A New Structural Motif for the Design of Potent Glucosidase Inhibitors

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An important field in glycobiology involves the design and synthesis of glycosidase inhibitors with potential therapeutic applications,[1] such as the treatment of metabolic disorders,[2] cancer,[3] and AIDS.[4] Retaining glucosidases hydrolyze sugar acetal linkages via a glucosylated enzyme intermediate[5] resulting in retention of configuration at the anomeric center. Both glucosylation and deglucosylation occur via transition states (TSs) that have oxacarbenium ion character[5−7] and a distorted six-membered ring.[8] Although no single compound effectively inhibits all glucosidases,[6,9] all inhibitors possess functionalities that mimic certain features of the glucosyl oxacarbenium ion (**1**). For instance, 1-deoxynojirimicin (**2**),[10] isofagomine (**3**),[11] and validamine (**4**)[12] mimic charge development at the TS by incorporating a basic nitrogen atom in place of *O*-5, *C*-1, and O-1, respectively (Figure 1).

Conformationally restricted compounds, e.g., nojiritetrazole (**5**),[13] where the fused tetrazole ring forces the six-membered ring into a half-chair conformation, are potent inhibitors. Also, compounds such as valienamine (**6**) potentially mimic both the charge and the ring distortion of the TS. Since several different sequence-based families of enzyme exist that are capable of hydrolyzing an α-glucoside linkage,[14] it is not too surprising that no single structural class of compounds yields tight binding inhibitors for all α-glucosidases.[15] Thus, constant motivation exists for the design of new structural motifs to be used as a basis for potent glycosidase inhibitors.[16]

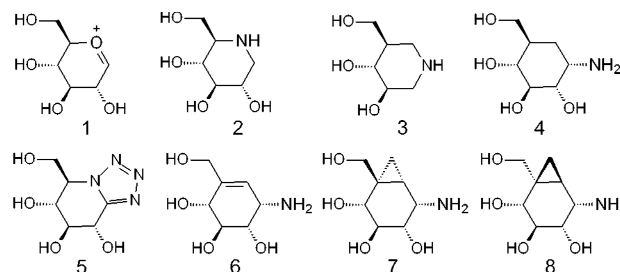
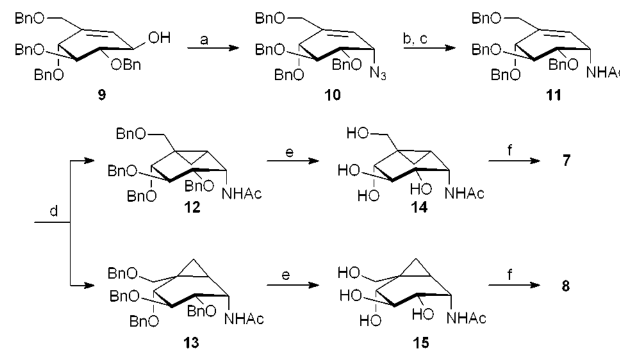


Figure 1. Structures of various glucosidase inhibitors and of the glucopyranosyl oxacarbenium ion.

Scheme 1. Synthesis of the Bicyclo[4.1.0]heptane Compounds **7** and **8**



(a) DBU, DPPA, toluene, room temperature; (b) PPh_3, pyridine, NH_4OH, room temperature; (c) pyridine, AcCl, toluene, room temperature; (d) $ZnMe_2$, CH_2I_2, toluene, −10 °C; (e) 10% Pd−C H_2 (12 psi), MeOH; (f) KOH, MeOH:H_2O (1:1).

The current report details the synthesis and biological activity for the two bicyclo[4.1.0]heptane derivatives **7** and **8**, the first example of bicyclo[4.1.0]heptane carbocyclic glucosidase inhibitors (Scheme 1). In addition, compound **7** is the tightest binding yeast α-glucosidase inhibitor reported to date.

