Synthesis, Conformational Analysis and Comparative Protein Binding of a Galabioside and Its Thioglycoside Analogues

Ulf Nilsson, Roger Johansson and Göran Magnusson*

Abstract: The two thio analogues (**2** and **3**) of TMSEt galabioside [2-(trimethylsilyl)ethyl 4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside, **1**], having anomeric sulfur instead of anomeric oxygen atoms, were synthesized and their conformations investigated by NMR and computational (MM3) methods. A spacer galabioside was covalently coupled to aminated microtiter plates, and binding of a bacterial pilus adhesin (PapG) to the plates was inhibited by the soluble ligands **1**, **2** and **3**. The ligand **2**, which has an intersaccharidic sulfur linkage, was a much less efficient inhibitor than **1**, which has the natural oxygen linkage. The inhibitory power of ligand **3** was only slightly less than that of **1**. An NMR experiment with **1** and **2**, in which hydroxyl-group hydrogens had been partially (50%) substituted by deuterium, demonstrated the presence (in **1**) and absence (in **2**) of an intramolecular (HO2′–HO6) hydrogen bond. This result indicates that the conformations of **1** and **2** are different and that the difference is sufficient to cause the observed (≈30 times) reduction of the saccharide–protein binding strength.

Keywords
conformational analysis · galabioside · hydrogen bonds · protein recognition · thioglycosides

Introduction

Glycolipids of the globo series[1] (Fig. 1) are natural saccharide ligands for various receptor proteins (e.g., bacterial and viral lectins, toxins and antibodies).[2] The virulence and organ tropism of uropathogenic *E. coli* is a thoroughly studied example, and it is thought that bacterial attachment is a prerequisite for infectivity.[3] Galabiose (α-D-Gal*p*-(1 → 4)-β-D-Gal*p*) is the smallest saccharide moiety to retain affinity towards several of these proteins. It constitutes a bent knee in the larger saccharides of the globo series, and it seems reasonable that the exposed convex side is the preferential epitope recognized by the proteins.

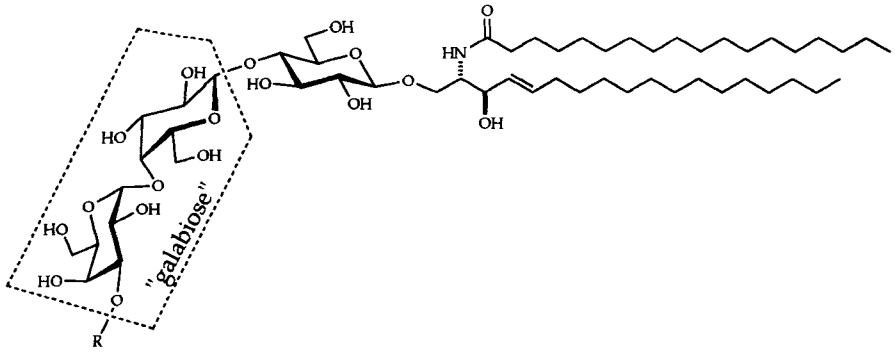

Fig. 1. Glycolipids of the globo series. R = H: globotriosyl ceramide (GbO_3); R = β-D-GalNAc: globotetraosyl ceramide (GbO_4); R = α-D-GalNAc-(1 → 3)-β-D-GalNAc: Forssman glycolipid; R = β-D-Gal-(1 → 3)-β-D-GalNAc: galactosylgloboside.

We have synthesized a large number of saccharides corresponding to the globo series of glycolipids (all di- to pentasaccharide fragments of the Forssman saccharide,[4] as well as all monodeoxy analogues of galabiose,[5] lactose[6] and globotriose[7]) and analyzed their conformational behaviour by NMR and computer simulations.[8] Each intersaccharidic Φ/Ψ angle was virtually the same in all the energy-minimized compounds; this shows that these structural alterations only had a minor influence on the overall conformations.

Several of the saccharides mentioned above were used as inhibitors in mapping of the receptor sites of four proteins with lectin activity, namely, the pilus-associated $PapG_{J96}$ adhesin of class I *E. coli*,[9] the $PapG_{AD110}$ adhesin of class II *E. coli*,[10] the surface lectin of *S. suis*[11] and verotoxin of *E. coli*.[12] It was thus demonstrated that the two bacterial species use different galabiose-related epitopes for their attachment. In the case of the $PapG_{J96}$ and $PapG_{AD110}$ adhesins, the four hydroxyls HO6, HO2′, HO4′ and HO6′ and the oxygen O3′ of the galabioside moiety were found to be essential for bindning, probably owing to the formation of intermolecular hydrogen bonds in the saccharide–protein complex.

The intersaccharidic oxygen atom (O1′) is buried in the concave side of the galabioside structure and is probably not in-

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volved in hydrogen bonding to the various proteins mentioned above. This is supported by the fact that in the larger saccharides (GbO_3 and GbO_4), the oxygen atom is even less accessible, but the binding strength is retained or even increased. Substituting the intersaccharidic oxygen in galabiosides by a sulfur atom would change the overall environment of the hydrogen-bonding hydroxyl groups slightly. The question that we want to address is whether such minor conformational changes would be sufficient for a significant change in binding strength to the $PapG_{J96}$ adhesin.

We now report the synthesis of two thiodisaccharide analogues (**2** and **3**) of the galabioside **1** (Fig. 2) and an investigation into their conformations and ability to inhibit the binding between the $PapG_{J96}$ adhesin and the galabioside **14** immobilized on microtiter plates.

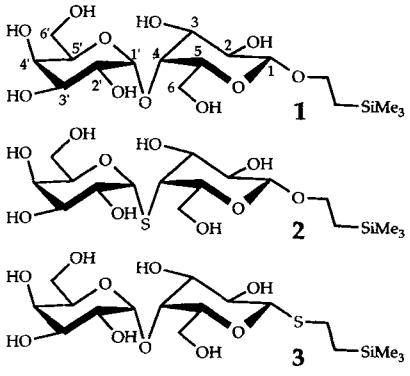


Fig. 2. TMSEt galabioside **1** and its thioanalogues **2** and **3**.

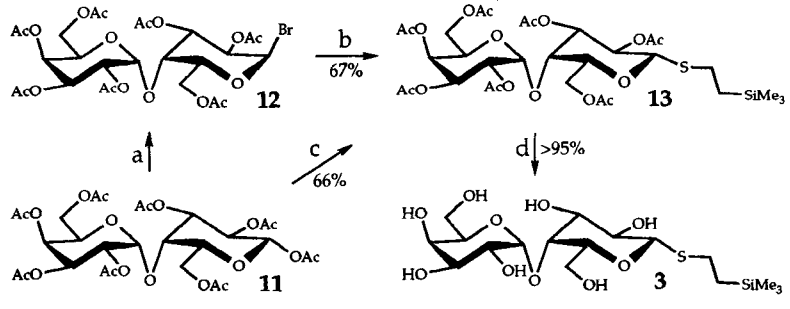
Results

Synthesis of the thiogalabiosides 2 and 3: Disaccharide analogues with an interglycosidic sulfur linkage have been synthesized by S_N2-type displacements with sulfur-containing sugars as nucleophiles. This area of carbohydrate chemistry has been investigated to a large extent by the group of Defaye, and a review of this and other work has appeared.[13] Recent examples of this reaction type show the unexpected formation of an α-thioglycoside, despite the use of a glycosyl donor having a participating acetate group in the 2-position,[14] and of a diglucoside in 87% yield, by displacement of a triflate ion from 1,3,4,6-tetra-*O*-acetyl-2-*O*-triflyl-β-D-mannopyranose by 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranose, induced by sodium hydride.[15]

