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Polyhydroxylated pyrrolidine and 2-oxapyrrolizidine as glycosidase inhibitors†

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Using D-serine as a chiral precursor, a polyhydroxylated pyrrolidine (**1**), its derivatives bearing carboxylate, phosphate and phosphonate groups (**2–4**) and an oxapyrrolizidine (**5**) were synthesized. The pyrrolidine ring was formed by intramolecular amino-mercuration. The bicyclic scaffold of oxapyrrolizidine was further constructed by an intramolecular attack of the carbamate group on the iodomethyl group. Compounds **1** and **5** were found to inhibit β-glucosidase and α-galactosidase, respectively, in a competitive manner, whereas compounds **2**, **3** and **4** did not produce significant inhibition against glycosidases.

Introduction

Glycosidases are the enzymes that catalyze the cleavage of glycosidic bonds and participate in a myriad of biological activities, including protein folding, bacterial/viral infections, tumor metastasis and metabolism. The development of glycosidase inhibitors has been demonstrated to be an effective way for therapeutic interventions.[1–6] For example, Miglitol® is prescribed for treating type II diabetes by inhibiting the intestinal α-glucosidase.[7] Celgosivir® is an oral prodrug of castanospermine, a natural indolizine alkaloid, used for the treatment of HCV infection by inhibiting α-glucosidase I.[8] Anti-influenza drugs zanamivir (Relenza®)[9] and oseltamivir (Tamiflu®)[10] are the inhibitors of influenza virus neuraminidase, which is a glycoprotein responsible for cleavage of sialic acid (Neu5Ac) in the linkage between the progeny virus and the surface sialoreceptor of host cells.[11–13] We have also utilized fucosidase inhibitors to discover a secreted human fucosidase that plays an important role in the infection, adhesion, growth and pathogenicity of *Helicobacter pylori*.[14]

Pyrrolidines and piperidines with five- and six-membered nitrogen rings are commonly found in natural products.[15] Polyhydroxylated pyrrolidines and piperidines are known as azasugars (or iminocyclitols),[16–20] which are often utilized as the inhibitors of glycosidases and glycotransferases. Azasugars are protonated at physiological pH to mimic the oxocarbenium transition state in enzymatic hydrolysis of the glycoside bond and in glycosyl transfer.[21,22] Some polyhydroxylated bicyclic alkaloids, such as pyrrolizidines and indolizidines having 5–5 and 5–6 fused rings, are also recognized as the azasugar mimetics possessing inhibitory activity against glycosidases.[16–20]

We report herein the synthesis of compounds **1–5** (Fig. 1) and their inhibitory activities against glycosidases. Compound **1** is a polyhydroxylated pyrrolidine, whereas compound **2** (bulgecinine)[23] is a polyhydroxylated pyrrolidine-2-carboxylic acid. Compounds **3** and **4** contain a phosphonic acid and a phosphoric acid, respectively, to act as the surrogate of the sulfonic acid in bulgencin A,[24] which is a natural inhibitor of lytic transglycosylase (LTase). The bicyclic compound **5** has a

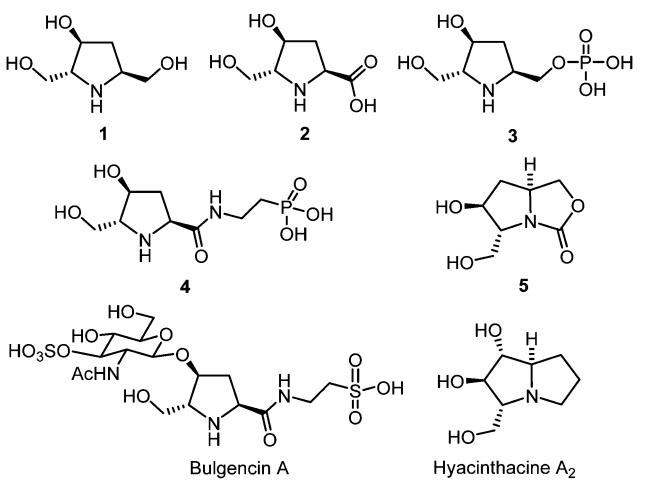

Fig. 1 Structures of the target polyhydroxylated pyrrolidine and pyrrolizidine compounds **1–5**. The natural alkaloids bulgencin A and hyacinthacine A_2, which possess inhibitory activities against glycosyltransferase and glycosidase, are shown for comparison.

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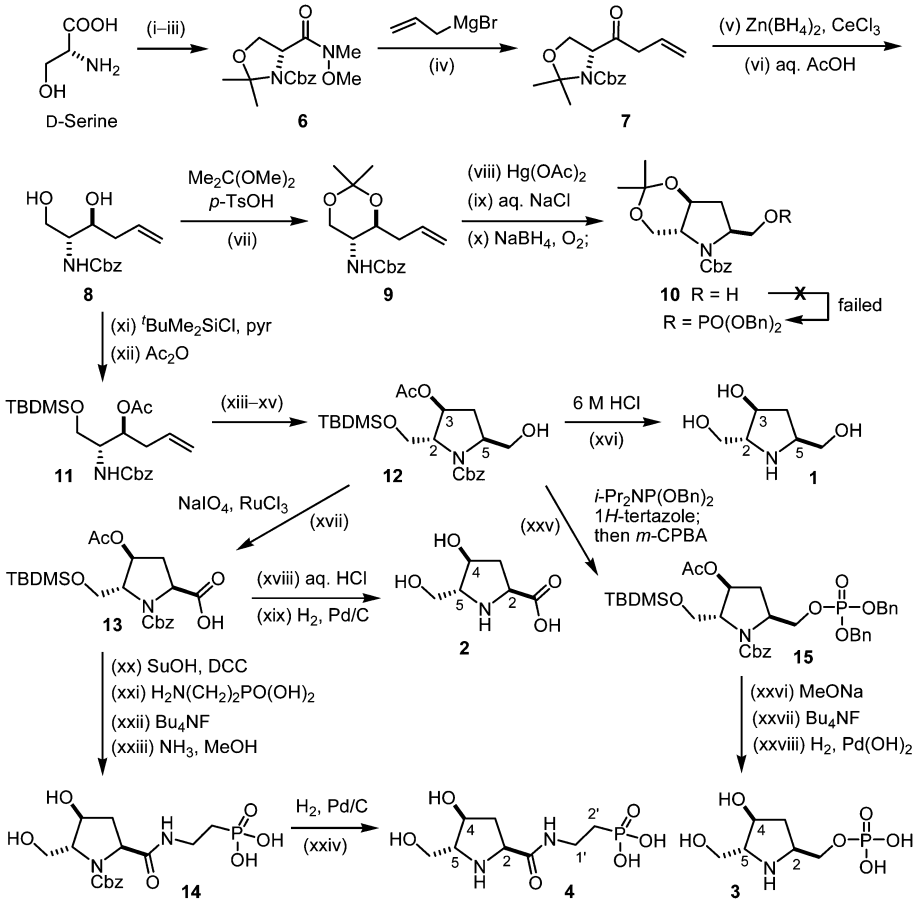
scaffold of 3-oxapyrrolizidine similar to the natural pyrrolizidine alkaloid hyacinthacine A_2 possessing inhibitory activity against amyloglucosidase.[25]

