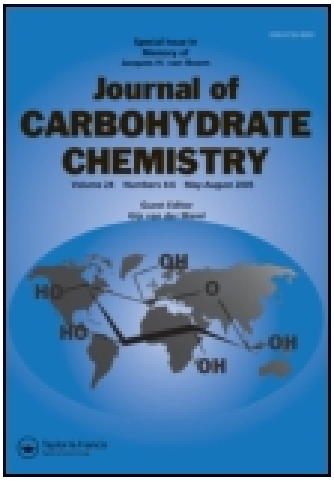

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

Synthesis, Conformational Analysis, and Evaluation as Glycosidase Inhibitors of Two Ether-Bridged Iminosugars

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Published online: 14 Dec 2011.

To cite this article: Bo Luo, Filipa Marcelo, Jérôme Désiré, Yongmin Zhang, Matthieu Sollogoub, Atsushi Kato, Isao Adachi, F. Javier Cañada, Jesús Jiménez-Barbero & Yves Blériot (2011) Synthesis, Conformational Analysis, and Evaluation as Glycosidase Inhibitors of Two Ether-Bridged Iminosugars, Journal of Carbohydrate Chemistry, 30:7-9, 641-654, DOI: 10.1080/07328303.2011.630547

To link to this article: http://dx.doi.org/10.1080/07328303.2011.630547

Journal of Carbohydrate Chemistry, 30:641–654, 2011

ISSN: 0732-8303 print / 1532-2327 online
DOI: 10.1080/07328303.2011.630547

Synthesis, Conformational Analysis, and Evaluation as Glycosidase Inhibitors of Two Ether-Bridged Iminosugars

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Two bicyclic iminosugars have been synthesized from polyhydroxylated azepanes by ring isomerization followed by debenzylative cyclization. Their conformations have been studied by NMR and molecular modeling and their glycosidase inhibition profiles were determined. They both adopt a chair conformation for the piperidine ring and display weak inhibition on α-glucosidases.

Keywords Conformational analysis; Iminosugar; Glycosidase inhibitor; Glycomimetics

INTRODUCTION

Iminosugars, sugar mimics in which the endocyclic oxygen has been replaced by nitrogen, represent the most promising class of sugar analogs as

Received September 4, 2011; accepted October 5, 2011.
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therapeutic agents.[1] Since the discovery of its most famous representative, 1-deoxynojirimycin (DNJ), a vast array of derivatives have been synthesized and assayed on numerous pathologies.[2] Promising results have been obtained in the field of cancer[3] and viral infections[4] including HIV,[5] but more significantly, an *N*-hydroxyethyl analog, miglitol, was approved by the U.S. Food and Drug Administration (FDA) in 1996 for the treatment of late-onset diabetes (marketed as Glyset).[6] Additionally, an *N*-butyl derivative, NB-DNJ or miglustat,[7] marketed as Zavesca, has been launched for the treatment of Gaucher disease, a rare lysosomal disorder. The same compound is in phase II trials for the treatment of cystic fibrosis.[8]

Most of the synthetic efforts have been directed toward five- and six-membered as well as bicyclic iminosugars because of their natural origin and/or therapeutic potency. Unnatural ether-bridged iminosugars have been poorly investigated, although their restricted conformation could lead to interesting biological activities. A dihydroxy-3,6-anhydro iminoheptitol **1** has been independently reported by Kilonda[9] and Poitout.[10] Fuentes has described the synthesis of a series of ether-bridged nortropane-like iminocyclitols **2** bearing various substituents at C-1.[11] Interestingly, N-substituted derivatives **3** and **4** display analgesic and anti-inflammatory activities in mice and rats,[12] while [2,2,2]-bicyclic iminosugar **5**[13] shows weak inhibition on several glycosidases. More recently, Wu disclosed the synthesis and biological evaluation of a series of N-substituted ether bridged bicyclic iminosugars **6a–d** (Fig. 1).[14] We report herein a skeletal rearrangement-based route to similar scaffolds and their evaluation as glycosidase inhibitors.

RESULTS AND DISCUSSION

Our group has reported a new access to six-membered homoiminosugars via the ring isomerization of polyhydroxylated azepanes.[15] In this sequence chloromethyl piperidines were generated by ring contraction of polyhydroxylated azepanes[16] and the chlorine atom was subsequently displaced with an acetate in moderate yield to furnish target homoiminosugars after deprotection. In order to improve the substitution step, we investigated the formation