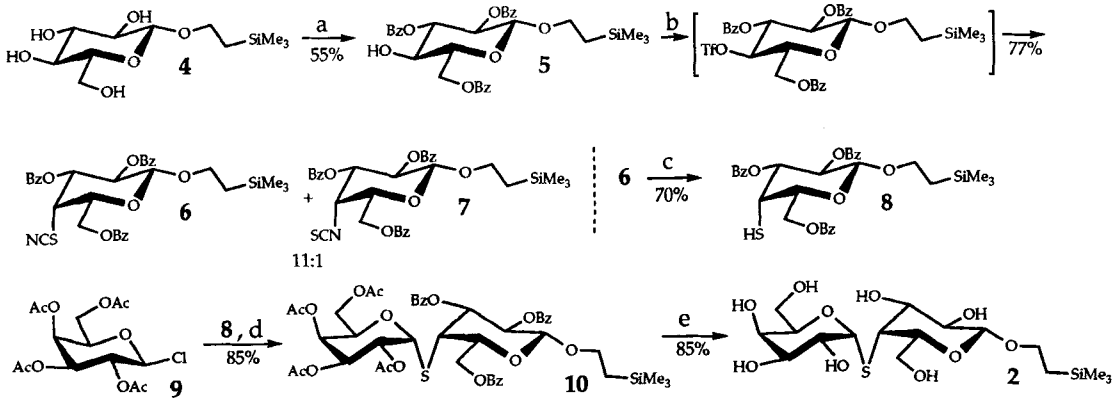
In the synthesis of **2**, the 2-(trimethylsilyl)ethyl (TMSEt) group was chosen for anomeric protection, because it is easily removed and transformed stereoselectively into a 1-O-acyl [16] or 1-chloro group,[17] thereby permitting further anomeric functionalization. The known TMSEt glucoside **4**[16] was regioselectively benzoylated with benzoyl chloride at low temperature. A mixture was obtained with the desired tribenzoate **5** as the major product (Scheme 1). Treatment of **5** with trifluoromethanesulfonic anhydride and addition of potassium thiocyanate to the crude 4-*O*-triflate gave the thiocyanate **6** (70%) and the isothiocyanate **7** (7%). The CN signals in the ^{13}C NMR spectra of **6** and **7** (at $\delta = 110.9$ and 140.2, respectively) are in accord with the thiocyanate and isothiocyanate structures, respectively.[18] Reduction of the thiocyanate group in **6** with zinc in acetic acid gave the thiol **8** (70%), ready for glycosylation.

Glycosylation of **8** with the galactosyl β-chloride[19] **9** was performed in the presence of caesium carbonate,[20] and the reaction seems to proceed by direct S_N2 displacement of chloride ion. The slightly basic conditions thus prevent the formation of an intermediate acetoxonium ion (which is an accepted intermediate towards β-glycosides), and the pure α-thioglycoside **10** (85%) was obtained. Attempted glycosylation of **8** with **9** failed to produce **10** when sodium hydride was used as base instead of caesium carbonate. The acetyl and benzoyl protecting groups in **10** were removed by treatment with methanolic sodium methoxide, which furnished the desired thiogalabioside **2** (85%).

Thioglycoside **3** was prepared from galabiose octaacetate (**11**), either directly or via the bromosugar **12**.[21] Thus, treatment of **12** with 2-(trimethylsilyl)ethanethiol and sodium hydride furnished the thioglycoside **13** (67%) (note that sodium hydride was successfully employed here, in contrast to the reaction with the β-chlorosugar **9** above). Alternatively, **13** was formed (66%) when the octaacetate **11** was treated with (trimethylsilyl)ethanethiol and borontrifluoride etherate.[22] Removal of the protecting groups in **13** gave the thiogalabioside **3** in quantitative yield (Scheme 2).



Scheme 2. a) HBr, HOAc, Ac_2O; see ref. [21]. b) $HSCH_2CH_2SiMe_3$, NaH, DMF, 22°C. c) $HSCH_2CH_2SiMe_3$, BF_3OEt_2, CH_2Cl_2, 22°C. d) MeONa, MeOH.



Scheme 1. a) BzCl, CH_2Cl_2, pyridine, −78°C → −50°C. b) $(F_3CSO_2)_2O$, CH_2Cl_2, pyridine, 0°C, then KSCN, DMF, 90°C. c) Zn, HOAc, reflux. d) Cs_2CO_3, DMF, 22°C. e) MeONa, MeOH.

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Conformational analysis of 1 and 2: The conformations of galabiosides, as well as larger saccharides where galabiose is an integral part (cf. Fig. 1), have been thoroughly investigated by NMR and computational methods.[8] It was found that the overall galabiose conformations were practically the same in all the structures, including deoxygalabiosides. The H5′ NMR signal of galabiosides was found to be a useful conformational probe, since H5′ is deshielded by its van der Waals contact with O3. The chemical shift of H5′ is $\delta \approx 4.4$ in galabiosides and ≈ 3.9 in galactosides or 3-deoxygalabiosides.[8]

An NMR investigation of the thiogalabioside **2** showed that its overall conformation is similar to that of the parent compound **1**, although the energy minimum of **2** is less distinct than that of **1** (Fig. 3). The chemical shifts and coupling constants (Table 1) for the ring protons of both compounds are very similar, revealing that the normal pyranoside $^4C_1$ conformation is maintained in **2**. Significant deviations are found for δ_{H4}, $\delta_{H1'}$, $\delta_{H2'}$, $J_{H3,4}$ and $J_{H1',2'}$, which reflect the expected influence of the sulfur atom. The $J_{5,6}$ values ($\approx 5-7$ Hz) are similar for both **1** and **2** and little information is obtained regarding the torsional angle O5-C5-C6-O6, other than that all conformations, including the *gauche–gauche* conformation (gg: torsional angle O5-C5-C6-O6 = 60 and C4-C5-C6-O6 = 60°) are possible for both compounds. Furthermore, H5′ of **2** is strongly deshielded ($\delta = 4.41$) by its van der Waals contact with O3, as is normally the case in galabiosides,[8] and strong NOE's are found for H4 on irradiation of H1′, and vice versa.

