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# Aryl *O*- and *S*-galactosides and lactosides as specific inhibitors of human galectins-1 and -3: Role of electrostatic potential at O-3

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**Abstract**—Phase transfer catalyzed reaction was used for the high yielding synthesis of aryl 1-thio-β-D-galacto- and lacto-pyranosides carrying a panel of substituents on the phenyl groups. Best galectin-1 inhibitors were simple *p*-nitrophenyl thiogalactoside **5a** for the monosaccharide and *o*-nitrophenyl thiolactoside **6f** or napthylsulfonyl lactoside **8c**, both being 20 times better relative to natural ligands. Relative inhibitory properties as low as 2500 and 40 μM were observed, respectively. The electronic effects of the lactoside aglycons directly influenced the electrostatic potential at O-3, which was associated with the inhibitory potencies against galectin-1.
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The galectins are a family of cytosolic β-D-galactoside binding proteins for which fourteen members have been identified in mammals.[1–4] Galectins-1 and -2 possess one carbohydrate recognition domain (CRD) per subunit and exist as dimers, in comparison to galectins-4, -8, and -9 which are connected to a short linker region. Galectin-3 (Gal-3) is unique and exists as chimera type composed of one CRD connected to non-CRD domain consisting of collagen-like repeats of a peptide sequence rich in proline and glycine, and is capable of self-association. The C-terminal CRD domain of Gal-3 is homologous to that of Gal-1.[5] The role of the galectins is not yet clear, but a striking common feature of all galectin members is the strong modulation of their expression during development, differentiation stages, and under various physiological or pathological conditions.[2] Gal-3 possesses diverse biological activities and is involved in colon cancer metastasis,[6] brain tumor progression,[7] inhibits metastasis-associated cancer cell adhesion,[8] and may play a key role in innate immunity.[9,10] Recent reports suggest that Gal-3[11] and Gal-1[12–14] can regulate cell apoptosis.[15] Also, Gal-1 acts as a soluble host factor that promotes HIV-1 infectivity through stabilization of virus attachment to host cells.[16] Thus, selective inhibition of galectins may lead to anti-cancer, anti-inflammatory, or even anti-HIV properties.[2,16–18]

Naturally occurring carbohydrate ligands for galectins have low affinities, are too polar to be used as drugs, and possess poor physiological stabilities due to their acid-sensitive glycosidic bonds.[19] For instance, galactose and lactose have inhibitory properties of 50 and 0.8 mM for both Gal-1 and Gal-3, respectively. Combining experimental information from the binding data and high resolution X-ray crystal structures of galectin–carbohydrate ligand complexes enables rational design approach for the development of new classes of glycomimetic inhibitors with high affinity, stability, and specificity.[20–25] Nilsson et al. have explored the 3′-position of a panel of lactoside derivatives for the synthesis of high affinity inhibitors of Gal-3 using benzamido[20–22] or triazole[23] functionalities. We report herein the efficient synthesis of small libraries of aryl thiogalactosides and lactosides using phase transfer catalysis reaction (PTC) with the aim to further explore the effect of the aglycons on the relative capacity toward galectins.[24,25] Encouraging inhibitory properties were obtained for inhibitors having anomeric sulfides that conferred better stability under physiological conditions. The attachment of aryl groups also increased the lipophilicities of the molecules, a desirable property for better cell permeability in in vivo assays. All the

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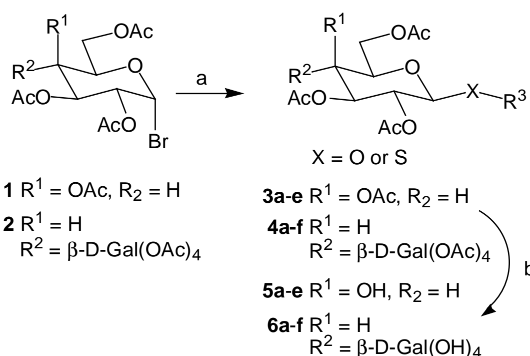
reactions were efficiently accomplished at room temperature under mildly basic conditions, proceeded with good to excellent yields, and were essentially completed within three hours under non-anhydrous conditions.[26] Exploration of anomeric sulfones from anomeric sulfide oxidation has also been accomplished.

