Lectin Inhibitors

DOI: 10.1002/anie.200500627

# $C_2$-Symmetrical Thiodigalactoside Bis-Benzamido Derivatives as High-Affinity Inhibitors of Galectin-3: Efficient Lectin Inhibition through Double Arginine–Arene Interactions**

*Ian Cumpstey, Anders Sundin, Hakon Leffler, and Ulf J. Nilsson**

Galectin-3 is a β-galactoside-binding protein[1] that has been implicated as playing an important role in cancer and inflammation processes.[2] Recently, a dominant negative C-terminal fragment of galectin-3 was shown to slow the growth of breast cancer in mice by blocking galectin-3 endogenous ligands.[3] Although many intriguing biological activities have been demonstrated,[2] the precise mode of action of galectin-3 remains unknown. The synthesis of potent, low-molecular-weight inhibitors of galectin-3 is therefore an important goal, as such molecules could be used both as tools to investigate the molecular biology of the protein and as lead compounds for drugs.

The β-galactoside binding site (termed subsite C in Ref. [1b]) is highly conserved across the galectins, whereas the neighboring sites on either side of the Gal group (B, toward the nonreducing terminus; D, toward the reducing terminus) are more variable, albeit important, contributors to the interaction. *N*-Acetyllactosamine (LacNAc, **1**), which binds in subsites C and D, is the best natural disaccharide ligand for galectin-3 (Figure 1a). We have previously reported the synthesis of potent inhibitors of galectin-3 based on modification of LacNAc at C3 of galactose to target subsite B.[5] The best inhibitors were aromatic amides of general structure **2** (Figure 1c) and according to the crystal structure of an inhibitor–galectin-3 complex, the increase in binding affinity results from particularly favorable interactions of the aromatic amide moiety with an arginine side chain (Arg 144).[5b] Arene–guanidinium interactions[6] have attracted considerable interest in recent years, as they can be as strong as cation–anion interactions[6c] and up to 70% of all arginine side chains are involved in such interactions.[6b]

[*] Dr. I. Cumpstey, A. Sundin, Dr. U. J. Nilsson
Organic Chemistry, Lund University
Box 124, 221 00 Lund (Sweden)
Fax: (+46) 46-222-8209
E-mail: ulf.nilsson@organic.lu.se
Dr. H. Leffler
Section MIG, Department of Laboratory Medicine
Lund University
Solvegatan 23, 223 62 Lund (Sweden)

[**] This work was supported by the Swedish Research Council and the programs "Chemistry for the Life Sciences" and "Glycoconjugates in Biological Systems" sponsored by the Swedish Foundation for Strategic Research.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

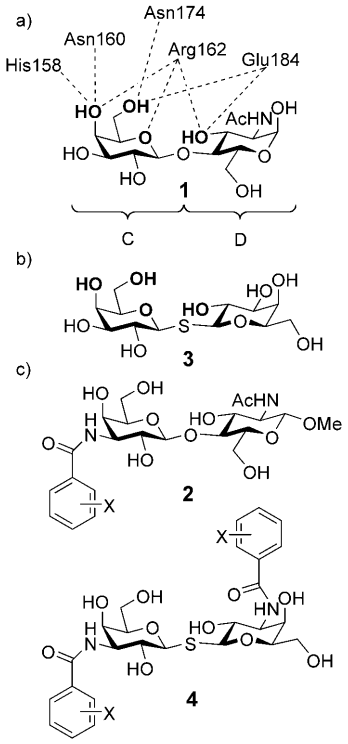



***Figure 1.*** a) Representation of the complex of *N*-acetyllactosamine **1** with galectin-3 in which dashed lines represent hydrogen bonds.[4] The areas known as subsites C and D are indicated, and the key polar interacting groups on the saccharide are shown in bold.[1b] b) Thiodigalactoside **3** with key polar groups for interaction with galectin-1 shown in bold.[8b] c) Lactosamine-based galectin inhibitors **2** and proposed thiodigalactoside-based inhibitors **4**, showing the similarity in structure and conformation.

The guanidinium ion is positively charged, has a π system, and is poorly solvated in water,[7] which makes it ideal for interactions with aromatic π systems. Consequently, the targeting of protein arginine residues with aromatic structures is emerging as an attractive strategy for protein inhibitor development.

