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Protein subtype-targeting through ligand epimerization: Talose-selectivity of galectin-4 and galectin-8

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ABSTRACT

A series of O2 and O3-derivatized methyl β-D-talopyranosides were synthesized and evaluated in vitro as inhibitors of the galactose-binding galectin-1, -2, -3, -4 (N- and C-terminal domains), 8 (N-terminal domain), and 9 (N-terminal domain). Galectin-4C and 8N were found to prefer the D-talopyranose configuration to the natural ligand D-galactopyranose configuration. Derivatization at talose O2 and/or O3 provided selective submillimolar inhibitors for these two galectins.

The family of about 15 different galectin proteins is characterized by high sequence homology and galactose-binding properties, which in general are related to their functions.[1,2] The functions of galectins have been discovered to be mainly in regulating inflammatory processes[3–5] and in cancer growth and metastasis.[6–10] The last few years have witnessed coherent pictures emerging on the mechanisms behind galectins' apparently multi-faceted influences on inflammation and cancer through modulating apoptosis, cell adhesion, angiogenesis, growth factor signaling, and differentiation. Di- or multimerization of galectins allows for ligand cross-linking and lattice formation,[11] which is believed to orchestrate receptor half-lives on cell surfaces.[12–15] A recent important example of this is that differences in cell-surface glycosylation patterns are decisive for galectin-1 lattice formation and subsequent T-cell apoptosis.[16] Intracellularly, galectins have been shown to direct raft-independent apical protein sorting[17] and intracellular targeting.[18] Altogether, a majority of the mechanisms depend on carbohydrate ligand binding, which strongly suggest that the carbohydrate-binding activity of galectins is an attractive therapeutic target.[19] This hypothesis is supported by in vivo experimental observations that inhibition of galectin functions suppresses cancer growth.[20,21]

The galectins are highly conserved and share a common recognition motif in that the α-face of the core galactoside residue of natural ligands stack face-to-face with a tryptophane side chain, while the β-face galactose hydroxyls form hydrogen bonds to polar side chains.[22] Noteworthy is that a large number of these polar amino acids are arginines that line up alongside the natural ligands to form hydrogen bonds (Fig. 1). However, the fine-structure in positioning these polar side chains differs between the galectins. An additional characteristic feature of galectin/ligand complexes is that the core galactose O2 does not interact with the protein, but rather extends out into the surrounding environment.

Following the observation that galactose O2 is directed out from the galectin perpendicular to the arginines that form polar interactions with ligands, D-talopyranose emerged as an attractive scaffold for the design of novel galectin inhibitors. The inverted C2 configuration, relative to D-galactose, offers possibilities for installing affinity-enhancing talose O2 substituents engaging in previously inaccessible interactions with polar amino acid functionalities. As the galectins typically have two or three arginine residues interacting with ligands, talose O2-substituents chosen should be electron-rich. The O2-substituted talosides should preferably also carry aromatic O3-sustituents due to the proven affinity-enhancing effects on several of the galectins by such substituents.[25,28–32] Furthermore, because talosides appear not to be naturally present in mammalians, and hence no endogenous talose-processing enzymes are present, they may offer desirable hydrolytic stability in talose-based drug candidates—the hydrolytic lability is an often-quoted drawback of carbohydrate-based drugs. Herein, the synthesis of a series of methyl 3-O-(4-methylbenzoyl)-β-D-talopyranoside O2 derivatives and their in vitro evaluation as inhibitors of galectin-1, -2, -3, -4N (N-terminal domain), -4C (C-terminal domain), -8N (N-terminal domain), and -9N (N-terminal domain) are presented.

