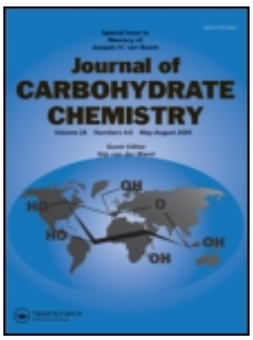

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Synthesis of Unsaturated Aminopyranosides as Possible Transition State Mimics for Glycosidases[†]

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ABSTRACT

Four unsaturated aminopyranosides have been prepared as possible transition-state mimics targeted towards carbohydrate processing enzymes. The conformations of the protonated aminosugars have been investigated by molecular modelling and their ability to inhibit α- and β-glucosidases and an α-mannosidase have been probed. Two targets proved moderate inhibitors of α-glucosidases from Brewer's yeast and *Bacillus stearothermophilus*.

Key Words: Glycosyl processing enzyme; Oligosaccharide; Inhibitor.

[†]This paper is dedicated to Professor Gérard Descotes on the occasion of his 70th birthday.

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INTRODUCTION

As progress in molecular biology and cellular biochemistry has developed, it has become increasingly apparent that the glycoproteins expressed on cell surfaces play fundamental roles in a vast array of biological processes and interactions, including cell-cell recognition, cell growth and development.[1–3] More recently, it has been found that a major pathway towards the malignancy of tumour cells is the decoration of these proteins with aberrant oligosaccharides.[4,5] These altered carbohydrates appear to confer a variety of properties on the cancerous cells, such as their ability to grow unchecked, evade the immune system and metastasise to secondary sites around the body.[6,7] Clearly, therefore, inhibiting the formation of these altered oligosaccharides offers an important method for therapeutic intervention. A multitude of natural and unnatural inhibitors of the carbohydrate processing enzymes have been reported, and their therapeutic properties are currently being assessed.[8]

During glycosidase mediated oligosaccharide processing, a half chair transition state with substantial sp^2 character at the anomeric position is formed and it has been postulated that compounds capable of mimicking this flattened transition state are good candidates for inhibition studies.[9–11] For example, unsaturated derivatives of the natural pyranoses are often good inhibitors of glycosidases in bacterial and fungal systems with K_i values several hundred times smaller than for their saturated analogues.[12,13] This is believed to be a result of unsaturation in the pyranose ring forcing them to adopt the half-chair conformation. The incorporation of amine substituents within monosaccharide derivatives has also been shown to further enhance inhibition.[14,15] For example, glycosylamines derived from D-glucose and D-mannose display affinites for their respective hydrolases up to 1000 times higher than the parent aldoses.[16] Similar findings have also been reported for D-galactosylamines.[17] Furthermore, it is worth noting that the amine need not be directly attached to the sugar ring, as β-D-glucosylmethylamine has also been shown to demonstrate moderate enzymatic inhibitory activity.[18]

RESULTS AND DISCUSSION

As part of a programme directed towards the identification of novel inhibitors for the glycoside processing enzymes, we were interested to determine whether unsaturated aminopyranosides would be of use as mimics of glycosidase transition states. Four compounds **5**, **12**, **15** and **18** that contained both *endo*cyclic double bonds and amine functionalities were identified as synthetic targets (Figure 1).

At the beginning of our programme, MOPAC molecular modelling studies were performed to probe the lowest energy conformations of the protonated targets and in each case this was found to be the half-chair conformation. Encouraged by these results, synthetic routes to **5**, **12**, **15** and **18** were devised. The partially protected mannopyranoside **1**[19] proved a key intermediate for entry to target **5** (Scheme 1). Oxidation of the alcohol using pyridinium chlorochromate (PCC) in toluene at reflux allowed one-pot entry to the required enal **2** in 58% yield.[20] Installation of the amine moiety at C-6 of enal **2** to afford intermediate **3** was achieved by reductive amination.

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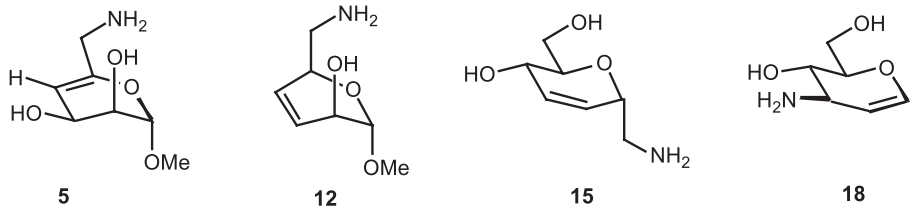


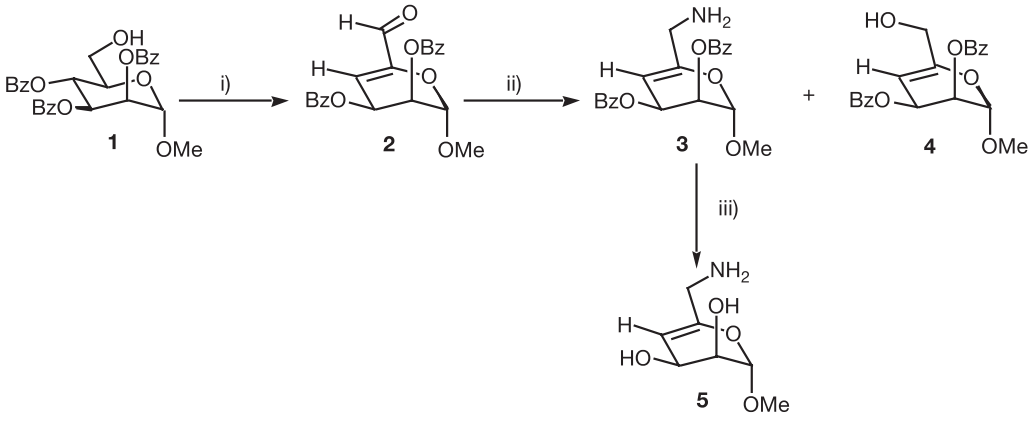
Figure 1.