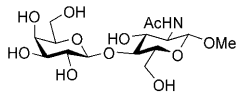
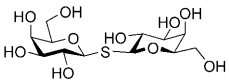
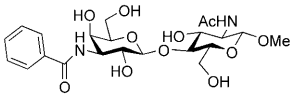
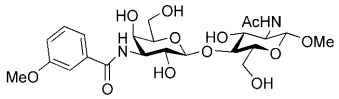
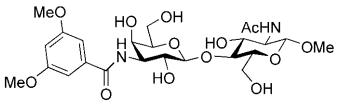
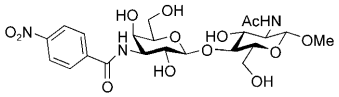
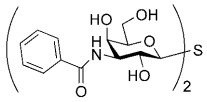
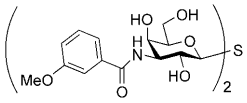
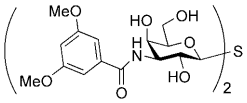
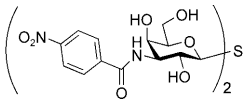
Thiodigalactoside **3** has been shown to bind to galectins with about the same affinity as LacNAc,[8] and it was hypothesized that it bound in a mode similar to that of LacNAc,[8a] that is, in subsites C and D, with a similar conformation and hydrogen-bonding network (Figure 1b). This notion has recently been confirmed for galectin-1, for which X-ray structures have been solved both with LacNAc **1** and with thiodigalactoside **3** as ligands.[8b] The galactose residues bind identically in subsite C for the two disaccharides, and in subsite D, the Gal of thiodigalactoside and GlcNAc of LacNAc show identical patterns of interaction with the protein.

We reasoned that derivatization with aromatic amides at C3 of galactose in thiodigalactoside, as for **2**, could increase the affinity for galectin-3 through favorable interactions with Arg144 in subsite B. Furthermore, similar derivatization at the second galactose C3 of **2** would give an amide that could interact with an arginine residue (Arg186) in subsite D (Figure 1c). The resulting molecules **4** are $C_2$-symmetrical, which simplifies the synthesis and could also lead to interesting thermodynamic properties as there are two degenerate binding modes.

Computer modeling[9] of the complex of galectin-3 with a thiodigalactoside bis-benzamido derivative **4a** (Table 1), which was performed by starting from the reported crystal structure of galectin-3 complexed with a C3-benzamido LacNAc-based inhibitor,[5b] gave an energy minimum with the two galactose units bound in subsites C and D (Figure 2). Moreover, the two aromatic rings of the benzamides were involved in stacking interactions with the two arginine residues, Arg144 and Arg186.

To test the potential of 3,3′-bis-amide-derivatized thiodigalactosides as inhibitors of galectin-3, we synthesized a selection of these compounds. The amide structures were

***Table 1:*** $K_d$ values for the inhibitors **4a–d** established by fluorescence polarization.[11]

| | Compound | $K_d$ [nM] | Relative activity[a] |
|---|---|---|---|
| **9** |  | 69000[5b] | 1 |
| **3** |  | 43000 | 1.6 |
| **2a** |  | 6700[5b] | 10 |
| **2b** |  | 2500[5b] | 28 |
| **2c** |  | 1100[5b] | 63 |
| **2d** |  | 950[5b] | 73 |
| **4a** |  | 3000 | 23 |
| **4b** |  | 61 | 1130 |
| **4c** |  | 50 | 1380 |
| **4d** |  | 33 | 2090 |

[a] Compounds **9**, **2a–d**, and **3** are included for reference.

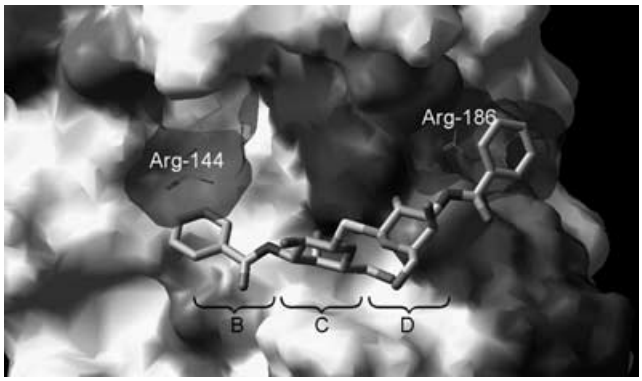



***Figure 2.*** Molecular model of **4a** bound in the galectin-3 active site, energy-minimized by using MacroModel MMFF/water. The two arginine residues, Arg144 and Arg186, in subsites B and D, respectively, are stacked against the two aromatic rings of the amide groups.

chosen based on the best results from our investigation into C3-benzamido LacNAc derivatives **2**.[5] Consequently, the bis-amide structures **4a–d** were synthesized starting from the known 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-D-galactopyranose[10] (Supporting Information).

