β-Glycosidase inhibitors mimicking the pyranoside boat conformation

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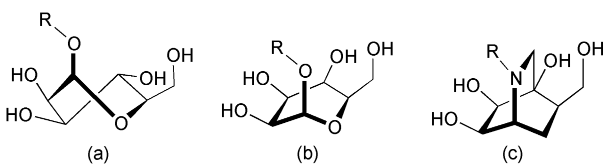
Polyhydroxylated isoquinuclidines mimicking the boat conformation of pyranosides are strong and selective inhibitors of a retaining β-mannosidase.

According to the principle of stereoelectronic control,[1] heterolytic cleavage of an acetal C–O bond requires an antiperiplanar orientation of a doubly occupied, non-bonding orbital. This antiperiplanar lone pair hypothesis (ALPH) means that hydrolysis of β-D-pyranosides involves a conformational change of the tetrahydropyran ring from a chair to a twist-boat or boat resulting in a pseudoaxial orientation of the aglycon (Fig. 1).[2] A



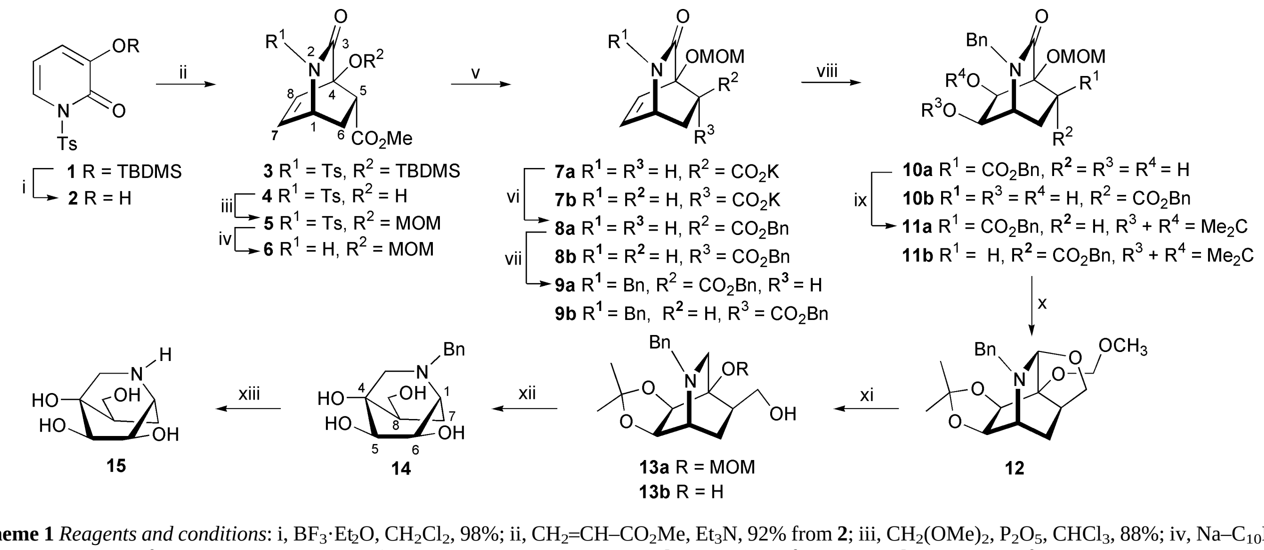
14 R = Bn
15 R = H

Fig. 1 Skew boat ($^1S_3$, a) and boat ($^{1,4}B$, b) conformers of a β-D-mannopyranoside and isoquinuclidines **14** and **15**.

similar conformational change is required for nucleophilic assistance of the cleavage according to an intermediate S_N1/S_N2 mechanism. The relevance of ALPH for enzymic glycoside cleavage has met with strong scepticism,[2c] but crystal structures of three *endo*-glycosidases in complex with substrate analogues show a skew boat or a flattened boat conformation of the tetrahydropyran ring.[3] A skew boat or boat-like conformer with a pseudoaxial (elongated) C(1)–O bond may conceivably be closer to the transition state of an enzymic β-glycoside cleavage than a reactive intermediate of an oxycarbenium cation type. If so, inhibitors mimicking the boat-like conformation of a glycoside, the proper location of the 'glycosidic heteroatom' (*i.e.* the heteroatom attached to C(1) of the glycoside), and the correct orientation of its lone pairs may possess a more strongly pronounced character as transition state analogues than inhibitors mimicking the cationic reactive intermediate. Inhibitors mimicking the shape of an oxycarbenium cation appear indeed to be only partial transition state analogues.[4] Since the conformational change from a chair to a twist-boat or boat should be induced by all β-glycosidases, we wanted to synthesize and evaluate a mimic of such a conformer of a glycoside that is cleaved by an *exo*-glycosidase. The cyclohexane ring of **14** and **15** (Fig. 1) mimics the tetrahydropyran ring of a β-mannoside in a $^{1,4}B$ conformation; it is substituted by a pseudoaxial amino group that should allow lateral protonation.[5] Only one[6] of the known bicyclic glycosidase inhibitors[7] mimicks a tetrahydropran ring in a ($^{2,5}B$) boat conformation. It has been designed in another conceptual context, and was not tested against β-mannosidases; it is a poor inhibitor of other glycosidases.