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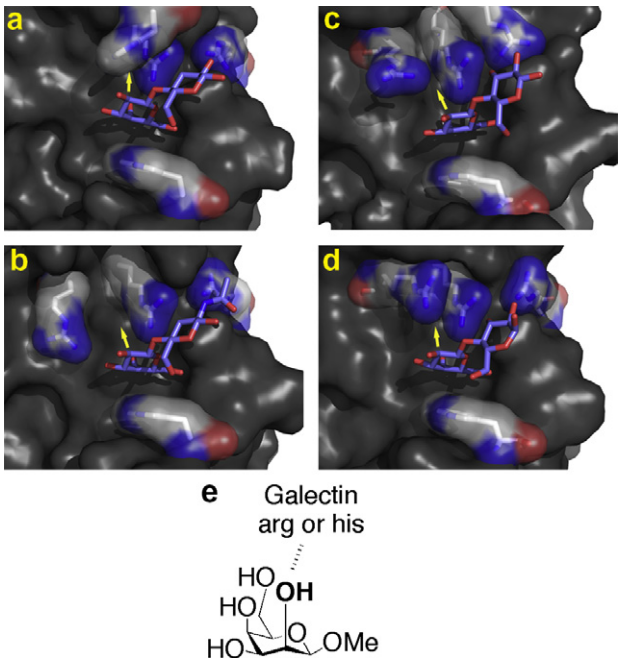


Figure 1. The galactose-binding sites[23] of human galectin-1,[24] -3,[25] -8N,[26] and -9N[27] complexed with lactose or lacNAc (a–d). Galectin-8N is a homology model, while the remaining galectins shown are crystal structures. Yellow arrows indicate the core galactose C2–H2 bond direction, which corresponds to a talose C2–O2 bond direction. (e) Talose HO2 (bold-face) is directed towards basic amino acid side chains of galectins.

The key 3-O-toluolyated intermediate **2** was obtained in a good 70% yield by a selective acylation on the equatorial O3 of the known benzylidene-protected methyl β-D-talopyranoside **1**[33] (Scheme 1). By employing a small excess of acid chloride, the 2,3-O-di-toluoylated **3** could be isolated from the same reaction. Acetylation of **2** gave **4** and sulfation of **2** to give **5** was uneventful as well. PCl_3-mediated installation of the H-phosphonate **6** under strictly anhydrous conditions went smoothly. Subsequently, **7** and **8** were obtained from **6** by pivaloyl chloride-mediated coupling of benzyl alcohol and methanol, respectively, followed by iodine oxidization and water/pyridine hydrolysis.[34] Deprotection of **1** with Dowex 50X8-400 in methanol gave methyl β-D-talopyranoside **9**, while treatment of **2**–**8** using aqueous acetic acid gave **10**–**16**.

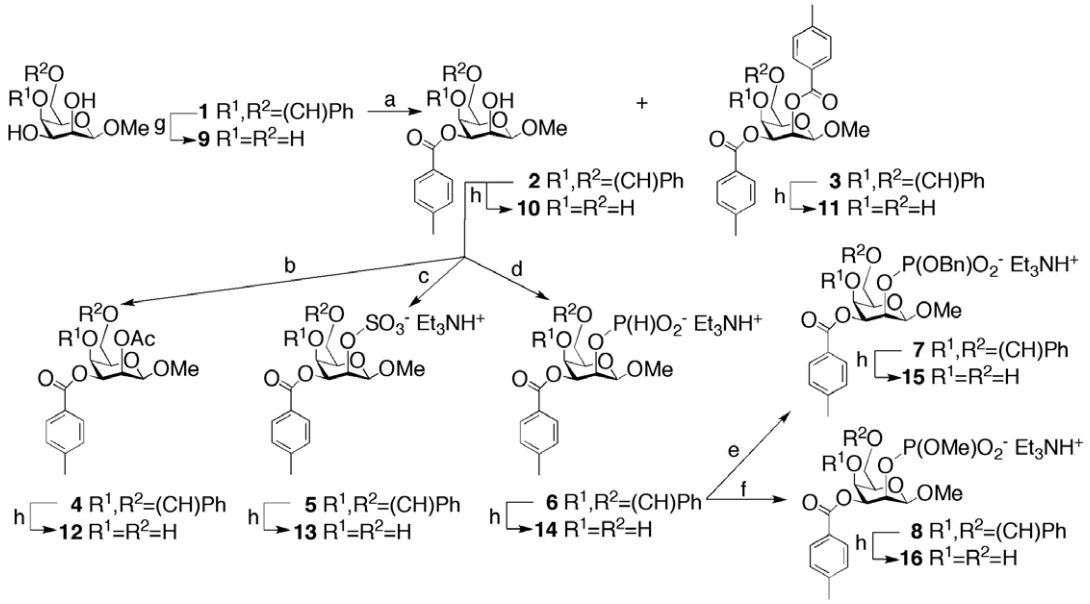
Evaluation of talosides **9**–**16**, together with methyl β-D-galactopyranoside **17** as reference, against galectin-1, -2, -3, -4N, -4C, -8N, and -9N was performed in a competitive in vitro fluorescence polarization assay (Table 1).[35–37] In general, but for a few notable exceptions, the galactose-binding galectins did not bind or bound only weakly to the talosides **9**–**16**.

