Noeuromycin,[1] A Glycosyl Cation Mimic that Strongly Inhibits Glycosidases

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Received January 29, 2001

Interest in design and synthesis of inhibitors of glycoside cleavage has been intense during the last years.[2−4] Such inhibitors are not only useful as potential drugs against a number of diseases such as diabetes or influenza but can also provide new insight in the widespread and important glycoside cleavage/formation process.

Some time ago it was found that a subtle change in the classical glycosidase inhibitor 1-deoxynojirimycin (**1a**, Figure 1), by moving the nitrogen to the anomeric position to form isofagomine (**2a**), gave a much more potent β-glucosidase inhibitor.[5] This appears to be general, and some of the strongest β-glycosidase inhibitors have been found among the isofagomines (such as **3a** and **4a**)[6−10] (see Figure 1). It is obvious that protonated **2a** mimics the cation **A**, an intermediate in the glycoside cleavage process, and this has been suggested to be the basis of its potent inhibition particularly if the transition state resembles **A**. Interestingly the transition state is not believed to resemble **A** but rather a resonance form of **A** the oxocarbenium ion **B** according to the Phillips mechanism for lysozyme action. Furthermore, recent work on acid-catalyzed glycoside hydrolysis concludes that an oxocarbenium ion transition state (like **B**) is much more important than charge development on anomeric carbon (like **A**).[11] These apparent inconsistencies are worth investigating.

It may be argued that isofagomine (**2**) is not a perfect mimic of **A** as it lacks the 2-hydroxyl group, which was omitted in the design because of the reported instability of hemiaminals,[12] and attempts to mimic the 2-hydroxyl group met limited succes.[13,14] However a recent study, where the thermodynamic functions of binding between **1a** or **2a** and β-glucosidase were examined, suggested that the 2-OH of **1a** contributed significantly to binding enthalpy, while a similar interaction was lacking for **2a**.[15] This accords with the 2-OH being crucial for binding of both the inhibitor **1a** and the substrate to many glycosidases.[16] It was therefore decided to attempt to synthesize an analogue of **2a** where the 2-hydroxyl group was present.

Our synthesis started from the known[17] 2-hydroxymethylglucose derivative **5** (Scheme 1). This compound was subjected to

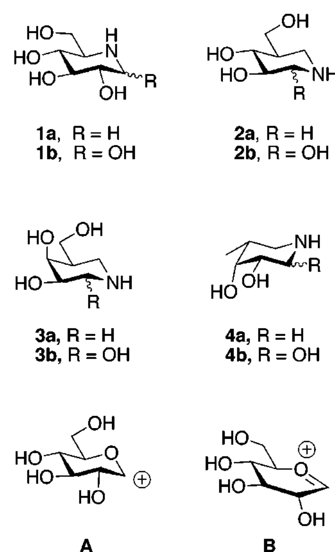
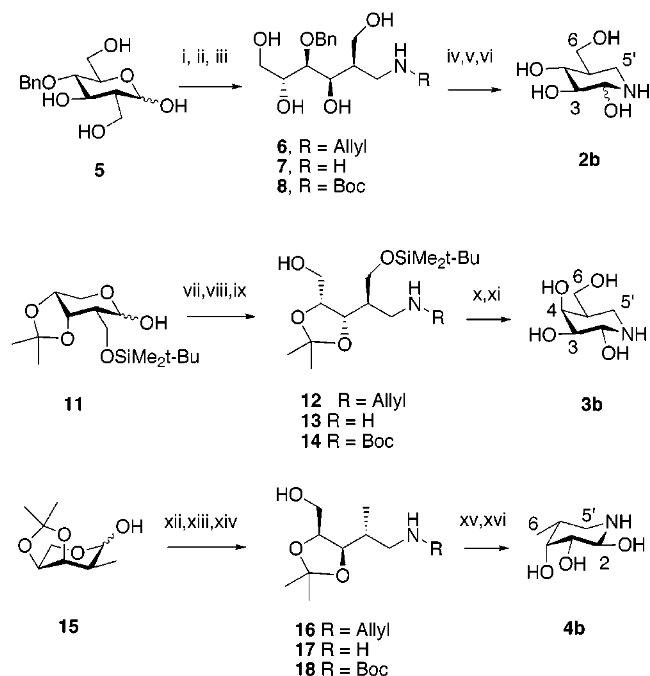


Figure 1. The chemical structures of 1-deoxynojirimycin (**1a**), nojirimycin (**1b**), isofagomine (**2a**), noeuromycin (**2b**), analogues **3a**-**4b**, and the resonance forms **A** and **B** of the glucosyl cation.