Bis-amides **4a–d** were tested for binding to galectin-3 with a fluorescence polarization assay[11] (Table 1). Pleasingly, our strategy was successful; bis-amides **4a–d** all have dissociation constant values ($K_d = 33$–$3000$ nM) significantly better than those of nonderivatized thiodigalactoside **3** and the methyl β-glycoside of *N*-acetyllactosamine **9**, both of which were included in the test as reference compounds. The substituted benzamides **4b–d** bind much better than the unsubstituted benzamide **4a**, as would be expected if the thiodigalactoside derivatives **4** bound in the galactose binding site of galectin-3 (subsite C) similarly to the LacNAc-derived amides **2**.[5b] Furthermore, these thiodigalactosides **4** have much higher affinities than the corresponding LacNAc derivatives **2** (compare **2a–d** and **4a–d**), and it is logical to conclude that this finding is the consequence of a favorable interaction between the second aromatic amide and Arg186, as suggested by our docking experiments (Figure 2). An alternative interpretation would be that compounds **4a–d** cross-link by binding to subsites B and C of two galectin-3 molecules. However, such a binding mode is unlikely as, for steric reasons, it would require a much longer galactose–galactose distance than that present in **4a–d**.

In conclusion, the targeting of arginine side chains with aromatic structures has been demonstrated as an efficient approach toward the discovery of monovalent high-affinity lectin inhibitors. The 3,3′-bis-benzamido-thiodigalactosides **4a–d** are the best monovalent galectin-3 inhibitors reported to date and, as such, constitute promising lead structures for the development of galectin-targeting drugs. In general, the targeting of arginine–arene interactions may be underexploited in drug design relative to targeting, for example, hydrogen-bonding and ionic interactions. Our observation that both of the arginine–arene interactions between galectin-3 and the inhibitors **4a–d** provide substantial affinity enhancements implies that systematic targeting of such interactions could find wider use in drug design.

Received: February 21, 2005
Published online: July 11, 2005

**Keywords:** arenes · drug design · inhibitors · proteins · stacking interactions

[1] a) D. Houzelstein, I. R. Gonçalves, A. J. Fadden, S. S. Sidhu, D. N. Cooper, K. Drickamer, H. Leffler, F. Poirier, *Mol. Biol. Evol.* **2004**, *21*, 1177; b) H. Leffler, S. Carlsson, M. Hedlund, Y. Qian, F. Poirier, *Glycoconjugate J.* **2002**, *19*, 433.
[2] a) H. Leffler, *Glycoconjugate J.* **2002**, *19*, 433; b) F.-T. Liu, G. A. Rabinovich, *Nat. Rev. Cancer* **2005**, *5*, 29.
[3] C. M. John, H. Leffler, B. Kahl-Knutson, I. Svensson, G. A. Jarvis, *Clin. Cancer Res.* **2003**, *9*, 2374.
[4] J. Seetharaman, A. Kanigsberg, R. Slaaby, H. Leffler, S. H. Barondes, J. Rini, *J. Biol. Chem.* **1998**, *273*, 13047.
[5] a) P. Sörme, Y. Qian, P.-G. Nyholm, H. Leffler, U. J. Nilsson, *ChemBioChem* **2002**, *3*, 183; b) P. Sörme, P. Arnoux, B. Kahl-Knutsson, J. M. Rini, H. Leffler, U. J. Nilsson, *J. Am. Chem. Soc.* **2005**, *127*, 1737.
[6] a) J. C. Ma, D. A. Dougherty, *Chem. Rev.* **1997**, *97*, 1303; b) J. P. Gallivan, D. A. Dougherty, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9459; c) J. P. Gallivan, D. A. Dougherty, *J. Am. Chem. Soc.* **2000**, *122*, 870; d) N. Zacharias, D. A. Dougherty, *Trends Pharmacol. Sci.* **2002**, *23*, 281.
[7] P. E. Mason, G. W. Neilson, C. E. Dempsey, A. C. Barnes, J. M. Cruickshank, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4557.
[8] a) H. Leffler, S. Barondes, *J. Biol. Chem.* **1986**, *261*, 10119; b) M. A. Bianchet, H. Ahmed, G. R. Vasta, L. M. Amzel, *Proteins* **2002**, *40*, 378; c) C. F. Brewer, *Glycoconjugate J.* **2002**, *19*, 459.
[9] MacroModel version 8.6: http://www.schrodinger.com/ See: F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liscamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440.
[10] T. L. Lowary, O. Hindsgaul, *Carbohydr. Res.* **1994**, *251*, 33.
[11] P. Sörme, B. Kahl-Knutson, M. Huflejt, U. J. Nilsson, H. Leffler, *Anal. Biochem.* **2004**, *334*, 36.