Inhibitors of Carbohydrate-Processing Enzymes: Design and Synthesis of Sugar-Shaped Heterocycles

BRUCE GANEM

Baker Laboratory, Department of Chemistry, Cornell University, Ithaca, New York 14853-1301

Received January 12, 1996

Introduction

Today it is widely apparent that all three major classes of nature's biopolymers play fundamental roles in life processes. Yet until recently, scientific studies devoted to proteins and nucleic acids far outweighed the corresponding focus of attention on carbohydrates. The long lag in research on saccharide chemistry and biochemistry can be attributed to a variety of causes. First, carbohydrates were for many decades thought to be important mainly as sources of simple metabolic energy. Other biochemical significance seemed limited to their role as relatively inert structural polymers in plants, fungi, insects, and crustaceans. Second, until the advent of sophisticated analytical instrumentation, it was usually impossible to detect the minuscule quantities of carbohydrate involved in triggering other metabolic phenomena. Third, the sheer chemical complexity of carbohydrates dwarfed that of peptides and nucleic acids, thus complicating the challenge of oligosaccharide synthesis and structural analysis. With their multiplicity of interconnective possibilities, simple four-, five-, and six-carbon sugars could give rise to a wide diversity of structural and stereochemical isomers even in quite low molecular weight assemblies.

In fact, carbohydrates and their conjugates such as glycoproteins and glycolipids are now recognized to play a wide variety of metabolic roles, both as free-standing chemical mediators and as cell surface receptors and markers.[1] By transmitting chemical signals, either alone or through carbohydrate−protein interactions, carbohydrates mediate an enormous repertoire of biologically important events, including cell−cell recognition, cell growth, cell development, cell−cell adhesion, inflammation, and metastasis. Gaining a molecular level understanding of the language and syntax of oligosaccharide signaling mechanisms is at the very heart of modern carbohydrate research, and promises to change the way many serious diseases are treated. For example, learning how to alter or intercept those signals could prove useful in treating diabetes, metastatic cancer, and lysozomal storage diseases. Redirecting such signals may ultimately make it possible to regulate the biosynthesis of N-linked glycoproteins and glycolipids. These glycoconjugates are principal components of cell membranes, and play prominent roles not only in antigen-directed immune responses, but also in other immune recognition phenomena such as lymphocyte recirculation, lymphocyte trafficking, and cell−cell or cell−matrix adhesion.

The first goal of such a program is to understand the reactions that assemble, trim, and shape carbohydrates into bioactive glycoprotein and glycolipid conjugates. Central among those reactions are glycosyl group transfer reactions, which are catalyzed by two families of enzymes: glycosidases and glycosyl transferases.[2] Overall, these processes involve cleavage of the glycoside bond linking a sugar's anomeric carbon with an oligo- or polysaccharide or a nucleoside diphosphate group. The liberated glycosyl group may then be transferred to water (by glycosidases) or to some other nucleophilic acceptor (by transferases). Cells constantly recycle certain metabolic intermediates and adjust concentrations of key components according to need. For that reason, the enzymes that catalyze glycosyl group transfer reactions are vital to the normal growth and development of all cells. Defects or genetic deficiencies of such enzymes are associated with several well-known inherited disorders of carbohydrate metabolism.

Glycosidases, in particular, play many fundamental roles in biochemistry and metabolism. *Exoglycosidases* remove sugars one at a time from the nonreducing end of an oligo- or polysaccharide, and are involved in the breakdown of starch and glycogen, the processing of eucaryotic glycoproteins, the biosynthesis and modification of glycosphingolipids, and the catabolism of peptidoglycans and other glycoconjugates. *Endoglycosidases* are capable of cleaving interior glycosidic bonds within polysaccharides. Besides being involved in the catabolism and clearance of aged glycoproteins, endoglycosidases also catalyze the alteration of bacterial and plant cell walls, and the hydrolysis of highly insoluble, structural polysaccharides like chitin and cellulose. Given the fact that cellulose and its derivatives constitute two-thirds of the biosphere's carbonaceous material, and a large-scale source of fuel ethanol,[3] exo- and endocellulases perform one of the world's most important biochemical reactions.

A major goal of our research, and of investigations in many other laboratories, has been to design and synthesize new generations of glycosidase inhibitors with which chemists might exert more potent and

Bruce Ganem was born in Boston, MA, in 1948. He received his B.A. degree from Harvard College in 1969 and completed his Ph.D. with Gilbert Stork at Columbia University. He then served as a National Institutes of Health Postdoctoral Fellow with the late W. S. Johnson at Stanford before joining the Cornell faculty in 1974, where he is now the Franz and Elisabeth Roessler Professor and Chairman of the Department of Chemistry. His research interests in natural products synthesis, synthetic methodology, and bioorganic chemistry have led to interdisciplinary work in biochemistry, enzymology, and structure-based drug design.

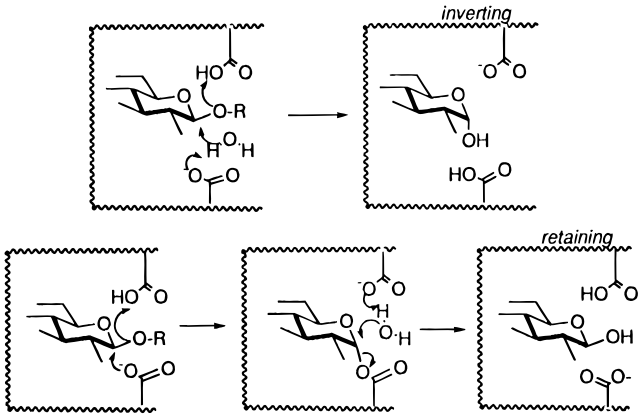
(1) *Glycoconjugates: Composition, Structure, and Function*; Allen, H. J., Kisailus, E. C., Eds.; Dekker: New York, 1992.

(2) (a) Sinnott, M. L. In *Enzyme Mechanisms*; Page, M. I., Williams, A., Eds.; The Royal Society of Chemistry: London, 1987; p 259. (b) Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319.

(3) Lynd, L. R.; Cushman, J. H.; Nichols, R. J.; Wyman, C. E. *Science* **1991**, *251*, 1318.

