



HAL
open science

Sugar-Derived Amidines and Congeners: Structures, Glycosidase Inhibition and Applications

Yves Blériot, Nicolas Auberger, Jérôme Désiré

► **To cite this version:**

Yves Blériot, Nicolas Auberger, Jérôme Désiré. Sugar-Derived Amidines and Congeners: Structures, Glycosidase Inhibition and Applications. *Current Medicinal Chemistry*, 2022, 29 (7), pp.1271-1292. 10.2174/0929867329666211222164545 . hal-04543430

HAL Id: hal-04543430

<https://hal.science/hal-04543430v1>

Submitted on 12 Apr 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

SUGAR-DERIVED AMIDINES AND CONGENERS: STRUCTURES, GLYCOSIDASE INHIBITION AND APPLICATIONS

Yves Blériot, Nicolas Auburger, Jérôme Désiré*

*Université de Poitiers, IC2MP, UMR CNRS 7285, Equipe “OrgaSynth” , Groupe Glycochimie
4 rue Michel Brunet, 86073 Poitiers cedex 9, France*

Introduction

1 Glycoamidines and derivatives: structure and glycosidase inhibition

1.1 Glycoamidines

1.2 Introduction of an alkyl or aryl aglycon moiety

1.3 Alteration of the hydroxyl pattern of the sugar moiety

1.4 Alteration of the amidine function

1.4.1 Alteration of the endocyclic amine

1.4.2 Alteration of the exocyclic amine: amidrazones and amidoximes

1.5 Guanidinosugars

1.6 Pseudodisaccharides

1.7 Glycosylamidines

2 Their use as TS analogues

2.1 Use as haptens to elicit catalytic antibodies

2.2 Use as templates to produce microgel-based catalysts

Table of inhibition

Conclusion

References

Introduction

Glycosidases, the enzymes responsible for the breakdown of glycoconjugates are ubiquitous through all kingdoms of life. The extreme chemical stability of the glycosidic bond combined with the catalytic rates achieved by glycosidases makes them among the most proficient of all enzymes.¹ They have been classified in families based on amino acid sequence similarities (<http://www.cazy.org>). Because there is a direct relationship between sequence and folding similarities, such a classification reflects the structural features of these enzymes better than their sole substrate specificity and provides a convenient tool to derive mechanistic information. Given their multitude of roles *in vivo*, inhibition of these enzymes is highly attractive with potential in the treatment of a vast array of pathologies ranging from lysosomal storage diseases^{2,3} and diabetes⁴ to viral infections⁵. Therefore great efforts have been invested in the last three decades to design and synthesize inhibitors of glycosidases^{6,7} leading to a number of drugs currently on the market.⁸ Glycosidase inhibitors are also extremely useful probes for dissecting the subtle differences in mechanisms employed by different glycosidase families. They can also be used as tools for chemical biology and selective inhibitors are invaluable for modulating effects in cells or *in vivo*.

Most of the mechanisms developed by glycosidases for glycoside hydrolysis proceed with either net retention or inversion of anomeric configuration. Traditionally, glycosidases possess two carboxylate-containing residues which are responsible for hydrolysis. Inversion of stereochemistry is a single step mechanism (Figure 1a), which allows both substrate and a water molecule to be bound simultaneously. One of the catalytic residues acts as a general acid and the other as a general base. Protonation of the glycosidic oxygen by the general acid and departure of the leaving group is accompanied by concomitant nucleophilic attack by a water molecule that has been deprotonated by the general base.⁹ Retention of stereochemistry is a double displacement mechanism, consisting of two inverting steps (Figure 1b); one of the catalytic residues acts as the acid/base residue and the other as a nucleophile. During the first (glycosylation) step of the reaction the acid/base protonates the glycosidic oxygen to assist leaving group departure, which is concomitant with attack of the enzyme nucleophile at the anomeric carbon, thus leading to a covalent glycosyl-enzyme intermediate. In the second (deglycosylation) step the acid/base residue deprotonates a water molecule that attacks at the anomeric carbon and displace the glycoside.

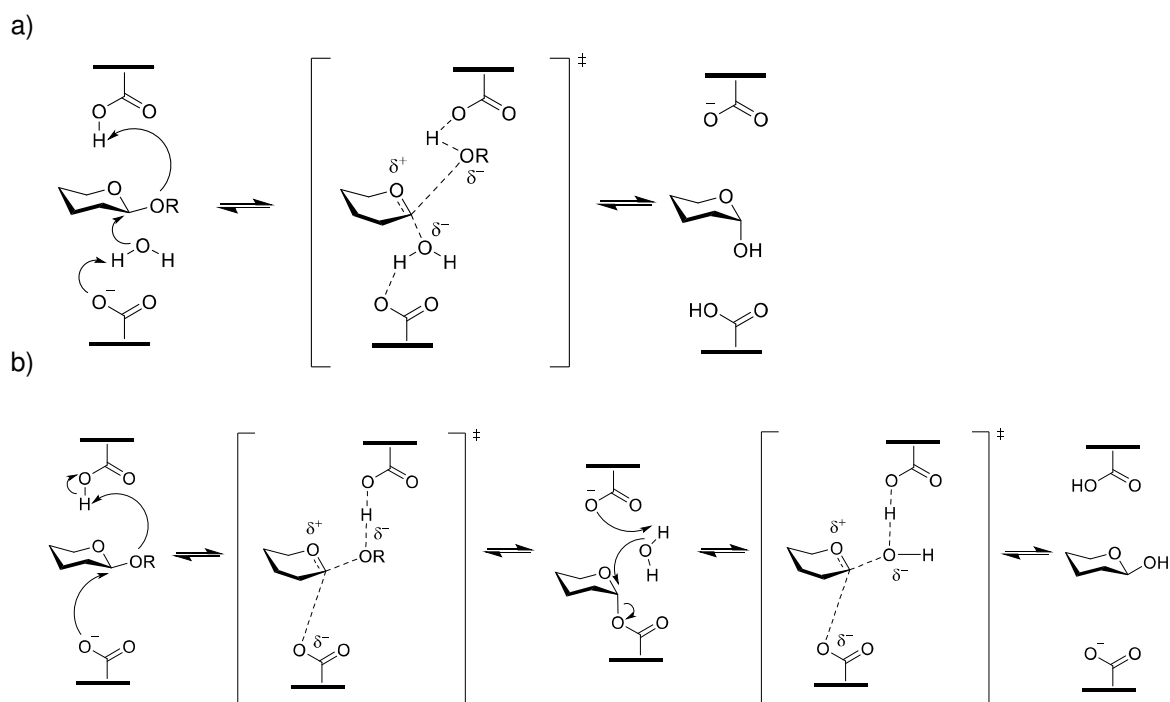


Figure 1: Classical glycosidase mechanisms with a) inversion of anomeric configuration and b) with retention of anomeric configuration.

Pauling was the first to postulate that the inhibitors with the highest affinity for an enzyme were likely to be those that mimicked more closely the transition state structure.^{10a} Accordingly, a large number of inhibitors have been reported and proposed to mimic the fleeting transition state of the hydrolysis reaction. However, their true transition state analogy has been validated only in a handful of cases.^{10b-d} As glycoside-degrading enzymes are able to enhance the rate of catalysis 10^{17} fold over the noncatalyzed reaction, this would translate to an estimated dissociation constant for the transition state to not be greater than 10^{-22} M, emphasizing a tremendous affinity between the transition state and the enzyme active site.¹¹ The short-lived transition state of most glycosidases has a substantial oxocarbenium character (Figure 1)¹² with a trigonal anomeric carbon which causes sp^2 hybridisation along the bond between the anomeric carbon and the endocyclic oxygen. The double bond character imposed on the pyranose ring forces a distortion from the substrate initial chair conformation to energetically unfavourable half chair or boat conformation where C1, C2, O5 and C5 lie in a plane (Figure 2).¹³ There is also a considerable build-up of positive charge on the pyranose ring at the transition state, which is delocalised along the bond between the anomeric carbon and endocyclic oxygen.¹¹

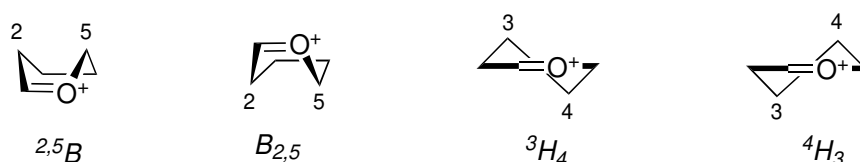


Figure 2: Range of transition state conformations adopted by classical glycosidases

Most of the putative glycosidase inhibitors reported in the literature are typically found in a relaxed chair conformation in solution, although some distortion may occur upon binding to an enzyme.¹⁴ The majority of them display a protonated secondary amine group and hence mimic the charge distribution at the transition state to some degree but not the distorted chair imposed by the anomeric carbon hybridization. In the early 1990s, an approach aimed at incorporating group(s) in the inhibitor which caused distortion of the compound to mimic the geometry of the transition state. Glycoamidines, introduced by Ganem *et al.*,^{15,16} combine the two key structural elements, charge and conformation, in a single compound. In this review, we discuss the various structures that have emerged since Ganem pioneering work as well as their inhibition profile and their use as transition state analogues.

1 Glycoamidines and derivatives: structure and glycosidase inhibition

1.1 Glycoamidines

Glycosylamines and iminosugars, with a basic nitrogen directly attached to the anomeric carbon, mimic the partial positive charge of the putative transition state for glycoside hydrolysis in their protonated forms (Figure 3). In their chair conformations, however, neither structure accurately reflects the ring distortion accompanying displacement reactions catalyzed by either retaining or inverting enzymes. Nucleophilic attack either by water or enzyme carboxylate is accompanied by rehybridization of the anomeric carbon to a sp^2 geometry, thus flattening the saccharide ring. The importance of this conformational distortion has been studied in depth¹⁷ and is illustrated by the inhibitory potency of simple aldonolactones¹⁸ which are unprotonated at neutral pH but which adopt a pseudo-half-chair conformation. Combining the correct charge and shape of the glycosyl cation into a stable structure seemed like an interesting new design target and amidines derivatives of glycopyranoses, that can be seen as promising but not perfect transition state mimics, have been developed according to the following considerations. First, their resonance-stabilized π -system was expected to prefer the endocyclic tautomer,¹⁹ thus recreating the flattened conformation of the hydrolysis transition state. In case of exocyclic tautomer, the C1 and the exocyclic amidine nitrogen would be sp^2 -hybridized, the aglycone of the glycoamidines being located approximately in the plane of the glycopyranosyl part, which resembles more closely its location in β -glycoside substrates than in α -glycosides. Second, unlike lactones, the basic amidines would be fully protonated at physiological pH, thus mimicking the positive charge of the glycosyl cation. Therefore, in this review, they will be represented in their protonated state.

Nevertheless, they will be thereby not capable of accepting a proton from a side-chain carboxylic acid in the enzyme active site as the natural substrates are alleged to do.²⁰ Finally, the presence of nitrogen atoms at both exo and endo positions flanking the anomeric site combined the essential structural features of both the iminosugar and glycosylamine families (Figure 3). The combination of all these features embodied in a sugar-like ring made the amidine targets particularly appealing in the 1990s.

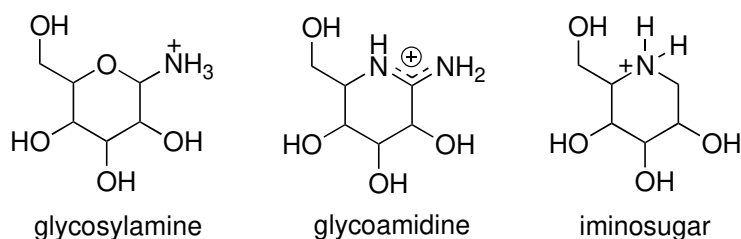


Figure 3: General structure of glycosylamine, glycoamidine and iminosugar in their protonated state

The first suggestion that a D-glucoamidine **1** might be a good candidate for study was proposed by Reese *et al.* in 1971.²¹ An unsuccessful synthetic approach to **1** was reported by Bird *et al.* in 1990.²² The same year, Ganem *et al.* reported the first synthesis of a glycoamidine.¹⁵ Starting from readily available persilylated D-gluconolactam the synthetic strategy involved its conversion to the thionolactam, which upon treatment with ammonia-saturated methanol, afforded amidine **1** (pK_a 10.6) as a moisture sensitive oil. Acidification with anhydrous HCl-CH₃OH gave its stable hydrochloride salt. As the free base, **1** is extremely sensitive to nucleophiles at higher pH and reverted to lactam by nucleophilic hydrolysis (pH 8) with a half-life of 1 h. As its hydrochloride salt, amidine **1** was found indefinitely stable in water. The synthetic sequence developed by Ganem was successfully applied to D-galactonolactam to afford galactoamidine **2** but failed to produce mannoamidine from D-mannonolactam as epimerization at C-2 occurred to solely produce glucoamidine **1**.²³ Amidine **1** proved to be a potent inhibitor of sweet almond β -glucosidase, displaying a pH-independent inhibition ($K_i \approx 10 \mu\text{M}$) between 4.5 and 7.0, a moderate inhibitor of bovine β -galactosidase but a good competitive inhibitor of jack bean α -mannosidase ($K_i 9 \mu\text{M}$). By comparison, *gem*-diamine **3**,²⁴ displaying the same combination of endocyclic and exocyclic nitrogens without the flattened half-chair conformation was found to be a good competitive inhibitor of sweet almond β -glucosidase ($K_i 40 \mu\text{M}$) but has no activity against other glycosidases, suggesting that the half-chair conformation of **1** might be responsible for its unusual broad spectrum activity (Figure 4).

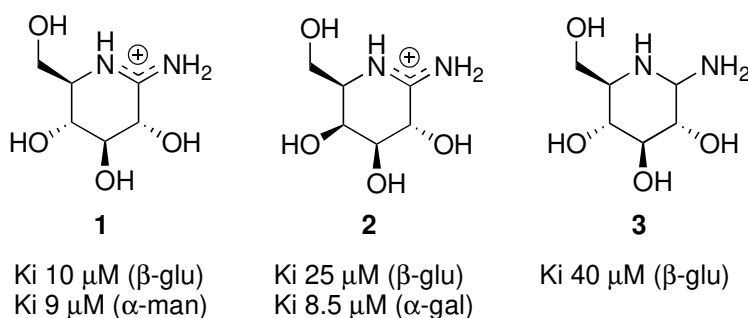


Figure 4: Structures and inhibitory potency of glucoamidine **1**, galactoamidine **2** and *gem*-diamine **3**

1.2 Introduction of an aryl or alkyl aglycon moiety

Efforts to improve the inhibitory potency of original glycoamidines mainly focused on the incorporation of supplementary electrostatic interaction to the glycoamidine core by using aliphatic and aromatic aglycons. To avoid the detrimental epimerization at C-2 position observed in the D-mannose series, Tellier *et al.* exploited a 2,3:4,6-di-*O*-isopropylidene-D-mannonolactam and installed a benzyl group as the aglycon moiety to produce mannoamidine **4** (Figure 5a) in which the CH₂ group was supposed to mimic the lengthening of the glycosidic bond during the enzymatic cleavage. As its hydrochloride salt,

amidine **4** proved to be a potent inhibitor of jack bean α -mannosidase (K_i 550 nM) and snail β -mannosidase (K_i 6 μ M).²⁵