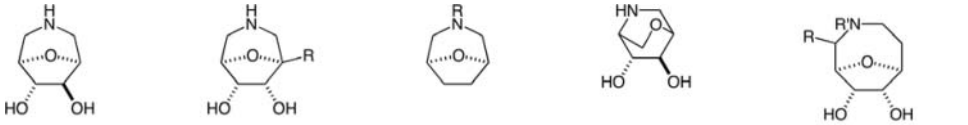
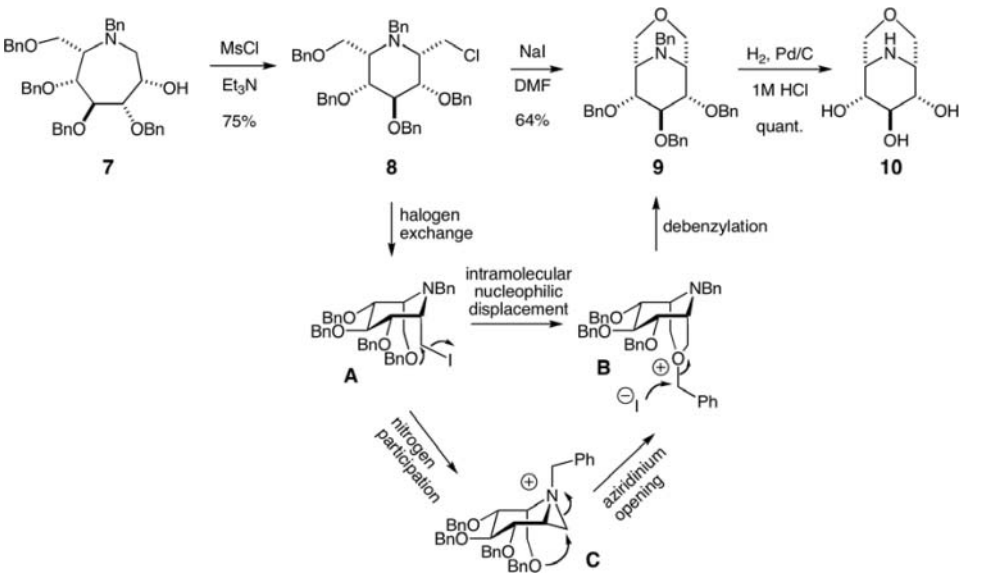


Figure 1: Structure of ether bridged bicyclic iminosugars **1–6.**

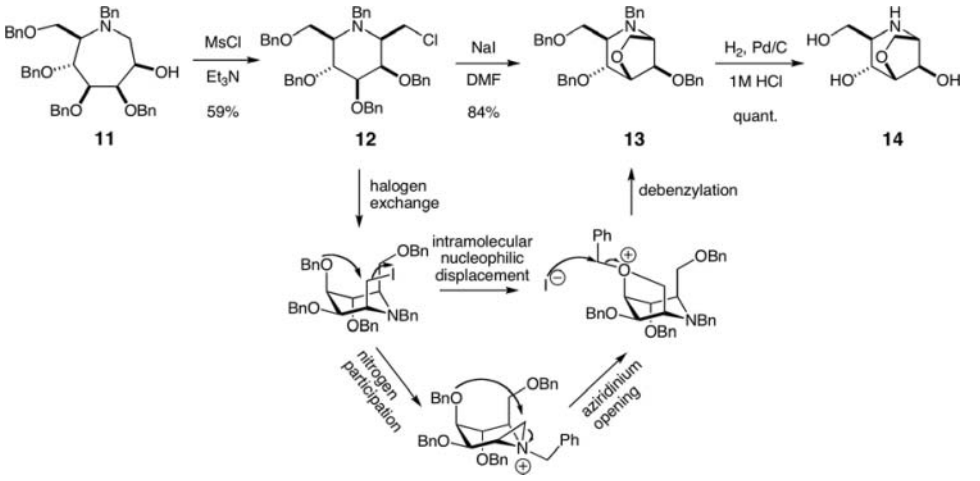
of the more reactive iodomethyl piperidine. Known azepane **7** was first treated with mesyl chloride to yield the corresponding piperidine **8** (75% yield). Reaction of **8** with NaI in DMF did not afford the corresponding iodinated piperidine but the symmetrical bicyclic derivative **9** as the major product (64% yield). Its formation can be tentatively explained by a halogen exchange to yield compound **A** followed by an intramolecular iodine displacement by the benzyl ether at C-6 to furnish intermediate **B** followed by O-debenzylation. Nitrogen participation leading to a fused aziridinium-piperidine scaffold **C** can also take place in this transformation. Final hydrogenolysis quantitatively furnished the bicyclic piperidine **10** (Sch. 1). Simplification of the NMR spectra for compounds **9** and **10** supports their symmetrical structure. Similar cyclizations mediated by iodine have been previously reported in sugar chemistry.[17] Compound **10** displays an interesting structure as it can be seen as a locked iminoxylitol, an iminosugar with therapeutic potential in Gaucher disease.[18] It can also be viewed as a calystegine[19] analog in which the two-carbon bridge locking the iminosugar conformation has been replaced by a dimethyleneoxy tether yielding a fused morpholine-iminosugar hybrid.



Scheme 1: Synthesis of bicycle **10**.