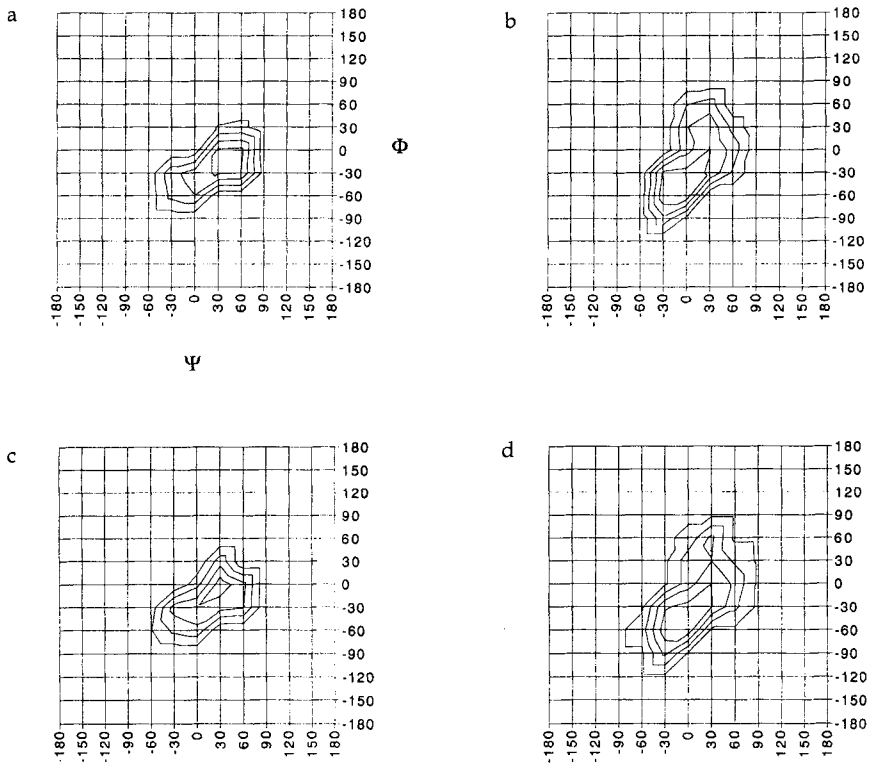

Fig. 3. Calculated energy diagrams [24] showing the Φ/Ψ angles of the various conformations of Galα4Gal and GalSα4Gal. Spacing between equi-energetic curves corresponds to 4.187 kJ mol^{-1}. a) Galα4Gal-gg; b) GalSα4Gal-gg; c) Galα4Gal-tg; d) GalSα4Gal-tg.

Table 1. ^{1}H NMR data [a] for compounds **1** and **2**.

Proton	Chemical shift (δ)		^3J(H,H)	Coupling constant	
	1	**2**		**1**	**2**
H1	4.47	4.40	J(1,2)	7.8	7.7
H2	3.52	3.25	J(2,3)	10.2	10.0
H3	3.71	3.92	J(3,4)	3.4	4.7
H4	4.03	3.39	J(4,5)	1.0	<1
H5	3.74	3.88	J(5,6a)	6.6	4.8
H6a	3.83	3.94	J(5,6b)	6.6	5.0
H6b	3.88	3.85	J(6a,6b)	11.8	8.6
H1′	4.97	5.40	J(1′,2′)	3.9	5.7
H2′	3.83	4.12	J(2′,3′)	10.5	10.4
H3′	3.91	3.76	J(3′,4′)	3.3	3.3
H4′	4.03	4.01	J(4′,5′)	1.0	<1
H5′	4.36	4.41	J(5′,6a′)	6.1	5.5
H6a′	3.70	3.74	J(5′,6b′)	6.9	5.5
H6b′	3.70	3.74	J(6a′,6b′)	nd [b]	nd

[a] Measured at 500 MHz in D_2O; data for compound **1** are taken from ref. [8b]. [b] nd, not determined.

The strong deshielding of H5′ in both **1** and **2** requires that the H5′–O3 distance be <3 Å in both compounds.[8] This was used as a conformational constraint in the analysis (see below) of whether an intramolecular hydrogen bond between HO2′ and HO6 exists in the two compounds.

The conformations of the disaccharides Galα4Gal (4-*O*-α-D-galactopyranosyl-β-D-galactopyranose) and GalSα4Gal (4-*S*-α-D-galactopyranosyl-4-thio-β-D-galactopyranose), corresponding to **1** and **2**, repectively, were calculated by molecular mechanics[23] [MM3(92)].[24] It is obvious from molecular modelling that the *gauche–gauche* and *trans–gauche* conformations (tg: torsional angle O5-C5-C6-O6 = 180°) are potentially amenable to intramolecular hydrogen-bond formation between HO2′ and HO6, whereas, in the gt conformation (torsional angle C4-C5-C6-O6 = 180°), HO6 is placed too far away from HO2′ (cf. Fig. 3 and 4, Table 2). Therefore, only the gg and tg conformations were used as input structures in the calculations. In order to obtain conformational energies that are directly comparable to each other, all hydroxyl-group protons were placed so as to avoid intramolecular hydrogen bonds. The calculations (dielectric constant setting: 80.0) were performed by varying the intersaccharidic glycosidic bond angles Φ and Ψ (30° increments, using the dihedral-driver option of the MM3 program[24]), thereby creating 144 conformations for each starting structure. The energy maps for the four calculations are shown in Figure 3 and a least-squares fit of low-energy conformations in Figure 4, using all ring atoms of the Galα moiety. The energy minimum of GalSα4Gal (cf. **2**) is less well defined than that of Galα4Gal (cf. **1**), which is not surprising since bond lengths are different (see below) and the anomeric effect is less pronounced in thioglycosides.[25]

The O2′–O6 and H5′–O3 distances (hydrogen bonding and ^{1}H NMR deshielding sites, respectively) were measured in the calculated conformations having total energies from 0 to 20 kJ mol^{-1} relative to the minumum-energy conformations (Table 2). Thus, only gg conformations of the Galα4Gal structure combines the required short distance ($< \approx 2.8$ Å) between H5′ and O3 with a distance between O2′ and O6 that allows

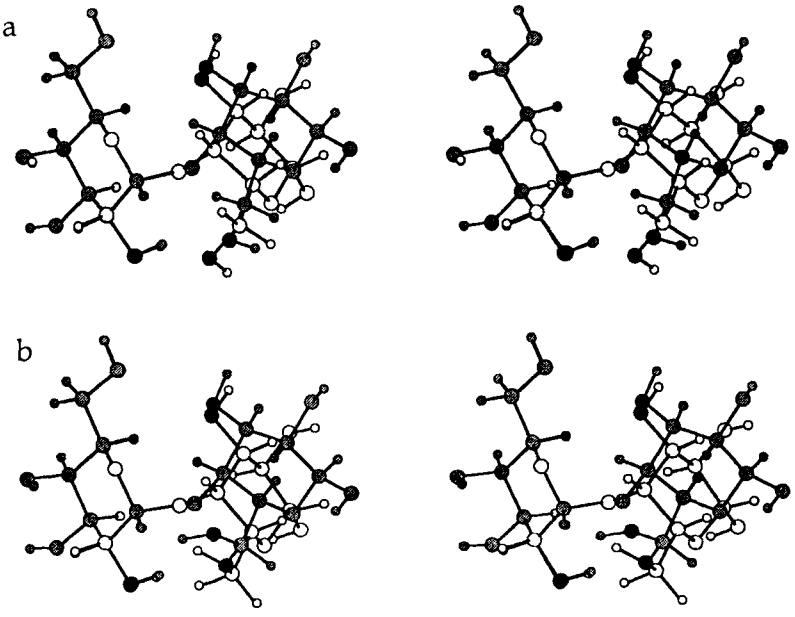

Fig. 4. Stereorepresentation of the least-squares fit between low-energy conformations of Galα4Gal (empty circles) and GalSα4Gal (grey circles), generated by using all ring atoms of the Galα moiety in the fitting. Atoms that are important for the interactions discussed in the text (O2′ and O6, bottom part of molecules; H5′ and O3, top part) are shown as black dots. a) Galα4Gal-gg (143.8 kJ mol^{-1}) and GalSα4Gal-gg (164.3 kJ mol^{-1}); b) Galα4Gal-tg (142.3 kJ mol^{-1}) and GalSα4Gal-tg (165.8 kJ mol^{-1}), see Table 2.

Table 2. Calculated energies [a] and selected interatomic distances for conformations of Galα4Gal and GalSα4Gal.

