Discovery of Potent Inhibitors of PapG Adhesins from Uropathogenic *Escherichia coli* through Synthesis and Evaluation of Galabiose Derivatives

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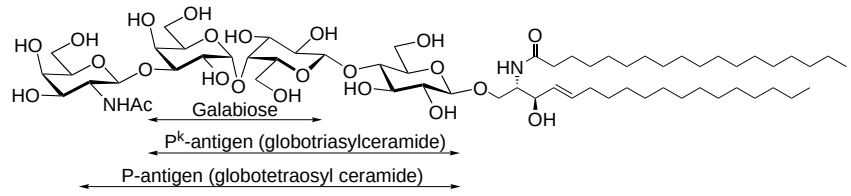
The synthesis of two galabioside (Galα1-4Gal) collections based on diversification at the O-1 and O-3′ atoms is reported. The galabiosides were evaluated as inhibitors of hemagglutination of human erythrocytes by two strains of Escherichia coli *that expressed the class I and class II PapG adhesins, respectively. The class I adhesin was found to prefer aromatic substituents both at the O-1 and the O-3′ position of the galabiose disaccharide. One galabioside,* p*-methoxyphenyl [3-*O*-(*m*-nitrobenzyl)-*α*-*D*-galacto-pyranosyl]-(1-4)-*β*-*D*-galactopyranoside], had an IC_{50} value of 4.1 μM, which is the best inhibition of the class I adhesin to date. In the case of the class II adhesin, one inhibitor, 2-[(*S*)-2-methoxycarbonyl-2-acetamido-ethylthio]ethyl {3-*O*-3-[2-(methoxy-carbonylphenylthio)propyl]-α-*D*-galactopyranosyl}-(1-4)-α-*D*-galac-topyranoside, was found to have an IC_{50} value of 68 μM, which is the best artificial inhibition of the class II adhesin reported so far with an affinity for the adhesin comparable to that of the natural tetrasaccharide ligand globotetraose.*

KEYWORDS:

antibiotics · bacterial adhesin · carbohydrates · galabiose · inhibitors

Introduction

Adhesion to host cell surfaces is an important virulence factor in many bacterial infections[1] and glycoconjugates present on the cell surface, such as glycolipids and glycoproteins, often function as receptors for extracellular bacterial proteins termed adhesins.[2, 3] Inhibitors of these bacterial adhesins constitute potential novel antibacterial agents since they would inhibit bacterial adhesion and thus prevent infection. Bacterial resistance towards such anti-adhesive drugs is believed to evolve slowly because the infecting bacteria are not killed and are consequently not under selection pressure.



Scheme 1. *P blood group antigens.*

One classical example of a pathogenic bacterium that adheres to glycoconjugates is uropathogenic *Escherichia coli*. The majority of uropathogenic *E. coli* strains that cause pyelonephritis (severe kidney infection) adhere to the Galα1-4Gal (galabiose; Gal = galactose) disaccharide moiety[4–6] present in the globoseries of glycolipids found in the upper urinary tract (Scheme 1). These *E. coli* strains use proteinaceous appendages called P-pili, which are terminated by an adhesin as a device for binding to the glycolipids.[7] The adhesin, called PapG, exists in three molecular variants (classes I – III), as classified by their slightly different agglutination patterns.[8, 9] In particular, the PapG class II adhesin has been shown to be associated with the occurrence of pyelonephritis in humans.[10, 11]

The structural requirements for class I and II PapG adhesins, which bind to globoseries glycolipids, have been investigated with a large number of synthetic saccharides.[12–14] The results from these studies unambiguously showed that the galabiose disaccharide was required for binding by both the class I and II adhesins. Furthermore, the class I adhesin was found to prefer hydrophobic substituents at the O-1 atom and to tolerate substituents at the O-3′ position of galabiose.[12, 14] The binding site of the class II adhesin was shown to be extended beyond the galabiose disaccharide unit to involve the entire GalNAcβ1-3Galα1-4Galβ1-4Glc (Gb4) tetrasaccharide, because the presence of the β glucose (Glc), and to a lesser extent, the β *N*-acetylgalactosamine (GalNAc), residues improved affinity.[13] This

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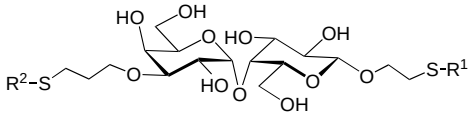
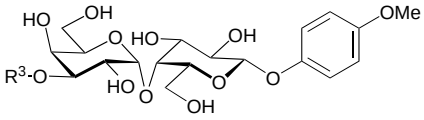
result was recently supported by the crystal structure of the class II adhesin complexed with the globotetarose (Gb4) tetrasaccharide.[15]

Most lectins bind natural carbohydrate ligands with low affinity (dissociation constants K_d normally in the 0.1 – 1 mM range) and rather large affinity enhancements are required to accept a compound as a lead inhibitor for drug development. One attractive strategy to enhance the affinity of a ligand for a lectin is to use a small key saccharide as a core structure and introduce substituents that interact with the lectin in a favourable manner.[16–19] In this context, galabiosides derivatised at the O-1 and O-3′ positions appear attractive as molecules to be used for the discovery of efficient PapG inhibitors.

We herein present the synthesis of two collections of galabiosides (Scheme 2) and the evaluation of these as inhibitors of the PapG class I and class II adhesins. The first collection of compounds (collection A) was derived from a galabioside



Galabioside collection A



Galabioside collection B

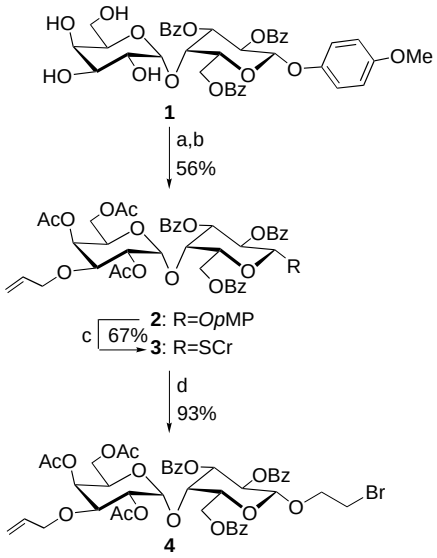
Scheme 2. *Schematic representation of galabiose collections A and B. R1 – R3 indicate regions of diversity discussed within the text.*

building block functionalised with a 2-bromoethyl group at the O-1 position and an allyl group at the O-3′ position. These two functionalities allowed orthogonal and sequential derivatisation with thiols (bromide substitution followed by radical addition to the alkene) to give thioethers. The second collection of galabiosides (collection B, Scheme 2) comprised *p*-methoxyphenyl galabiosides regioselectively alkylated at the O-3′ position through the use of the corresponding O-3′,4′-stannylidene acetal.

Results and Discussion

Synthesis

The building block **4** for galabioside collection A (Scheme 2) was prepared from *p*-methoxyphenyl α-D-galactopyranosyl-(1-4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (**1**; Scheme 3)[20]. Stannylidene acetal mediated regioselective alkylation of **1** at the O-3′ position with allyl bromide[21] gave **2** in 56% yield after acetylation. Treatment of **2** with thiocresol and $BF_3 \cdot Et_2O$ gave **3** as an anomeric mixture (α/β 1:10) in 67% yield. *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) promoted glycosylation of 2-bromoethanol with **3**



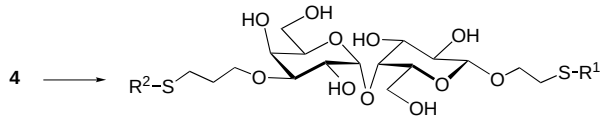
Scheme 3. *a) 1. Bu_2SnO, benzene, Δ, 15 h, 2. allylbromide, Bu_4NBr 6 h; b) Ac_2O, pyridine, 15 h, 22 °C, 55% from **1**; c) MePhSH, $BF_3 \cdot OEt_2$, toluene, CH_2Cl_2, 55 °C, 15 h, 67%; d) $BrCH_2CH_2OH$, NIS, TMSOTf, 0 °C, 10 min, 93%.* Op*MP* = O-p-*methoxyphenyl, Bz = benzyl.*

gave the building block **4** (93%), which carries two handles (an alkyl bromide and an alkene moiety) that possess orthogonal reactivities towards thiols.

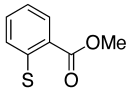
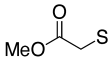
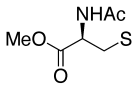
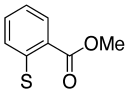
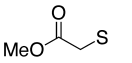
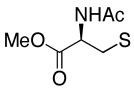
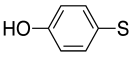
Five different thiols (cyclohexylmercaptane, methyl thiosalicylate, methyl thioglycolate, *N*-acetyl-L-cystein methyl ester and 4-mercaptophenol) were introduced at the aglycon of **4** by Cs_2CO_3 mediated bromide substitution[22] in 87 – 94% yields. Radical addition of a second set of four thiols (cyclohexylmercaptane, methyl thioglycolate, methyl thiosalicylate and *N*-acetyl-L-cystein methyl ester) to the allyl group at the O-3′ position proceeded smoothly and after deacylation afforded compounds **5** – **20** in 23 – 82% overall yield from **4** (Table 1). Somewhat lower yields were obtained with methyl thiosalicylate in the radical addition step. Intermediate galabiosides with a phenol-containing aglycon (after bromide substitution with 4-mercaptophenol) gave a number of byproducts under radical addition conditions. Reasonable yields could be achieved if the phenolic hydroxyl group was protected prior to addition to the O-3′ allyl ether. Thus, acetylation and thiol radical addition followed by deacylation yielded compounds **21** – **24** in 29 – 48% yield from **4**. The second set of galabiosides (collection B) was obtained by regioselective *O*-3′-alkylation of the O-3′,4′-stannylidene acetal of compound **1**, followed by deacetylation to give compounds **25** – **30** (Table 2).

