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Cyclic Amidine Sugars as Transition-State Analogue Inhibitors of Glycosidases: Potent Competitive Inhibitors of Mannosidases

Marie-Pierre Heck,*[†,⊥] Stéphane P. Vincent,[§,‡] Brion W. Murray,[§,#] François Bellamy,[||] Chi-Huey Wong,[§] and Charles Mioskowski*[†,⊥]

Contribution from the CEA, CE-Saclay, Service de Marquage Moléculaire et de Chimie Bioorganique, Bât 547, Département de Biologie Joliot-Curie, 91191 Gif Sur Yvette, France; Université Louis Pasteur, Laboratoire de Synthèse Bio-Organique associé au CNRS, Faculté de Pharmacie, 74 route du Rhin BP 24, 67401 Illkirch, France; The Scripps Research Institute, Department of Chemistry, 10550 North Torrey Pines Road, La Jolla, California 92037; Laboratoires Fournier, 50 rue de Dijon, 21121 Daix, France

Received August 8, 2003; E-mail: heck@dsvidf.cea.fr; mioskow@bioorga.u-strasbg.fr

Abstract: A series of monocyclic glycoamidines bearing different exocyclic amine, alcohol, or alkyl functionalities and bicyclic amidines derived from D-glucose and D-mannose were synthesized and tested as inhibitors of various glycosidases. All the prepared compounds demonstrated good to excellent inhibition toward glycosidases. In particular, the biscationic D-mannoamidine **9b** bearing an exocyclic ethylamine moiety proved to be a selective competitive inhibitor of α- and β-mannosidases (K_i = 6 nM) making it the most potent inhibitor of these glycosidases reported to date. A favorable $B_{2,5}$ boat conformation might explain the selectivity of mannosidase inhibition compared to other glycosidases.

Introduction

Glycosciences are emerging as a key research field at the frontiers of biology, synthetic and supramolecular chemistry, and enzymology.[1] Among the carbohydrate processing enzymes, glycosidases have been identified as an important class of therapeutic targets with applications in the treatment of influenza infection,[2] cancer,[3] AIDS,[4] and diabetes.[5] Thus, numerous classes of inhibitors have been discovered, some of them giving interesting insights into the mechanism of enzymatic glycoside hydrolysis.

Two general classes of glycosidase inhibitors can be defined: (i) natural products and synthetic analogues whose design has been inspired by the inhibitory activity of the natural inhibitors[6] and (ii) inhibitors whose design has been rationally conceived from the mechanism of the enzymatic reaction. The latter class of inhibitors comprises transition-state analogues of the glycoside cleavage process,[7] mechanism-based inactivators,[8] and conformationally locked molecules.[9]

The transition state of this enzymatic hydrolysis may strongly vary as a function of the type of glycosidase: for instance, glycosidases proceeding through retention or inversion of configuration at the anomeric center do not share the same mechanism.[8c,d] Furthermore, stereoelectronic factors may play a key role in the pathway through which a glycoside is enzymatically hydrolyzed.[10] Thus, the transition-state or the

† CEA, CE-Saclay.
⊥ Université Louis Pasteur.
§ The Scripps Research Institute.
‡ Current address: Ecole Normale Supérieure, Département de Chimie, 24 rue Lhomond, 75231 Paris, France.
# Current address: Pfizer La Jolla Laboratories, 10628 Science Center Drive, San Diego, CA 92121, USA.
|| Laboratoires Fournier.

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high-energy intermediates (such as oxycarbenium species) may adopt very different conformations depending on the structure of the sugar-substrate and the α/β-nature of the glycosidase.[11] The analysis of glycosidases sequences has allowed their classification into families and superfamilies on the basis of their sequence similarities and mechanism of action. To date, over 80 families have been defined,[12] which means that numerous distinct mechanistic pathways are likely occurring among the whole glycosidase family. Because of this multiplicity of mechanisms, it is difficult to define whether a given inhibitor is a transition-state analogue or an opportunistic binder of the catalytic pocket. Hundreds of natural or synthetic inhibitors have already been described,[6,13] among them some rationally designed mechanism-based inhibitors. However, only few molecules display inhibitory activity in the nanomolar range. In this study, we describe the synthesis of a new family of glycoamidines which display a low nanomolar and selective inhibition pattern for mannosidases.

Design of the Inhibitors: Targeting an Additional Ionic Interaction at the Vicinity of the Anomeric Center. Cationic glycosides can be viewed as early transition-state analogues (if they mimic the initial protonation of the exocyclic oxygen) or late transition-state analogues (if they mimic high-energy intermediates such as oxycarbenium species, after disruption of the glycosidic bond).[7d] Thus, glycoside analogues adopting a $^4H_3$ half-chair conformation tend to mimic the glycosyl oxycarbenium species often considered close to the transition state. The first inhibitors designed in this way were the glycoamidines, glycoamidrazones, and glycoamidoxime developed by Ganem et al.[14] Then, substituted amidines,[15] amidine pseudodisaccharides,[16] mannohydroximolactone,[15c] and fucoamidrazone[17] were reported as well and showed good inhibition levels for glycosidases. All these inhibitors were designed to mimic both the half-chair conformation and the charge developed around the anomeric center.

The glycoamidines reported by Ganem[14] displayed very good inhibition properties especially in the mannosidase series. We decided to explore this design by adding a supplementary electrostatic interaction to the glycoamidine core structure of the inhibitor. Indeed, most of the glycosidases have two carboxylic residues acting as general base and general acid at the vicinity of the substrate's anomeric center (or, in the case of retaining glycosidases, one carboxylate acts as a nucleophile to give a covalent intermediate) (Figure 1, left). Once the inhibitor is protonated, the amidine functionality is a monocation that can ionically interact with only one of the two carboxylates. Thus, the addition of a supplementary amino group through a short spacer attached to the exocyclic nitrogen might provide a new ionic interaction with the second carboxylate (Figure 1, right).

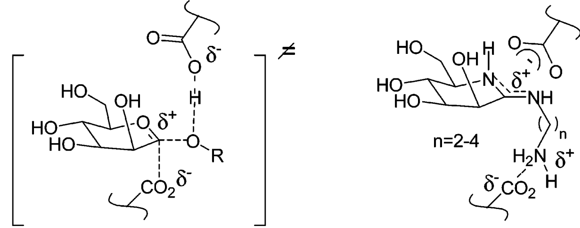

Figure 1. Expected transition state in a glycosidase reaction (left) and designed biscationic mannoamidines as transition-state analogues (right).

D-Glucose series: D-Mannose series: Bicyclic amidines:

7a-e 9a-e 12 13

a: n=2, R= CH_3
b: n=2, R= NH_2
c: n=3, R= NH_2
d: n=4, R= NH_2
e: n=3, R= OH

Figure 2. Designed inhibitors.

In principle, adding a properly placed electrostatic interaction should dramatically increase the binding strength of a given inhibitor. We then designed a set of monocyclic amidines in the gluco and the manno series, bearing an ethyl-, a propyl-, or a butylamino group linked to the exocyclic nitrogen (**7b**, **9b**, **7c**, **9c**, **7d**, **9d**; Figure 2). In an attempt to demonstrate the role of this additional amine, analogues bearing an alkyl chain (**7a**, **9a**), a hydroxyl function (**7e**, **9e**), and a six-membered ring cyclic amidine (**12**, **13**) were synthesized (Figure 2).

Synthesis of the Glycoamidines. The general synthetic route to protected glucoamidines **4a**–**e** and mannoamidines **5a**–**e** was carried out as summarized in Scheme 1, following the procedure reported by Ganem.[14c] A slight modification was applied using benzyl ethers as protective groups to improve compound stability and to facilitate purifications.

The sequence started with 2,3,4,6 tetra-*O*-benzyl-D-gluconolactam **1** which was prepared in four steps (42% overall yield) from commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose.[18] Lactam **1** was then reacted with Lawesson's reagent[19] (C_6H_6, reflux, 8 h, 84% yield) to afford the corresponding glucothionolactam **2** as previously reported.[20] Treat-

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Scheme 1. Preparation of Glycoamidines from Gluconolactam[a]

[a] Reagents and conditions: (a) Lawesson's reagent, C_6H_6, reflux, 8 h; (b) Meerwein's salt, CH_2Cl_2, 0 °C, 1.5 h; (c) R^1NH_2, CH_2Cl_2, 0 °C to rt, 12 h (for details see Table 1).

Table 1. Obtention of Glucoamidines **4** and Mannoamidines **5**

entry	R^1NH_2	equiv	4 (%)	5 (%)
1	$CH_3(CH_2)_2NH_2$	1	**4a** (69)	**5a** (23)
2	$CH_3(CH_2)_2NH_2$	1.8	**4a** (29)	**5a** (68)
3	$CH_3(CH_2)_2NH_2$	10	**4a** (25)	**5a** (70)
4	$BocNH(CH_2)_2NH_2$	1.8	**4b** (23)	**5b** (70)
5	$BocNH(CH_2)_3NH_2$	1.8	**4c** (26)	**5c** (66)
6	$BocNH(CH_2)_4NH_2$	1.8	**4d** (22)	**5d** (66)
7	$TBDMSO(CH_2)_3NH_2$	1.8	**4e** (16)	**5e** (67)

ment of **2** with Meerwein's salt yielded glucoiminothioethyl ether **3** (95% yield). Iminothioether **3** was then treated with a series of amines $\mathbf{R^1}NH_2$[21] and afforded a separable mixture of glucoamidine **4a**−**e**[22] and mannoamidine **5a**−**e**.[23] The results are summarized in Table 1.

