Amidine, Amidrazone, and Amidoxime Derivatives of Monosaccharide Aldonolactams: Synthesis and Evaluation as Glycosidase Inhibitors

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Received July 6, 1993[®]

Abstract: The synthesis of amidine, amidrazone, and amidoxime derivatives of D-glucono, D-mannono, and D-galactonolactams, which are potent glycosidase inhibitors, is described. With their sugar-like structures and resonance-stabilized, partially positively charged anomeric carbons, these monosaccharide analogs mimic key conformational and electrostatic features of the corresponding glycopyranosyl cations. In the D-*gluco* series, all three derivatives are potent inhibitors of sweet almond β-glucosidase. Levels of inhibition remain nearly constant despite a 10^5 change in basicity, indicating that conformational flattening of the hydrolysis intermediate is more important for transition-state binding by the enzyme than charge development. The same D-*gluco* derivatives also interact with mannose- and galactose-processing enzymes. Considerably weaker inhibition is observed with 1β-amino-1-deoxynojirimycin, which embodies similar endocyclic and exocyclic nitrogens in an undistorted chair conformation. In the D-*manno* series, the amidrazone and amidoxime are potent inhibitors of jackbean α-mannosidase, mung bean α-mannosidase, fungal β-mannosidase, Golgi α-mannosidase I, α-mannosidase II, and soluble (or endoplasmic reticulum) α-mannosidase. The mannoamidrazone also inhibits Golgi α-mannosidase I and the endoplasmic reticulum mannosidase *in vivo*. In the D-*galacto* series, significant inhibition of almond β-glucosidase, bovine liver β-galactosidase, and green coffee bean α-galactosidase is observed, but little or no inhibition of amyloglucosidase.

Background and Rationale

In the hierarchy of enzymes that process carbohydrates, those which hydrolyze glycosidase bonds to release mono or oligosaccharides are among the most important. Such ubiquitous enzymes, called glycosidases, are essential for the normal growth and development of all organisms. They are involved in a variety of important cellular functions including the breakdown of carbohydrate foodstuffs,[1] the processing of eukaryotic glycoproteins,[2] and the catabolism of polysaccharides and glycoconjugates.[3]

Over the past several years, interest in the design and synthesis of glycosidase inhibitors has surged. This is in part due to the discovery that plants produce nitrogen-containing natural products with structures and shapes highly reminiscent of monosaccharides. Many of these novel alkaloids competitively inhibit the glycosidases whose substrates they most closely resemble.[4] Numerous stereoisomers and analogs have been prepared by total synthesis, many of which have also displayed potent activity. Among the most effective glycosidase inhibitors are (a) polyhydroxylated piperidines such as nojirimycin **1**,[5] 1-deoxynojirimycin (1-dNM) **2**,[5] 1-deoxymannojirimycin **3**[6] and its galactose analog **4**;[7] (b) D-gluconolactone **5**;[8] its oxime **6**,[9] and the corresponding 5-amino-5-deoxylactam **7**;[10] (c) polyhydroxylated pyrrolidines such as **8–10**;[11] and (d) the indolizidine alkaloids swainsonine **11**[12] and castanospermine **12**[13] (Scheme I).[14]

The mechanism of enzymatic hydrolysis of glycosidic bonds has been the object of much research.[15,16] One proposal, based on X-ray crystallographic studies of lysozyme-inhibitor complexes,[17] implicates bilateral carboxylic acid groups as catalytic residues in a transition state embodying substantial positive charge buildup and significant flattening of the substrate's pyranose ring, as shown in Figure 1. The intermediacy of a full-fledged, point-charge stabilized oxocarbonium (glycosyl) ion has also been suggested.[18] Although the relative importance of such electrostatic and conformational changes remains controversial, structural analogs of the charged, half-chair glycosyl cation have long represented an attractive synthetic target for the design of potent glycosidase inhibitors.

All known inhibitors, including those depicted in Scheme I, have been imperfect structural mimics of the transition state shown in Figure 1. For example, protonated 1-deoxynojirimycin **2** and its congeners **3** and **4** may simulate the charge of the corresponding glycosyl cation, but their chair conformations do

® Abstract published in *Advance ACS Abstracts*, December 1, 1993.

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Scheme I

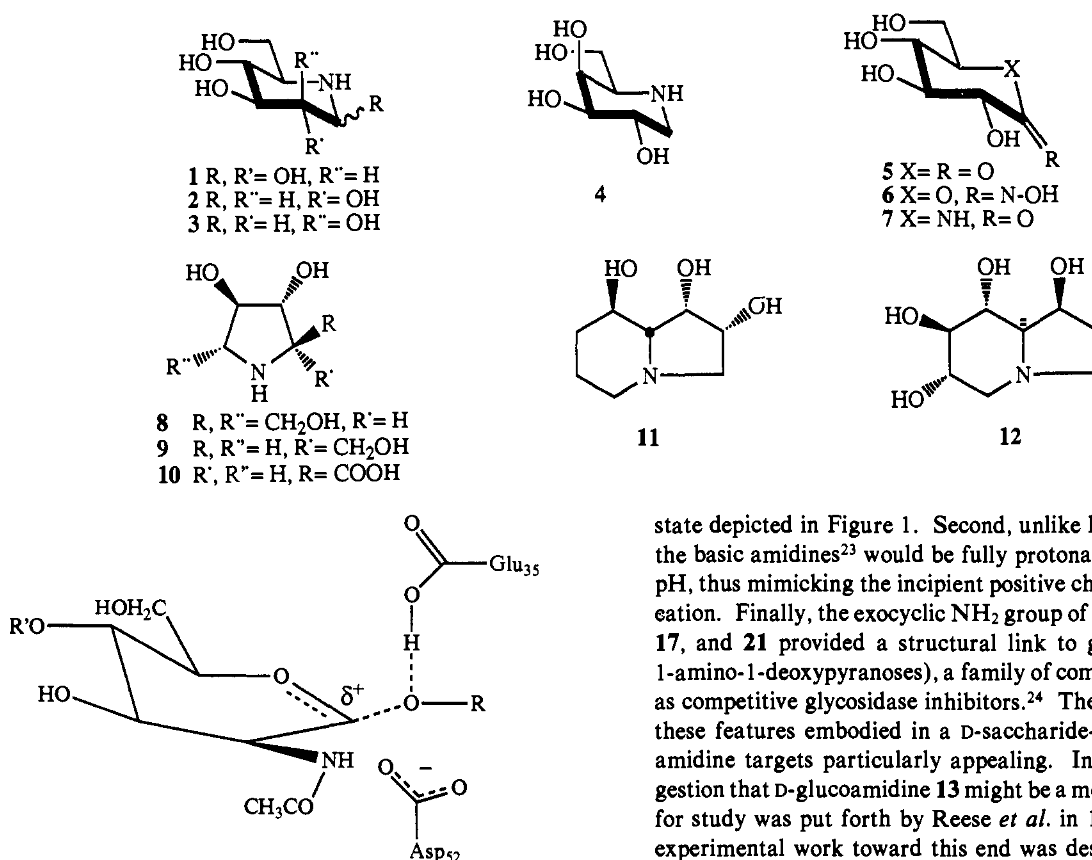

Figure 1. Role of bilateral carboxylic acid groups in the enzyme-assisted hydrolysis of glycosidic bonds, based on X-ray crystallographic studies of lysozyme-inhibitor complexes.

not mimic the flattening evident in Figure 1.[19] Alternatively, sp^2-based inhibitors **5–7** adopt distorted half-chair conformations which flatten the anomeric region somewhat,[20] but can only achieve the requisite charge and endocyclic π-electron density of the glycosyl cation in minor, dipolar resonance structures. Nevertheless, significant competitive inhibition is observed with **5–7**. Taken as a whole, these data suggest that *both* conformational and electrostatic factors may be important in inhibition. To our knowledge, no systematic approach to the synthesis of glycosidase inhibitors has mimicked in a stable, covalent structure both the requisite positive charge and flattened half-chair ring conformation evident in Figure 1, two key features of a promising design strategy. Here we present a full account of our studies[21] on the synthesis of saccharide amidines, amidrazones, and amidoximes (Scheme II) possessing the D-*gluco* (e.g. **13–16**), D-*manno* (e.g. **17–20**), and D-*galacto* (e.g. **21–24**) configurations.