Conversion of the known alcohol **9**[17] to azide **10** proceeded in a 68% yield with DPPA (diphenylphosphoryl azide) in the presence of DBU.[18] After reducing **10** with PPh_3 in aqueous pyridine,[19] acetylation gave acetamide **11** in a yield of 97% from **10**. The cyclopropyl group was introduced quantitatively by using Furakawa's protocol,[20] to give a 1:1 ratio of **12** and **13**.[21]

While both compounds have the same stereochemistry at four stereocenters, the 1*R* (**12**) and the 1*S* (**13**) diastereomers are stereochemically related to D-glucose and L-idose, respectively. The diastereomers were separated by column chromatography (see Supporting Information for details). After removal of the *O*-benzyl group (10% Pd−C and H_2) the acetamido groups were hydrolyzed with KOH (1.5 equiv) in refluxing MeOH:H_2O (1:1 v/v). After neutralization with H^+ ion-exchange resin, the final compounds were eluted from the resin with 1 N NH_4OH. The resultant syrups were crystallized as their HCl salts from acidic methanol (1.5 equiv of HCl) by the addition of acetone. The yields of **7** and **8** (from **14** and **15**) were 46% and 33%, respectively.

The configuration of both diastereomers was confirmed by single-crystal X-ray diffraction experiments. Structural diagrams

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(21) Two diagnostic doublets for the diastereomers appear in the ^1H NMR spectrum at δ 3.05 ppm (H-8_A) for **12** and δ 2.77 ppm (H-8_A) for **13** (see Supporting Information for full characterization of **12** and **13**).

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Table 1. Inhibition Kinetic Parameters[a,b]

enzyme	K_i (μM) for competitive inhibition						
	2[c]	**4**[d]	**6**[d]	**7**	**8**	**14**	**15**
yeast α-glucosidase	15 ± 1	580	18	0.107 ± 0.015	820 ± 70	4100 ± 490	800 ± 100
rice α-glucosidase	0.0024 ± 0.0004	NR[e]	NR[e]	103 ± 8	2640 ± 330	NI[f]	NI[g]

[a] All kinetic measurements were made by using the same experimental protocol as that reported in ref 22. [b] The kinetic data were fit to a standard competitive inhibition equation. [c] Reference 22. [d] IC_{50} ref 23. [e] Not reported. [f] No inhibition observed at 1.3 mM. [g] No inhibition observed at 1.2 mM.

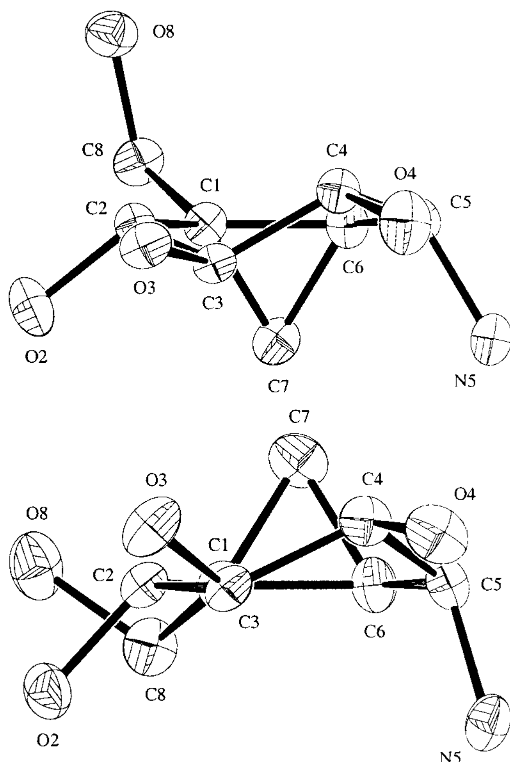

Figure 2. Molecular structures of the 1*R* (top) and 1*S* (bottom) diastereomeric cations; 50% enclosure ellipsoids are shown. All hydrogen atoms and the chloride ion for both structures are excluded for clarity.

for the two diastereomers (**7** and **8**) are shown in Figure 2. Both the 1*R*[24] (**7**) and the 1*S*[25] (**8**) diastereomers adopt half-chair conformations with the most marked difference between the two isomers being the relative orientation of the cyclopropyl ring and the hydroxymethyl substituent at C1 (IUPAC numbering scheme, Figure 2).

