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Access to Galectin-3 Inhibitors from Chemoenzymatic Synthons

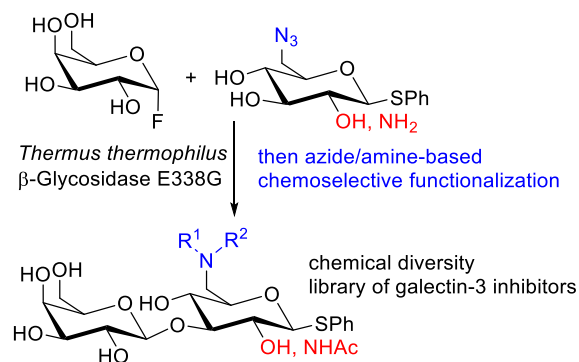
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ABSTRACT: Chemo-enzymatic strategies are useful to provide both regio- and stereoselective access to bioactive oligosaccharides. We show herein that a glycosynthase mutant of a *Thermus thermophilus* α -glycosidase can react with unnatural glycosides such as 6-azido-6-deoxy-D-glucose/glucosamine to lead to β -D-galactopyranosyl-(1 \rightarrow 3)-D-glucopyranoside or β -D-galactopyranosyl-(1 \rightarrow 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.^{1,2,3,4} The transfer catalyzed by some of these enzymes can occur efficiently not only with their natural donor substrates but also with synthetic analogs. 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 inhibitor 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.^{6,7,8,9} Interestingly a reverse strategy consisting in 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 natural¹² or mutant glycoside hydrolases obtained by directed evolution¹³ or rational design¹⁴ as well as glycosynthases¹⁵ 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 tri-saccharides from seven glycosynthases derived from these glycosidases to demonstrate the utility of this approach. Considering now the acceptor sub-

strate, 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 thio-glycosides.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 hapten for vaccination.²⁰ As an extension of the use of chemoenzymatic synthons, we herein report that 6-azido-glycosides 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 non-lectin collagen-like domain.²¹ Galectin-3 is found in the nucleus, the cytoplasm, in the vicinity of the cell surface, the extracellular matrices and even in 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 -Galp-(1-3)- β -D-GlcpN (lactosamine type I) and β -Galp-(1-4)- β -D-GlcpN (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 di-galactoside 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 μ M and 93 μ M, 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 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 C2-position are key since, for example, aryl substituents have been shown to enhance the binding affinity towards galectin-3 by one 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*-acetylglucosamine 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 thio-glycoside 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 (TT β Gly E338G) which was successfully used as a catalyst for the expeditious synthesis of the β -Galp-(1-3)- β -D-GlcNp / Glcp core.³³ We reasoned that if this glycosynthase accepts 6-azido-glycosides as substrate that would offer a unique opportunity to explore the effect of modifications at C6-position (equivalent to C2 position on type lactosamine / lactose) on galectin-3 recognition.

Access to β -Galp-(1-3)-6-azido-6-deoxy- β -D-Glcp/GlcNp 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 described³³ 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 3³⁴ 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 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 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 TT β Gly E338G mutant as described previously³³ gave access to phenyl α -D-galactopyranosyl-(1 \rightarrow 3)-2-amino-2-deoxy-1-thio- α -D-glucopyranoside 9 as a sole product in 40% yield (Scheme 2 and Ta-

ble 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).

Scheme 1. Preparation of the enzymatic synthons and chemoenzymatic access to β-Galp-(1-3)-6-azido-6-deoxy-β-D-Glcp/GlcNp motifs

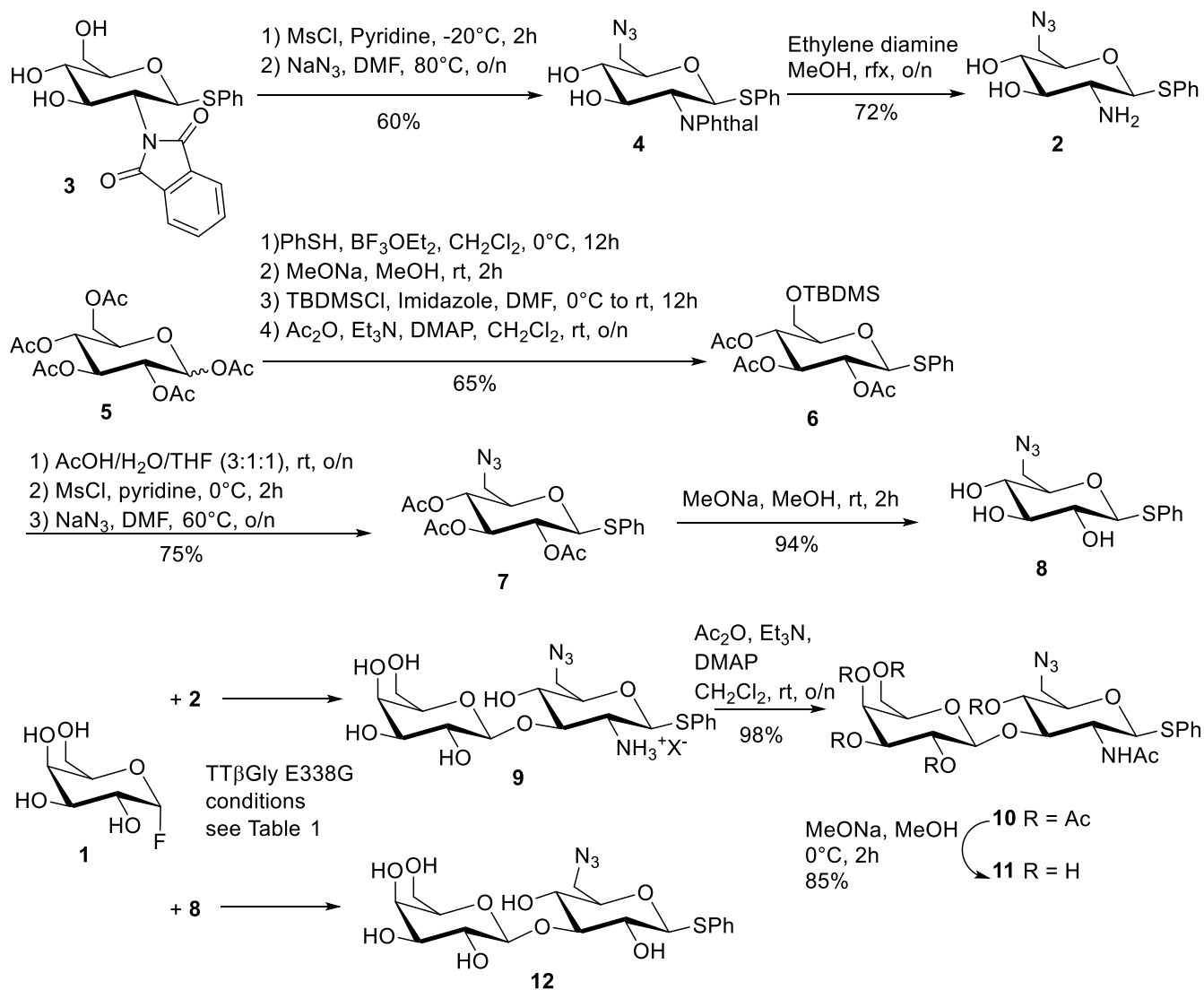


Table 1. Enzymatic glycosylation reaction^a

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 (150mM, pH 7.3), 37°C, 72h; ^b+ unseparable mixture of regioisomers and excess donor 1

Formation of by-products, presumably corresponding to the 1→2 or/and 1→4 regioisomer disaccharides together with some unreacted fluoride donor were detectable on TLC plates. Similar reaction 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 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).

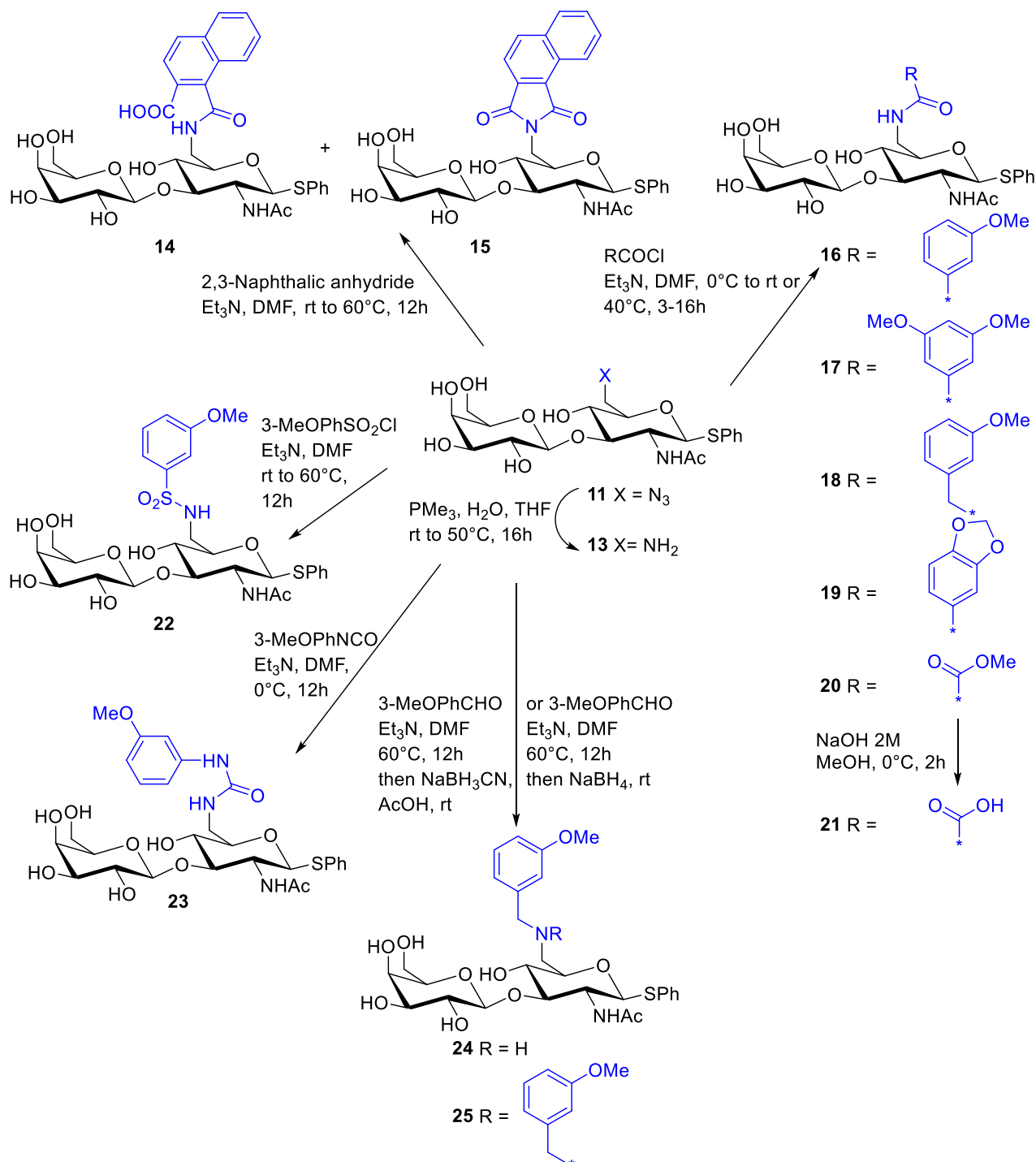
Along this line, our laboratory reported that the nature of the anomeric substituent could orient the regioselectivity of the glycosylation catalyzed by TTβGly E338G in particular towards the formation of the β-(1→2) disaccharides.³⁶

