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# A Cyclic Phosphonamidate Analogue of Glucose as a Selective Inhibitor of Inverting Glycosidases

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**Abstract**—Recent work in the synthesis of cyclic phosphonate analogues of glucose [Darrow, J. W.; Drueckhammer, D. G. (1994) *J. Org. Chem.* **1994**, *59*, 2976] has been extended to the synthesis of a corresponding phosphonamidate analogue. A phosphonate salt, phosphonate methyl ester, and phosphonamidate analogue were tested as inhibitors of two inverting α-glycosidases, (trehalase and glucoamylase), and two retaining glycosidases, (α-glucosidase and β-glucosidase). No inhibition of any of these enzymes was observed with the phosphonate salt or methyl ester. However, the phosphonamidate gave moderate competitive inhibition of the two inverting glycosidases and the retaining α-glucosidase but no inhibition of β-glucosidase. The phosphonamidate showed enhanced binding relative to a simple monosaccharide only with the inverting glycosidases. This enhanced binding is believed to be due to hydrogen bonding interactions between the phosphonamidate group and two active site carboxylate residues implicated in catalysis. The selectivity toward inverting glycosidases is consistent with differences in distance of an active site carboxylate from the anomeric carbon of the glycoside substrate for the inverting versus the retaining glycosidases. Copyright © 1996 Elsevier Science Ltd

## Introduction

A large number of both natural and designed inhibitors of glycosidase enzymes are known.[1–6] Glycosidase inhibitors have been valuable as tools for increasing our understanding of the biological roles of carbohydrates and carbohydrate processing[7,8] and have been useful in unraveling mechanistic details of glycosidase enzymes.[9] Glycosidase inhibitors are also potentially useful in the treatment of a wide range of medical disorders, and have been investigated as potential antiviral,[10] antitumor,[11] and antimicrobial agents.[12] While many of the known glycosidase inhibitors exhibit great potency, most show limited specificity.[1–6] This is a potential problem in the use of glycosidase inhibitors as pharmaceutical agents, as they would likely interfere with essential biological functions in addition to exhibiting the desired biological effect.

The glycosidases can be divided into two mechanistic classes.[9,13,14] The inverting glycosidases catalyse glycoside hydrolysis with inversion of configuration at the anomeric carbon while the retaining glycosidases catalyse hydrolysis with retention at the anomeric carbon. Despite these differing mechanisms, both classes of glycosidases are believed to proceed by similar transition states resembling the glycosyl cation.[9,15] Most known glycosidase inhibitors mimic the positive charge character and/or half-chair conformation of this glycosyl cation-like transition state. The consistency of transition state structure presents a major problem in the development of glycosidase inhibitors that possess greater enzyme specificity. Some specificity may be imparted by the orientation of hydroxyl groups on a sugar analogue-based inhibitor. For example the glucose configuration is employed for maximum inhibition of an enzyme that hydrolyses glucosidic linkages.[1] However, the distinction between different enzymes hydrolysing glycosides of the same monosaccharide unit by simple small molecule inhibitors is largely an unsolved problem. The identification of mechanistic and/or structural differences between different glycosidases that would permit design of inhibitors of greater specificity is a major challenge in glycosidase enzymology.

We[16] and others[17] recently described the synthesis of cyclic phosphonate (phostone) analogues of glucose (**1** and **2**) and related sugars. Despite these and other publications describing sugar analogues in which the anomeric carbon has been replaced with phosphorus,[18,19] the potential biological activity of these cyclic phosphonate sugar analogues has not been explored significantly. We envisioned that these compounds might provide an entry into a novel class of glycosidase inhibitors exhibiting greater enzyme specificity. We describe here the extension of our previous synthetic work to the synthesis of a phosphonamidate **3** and the testing of phosphonate and phosphonamidate analogues of glucose (Fig. 1) as inhibitors of four common glycosidases representing the two different mechanistic classes. While the phosphonate analogues gave no measurable inhibition of any of the enzymes studied, the phosphonamidate analogue gave modest selective inhibition of glycosidases that catalyse glycoside hydrolysis with inversion at the anomeric carbon. This information may serve as a paradigm for the design of more selective glycosidase inhibitors and

