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IMINOHEPTITOLS AS GLYCOSIDASE INHIBITORS: SYNTHESIS OF, AND MANNOSIDASE AND FUCOSIDASE INHIBITION BY, α-HOMOMANNOJIRIMYCIN AND 6-EPI-HOMOMANNOJIRIMYCIN

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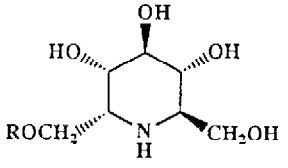
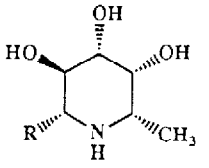
C-2 and C-6 of a heptonolactone are joined together by nitrogen to afford α-homomannojirimycin (HMJ) and 6-epi-α-homomannojirimycin; the inhibition of some α-mannosidases and α-fucosidases by these iminoheptitols is reported.

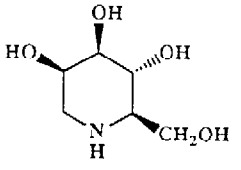
Iminoheptitols, such as α-homonojirimycin (1), may constitute a general class of glycosidase inhibitors in which it is possible to use the anomeric substituent to obtain additional potency and/or specificity in comparison to the corresponding azahexoses that lack such a substituent; for example, the β-glucopyranosyl derivative (2)[1,2] of α-homonojirimycin (1), the first example of a naturally occurring azaheptose,[3] is a powerful α-glucosidase inhibitor and is a drug candidate for antidiabetic therapy.[4] β-Homofuconojirimycin (3)[5] is as powerful an inhibitor of human liver α-fucosidase as is deoxyfuconojirimycin (4).[6] Deoxymannojirimycin (DMJ) (5) is a major biochemical tool for the investigation and inhibition of mannosidases of glycoprotein processing,[7] although it is a relatively weak inhibitor of α-mannosidases in general; it has been suggested that DMJ may attenuate the infectivity of HIV-1[8] due to its inhibition of processing mannosidases and molecular graphics studies have been reported in an attempt to design mannosidase inhibitors as potential anti-HIV agents.[9] In fact, DMJ is a better inhibitor of α-fucosidases than of α-mannosidases.[10] This is presumably due to the correspondence of the stereochemistry of the hydroxyl functions at C-2, C-3 and C-4 of DMJ with those in both mannose and fucose and the less stringent structural requirementsfor the inhibition of α-fucosidase than of α-mannosidase.[6] α-Homomannojirimycin (HMJ) (6) may be a more specific inhibitor of mannosidase than is DMJ (5), because of the additional interaction of the anomeric substituent with the active site of α-mannosidases. Furthermore, HMJ (6) should be a relatively weak inhibitor of α-fucosidases since the configurations at C-2 and C-6 are incorrect in relation to α-L-fuco configuration; the presence of the polar hydroxymethyl - rather than a methyl - substituent at C-6 should also decrease fucosidase inhibition.[6]



(1) R = H (2) R = β-glucopyranosyl



(3) R = CH_2OH (4) R = H



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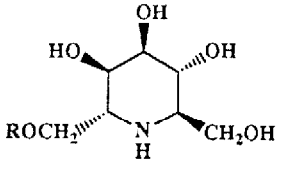
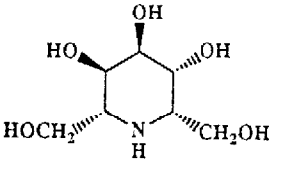


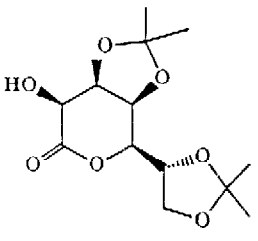
(6) R = H (7) R = linked mannose



(8)

Additionally, HMJ (6) should allow the preparation of a number of α-mannosyl derivatives (7) which might allow differential inhibition of the different mannosidases of glycoprotein processing, depending on the nature of the link to the mannose residue. This paper reports the synthesis of HMJ (6) and of 6-epi-HMJ (8)[11] from the readily available azidolactone (10),[12] and compares the inhibition of human liver mannosidases and fucosidases by DMJ, HMJ and 6-epi-HMJ.

Synthetic work. Carbohydrates are almost certain to be, at present, the starting materials of choice for the synthesis of homonojirimycin and its analogues such as HMJ which contain five adjacent chiral centres and seven adjacent carbon atoms bearing functional groups. Previous syntheses of this class of compounds,[1,2,5] begin with hexose derivatives and add the additional carbon atom relatively late in the synthesis; this paper starts with a protected heptonolactone (9)[10] in which the nitrogen may readily be introduced at C-2. The protected heptonolactone(9), in which only the C-2 hydroxyl group is free, is readily available from the Kiliani reaction on diacetone mannose. Esterification with triflic anhydride, followed by treatment with sodium azide, results in overall displacement at C-2 with *retention of configuration* to give the azido-lactone (10) in 76% yield.[10] Hydrolysis of the side chain acetonide in (10) by 80% aqueous acetic acid gave the diol (11),[13] m.p.126°-127°C [94% yield] which, with *tert*-butyldimethylsilyl chloride in dimethylformamide in the presence of imidazole, afforded the silyl ether (12), m.p. 138°-139°C, $[\alpha]_D^{20}$ +109.6° (*c*, 0.99 in $CHCl_3$), (79% yield). The silyl ether (12) is a divergent intermediate for both the synthesis of HMJ (6) and of 6-epi-HMJ.



