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Aziridines as a structural motif to conformational restriction of azasugars

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In order to investigate the hypothesis that the glycosidase inhibitor isofagomine was bound to α-or β-glucosidase in a $^{1,4}B$ conformation, a number of bicyclic aziridines that adopt the $^{1,4}B$ or $B_{1,4}$ conformations were synthesised and investigated. (1*R*)-2-*endo*,3-*exo*-2,3-Dihydroxy-4-*endo*-4-hydroxymethyl-6-azabicyclo[3.1.0]hexane (**5**) and its *N*-methyl and *N*-benzyl analogues and (1*S*)-2-*exo*-3-*endo*-2,3-dihydroxy-4-*endo*-4-hydroxymethyl-6-azabicyclo-[3.1.0]hexane (**6**) were synthesised. The aziridines **5** and **6** were found to be weak or not inhibitors of α-glucosidase, β-glucosidase and α-fucosidase.

Introduction

While the aziridine group is known as a useful reaction intermediate,[1] it is also an interesting structural motif in bioactive compounds. The aziridine's proton accepting properties, its rigidity and its potential reactivity can all contribute to specific molecular interactions with proteins, and indeed several important natural products such as Mitomycin C,[2] Porfiromycin[3] and Carzinophilin A[4] contain the aziridine functionality. A number of saccharide derivatives containing the aziridine group have been made, mostly as intermediates,[5–8] but also as glycosidase inhibitors.[9–11] The aziridines **1**[9] and **2**[10] have been reported to be irreversible inhibitors, while **3** was recently shown to be a reversible competitive inhibitor.[11]

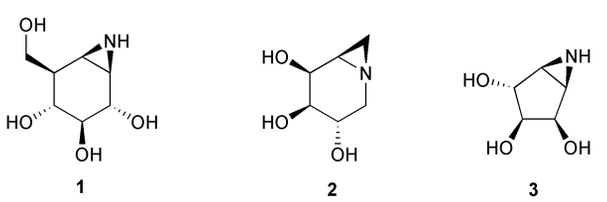

Fig. 1

Our long-standing interest in glycosidase inhibitors of the isofagomine-type[12] (**4**, Fig. 2) has led us to consider conformationally restrained analogues[13] as a means of providing information about the binding of these inhibitors. The isofagomines are strong β-glycosidase inhibitors.[12] However stereoelectronic effects dictate that β-glycosides must adopt a boat-like transition state during hydrolysis (**A**, Fig. 2).[14] It may therefore be considered paradoxical that **4** and analogues, while themselves in a chair conformation, are particularly potent against β-glycosidases. This led us to consider whether **4** might be binding in a boat conformation and/or whether **4b**, the boat conformer of **4**, would be a good inhibitor. Another reason to suppose that inhibitor **4** might not be binding to certain glycosidases in the favoured chair conformation is the peculiar slow-onset binding observed, particularly to β-glucosidase.[18,19] The association of **4** to β-glucosidase is much slower than the rate of diffusion control one would normally expect for such a process. The slow rate would however be consistent with an energetically unfavourable and little-populated conformation binding to the enzyme. In this paper we analyse the problem by synthesis of the bicyclic aziridine **5**, which adopts the desired boat $^{1,4}B$ conformation, and mimics **4b**. We also report the synthesis of an isomer **6** that adopts the $B_{1,4}$ conformation.

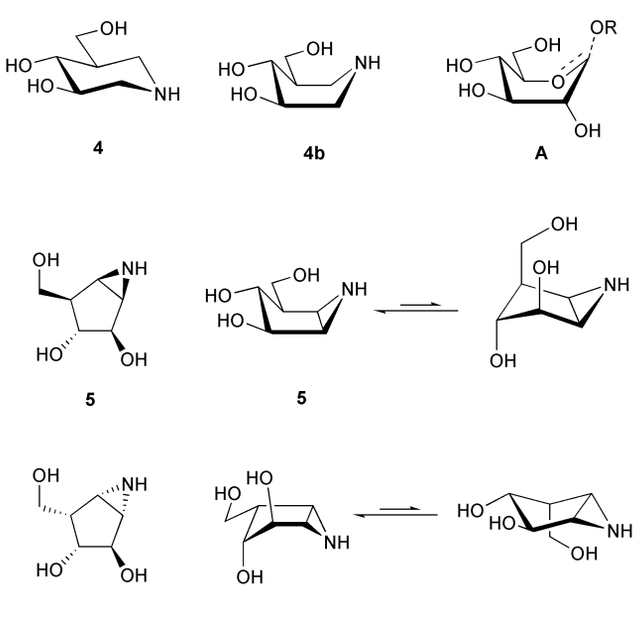

Fig. 2

Results and discussion

Our synthetic plan to obtain **5** and **6** relied on the chemistry of Ferrier *et al.*, who synthesised aziridine **12** from methyl D-glucopyranoside (**7**) as outlined in Scheme 1.[5] Conversion of **7** to a protected 2,6-ditosylate **8** followed by nucleophilic substitution with iodine and reductive elimination with Zn powder gave the alkenal **10**, which was shown to undergo 1,3-dipolar cycloaddition upon treatment with *N*-methylhydroxylamine to give **11**.[5] Reductive cleavage of the N–O bond resulted in spontaneous formation of **12**.[5] Since deprotection of **12** would give us the *N*-methyl analogue of the desired compound, our immediate goal was to modify Ferrier's synthesis to reach **5**. Initially, we uneventfully repeated the sequence to **12** and found that the benzoyl groups can be successfully removed with NaOMe in methanol to give new aziridine **13** in a 78% yield.

