ChemPubSoc Europe

DOI: 10.1002/cmdc.201700744

CHEMMEDCHEM Communications

Monosaccharide Derivatives with Low-Nanomolar Lectin Affinity and High Selectivity Based on Combined Fluorine–Amide, Phenyl–Arginine, Sulfur–π, and Halogen Bond Interactions

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The design of small and high-affinity lectin inhibitors remains a major challenge because the natural ligand binding sites of lectin are often shallow and have polar character. Herein we report that derivatizing galactose with un-natural structural elements that form multiple non-natural lectin–ligand interactions (orthogonal multipolar fluorine–amide, phenyl–arginine, sulfur–π, and halogen bond) can provide inhibitors with extraordinary affinity (low nanomolar) for the model lectin, galectin-3, which is more than five orders of magnitude higher than the parent galactose; moreover, is selective over other galectins.

Lectin binding events to glycoconjugates are rate-limiting steps in many pathophysiological processes,[1] including host–pathogen interactions, inflammation, immunity, and cancer. Consequently, the discovery of drug-like inhibitors of such interactions is receiving significant attention.[2] However, finding small, high-affinity lectin inhibitors is a major challenge because the lectin carbohydrate binding sites tend to be polar and shallow. Lectins typically bind natural glycans with multiple hydrogen bonds, sometimes enhanced by CH–π stacking of carbohydrate CH groups onto aromatic amino acid side chains, and with recently highlighted contributions from conformational entropy.[3] This usually results in weak-to-medium affinity (micromolar to millimolar) for a small mono- or disaccharides, although exceptions are known. The challenge, then, is to find lectin inhibitors with drug-like (low-nanomolar) affinities and pharmacological properties that are much better than those of the natural ligands. This has been achieved in some cases by modifying natural carbohydrate core structures with unnatural chemical groups,[2a] such as the heparin mimetic fondaparinux,[4] nanomolar-affinity inhibitors of the uropathogenic *E. coli* adhesin FimH obtained by optimized interactions with carbohydrate recognition domatin (CRD)-lining tyrosine side chains,[5] sodium glucose transporter (SGLT2) inhibitors of the glifozin family,[6] influenza neuramnidase inhibitors zanamivir and oseltamivir,[7] selectin inhibitors,[8] siglec inhibitors based on optimized substituents on a sialic acid core structure,[9] and galectins,[10] the topic of this report.

Similar to other lectins, the galectin carbohydrate binding site is shallow, found along the concave side of the ~130-residue β-sandwich CRD.[11] The carbohydrate binding site contains the galectin-defining galactoside binding site conferred by a conserved motif of about seven amino acids. By itself, this subsite has weak binding to galactosides, with K_d values in the millimolar range. Addition of saccharides on either side of the galactose can significantly enhance affinity, but also decrease it. In the most commonly used galectin saccharide inhibitors lactose, *N*-acetyl-lactosamine, and thiodigalactoside, the addition of a monosaccharide on the reducing side of the galactose increases affinity by 10- to 100-fold, to K_d values in the mid-micromolar range. Previously we achieved much higher (nanomolar) affinities by derivatizing such disaccharides with artificial moieties that target additional sites at either end, that is, C3-derivatization of *N*-acetyl-lactosamine and C3,C3′-derivatization of thiodigalactoside with aromatic ester,[12] amide,[10a,b,13] or triazole[10c,14] moieties. Recently we found that C3-multifluorinated phenyl groups providing orthogonal multipolar fluorine–amide interactions strongly enhanced affinity for galectin-3.[10c,14a] This inspired attempts to replace the monosaccharide at the reducing side of galactose with a less polar aglycon, with the aim of still reaching high affinity while keeping the galactose derivatized with a C3-trifluorophenyl group. Indeed, with 4-methylphenylthio as the aglycon **1a**, single-digit micromolar affinity for galectin-3 was reached.[14a]

Herein we report further optimization of the thioglycosidic aglycon that affords galectin-3 inhibitors with low-nanomolar

