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Homoisofagomines: Chemical-Enzymatic Synthesis and Evaluation as α- and β-Glucosidase Inhibitors

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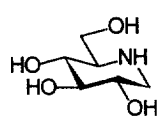
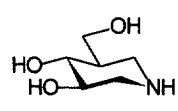
Abstract. Methyl- and hydroxymethyl derivatives of the highly potent glycosidase inhibitor isofagomine are accessible via aldolase-catalyzed C-C bond formation and competitively inhibit β-glucosidase at low micromolar concentrations.

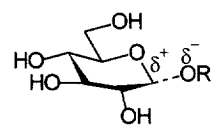
Polyhydroxylated piperidines are an important class of inhibitors of oligosaccharide processing enzymes.[1] Thus, deoxynojirimycin (**1**) and several of its derivatives potently inhibit both α-and β-glucosidases from various sources and exhibit interesting biological activities.[2] In an attempt to model the electrostatic interaction between the protonated nitrogen and the enzyme carboxylate assumed to stabilize the putative oxocarbenium transition state (**3**) Jespersen et al.[3] prepared the 1-*N*-iminosugar isofagomin (**2**) which was shown to be the most potent inhibitor of β-glucosidase from almonds yet described.[4] Meanwhile, several other sugar analogs with nitrogen in the anomeric position have been prepared.[5] A QSAR study[6] including eleven deoxynojirimycin-type glucose analogues revealed the importance of the presence of the 2-hydroxyl group (sugar numbering) in the proper topographical orientation as prerequisite for an effective inhibition. Thus, removal of the 2-hydroxyl group from **1** increased the K_i by more than two orders of magnitude. Introduction of a hydroxyl group into the corresponding position of **2** would create an unstable hemiaminal function. Therefore, homoisofagomines **4** and **5** were targeted as 2-hydroxyl surrogates. Notably, **4** resembles both nojirimycin-type (e.g. **1**) and isofagomin-type iminosugars (e.g. **2**).



deoxynojirimycin (**1**)



isofagomin (**2**)



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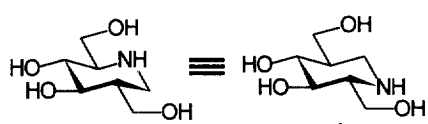

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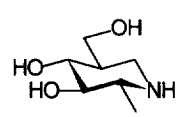

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Whereas previous syntheses of **2** and its analogs rely on the chiral pool, a stereoselective aldolase-catalyzed C,C bond formation[7] serves as the key step of the approach presented herein. A suitable *N*-protected β-aminoaldehyde

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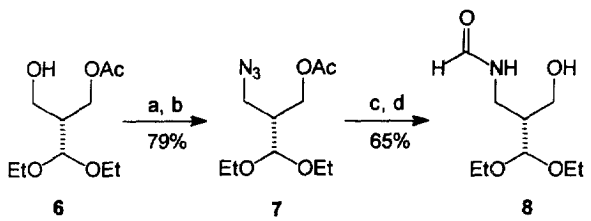
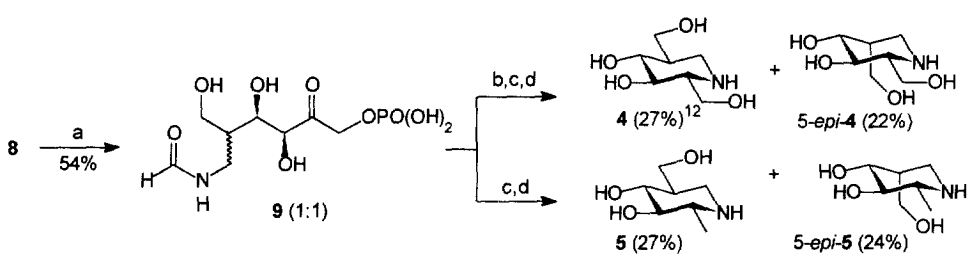


