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A New Model for the Stereoselective Construction of the Kdo Structure Through a Mechanism Similar to that Suggested for the Enzyme Kdo8P Synthase

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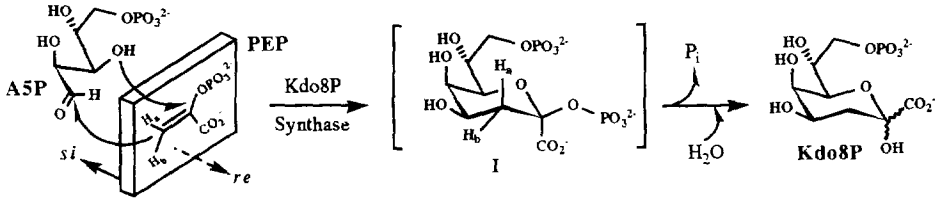
Abstract: Stereospecific chemical synthesis of the Kdo structure was demonstrated using a mechanism similar to that suggested for the enzyme Kdo8P synthase. The derivative of D-(-)-arabinose (compound **3**), in which the enolpyruvate moiety is attached at C-3 hydroxyl, was synthesized in 12 chemical steps and an intramolecular condensation of enolpyruvate and aldehyde functions was examined, under Lewis acid conditions. Copyright © 1996 Elsevier Science Ltd

Higher 3-deoxy-2-ulosonic acids, as well as sialic acids, are widely spread natural carbohydrates which participate in various important biological processes and contain either 7, 8 or 9 carbon atoms.[1] In this family of compounds the anomeric center possesses a carboxylate group, with the adjacent position not oxygenated. Interestingly, the biosynthesis of these compounds follows a similar general route which involves the stereospecific condensation of phosphoenolpyruvate (PEP) with an appropriate aldose.[2] The extraordinary feature of these enzyme-catalyzed reactions is that in most cases where enzymes utilize PEP as a substrate, cleavage of the P-O bond of PEP occurs, in the above case the PEP undergoes an unusual C-O bond cleavage.[3] Such a C-O bond cleavage is very rare for PEP-utilizing enzymes and a nonenzymatic analogy of this transformation in solution has yet to be demonstrated. In order to understand such an unusual condensation mechanism, we set out to construct an appropriate chemical model and demonstrate, using this model system, the similar chemistry in solution. For this purpose we first selected the formation of 8-carbon atom ulosonic acid 3-deoxy-D-*manno*-2-octulosonate (Kdo),[4] which is synthesized as an 8-phosphate derivative (Kdo8P) by the coupling of arabinose-5-phosphate (A5P) with PEP, and catalyzed by the enzyme Kdo8P synthase.[5]

Scheme 1.