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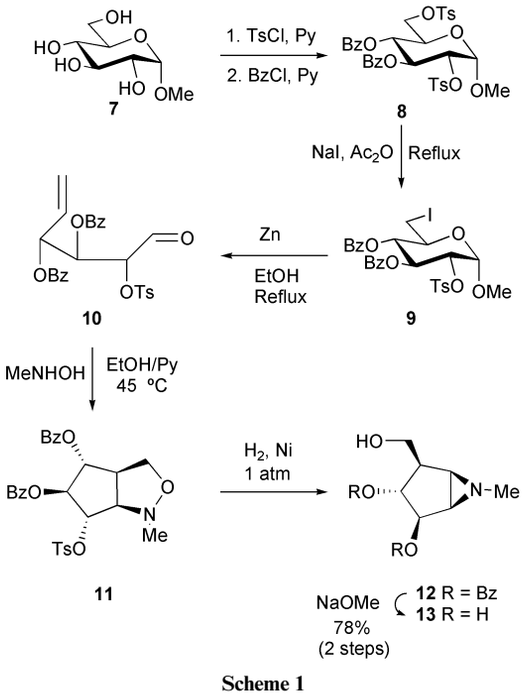
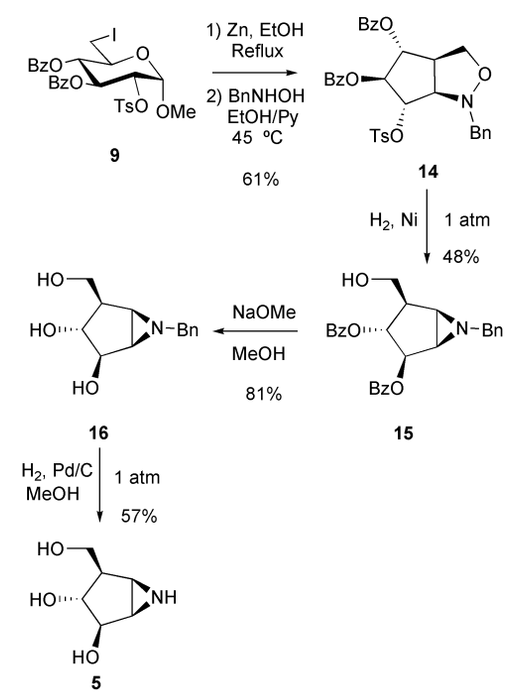
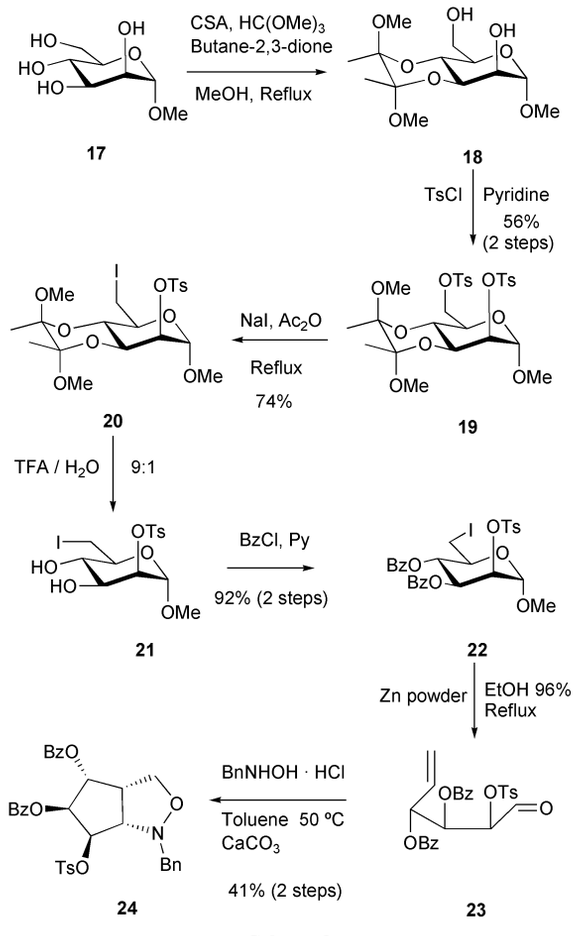


