Synthesis and Biological Studies of O3-Aryl Galactosides as Galectin Inhibitors

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Dedicated to Prof. Antonio Togni at the occasion of his 65th birthday

β-Galactose derivatives have recently been reported to selectively inhibit galectin-3, and a library of O3-arylated galactosides with varying substitution patterns was designed to study such inhibitions further. The O3-arylated galactosides were synthesized using diaryliodonium salts under mild and transition metal free conditions, providing the target products in moderate to good yields. An O3-trifluoroethylated galactoside was also synthesized using iodonium salt chemistry. Azido-substituted products were subsequently transformed into the corresponding triazoles. After deprotection, a selection of galactoside derivatives were evaluated for inhibitory potencies against galectins-1, 3, 4 N (N-terminal domain), 4 C (C-terminal domain), 7, 8 N, 8 C, 9 N, and 9 C and one compound with promising affinity and selectivity for both the N- and C-terminal domain of galectin-9 was discovered.

Keywords: arylation, carbohydrates, galactosides, galectin inhibitor, hypervalent compounds.

Introduction

Carbohydrates are among the most abundant biomacromolecules and play important roles in living organisms. They serve as energy storage, are found in structural components and facilitate cell signalling. Consequently, carbohydrates are of great interest within medicinal chemistry.

Derivatization of carbohydrates through introduction of functional groups at the oxygens gives rise to a vast array of products with potential biological activity, and methodology development to reach O-functionalized carbohydrates is thus of importance. Common methods include the stepwise addition of leaving groups followed by nucleophilic substitution, leading to inversion of carbohydrate carbon stereochemistry, and esterification, which is performed under harsh conditions causing scope limitations. Recently, a Cu-mediated site-selective O-arylation of carbohydrates with arylboronic acids was reported.

To increase the synthetic routes to these targets, we recently developed an efficient O-arylation of carbohydrates with retention of stereochemistry. The reactions were performed using diaryliodonium salts under mild and transition metal-free conditions and provided a broad substrate scope (Scheme 1). The reaction could also be extended to O-trifluoroethylolation using trifluoroethyl( mesityl)iodonium triflate. β-D-Galactopyranosides are found terminally or internally in glycoconjugates and have the ability to...
bind to the protein family of galectins. Such binding influences the cellular trafficking, localization, and molecular interactions of glycoconjugates and thereby regulate their functions. For example, galectins can interact with such cell surface glycoproteins on T cell receptors responsible for recognizing antigens, and the membrane transport protein CD98. Galectins can also interact with the transforming growth factor-β TGF-β-R, which is involved in paracrine signalling/cell-cell communication, and receptors for vascular endothelial growth (VEGF2-R), which are involved in the formation of the circulatory system and the growth of blood vessels.

In 2016, Nilsson and co-workers reported that a C3-derivatized β-galactose derivative could selectively inhibit galectin-3. The derivative proved successful in a bleomycin-induced mouse model of lung fibrosis and thus proved promising for the development of anti-fibrotic drugs. The O-arylated galactosides generated through the methodology in Scheme 1 were interesting targets for biological studies as galectin inhibitors. The previous scope only included one O3-arylated galactose, and we hypothesized that such compounds have the potential for discovery of novel galectin-inhibitory structural classes as C3-derivatised galactosides are known drug-like inhibitors. Herein, we describe the results from a targeted synthesis of a variety of O3-arylated galactosides and the investigation of their biological properties.

