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Synthesis of novel polyhydroxylated pyrrolidine–triazole/-isoxazole hybrid molecules†

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A straightforward synthesis of novel, 2-heterocyclyl polyhydroxylated pyrrolidines is described. Stereocontrolled additions of nucleophiles to cyclic nitrones generated the corresponding 2,3-*trans* adducts, allowing the synthesis of the corresponding pyrrolidines *via* key intermediates bearing an alkyne and a nitrile oxide. Three hybrid systems, including a pyrrolidine with two isoxazoles and one triazole, are efficiently prepared *via* 1,3-dipolar cycloaddition. Biological testing of the product alkaloids showed that subtle structural variations have drastic effects on their inhibitory activities against glucosidases.

Introduction

A new class of polyhydroxylated pyrrolidines, in which an aryl moiety is directly attached at the C-2 position of the pyrrolidine ring with a specific 2,3-*trans* configuration, was recently discovered.[1] The large variety of biological activities exhibited by molecules containing the 2-aryl pyrrolidine skeleton suggests that this motif is a privileged scaffold with potential use in combinatorial chemistry for drug discovery.[2] Members of this class including (–)-codonopsinol, radicamine A, and radicamine B have been isolated from plants known for their utility as diuretics, antidotes, hemostats and carcinostatic agents and for the treatment of liver diseases.[3] They have also been found to inhibit various glycosidases, especially α-glucosidases, and some synthetic 2-aryl pyrrolidines were found to exhibit better inhibitory potency than natural alkaloids against glycosidases. This interesting biological activity has motivated the development of various methods for their synthesis.[4]

In a previous study,[4a] we reported an efficient preparation of 2-aryl polyhydroxylated pyrrolidine alkaloids, in which excellent diastereoselectivity was achieved using a stereocontrolled addition of Grignard reagents to cyclic nitrones, and the five-membered chiral cyclic nitrone **1** and its enantiomer are key intermediates (Fig. 1). Inspired by many biomolecules containing a five-membered heterocycle such as a triazole or isoxazole,[5,6] we are curious whether we can develop a new chemical method to combine two scaffolds, a polyhydroxylated pyrrolidine and a heterocycle, to form a hybrid molecule and increase the molecular diversity.

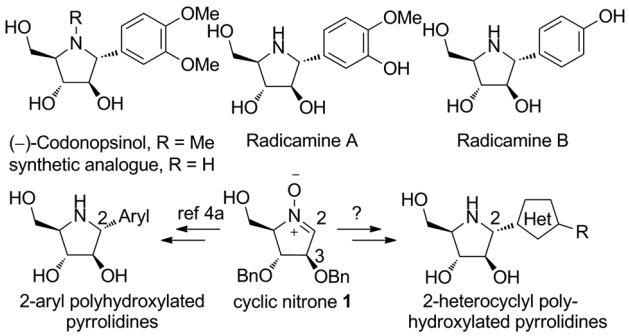

Fig. 1 Examples of bioactive 2-aryl polyhydroxylated pyrrolidines and the identification of cyclic nitrone **1** as a key intermediate for their synthesis.

Based on our literature search, a nucleophilic addition of organometallic reagents to cyclic nitrons has been reported,[7] but the direct use of heterocyclic lithium reagents[7e] is not practical for our further diversity. In contrast, isoxazoles and 1,2,3-triazoles can be accessed *via* a 1,3-dipolar cycloaddition of alkynes with nitrile oxides and azides, respectively, and the high yield and regioselectivity of this ring formation step make it a good choice for the efficient conjugation of two diverse fragments or even two molecules.[8] Therefore, we decided to install the desired functional groups such as an alkyne and a nitrile oxide on a pyrrolidine skeleton first and then undergo a heterocyclic ring formation with a concomitant increase in molecular diversity.

To the best of our knowledge, however, the synthesis of hybrid molecules containing both a polyhydroxylated pyrrolidine and a functionalized triazole or isoxazole remains

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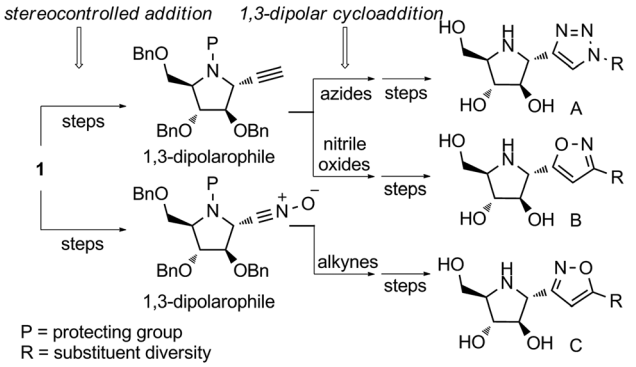


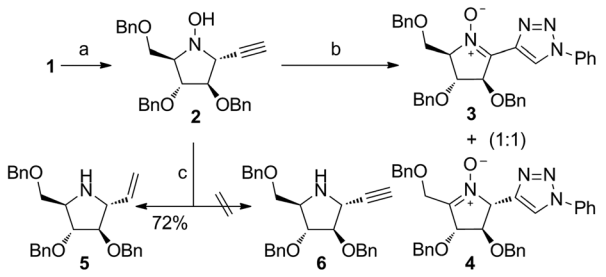
Scheme 1 General approaches towards the synthesis of polyhydroxylated pyrrolidine heterocycle hybrid molecules.

incompletely explored. Herein, we report a new approach for the installation of an alkynyl and oxime group at the C-2 position of the pyrrolidine ring from a chiral cyclic nitrone (Scheme 1). These alkynes and oximes are then elaborated to give functionalized or substituted isoxazole or triazole *via* 1,3-dipolar cycloadditon. Novel polyhydroxylated pyrrolidine heterocycle hybrid molecules were prepared using this method, and their inhibitory activities against glycosidases were studied.

