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An Isofagomine Analogue with an Amidine at the Pseudoanomeric Position

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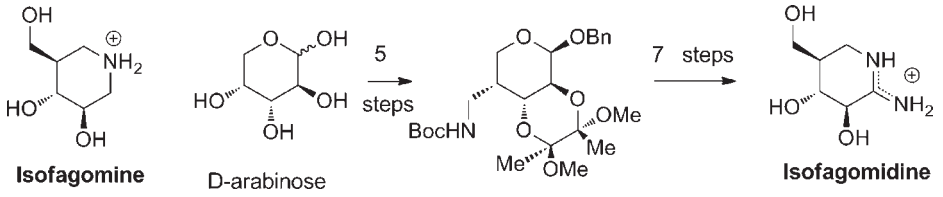
ABSTRACT



(3*R*,4*R*,5*R*)-2-Imino-3,4-dihydroxy-5-hydroxymethylpiperidine hydrocloride or isofagomidine was synthesized from D-arabinose in 12 steps and an overall yield of 9.9%. The synthesis proceeded by introduction of an aminomethyl group in the 4-position of D-arabinose and conversion of C-1 into a nitrile. The key step in the synthesis was a copper-catalyzed cyclization of aminonitrile to amidine. Isofagomidine was a potent α-mannosidase inhibitor (K_i = 0.75 μM).

Iminosugar glycosidase inhibitors of the 1-deoxynojirimycin type (**1**) are natural products that resemble monosaccharides;[1] in fact, the conjugate acids of these amines resemble the transition state of glycoside cleavage, which is the likely basis of their biological activity (Figure 1).[2] These compounds have therapeutic potential, which has been exploited in antidiabetes treatment,[3] and are also being evaluated for treatment of diseases ranging from cancer and AIDS to lysosomal storage disorders and hepatitis infections.[4] A number of interesting analogues of **1** have been made over the years. Particularly noteworthy is the glucoamidine **2** that was designed by Ganem and found to be a broad-spectrum glycosidase inhibitor.[5] Amidine **2** is obviously a much stronger base than **1**, and the ionic interaction between enzyme and inhibitor should be more profound.

A series of glycosidase inhibitors related to **1** are 1-azasugars, such as isofagomine **3**,[6] which in protonated form resemble the alternate resonance form of the oxocarbenium ion transition state (Figure 1). These compounds are potent β-glycosidase inhibitors presumably because they form a strong salt bridge with the nucleophilic carboxylate in these enzymes.[7] X-ray structures of the inhibitor−enzyme complex provide evidence of this, showing a protonated isofagomine.[8]

On the basis of the observation that ionic interaction is so important for the binding of **3**, the idea emerges that application of Ganem's idea of introducing an amidine into the isofagomine structure may lead to interesting inhibitors. The structure **4** (Figure 1), which has an amidine placed at the 1,2-positions, has the advantage that it has a polar group at the 2-position where NHAc[9] and OH

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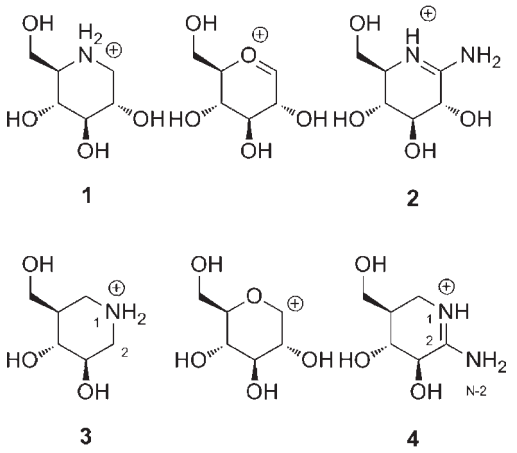
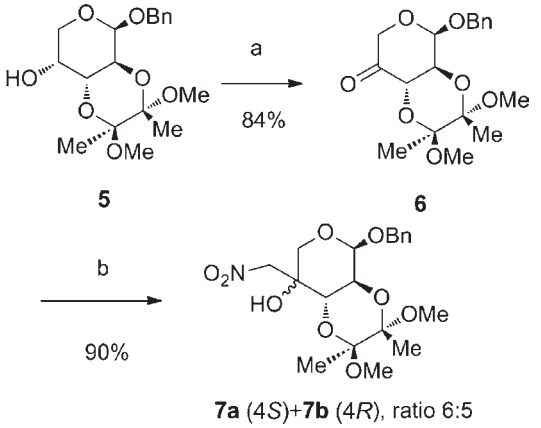


Figure 1. Nitrogen-containing glycosidase inhibitors 1-deoxynojirimycin (**1**), glucoamidine (**2**), isofagomine (**3**), and isofagomidine (**4**) and their similarity with the oxocarbenium ion intermediate.

groups are acceptable; the latter group has been shown to improve inhibition.[10] In this paper, we report the synthesis of **4** and some of its properties as a glycosidase inhibitor.

