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Eight Stereoisomers of Homonojirimycin from D-Mannose

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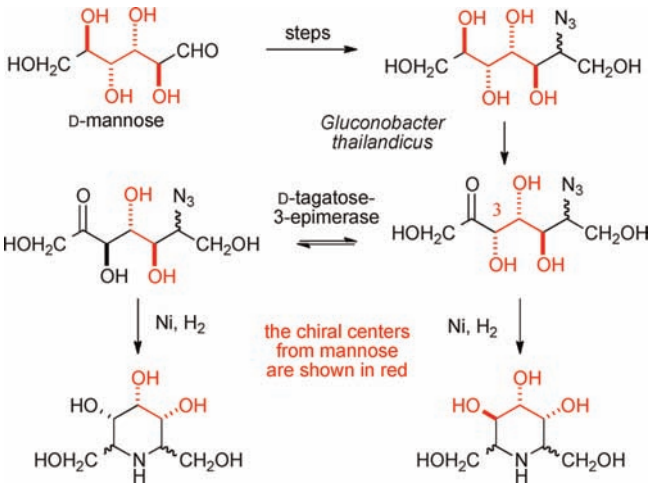
ABSTRACT



Although there are 32 6-azidoheptitols, there are only 16 homonojirimycin (HNJ) stereoisomers. Two epimeric azidoalditols derived from D-mannose allow the synthesis in water of eight stereoisomers of HNJ.

Homonojirimycin (HNJ) **1** is a seven carbon imino-sugar[1] which was made[2] before it was recognized as a natural product from *Omphalea diandra* (Euphorbiaceae).[3] Many of the stereoisomers of HNJ, as well as corresponding glycosides, have subsequently been isolated from a wide range of plants[4] and synthesized by many different routes.[5]

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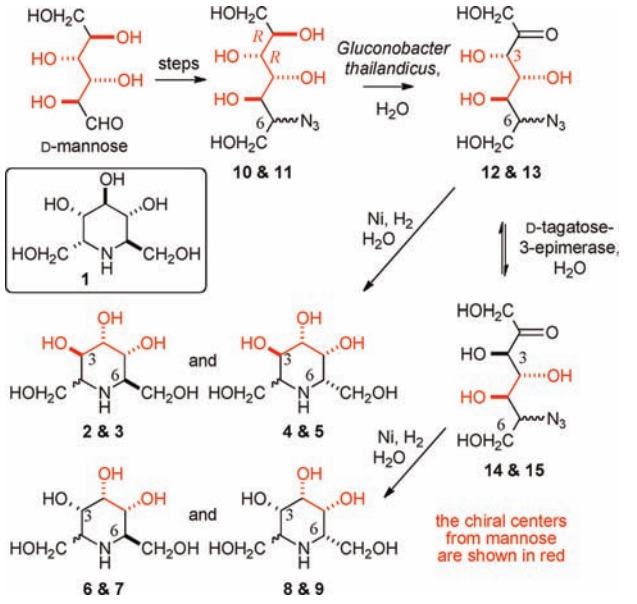
This paper describes the synthesis of eight (**2**–**9**) of the 15 diastereomers of HNJ from D-mannose by a divergent biotechnological strategy in water that depends on the isomerization of monosaccharides by the techniques of Izumoring [Scheme 1].[6]

Chain extension and functional group manipulation allowed the conversion of D-mannose to the two epimeric 6-azido-heptitols **10/11** in which all the chiral centers are preserved. *Gluconobacter thailandicus* specifically recognizes a terminal diol stereochemical motif with an *R,R* configuration adjacent to the terminal carbon and thus allowed the oxidation of **10/11** to the azidoketoses **12/13**. D-Tagatose-3-epimerase (DTE) is a promiscuous enzyme that epimerizes C3 of a ketose; there is no ketose so far reported that is not a substrate for DTE. DTE equilibrated the azidoketoses **12/13** with their C3 epimers **14/15**. Hydrogenation of each of the four azidoketoses by Raney nickel resulted in reduction of the azide to the corresponding amine which underwent intramolecular reductive amination to give two epimeric HNJs.