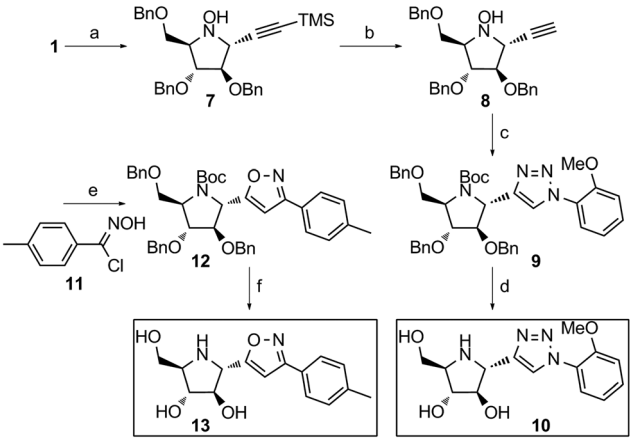
Results and discussion

As illustrated in Scheme 2, the synthesis of the desired alkyne **6** commenced with the elaboration of enantiopure tri-*O*-benzyl cyclic nitrone **1**, readily available from D-arabinose.[9] Nucleophilic addition of ethynyl lithium to cyclic nitrone **1** gave only a single 2,3-*trans* adduct **2** in good yield (90%), which underwent a copper catalyzed 'click' reaction with azidobenzene. Unexpectedly, a mixture of the triazolyl cyclic nitrones **3** and **4** was obtained, presumably by copper-mediated oxidation.[10] Cleavage of the N–O bond of **2** using Zn/AcOH conditions gave vinyl pyrrolidine **5** instead of the desired alkynyl pyrrolidine **6** (Scheme 2).[11]

These problems required our synthetic strategy to be redesigned (Scheme 3). Fortunately, the TMS-masked acetylene moiety in **7** was found to withstand the Zn/AcOH reductive



Scheme 2 Attempts for the preparation of the alkyne and triazoles. Reagents and conditions: (a) HC≡C–Li, THF, 0 °C, 3 h, 90%, (b) PhN_3, $CuSO_4$, Na ascorbate, *t*-BuOH, H_2O, rt, 14 h, 50%, (c) Zn, HOAc, rt, 3 h.



Scheme 3 Synthesis of 2-triazolyl- and 2-isoxazolyl polyhydroxylated pyrrolidines **10** and **13** from alkyne **8**. Reagents and conditions: (a) TMSC≡C–Li, THF, −78 °C, 1.5 h, 89%, (b) *i*. Zn, HOAc, DCM, rt, 24 h, *ii*. TBAF, THF, rt, 2 h, *iii*. Boc_2O, Et_3N, DCM, rt, 2 h, 81% over three steps from **7**, (c) 2-azidoanisole, $CuSO_4$, Na ascorbate, *t*-BuOH, H_2O, rt, 14 h, 88%, (d) H_2, $Pd(OH)_2/C$, MeOH, HCl, rt, 12 h, 90%, (e) *N*-hydroxy-4-methylbenzimidoyl chloride **11**, Et_3N, DCM, rt, 12 h, 70%, (f) BCl_3, DCM, −78 °C, 4 h, 73%.

conditions[12] and the key alkyne **8** was successfully obtained in 81% yield from **7** over three steps. With this 1,3-dipolarophile **8** in hand, the copper(I)-catalyzed 1,2,3-triazole ring formation between 2-azidoanisole and **8** was performed as a model reaction to generate the single adduct **9** in good yield (88%) with excellent regioselectivity.[13] Catalytic hydrogenation [$Pd(OH)_2/H_2$] of **9** under acidic conditions delivered C-2-triazolyl polyhydroxylated pyrrolidine **10** in good yield (90%). The ^1H NMR spectrum of **10** showed a characteristic peak at 8.3 ppm, corresponding to the methine proton on the triazole ring.

Attempts to react **8** with 4-methylbenzaldehyde oxime and bleach[14] under biphasic conditions were unsatisfactory due to the poor yield (<30%). In contrast, treatment of **8** with *N*-hydroxy-4-methylbenzimidoyl chloride (**11**), prepared by the treatment of 4-methylbenzaldehyde oxime with *N*-chlorosuccinimide (NCS) under basic homogeneous conditions,[15] afforded **12** in good yield (70%) as a single adduct with excellent regioselectivity. After global deprotection of **12** by treatment with BCl_3 in CH_2Cl_2 at −78 °C,[16] the C-2-isoxazolyl polyhydroxylated pyrrolidine **13** was obtained in 73% yield. Notably, catalytic hydrogenation [$Pd(OH)_2/H_2$] of **12** did not give **13**, presumably because the isoxazole ring of **12** was labile under these conditions.[17] In the ^1H NMR spectrum of **13**, the characteristic peak corresponding to the methine proton on the isoxazole ring was observed at 6.82 ppm.

Next, our attention turned to the development of a new route to the preparation of another type of C-2-isoxazolyl polyhydroxylated pyrrolidine (Scheme 4). N-protected aldehyde **15** (2,3-*trans* configuration)[18] was obtained from cyclic nitrone **1** in an overall yield of 54% over four steps *via* the selective addition of vinylmagnesium bromide, reduction of the N–O bond, *N*-Boc protection, and ozonolysis. Compound **15** was reacted with hydroxylamine hydrochloride in the presence of

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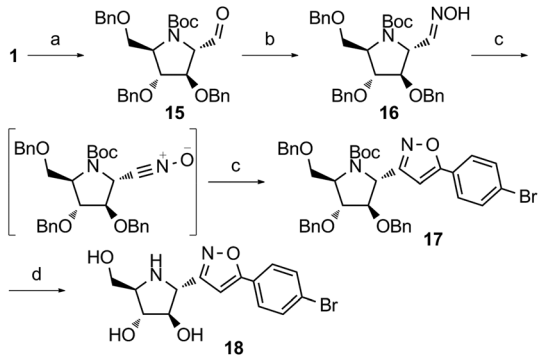

Scheme 4 Synthesis of the regioisomeric 2-isoxazolyl polyhydroxylated pyrrolidine **18** from oxime **16**. Reagents and conditions: (a) (i) Vinyl MgBr, THF, 0 °C, 2 h, (ii) Zn, HOAc, rt, 14 h, (iii) Boc_2O, Et_3N, DCM, rt, 2 h, (iv) O_3, MeOH, –78 °C, 10 min, 54% over four steps; (b) NaOMe, NH_2OH-HCl, MeOH, rt, 2 h, 90%; (c) NaOCl, Et_3N, DCM, H_2O, 1-bromo-4-ethynylbenzene, 0 °C, 12 h, 72%; (d) BCl_3, DCM, –78 °C, 4 h, 65%.