In parallel, Striegler *et al.* reported the synthesis of a series of substituted benzyl galactoamidines **5a-j** (Figure 5a) and studied the influence of the electronic properties of the aromatic aglycon through the incorporation of an electron-withdrawing fluorine or an electron-donating methyl group at various positions of the aromatic ring. All compounds behaved as very potent competitive inhibitors of β -galactosidase (*Aspergillus oryzae*) with inhibition constants (K_i) in the low nanomolar range (12-48 nM) that were influenced by the electronic nature of the substituent in *p*-position of the aglycon.²⁶ Encouraged by these results, the same group evaluated this library as putative transition-state analogs (TSA) leading to the identification of *p*-methylbenzyl-D-galactoamidine **5d** as a suitable candidate fulfilling the requirements as a TSA.²⁷ These preliminary results prompted an in-depth investigation on the contributions of the aglycon moiety toward the interaction with the active site of the enzyme that may include steric, electronic, hydrophobic, and spacer effects, π - π stacking of the benzyl ring, and van der Waals interactions, leading to assess 20 derivatives **5a-t** displaying either an alkyl or aryl substituent at the pseudoanomeric position.²⁸ Galactoamidines derived from cyclic aliphatic and linear amines showed higher inhibition activity on β -galactosidase (*Aspergillus oryzae*) than those derived from benzylamines. Hydrophobic interactions of the methyl group rather than π - π stacking interactions of the aromatic ring in *p*-methylbenzyl-D-galactoamidine **5d** (K_i 8 nM) were identified to cause its transition-state-like character. Interestingly, a flexible 3-carbon methylene spacer between the exocyclic amine of the sugar moiety and the phenyl group increased the inhibition drastically. This library was next used to examine similarities in the active sites of β -galactosidases within the GH35 family, demonstrating a significant higher inhibition in the picomolar range of bovine liver β -galactosidase than previously determined for *Aspergillus oryzae* β -galactosidase. Molecular dynamics studies suggested loop closing interactions as a rationale for the disparity of the active sites in the two β -galactosidases under investigation.²⁹ Interestingly, these galactoamidines proved to be also potent inhibitors of β -glucosidase from sweet almonds (10 < K_i < 300 nM). To provide a rationale for the observed structure-activity relationship and because of the lack of solid state data for this enzyme, another GH1 glycosidase from white clover, 1CBG β -glucosidase, was used for subsequent docking studies to visualize the interactions of **5d** in the active site.³⁰ Amidine **5d** interacts with both amino acids relevant for catalysis, Glu-183 (nucleophile) and Glu-397 (proton donor). Further interactions of **5d** as H-bond acceptor were noted with Trp-454 and Gln-33, as H-bond donor with Glu-453 in addition to electrostatic interactions between the protonated amidine binding site and Glu-453, and π - π stacking interactions between the *p*-methylbenzyl aglycon and Trp-369 (Figure 5b). Striegler *et al.* then attempted to target and competitively inhibit glucosyl hydrolases and transferases through the design of glucoamidines bearing an aromatic aglycon moiety. However, they failed to synthesize such compounds. Modeling studies suggested an intrinsic H-abstraction from C-4 in the glycon to the endocyclic N-atom in twisted perbenzylated glucoamidines leading to elimination of substituents at C-3 and C-4. To overcome the encountered synthetic pitfall, they decreased the ring size of the glycon from a six- to a five-membered ring and synthesized the first five-membered arabinoamidine **5u** (Figure 5a). Disappointingly, arabinoamidine **5u** was a poor β -glucosidase inhibitor, making fewer interactions within the active site of model 1CBG β -glucosidase and importantly no interaction with the amino acid acting as nucleophile (Glu-183) according to docking studies (Figure 5b).

a)

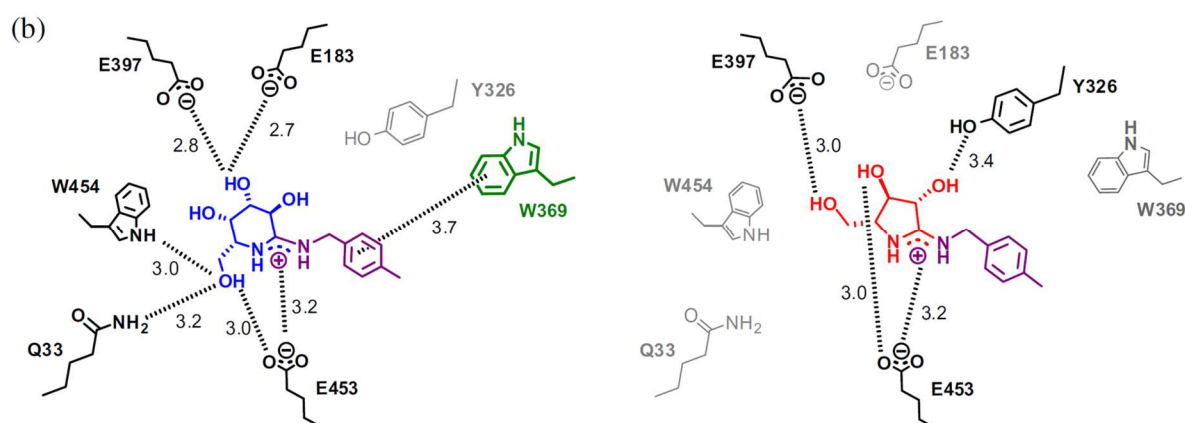
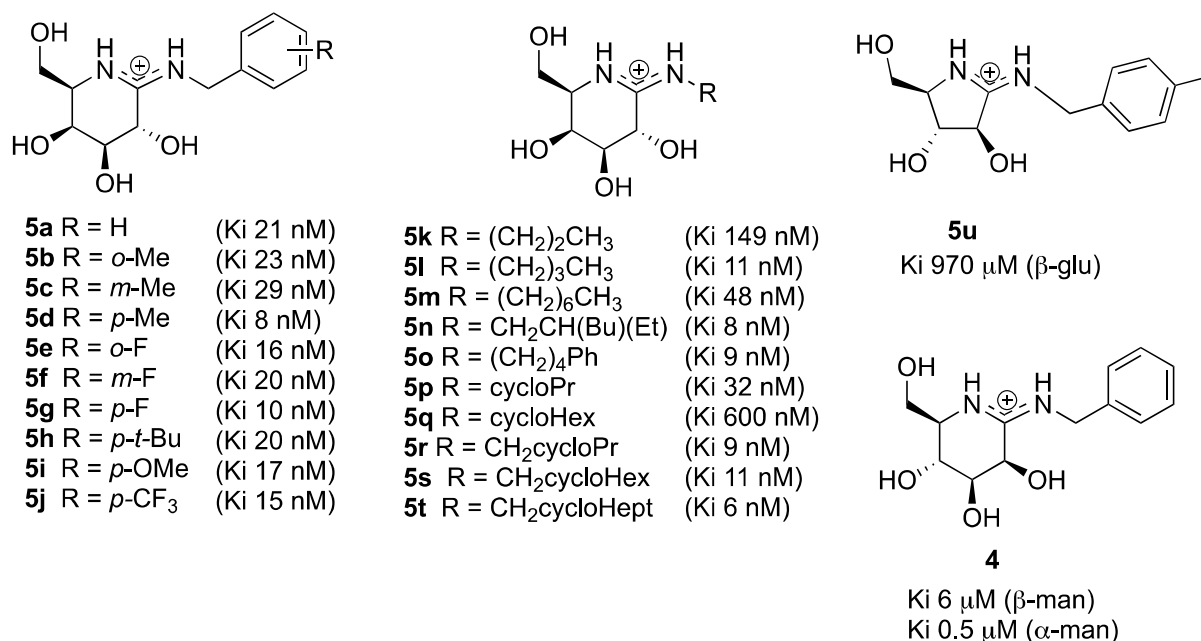


Figure 5: (a) Structures of benzyl mannoamidine **4**, galactoamidines **5a-t** and arabinamidines **5u**; (b) docking of galactoamidines **5d** and arabinamidines **5u** in the active site of 1CBG β-glucosidase

In line with the studies above aiming at deciphering and improving the impact of the aglycone moiety on the potency of glycoamidines as glycosidase inhibitors, Heck, Mioskowski *et al.* explored the effect of a supplementary electrostatic interaction to the glycoamidines core structure of the inhibitor to target both carboxylates of the glycosidase active site (Figure 6a).³¹ Indeed, once the inhibitor is protonated, the amidine functionality is a monocation that can ionically interact with only one of the two carboxylates (Figure 6b). Thus, the addition of a supplementary amino group through a short spacer attached to the exocyclic nitrogen was planned to provide a new ionic interaction via its protonated ammonium form with the second carboxylate (Figure 6c).

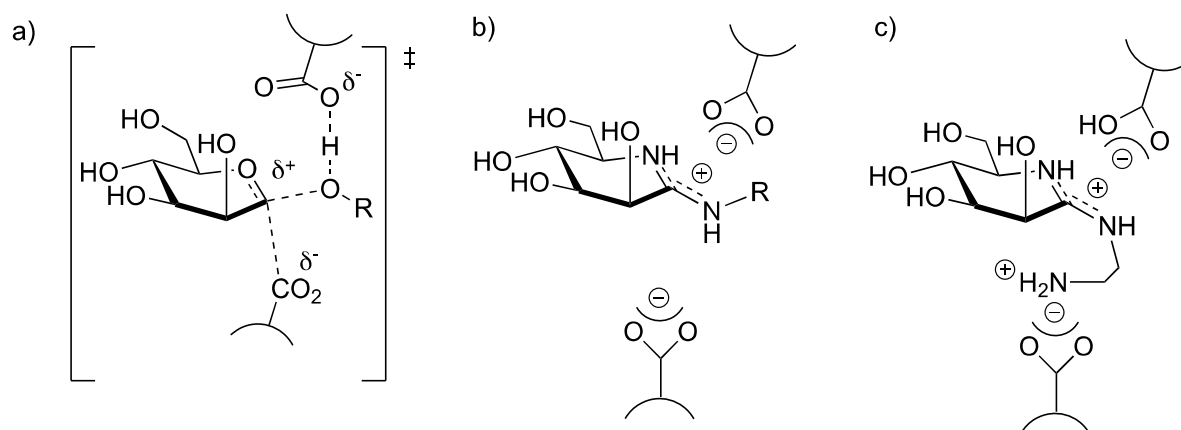
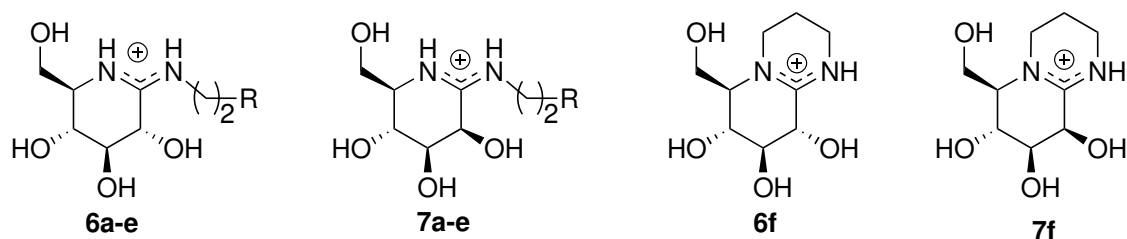


Figure 6: a) Postulated exploded transition state of a mannosidase; b) monocationic mannoamidinium and c) biscationic mannoamidinium as transition state analogues

A set of monocyclic amidines in the D-glucoside **6** and in the D-mannoside **7** series, bearing an ethyl-, a propyl-, or a butylamino group linked to the exocyclic nitrogen were synthesized. In an attempt to demonstrate the role of this additional amine, amidines **8** bearing an alkyl chain, a hydroxyl function or a fused six-membered ring were also produced (Figure 7). All the prepared compounds demonstrated good to excellent inhibition toward glycosidases. In particular, the biscationic D-mannoamidinium bearing an exocyclic ethylamine moiety proved to be a selective competitive inhibitor of α - and β -mannosidases (K_i 6 nM and 9 nM respectively) making it one of the most potent inhibitors of these glycosidases.³¹ A favorable $B_{2.5}$ boat conformation was invoked to explain the selectivity of mannosidase inhibition compared to other glycosidases. To reveal the significance of binding interactions and probe the degree of transition state mimicry of these molecules, these mannoamidines were the subject of linear free energy correlations studies. Despite their sp^2 anomeric centers and $B_{2.5}$ conformations in solution, these amidines were not classified as transition state mimics of *Bacteroides thetaiotaomicron* β -mannosidase BtMan2A.³² The reason for this most likely stems from the high (8–10) pK values of the amidines, which cannot accept a favorable hydrogen bond from the catalytic acid at pH 5.6. The interaction of catalytic acid with the glycosidic oxygen in catalysis is known to be highly significant. At pH 5.6, the protonated state of the amidinium ion is likely to hinder binding, which is consistent with the observation that the K_i for one of these amidines **7** bearing a $CH_2CH_2NH_2$ aglycon moiety decreases from 1 μ M at pH 5.6 to 49 nM at pH 7.5.



- 6a** R = CH₃ **7a** R = CH₃
6b R = NH₂ **7b** R = NH₂
6c R = CH₂NH₂ **7c** R = CH₂NH₂
6d R = CH₂CH₂NH₂ **7d** R = CH₂CH₂NH₂
6e R = CH₂OH **7e** R = CH₂OH

amidine	α -man	β -man	α -glu	β -glu	α -gal	β -gal	α -fuc	β -gluNAc
6a	2.3	3.3	13	3.6	184	31	295	374
6b	0.06	0.13	32	34	192	53	96	339
6c	0.09	0.41	14	71	44	44	91	252

6d	0.12	0.71	39	50	451	6	221	136
6e	1.7	0.87	31	20	28000	1000	470	4500
6f	8.8	nd	1100	nd	2000	1400	75	1100
7a	0.11	0.19	125	111	4700	2200	34	900
7b	0.006	0.009	81	6600	218	82	6	99
7c	0.019	0.15	2870	22	1400	119	8.5	85
7d	0.012	0.06	155	315	1800	156	10	112
7e	0.08	0.15	38	44	1200	235	79	1300
7f	6.0	nd	1800	nd	7400	6300	930	750

Figure 7: Structures and inhibitory potency of mannoamidines and glucoamidines designed by Heck and Mioskowski.

The concept of introducing an amidine moiety into known inhibitors has been applied to various structures. In its protonated form, glycosidase inhibitor isofagomine resembles the alternate resonance form of the oxocarbenium ion transition state, that is the glycosyl cation (Figure 8). Isofagomine is a potent β -glycosidase inhibitor presumably because it forms a strong salt bridge with the nucleophilic carboxylate in these enzymes,³³ an hypothesis supported by X-ray structures of the inhibitor-enzyme complex showing a protonated isofagomine.³⁴ Inspired by the importance of the ionic interaction for the binding of isofagomine, Bols *et al.* synthesized isofagomidine **9** (Figure 8), which has an amidine function placed at the 1,2-positions and the advantage to display a polar group at the 2-position.³⁵ Partial atomic and group charges of selected atoms in isofagomine and isofagomidine were calculated and showed that the more stable isomer of isofagomidine **9** has atom and group charges at N-1 that are more negative than isofagomine. Compound **9** was tested for inhibition of seven commercially available glycosidases. Compared to isofagomine, it is a weaker inhibitor of all the tested glycosidases except α -mannosidase, where it is a much stronger inhibitor (K_i 0.75 μ M), a result that can be tentatively explained by the unique charge distribution of isofagomidine.

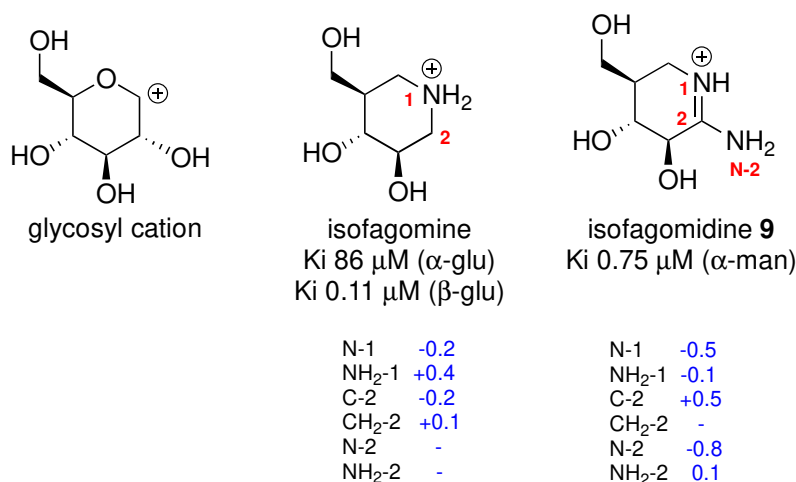


Figure 8: Structures of glycosyl cation, isofagomine and isofagomidine and their partial atomic and group charges

1.3 Alteration of the hydroxyl pattern of the sugar moiety

Beside the tuning of the aglycon structure to study its impact on the potency and selectivity of glycoamidines as glycosidase inhibitors, structural modification of the sugar moiety has been also explored. The contribution of the sugar hydroxyl groups on the overall inhibition of glycoamidines has

been evaluated. Striegler *et al.* reported three galactoamidines **10a-c** in which the hydroxyl groups at C-2 and C-4 were altered through either methylation or deoxygenation²⁹ (Figure 9) while Tellier *et al.* synthesized three benzylamidines **11a-c** with a reduced or absence of hydroxylation pattern.³⁶ All galactoamidines remained competitive *E. Coli* β -galactosidase inhibitors of the relevant glycosidases but showed reduced inhibition ability compared to the fully hydroxylated galactoamidine **5d**, thus suggesting strong hydrogen-bonding interactions between the hydroxyl group at C-2 of the galactoamidine and the active site of the enzyme. The lack of hydroxyl groups in benzylamidines **11a-c** led to very weak α -mannosidase and glycosidase inhibitors compared to fully hydroxylated mannoamidine **4**, demonstrating that the presence of a positive charge and aglycon moiety in the inhibitor cannot compensate the absence of recognition motif provided by the hydroxyl pattern.

