Articles

First Nonenzymatic Synthesis of Kdo8P through a Mechanism Similar to That Suggested for the Enzyme Kdo8P Synthase

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The mechanism of Kdo8P synthase, the enzyme that catalyzes the unusual condensation of D-arabinose 5-phosphate (A5P) with phosphoenolpyruvate (PEP) to form Kdo8P, remains a fascinating subject for bioorganic research. This paper describes the synthesis of two intramolecular models (**1** and **2**) bearing an enolpyruvate moiety at C-3 of the arabinose fraction. This means that their open-chain aldehyde forms closely mimic the proposed situation, whereby two substrates A5P and PEP evolve into a ternary complex with the synthase. Examination of **1** (in organic solvent) and **2** (in a water solution) under Lewis acid conditions establishes that they both undergo highly stereospecific intramolecular condensation of the enolpyruvate double bond with the carbonyl of sugar. This results in the required Kdo structure possessing the desired stereochemistry. Mechanistic studies suggest that the observed intramolecular condensation process takes place via a stepwise mechanism involving the formation of a transient oxocarbenium ion intermediate. The results obtained, uniquely demonstrate enzyme-like chemistry in the stereospecific synthesis of the Kdo system. Further investigation is certainly warranted, in order to facilitate the construction of other 3-deoxy-2-ulosonoc acids and sialic acids on the basis of the same general model. This is illustrated here in the case of Kdo. Furthermore, the results support the validity of the mechanism suggested for the Kdo8P synthase action, in particular, the possible role of the enzyme in the catalysis of the initial condensation step.

Introduction

Higher 3-deoxy-2-ulosonic acids are widely spread, natural carbohydrates. They participate in various important biological processes and contain either seven carbon atoms (3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate, DAHP),[1] eight carbon atoms (3-deoxy-D-*manno*-octulosonic acid, Kdo)[2] or nine carbon atoms (3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid, KDN, and sialic acids).[3] In these unusual carbohydrates, the anomeric center possesses a carboxylate group, and the adjacent position is not oxygenated. Interestingly, the biosynthesis of these compounds follows a similar general route, involving the stereospecific condensation of phosphoenolpyruvate (PEP) with an appropriate aldose.[4] The extraordinary feature of these enzyme-catalyzed reactions is that the PEP undergoes an unusual C−O bond cleavage.[5] This differs from the majority of cases where enzymes utilize PEP as a substrate and cleavage of the P−O bond of PEP occurs. The unusual 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. Many synthetic methodologies of 3-deoxy-2-ulosonic acids, both chemical[6] and enzymatic,[7] have been published over the last decade. However, the puzzling question of the preference of the C−O bond cleavage of PEP over the P−O bond cleavage, in these enzyme-catalyzed reactions, remains unanswered. In an attempt to understand this unusual condensation mechanism, we constructed an appropriate chemical model using this system to demonstrate similar chemistry in solution. The formation of eight-carbon ulosonic acid Kdo[8] was initially selected for this purpose. This sugar is synthesized as an 8-phosphate derivative (Kdo8P) by the coupling of arabinose 5-phosphate (A5P) with PEP and is catalyzed by the enzyme Kdo8P synthase.[9]

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(2) (a) Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 323. (b) *Bacterial Lipopolysaccharides: Structure, Synthesis and Biological Activities*; Anderson L., Unger, F. M., Eds.; ACS Symposium Series No. 231; American Chemical Society; Washington, DC, 1983.

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(5) (a) DeLeo, A. B.; Dayan, J.; Sprinson, D. B. *J. Biol. Chem.* **1973**, *248*, 2344. (b) Hedstrom, L.; Abeles, R. H. *Biochem. Biophys. Res. Comm.* **1988**, *157*, 816. (c) Schauer, R. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 131.

(6) For recent chemical syntheses, see: (a) Ramage, R.; MacLeod, A. M.; Rose, G. W. *Tetrahedron* **1991**, *47*, 5625. (b) Dondoni, A.; Marino, P. *J. Org. Chem.* **1991**, *56*, 5294. (c) Giese, B.; Carboni, B.; Gobel, T.; Muhn, R.; Wetterich, F. *Tetrahedron Lett.* **1992**, *33*, 2673. (d) Giese, B.; Linker, T. *Synthesis* **1992**, 46. (e) Chan, T.-H.; Li, C.-J. *J. Chem. Soc., Chem. Commun.* **1992**, 747. (f) Libineau, A.; Auge, J.; Lubin, N. *Tetrahedron* **1993**, *49*, 4639. (g) Dondoni, A.; Marra, A.; Merino, P. *J. Am. Chem. Soc.* **1994**, *116*, 3324. (h) Gao, J.; Harter, R.; Gordon, D. M.; Whitesides, G. M. *J. Org. Chem.* **1994**, *59*, 3714.

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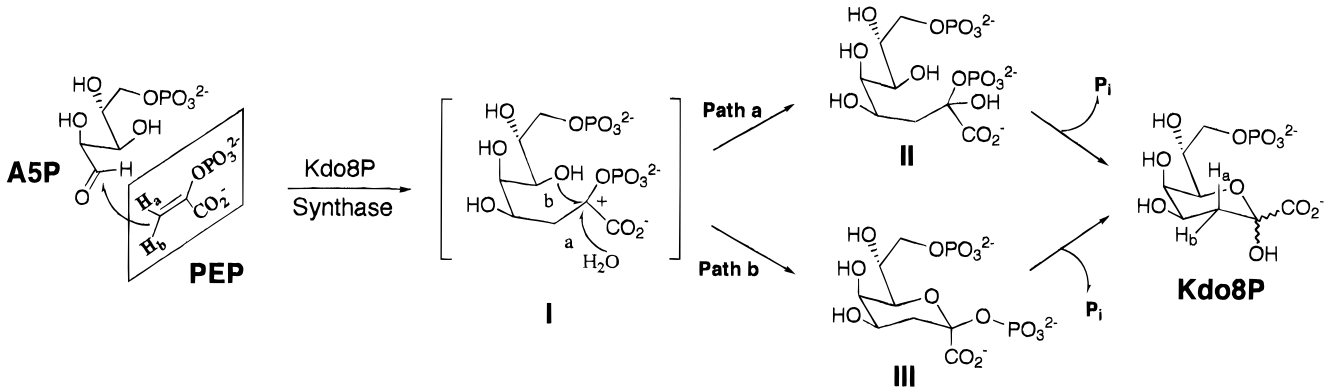
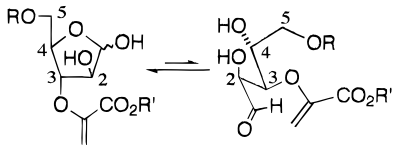
Scheme 1



Most recent suggestions regarding the mechanism of this enzyme-catalyzed reaction (Scheme 1) involve the formation of either the acyclic intermediate II (path a)[5b] or the cyclic intermediate III (path b).[10] The question as to which of these pathways is correct remains to be determined conclusively. The path leading to the formation of the transient oxocarbenium intermediate I is determined using the known stereochemical course of the reaction,[10] when condensation is a stepwise process. In this case, the initial attack of the π electrons, stemming from the *si* face of the PEP double bond, and directed toward the *re* face of the carbonyl of A5P, will result in the formation of the transient oxocarbenium intermediate I. In the next step, the cation I may be captured in two ways. The first is by the addition of water, which will lead to the formation of acyclic intermediate II (path a). A second path is the addition of the hydroxyl-3 of A5P (path b), which will result in the formation of the cyclic bisphosphate intermediate III. Both intermediates II and III are expected to undergo rapid elimination of the inorganic phosphate (P_i), through C−O bond cleavage of the C-2 phosphate, to produce the product Kdo8P.

From the mechanism in Scheme 1 it seems that the initial condensation step in the enzyme catalysis largely involves catalysis by proximity. This is due to the reactive groups of two substrates (A5P and PEP) being held proximal to each other within a bonding, critical distance.[11] The synthase is not a metalloenzyme and does not require the addition of metal cations for catalytic activity.[9] Instead, extra acceleration of the condensation step might be achieved through the activation of the aldehyde carbonyl by one or more active site electrophiles. This postulation, though, is not new or unusual. Indeed, over the last two decades, many groups have pointed out the possible relationship between enzyme catalysis and intramolecular catalysis. As shown recently by Menger,[12] the idea is attractive because huge rate acceleration sometimes occurs when an intermolecular reaction is converted into its intramolecular counterpart. Also, an enzyme reacting with its bound substrate resembles an intramolecular organic process. We have, therefore, remained true to the concept of catalysis by proximity of reactive groups. In an initial attempt to evaluate intramolecular models, we installed the enolpyruvate moiety of PEP onto the C-3 hydroxyl of arabinose and synthesized the derivatives **1** and **2**. One of the conformations of the open-chain, aldehyde form of **1** and **2** closely resembles the proposed situation, whereby two substrates, A5P and PEP, evolve into a ternary complex with the synthase. Using these models, we describe here the novel, stereospecific synthesis of the Kdo structure in organic solvents.[13] Also outlined is the first stereospecific, chemical synthesis of Kdo8P in a water solution by a mechanism similar to that suggested for the synthase. Our results provide additional insight into the manner in which PEP and A5P are recognized by the synthase and the nature of the catalytic events occurring at the enzyme active site.



1: R = Ts; R' = Me
2: R = PO_3H_2; R' = H

Results and Discussion

Prior to embarking on the multistep synthesis and examination of compound **2**, we constructed a simple model **1**. All initial studies on the subject of intramolecular condensation were undertaken in organic solvents using this model.

Synthesis and Evaluation of the Model Compound 1. The synthesis of **1** begins with the differentially protected arabinose derivative **3**. This derivative was prepared from D-(−)-arabinose in seven chemical steps following a previously reported procedure[14] (Scheme 2). The key step, introduction of the enolpyruvyl moiety to the C-3 hydroxyl of **3**, was successfully accomplished in four-stages, a method developed by Ganem.[15] Thus, treatment of alcohol **3** with dimethyl diazomalonate and rhodium(II) acetate yielded the corresponding malonate ether. Condensation of this product with dimethylmethyleneammonium iodide and triethylamine was followed by alkylation with iodomethane. Subsequent decarboxylative elimination of the corresponding ammonium salt afforded the enolpyruvate ester **4** in an overall 36% yield.

(10) (a) Sheffer-Dee-Noor, S.; Belakhov, V.; Baasov, T. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1583. (b) Baasov, T.; Sheffer-Dee-Noor, S.; Kohen, A.; Jakob, A.; Belakhov, V. *Eur. J. Biochem.* **1993**, *217*, 991.

(11) (a) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969. (b) Kirby, A. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 183. (c) Menger, F. M. *Acc. Chem. Res.* **1985**, *18*, 128.

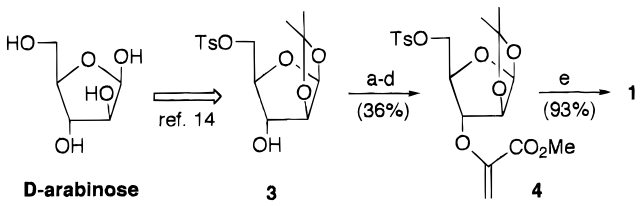
(12) Menger, F. M. *Acc. Chem. Res.* **1993**, *26*, 206.

(13) The preliminary results on the synthesis and examination of compound **1** in organic solvents have recently been published as a communication: Du, S.; Plat, D.; Baasov, T. *Tetrahedron Lett.* **1996**, *37*, 3545.

(14) Kalvoda, L.; Prystas, M.; Sorm, F. *Collect. Czech. Chem. Commun.* **1976**, *41*, 788.