Galα4Gal-gg [b]				GalSα4Gal-gg [b]			
E [c]	Φ/Ψ [d]	O2′–O6 [e]	H5′–O3 [e]	E [c]	Φ/Ψ [d]	O2′–O6 [e]	H5′–O3 [e]
138.9	−10/47	3.23	4.06	164.3	−52/−13	3.56	*2.75*
142.5	−17/45	3.13	3.88	167.5	−41/−20	3.13	3.29
143.1	−12/42	3.10	3.95	167.6	−42/−13	3.21	3.13
143.8	−44/−16	*2.81*	*2.42*	167.9	−60/−24	3.78	*2.88*
144.0	−43/15	*2.76*	*2.46*	168.9	−60/0	3.84	*2.48*
146.5	0/60	3.57	4.56	169.2	−60/−30	3.77	*2.90*
147.1	0/30	*2.99*	4.02	170.7	−31/1	3.16	3.33
147.1	−30/30	*2.90*	3.39	171.0	−30/0	3.14	3.34
147.8	−30/60	3.31	3.92	172.0	−30/−30	*2.87*	3.59
149.9	−30/0	*2.65*	*2.71*	172.3	0/31	3.56	4.30
152.1	−60/0	*2.96*	*2.27*	172.3	−27/30	3.79	3.58
153.3	−30/−30	*2.67*	*2.85*	173.5	30/30	3.56	5.07
154.8	−60/−30	3.32	*2.44*	177.0	30/0	*2.84*	5.00
159.1	30/60	4.15	5.18	177.8	−90/−30	4.58	*2.77*
				178.2	0/60	4.48	4.68
				178.3	0/0	*2.82*	4.21
				178.7	60/30	4.29	5.48
				181.2	60/0	3.57	5.50
				181.5	−30/60	4.71	3.99
				182.3	30/60	4.59	5.34

Galα4Gal-tg [b]				GalSα4Gal-tg [b]			
E [c]	Φ/Ψ [d]	O2′–O6 [e]	H5′–O3 [e]	E [c]	Φ/Ψ [d]	O2′–O6 [e]	H5′–O3 [e]
142.1	−10/39	3.33	3.93	165.8	−52/−11	5.15	*2.72*
142.3	−38/−13	4.55	*2.56*	168.8	−62/−25	5.49	*2.89*
144.4	0/30	3.19	4.01	168.9	−43/−11	4.90	3.09
146.3	−23/39	3.74	3.65	169.1	−60/0	5.24	*2.51*
147.2	−30/0	3.97	*2.77*	169.5	−60/−30	5.47	*2.90*
148.5	−36/−25	4.63	*2.83*	170.4	−31/−2	4.35	3.35
149.3	−30/30	3.81	3.41	171.2	−77/−32	5.75	*2.88*
149.9	0/60	4.00	4.57	171.7	−3/32	3.81	4.27
150.7	−30/60	4.05	3.93	171.8	−30/30	4.54	3.55
152.4	−60/0	4.84	*2.30*	172.2	0/30	3.77	4.31
152.8	30/30	3.05	4.78	172.6	−30/−30	4.73	3.63
154.3	−60/−30	5.25	*2.47*	175.2	−90/−30	5.86	*2.80*
157.1	0/0	3.05	3.54	175.3	60/30	3.28	5.50
159.7	−60/−60	5.66	3.02	176.4	30/30	3.52	5.09
162.0	−30/−60	5.16	3.11	177.2	0/60	4.52	4.70
				178.1	30/0	*2.84*	5.00
				178.2	0/0	3.32	4.23
				179.3	−30/60	5.16	4.01
				181.2	30/60	4.17	5.36
				182.4	−60/−60	5.76	3.69
				183.6	60/0	*2.86*	5.51
				185.2	−90/0	5.90	*2.26*
				185.4	0/180	3.01	5.58
				186.0	−90/−60	6.12	3.63

[a] Conformations with energies of >20 kJ mol^{-1} above the lowest energy were excluded. [b] The initial torsional angle O5-C5-C6-O6 was set at −60° for gg (gauche–gauche) and 180° for tg (trans–gauche). [c] Energy (kJ mol^{-1}) as calculated by the MM3 program. [d] Torsional angles H1′-C1′-O1′-C4 and C1′-O1′-C4-H4; degrees. [e] Interatomic distance (Å); values expected to imply reasonable opportunity for hydrogen bonding and oxygen-induced deshielding of H5′ in the ^{1}H NMR spectra are given in italics.

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hydrogen bonding ($< \approx 3$ Å). Galα4Gal-gg has five low-energy conformations fulfilling these criteria, whereas no conformations of Galα4Gal-tg, GalSα4Gal-gg or GalSα4Gal-tg display both O2′–O6 and H5′–O3 distances of less than 3 Å. Comparing Galα4Gal-gg with GalSα4Gal-gg and Galα4Gal-tg with GalSα4Gal-tg (Fig. 3 and 4, Table 2) shows that, in the conformations that display a reasonable H5′–O3 distance (as required by the ^{1}H NMR data), the O2′–O6 distance in GalSα4Gal is more than 0.8 Å larger than in Galα4Gal. This indicates that an O2′–O6 hydrogen bond can only be formed in the natural galabiosides and not in the thio analogue.

We have reported[8a] that an intramolecular hydrogen bond exists (in [D$_6$]DMSO solution) between HO2′ and HO6 in the methyl galabioside corresponding to **1**. To investigate whether this hydrogen bond had disappeared in **2** (due to the greater distance between HO2′ and HO6), we performed an NMR study (in [D$_6$]DMSO) of the isotope-induced chemical shifts for the hydroxyl protons in **1** and **2** by partly ($\approx 50\%$) replacing them with deuterium.[8,26] Where an intramolecular hydrogen bond existed, the proton signals involved were doubled, due to the slightly different inductive effects from hydrogen and deuterium. As can be seen from Figure 5, there is an intramolecular hydrogen bond present in **1** but not in **2**; this clearly demonstrates that there is a structural difference between them in [D$_6$]DMSO. The signal intensity of the hydroxyl protons were in both cases reduced to $\approx 50\%$ on addition of MeOD, corresponding to the degree of deuterium exchange. It should be noted that there was a significant deshielding of H5′ in **2**, both in D$_2$O ($\delta = 4.41$) and in [D$_6$]DMSO ($\delta = 4.26$), consistent with the situation for the corresponding methyl galabioside[8a] ($\delta = 4.34$ and 4.15, repectively). This increases the validity of the constraint of a short H5′–O3 distance (<3 Å) in both **1** and **2**.