Scheme 1. Synthesis of aldehyde precursor **8**. Reagents and conditions: (a) 1.3 eq MsCl/pyr/0° C/overnight; (b) 2.0 eq NaN_3/DMF/40 °C/overnight; (c) 3 eq $LiAlH_4/Et_2O$/reflux/60 min; (d) i) 5.0 eq $(HCO)_2O$[9]/Et_2O-pyr/rt/ overnight, ii) 5% NEt_3/MeOH/overnight

precursor (**8**) is obtained in few steps from **6** (Scheme 1). **6** was prepared in high yield and an enantiomer ratio of 85:15 by lipase-catalyzed monoacetylation of the corresponding diol.[8] Of several *N*-protective groups investigated, the formyl group was selected for its superiour water solubility and substrate behaviour.[10] The acetal of **8** is cleaved under exceptionally mild acidic conditions, which might be attributed to a neighbouring effect of the hydroxyl group. The resulting aldehyde solution is stable for several days.

Taking into account the absolute and relative stereochemistry required at the two chiral centres to be formed, rabbit muscle aldolase (RAMA) was chosen as catalyst for the subsequent C,C bond formation.[7] Prior to the addition of RAMA 0.7 eq of dihydroxyacetonephosphate (DHAP)[11] was added to the aldehyde solution and the pH was carefully adjusted to 6.8 (Scheme 2). Although the aldolase reaction proceeded with complete stereoselectivity as regards the two new chiral centres, unexpectedly a nearly 1:1 mixture of diastereomeric aldol products was isolated in 54% yield after ion-exchange chromatography on Dowex 2 X 8 (H^+-form). Since, no chiral information was lost from **6** to **8** as shown by chiral HPLC, racemization of the aldehyde substrate even under neutral conditions has to be assumed. It could be caused either by *N*-formyl-assisted α-hydrogen abstraction or by reversible 1,4- elimination. For the synthesis of **4**, the phosphate was hydrolyzed using acid phosphatase. The *N*-formyl group was removed in 0.6 M HCl. Subsequent catalytic hydrogenation of the crude imine gave a mixture of **4** and its 5-epimer after column chromatography on silica gel (CH_2Cl_2/CH_3OH/22% NH_4OH = 20/50/1). Except the omission of the phosphate hydrolysis step the synthesis of **5** and its 2-epimer followed exactly the same procedures. The 2-methyl group in **5** and 5-*epi*-**5** results from



Scheme 2. Aldolase-catalyzed synthesis of homoisofagomines. Reagents and conditions: (a) i) 20 mM HCl/rt/2 h, ii) 0.7eq DHAP/ RAMA (60 U/mmol)/25 mM BIS-TRIS (pH 6.8)/rt/overnight;(b) acid phosphatase (25 U/mmol)/pH 4.8/rt/3 d; (c) 0.6 M HCl/35°C/36 h; (d) H_2 (8 bar)/Pd-C/rt/overnight

Table 1. Competitive inhibition of α- and β-glucosidase (K_i [μM]).

enzyme	1	2	4	5
β-glucosidase (sweet almond)	47[5]	0.11[5]	6.6[a]	2.1[a]
α-glucosidase (yeast))	25[5]	86[5]	n.i.[b]	n.i.[b]

a) 50 mM phosphate, pH 7.0, 30 °C, 0.5 - 16 mM β-*p*-NPG; b) <20% inhibition at 0.1 mM inhibitor in 50 mM phosphate, pH 7.0, 30 °C,0.5 mM EDTA, 2.5 mM α-*p*-NPG

hydrogenolytic phosphate cleavage during the reductive amination step.[7] Finally, semipreparative HPLC afforded the pure diastereomers depicted in Scheme 2.[12]

