Recent Developments of Transition-State Analogue Glycosidase Inhibitors of Non-Natural Product Origin

Vinni H. Lillelund, Henrik H. Jensen, Xifu Liang, and Mikael Bols*

Department of Chemistry, Aarhus University, DK-8000 Aarhus C, Denmark

Received July 17, 2001

Contents

I. Introduction and Scope

Glycosidase inhibitors have been subject to extensive interest in the past decade. As glycoside cleavage is a biologically widespread process, glycosidase inhibitors have many potential medical applications. Inhibition of intestinal α-glycosidases can be used to treat diabetes through the lowering of blood glucose levels, and several α-glycosidase inhibitors are being marketed against type 2 diabetes.[1] An alternative way of treating diabetes that has been explored recently is through inhibition of glycogen phosphorylase, which also results in a lowering of blood glucose.[2] Glycosidases are also involved in the trimming of cell- and viral-surface oligosaccharides, which also can be used as a therapeutic target. Inhibition of these glycosidases can disrupt biosynthesis of oligosaccharides and hence cell−cell or cell−virus recognition processes. This principle is used in the anti-influenza neuraminidase inhibitors that have recently been marketed[3] and is also the basis for the potential use of α-glycosidase inhibitors against HIV,[4] Gauchers disease, hepatitis,[5] and cancer.[5]

A. Scope

A very large amount of literature on glycosidase inhibitors exists. Since the beginning of the 1960s a number of natural products that are potent glycosidase inhibitors have been found in plants and microorganisms. This, combined with the always large interest in natural products, led to a large body of work on synthesis of these natural products and their analogues. Thus, a very large fraction of the glycosidase inhibitor literature relates to these natural products, much of which have been reviewed (Tables 1−3). However, in the past decade several fundamentally new types of synthetic inhibitors have emerged that are very potent and which have led to new insight in the glycoside cleavage process. It is the aim of this review to cover this work, but it is necessary to limit the topic, and this has been done as follows. Eleven years ago two frequently cited comprehensive reviews on the glycosidases[6] and glycosidase inhibitors were published.[7] Since then many reviews (Tables 1−3) and accounts have been published on the subject covering various subsections of the field. In particular, the glycosidase inhibitors from natural products have been covered extensively, and also Arnold Stütz's book covers in table form all iminosugar glycosidase inhibitors known until 1998 comprehensively.[8] The present review will, therefore, limit itself to cover all new glycosidase inhibitor structures that appeared since 1990 of non-natural-product origin, provided they can be regarded as transition-state analogues of the glycoside cleavage process and provided they are not natural products or simple analogues of such. More specifically, we have not covered nojirimycin analogues, indolizidines, and five-membered iminosugars of the 1,4-iminoarabinitol type. Nor have we covered cyclopentane inhibitors of the mannostatin type, except the recent work to rationalize the inhibition of these compounds.

* To whom correspondence should be addressed. Phone: +458942-3963. Fax: +4586196199. E-mail: mb@chem.au.dk.

10.1021/cr000433k CCC: $39.75
Published on Web 01/29/2002

Vinni Høyer Lillelund, née Andreassen, was born in 1975 in Aalborg, Denmark. She received her M.Sc. degree in 2000 with Professor Mikael Bols at the University of Aarhus, Denmark, where she worked with azasugar glycosidase inhibitors. Currently she is a Ph.D. student under the supervision of Professor Mikael Bols. The topic of her studies is cyclodextrin chemistry and artificial enzymes.

Henrik Helligsø Jensen was born in 1975 in Herning, Denmark. He began studying chemistry and mathematics in 1995 at the University of Aarhus, where from he earned his M.Sc. degree in 2001 in bioorganic chemistry under the supervision of Professor Mikael Bols. He is currently working in the same group toward his Ph.D. degree in the field of design and synthesis of new glycosidase inhibitors.

Xifu Liang was born in 1960 in People's Republic of China. He received his B.Sc. degree in pharmaceutical chemistry at Shenyang College, China, in 1982, after which he joined the Northchina Pharmaceutical Corporation, Shijiazhuang, China, where he was a pharmaceutical chemist from 1982 to 1991. He then went on to a research assistantship with Professor Prinzbach at the University of Freiburg, where he worked on total synthesis of aminoglycoside antibiotics. In 1998 he began Ph.D. studies in Professor Bols' group on the subject of chemoenzymatic synthesis of azasugars. He received his Ph.D. degree in 2001 and is currently a research chemist at Leo Pharmaceutical Products, Copenhagen, Denmark.

Mikael Bols was born in 1961 in Copenhagen, Denmark. He received his M.Sc. (1985) and Ph.D. (1988) degrees from the Technical University of Denmark under the Supervision of Professor Inge Lundt. After a postdoctoral stay from 1988 to 1989 at Queen's University, Canada, with Professor Walter Szarek, he joined Leo Pharmaceutical Products, where he was a research chemist from 1989 to 1991. He then returned to an assistant professorship at the Technical University of Denmark. In 1994 he did a sabbatical stay at Columbia University in Professor Gilbert Stork's group. Finally, in 1995 he went to the University of Aarhus, where he, in 2000, became a Full Professor. His research interests are bioorganic chemistry, carbohydrates, and stereoselective synthesis.

B. Design of Transition-State Analogues

An attractive approach to potent inhibitors is to create compounds that mimic the transition state of the enzyme-catalyzed reaction. The rationale behind this method is the belief that the transition state is likely to be the point on the reaction trajectory that has the highest degree of enzymatic stabilization. For glycosidases it is believed that this stabilization is so huge that the transition state is bound with a dissociation constant of up to 10^{-20} M, which means that it is potentially possible to create transition-state analogue inhibitors with a K_i of a similar magnitude. However, no inhibitor has yet been discovered that comes even close to this value, due to the impossible task to precisely mimicking the shape and charge of the transition state.

To be able to design transition-state analogues of glycosidases one has to analyze the mechanism of glycoside cleavage. The mechanism of glycoside cleavage have been reviewed several times recently,[6,101] so only a brief extract will be made here.

Acidic glycoside hydrolysis is believed to be an A-1 process in which protonation of the exocyclic oxygen in the glycosidic bond converts it to a leaving group that is eliminated from the structure with formation of intermediate cation/oxocarbenium ion **1**. The transition state is believed to be late and quite closely resemble the ion **1**. This is supported by evidence of various degrees of sp^2 character of the anomeric carbon atom in the transition state (Scheme 1), as obtained from kinetic isotope effects measurements.[9]

Enzymatic glycoside hydrolysis can follow two mechanisms (Scheme 1). Glycosidases have two carboxylic acids in the active site that afford the catalysis. Retaining glycosidases convert the substrate to product with retention of anomeric configuration (Scheme 1b). The catalysis is believed to occur by protonation of the exocyclic oxygen by one acidic group, the catalytic general acid, which converts it

Table 1. Recent Reviews and Accounts about Glycosidases and Their Inhibitors

author	title	year
A. D. Elbein	Glycosidase inhibitors: inhibitors of N-linked oligosaccharide processing	1991[253]
Y. Nishimura	Glycosidase and Glycosyltransferase inhibitors	1992[254]
G. Mooser	Glycosidases and glycosyl transferases	1992[255]
B. Winchester et al.	Amino-sugar glycosidase inhibitors: versatile tools for glycobiologists	1992[256]
G. Legler	Sugar analog inhibitors for glycosidases, tools for the elucidation of enzymatic glycoside cleavage.	1993[257]
A. Vasella et al.	Synthesis and evaluation of new glycosidase inhibitors	1994[258]
G. S. Jacob	Glycosylation inhibitors in biology and medicine	1995[259]
B. Ganem	Inhibitors of Carbohydrate-Processing Enzymes: Design and Synthesis of Sugar-Shaped Heterocycles	1996[156]
B. Draeger	Glycosidase inhibitors. Biological activity and therapeutic significance	1996[260]
S. Picasso	Azasugar glycosidase inhibitors. Useful tools for glycobiologists	1996[261]
G. Legler	The catalytic efficiency of glycoside hydrolases and models of the transition state based on substrate related inhibitors	1998[262]
M. Bols	1-Azasugars, Apparent Transition State Analogs of Equatorial Glycoside Formation/cleavage	1998[263]
J. D. Esko	Natural and synthetic inhibitors of glycosylation	1999[264]
C. W. Ekhart et al.	Tables of glycosidase inhibitors with nitrogen in the sugar ring and their inhibitory activities	1999[265]
T. D. Heightman et al.	Recent insight into inhibition, structure, and mechanism of configuration-retaining glycosidases	1999[188]
C. S. Rye et al.	Glycosidase mechanisms	2000[266]
E. S. H. Ashry et al.	Glycosidase inhibitors and their chemotherapeutic value	2000[267,268,269]

Table 2. Recent Reviews and Accounts Focusing on Synthesis of Glycosidase Inhibitors

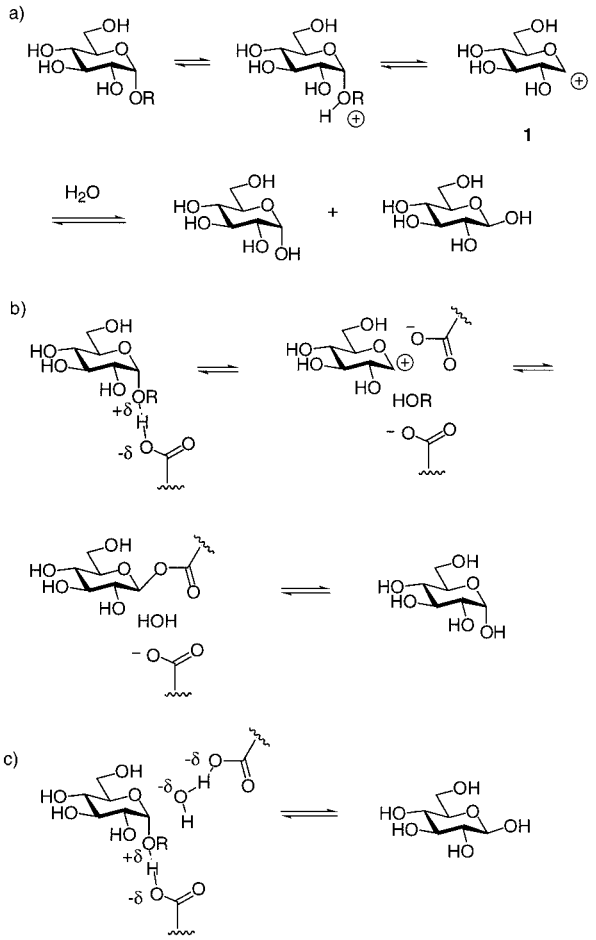
author	title	year
G. W. Fleet et al.	Plagiarizing plants: amino sugars as a class of glycosidase inhibitors	1990[270]
P. Vogel	Total, asymmetric synthesis of glycosidase inhibitors such as the aza sugars, castanospermine and analogs, from furan	1992[271]
F. Effenberger et al.	Enzymic carbon-carbon bonding	1993[272]
G. C. Look et al.	Enzyme-catalyzed Organic Synthesis: Practical Routes to Aza Sugars and Their Analogues for Use as Glycoprocessing Inhibitors	1993[273]
Y. Ichikawa et al.	Topochemistry and inhibition of selectin-mediated cell adhesion: Chemical-enzymatic synthesis of inhibitors related to E-selectin recognition	1994[274]
V. Jaeger et al.	Synthesis via isoxazolines. Part 23. Synthesis of glycosidase-inhibiting iminopolyols via isoxazolines	1994[275]
Y. Nishimura	Stereoselective synthesis and transformation of siastatin B. A novel glycosidase inhibitor, directed toward new drugs for viral infection and tumor metastasis	1995[137]
C. -H. Wong	Enzymic and chemo-enzymic synthesis of carbohydrates	1995[276]
C. -H. Wong et al.	Enzymes in organic synthesis: application to the problems of carbohydrate recognition. Part 2	1995[277]
A. De Raadt et al.	Chemical and chemo-enzymatic approaches to glycosidase inhibitors with basic nitrogen in the sugar ring	1997[278]
K. Tatsuta	Total Synthesis and chemical design of useful glycosidase inhibitors	1998[279]
A. Goti et al.	A Straightforward route to enantiopure pyrrolizidines by cycloaddition to pyrroline N-oxides derived from the chiral pool	1999[280]
M. H. Fechter et al.	Chemical and chemo-enzymatic approaches to unnatural ketoses and glycosidase inhibitors with basic nitrogen in the sugar ring	1999[281]

Table 3. Recent Reviews and Accounts Covering Natural Products That Inhibit Glycosidases

author	title	year
D. Lednicer et al.	Plants and other organisms as a source of anti-human immunodeficiency virus (HIV) drugs	1991[282]
R. J. Molyneux et al.	Swainsonine, slaframine, and castanospermine	1991[283]
L. E. Fellows et al.	Distribution and biological activity of alkaloidal glycosidase inhibitors from plants	1992[284]
B. Winchester	Natural and synthetic inhibitors of glycosidases	1992[285]
R. J. Molyneux	Isolation, characterization and analysis of polyhydroxy alkaloids	1993[286]
R. J. Nash et al.	Polyhydroxylated alkaloids that inhibit glycosidases	1996[287]
H. Yamada et al.	Pharmacologically active glycosidase inhibitors in medicinal plants	1998[288]
A. D. Elbein et al.	Alkaloid glycosidase inhibitors	1999[289]
A. D. Elbein et al.	Inhibitors of glycoprotein processing	1999[290]
N. Asano et al.	Sugar-mimic glycosidase inhibitors: natural occurrence, biological activity and prospects for therapeutic application	2000[291]
N. Asano	Alkaloidal sugar mimetics: Biological activities and therapeutic applications	2000[292]

to a leaving group with formation of a cation/oxo-carbenium ion intermediate similar to **1**. This ion reacts with the other catalytic carboxylates, the catalytic nucleophile, to form an intermediate ester. Subsequently, this ester is displaced by a molecule of water with general base catalysis to form the product hemiacetal with retention of configuration. Thus, this mechanism has two separate steps and, therefore, two transition states. In inverting glycosidases the general acid has the same function, while a general base acts by directing a water molecule to attack the anomeric carbon (Scheme 1c). Hence, the product is obtained with inversion of configuration. The transition states of the enzymatic reactions are

Scheme 1



likely to resemble that of non-enzymatic glycoside hydrolysis and there is evidence for this from linear free energy relationships.[10,11] The most significant difference is probably that structural distortion of the substrate is believed to occur in a number of cases of enzymatic hydrolysis as indicated by several X-ray structures that show distortion of bound substrate analogues.[12,13] Structural distortion may facilitate bond breakage (Figure 1).

Some difficulty and controversy is associated with the classification of inhibitors as transition-state analogues. Direct information about the transition state can be obtained from kinetic studies, which include isotope effects studies[6] (see also the discussion of compounds **266**−**269** in section V). These studies have indicated various degrees of sp^2 character at the anomeric carbon, which can imply

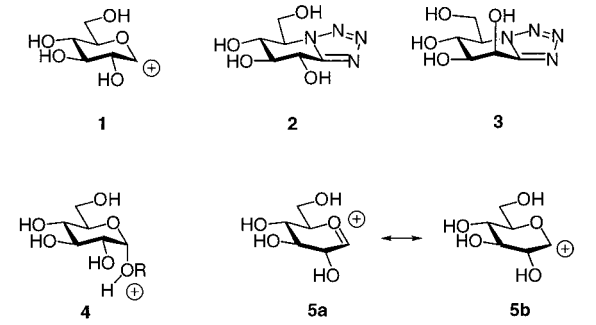


Figure 1.

formation of an oxocarbenium ion-like transition state. Much classification of glycosidase inhibitors as transition-state analogues has simply been to compare their conformation with the half-chair that oxocarbenium ion **5** is expected to adopt. A number of more "rigorous" criteria are available to help classify glycosidase inhibitors as transition-state analogues. These criteria are (a) strong inhibition, (b) slow inhibition, (c) pH profile, and (d) free energy relationships. It should be noted that these criteria will reveal a lack of a transition-state-like property. Fulfilling a criterion does, however, not necessarily mean that the inhibitor in question is a very good transition-state analogue. Criterion (a) must be used with caution. A good transition-state analogue must necessarily be a strong inhibitor, but a strong inhibitor does not have to be a transition-state analogue. In fact, a strong inhibitor may not even bind to the active site. Criterion (b) has been suggested to be associated with transition-state analogues based on the following hypothesis: Enzymes (or some) are generally in a conformation that binds the substrate in a ground-state conformation but can then undergo a conformational change in order to bind the transition state better. Transition-state analogue inhibitors can only bind to the conformationally changed enzyme, and the slow onset inhibition is associated with the time spent for the enzyme to change conformation.[14] Recent work shows, however, examples of glycosidase inhibitors that have slow onset inhibition that is not associated with a slow conformational change of the enzyme.[205,206] It is, in the opinion of these reviewers, likely that slow onset inhibition is associated with any very strong inhibitor and that it is too poorly understood to be a safe criterion. Criterion (c) has been used to disqualify inhibitors, whose binding profile as a function of pH did not correlate with that of catalysis.[157] This will typically occur when the inhibitor changes charge under the observed pH range, and though this may be regarded as an imperfection, the inhibitor could still be a transition-state analogue at one pH and not another. In criterion (d) the ln K_i values of an inhibitor toward a series of enzymes or enzyme mutants are compared with the ln(k_{cat}/K_M) values of a substrate toward those enzymes. Alternatively the same comparison is done with one enzyme but with a series of similar structural analogues of an inhibitor and the substrates. In both cases, a linear relationship should be obtained if the inhibitor or inhibitor-type is a transition-state analogue of the enzymatic reaction of that substrate provided that $k_{cat}/K_M \approx k_{cat}/K_S$, which is a good assumption. It should be noted that a linear relationship will only reveal similarity between the transition state and the inhibitor with regard to what has been varied.[15] Parts of the inhibitor that are not varied may not resemble the transition state. A classical study in this regard was made by Vasella's and Withers' groups, which found a linear relationship between ln(k_{cat}/K_M) for hydrolysis of a 4-nitrophenylglycoside by a series of different mannosidases, glucosidases, and galactosidases and the ln K_i value for gluco- and mannotetrazole **2** and **3**.[163] That a linear correlation was obtained is remarkable be-

cause hydrolysis of different substrates by different enzymes would be expected to have different transition states. However, the correlation shows that these transition states have a common denominator which **2** and **3** resemble. This could be the geometry of the glycon portion of the transition state and the position of the 2-OH, since the 2-OH was the only varied part of the inhibitor. However, since no variation in the charge of the inhibitor was made, it is, in the opinion of these reviewers, not possible to conclude that charge is relatively unimportant in the transition state, as was done in the paper. Similar free energy relationship studies have been made for other glycosidase inhibitors.[16,17]

In the design of transition-state analogues of glycoside hydrolysis, much of the focus has been on mimicking the assumed geometry of the transition state or its assumed charge. Most designs focusing on geometry have tried to create inhibitors that are in a half-chair conformation, which is commonly accepted to be the conformation of the transition state. It should be noted, however, that the most substantial evidence for this conformation is the isotope effects measurements that show various degrees of sp^2 character at the anomeric carbon of the transition state. Designs focusing on charge have mimicked charge build-up in a number of different places. This review will be organized according to where charge build-up is believed to occur. The first section will deal with inhibitors that mimic protonation at the exocyclic oxygen, thus a compound resembling **4** (section II). The second section will deal with inhibitors that mimic charge build-up at the endocyclic oxygen (**5a**, section III), while section IV deals with compounds that mimic the cation **1**. Section V deals with compounds that mimic two of **1**, **4**, and **5a** at the same time. Finally, section VI deals with neutral compounds. It should be made clear that **5a** and **5b** are resonance forms of the oxocarbenium ion, while **1** and **5b** are conformers. Conformer **1**, which in this review is termed the glycosyl cation, may have sp^2 or sp^3 hybridization at the anomeric carbon.

II. Inhibitors that Mimic a Positively Charged Exocyclic Oxygen

Since glycoside cleavage of an ordinary *O*-glycoside will involve protonation of the exocyclic oxygen of the glycosidic bond, an early transition state may have a considerable charge on this atom. Thus, stable compounds that resemble the conjugate acid of the glycoside, **4**, may be regarded as transition-state analogues. Compounds that have an amine in place of the exocyclic oxygen are the most common transition-state analogues of this kind.

Many of the inhibitors which contain an exocyclic nitrogen are isolated from nature or derivatives of these compounds. The main classes of this type of compounds are the trehazolins, the allosamidines, acarbose and derivatives thereof, and the mannostatins (Figure 2).

The trehazolins exhibit powerful specific inhibition of various trehalases, the allosamidines inhibit various chitinases, acarbose is a very potent α-glucosidase inhibitor,[18] and the mannostatins inhibit α- and β-mannosidases very strongly. Therefore, each main class inhibits mainly one group of enzymes.[19] In accordance with the topic of this review, these compounds are not discussed here, but a review by Berecibar et al.[19] can be recommended. However, one exception will be made, namely, the amino(hydroxymethyl)cyclopentanetriols, which have a stereochemistry similar to the stereochemistry of either gluco-, manno-, galacto-, fuco-, or xylopyranose. Great progress has been made in this field the past few years, and some very strong and specific inhibitors have been synthesized.

The relation between the structure of the aminocyclopentanes and their inhibitory properties is not yet completely understood. It has been proposed that mannostatin A mimics the mannosyl cation, and this has been supported by comparing the inhibitor with a molecular orbital-optimized half-chair form of the mannosyl cation.[20−22] By superimposing the assumed transition state on mannostatin A, it can be seen that there is a very good overlap of the 2- and 3-OH of the mannosyl oxocarbenium ion and two of the hydroxyl groups at mannostatin A and that the nitrogen of mannostatin A is placed near the oxocarbenium ion. However, whether the amino group of mannostatin A mimics charge at ring oxygen or anomeric carbon in the mannosyl oxocarbenium ion[22] or if it corresponds to the protonated exocyclic oxygen of the substrate has not been determined yet. However, the importance of having the basic amino group has been established by means of the *N*-acetyl-substituted mannostatin A which does not inhibit any of the mannosidases it has been tested against.[23]

From the similar K_i values of the α- and β-*manno*-configured inhibitors **6**, **8**, **9,** and **11** there is an indication that the inhibitors mimic the oxocarbenium ion because they are not anomer selective (Table 4).[24] If the protonated N atoms of these compounds mimicked protonated exocyclic oxygen, a difference in potency would be expected with **9**−**11**

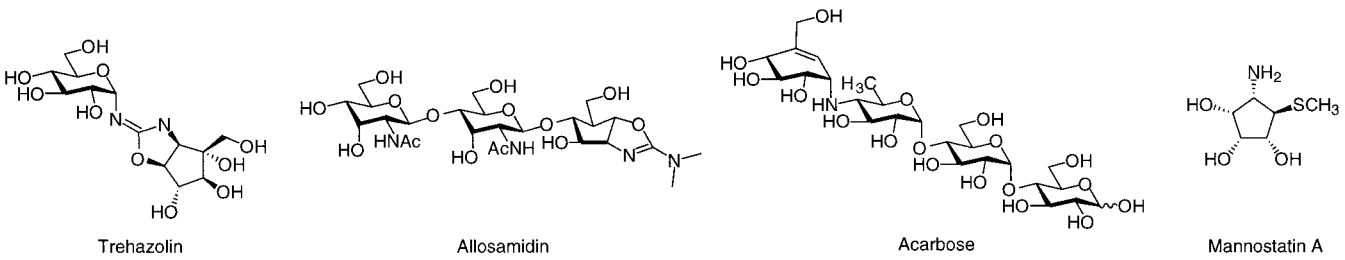
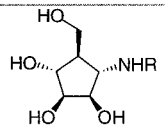
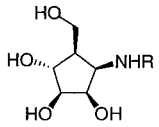


Figure 2.

Table 4. K_i values in μM from Ref 24

		R	α-man'ase[a]	α-man'ase[b]
6		H	0.41	0.61
7		CH_3	0.062*[c]	
8		Bn	1.0	0.5
9		H	0.074	0.12
10		CH_3	1.3	0.15
11		Bn	0.5	2.2

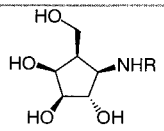
*IC_{50} value. [a] Jack bean α-mannosidase. [b] Almond α-mannosidase. [c] From ref 20.

being less potent. Whether this trend also is present with the *N*-methyl *manno*-configured inhibitors **7** and **10** is unclear, because their inhibitory properties have been determined at different conditions.

Looking at the series of *galacto*-configured amino-(hydroxymethyl)cyclopentanetriols **12**−**20,** it can be seen that only **12** and **18** inhibit α-galactosidase (Coffee bean) whereas the *β*-galactosidases are inhibited by almost all of the inhibitors (Table 5). This seems to show that this class of inhibitors is anomer selective; thus, the amino group might correspond to the exocyclic oxygen of the protonated substrate instead of mimicking the galactosyl cation.[25,26] However, this would have been more convincing if a series of α-*galacto*-configured inhibitors had been synthesized and their inhibitory properties had confirmed a similar trend. Second, it should be noted that *β*-glycosidases commonly prefer hydrophobic aglycons, perhaps because this mimics the hydrophobic pyranose core of a monosaccharide unit. The anomer selectivity observed can be explained by this aglycon affinity of *β*-glycosidases.

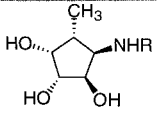
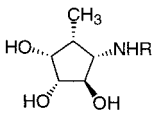
The same pattern shows by comparing the *fuco*-configured inhibitors **22** and **24** where the α-configured inhibitor **22** is the stronger inhibitor toward α-L-fucosidase (Table 6).[27,28] Such a difference cannot be observed between the unsubstituted inhibitors **21** and **23**. However, this is not surprising because if the *N*-substituent is large it may be required that it can fit into the aglycon subsite in the enzyme. Likewise the nonsubstituted α/*β*-*gluco*-configured inhibitors **25** and **26** have comparable K_i values against both α- and *β*-glycosidases while the N−$CH_2C_6H_{11}$-substituted *β*-*gluco*-configured inhibitor **27** is more selective between α- and *β*-glycosidases than the corresponding nonsubstituted *β*-*gluco*-configured inhibitor **26** (Table 7).[24,29] Accordingly, the influence of a hydrophobic N-substituent on the K_i value is similar in the gluco-, galacto-, and fucopyranose series. Hence, the main trend is if the substituent is bulky it enhances the inhibition toward the anomeric corresponding

Table 5. K_i values in μM from Ref 26

		R	β-glu[a]	β-glu[b]	α-gal[c]	β-gal[d]	β-gal[e]	β-gal[f]
12		H	2.2	0.17	12	4.5	3.3	0.145
13		CH_3	0.45	0.35	-	0.22	0.09	0.068
14		$(CH_2)_2OH$	5	14	-	0.087	0.48	0.16
15		Ph	113	23	-	185		
16		C_6H_{11}	0.83	7	-	2.4	3	0.035
17		Bn	1.39	0.165	-	0.002	0.006	0.14
18		$CH_2C_6H_{11}$	0.104	0.12	22	0.02	0.004	
19		$CH_2(4\text{-}C_6H_4Br)$	0.017	0.12	-	0.0006	0.0007	
20		$CH_2(4\text{-}C_6H_4I)$	0.053 –	0.187	-	0.008	0.003	

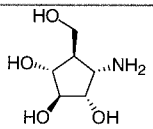
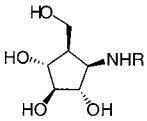
[−]No inhibition at 1000 μM. [a] Almond *β*-glucosidase. [b] *Caldocellum saccharolyticum* *β*-glucosidase. [c] Coffee bean α-galactosidase. [d] *E. coli* *β*-galactosidase. [e] Bovine liver *β*-galactosidase. [f] Jack bean *β*-galactosidase.