S0001-4842(95)00218-4 CCC: $12.00

Scheme 1



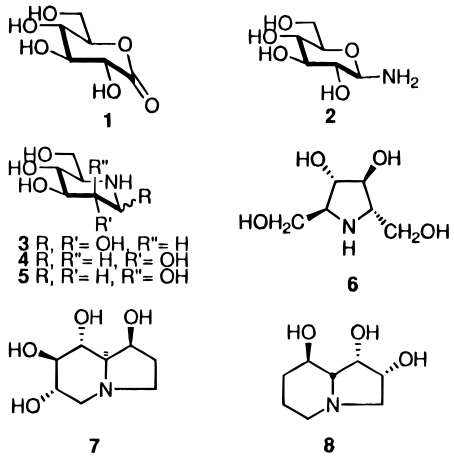
effective control over glycoside hydrolysis.[4] Aside from their potential value in basic biochemical research, several synthetic glycosidase inhibitors have already demonstrated promising therapeutic applications, in the areas of both diabetes management[5] and antiviral chemotherapy.[6]

Glycoside hydrolysis may be accomplished with either retention or inversion of configuration (Scheme 1).[1,2,7] While the catalytic apparatus of both retaining and inverting enzymes typically features bilateral carboxylic acid groups, the different stereochemical outcomes are achieved by mechanistically distinct pathways. Inverting enzymes use a combination of carboxylic acid and carboxylate groups to achieve acid and base catalysis of direct attack by a water molecule at the anomeric center, whereas in retaining enzymes, the carboxylate functions as a nucleophile in a process involving a glycosylenzyme intermediate. X-ray crystallographic studies of several glycosidases further establish that the two carboxyl residues are more widely separated in inverting enzymes than in retaining enzymes, presumably to create enough space for the participating water molecule.[8]

Classic Inhibitors of Glycosidases

Historically, the first glycosidase inhibitors were families of monosaccharide-derived δ-aldonolactones (such as D-gluconolactone (**1**), Scheme 2)[9] and glycosylamines (e.g., 1-amino-1-deoxypyranoses such as D-glucosylamine (**2**)).[10] Although lacking long-term stability in aqueous solution, these families of compounds typically displayed competitive inhibition against the glycosidases whose substrates they most closely resembled. More recently, several polyhydroxylated piperidine, pyrrolidine, and indolizine alkaloids have been identified as naturally-occurring glycosidase inhibitors in plants and microorganisms.[2] Many of these structures, for example, nojirimycin (**3**),

Scheme 2



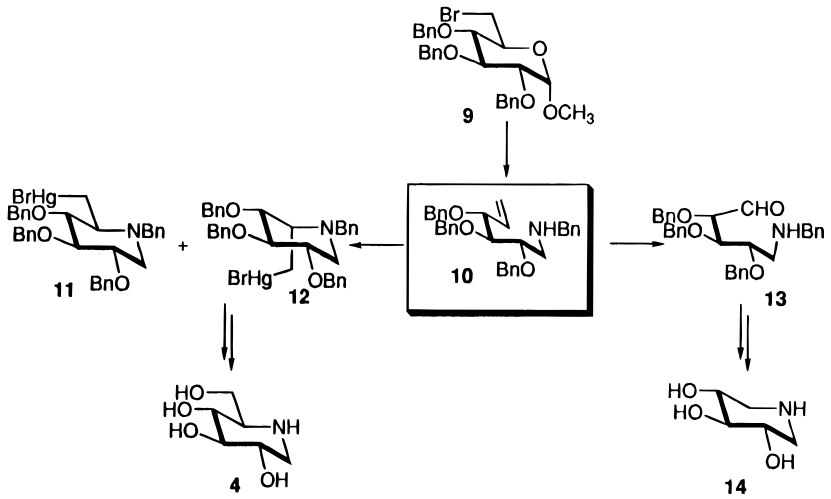
1-deoxynojirimycin (**4**), and 1-deoxymannonojirimycin (**5**), bear a striking resemblance to monosaccharides, and may be regarded as simple azasugar analogs of D-glucose and D-mannose, respectively, in which the pyranose oxygen is replaced with a basic nitrogen atom. In the five-membered series, 2,5-bis(hydroxymethyl)-3,4-dihydroxypyrrolidine (**6**) represents an azasugar analog of β-D-fructofuranose. The polyhydroxylated indolizidine alkaloids castanospermine (**7**) and swainsonine (**8**) can be seen to resemble D-glucopyranose and D-mannopyranose. By now, over two dozen naturally-occurring azasugars have been identified, and many additional analogs and homologs have been synthesized. Most are active inhibitors of glycosidases. As interest grew in the many and varied therapeutic uses of azasugars, these alkaloids have found a wide range of applications in the emerging field of glycobiology.[11]

Organomercurial Route to Azasugar Inhibitors

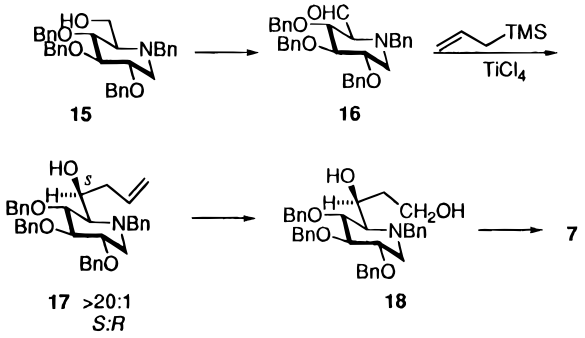
We devised an enantioselective synthetic route to naturally-occurring azasugars, as well as to many of their unnatural congeners, from readily available chiral monosaccharides. Our strategy hinged on breaking open the pyranose (or furanose) ring, and reforming the corresponding piperidine (or pyrrolidine) analogue with retention of the critical stereocenters by the process of intramolecular aminomercuration. The general approach, as well as some of the more useful ramifications of this strategy, are illustrated in Scheme 3 for the case of D-glucose. A one-pot, reductive ring opening and reductive amination of the pyranose were achieved by heating tri-*O*-benzyl-6-bromopyranoside (**9**) with acid-washed zinc dust in 19:1 propanol−water containing benzylamine and $NaBH_3CN$ to afford aminoalkene **10** in 91% overall yield. When this key intermediate was reacted with mercuric trifluoroacetate in anhydrous THF, a 3:2 mixture of bromomercurials **11** and **12** was isolated in good yield after ligand exchange (LiBr−THF). After chromatographic separation, the major cyclization product **11** could be transformed to 1-deoxynojirimycin (**4**) by reductive oxygenation ($NaBH_4$−DMF−O_2)[12] and hydrogenolytic deprotection. Additional quantities of **11** could be obtained by reequilibration of the minor

(4) Ganem, B. *Proc. Robert A. Welch Found. Conf. Chem. Res.* **1991**, *25*, 207.
(5) (a) Anzeveno, P. B.; Creemer, L. J.; Daniel, J. K.; King, C.-H. R.; Liu, P. S. *J. Org. Chem.* **1989**, *54*, 2539; (b) Elbein, A. D. *Annu. Rev. Biochem.* **1987**, *56*, 497.
(6) Fleet, G. W. J.; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Smith, P. W.; Son, J. C.; Wilson, F.; Witty, D. R.; Jacob, G. S.; Rademacher, T. W. *FEBS Lett.* **1988**, *237*, 128.
(7) Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171.
(8) Wang, Q.; Graham, R. W.; Trimbur, D.; Warren, R. A. J.; Withers, S. G. *J. Am. Chem. Soc.* **1994**, *116*, 11594.
(9) Conchie, J.; Hay, A. J.; Strachan, I.; Levvy, G. A. *Biochem. J.* **1967**, *102*, 929.
(10) Lai, H.-Y.; Axelrod, B. *Biochem. Biophys. Res. Commun.* **1973**, *54*, 463.
(11) Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992**, *2*, 199.
(12) Hill, C. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1974**, *96*, 870.

