Fragment-based development of triazole-substituted *O*-galactosyl aldoximes with fragment-induced affinity and selectivity for galectin-3†

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A fragment-based development of 3C-triazol-1-yl-O-galactopyranosyl aldoximes led to the discovery of highly selective and high affinity (K_d down to 11 μM) small monosaccharide based inhibitors of galectin-3. Galectin-7, 8 N-terminal CRD, and 9 N-terminal CRD bound the inhibitors only weakly. The galectin-3 selectivity was hypothesized to stem from interaction of the aldoxime moiety with a site not present in the other galectins.

Introduction

Galectins were defined in 1994 as proteins with "affinity for β-galactosides and a significant sequence similarity in the carbohydrate binding site. . .".[1] They are widely spread throughout nature from plants to invertebrates and now includes 15 mammalian proteins.[2] The galectins are suggested to control several important biological events, including intracellular trafficking,[3] cell signalling,[4–7] apoptosis,[8] and cell adhesion.[2] Observations of these events on a cellular or organismal level related to inflammation,[9,10] immunity,[11,12] and cancer progression[13] have been reported. A coherent picture on the molecular mechanisms of galectins underlying the cellular and organism level observations has begun to emerge during recent years. Central are the discoveries that galectin-1 regulates selective T helper cell apoptosis *via* selective recognition of glycans,[4] that intracellular targeting of proteins is related to galectin-8 activities,[14,15] and that cell-surface glycoprotein localization and residence time is controlled by lattices formed *via N*-glycan and galectin-3 cross-linking.[5–7] Recent important additions to knowledge about galectin molecular mechanisms are that glycan-mediated intracellular clustering with galectin-3 was shown to influence apical sorting of proteins[3] and that galectin-3-mediated lattice formation with T cell receptors prevented their co-localization with CD8 thus conferring anergy in tumor-infiltrating CD8+ lymphocytes.[16] Clearly, galectins are indeed emerging as attractive targets for new anti-cancer and anti-inflammatory drugs and development discovery of high-affinity and selective small-molecule inhibitors[17–31] is hence critical.[32–34]

The carbohydrate recognition domain (CRD) of galectins is a beta-sandwich of about 135 aa which forms a groove that binds up to a tetrasaccharide and can schematically be described as four subsites (A–D). Subsite C is built from most of the conserved sequence elements and binds galactose. When natural oligosaccharides bind, subsite D is occupied by another pyranoside that is glycosylated by the subsite C-binding galactose. Typically, this subsite D-binding pyranoside is (1→4)-linked Glc or GlcNAc or (1→3)-linked GlcNAc or GalNAc.

In order to investigate the proposed biological role of galectins, potent and selective inhibitors are essential. However, the use of natural ligands as inhibitors is hampered by their difficult synthesis, sensitivity to hydrolysis, and their high polarity. In attempts to circumvent these disadvantages, we have previously presented the replacement of the saccharide in subsite D with simpler organic fragments by introducing aldoxime ethers at β-galactose C1 *via* reaction of *O*-β-D-galactopyranosyl hydroxylamine with aldehydes.[25] We herein demonstrate a high selectivity of *O*-galactosyl aldoximes towards galectin-3 as a result of further evaluation of oxime ethers against galectin-1, 7, 8 N (N-terminal domain), and 9 N (N-terminal domain). Furthermore, transformations of the *O*-galactosyl aldoximes through a reduction–acylation protocol are demonstrated to lead to decreased inhibition, which emphasizes the importance of the oxime functionality for selectivity and affinity for galectin-3. Finally, we show that both selectivity and affinity for galectin-3 can be greatly improved by combining structural fragments earlier optimized as subsite B-binding entities (aromatic amides and 1,2,3-triazoles[17,18,23]) with the best subsite D-binding aldoxime structures in a fragment-based manner.

Results and discussion

Evaluation of oxime ethers against galectin-1, 3, 7, 8 N, and 9N

The first overall important observation when analyzing the dissociation constants for the panel of oxime ethers (Fig. 1) for galectin-1, 3, 7, 8 N, and 9 N was that the structure of the aldehyde component greatly influenced the affinity for the different galectins. When comparing the three best inhibitors for each of the five tested galectins (Table 1), structure preferences of the galectins were seen. Oxime ethers with selectivity for galectin-1, 3, 7, and 8 N were found, while the best inhibitors for galectin-9N displayed higher affinity for other galectins.

The oxime ether with highest affinity for galectin-1 (the aromatic **26**, K_d = 910 μM) showed 10 times affinity enhancement as compared to the reference methyl β-D-galactoside **51**, while

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Table 1 Galectin dissociation constants (mM) of oxime inhibitors and the three reference compounds methyl β-D-galactoside **51**, methyl β-lactoside **52**, and methyl β-LacNAc **53**. The three best inhibitors for each galectin are in bold-face

	Galectin				
	1	3	7	8N	9N
51[19]	10000	4400	4800	5200	3400
52[20]	190	160	110	62	23
53[20]	65	59	550	1000	490
10	**1000**	2800	1900	N.I.[a]	N.I.
26	**910**	6300	N.I.	N.I.	> 2000
34	**1200**	650	> 2000	> 2000	N.I.
35	8400	**360**	> 2000	> 2000	> 2000
41	> 5000	**370**	> 2000	N.I.	N.I.
49	> 5000	**330**	> 5000	> 5000	N.I
8	N.I.	4600	**340**	**780**	**950**
19	1200	> 5000	**390**	N.I.	N.I.
25	2200	550	**510**	**160**	**1200**
50	N.D.[b]	480	840	**370**	> 5000
39	N.I.	820	1000	> 2000	**1500**

[a] Not inhibitory. [b] Not determined.

both methyl β-lactoside **52** and methyl β-LacNAc **53** showed much stronger affinity for galectin-1. Larger ring systems seem to be disadvantageous for galectin-1 (*i.e.* naphthyl **41**, indol **49**, anthracene **8**, and naphthyl **50**), while bicyclic ring systems were preferred by galectin-3 (*i.e.* **41** and **49**). The anthracene moiety in **8** appeared to be too large for the CRD in galectin-3, but not for galectin-7, 8 N, and 9N. Galectin-7 also showed good affinity for other large ring systems, such as the tricyclic **16** or **39** (K_d = 780 μM, and 1000 μM, respectively). Two oxime ethers, the acetamidophenyl **19** and anthracene **8**, were as good as LacNAc as inhibitors for galectin-7 although not as good as lactose. Compound **19** showed 3-fold selectivity for galectin-7 over galectin-1 and no, or very small, affinity could be seen for the other galectins. Bicyclic ring systems were not preferred by galectin-7 and 9N. A 3-hydroxy-2-naphthyl oxime **50** turned out to be the second best inhibitor for galectin-8N, although not selective for this galectin. It is noteworthy that the corresponding unsubstituted naphthyl analogue **41** showed no binding to galectin-8N. Interestingly, the 2,5-dihydroxylated phenyl oxime **25**, which also had a hydroxyl group at the *ortho* position, had about three times higher affinity for galectin-8N than for galectin-3 and 7 and seven times higher than for galectin-9N. However, neither *ortho* nor *meta* hydroxylated phenyl oximes (*i.e.* **2** or **10**) showed affinity for galectin-8N. Galectin-9N prefers large substituents, but no selectivity was observed for this galectin.