Both **4** and **5** were evaluated as competitive inhibitors of α– and β-glucosidase, respectively.[13,14] Whereas, both compounds are inactive against yeast α-glucosidase, they are more potent inhibitors of β-glucosidase from almonds than **1** (Table 1). This finding as well as the inhibition pattern indicate, that both **4** and **5** bind to β-glucosidase in the same orientation as isofagomin (**2**). The introduction of 2-methyl and 2-hydroxymethyl substituents, respectively, affects the inhibition to a marked, although, not dramatic extent. This effect could be caused by unfavourable interactions between these substituents and the enzyme surface (e. g. steric hindrance). On the other hand, stabilization of the ground state-like chair conformation by additional equatorial substituents could impede the binding of **4** and **5** in a possibly more transition state-like half-chair conformation.

In summary, the applicability of aldolase chemistry towards the synthesis of seven-carbon isofagomin derivatives has been demonstrated. Using different aldolases other sugar analogs should be easily accessible. E.g., fuculose-1-phosphate aldolase could be used to obtain 1-*N*-iminosugars corresponding to the D-*galacto* series based on the same procedures and starting materials. The homoisofagomins presented are potent inhibitors of β-glucosidase from almonds. Their interaction with other α- and β-glucoside-processing enzymes is currently under investigation.