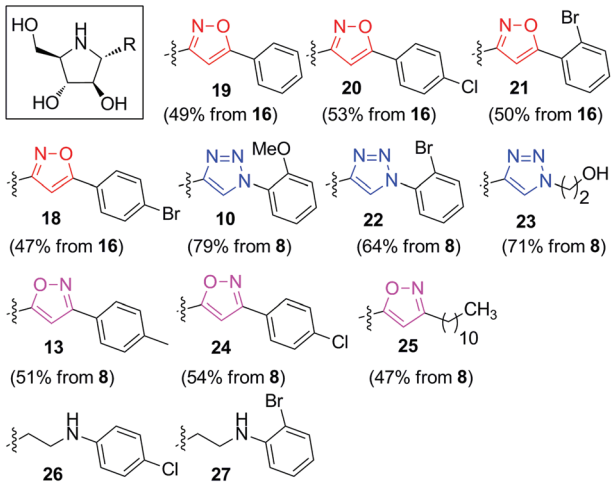

Fig. 2 Examples of the synthesized hybrid molecules. The specified yield refers to the overall yield from the corresponding alkyne **8** or oxime **16**.

sodium methoxide to give oxime **16** (90%), which was treated with bleach and 1-bromo-4-ethynylbenzene to afford isoxazole **17** (72%) *via in situ* generation of the corresponding nitrile oxide and 1,3-dipolar cycloaddition at low temperature. To avoid dehalogenation during palladium-catalyzed hydrogenation, deprotection of **17** was performed using BCl_3 to give pyrrolidine **18**. In the ^{1}H NMR spectrum of **18**, the characteristic peak for the methine proton on the isoxazole ring was observed at 6.9 ppm, which is much more similar to the chemical shift of the methine proton in **13** than **10**.

Several different polyhydroxylated pyrrolidine-heterocycle hybrid molecules were synthesized using these conditions (Fig. 2), and their inhibitory activity against glucosidases was studied (Table 1).

Biological evaluation

Inhibitory potency and selectivity was found to depend on the type of heterocyclic ring and its substituents. For example,

Table 1 Inhibitory activities of synthesized alkaloids against glucosidases[a]

Compound	IC_{50} (μM)	
	α-Glucosidase[b]	β-Glucosidase[c]
10	1.4 ± 0.1	NI[d]
13	5.8 ± 0.6	59 ± 7
18	1.6 ± 0.2	NI
20	1.1 ± 0.2	NI
21	0.2 ± 0.01 (K_i = 67 nM)[e]	50 ± 5
22	6.5 ± 0.4	75 ± 6
23	72 ± 6	NI
24	1.4 ± 0.1	39 ± 2
26	15 ± 1	6.3 ± 0.5
27	1.3 ± 0.02	8 ± 1

[a] IC_{50} and K_i values were measured in triplicates. [b] From *Bacillus stearothermophilus*. [c] From almonds. [d] No inhibition (less than 50% inhibition at 400 μM). [e] Competitive inhibition.

compounds **10**, **18**, **20** and **21** showed significant inhibitory selectivity between α-glucosidase and β-glucosidase. Also, **21** and **22**, which are similar in overall structure but have different heterocyclic rings, had very different biological activities: against α-glucosidase (*Bacillus*), the former, with an isoxazole ring, was approximately 30-fold more potent (IC_{50} = 0.2 μM) than the latter, with a triazole ring (IC_{50} = 6.5 μM). However, two types of regioisomeric isoxazoles, **20** and **24**, showed a similar inhibitory potency of α-glucosidase; their IC_{50} values were 1.1 and 1.4 μM, respectively. Compound **21** was the most potent inhibitor with a K_i value of 67 nM against α-glucosidase (*Bacillus*) (Fig. 3). For comparison purposes, **26** (*vs.* **20** or **24**) and **27** (*vs.* **21**) were prepared with an alkyl chain instead of a heterocyclic ring between the pyrrolidine and substituent moieties. Obviously, **20** and **24** showed much better activity (>10-fold) than **26** against α-glucosidase. The inhibitory activity of **21** was approximately 6.5-fold higher than that of **27** against α-glucosidase. Notably, though **26** and **27** had moderate inhibition activity against α- and β-glucosidases, they dramatically lost their selectivity to distinguish α- and β-glucosidases, possibly due to their flexible alkyl spacer. In contrast, our molecules containing a heterocyclic ring exhibited not

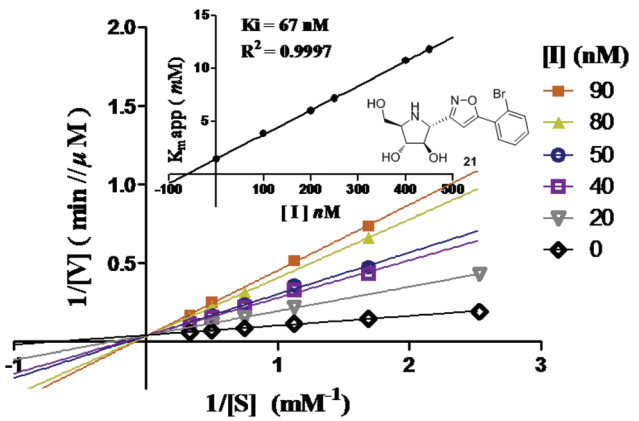

Fig. 3 Lineweaver–Burk double reciprocal plots of compound **21**.