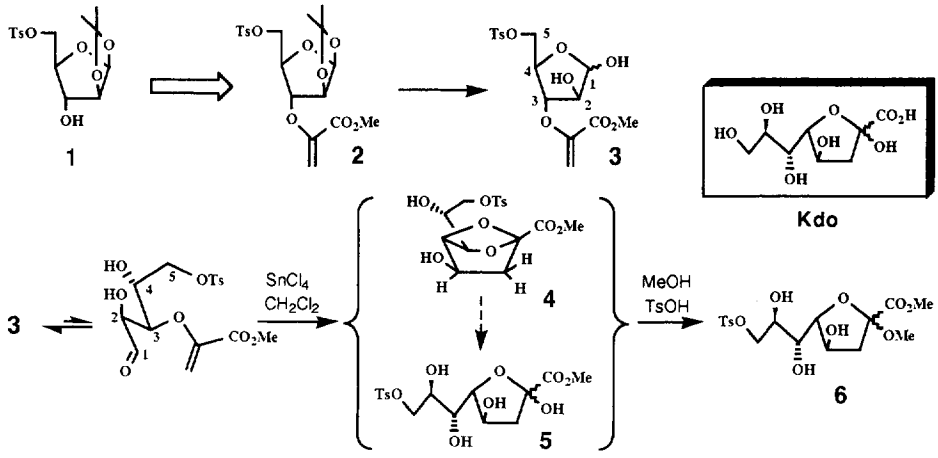


Recent studies of the mechanism of the Kdo8P-synthase-catalyzed reaction have suggested[6] that the enzyme catalysis may proceed through the formation of the cyclic bisphosphate intermediate I (Scheme 1). This intermediate might be formed either by a synchronous or by a stepwise generation of new C-C and C-O bonds between A5P and PEP. The question of which of these pathways is correct remains to be determined

conclusively. The intermediate I is expected to undergo rapid hydrolysis to produce the product Kdo8P and inorganic phosphate (P_i). From this mechanism it seems that at the initial condensation step the enzyme catalysis should mostly involve *catalysis by approximation* due to the reactive groups being held proximal to each other within a bonding, *critical distance*.[7] In addition, since the synthase is neither a metalloenzyme, nor does it require the addition of metal cations for catalytic activity,[5] extra acceleration of the condensation step might be achieved through the activation of aldehyde carbonyl by active site electrophile/s. This postulate though, provides nothing new or unusual. As a first and simple model in the investigation of the above hypothesis we have achieved a stereospecific chemical synthesis of the Kdo structure by a mechanism similar to that suggested for the synthase. This was attained by employing two general categories of chemical catalysis invoked by enzymes: catalysis by approximation and acid-base catalysis.

As seen from Scheme 1, the formation of intermediate I requires bonding distance not only between the aldehyde carbon and C-3 of PEP, but also between the C-3 oxygen of A5P and C-2 of PEP. Therefore, in keeping with the concept of catalysis by proximity of reactive groups, we have installed the enolpyruvate moiety of PEP onto the C-3 hydroxyl of arabinose and synthesized the aldose **3** as a mixture of anomers.[8] The open-chain aldehyde form of **3** resembles the proposed situation whereby two substrates A5P and PEP evolve into a ternary complex with the synthase (Scheme 1). Treatment of **3** with Lewis acid[9] ($SnCl_4$, CH_2Cl_2 or BF_3, Et_2O) afforded the mixture of products **4** and **5** (Scheme 2), whose structures were unambiguously determined by chemical, spectroscopic and X-ray diffraction analysis. Upon close investigation of the reaction conditions, we found that the first product formed during the condensation is the bicyclic **4**, which under the reaction conditions suffers the opening of its ketal ring, resulting in the formation of the anomeric mixture of **5**.[10] Further treatment of the condensation reaction mixture with acid (MeOH, TsOH) induces the formation of α- and β-methyl furanoside derivatives of Kdo (α-**6** and β-**6**),[11] whose structures were determined by comparison with the reported furanoside structures of Kdo.[12]

Scheme 2.



The formation of **4** is especially noteworthy. To the best of our knowledge, this represents the first example of nonenzymic condensation of the enolpyruvate moiety to the carbonyl group, leading to a required 3-

deoxy-2-ulosonic acid system possessing the desired stereochemistry. The absolute configuration at the newly formed stereogenic center (C-4) was determined by X-ray structure analysis of compound **4** and found to be of (*R*)-configuration (Figure 1), which is the same as for Kdo. Since no C-4 epimer was detected among the reaction products, it appears that the condensation step is highly stereospecific involving the attachment of the *re* face of enolpyruvate to the *re* face of the carbonyl. The experiments to quantify the intramolecular benefit and the mechanism of the condensation process in **3** are underway and will be reported in due course.