Reactions toward Gal-1 and -3 inhibitors started from commercially available acetobromogalactose **1** and peracetylated lactosyl bromide **2**, which was obtained in quantitative yield by treatment of lactose peracetate with HBr in AcOH. As expected, the phase transfer catalyzed nucleophilic displacements of both glycosyl bromides by the aryl alcohols or aryl thiols occurred with complete anomeric inversion to afford only the corresponding β-glycoside derivatives **3a–e** and **4a–f**, respectively (Scheme 1). After de-O-acetylations with methanolic sodium methoxide, compounds **5a–e**




**Scheme 1.** Reagents and conditions: (a) $HXR_3$ (X = O or S), TBAHS, 1 M $Na_2CO_3$, DCM; (b) NaOMe, MeOH.

and **6a–f** were obtained in good to excellent yields (Table 1) and were ready to be evaluated on the galectins using an inhibition of hemagglutination assay described below, with only two synthetic steps.

With aryl thioglycosides **3b**, **4c**, and **4d** in hand, synthesis of anomeric sulfones by oxidation of the sulfide groups using *m*CPBA (2.1 equiv, $CH_2Cl_2$) afforded sulfones **7b**, **8c**, and **8d**, respectively, after de-O-acetylation (Scheme 2) (Table 2).

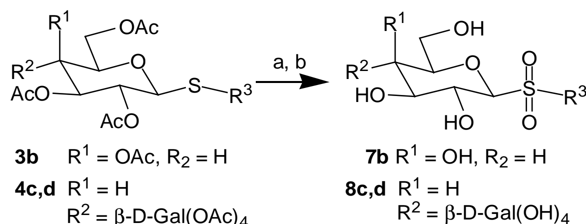
All new compounds and control **9** (galactose) and **10** (lactose) were tested by inhibition of hemagglutination assay at a concentration of 1 μM for both galectins. Hemagglutination assays were performed using red blood cells, type O, fixed with 3% glutaraldehyde–0.0025% $NaN_3$ in PBS,[16,27] to confer both lectins equal relative affinities. Table 3 shows inhibitory properties and relative activities of our derivatives toward Gal-1 and -3. The first overall observation was that none of our compounds bound to human Gal-4, indicating that phenol and thiophenol derivatives were better inhibitors and improved specifity toward Gal-1 and -3.[28] *S*-Galactosides improved the inhibitory properties against Gal-1 (**5a** vs **5d**) and the anomeric sulfones did not have beneficial effect (**7b**). Amongst all galactosides tested, *p*-nitrothiophenyl galactoside **5a** demonstrated the best affinity (2500 μM) with a relative inhibitory potency 20 times better than the control galactose **9**. Lactosides **6f** and **8c** were more specific toward Gal-1 than Gal-3.

Most *O*-aryl lactosides were estimated as being 3 times better than the natural analogue **10**, thus indicating a preference for aromatic aglycons.[24,25] *S*-Lactosides were

**Table 1.** Synthesis of different *O*- and *S*-galacto- and lacto-pyranosides **5–6** using PTC reactions followed by treatment with methanolic sodium methoxide

| Compound | $R^1$ | $R^2$ | $R^3$ | X | Yield (%) |
|---|---|---|---|---|---|
| **5a** | OH | H | $NO_2$ | S | 92[26] |
| **5b** | OH | H | OMe | S | 86 |
| **5c** | OH | H | | S | 78 |
| **5d** | OH | H | $NO_2$ | O | [a] |
| **5e** | OH | H | $O_2N$ | O | 73 |
| **6a** | H | β-D-Gal(OH)$_4$ | $NO_2$ | S | 91[24] |
| **6b** | H | β-D-Gal(OH)$_4$ | OMe | S | 98 |
| **6c** | H | β-D-Gal(OH)$_4$ | | S | 93 |
| **6d** | H | β-D-Gal(OH)$_4$ | Br | S | 88 |
| **6e** | H | β-D-Gal(OH)$_4$ | $NO_2$ | O | 85 |
| **6f** | H | β-D-Gal(OH)$_4$ | $O_2N$ | O | 75 |

[a] Commercially available.




