6aT,6bT}$ = 14.4 Hz, H-6aT), 4.43 (dd, 1 H, $J_{5T,6bT}$ = 8.0, $J_{6aT,6bT}$ = 14.4 Hz, H-6bT), 4.18 (m, 2 H, H-5′, H-5T), 4.08 (dd, 1 H, $J_{5',6'a}$ = 6.4, $J_{6'a,\ 6'b}$ = 11.2 Hz, H-6′a), 4.03 (dd, 1 H, $J_{5',6'b}$ = 6.6, $J_{6'a,\ 6'b}$ = 11.2 Hz, H-6′b), 3.82 (m, 3 H, H-5, CH_2-triazole), 2.78 (dd, 1 H, $J_{5,6a}$ = 2.3, $J_{6a,6b}$ = 14.5 Hz, H-6a), 2.52 (dd, 1 H, $J_{5,6b}$ = 7.0, $J_{6a,6b}$ = 14.5 Hz, H-6b), 2.09, 2.00, 1.98, 1.97 (2 ×), 1.96, 1.92, 1.91, 1.90, 1.88 (10 s, 30H, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ = 174.3, 174.7, 174.6, 174.4, 174.3, 174.2, 174.1, 174.0 (-$OCOCH_3$), 149.9 (C-4 triazole), 129.0 (C-5 triazole), 96.2 (C-1G), 85.6 (C-1′), 84.9 (C-1), 82.5 (C-5), 78.9 (C-5′), 78.4 (C-3), 76.3 (C-3′), 75.8 (C-2T), 75.4 (C-2), 74.7 (C-3T), 74.3 (C-4T), 73.6 (C-5T), 73.4 (C-4), 72.4, 72.3 (C-2′, C-4′), 66.2

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(C-6′), 54.9 (C-6T), 37.0 (C-6), 31.2 (CH_2−triazole), 25.7, 25.6, 25.5, 25.4, 25.3, 25.2, 25.1 (CH_3CO-). Anal. Calcd for $C_{82}H_{108}N_6O_{47}S_4$: C, 47.86; H, 5.29; N, 4.08; S, 6.23. Found: C, 48.09; H, 5.59; N, 3.87; S, 6.02. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{82}H_{109}N_6O_{47}S_4$: 2057.5206, found: 2057.5236.

Compound ***21***. Yield: 588 mg, 73%; $[\alpha]^{20}_D$ +22.6 (c = 0.3, $CHCl_3$); R_f = 0.31 (Hexane/EtOAc 1:8); ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6) δ = 7.99, 7.89 (2 × H-triazole), 5.37−5.25 (m, 6 H, 2 × H-3, 2 × H-4′, H-3C, H-3′C), 5.15 (m, 8 H, 2 × H-1, 2 × H-1′, 2 × H-3′, H-1C, H-1′C), 5.00−4.85 (m, 9 H, 2 × H-2, 2 × H-4, 2 × H-2′, H-2C, H-2′C, H-4′C), 4.81−4.50 (m, 4 H, H-6aC, H-6′aC, −6bC, H-6′bC), 4.30−3.85 (m, 12 H, 2 × H-5, 2 × H-5′, 2 × H-6′a, 2 × H-6′b, 2 × CH_2-triazole), 3.70−3.50 (m, 3 H, H-4C, H-5C, H-5′C), 2.85 (m, 2 H, 2 × H-6a), 2.62 (m, 2 H, 2 × H-6b), 2.13, 2.01, 2.00, 1.99, 1.98, 1.97 (2 ×), 1.96, 1.95, 1.94, 1.93, 1.92, 1.91, 1.90 (13 s, 57H, 19 × CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ = 170.4, 170.3, 170.2, 170.1, 170.0, 169.8, 169.7, 169.6 (-OCOCH$_3$), 145.1, 144.9 (2 × C-4 triazole), 124.5, 124.0 (2 × C-5 triazole), 99.8 (C-1′C), 81.0, 80.0 (2 × C-1′, 2 × C-1, C-1C), 77.6, 77.2, 76.9 (2 × C-5, C-4C, C-5C), 74.2 (2 × C-5′), 73.6 (2 × C-3), 73.1, 72.7 (C-3C, C-3′C, C-5′C), 71.5 (2 × C-3′), 71.3 (2 × C-2), 70.7 (2 × C-4), 70.5, 70.4, 70.3 (C-2C, C-2′C, C-4′C), 67.8 (2 × C-2′, 2 × C-4′), 61.5 (2 × C-6′), 50.7, 50.0 (C-6C, C-6′C), 32.7, 32.4 (2 × C-6), 26.5, 26.3 (2 × CH_2-triazole), 21.0, 20.6, 20.5, 20.4, 20.3, 20.2 (CH_3CO-). Anal. Calcd for $C_{160}H_{210}N_{12}O_{90}S_9$: C, 47.69; H, 5.25; N, 4.17; S, 7.16. Found: C, 47.42; H, 5.22; N, 4.45; S, 6.89. HRMS (ESI): m/z $[M + 2Na]^{2+}$ calcd for $C_{160}H_{210}N_{12}Na_2O_{90}S_9$: 2036.4748, found: 2036.4689.