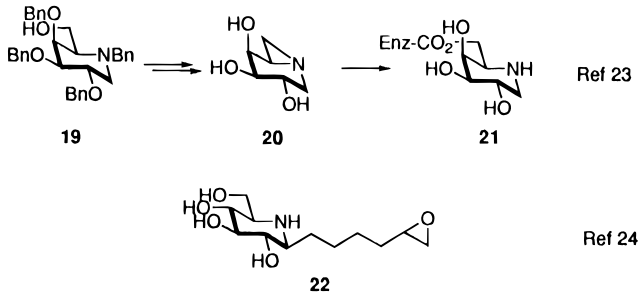
Scheme 3



Scheme 4



Scheme 5



organomercurial **12** with HBr, thus providing **4** in 35% overall yield from **9**.

This strategy was successful in producing not only azasugars **4** and **5**, and **7**,[13,14] but also several new piperidine analogs in the glucuronic acid,[15] glucosamine,[16] galactose,[17] and galacturonic acid[18] series. Pyrrolidine analogs of various pentofuranoses could also be obtained.[19] Alternatively, ozonolysis of aminoalkene **10** to amino aldehyde **13** followed by intramolecular reductive amination led to 1,5-dideoxy-1,5-iminoxylitol (**14**), representing a new family of five-carbon iminoalditols which are also potent glycosidase inhibitors.[20] These synthetic inhibitors could be further modified with *N*-carboxyalkyl tethers for anchoring to solid supports for the purification of respective glycosidases by affinity chromatographic techniques.[21]

Our laboratory also reported the first total synthesis of the indolizidine glycosidase inhibitor (+)-castanospermine by extending the chiral azasugar route outlined in Scheme 3. Reductive oxygenation of **4** generated a protected alcohol, **15** (Scheme 4), which could be oxidized to aldehyde **16**. Completion of the indolizidine framework hinged on a stereoselective allylation leading to **17**, and the desired *S*-configuration of the new stereocenter was obtained using allyltrimethylsilane−$TiCl_4$ in 66% yield. Ozonolysis of **17** followed by $NaBH_4$ reduction gave alcohol **18** which, upon mesylation and hydrogenolysis, gave (+)-castanospermine. This synthesis not only correlated the absolute configuration of **7** with that of D-glucose, but also established the enantiomeric relationship between the indolizidine ring systems of castanospermine and swainsonine (**8**).[22] In addition, stereocontrolled allylations of aldehyde **16** and its mannose and galactose congeners led to the synthesis of 1-epi-, 6-epi-, and 8-epicastanospermine for detailed structure−activity relationships.

Other Applications of Azasugar-Based Structures

With its considerable flexibility, the organomercurial approach to azasugars could also be adapted to the synthesis of several new saccharide-derived irreversible inactivators capable of alkylating selectively at the active site of glycosidases. For example, aziridinyl triol **20,** prepared from the D-galactose-derived piperidine **19** (Scheme 5), displayed potent, time-dependent inactivation of green coffee bean α-galactosidase, but had no effect on yeast α-glucosidase, jackbean α-mannosidase, or bovine β-galactosidase. When inactivated enzyme was treated with hydroxylamine and then $FeCl_3$, a strong absorbance at 510 nm characteristic of an iron−hydroxamate chelate was detected, suggesting that the aziridine had alkylated an active site carboxyl group, forming ester **21**. Judging from its apparent second-order rate constant for association of free enzyme and inhibitor, aziridine **20** represents the most potent and specific α-galactosidase inactivator yet reported.[23] In a similar vein,

(13) Bernotas, R. C.; Ganem, B. *Tetrahedron Lett.* **1984**, *25*, 165.
(14) Bernotas, R. C.; Ganem, B. *Tetrahedron Lett.* **1985**, *26*, 1123.
(15) Bernotas, R. C.; Ganem, B. *Tetrahedron Lett.* **1985**, *26*, 4981.
(16) Bernotas, R. C.; Pezzone, M. A.; Ganem, B. *Carbohydr. Res.* **1987**, *167*, 305.
(17) Bernotas, R. C.; Ganem, B. *Carbohydr. Res.* **1987**, *167*, 312.
(18) Tong, M. K.; Blumenthal, E. M.; Ganem, B. *Tetrahedron Lett.* **1990**, *31*, 1683.
(19) Liotta, L. J.; Ganem, B. *Synlett* **1990**, 503.
(20) Bernotas, R. C.; Papandreou, G.; Urbach, J.; Ganem, B. *Tetrahedron Lett.* **1990**, *31*, 3393.
(21) Bernotas, R. C.; Ganem, B. *Biochem. J.* **1990**, *270*, 539.
(22) Hamana, H.; Ikota, N.; Ganem, B. *J. Org. Chem.* **1987**, *52*, 5492.

