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Double Affinity Amplification of Galectin–Ligand Interactions through Arginine–Arene Interactions: Synthetic, Thermodynamic, and Computational Studies with Aromatic Diamido Thiodigalactosides

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Abstract: A series of aromatic mono- or diamido-thiodigalactoside derivatives were synthesized and studied as ligands for galectin-1, -3, -7, -8N terminal domain, and -9N terminal domain. The affinity determination in vitro with competitive fluorescence-polarization experiments and thermodynamic analysis by isothermal microcalorimetry provided a coherent picture of structural requirements for arginine–arene interactions in galectin–ligand binding. Computational studies were employed to explain binding preferences for the different galectins. Galectin-3 formed two almost ideal arene–arginine stacking interactions according to computer modeling and also had the highest affinity for the diamido-thiodigalactosides (K_d below 50 nM). Site-directed mutagenesis of galectin-3 arginines involved in binding corroborated the importance of their interaction with the aromatic diamido-thiodigalactosides. Furthermore, the arginine mutants revealed distinct differences between free, flexible, and solvent-exposed arginine side chains and tightly ion-paired arginine side chains in interactions with aromatic systems.

Keywords: carbohydrates · galectin · inhibitors · thioglycosides

Introduction

The galectins are a family of soluble proteins that are defined by their affinity for β-galactosides and a conserved sequence motif[1,2] with approximately 15 known in humans. Galectin-3 is a multifunctional protein,[3] but experiments in cell cultures and with null mutant mice clearly indicate rate-limiting roles in inflammatory conditions,[4–6] making it a potential target for the development of inhibitors as potential anti-inflammatory agents. Galectin-3 has also been suggested to play a role in cancer,[7] and an inhibitory galectin-3 fragment showed anticancer effects in a mouse model.[8] Galectin-1 has immunomodulatory effects,[9,10] may promote cancer, and molecules interacting with galectin-1 may inhibit cancer growth.[11] Also other galectins have been implicated in cancer[12–14] and are involved in inflammation.[15]

The mechanisms by which galectins influence inflammation and cancer include modulation of apoptosis, cell adhesion, angiogenesis, growth-factor signaling, and, possibly as a result, cell differentiation. Most of these effects appear to require the carbohydrate-binding activity of the galectins, making their carbohydrate-binding site a suitable target for inhibitor design. As galectins may form noncovalent di- or multimers of carbohydrate-recognition domains (CRDs) or have two CRDs within the same peptide chain, their binding to glycoconjugates results in cross-linking and lattice formation.[16] This in turn is thought to be their mechanism of action, resulting in signaling or regulation of receptor targeting or residence time at the cell surface.[17–20] For example, biosynthetic control of membrane-bound protein glycoforms has been demonstrated to modulate galectin-1 binding and T-cell apoptosis.[9] Moreover, it was recently demonstrated that intracellular glycan-dependent clustering involving galectin-3 plays a decisive role in orchestrating raft-independent apical sorting of proteins.[21]

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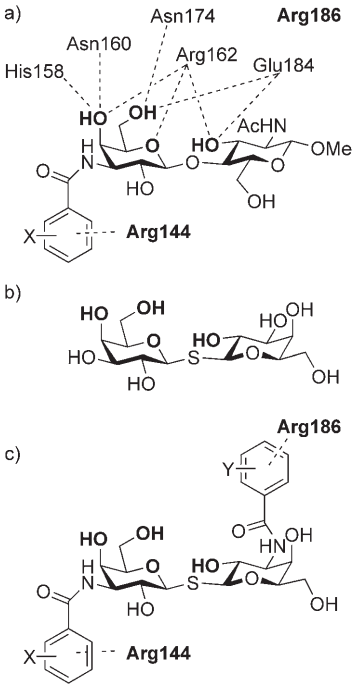
The above observations all point towards clinical implications of modulating galectin–carbohydrate binding activities. Galectin-3C, the cancer-inhibiting fragment of galectin-3 mentioned above, competes for the same carbohydrate ligands, but is incapable of forming aggregates or lattices, and hence, inhibits the function of intact galectin-3.[8] The fact that galectin-3C had no toxic effects in mice together with the fact that galectin-3 null mice are healthy under animal house conditions,[22] suggests that it should be possible to find a therapeutically favorable degree of inhibition of galectin-3 carbohydrate-binding activity without serious side effects. For this purpose, it is desirable that selective monovalent low-molecular-weight inhibitors could be synthesized and such molecules could also be of use as biomolecular tools to investigate the biological mechanisms of the galectins.[23]

We recently reported that large increases in affinity for galectin-3 could be achieved by substitution of the 3′-position of *N*-acetyllactosamine by various aromatic amide moieties.[24,25] An X-ray crystal structure of one of these ligands bound into the binding site of galectin-3 showed that to form the complex, an arginine residue (Arg144) had moved 3.5 Å to form a face-to-face interaction between its guanidinium functionality and the aromatic ring of the amide substituent on *N*-acetyllactosamine (LacNAc)[25] (Scheme 1a). Galectin-3 has another arginine residue in the vicinity of the bound disaccharide. This arginine appeared to be an attractive option to target with a second arene–arginine interaction and thus form even more strongly binding inhibitors. However, rather than use LacNAc as a skeleton to build on, we decided to investigate the simpler and non-natural hydrolytically stable disaccharide thiodigalactoside. Thiodigalactoside has been shown to bind to galectins with a similar affinity to lactose or LacNAc. In addition, X-ray crystal structures were recently published of a toad galectin in complex with LacNAc and with thiodigalactoside; the two galactose residues were bound identically in the β-galactoside binding site, whereas the second galactose of thiodigalactoside was bound in the same place as the GlcNAc of LacNAc, with identical hydrogen bonding networks between the disaccharides and the protein[26] (Scheme 1b). It seems logical to assume that derivatization of thiodigalactoside at the 3-position of the galactose in the β-galactoside binding site will give an aromatic amide that can interact with galectin arginine side chains, for example, Arg144 in galectin-3. However, the 3-position of the second galactose residue is positioned such that similar derivatization at this position will give an amide directed over another arginine side chain, for example, Arg186 in galectin-3 (Scheme 1c).



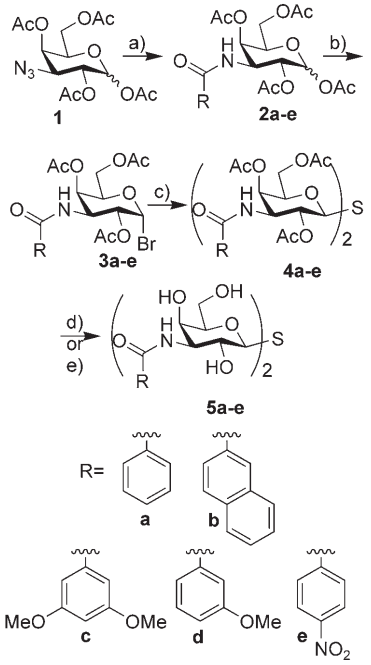
Scheme 1. a) Galectin-3 complex with a LacNAc 3′-benzamido derivative that has high affinity for galectin-3 owing to interactions between the aromatic moiety and Arg144. b) Thiodigalactoside with hydroxy groups that form key interactions with galectin-1 (shown in bold). c) 3,3′-Diamido-thiodigalactoside derivatives proposed to form double interactions with arginine side chains of galectin-3 (Arg144 and Arg186).

Following on from earlier studies,[27] we report herein details of the synthesis of thiodigalactoside derivatives that bear two identical amides at the two 3-positions (i.e. C_2-symmetrical compounds). In addition, we report thiodigalactosides having two different amides at the 3- and 3′-positions, or a single amide at the 3-position of one of the galactose residues, which provided the opportunity to undertake a systematic study to assess the importance of the presence of two aromatic amides and to try to optimize interaction with each of the two relevant arginine residues. We also disclose the results of fluorescence-polarization binding studies of the thiodigalactoside derivatives to galectins-1, -3, -7, -8N, and -9N, as well as microcalorimetry studies of the binding to galectin-3 (C-terminal domain). Furthermore, computational modeling of the interaction of the thiodigalactoside derivatives with galectins corroborated the hypothesis, and binding studies with galectin-3 mutants in which Arg144 and Arg186 were mutated to serine provided a picture of the relative importance of the two arginines in the structurally different interactions with aromatic amides.

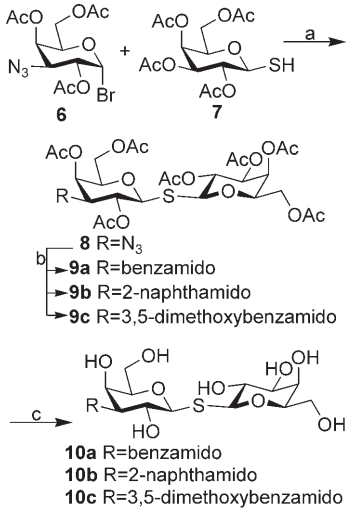
Results and Discussion

Chemical synthesis: Initial attempts to synthesize 3-amide-derivatized thiodigalactosides were based on a symmetrical synthesis of the disaccharide in which both glycosidic bonds are formed in a single reaction step by using two equivalents of the glycosyl bromide and sodium sulfide as a nucleophile. The synthesis of thiodigalactoside has been published by using procedures based on this concept and phase-transfer conditions.[28] To keep the common intermediate as long as possible in the synthesis before diversification and synthesis of different amides, we initially attempted to carry out a sodium sulfide mediated glycosylation by using 2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-D-galactosyl bromide (**6**).[29] Unfortu-

nately, but not unexpectedly, this reaction produced an intractable mixture of products, presumably owing to reduction of the azide functionality. Instead, an earlier diversification was necessary for the synthesis of symmetrical diamides. Thus, *galacto* azide **1**[29] (Scheme 2) was reduced by catalytic hydrogenation, and the resulting amine was acylated with the respective aromatic acyl chlorides to give the amides **2a–e** (Scheme 3). Bromination of **2a–e** was carried



Scheme 2. a) 1. H_2, Pd/C, EtOH, HCl (Et_2O); 2. RCOCl, DCM, pyridine, DMAP, 40–91% over two steps. b) HBr/AcOH, Ac_2O, DCM. c) Na_2S (dried), MeCN, 4-Å molecular sieves. d) NaOMe, MeOH. e) $MeNH_2$, H_2O, 7–27% over three steps. DCM = dichloromethane, DMAP = 4-dimethylaminopyridine.



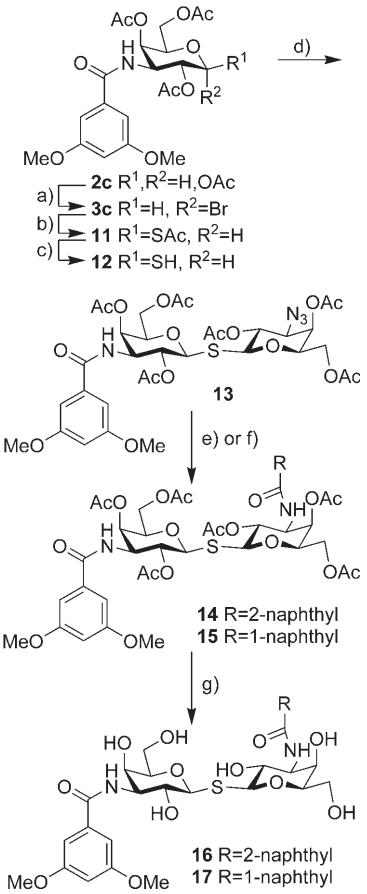
Scheme 3. a) K_2CO_3, MeCN, RT, 72%. b) 1. H_2, EtOH, Pd/C, HCl (Et_2O); 2. RCOCl, DCM, pyridine; **9a**, 43%; **9b**, 40%; **9c**, 66%. c) $MeNH_2$, H_2O; **10a**, 95%; **10b**, 66%; **10c**, 60%.

out by using HBr in AcOH, and the crude bromides **3a–e** were subjected to treatment with dried sodium sulfide in acetonitrile to give the thiodigalactosides **4a–e** in moderate yields. For the amides with more electron-rich aromatic systems, leaving the bromination reaction for longer resulted in the formation of side products. This was particularly pronounced for the 2-naphthamide derivative **3b**, which could help to explain the low yield in the dimerization reaction. In contrast, the electron-poor *para*-nitro derivative **3e** was formed very cleanly. Deprotection of the thiodigalactoside derivatives **4a–e** was carried out by using either an amine or sodium methoxide in methanol to give the desired symmetrical diamides **5a–e**.