Scheme 1. Synthesis of Noeuromycin and Analogues[a]



[a] i) $CH_2{=}CHCH_2NH_2$, $NaCNBH_3$, AcOH, 25 °C, 83%. ii) $(Ph_3P)_3RhCl$, $MeCN/H_2O$, reflux followed by TFA/H_2O, 25 °C. iii) $(t\text{-}BuOCO)_2O$, $NaHCO_3$, Me_2CO/H_2O, 25 °C, 87% from **6**. iv) $NaIO_4$. v) H_2 (1 atm.), Pd/C, MeCOOH, 25 °C. vi) TFA/H_2O, 25 °C, α:β 1:2, 55% from **8**. vii) $CH_2{=}CHCH_2NH_2$, $NaCNBH_3$, AcOH, 25 °C, 57%. viii) $(Ph_3P)_3RhCl$, $MeCN/H_2O$, reflux. ix) $(t\text{-}BuOCO)_2O$, $NaHCO_3$, Me_2CO/H_2O, 25 °C, 64% from **12**. x) TEMPO, MCPBA, 41%. xi) HCl, H_2O, α:β:pyranose ≈ 6:0:1, 99%. xii) $CH_2{=}CHCH_2NH_2$, $NaCNBH_3$, AcOH, 25 °C, 86%. xiii) $(Ph_3P)_3RhCl$, $MeCN/H_2O$, reflux. xiv) $(t\text{-}BuOCO)_2O$, $NaHCO_3$, MeCN, 25 °C, 86%. xv) TEMPO, MCPBA, Bu_4NBr, CH_2Cl_2, 25 °C, 99%. xvi) HCl, H_2O, α:β ≈ 1:0, 99%.

reductive amination with allylamine to **6**, deallylation using Wilkinson's catalyst followed by acidic hydrolysis to free amine **7** and Boc-protection to give the partially protected aminoglucitol derivative **8**. The glycol of **8** was subjected to periodate cleavage

(1) We propose the name noeuromycin to **2b** due to its resemblance to nojirimycin **1b** and to its discovery on the day of the Danish Euro Referendum (September 28, 2000).

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10.1021/ja010240u CCC: $20.00 © 2001 American Chemical Society
Published on Web 05/03/2001

Table 1. Inhibition Constants K_i in nM for Binding of Inhibitors **1**−**2**, **9**, and **10** to Various Enzymes[a]

enzyme	**1a**[d]	**1b**[d]	**2a**[d]	**2b**	**9**[d]	**10**[d]
α-glucosidase[b]	12600	6300	86000	22	59000	180[e]
isomaltase[b]	11000	−	7200	25	−	−
β-glucosidase[c]	47000	890	110	69	100	200000[e]

[a] −: inhibition not measured. [b] From yeast. [c] From almonds. [d] From ref 2. [e] IC_{50} value.

followed by deprotection to give noeuromycin[1] **2b** (α:β 1:2).[18] It was isolated as a hydrotrifluoroacetate and could as such be kept in solution for days without apparent decomposition.

Noeuromycin (**2b**) was tested for inhibition of glycosidases (Table 1) and was found to be a remarkably strong glucosidase inhibitor. The K_i values were all in the nanomolar range and between 2 and 4000 times smaller than those of **2a**. Evidently the incorporation of the 2-hydroxyl group increases inhibition profoundly; therefore, the inhibition of glucosidases is presumably caused mainly by the equatorial β-anomer. The inhibitor **2b** was also considerably more potent against glucosidases than the inhibitors **1a**, **1b**, **9**, and **10** resembling oxocarbenium ion intermediate **B** (Table 1). It is also remarkable that in contrast to **2a** the inhibitor **2b** inhibits both β- and α-glucosidases strongly.