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 towards galectin-3. The syntheses could be achieved upon exploiting the

unique reactivity of azide substituent either directly using click chemistry or after its reduction to the corresponding amine.

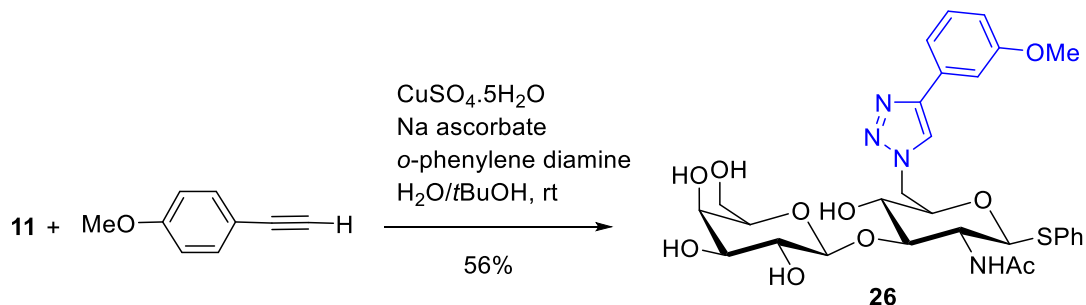
Access to inhibitors using azide specific reactivity in 11. Staudinger reaction or hydrogenation (NaBH₄/catalytic NiCl₂·6H₂O)³⁷ was accompanied with acetate migration when carried out with intermediate 10. Reduction was thus attempted on deprotected disaccharide 11 which was treated with trimethylphosphine. 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).

Scheme 2. Synthesis of galectin-3 inhibitors from disaccharide 13



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 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 towards galectin-3. This effect was particularly pronounced with 1-carboxy-2-naphthoyl and 3-methoxybenzoyl substituents.^{30,27,38,39} Based on 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

Scheme 3. Synthesis of compound 26 from disaccharide 11



Binding affinity measurements

We next carried out biochemical experiments to measure the binding affinities of these derivatives towards galectin-3 in order to validate the value of the chemoenzymatic approach for the discovery of new galectin inhibitors. Dissociation constants were determined by fluorescence polarization assay using known 2-[(fluoresceinyl)thio-ureido]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 \rightarrow 3)-2-acetamido-2-deoxy-1-thio- β -D-glucopyranoside 27³³ (Figures S1 and S2) were tested in parallel as positive controls. The head-to-head comparison towards known inhibitors allows to assessing the value of the new compounds for targeting galectin-3. TD139 is among the most potent disaccharide-based galectin-3 inhibitor 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.

K_d for reference compound 27 was found equal to 52 μ M by fluorescence polarization, in agreement with the value measured by isothermal microcalorimetry (32 μ M) (See Figure S2 & Table S1).

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

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 equivalent of 3-methoxybenzaldehyde in the presence of sodium cyanoborohydride as the reducing agent or 2.4 equivalent 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 intrinsic reactivity of compound 11 since, in our hands, these experimental conditions⁴⁰ 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 H₂O/THF mixture at rt afforded an inseparable mixture of regioisomers.

slightly improved affinities (amides 14, 19, 20 and 21, urea 23 and triazole 26 with K_d s in the range of 28-42 μ M and imide 15, amines 24 and 25 with K_d s in the range of 20 μ M) and, finally, 3-methoxysulfonamide 22 having a K_d equal to 10.4 μ M.

Table 2. Dissociation constants of compounds tested against galectin-3 at rt^{a,b}

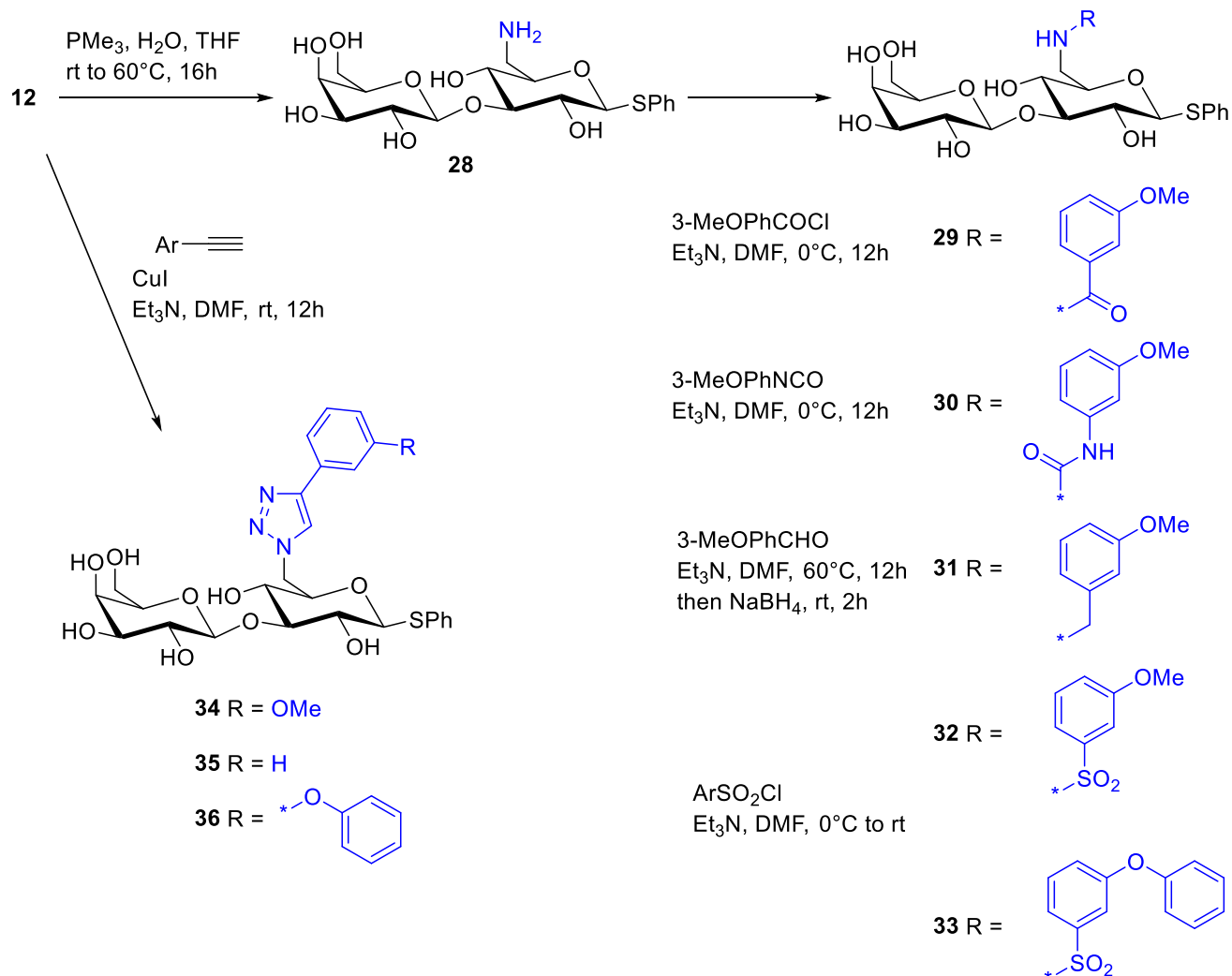
Inhibitor	K_d (μ M)	Inhibitor	K_d (μ M)
TD139	0.22 \pm 0.041	27	51.8 \pm 10.3
14	28.0 \pm 3.6	25	22.1 \pm 3.8
15	19.4 \pm 3.4	26	40.2 \pm 6.2
16	58.3 \pm 8.9 ^c	29	35.9 \pm 10.9
17	64.5 \pm 6.8	30	42.7 \pm 6.1
18	52.2 \pm 9.0	31	14.9 \pm 2.4
19	37.6 \pm 6.7	32	9.14 \pm 1.9
20	37.6 \pm 5.2 ^c	33	14.6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

^aConcentrations were as follows: probe 100 nM, hGal-3 1 μ M and inhibitors tested in serial dilutions from 800 μ M to 10 nM; ^bAssays 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.