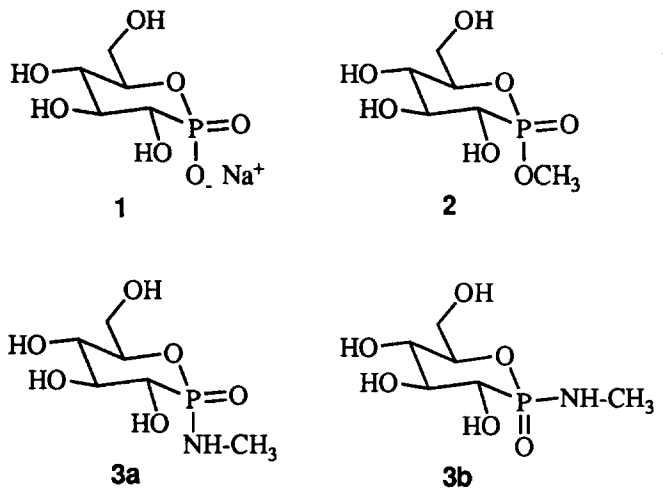
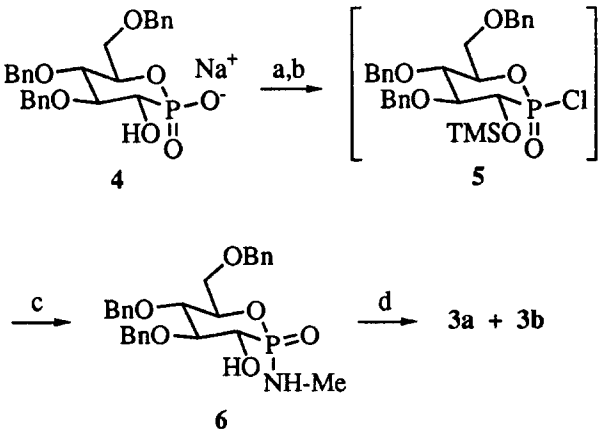



**Figure 1.** Structures of cyclic phosphonate and phosphonamidate analogues of glucose.

provides further support for mechanistic conclusions regarding glycosidase mechanisms previously derived from structural data.

## Results

The sodium phosphonate **1** and methyl phosphonate **2** analogs of D-glucopyranose were prepared as described previously.[16] The methyl phosphonamidate analogue **3** was prepared from the tribenzyl protected sodium phosphonate **4**[16] as shown in Scheme 1. Reaction of **4** with trimethylsilyl triflate and triethylamine in dry dichloromethane, followed by addition of oxalyl chloride and catalytic DMF formed the phosphoryl chloride **5**.[20] Compound **5** was not isolated but was reacted with methylamine in dichloromethane to form the protected *N*-methyl phosphonamidate **6**. Compound **6** was isolated by chromatography on silica gel in 30% overall yield from **4**. Most of the balance of material was the starting material **4**. When the reaction with oxalyl chloride was allowed to proceed for 2 h at room temperature, **6** was formed as a single isomer. However, when the reaction with oxalyl chloride was allowed to proceed for 18 h, **6** was formed as a 1:1 mixture of the two epimers of phosphorus. By comparison with spectral data of the corresponding methyl phosphonates, the structure of the single isomer of **6** formed with short reaction time was tentatively assigned as that shown in Scheme 1, with the methylamine group axial.




**Scheme 1.** Synthesis of a phosphonamidate analogue of glucose. a. TMS-OTf, $Et_3N$, $CH_2Cl_2$; b. $(COCl)_2$, DMF, $CH_2Cl_2$; c. $CH_3NH_2$, $CH_2Cl_2$; d. $H_2$, $PtO_2$, $CH_3OH$, 1500 psi.

Hydrogenolysis of **6** was very sluggish at atmospheric pressure. High pressure hydrogenolysis proceeded readily but gave a mixture of two isomeric products from a single isomer of **6**. The product was identified as a mixture of **3a** and **b** by $^1H$, $^{13}C$, and $^{31}P$ NMR. Attempts to separate **3a** and **b** by chromatography were unsuccessful. A mixture of **3a** and **b** in pH 7 buffer demonstrated a half life of about 18 h at room temperature. At pH 4.5, the mixture of **3a** and **b** was completely hydrolysed in less than 1 h to a single product which was identified as the previously characterized phosphonate salt **1** based on identical spectral data.

Compounds **1–3** were investigated as potential inhibitors of the glycosidases trehalase, glucoamylase and α- and β-glucosidase. Inhibition by **1** and **2** was investigated at pH 7.0 and 4.5 for trehalase and glucoamylase and at pH 6.0 and 4.5 for α- and β-glucosidase. Inhibition by **3** was only investigated at pH 7.0 and 6.0 for the respective enzymes due to the instability of **3** at lower pH. Enzyme activities at pH 6 and 7 were determined by monitoring the release of glucose from a disaccharide or methyl glycoside substrate using a hexokinase/glucose-6-phosphate dehydrogenase coupled enzyme assay system. In glucoamylase assays, glucokinase was substituted for hexokinase as the use of hexokinase in assays with maltose as substrate led to a slow glycosidase-independent increase in absorbance. The coupling enzymes were not sufficiently active for inhibition studies at pH 4.5 and alternate assays were employed. For trehalase and glucoamylase, aliquots were taken at specific time intervals and assayed for glucose concentration. For α- and β-glucosidase, the α- and β-*p*-nitrophenyl-D-glucopyranosides were used as substrates with aliquots removed at timed intervals, treated with base, and the concentration of *p*-nitrophenolate anion measured spectrophotometrically.

