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Copper-Free Huisgen 1,3-Dipolar Cycloaddition to 3-Benzotriazolo-3-Deoxy-β-D-Galactopyranoside: Cyclization of a Galactopyranoside Azide and Benzyne

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Abstract

Methyl 2-*O*-acetyl-3-benzotriazolo-4,6-*O*-benzylidene-3-deoxy-β-D-galactopyranoside was assembled by reacting benzyne, from 2-trimethylsilyl-trifluoromethansulfonylbenzene, and a secondary galactopyranoside azide in a copper-free Huisgen 1,3-dipolar cycloaddition. Following deprotection, the resulting benzotriazoles were evaluated as galectin inhibitors resulting in a 2-4 fold increase in affinity against galectin-1, -2 and -4 N-terminus compared to the parent methyl galactoside.

Keywords. Huisgen, Azide, Benzotriazole, Benzyne, Galactoside, Galectin.

Introduction

Our understanding of the pivotal roles of the mammalian galectin family of proteins has expanded rapidly over the last years.[1-3] Galectins have been implicated in cancer progression, immunity[4], and inflammation and the underlying molecular mechanisms, such as regulation of apoptosis, cell signalling, intracellular trafficking and cell adhesion, responsible have increasingly been understood. Recent important examples, such as that galectin-3 causes regulation of growth factor residence time at the plasma membrane[5] and anergy in tumour-infiltrating leukocytes by preventing CD8-TCR co-localization[6], point to the advantages in being able to modulate the effects of the galectins downwards. The galectins are defined by β-galactoside binding ability[7], even though the members differ in specificity for β-galactoside-containing glycans, which is crucial for their functions. Currently, it seems like most of the galectins' functions depend on their β-galactoside binding ability.[8] Thus, a viable route to modulating the galectins' functions is by competitive inhibitors. In our continuing efforts[9-16] to design and synthesize high-affinity and member-selective galectin inhibitors, we turned our interest towards benzotriazolo-substituted galactosides. While we had experience with the copper-catalyzed Huisgen 1,3-cycloaddition and ample literature precedence to go by, there was very little precedence of azide-benzyne cycloadditions to form benzotriazoles. The annulation of an azide and an alkyne was first reported in the late 19th century by A. Michael.[17] The thermal reaction was exhaustively developed by R. Huisgen[18] in the mid 20th century up to the point were it is now commonly referred to as the Huisgen 1,3-dipolar cycloaddition. The independent discovery of copper catalysis by the groups of Sharpless[19] and Meldal[20] helped immensely in popularizing the cycloaddition by allowing for ambient temperature and very high regioselectivities. The reaction is now the perhaps most prominent member of the "click" family of reactions. Due to the bio-orthogonal nature of organic azides and alkynes it has been an interesting means to conjugation in biological systems. However, use of the method in biological systems has been hampered by the toxicity of the copper and/or necessity to run at elevated temperatures. To circumvent copper-toxicity, activated alkynes have been developed that do not require copper catalysis. The activated alkynes are either electron-deficient[21] or strained ("spring-loaded").[22,23] The electron-deficient alkynes have met with less success in *in vitro* studies due to deleterious conjugate addition side reactions with nucleophiles.[22] The elegant use of a strained cyclooctyne in an *in vitro* cycloaddition with azido-glycan was first reported by Bertozzi and co-workers.[22] The first generation cycloalkynes were not as efficient as conjugating the azido-glycan in a Staudinger ligation, which prompted the groups of Bertozzi and Boons to successfully develop more activated cyclooctynes.[22,23]

Perhaps one may view the annulation of an azide and benzynes, or other arynes, as an extreme case of "spring-loaded" alkynes to give benzotriazoles. Although a reaction that has been known for long,[24-29] the problems in generating the benzynes precursor and the