General Procedure for the *O*-Deacetylation. Compounds **9**, **17**, **19**, and **21** (0.10 mmol) were suspended in a mixture of MeOH/Et_3N/H_2O 4:1:5 (10 mL) and stirred at room temperature. The solid was progressively dissolving, and after 4−6 h TLC (EtOAc or EtOAc/MeOH, 9:1) showed complete consumption of the starting material. The solution was concentrated, and the residue was dissolved in water (1 mL) and passed through a column filled with Dowex MR-3C mixed bed ion-exchange resin. The eluate was concentrated and further purified by filtration through an Octadecyl C18 minicolumn. Evaporation of the solvent afforded the free product, which showed a single spot by TLC (*n*-BuOH/EtOH/H_2O, 2.5:1:1) whose R_f are indicated in each case.

Compound ***18***. Yield: 54 mg, 85%; $[\alpha]^{20}_D$ −29.1 (c = 0.4, H_2O); R_f = 0.27 (BuOH/EtOH/H_2O, 2.5:1:1); ^{1}H NMR (500 MHz, D_2O) δ = 7.96 (s, 1 H, H-triazole), 4.80 (d, 1 H, $J_{1,2}$ = 10.1 Hz, H-1), 4.75 (dd, 1 H, $J_{5G,6aG}$ = 2.3, $J_{6aG,6bG}$ = 14.6 Hz, H-6aG), 4.69 (d, 1 H, $J_{1',2'}$ = 9.6 Hz, H-1′), 4.66 (d, 1 H, $J_{1G,2G}$ = 3.8 Hz, H-1G), 4.53 (dd, 1 H, $J_{5G,6bG}$ = 8.2, $J_{6aG,6bG}$ = 14.6 Hz, H-6bG), 3.91 (dd, 1 H, $J_{4',5'}$ < 1, $J_{3',4'}$ = 3.3 Hz, H-4′), 3.88 (d, 2 H, J = 5.6 Hz, CH_2-triazole), 3.83 (ddd, $J_{5G,6aG}$ = 2.3, $J_{5G,6bG}$ = 8.2, $J_{4G,5G}$ = 9.6 Hz, H-5G), 3.70 (dd, 1 H, $J_{5',6'a}$ = 8.9, $J_{6'a,6'b}$ = 12.4 Hz, H-6′a), 3.65 (m, 2 H, H-5′, H-6′b), 3.60 (dd, 1H, $J_{3',4'}$ = 3.3, $J_{2',3'}$ = 9.3 Hz, H-3′), 3.58 (t, 1 H, $J_{2G,3G}$ = $J_{3G,4G}$ = 9.7 Hz, H-3G), 3.53 (t, 1 H, $J_{1',2'}$ = $J_{2',3'}$ = 9.6 Hz, H-2′), 3.49 (m, 1 H, H-5), 3.46 (dd, 1 H, $J_{1G,2G}$ = 3.8, $J_{2G,3G}$ = 9.8 Hz, H-2G), 3.41 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 3.33 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 9.7 Hz, H-2), 3.32 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 3.17 (t, 1 H, $J_{3G,4G}$ = $J_{4G,5G}$ = 9.6 Hz, H-4G), 2.88 (dd, 1 H, $J_{5,6a}$ = 2.2, $J_{6a,6b}$ = 14.2 Hz, H-6a), 2.58 (dd, 1 H, $J_{5,6b}$ = 8.1, $J_{6a,6b}$ = 14.2 Hz, H-6b); ^{13}C NMR (125 MHz, D_2O) δ = 145.0 (C-4 triazole), 125.3 (C-5 triazole), 99.1 (C-1G), 83.2 (C-1′), 82.6 (C-1), 79.2 (C-5′), 79.1 (C-5), 77.0 (C-3), 74.0 (C-3G), 73.0 (C-3′), 72.5, 72.3 (C-2, C-4), 71.1 (C-2G), 70.9 (C-4G), 69.9 (C-5G), 69.7 (C-2′), 68.8 (C-4′), 61.2 (C-6′), 54.8 (-OCH$_3$), 50.9 (C-6G), 32.6 (C-6), 25.6 (CH_2-triazole). Anal. Calcd for $C_{22}H_{37}N_3O_{14}S_2{\cdot}H_2O$: C, 40.67; H, 6.05; N, 6.47; S, 9.87. Found: C, 40.90; H, 6.32; N, 6.62; S, 9.73. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{22}H_{38}N_3O_{14}S_2$: 632.1790, found: 632.1799.