The kinetic inhibition results are shown in Table 1. Neither **1** nor **2** gave measurable inhibition of any of the glycosidases studied at pH values of 4.5 and either 6.0 or 7.0 and inhibitor concentrations up to 100 mM. The phosphonamidate **3** demonstrated significant inhibition of trehalase, glucoamylase and α-glucosidase. In all three cases, inhibition was competitive. No activity was observed against the β-glucosidase. α-Methylglucopyranoside is not accepted as a substrate by trehalase or glucoamylase and was examined as a potential inhibitor of these enzymes to determine the affinity of the enzyme for a monosaccharide species.

**Table 1.** $K_i$ and $K_m$ values (mM) for glycosidase inhibitors and substrates

| Enzyme | 1[a] | 2[a] | 3 | α-Glc-OMe[a,b] | $K_m$[a] |
|---|---|---|---|---|---|
| Trehalase | ni | ni | 60 ± 20[d] | ni | 0.9[e] |
| Glucoamylase | ni | ni | 10 ± 4[d] | ni | 9.2[f] |
| α-Glucosidase | ni | ni | 14 ± 4[g] | — | 1.8[h] |
| β-Glucosidase | ni | ni | ni | — | 12[i] |

[a]No inhibition was detected at pH 4.5 or 7.0 for trehalase and glucoamylase or at pH 4.5 or 6.0 for α- or β-glucosidase. Lower limits for $K_i$ are estimated at 200 mM.
[b]α-D-Methyl glucopyranoside.
[c]$K_m$ for substrate used in inhibition assays with each enzyme.
[d]pH 7.0.
[e]Trehalose, pH 7.0.
[f]Maltose, pH 7.0.
[g]pH 6.0.
[h]α-D-Methyl glucopyranoside, pH 6.0.
[i]β-D-Methyl glucopyranoside, pH 6.0.

No measurable inhibition was observed with either enzyme.

## Discussion

Efforts to convert the phosphonate **4** to the phosphonamidate **6** using several coupling methods (DCC, $CCl_3CN$, CDI) were unsuccessful and gave only recovered starting material. Attempts to prepare a primary phosphonamidate by coupling of **4** to ammonia was hampered by difficulties in isolation and characterization of product. The reaction sequence of Scheme 1 gave the *N*-methyl phosphonamidate **6** in a modest overall yield of 30%. Formation of a single isomer of **6** is probably due to kinetic formation of a single isomer of the phosphoryl chloride **5** followed by displacement by methylamine with inversion. Longer reaction times with phosphoryl chloride apparently lead to scrambling of stereochemistry at phosphorus in **5**, resulting in formation of **6** as a mixture of isomers. The stereochemical assignment of the isomer of **6** formed with short reaction times with oxalyl chloride is based on comparison of H-4 and *N*-methyl $^1$H NMR shifts as well as $^{31}$P shifts of both isomers with the related 2-oxo-1,3,2-dioxaphosphorinanes[21,22] and the related phosphonate.[16] The single isomer of **6** formed with short reaction times exhibited the further upfield H-4 and *N*-methyl signals in the $^1$H NMR spectrum and the further upfield $^{31}$P shift relative to the second isomer formed upon longer reaction times. Similar upfield shifts are observed in both the 2-oxo-1,3,2-dioxaphosphorinane and the phostone isomers having the P=O bond equatorial, thus the initially formed isomers of **5** and **6** are tentatively assigned the configurations at phosphorus shown in Scheme 1.

The configuration at phosphorus is scrambled in the hydrogenolysis step, forming **3** as a mixture of isomers. Both $^{31}$P and $^1$H NMR spectra confirmed that the *N*-methyl phosphonamidate remained intact in the hydrogenolysis reaction, despite the scrambling. The scrambling of stereochemistry may be explained by nucleophile-assisted trigonal bipyramidal phosphorus scrambling, either turnstile-type[23,24] or Berry pseudorotation,[24,25] with solvent methanol or possibly the free 6-hydroxyl group serving as the nucleophile. It is also possible that platinum-assisted trigonal bipyramidal scrambling is occurring. Acid hydrolysis of the mixture of isomeric products lead to a single product identified as the phosphonate salt **1**, based on comparison of spectral data with a previously prepared and well characterized sample of **1**.[16] This confirms that both the ring size and configuration at C-1 are maintained in the hydrogenolysis reaction.

