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Synthesis of a Novel α-Glucoside of the Powerful Glucosidase Inhibitor 2,5-Dideoxy-2,5-imino-D-mannitol via Enzymatic Glucosylation of 5-Azido-5-deoxy-D-fructopyranose

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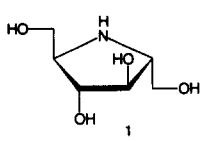
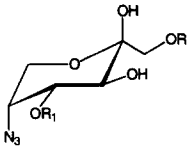
Abstract: Regio- and stereoselective enzymatic glucosylation of 5-azido-5-deoxy-D-fructopyranose with the aid of commerically available α-glucosidase from yeast allows easy access to the corresponding 4-O-α-D-glucopyranosyl derivative of this non-natural ketose. This disaccharide gives, in one step, access to 2,5-dideoxy-3-O-α-D-glucopyranosyl-2,5-imino-D-mannitol. Copyright © 1996 Elsevier Science Ltd

Various glycosylated derivatives of sugar related glycosidase inhibitors with basic nitrogen instead of oxygen in the ring have been found as natural products[1] or have been synthesized by chemical[2] as well as enzymatic[3] methods. Pioneering work in relation to the biochemical application of such glycosides as probes for a specific *endo*-mannosidase involved in glycoprotein trimming has been carried out by Spohr and co-workers[4] as well as Fleet and his group[5]. In a different context, Hasegawa and others have synthesized complex oligosaccharides containing the glucosidase inhibitor 1-deoxynojirimycin[6].

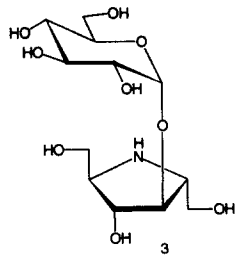
In the course of a project concerned with the synthesis of novel glycosidase inhibitors we have become interested in a simple, reasonably versatile, and efficient synthetic route to novel glycosides of 2,5-dideoxy-2,5-imino-D-mannitol (**1**), a powerful inhibitor of glucosidases and invertase[7], as well as derivatives thereof. These are potentially interesting inhibitors of glycoprotein processing glycosidases. Being a close structural relative to glucosides of 1-deoxymannojirimycin recently synthesized[4,5], our initial and main target was 2,5-dideoxy-3-O-α-D-glucopyranosyl-2,5-imino-D-mannitol (**3**).



1



2 R=R$_1$=H
4 R=H, R$_1$=α-Glu
5 R=α-Glu, R$_1$=H



3

With 5-azido-5-deoxy-D-fructopyranose (**2**), the direct precursor of compound **1**, available in gramm quantities[8], we have been looking for chemical as well as enzymatic methods to utilize this ketose for the preparation of desired pseudodisaccharide **3**. Incubation of compound **2** with commercially available α-glucosidase from yeast (SIGMA, Type VI) in the presence of maltose as the glucosyl donor over 6 days led to a mixture of products[9]. Separation of the interesting disaccharides from other sugars was achieved by

chromatography on charcoal employing water/EtOH as the eluant. Excess starting material can easily be recycled. Gratifyingly, a single glucoside of compound **2** was isolated in 6% yield (92% by conversion) and identified as the 4-O-α-D-glucopyranosyl derivative **4**.

Surprisingly, when a different preparation of α-glucosidase from yeast (SIGMA, Type III) was employed for comparison, the regioselectivity of the reaction dropped dramatically and two glucosylated azidosugars were obtained in approximately equal amounts (around 10% each) and identified as the 4-O-α-D-glucopyranosyl derivative **4** and the 1-O-α-D-glucopyranosyl derivative **5** of azidosugar **2**.[10] For analytical purposes, per-O-acetylated derivative **4a** and 3,4,2',3',4',6'-hexa-O-acetyl derivative **5a** were prepared from these free sugars.

Conventional hydrogenation of disaccharide **4** over palladium-on-carbon in dry methanol led smoothly and in excellent yield to the desired inhibitor derivative **3**.

In conclusion, commercially available α-glucosidases from yeast employed in this study were found to be capable to α-glucosylate non-natural substrates such as azidodeoxyfructose **2**. Employing this simple and short approach, the first glucosylated derivative of the powerful glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol could be synthesized.

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9 Typical experiment: **2** and a six-fold excess of maltose were stirred with α-glucosidase from yeast, SIGMA, in a potassium phosphate puffer at pH 5 until 60% of maltose were consumed. The reaction was quenched by brief heating to 90°C and the product mixture was separated on activated charcoal/celite employing a 0-30% (v/v) gradient water /EtOH. α-Glucosidase Type III (1000 u) gave around 10% isolated yields of each product **4** and **5**, while glucosidase Type VI (2000 u) exclusively gave **4**.

10 **4**: $[\alpha]_D^{20}$ +17.9 (c 0.8, MeOH), ^{13}C-NMR (MeOH-d_4) in δ: 103.1 (C-1'), 99.5 (C-2), 80.8 , 74.9, 74.6, 74.0, 71.8, 68.9, 65.5, 63.5, 62.9, 61.9; ^{1}H-NMR: 5.13 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1'), **4a**: $[\alpha]_D^{20}$ +7.7 (c 1.5, CH_2Cl_2), ^{13}C-NMR ($CDCl_3$): 103.1, 97.1, 75.3, 74.1, 69.7, 68.7, 68.5, 68.0, 63.2, 62.9, 62.3, 60.4; ^{1}H-NMR: 5.59 (d, 1 H, 9.9 Hz), 5.47 (dd, 1 H, 10.3 Hz, 9.5 Hz), 5.27 (d, 1 H, J 3.8 Hz), 5.08 (dd, 1 H, 9.5 Hz), 4.84 (dd, 1 H, 10.3 Hz, 3.8 Hz), 4.66 (d, 1 H, 12 Hz), 4.47 (d, 1 H, 12 Hz), 4.35-3.8 (m, 7 H). **5a**: ^{13}C-NMR ($CDCl_3$): 96.8 (C-1, C-1'), 71.0, 70.9, 70.7, 70.4, 68.7, 68.5, 68.0, 62.1, 61.4, 60.3; ^{1}H-NMR: 5.48 (dd, 1 H, 10 Hz, 10 Hz), 5.43 (dd, 1 H, 10.3 Hz, 3.7 Hz), 5.28 (d, 1 H, 10.3 Hz), 5.05 (dd, 1 H, 10 Hz, 10 Hz), 5.02 (d, 1 H, 10 Hz, 3.6 Hz), 4.28-4.08 (m, 6 H), 3.77 (d, 1 H, 10.2 Hz), 3.62 (s, 1 H, exchanges with D_2O), 3.35 (d, 1 H, 10.2 Hz). **3**: $[\alpha]_D^{20}$ +49.5 (c 0.1, H_2O), ^{13}C-NMR (D_2O): 98.5, 84.7, 78.2, 73.6, 73.1, 72.1, 70.3, 63.6, 62.7, 62.3, 62.2, 61.3.

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