A closer analysis of the structure–activity relationships reveals that aliphatic moieties had no effect on binding to any of the galectins. It is also apparent that an unsubstituted phenyl had no or only minor effects upon binding no matter if the oxime linker was one, two or three carbons long. However, hydroxyl substituents on various positions on the phenyl ring increase the affinity for all galectins. As natural ligands position a sugar moiety to form hydrogen bonds in subsite D, it is reasonable to assume that the affinity-enhancing effects are due to hydrogen bonding to subsite D.

Synthesis and evaluation of galactosyl hydroxylamines and *N*-acetylated hydroxylamines

Reducing the oxime ethers to hydroxylamines would lead to structural variations, as well as a secondary amino groups amenable for further derivatizations (*e.g.* acylations), which in turn was expected to provide potential novel galectin inhibitors with altered biological properties. Various ways to reduce the oxime ether were investigated. Pyridine–borane reduction[35] initially looked promising, but significant byproduct formations, including

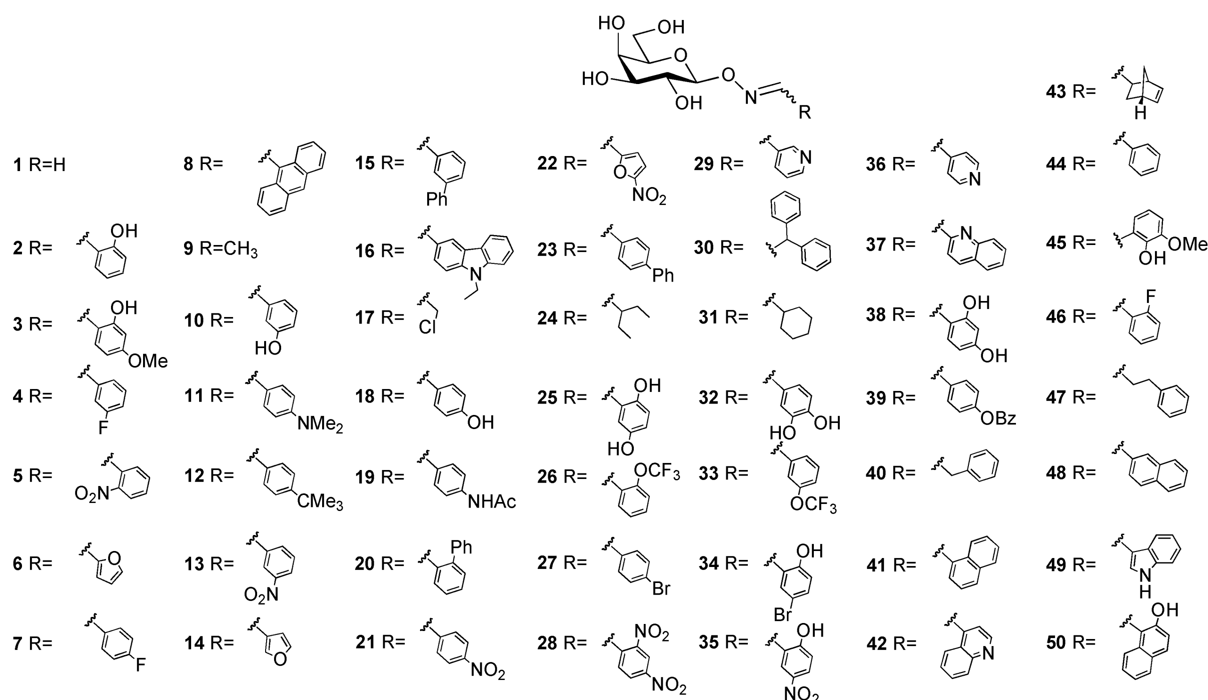
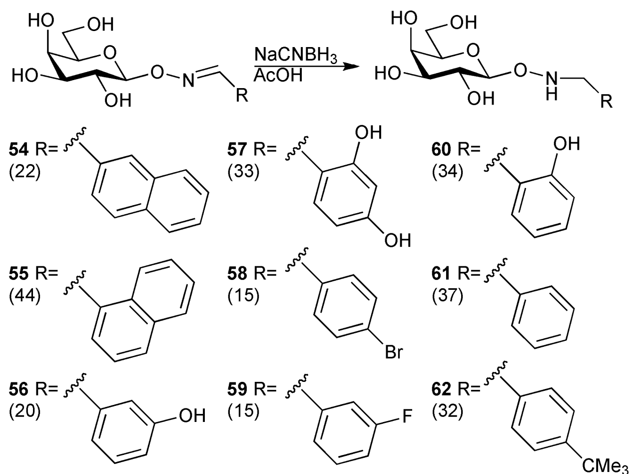
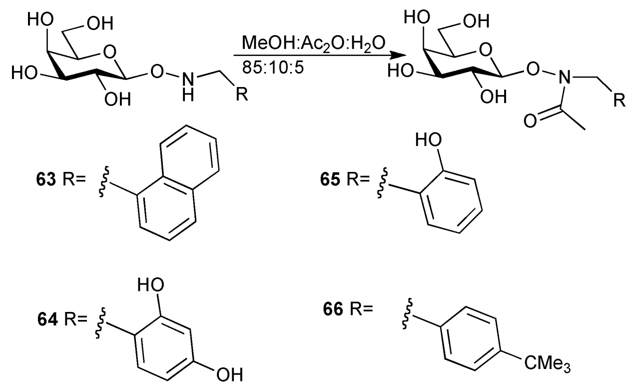


Fig. 1 Aldoximes **1–50**.[25]

O–N bond cleavage, were observed after work-up. The use of $NaCNBH_3$ in glacial acetic acid finally proved to be the best method, although varying amounts of unreacted starting material typically were recovered thus compromising isolated yields. Reasonable yields of the hydroxylamine could nevertheless be obtained with large excess of and subsequent additions of $NaCNBH_3$ (Scheme 1).[36,37] A panel of nine structurally diverse oxime ethers **2**, **4**, **10**, **12**, **27**, **38**, **41**, **44**, and **48** were selected for reduction to give the hydroxylamines **54–62** of which four (**55**, **57**, **60**, and **62**) underwent quantitative acylation to give **63–66** (Scheme 2).



Scheme 1 Synthesis of hydroxylamines **54–62** (yield in %).



Scheme 2 Synthesis of acylated hydroxylamines **63–66**. Yields were near quantitative.

Dissociation constants for hydroxylamines **54–62** and the acylated products **63–66** were determined for galectin-1, 3, 7, 8 N, and 9 N (Table 2), but in most cases the activity was lost as compared to the parent oxime ether. The only exception was galectin-7 towards which the naphthyl **54** was much more potent than the parent oxime ether (**48**, $K_d > 2$ mM).

As the biological evaluations of the hydroxyl amines **54–62** and their acetylated analogs **63–66** were disappointing and the oxime reduction–acylation protocol showed modest efficiency, this approach was not further pursued. Instead, project focus turned towards exploiting possibilities of combining selected galectin-3 subsite D-binding oximes with earlier reported subsite B-binding structural elements in order to develop monosaccharide derivatives with selectivity and high affinity for galectin-3.

