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SYNTHESIS AND BIOLOGICAL ACTIVITY OF C-6 MODIFIED DERIVATIVES OF THE GLUCOSIDASE INHIBITOR 1-DEOXYNOJIRIMYCIN.

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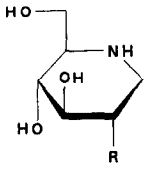
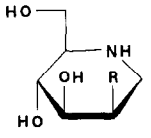
Abstract: A novel 1-deoxynojirimycin derivative, *(6S)*-6-C-ethyl-1-deoxynojirimycin, was synthesized and tested on a set of α- and β-glucosidases. Its enzyme inhibitory activity against α-glucosidases from yeast and rice was superior to those of 1,6-dideoxy-6-fluoronojirimycin, 1-deoxynojirimycin and its bicyclic analogue, castanospermine.

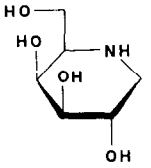
1-Deoxynojirimycin (1,5-dideoxy-1,5-imino-D-glucitol, **1**)[1], a natural product first discovered in the root bark of a *Morus* species[2] and also a component of fermentation broths of *Streptomyces lavendulae*[3], is an efficient inhibitor of various α- and β-glucosidases[4]. Its 2-deoxy-derivative, the natural product fagomine (**2**)[5], exhibits only very limited enzyme inhibitory activity, indicating the importance of OH-2 for recognition and/or binding to the receptor site. The synthetic 2-acetamido-2-deoxy derivative **3** inhibits *N*-acetyl-glucosaminidases[6] and the naturally occurring C-2 epimer, 1,5-dideoxy-1,5-imino-D-mannitol (**4**)[7], is a moderate inhibitor of various mannosidases[8]. The recently synthesized 2-*O*-ethyl- and 2-deoxy-2-fluoro-analogues of **4**, compounds **5** and **6**, do not exhibit reasonable activity against jack bean α-mannosidase or β-glucosidase from almonds[9]. A large variety of *N*-alkylated derivatives of **1** has been synthesized over the



1 R = OH
2 R = H
3 R = NHAc



4 R = OH
5 R = OEt
6 R = F



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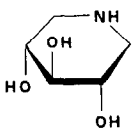

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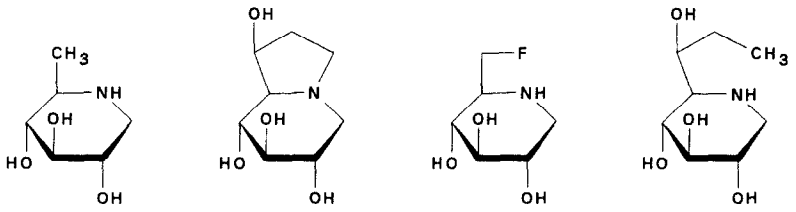
past years in context with diabetes research[10]. However, besides the syntheses of **3**, **5**, and **6**, derivatisation of ring positions has been confined to the synthesis of the D-*galacto* epimer **7**[11] (which is an inhibitor of the respective glycosidases), the formal removal of the hydroxymethyl group at C-5 (to give the *nor*-derivative 1,5-dideoxy-1,5-imino-xylitol **8**[12], a *meso*-compound, inhibiting almond β-glucosidase nearly as effectively

as **1**), and the recent chemoenzymatic synthesis of 1,6-dideoxynojirimycin (**9**), which was reported to be a poor inhibitor of glucosidases from yeast and almonds, respectively[9].



9 **10** **11** **12**

Configurationally the closest relative of 1-deoxynojirimycin in Nature is the indolizidine alkaloid castanospermine (**10**)[13], a powerful inhibitor of a large variety of α- and β-glucosidases[4], which can be considered a more rigid and lipophilic (because of two additional methylene groups) derivative of **1**, with the rotation of the hydroxymethyl group at C-5 locked by virtue of its bicyclic structure.

Castanospermine, being as active as **1** against β-glucosidase from *Asp. wentii*, is remarkably more efficient against β-glucosidase from almonds (1.5 μM versus 300 μM at pH 5). However, in contrast to **1**, compound **10** is virtually inactive against α-glucosidase from yeast[4].