Table 6. K_i values in μM from Ref 28

		R	α-fuc'ase[a]	α-fuc'ase[b]	α-fuc'ase[c]	α-fuc'ase[d]
21		H	15	12	14	125
22		Bn	0.68	0.57	0.30	6.0
23		H	28	18	22	58
24		Bn	110	28	18	180

[a] Bovine kidney α-L-fucosidase. [b] Bovine epididymis α-L-fucosidase. [c] Human placenta α-L-fucosidase. [d] *Fusarium oxysporum*377 α-L-fucosidase.

Table 7. K_i values in μM from Ref 24

		R	α-glu[a]	α-glu[b]	α-glu[c]	β-glu[d]	β-glu[e]	α-gal[f]	β-gal[g]	β-gal[h]	β-gal[i]	α-man[j]	β-man[k]
25			1.6	0.6	85	6.5	1.5		-[l]	21	-		
26		H	6.2	0.7	-	6.6	2.6	11[§m]	-	2.6	-	180[m]	
27		$CH_2C_6H_{11}$	67	100	-	0.024	2.7		-	0.18			
28		$COCH_3$					170[m]	16[§m]		23[§m]			19[§m]
29		CH_2CH_3	12[m]			2.6[m]	0.16[m]	20[§m]	9[§m]	37[m]		115[m]	9[§m]

*IC_{50} value. § % inhibition at 100 μM. [a] Yeast maltase. [b] Bakers yeast isomaltase. [c] Rice α-glucosidase. [d] Almond β-glucosidase. [e] *Caldocellum saccharolyticum* β-glucosidase. [f] Coffee bean α-galactosidase. [g] *E. coli* β-galactosidase. [h] Bovine liver β-galactosidase. [i] Jack bean β-galactosidase. [j] Jack bean α-mannosidase. [k] Snail β-mannosidase. [l] Inhibition < 50% at 1000 μM. [m] From ref 29.

glycosidase, and the opposite is true for the noncorresponding glycosidase. If the N-substituent is small, so is the effect on the inhibitory properties. These anomer selectivities can likewise be explained by the hydrophobic aglycon affinity of β-glycosidases mentioned above.

In the *manno* series such a relation cannot be deduced on the basis of the inhibitors which have been tested so far (Table 4).

The positive effect of a hydrophobic aglycon on β-glycosidase inhibition has also been investigated in a series of cyclophellitol analogues **30**−**34**. The hydrophobic aglycon enhanced the binding to almond β-glucosidase, and it appears that the opposite is true for inhibition of α-glucosidase from baker's yeast (Table 8).[30−32] However, the corresponding α-configured inhibitor **35** has also been synthesized and tested, and it is a stronger inhibitor of almond β-glucosidase than of α-glucosidase (baker's yeast); thus, the results should not be attached to much importance.

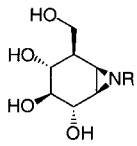
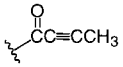
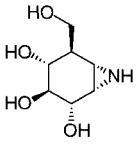
Classical mimics of the protonated substrate are glycosylamines.[7] However, these compounds hydrolyze very quickly under assay conditions (e.g., galactose derivatives of benzylamine and piperidine have a half-life about 20 min at pH 7 and 25 °C).[33] Nevertheless, a mannosylamine and a piperidinylmannose were recently synthesized and their biological activities investigated. None of them were very potent inhibitors (K_i = 300 and 700 μM, respectively).[34] A fucosylamine has also been investigated and found to be a potent inhibitor (K_i = 0.75 μM) against α-L-fucosidase (bovine kidney).[35]

Recently three glycosylamidines **36**−**38** have been synthesized, and they turned out to be stable in acidic solutions (pH 5−7).[36] They also were shown to be quite potent and selective inhibitors. For example, **36** only inhibits glucosidases and not galactosidases, and it is remarkably stronger toward the β-glucosidases than toward the α-glucosidases it has been tested against (Table 9). The importance of the positive charge was shown by synthesizing and testing the neutral glucosylamide **39** which was a very weak inhibitor.

To make some more stable inhibitors, various *C*-(glycopyranosyl)methylamines have been synthesized where the exocyclic oxygen has been replaced with an aminomethyl group. Indeed, the compounds are more stable but none were potent inhibitors (all had a K_i value in the millimolar range).[34,37,38] In 1993 BeMiller et al. investigated the inhibitory properties of various *N*-substituted *C*-(galactopyranosyl)methylamines, and the inhibition was in most cases enhanced compared with **41**.[39] As can been seen in Table 10 the best substituent was a benzyl group (in **42**), which enhanced the inhibition by a factor of 200 compared with **41**.

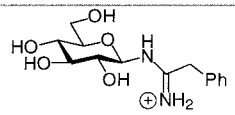
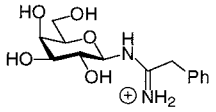
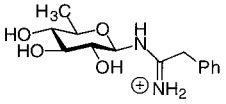
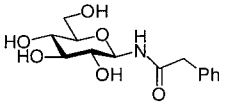
While this increase might be explained by β-glycosidases preferring hydrophobic aglycons, BeMiller et al. attempted to eliminate the influence of such effects. This was done by comparing the K_i values of **40**−**46** with the K_i values of the corresponding amide (i.e., CH_2NR_2 replaced with $CONR_2$) labeled K_i'. By analyzing K_i'/K_i for the inhibitors **40**−**46** it was found that **42** and **43** had the best K_i'/K_i ratio (Table 10). Since **42** and **43** have a pK_a value that is close to the pK_a of the catalytic acid of this enzyme (pK_a = 8.5), BeMiller et al. concluded that the optimal pK_a value

Table 8. % Inhibition at 100 μg/mL from Ref 31

		R	α-glu'ase[a]	β-glu'ase[b]
30		H		50[c]
31		CH_3	46	82
32		CH_2CH_3	38	18
33		Bu	20	98
34			66	95[d]
35			12	84

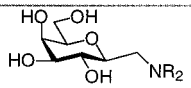
[a] Bakers yeast α-glucosidase. [b] Almond β-glucosidase. [c] % inhibition at 0.22 μg/mL, from ref 30. [d] % inhibition at 10 μg/mL.

Table 9. K_i values in μM from Ref 36

		α-glu[a]	α-glu[b]	β-glu[c]	β-glu[d]	α-gal[e]	β-gal[f]
36		21	>2500[g]	73	0.094	-	-
37		-	-	41	390	47	7.8
38		-	-	1100	11	-	-
39		>2500	>2500	-	770	>2500	>2500

– No inhibition at 2500 μM. [a] Yeast α-glucosidase. [b] *Aspergillus niger* α-glucosidase. [c] Almond β-glucosidase. [d] *Aspergillus niger* β-glucosidase. [e] *Aspergillus niger* α-galactosidase. [f] *E. coli* β-galactosidase. [g] Less than 50 % inhibition at 2500 μM.

Table 10. K_i values in μM from Ref 39

		R_2	pK_a	K_i	K_i'/K_i[a]
40		C_6H_{11},H	9.7	54	52
41		H, H	9.1	505	20
42		CH_2Ph, H	8.3	2.3	357
43		$(CH_2)_2OCH_3$, H	8.0	19	711
44		$(CH_2CH_2)_2O$	6.1	497	~1
45		CH_2CF_3, H	4.7	1597	2.0
46		Ph, H	4.0	171	11

E. coli lacZ β-galactosidase. [a] K_i' is the K_i value for the amide corresponding to the similar amine (i.e., $CH_2NR_2 = CONR_2$).

of such an inhibitor was one that was close to the pK_a of the proton-donating group of the enzyme.

BeMiller et al. also investigated the influence of pH on the inhibition of **41** against β-galactosidase (*E. coli*). The results showed that the inhibition was stronger at high pH values, and it was suggested that this was because the nonprotonated amine is the inhibitor and that the inhibitor becomes protonated in the active site.

Also, the *N*-(α-*C*-glucosylmethyl)aniline has been shown to be a stronger inhibitor ($K_i = 11$ μM)[40] than the corresponding nonsubstituted α-*C*-glucosylmethylamine.

Another way of mimicking the protonated glycoside has been approached by synthesizing the four amino-substituted (*R*/*S*)-phenylglucosides **47**,[41] **48**,[41] **49**,[40] and **50**[40] and bis-*C*-β-(glucopyranosyl)methylamine **51**.[37,42] Only **47** and **50** were reasonably potent inhibitors: Interestingly, **47** inhibited almond β-glucosidase ca. 100 times better than epimer **48** while **50** had a K_i value against α-glucosidase (yeast) that was ca. 30 times less than the K_i value of **49** (Figure 3).[40] The observation that **47** is a stronger inhibitor than **48** suggests that almond β-glucosidase is a *syn*-protonator according Vasellas protonation trajectory hypothesis (see section V).[188] The nitrogen atom is, however, not perfectly situated to mimic the exocyclic oxygen in these compounds.

A galactose analogue with a benzothiazole at the anomeric carbon, **56**, has been synthesized and tested in the hope of finding a strong glycosidase inhibitor (Figure 3). However, a $K_i = 650$ μM was found against β-galactosidase (*E. coli*).[43] Likewise, none of the compounds where the nitrogen is placed one atom father away from the anomeric carbon (**57**–**62**) were strong inhibitors (Figure 4).[44–46]

To mimic the assumed half-chair conformation of the transition state and the positive charge around the anomeric carbon, a series of 1-substituted galactals have been synthesized and tested against *E. coli* β-galactosidase.[43] The substituents were benzimidazole, imidazole, $C(NH)OCH_3$, $C(NH)NHCH_2Ph$, $C(NH)NH_2$, and $C(NH)NHNH_2$. With exception of the inhibitor with substituent R= $C(NH)NHCH_2Ph$, which had a $K_i = 6$ μM, all the inhibitors were weak (K_i between 570 μM (imidazole) and 15500 μM (benzimidazole)). There can be at least two explanations for this: (1) lack of the 2-OH group which is known to be important in binding to the enzyme[7] and (2) the aglycon might not fit well into the active site of the enzyme.

Another approach to mimic the charge development and the conformation of the assumed transition state has been made by synthesizing the two bicyclo-[4.1.0]heptane derivatives **63** and **65**.[47] The cyclohexane moiety of the compounds were supposed to adopt

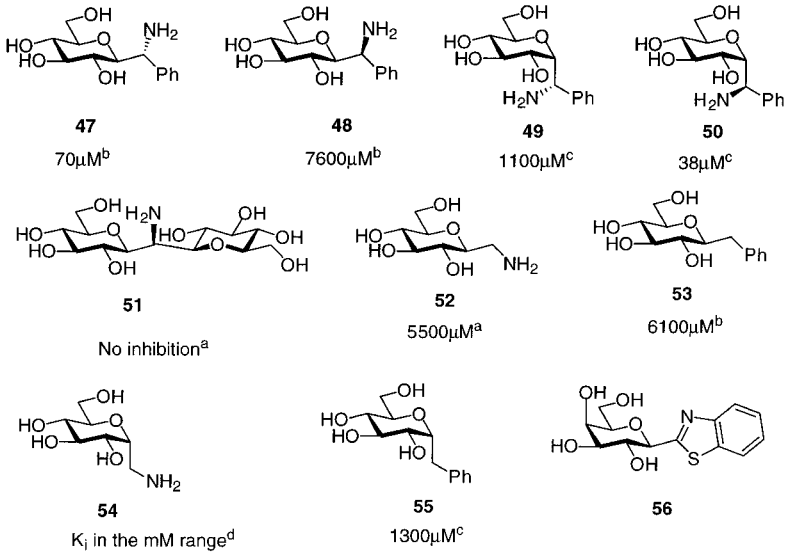


Figure 3. Inhibition of sweet almond β-glucosidase by **47**, **48**, and **51**−**53** and of yeast α-glucosidase by **49**, **50**, **54,** and **55**: [a]ref 37, [b]ref 41, [c]ref 40.

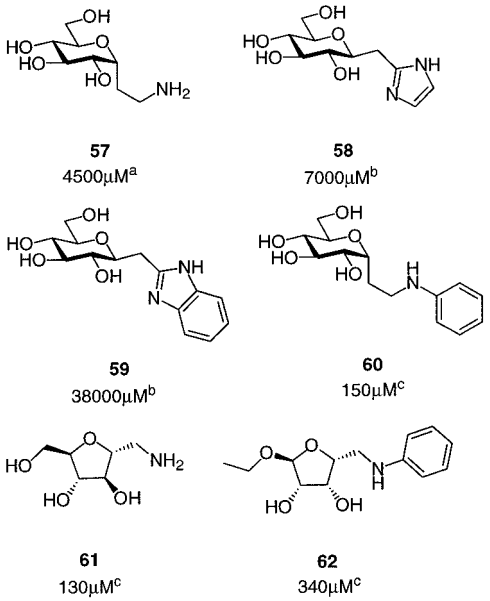
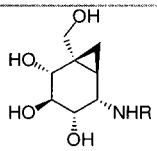
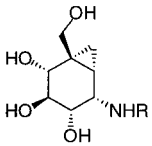


Figure 4. K_i values against sweet almond β-glucosidase: [a]ref 46, [b]ref 45, [c]ref 44.

Table 11. K_i values in μM from Ref 47

		R	α-glu'ase[a]	α-glu'ase[b]
63		H	820	2640
64		$COCH_3$	800	-[c]
65		H	0.107	103
66		$COCH_3$	4100	-[d]

[a] Yeast α-glucosidase. [b] Rice α-glucosidase. [c] No inhibition at 1200 μM. [d] No inhibition at 1300 μM.

a half-chair conformation by means of a fused cyclopropane. Crystal structures confirmed that this actually was the case. As could be expected, **65** was the stronger inhibitor and was remarkably potent against α-glucosidase (Table 11). The basic nitrogen was demonstrated to be essential, as the *N*-acetylated derivatives **64** and **66** were weak inhibitors.

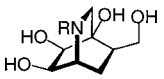
Instead of attempting to mimic a half-chair oxocarbenium ion, Lorthiois et al.[48] attempted to mimic the boat conformation which the substrates have been shown to adopt when binding to several β-glycosidases.[49−51] The conformation of the cyclohexane rings of **67** and **68** were, therefore, locked by a bridgehead. The bridgehead also contained a nitrogen which was supposed to interact with a carboxylic acid residue in the active site. The compounds were shown to be potent inhibitors of snail β-mannosidase, and the large difference in K_i between **67** and **68** suggests that the benzyl group is placed in the aglycon binding site of the enzyme (Table 12). Also, with these compounds the inhibitors are stronger at comparatively high pH, thus indicating that the inhibitors bind as free amines rather than as ammonium salts. It must be stressed, however, that the bound inhibitor can nevertheless be the ammonium salt.

Compounds with a nitrogen in the glycosidic position and with a sulfur in place of endocyclic oxygen have also been synthesized in the hope of preparing a stable and potent glycosidase inhibitor.[52,53] Five different compounds of this type have been synthesized and indeed found to be stable to hydrolysis. It was found that the inhibitor **69**, believed to be the α-anomer, was a remarkably better inhibitor than compounds with an aryl group as aglycon (**70**−**73**, Table 13).[53]

III. Inhibitors That Mimic a Positively Charged Endocyclic Oxygen

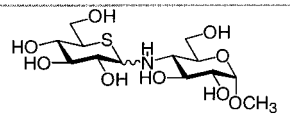
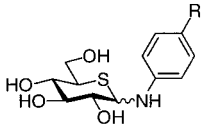
The transition state of acidic glycoside hydrolysis is believed to be late and thus resemble intermediates such as **1** and **5**.[54] Mimics of **5a** are thus potentially

Table 12. K_i values in μM from Ref 48

		R	β-glu'ase[a]	α-man'ase[b]	β-man'ase[c]
67		H	4300*	20000*	20
68		Bn	>5000*	9600*	0.17

*IC_{50} value. [a] *Caldocellum saccharolyticum* β-glucosidase. [b] Jack bean α-mannosidase. [c] Snail β-mannosidase.

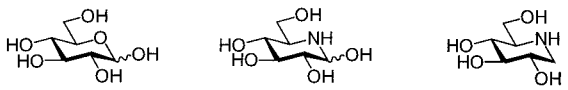
Table 13. K_i values in μM from Ref 53

		R	α-glu'ase[a]	glucoamylase[b]
69			500	4[c]
70		H	>10000	780
71		OCH_3	1050	470
72		NO_2		270
73		CF_3		870

[a] Brewer's yeast α-glucosidase. [b] *Aspergillus niger* G2 glucoamylase. [c] Only the α-anomer, ref 52.

transition-state analogues of glycoside cleavage processes and have been widely investigated. One of these mimics is nojirimycin (**74**).

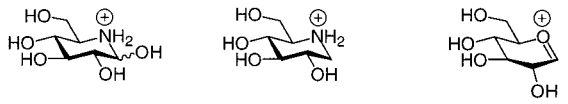
Nojirimycin **74** was isolated from microorganisms (*Streptomyces*) by Ishida et al.[55] Subsequently, 1-deoxynojirimycin **75** was obtained by Inouye et al.[56] by reduction of **74** or by isolation from bacterial culture[57] or mulberries.[58] Over three decades a large number of naturally occurring or synthesized nojirimycins have been reported and tested. **74** resembles glucose very closely, differing only by the presence of an endocyclic nitrogen rather than oxygen (Figure 5).



Glucose **74** **75**

Figure 5.

The protonated inhibitors **76** and **77** mimic the charge development of a transition state resembling glucosyl oxocarbenium ion **5a**, but they have a chair conformation instead of the expected half-chair conformation of **5a** (Figure 6). It is not possible for the

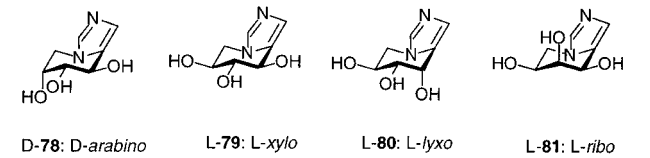


76 **77** **5a**

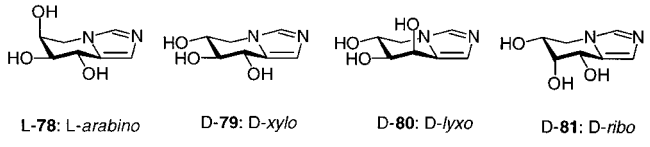
Figure 6.

transition state to have a charge on ring oxygen and a chair conformation at the same time, and therefore, nojirimycin **74** and 1-deoxynojirimycin **75** cannot be expected to be perfect transition-state analogues. The Withers group has made a linear free energy relationship analysis (k_{cat}/K_m vs K_i) of the resemblance of **75** with the transition state of β-glucosidase hydrolysis and concluded that **75** was not a transition-state analogue in that case.[17] Nevertheless, an *N*-substituted derivative of **75** has been used as a transition-state analogue to obtain catalytic antibodies so the compound must have some transition-state character.[59] Therefore, several groups have embarked on a quest to design and prepare glycoimidazole-, glycopyrrole-, and glycotriazole-type glycosidase inhibitors that mimic **5a** better. The following section will only discuss such types of transition-state analogues which mimic both charge and shape. Nojirimycin (**74**) and its five-, six-, and seven-membered analogues are considered natural-product analogues and will not be covered, and the reader is referred to Stütz's book.[8]

In 1991, Streith and collaborators synthesized the interesting series of imidazolopentoses D-**78**,[60,61] L-**79**,[62] L-**80**.[63], L-**78**, D-**79**, D-**80**, D-**81,** and L-**81**.[64] Of those eight stereoisomers, five (D-**78**, D-**79**, L-**79**, L-**80**, L-**81**) showed modest inhibitory properties of some glycosidases whereas the remaining three (L-**78**, D-**80**, D-**81**) were inactive. D-**78** inhibited α-mannosidase from jack beans with a K_i = 54 μM and a weak inhibition of α-mannosidase from almonds (K_i = 1000 μM). The L-**79** isomer proved to be a weak inhibitor of both α-mannosidase from jack beans (K_i = 360 μM) and α-galactosidase from *E. coli* (K_i = 380 μM). Its enantiomer, D-**79**, was a rather weak inhibitor of α-galactosidase from green coffee beans (IC_{50} = 1000 μM). L-**80** exhibited weak inhibition toward both β-galactosidase from jack beans (K_i = 1300) and α-glucosidase from baker's yeast (K_i = 580 μM). L-**81** was a moderate inhibitor of α-galactosidase from *E. coli* (K_i = 89 μM), Figure 7.



D-**78**: D-*arabino* L-**79**: L-*xylo* L-**80**: L-*lyxo* L-**81**: L-*ribo*



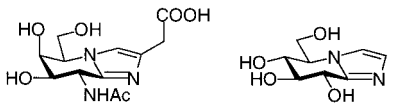
L-**78**: L-*arabino* D-**79**: D-*xylo* D-**80**: D-*lyxo* D-**81**: D-*ribo*

Figure 7.

Even though the half-chair conformation of imidazole analogues **78**−**81** may mimic a oxocarbenium transition state, they lack the hydroxymethyl group which may be important for binding to many glycosidases. As a consequence, it is unlikely that they will have optimal interactions with the glycosidase active

site. The same effect has been found in five-carbon iminoalditols.[65] The extent of the impairment by lacking the hydroxymethyl group seems to be dependent on the enzymes. It is worth noting that these azasugars showed more potent inhibition of α-glycosidases than β-glycosidases.

Compared with nagstatine **82**, a natural product (Figure 8),[66,67] the inhibitors **78**−**81** are quite poor



82 **83**

Figure 8.

glycosidase inhibitors and differ from **82** by having the most basic nitrogen atom one bond farther away. This suggests that it may be essential to have a basic nitrogen positioned as in **82**. This is supported by the report of Tatsuta et al. that an imidazole analogue **83** was a potent inhibitor of β-glucosidase from almonds (IC_{50} = 0.7 μM, see section V) and the findings of Vasella et al. regarding neutral tetrazole **2**, which is a relatively poor inhibitor (see section VI).

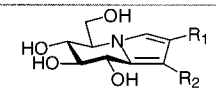
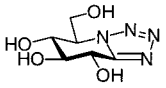
These observations led Heightman and Vasella to propose that β-glycosidases do not protonate their substrates from above but laterally, either anti or syn to the endocyclic C1−O bond (see section V).[188]

To support this hypothesis Vasella et al. designed analogues, **84**−**93**, which lack a nitrogen atom at the glycosidic position.[68,69] The inhibition data are summarized in Tables 14 and 15, respectively.

Indeed, all derivatives showed weaker inhibition than the corresponding tetrazoles **2** and **94**, respectively (Tables 14 and 15). The higher K_i values of these pyrroles indicate clearly a heteroatom, corresponding to the glycosidic O-atom, is required for strong inhibition. A remarkable increase of inhibition of β-glucosidase is observed for **89** and **92** compared to **84** and **85**. This possibly reflects that the interaction between a 2-NH_3^+ and the nucleophilic carboxylate contributes to an increase of the inhibitory strength. It is also worth noting that **85**, which possesses a =C−COOMe group at the glycosidic position, is a stronger inhibitor than **84**, in which the =C−COOMe group is one bond farther away, whereas **89** is a stronger inhibitor than **92**. The authors explained that the methoxycarbonyl group of **85** interacts with catalytic acid (see **A** and **B** in Figure 9) similar to the glycosidic heteroatom of **2** (see **C** in Figure 9), and the lower inhibitory activity of **85** than **2** is ascribed to the lower basicity and the cost of the required positional adjustment. This interaction is lost upon replacement of the OH group by an NH_3^+ group, perhaps by a competing intramolecular H-bond from the NH_3^+ to the methoxycarbonyl group or under the influence of the stronger π-acceptor on the basic properties of the ester (Figure 9). Surprisingly, β-glucosaminidases are inhibited about twice as strongly by the trifluoroacetamido analogues **90** and **93** than by the acetamides **88** and **91**.

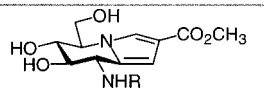
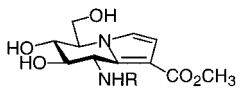
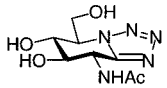
To further strengthen the postulate of in-plane protonation, triazoles **95**, **96,** and **97** were synthesized and their inhibition of β-glycosidases was compared with that of the tetrazoles **2** and **3** (see

Table 14. K_i values in μM from Ref 244

		R_1	R_2	β-glu'ase[a]	β-glu'ase[b]
84		CO_2CH_3	H	6000	1150* (pH6.8)
85		H	CO_2CH_3	300	410* (pH6.8)
86		CO_2CH_3	CO_2CH_3	25000	-
87		CH_2OH	CH_2OH	14000	-
2				200	5

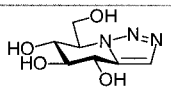
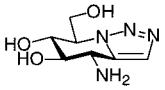
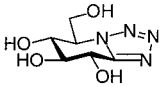
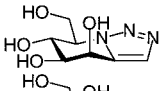
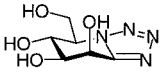
*Estimated values. [a] Almond β-glucosidase. [b] *Caldocellum saccharolyticum* β-glucosidase.

Table 15. K_i values in μM

		R	β-glucosaminidase[a]	β-glu'ase[b]
88		Ac	19	-
89		H	-	12 (pH 6.8)
90		CF_3CO	10	-
91		Ac	20	-
92		H	-	21 (pH 6.8)
93		CF_3CO	13	-
94			0.2	-

[a] Bovine kidney β-glucosaminidase. [b] *Caldocellum saccharolyticum* β-glucosidase.

Table 16. K_i values in μM

		β-glu'ase[a]	β-glu'ase[b]	β-man'ase[c]	β-gal'ase[d]
95		>8000	2000	-	>8000
96		>8000 (pH 6.8)	2800 (pH 6.8)	-	-
2		200	5	-	1.5
97		-	-	>8000	-
3		-	-	150	-

[–]No inhibition. [a] Almond β-glucosidase. [b] *Caldocellum saccharolyticum* β-glucosidase. [c] Snail β-mannosidase. [d] Bovine liver β-galactosidase.