A similar strategy was applied to the *β-D-manno*-like azepane **11**. Ring isomerization with mesyl chloride yielded the piperidine **12** (59% yield). Upon treatment with NaI in DMF the bicyclic derivative **13** was obtained in 84% yield. Unlike the formation of compound **10** involving the primary benzyl ether at C-6, compound **13** results from a debenzylative cyclization involving the benzyl ether at C-4. The regioselectivity of the cyclization can be rationalized by

the short distance between the benzyloxy oxygen at C-4 and the carbon at C-1 (Sch. 2). Final hydrogenolysis furnished the bicycle **14,** the structure of which was studied and confirmed by NMR and molecular modeling (see conformational analysis section).



Scheme 2: Synthesis of bicycle **14.**

Conformational Analysis

In order to confirm the structures of compounds **10** and **14** and deduce their conformations in solution, NMR experiments and molecular mechanics calculations were performed. The coupling constant analysis of iminosugar **10** indicates that it assumes a major conformation in solution that involves two fused piperidine rings, both adopting a chair conformation. In particular, the observed $J_{5,6}$ (5.3 Hz) and $J_{6,7b}$ (2.0 Hz) values are only in accordance with the calculated J values (4.6 Hz and 2.6 Hz, respectively) for such a chair/chair conformation and not with the other boat/chair, chair/boat, and boat/boat conformers (Table 1). Moreover, the combination of a chair/chair conformation is also the energetically preferred solution. Molecular dynamics studies were then conducted using the same force field, using the optimized chair/chair conformation (Fig. 2, left) as starting geometry. The MD simulations were carried out over 10 ns at 298 K, with a 0.05 fs time step and a 5 ps equilibration step; 250 structures were sampled for further analysis (see Fig. 2, right). The MD simulation showed that the chair/chair conformation is fairly stable and only displays minor fluctuations around its basic structure.

The coupling constant analysis of iminosugar **14** indicates that the piperidine ring adopts a chair conformation in solution (Fig. 3). In particular, the observed $J_{4,5}$ (4.6 Hz) and $J_{5,6}$ (1.6 Hz) values agree well with the calculated

Table 1: MM3*-based relative energy values (kJ/mol) and calculated and experimental vicinal coupling constant values (Hz) for the possible six-membered ring conformations of **10**

Conformation (piperidine/morpholine)	Chair/ chair	Boat/ chair	Chair/ boat	Boat/ boat	Experimental
Relative energy (MM3*)	−41.2	−17.6	−21.1	0	
$J_{4,5} = J_{4,3}$ (Hz)	7.1	3.2	7.4	4.3 and 1.9	9.1
$J_{5,6} = J_{3,2}$ (Hz)	4.6	8.2	4.5	7.2 and 8.0	5.3
$J_{6,7a} = J_{2,8a}$ (Hz)	0.9	0.8	5.2	2.3 and 8.1	<1.0
$J_{6,7b} = J_{2,8b}$ (Hz)	2.6	2.7	8.6	7.2 and 8.7	2.0

Figure 2: Left: View of the global minimum geometry for iminosugar **10,** as calculated by MM3* molecular mechanics calculations. Right: Superimposition of 250 structures of **10**, sampled during the 10 ns MD simulation at 298 K and using the GB/SA solvent model for water. Hydrogen atoms have been removed for sake of clarity (color figure available online).

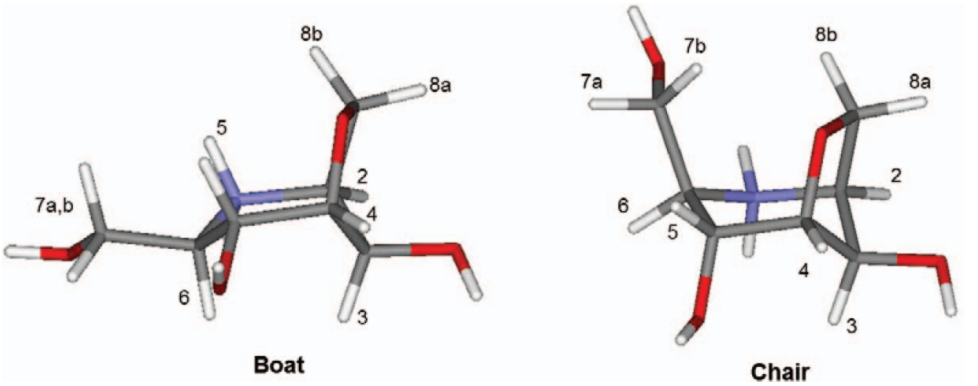


Figure 3: Left: The boat conformation of **14**, as calculated by MM3* molecular mechanics calculations (MM). Right: The chair conformation of **14**. The atomic numbering is also given (color figure available online).