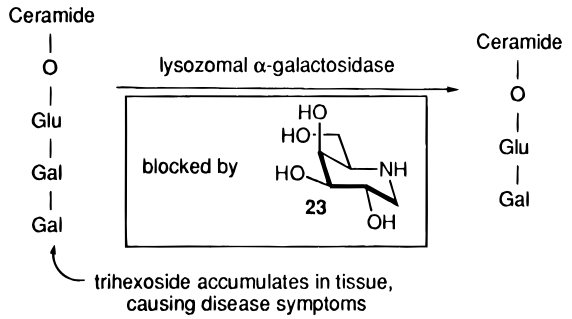


Figure 1. A Reversible Animal Model of Fabry's Disease

(epoxyalkyl)nojirimycin **22** showed time-dependent inactivation of almond emulsin β-glucosidase as well as yeast α-glucosidase, although its effect on the latter enzyme was an order of magnitude weaker.[24]

Azasugar **23**, a potent inhibitor of green coffee bean α-galactosidase ($K_I = 1.6 \times 10^{-8}$ M) and human placental ceramide trihexosidase ($IC_{50} = 4 \times 10^{-9}$ M), showed promise in developing a mouse model of Fabry's disease, a lysozomal storage disease for which no animal model exists (Figure 1). Fabry's disease is characterized by a deficiency of lysozomal α-galactosidase A that leads to storage of glycosphingolipids exhibiting terminal α-galactosyl residues in most visceral tissues, especially the vascular endothelium, heart, and kidney.[25] We reasoned that administration of a competitive inhibitor of α-galactosidase such as **23**[18] should create an apparent deficiency of the requisite galactosidase, and thus induce a reversible animal model of the disease. Administration of **23** to female C57 mice and male beige mice via their drinking water caused modest to substantial elevations in total kidney glycolipid and ceramide trihexoside levels, judging from HPLC analysis.[26] When administration of **23** was terminated, levels of these metabolites returned to normal. Azasugar **23** merits further investigation as a potential reversible model of Fabry's disease.

Simple azasugars like **4** are too small to inhibit endoglycosidases, which typically possess 4−6 subsites for binding individual sugar rings in a long polysaccharide chain. However, incorporation of an azasugar moiety into an oligosaccharide framework proved to be a useful approach to the design of endocellulase inhibitors.[27] The synthesis of such modified oligosaccharides was greatly simplified by the serendipitous discovery of an unusual 1,5-hydrogen rearrangement that selectively removed the C4-benzyl ether in the reductive oxygenation of mercurial **11**. Treatment of **11** with $NaBH_4$−DMF generated a radical that partitioned between O_2 capture and 1,5-H shift from the adjacent benzylic position. By simply changing the flow of oxidant to a slow stream of air (rather than a vigorous flux of pure O_2), gram quantities of alcohol **24** could be obtained in almost 70% yield. Coupling of **24** with cellobiose and cellotriose using the trichloroacetimidate method afforded oligomers **25** and **26** (Scheme 6), which were powerful mixed-competitive

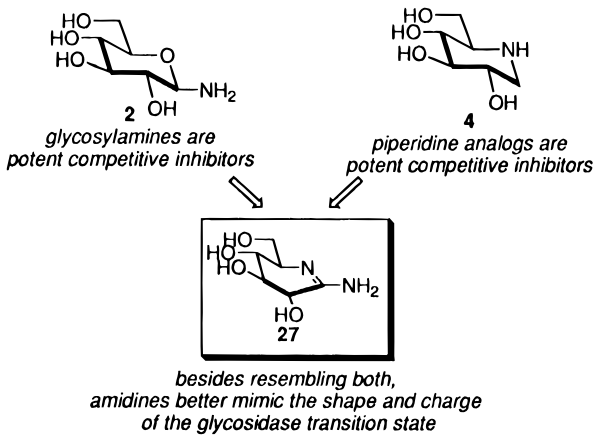
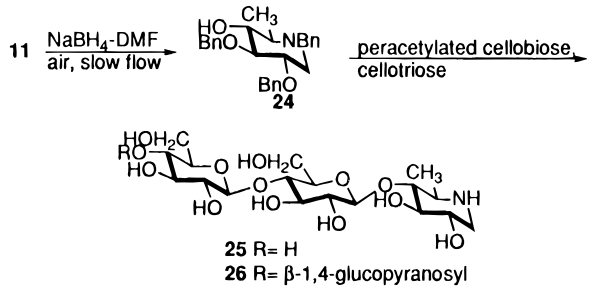


Figure 2.

Scheme 6



inhibitors of several endocellulases from the aerobic, thermophilic soil bacterium *Thermomonospora fusca*.

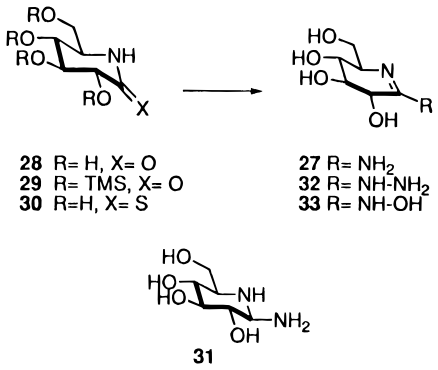
Beyond Azasugars: Amidines

With a basic nitrogen directly attached to the anomeric carbon, both glycosylamines and azasugars in their protonated forms can mimic the incipient partial positive charge of the putative transition state for glycoside hydrolysis (Scheme 1). In their chair conformations, however, neither structure accurately reflects the ring deformations accompanying displacement reactions catalyzed by either retaining or inverting enzymes. Backside attack by an incipient nucleophile (either water or carboxylate) would be accompanied by rehybridization of the anomeric carbon to an sp^2 geometry, thus flattening the saccharide ring. While the importance of this conformational distortion has been a matter of some controversy in the catalytic mechanism of lysozyme, simple aldonolactones[9] and aldonolactams[28] (which are unprotonated at normal pH but which exist in a pseudo-half-chair conformation) have long been known to inhibit glycosidases. An inhibitor possessing the correct *charge and shape* of the glycosyl cation seemed like a promising new design target.

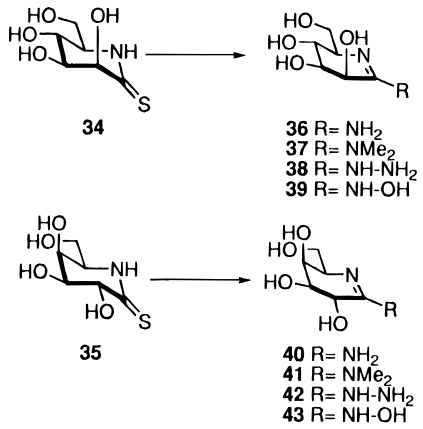
Our choice of **27**, an amidine analogue of D-glucose (Figure 2), as a synthetic target was guided by several important considerations. First, its resonance-stabilized π-system was expected to prefer the endocyclic tautomer as shown,[29] thus recreating the flattened conformation of the hydrolysis transition state. Second, unlike lactones or lactams, the basic amidines[30] would be fully protonated at physiological pH, thus

(23) Tong, M. K.; Ganem, B. *J. Am. Chem. Soc.* **1988**, *110*, 312.
(24) Liotta, L. J.; Lee, J.; Ganem, B. *Tetrahedron* **1991**, *47*, 2433.
(25) Desnick, R. J.; Sweeley, C. C. In *The Metabolic Basis of Inherited Disease*, 5th ed.; Stanbury, J. B., Wyngaarten, J. B., Frederickson. D. S., Goldstein, J. L., Brown, M. S., Eds.; McGraw Publications: Minneapolis, MN, 1982; p 906.
(26) Ganem, B.; Daniel, P. F. Unpublished studies.
(27) Liotta, L. J.; Bernotas, R. C.; Wilson, D. B.; Ganem, B. *J. Am. Chem. Soc.* **1989**, *111*, 785.
(28) Inouye, S.; Tsuruoka, T.; Ito, T.; Niida, T. *Tetrahedron* **1968**, *23*, 2125.
(29) Sieveking, H. U.; Luttke, W. *Justus Liebigs Ann. Chem.* ***1977***, 189.
(30) Hafelinger, G. In *The Chemistry of Amidines and Imidates;* Patai, S., Ed.; Wiley: London, 1975.