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 bearing aromatic substituents at 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 analogs of representative compounds (sulfonamide, amine, urea, amide) in the β -Galp-(1-3)- β -D-Glc_p series.

Access to inhibitors using azide specific reactivity of 12. To 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

Scheme 4. Synthesis of galectin-3 inhibitors from disaccharide 12



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

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 observed *i.e.* the K_{d} s of galectin-3 for urea/amide was 35-45 μM , 15-20 μM for amine and decreased to 8-10 μM for sulfonamides.

Binding affinity measurements

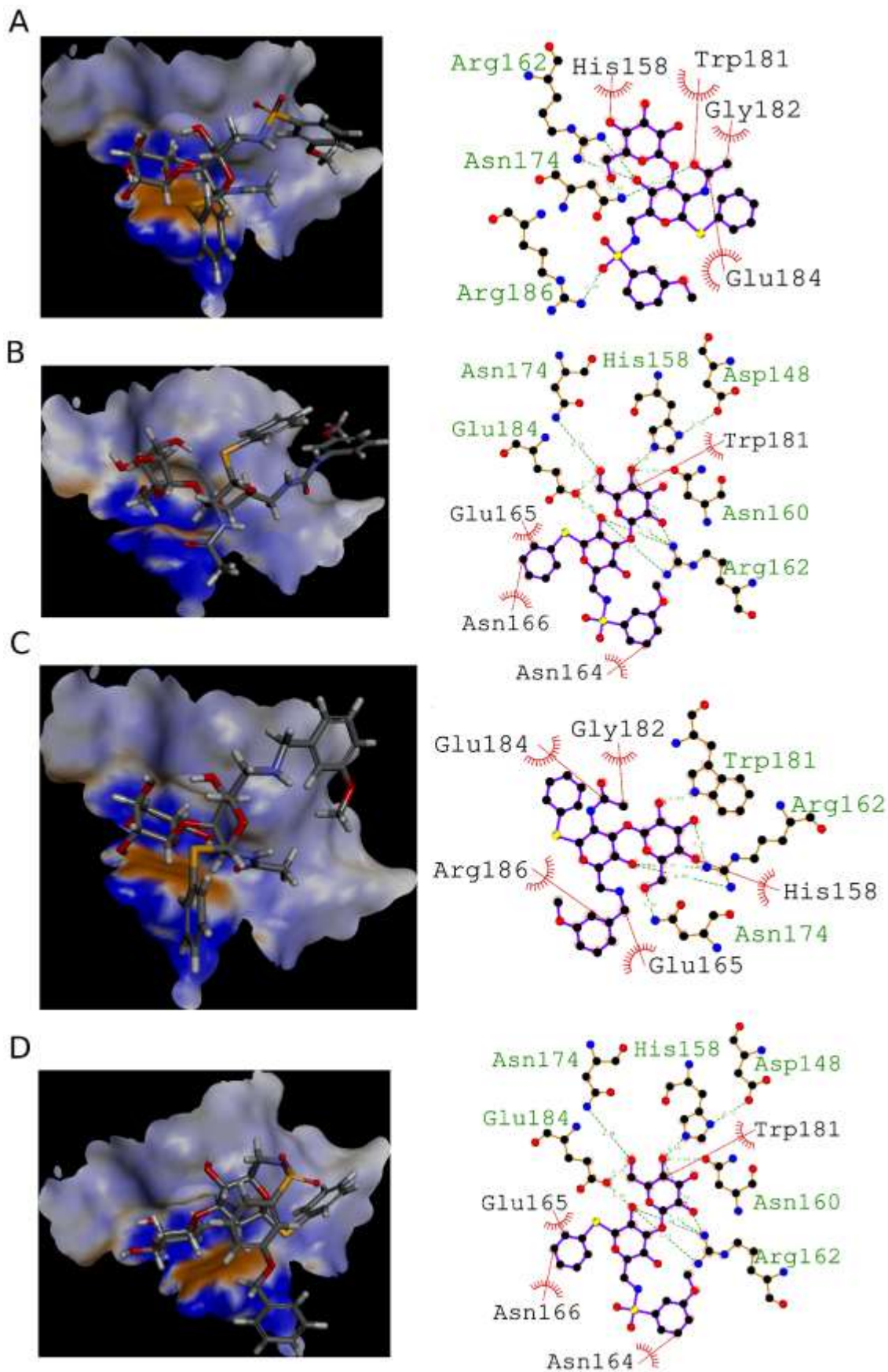


Figure 1. Molecular docking of selected compounds with the galectin-3. Overview of one representative binding pose after docking for 22 (A), 23 (B), 24 (C) and 32 (D). Ligands are displayed in sticks and colored by atom types: carbons in gray, hydrogen in white, oxygens in red, nitrogen in blue, sulfur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

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 interaction 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 a 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 outwards the CRD does not impact on 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 get 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 a 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)

Table 3. Dissociation constants (K_{ds}) of inhibitors for galectin-1, galectin-3 and galectin-7 at 4°C^a

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

^aConcentrations 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, 5μM; hGal-3, 1μM; hGal-7, 5μM and inhibitors tested in serial dilutions from 800 μM to 10 nM; ^bPositive control.

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 lack substituents at the C3 position of the galactose, this position being known to contribute significantly to the selectivity.⁴²

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 chemo-enzymatic pathways to synthesize complex oligosaccharides.

EXPERIMENTAL SECTION

General. 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 to 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+ 1H/13C 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 Waters SYNAPT G2-Si HDMS with detection with a hybrid quadrupole 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 whilst 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 five second auto-calibration 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 eq) was dissolved in anhydrous pyridine (120 mL) and the solution was cooled to -20 °C. Mesyl chloride (2.90 mL, 18.75 mmol, 3 eq) was added dropwise. After 2h, the reaction was quenched upon addition of methanol and the solvent was removed under reduced pressure. The residue was diluted with EtOAc (125 mL), washed with aqueous 1N HCl (125 mL), sat. aq. NaHCO₃ (125 mL) and brine (125mL). The organic layer was dried over Na₂SO₄ 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 NaN₃ (1.31 mg, 20.1 mmol, 5 eq). The resulting mixture was stirred at 80 °C for 16 h then was diluted by EtOAc (125 mL) and washed with H₂O (2 × 60 mL) and sat. aq. NaHCO₃ (60 mL). The organic layer was dried over Na₂SO₄ 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); ¹H NMR (400 MHz, CDCl₃) δ 7.87 (dd, *J* = 3.0, 5.4 Hz, 2H, 2 CH Phth), 7.76 (dd, *J* = 3.0, 5.4 Hz, 2 CH Phth), 7.44–7.41 (m, 2H, CH SPh), 7.32–7.25 (m, 3H, CH SPh), 5.59 (d, *J* = 10.2 Hz, 1H, H1), 4.28 (t, *J* = 9.7 Hz, 1H, H3), 4.16 (t, *J* = 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). ¹³C NMR (101 MHz, CDCl₃) 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 C₂₀H₁₈N₄O₅SiNa 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 eq) was stirred with a solution of ethylene diamine 0.8 M in MeOH (7.4 mL, 5.94 mmol, 1.7 eq). 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 (CH₂Cl₂/MeOH 95/5) to give compound 2 as a yellow oil (750 mg, 72%). *R*_f 0.81 (CH₂Cl₂/MeOH 9/1). ¹H 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). ¹³C{¹H} NMR (101 MHz, CD₃OD) δ 132.6 (2 CH, SPh), 132.1 (1 C_{quat}, SPh), 128.6 (2 CH, SPh), 127.8 (1 CH, 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 C₁₂H₁₆N₄O₅SiNa 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 eq) and BF₃OEt₂ (4.21 mL, 35.86 mmol, 0.7 eq) were added to a stirred solution of β-D-glucosapyranose pentaacetate 5 (20 g, 51.2 mmol, 1 eq) in CH₂Cl₂ (200 mL) at 0°C. After 12 h, the reaction mixture was quenched by addition of sat. aq. NaHCO₃ (150 mL). The aqueous layer was separated and extracted with CH₂Cl₂ (3 × 75 mL). The combined organic layer was washed with brine, dried over Na₂SO₄ 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, 1eq) in MeOH (220 mL), a solution of sodium methoxide 5.4M (8.24 mmol, 1.5 mL, 0.2 eq) was added at rt and stirred for 2h. Then the mixture was neutralized with an acidic resin (Amberlyte IR120-H⁺), 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), then a solution of *tert*-butyldimethylsilyl chloride (TBDMSCl, 6.8 g, 45.3 mmol, 1.1 eq) and imidazole (7.0 g, 103.0 mmol, 2.5 eq) in anhydrous DMF (40 mL) was added at 0°C. After stirring for 12h, the mixture was diluted by EtOAc (150 mL), washed with H₂O (2 × 100 mL) and sat. aq. NaHCO₃ (100 mL). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure; *R*_f 0.57 (Cyclohexane/EtOAc 1/9). Then the residue was solubilized in CH₂Cl₂ (100 mL) at 0°C, treated with Ac₂O (17.5 mL), Et₃N (20.1 mL), DMAP (cat.) and stirred for 16 h. The mixture was diluted by