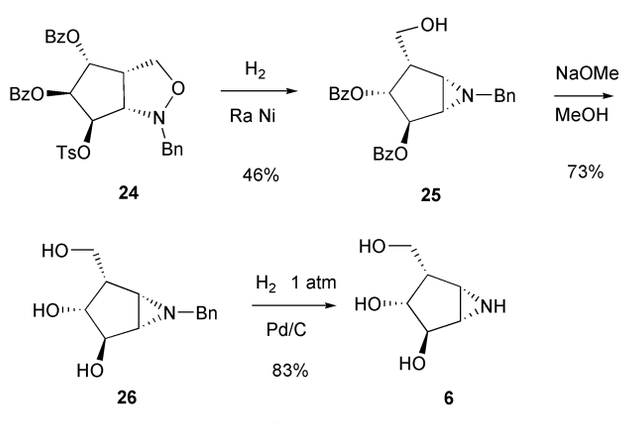
Scheme 1



Scheme 2



Scheme 3



Scheme 4

The more tricky synthesis of **5** was carried out as outlined in Scheme 2. The alkenal **10** was reacted with *N*-benzylhydroxylamine, which gave the 1,2-oxazine **14** in 61% yield. It was found that yields were improved when $CaCO_3$ and toluene were used in place of pyridine in this reaction. Reduction of the N–O bond was carried out with Raney nickel at 1 atm of hydrogen pressure and it was possible to avoid too much *N*-debenzylation. The aziridine **15** was obtained in 48% yield after chromatography. Debenzoylation using Zemplen conditions gave the *N*-benzylaziridine **16** in 81% yield. Finally hydrogenolysis in the presence of palladium on carbon gave the target compound **5** in 57% yield (Scheme 2). The aziridine is very sensitive, and the modest yield in this reaction is due to partial reductive opening of the aziridine.

The aziridine **5** may adopt either a conformation having the piperidine ring in the desired $^{3,6}B$ or in a $^{6}C_{3}$ conformation (Fig. 2). The NMR spectrum of **5** shows large couplings for $J_{2,3}$ and $J_{3,4}$ clearly identifying the boat conformation as the predominant one. Similarly the NMR data for **13** and **16** reveal the same conformational preference of these compounds.

We anticipated the isomer **6** might be synthesised from the methyl D-mannoside **17** in a similar manner since the intramolecular 1,3-dipolar cycloaddition has been shown to give opposite stereoselectivity in the mannose case relative to glucose.[15] However selective 2,6-ditosylation of **17** is not possible.[16] We therefore used Ley's method[17] of selectively protecting a diequatorial 1,2-diol. Tosylation of the crude **18** gave the new ditosylate **19** in 56% yield from **17**. Reaction of **19** with NaI in acetic anhydride gave the 6-iodo compound **20** in 74% yield. Hydrolysis of the diacetal with TFA to diol **21** and benzoylation gave the dibenzoate **22** in 92% yield from **20**.

The elimination–cycloaddition of **22** with *N*-benzylhydroxylamine proceeded satisfactorily. After treatment with Zn powder the formation of alkenal **23** was observed by NMR.

Table 1 K_i values in μM at pH 6.8, 25 °C (— = not investigated, NI = no inhibition)

	13	16	5	26	6
α-Glucosidase (yeast)	4000	4900	NI	NI	NI
β-Glucosidase (almonds)	240	280	NI	NI	NI
α-Fucosidase (bovine kidney)	—	—	—	1220[a]	2780[a]

[a] At 32 °C.

The reaction of **23** with *N*-benzylhydroxylamine in the presence of $CaCO_3$ resulted in **24** being obtained in 41% yield from **22**. No stereoisomers were observed.

The oxazine **24** was hydrogenolysed in the presence of Raney nickel giving the aziridine **25** in 46% yield. The internal nucleophilic substitution is in this case a relatively slow reaction and the initially formed monocyclic amine could be observed. Debenzoylation with NaOMe–MeOH gave the unprotected aziridine **26** in 73% yield. Finally hydrogenolysis of **26** gave **6** in an 83% yield.

The aziridine **6** may adopt a conformation with the piperidine ring in either a $B_{3,6}$ or in a $^3C_6$ conformation (Fig. 2), and indeed both conformations appear likely. However the NMR spectrum of **6** shows $J_{2,3}$ = 0 Hz, which is inconsistent with the chair conformation. The boat conformation must therefore be predominant. The NMR data for **26** reveal the same conformational preference.

The unprotected azidirines **13**, **16**, **5**, **26** and **6** were investigated for their ability to inhibit α- and β-glucosidase (Table 1). Weak competitive inhibition of α-glucosidase and intermediate inhibition of β-glucosidase were found for compounds **13** and **16**, but, surprisingly, no inhibition at all for the unsubstituted aziridine **5**. This suggests that these compounds bind distinctly differently from isofagomine (**4**) and similar compounds, because *N*-substitution of **4** decreases inhibition significantly. Inhibition was not found to be time-dependent for any of the compounds. Compounds **26** and **6** were also investigated for inhibition of an α-fucosidase due to their resemblance to L-fucose. Weak reversible, competitive inhibition was found for both compounds. Again the *N*-substituted aziridine **26** was more potent than unsubstituted **6** suggesting that binding was different from that of the corresponding isofagomine. Altogether the inhibition study disproves the hypothesis that 1-azasugars bind in a $^{1,4}B$ conformation.

In summary we have synthesised and investigated bicyclic aziridines as conformationally restricted analogues of isofagomine in a $^{1,4}B$ conformation. The aziridines are, in contrast to isofagomine, very poor or not glycosidase inhibitors and appear to bind to enzymes in a different mode than isofagomine. The results show that isofagomine does not bind the investigated glycosidases in the $^{1,4}B$ conformation.

Experimental section

General

Solvents were distilled under anhydrous conditions. All reagents were used as purchased without further purification. Pyridine was dried over potassium hydroxide before use. Evaporation was carried out on a rotary evaporator with the temperature kept below 40 °C. Glassware used for water-free reactions was dried for 2 hours min. at 130 °C before use. Columns were packed with silica gel 60 (230–400 mesh) as the stationary phase. TLC-plates (Merck, 60, F_{254}) were visualised by spraying with cerium sulfate (1%) and molybdic acid (1.5%) in 10 % H_2SO_4 and heating until coloured spots appeared. ^{1}H-NMR, ^{13}C-NMR and COSY were carried out on a Varian Gemini 200 instrument. In water the water-signal (δ 4.7) was used as reference. Mass spectra were carried out on a Micromass LC-TOF instrument. Optical rotations are given in 10^{-1} deg cm^2 g^{-1}.