The glycosidases employed in this study represent the two glycosidase mechanistic classes.[9] Trehalase and glucoamylase are inverting glycosidases, giving the β-anomer of glucopyranose as the initially released product of hydrolysis of the α-glucoside substrate trehalase and maltose, respectively. α- and β-glucosidase are both retaining glycosidases, giving products having the same anomeric configuration as the substrate. Crystal structural data of enzymes and enzyme–inhibitor complexes has revealed substantial insight into the mechanism and role of essential catalytic residues in the reaction of both the inverting (Fig. 2) and retaining (Fig. 3) glycosidases.[13,14,26–28] Both classes of glycosidases employ a protonated and a deprotonated carboxylate in catalysis. In the inverting glycosidases, the protonated carboxylate acts as an acid, protonating the leaving group while the deprotonated carboxylate acts as a base, deprotonating the nucleophilic water molecule. In the retaining glycosidases the protonated carboxylate again acts as an acid, protonating the leaving group and subsequently as a base to deprotonate the nucleophilic water molecule. The deprotonated carboxylate is believed to act as a nucleophile and subsequently as a leaving group. Both classes of glycosidases are believed to act via transition states having substantial glycosyl cation character.

The inverting glycosidases, much like the metalloproteases and aspartic proteases, catalyse the direct attack of water on electrophilic carbon. Based in part on the success of phosphonate and phosphonamidate compounds as inhibitors for enzymes catalysing hydro-

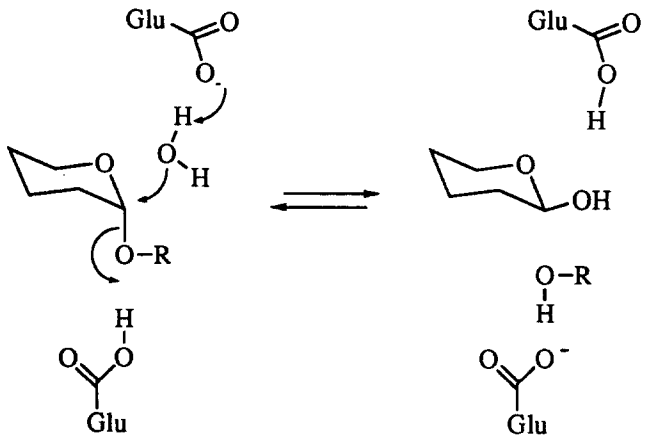



**Figure 2.** General mechanism for an inverting glycosidase.

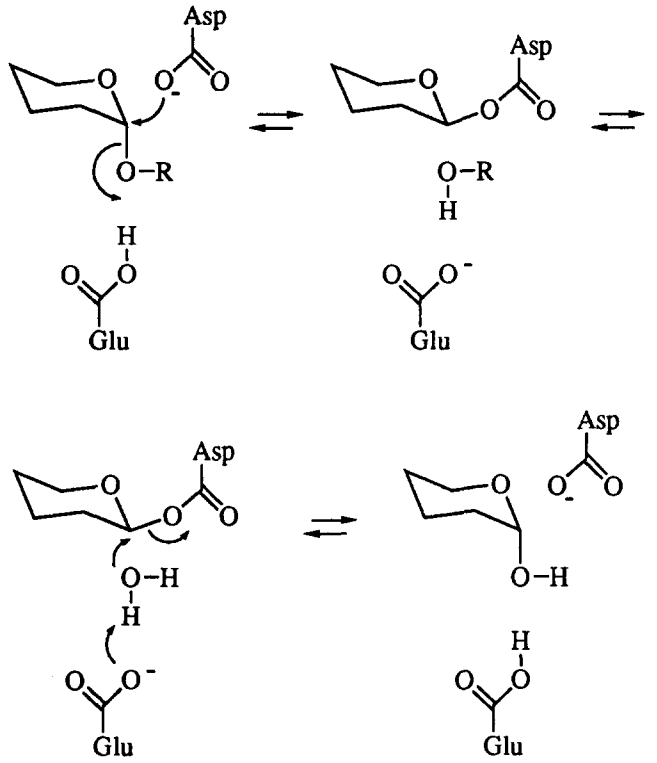



**Figure 3.** General mechanism for a retaining glycosidase.

lysis of amide bonds,[29,30] the cyclic phosphonate analogue of glucose **1** and its derivatives were targeted as potential inhibitors of inverting glycosidases. Though the proposed transition state for the inverting glycosidase reaction is trigonal bipyramidal and the phosphorus atom is tetrahedral, the phosphorus oxygens in **1–3** and nitrogen of **3** are in reasonable position to interact with active site carboxylate groups. The longer P=O and P—N bonds (1.48 and 1.77 Å)[31] relative to C—O bonds (1.42 Å)[32] may partially mimic the greater bond lengths in the transition state relative to the ground state between the anomeric carbon and the leaving group oxygen and between the anomeric carbon and the oxygen atom of the nucleophilic water molecule, though these distances are expected to be even greater in the proposed highly expanded transition state.[15] Thus, while an ideal transition state analogue for the inverting glycosidases would possess trigonal bipyramidal geometry about the anomeric carbon, compounds **1–3** represent a class of substrate analogues that possess certain aspects analogous to the transition state structure for glycoside hydrolysis with inversion.

