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Supplementary Material Available: Experimental details for the preparation of diazo esters, their catalytic reactions, and product characterization (5 pages). Ordering information is given on any current masthead page.

Mimicking the Glucosidase Transition State: Shape/Charge Considerations

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The enzyme-catalyzed hydrolysis of glucosidic bonds, a pivotal reaction in carbohydrate metabolism,[1] is thought to involve a transient, point-charge-stabilized oxocarbonium ion **1** (Scheme I) whose subsequent processing results in overall retention or inversion of glucoside stereochemistry. Analogues of this glucosyl cation have long represented an attractive synthetic target for the design of potent glucosidase inhibitors.

The transition state leading to **1** involves substantial positive charge buildup and significant flattening of the substrate's pyranose ring; however, the relative impact of these electrostatic and conformational changes on enzyme binding remains controversial. Here we report detailed kinetic studies on several new glucose derivatives which suggest that the shape, not the charge, of reactive intermediate **1** is a much more important determinant for binding to β-glucosidase.

Most known inhibitors until now have been imperfect structural mimics of **1**. For example, protonated 1-deoxynojirimycin **2** may simulate the charge of **1** but does not possess the same shape.[2] Alternatively D-gluconolactone **3**,[3] its oxime **4**,[4] and the corresponding 5-amino-5-deoxylactam **5**[5] adopt distorted half-chair conformations which flatten the anomeric region somewhat[6] but can only achieve the requisite charge and endocyclic π-electron density of **1** in minor, dipolar resonance structures. Nevertheless significant competitive inhibition is observed with **2–5**, suggesting that both conformational and electrostatic factors may be important in active site binding.

Recently we reported the synthesis of glucose analogue **6**, a highly basic amidine (pK_a 10.6) whose structure, shape, and charge in aqueous solution closely resembled that of cation **1**.[7] Unlike **2–5**, amidine **6** proved to be a potent, broad-spectrum competitive inhibitor of gluco-, manno-, and galactosidases, an observation which led us to hypothesize that many glycosidases experience strong H-bonding, electrostatic and/or other noncovalent interactions with this glycosyl cation mimic.[7] We now report the synthesis of amidrazone **7** and amidoxime **8**, two novel relatives of amidine **6** which exhibit enhanced stability at elevated pH. Both **7** and **8** are potent, broad-spectrum glycosidase inhibitors, thus

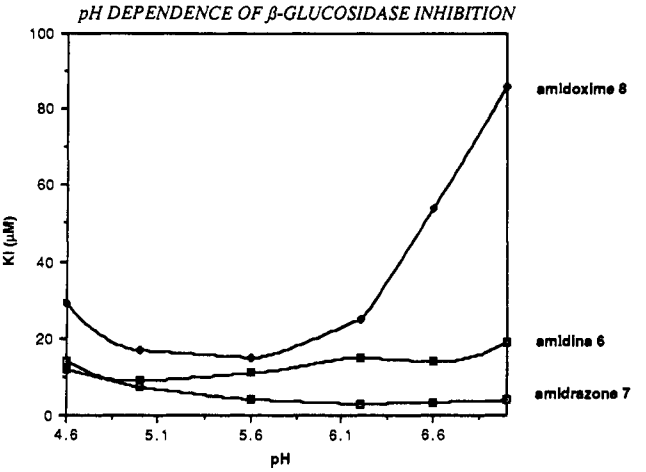
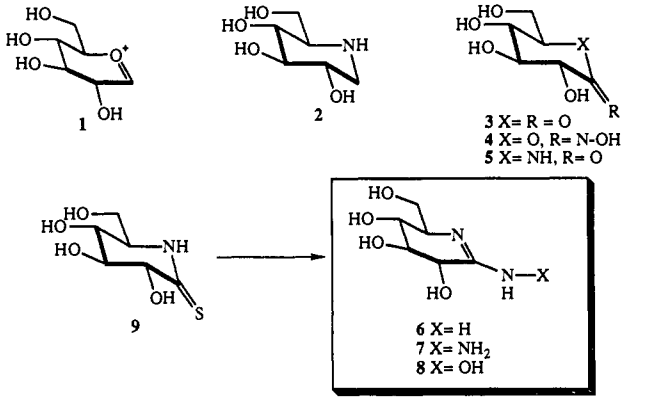


Figure 1.