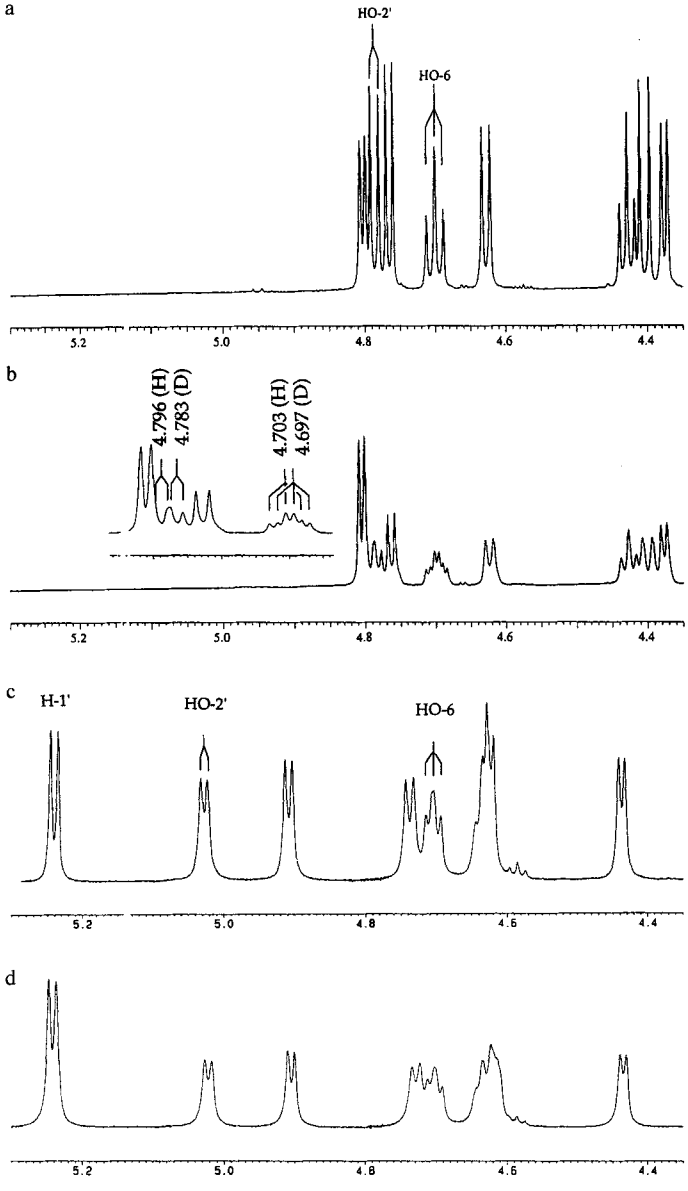

Fig. 5. ^{1}H NMR spectra of in [D$_6$]DMSO of a) **1**, b) **1** + CD$_3$OD, c) **2** and d) **2** + CD$_3$OD. Signal multiplicities demonstrate the presence of a hydrogen bond between HO2′ and HO6 in **1**, but not in **2**. Signal intensities for HO protons in the spectra with added CD$_3$OD were reduced to $\approx 50\%$ due to partial deuterium exchange.

Determination of the relative inhibitory power of compounds 1–3: The relative abilities of compounds **1–3** to inhibit the binding of PapG$_{J96}$ adhesin (as the PapG/PapD$_{J96}$ complex[27]) to galabiose were investigated by a newly developed ELISA method (see Experimental Procedure). Briefly, the spacer galabioside **14** was coupled covalently to microtiter plates (Fig. 6), and the

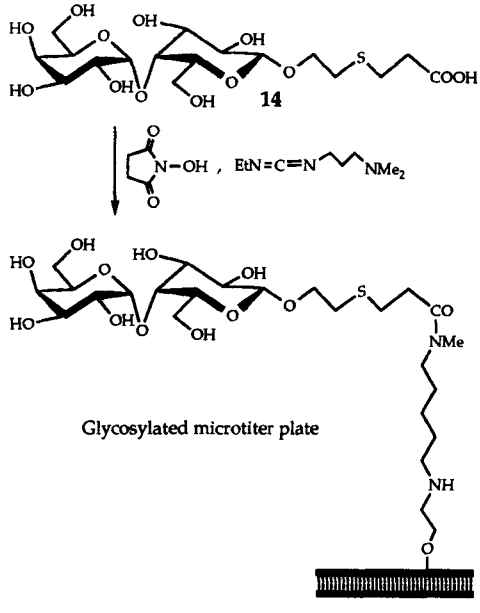

Fig. 6. Covalent coupling of galabioside **14** to aminated microtiter plates.

PapG/PapD$_{J96}$ complex and a series of diluted inhibitors **1–3** were added to the microtiter wells. The amount of bound protein was determined by using an anti-PapG/PapD$_{J96}$ antibody. As depicted in Figure 7, compounds **1** and **3** had similar inhibitory powers, whereas the thioanalogue **2** was much less efficient. With **1** as the reference inhibitor, the relative equilibrium constant K_{rel}[28] was obtained as the ratio of the IC_{50} value of **1** and each of the inhibitors **2** and **3**. The K_{rel} values were used for the calculation of difference free energies $\Delta\Delta G$, by using the expression $\Delta\Delta G = -RT\ln(K_{rel})$. Compound **2** has a $\Delta\Delta G$ value of $+8.0$ kJ mol^{-1}, which is consistent with loss of one strong intermolecular hydrogen bond in the interaction with PapG$_{J96}$ adhesin,[29] whereas $\Delta\Delta G = +1.6$ kJ mol^{-1} for **3** indicates that **1** and **3** interact in a similar way with the adhesin.

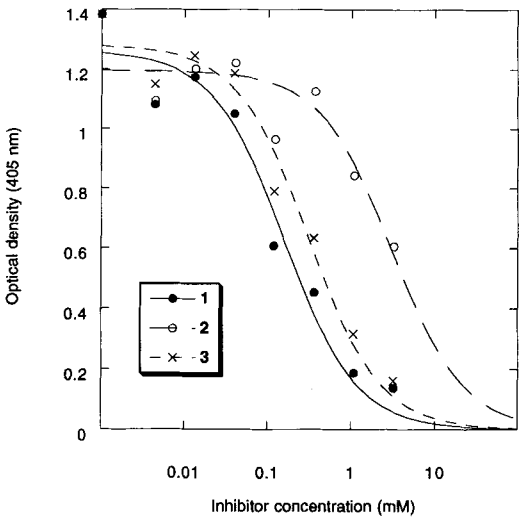

Fig. 7. Efficiency of compounds **1**–**3** as inhibitors of the binding of the PapG/PapD$_{J96}$ complex to microtiter plates carrying covalently bound **14**. IC_{50} and $\Delta\Delta G$ values: **1** (0.16 mM, 0.0 kJ mol^{-1}), **3** (0.31 mM, +1.6 kJ mol^{-1}) and **2** (4.4 mM, +8.0 kJ mol^{-1}).

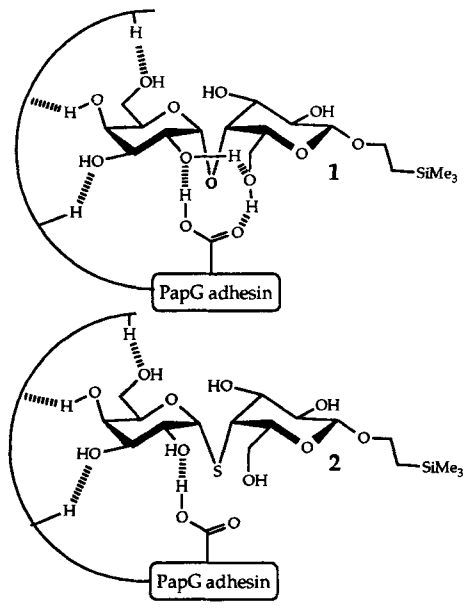

Fig. 8. Suggested hydrogen bonding between the PapG adhesin and **1** and **2**, respectively (cf. ref. [9]).

Discussion

The structural difference between the TMSEt galabioside **1** and its thio analogues **2** and **3** is limited to a single atom (O vs. S). Since the sulfur–carbon bond is longer than the oxygen–carbon bond (≈1.8 vs. ≈1.4 Å) and the C-S-C and C-O-C bond angles are different (≈105 vs. ≈111°), the relative spatial orientation of the galactopyranose rings is somewhat different in compounds **1** and **2**. This influences their efficiency as inhibitors of the PapG$_{J96}$ adhesin. We have earlier shown[9] that the hydrogen bonding between PapG$_{J96}$ and galabiosides requires three hydroxyl groups and one oxygen (HO2′, HO4′, HO6′ and O3′) in the Galα unit but only one hydroxyl group (HO6) in the Galβ unit. According to the molecular mechanics calculations, HO6 is moved away from HO2′ by approximately 1 Å in **2** as compared to **1**. This is confirmed by the NMR investigation (summarized in Fig. 5), which shows that the intramolecular hydrogen bond between HO6 and HO2′ in **1** is no longer present in **2**.