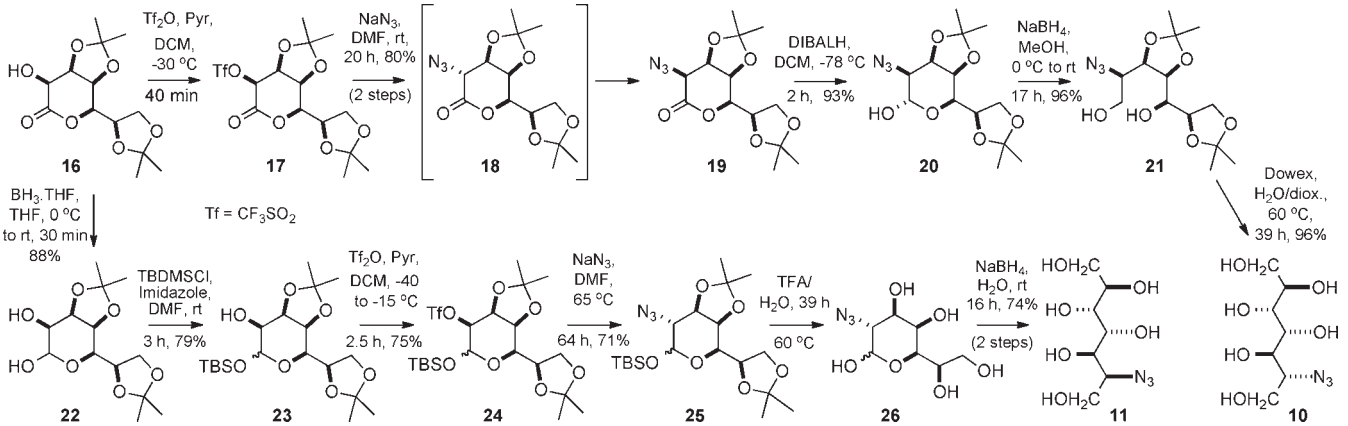
The seven carbon lactone diacetonide **16**, which may be prepared on a scale up to 10 kg by a Kiliani reaction on diacetone mannose,[7] is a key intermediate for the synthesis of the epimeric 6-azido-heptitols **10/11** [Scheme 2]. Esterification of the free alcohol in **16** with triflic anhydride afforded the crystalline triflate **17**; reaction of **17** with sodium azide in DMF resulted in nucleophilic substitution with retention to give **19** in an overall yield of 80% for the two steps.[8] The all *cis*-azide **19** is more stable than the epimeric azidolactone **18**;[9] it was not possible to readily isolate **18** from reaction of **17** with sodium azide. Reduction of the base-sensitive azidolactone **19** with DIBALH in dichloromethane gave the lactol **20**[10] [93% yield] which on further reduction with sodium borohydride in methanol afforded the protected diol **21** [96% yield]. Removal of the acetonides in **21** by acid hydrolysis with Dowex in water gave the azidoheptitol **10** [mp 136–138 °C, $[\alpha]_D^{25}$ +10.9 (*c* 1.0, H_2O)] in an overall yield of 69% from **16**. For the epimer **11**, the lactone **16** was reduced by THF/borane to give the lactol **22** [88% yield] in which the anomeric hydroxyl group was protected as the corresponding TBDMS ether **23** [79%]. Esterification of the hydroxyl group at C2 of **23** with triflic anhydride gave the triflate **24** [75%] which, with sodium azide in DMF, resulted in nucleophilic substitution with inversion to give **25** [71%]. Removal of the silyl ether and diacetonide groups in **25** by aqueous trifluoroacetic acid gave the unprotected lactols **26**; reduction of **26** with sodium borohydride in water gave the azidoheptitol **11** [mp 159–160 °C, $[\alpha]_D^{25}$ −8.5 (*c* 1.0, H_2O)] in an overall yield of 27% from **16**.

Oxidation of the (*R,R*) stereochemical motif adjacent to the terminal carbon in the epimeric azidoheptitols **10** and **11** by *Gluconobacter thailandicus* gave complete conversion to the azidoketoses **12** {$[\alpha]_D^{25}$ +21.7 (*c* 0.4, H_2O)} and **13** {$[\alpha]_D^{25}$ +25.7 (*c* 1.0, H_2O)} respectively [Scheme 3].

