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Synthesis and biological activity of divalent ligands based on 3-deoxy-4-thiolactose, an isosteric analogue of lactose†

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We report here the synthesis of divalent ligands containing 3-deoxy-4-thiolactose. This thiodisaccharide has been synthesized using the Michael addition of β-1-thiogalactose to the α,β-unsaturated system of sugar-derived dihydropyranones, followed by the reduction of the remaining carbonyl group. We were able to control the configuration (*S*) of the stereocenter linked to sulfur (C-4) of the reducing end by conducting the thioglycosylation at high temperature or by isomerization during the reduction of the 2-ulose thiodisaccharide with $NaBH_4$/THF. The energy profile for this reaction on a model compound was calculated. The anomeric position of the 3-deoxy-4-thiolactose was functionalized with a terminal alkyne, which was coupled to azide-containing sugar scaffolds through CuAAC reaction to afford mono- and divalent ligands. The final products were competitive inhibitors of *E. coli* β-galactosidase in the micromolar range. Their binding affinities to peanut agglutinin (PNA) were determined by isothermal calorimetry, which showed a clear decrease in the K_a values for monovalent derivatives compared to lactose. This report contributes to establishing the role of a particular hydroxyl group of lactose in sugar-protein recognition processes.

Introduction

Sugar recognition by proteins is determined by non-covalent interactions established between certain amino acid residues in the binding site of the protein and hydroxyl groups or hydrophobic moieties specifically disposed in the target oligosaccharide.[1,2] This means that the position and the stereochemistry of a restricted number of hydroxyl groups are important in terms of the recognition. Theoretical calculations provided information about the recognition processes at a molecular level, and drugs have been designed taking into account the three-dimensional structure of the protein–ligand complex.[3,4] However, the design of ligands cannot rely only on the knowledge of the protein structure. Sugar analogues are still needed to evaluate the participation of specific hydroxyl groups in the binding process. A main concern of organic chemists is the development of synthetic methodologies to provide these analogues, in the search for the molecular principles underlying the specific recognition process of complex carbohydrates. As an example, Leffler and coworkers have shown that the substitution of HO-3 of β-galactosides with an aromatic residue led to high affinity ligands of galectins.[5]

Although 2-deoxy-sugars occur widely in natural products and many synthetic methods for their preparation have been developed,[6] the impact of the removal of a ligand's hydroxyl group on the recognition process by a lectin, and the concomitant effect of its multivalent presentation, are aspects much less explored.

We have previously developed a direct methodology for the synthesis of 3-deoxy-4-thiodisaccharides. This approach involves the Michael addition of a 1-thioaldose to the enone system of a sugar-derived dihydropyranone,[7] and its further reduction to obtain a 3-deoxy-4-thio-disaccharide of *gluco* configuration for the reducing end.[8]

In this paper we report the synthesis of divalent ligands with 3-deoxy-4-(*S*-β-D-galactopyranosyl)-4-thio-α-D-*ribo*-hexopyranosides as isosteric analogues of lactose. So, the synthesis of these lactose-like structures was performed by a key modification of the reaction conditions in the Michael addition step, to achieve reverse stereoselectivity in comparison with that previously observed.[8] Sugar enones bearing a triple bond in the anomeric substituent were chosen as appropriate precursors, so that the resulting thiodisaccharide could be coupled to

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azide-containing sugar scaffolds, by means of the CuAAC reaction. In this way, mono- and divalent glycoclusters were obtained and their affinity to peanut agglutinin (PNA, *Arachis hypogaea*) was determined. A moderate cluster effect was established from the thermodynamic parameters of binding (K, ΔG, ΔH, ΔS and n). Additional information on the biological activity of the ligands was obtained by determination of their inhibitory activity against the β-galactosidase of *E. coli*.

Results and discussion

Dihydropyran-2-one **3a** was synthesized starting from 2-acetoxy-3,4,6-tri-*O*-acetyl-D-galactal **1** and alcohol **2** in the presence of $SnCl_4$ (Scheme 1).[8] Alcohol **2** was prepared by standard methods, starting from diethyleneglycol and propargyl bromide.[9]

Michael addition of **4** to dihydropyran-2-one **3a**, performed at −70 °C in CH_2Cl_2 under Et_3N catalysis, was highly diastereoselective, and the major isomer **5a** was isolated in 82% yield, in agreement with the tendency observed in our previous reports.[8] Indeed, the stereocontrol provided by the axially oriented anomeric substituent in the preferred $^0H_5$ conformation of the dihydropyranone directs the attack of **4** from the less hindered β face. The stereoisomeric thioulose **5b** was also isolated in 12% yield. An analysis of the coupling constant values $J_{3a,4}$, $J_{3b,4}$, $J_{4,5}$ of uloses **5a** and **5b** (Table 1) allowed the assignment of the stereochemistry of both products unequivocally: the large J-values observed for **5b** indicated the axial disposition of the H-4, which was in turn equatorial in **5a**.

As **5b** was the required precursor for the synthesis of lactose-like recognition elements, we decided to study the stereoselectivity of the Michael addition of **4** to **3a** in the search for optimized conditions to favor **5b**.

In a previous report, we observed the formation of two stereoisomeric products in the Michael addition of thiosugars to enones.[10] At low temperature, the product distribution depends on the steric hindrance provided by both the enone substituents and the nucleophile. We rationalized that the stereoselectivity could be inversed in favor of the thermodynamic product by increasing the reaction temperature. So, the addition was performed as indicated in Table 2. Indeed, at 80 °C under microwave irradiation, thiodisaccharide **5b** was obtained as the major product in 88% yield.

Table 1 Coupling constant values involving H-4 for **5a** and **5b**

^3J	**5a**	**5b**
$J_{3a,4}$	4.9	12.5
$J_{3b,4}$	2.6	5.1
$J_{4,5}$	1.9	10.8

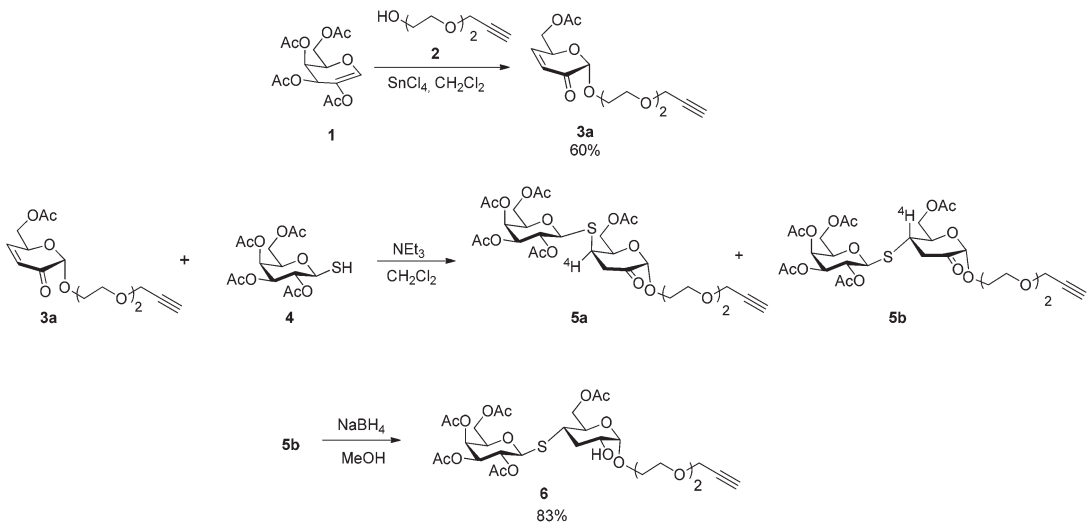
In order to obtain **6**, we conducted the reduction of **5b** under standard conditions ($NaBH_4$ in MeOH). Thiodisaccharide **6** (83%) was isolated as the only product, upon purification by column chromatography. The preferential approach of the hydride to the less hindered β-face of the ulose can be ascribed to the α-disposition of both the anomeric residue and the *S*-glycosidic substituent at C-4.[8,10]

We have also synthesized compound **8**, an analogue of **6**, in order to study the influence of the linker length on the biological activity of the final products. Thus, dihydropyran-2-one **3b** was synthesized from galactal **1** and propargyl alcohol under the same conditions as described above (Scheme 2).

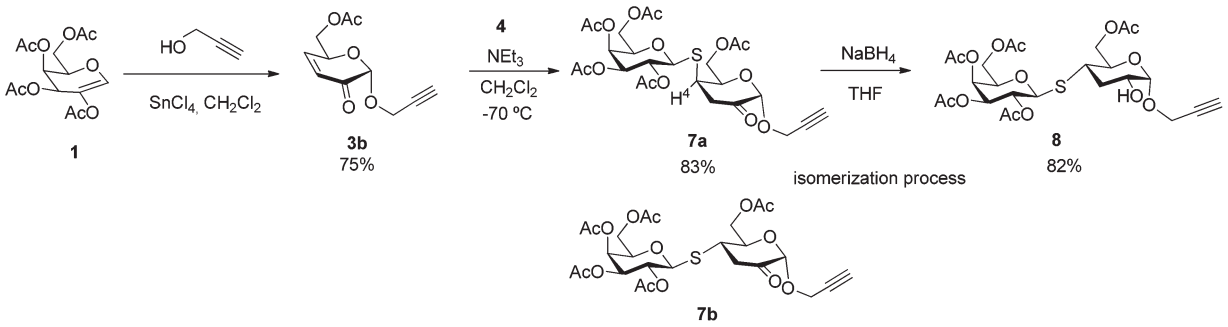
Michael addition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose **4** to the α,β-unsaturated system of the sugar enone

Table 2 Study of the stereoselectivity of the Michael addition in the synthesis of thiodisaccharide **6** (Scheme 1)

Conditions	**5a** (%)	**5b** (%)
−70 °C	82	12
−18 °C	63	30
25 °C	44	40
60 °C	16	65
80 °C (μW)	9	88



Scheme 1 Synthesis of thiodisaccharide **6**.



Scheme 2 Synthesis of thiodisaccharide **8**.

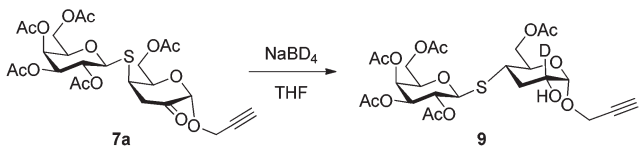
3b, under Et_3N catalysis at −70 °C, led to the thiopyranosid-2-ulose **7a** in 83% yield as the only isolated product (Scheme 2). The *R* configuration for the new stereocenter at C-4 in **7a** was confirmed by ^{1}H NMR spectroscopy, as the small coupling constant values ($J_{3a,4}$ = 4.9 Hz, $J_{3b,4}$ = 1.6 Hz) indicate that H-4 was equatorially oriented. In order to obtain the pursued stereoisomer having *S* configuration at C-4, the reaction temperature was raised to −18 °C. Under these conditions, only a small amount of **7b** was isolated in 4% yield, but at the same time, the yield of **7a** was significantly lowered (57%). When the reaction was conducted using MW at 70 °C, the only isolated product was **7a**, which was obtained in a poor 27% yield. This fact was attributed to the decomposition observed for the enone **3b** on heating.

