

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

Denis Giguère,^a Ramesh Patnam,^a Marc-André Bellefleur,^a Christian St-Pierre,^b Sachiko Sato^b and René Roy^{*a}

Received (in Bloomington, IN, USA) 9th December 2005, Accepted 28th February 2006

First published as an Advance Article on the web 16th March 2006

DOI: 10.1039/b517529a

Galactosides and lactosides bearing triazoles or isoxazoles, regioselectively 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 μ M.

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-3⁹ and Gal-1¹⁰ 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} Meldal¹⁵ and Sharpless¹⁶ 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

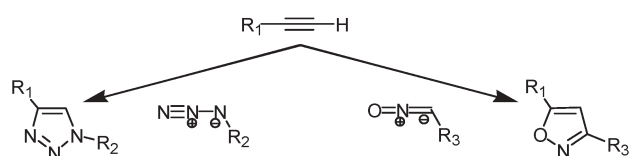
due to their stability.¹⁷ Isoxazoles are also useful from the point of view of their stability under physiological pH and are easy to make. 3,5-Disubstituted isoxazoles are more difficult to synthesise but new methods have recently been discovered that facilitate their synthesis (Scheme 1).^{16,18}

Naturally occurring carbohydrate ligands for galectins¹⁹ 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 displacement²² and de-O-acetylation using methanolic sodium methoxide afforded only phenyl 1-thio- β -D-galactoside **2**. Dibutylstannylene acetal formation with dibutyltin oxide²³ 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 Ohira²⁵ 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

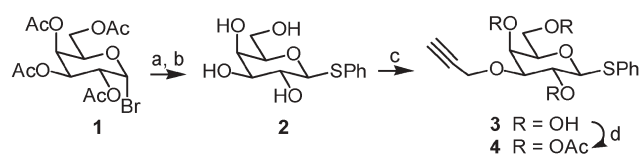


Scheme 1

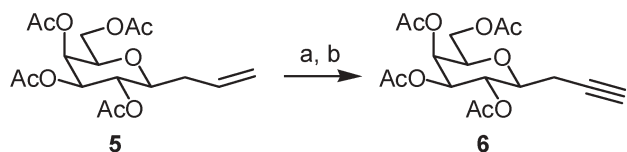
^a Department of Chemistry, Université du Québec à Montréal, P. O. Box 8888, Succ. Centre-Ville Montreal, Que., Canada H3C 3P8. E-mail: roy.rene@uqam.ca; Fax: +1 514 987 4054; Tel: +514 987 3000x2546

^b Research Center for Infectious Diseases, Faculty of Medicine, Université Laval, 2705 boul. Laurier, RC-9700 Sainte-Foy, Que., Canada G1V 4G2

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



Scheme 2 Reagents and conditions: a) HSPH, TBAHS, 1 M Na₂CO₃, AcOEt, 75%; b) NaOMe, MeOH, quant.; c) Bu₂SnO, MeOH, then Bu₄NI, propargyl bromide, benzene, 78%; d) Ac₂O, pyridine, 95%.



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 oxide²⁶ **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 $1 \mu M$ 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				92
2 ^a	4			97
3 ^a		10		94
4 ^b				78 ^e
5 ^c	7			61 ^e
6 ^a				98
7 ^a		13		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 C₃-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 C₃-symmetrical saccharide had good affinity with galectins³⁰ 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 C₃-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.

This work received support from the Natural Science and Engineering Research Council of Canada (NSERC) for a

Canadian Research Chair in Therapeutic Chemistry (RR) and from the Canadian Institutes for Health Research (SS).