Table 2: MM3*-based relative energy values (kJ/mol) and calculated and experimental vicinal coupling constant values (Hz) for the possible six-membered ring conformations of **14**

Conformation (piperidine)	Chair	Boat	Experimental
Relative energy (MM3*)	−13.2	0.0	
$J_{2,3}$ (Hz)	1.8	1.7	1.0
$J_{2,8a}$ (Hz)	3.7	3.8	3.1
$J_{2,8b}$ (Hz)	1.1	1.1	<1.0
$J_{4,5}$ (Hz)	4.9	0.6	4.6
$J_{4,3}$ (Hz)	0.8	0.9	<1.0
$J_{5,6}$ (Hz)	0.9	7.9	1.6
$J_{6,7a}$ (Hz)	6.6	4.5	6.1
$J_{6,7b}$ (Hz)	11.0	11.0	10.7

values (4.9 Hz and 0.9 Hz, respectively) for a chair conformation but not with the ones corresponding to a boat conformer (Table 2).

Further evidence of the exclusive existence of this conformation in compound **14** was confirmed by analysis of the NOE contacts. NOE values of 15% (H-7a/H-7b), 4% (H-6/H-7a), 4% (H-7b/H-8b), 1.5% (H-3/H-6), and 4.5% (H-4/H-5) were obtained and the corresponding distances between key protons calculated (Fig. 4, top right). The strong NOE observed between H-7b and H-8b supports a chair conformation for the piperidine ring. Molecular dynamics studies were then conducted with the same force field and using the optimized chair conformation (Fig. 3, right) as starting geometry. The MD simulations

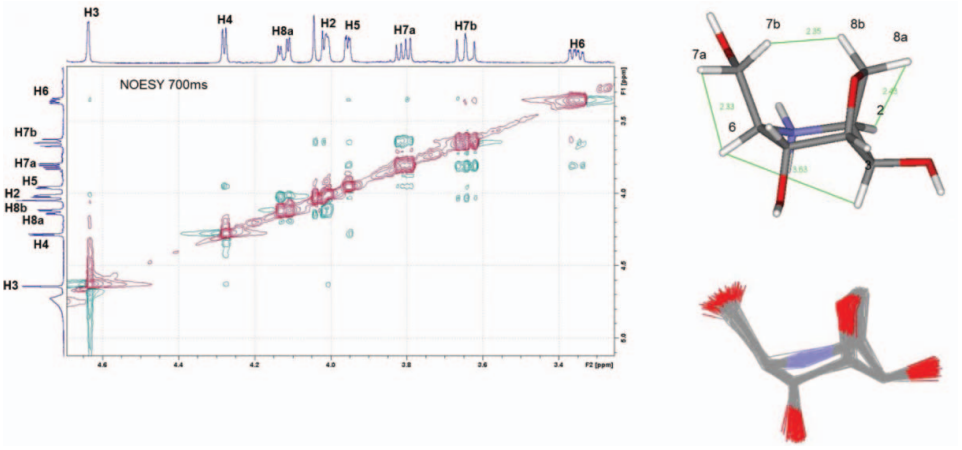


Figure 4: Left: 2D-NOESY (mixing time, 700 ms) of **14**. Right top: The chair conformation of **14,** showing the key interproton distances (in green) observed in the NOESY spectrum. Right bottom: Superimposition of 250 structures of **14,** sampled during the 10 ns MD simulation (MM3*, 298 K, and using the GB/SA solvent model for water). Hydrogen atoms have been removed for sake of clarity (color figure available online).

Table 3: Concentration of iminosugars giving 50% inhibition of glycosidases (IC_{50}, mM)

Enzyme	10	11
α-Glucosidase		
Rice	177	344
Yeast	NI[a] (0%)[b]	50
β-Glucosidase		
Almond	NI (15.8%)	NI (30.2%)
Bovine liver	NI (15.4%)	NI (30.9%)
α-Galactosidase		
Coffee beans	306	NI (15.4%)
β-Galactosidase		
Bovine liver	NI (12.2%)	962
α-Mannosidase		
Jack beans	NI (37.2%)	NI (16.1%)
β-Mannosidase		
Snail	NI (0.4%)	NI (27.9%)
α-L-Rhamnosidase		
P. decumbens	NI (22.3%)	NI (0%)
α-L-Fucosidase		
Bovine epididymis	NI (23.1%)	142
β-Glucuronidase		
Bovine liver	NI (22.8%)	NI (7.1%)
Trehalase		
Porcine kidney	NI (1.5%)	NI (11.4%)
Amyloglucosidase		
Aspergillus niger	NI (0%)	NI (3.1%)
β-Xylosidase		
Aspergillus niger	NI (1.1%)	NI (5.1%)

[a]NI: No inhibition (less than 50% inhibition at 1000 mM).
[b](): inhibition % at 1000 mM.
[c]ND: Not determined.

were carried out over 10 ns at 298 K, with a 0.05 fs time step and a 5 ps equilibration step; 250 structures were sampled for further analysis (see Fig. 4, bottom right). Again, the global minimum geometry was conformationally stable during the MD run, just displaying minor fluctuations around its basic structure.

