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Rabbit muscle aldolase (RAMA) as a catalyst in a new approach for the synthesis of 3-deoxy-D-*manno*-2-octulosonic acid and analogues

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Abstract

A new approach leading to 3-deoxy-D-*manno*-2-octulosonic acid (KDO) and 4-deoxy-KDO is described. The key step is the formation of the C_5–C_6 bond catalysed by fructose-1,6-bisphosphate aldolase, which controls the stereochemistry of these two centres. The important step of the aldehyde substrates (**1a**, **1b**, **1c**) synthesis is the Barbier reaction of ethyl-bromomethylacrylate or bromomethylacrylonitrile with the monoacetal of glyoxal in the presence of indium.

Keywords: Carbon–carbon bond formation; Aldolase; KDO; KDO analogues

1. Introduction

3-deoxy-D-*manno*-2-octulosonic acid (KDO) occurs as a ketosidic component in all lipopolysaccharides (LPS) of gram negative bacteria so far investigated, and it has also been identified in several acidic exopolysaccharides (k-antigens) [1,2]. This specific occurrence of KDO makes its biosynthetic pathway a possible therapeutic target. Some analogues have been tested as antimicrobial agents [3,4]. For this reason, syntheses of KDO and analogues have retained attention in the last few years. Total syntheses of KDO have been published [5,6], but the most convenient procedures involve hemisyntheses starting from D-arabinose or D-mannose [7–11].

Two enzymatic syntheses, based on the metabolic pathway of KDO, have been described: the first one uses KDO-8-phosphate synthetase which catalyses the condensation of phosphoenolpyruvate onto D-arabinose-5 phosphate [12]. The latter uses the KDO aldolase, acting in vivo on the biodegradation of KDO by reversible retroaldolisation into D-arabinose and pyruvate [13]. In both cases, C_3–C_4 bond is created with the control of the configuration in C_4 (Scheme 1).

Although these methods can afford KDO-8-phosphate or KDO in moderate to good yields, they do not allow the obtention of KDO analogues, due to the specificity of the enzymes for phosphoenolpyruvate or pyrurate and close analogues of D-arabinose [14]. In particular, 4-deoxy-KDO is not accessible.

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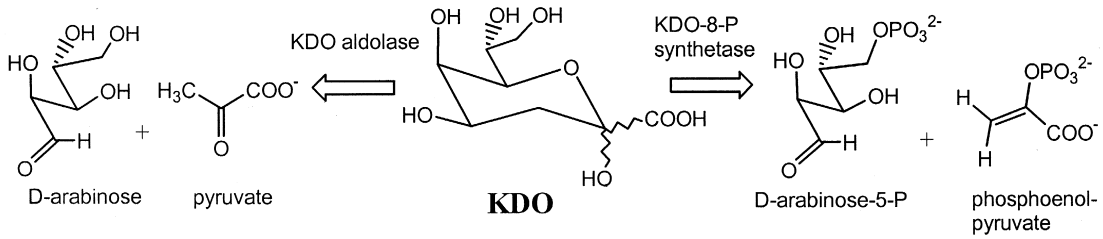


Scheme 1.

In the course of our studies on the utilisation of aldolase for the enzymatic synthesis of monosaccharides and analogues [15], we looked for a more versatile approach. Indeed, fructose-1,6-bisphosphate aldolase (RAMA), which catalyses the condensation of dihydroxyacetone phosphate (DHAP) onto a variety of aldehydes [16], can lead to KDO, according to Scheme 2. In this reaction, the aldolase allows the C_5–C_6 bond formation and fixes the stereochemistry on these centres in the configuration present in KDO. On the contrary, the configuration at C_4 can be chosen to lead to KDO or an epimer. Moreover, the substitution in C_4 can be omitted to provide 4-deoxy-KDO. The stereospecific reduction of the carbonyl group in C_7 could be obtained by chemical [17,18] or enzymatic reduction [19].

In this paper, we describe the synthesis of various aldehyde precursors, the activity of the aldolase towards them, and the synthesis of three KDO analogues.

