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β-D-Glycosylamidines: Potent, Selective, and Easily Accessible β-Glycosidase Inhibitors

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Abstract—β-D-Glycosylamidines, in which a glycon is connected via an *N*-glycoside linkage with a substituted amidine (aglycon), were synthesized in two steps from the corresponding sugars and served as stable and potent β-glycosidase inhibitors with high selectivity according to the glycon- and α, β-specificities of the enzymes.

Glycosidases are involved in a number of physiologically important processes such as intestinal digestion and the catabolism and post-translational processing of glycoproteins.[1] The inhibitors of glycosidases have attracted increasing research interest not only as chemotherapeutic agents,[2] but also as useful molecular probes to understand the function of glycoproteins[3] and to study the structure and the catalytic mechanism of glycosidases.[4–6] The inhibitors of glycosidases have generally been designed to mimic the charge and/or the shape of the oxocarbenium ion-like transition-state or intermediate:[7] incorporating a basic nitrogen center or a positive charge at or adjacent to C-1 (anomeric carbon) into a sugar surrogate is a common practice in designing glycosidase inhibitors.[5,6,8] However, the incorporation of a nitrogen positive center into a sugar pyranose ring requires a relatively long reaction sequence[9,10] and often disrupts the stereochemistry at C-1 and the spatial arrangement of the hydroxy groups, which affect the selectivity of the inhibitor. Endocyclic glyconamidines with a planar sp^2-geometry at C-1, for example, served as potent, but rather nonselective glycosidase inhibitors,[11] partly due to the deformed conformation of the pyranose ring. In addition, the design of aza sugars such as deoxynojirimycin- and isofagomin-based inhibitors[9,10] is always challenged by the dilemma that the replacement of the pyranose ring oxygen or the anomeric carbon (C-1) by a nitrogen atom would create an unstable hemiacetal function on the adjacent carbon (C-1 or C-2, respectively). We reasoned therefore that the incorporation of a positive charge near C-1 in such a way as to maintain the whole structure of the glycon, including the stereochemistry at the anomeric carbon and at C-2, should lead to potent and selective glycosidase inhibitors according to the glycon- and stereospecificities of the enzyme. Glycosylamines, in this regard, have been reported to be potent and selective glycosidase inhibitors,[5,12] but the susceptibility to spontaneous hydrolysis and the unstable nature of the α,β-configuration at the anomeric carbon[12] prevented them from serving as practical inhibitors. We report here that β-D-glycosylamidines **1a–c**, in which a glycon moiety is connected via an *N*-glycoside linkage with a substituted amidine (aglycon), were easily synthesized from the corresponding glycopyranoses and strongly inhibited the corresponding β-glycosidase with K_i as low as 0.1 μM with substantial selectivity according to the glycon- and α-, β-specificities of glycosidases. The β-D-glycosylamidines were hydrolytically stable around the optimum pH for most glycosidases (pH 5–7), where the parent glycosylamines are unstable.

The β-D-glucosylamidine **1a**, β-D-galactosylamidine **1b**, and β-D-xylosylamidine **1c** were synthesized in two steps from the corresponding sugar (D-glucose, D-galactose, and D-xylose, respectively) without protecting the hydroxy groups (Scheme 1). Thus, the β-D-glycosylamines **2a–c** prepared from the corresponding sugars[13] were allowed to react with the thioimidate **3** in dry pyridine to give the amidines **1a–c** in high yield after purification by

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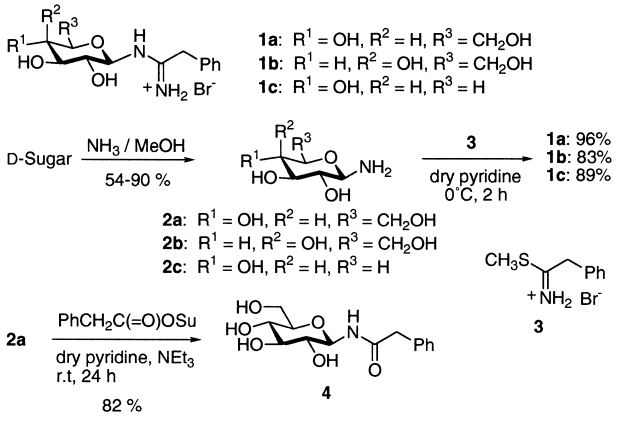