Glycosidase Inhibition

The two ether-bridged iminosugars **10** and **14** were assayed for their inhibitory activity toward 14 commercially available glycosidases. They did not inhibit the following enzymes at 1 mM concentration and optimal pH: almond and bovine liver β-glucosidases, Jack bean α-mannosidase, *Helix pomatia* snail β-mannosidase, *P. decumbens* α-L-rhamnosidase, bovine liver β-glucuronidase porcine kidney trehalase, *Aspergillus niger* amyloglucosidase,

and β-xylosidase. Compound **10** proved to be a weak inhibitor of rice α-glucosidase (IC_{50} 177 μM) and coffee bean α-galactosidase (IC_{50} 306 μM), while **14** was found to be a moderate inhibitor of yeast α-glucosidase (IC_{50} 50 μM) and a weak inhibitor of rice α-glucosidase (IC_{50} 344 μM), bovine liver β-galactosidase (IC_{50} 962 μM), and bovine epididymis α-L-fucosidase (IC_{50} 142 μM) (see Table 3).

CONCLUSION

In conclusion, two bicyclic ether-bridged iminosugars **10** and **14** have been prepared by ring isomerization of polyhydroxylated azepanes followed by debenzylative cyclization. They both adopt a chair-like spatial arrangement for the polyhydroxylated piperidine ring, compound **10** displaying a $^4C_1$ conformation analogous to glucose, while **14** showed a $^1C_4$ conformation analogous to mannose. Their glycosidase inhibition profile was determined, demonstrating that they are weak inhibitors of α-glucosidases.

EXPERIMENTAL SECTION

General Methods

Melting points (m.p.) were determined with a Büchi B-535 apparatus and are uncorrected. Optical rotations were measured at 20 $\pm$ 2°C with a Perkin Elmer Model 241 digital polarimeter, using a 10-cm, 1-mL cell. Chemical ionization mass spectra (CI-MS ammonia) and fast atom bombardment mass spectra (FAB-MS) were recorded on a JMS-700 spectrometer. ^{1}H NMR and ^{13}C NMR were performed on a Bruker DRX 400 spectrometer (400 MHz for ^{1}H, 100.6 MHz for ^{13}C). All chemical shifts (δ) are given in ppm relative to the residual deuterated solvent signals. Coupling constants (J) are reported in Hertz. Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F_{254} precoated plates and detection by charring with sulfuric acid. Flash column chromatography was performed on silica gel Merck 60 (230–400 mesh).

(2S,3R,4R,6S,5S)-1-Benzyl-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)azepan-6-ol **7**

R_f 0.26, Cy/EtOAc, 4:1; $[\alpha]_D$ −7.8 (c = 1.1 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): 7.38–7.27 (m, 25H, 5 × Ph), 4.77 (d, 1H, J = 11.0 Hz, CHPh), 4.73 (d, 1H, J = 11.7 Hz, CHPh), 4.71 (d, 1H, J = 11.0 Hz, CHPh), 4.70 (d, 1H, J = 11.4 Hz, CHPh), 4.60 (d, 1H, J = 11.7 Hz, CHPh), 4.52 (d, 1H, J = 12.0 Hz, CHPh), 4.50 (d, 1H, J = 11.4 Hz, CHPh), 4.47 (d, 1H, J = 12.0 Hz, CHPh), 4.05–3.95 (m, 5H, NCH_2Ph, H-6, H-4, H-8a), 3.89 (dd, 1H, J = 6.8, 3.2 Hz, H-3), 3.80 (dd, 1H,

J = 5.3, 2.1 Hz, H-5), 3.69 (dd, 1H, J = 9.8, 5.3 Hz, H-8b), 3.35 (m, 1H, H-2), 3.20 (dd, 1H, J = 14.1, 6.9 Hz, H-7a), 2.76 (dd, 1H, J = 14.1, 3.6 Hz, H-7b). ^{13}C NMR ($CDCl_3$, 100 MHz): 138.48, 138.43, 138.28, 138.21 (5 × Cipso), 128.46–126.85 (25 aromatic C), 83.66 (C-2), 82.16 (C-4), 81.44 (C-3), 73.96, 73.43, 72.95, 72.87 (4 × CH_2Ph), 70.27 (C-1), 67.77 (C-6), 60.82 (C-5), 59.09 (NCH_2Ph), 51.83 (C-7); ESI-HRMS: Calcd for $C_{42}H_{46}NO_5$ ($M + H^+$): 644.3376. Found 644.3383.

(2S,3R,4S,5S,6S)-1-Benzyl-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(chloromethyl) piperidine ***8***

To a stirred solution of alcohol **7** (61 mg, 0.09 mmol) in dry DCM (2 mL) was added Et_3N (38 μL, 0.27 mmol) at 0°C under an atmosphere of nitrogen, followed by addition of MsCl (11 μL, 0.138 mmol). The reaction mixture was stirred at 0°C for 1 h. TLC (Cy/EtOAc, 4:1) showed a complete reaction. The reaction mixture was concentrated under reduced pressure. Purification by preparative thin-layer plates (Cy/EtOAc, 4:1) gave chloromethyl piperidine **8** as a clear oil (45 mg, 75%).