We have previously shown that the reduction of thiouloses such as **7a** with $NaBH_4$ in non-protic solvents (THF) occurs with isomerization of C-4, and that the S-linked substituent adopts the equatorial disposition in the final product.[8b] This reduction–epimerization step was convenient for the synthesis of a 3-deoxy thio-analogue of lactose from **7a**. Thus, the reduction was conducted with an excess of $NaBH_4$ in anhydrous THF, which led, as expected, to the isomerized thiodisaccharide **8** in very good yield (82%). The structure of **8** was confirmed by ^{1}H and ^{13}C NMR spectroscopy, assisted by 2D ^{1}H COSY and ^{1}H–^{13}C HSQC experiments. To assign the stereochemistry, the large value of $J_{4,5}$ = 11.5 Hz and $J_{3ax,4}$ = 12.9 Hz, together with a small value for $J_{3eq,4}$ = 4.2 Hz, indicated that H-4 in **8** was axially disposed (*S*-configuration for C-4).

Mechanistic considerations and DFT studies for the isomerization step

In an attempt to explain the course of the isomerization process in the synthesis of thiodisaccharides such as **8**, we revised the initially proposed mechanism for this unusual result.[8b] This mechanism involved the 1,4-addition of a hydride to an unsaturated planar system formed under strong basic conditions in an aprotic solvent such as THF.

In order to obtain experimental evidence, the reduction of **7a** was conducted using $NaBD_4$ in THF, a reaction that led to compound **9** as the only isolated product in 80% yield (Scheme 3). The ^{1}H and ^{13}C NMR spectroscopic analysis of **9** confirmed the *S* configuration at C-4 and showed the

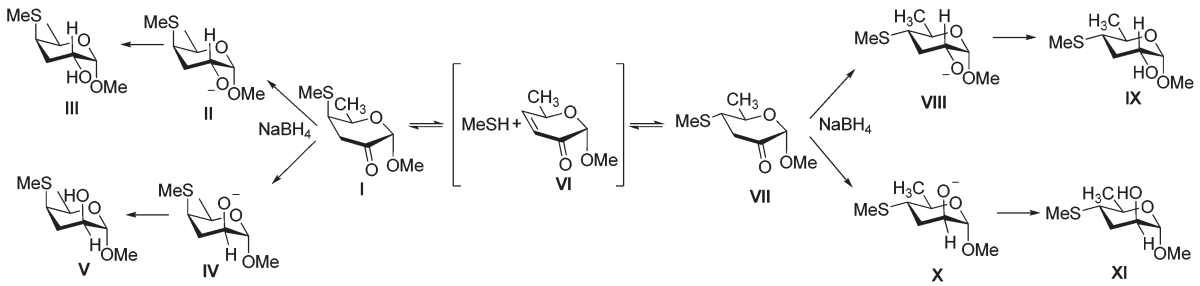


Scheme 3 Reduction of compound **7a** with $NaBD_4$.

incorporation of just a single deuterium atom into the molecule. Comparison of the ^{1}H NMR spectra of compounds **8** and **9** showed that the signal of H-2 was absent in the latter as well as the coupling constants of H-2 with H-1, H-3a and H-3b. These results brought into question the originally proposed mechanism. In fact, they suggest that the isomerization takes place prior to the reduction as no deuteration at C-4 was detected.

Therefore, an alternative mechanism is proposed, which is in agreement with the new findings. We now suggest a base-mediated retro-Michael reaction as the key step for the isomerization, followed by the attack of the 1-thiogalactose from the α face to afford the thermodynamically more stable ulopyranose **7b**, which is then rapidly reduced to give **8**. Acid-catalysed retro-Michael processes in favor of the more stable stereoisomer have been previously reported in hydroquinolinone systems.[11] These reports are consistent with the rapid equilibration of **7a** and **7b** prior to reduction.

This mechanism was examined computationally employing DFT calculations using the B3LYP hybrid functional (Scheme 4). In this computational study, compound **I** was used as a model molecule for thioulose **7a**, where the 6-acetoxy group was replaced by a methyl group, the thiogalactopyranose moiety by a thiomethyl group and the anomeric substituent by a methyl group. Reduction of the carbonyl group of **I** with $NaBH_4$ affords the epimeric alcohols **III** and **V** upon reaction work-up. This reduction is expected to be slow, due to the hindrance of the carbonyl group of **I** by the axially disposed –SMe and –OMe groups, located on opposite faces of the molecule. Therefore, ulose **I** could undergo a retro-Michael reaction to give enone **VI** as a precursor of **VII**, which results from the attack of MeSH from the α-face. On the basis of steric considerations (see below), compound **VII** should be rapidly reduced to **IX** (main product) and **XI**. The proposed mechanism has been studied by calculations, as follows.



Scheme 4 Proposed mechanism of C-4 isomerization during the $NaBH_4$/THF reduction of **7a**, using **I** as a model compound.

The energies of structures **I–XI** and the corresponding transition states for the reduction steps were calculated. The energy profile for the proposed mechanism is depicted in Fig. 1. The calculated energy barrier for the reduction of ulose **I**, with the axial thiomethyl group, was 9.08 kcal mol^{-1} for the attack of $NaBH_4$ from the β face and 11.46 kcal mol^{-1} from the α face. The ΔG values for the reduction of **I** to afford **II** and **IV** were 5.71 and 8.69 kcal mol^{-1}, respectively. Then, the reaction intermediates **II** and **IV** are protonated to give the corresponding thiodisaccharides **III** and **V**. These results are consistent with the experimental results found for the reduction of other uloses previously described in the literature, where the product having the HO-2 equatorially disposed (such as **III**) was the major product.[8]

However, if a retro-Michael step occurs, ulose **I** can regenerate enone **VI** and methanethiol (ΔG = 5.26 kcal mol^{-1}), and then the attack of the thiol from the α face affords ulose **VII** which is energetically favored ($\Delta G_{\mathbf{I,VII}}$ = −2.01 kcal mol^{-1}), due to the change of the thiomethyl group from an axial to an equatorial disposition. The carbonyl group of **VII** is then reduced to give thiodisaccharides **IX** and **XI**. In this case, a large difference in the energy barrier values, as well as in the ΔG values, was found when the attack of the BH_4^- group from each face of the ulose was considered: $\Delta G^{\ddagger}_{\beta\ face}$ = 5.60 kcal mol^{-1} and $\Delta G^{\ddagger}_{\alpha\ face}$ = 9.59 kcal mol^{-1}; $\Delta G_{\beta\ face}$ = −4.10 kcal mol^{-1} and $\Delta G_{\alpha\ face}$ = −7.48 kcal mol^{-1}. This behavior was expected as the thiomethyl group and the anomeric substituent hampered the approach of the hydride from the α face. In accordance with these results, thiodisaccharide **6** (structural analogue of **IX**) was the only isolated product in the reduction of ulose **5b**. This preliminary computational study provides a reasonable explanation for the reduction–isomerization reaction, consistent with the experimental results.

Synthesis of the glycoclusters

Two azide-containing scaffolds were prepared according to previously reported methods.[9,12] Coupling of the azide-functionalized thiodisaccharides **6** and **8** to the sugar scaffolds **10** and **13** was conducted by the "click" reaction with $CuSO_4$–sodium ascorbate, in dioxane–water, under microwave irradiation (Scheme 5).[13] The click reaction between **8** and the monoazide **10** afforded the monovalent compound **11** in 78% isolated yield. The ^1H NMR spectrum showed the signals of both sugar moieties, the glucose-scaffold and the thiodisaccharide. The assignments were further confirmed by 2D NMR techniques.

The symmetric divalent cycloadducts **14** and **15** were similarly prepared in 66% and 55% yields, respectively, by the click reaction of **6** or **8** with diazide **13** (Scheme 5). One major advantage of the use of scaffold **13**, in terms of the characterization of the products, was the simplicity of the

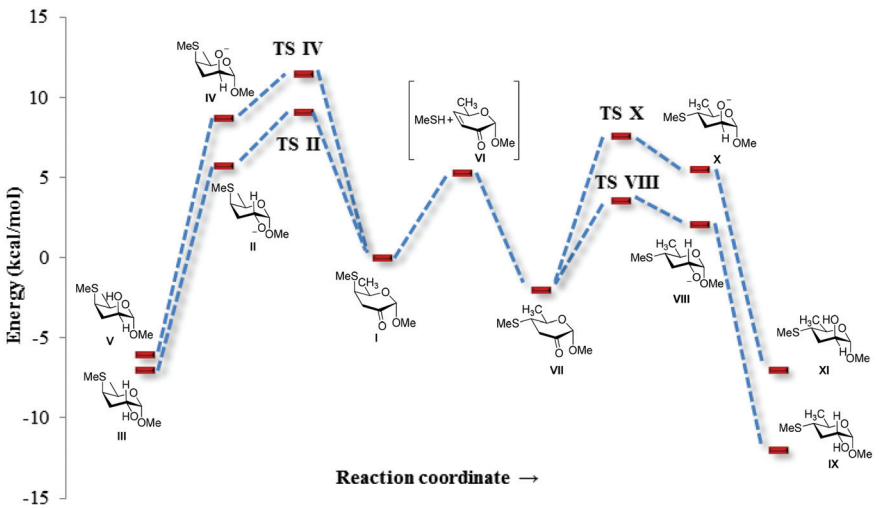
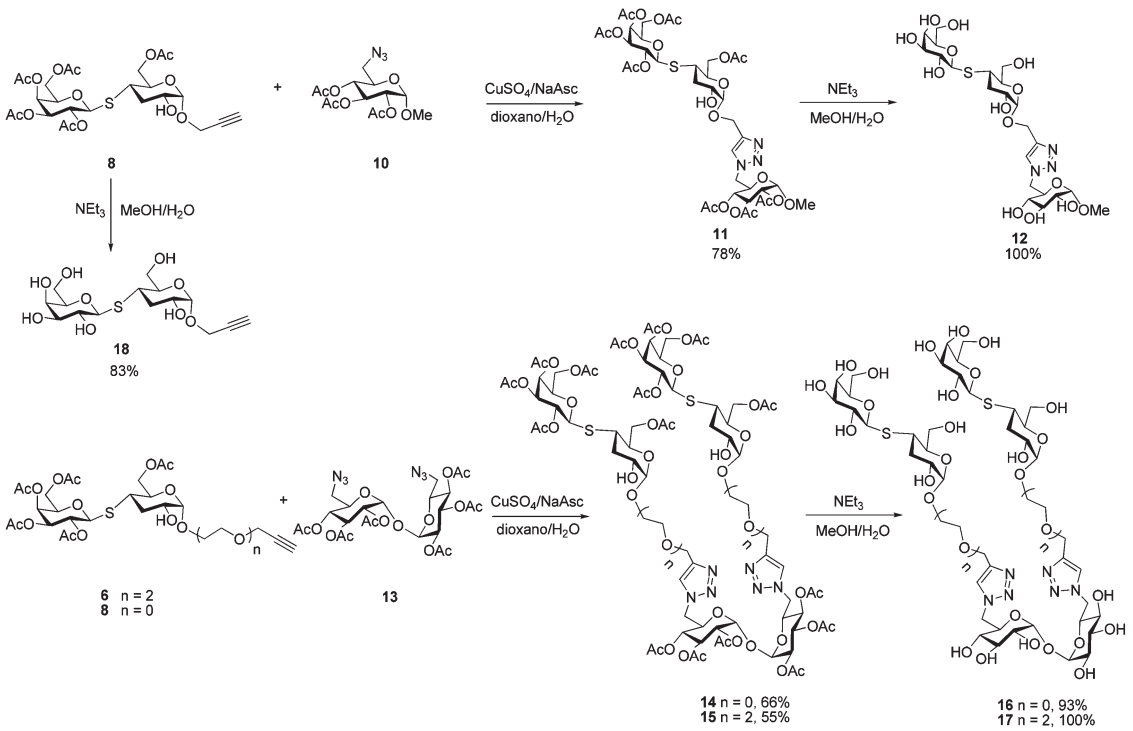


Fig. 1 Energy profile for the reduction–isomerization of ulose **I**.