The 6-hydroxy derivative **4** was also formed as a by-product in 10% yield. Removal of the benzoate groups from the C-2 and C-3 hydroxyl groups of **3** was achieved using K_2CO_3 or NaOMe in MeOH to afford the required target **5** in good yield.

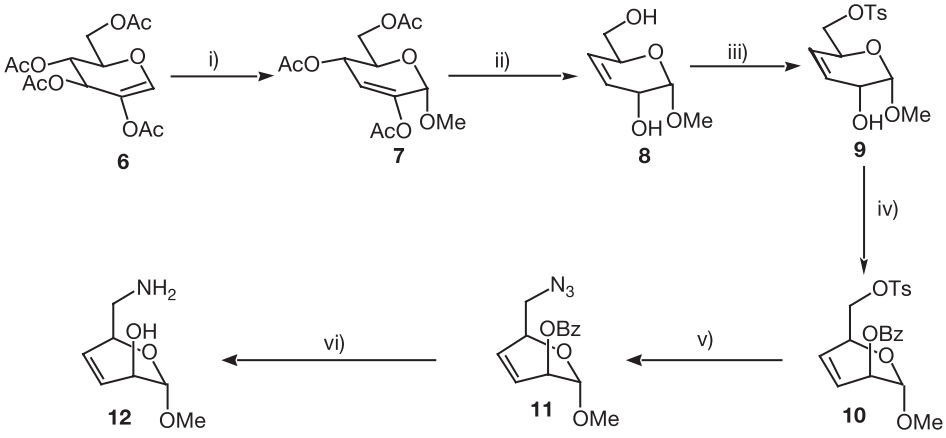
Glycal **6**[21] proved to be a convenient precursor to target **12** *via* a Ferrier glycosylation[22] with methanol (Scheme 2).

Optimum results for the Ferrier reaction were obtained using either $BF_3 \cdot OEt_2$ or $SnCl_4$ as catalyst, affording yields and ratios of methyl glycosides[23] **7α** and **7β** of 43% (8:1) and 49% (16:3) respectively. However, separation of the anomers proved impossible by column chromatography. The mixture of enopyranosides **7α** and **7β** thus formed was therefore treated with $LiAlH_4$ to induce reductive elimination of the acetates,[24] to afford diol **8**.[25] The reaction presumably proceeds *via* the formation of the intermediate allylic ketone and the highly stereoselective reduction of the ketone, as a result of hydride approach from the less hindered face of the molecule. Thus for the required methyl-α-glycoside **7α**, hydride attack occurs from the beta face. Separation of the anomers of **8** was possible at this stage, affording the α-D-erythro derivative **8α** in 61% yield.

In order to introduce the amine moiety at C-6, diol **8α** was converted to tosylate **9** in good yield *via* treatment with *p*-toluenesulfonyl chloride and triethyamine. Optimum



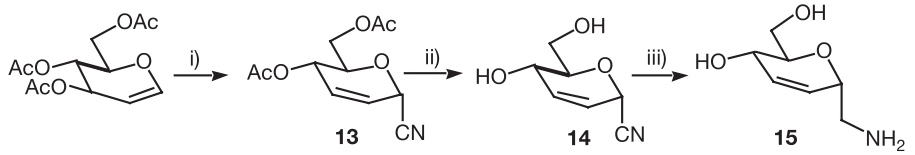
Scheme 1. i) PCC, silica gel, toluene, 58%; ii) NH_4OAc, $NaBH_3CN$, MeOH, 49% **3** and 10% **4**; iii) MeOH, K_2CO_3, 74%.



Scheme 2. i) MeOH, $BF_3{\cdot}OEt_2$, **7α:7β** 8: 1 43%; ii) $LiAlH_4$, THF, 61%; iii) *p*-TsCl, Et_3N, DCM, 73%; iv) PPh_3, $PhCO_2H$, DEAD, 90%; v) NaN_3, DMF, 42%; vi) $LiAlH_4$, Et_2O, 83%.

yields were obtained by performing the reaction under high dilution, but some of the di-tosylated derivative was consistently formed. Prior to introduction of the amine moiety at C-6, the hydroxyl group at C-2 was inverted *via* a Mitsunobu reaction, under standard reaction conditions, to afford the required α-D-*threo* derivative **10** in excellent yield. Pleasingly, no allylic rearrangement was observed, and the inversion of configuration at C-2 was confirmed by the reduced value of the $J_{1,2}$ coupling constant (0.8 Hz in **10** *versus* 4.4 Hz in **9**). Indirect introduction of the amine moiety to C-6 was achieved by displacement of the C-6 tosylate with sodium azide in DMF, at reflux, to afford azide **11** in moderate yield. Finally, reduction of the azide and deprotection of the C-2 benzoate ester was achieved in one-pot using lithium aluminium hydride in ether at reflux, to afford target *12* in an excellent 83% yield over two steps.

Synthetic entry to the 1-aminomethyl **15** and 3-amino **18** targets was possible from 3,4,6-tri-*O*-acetyl-D-glucal (Schemes 3 and 4). Lewis acid mediated reaction of 3,4,6-tri-*O*-acetyl glucal with trimethylsilylcyanide afforded the acetylated derivative **13** as a mixture of anomers.[26] Column chromatography allowed isolation of the required α-anomer, **13α**, in moderate yield. Removal of the acetate groups was achieved *via* acid mediated hydrolysis, to afford diol **14** in 76% yield. Subsequent reduction of the nitrile moiety using lithium aluminium hydride in ether, at reflux, gave the amine derivative **15** in excellent yield.



Scheme 3. i) TMSCN, $BF_3{\cdot}OEt_2$, DCM, 38%; ii) *p*-TsOH, MeOH, 76%; iii) $LiAlH_4$, Et_2O, 80%.

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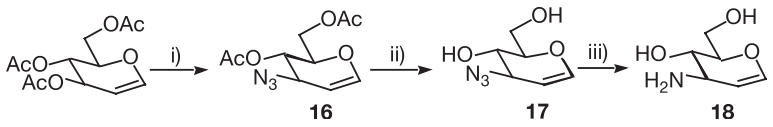

16 17 18

Scheme 4. i) $TMSN_3$, $BF_3{\cdot}OEt_2$, MeCN; ii) MeOH, Na, 36% over 2 steps; iii) $LiAlH_4$, Et_2O, 52%.

