Carbohydrate triazoles and isoxazoles as inhibitors of galectins-1 and -3†

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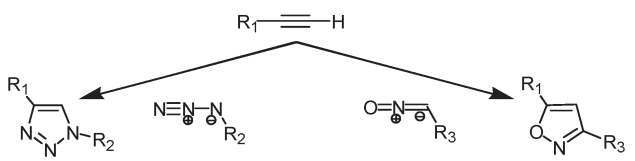
Galactosides and lactosides bearing triazoles or isoxazoles, regiospecifically prepared by [1,3]-dipolar cycloadditions between alkynes, azides or nitrile oxides, provided specific galectin-1 and -3 inhibitors with potencies as low as 20 μM.

Galectins are a family of cytosolic β-D-galactoside binding proteins of which fourteen members have been identified in mammals.[1,2] Galectin-1 (Gal-1) is a homodimer composed of subunits of approximately 130 amino acids and each subunit folds as one compact globular domain.[1] Galectin-3 (Gal-3) is quite unique and has one carbohydrate recognition domain (CRD) ending with a collagen-like repeat of peptides rich in proline and glycine capable of self association.[3,4] The roles of the galectin family are not yet clear, but a striking common feature of all galectins is the strong modulation of their expression during development, differentiation stages, and under different physiological or pathological conditions.[2] Recent studies have demonstrated that Gal-3 is involved in colon cancer metastasis,[5] brain tumor progression,[6] inhibition of metastasis-associated cancer cell adhesion,[7] and may play a key role in innate immunity.[8] Other reports suggest that Gal-3[9] and Gal-1[10] can regulate apoptosis processes.[11] It has also been reported that Gal-1 acts as an insoluble host factor that promotes HIV-1 infectivity through stabilization of virus attachment to host cells.[12]

Recent developments have been reported in the synthesis of carbohydrate-based 1,2,3-triazoles.[13,14] Meldal[15] and Sharpless[16] have solved the problem of 1,4-regioselectivity by using copper(I) catalysts (Scheme 1). This non-concerted cycloaddition is powerful for the synthesis of non-natural heterocycles which are attractive due to their stability.[17] Isoxazoles are also useful from the point of view of their stability under physiological pH and are easy to make. 3,5-Disubstituted isoxazoles are more difficult to synthesise but new methods have recently been discovered that facilitate their synthesis (Scheme 1).[16,18]



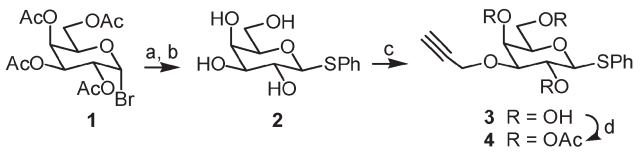
Scheme 1

Naturally occurring carbohydrate ligands for galectins[19] have low affinities, are too polar to be used as oral drugs, and possess low physiological stabilities due to their acid sensitive glycosidic bonds. A rational design approach for the development of new classes of glycomimetic inhibitors with high affinity, stability, and specificity is thus needed. Nilsson *et al.* have explored the 3′-position of lactoside derivatives toward the synthesis of high affinity inhibitors of galectin-3.[20,21] Some N-3′-triazole analogs provided high affinity enhancement. However, the lengthy synthetic scheme stimulated the impetus for a shorter synthesis. We thus report herein the straightforward synthesis and evaluation of O-3′ triazole and isoxazole analogs of both galactosides and lactosides. This strategy was also applied to the anomeric position.

The first alkyne adduct was synthesized from commercially available galactosyl bromide **1** shown in Scheme 2. Phase transfer catalyzed nucleophilic displacement[22] and de-*O*-acetylation using methanolic sodium methoxide afforded only phenyl 1-thio-β-D-galactoside **2**. Dibutylstannylene acetal formation with dibutyltin oxide[23] and *in situ* reaction with propargyl bromide allowed the regioselective formation of a 3-propynyl ether. Finally, protection under standard conditions provided intermediate **4**.

In order to synthesize more hydrolytically stable analogs, β-*C*-propynyl galactoside **6** was synthesised by ozonolysis of the known β-*C*-allyl derivative **5**[24] followed by the Ohira[25] modification of the Seyfert–Gilbert homologation reaction under mildly basic conditions (Scheme 3).