Scheme 5 Click coupling and *O*-deacetylation of thiodisaccharides and azide-containing scaffolds.

NMR spectra as a result of the symmetry. Molecular weights, determined by MS, confirmed the structures of **14** and **15**.

The glycoclusters **11**, **14** and **15** were *O*-deacetylated by treatment with Et_3N–MeOH–H_2O 1 : 4 : 5. After desalting with a mixed-bed ion exchange resin and purification by reverse-phase chromatography, the deprotected products were recovered in high yields (92–100%), and analyzed by NMR. Signals of the anomeric protons and carbons were diagnostic. HR-MS and elemental analysis of the products confirmed the structures of the free glycoclusters.

Compound **8** was also deacetylated under the same conditions to afford **18** in 83% yield. This product was used as a reference for the inhibitory activity and lectin binding assays, in order to determine the effect of the triazole ring on the biological activity of the ligands synthesized.

Evaluation of the inhibitory activity

The free products **12**, **16**, **17** and **18** were evaluated as inhibitors against β-galactosidase from *E. coli*. This enzyme has been extensively studied and the inhibitory activity of a variety of β-thiogalactosides has been determined.[14] The glycoclusters showed inhibitory activity of the enzyme in the micromolecular concentration range (Table 3).

The evaluation of the inhibitory behavior by the double reciprocal plot method, exemplified in Fig. 2 for compound **12**, showed that all the glycoclusters are competitive inhibitors of *E. coli* β-galactosidase. The inhibition constants are similar to those determined for other β-thiogalactosides previously reported.[8,9]

Table 3 K_i values for the inhibition of β-galactosidase from *E. coli* by glycoclusters, determined using *o*-nitrophenyl-β-D-galactopyranoside as a substrate, as described in the Experimental section. K_m = 1.38 mM

Valency	Compound	K_i value (μM)
1	**18**	648 ± 9
1	**12**	189 ± 7
2	**16**	196 ± 8
2	**17**	490 ± 9

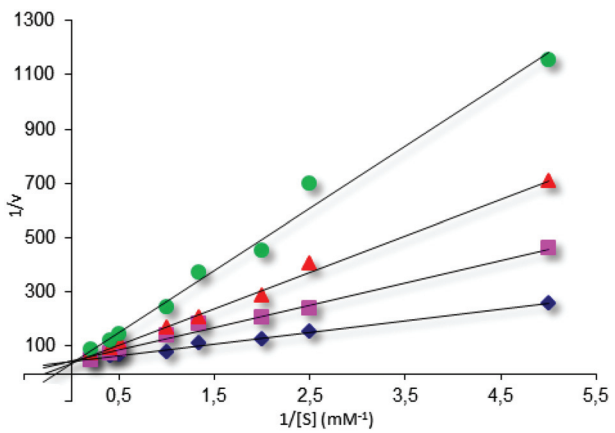


Fig. 2 Lineweaver–Burk plot for inhibition of *E. coli* β-galactosidase by compound **14** at concentrations ◆ = 0.00, ■ = 0.40, ▲ = 0.80 and ● = 1.20 mM of inhibitor.

The compounds evaluated, with the exception of the reference compound **18**, possess the 3-deoxylactose residue attached through a triazole ring to the C-6 of the sugar

scaffolds. In compounds **12** and **16** the 3-deoxylactose moiety and the scaffold are linked through a triazole ring, while for compound **17**, a diethyleneglycol chain separates the thioglycoside from the triazole and the scaffold. The presence of the triazole ring close to the 3-deoxylactose enhances the inhibition (compounds **12** and **16**), a result which is in agreement with previous reports.[8b,15]

The K_i values listed in Table 3 show that moving from the monovalent **12** to the divalent derivative **16**, bearing the same spacer to the platform, no increased inhibition and no cluster or multivalency effect are observed. Indeed, the inhibitory activities of **12** (monovalent) and **16** (divalent) are in the same order, and no statistical effect is observed.

Interestingly, the 3-deoxylactose analogues reported here are stronger inhibitors of β-galactosidase from *E. coli* than α-methyl thiolactoside (K_i = 7.7 mM)[16] and our previously synthesized multivalent thiogalactosides.[9] Their activities are of the same order as structurally related thiodisaccharides synthesized in our laboratory.[8]

Isothermal titration calorimetry

PNA is a homotetrameric legume lectin that binds specifically to β-galactose and displays a higher affinity towards lactose derivatives. It has been previously used as a model lectin to study the cluster effect in multivalent ligands.[17] In a recent report, we have studied the interaction of multi-thiolactosides with PNA by means of isothermal titration calorimetry (ITC).[18]

Compounds **12**, **16**, **17** and **18**, bearing 3-deoxy-4-thiolactosides as recognition elements, were evaluated as ligands for peanut (*Arachis hypogaea*) agglutinin (PNA) (Table 4). Lactose was used as a reference compound.

Compound **18** exhibits a lower binding affinity than the lactose reference, similar to that of galactose (K_a = 0.95 × 10^3 M^{-1}).[17] These results are consistent with the absence of the hydroxyl function at C-3 of the *S*-saccharide reducing-end sugar.[19] Monovalent glycoconjugate **12** shows a small improvement in the binding affinity compared to **18**, suggesting that the triazole ring or the sugar scaffold might have a positive effect in the binding process.

The two divalent glycoconjugates **16** and **17** show a moderate multivalent effect since they bind to PNA with a relative potency of 3.47 and 2.87, respectively, compared to the reference compound **18**.

The binding enthalpies of divalent glycoconjugates to the lectin are almost double the monovalent ones. As shown in Table 4, 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.

Conclusions

In conclusion, we have developed an efficient strategy, particularly in terms of atom-economy, for the synthesis of mono- and divalent ligands with isosteric analogs of lactose as recognition elements.

In this work we have extended the scope of the Michael addition of 1-thiogalactose to sugar enones and also the following reduction step of the carbonyl group, in order to obtain suitable lactose-like recognition elements for lectin-binding studies. First, reverse stereoselectivity was achieved for the Michael addition step by controlling the reaction conditions. Then, we also profited from the preliminary reported simultaneous isomerization–reduction reaction of 4-thioulosides. Faced with the new experimental evidence obtained, an alternative reaction mechanism was proposed, which is supported by theoretical calculations.

The recognition elements were grafted to azide-containing oligosaccharidic scaffolds and mono- and divalent ligands differing in the linker length were synthesized by CuAAC followed by deacetylation.

The resulting glycoclusters were tested as inhibitors of the β-galactosidase from *E. coli*, in order to estimate the stability of these compounds in biological fluids. These results confirmed that the absence of HO-3 enhances the hydrophobicity, and thus has a positive effect in terms of inhibition of the enzyme, which is in accordance with our previous work.[8]

The results from the lectin-binding affinity tests are consistent with the active participation of HO-3 of the glucose moiety in the recognition process. To give a quantitative example, we have previously determined that the K_a value of a divalent thiolactose ligand was 46.2 × 10^3 M^{-1}.[18] In the present report, we show that the binding affinities (given by K_a values) of divalent ligands **16** and **17**, lacking the aforementioned hydroxyl group, are approximately 15 times lower (3.64 × 10^3 M^{-1} and 3.01 × 10^3 M^{-1}, respectively).

The parameters associated with the interaction between these synthetic ligands and the peanut lectin, determined by ITC measurements, provided insights into the binding mode.

Table 4 Thermodynamic binding parameters of the synthetic ligands related to lactose, used as a reference. *Val* refers to the structural valency of the ligand; *n* is the stoichiometry of the binding; *Rel. pot. per lac.* is the corrected potency on a lactose molar basis

Compound	Valency	*n*	K_a (×10^{-3} M^{-1})	ΔH (kcal mol^{-1})	$T\Delta S$ (kcal mol^{-1})	ΔG (kcal mol^{-1})	*Rel. pot. per lac.*
Lactose	1	1.04	2.79	−9.56	−3.84	−5.72	1
18	1	0.98	1.05	−7.70	−3.10	−4.60	0.38
12	1	0.95	1.23	−7.54	−2.85	−4.69	0.44
16	2	0.48	3.64	−19.1	−11.89	−7.21	1.30
17	2	0.52	3.01	−21.0	−12.14	−8.86	1.08

Although PNA is a tetrameric lectin, a chelate effect does not seem to be possible since, from our preliminary calculations, the distance between the sugar epitopes is approximately 20 Å, much less than the smallest separation of the lectin binding sites (70 Å).[20] Thus, the increment in binding affinity observed may be attributed either to a multivalent effect of internal diffusion (sliding mechanism), where the proximity of the recognition elements allows their subsequent binding and recapture by a unique binding site, or to an aggregative process, where the distance between the ligands is long enough to allow different lectins to bind simultaneously to the same glycocluster without steric constraints.[21,22]

Experimental section

General methods

Materials and reagents were of the highest commercially available grade and used without further purification. $NaBD_4$ was purchased from Sigma-Aldrich. 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 per 100 mL). Microwave irradiation was carried out in a CEM Discover instrument, at 70 °C (power max. 300 W). High resolution mass spectra HRMS were obtained by electrospray ionisation (ESI) and Q-TOF detection. For ^1H, ^{13}C nuclear magnetic resonance (NMR) spectra, chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak ($CHCl_3$: ^1H: δ = 7.26 ppm; ^{13}C: δ = 77.2 ppm). Assignments of ^1H and ^{13}C were assisted by 2D ^1H-COSY and 2D ^1H–^{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 or trehalose scaffolds were labeled as "G" or "T", respectively. All the products were obtained as syrups.

General procedure for the synthesis of enones 3a and 3b

Propargyl alcohol or alkynyl alcohol **2** (2 mmol) and 2-acetoxy-3,4,6-tri-*O*-acetyl-D-galactal **1** (1 mmol) were dissolved in anhydrous CH_2Cl_2 (5 mL). The reaction mixture was cooled to −18 °C. $SnCl_4$ (177 μL) was added and the mixture was stirred for 2 h. When TLC showed complete conversion of the starting materials, the reaction mixture was diluted with CH_2Cl_2 and washed with a saturated solution of $NaHCO_3$ (3 × 30 mL), NaCl (30 mL) and water (30 mL). The resulting organic layers were dried over sodium sulfate, filtered and evaporated. The residue was purified by column chromatography.