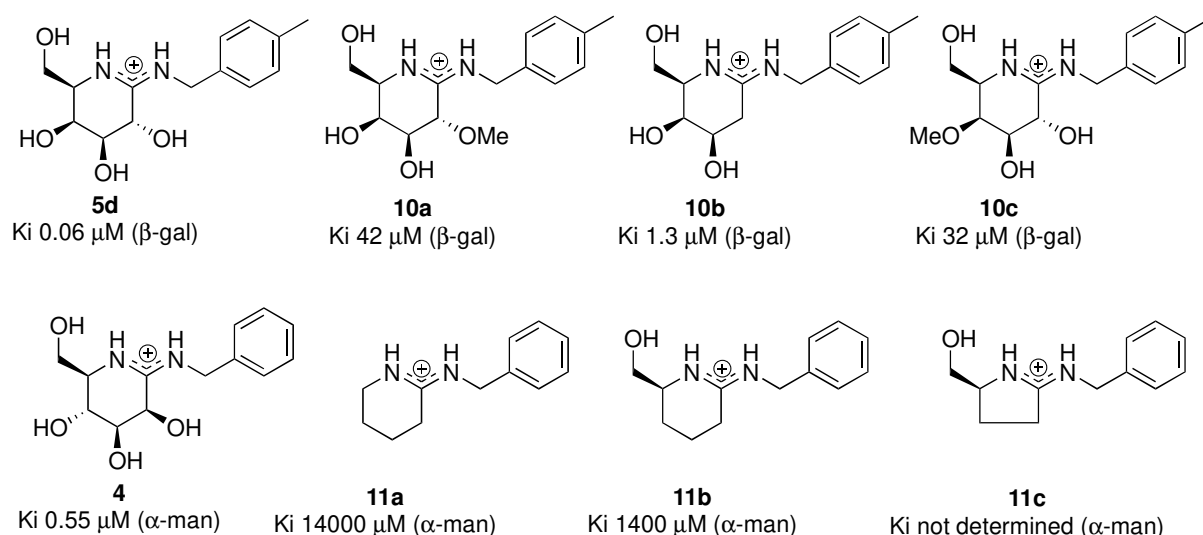


Figure 9: Structures of glycoamidines with altered hydroxyl pattern reported by Striegler and Tellier

1.4 Alteration of the amidine function

1.4.1 Alteration of the endocyclic nitrogen

Inspired by the beneficial alkylation of the nitrogen atom in deoxynojirimycin that has provided two approved drugs respectively for the treatment of Gaucher's disease³⁷ (miglustat, N-butyl-deoxynojirimycin) and type II diabetes³⁸ (miglitol, N-(2-hydroxyethyl)-deoxynojirimycin), Lindbäck, Lopéz, Sydnes *et al.* reported six-membered glucoamidines **12a-b** (Figure 10).³⁹ In these derivatives the endocyclic nitrogen of the amidine function has been armed with an amino- or methylamino group to obtain the corresponding hydrazide imides electronically similar to a sugar amidrazone that were found to be stable in D₂O for days at room temperature and to decompose upon treatment with aqueous NaOH (0.5 M). This structural modification proved of interest as it triggered some inhibitory selectivity in the compounds that demonstrated good α - and β -glucosidase inhibition in the low micromolar range but poor mannosidase inactivation unlike the unselective parent amidine **1** that inhibited glucosidases and mannosidases to the same extent. Invoking the ability of polyhydroxylated pyrrolidine DAB to inhibit various α -glucosidases⁴⁰ and the possibility to modulate its selectivity towards other glycosidases through chemical modification, they also examined the biological impact of the insertion of a hydrazide imide moiety into DAB through the synthesis of two derivatives **12c-d**.⁴¹ Indeed, this structural modification drastically changed the inhibition profile of these derivatives as they behaved as strong and selective α -mannosidase inhibitors (Ki 0.23 μ M for **12c** and 1.4 μ M for **12d**).

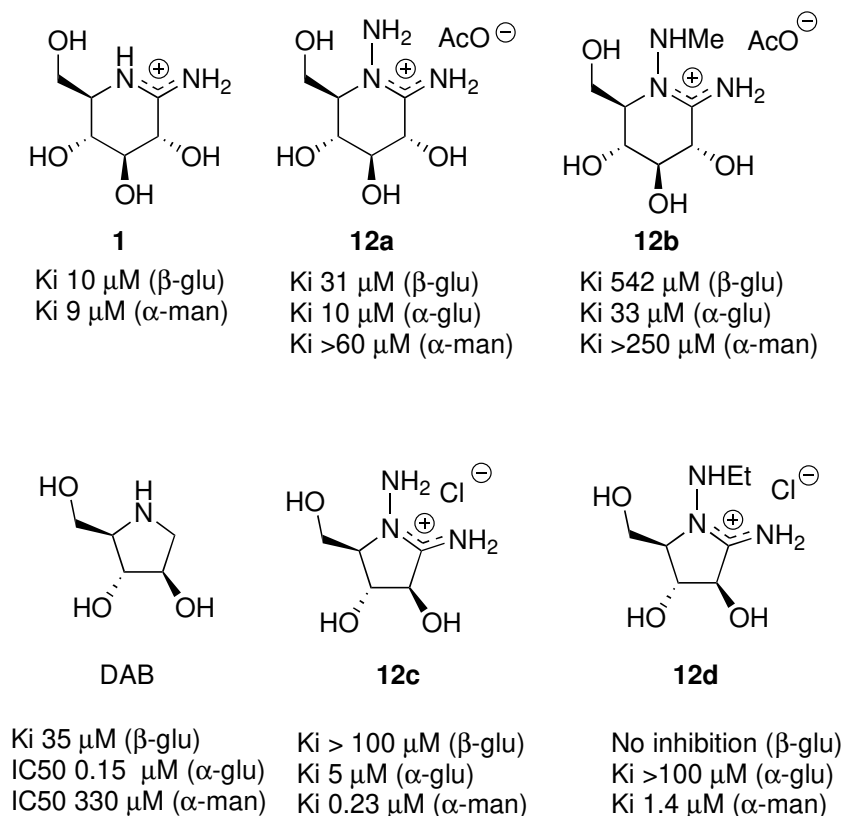


Figure 10: Structures and glycosidase inhibition of amidines **12a-d** developed by Lindbäck, Lopéz and Sydnes

1.3.3 Alteration of the exocyclic nitrogen: amidrazones and amidoximes

The hydrolytic instability of glucoamidine **1** at alkaline pH led to the design of other conformationally flattened saccharide analogs that might constitute more robust enzyme inhibitors. Exploiting the known correlation between the pK_a values of N-substituted amidines and the pK_a values of the corresponding N-substituted amines,⁴² Ganem *et al.* reasoned that amidrazone **13** and amidoxime **14** (Figure 11) should have lower pK_a values compared to **1**, since hydrazine (pK_a 8.10) and hydroxylamine (pK_a 5.97) are both less basic than ammonia (pK_a 9.21).⁴³ Indeed, potentiometric titrations of the acidic forms of compounds **1**, **13**, and **14** revealed pK_a values of 10.6, 8.7 and 5.6, respectively leading to improved half-lives at basic pH for **13** and **14**. Regarding the structure and the location of the C=N double bond in amidoxime **14**, Vasella *et al.* firmly established that the C=N bond was exocyclic whatever the amidrazone, exploiting nitrogen isotopomers and semiempirical and *ab initio* SCF-MO calculations.⁴⁴ The conformation of **14** both in the solid state (between 4C_1 and 4H_3) and in solution (flattened 4C_1) was also deciphered. Interestingly, calculations also predicted that protonation at the exocyclic N-atom would strengthen the conjugation between the endocyclic N-atom and the hydroxyimino group and lead to a half-chair conformation. In this work, a similar C=N exocyclic bond was postulated for amidrazone **13**. Importantly, all three glucosyl mimics **1**, **13**, and **14** inhibited almond β -glucosidase to a similar extent in the low micromolar range, despite the 10^5 range in basicity from amidine to amidoxime functionality, suggesting that, at least for this enzyme, conformational changes accompanying the rehybridization of the sugar's anomeric region are more important for inhibition than charge build-up on the glucopyranosyl oxygen.⁴⁵ Amidoxime **14** was also a potent yeast α -glucosidase inhibitor (Ki 2.9 μM). These encouraging data forced chemists to apply this structural modification to other stereoisomers. In the *D-manno* series, the amidrazone **15** developed a significant potency ranging from low micromolar to nanomolar inhibition on a set of different β -mannosidases (fungal) and α -mannosidases (jack bean, mung bean, Golgi α -man I and II, ER α -man), the last triad of mannosidases being involved in the trimming of highly mannosylated glycoproteins.⁴⁶ Interestingly, the mannoamidoxime **16** proved to be a potent jack bean α -mannosidase (Ki 0.15 μM) while its *D*-gluco configured epimer **14** was a 80 times weaker inhibitor, illustrating a dependence upon configuration unlike in the case of glucoamidine **1**. In the *D*-galacto series, amidrazone **17** inhibited green coffee bean α -galactosidase in the low micromolar range (Ki 8.3 μM).²³ Bovine β -galactosidase was competitively inhibited by amidrazone **17** (Ki 6.5 μM) and amidoxime **18**

(K_i 10 μM). By contrast, D-gluco- and D-manno-amidrazones **13** and **15** displayed non competitive inhibition of this enzyme. This strategy was extended to L-fucose, widely distributed in mammalian glycolipids and glycoproteins, where it plays notably an important role in cell-cell adhesion, making the synthesis of L-fucoamidrazone **19**, a prospective fucosidase inhibitor of interest.⁴⁷ Tested against recombinant human α -L-fucosidase, L-fucoamidrazone **19** displayed potent competitive inhibition (K_i 820 nM) (Figure 11).

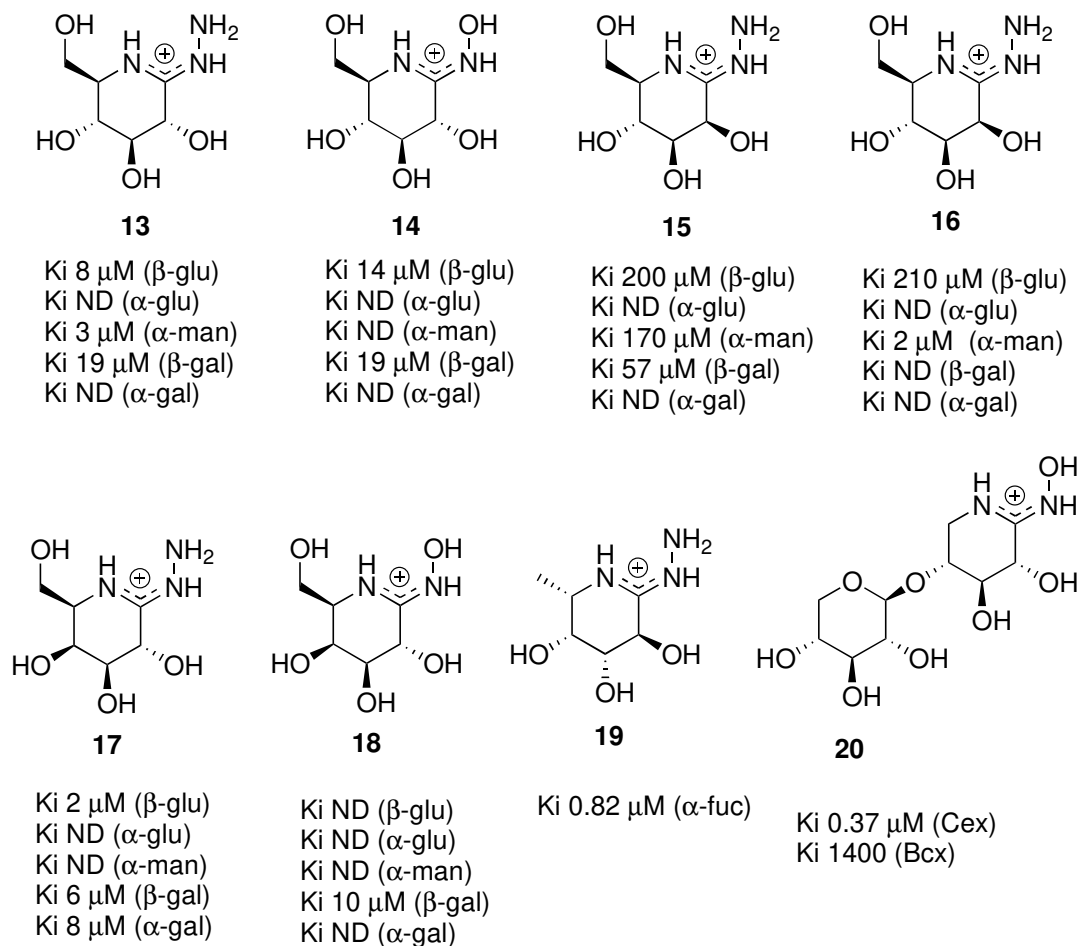


Figure 11: Structures and glycosidase inhibition of glycoamidrazones and glycoamidoximes **13-20**

Glycoamidoximes have also been incorporated in disaccharides. In their quest for detailed insights into the mode of binding of a series of tight-binding aza-sugar glycosidase inhibitors, Rose *et al.* performed X-ray crystallographic studies of a complex of xylobioamidoxime **20** (Figure 11) with the retaining family 10 xylanase Cex from *Cellulomonas fimi*.⁴⁸ Compound **20** proved to be a potent inhibitor (K_i 370 nM) and this study revealed lateral interaction⁴⁹ of the “glycosidic” nitrogen with the acid/base catalyst (Glu127) and hydrogen bonding of the sugar 2-hydroxyl with the catalytic nucleophile (Glu233) (Figure 12). Dramatically weaker binding of **20** to the family 11 xylanase Bcx from *Bacillus circulans* (K_i 1.4 mM) was recorded and rationalized on the basis that this enzyme utilizes a *syn* protonation trajectory and likely hydrolyses via a ^{2,5}B boat transition state, a conformation that can not be reached by this conformationally locked compound.

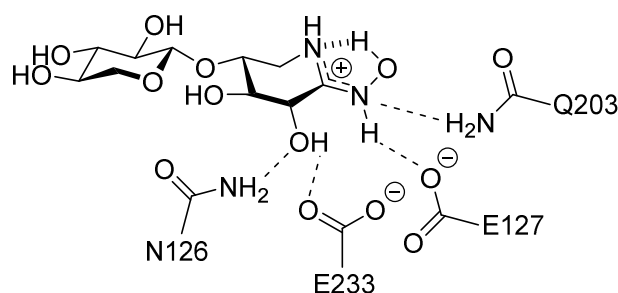


Figure 12: Schematic representation of relevant active site interactions with the proximal (-1) sugar and Cex.