(1*R*)-2-*endo*-3-*exo*-2,3-Dihydroxy-4-*endo*-4-hydroxymethyl-*N*-methyl-6-azabicyclo[3.1.0]hexane (13). To a solution of **12**[5] (50 mg, 0.14 mmol) in dry methanol (3 mL) was added methanolic NaOMe (pH 10). Reaction was kept at rt for 1 h. Then it was neutralised with IR-120(H^+) resin and the resin was washed with 5% aqueous ammonia and concentrated to dryness to give **13** (17 mg; 78%). ^{1}H NMR (200 MHz, D_2O): δ 3.98 (m, 1H, $J_{2,3}$ = 6.9 Hz, H-2), 3.78 (m, 2H, H-7a, H-7b), 3.12 (t, 1H, $J_{3,4}$ = 6.9 Hz, H-3), 2.22 (br s, 2H, H-1, H-5), 2.14 (s, 3H, Me), 2.0 (m, 1H, H-4).^{13}C NMR (50 MHz, D_2O): δ 77.8, 75.3 (C-2, C-3), 60.6 (C-7), 46.3, 45.1, 43.5, 42.4 (Me, C-1, C-4, C-5). HRFAB-MS: calcd. for $(M + H)^+$ $C_7H_{14}NO_3$: 160.0974, found: 160.0978.

(1*R*,5*R*)-6-*exo*,7-*endo*-6,7-Bis(benzoyloxy)-*N*-benzyl-8-*exo*-8-toluene-*p*-sulfonyloxy-3-oxa-2-azabicyclo[3.3.0]octane (14). To a suspension of methyl 3,4-di-*O*-benzoyl-6-deoxy-6-iodo-2-*O*-*p*-toluenesulfonyl-α-D-glucopyranoside (**9**)[5] (1.0 g, 1.50 mmol) in aqueous EtOH (20 mL, 96%) was added Zn dust (1.0 g, 15.30 mmol). The mixture was refluxed for 1 h and then filtered through Celite and concentrated to dryness to give a yellow oil that was dissolved in CH_2Cl_2 (20 mL), washed with water (2 × 10 mL), dried over Na_2SO_4, filtered and concentrated to dryness to give alkenal **10** as an oil. To a solution of compound **10** in toluene (8 mL) were added *N*-benzylhydroxylamine hydrochloride (359 mg, 2.25 mmol) and $CaCO_3$ (225 mg, 2.25 mmol), and the reaction was heated at 50 °C for 2 h. The mixture was then concentrated to dryness, and the residue was dissolved in CH_2Cl_2 (15 mL), washed with water (2 × 10 mL), dried over Na_2SO_4, filtered and concentrated to dryness. Compound **14** was crystallised from methanol (558 mg; 61%). Mp: 148–150 °C. $[\alpha]_D^{22}$ −18 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96–7.06 (m, 19 H, Ar–H), 5.91 (t, 1 H, $J_{6,7}$ = 8.2 Hz, $J_{7,8}$ = 8.2 Hz, H-7), 5.17 (dd, 1H, $J_{5,6}$ = 6.3 Hz, H-6), 5.10 (dd, 1H, $J_{1,8}$ = 5.6 Hz, H-8), 4.25 (dd, 1H, $J_{4a,5}$ = 4.2 Hz, $J_{4a,4b}$ = 9.6 Hz, H-4a), 4.21 (dd, 1H, $J_{4b,5}$ = 6.9 Hz, H-4b), 3.95, 3.83 (2d, 1H each, $^2J_{H,H}$ = 13.5 Hz, CH_2Ph), 3.84 (dd, 1H, $J_{1,5}$ = 9.6 Hz, H-1), 3.24 (m, 1H, H-5), 2.18 (s, 3H, Me). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ165.8, 164.6 (CO), 144.6, 136.2, 133.2, 133.0, 129.6, 129.5, 129.4, 128.7, 128.6, 128.2, 128.1, 128.0, 127.6, 127.3 (24 C, Ar), 83.5 (C-8), 78.7 (C-6), 76.3 (C-7), 70.4 (C-1), 69.8 (C-4), 59.0 (CH_2Ph), 49.8 (C-5), 21.2 (Me). HRMS (ES) calcd. for $(M + Na)^+$ $C_{34}H_{31}NNaO_8S$: 636.1668, found: 636.1667.