CH₂Cl₂ (100 mL), washed with H₂O (2 × 100 mL) and sat. aq. NaHCO₃ (100 mL). The organic layer was dried over Na₂SO₄ 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 4 steps). *R*_f 0.71 (Cyclohexane/EtOAc 7/3); ¹H NMR (400 MHz, CDCl₃) 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, COCH₃), 2.01 (s, 3H, COCH₃), 1.98 (s, 3H, COCH₃), 0.90 (s, 9H, 3CCH₃), 0.07 (s, 3H, CH₃, TBDMS), 0.05 (s, 3H, CH₃, TBDMS); ¹³C{¹H} NMR (101 MHz, CDCl₃) δ 170.2, 169.2, 169.2 (3 C_{quat}, COCH₃), 132.6 (2 CH, SPh), 133.2 (1 C_{quat}, SPh), 128.9 (2 CH, SPh), 128.0 (1 CH, SPh), 85.6 (C1), 78.9 (C5), 74.4 (C3), 70.1 (C2), 68.6 (C4), 62.4 (C6), 25.8 (3CCH₃), 20.7, 20.6 (3COCH₃), 18.4 (1 CCH₃), -5.4, -5.4 (2 CH₃, TBDMS); HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₂₄H₃₆O₈SiSNa 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, 1eq) was stirred for 16 h in a solution of AcOH/H₂O/THF (3/1/1; 100mL) at rt. Then THF was removed under reduced pressure and the mixture was neutralized with sat. aq. NaHCO₃ (3 × 70 mL). The organic layer was dried over Na₂SO₄ 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 eq) was added dropwise. After 2 h, the reaction was quenched 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 NaN₃ (944 mg, 14.5 mmol, 3 eq). The resulting mixture was stirred at 60 °C for 16 h, then diluted in EtOAc (100 mL) and washed with H₂O (2 × 50 mL) and sat. aq. NaHCO₃ (50 mL). The organic layer was dried over Na₂SO₄ 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 2 steps). *R*_f 0.43 (Cyclohexane/EtOAc 5/5). ¹H NMR (400 MHz, CDCl₃) 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, COCH₃), 2.03 (s, 3H, COCH₃), 2.00 (s, 3H, COCH₃); ¹³C{¹H} NMR (101 MHz, CDCl₃) δ 170.1, 169.4, 169.2 (3 C_{quat}, COCH₃), 133.7 (2 CH, SPh), 130.9 (C_{quat}, SPh), 129.0 (2 CH, SPh), 128.7 (1 CH, 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 (3 CH₃); HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₁₈H₂₁N₃O₇SiNa 446.0998; Found 446.1019.

Phenyl 6-*O*-azido-1-thio-β-D-glucopyranoside 8.⁴⁵ To a solution of compound 7 (690mg, 1.63 mmol, 1eq) in MeOH (10 mL) was added a solution of sodium methoxide 5.4M (0.49 mmol, 90 μL,

0.1 eq) at 0°C and stirred for 2 h. Then the mixture was neutralized with an acidic resin (Amberlyte IR120-H⁺), filtrated and the solvent removed under reduced pressure. The residue was purified by flash chromatography (Cyclohexane/AcOEt 4/6) 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); ¹H NMR (400 MHz, CD₃OD) 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); ¹³C{¹H} 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 C₁₂H₁₅N₃O₄SNa 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 eq) and acceptor 2 (150 mg, 0.5 mmol, 1 eq), 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 1M to pH 1 and stirred for 4h before neutralization with NaOH 1M. After removal of the solvent under reduced pressure, the crude residue was purified by flash chromatography (CH₂Cl₂/MeOH 90/10) to afford disaccharide 9 as a white solid (180 mg, 78%). *R*_f 0.75 (CHCl₃/MeOH/H₂O/CH₃COOH 60/30/5/3). ¹H NMR (400 MHz, D₂O) 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, 1H, H6b'), 2.83 (dd, *J* = 8.3, 11.1 Hz, 1H, H2); ¹³C{¹H} NMR (101 MHz, D₂O) δ 132.7 (2CH, SPh), 130.9 (C_{quat}, SPh), 129.4 (2CH, SPh), 128.5 (CH, 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 C₁₈H₂₆N₄O₈SNa 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, 1eq) in CH₂Cl₂ (2.5 mL) was treated with Ac₂O (350 μL), Et₃N (350 μL), DMAP (0.03 mmol, 4 mg, 0.1 eq) and stirred for 16h. The mixture was diluted by CH₂Cl₂ (25 mL), washed by H₂O (2 × 12 mL) and sat. aq. NaHCO₃ (12 mL). The organic layer was dried over Na₂SO₄ 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, CDCl₃) δ 7.52–7.45 (m, 2H, CH SPh), 7.33–7.27 (m, 3H, CH SPh), 6.07 (d, *J* = 8.1 Hz, 1H, NH), 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,

COCH₃), 2.05 (s, 3H, COCH₃), 2.04 (s, 3H, COCH₃), 2.04 (s, 3H, COCH₃), 2.03 (s, 3H, COCH₃), 1.95 (s, 3H, COCH₃); ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 170.5 (COCH₃), 170.4 (COCH₃), 170.1 (2COCH₃), 169.4 (COCH₃), 169.2 (COCH₃), 132.8 (2CH, SPh), 132.1 (C_{quat}, SPh), 129.0 (2CH, SPh), 128.2 (CH, 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 (COCH₃), 20.8 (2 COCH₃), 20.7 (COCH₃), 20.6 (COCH₃), 20.5 (COCH₃); HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₃₀H₃₈N₄O₁₄SNa 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, 1eq) in MeOH (3.5 mL), a solution of sodium methoxide 5.4M (0.035 mmol, 8 μL, 0.1 eq) was added at 0°C and stirred for 2 h. Then the mixture was neutralized with an acidic resin (amberlyte IR120-H⁺), filtrated and the solvent was removed under reduced pressure. The crude residue was purified by flash chromatography (CH₂Cl₂/MeOH 8/2) to give compound 11 as a white solid (135 mg, 85%); *R*_f 0.78 (CHCl₃/MeOH/H₂O/CH₃COOH 60/30/5/3); ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.93 (d, *J* = 7.6 Hz, 1H, NH), 7.46–7.44 (m, 2H, CH SPh), 7.35–7.31 (m, 2H, CH SPh), 7.28–7.25 (m, 1H, CH SPh), 5.01 (d, *J* = 7.7 Hz, 1H, H1), 4.89 (d, *J* = 1.6 Hz, 1H, OH), 4.79 (br s, 1H, OH), 4.64 (t, *J* = 5.4 Hz, 1H, OH), 4.48 (m, 2H, 2 × OH), 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, COCH₃); ¹³C{¹H} NMR (101 MHz, DMSO) δ 170.4 (COCH₃), 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 (COCH₃); HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₂₀H₂₈N₄O₉SNa 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 eq) and acceptor 8 (125 mg, 0.42 mmol, 1 eq), 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 72h. The mixture was acidified with HCl 1M to pH 1 and stirred for 4h before neutralization with NaOH 1M. After removal of the solvent under reduced pressure, the crude residue was purified by flash chromatography (CH₂Cl₂/MeOH 90/10) to afford a mixture of phenyl β-D-galactopyranosyl-(1→3)-6-azido-6-deoxy-1-thio-β-D-glucopyranoside, unreacted phenyl 6-azido-6-deoxy-1-thio-β-D-glucopyranoside. To facilitate the purification, the crude reaction mixture was suspended in CH₂Cl₂ (5 mL), treated with Ac₂O (1 mL), Et₃N (1 mL), DMAP (cat) and stirred for 16 h. Then the reaction mixture was diluted in CH₂Cl₂ (25 mL), washed with H₂O (2 × 15 mL) and sat. aq. NaHCO₃ (15 mL). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude residue was purified first by flash chromatography (Cyclohexane/EtOAc 65/35) then by reverse phase C-18 flash chromatography (H₂O/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, CD₃OD) 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, $COCH_3$), 1.92 (s, 3H, $COCH_3$); $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ 172.0 ($\underline{COCH_3}$), 171.9 ($\underline{COCH_3}$), 171.7 ($\underline{COCH_3}$), 171.4 ($\underline{COCH_3}$), 171.1 ($\underline{COCH_3}$), 171.0 ($\underline{COCH_3}$), 134.5 ($2C_{H}, SPh$), 132.6 (C_{quat}, SPh), 130.0 ($2C_{H}, SPh$), 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 (C_{CH_3}), 20.6 (C_{CH_3}), 20.5 (C_{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 \rightarrow 3)-2,4-*O*-di-acetyl-6-azido-6-deoxy-1-thio- β -D-glucopyranoside (141 mg, 0.20 mmol, 1 eq) in MeOH (2 mL), a solution of sodium methoxide 5.4M (0.02 mmol, 3 μ L, 0.1 eq) was added at 0°C and 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 ($2C_{H}, SPh$), 132.4 (C_{quat}, SPh), 128.5 ($2C_{H}, SPh$), 127.5 (C_{H}, 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 eq) in THF (5 ml), under an atmosphere of argon, PMe_3 1M in THF (200 μ L, 0.2 mmol, 2 eq) was added. 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 μ L) was added and the mixture was heated to 50°C in an oil bath for 16h. Then solvent was removed under reduced pressure to give crude phenyl β -D-galactopyranosyl-(1 \rightarrow 3)-2-acetyl-amino-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 \rightarrow 3)-2-acetamido-2,6-dideoxy-6-(1-carboxy-2-naphtoyl)amino-1-thio- β -D-glucopyranoside 14 and phenyl β -D-galactopyranosyl-(1 \rightarrow 3)-2-acetamido-2,6-dideoxy-6-(2,3-naphtal)imido-1-thio- β -D-glucopyranoside 15