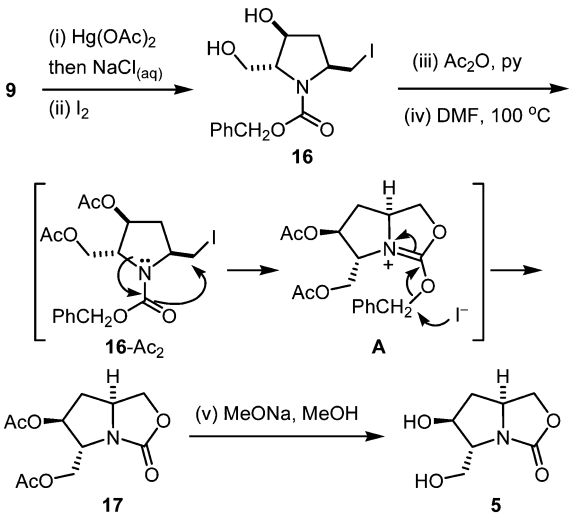
Results and discussion

We chose D-serine as the chiral precursor for the synthesis of polyhydroxylated pyrrolidines **1–4** (Scheme 1) and 3-oxapyrrolizidine **5** (Scheme 2). According to the previously reported procedures,[24] the benzyloxycarbonyl (Cbz) derivative of D-serine was converted to a Weinreb amide, and then protected as an *N*,*O*-acetal **6**. Further reaction of **6** with allylmagnesium bromide at −78 °C for 2 h afforded the desired product of β,γ-unsaturated ketone **7** in 74% overall yield. Excess allylmagnesium bromide or prolonged reaction should be avoided; otherwise, compound **7** might undergo isomerization to give the corresponding α,β-unsaturated ketone. The stereoselective reduction of ketone **7** was carried out by treatment with $Zn(BH_4)_2$ in the presence of $CeCl_3$, *via* a chelation-controlled mode, to give diol **8** after removal of the *N*,*O*-acetal protecting group. By the promotion of $Hg(OAc)_2$, the acetonide **9** derivative of **8** cyclized *via* a chair-like transition state to furnish a pyrrolidine compound. The subsequent oxidative demercuration afforded compound **10** having the (2*R*,3*S*,5*S*) configuration.[24] However, our attempts in phosphorylation of alcohol **10** by using dibenzyl *N*,*N*-diisopropylphosphite and 1*H*-tetrazole failed unexpectedly.

We thus searched for other methods to synthesize compounds **1–5**. The primary hydroxyl group in diol **8** was first protected as the *tert*-butyldimethylsilyloxy (TBDMS) group, and the secondary hydroxyl group was then protected as the acetoxy group, giving compound **11**. An intramolecular amino-mercuration of **11** followed by oxidative demercuration with $O_2/NaBH_4$ afforded a pyrrolidine product **12** in 78% overall yield with high



Scheme 1 Synthesis of pyrrolidines **1–4**. *Reagents and conditions*: (i) $PhCH_2OCOCl$, $NaHCO_3$, H_2O–THF (2 : 1), 25 °C, 3 h. (ii) MeNHOMe·HCl, *N*-methylmorpholine, EDCI, CH_2Cl_2, 0–25 °C, 1.5 h. (iii) $Me_2C(OMe)_2$, $Et_2O·BF_3$, Me_2CO, 25 °C, 15 h; 74% yield from D-serine. (iv) $H_2C{=}CHCH_2MgBr$, Et_2O, −78 °C, 2 h. (v) $Zn(BH_4)_2·Et_2O$, $CeCl_3·7H_2O$, MeOH, 0–25 °C, 2.5 h. (vi) AcOH–H_2O (3 : 1), 50 °C, 15 h; 67% yield from compound **6**. (vii) $Me_2C(OMe)_2$, *p*-TsOH, Me_2CO, 25 °C, 1 h; 84% yield. (viii) $Hg(OAc)_2$, MeCN, reflux, 1 h. (ix) Aq. NaCl, EtOAc, 25 °C, 1.5 h. (x) $NaBH_4$, O_2, DMF, 25 °C, 2.5 h; 51% yield for 3 steps. (xi) *t*-$BuMe_2SiCl$, pyr, 0 °C, 1 h. (xii) Ac_2O, 25 °C, 40 min; 86% yield for 2 steps. (xiii) $Hg(OAc)_2$, MeCN, 0–25 °C, 1.5 h. (xiv) Aq. NaCl, EtOAc, 25 °C, 0.5 h. (xv) $NaBH_4$, O_2, DMF, 25 °C, 3 h; 78% yield for 3 steps. (xvi) 6 M HCl, reflux, 5 h; 89% yield. (xvii) $NaIO_4$, cat. $RuCl_3$, MeCN–CCl_4–H_2O, 0 °C, 2.5 h; 92% yield. (xviii) 3 M HCl, THF, reflux. (xix) H_2, Pd/C, MeOH, 25 °C, 2 h; 73% yield for two steps. (xx) *N*-Hydroxysuccinimide, DCC, THF, 25 °C, 18 h. (xxi) $H_2N(CH_2)_2PO(OH)_2$, Et_3N, H_2O, DMF, 25 °C, 12 h. (xxii) Bu_4NF, THF, 25 °C, 1 h. (xxiii) NH_3 (2 M in MeOH), 25 °C, 15 h; 53% yield from compound **13**. (xxiv) H_2, Pd/C, MeOH, 25 °C, 3 h; 95% yield. (xxv) *i*-$Pr_2NP(OBn)_2$, 1*H*-tetrazole, CH_2Cl_2, 0–25 °C, 2 h; then *m*-$ClC_6H_4CO_3H$, 30 °C, 20 min; 93% yield. (xxvi) MeONa, MeOH–CH_2Cl_2, 25 °C, 30 min. (xxvii) Bu_4NF, THF, 25 °C, 30 min; 80% yield for 2 steps. (xxviii) H_2, $Pd(OH)_2/C$, MeOH, H_2O, 25 °C, 2 h. 84% yield.



Scheme 2 Synthesis of 3-oxapyrrolizidine **5**. *Reagents and conditions*: (i) $Hg(OAc)_2$, CH_3CN, 90 °C, 1 h; $NaCl_{(aq)}$, EtOAc, 25 °C, 1.5 h. (ii) I_2, 25 °C, 1.5 h; 50% yield from **9**. (iii) AcCl, pyr, 0 °C, 3 h; 80% yield. (iv) DMF, 100 °C, 8 h; 81% yield. (v) MeONa, MeOH, 25 °C, 10 min; 90% yield.

stereoselectivity (>95%) as shown by the HPLC analysis. The Hg(II)-assisted cyclization was best performed at 0 °C in acetonitrile solution. The structure of **12** was unambiguously determined to have the (2*R*,3*S*,5*S*) configuration by an X-ray diffraction analysis (Fig. S1 in the ESI†). As shown in the ^1H NMR spectrum, pyrrolidine **12** existed as a mixture of rotamers in $CDCl_3$ solution, presumably due to the restricted rotation of the carbamate group. Addition of $ZnCl_2$ to the $CDCl_3$ solution of **12** rendered a coalescence of the NMR signals (Fig. S2 in the ESI†) presumably due to chelation of the carbamate and hydroxyl groups with the Zn^{2+} ion to rigidify the molecular structure. The proton assignments were verified by two-dimensional ^1H–^1H COSY and NOESY (Fig. S3 and S4 in the ESI†).

In our synthetic scheme, compound **12** acted as a pivotal intermediate leading to the target molecules **1–4**. By heating **12** in aqueous HCl solution, a global deprotection occurred to give compound **1** in 89% yield. Oxidation of alcohol **12** with RuO_4 (generated *in situ* from $RuCl_3$ and $NaIO_4$)[26] at 0 °C proceeded smoothly to afford acid **13**. Compound **2** was obtained after removing all the protecting groups in **13** by acid-catalyzed hydrolysis and hydrogenolysis. Alternatively, acid **13** was activated as the *O*-succinimido ester for coupling with (2-aminoethyl)phosphonic acid. Compound **14** was then obtained by removal of the silyl and acetyl groups. Finally, the Cbz group was removed by hydrogenolysis to afford compound **4**. The ^{31}P NMR of **4** showed a signal at δ_P 21.3 for the phosphonate group. In the ^{13}C NMR spectrum, the signals at δ 58.4 ($J_{C-P} = 3.8$ Hz) and 28.0 ($J_{C-P} = 130$ Hz) were attributable to C-1′ and C-2′, respectively. Unlike alcohol **10**, compound **12** was successfully converted to phosphate diester **15** by treatment with dibenzyl *N*,*N*-diisopropylphosphoramidate at 0 °C in the presence of 1*H*-tetrazole, followed by oxidation with *m*-chloroperbenzoic acid. The desired phosphoric acid **3** was obtained by removing all the protecting groups. The ^{31}P signal of **3** occurring at δ_P 1.1 was consistent with the phosphate group. The C-1′ and C-5 of **3** appeared at δ_C 58.4 ($J_{C-P} = 7.6$ Hz) and 63.9 ($J_{C-P} = 4.6$ Hz), respectively.