Reference compounds that contain the invariable part of either galabioside collection A or B (that is, ethyl (3-*O*-propyl-α-D-galactopyranosyl)-(1-4)-β-D-galactopyranoside (**31**) and *p*-methoxyphenyl α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside (**32**), respectively) were needed in order to allow proper interpretation of the results from biological evaluations of the experimental compounds. Hydrogenolysis of **4** in aqueous KOH in methanol[23] gave **31** (77%) and conventional *O*-deacylation of **1** gave **32** (95%; Scheme 4).

Table 1. *Synthesis of galabiosides **5**–**24** (collection A).*[a]

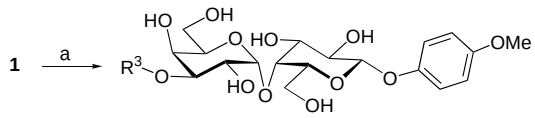


5–24

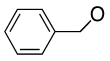
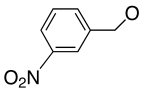
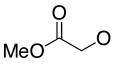
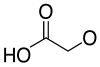
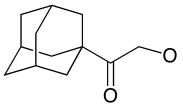
R^2–S → / R^1–S ↓				
	**5** (A, 64%)	**6** (C, 30%)	**7** (A, 67%)	**8** (A, 82%)
	**9** (A, 54%)	**10** (C, 47%)	**11** (A, 37%)	**12** (A, 60%)
	**13** (A, 58%)	**14** (C, 31%)	**15** (A, 65%)	**16** (A, 49%)
	**17** (A, 47%)	**18** (C, 23%)	**19** (A, 44%)	**20** (A, 43%)
	**21** (B, 48%)	**22** (D, 29%)	**23** (B, 36%)	**24** (B, 47%)

[a] Conditions: A) 1. R^1–SH, Cs_2CO_3, DMF, 2. R^2–SH, AIBN, hν, EtOAc, 3. NaOMe, MeOH; B) 1. R^1–SH, Cs_2CO_3, DMF, 2. Ac_2O, pyridine, 3. R^2–SH, AIBN, hν, EtOAc, 4. NaOMe, MeOH; C) 1. R^1–SH, Cs_2CO_3, DMF, 2. R^2–SH, AIBN, hν, 3. NaOMe, MeOH; D) 1. R^1–SH, Cs_2CO_3, DMF, 2. Ac_2O, pyridine, 3. R^2–SH, AIBN, hν, 4. NaOMe, MeOH.

Table 2. *Synthesis of galabiosides **25**–**30** (collection B).*[a]

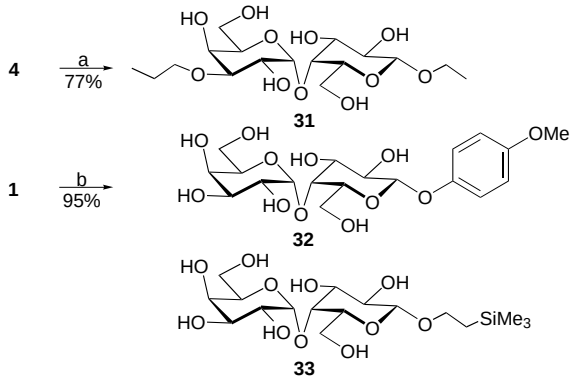


25–30

Product	R^3–O	Yield [%]
25		67
26		70
27		63
28		28[b]
29		50[b]
30		53

[a] Conditions: 1. Bu_2SnO, benzene, reflux, 2. R^3–Br, Bu_4NBr, benzene, reflux, 3. NaOMe, MeOH. [b] Isolated from same experiment.

The NMR spectroscopy data show a downfield shift of the H-5′ signal to 4.25–4.29 ppm in compounds **5**–**32**, which suggests that they adopt a conformation similar to that of known galabiosides,[24–26] an advantage in biological evaluations.



Scheme 4. *a) H_2(1 bar), 10% Pd/C, KOH, H_2O, MeOH, 4 h; b) NaOMe, MeOH, rt, 4 h.*

Inhibition of hemagglutination by HB101/pHMG93 (class I adhesin)

The two galabioside collections A and B (compounds **5**–**30**) and the reference compounds **31** and **32** were evaluated as inhibitors of hemagglutination by two different recombinant strains of *E. coli*, HB101/pHMG93 and HB101/pDC1, which express the class I and class II PapG adhesin, respectively. In addition, 2-(trimethylsilyl)ethyl galabioside (**33**; Scheme 4)[27] was included in the inhibition experiments and assigned the relative inhibition of 100% since this compound has been used as a reference in previous studies.[12–14]

The inhibition data for galabioside collection A against the class I adhesin is shown in Table 3. A comparison of the two

Table 3. *Inhibition data for galabioside collection A.*

Compound	HB101/pHMG93 (class I)		HB101/pDC1 (class II)	
	IC_{50} [μM]	Relative inhibition [%]	IC_{50} [μM]	Relative inhibition [%]
5[a]	–	–	–	–
6[a]	–	–	–	–
7	13	230	620	87
8	22	140	420	120
9	120	25	210	250
10[a]	–	–	–	–
11	35	86	84	620
12	43	70	170	310
13	20	150	1330	39
14	17	180	170	310
15	22	140	140	370
16	17	180	100	520
17	52	58	260	200
18	26	120	68	770
19	43	70	220	240
20	52	58	160	330
21	26	120	250	210
22	43	70	120	430
23	17	180	320	160
24	17	180	230	230
31	87	34	440	120
33	30	100[b]	520	100[b]

[a] Compounds **5**, **6** and **10** were not soluble in the concentrations required for inhibition. [b] The relative inhibitory power of the known inhibitor **33** (2-trimethylsilylethyl galabioside[12–14]) is defined as 100%.

reference compounds **31** and **33** suggests that the invariable spacer moieties of compounds **5** – **24** lower the inhibitory power because the known reference compound **33** exhibits three times better inhibition than **31**. Nevertheless, in ten of the compounds the inhibitory power is regained, mainly by the anomeric substituent R^1. The 2-(cyclohexylthio)ethyl (**7** – **8**), 2-(methoxycarbonylmethylthio)ethyl (**13** – **16**), and 2-(4-hydroxyphenylthio)ethyl (**21** – **24**) galabiosides proved to be among the more potent inhibitors. The substituents at the O-3′ atom had a minor effect, which suggests that the propyl linker is too long and positions the R^2 substituents outside the binding site of the class I adhesin. The compound with the best inhibition (**7**) is more than twice as potent as the hitherto best inhibitor (**33**) known against the class I adhesin.[14]

The compounds in galabioside collection B (**25** – **30**) have the substituents attached directly to the galabiose core structure and may thus be expected to be better suited as inhibitors of the class I adhesin. Indeed, large differences in inhibitory power were found within collection B (Table 4), which suggests that the substituents are positioned in the vicinity of, or in the combining site of the class I adhesin. The *p*-methoxyphenyl galabioside **32** was three times more powerful than the known reference 2-trimethylsilylethyl galabioside (**33**), which suggests that the aromatic aglycon of **32** interacts favourably with the adhesin.

Table 4. *Inhibition data for galabioside collection B.*

Compound	HB101/pHMG93 (class I)		HB101/pDC1 (class II)	
	IC_{50} [μM]	Relative inhibition [%]	IC_{50} [μM]	Relative inhibition [%]
25	4.9	610	120	430
26	7.6	320	260	200
27	4.1	730	180	290
28	35	86	> 6700	< 8
29	87	34	> 6700	< 8
30[a]	–	–	–	–
32	11	270	110	470
33	30	100[b]	520	100[b]

[a] Compound **30** was not soluble in the concentrations required for inhibition. [b] The relative inhibitory power of the known inhibitor **33** (2-trimethylsilylethyl galabioside[12–14]) is defined as 100%.

The *O*-3′-substitutions had significant impact on the inhibitory powers of compounds **25** – **30**. For example, compounds **28** and **29** showed decreased inhibitory power as compared to **32**, whereas two exceptionally potent inhibitors were found in compounds **25** and **27** (relative inhibition of 610 and 730%, respectively, as compared to **33**). These findings show that aromatic substituents at the O-3′ and O-1 position are preferred by the class I adhesin.