Synthetic Strategy

At the outset of this work, our choice of amidine targets was guided by several important considerations. First, their resonance-stabilized π-system preferred the endocyclic tautomer as shown,[22] thus recreating the desired flattened conformation of the transition state depicted in Figure 1. Second, unlike lactones or lactams, the basic amidines[23] would be fully protonated at physiological pH, thus mimicking the incipient positive charge of the glycosyl cation. Finally, the exocyclic NH_2 group of parent amidines **13**, **17**, and **21** provided a structural link to glycosylamines (i.e. 1-amino-1-deoxypyranoses), a family of compounds long known as competitive glycosidase inhibitors.[24] The combination of all these features embodied in a D-saccharide-like ring made the amidine targets particularly appealing. Indeed, the first suggestion that D-glucoamidine **13** might be a meritorious candidate for study was put forth by Reese *et al.* in 1971,[25] although no experimental work toward this end was described. An unsuccessful synthetic approach to **13** via 5-azido-5-deoxy-D-glucononitriles was reported by Bird *et al.* in 1990.[26]

Retrosynthetically, glucoamidine **13** and its congeners might be prepared from aminolysis of imino ether **25**, which itself should be readily available from D-gluconolactam **26** (Scheme III). Lactam **26** had previously been synthesized in 20% yield from the bisulfite adduct **27** of nojirimycin.[5] Basic hydrolysis of **27** (Dowex 1-2 × 200 OH resin) produced **1** *in situ*, which was oxidized (I_2, NaOH) and peracetylated to furnish tetra-O-acetyllactam **28** in 43% overall yield. Treatment of **28** with Meerwein's salt (Et_3OBF_4, CH_2Cl_2, room temperature, 36 h) afforded imino ether **25** (88%). Interestingly, exposure of **25** to excess anhydrous ammonia under scrupulously dry conditions (NH_3–CH_3OH, NH_3–$CHCl_3$, liquid NH_3) led only to D-gluconolactam **26** with no trace of the desired amidine **13**. The formation of **26** was likely coupled to an accompanying deacetylation: initial attack by ammonia at the C2 acetate ester of **26** and then subsequent intramolecular nucleophilic addition and elimination would form bicyclic imino ether **30**. Breakdown of **30** as shown (Scheme III) and exhaustive aminolysis would thus furnish **26**. Such neighboring group participation was precedented in numerous examples of anchimeric assistance by C2 ester-substituted sugars in Koenigs–Knorr and related glycosidic coupling reactions.[27] Use of the corresponding tetra-O-(trimethylsilyl)lactam **29**, readily prepared by silylation of **26**, might circumvent such participation; however, all attempts to O-alkylate the lactam carbonyl of **29** with Et_3OBF_4 led to immediate desilylation.

(19) Saul, R.; Molyneux, R. J.; Elbein, A. D. *Arch. Biochem. Biophys.* **1984**, *230*, 668.
(20) While **5–7** incorporate an sp^2 carbon at C-1, their conformations are distinctly different from the glycosyl cation, as methylenecyclohexane is from cyclohexene.
(21) Portions of this research have been reported in preliminary form: (a) Tong, M. K.; Papandreou, G.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112*, 6137. (b) Ibid. **1991**, *113*, 8984.
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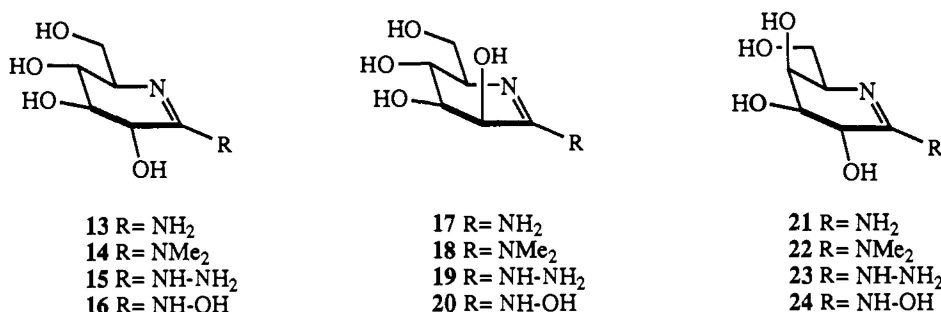
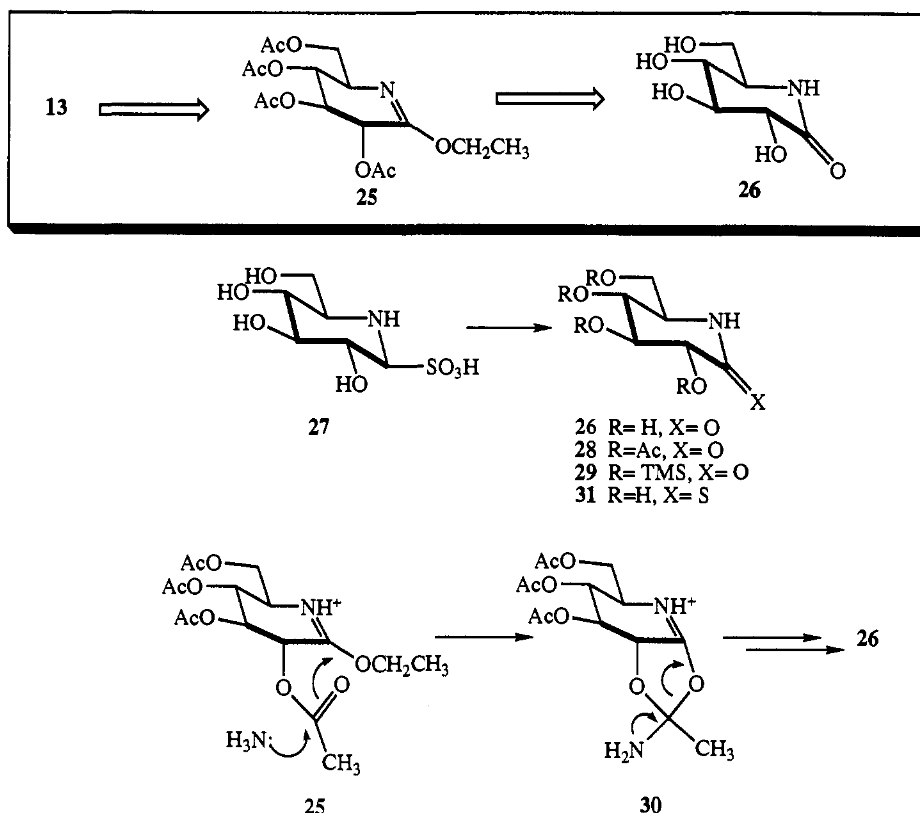
Scheme II



Scheme III



To circumvent this problem, silylated lactam **29** was reacted with Lawesson's reagent[28] to produce the corresponding thionolactam **31** after desilylation by aqueous acidic workup. Analytically pure samples of thionolactam **31** were obtained either by HPLC purification after flash silica gel chromatography or by adsorption onto activated charcoal with elution using 1:1 ethanol:H_2O.

When **31** was stirred with a saturated solution of Na-dried NH_3 in methanol, amidine **13** was obtained as a moisture-sensitive oil, along with 10–20% of D-gluconolactam **26** apparently resulting from hydrolysis of **13**. Acidification of the crude product with anhydrous HCl–CH_3OH gave a stable hydrochloride salt which could be chromatographed on silica (20:4:1 CH_3CN:H_2O:HOAc) with ion exchange to afford analytically pure acetate salt **13**·HOAc in 68% yield (Scheme IV). Similarly, when reacted with a saturated solution of dimethylamine–CH_3OH, **31** furnished pure **14**·HOAc in 85% yield after chromatography.

As salts, amidines **13** and **14** were stable indefinitely in water; however, as free bases, both amidines were extremely sensitive to nucleophiles at higher pH. For example, **13** decomposed to lactam **26** by nucleophilic hydrolysis (water, pH 8) with a half-life of 1 h. Amidine **13** also reacted with anhydrous dimethylamine to form **14** (71%), thus also providing a useful correlation of the two amidine structures. In a search for analogs of **13** with enhanced stability, the corresponding D-glucoamidrazone **15** and amidoxime **16** were also prepared by reacting thionolactam **31** with anhydrous hydrazine (CH_3OH, 5 °C, 2 h) or hydroxylamine (CH_3OH, room temperature, 14 h), respectively. Both **15** and **16** could be chromatographed on silica to afford **15**·HOAc (78% yield) and **16**·HOAc (75% yield), and both derivatives proved much more resistant to basic hydrolysis.

Oszczapowicz *et al.* established that pK_a values of N-substituted amidines varied directly with pK_a values of the corresponding N-substituted amines.[29] Hence, pK_a values of **15** and **16** were expected to be lower than the value for **13** since hydrazine (pK_a 8.10) and hydroxylamine (pK_a 5.97) were both less basic than ammonia (pK_a 9.21).[30] In fact, potentiometric titrations of the acidic forms of **13**, **15**, and **16** revealed pK_a values of 10.6, 8.7, and 5.6, respectively. Consistent with these observations, the half-life of amidrazone **15** was 8 h at pH 11, whereas glucoamidoxime **16** remained unchanged after several weeks in aqueous base at pH 11.