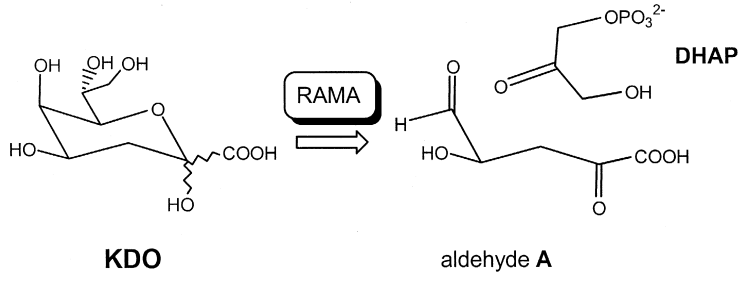
2. Results and discussion

The utilisation of aldehyde A (Scheme 2) could lead to a straight synthesis of 7-oxo-KDO, but we preferred to use analogues with a masked keto group namely a methylidene in C_2 in order to be able to reduce specifically the keto group in C_7 and to facilitate the structure determination of the products by limiting the hemiacetal cyclic forms. For the synthesis of 4-deoxy-KDO, we used the same strategy, but, since the number of possible cyclic forms is reduced in this case, we also synthesized the 2-oxo compound.

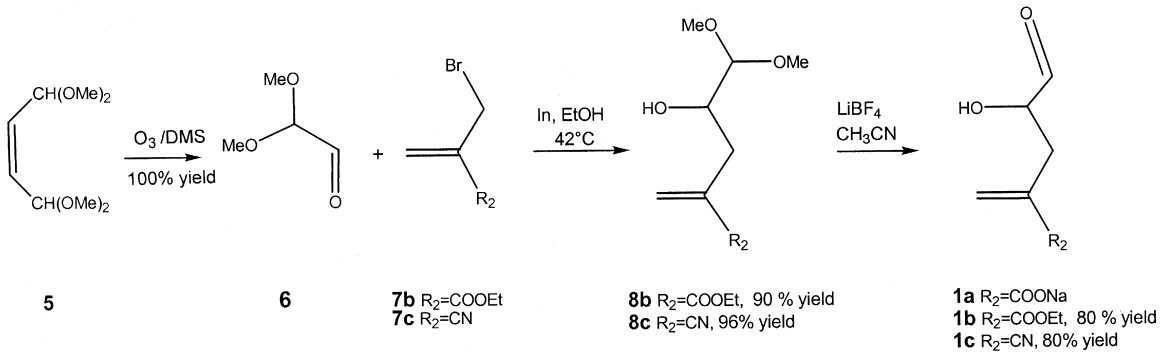
2.1. Synthesis of aldehyde substrates

Compounds **1a**, **1b**, **1c** were synthesized according to Scheme 3. The key step is the Barbier reaction of ethyl-bromomethylacrylate or bromomethylacrylonitrile with the monoacetal of glyoxal in the presence of indium [20].

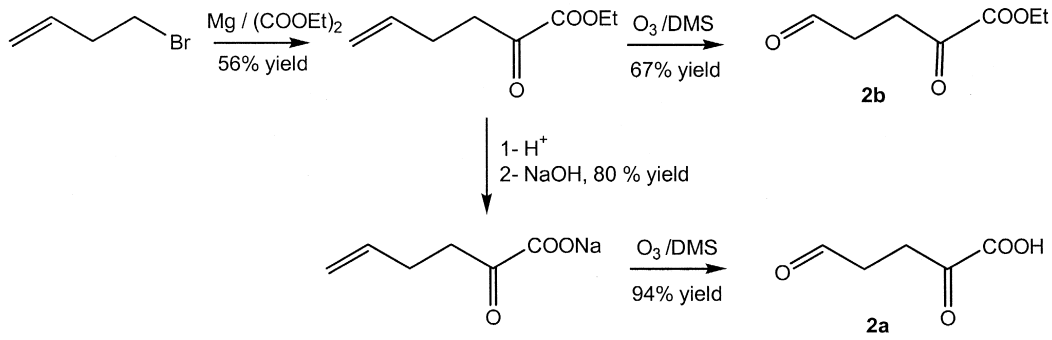
1,1,4,4-Tetramethoxybutene (**5**) was easily obtained from furan [21] and led to the desired aldehyde **6** by simple ozonolysis, thus, providing a convenient access to this interesting synthon. **7b** and **7c** were synthesized by known procedures from ethylacrylate [22] or acrylonitrile [23]. The Barbier reaction led to the desired compounds with satisfactory yields of 90% and 96% for **8b** and **8c** respectively. Usual acidic conditions for the acetal **8b** brought partial isomerisation of the α-hydroxyaldehyde into



Scheme 2.



Scheme 3.



Scheme 4.

α-hydroxyketone in the case of **1b**, thus, the aldehyde deprotection was carried out by the reaction of $LiBF_4$ in acetonitrile [24].

