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1-Aza Sugars,† Apparent Transition State Analogues of Equatorial Glycoside Formation/Cleavage

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For the last 50 years, there has been a strong belief in the scientific community that an important element of enzymatic catalysis was the ability of an enzyme to lower the energy of the transition state for the reaction it catalyzed.[1] For a long time, the only real evidence for this theory was the fact that stable compounds that resembled the transition state, transition state analogues, were competitive inhibitors of the enzyme. In the last decade, however, it has also been shown that transition state analogues can be used as haptens to raise antibodies with catalytic activity.[2]

These developments have led to a keen interest[3] in recent years for designing compounds that in terms of polarity and shape resemble the transition state of glycosidic cleavage or formation to create potent, selective enzyme inhibitors. (Due to space limitations, this Account will focus mainly on work from the author's laboratory.) Strong selective inhibitors of glycoside cleaving or synthesizing enzymes have many potential applications. These may be treatments of disorders that are affected by interfering with glycoprotein synthesis such as viral infections (HIV)[4] or inflammation or disorders related to carbohydrate metabolism such as diabetes.[5]

A fascinating aspect of this area of research is that controversy has existed regarding the exact structure of the transition state of glycoside cleavage,[6] and it has therefore not been straightforward to predict the structure of good transition state analogues. The generally accepted mechanism for acid-catalyzed cleavage (or formation) of a β-glycoside is shown in Scheme 1. According to this widely accepted mechanism, C−O cleavage is exocyclic and the transition state will be any of, or a hybrid of, the ions **1**−**3**. (The alternative possibility, endocyclic C−O bond cleavage,[7] is ignored in this treatment, as recent work[8] suggests it does not occur for enzymatic glycoside cleavage. It seem to occur to an extent in some other cases however.[9]) Assuming this mechanism to occur in enzymatic glycoside cleavage, a compound that resemble any of these ions should be an inhibitor of glycosidases (glycoside hydrolases).

There are a number of known compounds that fulfill these criteria. (Numerous competitive inhibitors of glycosidases are known,[10,8b] many of which are not good transition state analogues but resemble substrate or product in the ground state.) One group is compounds that resemble the natural product nojirimycin (Figure 1).[11,12] These compounds resemble monosaccharides, but

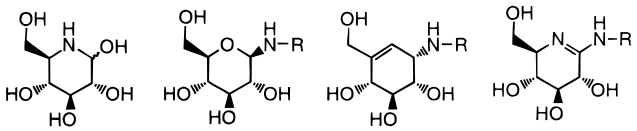

FIGURE 1.

the ring oxygen atom has been replaced with a nitrogen atom. Thus if protonated at the basic nitrogen atom, which may occur, these compounds are analogues of **3**.

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† The term 1-aza sugars is used to describe saccharide analogues having a nitrogen in place of anomeric carbon.

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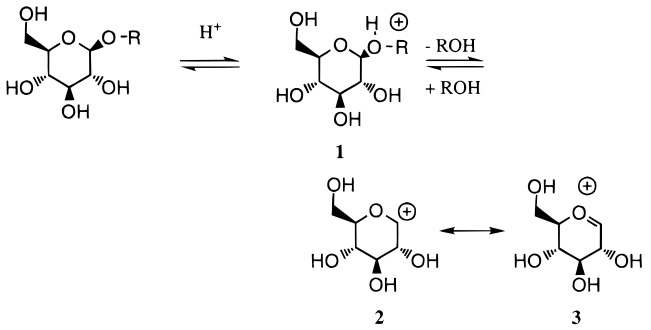
Scheme 1



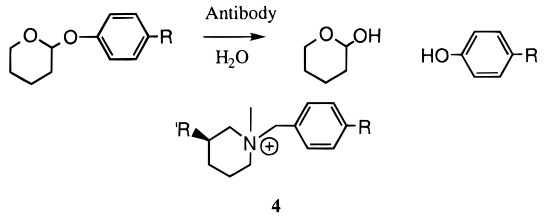
Another type of glycosidase inhibitor is the hydrolytically unstable glycosyl amine.[9] When protonated at the nitrogen atom, these compounds resemble **1**. Acarbose (a potent α-glucosidase inhibitor[13,5b]) and similar compounds, when protonated at nitrogen, also resemble **1**. Glucosamidines are a group of glycosidase inhibitors designed by Ganem and co-workers[3c] that resemble **3**.