We first studied the ratio of amine to use in the coupling reaction (Table 1, entries 1−3). Glucoiminothioethyl ether **3** reacted with 1 equiv of propylamine affording a 3:1 mixture of propylglucoamidine **4a** and propylmannoamidine **5a** (69% and 23% yields, respectively, entry 1) which were separated by chromatography on silica gel.[22] To our surprise, when glucoiminothioethyl ether **3** was treated with a slight excess of propylamine (1.8 equiv, entry 2), propylmannoamidine **5a** was isolated as a major product (68% yield) compared to propylglucoamidine **4a** (29% yield, entry 2). The same 3:7 ratio in manno/glucoamidine and the global yield were conserved when **3** was treated with an excess of propylamine (10 equiv, entry 3). The formation of D-mannoamidine **5a** starting from D-glucoiminothioether **3** is probably the result of an epimerization at C2 during the reaction with propylamine. Ganem et al.[14c] have previously observed such an epimerization when reacting D-mannothiolactam with a large excess of ammonia and have isolated glucoamidine as the sole product in 70% yield.

For the following experiments, glucoiminothioether **3** was reacted with 1.8 equiv of amines to obtain in one step the two epimers D-glucoamidine **4a**−**e** and D-mannoamidine **5a**−**e** (Table 1). Gluco- and mannoamidines bearing a Boc-protected aminoethyl, aminopropyl, and aminobutyl groups[24] (**4b**, **5b**, **4c**, **5c**, **4d**, **5d**; entries 4−6) were prepared in good yields (88−93%) in a ratio of 3:7 in favor of the mannoepimer. Similarly when 3-(*tert*-butyldimethylsiloxy)-1-propylamine[25] was reacted with glucoiminoether **3**, protected propanol glucoamidine **4e** and mannoamidine **5e** were isolated in 16% and 67% yields, respectively (entry 7).

(20) Hoos, R.; Naughton, A. B.; Thiel, W.; Vasella, A.; Weber, W.; Rupitz, K.; Withers, S. G. *Helv. Chim. Acta* **1993**, 76, 2666−2686. As reported, thiolactam **2** gave an inseparable crystalline 9:1 mixture of D-glucothiolactam and D-mannothiolactam.
(21) The amines were commercially available or synthesized; see refs 24 and 25.
(22) The less polar isomer (minor) was identified as D-glucoamidine, and the more polar isomer was assigned having a D-manno configuration.
(23) Theoretical calculations were not employed to predict the endo or exo constitution of the C═N bond. Based on previous studies reported by Vasella (ref 20), we postulate the presence of an exocyclic C═N bond.

Scheme 2. Preparation of Glycoamidines from Mannonolactam[a]

[a] Reagents and conditions: (a) Lawesson's reagent, C_6H_6, reflux, 8 h; (b) Meerwein's salt, CH_2Cl_2, 0 °C, 1.5 h, 95% yield; (c) $PrNH_2$, CH_2Cl_2, 0 °C to rt, 12 h.

To confirm and characterize the two epimers formed during the condensation of amine with D-glucoiminothioether **3**, we further studied the products obtained starting from D-mannoiminothioethyl ether **16** with propylamine. Iminothioether **16** was synthesized in two steps from tetrabenzylmannonolactam **14** as reported in Scheme 2.

Mannonolactam **14**[26] was treated with Lawesson's reagent to afford mannothionolactam **15** (89% yield), which was then reacted with Meerwein's salt to generate mannoiminothioether **16** (95% yield). Subsequent treatment of **16** with 1.8 equiv of propylamine gave a separable 9:1 mixture of two products which were identified as mannoamidine **5a** (90% yield) and glucoamidine **4a** (10% yield). This experiment demonstrated that under these conditions mannoiminothioether **16** is less epimerized. Removal of the protective groups to provide the final inhibitors was then performed as summarized in Scheme 3.

Hydrogenolysis of **4a** and **5a** with 30% palladium on activated carbon gave propylglucoamidine **7a** (93% yield) and propylmannoamidine **9a** (81% yield), respectively. Deprotection of the benzylglucoamidine carbamates **4b**, **4c**, **4d** and benzylmannoamidine carbamates **5b**, **5c**, **5d** with trifluoroacetic acid afforded the benzylglucoamidine amines **6b**, **6c**, **6d** (89%, 86%, 83% yields, respectively) and benzylmannoamidine amines **8b**, **8c**, **8d** (91%, 92%, 88% yields, respectively). Debenzylation reaction of benzylglucoamidines alkylamine **6b**, **6c**, **6d** gave glucoamidines alkylamine **7b**, **7c**, **7d** (95%, 89%, 91% yields, respectively), and benzylmannoamidines alkylamine **8b**, **8c**, **8d** afforded the mannoamidine alkylamines **9b**, **9c**, **9d** (89%, 88%, 91% yields, respectively).

Removal of TBDMS group in benzylglucoamidine **4e** and benzylmannoamidine **5e** using pyridinium-*p*-toluenesulfonate in EtOH gave benzylglucoamidine propyl alcohol **6e** (61% yield) and benzylmannoamidine propyl alcohol **8e** (80% yield). Hydrogenolysis of benzylglucoamidine **6e** and benzylmanoamidine **8e** yielded, respectively, glucoamidine propyl alcohol **7e** (92% yield) and mannoamidine propyl alcohol **9e** (86% yield).

(24) For the preparation of *N-tert*-butoxycarbonyl alkanediamines, see: Krapcho, A. P.; Kuell, C. S. *Synth. Commun.* **1990**, *20*, 2559−2564. Guo, H.; Naser, S. A.; Ghobrial, G.; Phanstiel, O., IV. *J. Med. Chem.* **2002**, *45*, 2056−2063.
(25) For the synthesis of 3-(*tert*-butyldimethylsiloxy)-1-propylamine, see: Grillot, A.-L.; Hart, D. J. *Tetrahedron* **1995**, *51*, 11377−11392.
(26) Tetrabenzylmannolactam **14** was prepared following the procedure reported by Pandit (ref 18b).

Scheme 3. Preparation of Final Glycoamidines[a]

4a: R=H, R'=OBn → **7a:** R=H, R'=OH
5a: R=OBn, R'=H → **9a:** R=OH, R'=H

n=2 **4b:** R=H, R'=OBn; **5b:** R=OBn, R'=H → **6b:** R=H, R'=OBn; **8b:** R=OBn, R'=H → **7b:** R=H, R'=OH; **9b:** R=OH, R'=H

n=3 **4c:** R=H, R'=OBn; **5c:** R=OBn, R'=H → **6c:** R=H, R'=OBn; **8c:** R=OBn, R'=H → **7c:** R=H, R'=OH; **9c:** R=OH, R'=H

n=4 **4d:** R=H, R'=OBn; **5d:** R=OBn, R'=H → **6d:** R=H, R'=OBn; **8d:** R=OBn, R'=H → **7d:** R=H, R'=OH; **9d:** R=OH, R'=H

4e: R=H, R'=OBn, R''=OTBDMS; **5e:** R=OBn, R'=H, R''=OTBDMS → **6e:** R=H, R'=OBn; **8e:** R=OBn, R'=H → **7e:** R=H, R'=OH; **9e:** R=OH, R'=H

[a] Reagents and conditions: (a) H_2, 30% Pd/C, EtOH, 12 h; (b) TFA, CH_2Cl_2, 0 °C to rt, 1.5 h; (c) PPTS, EtOH, 65 °C, 7 h.

Scheme 4 . Preparation of Bicyclic Amidines[a]

3 → a) 79% → **10** 10% R=Bn, b) → **12** 95% R=H; **11** 69% R=Bn, b) → **13** 90% R=H

[a] Reagents and conditions: (a) (i) $Br(CH_2)_3NH_2$, EtOH, 0 °C to rt, 8 h, (ii) K_2CO_3, 0 °C to rt, 6 h; (b) H_2, Pd/C, EtOH, 12 h.

Bicyclic amidines **12** and **13** were synthesized as illustrated in Scheme 4. Iminothioether **3** was treated with 3-bromopropylamine and K_2CO_3 in CH_2Cl_2 to yield a 1:7 mixture of protected bicyclic glucoamidine **10** (10% yield) and bicyclic mannoamidine **11** (69% yield) which were separated by chromatography on silica gel. Hydrogenolysis of **10** and **11** with 30% palladium on activated carbon gave D-gluco-1,5-diazabicyclo-[4,4,0]decene **12** (95% yield) and D-manno-1,5-diazabicyclo-[4,4,0] decene **13** (90% yield).

All the synthesized amidines (**7a**–**e**, **9a**–**e**, **12**, **13**) were shown to be stable for months at −40 °C.

Discussion of the Inhibitory Activity. Glucoamidines **7a**–**e** and **12** and mannoamidines **9a**–**e** and **13** were then evaluated against a wide variety of commercially available glycosidases: α-mannosidase (jack beans), β-mannosidase (snail acetone powder), α-glucosidase (yeast type III), β-glucosidase (almond), α-galactosidase (green coffee beans), β-galactosidase (*Aspergillus Orizae*), α-fucosidase (bovine kidney), *N*-acetyl-β-glucosaminidase (jack beans). The K_i values obtained are summarized in Table 2. Lineweaver–Burk plots were drawn, thus showing the competitive character of the inhibitions described in Table 2.