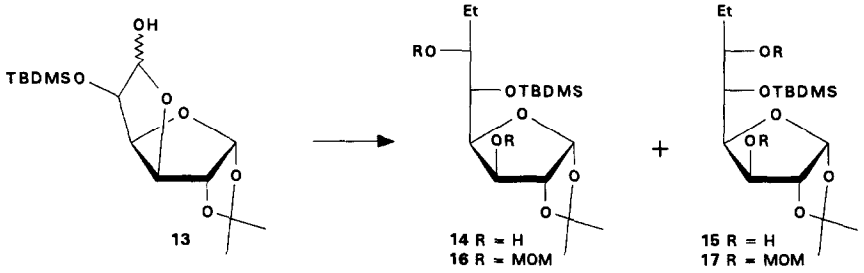
Both 1-deoxynojirimycin (**1**) and castanospermine (**10**) have been demonstrated to be efficient inhibitors of glucosidases involved in glycoprotein processing and by virtue of this have been shown to exhibit anti-HIV properties, **10** being the more active compound[14]. Interestingly, simple *N*-alkylated derivatives of **1** showed enhanced anti-HIV activities compared to the parent compound **1**, the *N*-(n-butyl)-derivative being the most efficient[15]. This again leads to the conclusion that increased lipophilicity and/or basicity can dramatically alter the inhibitor's biological activity.

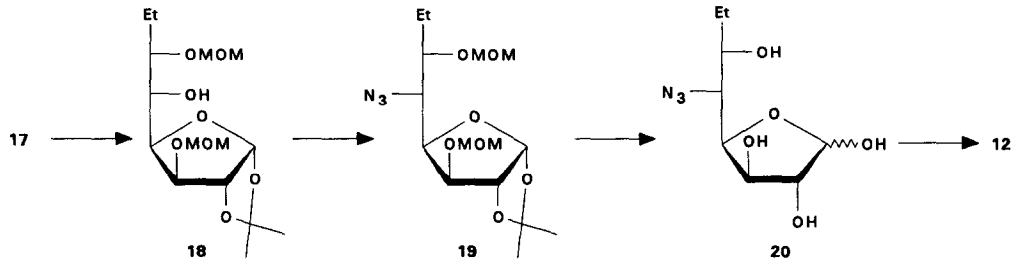
Compound **10** was also found to inhibit experimental metastasis and tumor growth in mice[16].

In context with a programme evaluating the influence of lipophilicity and basicity of the ring nitrogen on the biological activities of 1-deoxynojirimycin derivatives we had become interested in the role of the hydroxyl function at position C-6 during the interaction of **1** with the binding site of the respective glucosidase.

Our aim was to increase the lipophilicity of the molecule by relatively small amounts and not disturb the overall geometry of the molecule. Bearing in mind that a fluorine substituent is a virtually isosteric replacement for a hydroxy function, considerably decreasing the polarity of the respective molecule but still exhibiting hydrogen bridge accepting properties[17] (in contrast to a simple deoxy "function"), we had synthesized 1,5,6-trideoxy-6-fluoro-1,5-imino-D-glucitol (**11**)[18], the 6-deoxyfluoro derivative of **1**. To compare the properties of this compound with deoxynojirimycin derivatives with similarily increased lipophilicity but still bearing a hydroxy function at C-6 we prepared *(6S)*-6-*C*-ethyl-1-deoxynojirimycin **12**. This compound could also be considered a *seco*-derivative of castanospermine (**10**) and was expected to allow conclusions to be drawn concerning the entropic part of the free energy of binding for **10**.

The synthesis of *(6S)*-6-*C*-ethyl-1-deoxynojirimycin (1,5,7,8-tetradeoxy-1,5-imino-L-*glycero*-D-*gluco*-octitol, **12**) was started from 5-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-β-L-*ido*-hexodialdodifuranose (**13**)[19]. This smoothly reacted with freshly prepared ethylmagnesium bromide in ether to give an inseparable mixture of the two diastereomeric diols **14** and **15** in 82% combined yield. After protection of the hydroxyl