Compounds **2a** and **2b** were obtained from ethyl 2-oxo-hex-5-enoate [25] by hydrolysis and oznolysis (Scheme 4). **2a** was washed with ethyl acetate and methylene chloride, while **2b** was purified by flash chromatography; they were characterized by NMR.[1]

The synthesis of **2c**–**2e** have been previously described [26].

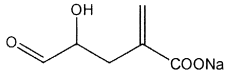
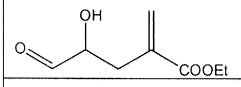
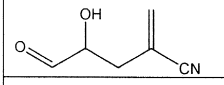
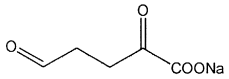
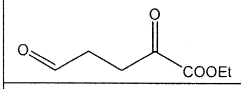
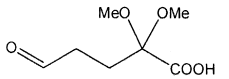
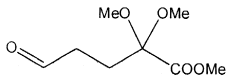
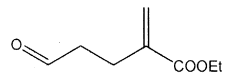
[1] **2b** ^{1}H NMR (400 MHz; $CDCl_3$) δ 9.57 (s, 1H, H5); 4.12 (q, 2H, CH_2, J = 7 Hz); 2.94 (t, 2H, H3, $J = 7$ Hz); 2.66 (t, 2H, H4, $J = 7$ Hz); 1.65 (t, 3H, CH_3, $J = 7$ Hz). ^{13}C NMR (100 MHz; $CDCl_3$) δ 199.57 (C5); 192.09 (C2); 159.97 (C1); 62.00 (CH_2); 36.49 (C4); 31.09 (C3); 13.37 (CH_3). **2a** ^{1}H NMR (400 MHz; D_2O) three forms in the presence of aldehyde–monohydrate–dihydrate δ 9.60 (s, 1H, H5); 5.1 (m, 2×1H, 2×CH5$(OH)_2$); 2.85 and 1.95 (m, 3×4H, H3 and H4, 3×2CH_2). ^{13}C NMR (100 MHz; D_2O) δ 210.2; 210.1; 208.8 (C5; C2); 185.4; 185.2; 183.9 (C1); 102.0; 93.5; 92.9 ($CH(OH)_2$); 37.5; 37.0; 36.7; 34.8; 33.2; 32.6 (2×CH_2, C3, C4).

2.2. Determination of the characteristic constants of the aldolase catalysed reactions

Aldehydes **1a**, **1b**, **1c** and **2a**–**2e** were submitted to the action of aldolase. The reaction was monitored by the enzymatic titration of residual DHAP (DHAP was prepared and assayed according to Gefflaut et al. [27] and Jung et al. [28]), and the characteristic constants of the reaction, the Michaelis constant K_m and the maximum rate V_{max}, were calculated. To facilitate the comparison between different substrates, the V_{max} value is given in percent of the activity of fructose-1,6-bisphosphate aldolase in the natural reaction ($V_{max\ rel}$). The results are reported in Table 1.

All tested aldehydes are substrates for aldolase. In the case of α-hydroxyaldehydes **1a**, **1b**, **1c**, the best substrate is the acid **1a**, probably due to the fact that the carboxylate group is comparable to the phosphate group present in

Table 1
Relative activities of aldehydes **1a**, **1b**, **1c**, **2a**–**2e** with DHAP in RAMA-catalyzed aldol condensations

Aldehyde substrates		Km* (mM)	Vmax* (% of the enzyme activity)	V at 50 mM (% of enz.activity)
	**1a**	28	33	18
	**1b**	53	21	10
	**1c**	57	27	20
	**2a**	47	26	13
	**2b**	74	26	15
	**2c**	63	31	14
	**2d**	380	68	8
	**2e**	127	49	14

The V_{max} values are given in percent of the activity of fructose-1,6-bisphosphate aldolase in the natural reaction.
* These are apparent values since the experimental conditions cannot allow to measure the initial rate of the reaction, but only after at least 10–20% DHAP consumption.