Glycoamidines Selectively Inhibit Mannosidases in the Low Nanomolar Range. Mannoamidines **9a**–**e** (entries 6–9) display a much better inhibitory activity toward α- and β-mannosidases than the corresponding glucoamidines **7a**–**7e** (entries 1–5) toward α- and β-glucosidases. This result is in good agreement with earlier studies.[14] Indeed, the glycoamidine core structure, even in the gluco series with an inverted configuration at C2, reaches a low nanomolar inhibition level with mannosidases, whereas all the other glycosidases were, at best, inhibited in the micromolar range.[27]

Table 2. Inhibitory Potencies of Synthesized Glycoamidines (K_i Values in μM)[27]

		glycosidases[a]							
entry	inhibitor	α-Man	β-Man	α-Glc	β-Glc	α-Gal	β-Gal	α-Fuc	β-NAcGl
1	**7a**	2.3	3.3	13	3.6	184	31	295	374
2	**7b**	0.06	0.13	32	34	192	53	96	339
3	**7c**	0.09	0.41	14	71	44	44	91	252
4	**7d**	0.12	0.71	39	50	451	6	221	136
5	**7e**	1.7	0.87	31	20	28 000	1000	470	4500
6	**9a**	0.11	0.19	125	111	4700	2200	34	900
7	**9b**	**0.006**	**0.009**	81	6600	218	82	6	99
8	**9c**	0.019	0.15	2870	22	1400	119	8.5	85
9	**9d**	0.012	0.06	155	315	1800	156	10	112
10	**9e**	0.08	0.15	38	44	1200	235	79	1300
11	**12**	8.8	nd	1100	nd	2000	1400	75	1100
12	**13**	6.0	nd	1800	nd	7400	6300	930	750

[a] Commercially available glycosidases: α-Man = α-Mannosidase (jack beans), β-Man = β-Mannosidase (snail acetone powder), α-Glc = α-Glucosidase (yeast type III), β-Glc = β-Glucosidase (almond), α-Gal = α-Galactosidase (green coffee beans), β-Gal = β-Galactosidase (*Aspergillus orizae*), α-Fuc = α-Fucosidase (bovine kidney), β-*N*-AcGl = *N*-Acetyl-β-glucosaminidase (jack beans), nd = not determined.

α-/β-Glycosidase Selectivity. Glucoamidines **7a**–**7e** (entries 1–5) and mannoamidines **9a**–**d** (entries 6–9) bearing alkylamino groups were almost always better competitive inhibitors of α-glycosidases than β-glycosidases. Nevertheless, this selectivity is moderate: depending on the length of the alkyl chain, K_i values for β-mannosidases are 1.5 to 7 times higher than values for α-mannosidases. This difference is not significant enough to draw any conclusion regarding a difference in the mechanistic pathways between α- and β-mannosidases. For instance, isofagomine derivatives,[7d,28] *N*-iminosugars,[29] as well as mannosyl derivatives locked in a $^{1,4}B$ conformation[9] display a greater α/β-glycosidase selectivity leading information in the difference of conformations or charge localization at the transition state of α- or β-glycosidases. The mannoamidines **9a**–**d** inhibiting α- and β-mannosidases in the nanomolar range, we can reasonably conclude that they should mimic a "*late*" high-energy intermediate common to α- and β-mannoside hydrolysis.

Effects of the Additional Amino Group and Spacer Length. The glycoamidines **7b**–**7d** and **9b**–**d** (entries 2–4, 6–8) were designed to engender two tight ionic interactions between the two catalytic carboxylates of the enzyme (the general protonator and the base/nucleophile), and both ami-

(27) K_i values were measured at pH 6.8 (pH 6 for the fucosidase). The pH dependency of glycoamidine inhibition against α-mannosidase and β-glucosidase has been previously reported. Ganem et al. have showed that, between pH 4.5 and 7, the inhibition of β-glucosidase is not pH dependent; see ref 14a–c. Blériot et al. have reported that the inhibition of α-mannosidase by *N*-benzylmannoamidine slightly increased between pH 5 (optimum mannosidase activity) and pH 6.8; see ref 15b. However the comparison of K_i values determined at different pH should be done with care. For instance, Davies et al. have recently reported that the pH dependency of the catalytic efficiency and the inhibition constant might not correspond: Varrot; A.; Tarling, C. A.; Macdonald, J. M.; Stick, R. V.; Zechel, D. L.; Withers, S. G.; Davies, G. J. *J. Am. Chem. Soc.* **2003**, *125*, 7496–7497.

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dinium and tethered amino group. The aminomannoamidines **9b**−**d** (entries 7−9) were potent inhibitors of α- and β-mannosidases in the low nanomolar range. The shortest length amine **9b** (entry 7), bearing an ethylene linker between the amidine and amine functions, was very potent toward α- and β-mannosidases (K_i = 6 nM and 9 nM, respectively), but **9b** was a moderate inhibitor against the other enzymes tested. These results make **9b** the most potent and selective inhibitor reported to date. Both propylamine mannoamidine **9c** (entry 8) and the butylamine mannoamidine **9d** (entry 9) also showed good potency and selectivity toward mannosidases with K_i in the range of 10−20 nM. For α-mannosidase, the K_i values of propyl derivative **9a** (entry 6) and alcohol **9e** (entry 10) are very similar compared to the corresponding propylamino derivative **9c** (entry 8): the presence of the amino group is very significant and has to be explained by an ionic interaction, otherwise the alcohol **9e** would be a more potent inhibitor than **9c**. As it was expected, the results showed that the amidine **9b** bearing the shortest spacer displayed the best inhibitory activity (entry 7). The effect of the tether length is particularly significant for the inhibition of the β-mannosidase. Nevertheless, the fact that butylaminoamidine **9d** (entry 9) was more potent than propylaminoamidine **9c** (entry 8) is still unclear. Although our results support a new beneficial interaction with one of the two catalytic carboxylates, we cannot exclude the possibility that the amino functionality interact with another properly placed residue. Only a cocrystallization of one of these inhibitors with the mannosidases would unambiguously answer this question at the molecular level. Derivatives of aminomethylpyrrolidine diol were previously reported as dicationic mimics of a transition or intermediate structure of an α-mannosidase-catalyzed hydrolysis and displayed a competitive and good selectivity toward α-mannosidase from jack bean (K_i = 7.4 μM).[30]

Effect of Glycobicyclic Amidine 12 and 13. Bicyclic glucoamidine **12** (entry 11) and mannoamidine **13** (entry 12) showed good inhibition properties for α-mannosidase (K_i = 9 μM and 6 μM respectively), but the inhibitions were much weaker for the other enzymes. The introduction of a fused six-membered ring on the glycoamidine core structure did not improved the inhibition level.

Effect of the Inversion of the Stereochemistry at the 2-Position. Inversion of the stereochemistry on C2 from manno to gluco configuration gives rise to an expected loss of inhibitory activity on the mannosidase inhibitions with a factor of 5 to 15 (Table 2: glucoamidines **7a**−**d** (entries 1−4) vs mannoamidines **9a**−**d** (entries 6−10)). The same effect was also observed for glucosidases: glucoamidines **7a**−**d** were 3 to 20 times more potent on glucosidases than the corresponding mannoamidines **9a**−**d**. The intriguing point was that glucoamidines only inhibit α- and β-glucosidases in the low micromolar range (4−70 μM), suggesting a major difference in the relative mechanism of glucosidases and mannosidases which might be explained by stereoelectronic and conformational factors.

Conformational Analysis. Due to the double bond between the anomeric carbon and the endocyclic oxygen, the oxycarbenium species (**a**, **b**; Figure 3), likely a high-energy intermediate of the enzymatic hydrolysis, is most often represented in its

Figure 3. Conformational analysis.

$^4H_3$ half-chair conformation (**b**, Figure 3). However, it does not imply that a molecule adopting this conformation will give rise to the tightest or the most selective binding to a given glycosidase. For instance, Vasella et al.[9a] have shown that polyhydroxylated isoquinuclidines locked in a $^{1,4}B$ boat conformation display strong and selective binding to β-mannosidase (**c**, Figure 3, K_i = 0.17 μM, IC_{50} = 0.68 μM for snail β-mannosidase, IC_{50} = 9.6 mM for jack bean α-mannosidase).

Since mannoamidines **9a**−**d** do not display significant selective inhibition between α- and β-mannosidases, they probably do not bind these glycosidases in a $^{1,4}B$ boat conformation. Indeed a stabilized $^4H_3$ transition state was reported for inverting α-mannosidase,[31] and other glycosides have been shown to adopt a $B_{2,5}$ boat conformation as mannonolactam in solution or solid state[32] (Figure 3, **d**) and mannonolactone.[33] Moreover, crystallographic studies of β-mannanase 26A from *P. cellulosa* bound to a fluorinated trisaccharide inactivator showed a distortion of the carbohydrate suggesting a $B_{2,5}$ boat conformation for the transition state, and the family 11-xylanases displayed a covalent xylobiosyl-enzyme intermediate in the same $B_{2,5}$ conformation.[34]

Since the double bond of the amidine functionality was proved to be exocyclic,[20] mannoamidines, **9a**−**d** should possess close structural features to mannonolactam **d**. Therefore, amidines **9a**−**d** might also bind mannosidases in a $B_{2,5}$ boat conformation (**f**, Figure 3) preferentially to a $^4H_3$ conformation (**e**, Figure 3). As reported for mannonolactam **d**, a significant NOE effect between H2 and H5 was observed for amidine **9a** in D_2O, showing that its conformation in aqueous solution is

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Table 3. Inhibitory Activities of Newly Synthesized and Known Mannosidase inhibitors toward Jack Bean α-Mannosidase[a]

Inhibitor	K_i (μM)	IC_{50} (μM)	Ref
1 (9b)	**0.006**	**0.011**	
2	9		14a
3	0.17		14c
4		0.07	34
5	0.07	0.08-0.1	35
6		0.06	36

[a] **1**, Amidine **9b**; **2**, Ganem's amidine; **3**, D-Mannoamidrazone; **4**, Mannostatin A; **5**, (−)-Swainsonine; **6**, Methylaminopentacyclitol.

closer to a $B_{2,5}$ boat (**f**, Figure 3) than to a $^4H_3$ half-chair (**e**, Figure 3). This conformational property should be responsible for the inhibitory potency and the selectivity of both manno- and glucoamidines against mannosidases and should also explain why the glucoamidines do not bind glucosidases in a low nanomolar range. In agreement with Davies,[34] we can assume that all glycosidases do not react through a $^4H_3$ half-chair transition state.