Five-membered glycoamidrazones have also been developed to target furanosidases. Nucleoside hydrolases, NAD hydrolases, , purine nucleoside phosphorylases, and purine or pyrimidine hydrolases promote the cleavage of C-N glycosidic bonds. These enzymes are notably involved in base-excision repair mechanisms of DNA⁵⁰ and ribosome inactivation.⁵¹ Schramm *et al.* have characterized the transition state structure of several nucleoside hydrolases⁵² including *Crithidia fasciculata* (CfNH) employing both experimental⁵³ and computational⁵³ methods. They concluded that, as the heterocyclic base is protonated, the ribofuranosyl ring is flattened to form a transition state structure with significant oxocarbenium ion character (Figure 13). This conformational change accommodates both an elongated (2.0 Å) C-N glycosidic bond and an incipient bond (ca. 3 Å) to the incoming oxygen nucleophile. With their delocalized charge and flattened structures, five-membered riboamidoxime **21a** and riboamidrazones **21b-d** were designed to resemble the putative transition state for this nucleoside hydrolase. While the riboamidoxime **21a** and riboamidrazones **21b** devoid of aglycon moiety inhibited *C. fasciculata* nucleoside hydrolase (CfNH) in the μM range, phenyl riboamidrazones **21b** (K_i 207 nM) and *p*-nitrophenyl riboamidrazones **21c** (K_i 2 nM) proved to be potent nucleoside hydrolase inhibitors.⁵⁴ Importantly, an isotope-edited resonance Raman study of CfNH in complex with the *p*-nitrophenyl riboamidrazones **21c** revealed that its molecular electrostatic potential, when bound to the catalytic site, closely resembles that of the transition state.⁵⁵ In parallel, a slow-onset, irreversible amidrazones transition state analogue **22** has been also reported for *Saccharomyces cerevisiae* Orotate phosphoribosyltransferase (OPRT). Compound **22** at 2 mM concentration, incubated with PPI, Mg^{2+} , and yeast OPRT, was reported to cause usually slow inactivation of catalytic activity with a $t_{1/2}$ of approximately 200 h ($k_{\text{obs}} = 0.004 \text{ h}^{-1}$). No recovery of activity could be detected, indicating extreme tight binding or irreversible inactivation of the enzyme even if no K_i value was reported for this compound.⁵⁶

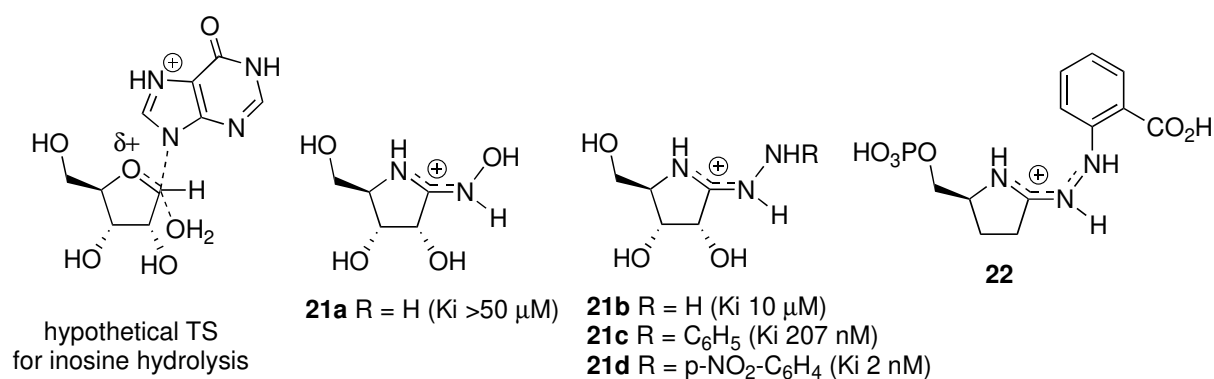


Figure 13: Hypothetical transition state for nucleoside hydrolase (left) and structures and glycosidase inhibition (CfNH) of five-membered amidoxime **21a** (middle) and amidrazones **21b-d** (middle) and **22** (right)

1.3 Guanidinosugars

The synthesis and biological evaluation of polyhydroxylated azaheterocycles closely related to glycoamidines and incorporating a cyclic guanidine resonance π -system have been reported. This type of compound, coined guanidinosugar, possesses a number of attractive features. Unlike amidines, guanidinosugars are stable over a wide pH range and their pK_a values can be modulated with appropriate substituents. The cyclic guanidine moiety may interact favorably with the two putative carboxylic residues in the active site of glycosidases (Figure 14) as observed for a potent inhibitor of

influenza virus sialidase.⁵⁷ Additionally, replacement of the C2-hydroxyl group with nitrogen can serve as a surrogate for either D-glucose or D-mannose, with electron density projected above and below the ring plane directly adjacent to the anomeric centre.

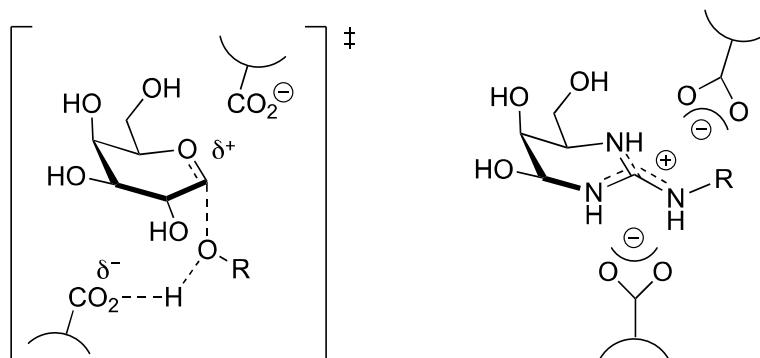


Figure 14: Postulated transition state of an α -galactosidase (left) and structure of a guanidinogalactose as transition state analogue (right).

The first guanidino-sugar **23a** was reported by Wong in 1994 and proved to be in equilibrium with acyclic guanidines **23b** as α - and β -anomers in D₂O at pH 5 (Figure 15a).⁵⁸ Raising the pH to 11 with NaOD displaced the equilibrium toward the formation of the expected cyclic guanidine. Inhibition studies carried out on green coffee bean α -galactosidase showed a modest inhibition (IC₅₀ 480 μ M at pH 10.7) that increased with pH corresponding to an increase of concentration of the biologically active species tetrahydropyrimidine **23a**. To solve this equilibrium issue that is triggered by the deprotonation of the OH group at C-3, this OH was removed leading to guanidino-trioses **24a** and **24b** that remained locked in the hexopyranose form whatever the pH. These compounds present bilateral N-C=N arrays for favorable H-bonding and electrostatic interactions with carboxylate functional groups but no inhibition data have been reported for these compounds to the best of our knowledge.⁵⁹ The same year, Lehmann *et al.* reported aryl D-gluco-like **25a-b** and aryl D-galacto-like guanidino-trioses **25c-d** that were found to be modest competitive and non-competitive inhibitors of β -galactosidase (*E. coli*), α -glucosidase (yeast) and β -glucosidase (emulsin) and that did not inhibit jack bean α -mannosidase (Figure 15b).⁶⁰ The incomplete hydroxyl topography of these 3-deoxy-guanidinosugars combined with their very basic character (pK_a > 10), leading to completely charged molecules under the biological assay conditions, forced Wong *et al.* to study fully substituted guanidino-galactoses **26a-c** bearing different pseudoanomeric substituents and displaying different pK_a values (Figure 15c).⁶¹ Compounds **26a-c** were assayed on coffee bean α -galactosidase (coffee bean) and β -galactosidase (*Aspergillus oryzae*) at various pH and were shown to be low micromolar inhibitors of these enzymes at basic pH. Regarding the mechanism of action of these molecules, it was suggested that the neutral six-membered cyclic guanidino-sugar is first bound to the enzyme active site, followed by protonation from one of the two catalytic carboxylic groups to form the tight complex shown in Figure 14. To closer mimic the glycosidase transition state, Wong *et al.* eventually explored the introduction of an extra hydroxyl group on the 3-N-position through the synthesis of **27a** and **27b** and their evaluation on α - and β -galactosidases but none exhibited significant inhibition, ruling out this type of motif (Figure 15d).⁶²

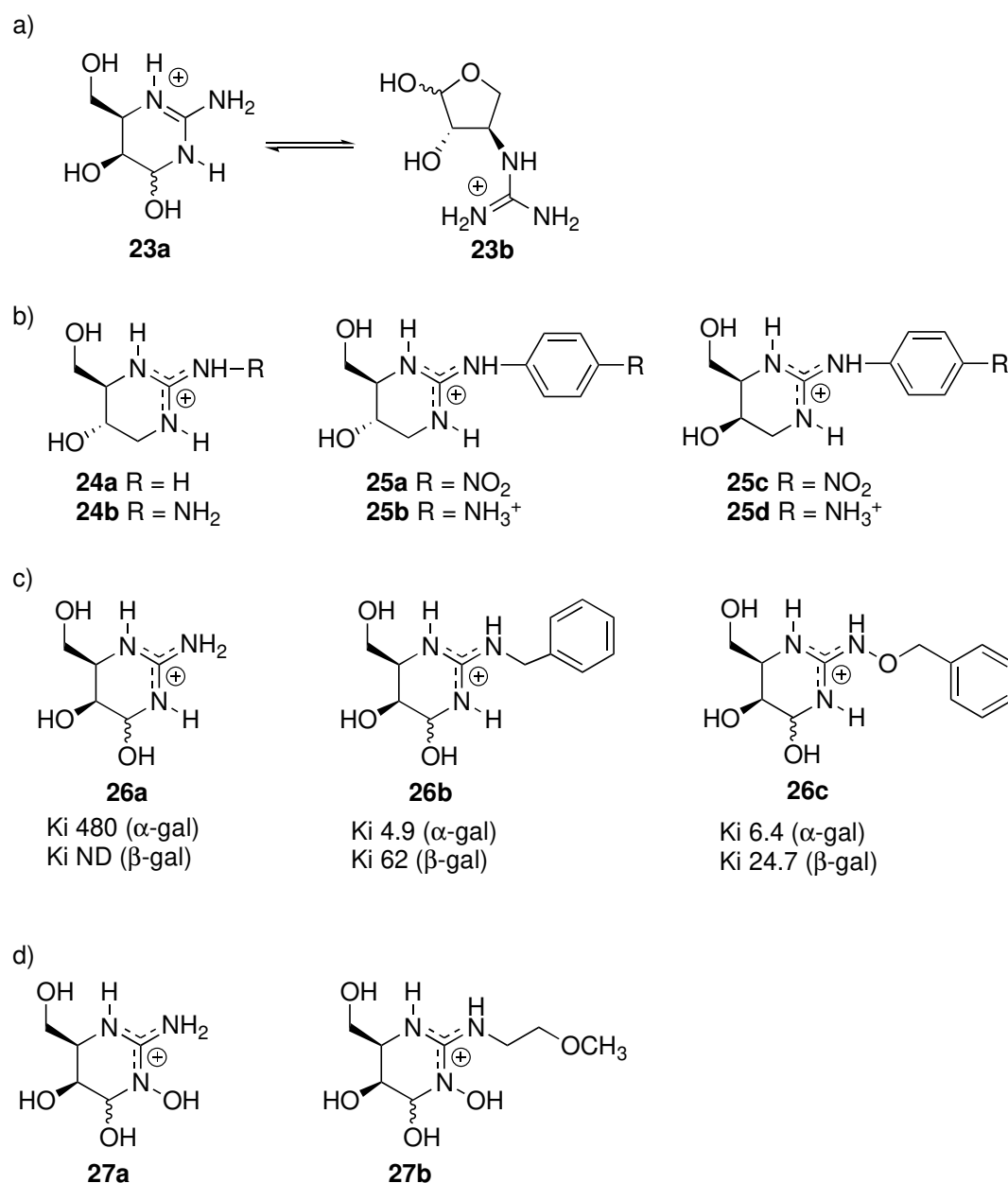


Figure 15: Guanidino-sugars a) Equilibrium between tetrahydropyrimidine **23a** and acyclic guanidine **23b** at acidic pH; b) Structures of 3-deoxy-guanidinosugars **24a-b** and **25a-d**; c) Structures and glycosidase inhibition of guanidino-galactoses **26a-c**; d) Structures of N3-substituted guanidino-galactoses **27a-b**.

1.4. Pseudodisaccharides

Many disaccharides incorporating an iminosugar have been reported to target specific glycosidases.⁶³ Glycoamidines and derivatives are no exception and several amidine- and guanidine-containing pseudodisaccharides have been disclosed as potentially superior inhibitors as they might bind both the -1 "glycopyranosyl" site and the +1 aglycone site of glycosidases hydrolyzing di- and oligosaccharides. This idea initially received support in the structure of the naturally-occurring pseudodisaccharide trehalase inhibitor trehalosin isolated from culture broth and bearing an amidine moiety (Figure 16).⁶⁴ Hypothesizing that the analogous 1,4-linked derivative might be an inhibitor of 1,4-glucosidases, Knapp *et al.* reported the synthesis of the pseudodisaccharide **28a** that was found to be a potent competitive inhibitor of yeast α -glucosidase (K_i 9.3 μ M) unlike trehalosin. The importance of the aglycon moiety was illustrated by the weaker inhibition (K_i 70 μ M) developed by compound **28b**. Although its aglycon portion was designed to bind α -glucosidase, **28a** showed competitive binding (K_i 40 μ M) to *Agrobacterium* β -

glucosidase.⁶⁵ A year before, the same group reported the synthesis of two other 1,4 and 1,6 amidine-linked pseudodisaccharides **28c-d** in which the sugar moiety was *N*-methylated and simplified to a piperidine or a hydroxymethyl-pyrrolidine scaffold for stability reasons.⁶⁶ The 1,6-linkage was also targeted by Tellier *et al.* who reported an amidine-1,6-linked pseudo-disaccharide **29** that was found to be a potent and very selective α -mannosidase inhibitor (K_i 2.6 μ M).⁶⁷ Interestingly, Lehmann *et al.* concomitantly published the synthesis of two cationic analogues of a disaccharide incorporating a guanidinium ion **30a-b** as gentobiose and allolactose surrogates but no inhibition data were provided.⁶⁸

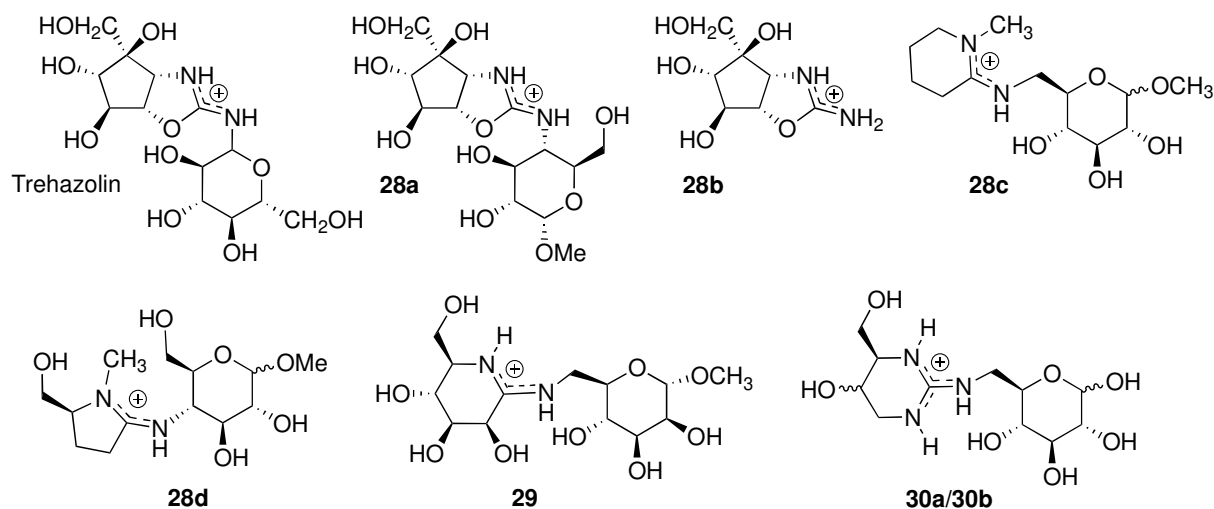


Figure 16: Structures of trehazolin and pseudodisaccharides **28-30**.

Glycosylamidines

Considering that glycoamidines, with a planar sp^2 -geometry at C-1, are potent but rather nonselective glycosidase inhibitors and that their synthetic access requires a relatively long synthetic sequence, Hiratake *et al.* reasoned that the exocyclic incorporation of a positive charge near the anomeric carbon in such a way as to maintain the whole structure of the glycon, including the stereochemistry at the anomeric carbon and at C-2 position, should lead to potent and selective glycosidase inhibitors according to the glycon- and stereospecificities of the enzyme. From the corresponding unprotected sugars, they produced in two synthetic steps the D-gluco, D-galacto and D-xylo-configured β -D-glycosylamidines **31a-c**, in which a glycon moiety is connected via a *N*-glycoside linkage to a substituted amidine (aglycon). These molecules proved stable between pH 5 and 7 and showed substantial potency and selectivity towards several β -glycosidases unlike the related endocyclic glycoamidines, a selectivity that was attributed in part to the chair-like conformation of these glycosylamidines that allows precise recognition of the hydroxyl pattern by the enzyme.⁶⁹ Compounds **31a** and **31b** were further exploited as ligand for affinity chromatography through their immobilization to a matrix via an appropriate spacer. This strategy allowed the purification of a β -glucosidase from tea leaves and a β -galactosidase from *Penicillium multicolor*.⁷⁰ In line with this approach, Ueda *et al.* designed two ligands **32a** and **32b** based on a D-glucoamidine scaffold⁷¹ aimed at purifying a leaf-opening factor β -glucosidase (LOFG) involved in the circadian rhythmic control of nyctinasty, a rhythmic movement of the leaves in leguminous plants. Both **32a** and **32b** proved stable up to pH 6.5. However, **32a** was unstable in an aqueous solution above pH 7, while **32b** was stable over a wide range of pH from 4.5 to 9.0. While affinity gels derived from these two ligands were able to purify LOFG to the same level, the higher stability of the gel derived from **32b** allowed a large scale purification of LOFG.⁷¹ (Figure 17).