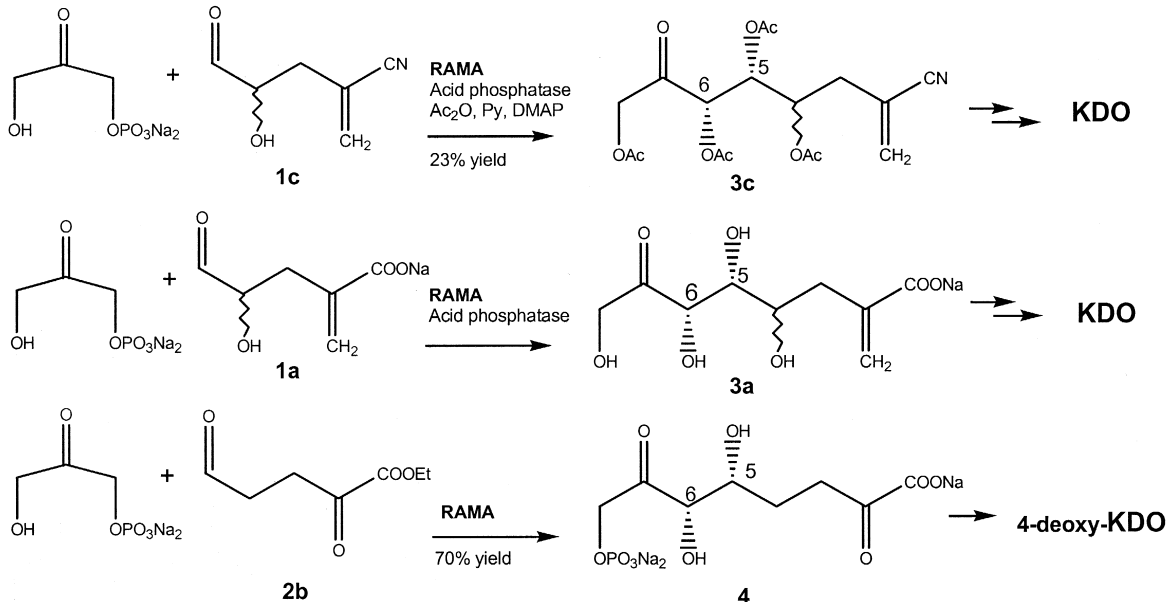
the natural substrate glyceraldehyde-3-phosphate. Moreover, in this case, the reaction is expected to proceed with some enantioselectivity as pointed out by Lees and Whitesides [29]. **1b** and **1c** present lower activities, although sufficient to make the synthesis possible.

In the case of aldehydes **2a** and **2b**, again, the results are better for the carboxylate **2a** than for the ester **2b**. The presence of the keto group in C_2 instead of a methylidene or gem-dimethoxy in the previously reported substrates [26], improves the efficiency of the catalysis.

2.3. Synthesis of KDO analogues

The syntheses were performed by already published protocols [15,26] (see Scheme 5).

•KDO analogue **3a**: Aldehyde **1a** was first put in reaction with 0.5 equivalent of DHAP. When DHAP



Scheme 5.

was totally consumed, the conversion rate of the aldehyde was near 50%, however, the residual aldehyde presented an eniantomeric excess of only 40%[2] which indicates that the enantioselectivity of aldolase is probably too low to insure a diastereospecific synthesis starting from racemic **1a**. Obtention of **3a** by this way needs a purification step probably very difficult with this salt, so, we decided to synthesize the nitrile analogue starting from **1c**.

•KDO analogue **3c**: After the aldolase catalysed reaction,[3] the phosphate group was hydrolysed in the presence of acid phosphatase, the mixture was concentrated in vacuo and acetylated. After column chromatography purification, **3c** was isolated as a mixture of the two diastereomers, in an overall yield of 23%. Analysis of the NMR spectra showed the presence of two diastereomers (1/1) which could not be separated by chromatography.[3]

•KDO analogue **4**: The enzymatic synthesis was run with 0.5 equivalent of DHAP in the same conditions as **3c**, but the phosphate was isolated by pre-

[2] The residual aldehyde was reduced by $NaBH_4$ and the alcohol, extracted by ethyl acetate, led to a lactone, which was analysed by chiral HPLC (Chiralcel OB), allowing to attribute the 40% ee value.