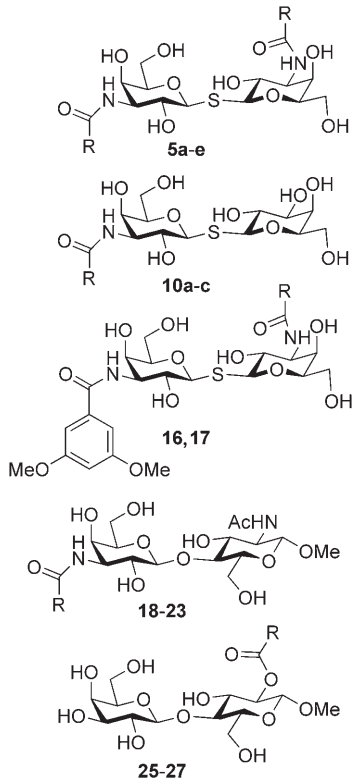
Even though the azide functionality in **6** was intolerant of sodium sulfide, it has been shown that an azide can survive similar reactions in which other sulfur nucleophiles are present. Thus, for the synthesis of unsymmetrical amides, we decided to try to substitute the azido bromide **6** with an anomeric thiol **7**. Treatment of the azido galactosyl bromide **6** with *galacto* thiol **7** under the same conditions gave the desired monoazido thiodigalactoside derivative **8**. Catalytic hydrogenolysis of the monoazido disaccharide **8** and subsequent acylation with aromatic acyl chlorides gave the monoamides **9a–c**, which were then deacetylated to give the deprotected monoamide-substituted thiodigalactosides **10a–c** (Scheme 3).

To synthesize unsymmetrical thiodigalactoside diamides, that is, derivatives bearing different amides at the 3- and 3′-positions, we planned to again use the azido galactosyl bromide **6** as the electrophile, however, a *galacto* thiol with an aromatic amide already in place at the 3-position (**12**) was used as a nucleophile this time. Thus, the dimethoxybenzamide-substituted galactose derivative **2c** was brominated as above to give **3c**, and an anomeric thioacetyl group was introduced by using potassium thioacetate to give **11** (Scheme 4). Selective hydrolysis of the thioacetate was achieved by using sodium methoxide at −40 °C, and the crude anomeric thiol **12** was treated with the azido bromide **6** under the same conditions as described earlier for the unsymmetrical glycosylations to give the desired disaccharide **13** as the only identified product. Catalytic hydrogenolysis and subsequent acylation with 2-naphthoyl chloride and 1-naphthoyl chloride gave the unsymmetrical diamides **14** and **15**, respectively, which were deacetylated to give the deprotected unsymmetrical diamide-substituted thiodigalactosides **16** and **17** (Scheme 5).

Binding of thiodigalactoside amides to galectin-1, -3, -7, -8N, and -9N: The set of five symmetrical diamides **5a–e**, three monoamides **10a–c**, and two unsymmetrical diamides **16** and **17** were evaluated for binding to galectin-1, -3, -7, -8N, and -9N by using a fluorescence polarization assay (Table 1).[31,32] Data for aromatic LacNAc C3′-amides **18–23**[25] and lactose C2 esters **24–27**[30] are included for reference as the they address the specific interactions of Arg144 and Arg186 from galectin-3 and the corresponding residues in galectin-1, -7, -8N, and -9N. The unsubstituted thiodigalactoside **28** was also included for reference, as were the



Scheme 4. a) HBr (33% in AcOH), DCM. b) KSAc, MeCN, 72% from **2c**. c) NaOMe (1 equiv), MeOH. d) **6**, K_2CO_3, MeCN, 39% from **1**. e) 1. H_2, Pd/C, EtOH, HCl; 2. 1-naphthoyl chloride, DCM, py. f) 1. H_2, Pd/C, EtOH, HCl; 2. 2-naphthoyl chloride, DCM, py. g) $MeNH_2$ H_2O, **16**, 67% from **13**, **17**, 50% from **13**.



Scheme 5. Generic structures of compounds evaluated against galectin-1, -3, -7, -8N, and -9N.

methyl glycosides of LacNAc **29** and lactose **30** (Scheme 5 and Table 1).

Galectin-1 showed some increased affinity for **5a–d** over thiodigalactoside **28**, presumably owing to formation of one arginine–arene interaction. This galectin lacks an arginine in close proximity to galactose C3 of the complexed lactose or LacNAc, which is reflected by the observation that aromatic LacNAc C3′ amides **18–23** show limited affinity enhancement over the parent LacNAc **29**. The aromatic C2 lactose esters **24–27** display significantly improved affinity over the parent lactoside **30** owing to arginine–arene interactions with Arg74,[30] suggesting that the interaction of the amido groups of **5a–d** with the galectin-1 Arg74 group is responsible for their improved affinity.

In the case of galectin-3, the increase in affinity of the amide-substituted derivatives over the parent thiodigalactoside **28** is particularly impressive, with increases as great as several hundred-fold for the best inhibitors. In addition, a progressive increase in affinity is observed for galectin-3 along each of the homologous series of unsubstituted, monosubstituted, and disubstituted thiodigalactosides studied (i.e. benzamide **5a** (1.7 μM) > **10a** (3.2 μM) > **28** (49 μM), 2-naphthamide **5b** (0.16 μM) > **10b** (1.8 μM) > **28** (49 μM), and 3,5-dimethoxybenzamide **5c** (0.046 μM) > **10c** (1.1 μM) > **28** (49 μM)). The question then emerged as to whether the two arginine side chains, Arg144 and Arg186, display different preferences for aromatic structures. To answer this question, the inclusion of the known LacNAc 3′-amides **18–23** and the lactose 2-esters **24–27** in addition to the unsymmetrical thiodigalactosides **16** and **17** in the analysis is important. This is because the unsymmetrical nature of these two classes of compounds places their aromatic moieties in a position that allows them to interact with only Arg144 or Arg186, respectively. Detailed insight into the preferences of Arg144 and Arg186 for different aromatic structures could provide guidance for the design of aromatic amides that are individually optimized to interact with either of the arginine residue. The aromatic moieties of the LacNAc amides, 3′-amides **18–23**, interact with Arg144 and a preference for the 3,5-dimethoxy and 4-nitrobenzamides **21** and **22** and the 2-naphthamide **20** is observed. The 1-naphthamide **19** is almost one order of magnitude worse than the 2-naphthamide **20**. In contrast, the 1-naphthoate **25** is twice as potent as the 2-naphthoate **26**, which suggests that Arg186 prefers the 1-naphthyl group, as the aromatic moieties of the lactose 2-esters **24–27** are bound to interact with Arg186. The 3,5-dimethoxybenzoate **27** binds well to galectin-3 and the 3,5-dimethoxyphenyl group can thus be concluded to interact well with both Arg144 and Arg186. The latter observation helps to explain why, although both unsymmetrical diamides **16**

Table 1. Affinity constants (μM) of thiodigalactoside derivatives and reference compounds for galectins-1, -3, -7, -8N, and -9N.[a]

		Galectin				
		-1	-3	-7	-8N	-9N
symmetrical diamido-thiodigalactosides						
5a	R = phenyl	35 ± 22	1.7 ± 0.4[b]	4.3 ± 3.5	high	7.0 ± 1.5
5b	R = 2-naphthyl	9.6 ± 7.2	0.16 ± 0.04[b]	1.7 ± 0.2	> 100	0.73 ± 0.14
5c	R = 3,5-dimethoxyphenyl	4.7 ± 1.4	0.046 ± 0.020[b]	17 ± 0.2	high	0.9 ± 0.3
5d	R = 3-methoxyphenyl	≈2	0.050 ± 0.012[b]	2.8 ± 1.1	≈100	1.8 ± 0.4
5e	R = 4-nitrophenyl	n.d.[c]	0.049 ± 0.029[b]	4.0 ± 1.0	> 100	0.68 ± 0.16
monoamido-thiodigalactosides						
10a	R = phenyl	n.d.[c]	3.2 ± 0.2	17 ± 5	350 ± 17	2.1 ± 0.8
10b	R = 2-naphthyl	n.d.[c]	1.8 ± 0.4	9.8 ± 4.4	30 ± 5	0.42 ± 0.18
10c	R = 3,5-dimethoxyphenyl	n.d.[c]	1.1 ± 0.1	10 ± 2	72 ± 12	0.69 ± 0.07
unsymmetrical diamido-thiodigalactosides						
16	R = 2-naphthyl	n.d.[c]	0.069 ± 0.13	3.5 ± 1.4	high	0.48 ± 0.15
17	R = 1-naphthyl	n.d.[c]	0.052 ± 0.021	1.0 ± 0.8	high	0.63 ± 0.02
LacNAc 3′-amides						
18	R = phenyl	22 ± 6	6.7[25]	41 ± 1	high	≈60
19	R = 1-naphthyl	25 ± 8	4.4[25]	7.6 ± 3.3	high	> 100
20	R = 2-naphthyl	11 ± 4	0.48[25]	≈100	high	high
21	R = 3,5-dimethoxyphenyl	30 ± 16	1.1[25]	≈100	high	> 100
22	R = 4-nitrophenyl	8.8 ± 4.4	0.95[25]	28 ± 6	high	47 ± 6
23	R = 3- methoxyphenyl	67 ± 22	2.5[25]	37 ± 5	high	> 100
lactose esters[30]						
24	R = phenyl	4.4	7.8	145	> 1000	40
25	R = 1-naphthyl	14	2.5	54	530	1.6
26	R = 2-naphthyl	8.7	5.2	124	740	2.7
27	R = 3,5-dimethoxyphenyl	21	2.2	86	> 1000	14
reference compounds						
28	thiodigalactoside	24 ± 11	49 ± 11[b]	160 ± 18	61 ± 17	38 ± 8
29	Me β-LacNAc[30]	65	59	550	1000	490
30	Me β-Lac[30]	187	160	110	62	23

[a] Determined by a fluorescence polarization assay. The location of R is shown in Scheme 5. Galectin-1, -7, and -9N were studied at 0 °C, whereas galectin-3 and -8N were studied at ambient temperature. [b] Average of 14 experiments provided basically the same K_d values but with improved data statistics as compared with previously reported data.[31] [c] Not determined.

In galectins-7 and -9N, both of the relevant arginine residues are present (i.e. Arg31 and Arg79 for galectin-7 and Arg44 and Arg87 for galectin-9N), and an increase in affinity is seen for the amide-substituted thiodigalactosides over the parent compound **28**. Notably, the increases in affinity were not as big for these galectins as they were for galectin-3. In addition, it was not the same compounds that had the highest affinity for all the different galectins. This means that the affinities of the thiodigalactosides for the galectins are governed by the local environment around the bound ligands, where, for example, steric and electronic factors can be very different between the different galectins. For example, the higher affinity of the mono-(3,5-dimethoxybenzamide) derivative **10c** than its bis-substituted analogue **5c** for both galectin-7 and -9N may arise from a steric clash by the second 3,5-dimethoxybenzamide moiety, which is avoided in the case of galectin-3.

and **17** bind strongly to galectin-3, the 2-naphthamide **16** binds more tightly. Thus, compounds **16** and **17** presumably bind galectin-3 in a manner that allows their naphthamides to interact with Arg144 and the 3,5-dimethoxybenzamide to interact with Arg186. Computational modeling, as discussed below, corroborated this hypothesis.

Galectin-7 and -9N show considerable affinity enhancements in response to the presence of one or two aromatic amides on thiodigalactoside. The case of galectin-8N is, however, in striking contrast. Here, all but one of the amide-substituted derivatives have lower affinity than the parent compound, and the one compound with an increased affinity (**10c**) only binds two times better than unsubstituted thiodigalactoside **28**. In interpreting this result, it is useful to compare the amino acid sequences of the different galectins (Figure 1).