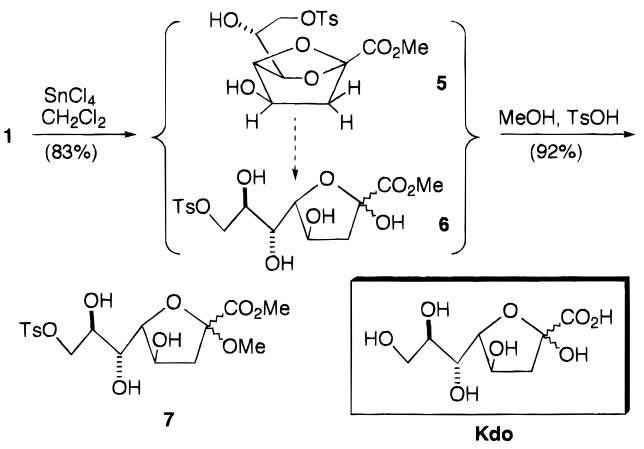
(15) Ganem, B.; Ikota, N.; Muralidharan, V. B.; Wade, W. S.; Young, S. D.; Yukimoto, Y. *J. Am. Chem. Soc.* **1982**, *104*, 6787.

Scheme 2[a]



[a] Key: (a) $N_2C(CO_2Me)_2$, $Rh_2(OAc)_4$, C_6H_6, reflux; (b) $[CH_2{=}NMe_2]^+ I^-$, Et_3N, CH_2Cl_2; (c) MeI, CH_2Cl_2; (d) DMSO, 100 °C, 0.5 h; (e) HOAc/H_2O/THF (65:35:10), 70 °C.

Scheme 3

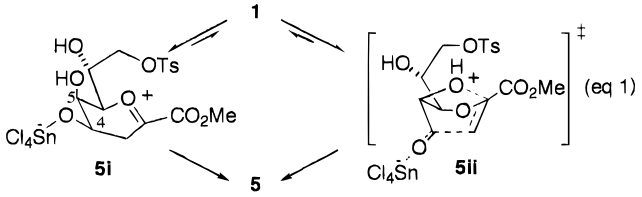


Removal of the acetonide moiety with a mild acid (65:35:10 HOAc:H_2O:THF) resulted in the target compound **1**, in a 93% isolated yield, as a mixture of anomers.

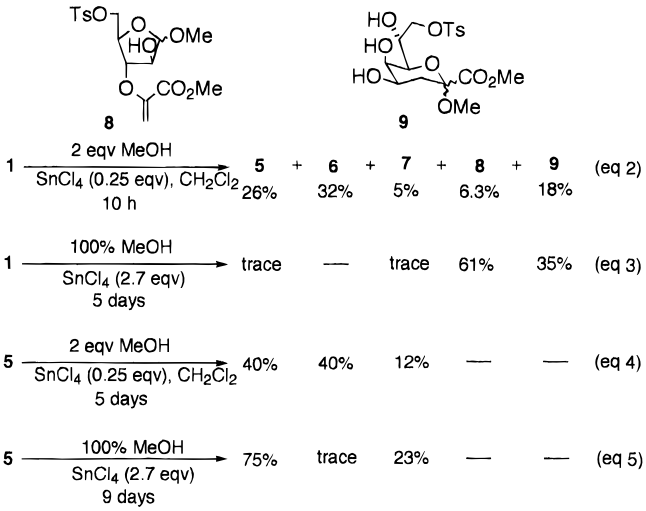
The intramolecular condensation of the aldehyde with the olefinic function in **1** was examined with different Lewis acids, and it was found that $SnCl_4$ in dichloromethane was the best mixture for this purpose. Thus, treatment of **1** with $SnCl_4$ at 0 °C afforded a mixture of products **5** (major, Scheme 3) and **6** (minor). However, when the reaction was carried out using the same stoichiometry, at room temperature, a predominance of mixture **6** is formed. Upon close investigation of the reaction, we found that the first product formed during the condensation process is the bicyclic **5**. The ketal ring of **5** opens under reaction conditions, resulting in the formation of the anomeric mixture **6**. This result was further corroborated by performing the above procedure on the chemically pure **5**. The same anomeric mixture **6** was afforded. Further treatment of the condensation reaction mixture with acid (MeOH, TsOH) induced the formation of α- and β-methyl furanoside derivatives of Kdo (α-**7** and β-**7**). The structures of these derivatives were determined by comparison with the reported furanoside structures of Kdo.[16] The predominant formation of the furanoside structures of Kdo is in accordance with the earlier reported results.[16a] Furanoside structures were seen to predominate when Kdo is sequentially protected, that is, when carboxylic acid protection is followed by the protection of hydroxyl groups.

The formation of bicyclic adduct **5** 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. It results in a required 3-deoxy-2-ulosonic acid system, possessing the desired stereochemistry. The structure of bicyclic **5** was unambiguously determined by chemical, spectroscopic, and X-ray diffraction analysis.[13] The newly formed stereogenic center (C-4) was found to have the same (*R*)-configuration as natural Kdo. The condensation step appeared to be highly stereospecific, as no C-4 epimer was detected among the reaction products.

Although more work is required to determine the true origin of the high diastereoselectivity observed in this intramolecular condensation, one rationalization of the results is shown in eq 1. Presumably, **5** arises via a



stepwise pathway involving the formation of the oxocarbenium ion intermediate **5i**.[17] A concerted process, involving a conformationally rigid, boatlike transition state such as **5ii**, is regarded as less likely. Indeed, an attempt to quench the cation **5i** by the addition of 2 mol equiv of methanol (0.25 equiv of $SnCl_4$, CH_2Cl_2, 10 h), resulted in the mixture of products (eq 2) containing the

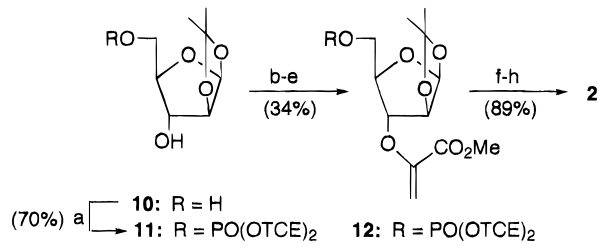


anomeric mixture (α and β) of methoxy pyranosides **9** (18%). This was also true for **5** (26%), **6** (32%), **7** (5%), and **8** (6.3%). Raising the amount of methanol to 4 mol equiv subsequently increased the percentage of the anomeric mixture of **9** (27%, 24 h). The reaction, using methanol (eq 3) as solvent (2.7 equiv of $SnCl_4$, 5 days), afforded a mixture of **8** (61%) and **9** (35%). Only trace amounts of **5** and **7** were detected by ^{1}H-NMR in this mixture. The same series of experiments were carried out using pure **5** (eqs 4 and 5). The aim of this series was to verify whether the methoxy pyranosides **9** form by direct quenching of the cationic intermediate **5i** with methanol, by acid-catalyzed methanolysis of bicyclic **5**, or by methylation of pyranoside derivatives of **6**. There was no discernible formation of methoxy pyranosides **9**, indicating that **9** should be formed only by the direct quenching of intermediate **5i** with methanol. The results

(16) (a) Charon, D.; Szabo, L. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2369. (b) Brade, H.; Zahringer, U.; Rietschel, E. T.; Christian, R.; Schulz, G.; Unger, F. M. *Carbohydr. Res.* **1984**, *134*, 157. (c) See also ref 2b.

(17) (a) Kresge, A. J.; Leibovitch, M.; Sikorski, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 2618. (b) Baasov, T.; Kohen, A. *J. Am. Chem. Soc.* **1995**, *117*, 6165.

Scheme 4[a]



[a] Key: (a) $(TCEO)_2P(O)Cl$, Py; (b) $N_2C(CO_2Me)_2$, $Rh_2(OAc)_4$, C_6H_6, reflux; (c) $[CH_2{=}NMe_2]^+ I^-$, Et_3N, CH_2Cl_2; (d) MeI, CH_2Cl_2; (e) DMSO, 100 °C, 4 h; (f) Zn, HOAc, MeOH; (g) Dowex 50W (H^+), D_2O; (h) 1 N KOH.

support the possibility that **5** is formed by a stepwise mechanism (eq 1), through the formation of a transient oxocarbenium intermediate **5i**, followed by the capture of the cation by the C-5 hydroxyl of the sugar moiety.

Synthesis and Examination of the Model Compound 2. We now had a new synthetic methodology in hand for the construction of the Kdo skeleton, via intramolecular condensation in an organic solvent. This was supported by the promising diastereoselectivity shown in the condensation step. In seeking to improve our model and more closely mimic the natural system, the following questions were addressed. (i) Can the same intramolecular condensation method be used in an aqueous solution, without the participation of protecting groups on the sugar substrate, for the direct chemical synthesis of the enzymatic reaction product, Kdo8P? (ii) What will the timing and stereochemical outcome of such a reaction be? In order to answer these questions, the model structure **2** was synthesized. Structure **2** satisfies most of the requirements of the solution studies. It bears the additional advantage of holding most of the functional groups of both enzyme substrates (A5P and PEP). Therefore, the prospect of examining its inhibitory and substrate properties in relation to Kdo8P synthase was very attractive.

Scheme 4 depicts our approach to the synthesis of **2**. First, the known[14] diol **10** was phosphorylated, with reasonable regioselectivity, to afford the protected phosphate **11**, in a 70% isolated yield. Compound **11** was subjected to a four-step sequence of reactions, similar to that employed in the preparation of tosylate **4**. The purpose of this was to introduce the enolpyruvate side chain on to the C-3 hydroxyl, resulting in enolpyruvyl phosphate **12**, in an overall 34% yield (for the four steps). In order to furnish the synthesis of target compound **2**, optimal conditions were achieved through stepwise deprotection of all the protecting groups. The best results were obtained using the following deprotection sequence: First, treatment of **12** with Zn−HOAc removed the trichloroethyl protections from the phosphate group. The isopropylidene protection was then removed by treatment of the observed phosphate with Dowex (H^+) resin in D_2O (36 h). Progress of the reaction was followed by ^{1}H-NMR. Finally, saponification of the carboxylic ester (KOH, H_2O, 4 °C) afforded the target **2** (89% after chromatography) as a mixture of anomers.

In order to devise appropriate conditions for the examination of the intramolecular condensation process in model compound **2**, the following arguments were considered: (i) We anticipated that if **2** was to undergo a stepwise condensation process similar to the previously examined compound **1** (eq 1) at pH≥5, the carboxylic acid group in **2** should be in its carboxylate form. Therefore, we would expect that a high rate of reaction may be caused due to stabilization of the positive charge generated on the transient, oxocarbenium ion intermediate. Recently reported acid-catalyzed hydrolysis studies of methyl α-methoxyacrylate[17a] and Kdo 2-phosphates[17b] have illustrated a similar inductive stabilizing effect of the α-carboxylate group. Therefore, when considering the possible positive role of the carboxylate function in the condensation process, we set out to use pH ≥ 5 as the working pH. (ii) In an attempt to accelerate the condensation step through the activation of the aldehyde carbonyl by a Lewis acid, we chose to add $ZnCl_2$ to the reaction mixture. Selection of Zn^{2+} as a Lewis acid seemed promising as zinc is a widely distributed ion and an essential component of many enzymes and proteins. However, the combination of the pH and Zn^{2+} restricted our work to a limited pH range (between 5 and 6). This is attributable to the fact that compound **2** precipitates at pH values >6, perhaps due to the formation of highly insoluble zinc phosphate salt. (iii) Compound **2** exists in water solutions as a mixture of two anomeric forms. The expected product of the intramolecular condensation, Kdo8P, also exists as a mixture of four isomers (α- and β-furanosides, 30%, and α- and β-pyranosides, 70%).[18] As the geminal C-3 protons of Kdo8P anomers are well separated in the region of 1.7−2.5 ppm, close monitoring of the condensation process by ^{1}H-NMR[19] was expected to be possible.