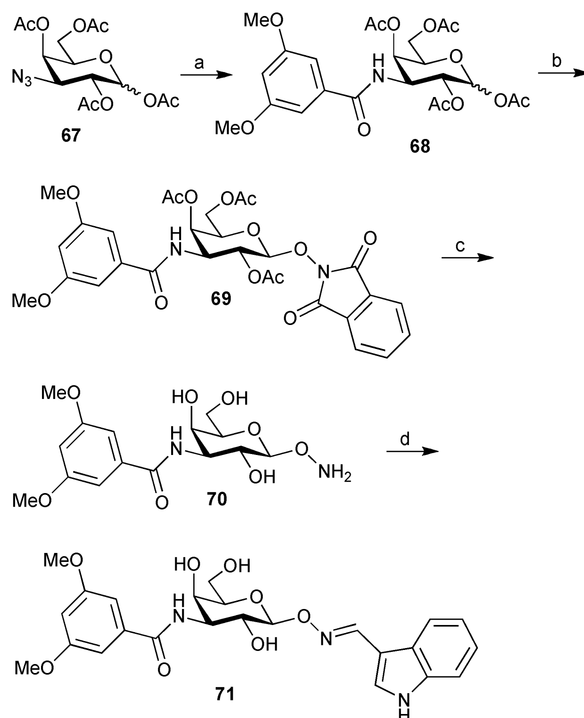
Table 2 Dissociation constants (μM) for hydroxylamines **54–62**

	Galectin				
	1	3	7	8N	9N
54	1900	820	490	N.D.[b]	N.D.[b]
55	N.I.[a]	3200	2500	> 5000	> 5000
56	N.I.[a]	4600	> 5000	> 5000	> 5000
57	N.I.[a]	3700	2400	> 5000	> 5000
58	N.I.[a]	2200	> 5000	> 5000	> 5000
59	N.I.[a]	4100	2100	> 5000	N.I.[a]
60	N.I.[a]	4600	> 5000	> 5000	N.I.[a]
61	> 5000	4100	1200	> 5000	> 5000
62	N.I.[a]	4600	> 5000	N.I.[a]	> 5000

[a] Not inhibitory. [b] Not determined.

Galactosyl oxime with an aromatic subsite B-binding amide at C3

Aromatic amides at C3′ of LacNAc have been shown to be efficient galectin-3 subsite-B binding fragments and detailed structure–activity relationships are available.[17,18] One of the optimized LacNAc C3′ amides, 3,5-dimethoxybenzamide **72**, was selected as the subsite B-binding fragment to combine with the best galectin-3-binding *O*-galactosyl oxime fragment, the indol derivative **49**. The 3,5-dimethoxybenzamide **68** was prepared in 91% yield by catalytic hydrogenation in ethanol/HCl over Pd/C of the azido group of the known **67**[38] (α/β 1/1) followed by acylation with 3,5-dimethoxybenzoyl chloride (Scheme 3). Conversion of **68** into the corresponding bromide, followed by substitution with *N*-hydroxyphthalimide under phase-transfer conditions gave **69**



Scheme 3 Synthesis of a galactosyl oxime **71** with an aromatic subsite B-binding amide at C3. (a) i. H_2, Pd/C, HCl, EtOH. ii. 3,5-dimethoxybenzoyl chloride, pyridine, CH_2Cl_2, 91%. (b) i. HBr, CH_2Cl_2. ii. *N*-hydroxyphthalimide, tetrabutylammonium hydrogensulfate, CH_2Cl_2, Na_2CO_3, 55%. (c) Hydrazine hydrate, MeOH, 72%. (d) Indole-3-carboxaldehyde, H_2O/THF, HCl, 53%, E/Z 5:1.

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Table 3 Dissociation constants (μM) for galactosyl oximes having a C3-amide (**71**) or C3-triazole (**76**) fragments

	Galectin			
	3	7	8N	9N
71	46	390	410	370
76	11	N.D.[a]	870	920
77	17	> 600	>> 3000	>> 3000

[a] Not determined.

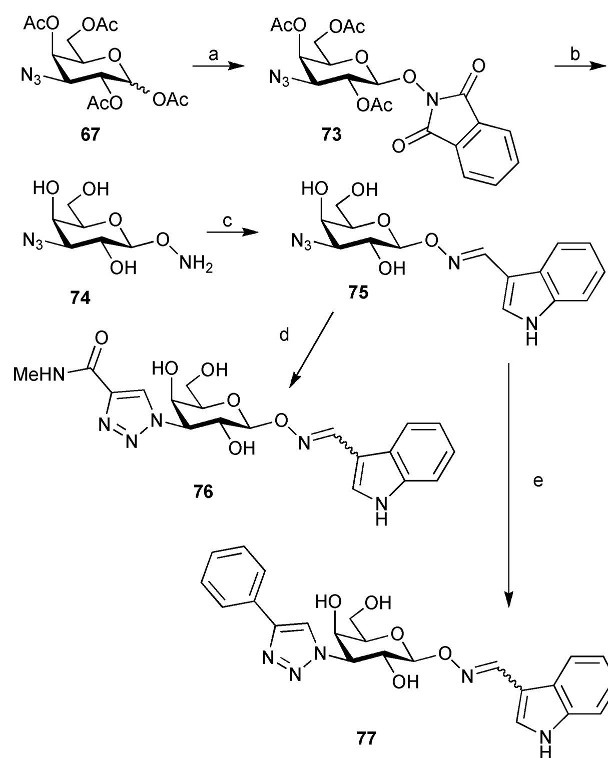
in 55% yield (no α-isomer was detected). Deprotection of the phthalimide and acetyl groups in **69** was accomplished using hydrazine hydrate in methanol to give the hydroxylamine **70** in 72% yield. The oxime ether **71** was formed in 53% yield after treating **70** with indole-3-carboxaldehyde in an acidified H_2O/THF mixture.

Compound **71** (K_d = 46 μM, Table 3) showed an affinity enhancement of 7 times for galectin-3 as compared to the oxime ether analogue **49** (Fig. 2), but the corresponding 3,5-dimethoxybenzamido LacNAc derivative **72** had 67 times higher affinity (K_d = 1 μM) for galectin-3 than the parent LacNAc structure, suggesting that the 3,5-dimethoxybenzamido moiety is not an optimal subsite B-binding fragment to combine with the subsite D-binding indole oxime. Further analysis showed that **71** showed some selectivity for galectin-3 with about 8 times higher affinity for galectin-3 as compared to galectin-7, 8 N, and 9 N (K_d = 390 μM, 410 μM and 370 μM).

Galactosyl oximes with subsite B-binding 1,2,3-triazoles at C3

Although the C3 amides described above indeed corroborated our hypothesis that a subsite B-binding aromatic amide fragment combined with a subsite D-binding oxime fragment could lead to efficient monosaccharide inhibitors of galectin-3, the absence of a synergistic affinity enhancement and thus lower than expected affinity of the derivative **71** was disappointing. The effects of the aromatic amide and the oxime ether were not even fully additive. Hence, we decided to investigate 1,2,3-triazoles as alternative subsite B-binding C3-fragments,[23] as they have been demonstrated to be about as efficient subsite B-binding fragments as aromatic amides (Scheme 4).