The synthesis of the C-3 aminoglycal **18** was achieved by Lewis acid mediated reaction of 3,4,6-tri-*O*-acetyl-D-glucal with trimethylsilylazide.[27] Purification was best achieved after the following deacetylation step, affording the azidoglucal **17**[27] in 36% yield over 2 steps. Selective reduction of the azide, in the presence of the glycal double bond, to afford the aminoglycal **18**[27] was then achieved employing lithium aluminium hydride, in ether, at reflux.

Enzyme Inhibition Studies

The abilities of compounds **5**, **12**, **15** and **18** to inhibit a range of glycosidases were analysed. Their activities against two α-glucosidases (EC 3.2.1.20 from brewers yeast and from *Bacillus stearothermophilus*), a β-glucosidase (EC 3.2.1.21 from almonds) and an α-mannosidase (EC 3.2.1.24 from Jack beans) were assessed spectrophotometrically using a *p*-nitrophenyl glycoside as substrate.[28,29] No inhibition of α-mannosidase or β-glucosidase was found with any of the inhibitors tested. Amine **15** displayed very weak inhibition of the α-glucosidases; a 4.1 mM concentration resulted in 16% reduction of activity of the *B. stearothermophilus* enzyme. Stronger inhibition of α-glucosidases from *B. stearothermophilus* and yeast was seen with target **5** (IC_{50} values of 27 mM and 1.1 mM respectively).

CONCLUSIONS

Synthesis of four unsaturated amine-containing carbohydrate derivatives has been achieved and preliminary glycosidase inhibition data have been collected. This has illustrated that targets **5** and **15** displayed moderate inhibitory activity against α-glucosidases from *B. stearothermophilus* and yeast.

EXPERIMENTAL

General methods. All NMR spectra were recorded on a Bruker WM250 or Bruker AMX400 spectrometer, using $CHCl_3$ as an internal standard unless stated otherwise (7.26 ppm for ^1H NMR, 77.0 ppm for ^{13}C NMR). Coupling constants are expressed in Hz. ^{13}C spectra were recorded using Distortionless Enhancement by Polarisation Transfer. Mass spectra were recorded on a Fisons VG Autospec. Infrared

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spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer. Optical activities were determined using a Perkin-Elmer 341 polarimeter. Melting points were determined using an Electrothermal digital melting point apparatus, and are uncorrected. All chemicals and materials were obtained from the Sigma-Aldrich Chemical Company, the B.D.H. Chemical Company or Lancaster Chemicals and were used as received. Anhydrous tetrahydrofuran was distilled from sodium and benzophenone immediately prior to use. Other anhydrous solvents were purchased and used as received. Molecular modeling was carried out using CS Chem3D Pro 4.0, on a Dell XPS T700r workstation. The semi-empirical MOPAC molecular computation application was employed, with PM3 (Parameterised Model revision 3) as the force-field. The structures discussed are the lowest energy conformations found after minimization. Compounds 3-azido-4,6-di-*O*-acetyl-3-deoxy-D-glucal **16**, 3-azido-3-deoxy-D-glucal **17** and 3-amino-3-deoxy-D-glucal **18** were all prepared as previously described in the literature.[27]

Methyl 2,3-di-*O*-benzoyl-4-deoxy-6-aldehydo-β-L-*erythro*-hex-4-enopyranoside (2). A dry 250 mL 3-necked round bottom flask was charged with Merck silica gel 60 (∼5 g). The flask was fitted with an efficient reflux condenser and a magnetic stirring bead, sealed and the system was flushed with nitrogen. A solution of **1**[19] (6.43 g, 12.7 mmol) dissolved in anhydrous toluene (100 mL) was added to the reaction vessel via syringe, followed by a further 60 mL anhydrous toluene. PCC (4.18 g, 19.4 mmol) was added portion-wise and the reaction mixture was heated at reflux for 36 h.[20] After cooling to rt the solution was filtered through a Celite® pad. The crude residue was washed with toluene (3 × 30 mL) and solvents removed in vacuo to yield a brown oil. After purification by flash column chromatography (hexane: Et_2O, 1:1, v/v) the desired product **2** was obtained as a white foam (2.82 g, 58%). $[\alpha]_D^{20}$ + 133.3 (*c* 1, chloroform); R_f 0.32 (hexane: Et_2O, 1:1, v/v); ν_{max} (liquid film)/cm^{-1} 3071 (C-Harom), 2936 (br, C-H), 2848 (w), 1729 (s, C═O), 1647 (w), 1602 and 1585 (C-Harom), 1452 (m), 1411 (w), 1316 (m), 1272 (s), 1177 (m), 1138 (m), 1113 (m), 1070 (m), 1026 (w), 989 (w), 915 (m), 709 (m); ^{1}H NMR (250 MHz; $CDCl_3$) δ 3.51 (3H, s, OCH_3), 5.26 (1H, d, *J* 2.8, H-1), 5.63 (1H, dd, *J* 4.4 and 1.7, H-2), 5.96 (10H, PhH, benzoyl), 9.24 (1H, s, H-6); ^{13}C NMR (62.8 MHz; $CDCl_3$) δ 57.3 (OCH_3), 64.8 (C-3), 65.4 (C-2), 99.2 (C-1), 118.6 (C-4), 128.7–130.5 (CHarom, Cquart, benzoyl), 133.9 and 134.0 (C═O, benzoyl), 149.8 (C-5), 186.3 (C-6); *m/z* (CI), 383 ($[M + H]^+$, 11%), 261 (100), 231 (69), 139 (24), 105 (74), 94 (7), 77 (16). HRMS (CI) Calcd for $C_{21}H_{19}O_7$: 383.1131. Found: $[M + H]^+$ 383.1162.