It is reasonable to believe that all of these compounds at least to some extent are transition state analogues of glycoside bond cleavage. It can and has been argued[7a] that glycosyl amines and nojirimycin analogues lack the sp^2 character of C-1 and ring-oxygen present in ion **3** and, thus, are imperfect analogues of the transition state. This may be true, but as these derivatives bind 10^3-10^4 times more strongly to glycosidases than glucose, the nitrogen must play an important role in the binding. This could be stronger hydrogen bonding or electrostatic binding of a protonated nitrogen. However, it is not reasonable that the endocyclic oxygen in glucose (or a glucoside) should form hydrogen bonds to the enzyme (counting out endocyclic C−O bond cleavage, see above) as that would lessen its ability to form an oxocarbenium ion. Therefore the strong and general inhibition of glycosidases by nojirimycin derivatives seems best explained by the nitrogen being protonated in the active site so that the compounds resemble transition state ion **3** in terms of charge.

It is noteworthy that no glycosidase inhibitor that was an analogue of **2** was known. This might be interpreted as meaning that that ion is insignificant in the glycoside cleavage process, and in many theoretical discussions of glycoside cleavage, **2** has indeed been ignored at the expense of **3**. It is also noteworthy that analogues that resemble several of the ions **1**−**3** were not known.

In 1991, Reymond et al.[14] reported that a catalytic antibody had been isolated that could catalyze the hydrolysis of a tetrahydropyranyl ether, a simple model of a glycosidic bond. To produce this antibody, transition state analogue **4** (Scheme 2) was used as the hapten. Compound **4** was an analogue of a carbocation at the anomeric center and thus in principle an analogue of ion **2** except for the lack of hydroxyl groups. This paper suggested that analogues of **2** were good transition state analogues of glycoside cleavage. We undertook a study to investigate whether analogues of **2**, that is a carbohydrate analogue of **4**, would inhibit glycosidases.

Scheme 2



This required synthesizing a new type of monosaccharide analogue with a nitrogen in place of an anomeric carbon atom, which presented a significant synthetic challenge. The challenge was that this type of chiral polyhydroxylated molecule was usually best obtained from carbohydrates; however, unlike molecules of the deoxynojirimycin type, it contained a branched C-chain not found commonly in carbohydrate building blocks.[15] This branched C-chain would have to be introduced.

The hydroxypiperidine **5**, named isofagomine after natural product fagomine,[16] was synthesized (Figure 2).

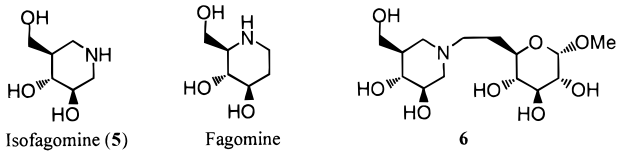
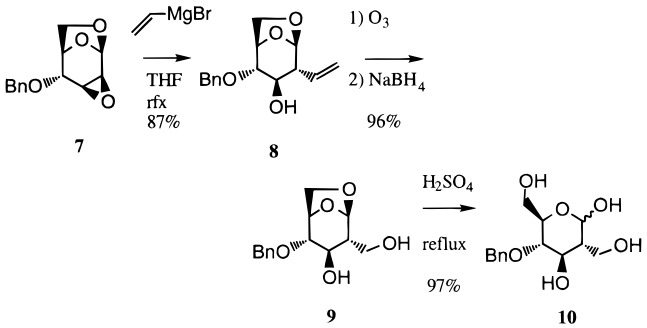


FIGURE 2.

We also made disaccharide analogues: An analogue **6** resembling isomaltose was chosen. To obtain **5** optically active we decided to carry out the synthesis from a carbohydrate.[17] However we decided to introduce the branched carbon chain by ring opening of a carbohydrate epoxide with a carbon nucleophile. Thus the synthesis commenced from known epoxide **7**,[18] which was reacted with vinyl magnesium bromide[19] to give the vinyl derivative **8**. The vinyl group was converted to the exocyclic hydroxymethyl group by ozonolysis followed by reduction with $NaBH_4$ to give the alcohol **9** (Scheme 3). This