Although galectin-1 neither bound the underivatized taloside **9** nor the reference galactoside **17**, talose 3-O-toluolyation gave weak but detectable binding when O2 was unsubstituted (**10**) or carried an aromatic substituent (**11** and **15**). This shows that talose-binding by galectin-1 is sensitive to the structure of the O2 substituent, which suggests that optimization of this substituent may provide an avenue towards improved galectin-1 inhibitors.

Galectin-2 recognized the galactoside **17** better than the corresponding taloside **9**. Furthermore, none of the 3-*O*-substituted talosides were recognized by this galectin, except for the H-phosphonate **14** and the benzyl phosphate **15**, which were weakly bound.

Galectin-3 did indeed bind the talose derivatives, although the talopyranose configuration (*i.e.*, **9**) was 2- to 3-fold worse than the galactopyranose configuration (*i.e.*, **17**). Interestingly, the galectin-3 preferences for the talose O2-substitutents parallels those for the analogous O2-substituted galactosides[37] in that the O2-sulfate **13** bound significantly stronger than other O2-substituents. This observation most likely reflects that both galactose and talose O2-substituents interact similarly with galectin-3 Arg144.

While galectin-4N did not bind any of the talosides **9**–**16**, galectin-4C bound the taloside **9** somewhat, but nevertheless significantly, better than the galactoside **17**. Furthermore, O3-toluoylation (**10**) greatly improved the affinity, while O2-substitution had marginal effect except for the 2-*O*-toluoate **11**. The affinity of **11** for galectin-4C (K_d 160 μM) is indeed remarkable, because **11** is a monosaccharide that should be compared to the virtually non-binding prototype galectin monosaccharide ligand **17**. Hence, the taloside **11** can be estimated to display more than two orders of



Scheme 1. Reagents and conditions: (a) 4-Toluoyl chloride, pyr. (**2**: 70% and **3**: 30%), (b) AcCl, pyr., CH_2Cl_2. (c) SO_3·NMe_3, DMF (86%). (d) PCl_3, CH_2Cl_2, MeCN, Imidazole, Et_3N, pyr., H_2O. (e) i—BnOH, pivaloyl chloride, pyr.; ii—I_2, H_2O, pyr. (82% from **2**). (f) i—MeOH, pivaloyl chloride, pyr.; ii—I_2, H_2O, pyr.; (g) Dowex 50X8-400, MeOH (80%); (h) AcOH (aq 70%), 70 °C (**10**: 84%, **11**: 67%, **12**: 91% from **2**, **13**: 75%, **14**: 89% from **2**, **15**: 57%, **16**: 65% from **2**).

