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Note

Synthesis and activity towards yeast α-glucosidase of 1,5-dideoxy-1,5-imino-L-iditol (1-deoxy-L-idonojirimycin)

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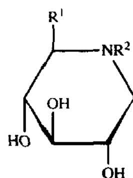
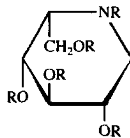
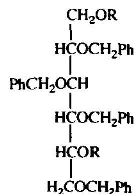
The synthesis and evaluation of glycosidase inhibitors is the subject of considerable current research[1]. Many glycosidases are involved in glycoprotein processing[2] and the breakdown of dietary carbohydrates[3], and control of such functions offers possibilities for chemotherapy in the treatment of certain diseases. Some glycosidase inhibitors, for example 1-deoxynojirimycin (**1**), which is a 1-deoxy-D-glucose analogue having an imino group in place of the ring oxygen, show anti-HIV activity[4] and *N*-butyl-1-deoxynojirimycin (**2**) is undergoing clinical trials[5]. Although some stereoisomers of **1** have been isolated or prepared, for example the D-*manno*[6–10] and D-*galacto* compounds[11–14] (C-2 and C-4 epimers, respectively), the C-3 epimer is apparently unknown and the C-5 epimer, 1,5-dideoxy-1,5-imino-L-iditol (1-deoxy-L-idonojirimycin) (**3**), has only been obtained, in the form of *N*-benzhydryl[15] and *N*-benzyl[9,15] derivatives, in low yields in non-stereospecific syntheses directed towards 1-deoxynojirimycin. We now report an efficient and simple synthesis of **3** and its inhibitory properties towards yeast α-glucosidase.



1 $R^1 = CH_2OH$, $R^2 = H$
2 $R^1 = CH_2OH$, $R^2 = n\text{-}C_4H_9$
7 $R^1 = R^2 = H$



3 R = H
6 $R = CH_2Ph$



4 R = H
5 $R = SO_2Me$

2,3,4,6-Tetra-*O*-benzyl-D-glucitol (**4**), prepared[16] by reduction of commercially available 2,3,4,6-tetra-*O*-benzyl-D-glucose, was converted[16] into the 1,5-di-*O*-

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methanesulphonyl derivative **5**, which was reacted with benzylamine for 4 days at 55°C under N_2 to afford, as an oil, *N*-benzyl-2,3,4,6-tetra-*O*-benzyl-1,5-dideoxy-1,5-imino-L-iditol (**6**) in 56% yield. Hydrogenolysis (10% Pd–C) of **6** in ethanol containing hydrochloric acid gave, on removal of catalyst and concentration, compound **3** as its hydrochloride (foam) in 97% yield.

Inhibition studies on **3** towards yeast α-glucosidase were conducted at pH 6.5 and at 30°C with 4-nitrophenyl α-D-glucopyranoside as substrate and similar measurements were conducted on 1-deoxynojirimycin (**1**). Lineweaver–Burk plots for the determination of inhibition constants (K_i) for **1** and **3** are shown in Figs. 1a and 1b, respectively. In agreement with other work[17,18], **1** was a potent competitive inhibitor of yeast α-glucosidase ($K_i = 14.6 \times 10^{-6}$ M). In contrast, **3** was found to be a non-competitive inhibitor of the enzyme ($K_i = 0.26 \times 10^{-3}$ M). Thus, inhibitor **3** and substrate bind to the enzyme reversibly, randomly, and independently at different sites but the resulting enzyme–substrate–inhibitor complex is inactive catalytically. It is possible that correct orientation of the catalytic site might be prevented in the presence of the inhibitor.

Clearly, inversion of configuration at C-5 in **1** to give **3** precludes acceptance of the inhibitor at the active site of the enzyme. Further, it has been noted by others[19] that 1,5-dideoxy-1,5-iminoxylitol (**7**), which is formally derived from **1** by removal of the hydroxymethyl group at C-5, has no effect whatsoever on yeast α-glucosidase. These data, in combination, indicate that the 5-CH_2OH group plays an important role in promoting effective binding of **1** at the active site of yeast α-glucosidase. It is pertinent, however, that the conformational preferences of the C-5 epimers **1** and **3** differ considerably, and therein may lie a partial explanation for their different inhibitory properties. Calculation[20] of the difference in free energy for the $^4C_1$ and $^1C_4$ conformers of **1** and **3** indicate that whereas for **1** the $^4C_1$ conformer is favoured to an overwhelming extent (> 98%), for **3** the corresponding figure is ~ 80%. Recent empirical correlations[18] have indicated the importance of certain topological features of this type of inhibitor in facilitating effective binding to enzymes.

