

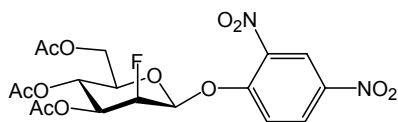
SUPPLEMENTARY MATERIAL FOR

Structure of the Michaelis complex of β -mannosidase, Man2A, provides insight into the conformational itinerary of mannoside hydrolysis

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Synthesis of Reagents

2,4-Dinitrophenyl 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro- β -D-mannopyranoside



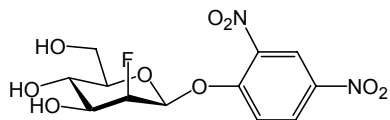
3,4,6-Tri-O-acetyl-D-glucal (5.62 g, 20.6 mmol) was dissolved in 3:1 DMF / H₂O (200 mL) followed by SelectfluorTM (10.5 g, 29.7 mmol, 1.4 eq.) and the whole mixture stirred overnight at 50°C. TLC analysis (1:1 PE/EtOAc) indicated complete conversion of the D-glucal to 2-deoxy-2-fluoro hexopyranoses at this time. The solvent was evaporated and the residue redissolved in EtOAc (300 mL), washed with brine and water, and then dried over MgSO₄. The solvent was evaporated and the crude, without further purification, re-dissolved in DMF (200 mL). DABCO (7.0 g, 62.4 mmol, ~ 3 eq.) was added and dissolved, followed by 2,4-dinitrofluorobenzene (6.0 g, 32.2 mmol, ~ 1.5 eq.). The vessel was purged with argon and stirred in the dark overnight at room temperature, whereupon TLC analysis (3:4 EtOAc / PE) indicated complete conversion to 2,4-dinitrophenyl 2-deoxy-2-fluoro-glycosides. The solvent was evaporated and the residue redissolved in EtOAc (300 mL). The solution was washed with 0.2 M H₂SO₄, saturated sodium bicarbonate and water. The organic layer was then dried over MgSO₄ then evaporated to afford a dark brown oil. Chromatography on silica gel (4:3 to 1:1 EtOAc / PE) and evaporation of the solvent afforded the alpha (1.49 g, 3.13 mmol, 15.1%) and beta (1.55 g, 3.26 mmol, 15.7%, $\beta/\alpha = 1:1$) anomers of the protected 2,4-dinitrophenyl 2-fluoro-mannosides, and an anomeric mixture of the protected 2,4-dinitrophenyl 2-fluoro-glucosides (2.58 g, 5.43 mmol, 26%, $\beta/\alpha = 3.7:1$ by ¹⁹F NMR) as pale yellow crystalline solids. ¹H and ¹⁹F NMR spectral data matched that reported previously (Ref 1S).

Data for 2,4-Dinitrophenyl 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro- β -D-mannopyranoside: Rf (3:4 EtOAc / PE) = 0.20; M.p. = 189.5-190.5°C; ¹H NMR (400 MHz, CDCl₃): δ 8.74 (d, 1 H, $J = 2.5$ Hz, H-3 Ph), 8.42 (dd, 1 H, $J = 2.5, 9.2$ Hz, H-5 Ph), 7.48 (d, 1 H, $J = 9.5$ Hz, H-6 Ph), 5.38 (dd, 1 H, $J = 14.6, < 1$ Hz, H-1), 5.37 (dd, 1 H, $J = 9.5, 9.5$ Hz, H-4), 5.14 (ddd, 1 H, $J = 24.3, 9.1, 2.6$ Hz, H-3), 5.12 (ddd, 1 H, $J = 51.4, 2.5, < 1$ Hz, H-2), 4.27 (apparent d, 2 H, $J = 4.4$ Hz, H-6_{a,b}), 3.87 (ddd, 1 H, $J = 4.4, 4.4, 8.8$ Hz, H-5), 2.14, 2.07, 2.06 (all s, 9 H, 3 \times OAc); ¹⁹F NMR (188.3 MHz, CDCl₃, referenced to CF₃CO₂H): δ -142.4 (ddd, $J_{F,H} = 50.3, 24.4, 15.2$ Hz); ¹³C NMR (100 MHz, CDCl₃): δ 170.4, 170.0, 169.2 (3 \times CH₃CO), 153.5, 142.6, 140.7 (3 \times C-Ph), 128.5, 121.6, 119.9 (3 \times CH-Ph), 97.2 (d, $J_{C,F} = 16.7$ Hz, C-1), 85.7 (d, $J_{C,F} = 194.7$ Hz, C-2), 73.3 (C-5), 70.2 (d, $J_{C,F} = 17.2$ Hz, C-3), 65.5 (C-4), 61.9 (C-6), 20.6 (3 lines, 3 \times CH₃CO); HRMS (DCI+): $m/z = 492.1269$; calcd. for C₁₈H₂₃FN₃O₁₂ [M + NH₄]⁺: $m/z = 492.1266$; Anal. calcd. for C₁₈H₁₉FN₂O₁₂ (474.35): C, 45.58; H, 4.04; N, 5.91; Found: C, 45.68; H, 4.15; N, 6.03.