Compound **73** was obtained in 33% yield (α/β 2/98) from glycosylation of **67** (α/β 1/1) using *N*-hydroxyphthalimide and boron trifluoride-diethyl etherate in CH_2Cl_2. The less reactive α-anomer of the starting material was recovered, which can explain



Scheme 4 Synthesis of galactosyl oximes **76** and **77** with subsite B-binding 1,2,3-triazoles at C3. (a) *N*-hydroxyphthalimide, boron trifluoride diethyl etherate, CH_2Cl_2, 33%. (b) 2.3 M $MeNH_2$ in MeOH, 88%. (c) Indole-3-carboxaldehyde, HCl (0.1 eq), H_2O/THF, 67%. (d) i. Cu(I), methyl propiolate, propanol. ii. 40% $MeNH_2$ in H_2O, 88%. (e) Cu(I), phenyl acetylene, propanol, 45%.

the moderate yield. Deprotection with methylamine in methanol gave hydroxylamine **74** in 88%. The oxime ether **75** was formed in 67% yield after reacting **74** with indole-3-carboxaldehyde in an acidified H_2O/THF mixture. Two triazoles were then prepared from the indole oxime ether **75**. First, Cu(I)-catalyzed 1,3-dipolar cycloaddition[39–42] with methyl propiolate, followed by treatment with methylamine furnished **76** in 88% yield. The second triazole was prepared in 45% by cycloaddition between phenyl acetylene and **75** to give **77**.

Compounds **76** (K_d = 11 μM) and **77** (K_d = 17 μM) were significantly better than the amide **71** as inhibitors of galectin-3 (Table 3). A comparison with the previously published results for galectin-3 for the methyl amide and the phenyl analogs of the

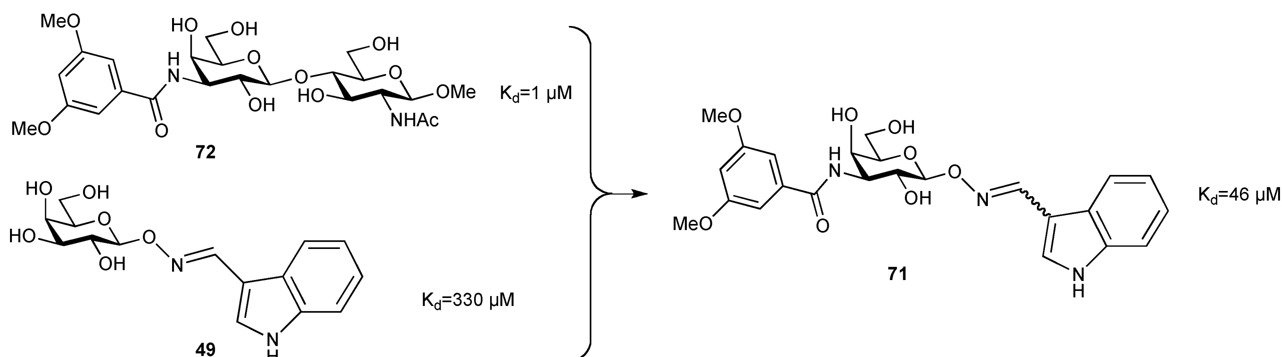


Fig. 2 Combination of a subsite B-binding aromatic amide with a subsite D-binding indole-3-carbaldoxime for selective galectin-3 inhibition.

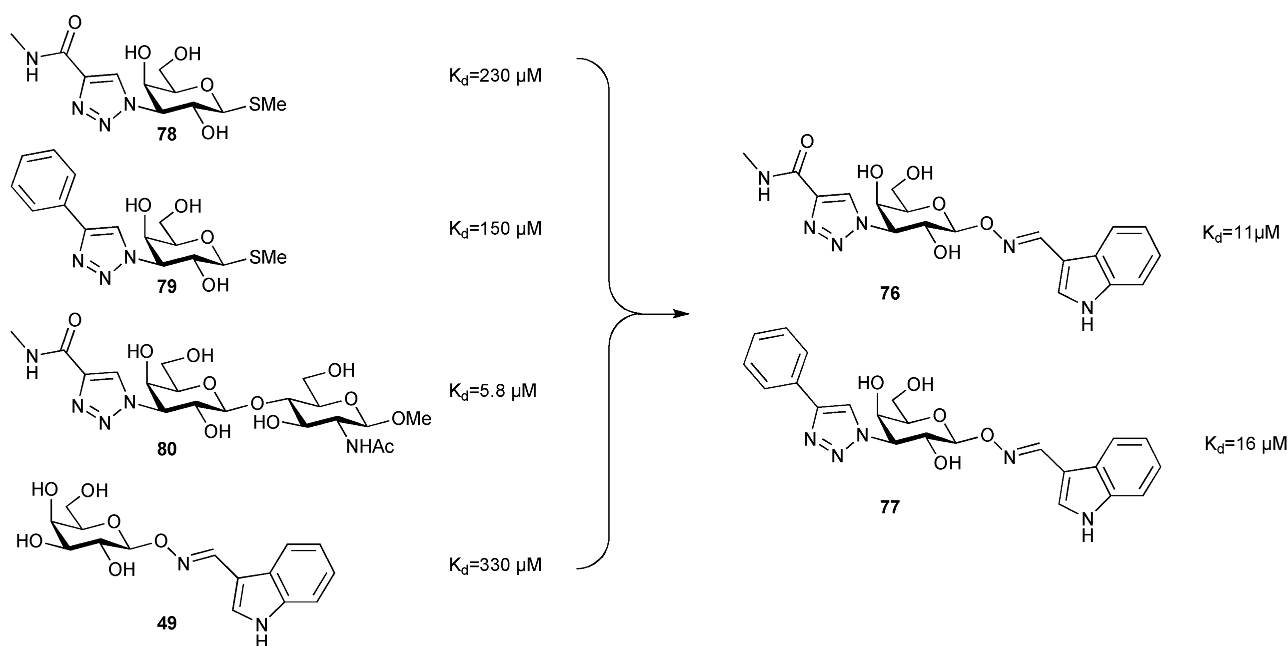


Fig. 3 Combination of subsite B-binding triazoles fragments with a subsite D-binding indole-3-carbaldoxime fragment for efficient and selective galectin-3 inhibition.

3-deoxy-3-(1*H*-1,2,3-triazol-1-yl)-1-thio-galactoside (*i.e.* **78** and **79**, K_d = 230 and 150 μM, respectively)[23] and oxime ether **49** (K_d = 330 μM)[25] clearly shows an additive effect between the substituents at galactose C1 and C3 (Fig. 3). Indeed, the 30-fold affinity-enhancement conferred by the triazole moiety of **76** (compare **76** with **49**) surpasses the 12-fold enhancement observed when the same triazole group was attached to a LacNAc disaccharide (*i.e.* **80**). This suggests a synergistic effect by the galactose C1 and C3 substituents in **76** and **77**. Furthermore, both monosaccharide derivatives **76** and **77** are superior to LacNAc (K_d = 67 μM) and lactose (K_d = 220 μM) methyl glycosides. Importantly, **76** and **77** have high selectivity for galectin-3 and show virtually no or very weak binding to galectin-7, 8 N, or 9N. Such high selectivity is very rarely observed for natural galectin ligands and the unnatural triazole and oxime fragments of **76** and **77** thus not only confer affinity but also high selectivity for galectin-3.

Molecular modeling

In an attempt to explain the improved affinity and selectivity of the triazoles **76** and **77** towards galectin-3, these two inhibitors were modeled into the binding sites of galectin-1, 3, 7, 8 N, and 9N. Energy minima of **76** and **77** in complex with the galectins did support the hypothesis that the substituted triazole fragments occupied the extended binding pocket near galactose C3 (subsite B). However, the indole oxime fragment of **76** and **77** adopted a bent conformation and consequently did not fully occupy subsite D extending from galactose C1 (Fig. 4). In the case of galectin-3, the indole aldoxime fragment was instead positioned in a cavity extending above the β-face of the galactose moiety, which resulted in arg144 being pinched by the two fragments. This cavity is not present in the other galectins investigated, which possibly explains the high galectin-3 selectivity conferred by the indole aldoxime fragment.