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only inhibitory potency but also selectivity between α- and β-glucosidases.

Conclusions

In summary, general and flexible synthetic routes to isoxazolyl and triazolyl polyhydroxylated pyrrolidines have been developed *via* protected pyrrolidines bearing an alkyne or oxime moiety at the C-2 position as key intermediates. A [3 + 2] cycloaddition reaction between alkynes and azides or nitrile oxides conveniently generates structurally diverse adducts in excellent yields and regioselectivities. The nature of the heterocyclic ring and its substituents was found to have a profound effect on inhibitory potency and glycosidase selectivity. This general and flexible chemistry should allow easy access to more structurally and stereogenically diverse polyhydroxylated pyrrolidine-heterocycle hybrid molecules, and therefore allow for more comprehensive studies of their biological functions in the future.

Experimental

General information

All solvents and reagents were obtained commercially and used without further purification. ^{1}H NMR spectra were recorded on a Bruker AVANCE 600 spectrometer in deuterium solvents such as chloroform-*d* (δ = 7.24), methanol-d_4 (δ = 3.31), and deuterium oxide (δ = 4.81) at ambient temperature. ^{13}C NMR spectra were recorded with a Bruker AVANCE 600 spectrometer and were assigned according to chloroform-*d* (δ = 77.0 ppm of the central line). Chemical shifts are given in ppm (δ) and coupling constants (J) are given in Hz. The splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (double of doublets). High resolution mass spectra were recorded using a Bruker Daltonics BioTOF III spectrometer (ESI-MS). Analytical HPLC spectra were recorded at 220 nm using a HITACHI L-2450 equipped with a photodiode array detector and a Mightysil column (ZORBOX XDB-C-18, 2.1 × 50 mm, 5 μm) eluted with a gradient from 90% H_2O/10% CH_3OH to 10% H_2O/90% CH_3OH with a flow rate = 0.2 mL min^{-1}. Flash column chromatography was carried out using a Merck Kieselgel Si60 (40–63 μm). IR spectra were recorded with a Thermo Nicolet 380. Optical rotations were measured with a Perkin-Elmer Model 341 polarimeter. Thin-layer chromatography (TLC) plates visualized by exposure to ultraviolet light at 254 nm and/or immersion in a staining solution (phosphomolybdic acid, ninhydrin, or potassium permanganate) followed by heating on a hot plate. Ozonolysis was performed on an ozone generator (Fischer Technology OZ 502/10). Reactions were monitored by analytical thin-layer chromatography (TLC) in silica gel 60 F254 plates and visualized under UV (254 nm) and by staining with *p*-anisaldehyde or acidic ninhydrin or phosphomolybdic acid. Concentration refers to rotary evaporation.

Synthesis

(2*R*,3*R*,4*R*)-3,4-Bis(benzyloxy)-2-((benzyloxy)methyl)-3,4-dihydro-2*H*-pyrrole 1-oxide (1). The cyclic nitrone **1** was prepared as a white solid in an overall yield of 56% over five steps following the known method. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.73 (dd, J = 2.9, 10.2 Hz, 1H), 4.02 (br, 1H), 4.05 (dd, J = 5.0, 10.2 Hz, 1H), 4.35 (t, J = 3.2 Hz, 1H), 4.50–4.53 (m, 5H), 4.60 (d, J = 12 Hz, 1H), 4.64 (br, 1H), 6.90 (s, 1H), 7.27–7.36 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.6, 137.2, 137.1, 132.8, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 82.7, 80.3, 77.5, 73.5, 71.9, 71.6, 66.0; HRMS: calculated for $[C_{26}H_{27}NO_4 + H]^+$ 418.2044, found 418.2049.

(2*R*,3*R*,4*R*,5*R*)-3,4-Bis(benzyloxy)-5-((benzyloxy)methyl)-2-((trimethylsilyl)ethynyl) pyrrolidin-1-ol (7). Compound **1** (5.0 g, 12.0 mmol) was dissolved in anhydrous tetrahydrofuran (10 mL) and then ((trimethylsilyl)ethynyl)lithium (3 equiv.) was added dropwise at −78 °C under argon. After stirring for 1.5 h, the reaction mixture was quenched with a saturated aqueous solution of ammonium chloride, extracted with ethyl acetate (15 mL × 3), dried over anhydrous magnesium sulfate, and concentrated. The crude product was purified by column chromatography (20% ethyl acetate in hexanes, silica gel) to give the title compound **7** (5.6 g, 89%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.18 (s, 9H), 3.35 (q, J = 4.3 Hz, 1H), 3.69 (qd, J = 4.4, 10.2 Hz, 2H), 3.94 (dd, J = 2.7, 6.4 Hz, 1H), 4.10 (t, J = 2.7 Hz, 1H), 4.23 (d, J = 2.7 Hz, 1H), 4.44–4.66 (m, 6H), 5.20 (br, 1H), 7.22–7.34 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.3, 138.2, 137.6, 128.6, 128.5, 128.4, 128.3, 128.0, 127.99, 127.92, 127.8, 127.7, 86.5, 82.9, 73.6, 72.2, 72.0, 69.5, 68.0, 62.8, 0.2; HRMS: calculated for $[C_{31}H_{37}NO_4Si + H]^+$ 516.7153, found 516.7141.