**Scheme 2.** Reagents and conditions: (a) *m*CPBA (2.1 equiv), DCM, 0 °C; (b) NaOMe, MeOH.

**Table 2.** Synthesis of sulfones **7**–**8** using *m*CPBA as an oxidizing agent

| Sulfides | Sulfones | $R^3$ | Yield (%) |
|---|---|---|---|
| **3b** | **7b** | *p*-MeOPh | 89 |
| **4c** | **8c** | β-Napht | 92 |
| **4d** | **8d** | *p*-BrPh | 98 |

**Table 3.** Inhibitory properties of compounds **5**–**10** against Gal-1 and -3

| Compound | Inhibitory properties (mM) | | Relative activity[a,b] | |
|---|---|---|---|---|
| | Galectin-1 | Galectin-3 | Galectin-1 | Galectin-3 |
| **5a** | 2.5 | >5 | 20 | >10 |
| **5b** | >5 | 2.5 | >10 | 20 |
| **5c** | 5 | 5 | 10 | 10 |
| **5d** | 10 | 10 | 5 | 5 |
| **5e** | >5 | >5 | >10 | >10 |
| **6a** | 0.313 | 0.313 | 2.6 | 2.6 |
| **6b** | 0.313 | 0.313 | 2.6 | 2.6 |
| **6c** | 0.313 | 0.313 | 2.6 | 2.6 |
| **6d** | 0.313 | 0.313 | 2.6 | 2.6 |
| **6e** | 0.313 | 0.625 | 2.6 | 1.3 |
| **6f** | 0.08 | 0.625 | 10 | 1.3 |
| **7b** | >5 | >5 | >10 | >10 |
| **8c** | 0.04 | 0.313 | 20 | 2.6 |
| **8d** | 0.313 | 0.313 | 2.6 | 2.6 |
| **9** (Gal) | 50 | 50 | 1 | 1 |
| **10** (Lac) | 0.8 | 0.8 | 1 | 1 |

[a] Compounds **5** and **7** were compared to galactose **9**, compounds **6** and **8** were compared to lactose **10**.
[b] Lactose is ~50× better than galactose.

less potent than *O*-lactosides for Gal-3 (**6a** compared to **6e**). For Gal-1 (**8c**,[29] 40 μM), the presence of an anomeric sulfone provided a compound 20 times better than lactose **10**. The position of the nitro group on the phenyl moiety seemed to play a key role for the specificity between Gal-1 and -3. There was no increase of inhibitory potency for Gal-3 when the nitro group was *para*- or *ortho*-substituted (**6e** and **6f**). Gal-1 was sensitive to this change and became 10 times more potent than the reference **10** when the nitro group was *ortho*-substituted (**6f**, 80 μM).

The nitro group position might be important not only from a steric point of view but also from an electrostatic one. Figure 1 shows the O-linked *o*-nitrophenyl lactoside **6f** extending out of the CRD pocket, thus lacking direct interactions with the lectin itself.[30]

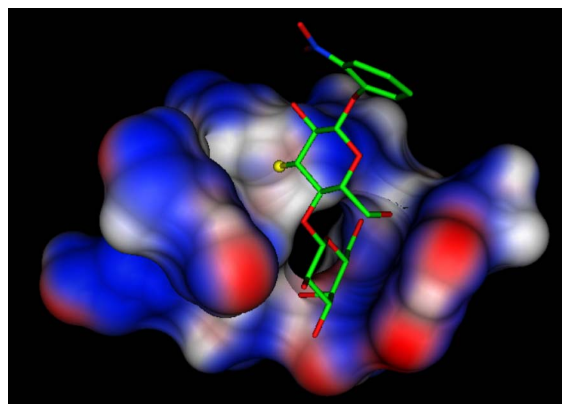


**Figure 1.** Connolly surface using a grid spacing of 0.75 colored by Active Lone Pair and docking of **6f** in Gal-1 showing the *o*-nitrophenyl aglycon pointing out of the CRD pocket and O-3 (yellow) into an H-bonding network. The blue regions are hydrophobic, the red are hydrophilic, while the white indicate regions through which hydrogen bonds are likely to form.

This suggested that the inhibitory specificity was provided from indirect contributions. Hydrogen bondings between Gal-1 and natural lactose only occurred through its O-3 hydroxyl group (Fig. 2) with two coming from Arg48, one from Glu71, and one from the endocyclic oxygen of the galactoside moiety.[31] These interactions create a network of three hydrogen bonds which are common features in carbohydrate–lectin recognitions.[32–34] Semi-empirical calculations correlated with the chemical modifications done at the anomeric position with the effect on charge density at the O-3 oxygen of the glucoside residue of lactose.

The O-3 electron density calculations were made on all lactoside derivatives evaluated on Gal-1. Electron withdrawing aglycons decreased the O-3 electronic densities and favored better interactions with Glu71 which accounted for 50% of the hydrogen bonding.