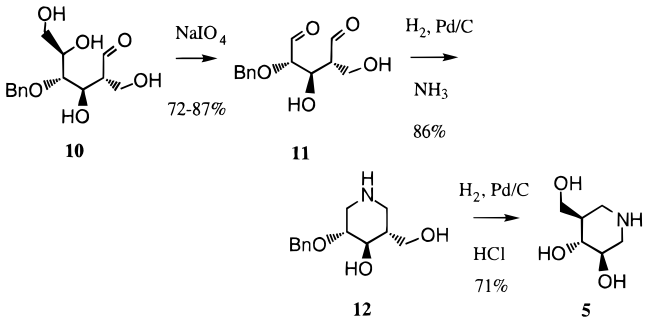
Scheme 3



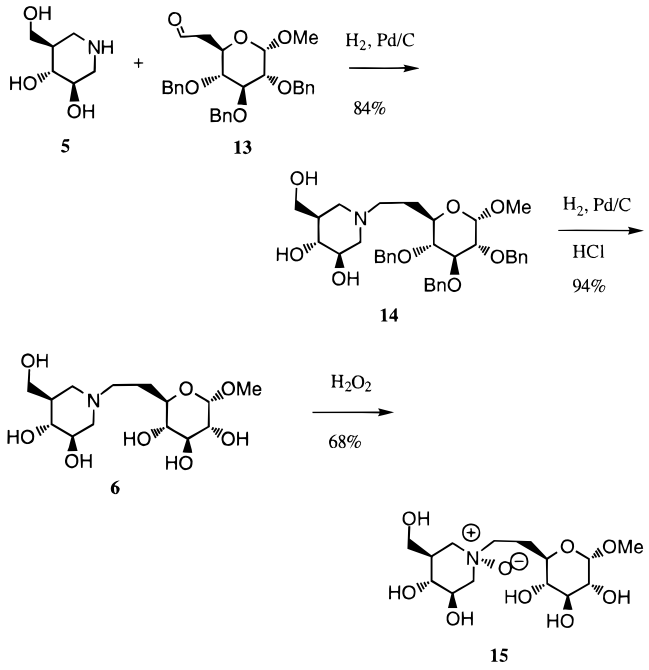
compound was hydrolyzed with dilute acid followed by sodium periodate cleavage and reductive amination of **11** to give the chiral piperidine **5** (Scheme 4).[20]

From **5** some disacccharide analogues were made.[21] Compound **5** (or **12**) was reacted with aldehyde **13** in the presence of hydrogen and palladium on carbon to give **6** after deprotection (Scheme 5). Disaccharide analogue **6** was converted to a number of charged derivatives. Oxi-

Scheme 4

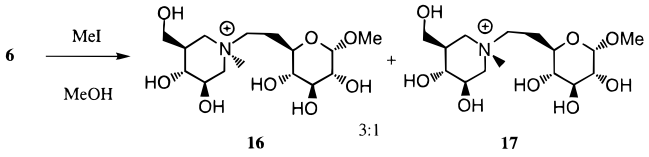


Scheme 5



dation with hydrogen peroxide gave a single *N*-oxide **15**, which was determined to have the structure with oxygen in the α-position. Reaction of **6** with methyl iodide gave both possible stereoisomeric *N*-methyl derivatives **16** and **17**. The compound with methyl in the α-position, **16**, was obtained over the *β*-isomer **17** in ratio 3:1[21] (Scheme 6).

Scheme 6



Isofagomine (**5**) and the disaccharide analogues (**6** and **15**) were found to be potent glycosidase inhibitors (Table 1). Indeed, **5** was to our knowledge the strongest inhibitor known of *β*-glucosidase and was 440 times more potent than 1-deoxynojirimycin. This is a remarkable potency, especially because **5** lacks the 2-hydroxyl group of glucose of 1-deoxynojirimycin. This OH is very important for the binding of 1-deoxynojirimycin, as 1,2-dideoxynojirimycin (fagomine) is a very poor glycosidase inhibitor.[16] Another remarkable observation was the large difference (780-fold) in K_i between the inhibition of α- and *β*-glucosidase by **5**.

Table 1. Inhibition Constants (*μ*M) of Isofagomine Analogues Versus Glycosidases[21]

enzyme	5	6	15	16	17	40
β-glucosidase	0.11	2.3	0.38	510	150	0.19[a]
α-glucosidase	86	59	70	>1000	280	>2000[a]
isomaltase	7.2	100	19	190	>1000	
glucoamylase	3.7	0.063	0.24	160	94	
α-mannosidase	770	1200	>1000	400	>1000	>2000[a]
β-galactosidase						0.004
α-galactosidase						200[a]

[a] IC_{50} value.

The disaccharide analogue **6** was generally a somewhat weaker inhibitor than **5** except against glucoamylase.[21] Glucoamylase requires at a minimum a disaccharide as substrate and thus has an active site where binding of another sugar residue is favored. Therefore, the stronger inhibition of this enzyme by **6** compared to **5** was understandable. On the other hand, the relatively poor binding of **6** to isomaltase, an enzyme specific to α-1,6-linkages, was puzzling. One explanation might be that the aglycon sugar residue was positioned in a somewhat wrong spatial position compared to the transition state because the nitrogen is tetrahedral and not trigonal as in C-1 of the transition state.