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vigorous conditions for the generation of benzyne itself has prevented its popularity. The development of 2-trimethylsilyl-trifluoromethanesulfonylbenzene[30] from which benzyne can be generated under mild conditions allowed the groups of Larock[31], Feringa[32] and Biehl[33] to very recently report on mild and practical cycloadditions to form benzotriazoles. Larock and co-workers studied the reaction extensively and settled on CsF-promoted reaction in acetonitrile under ambient temperature.[31] Feringa and co-workers shortly after contributed the use of CsF/crown ethers to allow for faster reactions at ambient temperature[32] while Biehl reported on a microwave-assisted version of the reaction.[33] Concurrently with these reports we have investigated a similar 2-trimethylsilyl-trifluoromethanesulfonylbenzene/ CH_3CN/TBAF system at ambient temperature for the azide annulation as reported below. Furthermore, this investigation was motivated by the search for biological activity, as we envisioned the benzotriazole structure would confer affinity to galactoside-based galectin inhibitors.

Formation of Secondary Benzotriazoles

3-Benzotriazolo-3-deoxy-galactoside **2** was readily obtained by a copper-free Huisgen 1,3-dipolar cycloaddition of azide **1**[34] and benzyne, obtained by treating 2-trimethylsilyl-trifluoromethanesulfonylbenzene with fluoride (TBAF) (Scheme1). Using 1.2 equiv of 2-trimethylsilyl-trifluoromethanesulfonylbenzene in THF gave 48% yield of product **2** and 49% yield of recovered azide **1** in a clean reaction. Alternatively, the product yield could be improved by using 3 eq of 2-trimethylsilyl-trifluoromethanesulfonylbenzene in CH_3CN to obtain product in 69% yield along with several by-products. Higher yields may likely be possible by reversal of our conditions, i.e. quenching the generated benzyne with excess azide. However, in our case, the azide was far more precious than the commercially available benzyne precursor. De-benzylidenation using aqueous HOAc followed by sodium methoxide mediated de-acetylation uneventfully gave benzotriazoles **3** and **4**, respectively.

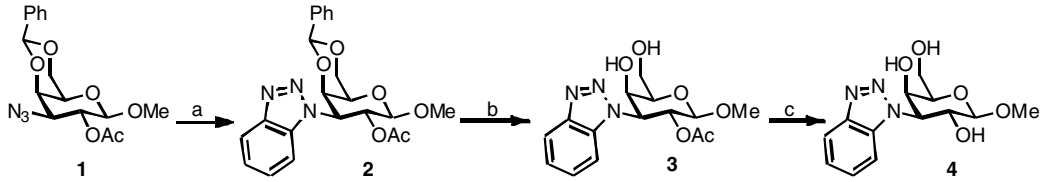
Benzotriazoles as Galectin Inhibitors

Benzotriazoles **3** and **4** were evaluated as galectin inhibitors in a competitive fluorescence polarization assay[13,14,36] (Table 1). Generally, acetylated **3** performed better than de-acetylated **4** by offering a 2-4 fold increase in affinity over parent methyl β-D-galactopyranoside for galectins 1, 2, 4N, and 7.

Although all galectins share a number of conserved amino acids, dubbed the galectin signature amino acids and critical for galactoside-binding, there are nevertheless variations in amino acid composition in and around the carbohydrate recognition domain (CRD) causing subtle or not-so-subtle specificity differences for the galactoside-containing oligosaccharides encountered *in vivo*. However, the modest affinity differences found for these benzotriazoles correspond to very small binding energy differences, making accurate predications and structure-based explanations difficult. Nevertheless, one may conclude that the galectin-1[37] and galectin-2[38] CRD:s are sufficiently similar to easily fit with affinity data. Galectin-4N[39] and -7[40] are very similar to each other but differs more from galectin-1 in CRD amino acid composition, however galectin-7 shows close topological similarity to galectin-1 and may accommodate the benzotriazole substituent in the same fashion. Interestingly, galectin-4C[41], which is much more similar to galectin-1 than are galectin-4N and -7, shows no measurable binding at all to **3** and weak binding to **4**.