EXPERIMENTAL

^1H NMR spectra were recorded (internal Me_4Si) at 400 MHz, and ^{13}C NMR spectra were recorded at 22.4 MHz. Rotations were measured with a Perkin–Elmer 141 polarimeter. TLC was performed on silica gel Machery-Nagel SIL G-25UV_{254} and column chromatography on Silica Gel 60 (Merck, 70–230 mesh). 2,3,4,6-Tetra-*O*-benzyl-1,5-di-*O*-methanesulphonyl-D-glucitol (**5**) was prepared[16] from 2,3,4,6-tetra-*O*-benzyl-D-glucose (Sigma Chemical Co.). Yeast α-glucosidase (type VI from brewer's yeast) was obtained from Sigma Chemical Co. Energy calculations were conducted using the MM2 force field in the Macromodel program[20].

N-Benzyl-2,3,4,6-tetra-O-benzyl-1,5-dideoxy-1,5-imino-L-iditol (**6**). — A mixture of **5**[16] (5.04 g, 7.2 mmol) and benzylamine (26.23 g, 0.24 mol) was stirred for 4 days

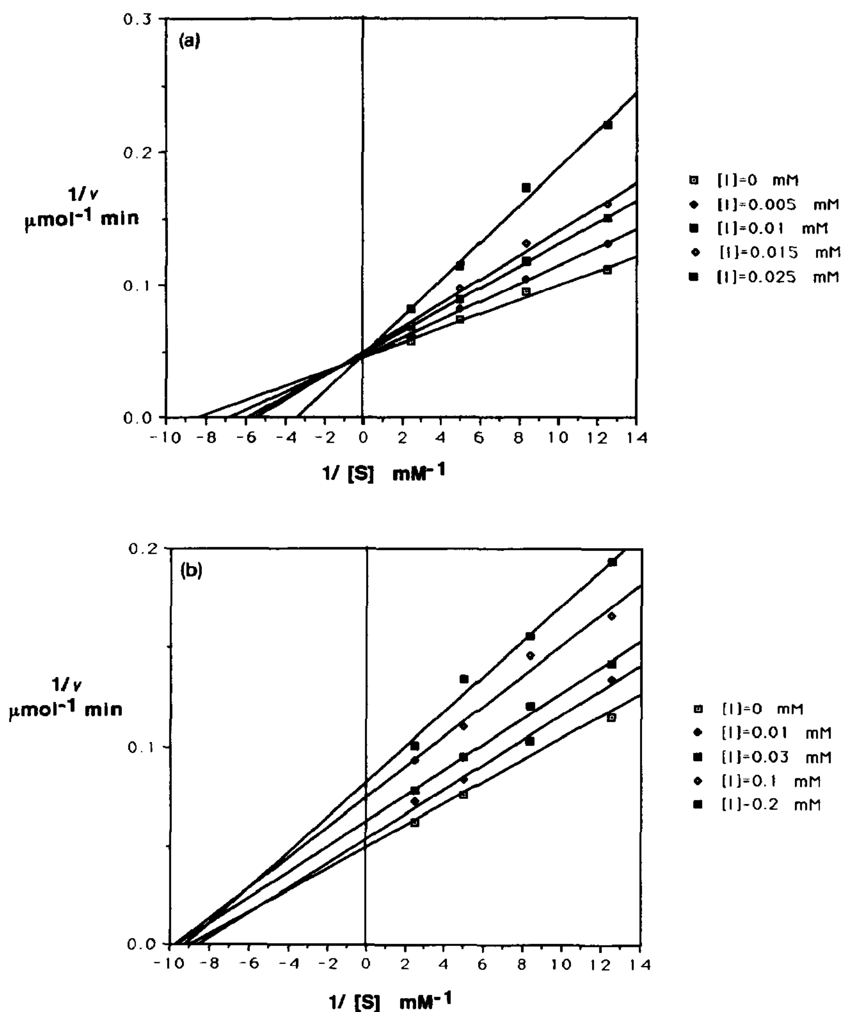

Fig. 1. Lineweaver–Burk reciprocal plots for the enzymic hydrolysis of 4-nitrophenyl α-D-glucopyranoside by yeast α-glucosidase in the presence of different fixed concentrations of (a) 1-deoxynojirimycin (1) (competitive inhibition) and (b) 1-deoxy-L-idonojirimycin (3) (non-competitive inhibition). Velocity (v) refers to the rate of liberation of 4-nitrophenol at 30°C and pH 6.5 in µmol min^{-1} per mg of enzyme.

