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Chemo-Enzymatic Synthesis of New Protected Aldoketoses: Intermediates in the Biosynthesis and Chemical Synthesis of Nojirimycin and Mannojirimycin

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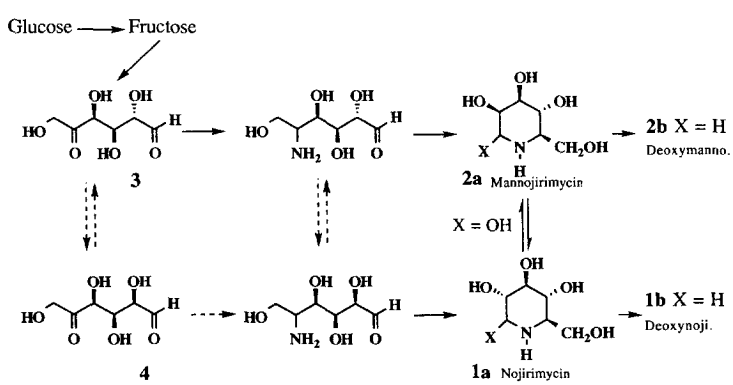
Abstract: Condensation of dihydroxy acetone phosphate (DHAP) on new hydroxy-aldehydes catalysed by fructose-1,6-diphosphate aldolases provides two aldoketoses presumed intermediates in the biosynthesis of nojirimycin and mannojirimycin, as well as useful precursors for the chemical synthesis of these aminosugars and some analogues.

In recent years, polyhydroxylated piperidines and pyrrolidines have emerged as potential therapeutic agents due to their ability to inhibit glycosidases.[1] They are active against disorders related to carbohydrate metabolism such as diabetes and obesity.[2] They also display significant activity against viral (including HIV) expression[3] and tumour growth.[4]

Of these compounds, the natural alkaloids nojirimycin **1a** and mannojirimycin **2a**, together with their 2-deoxy derivatives **1b** and **2b** have been especially studied. A number of chemical syntheses of **1** and **2** have been recently published, most of them starting from carbohydrate precursors[5]. Chemo-enzymatic strategies have also been described, often based on an aldolase-catalysed aldol reaction to generate the chirality.[6a,b]

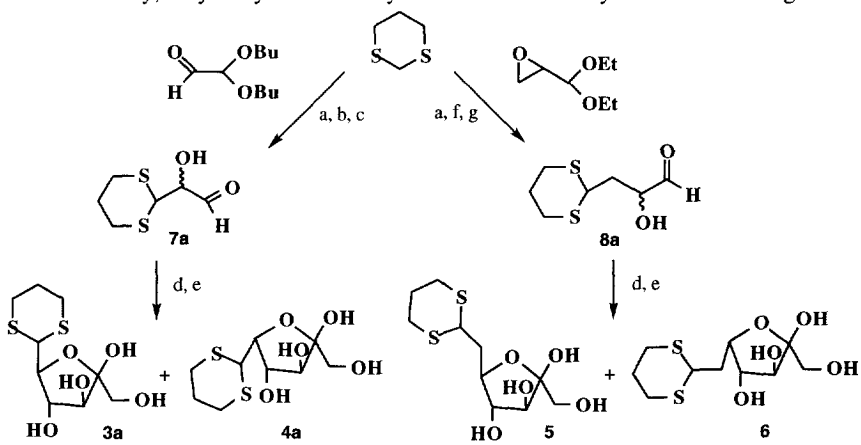
1 and **2** were isolated from several strains of *Streptomyces*, *Bacillus*[7] and from plants.[8] In a recent study of their biosyntheses in *Streptomyces subrutilus*, the biosynthetic pathway shown in scheme 1 was suggested[9]: intermediate 6-deoxy-6-oxo-D-fructose **3** coming from fructose directly would yield mannojirimycin. Fructose should also be the precursor of nojirimycin, the necessary epimerisation at C5 occuring on mannojirimycin itself or one of its precursors.