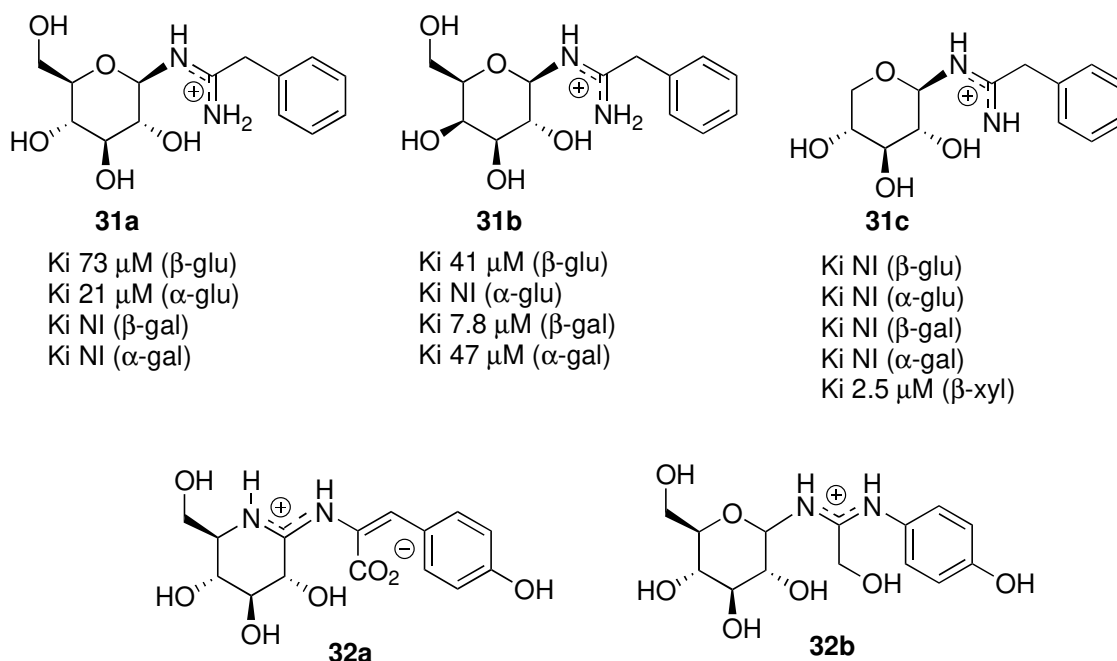


Figure 17: Structures and glycosidase inhibition of exocyclic glycoamidines **32a** and glycosylamidines **31a-c** and **32b**.

A disaccharidic glycosylamidine **33** was also designed to reveal the architecture of the active site of β -primeverosidase, a β -glycosidase found in tea leaves and involved in the aroma formation during the manufacturing process of oolong and black tea (Figure 18).⁷²

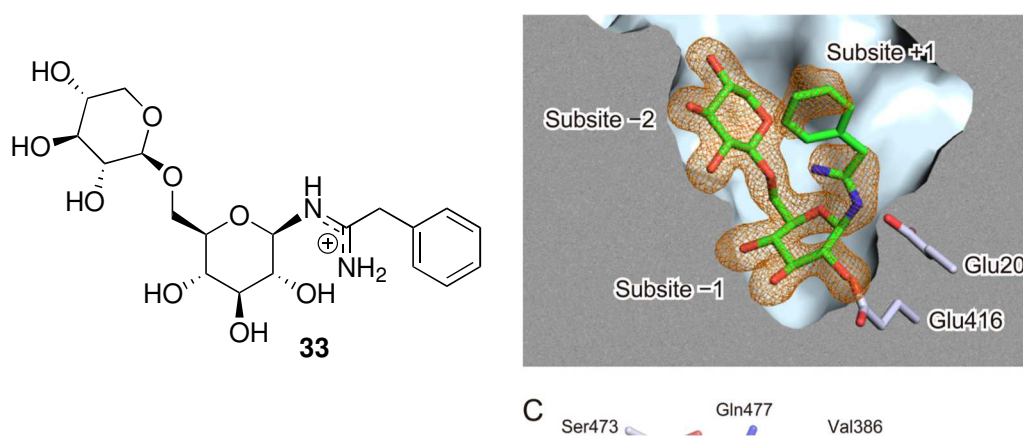


Figure 18: Structure of disaccharidic glycosylamidine **33** and its complex with β -primeverosidase.

Glycosylguanidines

Introduction of an exocyclic guanidine functionality, that is “outside” the sugar ring, exploiting the endocyclic nitrogen atom of iminosugars has been also explored to generate potent glycosidase inhibitors. To solve the lack of selectivity between different isoenzymes faced by deoxynojirimycin and castanospermine, Mellet *et al.* developed a new family of D-gluco- and D-galacto-configured glycomimetics such as **34a-f** in which the endocyclic amine and the parent primary alcohol were connected through their incorporation into a guanidine function (Figure 19a).⁷³ Interestingly, the resulting hemiaminal at the pseudoanomeric position proved stable in these molecules and its axial orientation was governed by the anomeric effect. The stereoelectronic alteration of the pseudoanomeric region in these castanospermine analogues led to potent and selective glycosidase inhibitors. This motif also allowed the modulation of the substituent (apolar, polar, linear or branched) on the exocyclic nitrogen. As an example, the amphiphilic sp^2 -iminosugar **34f** with a lipophilic octyl substituent efficiently stabilized β -glucocerebrosidase involved in Gaucher disease. Importantly, a crystal structure of its complex was

obtained to shed light on the molecular basis of β -glucocerebrosidase inhibition (Figure 19c).⁷⁴ Considering the cyclic framework in which the nitrogen resides, the likely reluctance of the nitrogen to adopt a full sp^2 geometry associated with the restrained movement of the side chain encouraged Pieters, Martin *et al.* to develop novel guanidinium derivatives of deoxynojirimycin such as **35** (Figure 19b), several compounds demonstrating distinct glycosidase inhibitory profiles.⁷⁵

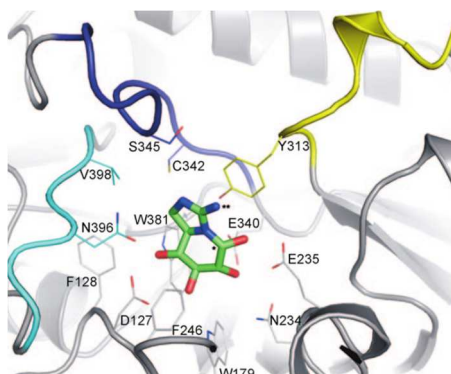
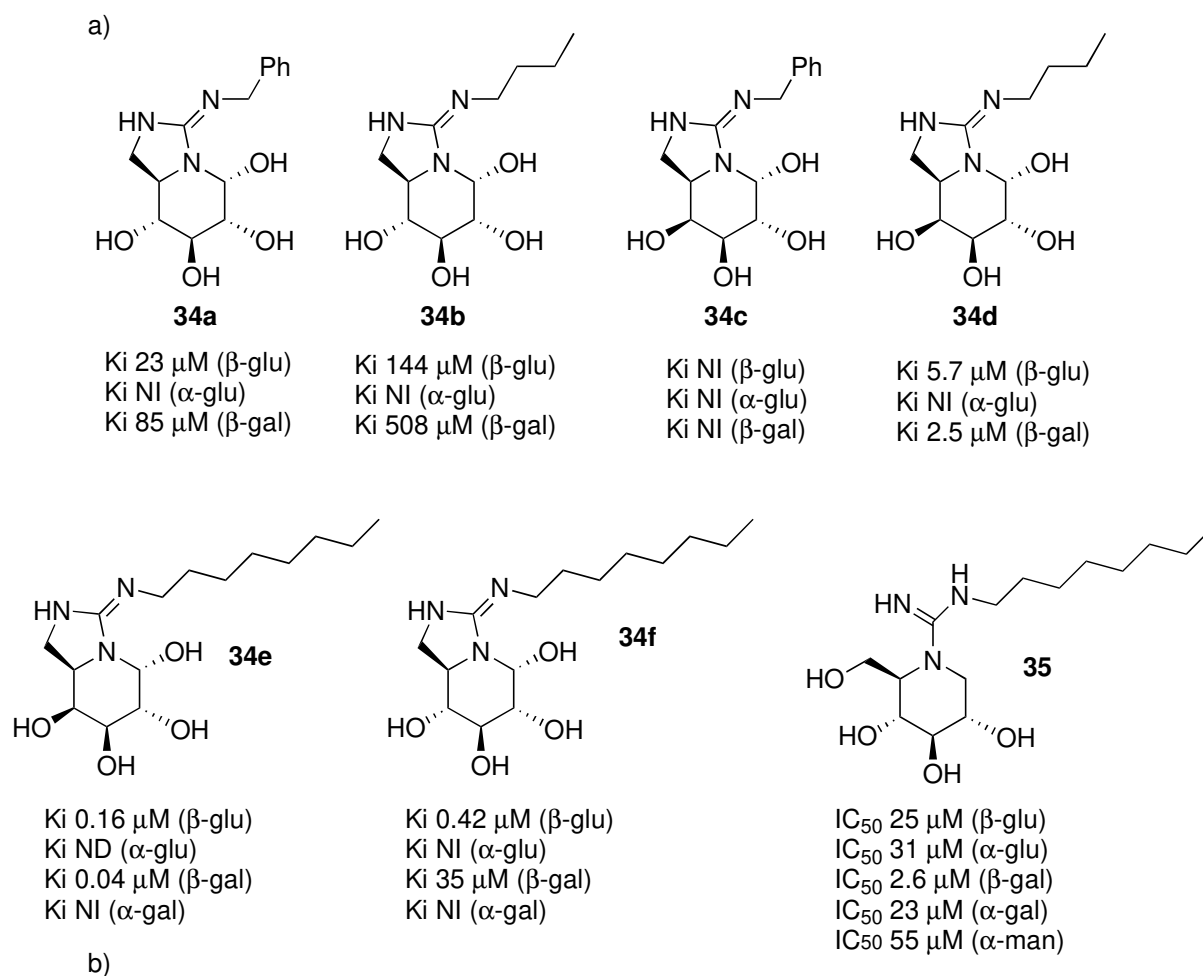


Figure 19 : a) Structures and glycosidase inhibition of sp^2 -iminosugars **34a-f** and of deoxynojirimycin analogue **35**; b) Structure of sp^2 -iminosugar **34f** in complex with recombinant β -glucocerebrosidase (the octyl chain was not detected in the electron density).

2. Their use as TS analogues

2.1 Use as haptens to elicit catalytic antibodies

Enzymes including glycosidases stabilize the transition states of chemical reactions to bring about large rate accelerations.^{10,53} The concept of transition state stabilization has been at the basis of the successful generation of numerous catalytic antibodies.⁷⁶ The strategy relies on the design of haptens as stable mimics of the transition state of a target reaction, which are used to elicit monoclonal antibodies with catalytic activity.⁷⁷ Monoclonal antibodies raised against transition state mimics have been shown to catalyze a number of chemical reactions and for this reason have been called abzymes (antibody enzymes). As part of a program aiming at generating glycosidase-like antibodies, Schultz *et al.* elicited monoclonal antibodies against several transition state analogues including glycoamidinium-derived hapten **36** and assayed their ability to catalyze the hydrolysis of simple acetals. While no activity above the uncatalyzed rate was observed whatever the substrate used with this hapten,⁷⁸ other haptens **37**, **38** and **39** containing a positive charge corresponding to the anomeric center of a cyclic acetal were able to elicit antibodies with hydrolytic activity. The absence of catalytic activity in the antibodies specific for hapten **36** was rationalized by the planar structure of these haptens that does not permit a favorable axial leaving group geometry in the corresponding substrates. This issue was finally solved by Masamune *et al.* who designed a half-chair transition-state analog **40** based on a fused cyclohexane-azetidinium scaffold that successfully generated catalytic antibodies with glycosidase capability (Figure 20).⁷⁹

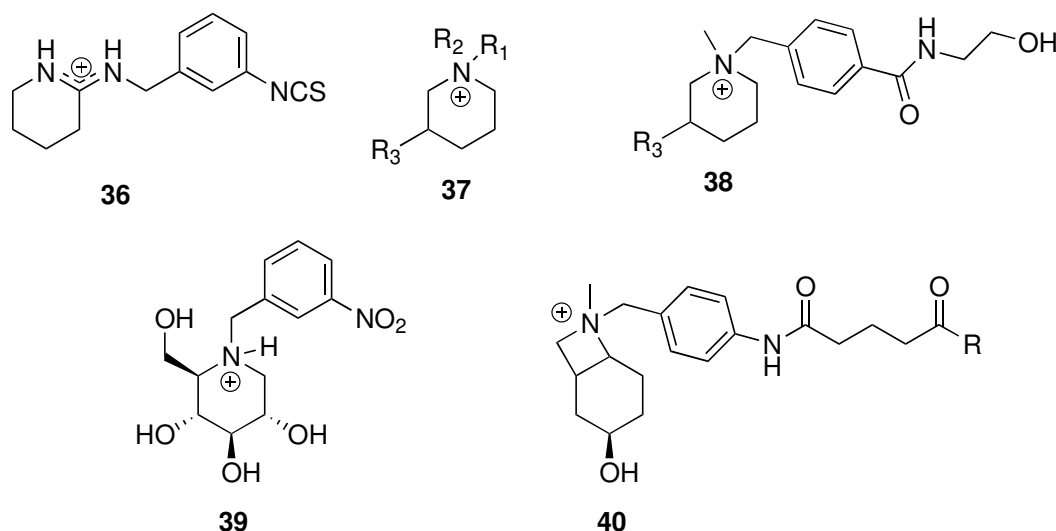


Figure 20: Structure of haptens **36-40** designed to elicit catalytic antibodies with glycosidase-like activity.

Repurposing of catalytic antibodies rationally designed to catalyze one reaction and used to catalyze another closely related reaction has been also successful. Indeed, cyclic amidinium salt **36** hapten, previously used by Tellier *et al.*⁸⁰ and by Schultz *et al.*⁷⁸ in unsuccessful attempts to generate antibodies with glycosidase activity, was therefore recycled to elicit abzymes with β -elimination ability. It was postulated that this hapten would induce a complementary charged group, presumably a carboxylate, in a hydrophobic antibody binding site able to perform elimination reactions. Several excellent hapten-binding monoclonal antibodies were identified and screened for their catalytic activity in the Kemp elimination (Figure 21a-c), and one antibody was relatively efficient, with k_{cat}/k_{uncat} up to 18,000. This work demonstrated that "orphan" antibodies - good natural binders rejected because they do not catalyze their specific target reaction - may yet find a catalytic role if introduced to the right reaction. Additionally, the crystal structure of the complex of a catalytic antibody with hapten **36** at 1.9-Å resolution demonstrated that the hapten amidinium group is stabilized through an ionic pair interaction with the carboxylate of a combining-site residue.⁸¹ The location of this carboxylate allows it to act as a general base in an allylic rearrangement. When compared with structures of other antibody complexes in which the positive moiety of the hapten is stabilized mostly by cation- π interactions, this structure shows that the amidinium moiety is a useful candidate to elicit a carboxylate in an antibody combining site at a predetermined location with respect to the hapten (Figure 21d).