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10. Although the azidoaldehyde prepared from **7** by deacetylation (PLE) and acid deacetalyzation was also accepted by RAMA, only decomposed products could be isolated.
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12. Since diastereomers were only partially separated on silica gel, pure 1-*N*-iminosugars were prepared by HPLC on a LiChrospher®-diol phase (8 x 250 mm) under elution with mixtures of MeOH and MTBE containing 0.1% of triethylamine. 15-20 mg of each compound (0.5-1 mg per injection) was isolated for kinetic and spectroscopic characterization. Yields are given in Scheme 2 and are based on **9**.
Selected spectroscopic data for 1-*N*-iminosugars: **4:** ^{1}H-NMR (400MHz, CD_3OD/D_2O): δ = 3.84 (dd, 1H *J* = 11Hz, 3.5Hz, NCHC$\underline{H}_2$OH), 3.79 (dd, 1H, *J* = 11Hz, 4Hz, $CH_2CHC\underline{H}_2OH$), 3.60 (dd, 1H, *J* = 11Hz 6.5Hz, NCHC$\underline{H}_2$OH), 3.54 (dd, 1H, *J* = 11Hz, 7Hz, $CH_2CHC\underline{H}_2OH$), 3.23 (t, 1H, *J* = 9.5Hz, NCHC$\underline{H}$OH), 3.19 (t, 1H, *J* = 9.5Hz, $NCH_2CHC\underline{H}OH$), 3.13 (dd, 1H, *J* = 12Hz, 4Hz, C$\underline{H}_2$NH), 2.46 (m, 1H, C$\underline{H}$NH), 2.45 (dd, 1H, *J* = 12Hz, 10Hz, C$\underline{H}_2$NH), 1.66 (m, 1H, $CH_2C\underline{H}CH_2OH$); ^{13}C-NMR (67.9 MHz, CD_3OD/D_2O): δ = 76.02 (CH), 74.79 (CH), 63.21 (CH_2), 63.06 (CH), 62.56 (CH_2), 47.75 (CH_2), 46.6 (CH); $[\alpha]^{25}_D$ +31.8° (*c* = 2.0, H_2O); FAB *m/z* (M + H^+) calcd. 178, obsd. 178; 5-*epi*-**4:** ^{1}H-NMR (400MHz, CD_3OD/D_2O): δ = 3.86 (dd, 1H, *J* = 11Hz, 5Hz, NCHC$\underline{H}_2$OH), 3.79-3.69 (m, 3H, NCHC$\underline{H}_2$OH $CH_2CHC\underline{H}_2OH$), 3.68 (dd, 1H, *J* = 9Hz, 5Hz, $NCH_2CHC\underline{H}OH$), 3.41 (t, 1H, *J* = 9Hz, NCHC$\underline{H}$OH), 3.1 (dd, 1H, *J* = 13Hz, 2.5Hz, C$\underline{H}_2$NH), 2.72 (dd, 1H, *J* = 13Hz, 3Hz, C$\underline{H}_2$NH), 2.46 (m, 1H, C$\underline{H}$NH), 2.09 (m, 1H, $CH_2C\underline{H}CH_2OH$); ^{13}C-NMR (67.9 MHz, CD_3OD/D_2O): δ = 75.73 (CH), 70.34 (CH), 62.75 (CH) 62.23 (CH_2), 59.39 (CH_2), 45.28 (CH_2), 43.6 (CH); FAB *m/z* (M + H^+) calcd. 178, obsd. 178; **5:** ^{1}H-NM (400MHz, CD_3OD): δ = 3.79 (dd, 1H, *J* = 11Hz, 4Hz, C$\underline{H}_2$OH), 3.53 (dd, 1H, *J* = 11Hz, 4Hz, C$\underline{H}_2$OH) 3.19 (dd, 1H, *J* = 10.5Hz, 9Hz, $CH_2CHC\underline{H}OH$), 3.07 (dd, 1H, *J* = 12.5Hz, 4Hz, C$\underline{H}_2$NH), 2.94 (t, 1H *J* = 9Hz , $CH_3CHC\underline{H}OH$), 2.45 (t, 1H, *J* = 12.5Hz, C$\underline{H}_2$NH), 2.44 (m, 1H, C$\underline{H}$NH), 1.68 (m, 1H, $C\underline{H}CH_2OH$), 1.19 (d, 3H, *J* = 4Hz); ^{13}C-NMR (67.9 MHz, CD_3OD): δ = 79.53 (CH), 75.63 (CH), 62.5 (CH_2), 57.77 (CH), 47.83 (CH_2), 46.74 (CH), 18.38 (CH_3); $[\alpha]^{25}_D$ +19.7° (*c* = 2.0, H_2O); FAB *m/z* (M + H^+) calcd. 162, obsd. 162; 5-*epi*-**5:** ^{1}H-NMR (400MHz, CD_3OD): δ = 3.87 (dd, 1H, *J* = 11Hz, 5Hz, C$\underline{H}_2$OH) 3.78 (dd, 1H, *J* = 11Hz, 7Hz, C$\underline{H}_2$OH), 3.62 (dd, 1H, *J* = 9Hz, 5Hz, $CH_2CHC\underline{H}OH$)), 3.20 (t, 1H, *J* = 9Hz, $CH_3CHC\underline{H}OH$), 3.14 (dd, 1H, *J* = 13Hz, 3Hz, C$\underline{H}_2$NH), 2.77 (dd, 1H, *J* = 13Hz, 3.5Hz, C$\underline{H}_2$NH), 2.52 (m 1H, C$\underline{H}$NH), 2.07 (m, 1H, $C\underline{H}CH_2OH$), 1.21 (d, 3H, *J* = 4Hz, C$\underline{H}_3$); ^{13}C-NMR (67.9 MHz, CD_3OD) δ = 75.56 (CH), 75.33 (CH), 60.13 (CH_2), 57.52 (CH), 45.75 (CH_2), 43.4 (CH), 17.87 (CH_3); FAB *m/z* (M + H^+) calcd. 162, obsd. 162.
13. Initial velocities of the hydrolysis of α-NPG and β-NPG by α- and β-glucosidase (SIGMA, G7256, G4511) were measured at concentrations of 0.5 - 16 mM. Enzymes were preincubated with the inhibitor for 5 min and reactions were started by substrate addition. Michaelis constants were obtained by non-linear regression using the program EnzymeKinetics® and K_i values by plotting K_M against [I].
14. The 5-epimers of **4** and **5** were inactive against α- and β-glucosidase as to be expected. 5-*epi*-**4** representing 2-homo-1-deoxy-*manno*-nojirimycin showed no activity against α-mannosidase from jack beans.