Scheme 1.

reversed phase column chromatography.[14] A common reagent for the preparation of amidines is an imidic ester,[15] but the glycosylamines **2a–c** did not react with ethyl phenylacetimidate hydrochloride, giving di-β-D-glycosylamine instead as the sole product. This was probably due to the low basicity of β-D-glycosylamines ($pK_a = 5.6$ of the conjugate acids)[12] and to the unstable nature of glycosylamines to a weak acid.[13] Since more reactive thioimidates are readily prepared by alkylation of the corresponding thioamides,[16] the present method is a general route to glycosylamidines with a wide variety of glycon and aglycon moieties. As a neutral analogue of glycosylamidines, β-D-glucosylamide **4** was synthesized by acylation of **2a** with phenylacetic acid *N*-hydroxysuccinimide ester.[17]

The inhibitory activities of **1a–c** were examined using several glycosidases with different glycon- and stereospecificities (Table 1).[18] The β-D-glycosylamidines **1a–c** served as potent and selective β-glycosidase inhibitors with high fidelity to enzyme's glycon- and stereospecificities: β-D-glucosylamidine **1a**, for example, selectively and competitively inhibited a β-glucosidase from *Aspergillus niger* with a K_i of less than 0.1 μM,[19] but did not inhibit the α-glucosidases,[20] α- and β-galactosidases, and β-xylosidase. Similarly, β-D-galactosylamidine **1b** and β-D-xylosylamidine **1c** inhibited the corresponding

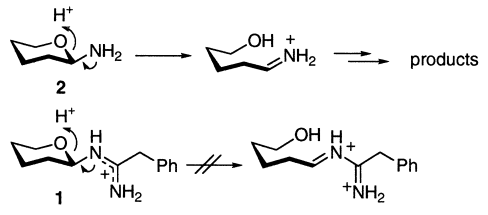
Table 1. Inhibitory activities of β-glycosylamidines **1a–c** and β-glucosylamide **4** (K_i [μM])

Glycosidases	Origin	1a	1b	1c	4
β-Glucosidase	*A. niger*[a]	0.094	390	11	770
	T. viride[b]	0.13	390	13	770
	Almond	73	41	1100	NI[c]
α-Glucosidase	*A. niger*	>2500[d]	NI	NI	>2500
	Bacillus sp.	>2500	NI	NI	NI
	Yeast	21	NI	NI	>2500
β-Galactosidase	*A. oryzae*	NI	2.4	NI	NI
	E. coli	NI	7.8	NI	>2500
α-Galactosidase	*A. niger*	NI	47	NI	>2500
β-Xylosidase	*A. pulverulentus*	NI	1000	2.5	NI

[a]*Aspergillus niger*.
[b]*Trichoderma viride*.
[c]No inhibition at 2.5 mM inhibitor.
[d]Less than 50% inhibition at 2.5 mM inhibitor.

β-glycosidase most potently, but showed little activity against α-glycosidases.[20] Sweet almond β-glucosidase was inhibited by both **1a** and **1b** with K_i of 73 and 41 μM, respectively, reflecting the broad substrate specificity of this enzyme with respect to the configuration of 4-OH.[21] Interestingly, a structurally homologous, but neutral amide **4** was an extremely poor inhibitor of β-glucosidases (K_i >770 μM), indicating that the positive charge of the amidine **1a** was essential for the inhibitory activity. The highly basic and permanently protonated amidines **1a–c** were probably bound tightly by charge–charge interaction with the catalytic carboxy group(s) in the enzyme active site[6,11,22] to display the inhibitory activity which almost rivaled those of the most potent β-glycosidase inhibitors.[6,8] In contrast to the endocyclic glyconamidines with flattened half-chair ring conformation,[11] the chair-shaped glycosylamidines **1a–c** successfully recruited the binding energy provided by the constellation of all the hydroxy groups including 2-OH[23] of the intact glycon pyranose ring, thereby attaining selective inhibition of the glycosidases whose substrates most closely resembled the inhibitor **1**. This notion is supported by the fact that the glycomimetics with undistorted chair conformation such as β-glycosylamines[5,12,24] and 1-β-amino-1-deoxynojirimycins[25] served as more selective β-glycosidase inhibitors than flattened endocyclic glyconamidines.[11]