Compound ***20***. Yield: 97 mg, 80%; $[\alpha]^{20}_D$ −49.6 (c = 0.4, H_2O); R_f = 0.10 (BuOH/EtOH/H_2O, 2.5:1:1); ^{1}H NMR (500 MHz, D_2O) δ = 7.92 (s, 1 H, H-triazole), 4.80 (d, 1 H, $J_{1,2}$ = 10.0 Hz, H-1), 4.73 (dd, 1 H, $J_{5T,6aT}$ = 2.4, $J_{6aT,6bT}$ = 14.7 Hz, H-6aT), 4.68 (d, 1 H, $J_{1',2'}$ = 9.6 Hz, H-1′), 4.49 (dd, 1 H, $J_{5T,6bT}$ = 8.4, $J_{6aT,6bT}$ = 14.7 Hz, H-6bT), 4.47 (d, 1 H, $J_{1T,2T}$ = 4.0 Hz, H-1T), 3.94 (ddd, 1 H, $J_{5T,6aT}$ = 2.3, $J_{5T,6bT}$ = 8.4, $J_{4T,5T}$ = 9.8 Hz, H-5T), 3.88 (m, 3 H, H-4′, CH_2-triazole), 3.69 (t, 1 H, $J_{2T,3T}$ = $J_{3T,4T}$ = 9.6 Hz, H-3T), 3.67 (dd, 1 H, $J_{5',6'a}$ = 8.9, $J_{6'a,6'b}$ = 12.3 Hz, H-6′a), 3.63 (m, 2 H, H-5′, H-6′b), 3.56 (dd, 1 H, $J_{3',4'}$ = 3.3, $J_{2',3'}$ = 9.5 Hz, H-3′), 3.52 (t, 1 H, $J_{1',2'}$ = $J_{2',3'}$ = 9.6 Hz, H-2′), 3.50 (m, 1 H, H-5), 3.42 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 3.39 (dd, 1 H, $J_{1T,2T}$ = 3.9, $J_{2T,3T}$ = 9.9 Hz, H-2T), 3.32 (m, 2 H, H-2, H-4), 3.15 (t, 1 H, $J_{3T,4T}$ = $J_{4T,5T}$ = 9.6 Hz, H-4T), 2.89 (dd, 1 H, $J_{5,6a}$ = 2.1, $J_{6a,6b}$ = 14.3 Hz, H-6a), 2.56 (dd, 1 H, $J_{5,6b}$ = 8.1, $J_{6a,6b}$ = 14.4 Hz, H-6b); ^{13}C NMR (125 MHz, D_2O) δ = 144.8 (C-4 triazole), 125.4 (C-5 triazole), 93.0 (C-1G), 83.3 (C-1′), 82.7 (C-1), 79.4 (C-5), 79.2 (C-5′), 77.0 (C-3), 73.9 (C-3′), 72.6 (C-3T), 72.4, 72.3 (C-2, C-4), 70.9 (C-4G), 70.7 (C-2G), 70.4 (C-5G), 69.7 (C-2′), 68.8 (C-4′), 61.2 (C-6′), 51.0 (C-6T), 32.3 (C-6), 25.6 (CH_2-triazole). Anal. Calcd for $C_{42}H_{68}N_6O_{27}S_4{\cdot}H_2O$: C, 40.84; H, 5.71; N, 6.80; S, 10.38. Found: C, 40.90; H, 6.02; N, 7.12; S, 10.33. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{42}H_{68}N_6NaO_{27}S_4$: 1239.2913, found: 1239.2899.