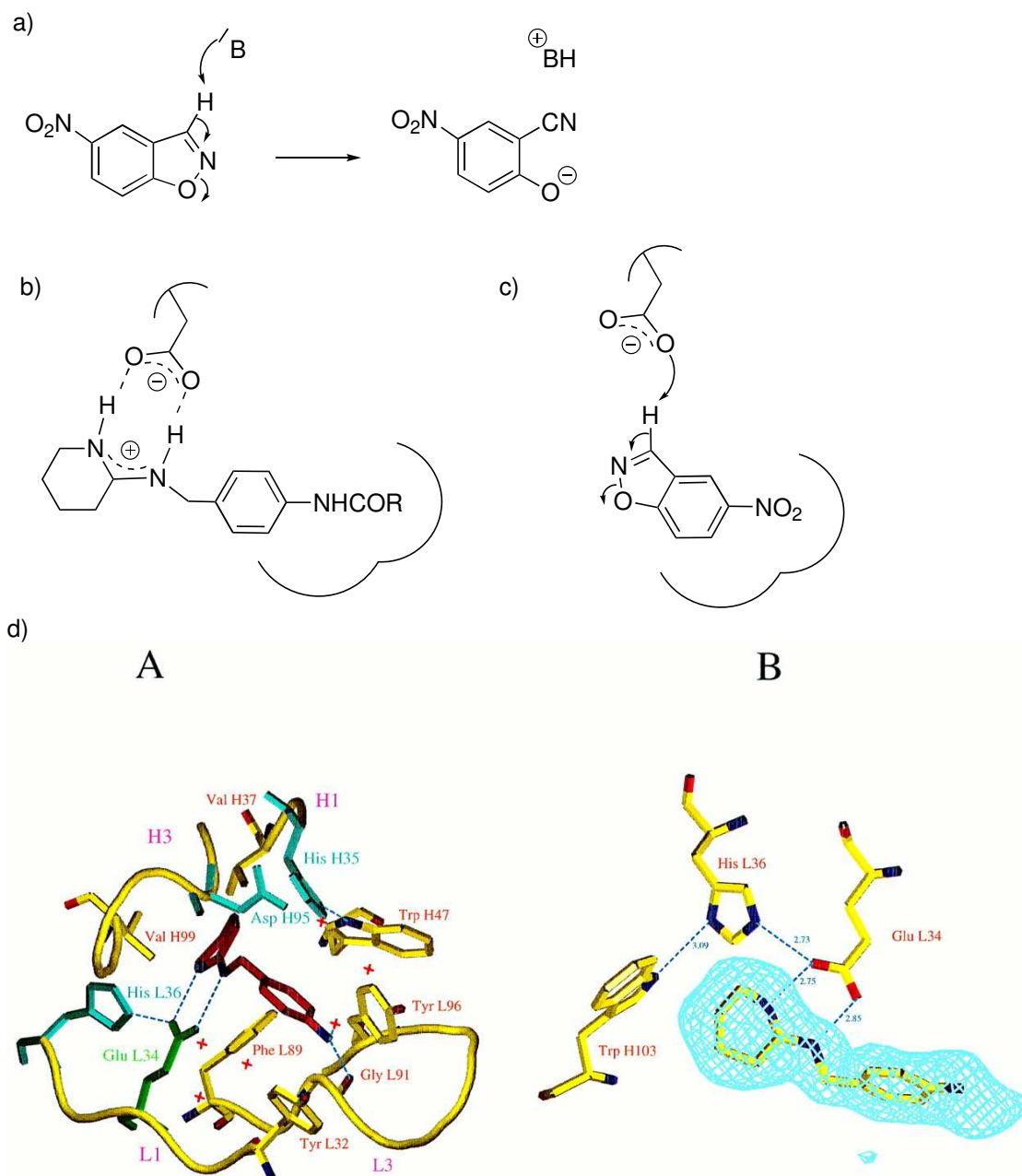



Figure 21: a) The Kemp elimination catalysed by a general base B; b)  The haptens used to elicit the antibodies in a schematic binding site; c) The substrate for the Kemp elimination reacting in the same binding site; d) (A) Schematic view of the active site of the catalytic antibody-**36** complex (B) Hydrogen bonding network established by **36** with catalytic residue Glu L34.

2.2 Use as templates to produce microgel-based catalysts

In line with the combined exploitation of the immune system and glycoamidinium salts to elicit glycosidase-like abzymes, Striegler *et al.* harvested the potential of polymers and microgels with embedded binuclear copper(II) complexes to develop biomimetic catalysts with hydrolytic activity. Unlike the recently disclosed molecularly imprinted glucosidase,⁸² the approach developed by Striegler *et al.* challenges the impact of a templating effect to the overall catalytic proficiency. While a biomimetic catalyst will probably never reach the sophistication level of an enzyme and consequently not hydrolyze glycosidic bonds with the same proficiency, anticipated key interactions of the enzymatic glycoside turnover can be implemented in enzyme mimics separately or used in selected combinations to probe their contributions to the overall catalytic performance. Glycosidases hydrolyze glycosidic bonds with one of the highest proficiencies known¹ and therefore contains very valuable models to study effective stabilizing interactions of transition states. Detailed mechanistic studies have disclosed a synergy of H-

bond, electrostatic, electronic, CH- π and π - π - stacking interactions of the glycon and aglycon during enzymatic glycoside hydrolyses.^{83,84,85,86,87,88} To synthesize the microgel, the instability of glycoamidines under basic conditions selected free radical polymerization under UV light as the method of choice in the presence of galactoamidine **5d**, previously experimentally characterized as transition state analogue.⁸⁹ The stepwise-built microgels utilize a synergy of interactions including metal complex catalysis in a hydrophobic microgel matrix, cross-linking, and shape recognition. To improve hydrolysis efficiency, additional immobilization of a polymerizable binuclear complex similar to Cu₂L **41** was achieved that led to the coordination of **5d** to **41** through the deprotonated amidine site at C-1, the deprotonated hydroxyl group at C-2, and the protonated hydroxyl group at C-3 (Figure 22).⁹⁰

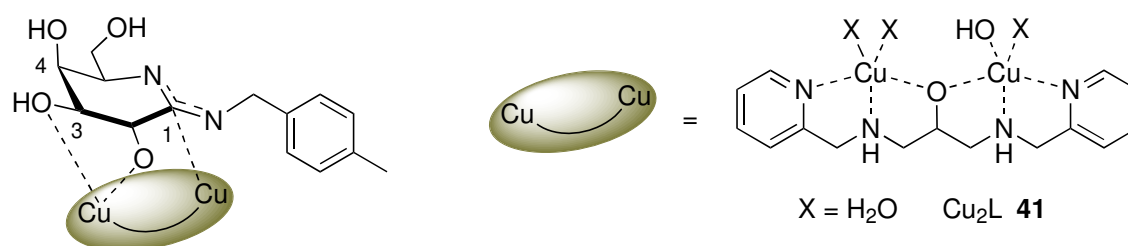


Figure 22: Chelation of Complex Cu₂L **41** and amidine **5d**

The resulting macromolecular catalysts, that contains independent catalytic sites, efficiently hydrolyzed model glycosides bearing a good leaving group with catalytic proficiency up to 3.3×10^6 in alkaline and 1×10^7 in neutral aqueous solution. While a very small templating effect due to the shape of the galactoamidine template contributed to the catalytic proficiency of the microgel catalyst, it did not control its performance as it was not specific toward a single substrate and allowed the hydrolysis of α - and β -glycosidic bonds. Capitalizing on this catalyst, cross-linked microgels were developed that hydrolyzed nonactivated 1,6- and 1,4-disaccharides under mild alkaline conditions at elevated temperatures.⁹¹ Computational analysis revealed the geometry of a putative transition state of the reaction and the location of intermolecular H-bonds that restrict movement and rotation of the glycosyl moieties during hydrolysis (Figure 23).

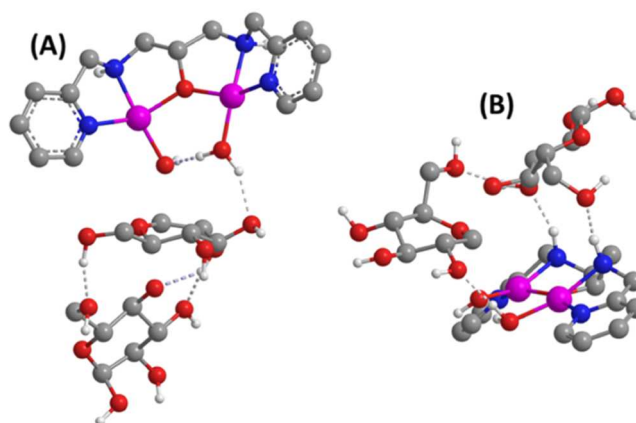


Figure 23: Structures of computed transition states for the Cu₂L **41**-catalyzed hydrolysis of (A) maltose and (B) cellobiose; C-atoms in gray, O-atoms in red, N-atoms in blue, Cu(II) ions in magenta, polar H-atoms and intermolecular H-bonds in light gray; and nonpolar H-atoms omitted for clarity.

These biomimetic catalysts are of interest as they operate with high proficiency under conditions where enzymes are not suitable, functional, or stable. They may also be used to transform glycosides that are not substrates of easily available natural enzymes.

Conclusion

The strong competitive inhibition of various glycosidases by sugar-based amidines has suggested amidines as transition state analogues of enzymatic glycoside hydrolysis in the 90's. This has led to the

disclosure of a vast array of related structures and their use as haptens to elicit glycosidase-like abzymes. We have to keep in mind that glycosidases accelerate hydrolysis of glycoside bond by a factor $> 10^{17}$ relative to the uncatalyzed reaction in water at neutral pH.¹ According to the transition state theory, a perfect transition state analogue should have a $K_i < 10^{-22}$ M since most of the K_m (K_s) are in the range 0.1 to 10 mM and such level of inhibition has never been reached by glycosidase inhibitors despite some impressive K_i s in the literature.^{92,93,94} The best inhibition observed to date with the sugar-based amidines and derivatives is in the nM range, far from the value expected for a perfect transition-state analogue. LFERs studies have shown that the strong binding of glycoamidines does not translate into true transition state analogy.⁹⁵ Nevertheless, glycoamidines have been a source of inspiration for the design of highly potent glycosidase inhibitors. Similar to the natural *N*-acetyl- β -D-glucosaminidase inhibitor nagstatin,⁹⁶ glycoimidazoles **42a-c** are closely related to glycoamidines. The connection of the two amine moieties by a C=C bond, leading to an imidazole motif, resulted in a flattening of the structure and a conjugated system (Figure 24). These compounds proved to be amongst the most potent inhibitors of glycosidase enzymes^{97,98} and are considered to be good glycosidase transition state mimics^{99,100,101} for which they provide strong support for Vasella's lateral protonation model.^{102,103} To the best of our knowledge, such structures have not been exploited as haptens or templates to generate glycosidase-like catalysts.

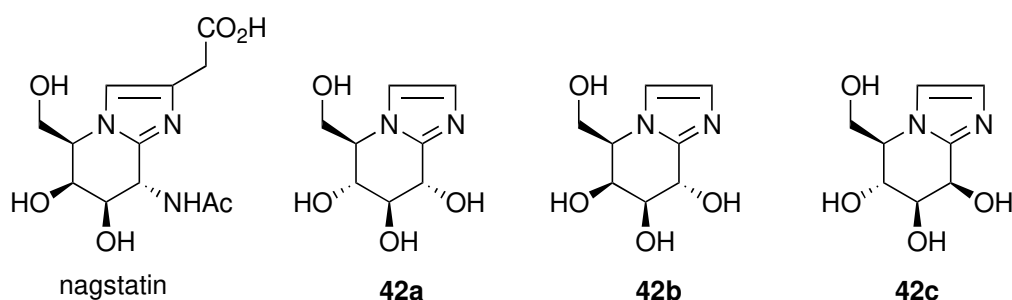


Figure 24: Structures of nagstatin and of glycoimidazoles **42a-c**

To conclude, despite disappointing results as TSA, glycoamidines still hold some interesting potential illustrated by their recent use as templates to generate microgels with hydrolytic activity. In addition, their convenient synthetic access from easily available glyconolactams and their simple exo- and endocyclic *N*-alkylation leading to improved glycosidase inhibition profiles make this class of compounds and their derivatives still worth investigating.

References

- (1) Wolfenden, R.; Lu, X.; Young, G. Spontaneous Hydrolysis of Glycosides. *J. Am. Chem. Soc.* **1998**, *120* (27), 6814–6815. <https://doi.org/10.1021/ja9813055>.
- (2) Pereira, D. M.; Valentão, P.; Andrade, P. B. Tuning Protein Folding in Lysosomal Storage Diseases: The Chemistry behind Pharmacological Chaperones. *Chemical Science* **2018**, *9* (7), 1740–1752. <https://doi.org/10.1039/C7SC04712F>.
- (3) Sánchez-Fernández, E. M.; García Fernández, J. M.; Ortiz Mellet, C. Glycomimetic-Based Pharmacological Chaperones for Lysosomal Storage Disorders: Lessons from Gaucher, G_{M1} - Gangliosidosis and Fabry Diseases. *Chemical Communications* **2016**, *52* (32), 5497–5515. <https://doi.org/10.1039/C6CC01564F>.
- (4) Krentz, A. J.; Bailey, C. J. Oral Antidiabetic Agents: Current Role in Type 2 Diabetes Mellitus. *Drugs* **2005**, *65* (3), 385–411. <https://doi.org/10.2165/00003495-200565030-00005>.
- (5) Chang, J.; Block, T. M.; Guo, J.-T. Antiviral Therapies Targeting Host ER Alpha-Glucosidases: Current Status and Future Directions. *Antiviral Research* **2013**, *99* (3), 251–260. <https://doi.org/10.1016/j.antiviral.2013.06.011>.
- (6) a) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. Recent Developments of Transition-State Analogue Glycosidase Inhibitors of Non-Natural Product Origin. *Chemical Reviews* **2002**, *102* (2), 515–554. <https://doi.org/10.1021/cr000433k>; b) Nash RJ, Kato A, Yu CY, Fleet GW. Iminosugars as therapeutic agents: recent advances and promising trends. *Future Med Chem.* 2011 Sep;*3*(12):1513-21. doi: 10.4155/fmc.11.117. PMID: 21882944; c) Asano N. Glycosidase inhibitors: update and perspectives on practical use. *Glycobiology.* 2003 Oct;*13*(10):93R-104R. doi: 10.1093/glycob/cwg090.