Scheme 7



Scheme 8



mimicking the incipient positive charge of the glycosyl cation. Finally, the presence of nitrogens at both exo and endo positions flanking the anomeric site combined the essential structural features of both the azasugar and glycosylamine families. 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 (**27**) might be a meritorious candidate for study was put forth by Reese *et al.* in 1971,[31] although no experimental work toward this end was described. An unsuccessful synthetic approach to **27** via 5-azido-5-deoxy-D-gluconononitriles was reported by Bird *et al.* in 1990.[32]

Our synthesis of amidine **27** began with readily available D-gluconolactam (**28**) as shown in Scheme 7. Silylated lactam **29** was reacted with Lawesson's reagent to afford D-gluconothionolactam (**30**) which, upon nucleophilic addition of ammonia in methanol, afforded **27** in 60−70% yield. As its hydrochloride salt, amidine **27** was stable indefinitely in water; however, as the free base, **27** was extremely sensitive to nucleophiles at higher pH. For example, **27** reverted to lactam **28** by nucleophilic hydrolysis (pH 8) with a half-life of 1 h. Amidine **27** was a potent inhibitor of sweet almond β-glucosidase (β-glu), and displayed pH-independent inhibition ($K_I \approx 10$ μM) between 4.5 and 7.0. Structure **27** was moderately active against bovine β-galactosidase (β-gal) and was a strong competitive inhibitor of jackbean α-mannosidase (α-man; $K_I = 9$ μM). By comparison, *gem*-diamine **31** embodied the same combination of endocyclic and exocyclic nitrogens, but without the flattened half-chair conformation. While **31** was a good competitive inhibitor of β-glu ($K_I = 40$ μM), no activity against other glycosidases was observed,[33] suggesting that the half-chair conformation of **27** might be responsible for its unusually broad spectrum of activity. The amidine design strategy has since been invoked by others to prepare pseudo-disaccharide analogs,[34,35] mannopyranose analogs,[36] and other pyranose-derived glycoamidines.[37] Syntheses of several more N-substituted polyhydroxylated five- and six-membered amidines have recently been reported, and accompanying bioassays expand the repertoire of exoglycosidases that are strongly inhibited by these monosaccharide analogs.[38]

More Stable than Amidines: Amidrazones and Amidoximes

The hydrolytic instability of amidine **27** led us to design other conformationally flattened saccharide analogs that might make more practical enzyme inhibitors. From the known correlation[39] between the pK_a values of N-substituted amidines and the pK_a values of the corresponding N-substituted amines, we reasoned that amidrazone **32** and amidoxime **33** (Scheme 7) should have lower pK_a values than that of **27**, since hydrazine (pK_a = 8.10) and hydroxylamine (pK_a = 5.97) were both less basic than ammonia (pK_a = 9.21).[40] Experimentally, D-glucoamidrazone (**32**) and D-glucoamidoxime (**33**) were readily prepared by reacting thionolactam **30** with anhydrous hydrazine (CH_3OH, 5 °C, 2 h, 78%), or hydroxylamine (CH_3OH, rt, 14 h, 75%), as shown in Scheme 7.

Potentiometric titrations of the acidic forms of **27**, **32**, and **33** revealed pK_a values of 10.6, 8.7 and 5.6, respectively. Consistent with these observations, the half-life of amidrazone **32** was 8 h at pH 11, whereas D-glucoamidoxime (**33**) remained unchanged after several weeks in aqueous base at pH 11. Interestingly, all three glucosyl mimics **27**, **32**, and **33** inhibited almond β-glu equally effectively, despite the 10^5 range in basicity from amidine to amidoxime functionality. The data suggest that, at least for this enzyme, conformational changes accompanying the rehybridization of the sugar's anomeric region are more important for inhibition than charge buildup on the glucopyranosyl oxygen.[41]

From D-mannono- and D-galactonothionolactams (**34** and **35**) (Scheme 8) it was also possible to prepare amidines, amidrazones, and amidoximes **36**−**43** related to mannose and galactose. A complete account of the synthesis, physical and spectroscopic properties, stability measurements, and biochemical assays of amidines, amidrazones, and amidoximes possessing the D-*gluco*, D-*manno*, and D-*galacto* configuration has been published, including a detailed tabulation of

(31) Reese, E. T.; Parrish, F. W.; Epplinger, M. *Carbohydr. Res.* **1971**, *18*, 381.
(32) Bird, P.; Dolphin, D. H.; Withers, S. G. *Can. J. Chem.* **1990**, *68*, 317.
(33) Yoon, H.; King, S. B.; Ganem, B. *Tetrahedron Lett.* **1991**, 32, 7199.
(34) Knapp, S.; Choe, Y. H.; Reilly, E. *Tetrahedron Lett.* **1993**, *34*, 4443.
(35) Blériot, Y.; Genre-Grandpierre, A.; Tellier, C. *Tetrahedron Lett.* **1994**, *35*, 1867.
(36) Blériot, Y.; Dintinger, T.; Guillo, N.; Tellier, C. *Tetrahedron Lett.* **1995**, *36*. 5175.
(37) Avalos, M.; Babiano, R.; Cintas, P.; Duran, C. J.; Jiménez, J. L.; Palacios, J. C. *Tetrahedron* **1995**, *51*, 8043.
(38) Blériot, Y.; Dintinger, T.; Genre-Grandpierre, A.; Padrines, M.; Tellier, C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2655.
(39) Oszczapowicz, J.; Orlinski, R.; Walczynska, H. *Pol. J. Chem.* **1979**, *53*, 2531.
(40) Hall, H. K., Jr. *J. Am. Chem. Soc.* **1957**, *79*, 5441.
(41) Ganem, B.; Papandreou, G. *J. Am. Chem. Soc.* **1991**, *113*, 8984.

inhibition constants for known and new glycosidase inhibitors.[42] Obviously, quite a wide variety of biological applications may be envisioned for these inhibitors, and by no means have all been explored at this writing. However, several noteworthy characteristics of our new inhibitors emerged, both from the type of kinetic behavior observed with readily available glycosidases (e.g., competitive versus noncompetitive inhibition) and from profiles of the pH dependence of activity and inhibition.