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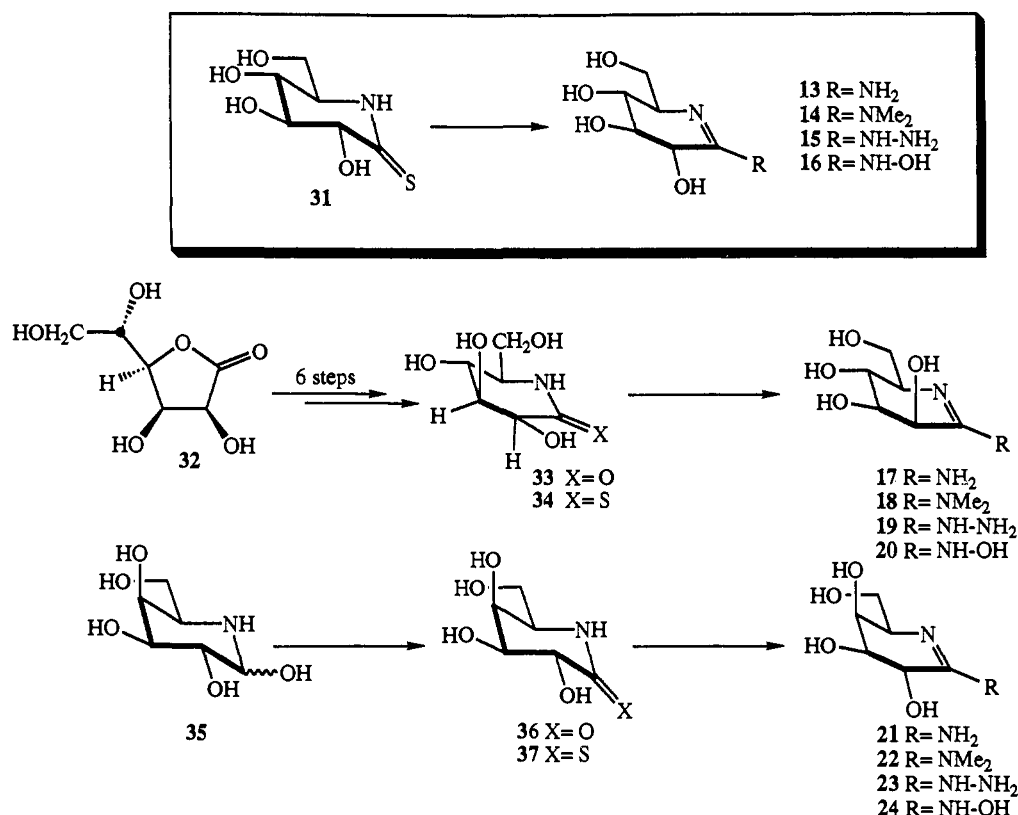
Scheme IV

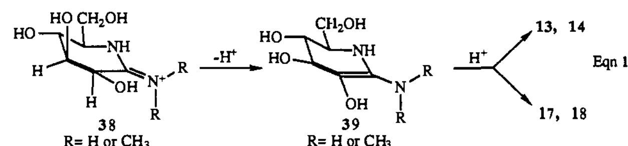


Thus, thionolactam **31**, easily prepared from D-gluconolactam, proved to be an extremely versatile intermediate for the synthesis of D-glucoamidines and their derivatives. We also envisioned synthesizing D-mannono and D-galactonothionolactams **34** and **37** (Scheme IV) from the corresponding lactams as precursors for **17–24**. In the D-*manno* series, an efficient and stereoselective synthesis of mannonolactam **33** from L-gulono-γ-lactone **32** was recently reported by Fleet *et al.*[31] When **33** was persilylated and then treated with Lawesson's reagent, workup under acidic conditions afforded D-mannonothionolactam **34** in 50% yield. Niwa *et al.* have established that lactam **33** adopted a twist-boat conformation both in solution and in the solid state.[32] Close parallels between the NMR spectra of **33** and **34** (particularly the relatively large values of $J_{5,6}$) suggested a similar conformation for D-mannonothionolactam.

When **34** was treated with anhydrous NH_3–CH_3OH, it unexpectedly afforded only glucoamidine **13** (70% yield), which was identified by comparison with an authentic sample derived from **31**. Moreover, when **34** was treated with Me_2NH–CH_3OH, a 2:1 mixture of dimethylamidines was isolated. The major, less polar product was identified as the previously characterized D-*gluco*-*N*,*N*-dimethylamidine **14**·HOAc (40%), while the minor product (20%) was assigned structure **18** having the D-*manno* configuration. Unlike **14**, amidine **18** displayed a small cis coupling (J = 4.0 Hz) for the C_2–C_3 hydrogens and was hydrolyzed to pure mannonolactam **33** in dilute NaOH (pH 10, 3 h). Controls using these basic conditions showed that no epimerization of either **26** or **33** occurred.

Several further experiments were designed to probe the mechanism of epimerization during the formation of amidines from D-mannonothionolactam **34**. To test whether the thionolactam ring underwent base-promoted epimerization, the reaction of **34** with NH_3-saturated methanol was terminated prematurely. Unreacted **34** was recovered intact, and no trace of epimerized D-gluconothionolactam **31** was detected. Compound **34** was also unaffected by Et_3N–CH_3OH and was unchanged upon standing in acidic methanol for several days. The parent lactams **26** and **33** were also stable to basic (NH_3–CH_3OH) or acidic (HCl–CH_3OH) conditions. Likewise, both *N*,*N*-dimethylamidines **14** and **18** were shown to be stable to Me_2NH–CH_3OH.

These findings ruled out any straightforward reactant or product isomerization. However, epimerization could occur via endocyclic bis-enamines like **39** which might arise as transient intermediates from initially formed iminium ions like **38**. Note that deprotonation of **38** as a twist-boat would be particularly facile, since it involves loss of an axial hydrogen adjacent to the iminium group (eq 1).



With more reactive nucleophiles such as anhydrous NH_2NH_2 or NH_2OH, thionolactam **34** was converted without detectable (HPLC) epimerization to the corresponding mannoamidrazone **19** and mannoamidoxime **20** in 75% and 73% yields, respectively.

In the D-*galacto* series, several syntheses of D-galactonolactam have been published.[33-35] However, the most expedient route to **37** involved the oxidation[36,37] of naturally occurring galactostatin **35** to **36**, followed by silylation and treatment in the now-standard

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(37) We wish to thank Dr. M. Narisada, of the Shionogi Research Laboratories, for a generous gift of galactostatin as its bisulfite addition compound.

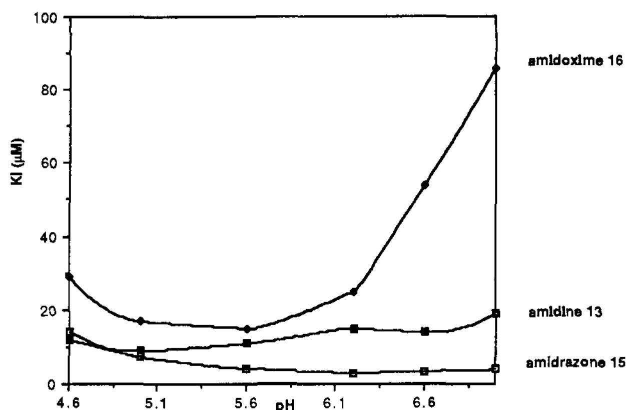

Figure 2. pH dependence of almond β-glucosidase and its inhibition by amidine **13**, amidrazone **15**, and amidoxime **16**.

fashion with Lawesson's reagent. Treatment of **37** under anhydrous conditions with NH_3–CH_3OH (room temperature, 7 h, 43%), Me_2NH–CH_3OH (room temperature, 3 h, 92%), NH_2-NH_2–CH_3OH (0 °C, 90 min, 85%), or NH_2OH–CH_3OH (room temperature, 2 h, 73%) gave the desired adducts **21–24** in the yields indicated (Scheme IV). In no cases were epimerized products detected.

Bioassays of Glycosidase Inhibition

Inhibitors Possessing the D-Gluco Configuration. Glucose analogs **13–16** were good competitive inhibitors of almond β-glucosidase (β-glu), a commercially available enzyme with broad substrate specificity and a catalytic mechanism like lysozyme's involving bilateral carboxyl functions.[38] The enzyme displayed a bell-shaped pH profile with maximum activity at pH 5.6. Enzyme-inhibitor dissociation constants using *p*-nitrophenyl-β-D-glucopyranoside as substrate (K_M = 2.5 mM, constant over pH range examined)[38] were 10 ± 2 μM for amidine **13**; 8.4 ± 0.9 μM for amidrazone **15**; and 13.8 ± 3 μM for amidoxime **16**. Weaker inhibition was observed for *N,N*-dimethylamidine **14** (K_I = 83 ± 8 μM). Plots of K_Is versus pH over the range 4.6–7.0 were constructed (Figure 2) to investigate the pH dependence of inhibition.

Over the pH range examined, amidine **13** (pK_a = 10.6) was fully protonated and the observed K_I (≈10 μM) was invariant. By contrast, 1-dNM **2** (pK_a = 6.7) displayed a bell-shaped inhibition profile (K_I range = 370-18 μM), with inhibition peaking at pH 6.75. Given the considerable overlap of pK_a values for protonated **2** and the enzyme's undissociated craboxyl group, it was not possible to distinguish from these data whether competitive inhibition arose from unprotonated **2** binding to singly protonated enzyme, or protonated **2** binding to unprotonated enzyme.[38] However, the pH independence of inhibition by **13** indicated that the protonated inhibitor clearly interacted with the more acidic, fully dissociated carboxyl group (pK_a = 4.4) in the active site. Inhibition of β-glu by amidrazone **15** was also pH-independent, whereas the observed pH dependence of inhibition by amidoxime **16** paralleled the variation in k_{cat}/K_M with pH for the enzyme. While such behavior would be expected for a transition state analog inhibitor,[39] the pH dependence might also reflect ionization of the free inhibitor, since the pK_a of **16** is likely within the pH range of the assay.[40]

The structures of **13–16** closely mimicked the flattened conformation of the glucopyranosyl cation. However, the special importance of the endocyclic double bond in these inhibitors became evident when **16** was compared with D-gluconohydroximinolactone **6** having an exocyclic imine group. Although both **6** and **16** incorporated an sp^2 hybridized carbon at C-1, corresponding to the substrate's anomeric center in Figure 1, the conformations of each inhibitor were distinctly different. While the exocyclic C=N bond in **6** created a distorted half-chair conformation which flattened the anomeric region somewhat, this oxime could only achieve the endocyclic π-electron density of the glucosidase transition state (Figure 1) in minor, dipolar resonance structures. As an inhibitor of β-glu, **6** (K_I = 98 μM) was 7 times weaker than **16**.[9] Clearly, the overall ring conformations as well as the flattened anomeric regions of these glucosyl mimics were significant in determining transition-state binding to the enzyme.