Amines **7** and **8**, and their corresponding acetamido compounds **14** and **15**, were tested as inhibitors against two commercially available α-glucosidase enzymes. Measured K_i values for these four bicyclo[4.1.0]heptane derivatives and the reported values for **2**, **4**, and **6** are listed in Table 1.

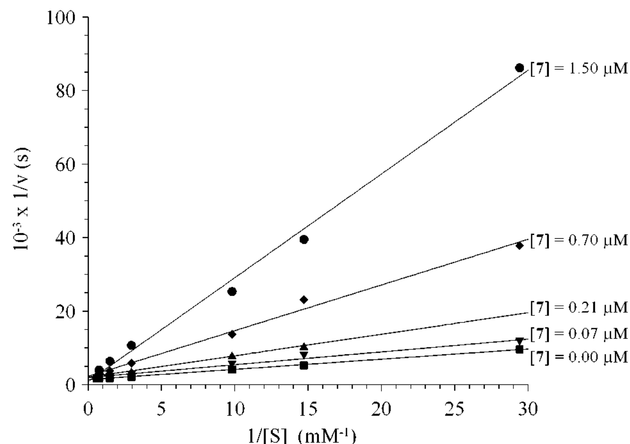

Figure 3. Lineweaver–Burk plot for inhibition of yeast α-glucosidase by **7**.

The Lineweaver–Burk plot (Figure 3) for inhibition of 4-nitrophenyl α-D-glucoside hydrolysis catalyzed by yeast α-glucosidase on the addition of **7** shows that this compound binds competitively to the active site of the enzyme. Moreover, the measured K_i value (107 nM) for inhibition of yeast α-glucosidase by compound **7** is, to the best of our knowledge, lower than all previously reported values.

The comparison between **6** and **7** is particularly interesting since these compounds are structurally very similar, the ~170-fold (12.7 kJ/mol) difference in binding of these compounds to the yeast enzyme could result from one or both of the following factors: (1) the introduction of a more favorable hydrogen bond(s) between **7**'s hydroxymethyl group and the active site or (2) the addition of an extra hydrophobic interaction between the cyclopropyl CH_2 group and the enzyme's active site. Finally, a comparison between amine **7** and acetamide **14** supports the current idea that a positive charge greatly enhances inhibitor potency,[7] although other factors such as hydrogen bonding may contribute to the 26 kJ/mol difference in the free energy of binding.

In summary, a new class of tight-binding competitive α-glucosidase inhibitors has been synthesized and this structural motif will be of utility in the design of other glycosyl transferring enzyme inhibitors.

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Supporting Information Available: Preparation and tables giving the full spectroscopic characterization of compounds **7**, **8**, and **10**–**15**, tables of experimental details, positional parameters, and thermal parameters for the X-ray analyses of **7** and **8** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) Crystal structure of (1*R*,2*R*,3*S*,4*S*,5*S*,6*S*)-5-ammonio-1-hydroxymethyl-2,3,4-bicyclo[4.1.0]heptanetriol chloride: colorless plate; $C_8H_{16}ClNO_4$; monoclinic, space group $P2_1$; $Z = 2$; $a = 7.2170(9)$ Å; $b = 9.1308(11)$ Å; $c = 7.6440(10)$ Å; $\beta = 102.427(10)°$; $V = 491.91(11)$ Å^3; T 293 K; $R_F = 0.026$; GoF = 1.34.

(25) Crystal structure of (1*S*,2*R*,3*S*,4*S*,5*S*,6*R*)-5-ammonio-1-hydroxymethyl-2,3,4-bicyclo[4.1.0]heptanetriol chloride: colorless blade; $C_8H_{16}ClNO_4$; orthorhombic, space group $P2_12_12_1$; $Z = 4$; $a = 7.7355(13)$ Å; $b = 6.6838(13)$ Å; $c = 19.6814(39)$ Å; $V = 1017.6$ Å^3; $T = 293$ K.; $R_F = 0.033$; GoF = 1.24.