R_f 0.73, Cy/EtOAc, 4:1; $[\alpha]_D$ +18 (c = 1.4 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): 7.40–7.30 (m, 25H, 5 × Ph), 4.98 (s, 2H, CH_2Ph), 4.73 (d, 1H, J = 11.2 Hz, CHPh), 4.62 (d, 1H, J = 11.2 Hz, CHPh), 4.61 (s, 2H, CH_2Ph), 4.57 (d, 1H, J = 12.0 Hz, CHPh), 4.53 (d, 1H, J = 12.0 Hz, CHPh), 4.24 (s, 2H, CH_2Ph), 3.94 (dd, 1H, J = 11.2, 5.7 Hz, H-8a), 3.86 (dd, 1H, J = 10.0, 5.8 Hz, H-7a), 3.85 (app. t, 1H, J = 5.4 Hz, H-8b), 3.78–3.71 (m, 3H, H-5, H-3, H-4), 3.65 (dd, 1H, J = 10.0, 7.0 Hz, H-7b), 3.60 (m, 1H, J = 5.5, 2.9 Hz, H-2), 3.48 (m, 1H, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz): 139.95, 138.81, 138.32, 138.16 (5 × Cipso), 128.34–126.98 (25 aromatic C), 79.99 (C-4), 79.70 (C-5), 78.55 (C-3), 75.08, 73.43, 72.98, 72.71 (4 × CH_2Ph), 70.07 (C-7), 61.67 (C-2), 58.06 (C-6), 57.97 (NCH_2Ph), 43.61 (C-1); ESI-HRMS ($M+H^+$): Calcd for $C_{42}H_{45}O_4NCl$: 662.3037. Found: 662.3020.

(1S,5R,6S,7R,8R)-9-Benzyl-6,7,8-tris(benzyloxy)-3-oxa-9-aza-bicyclo[3.3.1]nonane ***9***

A mixture of chloromethyl piperidine **8** (31 mg, 0.054 mmol) and NaI (33 mg, 0.216 mmol) in DMF (1 mL) was stirred at 130°C for 3 h. The reaction mixture was concentrated under reduced pressure. The resulting residue was purified by preparative thin-layer plates (Cy/EtOAc, 4:1) to give bicyclic derivative **9** (15 mg, 64%) as an oil.

R_f 0.5, petroleum ether/EtOAc, 9:1; ^{1}H NMR ($CDCl_3$, 400 MHz): 7.40–7.26 (m, 20H, 4 × Ph), 4.96 (s, 2H, 2 × CHPh), 4.69 (d, 2H, J = 11.8 Hz, 2 × CHPh), 4.60 (dd, 2H, J = 11.8 Hz, 2 × CHPh), 4.55 (t, 1H, J = 8.6 Hz, H-7), 4.00 (d, 2H, J = 11.3 Hz, H-2a, H-4a), 3.93 (m, 4H, H-6, H-8, NCH_2Ph), 3.74 (d, 2H, J = 11.2 Hz, H-2b, H-4b), 2.72 (d, 2H, J = 4.7 Hz, H-1, H-5); ^{13}C NMR

($CDCl_3$, 100 MHz): 139.36, 138.62, 138.50 (4 × Cipso), 128.44–127.27 (20 aromatic C), 84.67 (C-7), 80.26 (C-6, C-8), 75.56, 72.65 (3 × CH_2Ph), 61.90 (C-2, C-4), 55.68 (NCH_2Ph), 54.43 (C-1, C-5); ESI-HRMS: Calcd for $C_{35}H_{38}NO_4$ ($M + H^+$): 536.2801. Found 536.2804.

(1S,5R,6S,7R,8R)-3-Oxa-9-aza-bicyclo[3.3.1]nonan-6,7,8-triol ***10***

To a solution of **9** (12 mg, 0.027 mmol) in MeOH (2 mL) containing 1 M aq. HCl (20 μL) was added 10% Pd/C (5 mg). The suspension was stirred under H_2 atmosphere for 4 h at rt, filtered through a pad of Celite, and eluted with MeOH. The solvent was removed under reduced pressure to afford compound **10** (5.6 mg, quant.) as its hydrochloride salt.

$[\alpha]_D$ 0 (c = 0.3 in MeOH); ^{1}H NMR (D_2O, 400 MHz): 4.27–4.20 (m, 3H, J = 13.6, 9.2 Hz, H-4a, H-2a, H-7), 3.95 (dd, 2H, J = 9.2, 5.2 Hz, H-6, H-8), 3.86 (d, 2H, J = 13.6 Hz, H-4b, H-2b), 3.58 (d, 2H, J = 5.2 Hz, H-1, H-5); ^{13}C NMR (D_2O, 100 MHz): 74.05 (C-7), 69.56 (C-6, C-8), 61.87 (C-2, C-4), 51.53 (C-1, C-5); ESI-HRMS: Calcd for $C_7H_{14}O_4N$ ($M + H^+$): 176.0917. Found 176.0914.

