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SYNTHESIS OF AND GLYCOSIDASE INHIBITION BY α-L-HOMOFUCONOJIRIMYCIN

D.M. Andrews*, M.I. Bird*, M.M. Cunningham and P. Ward
Glaxo Group Research, Greenford Road, Greenford, Middlesex UB6 0HE, U.K.

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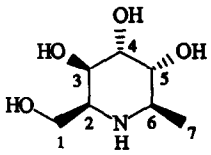
Abstract: The first synthesis of the novel azasugar α-L-homofuconojirimycin is reported. The biological activity of this compound is described and is shown to be more specific for α-L-fucosidase than compounds previously tested.

Glycosidase inhibitors based upon azasugars have attracted considerable interest as tools for the study of a number of biochemical pathways and disease states. Several groups have reported synthetic and semisynthetic approaches to azasugar inhibitors of α-L-fucosidases.[1] Fleet *et al* have reported the synthesis of 6-epi-α-L-homofuconojirimycin **1** and β-L-homofuconojirimycin **2**.[2] This paper describes the first synthesis of α-L-homofuconojirimycin **3**, a closer aza analogue of L-fucose **4** than **1** or **2**. We also report the ability of this compound to act as an inhibitor of glycosidases.



1 **2** **3** **4**

Synthesis

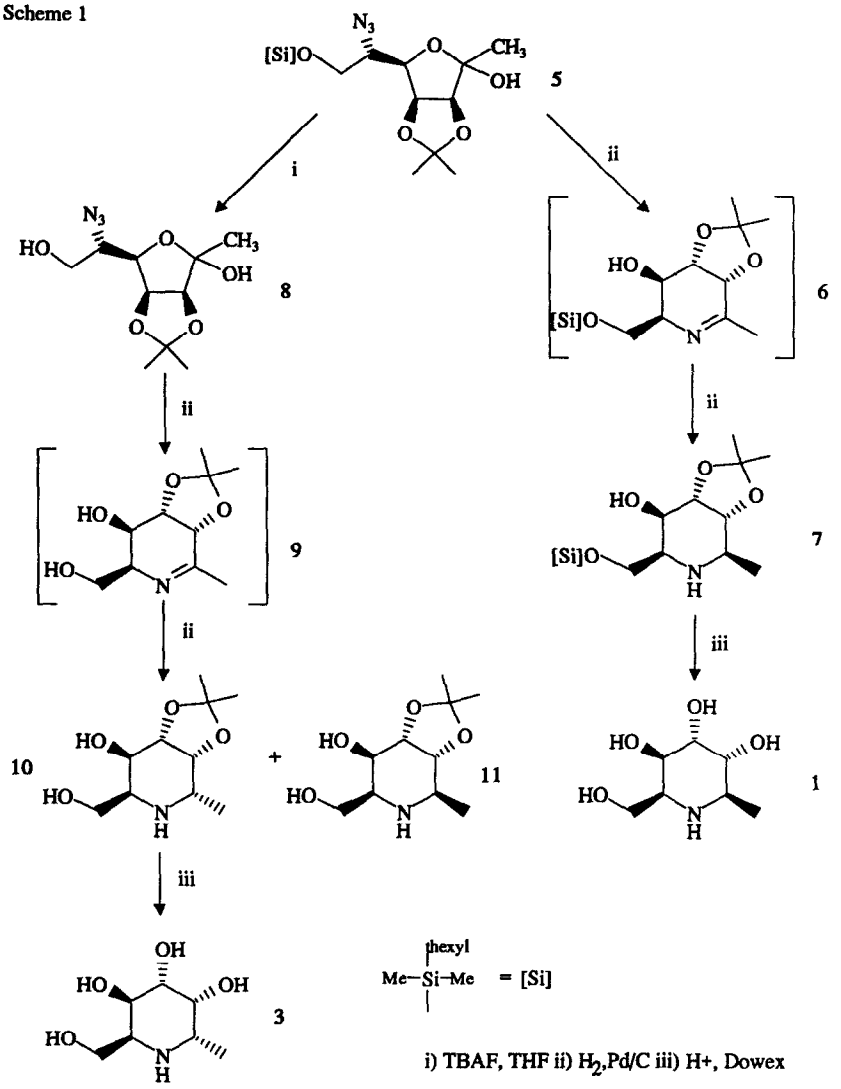
α-L-Homofuconojirimycin **3** can be obtained from the intermediate **5** which was synthesised by a route analogous to that described by Fleet.[2] This common intermediate can be used to make both **1** and **3** as shown in scheme 1.

Reduction of **5** by hydrogenation in ethyl acetate over palladium black gave **7** as the major product, confirming that the approach of hydrogen to the imine **6** is determined by the bulk of the silyl ether at C1. Initial fluoride-mediated deprotection of **5** gave the azide **8**, hydrogenation of which gave the partially protected imine intermediate **9**. In the absence of the bulky silyl ether, this transient intermediate can be hydrogenated on either face. The stereochemical outcome of the imine reduction showed considerable variation (Table 1), but the reaction proved amenable to optimisation.

