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Access to Galectin-3 Inhibitors from Chemoenzymatic Synthons

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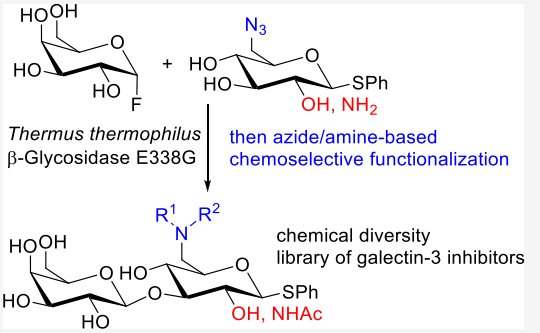
ABSTRACT: Chemoenzymatic strategies are useful for providing both regio- and stereoselective access to bioactive oligosaccharides. We show herein that a glycosynthase mutant of a *Thermus thermophilus* α-glycosidase can react with unnatural glycosides such as 6-azido-6-deoxy-D-glucose/glucosamine to lead to β-D-galactopyranosyl-(1→3)-D-glucopyranoside or β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucopyranoside derivatives bearing a unique azide function. Taking advantage of the orthogonality between the azide and the hydroxyl functional groups, the former was next selectively reacted to give rise to a library of galectin-3 inhibitors. Combining enzyme substrate promiscuity and bioorthogonality thus appears as a powerful strategy to rapidly access to sugar-based ligands.



INTRODUCTION

By essence glycosyltransferases are designed and thus widely used to achieve glycan synthesis with incomparable selectivity and efficiency.[1−4] The transfer catalyzed by some of these enzymes can occur efficiently not only with their natural donor substrates but also with synthetic analogues. This considerably increases the scope of their applications, which is therefore not limited to the sole preparation of natural glycans. A major achievement was the advent of metabolic functionalization.[5] Glycosyltransferase substrate promiscuity has now been exploited for the synthesis of complex glycans as well as for inhibitors or ligands. This was achieved starting from chemoenzymatic synthons, first enzymatically used either in cellulo or in vitro as substrates to form oligosaccharides prior to their chemical modifications.[6−9] Interestingly, a reverse strategy consisting of using temporary protecting groups on acceptor substrates to divert and control the reactivity of glycosyltransferases has been applied to synthesize complex glycoforms.[10,11] *trans*-Glycosidases, whether natural[12] or mutant glycoside hydrolases obtained by directed evolution[13] or rational design,[14] as well as glycosynthases[15] are powerful tools for the synthesis of oligosaccharides or glycoconjugates. For example, the design of a *trans*-β-*N*-acetylglucosaminidase engineered from a GH1 glycoside hydrolase able to hydrolyze galactose and glucose but not *N*-acetyl-D-glucosamine has been reported.[16] Glycosynthases arise from the mutation of the catalytic nucleophile residue of a retaining glycoside hydrolase to either Ala, Gly, or Ser. While glycosynthases have lost their hydrolytic capacity, they are able to catalyze the selective transfer of fluoro-activated sugar donors to appropriate acceptors.[17,15] However, despite their broad interest, such enzymes have scarcely been tested for their ability to accept chemoenzymatic synthons. Withers' laboratory has recently reported the screening of glycoside hydrolase activities from the GH1 family (CA-zyme) for substrates bearing either an amino or an azido group at the 3-, 4-, or 6-position of the glucose.[18] The panel of tested enzymes proved rather tolerant for the replacement of a hydroxyl with an amino group and when an azido group was present at the 6-position. They were much less permissive for 3-azido and 4-azido substrates due to the steric demand of the azido group because of the strong hydrogen bonds formed between substrates and highly conserved residues of GH1 enzyme catalytic domains. Finally, the authors succeeded in synthesizing taggable di- and trisaccharides from seven glycosynthases derived from these glycosidases to demonstrate the utility of this approach. Considering now the acceptor substrate, it is well established that a wide range of aglycons are tolerated albeit with a preference for arylated substituents in line with their capability to form stabilizing hydrophobic interactions with the residues within the +1,+2 enzyme subsites. Furthermore, the hydroxyl can be advantageously replaced by a thiol group which can next be used as the glycosylation site to give rise to thioglycosides.[19] Finally, site-specific enzymatic α-D-glucosylation has been performed on a disaccharide whereby an acetamido group has been replaced by a trichloroacetamide at a remote position to allow further chemical elongation of the resulting trisaccharide building block to form an important

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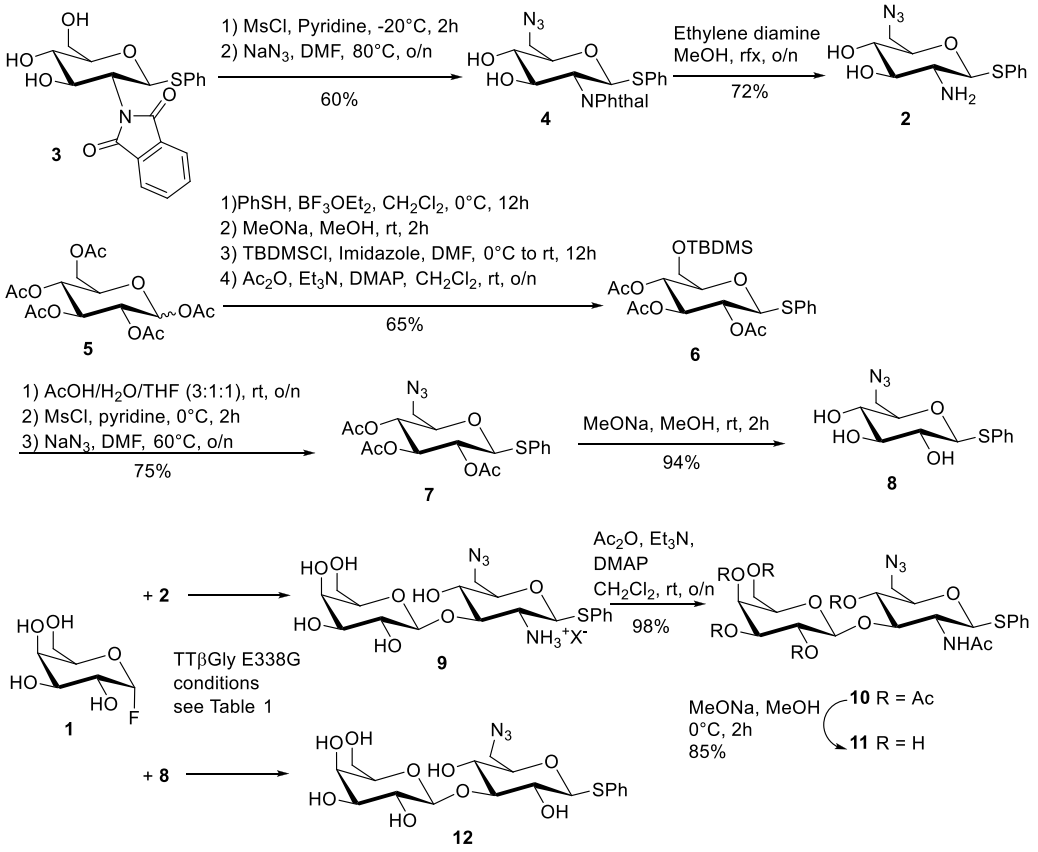
Scheme 1. Preparation of the Enzymatic Synthons and Chemoenzymatic Access to *β*-Gal*p*-(1-3)-6-azido-6-deoxy-*β*-D-Glc*p*/GlcN*p* Motifs



hapten for vaccination.[20] As an extension of the use of chemoenzymatic synthons, we herein report that 6-azidoglycosides are effective glycosynthase acceptor substrates, useful to give access to glycan-derived inhibitors of relevant therapeutic targets. In the present work, we focused on the galectin-3 protein as a representative example. Galectins form an evolutionary conserved family of animal lectins which bind *α*-galactoside motifs through one or two carbohydrate binding domains (CRDs). Human galectin-3 is the only chimera-type galectin composed of a single CRD at its C-terminus and of a nonlectin collagen-like domain.[21] Galectin-3 is found in the nucleus, the cytoplasm, the vicinity of the cell surface, the extracellular matrices, and even the circulation. Galectin-3 is expressed in various cell types including epithelial and endothelial cells, fibroblasts, as well as immune cells. Galectin-3 has been increasingly recognized as being involved in highly diverse physiological or pathophysiological processes such as adhesion, differentiation, apoptosis, cell division, proliferation, trafficking, growth or regulation of cell signaling, and gene expression.[22,23] Consequently, galectin-3 has emerged as a potential novel therapeutic target, and sugar-derived small-molecule inhibitors have been proposed as galectin-specific inhibitors and beyond as potential novel drug candidates.

RESULTS AND DISCUSSION

Rationale. Galectin-3 recognizes both *β*-Gal*p*-(1→3)-*β*-D-Glc*p*N (lactosamine type I) and *β*-Gal*p*-(1−4)-*β*-D-Glc*p*N (lactosamine type II) core structures. A close study of lactosamine I and II binding modes was performed recently.[24] In their work, Hsieh and co-workers found that galactose position for both lactosamine types was highly conserved in galectin-3. This orientation is also identical in more recent structures of digalactoside galectin inhibitors where the galactose binding position is strictly conserved.[25,26] This evolution-driven specificity for galactose recognition involves two chemical interactions. First, a characteristic direct or water-mediated hydrogen network is formed by the conserved amino acids H158, N160, R162, N174, and E184 with the oxygens O3, O4, O5, and O6 of the galactose moiety. Second, W181 tightens the binding via a hydrophobic interaction with galactose atoms C3, C4, C5, and C6. Concerning the glucose or NAc-glucose recognition for lactosamine type I or II, the authors observed a rotation of 240° around the glycosidic angle.[24] This rotation does not alter one important hydrogen bond with R162 and E184 since the lactosamine type II C4-OH and the lactosamine type I C3-OH are superimposable at this oxygen position. This rotation exchanges C2 and C6 positions between the lactosamines I and II leading to differences in their binding affinities, measured K_d being, respectively, 33 and 93 μM, in line with other studies.[24,27] While the carbohydrate core is involved in a dense hydrogen bond network making the presence of the 4-OH and 3-OH groups mandatory for the recognition as described above, the careful choice of the substituents at the other positions provides a significant contribution to both affinity and specificity. Tremendous synthetic efforts have been devoted to the decoration of the lactose/type II lactosamine core notably at the C2 position of the glucose/glucosamine residue.[28,29] Modifications at the C2-position are key since,

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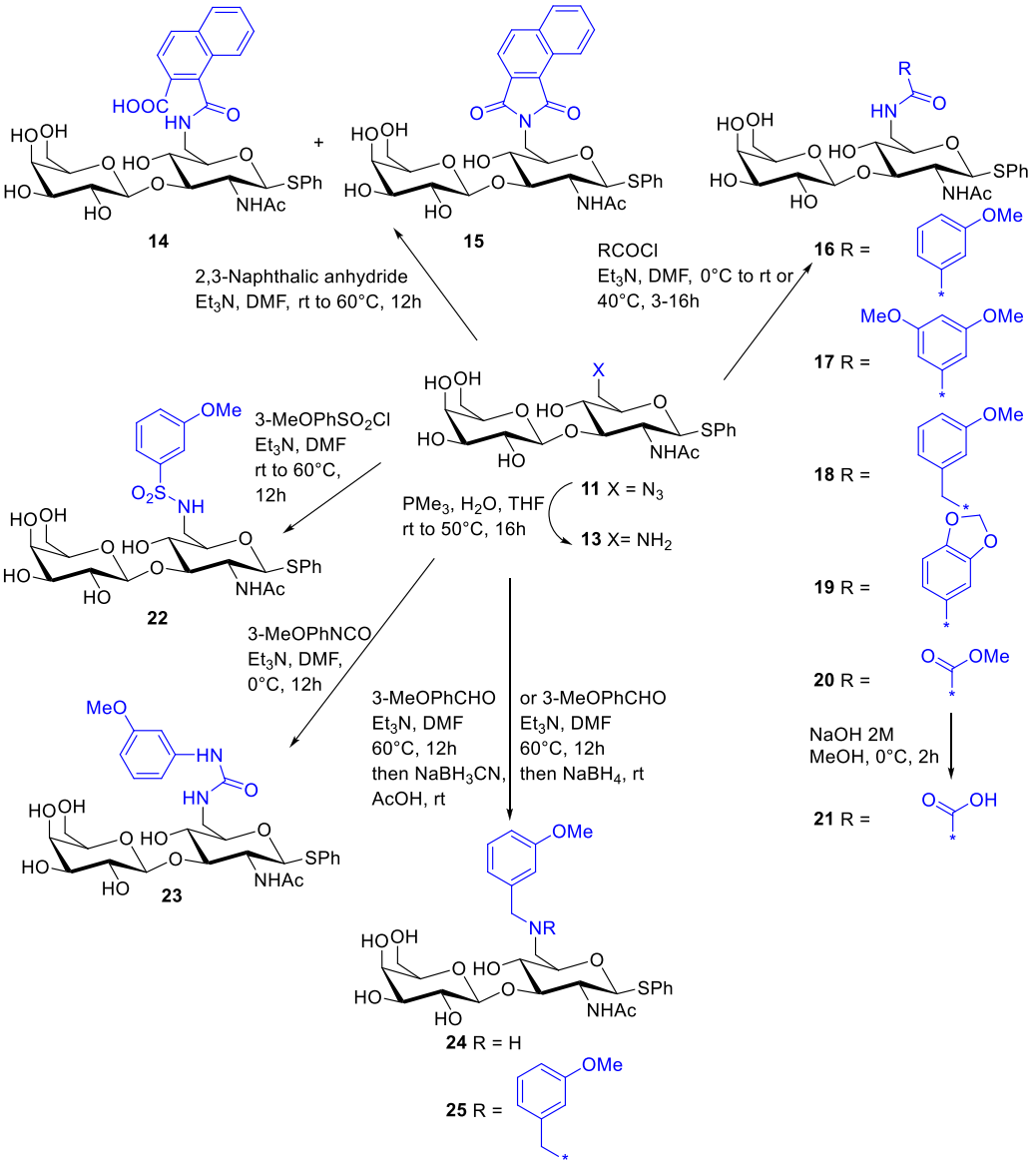
Scheme 2. Synthesis of Galectin-3 Inhibitors from Disaccharide 13



for example, aryl substituents have been shown to enhance the binding affinity toward galectin-3 by 1 order of magnitude through cation−π interactions with the residue R186 of the CRD.[30] By contrast, to our knowledge, only two reports describe the preparation of type I lactosamine-derived galectin inhibitors. The first one concerns the preparation of *N*-acetyllactosamine type 1 oligomers by iterative enzymatic synthesis.[31] The second one describes the synthesis of a series of inhibitors bearing modifications at either the 2′-OH position of the galactose or the amino position of the glucosamine residues, obtained according to a classical glycosylation strategy between a thioglycoside donor and a 4,6-*O*-benzylidene-protected glucosamine acceptor.[32] Our laboratory reported some years ago the preparation of the glycosynthase E338G mutant from *Thermus thermophilus* glycoside hydrolase (TTβGly E338G) which was successfully used as a catalyst for the expeditious synthesis of the β-Gal*p*-(1→3)-β-D-GlcN*p*/Glc*p* core.[33] We reasoned that if this glycosynthase accepts 6-azidoglycosides as substrate that would offer a unique opportunity to explore the effect of modifications at the C6-position (equivalent to C2 position on type lactosamine/lactose) on galectin-3 recognition.