functions by reaction with chloromethyl methylether in the presence of *N*-ethyldiisopropylamine, chromatographic separation yielded fully protected compounds **16** (47%, $[\alpha]_D$ -36, c 1.5, chloroform) and **17** (41%, $[\alpha]_D$ -42, c 1.4, chloroform). Their configurations at the newly introduced chiral centre, C-6, were assigned according to the results of a previous investigation[19]. Removal of the silyl protecting group at O-5 from less polar **17** with wet tetra-n-butylammonium fluoride in tetrahydrofuran gave syrupy alcohol **18** (94%, $[\alpha]_D$ -51, c 1.3, chloroform) and *O*-sulfonylation of the latter with triflic anhydride followed by treatment with sodium azide in *N,N*-dimethylformamide led to syrupy azidodeoxyoctose derivative **19** (80%, $[\alpha]_D$ -20.5, c 1.2, chloroform). Conventional simultaneous removal of the protecting groups with acidic ion exchange resin Amberlite IR 120 [H^+] in acetonitrile/ water (1:1, v/v) and subsequent reductive cyclization (PARR-apparatus, 4 bar hydrogen, palladium-on-charcoal 5%) of the resulting free azidosugar **20** (82% after chromatography) gave desired **12**[20] which was isolated in 65% yield as the free base after conventional purification on Amberlite CG 50.

The glucosidase inhibitory properties of compounds **11** and **12** are summarized in the following table.

Table. Competitive Inhibition Constants K_i (μM) at pH 5 unless stated otherwise; calculated with S/K_m.

Compound	Glucosidase			
	β (*Asp. wentii*)	β (almonds)	α (yeast)	α (rice)
1	2,0	300	460 23 (pH 6) 21 (pH 7)	0,16[b] 0,014[b] (pH 6)
9[a]	---	780	1560	---
11	380	600	340 29 (pH 6) 47 (pH 7)	15 8 (pH 6)
12	42	630	3 60 (pH 6) 2000 (pH 7)	≤0,023[b] ≤0,007[b] (pH 6)
10	0,9	1,5[b]	>1500	0,015[b]

[a] data given in ref. 9; at pH 6.5.

[b] slow approach to the inhibition equilibrium; K_i refers to the final state.

In contrast to the 6-deoxy-derivative **9**[9] the 6-deoxyfluoro analogue **11**, although being a comparatively (to **1**) poor inhibitor of β-glucosidase from *Asp. wentii*, is similarily active as parent compound **1** against β-glucosidase from almonds. This reflects differences in the glycon binding sites of the two enzymes which include hydrogen bonds to the C-6 hydroxyl group in the *Aspergillus* enzyme[21], whereas the enzyme from almonds shows about the same activity with β-glucosides and 6-deoxy-β-glucosides.[22] With the α-glucosidase from yeast, **1** and **11** show very similar inhibitory potencies which would point to a glycon binding site similar to that of the almond enzyme. *(6S)*-6-*C*-ethyl-1-deoxynojirimycin (**12**), comparable with the 6-deoxyfluoro derivative **11** in its activity against β-glucosidase from almonds but about ninefold more active against β-glucosidase from *Asp. wentii*, exhibits distinctly improved inhibitory power (at pH 5) against yeast α-glucosidase compared to **1** as well as its bicyclic, conformationally rigid analogue, castanospermine (**10**), which is known[4] to be practically inactive against this enzyme. Only minor effects of the structural alteration of **1** studied here are seen with the inhibition of the α-glucosidase from rice by **10** and **12**. Effects of the fluoro substituent at C-6 in **11** with the same enzyme are similar to those with β-glucosidase from *Asp. wentii*, i. e. these enzymes obviously require the C-6 hydroxyl group for efficient binding.

The much stronger inhibition of yeast α-glucosidase by **12** compared with **10** might be taken as support of a hypothesis by Hosie and coworkers[23] who had proposed that the active site of this enzyme had adopted to a transition state distinctly different from the rigid conformation of **10**. Basic glucose analogues with a flexible structure such as **12** would bind tightly because of their ability to adopt a conformation resembling this transition state. It should, however, be noted that the pH-dependence of K_i for **12** is different from that for **1** and **11**. Tight binding of basic glycose analogues can be explained by the formation of a close ion pair consisting of the protonated inhibitor and a carboxylate group of the catalytic site.[4] pH-Dependence of K_i

will thus be governed by the protonation of both the inhibitor and the catalytic carboxylate. As compounds **1**, **11**, and **12** have pK_a-values near 6.5 the inverse pH-dependence of K_i seen with **12** would indicate a different binding mode and interactions with another carboxylate group. The different inhibition of the two β-glucosidases by **10** and **12** might reflect entropy effects arising from the "freezing" of the side chain of compound **12** in a position required by the geometry of the active site which is already preformed in castanospermine (**10**).