Inhibitory Activities of Mannoamidine 9b Compared to Known Inhibitors. Comparison of the inhibitory potency of our best inhibitor **9b** with known inhibitors of mannosidases is reported in Table 3.[35] Amidine **9b** inhibited jack bean α-mannosidase in the nanomolar range (entry 1). Ganem's amidine[14a] (entry 2) and amidrazone[14c] (entry 3) were proved to be good inhibitors of α-mannosidase with a K_i in the low micromolar range. Mannostatin A[36] (entry 4), swainsonine[37] (entry 5), and methylaminopentacyclitol[38] (entry 6) were the most potent inhibitors of α-mannosidase reported thus far with K_i values comprised between 50 and 200 nM. To our knowledge, these results prove that mannoamidine **9b** is the first inhibitor displaying a K_i value below 10 nM against jack bean α-mannosidase.

Conclusion

In conclusion, we have designed and synthesized a series of biscationic glycoamidines in the gluco and manno series which displayed excellent inhibition properties against α- and β-mannosidases. Moreover, we have demonstrated the enhancement of binding interactions triggered by an additional amino functionality anchored to the amidine unit through a short spacer. A favorable $B_{2,5}$ boat conformation might explain the selectivity of the mannosidase inhibition compared to other glycosidases. To further improve the selectivity and the binding strength of these amidines, it can be envisaged attaching either an hydrophobic moiety or a second carbohydrate part to the terminal amino group.

Experimental Section

General Methods. All reagents were commercial grade and were used as received without further purification. All reactions were performed under inert atmosphere, and anhydrous solvents were dried and distilled over appropriate desiccant prior to use. Thin-layer chromatogry (TLC) and flash chromatography separations were, respectively, performed on precoated silica gel 60 F254 plates (Merck, 0.25 mm) and on Merck silica gel 60 (230−400 mesh). ^{1}H NMR spectra were recorded at 300 MHz, and ^{13}C NMR spectra were obtained at 75 MHz in the specified solvent. Mass spectra were recorded at 70 eV on a Finnigan-Mat 4600 using chemical ionization mode (CI−NH_3 or CI−CH_4). ESITOF (electrospray) mass spectra were mesured on a Mariner Perspective Biosystem apparatus. High-resolution mass spectra HRMS/LSIMS$^+$ were performed by the Centre Regional de Mesures Physiques de l'Ouest on a Zabspec TOF Micromass (matrix: 3-nitrobenzyl alcohol). IR spectra were recorded on NaCl plates as thin film on an FTIR instrument. Optical rotations were recorded on a Perkin-Elmer 241 MC polarimeter. Elemental analyses were measured at the Service de Microanalyze de Gif sur Yvette (ICSN).

2,3,4,6-Tetra-*O*-benzyl-D-gluconoiminothioether 3. To a solution of gluconothiolactam **2** (300 mg, 0.54 mmol) in dry CH_2Cl_2 (13 mL) at 0 °C was added triethyloxonium tetrafluoroborate (Meerwein's salt) (112 mg, 0.59 mmol). The mixture was stirred at 0 °C for 1 h 30 min and was used directly in the next step. The crude was concentratred under vacuum to give **3** (298 mg, 95% yield): ^{1}H NMR ($CDCl_3$) δ 7.40−7.21 (m, 20H), 4.80−4.31 (m, 10H), 4.08 (dd, J = 4.4, 6.9 Hz, 1H), 3.99 (dd, J = 2.7, 10.6 Hz, 1H), 3.87 (dd, J = 6.9, 9.0 Hz, 1H), 3.50 (dd, J = 2.2, 10.7 Hz, 1H), 3.18 (q, J = 7.5 Hz, 2H), 1.42 (t, J = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 192.7, 136.9, 136.6, 135.5, 128.9−128.0, 81.4, 78.6, 76.3, 74.9, 73.6, 73.4, 66.7, 63.6, 25.7, 11.8; IR (neat, cm^{-1}) 3565, 2930, 2874, 1674, 1604, 1455, 1072, 744, 700; MS (DCI/NH_3) m/z (M + H)$^+$ = 582.

General Procedure for the Preparation of Amidines 4a, 5a, 4b, 5b, 4c, 5c, 4d, 5d, 4e, 5e. To a stirred solution of freshly prepared glucoiminothioether **3** (0.17 mmol, 1 equiv) in 4 mL of dry CH_2Cl_2 at 0 °C was added a solution of amine (0.3 mmol, 1.8 equiv) in 2 mL of dry CH_2Cl_2. The reaction mixture was allowed to warm to room temperature and was stirred for 8 h. The solution was concentrated under vacuum and purified by flash column chromatography on silica gel eluting with CH_2Cl_2/MeOH (98:2) to give glucoamidine (less polar isomer) and mannoamidine (more polar isomer).

***N,N*-Propyl-2,3,4,6-tetra-*O*-benzyl-D-glucoamidine 4a and *N,N*-Propyl-2,3,4,6-tetra-*O*-benzyl-D-mannoamidine 5a.** Glucoiminothioether **3** (65 mg, 0.11 mmol) was reacted with propylamine (16 μL, 0.2 mmol) to give glucoamidine **4a** (19 mg, 29%) and mannoamidine **5a** (44 mg, 68%) following the general procedure. **4a**: ^{1}H NMR ($CDCl_3$) δ 7.40−7.16 (m, 20H), 4.93 (d, J = 11.6 Hz, 1H), 4.79 (s, 2H), 4.63−4.36 (m, 7H), 4.01−3.96 (m, 2H), 3.88−3.79 (m, 2H), 3.51 (dd, J = 2.8, 10.3 Hz, 1H), 3.22 (t, J = 6.9 Hz, 2H), 1.50 (q, J = 7.2 Hz, 2H), 0.87 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 162.4, 137.4, 137.2, 136.9, 136.0, 129.1−128.1, 81.4, 77.8, 75.9, 74.8, 74.4, 73.5, 73.0, 80.0, 57.6, 43.7, 21.1, 10.8; IR (neat, cm^{-1}) 3301, 2877, 1680, 1455, 1094, 743, 699; MS (ESITOF) m/z (M)$^+$ = 578; $[\alpha]^{25}_D$ = −20.6 (c = 1.95, CH_2Cl_2). **5a**: ^{1}H NMR ($CDCl_3$) δ 7.37−7.08 (m, 20H), 4.59−4.25 (m, 10H), 3.91 (t, J = 2.8 Hz, 1H), 3.78−3.61 (m, 4H), 3.38−3.36 (m, 2H), 1.62 (q, J = 7.2 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 162.1, 137.7, 136.8, 135.6, 128.9−127.8, 73.5, 73.2, 73.1, 72.5, 72.4, 71.9, 71.6, 68.2, 55.6, 43.6, 21.1, 10.8; IR (neat, cm^{-1})

(35) Except for amidines, inhibitors showing K_i < 0.2 μM were only reported in Table 2.

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3307, 2942, 2362, 1681, 1574, 1455, 1361, 1092, 819; MS (DCI/NH_3) *m/z* $(M)^+ = 579$; $[\alpha]^{24}_D = +6.1$ ($c = 2.77$, CH_2Cl_2).

***N,N*-Propyl-D-glucoamidine 7a.** To a stirred solution of amidine **4a** (17 mg, 0.03 mmol) in EtOH (2.5 mL) was added 30% Pd on activated carbon. The mixture was stirred under hydrogen (1 atm) for 12 h and was filtered through a pad of Celite and washed with EtOH. The solvent was concentrated under vacuum to afford the amidine **7a** (5.9 mg, 93% yield): ^{1}H NMR (CD_3OD) δ 4.17 (d, $J = 9.6$ Hz, 1H), 3.82−3.75 (m, 4H), 3.62−3.55 (m, 3H), 3.33−3.30 (m, 1H), 1.68 (q, $J = 7.2$ Hz, 2H), 0.98 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (CD_3OD) δ 165.9, 74.2, 69.9, 62.4, 61.5, 60.9, 44.5, 22.2, 11.3; HRMS calcd for $C_9H_{19}N_2O_4$ $(M + H)^+$ 219.1345, found 219.1337; $[\alpha]^{28}_D = +1.2$ ($c = 1.69$, CH_3OH).