Access to β-Gal*p*-(1→3)-6-azido-6-deoxy-β-D-Glc*p*/GlcN*p* Motifs. To test our working hypothesis, we first synthesized potential donor and acceptor substrates for the enzyme. α-Galactopyranosyl fluoride **1** was thus prepared in two steps (90% overall yield) from galactose-pentaacetate upon HF-pyridine treatment followed by Zemplén deprotection as described[33] except that we omitted the final neutralization step as we noticed that compound **1** could be stored longer at −20 °C. Synthesis of phenyl 2-acetamido-6-azido-2,6-dideoxy-1-thio-α-D-glucopyranoside **2** was preferentially carried out from a 2-phthalimido rather than a 2-acetamido precursor. Hence, derivative **3**[34] was selectively mesylated at the C-6 position and next subjected to S_N2 substitution using sodium azide to give compound **4**. Acceptor **2** was obtained after ethylene diamine treatment to remove the phthalimide protecting group from intermediate **4** (46% yield for the three steps) (Scheme 1).[35] In parallel, glucose pentaacetate **5** was glycosylated with thiophenol in the presence of boron trifluoride etherate, treated with sodium

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methoxide in methanol, silylated at the C-6 upon treatment with the TBDMS-imidazole complex, and submitted to peracetylation to afford the intermediate **6**. Selective deprotection of the 6-hydroxyl moiety of derivative **6**, next subjected to mesylation followed by sodium azide nucleophile substitution, conducted to the intermediate **7**. Final deacetylation of **7** gave rise to a second chemoenzymatic synthon, the phenyl 6-azido-6-deoxy-1-thio-β-D-glucopyranoside **8** in 8 steps and 45% overall yield (Scheme 1).

To our delight, the reaction of α-galactopyranosyl fluoride **1** with acceptor **2** at a 2:1 stoichiometry in phosphate buffer in the presence of TTβGly E338G mutant as described previously[33] gave access to phenyl α-D-galactopyranosyl-(1→3)-2-amino-2-deoxy-1-thio-α-D-glucopyranoside **9** as the sole product in 40% yield (Scheme 2 and Table 1). Addition of 5% or 10% DMSO in the reaction mixture favored the solubilization of the acceptor **2** without apparent alteration of TTβGly E338G activity, leading to improved 67% and 78% yields, respectively (Table 1). Finally, compound **9** was peracetylated to give intermediate **10** which was then treated with sodium methoxide in methanol to afford derivative **11**. The optimized enzymatic conditions were next applied to the enzymatic glycosylation between compounds **1** and **8** to give phenyl α-D-galactopyranosyl-(1→3)-1-thio-α-D-glucopyranoside **12** in 47% isolated yield (Scheme 1 and Table 1).

Table 1. Enzymatic Glycosylation Reaction[a]

donor (equiv)	acceptor (equiv)	DMSO (%)	yield (%)
1 (2)	**2** (1)		40
1 (2)	**2** (1)	5	67
1 (2)	**2** (1)	10	78
1 (1.5)	**8** (1)	10	47[b]

[a]TTβGly E338G (3−4.5 mg/mL), phosphate buffer (150 mM, pH 7.3), 37 °C, 72 h. [b]Plus inseparable mixture of regioisomers and excess donor **1**

Formation of byproducts, presumably corresponding to the 1→2 and/or 1→4 regioisomer disaccharides together with some unreacted fluoride donor, were detectable on TLC plates. Similar reactions led to a mixture of both β-(1→3) disaccharide (96−94%) and β-(1→6) regioisomer (4−6%) when phenyl 1-thio-β-D-glucopyranoside was used as the acceptor substrate. While the C6 position is no longer reactive due to the replacement of the hydroxyl by the azide, this observation suggests that the steric demand of the azide substituent modifies the position of the acceptor within the (+1 site).

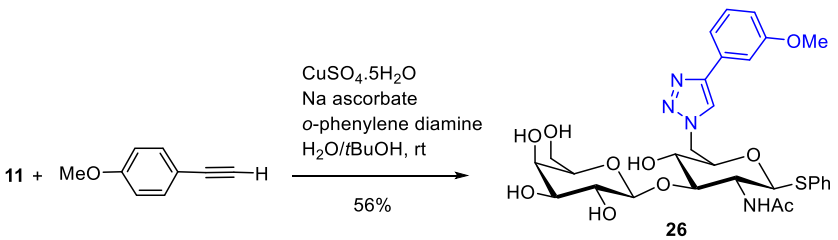
Along this line, our laboratory reported that the nature of the anomeric substituent could orient the regioselectivity of the glycosylation catalyzed by TTβGly E338G, in particular, toward the formation of the β-(1→2) disaccharides.[36]

Successful enzymatic access to disaccharides **11** and **12** offered a unique opportunity to further document structure−activity relationships within a series of sugar-based derivatives that were further evaluated in a biochemical test to assess their binding affinity toward galectin-3. The syntheses could be achieved upon exploiting the unique reactivity of azide subsituent either directly using click chemistry or after its reduction to the corresponding amine.

Access to Inhibitors Using Azide Specific Reactivity in 11. Staudinger reaction or hydrogenation ($NaBH_4$/catalytic $NiCl_2{\cdot}6H_2O$)[37] was accompanied by acetate migration when carried out with intermediate **10**. Reduction was thus attempted on deprotected disaccharide **11** which was treated with trimethylphosphine. Amine intermediate **13** was not purified but further reacted with 2,3-naphthalic anhydride to give amide **14** (19%) together with the corresponding imide **15** (18%) (Scheme 2).

Next, amine **13** was reacted in parallel with a series of acyl chlorides to give amides **16** (50%), **17** (38%), **18** (65%), **19** (55%), and **20** (60%) (Scheme 2). The methyl ester compound **20** was further saponified to give an additional carboxylic acid derivative **21** in 95% yield. On the one hand, a carboxylic acid substituent as in **21** was expected to interact with the Arg186 side-chain residue of galectin-3 CRD. On the other hand, aromatic substituents at the GlcN-C2 position in type II lactosamines have been shown to interact through cation−π interactions with Arg 186, contributing substantially to the affinity toward galectin-3. This effect was particularly pronounced with 1-carboxy-2-naphthoyl and 3-methoxybenzoyl substituents.[30,27,38,39] On the basis of this knowledge, the 3-methoxyaryl motif was next selected to synthesize a new set of derivatives simply differing by their connectivity to the sugar moiety. Hence, amine **13** was reacted with 3-methoxysulfonyl chloride or 3-methoxyisocyanate to afford sulfone **22** and urea **23** with 20% and 47% yield, respectively. Monobenzylated amine **24** or dibenzylated amine **25** were respectively obtained upon reductive amination of **13** with either 1.2 equiv of 3-methoxybenzaldehyde in the presence of sodium cyanoborohydride as the reducing agent or 2.4 equiv of the aldehyde using sodium borohydride in 56% and 37% yield. Finally, triazole derivative **26** was obtained with 23% yield according to a copper-catalyzed azide−alkyne cycloaddition (CuAAC) reaction from 3-methoxyphenylacetylene and disaccharide **11** (Scheme 3). The low isolated yield was ascribed to the intrinsic reactivity of compound **11** since, in our hands, these experimental conditions[40] led to the quantitative transformation of azide **4** used as a model reactant. Alternatively, using copper(II) sulfate, sodium ascorbate and sodium carbonate in a H_2O/THF mixture at rt afforded an inseparable mixture of regioisomers.

Scheme 3. Synthesis of Compound 26 from Disaccharide 11



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Binding Affinity Measurements. We next carried out biochemical experiments to measure the binding affinities of these derivatives toward galectin-3 in order to validate the value of the chemoenzymatic approach for the discovery of new galectin inhibitors. Dissociation constants were determined by a fluorescence polarization assay using known 2-[(fluoresceinyl)thioureido]ethyl 4-*O*-[3-*O*-(3-methoxybenzyl)-*β*-D-galactopyranosyl]-*β*-D-glucopyranoside as fluorescent probe (Figure S1) and recombinant human galectin-3 (hGal-3) protein (Table 2).[29] Two inhibitors previously described in the literature, the bis{3-deoxy-3-[4-(3-fluorophenyl)-1*H*-1,2,3-triazol-1-yl]-*β*-d-galactopyranosyl}sulfane (usually referred to as TD139)[25] and the phenyl *β*-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-1-thio-*β*-D-glucopyranoside **27**[33] (Figures S1 and S2), were tested in parallel as positive controls. The head-to-head comparison toward known inhibitors allows us to assess the value of the new compounds for targeting galectin-3. TD139 is among the most potent disaccharide-based galectin-3 inhibitors developed so far. This derivative has been evaluated in a Phase II clinical trial. Derivative **27** is not modified at the C6 position of the glucose residue representing the type I lactosamine core. This inhibitor is thus useful to assess the contribution of the substituent at the C6 position of the glucose to the binding.

Table 2. Dissociation Constants of Compounds Tested against Galectin-3 at rt[a,b]

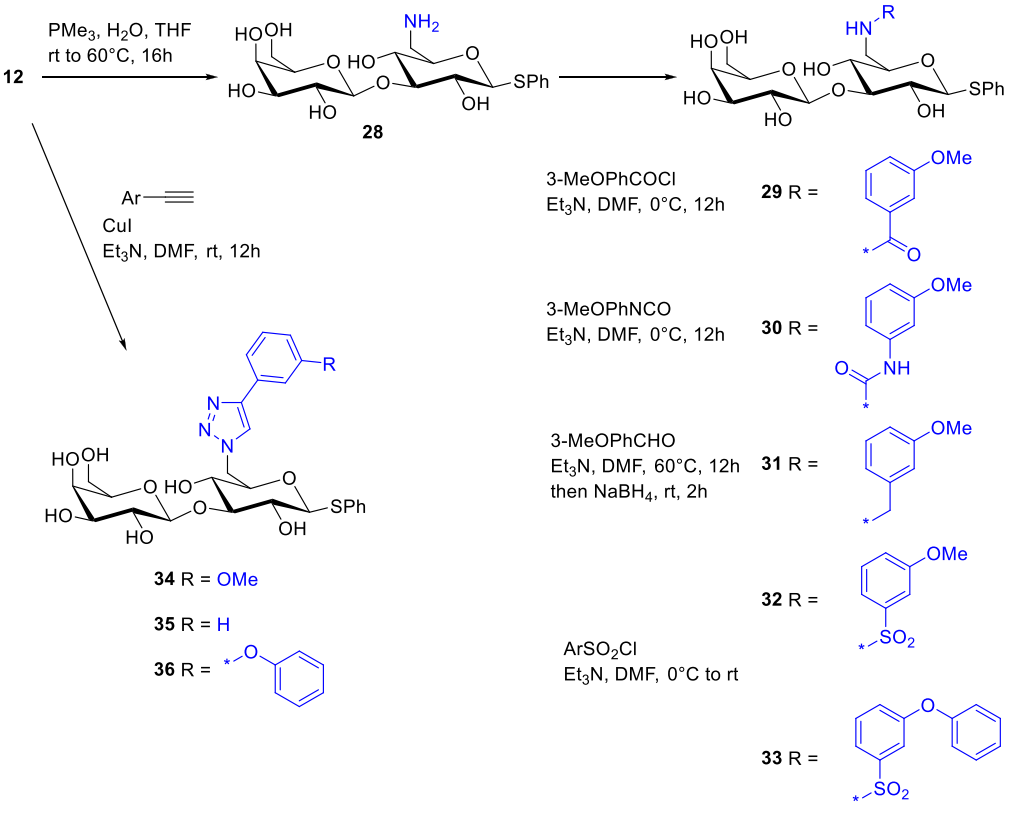
inhibitor	K_d (μM)	inhibitor	K_d (μM)
TD139	0.22 ± 0.041	**27**	51.8 ± 10.3
14	28.0 ± 3.6	**25**	22.1 ± 3.8
15	19.4 ± 3.4	**26**	40.2 ± 6.2
16	58.3 ± 8.9[c]	**29**	35.9 ± 10.9
17	64.5 ± 6.8	**30**	42.7 ± 6.1
18	52.2 ± 9.0	**31**	14.9 ± 2.4
19	37.6 ± 6.7	**32**	9.14 ± 1.9
20	37.6 ± 5.2[c]	**33**	14.6 ± 2.6
21	41.5 ± 7.0	**34**	68.5 ± 10.5
22	10.4 ± 2.3[c]	**35**	41.0 ± 5.4
23	36.8 ± 6.4[c]	**36**	225[d]
24	20.8 ± 3.4[c]		

[a]Concentrations were as follows: probe 100 nM, hGal-3 1 μM, and inhibitors tested in serial dilutions from 800 μM to 10 nM. [b]Assays were carried out in duplicate. [c]An independent assay was repeated in duplicate for this derivative and confirmed the K_d value determined in the first experiment. [d]K_d value might be overestimated as compound **36** precipitated during the assay.

The tested compounds could be ranked depending on their affinity to galectin-3: compounds with similar or less affinity than the reference **27** (e.g., methoxyphenyl amides **16**, **17**, and **18**), those with slightly improved affinities (amides **14**, **19**, **20**, and **21**, urea **23**, and triazole **26** with K_d's in the range of 28–42 μM and imide **15**, amines **24** and **25** with K_d's in the range of 20 μM), and finally, 3-methoxysulfonamide **22** having a K_d equal to 10.4 μM.

Introduction of aromatic substituents at the C6 position of the glucose residue does not impair the recognition between the compounds and the galectin-3 CRD, suggesting that the interactions established by the disaccharide core are maintained upon binding. Such observation was expected based on the results obtained from type II lactosamine derivatives

Scheme 4. Synthesis of Galectin-3 Inhibitors from Disaccharide 12



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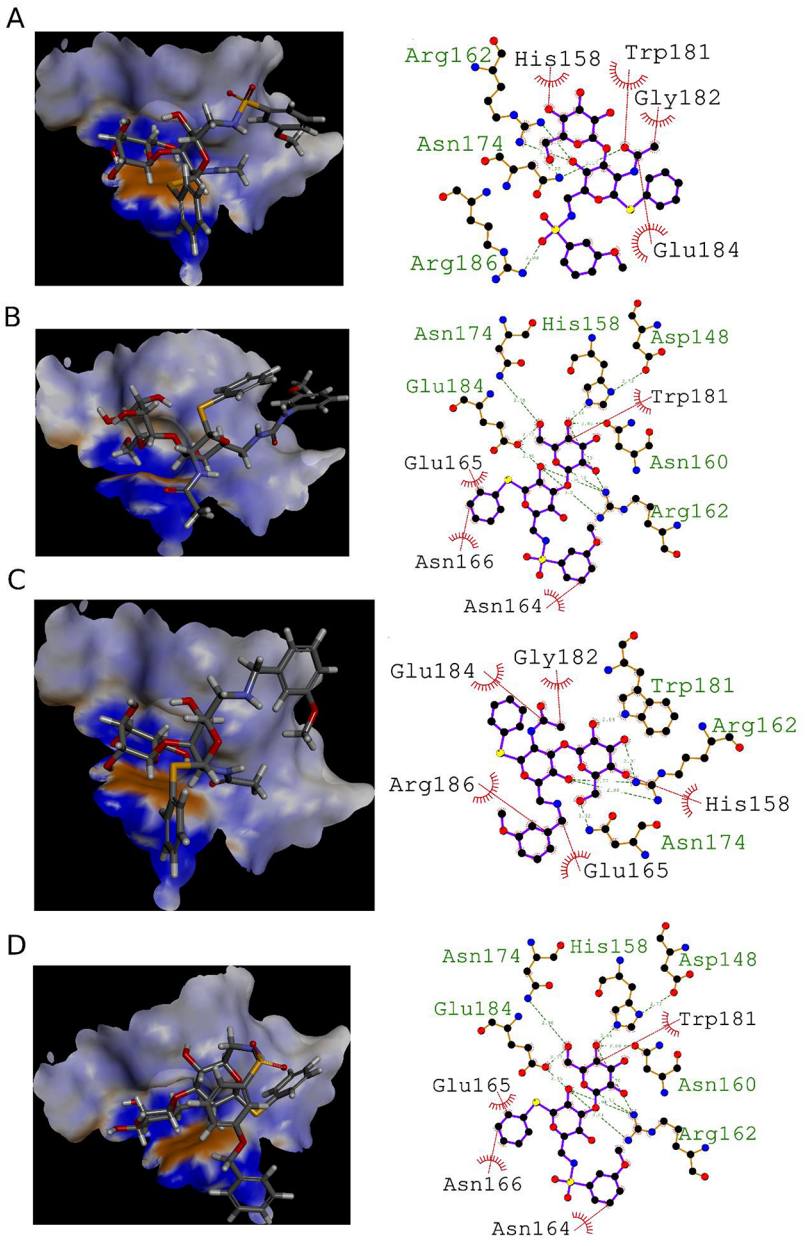

Figure 1. Molecular docking of selected compounds with the galectin-3. Overview of one representative binding pose after docking for **22** (A), **23** (B), **24** (C), and **32** (D). Ligands are displayed in sticks and colored by atom types: carbons in gray, hydrogen in white, oxygens in red, nitrogen in blue, sulfur in yellow. The receptor surface is colored by aromaticity with the aromatic face in orange (W181) and the aromatic edge in blue

bearing aromatic substituents a the C2 position of the glucose residue.[30,38] Analogously, the overall contribution of the substituents was equally modest. However, the nature of the linkage between the substituent and the C6 position of the glucose seems to modulate the affinity. To confirm the trends within the different structural classes we decided to synthesize analogues of representative compounds (sulfonamide, amine, urea, amide) in the *β*-Gal*p*-(1→3)-*β*-D-Glc*p* series.