Compound ***22***. Yield: 224 mg, 92%; $[\alpha]^{20}_D$ −41.3 (c = 0.4, H_2O); R_f = 0.15 (BuOH/EtOH/H_2O, 2.5:1:1, double development); ^{1}H NMR (500 MHz, D_2O) δ = 8.02, 7.81 (2s, 2 H, 2 × H-triazole), 4.80 (m, 4 H, 2 × H-1, H-6′aC, H-6aC), 4.66 (d, 2 H, $J_{1',2'}$ = 9.6 Hz, 2 × H-1′), 4.60 (dd, 1 H, $J_{5C,6bC}$ = 7.8, $J_{6aC,6bC}$ = 14.7 Hz, H-6bC), 4.50 (d, 1 H, $J_{1'C,2'C}$ = 7.9 Hz, H-1′C), 4.30 (dd, 1 H, $J_{5'C,6b'C}$ = 9.5, $J_{6a'C,6b'C}$ = 14.4 Hz, H-6b′C), 4.05 (d, 1 H, $J_{1C,2C}$ = 10.1 Hz, H-1C), 3.88 (m, 6 H, 2 × H-4′, 2 × CH_2-triazole), 3.78 (m, 1 H, H-5′C), 3.70 (m, 2 H, 2 × H-6′a), 3.64 (m, 4 H, 2 × H-5′, 2 × H-6′b), 3.60 (m, 2 H, 2 × H-3′), 3.56−3.46 (m, 7 H, H-3C, H-4C, H-3′C, 2 × H-2′, 2 × H-5), 3.41 (m, 4 H, 2 × H-3, H-2C, H-2′C), 3.34 (m, 4 H, 2 × H-2, 2 × H-4), 3.20 (m, 2 H, H-4′C, H-5C), 2.95 (m, 2 H, 2 × H-6a), 2.65 (m, 2 H, 2 × H-6b); ^{13}C NMR (125 MHz, D_2O) δ = 145.0, 143.9 (2 × C-4 triazole), 125.4, 125.1 (2 × C-5 triazole), 102.1 (C-1′C), 83.3 (2 × C-1′), 83.2 (2× C-1), 82.7 (C-1C), 79.2, 79.1 (2 × C-5, 2 × C-5′), 78.6 (C-3C), 77.0 (2 × C-3, C-5C), 75.6, 75.3 (C-4C, C-3′C), 74.0 (2 × C-3′), 72.8, 72.5, 72.4, 72.3, 72.0 (C-2C, C-2′C, C-5′C, 2 × C-2, 2× C-4), 70.7 (C-4′C), 69.7 (2 × C-2′), 68.8 (2 × C-4′), 61.2 (2 × C-6′), 50.9 (C-6C, C-6′C), 33.1, 32.6 (2 × C-6), 26.0, 25.8 (2 × CH_2-triazole). Anal. Calcd for $C_{84}H_{134}N_{12}O_{52}S_9{\cdot}H_2O$: C, 41.17; H, 5.59; N, 6.86; S, 11.78. Found: C, 40.90; H, 5.32; N, 7.02; S, 11.63. HRMS (ESI): m/z $[M + 2Na]^{2+}$ calcd for $C_{84}H_{134}N_{12}Na_2O_{52}S_9$: 1238.2740, found: 1238.2738.

Deacetylation of Compound 9. *Compound* ***23***. Thiodisaccharide **9** (100 mg, 0.14 mmol) was dissolved in 0.5 M NaOMe in MeOH (0.4 mL). The mixture was stirred for 2 h at room temperature. The solution was neutralized with acidic ion-exchange resin Dowex 50X [H^+], filtered, and concentrated. The residue obtained was purified by reverse-phase chromatography to afford compound **23** (52 mg, 89%). $[\alpha]^{20}_D$ −19.6 (c = 0.4, H_2O); R_f = 0.55 (BuOH/EtOH/H_2O, 2.5:1:1); ^{1}H NMR (500 MHz, D_2O) δ = 4.80 (d, 1 H, $J_{1,2}$ = 10.0 Hz, H-1), 4.69 (d, 1 H, $J_{1',2'}$ = 9.7 Hz, H-1′), 3.90 (dd, 1 H, $J_{4',5'}$ < 1, $J_{3',4'}$ = 3.3 Hz, H-4′), 3.70 (dd, 1 H, $J_{5',6'a}$ = 8.8, $J_{6'a,6'b}$ = 12.6 Hz, H-6′a), 3.65 (dd, 1 H, $J_{5',6'b}$ = 2.1, $J_{6'a,6'b}$ = 12.6 Hz, H-6′b), 3.63 (ddd, 1 H, $J_{4',5'}$ < 1, $J_{5',6'b}$ = 2.1, $J_{5',6'a}$ = 8.8 Hz, H-5′), 3.60 (dd, 1H, $J_{3',4'}$ = 3.3, $J_{4',5'}$ = 9.5 Hz, H-3′), 3.57 (ddd, 1 H, $J_{5,6a}$ = 2.5, $J_{5,6b}$ = 8.7, $J_{4,5}$ = 9.5 Hz, H-5), 3.53 (t, 1 H, $J_{1',2'}$ = $J_{2',3'}$ = 9.6 Hz, H-2′), 3.42 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3), 3.35 (dd, 2 H, J = 2.6 Hz, CH_2S), 3.34 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 10.0 Hz, H-2), 3.33 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4), 3.16 (dd, 1 H, $J_{5,6a}$ = 2.5, $J_{6a,6b}$ = 14.4 Hz, H-6a), 2.76 (dd, 1 H, $J_{5,6b}$ = 8.4, $J_{6a,6b}$ = 14.4 Hz, H-6b), 2.62 (t, 1 H, $J_{C\equiv CH,CH2C\equiv CH}$ = 2.6 Hz, C≡CH); ^{13}C NMR (125 MHz, D_2O) δ = 83.0 (C-1′), 82.3 (C-1), 80.8 (C≡CH), 79.2 (C-5′), 79.0 (C-5), 77.0 (C-3), 73.9 (C-3′), 72.5, 72.4 (C-2, C-4), 72.2 (C≡CH), 69.6 (C-2′), 68.8 (C-4′), 61.1 (C-6′), 32.6 (C-6), 19.3 (CH_2S). Anal. Calcd for $C_{15}H_{24}O_9S_2{\cdot}H_2O$: C, 41.85; H, 6.09; S, 14.90. Found: C, 42.00; H, 6.22; S,15.23. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{15}H_{24}NaO_9S_2$: 435.0759, found: 435.0756.