The study was extended to synthesis of the D-*galacto*- and L-*fuco*-isomers of **2b** (Scheme 1). The *galacto*-isomer was obtained from the known derivative **11**.[19] Reductive amination with allylamine to **12**, deallylation with Wilkinsons catalyst to **13** and Boc-protection gave **14**. Alcohol **14** was oxidized with TEMPO and MCPBA to the lactol, which was finally deprotected to the D-*galacto*-noeuromycin **3b** (α:β: pyranose ≈ 6:0:1, Scheme 1).[18] By a similar sequence (Scheme 1) the known derivative **15**,[20] was converted to L-*fuco*-noeuromycin **4b** (α:β ≈ 1:0)[18] in good overall yield. Both compounds were only in α-form, which has the 2-hydroxyl group equatorial. In the case of **3b** a small amount of a pyranose form was present.

3b and **4b** were, like **2b**, glycosidase inhibitors in the nanomolar range (Table 2). While the galactose analogue **3b** was 50 times more potent α-galactosidase inhibitor than isogalactofagomine **3a**, its inhibition of three β-galactosidases varied from 4 times more potent to 9 times less potent than **3a**. The contribution of the 2-hydroxyl group to binding varies obviously between β-galactosidases. The L-fucose analogue **4b** was an α-fucosidase inhibitor 1000 times more powerful than isofucofagomine **4a**. The comparison of **3b** and **4b** to the *galacto* and *fuco* analogues of 1-deoxynojirimycin, **19** and **20** (Figure 2) is also interesting. Compound **20** is known to be an extremely potent α-fucosidase inhibitor, but it is clear that **4b** is equally potent (Table 2). On the other hand compound **19** is much more potent than **3b** versus α-galactosidase, while the reverse is true for β-galactosidase much like what has been observed for **3a**[6] but less extreme.

It is obvious that incorporation of a 2-hydroxyl group in **2a**−**4a** creates very tight binding inhibitors of both α- and β-glycosidases. The increase in binding to α-glycosidases of **2b**−**4b** is particularly remarkable compared with **2a**−**4a**. It shows that, contrary to previous beliefs, the anomeric nitrogen atom can interact effectively with these enzymes as well. It is likely that a salt bridge is formed between this group of **2b** and the nucleophilic carboxylate of the enzyme as suggested for binding of **2a** to β-glycosidases (**C**, Figure 3).[3,9] A similar interaction of **2b** with the nucleophilic carboxylate of an α-glycosidase can be imagined (**D**, Figure 3).

The present work shows that 1-azasugars are extremely potent inhibitors of glycosidases. It also suggests that charge development at the anomeric center is involved in the glycosidase-catalyzed reaction. The very tight binding competitive inhibition observed suggests that positive charge is present at the anomeric carbon at the transition state or in an intermediate on the reaction trajectory that is close to the transition state.

Table 2. Inhibition Constants K_i in nM for Binding of **3**−**4**, **19**, and **20** to Various Enzymes[a]

enzyme	**3a**	**3b**	**19**	**4a**	**4b**	**20**
α-fucosidase[b]	−	−	−	4000	4.7	29[j]
α-fucosidase[c]	−	−	−	6400[k]	3.2	1.3[j]
α-galactosidase[d]	50000	742	1.6[j]	−	−	−
β-galactosidase[e]	328	91	81000	−	−	−
β-galactosidase[f]	4[h]	35	−	−	−	−
β-galactosidase[g]	200[i]	397	12500[j]	−	−	−

[a] −: Inhibition not measured. [b] From bovine kidney. [c] From human placenta. [d] From Green Coffee Bean. [e] From *Saccharomyces Fragilis*. [f] From *Aspargillus Oryzae*. [g] From *E. Coli*. [h] From ref 6. [i] Measured on the racemic compound, from ref 21. [j] From ref 2. [k] From ref 20.

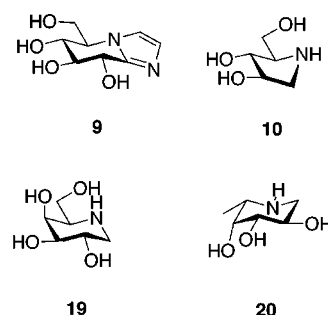

Figure 2. The chemical structure of the glycosidase inhibitors **9**, **10**, **19**, and **20**.