Scheme 2 shows the synthesis of compound **5**. The organomercuric product derived from mercuration of **9** was oxidized with iodine to give the iodo compound **16**. The hydroxyl groups in **16** were protected by acetylation, and the derivative **16**-Ac_2 was heated (100 °C) in DMF solution for 8 h to afford 2-oxapyrrolizidinone **17** in 81% yield. This reaction was presumably initiated by an intramolecular attack of the carbamate group in **16**-Ac_2 at the iodomethyl position, and a subsequent counter-attack of iodide ion on the benzyl group of the intermediate **A** would form compound **17**. Saponification of **17** produced compound **5**, whose structure was rigorously determined by spectroscopic methods (IR, HRMS, ^1H, ^{13}C, COSY and NOESY).

Compounds **1–5** were incubated with various glycosidases, including α-glucosidase, β-glucosidase, α-mannosidase, β-mannosidase, α-galactosidase, and β-galactosidase, to evaluate their inhibitory activities (Table 1). At a concentration of 1 mM, compounds **1** and **5** were found to inhibit β-glucosidase and α-galactosidase with 1.7% and 5.4% remaining activities, respectively. In contrast, compounds **2**, **3** and **4** containing acidic moieties (carboxylic, phosphoric or phosphonic acids) did not show any significant inhibition against the six glycosidases. The kinetic studies of compounds **1** and **5** further supported their specific enzymatic activities. The Lineweaver–Burk plots (Fig. 2) indicated that compounds **1** and **5** exhibited competitive inhibition for β-glucosidase ($K_i = 29.2$ μM) and α-galactosidase ($K_i = 40.8$ μM), respectively.

Table 1 Remaining activities of compounds **1–5** in inhibition of glycosidases[a]

	Remaining activity					
Compound	α-Glucosidase	β-Glucosidase	α-Mannosidase	β-Mannosidase	α-Galactosidase	β-Galactosidase
1	12.8	1.7[b]	101.9	97.0	50.4	53.5
2	110.1	115.1	82.0	99.3	95.2	90.7
3	97.0	96.1	76.8	100	91.9	114.0
4	97.1	83.6	83.9	93.7	107.0	55.8
5	39.1	75.7	72.2	98.7	5.4[c]	109.3
None[d]	100	100	100	100	100	100

[a] The test compound at 1 mM concentration was used along with the substrate (*p*-nitrophenyl glycoside) at a concentration of its Michaelis constant K_m. [b] Compound **1** is a competitive inhibitor of β-glucosidase with $K_i = 29.2$ μM. [c] Compound **5** is a competitive inhibitor of α-galactosidase with $K_i = 40.8$ μM. [d] Negative control, no compound was added.

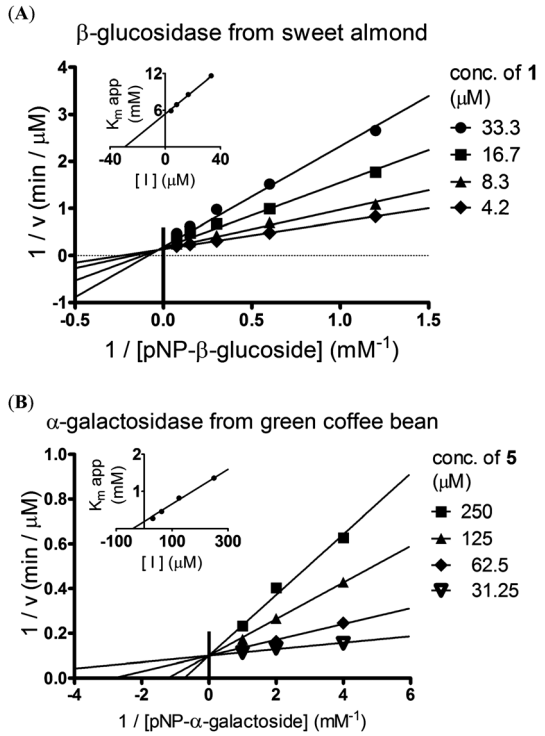


Fig. 2 Lineweaver–Burk plot analysis of the steady-state kinetics of β-glucosidase and α-galactosidase in the presence of compounds **1** and **5**, respectively. In both experiments, the resulting slopes were plotted against inhibitor concentrations (shown as insets). (A) Kinetics for β-glucosidase in the presence of **1**. The inset indicates that **1** is a competitive inhibitor of β-glucosidase with a K_i value of 29.2 μM. (B) Kinetics for α-galactosidase in the presence of **5**. The inset indicates that **5** is a competitive inhibitor of α-galactosidase with a K_i value of 40.8 μM.

A natural product (2*R*,3*R*,4*R*,5*R*)-bis(dihydroxymethyl)-dihydroxypyrrolidine (DMDP) has been found to be a potent β-glucosidase inhibitor with $K_i = 0.2$ μM.[27] Under physiological conditions, DMDP exists as a positively charged pyrrolidinium molecule that has the conformation similar to the oxocarbenium transition state (TS) in glucosidase-catalyzed hydrolysis (Fig. 3). DMDP also has four substituents corresponding to the substituents at the C2, C3, C4 and C5 positions of glucoside. Interestingly, compound **1** can be visualized as the **A**- and **B**-forms that have the structural features well correlated with DMDP except for lacking a hydroxyl substituent. This structural insight explains why compound **1** is also a competitive inhibitor against β-glucosidase, though **1** is less potent than DMDP.

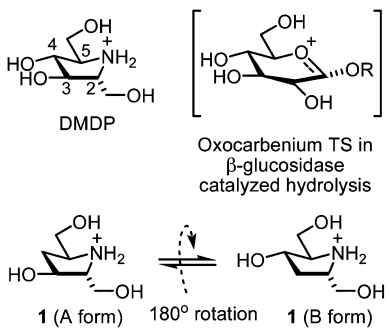


Fig. 3 Correlation of the structural features of compound **1** with DMDP and the oxocarbenium transition state in the β-glucosidase catalyzed hydrolysis.

To understand why compound **5** inhibited α-galactosidase in a competitive manner, we performed the molecular docking experiment. The available X-ray structure of rice α-galactosidase (PDB code: 1UAS)[28] was applied to the present study because this enzyme has reasonable sequence identity (62%) to the α-galactosidase from green coffee bean that was utilized in our activity assay (Table 1). In the complex structure (Fig. 4A), α-galactose displays eight hydrogen bond interactions with the amino acid residues in the enzyme active site. Our computational modeling (Fig. 4B) also reveals that the relatively rigid bicyclic compound **5** also exhibits four possible hydrogen bond interactions with the key amino acids of α-galactosidase, including Asp51, Lys128, Trp164 and Arg181. This result thus supports the function of compound **5** as a competitive inhibitor against α-galactosidase.