***N,N*-Propyl-D-mannoamidine 9a.** Amidine **5a** (35 mg, 0.05 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give deprotected amidine **9a** (10.5 mg, 81% yield): ^{1}H NMR (CD_3OD) δ 4.62 (d, $J = 3.1$ Hz, 1H), 3.98 (t, $J = 3.1$ Hz, 1H), 3.90 (dd, $J = 3.5$, 4.0 Hz, 1H), 3.84 (dd, $J = 4.9$, 11.3 Hz, 1H), 3.74 (dd, $J = 5.7$, 11.4 Hz, 1H), 3.38 (q, $J = 4.9$ Hz, 1H), 3.36−3.25 (m, 2H), 1.63 (q, $J = 7.3$ Hz, 2H), 0.94 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (CD_3OD) δ 166.0, 73.3, 69.6, 67.2, 62.4, 60.9, 44.3, 22.2, 11.2; IR (neat cm^{-1}) 3326, 2970, 2466, 1681, 1455, 1316, 1035; HRMS calcd for $C_9H_{19}N_2O_4$ $(M + H)^+$ 219.1345, found 219.1347; $[\alpha]^{27}_D = -9.7$ ($c = 1.32$, CH_3OH).

***N,N*-[3-(*tert*-Butyldimethylsiloxy)propyl]-2,3,4,6-tetra-*O*-benzyl-D-glucoamidine 4e and *N,N*-[3-(*tert*-Butyldimethylsiloxy)propyl]-2,3,4,6-tetra-*O*-benzyl-D-mannoamidine 5e.** Glucoiminothioether **3** (200 mg, 0.34 mmol) was reacted with 3-(*tert*-butyldimethylsiloxy)-1-propylamine[25] (117 mg, 0.6 mmol) to give glucoamidine **4e** (39 mg, 16%) and mannoamidine **5e** (163 mg, 67%) following the general procedure. **4e**: ^{1}H NMR ($CDCl_3$) δ 7.39−7.09 (m, 20H), 4.90 (d, $J = 11.8$ Hz, 1H), 4.77 (s, 2H), 4.62−4.28 (m, 6H), 3.94−3.85 (m, 3H), 3.79−3.67 (m, 4H), 3.58−3.49 (m, 1H), 3.40−3.36 (m, 2H), 1.74−1.69 (m, 2H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 162.2, 137.8, 136.8, 135.4, 129.1−127.4, 73.4, 73.1, 73.0, 72.4, 72.2, 72.1, 71.7, 68.3, 62.3, 55.7, 41.4, 29.8, 26.2, 18.7, 0.3; IR (neat, cm^{-1}) 3296, 2929, 1682, 1455, 1091, 741, 699; MS (DCI/NH_3) *m/z* $(M + H)^+ = 709$. **5e**: ^{1}H NMR ($CDCl_3$) δ 8.03−7.95 (br s, 1H), 7.39−7.07 (m, 20H), 4.61−4.23 (m, 9H), 3.87 (d, $J = 2.8$ Hz, 1H), 3.81−3.56 (m, 8H), 1.92−1.86 (m, 2H), 0.9 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 162.2, 137.8, 136.9, 135.4, 129.1, 127.4, 73.4, 73.1, 73.0, 72.2, 72.1, 71.8, 68.3, 62.3, 55.7, 41.4, 29.8, 26.2, 18.7, −4.9; IR (neat cm^{-1}) 3297, 2928, 1681, 1454, 1256, 1093, 837, 736; MS (DCI/NH_3) *m/z* $(M + H)^+$ 709. Anal. Calcd for $C_{43}H_{56}N_2O_5Si$: C, 72.88; H, 7.99. Found: C, 72.85; H, 7.89.

***N,N*-(3-Hydroxypropyl)-2,3,4,6-tetra-*O*-benzyl-D-glucoamidine 6e.** To a stirred solution of glucoamidine **4e** (30 mg, 0.04 mmol) in EtOH (3 mL) was added pyridinium *p*-toluenesulfonate (6 mg, 0.02 mmol). The reaction was heated at 60 °C for 6 h. The solvent was concentrated, and the crude product was purified by column chromatography CH_2Cl_2/MeOH (95:5) to yield compound **6e** (15.2 mg, 61% yield): ^{1}H NMR ($CDCl_3$) δ 7.60−7.56 (br s, 1H), 7.42−7.14 (m, 20H), 4.90 (d, $J = 11.3$ Hz, 1H), 4.89 (s, 1H), 4.65−4.32 (m, 7H), 3.98−3.90 (m, 2H), 3.75 (dd, $J = 2.4$, 9.8 Hz, 1H), 3.64 (t, $J = 5.0$ Hz, 2H), 3.51 (dd, $J = 2.8$, 10.2 Hz, 1H), 3.48−3.44 (m, 1H), 3.18−3.05 (br s, 1H), 1.72−1.55 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 162.4, 137.5, 137.1, 136.5, 129.0−128.0, 81.9, 78.2, 75.6, 75.1, 74.3, 74.2, 73.6, 70.4, 62.4, 55.2, 40.1, 26.4; IR (neat cm^{-1}) 3252, 2925, 1681, 1455, 1075, 738, 698; MS (DCI/NH_3) *m/z* $(M + H)^+$ 595.

***N,N*-(3-Hydroxypropyl)-2,3,4,6-tetra-*O*-benzyl-D-mannoamidine 8e.** In a manner similar to the preparation of hydroxyamidine **6e**, amidine **5e** (144 mg, 0.2 mmol) was converted to **8e** (97 mg, 80% yield). ^{1}H NMR ($CDCl_3$) δ 8.11−7.92 (br s, 1H), 7.36−7.10 (m, 20H), 4.72 (d, $J = 11.8$ Hz, 1H), 4.62−4.30 (m, 9H), 3.78 (dd, $J = 2.6$, 3.1 Hz, 1H), 3.74−3.54 (m, 8H), 1.85−1.79 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 162.6, 137.7, 137.0, 136.9, 135.9, 128.9−128.0, 73.7, 73.5, 73.3, 73.2, 72.9, 72.6, 71.7, 68.7, 60.1, 55.6, 40.4, 29.7; IR (neat cm^{-1}): 3364, 2366, 1681, 1455, 1076, 711; MS (DCI/NH_3) *m/z* $(M + H)^+ = 595$; $[\alpha]^{27}_D = +7.9$ ($c = 1.15$, CH_2Cl_2).

***N,N*-(3-Hydroxypropyl)-D-glucoamidine 7e.** Amidine **6e** (11 mg, 0.02 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give deprotected glucoamidine **7e** (4 mg, 92% yield): ^{1}H NMR (CD_3OD) δ 4.19 (d, $J = 9.7$ Hz, 1H), 3.79−3.71 (m, 3H), 3.65 (t, $J = 5.8$ Hz, 2H); 3.57−3.31 (m, 4H), 1.78−1.68 (m, 2H); ^{13}C NMR (CD_3OD) δ; 166.3, 73.5, 69.2, 67.1, 62.1, 60.8, 59.9, 40.4, 31.2, 18.2; IR (neat, cm^{-1}) 3331, 2466, 1667, 1064, 521; HRMS calcd $C_9H_{19}N_2O_5$ $(M + H)^+$ 235.1294, found 235.1290. $[\alpha]^{28}_D = +1.3$ ($c = 1.08$, CH_3OH).

***N,N*-(3-Hydroxypropyl)-D-mannoamidine 9e.** Amidine **8e** (58 mg, 0.097 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give deprotected mannoamidine **9e** (19 mg, 86% yield): ^{1}H NMR (CD_3OD) δ 4.65 (d, $J = 3.0$ Hz, 1H), 4.01 (t, $J = 3.1$ Hz, 1H), 3.89 (dd, $J = 3.2$, 4.0 Hz, 1H), 3.83 (dd, $J = 4.8$, 11.4 Hz, 1H), 3.75 (dd, $J = 5.3$, 11.4 Hz, 1H), 3.65 (t, $J = 5.8$ Hz, 2H), 3.55−3.41 (m, 3H), 1.34−1.26 (m, 2H); ^{13}C NMR (CD_3OD) δ 166.5, 73.5, 70.1, 67.2, 62.7, 61.0, 59.7, 40.1, 31.4; IR (neat, cm^{-1}) 3364, 2366, 1681, 1455, 1076, 711; HRMS cald for $C_9H_{19}N_2O_5$ $(M + H)^+$ 235.1294, found 235.1284; $[\alpha]^{27}_D = -5.8$ ($c = 1.29$, CH_3OH).

***N,N*-[2-(*tert*-Butoxycarbonylamino)ethyl]-2,3,4,6-tetra-*O*-benzyl-D-glucoamidine 4b and *N,N*-[2-(*tert*-Butoxycarbonylamino)ethyl]-2,3,4,6-tetra-*O*-benzyl-D-mannoamidine 5b.** Glucoiminothioether **3** (100 mg, 0.17 mmol) was reacted with 2-(*tert*-butoxycarbonylamino)-1-ethylamine (52 mg, 0.31 mmol) to give glucoamidine **4b** (28 mg, 23%) and mannoamidine **5b** (83 mg, 70%) following the general procedure. **4b**: ^{1}H NMR ($CDCl_3$) δ 8.71−8.60 (br s, 1H), 7.40−7.19 (m, 20H), 5.35−5.32 (br s, 1H), 4.81 (q, $J = 11.2$ Hz, 2H), 4.73 (s, 2H), 4.57−4.47 (m, 4H), 4.36 (d, $J = 11.8$ Hz, 1H), 3.99−3.68 (m, 3H), 3.76 (dd, $J = 2.9$, 10.4 Hz, 1H), 3.53 (dd, $J = 2.6$, 10.2 Hz, 1H), 3.51−3.48 (m, 2H), 3.38−3.34 (m, 2H), 1.40 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 163.2, 158.2, 137.4, 137.2, 137.0, 135.9, 128.7, 127.8, 81.1, 80.5, 77.4, 74.2, 74.1, 73.3, 73.1, 68.9, 57.4, 43.9, 38.3, 28.3; IR (neat, cm^{-1}) 2929, 1685, 1515, 1081, 738; MS (ESITOF) *m/z* $(M)^+$ 679, $(M + H)^+$ 680; $[\alpha]^{27}_D = -4.7$ ($c = 1.95$, CH_2Cl_2). **5b**: ^{1}H NMR ($CDCl_3$) δ 7.36−7.06 (m, 20H), 5.49−5.46 (br s, 1H), 4.78 (d, $J = 11.8$ Hz, 1H), 4.59−4.49 (m, 4H), 4.41−4.23 (m, 4H), 3.90 (dd, $J = 2.6$, 2.7 Hz, 1H), 3.78−3.72 (m, 3H), 3.68−3.41 (m, 6H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.9, 158.5, 137.8, 137.0, 136.9, 135.9, 129.0−127.9, 80.5, 73.6, 73.4, 73.2, 72.6, 72.3, 71.6, 68.5, 55.9, 44.0, 38.5, 28.4; IR (neat, cm^{-1}) 3292, 2929, 1685, 1515, 1456, 1081, 739; MS (ESITOF) *m/z* $(M)^+$ 679; $[\alpha]^{27}_D = -8.7$ ($c = 1.82$, CH_2Cl_2).