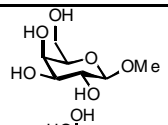
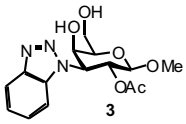
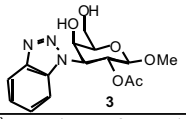
Conclusions

An efficient protocol towards benzotriazolo-substituted galactosides has been developed, which was motivated by our search for galectin inhibitors with improved affinity and selectivity. A 2-4 fold affinity increase by the benzotriazolo substituent on binding galectins 1, 2, 4N, and 7 were observed suggesting that the benzotriazole structure situated on galactose C3 is not optimal for these lectins. However, the synthetic protocol is mild, simple, efficient, and most probably



Scheme 1. Reagents and conditions for the formation of secondary benzotriazoles.
A) 2-trimethylsilyl-trifluoromethansulfonylbenzene, TBAF, CH_3CN (69%).
b) HOAc (aq., 67%) (66%). c) NaOMe, MeOH (95%).

Table 1. Affinity of compounds for different galectins as measured by a competitive fluorescence polarization assay.

Compound	Galectin							
	1	2	3	4C[a]	4N[a]	7	8N[a]	9N[a]
	>10[35]	13±3[13]	4.4±0.8[35]	>10[13]	6.6±0.2[13]	4.8[35]	6.3±1.5[13]	1.2±0.2[13]
 **3**	4.2±0.6	3.8±0.1	4.4±1.0	>4	1.4±0.5	2.6±1.1	>4	3.4±0.4
 **3**	5.6±0.2	6.7±0.2	17.1±1.6	10.6±0.9	2.7±0.8	>2	10-40	>20

[a] C and N refer to the C- and N-terminal CRD of galectins 4, 8 and 9.

compatible with other carbohydrate scaffolds, which holds promise for its use for the discovery of inhibitors of other classes of lectins.

5. Experimental Procedures

Materials and Methods.

NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer at ambient temperature. ^{1}H-NMR spectra were assigned using 2D-methods (COSY). Chemical shifts are given in ppm downfield from the signal for Me_4Si, with reference to residual $CHCl_3$, DMSO, HDO or CD_2HOD. HRMS was recorded on a Micromass Q-TOF micro spectrometer (ESI) and a JEOL SX-120 FAB spectrometer. Reactions were monitored by TLC using aluminum-backed silica gel plates (Merck $60F_{254}$) and visualized using UV light and by charring with ethanolic H_2SO_4 (7%). Preparative chromatography was performed using silica gel (Amicon Matrex 35-70 m, 60 Å) columns. Preparative TLC was performed using glass-backed silica gel plates (200*200*1 mm, $60F_{254}$). DMF was distilled and stored over 4 Å M.S. Other solvents were dried by storing over activated M.S. Reagents were from Acros and Sigma-Aldrich and used as supplied.

Methyl 2-*O*-acetyl-3-(*N*-benzotriazolo)-4,6-*O*-benzylidene-3-deoxy-β-D-galactopyranoside 2.

Method A: TBAF (1 M, THF, 857 μL) was added in portions to a stirred solution of the azide **1**[34] (100 mg, 286 μmol) and 2-trimethylsilyl-trifluoromethansulfonylbenzene (208 μL, 857 μmol) in CH_3CN (3 mL) over 1.25 h at rt. After another 1.5 h, the reaction was concentrated and purified by column chromatography (toluene:acetone 100:10) to give benzotriazole **2** in 69% yield (84 mg). Method B: 2-trimethylsilyl-trifluoromethansulfonylbenzene (19 μL, 78 μmol) was added dropwise to a stirred solution of the azide **1**[34] (22.8 mg, 65 μmol) and TBAF (20 mg, 78 μmol) in CH_3CN (1 mL) at rt. After 6 h, the reaction was concentrated and purified by column chromatography (toluene:acetone 100:10) to give benzotriazole **2** in 48% yield (13.4 mg) and recovered **1** in 49% yield (11.1 mg). ^{1}H NMR ($CDCl_3$) δ 7.97 (br d, J = 8.3 Hz, 1 H, Ar*H*), 7.79 (br d, J = 8.4 Hz, 1 H, Ar*H*), 7.38-7.15 (m, ~7 H, Ar*H*), 6.08 (dd, J = 11.7, 7.6 Hz, 1 H, *H*2), 5.53 (dd, J = 11.7, 3.4 Hz, 1 H, *H*3), 5.45 (s, 1 H, C*H*), 4.66 (d, J = 7.6 Hz, 1 H, *H*1), 4.53 (dd, J = 3.3, 0.8 Hz, 1 H, *H*4), 4.50 (dd, J = 12.6, 1.5 Hz, 1 H, *H*6), 4.16 (dd, J = 12.6, 1.8 Hz, 1 H, *H*6), 3.82-3.79 (m, 1 H, *H*5), 3.61 (s, 3 H, OC*H*$_3$), 1.69 (s, 3 H, *Ac*). ESIMS *m/z* calcd for $[C_{22}H_{24}N_3O_6]^+$, 426.1665; found: 426.1670.