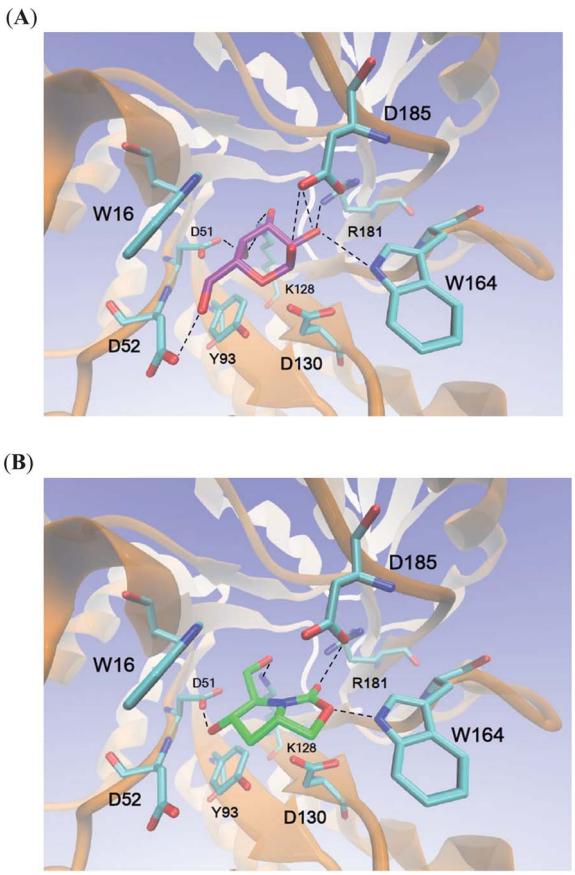


Fig. 4 (A) X-ray structure of rice α-galactosidase (PDB code: 1UAS) complexed with α-galactose. (B) Molecular modeling of rice α-galactosidase with compound **5** using the Autodock 4.2 program.[29] Both compounds are shown in stick representation. Trace of the α-galactosidase backbone is shown as orange ribbon, while side chains of the enzyme residues within 6 Å radius centered on α-galactose or **5** are shown in stick representation explicitly. The carbon atoms of **5**, galactose, and the adjacent residues are colored in green, purple and cyan, respectively. The dashed lines represent hydrogen-bonding interactions between the ligand and the enzyme residues. The maximum number of energy evaluations was set up to 8×10^7. All 50 docked structures obtained from independent runs were clustered using a tolerance of 0.5 Å rmsd and converged to only one cluster.

Conclusions

We have utilized D-serine as a chiral starting material to prepare azasugar **1** and 3-oxapyrrolizidine **5** involving a key reaction of intramolecular amino-mercuration, followed by oxidative demercuration with $O_2/NaBH_4$ or I_2 to attain the pivotal alcohol intermediate **12** and iodo compound **16**. The bicyclic scaffold of oxapyrrolizidine was further constructed by an intramolecular attack of the carbamate group on the iodomethyl group. The structures and stereochemistry of synthetic compounds were rigorously determined by NMR spectra and X-ray diffraction analysis. Compounds **1** and **5** are competitive inhibitors against β-glucosidase ($K_i = 29.2$ μM) and α-galactosidase ($K_i = 40.8$ μM), respectively, as shown by the Lineweaver–Burk plots. Under physiological conditions, compound **1** is considered to exist as a positively charged pyrrolidinium molecule having three hydroxyl groups to mimic the oxocarbenium transition state in the hydrolysis catalyzed by β-glucosidase. The molecular docking experiment also supports that 3-oxapyrrolizidine **5** can fit the active site of α-galactosidase to render substantial hydrogen bond interactions with several enzyme active-site residues.

In our current study, we have not yet investigated the possible activities of compounds **1–5** on lytic transglycosylases and *N*-acetyl-β-hexosaminidases. Lytic transglycosylases are cell-wall degrading enzymes that catalyze the cleavage of peptidoglycans at the glycosidic linkage between *N*-acetylmuramoyl (MurNAc) and *N*-acetylglucosaminyl (GlcNAc) residues with the concomitant formation of a 1,6-anhydromuramoyl product. The enzymatic reaction is suggested proceeding with an oxocarbenium ion transition state stabilized by the *N*-acetyl oxygen of MurNAc.[30] *N*-Acetyl-β-hexosaminidases are lysosomal hydrolases that catalyze the hydrolysis of *N*-acetyl-β-glucosamine and *N*-acetyl-β-galactosamine residues at the nonreducing terminals in glycoproteins, glycolipids and glycosaminoglycans. The enzymatic hydrolysis of terminal *N*-acetyl-β-hexosamine also involves an oxocarbenium ion, which can be stabilized by the neighboring acetamido group of the substrate.[31] Though compounds **1–5** can mimic the oxocarbenium transition state in enzymatic hydrolysis of the glycoside bond, and compounds **3** and **4** contain negatively charged phosphonic or phosphoric groups to mimic the sulfonic acid of bulgencin A, further studies are needed to understand whether compounds **1–5** having no *N*-acetyl group adjacent to the anomeric position will show reasonable inhibitory activities against lytic transglycosylases and *N*-acetyl-β-hexosaminidase.

Experimental

General

All the reagents and solvents were of reagent grade and used without further purification unless otherwise specified. All solvents were of anhydrous grade unless indicated otherwise. CH_2Cl_2, DMF, CH_3CN and Et_3N were distilled from CaH_2. All non-aqueous reactions were carried out in oven-dried glassware under a slight positive pressure of argon unless otherwise noted. Reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) on 0.25 mm silica gel plates. UV light, iodine vapor, *p*-anisaldehyde, ninhydrin and phosphomolybdic acid sprays are applied for visualization. Preparative thin-layer chromatography was performed on 1 or 2 mm silica gel glass plates. Silica gel (0.040–0.063 mm particle sizes) and LiChroprep RP-18 (0.040–0.063 mm particle sizes) were used for column chromatography. High-performance liquid chromatography (HPLC) was performed on an Agilent 1100 Series instrument equipped with a degasser, a Quat pump, and a UV detector.

Melting points were recorded on a Yanaco or Electrothermal MEL-TEMP 1101D apparatus in open capillaries and are not corrected. Optical rotations were measured on a digital polarimeter of Japan JASCO Co. DIP-1000. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Infrared (IR) spectra were recorded on Nicolet Magna 550-II or Thermo Nicolet 380 FT-IR spectrometers. UV-visible spectra were recorded on a Perkin Elmer Lambda 35 spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Varian Unity Plus-400 (400 MHz) spectrometer. ^{31}P NMR data were acquired at 162 MHz using a Bruker Avance-400 spectrometer. Chemical shifts (δ) are given in parts per million (ppm) relative to δ_H 7.26/δ_C 77.0 (central line of t) for $CHCl_3/CDCl_3$, δ_H 4.80 for H_2O/D_2O, or δ_H 3.31/δ_C 48.2 for CD_3OD-d_4. The splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double of doublets) and br (broad). Coupling constants (J) are given in Hz. Distortionless enhancement polarization transfer (DEPT) spectra were taken to determine the types of carbon signals. The ESI-MS experiments were conducted on a Bruker Daltonics BioTOF III high-resolution mass spectrometer. Elemental analysis (EA) data were acquired using a HERAEUS VarioEL-III.