- Epub 2003 Jul 8. PMID: 12851286; d) Asano N. Naturally occurring iminosugars and related compounds: structure, distribution, and biological activity. *Curr Top Med Chem.* 2003;3(5):471-84. doi: 10.2174/1568026033452438. PMID: 12570862; e) Wadood A, Ghufuran M, Khan A, Azam SS, Jelani M, Uddin R. Selective glycosidase inhibitors: A patent review (2012-present). *Int J Biol Macromol.* 2018 May;111:82-91. doi: 10.1016/j.ijbiomac.2017.12.148. Epub 2018 Jan 2. PMID: 29305216; f) Conforti I, Marra A. Iminosugars as glycosyltransferase inhibitors. *Org Biomol Chem.* 2021 Jun 30;19(25):5439-5475. doi: 10.1039/d1ob00382h. Erratum in: *Org Biomol Chem.* 2021 Jun 18;; PMID: 33881114.
- (7) *Iminosugars: From Synthesis to Therapeutic Applications*; Compain, P., Martin, O. R., Eds.; J. Wiley: Chichester, West Sussex, England ; Hoboken, NJ, USA, 2007.
- (8) Nash, R. J.; Kato, A.; Yu, C.-Y.; Fleet, G. W. Iminosugars as Therapeutic Agents: Recent Advances and Promising Trends. *Future Medicinal Chemistry* 2011, 3 (12), 1513–1521. <https://doi.org/10.4155/fmc.11.117>.
- (9) a) Vocadlo, D. J.; Davies, G. J. Mechanistic Insights into Glycosidase Chemistry. *Curr Opin Chem Biol* 2008, 12 (5), 539–555. <https://doi.org/10.1016/j.cbpa.2008.05.010>; b)
- (10) a) Pauling, L. Nature of Forces between Large Molecules of Biological Interest*. *Nature* 1948, 161 (4097), 707–709. <https://doi.org/10.1038/161707a0>; b) Wicki J, Williams SJ, Withers SG. Transition-state mimicry by glycosidase inhibitors: a critical kinetic analysis. *J Am Chem Soc.* 2007 Apr 18;129(15):4530-1. doi: 10.1021/ja0707254. Epub 2007 Mar 27. PMID: 17385869; c) Gloster TM, Davies GJ. Glycosidase inhibition: assessing mimicry of the transition state. *Org Biomol Chem.* 2010 Jan 21;8(2):305-20. doi: 10.1039/b915870g. Epub 2009 Nov 5. PMID: 20066263; PMCID: PMC2822703; d) Gloster TM, Meloncelli P, Stick RV, Zechel D, Vasella A, Davies GJ. Glycosidase inhibition: an assessment of the binding of 18 putative transition-state mimics. *J Am Chem Soc.* 2007 Feb 28;129(8):2345-54. doi: 10.1021/ja066961g. Epub 2007 Feb 6. PMID: 17279749.
- (11) Wolfenden, R.; Snider, M. J. The Depth of Chemical Time and the Power of Enzymes as Catalysts. *Acc. Chem. Res.* 2001, 34 (12), 938–945. <https://doi.org/10.1021/ar000058i>.
- (12) Martin, A.; Arda, A.; Désiré, J.; Martin-Mingot, A.; Probst, N.; Sinaý, P.; Jiménez-Barbero, J.; Thibaudeau, S.; Blériot, Y. Catching Elusive Glycosyl Cations in a Condensed Phase with HF/SbF₅ Superacid. *Nature Chem* 2016, 8 (2), 186–191. <https://doi.org/10.1038/nchem.2399>.
- (13) Davies, G. J.; Ducros, V. M.-A.; Varrot, A.; Zechel, D. L. Mapping the Conformational Itinerary of β -Glycosidases by X-Ray Crystallography. *Biochemical Society Transactions* 2003, 31 (3), 523–527. <https://doi.org/10.1042/bst0310523>.
- (14) Marcelo, F.; He, Y.; Yuzwa, S. A.; Nieto, L.; Jiménez-Barbero, J.; Sollogoub, M.; Vocadlo, D. J.; Davies, G. D.; Blériot, Y. Molecular Basis for Inhibition of GH84 Glycoside Hydrolases by Substituted Azepanes: Conformational Flexibility Enables Probing of Substrate Distortion. *J. Am. Chem. Soc.* 2009, 131 (15), 5390–5392. <https://doi.org/10.1021/ja809776r>.
- (15) Tong, M. K.; Papandreou, G.; Ganem, B. Potent, Broad-Spectrum Inhibition of Glycosidases by an Amidine Derivative of D-Glucose. *J. Am. Chem. Soc.* 1990, 112 (16), 6137–6139. <https://doi.org/10.1021/ja00172a045>.
- (16) Ganem, B. Inhibitors of Carbohydrate-Processing Enzymes: Design and Synthesis of Sugar-Shaped Heterocycles. *Acc. Chem. Res.* 1996, 29 (7), 340–347. <https://doi.org/10.1021/ar9502184>.
- (17) Speciale, G.; Thompson, A. J.; Davies, G. J.; Williams, S. J. Dissecting Conformational Contributions to Glycosidase Catalysis and Inhibition. *Current Opinion in Structural Biology* 2014, 28, 1–13. <https://doi.org/10.1016/j.sbi.2014.06.003>.
- (18) Conchie, J.; Levvy, G. A. Inhibition of Glycosidases by Aldonolactones of Corresponding Configuration. *Biochemical Journal* 1957, 65 (2), 389–395. <https://doi.org/10.1042/bj0650389>.
- (19) Aly, A. A.; El-Din, A. M. N. Functionality of Amidines and Amidrazones. *Arkivoc* 2008, 2008 (1), 153–194. <https://doi.org/10.3998/ark.5550190.0009.106>.
- (20) Sinnott, M. L. Catalytic Mechanism of Enzymic Glycosyl Transfer. *Chem. Rev.* 1990, 90 (7), 1171–1202. <https://doi.org/10.1021/cr00105a006>.
- (21) Reese, E. T.; Parrish, F. W.; Ettliger, M. Nojirimycin and D-Glucono-1,5-lactone as Inhibitors of Carbohydrases. *Carbohydrate Res.* 1971, 18 (3), 381-388. [https://doi.org/10.1016/S0008-6215\(00\)80274-8](https://doi.org/10.1016/S0008-6215(00)80274-8).
- (22) Bird, P.; Dolphin, D. H.; Withers, S. G. The Synthesis of Protected 5-Azido-5-Deoxy- D -Glucononitriles as Precursors of Glycosidase Inhibitors. *Can. J. Chem.* 1990, 68 (2), 317–322. <https://doi.org/10.1139/v90-045>.
- (23) Papandreou, G.; Tong, M. K.; Ganem, B. Amidine, Amidrazone, and Amidoxime Derivatives of Monosaccharide Aldonolactams: Synthesis and Evaluation as Glycosidase Inhibitors. *J. Am. Chem. Soc.* 1993, 115 (25), 11682–11690. <https://doi.org/10.1021/ja00078a004>.
- (24) Yoon, H.; King, S. B.; Ganem, B. Synthesis of 1- β -Amino-Deoxynojirimycins: a New Family of

- Glucosidase Inhibitors. *Tetrahedron Lett.* **1991** 32 (49) 7199-7202. [https://doi.org/10.1016/0040-4039\(91\)80475-L](https://doi.org/10.1016/0040-4039(91)80475-L).
- (25) Blériot, Y.; Genre-Grandpierre, A.; Tellier, C. Synthesis of a Benzylamide Derived from D-Mannose. A Potent Mannosidase Inhibitor. *Tetrahedron Letters* **1994**, 35 (12), 1867–1870. [https://doi.org/10.1016/S0040-4039\(00\)73182-0](https://doi.org/10.1016/S0040-4039(00)73182-0).
- (26) Kanso, R.; Yancey, E. A.; Striegler, S. N-Benzylgalactonoamidines as Potent β -Galactosidase Inhibitors. *Tetrahedron* **2012**, 68 (1), 47–52. <https://doi.org/10.1016/j.tet.2011.10.048>.
- (27) Fan, Q.-H.; Striegler, S.; Langston, R. G.; Barnett, J. D. Evaluating N-Benzylgalactonoamidines as Putative Transition State Analogs for β -Galactoside Hydrolysis. *Org. Biomol. Chem.* **2014**, 12 (17), 2792–2800. <https://doi.org/10.1039/C4OB00153B>.
- (28) Fan, Q.-H.; Claunch, K. A.; Striegler, S. Structure–Activity Relationship of Highly Potent Galactonoamidine Inhibitors toward β -Galactosidase (*Aspergillus Oryzae*). *J. Med. Chem.* **2014**, 57 (21), 8999–9009. <https://doi.org/10.1021/jm501111y>.
- (29) Pickens, J. B.; Wang, F.; Striegler, S. Picomolar Inhibition of β -Galactosidase (Bovine Liver) Attributed to Loop Closure. *Bioorganic & Medicinal Chemistry* **2017**, 25 (20), 5194–5202. <https://doi.org/10.1016/j.bmc.2017.07.020>.
- (30) Pickens, J. B.; Striegler, S.; Fan, Q.-H. Arabinoamidine Synthesis and Its Inhibition toward β -Glucosidase (Sweet Almonds) in Comparison to a Library of Galactonoamidines. *Bioorganic & Medicinal Chemistry* **2016**, 24 (16), 3371–3377. <https://doi.org/10.1016/j.bmc.2016.04.069>.
- (31) Heck, M.-P.; Vincent, S. P.; Murray, B. W.; Bellamy, F.; Wong, C.-H.; Mioskowski, C. Cyclic Amidine Sugars as Transition-State Analogue Inhibitors of Glycosidases: Potent Competitive Inhibitors of Mannosidases. *J. Am. Chem. Soc.* **2004**, 126 (7), 1971–1979. <https://doi.org/10.1021/ja037822r>.
- (32) Tailford, L. E.; Offen, W. A.; Smith, N. L.; Dumon, C.; Morland, C.; Gratien, J.; Heck, M.-P.; Stick, R. V.; Blériot, Y.; Vasella, A.; Gilbert, H. J.; Davies, G. J. Structural and Biochemical Evidence for a Boat-like Transition State in β -Mannosidases. *Nat Chem Biol* **2008**, 4 (5), 306–312. <https://doi.org/10.1038/nchembio.81>.
- (33) Williams, S. J.; Hoos, R.; Withers, S. G. Nanomolar versus Millimolar Inhibition by Xylobiose-Derived Azasugars: Significant Differences between Two Structurally Distinct Xylanases. *J. Am. Chem. Soc.* **2000**, 122 (10), 2223–2235. <https://doi.org/10.1021/ja993805j>.
- (34) Varrot, A.; Tarling, C. A.; Macdonald, J. M.; Stick, R. V.; Zechel, D. L.; Withers, S. G.; Davies, G. J. Direct Observation of the Protonation State of an Imino Sugar Glycosidase Inhibitor upon Binding. *J. Am. Chem. Soc.* **2003**, 125 (25), 7496–7497. <https://doi.org/10.1021/ja034917k>.
- (35) Lindbäck, E.; López, Ó.; Fernández-Bolaños, J. G.; Sauer, S. P. A.; Bols, M. An Isofagomine Analogue with an Amidine at the Pseudoanomeric Position. *Org. Lett.* **2011**, 13 (11), 2908–2911. <https://doi.org/10.1021/ol200942g>.
- (36) Blériot, Y.; Dintinger, T.; Genre-Grandpierre, A.; Padrines, M.; Tellier, C. Inhibition of Glycosidases by Substituted Amidines. *Bioorganic & Medicinal Chemistry Letters* **1995**, 5 (22), 2655–2660. [https://doi.org/10.1016/0960-894X\(95\)00474-8](https://doi.org/10.1016/0960-894X(95)00474-8).
- (37) Horne, G.; Wilson, F. X.; Tinsley, J.; Williams, D. H.; Storer, R. Iminosugars Past, Present and Future: Medicines for Tomorrow. *Drug Discov. Today* **2011**, 16 (3–4), 107–118. <https://doi.org/10.1016/j.drudis.2010.08.017>.
- (38) Campbell, L. K.; Baker, D. E.; Campbell, R. K. Miglitol: Assessment of Its Role in the Treatment of Patients with Diabetes Mellitus. *Annals of Pharmacotherapy* **2000**, 34 (11), 1291-1301. <https://doi.org/10.1345/aph.19269>.
- (39) Lindbäck, E.; Lopéz, Ó.; Tobiesen, Å.; Fernández-Bolaños, J. G.; Sydnes, M. O. Sugar Hydrazide Imides: A New Family of Glycosidase Inhibitors. *Org. Biomol. Chem.* **2017**, 15 (41), 8709–8712. <https://doi.org/10.1039/C7OB01673E>.
- (40) Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. Sugar-Mimic Glycosidase Inhibitors: Natural Occurrence, Biological Activity and Prospects for Therapeutic Application. *Tetrahedron: Asymmetry* **2000**, 11 (8), 1645–1680. [https://doi.org/10.1016/S0957-4166\(00\)00113-0](https://doi.org/10.1016/S0957-4166(00)00113-0).
- (41) Haarr, M. B.; Lopéz, Ó.; Pejov, L.; Fernández-Bolaños, J. G.; Lindbäck, E.; Sydnes, M. O. 1,4-Dideoxy-1,4-Imino- D -Arabinitol (DAB) Analogues Possessing a Hydrazide Imide Moiety as Potent and Selective α -Mannosidase Inhibitors. *ACS Omega* **2020**, 5 (29), 18507–18514. <https://doi.org/10.1021/acsomega.0c02466>.
- (42) Oszczapowicz, J.; Krawczyk, W.; Lyzwinski, P. Amidines. Part 30. Influence of substitution at amino nitrogen atom on pKa values of N2-phenylacetamidines and N2-phenylformamidines. *J. Chem. Soc., Perkin Trans. 2* **1990**, 2, 311-314. <https://doi.org/10.1039/P29900000311>.
- (43) Hall, H. K. Jr Correlation of the Base Strengths of Amines. *J. Am. Chem. Soc.* **1957**, 79 (20), 5441-5444. <https://doi.org/10.1021/ja01577a030>.
- (44) Hoos, R.; Vasella, A.; Rupitz, K.; Withers, S. G. D-Glyconhydroximolactams strongly inhibit α -

- glycosidases. *Carbohydr. Res.* **1991**, *298* (4), 291-298. [https://doi.org/10.1016/S0008-6215\(96\)00320-5](https://doi.org/10.1016/S0008-6215(96)00320-5).
- (45) Ganem, B.; Papandreou, G. Mimicking the Glucosidase Transition State: Shape/Charge Considerations. *J. Am. Chem. Soc.* **1991**, *113* (23), 8984-8985. <https://doi.org/10.1021/ja00023a078>.
- (46) Pan, Y. T.; Kaushal, G. P.; Papandreou, G.; Ganem, B.; Elbein, A. D. D-Mannanolactam Amidrazone. A New Mannosidase Inhibitor That Also Inhibits the Endoplasmic Reticulum or Cytoplasmic Alpha-Mannosidase. *Journal of Biological Chemistry* **1992**, *267* (12), 8313-8318. [https://doi.org/10.1016/S0021-9258\(18\)42444-1](https://doi.org/10.1016/S0021-9258(18)42444-1).
- (47) Schedler, D. J. A.; Bowen, B. R.; Ganem, B. A Novel Inhibitor of Human α -L-fucosidase: Enantioselective Synthesis of L-Fucoamidrazone. *Tetrahedron Lett.* **1994**, *35* (23), 3845-3848. [https://doi.org/10.1016/S0040-4039\(00\)76682-2](https://doi.org/10.1016/S0040-4039(00)76682-2).
- (48) Notenboom, V.; Williams, S. J.; Hoos, R.; Withers, S. G.; Rose, D. R. Detailed Structural Analysis of Glycosidase/Inhibitor Interactions: Complexes of Cex from *Cellulomonas fimi* with Xylobiose-Derived Aza-Sugars. *Biochemistry* **2000**, *39* (38), 11553-11563. <https://doi.org/10.1021/bi0010625>.
- (49) Vasella, A.; Davies, G. J.; Böhm, M. Glycosidase Mechanisms. *Curr. Op. Chem. Biol.* **2002** *6* (5), 619-629. [https://doi.org/10.1016/S1367-5931\(02\)00380-0](https://doi.org/10.1016/S1367-5931(02)00380-0).
- (50) McCullough, A. K.; Dodson, M. L.; Lloyd, R. S. Initiation of Base Excision Repair: Glycosylase Mechanisms and Structures. *Annu. Rev. Biochem.* **1999**, *68* (1), 255-285. <https://doi.org/10.1146/annurev.biochem.68.1.255>.
- (51) Walsh, M. J.; Dodd, J. E.; Hautbergue, G. M. Ribosome-Inactivating Proteins: Potent Poisons and Molecular Tools. *Virulence* **2013**, *4* (8), 774-784. <https://doi.org/10.4161/viru.26399>.
- (52) Zhou, G.-C.; Parikh, S. L.; Tyler, P. C.; Evans, G. B.; Furneaux, R. H.; Zubkova, O. V.; Benjes, P. A.; Schramm, V. L. Inhibitors of ADP-Ribosylating Bacterial Toxins Based on Oxacarbenium Ion Character at Their Transition States. *J. Am. Chem. Soc.* **2004**, *126* (18), 5690-5698. <https://doi.org/10.1021/ja038159+>.
- (53) Schramm, V. L.; Horenstein, B. A.; Kline, P. C. Transition State Analysis and Inhibitor Design for Enzymatic Reactions. *Journal of Biological Chemistry* **1994**, *269* (28), 18259-18262. [https://doi.org/10.1016/S0021-9258\(17\)32294-9](https://doi.org/10.1016/S0021-9258(17)32294-9).
- (54) Boutellier, M.; Horenstein, B. A.; Semenyaka, A.; Schramm, V. L.; Ganem, B. Amidrazone Analogs of D-Ribofuranose as Transition-State Inhibitors of Nucleoside Hydrolase. *Biochemistry* **1994**, *33* (13), 3994-4000. <https://doi.org/10.1021/bi00179a028>.
- (55) Deng, H.; Chan, A. W.-Y.; Bagdassarian, C. K.; Estupiñán, B.; Ganem, B.; Callender, R. H.; Schramm, V. L. Trypanosomal Nucleoside Hydrolase. Resonance Raman Spectroscopy of a Transition-State Inhibitor Complex. *Biochemistry* **1996**, *35* (19), 6037-6047. <https://doi.org/10.1021/bi9526544>.
- (56) Witte, J. F.; Bray, K. E.; Thornburg, C. K.; McClard, R. W. 'Irreversible' Slow-Onset Inhibition of Orotate Phosphoribosyltransferase by an Amidrazone Phosphate Transition-State Mimic. *Bioorganic & Medicinal Chemistry Letters* **2006**, *16* (23), 6112-6115. <https://doi.org/10.1016/j.bmcl.2006.08.109>.
- (57) von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Van Phan, T.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. Rational Design of Potent Sialidase-Based Inhibitors of Influenza Virus Replication. *Nature* **1993**, *363* (6428), 418-423. <https://doi.org/10.1038/363418a0>.
- (58) Fotsch, C. H.; Wong, C.-H. Synthesis of a Guanidino-Sugar as a Glycosyl Cation Mimic. *Tetrahedron Letters* **1994**, *35* (21), 3481-3484. [https://doi.org/10.1016/S0040-4039\(00\)73215-1](https://doi.org/10.1016/S0040-4039(00)73215-1).
- (59) Chan, A. W.-Y.; Ganem, B. Guanidine Analogs of a Deoxysugar. *Tetrahedron Letters* **1995**, *36* (6), 811-814. [https://doi.org/10.1016/0040-4039\(94\)02392-O](https://doi.org/10.1016/0040-4039(94)02392-O).
- (60) Lehmann, J.; Rob, B. Cyclische Guanidinium-Ionen als Glycosylkation-Analoga sind kompetitive und nichtkompetitive Inhibitoren für Glycosidhydrolasen. *Liebigs Ann. Chem.* **1994**, *1994* (8), 805-809. <https://doi.org/10.1002/jlac.199419940808>.
- (61) Grein, F.; Deslongchamps, P. The Anomeric and Reverse Anomeric Effect. A Simple Energy Decomposition Model for Acetals and Protonated Acetals. *Can. J. Chem.* **1992**, *70* (5), 1562-1572. <https://doi.org/10.1139/v92-192>.
- (62) a) Le, V.-D.; Wong, C.-H. Synthesis of 2-Substituted Polyhydroxytetrahydropyrimidines (*N*-Hydroxy Cyclic Guanidino-Sugars): Transition-State Mimics of Enzymatic Glycosidic Cleavage. *J. Org. Chem.* **2000**, *65* (8), 2399-2409. <https://doi.org/10.1021/jo9915574>; b) Jeong, J.-H.; Murray, B. W.; Takayama S.; Wong C.-H. Cyclic Guanidino-Sugars with Low pKa as Transition-State Analog Inhibitors of Glycosidases: Neutral Instead of Charged Species Are the Active Forms. *J. Am. Chem.*

