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Iminosugars

Influence of Side Chain Conformation on the Activity of Glycosidase Inhibitors

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Abstract: Substrate side chain conformation impacts reactivity during glycosylation and glycoside hydrolysis and is restricted by many glycosidases and glycosyltransferases during catalysis. We show that the side chains of *gluco* and *manno* iminosugars can be restricted to predominant conformations by strategic installation of a methyl group. Glycosidase inhibition studies reveal that iminosugars with the *gauche,gauche* side chain conformations are 6- to 10-fold more potent than isosteric compounds with the *gauche,trans* conformation; a *manno*-configured iminosugar with the *gauche,gauche* conformation is a 27-fold better inhibitor than 1-deoxymannojirimycin. The results are discussed in terms of the energetic benefits of preorganization, particularly when in synergy with favorable hydrophobic interactions. The demonstration that inhibitor side chain preorganization can favorably impact glycosidase inhibition paves the way for improved inhibitor design through conformational preorganization.

The biosynthesis and degradation of glycosides are catalyzed by two classes of enzymes: glycosyltransferases (GTs), which install glycosidic bonds, and glycoside hydrolases (GHs), or glycosidases, which cleave them. Inhibition of GTs or GHs has applications in the treatment of many diseases, including bacterial infection, viral infection, diabetes, cancer and genetic disorders.[1,2] Inhibitor design is often based on mimicry of the substrate conformation and/or of the positively charged transition state.[3–6] This is illustrated by the drugs: miglitol (Figure 1), a GH inhibitor used to treat type II diabetes by targeting intestinal α-glucosidases, is an iminosugar whose conjugate acid mimics the positive charge at the transition state for the enzymatic glucoside hydrolysis.[3] Zanamivir, a drug used to treat influenza by inhibiting a neuraminidase, on the other hand was designed to mimic the conformation of the natural substrate at the transition state for hydrolysis.[3] Finally, 4′-deaza-1′-aza-2′-deoxy-9-methylene Immucillin-G (DADMe-Immucillin-G), a purine nucleoside phosphorylase inhibitor with potential for the treatment for malaria, is a transition state analog that, like miglitol, is protonated and positively charged when bound to the enzyme.[4]

In chemical glycosylation and glycoside hydrolysis, the reactivity of glycosidic bond-forming or bond-breaking events is influenced by the carbohydrate side chain conformation: *gauche,gauche* (*gg*), *gauche,trans* (*gt*), or *trans,-gauche* (*tg*).[7–10] The relative population of these conformers in aqueous solution is configuration-dependent with D-glucopyranosides and D-mannopyranosides being ≈50:50 mixtures of *gg* and *gt* conformers at equilibrium (Scheme 1).[11,12]

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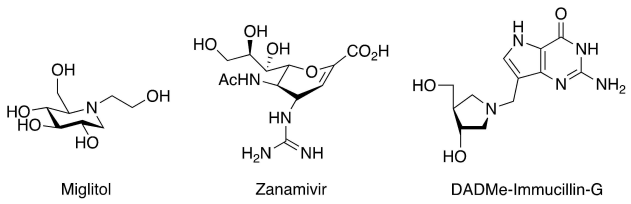
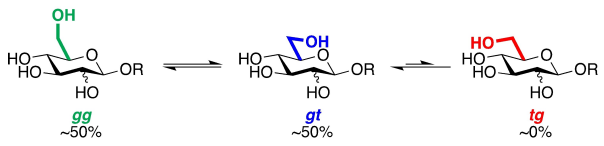


Figure 1. Structures of miglitol, zanamivir, and DADMe-Immucillin-G. Ac, acetyl.



Scheme 1. Approximate aqueous solution populations of three staggered conformations of D-glucopyranosides (equatorial OH at C2) and D-mannopyranosides (axial OH at C2).