Methyl 2-*O*-acetyl-3-(*N*-benzotriazolo)-3-deoxy-β-D-galactopyranoside 3.

Compound **2** (84 mg, 197 μmol) was stirred in HOAc (aq, 67%, 2.4 mL) at 70 °C for 6 h before being concentrated and purified by column chromatography (CH_2Cl_2:MeOH 100:5) to give **3** in 66% yield (44 mg). ^{1}H NMR (MeOD) δ 7.99 (app t, J = 7.8 Hz, 2 H, Ar*H*), 7.58-7.52 (m, 1 H, Ar*H*), 7.46-7.41 (m, 1 H, Ar*H*), 6.08 (dd, J = 11.4, 7.7 Hz, 1 H, *H*2), 5.43 (dd, J = 11.4, 3.0 Hz 1 H, *H*3), 4.70 (d, J = 7.7 Hz, 1 H, *H*1), 4.28 (br d, J = 2.2 Hz, 1 H, *H*4), 3.97 (ddd, J = 6.6, 5.7, 1.0 Hz, 1 H, *H*5), 3.85 (dd, J = 11.3, 6.6 Hz, 1 H, *H*6), 3.79 (dd, J = 11.3,

5.7 Hz, 1 H, *H*6), 3.58 (s, 3 H, OCH_3), 1.69 (s, 3 H, *Ac*). ESIMS *m/z* calcd for $[C_{15}H_{20}N_3O_6]^+$, 338.1352; found: 338.1345.

Methyl 3-(*N*-benzotriazolo)-3-deoxy-β-D-galactopyranoside 4.

Methanolic sodium methoxide (1 M, 100 μL) was added to as stirred solution of ester **3** (29 mg, 86 μmol) in MeOH (2 mL) at rt. After 17 h, the reaction was concentrated and purified by column chromatography (CH_2Cl_2:MeOH 100:5 -> 100:10 gradient) to give benzotriazole **4** in 95% yield (24 mg). ^{1}H NMR (MeOD) δ 8.01-7.93 (m, 2 H, Ar*H*), 7.57-7.51 (m, 1 H, Ar*H*), 7.46-7.41 (m, 1 H, Ar*H*), 5.11 (dd, *J* = 11.1, 3.1 Hz, 1 H, *H*3), 4.71 (dd, *J* = 11.1, 7.6, 1 H, *H*2), 4.48 (d, *J* = 7.6 Hz, 1 H, *H*1), 4.18 (br d, *J* = 3.0 Hz, 1 H, *H*4), 3.91 (ddd, *J* = 6.6, 5.6, 1.0 Hz, 1 H, *H*5), 3.83 (dd, *J* = 11.2, 6.5 Hz 1 H, *H*6), 3.77 (dd, *J* = 11.2, 5.7 Hz, 1 H, *H*6), 3.64 (s, 3 H, OCH_3). ESIMS *m/z* calcd for $[C_{13}H_{18}N_3O_5]^+$, 296.1246; found: 296.1252.

Galectin binding assay.

Affinities of compounds for each galectin were determined in a competitive fluorescence polarization assay as described previously.[13,14,36]

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