Methyl 2,3-di-*O*-benzoyl-4,6-dideoxy-6-amino-β-L-*erythro*-hex-4-enopyranoside (3) and methyl 2,3-di-*O*-benzoyl-4-deoxy-β-L-*erythro*-hex-4-enopyranoside (4). To a stirred solution of ammonium acetate (1.46 g, 18.9 mmol), sodium cyanoborohydride (82.7 mg, 1.32 mmol) and 3 Å activated molecular sieves in anhydrous methanol (35 mL), was added aldehyde **2** (0.72 g, 1.88 mmol) dissolved in anhydrous methanol (5 mL). The reaction was stirred at rt for 14.5 h and then filtered through a Celite® pad, washed with methanol (2 × 20 mL) and solvents removed in vacuo. The crude residue was taken up in a minimum of water (∼100 mL) and extracted with chloroform (6 × 30 mL). The combined organic layers were dried (Na_2SO_4), filtered and solvents

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removed in vacuo. Purification by flash column chromatography (hexane: Et_2O, 1:1, v/v) gave the desired product **3** as a pale orange foam (207 mg, 28%). $[\alpha]_D^{20}$ + 129.2 (*c* 1, chloroform); R_f 0.26 (Et_2O); ν_{max} (liquid film)/cm^{-1} 3065 (C-Harom), 2928 (br, C-H), 2842 (w), 1728 (s, C═O), 1681 (w), 1603 and 1586 (C-Harom), 1449 (m), 1313 (m), 1273 (s), 1173 (m), 1141 (m), 1116 (s), 1069 (m), 1026 (w), 978 (w), 915 (m), 711 (m); ^{1}H NMR (250 MHz; $CDCl_3$) δ 3.31 (2H, s, 2 × H-6), 3.48 (3H, s, OCH_3), 4.98 (1H, d, *J* 2.3, H-1), 5.13 (1H, d, *J* 4.9, H-4), 5.46–5.50 (1H, m, H-3), 5.79–5.83 (1H, m, H-2), 7.27–7.43 and 7.83–7.94 (10H, m, PhH, benzoyl); ^{13}C NMR (62.8 MHz; $CDCl_3$) δ 50.4 (C-6), 56.9 (OCH_3), 65.3 (C-2), 67.0 (C-3), 96.9 (C-1), 99.1 (C-4), 128.7–133.7 (CHarom, Cquart, benzoyl), 152.1 (C-5), 166.0 and 166.2 (C═O, benzoyl); *m/z* (CI), 384 ([M + H]$^+$, 16%), 232 (7), 140 (5), 105 (100), 78 (30). HRMS (CI) Calcd for $C_{21}H_{22}NO_6$: 384.1447. Found: [M + H]$^+$ 384.1444.

Further elution gave **4** (71 mg, 10%) as a yellow semi-solid. $[\alpha]_D^{20}$ + 117.6 (*c* 1, chloroform); R_f 0.51 (Et_2O); ν_{max} (liquid film)/cm^{-1} 3510 (br, O-H), 3062 (w, C-Harom), 2938 (br, C-H), 2841 (w), 1728 (s, C═O), 1601 and 1588 (w, C-Harom), 1450 (w), 1315 (m), 1274 (s), 1177 (w), 1116 (m), 1071 (m), 1027 (w), 913 (w), 710 (m); ^{1}H NMR (250 MHz; $CDCl_3$) δ 3.51 (3H, s, OCH_3), 4.09 (2H, s, 2 × H-6), 5.09 (1H, d, *J* 2.5, H-1), 5.15 (1H, d, *J* 5.0, H-4), 5.48–5.51 (1H, m, H-3), 5.82 (1H, dd, *J* 4.4 and 3.2, H-2), 7.29–7.37 and 7.85–8.01 (10H, m, PhH, benzoyl); ^{13}C NMR (62.8 MHz; $CDCl_3$) δ 57.0 (OCH_3), 62.7 (C-6), 65.2 (C-2), 67.1 (C-3), 95.9 (C-1), 99.1 (C-4), 128.7–133.7 (CHarom, Cquart, benzoyl), 153.4 (C-5), 165.9 and 166.2 (C═O, benzoyl); *m/z* (CI), 385 ([M + H]$^+$, 11%), 263 (15), 231 (14), 141 (23), 122 (10), 105 (100), 77 (13).

Methyl 4,6-dideoxy-6-amino-β-L-*erythro*-hex-4-enopyranoside (5). To a solution of hex-4-enopyranoside **3** (43 mg, 0.16 mmol) in absolute methanol (25 mL) was added anhydrous potassium carbonate (51 mg, 0.37 mmol). The solution was stirred at rt until TLC analysis showed an absence of any starting material (*ca.* 2.5 h). Solvents were removed in vacuo to yield the crude product and residual K_2CO_3, purification by flash column chromatography (EtOH: ammonium hydroxide, 9:1, v/v) furnished the target compound **5** as a yellow foam (14 mg, 48%). $[\alpha]_D^{20}$ + 87.0 (*c* 1, methanol); R_f 0.33 (EtOH: ammonium hydroxide, 9:1, v/v); ν_{max} (liquid film)/cm^{-1} 3420 (br, O-H), 2943 (m, C-H), 2852 (w, O-CH_3), 1648 (m, -NH_2), 1447 (w), 1347 (w), 1261 (w), 1182 (w), 1149 (m), 1065 (m), 1026 (m), 990 (m); ^{1}H NMR (400 MHz; CD_3OD) δ 3.16–3.26 (2H, m, 2 × H-6), 3.53 (3H, s, OCH_3), 3.67 (1H, dd, *J* 3.5 and 0.8, H-3), 4.20–4.23 (1H, m, H-2), 4.84 (1H, d, *J* 3.2, H-4), 4.86 (1H, d, *J* 0.9, H-1); ^{13}C NMR (100 MHz; CD_3OD) δ 50.6 (C-6), 56.8 (OCH_3), 63.9 (C-2), 68.7 (C-3), 101.8 (C-1), 102.3 (C-4), 150.3 (C-5); *m/z* (CI), 102 (34), 85 (100).