It is worth noting that the amidine **1a** was quite stable in acidic media around the pH optimum for most glycosidases. No decomposition or anomerization of **1a** was observed in two months at pH 5–7 at 25 °C.[26] This is in marked contrast to the unstable nature of the parent glycosylamines **2a–c**, which easily undergo hydrolysis, anomerization and dimerization catalyzed by a weak acid.[13] The stability of the glycosylamidine **1** probably comes from the fact that the highly basic amidine is completely protonated under acidic conditions, thereby preventing the acid-catalyzed opening of the pyranose ring as would easily occur with the parent glycosylamines (Scheme 2). Under basic conditions, however, the amidine **1a** was hydrolyzed to the amide **4** and ammonia with a half-life ($t_{1/2}$) of about 24 h in a carbonate buffer (pH 10) and with a $t_{1/2}$ of 5 h in 1M NaOH at 25 °C. The alkaline hydrolysis was quite selective, and no other products such as β-D-glucosylamine **2a** and phenylacetamide were observed. The glycosylamidines **1a–c** thus eliminated the dilemma associated with glycosylamines that the incorporation of an alkyl substituent on the nitrogen increased the basicity of the nitrogen and the inhibition potency accordingly, but simultaneously decreased the stability due to the ease of ring-opening, and vice versa;[12,27] it is possible with glycosylamidines to incorporate, in principle, any kind of aglycon structures



Scheme 2.

into a basic sugar template to improve the inhibition potency and selectivity without sacrificing its basicity and stability.

In conclusion, chemically stable β-D-glycosylamidines **1a**–**c** were prepared in two steps from the parent glycopyranoses.[28] Although the structure of the aglycon moiety was not optimized, the glycosylamidines **1a**–**c** showed substantial potency and selectivity towards several glycosidases. The predetermined nature of the selective glycosidase inhibition, along with the strong inhibition potency and the ease of synthesis, should promise the preparation of a wide variety of glycosylamidines, including the α-form, serving as "custom-made" glycosidase inhibitors with good prospects for applications such as a ligand for affinity purification and the gene cloning of glycosidases. These aspects of work are now in progress.