Condensation experiments using the model **2** were performed in water solutions containing 5 mol equiv of $ZnCl_2$. Progress of the reaction was monitored by ^{1}H-NMR (Figure 1A). As seen from these spectra, the characteristic resonances of **2** in the range of 5.1−5.35 ppm gradually decrease with time, and new resonances in the region of 1.7−2.5 ppm subsequently evolve. Careful analysis of the spectra reveals that the first product exclusively formed during the reaction is Kdo8P. Under the reaction conditions, the product suffers Lewis acid-catalyzed dehydration[20] to afford a mixture of bicyclic products **13** and **14** (in a ratio of 2:1). A trace amount of furoic acid derivative **15** was detected[21] during the later stage of the reaction (Scheme 5). To further support this conclusion, pure Kdo8P was synthesized enzymatically[22] and was subjected to the same reaction conditions as for the model **2** (Figure 1B). It is evident from the very

(18) Baasov, T.; Jacob, A. *J. Am. Chem. Soc.* **1990**, *112*, 4972.

(19) It is noteworthy that during early stages of our investigation, this procedure of monitoring the progress of the condensation reaction by ^{1}H-NMR (10 mM compound **2**, 50 mM $ZnCl_2$, 50 °C, pH 5, D_2O) was particularly troublesome. Initially, we could not discern any formation of expected resonances of Kdo8P in the region of 1.7−2.5 ppm, although the starting material (**2**) was monotonously converted to a mixture of products (as indicated by the disappearance of the resonances in the region of 5.1−5.35 ppm). This was very difficult to assign using the observed spectra. Upon close investigation we found that, under the reaction conditions, C-3 protons of Kdo8P undergo rapid exchange with the solvent (D_2O) protons to form 3,3-dideuterio-Kdo8P. This conclusion was further supported when the same reaction was performed using authentic Kdo8P (10 mM, 50 mM $ZnCl_2$, 50 °C, pH 5, D_2O), yielding a rapid exchange of geminal (C-3) protons (half-life of 10 min).

(20) The acid-catalyzed degradation of Kdo to form the furoic acid derivatives, as well as a bicyclic structure similar to **14**, has been previously reported: McNicolas, P. A.; Batley, M.; Redmond, J. W. *Carbohydr. Res.* **1987**, *165*, 17.

(21) The presence of furoic acid derivative **15** was determined by characteristic resonances of the furan ring protons (6.45 ppm, $J = 3.3$ Hz, and 6.89 ppm, $J = 3.3$ Hz) in ^{1}H-NMR of the reaction mixture and by comparison of these results with previously reported data for a similar structure.[20]

(22) Bednarski, M. D.; Crans, D. C.; DiCosimo, R.; Simon, E. S.; Stein, P. D.; Whitesides, G. M. *Tetrahedron Lett.* **1988**, *29*, 427.

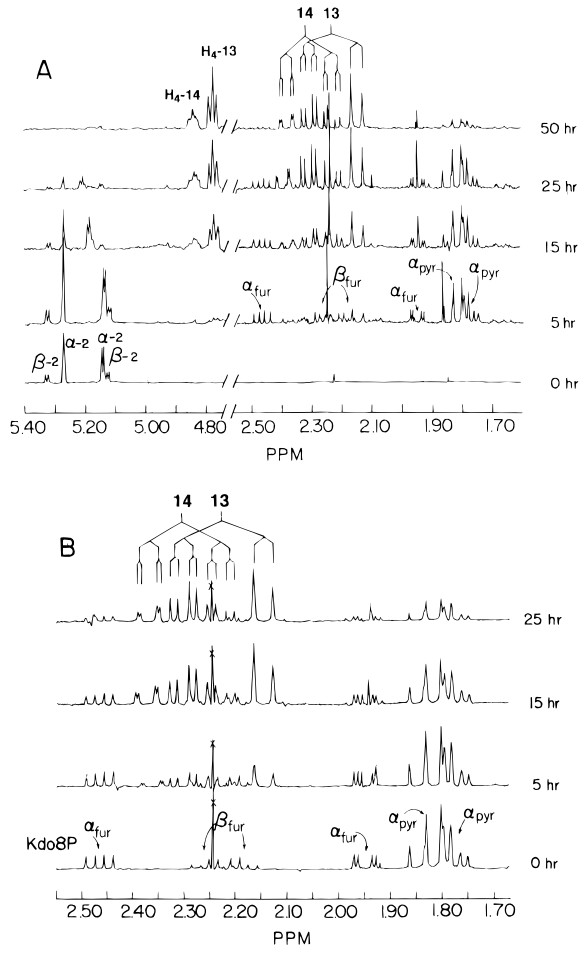


Figure 1. Time course of ^{1}H-NMR spectra showing (A) the conversion of **2** to Kdo8P followed by production of bicyclic products **13** and **14** and (B) the conversion of Kdo8P to a mixture of **13** and **14**. The experiments were performed in H_2O (pH = 5, 50 °C) in the presence of $ZnCl_2$ (50 mM) together with compound **2** (10 mM, spectra A) or with Kdo8P (10 mM, spectra B). Aliquots of the reaction mixture were withdrawn at various time intervals and immediately passed through a small (5 mL) column of Dowex 50W (H^+ form) in order to remove Zn^{2+} ions. The pH of the elute was adjusted to 3.8 (0.1 N NaOH) and rapidly concentrated under a high vacuum. The residue was dissolved in D_2O (0.5 mL), and the ^{1}H-NMR spectrum was recorded on a Bruker WH-400 (referenced to HOD at 4.63 ppm).

similar time course obtained in this experiment that Kdo8P is indeed unstable under the reaction conditions. It suffers a further transformation to form the mixtures **13** and **14**. In addition, the observed resonances in the region of the geminal C-3 protons (1.7−2.5 ppm, Figure 1A) following 5 h of treatment of compound **2** are identical to those of authentic Kdo8P (Figure 1B, the spectrum at zero time). This indicates that at first the model **2** undergoes intramolecular condensation between the enolpyruvate double bond and aldehyde functions to form Kdo8P. The bicyclic structures of **13** and **14** were assigned using a combination of ^{1}H NMR, ^{13}C NMR, and two-dimensional (^{1}H−^{1}H) COSY analyses and by comparison with other known, similar structures.[23]

The half-life ($t_{1/2}$) for the conversion of **2** to Kdo8P was estimated to be about 5 h. In the production of the mixture **13** and **14**, the half-life for the dehydration of Kdo8P was approximately 15 h. It is worth noting that a conversion such as this, the formation of **13** and **14** from Kdo8P, is largely catalyzed by Zn^{2+} ions. This is indicated by the fact that under the same reaction conditions, but without the inclusion of Zn^{2+}, Kdo8P was predominantly stable. The constant 2:1 ratio of these two bicyclic structures, observed during the entire course of the reaction, suggests that both are formed from the same reaction intermediate. This intermediate is most likely the furanose structure of Kdo8P, which after dehydration forms the stable oxocarbenium intermediate IV (Scheme 6). This intermediate cation may be captured intramolecularly, either by the addition of a side chain C-6 hydroxyl, leading to the formation of bicyclic **13** (path a), or by the addition of a C-7 hydroxyl, leading to the formation of bicyclic **14** (path b). Alternatively, IV can undergo the elimination of a second molecule of water to form the stable, furoic acid derivative **15**.

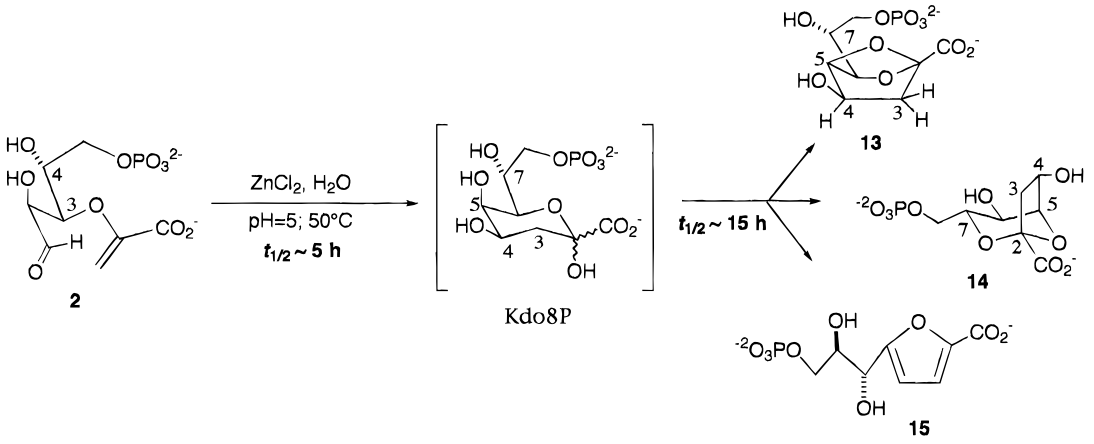
The Intramolecular Condensation Mechanism in a Water Solution. The observed results clearly demonstrate that **2** undergoes intramolecular condensation in a water solution to exclusively form the desired structure of Kdo8P. As formation of the C-4 epimer of Kdo8P was not detected among the reaction products, it appears that this condensation process is highly stereospecific. This was also demonstrated for the model **1** in organic solvents (see above). In order to further investigate the diastereoselectivity of this reaction, the same reaction (10 mM compound **2**, pH = 5, 50 °C) was induced without the addition of $ZnCl_2$, and its progress was monitored by ^{1}H-NMR. Under these conditions the condensation process was largely retarded (the half-life of the disappearance of **2** was estimated to be about 22 h). It was very interesting to find that in addition to the hydrolytic degradation products of **2** (A5P and pyruvate, both about 32% of total products), a mixture of Kdo8P and 4-*epi*-Kdo8P[24] was also detected (in a ratio of about 55:45, approximately 68% of total products). However, the same reaction (10 mM compound **2**, 50 °C, no $ZnCl_2$ added) at pH = 1.6 virtually abolished the condensation process. After 60 h of incubation, only trace amounts of Kdo8P and 4-*epi*-Kdo8P were obtained, alongside the main products, A5P and pyruvate—the hydrolytic degradation products of **2**.

The above results clearly demonstrate the dual role of Zn^{2+} ions in the condensation process of **2**. The addition of Zn^{2+} at pH = 5 accelerates the reaction roughly 5-fold

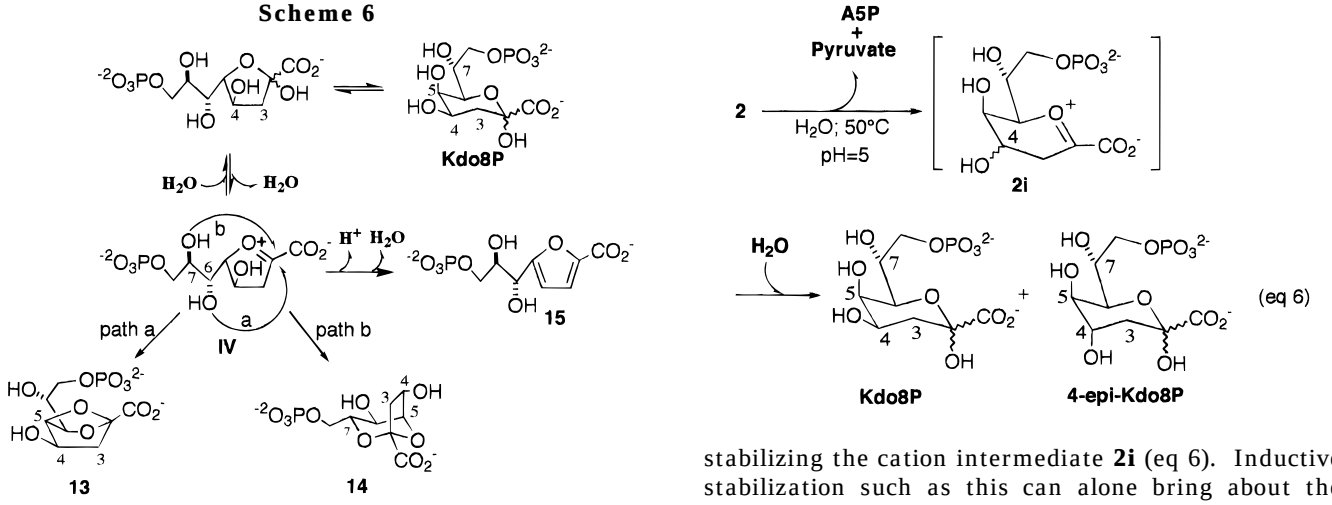
(23) The structure of **13** was assigned on the basis of a very characteristic broad doublet of $H_{3a'}$ (bd, ^2J = 14.8 Hz, $^3J \leq 1$ Hz), indicating the pseudoaxial orientation of this proton relative to H_4, with a dihedral angle of about 90°. The same couplings of $H_{3a'}$ were recorded for the bicyclic **5**, whose structure was unambigously determined by single-crystal X-ray analysis.[13] The structure of **14** was confirmed by the characteristic coupling constants of H-3 and H-3′ in the ^{1}H-NMR and by comparison of its ^{1}H- and ^{13}C-NMR data to that of a similar structure.[20]