Amine 13 was dissolved in anhydrous DMF (2 ml) at 0°C and treated with 2,3-naphtalic anhydride (34 mg, 0.17 mmol, 1.7 eq) and Et_3N (27 μ L, 0.2 mmol, 2.0 eq). The mixture was stirred 12 hours 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 reverse 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, $C_{H}Napht$), 8.09 (dd, $J = 2.3, 6.5$ Hz, 1H $C_{H}Napht$), 7.95–7.86 (m, 3H, $C_{H}Napht$), 7.69–7.60 (m, 2H, $C_{H}Napht$), 7.44–7.38 (m, 2H, $C_{H}SPh$), 7.11–6.98 (m, 3H, SPh), 5.06–4.88 (m, 2H, $H1$, OH), 4.81 (br s, 2H, $2 \times OH$), 4.51 (d, $J = 3.3$ Hz, 2H, $2 \times OH$), 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 ($C_{H}, Napht$), 129.5 ($2C_{H}, SPh$), 129.3 ($2C_{H}, SPh$), 129.1 ($C_{H}, Napht$), 128.6 ($C_{H}, Napht$), 128.4 ($C_{H}, Napht$), 128.0 ($C_{H}, Napht$), 127.6 ($C_{H}, Napht$), 126.6 (C_{H}, 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, $C_{H}Napht$), 8.29 (dd, $J = 3.3, 6.2$ Hz, 2H, $C_{H}Napht$), 7.87 (d, $J = 8.2$ Hz, 1H, NH), 7.81 (dd, $J = 3.3, 6.3$ Hz, 1H, $C_{H}Napht$), 7.07–7.02 (m, 2H, $C_{H}SPh$), 6.54–6.45 (m, 3H, $C_{H}SPh$), 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 (C_{H}, SPh), 130.1 ($2C_{H}, Napht$), 129.8 ($2C_{H}, SPh$), 128.8 ($2C_{H}, Napht$), 127.7 ($2C_{quat}, Napht$), 126.6 (C_{H}, SPh), 125.0 ($2C_{H}, Napht$), 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 \rightarrow 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 μ L, 0.11 mmol, 1.1 eq) and Et_3N (15 μ L, 0.11 mmol, 1.1 eq). The mixture was stirred 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, $C_{H}3MeOBz$), 7.40 (t, $J = 7.9$ Hz, 1H, $C_{H}3MeOBz$), 7.32 (t, $J = 4.5$ Hz, 2H, $C_{H}SPh$), 7.13 (dd, $J = 3.4, 8.5$ Hz, 1H, $C_{H}3MeOBz$), 7.04 (t, $J = 7.4$ Hz, 1H, $C_{H}SPh$), 6.91 (t, $J = 7.6$ Hz, 2H, $C_{H}SPh$), 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, $\text{H1}'$), 3.90–3.82 (m, 1H, H6a), 3.80 (s, 3H, CH_3 3MeOBz), 3.77–3.44 (m, 7H, H2 , H3 , $\text{H4}'$, H5 , $\text{H6a}'$, H6'b , $\text{H5}'$), 3.42–3.22 (m, 4H, $\text{H2}'$, $\text{H3}'$, H6b , H4), 1.84 (s, 3H, COCH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, $\text{DMSO}-d_6$) 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 (2 CH , SPh), 129.1 (2 CH , SPh), 126.7 (CH , SPh), 120.0 (CH , 3MeOBz), 117.5 (CH , 3MeOBz), 113.0 (CH , 3MeOBz), 104.3 ($\text{C1}'$), 86.2 (C1), 85.5 (C3), 78.0 (C5), 76.2 ($\text{C5}'$), 73.6 ($\text{C3}'$), 71.5 (C4), 71.1 ($\text{C2}'$), 68.7 ($\text{C4}'$), 61.1 ($\text{C6}'$), 55.8 (CH_3 , 3MeOBz), 53.5 (C2), 41.7 (C6), 23.5 (COCH_3); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{28}\text{H}_{36}\text{N}_2\text{O}_{11}\text{SNa}$ 631.1938; Found 631.1941.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 eq) and Et_3N (60 μL , 0.44 mmol, 4 eq). 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 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9/1) to give derivative 17 as a white solid (22 mg, 38%); R_f 0.67 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ 60/30/5/3); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.56 (t, $J = 5.7$ Hz, 1H, $\text{NH}(3\text{MeOBz})$), 7.98 (d, $J = 8.3$ Hz, 1H, $\text{NH}(\text{Ac})$), 7.32 (d, $J = 7.4$ Hz, 2H, CHSPh), 7.08 (d, $J = 2.3$ Hz, 2H, $\text{CH}3\text{MeOBz}$), 7.05 (t, $J = 7.7$ Hz, 1H, CHSPh), 6.92 (t, $J = 7.7$ Hz, 2H, CHSPh), 6.69 (t, $J = 2.3$ Hz, 1H, $\text{CH}3\text{MeOBz}$), 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, $\text{H1}'$), 3.90–3.81 (m, 1H, H6a), 3.78 (s, 6H, 2 \times CH_3 3MeOBz), 3.75–3.45 (m, 7H, H2 , H3 , $\text{H4}'$, H5 , $\text{H6a}'$, H6'b , $\text{H5}'$), 3.41–3.30 (m, 2H, $\text{H2}'$, $\text{H3}'$), 3.30–3.19 (m, 2H, H4 , H6b), 1.83 (s, 3H, COCH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, $\text{DMSO}-d_6$) δ 170.4 (1C, 1 C_{quat} , COCH_3), 166.1 (C_{quat} , COPh), 160.8 (2 C_{quat} , 3MeO-C), 136.8 (C_{quat} , 3MeOBz), 135.5 (C_{quat} , SPh), 129.5 (CH , 3MeOBz), 129.1 (2 CH , SPh), 126.7 (CH , SPh), 105.8 (2 CH , 3MeOBz), 104.4 ($\text{C1}'$), 103.6 (CH , 3MeOBz), 85.5 (2 CH , C1 and C3), 78.0 (C5), 76.3 ($\text{C5}'$), 73.6 ($\text{C3}'$), 71.5 (C4), 71.1 ($\text{C2}'$), 68.7 ($\text{C4}'$), 61.1 ($\text{C6}'$), 55.9 (2 CH_3 , 3MeOBz), 52.9 (C2), 41.8 (C6), 23.5 (COCH_3); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{29}\text{H}_{38}\text{N}_2\text{O}_{12}\text{SNa}$ 661.2043; Found 661.2037.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 eq) and Et_3N (14 μL , 0.10 mmol, 1.1 eq). The mixture was stirred 3 h and concentrated under reduced pressure. The residue was purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9/1) to give derivative 18 as a white solid (37 mg, 66%). R_f 0.65 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ 60/30/5/3); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.11 (t, $J = 5.8$ Hz, 1H, $\text{NH}(3\text{MeOPh})$), 8.01 (d, $J = 8.0$ Hz, 1H, $\text{NH}(\text{Ac})$), 7.42 (d, $J = 7.1$ Hz, 2H, CHSPh), 7.29 (d, $J = 8.0$ Hz, 2H, CHSPh), 7.23 (d, $J = 7.0$ Hz, 1H, CHSPh), 7.17 (t, $J =$

$J = 7.8$ Hz, 1H, $\text{CH}3\text{MeOPh}$), 6.83–6.79 (m, 2H, $\text{CH}3\text{MeOPh}$), 6.78 (dd, $J = 3.1$, 8.1 Hz, 1H, $\text{CH}3\text{MeOPh}$), 4.91 (d, $J = 9.8$ Hz, 1H, H1), 4.86–4.79 (m, 2H, 2 \times 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, $\text{H1}'$), 3.71 (s, 3H, CH_3 3MeOPh), 3.69–3.56 (m, 4H, H2 , H3 , H6a , $\text{H4}'$), 3.55–3.37 (m, 6H, $\text{H6a}'$, $\text{H6b}'$, $\text{H5}'$, H5 , CH_2 3MeOPh CH_2), 3.37–3.28 (m, 2H, $\text{H2}'$, $\text{H3}'$), 3.24–3.17 (m, 1H, $\text{H4}'$), 3.11 (dd, $J = 5.9$, 14.0 Hz, 1H, H6b), 1.84 (s, 3H COCH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, $\text{DMSO}-d_6$) 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 (2 CH , SPh), 129.6 (CH , 3MeOPh), 129.4 (2 CH , SPh), 127.0 (CH , SPh), 121.7 (CH , 3MeOPh), 115.2 (CH , 3MeOPh), 112.3 (CH , 3MeOPh), 104.3 ($\text{C1}'$), 86.0 (C1), 85.3 (C3), 78.4 (C5), 76.3 ($\text{C5}'$), 73.6 ($\text{C3}'$), 71.0 ($\text{C2}'$), 70.8 (C4), 68.7 ($\text{C4}'$), 61.0 ($\text{C6}'$), 55.4 (CH_3 , 3MeOPh), 53.4 (C2), 45.9 (CH_2 , 3MeOPh CH_2), 40.9 (C6), 23.5 (COCH_3); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{29}\text{H}_{38}\text{N}_2\text{O}_{11}\text{SNa}$ 645.2094; Found 645.2088.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 3)-2-acetamido-6-(piperonyloxy)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 piperonyloxy chloride (20 mg, 0.11 mmol, 1.1 eq) and Et_3N (15 μL , 0.11 mmol, 1.1 eq). The mixture was stirred 12 h and concentrated under reduced pressure. The residue was first purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 75/25) then by reverse phase C-18 flash chromatography using a $\text{H}_2\text{O}/\text{MeOH}$ gradient mixture as eluent to give derivative 19 as a white solid (33 mg, 55%); R_f 0.65 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ 60/30/5/3); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.38 (t, $J = 5.7$ Hz, 1H, $\text{NH}(\text{C}(\text{O})\text{Ph})$), 8.02 (d, $J = 8.2$ Hz, 1H, $\text{NH}(\text{Ac})$), 7.47 (d, $J = 8.0$ Hz, 1H, Ph), 7.41 (br s, 1H, Ph), 7.33 (d, $J = 7.7$ Hz, 2H, CHSPh), 7.08 (t, $J = 7.4$ Hz, 1H, CHSPh), 7.03–6.93 (m, 3H, CH , SPh, Ph), 6.11 (s, 2H, $\text{OC}_2\text{H}_2\text{O}$), 4.98–4.79 (m, 3H, 2 \times OH , H1), 4.71 (br s, 1H, OH), 4.52–4.50 (m, 2H, 2 \times OH), 4.21 (d, $J = 6.4$ Hz, 1H, $\text{H1}'$), 3.82 (dd, $J = 6.3$, 12.8 Hz, 1H, H6a), 3.76–3.60 (m, 3H, H2 , H3 , $\text{H4}'$), 3.60–3.44 (m, 4H, H5 , H6'a , H6'b , $\text{H5}'$), 3.44–3.29 (m, 2H, $\text{H2}'$, $\text{H3}'$), 3.29–3.18 (m, 2H, H4 , H6b), 1.84 (s, 3H, COCH_3); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, $\text{DMSO}-d_6$) 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 (2 CH , SPh), 129.1 (2 CH , SPh), 128.8 (C_{quat} , CONH), 126.8 (CH , SPh), 122.7 (CH , Ph), 108.2 (CH , Ph), 107.9 (CH , Ph), 104.3 ($\text{C1}'$), 102.1 (CH_2 , OCH_2O), 86.1 (C1), 85.5 (C3), 78.0 (C5), 76.3 (C5), 73.6 ($\text{C3}'$), 71.5 (C4), 71.0 ($\text{C2}'$), 68.7 ($\text{C4}'$), 61.0 ($\text{C6}'$), 53.5 (C2), 41.3 (C6), 23.5 (COCH_3); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{28}\text{H}_{34}\text{N}_2\text{O}_{12}\text{SNa}$ 645.1730; Found 645.1738.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 chlorooxacetate (10 μL , 0.11 mmol, 1.1 eq) and Et_3N (15 μL , 0.11 mmol, 1.1 eq). The mixture was stirred 12 h and concentrated under reduced pressure. The residue was purified firstly by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 7/3) then by reverse phase C-18 flash chromatography using a $\text{H}_2\text{O}/\text{MeOH}$ gradient as eluent to give derivative 20 as a white solid (33 mg, 60%); R_f 0.75 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ 60/30/5/3); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.91 (t, $J = 6.1$ Hz, 1H,