(1*R*)-2-*endo*,3-*exo*-2,3-Bis(benzoyloxy)-*N*-benzyl-4-*endo*-4-hydroxymethyl-6-azabicyclo[3.1.0]hexane (15). To a solution of **14** (211 mg, 0.34 mmol) in acetone (5 mL) was added Raney nickel and the mixture was hydrogenated at atmospheric pressure and room temperature for 4 days. Then it was filtered through a Celite bed, concentrated to dryness and purified by column chromatography (CH_2Cl_2 ⟶ CH_2Cl_2–MeOH 80 : 1 gradient) to yield **15** (73 mg; 48%). $[\alpha]_D^{25}$ −96 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.07–7.08 (m, 15H, Ar–H), 5.59 (dd, 1H, $J_{1,2}$ = 2,8 Hz, $J_{2,3}$ = 6.2 Hz, H-2), 5.45 (dd, 1H, $J_{3,4}$ = 6.8 Hz, H-3), 3.91 (2dd, 2H, $J_{4,7a}$ = 4.6, $J_{4,7b}$ = 6.2 Hz, $J_{7a,7b}$ = 11.0 Hz, H-7a, H-7b), 3.60, 3.25 (2d, 1H each, $^2J_{H,H}$ = 13.4 Hz, CH_2Ph), 2.66 (dd, 1H, $J_{1,5}$ = 5.2 Hz, $J_{4,5}$ = 3.4 Hz H-5), 2.54–2.39 (m, 2H, H-1, H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.4, 166.3 (2CO), 138.4, 133.2, 133.0, 129.8, 129.7, 129.5, 128.3, 128.2, 127.4, 127.0 (18C, Ar), 79.8, 76.6 (C-2,

C-3), 62.3 (CH_2Ph), 61.1 (C-7), 46.2, 42.8, 42.4 (C-1, C-4, C-5). HRMS (ES) calcd. for $(M + Na)^+$ $C_{27}H_{25}NNaO_5$: 466.1630, found: 466.1629.

(1*R*)-*N*-Benzyl-2-*endo*,3-*exo*-2,3-dihydroxy-4-*endo*-4-hydroxymethyl-6-azabicyclo[3.1.0]hexane (16). To a solution of **15** (69 mg, 0.16 mmol) in dry methanol (5 mL) was added methanolic NaOMe (pH 10). The reaction mixture was kept at rt for 3 h. Then it was neutralised with IR-120(H^+) resin and the resin was washed with 5% aqueous ammonia and concentrated to dryness to give **16** (30 mg; 81%). $[a]_D^{25}$ +39 (*c* 0.8, D_2O). ^{1}H NMR (200 MHz, D_2O): δ 7.36 (m, 5H, Ar–H), 4.05 (dd, 1H, $J_{1,2}$ = 2.7 Hz, H-2, $J_{2,3}$ = 6.5 Hz, H-2), 3.70 (dd, 1H, $J_{3,4}$ = 8.5 Hz, H-3), 3.64 (dd, 1H, $J_{4,7a}$ = 4.8 Hz, $J_{7a,7b}$ = 11.8 Hz, H-7a), 3.47, 3.30 (2d, 1H each, $^2J_{H,H}$ = 14.0 Hz, CH_2Ph), 3.22 (dd, 1H, $J_{4,7b}$ = 2.7 Hz, H-7b), 2.50 (br s, 2H, H-1, H-5), 2.06 (m, 1H, H-4).^{13}C NMR (50 MHz, D_2O): δ 138.0, 128.2, 127.6, 127.1 (5C, Ar), 77.8, 75.3 (C-2, C-3), 60.6 (CH_2Ph), 60.2 (C-7), 46.3, 44.6, 42.0 (C-1, C-4, C-5). HRFAB-MS: calcd. for $(M + H)^+$ $C_{13}H_{18}NO_3$: 236.1287, found: 236.1288.

(1*R*)-2-*endo*,3-*exo*-2,3-Dihydroxy-4-*endo*-4-hydroxymethyl-6-azabicyclo[3.1.0]hexane (5). To a solution of **16** (16 mg, 0.07 mmol) in water (5 mL) was added palladium over charcoal 10% (50 mg) and the mixture was hydrogenated at atmospheric pressure and rt for 20 minutes. Then it was filtered through Celite and concentrated to dryness to yield **5** (6 mg; 57%). ^{1}H NMR (200 MHz, D_2O): δ 4.07 (d, $J_{2,3}$ = 6.6 Hz, H-2), 3.69 (m, 2H, H-7a, H-7b), 3.19 (dd, 1H, $J_{3,4}$ = 8.0 Hz, H-3), 2.69 (s, H-1, H-5), 2.06 (m, 1H, H-4). HRCI-MS calcd. for $[M + H]^+$ $C_6H_{12}NO_3$: 146.0817, found: 146.0814.