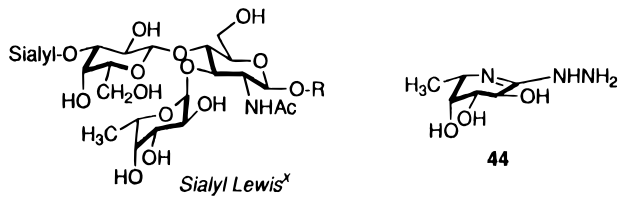
In the d-*manno* series, amidrazone **38** proved to be of particular biological interest. Since up to six mannose units are removed during the processing of oligosaccharides attached to arginines in nascent N-linked glycoproteins, it would be desirable to have inhibitors of the various mannosidases that trim those residues in order to elucidate the role of each type of glycosylated site in the function of various glycoproteins.

Amidrazone **38** was noteworthy for its effect on a range of different mannosidases. Besides jackbean α-man, **38** 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.[43] The latter triad of mannosidases constituted the principal mannose-processing pathway in the biosynthesis of glycoproteins. A further test of D-mannoamidrazone (**38**) as an *in vivo* mannosidase inhibitor was performed in animal cell cultures using Madin-Darby canine kidney cells infected with influenza virus. Addition of **38** 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 D-mannoamidrazone (**38**) also inhibited Golgi α-man I and the soluble, endoplasmic reticulum mannosidase *in vivo*. To date, no other inhibitor has exhibited such a broad spectrum of interference with the N-linked glycoprotein-processing pathway.

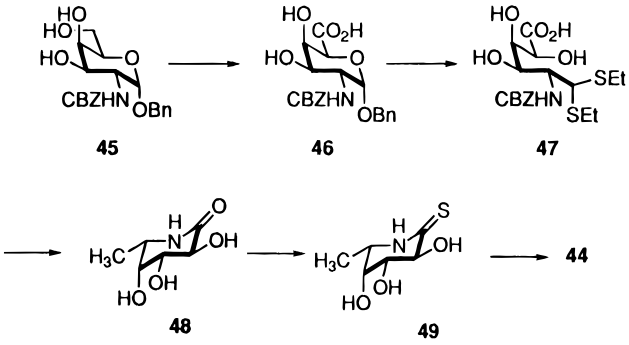
D-Galactoamidine (**40**) and D-galactoamidrazone (**42**) were active inhibitors of green coffee bean α-galactosidase, exhibiting K_I values of 8.5 and 8.3 ± 0.4 μM, respectively, at pH 6.6. As with almond β-glu, kinetic studies suggested the existence of two active site acidic groups having pK_a values of 2.8−3.5 and 5.1−5.9. Inhibition of α-galactosidase by both **42** and **43** was pH-independent, suggesting that the protonated form of the basic inhibitors interacted with the more dissociated of the two active site carboxylates. *N*,*N*-Dimethylamidine **41** was somewhat less potent than **40** (17% of control activity for **41** versus 3% of control activity for **40**).[42]

In some cases, inhibitors in different stereochemical series exerted quite different effects on certain glycosidases. For example, bovine β-galactosidase was competitively inhibited by D-galactoamidrazone (**42**) (K_I = 6.5 ± 0.1 μM) and D-galactoamidoxime (**43**) (K_I = 10 ± 0.5 μM). By contrast, D-gluco- and D-mannoamidrazones (**32** and **38**) displayed noncompetitive inhibition of this enzyme, which has evolved both a galactose and a glucose binding site to hydrolyze

Scheme 9



Scheme 10



lactose, with optimal activity at pH 4.5.[44] Inhibitors **32** and **38** apparently interacted strongly with the glucose site, causing weak noncompetitive inhibition by binding both free enzyme and the enzyme−substrate complex. Inhibitors **42** and **43**, which possessed the natural D-*galacto* configuration, bound competitively (and selectively) at the catalytic site.

Glycosides of L-fucose are widely distributed in mammalian glycolipids and glycoproteins, where they are thought to play important roles in cell−cell adhesion, and in mediating leukocyte trafficking and recruitment to inflammatory sites. Cytokines trigger endothelial cells to produce cell-surface endothelial−leukocyte adhesion molecules (ELAMs) which bind to a tetrasaccharide, sialyl Lewis^x, expressed on the leukocyte surface.[45] Sialyl Lewis^x (Scheme 9) is the minimal carbohydrate ligand for many ELAMs, and adhesion has been shown to depend critically on the presence of a 1,3-linked L-fucose residue. For these reasons, the corresponding L-fucoamidrazone (**44**), a prospective fucosidase inhibitor, was of interest as a prototype for new anti-inflammatory and antitumor drugs.

For the synthesis of **44**, a new route to L-fuconic-δ-lactam (**48**) was developed from D-galactosamine using a chain end interchange strategy (Scheme 10). Thus, galacturonic acid **46** was prepared by platinum−oxygen dehydrogenation of **45**. After hydrolysis of the glycosidic bond in **46**, thioacetalization afforded **47** in 60% overall yield. Upon exposure under acidic conditions to Raney nickel, desulfurization was accompanied with N-deprotection and concomitant cyclization to generate lactam **48** (76%). Exhaustive silylation of **48**, followed by treatment with Lawesson's reagent furnished thionolactam **49** after desilylation in the acidic workup. Reaction of **49** with hydrazine in CH_3OH gave the target amidrazone **44** in 90% yield.[46] Tested against recombinant human α-L-fucosidase under steady-state assay conditions, L-fucoamidrazone (**44**) displayed clean competitive inhibition, with K_i =

(42) Papandreou, G.; Tong, M. K.; Ganem, B. *J. Am. Chem. Soc.* **1993**, *115*, 11682.
(43) Pan, Y. T.; Kaushal, G. P.; Papandreou, G.; Ganem, B.; Elbein, A. D. *J. Biol. Chem.* **1992**, *267*, 8313.
(44) (a) Dey, P. M.; Pridham, J. B. *Adv. Enzymol.* **1972**, *36*, 91. (b) Huber, R. E.; Gaunt, M. T. **1982**, *60*, 608.
(45) Lasky, L. A. *Science* **1992**, *258*, 964.
(46) Schedler, D. J. A.; Bowen, B. R.; Ganem, B. *Tetrahedron Lett.* **1994**, *35*, 3845.

