Synthesis of an 1'-Azasugar Analogue of Maltose

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Received 3 October 2000

Abstract: Methyl 1'-azamaltoside (**3**) was synthesised from levoglucosane and D-galactose in a 16 step synthesis. Methyl 1'-azamaltoside (**3**) was found to inhibit glucoamylase with K_i of 0.63 μM.

Key words: glycosidase inhibitor, glucoamylase, reductive amination, isofagomine, glycosides, disaccharides, carbohydrates, nitrogen

Specific inhibitors of glycosidases and related enzymes are subject of much current interest[1–3] either as potential drugs against various diseases and disorders, as glycobiochemical tools or as agents that provide information about the chemistry of enzymatic glycoside cleavage. In particular, the various forms of azasugars have been found to be the most potent and specific inhibitors so far.[3]

Most potent azasugar glycosidase inhibitors have a monosaccharide structure. Nevertheless, the transition state for a reaction catalysed by a typical glycosidase is a di- or oligosaccharide structure, because the typical substrate is a di- or oligosaccharide. This means that a di- or oligosaccharide transition state analogue would be expected to be a more potent inhibitor than a monosaccharide analogue, because it would have more binding interactions to the enzyme. It is also likely that such molecules would be more selective inhibitors. However little is known about how to design effective disaccharide inhibitors.[4]

Previous work from our laboratory has shown that modification of the inhibitor isofagomine (**1**) at N-1 with another sugar residue to create **2** resulted in a 60 fold increase in the inhibition of glucoamylase (Figure).[5] This clearly suggested that substitution at N-1 of **1** with the extra glucose residue increased affinity by mimicking the sugar moiety of the leaving group. However, since the natural substrate of glucoamylase is the α-1,4-linked glucose units of starch, compound **2**, which mimics a 1,6-linked disaccharide, cannot be expected to be an optimal transition state mimic. The analogue **3**, which mimics the 1,4-linked disaccharide maltose was, on the other hand, expected to mimic the transition state better than **2** (Figure). In the present paper we report the synthesis of **3** and its inhibition of a series of glycosidases.

For the synthesis of **3** a number of approaches involving known building blocks and reductive amination strategies were envisaged. For this purpose the known nitrile **4** was synthesised in 6 steps from D-galactose.[6] It was converted to the primary amine **5** in 62% yield by reduction with excess $LiAlH_4$ in Et_2O. The nitrile **4** was also converted to the aldehyde **6** in 72% yield by reduction with DIBAL in THF/CH_2Cl_2 (Scheme 1). Compound **6** has previously been synthesised by another route.[7]

Building blocks **7**–**9** were taken from our isofagomine synthesis (Scheme 2).[8] In that synthesis **7** is obtained in 7 steps from levoglucosane (1,6-anhydroglucose), which is then converted to the dialdehyde **8** by periodate cleavage and to 3-benzylisofagomine **9** by reductive amination with ammonia. Substitution of ammonia with the amine **5** in the latter step appeared to be a particular attractive route to the target. However, reductive amination of equimolar amounts of **5** and **8** using H_2 at 45 atm and 5% Pd/C as catalyst in EtOH gave a complex mixture of products; however, the expected target **3a** did not appear to be among them (Scheme 3). We believe that reductive amination is slow because the amine cannot be employed in excess. This allows the sensitive dialdehyde to undergo aldol condensations and similar side reactions. To simplify the problem the reductive amination between **5** and monoaldehyde **7** was attempted with the plan to reverse the order of $NaIO_4$ cleavage and reductive amination steps in the synthesis. However, the reductive amination was very slow and hydrogenolysis of the benzyl groups took place with a greater rate making a subsequent $NaIO_4$ step impossible.