Methyl 2,4,6-tri-*O*-acetyl-3-deoxy-α-D-*erythro*-hex-2-enopyranoside (7).[23] To a stirred solution of 2,3,4,6-tetra-*O*-acetyl-D-glucal **6**[21] (5.10 g, 15.4 mmol) in anhydrous toluene (100 mL) was added anhydrous methanol (1.25 mL, 30.9 mmol), followed by boron trifluoride diethyl etherate (3.80 mL, 30.9 mmol).[22] After stirring under an inert atmosphere for 25 min the reaction was quenched by the dropwise addition of satd aq $NaHCO_3$ soln. (100 mL). The resulting solution was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine

(2×100 mL), dried ($MgSO_4$), filtered and the solvents removed in vacuo to yield a yellow oil. Purification by flash column chromatography (Et_2O) furnished the hex-2-enopyranoside **7** as a colourless syrup (2.01 g, 43%, inseparable anomeric mixture; 9:2, α:β). Characterisation for this mixture was in agreement with that reported in the literature.[23]

Methyl 3,4-dideoxy-α-D-*erythro*-hex-3-enopyranoside (8).[25] To a stirred suspension of $LiAlH_4$ (0.41 g, 10.8 mmol) in anhydrous THF (20 mL) cooled to rt was added a solution of hex-2-enopyranoside **7** (1.16 g, 3.85 mmol) in anhydrous THF (10 mL)[24] After stirring at 0°C under an inert atmosphere for 35 min the reaction was quenched by the dropwise addition of a sat aq potassium sodium tartrate solution (∼20 mL). The insoluble solids were filtered off through a Celite® pad and washed with EtOAc (100 mL). The resulting filtrate was dried ($MgSO_4$), filtered and solvents removed in vacuo to yield a crude mixture of anomers. Purification by flash column chromatography (EtOH: ammonium hydroxide, 9:1, v/v) allowed separation of the anomers, furnishing methyl-3,4-dideoxy-α-D-*erythro*-hex-3-enopyranoside **8α** as a colourless semi-solid (373 mg, 61%) whose data was in agreement with that reported in the literature.[25]

Methyl 3,4-dideoxy-6-*O*-tosyl-α-D-*erythro*-hex-3-enopyranoside (9). To a solution of hex-3-enopyranoside **8** (0.75 g, 4.68 mmol) in anhydrous DCM (50 mL) cooled to 0°C was added dropwise TEA (1.30 mL) followed by a solution of *p*-toluenesulfonyl chloride (recrystallised from hot ether) (893 mg, 4.68 mmol) in anhydrous DCM (10 mL). The reaction was stirred under an inert atmosphere and allowed to warm to rt overnight. After 48 h the reaction mixture was quenched by the addition of water (100 mL) and extracted with DCM (3×100 mL). The combined organic extracts were dried ($MgSO_4$), filtered and solvents removed in vacuo. Purification by flash column chromatography (toluene: EtOAc, 5:1, v/v) furnished the hex-3-enopyranoside **9** as a colourless syrup (788 mg, 73%). $[\alpha]_D^{20}$ + 0.9 (*c* 1, chloroform); R_f 0.32 (toluene: EtOAc, 5:1, v/v); ν_{max} (liquid film)/cm^{-1} 3509 (br, O-H), 3044 (w, C-Harom), 2935 (m, C-H), 2833 (m, O-CH3), 1596 (w), 1450 (w), 1402 (w), 1358 (m, S═O), 1174 (s, S═O), 1096 (m), 1049 (m), 1032 (m), 970 (m), 899 (w), 814 (w), 661 (m); ^{1}H NMR (250 MHz; $CDCl_3$) δ 1.92 (br, s, OH), 2.38 (3H, s, CH_3, tosyl), 3.38 (3H, s, OCH_3), 3.92–4.01 (2H, m, H-6), 4.03–4.06 (1H, m, H-2), 4.21–4.23 (1H, m, H-5), 4.73 (1H, d, *J* 4.4, H-1), 5.54 (1H, dddd, *J* 10.6, 1.0, 1.0 and 1.0, H-4), 5.68–5.73 (1H, dm, *J* 10.6, H-3), 7.26–7.29 and 7.70–7.75 (4H, m, PhH, tosyl); ^{13}C NMR (62.8 MHz; $CDCl_3$) δ 22.1 (CH_3, tosyl), 56.6 (OCH_3), 64.3 (C-2), 66.7 (C-5), 71.3 (C-6), 98.3 (C-1), 125.0 (C-4), 128.4 (CHarom, tosyl), 130.0 (C-3), 130.2 (CHarom, tosyl), 133.3 and 145.4 (Cquart, tosyl); *m/z* (CI), 332 ($[M + NH_4]^+$, 22%), 300 (31), 143 (11), 111 (51), 82 (100), 54 (24). HRMS (CI) Calcd for $C_{14}H_{22}NO_6S$: 332.1168. Found: 332.1169.

Methyl 2-*O*-benzoyl-3,4-dideoxy-6-*O*-tosyl-α-D-*threo*-hex-3-enopyranoside (10). To a solution of hex-3-enopyranoside **9** (0.70 g, 2.23 mmol), triphenylphosphine (2.63 g, 10.0 mmol) and benzoic acid (0.82 g, 6.68 mmol) in anhydrous THF (15 mL) cooled to 0°C was added diethyl azodicarboxylate (DEAD) (1.94 g, 1.76 mL, 10.9 mmol) dropwise. The reaction mixture was stirred at 0°C under an inert atmosphere for 30 min, then