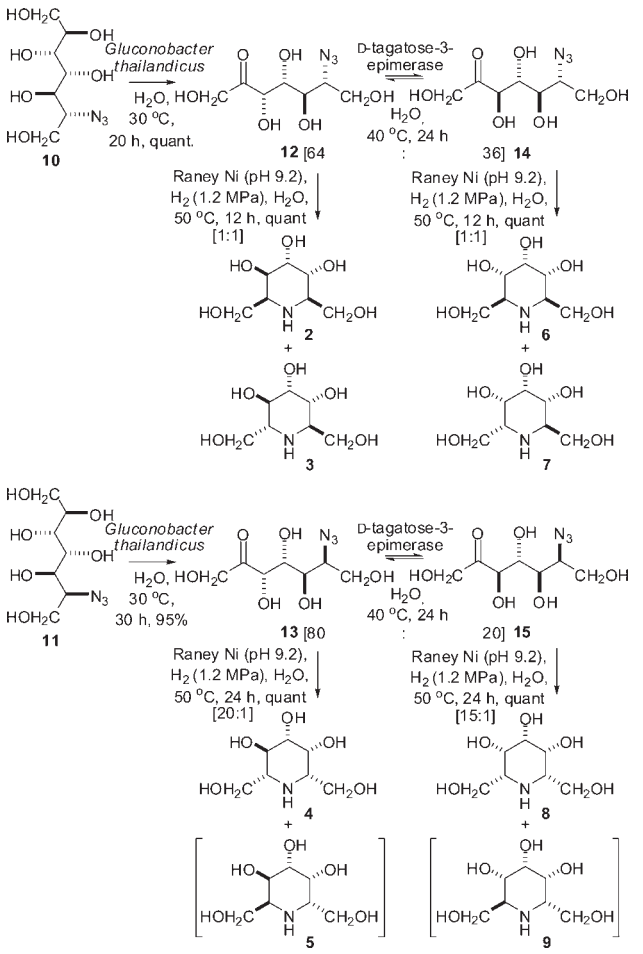
Scheme 1. Summary of Synthesis of HNJ Stereoisomers



Scheme 2. Synthesis of Azidoheptitols



Scheme 3. Biotechnological Synthesis of HNJ Stereoisomers



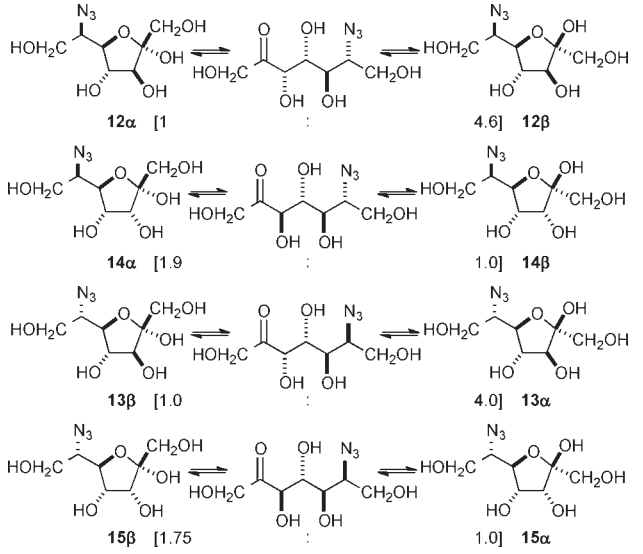


Figure 1. Ratio of anomers of azidoketoses.

Reaction of DTE with **12** caused equilibration with the C3 epimer **14** $\{[\alpha]_D^{25}$ +31.0 (*c* 0.7, H_2O)$\}$ in a ratio of 64:36. Similarly DTE caused equilibration of **13** with **15** $\{[\alpha]_D^{25}$ +22.3 (*c* 1.0, H_2O)$\}$.