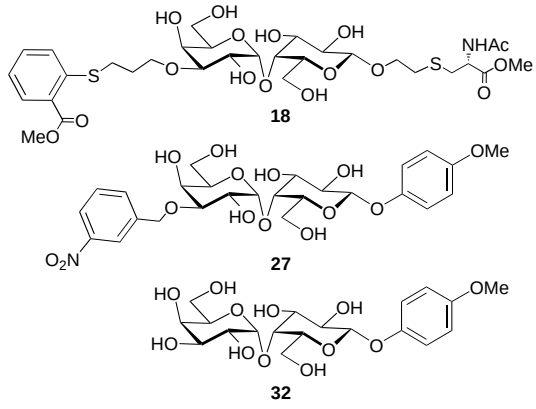
Inhibition of hemagglutination by HB101/pDC1 (class II adhesin)

The inhibition data for the first set of galabiosides (collection A) against the class II adhesin are given in Table 3. In contrast to the results with the class I adhesin described above, no difference was found between compounds **31** and **33**, which suggests that the ethyl and propyl spacer moieties at the O-1 and O-3′ atoms are well tolerated by the class II adhesin. Four galabiosides (**11**, **16**, **18** and **22**) displayed superior inhibitory power as compared to the reference **33** (relative inhibitory powers of 620, 520, 770 and 430%, respectively). Consequently, the substituents R^1 and R^2 in the galabioside collection A exert a large influence on the binding affinity. This result is in agreement with studies on Forssman pentasaccharide fragments[13] and with the recently published structure of the class-II-adhesin:Gb4 complex,[15] which revealed recognition of sugar moieties flanking the galabiose disaccharide by this adhesin. The best inhibitor (**18**, IC_{50} value 68 μM) shows 770% inhibitory power as compared to the known compound **33** and is thus at least as good as the best known ligand so far, the Gb4 tetrasaccharide (560%[13] as compared to **33**).

No members from galabioside collection B were better than the reference inhibitor **32** (Table 4). Compound **32** displayed inhibitory power (470% relative to **33**) in the same range as Gb4.[13] Thus, the same magnitude of inhibitory power could be obtained with the rather simple disaccharide **32** (readily prepared in high yields on a large scale[20]) as with the Gb4 tetrasaccharide. Although no affinity enhancement was obtained by substitutions at the O-3′ position (all *O*-3′-substituents in collection B had a negative influence on the inhibitory power) it is noteworthy that substituents at the O-3′ atom seem to have a large influence on the inhibitory power, which leads to the speculation that the right choice of substituent at the O-3′ position might lead to improved inhibitors.

Conclusions

We have synthesised and evaluated two collections of galabiosides as inhibitors of uropathogenic *E. coli* strains that express either class I or class II PapG adhesins. The two different adhesins (class I and II) were, as expected, found to display different recognition patterns. One galabioside **27** (Scheme 5) was found to inhibit the class I adhesin with an IC_{50} value as low as 4.1 μM. Compound **27** is thus 20 – 30 times more potent than the natural ligand Gb4[14] and is the best inhibitor to date against the class I



Scheme 5. *Compounds **27** (IC_{50} value 4.1 μM against class I) and **18** (IC_{50} value 68 μM against class II) are the best inhibitors known against the class I and II PapG adhesins. The* p*-methoxyphenyl galabioside **32** is a potent yet simple inhibitor against both the class I and class II PapG adhesins.*

adhesin. Other inhibitors (**18** and **32**) displayed IC_{50} values as low as 68 and 110 μM against the class II adhesin, which is at least as potent as the natural tetrasaccharide ligand, Gb4.[13] It is noteworthy that for both adhesins the presence of a *p*-methoxyphenyl galabioside aglycon results in potent inhibitors (**27** and **32**). This can most likely be explained by a favourable interaction between the aromatic aglycon and a conserved Trp residue found close to the galabiose O-1 atom in the crystal structure of the class II PapG adhesin.[15] These inhibitors represent the best inhibitors known against the PapG class I and II adhesins and constitute an advance towards anti-adhesion therapeutic agents that target urinary tract infections.

Experimental Section

General: NMR spectra were recorded on a Bruker DRX-400 instrument. Residual $CHCl_3$ or CD_2HOD were used as internal references at 7.27 and 3.31 ppm, respectively. ^{1}H NMR spectral assignments were made by using COSY. Solutions were concentrated by rotary evaporation with a bath temperature at or below 40 °C. Flash chromatography was performed on Grace Amicon Silica gel 60 (0.035 – 0.070 mm) and TLC was performed on Kieselgel 60 F_{254} plates (Merck). All nonaqueous reactions were run in septum-capped, oven-dried flasks under Ar (1 atm). CH_2Cl_2 and toluene were distilled from CaH_2. Et_2O was distilled from Na. UV-irradiations were performed with a water-cooled Original Hanau 70 W mercury high-pressure lamp. Recombinant *Escherichia coli* strains HB101/pHMG93[28] and HB101/pDC1,[29] which express class I and class II PapG adhesin respectively, were used to determine hemagglutination inhibition by selected substances. The plasmid pHMG93, which carries the *Pap* genes was constitutively expressed from the *ala*S promoter. The pDC1 vector expresses the Pap pili from *E. coli* IA2.

p-Methoxyphenyl (2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (2): Compound **1**[20] (7.47 g, 10.0 mmol) and Bu_2SnO (2.99 g, 12.0 mmol) were refluxed in benzene (180 mL) with azeotropic removal of water overnight then allyl bromide (17.3 mL, 0.2 mol) and Bu_4NBr (1.61 g, 5.0 mmol) were added. After 6 h reflux, the mixture was concentrated, flash chromatographed (SiO_2, 4:1 – 2:1 toluene/acetone gradient), concentrated again and treated with pyridine (75 mL) and acetic anhydride (60 mL) overnight. Coevaporation with toluene gave **2** (5.17 g, 56 %). Recrystallisation from EtOAc/Et_2O gave an analytical sample; mp: 161 – 162 °C; $[\alpha]_D^{23}$: +122 (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 8.10 – 7.95 (m, 6H, Ar–H), 7.67 – 7.35 (m, 9H, Ar–H), 6.99 (m, 2H, OC_6H_4OMe), 6.71 (m, 2H, OC_6H_4OMe), 5.97 (dd, 1H, J = 7.8, 10.6 Hz, H-2), 5.87 (m, 1H, CH_2=C*H*–CH_2), 5.52 (d, 1H, J = 2.0 Hz, H-4′), 5.40 (dd, 1H, J = 2.8, 10.5 Hz, H-3), 5.32 (m, 1H, CH_2=), 5.23 – 5.15 (m, 4H, H-1, H-1′, H-2′, CH_2=), 4.83 (m, 1H, H-6), 4.61 – 4.53 (m, 2H, H-6, H-5′), 4.47 (d, 1H, J = 2.4 Hz, H-4), 4.28 – 4.18 (m, 2H, H-5, OCH_2), 4.10 – 4.01 (m, 2H, H-3′, OCH_2), 3.88 (dd, 1H, J = 7.7, 11.0 Hz, H-6′), 3.74 (s, 3H, OMe), 3.63 (dd, 1H, J = 6.2, 11.0 Hz, H-6′), 2.20, 2.12, 1.92 (3 s, 3H each, OAc) ppm; ^{13}C NMR ($CDCl_3$): δ = 171.1, 170.7, 170.6, 166.6, 166.5, 165.8, 156.1, 151.6, 134.6, 134.2, 133.9, 133.8, 130.3, 130.2, 130.1, 128.8, 129.7, 129.1, 129.03, 129.00, 128.9, 119.1, 117.7, 114.9, 101.5, 99.4, 76.1, 73.9, 73.3, 72.9, 71.2, 70.6, 69.8, 68.1, 67.5, 63.2, 61.7, 56.0, 21.4, 21.2, 21.0 ppm; HRMS: calcd for $C_{49}H_{50}O_{18}Na$ (M+Na): 949.2895; found: 949.2903.

***p*-Tolyl (2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (3)**: $BF_3 \cdot Et_2O$ (1.0 mL, 7.1 mmol) was added to a mixture of **2** (5.08 g, 5.48 mmol) and thiocresol (2.83 g, 22 mmol) in toluene:CH_2Cl_2 (180 mL, 1:1) at room temperature. The mixture was stirred at 55 °C overnight then diluted with CH_2Cl_2 (200 mL), washed with satd aqueous $NaHCO_3$, (2 × 100 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (SiO_2, 1:2 – 1:3 heptane/diethylether gradient) gave **3**, (3.42 g, 67 %). Recrystallisation from EtOH gave an analytical sample; mp: 186 – 188 °C; $[\alpha]_D^{23}$: +85 (c = 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 8.09 – 7.93 (m, 6H, Ar–H), 7.65 – 7.34 (m, 11H, Ar–H), 7.07 (d, 2H, J = 8.1 Hz, Ar–H), 5.90 (m, 1H, =C*H*–CH_2), 5.68 (t, 1H, J = 10.1 Hz, H-2), 5.42 – 5.34 (m, 3H, H-3, H-4′, =CH_2), 5.25 (m, 1H, =CH_2), 5.11 (m, 2H, H-1′, H-2′), 4.92 (d, 1H, J = 9.8 Hz, H-1), 4.80 (dd, 1H, J = 7.4, 11.6 Hz, H-6), 4.50 (dd, 1H, J = 5.6, 11.4 Hz, H-6), 4.39 (d, 1H, J = 2.6 Hz, H-4), 4.25 – 4.13 (m, 3H, H-5, H-5′, OCH_2), 4.05 (m, 1H, OCH_2), 3.90 (m, 2H, H-3′, H-6′), 3.67 (dd, 1H, J = 3.6, 11.0 Hz, H-6′), 2.38 (s, 3H, Me), 2.19, 2.08, 1.95 (3 s, 3H each, OAc) ppm; ^{13}C NMR ($CDCl_3$): δ = 171.0, 170.8, 170.6, 166.53, 166.49, 165.6, 138.9, 134.6, 134.6, 134.5, 134.1, 133.9, 133.8, 133.2, 130.3, 130.22, 130.16, 130.0, 129.9, 129.8, 129.1, 129.0, 128.9, 128.2, 117.5, 99.2, 86.5, 76.9, 76.6, 75.2, 73.0, 71.1, 70.6, 68.2, 67.9, 67.4, 63.6, 61.7, 21.7, 21.4, 21.2, 21.1 ppm; HRMS calcd for $C_{49}H_{50}O_{16}SNa$ (M+Na): 949.2717; found: 949.2725.