Synthetic procedures

Compounds **6–10** were prepared according to the previously described methods.[24]

(2*R*,3*S*)-2-(Benzoxycarbonyl)amino-1-[(*tert*-butyldimethylsilyl)-oxy]hex-5-en-3-yl acetate (11). To a solution of diol **8** (1.65 g, 6.22 mmol) in pyridine (8 mL) was added *tert*-butyldimethylsilyl chloride (1.13 g, 7.46 mmol). The mixture was stirred at 0 °C for 1 h, and then acetic anhydride (3.2 mL) was added at 0 °C. The mixture was stirred for 40 min, and quenched by treatment with MeOH (1 mL) for 10 min. The mixture was concentrated under reduced pressure. The residue was diluted with EtOAc, and washed with 1 M HCl, $NaHCO_{3(aq)}$ and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc–hexane = 1 : 9) to yield compound **11** (2.76 g, 86%). $C_{22}H_{35}NO_5Si$; white solid; mp 62.0–63.8 °C; TLC (EtOAc–hexane = 1 : 9) $R_f = 0.36$; $[\alpha]_D^{22}$ −2.5 (*c* 1.01, EtOAc); IR ν_{max} (neat) 1731 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.25 (5H, m), 5.80–5.71 (1H, m), 5.10–4.96 (6H, m), 3.94 3.90 (1H, m), 3.72 (1H, dd, $J = 10.4$, 3.2 Hz), 3.62 (1H, dd, $J = 10.4$, 4.4 Hz), 2.52–2.45 (1H, m), 2.37–2.29 (1H, m), 2.02 (3H, s), 0.87 (9H, s), 0.03 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 155.8, 136.2, 133.4, 128.4 (2×), 128.03, 127.97 (2×), 117.7, 72.1, 66.9, 61.8, 53.7, 35.5, 25.9 (3×), 21.2, 18.3, −5.4, −5.5; ESI-HRMS (negative

mode) calcd for $C_{22}H_{34}NO_5Si$: 420.2206, found *m*/*z* 420.2211 $[M - H]^-$; anal. calcd for $C_{22}H_{35}NO_5Si$: C, 62.67; H, 8.37; N, 3.32%. Found: C, 62.47; H, 8.06; N 3.14%.

Benzyl (2*R*,3*S*,5*S*)-3-acetoxy-2-(*tert*-butyldimethylsilyloxy)-methyl-5-(hydroxymethyl)pyrrolidine-1-carboxylate (12). To a solution of **11** (2.24 g, 5.31 mmol) in anhydrous CH_3CN (50 mL) was added mercuric(II) acetate (3.38 g, 10.60 mmol) at 0 °C. The mixture was slowly warmed to room temperature over 1.5 h, and added a mixture of EtOAc–brine (v/v = 1 : 1, 44 mL). The resulting biphasic mixture was stirred at room temperature for 30 min, and diluted with EtOAc. The aqueous phase was extracted twice with EtOAc. The combined organic extract was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a crude organomercuric compound (3.87 g) as colorless oil, which was used in the next step without further purification.

A solution mixture of $NaBH_4$ (333 mg, 8.80 mmol) in anhydrous DMF (100 mL) was bubbled with oxygen at room temperature for 30 min. To this mixture was added dropwise a DMF (50 mL) solution of the above-prepared organomercuric compound *via* an addition funnel over 3 h with continuous bubbling of oxygen. The resulting gray mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc, and washed with H_2O and brine. The organic phase was dried over $MgSO_4$, filtered, concentrated, and purified by chromatography on a silica gel column (EtOAc–hexane, 2 : 8 to 4 : 6) to yield pyrrolidine **12** (2.52 g, 78%). $C_{22}H_{35}NO_6Si$; white solid; mp 94.9–96.8 °C; TLC (EtOAc–hexane, 4 : 6) $R_f = 0.37$; HPLC (Agilent HC-C18 column, 4.6 × 250 mm, 5 μm porosity) t_R 43.6 min (CH_3CN–H_2O (3 : 2) elution at a flow rate of 0.5 mL min^{-1}); $[\alpha]_D^{25}$ −29.2 (*c* 1.16, EtOAc); IR ν_{max} (neat) 3459, 1739, 1701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 7.34–7.31 (5H, m), 5.22–5.05 (3H, m), 4.39–3.60 (7H, m), 2.61–2.52 (1H, m), 2.04/1.99 (3H, s), 1.86–1.71 (1H, m), 0.84/0.82 (9H, s), 0.00/−0.07 (3H, s), −0.03/−0.08 (3H, s); ^1H NMR (400 MHz, with additive $ZnCl_2$ in $CDCl_3$) δ 7.35 (5H, br), 7.18 (1H, br, OH), 5.29 (1H, d, $J = 12.0$ Hz), 5.18–5.12 (2H, m), 4.56 (1H, m), 4.23 (1H, t, $J = 10.4$ Hz), 4.03–3.96 (2H, m), 3.76–3.68 (2H, m), 2.61 (1H, ddd, $J = 14.4$, 9.6, 5.2 Hz), 2.08 (3H, s), 1.72 (1H, d, $J = 14.4$ Hz), 0.81 (9H, s), −0.08 (3H, s), −0.10 (3H, s); ^{13}C NMR (100 MHz, with additive $ZnCl_2$ in $CDCl_3$) δ 170.9, 158.1, 134.2, 128.7 (2×), 128.6 (2×), 76.6, 70.1, 70.0, 67.1, 61.2, 59.8, 33.7, 25.8, 21.6 (3×), 18.1, −5.70 (2×), −5.74; ESI-HRMS calcd for $C_{22}H_{36}NO_6Si$: 438.2312, found *m*/*z* 438.2295 $[M + H]^+$; anal. calcd for $C_{22}H_{35}NO_6Si$: C, 60.38; H, 8.06; N, 3.20%. Found: C, 60.44; H, 7.95; N 2.91%.

Benzyl (2*S*,4*S*,5*R*)-4-acetoxy-5-[(*tert*-butyldimethylsilyloxy)-methyl]pyrrolidine-2-carboxylate (13). To a solution of alcohol **12** (207 mg, 0.47 mmol) in CH_3CN–CCl_4–H_2O (3 mL, v/v/v = 2 : 2 : 3) was added sodium periodate (405 mg, 1.89 mmol) and $RuCl_3 \cdot 3H_2O$ (6 mg, 0.022 mmol). The mixture was stirred at 0 °C (ice bath) for 3 h, and then filtered through a pad of Celite. The filtrate was diluted with EtOAc, and washed with 1 M HCl and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford acid **13** (196 mg, 92%), which was used in the next step without further purification. $C_{22}H_{33}NO_7Si$; brown oil; TLC (EtOAc–hexane = 7 : 3) $R_f = 0.26$; ^1H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 7.36–7.25 (5H, m), 5.27–5.05 (3H, m), 5.54/4.51 (1H, d, $J = 10.0$ Hz), 4.07–3.74 (3H, m), 2.74–2.59 (1H, m), 2.30/2.22 (1H, d, $J = 14.0$ Hz), 1.97/1.94 (3H, s), 0.84/0.82 (9H, s), 0.00/−0.03/−0.07/−0.10 (6H, s); ESI-HRMS (negative mode) calcd for $C_{22}H_{32}NO_7Si$: 450.1954, found *m*/*z* 450.1948 $[M - H]^-$.

Benzyl (2*S*,4*S*,5*R*)-4-hydroxy-5-hydroxymethyl-2-(2-phosphonylethylamino)carbonyl-pyrrolidine-1-carboxylate (14). To a solution of acid **13** (467 mg, 1.03 mmol) in anhydrous THF (5.0 mL) was added *N*-hydroxysuccinimide (146 mg, 1.26 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (DCC, 258 mg, 1.25 mmol) at 0 °C. The mixture was warmed to room temperature over 16 h, and filtered. The filtrate was concentrated under reduced pressure, and purified by chromatography on a silica gel column (EtOAc–hexane, 4 : 6 to 5 : 5) to yield **13**-OSu, the succinimide ester of **13** (480 mg, 85%). $C_{26}H_{36}N_2O_9Si$; colorless foam; TLC (EtOAc–hexane = 5 : 5) $R_f = 0.45$; $[\alpha]_D^{23}$ −53.3 (*c* 2.80, $CHCl_3$); IR ν_{max} (neat) 1824, 1788, 1741, 1714, 1215 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 7.34–7.26 (5H, m), 5.29–5.19 (2H, m), 5.07/5.06 (1H, d, $J = 12.0$ Hz), 4.83/4.78 (1H, dd, $J = 10.0$, 0.8 Hz), 4.10–4.00 (2H, m), 3.79–3.73 (1H, m), 2.85–2.74 (5H, m), 2.46/2.44 (1H, s), 2.02/2.01 (3H, s), 0.83/0.82 (9H, s), 0.00/−0.04/−0.07/−0.10 (6H, s); ^{13}C NMR (100 MHz, $CDCl_3$, a mixture of rotamers) δ 170.49, 170.45, 168.3, 167.2, 166.6, 153.8, 153.5, 135.8, 128.4, 128.3, 128.24, 128.19, 128.1, 127.9, 76.8, 75.7, 67.7, 67.5, 66.2, 65.4, 62.2, 61.2, 57.5, 57.2, 36.1, 34.8, 34.0, 25.88, 25.86, 25.7, 25.0, 21.1, 21.0, 18.2, −5.5, −5.6; ESI-HRMS calcd for $C_{26}H_{37}N_2O_9Si$: 549.2268, found *m*/*z* 549.2271 $[M + H]^+$.