Clearly, in galectin-8N, there is no arginine residue that corresponds to the Arg186 of galectin-3 according to sequence alignment. This fact, coupled with the low affinity of the LacNAc amide derivatives **18**–**23** for galectin-8N, supports our theory that the diamido-thiodigalactoside derivatives **5a**–**e** bind to galectin-3 with double arene–arginine interactions (with Arg144 and Arg186).

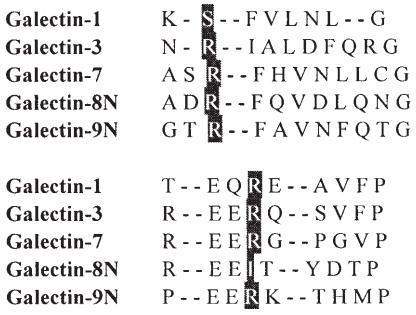


Figure 1. Partial sequence alignment of galectin-1, -3, -7, -8N, and -9N. The residues corresponding to Arg144 and Arg186 in galectin-3 are highlighted.

As the aromatic structures chosen had already been shown to produce derivatives with a high affinity for galectin-3 through interaction with Arg144, albeit on a LacNAc skeleton, it is perhaps not surprising that the resulting thiodigalactoside amides had the highest affinity for galectin-3. It is possible, therefore, that exploration of other analogues with aromatic amides or other structures would yield com-

pounds with higher affinities and selectivities for galectin-7 and -9N.

Computational studies of thiodigalactosides in complex with galectins: To obtain a deeper insight into the structural features of diamido-thiodigalactosides binding to galectins, computational studies were performed. Starting from published X-ray crystal structures of galectin-1, -7, and -9N in complex with lactose,[33–35] galectin-3 in complex with a C3′-amido-derivatised LacNAc-based inhibitor,[25] and a homology model of galectin-8N in complex with LacNAc,[36] the different ligand structures **5a–e**, **10a–e**, **16**, and **17** were built into the galectin binding sites and energy-minimization calculations were performed. Several starting conformations of the ligands with respect to amide conformation and conformations of substituents on the aromatic amides were used as the input in the calculations. All minimizations converged to low-energy complex structures and the complexes with best inhibitor of each of the galectins are depicted in Figure 2. In each case, one of the thiodigalactoside galactoside residues is bound in the conserved galactose site through stacking of its β face onto a tryptophan side chain and with HO4 and HO6 deeply buried and hydrogen bonded. An ion-pairing network of arginine groups and carboxylate groups, which results in an extended flat π-electron system, interacts, in each of the galectins, except galectin-8N, with one aromatic

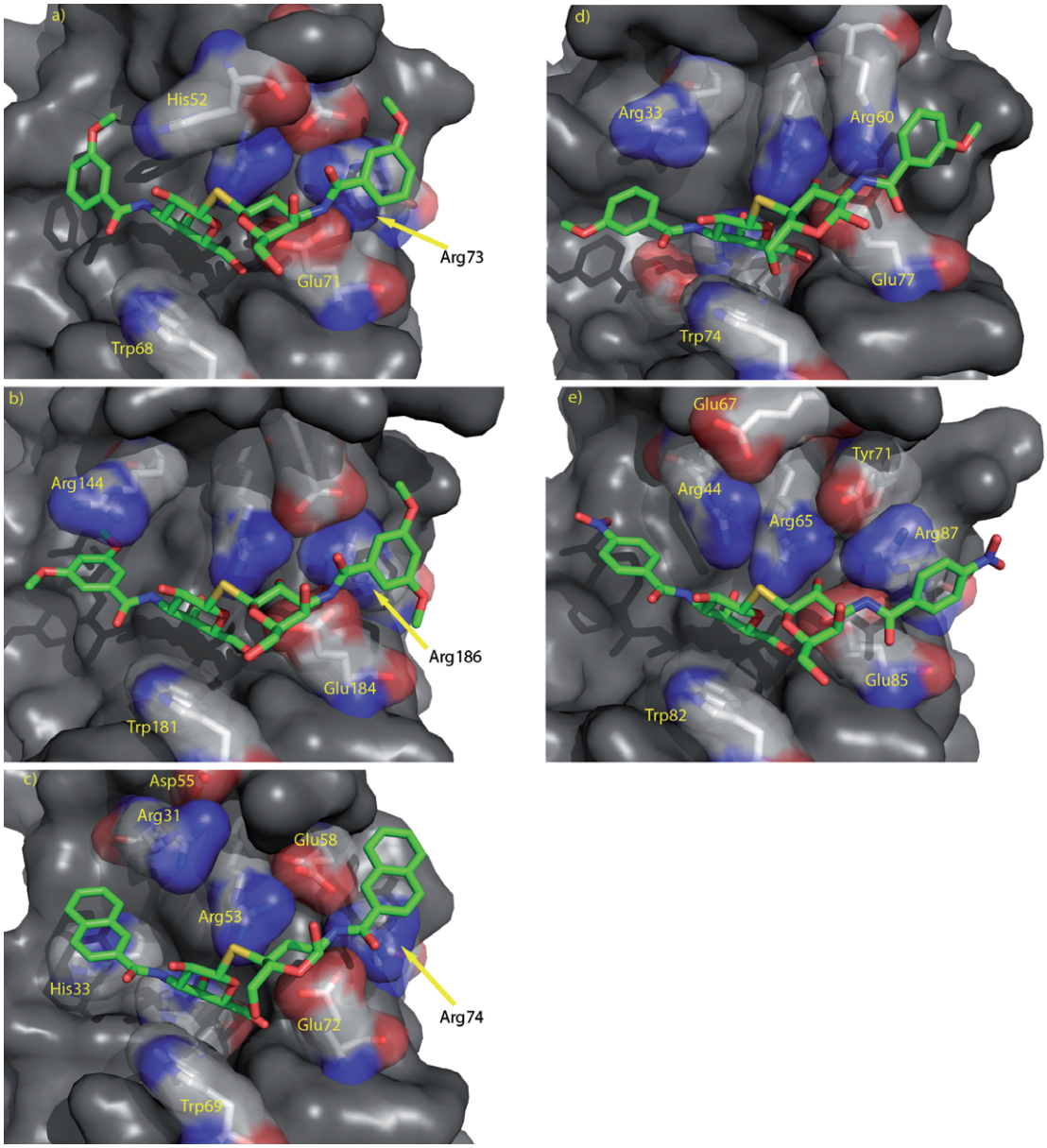


Figure 2. Modeled low-energy structures of galectins in complex with their respective best diamido-thiodigalactoside inhibitors. The side chains directly involved in complex formation with the inhibitors are depicted by transparent surfaces. a) Galectin-1 in complex with **5d**; b) galectin-3 in complex with **5c**; c) galectin-7 in complex with **5b**; d) galectin-8N in complex with **5d**; e) galectin-9N in complex with **5e**.

amide residue of the inhibitors with varying degrees of complementarity. This is in analogy with the observation that lactose 2-*O*-benzoates[30] were efficient inhibitors of galectin-1 and -3 and were tolerated by galectin-7 and -9N owing to stacking of the aromatic esters onto the ion-pair network of arginine and carboxylate groups. The remaining aromatic amide residues of the inhibitors are left to interact with variable and nonconserved structural elements near C3 of the buried galactose residue.

Detailed analysis of the binding to each of the galectins: In galectin-1, one aromatic amide of the amido-thiodigalactosides **5a–e**, **10a–c**, **16**, and **17** stacks onto an arginine (Arg73) side chain in an network of ion-pairing groups of arginine and carboxylate groups (Figure 2a). The corresponding aromatic amide oxygen group points away from the protein surface, thus avoiding repulsive van der Waals contacts with the Glu71 carboxylate. The remaining aromatic amide fills a cavity close to His52, however, only with moderate shape and electronic complementarity.

In the case of the tightest-binding galectin, galectin-3, all energy-minimized complexes supported the hypothesis of two simultaneous affinity-enhancing arginine–arene interactions (Figure 2b). Indeed, the complexes between galectin-3 and amido-thiodigalactosides **5a–e**, **10a–c**, **16**, and **17** all displayed high surface complementarities. The lowest-energy complexes had one aromatic moiety that stacked effectively onto Arg186, which was involved in an arginine–carboxylate ion-pairing network. As has been seen in the galectin-1 complexes, the corresponding aromatic amide oxygen groups point away from the protein surface in all cases, thus avoiding repulsive van der Waals contacts with a glutamate (Glu184) carboxylate group. The remaining aromatic moiety shows optimal complementarity with a cavity formed between the Arg144 side chain and the protein backbone. Within this cavity, Arg144 stacks in a face-to-face manner with the aromatic amide stacked in the same way. This has been observed in a published complex with an aromatic C3 amide of LacNAc.[25] The low-energy complexes with the unsymmetrical amides **16** and **17** both had the 3,5-dimethoxybenzamide moiety stacked onto Arg186. Thus, the naphthamides of **16** and **17** presumably interact with Arg144, which may explain why the 2-naphthamide **16** binds more tightly; 2-naphthamides are preferred over 1-naphthamides by Arg144.[25]

One aromatic moiety of the amido-thiodigalactosides **5a–e**, **10a–c**, **16**, and **17** also stacks face-to-face onto an arginine (Arg74) of the arginine–carboxylate ion-pair network of galectin-7 (Figure 2c). However, as a result of this arene–Arg74 stacking, the corresponding amide oxygen atom will end up in an unfavorable position close to the carboxylate group of either Glu58 or Glu72. The remaining aromatic moiety of the bis-amido-thiodigalactosides shows comparatively poor complementarity with galectin-7 and does not stack with Arg31 (corresponding to Arg144 in galectin-3), but instead interacts edge-to-face with His33. The reason for this is probably that Arg31 is interacting with Asp55 and Arg53,[34] thus preventing it from rearranging to stack with the aromatic amide of the inhibitor. The corresponding Arg144 of galectin-3 does not form such distinct interactions with other amino acids and thus has a lower barrier of rearrangement and stacking with the aromatic amide of the inhibitor.

An arginine residue corresponding to Arg86 in the arginine–carboxylate network of galectin-3 is absent in galectin-8N (mentioned above and shown in Figure 1). However, the modeling studies with the galectin-8N homology model suggested that one aromatic group of the amido-thiodigalactosides **5a–e**, **10a–c**, **16**, and **17** instead stacked face-to-face onto another arginine side chain, Arg60, albeit with moderate surface complementarity (Figure 2d). Furthermore, in such a low-energy conformation, the corresponding amide oxygen is placed in an unfavorable position close to Glu77, which is similar to the observation for galectin-7 discussed above and also in accordance with the observation that lactose 2-*O*-benzoates bind poorly[30] to this galectin. The remaining aromatic amide of the inhibitors **5a–e**, **10a–c**, **16**, and **17** appears not to be able to interact favorably with galectin-8N as it shows low shape complementarity and does not reach the Arg33 side chain (corresponding to Arg144 in galectin-3). Taken together, none of the aromatic amido moieties of the amido-thiodigalactosides **5a–e**, **10a–c**, **16**, **17**, the reference LacNAc amides **18–23**, or lactose esters **24–27** are involved in favorable interactions with galectin-8N.

The ion-paring network of the ligand-binding site of galectin-9N is different in that it has a tyrosine residue that corresponds to a carboxylate side chain in the other galectins. Nevertheless, one aromatic moiety of the amido-thiodigalactosides **5a–e**, **10a–c**, **16**, and **17** stacks face-to-face onto an arginine group (Arg87) of the ion-pair network of galectin-9N (Figure 2e). This interaction provides some affinity amplification, which is corroborated by the observation that lactose esters **24–27** display somewhat improved binding over lactose.[30] A possible explanation for the moderate affinity amplifications is that the corresponding amide oxygen is bound to come in contact with a glutamate (Glu85), which is a structural feature that is analogous to the calculated complexes with galectin-7 and -8N. The remaining aromatic amide moieties of the amido-thiodigalactosides **5a–e**, **16**, and **17** provide some affinity amplification as well, which is reflected in the results with the corresponding LacNAc amides **18–23**. This relatively limited effect may be attributed to the lack of interaction with Arg44 (corresponding to Arg144 in galectin-3). According to the calculation and the published structure,[35] Arg44 of galectin-9N preferentially takes part in interactions with Glu67 and Arg65, a structural feature that is similar to that observed in galectin-7. Nevertheless, the moderate individual affinity amplifications by the two aromatic amides of **5a–e**, **16**, and **17** result in a significantly improved total affinity amplification over the parent thiodigalactoside **28**.