Inhibition by **1** would require that both carboxylate residues be protonated (Fig. 4) while the active form of the enzyme has only one of these residues protonated. The diprotonated form of the enzyme is likely present in very low concentration, even at pH 4.5. Furthermore, the glycosidase active site is designed to stabilize the positively charged glycosyl cation character of the transition state. The negative charge of the phosphonate anion in this position is likely to result in unfavorable interactions. The phosphonate ester **2** should avoid the repulsive interactions of the negative charge. However, favorable interaction with both carboxylates involved in catalysis again requires that both carboxylates be protonated (Fig. 4). Also, the methyl-bearing oxygen is probably a poor hydrogen bond acceptor, forming only a weak hydrogen bond with the protonated carboxylate residue. The lack of inhibition by **1** and **2** is thus readily rationalized.

The phosphonamidate **3** was prepared, as it was expected that the phosphonamidate oxygen could act as a hydrogen bond acceptor and the nitrogen as a hydrogen bond donor, thus potentially binding to the more prevalent active form of the enzyme having one protonated and one unprotonated active site carboxylate. Analysis of NMR spectra indicated that compound **3** was formed as a mixture of the two isomers at phosphorus in an approximately 1:1 ratio. This mixture of phosphonamidate compounds exhibited modest competitive inhibition of trehalase, glucoamylase, and α-glycosidase. The $K_i$ for **3** with trehalase has a fairly large error range, as inhibitor concentrations used were well below $K_i$ due to limited amounts of material and the very modest inhibition. However, all assays were performed in duplicate and clearly showed inhibition of trehalase activity in the presence of **3**.

The inhibition of glycosidase activity by **3** is fairly poor relative to other known glycosidase inhibitors and the $K_i$ values are near the $K_m$ for the respective substrate of glucoamylase and well above the substrate $K_m$ for trehalase. One potential reason for the modest $K_i$ values for trehalase and glucoamylase relative to substrate $K_m$ values is that the substrates are both disaccharides, while the inhibitors are simple monosaccharide analogues.

Certainly the second monosaccharide unit of the substrates must contribute substantial binding energy that is lost in going to the monosaccharide analogue inhibitors. In order to compare the inhibitor binding to monosaccharide binding, β-methylglucopyranoside was

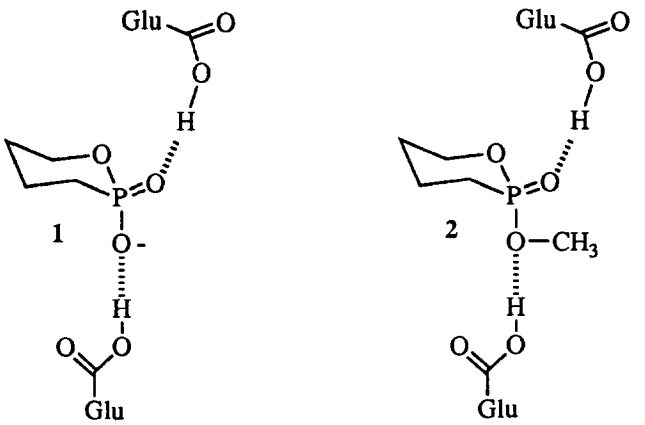



**Figure 4.** Proposed binding of phosphonate analogues to an inverting glycosidase. Dashed lines indicate hydrogen bonding interactions.

tested for inhibition of both inverting glycosidases. This glycoside is not a substrate for either glycosidase and does not interfere with the coupled assay. One or both of the isomers of the phosphonamidate **3** bind to the inverting glucosidases at least 10–20 fold better than does methyl glucopyranoside. In contrast, the isomers of **3** bind 10-fold more poorly to the retaining α-glucosidase than methyl glucopyranoside and show no measurable binding to the retaining β-glucosidase. These results suggest that the phosphonamidate functionality contributes significantly to binding to the inverting glycosidases but impedes binding to the retaining glycosidases.

The enhanced binding of the phosphonamidates to the inverting glycosidases may be explained by the proposed binding modes of the two isomers of **3** to glucoamylase and trehalase as illustrated in Figure 5. Compound **3b** is expected to bind to the active substrate-binding form of the enzyme (compare to the enzyme–substrate complex of Fig. 2), with phosphonamidate oxygen accepting a hydrogen bond from the protonated carboxylate and phosphonamidate nitrogen donating a hydrogen bond to the ionized carboxylate. Phosphonamide nitrogen as a hydrogen bond donor is similarly observed in binding of a phosphonamide peptide analogue to thermolysin.[30] Compound **3a** is expected to bind to the 'inactive' or 'product-binding' form of the enzyme (compare to the enzyme product complex of Fig. 2) in which the protonated and deprotonated carboxylates are reversed. The $pK_a$ values of the two acid residues are probably similar, though each may be greatly influenced by the protonation state of the other. Recent estimates place the $pK_a$ values in glucoamylase at 5.9 and 2.8 for the two carboxylates respectively.[33,34] For trehalase, $pK_a$ estimates range from 7.2 to 8.2, and 4.8 to 5.2, though the exact nature of the groups being titrated is less clear with this enzyme.[35,36] In binding to the product-binding form of the enzyme, the *N*-methyl group of **3a** could mimic the position of the aglycone of the natural substrate. It is not clear if space exists in the active site for the *N*-methyl group of **3b** in binding to the substrate binding form, as this position is occupied by a hydrogen atom during glycoside hydrolysis. It is thus likely that the inhibition observed is due primarily to binding of **3a** to the product-binding form of the enzyme.