(24) Purification of this mixture by ion-exchange chromatography [AG1 × 8 (HCO_3^- form), eluting with a linear gradient of triethylammonium bicarbonate buffer (0−0.7 M, pH 7.5] resulted in a good separation of A5P and pyruvate, but the Kdo8P and 4-*epi*-Kdo8P were still obtained as the same mixture (by ^{1}H-NMR) as in the crude reaction. Attempts to use other buffer systems or another ion-exchange resin (DEAE Sephadex) gave similar results. Nevertheless, the structure of 4-*epi*-Kdo8P was determined on the basis of coupling constants of the major α-pyranose anomer in the ^{1}H-NMR of the reaction mixture and by comparison of the observed data with those previously reported for a similar structure (Auge, C.; Bouxom, B.; Cavaye, B.; Gautheron, C. *Tetrahedron Lett.* **1989**, *30*, 2217). The resonances of this anomer in the region of C-3 protons were well separated from that of Kdo8P anomers and had small H_3−H_4 couplings (^3J = 2.7 and 3.8 Hz) and large geminal (H_{3a}−H_{3e}) couplings (^2J = 15.0 Hz), indicative of the presence of an axial C-4 hydroxyl group. In addition, the close and upfield signals of C-3 protons (1.70 and 2.06 ppm) were consistent with the pyranose structure of 4-*epi*-Kdo8P.

Scheme 5



Scheme 6



and results in very high diastereoselectivity. The observed *erythro* selectivity in aldose **2** at the carbon, with the newly generated hydroxyl group (C-1), relative to the hydroxyl group at C-2, is the same as that shown in the $SnCl_4$-assisted, intramolecular condensation in model **1**. Both are in accordance with the chelation−Cram model.[25]

As to whether the formation of new C−C and C−O bonds in Kdo8P is a synchronous or stepwise process, the results observed suggest that the intramolecular condensation of **2** in water is similar to that of model compound **1** in an organic solvent (eq 1). Both follow a stepwise process that involves the formation of a transient oxocarbenium intermediate (eqs 1 and 6). Such an intermediate, in an organic solvent (**5i**, eq 1), carries an α-linked carboxylic ester group. This causes destabilization of the cation, due to its electron-withdrawing character. Therefore, in order to observe condensation using **1**, we must employ a strong Lewis acid such as $SnCl_4$. The weaker acids, such as $LiClO_4$ and $ZnCl_2$, do not result in any condensation products, even at higher temperatures.[26] In contrast to model **1**, at pH = 5 the carboxylic acid in model **2** should be in its carboxylate form, thus stabilizing the cation intermediate **2i** (eq 6). Inductive stabilization such as this can alone bring about the condensation process, even without the addition of a Lewis acid (formation of a mixture of Kdo8P and 4-*epi*-Kdo8P in the absence of $ZnCl_2$). At pH = 1.6, however, the observed rate of retardation (as compared to that at pH = 5) most likely reflects the electron-withdrawing effect of the neutral carboxylic acid, which would destabilize the intermediate **2i**. These results are in agreement with the previously reported catalytic role of the α-carboxylate group in the acid-catalyzed hydrolysis of α-methoxyacrylate[17a] and Kdo 2-phosphates.[17b] They strongly support, therefore, the idea of a stepwise mechanism for the condensation of **2** in a water solution, as illustrated in eq 6.

Interaction of model 2 with Kdo8P Synthase. The acyclic form of **2** bears a strong similarity to the proposed situation (Scheme 1) whereby two substrates, A5P and PEP, evolve into a ternary complex with the synthase. It is, therefore, of considerable interest to verify whether this acyclic form may be accepted by the enzyme as a substrate. This would mean that the enzyme could catalyze intramolecular condensation in **2** and generate its natural product Kdo8P. Toward this goal, **2** was incubated (0.1 M Tris−HCl buffer, pH 7.3, 37 °C) with highly concentrated, homogeneous Kdo8P synthase. The synthase was at a level of concentration 1000 times greater than that normally introduced into an assay experiment. Progress of the reaction was monitored by ^{31}P-NMR over a 24-h period. However, no difference was detected between the observed spectra and those of the blank experiment (run parallel, under the same conditions minus the enzyme). Neither a further increase in enzyme concentration (up to 10 mg of enzyme per

(25) (a) Cram, D. J.; Abd Elhafez, *J. Am. Chem. Soc.* **1952**, *74*, 5828. (b) Reetz, M. T. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 556.

(26) During the initial steps of our investigation with compound **1**, we screened other Lewis acids, in addition to $SnCl_4$. These included $LiClO_4$ and $ZnCl_2$, which performed in ether and in THF/H_2O as solvents, and contained up to 5 mol equivs of each, relative to **1**. The reaction temperature was varied from rt up to the reflux of the corresponding solvent. No significant change was detected in the starting material **1** in either case, even after 12 h reflux, as determined by TLC.

experiment) nor the addition of inorganic phosphate[27] altered the results. No enzyme-catalyzed acceleration in the condensation process was detected. Competition experiments have revealed, however, that **2** is a competitive inhibitor against A5P binding ($K_i = 200 \pm 10$ μM) but is uncompetitive against PEP binding ($K_i = 10 \pm 1$ μM). The binding results indicate that **2** is a moderate inhibitor of the enzyme, and inhibition patterns suggest that **2** behaves as a simple A5P-based substrate−analog inhibitor.[28] This allows for the familiar, sequentially ordered kinetic mechanism of the synthase (PEP binding is followed by A5P binding).[29] It also indicates that **2** specifically interacts with the A5P binding site but has little, if any, interaction with the PEP binding site. The observed inability of **2** to serve as a substrate further supports this explanation.

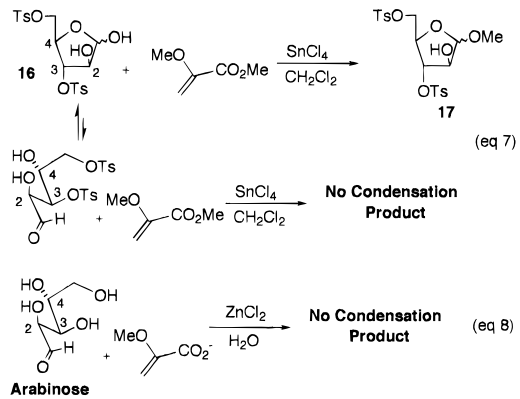
In summary, two model compounds (**1** and **2**) were prepared in order to test the hypothesis that catalysis by proximity is a predominate mechanism in the initial condensation step between A5P and PEP, catalyzed by Kdo8P synthase. We have shown that installation of the enolpyruvate moiety in precise proximity to the aldehyde function leads to a rapid, Lewis acid-promoted addition of the enolpyruvate double bond to the aldehyde carbonyl, in an intramolecular manner.[30] The condensation process was highly diastereospecific at the newly formed stereogenic center (C-4) and took place according to a stepwise mechanism, via the participation of a transient oxocarbenium ion intermediate. We have also shown that the carboxylic acid of the enolpyruvate moiety affords catalysis and is more effective when in the carboxylate form than when unionized. This suggests that the PEP may be recognized at the enzyme active site, so as to maintain the ionized form of its carboxylate group during catalysis. Our results are a unique demonstration of enzyme-like chemistry, apparent in the stereospecific synthesis of the Kdo system (in both organic solvents and a water solution). This certainly warrants further investigation in order to facilitate the construction of other 3-deoxy-2-ulosonic acids and sialic acids, based on the same general model as shown here in the case of Kdo. Furthermore, the results support the validity of the Kdo8P synthase mechanism in Scheme 1, particularly, the possible role of the enzyme at the initial condensation step and the feasibility of the formation of transient intermediate I. Thus, the fact that the catalytic groups of A5P and PEP are held at bonding distances, in conjunction with minor, 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. A more detailed characterization of the initial steps of this enzyme catalysis, using new models that will include the phosphate moiety of PEP, is the subject of an ongoing study.

(27) The coaddition of inorganic phosphate (up to 5 mM) to the reaction mixture containing compound **2** and the enzyme, was expected to cause a noncovalent occupation of the site of the enzyme which is naturally occupied by the phosphate of PEP and thus may promote enzyme catalysis.

(28) For a similar example of EPSP synthase inhibition, see: Marzabadi, M. R.; Gruys, K. J.; Pansegrau, P. D.; Walker, M. C.; Yuen, H. K.; Sikorski, J. A. *Biochemistry* **1996**, *35*, 4199.

(29) Kohen, A.; Jakob, A.; Baasov, T. *Eur. J. Biochem.* **1992**, *208*, 443.

(30) It is noteworthy that, in order to quantify the intramolecular benefit of the condensation process in the models **1** and **2**, we have attempted to measure the effective molarity[11] parameters of these systems by comparison to the corresponding intermolecular reactions. As an intramolecular counterpart of model **1**, we used 3,5-bistosyl arabinose **16** [prepared in two steps from **3**: TsCl, pyridine, 90%; HOAc/H_2O/THF, 70 °C, 12 h, 70%]. It was reacted with methyl α-methoxyacrylate[31] under the same conditions ($SnCl_4$, CH_2Cl_2, 0 °C). There was no discernible formation of the expected condensation product over a 24-h period, and when the reaction mixture was warmed to room temperature (with the presence of 3 mol equiv of $SnCl_4$), only methoxy furanosides of the starting bistosylate (α-**17** and β-**17**) were isolated. In order to test the intermolecular counterpart of model **2**, D-arabinose was treated with α-methoxyacrylate [5 mol equiv $ZnCl_2$, pH 5.0] at two different temperatures: 50 and 90 °C. After 50 h, we had not detected any possible condensation product in either reactions. During this time the arabinose remained unchanged and α-methoxyacrylate decomposed to form pyruvic acid. Thus, although at this stage of the investigation the observed results do not, unfortunately, allow us to provide any numerical value to represent the advantage of our intramolecular models (**1** and **2**) over their intermolecular counterparts, the data clearly demonstrate the importance of the proximity factor in the occurrence of condensation.



Experimental Section

General Methods. Kdo8P synthase (specific catalytic activity 9 U/mg) was isolated from overproducing strain *Escherichia coli* DH5α (pJU1). The plasmid pJU1, containing the *kdsA* gene,[32] was provided by Professor J. R. Knowles. The purification procedure followed the protocol of Ray[9] with some modifications, as previously described.[10b] A5P and Kdo8P were prepared enzymatically according to the procedure of Whitesides.[22] Methyl α-methoxyacrylate and α-methoxyacrylate were prepared by a previously reported procedure.[31] All other chemicals were received from Aldrich or from Sigma and used without further purification, unless noted. In all the synthetic work described, reactions were performed under dry argon atmosphere unless otherwise noted. All solvents were dried over standard drying agents[33] and freshly distilled prior to use. Flash column chromatography[34] was performed on silica gel 60 (70−230 mesh). Reactions were monitored by TLC on silica gel 60 F_{254} (0.25 mm, Merck) and detected by charring with a yellow solution containing $Na_2MoO_4 \cdot 4H_2O$ (5 g) and cerric ammonium nitrate (5 g) in 10% H_2SO_4 (300 mL). Soviet spray[35] solution was used for the detection or qualitative analysis of the compounds containing phosphate monoesters.