$\text{NH}(\text{C}(\text{O})\text{CO}_2\text{Me})$), 7.90 (d, $J = 7.8$ Hz, 1H, NHAc), 7.38–7.32 (m, 2H, CH SPh), 7.25–7.21 (m, 3H, CH 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_3MeO), 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); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, $\text{DMSO}-d_6$) 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 (2 CH , SPh), 129.3 (2 CH , 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 : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{23}\text{H}_{32}\text{N}_2\text{O}_{12}\text{SNa}$ 583.1574; Found 583.1582.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 3)-2-acetamido-6-(carboxyoacetamido-2,6-dideoxy-1-thio- β -D-glucopyranoside 21

A solution of ester derivative 20 (15 mg, 0.027 mmol, 1 eq) in a mixture of aq. NaOH 2M (1 ml) and MeOH (1 ml) was stirred 2 h at 0°C. Then the mixture was acidified with HCl 1M to pH 1 and concentrated under reduced pressure. The residue was purified by reverse phase C-18 flash chromatography using a $\text{H}_2\text{O}/\text{MeOH}$ gradient mixture to give acid 21 as a colorless solid (14 mg, 95%); R_f 0.25 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ 60/30/5/3); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.31 (br s, 1H, $\text{NH}(\text{C}(\text{O})\text{CO}_2\text{Na})$), 7.95 (d, $J = 8.2$ Hz, 1H, NHAc), 7.40 (d, $J = 7.6$ Hz, 2H, CH SPh), 7.31 (t, $J = 7.5$ Hz, 2H, CH SPh), 7.22 (t, $J = 7.2$ Hz, 1H, CH 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); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, $\text{DMSO}-d_6$) δ 170.3 (2 C_{quat} , COCH_3 , COONa), 165.9 (C_{quat} , NHCOCO_2Na), 135.0 (C_{quat} , SPh), 130.2 (2 CH , SPh), 129.6 (2 CH , 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 (2C, C4 and C2'), 68.6 (C4'), 61.0 (C6'), 53.5 (C2), 41.1 (C6), 23.4 (COCH_3); HRMS (ESI-TOF) m/z : $[\text{M} - \text{Na}]^-$ Calcd for $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_{12}\text{S}$ 545.1441; Found 545.1437.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 eq) and Et_3N (24 μL , 0.17 mmol, 1.6 eq). The mixture was stirred 24 h at rt and concentrated under reduced pressure. The residue was purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9/1) to give derivative 22 as a white solid (14 mg, 21%); R_f 0.70 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ 60/30/5/3); ^1H NMR (400 MHz, CD_3OD) δ 7.55–7.61 (m, 2H, CH SPh), 7.45–7.39 (m, 3H, CH 3MeOPh), 7.37–7.28 (m, 3H, CH SPh), 7.16 (dt, $J = 2.3, 7.4$ Hz, 1H, CH 3MeOPh), 4.74 (d, $J = 10.5$ Hz, 1H, H1), 4.27 (d, $J = 7.4$ Hz, 1H, H1'), 3.86 (s, 3H, $\text{CH}_3\text{3MeOPh}$), 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); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CD_3OD) δ 172.6 (C_{quat} , COCH_3), 160.1 (C_{quat} , 3MeO-C), 141.8 (C_{quat} , 3MeO-Ph), 133.5 (C_{quat} , SPh), 131.5 (2 CH , SPh), 129.9 (CH , 3MeOPh),

128.6 (2 CH , 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 : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{27}\text{H}_{36}\text{N}_2\text{O}_{12}\text{S}_2\text{Na}$ 667.1607; Found 667.1614.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 eq) and Et_3N (17 μL , 0.12 mmol, 1.1 eq). The mixture was stirred for 12 h and concentrated under reduced pressure. The residue was first purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 85/15) then by reverse phase C-18 flash chromatography using a $\text{H}_2\text{O}/\text{MeOH}$ gradient mixture to give derivative 23 as a white solid (29 mg, 47%); R_f 0.89 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ 60/30/5/3); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.77 (s, 1H, $\text{NH}(3\text{MeOPh})$), 7.99 (d, $J = 8.0$ Hz, 1H, NHAc), 7.44–7.38 (m, 2H, CH SPh), 7.26–7.20 (m, 2H, CH SPh), 7.20–7.10 (m, 2H, CH SPh , 3MeOPh), 7.13 (dd, $J = 5.7, 13.9$ Hz, 1H, CH 3MeOPh), 6.86 (dd, $J = 1.9, 8.1$ Hz, 1H, CH 3MeOPh), 6.48 (dd, $J = 2.5, 8.2$ Hz, 1H, CH 3MeOPh), 6.33 (t, $J = 6.0$ Hz, 1H, NHCONH), 4.94–4.77 (m, 3H, H1, 2 \times OH), 4.76–4.64 (m, 1H, OH), 4.52 (d, $J = 11.8$ Hz, 2H, 2 \times OH), 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); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, $\text{DMSO}-d_6$) 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 (2 CH , SPh), 129.9 (CH , 3MeOPh), 129.4 (2 CH , SPh), 127.0 (CH , SPh), 110.3 (CH , 3MeOPh), 106.9 (CH , 3MeO-Ph), 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 : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{28}\text{H}_{37}\text{N}_3\text{O}_{11}\text{SNa}$ 646.2046; Found 646.2043.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 eq) and Et_3N (7 μL , 0.05 mmol, 1 eq). 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 eq) was added. After completion of the reaction (2 h), the mixture was concentrated under reduced pressure. The residue was first purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 8/2) then by reverse phase C-18 flash chromatography using a $\text{H}_2\text{O}/\text{MeOH}$ gradient mixture to give derivative 24 as a white solid (16.7 mg, 56%); R_f 0.76 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ 60/30/5/3); ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.92 (d, $J = 8.1$ Hz, 1H, NHAc), 7.44–7.38 (m, 2H, CH SPh), 7.29–7.17 (m, 4H, CH SPh , 3MeOPh), 6.88 (dd, $J = 1.4, 2.8$ Hz, 1H, CH 3MeOPh), 6.85 (dt, $J = 1.2, 7.6$ Hz, 1H, CH 3MeOPh), 6.80 (ddd, $J = 1.0, 2.7, 8.3$ Hz, 1H, CH 3MeOPh), 4.95 (d, $J = 9.5$ Hz, 1H, H1), 4.85–4.72 (m, 2H, NHCH_2 , OH), 4.62 (t, $J = 5.1$ Hz, 1H, OH), 4.48 (d, $J = 4.7$ Hz, 1H, OH),

4.46 (br s, 1H, OH), 4.17 (d, $J = 7.1$ Hz, 1H, H1'), 3.73 (s, 3H, CH₃ 3MeOPh), 3.71–3.56 (m, 5H, NHCH₂, 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 COCH₃); ¹³C{¹H} NMR (101 MHz, DMSO-*d*₆) δ, 170.4 (C_{quat}, COCH₃), 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, 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 (CH₃, 3MeOPh), 53.6 (C2), 53.1 (CH₂, NHCH₂), 40.7 (C6), 23.5 (COCH₃); HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₂₈H₃₉N₂O₁₀SNa 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 eq) and Et₃N (28 μL, 0.2 mmol, 2 eq). 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, then NaBH₃CN (12.6 mg, 0.2 mmol, 2 eq) and AcOH (11.4 μL, 0.2 mmol, 2 eq) were added. After 2 h, the reaction mixture was concentrated under reduced pressure. The residue was first purified by flash chromatography (CH₂Cl₂/MeOH 8/2) then by reverse phase C-18 flash chromatography using a H₂O/MeOH gradient mixture to give derivative 25 as a white solid (26.6 mg, 37%); *R*_f 0.85 (CHCl₃/MeOH/H₂O/CH₃COOH 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, CHSPh), 7.29–7.22 (m, 2H, CHSPh), 7.22–7.13 (m, 3H, CHSPh, 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 × CH₃ 3MeOPh), 3.66–3.58 (m, 6H, N(CH₂)₂, H2, H3, H5, H4'), 3.53–3.41 (m, 5H, N(CH₂)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 COCH₃); ¹³C{¹H} NMR (101 MHz, DMSO-*d*₆) δ 170.4 (C_{quat}, COCH₃), 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 (2CH₂, N(CH₂)₂), 55.3 (2CH₃, 3MeOPh), 55.1 (C2), 40.7 (C6), 23.5 (COCH₃); HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₃₆H₄₇N₂O₁₁SNa 715.2901; Found 715.2868.

