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Structure–activity relationships in a series of C2-substituted gluco-configured tetrahydroimidazopyridines as β-glucosidase inhibitors

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ABSTRACT

Inhibition of glycoside hydrolases has widespread application in treatment of diabetes, viral infections, lysosomal storage diseases and cancers. Gluco-configured tetrahydroimidazopyridines are the most potent β-glucosidase inhibitors reported to date. Using transition state mimic strategy, a series of C2-substituted gluco-configured tetrahydroimidazopyridines were designed and synthesized. Compounds **3** (K_i = 0.64 nM) and **5** (K_i = 0.58 nM) showed stronger inhibitory potency against β-glucosidase. Maestro 9.1 was used to study the structure–activity relationships by docking the compounds into the β-glucosidase active sites.

1. Introduction

Glycosidase inhibition is important not only for the insightful understanding of enzyme mechanism, but also for questing promising therapeutics in treatment of disorders such as diabetes,[1] viral infections including HIV and influenza,[2] metastatic cancer[3] and lysosomal storage diseases.[4,5] The knowledge of the crystal structure of glycosidase and its complexes with known inhibitors provides a rich framework to design more potent inhibitors.[6] Currently, more than 20,000 glycosidase sequences are known and have been classified into over 100 glycosidase families based upon amino-acid sequence similarity (http://www.cazy.org/).[7] The β-glucosidases from Sweet almonds and *Thermotoga maritima* (*Tm*GH1) are both belonged to family GH1 of the CAZy system which hydrolyze glycosides with net retention of anomeric configuration using a double-displacement mechanism in which a covalent glycosyl-enzyme intermediate is formed.[8,9] Subsequently, the covalent intermediate is dissociated via a short-lived oxocarbenium ion-like transition state (Fig. 1). The catalytic mechanism involves two key catalytic carboxylates: an acid/base and a nucleophile. In the first step, the acid/base carboxylate provides an acid-catalyzed leaving group departure, simultaneously a nucleophilic attack by the other residue is responsible for the formation of the covalent glycosyl-enzyme intermediate. In the second step, the acid/base carboxylate functions as a general base assistance to nucleophilic attack by water, which hydrolyzes the glycosyl-enzyme intermediate.[6,10] The transition state for enzymatic glycoside hydrolysis displays sp^2 hybridization with a partial positive charge predominantly located across the bond between the anomeric carbon and endocyclic oxygen, and likely involves distortion of the pyranoside to half-chair ($^4H_3$ and $^3H_4$ or their equivalent ^4E and ^3E envelop forms) or boat ($^{2,5}B$ or $B_{2,5}$) conformations.[11,12]

Some of the most powerful glycosidase inhibitors are bicyclic compounds which contain imidazole moieties, such as the glucoimidazole β-glucosidase inhibitors **1** and **2** (Fig. 2a).[13,14] Both compounds possess a nonhydrolyzable glycosidic C=N bond between anomeric carbon of the 'sugar' and N1 of imidazole moieties, and their anomeric carbons display sp^2 hybridization. The conformations of both compounds in the ground state accurately mimic the assumed flattened half-chair/envelope conformation of the sugar ring in the transition state resulting from fusion of the planar imidazole ring to the 'glycon', and protonation of the imidazole ring effectively imitates the charge distribution in the oxocarbenium ion (Fig. 2b).[15] Furthermore, both inhibitors also possess a lone pair electrons in N1 atom of imidazole moieties for lateral 'anti-protonation' by the acid/base residue.[16] Compound **2** bearing C2 substituent with a phenethyl group shows that an aglycon mimicking group in this position leads to stronger inhibition, and compound **2** also is proposed to interact with active site residues in the +1 subsite which would result in increasing potency and affinity[9,14,16] Gluco-configured tetrahydroimidazopyridine **1** showed

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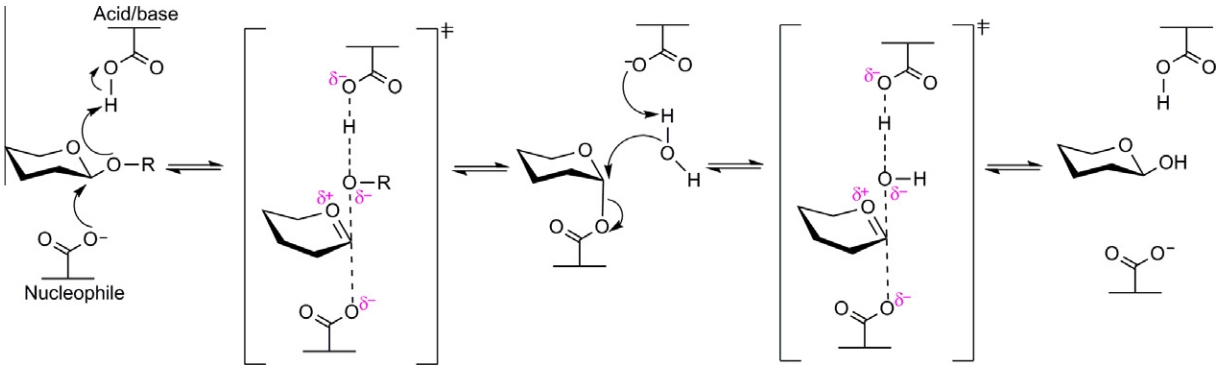


Figure 1. Canonical retaining mechanism for β-glycoside hydrolysis via a short-lived oxocarbenium ion-like transition state.

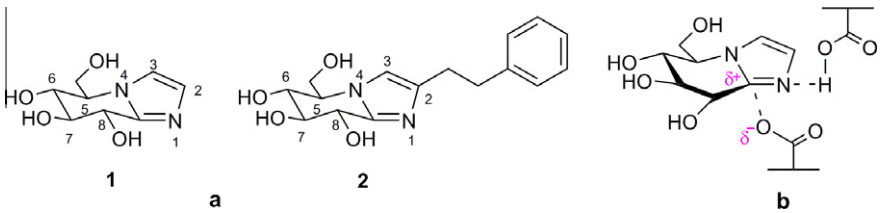


Figure 2. (a) Structure of gluco-configured tetrahydroimidazopyridine **1** and phenethyl-substituted gluco-configured tetrahydroimidazopyridine **2**; (b) proposed mechanism of inhibition of β-glucosidase by tetrahydroimidazopyridine **1**.

significant inhibition against β-glucosidase from Sweet almonds (K_i = 100 nM).[17] Provocatively, compound **2** with a phenethyl group substituted at C2 displayed a 50 times stronger inhibition (K_i = 1.9 nM) than compound **1** against β-glucosidase from Sweet almonds. Therefore, in our research, a novel series of C2-substituted gluco-configured tetrahydroimidazopyridines were designed and synthesized. Their inhibitory activities were evaluated and structure–activity relationships were further investigated.

2. Chemistry

A successful synthetic strategy toward gluco-configured tetrahydroimidazopridine derivatives was shown in Scheme 1. Commercially available methyl α-D-glucopyranoside **1a** was applied as the starting material, which was subjected to per-benzylation using NaH and BnBr to afford methyl 2,3,4,6-tetra-*O*-benzyl-alpha-D-glucopyranoside **2a**.[18] Subsequently, compound **2a** was treated with glacial acetic acid and sulphuric acid for hydrolyzing glycosidic bond to give 2,3,4,6-tetra-*O*-benzyl-D-glucose **3a**.[19] After Swern oxidation of **3a** with DMSO/Ac_2O, tetrabenzylgluconolactone **4a** was obtained with a high yield (96%).[20] Ammonolysis of **4a** with methanolic ammonia resulted in the hydroxy-amide **5a** in high yield (97%). Swern oxidation of **5a** with DMSO/Ac_2O gave the corresponding keto amide **6a** and isomers, which can be used for next reaction without further purification. Reduction of **6a** with sodium cyanoborohydride and formic acid afforded the lactam **7a**, and the two steps yield from **5a** to **7a** was 45%.[21] Lactam **7a** was reacted with Lawesson's reagent at 25 °C to afford thiolactam **8a** in 96% yield. Using $Hg(OAc)_2$ activation of the thiolactam **8a**, which was treated with highly nucleophilic aminoacetaldehyde dimethyl acetal to yield a mixture of the gluco-configurated amidine **9a** and manno-configurated amidine, which can be directly used for next reaction without further purification. Cyclization of the crude amidines with acidic condition ($TsOH{\cdot}H_2O$) in the presence of additional H_2O led to gluco-configurated imidazole **10a** with 48% overall yield from the thiolactam **8a**.[13]