Spectrophotometric measurements were made on a Hewlett-Packard 8452A diode array spectrophotometer using 1-cm pathlength cells with a thermostated cell holder and circulating water bath at desired temperature. Mass spectra were obtained using a TSQ-70B mass spectrophotometer (Finnigan Mat) under fast atom bombardment (FAB) in the glycerol matrices.

1,2-*O*-Isopropylidene-3-*O*-[1-(methoxycarbonyl)-ethenyl]-5-*O*-(toluenesulfonyl)-β-D-arabinofuranose (4). To a solution of tosylate **3**[14] (5.2 g, 15 mmol) in dry benzene (130 mL) was added rhodium tetraacetate (100 mg). The green mixture was heated at reflux, and then dimethyl diazomalonate (8.2 g, 30 mmol) was added dropwise for 30 min. This

(31) Ogata, N.; Nozakura, S.; Murahashi, S. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 2987.

(32) Woisetschlager, M.; Hogenauer, G. *J .Bacteriol.* **1986**, *168*, 437.

(33) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, 1988.

(34) Still, W. C.; Kahn, M; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(35) Vaskovsky, V. E.; Latyshev, N. A. *J. Chromatogr.* **1975**, *115*, 246.

mixture was refluxed for 3.5 h, cooled, and then filtered through Celite. After thorough washing of the Celite with ethyl acetate, the washes were combined and evaporated to afford 13.4 g of a brown oil. Purification by chromatography on silica gel (EtOAc/hexane 1:1) afforded 5.8 g of the corresponding malonate ester (80.6% yield): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.26 (s, 3H, isopropylidene protons), 1.35 (s, 3H, isopropylidene protons), 2.42 (s, 3H, CH_3 of Ts), 3.80 (s, 3H, CO_2CH_3), 3.81 (s, 3H, CO_2Me), 4.07 (d, 1H, J = 2.6 Hz, H-3), 4.12 (dd, 1H, J = 10.2, 5.8 Hz, H-5), 4.16 (dd, 1H, J = 10.2, 6.3 Hz, H-5′), 4.24 (ddd, 1H, J = 6.3, 5.8, 2.6 Hz, H-4), 4.62 (s, 1H, CHCO$_2$Me), 4.65 (d, 1H, J = 3.9 Hz, H-2), 5.83 (d, 1H, J = 3.9 Hz, H-1), 7.32 (d, 2H, J = 8.1 Hz, aromatic protons), 7.77 (d, 2H, J = 8.1 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ 21.57 (Me of Ts), 26.11 and 26.79 (Me of isopropylidene), 53.08 (CO_2CH_3), 68.07, 77.92, 81.81, 83.74, 84.81, 105.77 (C1), 113.18 ($C(CH_3)_2$), 128.05, 129.89, 132.70, 145.03 (aromatic carbons), 166.16 (CO_2CH_3), 166.22 (CO_2CH_3).

The above-mentioned malonate (5.8 g, 0.012 mol) was dissolved in dry dichloromethane (80 mL), to which triethylamine (2.8 mL) and dimethylmethyleneammonium iodide (2.8 g, 0.015 mol) were added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2, washed with water and saturated NaCl, dried, and evaporated to give the corresponding Mannich base. This material, without further purification, was dissolved in dry dichloromethane (140 mL), and 15 mL of freshly distilled methyl iodide was added. The reaction mixture was protected from the light and stirred at room temperature for 24 h. The mixture was then evaporated to dryness, and the residue obtained was dissolved in freshly distilled DMSO (6.5 mL). This mixture was heated at 100 °C for 30 min. The solvent was evaporated under reduced pressure and the residue dissolved in dichloromethane. The insoluble impurity (white powder) was filtered off, the solution was evaporated, and the compound was purified by chromatography on a silica gel column (EtOAc/hexane, 1:1) to give pure product **4** (2.34 g, 36% yield referring to compound **3**): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.27 (s, 3H, isopropylidene protons), 1.41 (s, 3H, isopropylidene protons), 2.42 (s, 3H, Me of Ts), 3.77 (s, 3H, CO_2CH_3), 4.12 (dd, 1H, J = 10.1, 5.0 Hz, H-5), 4.23 (dd, 1H, J = 10.1, 7.2 Hz, H-5′), 4.31 (ddd, 1H, J = 7.2, 5.0, 2.6 Hz, H-4), 4.37 (d, J = 2.6 Hz, H-3), 4.58 (d, 1H, J = 4.1 Hz, H-2), 4.81 (d, 1H, J = 3.3 Hz, vinylic proton), 5.47 (d, 1H, J = 3.3 Hz, vinylic proton), 5.86 (d, 1H, J = 4.1 Hz, H-1), 7.32 (d, 2H, J = 8.3 Hz, aromatic protons), 7.77 (d, 2H, J = 8.3 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 50.3 MHz), δ 21.57 (Me of Ts), 26.25 and 26.65 (2Me of isopropylidene), 52.41 (CO_2CH_3), 67.89, 80.92, 81.00, 83.95, 97.64 (β-vinylic carbon), 105.85 (C1), 113.18 ($C(CH_3)_2$), 128.02, 129.94, 132.64, 145.12 (aromatic carbons), 149.24 (α-vinylic carbon), 162.86 (CO_2Me); negative CIMS m/z 428.0 (M^-, $C_{19}H_{24}O_9S$ requires 428.45).

3-*O*-[1-(Methoxycarbonyl)ethenyl]-5-*O*-(toluenesulfonyl)-D-arabinofuranose (1). A stirred solution of acetonide **4** (1.26 g, 2.95 mmol) in 60 mL of a mixture of acetic acid/water/tetrahydrofuran (65:35:10) was heated at 70 °C for 12 h. The reaction mixture was diluted with EtOAc (60 mL), washed with saturated Na_2CO_3, dried ($MgSO_4$), and concentrated. The crude product was purified by column chromatography (EtOAc/hexane, 2:1) to afford an anomeric mixture of the target diol **1** as a clear oil (1.06 g, 93%): ^{1}H-NMR (400M Hz, $CDCl_3$) data for β-**1**, δ 2.42 (s, 3H Me of Ts), 3.76 (s, 3H, CO_2CH_3), 4.14−4.20 (m, 3H, H-2, H-5 and H-5′), 4.28−4.30 (m, 1H, H-4), 4.32 (bs, 1H, H-3), 4.91 (d, 1H, J = 3.2 Hz, vinyl proton), 5.41 (d, 1H, J = 4.2 Hz, H-1), 5.50 (d, 1H, J = 3.2 Hz, vinyl proton), 7.33 (d, 2H, J = 8.1 Hz, aromatic protons), 7.75 (d, 2H, J = 8.1 Hz, aromatic protons); α-**1**, 2.42 (s, 3H, Me of Ts), 3.76 (s, 3H, CO_2CH_3), 4.13 (dd, 1H, J = 8.4, 4.8 Hz, H-5), 4.15−4.19 (m, 2H, H-2 and H-3), 4.26 (t, 1H, J = 5.2 Hz, H-4), 4.51 (dd, 1H, J = 8.4, 4.8 Hz H-5′), 4.97 (d, 1H, J = 3.0 Hz, vinyl proton), 5.27 (s, 1H, H-1), 5.46 (d, 1H, J = 3.0 Hz, vinyl proton), 7.33 (d, 2H, J = 8.1 Hz, aromatic protons), 7.75 (d, 2H, J = 8.1 Hz, aromatic protons); ^{13}C-NMR (50.3 MHz, $CDCl_3$) data for the anomeric mixture of **1**, δ 21.46 (Me of Ts), 52.38 and 52.55 ($2CO_2CH_3$), 68.77 and 69.33 (2C5), 74.95, 77.99, 78.57, 79.92, 82.95, 83.03, [97.31, 98.58, 98.63, 103.49, (2C1 and 2β-vinylic carbons)], [127.86, 129.89, 132.38, 132.42, 145.16, (aromatic carbons)], 149.96 and 149.29 (α-vinylic carbons), 163.34 and 163.48 ($2CO_2CH_3$); CIMS m/z 389.0 (MH^+, $C_{16}H_{21}O_9S$ requires 389.4).

Intramolecular Condensation of Model 1. Procedure A. The anomeric mixture of **1** (88 mg 0.227 mmol) in CH_2Cl_2 (4 mL) was treated with stannic chloride (18.5 mg, 0.072 mmol) at 0 °C. After 5 h at 0 °C, the reaction mixture was diluted with CH_2Cl_2 (10 mL) and EtOAc (20 mL) and neutralized with diluted $NaHCO_3$. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried ($MgSO_4$) and concentrated. The silica gel chromatography of the crude (EtOAc/hexane, 1.5:1) afforded the bicyclic **5** (60 mg, 68.2%) along with unreacted diol **1** (13 mg, 14.8%). Data for **5**: ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.88 (d, 1H, J = 13.5 Hz, H-3a′), 2.43 (s, 3H, CH_3 of Ts), 2.48 (dd, 1H, J = 13.5, 6.6 Hz, H-3e′), 3.51 (d, 1H, J = 8.2 Hz, H-6), 3.66 (m, 1H, H-7), 3.83 (s, 3H, CO_2CH_3), 4.04 (dd, 1H, J = 10.8, 5.6 Hz, H-8), 4.19 (dd, 1H, J = 10.8, 2.2 Hz, H-8′), 4.22 (d, 1H, J = 6.6 Hz, H-4), 4.84 (s, 1H, H-5), 7.32 (d, 2H, J = 8.1 Hz, aromatic protons), 7.75 (d, 2H, J = 8.1 Hz, aromatic protons); Ha′ and He′ 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; ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ 21.61 (Me of Ts), 46.46 (C3), 53.09 (CO_2CH_3), 69.08, 70.73, 71.42, 74.96, 84.05, 104.59 (C2), 128.03, 129.94, 132.40, 145.18, 165.48 (C1); CIMS m/z 389.2 (MH^+, $C_{16}H_{21}O_9S$ requires 389.4).

Procedure B. To a solution of diol **1** (84.3 mg, 0.217 mmol) in CH_2Cl_2 (8 mL) at 0 °C was added a solution of $SnCl_4$ (14 mg, 0.053 mmol) in CH_2Cl_2 (0.3 mL). The reaction mixture was allowed to warm at room temperature, and the reaction progress was monitored by TLC [silica gel, EtOAc/hexane (4:1), the R_f of **1**, **5**, and **6** were 0.56, 0.35, and 0.12, respectively]. At the beginning of the reaction, mainly the bicyclic **5** was formed. This was in time gradually transformed to ketose **6**. After approximately 8 h all of the starting material (**1**) was gone, and a repetition of the workup and purification procedure used in A afforded the bicyclic **5** (17.8 mg, 20.2%) and the ketose **6** (57.4 mg, 62.3%). Data for **6**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.86 (dd, J = 12.8, 4.7 Hz, H-3e of α-Py), 2.12 (dd, J = 14.2, 2.2 Hz, H-3e′ of α-Fu), 2.12 (dd, J = 12.8, 11.4 Hz, H-3a of α-Py), 2.37−2.39 (m, Me of Ts, and H-3′ of β-Fu), 2.46 (dd, J = 13.9, 6.1 Hz, H-3′′ of β-Fu), 2.58 (dd, J = 14.2, 6.7 Hz, H-3a′ of α-Fu), 3.73 (CO_2CH_3), 3.75 (CO_2CH_3), 3.77 (CO_2CH_3), 3.92 (d, J = 9.0 Hz), 3.97−4.31 (m), 7.29 (d, J = 8.0 Hz, aromatic protons), 7.73−7.76 (m, aromatic protons); CIMS m/z 407.0 (MH^+, $C_{16}H_{23}O_{10}S$ requires 407.4).