The chemical and physical properties of **13**, **15**, and **16** shed additional light on the relative importance of electrostatic and conformational changes in the enzymatic hydrolysis of β-glucosides. Nojirimycin and its congeners (Scheme I) represented imperfect structural mimics of the glucopyranosyl cation, simulating the charge of the glucosyl cation, but not its flattened shape. Likewise, D-gluconolactone **5**,[8] its oxime **6**,[9] and the corresponding 5-amino-5-deoxylactam **7**[10] mimicked to some degree the half-chair conformation of the glucosidase transition state, but as uncharged species, they failed to simulate the putative electrostatic changes. Since both types of inhibitors displayed significant competitive inhibition against β-glu, it appeared that both conformational and electrostatic changes were important in active site binding. However, with binding of **13**, **15**, and **16** essentially constant despite a 10^5 change in basicity, the overall conformational changes accompanying the rehybridization of the sugar's anomeric region appeared to be more important for inhibition than achieving the full-fledged charge of the glucopyranosyl cation. In a separate series of experiments with imidazole-based glycosidase inhibitors, Li and Byers recently also concluded that shape was more important than charge for tight binding.[41]

Structures **13–16** were also found to inhibit bovine β-galactosidase (β-gal), an enzyme which, like β-glu, exhibits broad substrate specificity for both *gluco* and *galacto* substrates. For example, amidrazone **15** displayed comparable levels of noncompetitive inhibition against β-gal (K_I = 19 ± 1 μM; *vide infra*). Although additional K_I values were not obtained, comparative assays indicated that **16** was comparable to **15** in potency (both exhibited 4% of control activity against β-gal) and **13** was somewhat less potent (20% of control activity).[42] Surprisingly, **13** and **15** were also potent competitive inhibitors of jackbean mannosidase (α-man; K_I = 9.0 ± 1, 3.1 ± 0.6 μM, respectively), an enzyme which does not normally process substrates having the D-*gluco* configuration. However, the observed epimerization at C2 in reactions of D-mannonothionolactam **34** raised the possibility of a similar isomerization in reactions of glucothionolactam **31**. Although both NMR and HPLC analysis showed no evidence for D-*manno* isomers, trace contaminants which might affect the α-man assays could not be ruled out.[43]

Additional evidence linking the unusual potency of amidine **13** to its unique shape came from a new family of nojirimycin derivatives. When the bisulfite addition product of nojirimycin **40** was dissolved in ammonia-saturated methanol (room temperature, 17 h), 1β-amino-1-deoxynojirimycin **41** was formed in good yield (eq 2).[44] This *gem*-diamine was especially interesting, since it embodied the same highly desirable combination of endocyclic and exocyclic nitrogens found in amidine **13**, but without the flattened half-chair conformation. While the chair-shaped *gem*-diamine **41** did competitively inhibit β-glu rather well (K_I = 40 ± 3 μM), activity against other glycosidases was

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(41) Li, Y.-K.; Byers, L. D. *Biochim. Biophys. Acta* **1989**, *999*, 227.
(42) Percent control activity refers to the residual activity of an inhibited (1 mM) reaction mixture when compared to a control assay without inhibitor (substrate = 5 mM).
(43) Given the potency of **17** and **19** against their cognate enzyme, it is conceivable that 1% or less as a contaminant could explain the observed mannosidase inhibition. We thank one of the referees for this suggestion.
(44) Yoon, H.; King, S. B.; Ganem, B. *Tetrahedron Lett.* **1991**, *32*, 7199.

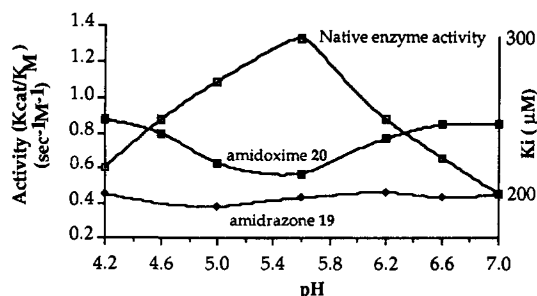
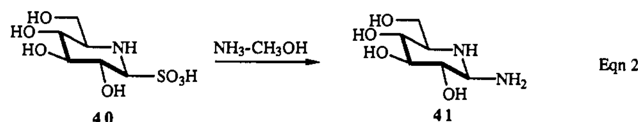


Figure 3. pH dependence of almond β-glucosidase and its inhibition by amidrazone **19** and amidoxime **20**. The pH dependence of native enzyme activity is given by K_{cat}/K_M values (left axis), while the pH dependence of inhibition is given by K_I values (right axis).

not observed. In fact, **41** had no effect on yeast α-glu, jackbean α-man, green coffee bean α-gal, or bovine liver β-gal.



Taken as a whole, these findings contrasted with molecular mechanics calculations by Kajimoto *et al.* using inhibition data from simple azasugars and amidine **13**.[45] Such calculations suggested that electrostatic attractions between the positive charge of the inhibitor and key sites on the enzyme were the most important factor for binding and recognition and that a good glycosidase inhibitor required both a half-chair conformation and positive charge character.

Inhibitors Possessing the D-Manno Configuration. Of mannose analogs **17–20** which were investigated in this study, amidrazone **19** and amidoxime **20** proved to be the most potent and stable inhibitors. Against the cognate enzyme for this series, jackbean α-man, inhibition constants for **19** and **20** were determined to be 170 ± 20 nM and 2 ± 0.1 μM, respectively, at pH 5.0. D-Mannoamidrazone **19** was particularly noteworthy for its effect on a range of different mannosidases. Besides jackbean α-man, **19** also inhibited mung bean α-man (IC_{50} = 400 nM), fungal β-man (IC_{50} = 150 nM), Golgi α-man I (IC_{50} = 4 μM), and α-man II (IC_{50} = 90–100 nM). It also proved to be the first potent inhibitor of the soluble (or endoplasmic reticulum) α-man, with an IC_{50} of 1 μM.[46] The latter triad of mannosidases constituted the principal mannose-processing pathway in the biosynthesis of glycoproteins. A further test of mannoamidrazone **19** as an *in vivo* mannosidase inhibitor was performed in animal cell cultures using Madin–Darby canine kidney cells infected with influenza virus. Addition of **19** to the culture medium prevented almost completely the formation of complex *N*-linked oligosaccharides and produced instead about equal amounts of $Man_9(GlcNAc)_2$ and $Man_8(GlcNAc)_2$ structures. These data indicated that mannoamidrazone **19** also inhibited Golgi α-man I and the soluble endoplasmic reticulum mannosidase *in vivo*.

Samples of **19** and **20** had similar, though modest, effects on almond β-glu (K_I = (200–210) ± 25 μM). The pH profile of β-glu inhibition established that inhibition by mannoamidrazone **19** was pH-independent, whereas the binding of mannoamidoxime to β-glu was pH-dependent, with a maximum at the enzyme's pH optimum (Figure 3). Compound **19** also exhibited a significant degree of linear, mixed, noncompetitive inhibition against bovine liver β-gal (K_I = 57 ± 2.5 μM at pH 7.0; *vide infra*). Although trace contamination by the D-*gluco* isomers could not be ruled out, the very significant effect evident against β-gal would have required D-*gluco* contaminants well above the threshold of NMR detection.

Other chair-shaped inhibitors exhibit little cross-reactivity between gluco and mannosidases. For example, 1-deoxynojirimycin ligands have been used for affinity purification of processing glucosidases, but not mannosidases, in the mammalian glycoprotein biosynthetic pathway.[47] Moreover, 1-deoxymannojirimycin **3** specifically inhibits only mannosidases (K_I = 68 and 83 μM for jackbean and calf liver α-man, respectively), but not almond β-glu (K_I = 5.3 mM at pH 5.0).[48]

Inhibitors Possessing the D-Galacto Configuration. Preliminary screening of D-galactopyranosyl derivatives **21–24** revealed significant inhibition of almond β-glu, bovine liver β-gal, and green coffee bean α-gal, but no inhibition of amyloglucosidase. Only galactoamidrazone **23** showed a modest level of inhibition of jackbean α-man.

Compound **23** was a competitive inhibitor of almond β-glu, with a pH-independent enzyme-inhibitor dissociation constant of 2.4 ± 0.7 μM at pH 5.6. The fact that **23** was 3 times more potent than glucoamidrazone **15** (K_I = 8.4 μM) against the latter's cognate enzyme was quite surprising. While gluco- and galactosidase substrate profiles are similar to some extent (judging from K_M values), none of the classic inhibitors such as 1-deoxynojirimycin **2**,[49,50] 1-deoxygalactonojirimycin **4**,[51] or castanospermine **12**[19] inhibit both enzymes well. Table I presents relevant kinetic data on **2**, **3**, **4**, and **12** and summarizes K_I values reported here for the title compounds **13–24**.