Scheme I



adding further support to the above-mentioned hypothesis.[8] Moreover their effect on sweet almond β-glucosidase (β-Glu) casts a revealing light on the relative importance of electrostatic effects and conformational changes in the glucosidase transition state.

Following the method for **6**, enantiomerically pure **7** and **8** were synthesized by reacting thionolactam **9** with anhydrous hydrazine (CH_3OH, 5 °C, 2 h) or hydroxylamine[9] (CH_3OH, room temperature, 14 h), respectively. Compared to **1**, amidrazone **7** (73% yield, pK_a = 8.7) was remarkably stable at basic pH ($t_{1/2}$ = 8 h at pH 11), while amidoxime **8** (75% yield; pK_a = 5.6)[10] was unchanged after several weeks in aqueous base at pH 11.

Glucoamidrazone **7**, when assayed against a wide variety of enzymes, proved in all respects similar to amidine **6**. Under steady-state conditions, **7** competitively inhibited β-glu with a K_I of 8.4 ± 0.9 μM (*p*-nitrophenyl-β-D-glucopyranoside as substrate; 37 °C; K_M = 2.1–3.5 mM). Like **6** (K_I = 10 ± 2 μM), inhibition of β-glu by **7** was pH-independent between 4.6 and 7.0 (Figure 1), suggesting that the protonated amidrazone interacted with the more dissociated of the two active site carboxyl residues (pK_a values for β-glu: 4.4, 6.7).[11] Glucoamidoxime **8** also exhibited broad-spectrum inhibition but with pH-dependent behavior (for

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(5) Inouye, S.; Tsuruoka, T.; Ito, T.; Niida, T. *Tetrahedron* **1968**, *23*, 2125.
(6) While **3–5** incorporate an sp^2 carbon at C-1 (corresponding to the anomeric center of **1**), their conformations are distinctly different from **1**, as methylenecyclohexane is from cyclohexene.
(7) Tong, M. K.; Papandreou, G.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112*, 6137.
(8) The corresponding D-mannoamidrazone and D-mannoamidoxime, synthesized in analogous fashion, also exhibit the same pattern of indiscriminate (i.e., broad-spectrum) inhibition. Besides inhibiting jackbean α-mannosidase (K_I = 166 ± 13 nM), mung bean α-mannosidase (IC_{50} = 400 nM), fungal β-mannosidase (IC_{50} = 150 nM), and bovine β-galactosidase (K_I = 57 ± 3 μM), the mannoamidrazone also inhibits Golgi α-mannosidase I (IC_{50} = 4 μM), α-mannosidase II (IC_{50} = 90–100 nM), and endoplasmic reticulum α-mannosidase (IC_{50} = 1 μM): Pan, Y. T.; Kaushal, G. P.; Papandreou, G.; Ganem, B.; Elbein, A. D. *J. Biol. Chem.*, submitted for publication.
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(11) Dale, M. P.; Ensley, H. E.; Kern, K.; Sastry, K. A. R.; Byers, L. D. *Biochemistry* **1985**, *24*, 3530.

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β-glu, K_I = 13.8 ± 3 μM at pH 5.6). Moreover the observed pH dependence of inhibition by **8** paralleled the variation in k_{cat}/K_M with pH for the enzyme (pH optimum = 5.6),[11] as expected for an ideal transition-state analogue inhibitior (Figure 1).

To our knowledge, amidoxime **8** is the first nonbasic saccharide analogue which mimics the true half-chair conformation of the glucosyl intermediate. The importance of the amidoxime's endocyclic double bond is evident when one compares **8** with D-gluconohydroximinolactone **4**,[4] whose functionality closely resembles **8** but whose exocyclic C=N bond creates a lactone-like conformation.[6] Compound **4** is a 7-fold weaker inhibitor of β-glu (K_I = 98 μM).

Two important conclusions emerge from this work. First, it is apparent that the common structural elements of glucosyl mimics **6–8** (and their mannose analogues)[8] represent an "Achilles heel" in carbohydrate enzymology to which many simple glycosidases are vulnerable. Moreover levels of β-glu inhibition remain nearly constant despite a 10^5 change in basicity over the amidoxime–amidrazone–amidine series. Assuming that the exocyclic NH_2, $NHNH_2$, and NHOH groups of **6–8**, respectively, interact in similar ways with the β-glu active site (an assumption not devoid of risk),[12] we conclude that adopting the flattened anomeric conformation of the glucosyl intermediate is more important for transition-state binding by the enzyme than achieving the full-fledged charge of the glucopyranosyl cation. This observation should be useful in designing potent new inhibitors of glucoside hydrolysis.