Hence, a direct correlation exists between the inhibitory potencies and the charge density at O-3 calculated on electrostatic potential. Figure 3 reveals the correlation between the inhibitory properties and the electron densi-

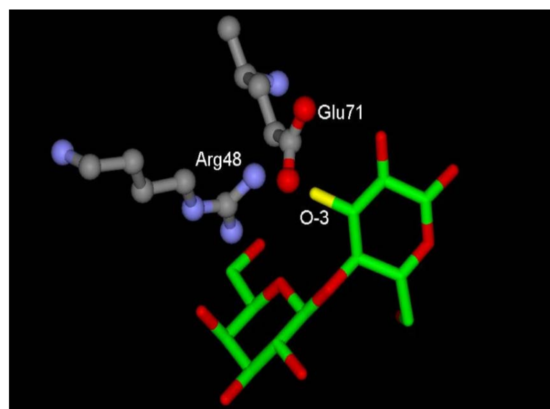



**Figure 2.** O-3 (yellow) hydrogen bonding interactions from lactose **10**, and Arg48 and Glu71 residues of Gal-1 based on the X-ray crystal structure.[30]

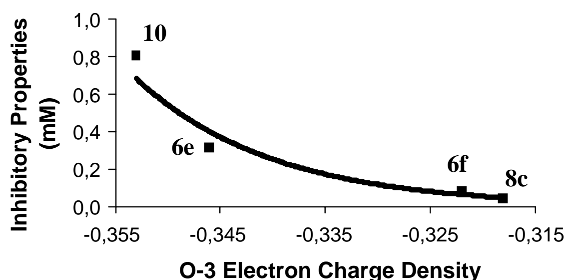



**Figure 3.** Correlation between O-3 electron charge density calculated on electrostatic potential of lactosides **6e**, **6f**, **8c**, and reference **10**, and the inhibitory properties (mM) of those compounds evaluated on Gal-1.

ty of compounds **6e**, **6f**, **8c**, and reference **10** (**6a–d** not shown).[35] *O*-Lactoside **6e** (*p*-nitrophenyl) possesses a theoretical electron density of −0.346 compared to that of *o*-nitrophenyl **6f** of −0.322. This difference of electron density could explain the relative inhibitory potency variation on Gal-1. Compound **8c**, having the lowest O-3 electron density, has also the highest inhibitory potency.

In conclusion, preliminary data showed a novel direction toward more specific Gal-1 and -3 inhibitors. Most compounds were made in high yields with only 2 or 3 synthetic steps from commercially available starting materials. The PTC reaction has proven its practicality for the synthesis of more stable and less polar inhibitors compared to natural ligands. The best inhibitors **5a** (2500 μM) and **8c** (40 μM) have shown 20 times better affinity toward Gal-1 as compared to galactose **9** and lactose **10**, respectively. The strategy used for the synthesis of inhibitors provides a large potential for further improvements by changing the nucleophiles in the PTC reaction. Additionally, the actual modifications, combined to those already published,[20–25,36–38] can lead to improved pharmacological properties. Finally, inhibitory data correlated with the electron density at the O-3 group of the glucose unit within the lactoside ligand that led to a greater inhibitory capacity for Gal-1. Current progresses are made by taking advantage of this effect and are guiding the design of more selective inhibitors against human galectins.[37] Although the above compounds are notably less efficient than those described by Nilsson et al.,[20–23] we used inhibition of hemagglutination assays, known to require higher concentrations of ligand.

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38. Compound **8c**: mp 181.5–182.5 °C; $[\alpha]_D$ + 6.5 ($c$ = 1, DMSO); $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 8.51 (s, 1H, $H_{AR}$), 8.03–7.87 (m, 4H, $H_{AR}$), 7.66–7.58 (m, 2H, $H_{AR}$), 4.42 (d, $J$ = 9.34 Hz, H-1), 4.21 (d, $J$ = 7.42 Hz, 1H, H-1′), 3.65–3.26 (m, 12H, H-2-6, H-2′-6′); $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm) : 135.2, 133.5, 132.8, 130.7, 130.6, 129.9, 129.04, 128.7, 128.7, 125.5 (7 C=O), 104.9 (C-1′), 92.9 (C-1), 81.1 (C-4), 78.9 (C-3), 77.4 (C-2), 77.1 (C-5), 74.8 (C-3′), 72.5 (C-5′), 71.3 (C-2′), 70.3 (C-4′), 62.5 (C-6), 61.5 (C-6′).