(2*S*,6*S*)-6-Acetoxymethyl-2-[2-(2-propargyloxyethoxy)ethoxy]-2*H*-pyran-3(6*H*)-one (3a). Yield 60%; R_f 0.41 (hexane–EtOAc 1 : 1); $[\alpha]_D^{20}$ −30.5 (*c* 0.4 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.92 (1 H, dd, $J_{3,5}$ = 1.7, $J_{3,4}$ = 10.6 Hz, H-4), 6.10 (1 H, dd, $J_{4,5}$ = 2.6, $J_{3,4}$ = 10.6 Hz, H-3), 4.85 (1 H, s, H-1), 4.82 (1 H, m, H-5), 4.30 (1 H, dd, $J_{5,6a}$ = 5.3, $J_{6a,6b}$ = 11.7 Hz, H-6a), 4.19 (1 H, dd, $J_{5,6b}$ = 4.4, $J_{6a,6b}$ = 11.7 Hz, H-6b), 4.13 (2 H, d, $J_{CH2-C\equiv CH,C\equiv CH}$ = 2.3 Hz, CH_2–C≡CH), 4.35, 4.29 (2 H, 2 m, CH_2O, C1), 3.65–3.58 (6 H, m, 3 × CH_2O), 2.38 (1 H, t, $J_{CH2O-C\equiv CH,C\equiv CH}$ = 2.4 Hz, C≡C*H*), 2.03 (3 H, s, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.2 (C-2), 170.6 (–*C*OCH$_3$), 147.7 (C-3), 126.0 (C-4), 97.6 (C-1), 79.5 (*C*≡CH), 74.6 (C≡*C*H), 70.4, 70.1, 69.0 (3 × CH_2O), 68.4 (CH_2O, C1), 66.9 (C-5), 64.6 (C-6), 58.3 (CH_2O–C≡CH), 20.7 (CH_3CO). Anal. Calcd for $C_{15}H_{20}O_7$: C, 57.69; H, 6.45. Found: C, 57.42; H, 6.61. HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{15}H_{20}NaO_7$: 335.11012, found: 335.11036.

(2*S*,6*S*)-6-Acetoxymethyl-2-(2-propargyloxy)-2*H*-pyran-3(6*H*)-one (3b). Yield 75%; R_f 0.59 (hexane–EtOAc 1 : 1); $[\alpha]_D^{20}$ −12.6 (*c* 0.3 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 6.92 (1 H, dd, $J_{3,5}$ = 1.7, $J_{3,4}$ = 10.7 Hz, H-4), 6.15 (1 H, dd, $J_{4,5}$ = 2.3, $J_{3,4}$ = 10.6 Hz, H-3), 5.01 (1 H, s, H-1), 4.74 (1 H, dddd, $J_{3,5}$ = 1.7, $J_{4,5}$ = 2.3, $J_{5,6b}$ = 4.6, $J_{5,6a}$ = 5.4 Hz, H-5), 4.35, 4.29 (2 H, 2 dd, $J_{CH2O,C\equiv CH}$ = 2.4, *J* = 15.7 Hz, CH_2O), 4.30 (1 H, dd, $J_{5,6a}$ = 5.4, $J_{6a,6b}$ = 11.7 Hz, H-6a), 4.19 (1 H, dd, $J_{5,6b}$ = 4.6, $J_{6a,6b}$ = 11.6 Hz, H-6b), 2.46 (1 H, t, $J_{CH2O,C\equiv CH}$ = 2.4 Hz, C≡C*H*), 2.03 (3 H, s, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.5 (C-2), 170.6 (–*C*OCH$_3$), 147.0 (C-3), 126.3 (C-4), 96.4 (C-1), 78.2 (*C*≡CH), 75.7 (C≡*C*H), 67.2 (C-5), 64.4 (C-6), 56.3 (CH_2O), 20.7 (CH_3CO). Anal. Calcd for $C_{11}H_{12}O_5$: C, 58.93; H, 5.39. Found: C, 58.99; H, 5.48. HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{11}H_{12}NaO_5$: 247.05769, found: 247.05822.

2-(2-Propargyloxyethoxy)ethyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-*threo*-hexopyranosid-2-ulose (5a). (2*S*,6*S*)-6-Acetoxymethyl-2-[2-(2-propargyloxyethoxy)ethoxy]-2*H*-pyran-3(6*H*)-one (**3a**, 100 mg, 0.321 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose **4** (116 mg, 0.321 mmol) were dissolved in anhydrous CH_3CN (1.5 mL). The reaction mixture was flushed with a stream of nitrogen, sealed, and cooled to −70 °C. NEt_3 (6 μL) was added, and the mixture was stirred at −70 °C for 4 h. TLC (EtOAc–hexane, 1 : 1) showed complete conversion of the starting materials. The reaction mixture was concentrated, and the residue purified by flash chromatography to give **5a** (178 mg). Yield 82%; R_f 0.54 (hexane–EtOAc, 1 : 2.5); $[\alpha]_D^{20}$ −20.6 (*c* 0.3 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 5.36 (1 H, dd, $J_{4',5'}$ = 1.0, $J_{3',4'}$ = 3.4 Hz, H-4′), 5.11 (1 H, t, $J_{1',2'}$ = $J_{2',3'}$ = 10.0 Hz, H-2′), 4.97 (1 H, dd, $J_{3',4'}$ = 3.4, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.73 (1 H, ddd, $J_{4,5}$ = 1.9, $J_{5,6a}$ = 4.5, $J_{5,6b}$ = 7.5 Hz, H-5), 4.63 (1 H, s, H-1), 4.50 (1 H, d, $J_{1',2'}$ = 10.0 Hz, H-1′), 4.23 (1 H, dd, $J_{5,6a}$ = 4.5, $J_{6a,6b}$ = 11.8 Hz, H-6a), 4.18 (1 H, dd, $J_{5,6b}$ = 7.5, $J_{6a,6b}$ = 11.8 Hz, H-6b), 4.14 (2 H, d, $J_{CH2-C\equiv CH,C\equiv CH}$ = 2.4 Hz, CH_2–C≡CH), 4.10 (1 H, dd, $J_{5',6'a}$ = 6.4, $J_{6'a,6'b}$ = 11.3 Hz, H-6′a), 4.04 (1 H, dd, $J_{5',6'b}$ = 6.9, $J_{6'a,6'b}$ = 11.3 Hz, H-6′b), 3.83 (1 H, ddd, $J_{4',5'}$ = 1.0, $J_{5',6'a}$ = 6.4, $J_{5',6'b}$ = 6.9 Hz, H-5′), 3.69–3.59 (9 H, m, H-4, 4 × CH_2O), 3.16 (1 H, dd, $J_{3a,4}$ = 4.9, $J_{3a,3b}$ = 15.2 Hz, H-3a), 2.74 (1 H, dd, $J_{3b,4}$ = 2.6, $J_{3a,3b}$ = 15.2 Hz, H-3b), 2.39 (1 H, t, $J_{CH2-C\equiv CH,C\equiv CH}$ = 2.4 Hz, C≡C*H*), 2.10, 2.03, 2.02, 1.99, 1.91 (15 H, 5 s, 5 CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ 198.4 (C-2), 170.5, 170.3, 170.1,

169.9, 169.7 (–*C*OCH$_3$), 98.9 (C-1), 82.6 (C-1′), 79.6 (*C*≡CH), 74.6 (C≡*C*H), 74.4 (C-5′), 71.7 (C-3′), 70.4, 69.9, 69.1, 67.3 (4 × *C*H$_2$O), 68.7 (C-5), 67.0, 66.9 (C-2′, C-4′), 64.6 (C-6), 61.1 (C-6′), 58.4 (*C*H$_2$–C≡CH), 44.5 (C-4), 43.4 (C-3), 20.8, 20.7, 20.6 (×2), 20.5 (*C*H$_3$CO). Anal. Calcd for $C_{29}H_{40}O_{16}S$: C, 51.47; H, 5.96; S, 4.74. Found: C, 51.61; H, 6.23; S, 4.56. HRMS (ESI): *m*/*z* [*M* + Na]$^+$ calcd for $C_{29}H_{40}NaO_{16}S$: 699.19293, found: 699.19244.

2-(2-Propargyloxyethoxy)ethyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-*erythro*-hexopyranosid-2-ulose (5b). (2*S*,6*S*)-6-Acetoxymethyl-2-[2-(2-propargyloxyethoxy)ethoxy]-2*H*-pyran-3(6*H*)-one (**3a**, 100 mg, 0.321 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose **4** (116 mg, 0.321 mmol) were dissolved in anhydrous CH$_3$CN (1 mL). The vial was flushed with a stream of nitrogen and sealed. Et$_3$N (6 μL) was added, and the mixture was heated at 80 °C under microwave irradiation for 30 min. TLC (EtOAc–hexane, 1 : 1) showed complete conversion of the starting materials. The reaction mixture was concentrated, and the residue purified by flash chromatography to give **5b** (191 mg). Yield 88%; R_f 0.26 (hexane–EtOAc, 1 : 2.5); $[\alpha]_D^{20}$ −20.6 (*c* 0.3 in CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.36 (1 H, dd, $J_{4',5'}$ = 0.9, $J_{3',4'}$ = 3.3 Hz, H-4′), 5.09 (1 H, t, $J_{1',2'} = J_{2',3'}$ = 9.9 Hz, H-2′), 4.97 (1 H, dd, $J_{3',4'}$ = 3.3, $J_{2',3'}$ = 9.9 Hz, H-3′), 4.67 (1 H, s, H-1), 4.56 (1 H, d, $J_{1',2'}$ = 10.0 Hz, H-1′), 4.46 (1 H, dd, $J_{5,6a}$ = 1.9, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.33 (1 H, dd, $J_{5,6b}$ = 5.0, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.28 (1 H, ddd, $J_{5,6a}$ = 2.0, $J_{5,6b}$ = 5.0, $J_{4,5}$ = 11.0 Hz, H-5), 4.14 (2 H, d, $J_{CH2-C\equiv CH,C\equiv CH}$ = 2.4 Hz; C*H*$_2$–C≡CH), 4.06 (2 H, m, H-6′a, H-6′b), 3.90 (1 H, m, H-5′), 3.83, 3.69 (2 H, 2 m, C*H*$_2$O, C1), 3.65–3.57 (6 H, m, 3 × C*H*$_2$O), 3.26 (1 H, ddd, $J_{3b,4}$ = 5.1, $J_{4,5}$ = 10.8, $J_{3a,4}$ = 12.5 Hz, H-4), 2.90 (1 H, dd, $J_{3a,4}$ = 12.5, $J_{3a,3b}$ = 14.7 Hz, H-3a), 2.80 (1 H, dd, $J_{3b,4}$ = 5.1, $J_{3a,3b}$ = 14.8 Hz, H-3b), 2.42 (1 H, t, $J_{CH2-C\equiv CH,C\equiv CH}$ = 2.4 Hz, C≡C*H*), 2.10, 2.03, 2.02, 1.99, 1.91 (5 s, 15H; 5 C*H*$_3$CO); ^{13}C NMR (125 MHz, CDCl$_3$) δ 198.7 (C-2), 170.5, 170.3, 170.1, 169.9, 169.4 (–*C*OCH$_3$), 98.8 (C-1), 83.8 (C-1′), 79.5 (*C*≡CH), 74.7 (C≡*C*H), 74.4 (C-5′), 71.5 (C-3′), 70.3, 69.8, 68.9, 67.4 (4 × *C*H$_2$O), 69.6 (C-5), 67.1 (C-2′), 67.0 (C-4′), 63.4 (C-6), 61.5 (C-6′), 58.2 (*C*H$_2$–C≡CH), 43.3 (C-3), 42.9 (C-4), 20.8, 20.7, 20.6 (×2), 20.5 (*C*H$_3$CO). Anal. Calcd for $C_{29}H_{40}O_{16}S$: C, 51.47; H, 5.96; S, 4.74. Found: C, 51.49; H, 6.14; S, 4.66. HRMS (ESI): *m*/*z* [*M* + Na]$^+$ calcd for $C_{29}H_{40}NaO_{16}S$: 699.19293, found: 699.19230.