(2′*S*,3′*S*)-Methyl 3,4-*O*-[2′,3′-dimethoxybutane-2′,3′-diyl]-2,6-bis(*O*-toluene-*p*-sulfonyl)-α-D-mannopyranoside (19). To a stirred solution of methyl α-D-mannopyranoside (**17**, 5.0 g, 25.75 mmol) and (±)-camphorsulfonic acid (692 mg, 2.98 mmol) in methanol (50 mL) were added trimethyl orthoformate (12 mL, 110.22 mmol) and butane-2,3-dione (3.6 mL, 41.2 mmol) and the solution was refluxed for 16 h. On cooling to room temperature, the mixture was quenched with Et_3N (0.5 mL) and then concentrated to dryness. To a stirred solution of the residue in anhydrous pyridine (30 mL) at 0 °C was added toluene-*p*-sulfonyl choride (14.7 g, 77.25 mmol). The mixture was warmed to rt and stirring was continued for 24 h before the reaction mixture was partitioned between ice-cold water and dichloromethane. The organic layer was washed with 1 M aqueous HCl (3 × 30 mL), saturated aqueous $NaHCO_3$ (20 mL), water (20 mL), dried over $MgSO_4$, filtered and concentrated to dryness. Crystallisation from methanol yielded **19** (8.85 g; 56%). Mp: 138–142 °C. $[a]_D^{24}$ +120 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80–7.27 (m, 8H, Ar–H), 4.70 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1), 4.44 (dd, 1H, $J_{2,3}$ = 3.1 Hz, H-2), 4.25 (dd, 1H, $J_{5,6a}$ = 1.5 Hz, $J_{6a,6b}$ = 10.6 Hz, H-6a), 4.14 (dd, 1H, $J_{5,6b}$ = 5.5 Hz, H-6b), 3.87 (dd, 1H, $J_{3,4}$ = 10.0 Hz, H-3), 3.79 (m, 1H, H-5), 3.74 (t, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 3.23, 3.10, 2.99 (s, 3 H each, 3 OMe), 2.42, 2.40 (s, 3 H each, 2 C$H_3C_6H_4$), 1.14, 0.94 (s, 3 H each, 2 Me). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 144.7, 144.4, 129.7, 129.2, 128.3, 127.9 (12C, Ar), 100.0, 99.7 (C-2′, C-3′), 99.4 (C-1), 76.4 (C-2), 68.5 (C-5), 67.9 (C-6), 65.5 (C-3), 62.8 (C-4), 55.1 (CH_3OC-1), 48.0, 47.8 (2 OMe), 21.6, 21.5 (2$CH_3C_6H_4$), 17.5, 17.3 (2 Me). CI-MS *m*/*z*: 585 ($[M - OMe]^+$). Anal. Calcd. for $C_{27}H_{36}O_{12}S_2$: C, 52.58, H, 5,88. Found: C, 52.59, H, 5.91%.

(2′*S*,3′*S*)-Methyl 6-deoxy-3,4-*O*-[2′,3′-dimethoxybutane-2′,3′-diyl]-6-iodo-2-*O*-toluene-*p*-sulfonyl-α-D-mannopyranoside (20). To a solution of **19** (4.06 g, 6.59 mmol) in acetic anhydride (40 mL) was added sodium iodide (1.48 g, 9.87 mmol), and the mixture was refluxed for 1.5 h. After filtration of the solids, the filtrate was concentrated to dryness and the residue was dissolved in CH_2Cl_2 (40 mL) and washed with saturated aqueous $Na_2S_2O_3$ (2 × 20 mL), water (20 mL), dried over $MgSO_4$, filtered and concentrated to dryness. Crystallisation from methanol yielded **20** (2.78 g; 74%). Mp: 132–136 °C. $[a]_D^{22}$ = +125 (*c* 1.1, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84–7.24 (m, 4H, Ar–H), 4.88 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.51 (dd, 1H, $J_{2,3}$ = 3.1 Hz, H-2), 3.93 (dd, 1H, $J_{3,4}$ = 9.7 Hz, H-3), 3.69 (t, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 3.62 (ddd, 1H, $J_{5,6a}$ = 2.1 Hz, $J_{5,6b}$ = 8.2 Hz, H-5), 3.49 (dd, 1H, $J_{6a,6b}$ = 10.6 Hz), 3.40, 3.18 (s, 3 H each, 2 OMe), 3.17 (dd, 1H, H-6b), 3.03 (s, 3H, OMe), 2.41 (s, 3H, C$H_3C_6H_4$), 1.18, 0.98 (s, 3 H each, 2 Me). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 144.4, 133.6, 129.3, 128.4 (6 C, Ar). 100.0, 99.8 (C-2′, C-3′), 99.5 (C-1), 76.8 (C-2), 70.4 (C-5), 67.0 (C-4), 65.43 (C-3), 55.3, 48.2, 47.9 (3 OMe), 21.6 ($CH_3C_6H_4$), 17.6, 17.3 (2 Me), 4.18 (C-6). FAB-MS *m*/*z*: 595 ($[M + Na]^+$). Anal. Calcd. for $C_{20}H_{29}IO_9S$: C, 41.96; H, 5.10; S, 5.60. Found: C, 42.11; H, 5.09; S, 5.59%.

Methyl 3,4-di-*O*-benzoyl-6-deoxy-6-iodo-2-*O*-toluene-*p*-sulfonyl-α-D-glucopyranoside (22). A solution of **20** (2.43 g, 4.25 mmol) in trifluoroacetic acid–H_2O 9 : 1 (13 mL) was kept at room temperature for 1.5 h. Concentration to dryness and co-evapourating with ethanol gave methyl 6-deoxy-6-iodo-2-*O*-toluene-*p*-sulfonyl-α-D-glucopyranoside (**21**), which was directly used for the next reaction, without further purification. Data for compound **21**: HRCI-MS: calcd. for $[M + H]^+$ $C_{14}H_{20}IO_7S$: 458.9974, found: 458.9974. To a solution of **21** in pyridine (15 mL) was added benzoyl chloride (2.96 mL, 25.5 mmol) and the reaction was kept at rt for 24 h. The solution was then poured over water–ice, diluted with CH_2Cl_2 (25 mL) and the organic layer was washed with 1 M aqueous HCl (2 × 20 mL), saturated aqueous $NaHCO_3$ (20 mL), water (20 mL), dried over $MgSO_4$, filtered and concentrated to dryness. Crystallisation from methanol yielded **22** (2.62 g; 92%). Mp: 172–174 °C. $[a]_D^{22}$ −8 (*c* 1.2, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90–6.86 (m, 14H, Ar–H), 5.53 (t, 1H, $J_{4,5}$ = 10.2 Hz, H-4), 5.46 (dd, 1H, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 10.1 Hz, H-3), 5.04 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.90 (dd, 1H, H-2), 4.00 (td, 1H, $J_{5,6a}$ = 2.6 Hz, $J_{5,6b}$ = 9.2 Hz, H-5), 3.54 (s, 3H, OMe), 3.35 (dd, 1H, $J_{6a,6b}$ = 10.8 Hz, H-6a), 3.27 (dd, 1H, H-6b), 2.09 (s, 3H, C$H_3C_6H_4$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 164.5 (2 CO), 154.4, 133.5, 133.2, 129.7, 128.6, 128.4, 128.1, 127.8 (18C, Ar), 98.9 (C-1), 75.8 (C-2), 70.6 (C-5), 69.6 (C-4), 69.2 (C-3), 55.8 (OMe), 21.5 ($CH_3C_6H_4$), 3.3 (C-6). FAB-MS *m*/*z*: 689 ($[M + Na]^+$). Anal. Calcd. for $C_{28}H_{27}IO_9S$: C, 50.46; H, 4.08; S, 4.81. Found: C, 50.43, H, 3.96; S, 4.90%.