Scheme 1. Synthesis of Henry Adducts **7a** and **7b**[a]



[a] Key: (a) Dess–Martin periodinane; (b) $MeNO_2$, Et_3N.

Synthesis of **4** started with known building block **5** that can be made from inexpensive D-arabinose in two steps.[11] Alcohol **5** was oxidized to the ketone **6** with Dess–Martin periodinane giving 84% yield. The ketone **6** was then reacted with Et_3N in nitromethane giving the two diastereomeric Henry adducts **7a** and **7b** in a ratio of 6:5 and in a combined yield of 90% (Scheme 1).

The subsequent elimination/reduction was a little more tricky than anticipated. When the mixture of **7a**/**7b** was acetylated under acidic conditions (using Ac_2O and TsOH), spontaneous elimination occurred when the compound

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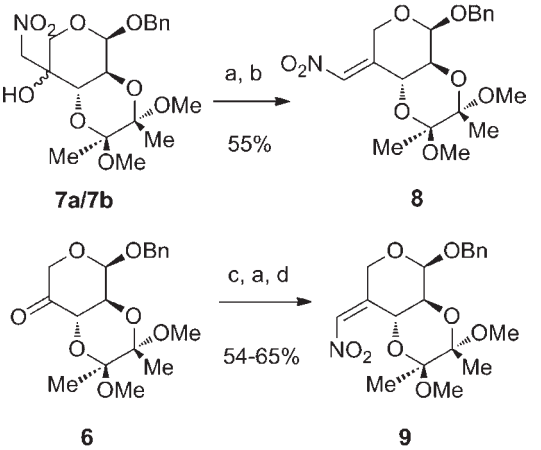
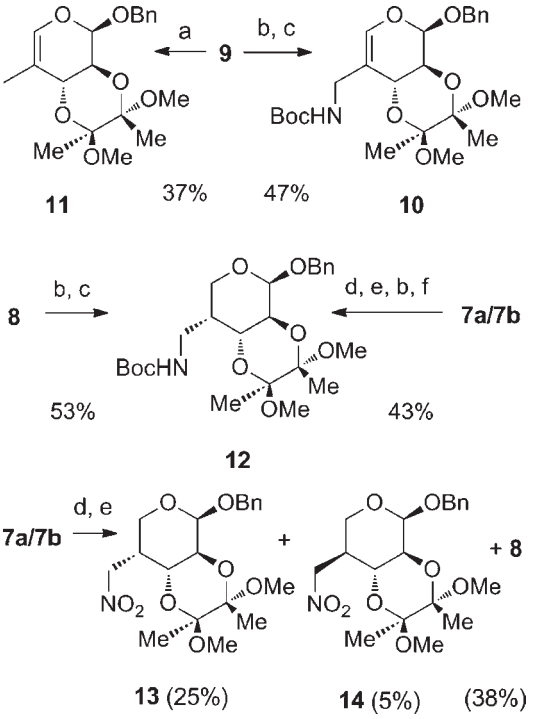
Scheme 2. Elimination Reactions from the Henry Adducts[a]



[a] Key: (a) Ac_2O/TsOH; (b) silica; (c) $MeNO_2/Et_3N$; (d) Et_3N.

came in contact with silica gel during chromatography to give the *E*-alkene **8** in 55% yield (Scheme 2). If a more conventional elimination with triethylamine was chosen the Z-alkene **9** was obtained in a more favorable yield (Scheme 2). However, only reduction of the former led to the desired product: when **9** was treated with lithium aluminum hydride conjugate reduction was not observed, but double bond migration (Scheme 3).

Scheme 3. Reduction of Nitroalkenes **8** and **9**[a]



[a] Key: (a) H_2, Pd/C, Boc_2O; (b) $LiAlH_4$; (c) Boc_2O; (d) Ac_2O, TsOH; (e) $NaBH_4$; (f) Boc_2O, Et_3N.

The nitro group was reduced, and upon Boc-protection the derivative **10** was isolated in 47% yield; reduction under hydrogenation conditions furnished **11** (37% yield).