***N,N*-(2-Aminoethyl)-2,3,4,6-tetra-*O*-benzyl-D-glucoamidine 6b.** To a solution of glucoamidine **4b** (22 mg, 0.03 mmol) in CH_2Cl_2 (1.5 mL) was added trifluoroacetic acid (50 μL, 1.57 mmol) at 0 °C, and the reaction was stirred 6 h at room temperature. The mixture was concentrated to yield **6b** (16 mg, 89% yield) which was pure enough to be used in the next step without further purification. ^{1}H NMR (CD_3OD) δ 7.33−7.21 (m, 20H), 4.90−4.67 (m, 2H), 4.64−4.36 (m, 7H), 4.04−3.97 (m, 2H), 3.94−3.91 (m, 1H), 3.71−3.57 (m, 4H), 3.18 (t, $J = 6.3$ Hz, 2H); ^{13}C NMR (CD_3OD) δ 165.7, 139.0, 138.8, 137.9, 129.7, 129.2, 81.9, 77.5, 75.9, 74.6, 74.4, 73.7, 69.4, 58.5, 40.9, 38.4; MS (ESITOF) *m/z* $(M + Na)^+$ 602; $[\alpha]^{27}_D = +5.9$ ($c = 1.19$, CH_3OH).

***N,N*-(2-Aminoethyl)-2,3,4,6-tetra-*O*-benzyl-D-mannoamidine 8b.** From **5b** (82 mg, 0.12 mmol), compound **8b** was obtained (63 mg, 92% yield) following the preparation reported for **6b** from **4b**. ^{1}H NMR (CD_3OD) δ 7.39−7.12 (m, 20H), 4.82 (s, 1H), 4.68−4.64 (m, 2H), 4.52−4.24 (m, 6H), 3.87−3.85 (m, 2H), 3.69 (t, $J = 9.9$ Hz, 2H), 3.62−3.59 (m, 3H), 3.20−3.17 (m, 2H); ^{13}C NMR (CD_3OD) δ 165.7, 138.9, 137.7, 130.1−128.9, 75.5, 74.6, 74.5, 74.1, 73.9, 73.7, 72.6, 68.8, 57.2, 40.4, 38.5; MS (ESITOF) *m/z* $(M + Na)^+$ 602; $[\alpha]^{27}_D = -2.5$ ($c = 1.81$, CH_3OH).

***N,N*-(2-Aminoethyl)-D-glucoamidine 7b.** Glucoamidine **6b** (15 mg, 0.025 mmol) was hydrogenated in a manner similar to the preparation

of **7a** to give glucoamidine **7b** (5.2 mg, 95% yield): ^{1}H NMR (CD_3OD) δ 4.22 (d, J = 9.7 Hz, 1H), 3.91−3.61 (m, 6H), 3.50−3.47 (m, 1H), 3.29−3.25 (m, 2H); ^{13}C NMR (CD_3OD) δ 167.4, 74.0, 70.3, 69.1, 62.6, 61.0, 40.4, 38.5; HRMS calcd for $C_8H_{18}N_3O_4$ $(M + H)^+$ 220.1297, found 220.1294; $[\alpha]^{27}_D = +3.9$ (c = 1.11, CH_3OH).

***N*,*N*-(2-Aminoethyl)-D-mannoamidine 9b.** Mannoamidine **8b** (80 mg, 0.13 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give deprotected mannoamidine **9b** (27 mg, 89% yield): ^{1}H NMR (CD_3OD) δ 4.71 (d, J = 3.2 Hz, 1H), 4.05 (t, J = 3.0 Hz, 1H), 3.94−3.91 (m, 2H), 3.89−3.74 (m, 3H), 3.46−3.42 (m, 1H), 3.26 (t, J = 6.5 Hz, 2H); ^{13}C NMR (CD_3OD) δ 167.7, 73.8, 69.8, 67.5, 62.0, 61.2, 40.3, 38.7; HRMS calcd for $C_8H_{18}N_3O_4$ $(M + H)^+$ 220.1297, found 220.1298; $[\alpha]^{27}_D = -4.7$ (c = 1.22, CH_3OH).

***N*,*N*-[3-(*tert*-Butoxycarbonylamino)propyl]-2,3,4,6-tetra-*O*-benzyl-D-glucoamidine 4c and *N*,*N*-[3-(*tert*-Butoxycarbonylamino)-propyl]-2,3,4,6-tetra-*O*-benzyl-D-mannoamidine 5c.** Glucoiminothioether **3** (200 mg, 0.34 mmol) was reacted with 3-(*tert*-butoxycarbonyl-amino)-1-propylamine (108 mg, 0.61 mmol) to give glucoamidine **4c** (62 mg, 26%) and mannoamidine **5c** (157 mg, 66%) following the general procedure. **4c**: ^{1}H NMR ($CDCl_3$) δ 7.36−7.23 (m, 20H), 5.08−4.95 (br s, 1H), 4.88 (d, J = 11.2 Hz, 1H), 4.77 (s, 2H), 4.76−4.49 (m, 5H), 4.35 (d, J = 11.2 Hz, 1H), 4.01−3.89 (m, 2H), 3.87 (dd, J = 6.1, 9.5 Hz, 1H), 3.83 (dd, J = 2.7, 10.1 Hz, 1H), 3.52 (dd, J = 2.8, 9.9 Hz, 1H), 3.42−3.38 (br s, 2H), 3.11−3.07 (m, 2H), 1.74−1.70 (m, 2H), 1.63−1.60 (br s, 1H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.7, 157.8, 137.6, 137.4, 137.2, 136.2, 130.0−127.8, 81.6, 79.9, 75.5, 74.5, 74.3, 73.5, 72.6, 69.3, 57.5, 39.2, 36.9, 29.1, 28.5; IR (neat, cm^{-1}) 3297, 2933, 1682, 1519, 1455, 1074, 738, 699; MS (DCI/NH_3) m/z $(M + H)^+$ 694; $[\alpha]^{26}_D = -14.6$ (c = 1.44, CH_2Cl_2). **5c**: ^{1}H NMR ($CDCl_3$) δ 8.62−8.55 (br s, 1H), 7.43−7.12 (m, 20H), 5.34 (t, J = 3.5 Hz, 1H), 5.67 (d, J = 11.8 Hz, 1H), 4.77−4.30 (m, 8H), 3.98 (s, 1H), 3.85−3.70 (m, 4H), 3.61−3.43 (m, 2H), 3.18−3.07 (m, 2H), 1.61 (br s, 2H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.5, 157.8, 137.9, 137.1, 136.0, 128.9−127.9, 79.8, 73.6, 73.3, 73.4, 72.6, 71.8 68.3, 55.8, 39.2, 37.0, 29.0, 28.5; IR (neat, cm^{-1}) 3295, 2977, 1695, 1517, 1453, 1367, 1014, 738, 700; MS (DCI/NH_3) m/z $(M + H)^+$ 694; $[\alpha]^{26}_D = +1.1$ (c = 2.55, CH_2Cl_2).

***N*,*N*-(3-Aminopropyl)-2,3,4,6-tetra-*O*-benzyl-D-glucoamidine 6c.** From **4c** (24 mg, 0.03 mmol), compound **6c** was obtained (18 mg, 86% yield) following the same procedure reported for **6b** from **4b**. ^{1}H NMR (CD_3OD) δ 7.37−7.21 (m, 20H), 4.85−4.34 (m, 10H), 4.01−3.94 (m, 2H), 3.91−3.88 (m, 1H), 3.66 (dd, J = 3.6, 10.3 Hz, 1H), 3.58 (dd, J = 3.8, 10.2 Hz, 1H), 3.39 (t, J = 6.7 Hz, 2H), 2.94 (t, J = 7.6 Hz, 2H), 1.96−1.91 (m, 2H); ^{13}C NMR (CD_3OD) δ 164.8, 138.9, 138.7, 137.8, 130.1−129.1, 81.5, 77.6, 75.5, 75.4, 74.2, 73.4, 73.7, 69.3, 57.9, 40.4, 38.0, 26.7; IR (NaCl, cm^{-1}) 3261, 3032, 1678, 1455, 1074, 744, 699; MS (DCI/NH_3) m/z $(M + H)^+$ 594; $[\alpha]^{23}_D = +0.9$ (c = 1.38, MeOH).