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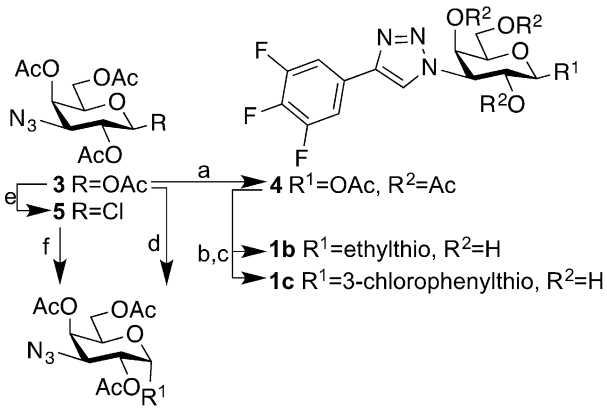
Supporting information (synthesis procedures, crystallization experiments, and data collection and refinement) and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/cmdc.201700744.

affinities—unprecedented for a monosaccharide galectin inhibitor. A series of β- (**1b**,**c**) and α-thio-D-galactopyranosides (**2a**–**k**) carrying the same C3 4-(3,4,5-trifluorophenyl)-1*H*-triazole substituent were synthesized (Scheme 1), and affinities were compared with that of **1a** using a competitive fluorescence anisotropy assay (Tables 1–3).[10c]

Influence of the anomeric configuration. Much to our surprise, α-D-thio-galactopyranoside **2a** had one order of magnitude higher affinity for galectin-3 than the reference β-D-thio-galactopyranoside **1a**. For an aliphatic aglycon, the α-anomer **2b** also had enhanced affinity over the corresponding β-anomer **1b**, albeit with a smaller fivefold difference. Because the methyl α- and β-D-galactopyranosides have similar affinities for galectin-3,[15] this suggests that the larger α-aglycons find new interactions with the galectin that are not typically exploited by natural ligands and that have not previously been explored for artificial ligand design.

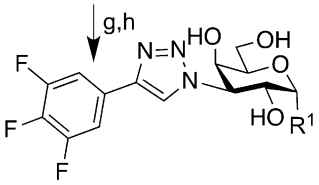


via route e,f

6c R^1=3-chlorophenylthio
6e R^1=phenylthio
6f R^1=3-bromophenylthio
6g R^1=3-iodophenylthio
6h R^1=3,4-dichlorophenylthio
6i R^1=3-chloro-4-cyanophenylthio
6j R^1=2,3-dichlorophenylthio

via route d

6a R^1=4-methylphenylthio
6b R^1=ethylthio
6d R^1=4-chlorophenylthio
6k R^1=3,4-dichlorophenoxy

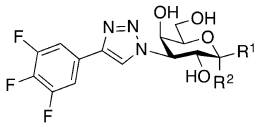


2a R^1=4-methylphenylthio
2b R^1=ethylthio
2c R^1=3-chlorophenylthio
2d R^1=4-chlorophenylthio
2e R^1=phenylthio
2f R^1=3-bromophenylthio
2g R^1=3-iodophenylthio
2h R^1=3,4-dichlorophenylthio
2i R^1=3-chloro-4-cyanophenylthio
2j R^1=2,3-dichlorophenylthio
2k R^1=3,4-dichlorophenoxy

Scheme 1. Synthesis of compounds **1a**–**c** and **2a**–**k**: a) 3,4,5-trifluorophenylacetylene, CuI, DIPEA, toluene, 40 °C, 49%; b) R^1-SH, BF_3·OEt_2, mol. sieves (3 Å), CH_2Cl_2, overnight, 29–50%; c) NaOMe, MeOH, 2 h, 25–87%; d) R^1-SH or R^1-OH, BF_3·OEt_2, mol. sieves (3 Å), CH_2Cl_2 or 1,2-dichloroethane, overnight, 0 °C→RT, 4 days, 21–48%; e) PCl_5, BF_3·OEt_2, CH_2Cl_2, 20 min, 91%; f) R^1-SH, $CsCO_3$ or NaH, 50 °C, DMF 25–66%; g) 1,2,3-trifluoro-5-[2-(trimethylsilyl)ethynyl]benzene, CuI, DIPEA, toluene, 40 °C; h) NaOMe, MeOH, 2 h, 23–91% over two steps.