Methyl [2-Methoxy-8-*O*-(toluenesulfonyl)-3-deoxy-D-*manno*-2-octulofuranosid]onate (7). *p*-Toluenesulfonic acid (20 mg, 0.1 mmol) was added to a solution of bicyclic **5** (26.5 mg, 0.068 mmol) in dry MeOH (2 mL). After being stirred for 12 h at room temperature, the reaction mixture was diluted with EtOAc, washed with saturated Na_2CO_3 solution, dried over $MgSO_4$, and concentrated under a vacuum. Chromatography of the oily residue (EtOAc/hexane, 2:1) furnished **7** (26.4 mg, 92%) as mixture of α- and β-anomers [R_f 0.29, EtOAc/hexane (4:1)]. This anomeric mixture could only be separated using preparative TLC plates (silica gel, 5% MeOH/$CHCl_3$, four runs). Data for α-**7**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.23 (dd, 1H, J = 14.7, 1.2 Hz, H-3a′), 2.41 (dd, 1H, J = 14.7, 6.6 Hz, H-3e′), 2.44 (s, 3H, CH_3 of Ts), 3.33 (s, 3H, OMe), 3.56−3.62 (m, 1H, H-6), 3.80 (s, 3H, CO_2CH_3), 3.82−3.88 (m, 1H, H-7), 4.18 (dd, 1H, J = 10.4, 6.1 Hz, H-8), 4.32 (dd, 1H, J = 10.4, 3.3 Hz, H-8′), 4.34 (d, 1H, J = 6.5 Hz, H-4), 4.49 (s, 1H, H-5), 7.34 (d, 2H, J = 8.1 Hz, aromatic protons), 7.79 (d, 2H, J = 8.1 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ 21.63 (Me of Ts), 45.72 (C3), 51.81 and 53.13 (OCH_3 and CO_2CH_3), 70.65, 71.09, 71.64,73.95, 88.73, 106.98 (C2), 128.04, 129.97, 132.67, 145.15 (aromatic carbons), 170.08 (C1). Data for β-**7**: ^{1}H NMR($CDCl_3$, 400 MHz) δ 2.32 (dd, 1H, J = 13.6, 6.8 Hz, H-3a′), 2.54 (dd, 1H, J = 13.6, 7.0 Hz, H-3e′), 2.44 (s, 3H, CH_3 of Ts), 3.29 (s, 3H, OMe), 3.55−3.61 (m, 1H, H-6), 3.81 (s, 3H, CO_2CH_3), 3.82−3.85 (m, 1H, H-7), 4.19 (dd, 1H, J = 10.5, 6.1 Hz, H-8), 4.26 (t, 1H, J = 5.0 Hz, H-5), 4.34 (dd, 1H, J = 10.5, 2.0 Hz, H-8′), 4.49 (ddd, 1H, J = 7.0, 6.8, 5.0 Hz, H-4), 7.34 (d,

2H, J = 8.1 Hz, aromatic protons), 7.79 (d, 2H, J = 8.1 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ 21.64 (Me of Ts), 44.58 (C3), 51.95, 52.86 (OCH_3 and CO_2CH_3)), 71.09, 71.75, 72.04, 88.73 (C4, C5, C6, C7, C8), 106.05 (C2), 128.04, 130.00, 132.77, 145.24 (aromatic carbons), 169.31 (C1); CIMS m/z 421.0 (MH^+, $C_{17}H_{25}O_{10}S$ requires 421.4).

Quenching Experiments with MeOH. (i) Intramolecular Condensation of 1 in the Presence of 2 Mol Equiv MeOH. To a solution of **1** (54.5 mg, 0.14 mmol) in dry CH_2Cl_2 (4.5 mL) was added freshly distilled dry MeOH (9 mg, 0.28 mmol) in CH_2Cl_2 (0.5 mL). The mixture was cooled at 0 °C, and $SnCl_4$ (9.2 mg, 0.037 mmol) in CH_2Cl_2 (0.5 mL) was introduced dropwise. The reaction progress was monitored by TLC [silica gel, EtOAc/hexane (4:1), R_f 0.56 (**1**), 0.35 (**5**), 0.12 (**6**), 0.29 (α-**7** + β-**7**), 0.70 (α-**8**), 0.77 (β-**8**), 0.12 (α-**9**), and 0.18 (β-**9**)], and the mixture was stirred at room temperature until all the starting material had been consumed (10 h). After an usual workup, as in the original procedure, the ^{1}H-NMR spectrum of the crude showed the following distribution of products (as was determined by the integration of the characteristic protons of each isomer): **5** (26%), **6** (32%), **7** (5%), **8** (6.3%), **9** (18%). This mixture was completely separated by twice repeating the chromatography on silica gel columns with EtOAc/hexane (2:1) as an eluent, in conjunction with one preparative TLC (silica gel, 5% MeOH/$CHCl_3$). Data for α-**8**: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.43 (s, 3H, Me of Ts), 3.32 (s, 3H, OMe), 3.76 (s, 3H, CO_2CH_3), 4.09−4.13 (m, 2H, H-2, H-5), 4.21 (dd, 1H, J = 11.2, 3.3 Hz, H-5′), 4.37−4.40 (m, 2H, H-3, H-4), 4.85 (s, 1H, H-1), 4.88 (d, 1H, J = 2.6 Hz, vinyl proton), 5.46 (d, 1H, J = 2.6 Hz, vinyl proton), 7.33 (dd, 2H, J = 8.1 Hz, aromatic protons), 7.78 (dd, 2H, J = 8.1 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ 21.62 (Me of Ts), 52.39 (CO_2CH_3), 54.95 (OMe), 68.56 (C5), 78.97, 79.66, 84.05, 98.96 (β-vinylic carbon), 109.37 (C1), 128.00, 129.94, 132.94, 145.12 (aromatic carbons), 150.24 (α-vinylic carbon), 163.28 (CO_2CH_3). Data for β-**8**: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.43 (s, 3H, Me of Ts), 3.39 (s, 3H, OMe), 3.76 (s, 3H, CO_2CH_3), 4.08 (dd, 1H, J = 10.1, 6.0 Hz, H-5), 4.14 (dd, 1H, J = 10.1 and 5.4 Hz, H-5′), 4.21−4.26 (m, 2H, H-2 and H-4), 4.29 (dd, 1H, J = 4.0, 3.6 Hz, H-3), 4.93 (d, 1H, J = 4.7 Hz, H-1), 5.04 (d, 1H, J = 2.6 Hz, vinyl proton), 5.48 (d, 1H, J = 2.6 Hz, vinyl proton), 7.33 (dd, 2H, J = 8.1 Hz, aromatic protons), 7.78 (dd, 2H, J = 8.1 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ 21.61 (Me-Ts), 52.37 (CO_2CH_3), 54.90 (OMe), 69.38 (C5), 76.75, 79.15, 83.95, 99.40 (β-vinylic carbon), 103.19 (C1), 127.98, 129.91, 132.86, 145.04 (aromatic carbons), 149.77 (α-vinyl carbon), 163.28 (CO_2CH_3); CIMS m/z 403.0 (MH^+, $C_{17}H_{23}O_9S$ requires 403.4).

Data for α-**9**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.83 (dd, 1H, J_1 = J_2 = 12.5 Hz, H-3a), 2.06 (dd, 1H, J = 12.5, 5.0 Hz, H-3e), 2.44 (s, 3H, Me of Ts), 3.13 (s, 3H, OMe), 3.61 (d, 1H, J = 8.5 Hz, H-6), 3.77 (s, 3H, CO_2CH_3), 4.00 (dd, 1H, J = 12.5, 5.0 Hz, H-4), 4.02 (bs, 1H, H-5), 4.18 (ddd, 1H, J = 8.5, 4.5, 4.5 Hz, H-7), 4.26−4.27 (m, 2H, H-8 and H-8′), 7.34 (d, 2H, J = 8.0 Hz, aromatic protons), 7.79 (d, 2H, J = 8.0 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 21.63 (Me of Ts), 34.61 (C-3), 51.30 (OMe), 52.74 (CO_2CH_3), 65.69 (C-8), 66.25 (C-4), 67.80 (C-5), 70.78, 70.96, 99.42 (C-2), 128.00, 129.97, 132.65, 145.15 (aromatic carbons), 168.87 (C-1). Data for β-**9**: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.89 (dd, 1H, J_1 = J_2 = 12.5 Hz, H-3a), 2.37 (dd, 1H, J = 12.5, 4.6 Hz, H-3e), 2.43 (s, 3H, Me of Ts), 3.24 (s, 3H, OMe), 3.62 (d, 1H, J = 8.3 Hz, H-6), 3.67−3.70 (m, 1H, H-4), 3.79 (s, 3H, CO_2CH_3), 3.99 (bs, 1H, H-5), 4.15 (dd, 1H, J = 8.3, 6.0 Hz, H-7), 4.19 (dd, 1H, J = 10.0, 6.0 Hz, H-8), 4.31 (d, 1H, J =10.0 Hz, H-8′), 7.33 (d, 2H, J = 8.0 Hz, aromatic protons), 7.80 (d, 2H, J = 8.0 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ 21.61 (Me of Ts), 34.72 (C-3), 51.64 (OMe), 52.55 (CO_2CH_3), 65.89 (C8), 66.99 (C5), 68.43 (C4), 71.61, 73.56, 99.62 (C2), 128.00, 129.91, 132.76, 145.10 (aromatic carbons), 168.66 (C1); FAB mass spectrum m/z 420.9 (MH^+, $C_{17}H_{25}O_{10}S$ requires 421.4).

(ii) Intramolecular Condensation of 1 in MeOH. The diol **1** (29.3 mg, 0.076 mmol) was dissolved in freshly distilled dry MeOH (2 mL). $SnCl_4$ (5.0 mg, 0.019 mmol) was was then added dropwise at 0 °C. The mixture was stirred at room temperature, and the reaction progress was monitored by TLC. After 24 h, an additional amount of $SnCl_4$ (50 mg, 0.19 mmol) was added, and the reaction was continued until all the starting material had been consumed (total 5 days). After the usual workup, as in the original procedure, and subsequent chromatography on a silica gel column [EtOAc/hexane (2:1)], **8** (18.4 mg, 60.6%), α-**9** (11.0 mg, 34.8%), and trace amounts of bicylic **5** and furanosides **7** were also isolated.

1,2-*O*-Isopropylidene-5-[bis(2,2,2-trichloroethoxy)phosphoryl]-β-D-arabinofuranose (11). To a solution of the diol **10**[14] (45.9 mg, 0.241 mmol) in dry pyridine (1 mL) was added bis(2,2,2-trichloroethyl)phosphochloridate (92 mg, 0.241 mmol) in 20 mg portions every 1 h. The mixture was stirred at room temperature, and progress was monitored by TLC. After more than 90% of the starting material had been consumed (5 h), the solvent was removed by evaporation under a high vacuum and the residue was purified on a silica gel column [EtOAc/hexane (2:1)] to afford **11** (90 mg, 70%) as an oil: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.31 (s, 3H, isopropylidene protons), 1.52 (s, 3H, isopropylidene protons), 4.1−4.4 (m, 4H, H-3, H-4, H-5, H-5′), 4.56 (d, 1H, J = 3.79 Hz, H-2), 4.63 (d, 2H, J = 1.49 Hz, CCl_3CH_2), 4.66 (d, 2H, J = 1.40 Hz, CCl_3CH_2), 5.93 (d, 1H, J = 3.79 Hz, H-1); proton-decoupled ^{31}P-NMR ($CDCl_3$, 81.03 MHz) δ −6.32 (s); CIMS m/z 530.6 (MH^+, $C_{12}H_{18}O_8Cl_6P$ requires 531.2).