Seminal studies by Bols and co-workers demonstrated the influence of side chain conformation on glycoside reactivity by evaluation of spontaneous hydrolysis at pH 6.5 of a series of conformationally locked dinitrophenyl glucopyranosides (Figure 2).[8] The rate of glycoside hydrolysis was found to be *gg* > *gt* > *tg*, as corroborated in the *galacto* series by our laboratory.[9] In addition, our recent findings have revealed that the influence of side chain conformation on reactivity is exploited by nature, where many GHs and GTs bind their ligands with specific side chain conformations.[13,14] For example, >80% of glucosidases restrict the side chain of glucose in their active sites to the *gg* conformation: considering the ≈50:50 unbound population of *gg*:*gt* conformers, these enzymes appear to have evolved to take the advantage of the substrate side chain conformation to gain additional transition state stabilization.[13] Davies and co-workers studied the GH family 1 β-glucosidase from *Thermotoga maritima* (*Tm*GH1) and found that the conformationally rigid castanospermine **1** (Figure 3) is a four-fold better inhibitor than 1-deoxynojirimycin **2**, whose side chain adopts the *gg* conformation in complex with *Tm*GH1 (Figure 4a),[5,15,16] suggesting that nature has already benefitted from the concept of side chain conformational control to evolve improved inhibitors.[13] Collectively, these studies suggest that preorganization of inhibitor side chain conformation can be used to influence their activity.

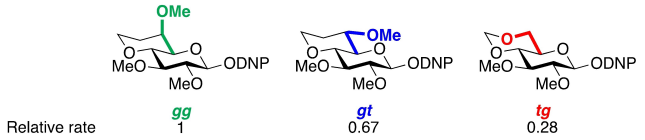


Figure 2. Relative rates of spontaneous hydrolysis of dinitrophenyl glycosides. DNP, 2,4-dinitrophenyl.

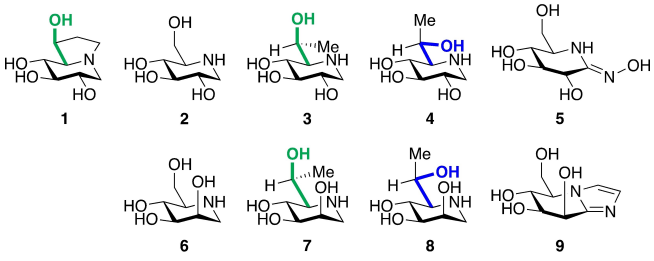


Figure 3. Structures of castanospermine **1**, 1-deoxynojirimycin **2**, glucohydroximolactam **5**, 1-deoxymannojirimycin **6**, mannoimidazole **9**, and the targeted inhibitors **3**, **4**, **7** and **8**.

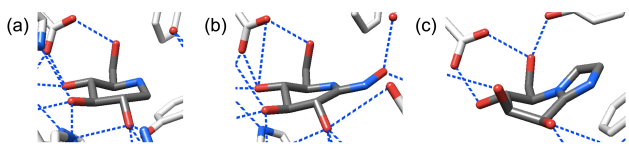
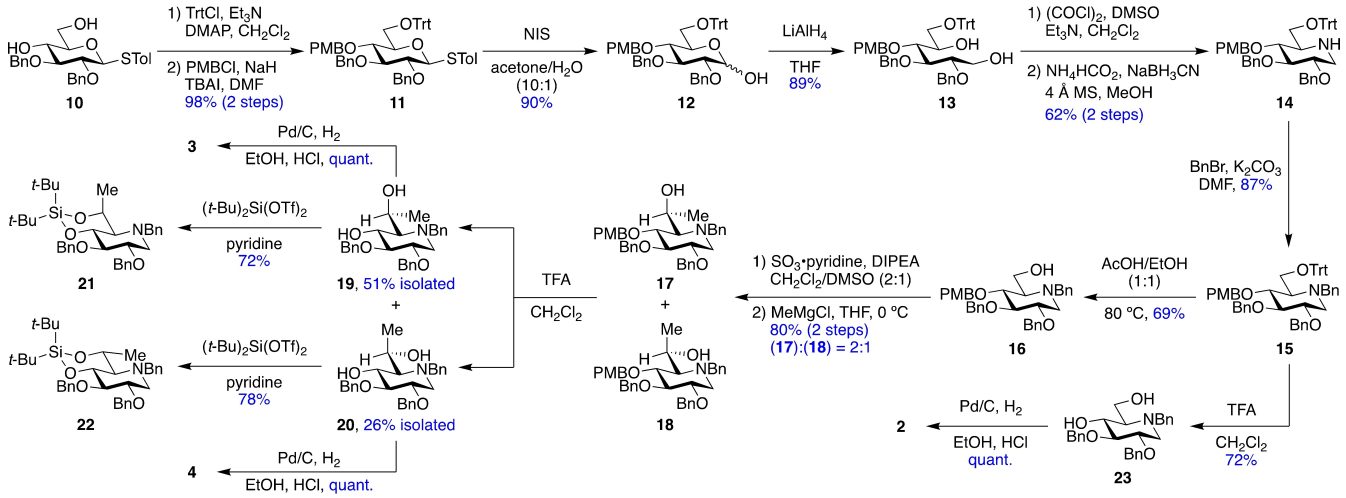


Figure 4. Partial X-ray crystal structures of a) *Tm*GH1 in complex with **2** (PDB ID: 2 J77), b) *Ss*GH1 in complex with **5** (PDB ID: 1UWU), and c) *Bt*Man2A in complex with **9** (PDB ID: 2VMF).