All terminal alkynes **4** and **6–9** reacted with a panel of azides (**10**, **11**, and **13**) or nitrile oxide **12** to give product containing only one regioisomer, summarized in Table 1. Alkyne **4** was treated with two different azides (**10** and **11**) for the formation of triazoles **14** and **15**, respectively, designed to maximize binding interactions with arginine 144.[20] Anomeric *C*-propynyl galactoside **6** reacted



Scheme 2 *Reagents and conditions*: a) HSPh, TBAHS, 1 M Na_2CO_3, AcOEt, 75%; b) NaOMe, MeOH, quant.; c) Bu_2SnO, MeOH, then Bu_4NI, propargyl bromide, benzene, 78%; d) Ac_2O, pyridine, 95%.

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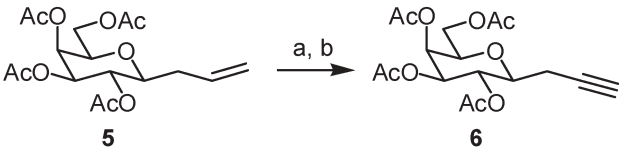
† Electronic supplementary information (ESI) available: Experimental procedure and compound characterization data. See DOI: 10.1039/b517529a

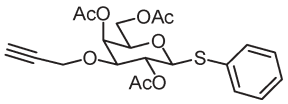
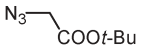
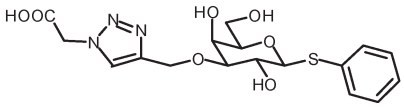
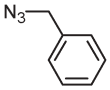
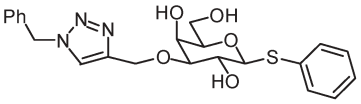
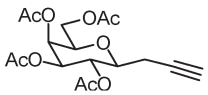
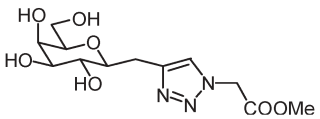
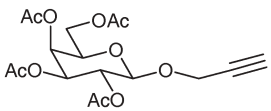
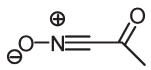
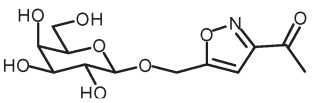
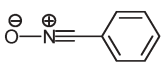
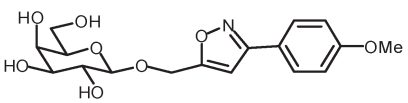
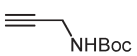
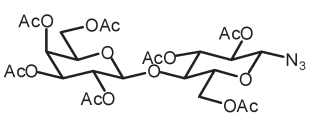
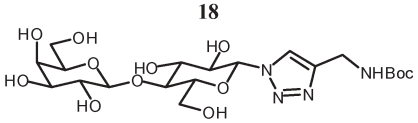
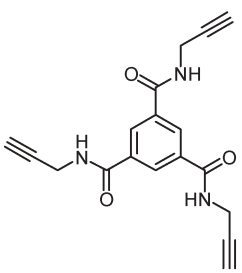
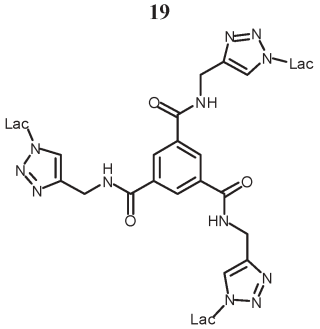


Scheme 3 *Reagents and conditions*: a) O_3, Me_2S, MeOH; b) $(MeO)_2P(O)CHN_2C(O)CH_3$, Na_2CO_3, MeOH, then Ac_2O, pyridine, 86% over 3 steps.

with azide **10** to form stable triazole **16** while *O*-propynyl galactoside **7** reacted with acetone nitrile oxide generated *in situ* from acetone and ceric ammonium nitrate (CAN)[18] and benzonitrile oxide[26] **12** (prepared from benzhydroximoyl chloride and pyridine) to provide the corresponding isoxazole heterocycles **17** and **18**, respectively. To synthesize and evaluate anomeric triazoles, lactosyl azide **13** reacted with N-Boc protected propargyl amine **8** to afford triazole **19** in good yield. Finally, C_3-symmetric tris-lactoside **20** was prepared from the cycloaddition of **13** with *N*,*N*′,*N*″-tripropargyl-1,3,5-carboxamidobenzene **9** (obtained in 82% yield by treatment of 1,3,5-benzenetricarboxylic acid with oxalyl chloride then propargyl amine added dropwise).