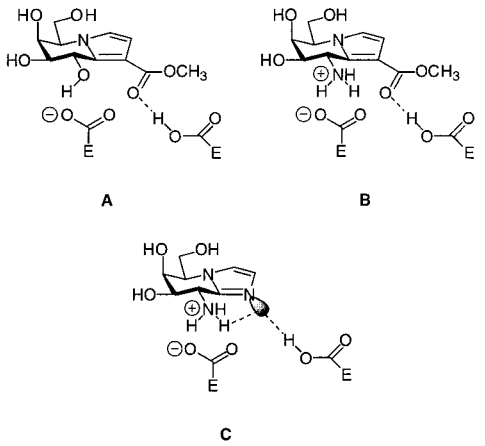
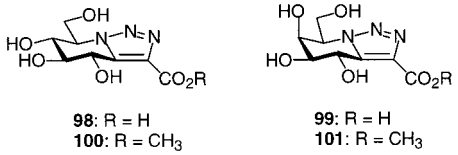


Figure 9.

section VI). A dramatically different inhibitory potency was observed. At a concentration of 8000 μM, **95** showed no activity against the β-glucosidase from sweet almonds and was 400 times weaker than the tetrazole **2** against β-glucosidase from *Caldocellum saccharolyticum*. Interestingly, **96** showed similar inhibitory strength to **95**. The mannotriazole **97** is inactive at 8000 μM against snail β-mannosidase, Table 16.

Fleet et al. also prepared glycotriazoles **98** and **99** and their corresponding esters **100** and **101** and tested these on a number of glycosidases (Figure 10).



98: R = H
100: R = CH_3

99: R = H
101: R = CH_3

Figure 10.

However, no significant inhibition of glycosidases was observed by either of the triazole carboxylic acids **98** and **99** or the methyl esters **100** and **101** at the concentration of over 500 μM, but the compounds showed weak inhibition of glycogen phosphorylase b with a K_i 7400 μM.[70]

Though pyranose imidazole **106** was first prepared by Streith et al. in 1991,[60] its inhibitory activity was not determined until 1999,[71] when related derivatives were also reported. Toward β-glycosidase all analogues were inactive. The dihydroxy compounds **102** and **103** exhibited practically no inhibition of α-glycosidases either, while **104** showed slight inhibitory activity toward α-mannosidase from jack bean with IC_{50} = 500 μM. These low activities can partly be ascribed to the absence of hydroxymethyl group. Among the trihydroxy compounds **106** had no inhibition of α-glycosidase, **104** exhibited moderate inhibition of α-mannosidase (IC_{50} = 150 μM), whereas **105** showed strong inhibition of this enzyme (IC_{50} = 10 μM, K_i = 5 μM). This is clearly due to the correct configuration of hydroxyl groups in **105**, Figure 11.

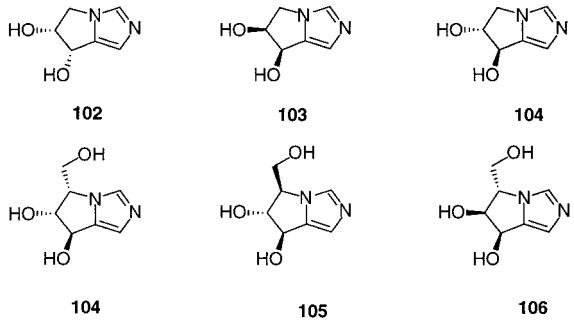


Figure 11.

Recently, a tetrahydro-4*H*-1,2,3-trizolo[1,5-*a*]azepin **107** was synthesized by Martin and co-workers.[72] The effect of this compound on a number of glycosidases was investigated. Homonojiritriazole **107** showed only weak inhibition of α-galactosidase from *E. coli* (inhibition 36% at 1000 μM) and isomaltase from bakers yeast (37% at 1000 μM). In addition, it appears that insertion of a β-configured hydroxymethine group in the saturated ring is not sufficient to restore inhibition: the additional OH group does not

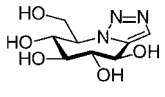


Figure 12.

play the role of a surrogate of a glycosidic heteroatom, Figure 12.

A. Glycosidase Inhibitors in the Form of Cyclic Sulfonium Ions

Mimicry of the developing positive charge on the endocyclic oxygen has been accomplished by using sulfonium ions. The first compound synthesized of this class was a salt **108** closely related to the alkaloid swainsonine (Figure 13). Compound **108** was tested

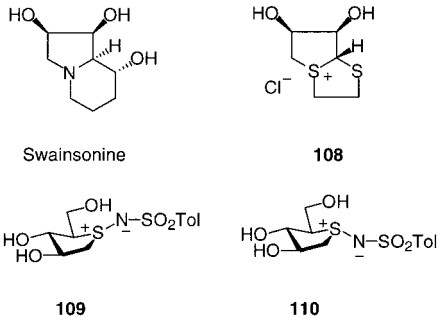


Figure 13.

against 12 different human enzymes but showed only appreciable inhibition of two enzymes. Inhibition of α-mannosidase (76% at 1 mM, pH 6.5) was somewhat smaller than by swainsonine but comparable with that of 1-deoxymannonojirimycin. α-Fucosidase was also moderately inhibited (41% at 1 mM, pH 5.5) by the cyclic sulfonium ion **108**.[73]

Two diastereomeric compounds, **109** and **110**, containing a trivalent sulfur were also investigated as glycosidase inhibitors by Wong and co-workers. Both compounds were tested for inhibition against almond *β*-glucosidase, bovine kidney *N*-acetyl-*β*-glucoaminidase, and brewer's yeast α-glucosidase. Only *β*-glucosidase was weakly inhibited (K_i = 1.7 mM) and only by one of the diastereoisomers.[74]

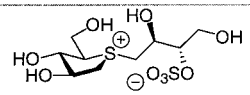
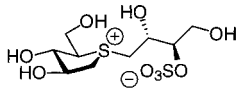
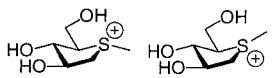
The most interesting member of this class of glycosidase inhibitors is perhaps the natural compounds salacinol **111** and kotalanol **115** (Tables 17 and 18). These natural products are beyond the scope of this review. However, they deserve to be mentioned because they are potent natural inhibitors of α-glucosidases. Because of this powerful affinity, they have been used as a treatment of diabetes in traditional Indian medicine. Two recent total syntheses of salacinol have appeared[75,76] as well as synthesis of a sulfonium-ion analogue of natural compound castanospermine.[77]

IV. Transition-State Analogues with Nitrogen in the Anomeric Position

The transition state of enzymatic glycoside cleavage may involve charge on the anomeric carbon through either resemblance of the intermediates carbocation **1** or oxocarbenium ion resonance form **5b** (Figure 1). Whether the transition state has more charge on the ring oxygen and resembles resonance form **5a** or has more charge on the anomeric carbon and resembles resonance form **5b** (or conformer **1**) may depend on the enzyme studied. The question was recently addressed for nonenzymatic glycoside hydrolysis with the claim being made that in that case 97% of the positive charge in the transition state resides on the oxygen atom.[78] This claim has been questioned however.[79]

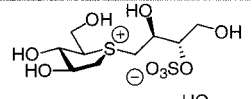
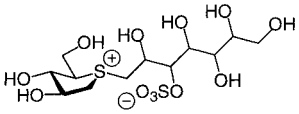
One way of mimicking **1** would be to incorporate a nitrogen atom in place of C1.[80−82] This idea has been investigated mainly by the groups of Bols, Ichikawa, and Nishimura. Nishimura has thoroughly investi-

Table 17. IC_{50} values in mM from Ref 245

		α-glu[a]	α-glu[b]	α-glu[c]	α-glu[d]	β-glu[e]	α-man[f]
111		0.0011			2.5		2.1
112		0.38				3.4	3.6
113/ 114[g]		2.0	2.2	2.1	1.4		1.2

[a] Rice α-glucosidase. [b] Baker's yeast α-glucosidase. [c] Yeast α-glucosidase. [d] *B. Stearothermophilus* α-glucosidase. [e] Almond *β*-glucosidase. [f] Almond α-mannosidase. [g] 4:6 mixture of diastereoisomers.

Table 18. K_i values in *μ*M

	Structure	Sucrase[a]	Maltase[a]	Isomaltase[a]
111		0.96[b]	0.93[b]	1.41[b]
115		0.43[c]	0.54[c]	4.25[c]

[a] Rat α-glucosidase. [b] Reference 246. [c] Reference 247.

gated Siastatin B analogues, which are in fact 2-aminoisofagomine analogues.

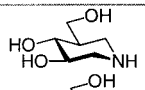
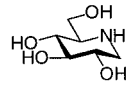
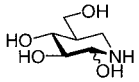
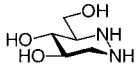
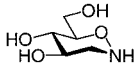
A. The Isofagomines

In 1994 the first synthetic azasugar **116** with nitrogen in the pseudo-anomeric position was prepared.[83,84] For stability reasons the 2-hydroxy group had been omitted, and because of the close resemblance between **116** and the natural product fagomine, the compound was named isofagomine. Isofagomine is a potent inhibitor of β-glucosidase, while α-glucosidase inhibition is moderate (Table 19). The inhibition of sweet almond β-glucosidase by **116** has been more deeply investigated. The onset of inhibitor was found to be slow, and the binding was found to be driven by a large increase in entropy. This was in striking contrast to the binding of **75**, which was found to bind due to a decrease in enthalpy.[85] Isofagomine **116** has been recognized as a potential drug in the treatment of type-II diabetes due to its powerful inhibition of glycogen phosphorylase.[86] Recently, an exhaustive study of analogues was conducted but only resulted in poorer inhibitors.[87] Isofagomine **116** has also been shown to inhibit glycogen phosphorylase activity in cultured astrocytes and mouse brain, where it demonstrated IC_{50} values of 3.3 and 1.0 μM, respectively.[88]

Soon after the first synthesis of **116** (for subsequent syntheses of **116**[89−92,94]), other 1-iminosugars-type inhibitors were prepared. They all showed potent inhibition of their corresponding β-glycosidases. The α-glycosidases, however, were generally more powerfully inhibited by the 1-deoxynojirimycin analogues. Hence, β-glucosidases, β-galactosidases,[93,94] and β-glucuronidases[94,95] were all strongly inhibited by isofagomines **120** (Table 20, alternative synthesis[96]) and **128** (Table 22, alternative synthesis[89]), respectively. L-*Fuco*-isofagomine **125** (Table 21),[94,97−99] however, was a relatively modest inhibitor of α-fucosidases when compared to the corresponding 1-deoxynojirimycin analogue **126**.

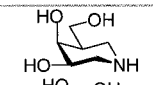
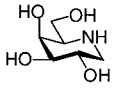
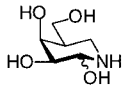
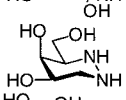
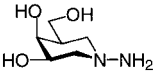
Several explanations of the observed inhibitory profile of isofagomines compared to that of the related 1-deoxynojirimycins have been offered. It has been proposed that strong interactions between the protonated form of the piperidine and the active site nucleophilic carboxylate are the reason for the observed potent inhibition. The differentiation between isofagomines and 1-deoxynojirimycins by α- and β-glycosidases has been proposed to be due to the

Table 19. K_i values in μM

		α-glu'ase[a]	β-glu'ase[b]	isomaltase[c]	glucoamylase[d]	α-man'ase[e]	GP'ase[f]
116		86[g]	0.11[g]	7.2[g]	3.7[h]	770[h]	0.7*,[i]
75		25[h]	47[h]	11[h]	9.8[h]	270[h]	55000[j]
117		0.022[k]	0.069[k]	0.025[k]			
118		6.9[l]	0.32[l]	0.27[l]		3306#,[j]	13.5*,#,[j]
119		-[m]	60#,[m]				

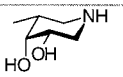
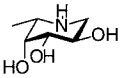
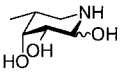
*IC_{50} value. # Inhibitor is racemic. − No inhibition. [a] Yeast α-glucosidase. [b] Sweet almond β-glucosidase. [c] Yeast isomaltase. [d] *Aspergillus awamori* glucoamylase. [e] Jack bean α-mannosidase. [f] Glycogen phosphorylase. [g] Reference 83. [h] Reference 110. [i] Reference 87. [j] Reference 100. [k] Reference 126. [l] Reference 204. [m] Reference 154.

Table 20. K_i values in μM

		α-gal'ase[a]	β-gal'ase[b]	β-gal'ase[c]	β-gal'ase[d]	β-glu'ase[e]
120		50[f]	0.004[g]	0.2#,[f]	0.33[f]	0.097[h]
121		0.0016[i]		12.5[i]	81[f]	540[i]
122		0.74[j]	0.035[j]	0.40[j]	0.091[j]	
123		0.28[f]	0.04[f]	0.30[f]	7.8[f]	0.13[f]
124		73[k]	4.5[k]			410[k]

Inhibitor is racemic. [a] Green coffee bean α-galactosidase. [b] *Aspergillus oryzae* β-galactosidase. [c] *E. coli* β-galactosidase. [d] *Saccharomyces fragilis* β-galactosidase. [e] Sweet almond β-glucosidase. [f] Reference 211. [g] Reference 93. [h] Reference 85. [i] Reference 7. [j] Reference 126. [k] Reference 217.

Table 21. K_i values in μM

		α-fuc'ase[a]	α-fuc'ase[b]
125		8.4[c]	6.4[d]
126		0.0013[e]	
127		0.0047[f]	0.0032[f]

[a] Bovine kidney α-L-fucosidase. [b] Human placenta α-L-fucosidase. [c] Reference 97. [d] Reference 98. [e] Reference 248. [f] Reference 126.

location of the active site carboxylate. Hence, the catalytic carboxylate in, e.g., β-glucosidase is located beneath the sugar ring, thereby interacting strongly with the protonated isofagomine, whereas no favorable interaction can be made directly with the protonated 1-deoxynojirimycin. In α-glucosidase, on the other hand, the catalytic carboxylate in α-glucosidase is located slightly above the sugar ring, thereby being able to favorably interact with the protonated 1-deoxynojirimycin but not the protonated isofagomine.[188] This does not explain, however, why the 2-hydroxy analogue **117** (noeuromycin) is so strong an α-glucosidase inhibitor. It was, therefore, recently proposed that the catalytic nucleophile of both α- and β-glycosidases can interact with the anomeric nitrogen atom and that the weak inhibition of α-glycosidases by the isofagomines is due to their lack of an essential hydroxyl group.[126]

Another explanation is simply that isofagomines in their protonated form imitate the transition state of enzymatic β-glycoside hydrolysis. Because of the lack of sp^2 character by the piperidines, this has been a much-challenged view. However, it has been suggested that β-glycoside hydrolysis happens through a simple dissociation in the chair ground-state conformation giving a C1 cation that later rehybridizes to the resonance-stabilized oxocarbenium ion. Thereby a protonated isofagomine would be a good analogue of both charge and shape of a high-energy species.[94,100,263] Isofagomine **116** has also been suggested to mimic the intermediate glycosyl−enzyme intermediate rather than the transition state. This proposal was based on the similarity between the X-ray structure of an isofagomine−enzyme complex and a 2-deoxy-2-fluoroglycosyl−enzyme complex.[219] It is also supported by the accepted view that the transition-state structure in the enzymatic β-glycoside hydrolysis differs from that of the α-glycoside.[101]

Cleavage of the α-glycosidic bond has been suggested to be facilitated by electron donation from the endocyclic oxygen lone pair. Thereby a transition state that resembles oxocarbenium ion resonance form **5a** would be the direct result. The protonated form of 1-deoxynojirimycin (**77**, Figure 6) is only a partial mimic of this ion closely related to the proposed transition state, since it imitates only charge but not shape.[94,263]

The 2-deoxyribofuranose analogue **133** and the *N*-acetylneuraminic acid (**132**) analogue **131** have also been made in the hope of obtaining potent inhibitors of human purine nucleoside phosphorylase and bacterial and viral sialidases, respectively (Figure 14). Imitation of aglycon was attempted in both

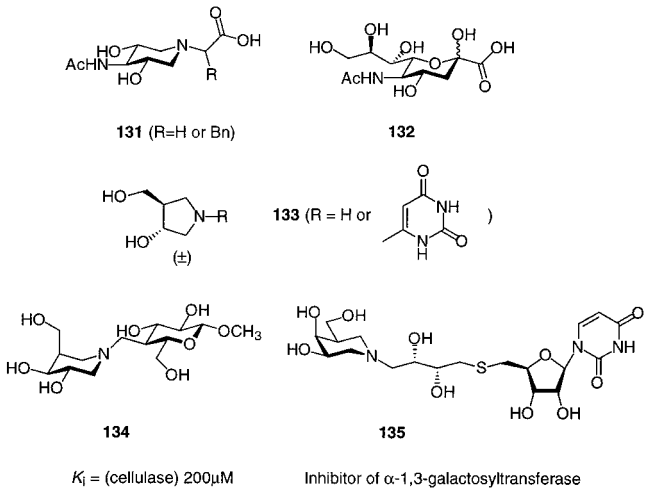
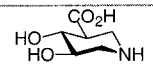
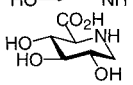
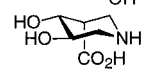


Figure 14.

cases, which resulted in inhibition constants in the micromolar region. Better aglycon mimicry and incorporation of a glycerol side chain in *N*-acetylneuraminic acid analogue **131** was suggested to be able to improve inhibition.[101−104]

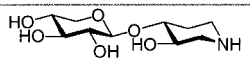
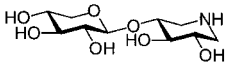
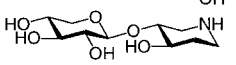
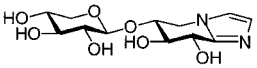
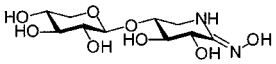
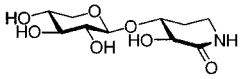
A xylobiose analogue of isofagomine, **136**, has been made by the Withers group together with five other xylobiose analogues (**137**−**141**), and they have been investigated as inhibitors (Table 23) of two different xylanases, namely, the retaining enzymes Cex (*Cellulomonas fimi*) and Bcx (*Bacillus circulans*). These enzymes belong to different glycosidase families; Cex is an *anti*-protonator of family 10, while Bcx is a *syn*-protonator of family 11.

Table 22. K_i values in μM

		β-gluu'ase[a]	β-gluu'ase[b]	α-glu'ase[c]	β-glu'ase[d]
128		0.079[e]		>1000*,[f]	>1000*,[f]
129			80[g]	6.1%[§,h]	89.4%[§,h]
130		1.3*,[e]		>1000*,[f]	>1000*,[f]

*IC_{50} value. § Inhibition at 0.56 mM. [a] Bovine liver β-glucuronidase. [b] Human liver β-glucuronidase. [c] Yeast α-glucosidase. [d] Sweet almond β-glucosidase. [e] Reference 95. [f] Reference 94. [g] Reference 249. [h] Reference 250.

Table 23. K_i values in μM

		Bcx[a]	Cex[b]	Cex-mutant[c]
136		1100[d]	0.13[d]	1.6[e]
137		1500[d]	5.8[d]	4800[e]
138		3100[d]	110[d]	
139		520[d]	0.15[d]	200[e]
140		1400[d]	0.37[d]	1300[e]
141		9000[e]	0.34[e]	2000[e]

[a] *Bacillis circulans* β-xylosidase. [b] *Cellulomonas fimi* β-xylosidase. [c] N126A Cex-mutant. [d] Reference 105. [e] Reference 124.

The inhibitor **136** was an inhibitor of Cex-xylanase in the nanomolar region ($K_i = 0.13$ μM) and as such the strongest among the inhibitors toward this enzyme. All the inhibitors studied were poor inhibitors of Bcx-xylanase with inhibition constants ranging from 520 to 9000 μM. This low activity against the family 11 xylanase was explained by the enzyme being a *syn*-protonator that favors boat conformers in the enzyme-catalyzed transition state, while Cex was believed to prefer a half-chair conformation.

From X-ray structures of the 2-fluorocellobiosyl covalent intermediate of Cex, it has been suggested that residue Asn126 interacts with the 2-OH. To investigate the effect of this aspargine residue, the Asn126 was changed by mutation to an alanyl residue. Many of the inhibitors of the natural enzyme which had a 2-hydroxyl group were poor inhibitors of this mutant, which showed that the 2-hydroxyl group contributed significantly to the binding of these inhibitors. However, the xylobiose isofagomine **136** was still a strong inhibitor,[105,106,124] which is consistent with its lack of a 2-OH.

All of the above-mentioned xylobiose-derived azasugars except the fagomine (1,2-dideoxynojirimycin, **138**) analogue were viewed as inhibitors complexed in the Cex active site through X-ray crystallography. It was shown that, as expected, all inhibitors were bound in the -2 and -1 subsites. It was further revealed that for deoxynojirimycin analogue **137** together with the isofagomine **136**, the azasugar moiety adopted a $^4C_1$ conformation. Even though xylobiose isofagomine **136** is a very potent inhibitor of Cex, it demonstrated only a few contacts with the enzyme. The most important interaction was proposed to be between the protonated anomeric nitrogen and the oxygen of the catalytic nucleophile. Because of this minority of inhibitor enzyme interactions and the conformation of the isofagomine, its strong binding was proposed to be fortuitous. 1-Deoxynojirimycin analogue **137** binds 830 times better than xylobiose, although no direct interactions were observed to the azasugar's nitrogen atom. Two water molecules, however, were found to form hydrogen bonds to the basic center, one of which forms a hydrogen bond back to the enzyme.[219]

B. N-Substituted Isofagomines

The endocyclic amine functionality of isofagomines provides an attractive handle for making analogues by alkylating the nitrogen atom. This has in the 1-deoxynojirimycin (**75**) cases produced highly active compounds such as *N*-2-hydroxyethyl-1-deoxynojirimycin (miglitol) and *N*-butyl-1-deoxynojirimycin.[107,108]

Inhibition of glycogen phosphorylase (pig liver) was investigated with a series of *N*-alkylated isofagomines including ring-substituted *N*-benzoylmethyl isofagomines. Together with different isomeric isofagomines inhibition results only showed loss of activity.[87]

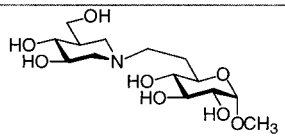
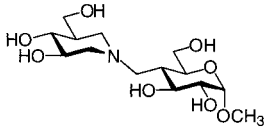
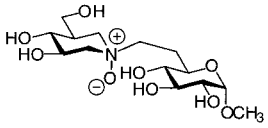
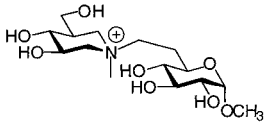
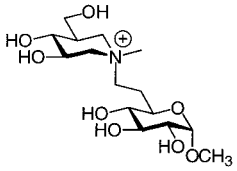
Isofagomines with stereochemistry resembling D-glucose (**116**), D-galactose (**120**), L-galactose, and L-fucose (**125**) have been studied as *N*-butyl derivatives. D-Isogalactofagomine (**120**) was also investigated for glycosidase inhibition in its *N*-butyl-*N*-oxide form. A moderate loss of inhibition was observed in each case toward the corresponding β-glycosidase for the D-*galacto* (IC_{50} (*N*-butyl) = 5 μM, IC_{50} (*N*-butyl-*N*-oxide) = 9.5 μM) and D-*gluco* (IC_{50} (*N*-butyl) = 38 μM) isofagomines. In the L-*galacto* case, however, both the isofagomine and the butylated version showed equal potency (IC_{50} = 300 μM) toward β-galactosidase. Inhibition of the corresponding α-glycosidases was roughly unchanged for *N*-butylated versions of isofagomines compared to the secondary amines; D-*gluco* (IC_{50} = 140 μM), D-*galacto* (IC_{50} (*N*-butyl) = 45 μM; IC_{50} (*N*-butyl-*N*-oxide) = 90 μM), L-*galacto* ($IC_{50} > 1000$ μM). L-Fuco-analogue, however, demonstrated a moderate loss in enzyme affinity upon butylation (IC_{50} = 270 μM) compared to **125**. The *N*-butyl derivative demonstrated an enhancement in inhibition of β-glucosidase (IC_{50} = 80 μM) by an order of magnitude compared to **125**.[94]

Racemic **120** has been studied as its *N*-methyl derivative. α-Galactosidase inhibition was roughly maintained (K_i = 74 μM) compared to the demethylated inhibitor (**120**), but inhibition at β-galactosidase was negatively affected upon *N*-methylation (K_i = 77 μM).[109]

C. Mimicry of the Aglycon Segment

A way of enhancing inhibitor binding to enzyme could be to augment the number of enzyme inhibitor interactions. Since the natural substrates for representative glycosidases are di- or oligosaccharides, imitation of the aglycon part is believed to be important. Targeting α-glucosidases and glucoamylase, isomaltose, and maltose analogues with isofagomine as the glycon moiety have been prepared.[83,110,111] They showed that inhibition of almond β-glucosidase and yeast isomaltase deteriorated upon introduction of aglycon (Table 24), except in the case of isomaltose analogue *N*-oxide where inhibition was essentially unchanged compared to the parent compound **116**. Furthermore, inhibition of α-glucosidase was basically unaffected by isomaltose analogue and its *N*-oxide compared to that of isofagomine itself. Toward glucoamylase (*Aspergillus niger*), an enzyme powerfully inhibited by the pseudo-tetrasaccharide acarbose (K_i = 0.024 μM, Figure 2)[110] activity was considerably enhanced in the cases of maltose ana-

Table 24. K_i values in μM

		α-glu'ase[a]	β-glu'ase[b]	isomaltase[c]	glucoamylase[d]
142		59[e]	2.3[e]	100[e]	0.063[e]
143		>500[f]	28[f]	480[f]	0.54[f]
144		70[e]	0.38[e]	19[e]	0.24[e]
145		280[e]	150[e]	>1000[e]	94[e]
146		>1000[e]	510[e]	190[e]	160[e]

[a] Yeast α-glucosidase. [b] Sweet almond β-glucosidase. [c] Yeast isomaltase. [d] *Aspergillus awamori* glucoamylase. [e] Reference 110. [f] Reference 111.

logue, isomaltose analogue, and the *N*-oxide of the latter. The distance between the sugar residues was used as an explanation of the stronger inhibition by isomaltose analogues, compared to that of maltose analogue. It was argued that lengthening of the glycosidic bond occurs in the transition state of hydrolysis; thus, the methylene spacer of the maltose analogue would be too short. The good inhibition by the *N*-oxide compared to other disaccharide analogues may be due its potential ability to mimic the negative charge of a departing negative oxygen.

The two diastereomeric ammonium ions of the isomaltose analogue showed a significant loss of activity toward all enzymes investigated compared to isofagomine **116**. The reason for this was proposed to be sterical hindrance by methyl groups and restricted flexibility of the ammonium ions.