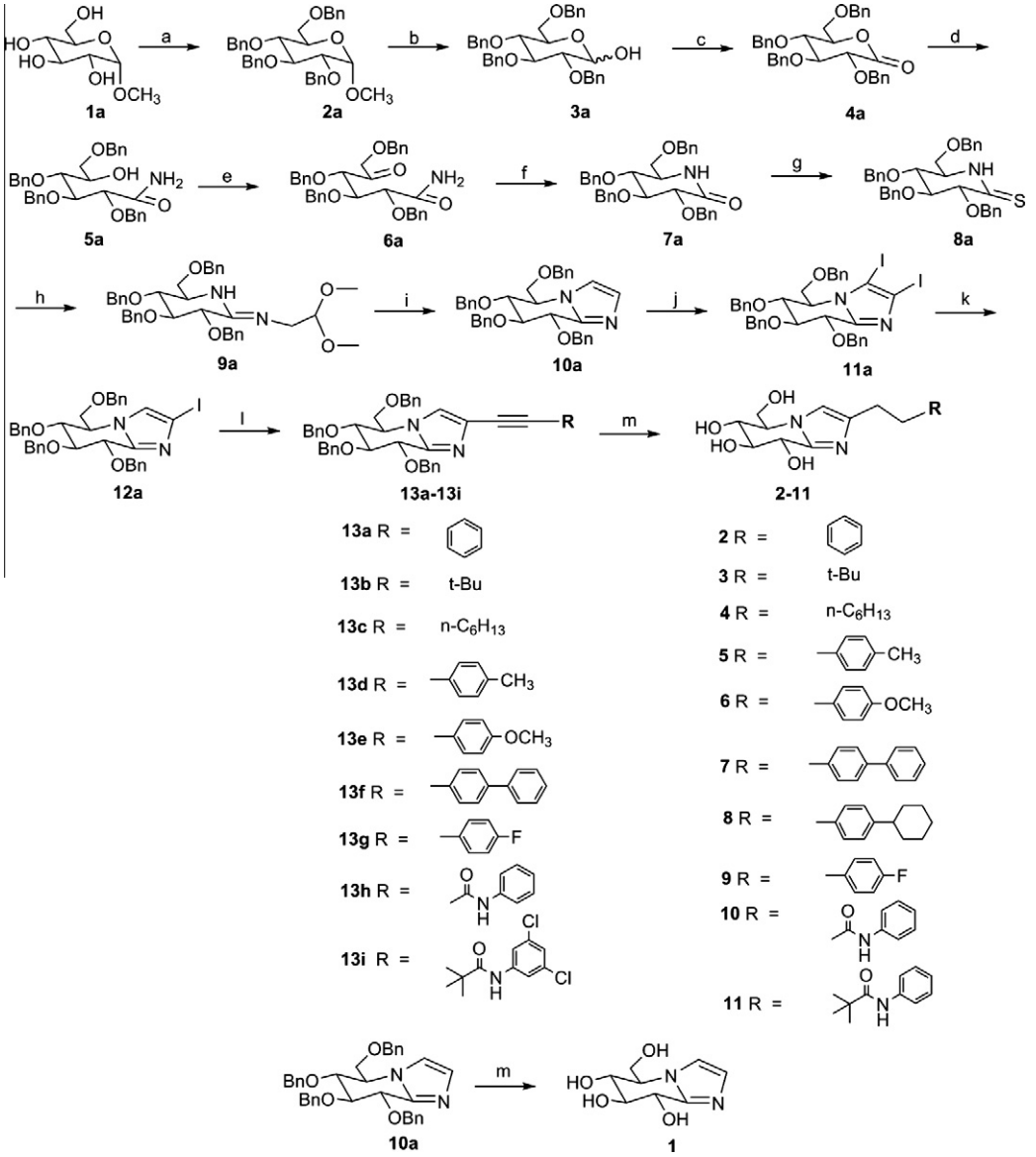
Treatment of gluco-configurated imidazole **10a** with excess NIS resulted in the 2,3-diiodo substituted imidazole **11a** in high yield (90%). Mono-deiodination of **11a** by sequential treatment with EtMgBr afforded the 2-iodo substituted imidazole **12a** as a key intermediate in 88% yield. Moreover, the structure of the compound **12a** was established by X-ray (Fig. 3). Then **12a** was subjected to Sonogashira coupling reaction, which was treated with monosubstituted alkynes in the presence of $Pd(PPh_3)_4$, CuI and Et_3N. These reactions led to the corresponding coupling products **13a–13i** with yields ranging from 64% to 76%. Finally, treatment of **10a** and **13a–13i** using H_2 and $Pd(OH)_2/C$ resulted in debenzylated products **1–11** with yields ranging from 42% to 72%.[22]

3. Enzymatic assays

Inhibitory activities of C2-substituted gluco-configured tetrahydroimidazopyridines **1–11** were tested against β-glucosidase from Sweet almonds. The K_i values were displayed in Table 1. Compound **2** with C2 substituted using a phenethyl group displayed a 50 times stronger inhibition (K_i = 1.9 nM) than compound **1** (K_i = 100 nM). Compound **3** bearing a 2,2-dimethylbutyl group at C2 showed a 156-fold higher potency (K_i = 0.64 nM) than compound **1**. And compound **5** with C2 substituted using a *p*-methyl-phenethyl group (K_i = 0.58 nM) showed a 3-fold higher potency than compound **2**.

4. Structure–activity relationship

For C2-substituted gluco-configured tetrahydroimidazopyridines in Table 1, compound **2** (with phenethyl, K_i = 1.9 nM) and **3** (with 2,2-dimethylbutyl, K_i = 0.64 nM) showed stronger inhibition than their parent compound **1**. Although compound **10** (K_i = 6.8 nM) and **11** (K_i = 3.5 nM) also displayed stronger inhibitory potency than parent compound **1**, their inhibitory potencies were weaker than compounds **2** and **3**. This suggested that hydrophobic substituted group at C2-position contributed to improving the enzyme inhibitory activity. However, when the hydrophobic chain on C2-substituted group became longer, the inhibitory potency became weaker as the compound **4** (K_i = 10.7 nM) had a



Scheme 1. Reagents and conditions: (a) NaH, BnBr, DMF, 0 °C→rt, 85%; (b) 80% AcOH, 1 M H_2SO_4, reflux, 60%; (c) Ac_2O, DMSO, rt, 96%; (d) NH_3, MeOH, 0 °C→rt, 97%; (e) Ac_2O, DMSO, rt; (f) $NaCNBH_3$, HCOOH, MeCN, reflux, 45% (over two steps); (g) Lawesson's reagent, toluene, 25 °C, 96%; (h) aminoacetaldehyde dimethyl acetal, THF, 0 °C; (i) TsOH·H_2O, toluene, 65 °C, 48% (over two steps); (j) NIS, DMF, 80 °C, 90%; (k) 1 M EtMgBr, THF, 0 °C, 88%; (l) RCCH, $Pd(PPh_3)_4$, DMF, 80 °C, 64–76%; (m) H_2, $Pd(OH)_2$/C, EtOAc/MeOH/H_2O/AcOH, 42–72%.

lower inhibitory potency than compounds **2** and **3**, which demonstrated the length of the chain had a great effect on inhibition against β-glucosidase. To find better inhibitors, we also introduced substituents onto the 4-position of the phenyl as compared to the compound **2**. A fascinating discovery here was that introducing a small electron-donating group improved the enzyme inhibitory potency, and introducing a big electron-donating group or electro-withdrawing group reduced the inhibition potency. Compound **5** (K_i = 0.58 nM) showed a 3-fold inhibitory potency increase than compound **2**, while compound **6** (K_i = 2.0 nM) and **9** (K_i = 3.2 nM) showed slightly weaker potency than compound **2**. Surprisingly, compound **7** (K_i = 24.5 nM) and **8** (K_i = 56.1 nm) with big electron-donating group showed about 12-fold and 28-fold weaker potency respectively than compound **2**, which may be caused by the substitution groups that were too big to make the inhibitors well accommodated into the enzyme pocket.