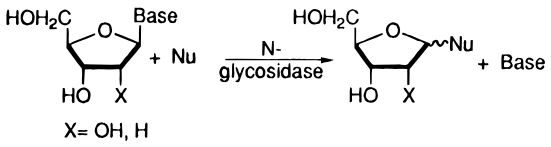
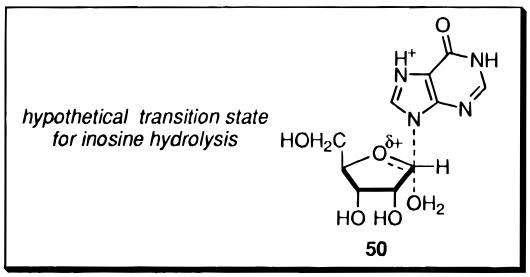
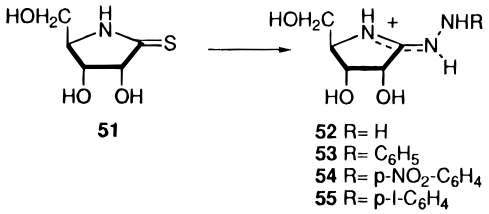


Figure 3.

Scheme 11





820 nM, and thus represents a promising lead in the design of new fucosidase inhibitors.

Extensions to Pentoses: D-Riboamidrazones

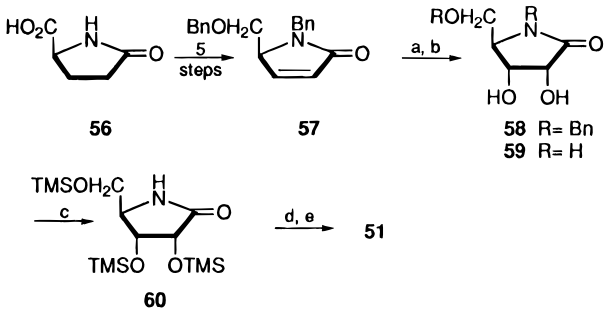
Distinct from the glycosidases discussed so far, a separate family of enzymes promotes the cleavage of C−N glycosidic bonds (Figure 3). This family includes the NAD hydrolases, nucleoside hydrolases, purine nucleoside phosphorylases, and purine or pyrimidine hydrolases which are involved, *inter alia*, in base-excision repair mechanisms of DNA,[47] ribosome inactivation,[48] and heterocyclic base salvage and recovery pathways.[49] Schramm *et al.* have characterized the transition structure of nucleoside hydrolase from *Crithidia fasciculata* employing both experimental[48b] and computational[50] methods.

Using labeled inosine and adenosine as substrates, multiple kinetic isotope effects and bond energy bond order vibrational analysis indicate that, as the heterocyclic base is protonated, the ribofuranosyl ring is flattened to form a transition structure with significant oxocarbenium character (viz., **50**, Scheme 11). The conformational change accommodates both an elongated (2.0 Å) C−N glycosidic bond and an incipient bond (ca. 3 Å) to the incoming oxygen nucleophile. An unusually strong hyperconjugative interaction with the adjacent hydrogen (H2′) is also evident.

With their delocalized charge and flattened structures, the family of amidrazones **52**−**55** can be seen to resemble the putative transition state **50** for nucleoside hydrolase. A synthetic route to **52**−**55** was therefore designed from a common intermediate,

(47) Sancar, A.; Sancar, G. B. *Annu. Rev. Biochem.* **1988**, *57*, 29.
(48) Endo, Y.; Tsurugi, K.; Lambert, J. M. *Biochem. Biophys. Res. Commun.* **1988**, *150*, 1032.
(49) (a) Parkin, D. W.; Horenstein, B. A.; Abdulah, D. R.; Estupinan, B.; Schramm, V. L. *J. Biol. Chem.* **1991**, *266*, 20658. (b) Horenstein, B. A.; Parkin, D. W.; Estupinan, B.; Schramm, V. L. *Biochemistry* **1991**, *30*, 10788. (c) Kline, P. C.; Schramm, V. L. *Biochemistry* **1992**, *31*, 5964.
(50) (a) Horenstein, B. A.; Schramm, V. R. *Biochemistry* **1993**, *32*, 7089. (b) Schramm, V. R.; Horenstein, B. A.; Kline, P. C. *J. Biol. Chem.* **1994**, *269*, 18259.

Scheme 12



(a) OsO_4-NMO, 3:1 acetone:H_2O, 59%; (b) Na/NH_3, then EtOH, 60%; (c) 10 equiv $(TMS)_2NH$, 10 equiv TMSCl, pyr; (d) 1.1 equiv Lawesson's reagent, C_6H_6, reflux, 1 h; (e) HCl-MeOH, rt, 20 min.

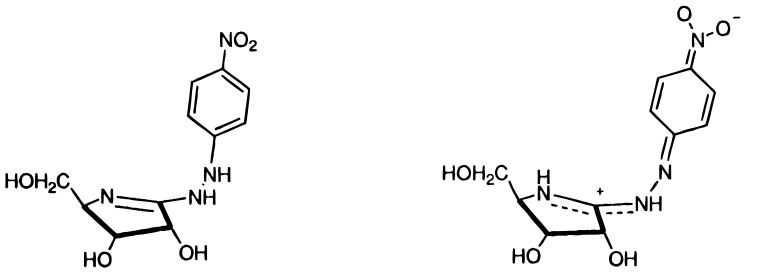
thionolactam **51**, itself readily prepared from (*S*)-pyroglutamic acid (Scheme 12). The key step, osmylation of unsaturated lactam **57**, furnished diol **58** having the D-*ribo* configuration as the major product (8:1 ratio).

The parent amidrazone inhibited *C. fasciculata* nucleoside hydrolase with an apparent dissociation constant of 10 μM. *N*-Phenyl amidrazone **53** was a slope-linear competitive inhibitor, for which full kinetic analysis revealed a K_i of 207 ± 13 nM. *p*-Nitrophenylamidrazone **54** exhibited slow-onset, tight binding inhibition, with an overall dissociation constant of 2 nM, making it the most potent nucleoside hydrolase inhibitor known to date.[51]

To better understand the origin of such potent inhibition, MOPAC-1 calculations using the AM1 Hamiltonian were used to identify the three lowest-energy conformations of both protonated and unprotonated **54**. In none of the minima was the *p*-nitrophenyl group situated above the plane of the ribose ring where a departing heterocyclic base would be positioned in the transition state for hydrolysis. However, the energy barrier for rotating the *p*-nitrophenyl group into a conformation like **56** (Figure 4) mimicking the transition state **50** was calculated to be 13.6 and 7.4 kcal/mol, respectively, for unprotonated and protonated **54**. Interestingly, superposition of the five-membered ring of unprotonated **56** with the calculated transition state structure **50** representing inosine stabilized by nucleoside hydrolase reveals that the *p*-nitrophenyl group of **56** lies in approximately the same plane as the departing hypoxanthine in **50.** By contrast, the *p*-nitrophenyl plane in protonated **56** is displaced 2.5 Å relative to the hypoxanthine plane calculated for **50** at the transition state.