2-(2-Propargyloxyethoxy)ethyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-*ribo*-hexopyranoside (6). To a solution of **5b** (95 mg, 0.141 mmol) in MeOH (2 mL) was added NaBH$_4$ (5.6 mg, 0.141 mmol) and stirred at room temperature. After 20 min, TLC showed no starting material remaining. The mixture was diluted with MeOH and treated with Dowex 50 H$^+$, then concentrated and subjected to column chromatography. Thiodisaccharide **6** (79 mg) was isolated as a pure product. Yield 83%; R_f 0.24 (hexane–EtOAc, 1 : 3); $[\alpha]_D^{20}$ −20.6 (*c* 0.3 in CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.36 (1 H, dd, $J_{4',5'}$ = 0.8, $J_{3',4'}$ = 3.2 Hz, H-4′), 5.09 (1 H, t, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2′), 4.97 (1 H, dd, $J_{3',4'}$ = 3.3, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.80 (1 H, d, $J_{1,2}$ = 3.5 Hz, H-1), 4.55 (1 H, d, $J_{1',2'}$ = 10.0 Hz, H-1′), 4.38 (1 H, dd, $J_{5,6a}$ = 0.9, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.28 (1 H, dd, $J_{5,6b}$ = 5.0, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.14 (2 H, d, $J_{CH2-C\equiv CH,C\equiv CH}$ = 2.3 Hz, C*H*$_2$–C≡CH), 4.09 (1 H, dd, $J_{5',6'a}$ = 7.0, $J_{6'a,6'b}$ = 11.4 Hz, H-6′a), 4.04 (1 H, dd, $J_{5',6'b}$ = 6.3, $J_{6'a,6'b}$ = 11.4 Hz, H-6′b), 3.88 (1 H, m, H-5′), 3.81 (1 H, m, H-5), 3.80, 3.67 (2 H, 2 m, C*H*$_2$O, C1), 3.66–3.57 (7 H, m, H-2, 3 × C*H*$_2$O), 2.83 (1 H, ddd, $J_{3a,4}$ = 4.1, $J_{4,5}$ = 11.3, $J_{3b,4}$ = 12.5 Hz, H-4), 2.41 (1 H, t, $J_{CH2-C\equiv CH,C\equiv CH}$ = 2.4 Hz, C≡C*H*), 2.33 (1 H, br s, O*H*), 2.20 (1 H, ddd, $J_{2,3a}$ = 4.5, $J_{3a,4}$ = 4.5, $J_{3a,3b}$ = 12.5 Hz, H-3a), 2.11, 2.04, 2.01, 1.99, 1.92 (15 H, 5 s, 5 C*H*$_3$CO), 1.85 (1 H, q, $J_{2,3a} = J_{3b,4} = J_{3a,3b}$ = 12.4 Hz, H-3b); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.5, 170.3, 170.1, 169.9, 169.5 (–*C*OCH$_3$), 98.2 (C-1), 83.6 (C-1′), 79.4 (*C*≡CH), 74.8 (C≡*C*H), 74.3 (C-5′), 71.7 (C-3′), 70.2, 70.0, 68.9, 67.4 (4 × *C*H$_2$O), 69.8 (C-5), 67.7 (C-2), 67.2 (C-2′), 67.0 (C-4′), 64.1 (C-6), 61.4 (C-6′), 58.3 (*C*H$_2$–C≡CH), 40.1 (C-4), 35.6 (C-3), 20.8, 20.7, 20.6 (×2), 20.5 (*C*H$_3$CO). Anal. Calcd for $C_{29}H_{42}O_{16}S$: C, 51.32; H, 6.24; S, 4.72. Found: C, 51.28; H, 6.54; S 5.03. HRMS (ESI): *m*/*z* [*M* + Na]$^+$ calcd for $C_{29}H_{42}NaO_{16}S$: 701.20858, found: 701.21112.

Propargyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-*threo*-hexopyranosid-2-ulose (7). (2*S*,6*S*)-6-Acetoxymethyl-2-(2-propargyloxy)-2*H*-pyran-3(6*H*)-one (**3b**, 135 mg, 0.602 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose **4** (219 mg, 0.602 mmol) were dissolved in anhydrous CH$_3$CN (3 mL). The reaction vial was flushed with nitrogen, sealed, and cooled to −70 °C. Et$_3$N (4 μL) was added, and the mixture was stirred at −70 °C for 4 h. TLC (EtOAc–hexane, 1 : 1) showed complete conversion of the starting materials into a major spot of R_f 0.33. The reaction mixture was concentrated, and the residue purified by flash chromatography (hexane–EtOAc, 1 : 1) to give **7** (294 mg). Yield 83%; R_f 0.33 (hexane–EtOAc, 1 : 1); $[\alpha]_D^{20}$ −15.1 (*c* 0.3 in CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 5.36 (1 H, m, H-4′), 5.11 (1 H, t, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2′), 4.97 (1 H, dd, $J_{3',4'}$ = 3.3, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.78 (1 H, s, H-1), 4.65 (1 H, m, H-5), 4.52 (1 H, d, $J_{1',2'}$ = 9.9 Hz, H-1′), 4.27 (2 H, m, C*H*$_2$O), 4.22 (2 H, m, H-6a, H-6b), 4.10 (1 H, dd, $J_{5',6'a}$ = 6.4, $J_{6'a,6'b}$ = 11.3 Hz, H-6′a), 4.04 (1 H, dd, $J_{5',6'b}$ = 6.8, $J_{6'a,6'b}$ = 11.3 Hz, H-6′b), 3.83 (1 H, m, H-5′), 3.65 (1 H, m, H-4), 3.11 (1 H, dd, $J_{3a,4}$ = 4.9, $J_{3a,3b}$ = 15.4 Hz, H-3a), 2.79 (1 H, dd, $J_{3b,4}$ = 1.6, $J_{3a,3b}$ = 15.4 Hz, H-3b), 2.47 (1 H, t, $J_{CH2O,C\equiv CH}$ = 2.4 Hz, C≡C*H*), 2.09, 2.03, 2.01, 1.99, 1.91 (15 H, 5 s, 5 C*H*$_3$CO); ^{13}C NMR (125 MHz, CDCl$_3$) δ 197.8 (C-2), 170.4, 170.3, 170.0, 169.9, 169.6 (–*C*OCH$_3$), 97.1 (C-1), 82.5 (C-1′), 77.7 (*C*≡CH), 75.9 (C≡*C*H), 74.5 (C-5′), 71.7 (C-3′), 69.2 (C-5), 67.0, 66.9 (C-2′, C-4′), 64.4 (C-6), 61.1 (C-6′), 54.8 (*C*H$_2$O), 44.1 (C-4), 43.5 (C-3), 20.6 (×5) (*C*H$_3$CO). Anal. Calcd for $C_{25}H_{32}O_{14}S$: C, 51.02; H, 5.48; S, 5.45. Found: C, 50.94, H, 5.66, S, 5.19. HRMS (ESI): *m*/*z* [*M* + Na]$^+$ calcd for $C_{25}H_{32}NaO_{14}S$: 611.14050, found: 611.13995.

Propargyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-*ribo*-hexopyranoside (8). To a solution of **7** (128 mg, 0.218 mmol) in anhydrous THF (2.5 mL) was added NaBH$_4$ (26 mg, 0.650 mmol) and stirred at room temperature. After 20 min, TLC showed no starting material remaining and one major product of R_f 0.36 (hexane–EtOAc, 1 : 1.5). The mixture was diluted with MeOH and treated with Dowex 50 H$^+$, then concentrated and subjected to column chromatography. The major product was identified as

8 (105 mg). Yield 82%; R_f 0.36 (hexane–EtOAc 1 : 1.5); $[\alpha]_D^{20}$ −63.1 (*c* 0.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.44 (1 H, m, H-4′), 5.16 (1 H, t, $J_{1',2'} = J_{2',3'} = 10.0$ Hz, H-2′), 5.05 (1 H, dd, $J_{3',4'} = 3.4$, $J_{2',3'} = 9.9$ Hz, H-3′), 5.01 (1 H, d, $J_{1,2} = 3.5$ Hz, H-1), 4.63 (1 H, d, $J_{1',2'} = 10.0$ Hz, H-1′), 4.47 (1 H, dd, $J_{5,6a} = 2.0$, $J_{6a,6b} = 12.0$ Hz, H-6a), 4.31 (3 H, m, CH_2O, H-6b), 4.16 (1 H, dd, $J_{5',6'a} = 6.8$, $J_{6'a,6'b} = 11.3$ Hz, H-6′a), 4.10 (1 H, dd, $J_{5',6'b} = 6.5$, $J_{6'a,6'b} = 11.3$ Hz, H-6′b), 3.92 (1 H, ddd, $J_{4',5'} = 1.0$, $J_{5',6'a} \cong J_{5',6'b} \cong 6.6$ Hz, H-5′), 3.84 (1 H, ddd, $J_{5,6a} = 2.0$, $J_{5,6b} = 5.2$, $J_{4,5} = 11.0$ Hz, H-5), 3.77 (1 H, ddd, $J_{1,2} = 3.8$, $J_{2,3a} = 4.9$, $J_{2,3b} = 8.7$ Hz, H-2), 3.35 (1 H, br s, O*H*), 2.91 (1 H, ddd, $J_{3eq,4} = 4.2$, $J_{4,5} = 11.0$, $J_{3ax,4} = 12.9$ Hz, H-4), 2.50 (1 H, t, $J_{CH2O,C\equiv CH} = 2.4$ Hz, C≡C*H*), 2.30 (1 H, ddd, $J_{2,3eq} = 9.2$, $J_{3eq,4} = 4.5$, $J_{3eq,3ax} = 12.3$ Hz, H-3eq), 2.17, 2.11, 2.08, 2.07, 2.00 (15 H, 5 s, 5 CH_3CO), 1.90 (1 H, q, $J_{3ax,2} \cong J_{3ax,3eq} \cong J_{3ax,4} \cong 12.2$ Hz, H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.4, 170.2, 170.0, 169.5 (–*C*OCH$_3$), 96.7 (C-1), 83.4 (C-1′), 78.7 (*C*≡CH), 75.2 (C≡*C*H), 74.4 (C-5′), 71.7 (C-3′), 70.3 (C-5), 67.5 (C-2), 67.3 (C-2′), 67.1 (C-4′), 63.9 (C-6), 61.4 (C-6′), 55.0 (*C*H$_2$O), 39.9 (C-4), 35.5 (C-3), 20.9, 20.7 (×2), 20.6, 20.5 (*C*H$_3$CO). Anal. Calcd for $C_{25}H_{34}O_{14}S$: C, 50.84; H, 5.80; S, 5.43. Found: C, 51.06; H, 5.66; S, 5.24. HRMS (ESI): *m*/*z* [*M* + Na]$^+$ calcd for $C_{25}H_{34}NaO_{14}S$: 613.15615, found: 613.15557.