(2*R*,3*R*,4*R*,5*R*)-*tert*-Butyl-4-bis(benzyloxy)-5-((benzyloxy)-methyl)-2-ethynylpyrrolidine -1-carboxylate (8). Zinc dust (2.75 g, 10 equiv.) was suspended in acetic acid (10 mL). The mixture was stirred at room temperature for 15 min, after which the color of the solution turned brown. A solution of compound **7** (2.18 g, 4.23 mmol) in dichloromethane (10 mL) was added. After stirring at room temperature for 24 h, acetic acid was removed under reduced pressure, and the solution was adjusted to pH = 7 with saturated sodium bicarbonate aqueous solution and filtered through a pad of celite. The filtrate was washed with ethyl acetate, dried over anhydrous magnesium sulfate, and concentrated to give the crude product, which was treated with tetrabutylammonium fluoride (1 M solution in tetrahydrofuran, 4.9 mL, 1.17 equiv.). The mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated and purified by column chromatography (33% ethyl acetate in hexanes, silica gel) to give the pyrrolidine (1.5 g, 92%) as a colorless oil. The pyrrolidine (1.50 g, 3.51 mmol) was reacted with di-*tert*-butyl dicarbonate (1.11 mL, 1.3 equiv.) in dichloromethane (7 mL) in the presence of triethylamine (660 μL) at room temperature. After stirring for 2 h, water was added to the reaction mixture, followed by extraction with dichloromethane, dried over anhydrous magnesium sulfate, and concentrated. The crude mixture was

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purified by column chromatography (10% ethyl acetate in hexanes, silica gel) to give the title compound **8** (1.63 g, 88%) as a colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$; rotamers were observed) δ 1.45 + 1.50 (ss, 9H), 2.36 + 2.42 (ss, 1H), 3.45 (t, J = 9.6 Hz, 1H), 3.75 + 3.92 (dd dd, J = 3.6, 8.4 Hz, J = 3.6, 8.4 Hz, 1H), 4.12–4.29 (m, 3H), 4.40–4.67 (m, 7H), 7.20–7.37 (m, 15H); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers were observed) δ 154.1 + 153.6, 138.6 + 138.3, 138.0 + 138.9, 137.2, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 127.7, 127.6, 86.4 + 85.3, 82.9, 81.6 + 81.5, 80.8, 80.7, 80.6, 73.1, 72.3, 71.8, 71.7, 71.6, 71.2, 71.0, 68.7 + 68.3, 62.8 + 62.5, 54.6 + 54.0, 28.5; HRMS: calculated $[C_{33}H_{37}NO_5 + H]^+$ 528.2672, found 528.2672.

(2*R*,3*R*,4*R*,5*R*)-*tert*-Butyl 3,4-bis(benzyloxy)-5-((benzyloxy)-methyl)-2-(1-(2-methoxy phenyl)-1*H*-1,2,3-triazol-4-yl)pyrrolidine-1-carboxylate (9). To a solution of compound **8** (60 mg, 0.11 mmol) and 2-azidoanisole (0.5*M* solution in *tert*-butyl methyl ether, 0.29 mL, 1.09 equiv.) in *tert*-butanol (2 mL) was added copper(II) sulfate pentahydrate (2.8 mg, 0.1 equiv.), sodium ascorbate (2.2 mg, 0.1 equiv.), and water (1 mL). The mixture was stirred at 40 °C for 12 h. The mixture was then diluted with water and extracted with ethyl acetate (10 mL × 3). The combined organic layers were dried over anhydrous magnesium sulfate, and concentrated. The crude mixture was purified by column chromatography (10% ethyl acetate in hexanes, silica gel) to give the title compound **9** (69 mg, 88%) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$; rotamers were observed) δ 1.23 + 1.40 (ss, 1H), 3.64 (m, 1H), 3.66 + 3.37 (ss, 1H), 3.98 (dd, J = 4.2, 8.8 Hz, 1H), 4.17 + 4.18 (ss, 1H), 4.24 + 4.26 (ss, 1H), 4.35–4.63 (m, 6 H), 4.65 + 4.75 (dd, J = 5.1 Hz, J = 11.7 Hz, 1H), 5.27 + 5.34 (ss, 1H), 6.89–7.15 (m, 7H), 7.24–7.39 (m, 13 H), 7.58 + 7.22 (dd br, J = 1.3, 7.9 Hz, 1H), 7.82 + 7.86 (ss, 1H); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers were observed) δ 154.3, 151.5, 148.6, 138.8, 137.8, 137.7, 130.2, 129.9, 128.7, 128.64, 128.60, 128.5, 128.4, 128.0, 127.9, 127.8, 127.76, 127.73, 126.6, 125.9, 125.6, 124.2, 121.3 + 121.2, 112.4 + 112.2, 87.0 + 86.6, 84.9 + 83.6, 82.6 + 80.3, 73.3, 71.9 + 71.8, 71.1, 68.9 + 68.3, 63.9 + 63.5, 61.3 + 60.6, 56.0 + 55.8, 28.6 + 28.3; HRMS: calculated for $[C_{40}H_{44}N_4O_6 + H]^+$ 676.3261, found 676.3251.

(2*R*,3*R*,4*R*,5*R*)-5-(Hydroxymethyl)-2-(1-(2-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)pyrrolidine-3,4-diol (10). A mixture of compound **9** (68 mg, 0.1 mmol), concentrated hydrochloric acid (5 drops), and $Pd(OH)_2$ (5 mg, 0.01 equiv.) in methanol (4 mL) was stirred at room temperature under hydrogen. After 12 h, the reaction mixture was filtered through a pad of celite and concentrated. The crude product was purified by column chromatography (10% methanol in dichloromethane, silica gel) to give the title compound **10** (28 mg, 90%) as a white solid. $[\alpha]_D^{20}$ +10.16 (c 0.16 in MeOH); IR (neat): 3313 (br), 2934 (m), 1658 (m), 1475 (m) cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 3.27 (dd, J = 6.1, 10.4 Hz, 1H), 3.70 (dd, J = 3.7, 11.3 Hz, 1H), 3.76 (dd, J = 4.0, 11.3 Hz, 1H), 3.90 (s, 3H), 3.97 (t, J = 6.5 Hz, 1H), 4.27 (t, J = 6.1 Hz, 1H), 4.30 (d, J = 7.3 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 153.4, 148.5, 132.1, 127.5, 126.8, 126.2, 122.2, 113.9, 83.5, 79.4, 65.2, 63.2, 59.3, 56.7; HRMS: calculated for $[C_{14}H_{18}N_4O_4 + H]^+$ 307.1401 found 307.1402.