2-Bromoethyl (2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (4): 2-Bromoethanol (0.54 mL, 7.8 mmol), TMSOTf (0.137 mL, 0,69 mmol) and finally NIS (1.0 g, 4.5 mmol) were added to a solution of **3** (3.22 g, 3.37 mmol) in CH_2Cl_2 (140 mL) at 0 °C and the resulting solution was stirred for 40 min. Et_3N (2 mL) was added, the mixture was diluted with CH_2Cl_2 (200 mL), washed with 10 % aqueous $Na_2S_2O_3$ (100 mL) and satd aqueous $NaHCO_3$ (100 mL), dried ($MgSO_4$), evaporated and flash chromatographed (SiO_2, 3:1 – 2:1 heptane/EtOAc gradient) to give **4** (3.0 g, 93 %). Recrystallisation from EtOAc/Et_2O gave an analytical sample; mp: 144 – 145 °C; $[\alpha]_D^{23}$: +109 (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 8.05 – 7.92 (m, 6H, Ar–H), 7.63 – 7.33 (m, 9H, Ar–H), 5.93 – 5.80 (m, 1H, =CH), 5.75 (dd, 1H, J = 7.7, 10.6 Hz, H-2), 5.48 (dd, 1H, J = 1.5, 3.2 Hz, H-4′), 5.35 – 5.30 (m, 2H, H-3, H_2C=), 5.25 – 5.11 (m, 3H, H-1′, H-2′, H_2C=), 4.82 (d, 1H, J = 7.7 Hz, H-1), 4.77 (dd, 1H, J = 7.0, 11.3 Hz, H-6), 4.50 (m, 2H, H-6, H-5′), 4.41 (d, 1H, J = 2.8 Hz, H-4), 4.24 – 4.11 (m, 3H, H-5, OCH_2CH_2Br, =CH–CH_2), 4.05 (m, 2H, H-3′, =CH–CH_2), 3.91 (dt, 1H, J = 7.1, 11.3 Hz, OCH_2CH_2Br), 3.81 (dd, 1H, J = 7.7, 11.0 Hz, H-6′), 3.53 (dd, 1H, J = 6.2, 10.8 Hz, H-6′), 3.45 (m, 2H, CH_2Br), 2,17, 2.07, 1.86 (s, 3H each, OAc) ppm; ^{13}C NMR ($CDCl_3$): δ = 171.1, 170.7, 170.6, 166.6, 166.5, 165.9, 134.6, 134.1, 134.0, 133.8, 130.3 – 128.9 (Ar), 117.7, 102.2, 99.2, 75,7, 73.7, 73.1, 72.8, 71.1, 70.5, 70.3, 69.7, 69.0, 67.4, 62.8, 61.7, 30.1, 21.4, 21.2, 21.0 ppm; HRMS calcd for $C_{44}H_{47}O_{17}BrNa$ (M+Na): 949.1894; found: 949.1902

General procedures for preparation of galabioside collection A: Method A (compounds **5**, **7** – **9**, **11** – **13**, **15** – **17** and **19** – **20**): Cs_2CO_3 (46 mg, 0.140 mmol) and the appropriate thiol (0.162 mmol) were added to a solution of **4** (100 mg, 0.108 mmol) in deoxygenated dimethylformamide (DMF; 5 mL). The mixture was stirred at ambient temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (15 mL), washed with satd aqueous $NaHCO_3$ (5 mL) and water (5 mL), dried ($MgSO_4$), concentrated and flash chromatographed (SiO_2, heptane/EtOAc). The thiol (5 equiv) and azobisisobutyronitrile (AIBN; cat) were added to the residue (25 mg) in EtOAc (0.5 mL) and Ar was bubbled through the mixture for 15 min. The reaction flask was sealed with a septum and UV irradiated for 48 h. Concentration and flash chromatography (SiO_2, heptane/EtOAc) gave the protected intermediate, which was *O*-deacylated in methanolic NaOMe (0.01 M, 1.5 mL) for 5 h. Methanolic acetic acid (10 %) was added until a neutral result was seen on moist pH paper. The mixture was then concentrated and flash chromatographed (SiO_2, CH_2Cl_2/MeOH) to give compounds **5**, **7** – **9**, **11** – **13**, **15** – **17**, and **19** – **20**.

Method B (compounds **21**, **23** and **24**): Cs_2CO_3 (69 mg, 0.211 mmol) and 4-mercaptophenol (31 mg, 0.243 mmol) were added to a

solution of **4** (150 mg, 0.162 mmol) in deoxygenated DMF (8 mL). The mixture was stirred at ambient temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with satd aqueous $NaHCO_3$ (8 mL) and water (8 mL), dried ($MgSO_4$), concentrated and flash chromatographed (SiO_2, 3:1 – 2:1 heptane/EtOAc gradient) to give the intermediate (151 mg; 95%). Pyridine (10 mL) and acetic anhydride (5 mL) were added to the residue and the mixture was stirred overnight, concentrated, coconcentrated with toluene and flash chromatographed (SiO_2, 3:1 – 2:1 heptane/EtOAc gradient) to give the corresponding 4-acetoxyphenylthio derivative (152 mg, 97%). The appropriate thiol (5 equiv) and AIBN (cat) were added to a solution of a portion of the residue (25 mg) in EtOAc (0.5 mL) and Ar was bubbled through the mixture for 15 min. The reaction flask was sealed with a septum and UV irradiated for 48 h. Concentration and flash chromatography (SiO_2, heptane/EtOAc) gave the protected intermediate, which was deacylated in methanolic NaOMe (0.01 M, 1.5 mL) for 5 h. Methanolic acetic acid (10%) was added until a neutral result was seen on moist pH paper. The resulting mixture was concentrated and flash chromatographed (SiO_2, CH_2Cl_2/MeOH) to give compounds **21**, **23** and **24**.

Method C (compounds **6**, **10**, **14** and **18**): Cs_2CO_3 (46 mg, 0.140 mmol) and the appropriate thiol (0.162 mmol) were added to a solution of **4** (100 mg, 0.108 mmol) in deoxygenated DMF (5 mL). The mixture was stirred at ambient temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (15 mL), washed with satd aqueous $NaHCO_3$ (5 mL) and water (5 mL), dried ($MgSO_4$), concentrated and flash chromatographed (SiO_2, heptane/EtOAc) to give the intermediate monosubstituted galabioside. Methyl thiosalicylate (0.20 mL) and AIBN (cat) were added to a solution of the monosubstituted galabioside (25 mg) and Ar was bubbled through the mixture for 15 min. The reaction flask was sealed with a septum and UV irradiated for 48 h. Flash chromatography (SiO_2, heptane/EtOAc) gave the protected intermediate, which was deacylated in methanolic NaOMe (0.01 M, 1.5 mL) for 5 h. Methanolic acetic acid (10%) was added until a neutral result was seen on moist pH paper. The resulting mixture was concentrated and flash chromatographed (SiO_2, CH_2Cl_2/MeOH) to give compounds **6**, **10**, **14**, and **18**.

Method D (compound **22**): Cs_2CO_3 (69 mg, 0.211 mmol) and 4-mercaptophenol (31 mg, 0.243 mmol) were added to a solution of **4** (150 mg, 0.162 mmol) in deoxygenated DMF (8 mL). The mixture was stirred at ambient temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with satd aqueous $NaHCO_3$ (8 mL) and water (8 mL), dried ($MgSO_4$), concentrated and flash chromatographed (SiO_2, 3:1 – 2:1 heptane/EtOAc gradient) to give the intermediate (151 mg, 95%). Pyridine (10 mL) and acetic anhydride (5 mL) were added to the residue and the mixture was stirred overnight, concentrated and coconcentrated with toluene and flash chromatographed (SiO_2, 3:1 – 2:1 heptane/EtOAc gradient) to give the 4-hydroxybenzyl substituted galabioside (152 mg, 97%). Methyl thiosalicylate (0.20 mL) and AIBN (cat) were added to a solution of the monosubstituted galabioside (25 mg) and Ar was bubbled through the mixture for 15 min. The reaction flask was sealed with a septum and UV irradiated for 48 h. Flash chromatography (SiO_2, heptane/EtOAc) gave the protected intermediate, which was deacylated in methanolic NaOMe (0.01 M, 1.5 mL) for 5 h. Methanolic acetic acid (10%) was added until a neutral result was seen on moist pH paper. The resulting mixture was concentrated and flash chromatographed (SiO_2, CH_2Cl_2/MeOH) to give compound **22**.