Galactoamidine **21** and galactoamidrazone **23** were active inhibitors of α-gal, exhibiting K_I values of 8.5 and 8.3 ± 0.4 μM, respectively, at pH 6.6. This enzyme (MW 40 000; the major component in commercially available enzyme from Sigma Chemical Co.) exhibited optimal activity at pH 6.1.[52] As with almond β-glu, kinetic studies suggested the existence of two active site acidic groups having pK_a values of 2.8–3.5 and 5.1–5.9. Inhibition of α-gal by both **23** and **24** was pH-independent, again suggesting that the protonated form of the basic inhibitors interacted with the more dissociated of the two active site carboxylates. *N,N*-Dimethylamidine **22** was somewhat less potent than **21** (17% of control activity for **22** versus 3% of control activity for **21**).[42]

Inhibition of α-gal by galactoamidoxime **24** was much weaker than expected (59% of control activity),[42] and plots of absorbance versus time as a function of inhibitor concentration uncovered no kinetic evidence for a slow approach to steady-state binding. However, further investigation revealed other anomalous behavior. With its pK_a of 5.8, as determined by potentiometric titration, **24** was only partially protonated at physiological pH and was therefore expected to inhibit its cognate glycosidase in pH-dependent fashion, like **16** and **20**. In fact, amidoxime **24** exhibited pH-independent inhibition of α-gal between pH 4.6 and 7.0. The significance of these observations is presently unclear, but may be revealed by an X-ray crystallographic analysis of the α-gal/**24** complex. Work toward this objective is currently underway.

Bovine β-gal was competitively inhibited by galactoamidrazone **23** (K_I = 6.5 ± 0.1 μM) and galactoamidoxime **24** (K_I = 10 ± 0.5 μM). Besides processing simple β-galactosides, this enzyme (M_r = 67 000; monomeric at pH 7.0 or dimeric at pH 4.5) has evolved both a galactose- and a glucose-binding site to hydrolyze

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Table I. Comparison of Glycosidase Inhibition Constants for Various Known and New Inhibitors

inhibitor	K_I value (reference)			
	β-glu	α-man	β-gal	α-gal
1-dNM **2**	18 μM (49)		8% inhibition at 1 mM (50)	8% inhibition at 1 mM (50)
3	5.3 mM (48)	68 μM (48)		
1-dGNM **4**	540 μM (51)		12.5 μM (51)	0.0016 μM (51)
castanospermine **12**	10–20 μM (19)		no effect at 1.3 mM (49)	no effect at 1.3 mM (49)
13	10 ± 2 μM	9.0 ± 1 μM		
14	83 ± 8 μM			
15	8.4 ± 0.9 μM	3.1 ± 0.6 μM	19 ± 1 μM	
16	13.8 ± 3 μM		comparable to **15**	
19	200 ± 25 μM	170 ± 20 nM	57 ± 2.5 μM	
20	210 ± 25 μM	2 ± 1 μM		
21	25 ± 5 μM			8.5 ± 0.4 μM
23	2.4 ± 0.7 μM		6.5 ± 0.1 μM	8.3 ± 0.4 μM
24			10 ± 0.5 μM	
41	40 ± 3 μM	no inhibition at 1 mM	no inhibition at 1 mM	no inhibition at 1 mM

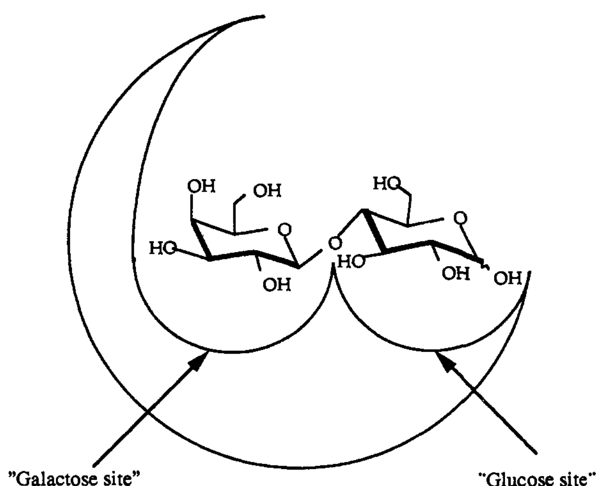

Figure 4. Representation of the binding domain of bovine β-galactosidase.

lactose,[53] with optimal activity at pH 4.5 (Figure 4). Competitive inhibitors such as isopropyl-β-D-thiogalactoside and D-galactonolactam target the catalytic (galactose-binding) site of β-gal. Only a few substances (notably D-arabinose, D-lyxose, cellobiose, maltose) target and glucose-binding site of the enzyme, causing weak noncompetitive inhibition by binding both free β-gal and the enzyme–substrate complex.

In contrast to galactoamidrazone **23**, gluco and mannoamidrazones **15** and **19** displayed noncompetitive inhibition of β-gal. These structures apparently interacted strongly with the glucose site, whereas **23** and **24**, which possessed the natural D-galacto configuration, bound competitively (and selectively) at the catalytic site.

In summary, an efficient and general synthetic route to novel amidine, amidrazone, and amidoxime analogs of simple monosaccharides has been developed. The design of such structures as prospective glycosidase inhibitors was based on the hypothesis that good transition-state analogs of glycoside hydrolysis should develop partial positive charge as well as a flattened chair conformation characteristic of the glycosyl cation (Figure 1). In fact, however, mimicking the incipient charge buildup proved less important for enzyme inhibition, since even the relatively neutral amidoximes **16**, **20**, and **24** effectively blocked glycoside hydrolysis. Studies of secondary deuterium kinetic isotope effects have also demonstrated that glycosidase transition states involve little charge development on the anomeric carbon.[54,55] Moreover, full-fledged glycosyl cations have been shown to have no significant lifetimes in the presence of enzyme-bound nucleophiles, suggesting that, at least with anionic nucleophiles such as carboxylates, glycosides undergo substitution by concerted displacement mechanisms.[56]

Experimental Section

General Methods. All reactions were run in flame-dried glassware under an inert atmosphere (argon or nitrogen). Melting points are uncorrected. Proton and ^{13}C NMR spectra were recorded on Bruker WM-300, WM-300, or Varian XL-400 spectrometers. Infrared spectra were taken on a Mattson Galaxy Model infrared spectrometer. UV-visible absorption spectra were obtained on a Hewlett-Packard HP 8451A diode array spectrometer. Mass spectra were obtained on a Finnigan 3300 or a VG-70-VSE mass spectrometer. Fast atom bombardment spectra were obtained in a glycerol or 3-nitrobenzyl alcohol matrix on a Kratos MS-890, VG-ZAB-SE, or VG-70-4F spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Flash chromatography was performed using Silica Gel 60 (Merck, 230–400 mesh). High-performance liquid chromatography was performed with an Eldex 9600 system using a μ-Porasil column.

Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl. Dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) were distilled from the stored over microwave-activated 4-Å molecular sieves. Triethylamine was distilled from the stored over barium oxide. Lawesson's reagent (*p*-(methoxyphenyl)thionophosphine sulfide dimer) was recrystallized from benzene. Anhydrous methanol was prepared by distillation from magnesium methoxide. Ammonia was dried by distillation over sodium. Anhydrous hydrazine was dried and distilled over sodium hydroxide. Anhydrous hydroxylamine in methanol was prepared by dissolving hydroxylamine sulfate in liquid ammonia, evaporating the solvent and triturating the residue with anhydrous methanol under Ar. Benzene, pyridine, oxalyl chloride, acetonitrile, methylene chloride, chlorotrimethylsilane, and hexamethyldisilazane were dried by distillation from CaH_2. All enzymes were obtained from Sigma. All other commercially obtained reagents were used as received.

2,3,4,6-Tetra(trimethylsilyl)-D-gluconolactam 29. To a stirred suspension of nojirimycin bisulfite adduct **27** (1.02 g, 4.22 mmol; Baeyer AG) in water (25 mL) was added activated Dowex 1 × 2–200 resin (HO^- form, 10 g, Aldrich) to make the pH 8–10. After stirring for 30 min at room temperature, the resin was filtered and rinsed with distilled water (160 mL), and the combined filtrates were lyophilized to afford a crude sample of nojirimycin **1** (0.89 g, R_f 0.58 in 4:1 ethanol:H_2O), which was dissolved in distilled water (15 mL) and used immediately in the next step.

