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Glycosidase inhibition by 1-glycosyl-4-phenyl triazoles

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Abstract—1-Glycosyl-4-phenyl triazoles have been prepared via a copper-mediated [3 + 2] cycloaddition of glycosyl azides with phenylacetylene. These triazoles have been evaluated for their ability to inhibit the enzymatic activity of glycosidases. © 2005 Elsevier Ltd. All rights reserved.

1. Introduction

Glycosidase inhibitors are useful anti-viral, anti-proliferative, and anti-diabetic agents.[1] Enzyme-catalyzed glycosidic bond cleavage of pyranosides is usually mediated by two catalytic carboxylic acid residues and proceeds typically via a positively charged oxocarbenium ion transition state.[2] The charge buildup is accompanied by planarization of the anomeric carbon and concomitant distortion of the pyranose ring out of its chair conformation. Many natural and synthetic glycosidase inhibitors mimic the charge buildup and/or the conformational distortion of the transition state.[3] Glycosyl amines with the general structures **A** or **B** inhibit a variety of glycosidases by positioning a positive charge in the active site, which can interact with the carboxylate moieties.[4] Introduction of substituents on the amino functionalities can generate better inhibitors than the parent amines, presumably through favorable binding interactions between the R substituent and residues immediately adjacent to the active site.[4,5] In this paper, we report that glycosyl triazoles, **C**, are glycosidase inhibitors[6] (Fig. 1).

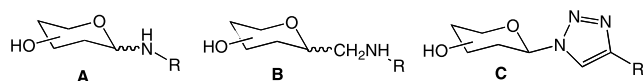Based on the known affinities of many glycosidases for aryl glycosides, we prepared and tested a pair of triazoles

derived from phenylacetylene. These glycosyl triazoles were synthesized via a copper-catalyzed [3+2] cycloaddition between the appropriate acetyl-protected β-azido glycosides (**1/2**) and phenylacetylene (Scheme 1).[7,8] Subsequent Zemplen deprotection of the esters afforded the target compounds **5** and **6** as single regioisomers.

The glycosyl triazoles were screened for inhibitory activity against three β-glycosidases—sweet almond glucosidase (SAG), *Escherichia coli* galactosidase (ECG), and bovine liver galactosidase (BLG).[9] A percentage inhibition assay was performed as a preliminary screen. In this experiment, the activity of the enzymes was compared in the absence and presence of the triazoles (Table 1). Two known glycosidase inhibitors, deoxygalactonojirimycin (DNJGal) and deoxynojirimycin (DNJ, gluco configuration), were used as positive controls for comparison.[10] Neither triazole was a good inhibitor of SAG, while the galactose derivative **5** showed a modest inhibition of ECG. The glucose triazole **6** is a better

Figure 1. Aglycone-modified glycosidase inhibitors.
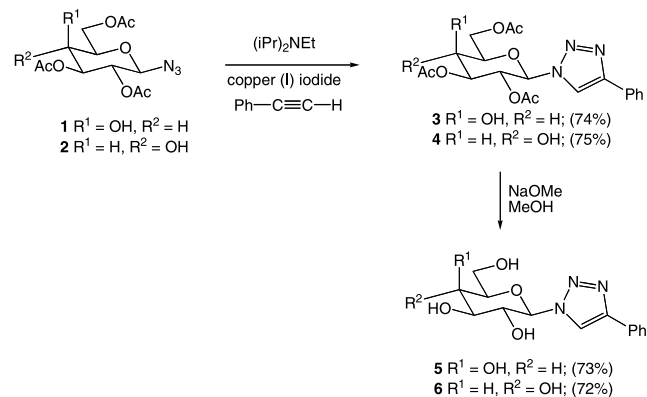
Scheme 1.