5: ^{1}H NMR (CD_3OD): $\delta = 4.96$ (d, 1H, $J = 4.0$ Hz, H-1′), 4.32 (d, 1H, $J =$ 7.3 Hz, H-1), 4.27 (t, 1H, $J = 6.3$ Hz, H-5′), 4.12 (d, 1H, $J = 1.9$ Hz, H-4′), 4.01 – 3.94 (m, 2H, H-4, OCH_2CH$_2$S), 3.89 – 3.47 (m, 12H), 2.79 (t, 2H, $J = 6.8$ Hz, OCH$_2$CH_2S), 2.75 – 2.65 (m, 4H, OCH$_2$CH$_2$CH_2S, SCH, SCH), 1.99 (m, 4H, CH_2), 1.88 (m, 2H, OCH$_2$CH_2CH$_2$S), 1.77 (m, 4H, CH_2), 1.63 (m, 2H, CH_2), 1.39 – 1.25 (m, 10H, CH_2) ppm; HRMS calcd for $C_{29}H_{52}O_{11}S_2Na$ (M+Na): 663.2849; found: 663.2844.

6: ^{1}H NMR (CD_3OD): $\delta = 7.88$ (m, 1H, Ar–H), 7.51 (m, 2H, Ar–H), 7.20 (m, 1H, Ar–H), 4.97 (d, 1H, $J = 4.0$ Hz, H-1′), 4.32 (d, 1H, $J = 7.3$ Hz, H-1), 4.26 (t, 1H, $J = 5.8$ Hz, H-5′), 4.13 (d, 1H, $J = 2.1$ Hz, H-4′), 4.01 (d, 1H, $J = 3.1$ Hz, H-4), 3.98 – 3.47 (m, 16H), 3.13 (m, 2H, OCH$_2$CH$_2$CH_2S), 2.78 (t, 1H, $J = 7.2$ Hz, OCH$_2$CH_2S), 2.72 (m, 1H, CHS), 1.99 (m, 4H, CH_2, OCH$_2$CH_2CH$_2$S), 1.76 (m, 2H, CH_2), 1.62 (m, 1H, CH_2), 1.37 – 1.25 (m, 5H, CH_2) ppm; HRMS calcd for $C_{31}H_{48}O_{13}S_2Na$ (M+Na): 715.2434; found: 715.2429.

7: ^{1}H NMR (CD_3OD): $\delta = 4.97$ (d, 1H, $J = 4.0$ Hz, H-1′), 4.32 (d, 1H, $J =$ 7.3 Hz, H-1), 4.27 (t, 1H, $J = 6.5$ Hz, H-5′), 4.12 (d, 1H, $J = 2.3$ Hz, H-4′), 4.02 – 3.94 (m, 2H, H-4, OCH_2CH$_2$S), 3.89 – 3.46 (m, 15H), 3.30 (s, 2H, SCH_2COOMe), 2.81 – 2.71 (m, 4H, CH_2S), 1.99 (m, 2H, CH_2), 1.92 (m, 2H, OCH$_2$CH_2CH$_2$S), 1.77 (m, 2H, CH_2), 1.63 (m, 1H, CH_2), 1.40 – 1.25 (m, 5H, CH_2) ppm; HRMS calcd for $C_{26}H_{46}O_{13}S_2Na$ (M+Na): 653.2278; found: 653.2277.

8: ^{1}H NMR (CD_3OD): $\delta = 4.97$ (d, 1H, $J = 4.0$ Hz, H-1′), 4.63 (m, 1H, SCH$_2$CH(NHAc)), 4.32 (d, 1H, $J = 7.4$ Hz, H-1), 4.26 (t, 1H, $J = 5.9$ Hz, H-5′), 4.12 (d, 1H, $J = 2.4$ Hz, H-4′), 4.00 (d, 1H, $J = 3.0$ Hz, H-4), 3.96 (m, 1H, OCH_2CH$_2$S), 3.88 – 3.46 (m, 15H), 3.00 (m, 1H, SCH_2CH(NHAc)), 2.88 – 2.70 (m, 6H, OCH$_2$CH$_2$CH_2S, OCH$_2$CH_2S, SCH_2CH(NHAc), SCH), 2.00 (m, 5H, CH_2, Ac), 1.88 (m, 2H, OCH$_2$CH_2CH$_2$S), 1.76 (m, 2H, CH_2), 1.62 (m, 1H, CH_2), 1.30 (m, 5H, CH_2) ppm; HRMS calcd for $C_{29}H_{51}O_{14}NS_2Na$ (M+Na): 724.2649; found: 724.2661.

9: ^{1}H NMR (CD_3OD): $\delta = 7.89$ (d, 1H, $J = 7.8$ Hz, Ar–H), 7.51 (d, 2H, $J =$ 3.6 Hz, Ar–H), 7.23 (m, 1H, Ar–H), 4.96 (d, 1H, $J = 3.9$ Hz, H-1′), 4.34 (d, 1H, $J = 7.0$ Hz, H-1), 4.27 (t, 1H, $J = 5.9$ Hz, H-5′), 4.12 (d, 1H, $J = 2.0$ Hz, H-4′), 4.05 (m, 1H, OCH_2CH$_2$S), 4.00 (d, 1H, $J = 2.1$ Hz, H-4), 3.89 – 3.50 (m, 15H), 3.25 (t, 2H, $J = 7.0$ Hz, OCH$_2$CH_2S), 2.70 – 2.64 (m, 3H, OCH$_2$CH$_2$CH_2S, SCH), 1.98 (m, 2H, CH_2), 1.88 (m, 2H, OCH$_2$CH_2CH$_2$S), 1.75 (m, 2H, CH_2), 1.62 (m, 1H, CH_2), 1.38 – 1.23 (m, 5H, CH_2) ppm; HRMS calcd for $C_{31}H_{48}O_{13}S_2Na$ (M+Na): 715.2434; found: 715.2440.

10: ^{1}H NMR (CD_3OD): $\delta = 7.88$ (m, 2H, Ar–H), 7.50 (m, 4H, Ar–H), 7.20 (m, 2H, Ar–H), 4.97 (d, 1H, $J = 3.9$ Hz, H-1′), 4.34 (d, 1H, $J = 6.9$ Hz, H-1), 4.27 (t, 1H, $J = 6.6$ Hz, H-5′), 4.13 (d, 1H, $J = 2.6$ Hz, H-4′), 4.06 (dt, 1H, $J = 7.3$, 10.6 Hz, OCH_2CH$_2$S), 4.01 (d, 1H, $J = 2.3$ Hz, H-4), 3.92 – 3.50 (m, 18H), 3.26 (t, 2H, $J = 7.0$ Hz, OCH$_2$CH_2S), 3.12 (dt, 2H, $J = 1.3$, 7.8 Hz, OCH$_2$CH$_2$CH_2S), 1.99 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for C33H44O15S2Na (M+Na): 767.2019; found: 767.2030.

11: ^{1}H NMR (CD_3OD): $\delta = 7.89$ (m, 1H, Ar–H), 7.51 (m, 2H, Ar–H), 7.22 (m, 1H, Ar–H), 4.96 (d, 1H, $J = 3.9$ Hz, H-1′), 4.34 (d, 1H, $J = 7.0$ Hz, H-1), 4.27 (t, 1H, $J = 6.0$ Hz, H-5′), 4.12 (d, 1H, $J = 2.1$ Hz, H-4′), 4.06 (dt, 1H, $J = 7.3$, 10.6 Hz, OCH_2CH$_2$S), 4.00 (d, 1H, $J = 2.0$ Hz, H-4), 3.89 – 3.49 (m, 18H), 3.30 (s, 2H, OCH_2COOMe), 3.25 (t, 2H, $J = 7.0$ Hz, OCH$_2$CH_2S), 2.78 (t, 2H, $J = 7.0$ Hz, OCH$_2$CH$_2$CH_2S), 1.91 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{28}H_{42}O_{15}S_2Na$ (M+Na): 705.1863; found: 705.1868.

12: ^{1}H NMR (CD_3OD): $\delta = 7.89$ (m, 1H, Ar–H), 7.51 (m, 2H, Ar–H), 7.22 (m, 1H, Ar–H), 4.97 (d, 1H, $J = 4.0$ Hz, H-1′), 4.63 (ddd, 1H, $J = 1.2$ Hz, 4.0, 8.0, SCH$_2$CH(NHAc)), 4.34 (d, 1H, $J = 7.1$ Hz, H-1), 4.27 (t, 1H, $J =$ 6.1 Hz, H-5′), 4.12 (d, 1H, $J = 2.5$ Hz, H-4′), 4.06 (dt, 2H, $J = 7.3$ Hz, 10.6, OCH_2CH$_2$S), 4.01 (d, 1H, $J = 2.2$ Hz, H-4), 3.90 – 3.50 (m, 18H), 3.26 (t, 2H, $J = 6.9$ Hz, OCH$_2$CH_2S), 3.01 (ddd, 1H, $J = 1.9$, 5.2, 13.8 Hz, SCH_2CH(NHAc)), 2.84 (ddd, 1H, $J = 1.1$, 8.1, 13.8 Hz, SCH_2CH(NHAc)), 2.71 (m, 2H, OCH$_2$CH$_2$CH_2S), 2.00 (s, 3H, Ac), 1.89 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{31}H_{47}O_{16}NS_2Na$ (M+Na): 776.2234; found: 776.2246.