The quaternery ammonium derivatives **16** and **17** were very poor inhibitors. The *N*-oxide **15** was generally a good inhibitor, generally with a inhibition profile like that of isofagomine (**5**) (Figure 3). Though it appeared from the

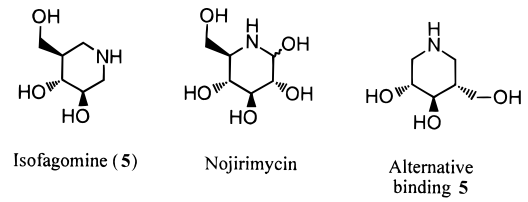


FIGURE 3.

above that 1-aza sugar analogues of monosaccharides in general inhibited glycosidases, the inhibition by **5**, and to some extent the disaccharide analogues, could be a result of a different binding mode than glucose. Thus **5** could be binding to the enzyme with the nitrogen in the same spatial position as 1-deoxynojirimycin as it also was a 2-deoxy-2-(hydroxymethyl)-5-(norhydroxymethyl) analogue of the latter. A good way to investigate whether **5** was binding in a manner similar to that of glucose with nitrogen in the spatial position of glucose anomeric carbon was to prepare the corresponding 1-aza sugar analogues of other monosaccharides and see if they inhibited the corresponding glycosidases. We chose to make the corresponding analogue **18** (Figure 4) of L-

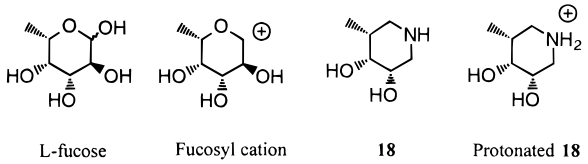
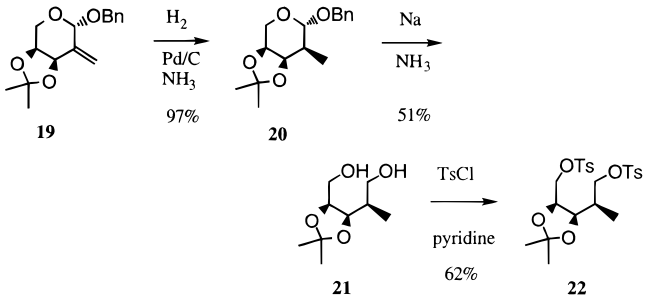


FIGURE 4.

fucose, which we decided to synthesize from L-arabinose.[22]

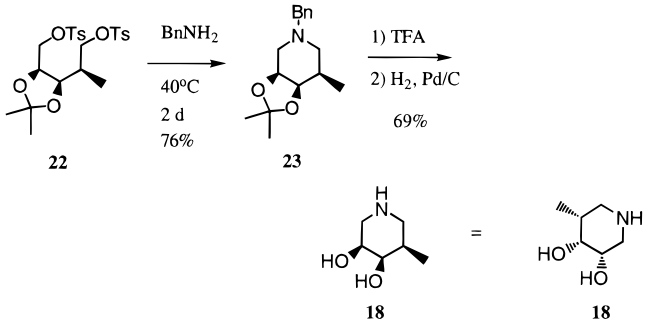
L-Arabinose was converted to the known vinyl compound **19**. Stereoselective hydrogenation of **19** gave methyl derivative **20** having the correct stereochemistry, which after reduction to the polyol **21** and tosylation could be cyclized to piperidine **23** by reaction with neat benzylamine[23] (Scheme 7). Deprotection gave **18** (Scheme

Scheme 7



8). Piperidine **18** was found to be a potent α-fucosidase

Scheme 8

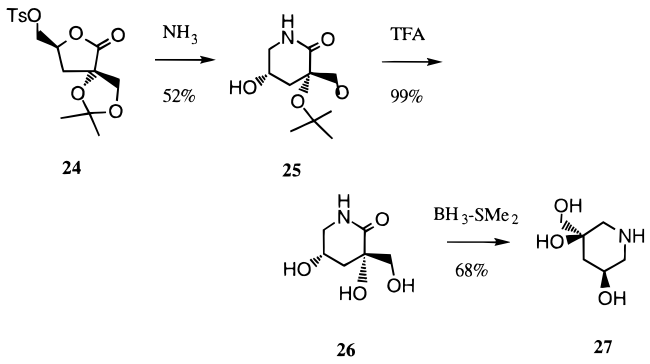


inhibitor with a K_i of 6.4 μM.[22] Equally important, the inhibition was selective, as significant inhibition of other glycosidases were not observed. Unlike that of **5**, the stereochemistry of **18** cannot be brought to superimpose with the nojirimycin analogue of L-fucose, so the inhibition of fucosidase by **18** is strong evidence for binding of these compounds with nitrogen in the place of the anomeric carbon of the glycosidase substrate. Fucose analogue **18** has also been made by Ichikawa's group at John Hopkins.[24]

More evidence was obtained by preparing other analogues of isofagomine (**5**), from the pool of known branched chain carbohydrate derivatives.[25] Isosaccharinic acid was converted to known derivative **24**,[26] which by treatment with ammonia gave lactam **25**. After removal of the acetonide with aqueous TFA and reduction of the lactam with borane−dimethyl sulfide, analogue **27** was obtained (Scheme 9). This compound should not be and was not a glycosidase inhibitor because it does not have the correct stereochemistry. Yet it provided more evidence against non-glucose-like binding.