The Glide from Maestro 9.1 was used to perform the docking simulation to study the structure–activity relationships of designed inhibitors with the β-glucosidase activity sites.[23] Since structure and sequence information of β-glucosidase from Sweet almonds was yet unknown, structure of β-glucosidase from the *Thermotoga maritima* (*Tm*GH1) (PDB ID: 2J7D) was used as our initial protein model for docking under the assumption that β-glucosidase from *Thermotoga maritima* had similar active sites as that from Sweet almonds because they both belonged to β-glucosidase family 1.[6,8] Figure 4 showed the best docked pose of compound **5** (the best binder among the tested compounds in this paper with a K_i of 0.58 nM) with the enzyme. In this simulated structure, the sugar moiety of the inhibitor occupied the active site of the enzyme, establishing hydrogen bond interactions mainly with important residue N165, E351, E405. The sp^2 hybridization of carbon contributed to the pyranose ring adopting an envelope (^{4}E) conformation which was similar to the transition state. The N1 atom of the imidazole moiety formed hydrogen bond with the lateral 'anti-protonating' catalytic acid/base residue (E166). The 4-methylphenethyl group stretched away from the active site,

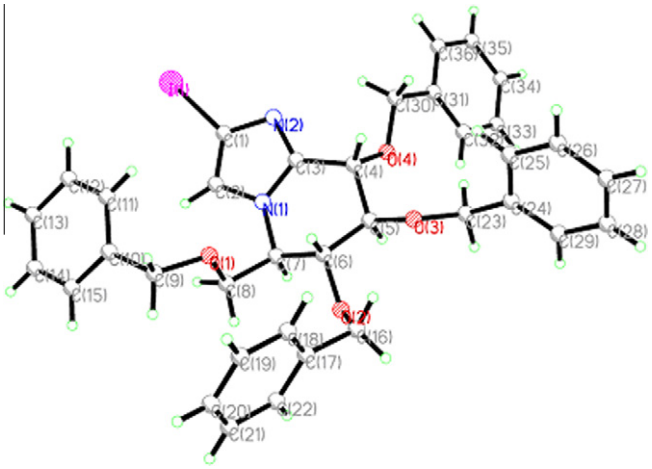


Figure 3. X-ray crystal structure of the compound **12a**.

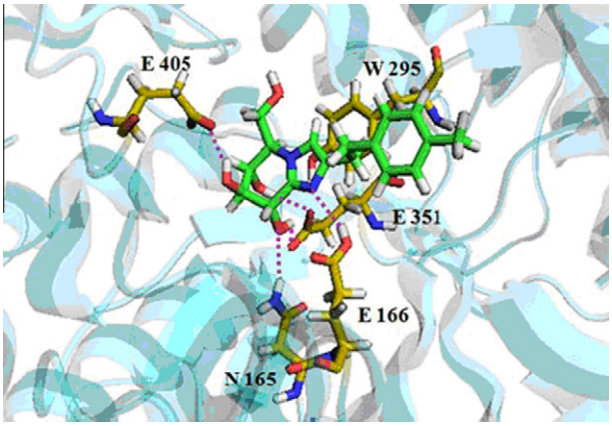
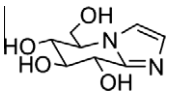
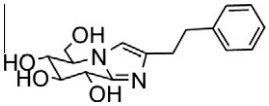
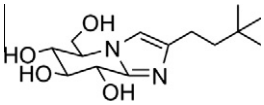
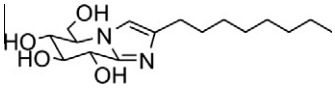
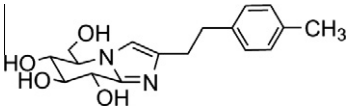
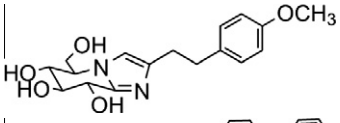
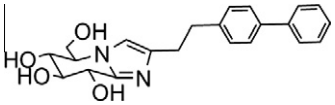
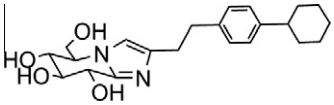
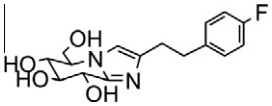
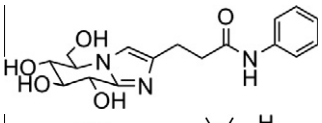
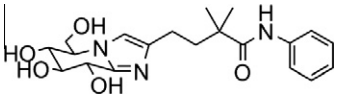


Figure 4. The best docked pose of compound **5** against *Tm*GH1 structure 2J7D.

Table 1
Inhibition constants K_i (nM) of gluco-configured tetrahydroimidazopridine derivatives against β-glucosidase from Sweet almonds

	Compound	K_i (nM) (β-glucosidase from almonds)
1		100
2		1.9
3		0.64
4		10.7
5		0.58
6		2.0
7		24.5
8		56.1
9		3.2
10		6.8
11		3.5

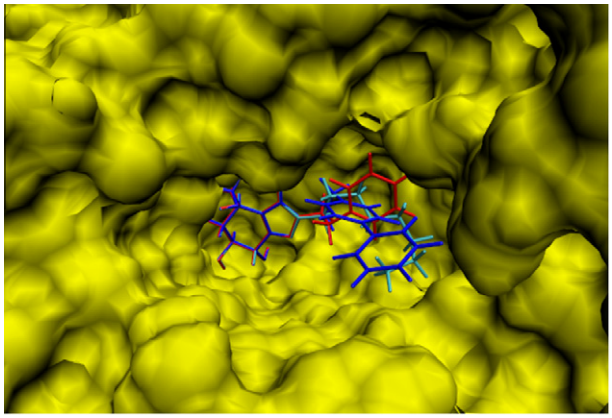


Figure 5. 3D-Superimpostion the docked conformations of compound **4** (sticks with baby blue), **5** (sticks with red), **7** (sticks with blue) into *Tm*GH1 active site.

interacting with the solvent-exposed W295. 3D-Superimpostion comparison of compounds **4**, **5** and **7** was shown in Figure 5. The octyl of compound **4** and the 2-(biphenyl-4-yl)ethyl of compound **7** protruded out of the active site onto the surface of *Tm*GH1. The octyl and 2-(biphenyl-4-yl)ethyl were more solvent exposed than the 4-methylphenethyl of compound **5**, which made **4** and **7** having fewer productive contacts with the enzyme. Alternatively, the conformational rigidity imposed by two ring system (compounds **7** and **8)** did not allow the this type of inhibitor to adopt a conformation that was either sufficiently close to that of the transition state or one which maximized adventitious interactions with the enzyme.

5. Conclusion

In summary, a series of C2-substituted gluco-configured tetrahydroimidazopyridines were synthesized as β-glucosidase inhibitors. Compounds **3** (K_i = 0.64 nM) and **5** (K_i = 0.58 nM) showed stronger inhibitory potency than parent compounds **1** and **2**. The structure–activity relationships indicated the hydrophobic chain length of C2-substituted group could significantly affect the inhibitory potency. For compound **2** derivatives, a small electron-donating substituent group at the 4-position of the phenyl could make inhibitors well accommodated into the enzyme pocket. Overall, these new compounds represent a novel chemical scaffolds for developing highly potent and specific drugs.

6. Experimental section

6.1. Chemistry

All reagents were purchased from commercially sources and were used without further purification. All solvents were available commercially dried or freshly dried and distilled prior to use. Reactions were monitored by Thin Layer Chromatography (TLC) using silica gel GF_{254} plates with detection by short wave UV fluorescence (λ = 254 nm) and staining with 10% phosphomolybdic acid in EtOH. Column chromatography was conducted by silica gel (200–300 mesh) with ethyl acetate and petroleum ether (60–90 °C) or dichloromethane and methanol as eluent. ^{1}H NMR and ^{13}C NMR were recorded with Bruker AV 400 spectrometer at 400 MHz (^{1}H NMR), 100 MHz (^{13}C NMR) using $CDCl_3$, DMSO-d_6 or CD_3OD as solvents. Chemical shifts were reported in δ (ppm) from TMS internal standard (0.00 ppm). Coupling constants were reported in hertz. High-resolution mass spectra (HRMS) were obtained on a Varian QFT-ESI mass spectrometer.