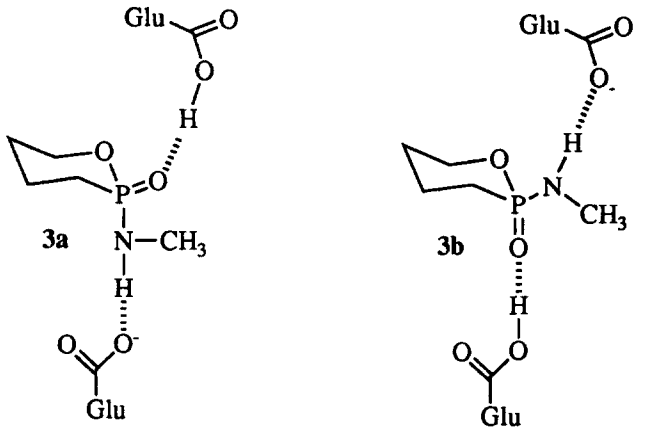



**Figure 5.** Proposed binding of stereoisomeric phosphonamidate analogues to an inverting glycosidase. Dashed lines indicate hydrogen bonding interactions.

Nitrogen could perhaps act as a hydrogen bond acceptor rather than donor or the inhibitor could even exist as the *N*-protonated cation in the enzyme inhibitor complex, in keeping with the presumed binding mode of various amino sugars to glycosidases. However, the phosphonamidate nitrogen is much less basic than amine nitrogen. A $pK_a$ value of 3.6 for an *N*-alkyl phosphonamide zwitterion has been reported.[37] The $pK_a$ for protonated phosphonamidate ester is expected to be substantially lower than this value, based on the differences in the first and second $pK_a$ values of a phosphonic acid[38] and a $pK_a$ of −1.7 reported for a phosphinamide.[39] Thus N-protonation of inhibitor appears very unlikely and the phosphonamide nitrogen should also be a very weak hydrogen bond acceptor due to its low basicity. Formation of a covalent adduct with the enzyme also appears unlikely, based on the apparent lack of a nucleophilic group positioned near the anomeric carbon of the substrate in the active site of the inverting glycosidases and the absence of time-dependent inhibition. Enzyme–inhibitor complexes as shown in Figure 5 and described above seem more likely.

The observed selectivity of the phosphonamidate compounds for the inverting enzymes may be rationalized based on the active site structures of the inverting and retaining glycosidases. Crystal structures of inhibitor complexes of glucoamylase and other inverting glycosidases indicate that the basic carboxylate oxygen is positioned far enough from the reactive anomeric center (about 4.0 Å) to allow a water molecule (the proposed nucleophile) to be bound 3.3 Å from the anomeric center, between the carboxyl residue and the anomeric center.[13,14,26] The corresponding active site deprotonated carboxylate in the retaining glycosidases lysozyme and α-amylase is positioned significantly closer to the anomeric center, with a carboxylate oxygen–anomeric carbon distance of 2.3–3.3 Å.[13,14,27,28] The greater carboxylate to C-1 distance in the inverting glycosidases should permit the methylamine nitrogen of **3b** or the phosphoryl oxygen of **3a** to occupy a position corresponding to the carboxylate-bound water molecule. In the retaining glycosidase, the closer proximity of the carboxylate would not appear to allow space for an intervening oxygen or nitrogen atom. The binding observed to the α-glucosidase presumably requires movement of the nucleophilic carboxylate, resulting in reduced affinity relative to a simple monosaccharide derivative.

Structural homology between the natural glycoside substrate and the cyclic phosphonamidate inhibitor allows the native hydrogen bond contacts with the enzyme active site to be maintained, with the

phosphonamidate moiety providing additional binding interactions not formed by the substrate. The interactions provided by the phosphonamidate moiety are probably similar to the key interactions of the rather non-selective imidazole-based inhibitors, which appear to bridge the two acidic and basic carboxylate residues but do not possess a sugar-like structure.[40] The cyclic phosphonamidate is unique among previously studied glycosidase inhibitors in that it incorporates sugar hydroxyl homology for both increasing enzyme selectivity and maximizing favorable native hydrogen bond contacts, while potentially binding to both the catalytic acid residue and the catalytic base residue. It is presumably this potential to form hydrogen bonds to both catalytic residues in the inverting glycosidase active site, as well as the homology with the native sugar substrate, that imparts inhibitor binding and thus selective glycosidase inhibition. The phosphorus in the anomeric position may also provide other benefits to binding. Crystallographic studies of cyclic phosphate systems suggest that phosphorus in a six-member ring tends to adopt a chair-like conformation, with some flattening toward a half-chair conformation about the phosphorus atom.[41] This could provide some similarity to the half-chair conformation of the presumed glycosyl cation-like transition state.