Following established principles for the restriction of side chain conformation,[17,18] to investigate whether side chain preorganization can influence inhibitor potency, we designed two derivatives (**3** and **4**, Figure 3) of 1-deoxynojirimycin **2** carrying a methyl group at C6. In addition to *Tm*GH1, whose crystal structure in complex with **2** is known (Figure 4a),[5] we also selected a GH family 1 β-glycosidase from *Sulfolobus solfataricus* (*Ss*GH1) for our studies on these compounds: X-ray studies (Figure 4b) indicate that glucohydroximolactam-type inhibitor **5** adopts the *gg* side chain conformation when bound to this enzyme.[19,20] Additionally, we designed two *manno*-configured derivatives (**7** and **8**) of 1-deoxymannojirimycin **6**, and a GH family 2 β-mannosidase from *Bacteroides thetaiotaomicron* (*Bt*Man2A) was selected for studies on this series of compounds: the crystal structure of the mannoimidazole **9**:*Bt*Man2A complex reveals the side chain of **9** to be bound in the *gg* conformation (Figure 4c).[21] Compound **3** with the L-*glycero*-D-*gluco* configuration and compound **7** with the L-*glycero*-D-*manno* configuration, whose side chains were predicted to predominantly adopt the *gg* conformation,[17,18] were anticipated to be better inhibitors of the respective glycosidases than their respective isomers **4** and **8** with the *gt* conformation of their side chains.

The synthesis of compounds **2**–**4** commenced with known[22,23] intermediate **10** (Scheme 2). Selective tritylation of O6, followed by protection of O4 with the *p*-methoxybenzyl (PMB) group provided the fully protected sugar **11** in 98% yield. Thioglycoside **11** was hydrolyzed to hemiacetal **12** in 90% yield using *N*-iodosuccinimide (NIS) in wet acetone. Reduction of **12** with lithium aluminum hydride afforded diol **13** in 89% yield. Swern oxidation of **13**, followed by double reductive amination[24] afforded iminosugar **14** in 62% yield. The basic amine in **14** was protected with a benzyl group to form intermediate **15**, and then selective cleavage of the trityl ether with a 1:1 mixture of acetic acid and ethanol at 80 °C provided alcohol **16** in 60% yield over two steps. Parikh-Doering oxidation[25] of **16** and immediate treatment with methyl magnesium chloride in THF at 0 °C furnished the C6-methyl-substituted products **17** and **18** as an inseparable 2:1 mixture in 80% yield over two steps. Cleavage of the PMB ethers in **17** and **18** was achieved with trifluoroacetic acid (TFA) in dichloromethane to give diol **19** in 51% isolated yield and isomer **20** in 26% yield. To determine the configuration at C6, compounds **19** and **20** were converted to rigid bicyclic derivatives **21** in 72% yield and **22** in 78% yield, respectively, by installation of the 4,6-*O*-di-*tert*-butylsilylene acetal group. Bicyclic compound **21** displayed a $^{3}J_{H5,H6}$ of 5.0 Hz and nuclear Overhauser effect (NOE) correlations of H4 with the methyl group, a *tert*-butyl group and H2, indicative of an axial methyl group and the L-*glycero*-D-*gluco* configuration. Compound **22** had a $^{3}J_{H5,H6}$ of 9.5 Hz and NOE correlations of H4 with H6, H2 and a *tert*-butyl group, indicating an equatorial methyl group and the D-*glycero*-D-*gluco* configuration. Diols **19** and **20** were subjected to hydrogenolysis to provide iminosugars **3** and **4** as hydrochloride (HCl) salts in quantitative yields. The L-*glycero*-D-*gluco* isomer **3** had an NOE correlation between the methyl group and H5 and a