6.1.1. Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (2a)

Benzyl bromide (9.19 mL, 77 mmol) was added to a stirred solution of methyl α-D-glucopyranoside (3.0 g, 15 mmol) in anhydrous DMF (30 mL). The reaction mixture was cooled to 0 °C and sodium hydride (3.6 g, 90 mmol, 60% dispersion in oil) was then added portion-wise. The mixture was then allowed to warm to room temperature. After 17 h, TLC (petroleum ether/ethyl acetate = 5:1) indicated complete conversion of starting material to a major product (R_f = 0.50). The reaction mixture was quenched with methanol, diluted with diethyl ether (200 mL) and washed with water (100 mL). The aqueous phase was extracted with diethyl ether (200 mL) and the combined organic was dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 20:1) to give **2a** (7.2 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.37 (s, 3H, OCH_3), 3.54–3.65 (m, 3H), 3.70–3.75 (m, 2H), 3.98 (t, J = 9.6 Hz, 1H), 4.45–4.48 (m, 2H), 4.58–4.67 (m, 3H), 4.78–4.84 (m, 3H), 4.98 (d, J = 10.8 Hz, 1H), 7.12–7.36 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.20, 68.51, 70.08, 73.42, 73.51, 75.06, 75.78, 77.70, 79.87, 82.16, 98.24, 127.61, 127.71, 127.88, 127.93, 128.00, 128.17, 128.38, 128.42, 128.47, 137.95, 138.20, 138.29, 138.83; HRMS (ESI) calcd for $[C_{35}H_{38}O_6+Na]^+$ 577.2561, found 577.2564.

6.1.2. 2,3,4,6-Tetra-*O*-benzyl-D-glucopyranoside (3a)

A solution of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside **2a** (3.7 g, 6.7 mmol) in 80% AcOH (100 mL) was added 1 M H_2SO_4 (25 mL). After the solution was refluxed for 6 h, the reaction mixture was neutralized by ice water containing 2.5 g NaOAc. This mixture was concentrated to dryness on the rotoevaporator. Then the mixture was suspended in water (100 mL) and the crude product was extracted using CH_2Cl_2 (2 × 100 mL), the extracts were washed with saturated aqueous solutions of $NaHCO_3$ (100 mL), followed by saturated aqueous solutions of NaCl (100 mL), dried over Na_2SO_4, filtered and concentrated to dryness. The crude product was recrystallized with EtOAc, affording a white solid **3a** (2.2 g, 60%). The white solid was used for the next step.

6.1.3. 2,3,4,6-Tetra-*O*-benzyl-D-gluconolactone (4a)

Acetic anhydride (7.6 mL, 80 mmol) was added under argon atmosphere to a solution of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside **3a** (2.2 g, 4 mmol) in anhydrous DMSO (11.4 mL, 160 mmol). The mixture was stirred for 16 hours at ambient temperature. TLC analysis showed complete conversion of starting material to a major product R_f = 0.66 (petroleum ether/ethyl acetate = 5:1). The reaction mixture was diluted with diethyl ether (150 mL), washed thoroughly with water (2 × 60 mL), 10% aqueous solution of $NaHCO_3$ (3 × 50 mL), and twice with saturated NaCl (2 × 50 mL) solution. After drying on Na_2SO_4, the organic layer was concentrated in vacuo. The crude product was purified by silica gel column chromatography. Elution was performed with petroleum ether/ethyl acetate = 25:1 to give **4a** (2.06 g, 96%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.67–3.74 (m, 2H), 3.89 (t, J = 6.4 Hz, 1H), 4.02 (t, J = 6.4 Hz, 1H), 4.37 (d, J = 6.4 Hz, 1H), 4.47–4.56 (m, 3H), 4.60–4.72 (m, 5H), 4.87 (d, J = 11.6 Hz, 1H), 7.22–7.36 (m, 20H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 68.29, 72.29, 72.37, 72.73, 75.29, 77.38, 77.57, 79.60, 127.57, 127.66, 127.71, 127.74, 127.79, 127.86, 128.24, 137.50, 137.60, 137.81, 137.86, 169.07; HRMS (ESI) calcd for $[C_{34}H_{34}O_6+Na]^+$ 561.2248, found 561.2254.

6.1.4. 2,3,4,6-Tetra-*O*-benzyl-D-gluconamide (5a)

A solution of 2,3,4,6-tetra-*O*-benzyl-D-gluconolactone **4a** (5.3 g, 10 mmol) in saturated methanolic ammonia (60 mL) was stirred at 0 °C for 2 h. TLC analysis showed complete conversion of starting material to a major product R_f = 0.25 (petroleum ether/ethyl acetate = 1:1). Subsequently, it was concentrated in vacuo. The residue was recrystallized with EtOAc and hexane, resulting in a white

solid **5a** (5.4 g, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.53 (dd, J = 5.6 Hz, J = 10.0 Hz, 1H), 3.63–3.71 (m, 2H), 3.81–3.86 (m, 1H), 4.05–4.09 (m, 2H), 4.45–4.50 (m, 4H), 4.61–4.72 (m, 4H), 5.09 (d, J = 5.2 Hz, 1H), 7.19–7.51 (m, 22H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 69.79, 71.67, 72.34, 73.52, 74.94, 79.61, 80.30, 81.78, 127.19, 127.25, 127.33, 127.55, 127.61, 127.78, 127.95, 128.01, 128.13, 128.16, 137.81, 138.42, 138.83, 138.85, 172.51; HRMS (ESI) calcd for $[C_{34}H_{37}NO_6+Na]^+$ 578.2513, found 578.2518.

6.1.5. 2,3,4,6-Tetra-*O*-benzyl-5-dehydro-5-oxo-D-gluconamide (6a)

Acetic anhydride (10.3 mL, 0.108 mol) was added under argon atmosphere to a solution of the compound **5a** (3.0 g, 5.4 mmol) in anhydrous DMSO (20.6 mL, 0.216 mol). The mixture was stirred for 8 h at room temperature. TLC analysis showed complete conversion of starting material to a major product with R_f = 0.5 (petroleum ether/ethyl acetate = 1:1). The reaction mixture was diluted with diethyl ether (150 mL), washed thoroughly with water (2 × 60 mL), 10% aqueous solution of $NaHCO_3$ (3 × 60 mL), and twice with saturated NaCl (2 × 60 mL) solution. After drying on Na_2SO_4, the organic layer was concentrated in vacuo to give a yellow syrup (2.6 g), which can be directly used for next reaction without further purification.

6.1.6. 2,3,4,6-Tetra-*O*-benzyl-D-gluconolactam (7a)

Formic acid (10.6 mL, 0.28 mol) was slowly added to a solution of $NaCNBH_3$ (0.59 g, 9.4 mmol) and the compound **6a** (2.6 g, 4.7 mmol) in anhydrous acetonitrile (40 mL). The reaction mixture was refluxed for 2.5 h. TLC analysis indicated the complete disappearance of the starting material to a major product with R_f = 0.63 (petroleum ether/ethyl acetate = 1:1). After cooling to 0 °C, the mixture was quenched by addition of aqueous HCl (1 M, 9.4 mL). After stirring for 15 min, the mixture was poured into a stirred mixture of EtOAc:10% aqueous $NaHCO_3$ solution (200 mL, 1:1). The aqueous layer was separated and extracted with EtOAc (3 × 100 mL). The organic fractions were combined and washed with saturated aqueous NaCl (150 mL) solution. After drying on Na_2SO_4, the organic layer was filtered and concentrated to dryness. The residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 5:1) to give a white solid **7a** (1.31 g). The two steps yield was 45%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.25 (t, J = 8.0 Hz, 1H), 3.50–3.61 (m, 3H), 3.91 (t, J = 8.0 Hz, 1H), 3.99 (d, J = 8.0 Hz, 1H), 4.41–4.50 (m, 3H), 4.71–4.78 (m, 2H), 4.82–4.87 (m, 2H), 5.18 (d, J = 11.2 Hz, 1H), 5.97 (br s, 1H, NH), 7.17–7.43 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.75, 70.02, 73.32, 74.63, 74.74, 77.08, 78.79, 82.31, 127.79, 127.88, 128.00, 128.13, 128.36, 128.41, 128.47, 128.55, 137.24, 137.53, 137.81, 138.00, 170.48; HRMS (ESI) calcd for $[C_{34}H_{35}NO_5+Na]^+$ 560.2407, found 560.2406.