The loss of inhibitory power of **2**, as compared to **1**, may be explained as follows: 1) The difference in the conformation of **2** vs. **1** may be directly responsible for the loss of an intermolecular hydrogen bond between HO6 and PapG$_{J96}$. 2) The intramolecular hydrogen bond in **1** is of a cooperative nature[30] and therefore necessary for strong binding between **1** and PapG$_{J96}$. In other words, the close proximity of HO6 and HO2′ in **1** means that they behave like vicinal diols; this is not the case in **2**, since the distance between HO6 and HO2′ is too long (Fig. 8). It has been pointed out by Quiocho[30] that the majority of intermolecular hydrogen bonds in crystals of protein–carbohydrate complexes are formed between vicinal diols and polar amino acid side chains (e.g., a carboxyl, amido, or guanidino group). It is probable that such structural preferences also operate in complexes formed in solution.

It should be noted that the evidence for the loss of a crucial intramolecular hydrogen bond on moving from **1** to **2** rests on distance measurements in energy-minimized conformations and on NMR data obtained in [D$_6$]DMSO solution. The situation might be different in an aqueous environment; on the other hand, the DMSO environment might be a good model of the semipolar interior of a hydrated protein receptor site.

Finally, the GalSα4Gal structure (cf. **2**) seems to have a somewhat less well-defined Φ/Ψ energy surface than Galα4Gal (cf. **1**; Fig. 3). In addition, breaking the O2′–O6 hydrogen bond in **2** lowers the rotational barrier of the $HOCH_2$-6 group. These factors would imply that **2** has a somewhat less favourable entropy of binding to PapG$_{J96}$ than **1**. These are, however, subtle effects that largely derive from loss of the intramolecular hydrogen bond in **2**.

The possibility that PapG$_{J96}$ needs the intersaccharidic oxygen atom (O1′) in **1** for direct recognition can probably be ruled out since the oxygen atom is buried deep into the concave side of the galabiose structure; the binding site of PapG$_{J96}$ is thought to recognize the convex side of galabiose.[9, 31]

Conclusions

We have synthesized a sulfur analogue of an inhibitory galabioside. The analogue has a slightly altered conformation, which seems to preclude hydrogen bond(s) of importance for the recognition of galabiosides by the PapG$_{J96}$ adhesin. The loss of binding strength for the thiogalabioside **2**, compared to the parent O-galabioside **1**, corresponds well with what is expected for loss of one hydrogen bond between saccharide and protein.

Our findings question the general use of thioglycosides as hydrolytically stabilized analogues of receptor-active saccharidic ligands. A subtle structural change in the ligand (**1** → **2**) was found to be detrimental to recognition by the receptor protein. The results also demonstrate what high degree of structural precision is needed in the design of ligands for recognition by proteins and other receptor molecules.

Experimental Procedure

General methods: Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with a Varian XL-300 spectrometer, except for compounds **1**, **2**, and **10**, which were investigated with a Bruker ARX 500 spectrometer. 1,4-Dioxane or acetone was used as internal reference in ^{13}C NMR experiments in D_2O. Solutions were concentrated by using rotary evaporation with bath temperature at or below 40 °C. Anhydrous

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Na_2SO_4 was used as drying agent for the organic extracts in the workup procedures. TLC was performed on Kieselgel $60F_{254}$ plates (Merck). Column chromatography was performed with SiO_2 (Matrex LC-gel; 60A, 35-70 MY, Grace).

2-(Trimethylsilyl)ethyl 4-*S*-(α-D-galactopyranosyl)-4-thio-β-D-galactopyranoside (2): Compound **10** (22.5 mg, 0.0239 mmol) was treated with methanolic sodium methoxide (3 mL, 10 mM) for 5 h and 20 min, and then neutralized with Duolite C26 (H^+) resin, filtered, and concentrated. Flash chromatography (CH_2Cl_2/MeOH 4:1) of the residue gave **2** (9.3 mg, 85%); $[\alpha]_D^{25} = +128$ ($c = 0.14$, H_2O). ^1H NMR: see Table 1. ^{13}C NMR (D_2O): $\delta = 102.0, 86.6, 74.5, 71.9, 71.8, 71.0, 69.4, 68.5, 67.8, 67.7, 61.1, 60.4, 50.0, 17.2, -2.9$. HRMS: calcd for $C_{17}H_{35}O_{10}SSi$ ($M+1$): 459.1720; found: 459.1737.

2-(Trimethylsilyl)ethyl 4-*O*-(α-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (3): Compound **13** (303.2 mg, 0.40 mmol) was treated with methanolic sodium methoxide (40 mL, 0.0015 mM) for 5 h, neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated to give a quantitative yield of **3**; $[\alpha]_D^{25} = +45$ ($c = 1$, H_2O). ^1H NMR (D_2O): $\delta = 4.92$ (d, 1H, $J = 3.7$ Hz, H1'), 4.52 (d, 1H, $J = 9.5$ Hz, H1), 4.30 (brt, 1H, $J = 6.2$ Hz, H5'), 3.53 (t, 1H, $J = 9.5$ Hz, H2), 2.79 (m, 2H, SCH_2), 0.89 (m, 2H, CH_2Si), −0.02(s, 9H, $SiMe_3$). ^{13}C NMR (D_2O): $\delta = 101.3, 86.5, 79.6, 78.5, 74.7, 71.7, 70.8, 70.0, 69.8, 69.6, 61.3, 60.9, 27.1, 18.1, -1.8$. HRMS: calcd for $C_{17}H_{34}O_{10}SSiNa$ (M +Na): 481.1540; found: 481.1535.

2-(Trimethylsilyl)ethyl 2,3,6-tri-O-benzoyl-β-D-glucopyranoside (5): To a cooled (−78 °C) solution of **4** [16] (0.96 g, 3.41 mmol) in CH_2Cl_2 (9 mL) and pyridine (4.5 mL) was slowly added benzoyl chloride (1.31 mL, 12.5 mmol). The temperature was raised to −50 °C, and the reaction was carefully monitored by TLC (SiO_2, toluene/Et_2O 2:1). When the formation of **5** was at its optimum, the reaction mixture was quenched with MeOH (4.5 mL). The mixture was diluted with $CHCl_3$ (20 mL), washed twice with saturated aqueous NaCl (15 mL), dried, filtered, and concentrated. The residue was chromatographed (SiO_2, toluene/Et_2O 10:1 → 4:1) to give **5** (1.12 g, 55%). $[\alpha]_D^{23} = +48$ ($c = 1.3$, $CHCl_3$). ^1H NMR ($CDCl_3$): $\delta = 5.43$ (m, 2H, H2,3), 4.75 (dd, 1H, $J = 12.0$, 4.5 Hz, H6), 4.74 (d, 1H, $J = 7.2$ Hz, H1), 4.67 (dd, 1H, $J = 12.0$, 2.3 Hz, H6), 4.06–3.96 (m, 1H, OCH_2CH_2), 3.92 (m, 1H, H4), 3.81 (ddd, 1H, $J = 9.7$, 4.4, 2.3 Hz, H5), 3.66–3.56 (m, 1H, OCH_2CH_2), 3.43 (d, 1H, $J = 4.1$ Hz, HO4), 0.95–0.80 (m, 2H, CH_2CH_2Si), −0.05 (s, 9H, $SiMe_3$). HRMS: calcd for $C_{32}H_{37}O_9Si$ ($M+1$): 593.2207; found: 593.2218. Anal. calcd for $C_{32}H_{36}O_9Si$: C 64.9, H 6.1; found: C 65.3, H 6.5.