Notes and references

- S. H. Barondes, D. N. Cooper, M. A. Gitt and H. Leffler, *J. Biol. Chem.*, 1994, **269**, 20807.
- L. Chiariotti, P. Salvatore, R. Frunzio and C. B. Bruni, *Glycoconjugate J.*, 2004, **19**, 441; H. Horrie, *Curr. Drug Targets*, 2005, **6**, 373.
- D. N. W. Cooper and S. H. Barondes, *Glycobiology*, 1999, **9**, 979.
- J. Hirabayashi and K. Kasai, *Glycobiology*, 1993, **3**, 297.
- R. S. Bresalier, N. Mazurek, L. R. Stenberg, J. C. Byrd, C. K. Yunker, P. Nangia-Makker and A. Raz, *Gastroenterology*, 1998, **115**, 287.
- B. N. Stillman, P. S. Mischel and L. G. Baum, *Brain Pathol.*, 2005, **15**, 124.
- J. Zou, V. V. Glinsky, L. A. Landon, L. Matthews and S. L. Deutscher, *Carcinogenesis*, 2005, **26**, 309.
- S. Sato and J. Nieminem, *Glycoconjugate J.*, 2004, **19**, 441; F. T. Liu, *Int. Arch. Allergy Immunol.*, 2005, **136**, 385.
- S. Califice, V. Castronovo and F. Van Den Brule, *Int. J. Oncol.*, 2004, **25**, 983; S. Nakahara, N. Oka and A. Raz, *Apoptosis*, 2005, **10**, 267.
- N. L. Perillo, K. E. Pace, J. J. Seilhamer and L. G. Baum, *Nature*, 1995, **378**, 736; T. Szoke, K. Kayser, J. D. Baumhake, I. Trojan, J. Furak, L. Tislaviez, A. Horvath, K. Szluha, H.-J. Gabius and S. André, *Oncology*, 2005, **69**, 167.
- D. K. Hsu and F. T. Liu, *Glycoconjugate J.*, 2004, **19**, 507; J. D. Hermandes and L. G. Baum, *Glycobiology*, 2002, **12**, 127R.
- M. Ouellet, S. Mercier, I. Pelletier, S. Bounou, J. Roy, J. Hirabayashi, S. Sato and M. J. Tremblay, *J. Immunol.*, 2005, **174**, 4120.
- J. A. F. Joosten, N. T. H. Tholen, F. A. E. Maate, A. J. Brouwer, G. W. van Esse, D. T. S. Rijkers, R. M. J. Liskamp and R. J. Pieters, *Eur. J. Org. Chem.*, 2005, 3182; S. Hotha and S. Kashyap, *J. Org. Chem.*, 2006, **71**, 364.
- Y. Gao, A. Eguchi, K. Takehi and Y. C. Lee, *Bioorg. Med. Chem.*, 2005, **13**, 6151.
- C. M. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057.
- F. Himo, T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless and V. V. Fokin, *J. Am. Chem. Soc.*, 2005, **127**, 210.
- H. Wamho, in *Comprehensive Heterocyclic Chemistry*; A. R. Katritzky, C. W. Rees and K. T. Potts, eds., Pergamon: Oxford, 1984, Vol. **5**, p. 669.
- K.-I. Itoh, S. Takahashi, T. Ueki, T. Sugiyama, T. Takahashi and C. A. Horiuchi, *Tetrahedron Lett.*, 2002, **43**, 7035.
- H. Leffler and S. H. Barondes, *J. Biol. Chem.*, 1986, **22**, 10119.
- P. Sörme, Y. Qian, P.-G. Nyholm, H. Leffler and U. J. Nilsson, *ChemBioChem*, 2002, **3**, 183; P. Sörme, P. Arnoux, B. Kahl-Knutsson, H. Leffler, J. M. Rini and U. J. Nilsson, *J. Am. Chem. Soc.*, 2005, **127**, 1737; I. Cumpstey, A. Sundin, H. Leffler and U. J. Nilsson, *Angew. Chem., Int. Ed.*, 2005, **44**, 5110.
- B. A. Salameh, H. Leffler and U. J. Nilsson, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3344.
- R. Roy, F. D. Tropper, S. Cao and J. M. Kim, Phase Transfer Catalyst Mechanism and Syntheses, *ACS Symp. Ser.*, 1997, **659**, 163.
- T. B. Grindley, Synthetic oligosaccharides, *ACS Symp. Ser.*, 1994, **560**, 51.
- T. Uchiyama, V. P. Vassilev, T. Kajimoto, W. Wong, H. Huang, C.-C. Lin and C.-H. Wong, *J. Am. Chem. Soc.*, 1995, **117**, 5395.
- S. Ohira, *Synth. Commun.*, 1989, **19**, 561.
- F. M. Hausser and X. Hu, *Org. Lett.*, 2002, **4**, 977.
- W. T. Butler, *J. Immunol.*, 1963, **90**, 663.
- Concentration at 0.5 μM for galectin-4.
- T. K. Dam, H.-J. Gabius, S. André, H. Kaltner, M. Lensch and C. F. Brewer, *Biochemistry*, 2005, **44**, 12564; N. Ahmad, H.-J. Gabius, S. Sabesan, S. Oscarson and C. F. Brewer, *Glycobiology*, 2004, **14**, 817.
- S. André, B. Liu, H.-J. Gabius and R. Roy, *Org. Biomol. Chem.*, 2003, **1**, 3909.
- R. T. Lee, Y. Ichikawa, H. J. Allen and Y. C. Lee, *J. Biol. Chem.*, 1990, **265**, 7874.
- H. Ahmed, H. J. Allen, A. Sharma and K. L. Matta, *Biochemistry*, 1990, **29**, 5315.
- D. Giguère, S. Sato, C. St-Pierre, S. Sirois and R. Roy, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1668.