Preliminary tests with the fluorinated compound **11** on glucosidase I of glycoprotein trimming from pig liver showed 50% inhibition at 1 mM whereas only slight inhibition of glucosidase II was observed at concentrations up to 2.5 mM.

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10 For one of the recent examples see Junge, B.; Böshagen, H.; Kinast, G.; Köbernick, H.; Krause, H.-P.; Müller, L.; Puls, W. *6th European Carbohydrate Meeting*, **1989**, Prague, Abstr. *L 42*.

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[20] All new compounds gave satisfactory analytical data in full agreement with the structures proposed. NMR-data:
18: ^{13}C NMR (75.47 MHz, $CDCl_3$, δ in ppm): 104.7 (C-1); 83.4, 82.6, 78.6, 78.1, 70.3 (C-2,3,4,5,6); 22.8 (C-7); 8.6 (C-8); 111.9, 26.8, 26.4 (isopropylidene); 96.3, 96.2, 56.2, 55.9 (methoxymethyl); ^1H NMR (300 MHz, $CDCl_3$, δ in ppm, coupling constants J in Hz): 5.91 (d, $J_{1,2}$ 3.8, H-1), 4.54 (d, J 3.8, H-2), 4.16 (d, $J_{3,4}$ 3.3, H-3), 4.31 (dd, $J_{4,5}$ 3.3, H-4), 3.87 (broad signal, H-5), 3.59 (m, H-6), 1.66 (m, 2 H, H-7,7´), 0.91 (3 H, t, J 7.4, H-8), 3.18 (broad signal, OH-5), 4.72-4.60 (m, 4 H) and 3.35, 3.34 (2 s, 3 H each, methoxymethyl); 1.40 and 1.25 (2 s, 3 H each, isopropylidene).
9: ^{13}C NMR ($CDCl_3$): 105.1 (C-1); 83.1, 81.1, 78.8, 77.5 (C-2,3,4,6); 60.4 (C-5); 25.1 (C-7); 10.2 (C-8); 112.1, 26.9, 26.5 (isopropylidene); 97.1, 96.7, 56.2, 56.0 (methoxymethyl); ^1H NMR ($CDCl_3$): 5.84 (d, $J_{1,2}$ 3.6, H-1), 4.63 (d, J 3.6, H-2), 4.16 (d, $J_{3,4}$ 3.0, H-3), 4.35 (dd, $J_{4,5}$ 10.0, H-4), 3.50 (dd $J_{5,6}$ 1.7, H-5), 3.81 (m, H-6), 1.87-1.62 (m, 2 H, H-7,7´), 0.93 (t, 3 H, J 7.5, H-8), 4.73-4.65 (m, 4 H) and 3.39, 3.36 (2 s, 3 H each, methoxymethyl), 1.42 and 1.27 (2 s, 3 H each, isopropylidene).
12 (free base in D_2O): ^{13}C NMR: 79.5 (C-3); 72.5, 72.1, 71.1 (C-2,4,6); 62.6 (C-5); 49.5 (C-1); 27.3 (C-7); 11.0 (C-8); ^1H NMR: 3.88 (dd, $J_{6,7}$ 7, $J_{6,7'}$ 7, H-6), 3.48 (m, $J_{1a,2}$ 10.4, H-2), 3.39, 3.32 (2 t, 1 H each, $J_{2,3}=J_{3,4}$ ca. 9.2, H-3,4), 3.10 (dd, $J_{1a,1e}$ 12.8, $J_{1e,2}$ 5.0, H-1e), 2.46 (d, $J_{4,5}$ 9.5, H-5), 2.45 (dd, H-1a), 1.69-1.49 (m, 2 H, H-7,7´), 0.95 (t, 3 H, J 7.4, H-8).

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