Enzymatic Assays. Stability of the Glycoclusters toward β-Galactosidase. *Escherichia coli* β-galactosidase (grade VIII, EC 3.2.1.23, 117 U/mg) was purchased from Sigma-Aldrich. A solution of the glycoclusters (1 mM) in sodium phosphate buffer (100 mM, pH 7.3, $MgCl_2$ 1.2 mM, 2-mercaptoethanol 100 mM) was incubated with the β-galactosidase from *E. coli* (0.6 U/mL) at 37 °C. Aliquots of the solution were taken after 8, 16, and 24 h, evaporated, and examined by TLC and NMR. *o*-Nitrophenyl-β-D-galactopyranoside was used for the control experiment.

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Isothermal Titration Calorimetry (ITC). Recombinant human galectin-3 was purchased from Interchim Acris Antibodies (Lyophilized, PA274X, Uniprot ID P17931). Peanut agglutinin from *Arachis hypogaea* was purchased from Sigma (lyophilized powder, affinity-purified, agglutination activity <0.1 μg mL^{-1}). An ITC instrument was used for the titrations at 298 K.[35] Respective concentrations and molar ratios in needle and cell, injection volumes, and time intervals between injections were varied to obtain (1) inflection and saturation about halfway through the experiment, (2) sufficient heat production per injection to allow good peak integration, and (3) sufficient time between the injections to allow a return to equilibrium. A typical titration involved 16 injections at 3 min intervals of 2.5 μL aliquots of ligand solution into the sample cell (200 μL) containing PNA (150 μM) or hGal-3 (15 μM). The solutions were prepared by dissolving the ligand in 20 mM phosphate buffer, pH 7.4, and 150 mM NaCl at 298 K. The ligand concentration was 1.5 mM for PNA or 0.15 mM for hGal-3. The titration cell was continuously stirred at 400 rev/min. The heats of dilution of the ligands in the buffer were subtracted from the titration data. Fitting was performed using the Origin software to determine the binding stoichiometry (n), association constant, and the enthalpy change (ΔH).

Docking Calculations. The more stable conformers of compounds **1**, **23**, and **25** (as determined by NMR) were manually docked into the carbohydrate-binding sites of the lectins by superimposing the terminal Gal residue with that of the crystallographic coordinates (PDB codes 1A3K for the CRD of human galectin-3 and 1CR7 for peanut agglutinin). Then, different possibilities of arranging the disaccharides were used as input geometries for AUTODOCK 4.2 simulations with the multiple Lamarckian Genetic Algorithm.[47] The AUTOGRID4 program present in AUTODOCK 4.2 generated grids of probe atom interaction energies and electrostatic potential. Grid spacings of 0.375 Å were used for the local searches. For each calculation, 100 docking runs were performed using a population of 250 individuals and an energy evaluation number of 3 × 106.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra for compounds **8**, **9**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **23**, and **24**. ITC data for compounds **1**, **18**, **20**, **22**, and **23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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