Propargyl [2-^{2}H]-6-*O*-acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-*ribo*-hexopyranoside (9). To a solution of **7** (95 mg, 0.162 mmol) in anhydrous THF (2.0 mL) was added $NaBD_4$ (20 mg, 0.486 mmol) and stirred at room temperature. After 20 min, TLC showed no starting material remaining and one major product of R_f 0.32 (hexane–EtOAc, 1 : 1.5). The mixture was diluted with MeOH and treated with Dowex 50 H$^+$, then concentrated and subjected to column chromatography. The major product was identified as **9** (76 mg). Yield 80%; R_f 0.32 (hexane–EtOAc 1 : 1.5); $[\alpha]_D^{20}$ −62.5 (*c* 0.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.42 (1 H, m, H-4′), 5.14 (1 H, t, $J_{1',2'} = J_{2',3'} = 10.0$ Hz, H-2′), 5.05 (1 H, dd, $J_{3',4'} = 3.4$, $J_{2',3'} = 9.9$ Hz, H-3′), 4.98 (1 H, s, H-1), 4.61 (1 H, d, $J_{1',2'} = 10.0$ Hz, H-1′), 4.45 (1 H, dd, $J_{5,6a} = 2.0$, $J_{6a,6b} = 12.0$ Hz, H-6a), 4.31 (3 H, m, CH_2O, H-6b), 4.16 (1 H, dd, $J_{5',6'a} = 6.8$, $J_{6'a,6'b} = 11.3$ Hz, H-6′a), 4.10 (1 H, dd, $J_{5',6'b} = 6.5$, $J_{6'a,6'b} = 11.3$ Hz, H-6′b), 3.92 (1 H, ddd, $J_{4',5'} = 1.0$, $J_{5',6'a} \cong J_{5',6'b} \cong 6.6$ Hz, H-5′), 3.84 (1 H, ddd, $J_{5,6a} = 2.0$, $J_{5,6b} = 5.2$, $J_{4,5} = 11.0$ Hz, H-5), 3.34 (1 H, br s, O*H*), 2.90 (1 H, ddd, $J_{3eq,4} = 4.2$, $J_{4,5} = 11.0$, $J_{3ax,4} = 12.9$ Hz, H-4), 2.50 (1 H, t, $J_{CH2O,C\equiv CH} = 2.4$ Hz, C≡C*H*), 2.30 (1 H, dd, $J_{3eq,4} = 4.1$, $J_{3eq,3ax} = 12.3$ Hz, H-3eq), 2.17, 2.11, 2.08, 2.07, 2.00 (15 H, 5 s, 5 CH_3CO), 1.90 (t, 1 H, $J_{3ax,3eq} \cong J_{3ax,4} \cong 12.2$ Hz, H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.5, 170.3, 170.2, 170.0, 169.5 (–*C*OCH$_3$), 96.6 (C-1), 83.4 (C-1′), 78.7 (*C*≡CH), 75.2 (C≡*C*H), 74.4 (C-5′), 71.7 (C-3′), 70.3 (C-5), 67.2 (C-2′), 67.0 (C-4′), 63.9 (C-6), 61.4 (C-6′), 54.9 (*C*H$_2$O), 39.9 (C-4), 35.4 (C-3), 20.9, 20.7 (×2), 20.6, 20.5 (*C*H$_3$CO).

General procedure for the click reaction: synthesis of compounds 11, 14, 15

The corresponding azido-saccharide **10** or **13** (1.00 mmol) and the selected alkynyl-thiodisaccharide **6** or **8** (1.00 mmol per mol of reacting azide) were dissolved in a dioxane–H_2O mixture (8 : 2 mL, 35 mL). Copper sulfate (0.25 mmol per mol of reacting azide) and sodium ascorbate (0.50 mmol per mol of azide reacting group) were added, and the mixture was stirred at 70 °C under microwave irradiation, until MS indicated the disappearance of starting materials and intermediates (40 min). The mixture was then poured into a 1 : 1 H_2O–NH_4Cl solution (60 mL) and extracted with EtOAc (4 × 30 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 11. Yield 78%; R_f 0.39 (EtOAc); $[\alpha]_D^{20}$ −33.4 (*c* 0.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (1 H, s, H-triazole), 5.44 (1 H, dd, $J_{3G,4G} = 9.4$, $J_{2G,3G} = 10.2$ Hz, H-3G), 5.42 (1 H, dd, $J_{4',5'} = 1.0$, $J_{3',4'} = 3.4$ Hz, H-4′), 5.12 (1 H, t, $J_{1',2'} = J_{2',3'} = 10.0$ Hz, H-2′), 5.02 (1 H, dd, $J_{3',4'} = 3.4$, $J_{2',3'} = 10.0$ Hz, H-3′), 4.92 (1 H, d, $J_{1,2} = 3.6$ Hz, H-1), 4.89 (1 H, d, $J_{1G,2G} = 3.6$ Hz, H-1G), 4.85, 4.66 (2 H, 2 d, $J = 12.4$ Hz, CH_2-triazole), 4.80 (1 H, dd, $J_{1G,2G} = 3.6$, $J_{2G,3G} = 10.2$ Hz, H-2G), 4.78 (1 H, dd, $J_{3G,4G} = 9.4$, $J_{4G,5G} = 10.2$ Hz, H-4G), 4.60 (1 H, d, $J_{1',2'} = 9.9$ Hz, H-1′), 4.57 (1 H, dd, $J_{5G,6aG} = 2.5$, $J_{6aG,6bG} = 14.4$ Hz, H-6aG), 4.45 (1 H, dd, $J_{5,6a} = 2.0$, $J_{6a,6b} = 12.0$ Hz, H-6a), 4.39 (1 H, dd, $J_{5G,6bG} = 7.8$, $J_{6aG,6bG} = 14.4$ Hz, H-6bG), 4.32 (1 H, dd, $J_{5,6b} = 5.2$, $J_{6a,6b} = 12.0$ Hz, H-6b), 4.19 (1 H, ddd, $J_{5G,6aG} = 2.5$, $J_{5G,6bG} = 8.0$, $J_{4G,5G} = 10.3$ Hz, H-5G), 4.15 (1 H, dd, $J_{5',6'a} = 6.6$, $J_{6'a,6'b} = 11.2$ Hz, H-6′a) 4.09 (1 H, dd, $J_{5',6'b} = 6.6$, $J_{6'a,6'b} = 11.2$ Hz, H-6′b), 3.97 (1 H, ddd, $J_{4',5'} = 0.9$, $J_{5',6'a} = J_{5',6'b} = 6.6$ Hz, H-5′), 3.90 (1 H, ddd, $J_{5,6a} = 1.9$, $J_{5,6b} = 5.0$, $J_{4,5} = 10.9$ Hz, H-5), 3.70 (1 H, m, H-2), 3.14 (3 H, s, –OCH_3), 2.87 (1 H, ddd, $J_{3a,4} = 4.1$, $J_{4,5} = 11.1$, $J_{3b,4} = 12.2$ Hz, H-4), 2.29 (1 H, d, O*H*), 2.22 (1 H, ddd, $J_{2,3a} = 4.6$, $J_{3a,4} = 4.1$, $J_{3a,3b} = 12.6$ Hz, H-3a), 2.13, 2.08, 2.03, 2.02, 2.01, 2.00, 1.97, 1.94 (8 s, 24H; 8 CH_3CO), 1.87 (1 H, q, $J_{3a,2} \cong J_{3a,3b} \cong J_{3b,4} \cong 12.4$ Hz, H-3b); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.3, 170.2, 170.1, 169.9, 169.7, 169.6, 169.5 (–*C*OCH$_3$), 143.9 (C-4 triazole), 124.2 (C-5 triazole), 97.0 (C-1), 96.7 (C-1G), 83.3 (C-1′), 74.3 (C-5′), 71.7 (C-3′), 70.6, 70.0, 69.7, 69.6, 67.7, 67.6, 67.3, 67.1 (C-2G, C-3G, C-4G, C-5G, C-2, C-5, C-2′, C-4′), 64.0 (C-6), 61.3 (C-6′), 60.4 (*C*H$_2$-triazole), 55.5 (–O*C*H$_3$), 50.6 (C-6G), 39.9 (C-4), 35.5 (C-3), 20.9, 20.7 (×2), 20.6, 20.5 (*C*H$_3$CO). Anal. Calcd for $C_{38}H_{53}N_3O_{22}S$: C, 48.77; H, 5.71; N, 4.49; S, 3.43. Found: C, 48.88; H, 5.61; S, 3.65. HRMS (ESI): *m*/*z* [*M* + H]$^+$ calcd for $C_{38}H_{54}N_3O_{22}S$: 936.29142, found: 936.28842.

Compound 14. Yield 66%; R_f 0.55 (EtOAc–MeOH, 96 : 4); $[\alpha]_D^{20}$ −20.6 (*c* 0.3 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (1 H, s, H-triazole), 5.44 (2 H, m, H-3 T, H-4′), 5.05 (2 H, m, H-1, H-3′), 4.96 (3 H, m, H-2 T, H-4 T, H-2′), 4.90, 4.69 (4 H, 2 d, $J = 11.8$ Hz, 2 × CH_2-triazole), 4.73 (1 H, d, $J_{1T,2T} = 3.7$ Hz, H-1 T), 4.62 (2 H, m, H-6aT, H-1′), 4.49 (1 H, dd, $J_{5,6a} = 1.0$, $J_{6a,6b} = 11.8$ Hz, H-6a), 4.39 (2 H, m, H-6bT, H-6b), 4.25–4.05 (3 H, m, H-5 T, H-6′a, H-6′b), 3.90 (1 H, ddd, $J_{4',5'} = 1.0$, $J_{5',6'a} \cong J_{5',6'b} \cong 6.6$ Hz, H-5′), 3.89 (1 H, ddd, $J_{5,6a} = 2.0$, $J_{5,6b} = 5.2$, $J_{4,5} = 11.0$ Hz, H-5), 3.77 (1 H, ddd, $J_{1,2} = 3.8$, $J_{2,3a} = 4.9$, $J_{2,3b} = 8.7$ Hz, H-2), 2.96 (1 H, ddd, $J_{3eq,4} = 4.1$, $J_{4,5} = 11.0$, $J_{3ax,4} = 12.9$ Hz, H-4), 2.37 (1 H, ddd, $J_{2,3eq} = 4.5$, $J_{3eq,4} = 9.2$, $J_{3eq,3ax} = 12.3$ Hz, H-3eq), 2.14, 2.13, 2.08, 2.05, 2.04, 2.03, 2.00, 1.96 (24 H, 8 s, 8 × CH_3CO), 1.91 (1 H, q, $J_{3ax,2} \cong J_{3ax,3eq} \cong J_{3ax,4} \cong 12.2$ Hz,

H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.4, 170.1, 169.9, 169.8, 169.7, 169.6, 169.5 (–*C*OCH$_3$), 144.5 (C-4 triazole), 124.5 (C-5 triazole), 97.5 (C-1), 91.5 (C-1 T), 85.2 (C-1′), 74.5 (C-5′), 71.6 (C-3′), 69.9, 69.8, 69.5, 69.3, 68.6, 67.5, 67.2, 67.1 (C-2 T, C-3 T, C-4 T, C-5 T, C-2, C-5, C-2′, C-4′), 64.1 (C-6), 61.4 (C-6′), 60.5 (*C*H$_2$-triazole), 50.7 (C-6 T), 41.7 (C-4), 36.5 (C-3), 20.9, 20.7 (×3), 20.6, 20.5 (*C*H$_3$CO). Anal. Calcd for $C_{74}H_{100}N_6O_{43}S_2$: C, 48.68; H, 5.52; N, 4.60; S, 3.51. Found: C, 48.35; H, 5.31; N, 4.89; S, 3.81. HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{74}H_{100}N_6NaO_{43}S_2$: 1847.51564, found: 1847.51266.