The reduction of **8**, on the other hand, gave the desired reaction and stereoisomer. When lithium aluminum hydride was used, a 53% yield of **12** was obtained after Boc protection of the amine. Yet since the silica-promoted elimination reaction also gave a modest yield, the synthesis of **12** from **7a**/**7b** by this route had on overall yield below 30%. A problem may be that $LiAlH_4$ can reduce the nitro group before conjugate reduction has occurred. Therefore, experimentation with sodium borohydride was also done with the crucial difference here being that $NaBH_4$ does promote elimination of the acetate and subsequent conjugate addition but does not reduce the nitro group. Treatment of the acetylated **7a**/**7b** (Scheme 3) with $NaBH_4$ led to three compounds: the epimeric pair of saturated nitro derivatives **13** and **14** was obtained in a 5:1 ratio and a 30% combined yield, while the alkene **8** was obtained in 38% yield.

Compound **13** could be converted to **12** by reduction with $LiAlH_4$ and Boc protection, confirming its stereochemistry. With the rather high selectivity toward **13**, a one-pot procedure was devised from **7a**/**7b** consisting of acetylation, $NaBH_4$ treatment, $LiAlH_4$ reduction, and Boc protection (Scheme 3). This gave reproducibly **12** in 43% yield, which is significantly better than via **8**.

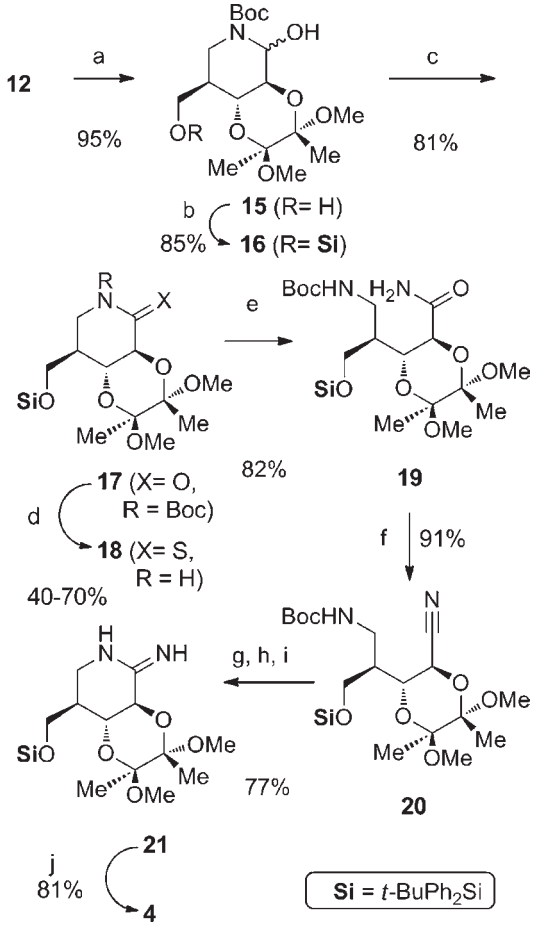
From **12**, hydrogenolysis with palladium on carbon gave the hemiaminal **15** with the Boc group on nitrogen and a yield of 95% (Scheme 4). The released C-5 hydroxyl could now be silylated with *tert*-butyldiphenylsilyl chloride and imidazole in DMF, giving silyl ether **16** in 85% yield. Oxidation of the aminal to lactam **17** was carried out with TEMPO, *m*-chloroperbenzoic acid, and tetrabutylammonium bromide in dichloromethane to give the product in 81% yield. This lactam was reacted with Lawesson's reagent, which led to formation of thiolactam with loss of the Boc group. Yields in this reaction were variable, giving from 40 to 70% of **18**.

Disappointingly, we were not able to convert **18** to amidine in a manner similar to Papandreau and Ganem.[5] Reaction of **18** with ammonia under a variation of conditions, including benzylamine, hydrazine, or hydroxylamine, led to complex mixtures apparently caused by partial opening of the lactam ring and possibly epimerization as well. Eventually, the strategy using the thiolactam **18** had to be abandoned, which was not a big loss since its synthesis was not very reliable.

Fortunately, it was found that the lactam **17** quite easily reacts with ammonia with ring-opening and formation of a primary amide. Reaction of **17** with a mixture of aqueous ammonia–dioxane gave **19** in 82% yield (Scheme 4). Dehydration of **19** using Swern conditions led to a 91% yield of nitrile **20**. Now removal of the Boc group with trifluoroacetic acid led to the aminonitrile that with Cu-catalysis cyclized to the amidine. The copper salt complexes the amidine quite powerfully but with a subsequent treatment with H_2S gas the copper is precipitated as the sulfide and removed conveniently so that the amidine product **21** can be obtained pure in 77% yield. Finally, deprotection was carried out using concentrated hydrochloric acid, which led to **4** (as the hydrochloride) in 81% yield (Scheme 4).