Interestingly, methyl glucopyranoside demonstrated no measurable binding to either of the two inverting glycosidases despite its structural homology to the respective substrates. This suggests that incorporation of the second saccharide unit of the disaccharide substrate is essential for optimal binding. The cyclic phosphonamidate structure described here can potentially serve as a framework for additional modifications that could lead to further enhanced binding. In particular, attachment to other amines or amino sugars to form glycoside or disaccharide analogues could provide additional binding interactions and potentially lead to enhanced binding and specificity.

Inhibition studies with a cyclic phosphonamidate analogue of glucose provide further evidence for mechanistic differences between retaining and inverting glycosidases. These results support previous conclusions based on structural data regarding the position and role of active site carboxylate residues in catalysis. These studies also provide insights into a new basis for selectivity in the design of glycosidase inhibitors.

## Experimental

Trehalase (EC 3.2.1.28) from porcine kidney, glucoamylase (EC 3.2.1.3) from *Aspergillus niger*, α-glucosidase (EC 3.2.1.20) from baker's yeast, β-glucosidase (EC 3.2.1.21) from almonds, and cofactors, coupling enzymes, and other reagents for enzyme assays were obtained from Sigma. The cyclic phosphonate sodium salt **1** and methyl ester **2** were prepared as described previously.[16]

**(1*S*)-2,3,5-Tribenzyl-D-arabinose 1-(methylphosphonamidate) 6.** To a solution of 2,3,5-tribenzyl-D-*arabit*-δ-phostone, sodium salt **3** (40.0 mg, $8.24 \times 10^{-5}$ mol) in dichloromethane was added freshly distilled triethylamine (18.4 mg, $1.81 \times 10^{-4}$ mol, 25.3 μL). Trimethylsilyl triflate (42.9 mg, $1.81 \times 10^{-4}$ mol, 36.0 μL, 2.2 equiv) was added to the stirring solution at rt via dry syringe. The reaction was allowed to stir at rt for 2 h and the reaction flask was evacuated under red. pres. (0.5 torr) to remove solvent, triethylamine, and residual TMS-triflate. The system was flushed with dry $N_2$ and left under vacuum (0.5 torr) at rt for 30 min. The resulting residue was dissolved in dry dichloromethane (1 mL) and the solution cooled to 0 °C while stirring under dry $N_2$. Oxalyl chloride (100 μL, 2.0 M soln in dichloromethane, $2.0 \times 10^{-4}$ mol) was syringed directly into the stirring solution at 0 °C under dry $N_2$. Dry DMF (0.005 mL) was added, upon which vigorous bubbling ensued. The reaction was stirred for 15 min at 0 °C, and then allowed to slowly warm to rt. After 2 h at rt, the reaction flask was evacuated under red pres. (0.5 torr) to remove residual oxalyl chloride. The system was flushed with dry $N_2$ and left under vacuum (0.5 torr) at rt for 30 min. The resulting residue was dissolved in dry dichloromethane (1 mL) and the resulting soln cooled to 0 °C while stirring. After 10 min, a soln of methylamine in dry THF (2.0 mL, 2.0 M) was introduced into the 0 °C reaction soln (under dry $N_2$) via dry syringe. The reaction was stirred for 15 min at 0 °C, and then allowed to warm to rt. After 2 h at rt, the reaction flask was evacuated under red press. (0.5 torr) to remove solvent and residual methylamine. The product was purified by preparative TLC in EtOAc ($R_f$ 0.4) to give **6** (12.5 mg, 0.025 mmol, 30% overall yield for the three steps). Only one diastereomer (at phosphorus) was obtained as indicated by spectral data. 400 MHz $^1$H NMR ($CD_3OD$): δ 7.3 (m, 15H), 4.95 (d, 1H, $J$ = 10.88 Hz), 4.76 (d, 1H, $J$ = 10.99 Hz), 4.73 (d, 1H, $J$ = 10.85 Hz), 4.51 (d, 1H, $J$ = 12.05 Hz), 4.50 (d, 1H, $J$ = 10.85 Hz), 4.44 (d, 1H, $J$ = 12.05 Hz), 4.12 (dddd, 1H, $J$ = 1.96, 3.68, 3.71, 10.02 Hz), 3.89 (dd, 1H, $J$ = 6.52, 10.07 Hz), 3.86 (dt, 1H, $J$ = 1.86, 10.2 Hz), 3.75 (ddd, 1H, $J$ = 2.15, 3.71, 11.12 Hz), 3.65 (m, 2H); 2.59 (d, 3H, $J$ = 11.81 Hz). 100.6 MHz $^{13}$C NMR ($CD_3OD$): δ 37.83, 137.24, 137.08, 127.87, 127.82, 127.42, 127.35, 127.30, 127.23, 83.89, 83.77, 77.32, 77.15, 77.00, 76.68, 75.68, 74.86, 74.06, 72.96, 68.18, 68.06, 66.88, 25.73. 162.0 MHz $^{31}$P NMR ($CD_3OD$) (vs $H_3PO_4$ in $CD_3OD$ external ref. δ 0.0 ppm): δ 31.27 ppm. MS $[MH]^+$ Exact mass 498.21 (calcd for $C_6H_{15}NO_6P^+$, 498.20).