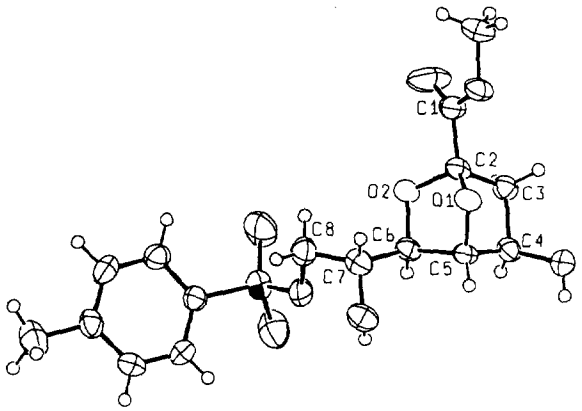


Figure 1. ORTEP drawing of **4** from single-crystal X-ray analysis. Crystallographic data: monoclinic $P2_1$, a=15.307(4)Å, b=7.642(2)Å, c=7.559(2)Å, β=95.65(3)°, V=879.9Å^3, Z=2 provide a calculated density ρ=1.466 gcm^3, T=293K, λ=0.71069Å, R=0.030 for 1538 reflections. Bond lengths (Å): C(1)-C(2)=1.508(5), C(2)-C(3)=1.544(5), C(2)-O(1)=1.420(4), C(2)-O(2)=1.431(4), C(3)-C(4)=1.527(5), C(4)-C(5)=1.528(5), C(5)-C(6)=1.521(4), C(5)-O(1)=1.457(4), C(6)-O(2)=1.444(4), C(6)-C(7)=1.520(5), C(7)-C(8)=1.511(6). Bond angles (°): C(1)-C(2)-C(3)=115.4(3), C(1)-C(2)-O(1)=115.3(3), C(1)-C(2)-O(2)=109.5(3), O(1)-C(2)-C(3)=102.7(2), O(2)-C(2)-C(3)=108.6(3), O(1)-C(2)-O(2)=104.6(2), C(3)-C(4)-C(5)=101.5(3), C(4)-C(5)-C(6)=108.2(3), C(4)-C(5)-O(1)=103.0(3), C(6)-C(5)-O(1)=100.3(2), C(2)-O(1)-C(5)=94.3(2), C(2)-O(2)-C(6)=104.4(2), C(6)-C(7)-C(8)=111.3(3). The e.s.d. in parenthesis is in the unit of the least significant digit.

The results obtained so far provide a unique demonstration of enzyme like chemistry in the stereospecific synthesis of the Kdo system and certainly warrant further investigation to facilitate the construction of other 3-deoxy-2-ulosonoc acids and sialic acids based on the same general model, as shown here in the case of Kdo. Furthermore, the results also support the validity of the mechanism recently suggested by us, for the action of Kdo8P synthase, especially as to the possible role of the enzyme at the initial condensation step as hypothesized above. Thus, the holding of the catalytic groups of A5P and PEP at bonding distances, plus a small general acid catalysis, could be sufficient to explain the high rate of acceleration attributed to the initial condensation step in the Kdo8P-synthase-catalyzed reaction.

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8. First, D-(-) arabinose was converted to compound **1** in seven chemical steps according to the published method: Kalvoda, L.; Prystas, M.; Sorm, F. *Collec. Czechoslov. Chem. Comm.* **1976**, *41*, 788-799. Next, the introduction of enolpyruvyl moiety on the hydroxyl-3 of **1** was accomplished according the procedure developed by Ganem (Ganem, B.; Ikota, N.; Muralidharan, V. B.; Wade, W. S.; Young, S. D.; Yukimoto, Y. *J. Am. Chem. Soc.* **1982**, *104*, 6787-6788). In a four-step sequence [$N_2C(CO_2Me)_2$, $Rh_2(OAc)_4$, benzene; $CH_2{=}^+N(CH_3)_2I^-$, CH_2Cl_2, Et_3N; CH_3I, CH_2Cl_2; DMSO, 95°C] the acetonide **1** was converted to enolpyruvate ester **2** in overall 36% yield. Removal of the acetonide moiety with mild acid (65:35:10; HOAc:H_2O:THF, 93%) then provided the target compound **3**. Satisfactory spectral data were obtained for all new compounds.