13: ^{1}H NMR (CD_3OD): $\delta = 4.96$ (d, 1H, $J = 4.0$ Hz, H-1′), 4.33 (d, 1H, $J =$ 7.3 Hz, H-1), 4.27 (t, 1H, $J = 6.6$ Hz, H-5′), 4.12 (d, 1H, $J = 1.9$ Hz, H-4′), 4.08 – 3.99 (m, 2H, H-4, OCH_2CH$_2$S), 3.89 – 3.47 (m, 15H), 3.27 (s, 2H,

SCH_2COOMe), 2.89 (t, 2H, J = 6.8 Hz, OCH$_2$CH_2S), 2.72 – 2.63 (m, 3H, OCH$_2$CH$_2$CH_2S, SCH), 2.00 (m, 2H, CH$_2$), 1.88 (m, 2H, OCH$_2$CH_2CH$_2$S), 1.76 (m, 2H, CH$_2$), 1.62 (m, 1H, CH$_2$), 1.40 – 1.25 (m, 5H, CH$_2$) ppm; HRMS calcd for $C_{26}H_{46}O_{13}S_2Na$ (M+Na): 653.2278; found: 653.2285.

14: ^{1}H NMR (CD$_3$OD): δ = 7.88 (m, 1H, Ar–H), 7.51 (m, 2H, Ar–H), 7.19 (m, 1H, Ar–H), 4.97 (d, 1H, J = 3.9 Hz, H-1′), 4.33 (d, 1H, J = 7.3 Hz, H-1), 4.28 (t, 1H, J = 6.4 Hz, H-5′), 4.13 (d, 1H, J = 2.0 Hz, H-4′), 4.05 (dt, 1H, J = 6.7, 10.4 Hz, OCH_2CH$_2$S), 4.01 (d, 1H, J = 2.6 Hz, H-4), 3.91 – 3.48 (m, 18H), 3.37 (s, 2H, SCH_2COOMe), 3.12 (t, 2H, J = 6.7 Hz, OCH$_2$CH$_2$CH_2S), 2.88 (t, 2H, J = 6.8 Hz, OCH$_2$CH_2S), 1.99 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{28}H_{42}O_{15}S_2Na$ (M+Na): 705.1863; found: 705.1874.

15: ^{1}H NMR (CD$_3$OD): δ = 4.97 (d, 1H, J = 4.0 Hz, H-1′), 4.32 (d, 1H, J = 7.3 Hz, H-1), 4.27 (t, 1H, J = 6.5 Hz, H-5′), 4.12 (d, 1H, J = 2.3 Hz, H-4′), 4.05 (m, 1H, OCH_2CH$_2$S), 4.01 (d, 1H, H-4), 3.89 – 3.47 (m, 18H), 3.38 (s, 2H, SCH_2COOMe), 3.30 (s, 2H, SCH$_2$COOMe), 2.89 (t, 2H, J = 6.8 Hz, OCH$_2$CH_2S), 2.79 (t, 2H, J = 7.1 Hz, OCH$_2$CH$_2$CH_2S), 1.91 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{23}H_{40}O_{15}S_2Na$ (M+Na): 643.1706; found: 643.1716.

16: ^{1}H NMR (CD$_3$OD): δ = 4.96 (d, 1H, J = 4.0 Hz, H-1′), 4.63 (m, 1H, SCH$_2$CH(NHAc)), 4.33 (d, 1H, J = 7.3 Hz, H-1), 4.27 (t, 1H, J = 6.3 Hz, H-5′), 4.12 (d, 1H, J = 2.0 Hz, H-4′), 4.05 (dt, 1H, J = 6.7, 10.4 Hz, OCH_2CH$_2$S), 4.00 (d, 1H, J = 2.7 Hz, H-4), 3.90 – 3.47 (m, 18H), 3.38 (s, 2H, SCH_2COOMe), 3.00 (ddd, 1H, J = 1.9, 5.2, 13.8 Hz, SCH_2CH(NHAc)), 2.91 – 2.81 (m, 3H, OCH$_2$CH_2S, SCH_2CH(NHAc)), 2.71 (m, 2H, OCH$_2$CH$_2$CH_2S), 2.00 (s, 3H, Ac), 1.89 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{26}H_{45}O_{16}NS_2Na$ (M+Na): 714.2077; found: 714.2091.

17: ^{1}H NMR (CD$_3$OD): δ = 4.96 (d, 1H, J = 4.0 Hz, H-1′), 4.65 (m, 1H, CH(NHAc)), 4.33 (d, 1H, J = 7.2 Hz, H-1), 4.27 (t, 1H, J = 6.1 Hz, H-5′), 4.13 (d, 1H, J = 2.2 Hz, H-4′), 4.06 – 4.00 (m, 2H, H-4, OCH_2CH$_2$S), 3.89 – 3.47 (m, 15H), 3.08 (ddd, 1H, J = 1.9, 7.5, 21.4 Hz, SCH_2CH(NHAc)), 2.90 (dd, 1H, J = 8.4, 13.8 Hz, SCH_2CH(NHAc)), 2.80 (m, 2H, OCH$_2$CH_2S), 2.68 (m, 3H, SCH, OCH$_2$CH$_2$CH_2S), 2.00 (m, 5H, CH$_2$, OMe), 1.88 (m, 2H, OCH$_2$CH_2CH$_2$S), 1.76 (m, 2H, CH$_2$), 1.62 (m, 1H, CH$_2$), 1.37 – 1.25 (m, 5H, CH$_2$) ppm; HRMS calcd for $C_{29}H_{51}O_{14}NS_2Na$ (M+Na): 724.2649; found: 724.2659.

18: ^{1}H NMR (CD$_3$OD): δ = 7.89 (m, 1H, Ar–H), 7.51 (m, 2H, Ar–H), 7.20 (m, 1H, Ar–H), 4.97 (d, 1H, J = 4.0 Hz, H-1′), 4.65 (m, 1H, SCH$_2$CH(NHAc)), 4.33 (d, 1H, J = 7.1 Hz, H-1), 4.27 (t, 1H, J = 6.0 Hz, H-5′), 4.13 (d, 1H, J = 2.1 Hz, H-4′), 4.06 – 3.99 (m, 2H, H-4, OCH_2CH$_2$S), 3.88 – 3.50 (m, 18H), 3.15 – 3.06 (m, 3H, OCH$_2$CH$_2$CH_2S, SCH_2CH(NHAc)), 2.89 (ddd, 1H, J = 1.9, 8.3, 13.8 Hz, SCH$_2$CH(NHAc)), 2.80 (m, 2H, OCH$_2$CH_2S), 2.00 (m, 5H, Ac, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{31}H_{47}O_{16}S_2Na$ (M+Na): 776.2234; found: 776.2236.

19: ^{1}H NMR (CD$_3$OD): δ = 4.96 (d, 1H, J = 4.1 Hz, H-1′), 4.65 (m, 1H, SCH$_2$CH(NHAc)), 4.33 (d, 1H, J = 7.1 Hz, H-1), 4.26 (t, 1H, J = 5.7 Hz, H-5′), 4.12 (d, 1H, J = 2.4 Hz, H-4′), 4.06 – 3.99 (m, 2H, OCH_2CH$_2$S, H-4), 3.88 – 3.48 (m, 18H), 3.30 (s, 2H, SCH_2COOMe), 3.09 (dd, 1H, J = 5.2, 14.1 Hz, SCH_2CH(NHAc)), 2.89 (m, 1H, SCH_2CH(NHAc)), 2.83 – 2.77 (m, 4H, OCH$_2$CH$_2$CH_2S, OCH$_2$CH_2S), 2.00 (s, 3H, Ac), 1.91 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{26}H_{45}O_{16}NS_2Na$ (M+Na): 714.2077; found: 714.2091.

20: ^{1}H NMR (CD$_3$OD): δ = 4.96 (d, 1H, J = 4.0 Hz, H-1′), 4.64 (m, 2H, SCH$_2$CH(NHAc)), 4.33 (d, 1H, J = 7.2 Hz, H-1), 4.26 (t, 1H, J = 6.0 Hz, H-5′), 4.12 (d, 1H, J = 2.2 Hz, H-4′), 4.06 – 3.99 (m, 2H, OCH_2CH$_2$S, H-4), 3.88 – 3.48 (m, 18H), 3.09 (dd, 1H, J = 5.3, 13.9 Hz, SCH_2CH(NHAc)), 2.92 – 2.89 (m, 4H, SCH_2CH(NHAc), OCH$_2$CH_2S), 2.71 (m, 2H, OCH$_2$CH$_2$CH_2S), 2.01 (s, 6H, Ac), 1.89 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{29}H_{50}O_{17}N_2S_2Na$ (M+Na): 785.2449; found: 785.2446.

21: ^{1}H NMR (CD$_3$OD): δ = 7.30 (m, 2H, Ar–H), 6.74 (m, 2H, Ar–H), 4.96 (d, 1H, J = 4.1 Hz, H-1′), 4.26 (m, 2H, H-1, H-5′), 4.11 (m, 1H, H-4′), 3.99 (m, 1H, H-4), 3.89 – 3.47 (m, 13H), 3.01 (t, 2H, J = 7.2 Hz, OCH$_2$CH_2S), 2.67 (m, 3H, OCH$_2$CH$_2$CH_2S, CHS), 1.97 (m, 2H, CH$_2$), 1.87 (m, 2H, OCH$_2$CH_2CH$_2$S), 1.76 (m, 2H, CH$_2$), 1.62 (m, 1H, CH$_2$), 1.37 – 1.25 (m, 5H, CH$_2$) ppm; HRMS calcd for $C_{29}H_{46}O_{12}S_2Na$ (M+Na): 673.2328; found: 673.2333.