An isofagomine isomer analogous to D-gulo- or D-idopyranose linked to a glucosidic unit **134** has been investigated as an inhibitor of cellulase from *Humicola insolens* (Figure 14). On the basis of the X-ray structure of a non-hydrolyzable thiooligosaccharide/cellulase complex, the isofagomine isomer unit was chosen since it was believed to imitate the ring-distorted substrate. The disaccharide analogue was demonstrated to be a competitive inhibitor with an inhibition constant (K_i) of 200 μM.[112]

An effort to rationally design an inhibitor of α-1,3-galactosyl transferase was made by Ichikawa and coworkers.[113] By building a mimic of UDP-galactose using isogalactofagomine, a diol from L-tartric acid, and 5′-thio-uridine moiety, a more selective (mixed) inhibitor **135** of the target enzyme was found (Figure 14). Galactosyltranferase inhibition compared to that from the azasugar **120** itself was doubled, and the unwanted inhibition of β-galactosidase was considerably diminished. Neither **120** nor **135** affected bovine β-1,4-galactosyltransferase. Interestingly, 1-deoxygalactonojirimycin **121** inhibits neither the α-1,3- nor the β-1,4-transferase.[114]

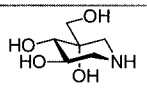
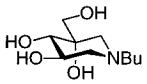
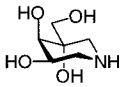
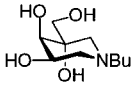
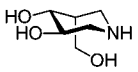
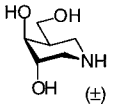
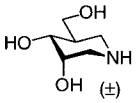
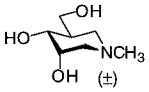
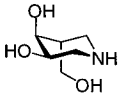
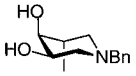
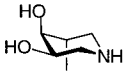
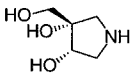
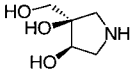
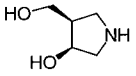
D. Exchange or Addition of Isofagomine Hydroxyl Groups

The early chiral pool syntheses of **116** and **120** involved many steps. More easily obtained, both from D-mannose, were the isofagomine analogues **147** and **149**, which have an extra hydroxyl group in the 5-position[115−117] The compounds showed that a high degree of inhibitory activity was lost toward all glycosidases investigated when this OH was incorporated. Activity was also lost upon *N*-butylation (Table 25). 5-Hydroxyisofagomines with a *N*-butyl or *N*-octyl substituent, however, demonstrated some inhibition of glycolipid biosynthesis.[115]

Isofagomine analogues of both enantiomers of 2-deoxyribose with an extra 4-hydroxy group, **158** and **159**, were prepared. Analogue **158** demonstrated essentially little change in activity (K_i = 180 μM),[116] compared to the analogous nonhydroxylated racemic diol (**133**, R = H, K_i = 160 μM), toward purine nucleoside phosphorylase from human blood.[102,103] Triols were also tested for inhibitory activity against α- and β-glucosidase. Neither enantiomer had any significant activity,[116] which was also the case for iminosugar **160**, which additionally was tested against galactosidases, mannosidases, xylosidases, and an α-L-fucosidase.[118]

As a potential inhibitor of fucosidases, the analogue of **125** was prepared with the 5-methyl group ex-

Table 25. K_i values in μM

		α-glu'ase[a]	β-glu'ase[b]	α-gal'ase[c]	β-gal'ase[d]
147		230[f]	4.3[g]	>5000*[,g]	>5000*[,g]
148		1500*[,h]	850*[,h]		
149		>2000*[,i]	420*[,i]	610*[,i]	5.7[i]
150			400*[,h]		
151		>1000*[,h]	8*[,h]	>1000*[,h]	>1000*[,h]
152		>1000[j]	22[j]	>1000[j]	405[§,j]
153		>1000[j]	76[j]	37[j]	9[j]
154		>1000[j]	>1000[j]	>1000[j]	530[j]
155		-[k]	-[k]	-[k]	
156		60[k]	1700[k]	89[k]	180[e,k]
157		-[k]	140[k]	-[k]	-[e,k]
158		3800[f]	1400[f]		
159		2000[f]	1130[f]		
160		800[l]	1000[l]	-[l]	

*IC_{50} values. – No inhibition. § Noncompetitive inhibition. [a] Yeast α-glucosidase. [b] Sweet almond β-glucosidase. [c] Green coffee bean α-galactosidase. [d] *Aspergillus oryzae* β-galactosidase. [e] *E. coli* β-galactosidase. [f] Reference 116. [g] Reference 115. [h] Reference 94. [i] Reference 117. [j] Reference 109. [k] Reference 120. [l] Reference 118.

changed for a methyleno or a methoxy substituent. No inhibition of fucosidase or other glycosidases by these azasugars was observed below 1 mM.[97]

All stereoisomers of isofagomine **116** have been prepared.[94,109,119,120] Generally, inhibitors with an isomeric configuration compared to the natural substrate for the enzyme show considerably less activity compared to that of rightly configured inhibitors. The only exceptions are isogalactofagomine **120**, which inhibits almond β-glucosidase as well as isofagomine **116**,[85] and racemic isoallofagomine **153**, which is more potent than isogalactofagomine **120** toward α-galactosidase.[109]

A series of racemic isofagomine analogues **161**–**174** has been prepared, where the 3-hydroxy group was exchanged for other polar groups such as amino group, carboxylic acid, and hydroxymethyl (Figure 15). The result of this study was in most cases complete loss of activity. Only a few moderate inhibitors of β-glucosidase were identified. Surprisingly the inhibitors **169** and **170** which have an epimeric configuration, compared to that of **116**, showed the greatest activity. It was, therefore, proposed that almond β-glucosidase had a previously unexploited binding site beneath the sugar ring to which the 3-hydroxy methyl or 3-amino group could interact.[121,122] Investigating the inhibition by 3-deoxyisofagomine **173** proved that the contribution to inhibitor binding of the 3-substituent was essential, since the deoxy compound showed no activity. A hybrid compound **174** of **116** and **170** was thought to exploit the proposed two binding sites, thereby being a

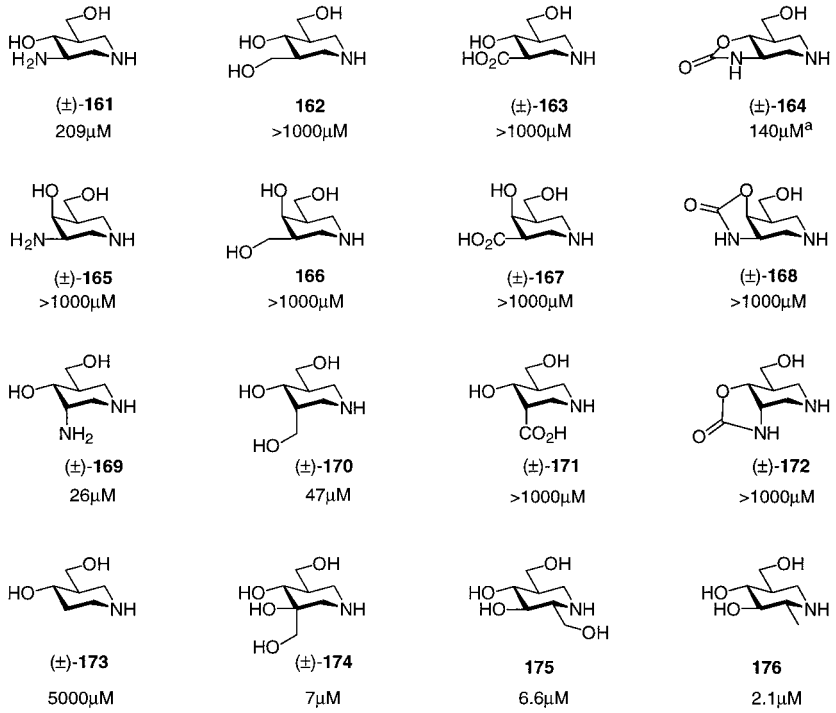


Figure 15. Inhibition constants (K_i) against sweet almond β-glucosidase. From refs 121, 123, and 125. [a]Uncompetitive inhibition.

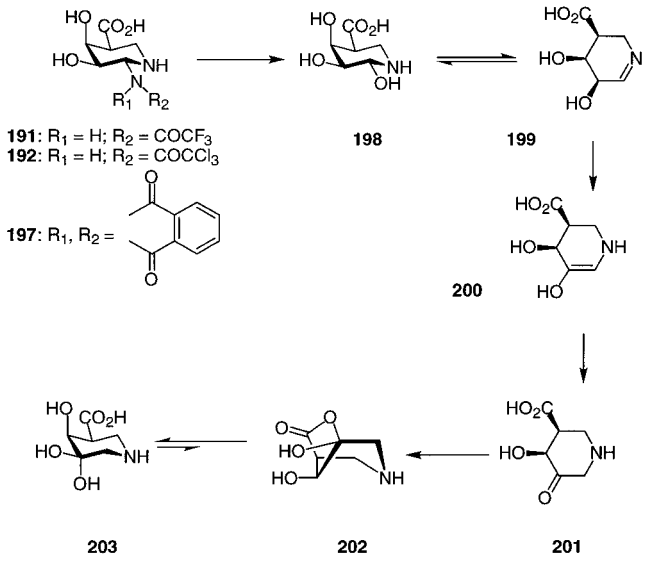
stronger inhibitor than isofagomine (**116**) itself. The inhibition of β-glucosidase by **174**, however, turned out to be 32 times weaker than **116**. This was explained by competition of the two 3-substituents to the same binding site.[123]

E. 2-Hydroxy Analogues

It is believed that the 2-OH of natural compound 1-deoxynojirimycin **75** greatly contributes to enzyme binding, since removal of this group results in a significant decrease of inhibitory activity.[8] Although desirable, it was speculated that direct introduction of a 2-OH into **116** would result in a highly unstable hemiaminal.[85,105,124] As a replacement, a 2-hydroxymethyl and a methyl group were introduced to form homoisofagomines **175** and **176**, which were prepared together with their 5-epimers (Figure 15). The azasugars **175** and **176** were investigated for inhibition of yeast α-glucosidase, jack bean α-mannosidase, and almond β-glucosidase. Only **175** and **176** were inhibitors and only of the latter enzyme, which was competitively inhibited in the low micromolar region. The 20−60 times lower enzyme affinity compared to **116** was attributed to steric hindrance by the 2-substituents.[125]

Although an isofagomine with a hydroxy group neighboring the nitrogen was believed not to be stable, synthesis was attempted in order to test this proposed instability. The isofagomine-like hemiaminal **117** was discovered on the day of the Danish Euro Referendum (Sept 28, 2000), and because of its similarity with natural compound nojirimycin, it was given the name noeuromycin. It seemed to be fairly stable in acidic solution, but at neutral pH it underwent the Amadori rearrangement (see Scheme 2). The compound, however, was stable enough under assay conditions, and the activity of **117** was demonstrated to be in the nanomolar region for α-glucosidase, isomaltase, and β-glucosidase (Table 19). The inhibition of the α-glucosidases was much higher than that of the 2-deoxy compound **116**. To further elaborate on this important finding, the study was extended to involve D-galactose and L-fucose analogues as well. They also showed from submicromolar to low nanomolar inhibition of their corresponding glycosidases. L-*Fuco*-analogue **127** was a strong inhibitor of both bovine kidney and human placenta α-L-fucosidase (Table 21). D-*Galacto* analogue **122** was especially a strong inhibitor against β-galactosidase from *Sacc. fragilis*. Other β-galactosidases were not inhibited as strongly as by **120**, and α-galactosidase inhibition was considerable though still 500 times weaker than that of 1-deoxygalactonojirimycin

Scheme 2



(**121**, Table 20). This result showed, however, that contrary to past beliefs, inhibitors with nitrogen in the anomeric position can effectively inhibit α-glycosidases.[126] Independently of this work done in the Bols group, Nishimura and co-workers discovered that **127** was an extremely strong fucosidase inhibitor. This was realized by following the degradation of the corresponding 2-aminoisofagomine by measuring the inhibitory activity, at pH 6.3. The 2-aminoisofagomine was shown to turn into hemiaminal, which later formed a hydrated ketone through an Amadori rearrangement. This was also observed for the galacturonic-type 2-aminoisofagomine, which also had a high activity toward *β*-glucuronidase after degradation.[127]

F. 2-Amino Analogues

Close relatives of isofagomine **116** are siastatin B (**177**) and its derivatives. Siastatin B (**177**) was isolated by Umezawa et al. in 1974 from *Streptomyces verticillus var. quantum* and found to inhibit sialidases, *β*-glucuronidase, and *N*-acetyl-*β*-D-glucosaminidase.[128] The relative configuration of **177** was determined as (2*R*/*S*,3*R*/*S*,4*S*/*R*,5*S*/*R*)-2-acetamido-3,4-dihydroxypiperidine-5-carboxylic acid by ^{1}H NMR and X-ray crystallography. **177** is clearly related to **116**, though this was apparently not realized at first. The absolute configuration of **177** was established as the (2*R*,3*R*,4*S*,5*S*)-stereoisomer unambiguously through first total synthesis of **177** and its enantiomer by Nishimura and co-workers.[129−131] Another total synthesis was accomplished by Knapp and coworker.[132] Three other diastereomers **178**, **179,** and **180** were isolated by Takatsu et al. in 1996 from the culture filtrate of *Streptomyces nobilis* SANK 60192 (Figure 16).[133,134]

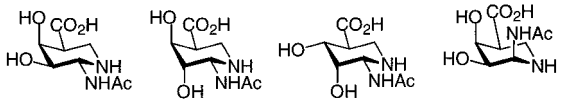


Figure 16.

As shown in Figure 17, **177** has the same topographical orientation of the functional groups as

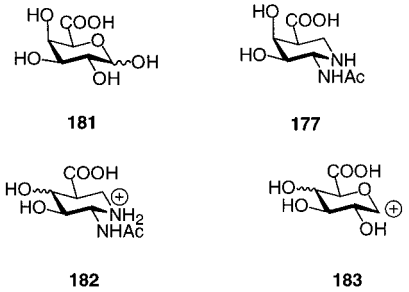


Figure 17.

galacturonic acid **181**. It was suggested by Nishimura et al. that **177** mimics glucuronic acid, **181**, in ground-state binding to *β*-glucuronidase.[135] Later they modified the postulate stating that the protonated form **182** of gem-diamine 1-iminosugars (or 1-azasugars) may mimic glycopyranosyl cation **183**, which may resemble the transition state of enzymatic glycosidic hydrolysis.[136]

Over a decade Nishimura and co-workers synthesized a number of derivatives of **177** in order to investigate the structure−activity relationship. In turn, they also designed and synthesized related compounds and **177** has been modified as shown in Figure 18.[137] Considering the topic of this review, we

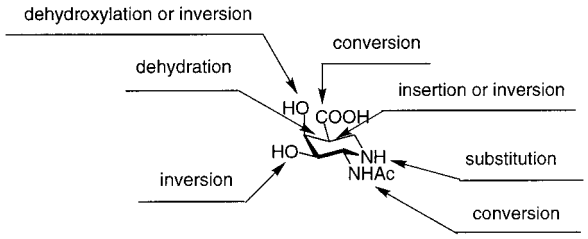


Figure 18.

will not discuss the inhibition of sialidases by **177** and analogues.

The parent compound **177** exhibited inhibition of *β*-glucuronidase from bovine liver with $IC_{50} = 71\ \mu M$ but no inhibition of α-glucosidase from baker's yeast (inhibition: 3% at 100 *μ*g/mL). In contrast, 1-*N*-alkylated derivatives **184**−**190** had practically no activity against glucuronidase (Figure 19). The activ-

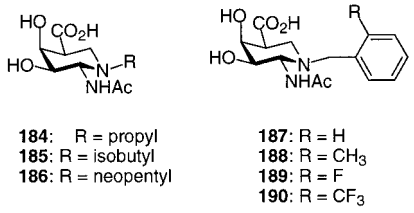


Figure 19.

ity against α-glucosidase varied with substitution. 1-Propyl- and benzylsiastatin B derivatives, **184** and **187**, exhibited enhanced inhibition of glucosidase (inhibition: 40% and 93% at 100 *μ*g/mL, respectively). This phenomenon has been explained with hydrophobic interaction between the inhibitor and an aglycon binding site within the enzyme. There are a number of precedents for such an interaction.[24,26] However, not all 1-N-alkylated derivatives showed the same effect, and small changes of the structure disrupted the inhibition completely.

As shown in Table 26, modification of the amide function at C-2 of **177** had a remarkable effect on the

Table 26. IC_{50} values in *μ*M

	R	β-glucuronidase[a]	β-glu'ase[b]
177	-$COCH_3$	71	>460
191	-$COCF_3$	0.065	13
192	-$COCCl_3$	4.7	137
193	-$NHC(=NH)NH_2$	4.1	96

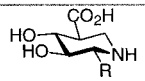
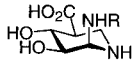
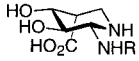
[a] Bovine liver *β*-glucuronidase. [b] Almond *β*-glucosidase.

inhibitory activity. Replacement of the acetamide group by trifluoroacetamide, trichloroacetamide, or guanidine groups clearly enhanced the inhibitory

activity toward β-glucuronidase and β-glucosidase.[135,138,139] In particular, **191** was found to be an extremely potent inhibitor of β-glucuronidase from bovine liver ($IC_{50} = 0.065\ \mu M$) and a potent inhibitor of β-glucosidase from almond ($IC_{50} = 13\ \mu M$). The inhibition of β-glucuronidase from bovine liver by **191** is over 1000 times stronger than that of **177**.

Glucuronic acid-type 2-trifluoroacetamidoisofagomine **194** is also a very potent inhibitor of β-glucuronidase from bovine liver with $IC_{50} = 0.065\ \mu M$ and is a moderate inhibitor of β-glucosidase with $IC_{50} = 100\ \mu M$[136] (Table 27). Although the IC_{50} values of siastatin analogues were not determined under the same conditions as the K_i value of the corresponding isofagomine **128** ($K_i = 0.079\ \mu M$), the siastatin analogue **194** seems to be more potent than the isofagomine analogue **128**.[94] Furthermore, the values of the IC_{50}s showed clearly that **194** is more potent toward the β-glucosidase from almonds than **128**.

Table 27. IC_{50} values in μM from Ref 136

		R	β-glucuronidase[a]	β-glu'ase[b]
194		-NHCOCF$_3$	0.065	98
128		-H	0.079*,[c]	>1000[c]
195		-COCF$_3$	0.065	36
196		-COCF$_3$	130	

*K_i value. [a] Bovine liver β-glucuronidase. [b] Almond β-glucosidase. [c] Reference 94.

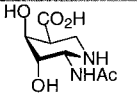
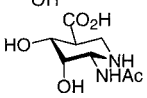
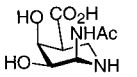
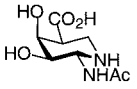
Trifluoroacetamide and trichloroacetamide analogues of **177** are unstable under the biological condition, although they are stable for several weeks in hydrochloric acid (pH < 1) according to the report from Nishimura et al.[127,140] For example, **191**, **192,** and **197** were shown to rearrange in the media (pH 5.0−6.3) to a ketone hydrate **203** or lactone **202** via hemiaminal **198**. In contrast to **191** and **192**, 2-phthalimide **197** is unstable in a solution of strong acid (Scheme 2). The IC_{50} values of **191**, **192,** and **197** against β-glucuronidase are 0.092, 0.096, and 0.073 μM, respectively, while the values of **198** and **202** are 0.16 and 3.1 μM. Inhibition studies indicated that the 2-trifluoroacetamide **191**, 2-trichloroacetamide **192,** and **198** are equally potent inhibitors of β-glucuronidase and that all contribute significantly to the overall inhibitory activity. However, **197** proved to be very unstable in the test medium. Therefore, the IC_{50} value of **197** must be expected to be that of **198**. The importance of the 2-hydroxy group has also been affirmed in the case of noeuromycin (**117**) and its analogues **122** and **127**.[126] Therefore, it is understandable that hemiaminal **198** showed strong inhibition of β-glucuronidase. As bioisosteres of OH, 2-trifluoroacetamide and trichloroacetamide may act as H donors and, therefore, enhance the interaction between the inhibitor and active-site residues.

Interestingly, D-mannuronic acid analogue **195** also inhibited β-D-glucuronidase very strongly. This result is consistent with the reports that mannojirimycin and D-mannono-1,5-lactam were slightly stronger against glucosidases than **74** and D-glucono-1,5-lactam (**204**, Figure 27).[141−144] The conformation of **195** is indicated to be a boat conformation by its ^1H NMR spectrum. Nishimura et al. explained that **195** is likely a mimic of the glycopyranosyl cation, the flattened conformational cation in the course of the enzymatic reaction. This explanation seems to be in conflict with the result achieved by Arai et al. **180** showed over 20 times higher inhibition toward β-glucuronidase from bovine liver than that of **177** and over 30 times higher inhibition toward heparanase than that of **177**. However, it was proved that the conformation of **180** was chair form and the acetyl substituent at the C-2 position on the piperidine ring was oriented axially according to ^1H NMR.[133,134] The conformational discrepancy between **180** and **195** can, however, be explained by stereoelectronic substituent effects.[145] The NMR data published by Nishimura et al.[136] are also consistent with **195** being in a $^1C_4$ conformation in protonated form, and conformational change from $^4C_1$ to $^1C_4$ conformation on protonation has recently been observed in similar compounds.[145] Stereoelectronic substituent effects predict that **180** will be less likely than **195** to undergo conformational change on protonation because of the axial 4-hydroxyl group.

The other two natural heparanase inhibitors **178** and **179** have contributed to deepening the knowledge of the relationship between the structure and biological activity of **177**. Comparing the data obtained, it was found that the 3-epimer did not diminish the inhibition against β-glucuronidase clearly; however, inhibitory activity of 3,4-diepisiastatin **179** is 10 times weaker than siastatin B. Against heparanase (endo-β-glucuronidase), only **180** exhibited potent inhibition with $IC_{50} = 12\ \mu M$.

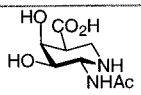
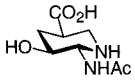
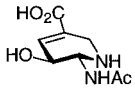
The role of the 4-OH was investigated (Table 29). Because the β-glucosidase and β-glucuronidase belong to family 1 and family 2, respectively, according to the classification of glycosidases by Henrissat,[146] they show little preference for a specific configuration at C-4.[188] Indeed, both **191** and **194** have the same inhibition value toward this enzyme ($IC_{50} = 0.065\ \mu M$) (see Tables 26 and 27), whereas **191** inhibited β-glucosidase even about 8 times stronger than **194**. It is interesting to note that **177** showed 20 times

Table 28. IC_{50} values in μM from Ref 134

		β-glucuronidase[a]	heparanase[b]
178		50	>350
179		>350	>350
180		1.6	12
177		39	>350

[a] Bovine liver β-glucuronidase. [b] B16−BL6 melanoma cell heparanase.

Table 29. IC_{50} values in μM

		α-glu'ase [a]	β-glucuronidase [b]
177		>460	71
205		26	60
206		80	115

[a] Baker's yeast α-glucosidase. [b] Bovine liver β-glucuronidase.

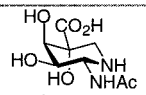
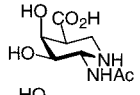
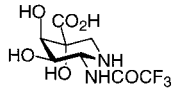
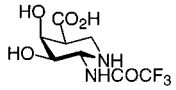
weaker activity toward α-glucosidase from baker's yeast than its 4-deoxy analogue **205** (Table 29). The result showed that the presence of the 4-OH may not contribute significantly to the binding interaction. Instead, the basicity of the aminal group can be weakened by 4-OH through a field effect.[79] 4,5-Olefin **206** showed similar activity. The three compounds **177**, **205,** and **206** exhibited similar activity against glucuronidase from bovine liver (Table 29).[147]

Compounds **191**, **194,** and **195** were also evaluated as inhibitors of recombinant human heparanase (endo-α-glucuronidase) from human melonoma A375M cell transfected with pBK−CMV expression vector containing the heparanase cDNA.[136] The IC_{50} values are 1.02, 10.6, and 29.0 μM, respectively. Heparanase cleaves the β-1,4-linkage between glucuronic acid (GlcUA) and *N*-acetylglucosamine (GlcNAc) in heparin sulfate. The weak activities against heparanase compared with exo-α-glucuronidase indicate that heparanase should recognize simultaneously glucuronic acid and adjacent glycoses on both sides of the scissile glycosidic bond.

As shown in Table 30, introduction of a 5-OH in compounds **207** and **208** decreased activity against β-glucuronidase slightly compared with analogues **177** and **191**. It is clear that the 5-OH group plays a minor role in inhibition.[137,148]

However, the 5-COOH was found to be an essential functional group for inhibition of glucuronidase from bovine liver. Replacement with any other functional group to form analogues **209**−**217** diminished the inhibition to IC_{50} > 100 μg/mL (Figure 20).[137,148]

Table 30. IC_{50} values in μM from Ref 137: IC_{50} Values Calculated According to IC_{50} in μg/mL

		α-glu'ase [a]	β-glucuronidase [b]
207		-	107
177		-	61
208		24	0.062
191		13	0.026

[a] Baker's yeast α-glucosidase. [b] Bovine liver β-glucuronidase.

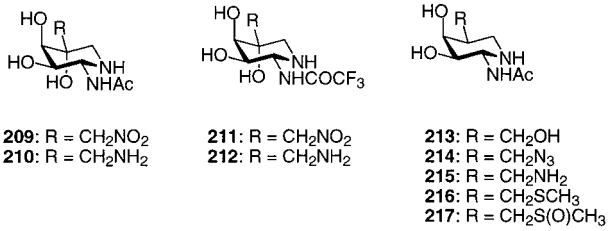


Figure 20.

These compounds have also been studied in vivo against cancer metastasis. It was supposed that the metabolism of α-L-iduronide from basement membranes and/or extracellular matrix was responsible for tumor metastasis.[149,150] According to this proposal, Nishimura and co-workers accordingly synthesized L-iduronic acid analogues **218**−**222**. By pretreatment of B16BL6 cells with these compounds in culture, pulmonary metastasis in mice was remarkably inhibited.

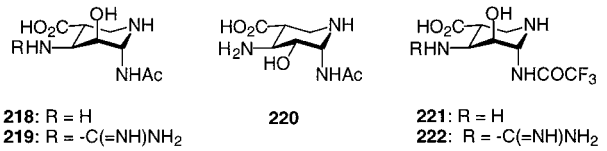
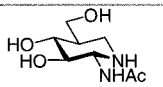
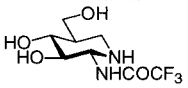
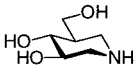
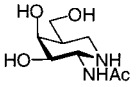
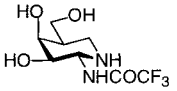
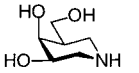


Figure 21.

Glucose and galactose analogues of **177** were reported in 1999 (Table 31).[140,151] Trifluoroacetamide analogues **224** and **225** were found to inhibit glucosidase and galactosidase stronger than acetamide analogues **223** and **213**, while **223** and **213** are better inhibitors of both β-D-*N*-acetylgalactosaminidase from chicken liver and β-D-*N*-acetylglucosaminidase from bovine liver. This result is consistent with those of siastatin B derivatives we have discussed earlier. Both *gluco*- and *galacto*-configured iminosugars **224** and **225** showed similar inhibition of β-glucosidase from almonds with IC_{50} = 0.42 and 0.47 μM, respectively, and 4 times weaker than the isofagomine (K_i = 0.1 μM). **223** and **213** affected β-D-*N*-acetylglucosaminidase at IC_{50} = 12 and 2.1 μM, respectively, but **224** and **225** have practically no inhibitory effect against this enzyme. The binding groups equivalent to the 2-NHAc groups in *N*-acetylgalacto- and glucosamine are likely to play important roles for specificity and potency of the inhibitors for the corresponding enzymes. For inhibition of α-glucosidase, α-galactosidase, and β-D-*N*-acetylgalactosaminidase, the correct configuration of 4-OH proved to be crucial. **224** inhibited α-glucosidase with IC_{50} = 0.19 μM, while **225** showed only weak inhibition toward this enzyme (IC_{50} > 340 μM). Similarly, **225** affected α-galactosidase from *Aspergillus niger* (IC_{50} = 0.34 μM), while **224** showed practically no inhibition toward this enzyme (IC_{50} > 320 μM). Isofagomines **116** and **120** showed the same trend but much weaker inhibition of α-glucosidases.