6.1.7. 2,3,4,6-Tetra-*O*-benzyl-D-glucothionolactam (8a)

2,3,4,6-Tetra-*O*-benzyl-D-gluconolactam **7a** (2.15 g, 4.0 mmol) and Lawesson's reagent (1.62 g, 4.0 mmol) in toluene (60 mL) was stirred at 25 °C for 28 h. TLC analysis showed complete conversion of starting material to a major product (R_f = 0.27, diethyl ether/ethyl acetate = 3:1). Then the mixture was concentrated in vacuo, the crude product was purified by silica gel column chromatography (diethyl ether/ethyl acetate = 5:1) to give a white solid **8a** (2.14 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.37 (dd, J = 8.0 Hz, J = 9.2 Hz, 1H), 3.57 (dd, J = 4.8 Hz, J = 9.2 Hz, 1H), 3.63 (dd, J = 3.2 Hz, J = 9.2 Hz, 1H), 3.86–3.91 (m, 2H), 4.35 (d, J = 11.6 Hz, 1H), 4.43–4.49 (m, 4H), 4.58 (d, J = 11.6 Hz, 1H), 4.67 (d, J = 11.6 Hz, 1H), 4.74 (d, J = 11.2 Hz, 1H), 5.02 (d, J = 11.6 Hz, 1H), 7.14–7.42 (m, 20H), 8.13 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.93, 68.38, 72.54, 72.61, 72.79, 73.43, 78.41, 81.31, 82.47, 127.92, 128.03, 128.13, 128.24, 128.34, 128.42, 128.50, 128.62, 137.09, 137.39, 137.52, 200.42; HRMS (ESI) calcd for $[C_{34}H_{35}NO_4S+Na]^+$ 576.2179, found 576.2183.

6.1.8. 2,3,4,6-Tetra-*O*-benzyl-1,5-dideoxy-1-[(2',2'-dimethoxyethyl)imino]-1,5-imino-D-glucitol (9a)

A solution of 2,3,4,6-tetra-*O*-benzyl-D-glucothionolactam **8a** (1.0 g, 1.8 mmol) in anhydrous THF (15 mL) was treated with aminoacetaldehyde dimethyl acetal (l.0 mL, 9.3 mmol) and $Hg(OAc)_2$ (0.80 g, 2.5 mmol), and kept at 0 °C for 50 min. TLC analysis showed complete conversion of starting material to a major product with R_f = 0.20 and a by-product with R_f = 0.18 (petroleum ether/ethyl acetate = 1:2). The mixture was filtered, washed with water, extracted with ethyl acetate, dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 1:1→1:5) to afford a yellow oil which was a mixture of the gluco/manno-configurated amidines (1.0 g).

6.1.9. (5*R*,6*R*,7*S*,8*S*)-6,7,8-Tris(benzyloxy)-5-[(benzyloxy)-methyl]-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (10a)

A mixture of the gluco/manno-configurated amidines (1.0 g, 1.6 mmol) was dissolved in toluene (30 mL), treated with $TsOH·H_2O$ (0.95 g, 5.0 mmol) and H_2O (1.0 mL), and stirred at 65 °C for 18 h. TLC analysis showed complete conversion of starting material to a major product (R_f = 0.45, petroleum ether/ethyl acetate = 1:1). Then the mixture was concentrated in vacuo, the crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate/Et_3N = 500:80:10) affording a yellow syrup **10a** (484.4 mg), and the yield of the two steps was 48%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.73 (dd, J = 5.6 Hz, J = 10.4 Hz, 1H), 3.83–3.87 (m, 2H), 4.08 (dd, J = 5.6 Hz, J = 7.6 Hz, 1H), 4.18 (ddd, J = 2.8 Hz, J = 5.6 Hz, J = 8.0 Hz, 1H), 4.42–4.51 (m, 3H), 4.68 (d, J = 11.6 Hz, 1H), 4.75 (d, J = 6.0 Hz, 1H), 4.80–4.90 (m, 3H), 5.18 (d, J = 11.6 Hz, 1H), 7.04 (d, J = 1.2 Hz, 1H), 7.12 (d, J = 1.2 Hz, 1H), 7.17–7.44 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 58.20, 68.39, 72.80, 73.29, 74.06, 74.21, 75.95, 81.84, 117.45, 127.63, 127.87, 127.91, 127.97, 128.02, 128.11, 128.21, 128.35, 128.48, 128.55, 128.96, 137.29, 137.58, 137.83, 138.18, 143.90; HRMS (ESI) calcd for $[C_{36}H_{36}N_2O_4+H]^+$ 561.2748, found 561.2752.

6.1.10. (5*R*,6*R*,7*S*,8*S*)-6,7,8-Tris(benzyloxy)-5-[(benzyloxy)-methyl]-2,3-diiodo-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (11a)

A solution of **10a** (2.0 g, 3.57 mmol) in DMF (40 mL) was treated with *N*-iodosuccinimide (NIS) (8.0 g, 35.7 mmol) and kept at 80 °C for 6 h. TLC analysis showed complete conversion of starting material with R_f = 0.08 to a major product with R_f = 0.56 (petroleum ether/ethyl acetate = 5:1). The reaction mixture was diluted with Et_2O (100 mL), washed with saturated aqueous NH_4Cl solution (2 × 30 mL), 5% aqueous $Na_2S_2O_3$ solution (30 mL) and water (2 × 30 mL), dried over Na_2SO_4, filtered and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 1:0→10:1) affording a yellow oil **11a** (2.56 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.64 (dd, J = 4.8 Hz, J = 9.2 Hz, 1H), 3.71 (t, J = 9.2 Hz, 1H), 4.08 (t, J = 4.0 Hz, 1H), 4.33 (ddd, J = 2.4 Hz, J = 4.8 Hz, J = 8.0 Hz, 1H), 4.42–4.48 (m, 4H), 4.54–4.58 (m, 2H), 4.66–4.70 (m, 2H), 4.82 (d, J = 11.6 Hz, 1H), 5.15 (d, J = 11.6 Hz, 1H), 7.19–7.43 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 60.79, 69.33, 71.95, 72.18, 72.81, 72.95, 73.05, 73.14, 77.85, 81.24, 96.72, 127.64, 127.81, 127.88, 127.95, 128.00, 128.08, 128.15, 128.33, 128.48, 137.30, 137.39, 137.49, 138.06, 148.94; HRMS (ESI) calcd for $[C_{36}H_{34}I_2N_2O_4+H]^+$ 813.0681, found 813.0687.

6.1.11. (5*R*,6*R*,7*S*,8*S*)-6,7,8-Tris(benzyloxy)-5-[(benzyloxy)-methyl]-2-iodo-5,6,7,8-tetrahydroimidazo[1,2-*a*]pyridine (12a)

A solution of **11a** (2.5 g, 3.13 mmol) in THF (25.0 mL) was treated with a 1 M solution of EtMgBr in anhydrous THF (4.7 mL, 4.70 mmol), stirred at 0 °C for 10 min. TLC analysis showed complete conversion of starting material with R_f = 0.68 to a major product with R_f = 0.56 (petroleum ether/ethyl acetate = 4:1). The reaction mixture was treated with a saturated aqueous NH_4Cl solution (10 mL) and extracted with EtOAc (2 × 40 mL). The organic fractions were combined, dried over Na_2SO_4, filtered and concentrated in vacuum. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 20:1→15:1) to afford a white solid **12a** (1.85 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.68 (dd, J = 5.6 Hz, J = 10.4 Hz, 1H), 3.76- 3.82 (m, 2H), 4.06 (dd, J = 5.2 Hz, J = 6.8 Hz, 1H), 4.17 (ddd, J = 2.4 Hz, J = 5.6 Hz, J = 8.0 Hz, 1H), 4.40–4.49 (m, 3H), 4.62 (d, J = 11.2 Hz, 1H), 4.71–4.82 (m, 4H), 5.11 (d, J = 11.6 Hz, 1H), 7.09 (s, 1H), 7.15–7.41 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 58.36, 68.27, 72.61, 73.30, 73.41, 73.80, 73.98, 75.81, 81.29, 123.30, 127.63, 127.91, 127.99, 128.08, 128.18, 128.32, 128.46, 128.52, 128.59, 137.12, 137.47, 137.65, 138.05, 145.82; HRMS (ESI) calcd for $[C_{36}H_{35}IN_2O_4+H]^+$ 687.1714, found 687.1718.

6.1.12. General procedure for synthesis of compounds 13a–13i

A mixture of **12a** (201.8 mg, 0.3 mmol), $Pd(PPh_3)_4$ (17.3 mg, 0.015 mmol), Et_3N (0.22 mL, 1.5 mmol), CuI (5.7 mg, 0.03 mmol) and monosubstituted alkynes **13a–13i** (0.9 mmol) in DMF (6.0 mL) was stirred at 80 °C for 3 h under argon atmosphere. TLC analysis showed complete conversion of starting material to a major product. The reaction mixture was cooled to room temperature, diluted with Et_2O (25 mL), washed with a saturated aqueous NH_4Cl solution (3 × 10 mL), dried over Na_2SO_4, filtered and concentrated in vacuum. The crude product was purified by silica gel column chromatography to give the pure product.