(2*R*,3*R*,4*R*,5*S*)-5-(Hydroxymethyl)-2-(3-(*p*-tolyl)isoxazol-5-yl)-pyrrolidine-3,4-diol (13). A mixture of compound **8** (60 mg, 0.12 mmol), *N*-hydroxy-4-methylbenzimidoyl chloride **11** (1 M solution in dichloromethane, 150 μL, 3 equiv.), and triethylamine (20 μL, 1.17 equiv.) in dichloromethane (3 mL) was stirred at room temperature for 12 h. The mixture was evaporated and then water was added. The aqueous layer was extracted with ethyl acetate (10 mL × 3). The combined organic layer was dried over anhydrous magnesium sulfate, and concentrated. The crude mixture was dissolved in dry dichloromethane under an argon atmosphere at −78 °C. Boron trichloride (1 M solution in hexanes, 1.8 mL, 15 equiv.) was added dropwise at the same temperature. The reaction mixture was allowed to warm to 0 °C and stirred for 4 h. The reaction mixture was quenched with methanol, and concentrated. The crude mixture was purified by column chromatography (10% methanol in dichloromethane, silica gel) to give the title compound **13**. $[\alpha]_D^{20}$ +11.90 (c 0.13 in MeOH); IR (neat): 3313 (br), 2955 (m), 2924 (s), 1612 (m), 1403 (s) cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 2.39 (s, 3 H), 3.16–3.18 (m, 1H), 3.66 (dd, J = 5.2, 11.2 Hz, 1H), 3.70 (dd, J = 4.5, 11.2 Hz, 1H), 4.01 (t, J = 5.0 Hz, 1H), 4.16 (t, J = 5.8 Hz, 1H), 4.34 (d, J = 6.5 Hz, 1H), 6.82 (s, 1H), 7.28 (d, J = 8.1 Hz, 1H), 7.70 (d, J = 8.1 Hz, 1H); ^{13}C (150 MHz, CD_3OD) δ 174.7, 163.9, 141.8, 130.8, 127.9, 127.5, 101.1, 77.3, 73.7, 66.6, 63.7, 60.6, 21.5; HRMS: calculated for $[C_{15}H_{18}N_2O_4 + H]^+$ 291.1267, found 291.1262.

(2*R*,3*R*,4*R*,5*R*)-*tert*-Butyl 3,4-bis(benzyloxy)-5-((benzyloxy)-methyl)-2-((hydroxyimino)methyl)pyrrolidine-1-carboxylate (16). Compound **1** (2 g, 5 mmol) was dissolved in tetrahydrofuran (20 mL) and then vinyl magnesium bromide (1 M solution in tetrahydrofuran, 14 mL, 3 equiv.) was added dropwise at 0 °C under an argon atmosphere. After 14 h, the reaction mixture was quenched with saturated ammonium chloride aqueous solution, extracted with dichloromethane (20 mL × 3), dried over anhydrous magnesium sulfate, and concentrated. The crude mixture was purified by column chromatography (25% ethyl acetate in hexanes, silica gel) to give the hydroxylamine (1.91 g, 90%) as a brown solid. A mixture of the hydroxylamine (170 mg, 0.38 mmol) and zinc dust (248 mg, 10 equiv.) in acetic acid (3 mL) was stirred at room temperature overnight. The reaction mixture was filtered through a pad of celite and the filtrate was neutralized with saturated sodium bicarbonate aqueous solution, and extracted with dichloromethane (5 mL × 3). The combined organic layers were dried over anhydrous magnesium sulfate, concentrated, and then reacted directly with di-*tert*-butyl dicarbonate (437 μL, 5 equiv.) and triethylamine (265 μL, 5 equiv.) in dichloromethane (4 mL) at room temperature. After stirring for 2 h, water (20 mL) was added to the reaction mixture, which was then extracted with dichloromethane, dried over anhydrous magnesium sulfate, and concentrated to give a crude *tert*-butoxycarbonyl-protected product. After the ozonolysis of the crude *tert*-butoxycarbonyl-protected product in methanol (20 mL) at −78 °C for 10 min, the crude aldehyde was reacted with hydroxylamine hydrochloride (132 mg, 5 equiv.) and sodium methoxide (5.4 M solution in methanol, 352 μL, 5 equiv.) for 2 h. The solvent was evapor-

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ated, and after the addition of water, the aqueous layer was extracted with ethyl acetate (5 mL × 3). The combined organic layers were dried over anhydrous magnesium sulfate, concentrated, and purified by column chromatography (25% ethyl acetate in hexanes, silica gel) to give compound **16** (112 mg, 54% over four steps) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$; rotamers were observed) δ 1.47 (s, 13H), 3.61 + 3.69 (m, 1H), 3.67 (m, 1H), 4.02 (m, 1H), 4.03–4.37 (m, 3H), 4.44 (m, 1H), 4.47–4.80 (m, 9H), 5.12 + 5.24 (dd, J = 4.8, 5.2 Hz), 6.75 + 6.76 (ss, 1H), 7.31–7.51 (m, 23H), 8.99 + 9.10 (ss, 1H), 9.45 + 9.49 (ss, 1H); ^{13}C NMR (150 MHz, $CDCl_3$; rotamers were observed) δ 154.2, 153.2, 154.0, 153.9, 152.4, 152.3, 150.8, 150.7, 138.6, 138.5, 138.3, 138.2, 137.9, 137.8, 137.6, 137.5, 137.3, 137.2, 128.6, 128.5, 128.47, 128.44, 128.41, 128.3, 128.0, 127.9, 127.8, 127.7, 127.68, 127.65, 127.59, 127.56, 127.4, 85.9, 84.7, 84.6, 83.3, 82.4, 82.2, 81.1, 80.7, 80.67, 80.63, 73.1, 71.64, 71.62, 71.60, 71.2, 71.1, 71.0, 70.9, 68.5, 67.9, 63.3, 63.1, 63.0, 62.7, 62.6, 59.2, 59.1, 28.5, 28.4; HRMS: calculated for $[C_{32}H_{38}N_2O_6 + H]^+$ 547.2730, found 547.2731.