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14. A typical method for the synthesis of N^1-β-D-glucopyranosylphenylacetamidine hydrobromide (**1a**): β-D-Glucosylamine (**2a**) (0.90 g, 5.0 mmol) suspended in dry pyridine (20 mL) was cooled at 0 °C. The thioimidate **3** (1.27 g, 5.15 mmol) was added at once to the suspension, and the mixture was stirred at 0 °C for 2 h under an argon atmosphere. The turbid solution became clear in about 20 min after the addition of **3**, and the reaction was completed in 2 h (^{13}C NMR). The reaction mixture was concentrated to dryness, and the residual oil was washed with Et_2O (20 mL). The crude product was dissolved in water (2 mL) and applied to a medium-pressure ODS column (ODS-S-50D, Yamazen Co., Osaka, Japan) eluted with water (6 mL/min). The product was eluted at 0.55–0.75 bed volume. The fractions were collected and freeze-dried to afford **1a** as colorless and hygroscopic powder (1.80 g, 96%): mp 109–110 °C (decomp., uncorrected); $[\alpha]_D^{22}$ −37.5° (c = 2.01, H_2O); IR (KBr) ν_{max} 3200 (br), 1670, 1607, 1320, 1030 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 7.49–7.37 (m, 5H, phenyl), 4.87 (d, J = 8.5 Hz, 1H, H-1), 3.97 (s, 2H, CH_2Ph), 3.89 (dd, J = 12.6 and 2.2 Hz, 1H, H-6b), 3.75 (dd, J = 12.6 and 5.3 Hz, 1H, H-6a), 3.60 (ddd, J = 9.7, 5.3 and 2.2 Hz, 1H, H-5), 3.58–3.45 (m, 3H, H-2, H-3 and H-4); ^{13}C NMR (100 MHz, D_2O) δ 171.2 (C=N), 134.4, 131.4, 131.5, 130.8 (phenyl), 83.8 (C-1), 80.1 (C-5), 78.6 (C-3), 74.1 (C-2), 71.2 (C-4), 62.8 (C-6), 41.1 (*C*H_2Ph). Anal. calcd for $C_{14}H_{21}BrN_2O_5 \cdot 0.4H_2O$: C, 43.74; H, 5.72; N, 7.29. Found: C, 43.68; H, 5.77; N, 7.31. HRMS (FAB) calcd for $C_{14}H_{21}N_2O_5$ (MH^+) 297.1451, found 297.1450.
Amidine **1b**: mp 108–109 °C; $[\alpha]_D^{23}$ −14.0° (c = 1.97, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.50–7.37 (m, 5H, phenyl), 4.81 (d, J = 8.4 Hz, 1H, H-1), 4.01 (d, J = 2.4 Hz, 1H, H-4), 3.98 (s, 2H, CH_2Ph), 3.86 (t, J = 6.0 Hz, 1H, H-6a), 3.82–3.67 (m, 4H, H-2, H-3, H-5 and H-6b); ^{13}C NMR (50 MHz, D_2O) δ 169.0 (C=N), 132.1, 129.4, 129.2, 128.5 (phenyl), 82.0 (C-1), 77.1 (C-5), 73.1 (C-3), 69.0, 68.5 (C-2 and C-4), 60.9 (C-6), 38.6 (C-2).
Amidine **1c**: mp 98–99 °C, $[\alpha]_D^{24}$ −22.3° (c = 2.01, H_2O); ^{1}H NMR (200 MHz, D_2O) δ 7.50–7.37 (m, 5H, phenyl), 4.82 (d, J = 8.4 Hz, 1H, H-1), 4.00 (dd, J = 11.6 and 5.3 Hz, 1H, H-5), 3.96 (s, 2H, CH_2Ph), 3.85–3.64 (m, 1H, H-5), 3.58–3.51 (m, 1H, H-2), 3.55 (t, J = 6.0 Hz, H-3), 3.46 (t, J = 11 Hz, 1H, H-4); ^{13}C NMR (50 MHz, D_2O) δ 169.1 (C=N), 132.1, 129.4, 129.1, 128.5 (phenyl), 82.0 (C-1), 76.2 (C-3), 71.5 (C-2), 68.6 (C-4), 67.0 (C-5), 38.5 (*C*H_2Ph).
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17. This method enabled the synthesis of the amide **4** without protecting the hydroxy groups, but the addition of NEt_3 was necessary to prevent the dimerization of glucosylamine **2a** catalyzed by an acidic by-product *N*-hydroxysuccinimide.
18. All the glycosidases used were chromatographically pure. Each enzyme was assayed at 30 °C in either acetate buffer (50 mM, pH 4 and 5) or in phosphate buffer (50 mM, pH 6 and 7) using *p*-nitrophenyl α- and β-D-glucopyranosides, *p*-nitrophenyl α-D-galactopyranoside, *o*-nitrophenyl β-D-galactopyranoside, and *p*-nitrophenyl β-D-xylopyranoside as substrates, according to a reported standard assay method (ref 10).

19. The inhibition was reversible, and no time-dependent or slow-binding inhibition was observed.
20. The α-glucosidase from yeast and α-galactosidase from *A. niger* were inhibited moderately by **1a** and **1b**, respectively, but the inhibition was selective with respect to the glycon structure.
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