6.1.12.1. (5R,6R,7S,8S)-6,7,8-Tris(benzyloxy)-5-[(benzyloxy)-methyl]-2-(2-phenylethynyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine (13a). A yellow solid (150.6 mg, 76%, R_f = 0.49, petroleum ether/ethyl acetate = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.73 (dd, J = 5.6 Hz, J = 10.4 Hz, 1H), 3.82–3.85 (m, 2H), 4.11 (dd, J = 5.2 Hz, J = 7.2 Hz, 1H), 4.20 (ddd, J = 2.8 Hz, J = 5.6 Hz, J = 7.6 Hz, 1H), 4.44–4.51 (m, 3H), 4.64 (d, J = 11.2 Hz, 1H), 4.73–4.86 (m, 4H), 5.17 (d, J = 11.6 Hz, 1H), 7.16–7.55 (m, 26H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 58.30, 68.29, 72.67, 73.31, 73.60, 73.79, 73.99, 75.89, 81.44, 83.29, 89.20, 121.85, 123.37, 127.60, 127.90, 127.97, 128.08, 128.15, 128.22, 128.32, 128.46, 128.56, 131.54, 137.14, 137.44, 137.65, 138.07, 144.11; HRMS (ESI) calcd for $[C_{44}H_{40}N_2O_4+H]^+$ 661.3061, found 661.3067.

6.1.12.2. (5R,6R,7S,8S)-6,7,8-Tris(benzyloxy)-5-[(benzyloxy)-methyl]-2-(3,3-dimethylbut-1-ynyl)-5,6,7,8-tetrahydroimidazo-[1,2-a]pyridine (13b). A yellow solid (138.3 mg, 72%, R_f = 0.42, petroleum ether/ethyl acetate = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.32 (s, 9H), 3.71 (dd, J = 5.2 Hz, J = 10.4 Hz, 1H), 3.78–3.83 (m, 2H), 4.08 (dd, J = 5.2 Hz, J = 6.8 Hz, 1H), 4.16 (ddd, J = 2.8 Hz, J = 5.2 Hz, J = 7.6 Hz, 1H), 4.42–4.50 (m, 3H), 4.61 (d, J = 11.2 Hz, 1H), 4.70–4.80 (m, 4H), 5.10 (d, J = 11.6 Hz, 1H), 7.12 (s, 1H), 7.14–7.40 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.97, 31.01, 58.04, 68.32, 72.45, 73.24, 73.64, 73.89, 76.14, 81.63, 120.85, 127.52, 127.87, 127.93, 127.98, 128.05, 128.28, 128.44, 128.53, 137.26, 137.54, 137.71, 138.24, 143.46; HRMS (ESI) calcd for $[C_{42}H_{44}N_2O_4+H]^+$ 641.3374, found 641.3377.

6.1.12.3. (5R,6R,7S,8S)-6,7,8-Tris(benzyloxy)-5-(benzyloxymethyl)-2-(oct-1-ynyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine (13c). A yellow solid (143.3 mg, 70%, R_f = 0.39, petroleum ether/ethyl acetate = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, J = 6.8 Hz, 3H), 1.23–1.61 (m, 9H), 2.40 (t, J = 6.8 Hz, 1H), 3.68 (dd, J = 5.6 Hz, J = 10.4 Hz, 1H), 3.77–3.82 (m, 2H), 4.05–4.19 (m, 2H), 4.40–4.49 (m, 3H), 4.62–4.71 (m, 2H), 4.75–4.83 (m, 3H), 5.11 (d, J = 11.6 Hz, 1H), 7.08–7.40 (m, 21H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.12, 19.57, 22.60, 28.69, 28.71, 31.45, 58.31, 68.37, 72.64, 73.29, 73.50, 73.85, 74.03, 75.96, 81.70, 90.25, 120.66, 123.25, 127.86, 127.91, 128.00, 128.14, 128.18, 128.29, 128.47, 128.60, 137.25, 137.55, 137.77, 138.23, 143.61; HRMS (ESI) calcd for $[C_{44}H_{48}N_2O_4+H]^+$ 669.3687, found 669.3689.

6.1.12.4. (5R,6R,7S,8S)-6,7,8-Tris(benzyloxy)-5-(benzyloxymethyl)-2-((4-methylphenyl)ethynyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine (13d). A yellow solid (139.7 mg, 69%, R_f = 0.49, petroleum ether/ethyl acetate = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H), 3.73 (dd, J = 5.6 Hz, J = 10.4 Hz, 1H), 3.81–3.85 (m, 2H), 4.10 (dd, J = 5.2 Hz, J = 6.8 Hz, 1H), 4.20 (ddd, J = 2.8 Hz, J = 5.6 Hz, J = 8.0 Hz, 1H), 4.43–4.51 (m, 3H), 4.63 (d, J = 11.2 Hz, 1H), 4.74–4.86 (m, 4H), 5.17 (d, J = 12.0 Hz, 1H), 7.12–7.45 (m, 25H); ^{13}C NMR (100 MHz, $CDCl_3$): 21.55, 58.33, 68.30, 72.72, 73.33, 73.60, 73.82, 74.02, 75.89, 81.45, 120.27, 121.65, 127.63, 127.93, 128.00, 128.08, 128.11, 128.19, 128.34, 128.49, 128.59, 129.03, 131.49, 137.17, 137.46, 137.67, 138.11, 138.15, 144.04; HRMS (ESI) calcd for $[C_{45}H_{42}N_2O_4+H]^+$ 675.3217, found 675.3219.

6.1.12.5. (5R,6R,7S,8S)-6,7,8-Tris(benzyloxy)-5-(benzyloxymethyl)-2-[(4-methoxyphenyl)ethynyl]-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine (13e). A yellow solid (149.2 mg, 72%, R_f = 0.37, petroleum ether/ethyl acetate = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.73 (dd, J = 5.6 Hz, J = 10.4 Hz, 1H), 3.81–3.85 (m, 5H), 4.10 (dd, J = 5.6 Hz, J = 7.2 Hz, 1H), 4.20 (ddd, J = 2.8 Hz, J = 5.6 Hz, J = 8.0 Hz, 1H), 4.43–4.51 (m, 3H), 4.64 (d, J = 11.2 Hz, 1H), 4.73–4.86 (m, 4H), 5.17 (d, J = 11.6 Hz, 1H), 6.85–6.87 (m, 2H), 7.16–7.49 (m, 23H); ^{13}C NMR (100 MHz, $CDCl_3$): 55.30, 58.29, 68.31, 72.70, 73.32, 73.84, 74.04, 75.91, 81.56, 113.90, 115.51, 121.43, 127.61, 127.92, 127.96, 128.00, 128.07, 128.11, 128.18, 128.34, 128.48, 128.58, 133.06, 137.18, 137.47, 137.69, 138.13, 144.01, 159.45; HRMS (ESI) calcd for $[C_{45}H_{42}N_2O_5+H]^+$ 691.3167, found 691.3174.

6.1.12.6. (5R,6R,7S,8S)-6,7,8-Tris(benzyloxy)-5-(benzyloxymethyl)-2-(biphenyl-4-ylethynyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine (13f). A yellow solid (154.7 mg, 70%, R_f = 0.42, petroleum ether/ethyl acetate = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.74 (dd, J = 5.6 Hz, J = 10.8 Hz, 1H), 3.82–3.86 (m, 2H), 4.11 (dd, J = 5.2 Hz, J = 6.8 Hz, 1H), 4.22 (ddd, J = 2.8 Hz, J = 5.6 Hz, J = 7.6 Hz, 1H), 4.44–4.52 (m, 3H), 4.65 (d, J = 11.2 Hz, 1H), 4.74–4.87 (m, 4H), 5.17 (d, J = 12.0 Hz, 1H), 7.17–7.18 (m, 2H), 7.25–7.63 (m, 28H); ^{13}C NMR (100 MHz, $CDCl_3$): 58.32, 68.32, 72.67, 73.31, 73.62, 73.79, 73.98, 75.91, 81.46, 121.91, 122.31, 126.92, 127.01, 127.54, 127.60, 127.90, 127.97, 128.08, 128.15, 128.32, 128.46, 128.57, 128.83, 131.94, 137.16, 137.45, 137.65, 138.08, 140.45, 140.68, 144.15; HRMS (ESI) calcd for $[C_{50}H_{44}N_2O_4+H]^+$ 737.3374, found 737.3366.