Access to Inhibitors Using Azide Specific Reactivity of 12. Toward this aim, azide **12** was reduced into the amine **28** upon treatment with trimethylphosphine and next reacted with 3-methoxybenzoyl chloride, 3-methoxyphenylisocyanate, 3-methoxybenzaldehyde, and 3-methoxysulfonyl chloride to give derivatives **29**, **30**, **31**, and **32** in 57%, 65%, 61%, and 70% yields, respectively (Scheme 4). In addition, amine **28** was condensed with 4-phenyloxyphenylsulfonyl chloride to afford compound **33** in 55% yield to further exemplify the most promising sulfonamide family of inhibitor. Moreover, it had been suggested that a phenyloxyphenyl substituent (at the C2 position of the type II lactosamine) might be oriented such as to establish cation−*π* interactions with both Arg186 and Arg168 residues of the galectin-3 CRD.[41]

Furthermore, CuAAC reactions were carried out with azide **12** to prepare triazole inhibitors from methoxyphenylacetylene, acetylene, or 1-ethynyl-4-phenoxybenzene using either a catalytic or a stoichiometric amount of copper(I) iodide.[25] Derivatives **34**, **35**, and **36** were obtained in 36%, 85%, and 62% yields.

Binding Affinity Measurements. Galectin-3 binding affinities of the new analogues were measured by a fluorescence polarization assay as previously described (Table 2). The same ranking as that reported in the first series of compounds was

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observed; i.e., the K_d of galectin-3 for urea/amide was 35−45 μM, 15−20 μM for amine, and decreased to 8−10 μM for sulfonamides.

In silico analysis of docked compounds **22** (sulfonamide), **23** (urea), and **24** (amine) confirms that the core conserved binding motif characteristic of the lactosamine moieties is recovered via interactions of all compounds with H158, N160/R162, N174, and W181 (Figure 1). In all binding modes observed, the thiophenyl anomeric modification does not seem to bring critical contacts explaining the differences in binding energies. The residue E184 is found in interactions with **22** and **24**, with small additional hydrophobic interactions provided by E165 and R186 for **24**. The urea part of **23** is also involved in E184 targeting but through a hydrogen bond. Two small hydrophobic interactions are detected for **23** with R183 and R186. In total, the number of interactions between compound **22** (7), **23** (9), and **24** (8) does not seem to be enough to explain the relative differences in binding efficiency on galectin. The galactose binding position is, however, displaced from its location in lactosamine type I structure with an RMSD of 1.58 Å (**22**), 3.23 Å (**23**), and 1.55 Å (**24**), indicative of a counterbalance between galectin affinity provided by the galactose and by additional interaction energy provided by substituents. The equatorial substituent at the C2 position of Glc/GlcN, which points outward on the CRD, does not impact the binding. The acetamido present at the C2 position of the GlcN is not involved in direct interactions with galectin, but it reduces the potential rotation between Gal and GlcN compared to Glc. When the acetamido is removed (**32**), the galactose can rotate more (Figure 1 D). The substituent in position 6 is therefore free to have extra hydrophobic interactions with galectin (close to R186). In this situation, the lactosamine core is less displaced with a galactose RMSD of only 0.62 Å. This geometric observation could explain why compound **32** is measured to be (relatively) better than **22**. The bulky sulfonamide **33** has a similar behavior as inhibitors **22** and **32**. The best pose for the bulkier compound **33** is also less displaced than its acetamido counterpart with an RMSD of 0.33 Å (not shown).

Sulfonamides **22** and **32** were eventually selected to document the selectivity. To this aim, their dissociation constants were determined for galectin-1 C3S (a stable mutant of galectin-1) (hGal-1) and galectin-7 (hGal-7)[42] in addition to galectin-3 (Table 3)

Lower affinities were observed for both sulfonamides **22** and **32** for hGal-1 and hGal-7 compared with hGal-3. This resulted in selectivity factors of 4 and 14 in favor of hGal-3 for compound **22**. Selectivities were further increased to 9 (hGal-3 vs hGal-1) and 18 (hGal-3 vs hGal-7) for compound **32**. Of note, derivatives **22** and **32** lacks substituents at the C3 position of the galactose, this position being known to contribute significantly to the selectivity.[42]

Table 3. Dissociation Constants (K_ds) of Inhibitors for Galectin-1, Galectin-3, and Galectin-7 at 4 °C[a]

inhibitor	hGal-1 (μM)	hGal-3 (μM)	hGal-7 (μM)
TD139[b]	2.0 ± 0.8	0.01 ± 0.003	15.5 ± 6.2
22	11.7 ± 4.1	2.66 ± 0.5	39.4 ± 14.4
32	23.5 ± 9.4	2.51 μ ± 0.2 μM	46.1 ± 18.0

[a]Concentrations were as follows: 2-(fluorescein-5-thiourea)ethyl [3-*O*-(2-naphthyl)methyl-β-D-galactopyranosyl]-(1−4)-2-deoxy-2-(3-methoxybenzamido)-β-D-glucopyranoside42 used as probe (500 nM); hGal-1, 5 μM; hGal-3, 1 μM; hGal-7, 5 μM, and inhibitors tested in serial dilutions from 800 μM to 10 nM. [b]Positive control.

CONCLUSION

Substrate promiscuity of enzymes, in particular, that of glycoenzymes, remains largely overlooked. Glycosyltransferases, glycoside hydrolases, or transglycosidases can all show exquisite reactivity not limited to their known natural substrates but extended to sugars lightly protected or modified with biorthogonal functionalities. This peculiarity has been herein exploited and illustrated using a GH1 glycosynthase to rapidly access to a library of potential galectin inhibitors. Further glyco-engineering will feed this enzyme toolbox, expanding the scope of chemoenzymatic pathways to synthesize complex oligosaccharides.

EXPERIMENTAL SECTION

General Methods. All reagents were purchased from commercial sources and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates with fluorescent indicator (GF254) and visualized under UV light. Detection was further achieved by charring with vanillin (1.5 g) in sulfuric acid/ethanol (1.5:95 mL). Flash chromatography purifications were carried out on silica gel columns (4−80 g, 240−400 mesh) using an automated chromatography system equipped by both ELS (evaporative light scattering) and UV/diode array allowing the simultaneous use of two customizable wavelengths detectors.

All NMR experiments were performed at 400.13 MHz using a 400 MHz spectrometer equipped with a DUAL+ $^{1}H/^{13}C$ ATMA grad 5 mm probe. Structural assignments were made with additional information from gCOSY and gHSQC experiments using standard pulse programs from the Bruker library. Chemical shifts are given relative to external TMS with calibration involving the residual solvent signals. High-resolution mass spectra were recorded in positive mode on a Waters SYNAPT G2-Si HDMS with detection with a hybrid quadripole time-of-flight (Q-TOF) detector. The compounds were individually dissolved in MeOH at a concentration of 1 mg·mL^{-1} and then infused into the electrospray ion source at a flow rate of 10 μL·min^{-1} at 100 °C. The mass spectrometer was operated at 3 kV while scanning the magnet at a typical range of 4000−100 Da. The mass spectra were collected as continuum profile data. Accurate mass measurement was achieved based on every 5 s autocalibration using leucine-enkephalin ($[M + H]^+$ = 556.2771 *m*/*z*).

Phenyl 6-Azido-2,6-dideoxy-2-phthalimido-1-thio-β-D-glucopyranoside **4**. Phenyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **3** (2.5 g, 6.25 mmol, 1 equiv) was dissolved in anhydrous pyridine (120 mL), and the solution was cooled to −20 °C. Mesyl chloride (2.90 mL, 18.75 mmol, 3 equiv) was added dropwise. After 2 h, the reaction was quenched upon addition of methanol, and the solvent was removed under reduced pressure. The residue was diluted with EtOAc (125 mL) and washed with aqueous 1 N HCl (125 mL), satd aq $NaHCO_3$ (125 mL) and brine (125 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/EtOAc 5/5) to give the mesylated intermediate as a yellow oil (1.93 g, 65%). R_f 0.48 (cyclohexane/EtOAc 2/8). This compound was directly dissolved in anhydrous DMF (25 mL) and treated with NaN_3 (1.31 mg, 20.1 mmol, 5 equiv). The resulting mixture was stirred at 80 °C for 16 h then was diluted with EtOAc (125 mL) and washed with H_2O (2 × 60 mL) and satd aq $NaHCO_3$ (60 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/EtOAc 7/3) to give compound **4** as a yellow oil (1.54 g, 92%): R_f 0.45 (cyclohexane/EtOAc 3/7); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (dd, *J* = 3.0, 5.4 Hz 2H, 2 C*H* Phth), 7.76 (dd, *J* = 3.0, 5.4 Hz, 2 C*H* Phth), 7.44−7.41 (m, 2H, C*H* SPh), 7.32−7.25 (m, 3H, C*H* SPh), 5.59 (d, *J* = 10.2 Hz, 1H, H1), 4.28 (t, *J* = 9.7 Hz, 1H, H3), 4.16 (t, *J*

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= 10.2 Hz, 1H, H2), 3.63−3.58 (m, 2H, H5, H6a), 3.52 (t, J = 8.8 Hz, 1H, H4), 3.48 (dd, J = 6.2, 13.6 Hz, 1H, H6b). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.9 (2 C(O), Phth), 134.3 (2 CH, Phth, 1 C_{quat}, SPh), 133.1 (2 CH, SPh), 131.6 (2 C_{quat}, Phth), 128.9 (2 CH, SPh), 128.2 (1 CH, SPh), 123.9 and 123.4 (2 CH, Phth), 83.7 (C1), 78.9 (C5), 72.9 (C3), 72.1 (C4), 55.6 (C6), 51.6 (C2); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{20}H_{18}N_4O_5SNa$ 449.0896, found 449.0902.

Phenyl 2-Amino-6-azido-2,6-dideoxy-1-thio-β-D-glucopyranoside **2**. Compound **4** (1.49 g, 3.5 mmol, 1 equiv) was stirred with a solution of ethylene diamine 0.8 M in MeOH (7.4 mL, 5.94 mmol, 1.7 equiv). The mixture was heated at 65 °C for 16 h, and then the solvent was removed under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 95/5) to give compound **2** as a yellow oil (750 mg, 72%): R_f 0.81 (CH_2Cl_2/MeOH 9/1); ^{1}H NMR (400 MHz, CD3OD) δ 7.63−7.59 (m, 2H, CH SPh), 7.37−7.31 (m, 3H, CH SPh), 4.54 (d, J = 9.8 Hz, 1H, H1), 3.57 (dd, J = 1.7, 12.7 Hz, 1H, H6a), 3.47−3.42 (m, 1H, H5), 3.41 (dd, J = 5.7, 12.7 Hz, 1H, H6b), 3.30−3.20 (m, 2H, H3, H4), 2.64−2.57 (m, 1H, H2). ^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ 132.6 (2CH, SPh), 132.1 (1C_{quat}, SPh), 128.6 (2CH, SPh), 127.8 (1CH, SPh), 88.3 (C1), 79.1 (C5), 77.5 (C3), 70.8 (C4), 55.6 (C2), 51.6 (C6); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{12}H_{16}N_4O_3SNa$ 319.0840, found 319.0834.

Phenyl 2,3,4-Tri-O-acetyl-6-O-tert-butyldimethylsilyl-1-thio-β-D-glucopyranoside **6**.[43] Thiophenol (6 mL, 61.50 mmol, 1.2 equiv) and $BF_3{\cdot}OEt_2$ (4.21 mL, 35.86 mmol, 0.7 equiv) were added to a stirred solution of β-D-glucosapyranose pentaacetate **5** (20 g, 51.2 mmol, 1 equiv) in CH_2Cl_2 (200 mL) at 0 °C. After 12 h, the reaction mixture was quenched by addition of satd aq $NaHCO_3$ (150 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (3 × 75 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by precipitation in a mixture of warm EtOAc/cyclohexane to give phenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (18.2 g, 80%); R_f = 0.50 (cyclohexane/EtOAc 7/3). To a solution of this intermediate (18.2 g, 41.2 mmol, 1 equiv) in MeOH (220 mL) was added a solution of sodium methoxide 5.4 M (8.24 mmol, 1.5 mL, 0.2 equiv) at rt and stirred for 2 h. Then the mixture was neutralized with an acidic resin (Amberlyte IR120-H^+) and filtrated, and the solvent was removed under reduced pressure to give crude phenyl 1-thio-β-D-glucopyranoside which was further used without purification.

The triol was solubilized in anhydrous DMF (100 mL), and then a solution of *tert*-butyldimethylsilyl chloride (TBDMSCl, 6.8 g, 45.3 mmol, 1.1 equiv) and imidazole (7.0 g, 103.0 mmol, 2.5 equiv) in anhydrous DMF (40 mL) was added at 0 °C. After being stirred for 12 h, the mixture was diluted with EtOAc (150 mL) and washed with H_2O (2 × 100 mL) and satd aq $NaHCO_3$ (100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure; R_f 0.57 (cyclohexane/EtOAc 1/9). Then the residue was solubilized in CH_2Cl_2 (100 mL) at 0 °C, treated with Ac_2O (17.5 mL), Et_3N (20.1 mL), DMAP (cat.), and stirred for 16 h. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with H_2O (2 × 100 mL) and satd aq $NaHCO_3$ (100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/EtOAc 9/1) to give phenyl 2,3,4-tri-O-acetyl-6-O-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside **6** as a white solid (16.64 g, 65% over four steps): R_f 0.71 (cyclohexane/EtOAc 7/3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51−7.49 (m, 2H, CH SPh), 7.30−7.28 (m, 3H, CH SPh), 5.22 (t, J = 9.3 Hz, 1H, H3), 5.03 (t, J = 9.8 Hz, 1H, H4), 4.96 (t, J = 9.3, 10.0 Hz, 1H, H2), 4.72 (d, J = 10.0 Hz, 1H, H1), 3.75 (dd, J = 2.5, 11.6 Hz, 1H, H6a), 3.70 (dd, J = 5.0, 11.6 Hz, 1H, H6b), 3.58 (ddd, J = 2.5, 5.0, 10.0 Hz, 1H, H5), 2.07 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 0.90 (s, 9H, $3CCH_3$), 0.07 (s, 3H, CH_3, TBDMS), 0.05 (s, 3H, CH_3, TBDMS); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.2, 169.2, 169.2 (3C_{quat}, $COCH_3$), 132.6 (2CH, SPh), 133.2 (1C_{quat}, SPh), 128.9 (2CH, SPh), 128.0 (1CH, SPh), 85.6 (C1), 78.9 (C5), 74.4 (C3), 70.1 (C2), 68.6 (C4), 62.4 (C6), 25.8 ($3CCH_3$), 20.7, 20.6 ($3COCH_3$), 18.4 ($1CCH_3$), −5.4, −5.4 ($2CH_3$, TBDMS); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{24}H_{36}O_8SiSNa$ 535.1798, found 535.1790.