[3] **3c** ^{1}H NMR (400 MHz; $CDCl_3$) (diastereomer **1**) δ 5.98 (s, 1H, $=CH_2$); 5.77 (s, 1H, $=CH_2$); 5.63 (d, 1H, H6, $J = 3.6$ Hz); 5.28 (dd, 1H, H5, $J = 8.3$ and 2.2 Hz); 4.82 (d, 1H, H8a, $J = 16.1$ Hz); 4.75 (d, 1H, H8b, $J = 16.1$ Hz); 4.24 (m, 1H, H4); 2.53 (dd, 1H, H3a, $J = 13.6$ and 2.8 Hz); 2.49 (dd, 1H, H3b, $J = 15.7$ and 2.3 Hz); 2.08 (s, 3H); 2.05 (s, 3H); 2.03 (s, 3H); 2.00 (s, 3H). ^{13}C NMR (100 MHz; $CDCl_3$): 198.40 (C7); 170.48; 170.30; 170.06; 169.78 (4×C=O Ac); 133.38 ($=CH_2$); 118.83 (C2); 118.32 (C1); 74.41 (C6); 73.12 (C5); 71.52 (C8); 66.49 (C4); 33.92 (C3); 20.98; 20.89; 20.61; 20.34 (4×CH_3 Ac). (diastereomer **2**) δ 5.90 (s, 1H, $=CH_2$); 5.72 (s, 1H, $=CH_2$); 5.39 (d, 1H, H6, $J = 4.6$ Hz); 5.31 (t, 1H, H5, $J = 4.7$ Hz); 4.80 (d, 1H, H8a, $J = 17.0$ Hz); 4.76 (d, 1H, H8b, $J = 17.0$ Hz); 4.18 (m, 1H, H4); 2.63 (dd, 1H, H3a, $J = 15.7$ and 2.3 Hz); 2.59 (dd, 1H, H3b, $J = 15.0$ and 3.3 Hz); 2.15 (s, 3H); 2.10 (s, 3H); 2.08 (s, 3H) ; 2.05 (s, 3H). ^{13}C NMR (100 MHz; $CDCl_3$): 197.55 (C7); 169.94; 169.70; 169.64; 169.48 (4×C=O Ac); 131.98 (=CH2); 119.18 (C2); 117.97 (C1); 74.19 (C6); 73.13 (C5); 72.70 (C8); 66.75 (C4); 34.18 (C3); 20.68; 20.39; 20.35; 20.05 (4×CH3 Ac).

cipitation of its barium salt with a 70% yield.[4] The yield of synthesis was drastically increased when compared to the obtention of the 2-methylidene or 2,2-dimethoxy analogues previously reported [26].

3. Conclusion

These results show that fructose-1,6-bisphophate aldolase provides a new and versatile approach to the chemo enzymatic synthesis of ulosonic acids. To accede to KDO itself, it will be necessary to start with optically pure **1c** or **1a** whose synthesis is under investigation.

This method allowed us to obtain 4-deoxy-7-ox-KDO-8-phosphate, which can already be an interesting analogue. Hydrolysis of the phosphate group and specific reduction of the carbonyl in C_7 will lead to 4-deoxy-KDO.

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[4] Experimental procedure for the synthesis of **4**: to a solution of **2b** (1 g; 6.32 mmol) in water:EtOH (95:5; 1 ml) was added 14.55 ml of a solution containing 3.16 mmol of DHAP, and the mixture was adjusted to pH 7 with 1 N NaOH. 280 U of RAMA were added; the total volume was ca. 42 ml. After 15.5 h, the mixture was concentred to a volume of 20 ml, the pH was adjusted to 8.2, a solution of barium chloride (12.64 mmol in 20.5 ml) was added, and the solution was readjusted to pH 8.2. Ethyl alcohol (200 ml) was added, and the resulting suspension was kept at 4°C for 5 h. The precipitate formed was separated by centrifugation, washed with EtOH and dried in vacuo to give barium salt. Then, it was stirred with ion-exchange resin (Dowex 50W×H8, Na^+ form). The resin was removed by filtration and washed with water. The combined filtrated were lyophilised to provide **4** (756 mg, 70% from DHAP) as white powder. $[\alpha]_D^{25} = -9.43$ (H_2O, c 0.026); ^{31}P NMR (121 MHz; D_2O): 3.10 (100%); ^{13}C NMR (100 MHz; D_2O) 7(α-pyranose) 208.40 (d, C7, $J = 5$ Hz); 172.32 (C1); 97.37 (C2); 81.90 (C6); 76.93 (C5); 69.99 (d, C8, $J = 4$ Hz); 33.12 (C3); 31.39 (C4). 7(β-furanose) 208.40 (d, C7, $J = 5$ Hz); 173.32 (C1); 101.97 (C2); 86.42 (C5); 73.44 (C6); 70.10 (d, C8, $J = 4$ Hz); 38.62 (C3); 36.68 (C4). MS (ES^-) = m/z 321 $(MH-2Na)^-$.