Clearly, these gas-phase calculations provide only qualitative values for predictions about conformational energetics. An X-ray crystallographic analysis of a nucleoside hydrolase complex with *p*-iodophenyl amidrazone **55** (K_i = 8 nM, in progress) may shed light on the true conformation of bound inhibitor. Independently, resonance Raman and ultraviolet−visible absorbance spectroscopies have established that inhibitor **54** binds to nucleoside hydrolase as the neutral, zwitterionic species **57** (Figure 4). Moreover, the tautomeric structure depicted in **57** can be established by isotope-edited resonance Raman spectroscopy on

(51) Boutellier, M.; Horenstein, B. A.; Semenyaka, A.; Schramm, V. L.; Ganem, B. *Biochemistry* **1994**, *33*, 3994.



56
from MOPAC-AM1 calculations:
7-13 kcal/mol above global conformational minimum

57
from isotope edited Raman spectroscopy:
neutral, but zwitterionic, bound inhibitor

Figure 4.

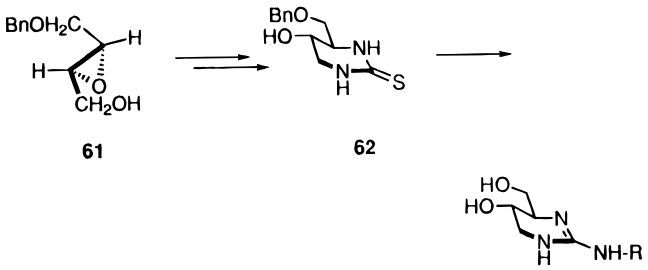
inhibitor **54** specifically ^{15}N-enriched at each nitrogen of the amidrazone linkage.[52]

Future Prospects

Carbohydrate structures play profound roles in mediating cell−cell recognition, cell growth, development, and cell−cell or cell−matrix adhesion, as well as inflammation and a wide range of other immunological phenomena. With the growth of our understanding of the biological roles played by carbohydrates, the usefulness of azasugars and other saccharide mimics may extend well beyond the inhibition of glycosidases and other carbohydrate-processing enzymes to the design of new regulatory agents and potential drug leads. For example, synthetic analogs of glycosaminoglycans and glycosphingolipids will likely become important new fields of research. The family of glycosaminoglycan glycopolymers includes hyaluronic acid, which is thought to promote a wide range of adhesion phenomena,[53] as well as heparin and heparan sulfate, which are potent anticoagulants and also implicated in the control of cell growth, transformation, and differentiation in mammalian organisms.[54] Glycosphingolipids are ubiquitous components of eucaryotic cell membranes, where they are thought to modulate functional membrane proteins, regulate transmembrane signaling, and play a role in unregulated cellular proliferation.[55]

We and others have continued to create new glycomimetic structures with the hope of discovering such uses in research and medicine.[56,57] One major challenge for synthetic chemists is to devise sugar-shaped heterocycles and other non-carbohydrate templates with which to assemble bioactive oligosaccharide analogs. Recently we described an enantioselective route to guanidine **63** and *N*-aminoguanidine **64** (Scheme 13) whose molecular frameworks bear close spatial relationships to a typical saccharide.[58] Like

Scheme 13



61 **62**

63 R= H
64 R= NH_2

amidrazones and amidoximes, compounds **63** and **64** mimic a conformationally flattened hexopyranose. Additional sugar residues can be attached at the commonly substituted 1, 2, 4, and 6 positions on the ring. Moreover, by replacing the C2-hydroxyl group with nitrogen, both **63** and **64** can serve as a surrogate for either D-glucose or D-mannose, with electron density projected above and below the ring plane directly adjacent to the anomeric center. Such saccharide "look-alikes" might be used to form the core frameworks of artificially assembled oligosaccharides.

Conclusion

With an enormous diversity of structure and function among bioactive glycopolymers, glycoproteins, and glycolipids, the challenge of tackling the most formidable problems in glycobiology requires ever more chemical expertise. Organic chemists who have taken up this challenge are forging an important area of glycotechnology, combining at times both synthetic chemical methods, as highlighted in this Account, and chemoenzymatic approaches.[59] Just as the discovery of biologically active peptides prompted the development of peptidomimetics, growing interest in the key metabolic roles played by mono- and oligosaccharides ensures that research on new glycomimetics will remain solidly at the frontiers of modern carbohydrate chemistry.

It is a pleasure to acknowledge the very significant contributions and hard work of my graduate and undergraduate students, postdoctoral associates, and other collaborators whose names appear in the references. Continuous support of this project by the National Institutes of Health (Grant GM 35712) is gratefully acknowledged.

AR9502184

(52) H. Deng, A. W.-Y. Chan, C. K. Bagdassarian, B. Estupinan, B. Ganem, V. L. Schramm, R. H. Callender, *Biochemistry* **1996**, *35*, 6037.
(53) Roden. L. In *The Biochemistry of Glycoproteins and Proteoglycans*; Lennarz, W., Ed.; Plenum Press, New York, 1980,; p 267.
(54) Karllson, K. *Annu. Rev. Biochem.* **1989**, *58*, 309.
(55) Radin, N. S.; Inokuchi, J.-I. *Biochem. Pharmacol.* **1988**, *37*, 2879.
(56) Carson, K. G.; Ganem, B.; Radin, N. S.; Abe, A.; Shayman, J. A. *Tetrahedron Lett.* **1994**, *35*, 2659.
(57) Abe, A.; Radin, N. S.; Shayman, J. A.; Wotring, L. L.; Zipkin, R. E.; Sivakumar, R.; Ruggieri, J. M.; Carson, K. G.; Ganem, B. *J. Lipid Res.* **1995**, *36*, 611.
(58) Chan, A. W.-Y.; Ganem, B. *Tetrahedron Lett.* **1995**, *36*, 811.
(59) Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, Jr., J. A.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6187.