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concentrated in vacuo. The resulting residue was diluted with diethyl ether (100 mL) and washed successively with satd aq $NaHCO_3$ soln. (3 × 100 mL) and brine (2 × 100 mL). The organic extract was dried ($MgSO_4$), filtered and solvents removed in vacuo to yield an orange/pink syrup. Purification by flash column chromatography (toluene: Et_2O, 3:1, v/v) furnished the benzoate **10** as a pale pink oil (835 mg, 90%). $[\alpha]_D^{20}$ + 73.7 (*c* 1, chloroform); R_f 0.24 (toluene: Et_2O, 3:1, v/v); ν_{max} (liquid film)/cm^{-1} 3051 (w, C-Harom), 2976 (m, C-H), 2935 (m, C-H), 2833 (w, O-CH_3), 1759 (s, C═O), 1715 (s), 1599 (m), 1450 (m), 1365 (s, S═O), 1314 (m), 1252 (m), 1174 (s, S═O), 1110 (m), 1069 (m), 977 (w), 811 (w), 712 (m); ^{1}H NMR (400 MHz; $CDCl_3$) δ 2.42 (3H, s, CH_3, tosyl), 3.46 (3H, s, OCH_3), 4.16–4.25 (2H, m, H-6), 4.34–4.44 (1H, m, H-5), 4.87 (1H, d, *J* 0.8, H-1), 5.13–5.16 (1H, m, H-2), 5.98 (1H, dd, *J* 11.8 and 1.38, H-4), 6.01–6.06 (1H, m, H-3), 7.29–7.33, 7.44–7.48, 7.71–7.83 and 8.02–8.06 (9H, m, PhH, tosyl and benzoyl); ^{13}C NMR (100 MHz; $CDCl_3$) δ 21.6 (CH_3, tosyl), 56.1 (OCH_3), 65.3 (C-2), 65.9 (C-5), 70.5 (C-6), 98.6 (C-1), 122.9 (C-3), 129.6 (C-4), 127.9–134.3 (CHarom, tosyl and benzoyl), 144.3 and 152.3 (Cquart, tosyl), 165.7 (Cquart, benzoyl), 169.1 (C═O, benzoyl); *m/z* (CI), 436 ([M + NH_4]$^+$, 20%), 387 (90%, M$^+$-OMe), 353 (8), 295 (15), 264 (14), 233 (18), 177 (6), 155 (7), 105 (100), 77 (43). HRMS (CI) Calcd for $C_{21}H_{26}NO_7S$: 436.1430. Found: 436.1425.

Methyl 2-*O*-benzoyl-3,4,6-trideoxy-6-azido-α-D-*threo*-hex-3-enopyranoside (11). A solution of hex-3-enopyranoside **10** (813 mg, 1.94 mmol) and NaN_3 (1.26 g, 19.4 mmol) in anhydrous *N*,*N*-dimethylformamide (20 mL) was stirred under an inert atmosphere at rt overnight. The progress of the reaction was studied by TLC analysis. After 44 h TLC analysis (hexane: EtOAc, 3:1, v/v) indicated that no change had occurred. The reaction mixture was then heated at 75–80°C for 2 h. The solution was cooled to rt and solvents removed in vacuo. The resulting residue was taken up in Et_2O (50 mL) and washed with water (3 × 50 mL). The organic extract was dried ($MgSO_4$), filtered and solvents removed in vacuo to yield a yellow syrup. Purification by flash column chromatography (hexane: EtOAc, 3:1, v/v) yielded the azide **11** as a colourless oil (235 mg, 42%). $[\alpha]_D^{20}$ + 262.7 (*c* 1, chloroform); R_f 0.35 (hexane: EtOAc, 3:1, v/v); ν_{max} (liquid film)/cm^{-1} 3058 (w, C-Harom), 2928 (m, C-H), 2833 (w, O-CH_3), 2096 (s, N_3), 1718 (s, C═O), 1599 (m), 1534 (w), 1443 (m), 1314 (m), 1263 (m), 1106 (m), 1062 (m), 1025 (w), 998 (w), 950 (w), 712 (m); ^{1}H NMR (400 MHz; $CDCl_3$) δ 3.25 (1H, dd, *J* 11.9 and 6.0, H-6), 3.43 (1H, dd, *J* 11.9 and 4.0, H-6), 3.44 (3H, s, OCH_3), 4.31–4.36 (1H, m, H-5), 4.88 (1H, d, *J* 0.8, H-1), 5.09–5.12 (1H, m, H-2), 5.91 (1H, dd, *J* 10.9 and 1.1, H-4), 5.95–6.00 (1H, m, H-3), 7.31–7.38, 7.45–7.48 and 7.98–8.02 (5H, m, PhH, benzoyl); ^{13}C NMR (100 MHz; $CDCl_3$) δ 54.5 (C-6), 55.2 (OCH_3), 62.1 (C-2), 66.4 (C-5), 99.8 (C-1), 123.4 (C-3), 132.5 (C-4), 129.3–139.0 (CHarom, Cquart, benzoyl), 166.8 (C═O, benzoyl); *m/z* (CI), 290 ([M + H]$^+$, 9%), 258 (81), 202 (10), 105 (100), 77 (11). HRMS (CI) Calcd for $C_{14}H_{16}N_3O_4$: 290.1141. Found: 290.1134.

Methyl 3,4,6-trideoxy-6-amino-α-D-*threo*-hex-3-enopyranoside (12). $LiAlH_4$ (65 mg, 1.80 mmol) was dissolved in anhydrous Et_2O (8 mL) and brought to reflux. A solution of azide **11** (55 mg, 0.19 mmol) dissolved in anhydrous diethyl ether (4 mL) was added *via* syringe to the refluxing suspension of $LiAlH_4$. The reaction mixture was heated at reflux for 2.5 h. After cooling to rt the reaction was quenched by the