(1*S*,5*S*)-6-*endo*,7-*exo*-6,7-Bis(benzoyloxy)-*N*-benzyl-8-*exo*-8-toluene-*p*-sulfonyloxy-3-oxa-2-azabicyclo[3.3.0]octane (24). To a suspension of **22** (355 mg, 0.53 mmol) in aqueous EtOH (8 mL, 96%) was added Zn dust (374 mg, 6.10 mmol). The mixture was refluxed for 1 h and then filtered through Celite and concentrated to dryness to give an oil that was dissolved in CH_2Cl_2 (15 mL), washed with water (2 × 10 mL), dried over $MgSO_4$, filtered and concentrated to dryness to give (2*S*,3*S*,4*R*)-3,4-bis-*O*-benzoyloxy-2-*O*-toluene-*p*-sulfonylhex-5-enal (**23**) as an oil, that was used without further purification in the next step. HRFAB-MS calcd. for $[M + H]^+$ $C_{27}H_{25}O_8S$: 509.1263, found: 509.1270. To a solution of **23** in toluene (5 mL) were added *N*-benzylhydroxylamine hydrochloride (131 mg, 0.80 mmol) and $CaCO_3$ (80 mg, 0.80 mmol) and the reaction mixture was heated at 50 °C for 1.5 h. The mixture was then filtered and the filtrate was concentrated to dryness. The residue was dissolved in CH_2Cl_2 (10 mL), washed with water (2 × 10 mL), dried over Na_2SO_4, filtered and concentrated to dryness. Column chromatography (CH_2Cl_2) gave **24** (146 mg; 41%), which was crystallised from methanol. Mp: 126–130 °C (decomp.). $[a]_D^{22}$ −95 (*c* 0.8, DMSO). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97–6.90 m, 19H, Ar–H), 5.72 (dd, 1H, $J_{5,6}$ = 8.3 Hz, $J_{6,7}$ = 9.9 Hz, H-6), 5.56 (dd, 1H, $J_{7,8}$ = 4.5 Hz, H-7), 4.90 (d, 1H, $J_{1,8} \approx$ 0.0 Hz, H-8),

4.05, 3.95 (2d, 1H each, $^2J_{H,H}$ = 13.5 Hz, CH_2Ph), 3.98 (dd, 1H, $J_{4a,5}$ = 4.6 Hz, $J_{4a,4b}$ = 9.5 Hz, H-4a), 3.93 (dd, 1H, $J_{4b,5}$ = 6.4 Hz, H-4b), 3.84 (d, 1H, $J_{1,5}$ = 8.1 Hz, H-1), 3.76 (m, 1H, H-5), 2.20 (s, 3H, Me) . ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 165.7, 165.1 (2 CO), 144.8, 136.4, 133.5, 133.3, 132.6, 129.9, 129.8, 129.7, 128.9, 128.5, 128.2, 127.7, 127.6 (24C, Ar), 79.6 (C-8), 74.3 (C-6), 73.8 (C-7), 72.2 (C-1), 65.4 (C-4), 60.5 (*C*H_2Ph), 45.4 (C-5), 21.6 (Me). HRCI-MS calcd. for $[M + H]^+$ $C_{34}H_{32}NO_8S$: 614.1849, found: 614.1834.