9. In a typical condensation experiment, the aldose **3** (88 mg, 0.227 mmol) in CH_2Cl_2 (4 mL) was treated with $SnCl_4$ (14 mg, 0.053 mmol) at 0°C. After 5 hours the reaction mixture was diluted with CH_2Cl_2 (10 mL) and EtOAc (20 mL), and neutralized with saturated $NaHCO_3$. The usual workup and column chromatography then provided the bicyclic **4** (60 mg, 68.2%) and the anomeric mixture of **5** (13 mg, 14.8%). Similar results were obtained when the BF_3 etherate was used instead of $SnCl_4$ as a Lewis acid. ^1H NMR of **4** ($CDCl_3$, 400MHz) δ 1.88 (d,1H, J = 13.5 Hz, C_3-H_a'), 2.43 (s, 3H, CH_3 of Ts), 2.48 (dd,1H, J = 13.5 and 6.6 Hz, C_3-H_e'), 3.51 (d,1H, J = 8.2 Hz, C_6-H), 3.66 (m, 1H, C_7-H), 3.83 (s, 3H, CO_2Me), 4.04 (dd,1H, J = 10.8 and 5.6 Hz, C_8-H), 4.19 (dd,1H, J = 10.8 and 2.2 Hz, C_8-H'), 4.22 (d, 1H, J = 6.6 Hz, C_4-H), 4.84 (s, 1H, C_5-H), 7.32 (d, 2H, J = 8.1 Hz, aromatic protons), 7.75 (d, 2H, J = 8.1 Hz, aromatic protons). H_a' and H_e' refer to the geminal protons of the furanose anomers of Kdo that have similar orientations to the axial and equatorial protons, respectively, in the pyranose anomers.

10. This result was further supported by performing the same reaction ($SnCl_4$, CH_2Cl_2) on the chemically pure **4**, which afforded the same anomeric mixture of **5**.

11. ^1H NMR of α-**6** ($CDCl_3$, 400MHz) δ 2.23 (dd,1H, J = 14.7 and 1.2 Hz, C_3-H_a'), 2.41 (dd,1H, J = 14.7 and 6.6 Hz, C_3-H_e'), 2.44 (s, 3H, CH_3 of Ts), 3.33 (s, 3H, OMe), 3.56-3.62 (m, 1H, C_6-H), 3.80 (s, 3H, CO_2Me), 3.82-3.88 (m, 1H, C_7-H), 4.18 (dd,1H, J = 10.4 and 6.1 Hz, C_8-H), 4.32 (dd,1H, J = 10.4 and 3.3 Hz, C_8-H'), 4.34 (d,1H, J = 6.5 Hz, C_4-H), 4.49 (s, 1H, C_5-H), 7.34 (d, 2H, J = 8.1 Hz, aromatic protons), 7.79 (d, 2H, J = 8.1 Hz, aromatic protons). ^1H NMR of β-**6** ($CDCl_3$, 400MHz) δ 2.32 (dd,1H, J = 13.6 and 6.8 Hz, C_3-H_a'), 2.54 (dd,1H, J = 13.6 and 7.0 Hz, C_3-H_e'), 2.44 (s, 3H, CH_3 of Ts), 3.29 (s, 3H, OMe), 3.55-3.61 (m, 1H, C_6-H), 3.81 (s, 3H, CO_2Me), 3.82-3.85 (m, 1H, C_7-H), 4.19 (dd,1H, J = 10.5 and 6.1 Hz, C_8-H), 4.26 (t,1H, J = 5.0 Hz, C_5-H), 4.34 (dd,1H, J = 10.5 and 2.0 Hz, C_8-H), 4.49 (ddd, 1H, J = 7.0, 6.8 and 5.0 Hz, C_4-H), 7.34 (d, 2H, J = 8.1 Hz, aromatic protons), 7.79 (d, 2H, J = 8.1 Hz, aromatic protons).

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