- Soc.* **1996**, *118* (18), 4227-4234. <https://doi.org/10.1021/ja951602z>
- (63) Marra, A.; Zelli, R. Synthesis and Biological Properties of Imino-Disaccharides and -Oligosaccharides. In *Carbohydrate Chemistry*; Pilar Rauter, A., Lindhorst, T., Queneau, Y., Eds.; Royal Society of Chemistry: Cambridge, **2017**; Vol. 43, pp 1–70. <https://doi.org/10.1039/9781788010641-00001>.
- (64) Goering, B. K.; Li, J.; Ganem, B. Aminocyclopentitols from fulvenes: syntheses of (+)-trehazolin and the pentasubstituted cyclopentane of keruffaride. *Tetrahedron Lett.* **1995**, *36* (49) 8905-8908. [https://doi.org/10.1016/0040-4039\(95\)01927-A](https://doi.org/10.1016/0040-4039(95)01927-A).
- (65) Knapp, S.; Purandare, A.; Rupitz, K.; Withers, S. G. A (1.Fwdarw.4)-"trehazolid" Glucosidase Inhibitor with Aglycon Selectivity. *J. Am. Chem. Soc.* **1994**, *116* (16), 7461–7462. <https://doi.org/10.1021/ja00095a081>.
- (66) Knapp, S.; Choe, Y. H.; Reilly, E. Amidine Pseudodisaccharides. *Tetrahedron Letters* **1993**, *34* (28), 4443–4446. [https://doi.org/10.1016/0040-4039\(93\)88054-M](https://doi.org/10.1016/0040-4039(93)88054-M).
- (67) Blériot, Y.; Dintinger, T.; Guillo, N.; Tellier, C. Synthesis of an Amidine Pseudo-(1-->6)-Dimannoside and Evaluation as a Glycosidase Inhibitor. *Tetrahedron Letters* **1995**, *36* (20), 5175-5178.
- (68) Lehmann, J.; Rob, B. Two Cationic Analogues of Disaccharide Heterolysis. Potential Epitopes for Preparing Antibodies with Saccharidase Activity. *Tetrahedron: Asymmetry* **1994**, *5* (11), 2255–2260. [https://doi.org/10.1016/S0957-4166\(00\)86302-8](https://doi.org/10.1016/S0957-4166(00)86302-8).
- (69) Guo, W.; Hiratake, J.; Ogawa, K.; Yamamoto, M.; Ma, S.-J.; Sakata, K. β -D-Glycosylamidines: Potent, Selective, and Easily Accessible β -Glycosidase Inhibitors. *Bioorg. Med. Chem. Lett.* **2001**, *11* (4), 467-470. [https://doi.org/10.1016/S0960-894X\(00\)00706-X](https://doi.org/10.1016/S0960-894X(00)00706-X).
- (70) Inoue, K.; Hiratake, J.; Mizutani, M.; Takada, M.; Yamamoto, M.; Sakata, K. β -Glycosylamidine as a Ligand for Affinity Chromatography Tailored to the Glycon Substrate Specificity of β -Glycosidases. *Carbohydrate Research* **2003**, *338* (14), 1477–1490. [https://doi.org/10.1016/S0008-6215\(03\)00201-5](https://doi.org/10.1016/S0008-6215(03)00201-5).
- (71) Kato, E.; Sasaki, T.; Ueda, M. Affinity Purification and Characterization of a Key Enzyme Responsible for Circadian Rhythmic Control of Nyctinasty in *Lespedeza Cuneata* L. *Bioorganic & Medicinal Chemistry* **2008**, *16* (8), 4600–4616. <https://doi.org/10.1016/j.bmc.2008.02.035>.
- (72) Saino, H.; Shimizu, T.; Hiratake, J.; Nakatsu, T.; Kato, H.; Sakata, K.; Mizutani, M. Crystal Structures of β -Primeverosidase in Complex with Disaccharide Amidine Inhibitors. *Journal of Biological Chemistry* **2014**, *289* (24), 16826–16834. <https://doi.org/10.1074/jbc.M114.553271>.
- (73) Aguilar-Moncayo, M.; García-Moreno, M. I.; Trapero, A.; Egido-Gabás, M.; Llebaria, A.; García Fernández, J. M.; Ortiz Mellet, C. Bicyclic (Galacto)Nojirimycin Analogues as Glycosidase Inhibitors: Effect of Structural Modifications in Their Pharmacological Chaperone Potential towards β -Glucocerebrosidase. *Org. Biomol. Chem.* **2011**, *9* (10), 3698-3713. <https://doi.org/10.1039/c1ob05234a>.
- (74) Brumshtein, B.; Aguilar-Moncayo, M.; García-Moreno, M. I.; Ortiz Mellet, C.; García Fernández, J. M.; Silman, I.; Shaaltiel, Y.; Aviezer, D.; Sussman, J. L.; Futerman, A. H. 6-Amino-6-Deoxy-5,6-Di- *N*-(*N*'-Octyliminomethylidene)Nojirimycin: Synthesis, Biological Evaluation, and Crystal Structure in Complex with Acid β -Glucosidase. *ChemBioChem* **2009**, *10* (9), 1480–1485. <https://doi.org/10.1002/cbic.200900142>.
- (75) Kooij, R.; Branderhorst, H. M.; Bonte, S.; Wieclawska, S.; Martin, N. I.; Pieters, R. J. Glycosidase Inhibition by Novel Guanidinium and Urea Iminosugar Derivatives. *Med. Chem. Commun.* **2013**, *4* (2), 387–393. <https://doi.org/10.1039/C2MD20343J>.
- (76) Stevenson, J. D.; Thomas, N. R. Catalytic Antibodies and Other Biomimetic Catalysts. *Nat. Prod. Rep.* **2000**, *17* (6), 535–577. <https://doi.org/10.1039/b006389o>.
- (77) Mader, M. M.; Bartlett, P. A. Binding Energy and Catalysis: The Implications for Transition-State Analogs and Catalytic Antibodies. *Chem. Rev.* **1997**, *97* (5), 1281–1302. <https://doi.org/10.1021/cr960435y>.
- (78) Yu, J.; Hsieh, L. C.; Kochersperger, L.; Yonkovich, S.; Stephans, J. C.; Gallop, M. A.; Schultz, P. G. Progress toward an Antibody Glycosidase. *Angew. Chem. Int. Ed. Engl.* **1994**, *33* (3), 339–341. <https://doi.org/10.1002/anie.199403391>.
- (79) Suga, H.; Tanimoto, N.; Sinskey, A. J.; Masamune, S. Glycosidase Antibodies Induced to a Half-Chair Transition-State Analog. *J. Am. Chem. Soc.* **1994**, *116* (24), 11197–11198. <https://doi.org/10.1021/ja00103a061>.
- (80) Blériot, Y.; Genre-Grandpierre, A.; Imbert, A.; Tellier, C. Structure and Conformation of Mannoamidines by Nmr and Molecular Modeling: Are They Good Transition State Mimics? *Journal of Carbohydrate Chemistry* **1996**, *15* (8), 985–1000. <https://doi.org/10.1080/07328309608005704>.
- (81) Golinelli-Pimpaneau, B.; Goncalves, O.; Dintinger, T.; Blanchard, D.; Knossow, M.; Tellier, C.

- Structural Evidence for a Programmed General Base in the Active Site of a Catalytic Antibody. *Proceedings of the National Academy of Sciences* **2000**, *97* (18), 9892–9895. <https://doi.org/10.1073/pnas.97.18.9892>.
- (82) Li, X.; Zangiabadi, M.; Zhao, Y. Molecularly Imprinted Synthetic Glucosidase for the Hydrolysis of Cellulose in Aqueous and Nonaqueous Solutions. *J. Am. Chem. Soc.* **2021**, *143* (13), 5172–5181. <https://doi.org/10.1021/jacs.1c01352>.
- (83) Danby, P. M.; Withers, S. G. Glycosyl Cations versus Allylic Cations in Spontaneous and Enzymatic Hydrolysis. *J. Am. Chem. Soc.* **2017**, *139* (31), 10629–10632. <https://doi.org/10.1021/jacs.7b05628>.
- (84) Jongkees, S. A. K.; Withers, S. G. Unusual Enzymatic Glycoside Cleavage Mechanisms. *Acc. Chem. Res.* **2014**, *47* (1), 226–235. <https://doi.org/10.1021/ar4001313>.
- (85) Namchuk, M. N.; McCarter, J. D.; Becalski, A.; Andrews, T.; Withers, S. G. The Role of Sugar Substituents in Glycoside Hydrolysis. *J. Am. Chem. Soc.* **2000**, *122* (7), 1270–1277. <https://doi.org/10.1021/ja992044h>.
- (86) Pedersen, C. M.; Bols, M. On the Nature of the Electronic Effect of Multiple Hydroxyl Groups in the 6-Membered Ring – the Effects Are Additive but Steric Hindrance Plays a Role Too. *Org. Biomol. Chem.* **2017**, *15* (5), 1164–1173. <https://doi.org/10.1039/C6OB02427K>.
- (87) Duo, T.; Robinson, K.; Greig, I. R.; Chen, H.-M.; Patrick, B. O.; Withers, S. G. Remarkable Reactivity Differences between Glucosides with Identical Leaving Groups. *J. Am. Chem. Soc.* **2017**, *139* (44), 15994–15999. <https://doi.org/10.1021/jacs.7b09645>.
- (88) Wang, B.; Olsen, J. I.; Laursen, B. W.; Navarro Poulsen, J. C.; Bols, M. Determination of Protonation States of Iminosugar–Enzyme Complexes Using Photoinduced Electron Transfer. *Chem. Sci.* **2017**, *8* (11), 7383–7393. <https://doi.org/10.1039/C7SC01540B>.
- (89) Pickens, J. B.; Mills, L. G.; Wang, F.; Striegler, S. Evaluating Hydrophobic Galactonoamidines as Transition State Analogs for Enzymatic β -Galactoside Hydrolysis. *Bioorganic Chemistry* **2018**, *77*, 144–151. <https://doi.org/10.1016/j.bioorg.2018.01.012>.
- (90) Sharma, B.; Pickens, J. B.; Striegler, S.; Barnett, J. D. Biomimetic Glycoside Hydrolysis by a Microgel Templated with a Competitive Glycosidase Inhibitor. *ACS Catal.* **2018**, *8* (9), 8788–8795. <https://doi.org/10.1021/acscatal.8b02440>.
- (91) Striegler, S.; Sharma, B.; Orizu, I. Microgel-Catalyzed Hydrolysis of Nonactivated Disaccharides. *ACS Catal.* **2020**, *10* (24), 14451–14456. <https://doi.org/10.1021/acscatal.0c03401>.
- (92) Thomas, K.; Haapalainen, A. M.; Burgos, E. S.; Evans, G. B.; Tyler, P. C.; Gulab, S.; Guan, R.; Schramm, V. L. Femtomolar Inhibitors Bind to 5'-Methylthioadenosine Nucleosidases with Favorable Enthalpy and Entropy. *Biochemistry* **2012**, *51* (38), 7541–7550. <https://doi.org/10.1021/bi3009938>.
- (93) Singh, V.; Evans, G. B.; Lenz, D. H.; Mason, J. M.; Clinch, K.; Mee, S.; Painter, G. F.; Tyler, P. C.; Furneaux, R. H.; Lee, J. E.; Howell, P. L.; Schramm, V. L. Femtomolar Transition State Analogue Inhibitors of 5'-Methylthioadenosine/S-Adenosylhomocysteine Nucleosidase from *Escherichia Coli*. *Journal of Biological Chemistry* **2005**, *280* (18), 18265–18273. <https://doi.org/10.1074/jbc.M414472200>.
- (94) Lee, J. E.; Singh, V.; Evans, G. B.; Tyler, P. C.; Furneaux, R. H.; Cornell, K. A.; Riscoe, M. K.; Schramm, V. L.; Howell, P. L. Structural Rationale for the Affinity of Pico- and Femtomolar Transition State Analogues of *Escherichia Coli* 5'-Methylthioadenosine/S-Adenosylhomocysteine Nucleosidase. *Journal of Biological Chemistry* **2005**, *280* (18), 18274–18282. <https://doi.org/10.1074/jbc.M414471200>.
- (95) Gloster, T. M.; Davies, G. J. Glycosidase Inhibition: Assessing Mimicry of the Transition State. *Org. Biomol. Chem.* **2010**, *8* (2), 305–320. <https://doi.org/10.1039/B915870G>.
- (96) Aoyama, T.; Naganawa, H.; Suda, H.; Uotani, K.; Aoyagi, T.; Takeuchi, T. The structure of Nagstatin, a New Inhibitor of N-Acetyl- β -D-Glucosaminidase. *The Journal of Antibiotics* **1992**, *45* (9), 1557–1558.
- (97) Terinek, M.; Vasella, A. Synthesis of N-Acetylglucosamine-Derived Nagstatin Analogues and Their Evaluation as Glycosidase Inhibitors. *Helvetica Chimica Acta* **2005**, *88* (1), 10–22.
- (98) Terinek, M.; Vasella, A. Synthesis and Evaluation of Two Mannosamine-Derived Lactone-Type Inhibitors of Snail β -Mannosidase. *Tetrahedron: Asymmetry* **2005**, *16* (2), 449–469. <https://doi.org/10.1016/j.tetasy.2004.11.068>.
- (99) Males, A.; Speciale, G.; Williams, S. J.; Davies, G. J. Distortion of Mannoimidazole Supports a $B_{2.5}$ Boat Transition State for the Family GH125 α -1,6-Mannosidase from *Clostridium Perfringens*. *Org. Biomol. Chem.* **2019**, *17* (34), 7863–7869. <https://doi.org/10.1039/C9OB01161G>.
- (100) Voadlo, D. J.; Davies, G. J. Mechanistic Insights into Glycosidase Chemistry. *Current Opinion in Chemical Biology* **2008**, *12* (5), 539–555. <https://doi.org/10.1016/j.cbpa.2008.05.010>.

- (101) Gloster, T. M.; Vocadlo, D. J. Developing Inhibitors of Glycan Processing Enzymes as Tools for Enabling Glycobiology. *Nat Chem Biol* **2012**, *8* (8), 683–694.
<https://doi.org/10.1038/nchembio.1029>.
- (102) Heightman, T. D.; Vasella, A. T. Recent Insights into Inhibition, Structure, and Mechanism of Configuration-Retaining Glycosidases. *Angew. Chem. Int. Ed.* **1999**, *38*, 750-770.
- (103) Varrot, A.; Schülein, M.; Pipelier, M.; Vasella, A.; Davies, G. J. Lateral Protonation of a Glycosidase Inhibitor. Structure of the *Bacillus Agaradhaerens* Cel5A in Complex with a Cellobiose-Derived Imidazole at 0.97 Å Resolution. *J. Am. Chem. Soc.* **1999**, *121* (11), 2621–2622.
<https://doi.org/10.1021/ja984238n>.