Results and Discussion

Synthesis of O3-Arylated Galactosides

Benzyl-protected galactoside 1 was chosen as the key starting material for diversifying 3-O-arylations and the reaction with diphenyliodonium triflate (2a) was examined first. Product 3a was obtained in 65% yield together with recovered starting material under the previously developed conditions. To increase the conversion, additional 2a and base were added to the reaction after 1 h, resulting in 80% yield of 3a. This stepwise addition set-up was next applied to a series of functionalized diaryliodonium salts 2, which were either symmetric or unsymmetric with a phenyl, mesityl, anisyl or trimethoxyphenyl (TMP) dummy group. The choice of iodonium reagent and the observed chemoselectivities are detailed in the Supporting Information. The reactions were performed without individual optimizations of the reaction conditions, as the main focus was to obtain products 3 for biological investigations (Scheme 2).
Electron-donating alkyl-substituted aryl groups could be transferred to provide products 3a–3d. Arylated products with a variety of electron-withdrawing functional groups in the para-position (3e–3j) were easily obtained, and the yield of nitro-substituted 3e increased from 78% to quantitative under these conditions. Even the p-methoxy-substituted product 3k was successfully obtained, although the arylation resulted in a regiosomeric mixture (3.7 : 1) of 3k : 3o, likely formed through an aryne mechanism. The scope with meta-substituted aryl groups was subsequently examined and delivered products 3l–3o. The azido-functionalized products 3p–3t, which are very interesting for further derivatization, were formed in moderate yields that could not be improved by changing the reaction temperature. Reactions with the 4-azidophenyl (TMP) salt proved to give better yield than the corresponding anisyl salt, see the Supporting Information for further details.

**Derivatization of the O-Arylated Galactosides 3**

The azido-functionalized galactosides 3h and 3n were efficiently transformed into triazoles 4h and 4n through CuAAC reactions. The benzyl protecting groups in O3-aryl galactosides 3 and 4 were subsequently removed through hydrogenation to provide target compounds 5 and 6 (Scheme 4,a). Most products were obtained in good yields, but the deprotection of substrates 3e and 3v failed. The arylation and hydrogenation could also be combined into a sequential one-pot procedure without purification of compound 3. In this fashion, product 5k was obtained in improved overall yield (Scheme 4,b). Basic hydrolysis of methyl ester 5f provided carboxylic acid 7f (Scheme 4,c).

**Synthesis of an O3-Trifluoroethylated Galactoside**

We have previously demonstrated that trifluoroethyldimethyl (mesityl)iodonium triflate (8) can be utilized to transfer a trifluoroethyl moiety to carbohydrates. This methodology was applied to synthesize target product 9 (Scheme 5), but the trifluoroethylation of substrate 1 did not proceed under our reported conditions. A small optimization revealed that product 9 could be obtained by addition of the reagents at −78°C followed by reaction at room temperature overnight. Subsequent deprotection by hydrogenation delivered target product 10.

**Evaluation of 5–7 and 10 as Galectin Inhibitors**

With a variety of diverse 3-O-arylated galactoside derivatives at hand we proceeded to evaluate selected compounds (5a–5f, 5k, 5m, 5o, 5q, 6h, 6n, 7f and 10) for their galectin inhibitory properties (Figure 1). The compounds were evaluated for binding to galectins-1, 3, 4 N (N-terminal domain), 7, 8 N,
8 C (C-terminal domain), 9 N, and 9 C in a reported competitive fluorescence anisotropy assay\cite{18,19} with specific experimental conditions as earlier described\cite{20,21} (Table 1). Analysis of the affinities for galectin-1 in comparison to the unsubstituted methyl β-D-galactopyranoside (11) shows that several 3-O-arylated derivatives were significantly better ligands. Affinities were nevertheless mediocre with $K_d$ values in the range of 1–2 mM. A similar trend was observed for galectin-3, 4 N, 4 C, 7, and 8 C, however with fewer compounds binding with $K_d$ 1–3 mM and more non-binding compounds. Interestingly, galectin-9 N and galectin-9 C found the PMP-derivative 5k as a lower μM inhibitor with a $K_d$ of 260 and 250 μM, respectively, which is significantly better than the unsubstituted reference 11. Furthermore, the tert-butyl derivative 5d also revealed a reasonably good affinity for galectin-9 N. Hence, among the galectins investigated, galectin-9 galactoside ligand binding is discovered to benefit from 3-O-arylation as both carbohydrate-recognizing domains, the N-terminal and the C-terminal, show low μM-affinity for 5k. Other evaluated galactosides presented less pronounced affinity enhancements upon binding to the galectin-9 N- and C-terminal domains than 5k, their interactions with the tested compound are highly dependent on the position and chemical nature of the aryl substituent. The meta-OMe analog 5o is 3–4-fold worse inhibitor than the para-OMe 5k and other para substituents are less efficient than methoxy. Intriguingly, this methoxy-substituent effect is reverse to that recently reported for the corresponding 3-N-arylated galactosides, for which the meta-OMe ($K_d$ 140 μM) was three times more potent inhibitor than the para-OMe ($K_d$ 440 μM).\cite{22}

**Conclusions**

A series of O3-functionalized galactosides was successfully synthesized using iodonium salts under Scheme 5. Synthesis of O3-trifluoroethylated target product 10.