The isoquinuclidines **14** and **15** were synthesised in 14 and 15 steps and in 6.6 and 5.4% overall yields, respectively, from 3-hydroxypyridone *via* the known tosylate **1**[8] as shown in Scheme 1. Notable features are the diastereoselective high yielding Michael addition and aldolisation of **2** to **4**, the epimerisation and hydrolysis (dealkylation?) of the ester **6** to **7a**, and the two step reduction of **11a** *via* **12** to **13**.†

Both **14**‡ (K_i = 0.17 μM; IC_{50} = 0.69 μM)§ and **15**¶ (K_i = 20 μM; IC_{50} = 29.4 μM) inhibit snail β-mannosidase competitively, as determined from a Lineweaver–Burk plot, **14** being about 120 times stronger than **15**. Jack bean α-mannosidase is inhibited *ca*. 10^4 times more weakly by **14** (IC_{50} = 9.6 mM) than snail β-mannosidase and about 700 times more weakly by **15** (IC_{50} = 20 mM). Both **14** (IC_{50} > 5.0 mM) and **15** (IC_{50} = 4.3 mM) are poor inhibitors of β-glucosidase from *Caldocellum saccharolyticum*. The value of IC_{50} for β-mannosidase dropped from 4.08 to 0.69 μM for **14** and from 183 to 29.4 μM for **15** as the preincubation was prolonged from 10



Scheme 1 *Reagents and conditions*: i, BF_3·Et_2O, CH_2Cl_2, 98%; ii, CH_2=CH–CO_2Me, Et_3N, 92% from **2**; iii, $CH_2(OMe)_2$, P_2O_5, $CHCl_3$, 88%; iv, Na–$C_{10}H_8$, DME, −78 °C, 88% from **5**; v, K_2CO_3, MeOH, Δ; vi, BnBr, $NaHCO_3$, DMF, **8a**/**8b** 80 : 20 (49% from **6**), **9a**/**9b** 80 : 20 (18% from **6**); vii, NaH, BnBr, DMF, 89%; viii, OsO_4, acetone–H_2O, 89%; ix, $Me_2C(OMe)_2$, acetone, CSA, 94%; x, THF, Δ, **12** (57%), **13a** (17%), **13b** (8%); xi, $LiAlH_4$, dioxane, Δ, **13a** (67%); xii, TFA, H_2O, Δ, 84%; xiii, H_2/Pd(OH)$_2$/C, conc. HCl, MeOH/H_2O, 82%.

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min to 2 h. The inhibition by **14** and **15**, as represented by $1/IC_{50}$, shows a linear dependence on pH, revealing inhibition by the free amines rather than by the ammonium salts. The inhibition is *ca*. 4–6 times stronger at pH 5.6 than at pH 4.5 and again *ca*. 4–6 times weaker at pH 3.6.‖

Since **15** (pK_{HA} = 8.4) is a stronger base than **14** (pK_{HA} = 7.5), one expects **14** to be a stronger inhibitor than **15** at pH 4.5 by a factor of about 10. That **14** is 120 times stronger than **15** evidences a hydrophobic interaction of the benzyl group of **14** with the aglycon binding site. Such interactions are well precedented.[10,11]

The pH dependence of the inhibition by **14** and **15** evidences the essential interaction with the catalytic acid, and confirms its flexibility.[12] In contrast to the inhibition by the azole type inhibitors,[4,13–15] that is characterised by a cooperative interaction of the inhibitor with the catalytic acid and the catalytic nucleophile[2b] the interaction of **14** and **15** with the catalytic nucleophile appears to play at best a minor role. The inhibitory activity of **14** and **15** is in agreement with the postulate that a conformational change of the pyranose ring precedes or accompanies the enzymatic cleavage of β-glycosides.

Notes and references

† The esters **9a** and **9b** were isolated in yields of 41 and 10% from **5**. Selected ^{1}H-NMR data for **8a**: 4.00 (ddt, J = 5.6, 3.4, 2.1, H-C(1)), 2.93 (dd, J ≈ 10.5, 4.9, H-C(5)), 1.95 (ddd, J = 12.5, 5.0, 3.4, H_{exo}-C(6)), 1.79 (ddd, J = 12.5, 10.6, 2.2, H_{endo}-C(6)); selected ^{1}H-NMR data for **8b**: 4.05 (ddt, J ≈ 5.3, 3.6, 1.4, H-C(1)), 3.06 (ddd, J = 10.0, 5.1, 0.9, H-C(5)), 2.09 (ddd, J = 12.8, 10.0, 3.7, H_{exo}-C(6)), 1.56 (ddd, J = 12.5, 5.0, 1.6, H_{endo}-C(6)). The *exo* configuration of the diols **10** (*exo* refers to the face *syn* to the C(1)–N bond) was assigned on the basis that $J_{1,6}$ = 2.1 Hz (**10a** and **10b**) is smaller than $J_{1,7exo}$ = 3.6 (**10a**) and 4.4 Hz (**10b**) and closer to $J_{1,7endo}$ = 2.0 (**10a**) and 1.7 Hz (**10b**).[8] An attempt to reduce **11a** to **13** in one step was not successful.