***N*,*N*-(3-Aminopropyl)-2,3,4,6-tetra-*O*-benzyl-D-mannoamidine 8c.** From **5c** (146 mg, 0.21 mmol), mannoamidine **8c** was obtained (115 mg, 92% yield) following the preparation reported for **6b** from **4b**. ^{1}H NMR (CD_3OD) δ 7.38−7.09 (m, 20H), 4.81 (d, J = 11.9 Hz, 1H), 4.64 (d, J = 12.0 Hz, 2H), 4.50−4.21 (m, 6H), 3.89−3.81 (m, 2H), 3.66−3.64 (m, 1H), 3.61−3.54 (m, 2H), 3.51−3.46 (m, 2H), 2.93 (t, J = 7.1 Hz, 2H), 1.99−1.89 (m, 2H); ^{13}C NMR (CD_3OD) δ 164.9, 138.9, 138.7, 138.5, 137.8, 130.1−128, 96, 75.6, 74.4, 74.1, 73.7, 72.6, 68.8, 57.1, 39.9, 37.9, 26.8; IR (neat, cm^{-1}) 3298, 2875, 2101, 1681, 1455, 1092; MS (DCI/NH_3) m/z $(M + H)^+$ 594.

***N*,*N*-(3-Aminopropyl)-D-glucoamidine 7c.** Glucoamidine **6c** (15 mg, 0.025 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give glucoamidine **7c** (5.3 mg, 89% yield): ^{1}H NMR (CD_3OD) δ 4.20 (d, J = 6.5 Hz, 1H), 3.88−3.77 (m, 3H), 3.66−3.55 (m, 2H), 3.49−3.45 (m, 2H), 2.98 (t, J = 10.2 Hz, 2H), 2.06−1.95 (m, 2H); ^{13}C NMR (CD_3OD) δ 166.6, 74.2, 70.1, 69.3, 62.5, 61.2, 39.7, 38.1, 26.8; IR (neat, cm^{-1}) 3330, 2528, 1681, 1434, 1061; HRMS calcd for $C_9H_{20}N_3O_4$ $(M + H)^+$ 234.1454, found 234.1455; $[\alpha]^{27}_D = +3.3$ (c = 1.57, MeOH).

***N*,*N*-(3-Aminopropyl)-D-mannoamidine 9c.** Mannoamidine **8c** (96 mg, 0.135 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give mannoamidine **9c** (28.2 mg, 88% yield). ^{1}H NMR (CD_3OD) δ 4.64 (d, J = 3.0 Hz, 1H), 3.99 (t, J = 2.9 Hz, 1H), 3.90−3.73 (m, 3H), 3.57−3.40 (m, 3H), 2.98 (t, J = 7.8 Hz, 2H), 2.01−1.92 (m, 2H); ^{13}C NMR (CD_3OD) δ 166.9, 73.7, 69.8, 67.3, 62.1, 61.0, 39.7, 38.0, 26.9; IR (neat, cm^{-1}) 2980, 1681, 1434, 1061; HRMS calcd for $C_9H_{19}N_3O_4$ $(M + H)^+$ 234.1454, found 234.1456. $[\alpha]^{26}_D = +0.7$ (c = 1.34, CH_3OH).

***N*,*N*-[4-(*tert*-Butoxycarbonylamino)butyl]-2,3,4,6-tetra-*O*-benzyl-D-glucoamidine 4d and *N*,*N*-[4-(*tert*-Butoxycarbonylamino)butyl]-2,3,4,6-tetra-*O*-benzyl-D-mannoamidine 5d.** Glucoiminothioether **3** (200 mg, 0.34 mmol) was reacted with 4-(*tert*-butoxycarbonylamino)-1-butylamine (116 mg, 0.62 mmol) to give glucoamidine **4d** (53 mg, 22%) and mannoamidine **5d** (160 mg, 66%) following the general procedure. **4d**: ^{1}H NMR ($CDCl_3$) δ 7.35−7.22 (m, 21H), 4.89 (d, J = 11.5 Hz, 1H), 4.82−4.36 (m, 9H), 3.96−3.78 (m, 4H), 3.53 (dd, J = 2.4, 10.6 Hz, 1H), 3.31 (t, J = 6.7 Hz, 2H), 3.07−3.05 (m, 2H), 1.57−1.46 (m, 4H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.7, 156.7, 137.5, 137.2, 137.1, 136.1, 129.1, 127.9, 81.1, 77.6, 75.6, 74.7, 74.1, 73.5, 73.1, 69.2, 57.2, 42.1, 39.5, 28.5, 27.2, 24.5; IR (neat, cm^{-1}) 2933, 1683, 1454, 1074; MS (ESITOF) m/z $(M + H)^+$ 707; $[\alpha]^{26}_D = -17.1$ (c = 1.3, CH_2Cl_2). **5d**: ^{1}H NMR ($CDCl_3$) δ 7.62−7.51 (br s, 1H), 7.38−7.09 (m, 20H), 4.87−4.85 (br s, 1H), 4.74 (d, J = 11.8 Hz, 1H), 4.64−4.27 (m, 8H), 3.91 (t, J = 2.9 Hz, 1H), 3.76−3.67 (m, 4H), 3.44 (q, J = 6.9 Hz, 2H), 3.09−3.05 (m, 2H), 1.69−1.64 (m, 2H), 1.56−1.49 (m, 2H), 1.43 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.35, 156.8, 137.8, 136.9, 135.9, 129.0−127.9, 79.5, 73.8, 73.5, 73.3, 72.6, 72.3, 71.8, 61.4, 55.9, 41.9, 39.4, 28.5, 27.2, 24.6; IR (neat, cm^{-1}) 3302, 2933, 1683, 1454, 1074, 743, 700; MS (ESITOF) m/z $(M)^+$ 707; $[\alpha]^{26}_D = -8.9$ (c = 1.94, CH_2Cl_2).

***N*,*N*-(4-Aminobutyl)-2,3,4,6-tetra-*O*-benzyl-D-glucoamidine 6d.** From **4d** (50 mg, 0.07 mmol) compound, **6d** was obtained (36 mg, 83% yield) following the same procedure reported for the synthesis of **6b** from **4b**. ^{1}H NMR (CD_3OD) δ 7.37−7.22 (m, 20H), 4.95−4.35 (m, 12H), 4.00−3.94 (m, 2H), 3.89−3.87 (m, 2H), 3.66 (dd, J = 3.6, 10.3 Hz, 1H), 3.57 (dd, J = 3.8, 10.2 Hz, 1H), 2.88−2.84 (m, 2H), 1.65−1.62 (m, 2H); ^{13}C NMR (CD_3OD) δ 164.4, 138.9, 138.7, 137.9, 129.7−129.1, 81.5, 77.6, 75.6, 75.4, 74.2, 74.1, 73.3, 69.3, 57.7, 42.7, 40.1, 25.6, 25.5; IR (neat, cm^{-1}) 3032, 1678, 1455, 1074. MS (DCI/NH_3) m/z $(M + H)^+$ 608; $[\alpha]^{26}_D = +1.9$ (c = 1.42, CH_3OH).

***N*,*N*-(4-Aminobutyl)-2,3,4,6-tetra-*O*-benzyl-D-mannoamidine 8d.** From **5d** (134 mg, 0.19 mmol) compound, **8d** was obtained (101 mg, 88% yield) following the preparation reported for **6b** from **4b**. ^{1}H NMR (CD_3OD) δ 7.39−7.12 (m, 20H), 4.82 (d, J = 12.0 Hz, 1H), 4.68 (s, 1H), 4.64−4.62 (m, 3H), 4.52−4.25 (m, 6H), 3.88−3.85 (m, 2H), 3.68−3.65 (m, 1H), 3.61−3.54 (m, 2H), 3.42−3.39 (m, 2H), 2.86−2.84 (m, 2H), 1.58−1.53 (m, 2H); ^{13}C NMR (CD_3OD) δ 164.6, 139.1, 138.8, 138.5, 137.7, 130.0−128.9, 75.7, 74.4, 74.1, 73.7, 72.7, 68.9, 57.1, 42.2, 40.2, 25.7, 25.6; IR (neat, cm^{-1}) 2929, 1680, 1455, 1074, 1028; MS (DCI/NH_3) m/z $(M + H)^+$ 608; $[\alpha]^{27}_D = +5.2$ (c = 2.21, CH_3OH).

***N*,*N*-(4-Aminobutyl)-D-glucoamidine 7d.** Glucoamidine **6d** (30 mg, 0.049 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give glucoamidine **7d** (11.1 mg, 91% yield): ^{1}H NMR (CD_3OD) δ 4.19 (d, J = 9.7 Hz, 1H), 3.88−3.78 (m, 4H), 3.58 (t, J = 9.3 Hz, 1H), 3.46−3.43 (m, 4H), 2.99−2.96 (m, 2H), 1.74−1.72 (m, 2H); ^{13}C NMR (CD_3OD) δ 166.2, 74.2, 70.1, 69.4, 62.3, 61.3, 42.3, 40.3, 25.7, 25.6; IR (neat, cm^{-1}) 3326, 2970, 1681, 1455, 1035; HRMS calcd for $C_{10}H_{22}N_3O_4$ $(M + H)^+$ 248.1610, found 248.1610; $[\alpha]^{27}_D = -0.8$ (c = 1.62, CH_3OH).