Thermodynamic characterization of galectin-3 complexes with microcalorimetry: To investigate the thermodynamic characteristics of the interactions, we decided to carry out

an isothermal calorimetry experiment with the galectin that was most efficiently inhibited by the thiodigalactoside amides. Thus, the binding of the five C_2-symmetrical diamides **5a–e**, the three monoamides **10a–c**, and thiodigalactoside **28** to galectin-3C (C-terminal) was measured by using microcalorimetry (Figure 3 and Table 2). Galectin-3C was used to avoid interfering self-aggregation through the N-terminal, which was observed with intact galectin-3.[37] In general, the affinity constants obtained by microcalorimetry correspond well to those obtained in the fluorescence polarization assay. Increases in enthalpic contributions by the aromatic amide moieties were responsible for the affinity amplifications observed. Partial entropy compensations occurred for the tighter-binding diamides **5b**, **5d**, and **5e**, which suggests that desolvation effects are significant.

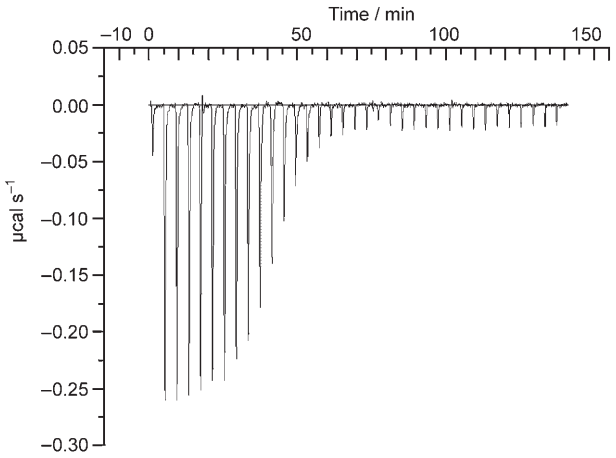


Figure 3. Isothermal titration calorimetry of galectin-3 with **5e**.

Table 2. Isothermal microcalorimetry data for **5a–e**, **10a–c**, and **28** binding to galectin-3C.

	$-\Delta H$ [kcal mol^{-1}]	$-T\Delta S$ [kcal mol^{-1}]	n	K_d [μM]
28	8.3 ± 0.4	2.9	0.93	118 ± 3
5a	9.6 ± 0.06	1.5	1.04	1.1 ± 0.1
10a	10.9 ± 0.1	3.7	0.94	5.9 ± 0.2
5b	15.7 ± 0.1	5.2	0.93	0.020 ± 0.003
10b	10.2 ± 0.04	2.3	0.96	1.8 ± 0.1
5c	12.8 ± 0.2	2.5	1.00	0.029 ± 0.005
10c	9.4 ± 0.05	1.8	1.03	2.7 ± 0.1
5d	15.9 ± 0.1	6.7	0.93	0.19 ± 0.01
5e	13.4 ± 0.2	3.5	1.03	0.055 ± 0.006

Interestingly, stepwise addition of amides in the 2-naphthoyl (**28**, **10b**, **5b**) and 3,5-dimethoxybenzoyl (**28**, **10c**, **5c**) series gave an increase in ΔH for each added amide, which can be interpreted as a stepwise addition of arginine–arene interactions between galectin and ligand. In the unsubstituted benzamide series (**28**, **10a**, **5a**), however, there is an increase in ΔH between **28** and **10a**, but a decrease upon the addition of the second benzamide to form **5a**. It is possible that the benzamide moiety is too small to be able to establish good contact simultaneously with both arginine residues (Arg144 and Arg186), which results in a loss in ΔH. This could explain the rather marginal increase in affinity on going from a mono- to a diamide in this series compared with the much bigger increases for **10b**→**5b** and **10c**→**5c**.

Binding of the thiodigalactosides to galectin-3 arginine mutants: Two arginine mutants of galectin-3, R144S and R186S,[30] were evaluated for binding to the thiodigalactoside amides **5a–e**, **16**, and **17** to obtain information regarding the relative importance of the two arginine–arene interactions (Table 3). Differential scanning calorimetry with the two mutants showed that they are as stable as the wild type, which suggests that no major conformational rearrangements occur upon Arg–Ser mutation.[38] Furthermore, the stability of the mutants, as determined by differential scanning calorimetry, was increased by the presence of lactose to the same extent as the wild type.

Table 3. Dissociation constants (μM) at 4 °C of thiodigalactoside derivatives and reference compounds for wild-type galectins-3 and R144S and R186S mutants.[a]

		Wt	R144S	R186S
symmetrical diamido-thiodigalactosides				
5a	R = phenyl	0.62 ± 0.006	1.4 ± 0.2	> 500
5b	R = 2-naphthoyl	0.044 ± 0.020	0.059 ± 0.027	2.6 ± 0.8
5c	R = 3,5- dimethoxyphenyl	0.027 ± 0.009	0.032 ± 0.010	4.6 ± 0.4
5d	R = 3-methoxyphenyl	0.078 ± 0.017	0.076 ± 0.028	15 ± 2.9
5e	R = 4-nitrophenyl	0.012 ± 0.002	0.030 ± 0.005	2.8 ± 0.2
unsymmetrical diamido-thiodigalactosides				
16	R = 2-naphthyl	0.027 ± 0.014	0.021 ± 0.003	4.3 ± 1.4
17	R = 1-naphthyl	0.061 ± 0.026	0.079 ± 0.015	12 ± 2.4
LacNAc 3′-amides				
18	R = phenyl	1.7 ± 0.2	4.2 ± 0.7	n.d.[b]
19	R = 1-naphthyl	0.94 ± 0.16	7.6 ± 2.3	n.d.[b]
20	R = 2-naphthyl	0.24 ± 0.05	1.4 ± 0.1	n.d.[b]
21	R = 3,5- dimethoxyphenyl	0.38 ± 0.08	1.1 ± 0.3	n.d.[b]
22	R = 4-nitrophenyl	0.16 ± 0.03	0.66 ± 0.10	n.d.[b]
23	R = 3-methoxyphenyl	0.67 ± 0.14	58 ± 7.9	n.d.[b]
lactose esters[30]				
24	R = phenyl	1.3 ± 0.1	n.d.[b]	high
25	R = 1-naphthyl	0.28 ± 0.02	n.d.[b]	high
26	R = 2-naphthyl	0.97 ± 0.47	n.d.[b]	480 ± 54
27	R = 3,5- dimethoxyphenyl	0.39 ± 0.06	n.d.[b]	high
reference compounds				
28	thiodigalactoside	9.5 ± 2.2	18 ± 3.3	186 ± 51
29	Me β-LacNAc[30]	21 ± 5.0	26 ± 4.3	1400 ± 600
30	Me β-Lac[30]	38 ± 6.8	54 ± 11	146 ± 24

[a] R is defined in Scheme 5. [b] Not determined.

The conclusion that no major conformational changes occur upon R144S mutation is supported by the observations that affinities of the compounds (**28–30**) not interacting with Arg144 are barely affected. The effect of mutating Arg144 to a serine had surprisingly small effect on the affinity for compounds interacting with this residue. However, a closer look at a complex between a diamido-thiodigalactoside and galectin-3 (Figure 4a) shows that aromatic amides

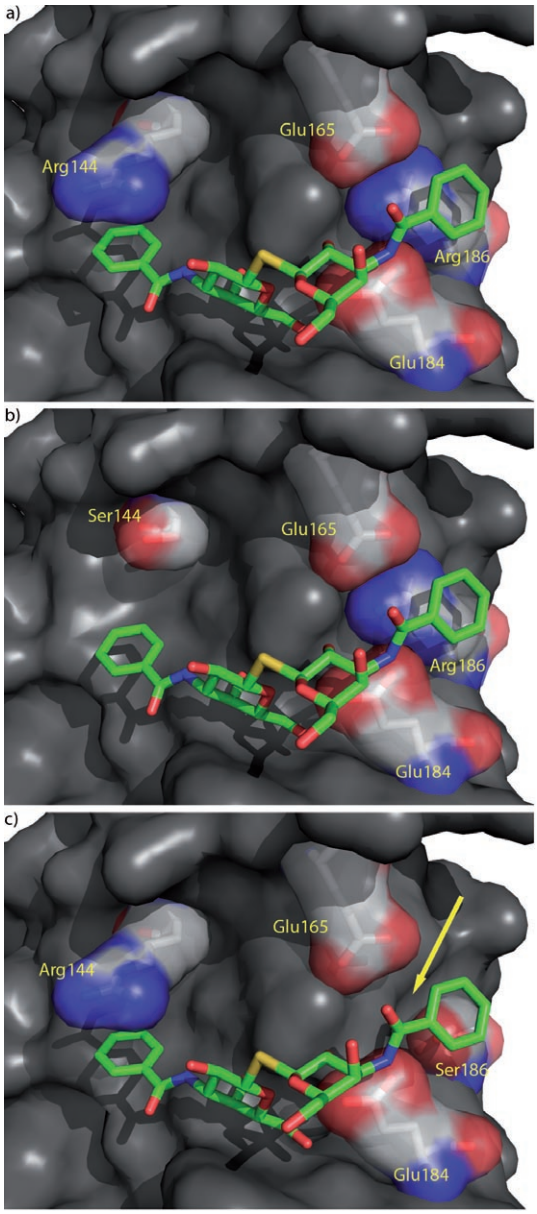


Figure 4. Modeled low-energy structures of compound **5a** in complex with a) wild type, b) R144S mutant, and c) R186S mutant galectin-3. A void volume between Glu165 and Glu184 and below one benzamide is indicated with an arrow in (c).

interacting with Arg144 are placed in between the Arg144 guanidinium ion and the protein surface. Hence, removal of the Arg144 side chain results in a complex in which the aromatic moiety of the ligand retains good surface complementarity with the protein surface (Figure 4b). Hence, the affinity enhancement achieved from interactions between the ligand aromatic amides and the free and relatively flexible Arg144 appears to be driven not only by the Arg144–arene interaction itself, but also to a large extent by desolvation effects of the Arg144 side chain and favorable van der Waals contact between the aromatic amide and the protein surface. Furthermore, the limited contribution to the free energy of a direct Arg144 guanidinium–arene interaction with a favorable enthalpy may be counterbalanced by an entropic penalty in which Arg144 conformational freedom becomes more restricted.

In contrast, the R186S mutant shows greatly reduced affinity for all ligands. Again, a closer look at the galectin-3 structure provides a possible explanation. The Arg186 side chain forms ion pairs with Glu165 and Glu184 and is situated between one aromatic amide of **5a–e** and the protein (Figure 4a). Replacing Arg186 with the smaller serine leaves a void space to be filled and, as a result, the ion pair with Glu165 is disrupted (Figure 4c). This leads to a much poorer surface complementarity between the ligand aromatic moiety and the protein and significantly altered solvation. In addition, the charge of Glu165 is not neutralized by an arginine side chain and is located close to and possibly repels the π system of the aromatic moiety. Affinities for the unsubstituted references **28–30** are also greatly reduced, indicating that this mutation also affects interactions with the carbohydrate core structure. This is not surprising as one hydroxy group of the disaccharides **28–30** (one galactose HO2 in **28**, GlcNAc HO3 in **29**, and Glc HO3 in **30**) bridges the Arg162–Glu185 ion pair that is part of the planar ion-pair network[30] created by Arg162, Glu165, Arg186, and Glu185. However, a major conformational change in R186S compared to the wild type is unlikely as the R186S mutant, in addition to having the same melting temperature in differential-scanning calorimetry experiments, display the same recognition pattern towards natural ligands that are extended at galactose O3 with Arg144-interacting mono- or oligosaccharide structures.[38]

Conclusions

In conclusion, we have synthesized C_2-symmetrical and unsymmetrical thiodigalactoside derivatives bearing one or two aromatic amide substituents at the 3-positions. The diamides **5a–e** and **16**,**17** showed greatly enhanced affinity for galectin-3 over the parent thiodigalactoside **28**, as measured by fluorescence polarization and isothermal microcalorimetry, whereas the monosubstituted compounds **10a–c** showed intermediate affinity for galectin-3. This result can be explained as being due to a double arene–arginine interaction, that is, interaction of the two aromatic amide moieties with Arg144 and Arg186. Mutant studies and computer modeling have confirmed that this is likely to be the case. Moreover, fluorescence polarization assays showed that binding to galectins-7 and -9N, both of which also have arginine residues in the relevant positions, was also increased by the derivatization, albeit to a lesser extent than for galectin-3 and with different compounds showing the highest affinities. Galectin-1 showed only modestly improved affinity for the diamides **5a–d**, which reflects that this galectin lacks an arginine residue close to galactose C3 of the bound ligands (corresponding to, for example, Arg144 in galectin-3). Galectin-8N, on the other hand, is missing the arginine corresponding to Arg186 and, in this case, derivatization with aromatic

amides resulted in dramatic loss of affinity, or, in one case, a marginal gain.