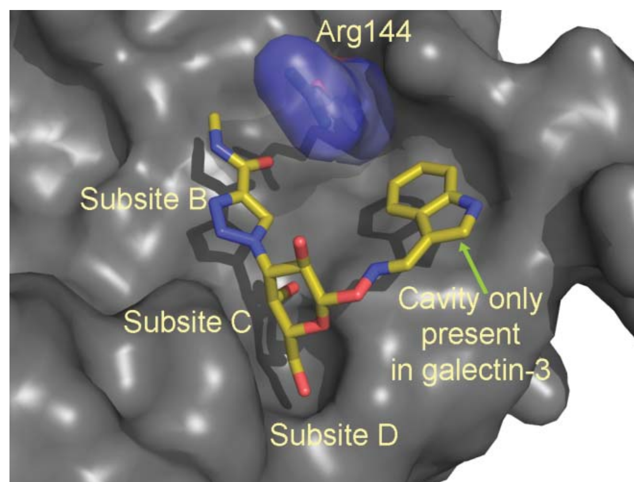


Fig. 4 Calculated energy minima of **76** in complex with galectin-3.

Conclusions

In conclusion, we have from a panel of structurally simple galactosyl oxime ethers identified potent and selective ligands for galectin-3 and 7. Furthermore, ligands were synthesized using a fragment based approach where amide and triazole moieties optimized for subsite B in galectin-3 were combined with a subsite D-binding oxime ether. This approach proved particularly successful for galactose C3-triazoles as two compounds, **76** and **77**, displayed low-micromolar affinity and indeed high selectivity for galectin-3. Hence, the results herein represent a significant progress towards potentially important research tools and towards a lead for the development of galectin-3-targeting drugs as the compounds are small, selective, show promising affinities, and are less polar than natural saccharide ligands (*e.g.* **77** has K_d = 17 μM, MW 449, and clogP 2.3).

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Table 4 Yields (%) and ^{1}H NMR for hydroxylamines **54–62**. Chemical shifts for substituents and H-1

	Yield	^{1}H NMR δ:	HRMS calcd/found
54	22	7.87 (br s, 1H, Ar-H), 7.84–7.81 (m, 3H, Ar-H), 7.54 (dd, 1H, *J* 8.5 Hz, 1.7 Hz, Ar-H), 7.47–7.44 (m, 2H, Ar-H), 4.54 (d, 1H, *J* 7.9 Hz, H-1), 4.30 (s, 2H, NCH_2).	336.1447/336.1441^{a}
55	44	8.26 (d, 1H, *J* 8.3 Hz, Ar-H), 7.87–7.79 (m, 2H, Ar-H), 7.57–7.48 (m, 3H, Ar-H), 7.42 (dd, 1H, *J* 8.2 Hz, *J* 7.1 Hz, Ar-H), 4.63 (d, 2H, *J* 5.8 Hz, NCH_2), 4.58 (d, 1H, *J* 7.9 Hz, H-1).	336.1447/336.1445^{a}
5f	20	7.12 (t, 1H, *J* 8.0 Hz, Ar-H), 6.85–6.83 (m, 2H, Ar-H), 6.69 (ddd, 1H, *J* 8.1 Hz, *J* 2.4 Hz, *J* 1.1 Hz, Ar-H), 4.51 (d, 1H, *J* 7.7 Hz, H-1), 4.06 (S, 2H, NCH_2).	302.1240/302.1237^{a}
57	33	6.99 (d, 1H, *J* 8.2 Hz, Ar-H), 6.28 (d, 1H, *J* 2.3 Hz, Ar.H), 6.24 (dd, 1H, *J* 8.1 Hz, *J* 2.3 Hz, Ar-H), 4.51 (d, 1H, *J* 7.6 Hz, H-1), 4.11 (d, 1H, *J* 12.0 Hz, NCH_2), 4.04 (d, 1H, *J* 11.3 Hz, NCH_2).	318.1189/318.1189^{a}
58	15	7.46 (br d, 2H, *J* 8.3 Hz, Ar-H), 7.32 (br d, 2H, *J* 8.3 Hz, Ar-H), 4.48 (d, 1H, *J* 7.6 Hz, H-1), 4.09 (d, 2H, *J* 1.1 Hz, NCH_2).	386.0215/386.0211^{b}
59	15	7.34–7.28 (m, 1H, Ar-H), 7.21–7.17 (m, 2H, Ar-H), 7.01–6.96 (m, 1H, Ar-H), 4.50 (d, 1H, *J* 7.7 Hz, H-1), 4.13 (s, 2H, NCH_2).	304.1196/304.1198^{a}
60	34	7.21 (dd, 1H, *J* 7.7 Hz, *J* 1.5 Hz, Ar-H), 7.11 (dd, 1H, *J* 7.8 Hz, *J* 1.6 Hz, Ar-H), 6.81–6.76 (m, 2H, Ar-H), 4.53 (d, 1H, *J* 7.52 Hz, H-1), 4.20 (d, 1H, *J* 12.4 Hz, NCH_2), 4.13 (d, 1H, *J* 12.6, NCH_2).	302.1240/302.1246^{a}
61	37	7.39 (br dd, 2H, *J* 8.3 Hz, *J* 1.6 Hz, Ar-H), 7.33–7.24 (m, 3H, Ar-H), 4.51 (d, 1H, *J* 7.7 Hz, H-1), 4.13 (s, 2H, NCH_2).	286.1291/286.1293^{a}
62	32	7.36 (br d, 2H, *J* 8.54 Hz, Ar-H), 7.31 (br d, 2H, *J* 8.5 Hz, Ar-H), 4.51 (d, 1H, *J* 7.7 Hz, H-1), 4.10 (s, 2H, NCH_2), 1.30 (s, 9H, CH_3).	342.1917/342.1920^{a}

^{a} $[M + H]^+$. ^{b} $[M + Na]^+$.

Experimental

General methods

All commercial chemicals were used without further purification. Thin layer chromatography (TLC) was carried out on $60F_{254}$ silica (Merck) and visualization was made by UV light followed by heating with aqueous sulfuric acid. Column chromatography (CC) was performed on silica (Amicon 35–70 μm, 60 Å). Reversed phase chromatography was performed on Waters Sep-Pack Vac 35 cc C_{18}–5 g columns. NMR experiments were recorded with Bruker ARX 300 MHz or Bruker DRX 400 MHz spectrometers at ambient temperature. ^{1}H-NMR assignments were derived from COSY experiments. Chemical shifts are given in ppm relative to TMS, using the solvent residual peaks of CD_2HOD at 3.31, HDO at 4.79 and $CHCl_3$ at 7.26. The optical rotations were measured with a Perkin-Elmer 341 or a 241 polarimeter. HRMS (FAB) were recorded with a JEOL SX-120 instrument or (ESI) with a Micromass Q-TOF micro spectrometer. MALDI TOF MS was recorded with a Bruker Biflex III. Fluorescence polarization experiments and calculations were performed as described[19,43] and performed at 4 °C except for galectin-3 and -8N, which were done at ambient temperature. Concentrations and probes used for galectins-1, -3, -7, -8N and -9N were as described.[19,43] The probes were used at 0.1 μM.

