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Conformational studies on phenyl thioglycosides: a remote effect on disaccharide linkage by phenyl aglycons attenuates recognition of galabiosides by a bacterial adhesin

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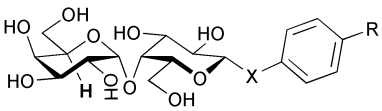
Phenyl *S*-galabiosides display altered conformational properties, as compared to phenyl *O*-galabiosides, characterised by a remote effect on the galabiose intersaccharidic glycoside bond by the phenyl aglycon, resulting in significantly lowered affinity for the PapG class II adhesin of uropathogenic *E. coli*.

In an ongoing project aiming at the discovery of inhibitors of PapG adhesins, proteins used by uropathogenic *Escherichia coli* for adhesion to glycolipids of the globoseries present on human uroepithelial cells,[1–3] we have prepared panels of galabiose derivatives,[4–8] which led to the discovery that the rather simple *p*-methoxyphenyl galabioside **3** (Fig. 1) was an inhibitor as potent as the natural tetrasaccharide ligand globotetraose.[8,9] Computational docking experiments with *p*-methoxyphenyl galabioside **3** and the PapG adhesin suggested favourable interactions between the *p*-methoxyphenyl moiety and Trp107 and Arg170 side-chains of the adhesin.[9] This result encouraged us to synthesise aryl *S*-galabiosides, because thioglycosides are hydrolytically stable[10,11] and because they were readily prepared by straightforward nucleophilic substitution of per-*O*-acylated galabiosyl bromide with thiolate anions. However, to our disappointment, phenyl *S*-galabiosides turned out to be rather poor inhibitors of the class II PapG adhesin.[12]† This somewhat unexpected and discouraging result prompted us to investigate in detail the conformations of phenyl *S*-galabiosides and compare them to those of the corresponding phenyl *O*-galabiosides.



	δ (H-5′)	K_d (μM)
1 X=O, R=H	4.33	140
2 X=S, R=H	3.87	340
3 X=O, R=OMe	4.32	170
4 X=S, R=OMe	3.79	488
5 X=O, R=Me	4.32	180
6 X=S, R=Me	3.67	412

Fig. 1 Structures, H-5′ (bold) chemical shifts, and K_d (against an *E. coli* class II PapG adhesin) of phenyl *O*- and *S*-galabiosides **1**–**6**.

A diagnostic signal for the overall conformation of galabiose disaccharides is a downfield shift of H5′ of about 0.5 ppm (~3.8 to ~4.3 ppm), which is a result of a van der Waals contact between O3 and H5′.[6,13] However, this downfield shift was not observed in phenyl *S*-galabiosides **2**, **4**, and **6**, indicating different conformational behaviour as compared to the corresponding *O*-galabiosides **1**, **3**, and **5** (Fig. 1). In addition to a large upfield shift for H5′ (~0.6 ppm), small upfield shifts were observed for H3′ and H4′ (~0.2 ppm) of **2** as compared to **1** (Table 1).‡

Monte Carlo simulations§ were performed in order to search for low energy conformations of **1** and **2**. In all low energy conformations found for **1**, the phenyl aglycon adopts an outstretched position away from the galabiose disaccharide moiety (represented by conformer I in Fig. 2A). However, for the corresponding phenyl *S*-galabioside **2**, two groups of low energy conformations could be identified. One group of conformers resembled those found for the phenyl *O*-galabioside **1** (2 of 8 lowest energy conformations, represented by

Table 1 ^{1}H-NMR[a] δ for **1** and **2**

Proton	**1**	**2**	Proton	**1**	**2**
H1	5.08	4.64	H1′	4.92	4.85
H2	3.78	3.44	H2′	3.77	3.73
H3	3.84	3.63	H3′	3.87	3.63
H4	4.04	3.96	H4′	3.97	3.82
H5	~3.80	3.68	H5′	4.32	3.69
H6a	~3.80	3.73	H6a′	3.65	3.54
H6b	~3.80	3.79	H6b′	3.65	3.56

[a] Measured at 500 MHz in D_2O.