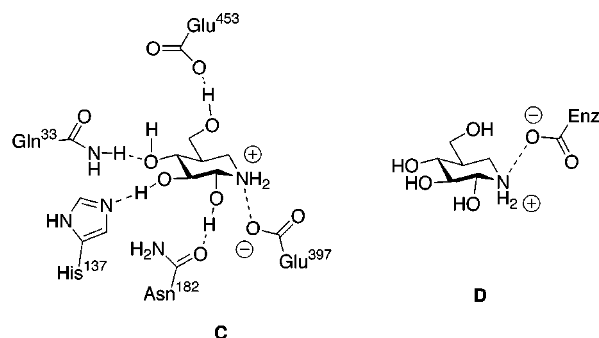

Figure 3. Proposed binding of **2b** in the active site of a β-glucosidase (**C**), in this case from white clover and a retaining α-glucosidase (**D**).

Acknowledgment. We thank the Danish Research Councils for financial support through the THOR program, Vinni Andreassen for preliminary experiments, and Professor A. E. Stütz for kindly providing us a sample of **19**.

Supporting Information Available: Experimental procedures for the preparation and characterization of **2b**, **3b** and **4b**, and NMR spectra of **2b**, **3b** and **4b** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Compounds **2b**, **3b**, and **4b** were isolated as hydrochlorides or hydrotrifluoroacetates. NMR data of the noeuromycins (α is α-anomer, β is β-anomer, p is pyranose form): **2b**, ^{13}C NMR (50 MHz, D_2O): δ 162.9 (m, CF_3COO^-), 115.7 (q, CF_3COO^-), 81.1 [C-2 (β)], 78.1 [C-2 (α)], 74.3 [C-3 (β)], 71.4 [C-3 (α)], 69.8 [C-4 (β)], 66.8 [C-4 (α)], 59.2 [C-6 (β)], 58.9 [C-6 (α)], 41.4 [C-5′ (β)], 41.0 [C-5′ (α)], 40.8 [C-5 (β)], 38.5 [C-5 (α)]. ^1H NMR (200 MHz, D_2O): δ 4.96 [d, $J_{2,3}$ 3.0, H-2 (β)], 4.30 [d, $J_{2,3}$ 9.0, H-2(α)], 3.6−2.6 (m, H-3, H-4, H-5′a, H-5′b, H-6a, H-6b), 2.1−1.5 (m, H-5). **3b**, ^{13}C NMR (50 MHz, D_2O): δ 81.2 (C-2), 73.9 (C-3), 70.1 (C-4), 62.4 (C-6), 42.0 (C-5), 41.8 (C-5′). ^1H NMR (400 MHz, D_2O): δ 5.08 [d, $J_{1,2}$ 2.4, H-1 (p)], 4.76−4.82 [H-2 (α)], 4.15 [bs, H-4 (α)], 3.78 [bs, H-3 (p)], 3.61−3.71 [m, H-3 (α), H-6 (α), H-2 (p), H-5a (p), H-5b (p)], 3.54 [dd, $J_{5,6a}$ 7.2, $J_{6a,6b}$ 11 Hz, H-6a (α)], 3.23 [dd, $J_{5,5'eq}$ 3.8, $J_{5'ax,5'eq}$ 13 Hz, H-5′eq (α)], 3.06−3.14 [m, H-4′a (p)], 3.03 [t, $J_{5,5'ax}$ 13.0 Hz, H-5′ax (α)], 2.86−2.96 [m, H-4′b (p)], 2.19−2.28 [m, H-4 (p)], 2.05−2.16 (m, H-5 (α)]. **4b**, ^{13}C NMR (50 MHz, D_2O): δ 77.7 (C-2), 71.0 (C-3), 69.8 (C-4), 42.3 (C-5′), 31.3 (C-5), 12.7 (C-6). ^1H NMR (200 MHz, D_2O): δ 4.70 (d, $J_{2,3}$ 9.1, H-2), 3.97 (br s, H-4), 3.68 (dd, $J_{3,4}$ 2.5 Hz, H-3), 3.09 (dd, $J_{5,5'eq}$ 5.1, $J_{5'ax,5'eq}$ 12.8 Hz, H-5′eq), 2.95 (t, $J_{5,5'ax}$ 12.8 Hz, H-5′ax), 2.06 (m, H-5), 0.98 (d, $J_{5,6}$ 7.3 Hz, CH_3).

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