Phenyl 2,3,4-Tri-O-acetyl-6-O-azido-1-thio-β-D-glucopyranoside **7**.[44] Compound **6** (2.96 g, 5.77 mmol, 1 equiv) was stirred for 16 h in a solution of AcOH/H_2O/THF (3/1/1; 100 mL) at rt. Then THF was removed under reduced pressure, and the mixture was neutralized with satd aq $NaHCO_3$ (3 × 70 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. R_f 0.57 (cyclohexane/EtOAc 1/9). The residue was purified by flash chromatography (cyclohexane/EtOAc 75/25) to give phenyl 2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranoside as a colorless oil (1.94 g, 85%). R_f 0.32 (cyclohexane/EtOAc 7/3). This intermediate was dissolved in anhydrous pyridine (32 mL), and the solution was cooled to 0 °C. Mesyl chloride (750 μL, 9.7 mmol, 2 equiv) was added dropwise. After 2 h, the reaction was quench by adding methanol, and the solvent was removed under reduced pressure. R_f 0.88 (cyclohexane/EtOAc 5/5). The residue was directly dissolved in anhydrous DMF (50 mL) and treated with NaN_3 (944 mg, 14.5 mmol, 3 equiv). The resulting mixture was stirred at 60 °C for 16 h, then diluted in EtOAc (100 mL) and washed with H_2O (2 × 50 mL) and satd aq$NaHCO_3$ (50 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/EtOAc 8/2) to give phenyl 2,3,4-tri-O-acetyl-6-azido-6-deoxy-1-thio-β-D-glucopyranoside **7** as a white solid (1.8 g, 88% over two steps): R_f 0.43 (cyclohexane/EtOAc 5/5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56−7.50 (m, 2H, CH SPh), 7.37−7.32 (m, 3H, CH SPh), 5.23 (t, J = 9.3 Hz, 1H, H3), 4.99−4.92 (m, 2H, H2, H4), 4.73 (d, J = 10.0 Hz, 1H, H1), 3.70−3.64 (m, 1H, H5), 3.37 (dd, J = 2.5, 11.5 Hz, 1H, H6a), 3.31 (dd, J = 5.0, 11.5 Hz, 1H, H6b), 2.10 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.1, 169.4, 169.2 (3C_{quat}, $COCH_3$), 133.7 (2CH, SPh), 130.9 (C_{quat}, SPh), 129.0 (2CH, SPh), 128.7 (1CH, SPh), 85.7 (C1), 77.0 (C5), 73.8 (C3), 69.9 and 69.3 (C2 and C4), 51.3 (C6), 20.7, 20.6, and 20.6 ($3CH_3$,); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{18}H_{21}N_3O_7SNa$ 446.0998, found 446.1019.

Phenyl 6-O-Azido-1-thio-β-D-glucopyranoside **8**.[45] To a solution of compound **7** (690 mg, 1.63 mmol, 1 equiv) in MeOH (10 mL) was added a solution of sodium methoxide 5.4 M (0.49 mmol, 90 μL, 0.1 equiv) at 0 °C and the mixture stirred for 2 h. Then the mixture was neutralized with an acidic resin (Amberlyte IR120-H^+) and filtrated and the solvent removed under reduced pressure. The residue was purified by flash chromatography (cyclohexane/AcOEt 45/55) to give phenyl 6-azido-6-deoxy-1-thio-β-D-glucopyranoside **8** as a white solid (455 mg, 94%): R_f 0.28 (cyclohexane/AcOEt 4/6); ^{1}H NMR (400 MHz, CD_3OD) δ 7.62−7.57 (m, 2H, CH SPh), 7.35−7.27 (m, 3H, CH SPh), 4.60 (d, J = 9.7 Hz, 1H, H1), 3.56 (dd, J = 2.0, 12.9 Hz, 1H, H6a), 3.46−3.35 (m, 3H, H5, H6b, H3), 3.27 (t, 1H, J = 9.0 Hz, H4), 3.22 (dd, J = 8.7, 9.8 Hz, 1H, H2); ^{13}C{^{1}H} NMR (101 MHz, MeOD) δ 132.9 (C_{quat}, SPh), 132.4 (2CH, SPh), 128.5 (2CH, SPh), 127.4 (CH, SPh), 87.8 (C1), 78.9 (C5), 78.0 (C3), 72.2 (C2), 70.6 (C4), 51.6 (C6); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{12}H_{15}N_3O_4SNa$ 320.0681, found 320.0694.

Phenyl β-D-Galactopyranosyl-(1→3)-2-amino-6-azido-2,6-dideoxy-1-thio-β-D-glucopyranoside **9**. Donor **1** (184 mg, 1.0 mmol, 2 equiv) and acceptor **2** (150 mg, 0.5 mmol, 1 equiv) were dissolved in a phosphate buffer (150 mmol/L, pH 7, 6 mL) and DMSO (1 mL). Then 3 mL of glycosynthase E338G (4.2 mg·mL^{-1}) was added, and the reaction was allowed to proceed at 37 °C until complete consumption of the fluoride (about 72 h). The mixture was acidified with HCl 1 M to pH 1 and stirred for 4 h before neutralization with NaOH 1 M. After removal of the solvent under reduced pressure, the crude residue was purified by flash chromatography (CH_2Cl_2/MeOH 90/10) to afford disaccharide **9** as a white solid (180 mg, 78%): R_f 0.75 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^{1}H NMR (400 MHz, D_2O) δ 7.57−7.52 (m, 2H, CH SPh), 7.36−7.28 (m, 3H, CH SPh), 4.66 (d, J = 8.3 Hz, 1H, H1), 4.51 (d, J = 7.7 Hz, 1H, H1′), 3.89 (d, J = 3.3 Hz, 1H, H4′), 3.76−3.66 (m, 3H, H5′, H6a′, H6b′), 3.65 (dd, J = 3.3, 9.9 Hz, 1H, H3′), 3.62−3.52 (m, 4H, H2′, H6a, H5, H3), 3.49 (dd, J = 8.1, 18.5 Hz, 1H, H4), 3.44 (dd, J = 5.7, 12.9 Hz,

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1H, H6b′), 2.83 (dd, J = 8.3, 11.1 Hz, 1H, H2); $^{13}C\{^1H\}$ NMR (101 MHz, D_2O) δ 132.7 (2*C*H, SPh), 130.9 (C_{quat}, SPh), 129.4 (2*C*H, SPh), 128.5 (*C*H, SPh), 104.1 (C1′), 87.8 (C1), 87.6 (C3), 78.2 (C5), 75.5 (C5′), 72.8 (C3′), 71.2 (C2′), 69.0 (C4), 68.6 (C4′), 60.9 (C6′), 54.6 (C2), 51.3 (C6); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{18}H_{26}N_4O_8SNa$ 459.1550, found 459.1558.

Phenyl 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-4-O-acetyl-2-acetamido-6-azido-2,6-dideoxy-1-thio-β-D-glucopyranoside ***10***. A solution of compound **9** (150 mg, 0.33 mmol, 1 equiv) in CH_2Cl_2 (2.5 mL) was treated with Ac_2O (350 μL), Et_3N (350 μL), and DMAP (0.03 mmol, 4 mg, 0.1 equiv) and stirred for 16 h. The mixture was diluted with CH_2Cl_2 (25 mL) and washed with H_2O (2 × 12 mL) and satd aq $NaHCO_3$ (12 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (cyclohexane/EtOAc 6/4) to give compound **10** as a yellow oil (233 mg, 98%): R_f 0.50 (cyclohexane/EtOAc 3/7); ^1H NMR (400 MHz, $CDCl_3$) δ 7.52−7.45 (m, 2H, C*H* SPh), 7.33−7.27 (m, 3H, C*H* SPh), 6.07 (d, J = 8.1 Hz, 1H, N*H*), 5.33 (dd, J = 1.1, 3.4 Hz, 1H, H4′), 5.12 (d, J = 10.2 Hz, 1H, H1), 5.03 (dd, J = 7.5, 10.4 Hz, 1H, H2′), 4.97 (dd, J = 3.3, 10.4 Hz, 1H, H3′), 4.81 (t, J = 9.2 Hz, 1H, H4), 4.62 (d, J = 7.6 Hz, 1H, H1), 4.33 (t, J = 9.2 Hz, 1H, H3), 4.14−4.02 (m, 2H, H6a′, H6b′), 3.88 (td, J = 1.02, 6.6 Hz, 1H, H5′), 3.63 (ddd, J = 3.0, 6.8, 9.7 Hz, 1H, H5), 3.51 (dd, J = 8.1, 9.4 Hz, 1H, H2), 3.33 (dd, J = 6.8, 13.4 Hz, 1H, H6a), 3.27 (dd, J = 3.0, 13.4 Hz, 1H, H6b), 2.13 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 170.5 ($COCH_3$), 170.4 ($COCH_3$), 170.1 (2$COCH_3$), 169.4 ($COCH_3$), 169.2 ($COCH_3$), 132.8 (2*C*H, SPh), 132.1 (C_{quat}, SPh), 129.0 (2*C*H, SPh), 128.2 (*C*H, SPh), 100.6 (C1′), 85.4 (C1), 78.0 (C3), 77.2 (C5), 70.0 (C3′), 70.6 (C5′), 70.1 (C4), 69.3 (C2′), 66.9 (C4′), 61.0 (C6′), 55.9 (C2), 51.6 (C6), 23.6 ($COCH_3$), 20.8 (2 $COCH_3$), 20.7 ($COCH_3$), 20.6 ($COCH_3$), 20.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{30}H_{38}N_4O_{14}SNa$ 733.2003, found 733.2011.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-6-azido-2,6-dideoxy-1-thio-β-D-glucopyranoside ***11***. To a solution of compound **10** (233 mg, 0.32 mmol, 1 equiv) in MeOH (3.5 mL) was added a solution of sodium methoxide 5.4 M (0.035 mmol, 8 μL, 0.1 equiv) at 0 °C and the mixture stirred for 2 h. Then the mixture was neutralized with an acidic resin (amberlyte IR120-H^+) and filtrated, and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (CH_2Cl_2/MeOH 8/2) to give compound **11** as a white solid (135 mg, 85%): R_f 0.78 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 7.93 (d, J = 7.6 Hz, 1H, N*H*), 7.46−7.44 (m, 2H, C*H* SPh), 7.35−7.31 (m, 2H, C*H* SPh), 7.28−7.25 (m, 1H, C*H* SPh), 5.01 (d, J = 7.7 Hz, 1H, H1), 4.89 (d, J = 1.6 Hz, 1H, O*H*), 4.79 (br s, 1H, O*H*), 4.64 (t, J = 5.4 Hz, 1H, O*H*), 4.48 (m, 2H, 2 × O*H*), 4.17 (d, J = 6.3 Hz, 1H, H1′), 3.68−3.59 (m, 3H, H2, H3, H4′), 3.56−3.49 (m, 4H, H6a, H5, H6a′, H6b′), 3.49−3.39 (m, 2H, H5′, H6b), 3.37−3.22 (m, 3H, H2′, H3′, H4), 1.84 (s, 3H, $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO) δ 170.4 ($COCH_3$), 134.4 (C_{quat}, SPh), 130.6 (2CH, SPh), 129.4 (2CH, SPh), 127.4 (CH, SPh), 104.3 (C1′), 85.8 (C1), 85.1 (C3), 78.6 (C5), 76.3 (C5′), 73.5 (C3′), 71.0 (C2′), 70.0 (C4), 68.7 (C4′), 61.1 (C6′), 51.9 (C2), 51.4 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{20}H_{28}N_4O_9SNa$ 523.1474, found 523.1506.

Phenyl β-D-Galactopyranosyl-(1→3)-6-azido-6-dideoxy-1-thio-β-D-glucopyranoside ***12***. Donor **1** (115 mg, 0.63 mmol, 1.5 equiv) and acceptor **8** (125 mg, 0.42 mmol, 1 equiv) were dissolved in a phosphate buffer (200 mmol/L, pH 6.8, 6.5 mL) and DMSO (1 mL). Then glycosynthase E338G (2.5 mL, c = 4.0 mg·mL^{-1}) was added, and the reaction was allowed to proceed at 37 °C for 72 h. The mixture was acidified with HCl 1 M to pH 1 and stirred for 4 h before neutralization with NaOH 1 M. After removal of the solvent under reduced pressure, the crude residue was purified by flash chromatography (CH_2Cl_2/MeOH 90/10) to afford a mixture of phenyl β-D-galactopyranosyl-(1→3)-6-azido-6-deoxy-1-thio-β-D-glucopyranoside and unreacted phenyl 6-azido-6-deoxy-1-thio-β-D-glucopyranoside. To facilitate the purification, the crude reaction mixture was suspended in CH_2Cl_2 (5 mL), treated with Ac_2O (1 mL), Et_3N (1 mL), and DMAP (cat.), and stirred for 16 h. Then the reaction mixture was diluted in CH_2Cl_2 (25 mL) and washed with H_2O (2 × 15 mL) and satd aq $NaHCO_3$ (15 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified first by flash chromatography (cyclohexane/EtOAc 65/35) then by reversed-phase C-18 flash chromatography (H_2O/MeOH 4/6) to give phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2,4-di-*O*-acetyl-6-azido-6-deoxy-1-thio-β-D-glucopyranoside as a colorless oil (141 mg, 48% over 2 steps): R_f 0.50 (cyclohexane/EtOAc 5/5); ^1H NMR (400 MHz, CD_3OD) δ 7.55−7.49 (m, 2H, C*H* SPh), 7.36−7.30 (m, 3H, C*H* SPh), 5.34 (dd, J = 1.1, 3.4 Hz, 1H, H4′), 5.21 (t, J = 9.0 Hz, 1H, H3), 5.10 (dd, J = 3.5, 10.4 Hz, 1H, H3′), 4.98 (dd, J = 7.9, 10.4 Hz, 1H, H2′), 4.92 (d, J = 10.4 Hz, 1H, H1), 4.81 (dd, J = 9.1, 10.1 Hz, 1H, H2), 4.69 (d, J = 7.9 Hz, 1H, H1′), 4.16−4.06 (m, 3H, H5′, H6a′, H6b′), 3.79 (dd, J = 8.9, 9.8 Hz, 1H, H4), 3.73 (ddd, J = 2.1, 5.3, 9.8 Hz, 1H, H5), 3.63 (dd, J = 2.2, 13.5 Hz, 1H, H6a), 3.42 (dd, J = 5.3, 13.5 Hz, 1H, H6b), 2.12 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 1.92 (s, 3H, $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ 172.0 ($COCH_3$), 171.9 ($COCH_3$), 171.7 ($COCH_3$), 171.4 ($COCH_3$), 171.1 ($COCH_3$), 171.0 ($COCH_3$), 134.5 (2*C*H, SPh), 132.6 (C_{quat}, SPh), 130.0 (2*C*H, SPh), 129.5 (*C*H, SPh), 101.9 (C1′),86.1 (C1), 79.2 (C5), 77.5 (C4), 75.5 (C3), 72.4 (C3′), 71.8 and 71.7 (C2 and C5′), 70.7 (C2′), 68.6 (C4′), 62.3 (C6′), 51.9 (C6), 21.1 ($COCH_3$), 20.8 ($COCH_3$), 20.7 (CH_3), 20.6 (CH_3), 20.5 (CH_3); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{18}H_{25}N_3O_9SNa$ 734.1843, found 734.1854.