1,2-*O*-Isopropylidene-3-*O*-[1-(methoxycarbonyl)-ethenyl]-5-[bis(2,2,2-trichloroethoxy)phosphoryl]-β-D-arabinofuranose (12). To a solution of **11** (1.4 g, 2.63 mmol) in dry benzene (50 mL)was added rhodium tetraacetate (18.5 mg). The green mixture was heated at reflux, and then dimethyl diazomalonate (2.6 g, 18.05 mmol) was added dropwise for 30 min. This mixture was refluxed for 3 h and then cooled and filtered through Celite. After thorough washing of Celite with ethyl acetate, the washes were combined and evaporated. Purification by chromatography on silica gel (EtOAc/hexane, 2:1) afforded 1.78 g (54% yield) of the corresponding malonate ester: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.31 (s, 3H, isopropylidene protons), 1.51 (s, 3H, isopropylidene protons), 3.80 (s, 6H, CO_2CH_3), 4.07 (d, 1H, J = 2.6 Hz, H-3), 4.0−4.49 (m, 4H, H-3, H-4, and 2H-5), 4.63−4.70 (m, 5H, H-2, $2CCl_3CH_2$), 5.90 (d, 1H, J = 3.91 Hz, H-1); proton-decoupled ^{31}P-NMR ($CDCl_3$, 81.03 MHz) δ −4.24 (s).

The above malonate (1.64 g, 2.47 mmol) was dissolved in dry CH_2Cl_2 (30 mL) to which triethylamine (0.5 mL) and dimethylmethyleneammonium iodide (0.55 g, 2.97 mmol) were added, and the mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with CH_2Cl_2 (60 mL) and washed with 10% Na_2CO_3. The organic fraction was then dried and evaporated to give the corresponding Mannich base (1.66 g, 93%) as an oil: ^{1}H-NMR ($CDCl_3$, 200 MHz), δ 1.26 (s, 3H, isopropylidene protons), 1.49 (s, 3H, isopropylidene protons), 2.24 [s, 6H, $N(CH_3)_2$], 2.82−2.97 (m, 2H, CH_2N), 3.76 (s, 3H, CO_2CH_3), 3.78 (s, 3H, CO_2CH_3), 4.37−4.41 (m, 4H, H-3, H-4 and 2H-5), 4.58 (d, 1H, J = 3.84 Hz, H-2), 4.65 (m, 4H, $2CCl_3CH_2$), 5.86 (d, 1H, J = 3.76 Hz, H-1); proton-decoupled ^{31}P-NMR ($CDCl_3$, 81.03 MHz) δ −4.24 (s).

This Mannich base was not further purified but was dissolved in dry dichloromethane (20 mL), and 9 mL of freshly distilled methyliodide was added. The reaction mixture was protected from the light and stirred at room temperature for 24 h. The mixture was evaporated to dryness, and the residue obtained was dissolved in freshly distilled DMSO (3.0 mL). This mixture was heated at 100 °C for 4 h. The solvent was evaporated under reduced pressure, and the residue was purified by chromatography on a silica gel column (EtOAc/hexane 1:1) to give 0.96 g of pure **12** (34% yield referring to compound **11**): ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.30 (s, 3H, isopropylidene protons), 1.53 (s, 3H, isopropylidene protons), 3.77 (s, 3H, CO_2CH_3), 4.38−4.39 (m, 4H, H-3, H-4, H-5, H-5′), 4.60−4.63 (m, 5H, H-2, $2CCl_3CH_2$), 4.87 (d, J = 3.1 Hz, vinylic proton), 5.50 (d, J = 3.1 Hz, vinylic proton), 5.90 (d, 1H, J = 3.9 Hz, H-1); ^{13}C-NMR ($CDCl_3$, 50.3 MHz) δ 26.25 (CH_3 of isopropylidene), 27.14 (CH_3 of isopropylidene), 52.55 (CH_3 of CO_2CH_3), 60.38 (CCl_3), 67.36 (d, J = 5.2 Hz, C5), 77.23 (bs, CH_2-CCl_3), 80.91 (C2), 81.51 (d, J = 7.4 Hz, C4), 83.99 (C3), 97.79 (β-vinylic carbon), 105.76 (C1), 113.49 ($C(CH_3)_2$), 149.28 (α-vinylic carbon), 162.81 (CO_2CH_3); proton-decoupled ^{31}P-

NMR ($CDCl_3$, 81.03 MHz), δ −4.17 (s); negative CIMS *m/z* 613.0 [$(M - H)^-$, $C_{16}H_{20}O_{10}Cl_6P$ requires 613.3).

3-*O*-[1-(Methoxycarbonyl)ethenyl]-5-*O*-phosphono-D-arabinofuranose (2α and 2*β*). To a solution of **12** (198.6 mg, 0.322 mmol) in dry MeOH (5 mL) were added activated Zn powder (0.4 g) and acetic acid (1 mL). The mixture was stirred at room temperature for 2 h and then filtered through Celite. After thorough washing of Celite with MeOH, the washes were combined and evaporated to dryness. The observed residue was dissolved in D_2O (5 mL), and sufficient Dowex 50W (H^+ form) resin (which had been thoroughly washed with D_2O) was added with stirring to bring the solution to pH = 1. Progress of the reaction was monitored by ^{1}H-NMR (the disappearance of isopropylidene methyl resonances), and after 24 h the mixture was filtered. The pH of the resulting filtrate was adjusted to 14 with KOH (1 N), and the resulting base solution was stirred at room temperature for 2 h. After this time, the pH was adjusted to 7.3 with Dowex 50W resin (H^+ form), the solution was filtered, and the product was purified through ion-exchange chromatography on AG1 × 8 (HCO_3^- form), eluting with a linear gradient of triethylammonium bicarbonate buffer (0−0.7 M, pH = 7.5). Fractions were analyzed for inorganic phosphate after digestion with $HClO_4$. The active fractions were then combined and concentrated until dry. The residue was dissolved in water, passed through a column of Dowex 50W (K^+ form), and concentrated again by lyophilization to give the highly purified phosphate **2** (85 mg, 89%) as a mixture of anomers. Data for *β*-**2**: ^{1}H-NMR (potassium salt, pD = 7.1, D_2O, 400 MHz) δ 3.70−3.82 (m, 2H, H-5, H-5′), 3.99−4.01 (m, 2H, H-2 and H-3), 4.35−4.37 (m, 1H, H-4), 4.58 (d, 1H, *J* = 2.90 Hz, vinylic proton), 5.03 (d, 1H, *J* = 2.90 Hz, vinylic proton), 5.23 (d, 1H, *J* = 3.9 Hz, H-1); ^{13}C-NMR (D_2O, 100.6 MHz) δ 67.05 (C5), 76.51 (C2), 83.94 (C3), 82.50 (d, *J* = 7.85 Hz, C4), 96.10 (*β*-vinylic carbon), 99.63 (C1), 162.86 (α-vinylic carbon), 172.79 (CO_2^-). Data for α-**2**: ^{1}H-NMR (potassium salt, pD = 7.1, D_2O, 400 MHz) δ 3.70−3.82 (m, 2H, H-5, H-5′), 3.98 (s, 1H, H-2), 4.20 (d, 1H, *J* = 3.29 Hz, H-3), 4.35−4.37 (m, 1H, H-4), 4.52 (d, 1H, *J* = 2.90 Hz, vinylic proton), 5.05 (d, 1H, *J* = 2.90 Hz, vinylic proton), 5.20 (s, 1H, H-1); ^{13}C-NMR (D_2O, 100.6 MHz) δ 66.51 (C5), 80.74 (C2), 84.94 (C3), 85.40 (d, *J* = 8.95 Hz, C4), 96.10 (*β*-vinylic carbon), 104.88 (C1), 156.35(α-vinylic carbon), 162.86 (CO_2^-). Proton-decoupled ^{31}P-NMR (D_2O, 162 MHz) δ 4.52 (s); proton-coupled ^{31}P-NMR (D_2O, 162 MHz) δ 4.47−4.35 (m); FAB mass spectrum *m/z* 415.3 (MH^+, $C_8H_{11}O_{10}PK_3$ requires 415.4).

Intramolecular Condensation of Model 2. In a typical condensation experiment, the phosphate **2** (52 mg, 0.125 mmol) was dissolved in water (1.3 mL), and $ZnCl_2$ (80 mg, 0.592 mmol, in 80 μL of H_2O) was added at room temperature. The pH of this solution was carefully adjusted to pH 5.0 with diluted H_2SO_4. The mixture was heated to 50 °C, and the reaction progress was monitored by ^{1}H-NMR in the following manner: Aliquots (0.3 mL) of the reaction mixture were withdrawn at various time intervals and immediately passed through a small (5 mL) column of Dowex 50W (H^+ form) in order to remove Zn^{2+} ions. The column was then washed with distilled water (15 mL), and the fractions were analyzed for inorganic phosphate after digestion with $HClO_4$. The active fractions were combined and the pH carefully adjusted to 3.8 with NaOH (0.1 N). The solution was then rapidly concentrated under a high vacuum. The residue was dissolved in D_2O (0.4 mL), and the ^{1}H-NMR spectrum was recorded. Analysis of the observed ^{1}H-NMR spectra revealed, in the presence of Kdo8P, bicyclic structures **13** and **14** and a trace amount of furoic acid derivative **15**. For the quantitative production of bicyclic structures **13** and **14** alone, the experiment was performed as above but the incubation at 50 °C was continued for 30 h. Data of **13**: ^{1}H-NMR (D_2O, 400 MHz) δ 2.10 (bd, 1H, *J* = 14.8 Hz, H-3a′), 2.26 (dd, 1H, *J* = 14.8, 5.5 Hz, H-3e′), 3.77−3.98 (m, 3H, H-7, H-8, H-8′), 4.10 (dd, 1H, *J* = 6.5, 1.8 Hz, H-6), 4.54 (dd, 1H, *J* = 4.8, 1.8 Hz, H-5), 4.73 (dd, 1H, *J* = 4.8, 5.5 Hz, H-4); ^{13}C-NMR (D_2O, 100.6 MHz) δ 45.12 (C3), 67.83 (C8), 85.60 (C4), 87.87 (C7), 80.44 (C6), 94.94 (C5), 108.55 (C2), 179.06 (C1). Data of **14**: ^{1}H-NMR (D_2O, 400 MHz) δ 2.19 (dd, 1H, *J* = 14.7, 6.5 Hz, H-3a′), 2.31 (dd, 1H, *J* = 14.7, 2.3 Hz, H-3e′), 3.77−3.98 (m, 3H, H-7, H-8, H-8′), 4.11 (dd, 1H, *J* = 3.4, 1.5 Hz, H-6), 4.52 (dd, 1H, *J* = 4.7, 1.5 Hz, H-5), 4.80 (dd, 1H, *J* = 6.5, 4.7 Hz, H-4); ^{13}C-NMR (D_2O, 100.6 MHz) δ 46.20 (C3), 68.20 (C8), 79.06 (C6), 85.60 (C4), 89.10 (C7), 92.72 (C5), 109.16 (C2), 178.43 (C1). FAB mass spectrum for the mixture **13** and **14** *m/z* 415.0 (MH^+, $C_8H_{11}O_{10}PK_3$ requires 415.4). The furoic acid derivative **15** was identified by the presence of the following characteristic resonances: ^{1}H-NMR (D_2O, 400 MHz) δ 6.45 (d, 1H, *J* = 3.3 Hz, H-4), 6.89 (d, 1H, *J* = 3.3 Hz, H-3).

A time course of the $ZnCl_2$-catalyzed dehydration of Kdo8P was performed in the same manner as described for **2**, which provided the same mixture of the bicyclic **13** and **14** (in the ratio of 2:1, respectively), along with a trace amount of furoic acid derivative **15**.

The experiments carried out without the presence of $ZnCl_2$ took place as follows: Compound **2** (52 mg, 0.125 mmol) was dissolved in water (1.3 mL), and the pH was adjusted to 5.0 with diluted H_2SO_4. The solution was heated at 50 °C. The reaction progress was monitored using ^{1}H-NMR by removing aliquots (0.3 mL) at various time intervals, concentrating the solution under high vacuum, and then dissolving the residue in D_2O (0.4 mL). Analysis of the observed ^{1}H-NMR spectra revealed that the reaction mixture contaned Kdo8P, 4-*epi*-Kdo8P, A5P, and pyruvate.