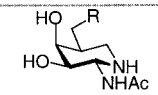
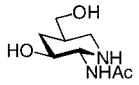
To investigate the electrostatic effect of substituents at C-5 on affinity for glycosidases, the hydroxymethyl group of **213** was replaced by azidomethyl, aminomethyl, methylthiomethyl, and methylsulfinylmethyl groups in analogues **214**−**217**.[152] The

Table 31. IC_{50} values in μM from Refs 94, 151, and 136

		α-glu[a]	β-glu[b]	α-gal[c]	β-gal[d]	N-Ac-α-glu[e]	N-Ac-β-glu[f]
223		2.9	5.4	>390	>390	>390	12
224		0.19	0.42	>320	190	>320	>320
116		150	0.1	>2000	270		
213		>420	79	25	17	0.33	2.1
225		>340	0.47	0.34	0.17	2.2	340
120		>2000	0.19	200	0.012		

[a] Baker's yeast α-glucosidase. [b] Almond *β*-glucosidase. [c] *Aspergillus niger* α-galactosidase. [d] *Aspergillus niger β*-galactosidase. [e] Chicken liver α-D-*N*-acetylgalactosaminidase. [f] Bovine liver *β*-D-*N*-acetylgalactosaminidase.

Table 32. IC_{50} values in μM from Ref 152

		R	β-glu'ase[a]	NAc-α-glu'ase[b]	NAc-β-glu'ase[c]
213		OH	>530	1.3	2.1
214		N_3	>530	2.6	197
215		NH_2	>530	187	>530
216		SMe	>530	85	1.2
217		S(O)Me	>530	224	>530
226			46	>530	>530

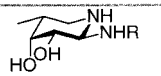
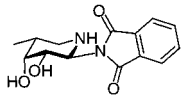
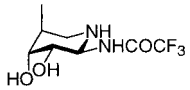
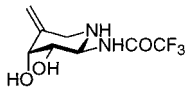
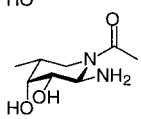
[a] Almond *β*-glucosidase. [b] Chicken liver α-D-*N*-acetylgalactosaminidase. [c] Bovine liver *β*-D-*N*-acetylgalactosaminidase.

inhibitory activities of synthesized derivatives for three different glycosidases are summarized in Table 32. Interestingly, the 4-deoxy analogue **226** showed potent inhibition of *β*-glucosidase (almonds). This result suggests that 4-OH may not be crucial for this enzyme.

The last series of inhibitors synthesized by Nishimura et al. were Siastatin analogues of L-fucose.[140,153] Strikingly, **229**, **230,** and **231** affected very potently α-L-fucosidase equivalent to the trifluoroacetamide **228**. As discussed above (Scheme 2), they are unstable in the test media and slowly change their structures to a ketone hydrate, similar to **203,** via the hemiaminal, fuconoeuromycin **127**. A mixture of three different compounds proved to contribute to the inhibitory activity. **234**, derived from **230**, showed practically no inhibition. As expected, the correct configuration of the 5-methyl group turned out to be essential. Changing the configuration to form **232** diminished inhibitory activity drastically. Compared with isofagomine **125**, 2-amino analogues showed inhibitory values 1000−2000 times higher and comparable to **127**, Table 33.

A more perfect mimic of **1** or **5b** than isofagomine (**116**) is presumably the oxazine **119** (Table 19), a compound with a weakly, basic nitrogen in the anomeric position and the sugar ring oxygen retained. It had no significant inhibition of α-glucosidase and galactosidases, however. It was an inhibitor of sweet almond *β*-glucosidase with an inhibition

Table 33. IC_{50} values in μM

		R	α-L-fucosidase[a]
227		$COCH_3$	0.48
228		$COCF_3$	0.011
229		$COCCl_3$	0.009
230		H	0.016
231			0.013
232			1.8
233			0.7
234			>270

[a] Bovine kidney α-L-fucosidase.

constant (K_i) of 60 μM. A reason for the considerable loss of activity compared to 1-azafagomine (**118**, pK_a 5.3),[211] which is similar to the oxazine in many ways, was suggested to be the lower base strength of the oxazine (pK_a 3.6).[154]

V. Inhibitors that Mimic Charge in Several Places

The transition state of glycoside cleavage is likely to have charge distributed over several atoms. Transition-state analogues that not only mimic charge in one position but in several places may mimic the transition state better. Mimics that combine two of **1**, **4,** and **5a** have been investigated.

A. Mimics of 4 and 5a

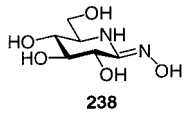
In 1990 the Ganem group synthesized the first transition-state analogues which mimicked both the partial positive charge on the endo- and exocyclic oxygen, the sp^2-hybridized anomeric carbon, and the flattened chair conformation of the assumed transition state.[155,156] They synthesized the amidine analogues of D-glucose (**235** and **236**, Table 34).

The amidine **235** was shown to be a relatively strong competitive inhibitor of β-glucosidase (sweet almond), having a K_i value (K_i = 8 μM) on the same scale as the K_i value of 1-deoxynojirimycin **75** (K_i = 18 μM).[157] The amidine **236** was shown to be less potent than **235**.

A special feature of those inhibitors was that they also inhibited α-mannosidase (jack bean) and β-galactosidase (bovine liver) in contrast to 1-deoxynojirimycin **75** and most other glycosidase inhibitors. Therefore, M. K. Tong et al. called the inhibitors broad spectrum.

Since the synthesis of **235** and **236,** the *galacto*-(**241**) and *manno*-analogues have been synthesized.[158] Some amidrazones and amidoximes (**237**−**240**, **242**−**243**) have also been made, and all are broad spectra inhibitors of glycosidases (Table 34).[159,160]

Also worth noting is that the K_i values of **235** (pK_a = 10.6), amidrazone **237** (pK_a = 8.7), and amidoxime **238** (pK_a = 5.6), (Figure 22) against β-glucosidase (sweet almond) are on the same scale despite the large difference in pK_a. This has lead G.

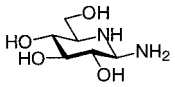


238

Figure 22.

Papandreou et al. to assume that the flattened chair conformation and the sp^2 hybridization of the anomeric carbon are more important for inhibition than having a positive charge.

An observation that supports this hypothesis is that gemdiamine **244** is an inhibitor of β-glucosidase (sweet almond, K_i = 40 μM, Figure 23) but does not



244

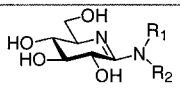
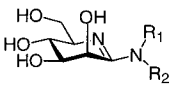
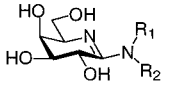
Figure 23.

inhibit α-glucosidase (yeast), α-mannosidase (jack bean), α-galactosidase (coffee bean), nor β-galactosidase (bovine liver).[161]

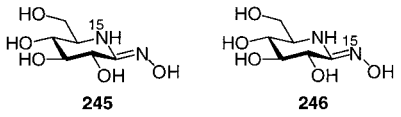
A fucose analogue has also been synthesized and was shown to be a potent inhibitor of α-L-fucosidase (human, K_i = 0.82 μM).[162]

P. Ermert et al. questioned if amidines and their derivatives were transition-state analogues.[163] They argued that when glycosidases were specific regarding substrates, then transition-state analogues should also be specific. Likewise, it was shown that the most stable tautomer of the amidoxime was **238** both in the solid state and in solution (Figure 22), and thus the double bond was not endocyclic as assumed by M. K. Tong et al. but exocyclic.[164] This has been proven by X-ray, ^{1}H NMR, and by ^{15}N NMR of the ^{15}N-labeled isotopomers **245** and **246** (Figure 24).[164] The location of the double bond is interesting to study, particularly because it has a large effect on

Table 34. K_i values in μM

		R_1	R_2	β-glu'ase[a]	α-gal'ase[b]	β-gal'ase[c]	α-man'ase[d]
235		H	H	8[f]		(8)[f]	9[f]
236		CH_3	CH_3	83[f]		(83)[f]	(40)[f]
237		H	NH_2	8.4[g]		19[§h]	3.1[h]
238		H	OH	13.8[g]		(19)[h]	12[i]
239		H	NH_2	200[h]		57[§g]	0.17[g]
240		H	OH	210[h]			2[h]
241		H	H	25[h]	8.5[h]		
242		H	NH_2	2.4[h]	8.3[h]	6.5[h]	
243		H	OH		5[j]	10[h]	

*IC_{50} value. § Noncompetitive inhibition, values in parentheses estimated from IC_{50} values. [a] Sweet almond β-glucosidase. [b] Coffee bean α-galactosidase. [c] Bovine liver β-galactosidase. [d] Jack bean α-mannosidase. [e] *Aspergillus niger* β-mannosidase. [f] Reference 155. [g] Reference 159. [h] Reference 158. [i] Reference 171. [j] Reference 170.



245 246

Figure 24.

the conformation of the compound and thereby also on the interpretation of the inhibitory properties. However, when the compounds are protonated they will all adopt a half-chair-like conformation because of a stronger conjugative interaction between the exo- and endocyclic nitrogens.

Various groups have synthesized and tested amidine and amidoxime derivatives where the substituent at the exocyclic nitrogen has been varied[164−171] These substituents are supposed to mimic the aglycon in the natural substrates, thereby enhancing the selectivity and inhibitory properties of the compounds. However, they have only succeeded in improving the selectivity, and the K_i values are comparable with the K_i value of the corresponding monosaccharide analogues (Tables 35−37). By means of some theoretical calculations and interresidue NOE experiments it was shown that the orientation of the phenyl group in **249** differed significantly from the position of the leaving group in the transition state.[172] Thus, this can be an explanation of why the inhibitory properties did not improve significantly by mimicking the aglycon.

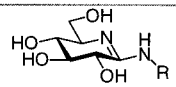
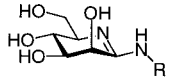
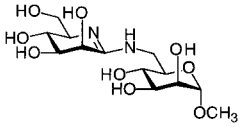
Y. Blériot et al. synthesized and tested some amidine derivatives which contained no or one hydroxy group. They showed some inhibition against different glycosidases; however, their K_i values were all in the range of 14000−700 μM.[167]

Recently a disaccharide analogue (**261**) was synthesized which consisted of a 1−4 linked glucosyl and amidoxime moiety. Thus, the analogue was supposed to bind at the −1/−2 subsite in the enzyme. Its biological activity against two cellulases was investigated,[173] and as expected it was a stronger inhibitor than the corresponding monosaccharide analogue (Table 38). However, the inhibition was of the mixed type; thus, the inhibitor is not binding at the subsites occupied by the substrate (−2, −1, +1), and therefore, it can hardly be a transition-state analogue. The influence of the glycosyl moiety was particularly evident when tested against Cel6A (*Trichoderma reesei*) (K_i = 5 μM), which the amidoxime does not inhibit at all.

A disaccharide analogue (**262**) which was supposed to bind at the −1/+1 subsite in the enzyme has also been synthesized, but it did not inhibit the two cellulases it was tested against.

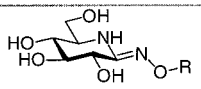
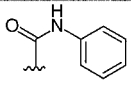
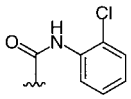
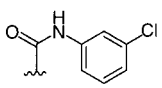
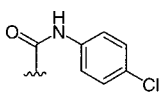
Compounds which mimic trisaccharides (**263**−**265**) have also been synthesized and tested against the two cellulases, but the K_i values were at best comparable with the inhibitory properties of the disaccharide analogue (Table 38).[173]

Table 35. K_i values in μM

		R	α-glu[a]	β-glu[b]	β-glu[c]	β-gal[d]	α-man[e]	β-man[f]
247		butyl	9.0[g]	4.2[g]	0.13[g]			
248		dodecyl			0.0002[g§]			
249		benzyl		5[h]		-[h]	0.55[h]	6[h]
250			100[h]			-[h]	2.6[h]	120[h]

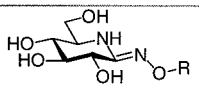
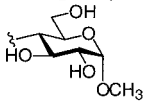
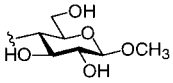
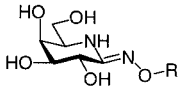
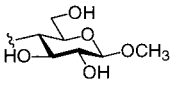
*IC_{50} value. − No inhibition at 1000 μM. § Competitive component of mixed competitive/noncompetitive inhibition. [a] Yeast α-glucosidase. [b] Sweet almond β-glucosidase. [c] Cytostolic bovine β-glucosidase. [d] Bovine liver β-galactosidase. [e] Jack bean α-mannosidase. [f] Snail β-mannosidase. [g] Reference 166. [h] Reference 168.

Table 36. K_i values in μM from Ref 164

		R	β-glu'ase[a]	β-glu'ase[b]
251			13	1.2
252			8	0.15
253			12	0.9
254			21	0.8

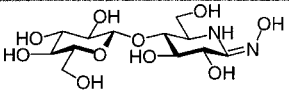
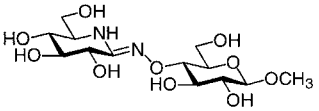
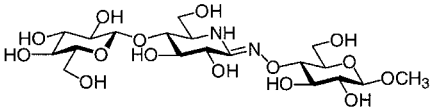
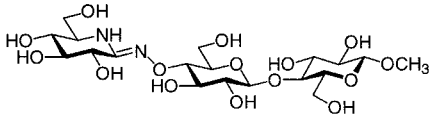
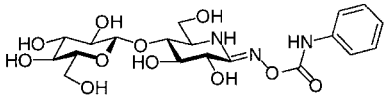
[a] Sweet almond β-glucosidase. [b] *Agrobacterium faecalis* β-glucosidase.

Table 37. K_i values in μM from Ref 170

		R	α-glu[a]	β-glu[b]	β-glu[c]	α-gal[d]	β-gal[e]
255		$C_{12}H_{25}$	40*	2*	0.3*		
256		$CH(CH_2OH)_2$	4000*	150*	8*		
257		CH_2SCH_3	50[f]	24[f]	2.4[f]		
258			>5000*	1000*	2*		
259			2500*	60*	3.6*		
260					3.3	250	2500*

*IC_{50} value. [a] Brewer's yeast α-glucosidase. [b] Sweet almond β-glucosidase. [c] *Caldocellum saccharolyticum* β-glucosidase. [d] Coffee bean α-galactosidase. [e] Bovine liver β-galactosidase. [f] Reference 171.

Table 38. K_i values in μM Taken from Ref 173

		Cel6A[a]	Cel7A[b]
261		5	190(α=2.3)
262		-	-
263		14	6000
264		700	40
265		1	740

[–] No inhibition at 2000 μM. [a] *Trichoderma reesei* cellobiase Cel6A. [b] *Trichoderma reesei* cellobiase Cel7A.

The transition state of inosine during hydrolysis by nucleoside hydrolase has been characterized by kinetic isotope effects, bond-energy/bond-order vibrational analyses, and molecular electrostatic potential surface calculations.[174−177] On this basis various transition-state analogues have been synthesized, and among these are four amidrazone derivatives (**266**−**269**). The analogues have been tested against a nucleoside hydrolase (*Crithidia fasciculata*), and they all were shown to be inhibitors of the nucleoside hydrolase (Figure 25).[176] **269** was remarkably stronger than the others. It has been found through resonance Raman and ultraviolet−visible absorbance spectroscopy experiments that **269** binds to the enzyme as the neutral zwitterionic species with a negative charge positioned at the nitro group and the positive charge is centered at the anomeric carbon.[178] **269** is the only compound of the four which has the possibility to form a zwitterion; thus, this can be an explanation of why it is such a strong inhibitor compared with the similar compounds.

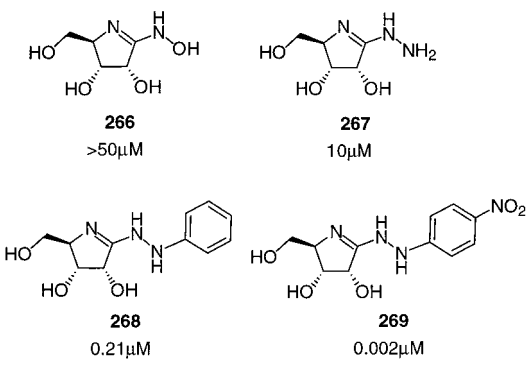
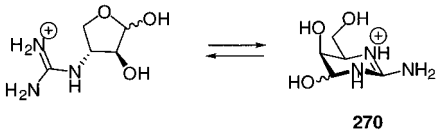


Figure 25.

Another class of compounds which mimics a flattened chair conformation, the sp^2-hybridized anomeric carbon, and a positive charge are compounds containing a cyclic guanidinium structure such as **270** (Figure 26).[179] Most of these compounds lack the 3-hydroxy group in order to restrict the compound in the pyranose form, because only this form mimics the natural sugars well.[180−182] The inhibitors have been varied by substituting the exocyclic nitrogen



270

Figure 26.

with different groups that are supposed to mimic the aglycon, by mimicking gluco-, galacto-, or fucopyranosides and by substituting the exocyclic nitrogen by sulfur.[183,184]

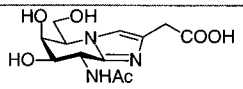
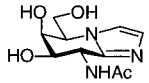
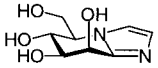
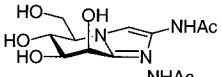
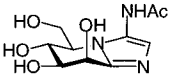
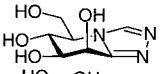
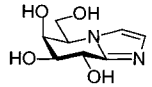
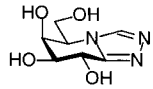
A series of 2-hydroxy guanidino sugars[184] and a series consisting of seven- and nine-membered cyclic guanidinium structures[185,186] have also been synthesized. None of the cyclic guanidino sugars were potent inhibitors toward the glycosidases they have been tested against.

Another approach to mimic both the flattened chair conformation and the positive charge of the assumed transition state was begun in 1991 by Vasella's group.[187] They tried to mimic the conformation and charge of the transition state by synthesizing compounds which consisted of an azasugar fused with heterocycles such as triazoles (section III) or imidazoles.[188] In this way it was possible to make a series of compounds which mimicked the transition state in the desired manner and which had a wide range of pK_a values (tetrazoles pK_a ca. −4, imidazoles pK_a ca. 6).[189] Yet another benefit was that the azoles were expected to be more stable than the amidines and amidine derivatives which were already known. It should also be mentioned that imidazole itself and derivatives of L-histidine also have turned out to be moderate to strong inhibitors of different glycosidases.[190] In this section only the azoles which are likely to be protonated both in the position of the endo- and exocyclic oxygen of the transition state are referred to. That is, the tri and imidazoles which do not have a nitrogen in the position of the exocyclic oxygen and the tetrazoles are discussed in section VI.

In 1992 the potent and selective inhibitor nagstatin (**82**) was isolated in the fermentation broth of *Streptomyces amakusaensis* MG846 fF3 by Aoyagi et al.[66] Nagstatin was shown to be a *N*-acetylgalactosamine fused with a substituted imidazole;[67] thus, the structure was similar to the tetrazoles synthesized by Vasella's group. By comparing IC_{50} values of nagstatin with IC_{50} values of **271** and **276** against *N*-acetyl-*β*-D-glucosaminidase and *N*-acetyl-α-galactosidase, it can be seen that the substituent at the imidazole does not contribute to the binding (Table 39). The NHAc group at C2 (sugar numbering) on the other hand must, not surprisingly, be very important since the IC_{50} value of **271** is a factor of 104 smaller than the IC_{50} value of **276** against *N*-acetyl-*β*-D-glucosaminidase and a factor ca. 10 smaller against *N*-acetyl-α-D-galactosaminidase.

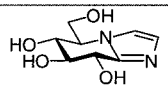
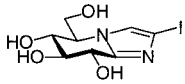
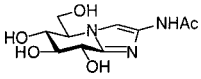
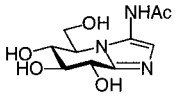
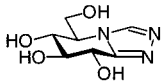
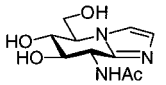
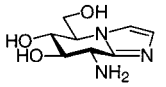
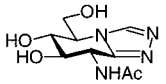
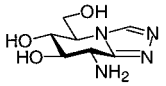
Subsequently, Tatsuta's group also synthesized compounds of this class (**82**, **83,** and **271**−**277**),[32,191−195] As can be seen in Tables 39 and 40, the imidazoles are potent inhibitors of various glycosidases and are more selective than the amidines and their derivatives. Also, the 1,2,4-triazoles are potent and selective inhibitors though they are not as strong as the imidazoles. This may be due to their lower basicity. Probably for the same reason the gluco- and mannoimidazoles with a *N*-acetyl substituent at C2 or C3 (**273**, **274, 279**, **280**) (systematic numbering)[189] and the glucoimidazole with an iodine at C2 (**278**)[196] were not very strong inhibitors (Tables 39 and 40). In light of a pK_a/K_i correlation, with **273**−**274** and **279**− **80** it could also be seen that it was not favorable to have a substituent at C3. This may be due to two things: (1) An unfavorable position of the C3 substituent or

Table 39. IC_{50} values in μM

		β-glu'ase[a]	β-gal'ase[b]	α-man'ase[c]	β-man'ase[d]	NAc-β-glu'ase[e]	NAc-α-gal'ase[f]
82		>334[§g]	>334[§g]			0.013[§g]	63[§g]
271		319[§g]	11[§g]			0.006[§g]	10[§g]
272		17[§g]	>500[§g]	0.6[§g]	0.1[§g]	>500[§g]	>500[§g]
273				85[h]	14[h]		
274				130[h]	275[h]		
275		285[§i]		65[§i]	0.4[§i]		
276		0.5[§g]	0.008[§g]	>500[§g]	370[§g]	60[§g]	125[§g]
277		12[§i]	0.4[§i]	>500[§i]	>500[§i]		

[§] Recalculated from μg/mL. [a] Sweet almond *β*-glucosidase. [b] *E. coli* *β*-galactosidase. [c] Jack bean α-mannosidase. [d] Snail *β*-mannosidase. [e] Bovine kidney *N*-acetyl-*β*-glucosaminidase. [f] Chicken liver *N*-acetyl-α-galactosaminidase. [g] Reference 194. [h] Reference 189. [i] Reference 195.

Table 40. K_i values in μM

		α-glu'ase[a]	β-glu'ase[b]	β-glu'ase[c]	N-Ac-β-glu'ase[d]
83		59[e]	0.1[e]	0.02[e]	
278		1850*[f]	1.28*[f]	0.17[f]	
279		71*[g]	3*[g]	1.5*[g]	
280		136*[g]	250[g]	91[g]	
281		870[e]	19[e]	0.17[e]	
282			133*[§h]		0.007*[§h]
283			68[i]	20[i]	
284					0.034[j]
285			210[i]	12[i]	

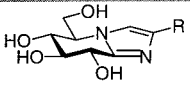
*IC_{50} value. § Recalculated from μg/mL. [a] Brewer's Yeast α-glucosidase. [b] Almond β-glucosidase. [c] *Caldocellum saccharolyticum* β-glucosidase. [d] Bovine kidney *N*-acetyl-β-glucosaminidase. [e] Reference 251. [f] Reference 196. [g] Reference 189. [h] Reference 194. [i] Reference 220. [j] Reference 244.

(2) the conformation of the C3-substituted imidazoles deviates slightly from the $^7H_6$ conformation the C2-substituted and the unsubstituted imidazoles have adopted because of an 1,5-interaction between the acetamido and the hydroxymethyl groups. This deviation may result in the C3-substituted imidazoles **274** and **280** mimic the transition state to a lesser extent.

A manno- and a glucoimidazole analogue in which the stereochemistry of C5 is inverted,[197] a *N*-acetyl-L-galactosamineimidazole and a *N*-acetyl-L-galactoimidazole,[194] have also been synthesized but, not surprisingly, neither were strong inhibitors toward the glycosidases they were tested against.

To enhance the K_i values and the selectivity toward β-glucosidases, several imidazoles with a substituent at C2 (systematic numbering) which was supposed to mimic the aglycon were synthesized (**286**−**298**). As can be seen in Table 41 that the selectivity of the inhibitors between β-glucosidases and α-glucosidase

Table 41. K_i values in μM from Ref 196

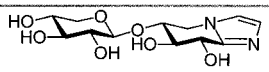
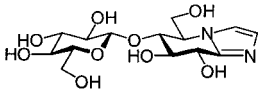
		R	α-glu'ase[a]	β-glu'ase[b]	β-glu'ase[c]
83		H	59	0.1	0.02(α=3.2)
286		CH_3	72*	0.26*	0.025*
287		CH_2OH	69*	0.011	0.005
288		CH_2NH_2	2060*	1.6*	0.15*
289		$(CH_2)_3NH_2$	185*	0.07*	0.025*
290			>3400*	3*	0.6
291		$(CH_2)_2COOH$	242*	0.055*	0.018*
292		$(CH_2)_2COOCH_3$	25*	0.0099[§]	0.0018(α=2.5)
293		Ph	554*	0.1(α=7.0)	0.018
294		$(CH_2)_2Ph$	0.5*	0.0012	0.00011(α=15)
295		(R)-$CH(OH)CH_2Ph$	3	0.063	0.016
296		(S)-$CH(OH)CH_2Ph$	10	0.041	0.007
297		CONHPh	2150*	3	0.14
298		$SO_2C_6H_4CH_3$	60[§d]	5[d]	0.04(α=2.2)[d]

*IC_{50} values. § Noncompetitive inhibition. [a] Brewer's yeast α-glucosidase. [b] Almond β-glucosidase. [c] *Caldocellum saccharolyticum* β-glucosidase. [d] Reference 68.

has only been enhanced by a maximal factor of ca. 10 (**287**) compared with **83,** and in some cases it has even reduced the selectivity (e.g., **295**). In an apparent contradiction, the K_i values for β-glycosidases are, in the case of **292** and **294**, lowered by a factor of ca. 10 (almond and *Caldocellum saccharolyticum*) and ca. 100 (almond) and 200 (*Caldocellum saccharolyticum*), respectively. This is similar to what was found when investigating the N-substituted amino(hydroxymethyl)pentanetriols (section II), and it has been explained by the flexibility of the substituents so they can make favorable interactions with the aglycon binding subsite.[196]

Two disaccharide analogues **139** and **299** which were supposed to bind to the −2/−1 subsites in the enzymes have also been synthesized (Table 42).[105,173]

Table 42. K_i values in μM

		Cex[a]	Bcx[b]	Cel6A[c]	Cel7A[d]
139		0.15[e]	520[e]		
299				1[f]	130[§f]

§ Noncompetitive inhibition. [a] *Cellulomonas fimi* xylanase. [b] *Bacillus circulans* xylanase. [c] *Trichoderma reesei* cellobiase Cel6A. [d] *Trichoderma reesei* cellobiase Cel7A. [e] Reference 105. [f] Reference 173.