Influence of phenyl aglycon halogen substituents. To explore these potential new interactions of the α-aglycon, we used the phenyl **2e** (similar affinity as **2a**) as a scaffold and examined different halogen substituents. The 3-chloro **2c** enhanced affinity for galectin-3 by another order of magnitude, leading to K_d ~50 nM, whereas the 4-chloro **2d** did not. 3-Bromo (**2f**) and 3-iodo (**2g**) also lead to enhanced affinity. Introduction of an electron-withdrawing substituent, 4-chloro (**2h**) or 4-cyano (**2i**), next to the 3-chloro group enhanced affinity further by a factor of about two, reaching a remarkable K_d value of 23 nM for **2i**. In contrast, the addition of a 2-chloro to the 3-chloro **2j** decreased affinity by one order of magnitude. The β-anomer **1c** of **2c** had 30-fold lower affinity (K_d: 1.60 ± 0.078 μM) for galectin-3, but was among the best β-anomers (cf. **1a** and **1b** in Table 1).

Table 1. K_d values for **1a**,**b** and **2a**,**b** for human galectin-3 determined by competitive fluorescence polarization.[10c]

Compd			K_d [μM][a]
	R^1	R^2	
1a	4-methylphenylthio	H	5.2[14a]
2a	H	4-methylphenylthio	0.33 ± 0.033
1b	ethylthio	H	5.1 ± 0.53
2b	H	ethylthio	1.0 ± 0.087

[a] Values are the mean ± SEM calculated from 4 to 25 single point measurements from at least two independent experiments.

Influence of the anomeric sulfur. The importance of the α-anomeric sulfur is apparent, as the *O*-glycoside **2k** had ~15-fold lower affinity (K_d: 0.49 ± 0.024 μM) than the corresponding *S*-glycoside **2h**.

Structural analysis. The structure–activity relationships (SAR) described above suggests that the strong affinity enhancement of some compounds with α-linked aglycons is due to a subtle combination of different types of sterically precise interactions with galectin-3. To analyze these further, complexes of the galectin-3 CRD (galectin-3C) with one of the best α-glycosides (**2h**) and an analogous β-compound (**1c**) were compared by X-ray crystallography. The crystals diffracted to 1.2 and 1.5 Å, respectively, and clear ligand density was observed for the galactose residue and its 3C substituent. As expected, their positions were essentially identical for the two compounds, including the orthogonal multipolar ligand fluorine–amide interaction with R144, I145, and S237, and the ligand phenyl–R144 side-chain stacking, and similar as observed earlier for disaccharide derivatives[10c] (Figure 1c,d). The electron density maps were weaker for the aglycons replacing glucose, and these showed double conformations for **1c** (Fig-

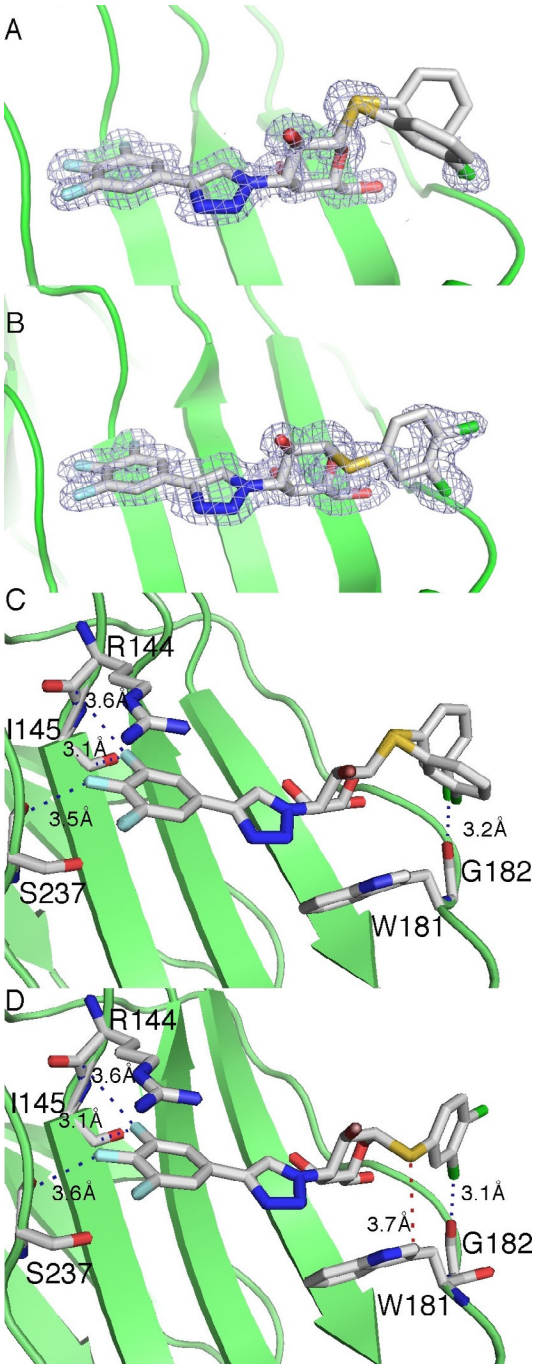
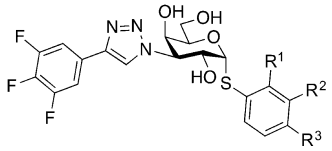