Table 1
Galectin dissociation constants (mM) for compounds methyl β-D-galactopyranoside **17** and **9**–**16** as measured by a fluorescence polarization assay[35–37]

Compound	Galectin						
	1	2	3	4N	4C	8N	9N
17	>10[b]	13 ± 3[a]	4.4 ± 0.8	6.6 ± 0.2	>10	6.3 ± 1.5	1.2 ± 0.2
9	>10	>10	10 ± 3.6	>10	10 ± 2.0	1.5 ± 0.5	4.6 ± 0.1
10	≈2	>4[c]	1.4 ± 0.6	>4	1.4 ± 0.7	0.40 ± 0.13	>4
11	1.8 ± 0.6	>4	0.70 ± 0.18	>4	0.16 ± 0.06	3.6 ± 0.1	>4
12	>4	>4	0.55 ± 0.15	>4	1.6 ± 0.1	>4	>4
13	>4	>4	0.25 ± 0.08	>4	3.0 ± 0.2	1.1 ± 0.3	>4
14	>4	1.5 ± 0.5	2.3 ± 0.6	>4	>4	1.5 ± 0.5	>4
15	4.4 ± 1.2	4.6 ± 2.0	2.5 ± 1.3	>4	3.8 ± 0.2	2.0 ± 0.3	>4
16	>4	>4	0.60 ± 0.33	>4	>4	1.0 ± 0	>4

[a] Mean values and standard deviations are from 2 to 12 single-point measurements (nd, not determined).
[b] Compounds **9** and **17** were evaluated at concentrations up to 5 mM and non-inhibitory compounds are estimated to have $K_d > 10$.
[c] Compounds **10**–**16** were evaluated at concentrations up to 2 mM and non-inhibitory compounds are estimated to have $K_d > 4$.

magnitude-improved affinity for galectin-4C, as compared to the galactoside **17**. This result holds promise for the development of efficient monosaccharide inhibitors toward galectin-4C. Such inhibitors may find use in in vivo situations, because activity of a full-length tandem-repeat galectin (galectin-4) can be expected to depend on both individual domains being fully functional, as seen for galectin-8.[26]

Galectin-8N perhaps provided the most interesting results, because this galectin bound the taloside **9** about fourfold tighter than the galactoside **17**. Toluoylation at O3 (**10**) resulted in the first submillimolar inhibitory activity discovered for a monosaccharide against galectin-8N. However, all of the O2-substituents (**11**–**16**) conferred decreased affinities. As galectin-8N indeed binds the taloside configuration well, an investigation of other O2 structures can be expected to lead to improved inhibitors. Again, inhibitors against one domain (galectin-8N) can be expected to block in vivo activity of the full-length protein (galectin-8) because function of both domains (galectin-8 N- and C-terminal domains) is required for activity. This has indeed been demonstrated for galectin-8 binding to cell surfaces.[26]

Finally, galectin-9N did not prefer the talopyranose configuration, because this galectin bound the galactoside **17** four times tighter than the taloside **9**. Substitutions at O3 and O2 (**10**–**16**) depleted binding to galectin-9N. For galectin-9 inhibition by talosides to be viable, presumably talosides inhibiting the C-terminal domain have to be identified.

In conclusion, evaluation of the synthetic O2- and O3-substituted talosides for galectin binding provided interesting clues to the development of selective inhibitors. Galectin-4C and -8N did prefer the talopyranose to the galactopyranose configuration, suggesting that optimized and selective inhibitors of these two galectins may be obtained based on a talopyranose scaffold. In this context, it is particularly noteworthy that galectin-4C and -8N display submillimolar affinity for talopyranosides **11** and **10**, respectively, which is more than one order of magnitude better compared to the prototype galectin ligand **17**. In light of the importance of galectin-8N in intracellular sorting,[18] neutrophile activation,[38] and cancer[39] and of galectin-4 in cancer,[40] the discovery of routes towards efficient inhibitors of these galectins is particularly promising. Replacing galactose by optimized talopyranoside residues in disaccharide molecules (e.g., lacNAc,[25,28] thiodigalactoside,[31,32] or lactose, [41]) and/or attaching additional affinity enhancing structural elements can be expected to provide improved inhibitory potencies.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.066.

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