Table 1: Reduction of **8** - Stereochemical outcome

Solvent	Catalyst	Ratio		
		9:	**10**:	**11**:
EtOH	Pd black	1	5	1
EtOAc	Pd black	0.5	3	1
EtOH	Pd/Carbon 5%	0.7	8	1

Scheme 1



The 400 MHz nmr of these mixtures is complex, but a reliable estimate of the ratio of products may be made by comparing the integral for the C7 protons, which resonate at 1.02ppm and 1.10ppm for **10** and **11** respectively. Table 1 illustrates the influence of solvent polarity and catalyst activity on stereochemical outcome. This suggests that in the absence of the silyl protecting group, chelation of the free hydroxyls to the catalyst, or the steric bulk of the isopropylidene ketal determines the approach of the substrate to the catalyst [3]

Removal of the isopropylidene protecting group by aqueous TFA gave **3**, which was purified by elution through Dowex 50x8-100 using 0.5 M ammonium hydroxide. This yielded the hydrochloride salt of **3** (66% from **8**). Nmr of **3** showed the coupling constants [$J_{5,6}$ = 2 Hz], indicating the equatorial-axial relationship of H-5 and H-6, compared to the hydrochloride of **1**, which shows a trans-diaxial relationship [$J_{5,6}$ = 10.7 Hz],[2] confirming the structure to be as shown.

α-L-Homofuconojirimycin **3** and deoxyfuconojirimycin were evaluated as inhibitors of the α-L-fucosidases from bovine epididymus (Sigma) and solubilised human-neutrophils. In each case, enzyme activity was linear with time for at least one hour in the absence or presence of inhibitor, and the inhibition was fully competitive with respect to the substrate 4-methylumbelliferyl-fucoside. There were no significant differences in the inhibitory properties of the two compounds (Table 2), and the Ki values for inhibition of neutrophil α-L-fucosidase were similar to those reported for inhibition of human liver α-L-fucosidase by deoxyfuconojirimycin and β-L-homofuconojirimycin[5]. Thus it is likely that the α- and β- isomers of homofuconojirimycin will have comparable inhibitory potencies for the human enzyme. It was also observed that the apparent affinity of the epididymal α-L-fucosidase for its substrate and both inhibitors was greater than for the neutrophil enzyme (Table 2).

Table 2: Inhibition of α-L-Fucosidases by α-L-Homofuconojirimycin **3** and Deoxyfuconojirimycin

Compound	Fucosidase[a]	
	Neutrophil	Epididymal
	Ki (nM)	
α-L-Homofuconojirimycin **3**	11.3 ± 0.66	5.8 ± 1.34
Deoxyfuconojirimycin	9.8 ± 0.54	6.2 ± 0.88
K_m value for 4-MU-Fucoside (mM)	0.25 ± 0.01	0.05 ± 0.01

[a] Assays were conducted at pH 5.5 at 30°C with 4-methylumbelliferyl-fucoside (4-MU-fucoside) as substrate. Similar results were obtained when using *p*-nitrophenyl-fucoside as a substrate.

Glycosidase inhibition by α-L-homofuconojirimycin **3** was highly selective for α-L-fucosidase. At 1mM, no inhibition of α-D-mannosidase, β-D-galactosidase or neuraminidase was observed (Table 3). However, the compound **3** weakly inhibited (IC_{50} = 10mM) the α1,3-fucosyltransferase from human amniotic fluid, when assayed with 10mM *N*-acetyllactosamine as substrate. In addition, there was no synergy of inhibition of the latter enzyme in the presence of 50μM GDP, as has been reported for two 5-member azasugars which inhibit a recombinant human α1,3-fucosyltransferase[6]. Interestingly, α-L-homofuconojirimycin **3** did not inhibit the α 1,3-fucosyltransferase extracted from solubilised human-neutrophils.

Table 3: Specificity of α-L-Homofuconojirimycin **3** as an Inhibitor of Glycosidases

Glycosidase[a]	Inhibitor	% Inhibition at 1mM
α-L-Fucosidase (Bovine epididymis)	α-L-homofuconojirimycin	99
	Deoxyfuconojimycin	99
α-D-Mannosidase (Jack Bean)	α-L-homofuconojirimycin	0
	Deoxymannojirimycin	83
β-D-Galactosidase (Type VII, Jack Bean)	α-L-homofuconojirimycin	0
	Deoxygalactonojirimycin	74
Neuraminidase (Type X, *C. perfringens*)	α-L-homofuconojirimycin	0
	2,3-dehydro-2-deoxy N- acetyl neuraminic acid	50

[a]The glycosidases (all ex Sigma) were assayed with their respective 4-methylumbelliferyl-glycoside substrates.

Conclusions

6-Epi-α-L-homofuconojirimycin **1** and β-L-homofuconojirimycin **2** have been shown to be weak inhibitors of other glycosidases.[5] α-L-Homofuconojirimycin **3** is a highly selective inhibitor for α-L-fucosidase, and may complement **1** and **2** as a useful biochemical tool. The configuration of the methyl substituent on C-6 confers specificity to the inhibitor but its potency is unchanged relative to deoxyfuconojirimycin and the other fuconojirimycin analogues.

References and Notes

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4 **3**: ^{1}H NMR (400 MHz, DMSO-d_6/D_2O, δ in ppm, coupling constants J in Hz): 3.38, 3.48 (m, H-1), 2.87 (m, $J_{1,2}$ 6.5, $J_{2,3}$ 2, H-2), 3-5-3.8 (m, H- 3,4,5), 2.96 (m, $J_{6,7}$ 7, $J_{5,6}$ 2, H-6), 1.01 (d, $J_{6,7}$ 7, H-7),. m.p. 160-170 ^{o} (dec.), $[\alpha]^{20}_D$ -28^o (*c*, 1.0, $CHCl_3$), acc. mass: calc. 178.1079; found 178.1071

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