To a solution of phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2,4-*O*-diacetyl-6-azido-6-deoxy-1-thio-β-D-glucopyranoside (141 mg, 0.20 mmol, 1 equiv) in MeOH (2 mL) was added a solution of sodium methoxide 5.4 M (0.02 mmol, 3 μL, 0.1 equiv) at 0 °C and the mixture stirred for 2 h. Then the solvent was removed under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 9/1) to give to give disaccharide **12** as a white solid (90 mg, 100%): R_f 0.60 (CH_2Cl_2/MeOH 9/1); ^1H NMR (400 MHz, CD_3OD) δ 7.61−7.57 (m, 2H, C*H* SPh), 7.36−7.30 (m, 3H, C*H* SPh), 4.64 (d, J = 9.8 Hz, 1H, H1), 4.53 (d, J = 7.7 Hz, 1H, H1′), 3.81 (dd, J = 1.0, 3.3 Hz, 1H, H4′), 3.78 (dd, J = 7.6, 11.4 Hz, 1H, H6a′), 3.69 (dd, J = 4.5, 11.4 Hz, 1H, H6b′), 3.63−3.55 (m, 4H, H2′, H3, H5′, H6a), 3.54−3.47 (m, 2H, H3′, H5), 3.44−3.33 (m, 3H, H6b, H2, H4); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 132.7 (2CH, SPh), 132.4 (C_{quat}, SPh), 128.5 (2CH, SPh), 127.5 (CH, SPh), 104.2 (C1′), 87.4 (C3), 87.1 (C1), 78.6 (C5), 75.7 (C5′), 73.3 (C3′), 71.6 (C2′), 17.3 (C2), 69.3 and 68.9 (C4 and C4′), 61.1 (C6′), 51.6 (C6); HRMS (ESI-TOF) m/z $[M + Na]^+$calcd for $C_{18}H_{25}N_3O_9SNa$ 482.1209, found 482.1181.

General Procedure for the Reduction of Azide Derivative 11. To a solution of disaccharide **11** (50 mg, 0.1 mmol, 1 equiv) in THF (5 mL), under an atmosphere of argon, was added PMe_3 1 M in THF (200 μL, 0.2 mmol, 2 equiv). The reaction was stirred at rt and monitored by TLC ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3). After completion, H_2O (10 μL) was added, and the mixture was heated to 50 °C in an oil bath for 16 h. Then solvent was removed under reduced pressure to give crude phenyl β-D-galactopyranosyl-(1→3)-2-acetylamino-6-amino-2,6-dideoxy-1-thio-β-D-glucopyranoside **13** used without further purification: R_f 0.78 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3).

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-2,6-dideoxy-6-(1-carboxy-2-naphthoyl)amino-1-thio-β-D-glucopyranoside ***14*** *and Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-2,6-dideoxy-6-(2,3-naphthal)imido-1-thio-β-D-glucopyranoside* ***15***. Amine **13** was dissolved in anhydrous DMF (2 mL) at 0 °C and treated with 2,3-naphthalic anhydride (34 mg, 0.17 mmol, 1.7 equiv) and Et_3N (27 μL, 0.2 mmol, 2.0 equiv). The mixture was stirred 12 h at rt and then heated at 60 °C in an oil bath. After 12 h, formation of two products was observed. The reaction was stopped and concentrated under reduced pressure. The residue was purified by

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reversed-phase C-18 flash chromatography using H_2O/MeOH gradient mixture as eluant to successively give **14** as a white solid (11.7 mg, 19%) and **15** as a white solid (12.3 mg, 18%). Compound **14**: R_f 0.67 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 8.64 (br s, 1H, NHCONapht), 8.36 (s, 1H, CH Napht), 8.09 (dd, J = 2.3, 6.5 Hz, 1H CH Napht), 7.95−7.86 (m, 3H, CH Napht), 7.69−7.60 (m, 2H, CH Napht), 7.44−7.38 (m, 2H, CH SPh), 7.11−6.98 (m, 3H, SPh), 5.06−4.88 (m, 2H, H1, OH), 4.81 (br s, 2H, 2 × OH), 4.51 (d, J = 3.3 Hz, 2H, 2 × OH), 4.28−4.19 (m, 1H, H1′), 3.88 (dd, J = 7.2, 13.2 Hz, 1H, H6a), 3.78−3.45 (m, 7H, H2, H3, H5, H4′, H6′a, H6′b, H5′), 3.45−3.14 (m, 4H, H2′, H3′, H4, H6b), 1.83 (s, 3H $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ, 170.4 (2C_{quat}, $COCH_3$, COOH), 169.0 (C_{quat}, CONapht), 135.3, 134.9, 133.3, 132.7 (4C_{quat}, Napht, SPh), 130.0 (CH, Napht), 129.5 (2CH, SPh), 129.3 (2CH, SPh), 129.1 (CH, Napht), 128.6 (CH, Napht), 128.4 (CH, Napht), 128.0 (CH, Napht), 127.6 (CH, Napht), 126.6 (CH, SPh), 104.3 (C1′), 85.6 (C1), 85.3 (C3), 78.4 (C5), 76.3 (C5′), 73.6 (C3′), 71.1, 71.0 (C2′,C4), 68.8 (C4′), 61.1 (C6′), 53.5 (C2), 41.7 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M - H]^-$ calcd for $C_{32}H_{35}N_2O_{12}S$ 671.1911, found 671.1920.

Compound **15**: R_f 0.88 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 8.52 (s, 2H, CH Napht), 8.29 (dd, J = 3.3, 6.2 Hz, 2H, CH Napht), 7.87 (d, J = 8.2 Hz, 1H, NH), 7.81 (dd, J = 3.3, 6.3 Hz, 1H, CH Napht), 7.07−7.02 (m, 2H, CH SPh), 6.54−6.45 (m, 3H, CH SPh), 5.03 (d, J = 1.7 Hz, 1H, OH), 4.82 (d, J = 4.9 Hz, 1H, OH), 4.78 (d, J = 8.0 Hz, 1H, H1), 4.70 (t, J = 5.1 Hz, 1H, OH), 4.56 (d, J = 3.7 Hz, 1H, OH), 4.53 (d, J = 4.7 Hz, 1H, OH), 4.21 (d, J = 6.8 Hz, 1H, H1′), 3.96 (dd, J = 3.4, 14.0 Hz, 1H, H6a), 3.89 (dd, J = 9.6, 14.1 Hz, 1H, H6b), 3.75−3.59 (m, 4H,H2, H3, H5, H4′), 3.57−3.46 (m, 3H, H6′a, H6′b, H5′), 3.40−3.30 (m, 3H, H2′, H3′, H4), 1.83 (s, 3H $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 170.3 (C_{quat}, $COCH_3$), 167.7 (2C_{quat}, $N(CO)_2$), 135.5 (2C, 2C_{quat}, Napht), 134.4 (1C, 1C_{quat}, SPh), 130.8 (CH, SPh), 130.1 (2CH, Napht), 129.8 (2CH, SPh), 128.8 (2CH, Napht), 127.7 (2C_{quat}, Napht), 126.6 (CH, SPh), 125.0 (2CH, Napht), 104.4 (C1′), 86.0 (C1), 85.3 (C3), 76.3 (C5), 76.2 (C5′), 73.6 (C3′), 71.8 and 71.0 (C2′ and C4), 68.8 (C4′), 61.5 (C6′), 53.3 (C2), 40.6 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{32}H_{34}N_2O_{11}SNa$ 677.1781, found 677.1773.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-2,6-dideoxy-6-(3-methoxybenzamido)-1-thio-β-D-glucopyranoside ***16***. Amine **13** was dissolved in anhydrous DMF (2 mL) at 0 °C and treated with 3-methoxybenzoyl chloride (15 μL, 0.11 mmol, 1.1 equiv) and Et_3N (15 μL, 0.11 mmol, 1.1 equiv). The mixture was stirred for 2 h and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 9/1) to give derivative **16** as a white solid (29 mg, 50%): R_f 0.72 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 8.55 (t, J = 5.7 Hz, 1H, NH(3MeOBz)), 7.98 (d, J = 8.3 Hz, 1H, NHAc), 7.50−7.44 (m, 2H, CH 3MeOBz), 7.40 (t, J = 7.9 Hz, 1H, CH 3MeOBz), 7.32 (t, J = 4.5 Hz, 2H, CH SPh), 7.13 (dd, J = 3.4, 8.5 Hz, 1H, CH 3MeOBz), 7.04 (t, J = 7.4 Hz, 1H, CH SPh), 6.91 (t, J = 7.6 Hz, 2H, CH SPh), 4.92−4.90 (m, 1H, OH), 4.86 (d, J = 9.5 Hz, 1H, H1), 4.81 (d, J = 4.4 Hz, 1H OH), 4.69 (t, J = 5.2 Hz, 1H, OH), 4.52 (d, J = 4.7 Hz, 1H, OH), 4.49 (d, J = 3.3 Hz, 1H, OH), 4.22 (d, J = 6.4 Hz, 1H, H1′), 3.90−3.82 (m, 1H, H6a), 3.80 (s, 3H, CH_3 3MeOBz), 3.77−3.44 (m, 7H, H2, H3, H4′, H5, H6a′, H6′b, H5′), 3.42−3.22 (m, 4H, H2′, H3′, H6b, H4), 1.84 (s, 3H $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 170.3 (C_{quat}, $COCH_3$), 166.3 (C_{quat}, COPh), 159.6 (C_{quat}, 3MeO-C), 136.2 (C_{quat}, 3MeOBz), 135.5 (C_{quat}, SPh), 129.8 (CH, 3MeOBz), 129.6 (2CH, SPh), 129.1 (2CH, SPh), 126.7 (CH, SPh), 120.0 (CH, 3MeOBz), 117.5 (CH, 3MeOBz), 113.0 (CH, 3MeOBz), 104.3 (C1′), 86.2 (C1), 85.5 (C3), 78.0 (C5), 76.2 (C5′), 73.6 (C3′), 71.5 (C4), 71.1 (C2′), 68.7 (C4′), 61.1 (C6′), 55.8 (CH_3, 3MeOBz), 53.5 (C2), 41.7 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{28}H_{36}N_2O_{11}SNa$ 631.1938, found 631.1941.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-2,6-dideoxy-6-(3,5-dimethoxybenzamido)-1-thio-β-D-glucopyranoside ***17***. Amine **13** was dissolved in anhydrous DMF (2 mL) at 0 °C and treated with 3,5-dimethoxybenzoyl chloride (80 mg, 0.44 mmol, 4 equiv) and Et_3N (60 μL, 0.44 mmol, 4 equiv). The mixture was stirred 8 h at rt where 20% of conversion was observed and at 40 °C for 16 h where all the starting product was converted. Then the mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 9/1) to give derivative **17** as a white solid (22 mg, 38%); R_f 0.67 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 8.56 (t, J = 5.7 Hz, 1H, NH(3MeOBz)), 7.98 (d, J = 8.3 Hz, 1H, NH(Ac)), 7.32 (d, J = 7.4 Hz, 2H, CH SPh), 7.08 (d, J = 2.3 Hz, 2H, CH 3MeOBz), 7.05 (t, J = 7.7 Hz, 1H, CH SPh), 6.92 (t, J = 7.7 Hz, 2H, CH SPh), 6.69 (t, J = 2.3 Hz, 1H, CH 3MeOBz), 4.92−4.90 (m, 1H, OH), 4.86 (d, J = 9.3 Hz, 1H, H1), 4.81 (d, J = 4.9 Hz, 1H OH), 4.69 (t, J = 5.2 Hz, 1H, OH), 4.51 (d, J = 4.7 Hz, 1H, OH), 4.48 (d, J = 3.2 Hz, 1H, OH), 4.21 (d, J = 6.6 Hz, 1H, H1′), 3.90−3.81 (m, 1H, H6a), 3.78 (s, 6H, 2 × CH_3 3MeOBz), 3.75−3.45 (m, 7H, H2, H3, H4′, H5, H6a′, H6b′, H5′), 3.41−3.30 (m, 2H, H2′, H3′), 3.30−3.19 (m, 2H, H4, H6b), 1.83 (s, 3H $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 170.4 (1C, 1C_{quat}, $COCH_3$), 166.1 (C_{quat}, COPh), 160.8 (2C_{quat}, 3MeO-C), 136.8 (C_{quat}, 3MeOBz), 135.5 (C_{quat}, SPh), 129.5 (CH, 3MeOBz), 129.1 (2CH, SPh), 126.7 (CH, SPh), 105.8 (2CH, 3MeOBz), 104.4 (C1′), 103.6 (CH, 3MeOBz), 85.5 (2CH, C1 and C3), 78.0 (C5), 76.3 (C5′), 73.6 (C3′), 71.5 (C4), 71.1 (C2′), 68.7 (C4′), 61.1 (C6′), 55.9 (2CH_3, 3MeOBz), 52.9 (C2), 41.8 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{29}H_{38}N_2O_{12}SNa$ 661.2043, found 661.2037.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-2,6-dideoxy-6-(3-methoxyphenylacetamido)-1-thio-β-D-glucopyranoside ***18***. Amine **13** was dissolved in anhydrous DMF (2 mL) at 0 °C and treated with 3-methoxyphenylacetyl chloride (16 μL, 0.10 mmol, 1.1 equiv) and Et_3N (14 μL, 0.10 mmol, 1.1 equiv). The mixture was stirred 3 h and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 9/1) to give derivative **18** as a white solid (37 mg, 66%): R_f 0.65 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 8.11 (t, J = 5.8 Hz, 1H, NH(3MeOPh)), 8.01 (d, J = 8.0 Hz, 1H, NHAc), 7.42 (d, J = 7.1 Hz, 2H, CH SPh), 7.29 (d, J = 8.0 Hz, 2H, CH SPh), 7.23 (d, J = 7.0 Hz, 1H, CH SPh), 7.17 (t, J = 7.8 Hz, 1H, CH 3MeOPh), 6.83−6.79 (m, 2H, CH 3MeOPh), 6.78 (dd, J = 3.1, 8.1 Hz, 1H, CH 3MeOPh), 4.91 (d, J = 9.8 Hz, 1H, H1),4.86−4.79 (m, 2H, 2 × OH), 4.68 (t, J = 5.3 Hz, 1H, OH), 4.51 (d, J = 4.7 Hz, 1H, OH), 4.48 (d, J = 3.7 Hz, 1H, OH), 4.19 (d, J = 6.3 Hz, 1H, H1′), 3.71 (s, 3H, CH_3 3MeOPh), 3.69−3.56 (m, 4H, H2, H3, H6a, H4′), 3.55−3.37 (m, 6H, H6a′, H6b′, H5′, H5, CH_2 3MeOPhCH_2), 3.37−3.28 (m, 2H, H2′, H3′), 3.24−3.17 (m, 1H, H4′), 3.11 (dd, J = 5.9, 14.0 Hz, 1H, H6b), 1.84 (s, 3H $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 170.1 (C_{quat}, $COCH_2Ph$), 170.4 (C_{quat}, $COCH_3$), 159.6 (C_{quat}, 3MeO-C), 138.3 (C_{quat}, 3MeOPh), 135.3 (C_{quat}, SPh), 129.8 (2CH, SPh), 129.6 (CH, 3MeOPh), 129.4 (2CH, SPh), 127.0 (CH, SPh), 121.7 (CH, 3MeOPh), 115.2 (CH, 3MeOPh), 112.3 (CH, 3MeOPh), 104.3 (C1′), 86.0 (C1), 85.3 (C3), 78.4 (C5), 76.3 (C5′), 73.6 (C3′), 71.0 (C2′), 70.8 (C4), 68.7 (C4′), 61.0 (C6′), 55.4 (CH_3, 3MeOPh), 53.4 (C2), 45.9 (CH_2, 3MeOPhCH_2), 40.9 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M + Na]^+$calcd for $C_{29}H_{38}N_2O_{11}SNa$ 645.2094, found 645.2088.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-6-(piperonyloyl)amido-2,6-dideoxy-1-thio-β-D-glucopyranoside ***19***. Amine **13** was dissolved in anhydrous DMF (2 mL) at 0 °C and treated with piperonyloyl chloride (20 mg, 0.11 mmol, 1.1 equiv) and Et_3N (15 μL, 0.11 mmol, 1.1 equiv). The mixture was stirred for 12 h and concentrated under reduced pressure. The residue was first purified by flash chromatography (CH_2Cl_2/MeOH 75/25) and then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture as eluent to give derivative **19** as a white solid (33 mg, 55%): R_f 0.65 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 8.38 (t, J = 5.7 Hz, 1H, NH(C(O)Ph)), 8.02 (d, J = 8.2 Hz, 1H, NH(Ac)), 7.47 (d, J = 8.0 Hz, 1H, Ph), 7.41 (br s, 1H, Ph), 7.33 (d, J = 7.7 Hz, 2H, CH SPh), 7.08 (t, J = 7.4 Hz, 1H, CH SPh), 7.03−6.93 (m, 3H, CH, SPh, Ph),