A solution of the above-prepared **13**-OSu (87.0 mg, 0.17 mmol) in DMF (0.4 mL) was added slowly to a stirring solution of (2-aminoethyl)phosphonic acid (26.0 mg, 0.21 mmol) in H_2O–Et_3N (0.2 mL, v/v = 1 : 1) at room temperature. The mixture was stirred at room temperature for 5 h, and then concentrated under reduced pressure. The crude product was dissolved in THF (5.0 mL), and treated with tributylammonium fluoride (TBAF, 0.3 mL of 1 M solution in THF). The mixture was stirred at room temperature for 1 h, and concentrated under reduced pressure. The residue was stirred in NH_3 (3 mL of 2 M methanolic solution) at room temperature for 15 h, concentrated under reduced pressure, and separated by anion-exchange chromatography (diethylaminoethyl cellulose, DE52) with elution of 0.5 M ammonium acetate in methanol. The product fraction was concentrated under reduced pressure, desalted and purified by chromatography on a reversed-phase RP-C18 column (1% NH_4OH in H_2O) to afford phosphonate **14** (47.0 mg, 62%). $C_{16}H_{29}N_4O_8P$; colorless foam; TLC (*i*-PrOH–NH_4OH–H_2O = 10 : 3 : 2) $R_f = 0.28$; $[\alpha]_D^{24}$ −19.4 (*c* 0.60, MeOH); IR ν_{max} (KBr) 3212, 1686 cm^{-1}; ^1H NMR (400 MHz, D_2O, a mixture of rotamers) δ 7.46–7.34 (5H, m), 5.22–5.02 (2H, m), 4.38–3.97 (2H, m), 3.95–3.73 (1H, br), 3.73–3.36 (3H, m), 3.22 (1H, dd, $J = 16.0$, 8.8 Hz), 2.63–2.51 (1H, m), 1.99–1.57 (3H, m); ^{13}C NMR (100 MHz, D_2O, a mixture of rotamers) δ 174.6, 174.2, 156.1, 155.6, 135.7, 128.9, 128.8, 128.74, 128.67, 128.6, 128.2, 128.1, 127.6, 73.3, 73.0, 72.5, 68.8, 68.4, 68.2, 68.2, 61.8, 60.9, 60.6, 59.7, 36.9, 36.3, 35.5, 28.7, 27.4; ^{31}P NMR (162 MHz, D_2O,

a mixture of rotamers) δ 20.9, 20.8; ESI-HRMS (negative mode) calcd for $C_{16}H_{22}N_2O_8P$: 401.1114; found *m*/*z* 401.1112 $[M - H]^-$.

Benzyl (2*R*,3*S*,5*S*)-3-acetoxy-5-(dibenzylphosphoryloxy)methyl-2-(*tert*-butyldimethylsilyloxy)methylpyrrolidine-1-carboxylate (15). To a mixture of alcohol **12** (1.39 g, 3.17 mmol) and 1*H*-tetrazole (653 mg, 9.32 mmol) in anhydrous CH_2Cl_2 (60 mL) was added dibenzyl *N*,*N*-diisopropylphosphoramide (2.4 mL, 7.30 mmol) at 0 °C. The mixture was warmed slowly to room temperature over 2 h to furnish a phosphite intermediate, which was treated with *m*-chloroperbenzoic acid (14.7 mmol, 3.3 g of 77% content) at −30 °C for 20 min. The reaction mixture was diluted with CH_2Cl_2, and washed with saturated $NaHCO_{3(aq)}$ and brine. The organic phase was dried over $MgSO_4$, filtered, concentrated, and purified by chromatography on a silica gel column (EtOAc–hexane, 3 : 7 to 4 : 6) to yield phosphate **15** (2.28 g, 93%). $C_{36}H_{48}NO_9PSi$; colorless oil; TLC (EtOAc–hexane = 3 : 7) R_f = 0.32; $[\alpha]_D^{23}$ −37.1 (*c* 1.02, $CHCl_3$); IR ν_{max} (neat) 1741, 1703 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 7.35–7.25 (15H, m), 5.18–4.94 (7H, m), 4.51/4.32 (1H, m), 4.16–3.68 (5H, m), 2.41–2.30 (1H, m), 2.09–2.04 (1H, m), 1.95/1.92 (3H, s), 0.83/0.81 (9H, s), −0.02/−0.08 (3H, s), −0.06/−0.10 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$, a mixture of rotamers) δ 170.2, 170.1, 153.8, 153.6, 136.0, 135.9, 135.6, 128.5, 128.41, 128.39, 128.34, 128.26, 128.1, 128.02, 127.99, 127.80, 127.78, 127.7, 69.3, 69.2, 67.33, 67.27, 67.2, 67.1, 66.6, 66.5, 65.8, 62.1, 60.6, 57.8, 57.7, 57.1, 57.0, 33.1, 32.1, 25.87, 25.85, 21.3, 21.2, 18.2, −5.50, −5.53, −5.56; ^{31}P NMR (162 MHz, $CDCl_3$, a mixture of rotamers) δ −0.51, −0.56; ESI-HRMS calcd for $C_{36}H_{48}NO_9PSi$: 698.2914; found *m*/*z* 698.2921 $[M + H]^+$.

(2*R*,3*S*,5*S*)-2,5-Bis(hydroxymethyl)-3-hydroxypyrrolidine (1). A solution of **12** (156 mg, 0.356 mmol) in 6 M HCl was heated at 100 °C for 5 h, and then concentrated under reduced pressure. The residue was rinsed with CH_2Cl_2 three times to afford pyrrolidine **1** as the hydrochloric salt (58 mg, 89%). $C_6H_{14}ClNO_3$; yellow oil; $[\alpha]_D^{20}$ +22.6 (*c* 1.12, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.41 (1H, q, *J* = 6.4 Hz), 3.95–3.89 (3H, m), 3.84–3.80 (2H, m), 3.64 (1H, q, *J* = 6.4 Hz), 2.51 (1H, dt, *J* = 13.6, 6.4 Hz), 1.85 (1H, dt, *J* = 13.6, 7.6 Hz); ^{13}C NMR (100 MHz, D_2O) δ 70.2, 66.2, 60.6, 59.4, 58.2, 34.0; ESI-HRMS calcd for $C_6H_{14}NO_3$: 148.0974; found *m*/*z* 148.0980 $[M + H]^+$.