The thiodigalactoside diamides were shown herein to be the best carbohydrate-based galectin-3, -7, and -9N inhibitors that have been reported to date and clearly demonstrate the success of our strategy of targeting arginine residues with aromatic substituents to increase ligand affinity for proteins. The galectin inhibition efficiency of the thiodigalactoside diamides makes them promising leads for the development of galectin-targeting drugs. Indeed, the protein-binding affinity demonstrated herein was recently conferred in a clinically relevant fibrosis model,[39] thus further suggesting the potential of the compounds as galectin-targeting leads. Finally, the studies significantly contribute to our knowledge about fundamental aspects of arginine–arene interactions and provide a basis for successfully taking advantage of the relatively poorly exploited arginine–arene interactions in drug development.

Experimental Section

General methods: Proton nuclear magnetic resonance (^{1}H) spectra were recorded on a Bruker DRX 400 (400 MHz) or a Bruker ARX 300 (300 MHz) spectrometer; multiplicities are quoted as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), apparent triplet (at) or apparent triplet of doublets (atd). Carbon nuclear magnetic resonance (^{13}C) spectra were recorded on a Bruker DRX 400 (100.6 MHz) spectrometer. Spectra were assigned using COSY, HMQC, and DEPT experiments. All chemical shifts are quoted on the δ scale in parts per million (ppm). Low- and high-resolution (HRMS) fast atom bombardment mass spectra were recorded by using a JEOL SX-120 instrument. Optical rotations were measured on a Perkin–Elmer 341 polarimeter with a path length of 1 dm; concentrations are given in grams per 100 mL. TLC was carried out on Merck Kieselgel sheets that were precoated with $60F_{254}$ silica. Plates were developed by using 10% sulfuric acid. Flash column chromatography was carried out on silica (Matrex, 60 Å, 35–70 μm, Grace Amicon). Dichloromethane was distilled from calcium hydride immediately before use. Acetonitrile was distilled from calcium hydride and stored over 4-Å molecular sieves. All other reagents and solvents were used as supplied. Isothermal microcalorimetry was preformed as described.[25] Fluorescence polarization experiments with rat galectin-1 and human galectin-3, -7, -8N, and -9N were performed as described.[31,32] K_d values were calculated as averages of 4 to 25 single-point measurements. The deprotected symmetrical diamides **5a–e** were prepared as described.[27]

Bis[3-deoxy-3-(3,5-dimethoxybenzamido)-β-D-galactopyranosyl]sulfane (5c): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 3.48–3.51 (m, 6H; H-5, H-6, 6′-H), 3.69 (atd, J = 10.0, $J_{OH,2}$ = 6.3 Hz, 2H; 2-H), 3.79 (s, 12H; 2× OCH_3), 3.85 (d, $J_{3,4}$ = 2.9, $J_{OH,4}$ = 5.5 Hz, 2H; 4-H), 3.94 (ddd, $J_{2,3}$ = 10.4, $J_{NH,3}$ = 7.9 Hz, 2H; 3-H), 4.60 (at, J = 5.6 Hz, 2H; 6-OH), 4.72 (d, $J_{1,2}$ = 9.6 Hz, 2H; 1-H), 4.87 (d, 2H; 4-OH), 4.93 (d, 2H; 2-OH), 6.63 (t, J = 2.2 Hz, 2H; Ar-H), 7.07 (d, 2H; Ar-H), 8.07 ppm (d, 2H; NH); FAB$^+$ (m/z): 707 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{30}H_{40}O_{14}N_2SNa$ [MNa$^+$] 707.2098; found 707.2095.

Bis[3-deoxy-3-(benzamido)-β-D-galactopyranosyl]sulfane (5a): ^{1}H NMR (300 MHz, D_2O): δ = 3.70–3.91 (m, 8H; 2-H, 5-H, 6-H, 6′-H), 4.07 (d, $J_{3,4}$ = 3.0 Hz, 2H; 4-H), 4.23 (dd, $J_{2,3}$ = 10.3 Hz, 2H; 3-H), 4.99 (d, $J_{1,2}$ = 9.9 Hz, 2H; 1-H), 7.51 (at, J = 7.4 Hz, 4H; Ar-H), 7.61 (at, J = 7.4 Hz, 2H; Ar-H), 7.78 ppm (at, J = 7.2 Hz, 4H; Ar-H); ^{1}H NMR (400 MHz, CD_3OD): δ = 3.67–3.74 (m, 4H; 5-H, 6-H), 3.80 (dd, $J_{5,6'}$ = 6.6, $J_{6,6'}$ = 10.5 Hz, 2H; 6′-H), 3.89 (at, J = 10.0 Hz, 2H; 2-H), 4.03 (d, $J_{3,4}$ = 2.9 Hz, 2H; 4-H), 4.17 (dd, $J_{2,3}$ = 10.3 Hz, 2H; 3-H), 4.83 (d, $J_{1,2}$ = 9.7 Hz, 2H; 1-H), 7.47 (at, J = 7.4 Hz, 4H; Ar-H), 7.54 (at, J = 7.4 Hz, 2H; Ar-H), 7.88 ppm (at, J = 7.1 Hz, 4H; Ar-H); ^{13}C NMR (100.6 MHz, CD_3OD): δ = 58.8 (d, C3), 62.9 (t, C6), 69.2, 69.4 (2× d, C2, C4), 81.7 (d, C5), 86.4 (d, C1), 128.6, 129.5, 132.7 (3× d, Ar-CH), 135.9 (s, Ar-C), 170.6 ppm (s, C=O); FAB$^+$ (m/z): 587 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{26}H_{32}O_{10}N_2SNa$ [MNa$^+$] 587.1675; found 587.1676.

Bis[3-deoxy-3-(2-naphthamido)-β-D-galactopyranosyl]sulfane (5b): ^{1}H NMR (400 MHz, $CDCl_3$:CD_3OD, 1:1): δ = 3.67–3.76 (m, 4H; 5-H, 6-H), 3.83 (dd, $J_{5,6'}$ = 6.8 Hz, $J_{6,6'}$ = 1.2 Hz, 2H; 6′-H), 3.93 (at, J = 10.0 Hz, 2H; 2-H), 4.08 (d, $J_{3,4}$ = 3.0 Hz, 2H; 4-H), 4.22 (dd, $J_{2,3}$ = 10.3 Hz, 2H; 3-H), 4.78 (d, $J_{1,2}$ = 9.7 Hz, 2H; 1-H), 7.51–7.56 (m, 4H; Ar-H), 7.86–7.95 (m, 8H; Ar-H), 8.41 ppm (s, 2H; Ar-H); FAB$^+$ (m/z): 687 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{34}H_{36}O_{10}N_2SNa$ [MNa$^+$] 687.1988; found 687.1987.

Bis[3-deoxy-3-(3-methoxybenzamido)-β-D-galactopyranosyl]sulfane (5d): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 3.46–3.63 (br m, 12H; OH-2, OH-4, OH-6, H-5, H-6, H-6′), 3.70 (at, J = 10.0 Hz, 2H; H-2), 3.81 (s, 6H; OCH_3), 3.86 (d, $J_{3,4}$ = 2.9 Hz, 2H; 4-H), 3.95 (m, 2H; 3-H), 4.72 (d, $J_{1,2}$ = 9.7 Hz, 2H; 1-H), 7.08 (dd, J = 2.2 Hz, J = 8.1 Hz, 2H; Ar-H), 7.37 (at, J = 7.9 Hz, 2H; Ar-H), 7.46 (d, J = 2.2 Hz, 2H; Ar-H), 7.48 (d, J = 7.8 Hz, 2H; Ar-H), 8.07 ppm (d, $J_{NH,3}$ = 7.9 Hz, 2H; NH); FAB$^+$ (m/z): 647 ([M+Na]$^+$, 100), 625 ([M+H]$^+$, 12%); HRMS (m/z): calcd for $C_{28}H_{36}O_{12}N_2SNa$ [MNa$^+$] 647.1887; found 647.1888.

Bis[3-deoxy-3-(4-nitrobenzamido)-β-D-galactopyranosyl]sulfane (5e): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 3.51–3.52 (m, 6H; 5-H, 6-H, 6′-H), 3.72 (atd, J = 10.0 Hz, $J_{OH,2}$ = 6.1 Hz, 2H; 2-H), 3.89 (m, 2H; 4-H), 3.97 (ddd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.0 Hz, $J_{NH,3}$ = 7.9 Hz, 2H; 3-H), 4.61 (at, J = 5.5 Hz, 2H; 6-OH), 4.73 (d, $J_{1,2}$ = 9.8 Hz, 2H; 1-H), 4.96–4.98 (m, 4H; 2-OH, 4-OH), 8.15, 8.32 (2× d, J = 8.9 Hz, 8H; Ar-H), 8.56 ppm (d, 2H; NH); FAB$^+$ (m/z): 677 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{26}H_{30}O_{14}N_4SNa$ [MNa$^+$] 677.1377; found 677.1367.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl 2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-1-thio-β-D-galactopyranoside (8): 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranose (23 mg, 0.063 mmol) and 2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-β-D-galactopyranosyl bromide[29] (25 mg, 0.063 mmol) were dissolved in acetonitrile (2 mL) and potassium carbonate (18 mg, 0.13 mmol) was added. The reaction mixture was stirred at room temperature under N_2 for 2.5 h after which time TLC (4:5 heptane/ethyl acetate) showed the formation of a major product (R_f = 0.2) and very little remaining of either starting material (R_f = 0.4 and 0.7). The reaction mixture was diluted with dichloromethane (30 mL) and washed with H_2SO_4 (30 mL of a 10% aqueous solution). The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (1:2 heptane/ethyl acetate) to give **8** (26 mg, 61%) as a colorless oil; $[\alpha]_D^{22}$ = −14 (c = 0.5 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.99, 2.06, 2.06, 2.08, 2.14, 2.17, 2.18 (7× s, 21H; 7× CH_3), 3.64 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.3 Hz, 1H; 3_b-H), 3.85–3.91 (m, 2H; 5_a-H, 5_b-H), 4.07–4.14 (m, 3H; 6_a-H, 6_b-H, $6'_b$-H), 4.19 (dd, $J_{5,6'}$ = 6.4 Hz, $J_{6,6'}$ = 11.3 Hz, 1H; $6'_a$-H), 4.79 (d, $J_{1,2}$ = 10.0 Hz, 1H; 1_b-H), 4.80 (d, $J_{1,2}$ = 10.1 Hz, 1H; 1_a-H), 5.05 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.4 Hz, 1H; 3_a-H), 5.17 (at, J = 10.0 Hz, 1H; 2_b-H), 5.22 (at, J = 10.0 Hz, 1H; 2_a-H), 5.44 (d, 1H; 4_a-H), 5.47 ppm (d, 1H; 4_b-H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 20.7, 20.8, 20.8, 20.9, 20.9, 21.0 (6× q, 7× C(O)CH_3), 61.4, 61.7 (2× t, $C6_a$, $C6_b$), 63.0 (d, $C3_b$), 67.2, 67.4, 67.8, 68.6 (4× d, $C2_a$, $C4_a$, $C2_b$, $C4_b$), 72.0 (d, $C3_a$), 74.8, 75.6 (2× d, $C5_a$, $C5_b$), 81.4, 81.5 (2× d, $C1_a$, $C1_b$), 169.6, 169.6, 170.1, 170.2, 170.3, 170.5, 170.5 ppm (7× s, 7× C=O); FAB$^+$ (m/z): 700 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{26}H_{35}O_{16}N_3SNa$ [MNa$^+$] 700.1636; found 700.1635.

