SUPPLEMENTARY INFORMATION

A new, simple, high affinity glycosidase inhibitor: analysis of binding through x-ray crystallography, mutagenesis and kinetic analysis

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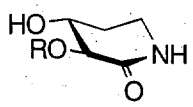
Experimental

General

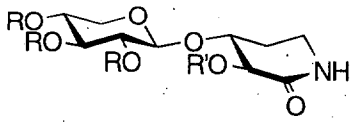
All melting points are uncorrected. Organic extracts were dried over $MgSO_4$ and concentrated *in vacuo*. ^1H and ^{13}C NMR spectra were recorded on Bruker instruments and were referenced using the solvent peak. NMR spectra were in $CDCl_3$ unless otherwise noted. Flash chromatography was performed on Merck silica gel 60 (a) Still, W.C.; Kahn, M.; Mitra, A.J. *J. Org. Chem.* **1978**, *43*, 2923-2925. Thin layer chromatography was performed on Merck silica gel 60 F_{254} plates. Microanalyses were performed by Mr Peter Borda at the University of British Columbia.



6 R = H
7 R = Bz

*5-Amino-4,5-dideoxy-2-*O*-benzoyl-*D-threo*-pentono-1,5-lactam* **7**

A solution of the lactam **6** (222 mg, 1.69 mmol) in dry pyridine (5 ml) was treated with benzoyl chloride (237 μL, 2.03 mmol) at -40° and the solution was allowed to warm to room temperature overnight. The solvent was evaporated and the residue was purified by flash chromatography (80-100% EtOAc/petrol → 10% MeOH/EtOAc) to give the monobenzoate **6** as a white solid (269 mg, 68%). A small portion was crystallized to give colorless needles, m.p. 137-139° ($CHCl_3/Et_2O$). ^{1}H NMR (400 MHz, d_6-DMSO): δ 1.83-1.95, 2.03-2.12 (2m, H4,4), 3.13-3.26 (m, H5,5), 4.02-4.11 (m, H3), 5.18 (d, $J_{2,3}$ 8.7 Hz, H2), 5.49 (d, $J_{3,OH}$ 4.8 Hz, OH), 7.54-7.74, 8.00-8.05 (2m, Ph), 7.84 (br s, NH). ^{13}C NMR (75.5 MHz, d_6-DMSO) δ 29.75 (C4), 37.32 (C5), 66.84 (C3), 75.43 (C2), 128.71, 129.45, 133.41 (Ph), 165.31 (NCO), 167.21 (PhCO). Anal. calc. for $C_{12}H_{13}NO_6$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.26; H, 5.64; N, 6.02. DCI HRMS (NH_3/CH_4): calc. for *m/z* $[M + H]^+$: 236.0923; found: 236.0922.



8 R = Ac, R' = Bz
1 R, R' = H

*3-*O*-(Tri-*O*-acetyl-β-*D*-xylopyranosyl)-5-amino-2-*O*-benzoyl-*D-threo*-pentono-1,5-lactam* **8**

$BF_3 \cdot Et_2O$ (200 μL, 1.6 mmol) was added to a solution the alcohol **7** (157 mg, 668 μmol) and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl trichloroacetimidate (393 mg, 935 μmol) in 1,2-dichloroethane (10 mL) at room temperature under N_2 and allowed to stand for 2 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$, separated, dried ($MgSO_4$) and the

solvent evaporated. The residue was purified by flash chromatography (80-100% EtOAc/petrol → 10% MeOH/EtOAc) to give the disaccharide **8** as a colourless solid (233 mg, 71%). Recrystallization afforded colorless needles, m.p. 147-149° (EtOH/Pr^i_2O). ^{1}H NMR (400 MHz): δ1.90-2.05, 2.15-2.23 (2m, H4,4), 1.97, 1.99, 2.02 (3s, Ac), 3.24 (dd, $J_{4',5'}$ 6.8, $J_{5',5'}$ 12.2 Hz, H5'), 3.28-3.42 (m, H5,5), 3.90 (dd, $J_{4',5'}$ 4.2 Hz, H5'), 4.28 (ddd, $J_{2,3}$ 7.7, $J_{3,4}$ 3.9, 9.6, Hz, H3), 4.68 (d, $J_{1',2'}$ 5.3 Hz, H1'), 4.73 (ddd, $J_{3',4'} \approx J_{4',5'}$ 6.8, $J_{4',5'}$ 4.3 Hz, H4'), 4.80 (dd, $J_{2',3'}$ 7.0 Hz, H2'), 5.01 (dd, H3'), 5.38 (d, H2), 6.34 (br s, NH), 7.39-8.08 (3m, Ph). ^{13}C NMR (75.5 MHz) δ 20.60, 20.68, 20.72 (Me), 26.63 (C4), 37.81 (C5), 60.79 (C5'), 67.94, 69.73, 69.83, 72.81, 74.31 (C2,3,2',3',4'), 98.01 (C1'), 128.40, 129.89, 133.34 (Ph), 165.60 (NCO), 167.71 (PhCO), 169.26, 169.69, 169.89 (MeCO). Anal. calc. for $C_{23}H_{27}NO_{11}$: C, 55.98; H, 5.51; N, 2.84. Found: C, 55.58; H, 5.41; N, 2.76.

