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3-(1,2,3-Triazol-1-yl)-1-thio-galactosides as small, efficient, and hydrolytically stable inhibitors of galectin-3

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Abstract—Copper(I)-catalyzed addition of alkynes to methyl 3-azido-3-deoxy-1-thio-β-D-galactopyranoside afforded stable and structurally simple 3-deoxy-3-(1*H*-1,2,3-triazol-1-yl)-1-thio-galactosides carrying a panel of substituents at the triazole C4 in high yields. The 3-(1*H*-[1,2,3]-triazol-1-yl)-1-thio-galactoside collection synthesized contained inhibitors of the tumor- and inflammation-related galectin-3 with K_d values as low as 107 μM, which is as potent as the natural disaccharide inhibitors lactose and *N*-acetyllactosamine.

Galectins are a family of carbohydrate-binding proteins (i.e., lectins), which are defined by having at least one carbohydrate recognition domain (CRD) with specificity for β-D-galactosides.[1,2] Fourteen different galectins have been characterized so far; they are numbered according to the chronology of their discovery (galectin-1 to galectin-14) and are widely distributed from lower to higher vertebrates. Although the exact functions of galectins are still unknown, it is clear that they play a role in cell–cell communication, cell-matrix adhesion, cell growth regulation, and intracellular processes, such as pre-mRNA splicing. They show a combination of the properties expected for both intra- and extracellular proteins. Their activity as extracellular proteins, where they usually bind glycoconjugates, has attracted most attention.[3–11] Strong evidence from several reports suggests that galectins play an important role in inflammation and immunity,[5,12–14] as well as cancer.[6,7,10] Recently, it was demonstrated that a C-terminal fragment of galectin-3 containing the CRD decreased tumor growth in a human breast cancer mouse model by acting as a dominant negative inhibitor.[15] Moreover, glycoconjugates, which decrease metastasis in mice, have been suggested to act by the inhibition of galectins.[16,17] Thus, efficient inhibition of galectins may lead to anti-inflammatory and anti-cancer properties.

Natural small ligands of the galectins, such as *N*-acetyl lactosamine (LacNAc) and lactose, show low inhibition potency[18] and development of new inhibitors with a higher affinity for galectins has therefore emerged as an important task. The crystal structure of a LacNAc: galectin-3 complex[19] shows a possible extended binding groove close to HO-3(Gal). This binding groove has been recently exploited in the design of high-affinity inhibitors through synthesis of 3′-benzamido derivatives of LacNAc from a 3-azido-3-deoxy galactose precursor.[20,21] Such a 3-azido-3-deoxy-galactose derivative is particularly versatile, as the azide functionality potentially allows for various different chemical transformations. Cycloaddition of azides with acetylenes appears to be attractive in this context, as the resulting [1,2,3]-triazoles are non-natural heterocycles well known for their stability towards oxidation, reduction, hydrolysis, etc.[22] They have been traditionally prepared through thermal Huisgen 1,3-dipolar cycloaddition[23] between azides and monosubstituted alkynes, yielding both 1,4- and 1,5-disubstituted triazoles. In recent years, an important development in triazole chemistry has been reported,[24–27] in that the presence of a copper catalyst leads to a regioselective reaction toward 1,4-disubstituted triazoles. Moreover, mild conditions and high yield of the reaction attract attention, as neither complex reagents nor anhydrous conditions are needed.

In this communication, we report on the discovery of novel galectin-3 inhibitors based on Cu(I)-catalyzed cycloaddition of methyl 3-azido-3-deoxy-1-thio-β-D-

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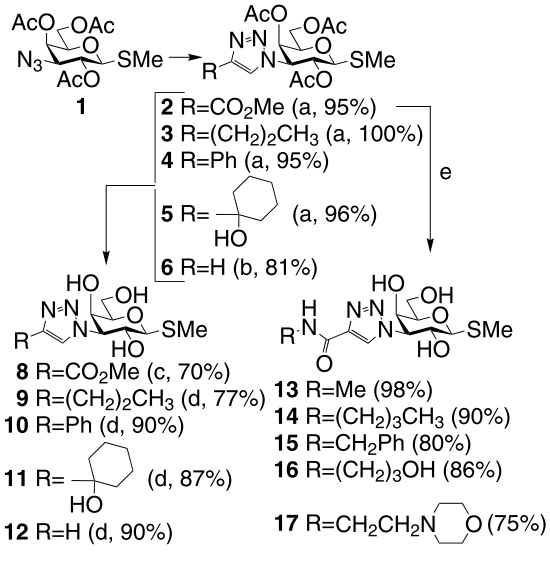
galactoside **1**[20] with acetylene derivatives. The expected 1,4-disubstituted triazoles **2–5** were obtained in high yield and as single regioisomers (Scheme 1). The reaction took place at room temperature in the case of methyl propiolate (→**2**), whereas for aliphatic or aromatic substituted acetylenes (→**3–5**) long reaction times and heating at 45° were necessary. This was probably due to a lower dipolarophilicity of these acetylenes combined with steric hindrance of the azide **1**. The monosubstituted triazole **6** was obtained by heating **1** and propiolic acid without Cu(I) at 65° in toluene; the initially formed 4-carboxy-triazole was decarboxylated in situ.[28] Deprotection of **3–6** with methylamine in water gave **9–12**, whereas the ester **8** was obtained by the treatment of **2** with methanolic sodium methoxide. The reaction of methyl ester **2** with different amines gave a panel of 4-carbamoyltriazoles (**13–17**). Amide formation was fast in the case of aliphatic primary amines, while heating at 40° was necessary in the case of benzylamines (→**15**).