Table 1. Percentage inhibition of glycosidases by triazoles[a]

Compound[b]	Enzymes		
	SAG (%)	ECG (%)	BLG (%)
5	<20	30 ± 11	<20
6	<20	<20	48 ± 11
DNJGal	<20	91 ± 1	<20
DNJGlu	50 ± 8	<20	<20

[a] Values are the averages of at least three experiments, with the exception of the screenings of **6** and DNJGal with ECG, which were only carried out twice.

[b] All compounds were screened at a concentration of 0.24 mM.

inhibitor of BLG than the galactose derivative **5**, an observation which is consistent with the known ability of BLG to bind glucosides.[11]

The inhibition of ECG by the triazole **5** was examined in greater detail at various substrate and inhibitor concentrations. These experiments indicated that **5** inhibited ECG with a K_i value of 330 ± 110 μM. In a similar manner, the K_i of **6** for BLG was determined to be 1.6 ± 0.4 mM. The K_i value of **5** is comparable to that reported for the inhibition of ECG by the *N*-phenyl derivative of **B** (171 μM).[4b] However, the isosteric 5-phenyl-1,3,4-oxadiazole derivative of galactose is a better inhibitor of ECG (15 μM), indicating that subtle changes to heteroatom positioning within the five-membered ring affect the inhibitory potencies.[4c]

Anomeric triazoles can be converted into better nucleofuges upon protonation. Bröder and Kunz have shown that electron-deficient glycosyl triazoles are converted to the corresponding glycosyl fluorides upon treatment with HF·pyridine.[8h] Thus, it is possible that glycosyl triazoles also serve as substrates for these enzymes. To rule out this possibility, we incubated the triazoles with the enzymes and subsequently examined the solutions for the presence of phenyl triazole and glucose/galactose, which were not detected by thin-layer chromatography or by ESI mass spectrometry. Furthermore, we have evaluated the inhibitory properties of the potential hydrolysis products phenyl triazole and the parent sugars, and they do not exhibit any appreciable inhibitory activity against these enzymes.

Modular synthesis of glycosyl triazoles is well-suited for rapid generation of combinatorial libraries.[5c,12] We are currently preparing libraries of glycosyl triazoles in an effort to identify higher affinity inhibitors, as well as those that are highly selective for individual glycosidases.

2. Experimental procedures

β-Azido-galactopyranoside tetraacetate **1** and β-azido-glucopyranoside tetraacetate **2** were prepared according to the method of Tropper et al.[13]

2.1. Representative procedure for the preparation of glycosyl triazoles via 3 + 2 cycloaddition

β-Azido-glycopyranoside tetraacetate (0.5 mmol), which had been azeotropically dried by rotary evaporation of toluene (3×), was dissolved in dry toluene (0.1 M), followed by the addition of phenylacetylene (1.3 equiv) and copper(I) iodide (0.1 equiv). Diisopropylethylamine (1 equiv) was then added to the solution. The reaction was allowed to stir at room temperature until *tlc* analysis (1:1; ethyl acetate/hexane) indicated completion. The solution was concentrated in vacuo and purified by flash chromatography (1:1 ethyl acetate:hexane or 3:1 toluene:ethyl acetate as the eluent). A slight yellow coloration in the resultant product was removed by trituration with cold methanol to provide the purified product.

2.1.1. 4-Phenyl-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galacto-pyranosyl)-1,2,3-triazole (3). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 8.05, 1H, s; 7.86, 2H, d, *J* = 8.2 Hz; 7.47–7.33, 3H, m; 5.90, 1H, d, *J* = 9.3 Hz; 5.64, 1H, t, *J* = 9.8 Hz; 5.58, 1H, d, *J* = 3.2 Hz; 5.28, 1H, dd, *J* = 3.3, 10.3 Hz; 4.28–4.11, 3H, m; 2.26, 2.05, 2.02, 1.90, 12H, s. ^{13}C NMR (CDCl$_3$, 300 MHz): δ 170.4, 170.0, 169.8, 169.2, 148.4, 129.9, 128.8, 128.5, 128.2, 125.9, 117.8, 86.3, 74.0, 70.8, 67.7, 66.9, 61.2, 20.7, 20.5, 20.3. HRMS (FAB) Calcd for C$_{22}$H$_{25}$N$_3$O$_9$Na [M+Na]$^+$ 498.1489. Found 498.1470. Mp: 197–199 °C.