Typical procedure for reduction of oxime ethers

Compound **44** (25 mg, 88 μmol) was dissolved in glacial acetic acid (2 mL) and stirred under a nitrogen atmosphere. $NaCNBH_3$ (28 mg, 446 μmol) was added, the reaction mixture was stirred for 75 minutes, and then lyophilized. Flash chromatography gave **61** (9.3 mg, 37%): ^{1}H NMR (400 MHz, MeOD): δ 7.39 (br dd, 2H, *J* 8.3 Hz, *J* 1.6 Hz, Ar-H), 7.33–7.24 (m, 3H, Ar-H), 4.51 (d, 1H, *J* 7.7 Hz, H-1), 4.13 (s, 2H, NCH_2), 3.81 (dd, 1H, *J* 1.0 Hz, *J* 3.2 Hz, H-4), 3.77 (dd, 1H, *J* 11.4 Hz, *J* 7.1 Hz, H-6), 3.69 (dd, 1H, *J* 11.3 Hz, *J* 5.1 Hz, H-6′), 3.59–3.49 (m, 2H), 3.46 (dd, 1H, *J* 9.6 Hz, *J* 3.2 Hz, H-3); ESIMS *m/z* calcd. for $[C_{13}H_{19}NO_6 + H]^+$: 286.1291. Found: 286.1293.

Typical procedure for acylation of hydroxylamines

Compound **55** was dissolved in a freshly prepared mixture of methanol, acetic acid anhydride and water (85:10:5, 2 mL) and the reaction mixture was stirred overnight. Lyophilization gave **63** in quantitative yield: ^{1}H NMR (300 MHz, MeOD): δ 8.18–8.16 (m, 1H, Ar-H), 7.89–7.79 (m, 2H, Ar-H), 7.55–7.41 (m, 4H, Ar-H), 5.61 (d, 1H, *J* 16.2 Hz, NCH_2), 5.35 (d, 1H, *J* 16.1 Hz, NCH_2), 4.72 (d, 1H, *J* 7.9 Hz, H-1), 3.84 (d, 1H, *J* 3.0 Hz, H-4), 3.72–3.54 (m, 3H), 3.49–3.43 (m, 2H), 2.31 (s, 3H, NAc); ESIMS *m/z* calcd. for $[C_{19}H_{24}NO_7 + H]^+$: 378.1553. Found: 378.1555.

1,2,4,6-Tetra-*O*-acetyl-3-(3,5-dimethoxybenzamido)-3-deoxy-D-galactopyranose 68

To a stirred solution of 3-azido-1,2,4,5-tetra-*O*-acetyl-3-deoxy-D-galactopyranose **67**[38] (102 mg, 0.27 mmol) in EtOH (15 mL) was added 1 M HCl (2.7 mL) and Pd/C (10%, 107 mg). The mixture was hydrogenated (H_2, 1 atm) for 70 minutes, filtered through Celite, and concentrated under reduced pressure to give the intermediate 3-amino-1,2,4,5-tetra-*O*-acetyl-3-deoxy-D-galactopyranoside, which was immediately dissolved in dry CH_2Cl_2 (10 mL). To the solution was added 3,5-dimethoxybenzoyl chloride (546 mg, 2.7 mmol) dissolved in CH_2Cl_2 (10 mL) followed by pyridine (1.64 mL). The reaction mixture was stirred under a nitrogen atmosphere overnight, concentrated, and co-concentrated with toluene. Flash chromatography (EtOAc/heptane, 5:4) gave **68** (126 mg, 91%) as a mixture of anomers: $[\alpha]_D^{24}$ 76 (*c* 0.5, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.77 (t, 4H, *J* 2.8 Hz, Ar-H), 6.56 (t, 2H *J* 2.3 Hz, Ar-H), 6.46 (t, 2H, *J* 7.4 Hz, α and β NH), 6.34 (d, 1H, *J* 3.6 Hz, αH-1), 5.84 (d, 1H, *J* 8.2 Hz, βH-1), 5.61 (br d, 1H, *J* 2.1 Hz, αH-4), 5.54 (d, 1H, *J* 3.2 Hz, βH-4), 5.36 (dd, 1H, *J* 11.5 Hz, *J* 3.6 Hz, αH-2), 5.17 (dd, 1H, *J* 11.2 Hz, *J* 8.3 Hz, βH-2), 4.81 (ddd, 1H, *J* 11.2 Hz, *J* 7.9 Hz, *J* 3.0 Hz, αH-3), 4.53 (ddd, 1H, *J* 11.1 Hz, *J* 7.9 Hz, *J* 3.3 Hz, βH-3), 4.41 (br t, 1H, αH-5),

Table 5 ^{1}H NMR for acetylated hydroxylamines **63–66**. Chemical shifts for substituents and H-1

	^{1}H NMR δ:	HRMS calcd/found
63	8.18–8.16 (m, 1H, Ar-H), 7.89–7.79 (m, 2H, Ar-H), 7.55–7.41 (m, 4H, Ar-H), 5.61 (d, 1H, *J* 16.2 Hz, NCH_2), 5.35 (d, 1H, *J* 16.1 Hz, NCH_2), 4.72 (d, 1H, *J* 7.9 Hz, H-1), 2.31 (s, 3H, NAc).	378.1553/378.1555[a]
64	7.09 (d, 1H, *J* 7.8 Hz, Ar-H), 6.27–6.24 (m, 2H, Ar-H), 4.74 (d, 1H, *J* 8.0 Hz, H-1), 4.85 (d, 1H, NCH_2, partly obscured by solvent peak), 4.81 (d, 1H, *J* 15.8 Hz, NAc), 2.26 (s, 3H, CH_3).	382.1114/382.1116[b]
65	7.22 (dd, 1H, *J* 7.9 Hz, *J* 1.1 Hz, Ar-H), 7.10 (dt, 1H, *J* 7.7 Hz, *J* 1.7 Hz, Ar-H), 6.79 (br t, 2H, *J* 8.6 Hz, Ar.H), 4.93 (d, 1H, *J* 16.2 Hz, NCH_2), 4.92 (d, 1H, *J* 16.4 Hz, NCH_2), 4.74 (d, 1H, *J* 8.0 Hz, H-1), 2.29 (s, 3H, NAc).	366.1165/366.1155[b]
66	7.34 (d, 2H, *J* 8.4 Hz, Ar-H), 7.27 (d, 2H, *J* 8.47 Hz, ArH), 4.98 (d, 1H, *J* 15.8 Hz, NCH_2), 4.88 (d, 1H, NCH_2, partly obscured by solvent peak), 4.72 (d, 1H, *J* 8.0 Hz, H-1), 2.25 (s, 3H, NAc), 1.30 (s, 9H, CCH_3).	384.2022/384.2023[a]

[a] $[M + H]^+$. [b] $[M + Na]^+$.

4.17–3.97 (m, 5H), 3.80 (s, 12H, OMe), 2.20 (s, 3H, OAc), 2.14 (s, 6H, OAc), 2.13 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.04 (s, 9H, OAc); FAB-HRMS *m/z* calcd. for $[C_{23}H_{29}NO_{12}Na]^+$: 534.1587. Found: 534.1591.