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6.11 (s, 2H, OCH_2O), 4.98−4.79 (m, 3H, 2 × O*H*, H1), 4.71 (br s, 1H, O*H*), 4.52−4.50 (m, 2H, 2 O*H*), 4.21 (d, *J* = 6.4 Hz, 1H, H1′), 3.82 (dd, *J* = 6.3, 12.8 Hz, 1H, H6a), 3.76−3.60 (m, 3H, H2, H3, H4′), 3.60−3.44 (m, 4H, H5, H6′a, H6′b, H5′), 3.44−3.29 (m, 2H, H2′, H3′), 3.29−3.18 (m, 2H, H4, H6b), 1.84 (s, 3H, $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 170.4 (C_{quat}, $COCH_3$), 165.7 (C_{quat}, COPh), 150.1 (C_{quat}, $COCH_2$), 147.7 (C_{quat}, $COCH_2$), 135.4 (C_{quat}, SPh), 129.6 (2CH, SPh), 129.1 (2CH, SPh), 128.8 (C_{quat}, CCONH), 126.8 (CH, SPh), 122.7 (CH, Ph), 108.2 (CH, Ph), 107.9 (CH, Ph), 104.3 (C1′), 102.1 (CH_2, OCH_2O), 86.1 (C1), 85.5 (C3), 78.0 (C5), 76.3 (C5), 73.6 (C3′), 71.5 (C4), 71.0 (C2′), 68.7 (C4′), 61.0 (C6′), 53.5 (C2), 41.3 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) *m*/*z* $[M + Na]^+$calcd for $C_{28}H_{34}N_2O_{12}SNa$ 645.1730, found 645.1738.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-6-(methoxyoxoacetamido)-2,6-dideoxy-1-thio-β-D-glucopyranoside ***20***. Amine **13** was dissolved in anhydrous DMF (2 mL) at 0 °C and treated with methyl chlorooxoacetate (10 μL, 0.11 mmol, 1.1 equiv) and Et_3N (15 μL, 0.11 mmol, 1.1 equiv). The mixture was stirred 12 h and concentrated under reduced pressure. The residue was purified first by flash chromatography (CH_2Cl_2/MeOH 7/3) then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient as eluent to give derivative **20** as a white solid (33 mg, 60%): R_f 0.75 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 8.91 (t, *J* = 6.1 Hz, 1H, N*H*(C(O)CO_2Me)), 7.90 (d, *J* = 7.8 Hz, 1H, N*H*Ac), 7.38−7.32 (m, 2H, C*H* SPh), 7.25−7.21 (m, 3H, C*H* SPh), 4.90 (br s, 1H, OH), 4.85−4.80 (m, 2H, H1 and OH), 4.68 (t, *J* = 5.0 Hz, 1H, OH), 4.17 (d, *J* = 6.8 Hz, 1H, H1′), 3.79 (s, 3H, CH_3 MeO), 3.72−3.44 (m, 8H, H3, H4′, H6a, H2, H5′, H6′a, H6′b, H5), 3.34−3.22 (m, 3H, H2′, H3′, H6b), 3.22 (t, *J* = 8.6 Hz, 1H, H4), 1.83 (s, 3H, $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 170.3 (C_{quat}, $COCH_3$), 161.5 (C_{quat}, COOMe), 157.3 (C_{quat}, $NHCOCO_2Me$), 135.1 (C_{quat}, SPh), 129.9 (2CH, SPh), 129.3 (2CH, SPh), 127.0 (CH, SPh), 104.4 (C1′), 85.8 (C1), 85.5 (C3), 77.4, 76.3 (C5′, C5), 73.5 (C3′), 71.2 (C4), 71.0 (C2′), 68.7 (C4′), 61.1 (C6′), 53.3 (CH_3, MeO), 50.9 (C2), 41.4 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) *m*/*z* $[M + Na]^+$ calcd for $C_{23}H_{32}N_2O_{12}SNa$ 583.1574, found 583.1582.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-6-(carboxyoxoacetamido-2,6-dideoxy-1-thio-β-D-glucopyranoside ***21***. A solution of ester derivative **20** (15 mg, 0.027 mmol, 1 equiv) in a mixture of aq. NaOH 2 M (1 mL) and MeOH (1 mL) was stirred 2 h at 0 °C. Then the mixture was acidified with HCl 1 M to pH 1 and concentrated under reduced pressure. The residue was purified by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give acid **21** as a colorless solid (14 mg, 95%); R_f 0.25 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 8.31 (br s, 1H, N*H*(C(O)CO_2Na)), 7.95 (d, *J* = 8.2 Hz, 1H, N*H*Ac), 7.40 (d, *J* = 7.6 Hz, 2H, C*H* SPh), 7.31 (t, *J* = 7.5 Hz, 2H, C*H* SPh), 7.22 (t, *J* = 7.2 Hz, 1H, C*H* SPh), 4.92−4.83 (m, 2H, H1 and OH), 4.52 (br s, 1H, OH), 4.17 (d, *J* = 6.4 Hz, H1′), 3.73−3.55 (m, 4H, H3, H4′, H6a, H2), 3.55−3.41 (m, 4H, H5′, H6′a, H6′b, H5), 3.40−3.28 (m, 2H, H2′, H3′), 3.26−3.14 (m, 2H, H4, H6b), 1.83 (s, 3H, $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 170.3 (2C_{quat}, $COCH_3$, COONa), 165.9 (C_{quat}, $NHCOCO_2Na$), 135.0 (C_{quat}, SPh), 130.2 (2CH, SPh), 129.6 (2CH, SPh), 127.1 (CH, SPh), 104.4 (C1′), 86.1 (C1), 85.3 (C3), 78.0 (C5), 76.3 (C5′), 73.4 (C3′), 71.0 (2*C*, C4 and C2′), 68.6 (C4′), 61.0 (C6′), 53.5 (C2), 41.1 (C6), 23.4 ($COCH_3$); HRMS (ESI-TOF) *m*/*z* $[M - Na]^-$calcd for $C_{22}H_{28}N_2O_{12}S$ 545.1441, found 545.1437.

Phenyl β-D-galactopyranosyl-(1→3)-2-acetamido-2,6-dideoxy-6-(3-methoxyphenylsulfonamido)-1-thio-β-D-glucopyranoside ***22***. Amine **13** was dissolved in anhydrous DMF (2 mL) at 0 °C and treated with 3-methoxyphenylsulfonyl chloride (24 μL, 0.17 mmol, 1.6 equiv) and Et_3N (24 μL, 0.17 mmol, 1.6 equiv). The mixture was stirred 24 h at rt and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 9/1) to give derivative **22** as a white solid (14 mg, 21%): R_f 0.70 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, CD_3OD) δ 7.55−7.61 (m, 2H, C*H* SPh), 7.45−7.39 (m, 3H, C*H* 3MeOPh), 7.37−7.28 (m, 3H, C*H* SPh), 7.16 (dt, *J* = 2.3, 7.4 Hz, 1H, C*H* 3MeOPh), 4.74 (d, *J* = 10.5 Hz, 1H, H1), 4.27 (d, *J* = 7.4 Hz, 1H, H1′), 3.86 (s, 3H, CH_3 3MeOPh), 3.82−3.73 (m, 3H, H2, H4′, H6a′), 3.73−3.61 (m, 2H, H6b′, H3), 3.59−3.49 (m, 2H, H5′, H2′), 3.49−3.40 (m, 2H, H3′, H6a), 3.38−3.33 (m, 1H, H5), 3.22 (dd, *J* = 8.1, 9.4 Hz, 1H, H4), 3.02 (dd, *J* = 7.6, 13.8 Hz, 1H, H6b), 1.99 (s, 3H, $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ 172.6 (C_{quat}, $COCH_3$), 160.1 (C_{quat}, 3MeO-*C*), 141.8 (C_{quat}, 3MeOPh), 133.5 (C_{quat}, SPh), 131.5 (2CH, SPh), 129.9 (CH, 3MeOPh), 128.6 (2CH, SPh), 127.2 (CH, SPh), 118.7 (CH, 3MeOPh), 118.2 (CH, 3MeOPh), 111.6 (CH, 3MeOPh), 104.2 (C1′), 86.4 (C1), 84.3 (C3), 78.4 (C5), 75.7 (C5′), 73.2 (C3′), 70.9 (C2′), 70.4 (C4), 68.8 (C4′), 61.1 (C6′), 54.8 (CH_3, 3MeOPh), 55.0 (C2), 44.2 (C6), 21.7 ($COCH_3$); HRMS (ESI-TOF) *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{36}N_2O_{12}S_2Na$ 667.1607, found 667.1614.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-2,6-dideoxy-6-(3-methoxyphenylureido)-1-thio-β-D-glucopyranoside ***23***. Amine **13** was dissolved in anhydrous DMF (2 mL) at 0 °C and treated with 3-methoxyphenyl isocyanate (16 μL, 0.12 mmol, 1.2 equiv) and Et_3N (17 μL, 0.12 mmol, 1.1 equiv). The mixture was stirred for 12 h and concentrated under reduced pressure. The residue was first purified by flash chromatography (CH_2Cl_2/MeOH 85/15) then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **23** as a white solid (29 mg, 47%): R_f 0.89 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 8.77 (s, 1H, N*H*(3MeOPh)), 7.99 (d, *J* = 8.0 Hz, 1H, N*H*Ac), 7.44−7.38 (m, 2H, C*H* SPh), 7.26−7.20 (m, 2H, C*H* SPh), 7.20−7.10 (m, 2H, C*H* SPh, 3MeOPh), 7.13 (dd, *J* = 5.7, 13.9 Hz,1H, C*H* 3MeOPh), 6.86 (dd, *J* = 1.9, 8.1 Hz, 1H, C*H* 3MeOPh), 6.48 (dd, *J* = 2.5, 8.2 Hz, 1H, C*H* 3MeOPh), 6.33 (t, *J* = 6.0 Hz, 1H, N*H*CONH), 4.94−4.77 (m, 3H, H1, 2 × O*H*), 4.76−4.64 (m, 1H, O*H*), 4.52 (d, *J* = 11.8 Hz, 2H, 2 × O*H*), 4.20 (d, *J* = 5.2 Hz, 1H, H1′), 3.71 (s, 3H, CH_3 3MeOPh), 3.68−3.58 (m, 4H, H2, H3, H6a, H4′), 3.52 (d, *J* = 6.1 Hz, 2H, H6′a, H6′b), 3.49−3.28 (m, 4H, H5′, H5, H2′, H3′), 3.22 (t, *J* = 9.3 Hz, 1H, H4), 3.19−3.10 (m, 1H, H6b), 1.83 (s, 3H $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ, 170.4 (C_{quat}, $COCH_3$), 160.1 (C_{quat}, 3MeO-*C*), 155.6 (C_{quat}, CONHPh), 142.3 (C_{quat}, 3MeOPh), 135.2 (C_{quat}, SPh), 130.0 (2CH, SPh), 129.9 (CH, 3MeOPh), 129.4 (2CH, SPh), 127.0 (CH, SPh), 110.3 (CH, 3MeOPh), 106.9 (CH, 3MeOPh), 104.3 (C1′), 103.7 (CH, 3MeOPh), 86.2 (C1), 85.4 (C3), 78.6 (C5), 76.3 (C5′), 73.5 (C3′), 71.0 (C2′), 70.7 (C4), 68.7 (C4′), 61.0 (C6′), 55.3 (CH_3, 3MeOPh), 53.4 (C2), 41.1 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) *m*/*z* $[M + Na]^+$calcd for $C_{28}H_{37}N_3O_{11}SNa$ 646.2046, found 646.2043.

Phenyl β-D-Galactopyranosyl-(1−3)-2-acetamido-6-N-(3-methoxybenzyl)amino-2,6-dideoxy-1-thio-β-D-glucopyranoside ***24***. Amine **13** was dissolved in anhydrous DMF (1 mL) and treated with 3-methoxybenzaldehyde (7.3 μL, 0.06 mmol, 1.2 equiv) and Et_3N (7 μL, 0.05 mmol, 1 equiv). The mixture was stirred for 12 h at 60 °C, and the reaction was monitored by TLC. When all starting product was consumed, the mixture was cooled to rt and $NaBH_4$ (2.3 mg, 0.06 mmol, 1.2 equiv) was added. After completion of the reaction (2 h), the mixture was concentrated under reduced pressure. The residue was first purified by flash chromatography (CH_2Cl_2/MeOH 8/2) and then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **24** as a white solid (16.7 mg, 56%): R_f 0.76 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 7.92 (d, *J* = 8.1 Hz, 1H, N*H*Ac), 7.44−7.38 (m, 2H, C*H* SPh), 7.29−7.17 (m, 4H, C*H* SPh, 3MeOPh), 6.88 (dd, *J* = 1.4, 2.8 Hz, 1H, C*H* 3MeOPh), 6.85 (dt, *J* = 1.2, 7.6 Hz, 1H, C*H* 3MeOPh), 6.80 (ddd, *J* = 1.0, 2.7, 8.3 Hz, 1H, C*H* 3MeOPh), 4.95 (d, *J* = 9.5 Hz, 1H, H1), 4.85−4.72 (m, 2H, N*H*CH_2, OH), 4.62 (t, *J* = 5.1 Hz, 1H, O*H*), 4.48 (d, *J* = 4.7 Hz, 1H, O*H*), 4.46 (br s, 1H, OH), 4.17 (d, *J* = 7.1 Hz, 1H, H1′), 3.73 (s, 3H, CH_3 3MeOPh), 3.71−3.56 (m, 5H, NHCH_2, H2, H3, H4′), 3.55−3.38 (m, 4H, H6′a, H6′b, H5′, H5), 3.38−3.28 (m, 2H, H2′, H3′), 3.22 (t, *J* = 8.8 Hz, 1H, H4), 2.92 (dd, *J* = 2.6, 12.7 Hz, 1H, H6a), 2.64 (dd, *J* = 7.8, 12.7 Hz, 1H, H6b), 1.83 (s, 3H $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ, 170.4 (C_{quat}, $COCH_3$), 159.7 (C_{quat}, 3MeO-*C*), 134.7 (C_{quat}, 3MeOPh), 130.4 (2CH, SPh), 129.6 (CH, 3MeOPh), 129.4 (2CH, SPh), 127.2 (CH, SPh), 120.5 (CH,