5-Amino-3-O-*(β*-D-*xylopyranosyl)*-D-threo-*pentono-1,5-lactam* **1**

A suspension of the disaccharide **8** (200 mg) in dry MeOH (10 mL) was treated with a small piece of sodium metal and the suspension was stirred overnight. The solution was neutralized with cation exchange resin (IR-120, H^+ form) and the solvent was evaporated. The residue was purified by flash chromatography (17:2:1 EtOAc/MeOH/H_2O) to give the lactam **1** as a colorless foam (81 mg, 76%). ^{1}H NMR (400 MHz, D_2O): δ1.84-1.96, 2.16-2.25 (2m, H4,4), 3.20-3.38 (m, 4H, H5,5,2',3'), 3.43 (dd, $J_{4',5'}$ 9.2, $J_{5',5'}$ 11.6 Hz, H5'), 3.61 (ddd, $J_{3',4'}$ 10.4, $J_{4',5'}$ 5.4 Hz, H4'), 3.94 (dd, H5'), 4.01-4.10 (m, H2,3), 4.53 (d, $J_{1',2'}$ 7.8 Hz, H1'). ^{13}C NMR (75 MHz, D_2O) δ25.27 (C4), 37.77 (C5), 65.41 (C5'), 69.44, 71.13, 73.02, 75.90, 76.86 (C2,3,2',3',4'), 100.92, (C1'), 173.36 (CO). Anal. calc. for $C_{10}H_{17}NO_7.H_2O$: C, 42.72; H, 6.81; N, 4.98. Found: C, 42.72; H, 6.65; N, 5.00.

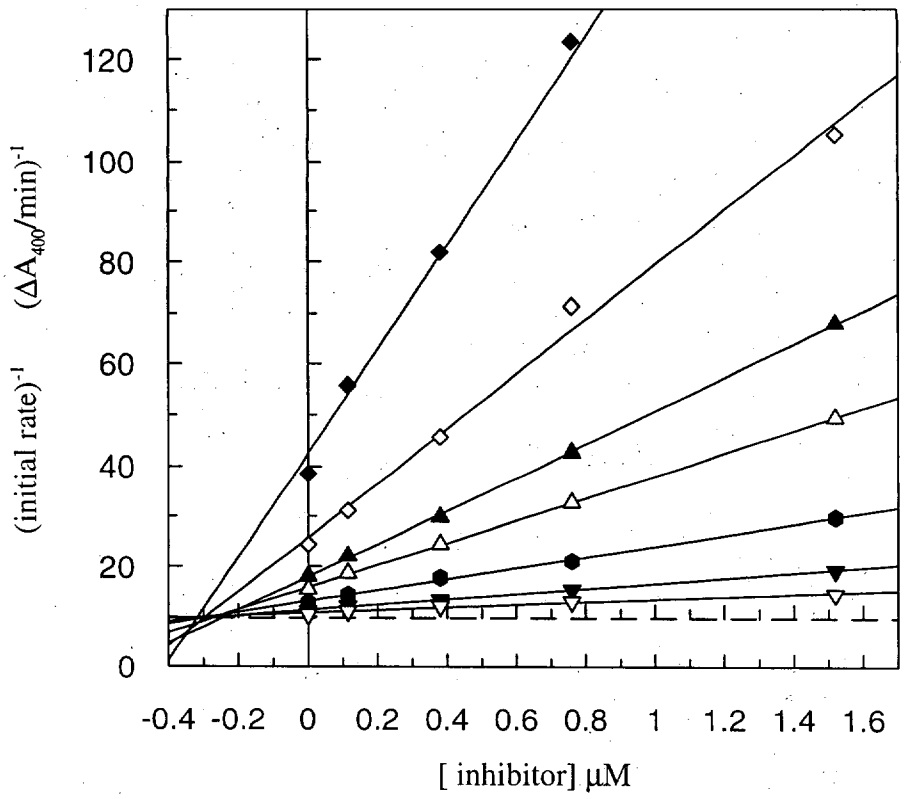


Figure 1. Dixon plot of inhibition of *Cellulomonas fimi* xylanase by the xylobiose-derived lactam **1**. The concentrations of the substrate, 2,4-dinitrophenyl β-cellobioside, used were 0.028 (◆), 0.056 (◇), 0.097 (▲), 0.139 (△), 0.278 (⬢), 0.556 (▼), 1.11 (▽) mM.

Kinetic Analysis

a) Inhibition of *Cellulomonas fimi* xylanase, Cex. Cex was purified as described previously (a) Gilkes, N.R.; Langford, M.L.; Kilburn, D.G.; Miller, R.C.; Warren, R.A.J. *J. Biol. Chem.* **1984**, *259*, 10455-10459. Inhibition constants were determined at 37°C using a 0.05 M NaH_2PO_4/Na_2PO_4 buffer (pH 7.0) and 2,4-dinitrophenyl β-cellobioside as a substrate. Measurements were started by addition of Cex. Measurements of the increase of absorption at

400 nm per min in a continuous assay yielded reaction rates. This increase was linear during all measurements (1-3 min). Michaelis parameters (V_{max} and K_m) were extracted from these data by best fit to the Michaelis-Menten equation using the program *Grafit* (b) Leatherbarrow, R.J. *Grafit 4.0*; Erithacus Software: Staines. Estimates of K_i values were obtained by measuring rates in a series of cells at a fixed substrate concentration in the presence of a range of inhibitor concentrations (6-10 concentrations) which encompassed the K_i value ultimately determined, generally from 0.3 K_i to 3 K_i. The observed rates were plotted in the form of a Dixon plot and the K_i value was determined by an intersection of this line with a horizontal line drawn through $1/V_{max}$. Full K_i determinations were performed by measurement of rates at a series of 7 substrate concentrations (generally from 0.3 K_i to 3 K_i) in the presence of a range of inhibitor concentrations (typically 5 concentrations) which bracket the K_i value ultimately determined. Full K_i determinations were usually within 25% of the estimated K_i values. K_i values were calculated from these data by 3-dimensional non-linear regression analysis using the program *Grafit*.

The generation of the N126A Cex mutant and the kinetic analysis performed with this mutant will be described elsewhere.