Both inhibit the glycosidases they have been tested against, and a crystal structure of Cel5A (*Bacillus agaradhaerens*) complexed with **299** showed that the inhibitor indeed binds to the −2/−1 subsites in the enzyme.[198] Worth noting also is the very strong inhibitor **139** of *Cellulomonas fimi* xylanase (K_i = 0.15 μM) which is one of the best small inhibitors seen for xylanases.

The azoles are not only interesting because of their strong inhibitory properties, but also have provided a better understanding of the enzymatic mechanism of the hydrolysis of glycosides.[188]

In 1996 Heightman et al. noticed that the moderate to strong β-glycosidase inhibitors **2**, **3**, **83**, **204**, **237**−**238**, **270**, and **300**−**302** all had a heteroatom (N or O) at the glycosidic position (Figure 27) and that the similar compounds **95**, **97**, and **303**−**305** which did not have a heteroatom in this position (Figure 28) were weak inhibitors; therefore, they suggested that there is an important interaction with the enzyme at this position.[199] Assuming that the inhibitors are bound in the same way in the enzyme as the substrate, it is reasonable to propose that the enzyme−inhibitor interaction is an interaction between the catalytic acid and the heteroatom, thus mimicking the protonation of the glycosidic oxygen at the substrate.

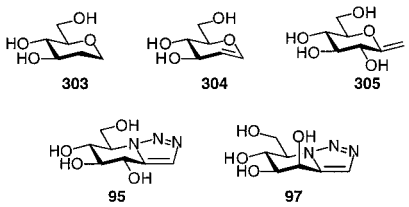


Figure 28.

According to Koshland's mechanism of the hydrolysis by retaining glycosidases,[200] the catalytic acid is placed above the saccharide ring plane, but this disagrees with the hypothesis above, e.g., in the case of the tetrazole as inhibitor because the lone pair at the glycosidic nitrogen is positioned in the plane of the ring.

To investigate the importance of the heteroatom, Heightman et al. synthesized a *gluco*- and a *manno*-configured 1,2,3-triazole (**95** and **97**, see section III) of which the structure is similar to the corresponding tetrazoles (**2** and **3**) except they lack a heteroatom in the glycosidic position.[199] As expected, the triazoles did not inhibit β-glucosidases as good as the corresponding tetrazoles; thus, the results supported that the heteroatom is important, and on the basis of this Heightman et al. proposed that the catalytic acid is placed in the plane of the ring in β-glycosidases (Figure 29).

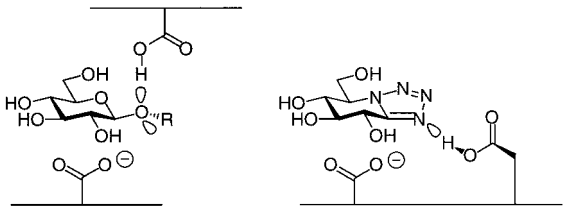


Figure 29.

To verify this they also manually docked glucotetrazole **2** into the active site of cyanogenic β-glucosidase (white clover) and galactotetrazole **302** into the active site of β-galactosidase (*E. coli*) and the 6-phospho-β-galactosidase (*Lactococcus lactis*).[199] The modeling did indeed show that the catalytic acid was placed close to the saccharide ring plane in all three cases. Also, the crystal structure of Cel5A (*Bacillus agaradhaerens*) complexed with **299**[198] and crystal structures of various other β-glycosidases complexed with substrates, products, or inhibitors have supported this finding.[188]

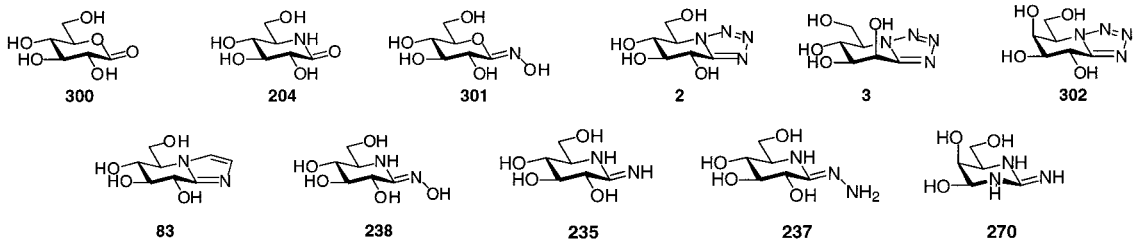


Figure 27.

Heightman et al. were aware that the interaction between the heteroatom and the catalytic acid could not be an important interaction with all the inhibitors. For example, they noted that the glycosidic nitrogen in the amidinium salt **236** was not able to interact with the catalytic acid and proposed that a charge−charge interaction with the nucleophile played an important role for the inhibition by such salts (Figure 30).[201] This raised the question to what extent

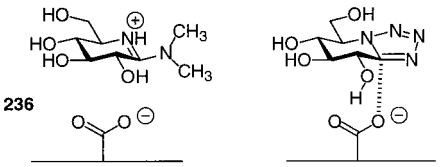


Figure 30.

does a charge−dipole interaction between the nucleophile and the anomeric carbon contribute to the binding of neutral inhibitors such as the tetrazole? To be able to estimate this they calculated the distance-dependent heats of formation of various azole acetate complexes using theoretical calculations (AM1). The results showed that about one-half of the binding energy came from the charge−dipole interaction and the other half must be due to interactions with the catalytic acid.

Another finding concerning the position of the catalytic acid in β-glycosidases was that the partial protonation can occur either syn or anti to the O5−C1 bond dependent on the orientation of the aglycon (Figure 31).

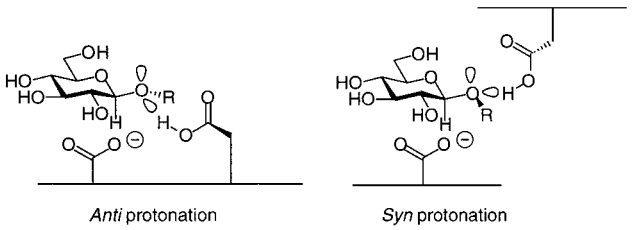


Figure 31.

In most cases it can be seen from the crystal structures whether an enzyme is a *syn*- or an *anti*-protonator, and not very surprisingly there is a correlation between this and the glycosidase families defined by the amino acid sequence to which the enzyme belongs.

Whether a glycosidase is a *syn*- or *anti*-protonator should also be possible to determine by investigating the inhibitory properties of a series of carefully selected compounds. For example, the imidazoles which contain a heteroatom at the glycosidic position would be expected to be selective *anti*-protonators because the orientation of the lone pair at the glycosidic nitrogen should be able to interact with the catalytic acid only in this type of enzymes. Unfortunately only a few transition-state analogues have been tested against both *syn*- and *anti*-protonators, and in those cases the results have not been very convincing, probably because the inhibitors did not mimic the transition state well enough.[173]

B. Mimics of 1 and 5

On the basis of the observation that the position of the nitrogen atom greatly influences whether α- or β-glycosidases are strongly inhibited, compound **118** was synthesized (Table 19). This compound, first prepared as a racemate, was a hybrid of fagomine (1,2-dideoxynojirimycin) and isofagomine (**116**), since it contained a nitrogen atom in a position corresponding to both an endocyclic oxygen and anomeric center. It was, therefore, capable of being protonated in two different positions, thereby being able to mimic a proposed transition state for both α- and β-glucoside hydrolysis. The hydrazine **118**, which was given the name 1-azafagomine, indeed turned out to be a potent inhibitor of α- and β-glucosidase (Table 19). Activity toward isomaltase and glycogen phosphorylase was also demonstrated to be powerful.[100] Later, both enantiomers of the 1-azafagomine were prepared,[202−204] and along with this, a careful study of its mode of slow binding to glycosidases was undertaken. The process of slow inhibition was established to be due to slow association/dissociation of enzyme and inhibitor and not a consequence of slow interchanges between enzyme conformations.[205] The slow rates of inhibitor−enzyme association were later explained from thermodynamic and kinetic measurements that an enthalpy barrier had to be crossed in order to get enzyme−inhibitor binding. As in the case of β-glucosidase inhibition by isofagomine **116**, entropy was shown to be the driving force for formation of the enzyme−inhibitor complex. This was in contrast to the binding of the natural compound 1-deoxynojirimycin **75**, which was demonstrated to bind to almond β-glucosidase because it was energetically favored.[85,206]

By using ^{13}C-labeled 1-azafagomine **118** ([5-^{13}C]-1-azafagomine), a way of measuring the concentration of enzyme active sites was reported. This led to an alternative way of determining the k_{cat} value of glycosidases inhibited by 1-azafagomine, even of impure enzyme samples. The method was based on monitoring the decrease in signal intensity for unbound inhibitor in a series of binding experiments by ^{13}C NMR spectroscopy relative to that of a reference.[207]

Racemic 1-azafagomine **118** has furthermore been used as the glycon part in the synthesis of the first combinatorial library of 125 potential glycosidase inhibitors (Figure 32).[208,209] The hydrazine was coupled via an acetic acid linker on N-1 to a variable tripeptide chain. The library was screened for activity toward α- and β-glucosidase as well as glycogen phosphorylase, and more selective compounds were found since essentially only β-glucosidase was inhibited. The library member with the lowest inhibition constant (**307**) was identified via decomposition to contain a tripeptide consisting of three hydroxyproline residues. This compound had a K_i of 40 μM, which was considerably lower than the K_i values for the corresponding compound containing only the linker (**306**) or the linker with one hydroxyproline residue (**308**). This suggested that a longer chain enhances inhibitory activity toward almond β-glucosidase.

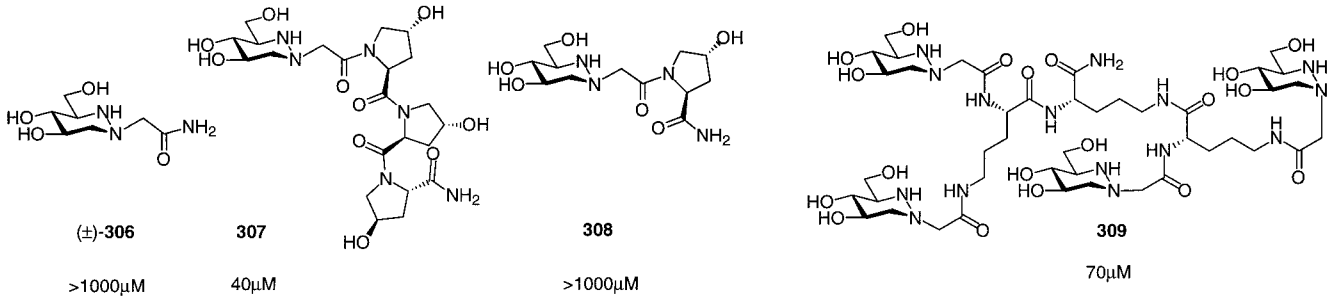


Figure 32. Inhibition constant (K_i) against sweet almond β-glucosidase of the strongest library member and dendrimer. The glycon moeity is racemic.

In this study a dendrimer (**309**) in the form of a tetravalent azasugar was prepared (Figure 32). The synthesis of this compound was also performed on a solid phase using diamino acid ornithine. The target compound showed an inhibition constant toward almond β-glucosidase of 70 μM.[209]

1-Azafagomine (**118**) was studied as a potential inhibitor of processing glycosidases, and the activity was compared to that of 1-deoxynojimycin (**75**) and castanospermine (**312**). The potency of 1-azafagomine (**118**) was determined to be considerably lower than that of the alkaloids.[210]

To further test the generality of glycosidase inhibition by azafagomines, analogues of the naturally occurring galactostatin (galactonojirimycin) and castanospermine (**312**) have been synthesized.

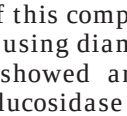
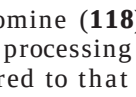
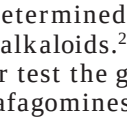
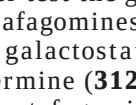
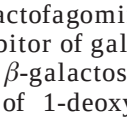
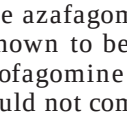
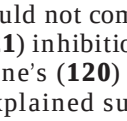
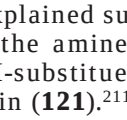
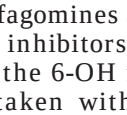
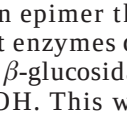
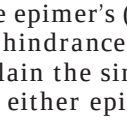
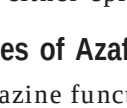
1-Azagalactofagomine (**123**) was found to be a strong inhibitor of galactosidases (Table 20). Inhibition toward β-galactosidases was noticeably stronger than that of 1-deoxygalactonojirimycin (**121**). In addition, the azafagomine's inhibition of α-galactosidase was shown to be superior to that of the corresponding isofagomine analogue **120**. The hydrazine, however, could not compete with 1-deoxygalactonojirimycin's (**121**) inhibition of α-galactosidase and isogalactofagomine's (**120**) inhibition of β-galactosidases. This was explained supposedly to be due to a higher basicity of the amines and the presence of an additional OH-substituent in the case of 1-deoxygalactonojirimycin (**121**).[211]

Since azafagomines apparently are broad-spectrum glycosidase inhibitors, a study of the preferred orientation of the 6-OH for both α- and β-glycosidases was undertaken with a castanospermin analogue (**310**) and an epimer thereof (**311**). Inhibition results showed that enzymes α-glucosidase (rice), isomaltase (yeast), and β-glucosidase (sweet almonds) all favored an axial 6-OH. This was concluded since the inhibition by castanospermine analogue **310** was moderate whereas the epimer's (**311**) activity was significantly less. Steric hindrance from the ethylene bridge was used to explain the similar inhibition of yeast α-glucosidase by either epimer.[212]

C. Analogues of Azafagomines

The hydrazine functionality of 1-azafagomine has been oxidized and thereby transformed into two different hydrazones **315** and **316** (Table 43). By studying these compounds as glycosidase inhibitors it was suggested that mimicry of transition-state charge rather than shape is more important for

Table 43. K_i values in μM

		α-glu[a]	β-glu[b]	isomaltase[c]
310	 (±)	600[e], 15[d,e]	10[e]	79[e]
311	 (±)	570[e], >1000[d,e]	690[e]	550[e]
312		>1000[f], 0.015[d,g]	1.5[g]	
313	 (±)	275[e], 250[d,e]	660[e]	>1000[e]
314	 (±)	380[e], 150[d,e]	820[e]	>1000[e]
315		182[h]	>1000[h]	237[h]
316		244[h]	>1000[h]	758[h]
317	 (±)	340[i]	56[j]	79[j]
318	 (±)	>1000[k]	128[j]	3080[k]
319	 (±)	>1000[k]	28[j]	>3000[k]
320	 (±)	1541[l]	153[l]	
321	 (±)	>1000[l]	79[l]	

[a] Yeast α-glucosidase. [b] Sweet almond β-glucosidase. [c] Yeast isomaltase. [d] Rice α-glucosidase. [e] Reference 212. [f] Reference 8. [g] Reference 7. [h] Reference 213. [i] Reference 121. [j] Reference 205. [k] Reference 100. [l] Reference 252.

strong inhibitor−enzyme binding. The argument was based on the less basic hydrazones being poor inhibitors, although they were believed to possess a half-chair conformation.[213] Among analogues of 1-azafagomine mainly the 3-position (carbohydrate numbering) has been explored. Interestingly, 3-deoxy-3-fluoro analogue **321** was a much poorer inhibitor than 1-azafagomine (**118**) itself. This suggests that the 3-OH of 1-azafagomine (**118**) is involved in hydrogen bonding.[214] It was proposed in the paper that the 3-OH act as a hydrogen-bond donor based on the belief that a fluoro substituent was capable of acting as a hydrogen-bond acceptor as well as the OH group. However, new results show that fluorine generally is a very poor hydrogen-bond acceptor.[215,216]

The consequence of epimerizing the 3-OH was drastic on inhibition of glucosidases as it resulted in decreased activity. However, additional removal of the 6-OH resulted in a slight regain of inhibition toward β-glucosidase.[100,205] The strongest inhibitor of the 1-azafagomine analogues was the 3-deoxy-3-amino version of the hexahydropyridazine inhibitor (**317**), which was the only modified 1-azafagomine that demonstrated α-glucosidase inhibition.[121,205]

D. Mimics of 1 and 4

As a hybrid compound of the galactosidase inhibitors 1-aminogalactose and isogalactofagomine (**120**), the hydrazine **124** was prepared (Table 20). This compound was a micromolar inhibitor of α- and β-galactosidase. The inhibition constant toward α-galactosidase (coffee beans) was determined to be 73 μM, comparable to inhibition by isofagomine analogue **120** (K_i = 50 μM).[211] The activity is about 10 times less than 1-aminogalactose.[7] The hydrazine inhibited β-galactosidase (*Aspergillus oryzae*) with a K_i value of 4.5 μM and β-glucosidase (almonds) with a K_i value of 410 μM, which is about 1000 and 4000 times less potent than isogalactofagomine (**120**), respectively.[217]

VI. Inhibitors That Do Not Mimic Charge

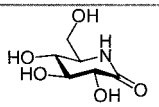
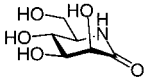
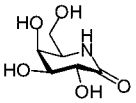
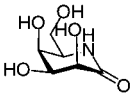
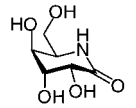
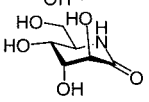
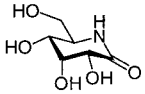
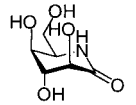
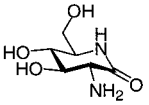
There is evidence from kinetic isotope effects studies that the anomeric carbon has various degrees of sp^2 hybridization in the transition state of glycoside hydrolysis. This is consistent with a transition state that has half-chair geometry. Confirming this hypothesis was the quite old observation that δ-aldonolactones and lactams are glycosidase inhibitors. These molecules are uncharged yet inhibit glycosidases much better than aldoses, which has been interpreted as these molecules geometrically more closely resemble the transition state. Aldonolactones and lactams have a sp^2-hybridized anomeric carbon, and their resonance forms may be able to resemble a double bond between C_1-O_5. Interestingly it has recently been suggested that the increased binding of these molecules is due to not only geometry but also binding between the carbonyl group and the catalytic acid.[195]

The aldonolactams have recently been revisited. Nishimura's group synthesized all possible D-stereoisomers of hexono-1,5-lactam **204** and **322**−**328**, obtained their X-ray structures, and studied the inhibition of a number of glucosidases, galactosidases, and mannosidases.[218] It was found that most of the stereoisomers were not inhibitors, with two exceptions: The D-*manno* isomer **322** was a good inhibitor of both α- and β-mannosidase, β-glucosidase, and β-galactosidase and five different stereoisomers (*gluco* **204**, *manno* **322**, *galacto* **323**, *talo* **324**, and *ido* **328**) (Table 44) were able to inhibit almond β-glucosidase. Interestingly, all the lactams that are in the boat conformation (**322**, **324**, **328**) are among those, suggesting that this conformation was preferred. However, the energy difference between half-chair, boat, and scew-boat is likely to be small, and it is conceivable that all these inhibitors bind in a different conformation than found unbound. Thus, it has recently been shown that several xylanase inhibitors shifted from half-chair to envelope conformation when bound to the enzyme,[219] and this shows that caution must be taken when evaluating the effect of an inhibitor's geometry on its enzyme inhibition.

Panday et al. recently made the D-glucosamino lactam **329** and found that it was a relatively good β-glucosidase inhibitor, Table 44.[220]

Interesting new types of lactams were introduced by Withers' group in which the nitrogen is in its

Table 44. K_i values in μM

	Structure	Conformation	β-glu'ase[a]	β-gal'ase[b]
204		H	51	-
322		$B_{5,2}$	0.51	4.5
323		H	85	-
324		$B_{5,2}$	120	15
325		H	-	-
326		S	-	-
327		H	-	-
328		$B_{5,2}$	46	-
329			6.6	

[−]No inhibition. Mannolactam **322** also inhibited snail β-mannosidase with K_i = 9 μM and jack bean α-mannosidase with K_i = 68 μM, while **329** inhibited β-glucosidase from *C. saccharomyces* with K_i = 0.7 μM. [a] Almond β-glucosidase. [b] *Aspergillus niger* β-galactosidase.

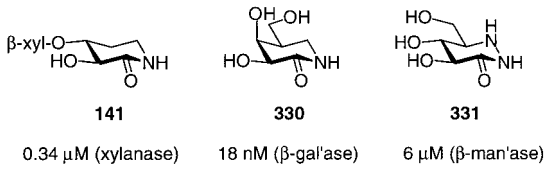


Figure 33.

anomeric position similar to the isofagomines and the carbonyl group in the 2-position (see also section IV).[125] The rationale behind this inhibitor was to try to exploit a strong interaction between the enzyme and a substrate 2-OH. They made the xylobiose analogue **141** and found that it bound to Cex-xylanase with $K_i = 0.34\ \mu$M. They also showed that Cex mutant having the amino acid supposed to bind the 2-OH replaced bound **141** poorly but bound the corresponding isofagomine showing that the carbonyl group was important for binding. It was suggested that this isofagomine lactam was binding as its iminol tautomer, since this tautomer resembled an isofagomine with a 2-OH, and this hypothesis was not inconsistent with an X-ray crystal structure of **141** bound to Cex.[125] Nevertheless, this theory must be regarded as questionable since the free energy cost of amide−iminol isomerization is very large (∼45 kJ/mol).

Subsequently, the D-*galacto* analogue **330** was made.[221] This compound is also a strong inhibitor with $K_i = 18$ nM toward β-galactosidase from *Aspergillus oryzae*. Other galactosidases are inhibited less by **330,** but it is definitely much more potent than galactonolactam **323** (Table 44).

Vasella's group has made the analogues tetrahydropyridazinone **331**, which can be regarded as an azafagomine lactam.[222] This compound was found to inhibit β-glucosidase and β-mannosidase with a K_i of 13 and 6 μM, respectively. The corresponding 5-ene was considerably weaker against both enzymes.

A series of ureas **332**−**334** have been made that quite closely resemble hexonolactams. These compounds lack, however, both the 2- and 3-OH and are consequently very poor inhibitors (15−90 mM) of β-glucosidase and β-galactosidase, Figure 34.[223]

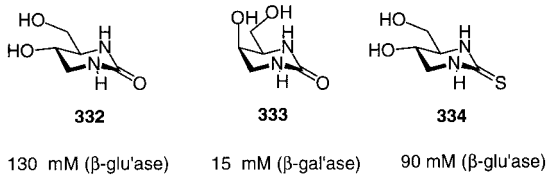


Figure 34.

Aldonolactone oximes are compounds that are closely related to the lactones and were first reported by Vasella more than 15 years ago. These molecules have a very low basicity and are, therefore, regarded as neutral inhibitors here. While the unsubstituted oxime was originally found to be a moderate inhibitor, substituted analogues were found to inhibit almond β-glucosidase in the low micromolar range.[224] It has now been found that an *N*-*O*-dinitrophenyl derivative **335** (Figure 35) inhibits α-glucosidase with a similar potency.[225]

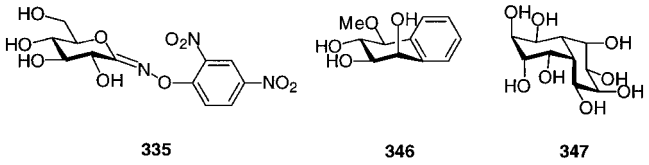


Figure 35.

Vasella's group has in the glycotetrazoles made a series of neutral transition-state analogues that both resemble the hexonolactams and the Ganem-type aminidines.[194] These compounds have an estimated pK_a value of −4 and cannot be expected to be fully protonated in the active site. The first two examples of these compounds, the glucose and mannose analogues **2** and **3** (Table 45), were found to have a half-chair conformation and inhibit glycosidases moderately, though **2** inhibited β-glucosidase and β-galactosidase with a K_i of about 1.5 μM.[170] Interestingly, a log(k_{cat}/K_M) vs log K_i plot for the inhibitors **2** and **3** and for the hydrolysis reaction of 4-nitrophenyl glucoside or mannoside by a series of different glycosidases gave a linear relationship. This suggests a similarity between the transition state of these different enzyme-catalyzed reactions and the inhibitors at least in regard to the changes made in the inhibitor and substrate/enzyme pair.

Mannotetrazole **3** has been found to be a poor α-fucosidase inhibitor,[226] while glucotetrazole **2** has been found to inhibit glycogen phosphorylase with a K_i of 53 μM. An X-ray structure of the inhibitor binding to the enzyme was also reported and showed that it bound together with phosphate.[227]

Several stereoisomers and analogues of **2** and **3** have been reported. The galactose and *N*-acetylglucosamine analogues **302** and **94** have been made; the former inhibit β-galactosidase with $K_i = 0.8-1\ \mu$M, while **94** inhibits β-glucosaminidase with $K_i = 0.2\ \mu$M (Table 15).[228]

The 6-deoxy analogue of **3**, **336** has been made together with some furanoside analogues (Figure 36).

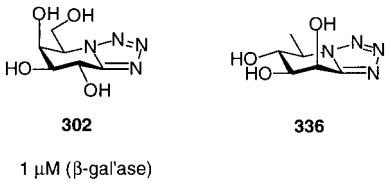
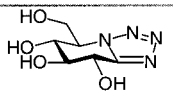
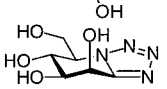


Figure 36.

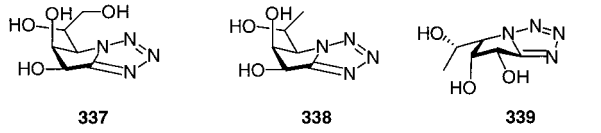
These compounds were in general poor glycosidase inhibitors except that **336** was found to be a better inhibitor of α-mannosidase than **3**.[229,233] Another furanose analogue, the L-rhamnofuranose tetrazole **337**, inhibited naranginase with $K_i = 56\ \mu$M (Figure 37).[230] Curiously, the corresponding L-rhamnopyranose analogue **340** inhibited this enzyme less than **337**. The 5-epimer of **340**, **341** was even weaker (Figure 38).[231,236] A cellobiosyl tetrazole **342** has also been made, but inhibition studies have apparently not been made.[232]

A series of compounds that can be regarded as related to the lactones and lactams are the glucose-resembling phosphonates and phosphonamides **343**−

Table 45. K_i values in μM from Ref 163

	Structure	β-glu[a]	α-glu[b]	β-gal[c]	β-man[d]	α-man[e]	α-man[f]
2		1.4	1300-5600	1.5	47000	8500	13800
3		6000	100000	14000	160	180	700

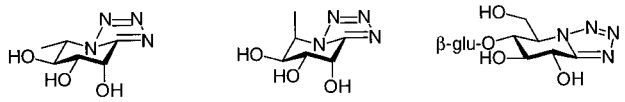
[a] *Agrobacter* β-glucosidase. [b] Yeast α-glucosidase. [c] Bovine liver β-galactosidase. [d] Snail β-mannosidase. [e] Jack bean α-mannosidase. [f] Almond α-mannosidase.