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3MeOPh), 113.8 (CH, 3MeOPh), 112.6 (CH, 3MeOPh), 104.3 (C1′), 85.6 (C1), 85.5 (C3), 79.3 (C5), 76.3 (C5′), 73.5 (C3′), 71.0 (C2′), 70.9 (C4), 68.7 (C4′), 61.1 (C6′), 55.4 (CH_3, 3MeOPh), 53.6 (C2), 53.1 (CH_2, $NHCH_2$), 40.7 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{28}H_{39}N_2O_{10}SNa$ 595.2325, found 595.2352.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetamido-6-N,N′-di-(3-methoxybenzyl)amino-2,6-dideoxy-1-thio-β-D-glucopyranoside ***25***. Amine **13** was dissolved in anhydrous DMF (2 mL) and treated with 3-methoxybenzaldehyde (29.0 μL, 0.24 mmol, 2.4 equiv) and Et_3N (28 μL, 0.2 mmol, 2 equiv). The mixture was stirred for 12 h at 60 °C, and the reaction was monitored by TLC. When all starting product was consumed, the mixture was cooled to rt, and then $NaBH_3CN$ (12.6 mg, 0.2 mmol, 2 equiv) and AcOH (11.4 μL, 0.2 mmol, 2 equiv) were added. After 2 h, the reaction mixture was concentrated under reduced pressure. The residue was first purified by flash chromatography (CH_2Cl_2/MeOH 8/2) and then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **25** as a white solid (26.6 mg, 37%): R_f 0.85 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^1H NMR (400 MHz, DMSO-d_6) δ 7.93 (d, J = 7.8 Hz, 1H, NHAc), 7.48−7.43 (m, 2H, CH SPh), 7.29−7.22 (m, 2H, CH SPh), 7.22−7.13 (m, 3H, CH SPh, 3MeOPh), 6.90−6.84 (m, 4H, CH 3MeOPh), 6.79−6.73 (m, 2H, CH 3MeOPh), 5.06 (d, J = 8.6 Hz, 1H, H1), 4.83 (br s, 1H, OH), 4.72 (s, 1H, OH), 4.63 (d, J = 7.6 Hz, 1H, OH), 4.54−4.51 (br s, 2H, 2x OH), 4.18 (d, J = 6.4 Hz, 1H, H1′), 3.69 (s, 6H, 2 × CH_3 3MeOPh), 3.66−3.58 (m, 6H, $N(CH_2)_2$, H2, H3, H5, H4′), 3.53−3.41 (m, 5H, $N(CH_2)_2$, H6′a, H6′b, H5′), 3.39−3.26 (m, 2H, H2′, H3′), 3.16−3.08 (m, 1H, H4), 2.91 (d, J = 13.8 Hz, 1H, H6a), 2.58 (dd, J = 8.1, 14.1 Hz, H6b, 1H), 1.84 (s, 3H $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 170.4 (C_{quat}, $COCH_3$), 159.6 (2C_{quat}, 3MeO-C), 141.5 (2C_{quat}, 3MeOPh), 134.9 (C_{quat}, SPh), 130.0 (2CH, SPh), 129.5 (2CH, 3MeOPh), 129.4 (2CH, SPh), 127.0 (CH, SPh), 121.0 (2CH, 3MeOPh), 114.1 (2CH, 3MeOPh), 112.7 (2CH, 3MeOPh), 104.2 (C1′), 85.7 (C1), 85.4 (C3), 78.8 (C5), 76.2 (C5′), 73.5 (C3′), 71.0 (C2′), 71.0 (C4), 68.7 (C4′), 61.0 (C6′), 58.1 (2CH_2, $N(CH_2)_2$), 55.3 (2CH_3, 3MeOPh), 55.1 (C2), 40.7 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{36}H_{47}N_2O_{11}SNa$ 715.2901, found 715.2868.

Phenyl β-D-Galactopyranosyl-(1→3)-2-acetylamino-2,6-dideoxy-6-[4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl]-1-thio-β-D-glucopyranoside ***26***. To a solution of azide **11** (20 mg, 0.04 mmol) in H_2O/^{t}BuOH (1/1 2 mL) were added 3-methoxyphenylacetylene (11.4 μL, 0.08 mmol, 2 equiv), *o*-phenylenediamine (16 μL of a 375 mM solution in H_2O, 6 μmol, 0.15 equiv), sodium ascorbate (16 μL of a 250 mM solution in H_2O, 4 μmol, 0.1 equiv), and copper sulfate pentahydrate (16 μL of a 125 mM solution in H_2O, 2.0 μmol, 0.05 equiv). The reaction mixture was stirred at rt under an argon atmosphere for 12 h, by which time TLC (cyclohexane/EtOAc 9/1) showed complete conversion. Then activated charcoal was added to the reaction mixture, which was stirred for 16 h. The reaction mixture was then filtered through a Celite plug and eluted with water, and the solvent evaporated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 1/0 to 5/5) to give derivative **26** as a white solid (6.7 mg, 23%): R_f 0.52 (CH_2Cl_2/MeOH 85/15); ^1H NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H, C = CH triazole), 7.95 (d, J = 8.0 Hz, 1H, NH(3MeOBz)), 7.44−7.34 (m, 3H, CH 3MeOBz), 7.05−7.00 (m, 2H, CH SPh), 6.99−6.90 (m, 4H, CH 3MeOBz, 3 CH SPh), 5.13 (br s, 1H, OH), 4.89−4.77 (m, 3H, H1, H6a, OH), 4.71 (t, J = 5.0 Hz, 1H, OH), 4.58−4.52 (m, 3H, H6b, 2 OH), 4.21 (d, J = 5.9 Hz, 1H, H1′), 3.89−3.82 (m, 1H, H5), 3.81 (s, 3H, CH_3 3MeOBz), 3.74−3.64 (m, 2H, H2, H3), 3.62 (d, J = 3.7 Hz, 1H, H4′), 3.58−3.51 (m, 2H, H6a′, H6b′), 3.51−3.46 (m, 1H, H5′), 3.40−3.29 (m, 3H, H2′, H3′, H4), 1.82 (s, 3H, $COCH_3$).; $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 170.4 (C_{quat}, $COCH_3$), 160.1 (C_{quat}, 3MeO-C), 146.6 (C=CH triazol), 134.6 (C_{quat}, 3MeOBz), 132.6 (C_{quat}, SPh), 130.5 (CH, 3MeOBz), 130.0 (2CH, SPh), 129.1 (2CH, SPh), 127.0 (CH, SPh), 123.0 (C = CH triazol), 118.0 (CH, 3MeOBz), 114.0 (CH, 3MeOBz), 110.9 (CH, 3MeOBz), 104.5 (C1′), 86.1 (C1), 85.1 (C3), 78.2 (C5), 76.3 (C5′), 73.5 (C3′), 71.0 and 70.6 (C2′ and C4), 68.7 (C4′), 61.1 (C6′), 55.6 (CH_3, 3MeOBz), 53.2 (C2), 51.6 (C6), 23.5 ($COCH_3$); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{29}H_{36}N_4O_{10}SNa$ 655.2050, found 655.2053.

General Procedure for the Reduction of Azide Derivative 12. To a solution of disaccharide **12** (30 mg, 0.065 mmol, 1 equiv) in THF (3 mL), under an atmosphere of argon, was added PMe_3 1 M in THF (130 μL, 0.13 mmol, 2 equiv). The reaction was stirred at rt and monitored by TLC ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3). After completion, H_2O (60 μL) was added, and the mixture was heated to 60 °C in an oil bath for 16 h. Then solvent was removed under reduced pressure to give crude phenyl β-D-galactopyranosyl-(1→3)-6-amino-6-deoxy-1-thio-β-D-glucopyranoside **28**, used without further purification: R_f 0.60 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3).

Phenyl β-D-Galactopyranosyl-(1→3)-6-deoxy-6-(3-methoxybenzamido)-1-thio-β-D-glucopyranoside ***29***. Amine **28** was dissolved in anhydrous DMF (1.5 mL) at 0 °C and treated with 3-methoxybenzoyl chloride (11 μL, 0.08 mmol, 1.2 equiv) and Et_3N (11 μL, 0.08 mmol, 1.2 equiv). The mixture was stirred for 12 h and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 9/1) then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **29** as a white solid (21.2 mg, 57%): R_f 0.42 (CH_2Cl_2/MeOH 9/1); ^1H NMR (400 MHz, DMSO-d_6) δ 8.51 (t, J = 5.8 Hz, 1H, NH(3MeOBz)), 7.51−7.35 (m, 5H, 3 CH 3MeOBz, 2 CH SPh), 7.16−7.11 (m, 1H, CH 3MeOBz), 7.08 (m, 1H, CH SPh), 7.00−6.93 (m, 2H, CH SPh), 5.49 (d, J = 4.6 Hz, 1H, OH), 5.01 (d, J = 3.3 Hz, 1H, OH), 4.97 (d, J = 2.5 Hz, 1H, OH), 4.83 (d, J = 5.5 Hz, 1H, OH), 4.72−4.68 (m, 1H, OH), 4.68 (d, J = 9.6 Hz, 1H, H1), 4.53 (d, J = 4.5 Hz, 1H, OH), 4.31 (d, J = 7.5 Hz, 1H, H1′), 3.87−3.80 (m, 1H, H6a), 3.80 (s, 3H, CH_3 3MeOBz), 3.64−3.54 (m, 2H, H4′, H5), 3.51 (m, 2H, H6a′, H6b′), 3.50−3.38 (m, 3H, H3, H5′, H2′), 3.38−3.29 (m, 2H, H3′, H2), 3.28−3.15 (m, 2H, H6b, H4); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6) δ 166.3 (C_{quat}, COPh), 159.6 (C_{quat}, 3MeO-C), 136.2 (C_{quat}, 3MeOBz), 134.9 (C_{quat}, SPh), 130.3 (CH, 3MeOBz), 129.8 (2CH, SPh), 129.1 (2CH, SPh), 126.9 (CH, SPh), 120.0 (CH, 3MeOBz), 117.5 (CH, 3MeOBz), 113.0 (CH, 3MeOBz), 104.6 (C1′), 89.2 (C3), 86.6 (C1), 78.0 (C5), 76.1 (C5′), 73.3 (C3′), 71.6 and 71.5 (C2 and C2′), 71.1 (C4), 68.5 (C4′), 60.9 (C6′), 55.8 (CH_3, 3MeOBz), 41.7 (C6); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{26}H_{33}NO_{11}SNa$ 590.1672, found 590.1671.

Phenyl β-D-Galactopyranosyl-(1→3)-6-deoxy-6-(3-methoxyphenylureido)-1-thio-β-D-glucopyranoside ***30***. Amine **28** was dissolved in anhydrous DMF (1.5 mL) at 0 °C and treated with 3-methoxyphenyl isocyanate (10 μL, 0.08 mmol, 1.2 equiv) and Et_3N (10 μL, 0.08 mmol, 1.2 equiv). The mixture was stirred for 12 h and concentrated under reduced pressure. The residue was first purified by l-phase flash chromatography (CH_2Cl_2/MeOH 9/1) then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **30** as a white solid (24.3 mg,65%): R_f 0.40 (CH_2Cl_2/MeOH 9/1); ^1H NMR (400 MHz, DMSO) δ 8.69 (s, 1H, NH(3MeOPh)), 7.53−7.44 (m, 2H, CH SPh), 7.32−7.07 (m, 5H, 3 CH SPh, 2 CH 3MeOPh), 6.90−6.82 (m, 1H, CH 3MeOPh), 6.52−6.44 (m, 1H, CH 3MeOPh), 6.22 (t, J = 6.2 Hz, 1H, NHCONH), 5.44 (d, J = 4.5 Hz, 1H, OH), 4.98 (d, J = 3.3 Hz, 1H), 4.91 (br s, 1H, OH), 4.82 (d, J = 5.3 Hz, 1H, OH), 4.74 (d, J = 9.7 Hz, 1H, H1), 4.69 (t, J = 5.3 Hz, 1H, OH), 4.50 (d, J = 4.6 Hz, 1H, OH), 4.28 (d, J = 7.4 Hz, 1H, H1′), 3.71 (s, 3H, CH_3 3MeOPh), 3.69−3.57 (m, 2H, H4′, H6a), 3.52−3.50 (m, 2H, H6a′, H6b′), 3.49−3.39 (m, 4H, H5′, H3, H5, H2′), 3.39−3.25 (m, 2H, H3′, H2), 3.21−3.06 (m, 2H, H4, H6b); $^{13}C\{^1H\}$ NMR (101 MHz, DMSO) δ 160.1 (C_{quat}, 3MeO-C), 155.5 (C_{quat}, CONHPh), 142.2 (C_{quat}, 3MeOPh), 134.4 (C_{quat}, SPh), 130.9 (2CH, SPh), 129.9 (CH, 3MeOPh), 129.4 (2CH, SPh), 127.2 (CH, SPh), 110.3 (CH, 3MeOPh), 106.9 (CH, 3MeOPh), 105.1 (C1′), 103.7 (CH, 3MeOPh), 89.2 (C3), 86.5 (C1), 78.6 (C5), 76.1 (C5′), 73.3 (C3′), 71.5 and 71.3 (C2 and C2′), 70.3 (C4), 68.6 (C4′), 61.0 (C6′), 55.3 (CH_3, 3MeOPh), 40.9 (C6); HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{26}H_{34}N_2O_{11}SNa$ 605.1781, found 605.1785.

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Phenyl β-D-Galactopyranosyl-(1→3)-6-deoxy-6-N-(3-methoxybenzyl)-1-thio-β-D-glucopyranoside ***31***. Amine **28** was dissolved in anhydrous DMF (1.5 mL) and treated with 3-methoxybenzaldehyde (9.6 μL, 0.08 mmol, 1.2 equiv) and Et_3N (11 μL, 0.08 mmol, 1.2 equiv). The mixture was stirred for 12 h at 60 °C, and the reaction was followed by TLC. When all starting product was consumed, the mixture was cooled to rt and $NaBH_4$ (5.9 mg, 0.16 mmol, 2.4 equiv) was added. After 2 h, the reaction mixture was concentrated under reduced pressure. The residue was first purified by flash chromatography (CH_2Cl_2/MeOH 9/1) and then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **31** as a white solid (22.1 mg, 61%): R_f 0.75 ($CHCl_3$/MeOH/H_2O/CH_3COOH 60/30/5/3); ^{1}H NMR (400 MHz, CD_3OD) δ 7.56−7.50 (m, 2H, C*H* SPh), 7.29−7.22 (m, 4H, C*H* SPh, 3MeOPh), 6.92−6.84 (m, 1H, 3H, C*H* 3MeOPh), 4.67 (d, *J* = 9.8 Hz, 1H, H1), 4.53 (d, *J* = 7.6 Hz, 1H, H1′), 3.86−3.73 (m, 7H, H6a′, H4′ C*H*$_3$ 3MeOPh, NHC*H*$_2$), 3.69 (dd, *J* = 4.5, 11.4 Hz, 1H, H6b′), 3.65−3.54 (m, 3H, H2′, H5′, H3), 3.5−3.45 (m, 2H, H3′, H5), 3.43 (dd, *J* = 8.5, 9.9 Hz, 1H, H2), 3.26 (t, *J* = 9.2 Hz, 1H, H4), 3.11 (dd, *J* = 2.0, 12.9 Hz, 1H, H6a), 2.78 (dd, *J* = 8.6, 12.7 Hz, 1H, H6b); ^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ, 160.0 (*C*$_{quat}$, 3MeO-*C*), 139.3 (*C*$_{quat}$, 3MeOPh), 132.6 (2*C*H, SPh), 132.5 (*C*$_{quat}$, SPh), 129.3 (*C*H, 3MeOPh), 128.6 (2*C*H, SPh), 127.5 (*C*H, SPh), 120.5 (*C*H, 3MeOPh), 113.8 (*C*H, 3MeOPh), 112.7 (*C*H, 3MeOPh), 104.2 (C1′), 87.3 (C3), 86.9 (C1), 77.6 (C5), 75.7 (C5′), 73.3 (C3′), 71.6 and 71.6 (C2 and C2′), 70.6 (C4), 68.9 (C4′), 61.2 (C6′), 54.3 (*C*H$_3$, 3MeOPh), 53.4 (C2), 52.3 (*C*H$_2$, NHCH$_2$), 49.6 (C6); HRMS (ESI-TOF) *m*/*z* [M + H]$^+$ calcd for $C_{26}H_{36}NO_{10}S$ 554.2060, found 554.2068.