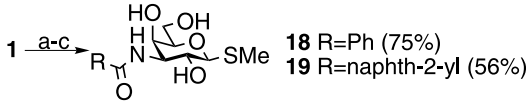
The triazoles **8–17** and the references **18–22** were evaluated as inhibitors of galectin-3 in a fluorescence polarization assay that had been recently developed by us[30,31] (Table 1). As reference compounds in the evaluation of triazoles **8–17** against galectins were included methyl 3-benzamido-3-deoxy-1-thio-β-D-galactoside **18** and methyl 3-(2-naphthamido)-3-deoxy-1-thio-β-D-galactoside **19** (Scheme 2), together with methyl β-D-galactoside **20**, methyl β-lactose **21**, and methyl β-LacNAc **22**. The references **18** and **19** are monosaccharide analogues of earlier reported high-affinity 3′-benzamido LacNAc-derived galectin-3 inhibitors.[20,21] A first and rather discouraging observation was that the triazole ring itself appeared to be detrimental to the interaction with galectin-3, as the monosubstituted 3-(triazol-1-yl)-galactoside **12** was virtually non-inhibitory. Fortunately, substituents at C4 of the triazole regain, as for the propyl substituted one (**9**), and even improve the inhibitory power (**8**, and **10**, **11**, and **13–17**) compared to that of the reference galactoside (**20**). In particular, a phenyl substituent (**10**) or amides (**13–17**) at C4 of the triazole enhance the affinity for galectin-3. The triazole ring of **8–17** can thus be regarded as an 'ambivalent spacer,' because it decreases inhibitory potency, but places its C4-substituents in positions allowing for favorable interactions with galectin-3. The affinity-enhancing effects of triazole C4-substituents clearly surpass the affinity-decreasing effect of the triazole ring itself.



Scheme 1. Reagents and conditions: (a) Cu(I), DIPEA, toluene, 40 °C; (b) Propiolic acid, toluene, 80 °C; (c) NaOMe/MeOH; (d) $MeNH_2$ 40% in H_2O; (e) RNH_2, MeOH.

Table 1. K_d values for galectin-3 with inhibitors **8–19** and the methyl β-glycosides of D-galactose **20**, lactose **21**, and LacNAc **22**

Compound	K_d (μM)	Affinity relative to **20**
8	1408	3.2
9	4600	1.0
10	147	29
11	1750	2.5
12	>20000	<0.2
13	230	19
14	124	36
15	107	42
16	386	12
17	571	7.5
18	2692	1.7
19	272	16
20	4400[29]	1
21	222[29]	20
22	67[21]	64



Scheme 2. Reagents and conditions: (a) H_2/C-Pd, MeOH; (b) Acyl chloride, pyridine; (c) $MeNH_2$ 40% in H_2O.

The three best inhibitors, the phenyl-substituted **10**, and the butyl and benzyl amides **14** and **15**, are about 40 times better than the simple methyl β-D-galactoside **20**, which indicates that the interactions between galectin-3 and the triazole C4-substituent are indeed strong. Furthermore, the K_d values of **10** (147 μM), **14** (124 μM), and **15** (107 μM) are significantly lower than those of the methyl lactoside **21** (222 μM) and almost as low as the LacNAc methyl glycoside **22** (67 μM). LacNAc is the most potent natural disaccharide inhibitor of galectin-3.[18] However, it should be emphasized that modified monosaccharide derivatives, such as the triazoles **10**, **14**, and **15**, have several advantages over oligosaccharides as inhibitors of galectin-3, as they may possess a longer half-life in vivo due to lack of hydrolytically labile glycosidic bonds, they are often easier to prepare, and they are typically less polar, which could improve cell membrane permeability. Finally, several triazole-derivatives (**10** and **13–15**) are better than the C3-naphthamido reference compound **19**. The C3-naphthamido group has been reported to be the most efficient affinity-enhancing C3-substituent on LacNAc derivatives,[21] much better than natural saccharides,

and the 4-substituted triazole moiety thus appears to be promising as an even more potent affinity-enhancing structural motif at galactose C3.

In conclusion, we have developed a new class of 3-[1,2,3]-triazol-1-yl galactosides as highly potent galectin-3 inhibitors. The inhibitors are the best monosaccharide inhibitors of galectin-3 known to date and are distinguished by their easy synthesis, high stability, and large potential for further improvement by optimizing the triazole substituents and by attaching carbohydrates or other structures to the galactose residue. Thus, the triazoles **8–17** are promising lead structures for the development of high-affinity galectin-3 inhibitors with potential as tools for studies of galectin-3 functions in vivo and as galectin-3 blocking drugs.

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