O-(2,4,6-Tri-*O*-acetyl-3-(3,5-dimethoxybenzamido-3-deoxy-β-D-galactopyranosyl)-*N*-hydroxyphthalimide 69

To a stirred solution of **68** (92 mg, 0.18 mmol) in dry CH_2Cl_2 (2 mL) was added Ac_2O (34 μL) and after 10 minutes 33% HBr in glacial acetic acid (1.2 mL). After 2 h, more CH_2Cl_2 (15 mL) was added, the solution washed using H_2O (15 mL), followed by saturated $NaHCO_3$ (20 mL), dried over Na_2SO_4, filtered, and concentrated at ambient temperature. The intermediate was immediately dissolved in 20 mL CH_2Cl_2 and tetrabutylammonium hydrogen sulfate (61 mg, 0.18 mmol), *N*-hydroxyphthalimide (146 mg, 0.90 mmol) and finally 1 M Na_2CO_3 (1.8 mL) were added. After 2 h, 20 mL CH_2Cl_2 were added, the organic layer washed with 15 mL H_2O, 15 mL saturated NaCl, and 15 mL H_2O. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (EtOAc/heptane, 5:4) gave **69** (60 mg, yield 55%): $[\alpha]_D^{22}$ 47 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88–7.78 (m, 4H, H-Ar), 6.78 (d, 2H, *J* 2.3 Hz, Ar-H), 6.59 (d, 1H, *J* 7.8 Hz, NH), 6.55 (t, 1H, *J* 2.3 Hz, H-Ar), 5.57 (br d, 1H, *J* 2.4 Hz, H-4), 5.35 (dd, 1H, *J* 10.9 Hz, *J* 8.1 Hz, H-2), 5.25 (d, 1H, *J* 8.1 Hz, H-1), 4.59 (ddd, 1H, *J* 10.9 Hz, *J* 7.8 Hz, *J* 3.3 Hz, H-3), 4.19–4.07 (m, 3H), 3.81 (s, 6H, OMe), 2.26 (s, 3H, OAc), 2.17 (s, 3H, OAc), 2.01 (s, 3H, OAc); FAB-HRMS *m/z* calcd. for $[C_{29}H_{30}N_2O_{13}Na]^+$: 637.1646. Found: 637.1660.

O-[3-Deoxy-3-(3,5-dimethoxybenzamido)-β-D-galactopyranosyl]-hydroxylamine 70

To **69** (26 mg, 43 μmol) dissolved in MeOH (2 mL) was added hydrazine hydrate (0.5 mL), and the reaction mixture was stirred overnight, and then concentrated. The residue was dissolved in H_2O and applied on to C-18 silica (5 g). Elution with a gradient of MeOH in H_2O and lyophilization gave **70** (15 mg, 72%): ^{1}H NMR (300 MHz, MeOD): δ 7.05 (d, 2H, *J* 2.3 Hz, Ar-H), 6.94 (d, 0.4H, *J* 2.3 Hz, NH partly exchanged with solvent), 6.64 (t, 1H, *J* 2.3 Hz, Ar-H), 4.53 (d, 1H, *J* 8.0 Hz, H-1), 4.10 (dd, 1H, *J* 10.8 Hz, *J* 3.1 Hz, H-3), 3.97 (br d, 1H, *J* 2.7 Hz, H-4), 3.81 (s, 6H, OMe), 3.75–3.69 (m, 3H); FAB-HRMS *m/z* calcd. for $[C_{15}H_{22}N_2O_8Na]^+$: 381.1274. Found: 381.1283.

O-[3-Deoxy-3-(3,5-dimethoxybenzamido)-β-D-galactopyranosyl]-indole-3-carbaldoxime 71

To **70** (11 mg, 31 μmol) and indole-3-carboxaldehyde (6.2 mg, 43 μmol) dissolved in H_2O (3 mL) and THF (1 mL) was added 0.1 M HCl (100 μL). The reaction mixture was stirred overnight, neutralized with 0.1 M $NaHCO_3$, and concentrated under reduced pressure. The residue was dissolved in H_2O and purified with C-18 RP HPLC. Elution with a gradient of acetonitrile in H_2O and lyophilization gave **71** (8 mg, 53%) as a E/Z (4:1) mixture: $[\alpha]_D^{22}$ 36 (*c* 0.3, MeOH; ^{1}H NMR (400 MHz, MeOD) for E isomer: δ 8.48 (s, 1H, NCH), 8.09 (br d, 1H, *J* 7.7 Hz, Ar-H), 7.56 (s, 1H, Ar-H), 7.40 (br d, 1H, *J* 8.1 Hz, Ar-H), 7.22–7.11 (m, 2H, Ar-H), 7.08 (d, 2H, *J* 2.3 Hz, Ar-H), 6.65 (br t, 1H, *J* 2.3 Hz, Ar-H), 5.19 (d, 1H, *J* 8.2, H-1), 4.23 (dd, 1H, *J* 10.9 Hz, *J* 3.1 Hz, H-3), 4.06 (d, 1H, *J* 3.0 Hz, H-4), 3.99 (dd, 1H, *J* 10.9 Hz, *J* 8.2 Hz, H-2), 3.84 (s, 6H), 3.84–3.72 (m, 3H). For Z isomer: 8.35 (s, 1 J, NCH), 7.91 (s, 1H, Ar-H), 7.82 (br d, 1H, *J* 7.1 Hz, Ar-H), 5.19 (d, 1H, *J* 8.1, H-1): FAB-HRMS *m/z* calcd. for $[C_{24}H_{27}N_3O_8Na]^+$: 508.1696. Found: 508.1700.

O-(3-Azido-3-deoxy-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-*N*-hydroxyphthalimide 73

To **67** (0.31 g, 0.84 mmol) and *N*-hydroxyphthalimide (0.68 g, 4.18 mmol) was added dry CH_2Cl_2 (15 mL). The mixture was cooled on an ice bath under a nitrogen atmosphere. A mixture of boron trifluoridediethyl etherate (2.1 mL, 16.7 mmol) and CH_2Cl_2 (5 mL) was slowly added and the reaction mixture was left at 4 °C for 48 hours. After filtration through Celite, the filtrate was washed with saturated $NaHCO_3$ and H_2O. The combined organic layer was dried (Na_2SO_4) and concentrated. Flash chromatography (heptane/EtOAc, 1:1) gave **73** (130 mg, 33%): $[\alpha]_D^{22}$ 1.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88–7.77 (m, 4H, Ar-H), 5.45–5.42 (m, 2H, *J* 3.5 Hz, *J* 1.1 Hz, *J* 2.3 Hz, *J* 8.0 Hz, H-2, H-4), 4.99 (d, 1H, *J* 8.2 Hz, H-1), 4.17 (br dd, 2H, *J* 7.0 Hz, *J* 0.7 Hz, H-6, H-6′), 3.90 (dt, 1H, *J* 6.7 Hz, *J* 1.2 Hz, H-5), 3.74 (dd, 1H, *J* 10.5 Hz, *J* 3.4 Hz, H-3), 2.28 (s, 3H, OAc), 2.21 (s, 3H, OAc), 2.00 (s, 3H, OAc); FAB-HRMS *m/z* calcd. for $[C_{20}H_{20}N_{24}O_{10}Na]^+$: 499.1077. Found: 499.1072.

O-(3-Azido-3-deoxy-β-D-galactopyranosyl)-hydroxylamine 74

Compound **73** (0.19 g, 0.41 mmol) dissolved in 2.3 M $MeNH_2$ in MeOH was stirred overnight and concentrated under reduced pressure. Flash chromatography (CH_2Cl_2/MeOH, 5:1–4:1) gave **74** (80 mg, 88%): $[\alpha]_D^{22}$ −5.3 (*c* 1, MeOH); ^{1}H NMR (300 MHz,

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D_2O): δ 4.58 (d, 1H, *J* 8.0 Hz, H-1), 4.07 (d, 1H, *J* 2.9 Hz, H-4), 3.76–3.72 (m, 3H), 3.67 (dd, 1H, *J* 10.5 Hz, *J* 8.0 Hz, H-2), 3.56 (dd, 1H, *J* 10.5 Hz, *J* 3.2 Hz, H-3); FAB-HRMS *m*/*z* calcd. for $[C_6H_{12}N_4O_5Na]^+$: 243.0705. Found: 243.0706.