Phenyl β-D-galactopyranosyl-(1→3)-2-acetyl-amino-2,6-dideoxy-6-[4-(3-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio-β-D-glucopyranoside 26

To a solution of azide 11 (20 mg, 0.04 mmol) in H₂O/^tBuOH (1/1 2 mL) were added 3-methoxyphenylacetylene (11.4 μL, 0.08 mmol, 2 eq), *o*-phenylenediamine (16 μL of a 375 mM solution in H₂O, 6 μmol, 0.15 eq), sodium ascorbate (16 μL of a 250mM solution in H₂O, 4 μmol, 0.1 eq) and copper sulfate pentahydrate (16 μL

of a 125mM solution in H₂O, 2.0 μmol, 0.05 eq). 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 16h. The reaction mixture was then filtered through a Celite® plug, eluted with water, and the solvent evaporated. The residue was purified by flash chromatography (CH₂Cl₂/MeOH 1/0 to 5/5) to give derivative 26 as a white solid (6.7 mg, 23%); *R*_f 0.52 (CH₂Cl₂/MeOH 85/15); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.51 (s, 1H, C=CH triazol), 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, CH₃ 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, COCH₃); ¹³C{¹H} NMR (101 MHz, DMSO-*d*₆) δ 170.4 (C_{quat}, COCH₃), 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 (CH₃, 3MeOBz), 53.2 (C2), 51.6 (C6), 23.5 (COCH₃); HRMS (ESI-TOF) *m/z*: [M + Na]⁺ Calcd for C₂₉H₃₆N₄O₁₀SNa 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 eq) in THF (3 ml), under an atmosphere of argon, PMe₃ 1M in THF (130 μL, 0.13 mmol, 2 eq) was added. The reaction was stirred at rt and monitored by TLC (CHCl₃/MeOH/H₂O/CH₃COOH 60/30/5/3). After completion, H₂O (60 μL) was added and the mixture was heated to 60°C in an oil bath for 16h. 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 (CHCl₃/MeOH/H₂O/CH₃COOH 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 eq) and Et₃N (11 μL, 0.08 mmol, 1.2 eq). The mixture was stirred 12 h and concentrated under reduced pressure. The residue was purified by flash chromatography (CH₂Cl₂/MeOH 9/1) then by reverse phase C-18 flash chromatography using a H₂O/MeOH gradient mixture to give derivative 29 as a white solid (21.2mg, 57%); *R*_f 0.42 (CH₂Cl₂/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 CHSPh), 7.16–7.11 (m, 1H, CH 3MeOBz), 7.08 (m, 1H, CHSPh), 7.00–6.93 (m, 2H, CHSPh), 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, CH₃ 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);

$^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, DMSO- d_6) δ 166.3 (C_{quat} , C_{OPH}), 159.6 (C_{quat} , 3MeO- C), 136.2 (C_{quat} , 3MeOBz), 134.9 (C_{quat} , SPH), 130.3 (CH , 3MeOBz), 129.8 (2 CH , SPH), 129.1 (2 CH , SPH), 126.9 (CH , SPH), 120.0 (CH , 3MeOBz), 117.5 (CH , 3MeOBz), 113.0 (CH , 3MeOBz), 104.6 ($\text{C}1'$), 89.2 (C3), 86.6 (C1), 78.0 (C5), 76.1 ($\text{C}5'$), 73.3 ($\text{C}3'$), 71.6 and 71.5 (C2 and $\text{C}2'$), 71.1 (C4), 68.5 ($\text{C}4'$), 60.9 ($\text{C}6'$), 55.8 (CH_3 , 3MeOBz), 41.7 (C6); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{26}\text{H}_{33}\text{NO}_{11}\text{SNa}$ 590.1672; Found 590.1671.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 eq) and Et_3N (10 μL , 0.08 mmol, 1.2 eq). The mixture was stirred 12 h and concentrated under reduced pressure. The residue was first purified by I phase flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9/1) then by reverse phase C-18 flash chromatography using a $\text{H}_2\text{O}/\text{MeOH}$ gradient mixture to give derivative 30 as a white solid (24.3 mg, 65%); R_f 0.40 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 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); $^{13}\text{C}\{^1\text{H}\}$ 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 (2 CH , SPH), 129.9 (CH , 3MeOPh), 129.4 (2 CH , SPH), 127.2 (CH , SPH), 110.3 (CH , 3MeOPh), 106.9 (CH , 3MeOPh), 105.1 ($\text{C}1'$), 103.7 (CH , 3MeOPh), 89.2 (C3), 86.5 (C1), 78.6 (C5), 76.1 ($\text{C}5'$), 73.3 ($\text{C}3'$), 71.5 and 71.3 (C2 and $\text{C}2'$), 70.3 (C4), 68.6 ($\text{C}4'$), 61.0 ($\text{C}6'$), 55.3 (CH_3 , 3MeOPh), 40.9 (C6); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{26}\text{H}_{34}\text{N}_2\text{O}_{11}\text{SNa}$ 605.1781; Found 605.1785.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 eq) Et_3N (11 μL , 0.08 mmol, 1.2 eq). The mixture was stirred 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 eq) was added. After 2 h, the reaction mixture was concentrated under reduced pressure. The residue was first purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9/1) then by reverse phase C-18 flash chromatography using a $\text{H}_2\text{O}/\text{MeOH}$ gradient mixture to give derivative 31 as a white solid (22.1 mg, 61%); R_f 0.75 ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$ 60/30/5/3); ^1H NMR (400 MHz, CD_3OD) δ 7.56–7.50 (m, 2H, CH SPH), 7.29–7.22 (m, 4H, CH SPH, 3MeOPh), 6.92–6.84 (m, 1H, 3H, CH 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' CH_2 3MeOPh, NHCH_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); $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CD_3OD) δ , 160.0 (C_{quat} , 3MeO- C), 139.3 (C_{quat} , 3MeOPh), 132.6 (2 CH , SPH), 132.5 (C_{quat} , SPH), 129.3 (CH , 3MeOPh), 128.6 (2 CH , SPH), 127.5 (CH , SPH), 120.5 (CH , 3MeOPh), 113.8 (CH , 3MeOPh), 112.7 (CH , 3MeOPh), 104.2 ($\text{C}1'$), 87.3 (C3), 86.9 (C1), 77.6 (C5), 75.7 ($\text{C}5'$), 73.3 ($\text{C}3'$), 71.6 and 71.6 (C2 and $\text{C}2'$), 70.6 (C4), 68.9 ($\text{C}4'$), 61.2 ($\text{C}6'$), 54.3 (CH_3 , 3MeOPh), 53.4 (C2), 52.3 (CH_2 , NHCH_2), 49.6 (C6); HRMS (ESI-TOF) m/z : $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{26}\text{H}_{36}\text{NO}_{10}\text{S}$ 554.2060; Found 554.2068.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 eq) and Et_3N (12 μL , 0.08 mmol, 1.2 eq). The mixture was stirred for 8 h at rt and concentrated under reduced pressure. The residue was purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 88/12) then by reverse phase C-18 flash chromatography using a $\text{H}_2\text{O}/\text{MeOH}$ gradient mixture to give derivative 32 as a white solid (27.8 mg, 70%); R_f 0.48 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9/1); ^1H NMR (400 MHz, CD_3OD) δ 7.59–7.55 (m, 2H, CH SPH), 7.47–7.39 (m, 3H, CH 3MeOPh), 7.37–7.29 (m, 3H, CH SPH), 7.17 (dt, J = 2.4, 7.2 Hz, 1H, CH 3MeOPh), 4.51 (d, J = 7.5 Hz, 1H, H1'), 4.48 (d, J = 9.89 Hz, 1H, H1), 3.86 (s, 3H, CH_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); $^{13}\text{C}\{^1\text{H}\}$ 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 CH , SPH), 130.0 (CH , 3MeOPh), 128.6 (2 CH , SPH), 127.3 (CH , SPH), 118.7 (CH , 3MeOPh), 118.2 (CH , 3MeOPh), 111.5 (CH , 3MeOPh), 104.2 ($\text{C}1'$), 87.3 (C3), 87.1 (C1), 78.2 (C5), 75.7 ($\text{C}5'$), 73.3 ($\text{C}3'$), 71.6 and 71.3 (C2 and $\text{C}2'$), 70.0 (C4), 68.9 ($\text{C}4'$), 61.2 ($\text{C}6'$), 54.8 (CH_3 , 3MeOPh), 44.2 (C6); HRMS (ESI-TOF) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{25}\text{H}_{33}\text{NO}_{12}\text{S}_2$ 626.1342; Found 626.1337.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 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 eq) and Et_3N (18 μL , 0.13 mmol, 1.2 eq). The mixture was stirred for 8 h at rt and concentrated under reduced pressure. The residue was purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 93/7) then by reverse phase C-18 flash chromatography using a $\text{H}_2\text{O}/\text{MeOH}$ gradient mixture to give derivative 33 as a white solid (38.9 mg, 54%); R_f 0.31 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 9/1); ^1H NMR (400 MHz, CD_3OD) δ 7.83–7.77 (m, 2H, CH PhenoxyPh), 7.58–7.56 (m, 2H, CH SPH), 7.47–7.42 (m, 2H, CH PhenoxyPh), 7.35–7.27 (m, 3H, CH SPH), 7.27–7.22 (m, 1H, CH PhenoxyPh), 7.12–7.07 (m, 2H, CH PhenoxyPh), 7.03–6.98 (m, 2H, CH 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); $^{13}\text{C}\{^1\text{H}\}$ 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 (2 CH , SPH), 129.2 (2 CH , PhenoxyPh), 128.9