(2*R*,3*R*,4*R*,5*R*)-2-(5-(4-Bromophenyl)isoxazol-3-yl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (18). A mixture of the oxime **16** (700 mg, 1.3 mmol) and 4-bromo-1-ethynylbenzene (1 g, 4.2 equiv.) in dichloromethane (6 mL) was stirred at 0 °C, and then a mixture of bleach (18 mL, 12 equiv.) and water (26 mL) was added dropwise. The reaction mixture was warmed to room temperature. After 12 h, the reaction mixture was quenched with saturated ammonium chloride aqueous solution, extracted with dichloromethane, dried over anhydrous magnesium sulfate, and concentrated. The crude product without purification was directly used in the next step. The crude material was dissolved in dichloromethane (80 mL) at −78 °C and then boron trichloride (20 mL, 15 equiv.) was added dropwise under an argon atmosphere. After stirring for 4 h, the mixture was quenched with methanol. Solvents were removed under reduced pressure and the residue was purified by column chromatography (10% methanol in dichloromethane) to give the title compound **18** (218 mg, 47%) as a white solid. $[\alpha]_D^{20}$ +17.05 (c 0.13 in MeOH); IR (neat): 3305 (br), 3124 (m), 2919 (m), 1610 (s), 1465 (s) cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 3.18–3.20 (m, 1H), 3.67 (dd, J = 5.8, 11.2 Hz, 1H), 3.74 (dd, J = 3.9, 11.2 Hz, 1H), 3.93 (t, J = 6.7 Hz, 1H), 4.13 (t, J = 6.7 Hz, 1H), 4.18 (d, J = 7.3 Hz, 1H), 4.6 (s, 1H), 6.90 (s, 1H), 7.67 (d, J = 8.2 Hz, 2H), 7.75 (d, J = 8.2 Hz, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 170.5, 167.4, 133.6, 128.6, 127.9, 125.6, 100.0, 83.6, 79.4, 65.2, 63.4, 59.8; HRMS: calculated for $[C_{14}H_{15}BrN_2O_4 + H]^+$ 356.1839, found 356.1829.

(2*R*,3*R*,4*R*,5*R*)-5-(Hydroxymethyl)-2-(5-phenylisoxazol-3-yl)-pyrrolidine-3,4-diol (19). The title compound **19** was synthesized by the procedure described for the preparation of compound **16** in 49% yield over three steps. $[\alpha]_D^{20}$ +11.43 (c 0.12 in MeOH); IR (neat): 3310 (br), 2927 (m), 1610 (m), 1463 (s) cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 3.21 (br, 1H), 3.67 (dd, J = 5.8, 11.2 Hz, 1H), 3.74 (dd, J = 3.2, 11.2 Hz, 1H), 3.94 (t, J = 6.4 Hz, 1H), 4.15 (t, J = 6.4 Hz, 1H), 4.18 (d, J = 6.9 Hz, 1H), 6.86 (s, 1H), 7.46–7.51 (m, 3H), 7.82 (d, J = 7.6 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 171.7, 167.1, 131.6, 130.4, 128.9, 126.9, 99.4, 83.6, 79.5, 65.2, 63.4, 59.8; HRMS: calculated $[C_{14}H_{16}N_2O_4 + H]^+$ 276.1110, found 276.1111.

(2*R*,3*R*,4*R*,5*R*)-2-(5-(4-Chlorophenyl)isoxazol-3-yl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (20). The title compound **20** was synthesized by the procedure described for the preparation of compound **16** in 53% yield over three steps. $[\alpha]_D^{20}$ +9.76 (c 0.04 in MeOH); IR (neat): 3307 (br), 2924 (m), 1613 (m), 1466 (m) cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 3.18–3.20 (m, 1H), 3.67 (dd, J = 5.8, 11.2 Hz, 1H), 3.73 (dd, J = 3.9, 11.2 Hz, 1H), 3.93 (t, J = 6.5 Hz, 1H), 4.13 (t, J = 6.4 Hz, 1H), 4.17 (d, J = 7.3 Hz, 1H), 7.51–7.53 (m, 2H), 7.81–7.83 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 170.4, 167.4, 137.5, 130.6, 128.5, 127.6, 99.9, 83.6, 79.4, 65.2, 63.4, 59.8; HRMS: calculated for $[C_{14}H_{15}ClN_2O_4 + H]^+$ 311.0720, found 311.0711.

(2*R*,3*R*,4*R*,5*R*)-2-(5-(2-Bromophenyl)isoxazol-3-yl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (21). The title compound **21** was synthesized by the procedure described for the preparation of compound **16** in 50% yield over three steps. $[\alpha]_D^{20}$ −7.10 (c 0.18 in MeOH); IR (neat): 3317 (br), 2925 (m), 1601 (s), 1435 (s) cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 3.18–3.21 (m, 1H), 3.67 (dd, J = 5.8, 11.3 Hz, 1H), 3.75 (dd, J = 4.0, 11.3 Hz, 1H), 3.94 (t, J = 6.5 Hz, 1H), 4.15 (t, J = 7.1 Hz, 1H), 4.22 (d, J = 7.1 Hz, 1H), 7.12 (s, 1H), 7.38 (td, J = 1.7, 8.1 Hz, 1H), 7.50 (td, J = 1.0, 7.7 Hz, 1H), 7.77 (dd, J = 1.0, 8.1 Hz, 1H), 7.81 (dd, J = 1.7, 7.7 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 169.5, 166.8, 135.6, 132.8, 131.4, 129.8, 129.2, 122.2, 104.1, 83.6, 79.5, 65.2, 63.4, 59.9; HRMS: calculated for $[C_{14}H_{15}BrN_2O_4 + H]^+$ 355.0215, found 355.0210.