at 55°C under N_2. Excess of benzylamine was removed by vacuum distillation (bath temperature 55°C/0.5 mmHg) and the residue was subjected to column chromatography (EtOAc–hexane, 1:19) to give, as an oil, **6** (2.48 g, 56%), $[\alpha]_D$ −34.5° (c 1, $CHCl_3$). NMR data ($CDCl_3$): ^1H, δ 2.550–2.640 (m, 1 H, H-1), 2.842 (dd, 1 H, $J_{1,1'}$ 11.7, $J_{1',2}$ 4.4 Hz, H-1′), 3.425 (m, 1 H, H-5), 3.520–3.610 (complex, 2 H, H-2,3), 3.729 and 3.990 (2 d, each 1 H, $J_{A,B}$ 14.3 Hz, NCH_2Ph), 3.700–3.790 (complex, 2 H, H-4,6), 3.938 (dd, 1 H, $J_{5,6'}$ 7.3, $J_{6,6'}$ 10.3 Hz, H-6′), 4.520, 4.533, 4.542, 4.611, 4.619, 4.637, 4.814, 4.848 (8 d, each 1 H, 4 OCH_2Ph), 7.200–7.370

(complex, 25 H, ArH); ^{13}C, δ 48.8 [NCH$_2$CH(OR)], 59.0 (NCH$_2$Ph), 59.8 (NCH), 64.7 (CH$_2$OCH$_2$Ph), 72.5, 72.7, 73.2, 75.3 (OCH$_2$Ph), 79.0, 80.3, 83.1 (CHOCH$_2$Ph), 126.8, 127.3, 127.5, 127.7, 127.8, 128.1, 128.2, 128.3, 138.5, 139.2, and 139.6 (aromatic C). Anal. Calcd for $C_{41}H_{43}NO_4$: C, 80.2; H, 7.1; N, 2.3. Found: C, 80.1; H, 7.2; N, 2.5.

1,5-Dideoxy-1,5-imino-L-iditol hydrochloride (**3** · HCl). — A solution of **6** (0.44 g, 0.72 mmol) in EtOH (11 mL) containing concd HCl (0.16 mL) was stirred under H_2 in the presence of 10% Pd–C (0.2 g) until uptake of H_2 ceased (6 days). The filtered solution was concentrated to leave, as a foam, the hydrochloride **3** · HCl (0.14 g, 97%), $[\alpha]_D$ −0.16°, $[\alpha]_{436}$ −0.74° (c 1.9, MeOH). NMR data (CD_3OD): ^{1}H, δ 3.244 (dd, 1 H, $J_{1,1'}$ 13.2, $J_{1,2}$ 1.5 Hz, H-1), 3.363 (dd, 1 H, $J_{1',3}$ 1.8 Hz, H-1'), 3.476 (ddd, 1 H, $J_{4,5}$ 1.8, $J_{5,6}$ 4.8, $J_{5,6'}$ 9.2 Hz, H-5), 3.808 (dd, 1 H, $J_{6,6'}$ 11.7 Hz, H-6), 3.852 (dd, 1 H, H-6'), 3.891 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 3.910–3.975 (m, 2 H, H-2,3); ^{13}C, δ 47.1 (NCH_2), 58.3 (NCH), 60.6 (CH_2O), 67.9, 68.4, and 69.2 (CHO). Mass spectrum: m/z 164.0923 ($C_6H_{14}NO_4$). Anal. Calcd for $C_6H_{14}ClNO_4$: C, 36.1; H, 7.1. Found: C, 36.2; H, 7.0.

Enzyme assays. — Assays were carried out at 30°C and pH 6.5 (10 mM PIPES–20 mM sodium acetate–0.1 mM EDTA, pH adjusted with 5 M HCl) with 4-nitrophenyl α-D-glucopyranoside as substrate (K_m = 111 μM) and with substrate concentrations in the range 0.08 to 0.4 mM. Liberation of 4-nitrophenol was measured at 400 nm and experiments were conducted so that less than 10% of the substrate was consumed. The slopes of Lineweaver–Burk reciprocal plots of $1/v$ against 1/[S] in the presence of increasing amounts of inhibitor (Figs. 1a and 1b) were plotted against the corresponding inhibitor concentrations [I], to which they are linearly related, and inhibition constants (K_i) were calculated from the intercept of this graph on the [I] axis[21].

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