Reductive amination was then performed between **6** and **9** (Scheme 3). Also in this case the reaction was extremely slow and partial hydrogenolysis of benzyl groups oc-

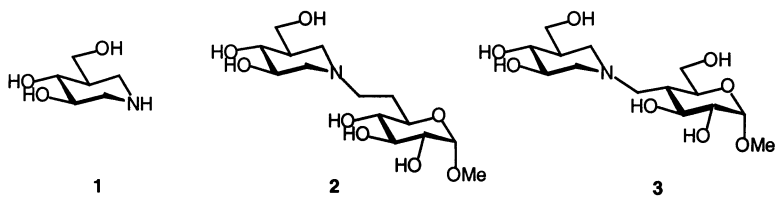
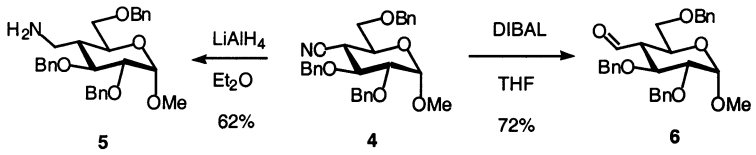
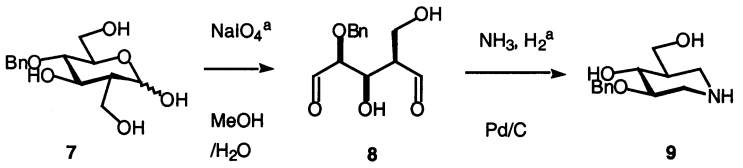


Figure The chemical structures of isofagomine (**1**), its isomaltose (**2**) and maltose (**3**) analogues



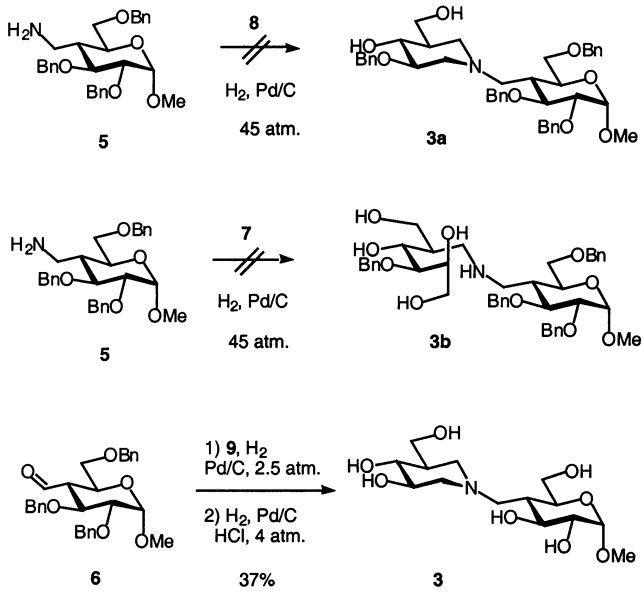
Synthesis of building blocks **5** and **6**

Scheme 1



Synthesis of building blocks **8** and **9**
a = see Ref. 8

Scheme 2



Synthesis of **3**

Scheme 3

curred leading to several products. However, as mass spectral analysis showed that reductive amination had taken place, the synthesis was modified so that the product mixture was subjected to an immediate hydrogenolysis with H_2 and Pd/C in the presence of HCl. The presence of acid is known to increase the rate of hydrogenolysis of the benzyl groups. This gave the target compound **3** in 37% yield (Scheme 3). It appears that due to the long reaction time much of the aldehyde undergo aldol condensation, which results in a low yield.

Compound **3** was found to be a competitive inhibitor of a series of glycosidases. The K_i values were determined and compared to those of **1** and **2** (Table). As can be seen **3** is a stronger inhibitor of glucoamylase than **1** suggesting that the second sugar increases binding. It is also a more selective inhibitor relative to **1**, while the latter is a poor inhibitor of α- and β-glucosidase and isomaltase. Nevertheless, the K_i of **3** vs glucoamylase is still 10 fold higher than that of **2**. This reveals that the second sugar residue of **3** does not have an optimal position for binding. Isomaltose analogue **2** should not be optimal for binding either. However, it is conceivable that in the transition state the distance between the two sugar residues is increased. In that case **2** with its longer linkage between the pyranose rings would be expected to fit better than **3**. This suggests that an improved inhibitor could be obtained by increasing the length of the linkage in **3**.