(2R,3R,4S,5R,6R)-1-Benzyl-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)azepane-6-ol ***11***

R_f 0.46, Cy/EtOAc, 4:1; $[\alpha]_D$ +26 (c = 1.1 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): 7.43–7.28 (m, 25H, 5 × Ph), 4.81 (d, 1H, J = 11.6 Hz, CHPh), 4.74 (d, 1H, J = 11.4 Hz, CHPh), 4.71 (d, 2H, J = 11.6 Hz, CH_2Ph), 4.63 (d, 1H, J = 11.4 Hz, CHPh), 4.55 (d, 1H, J = 12.0 Hz, CHPh), 4.50 (d, 1H, J = 12.0 Hz, CHPh), 4.49 (d, 1H, J = 11.4 Hz, CHPh), 4.19 (dd, 1H, J = 7.0, 4.2 Hz), 4.09 (d, 1H, J = 13.9 Hz, NCHPh), 4.07 (m, 1H), 3.91 (d, 1H, J = 14.0 Hz, NCHPh), 3.85 (dd, 1H, J = 10.0, 5.9 Hz), 3.82 (dd, 1H, J = 6.5, 4.2 Hz), 3.80 (dd, 1H, J = 7.0, 4.4 Hz), 3.75 (dd, 1H, J = 10.0, 4.1 Hz), 3.43 (br. dd, 2H, J = 10.8, 6.1 Hz), 3.33 (br. S), 3.03 (dd, 1H, J = 13.5, 2.8 Hz), 2.92 (dd, 1H,break; J = 13.5, 7.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): 139.92, 138.51, 138.40, 138.26, 137.85 (5 × Cipso), 128.65–126.90 (25 aromatic C), 85.55 (C-2), 83.17 (C-3), 78.63 (C-4), 73.54, 73.20, 72.72, 72.51 (4 × CH_2Ph), 68.55 (C-1), 67.32 (C-6), 62.42 (C-5), 59.19 (NCH_2Ph), 52.70 (C-7); ESI-HRMS: Calcd for $C_{35}H_{38}NO_4$ ($M + H^+$): 644.3376. Found 644.3369.

(2R,3R,4S,5R,6R)-1-Benzyl-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-6-(chloromethyl) piperidine ***12***

To a stirred solution of alcohol **11** (38 mg, 0.59 mmol) in dry DCM (2 mL) was added Et_3N (25 μL, 0.177 mmol) at 0°C under an atmosphere of nitrogen, followed by addition of MsCl (7 μL, 0.088 mmol). The reaction mixture was stirred at 0°C for 1 h and was concentrated under reduced pressure. Purification by preparative thin layer chromatography (Cy/EtOAc, 4:1) furnished the chloropiperidine **12** (23 mg, 59%) as an oil.

R_f 0.68, Cy/EtOAc, 4:1; $[\alpha]_D$ −34 (c = 1.0 in $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): 7.29–7.08 (m, 25H, 5 × Ph), 4.72 (d, 1H, J = 11.4 Hz, CHPh), 4.58 (ddd, 3H, J = 11.6, 11.3, 11.0 Hz, 3 × CHPh), 4.47 (dd, 2H, J = 11.6 Hz, 2 × CHPh), 4.21–4.13 (m, 4H, 2 × CHPh, NCHPh, H-5), 3.96–3.90 (m, 2H, NHPh, H-3), 3.77 (dd, 1H, J = 11.1, 6.7 Hz, H-7a), 3.68 (dd, 1H, J = 6.6, 2.6 Hz, H-4), 3.64 (dd, 1H, J = 9.3, 7.2 Hz, H-8a), 3.57–3.52 (m, 2H, H-7b, H-8b), 3.19–3.15 (m, 1H, H-6), 3.06–3.02 (m, 1H, H-2); ^{13}C NMR ($CDCl_3$, 100 MHz): 140.86, 138.73, 138.59, 138.49, 138.40 (5 × Cipso), 128.33–126.71 (25 aromatic C), 80.87 (C-4), 75.97 (C-5), 74.67 (C-3), 73.18, 72.87, 72.73, 72.47 (4 × CH_2Ph), 70.09 (C-7), 63.67 (C-2), 61.25 (C-6), 57.87 (NCH_2Ph), 45.13 (C-1); ESI-HRMS ($M + H^+$): Calcd for $C_{42}H_{45}O_4NCl$: 662.3037. Found: 662.3025.

(1S,3R,4R,5R,8R)-2-Benzyl-4,8-bis(benzyloxy)-3-((benzyloxy)methyl)-6-oxa-2-aza-bicyclo [3.2.1]octane ***13***

A mixture of chloropiperidine **12** (23 mg, 0.035 mmol) and NaI (21 mg, 0.139 mmol) in DMF (1 mL) was stirred at 130°C for 3 h. The reaction mixture was then concentrated under reduced pressure and the resulting residue purified by preparative TLC (Cy/EtOAc, 4:1) to give bicyclic compound **13** (16 mg, 84%) as an oil.