The solution of **1** was treated with alternating portions of 0.1 M I_2–0.5 M KI solution (83 mL; 2-mL aliquots) and 0.1 M NaOH (100 mL, 2.5-mL aliquots) at room temperature over a period of 90 min. After 24 h, the brown solution was decolorized by addition of aqueous $NaHSO_3$ (1 M, 4 mL), and then Amberlite IR-120 (H^+) resin was added (0.5 g, Aldrich) to bring the pH to 1. After stirring for 4 h at room temperature, the resin was filtered and rinsed with water (50 mL). The combined filtrates were then neutralized with Dowex MWA-1 (Serva Corp), the resin was filtered and washed, and the combined filtrates (ca. 400 mL) were concentrated at the rotary evaporator to afford the known[5] D-gluconolactam **26** as a white solid. Crude **26** (5–6 g) was suspended

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in dry pyridine (20 mL), then $(TMS)_2NH$ (5 mL) and TMSCl (3 mL) were added, and the dark brown reaction mixture was stirred at room temperature for 90 min. Concentration *in vacuo* afforded a brownish foam (1.6 g), which was flash chromatographed (40 mm × 4.5 in. column; 7:1 hexanes:ethyl acetate; 8-mL fractions) to furnish persilylated gluconolactam **29** (1.14 g, 2.46 mmol, 59%): R_f 0.27 (1:7 hexanes:ethyl acetate); $[\alpha]_D$ + 71° (c = 0.54, $CHCl_3$); ^{1}H-NMR δ ($CDCl_3$) 5.88 (br s, 1 H), 3.87 (d, 1 H, J = 8.6 Hz, H_3), 3.78 (dd, 1 H, J = 8.3, 1.8 Hz, H_7), 3.69 (dd, 1 H, J = 8.7, 8.6 Hz, H_4), 3.43 (dd, 1 H, J = 8.4, 8.4 Hz, H_5), 3.35–3.25 (m, 2 H, H_6,$H_{7'}$), 0.18 (s, 9 H), 0.14 (s, 18 H), 0.09 (s, 9 H); ^{13}C-NMR ($CDCl_3$) 170.9, 76.2, 74.0, 71.4, 63.7, 57.0, 0.91, 0.73, –0.70; IR (film) 3210, 3110, 2980, 2905, 1690, 1320, 1250, 1130, 950, 840 cm^{-1}; CIMS (methane) m/e 467 (M + 2, 65), 466 (M + 1, 59), 450 ($M^+ - CH_3$, 100); EIMS m/e 466 (M + 1, 5), 465 (M^+, 5), 155 (100), 127 (40).

D-Gluconothionolactam 31. To a solution of **29** (0.284 g, 0.61 mmol) in benzene (15 mL) under argon at room temperature was added Lawesson's reagent (0.148 g, 0.6 equiv), and the suspension was warmed (65 °C) until homogeneous and then brought to reflux for 30 min. After concentrating *in vacuo*, the residue was dissolved in CH_3OH (16 mL), acidified (1:9 concentrated HCl:CH_3OH; 10 drops), and stirred for 35 min at room temperature. Concentration afforded a white solid (0.25 g), which was flash chromatographed over SiO_2 (20 mm × 6 in. column; 7:3:1 CH_2Cl_2:CH_3OH:NH_4OH; 3-mL fractions) to afford **31** (0.105 g), which was purified by dissolving in distilled water (10 mL) and stirring with Norit A (1.0 g) for 30 min. The Norit was filtered, rinsed with water (10 mL, discarded), and then eluted with 1:1 ethanol:H_2O, and the eluant was concentrated *in vacuo* to give analytically pure **31** (74 mg, 0.38 mmol, 63%): R_f 0.33 (7:3:1 CH_2Cl_2:CH_3OH:NH_4OH); mp 126–128 °C; $[\alpha]_D$ +31° (c = 0.72, CH_3OH); ^{1}H-NMR δ (D_2O) 3.90 (d, 1 H, J = 9.5 Hz, H_3), 3.78 (dd, 1 H, J = 12.3, 2.4 Hz, H_7), 3.74 (dd, 1 H, J = 9.8, 8.7 Hz, H_5), 3.67 (dd, 1 H, J = 12.4, 4.1 Hz, $H_{7'}$) 3.60 (dd, 1 H, J = 9.7 Hz, H_4), 3.33 (m, 1 H, H_6); ^{13}C-NMR (D_2O, acetone ref) 214.8, 74.2, 72.6, 67.4, 61.6, 59.7; IR (KBr) 3360, 2940, 2900, 1660, 1560, 1450, 1292, 1075, 1030 cm^{-1}. High-resolution FAB-MS: calcd for $C_6H_{11}NO_4S$ 193.0409, found 193.0403.

D-Glucoamidine 13. Saturated anhydrous NH_3–CH_3OH (1.5 mL) was added dropwise to a solution of thionolactam **31** (14 mg, 0.072 mmol) in anhydrous CH_3OH (1 mL) at room temperature under argon. After 11 h, two new spots were visible by TLC (base line and R_f 0.08 in 7:3:1 CH_2Cl_2:CH_3OH:NH_4OH). The solution was concentrated *in vacuo*, redissolved in fresh CH_3OH (2.5 mL), and acidified to pH 3.5 with anhydrous HCl–CH_3OH (0.5 mL, prepared from 0.3 mL of AcCl in 8 mL of CH_3OH). Concentration *in vacuo* afforded a brown oil, which was purified by flash chromatography (8 mm × 2.5 in. column; 20:4:1 CH_3CN:H_2O:HOAc); 2-mL fractions) to afford **13**·HOAc (11.6 mg, 68%): R_f 0.14 (20:4:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ +27° (c = 0.17, CH_3OH); ^{1}H-NMR δ (D_2O) 4.24 (d, 1 H, J = 9.6 Hz, H_3), 3.77–3.56 (m, 4 H, H_7, H_4, H_5, $H_{7'}$), 3.34 (m, 1 H, H_6), 1.94–1.76 (s, 3 H); ^{13}C-NMR (D_2O, dioxane ref), 179.0, 167.5 , 72.0, 68.2, 67.3, 60.1, 59.3, 21.9; IR (KBr) 3390, 3240, 2930, 1685, 1575, 1555, 1415, 1075 cm^{-1}. High-resolution FAB-MS: calcd for $C_6H_{13}N_2O_4$ 177.0875, found 177.0875.

***N,N*-Dimethyl-D-glucoamidine 14.** Anhydrous dimethylamine was bubbled into dry CH_3OH (5 mL) under argon at 0 °C until the volume of the solution doubled. Four milliliters of this solution was transferred by Teflon cannula to a solution of thionolactam **31** (11 mg, 0.057 mmol) in CH_3OH (0.5 mL), and the resulting solution was stirred for 10 min at 0 °C before warming to rt. One new spot was visible by TLC after 8 h (R_f 0.17 in 20:4:1 CH_3CN:H_2O:HOAc). Workup and chromatography as for **13** afforded **14**·HOAc (10.6 mg, 71%): R_f 0.21 (20:4:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$+ 15° (c = 0.57, CH_3OH); ^{1}H-NMR δ (D_2O) 4.46 (d, 1 H, J = 7.0 Hz, H_3), 3.86 (dd, 1 H, J = 12.2, 3.1 Hz, H_7), 3.77 (dd, 1 H, J = 9.0, 7.0 Hz, H_4), 3.71 (dd, 1 H, J = 12.2, 3.8 Hz, $H_{7'}$), 3.60 (dd, 1 H, J = 11.6, 9.0 Hz, H_5), 3.45 (m, 1 H, H_6), 3.21 (s, 3 H), 3.04 (s, 3 H), 1.85 (s, 3 H); ^{13}C-NMR (D_2O, di oxane ref), 179.7, 163.2, 74.5, 68.9, 65.9, 58.9, 58.6, 41.7, 39.4, 22.2; IR (KBr) 3320, 2925, 1660, 1565, 1420, 1060 cm^{-1}. High-resolution EIMS: calcd for $C_8H_{16}N_2O_4$ 204.1111, found 204.1110.

D-Glucoamidrazone 15. Anhydrous NH_2NH_2 (70 μL, 2.208 mmol, distilled from NaOH) was added dropwise to a stirred solution of gluconothionolactam **31** (20.5 mg, 0.106 mmol) in anhydrous CH_3OH (3 mL) in an ice–H_2O bath under Ar. TLC monitoring (CH_3CN:HOAc:H_2O 20:1:4) indicated that **31** disappeared after 90 min. The solution was concentrated *in vacuo*, and the residue (24 mg) was purified by flash chromatography (3.5 in. × 12 mm column; 25:3:1 CH_3CN:H_2O:HOAc; 2-mL fractions) to afford **15**·HOAc (19.3 mg, 78%): R_f 0.33 (CH_3CN:AcOH:H_2O 10:1:4); $[\alpha]_D$ + 15.6° (c = 0.45, MeOH); ^{1}H-NMR δ (D_2O) 4.27 (m, 1 H, J = 2.9, 6.9 Hz, H_3), 3.81 (dd, 1 H, J = 2.8, 12.2 Hz, H_7), 3.72–3.67 (m, 3 H, H_4, H_5, $H_{7'}$), 3.41 (m, 1 H, H_6), 1.78 (s, 3 H); ^{13}C-NMR (D_2O), 179.1, 164.4, 72.4, 67.7, 60.2, 59.1, 22.2; IR (KBr), 3310, 2920, 1700, 1665, 1560, 1410, 1110, 1070, 1020 cm^{-1}. High-resolution FAB-MS: calcd for $C_6H_{14}O_4N_3$ 192.0984, found 192.0989.