(2*S*,4*S*,5*R*)-4-Hydroxy-5-(hydroxymethyl)pyrrolidine-2-carboxylate (2). A solution of acid **13** (93.0 mg, 0.206 mmol) in 3 M HCl–THF (2 mL, v/v = 2 : 3) was heated at 90 °C for 12 h to remove the acetyl and silyl protecting groups. The crude product was dried under reduced pressure, and rinsed with Et_2O twice. This sample (53.2 mg) in H_2O–MeOH (1 : 4, 4 mL) was subjected to hydrogenation (1 atm H_2) at room temperature for 2 h in the presence of Pd/C (4 mg). The mixture was filtered through a pad of Celite, and concentrated under reduced pressure. The crude product was dissolved in H_2O (1 mL) containing 2 drops of 10% $HCl_{(aq)}$, and purified by ion-exchange chromatography (Dowex 50WX8 resin) with successive elution of H_2O (25 mL) and 1 M NH_4OH (25 mL). The NH_4OH fraction was collected and concentrated *in vacuo* to afford acid **2** (24.3 mg, 73%). $C_6H_{11}NO_4$; colorless oil; $[\alpha]_D^{20}$ −15.1 (*c* 1.10, H_2O) {lit. (ref. 23) $[\alpha]_D^{23}$ −13.8 (*c* 0.21, H_2O)}; ^1H NMR (400 MHz, D_2O) δ 4.36 (1H, q, *J* = 4.8 Hz), 4.19 (1H, dd, *J* = 8.0, 7.6 Hz), 3.88 (1H, dd, *J* = 14.0, 6.8 Hz), 3.76–3.71 (2H, m), 2.65 (1H, m), 2.14 (1H, dt, *J* = 13.6, 5.2 Hz); ^{13}C NMR (100 MHz, D_2O) δ 174.0, 70.7, 66.9, 59.4, 58.2, 36.7; ESI-HRMS (negative mode) calcd for $C_6H_{10}NO_4$: 160.0610; found *m*/*z* 160.0212 $[M - H]^-$.

[(2*S*,4*S*,5*R*)-4-Hydroxy-5-(hydroxymethyl)pyrrolidin-2-yl]methyl phosphate (3). To a solution of phosphate **15** (153.0 mg, 0.219 mmol) in MeOH–CH_2Cl_2 (4 mL, v/v = 1 : 1) was added NaOMe (43.0 mg, 0.80 mmol). The mixture was stirred at room temperature for 30 min until the deacetylation product was detected by TLC (R_f = 0.45, EtOAc–hexane = 5 : 5) along with the disappearance of **15**. The reaction mixture was neutralized with Dowex 50WX8 resin to pH 6–7. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in THF (1.5 mL), and treated with a solution of tetrabutylammonium fluoride in THF (0.55 mmol, 0.55 mL of 1.0 M solution) at room temperature for 30 min. The mixture was concentrated under reduced pressure, and separated by preparative TLC (MeOH–CH_2Cl_2 = 1 : 19) to afford a diol product (**3**-Cbz dibenzyl ester, 94.4 mg, 80%). $C_{28}H_{32}NO_8P$; colorless oil; TLC (MeOH–CH_2Cl_2 = 1 : 19) R_f = 0.27; $[\alpha]_D^{23}$ −24.03 (*c* 1.97, EtOAc); IR ν_{max} (neat) 3405, 1697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, a mixture of rotamers, D_2O exchange) δ 7.32–7.29 (15H, m), 5.15–4.91 (6H, m), 4.42–4.05 (4H, m), 3.90–3.83 (1H, m), 3.69–3.48 (2H, m), 2.25–2.05 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$, a mixture of rotamers) δ 155.0, 154.4, 136.0, 135.8, 135.4, 135.3, 128.4, 128.1, 127.97, 127.93, 127.84, 127.79, 73.7, 72.8, 69.5, 69.4, 68.5, 67.7, 67.6, 67.4, 67.1, 66.9, 62.7, 62.1, 57.6, 57.5, 57.1, 57.0, 34.6, 34.2; ^{31}P NMR (162 MHz, $CDCl_3$, a mixture of rotamers) δ −0.34, −0.40; ESI-HRMS calcd for $C_{28}H_{32}NO_8PNa$: 564.1763; found *m*/*z* 564.1763 $[M + Na]^+$.

The above-prepared diol compound (299 mg, 0.55 mmol) in MeOH–H_2O (v/v 3 : 1, 20 mL) was subjected to hydrogenation (1 atm H_2) at room temperature for 2 h in the presence of 20% $Pd(OH)_2$/C (73 mg). The mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to afford phosphate **3** (167 mg, 84%). $C_6H_{14}NO_6P$; white solid; mp 118.2–120.9 °C; TLC (*i*-PrOH–NH_4OH–H_2O = 6 : 3 : 2) R_f = 0.31; $[\alpha]_D^{22}$ +16.1 (*c* 1.48, H_2O); IR ν_{max} (KBr) 3407, 1664 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 4.39 (1H, q, *J* = 6.4 Hz), 4.18–3.99 (3H, m), 3.90 (1H, dd, *J* = 12.8, 5.0 Hz), 3.78 (1H, dd, *J* = 12.8, 6.4 Hz), 3.64–3.60 (1H, m), 2.55–2.48 (1H, m), 1.94–1.87 (1H, m); ^{13}C NMR (100 MHz, D_2O) δ 70.1, 66.1, 63.9 ($^3J_{C-P}$ = 4.6 Hz), 58.1 ($^2J_{C-P}$ = 7.6 Hz), 58.0, 33.7; ^{31}P NMR (162 MHz, D_2O) δ 1.11; ESI-HRMS (negative mode) calcd for $C_6H_{13}NO_6P$: 226.0481; found *m*/*z* 226.0476 $[M - H]^-$; anal. calcd for $C_6H_{14}NO_6P$: C, 31.73; H, 6.21; N, 6.17%. Found: C, 31.44; H, 6.20; N, 6.07%.

2-[(2*S*,4*S*,5*R*)-4-Hydroxy-5-(hydroxymethyl)pyrrolidin-2-carbamido]ethylphosphonate (4). Carbamate **14** (31.0 mg, 0.071 mmol) in MeOH (5 mL) was subjected to hydrogenation (1 atm H_2) at room temperature for 3 h in the presence of 10% Pd/C (*ca.* 10 mg). The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to afford compound **4** (20.3 mg, 95%). $C_8H_{20}N_3O_6P$; white solid (hygroscopic); $[\alpha]_D^{24}$ −6.4 (*c* 0.58, H_2O); IR ν_{max} (KBr) 3370, 1673 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 4.45–4.38 (2H, m), 3.88 (1H, q, *J* = 7.6 Hz), 3.77–3.71 (2H, m), 3.51–3.44 (2H, m), 2.70 (1H, ddd, *J* =

14.0, 5.6, 5.2 Hz), 2.19–2.13 (1H, m), 1.93–1.82 (2H, m); ^{13}C NMR (100 MHz, D_2O) δ 169.1, 70.9, 70.6, 67.3, 58.44/58.41 (d, $^2J_{C-P}$ = 3.8 Hz), 37.3, 35.7, 28.6/27.3 (d, $^1J_{C-P}$ = 130.0 Hz); ^{31}P NMR (162 MHz, D_2O) δ 21.3; ESI-HRMS (negative mode) calcd for: $C_8H_{16}N_2O_6P$: 267.0746; found m/z 267.0750 $[M - H]^-$.

Benzyl (2*R*,3*S*,5*S*)-3-hydroxy-2-hydroxymethyl-5-(iodomethyl)-pyrrolidine-1-carboxylate (16). To a solution of carbamate **9** (73.0 mg, 0.24 mmol) in anhydrous CH_3CN (2 mL) was added mercuric(II) acetate (190 mg, 0.59 mmol). The mixture was heated at 90 °C for 1 h, cooled to room temperature, and a solution of EtOAc–brine (v/v 1 : 1, 2 mL) was added. The biphasic mixture was stirred at room temperature for 1.5 h, and filtered to remove the precipitates. The filtrate was extracted with EtOAc (3×). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford an organomercury compound (104 mg) as white foam.