Phenyl β-D-Galactopyranosyl-(1→3)-6-deoxy-6-(3-methoxyphenylsulfonamido)-1-thio-β-D-glucopyranoside ***32***. Amine **28** was dissolved in anhydrous DMF (1.5 mL) at 0 °C and treated with 3-methoxyphenylsulfonyl chloride (12 μL, 0.08 mmol, 1.2 equiv) and Et_3N (12 μL, 0.08 mmol, 1.2 equiv). The mixture was stirred for 8 h at rt and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 88/12) and then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **32** as a white solid (27.8 mg, 70%): R_f 0.48 (CH_2Cl_2/MeOH 9/1); ^{1}H NMR (400 MHz, CD_3OD) δ 7.59−7.55 (m, 2H, C*H* SPh), 7.47−7.39 (m, 3H, C*H* 3MeOPh), 7.37−7.29 (m, 3H, C*H* SPh), 7.17 (dt, *J* = 2.4, 7.2 Hz, 1H, C*H* 3MeOPh), 4.51 (d, *J* = 7.5 Hz, 1H, H1′), 4.48 (d, *J* = 9.89 Hz, 1H, H1), 3.86 (s, 3H, C*H*$_3$ 3MeOPh), 3.80 (dd, *J* = 1.0, 3.4 Hz, 1H, H4′), 3.77 (dd, *J* = 7.2, 10.9 Hz, 1H, H6a′), 3.69 (dd, *J* = 4.4, 11.5 Hz, 1H, H6b′), 3.61−3.47 (m, 4H, H2′, H5′, H3, H3′), 3.42 (dd, *J* = 2.6, 13.8 Hz, 1H, H6a), 3.38−3.29 (m, 2H, H2, H5), 3.21 (dd, *J* = 8.6, 9.7 Hz, 1H, H4), 2.99 (dd, *J* = 7.7, 13.8 Hz, 1H, H6b); ^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ 160.1 (*C*$_{quat}$, 3MeO-*C*), 141.8 (*C*$_{quat}$, 3MeOPh), 132.9 (*C*$_{quat}$, SPh), 132.1 (2*C*H, SPh),130.0 (*C*H, 3MeOPh), 128.6 (2*C*H, SPh), 127.3 (*C*H, SPh), 118.7 (*C*H, 3MeOPh), 118.2 (*C*H, 3MeOPh), 111.5 (*C*H, 3MeOPh), 104.2 (C1′), 87.3 (C3), 87.1 (C1), 78.2 (C5), 75.7 (C5′), 73.3 (C3′), 71.6 and 71.3 (C2 and C2′), 70.0 (C4), 68.9 (C4′), 61.2 (C6′), 54.8 (*C*H$_3$, 3MeOPh), 44.2 (C6); HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{25}H_{33}NO_{12}S_2$ 626.1342, found 626.1337.

Phenyl β-D-Galactopyranosyl-(1→3)-6-deoxy-6-[(4-phenoxy)-phenylsulfonamido]-1-thio-β-D-glucopyranoside ***33***. Amine **28** was dissolved in anhydrous DMF (5 mL) at 0 °C and treated with 4-phenoxyphenylsulfonyl chloride (35 mg, 0.13 mmol, 1.2 equiv) and Et_3N (18 μL, 0.13 mmol, 1.2 equiv). The mixture was stirred for 8 h at rt and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 93/7) and then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **33** as a white solid (38.9 mg, 54%): R_f 0.31 (CH_2Cl_2/MeOH 9/1); ^{1}H NMR (400 MHz, CD_3OD) δ 7.83−7.77 (m, 2H, C*H* PhenoxyPh), 7.58−7.56 (m, 2H, C*H* SPh), 7.47−7.42 (m, 2H, C*H* PhenoxyPh), 7.35−7.27 (m, 3H, C*H* SPh), 7.27−7.22 (m, 1H, C*H* PhenoxyPh), 7.12−7.07 (m, 2H, C*H* PhenoxyPh), 7.03−6.98 (m, 2H, C*H* PhenoxyPh), 4.51 (d, *J* = 7.6 Hz, 1H, H1′), 4.47 (d, *J* = 9.8 Hz, 1H, H1), 3.81−3.76 (m, 2H, H4′, H6a′), 3.70 (dd, *J* = 4.4, 11.5 Hz, 1H, H6b′), 3.61−3.55 (m, 2H, H2′, H5′), 3.55−3.49 (m, 2H, H3, H3′), 3.45 (dd, *J* = 2.5, 13.9 Hz, 1H, H6a), 3.37 (dd, *J* = 8.6, 9.8 Hz, 1H, H2), 3.32−3.25 (m, 1H, H5), 3.23 (dd, *J* = 8.3, 9.7 Hz, 1H, H4), 3.02 (dd, *J* = 7.4, 13.8 Hz, 1H, H6b); ^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ 161.3 and 155.4 (2C, 2*C*$_{quat}$, PhenoxyPh), 134.5 (1C, 1*C*$_{quat}$, PhenoxyPh), 132.9 (*C*$_{quat}$, SPh), 132.3 (2CH, SPh), 129.2 (2CH, PhenoxyPh), 128.9 (2CH, PhenoxyPh), 128.6 (2CH, SPh), 127.4 (2CH, SPh), 124.6 (CH, PhenoxyPh), 119.9 (2CH, PhenoxyPh), 117.3 (2CH, PhenoxyPh), 1074.2 (C1′), 87.4 (C3), 87.2 (C1), 78.0 (C5), 75.7 (C5′), 73.3 (C3′), 71.6 and 71.4 (C2′ and C2), 70.0 (C4), 68.9 (C4′), 61.2 (C6′), 44.2 (C6); HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{30}H_{35}NO_{12}S_2Na$ 688.1498, found 688.1515.

Phenyl β-D-Galactopyranosyl-(1→3)-6-deoxy-6-[4-(3-methoxyphenyl)-1H-1,2,3-triazol-1-yl]-1-thio-β-D-glucopyranoside ***34***. To a solution of azide **12** (30 mg, 65 μmol) in DMF (5 mL) were added 3-methoxyphenylacetylene (27.9 μL, 0.20 mmol, 3 equiv), copper iodide (1.24 mg, 6.5 μmol, 0.1 equiv), and Et_3N (9.1 μL, 65 μmol, 1 equiv). The reaction mixture was stirred at rt under an argon atmosphere for 12 h. After completion, the mixture was concentrated under reduced pressure and purified by flash chromatography (CH_2Cl_2/MeOH 1/0 to 9/1) to give derivative **34** as a white solid (13.9 mg, 36%): R_f 0.31 (CH_2Cl_2/MeOH 9/1); ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (s, 1H, C = C*H* triazol), 7.40−7.32 (m, 3H, C*H* 3MeOBz), 7.28−7.20 (m, 2H, C*H* SPh), 7.05−6.97 (m, 3H, C*H* SPh), 6.97−6.93 (m, 1H, C*H* 3MeOBz), 4.96 (dd, *J* = 2.4, 14.4 Hz, 1H, H6a), 4.64 (d, *J* = 9.9 Hz, 1H, H1), 4.62−4.54 (m, 2H, H6b and H1′), 3.87 (s, 3H, C*H*$_3$ 3MeOBz), 3.87−3.78 (m, 3H, H6a′, H4′, H5), 3.74 (dd, *J* = 4.4, 11.4 Hz, 1H, H6b′), 3.68 (t, *J* = 8.7 Hz, 1H, H3), 3.65−3.60 (m, 2H, H2′, H5′), 3.54 (dd, *J* = 3.4, 9.7 Hz, 1H, H3′), 3.44 (dd, *J* = 8.7, 9.9 Hz, 1H, H2), 3.40−3.34 (m, 1H, H4); ^{13}C{^{1}H} NMR (101 MHz, CD_3OD) δ 160.2 (*C*$_{quat}$, 3MeO-*C*), 147.2 (*C*=CH triazol), 132.6 (*C*$_{quat}$, 3MeOBz), 131.5 (*C*$_{quat}$, SPh), 131.5 (2*C*H, SPh), 129.7 (*C*H, 3MeOBz), 128.5 (2*C*H, SPh), 127.1 (*C*H, SPh), 122.4 (C = *C*H triazol), 117.8 (*C*H, 3MeOBz), 113.7 (*C*H, 3MeOBz), 110.7 (*C*H, 3MeOBz), 104.3 (C1′), 87.2 (C1 and C3), 77.8 (C5), 75.7 (C5′), 73.3 (C3′), 71.6 and 71.5 (C2′ and C2), 69.9 (C4), 68.9 (C4′), 61.2 (C6′), 54.5 (*C*H$_3$, 3MeOBz), 51.4 (C6); HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$calcd for $C_{27}H_{33}N_3O_{10}SNa$ 614.1784, found 614.1803.

Phenyl β-D-Galactopyranosyl-(1→3)-6-deoxy-6-(4-phenyl-1H-1,2,3-triazol-1-yl)-1-thio-β-D-glucopyranoside ***35***. To a solution of azide **12** (30 mg, 65 μmol) in DMF (1 mL) were added phenylacetylene (28.0 μL, 0.20 mmol, 3 equiv), copper iodide (12.45 mg, 65.4 μmol, 1 equiv), and Et_3N (10 μL, 72 μmol, 1.1 equiv). The reaction mixture was stirred at rt under an argon atmosphere for 12 h. After completion the mixture was concentrated under reduced pressure and purified by flash chromatography (CH_2Cl_2/MeOH 1/0 to 9/1) and then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **35** as a white solid (30 mg, 85%): R_f 0.18 (CH_2Cl_2/MeOH 9/1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (s, 1H, C = C*H* triazol), 7.82−7.79 (m, 2H, C*H* Ph), 7.46 (t, *J* = 7.6 Hz, 2H, C*H* Ph), 7.35 (t, *J* = 7.4 Hz, 1H, C*H* Ph), 7.17−7.12 (m, 2H, C*H* SPh), 7.00−6.95 (m, 3H, C*H* SPh), 5.51 (d, *J* = 4.7 Hz, 1H, O*H*), 5.18 (br s, 1H, O*H*), 4.98 (br s, 1H, O*H*), 4.84−4.74 (m, 3H, 2 O*H*, H6a and H1), 4.69 (t, *J* = 5.2 Hz, 1H, O*H*), 4.54−4.47 (m, 2H, O*H*, H6b), 4.31 (d, *J* = 7.4 Hz, 1H, H1′), 3.90−3.84 (m, 1H, H5), 3.64−3.62 (m, 1H, H4′), 3.59−3.46 (m, 4H, H6a′, H6b′, H3, H5′), 3.46−3.35 (m, 2H, H2′, H3′), 3.35−3.22 (m, 2H, H2, H4); ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6) δ 146.6 (*C*=CH triazol), 133.9 (*C*$_{quat}$, SPh), 131.3 (*C*$_{quat}$, Ph), 130.8 (2*C*H, SPh), 129.3 and 129.1 (2 × 2*C*H, SPh and Ph), 128.3 (CH, Ph), 127.1 (*C*H, SPh), 125.6 (2*C*H, Ph), 122.7 (C = *C*H triazol), 105.2 (C1′), 88.9 (C3), 86.3 (C1), 77.9 (C5), 76.2 (C5′), 73.3 (C3′), 71.5 and 71.3 (C2′ and C2), 70.2 (C4), 68.6 (C4′), 61.0 (C6′), 51.5 (C6); HRMS (ESI-TOF) *m*/*z* [M + Na]$^+$ calcd for $C_{26}H_{31}N_3O_9SNa$ 584.1679, found 584.1684.

Phenyl β-D-Galactopyranosyl-(1→3)-6-deoxy-6-[4-(4-phenoxyphenyl)-1H-1,2,3-triazol-1-yl]-1-thio-β-D-glucopyranoside ***36***. To a solution of azide **12** (30 mg, 65 μmol) in DMF (1 mL) were added 4-

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phenoxyphenylacetylene (23.7 μL, 0.13 mmol, 2 equiv), copper iodide (12.45 mg, 65.4 μmol, 1 equiv), and Et_3N (10 μL, 72 μmol, 1.1 equiv). The reaction mixture was stirred at rt under an argon atmosphere for 12 h. After completion, the mixture was concentrated under reduced pressure and purified by normal phase flash-chromatography (DCM/MeOH 1/0 to 9/1) and then by reversed-phase C-18 flash chromatography using a H_2O/MeOH gradient mixture to give derivative **36** as a white solid (26.7 mg, 63%): R_f 0.18 (CH_2Cl_2/MeOH 9/1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1H, C = C*H* triazol), 7.84−7.77 (m, 2H, C*H* PhenoxyPh), 7.45−7.38 (m, 2H, C*H* PhenoxyPh), 7.22−7.12 (m, 3H, C*H* SPh), 7.12−7.03 (m, 4H, C*H* PhenoxyPh), 7.03−6.96 (m, 3H, C*H* PhenoxyPh, SPh), 5.60 (br s, 1H, O*H*), 5.21 (br s, 1H, O*H*), 4.83−4.71 (m, 1H, H6a), 4.74 (d, J = 9.8 Hz, 1H, H1), 4.50 (dd, J = 8.8, 14.4 Hz, 1H, H6b), 4.32 (d, J = 7.4 Hz, 1H, H1′), 3.86 (ddd, J = 1.9, 6.0, 11.1 Hz, 1H, H5), 3.64 (dd, J = 1.3, 3.2 Hz, 1H, H4′), 3.58−3.47 (m, 4H, H6a′, H6b′, H5′, H3), 3.47−3.34 (m, 2H, H2′, H3′), 3.34−3.23 (m, 2H, H2, H4); ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6) δ 157.1 and 156.7 ($2C_{quat}$, PhenoxyPh), 146.2 (*C*=CH triazol), 134.0 (C_{quat}, SPh), 130.7 (2CH, SPh), 130.6 (2CH, PhenoxyPh), 129.1 (2CH, SPh), 127.4 (2CH, PhenoxyPh), 127.1 (CH, PhenoxyPh), 126.7 (C_{quat}, PhenoxyPh), 124.0 (CH, SPh), 122.4 (C = *C*H triazol), 119.5 and 119.1 4CH, PhenoxyPh), 105.2 (C1′), 89.0 (C3), 86.4 (C1), 77.9 (C5), 76.2 (C5′), 73.3 (C3′), 71.5 and 71.3 (C2′ and C2), 70.2 (C4), 68.6 (C4′), 61.0 (C6′), 51.5 (C6); HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{32}H_{35}N_3O_{10}SNa$ 676.1941, found 676.1918.

Molecular Modeling. Lactosamine derivatives were built using Discovery Studio 4.1, Dassault Systèmes BIOVIA, San Diego or from the Glycam Web Server.[46] The docking experiments were performed using the CDOCKER protocol[47] as previously described.[30,29] Briefly, the lactosamine binding domain of the 3ZSJ structure[48] was defined as the center of a 18 Å sphere where the ligand could freely explore the protein hypersurface. The protocol has a two-step procedure: the first phase involves a rapid ligand−protein evaluation with limited precision for interaction energy evaluation, and the second phase involves a full molecular dynamics simulation using the CHARMm force field. During the first phase, 250 binding modes were evaluated, 25 poses were refined in the second full potential phase, and the 10 best poses were kept. The best pose for each compound was selected as presenting the best binding interaction energy in the CDOCKER protocol and the lowest deviation of the galactose moiety from the location found in the original crystal structure. The root-mean-square deviation of the galactose location compared to the crystallographic galactose was assessed using the Find Most Common Sub-Structure implemented in rdkit.[49] Poses analysis and illustrations were performed in Discovery Studio and PyMol.[50] Detailed ligand−receptor interaction analysis were done with LigPlot+.[51]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c01927.

Experimental procedures for compound **1** precursors, fluorescence anisotropy experiment, and ^{1}H, ^{13}C, COSY and HSQC NMR spectra for compounds **2**, **4**−**12**, **14**−**27**, and **29**−**36** (PDF)

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Notes

The authors declare no competing financial interest.

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