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Supplementary Material Available: Experimental details (including physical properties, spectral and analytical data) for preparing **7** and **8** plus kinetic plots from enzymatic assays (6 pages). Ordering information is given on any current masthead page.

(12) One referee has noted, for instance, that the absence of a positive charge on **8** might be compensated for by additional H-bonding to the enzyme. An X-ray crystal structure of the β-glu E-I complex should resolve these issues.

Interception of Dimethylcarbene with Pyridine: A Laser Flash Photolysis Study

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Dialkylcarbenes are notoriously difficult to intercept with external chemical trapping agents[1] and to detect by matrix isolation spectroscopy.[2] Presumably, this is due to their ability to undergo rapid intramolecular rearrangements. In fact these rearrangements have at times been considered to have zero enthalpic barrier.

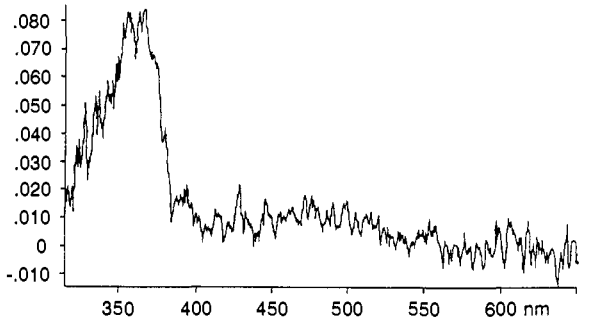
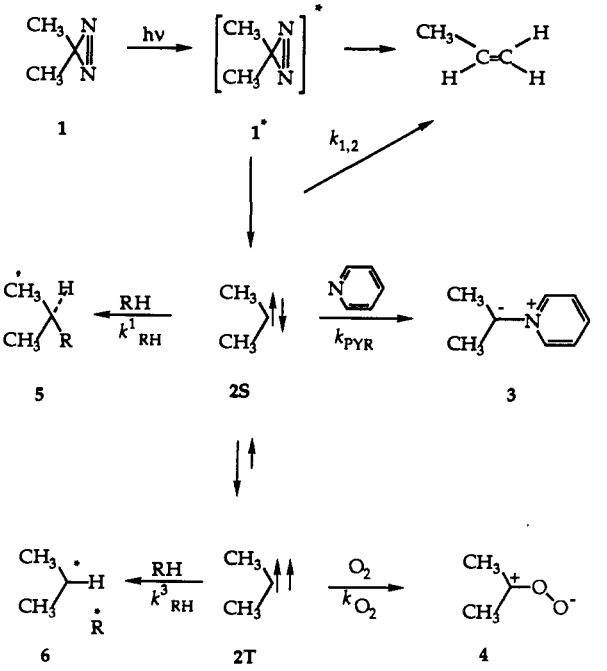


Figure 1. The transient spectrum of ylide **3** produced by LFP of 3,3-dimethyldiazirine (**1**) in *n*-pentane containing pyridine at 25 °C.

Scheme I



Indeed the question has been posed whether dialkylcarbenes are true reactive intermediates, or transition structures, or even nonstationary points on a potential surface connecting a carbene precursor with the product of rearrangement.[3]

We have recently demonstrated that two dialkylcarbenes, adamantanylidene,[4] and homocubanylidene[5] can be intercepted with pyridine to form ylides which are easily detected by laser flash photolysis (LFP) techniques. These two carbenes can only rearrange to highly strained products which may account for carbene lifetimes that are sufficiently long to permit their capture with pyridine. However, Houk and Evanseck[6] have recently calculated that the activation barrier to isomerization of dimethylcarbene is 6.4 kcal/mol, which has prompted this study of this carbene by the pyridine probe method.

Laser flash photolysis (LFP)[7] of diazirine **1** (Lumonics excimer laser, XeF, 351 nm, 50 mJ) in *n*-pentane does not produce any detectable transient absorbance. However, LFP of **1** in the presence of pyridine produces the transient of Figure 1 which is

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