To the crude organomercury compound in CH_2Cl_2 (2 mL) was added I_2 (83 mg, 0.33 mmol). After stirring at room temperature for 1.5 h, yellow solids precipitated from the deep red solution. The solids were filtered off. The filtrate was diluted with CH_2Cl_2, and washed with saturated $Na_2S_2O_{3(aq)}$. The organic phase was dried over $MgSO_4$, filtered, concentrated, and purified by flash chromatography on a silica gel column (EtOAc–hexane, 5 : 5) to yield the iodo-compound **16** (46.7 mg, 50%). $C_{14}H_{18}INO_4$; colorless oil; TLC (EtOAc–hexane, 5 : 5) R_f = 0.23; ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 7.29–7.14 (5H, m), 5.10–4.97 (2H, m), 4.30–4.16 (1H, m), 4.10–3.31 (6H, m), 3.02/2.62 (1H, s), 2.48–1.96 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$, a mixture of rotamers) δ 154.7, 153.9, 135.7, 135.3, 128.3, 128.2, 127.94, 127.89, 127.7, 127.5, 73.6, 72.6, 70.1, 69.0, 67.6, 67.4, 62.5, 62.1, 59.7, 37.2, 37.0, 10.5, 10.1; ESI-HRMS calcd for $C_{14}H_{18}INO_4Na$: 414.0153, found m/z 414.0173 $[M + Na]^+$.

(5*S*,7*S*,8*R*)-7-*O*-Acetyl-8-acetoxymethyl-1-aza-3-oxabicyclo[3.3.0]-octan-2-one (17). A solution of diol **16** (880 mg, 2.25 mmol) in anhydrous CH_2Cl_2 (30 mL) was treated with acetyl chloride (0.8 mL, 11.2 mmol) and pyridine (1.82 mL, 22.5 mmol) at 0 °C for 3 h. The mixture was diluted with CH_2Cl_2, and washed with H_2O and brine. The organic phase was dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography (EtOAc–hexane, 2 : 8 to 3 : 7) to yield the acetylation product **16**-Ac_2 (861 mg, 80%). $C_{18}H_{22}INO_6$; colorless oil; TLC (EtOAc–hexane, 2 : 8) R_f = 0.23; $[\alpha]_D^{23}$ −14.8 (*c* 2.51, $CHCl_3$); IR ν_{max} (neat) 1744, 1703 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, a mixture of rotamers) δ 7.39–7.30 (5H, m), 5.22–5.11 (3H, m), 4.35–4.05 (4H, m), 3.87/3.59 (1H, m/m), 3.18–3.13 (1H, m), 2.41–2.25 (2H, m), 2.06/2.05/2.04 (6H, s); ESI-HRMS calcd for $C_{18}H_{22}INO_6Na$: 498.0384, found m/z 498.0360 $[M + Na]^+$.

The above-prepared **16**-Ac_2 compound was dissolved (190 mg, 0.40 mmol) in DMF (4.0 mL) and heated at 100 °C for 8 h. The mixture was concentrated under reduced pressure. The residue was diluted with EtOAc, and washed with H_2O and brine. The organic phase was dried over $MgSO_4$, filtered, concentrated, and purified by silica gel column chromatography (EtOAc–hexane, 3 : 7 to 6 : 4) to afford the bicyclic compound **17** (83.1 mg, 81%). $C_{11}H_{15}NO_6$; colorless oil; TLC (EtOAc–hexane = 5 : 5) R_f = 0.23; $[\alpha]_D^{22}$ −41.6 (*c* 1.98, $CHCl_3$); IR ν_{max} (neat) 1743 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.18–5.15 (1H, m), 4.54 (1H, dd, J = 8.8, 8.0 Hz), 4.21–4.08 (5H, m), 2.58 (1H, dt, J = 13.2, 5.6 Hz), 2.07 (3H, s), 2.03 (3H, s), 1.78 (1H, ddd, J = 13.2, 8.0, 4.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7 (2×), 160.6, 76.6, 68.6, 63.7, 63.5, 57.2, 37.9, 21.24, 21.17; ESI-HRMS calcd for $C_{11}H_{16}NO_6$: 258.0978, found m/z 258.0974 $[M + H]^+$.

(5*S*,7*S*,8*R*)-1-Aza-7-hydroxy-8-hydroxymethyl-3-oxabicyclo-[3.3.0]octan-2-one (5). To a solution of diacetate **17** (77 mg, 0.30 mmol) in anhydrous MeOH (3.0 mL) was added NaOMe (6.2 mg). The mixture was stirred at room temperature for 10 min, and neutralized with Dowex 50W-X8 resin to pH 6–7. The resin was filtered off, and the filtrate was concentrated and purified by silica gel column chromatography (MeOH–CH_2Cl_2, 1 : 9) to yield compound **5** (46.5 mg, 90%). $C_7H_{11}NO_4$; white solid; mp 97.3–98.7 °C; TLC (MeOH–CH_2Cl_2, 1 : 19) R_f = 0.28; $[\alpha]_D^{20}$ −25.8 (*c* 1.23, MeOH); IR ν_{max} (neat) 3385, 1729 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 4.56 (1H, t, J = 8.8 Hz), 4.40 (1H, td, J = 6.0, 3.2 Hz), 4.22 (1H, dd, J = 8.8, 4.8 Hz), 4.16–4.09 (1H, m), 3.70–3.58 (3H, m), 2.38 (1H, ddd, J = 13.2, 6.0, 5.6 Hz), 1.66 (1H, ddd, J = 13.2, 7.6, 5.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 164.5, 75.3, 71.0, 70.3, 62.6, 58.9, 41.0; ESI-HRMS calcd for $C_7H_{11}NO_4Na$: 196.0580, found m/z 196.0577 $[M + Na]^+$.

Determination of glycosidase activity

All kinetics experiments of α-glucosidase (*Bacillus stearothermophilus*), β-glucosidase (sweet almond), α-mannosidase (Jack bean), β-mannosidase (*Helix pomatia*), α-galactosidase (green coffee bean), and β-galactosidase (*Escherichia coli*) were carried out using the corresponding 4-nitrophenyl glycosides (Sigma) as the chromogenic substrates. Standard reactions of each glycosidase (200 μL) contained ~0.05 nM enzyme in a suitable buffer, and 1 mM substrate for the initial screening shown in Table 1 (several concentrations prepared in the range of 4–250 μM when determining K_i values). These glycosidases required different buffers and pH for the activity assay under optimal conditions, including α-glucosidase (150 mM KH_2PO_4–K_2HPO_4, pH 6.8), β-glucosidase (50 mM PIPES–NaOAc, pH 6.2), α-mannosidase (50 mM sodium citrate, pH 4.5), β-mannosidase (100 mM sodium acetate, pH 4.5), α-galactosidase (50 mM citric acid–Na_2HPO_4, pH 6.5), and β-galactosidase (50 mM Tris, pH 7.0). All assays were performed in triplicate at 30 °C for 25 min. All enzymes were stable over the time course. The enzyme activities were measured, in a time-dependent manner, by a spectrophotometer for the release of 4-nitrophenol at 400 nm. The data were fit to the Michaelis–Menton equation using the software Kaleidagraph to determine the K_m values.

Molecular modeling

All docking simulations were performed using Autodock version 4.2 with the Lamarckian Genetic algorithm (LGA) method.[29] The structure of rice α-galactosidase in complex with an α-galactose was obtained from PDB (code: 1UAS).[28] The enzyme has 62.2% sequence identity as compared to green coffee bean α-galactosidase. An initial structure and atom partial charges of compound **5** were generated using PRODRG.[32] The grid box was centered on the active site with a dimension of 40 × 40 × 40 Å^3 where **5** was treated as a flexible ligand and the maximum number of energy evaluations was set up to 8 × 10^7.

Each docking simulation was repeated 50 times using different random generator seeds. All 50 docked structures obtained from independent runs were clustered using a tolerance of 0.5 Å rmsd and converged to only one cluster.

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