dropwise addition of satd aq potassium sodium tartrate soln. (~10 mL). The insoluble solids were filtered off through a Celite® pad and washed with warm methanol (~50 mL). The resulting filtrate was dried ($MgSO_4$), filtered and solvents removed in vacuo to yield a yellow oil. Purification by flash column chromatography (EtOH: ammonium hydroxide, 9:1, v/v) gave the target compound **12** as a pale yellow oil (25 mg, 83%). $[\alpha]_D^{20}$ + 8.5 (*c* 1, methanol); R_f 0.52 (EtOH: ammonium hydroxide, 9:1, v/v); ν_{max} (liquid film)/cm^{-1} 3392 (br, O-H), 2934 (m, C-H), 2843 (w), 1642 (m, -NH_2), 1567 (m), 1488 (m), 1384 (w), 1322 (w), 1195 (w), 1124 (m), 1065 (m), 970 (w), 905 (w); ^{1}H NMR (400 MHz; Acetone-d_6) δ 3.29–3.43 (2H, m, H-6), 3.39 (3H, s, OCH_3), 3.62–3.66 (1H, m, H-2), 4.35–4.40 (1H, m, H-5), 4.66 (1H, d, *J* 0.8, H-1), 5.86 (1H, dd, *J* 10.3 and 2.0, H-4), 5.95–6.01 (1H, m, H-3); ^{13}C NMR (100 MHz; Acetone-d_6) δ 54.8 (C-6), 55.7 (OCH_3), 64.3 (C-2), 69.8 (C-5), 103.1 (C-1), 127.2 (C-3), 131.7 (C-4); *m/z* (CI), 160 ([M + H]$^+$, 100%,), 128 (38), 112 (50), 81 (53), 43 (18). HRMS (CI) Calcd for $C_7H_{14}NO_3$: 160.0974. Found: 160.0977.

2,3-Dideoxy-4,6-di-*O*-acetyl-α-D-*erythro*-hex-2-enopyranosyl cyanide (13).[26] To a stirred solution of 3,4,6-tri-*O*-acetyl-D-glucal (0.2 g, 0.73 mmol) in anhydrous dichloromethane (3.5 mL) was added trimethylsilylcyanide (0.08 g, 0.1 mL 0.8 mmol), followed by boron trifluoride diethyl etherate (1 drop)[26] After stirring for five minutes, the solution was poured into diethyl ether (20 mL), washed with saturated sodium bicarbonate solution (5 mL) and water (10 mL) and dried (magnesium sulfate). After filtration through a short pad of silica gel, the solution was concentrated in vacuo and purified by column chromatography (hexane:ethyl acetate, 4:1 v/v) to give the α-cyanoglycal **13** as a white powder (0.31 g, 38%). Data for **13** agreed with that reported previously in the literature.[26]

2,3-Dideoxy-α-D-*erythro*-hex-2-enopyranosyl cyanide (14). To a stirred solution of **13** (0.2 g, 0.89 mmol) in anhydrous methanol (10 mL) was added *p*-toluenesulfonic acid (0.04 g) and the solution was stirred overnight. After neutralisation with barium carbonate, the solution was filtered and concentrated in vacuo. Column chromatography on silica gel afforded the diol **14** as a colourless oil (0.05 g, 40%). $[\alpha]_D^{20}$ − 50.0 (*c* 0.25, MeOH), R_f 0.19 (ethyl acetate:hexane 2:1 v/v); ν_{max} (KBr disc)/cm^{-1} 3419, 2928, 1714, 1684, 1243, 1186, 1089, 971, 901; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (1H, m, H-5), 3.75 (1H, dd, *J* 5.9, H-6), 3.94 (1H, d, *J* 12.0, H-6), 4.12 (1H, d, *J* 8.8, H-4), 5.25 (1H, br s, H-3), 5.91 (1H, d, *J* 9.9, H-2), 6.09 (1H, d, *J* 9.9, H-1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 62.3 (CH_2), 62.5 (CH), 63.7 (CH), 81.6 (CH), 117.8 (C), 123.7 (CH), 134.8 (CH); *m/z* (CI) 173 ([M + NH_4]$^+$, 15%), 129 (25), 111 (20), 81 (1), 68 (100), 55 (5), 43 (30). HRMS (CI) Calcd for $C_7H_9NO_3$: 173.0926. Found: 173.0919.

1-Aminomethyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranose (15). A solution of glycal **14** (1.0 g, 4.16 mmol) in anhydrous diethyl ether (3 mL) was added dropwise to a suspension of $LiAlH_4$ in anhydrous diethyl ether (5 mL) at reflux. After heating at reflux for 2.5 hours, the solution was cooled and crushed ice was added until effervescence ceased. The insoluble solids were filtered off and washed with warm methanol. The combined organics were concentrated to dryness and submitted to column chromatography (ethanol:ammonium hydroxide 9:1 v/v) to afford the aminoglycal **15** as a colourless oil (0.53 g, 80 %); $[\alpha]_D^{20}$+ 53 (*c* 0.4, chloroform);

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R_f 0.19 (silica, ethanol:ammonium hydroxide 9:1 v/v); ν_{max} (KBr disc)/cm^{-1}3420 (br), 2924, 1700, 1558, 1040; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.63 and 2.78 (2H, 2 × br s, CH_2NH_2), 3.39 (1H, br t, *J* 7.0, H-6), 3.51 (1H, dd, *J* 12.1, 7.0, H-5), 3.75 (2H, m, H-4 and H-6) 4.06 (1H, br d, *J* 9.2, H-1), 5.67 (1H, d, *J* 10.6, H-2), 5.76 (1H, d, *J* 10.6, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.1 (CH_2), 64.0 (CH), 75.7 (CH), 128.8 (CH), 131.5 (CH); *m/z* (CI) 160 ($[M + H]^+$, 80%), 142 (5), 112 (15), 94 (10), 81 (100). HRMS (CI) Calcd for $C_7H_{17}NO_3$: 160.0973. Found: 160.0970.

Enzyme Inhibition Studies

These studies were conducted essentially as described in the literature.[28,29] Enzymes were obtained from Sigma, and diluted in 0.02 M sodium acetate buffer, except for Jack bean mannosidase, which was diluted in 0.05 M sodium acetate buffer containing 0.1 mM zinc sulfate. α-Mannosidase and β-glucosidase experiments were conducted at pH 5.0 whilst α-glucosidase experiments were conducted at pH 6.8. Enzyme assays were incubated over a period of 30 min. α-Mannosidase inhibition assays employed *p*-nitrophenol α-D-mannopyranoside and other enzymes were assayed using the corresponding conjugate at 5 mM concentration in an appropriate buffer. Absorbances at 400 nm were recorded using a Perkin Elmer Lambda Bio UV/Visible spectrophotometer.