Typical procedure for the synthesis of 9a–c: Compound **8** (5 mg, 7.4 μmol) was dissolved in ethanol (2 mL) and palladium (10% on carbon, 5 mg) and HCl (0.4 mL of a 1 M solution in diethyl ether) were added. The mixture was degassed and stirred at room temperature under a hydrogen atmosphere. After 20 min, the mixture was filtered through Celite and concentrated in vacuo. The residue was dissolved in dichloromethane (2 mL) and pyridine (0.5 mL). The acid chloride (24 μmol) was added and the mixture was stirred at room temperature. After 2 h and 40 min, TLC (1:2 heptane/ethyl acetate) showed the presence of a single carbohydrate product (R_f = 0.3). The reaction mixture was diluted with dichloromethane (20 mL) and washed with H_2SO_4 (20 mL of a 10%

aqueous solution) and $NaHCO_3$ (20 mL of a saturated aqueous solution), dried over $MgSO_4$, filtered, and then concentrated in vacuo. The residue was purified by flash column chromatography to give the following amides:

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl 2,4,6-tri-*O*-acetyl-3-deoxy-3-benzamido-1-thio-β-D-galactopyranoside (9a): Yield: 43% (colorless oil); 1H NMR (400 MHz, $CDCl_3$): $\delta = 1.99$, 2.07, 2.08, 2.08, 2.15, 2.18 (6×s, 21H; 7×CH_3), 3.92 (at, $J = 6.7$ Hz, 1H; 5_a-H), 3.99 (at, $J = 6.4$ Hz, 1H; 5_b-H), 4.08–4.22 (m, 4H; 6_a-H, $6'_a$-H, 6_b-H, $6'_b$-H), 4.51 (ddd, $J_{2,3} = 10.7$ Hz, $J_{3,4} = 3.1$ Hz, $J_{NH,3} = 7.7$ Hz, 1H; 3_b-H), 4.84 (d, $J_{1,2} = 10.1$ Hz, 1H; 1_a-H), 4.94 (d, $J_{1,2} = 10.1$ Hz, 1H; 1_b-H), 5.06 (dd, $J_{2,3} = 10.0$ Hz, $J_{3,4} = 3.4$ Hz, 1H; 3_a-H), 5.14 (at, $J = 10.3$ Hz, 1H; 2_b-H), 5.28 (at, $J = 10.0$ Hz, 1H; 2_a-H), 5.45 (d, 1H; 4_a-H), 5.58 (d, 1H; 4_b-H), 6.47 (d, 1H; NH), 7.42 (at, $J = 7.5$ Hz, 2H; Ar-H), 7.52 (t, J 7.4 Hz, 1H; Ar-H), 7.65 ppm (d, $J = 7.2$ Hz, 2H; Ar-H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 20.7$, 20.9, 20.9, 20.9, 21.0, 21.1 (6×q, 7×C(O)CH_3), 54.3 (d, $C3_b$), 61.7, 62.0 (2×t, $C6_a$, $C6_b$), 67.4, 67.7 (2×d, $C2_a$, $C4_a$), 68.7, 68.9 (2×d, $C2_b$, $C4_b$), 72.0 (d, $C3_a$), 75.1 (d, $C5_a$), 76.1 (d, $C5_b$), 81.4 (d, $C1_a$, $C1_b$), 127.0, 129.0, 132.2 (3×d, Ar-CH), 133.4 (s, Ar-C), 167.4, 169.7, 169.9, 170.2, 170.3, 170.6, 172.0 ppm (7×s, C=O); FAB$^+$ (m/z): 778 (M+Na$^+$, 100%); HRMS FAB$^+$ (m/z): calcd for $C_{33}H_{41}O_{17}NSNa$ [MNa$^+$] 778.1993; found 778.1987.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl 2,4,6-tri-*O*-acetyl-3-deoxy-3-(2-naphthamido)-1-thio-β-D-galactopyranoside (9b): Yield: 40% (colorless oil); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.99$, 2.08, 2.08, 2.09, 2.16, 2.19 (6×s, 21H; 7×CH_3), 3.94 (at, $J = 6.5$ Hz, 1H; 5_a-H), 4.02 (at, $J = 6.4$ Hz, 1H; 5_b-H), 4.09–4.24 (m, 4H; 6_a-H, $6'_a$-H, 6_b-H, $6'_b$-H), 4.57 (ddd, $J_{2,3} = 10.7$ Hz, $J_{3,4} = 3.1$ Hz, $J_{NH,3} = 7.6$ Hz, 1H; 3_b-H), 4.85 (d, $J_{1,2} = 10.1$ Hz, 1H; 1_a-H), 4.97 (d, $J_{1,2} = 10.1$ Hz, 1H; 1_b-H), 5.07 (dd, $J_{2,3} = 9.9$ Hz, $J_{3,4} = 3.4$ Hz, 1H; 3_a-H), 5.19 (at, $J = 10.2$ Hz, 1H; 2_b-H), 5.30 (at, $J = 10.0$ Hz, 1H; 2_a-H), 5.46 (d, 1H; 4_a-H), 5.63 (d, 1H; 4_b-H), 6.62 (d, 1H; NH), 7.53–7.60 (m, 2H; Ar-H), 7.70 (dd, $J = 1.7$ Hz, $J = 8.6$ Hz, 1H; Ar-H), 7.86–7.94 (m, 3H; Ar-H), 8.20 ppm (s, 1H; Ar-H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 20.7$, 20.9, 21.0, 21.0, 21.1 (5×q, 7×CH_3), 54.4 (d, $C3_b$), 61.7, 62.0 (2×t, $C6_a$, $C6_b$), 67.4, 67.7 (2×d, $C2_a$, $C4_a$), 68.7, 69.0 (2×d, $C2_b$, $C4_b$), 72.0 (d, $C3_a$), 75.1 (d, $C5_a$), 76.1 (d, $C5_b$), 81.4, 81.4 (2×d, $C1_a$, $C1_b$), 123.3, 127.1, 127.9, 128.1, 128.9, 129.3 (6×d, 7×Ar-CH), 130.6, 132.7, 135.1 (3×s, 3×Ar-C), 167.5, 169.8, 169.9, 170.2, 170.3, 170.6, 172.1 ppm (7×s, C=O); FAB$^+$ (m/z): 828 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{37}H_{43}O_{17}NSNa$ [MNa$^+$] 828.2149; found 828.2142.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl 2,4,6-tri-*O*-acetyl-3-deoxy-3-(3,5-dimethoxybenzamido)-1-thio-β-D-galactopyranoside (9c): Yield: 66% (colorless oil); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.99$, 2.07, 2.08, 2.08, 2.16, 2.18 (6×s, 21H; 7×C(O)CH_3), 3.81 (s, 6H; 2×OCH_3), 3.92 (at, $J = 6.7$ Hz, 1H; 5_a-H), 3.99 (at, $J = 6.6$ Hz, 1H; 5_b-H), 4.08–4.23 (m, 4H; 6_a-H, $6'_a$-H, 6_b-H, $6'_b$-H), 4.48 (ddd, $J_{2,3} = 10.7$ Hz, $J_{3,4} = 3.0$ Hz, $J_{NH,3} = 7.7$ Hz, 1H; 3_b-H), 4.83 (d, $J_{1,2} = 10.1$ Hz, 1H; 1_a-H), 4.93 (d, $J_{1,2} = 9.9$ Hz, 1H; 1_b-H), 5.06 (dd, $J_{2,3} = 10.0$ Hz, $J_{3,4} = 3.4$ Hz, 1H; 3_a-H), 5.12 (at, $J = 10.3$ Hz, 1H; 2_b-H), 5.28 (at, $J = 10.0$ Hz, 1H; 2_a-H), 5.45 (d, 1H; 4_a-H), 5.56 (d, 1H; 4_b-H), 6.42 (d, 1H; NH), 6.58 (d, $J = 2.2$ Hz, 1H; Ar-H), 6.78 ppm (d, 2H; Ar-H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 20.7$, 20.9, 20.9, 20.9, 21.1 (5×q, 7×C(O)CH_3), 54.3 (d, $C3_b$), 55.7 (q, 2×OCH_3), 61.7, 62.0 (2×t, $C6_a$, $C6_b$), 67.4, 67.6 (2×d, $C2_a$, $C4_a$), 68.7, 68.9 (2×d, $C2_b$, $C4_b$), 72.0 (d, $C3_a$), 75.1 (d, $C5_a$), 76.0 (d, $C5_b$), 81.4 (d, $C1_a$, $C1_b$), 104.3, 104.9 (2×d, 7×Ar-CH), 161.1 (s, Ar-C), 169.9 ppm (s, C=O); FAB$^+$ (m/z): 838 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{35}H_{45}O_{19}NSNa$ [MNa$^+$] 838.2204; found 838.2200.

Typical procedure for the synthesis of 10a–c:

β-D-Galactopyranosyl 3-deoxy-3-benzamido-1-thio-β-D-galactopyranoside (10a): Compound **9a** (5 mg, 7.0 μmol) was dissolved in methanolic methylamine (40%, 2 mL). After 15 h, the reaction mixture was diluted with methanol, concentrated in vacuo, and purified on RP-HPLC (C18 H_2O/MeCN) to give **10a** (2.9 mg, 95%) as a colorless oil; ^{1}H NMR (400 MHz, D_2O): $\delta = 3.61$–3.90 (m, 9H; 2_a-H, 3_a-H, 5_a-H, 6_a-H, $6'_a$-H, 2_b-H, 5_b-H, H-6_b, H-$6'_b$), 3.99 (d, $J_{3,4} = 3.2$ Hz, 1H; H-4_a), 4.08 (d, $J_{3,4} = 3.0$ Hz, 1H; 4_b-H), 4.23 (dd, $J_{2,3} = 10.3$ Hz, 1H; 3_b-H), 4.84 (m, 1H; 1_a-H), 4.97 (d, $J_{1,2} = 9.9$ Hz, 1H; 1_b-H), 7.53 (at, $J = 7.6$ Hz, 2H; Ar-H), 7.63 (t, $J = 7.5$ Hz, 1H; Ar-H), 7.80 ppm (d, $J = 7.2$ Hz, 2H; Ar-H); ^{13}C NMR (100.6 MHz, D_2O): $\delta = 57.4$ (d, $C3_b$), 61.9 (t, $C6_a$, $C6_b$), 68.2, 68.3 (2×d, $C2_b$, $C4_b$), 69.5 (d, $C4_a$), 70.3 (d, $C2_a$), 74.6 (d, $C3_a$), 79.8, 80.4 (2×d, $C5_a$, $C5_b$), 84.2 (d, $C1_a$), 85.0 (d, $C1_b$), 127.9, 129.4, 132.9 ppm (3×d, Ar-CH); FAB$^+$ (m/z): 484 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{19}H_{27}O_{10}NSNa$ [MNa$^+$] 484.1253; found 484.1248.