Scheme 1 Biosynthetic pathway of nojirimycin and mannojirimycin

Such dioxo compounds can lead to piperidines by reductive amination. Actually, **4** is also the precursor of **1b** in one chemical synthesis: reductive amination of **4** with benzhydrylamine in the presence of cyanoborohydride, followed by hydrogenolysis leads to **1b** in 63 % yield.[10]

Due to the potential interest of aldoketoses such as **3** and **4** and related structures, we decided to develop a new route for their synthesis based on aldolase catalysis. We report here a fructose-1,6-diphosphate aldolase-based synthesis of **3** and **4** in a protected form (**3a** and **4a**) and of two homologues (**5** and **6**) (scheme 2).[11] Fructose-1,6-diphosphate aldolase catalyses the condensation of DHAP to various aldehydes, affording ketose-1-phosphates with the 3S,4R configuration.[12,13] Reaction of racemic 2-hydroxy-malonaldehyde in a suitably protected form should produce a mixture of **3** and **4** since aldolase is not enantioselective towards its aldehyde substrate. In the same way, 2-hydroxy-succinaldehyde should allow the synthesis of homologues of **3** and **4**.



Scheme 2 a) *n*-BuLi, THF, -78 °C -> -25 °C, 2 hours; b) aldehyde ($(BuO)_2CHCHO$), -70 °C 2 hours -> -50 °C 1 hour -> r. t.; c) HCl-KCl buffer pH 1 / ethanol 80/20, 70 °C, 12 hours; d) DHAP, fructose-1,6-diphosphate aldolase pH 6.8, r. t.; e) acid phosphatase pH 4.7, 37 °C, 2 days; f) epoxide, -50 °C -> -35 °C 3 hours -> r. t.; g) HCl-KCl buffer pH 1 / ethanol 70/30, 60 °C, 12 hours.

Compounds **3a** and **4a** were synthesised according to scheme 2. Addition of 1,3-dithiane anion on monoacetal of glyoxal[14] afforded acetal **7** in 68 % yield. After hydrolysis of **7**, no attempt was made to isolate the aldehyde **7a**, the solution was neutralised and directly used in the enzymatic reaction. An equimolar amount of DHAP prepared according to Effenberger[15] and 200 units of fructose-1,6-diphosphate aldolase were added. Both commercial rabbit muscle aldolase and spinach leaf aldolase[16] were used with the same efficiency. After 24 hours, no more than 5 % of the initial DHAP was present in the solution.[17] The pH was adjusted to 4.7 by HCl addition and acid phosphatase (120 units) was added. After two days, the mixture was concentrated under reduced pressure and the residue extracted with methanol. Equal amounts of **3a** and **4a** were isolated in 22.5 % yield after purification by chromatography on silica gel. Their configurations were established by ^1H and ^{13}C NMR spectroscopy.[18] A coupling constant of 6.9 Hz was observed between H4 and H5 for **3a** as observed in fructose derivatives. A 3.5 Hz value was measured for **4a** and corresponds to a sorbose-like configuration.[19] ^{13}C NMR showed that the anomers represented in scheme 2 for **3a** (β form) and **4a** (α form) were predominant in methanol solution with a C2 signal at respectively 104.0 and 105.5 ppm. For the minor form of **3a** and **4a** present in proportions of respectively 20 % and 15 %, values of 106.2 and 108.2 ppm were found.

The coupling condensation with aldolase was also performed on aldehyde **8**. This product is easily prepared by reaction of the dithiane anion on glycidaldehyde diethyl acetal. The same protocol as previously described was applied enabling us to isolate **5** and **6** in respectively 16 % and 18 % yield. Stereochemical attributions of aldol products were determined by ^{1}H and ^{13}C NMR spectroscopy.[20] A coupling constant of 4.5 Hz was observed between H4 and H5 for **6**, but could not be measured for **5** (AB system). In methanol solution, ^{13}C NMR showed a major β form for **5** and a major α form for **6** from the chemical shifts of C2. The minor form was present in only 18 % for **5** and 10 % for **6**.