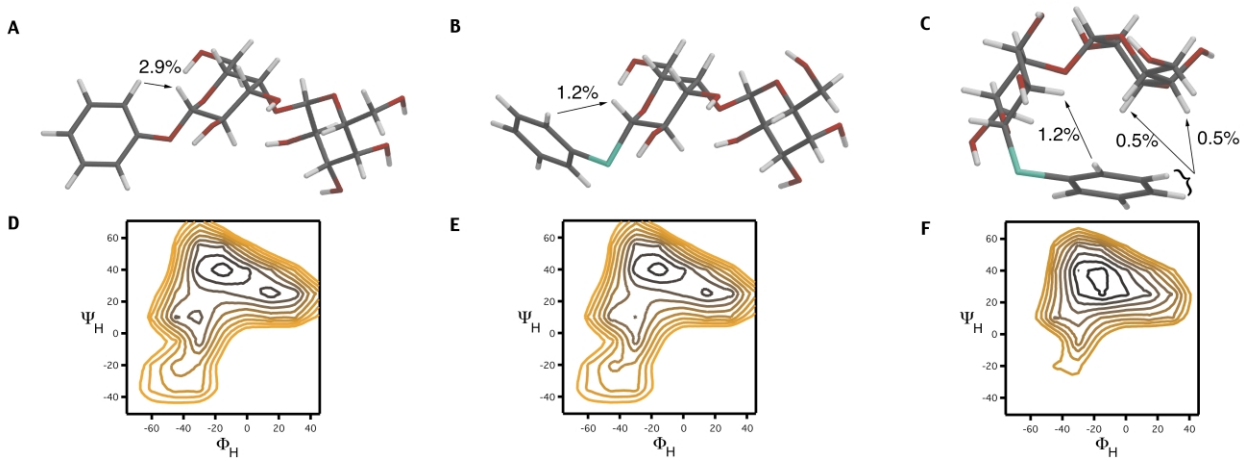


Fig. 2 A–C show representative conformers from Monte Carlo simulations (2 kJ mol^{-1} contour interval). Arrows indicate NOE. (A) Conformer I of phenyl *O*-galabioside **1**. (B) Conformer II of phenyl *S*-galabioside **2**. (C) Conformer III of phenyl *S*-galabioside **2**. (D) Energy contour map (Φ_H/Ψ_H) of the interglycosidic linkage of conformer I of phenyl *O*-galabioside **1**. (E) Energy contour map (Φ_H/Ψ_H) of the interglycosidic linkage of conformer II of phenyl *S*-galabioside **2**. (F) Energy contour map (Φ_H/Ψ_H) of the interglycosidic linkage of conformer III of phenyl *S*-galabioside **2**.

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conformer II in Fig. 2B), while the second and predominant group of conformers had the phenyl aglycon folded back towards the galabiose disaccharide moiety of **2** (6 of 8 lowest energy conformations, represented by conformer III in Fig. 2C). The folded conformer III is made possible by the longer C–S bond as compared to C–O (1.8 Å and 1.4 Å for C–S and C–O, respectively) and the smaller angle (101° and 118°, respectively). Conformer III of **2** was somewhat favoured over conformer II, which could be explained by an interaction between the phenyl ring and a hydrophobic patch made up by H3′, H4′ and H5′ of the galabiose moiety.

Two-dimensional dihedral drive calculations of the galabiose disaccharide bond¶ provided energy potential maps for each of the three conformers I–III (Fig. 2D–F). For conformer I (*O*-galabioside **1**), an energy map (Fig. 2D) similar to that reported for Galα1–4GalβOTMSEt[6,14] was found, displaying two energy minima (ϕ_H/ψ_H = −14/39 and −39/−3, respectively). The strong deshielding of H5′ requires that the H5′–O3 distance is less than 3 Å, a requirement met by the minimum at ϕ_H/ψ_H = −39/−3. In addition, the minimum at ϕ_H/ψ_H = −39/−3 was closer to that observed for the galabiose disaccharide in the crystal structure of globotetraose in complex with the class II adhesin.[15] The dihedral drive calculations of conformer II (of *S*-galabioside **2**) provided an energy map (Fig. 2E) similar to that found for conformer I of the *O*-galabioside **1**. In contrast, the minimum at ϕ_H/ψ_H = −39/−3 was absent in the energy contour map of the more populated‖ conformer III of the *S*-galabioside **2** (Fig. 2F). Thus, the upfield shifts of H3′and H4′ in the *S*-galabioside **2** can be explained by a field effect from the aromatic ring stacked towards the α-face of the Galα-residue in conformer III, while the large upfield shift for H5′ can be explained by the same field effect *and* the absence of the minimum at ϕ_H/ψ_H = −39/−3 in the more populated conformer III of the *S*-galabioside **2** (and thus a larger average distance between H5′ and O3). Furthermore, the calculations were supported by NOE-difference spectra of compounds **1** and **2**. Irradiation of the unresolved *o*- and *p*-hydrogens of the phenyl *O*-galabioside **1** resulted in 2.9% NOE of H1, while irradiation of the *m*-hydrogens did not have any effect (Fig. 2A). In contrast, irradiation of the unresolved *m*- and *p*-hydrogens of the phenyl *S*-galabioside **2** resulted in 0.5% NOE of H3′ and H4′ (Fig. 2C), while irradiation of the *o*-hydrogens resulted in 1.4% NOE of H2 and 1.2% NOE of H1 (Fig. 2B and C). The NOE of H1 could be explained by conformers I and II of compounds **1** and **2**, respectively, while the NOE of H2, H3′, and H4′ in the *S*-galabioside **2** can be explained by conformer III. The data indicate that for the *S*-galabioside **2**, conformer III is at least as populated as the bioactive conformer II.