Partial atomic and group charges of selected atoms in isofagomine (**3**), isofagomidine (**4**), and the lactam **22** were calculated with the CHelpG procedure of the Gaussian 03 program, which fits atomic charges to the molecular electrostatic potential with constraints on the electric dipole

Scheme 4. Synthesis of Isofagomidine **4**[a]



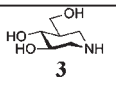
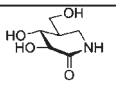
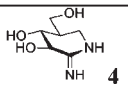
[a] Key: (a) H_2, Pd/C; (b) TBDMSCl, imidazole, DMF; (c) MCPBA, TEMPO; (d) Lawesson's reagent; (e) NH_3; (f) $(COCl)_2$, DMSO, Et_3N; (g) TFA; (h) CuCl; (i) H_2S; (j) HCl, 60 °C, 24 h.

Table 1. Atomic and Group Charges Calculated with the CHelpG Procedure at the DFT-B3LYP/6-31G Level on DFT-B3LYP/6-31G-Optimized Geometries

structure	N-1	NH_2-1	C-2	CH_2-2	N-2	NH_2-2
isofagomine (**3**)	−0.2	0.4	−0.2	0.1		
isofagomidine (**4**)	−0.5	−0.1	0.5		−0.8	0.1
lactam **22**	−0.7	−0.3	0.6		−0.6[a]	

[a] Partial change of the carbonyl oxygen in the 2-position.

Table 2. Inhibition Constants (K_i, μM) of **3**, **22**, and **4**

Enzyme	 **3**	 **22**	 **4**
β-Glucosidase,[a] pH 6.8	0.11	29	450
β-Glucosidase,[a] pH 6.8[h]	-	-	520
β-Glucosidase,[a] pH 6.8[i]	-	-	210
β-Glucosidase,[a] pH 8.0	-	-	270
α-Glucosidase,[b] pH 6.8	85.9	>250	840
α-Galactosidase,[c] pH 6.8	-	-	NI
β-Galactosidase,[d] pH 6.8	-	-	17400
β-Galactosidase,[e] pH 6.8	-	-	NI
α-Mannosidase,[f] pH 5.6	-	>250	0.75
β-Mannosidase,[g] pH 5.6	-	9[j]	280

[a] From almonds. [b] From baker's yeast. [c] From green coffee beans. [d] From *Aspergillus oryzae*. [e] From *E. coli*. [f] From jack beans. [g] From *Helix pomatia*. [h] Enzyme and inhibitor preincubated for 30 min. [i] Enzyme and inhibitor preincubated for 20 h. [j] At pH 4.0.

moment. Atomic and group charges for the atoms in the 1- and 2-positions (see Figure 1) are shown in Table 1. The calculations show that the more stable isomer of isofagomidine (**4**) has atom and group charges at N-1 that are more negative than **3** and more similar to those of the lactam **22**. The partial charge at C-2 is also similar to the lactam, but **4** has a slightly positive group charge (+0.1) for NH_2-2 contrary to that of O-2 of **22**. Thus, the compound resembles neither **3** nor **22** completely, but is more akin to the latter.

Compound **4** was tested for inhibition of seven commercially available glycosidases (Table 2), and the values were compared with structurally related isofagomine **3** and isofagomine lactam **22**. Although compound **4** quite resembles the structure of both **3** and **22**, it is clear from the data inhibitory properties of **4** is very different: Isofagomidine **4** is basically a weaker inhibitor than **3** and **22** of all the tested glycosidases except for α-mannosidase, where it is much stronger (K_i = 0.75 μM). Change in pH and incubation time (to see the role of slow onset inhibition[12]) had no major effect (Table 2).

The cause of the remarkable differences in binding between **3**, **4**, and **22** seems to be caused by the very different charge distribution in the three compounds (Table 1). The slightly positive group charge on the exocyclic amino group in **4** is the most profound difference in the charge distributions and is therefore likely to play a role.

In summary, this paper shows a successful synthesis of novel mannosidase inhibitor isofagomidine **4**. Sugar amidines of this type are rare, and our synthesis presents a new way of reaching such compounds. As an inhibitor **4** is strong versus α-mannosidase and in other ways is different from the structurally similar **3** and **22**.

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Supporting Information Available. Experimental procedures and copies of NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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