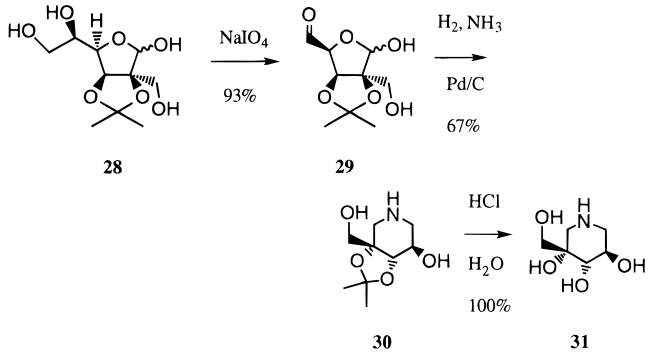
Other analogues were made from branched carbohydrate derivatives that could be obtained from formaldehyde alkylation of acetonides.[27] From this type of analogue, isofagomine derivatives could be obtained optically active in a short synthesis, but with an unneeded hydroxyl group at the tertiary center. Thus, the 5-OH analogue of

Scheme 9



isofagomine **31** was prepared from the known branched derivative **28**[27] (Scheme 10), which can be prepared in

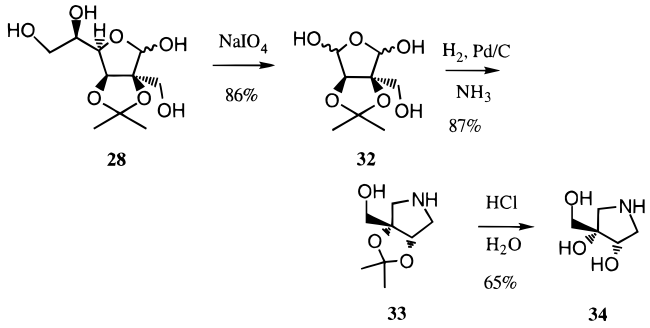
Scheme 10



three steps from mannose. Periodate cleavage of **28** gave a dialdehyde **29**, which by reductive amination gave piperidine **30** in 67% yield. Finally hydrolysis removed the acetonide to give **31**.[28] This compound has also been made by Ichikawa *et al.* by a similar route.[29] Compound **31** was considerably less potent than **5**, suggesting that the 5-hydroxyl group interfered unfavorably with the enzymes.

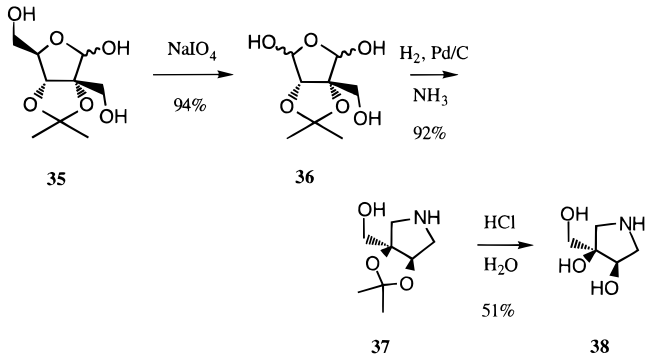
A five-membered analogue **34** was obtained when **28** was treated with 2 equiv of $NaIO_4$ to give dialdehyde **32** (Scheme 11). Reductive amination followed by hydrolysis

Scheme 11



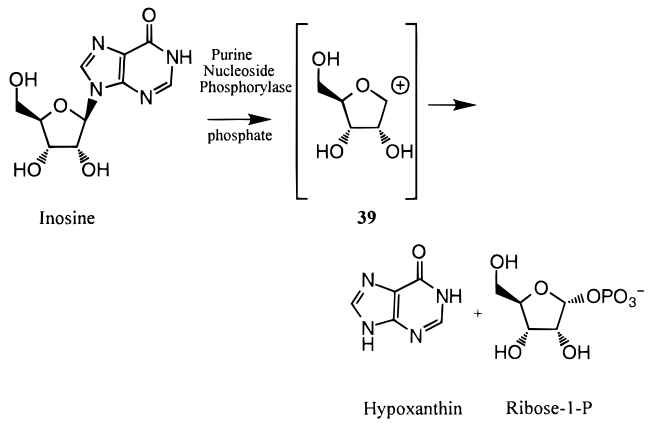
of the acetonide gave **34**. Its enantiomer, **38**, could be prepared from D-ribose. The known derivative **35** was prepared in two steps from ribose.[27] Periodate cleavage of **35** gave dialdehyde **36**, the enantiomer of **32**. Reductive amination and hydrolysis as above then led to **38**, the enantiomer of **34**[28] (Scheme 12).