22: ^{1}H NMR (CD$_3$OD): δ = 7.88 (m, 1H, Ar–H), 7.50 (m, 2H, Ar–H), 7.30 (m, 2H, Ar–H), 7.19 (m, 1H, Ar–H), 6.74 (m, 2H, Ar–H), 4.96 (d, 1H, J = 4.0 Hz, H-1′), 4.27 (m, 2H, H-1, H-5′), 4.13 (d, 1H, J = 2.1 Hz, H-4′), 4.99 (d, 1H, J = 2.3 Hz, H-4), 3.94 – 3.45 (m, 16H), 3.12 (dt, 2H, J = 1.6, 7.6 Hz, OCH$_2$CH$_2$CH_2S), 3.01 (t, 2H, J = 7.3 Hz, OCH$_2$CH_2S), 1.99 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{31}H_{42}O_{14}S_2Na$ (M+Na): 725.1914; found: 725.1906.

23: ^{1}H NMR (CD$_3$OD): δ = 7.31 (m, 2H, Ar–H), 6.74 (m, 2H, Ar–H), 4.96 (d, 1H, J = 3.9 Hz, H-1′), 4.26 (m, 2H, H-1, H-5), 4.12 (d, 1H, J = 2.0 Hz, H-4′), 3.99 (d, 1H, J = 2.3 Hz, H-4), 3.89 – 3.50 (m, 16H), 3.29 (s, 2H, SCH_2COOMe), 3.01 (t, 2H, J = 7.8 Hz, OCH$_2$CH_2S), 2.78 (dt, 2H, J = 1.3, 7.4 Hz, OCH$_2$CH$_2$CH_2S), 1.88 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{26}H_{40}O_{14}S_2Na$ (M+Na): 663.1757; found: 663.1771.

24: ^{1}H NMR (CD$_3$OD): δ = 7.31 (m, 2H, Ar–H), 6.75 (m, 2H, Ar–H), 4.96 (d, 1H, J = 3.9 Hz, H-1′), 4.63 (m, 1H, SCH$_2$CH_2(NHAc)COOMe), 4.26 (m, 2H, H-1, H-5′), 4.12 (d, 1H, J = 2.2 Hz, H-4′), 3.99 (d, 1H, J = 2.5 Hz, H-4), 3.93 (dt, 1H, J = 7.2, 10.2 Hz, OCH_2CH$_2$S), 3.89 – 3.45 (m, 15H), 3.02 (m, 3H, OCH$_2$CH_2S, SCH_2CH(NHAc)COOMe), 2.85 (ddd, 1H, J = 1.4, 8.1, 13.6 Hz, SCH_2CH(NHAc)COOMe), 2.72 (m, OCH$_2$CH$_2$CH_2S), 2.00 (s, 3H, OMe), 1.91 (m, 2H, OCH$_2$CH_2CH$_2$S) ppm; HRMS calcd for $C_{29}H_{45}O_{15}NS_2Na$ (M+Na): 734.2128; found: 734.2130.

General procedures for preparation of galabioside collection B: Bu_2SnO (36 mg, 0.144 mmol) was added to compound **1** (100 mg, 0.132 mmol) in benzene (12 mL). Benzene (10 mL) was removed by distillation followed by reflux overnight then the appropriate alkyl bromide (0.66 mmol) and Bu_4NBr (22 mg, 0.066 mmol) were added. After 5 h, the reaction was quenched by addition of MeOH (1 mL) and the mixture was concentrated and flash chromatographed (SiO_2, toluene/acetone) to give the protected intermediate, which was deacylated in methanolic NaOMe (0.01 M, 5 mL). Methanolic acetic acid (10%) was added until a neutral result was seen on moist pH paper. The resulting mixture was concentrated and flash chromatographed (SiO_2, CH_2Cl_2/MeOH) to give compounds **25** – **30**.

25: ^{1}H NMR (CD$_3$OD): δ = 7.46 – 7.25 (m, 5H, Ar–H), 7.05 (m, 2H, Ar–H), 6.87 (m, 2H, Ar–H), 5.00 (d, 1H, J = 3.9 Hz, H-1′), 4.82 (d, 1H, J = 7.5 Hz, H-1), 4.76/4.68 (q, 2H, J_{AB} = 11.3, 33.9 Hz, CH$_2$), 4.27 (t, 1H, J = 6.4 Hz, H-5′), 4.13 (d, 1H, J = 2.2 Hz, H-4′), 4.07 (d, 1H, J = 3.0 Hz, H-4), 3.99 (dd, 1H, J = 3.9, 10.2 Hz, H-2′), 3.85 – 3.63 (m, 11H) ppm; HRMS calcd for $C_{26}H_{34}O_{12}Na$ (M+Na): 561.1998; found: 561.1957.

26: ^{1}H NMR (CD$_3$OD): δ = 7.05 (m, 2H, Ar–H), 6.85 (m, 2H, Ar–H), 6.00 (m, 1H, =CH), 5.34 (ddd, 1H, J = 1.7, 3.4, 17.3 Hz, H_2C=), 5.16 (ddd, 1H, J = 1.3, 3.1, 10.4 Hz, H_2C=), 5.02 (d, 1H, J = 3.9 Hz, H-1′), 4.81 (d, 1H, J = 7.5 Hz, H-1), 4.30 (t, 1H, J = 7.5 Hz, H-5′), 4.26 (ddt, 1H, J = 1.4, 5.7, 12.8 Hz, =CH–CH$_2$), 4.17 – 4.12 (m, 2H, H-4′, =CH–CH_2), 4.07 (d, 1H, J = 2.9 Hz, H-4), 3.92 (dd, 1H, J = 3.9, 10.2 Hz, H-2′), 3.90 – 3.65 (m, 11H) ppm; HRMS calcd for $C_{22}H_{32}O_{12}Na$ (M + Na): 511.1791; found: 511.1812.

27: ^{1}H NMR (CD$_3$OD): δ = 8.39 (m, 1H, Ar–H), 8.15 (m, 1H, Ar–H), 7.88 (m, 1H, Ar–H), 7.59 (t, 1H, Ar–H), 7.06 (m, 2H, Ar–H), 6.85 (m, 2H, Ar–H), 5.06 (d, 1H, J = 4.0 Hz, H-1′), 4.83 (d, 1H, J = 7.5 Hz, H-1), 4.91/4.79 (q, 2H, J_{AB} = 16.6, 48.0 Hz, CH$_2$), 4.33 (t, 1H, J = 6.4 Hz, H-5′), 4.23 (d, 1H, J = 2.1 Hz, H-4′), 4.09 (d, 1H, J = 2.8 Hz, H-4), 4.05 (dd, 1H, J = 3.9, 10.2 Hz, H-2′), 3.85 – 3.63 (m, 11H) ppm; HRMS calcd for $C_{26}H_{33}O_{14}NNa$ (M+Na): 606.1799; found: 606.1799.

28: ^{1}H NMR (CD$_3$OD): δ = 7.05 (m, 2H, Ar–H), 6.84 (m, 2H, Ar–H), 5.01 (d, 1H, J = 4.0 Hz, H-1′), 4.81 (d, 1H, J = 7.5 Hz, H-1), 4.41/4.35 (q, 2H,

J_{AB} = 16.8, 28.3 Hz, CH_2), 4.29 (m, 1H, H-5′), 4.15 (dd, 1H, J = 1.2, 3.1 Hz, H-4′), 4.05 (d, 1H, J = 3.1 Hz, H-4), 3.99 (dd, 1H, J = 3.9, 10.2 Hz, H-2′), 3.85 – 3.63 (m, 14H) ppm; HRMS calcd for $C_{22}H_{32}O_{14}Na$ (M+Na): 543.1690; found: 543.1697.

29: ^{1}H NMR (CD_3OD): δ = 7.04 (m, 2H, Ar–H), 6.87 (m, 2H, Ar–H), 4.97 (d, 1H, J = 3.9 Hz, H-1′), 4.78 (d, 1H, J = 7.4 Hz, H-1), 4.28 (t, 1H, J = 6.1 Hz, H-5′), 4.14 (d, 1H, J = 2.4 Hz, H-4′), 4.12/4.07 (q, 2H, J_{AB} = 15.7, 25.9 Hz, CH_2), 4.03 (m, 1H, H-4), 3.94 (dd, 1H, J = 3.8, 10.3 Hz, H-2′), 3.79 – 3.66 (m, 14H) ppm; HRMS calcd for $C_{21}H_{29}O_{14}Na_2$ (M – H+2Na): 551.1353; found: 551.1343.

30: ^{1}H NMR (CD_3OD): δ = 7.05 (m, 2H, Ar–H), 6.84 (m, 2H, Ar–H), 5.01 (d, 1H, J = 3.9 Hz, H-1′), 4.81 (d, 1H, J = 7.5 Hz, H-1), 4.76/4.36 (q, 2H, J_{AB} = 18.4, 59.4 Hz, $OCH_2C(O)$), 4.27 (t, 1H, J = 5.8 Hz, H-5′), 4.06 (m, 2H, H-4′, H-4), 3.99 (dd, 1H, J = 4.0, 10.2 Hz, H-2′), 3.88 (m, 1H), 3.81 – 3.69 (m, 8H), 3.61 (m, 2H, H-3′, H-5), 2.02 (m, 3H, CH_2), 1.88 (m, 6H, CH_2), 1.77 (m, 6H, CH_2) ppm; HRMS calcd for $C_{31}H_{44}O_{13}Na$ (M+Na): 647.2680; found: 647.2686.