Figure 1. A,B) Electron density maps (grey mesh) $2|F_o|-|F_c|$ αc contoured at 1σ for **1c** and **2h** in complex with galectin-3C. Galectin-3C in complex with: C) **1c** revealing fluorine–amide carbonyl interactions with residues R144, I145, and S237 and an X-bond interaction with G182 backbone carbonyl oxygen atom, and D) **2h** revealing fluorine–amide carbonyl interactions with residues R144, I145, and S237, a S–π interaction with W181, and an X-bond interaction with G182 backbone carbonyl oxygen atom.

ure 1a), modeled with 0.3 and 0.7 occupancy. Despite this, the key affinity-enhancing atoms—the 3-chloro substituent of the phenyl aglycon and the glycosidic sulfur—could be clearly identified, and the phenyl ring modeled.

For both compounds **1c** and **2h**, the 3-chloro substituent has the direction and position expected to form a halogen bond with the backbone carbonyl oxygen atom of G182 in the protein, which provides an explanation for the tenfold higher affinity of **2c** and **2h** over **2d** and **2e** (Table 2). The 4-chloro group of **2d** and **2h** points out in solution and cannot form a

Table 2. K_d values for **2c–j** for human galectin-3 determined by competitive fluorescence polarization.[10c]

Compd				K_d [μM][a]
	R^1	R^2	R^3	
2c	H	Cl	H	0.049 ± 0.0027
2d	H	H	Cl	0.38 ± 0.022
2e	H	H	H	0.52 ± 0.038
2f	H	Br	H	0.031 ± 0.0024
2g	H	I	H	0.058 ± 0.0043
2h	H	Cl	Cl	0.037 ± 0.0010
2i	H	Cl	CN	0.047 ± 0.0063
2j	Cl	Cl	H	0.85 ± 0.031

[a] Values are the mean ± SEM calculated from 4 to 25 single point measurements from at least two independent experiments.

halogen bond with the protein, and does not enhance affinity by itself (cf. **2d**/**2e**). Halogen bond strengths can be enhanced by increasing the size of the halide σ-hole.[16] One way is to replace the chloro substituent with a larger halide; a small affinity enhancement was found here with bromide **2f**, but not with iodide **2g** which may have been too large to fit. Another way to increase the size of the halide σ-hole is to introduce an electron-withdrawing group in the vicinity. Adding 4-chloro **2h** or a 4-cyano group **2i** enhanced affinity over the 3-chloro **2c** by about twofold. In contrast, adding 2-chloro **2j** decreased affinity by tenfold, possibly due to steric conflict with the protein.