Scheme 12



Pyrrolidine **34** inhibited purine nucleoside phosphorylase with a K_i of 180 μM, which might be explained by it being an analogue of the ribofuranosyl cation **39**. The inhibition was moderate, but clearly stronger than that of the other analogues, which showed no inhibition of this enzyme (Scheme 13).

Scheme 13



As mentioned above, Ichikawa *et al.* prepared transition state analogues following similar ideas. This group made **18**,[24] **31**,[29] **40**,[30] **41**,[31] and **42**[32] (Figure 5). Particularly

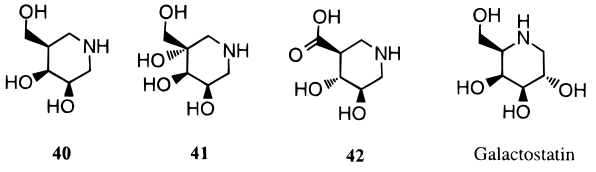


FIGURE 5.

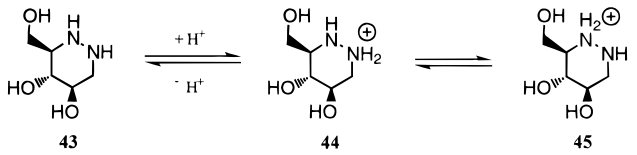
remarkable was galactose analogue **40**, which was a very potent inhibitor of β-galactosidase, with a K_i of 4.1 nM, but was an extremely weak inhibitor of α-galactosidase, with a IC_{50} of 200 μM (Table 1).[30] Interestingly, the galactose analogue of 1-deoxynojirimycin, galactostatin, has the reverse selectivity: strong inhibtion of α-galactosidase and weak inhibition of β-galactosidase. A 5-hydroxy analogue of **40**, **41**, was a considerably weaker inhibitor than **40**, while glucuronic acid analogue, **42**, was very potent. It had a K_i vs β-glucuronidase of 79 nM.

Together with our results, this clearly shows that sugar derivatives with a nitrogen in place of anomeric carbon are potent inhibitors of the corresponding glycosidases but are particularly potent against β-glycosidases. Thus, **5** was 780 times more potent as an inhibitor of almond β-glucosidase than of yeast α-glucosidase, while **40** was 16 700 times more potent as an inhibitor of β-galactosidase than of α-galactosidase. On the other hand, nojirimycin analogues have the reverse selectivity to a smaller degree: 1-deoxynojirimycin was twofold less potent inhibitor of almond β-glucosidase than of yeast α-glucosidase, while galactostatin was 1000 times less potent against β-galactosidase than against α-galactosidase.

Under the assumption that these inhibitors were protonated in the active site, this sugggested that a C-1 cation (**2**) was a more important transition state for enzymes catalyzing equatorial glycoside cleavage (β-glucosidase), while an oxocarbenium ion **3** was the more important for axial glycoside cleavage (α-glucosidase).

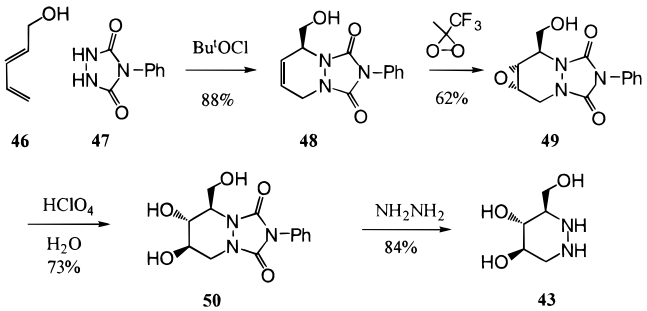
A deduction from the above considerations was that a transition state analogue that would mimic both **2** and **3** should be able to inhibit both α- and β-glycosidases strongly. Hydrazine derivative **43** was conceived to be able do that. When protonated, two ions **44** and **45**, were formed, and these were in equilibrium. Ion **44** resembled **2**, and ion **45** resembled **3** (Scheme 14). The new

Scheme 14



compound **43** was synthesized racemically by a Diels–Alder strategy.[33,34] Synthesis started from penta-2,4-dienol **46** and 4-phenylurazol **47**. The latter was preoxidized with *tert*-butyl hypochlorite to 4-phenyl-1,2,4-triazoline-3,5-dione, which underwent an ultrafast Diels–Alder reaction with the diene to give adduct **48**. Epoxidation of the double bond with (trifluoromethyl)methyldioxirane gave the trans epoxide **49** in good yield, which was selectively hydrolyzed and deprotected to give **43** (Scheme 15).