2.1.2. 4-Phenyl-1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyr-anosyl)-1,2,3-triazole (4). ^{1}H NMR (CDCl$_3$, 300 MHz): δ 8.00, 1H, s; 7.84, 2H, d, *J* = 7.3 Hz; 7.46–7.33, 3H, m; 5.93, 1H, d, *J* = 9.1 Hz; 5.53, t, 1H, *J* = 9.5 Hz; 5.44, 1H, *J* = 9.5 Hz; 5.27, 1H, t, *J* = 9.7 Hz; 4.34, 1H, dd, *J* = 5.1, 12.6 Hz; 4.16, 1H, d, *J* = 12.8 Hz; 4.03, 1H, m; 2.09, 2.04, 1.89, 12H, s. ^{13}C NMR (CDCl$_3$, 300 MHz): δ 170.5, 169.9, 169.4, 169.0, 148.5, 129.8, 128.9, 128.6, 125.9, 117.7, 85.8, 75.2, 72.7, 70.1, 67.7, 61.5, 20.7, 20.5, 20.2. HRMS (FAB) Calcd for C$_{22}$H$_{25}$N$_3$O$_9$Na [M+Na]$^+$ 498.1489. Found 498.1480. Mp: 213–215 °C.

2.2. Representative procedure for the deacetylation of glycosyl triazoles

The 4-phenyl-1-(tetraacetyl glycopyranosyl)-triazole (0.40 mmol) was dissolved in methanol (50 mM). Freshly prepared sodium methoxide (5 equiv) was then added portionwise and the reaction was monitored by *tlc* (1:1 ethyl acetate/hexanes or 5:10:1 ethyl acetate/acetone/water). Upon completion, Amberlite resin (H$^+$ form) was added until the solution was at neutral pH, and the solution was filtered and concentrated in vacuo. The crude product was taken up in water and treated with activated charcoal. The product was recrystallized from water to give a white solid.

2.2.1. 4-Phenyl-1-β-D-glucopyranosyl-1,2,3-triazole (5). ^{1}H NMR (CD$_3$OD, 300 MHz): δ 8.57, 1H, s; 7.84, 2H, d, *J* = 8.4 Hz, 7.48–7.36, 3H, m; 5.61, 1H, d, *J* = 9.3 Hz; 4.20, 1H, t, *J* = 9.3 Hz; 4.00, 1H, d, *J* = 2.4 Hz; 3.89–3.70, 4H, m. ^{13}C NMR (CD$_3$OD, 300 MHz): δ 131.3, 130.1, 129.7, 126.8, 121.2, 90.6, 80.1, 75.3, 71.6, 70.4, 62.5. HRMS (FAB) Calcd for C$_{14}$H$_{17}$O$_5$N$_3$Na [M+Na]$^+$ 330.1058. Found 330.1066. Mp: 201–203 °C.

2.2.2. 4-Phenyl-1-β-D-galactopyranosyl-1,2,3-triazole (6). ^{1}H NMR (CD$_3$OD, 300 MHz): δ: 8.58, 1H, s; 7.86, 2H, d, *J* = 7.8 Hz; 7.49–7.35, 3H, m; 5.67, 1H, d, *J* = 9.2 Hz;

4.00–3.90, 2H, m; 3.76, 1H, dd, $J = 5.1, 12.1$ Hz; 3.64–3.51, 3H, m. ^{13}C NMR (CD$_3$OD, 300 MHz): δ 131.6, 130.1, 129.5, 126.7, 89.8, 81.2, 78.5, 74.1, 70.9, 62.4. HRMS (FAB) Calcd for C$_{14}$H$_{17}$O$_5$N$_3$Na [M+Na]$^+$ 330.1058. Found 330.1066. Mp: 228–230 °C.