β-D-Galactopyranosyl 3-deoxy-3-(2-naphthamido)-1-thio-β-D-galactopyranoside (10b): Yield: 66% (colorless oil); ^{1}H NMR (400 MHz, D_2O): $\delta = 3.61$–3.92 (m, 9H; 2_a-H, 3_a-H, 5_a-H, 6_a-H, $6'_a$-H, 2_b-H, 5_b-H, 6_b-H, $6'_b$-H), 3.99 (d, $J_{3,4} = 3.1$ Hz, 1H; 4_a-H), 4.12 (d, $J_{3,4} = 3.0$ Hz, 1H; 4_b-H), 4.29 (dd, $J_{2,3} = 10.5$ Hz, 1H; 3_b-H), 4.84 (d(obs), 1H; 1_a-H), 4.99 (d, $J_{1,2} = 9.8$ Hz, 1H; 1_b-H), 7.63–7.69 (m, 2H; Ar-H), 7.85 (dd, $J = 1.5$ Hz, $J = 8.5$ Hz, 1H; Ar-H), 8.80–8.07 (m, 3H; Ar-H), 8.39 ppm (s, 1H; Ar-H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 58.9$ (d, $C3_b$), 62.9 (t, $C6_a$, $C6_b$), 69.2, 69.4, 70.7, 71.8 (4×d, $C2_a$, $C4_a$, $C2_b$, $C4_b$), 76.3 (d, $C3_a$), 81.0, 81.6 (2×d, $C5_a$, $C5_b$), 85.4, 86.5 (2×d, $C1_a$, $C1_b$), 125.2, 127.8, 128.8, 129.0, 129.2, 130.0 (6×d, Ar-CH), 133.1, 134.1, 136.3 ppm (3×s, Ar-C); FAB$^+$ (m/z): 534 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{23}H_{29}O_{10}NSNa$ [MNa$^+$] 534.1410; found 534.1428.

β-D-Galactopyranosyl 3-deoxy-3-(3,5-dimethoxybenzamido)-1-thio-β-D-galactopyranoside (10c): Yield: 60% (colorless oil); ^{1}H NMR (300 MHz, CD_3OD): $\delta = 3.49$ (dd, $J = 3.2$ Hz, $J = 9.2$ Hz; 1H), 3.56–3.88 (m, 9H), 3.82 (s, 6H; 2×OCH_3), 4.00 (d, $J_{3,4} = 2.9$ Hz, 1H; 4_a-H), 4.13 (dd, $J_{2,3} = 10.3$ Hz, $J_{3,4} = 3.0$ Hz, 1H; 3_b-H), 4.67 (d, $J_{1,2} = 9.7$ Hz, 1H; 1_a-H), 4.80 (d, $J_{1,2} = 9.8$ Hz, 1H; 1_b-H), 6.64 (t, $J = 2.2$ Hz, 1H; Ar-H), 7.03 ppm (d, 2H; Ar-H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 56.0$ (q, 2×OCH_3), 58.8 (d, $C3_b$), 62.9 (t, $C6_a$, $C6_b$), 69.2, 69.3, 70.7, 71.7 (4×d, $C2_a$, $C4_a$, $C2_b$, $C4_b$), 76.3 (d, $C3_a$), 81.0, 81.6 (2×d, $C5_a$, $C5_b$), 85.3, 86.4 (2×d, $C1_a$, $C1_b$), 104.5, 106.5 (2×d, Ar-CH), 137.9, 162.4 ppm (2×s, Ar-C); FAB$^+$ (m/z): 544 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{21}H_{31}O_{12}NSNa$ [MNa$^+$] 544.1465; found 544.1463.

1-(*S*)-Acetyl-2,4,6-Tri-*O*-acetyl-3-deoxy-3-(3,5-dimethoxybenzamido)-1-thio-β-D-galactopyranose (11): Compound **2c** (180 mg, 0.35 mmol) was dissolved in dichloromethane (4 mL) that had been dried over 4-Å molecular sieves. Acetic anhydride (67 μL, 0.71 mmol) and HBr (0.8 mL of a 33% solution in AcOH) were added and the mixture was stirred under N_2 at room temperature. After 2.5 h, the reaction mixture was diluted with dichloromethane (50 mL) and poured into ice water (50 mL). The organic phase was washed with saturated $NaHCO_3$ (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to give crude **3c**. The crude **3c** was immediately dissolved in distilled acetonitrile (2 mL). Potassium thioacetate (40 mg, 0.35 mmol) was added and the mixture was stirred under N_2 at room temperature. After 1 h and 40 min, TLC (1:1 heptane/ethyl acetate) showed the presence of a major product ($R_f = 0.2$). The reaction mixture was diluted with dichloromethane (50 mL) and washed with saturated $NaHCO_3$ (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (1:1 heptane/ethyl acetate) to give **11** (133 mg, 72%) as a white solid; $[\alpha]_D^{21} = +78.6$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.03$ (s, 6H; 2×OC(O)CH_3), 2.12 (s, 3H; OC(O)CH_3), 2.39 (s, 3H; SC(O)CH_3), 3.78 (s, 6H; 2×OCH_3), 4.01 (m, 1H; 5-H), 4.07–4.15 (m, 2H; 6-H, 6′-H), 4.58 (dd, $J_{2,3} = 10.9$ Hz, $J_{3,4} = 3.2$ Hz, $J_{NH,3} = 8.1$ Hz, 1H; 3-H), 5.16 (at, $J = 10.4$ Hz, 1H; H-2), 5.39 (d, $J_{1,2} = 10.2$ Hz, 1H; 1-H), 5.54 (d, 1H; 4-H), 6.38 (d, 1H; NH), 6.53 (t, $J = 2.2$ Hz, 1H; Ar-H), 6.73 ppm (d, 1H; Ar-H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 20.8$, 20.9 (2×q, 3×OC(O)CH_3), 31.0 (q, SC(O)CH_3), 54.0 (d, C3), 61.7 (t, C6), 67.7 (d, C2), 68.8 (d, C4), 76.2 (d, C5), 81.0 (d, C1), 104.1, 104.9 (2×d, Ar-CH), 135.6, 161.0 (2×s, Ar-C), 167.2, 169.9, 170.5, 171.6 (4×s, 3×OC=O, NC=O), 191.9 ppm (s, SC=O); FAB$^+$ (m/z): 550 ([M+Na]$^+$, 80%); HRMS (m/z): calcd for $C_{23}H_{29}O_{11}NSNa$ [MNa$^+$] 550.1359; found 550.1362.

2′,4′,6′-Tri-*O*-acetyl-3′-azido-3′-deoxy-β-D-galactopyranosyl 2,4,6-tri-*O*-acetyl-3-(3,5-dimethoxybenzamido)-3-deoxy-1-thio-β-D-galactopyranoside (13): Compound **11** (131 mg, 0.25 mmol) was dissolved in methanol (15 mL) and cooled to −40 °C. Freshly prepared sodium methoxide (0.24 mmol in 0.810 mL methanol) was transferred to the sugar solution and the reaction mixture was stirred at −40 °C. After 25 min, TLC (1:1 heptane/ethyl acetate) showed very little remaining starting material ($R_f = 0.2$) and the presence of a major product ($R_f = 0.15$). The reaction was quenched by the addition of Duolite C436 and stirred until the pH value reached 7. The mixture was filtered and concentrated in vacuo to

give the crude thiol **12**. The crude **12** was dissolved in acetonitrile (8 mL) and the bromide **6** (109 mg, 0.28 mmol) and potassium carbonate (67 mg, 0.48 mmol) were added. The reaction mixture was stirred at room temperature for 3 h and 20 min, after which time TLC analysis (1:2 heptane/ethyl acetate) showed the formation of a major product ($R_f = 0.05$) and the absence of the thiol **12** ($R_f = 0.15$) as well as remaining excess bromide **6** ($R_f = 0.7$). The reaction mixture was diluted with dichloromethane (50 mL) and washed with H_2SO_4 (50 mL of a 10% aqueous solution). The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (2:5 heptane/ethyl acetate) to give **13** (78 mg, 39%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.07$, 2.08, 2.09, 2.14, 2.16, 2.19 (6×s, 18H; 6×C(O)CH_3), 3.65 (dd, $J_{2,3} = 10.1$ Hz, $J_{3,4} = 3.3$ Hz, 1H; 3_a-H), 3.81 (s, 6H; 2×OCH_3), 3.87 (at, $J = 6.7$ Hz, 1H; 5_a-H), 3.97 (at, $J = 6.4$ Hz, 1H; 5_b-H), 4.09–4.15 (m, 4H; 6_a-H, $6'_a$-H, 6_b-H, $6'_b$-H), 4.48 (ddd, $J_{2,3} = 10.6$ Hz, $J_{3,4} = 3.0$ Hz, $J_{NH,3} = 7.7$ Hz, 1H; 3_b-H), 4.82 (d, $J_{1,2} = 10.0$ Hz, 1H; 1_a-H), 4.93 (d, $J_{1,2} = 10.0$ Hz, 1H; 1_b-H), 5.10 (at, $J = 10.2$ Hz, 1H; 2_b-H), 5.22 (at, $J = 10.0$ Hz, 1H; 2_a-H), 5.48 (d, 1H; 4_a-H), 5.56 (d, 1H; 4_b-H), 6.40 (d, 1H; NH), 6.57 (t, $J = 2.2$ Hz, 1H; Ar-H), 6.77 ppm (d, 2H; Ar-H); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 20.8$, 20.8, 20.9, 21.0 (4×q, 6×C(O)CH_3), 54.1 (d, $C3_b$), 55.6 (q, 2×OCH_3), 61.8 (t, $C6_a$, $C6_b$), 63.0 (d, $C3_a$), 67.9 (d, $C4_a$), 68.6, 68.7, 68.8 (3×d, $C2_a$, $C2_b$, $C4_b$), 75.8, 75.9 (2×d, $C5_a$, $C5_b$), 81.2, 81.4 (2×d, $C1_a$, $C1_b$), 104.1, 104.9 (2×d, Ar-CH), 135.5, 161.0 (2×s, Ar-C), 167.3, 169.6, 169.9, 170.0, 170.5, 170.5, 171.8 ppm (7×s, 7×C=O); FAB$^+$ (m/z): 821 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{33}H_{42}O_{17}N_4SNa$ [MNa$^+$] 821.2163; found 821.2173.