‡ Data for **14**: R_f (AcOEt–MeOH 5:1), 0.40; δ_H(300 MHz, CD_3OD): 7.40–7.13 (m, arom. H), 3.88 (dd, J = 12.1, 6.5, CH_2OH), 3.85 (dd, J = 8.4, 2.1, irrad. at 2.66 → d, J = 8.4, H-C(6), 3.81, 3.72 (2d, J = 13.1, N-CH_2Ph), 3.65 (dd, J = 10.6, 6.5, CH_2OH), 3.60 (dd, J = 8.4, 1.3, H-C(5)); 2.89 (dd, J ≈ 9.5, 1.8, H_b-C(3)), 2.66 (br q, J ≈ 2.6, H-C(1)), 2.47 (dd, J ≈ 9.6, 1.6, irrad. at 2.89 → d, J = 4.4, H_a-C(3)), 1.84–1.61 (m, irrad. at 2.66 → change, irrad. at 2.89 → change, H_{exo}-C(7), H-C(8)), 1.59 (ddd, J ≈ 13.7, 10.6, 2.8, irrad. at 2.66 → change, H_{endo}-C(7)); δ_C(75 MHz, CD_3OD): 140.54 (s), 130.22 (d), 129.60 (d); 128.38 (arom. C); 73. 56 (s, C(4)); 73.45 (d, C(6)); 70.33 (d, C(5)); 63.81 (t, CH_2OH), 61.29 (t, N-CH_2Ph), 56.82 (d, C(1)), 51.32 (t, C(3)), 40.14 (d, C(8)), 24.89 (d, C(7)); ESI–MS: 280 ([M + 1]$^+$), 302 ([M + Na]$^+$). Anal. calc. for $C_{15}H_{21}NO_4$·$0.5H_2O$: C 62.48, H 7.69, N 4.86%. Found: C 62.24, H 7.47, N 4.83%.

§ Snail β-mannosidase: at 25 °C and pH 4.5; jack bean α-mannosidase: at 37 °C and pH 4.5; β-glucosidase from *Caldocellum saccharolyticum*: at 55 °C and pH 6.8.

¶ Data for **15**: R_f (AcOEt–MeOH 5:1): 0.40; δ_H(300 MHz, D_2O): 3.99 (dd, J ≈ 8.6, 2.1, irrad. at 2.75 → d, J = 8.7, H-C(6)), 3.85 (dd, J = 10.9, 5.3, irrad. at 1.85 → d, J = 10.6, CH_b–C(8)), 3.73 (dd, J ≈ 8.6, 1.8, irrad. at 2.65→ d, J = 8.7, irrad. at 3.99 → t, J = 1.9, H–C(5)), 3.62 (dd, J ≈ 11.1, 8.0, irrad. at 1.85 → d, J = 10.6, –CH_a–C(8)), 2.88, br. dd, J ≈ 11.2, 2.0, irrad. at 1.85 → d, J = 11.2, irrad. at 2.65 → d, J ≈ 5.0, H_a–C(3)), 2.75 (br q, J ≈ 2.4, irrad. at 1.52 → br. t, J ≈ 2.5, irrad. at 1.85 → t, J = 2.2, irrad. at 3.99 → change, H–C(1)); 2.65 (dd, J ≈ 11.4, 1.8, H_b–C(3)), 1.76–1.95 (m, irrad. at 1.52 → change, irrad. at 2.75 → change, H_{endo}–C(7), H–C(8)), 1.52 (dd, J = 7.8, 3.4, irrad. at 1.85 → d, J = 2.5, irrad. at 2.75 → d, J = 7.8, H_{exo}–C(7)). δ_C(75 MHz, D_2O): 73.49 (d, C(6)), 73.35 (s, C(4)), 70.24 (d, C(5)), 65.00 (t, CH_2–C(8)), 41.58 (t, C(3)), 40.53 (d, C(1)), 29.01 (t, C(7)); MALDI-MS: 190 (100, [M + 1]$^+$), 212 (10, [M + Na]$^+$). Anal. calc. for $C_8H_{15}NO_4$·0.5 H_2O: C 48.48, H 8.14, N 7.07%. Found: C 48.28, H 7.91, N 6.77%.

‖ The enzyme loses *ca*. 50% activity at pH 5.5 and *ca*. 10% at pH 3.5.[9]

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