**(1*S*)-D-Arabinose 1-(methylphosphonamidate) 3.** A soln of **6** (12.5 mg, 0.025 mmol) containing suspended $PtO_2$ (0.6 mg, 0.0026 mmol) in dry MeOH (5 mL) was submitted to hydrogenation under high pressure (1500–2000 psi) at rt for 6 h. Filtration and evapn of solvent gave **3** (4.8 mg, 0.021 mmol, 85% yield). NMR spectra indicated two isomers due to apparent scrambling of stereochemistry at phosphorus in the hydrogenolysis step. 400 MHz $^1$H NMR ($CD_3OD$): δ 4.23 (m, 1H), 4.02–4.10 (m, 1H), 3.88–4.00 (m, 1H),

3.66–3.87 (m, 7H), 2.62 (d, 3H, $J=11.86$ Hz, isomer a), 2.61 (d, 3H, $J=11.82$ Hz, isomer b). 100.6 MHz $^{13}C$ NMR ($CD_3OD$): δ 85.67, 85.56, 85.52, 85.40, 80.76, 80.68, 80.00, 79.95, 78.57, 78.30, 78.03, 77.28, 76.88, 76.78, 71.43, 71.36, 68.28, 69.60, 62.73, 62.63, 27.73 (d, $J=9.86$ Hz), 26.84 (d, $J=26.27$ Hz). 162.0 MHz $^{31}P$ NMR ($CD_3OD$) (vs $H_3PO_4$ in $CD_3OD$ external ref. δ 0.0 ppm): δ 31.61, 31.37 ppm.

### Inhibition studies

Inhibition studies of trehalase at pH 7.0 were conducted in 1.0 mL 0.1 M Hepes buffer containing hexokinase (5 units), glucose-6-phosphate dehydrogenase (0.625 units), $MgCl_2$ (1 mM), NAD (1 mM) and ATP (1 mM). The concentration of trehalose was varied from $5.0\times10^{-5}$ to $1.0\times10^{-3}$ M at concns of **1** and **2** from 1.0 to 100 mM and concn of **3** from 1.0 to 5.0 mM. $K_m$ and $K_i$ values were calculated from double-reciprocal plots of $1/v$ vs 1/[trehalose] at varying concentrations of the inhibitors. Inhibition studies at pH 4.5 were conducted in sodium acetate buffer (0.1 M) with trehalose and inhibitor concentrations varied as at pH 7. Aliquots of 50 μL were removed at timed intervals, added to 1.0 ml samples of pH 7.0 Hepes buffer (0.1 M) containing hexokinase (5 units), glucose-6-phosphate dehydrogenase (0.625 units), $MgCl_2$ (1 mM), NAD (1 mM) and ATP (1 mM), and the increase in absorbance at 340 nm measured. Inhibition studies of glucoamylase were conducted as described for trehalase but with substitution of glucokinase for hexokinase and using concentrations of maltose from $5.0\times10^{-5}$ to $5.0\times10^{-4}$ M.

Inhibition studies of α-glucosidase at pH 6.0 were conducted in MES buffer (0.1 M) with other conditions as described above for pH 7 trehalase assays. The concentration of α-methylglucopyranoside was varied from $2.5\times10^{-4}$ to $2.0\times10^{-3}$ M. Inhibition studies of α-glucosidase at pH 4.5 were conducted in sodium acetate buffer (0.1 M) using *p*-nitrophenyl-α-D-glucopyranoside as substrate at concns from $2.5\times10^{-4}$ to $2.0\times10^{-3}$ M. Aliquots of 50 μL were removed at timed intervals, diluted into 1.0 mL sodium carbonate buffer (0.1 M, pH 9.9), and the concn of *p*-nitrophenolate anion determined from absorbance measurement at 405 nm.[42] Inhibition studies of β-glucosidase were conducted as described for α-glucosidase but using β-methylglucopyranoside and *p*-nitrophenyl-β-D-glucopyranoside as substrates at pH 6.0 and 4.5, respectively.

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