6.1.12.7. (5R,6R,7S,8S)-6,7,8-Tris(benzyloxy)-5-(benzyloxymethyl)-2-((4-fluoroph-enyl)ethynyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine (13g). A yellow solid (136.4 mg, 67%, R_f = 0.50, petroleum ether/ethyl acetate = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.73 (dd, J = 5.6 Hz, J = 10.4 Hz, 1H), 3.81–3.85 (m, 2H), 4.10 (dd, J = 5.2 Hz, J = 7.2 Hz, 1H), 4.21 (ddd, J = 2.8 Hz, J = 5.6 Hz, J = 8.0 Hz, 1H), 4.43–4.51 (m, 3H), 4.63 (d, J = 11.2 Hz, 1H), 4.73–4.86 (m, 4H), 5.15 (d, J = 11.6 Hz, 1H), 7.00–7.04 (m, 2H), 7.16–7.42 (m, 21H), 7.50–7.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 58.33, 68.32, 72.69, 73.29, 73.53, 73.79, 73.97, 75.82, 81.31, 115.41, 115.63, 121.85, 127.62, 127.92, 127.97, 128.08, 128.14, 128.32, 128.46, 128.56, 133.36, 133.44, 137.13, 137.40, 137.60,

138.02, 144.13, 161.12; HRMS (ESI) calcd for $[C_{44}H_{39}FN_2O_4+H]^+$ 679.2967, found 679.2973.

6.1.12.8. N-Phenyl-3-(5R,6R,7S,8S)-6,7,8-Tris(benzyloxy)-5-(benzyloxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridin-2-yl)propiolamide (13h). A yellow solid (137.1 mg, 65%, R_f = 0.39, petroleum ether/ethyl acetate = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.70 (dd, J = 6.0 Hz, J = 10.4 Hz, 1H), 3.79–3.83 (m, 2H), 4.09 (dd, J = 5.6 Hz, J = 6.4 Hz, 1H), 4.21 (ddd, J = 2.8 Hz, J = 6.0 Hz, J = 8.0 Hz, 1H), 4.41–4.50 (m, 3H), 4.62 (d, J = 11.2 Hz, 1H), 4.69–4.82 (m, 4H), 5.11 (d, J = 11.6 Hz, 1H), 7.13–7.17 (m, 3H), 7.26–7.47 (m, 21H), 7.54–7.56 (m, 2H), 7.82 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 58.64, 68.18, 72.76, 73.34, 73.45, 73.83, 73.98, 75.65, 80.52, 81.03, 84.39, 119.73, 121.46, 124.70, 125.83, 127.79, 127.98, 128.02, 128.08, 128.13, 128.23, 128.41, 128.52, 128.66, 129.12, 136.92, 137.28, 137.49, 137.60, 137.77, 144.92, 151.11; HRMS (ESI) calcd for $[C_{45}H_{41}N_3O_5+H]^+$ 704.3119, found 704.3114.

6.1.12.9. N-(3,5-Dichlorophenyl)-2,2-dimethyl-4-((5R,6R,7S,8S)-6,7,8-tris(benzyloxy)-5-(benzyloxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridin-2-yl)but-3-yn-amide (13i). A yellow solid (165.9 mg, 68%, R_f = 0.30, petroleum ether/ethyl acetate = 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.85 (s, 6H), 3.70–3.82 (m, 3H), 4.06–4.18 (m, 2H), 4.43–4.50 (m, 3H), 4.61–4.79 (m, 5H), 5.08 (d, J = 11.6 Hz, 1H), 6.55 (br s, 1H), 7.15–7.65 (m, 24H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.48, 49.96, 58.30, 68.27, 72.59, 73.29, 73.43, 73.68, 73.87, 75.94, 81.22, 85.19, 91.13, 122.17, 125.71, 127.63, 127.97, 128.04, 128.33, 128.47, 128.56, 131.18, 135.36, 137.15, 137.40, 137.56, 137.62, 137.99, 138.10, 143.88, 163.89; HRMS (ESI) calcd for $[C_{48}H_{45}Cl_2N_3O_5+H]^+$ 814.2809, found 814.2809.

6.1.13. General procedure for synthesis of compounds 1–11

A solution of **13a**–**13i** (0.2 mmol) in EtOAc/MeOH/H_2O (3:1:1, 2.5 mL) was treated with AcOH (2.5 mL) and $Pd(OH)_2$/C (100 mg), hydrogenated at 6 bar for 56 h at room temperature, filtered through Celite, and washed with MeOH/H_2O (9:1, 25 mL). The combined filtrates were concentrated in vacuum, the residue was purified by silica gel column chromatography to give the pure product.

6.1.13.1. (5R,6R,7S,8S)-5-(Hydroxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine-6,7,8-triol (1). A white solid (24.8 mg, 62%). ^{1}H NMR (400 MHz, CD_3OD): δ 3.59 (t, J = 8.4 Hz, 1H), 3.72 (t, J = 8.4 Hz, 1H), 3.78–3.87 (m, 2H), 4.09 (dd, J = 2.4 Hz, J = 12.0 Hz, 1H), 4.39 (d, J = 8.4 Hz, 1H), 6.94 (s, 1H), 7.21 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 61.39, 62.84, 69.31, 69.79, 76.69, 118.68, 128.99, 147.79. HRMS (ESI) calcd for $[C_8H_{12}N_2O_4+H]^+$ 201.0870, found 201.0873.

6.1.13.2. (5R,6R,7S,8S)-5-(Hydroxymethyl)-2-(2-phenylethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine-6,7,8-triol (2). A white solid (42 mg, 69%). ^{1}H NMR (400 MHz, CD_3OD): δ 2.79–2.83 (m, 2H), 2.90–2.94 (m, 2H), 3.68 (t, J = 8.4 Hz, 1H), 3.77–3.84 (m, 2H), 3.91 (dd, J = 3.6 Hz, J = 12.0 Hz, 1H), 4.13 (dd, J = 2.0 Hz, J = 12.0 Hz, 1H), 4.48 (d, J = 7.6 Hz, 1H), 6.97 (s, 1H), 7.12–7.26 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD): δ 31.34, 36.87, 61.53, 62.68, 69.37, 69.87, 76.75, 114.76, 126.88, 129.32, 129.44, 143.01, 143.28, 147.03; HRMS (ESI) calcd for $[C_{16}H_{20}N_2O_4+H]^+$ 305.1496, found 305.1492.

6.1.13.3. (5R,6R,7S,8S)-2-(3,3-Dimethylbutyl)-5-(hydroxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine-6,7,8-triol (3). A white solid (39.8 mg, 72%). ^{1}H NMR (400 MHz, CD_3OD): δ 0.86 (s, 9H), 1.42–1.46 (m, 2H), 2.39–2.44 (m, 2H), 3.57 (t, J = 8.0 Hz, 1H), 3.67–3.76 (m, 2H), 3.82 (dd, J = 4.0 Hz, J = 12.0 Hz, 1H), 4.05 (dd, J = 2.4 Hz, J = 12.0 Hz, 1H), 4.37 (d, J = 7.6 Hz, 1H), 6.90 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 24.30, 29.71, 31.14, 44.82, 61.43, 62.75, 69.31, 69.76, 76.69, 114.17, 144.11, 146.84; HRMS (ESI) calcd for $[C_{14}H_{24}N_2O_4+H]^+$ 285.1809, found 285.1810.

6.1.13.4. (5R,6R,7S,8S)-5-(Hydroxymethyl)-2-octyl-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine-6,7,8-triol (4). A white solid (42.5 mg, 68%). ^{1}H NMR (400 MHz, CD_3OD): δ 0.80 (t, J = 6.8 Hz, 3H), 1.20–1.23 (m, 10H), 1.48–1.53 (m, 2H), 2.39–2.43 (m, 2H), 3.57 (t, J = 8.0 Hz, 1H), 3.66–3.75 (m, 2H), 3.82 (dd, J = 4.0 Hz, J = 12.0 Hz, 1H), 4.05 (dd, J = 2.4 Hz, J = 12.0 Hz, 1H), 4.36 (d, J = 8.0 Hz, 1H), 6.89 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 14.47, 23.76, 29.13, 30.45, 30.54, 30.62, 33.09, 61.52, 62.66, 69.36, 69.85, 76.76, 114.39, 143.80, 146.86; HRMS (ESI) calcd for $[C_{16}H_{28}N_2O_4+H]^+$ 313.2122, found 313.2115.