Scheme 15



1-Azafagomine inhibited α-glucosidase with a K_i of 3.9 μM and β-glucosidase with a K_i of 0.65 μM. Isomaltase and glycogen phosphorylase were also strongly inhibited. The inhibition of the α-glucosidases by **43** was stronger than that by both 1-deoxynojirimycin and **5**. Inhibition of β-glucosidase was somewhat less potent than that of **5**, but still more potent than 1-deoxynojirimycin. Thus

Table 2. Inhibition Constants (μM) of Azafagomine Analogues versus Glycosidases[33]

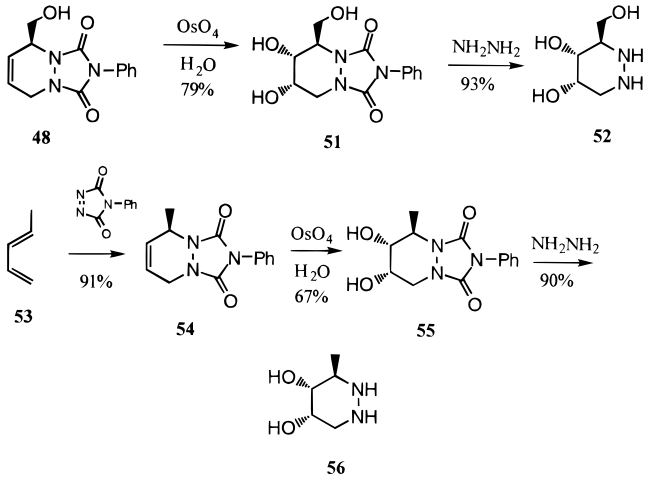
enzyme	**43**	deoxy-nojirimycin	**5**	**56**	**52**
α-glucosidase	3.9	25	86	>1000	>1000
β-glucosidase (pH 6.8)	0.65	47	0.11	41	137
β-glucosidase (pH 5.0)	0.76	330			
β-glucosidase (pH 7.5)	1.09				
isomaltase	1.06	11	7.2	>3000	3080
α-galactosidase	934	>1000			
β-galactosidase	702	>1000		>1000	149
α-mannosidase	3306	270	770	185	323
phosphorylase A	13.5[a]	55000			

[a] IC_{50} value.

displacing the C-1 of deoxynojirimycin with nitrogen increased β-glucosidase inhibition significantly, while displacement of the CH_2 in the ring oxygen atom space in **5** with nitrogen increased its α-glucosidase inhibition.

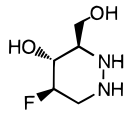
To collect evidence for a substrate-like binding of **43** with the two nitrogen atoms spatially positioned in place of C-1 and ring oxygen in the substrate, we investigated the importance of the stereochemistry of hydroxyl groups for the compound binding. For a substrate-like binding, stereoisomers of **43** with the wrong configuration should be poor inhibitors. Therefore isomers of **43** were prepared. A stereoisomer was obtained by syn dihydroxylation of the alkene **48** with the OsO_4-catalyzed reaction, giving stereoselectively **51**. This was hydrazinolyzed to hexahydropyridazine **52**. Similarly, a 6-deoxyhexose analogue was prepared by Diels−Alder reaction of pentadiene **53** with the azadienophile to adduct **54**, which was then dihydroxylated with OsO_4 and N-methylmorpholine-N-oxide and deprotected to hexahydropyridazine **56** (Scheme 16).

Scheme 16



Both **52** and **56** were much weaker inhibitors than **43** against various glycosidases (see Table 2). Both compounds (particularly **56**) inhibited β-glucosidase. This is an enzyme that has a relatively broad substrate specificity,[35] and therefore, the relatively strong inhibition of this enzyme by these compounds with a "wrong" stereochemistry was reasonable. These compounds also inhibited α-mannosidase much more than **43**. This may be contributed to the presence of a syn diol in the molecules which resemble the 2,3-diol of mannose. In any case, the weak inhibition of these compounds suggested that the glucoconfiguration of the hydroxy groups in **43** was essential for binding and was thus strong evidence for a substrate like binding of **43**.