337 **338** **339**

56 μM (Naranginase)

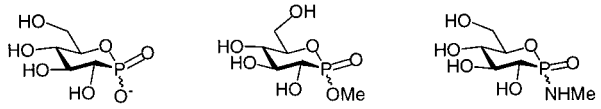
Figure 37.



340 **341** **342**

Figure 38.

345 recently reported by Darrow and Drueckhammer (Figure 39), albeit the geometry is quite different. These compounds were, however, very poor inhibitors of a number of glycosidases.[233]



343 **344** **345**

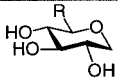
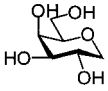
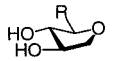
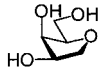
Figure 39.

A carbocyclic analogue of a mannosyl oxocarbenium ion has been made by Hudlicky and collaborators, who synthesized hydroxylated tetrahydronaphthalene **346** (Figure 35).[234] This compound displayed some inhibition of β-galactosidase but not of mannosidase. However, recently a surprisingly high affinity has been found in the hydroxylated decahydronaphthalene **347**.[235] Octol **347** inhibited α-glucosidase with $K_i = 12$ μM but did not inhibit β-glucosidase.

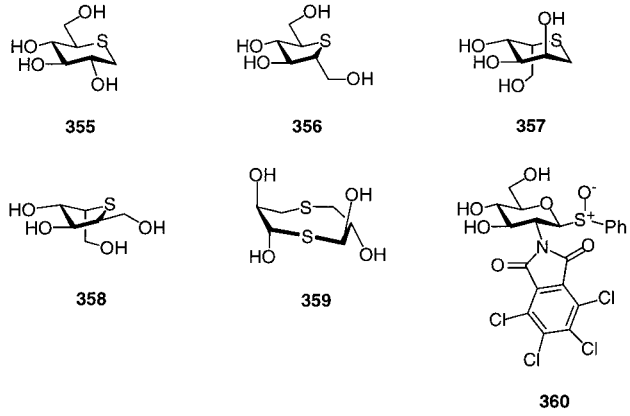
Field et al. studied the β-glucosidase and β-galactosidase inhibition of a series of five- and six-membered anhydroalditols (Table 46), and the five-membered anhydroalditols may possibly have a transition-state-like geometry. The D-*gluco*, D-*galacto*, and D-*xylo* stereoisomers of both pyranose and furanose form were made, but all compounds only inhibited the enzymes in the millimolar range, which is comparable to the inhibition displayed by monosaccharides.[236]

In a related study glycosidase inhibition of a series of 1,4-, 1,5-, and 1,6-thioalditols was investigated (Figure 40), and again the five-membered alditols may be considered transition-state analogues in terms of geometry.[237] The corresponding cyclic sulfoxides and sulfones were also studied. All compounds displayed weak inhibition comparable in strength to that of the anhydroalditols. The strongest inhibitor was the mannose analogue **357**, which had $K_i = 700$ μM toward α-mannosidase.

Table 46. K_i values in μM from Ref 236

	Structure	R	β-glu'ase[b]	β-gal'ase[c]
348		CH_2OH	60000	71000
349		H	39000	85000
350		CH_3	-	42000
351			140000	7400
352		CH_2OH	36200	51000
353		H	390000	77000
354			14800	1900

[a] Almond β-glucosidase. [b] *E. coli* β-galactosidase.



355 **356** **357**

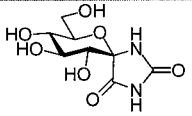
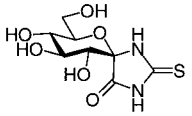
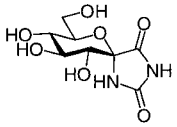
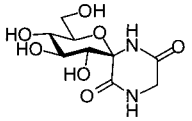
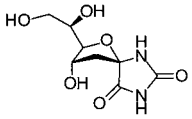
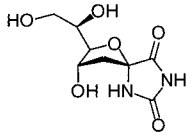
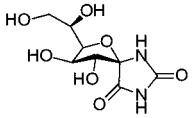
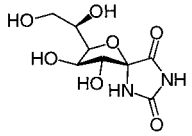
358 **359** **360**

Figure 40.

Thioglycosides are known to be weak to moderate glycosidase inhibitors by being nonhydrolyzable substrate analogues and are as such not covered here. It should be mentioned, however, that glycosyl sulfoxides have been investigated for glycosidase inhibition. One of the more potent of these compounds was the glucosamine derivative **360**, which inhibited β-glucosidase with $K_i = 166$ μM.[238]

A neutral series of glycogen phosphorylase inhibitors (**361**−**368**) has recently emerged that may resemble elements of the transition state. Bichard et al. found that the spirohydantoin **361** inhibited the muscle glycogen phosphorylase b with a K_i of 3.1

Table 47. K_i values in μM toward Glycogen Phosphorylase (GP)

	Structure	Liver GP a	Liver GP b	Muscle GP a	Muscle GP b
361		16.5	12.8	26	4.2
362		29.8	7	10.9	5.1
363		2050			105
364					59.7
365					8200
366					220000
367					-
368					-

[-]No inhibition.

μM.[239] Isomer **363** was somewhat less potent (K_i = 28.6 μM). As **363** contained a contamination of **361,** it was entirely possible that the impurity was causing the inhibition. The inhibition of both muscle and liver glycogen phosphorylase of **361** and **363** and thio analogue **362** has recently been subject to renewed study (Table 47).[240] While the K_i of **361** against muscle glycogen phosphorylase was essentially confirmed, **363** was found to be weaker than previously reported having K_i = 105 μM, which suggests that the first sample of **363** may indeed have been impure. The thiohydantoin **362** had a similar potency as **361**.

The diketopiperazin analogue **364** has also been made but was weaker than **361** (Table 47).[241] A series of furanose hydantoins have also been reported but had disappointing activity (**365**−**368**, Table 47).[242,243] The 3-deoxy analogues of **365** and **366**[249] and the *N*-phenyl analogues of **367** and **368**[250] were also studied but were not inhibitors.

VII. Conclusion

This review clearly shows that a large number of potential transition-state analogues have been evaluated for inhibition of glycosidases and that inhibitors with nanomolar affinity for glycosidases have been found. The most potent inhibitors have been found in all five inhibitor categories II−VI. This can be summarized as follows.

Among the inhibitors that emulate protonation of exocyclic oxygen (category II), the aminobicyclohexane **65** is a very strong α-glucosidase inhibitor and also the aminocyclopentanes **12**, **25**, and **26** have submicromolar affinity. Inhibition in the low nanomolar range has been obtained by substituting lipophilic groups on some of the aminocyclopentanes.

Strong inhibitors are also found among compounds that emulate an oxocarbenium ion (category III), though this has not been deeply covered in this review. Some 1-deoxynojirimycin analogues, such as **126** and **121**, are extremely potent α-glycosidase inhibitors with K_i values in the low nanomolar range. Strong inhibition by this type of inhibitor seems to be isolated to certain enzymes, however. Thus, 1-deoxynojirimycin **75** itself is a rather poor inhibitor of many glycosidases.

Inhibitors that mimic charge at the anomeric position (category IV) have been found to be potent inhibitors of many glycosidases. Particularly the noeuromycins, **117**, **122,** and **127**, are remarkable by being nanomolar inhibitors of both α- and β-glycosidases. The inhibition of the 2-deoxy analogues, the

isofagomines, are strong against some enzymes particularly β-glycosidases.

Category V, inhibitors that mimic charge in several places, also contain very strong inhibitors. In particular, the imidazoles such as **83** and **272** are very potent inhibitors. Substitution of imidazoles with lipophilic groups has been used to create low nanomolar inhibitors.

The neutral inhibitors (category VI) are generally weaker glycosidase inhibitors than inhibitors mimicking charge development. An exception is the isofagomine lactam **141** and analogues which are submicromolar inhibitors of some enzymes though the activity appears very confined to some enzymes.

Altogether it can be concluded that the best glycosidase inhibitors are compounds that have a nitrogen in place of the exo- or endocyclic oxygen or anomeric carbon and thus potentially mimic a charged transition state or intermediate. This suggests that it is more important to mimic the charge development in the transition state than the shape.

It has been argued that some of these potentially charged inhibitors are not transition-state analogues but rather so-called fortuitous binders that owe their binding to a coincidental favorable interaction between the charged center and a residue in the enzyme.[17] However, the fact that it is particularly favored to have a possibly charged group, where charge is anticipated to develop during the reaction, does not appear coincidental. Indeed, since these potentially charged inhibitors bind strongly (and if they are indeed charged), the corresponding charged reaction intermediates must also bind strongly.

Nevertheless, the transition state must be bound much stronger by a glycosidase than any known glycosidase inhibitor, and as such even the best glycosidase inhibitors are imperfect transition-state analogues. It seems that inhibitors that mimic charge in exocyclic, endocyclic, and anomeric positions all mimic features of the transition state. Which feature is the more important to mimic may depend on the enzyme.

VIII. Acknowledgments

We thank the Danish Research Councils (THOR program) and the Lundbeck foundation for financial support.

IX. References

(1) Truscheit, E.; Hillebrand, I.; Junge, B.; Müller, L.; Puls, W.; Schmidt, D. *Prog. Clin. Biochem. Med.* **1988,** *7,* 17−99.
(2) Treadway, J. L.; Mendys, P.; Hoover, D. J. *Expert Opin. Invest. Drugs* **2001,** *10,* 439−454.
(3) Laver, W. G.; Bischofberger, N.; Webster, R. G. *Sci. Am.* **1999,** *Jan,* 78−87.
(4) Groopman, J. E. *Rev. Infect.. Dis.* **1990,** *12,* 908−911.
(5) Zitzmann, N.; Mehta, A. S.; Carrouée, S.; Butters, T. D.; Platt, F. M.; McCauley, J.; Blumberg, B. S.; Dwek, R. A.; Block, T. M. *PNAS* **1999,** *96,* 11878−11882.
(6) Sinnott, M. L. *Chem. Rev.* **1990**, *90*, 1171−1202.
(7) Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990,** *48,* 319−385.
(8) *Iminosugars as glycosidase inhibitors;* Stütz, A., Ed.; Wiley-VCH: Weinheim, 1999.
(9) Bennet, A. J.; Sinnott, M. L. *J. Am. Chem. Soc.* **1986**, *108*, 7287−7294.
(10) Namchuk, M. N.; Withers, S. G. *Biochemistry* **1995**, *34*, 16194−16202.
(11) McCarter, J. D.; Adam, M. J.; Withers, S. G. *Biochem. J.* **1992**, *286*, 721−727.
(12) Davies, G. J.; Mackenzie, L.; Varrot, A.; Dauter, M.; Brzozowski, A. M.; Schülein, M.; Withers, S. G. *Biochemistry* **1998**, *37*, 11707−11713.
(13) Sulzenbacher, G.; Driquez, H. Henrissat, B.; Schülein, M.; Davies, G. J. *Biochemistry* **1996**, *35*, 15280−15287.
(14) Legler, G. *Iminosugars as glycosidase inhibitors*; Stütz, A., Ed.; Wiley-VCH: Weinheim, 1999; pp 31−67.
(15) Mader, M. M.; Bartlett P. A. *Chem. Rev.* **1997**, *97*, 1281−1301.
(16) Mosi, R.; Sham, H.; Uitdehaag, J. C. M.; Ruiterkamp, R.; Dijkstra, B. W.; Withers, S. G. *Biochemistry* **1998**, *37*, 17192−17198.
(17) Withers, S. G. Namchuk, M.; Mosi, R. *Iminosugars as glycosidase inhibitors: nojirimycin and beyond*; Stütz, A. E., Ed.; Wiley-VCH: Weinheim, 1999; pp 188−206.
(18) Truscheit, V. E.; Frommer, W.; Junge, B.; Müller, L.; Schmidt, D. D.; Wingender, W. *Angew. Chem.* **1981,** *93,* 738−755.
(19) Berecibar, A.; Grandjean, C.; Siriwardena, A. *Chem. Rev.* **1999**, *99*, 779−844.
(20) Farr, R. A.; Peet, N. P.; Kang, M. S. *Tetrahedron Lett.* **1990,** *31,* 7109−7112.
(21) Winkler, D. A.; Holan, G. *J. Med. Chem.* **1989**, *32*, 2084−2089.
(22) Winkler, D. A. *J. Med. Chem.* **1996**, *39*, 4332−4334.
(23) Tropea, J. E.; Kaushal, G. P.; Pastuszak, I.; Mitchell, M.; Aoyagi, T.; Molyneux, R. J.; Elbein, A. D. *Biochemistry* **1990**, *29*, 10062−10069.
(24) Kleban, M.; Hilgers, P.; Greul, J. N.; Kugler, R. D.; Li, J.; Picasso, S.; Vogel, P.; Jäger, V. *ChemBioChem* **2001,** 365−368.
(25) Leroy, E.; Reymond, J.-L. *Org. Lett.* **1999,** *1,* 775−777.
(26) Greul, J. N.; Kleban, M.; Schneider, B.; Picasso, S.; Jäger, V. *ChemBioChem* **2001,** 368−370.
(27) Blaser, A.; Reymond, J.-L. *Helv. Chim. Acta* **1999,** *82,* 760−768.
(28) Blaser, A.; Reymond, J.-L. *Org. Lett.* **2000,** *2,* 1733−1736.
(29) Boss, O.; Leroy, E.; Blaser, A.; Reymond, J.-L. *Org. Lett.* **2000,** *2,* 151−154.
(30) Tatsuta, K.; Niwata, Y.; Umezawa, K.; Toshima, K.; Nakata, M. *J. Antibiot.* **1991,** *44,* 912−914.
(31) Nakata, M.; Chong, C.; Niwata, Y.; Toshima, K.; Tatsuta, K. *J. Antibiot.* **1993,** *46,* 1919−1922.
(32) Tatsuta, K. *Pure Appl. Chem.* **1996,** *68,* 1341−1346.
(33) Legler, G.; Herrchen, M. *Carbohydr. Res.* **1983,** *116,* 95−103.
(34) Maity, S. K.; Dutta, S. K.; Banerjee, A. K.; Achari, B.; Singh, M. *Tetrahedron* **1994,** *50,* 6965−6974.
(35) Legler, G.; Stütz, E.; Immich, H. *Carbohydr. Res.* **1995,** *272,* 17−30.
(36) Guo, W.; Hiratake, J.; Ogawa, K.; Yamamoto, M.; Ma, S.-J.; Sakata, K. *Bioorg. Med. Chem. Lett.* **2001,** *11,* 467−470.
(37) Lai, W.; Martin, O. R. *Carbohydr. Res.* **1993,** *250,* 185−193.
(38) BeMiller, J. N.; Yadav, M. P. *Carbohydr. Res.* **1990,** *200,* 111−126.
(39) BeMiller, J. N.; Gilson, R. J.; Myers, R. W.; Santoro, M. M.; Yadav, M. P. *Carbohydr. Res.* **1993,** *250,* 93−100.
(40) Dietrich, H.; Schmidt, R. R. *Carbohydr. Res.* **1993,** *250,* 161−176.
(41) Schmidt, R. R.; Dietrich, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1328−1329.
(42) Martin, O. R.; Lai, W. *J. Org. Chem.* **1993**, *58*, 176−185.
(43) Kiss, L.; Somsák, L. *Carbohydr. Res.* **1996,** *291,* 43−52.
(44) Abdel-Rahman, A. A.-H.; El Ashry, E. S. H.; Schmidt, R. R. *Carbohydr. Res.* **1999,** *315,* 106−116.
(45) Streicher, H.; Reiner, M.; Schmidt, R. R. *J. Carbohydr. Chem.* **1997,** *16,* 277−298.
(46) Martin, J. L.; Veluraja, K.; Ross, K.; Johnson, L. N.; Fleet, G. W. J.; Ramsden, N. G.; Bruce, I.; Orchard, M. G.; Oikonomakos, N. G.; Papageorgiou, A. C.; Leonidas, D. D.; Tsitoura, H. S. *Biochemistry* **1991,** *30,* 10101−10116.
(47) Tanaka, K. S. E.; Winters, G. C.; Batchelor, R. J.; Einstein, F. W. B.; Bennet, A. J. *J. Am. Chem. Soc.* **2001,** *123,* 998−999.
(48) Lorthiois, E.; Meyyappan, M.; Vasella, A. *Chem. Commun.* **2000,** 1829−1830
(49) Davies, G. J.; Mackenzie, L.; Varrot, A.; Dauter, M.; Brzozowski, A. M.; Withers, S. G. *Biochemistry* **1998**, *37*, 11707−11713.
(50) Tews, I.; Perrakis, A.; Oppenheim, A.; Dauter, Z.; Wilson, K. S.; Vorgias, C. E. *Nat. Struct. Biol.* **1996,** *3,* 638−648.
(51) Sulzenbacher, G.; Driguez, H.; Henrissat, B.; Schülein, M.; Davies, G. *Biochemistry* **1996**, *35*, 15280−15287.
(52) Andrews, J. S.; Weimar, T.; Frandsen, T. P.; Svensson, B.; Pinto, B. M. *J. Am. Chem. Soc.* **1995**, *117*, 10799−10804.
(53) Randell, K. D.; Frandsen, T. P.; Stoffer, B.; Johnson, M. A.; Svensson, B.; Pinto, B. M. *Carbohydr. Res.* **1999,** *321,* 143−156.
(54) Overend, W. G.; Rees, C. W.; Sequeira, J. S. *J. Chem. Soc.* **1962,** 3429−3440.
(55) Ishida, N.; Kumagai, K.; Niida, T.; Tsuruaka, T.; Yumato, H. *J. Antibiot.* **1967,** *20,* 66−71.
(56) Inouye, S.; Tsuruaka, T.; Ito, T.; Niida, T. *Tetrahedron* **1968,** *24,* 2125−2144.

(57) Murro, S.; Miyata, S. *Agric. Biol. Chem*. **1980,** *44*, 219−221
(58) Yagi, M.; Kuono, T.; Aoyagi, Y.; Murai, H. *Nippon Nogei Kagaku Kaishi* **1976,** *50*, 571−572.
(59) Yu, J.; Choi, S. Y.; Moon, K.-D.; Chung, H.-H.; Youn, H. J.; Jeong, S.; Park, H.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1998,** *95*, 2880−2884.
(60) Frankowski, A.; Seliga, C.; Bur, D.; Streith, J. *Helv. Chim. Acta* **1991,** *74*, 934−940.
(61) Streith, J.; Boiron, A.; Frankowski, A.; Le Nouen, D.; Rudyk, H.; Tschamber, T. *Synthesis* **1995,** 944−946.
(62) Frankowski, A.; Deredas, D.; Le Nouen, D.; Tschamber, T.; Streith, J. *Helv. Chim. Acta* **1995,** *78*, 1837−1842.
(63) Frankowski, A.; Deredas, D.; Streith, J.; Tschamber, T. *Tetrahedron* **1998,** *54*, 9033−9042.
(64) Tschamber, T.; Siendt, H.; Boiron, A.; Gessier, F.; Deredas, D.; Frankowski, A.; Picasso, S.; Steiner, H.; Aubertin, A.-M.; Streith, J. *Eur. J. Org. Chem*. **2001,** 1335−1347.
(65) Bernotas, R. C.; Papandreou, G.; Urbach, J.; Ganem, B. *Tetrahedron Lett.* **1990,** *31*, 3393−3396.
(66) Aoyagi, T.; Suda, H.; Uotani, K.; Kojima, F.; Aoyama, T.; Horiguchi, K.; Hamada, M.; Takeuchi, T. *J. Antibiot*. **1992,** *45*, 1404−1408.
(67) Aoyama, T.; Naganawa, H.; Suda, H.; Uotani, K.; Aoyagi, T.; Takeuchi, T. *J. Antibiot*. **1992,** *45*, 1557−1558.
(68) Granier, T.; Gaiser, F.; Hintermann, L.; Vasella, A. *Helv. Chim. Acta* **1997,** *80*, 1443−1456.
(69) Panday, N.; Granier, T.; Vasella, A. *Helv. Chim. Acta* **1998,** *81*, 475−490.
(70) Krülle, T. M.; de la Fuente, C.; Pickering, L.; Aplin, R. T.; Tsitsanou, K. E.; Zographos, S. E.; Oikonomakos, N. G.; Nash, R. J.; Griffiths, R. C.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **1997,** *8*, 3807−3820.
(71) Streith, J.; Rudyk, H.; Tschamber, Tarnus, C.; Strehler, C.; Deredas, D.; Frankowski, A. *Eur. J. Org. Chem*. **1999,** 893−898.
(72) Tezuka, K.; Compain, P.; Martin, O. R. *Synlett* **2000,** *12*, 1837−1839.
(73) Siriwardena, A. H.; Chiaroni, A.; Riche, C.; El-Daher, S.; Winchester, B.; Grierson, D. S. *J. Chem. Soc., Chem. Commun* **1992,** 1531−1533.
(74) Yuasa, H.; Kajimoto, T.; Wong, C.-H. *Tetrahedron Lett*. **1994,** *35*, 8243−8246.
(75) Yuasa, H.; Takada, J.; Hashimoto, H. *Tetrahedron Lett.* **2000,** *41*, 6615−6618.
(76) Ghavami, A.; Johnston, B. D.; Pinto, B. M. *J. Org. Chem*. **2001,** *66*, 2312−2317.
(77) Svansson, L.; Johnston, B. D.; Gu, J.-H.; Patrick, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2000,** *122*, 10769−10775.
(78) Namchuk, M. N.; McCarter, J. D.; Becalski, A.; Andrews, T.; Withers, S. G.*J. Am. Chem. Soc.* **2000,** *122*, 1270−1277.
(79) Jensen, H. H.; Lyngbye, L.; Bols, M. *Angew. Chem., Int. Ed.* **2001**, *40*, 3447−3449.
(80) Kajimoto, T.; Liu, K. K.-C.; Pederson, R. L.; Zhong, Z.; Ichikawa, Y.; Porco, J. A Jr.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6187−6196.
(81) Reymond, J.-L.; Jahangiri, G. K.; Stoudt, C.; Lerner, R. A. *J. Am. Chem. Soc.* **1993,** *115*, 3909−3918.
(82) Reymond, J.-L.; Janda, K. D.; Lerner, R. A.*Angew. Chem., Int. Ed. Engl.* **1991,** *30*, 1711−1713.
(83) Jespersen, T. M.; Dong, W.; Sierks, M. R.; Skrydstrup, T.; Lundt, I.; Bols, M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1778−1779.
(84) Jespersen, T. M.; Bols, M.; Sierks, M. R.; Skrydstrup, T. *Tetrahedron* **1994,** *50*, 13449−13460.
(85) Bülow, A.; Plesner, I. W.; Bols, M. *J. Am. Chem. Soc.* **2000**, *122*, 8567−8568.
(86) Lundgren, K.; Rassov, A.; Bols, M. *Diabetes* **1996,** *45* (Suppl 12), 521.
(87) Jakobsen, P.; Lundbeck, J. M Kristiansen, M.; Breinholt, J.; Demuth, H.; Pawlas, J.; Candela, M. P. T.; Andersen, B.; Westergaard, N.; Lundgren, K.; Asano, N. *Bioorg. Med. Chem.* **2001,** *9*, 733−744.
(88) Waagepetersen, H. S.; Westergaard, N.; Schousboe, A. *Neurochem. Int.* **2000,** *36*, 435−440.
(89) Kim, Y. J.; Ichikawa, M.; Ichikawa, Y. *J. Org. Chem*. **2000**, *65*, 2599−2602.
(90) Pandey, G.; Kapur, M. *Tetrahedron Lett* **2000,** *41*, 8821−8824.
(91) Zhao, G.; Deo, U. C.; Ganem, B. *Org. Lett.* **2001,** *3*, 201−203.
(92) Andersch, J.; Bols, M. *Chem. Eur. J*. **2001**, *7*, 3744−3747.
(93) Ichikawa, Y.; Igarashi, Y. *Tetrahedron Lett.* **1995,** *36*, 4585−4586.
(94) Ichikawa, Y.; Igarashi; Y.; Ichikawa, M.; Suhara, Y. *J. Am. Chem. Soc.* **1998,** *120*, 3007−3018.
(95) Igarashi, Y.; Ichikawa, M.; Ichikawa, Y. *Tetrahedron Lett.* **1996,** *37*, 2707−2708.
(96) Liang, X.; Lohse, A.; Bols, M. *J. Org. Chem*. **2000,** *65*, 7432−7437.
(97) Igarashi, Y.; Ichikawa, M.; Ichikawa, Y. *Bioorg. Med. Chem. Lett.* **1996,** *6*, 553−558.
(98) Hansen, A.; Tagmose, T. M.; Bols, M. *J. Chem. Soc., Chem. Commun*. **1996,** 2649−2650.
(99) Hansen, A.; Tagmose, T. M.; Bols, M. *Tetrahedron* **1997,** *53*, 697−706.
(100) Bols, M.; Hazell, R. G.; Thomsen, I. B. *Chem. Eur. J*. **1997,** *3*, 940−947.
(101) Zechel, D. L.; Withers, S. G. *Acc. Chem. Res*. **2000,** *33*, 11−18.
(102) Hansen, S. U.; Bols, M. *Acta Chem. Scand*. **1998,** *52*, 1214−1222.
(103) Makino, K.; Ichikawa, Y. *Tetrahedron Lett.* **1998,** *39*, 8245−8248.
(104) Parr, I. B.; Horenstein, B. A. *J. Org. Chem*. **1997**, *62*, 7489−7494.
(105) Williams, S. J.; Hoos, R.; Withers, S. G. *J. Am. Chem. Soc.* **2000,** *122*, 2223−2235.
(106) Notenboom, V.; Birsan, C.; Warren, R. A. J.; Withers, S. G.; Rose, D. R. *Biochemistry* **1998**, *37*, 4751−4758.
(107) Cauderay, M.; Tappy, L.; Temler, E.; Jequier, E.; Hillebrand, I.; Felber, J.-P. *Metabolism* **1986,** *35*, 472−477.
(108) Karpas, A.; Fleet, G. W.; Dwek, R. A.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W. *Proc. Natl. Acad. Sci.* **1998**, 85, 9229−9233.
(109) Hansen, S. U.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2000,** 911−915.
(110) Dong, W.; Jespersen, T.; Bols, M.; Skrydstrup, T.; Sierks, M. R. *Biochemistry* **1996**, *35*, 2788−2795.
(111) Andreassen, V.; Svensson, B.; Bols, M. *Synthesis* **2001,** 339−342.
(112) Fort, S.; Coutinho, P. M.; Schülein, M.; Nardin, R.; Cottaz, S.; Driguez, H. *Tetrahedron Lett.* **2001,** *42*, 3443−3446.
(113) Kim, Y. J.; Ichikawa, M.; Ichikawa, Y. *J. Am. Chem. Soc.* **1999**, *121*, 5829−5830.
(114) Takayama, S.; Chung, S. J.; Igarashi, Y.; Ichikawa, Y.; Sepp. A.; Lechler, R. I.; Wu, J.; Hayashi, T.; Siuzdak, G.; Wong, C.-H. *Bioorg. Med. Chem*. **1999,** *7*, 401−409.
(115) Ichikawa, M.; Igarashi, Y.; Ichikawa, Y. *Tetrahedron Lett.* **1995,** *36*, 1767−1770.
(116) Bols, M.; Persson, M. P.; Butt, W. M.; Jørgensen, M.; Christensen, P.; Hansen, L. T. *Tetrahedron Lett.* **1996,** *37*, 2097−2100.
(117) Ichikawa, M.; Ichikawa, Y. *Bioorg. Med. Chem*. **1995,** *3*, 161−165.
(118) Godskesen, M.; Lundt, I. *Tetrahedron Lett.* **1998,** *39*, 5841−5844.
(119) Schneider, C.; Kazmaier, U. *Eur. J. Org. Chem*. **1998**, *1155*, 5−1159.
(120) Metha, G.; Modal, N. *Tetrahedron Lett.* **2000,** *41*, 5747−5751.
(121) Lohse, A.; Jensen, H. H.; Bach, P.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2000,** 659−665.
(122) Bach, P.; Lohse, A.; Bols, M. *Tetrahedron Lett.* **1999,** *40*, 367−370.
(123) Jensen, H. H.; Lohse, A.; Petersen, B. O.; Duss, J. Ø.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2000,** 667−670.
(124) Williams, S. J.; Notenboom, V.; Wicki, J.; Rose, D. R.; Withers, S. G. *J. Am. Chem. Soc.* **2000,** *122*, 4229−4230.
(125) Schuster, M. *Biorg. Med. Chem. Lett.* **1999,** *9*, 615−618.
(126) Liu, H.; Liang, X.; Søhoel, H.; Bülow, A.; Bols, M. *J. Am. Chem. Soc.* **2001,** *123*, 5116−5117.
(127) Kondo, K.; Adachi, H.; Shitara, E.; Kojima, F.; Nishimura, Y. *Bioorg. Med. Chem*. **2001,** *9*, 1091−1095.
(128) Umezawa, H.; Aoyagi, T.; Komiyama, T.; Morishima, H.; Hamada, M.; Takeuchi, T. *J. Antibiot*. **1974,** *27*, 963−969.
(129) Nishimura, Y.; Wang, W.-M.; Kondo, S.; Aoyagi, T.; Umezawa, H. *J. Am. Chem. Soc.* **1988,** *110*, 7249−7250.
(130) Nishimura, Y.; Wang, W.-M.; Kondo, S.; Aoyagi, T.; Umezawa, H *Zhongguo Yiyao Gongye Zazhi* **1989,** *20*, 307−313.
(131) Nishimura, Y.; Wang, W.-M.; Kudo, T.; Kondo, S. *Bull. Chem. Soc. Jpn*. **1992**, *65*, 978−986.
(132) Knapp, S.; Zhao, D. *Org. Lett*. **2000,** *2*, 4037−4040.
(133) Takatsu, T.; Takahashi, M.; Kawase, Y.; Enokita, R.; Okazaki, T.; Matsukawa, H.; Ogawa, K.; Sakaida, Y.; Kagasaki, T.; Kinoshita, T.; Nakajima, M.; Tanzawa, K. *J. Antibiot*. **1996,** *49*, 54−60.
(134) Kawase, Y.; Takahashi, M.; Takatsu, T.; Arai, M.; Nakajima, M.; Tanzawa, K. *J. Antibiot*. **1996,** *49*, 61−64.
(135) Nishimura, Y.; Kudo, T.; Kondo, S.; Takeuchi, T. *J. Antibiot*. **1994,** *47*, 101−107.
(136) Nishimura, Y.; Shitara, E.; Adachi, H.; Toyoshima, M.; Nakajima, M.; Okami, Y.; Takeuchi, T. *J. Org. Chem*. **2000,** *65*, 2−11.
(137) Nishimura, Y. *In Studies in Natural Products Chemistry*; Attaur-Rahman, Ed.; Elsevier: Amsterdam, 1995; Vol. 16, pp 75−121.
(138) Satoh, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *J. Antibiot*. **1996,** *49*, 321−325.
(139) Satoh, T.; Nishimura, Y.; Kondo, S.; Takeuchi, T. *Carbohydr. Res*. **1996,** *286*, 173−178.
(140) Nishimura, Y.; Shitara, E.; Kojima, F.; Takeuchi, T. *Bioorg. Med. Chem*. **1999,** *7*, 1241−1246.
(141) Niwa, T.; Inouye, S.; Tsuruoka, T.; Koaze, Y.; Niida, T. *Agric. Biol. Chem*. **1970,** *34*, 966−968.
(142) Reese, E. T.; Parrish, F. W.; Ettlinger, M. *Carbohydr. Res*. **1971,** *18*, 381−388.