2.3. Enzymatic inhibition

All enzymes, substrates, and nojirimycins were purchased from Sigma. Inhibition assays were carried out in 300-µL volumes in a 96-well plate on a multiwell plate reader held at 28 °C. Initial screening of the triazoles was carried out at triazole concentrations of 240 µM under the following conditions—SAG, 1.1 U/mL in 100 mM sodium acetate buffer, pH 5.0, 2 mM p-NO$_2$-phenyl-β-glucoside; ECG, 0.84 U/mL in 100 mM sodium phosphate buffer containing 10 mM MgCl$_2$, and 10 mM mercaptoethanol, pH 7.3, 0.2 mM o-NO$_2$-phenyl-β-galactoside; BLG, 0.39 U/mL in 100 mM sodium phosphate buffer containing 1 mM MgCl$_2$, and 10 mM mercaptoethanol, pH 7.3, 0.64 mM o-NO$_2$-phenyl-β-galactoside. All percentage inhibition screening experiments contained 8% DMSO. The rates of reactions were determined by continuously monitoring the release of nitrophenol (o-NO$_2$ at 410 nm and p-NO$_2$ at 405 nm) up to 10% substrate consumption. K_i values represent the average of at least three experiments using inhibitor concentrations between ~0.5 and 2 times K_i, and substrate concentrations between ~0.5 and 4 times K_m. The inhibitor, substrate, and buffer solutions were mixed

Figure 2. Lineweaver–Burk plots showing the inhibition of β-galactosidase-catalyzed hydrolysis of o-NO$_2$-phenyl-β-galactoside. (A) ECG inhibition by **5** at concentrations of 0.12 mM (▲), 0.24 mM (●), 0.32 mM (♦), and 0.48 mM (■). (B) BLG inhibition by **6** at concentrations of 0.48 mM (∗), 0.96 mM (■), 1.92 mM (▲), and 3.84 mM (●).

and prewarmed (<3 min) before the addition of enzyme. Analysis of Lineweaver–Burk plots (Fig. 2) indicated that **5** and **6** were both competitive inhibitors of ECG and BLG, respectively.

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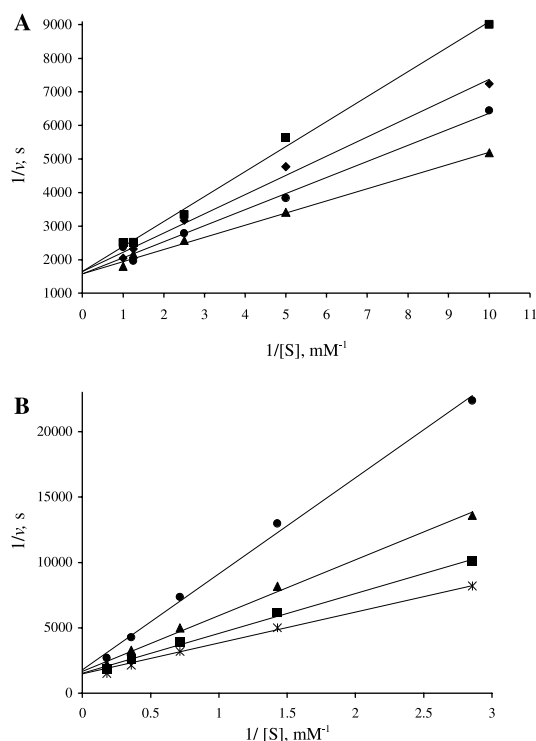
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9. Compounds **5** and **6** were also screened for activity against the β-galactosidase from *Asperigillus oryzae* and the α-galactosidase from green coffee beans. In neither case was any appreciable inhibition observed at a glycosyl triazole concentration of 240 μM.

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