Compound 15. Yield 55%; R_f 0.44 (EtOAc–MeOH, 9 : 1); $[\alpha]_D^{20}$ −20.6 (*c* 0.3 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d$_6$) δ 7.79 (1 H, s, H-triazole), 5.30 (2 H, m, H-3 T, H-4′), 5.09 (1 H, dd, $J_{3',4'}$ = 3.5, $J_{2',3'}$ = 9.9 Hz, H-3′), 4.96 (m2 H, H-2 T, H-2′), 4.88 (1 H, t, $J_{3T,4T} = J_{4T,5T}$ = 9.7 Hz, H-4 T), 4.80 (1 H, d, $J_{1T,2T}$ = 3.6 Hz, H-1 T), 4.73 (1 H, d, $J_{1',2'}$ = 10.0 Hz, H-1′), 4.70 (1 H, d, $J_{1,2}$ = 3.3 Hz, H-1), 4.52 (3 H, m, CH$_2$-triazole, H-6aT), 4.39 (1 H, dd, $J_{5T,6bT}$ = 8.1, $J_{6aT,6bT}$ = 14.4 Hz, H-6bT), 4.34 (1 H, dd, $J_{5,6a}$ = 1.9, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.22–4.14 (2 H, m, H-5 T, H-6b), 4.09–3.95 (3 H, m, H-5′, H-6′a, H-6′b), 3.74 (3 H, m, H-5, C*H*$_2$O), 3.60 (7 H, m, H-2, 3 × C*H*$_2$O), 2.84 (1 H, ddd, $J_{3eq,4}$ = 4.2, $J_{4,5}$ = 11.2, $J_{3ax,4}$ = 12.9 Hz, H-4), 2.04, 1.94 (2 H, 2 m, H-3eq, H-3ax), 2.08, 2.00, 1.99, 1.97, 1.96, 1.95, 1.91, 1.88 (24 H, 8 s, 8 C*H*$_3$CO); ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d$_6$) δ 169.8, 169.5, 169.1, 169.0, 168.9, 168.8, 168.6 (–*C*OCH$_3$), 144.3 (C-4 triazole), 124.2 (C-5 triazole), 97.8 (C-1), 90.9 (C-1 T), 82.5 (C-1′), 73.4 (C-5′), 70.9 (C-3′), 69.7, 69.4, 69.3, 69.1, 69.0, 68.8, 68.3, 68.0, 67.0, 66.9, 66.8, 66.4 (C-2 T, C-3 T, C-4 T, C-5 T, C-2, C-5, C-2′, C-4′, 4 × *C*H$_2$O), 63.6 (C-6, *C*H$_2$-triazole), 61.0 (C-6′), 49.6 (C-6 T), 34.7 (C-4), 34.7 (C-3), 20.3, 20.2 (×3), 20.1, 20.0 (*C*H$_3$CO). Anal. Calcd for $C_{82}H_{116}N_6O_{47}S_2$: C, 49.20; H, 5.84; N, 4.20; S, 3.20. Found: C, 49.28; H, 5.61; N, 4.45; S, 2.98. HRMS (ESI): *m*/*z* $[M + Na + H]^{2+}$ calcd for $C_{82}H_{117}N_6NaO_{47}S_2$: 1012.31389, found: 1012.31576.

General procedure for the *O*-deacetylation

Compounds **8**, **11**, **14**, and **15** (0.10 mmol) were suspended in a mixture of MeOH–Et$_3$N–H$_2$O 4 : 1 : 5 (10 mL) and stirred at room temperature. The solid was progressively dissolved 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$_2$O, 2.5 : 1 : 1) whose R_f are indicated in each case.

Compound 12. Yield 100%; R_f 0.32 (BuOH–EtOH–H$_2$O, 2.5 : 1 : 1); $[\alpha]_D^{20}$ −59.6 (*c* 0.4 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O) δ 8.06 (1 H, s, H-triazole), 4.88 (1 H, d, $J_{1,2}$ = 3.4 Hz, H-1), 4.76 (3 H, m, H-6aG, C*H*$_2$-triazole), 4.66 (1 H, d, $J_{1G,2G}$ = 3.7 Hz, H-1G), 4.56 (1 H, dd, $J_{5,6bG}$ = 7.9, $J_{6aG,6bG}$ = 14.6 Hz, H-6bG), 4.50 (1 H, d, $J_{1',2'}$ = 9.8 Hz, H-1′), 3.88 (2 H, m, H-5G, H-4′), 3.80 (3 H, m, H-6a, H-6b, H-2), 3.70 − 3.52 (5 H, m, H-3G, H-5, H-5′, H-6a′, H-6b′), 3.54 (1 H, dd, $J_{3',4'}$ = 3.3, $J_{4',5'}$ = 9.5 Hz, H-3′), 3.45 (2 H, m, H-2′, H-2G), 3.14 (1 H, dd, $J_{3G,4G} = J_{4G,5G}$ = 9.5 Hz, H-4G), 3.08 (3 H, s, –OC*H*$_3$), 3.00 (1 H, ddd, $J_{3a,4}$ = 4.3, $J_{4,5}$ = 11.3, $J_{3b,4}$ = 12.8 Hz, H-4), 2.13 (1 H, ddd, $J_{2,3a} = J_{3a,4}$ = 4.4, $J_{3a,3b}$ = 12.1 Hz, H-3a), 1.86 (1 H, q, $J_{2,3b} = J_{3b,4} = J_{3a,3b}$ = 12.3 Hz, H-3b); ^{13}C NMR (125 MHz, D$_2$O) δ 143.9 (C-4 triazole), 126.2 (C-5 triazole), 99.1 (C-1G), 97.2 (C-1), 84.7 (C-1′), 78.8 (C-5′), 73.9 (C-3′), 73.0, 72.7 (C-3G, C-5), 71.0 (C-2G), 70.8 (C-4G), 69.8 (C-5G), 69.7 (C-2′), 68.6 (C-4′), 67.1 (C-2), 61.2 (C-6), 61.0 (C-6′), 59.9 (*C*H$_2$-triazole), 54.9 (–O*C*H$_3$), 50.9 (C-6G), 38.5 (C-4), 33.5 (C-3). Anal. Calcd for $C_{22}H_{37}N_3O_{14}S{\cdot}H_2O$: C, 42.78; H, 6.36; N, 6.80; S, 5.19. Found: C, 42.90; H, 6.32; N, 7.02; S, 5.33. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{38}N_3O_{14}S$: 600.20690, found: 600.20919.

Compound 16. Yield 93%; R_f 0.25 (BuOH–EtOH–H$_2$O, 2.5 : 1 : 1); $[\alpha]_D^{20}$ −69.6 (*c* 0.4 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O) δ 8.00 (1 H, s, H-triazole), 4.88 (1 H, d, $J_{1,2}$ = 3.4 Hz, H-1), 4.75 (3 H, m, H-6aT, C*H*$_2$-triazole), 4.54 (3 H, m, H-1 T, H-6bT, H-1′), 4.00 (1 H, ddd, $J_{5T,6aT}$ = 2.4, $J_{5T,6bT}$ = 7.9, $J_{4,5}$ = 10.2 Hz, H-5 T), 3.89 (1 H, m, H-4′), 3.82 (2 H, m, H-6a, H-6b), 3.78 (m1 H, H-2), 3.70 (1 H, t, $J_{2T,3T} \cong J_{3T,4T}$ = 9.2 Hz, H-3 T), 3.67–3.59 (4 H, m, H-5, H-5′, H-6a′, H-6b′), 3.56 (1 H, dd, $J_{3',4'}$ = 3.4, $J_{4',5'}$ = 9.4 Hz, H-3′), 3.46 (1 H, t, $J_{1',2'} = J_{2',3'}$ = 9.6 Hz, H-2′), 3.41 (1 H, dd, $J_{1T,2T}$ = 3.8, $J_{2T,3T}$ = 9.8 Hz, H-2 T), 3.18 (1 H, dd, $J_{3T,4T}$ = 9.1, $J_{4T,5T}$ = 10.0 Hz, H-4 T), 3.02 (1 H, m, H-4), 2.16 (1 H, ddd, $J_{2,3a} = J_{3a,4}$ = 4.6, $^2J_{3a,3b}$ = 12.3 Hz, H-3a), 1.89 (1 H, q, $J_{2,3b} = J_{3b,4} = J_{3a,3b}$ = 12.3 Hz, H-3b); ^{13}C NMR (125 MHz, D$_2$O) δ 143.8 (C-4 triazole), 126.1 (C-5 triazole), 97.1 (C-1), 93.3 (C-1 T), 84.8 (C-1′), 78.9 (C-5′), 73.9 (C-3′), 72.7, 72.6 (C-3 T, C-5), 70.8 (C-4 T), 70.6 (C-2 T), 70.4 (C-5 T), 69.7 (C-2′), 68.9 (C-4′), 67.1 (C-2), 61.3 (C-6), 61.0 (C-6′), 59.9 (*C*H$_2$-triazole), 50.9 (C-6 T), 38.1 (C-4), 33.7 (C-3). Anal. Calcd for $C_{42}H_{68}N_6O_{27}S_2{\cdot}H_2O$: C, 43.07; H, 6.02; N, 7.18; S, 5.48. Found: C, 42.98; H, 6.31; N, 7.01; S, 5.31. HRMS (ESI): *m*/*z* $[M + 2Na]^{2+}$ calcd for $C_{42}H_{68}N_6Na_2O_{27}S_2$: 599.16791, found: 599.17053.

Compound 17. Yield 100%; R_f 0.22 (BuOH–EtOH–H$_2$O, 2.5 : 1 : 1); $[\alpha]_D^{20}$ +38.9 (*c* 0.3 in H$_2$O); ^{1}H NMR (500 MHz, D$_2$O) δ 7.91 (1 H, s, H-triazole), 4.73 (1 H, d, $J_{1,2}$ = 3.4 Hz, H-1), 4.67 (1 H, dd, $J_{5T,6aT}$ = 2.2, $J_{6aT,6bT}$ = 14.6 Hz, H-6aT), 4.55 (2 H, s, C*H*$_2$-triazole), 4.46 (3 H, m, H-1 T, H-6bT, H-1′), 3.92 (1 H, ddd, $J_{5T,6aT}$ = 2.4, $J_{5T,6bT}$ = 7.8, $J_{4,5}$ = 10.5 Hz, H-5 T), 3.82 (2 H, m, H-6a, H-4′), 3.75–3.66 (2 H, m, H-2, H-6b), 3.65–3.53 (13 H, m, H-3 T, H-5, H-5′, H-6a′, H-6b′, 4 × C*H*$_2$O), 3.49 (1 H, dd, $J_{3',4'}$ = 3.4, $J_{4',5'}$ = 9.4 Hz, H-3′), 3.39 (1 H, t, $J_{1',2'} = J_{2',3'}$ = 9.6 Hz, H-2′), 3.29 (1 H, dd, $J_{1T,2T}$ = 3.8, $J_{2T,3T}$ = 9.9 Hz, H-2 T), 3.05 (1 H, t, $J_{3T,4T} = J_{4T,5T}$ = 9.9 Hz, H-4 T), 2.94 (1 H, ddd, $J_{3a,4}$ = 4.6, $J_{4,5}$ = 10.0, $J_{3b,4}$ = 12.3 Hz, H-4), 2.08 (1 H, ddd, $J_{2,3a} = J_{3a,4}$ = 4.6, $J_{3a,3b}$ = 12.3 Hz, H-3a), 1.84 (1 H, q, $J_{2,3b} = J_{3b,4} = J_{3a,3b}$ = 12.3 Hz, H-3b); ^{13}C NMR (125 MHz, D$_2$O) δ 141.6 (C-4 triazole), 123.9 (C-5 triazole), 95.5 (C-1), 91.1 (C-1 T), 82.8 (C-1′), 76.7 (C-5′), 71.7 (C-3′), 70.3, 70.2, 68.6, 68.5, 68.1, 67.5, 67.4, 66.6, 66.5, 65.1, 64.3 (C-2 T, C-3 T, C-4 T, C-5 T, C-2, C-5, C-2′, C-4′, 4 × *C*H$_2$O), 60.8 (*C*H$_2$-triazole), 59.1 (C-6), 58.9 (C-6′), 48.6 (C-6 T), 36.5 (C-4), 31.6 (C-3). Anal. Calcd for $C_{50}H_{84}N_6O_{31}S_2{\cdot}H_2O$: C, 44.57; H, 6.43; N, 6.24; S, 4.76. Found: C, 44.48; H, 6.44; N, 6.35; S, 4.50. HRMS (ESI): *m*/*z* $[M + 2Na]^{2+}$ calcd for $C_{50}H_{84}N_6Na_2O_{31}S_2$: 687.22034, found: 687.21795.