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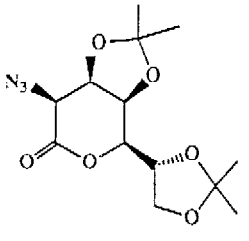

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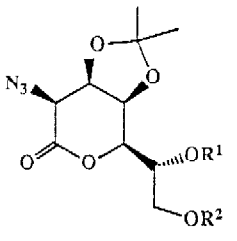
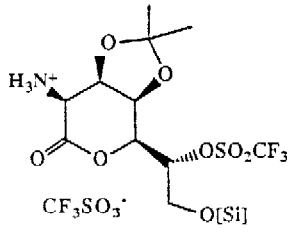


(11)$R^1=R^2=H$
(12)$R^1=H;\ R^2=[Si]$
(13)$R^1=O_2SCF_3;\ R^2=[Si]$

[Si] = Me_2Bu^tSi



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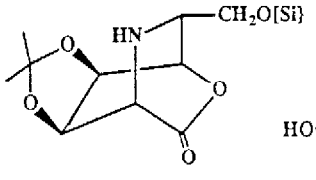

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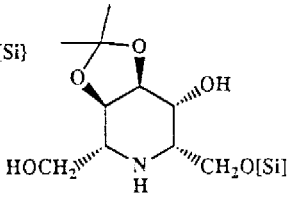

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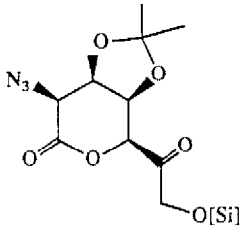
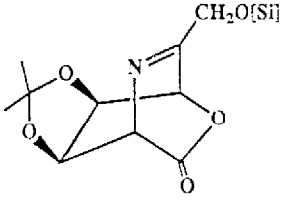


(17)



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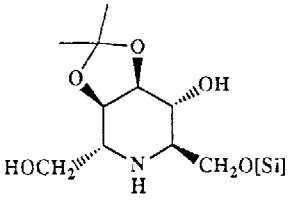

(19)

For the synthesis of 6-epi-HMJ (8), the piperidine ring is constructed by joining the nitrogen function at C-2 in (12) to C-6, with inversion of configuration at C-6. Treatment of (12) with trifluoromethanesulphonic anhydride in the presence of pyridine in dichloromethane at -20°C gave the triflate (13), m.p. 79°-80°C [95% yield]. Hydrogenation of the azidotriflate (13) in ethyl acetate in the presence of 10% palladium on carbon in the presence of sodium acetate resulted in reduction of azide to the corresponding amine followed by easy cyclisation to the bicyclic piperidine (15), waxy solid, $[\alpha]_D^{20}$ -15.4° (*c*, 1.2 in $CHCl_3$) in 96% yield; when the hydrogenation of (13) was carried out in the absence of sodium acetate, the bicyclic amine (15) was formed in 52% yield, together with the aminotriflate triflate salt (14), m.p. 77°-79°C, $[\alpha]_D^{20}$ +34.7° (*c*, 0.63 in $CHCl_3$), in 43% yield. Reduction of the bicyclic lactone (13) with lithium aluminum hydride in tetrahydrofuran gave the protected iminoheptitol (16), m.p. 112°-114°C, $[\alpha]_D^{20}$ +52.7° (*c*, 1.0 in $CHCl_3$), in 54% yield. Removal of the protecting groups from (16) by treatment with aqueous trifluoroacetic acid gave, after purification by ion exchange chromatography, 6-epi-HMJ (8),[14] $[\alpha]_D^{20}$ +26.4° (*c*, 0.5 in H_2O) as a very hygroscopic solid in 85% yield [42% overall yield from (12)]; the corresponding hydrochloride of (8),[15] m.p. 203°-205°C, $[\alpha]_D^{20}$ +31.1° (*c*, 1.0 in H_2O), is an easily crystallised solid.

The synthesis of HMJ (6) from the silyl ether (12) requires the formation of the piperidine ring with retention of configuration at C-6. Oxidation of the secondary alcohol function in (12) with pyridinium chlorochromate in dichloromethane gave the ketone (17), m.p. 120°-122°C, $[\alpha]_D^{20}$ +5.4° (*c*, 1.0 in $CHCl_3$), in 74% yield. Reduction of the ketoazide (17) with triethylphosphite[16] gave an intermediate aza-ylid which underwent an intramolecular aza-Wittig reaction[17] to give the bicyclic imine (18), oil, $[\alpha]_D^{20}$ +98.3° (*c*, 1.0 in $CHCl_3$) in 89% yield. Reduction of the imine (18) by lithium borohydride in tetrahydrofuran gave predominant reduction from the less hindered face of the carbon-nitrogen double bond[18] to afford the protected HMJ (19), m.p. 165°-166°C, $[\alpha]_D^{20}$ -28.5° (*c*, 1.0 in $CHCl_3$) [46% yield], together with a small amount of the C-6 epimer (16) [2% yield]. Removal from (19) of all the protecting groups by aqueous trifluoroacetic acid gave HMJ (6)[19] as a very hygroscopic solid, $[\alpha]_D^{20}$ +7.45° (*c*, 0.55 in H_2O), in 92% yield [28% overall yield from (12)]; the hydrochloride of HMJ is also a hygroscopic solid.