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REFERENCES

1. Dwek, R.A.; Butters, T.D. Glycobiology—understanding the language and meaning of carbohydrates. Chem. Rev. **2002**, *102*, 283–284.
2. Bertozzi, C.R.; Kiessling, L.L. Chemical glycobiology. Science **2001**, *291*, 2357–2364.
3. Seitz, O. Glycopeptide synthesis and the effects of glycosylation on protein structure and activity. Chem. Biochem. **2000**, *1*, 215–246.
4. Hounsell, E.F.; Young, M.; Davies, M.J. Glycoprotein changes in tumours: a renaissance in clinical applications. Clin. Sci. **1997**, *93*, 287–293.
5. Lloyd, K.O. Carbohydrate vaccines for the immunotherapy of cancer. Drug News Perspect. **2000**, *13*, 463–470.
6. Fukuda, M. Possible roles of tumour-associated carbohydrate antigens. Cancer Res. **1996**, *56*, 2237–2244.
7. Bironaite, D.; Nesland, J.M.; Dalen, H.; Risberg, B.; Bryne, M. *N*-glycans influence the in vitro adhesive and invasive behaviour of three metastatic cell lines. Tumour Biol. **2000**, *21*, 165–175.
8. Lillelund, V.H.; Jensen, H.H.; Liang, X.F.; Bols, M. Recent developments of

transition-state analogue glycosidase inhibitors of non-natural product origin. Chem. Rev. **2002**, *102*, 515–546.
9. Sinnott, M.L. Catalytic mechanisms of enzymatic glycosyl transfer. Chem. Rev. **1990**, *90*, 1171–1202.
10. Wong, C-H.; Halcomb, R.L.; Ichikawa, Y.; Kajimoto, T. Enzymes in organic synthesis—application to the problems of carbohydrate recognition 2. Angew. Chem., Int. Ed. Engl. **1995**, *34*, 521–546.
11. Legler, G. Glycoside hydrolases—mechanistic information from studies with reversible and irreversible inhibitors. Adv. Carbohydr. Chem. Biochem. **1990**, *48*, 319–384.
12. Kessler, J.; Scott, M.E.; Patwadhan, B.H.; Kumar, V.; Tanenbaum, S.W.; Flashner, M. Synthesis of glycal antagonists 1. Carbohydr. Res. **1981**, *94*, 123–130.
13. Kumar, V.; Tanenbaum, S.W.; Flashner, M. Synthesis of glycal neuraminidase inhibitors 2. Carbohydr. Res. **1982**, *103*, 281–285.
14. Kleban, M.; Hilgers, P.; Greul, J.N.; Kugler, R.D.; Li, J.; Picasso, S.; Vogel, P.; Jager, V. Amino (hydroxymethyl)cyclopentanetriols, an emerging class of potent glycosidase inhibitors Part 1. ChemBiochem **2001**, *2*, 365–368.
15. Greul, J.N.; Kleban, M.; Schneider, B.; Picasso, S.; Jager, V. Amino (hydroxymethyl)cyclopentanetriols, an emerging class of potent glycosidase inhibitors Part 2. ChemBiochem **2001**, *2*, 368–370.
16. Lai, H.-Y.L.; Axelrod, B. 1-Aminoglycosides, a new class of specific inhibitors of glycosidases. Biochem. Biophys. Res. Commun. **1973**, *54*, 463–468.
17. Legler, G.; Herrchen, M. *N*-Substituted D-galactosylamines as probes for the active-site of β-D-galactosidase from *Escherichia coli*. Carbohydr. Res. **1983**, *116*, 95–103.
18. Lai, W.; Martin, O.R. Synthesis and inhibition studies of *C*-(D-glycopyranosyl)-methylamines. Carbohydr. Res. **1993**, *250*, 185–193.
19. Ziegler, T.; Kovac, P.; Glaudemans, C. Selective bromoacetylation of alkyl hexopyranosides. Carbohydr. Res. **1989**, *194*, 185–198.
20. Mathad, V.T.; Shelfali, R.K.; Bhaduri, A.P. A convenient method for the synthesis of 4,5-glycals. Indian J. Chem. **1997**, *36B*, 808–809.
21. Chretien, F. A convenient preparation of hex-5,6-enopyranosides. Synth. Commun. **1989**, *19*, 1015–1024.
22. Ferrier, R.J. Substitution-with-allylic rearrangement reactions of glycal derivatives. Top. Curr. Chem. **2001**, *215*, 153–175.
23. Ferrier, R.J.; Prasad, N.; Sankey, G.H. Unsaturated carbohydrates Part XII. Synthesis and properties of 3-deoxy-α- and-β-D-*erythro*-hex-2-enopyranoside esters. J. Chem. Soc., C **1969**, 587–591.
24. Ichikawa, Y.; Isobe, M.; Bai, D.L.; Goto, T. Synthesis of a marine polyether toxin, okadaic acid. Tetrahedron **1987**, *43*, 4749–4758.
25. Bognár, R.; Herczegh, P.; Zsély, M.; Batta, G. Synthesis of 3,4-dideoxy-DL-hex-3-enopyranosides from 5-hydroxymethyl-2-furaldehyde. Carbohydr. Res. **1987**, *164*, 465–469.
26. Grynkiewicz, G.; BeMiller, J.N. Synthesis of 2,3-dideoxy-D-hex-2-enopyranosyl cyanides. Carbohydr. Res. **1982**, *108*, 229–235.
27. Guthrie, R.D.; Irvine, R.W. Allylic substitutions in tri-*O*-acetyl glycals and related compounds. Carbohydr. Res. **1980**, *82*, 207–224.

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28. Burrows, H.M.; Rastall, R.A. Essential carboxyl and tryptophan residues in Jack Bean α-D-mannosidase. Phytochemistry **1998**, *48*, 257–260.
29. Li, Y.-T. Studies on the glycosidases in Jack Bean Meal. J. Biol. Chem. **1967**, *242*, 5474–5480.

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