D-Glucoamidoxime 16. Anhydrous hydroxylamine (200 μL of a 1.25 M CH_3OH solution) was added under Ar to a stirred solution of glucothionolactam **31** (10 mg, 0.052 mmol) in CH_3OH (2.5 mL). TLC after 14 h showed a new spot at R_f 0.14 (CH_2Cl_2:CH_3OH:NH_4OH 7:3:1). The solution was concentrated *in vacuo*, and the residue (17 mg) was purified by SiO_2 chromatography (CH_3CN:H_2O:HOAc 30:3:1, 2-mL fractions) to afford glucoamidoxime **16**·HOAc (10 mg, 75%): R_f 0.41 (CH_3CN:H_2O:HOAc 10:4:1); $[\alpha]_D$ +62° (c = 0.39, CH_3OH); ^{1}H-NMR δ (D_2O) 4.09 (d, 1 H, J = 8.8 Hz, H_3), 3.75 (dd, 1 H, J = 2.5, 11.8 Hz, H_7), 3.64–3.49 (m, 3 H, $H_{7'}$, H_4, H_5), 3.16 (m, 1 H, H_6), 1.80 (s, 3 H); ^{13}C-NMR δ (D_2O, CH_3OH ref) 179.1, 156.4, 74.3, 68.4, 60.9, 57.8, 22.0; IR (KBr) 3400, 2920, 1660, 1590, 1570, 1420, 1335, 1110, 1020 cm^{-1}. High-resolution FAB-MS: calcd for $C_6H_{13}O_5N_2$ 193.0824, found 193.0821.

D-Mannonothionolactam 34. Mannonolactam **33**[31] (0.203 g, 1.14 mmol) was dissolved in dry pyridine (15 mL), and then $(TMS)_2NH$ (5 mL) and TMSCl (2.5 mL) were added dropwise. The resulting suspension was stirred at room temperature under Ar for 90 min. Concentration *in vacuo* afforded a white residue (613 mg), which was triturated with hexanes (60 mL in 3-mL fractions). The combined triturants were concentrated using a rotary evaporator to yield persilylated mannonolactam (0.533 g): R_f 0.20 (hexanes:EtOAc 7:1); ^{1}H-NMR δ ($CDCl_3$) 6.11 (s, 1 H, broad), 4.30 (d, 1 H, J = 2.7 Hz, H_3), 3.76 (dd, 1 H, J = 4.5, 2.7 Hz, H_4), 3.70 (m, 1 H, H_5), 3.56–3.51 (m, 2 H, H_7, $H_{7'}$), 3.26 (m, 1 H, H_6), 0.12–0.0 (m, 36 H); IR ($CHCl_3$), 3390, 2970, 1675, 1460, 1310, 1250, 1100 cm^{-1}; ^{13}C-NMR δ ($CDCl_3$) 17 0.5, 74.9, 69.3, 68.8, 64.1, 60.6, 0.10, 0.05, –0.21, –0.87. A solution of persilylated mannonolactam (176 mg, 0.378 mmol) in benzene (12 mL) under Ar was mixed with Lawesson's reagent (136 mg, 0.337 mmol) and the suspension heated to reflux for 1 h. The homogeneous reaction was concentrated *in vacuo*, and the residue was suspended in CH_3OH (10 mL) and acidified with methanolic HCl. Concentration afforded a white solid (230 mg), which was triturated with $CHCl_3$ (30 mL). The residue (81 mg) was purified by chromatography (5 in. × 15 mm column; CH_3CN:H_2O:AcOH 200:4:1; 3-mL fractions) to afford mannothionolactam **34** (38 mg, 50% from **33**): R_f 0.30 (CH_2Cl_2:CH_3OH:NH_4OH 7:3:1); $[\alpha]_D$ + 53.3° (c = 0.69, MeOH); ^{1}H-NMR δ (D_2O) 4.29 (d, 1 H, J = 3.7 Hz, H_3), 3.94 (dd, 1 H, J = 3.8, 5.4 Hz, H_4), 3.80 (dd, 1 H, J = 12.0, 3.6 Hz, H_7), 3.77 (dd, 1 H, J = 5.3, 7.1 Hz, H_5), 3.66 (dd, 1 H, J = 5. 6, 12.0 Hz, $H_{7'}$), 3.32 (m, 1 H, H_6); ^{13}C-NMR δ (D_2O, dioxane ref) 202.8, 72.7, 72.0, 67.7, 60.9, 60.5; IR (KBr) 3390, 2910, 1620, 1540, 1390, 1120, 1060; CIMS 194 (M + 1, 100); EIMS 193 (M^+, 100), 157 (96), 140 (29), 139 (26), 111 (54), 102 (32).

***N,N*-Dimethyl-D-mannoamidine 18.** The general procedure for **14** using thionolactam **34** (16.3 mg, 0.0844 mmol) gave, after flash chromatography, **18**·HOAc (4.8 mg, 21%) and **14**·HOAc (8.3 mg, 40%). For **18**·HOAc: R_f 0.18 (20:4:1 CH_3CN:H_2O:HOAc); $[\alpha]_D$ –13.6° (c = 0.22, CH_3OH); ^{1}H-NMR δ (D_2O) 4.76 (d, 1 H, J = 4 Hz, H_3), 3.91–3.80 (m, 2 H, H_7, H_5), 3.74–3.70 (m, 2 H, H_4, $H_{7'}$), 3.36 (m, 1 H, H_6), 3.23 (s, 3 H), 3.06 (s, 3 H), 1.80 (s, 3 H); ^{13}C-NMR δ (D_2O, CH_3OH ref), 181.6, 162.1, 69.7, 64.4, 63.2, 60.2, 59.6, 40.1, 39.2, 23.4; IR (KBr) 3390, 2920, 1670, 1560, 1540, 1420, 1100 cm^{-1}; FAB-MS 205.1 (M + 1, 100).

D-Mannoamidrazone 19. Anhydrous NH_2NH_2 (60 μL, 1.89 mmol) was added dropwise to a stirred solution of **34** (14 mg, 0.072 mmol) following the procedure for **15**. Chromatography afforded **19**·HOAc (14 mg, 75%): R_f 0.35 (CH_3CN:H_2O:AcOH 10:1:4); $[\alpha]_D$ + 10.9° (c = 0.46, CH_3OH); ^{1}H-NMR δ (D_2O) 4.64 (d, 1 H, J = 3.4 Hz, H_3), 3.97 (dd, 1 H, J = 3.6, 4.8 Hz, H_4), 3.89 (dd, 1 H, J = 4.9 Hz, H_5), 3.78 (dd, 1 H, J = 4.5, 11.8 Hz, H_7), 3.67 (dd, 1 H, J = 5.9, 11.8 Hz, $H_{7'}$), 3.37 (m, 1 H, H_6), 1.84 (s, 3 H); ^{13}C-NMR δ (D_2O, dioxane ref) 179.1, 164.1, 70.8, 67.5, 64.7, 61.1, 58.2, 2 2.1; IR (KBr) 3290, 2930, 1705, 1575, 1410, 1350, 1120, 1065, 1010 cm^{-1}. High-resolution FAB-MS: calcd for $C_6H_{14}O_4N_3$ 192.0984, found 192.0980.

D-Mannoamidoxime 20. Following the procedure for **16**, reaction of anhydrous hydroxylamine (280 μL of a 1.25 M CH_3OH solution) with 34 (12.2 mg, 0.063 mmol) afforded **20**·HOAc (11.6 mg, 73%): R_f 0.60 (CH_3CN:H_2O:HOAc 10:4:1); $[\alpha]_D$ –1.0° (c = 0.40, CH_3OH); ^{1}H-NMR δ (D_2O) 4.67 (d, 1 H, J = 2.2 Hz, H_3), 3.98–3.91 (m, 2 H, H_4, H_5), 3.80 (dd, 1 H, J = 4.4, 12 Hz, H_7), 3.70 (dd, 1 H, J = 5.3, 11.9 Hz, $H_{7'}$), 3.38

(m, 1 H, H_6); ^{13}C-NMR δ (D_2O, CH_3OH ref) 178.8, 156.0, 71.6, 66.5, 66.4, 61.7, 58.1, 21.8; IR (KBr) 3390, 2930, 1660, 1560, 1545, 1420, 1340, 1100, 1055, 1010 cm^{-1}. High-resolution FAB-MS: calcd for $C_6H_{13}O_5N_2$ 193.0824, found 193.0825.

D-Galactothionolactam 37. D-Galactonolactam **36** (88 mg, 0.5 mmol), prepared from galactostatin **35** (Meiji Seika Kaisha Ltd.) by a published procedure,[36] was suspended in pyridine (8 mL), and then $(TMS)_2NH$ (2 mL) and TMSCl (1 mL) were added at room temperature. After stirring for 1 h, the suspension was cooled to 0 °C and quenched with 50 mM phosphate buffer pH 7.0 (10 mL). The mixture was further diluted with H_2O (10 mL) and extracted with Et_2O (4 × 20 mL). The organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue (165 mg) was purified by SiO_2 chromatography (10 mm × 6 in. column; hexanes:EtOAc 6:1) to afford the corresponding persilylated lactam (125 mg, 56%), which was reacted directly with Lawesson's reagent (108 mg, 0.266 mmol) as described for **31** and **34** to afford galactothionolactam **37** (40 mg, 71%): R_f 0.35 (CH_2Cl_2:CH_3OH:NH_4OH 7:3:1); $[\alpha]_D$ +85° (*c* = 1.33, CH_3OH); ^{1}H-NMR δ (D_2O) 4.15 (dd, 1 H, *J* = 2.4 Hz, H 5), 4.09 (d, 1 H, *J* = 9.7 Hz, H_3), 3.75 (dd, 1 H, *J* = 2.4, 9.7 Hz, H_4), 3.67 (m, 2 H, H_7, $H_{7'}$), 3.58 (m, 1 H, H_6); ^{13}C-NMR δ (D_2O, acetone ref) 203.2, 72.6, 70.9, 67.3, 59.6, 58.9; IR (KBr) 3390, 2960, 2850, 1680, 1548, 1385, 1290, 1190, 1150 cm^{-1}; CIMS (isobutane) *m/e* 195 (M + 2, 12), 194 (M + 1, 100), 193 (M^+, 20), 178 (20).