R_f 0.47, Cy/EtOAc, 4:1; $[\alpha]_D$ −2 (c = 1.0 in $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): 7.35–7.25 (m, 20H, 4 × Ph), 4.60 (dd, 2H, J = 12.0 Hz, 2 × CHPh), 4.54 (d, 1H, J = 12.0 Hz, CHPh), 4.49–4.39 (m, 5H, 3 × CHPh, H-5, H-4), 4.02 (dd, 1H, J = 8.6, 3.2 Hz, H-9a), 3.97 (s, 2H, NCH_2Ph), 3.91 (d, 1H, J = 4.8 Hz, H-8), 3.66 (d, 1H, J = 8.6 Hz, H-9b), 3.60–3.49 (m, 2H, H-7a, H-7b), 3.29 (d, 1H, J = 3.2 Hz, H-3), 3.04 (dd, 1H, J = 9.9, 4.8 Hz, H-1); ^{13}C NMR ($CDCl_3$, 100 MHz): 139.86, 138.74, 138.74, 138.43 (4 × Cipso), 128.88–127.38 (20 aromatic C), 79.15 (C-5), 79.08 (C-8), 76.06 (C-4), 73.98 (C-7), 72.98, 71.40, 70.81 (3 × CH_2Ph), 71.70 (C-9), 61.31 (C-3), 61.14 (NCH_2Ph), 60.55 (C-1); ESI-HRMS: Calcd for $C_{35}H_{38}O_4N$ ($M + H^+$): 536.2795. Found 536.2796.

(1R,3R,4R,5R,8R)-3-(Hydroxymethyl)-6-oxa-2-azabicyclo[3.2.1]octane-4,8-diol ***14***

To a solution of compound **13** (16 mg, 0.030 mmol) in MeOH (3 mL) containing 1M aq. HCl (20 μL) was added 10% Pd/C (16 mg). The suspension was stirred under H_2 atmosphere for 4 h at rt, filtered through a Celite plug, and eluted with MeOH. The solvent was removed under reduced pressure to afford bicyclic derivative **14** (4.4 mg, 70%) as its hydrochloride salt.

$[\alpha]_D$ +6 (c = 0.34 in MeOH); ^1H NMR (D_2O, 400 MHz): 4.55 (d, 1H, J = 1.4 Hz, H-8), 4.20 (d, 1H, J = 4.5 Hz, H-5), 4.04 (dd, 1H, J = 11.4, 3.4 Hz, H-7a), 3.96–3.93 (m, 2H, J = 11.4, 3.4 Hz, H-7b, H-1), 3.87 (dd, 1H, J = 4.5 Hz, H-4), 3.73 (dd, 1H, J = 12.1, 6.1 Hz, H-9a), 3.56 (dd, 1H, J = 12.1, 10.6 Hz, H-9b), 3.30–3.25 (dddd, 1H, J = 10.6, 6.1, 1.4 Hz, H-3); ^{13}C NMR (D_2O, 100 MHz):

80.70 (C-5), 68.22 (C-8), 67.67 (C-4), 67.13 (C-7), 59.23 (C-9), 58.41 (C-3), 57.42 (C-1); ESI-HRMS: Calcd for $C_7H_{14}O_4N$ ($M + H^+$): 176.0917. Found 176.0916.

Conformational analysis

NMR experiments were performed on a Bruker AVANCE 500 spectrometer at 298 K and using D_2O as solvent. NMR assignments were performed using standard COSY and NOESY experiments. The obtained NMR parameters (J and NOE data) were compared to those expected for the possible geometries of the molecules. Thus, all possible arrangements for the fused six-membered rings were investigated using Macromodel 9.6[20] as implemented in the Maestro suite of programs (version 8.5.110).[21] The conformational search calculations were performed using the MM3* force field, with the GB/SA water solvent model[22] at 298 K. The general PRCG (Polak-Ribiere Conjugate Gradient) method for energy minimization was applied for a maximum iteration number of 5000 or a convergence threshold of 0.05. For all the obtained geometries, the expected vicinal coupling constants were estimated from the calculated torsion angles by using the empirical Karplus equation proposed by Haasnoot et al.[23] and compared to those obtained experimentally.

Glycosidase inhibition assay

For rice α-glucosidase and rat intestinal maltase activities, the reaction mixture contained 25 mM maltose and the appropriate amount of enzyme, and the incubations were performed for 10 to 30 min at 37°C. The reaction was stopped by heating at 100°C for 3 min. After centrifugation (600 g; 10 min), 0.05 mL of the resulting reaction mixture was added to 3 mL of the Glucose CII-test Wako (Wako Pure Chemical Ind., Osaka, Japan). The absorbance at 505 nm was measured to determine the amount of the released D-glucose. Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. The reaction mixture contained 2 mM of the substrate and the appropriate amount of enzyme. The reaction was stopped by adding 2 mL of a 400-mM Na_2CO_3 solution. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

ACKNOWLEDGMENTS

Bo Luo is the recipient of a CSC Fellowship.

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