Compound 18. Yield 83%; R_f 0.58 (BuOH–EtOH–H_2O, 2.5 : 1 : 1); $[\alpha]_D^{20}$ −48.3 (*c* 0.2 in H_2O); ^{1}H NMR (500 MHz, D_2O) δ 4.94 (1 H, d, $J_{1,2}$ = 3.4 Hz, H-1), 4.56 (1 H, d, $J_{1',2'}$ = 10.0 Hz, H-1′), 4.25 (2 H, m, CH_2O), 3.89 (1 H, m, H-4′), 3.81 (2 H, m, H-6a, H-6b), 3.76 (1 H, m, H-2), 3.75 (1 H, m, H-5), 3.65–3.55 (4 H, m, H-3′, H-5′, H-6′a, H-6′b), 3.46 (1 H, t, $J_{1',2'} = J_{2',3'}$ = 9.6 Hz, H-2′), 3.03 (1 H, m, H-4), 2.81 (1 H, t, $J_{CH2O,C\equiv CH}$ = 2.4 Hz, C≡C*H*), 2.14 (1 H, m, H-3a), 1.91 (1 H, q, $J_{2,3b} = J_{3b,4} = J_{3a,3b}$ = 12.3 Hz, H-3b); ^{13}C NMR (125 MHz, D_2O) δ 96.6 (C-1), 84.5 (C-1′), 78.8 (C-5′), 77.5 (*C*≡CH), 75.8 (C≡*C*H), 73.9 (C-3′), 72.8 (C-5), 69.7 (C-2′), 68.6 (C-4′), 66.9 (C-2), 61.1, 61.0 (C-6, C-6′), 54.7 (*C*H_2O), 38.4 (C-4), 33.3 (C-3). Anal. Calcd for $C_{15}H_{24}O_9S$: C, 47.36; H, 6.36; S, 8.43. Found: C, 47.66; H, 6.21; S, 8.76. HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{15}H_{24}NaO_9S$: 403.10332, found: 403.10346.

Isothermal titration calorimetry (ITC)

Peanut agglutinin from *Arachis hypogaea* was purchased from Sigma (lyophilized powder, affinity-purified, agglutination activity < 0.1 μg mL^{-1}). A VP-ITC (Microcal) instrument was used for the titrations at 298 K. 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). 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. The titration cell was continuously stirred at 400 rev min^{-1}. 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 enthalpy change (ΔH).

Enzymatic assays

Inhibition of β-galactosidase. The inhibitory activity of compounds **12**, **16**, **17** and **18** towards *E. coli* β-galactosidase (grade VIII, Sigma, EC 3.2.1.23, 117 U mg^{-1}) was determined under standard conditions. The enzyme (0.3 U; 1 U = 1 enzyme unit hydrolyses 1 μmol of *o*-nitrophenyl galactopyranoside per minute) was incubated with *o*-nitrophenyl-β-D-galactopyranoside (concentration range: 0.2–5.0 mM) in sodium phosphate buffer (100 mM, pH = 7.3, $MgCl_2$ 1.2 mM, 2-mercaptoethanol 100 mM) in the absence or presence of the synthetic inhibitors (concentration range: 0.6–4.0 mM); the final volume was 0.50 mL. After incubation for 10 min at 37 °C, the reaction was quenched by addition of sodium borate buffer 0.2 M (4.0 mL, pH = 10.0). The release of *o*-nitrophenol was measured by visible absorption spectroscopy at 410 nm. The K_i and K_m values and the inhibition type were determined from Lineweaver–Burk plots. Data manipulation to obtain K_i values was performed as described previously,[23] using the Microsoft Excel software to fit the Michaelis–Menten model.

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References

1 (*a*) Y. Hideya and H. Hironobu, *Trends Glycosci. Glycotech.*, 2001, **13**, 31; (*b*) H. J. Gabius, *Biochem. Soc. Trans.*, 2008, **36**, 1491; (*c*) N. Jayaraman, *Chem. Soc. Rev.*, 2009, **38**, 3463.
2 D. Schwefel, C. Maierhofer, J. G. Beck, S. Seeberger, K. Diederichs, H. M. Möller, W. Welte and V. Wittmann, *J. Am. Chem. Soc.*, 2010, **132**, 8704.
3 (*a*) E. Freire, *Drug Discovery Today*, 2008, **13**, 869; (*b*) M. Reynolds and S. Pérez, *C. R. Chim.*, 2011, **14**, 74.
4 C. Diehl, O. Engström, T. Delaine, M. Håkansson, S. Genheden, K. Modig, H. Leffler, U. Ryde, U. J. Nilsson and M. Akke, *J. Am. Chem. Soc.*, 2010, **132**, 14577.
5 (*a*) J. Tejler, E. Tullberg, T. Frejd, H. Leffler and U. J. Nilsson, *Carbohydr. Res.*, 2006, **341**, 1353; (*b*) B. A. Salameh, I. Cumpstey, A. Sundin, H. Leffler and U. J. Nilsson, *Bioorg. Med. Chem.*, 2010, **18**, 5367; (*c*) K. A. Stannard, P. M. Collins, K. Ito, E. M. Sullivan, S. A. Scott, E. Gabutero, I. D. Grice, P. Low, U. J. Nilsson, H. Leffler, H. Blanchard and S. J. Ralph, *Cancer Lett.*, 2010, **299**, 95.
6 (*a*) R. M. Lederkremer and C. Marino, *Adv. Carbohydr. Chem. Biochem.*, 2007, **61**, 143; (*b*) D. Hou and T. L. Lowary, *Carbohydr. Res.*, 2009, **344**, 1911.
7 G. Defina, O. Varela and R. M. Lederkremer, *Synthesis*, 1988, 891.
8 (*a*) M. L. Uhrig, V. E. Manzano and O. Varela, *Eur. J. Org. Chem.*, 2006, 162; (*b*) A. J. Cagnoni, M. L. Uhrig and O. Varela, *Bioorg. Med. Chem.*, 2009, **17**, 6203.
9 A. J. Cagnoni, O. Varela, S. G. Gouin, J. Kovensky and M. L. Uhrig, *J. Org. Chem.*, 2011, **76**, 3064.
10 M. L. Uhrig, L. Szilágyi, K. E. Kövér and O. Varela, *Carbohydr. Res.*, 2007, **342**, 1841.
11 (*a*) A. I. Meyers and D. Berney, *J. Org. Chem.*, 1989, **54**, 4673; (*b*) E. Vázquez, A. Galindo, D. Gnecco, S. Bernès, J. L. Terán and R. G. Enriquez, *Tetrahedron: Asymmetry*, 2001, **12**, 3209.

12 S. G. Gouin and J. Kovensky, *Tetrahedron Lett.*, 2007, **48**, 2875.
13 (*a*) S. G. Gouin, E. Vanquelef, J. M. García Fernández, C. Ortiz Mellet, F. Y. Dupradeau and J. Kovensky, *J. Org. Chem.*, 2007, **72**, 9032; (*b*) J. C. Bouillon, A. Meyer, S. Vidal, A. Jochum, Y. Chevolot, J. P. Cloarec, J. P. Praly, J. J. Vasseur and F. Morvan, *J. Org. Chem.*, 2006, **71**, 4700; (*c*) P. Appukkuttan, W. Dehaen, V. V. Fokin and E. Van der Eycken, *Org. Lett.*, 2004, **6**, 4223.
14 (*a*) J. P. Richard, *Biochemistry*, 1998, **37**, 4305; (*b*) M. L. Sinnot, *Chem. Rev.*, 1990, **90**, 1171; (*c*) H. W. Griesser, B. Müller-Hill and P. Overath, *Eur. J. Biochem.*, 1983, **137**, 567; (*d*) B. W. Matthews, *C. R. Biol.*, 2005, **328**, 549; (*e*) D. H. Juers, T. D. Heightman, A. Vasella, J. D. McCarter, L. Mackenzie, S. G. Withers and B. W. Matthews, *Biochemistry*, 2001, **40**, 14781; (*f*) R. H. Jacobson, X. J. Zhang, R. F. DuBose and B. W. Matthews, *Nature*, 1994, **369**, 761; (*g*) A. García-Herrero, E. Montero, J. L. Muñoz, J. F. Espinosa, A. Vián, J. L. García, J. L. Asensio, F. J. Cañada and J. Jiménez-Barbero, *J. Am. Chem. Soc.*, 2002, **124**, 4804; (*h*) E. Montero, A. García-Herrero, J. L. Asensio, K. Hirai, S. Ogawa, F. Santoyo-González, F. J. Cañada and J. Jiménez-Barbero, *Eur. J. Org. Chem.*, 2000, 1945.
15 L. Calle, V. Roldós, F. J. Cañada, M. L. Uhrig, A. J. Cagnoni, V. E. Manzano, O. Varela and J. Jiménez-Barbero, *Chem.–Eur. J.*, 2013, **19**, 4262.
16 E. Montero, A. Gracía-Herrero, J. L. Asonsia, K. Hirai, S. Ogawa, F. Santoyo-González, F. J. Cañada and J. Jiménez-Barbero, *Eur. J. Org. Chem.*, 2000, 1945.
17 M. Ambrosi, N. R. Cameron, B. G. Davis and S. Stolnik, *Org. Biomol. Chem.*, 2005, **3**, 1476.
18 A. J. Cagnoni, O. Varela, M. L. Uhrig and J. Kovensky, *Eur. J. Org. Chem.*, 2013, 972.
19 I. Cumpstey, A. Sundin, H. Leffler and U. J. Nilsson, *Angew. Chem., Int. Ed.*, 2005, **44**, 5110.
20 (*a*) R. Barenjee, S. C. Mande, V. Ganesh, K. Das, V. Dhanaraj, S. K. Mahanta, K. Suguna, A. Surolia and M. Vijayan, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 227; (*b*) R. Ravishankar, C. J. Thomas, K. Suguna, A. Surolia and M. Vijayan, *Proteins*, 2001, **43**, 260.
21 S. K. Choi, *Synthetic multivalent molecules. Concepts and Biomedical Applications*, Wiley-Interscience, New Jersey, 2004, pp. 1–22.
22 (*a*) T. K. Dam and C. F. Brewer, *Biochemistry*, 2008, **47**, 8470; (*b*) E. Fan, Z. Zhang, W. E. Minke, Z. Hou, C. L. M. J. Verlinde and W. G. J. Hol, *J. Am. Chem. Soc.*, 2000, **122**, 2663; (*c*) S. M. Dimick, S. C. Powell, S. A. McMahon, D. N. Moothoo, J. H. Naismith and E. Toone, *J. Am. Chem. Soc.*, 1999, **121**, 10286.
23 I. H. Segel, in *Enzyme Kinetics. Behavior and analysis of rapid equilibrium and steady-state enzyme systems*, John Wiley & Sons Inc, New York, 1993, pp. 100–201.