6.1.13.5. (5R,6R,7S,8S)-5-(Hydroxymethyl)-2-(4-methylphenethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine-6,7,8-triol (5). A white solid (45.2 mg, 71%). ^{1}H NMR (400 MHz, CD_3OD): δ 2.18 (s, 3H), 2.70–2.81 (m, 4H), 3.59 (t, J = 8.4 Hz, 1H), 3.71 (t, J = 8.4 Hz, 1H), 3.77–3.85 (m, 2H), 4.05 (dd, J = 2.4 Hz, J = 12.0 Hz, 1H), 4.43 (d, J = 8.0 Hz, 1H), 6.95–7.01 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD): δ 21.10, 30.30, 36.04, 61.13, 63.20, 69.11, 69.33, 76.34, 115.66, 129.32, 130.03, 136.57, 139.56, 140.88, 146.99; HRMS (ESI) calcd for $[C_{17}H_{22}N_2O_4+H]^+$ 319.1652, found 319.1658.

6.1.13.6. (5R,6R,7S,8S)-5-(Hydroxymethyl)-2-(4-methoxyphenethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine-6,7,8-triol (6). A white solid (42.8 mg, 64%). ^{1}H NMR (400 MHz, CD_3OD): δ 2.78 (m, 4H), 3.57–3.66 (m, 4H), 3.72–3.86 (m, 3H), 4.06 (dd, J = 2.4 Hz, J = 11.6 Hz, 1H), 4.48 (d, J = 8.4 Hz, 1H), 6.72–6.74 (m, 2H), 7.01–7.03 (m, 2H), 7.14 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 29.37, 35.23, 55.62, 60.83, 63.72, 68.81, 68.89, 75.96, 114.87, 116.54, 130.37, 134.03, 138.77, 146.99, 159.66; HRMS (ESI) calcd for $[C_{17}H_{22}N_2O_5+H]^+$ 335.1602, found 335.1602.

6.1.13.7. (5R,6R,7S,8S)-2-(2-(Biphenyl-4-yl)ethyl)-5-(hydroxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine-6,7,8-triol (7). A white solid (37.1 mg, 48%). ^{1}H NMR (400 MHz, CD_3OD): δ 2.75–2.90 (m, 4H), 3.61 (t, J = 8.4 Hz, 1H), 3.73 (t, J = 8.4 Hz, 1H), 3.79–3.86 (m, 2H), 4.05 (dd, J = 2.0 Hz, J = 11.6 Hz, 1H), 4.45 (d, J = 8.0 Hz, 1H), 7.00–7.06 (m, 3H), 7.18–7.32 (m, 4H), 7.41–7.48 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ 29.96, 35.80, 61.10, 63.30, 69.09, 69.26, 76.28, 115.92, 127.84, 128.02, 128.16, 129.34, 129.85, 130.00, 139.84, 140.33, 140.39, 141.74, 142.30, 147.07; HRMS (ESI) calcd for $[C_{22}H_{24}N_2O_4+H]^+$ 381.1809, found 381.1811.

6.1.13.8. (5R,6R,7S,8S)-2-((4-Cyclohexyl)phenethyl)-5-(hydroxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine-6,7,8-triol (8). A solution of **13f** (147.3 mg, 0.2 mmol) in EtOAc/MeOH/H_2O (3:1:1, 2.5 mL) was treated with AcOH (2.5 mL) and $Pd(OH)_2$/C (100 mg), hydrogenated at 8 bar for 68 hours at room temperature, filtered through Celite and washed with MeOH/H_2O (9:1, 25 mL). The combined filtrates were concentrated in vacuum, the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) affording a white solid (32.5 mg, 42%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.27–1.38 (m, 4H), 1.61–1.73 (m, 6H), 2.35–2.49 (m, 1H), 2.73–2.79 (m, 4H), 3.59 (t, J = 8.4 Hz, 1H), 3.71 (t, J = 8.4 Hz, 1H), 3.77–3.84 (m, 2H), 4.05 (dd, J = 2.0 Hz, J = 12.0 Hz, 1H), 4.43 (d, J = 8.0 Hz, 1H), 6.99–7.01 (m, 5H). ^{13}C NMR (100 MHz, CD_3OD): δ 27.31, 28.07, 30.05, 30.35, 35.81, 36.08, 45.68, 61.16, 63.19, 69.12, 69.35, 76.38, 115.59, 127.83, 129.33, 140.05, 141.02, 147.00. HRMS (ESI) calcd for $[C_{22}H_{30}N_2O_4+H]^+$ 387.2278, found 387.2276.

6.1.13.9. (5R,6R,7S,8S)-2-(4-Fluorophenethyl)-5-(hydroxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridine-6,7,8-triol (9). A white solid (46.4 mg, 72%). ^{1}H NMR (400 MHz, CD_3OD): δ 2.70–2.80 (m, 4H), 3.58 (t, J = 8.0 Hz, 1H), 3.67–3.83 (m, 3H), 4.04 (dd, J = 2.0 Hz, J = 11.6 Hz, 1H), 4.39 (d, J = 8.0 Hz, 1H), 6.84–7.10 (m, 5H); ^{13}C NMR (100 MHz, CD_3OD): δ 31.06, 35.89, 61.35, 62.79, 69.24, 69.69, 76.65, 115.10, 115.76, 115.97, 131.05, 131.12, 138.99, 139.02, 142.10, 147.13; HRMS (ESI) calcd for $[C_{16}H_{19}FN_2O_4+H]^+$ 323.1402, found 323.1406.

6.1.13.10. N-Phenyl-3-((5R,6R,7S,8S)-6,7,8-trihydroxy-5-(hydroxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]pyridin-2-yl)propanamide (10). A white solid (44.6 mg, 65%). ^{1}H NMR (400 MHz, CD_3OD): δ 2.63–2.90 (m, 4H), 3.60 (t, J = 8.4 Hz, 1H), 3.73 (t, J = 8.4 Hz, 1H), 3.82–3.86 (m, 2H), 4.06 (dd, J = 2.0 Hz, J = 11.6 Hz, 1H), 4.46 (d, J = 8.0 Hz, 1H), 6.96–7.43 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 33.76, 36.80, 61.03, 63.57, 69.05, 69.12, 76.10, 116.41, 121.34, 125.27, 129.81, 139.11, 139.73, 147.25, 172.97; HRMS (ESI) calcd for $[C_{17}H_{21}N_3O_5-H]^-$ 346.1408, found 346.1415.

6.1.13.11. 2,2-Dimethyl-N-phenyl-4-((5R,6R,7S,8S)-6,7,8-trihydroxy-5-(hydroxymethyl)-5,6,7,8-tetrahydroimidazo[1,2-a]-pyridin-2-yl)butanamide (11). A white solid (51.4 mg, 66%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.38 (s, 6H), 2.16–2.20 (m, 2H), 2.59–2.63 (m, 2H), 3.58 (t, J = 8.8 Hz, 1H), 3.73 (t, J = 8.8 Hz, 1H), 3.83–3.88 (m, 2H), 4.08 (dd, J = 2.0 Hz, J = 11.6 Hz, 1H), 4.46 (d, J = 8.4 Hz, 1H), 7.33–7.43 (m, 4H), 7.66–7.70 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD): δ 21.12, 27.49, 38.78, 54.90, 60.36, 64.35, 68.17, 68.61, 75.40, 117.29, 128.38, 129.55, 132.56, 136.68, 136.85, 146.94, 170.55. HRMS (ESI) calcd for $[C_{20}H_{27}N_3O_5+H]^+$ 390.2029, found 390.2032.

6.2. Enzyme kinetics

Inhibition constants (K_i) were determined at 37 °C, using 0.08 M KH_2PO_4/K_2HPO_4 solution as buffer (pH 6.8) and 4-nitrophenyl β-D-glucopyranoside (Sigma) as substrate. The assays were initiated by addition of β-glucosidase from Sweet almonds (Sigma, K_m = 3.5 mM) to a solution of the substrate (concentrations used: 1.17, 1.75, 3.50, 7.00, 10.50 mM) in the absence or presence of various concentrations of inhibitors. After the mixture was incubated for 10 min at 37 °C, the reactions were quenched by adding 1 M Na_2CO_3. The absorption of the resulting mixture was determined at 400 nm. Inhibition constants (K_i) were determined by taking the slopes from the Lineweaver–Burk plots and double reciprocal analysis.[24,25]

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Supplementary data

Supplementary data (^{1}H NMR and ^{13}C NMR spectra of new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.02.043.

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