![Scheme 5](image)

Figure 1. 3-O-Arylated and alkylated compounds evaluated for galectin binding affinities.
Table 1. $K_{d}$ Values [μmol of Sa – Sf, Sa, Sm, So, Sg, Sm, 6N, 7F, and 10 against human galectin-1, 3, 4N, 4C, 7N, 8C, 9N, and 9C as measured by a fluorescence anisotropy](a)

<table>
<thead>
<tr>
<th>Galectin</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sa</td>
<td>1500 ± 340</td>
<td>1900 ± 80</td>
<td>460 ± 68</td>
<td>1300 ± 50</td>
<td>620 ± 30</td>
<td>250 ± 90</td>
<td>750 ± 55</td>
<td>1500 ± 100</td>
<td>1400 ± 10</td>
<td>1700 ± 190</td>
</tr>
<tr>
<td>Sf</td>
<td>2700 ± 210</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>3200 ± 170</td>
<td>n.b.</td>
<td>n.b.</td>
<td>3000 ± 440</td>
<td>2400 ± 200</td>
<td>n.b.</td>
</tr>
<tr>
<td>Sm</td>
<td>2200 ± 250</td>
<td>2700 ± 50</td>
<td>1900 ± 90</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>So</td>
<td>1600 ± 290</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>2500 ± 170</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>Sg</td>
<td>1100 ± 160</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>1200 ± 130</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>Sm</td>
<td>770 ± 170</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>1300 ± 270</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>6N</td>
<td>930 ± 20</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>1500 ± 110</td>
<td>1800 ± 190</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>7F</td>
<td>1600 ± 70</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>&gt;10000 n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
</tbody>
</table>

(a) n.b. = Non-binding at the highest concentration tested (10 μM) indicating that the $K_{d}$ > 2 μM. μM = not tested.

Experimental Section

Arylation of Galactoside 1

Galactoside 1 (0.1 mmol) was added to a microwave vial and dissolved in toluene (2 mL) followed by addition of diaryliodonium salt 2 (2 equiv.) and 1BuOK (2 equiv.). The mixture was stirred at r.t. for 1 h, then additional 2 (1–2 equiv.) and 1BuOK (1–2 equiv.) were added. The mixture was stirred at r.t. until it was deemed complete by TLC (1–2 h). The mixture was then concentrated onto Celite under reduced pressure and purified by column chromatography to deliver target product 3.

CuAAC Reaction of Azido-Substituted Galactosides 3h and 3n

Azide 3h (21.0 mg, 36 μmol) was dissolved in CH$_2$Cl$_2$ (2 mL). Methyl propiolate (6.4 μL, 72 μmol), CuI (1 mg, 10 mol%) and $t$Pr$_2$NEt (13 μL, 72 μmol) were added, and the mixture was stirred at r.t. for 48 h. The solvent was removed under reduced pressure, the residue was dissolved in AcOEt and the solution was washed with brine, dried over Na$_2$SO$_4$ and concentrated in vacuo. The product was purified by column chromatography (hexane/AcOEt 7:1–2:1) to give triazole 4h as a colorless oil (19.0 mg, 29 μmol, 79%).

Hydrogenation of Galactosides 3 and 4

A solution of O-aryl galactoside 3 or 4 (28 μmol) in AcOEt/PrOH (1:3, 2 mL) was stirred with Pd(OH)$_2$/C
(10 wt-%, 4 mg) under hydrogen atmosphere at r.t. for 12 h. The mixture was then filtered through Celite and washed with MeOH. The filtrate was concentrated under reduced pressure and purified by column chromatography (CH₂Cl₂/MeOH) to give the desired compound 5 or 6.

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Author Contribution Statement

G. K., K. B. P., G. L. T. and M. M. all participated in the synthesis and characterization of the products. H. L. designed and supervised the fluorescence anisotropy experiments. B. O. and U. J. N. designed the study, supervised the project, participated in data interpretation and wrote the article.

References