All new compounds and references **21** (galactose) and **22** (lactose) were tested by inhibition of hemagglutination assay at a concentration of 1 μM for both galectins. Assays were performed using red blood cells, type O, fixed with 3% glutaraldehyde–0.0025% NaN_3 in PBS.[12,27] Table 2 shows inhibitory properties and relative affinity of our derivatives toward Gal-1 and -3. The first overall observation was that none of our compounds bound to human Gal-4, indicating that triazole and isoxazole derivatives have better affinities and selectivities for Gal-1 and -3.[28] Triazoles prepared from a 3-*O*-propynyl spacer showed the most promising family of specific Gal-3 inhibitors (**3** and **14**) among the tested

Table 1 Synthesis of triazoles and isoxazoles from various alkynes, azides, and nitrile oxides

Entry	Alkynes	Azides or nitrile oxides	Products[d]	Yields (%)[d]
1[a]	 **4**	 **10**	 **14**	92
2[a]	**4**	 **11**	 **15**	97
3[a]	 **6**	**10**	 **16**	94
4[b]	 **7**		 **17**	78[e]
5[c]	**7**	 **12**	 **18**	61[e]
6[a]	 **8**	 **13**	 **19**	98
7[a]	 **9**	**13**	 **20**	83

[a] CuI, DIPEA, THF. [b] CAN, acetone, molecular sieves, DCM. [c] NCS, pyridine, $CHCl_3$. [d] Yields and products are for cycloaddition and deprotection steps (NaOMe, MeOH, except for entry 1: NaOH/MeOH/H_2O). [e] Based on recovered starting material.

Table 2 Inhibitory properties and relative activity for Gal-1 and -3

Compound no.	Inhibitory properties (mM)		Relative activity[a]	
	Galectin-1	Galectin-3	Galectin-1	Galectin-3
3	> 5	1.25	> 10	40
14	1.25	5	40	10
15	> 5	> 5	> 10	> 10
16	5	> 5	10	> 10
17	2.5	> 5	20	> 10
18	1.25	> 5	40	> 10
19	not tested			
20	0.02	0.25	40 (13.3)[c]	3.2 (1.1)[b]
21 Gal	50	50	1	1
22 Lac[c]	0.8	0.8	1	1

[a] Compounds **3** and **14–18** were compared to reference galactose **21** and compound **20** was compared to lactose **22**. [b] Number in parentheses expresses the relative potency of each lactose unit in the trivalent derivative compared to lactose. [c] Lactose is ~50× better than Gal.

compounds, while **15** did not have any activity, probably due to the large size of the substituent on the triazole. The more stable *C*-galactoside derivative **16** had inhibitory properties of 5 mM against Gal-1 but no inhibition toward Gal-3. Isoxazoles carrying two different substituents and aromatic **18** showed the best results (1250 μM) having 40 times better affinity than the natural analog **21**. No inhibition was observed against Gal-3 for **15–18**, indicating that no anomeric triazoles or isoxazoles had higher inhibitory potency against Gal-3.

Unfortunately, anomeric triazole **19** wasn't soluble enough for testing even with 5% DMSO added. The C_3-symmetrical lactoside **20** was designed for the reason described below. First, studies have demonstrated that some galectins are dimeric and create a soluble network in the presence of a multivalent ligand.[29] Thus, glycoclusters may increase affinity enhancement due to multivalent effects and formation of soluble cross-linked lattices. Glycoclusters with a valency of three were synthesized because it was previously demonstrated that C_3-symmetrical saccharide had good affinity with galectins[30] and symmetrical analogs provided simpler analysis due to their intrinsic symmetry. As expected, trivalent lactoside **20** provided inhibitory properties of 20 μM against Gal-1 for relative affinity of 40 that are 13 times better for each lactose unit. Surprisingly, the multivalent effect did not exist for Gal-3 with inhibitory properties of 250 μM and relative affinity of 3.2 which is almost one lactose unit by galectins.

In conclusion, isoxazoles and triazoles have potential as Gal-1 selective inhibitors over other galectins and compared well with known inhibitors.[20,21,31–33] The best inhibitors among the tested series were triazole **14** and anomeric isoxazole **18** with inhibitory properties of 1250 μM for both inhibitors. Simple 3-propynyl galactoside **3** was a good candidate against Gal-3 and is a potential lead structure for the further development of novel inhibitors. Finally, we developed a potent trivalent inhibitor (**20**) of galectins with inhibitory properties of 20 μM. It is probable that formation of C_3-symmetric analogs of **15** or **18** would provide even better results. Although the above compounds are notably less efficient than those described by Nilsson *et al.*,[20,21] we used inhibition of hemagglutination assays known to require higher concentrations.

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