In conclusion, replacing an anomeric oxygen with a sulfur in phenyl galabiosides changes the position of the phenyl aglycon in space, which leads to a remote distorting effect on the interglycosidic bond. This results in slightly lower affinity of phenyl *S*-galabiosides for the PapG II adhesin of uropathogenic *E. coli*, because the dominating low energy conformations (*e.g.* III) of phenyl *S*-galabiosides presumably fit poorly into the combining site of the adhesin, while the minimum (ϕ_H/ψ_H = −39/−3) closest to the conformation in the crystal complex with the adhesin[15] is disfavoured. An additional factor affecting the affinity of the PapG adhesin for the thioglycosides **2**, **4**, and **6** is that the suggested favourable interactions between the phenyl aglycons of **1**, **3**, and **5** and Trp107 and Arg170 of the adhesin[9] are obviously diminished in the conformationally altered thioglycosides. The anomeric oxygen of the galabiose disaccharide is not directly involved in interaction with the adhesin,[15] which makes it less likely that interactions between the sulfurs of the thioglycosides **2**, **4**, and **6** and the PapG adhesin affect the affinity. Hence, replacing a glycosidic oxygen with sulfur, in order to construct hydrolytically stable glycoside mimics can lead to loss of biological activity due to altered conformational properties (as compared to their parent *O*-glycosides) not only of the thioglycoside bond itself, but also of *remote* glycoside bonds elsewhere in the saccharide. Consequently, replacing a glycosidic oxygen with a sulfur does not *per se* lead to the discovery of hydrolytically stable *O*-glycoside mimics.**

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Notes and references

† Loss of biological activity of *S*-glycosides as compared to the parent *O*-glycosides has been reported for other systems; see ref. 6.

‡ These upfield shifts were not observed for the aliphatic 2-(trimethylsilyl)ethyl *S*-galabioside when compared to the corresponding aliphatic *O*-glycoside 2-(trimethylsilyl)ethyl galabioside; see ref. 15.

§ MMFF/water force field implemented in MacroModel. 6-OH and 6′-OH were locked in a *gauche–trans* and *trans–gauche* conformation, respectively, in order to avoid formation of intramolecular hydrogen bonds. 300 conformers were collected within 50 kJ mol^{-1}.

¶ Intersaccharidic glycoside bond angles ϕ_C (O5′–C1′–O1′–C4) and ψ_C (C1′–O1′–C4–C3) were varied in 15° increments using the MMFF/water force field implemented in MacroModel. 81 structures were obtained and minimised. ϕ_C and ψ_C were converted to ϕ_H (H1′–C1′–O1′–C4) and ψ_H (C1′–O1′–C4–H4) by subtracting 120° from ϕ_C and adding 120° to ψ_C

‖ The bent low energy conformation III was 5.1 kJ mol^{-1} lower as compared to the outstretched low energy conformation II.

** Conformationally altered glycoside mimics are often recognised poorly, because lectin specificities are commonly conferred by forming key interactions with two or more monosaccharide moieties within an oligosaccharide structure.[16–18] The success of thioglycosides as glycosidase inhibitors presumably relies on the fact that most of these enzymes typically recognise and display specificities towards the non-reducing monosaccharide residue of the disaccharide bond to be cleaved.[10,11]

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