(143) Niwa, T.; Tsuruoka, T.; Goi, H.; Kodama, Y.; Itoh, J.; Inouye, S.; Yamada, Y.; Niida, T.; Nobe, M.; Ogawa, Y. *J. Antibiot.* **1984,** *37*, 1579−1586.
(144) Legler, G.; Jülich, E. *Carbohydr. Res.* **1984,** *128*, 61−72.
(145) Jensen, H. H.; Lyngbye, L.; Jensen, A.; Bols, M. *Chem. Eur. J.*, in press.
(146) Henrissat, B.; Davies, G. *Curr. Opin. Struct. Biol.* **1997**, *7*, 637−644.
(147) Kudo, T.; Nishimura, Kondo, S.; Takeuchi, T. *J. Antibiot.* **1993,** *46*, 300−309.
(148) Nishimura, Y.; Kudo, T.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1992,** *45*, 963−970.
(149) Nishimura, Y.; Satoh, T.; Adachi, H.; Kondo, S.; Takeuchi, T.; Azetaka, M.; Fukuyasu, H.; Iizuka, Y. *J. Am. Chem. Soc.* **1996**, *118*, 3051−3052.
(150) Nishimura, Y.; Satoh, T.; Adachi, H.; Kondo, S.; Takeuchi, T.; Azetaka, M.; Fukuyasu, H.; Iizuka, Y. *J. Med. Chem.* **1997**, *40*, 2626−2633.
(151) Shitara, E.; Nishimura, Y.; Kojima, F.; Takeuchi, T. *J. Antibiot.* **1999,** 348−350.
(152) Nishimura, Y.; Satoh, T.; Kudo, T.; Kondo, S.; Takeuchi, T. *Bioorg. Med. Chem.* **1996,** *4*, 91−96.
(153) Nishimura, Y.; Shitara, E.; Takeuchi, T. *Tetrahedron Lett.* **1999,** *40*, 2351−2354.
(154) Bach, P.; Bols, M. *Tetrahedron Lett.* **1999,** *40*, 3461−3464.
(155) Tong, M. K.; Papandreou, G.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112*, 6137−6139.
(156) Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340−347.
(157) Dale, M. P.; Ensley, H. E.; Kern, K.; Sastry, K. A. R.; Byers, L. D. *Biochemistry* **1985**, *24*, 3530−3539.
(158) Papandreou, G.; Tong, M. K.; Ganem, B. *J. Am. Chem. Soc.* **1993**, *115*, 11682−11690.
(159) Ganem, B.; Papandreou, G. *J. Am. Chem. Soc.* **1991**, *113*, 8984−8985.
(160) Pan, Y.-T.; Kaushal, G. P.; Papandreou, G.; Ganem, B.; Elbein, A. D. *J. Biol. Chem.* **1992,** *267*, 8313−8318.
(161) Yoon, H.; King, S. B.; Ganem, B. *Tetrahedron Lett.* **1991,** *32*, 7199−7202.
(162) Schedler, D. J. A.; Bowen, B. R.; Ganem, B. *Tetrahedron Lett.* **1994,** *35*, 3845−3848.
(163) Ermert, P.; Vasella, A.; Weber, M.; Rupitz, K.; Withers, S. G. *Carbohydr. Res.* **1993,** *250*, 113−128.
(164) Hoos, R.; Naughton, A. B.; Thiel, W.; Vasella, A.; Weber, W.; Rupitz, K.; Withers, S. G. *Helv. Chim. Acta* **1993,** *76*, 2666−2686.
(165) Legler, G.; Finken, M.-T.; Felsch, S. *Carbohydr. Res.* **1996,** *292*, 91−101.
(166) Legler, G.; Finken, M.-T. *Carbohydr. Res.* **1996,** *292*, 103−115.
(167) Blériot, Y.; Genre-Grandpierre, A.; Tellier, C. *Tetrahedron Lett.* **1994,** *35*, 1867−1870.
(168) Blériot, Y.; Dintinger, T.; Genre-Grandpierre, A.; Padrines, M.; Tellier, C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2655−2660.
(169) Blériot, Y.; Dintinger, T.; Guillo, N.; Tellier, C. *Tetrahedron Lett.* **1995,** *36*, 5175−5178.
(170) Vonhoff, S.; Heightman, T. D.; Vasella, A. *Helv. Chim. Acta* **1998,** *81*, 1710−1725.
(171) Hoos, R.; Vasella, A.; Rupitz, K.; Withers, S. G. *Carbohydr. Res.* **1997,** *298*, 291−298.
(172) Blériot, Y.; Genre-Grandpierre, A.; Imberty, A.; Tellier, C. *J. Carbohydr. Chem.* **1996,** *15*, 985−1000.
(173) Vonhoff, S.; Piens, K. Pipelier, M.; Braet, C.; Claeyssens, M.; Vasella, A. *Helv. Chim. Acta* **1999,** *82*, 963−980.
(174) Horenstein, B. A.; Parkin, D. W.; Estupinan, B.; Schramm, V. L. *Biochemistry* **1991**, *30*, 10788−10795.
(175) Horenstein, B. A.; Schramm, V. L. *Biochemistry* **1993**, *32*, 7089−7097.
(176) Schramm, V. L.; Horenstein, B. A.; Kline, P. C. *J. Biol. Chem.* **1994,** *269*, 18259−18262.
(177) Boutellier, M.; Horenstein, B. A.; Semenyaka, A.; Schramm, V. L.; Ganem, B. *Biochemistry* **1994**, *33*, 3994−4000.
(178) Deng, H.; Chan, A. W.-Y.; Bagdassarian, C. K.; Estupinán, B.; Ganem, B.; Callender, R. H.; Schramm, V. L. *Biochemistry* **1996**, *35*, 6037−6047.
(179) Fotsch, C. H.; Wong, C.-H. *Tetrahedron Lett.* **1994,** *35*, 3481−3484.
(180) Lehmann, J.; Rob, B. *Tetrahedron: Asymmetry* **1994,** *5*, 2255−2260.
(181) Lehmann, J.; Rob, B. *Liebigs Ann. Chem.* **1994,** 805−809.
(182) Lehmann, J.; Rob, B.; Wagenknecht, H.-A. *Carbohydr. Res.* **1995,** *278*, 167−180.
(183) Jeong, J.-H.; Murray, B. W.; Takayama, S.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 4227−4234.
(184) Le, V.-D.; Wong, C.-H. *J. Org. Chem.* **2000,** *65*, 2399−2409.
(185) Merrer, Y. L.; Gauzy, L.; Gravier-Pelletier, C.; Depezay, J.-C. *Bioorg. Med. Chem.* **2000,** *8*, 307−320.
(186) Gauzy, L.; Merrer, Y. L.; Cepezay, J.-C. *Synlett* **1998,** 402−404.
(187) Ermert, P.; Vasella, A. *Helv. Chim. Acta* **1991,** *74*, 2043−2053.
(188) Heightman, T. D.; Vasella, A. T. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 750−770.
(189) Panday, N.; Vasella, A. *Synthesis* **1999,** 1459−1468.
(190) Field, A.; Haines, A. H.; Chrystal, E. J. T.; Luszniak, M. C. *Biochem. J.* **1991,** *274*, 885−889.
(191) Tatsuta, K.; Miura, S.; Ohta, S.; Gunji, H. *Tetrahedron Lett.* **1995,** *36*, 1085−1088.
(192) Tatsuta, K.; Miura, S. *Tetrahedron Lett.* **1995,** *36*, 6721−6724.
(193) Tatsuta, K.; Miura, S.; Gunji, H. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 427−436.
(194) Tatsuta, K.; Miura, S.; Ohta, S.; Gunji, H. *J. Antibiot.* **1995,** *48*, 286−288.
(195) Tatsuta, K.; Ikeda, Y.; Miura, S. *J. Antibiot.* **1996,** *49*, 836−838.
(196) Panday, N.; Canac, Y.; Vasella, A. *Helv. Chim. Acta* **2000,** *83*, 58−79.
(197) Burgess, K.; Chaplin, D. A. *Heterocycles* **1994,** *37*, 673−677.
(198) Varrot, A.; Schülein, M.; Pipelier, M.; Vasella, A.; Davies, G. J. *J. Am. Chem. Soc.* **1999**, *121*, 2621−2622.
(199) Heightman, T. D.; Locatelli, M.; Vasella, A. *Helv. Chim. Acta* **1996,** *79*, 2190−2200.
(200) Koshland, D. E. *Biol. Rev.* **1953,** *28*, 416−436.
(201) Heightman, T. D.; Vasella, A.; Tsitsanou, K. E.; Zographos, S. E.; Skamnaki, V. T.; Oikonomakos, N. G. *Helv. Chim. Acta* **1998,** *81*, 853−864.
(202) Liang, X.; Bols, M. *J. Org. Chem.* **1999**, *64*, 8485−8488.
(203) Ernholt, B. V.; Thomsen, I. B.; Jensen, K. B.; Bols, M. *Synlett* **1999,** *6*, 701−704.
(204) Ernholt, B. V.; Thomsen, I. B.; Lohse, A.; Plesner, I. W.; Jensen, K. B.; Hazell, R. G.; Liang, X.; Jakobsen, A.; Bols, M. *Chem. Eur. J.* **2000,** *6*, 278−287.
(205) Lohse, A.; Hardlei, T.; Jensen, A.; Plesner, I. W.; Bols, M. *Biochem. J.* **2000,** *349*, 211−215.
(206) Bülow, A.; Plesner, I. W.; Bols, M. *Biochim. Biophys. Acta* **2001,** *1545*, 207−215.
(207) Hansen, S. U.; Plesner, I. W.; Bols, M. *Chembiochem* **2000,** *1*, 177−180.
(208) Lohse, A.; Jensen, K. B.; Bols, M. *Tetrahedron Lett.* **1999,** *40*, 3033−3036.
(209) Lohse, A.; Jensen, K. B.; Lundgren, K.; Bols, M. *Bioorg. Med. Chem.* **1999,** *7*, 1965−1971.
(210) Muroi, M.; Ando, O.; Bols, M.; Takatsuki, A. *Biosci. Biotechnol. Biochem.* **2000,** *64*, 1103−1105.
(211) Jensen, H. H.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001,** 905−909.
(212) Søndergaard, K.; Liang, X.; Bols, M. *Chem. Eur. J.* **2001,** *7*, 2324−2331.
(213) Hansen, S. U.; Bols, M. *J. Chem. Soc., Perkin Trans. 2* **2000,** 665−667.
(214) Thomsen, I.; Ernholt, B. V.; Bols, M. *Tetrahedron* **1997,** *53*, 9357−9364.
(215) Dunitz, J. D.; Taylor, R. *Chem. Eur. J.* **1997,** *3*, 89−98.
(216) O'Hagan, D.; Rzepa, H. S. *Chem. Commun.* **1997,** 645−652.
(217) Søhoel, H.; Liang, X.; Bols, M. *Synlett* **2000,** *3*, 347−348.
(218) Nishimura, Y.; Adachi, H.; Satoh, T.; Shitara, E.; Nakamura, H.; Kojima, F.; Takeuchi, T. *J. Org. Chem.* **2000,** *65*, 4871−4882.
(219) Notenboom, V.; Williams, S. J.; Hoos, R.; Withers, S. G.; Rose, D. R. *Biochemistry* **2000,** *39*, 11553−11563.
(220) Panday, N.; Meyyappan, M.; Vasella, A. *Helv. Chim. Acta* **2000,** *83*, 513−538.
(221) Søhoel, H.; Liang, X.; Bols, M. *J. Chem. Soc., Perkin 1* **2001,** 1584−1585.
(222) Ramana, C. V.; Vasella, A. *Helv. Chim. Acta* **2000,** *83*, 1599−1610.
(223) Jiriciek, R.; Lehman, J.; Rob, B.; Scheuring, M. *Carbohydr. Res.* **1993,** *250*, 31−43.
(224) Beer, D.; Vasella, A. *Helv. Chim. Acta* **1986,** *69*, 267−270.
(225) Therisod, M.; Therisod, H.; Lubineau, A. *Bioorg. Med. Chem. Lett.* **1995,** *5*, 2055−2058.
(226) Brandstetter, T. W.; Davis, B.; Hyett, D.; Smith, C.; Hackett, L.; Winchester, B. G.; Fleet, G. W. J. *Tetrahedron Lett.* **1995,** *36*, 7511−7514.
(227) Mitchell, E. P.; Withers, S. G.; Ermert, P.; Vasella, A. T.; Garman, E. F.; Oikonomakos, N. G.; Johnson, L. N. *Biochemistry* **1996**, *35*, 7341−7355.
(228) Heightman, T. D.; Ermert, P.; Klein, D.; Vasella, A. *Helv. Chim. Acta* **1995,** *78*, 514−532.
(229) Davis, B. G.; Brandstetter, T. W.; Hackett, L.; Winchester, B. G.; Nash, R. J.; Watson, A. A.; Griffiths, R. C.; Smith, C.; Fleet, G. W. J. *Tetrahedron* **1999,** *55*, 4489−4500.
(230) Davis, B.; Bell, A. A.; Nash, R. J.; Watson, A. A.; Griffiths, R. C.; Jones, M. G.; Smith, C.; Fleet, G. W. J. *Tetrahedron Lett.* **1996,** *37*, 8565−8568.
(231) Shilvock, J. P.; Wheatley, J. R.; Davis, B.; Nash, R. J.; Griffiths, R. C.; Jones, M. G.; Müller, M.; Crook, S.; Watkin, D. J.; Smith, C.; Besra, G. S.; Brennan, P. J.; Fleet, G. W. J. *Tetrahedron Lett.* **1996,** *37*, 8569−8572.
(232) Vonhoff, S.; Vasella, A. *Synth. Commun.* **1999,** *29*, 551−560.
(233) Darrow, J. W.; Drueckhammer, D. G. *Bioorg. Med. Chem. Lett.* **1996**, *4*, 1341−1348.

(234) Desjardins, M.; Lallemand, M.-C.; Freeman, S.; Hudlicky, T.; Abboud, K. A. *J. Chem. Soc., Perkin 1* **1999,** 621−628.
(235) Mehta, G.; Ramesh, S. S. *Chem. Commun.* **2000,** 2429−2430.
(236) Field, R. A.; Haines, A. H.; Chrystal, E. J. T. *Bioorg. Med. Chem. Lett.* **1991,** *1,* 667−672.
(237) Merrer, Y. L.; Fuzier, M.; Dosbaa, I.; Foglietti, M.-J.; Depezay, J.-C. *Tetrahedron* **1997,** *53,* 16731−16746.
(238) Robina, I.; Gomez-Bujedo, S.; Fernandez-Bolanos, J. G.; Pozo, L. D.; Demange, R.; Picasso, S.; Vogel, P. *Carbohydr. Lett.* **2000,** *3,* 389−396.
(239) Bichard, C. J. F.; Mitchell, E. P.; Wormald, M. R.; Watson, K. A.; Johnson, L. N.; Zographos, S. E.; Koutra, D. D.; Oikonomakos, N. G.; Fleet, G. W. *Tetrahedron Lett.* **1995,** *36,* 2145−2148.
(240) Osz, E.; Somsák, L.; Szilágyi, L.; Kovács, L.; Docsa, T.; Tóth, B.; Gergely, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1385−1390.
(241) Krülle, T. M.; Watson, K. A.; Gregoriou, M.; Johnson, L. N.; Crook, S.; Watkin, D. J.; Griffith, R. C.; Nash, R. J.; Tsitsanou, K. E.; Zographos, S. E.; Oikonomakos, N. G.; Fleet, G. W. *Tetrahedron Lett.* **1995,** *36,* 8291−8294.
(242) Agasimundin, Y. S.; Mumper, M. W.; Hosmane, R. S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 911−923.
(243) Brandstetter, T. W.; Fuente, C. de la; Kim, Y.; Johnson, L. N.; Crook, S.; Lilley, P. M.; Watkin, D. J.; Tsitsanou, K. E.; Zographos, S. E.; Chrysina, E. D.; Oikonomakos, N. G.; Fleet, G. W. J. *Tetrahedron* **1996,** *52,* 10721−10736.
(244) Panday, N.; Vasella, A. *Helv. Chim. Acta* **2000,** *83,* 1205−1208.
(245) Yuasa, H.; Takada, J.; Hashimoto, H. *Bioorg. Med. Chem. Lett.* **2001,** *11,* 1137−1139.
(246) Yoshikawa, M.; Murakami, T.; Shimada, H.; Matsuda, H.; Yamahara, J.; Tanabe, G.; Muraoka, O. *Tetrahedron Lett.* **1997,** *38,* 8367−8370.
(247) Yoshikawa, M.; Murakami, T.; Yashiro, K.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 1339−1340.
(248) Legler, G.; Stütz, A. E.; Immich, H. *Carbohydr. Res.* **1995,** *272,* 17−30.
(249) di Bello, I. C.; Dorling, P.; Fellows, L.; Winchester, B. *FEBS Lett.* **1984,** *176,* 61−64.
(250) Tsusuoka, T.; Fukuyasu, H.; Ishi, M.; Usui, T.; Shibahara, S.; Inouye, S. *J. Antibiot.* **1996,** *49,* 155−161.
(251) Granier, T.; Panday, N.; Vasella, A. *Helv. Chim. Acta* **1997,** *80,* 979−987.
(252) Thomsen, I.; Ernholt, B. V.; Bols, M. *Tetrahedron* **1997,** *53,* 9357−9364.
(253) Elbein, A. D. *FASEB J.* **1991,** *5,* 3055−63.
(254) Nishimura, Y. *Stud. Nat. Prod. Chem.* **1992,** *10,* 495−583.
(255) Mooser, G. *Enzymes* **1992,** *20,* 187−233.
(256) Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992,** *2,* 199−210.
(257) Legler, G. *Naturwissenschaften* **1993,** *80,* 397−409.
(258) Vasella, A.; Ermert, P.; Hoos, R. Naughton, A. B.; Rupitz, K.; Thiel, W.; Weber, M.; Weber, W.; Withers, S. G. *Alfred Benzon Symp.* **1994,** *36,* 134−152.
(259) Jacob, G. S. *Curr. Opin. Struct. Biol.* **1995,** *5,* 605−11.
(260) Draeger B. *Dtsch. Apoth. Ztg.* **1996,** *136,* 17−24.
(261) Picasso, S. *Chimia* **1996,** *50,* 648−649.
(262) Legler, G. In *Carbohydrate Mimics*; Chapleur, Y., Ed.; Wiley-VCH: Weinheim, 1998; pp 463−490.
(263) Bols, M. *Acc. Chem. Res.* **1998**, *31*, 1−8.
(264) Esko, J. D. In *Essential Glycobiology*; Varki, A., Ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1999; pp 609−623.
(265) Ekhart, C. W.; Fechter, M. H.; Hadwiger, P.; Mlaker, E.; Stütz, A. E. Tauss, A. Wrodnigg, T. M. In *Iminosugars as glycosidase inhibitors;* Stütz, A., Ed.; Wiley-VCH: Weinheim, 1999; pp 253−390.
(266) Rye, C. S.; Withers, S. G. *Curr. Opin. Chem. Biol.* **2000,** *4,* 573−580.
(267) El Ashry, E. S. H.; Rashed, N.; Shobier, A. H. S. *Pharmazie* **2000,** *55,* 251−262.
(268) El Ashry, E. S. H.; Rashed, N.; Shobier, A. H. S. *Pharmazie* **2000,** *55,* 331−348.
(269) El Ashry, E. S. H.; Rashed, N.; Shobier, A. H. S. *Pharmazie* **2000,** *55,* 403−415.
(270) Fleet, G. W.; Fellows, L. E.; Winchester, B. *Ciba Found. Symp.* **1990,** *154,* 112−125.
(271) Vogel, P. *Chim. Oggi.* **1992,** *10,* 9−15.
(272) Effenberger, F.; Null, V.; Straub, A. *Dechema Monogr.* **1993,** *129,* 197−207.
(273) Look, G. C.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182−190.
(274) Ichikawa, Y.; Wong, C.-H. *Alfred Benzon Symp.* **1994,** *36,* 118−33.
(275) Jaeger, V.; Mueller, R.; Leibold, T.; Hein, M.; Schwartz, M.; Fengler, M.; Jaroskova, L.; Paetzel, M.; LeRoy, P.-Y. *Bull. Soc. Chim. Belg.* **1994,** *103,* 491−507.
(276) Wong, C.-H. *Pure Appl. Chem.* **1995,** *67,* 1609−1616.
(277) Wong, C.-H.; Halcomb, R. L.; Ichikawa, Y.; Kajimoto, T. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 521−546.
(278) De Raadt, A.; Ekhart, C. W.; Ebner, M.; Stütz, A. E. *Top. Curr. Chem.* **1997,** *187,* 157−186.
(279) Tatsuta, K. In *Carbohydrate Mimics*; Chapleur, Y., Ed.; Wiley-VCH: Weinheim, 1998; pp 283−305.
(280) Goti, A.; Cicchi, S.; Cordero, F. M.; Fedi, V.; Brandi, A. *Molecules* **1999,** *4,* 1−12.
(281) Fechter, M. H.; Stütz, A. E.; Tauss, A. *Curr. Org. Chem.* **1999,** *3,* 269−285.
(282) Lednicer, D.; Snader, K. M. *Econ. Med. Plant Res.* **1991,** *5,* 1−20.
(283) Molyneux, R. J.; James, L. F. In *Mycotoxins Phytoalexins*; CRC Press: Boca Raton, FL, 1991; pp 637−656.
(284) Fellows, L. E.; Kite, G. C.; Nash, R. J.; Simmonds, M. S. J.; Scofield, A. M. *Proc. Phytochem. Soc. Eur.* **1992,** *33,* 271−82.
(285) Winchester, B. *Biochem. Soc. Trans.* **1992**, *20*, 699−705.
(286) Molyneux, R. J. *Phytochem. Anal.* **1993,** *4,* 193−204.
(287) Nash, R. J.; Watson. A. A.; Asano, N. *Alkaloids: Chem. Biol. Perspect.* **1996,** *11,* 345−376.
(288) Yamada, H.; Nagai, T. *Recent Res. Dev. Phytochem.* **1998,** *2,* 413−428.
(289) Elbein, A. D.; Molyneux, R. J. *Compr. Nat. Prod. Chem.* **1999,** *3,* 1−11.
(290) Elbein, A. D.; Molyneux, R. J. In *Iminosugars as glycosidase inhibitors;* Stütz, A., Ed.; Wiley-VCH: Weinheim, 1999; pp 216−251.
(291) Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000,** *11,* 1645−1680.
(292) Asano, N. *J Enzyme Inhib.* **2000,** *15,* 215−234.

CR000433K