Each of the azidoketoses is predominantly an equilibrium mixture of the α and *β* furanose anomers,[11] with very minor amounts (<2%) of other forms [Figure 1]. As shown in Figure 2, **12** exists in an α:*β* ratio of 1.0:4.6, **14**

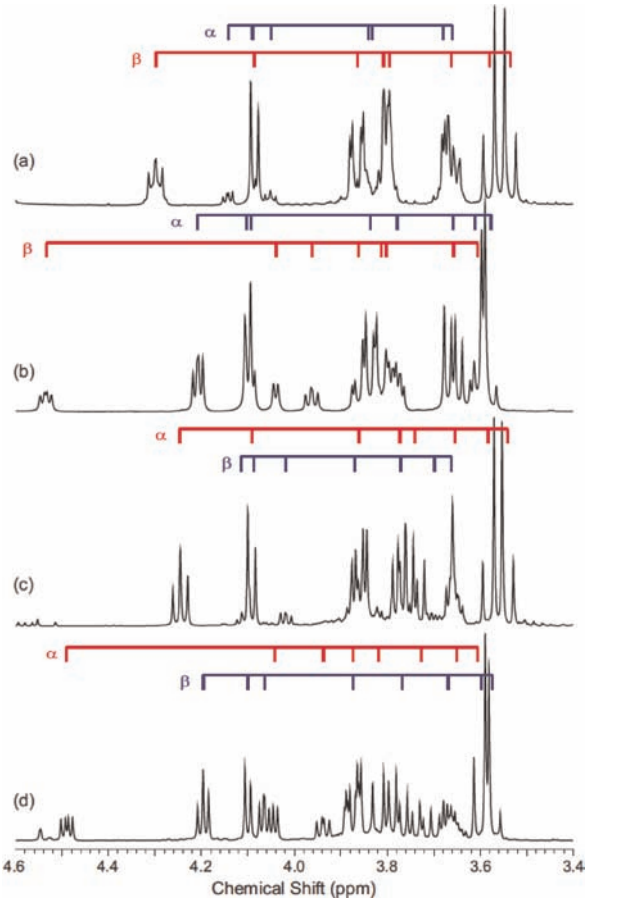


Figure 2. ^{1}H NMR spectra of azidoketoses (500 MHz) in D_2O: (a) **12**, (b) **14**, (c) **13**, (d) **15**. Full NMR analysis is reported in Supporting Information.

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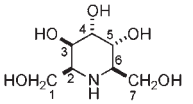
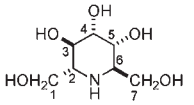
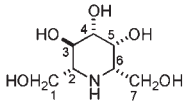
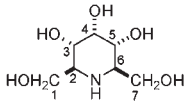
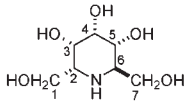
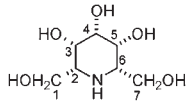
Table 1. Properties of HNJ Diastereomers[a]