In the present paper we have reported the synthesis of the first example of an 1-azasugar (isofagomine) linked to a secondary position of a monosaccharide. The results show that such a compound can be more potent and selective than the corresponding monosaccharide type azasugar, but also that the mimicry of the transion state is still not perfect.

^{13}C NMR and ^1H NMR spectra were recorded on a Varian Gemini 2000 (200 MHz) instrument. D_2O was used as solvent with DHO (^1H NMR: δ = 4.79) and acetone (^1H NMR: δ = 2.05; ^{13}C NMR: δ = 29.8) as reference. With $CHCl_3$ as solvent TMS and $CHCl_3$ (^{13}C NMR: δ = 76.93) were used as references. Mass spectra were ob-

Table Inhibition Constants (K_i) in μM for the Competitive Inhibition of Four Glycosidases by **1**–**3** at 25°C and pH 6.8

Enzyme	**1**[a]	**2**[a]	**3**
Glucoamylase (*A. Niger*)	3.7[b]	0.063[b]	0.63[c] 0.54[d]
β-Glucosidase (almond)	0.11[e]	2.3[e]	28
α-Glucosidase (yeast)	86[e]	58.8[e]	>500
Isomaltase (yeast)	7.2[e]	103[e]	480

[a] Taken from Ref. 5.
[b] Temperature 50°C, pH 4.5, and maltose as substrate.
[c] Temperature 37°C, pH 5.7, and nitrophenyl glucoside as substrate.
[d] Temperature 44°C, pH 4.5, and maltose as substrate.
[e] Temperature 37°C.

tained on a Micromass LCT instrument. Concentrations were performed on a rotary evaporator at a temperature below 40 °C.

Methyl 4-Aminomethyl-2,3,6-tri-*O*-benzyl-4-deoxy-α-D-glucopyranoside (5)

To a stirred solution of **4** (0.200 g, 0.42 mmol) in anhyd Et_2O (3.5 mL) at 0 °C and under anhydrous conditions was added dropwise a suspension of $LiAlH_4$ (0.065 g, 1.71 mmol) in anhyd Et_2O (2 mL). After stirring at r.t. for 3.5 h, the reaction was quenched with a mixture of H_2O (1 mL) and 1 N NaOH (1 mL). The aqueous phase was extracted repeatedly with Et_2O. The combined organic phases were washed with H_2O (4 mL), dried (Na_2SO_4), filtered and evaporated. The crude product was subjected to chromatography with EtOAc containing 1% Et_3N as eluent; yield: 0.126 g (62%); $[a]_D^{20}$ +4.6 (*c* = 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 7.42−7.28 (m, 15 H, C_6H_5), 5.05−4.45 (m, 7 H, PhC*H*$_2$, H-l), 3.94−3.81 (m, 2 H, H-3, H-5), 3.65−3.58 (m, 3 H, H-2, H-6a, H-6b), 3.39 (s, 3 H, OCH_3), 2.89 (dd, 1 H, H-4'a, $J_{4,4'a}$ = 3.5 Hz, $J_{4a,4b}$ = 13.5 Hz), 2.65 (dd, 1 H, H-4'b, $J_{4,4b}$ = 3.5 Hz, $J_{4a,4b}$ = 13.5 Hz), 1.79 (tt, 1 H, H-4, $J_{4,4'a/4'b}$ = 3.0 Hz, $J_{4,3/5}$ = 11.0 Hz).

^{13}C NMR ($CDCl_3$): δ = 138.5, 138.2, 137.9, 128.3−127.6 (several peaks, C_6H_5), 98.3 (C-l), 81.7 (C-2), 75.3 (Ph*C*H$_2$), 75.1 (C-3), 75.3, 72.7 (Ph*C*H$_2$), 70.1 (C-6), 68.5 (C-5), 55.1 (OCH_3), 45.2 (C-4), 38.4 (C-4').

HRMS (ES): *m*/*z* calcd for $C_{29}H_{35}NO_5$ (M + H^+): 478.2593, Found: 478.2560.