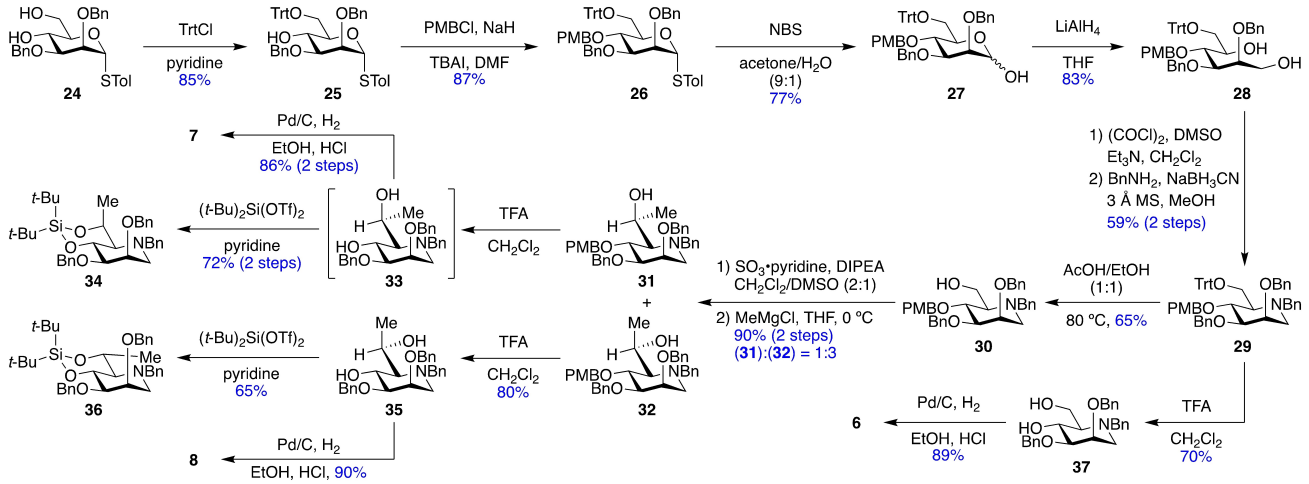


Scheme 2. Synthesis of *gluco* iminosugars **2**–**4**. Bn, benzyl; Tol, *p*-tolyl; Trt, triphenylmethyl; DMAP, 4-(dimethylamino)pyridine; PMB, *p*-methoxybenzyl; TBAI, tetrabutylammonium iodide; DMF, *N*,*N*-dimethylformamide; NIS, *N*-iodosuccinimide; DMSO, dimethyl sulfoxide; MS, molecular sieves; DIPEA, *N*,*N*-diisopropylethylamine; TFA, trifluoroacetic acid; Tf, trifluoromethanesulfonyl.

$^{3}J_{H5,H6}$ of 2.3 Hz consistent with the predicted predominant *gg* conformation, while the D-*glycero*-D-*gluco* isomer **4** had an NOE correlation between the methyl group and H4 and a $^{3}J_{H5,H6}$ of 3.3 Hz indicative of the anticipated predominant *gt* conformation. The HCl salts of **3** and **4** were also converted to the free bases, whose side chain conformations were determined in the same manner to be predominantly *gg* and *gt*, respectively (Figure S1). In parallel, intermediate **15** was treated with TFA to form diol **23**, which was debenzylated to afford 1-deoxynojirimycin **2**[26] in 72 % yield.

The synthesis and establishment of relative configuration and side chain conformation of *manno* iminosugars **6**–**8** were accomplished analogously, starting with known[27] diol **24** (Scheme 3). Alcohol **30** was oxidized and then reacted with methyl magnesium chloride in THF at 0 °C to give the separable C6-methyl-substituted products **31** and **32**.[28] Compound **31** was used to generate iminosugar **7** as an HCl salt via deprotection of the PMB group and hydrogenolysis in 86 % yield over two steps, while compound **32** was converted to the HCl salt of iminosugar **8** in 72 % yield. As expected, the L-*glycero*-D-*manno* iminosugar **7** showed an NOE correlation between the methyl group and H5 and a $^{3}J_{H5,H6}$ of 3.1 Hz, consistent with the predominant *gg* conformation, while the D-*glycero*-D-*manno* isomer **8** had an NOE correlation between the methyl group and H4 and a $^{3}J_{H5,H6}$ of 3.5 Hz, indicative of the *gt* side chain conformation. The HCl salts of **7** and **8** were also converted to their conjugate bases, whose side chain conformations were assigned analogously as *gg* and *gt*, respectively (Figure S2). Finally, intermediate **29** was used to prepare 1-deoxymannojirimycin **6**[29] in 62 % yield.