2-(Trimethylsilyl)ethyl 2,3,6-tri-O-benzoyl-4-deoxy-4-thiocyanato-β-D-galactopyranoside (6) and 2-(trimethylsilyl)ethyl 2,3,6-tri-O-benzoyl-4-deoxy-4-isothiocyanato-β-D-galactopyranoside (7): To an ice-cooled solution of **5** (2.7 g, 4.56 mmol) in CH_2Cl_2 (20 mL) was added pyridine (9.74 mL, 120 mmol) and trifluoromethanesulfonic anhydride (5.06 mL, 30 mmol) in CH_2Cl_2 (30 mL). After 30 min, $CHCl_3$ (50 mL) was added and the mixture was washed with 10% aqueous HCl (20 mL), saturated aqueous $NaHCO_3$ (20 mL), and saturated aqueous NaCl (20 mL). The organic phase was dried, filtered, and concentrated. The residue and KSCN (5.0 g, 0.05 mol) were dissolved in DMF (50 mL). The mixture was left overnight at 90 °C, cooled, CH_2Cl_2 (250 mL) and $CHCl_3$ (250 mL) were added, and the mixture was extracted with saturated aqueous NaCl. The organic phases were pooled, dried, filtered, and concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 10:1 → 8:1) to give **6** (1.90 g, 70%) and **7** (0.19 g, 7%).
6: $[\alpha]_D^{23} = -34$ ($c = 1.2$, $CHCl_3$). ^1H NMR (C_6D_6): $\delta = 5.98$ (dd, 1H, $J = 10.1$, 7.9 Hz, H2), 5.56 (dd, 1H, $J = 10.1$, 4.1 Hz, H3), 4.51 (dd, 1H, $J = 11.7$, 7.4 Hz, H6), 4.26 (d, 1H, $J = 7.9$ Hz, H1), 4.15 (dd, 1H, $J = 11.7$, 4.9 Hz, H6), 3.96–3.86 (m, 1H, OCH_2CH_2), 3.88 (dd, 1H, $J = 4.1$, 1.2 Hz, H4), 3.64 (ddd, 1H, $J = 7.3$, 4.9, 1.2 Hz, H5), 3.46–3.37 (m, 1H, OCH_2CH_2), 0.80–0.70 (m, 2H, CH_2CH_2Si), −0.10 (s, 9H, $SiMe_3$). ^{13}C NMR ($CDCl_3$): $\delta = 165.9, 165.7, 164.9, 110.8, 101.5, 72.3, 70.9, 69.7, 67.9, 62.9, 53.7, 17.9, -1.6$. HRMS: calcd for $C_{33}H_{36}O_8NSSi$ ($M+1$): 634.1931; found: 634.1918. Anal. calcd for $C_{33}H_{35}O_8NSSi$: C 62.5, H 5.6, N 2.2; found: C 62.8, H 5.7, N 1.9.
7: ^1H NMR ($CDCl_3$): $\delta = 5.71$ (dd, 1H, $J = 10.3$, 7.9 Hz, H2), 5.45 (dd, 1H, $J = 10.2$, 4.0 Hz, H3), 4.76 (d, 1H, $J = 7.9$ Hz, H1), 4.72 (dd, 1H, $J = 11.1$, 6.1 Hz, H6), 4.61 (dd, 1H, $J = 4.0$, 1.2 Hz, H4), 4.50 (dd, 1H, $J = 11.4$, 7.0 Hz, H6), 4.09 (m, 1H, H5), 4.08–3.98 (m, 1H, OCH_2CH_2), 3.68–3.58 (m, 1H, OCH_2CH_2), 0.90–0.70 (m, 2H, CH_2CH_2Si), −0.10 (s, 9H, $SiMe_3$). ^{13}C NMR ($CDCl_3$): $\delta = 140.2$. HRMS: calcd for $C_{33}H_{36}O_8NSSi$ ($M+1$): 634.1931; found: 634.1949.

2-(Trimethylsilyl)ethyl 2,3,6-tri-O-benzoyl-4-thio-β-D-galactopyranoside (8): To a solution of **6** (117.5 mg, 0.185 mmol) in HOAc (3 mL) was added Zn (1 g, 15.3 mmol). The mixture was refluxed at 130 °C for 3 h, allowed to cool to room temperature, then filtered, and $CHCl_3$ (25 mL) was added. The mixture was extracted with saturated aqueous $NaHCO_3$ (10 mL) and saturated aqueous NaCl (10 mL). The organic phase was dried, filtered, and concentrated. The residue was chromatographed (SiO_2, heptane/EtOAc 7:1) to give **8** (79 mg, 70%); $[\alpha]_D^{23} = +54$ ($c = 1.1$, $CHCl_3$). ^1H NMR ($CDCl_3$): $\delta = 5.73$ (dd, 1H, $J = 10.1$, 7.9 Hz, H2), 5.41 (dd, 1H, $J = 10.1$, 4.6 Hz, H3), 4.77 (d, 1H, $J = 7.9$ Hz, H1), 4.74 (dd, 1H, $J = 11.5$, 6.8 Hz, H6), 4.64 (dd, 1H, $J = 11.5$, 5.6 Hz, H6), 4.29 (ddd, 1H, $J = 6.8$, 5.6, 1.5 Hz, H5), 4.10–4.00 (m, 1H, OCH_2CH_2), 3.88 (ddd, 1H, $J = 9.7$, 4.6, 1.5 Hz, H4), 3.70–3.60 (m, 1H, OCH_2CH_2), 1.83 (d, 1H, $J = 9.7$ Hz, SH), 0.97–0.82 (m, 2H, CH_2CH_2Si), −0.05 (s, 9H, $SiMe_3$). The peak at $\delta = 1.83$ disappeared upon addition of D_2O. HRMS: calcd for $C_{32}H_{37}O_8SSi$ ($M+1$): 609.1978; found: 609.1964. Anal. calcd for $C_{32}H_{36}O_8SSi$: C 63.1, H 6.0, S 5.3; found: C 63.3, H 6.1, S 5.0.

2-(Trimethylsilyl)ethyl 2,3,6-tri-O-benzoyl-4-*S*-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-4-thio-β-D-galactopyranoside (10): To a solution of **9** [19] (120 mg, 0.0328 mmol) and Cs_2CO_3 (107 mg, 0.0328 mmol) in DMF (1 mL) was added **8** (100 mg, 0.0164 mmol) in DMF (1 mL). The mixture was stirred for 3 h and then concentrated. The residue was chromatographed (SiO_2, toluene/EtOAc 6:1) to give **10** (127.2 mg, 85%). $[\alpha]_D^{25} = +83$ ($c = 0.15$, $CHCl_3$). ^1H NMR ($CDCl_3$): $\delta = 5.80$ (d, 1H, $J = 5.1$ Hz, H1'), 5.60 (dd, 1H, $J = 10.4$, 4.7 Hz, H3), 5.43 (dd, 1H, $J = 10.4$, 7.6 Hz, H2), 5.31 (dd, 1H, $J = 11.0$, 3.1 Hz, H3'), 5.23 (dd, 1H, $J = 11.0$, 5.1 Hz, H2'), 5.15 (dd, 1H, $J = 3.0$, 1.2 Hz, H4'), 4.88 (dd, 1H, $J = 11.0$, 6.1 Hz, H6), 4.70 (d, 1H, $J = 7.6$ Hz, H1), 4.40 (dd, 1H, $J = 11.0$, 6.1 Hz, H6), 4.37 (dd, 1H, $J = 7.2$, 6.7 Hz, H5'), 4.22 (dd, 1H, $J = 7.4$, 6.1 Hz, H5), 4.06–3.96 (m, 1H, OCH_2CH_2), 3.78 (d, 1H, $J = 4.6$ Hz, H4), 3.66–3.56 (m, 1H, OCH_2CH_2), 3.48 (dd, 1H, $J = 11.0$, 7.1 Hz, H6'), 3.25 (dd, 1H, $J = 11.0$, 6.8 Hz, H6'), 2.17, 2.03, 1.99, 1.75 (4s, 3H each, OAc), 0.95–0.81 (m, 2H, CH_2CH_2Si), −0.05 (s, 9H, $SiMe_3$). Anal. calcd for $C_{46}H_{54}O_{17}S$: C 58.8, H 5.8, S 3.4; found: C 59.2, H 5.7, S 3.3.