Methyl 2,3,6-Tri-*O*-benzyl-4-deoxy-4-formyl-α-D-glucopyranoside (6)

Compound **4** (0.400 g, 0.84 mmol) was dissolved in a mixture of THF/CH_2Cl_2 (7:23, 2.4 mL) under anhydrous conditions. Then 1.5 M DIBAL in toluene (0.9 mL, 1.35 mmol) was added to the solution over 1 min at −20 °C. The reaction mixture was stirred at −20 °C for 2 h, and then stirred at 20 °C for 5 h. Subsequently, a mixture of THF/MeOH (1:1, 1.6 mL) and 1 M HCl (1.6 mL) were carefully added at −10 °C. The mixture was then stirred for 10 min at r.t. Et_2O (50 mL) was added, and the mixture was washed with 1 M HCl (2 × 12 mL) and H_2O (3 × 12 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated. Column chromatography was performed with CH_2Cl_2 containing 1% EtOAc as eluent to afford **6**[7]; yield: 0.290 g (72%).

^{1}H NMR ($CDCl_3$): δ = 9.64 (d, 1 H, H-4', $J_{4,4'}$ = 2.5Hz), 7.40−7.23 (m, 15 H, C_6H_5), 4.95−4.43 (m, 6 H, PhC*H*$_2$), 4.66 (d, 1 H, H-1, $J_{1,2}$ = 3.5 Hz), 4.25 (dd, 1 H, H-3, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 11.0 Hz), 4.04 (dt, 1 H, H-5, $J_{5,6a/6b}$ = 4.0 Hz, $J_{4,5}$ = 10.5 Hz), 3.61 (dd, 1 H, H 2, $J_{1,2}$=3.5 Hz, $J_{2,3}$ = 9.5 Hz), 3.55 (d, 2 H, H-6a, H-6b, $J_{5,6a}$ = $J_{5,6b}$ 4.0 Hz), 3.39 (s, 3 H, OCH_3), 2.98 (dt, 1 H, H-4, $J_{4,4'}$ = 2.5 Hz, $J_{4,3}$ = $J_{4,5}$ 10.5 Hz).

^{13}C NMR ($CDCl_3$): δ = 200.5 (C-4'), 138.1, 138.0, 137.6, 128.6−127.8 (C6H5), 98.4 (C-l), 80.7 (C-2), 75.4 (2 C, C-3, Ph*C*H$_2$), 73.6, 73.2 (Ph*C*H$_2$), 70.3 (C-6), 67.1 (C-5), 56.8, 55.5 (C-4, OCH_3).

MS (ES): *m*/*z* = 531.0 (M + MeOH + Na^+).

Methyl 4-Deoxy-4-[(3*R*, 4*R*, 5*R*)-3,4-dihydroxy-5-hydroxymethyl-piperidin-1-yl)-methylen]-α-D-glucopyranoside (3)

A solution of **9** (98 mg, 0.41 mmol) in MeOH (6 mL) and 5% Pd/C (200 mg) was added to a solution of **6** (280 mg, 0.55 mmol) in MeOH (6 mL). The reaction mixture was hydrogenated for 72 h at 2.5 bar. Subsequently, 1 M HCl (0.6 mL) and 10% Pd/C (100 mG) were added, and the mixture was hydrogenated for another 72 h at 4 bar. The mixture was filtered through Celite® and ion-exchanged with Amberlite IR 120 (plus). The product was liberated with 2.5% NH_4OH (250 mL). It was concentrated and then chromatographed using EtOH/EtOAc (1:4) as eluent; yield: 51 mg (37%); $[\alpha]_D$ +0.98 (*c* = 1, MeOH).