(2 $\underline{\text{C}}_{\text{H}}$, PhenoxyPh), 128.6 (2 $\underline{\text{C}}_{\text{H}}$, SPh), 127.4 (2 $\underline{\text{C}}_{\text{H}}$, SPh), 124.6 ($\underline{\text{C}}_{\text{H}}$, PhenoxyPh), 119.9 (2 $\underline{\text{C}}_{\text{H}}$, PhenoxyPh), 117.3 (2 $\underline{\text{C}}_{\text{H}}$, 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 C₃₀H₃₅NO₁₂S₂Na 688.1498; Found 688.1515.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 3)-6-deoxy-6-[4-(3-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio- β -D-glucopyranoside 34

To a solution of azide 12 (30 mg, 65 μ mol) in DMF (5 mL) were added 3-methoxyphenylacetylene (27.9 μ L, 0.20 mmol, 3 eq.), copper iodide (1.24 mg, 6.5 μ mol, 0.1 eq) and Et₃N (9.1 μ L, 65 μ mol, 1 eq). 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 (CH₂Cl₂/MeOH 1/0 to 9/1) to give derivative 34 as a white solid (13.9 mg, 36%); R_f 0.31 (CH₂Cl₂/MeOH 9/1); ¹H NMR (400 MHz, CD₃OD) δ 8.22 (s, 1H, C= $\underline{\text{C}}_{\text{H}}$ triazol), 7.40–7.32 (m, 3H, $\underline{\text{C}}_{\text{H}}$ 3MeOBz), 7.28–7.20 (m, 2H, $\underline{\text{C}}_{\text{H}}$ SPh), 7.05–6.97 (m, 3H, $\underline{\text{C}}_{\text{H}}$ SPh), 6.97–6.93 (m, 1H, $\underline{\text{C}}_{\text{H}}$ 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, $\underline{\text{C}}_{\text{H}}$ 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', HS'), 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); ¹³C{¹H} NMR (101 MHz, CD₃OD) δ 160.2 ($\underline{\text{C}}_{\text{quat}}$, 3MeO- $\underline{\text{C}}$), 147.2 ($\underline{\text{C}}_{\text{=CH}}$ triazol), 132.6 ($\underline{\text{C}}_{\text{quat}}$, 3MeOBz), 131.5 ($\underline{\text{C}}_{\text{quat}}$, SPh), 131.5 (2 $\underline{\text{C}}_{\text{H}}$, SPh), 129.7 ($\underline{\text{C}}_{\text{H}}$, 3MeOBz), 128.5 (2 $\underline{\text{C}}_{\text{H}}$, SPh), 127.1 ($\underline{\text{C}}_{\text{H}}$, SPh), 122.4 (C= $\underline{\text{C}}_{\text{H}}$ triazol), 117.8 ($\underline{\text{C}}_{\text{H}}$, 3MeOBz), 113.7 ($\underline{\text{C}}_{\text{H}}$, 3MeOBz), 110.7 ($\underline{\text{C}}_{\text{H}}$, 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 ($\underline{\text{C}}_{\text{H}}$ 3, 3MeOBz), 51.4 (C6); HRMS (ESI-TOF) m/z : [M + Na]⁺ Calcd for C₂₇H₃₃N₃O₁₀SNa 614.1784; Found 614.1803.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 3)-6-deoxy-6-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-1-thio- β -D-glucopyranoside 35

To a solution of azide 12 (30 mg, 65 μ mol) in DMF (1 mL) were added phenylacetylene (28.0 μ L, 0.20 mmol, 3 eq.), copper iodide (12.45 mg, 65.4 μ mol, 1 eq) and Et₃N (10 μ L, 72 μ mol, 1.1 eq). 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 (CH₂Cl₂/MeOH 1/0 to 9/1) then by reverse phase C-18 flash chromatography using a H₂O/MeOH gradient mixture to give derivative 35 as a white solid (30 mg, 85%); R_f 0.18 (CH₂Cl₂/MeOH 9/1); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.38 (s, 1H, C= $\underline{\text{C}}_{\text{H}}$ triazol), 7.82–7.79 (m, 2H, $\underline{\text{C}}_{\text{H}}$ Ph), 7.46 (t, J = 7.6 Hz, 2H, $\underline{\text{C}}_{\text{H}}$ Ph), 7.35 (t, J = 7.4 Hz, 1H, $\underline{\text{C}}_{\text{H}}$ Ph), 7.17–7.12 (m, 2H, $\underline{\text{C}}_{\text{H}}$ SPh), 7.00–6.95 (m, 3H, $\underline{\text{C}}_{\text{H}}$ SPh), 5.51 (d, J = 4.7 Hz, 1H, $\underline{\text{O}}_{\text{H}}$), 5.18 (br s, 1H, $\underline{\text{O}}_{\text{H}}$), 4.98 (br s, 1H, $\underline{\text{O}}_{\text{H}}$), 4.84–4.74 (m, 3H, 2 $\underline{\text{O}}_{\text{H}}$, H6a and H1), 4.69 (t, J = 5.2 Hz, 1H, $\underline{\text{O}}_{\text{H}}$), 4.54–4.47 (m, 2H, $\underline{\text{O}}_{\text{H}}$, 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, HS'), 3.46–3.35 (m, 2H, H2', H3'), 3.35–3.22 (m, 2H, H2, H4); ¹³C{¹H} NMR (101 MHz, DMSO-*d*₆) δ 146.6 ($\underline{\text{C}}_{\text{=CH}}$ triazol), 133.9 ($\underline{\text{C}}_{\text{quat}}$, SPh), 131.3 ($\underline{\text{C}}_{\text{quat}}$, Ph), 130.8 (2 $\underline{\text{C}}_{\text{H}}$, SPh), 129.3 and 129.1 (2 \times 2 $\underline{\text{C}}_{\text{H}}$, SPh and Ph), 128.3 ($\underline{\text{C}}_{\text{H}}$, Ph), 127.1 ($\underline{\text{C}}_{\text{H}}$, SPh), 125.6 (2 $\underline{\text{C}}_{\text{H}}$, Ph), 122.7 (C= $\underline{\text{C}}_{\text{H}}$ 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 C₂₆H₃₁N₃O₉SNa 584.1679; Found 584.1684.

Phenyl β -D-galactopyranosyl-(1 \rightarrow 3)-6-deoxy-6-[4-(4-phenoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-1-thio- β -D-glucopyranoside 36

To a solution of azide 12 (30 mg, 65 μ mol) in DMF (1 mL) were added 4-phenoxyphenylacetylene (23.7 μ L, 0.13 mmol, 2 eq.), copper iodide (12.45 mg, 65.4 μ mol, 1 eq) and Et₃N (10 μ L, 72 μ mol, 1.1 eq). 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 phase flash chromatography (DCM/MeOH 1/0 to 9/1) then by reverse phase C-18 flash chromatography using a H₂O/MeOH gradient mixture to give derivative 36 as a white solid (26.7 mg, 63%); R_f 0.18 (CH₂Cl₂/MeOH 9/1); ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.33 (s, 1H, C= $\underline{\text{C}}_{\text{H}}$ triazol), 7.84–7.77 (m, 2H, $\underline{\text{C}}_{\text{H}}$ PhenoxyPh), 7.45–7.38 (m, 2H, $\underline{\text{C}}_{\text{H}}$ PhenoxyPh), 7.22–7.12 (m, 3H, $\underline{\text{C}}_{\text{H}}$ SPh), 7.12–7.03 (m, 4H, $\underline{\text{C}}_{\text{H}}$ PhenoxyPh), 7.03–6.96 (m, 3H, $\underline{\text{C}}_{\text{H}}$ PhenoxyPh, SPh), 5.60 (br s, 1H, $\underline{\text{O}}_{\text{H}}$), 5.21 (br s, 1H, $\underline{\text{O}}_{\text{H}}$), 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', HS', H3), 3.47–3.34 (m, 2H, H2', H3'), 3.34–3.23 (m, 2H, H2, H4); ¹³C{¹H} NMR (101 MHz, DMSO-*d*₆) δ 157.1 and 156.7 (2 $\underline{\text{C}}_{\text{quat}}$, PhenoxyPh), 146.2 ($\underline{\text{C}}_{\text{=CH}}$ triazol), 134.0 ($\underline{\text{C}}_{\text{quat}}$, SPh), 130.7 (2 $\underline{\text{C}}_{\text{H}}$, SPh), 130.6 (2 $\underline{\text{C}}_{\text{H}}$, PhenoxyPh), 129.1 (2 $\underline{\text{C}}_{\text{H}}$, SPh), 127.4 (2 $\underline{\text{C}}_{\text{H}}$, PhenoxyPh), 127.1 ($\underline{\text{C}}_{\text{H}}$, PhenoxyPh), 126.7 ($\underline{\text{C}}_{\text{quat}}$, PhenoxyPh), 124.0 ($\underline{\text{C}}_{\text{H}}$, SPh), 122.4 (C= $\underline{\text{C}}_{\text{H}}$ triazol), 119.5 and 119.1 4 $\underline{\text{C}}_{\text{H}}$, 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 C₃₂H₃₅N₃O₁₀SNa 676.1941; Found 676.1918.

Molecular modelling. 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 protocol⁴⁷ as previously described.^{30,29} Briefly, the lactosamine binding domain of the 3ZSJ structure⁴⁸ was defined as the center of a 18 Angstrom sphere where the ligand could freely explore the protein hypersurface. The protocol has a two-step procedure, the first one involves a rapid ligand-protein evaluation with limited precision for interaction energy evaluation, and a second phase where a full molecular dynamics simulation using the CHARMM force field is performed. During the first phase, 250 binding modes were evaluated then 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 (RMSD) of the galactose location compared to the crystallographic galactose was assessed using the Find Most Common Sub-Structure (MCSS) 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. Experimental part for compound 1 precursors, fluorescence anisotropy experiment, ¹H, ¹³C, COSY and HSQC NMR spectra for compounds 2, 4-12, 14-27 and 29-36 "This material is available free of charge via the Internet at <http://pubs.acs.org>."

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The manuscript was written through contributions of all authors. / All authors have given approval to the final version of the manuscript.

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