2-(Trimethylsilyl)ethyl 2,3,6-tri-O-acetyl-4-*O*-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (13): **Method a**: To a solution of 2-(trimethylsilyl)ethanethiol (0.044 mL, 0.27 mmol) in dry DMF (0.35 mL) was added NaH (15 mg, 0.3 mmol, 50% in mineral oil) under N_2. After 6 min, the mixture was added to a solution of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranosyl bromide [21] (**12**, 146.5 mg, 0.21 mmol) in dry DMF (1.8 mL). After 80 min, the mixture was diluted with CH_2Cl_2, washed with saturated aqueous NaCl, dried, and concentrated. Flash chromatography (heptane/EtOAc, 2:1) gave **13** (105.4 mg, 67%); $[\alpha]_D^{25} = +62$ ($c = 1$ $CHCl_3$).
Method b: To a solution of 2-(trimethylsilyl)ethanethiol (0.091 mL, 1.23 mmol) and 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranose [21] (**11**, 416.2 mg, 0.61 mmol) in dry CH_2Cl_2 (1.8 mL) was added BF_3OEt_2 (0.093 mL, 0.73 mmol) under N_2. After 1 h, the mixture was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, dried, and concentrated. Flash chromatography (heptane/EtOAc, 2:1) gave **13** (303.2 mg, 66%) together with 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-1-thio-α-D-galactopyranoside (56.7 mg, 12%).
13: ^1H NMR ($CDCl_3$): $\delta = 5.56$ (brd, 1H, $J = 3.2$ Hz, H4'), 5.35 (dd, 1H, $J = 3.2$, 11.0 Hz, H3'), 5.25 (t, 1H, $J = 10.4$ Hz, H2), 5.20 (dd, 1H, $J = 3.5$, 11.0 Hz, H2'), 5.00 (d, 1H, $J = 3.7$ Hz, H1'), 4.88 (dd, 1H, $J = 2.7$, 10.4 Hz, H6), 4.49 (d, 1H, $J = 9.8$ Hz, H1), 2.76 (t, 2H, $J = 8.8$ Hz, SCH_2), 2.13, 2.11, 2.08, 2.062, 2.056, 2.04, 1.97 (6s, 3H each, OAc), 0.86 (m, 2H, CH_2Si), 0.04 (s, 9H, $SiMe_3$). HRMS: calcd for $C_{31}H_{48}O_{17}SSiNa$ (M +Na): 775.2289; found: 775.2279. Anal. calcd for $C_{31}H_{48}O_{17}SSi$: C 49.5, H 6.4; found: C 49.5, H 6.4.

2-(2-Carboxyethylthio)ethyl 4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside (14): 2-(2-Methoxycarbonylethylthio)ethyl 4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside [32] (257 mg, 0.53 mmol) was hydrolyzed in aqueous NaOH (30 mL, 0.04 mM) for 1 h, neutralized with Duolite C26 (H^+) resin, filtered, and concentrated. Flash chromatography (CH_2Cl_2/MeOH/H_2O, 65:35:5, 1% AcOH) of the residue gave a quantitative yield of **14**, $[\alpha]_D^{25} = +76$ ($c = 0.53$, H_2O). ^1H NMR (D_2O): $\delta = 4.96$ (d, 1H, $J = 3.9$ Hz, H1'), 4.49 (d, 1H, $J = 7.8$ Hz, H1), 4.37 (brt, 1H, $J = 6.0$ Hz, H5'), 2.84 (m, 4H, CH_2SCH_2), 2.58 (t, 2H, $J = 7.2$ Hz, CH_2CO). ^{13}C NMR (D_2O): $\delta = 103.8, 101.1, 77.9, 75.9, 73.2, 71.8, 71.6, 69.8, 69.6, 61.4, 60.8, 31.7, 27.7$. HRMS: calcd for $C_{17}H_{31}O_{13}S$ ($M+1$): 475.1485; found: 475.1493.

Covalent coupling of 14 to microtiter plates: Microtiter plates functionalized with secondary amino groups (CovaLink® microtiter plates, A/S Nunc, P. O. Box 280, Kamstrup, DK-4000 Roskilde, Denmark) were used for covalent coupling of the carboxylic acid functionalized galabioside **14**, essentially as described by Nunc (Fig. 6). To each well was added 0.050 mL of an aqueous solution containing **14** (4 mM) and *N*-hydroxy succinimide (8 mM) followed by 0.050 mL of a solution containing 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (8 mM). The plate was shaken for 2–6 h and then washed three times with Covabuffer. In order to determine the coupling concentration of **14** needed for the inhibition experiments described below, a dilution series of **14** in the coupling reaction was made. The optimal coupling concentration of **14** was found to be ≈1 mM.

ELISA assay—competitive inhibition of the PapG/PapD$_{J96}$ complex by 1, 2, and 3: The PapG/PapD$_{J96}$ complex was purified as previously described [27]. The complex (0.1 mg mL^{-1} in 2% BSA/PBS, 0.050 mL per microtiter well) was added to the microtiter plates carrying covalently linked **20** and containing 0.050 mL of the inhibitors **1–3** serially diluted three times between each well. Every inhibitor was investigated two or three times in order to obtain mean values. After 45 min incubation, the plates were washed three times with PBS and then blocked for nonspecific binding by a 1 h incubation with 0.4 mL of 2% BSA in PBS. The PapG/PapD$_{J96}$ complex was detected by incubation with primary anti-PapG/PapD$_{J96}$ rabbit antiserum (diluted 1/500 in 2% BSA in PBS, 100 μL per well) for 1 h, followed by washing (three times) with Covabuffer, and addition of alkaline phosphatase–conjugated antirabbit IgG (Sigma A7539, diluted 1/5000) and phosphatase substrate

104 (Sigma 104). The optical density was read at 405 nm. Incubations were at 24 °C unless otherwise stated.

NMR investigation of intramolecular hydrogen bonding in 1 and 2: Compounds **1** and **2** were dried under vacuum in NMR tubes for several days and dry [D_6]DMSO was added. The ^{1}H NMR spectra of 0.03 M solutions of **1** and **2** in [D_6]DMSO were recorded with a Bruker ARX500 instrument at 294 K. Assignments were based on 2D COSY experiments. To observe isotope-induced doubling of HO signals, CD_3OD was added in 0.010 mL increments until half of the hydroxyl protons had been exchanged by deuterium (the intensity of the HO signals were reduced to ≈50%).

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