(2*R*,3*R*,4*R*,5*R*)-2-(1-(2-Bromophenyl)-1*H*-1,2,3-triazol-4-yl)-5-(hydroxymethyl) pyrrolidine-3,4-diol (22). The title compound **22** was synthesized by the procedure described for the preparation of compound **8** in 64% yield over three steps. $[\alpha]_D^{20}$ +12.72 (c 0.12 in MeOH); IR (neat): 3318 (br), 2923 (m), 1493 (s) cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ 3.26 (br, 1H), 3.71 (br, 1H), 3.77 (br, 1H), 3.96 (br, 1H), 4.27 (br, 1H), 4.32 (br, 1H), 7.50–7.53 (m, 1H), 7.57–7.60 (m, 2H), 7.86 (d, J = 8.2 Hz, 1H), 8.27 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 149.2, 138.1, 135.2, 133.1, 130.1, 129.7, 126.3, 120.2, 83.7, 79.6, 65.2, 63.3, 59.4; HRMS: calculated for $[C_{13}H_{15}BrN_4O_3 + H]^+$ 355.0328, found 355.0377.

(2*R*,3*R*,4*R*,5*R*)-2-(1-(2-Hydroxyethyl)-1*H*-1,2,3-triazol-4-yl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (23). The title compound **23** was synthesized by the procedure described for the preparation of compound **8** in 71% yield over three steps. $[\alpha]_D^{20}$ +37.16 (c 0.22 in MeOH); IR (neat): 3285 (br), 2949 (m), 1616 (m), 1425 (m) cm^{-1}; ^{1}H NMR (600 MHz, D_2O) δ 3.26–3.28 (m, 1H), 3.72 (dd, J = 6.2, 11.8 Hz, 1H), 3.78 (dd, J = 4.3, 11.8 Hz, 1H), 3.99–4.01 (m, 3H), 4.25 (d, J = 8.3 Hz, 1H), 4.30 (t, J = 8.3 Hz, 1H), 4.55–4.56 (m, 2H), 8.0 (s, 1H); ^{13}C NMR (150 MHz, D_2O) δ 146.1, 124.2, 80.8, 76.9, 61.8, 61.6, 60.1, 55.9, 52.5; HRMS: calculated $[C_9H_{16}N_4O_4 + Na]^+$ 267.1069, found 267.1061.

(2*S*,3*R*,4*R*,5*R*)-2-(3-(4-Chlorophenyl)isoxazol-5-yl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (24). The title compound **24** was synthesized by the procedure described for the preparation of compound **8** in 54% yield over three steps. $[\alpha]_D^{20}$ +8.03 (c 0.11

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in MeOH); IR (neat): 3311 (br), (m), 2925 (m), 1633 (s), 1463 (s) cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 3.16–3.18 (m, 1H), 3.66 (dd, J = 5.4, 11.2 Hz, 1H), 3.73 (dd, J = 3.8, 11.2 Hz, 1H), 3.91 (t, J = 6.7 Hz, 1H), 4.21 (t, J = 6.4 Hz, 1H), 4.26 (d, J = 6.8 Hz, 1H), 6.81 (s, 1H), 7.48 (d, J = 8.6 Hz, 2H), 7.81 (d, J = 8.6 Hz, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 175.7, 163.0, 137.3, 130.4, 129.5, 129.2, 100.9, 83.1, 79.3, 65.2, 63.0, 60.2, 50.0; HRMS: calculated $[C_{14}H_{15}ClN_2O_4 + H]^+$ 311.0720, found 311.0721.

(2*R*,3*R*,4*R*,5*S*)-5-(Hydroxymethyl)-2-(3-undecylisoxazol-5-yl)-pyrrolidine-3,4-diol (25). The title compound **25** was synthesized by the procedure described for the preparation of compound **8** in 47% yield over three steps. $[\alpha]_D^{20}$ +15.23 (*c* 0.14 in MeOH); IR (neat): 3329 (br), 2929 (m), 1622 (s), 1459 (s) cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ 0.90 (t, J = 6.8 Hz, 3H), 1.29–1.34 (br, 18H), 1.65–1.68 (m, 2H), 2.65 (t, J = 7.4 Hz, 1H), 3.30–3.32 (m, 1H), 3.65 (dd, J = 5.5, 11.4 Hz, 1H), 3.71 (dd, J = 3.8, 11.4 Hz, 1H), 3.89 (t, J = 6.6 Hz, 1H), 4.15 (t, J = 6.5 Hz, 1H), 4.18 (d, J = 7.1 Hz, 1H), 6.32 (s, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 174.1, 165.8, 102.7, 82.8, 79.0, 64.9, 62.8, 59.7, 33.3, 30.9, 30.8, 30.7, 30.6, 30.4, 29.5, 23.9, 14.7; HRMS: calculated $[C_{19}H_{34}N_2O_4 + H]^+$ 355.2597, found 355.2584.

Assay for glycosidase inhibitory activity[19]

The inhibitory activities of α-glucosidase from *Bacillus stearothermophilus* (Sigma, G3651) and β-glucosidase from almonds (Sigma, G0395) were determined by measuring the absorbance of 4-nitrophenol at 405 nm. To evaluate the inhibition against α-glucosidase, the reaction mixture consisted of 10 μL of enzyme (1 U mL^{-1}), 20 μL of a synthesized molecule, 50 μL of 100 mM sodium phosphate buffer (pH 6.8) and 20 μL of 15 mM 4-nitrophenyl-α-D-glucopyranoside. To evaluate the inhibition against β-glucosidase, the reaction mixture consisted of 10 μL of enzyme (1 U mL^{-1}), 20 μL of a synthesized molecule, 45 μL of 100 mM sodium citrate buffer (pH 5.2) and 25 μL of 4 mM 4-nitrophenyl-β-D-glucopyranoside. After incubating at 37 °C for 30 minutes, 100 μL of 0.5 M glycine buffer (pH 10.2) was added to the reaction mixture to stop the reaction. The concentration of inhibitors required for inhibiting 50% of glycosidase activity under the assay conditions was defined as the IC_{50} value. The IC_{50} value was measured graphically by a plot of percent inhibition *versus* log of the test compound. The K_m value was determined using Michaelis–Menten kinetics.

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