Intermolecular Condensation Experiments. As an intermolecular counterpart for the model **1** we used 3,5-bis-(toluenesulfonyl)-D-arabinofuranose (**16**), which was prepared from **3** by following procedure. To a solution of acetonide **3** (511 mg, 1.49 mmol) in dry pyridine (2.5 mL) at 0 °C were added, *p*-toluenesulfonyl chloride (730 mg, 2.97 mmol) and 4-(dimethylamino)pyridine (50 mg). After the mixture was stirred at room temperature for 2 days, it was diluted with EtOAc (10 mL), washed with diluted H_2SO_4 and saturated NaCl, dried ($MgSO_4$), and concentrated. Column chromatography (EtOAc/hexane 1:1) afforded the target bistosyl acetonide (629.6 mg, 92%, based on unreacted starting material recovered) along with unreacted acetonide **3** (37.5 mg, 7.3%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.22 (s, 3H, isopropylydene protons), 1.31 (s, 3H, isopropylydene protons), 2.42 (s, 3H, Me of Ts), 2.45 (s, 3H, Me of Ts), 3.96 (dd, 1H, *J* = 10.1, 5.8 Hz, H-5), 4.01 (dd, 1H, *J* = 10.1, 7.6 Hz, H-5′), 4.14 (ddd, 1H, *J* = 7.6, 5.8, 1.5 Hz, H-4), 4.65 (d, 1H, *J* = 3.8 Hz, H-2), 4.74 (d, 1H, *J* = 1.5 Hz, H-3), 5.83 (d, 1H, *J* = 3.8 Hz, H-1), 7.32 (d, 2H, *J* = 8.2 Hz, aromatic protons), 7.35 (d, 2H, *J* = 8.2 Hz, aromatic protons), 7.71 (d, 2H, *J* = 8.2 Hz, aromatic protons), 7.77 (d, 2H, *J* = 8.2 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 100.6 M Hz) δ 21.62 (Me of Ts), 21.71 (Me of Ts), 25.71 (Me of isopropylidene), 26.35 (Me of isopropylidene), 67.32 (C-5), 81.79, 81.87, 84.25, 105.81 (C-1), 113.19 ($C(CH_3)_2$), 127.98, 129.90, 130.23, 132.26, 145.14, 145.78 (aromatic carbons). The bistosyl acetonide from the above (300 mg, 0.60 mmol) was dissolved in a mixture of acetic acid/water/tetrahydrofuran (8:2:1, 20 mL). After the mixture was stirred at 70 °C for 12 h, and subjected to the same workup and column chromatography as for the diol **1**, the bistosyl **16** was obtained as a clear oil (192 mg, 70%). Data for α-**16**: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.42 (s, 3H, Me of Ts), 4.05−4.09 (m, 2H), 4.18−4.21 (m, 1H), 4.36 (dd, 1H, *J* = 8.5, 4.3 Hz, H-5), 4.51 (dd, 1H, *J* = 4.8, 2.3 Hz), 5.25 (s, 1H, H-1), 7.31−7.34 (m, 4H, aromatic protons), 7.70−7.76 (m, 4H, aromatic protons). Data for *β*-**16**: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.41 (s, 3H, Me of Ts), 4.01 (dd, 1H, *J* = 11.0, 4.4 Hz, H-5), 4.05−4.09 (m, 1H, H-2), 4.11 (dd, 1H, *J* = 11.0, 3.6 Hz, H-5′), 4.18−4.21 (m, 1H, H-4), 4.64 (t, 1H, *J* = 4.3 Hz, H-3), 5.28 (d, *J* = 4.3 Hz, H-1), 7.31−7.34 (m, 4H, aromatic protons), 7.70−7.76 (m, 4H, aromatic protons); ^{13}C-NMR data for the anomeric mixture of **16** ($CDCl_3$, 100.6 MHz) δ 21.67 (2Me of Ts), 67.90 (C-5), 69.06 (C-5′), 75.49, 77.70, 79.27, 79.98, 83.61, 96.20 (C-1), 102.33 (C-1′), 127.90, 128.01, 129.90, 130.11, 130.17, 132.12, 132.29, 145.22, 145.70 (aromatic carbons).

Intermolecular Condensation in Organic Solvent. To a solution of bistosyl **16** (34.8 mg, 0.076 mmol) and methyl α-methoxyacrylate[31] (8.8 mg, 0.076 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added $SnCl_4$ (5.0 mg, 0.019 mmol, in 0.3 mL of CH_2Cl_2). The mixture was stirred at room temperature for 2 days. Using the same workup and purification as for intramo-

lecular condensation (procedure A), pure α- and β-methoxy furanosides of **17** (23.3 mg, 65%) along with unreacted **16** (10.5 mg, 30%) were afforded. Data for α-**17**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.45 (s, 3H, Me of Ts), 2.47 (s, 3H, Me of Ts), 2.73 (d , 1H, J = 6.5 Hz, OH-2), 3.31 (s, 3H, OMe), 4.10 (dd, 1H, J = 11.5, 4.0 Hz, H-5), 4.16 (dd, 1H, J = 11.5, 3.0 Hz, H-5′), 4.18 (dd, 1H, J = 6.5, 2.5 Hz, H-2), 4.23 (ddd, 1H, J = 5.5, 3.0, 4.0 Hz, H-4), 4.45 (dd, 1H, J = 5.5, 2.5 Hz, H-3), 4.79 (s, 1H, H-1), 7.35 (d, 2H, J = 8.0 Hz, aromatic protons), 7.37 (d, 2H, J = 8.5 Hz, aromatic protons), 7.74 (d, 2H, J = 8.5 Hz, aromatic protons), 7.78 (d, 2H, J = 8.0 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 100.6 MHz) δ 21.71 (Me of Ts), 21.77 (Me of Ts), 55.21 (OMe), 67.54 (C-5), 78.76, 79.98, 83.76, 108.53 (C-1), 127.96, 128.05, 129.90, 130.20, 132.17, 132.54, 145.09, 145.79 (aromatic carbons). Data for β-**17**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.46 (s, 6H, Me of Ts), 2.58 (d, 1H, J = 8.5 Hz, OH-2), 3.34 (s, 3H, OMe), 4.02 (dd, 1H, J = 10.5, 6.0 Hz, H-5), 4.09 (dd, 1H, J = 10.5, 4.0 Hz, H-5′), 4.18 (ddd, 1H, J = 6.0, 6.0, 4.0 Hz, H-4), 4.26 (ddd, 1H, J = 8.5, 6.0, 4.5 Hz, H-2), 4.58 (t, 1H, J = 6.0 Hz, H-3), 4.81 (d, 1H, J = 4.5 Hz, H-1), 7.36 (dd, 4H, J = 8.0 Hz, aromatic protons), 7.78 (dd, 4H, J = 8.0 Hz, aromatic protons); ^{13}C-NMR ($CDCl_3$, 100.6 MHz) δ 21.66 (Me of Ts), 21.71 (Me of Ts), 55.56 (OMe), 69.13 (C-5), 76.24, 78.17, 84.00, 101.96 (C-1), 127.99, 128.16, 129.92, 130.08, 132.50, 132.88, 145.05, 145.58 (aromatic carbons).

Intermolecular Condensation in Water Solution. To a solution of D-arabinose (208 mg, 1.39 mmol) and α-methoxyacrylic acid[31] (140 mg, 1.37 mmol) in H_2O (15 mL), was added $ZnCl_2$ (933 mg, 6.90 mmol). The pH of the mixture was adjusted to 5.0 using KOH (0.1 N). This solution was divided in two equal volumes, with one being incubated at 50 °C and the other at 90 °C. The reaction progress in both experiments was monitored by ^{1}H-NMR in the same manner as described above for model **2** in the presence of $ZnCl_2$. We did not detect any possible condensation product in either experiment, even after 50 h. During this time the arabinose was remained unchanged, while the α-methoxyacrylate decomposed to pyruvic acid.

Enzyme Assays. Unless otherwise stated, the enzyme activity was assayed in a 0.8 mL reaction buffer consisting of 0.1 M Tris-acetate, pH = 7.3, PEP (0.2 mM, = 20K_m), and A5P (0.5 mM, = 20K_m). All solutions except the enzyme were filtered through Millipore type-HA filters (0.45 μM) before use. Following equilibration at 37 °C for 2 min, Kdo8P synthase (20 μL, at a final concentration of about 30 nM) was added. The decrease in the absorbance difference between 232 and 350 nm (as internal reference) was monitored with time (MS-DOS UV/vis software). This method[36] is based on the absorbance difference at 232 nm between PEP (ϵ = 2840 M^{-1} cm^{-1}) and the other substrates and products (ϵ < 60 M^{-1} cm^{-1}) under the assay conditions. The initial rate was calculated from a linear, least-squares fit of the first 30 s of the progress curve. The concentrations of PEP, A5P, and **2** were determined precisely by quantitative assaying of the P_i released by alkaline phosphatase.[37] In each case, to ensure complete hydrolysis of the phosphate monoester, the aliquots of the incubation mixture with alkaline phosphatase were tested by ^{31}P NMR. One unit of the enzyme activity is the amount that catalyzes the consumption of 1 μmol PEP/min at 37 °C. During the enzyme purification, the enzyme activity was assayed by the thiobarbituric acid assay.[9]

Examination of Substrate and Inhibitory Activities of Model 2 with Kdo8P Synthase. In order to examine the substrate activity of **2**, we used the proton-decoupled ^{31}P-NMR assay in which the conversion of starting material could be clearly monitored. Reaction mixtures contained a 0.1 M Tris−HCl buffer (prepared in D_2O, pD = 7.3, 37 °C), bovine serum albumin (1.5 mg/mL, for the stabilization of the enzyme), 10 mM substrate (**2**), and 225 units (approximately 25 mg) of Kdo8P synthase in a total volume of 0.6 mL. A control experiment, containing all of the above but without the enzyme, was run parallel to the above. The resonance of **2** (4.5 ppm) did not change significantly during the first 3 h, but some new resonances (about 25% of the starting material) were evident after 24 h of incubation, as judged by the integration of signals. Although the identity of these new resonances were not precisely determined, a very similar time course for the formation of these new peaks was also seen in the corresponding control experiment. Thus, the model **2** failed to show any detectable enzyme-catalyzed acceleration in the expected condensation process, after 24 h. Similar results were obtained when the above experiments (with and without the enzyme) were performed in the presence of 5 mM inorganic phosphate.

In order to determine the K_i value of **2** against A5P binding, the reaction solutions were prepared as described above but with constant A5P (600 μM, = 23K_m) and variable PEP (9.8−29.5 μM). The concentrations of **2** in these experiments were in the range of 0−118 μM. Similarly, for the determination of the K_i value of **2** against PEP binding, the constant concentration of PEP (200 μM, = 20K_m) and variable A5P (15−54.7 μM) were used, while concentrations of the inhibitor (**2**) were in the range of 0−90 μM. Rate measurements were made as described above. A 5 s delay was allowed following initiation of the reaction. The initial rate was then determined by least-squares fitting of the first 10% of the progress curve to a straight line (between 20 and 80 s, depending on the initial concentration of PEP). All samples were assayed in triplicate, and analogous results were obtained in two to four different experiments. The K_i values were calculated from the secondary replots of the slopes from initial double-reciprocal plots ($1/v$ vs 1/[S]) versus inhibitor concentration.[38] These values were found to be 200 ± 10 μM and 10 ± 1 μM, against A5P and PEP, respectively.

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Supporting Information Available: Copies of ^{1}H-NMR, ^{13}C-NMR, and ^{31}P-NMR spectra for new compounds **1**, **2**, **4**, **5**, **7**−**9** (α and β), **11**, **12**, **16**, and **17** (α and β) and ^{1}H-NMR spectra of the mixtures of **13** and **14** and Kdo8P and 4-*epi*-Kdo8P (condensation products of **2** at pH = 5 and pH = 1.6) (38 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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