***N*,*N*-(4-Aminobutyl)-D-mannoamidine 9d.** Mannoamidine **8d** (96 mg, 0.158 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give mannoamidine **9d** (28.7 mg, 89% yield): ^{1}H

NMR (CD_3OD) δ 4.67 (d, J = 3.2 Hz, 1H), 4.02 (t, J = 3.1 Hz, 1H), 3.94−3.85 (m, 2H), 3.78 (dd, J = 5.9, 11.3 Hz, 1H), 3.46−3.40 (m, 3H), 2.99−2.94 (m, 2H), 1.73−1.71 (m, 4H); ^{13}C NMR (CD_3OD) δ 166.5, 73.6, 69.7, 67.2, 62.4, 61.1, 42.0, 40.2, 25.7, 25.6; IR (neat, cm^{-1}) 2970, 1681, 1455, 1030; HRMS calcd for $C_{10}H_{22}N_3O_4$ $(M + H)^+$ 248.1610, found 248.1616; $[\alpha]^{27}_D = -2.1$ (c = 1.0, CH_3OH).

2,3,4,6-Tetra-*O*-benzyl-D-gluco-1,5-diazabicyclo[4,4,0]decene 10 and 2,3,4,6-Tetra-*O*-benzyl-D-manno-1,5-diazabicyclo[4,4,0]decene 11. To a stirred solution of 3-bromopropylamine hydrobromide (68 mg, 0.31 mmol) and triethylamine (43 μL, 0.31 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C was added dropwise a solution of glucoiminothioether **3** (100 mg, 0.17 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 6 h. Powdered K_2CO_3 (24 mg 0.17 mmol) was added at 0 °C, and the reaction was stirred at room temperature for 6 h. The solution was concentrated and submitted to column chromatography (CH_2Cl_2/MeOH, 98:2) to yield glucoamidine **10** (10 mg, 10% yield) and mannoamidine **11** (68.3 mg, 69% yield). **10**: ^1H NMR ($CDCl_3$) δ 7.36−7.15 (m, 20H), 4.86 (d, J = 2.8 Hz, 1H), 4.70−4.34 (m, 8H), 3.86−3.82 (m, 2H), 3.70−3.58 (m, 5H), 3.44−3.22 (m, 2H), 2.31−2.15 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 160.0, 137.0, 136.9, 136.2, 136.0, 129.0−127.8, 73.9, 73.8, 73.4, 73.2, 73.0, 72.6, 72.5, 72.2, 71.1, 67.9, 63.1, 45.5, 38.8, 18.3; IR (neat, cm^{-1}) 3337, 1662, 1454, 1070, 748, 700; MS (ESITOF) m/z $(M)^+$ 576; $[\alpha]^{24}_D = -10$ (c = 1.8, $CHCl_3$). **11**: ^1H NMR ($CDCl_3$) δ 7.40−7.12 (m, 20H), 4.89−4.31 (m, 9H), 3.84 (dd, J = 3.1, 4.3 Hz, 1H), 3.71−3.58 (m, 6H), 3.45−3.39 (m, 2H), 2.03−1.94 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 160.5, 136.9−135.3, 129.1−128.0, 80.9, 75.7, 74.5, 74.1, 73.6, 73.4, 72.5, 67.3, 65.2, 45.5, 38.9, 18.5; IR (neat, cm^{-1}) 2876, 1660, 1455, 1361, 1067, 745, 700; MS (ESITOF) m/z $(M)^+$ 576; $[\alpha]^{24}_D = +7.2$ (c = 0.4, CH_3OH).

D-Gluco-1,5-diazabicyclo[4,4,0]decene 12. Glucoamidine **10** (10 mg, 0.017 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give mannoamidine **12** (3.5 mg, 95% yield): ^1H NMR (CD_3OD) δ 4.21 (d, J = 9.8 Hz, 1H), 4.09−4.01 (m, 3H), 3.98−3.79 (m, 4H), 2.15−2.00 (m, 2H), 0.91 (t, J = 5.4 Hz, 2H); ^{13}C NMR (CD_3OD) δ 163.2, 74.1, 70.1, 69.4, 62.3, 59.5, 46.7, 39.1, 19.4; IR (neat, cm^{-1}) 3343, 2894, 2484, 1658, 1323, 1061; HRMS calcd for $C_9H_{17}N_2O_4$ $(M + H)^+$ 217.1188, found 217.2000; $[\alpha]^{23}_D= -3.7$ (c = 1.9, MeOH).

D-Manno-1,5-diazabicyclo[4,4,0]decene 13. Mannoamidine **11** (60 mg, 0.104 mmol) was hydrogenated in a manner similar to the preparation of **7a** to give mannoamidine **13** (20.2 mg, 90% yield): ^1H NMR (CD_3OD) δ 4.66 (d, J = 3.2 Hz, 1H), 3.99 (t, J = 2.9 Hz, 1H), 3.92−3.71 (m, 3H), 3.58−3.45 (m, 3H), 2.15−2.09 (m, 2H), 0.89 (t, J = 6.4 Hz, 2H); ^{13}C NMR (CD_3OD) δ 163.4, 73.7, 69.5, 66.7, 61.1, 59.5, 46.8, 39.1, 19.7. IR (neat, cm^{-1}) 3348, 2951, 2478, 1659, 1324, 1059; HRMS cald for $C_9H_{17}N_2O_4$ $(M + H)^+$ 217.1188, found 217.1180; $[\alpha]^{23}_D = +0.95$ (c = 1.15, MeOH).

2,3,4,6-Tetra-*O*-benzyl-D-mannothiolactam 15. To a solution of mannolactam **14** (144 mg, 0.26 mmol) in dry benzene (5 mL) was added Lawesson's reagent (65 mg, 0.16 mmol), and the resulting solution was refluxed for 2 h. The mixture was concentrated under vacuum. Flash chromatography of the crude product (hexanes/EtOAc 8:2) gave the thiolactam **15** (134 mg, 89% yield): ^1H NMR ($CDCl_3$) δ 8.15 (br s, 1H), 7.48−7.18 (m, 20H), 5.06 (d, J = 12 Hz, 1H), 4.85−4.44 (m, 7H), 4.40 (d, J = 2.4 Hz, 1H), 3.91−3.79 (m, 2H), 3.59−3.52 (m, 2H), 3.42 (d, J = 9.0, 9.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 199.4, 137.8, 137.5, 137.3, 128.5−127.7, 79.6, 78.3, 73.9, 73.6, 73.2, 72.9, 72.3, 70.5, 59.6; IR (neat cm^{-1}) 3194, 2921, 2865, 1529, 1454, 1098, 739, 698; MS (DCI/NH_3) m/z $(M + H)^+$ 554, $(M + NH_4)^+$ 571. Anal. Calcd for $C_{34}H_{35}NO_4S$: C, 73.75; H, 6.37. Found: C, 73.75; H, 6.35. $[\alpha]^{24}_D = -31.3$ (c = 1.67, CH_2Cl_2).

2,3,4,6-Tetra-*O*-benzyl-D-mannoimino Thioether 16. To a solution of **15** (125 mg, 0.25 mmol) in dry CH_2Cl_2 (7 mL) at 0 °C was added Meerwein's salt (52 mg, 0.275 mmol). The mixture was stirred at 0 °C for 1 h 30 min and was used directly in the next step. To be analyzed, the crude was concentratred under vacuum to give **16** (125 mg, 95% yield); ^1H NMR ($CDCl_3$) δ 7.4−7.07 (m, 20H), 4.76−4.27 (m, 10H), 4.10−4.04 (m, 2H), 3.92 (dd, J = 2.7, 5.7 Hz, 1H), 3.82−3.77 (m, 1H), 3.33−3.17 (m, 2H), 1.42 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 192.7, 137.4, 136.5, 136.4, 128.7−127.8, 81.3, 78.6, 76.6, 74.0, 73.5, 73.2, 72.7, 71.6, 68.5, 60.9, 25.1, 11.5; IR (neat, cm^{-1}) 3568, 2874, 1603, 1454, 1070, 1026, 749, 699, 600; MS (DCI/NH_3) m/z $(M + H)^+$ 582.

Inhibition Analysis. α-Mannosidase (jack beans), β-mannosidase (snail acetone powder), α-glucosidase (yeast type III), β-glucosidase (almond), α-galactosidase (green coffee beans), β-galactosidase (*Aspergillus orizae*), α-fucosidase (bovine kidney), and *N*-acetyl-β-glucosaminidase (jack beans) were purchased from Sigma Chemical Company and used without further purification. The substrates for the glycosidases were the appropriate *p*-nitrophenylglycosides and were purchased from Sigma Chemical Company. The 1 mL enzymatic assays typical contained 0.05 units of enzyme. The assay buffer for all the enzymatic reactions except for α-L-fucosidase was 50 mM *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES, pH 6.8). An α-L-fucosidase assay was conducted in a 50 nM sodium acetate buffer, pH 6.0. Enzyme activity was determined by monitoring the production of the *p*-nitrophenylate anion at 400 nm on a Beckman DU70 spectrophotometer. K_m values for all the substrates were determined before K_i measurements. Substrate concentrations for the inhibition studies were chosen to be in the neighborhood of the determined K_m. K_i values were determined from five inhibitor concentrations. Double reciprocal analysis was used to establish that the inhibitors were competitive. The precise K_i values were derived from nonlinear least-squares fits of the data to the kinetic equation for competitive inhibition.

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Supporting Information Available: ^1H NMR and ^{13}C NMR spectra for compounds **7a**, **9a**, **7b**, **9b**, **7c**, **9c**, **7d**, **9d**, **7e**, **9e**, **12**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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