Further evidence for a binding of **43** similar to glucose came from synthesis of the 3-fluoro analogue. Epoxide **49** was reacted with pyridinum polyhydrogen fluoride, which selectively gave one fluoride that was deprotected to **57** (Figure 6). This compound was a 100-fold weaker



57

FIGURE 6.

inhibitor than **43**, which suggests that hydrogen bond donation of the 3-hydroxyl group of **43** is an important element for its binding.[36]

The β-glucosidase inhibition by **43** was independent of pH, which was in contrast to the pH-inhibition profiles of both 1-deoxynojirimycin and **31**. In the case of the latter two compounds, inhibition dropped with pH. This indicated that only the unprotonated inhibitors inhibited β-glucosidase because these compounds had a pK_a of ~7 (6.8 in the case of deoxynojirimycin). However, **43** was found to have a pK_a of 3.9 and was thus essentially unprotonated in most of β-glucosidase's working pH range. The inhibition was thus, like deoxynojirimycin and **5**, caused by the unprotonated inhibitor.

Conclusion

The work described in this Account presents evidence for the observation that 1-aza analogues of monosaccharides are strong glycosidase inhibitors and particularly strong inhibitors of glycosidases that cleave equatorial glycoside bonds (β-glycosidases). The inhibition by these compounds may be explained by their being transition state analogues; if protonated they resemble carbocation **2**. It may be argued that, since these compounds, and also nojirimycin analogues, are unprotonated at the pH where they act as inhibitors, it is unlikely that they should become protonated inside the active site of the enzyme. This argument is particularly valid in the case of **43** which has a pK_a of only 3.9, while the protonating acid in β-glucosidase has a pK_a of 6.7. However, if the enzyme is able to stabilize **2** considerably, the basicity of the inhibitor should also increase because the as protonated inhibitor is stabilized. Such a stabilization could come from a carboxylate group present in the active site (Figure 7).

So why should **2** be particularly important to stabilize for β-glucosidase? Stereoelectronic effects suggest[37] that a glycoside with an equatorial glycoside bond, unlike axial ones, cannot receive aid in glycoside bond breakage from the lone pairs of the ring oxygen. None of these lone pairs are antiperiplanar to the carbon−oxygen σ bond and, therefore, cannot overlap with its antibonding orbital.

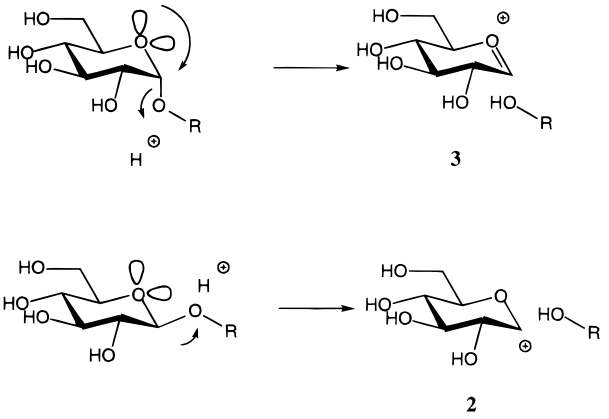


FIGURE 7. Stereoelectronic requirements for cleavage of α- and β-glucosides.

Thus an oxocarbenium ion (**3**) cannot be formed directly, but instead, unstabilized carbocation **2** has to be formed first. Ion **2** then has to rehybridize to receive resonance stabilization from the ring oxygen, but **2** will be the absolute high-energy transition state of the uncatalyzed reaction. The most essential ion for β-glucosidase to stabilize is therefore **2**. According to this theory, **44** and protonated **5** are very good transition state analogues of glycoside cleavage by a β-glucosidase. Ions **44** and **45**, protonated **5**, and protonated 1-deoxynojirimycin are imperfect transition state analogues of glycoside cleavage of an α-glucoside as they have the charge but lack the geometry of the resonance forms of the oxocarbenium ion.

These considerations assume that the enzyme hydrolyzes the glycoside in the ground state conformation. This actually conflicts with present theory for enzymatic (and nonenzymatic) hydrolysis of equatorial glycosides, according to which a conformational flip of the glycoside to the boat form occurs. This facilitates bond cleavage because the glycoside bond becomes axial so that one ring oxygen lone pair can overlap with its antibonding orbital. There is strong evidence that this occurs for lysozyme, as X-ray structural studies of this enzyme complexed with a trisaccharide have shown a twisted conformation of the glycon.[38] However, the discovery of 1-aza sugar inhibitors such as **5**, **43**, and **40** suggests that β-glucosidase and β-galactosidase and a number of other glycosidases hydrolyze the substrates in their ground state conformations. It may be that lysozyme is a rather untypical glycosidase that distorts its substrates, while other glycosidases accomplish the hydrolysis without forcing the substrate into a boat conformation.

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