compound	 **2**	 **3**	 **4**	 **6**	 **7**	 **8**
$[\alpha]_D^{25}$	+44.5 (*c* 0.85, H_2O) [Lit.[5a] +41.9 (*c* 0.43, H_2O)]	+4.3 (*c* 0.44, H_2O) [Lit.[5a] +4.3 (*c* 0.62, H_2O)]	-4.2 (*c* 0.27, MeOH) [Lit.[5e] -4.3 (*c* 1.3, MeOH)]	+0.1 (*c* 0.70, H_2O)	+27.7 (*c* 0.75, H_2O)	-0.1 (*c* 0.44, H_2O)
m.p.	140-144 °C	syrup	syrup	210-212 °C	syrup	>340 °C
δ_C (D_2O, 125 MHz) **C1**	62.1	61.7	61.7	62.1	60.5	62.2
C2	55.9	56.3	60.7	55.3	55.9	59.5
C3	66.7	69.2	69.2	69.4	70.0	70.3
C4	71.6	72.4	75.7	72.5	69.9	70.6
C5	70.0	69.8	69.6	69.4	69.8	70.3
C6	54.4	59.2	58.5	55.3	54.9	59.5
C7	62.1	60.0	62.0	62.1	61.3	62.2
δ_H (D_2O, 500 MHz) **H1**	3.71 (dd, *J* 5.5, 11.7)	3.73 (dd, *J* 5.6, 11.5)	3.72 (dd, *J* 5.5, 11.6)	3.65 (dd, *J* 6.2, 11.7)	3.72 (dd, *J* 4.6, 11.5)	3.67 (dd, *J* 6.9, 11.0)
H1′	3.80 (dd, *J* 3.0, 11.6)	3.78 (dd, *J* 3.2, 11.6)	3.82 (dd, *J* 3.0, 11.6)	3.84 (dd, *J* 3.0, 11.6)	3.83 (dd, *J* 8.2, 11.5)	3.72 (dd, *J* 6.5, 11.0)
H2	2.86 (ddd, *J* 3.1, 5.4, 10.5)	2.71 (ddd, *J* 3.3, 5.4, 8.8)	2.54-2.58 (m)	2.86 (ddd, *J* 3.0, 6.1, 10.3)	3.04 (a-dt, *J* 4.3, 8.4)	2.80 (a-dt, *J* 1.1, 6.7)
H3	3.76 (dd, *J* 3.2, 10.6)	3.62 (a-t, *J* 9.1)	3.57 (a-t, *J* 9.8)	3.47 (dd, *J* 2.9, 10.4)	3.89-3.91 (m)	3.95 (dd, *J* 1.1, 3.1)
H4	3.99 (a-t, *J* 3.5)	3.66 (dd, *J* 3.0, 9.1)	3.53-3.55 (m)	4.09 (t, *J* 2.9)	3.89 (a-t, *J* 2.9)	3.65 (t, *J* 3.1)
H5	3.92 (dd, *J* 1.5, 3.7)	4.00 (a-t, *J* 2.8)	4.00 (dd, *J* 1.4, 2.7)	3.47 (dd, *J* 2.9, 10.4)	3.67 (dd, *J* 2.6, 5.9)	3.95 (dd, *J* 1.1, 3.1)
H6	3.06 (a-dt, *J* 1.5, 6.6)	3.12 (ddd, *J* 2.7, 6.8, 7.8)	2.84 (dt, *J* 1.4, 6.7)	2.86 (ddd, *J* 3.0, 6.1, 10.3)	3.10 (a-dt, *J* 5.0, 6.9)	2.80 (a-dt, *J* 1.1, 6.7)
H7	3.62 (dd, *J* 6.7, 11.2)	3.67 (dd, *J* 6.7, 11.7)	3.63 (dd, *J* 6.7, 11.2)	3.65 (dd, *J* 6.2, 11.7)	3.63 (dd, *J* 7.6, 11.5)	3.67 (dd, *J* 6.9, 11.0)
H7′	3.66 (dd, *J* 6.5, 11.2)	3.73 (dd, *J* 7.9, 11.6)	3.67 (dd, *J* 6.7, 11.2)	3.84 (dd, *J* 3.0, 11.6)	3.77 (dd, *J* 4.8, 11.5)	3.72 (dd, *J* 6.5, 11.0)

[a] a-t = apparent triplet; a-dt = apparent double triplet.

in an α:*β* ratio of 1.9:1.0, **13** in an α:*β* ratio of 4.0:1.0, and **15** in an α:*β* ratio of 1.0:1.75.

Hydrogenation of the azidoketoses in the presence of Raney nickel in each case caused reduction of the azide to the corresponding amine, followed by intramolecular amination to give iminoheptitols; examination of the crude product from the reduction showed the clean formation of two iminosugars. The reduction of azidoketoses **12** [to **2** and **3**] and **14** [to **6** and **7**] showed no stereoselectivity in the reduction of the intermediate imine. In contrast, hydrogenation of **13** gave **4** together with **5** in a 20:1 ratio. A similar highly stereoselective reduction of **15** gave **8** and **9** in a ratio of 15:1.

The stereoselectivity in the cyclic reductive aminations may be rationalized by the arguments of Wong;[12] greater selectivity was found with synergy in steric hindrance and torsional strain, with the least selectivity where these effects were opposed. The crude reaction mixtures were separated by ion exchange chromatography by a combination of Dowex 1 × 2 (OH^- form) and Amberlite CG-50(NH_4^+ form) to afford six iminoheptitols for which the properties are reported in Table 1. It was not possible to obtain pure samples of iminoheptitols **5** and **9** formed as the minor products from the hydrogenation of **13** and **15**, respectively; further investigations for the separation of the iminoheptitols is in progress.

In summary, biotechnological syntheses of eight stereoisomers of HNJ from D-mannose are described; a similar sequence from D-glucose, which should provide the other eight stereoisomers, is under investigation.

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Supporting Information Available. Experimental procedures and full spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.