(1*S*)-*N*-Benzyl-2-*exo*,3-*endo*-2,3-bis(benzoyloxy)-4-*endo*-4-hydroxymethyl-6-azabicyclo[3.1.0]hexane (25). To a solution of **24** (70 mg, 0.11 mmol) in EtOAc–EtOH 3 : 1 (8 mL) was added Raney nickel and the mixture was hydrogenated at atmospheric pressure and rt for 18 h. Then it was filtered through Celite and concentrated to dryness. The residue was dissolved in MeOH (5 mL) and stirred at room temperature for 3 days. Finally, the solution was concentrated to dryness and compound TLC (CH_2Cl_2–MeOH 80 : 1) of the residue gave **25** (23 mg; 46%). $[\alpha]_D^{22}$ −77 (*c* 1.0, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10–7.24 (m, 15 H, Ar–H), 5.57 (d, 1H, $J_{2,3} \approx$ 0.0 Hz, $J_{3,4}$ = 7.6 Hz, H-3), 5.42 (s, 1H, $J_{1,2} \approx$ 0.0 Hz, H-2), 3.73 (m, 2H, H-7a, H-7b), 3.73, 3.34 (2d, 2H each, $^2J_{H,H}$ = 13.5 Hz, CH_2Ph), 2.82 (m, 1H, $J_{4,5}$ = 2.5 Hz, H-4), 2.51 (d, 1H, $J_{1,5}$ = 4.2 Hz, H-1), 2.44 (dd, 1H, H-5). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166.4, 165.5 (2 CO), 138.9, 133.4, 129.9, 129.8, 129.7, 128.5, 127.8, 127.3, 123.0 (18 C, Ar), 79.5 (C-2), 78.3 (C-3), 61.4 (*C*H_2Ph), 60.0 (C-7), 45.5 (C-4), 45.4 (C-1), 44.6 (C-5). HRFAB-MS calcd. for $[M + H]^+$ $C_{34}H_{32}NO_8S$ 444.1805, found: 444.1811.

(1*S*)-*N*-Benzyl-2-*exo*,3-*endo*-2,3-dihydroxy-4-*endo*-4-hydroxymethyl-6-azabicyclo[3.1.0]hexane (26). To a solution of **25** (96 mg, 0.22 mmol) in dry methanol (2 mL) was added methanolic NaOMe (pH 10). The reaction was kept at rt for 4 h. Then 1 M aqueous HCl was added (pH 8) and the solution was concentrated to dryness. Column chromatography of the residue (CH_2Cl_2 ⟶ CH_2Cl_2–MeOH 10 : 1 gradient) gave **26** (37 mg; 73%). $[\alpha]_D^{27}$ = −27 (*c* 0.4, CH_3OH). ^{1}H NMR (300 MHz, CD_3OD): δ 7.31 (m, 5H, Ar–H), 3.92 (s, 1H, $J_{1,2} \approx$ 0.0, Hz $J_{2,3} \approx$ 0.0 Hz, H-2), 3.82 (dd, 1H, $J_{4,7a}$ = 7.1 Hz, $J_{7a,7b}$ = 11.0 Hz, H-7a), 3.66 (ddd, 1H, $J_{3,4}$ = 5.5 Hz, $J_{1,3}$ = 1.8 Hz, $J_{3,5}$ = 1.3 Hz, H-3), 3.65 (dd, 1H, $J_{4,7b}$ = 8.1 Hz, H-7b), 3.42 (s, 2H, CH_2Ph), 2.52 (dd, 1H, $J_{1,5}$ = 4.3 Hz, $J_{4,5}$ = 2.0 Hz, H-5), 2.42 (dd, 1H, H-1), 2.37 (m, 1H, H-4). ^{13}C NMR (75.5 MHz, CD_3OD): δ 129.5, 128.9, 128.3, 104.2 (6C, Ar), 77.6 (C-3), 77.5 (C-2), 61.8 (*C*H_2Ph), 60.0 (C-7), 47.3 (C-1), 46.6 (C-4), 45.7 (C-5). HRFAB-MS cald. for $[M + H]^+$ $C_{13}H_{18}NO_3$: 236.1287, found: 236.1286.

(1*S*)-2-*exo*,3-*endo*-2,3-Dihydroxy-4-*endo*-4-hydroxymethyl-6-azabicyclo[3.1.0]hexane (6). To a solution of **26** (14 mg, 0.06 mmol) in ethanol (1.5 mL) was added palladium over charcoal 10% (30 mg) and the mixture was hydrogenated at atmospheric pressure and room temperature for 10 minutes. Then it was filtered through a Celite bed and concentrated to dryness to give **6** (7.2 mg; 83%). ^{1}H NMR (300 MHz, D_2O): δ 4.07 (s, 1H, $J_{2,3} \approx$ 0.0 Hz, H-2), 3.91 (m, 1H, H-3), 3.86 (dd, 1H, $J_{4,7a}$ = 7.3 Hz, $J_{7a,7b}$ = 11.0 Hz, H-7a), 3.79 (dd, 1H, $J_{4,7b}$ = 7.6 Hz, H-7b), 2.65 (br s, 1H, H-5), 2.60 (br s, 1H, H-1), 2.52 (m, 1H, H-4). ^{13}C NMR (75.5 MHz, D_2O): δ 77.1 (C-2), 75.7 (C-3), 59.0 (C-7), 44.7 (C-4), 37.5 (C-1), 36.1 (C-5). HRCI-MS calcd. for $[M + H]^+$ $C_6H_{12}NO_3$: 146.0817, found: 146.0814.

Enzyme kinetics

The enzyme assays were carried out as described previously.[18] All assays were performed at pH 6.8 and 25 °C. Steady state kinetics was performed and reaction rates were measured after possible slow-onset inhibition was essentially complete. The inhibition constants (K_i) were obtained from the formula $K_i = [I]/(K_{M'}/K_M\text{-}1)$, where $K_{M'}$ and K_M are Michaelis–Menten constants with and without inhibitor present respectively. $K_{M'}$ and K_M were obtained from a Hanes plot, which was also used to ensure that inhibition was competitive. The following K_M values (without inhibitor) were obtained using 4-nitrophenyl glycosides as substrates and the above conditions: α-glucosidase (yeast): 0.25 mM, β-glucosidase (almonds): 4 mM, α-fucosidase (bovine kidney): 0.24 mM.

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