^{1}H NMR ($CDCl_3$): δ = 4.8 (d, 1 H, H-1), 3.79 (dd, 1 H, H-5"a, $J_{5',5"a}$ = 3.5 Hz, $J_{5"a,5"b}$ = 11.5 Hz), 3.75−3.53 (m, 6 H, H-2, H-3, H-5, H-5"b, H-6a, H-6b), 3.46 (dt, 1-H, H-3', $J_{3',2'eq}$ = 4.0 Hz, $J_{3',2'ax}$ = $J_{3',4'}$ = 10.0 Hz), 3.37 (s, 3 H, OCH_3), 3.22 (t, 1 H, H-4', $J_{4',3'}$ = 10.0 Hz, $J_{4',5'}$ = 10.0 Hz), 3.10 (br dd, 2 H, H-2'eq, H-6'eq, $J_{2'eq,3'}$ = $J_{6'eq,5'}$ = 4.0 Hz, $J_{2'eq,2'ax}$ = $J_{6'eq,6'ax}$ = 11.5 Hz), 2.70 (dd, 1 H, H-4"a, $J_{4"a,4}$ = 6.5 Hz, $J_{4"a,4"b}$ = 13.0 Hz), 2.54 (dd, 1 H, H-4"b, $J_{4"a,4}$ = 6.5 Hz, $J_{4"a,4"b}$ = 13.0 Hz), 2.04 (t, 2 H, H-2'ax, H-6'ax, $J_{2'ax,2'eq}$ = $J_{6'ax,6'eq}$ = $J_{2'ax,3'}$ = $J_{6'ax,5'}$ = 11.0 Hz), 1.90 (m, 1 H, H-4), 1.71 (m, 1 H, H-5').

^{13}C NMR ($CDCl_3$): δ = 100.2 (C-1); 74.1, 72.6, 72.4, 71.9, 71.8 (C-2, C-3, C-5, C-3', C-4'), 62.7, 61.2 (C-6, C-5"), 58.3, 57.4 (C-2', C-6'), 55.6 (OCH_3), 55.2 (C-4"), 44.0, 41.3 (C-4, C-5').

HRMS (ES): *m*/*z* calcd for $C_{14}H_{27}NO_8$ (M + Na^+): 360.1634, Found: 360.1632.

Determination of Enzyme Inhibition Constants

Each glycosidase assay was performed by preparing ca 2 mL samples in cuvettes consisting of 1 mL of sodium phosphate buffer (0.1 M) of pH 6.8 (or as noted in the Table), 0.2 mL to 0.8 mL of a 1.0 mM or 10 mM solution of either 4-nitrophenyl α-D-glucopyranoside or 4-nitrophenyl β-D-glucopyranoside, and 0.1 mL of a solution of either the potential inhibitor or H_2O, and distilled H_2O to a total volume of 1.9 mL. Eight of the samples contained the potential inhibitor at fixed concentration, but with variant nitrophenyl glycoside concentration. Other 8 samples contained no inhibitor, but also variant nitrophenyl glycoside concentration. Finaly the reaction was started by adding 0.1 mL of a diluted solution of either α-glucosidase from bakers yeast (EC 3.2.1.20, Sigma G-5003), isomaltase from yeast, glucoamylase from aspargillus niger or β-glucosidase from almonds (EC 3.2.1.21, Sigma G-0395), and the formation of 4-nitrophenol was followed for 2 min at 25 °C (or as noted in the Table) by measuring absorbance at 400 nm. Initial velocities were calculated from the slopes for each of the eight reactions and used to construct two Hanes plots; one with and without inhibitor. From the two Michaelis−Menten constants (K_m) thus obtained, the inhibition constant (K_i) was calculated.

Glucoamylase was also assayed with a maltose substrate. Glucoamylase G2 from A. niger prepared as described earlier[9,10] was added to a final concentration of 0.070 μM to preincubated 1 mM maltose containing 12 different concentrations of inhibitor in the range 0− 2 μM in 0.1 M NaOAc (pH 4.5) at 44 °C. Linear rates of hydrolysis were determined by measuring glucose released in aliquots removed at 3 min intervals using the glucose oxidase method essentially as described.[11] The K_i was calculated from the equation 1/v = i/Vmax + (Km/Vmax[S]Ki)[I], where (*S*) and (I) are substrate and inhibitor concentrations, respectively, Km was 1.7 mM and kcat (Vmax/[E]) was 11.3 s^{-1} as determined from the Michaelis−Menten equation.

Acknowledgement

We thank the Danish National Research Councils for support through the THOR program and Sielsel Ehlers for glycoamylase inhibition experiments.

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Article Identifier:
1437-210X,E;2001,0,02,0339,0342,ftx,en;C13100SS.pdf