Data for 2,4-Dinitrophenyl 3,4,6-tri-O-acetyl-2-deoxy-2-fluoro- α -D-mannopyranoside: Rf (3:4 EtOAc / PE) = 0.70; M.p. = 217-219°C; ¹H NMR (200 MHz, CDCl₃): δ 8.80 (d, 1 H, $J = 2.7$ Hz, H-3 Ph), 8.45 (dd, 1 H, $J = 2.7, 9.3$ Hz, H-5 Ph), 7.54 (d, 1 H, $J = 9.3$ Hz, H-6 Ph), 5.92 (dd, 1 H, $J = 1.9, 6.1$ Hz, H-1), 5.47 (dd, 1 H, $J = 10.0, 10.0$ Hz, H-4), 5.41 (ddd, $J = 2.2, 10.0, 29.5$ Hz, H-3), 5.08 (ddd, 1 H, $J = 2.1, 2.1, 48.8$ Hz, H-2), 4.25 (dd, 1 H, $J = 5.1, 12.7$ Hz, H-6_a), 4.07 (m, 2 H, H-5, H-6_b), 2.11, 2.05, 2.03 (all s, 9 H,

3 × OAc); ^{19}F NMR (188.3 MHz, CDCl_3 , referenced to $\text{CF}_3\text{CO}_2\text{H}$): δ -129.5 (ddd, $J_{F,H}$ = 48.8, 29.0, 6.1 Hz); HRMS (DCI+): m/z = 492.1262; ; calcd. for $\text{C}_{18}\text{H}_{23}\text{FN}_3\text{O}_{12}$ [$\text{M} + \text{NH}_4$] $^+$: m/z = 492.1266.

2,4-Dinitrophenyl 2-deoxy-2-fluoro- β -D-mannopyranoside



The per-*O*-acetate (1.69 g, 3.57 mmol) was suspended in dry MeOH (50 mL) and cooled to 0°C under argon. Acetyl chloride (2 mL) was added dropwise to afford a 4% solution of HCl. The mixture was stirred for 2 days at 4°C. The solvent was evaporated followed by repeated evaporation of dry diethyl ether from the residue to remove excess HCl. The crude was columned on silica gel (95:5 EtOAc/MeOH). Subsequent evaporation of the solvent and recrystallization of the residue from acetone / PE to afforded white needles (1.08 g, 3.10 mmol, 87%). R_f (30:2:1 EtOAc/MeOH/H₂O = 0.50; ^1H NMR (400 MHz, D₂O): δ 8.95 (d, 1 H, J = 2.8 Hz, H-3 Ph), 8.59 (dd, 1 H, J = 2.8, 9.4 Hz, H-5 Ph), 7.67 (d, 1 H, J = 9.6 Hz, H-6 Ph), 5.82 (dd, 1 H, J = 18.4, < 1 Hz, H-1), 5.19 (ddd, 1 H, J = 51.5, 2.2, < 1 Hz, H-2), 4.05 (dd, 1 H, J = 2.2, 12.5 Hz, H-6a), 3.96 (ddd, 1 H, J = 30.1, 9.4, 2.4 Hz, H-3), 3.87 (dd, 1 H, J = 5.9, 12.5 Hz, H-6b), 3.84 (ddd, J = 1.1, 9.7, 9.7 Hz, H-4), 3.78 (m, 1 H, H-5); ^{19}F NMR (188 MHz, D₂O, ref. to $\text{CF}_3\text{CO}_2\text{H}$): δ -144.8 (ddd, J = 48.8, 29.2, 18.4 Hz); ^{13}C NMR (100 MHz, D₂O): δ 154.1, 142.0, 139.1 (C-1', 2', 4'), 130.1, 122.5, 118.1 (C-3', 5', 6'), 96.9 (d, $J_{C,F}$ = 14.9 Hz, C-1), 90.2 (d, $J_{C,F}$ = 183.9 Hz, C-2), 77.1 (C-5), 71.7 (d, $J_{C,F}$ = 17.5 Hz, C-3), 66.6 (C-4), 60.7 (C-6). Anal. calcd. for $\text{C}_{12}\text{H}_{13}\text{FN}_2\text{O}_9$ (348.24) + 0.4 mol acetone: C, 42.92; H, 4.87; N, 7.59. Found: C, 42.92; H, 4.27; N, 7.26.

References:

1S. Ortner, J.; Albert, M.; Weber, H.; Dax, K. *J. Carbohydr. Chem.* **1999**, *18*, 297-316.