3′-Deoxy-3-(2-naphthamido)-β-D-galactopyranosyl 3-deoxy-3-(3,5-dimethoxybenzamido)-1-thio-β-D-galactopyranoside (16) and 3′-deoxy-3-(1-naphthamido)-β-D-galactopyranosyl 3-deoxy-3-(3,5-dimethoxybenzamido)-1-thio-β-D-galactopyranoside (17): Compound **13** (28 mg, 0.035 mmol) was dissolved in ethanol (4 mL) and palladium (10% on carbon, 20 mg), acetic acid (10 μL), and HCl (0.8 mL of a 1 M solution in diethyl ether) were added. The mixture was degassed and stirred at room temperature under a hydrogen atmosphere. After 1 h and 15 min, the mixture was filtered through Celite and concentrated in vacuo to give the intermediate amine. Half of the intermediate amine was dissolved in dichloromethane (2 mL) and pyridine (0.5 mL). 1-Naphthoyl chloride (17 μL, 0.12 mmol) was added, and the mixture was stirred at room temperature. After 2 h, ethyl acetate (1 mL) was added and the reaction mixture was concentrated in vacuo to give the crude **15**. Methylamine (1.5 mL of a 40% aqueous solution) was added and the mixture was stirred further at room temperature. After 12 h, TLC analysis (5:1 chloroform/methanol) showed the presence of a single carbohydrate product ($R_f = 0.2$). The reaction mixture was diluted with methanol (1 mL) and concentrated in vacuo. The residue was purified by flash column chromatography (5:1 chloroform/methanol) to give **17** (6 mg, 50%) as a white solid; ^{1}H NMR (400 MHz, [D$_6$]DMSO with a drop of D_2O, the sugar residues are arbitrarily assigned as "x" and "y"): $\delta = 3.60$–3.74 (m (obs), 8H; 2_x-H, 5_x-H, 6_x-H, $6'_x$-H, 2_y-H, 5_y-H, 6_y-H, $6'_y$-H), 3.77 (s, 6H; 2×OCH_3) 3.83 (d, $J_{3,4} = 3.1$ Hz, 1H; 4_y-H), 3.94 (dd, $J_{2,3} = 10.3$ Hz, 1H; 3_y-H), 3.96 (d, $J_{3,4} = 3.1$ Hz, 1H; 4_x-H), 4.06 (dd, $J_{2,3} = 10.2$ Hz, 1H; 3_x-H), 4.72 (d, $J_{1,2} = 9.6$ Hz, 1H; 1_y-H), 4.75 (d, $J_{1,2} = 9.6$ Hz, 1H; 1_x-H), 6.62 (t, $J = 2.2$ Hz, 1H; dimethoxyphenyl-Ar-H), 7.03 (d, 2H; dimethoxyphenyl-Ar-H), 7.51–7.56 (m, 3H; naphthyl-H), 7.70 (d, $J = 7.1$ Hz, 1H; naphthyl-H), 7.95 (dd, $J = 3.3$ Hz, $J = 6.4$ Hz, 1H; naphthyl-H), 7.99 (d, $J = 8.3$ Hz, 1H; naphthyl-H), 8.26 ppm (dd, $J = 3.4$ Hz, $J = 6.2$ Hz, 1H; naphthyl-H); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO with a drop of D_2O): $\delta = 55.8$ (q, 2×OCH_3), 57.3, 57.6 (2×d, $C3_a$, $C3_b$), 60.7 (t, $C6_a$, $C6_b$), 66.8, 67.0, 67.5, 67.8 (4×d, $C2_a$, $C4_a$, $C2_b$, $C4_b$), 79.9, 80.0 (2×d, $C5_a$, $C5_b$), 84.1, 84.1, (2×d, $C1_a$, $C1_b$), 103.5, 105.8 (2×d, dimethoxyphenyl-Ar-CH), 125.3, 125.9, 126.5, 127.0, 128.5, 130.1 (6×d, naphthyl-CH), 133.4, 135.0, 137.1, 160.6 (4×s, Ar-C), 166.6, 169.1 ppm (2×s, 2×C=O); FAB$^+$ (m/z): 697 ([M+Na]$^+$, 100%); HRMS (m/z): calcd for $C_{32}H_{38}O_{12}N_2SNa$ [MNa$^+$] 697.2043; found 697.2048. The other half of the intermediate amine was treated similarly by using 2-naphthoyl chloride (22 mg, 0.12 mmol) to give **16** (8 mg, 67%) as a white solid; ^{1}H NMR (400 MHz, [D$_6$]DMSO with a drop of D_2O, the sugar residues are arbitrarily assigned as "x" and "y") 3.66–3.84 (m (obs), 15H; 2_x-H, 5_x-H, 6_x-H, $6'_x$-H, 2_y-H, 4_y-H, 5_y-H, 6_y-H, $6'_y$-H, 2×OCH_3) 3.88 (d, $J_{3,4} = 3.0$ Hz, 1H; 4_x-H), 3.94 (dd, $J_{2,3} = 10.3$ Hz, $J_{3,4} = 2.9$ Hz, 1H; 3_y-H), 4.02 (dd, $J_{2,3} = 10.3$ Hz, 1H; 3_x-H), 4.72 (d, $J_{1,2} = 9.6$ Hz, 1H; 1_y-H), 4.74 (d, $J_{1,2} = 9.6$ Hz, 1H; 1_x-H), 6.61 (t, $J = 2.2$ Hz, 1H; dimethoxyphenyl-Ar-H), 7.03 (d, 2H; dimethoxyphenyl-Ar-H), 7.58–7.61 (m, 2H; naphthyl-H), 7.93–8.01 (m, 4H; naphthyl-H), 8.48 ppm (s, 1H; naphthyl-H); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO with a drop of D_2O): $\delta = 56.0$ (q, 2×OCH_3), 57.7 (d, $C3_a$, $C3_b$), 60.9, 61.1 (2×t, $C6_a$, $C6_b$), 67.1, 67.2, 67.6, 67.8 (4×d, $C2_a$, $C4_a$, $C2_b$, $C4_b$), 80.1 (d, $C5_a$, $C5_b$), 84.2, (d, $C1_a$, $C1_b$), 103.7, 105.9 (2×d, dimethoxyphenyl-Ar-CH), 124.9, 127.4, 128.2, 128.3, 129.3 (5×d, naphthyl-CH), 132.2, 132.6, 134.7, 137.1, 160.8 (5×s, Ar-C), 166.9, 167.4 ppm (2×s, 2×C=O); FAB$^+$ (m/z): 697 ([M+Na]$^+$, 100%; HRMS FAB$^+$ (m/z): calcd for $C_{32}H_{38}O_{12}N_2SNa$ [MNa$^+$] 697.2043; found 697.2056.

Molecular modeling: Molecular modeling was performed with the MMFFs force field and the GB/SA solvation model for water implemented in MacroModel (version 9.1, Schrödinger, LLC, New York, 2005). All backbone torsions were selected for random variation. Starting conformations were built from the published crystal structures of galectin-1, -7, and -9N in complex with lactose,[33–35] of galectin-3 in complex with a C3′-amido-derivatised LacNAc-based inhibitor,[25] and a homology model of galectin-8N in complex with LacNAc.[36] Starting conformations of the amides were positioned in the two possible orientations. Complexes with naphthoates were minimized starting from both of the two alternative planar conformations and arene substituent starting conformations were systematically varied. The minimizations converged in all cases and the binding modes and overall structures of the minimized complexes closely resembled that of the crystal structures used for building starting conformations.

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[1] S. H. Barondes, V. Castronovo, D. N. W. Cooper, R. D. Cummings, K. Drickamer, T. Feizi, M. A. Gitt, J. Hirabayashi, C. Hughes, K. Kasai, H. Leffler, F. T. Liu, R. Lotan, A. M. Mecurio, M. Monsigny, S. Pillai, F. Poirer, A. Raz, P. W. J. Rigby, J. M. Rini, J. L. Wang, *Cell* **1994**, *76*, 597–598.
[2] H. Leffler, S. Carlsson, M. Hedlund, Y. Qian, *Glycoconjugate J.* **2004**, *19*, 433–440.
[3] J. Dumic, S. Dabelic, M. Flögel, *Biochim. Biophys. Acta Gen. Subj.* **2006**, *1760*, 616–635.
[4] F. T. Liu, D. K. Hsu, *Drug News Perspect.* **2007**, *20*, 455–460.
[5] N. E. Rhaleb, S. Pokharel, P. Harding, S. Rasoul, H. Peng, O. A. Carretero, *Am. J. Pathol.* **2008**, *172*, 247–255.
[6] N. C. Henderson, A. C. Mackinnon, S. L. Farnworth, T. Kipari, C. Haslett, J. P. Iredale, F. T. Liu, J. Hughes, T. Sethi, *Am. J. Pathol.* **2008**, *172*, 288–298.
[7] F.-T. Liu, G. A. Rabinovich, *Nat. Rev. Cancer* **2005**, *5*, 29–41.
[8] C. M. John, H. Leffler, B. Kahl-Knutsson, I. Svensson, G. A. Jarvis, *Clin. Cancer Res.* **2003**, *9*, 2374–2383.
[9] M. A. Toscano, G. A. Bianco, J. M. Ilarregui, D. O. Croci, J. Correale, J. D. Hernandez, N. W. Zwirner, F. Poirer, E. M. Riley, L. Baum, G. A. Rabinovich, *Nat. Immun.* **2007**, *825–834*.
[10] M. Salatino, D. O. Croci, G. A. Bianco, J. M. Ilarregui, M. A. Toscano, G. A. Rabinovich, *Expert Opin. Biol. Ther.* **2008**, *8*, 45–57.
[11] V. L. Thijssen, F. Poirer, L. G. Baum, A. W. Griffioen, *Blood* **2007**, *110*, 2819–2827.
[12] I. Camby, M. Le Mercier, F. Lefranc, R. Kiss, *Glycobiology* **2006**, *16*, 137R–157R.
[13] H. Lahm, S. Andre, A. Hoeflich, H. Kaltner, H. C. Siebert, B. Sordat, C.-W. von der Lieth, E. Wolf, H.-J. Gabius, *Glycoconjugate J.* **2003**, *20*, 227–238.
[14] S. Saussez, R. Kiss, *Cell. Mol. Life Sci.* **2006**, *63*, 686–697.

[15] G. A. Rabinovich, F.-T. Liu, A. Anderson, *Scand. J. Immunol.* **2007**, *66*, 143–158.
[16] J. Nieminen, A. Kuno, J. Hirabayashi, S. Sato, *J. Biol. Chem.* **2006**, *282*, 1374–1383.
[17] G. A. Rabinovich, M. A. Toscano, S. S. Jackson, G. R. Vasta, *Curr. Opin. Struct. Biol.* **2007**, *17*, 513–520.
[18] M. Demetriou, M. Granovsky, S. Quaggin, J. W. Dennis, *Nature* **2001**, *409*, 733–739.
[19] E. A. Partridge, C. Le Roy, G. M. Di Guglielmo, J. Pawling, P. Cheung, M. Granovsky, I. R. Nabi, J. L. Wrana, J. W. Dennis, *Science* **2004**, *306*, 120–124.
[20] K. S. Lau, E. A. Partridge, A. Grigorian, C. I. Silvescu, V. N. Reinhold, M. Demetriou, J. W. Dennis, *Cell* **2007**, *129*, 123–134.
[21] D. Delacour, C. Greb, A. Koch, E. Salomonsson, H. Leffler, A. Le Bivic, R. Jacob, *Traffic* **2007**, *8*, 379–388.
[22] F. Poirer, *Biochem. Soc. Symp.* **2002**, *69*, 95–103.
[23] R. J. Pieters, *ChemBioChem* **2006**, *7*, 721–728.
[24] P. Sörme, Y. Qian, P.-G. Nyholm, H. Leffler, U. J. Nilsson, *ChemBioChem* **2002**, *3*, 183–189.
[25] P. Sörme, P. Arnoux, B. Kahl-Knutsson, H. Leffler, J. M. Rini, U. J. Nilsson, *J. Am. Chem. Soc.* **2005**, *127*, 1737–1743.
[26] M. A. Bianchet, H. Ahmed, G. R. Vasta, L. M. Amzel, *Proteins* **2002**, *40*, 378–388.
[27] I. Cumpstey, A. Sundin, H. Leffler, U. J. Nilsson, *Angew. Chem.* **2005**, *117*, 5240–5242; *Angew. Chem. Int. Ed.* **2005**, *44*, 5110–5112.
[28] J. Bogusiak, W. Szeja, *Carbohydr. Res.* **1985**, *141*, 165–167.
[29] T. L. Lowary, O. Hindsgaul, *Carbohydr. Res.* **1994**, *251*, 33–67.
[30] I. Cumpstey, E. Salomonsson, A. Sundin, H. Leffler, U. J. Nilsson, *ChemBioChem* **2007**, *8*, 1389–1398.
[31] I. Cumpstey, S. Carlsson, H. Leffler, U. J. Nilsson, *Org. Biomol. Chem.* **2005**, *3*, 1922–1932.
[32] P. Sörme, B. Kahl-Knutsson, M. Huflejt, U. J. Nilsson, H. Leffler, *Anal. Biochem.* **2004**, *334*, 36–47.
[33] M. F. López-Lucendo, D. Solís, S. André, J. Hirabayashi, K.-i. Kasai, H. Kaltner, H.-J. Gabius, A. Romero, *J. Mol. Biol.* **2004**, *343*, 957–970.
[34] D. D. Leonidas, E. H. Vatzaki, H. Vorum, J. E. Celis, P. Madsen, K. R. Acharya, *Biochemistry* **1998**, *37*, 13930–13940.
[35] M. Nagae, N. Nishi, S. Nakamura-Tsuruta, J. Hirabayashi, S. Wataksuki, R. Kato, *J. Mol. Biol.* **2007**, *375*, 119–135.
[36] S. Carlsson, C. T. Öberg, M. C. Carlsson, A. Sundin, U. J. Nilsson, D. Smith, R. D. Cummings, J. Almkvist, A. Karlsson, H. Leffler, *Glycobiology* **2007**, *17*, 663–676.
[37] N. Ahmad, H.-J. Gabius, S. Andre, H. Kaltner, S. Sabesan, R. Roy, B. Liu, F. Macaluso, C. F. Brewer, *J. Biol. Chem.* **2004**, *279*, 10841–10847.
[38] E. Salomonsson, M. C. Carlsson, R. Hendus-Altenburger, C. T. Öberg, R. Nilsson, E. Nordberg-Karlsson, V. Johansson, A. Karlsson, U. J. Nilsson, H. Leffler, **2008**, unpublished work.
[39] A. C. MacKinnon, S. L. Farnworth, N. C. Henderson, P. S. Hodkinson, T. Kipari, H. Leffler, U. J. Nilsson, C. Haslett, J. Hughes, T. Sethi, *J. Immunol.* **2008**, *180*, 3650–2658.

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