TABLE % Inhibition of liver α-fucosidase and α-mannosidase

Inhibitor (1 mM)	α-mannosidases			α-fucosidase
	lysosomal	Golgi II	Neutral	
deoxymannojirimycin (5)	58%	45%	21%	91% [Ki 5.0μM]
α-homomannojirimycin (6)	49%	56%	30%	29%
6-epi-homomannojirimycin (8)	0%	0%	0%	96% [Ki 4.5μM]

Glycosidase Inhibition Studies. The iminoheptitols (6) and (8) were assayed as inhibitors of 14 human liver glycosidases and the effects compared with those of DMJ (5) [TABLE].[20] As would be predicted from their structures, only α-mannosidases and α-fucosidases were inhibited. The specificity and potency of inhibition of human α-mannosidases by HMJ (6) and DMJ (5) are very similar. Neither compound inhibited β-mannosidase. 6-epi-HMJ (8) did not inhibit any α-mannosidase, indicating that the correct configuration at

C-5 is essential for the inhibition of α-mannosidases.[6] In contrast, both DMJ (5) and 6-epi-HMJ (8) were powerful inhibitors of α-fucosidase, whereas HMJ (6) is only a weak inhibitor of this enzyme. The relative potencies of these compounds as fucosidase inhibitors may be understood by considering them as analogues of α-L-fucose; all three compounds have the correct chirality of the secondary hydroxyl groups - the minimum requirement for inhibition of α-fucosidase. However, their relative effectiveness as α-fucosidase inhibitors is determined by the stereochemistry of the substituents at C-2 and C-6; while DMJ (5) and 6-epi-HMJ (8) have only one substituent with the wrong configuration relative to α-L-fucose, both substituents at C-2 and C-6 of HMJ (6) are different from those in α-L-fucose. All the compounds are weaker inhibitors of α-fucosidase than is deoxyfuconojirimycin, since they all lack a lipophilic methyl substituent with the correct configuration.[6]

Thus HMJ (6) is a more selective inhibitor of α-mannosidases than DMJ (5). Its effect on the processing α-mannosidases using natural substrates remains to be investigated. The enhanced specificity of HMJ (6), relative to DMJ and the possibility of the formations of α-1,2-, α-1,3- and α-1,6-mannosyl derivatives attached to the anomeric hydroxymethyl group should make this a valuable compound for exploring the function and specificity of the processing mannosidases. In summary, this paper demonstrates the use of the readily available heptonolactone (9) in the synthesis of highly functionalised compounds and further indicates the potential of iminoheptitols as glycosidase inhibitors.

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[11] The respective systematic names for α-homomannojirimycin (6) and 6-epi-α-homomannojirimycin (8) are 2,6-dideoxy-2,6-imino-D-*glycero*-D-*talo*-heptitol and 2,6-dideoxy-2,6-imino-L-*glycero*-D-*talo*-heptitol.
[12] Bruce, I., Fleet, G. W. J., Girdhar, A., Haraldsson, M., Peach, J. M., Watkin, D. J., *Tetrahedron*, in press.
[13] Physical data for all new compounds reported in this paper was consistent with the structures proposed and satisfactory microanalytical data (CHN) was obtained for the following compounds: (6) [as the hydrochloride], (11) to (19) inclusive.
[14] ^{13}C NMR of 6-epi-HMJ (8) as free base (D_2O): δ 54.6 and 56.0(2 x d, C-2 and C-6), 62.1(two overlapping t, C-1 and C-7), 66.8, 70.1 and 71.7 (3 x d, C-3, C-4 and C-5).
[15] ^{13}C NMR of 6-epi-HMJ (8) as hydrochloride (D_2O): δ 56.1 and 56.4(2 x d, C-2 and C-6), 58.3 and 59.1 (2 x t, C-1 and C-7), 63.5, 67.4 and 69.4(3 x d, C-3, C-4 and C-5).
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[19] ^{13}C NMR of HMJ (6) as free base (D_2O): δ 56.2 and 59.0(2 x d, C-2 and C-6), 59.6 and 61.3(2 x t, C-1 and C-7), 68.9, 69.4 and 72.2 (3 x d, C-3, C-4 and C-5).
[20] For assay methods, see Daher, S. A., Fleet, G., Namgoong, S. K., Winchester, B., *Biochem. J.*, **258**, 613 (1989).

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