The anomeric sulfur atom of the α-D-galactopyranoside **2h** is positioned near W181, suggesting a beneficial sulfur–π interaction[17] not shared by the β-galactosides. This could be an important contribution to the affinity differences in the α/β anomeric pairs **1a**/**2a** (10-fold), **1b**/**2b** (5-fold), and **1c**/**2c** (30-fold). In addition, the oxygen analogue **2k** has a 15-fold lower affinity than the corresponding thiogalactoside **2h**, which also supports the hypothesis of affinity-enhancing effects of the α-anomeric sulfur. The longer C–S bonds and/or smaller C–S–C bond angle of the α-thiogalactosides **2a–2j** may also be important to place the aglycon in a favorable position relative to the equivalent values for O to interact with galectin-3, particularly for 3-chloro to form an optimal halogen bond.

The phenyl aglycon itself did not show evidence for any strong interactions with the protein, which may explain why replacing it with an aliphatic ethyl aglycon in **2b** only led to a twofold decrease in affinity (relative to **2a**). Instead, it may be more important as a scaffold to position the phenyl 3-chloro substituent to form a halogen bond with G182.

Table 3. K_d values of **2h** and methyl α-D-galactopyranoside for human galectins determined by competitive fluorescence polarization.[10c]

Galectin	K_d [μM][c]	
	2h	Me α-gal
1	3.7 ± 0.15	> 10 000[15]
2	0.64 ± 0.11	> 20 000
3	0.037 ± 0.0010	2700[15]
4C[a]	0.13 ± 0.012	> 20 000
4N[b]	2.9 ± 0.40	> 20 000
7	31 ± 3.7	11 000[15]
8C[a]	11 ± 1.9	> 20 000
8N[b]	83 ± 17	6300[15]
9C[a]	2.4 ± 0.41	6200 ± 220
9N[b]	2.7 ± 0.24	2800[15]

[a] C-terminal domain. [b] N-terminal domain. [c] Values are the mean ± SEM calculated from 4 to 25 single point measurements from at least two independent experiments.

Galectin selectivity. Having arrived at monosaccharide inhibitors with exceptional affinities for galectin-3, the important question of selectivity for different members of the galectin family was addressed (Table 3). In comparison with galectin-3, compound **2h** had > 100-fold lower affinity for most of the tested human galectins, and 20- and 4-fold lower affinity for galectin-2 and the C-terminal CRD of galectin-4, respectively. In contrast, methyl α-D-galactopyranoside shows both poor affinity and selectivity for the galectins investigated. Hence, at least some of the specific interactions of the artificial substituents contributing to the high galectin-3 affinity also contribute to the selectivity over other galectins.

Conclusions. Derivatizing a low-affinity monosaccharide with functionalities forming a combination of orthogonal multipolar fluorine–amide, phenyl–arginine, sulfur–π, and halogen bond interactions results in lectin ligands with affinities far surpassing those of common natural ligand fragments (e.g., ~100 000-fold more potent than methyl β-D-galactoside[18] and 5000-fold more potent than methyl β-lactoside);[19] removal of any of these interactions results in a significant loss of affinity. The compounds are the smallest high-affinity galectin-3 inhibitors described and thus constitute a new class of promising drug lead structures. We suggest that systematic introduction of interactions, as those described herein, can be a very useful strategy for the discovery of small ligands that target shallow and polar lectin carbohydrate binding sites, increasing the drugability for any such target. Polar and sp^3-rich monosaccharide scaffolds as drug discovery starting points differ substantially from the small, aromatic, and lipophilic starting scaffolds typically generated by fragment-based lead generation or high-throughput screening strategies, and hence may also provide a useful alternative strategy for a broader range of targets.

Acknowledgements

The authors gratefully acknowledge Barbro Kahl-Knutsson for screening of compounds in the fluorescence polarization assay. This work was supported by the Swedish Research Council (Grant No. 621-2012-2978), the Royal Physiographic Society (Lund, Sweden), a project grant awarded by the Knut and Alice Wallenberg Foundation (KAW 2013.0022), and Galecto Biotech AB, Sweden.

Conflict of interest

F.Z. is an employee and option holder of GalectoBiotech AB. H.L. and U.J.N. are shareholders in GalectoBiotech AB, a company that develops drugs targeting galectins.

Keywords: fluorine multipolar interactions · galectin-3 · halogen bonds · inhibitors · lectins · sulfur–π

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Manuscript received: November 28, 2017
Accepted manuscript online: December 1, 2017
Version of record online: January 15, 2018