This method allows a versatile synthesis of enantiomerically pure aldoketoses which are of interest for biosynthetic studies and to improve the production of nojirimycin and mannojirimycin by micro-organisms.These compounds can easily be labelled with ^{13}C or ^{14}C starting from available labelled dihydroxy acetone phosphate (DHAP) or fructose-1,6-diphosphate. Moreover, they are also useful key-intermediates in a new synthesis of these important natural compounds and some analogues.This approach is different from that developed previously where aldolase is also involved[6b] since nitrogen atom is introduced in the last step. This allows to access to nojirimycin and mannojirimycin **1a** and **2a** besides the deoxy compounds **1b** and **2b**. In a similar approach, **7a** and **8a** proved to be substrates of transketolase, allowing the synthesis of precursors of polyhydroxylated piperidines and pyrrolidines.[21] This work further illustrates the usefulness and versatility of aldolases as chiral catalysts, providing in this special case new aldoses.[22]

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18 For **3a** (major form): $[\alpha]_J^{25}$ + 10 (*c* 0.014, MeOH), ^{1}H NMR (400 MHz, CD_3OD) δ 2.11-2.28 (2H, 2 m, H8a and H8b); 3.00-3.17 (4H, 2 m, H7a,b and H9a,b); 3.67 (2H, s, H1); 4.10 (1H, pt, H5, *J* 6.9, 7.2 Hz); 4.23 (1H, d, H3, *J* 7.5 Hz); 4.32 (1H, d, H6, *J* 7.2 Hz); 4.49 (1H, dd, H4, *J* 6.9, 7.5 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ 27.4 (C8), 29.5-30.0 (C7 and C9), 50.9 (C6), 62.6 (C1), 77.4-79.3-84.3 (C3, C4, C5), 104.0 (C2). For **4a** (major form): $[\alpha]_J^{25}$ + 3 (*c* 0.011, MeOH), ^{1}H NMR (400 MHz, CD_3OD) δ 2.12-2.24 (2H, 2 m, H8a and H8b); 3.00-3.11 (4H, 2 m, H7a,b and H9a,b); 3.76 (2H, d, H1); 4.24 (1H, d, H6, *J* 1.5 Hz); 4.31 (1H, dd, H5, *J* 1.5, 3.4 Hz); 4.35 (1H, d, H3, *J* 9.5 Hz); 4.53 (1H, dd, H4, *J* 3.5, 9.5 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ 27.4 (C8), 29.0-29.6 (C7 and C9), 45.5 (C6), 65.2 (C1), 78.0-79.1-80.0 (C3, C4, C5), 105.5 (C2).

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20 **5** (major form): $[\alpha]_J^{25}$ + 28 (*c* 0.016, MeOH), ^{1}H NMR (400 MHz, CD_3OD) δ 2.03-2.29 (2H, 2 m, H9a,b); 2.20 (2H, m, H6, *J* 4.5, 9.5, 1.4 Hz); 3.03 (4H, m, H8a,b and H10a,b); 3.64 (2H, s, H1); 4.10 (1H, m, H3 or H4); 4.15 (1H, m, H5 *J* 1.4 Hz); 4.20 (1H, m, H4 or H3); 4.45 (1H, dd, H7, *J* 4.5, 9.5 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ 27.2 (C9), 30.6-31.0 (C8 and C10), 42.9 (C6), 45.0 (C7), 64.9 (C1), 77.4-78.7-81.1 (C3, C4, C5), 103.5 (C2). **6** (major form): $[\alpha]_J^{25}$ - 10 (*c* 0.022, MeOH), ^{1}H NMR (400 MHz, CD_3OD) δ 2.03-2.29 (2H, 2 m, H9a,b); 2.14 (2H, m, H6, *J* 2.0, 5.5, 7.5, 8.5 Hz); 3.15 (4H, m, H8a,b and H10a,b); 3.70 (2H, s, H1); 4.18 (1H, d, H3, *J* 3.0 Hz); 4.22 (1H, dd, H4, *J* 3.0, 4.5 Hz); 4.40 (1H, dd, H7, *J* 7.5 8.5 Hz); 4.64 (1H, ddd, H5, *J* 4.5, 5.5, 7.5 Hz). ^{13}C NMR (100 MHz, CD_3OD) δ 28.0 (C9), 30.1-32.0 (C8 and C10), 36.6 (C6), 45.0 (C7), 66.0 (C1), 77.4-79.0-79.5 (C3, C4, C5), 104.1 (C2).

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