O-(3-Azido-3-deoxy-β-D-galactopyranosyl)-indole-3-carbaldoxime 75

To **74** (27 mg, 123 μmol) and indole-3-carboxaldehyde (23 mg, 159 μmol) dissolved in H_2O (2 mL) and THF (2 mL) was added 0.1 M HCl (123 μL). The reaction mixture was stirred overnight, neutralized with 0.1 M $NaHCO_3$, and concentrated under reduced pressure. Flash chromatography (CH_2Cl_2/MeOH, 4:1) gave **75** (13 mg, 67%) as a E/Z (10:1) mixture: $[\alpha]_D^{22}$ −27 (*c* 0.5, MeOH); ^1H NMR (400 MHz, MeOD) for E isomer: δ 8.48 (s, 1H, NCH), 8.06 (d, 1H, *J* 7.9 Hz, Ar-H), 7.57 (s, 1H, Ar-H), 7.41 (d, 1H, *J* 8.1 Hz, Ar-H), 7.20 (br dt, 1H, *J* 7.6 Hz, *J* 1.1 Hz, Ar-H), 7.12 (dt, 1H, *J* 1.0 Hz, *J* 7.5 Hz, Ar-H), 5.11 (d, 1H, *J* 8.2 Hz, H-1), 3.99 (d, 1H, *J* 3.0 Hz, H-4), 3.95 (dd, 1H, *J* 10.3 Hz, *J* 8.2 Hz, H-2), 3.81–3.69 (m, 2H), 3.45 (dd, 1H, *J* 10.4 Hz, *J* 3.1 Hz, H-3). For Z isomer: 8.35 (s, 1H, NCH), 7.90 (s, 1H, Ar-H), 7.80 (br d, 1H, *J* 7.8 Hz, Ar-H), 7.45 (br d, 1H, *J* 7.3 Hz, Ar-H), 5.10 (d, 1H, *J* 8.1 Hz, H-1); FAB-HRMS *m*/*z* calcd. for $[C_{15}H_{17}N_5O_5Na]^+$: 370.1127. Found: 370.1137.

O-[3-Deoxy-3-(4-methylaminocarbonyl-1*H*-1,2,3-triazol-1-yl)-β-D-galactopyranosyl]-indole-3-carbaldoxime 76

To **75** (0.9 mg, 2.6 μmol) dissolved in propanol (0.5 mL) was added Cu wire, followed by methyl propiolate (0.5 μL). The reaction mixture was stirred for 24 hours then concentrated. The residue was dissolved in 40% $MeNH_2$ in H_2O, stirred overnight, and concentrated. Flash chromatography (CH_2Cl_2/MeOH, 10:1) gave **76** (0.72 mg, 65%) as a E/Z (10:1) mixture: ^1H NMR (400 MHz, MeOD) for E isomer: δ 8.48 (s, 1H, NCH), 8.48 (s, 1H, triazole-H), 8.20 (d, 1H, *J* 7.8 Hz, Ar-H), 7.57 (s, 1H, Ar-H), 7.40 (d, 1H, *J* 8.1 Hz, Ar-H), 7.21–7.18 (m, 1H, Ar-H), 7.16–7.11 (m, 1H, Ar-H), 5.21 (d, 1H, *J* 8.0 Hz, H-1), 4.98 (dd, 1H, *J* 11.1 Hz, *J* 3.1, H-3), 4.35 (dd, 1H, *J* 11.1 Hz, *J* 8.0 Hz, H-2), 4.11 (d, 1H, *J* 2.8 Hz, H-4), 3.82 (dd, 1H, *J* 11.4 Hz, *J* 6.4 Hz, H-6), 3.74 (dd, 1H *J* 11.5 Hz, *J* 5.8 Hz, H-6′), 3.84–3.72 (m, 2H), 2.94 (s, 3H, CH_3). For Z isomer: 8.52 (s, 1H, triazole-H), 8.37 (s, 1H, NCH), 7.92 (s, 1H, Ar-H), 7.81 (br d, 1H, *J* 7.1 Hz, Ar-H), 5.28 (d, 1H, *J* 7.9 Hz, H-1); MALDI TOF *m*/*z* calcd. for $[C_{19}H_{22}N_6O_6Na]^+$: 454.15. Found: 454.67; ESIMS *m*/*z* calcd. for $[C_{19}H_{23}N_6O_6]^+$: 431.1679. Found: 431.1678.

O-[3-Deoxy-3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)-β-D-galactopyranosyl]-indole-3-carbaldoxime 77

To **75** (2.2 mg, 6.3 μmol) dissolved in propanol (1 mL) was added Cu wire (about 1 g) followed by phenyl acetylene (2 μL). The reaction mixture was stirred for three days and then concentrated. Flash chromatography (CH_2Cl_2/MeOH, 10:1) gave **77** (1.26 mg, 44%) as a E/Z (5:1) mixture: ^1H NMR (400 MHz, MeOD) for E isomer: δ 8.49 (s, 1H, NCH), 8.45 (s, 1H, triazole-H), 8.10 (br d, 1H, *J* 6.6 Hz, Ar-H), 7.87–7.84 (m, 2H, Ar-H), 7.57 (s, 1H, Ar-H), 7.47–7.32 (m, 4H, Ar-H), 7.20 (dt, 1H, *J* 7.0 Hz, *J* 1.1 Hz, Ar-H), 7.14 (dt, 1H, *J* 7.7 Hz, *J* 1.0 Hz. Ar-H), 5.32 (d, 1H, *J* 8.0 Hz, H-1), 4.96 (dd, 1H, *J* 11.1 Hz, *J* 3.1 Hz, H-3), 4.44 (dd, 1H, *J* 11.1 Hz, *J* 8.0 Hz, H-2), 4.16 (d, 1H, *J* 3.0 Hz, H-4). 3.99–3.95 (m, 1H, H-5), 3.84 (dd, 1H, 11.4 Hz, H-6), 3.76 (dd, 1H, *J* 11.4 Hz, *J* 5.8 Hz, H-6′). For Z isomer: 8.38 (s, 1H, NCH), 7.93 (s, 1H, Ar-H), 7.82–7.80 (m, 2H, Ar-H), 5.30 (d, 1H, *J* 7.8 Hz, H-1), 4.59 (dd, 1H, *J* 11.0 Hz, *J* 8.0 Hz, H-2); ESIMS *m*/*z* calcd. for $[C_{23}H_{24}N_5O_5]^+$: 450.1777. Found: 450.1754.

Computational methods

Molecular modeling was performed with the MMFFs force field with water implemented in MacroModel (version 9.1, Schrödinger, LLC, New York, 2005). All triazole and oxime torsions were systematically varied, minimized, and finally minimized in complex with galectins. Starting conformations were built from the published crystal structures of galectin-1, 7, and 9 N in complex with lactose,[44–46] of galectin-3 in complex with a C3′-amido-derivatised LacNAc-based inhibitor,[17] and a homology model of galectin-8N in complex with LacNAc.[14] Starting conformations of the amides were positioned in the two possible orientations.

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