Ethyl (3-*O*-propyl-α-D-galactopyranosyl)-(1-4)-β-D-galactopyranoside (31): Compound **4** (50 mg, 0.054 mmol) was dissolved in MeOH (4.0 mL) and 0.3 M aqueous KOH (4.0 mL) and hydrogenolysed (H_2, 10% Pd–C, 0.025 g) for 5 h. The mixture was neutralised with methanolic acetic acid (10%), filtered through Celite, evaporated and flash chromatographed (SiO_2, 60:10:0 – 66:33:4 CH_2Cl_2/MeOH/H_2O gradient) to give **31** (17 mg, 77%). $[\alpha]_D^{23}$: +59 (c = 0.8, MeOH); ^{1}H NMR (CD_3OD): δ = 4.97 (d, 1H, J = 3.8 Hz, H-1′), 4.27 (m, 2H, H-1, H-5′), 4.11 (d, 1H, J = 2.4 Hz, H-4′), 4.01 (d, 1H, J = 2.6 Hz, H-4), 3.95 (m, 1H, OCH_2CH_3), 3.85 (m, 2H, H-2′), 3.77 – 3.46 (m, 10H), 1.65 (m, 2H, $OCH_2CH_2CH_3$), 1.25 (t, 3H, J = 7.1 Hz, OCH_2CH_3), 0.97 (t, 3H, J = 7.4 Hz, $OCH_2CH_2CH_3$) ppm; ^{13}C NMR (CD_3OD): δ = 105.3, 102.9, 79.9, 79.3, 76.5, 75.1, 73.2, 73.0, 72.9, 70.4, 68.4, 67.0, 63.1, 61.3, 24.5, 15.9, 11.3 ppm; HRMS calcd for $C_{17}H_{32}O_{11}Na$ (M+Na): 435.1842; found: 453.1845.

p-Methoxyphenyl α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside (32): Methanolic NaOMe (1.0 mL, 1 M) was added to compound **1** (8.20 g, 11.0 mmol) in MeOH (200 mL) and the resulting mixture was stirred overnight. Methanolic acetic acid (10%) was added until a neutral result was seen on moist pH paper. The resulting mixture was concentrated and flash chromatographed (SiO_2, 5:1:0.1 – 2:1:0.1 CH_2Cl_2/MeOH/H_2O) to give **32** (4.63 g, 95%). $[\alpha]_D^{23}$: +38 (c = 0.8, MeOH); ^{1}H NMR (CD_3OD): δ = 7.05 (m, 2H, Ar–H), 6.85 (m, 2H, Ar–H), 5.00 (m, 1H, H-1′), 4.82 (d, 1H, J = 7.5 Hz, H-1), 4.32 (t, 1H, J = 6.0 Hz, H-5′), 4.06 (d, 1H, J = 3.0 Hz, H-4), 3.94 (d, 1H, H-4), 3.88 – 3.63 (m, 12H) ppm; ^{13}C NMR (CD_3OD): δ = 156.9, 153.1, 119.4, 115.6, 104.1, 102.8, 79.2, 76.4, 74.8, 72.9, 72.8, 71.5, 71.2, 71.0, 62.8, 61.1, 56.2 ppm; HRMS calcd for $C_{19}H_{28}O_{12}Na$ (M + Na): 471.1478; found: 471.1464.

Hemagglutination: The hemagglutination assays were performed as previously described.[13] Briefly, cultures grown overnight on TYS agar plates (with appropriate antibiotic) were resuspended in phosphate buffered saline (PBS, pH 7.4, 5 mL) to an absorbance of 1.0 at 540nm. The bacterial cells were centrifuged (5000 rpm for 10 min) and resuspended in 1/10 volume of PBS. Suspended bacteria (25 μL) were serially diluted in PBS (25 μL) in a U-bottom 96-well microtiter plate. Human erythrocytes (serotype O, 25 μL), either heparin-treated or washed directly 3 – 5 times and resuspended in PBS to an absorbance of 1.9 at 640nm, were added to each well. The plates were incubated for 16 h at 4 °C, after which they were evaluated for hemagglutination. The first dilution that contained a pellet was the hemagglutination endpoint and was recorded as the inverse of the dilution.

Hemagglutination inhibition: The bacterial suspensions were diluted fourfold for HB101/pHMG93 and 32-fold for HB101/pDC1, that is, approximately 32-fold less than the dilution that results in their hemagglutination titration endpoints. The compounds (**5** – **33**) were serially diluted in PBS (25 μL) and incubated with diluted bacterial culture (25 μL) for 15 min at room temperature. Erythrocytes (25 μL) were added to each well and the plates were incubated at 4 °C for 16 h. The results were recorded as the first well that contained 50% hemagglutination, which was the first well that contained a pellet. The results are presented as an average of three separate experiments.

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[1] E. H. Beachey in *Bacterial Adherence. Receptors and Recognition*, *Vol. 8*, (Ed.: E. H. Beachey), Chapman and Hall, New York, **1980**.
[2] K.-A. Karlsson, *Curr. Opin. Struct. Biol.* **1995**, *5*, 622 – 635.
[3] D. Mirelman, I. Ofek, *Microbial Lectins and Agglutinins*, Wiley, New York, **1986**.
[4] H. Leffler, C. Svanborg-Edén, *FEMS Microbiol. Lett.* **1980**, *8*, 127 – 134.
[5] G. Källenius, R. Möllby, S. B. Svensson, J. Winberg, A. Lundblad, S. Svensson, B. Cedergren, *FEMS Microbiol. Lett.* **1980**, *7*, 297 – 302.
[6] K. Bock, M. E. Breimer, A. Brignole, G. C. Hansson, K.-A. Karlsson, G. Larson, H. Leffler, B. E. Samuelsson, N. Strömberg, C. Svanborg-Edén, J. Thurin, *J. Biol. Chem.* **1985**, *260*, 8545 – 8551.
[7] S. J. Hultgren, S. N. Abraham, M. Caparon, P. Falk, J. W. St Geme III, S. Normark, *Cell* **1993**, *73*, 887 – 901.
[8] N. Strömberg, B.-I. Marklund, B. Lund, D. Ilver, A. Hamers, W. Gaastra, K.-A. Karlsson, S. Normark, *EMBO J.* **1990**, *9*, 2001 – 2010.
[9] N. Strömberg, P.-G. Nyholm, I. Pascher, S. Normark, *Proc. Natl. Acad. Sci. USA.* **1991**, *88*, 9340 – 9344.
[10] I. M. Johanson, K. Plos, B.-I. Marklund, C. Svanborg, *Microb. Pathog.* **1993**, *15*, 121 – 129.
[11] G. Otto, T. Sandberg, B.-I. Marklund, P. Ulleryd, C. Svanborg, *Clin. Infect. Dis.* **1993**, *17*, 448 – 456.
[12] J. Kihlberg, S. J. Hultgren, S. Normark, G. Magnusson, *J. Am. Chem. Soc.* **1989**, *111*, 6364 – 6368.
[13] R. Striker, U. Nilsson, A. Stonecipher, G. Magnusson, S. J. Hultgren, *Mol. Microbiol.* **1995**, *16*, 1021 – 1029.
[14] U. Nilsson, R. T. Striker, S. J. Hultgren, G. Magnusson, *Bioorg. Med. Chem.* **1996**, *4*, 1809 – 1817.
[15] K. W. Dodson, J. S. Pinkner, T. Rose, G. Magnusson, S. J. Hultgren, G. Waksman, *Cell* **2001**, *105*, 733 – 743.
[16] U. J. Nilsson, E. J.-L. Fournier, O. Hindsgaul, *Bioorg. Med. Chem.* **1998**, *6*, 1563 – 1575.
[17] U. J. Nilsson, E. J.-L. Fournier, E. J. Fryz, O. Hindsgaul, *Comb. Chem. High Throughput Screening* **1999**, *2*, 335 – 352.
[18] A. Barkely, P. Arya, *Chem.-Eur. J.* **2001**, *7*, 555 – 563.
[19] T. Kanemitsu, O. Kanie, *Trends Glycosci. Glycotechnol.* **1999**, *11*, 267 – 276.
[20] J. Ohlsson, G. Magnusson, *Carbohydr. Res.* **2000**, *329*, 49 – 55.
[21] S. David, S. Hanessian, *Tetrahedron* **1985**, *41*, 643 – 663.
[22] J. Dahmén, T. Frejd, G. Magnusson, G. Noori, *Carbohydr. Res.* **1982**, *111*, C1 – C4.
[23] J. Dahmén, T. Frejd, G. Grönberg, T. Lave, G. Magnusson, G. Noori, *Carbohydr. Res.* **1983**, *118*, 293 – 301.
[24] K. Bock, T. Frejd, J. Kihlberg, G. Magnusson, *Carbohydr. Res.* **1988**, *176*, 253 – 270.
[25] U. Nilsson, R. Johansson, G. Magnusson, *Chem.-Eur. J.* **1996**, *2*, 295 – 302.
[26] M. Wilstermann, J. Balogh, G. Magnusson, *J. Org. Chem.* **1997**, *62*, 3659 – 3665.
[27] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmén, G. Noori, K. Stenvall, *J. Org. Chem.* **1988**, *53*, 5629 – 5647.
[28] M. Båga, M. Göransson, S. Normark, B.-E. Uhlin, *Cell* **1988**, *52*, 197 – 206.
[29] S. Clegg, *Infect. Immun.* **1982**, *38*, 739 – 744.

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