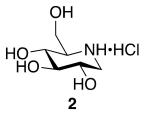
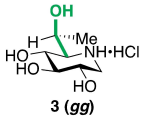
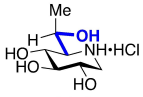
We evaluated the inhibition potency of these six compounds against the selected glycosidases by determining inhibition constants (K_i) of *gluco* iminosugars **2**–**4** for



Scheme 3. Synthesis of *manno* iminosugars **6**–**8**. NBS, *N*-bromosuccinimide.

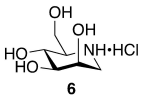
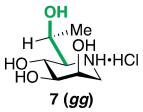
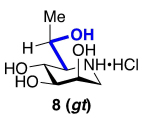
*Tm*GH1 and *Ss*GH1 (Table 1) and of *manno* compounds **6**–**8** for *Bt*Man2A (Table 2). Gratifyingly, a consistent pattern emerged according to which iminosugars **3** and **7**, with the predominant *gg* side chain conformation, are between six- and ten-fold better inhibitors than the isosteric **4** and **8**, respectively, with the enforced *gt* conformation validating the design hypotheses that side chain conformation can be used to influence activity and that the *gg* conformation is optimal. We attribute the weaker activity of the *gluco* isomer **3** with the *gg* conformation relative to the unrestricted 1-deoxynojirimycin **2** against the two glucosidase enzymes (Table 1) to an unfavorable steric or hydrophobic interaction of the conformation-controlling methyl group with the active site. In contrast, the *manno* isomer **7** with the *gg* conformation is a significantly better inhibitor of the mannosidase tested (Table 2) than 1-deoxymannojirimycin **6** itself, reflecting a synergistic combination of the optimal side chain conformation with a favorable hydrophobic or other interaction of the added methyl group with the active site.

Table 1: Inhibition constants (K_i) of inhibitors **2**–**4** for *Tm*GH1 and *Ss*GH1.

Compound	*Tm*GH1	*Ss*GH1
 **2**	5.4 ± 0.5 μM[a]	3.1 ± 0.2 μM
 **3 (*gg*)**	16 ± 2 μM	17 ± 1 μM
 **4 (*gt*)**	135 ± 11 μM	105 ± 10 μM

[a] Reported $K_i = 3.8$ μM with 2,4-dinitrophenyl β-D-glucopyranoside as a substrate.[15]

Table 2: Inhibition constants (K_i) of inhibitors **6**–**8** for *Bt*Man2A.

Compound	*Bt*Man2A
 **6**	38 ± 5 mM[a]
 **7 (*gg*)**	1.4 ± 0.2 mM
 **8 (*gt*)**	14 ± 1 mM

[a] Reported $K_i = 33$ mM with 2,4-dinitrophenyl β-D-mannopyranoside as a substrate.[21]

The weaker potency of the isomers **4** and **8**, whose side chains are restricted to the *gt* conformation, likely arises from the greater penalty that must be paid for the enzymes to force their side chains to adopt their higher energy *gg* conformations, and/or from a reduced contribution to binding by their ground state *gt* conformations. The *manno* isomer **8** is nevertheless a stronger inhibitor than 1-deoxymannojirimycin **6**, indicating that the methyl group in **8** must afford a favorable interaction, possibly a CH-π interaction with Trp656, with the glycosidase active site that more than offsets the effect of the less favorable nature of the *gt* side chain conformation in this compound. Overall, these results are fully consistent with i) the design hypotheses, with the imposed *gg* conformation affording better inhibitors than the *gt* conformation in each case studied, and ii) the well-appreciated and wide-ranging influence of methyl groups in medicinal chemistry that is maximized when conformational restriction and hydrophobic effects are in synergy.[30–32]

In summary, we have synthesized and evaluated *gluco* iminosugars **2**–**4** as inhibitors of *Tm*GH1 and *Ss*GH1 and *manno* iminosugars **6**–**8** as inhibitors of *Bt*Man2A. Inhibition studies with these three glycosidases consistently showed that compounds **3** and **7** with the *gg* side chain conformation are better inhibitors than the isosteric compounds **4** and **8**, respectively, with the *gt* side chain conformation. Moreover, preorganization of the side chain into the *gg* conformation by an ideally located methyl group gives the *manno*-configured iminosugar **7** 27-fold greater potency over 1-deoxymannojirimycin **6** itself, underlining the benefits to be gained from the synergy of conformational restriction and hydrophobic interactions, consistent with well-established principles in medicinal chemistry.[30–32] We anticipate this concept of conformational preorganization will be beneficial for the design of future inhibitors.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords: Glycosidase · Iminosugar · Inhibitor · Methyl Group · Side Chain Conformation

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