D-Galactoamidine 21. Following the procedure for **13**, saturated anhydrous NH_3–CH_3OH (1 mL) was added to a stirred solution of galactothionolactam **37** (7.1 mg, 0.037 mmol) in CH_3OH (1 mL). Workup and chromatography as described earlier afforded **21**·HOAc (3.7 mg, 43%): R_f 0.49 (CH_3CN:H_2O:HOAc 5:4:1); $[\alpha]_D$ +59.2° (*c* = 0.12, CH_3OH); ^{1}H-NMR δ (D_2O) 4.45 (d, 1 H, *J* = 10.1 Hz, H_3), 4.13 (s, 1 H, H_5), 3.86 (dd, 1 H, *J* = 2.1, 10.1 Hz, H_4), 3.71–3.55 (m, 3 H, H_7, $H_{7'}$, H_6), 1.89 (s, 3 H); ^{13}C-NMR δ (D_2O, CH_3OH ref) 181.6, 167.5, 70.8, 67.4, 66.2, 60.5, 57.2, 23.4; IR (KBr) 3420, 2950, 1690, 1570, 1437, 1103 cm^{-1}; FAB-MS (3-NBA matrix) 177.1 (M + 1, 30). High-resolution FAB-MS: calcd for $C_6H_{13}N_2O_4$ 177.0875, found 177.0870.

***N,N*-Dimethyl-D-galactoamidine 22.** The general procedure for **14** using galactothionolactam **37** (7.1 mg, 0.037 mmol) afforded, after chromatography, **22**·HOAc (9.0 mg, 92%): R_f 0.47 (CH_3CN:H_2O:HOAc 5:4:1); $[\alpha]_D$ + 45.3° (*c* = 0.32, CH_3OH); ^{1}H-NMR δ (D_2O) 4.51 (d, 1 H, *J* = 7.5 Hz, H_3), 4.11 (s, 1 H, H_5), 3.90 (dd, *J* = 7.3, 1.5 Hz, H_4), 3.70 (s, 3 H, H_7, $H_{7'}$, H_6), 3.22 (s, 3 H), 3.04 (s, 3 H), 1.78 (s, 3 H); ^{13}C-NMR δ (D_2O, CH_3OH ref) 180.1, 163.1, 73 .1, 67.5, 66.6, 60.2, 57.2, 41.7, 39.4, 23.2; IR (KBr) 3390, 3275, 2940, 1652, 1560, 1405, 1350, 1120, 1070 cm^{-1}; EIMS *m/e* 205 (M + 1, 8), 204 (M^+, 15), 174 ($M^+ - 2CH_3$, 9), 173 (100), 127 (11), 113 (30), 97 (12), 86 (12); CIMS (isobutane) *m/e* 206 (M + 2, 12), 205 (M + 1, 100), 187 (M + 1 – H_2O, 13), 173 (13), 169 (12).

D-Galactoamidrazone 23. Anhydrous NH_2NH_2 (35 µL, 1.10 mmol) was added dropwise to a stirred solution of galactothionolactam **37** (11 mg, 0.057 mmol) in CH_3OH (1.7 mL) following the procedure for **15**. Chromatography afforded **23**·HOAc (12.0 mg, 85%): R_f 0.38 (CH_3CN:H_2O:HOAc 10:4:1); $[\alpha]_D$ + 58.2° (*c* = 0.49, CH_3OH); ^{1}H-NMR δ (D_2O) 4.49 (d, 1 H, *J* = 10.1 Hz, H_3), 4.18 (dd, 1 H, *J* = 2.2 Hz, H_5), 3.86 (dd, 1 H, *J* = 2.3, 10.1 Hz, H_2, H_4), 3.69 (m, 3 H, H_6, H_7, $H_{7'}$), 1.83 (s, 3 H); ^{13}C-NMR δ (D_2O, CH_3OH ref) 179.3, 164.8, 71.0, 67.6, 65.6, 60.3, 56.6, 22.1; IR (KBr) 3500, 3300, 3040, 1700, 1560, 1420, 1340, 1135, 1060 cm^{-1}; EIMS *m/e* 191 (M^+, 19), 160 (40), 149 (39), 146 (34), 137 (79), 129 (31), 115 (29), 91 (49), 83 (50), 75 (100); CIMS (isobutane) *m/e* 192 (M + 1, 12), 169 (13), 81 (10), 79 (10), 75 (100). High-resolution CIMS: calcd for $C_6H_{14}O_4N_3$ 192.0984, found 192.0989.

D-Galactoamidoxime 24. Following the procedure for **16**, anhydrous NH_2OH (500 µL of a 1.5 M CH_3OH solution) was added under Ar at 0 °C to a solution of galactothionolactam **37** (9.8 mg, 0.051 mmol) in CH_3OH (1 mL). Workup and chromatography afforded **24**·HOAc (9.3 mg, 73%): R_f 0.42 (CH_3CN:H_2O:HOAc 10:4:1); $[\alpha]_D$ + 58.7° (*c* = 0.46, CH_3OH); ^{1}H-NMR δ (D_2O) 4.40 (d, 1 H, *J* = 9.6 Hz, H_3), 4.10 (s, 1 H, H_5), 3.76 (dd, 1 H, *J* = 2.2, 9.9 Hz, H_4), 3.70–3.59 (m, 2 H, H_7, $H_{7'}$), 3.52 (m, 1 H, H_6), 1.83 (s, 3 H); ^{13}C-NMR δ (D_2O, CH_3OH ref) 179.5, 157.9, 72.2, 67.7, 65.9, 60.9, 56.0, 22.2; IR (KBr) 3390, 2930, 1660, 1560, 1420, 1300, 1125, 1060 cm^{-1}; EIMS *m/e* 192 (M^+, 10), 167 (23), 161 (40), 145 (16), 140 (21), 126 (16), 115 (20), 111 (43), 110 (100); CIMS (isobutane) *m/e* 193 (M + 1, 22), 141 (30), 111 (100). High-resolution CIMS: calcd for $C_6H_{13}O_5N_2$ 193.0824, found 193.0827.

General Biological Procedures. The enzymes α-glucosidase (yeast), α-amyloglucosidase (*Aspergillus niger*), β-glucosidase (almonds), α-galactosidase (green coffee beans), β-galactosidase (bovine liver), and α-mannosidase (jackbean) were purchased from Sigma Chemical Co. Assays were performed using *p*-nitrophenyl α-(or β)-D-glycopyranosides as substrates at 37 °C in phosphate–citrate buffers. Yeast α-glucosidase and green coffee bean α-galactosidase were assayed at pH 6.6. Almond β-glucosidase was assayed at pH 5.0 and 5.6. Jackbean α-mannosidase was assayed at pH 5.0. Bovine liver β-galactosidase was assayed at pH 7.0. Typical enzyme concentrations for inhibitor screening were as follows: yeast α-glucosidase, 10 µL of enzyme suspension in 2.00 mL of pH 6.6 phosphate–citrate buffer; almond solution β-glucosidase, 25 µL of enzyme solution (0.4 mg of solid enzyme in 70 µL of buffer) in 5.00 mL of pH 5.0 or 5.6 buffer; green coffee bean α-galactosidase, 15 µL of enzyme suspension in 1.2 mL of pH 6.6 buffer; bovine liver β-galactosidase, 1.7 mg of solid enzyme in 850 µL of pH 7.0 buffer. Three sets of data were collected and averaged for each enzyme. The enzyme, buffer, and inhibitor were incubated for 5 min, and then substrate was added. Final inhibitor and substrate concentrations were 1 and 5 mM, respectively. The reaction was quenched with a pH 10 glycine buffer after 15 min. Absorbance readings were taken at 400 nm using distilled deionized water as a control. K_I values were determined using five substrate (usually 2–20 mM) and four inhibitor (usually 0–200 µM) concentrations. Substrate hydrolysis velocities (*V*) were determined by plotting the absorbance values with respect to time and then calculating the slopes of the lines. Double reciprocal plots of $1/V$ versus $1/[S]$ at different [I] were then generated (Lineweaver–Burk plots). The slopes of each of these lines (i.e. L–B slopes) were then plotted against [I], and the data were fitted to a straight line. The [I]-intercept gave the enzyme–inhibitor dissociation constant. K_I values were also calculated from Hanes–Woolf plots of $[S]/V$ versus [S] by replotting the $[S]/V$ intercepts versus [I] and ascertaining the [I] intercept. Reported K_I values represent an average of the two calculations.

Acknowledgment. We thank the National Institutes of Health (GM 35712) for generous financial support. Grants to the Cornell Nuclear Magnetic Resonance Facility from the NSF (CHE 7904825; PGM 8018643) and the NIH (RR02002) are also gratefully acknowledged.

Supplementary Material Available: Representative plots of kinetic data from enzymatic assays for K_I determinations (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead-page for ordering information.