Enzymatic Synthesis of a Branched-Chain Hexulose: 5-Deoxy-5-*C*-hydroxymethyl-β-L-*xylo*-hex-2-ulopyranose

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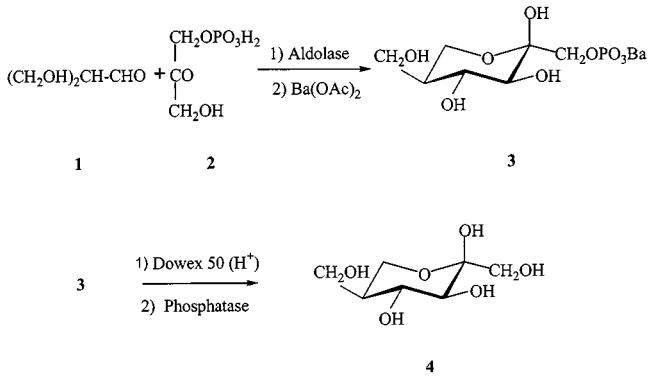
The coupling of dihydroxyacetone phosphate with 3-hydroxy-2-(hydroxymethyl)propanal (**1**) in the presence of rabbit muscle aldolase afforded a branched-chain hexulose phosphate isolated in 64% yield as the barium salt **3**.The parent, dephosphorylated sugar **4** was converted to its *anhydro* derivative **8** and glycoside **9**.

Introduction

The difficulties linked with the introduction of a branch of the >CHR type with a determined configuration in a sugar are well known.[1] On the other hand this mode of branching is the more useful when sugars are used as chiral units in total synthesis. As a new approach to the problem, we report the preparation (Scheme 1) of the branched-chain ketose **4** by the enzymatic condensation of aldehyde **1** with dihydroxyacetone phosphate (DHAP, **2**) catalyzed by rabbit muscle aldolase (from now on simply called aldolase in this paper). Aldehyde **1** is one of a collection of 64 aldehydes which were tested by the Whitesides group as potential substrates for aldolase by V_{max} estimation.[2] The V_{max} was 0.34 times that of D-glyceraldehyde phosphate, the natural substrate, and thus the reaction was considered potentially useful in synthesis. However, although the handling of an elusive aldehyde such as **1** cannot be straightforward, no details were given nor was the isolation of the product ketose phosphate reported. In this paper, we describe a preparative-scale synthesis of this compound, and some chemical transformations.



Scheme 1. The aldolase reaction with aldehyde **1**

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Discussion

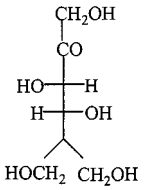
Presumably, the authors[2] had examined an aqueous solution of **1** directly obtained by acidic hydrolysis of its diethyl acetal, 1,1-diethoxy-3-hydroxy-2-(hydroxymethyl)propane, since the isolation of **1** appears problematic. The preparation of such a solution with the help of an ion-exchange resin in the H^+ form (26 mL per mmol of acetal) has been already reported.[3] Removal of the water gave an oil with the expected composition; however the authors considered it difficult to separate this concentration step from a subsequent chemical transformation, the elimination of water from this β-hydroxylated aldehyde, yielding the substituted acrylic acid.[4] For this reason some authors adopted a 1-h standing at room temperature in aqueous sulfuric acid, pH = 2.5, as conditions for the hydrolysis of the acetal. Catalytic hydrogenation of the crude product gave 2,2-dihydroxy-1,3-propanediol in quantitative yield.[5] In our system, these conditions did not result in a quantitative yield, as 65% of unreacted acetal could be recovered by ether extraction. The acidic solvent was a 0.1 M aqueous solution of DHAP, partially neutralized to the required pH with 1 M KOH. Acceptable conditions are reported below.

The precursor acetal has been prepared by reduction of diethyl 2,2-diethoxymethylmalonate, a compound easily available on the mole scale.[3] For this reduction, the authors utilized 3 mol of $LiAlH_4$ per mol of diester. Such excess is not necessary: We obtained this acetal in 82% yield with a 1.5:1 ratio and conventional distillation. We found that the hydrolysis of this acetal was practically complete after 2 h at pH = 2.00 at room temperature by enzymatic estimation of released ethanol with alcohol dehydrase and oxidized diphosphopyridine nucleotide.

Aqueous solutions of DHAP were prepared by the method of Effenberger.[6] Their molarities were about 0.1 M but this figure coud be somewhat raised by more sparingly using water in the last steps. The pH was about 1.0. Three equivalents of acetal were added to the solution of DHAP, and the pH was quickly raised to 2.0 with 1 M KOH and kept for 2 h at this value. There was a slight turbidity, but this had no adverse effect on the enzyme. Then the pH was

  1434–193X/99/0606–1415 $ 17.50+.50/0

raised to 7.20 with 1 M KOH and aldolase was added. After 2 d at room temperature, the product ketose phosphate **3** was isolated as the barium salt in about 70% yield, on a 20-mmol scale. Treatment with phosphatase gave the free sugar **4** in nearly quantitative yield as a hygroscopic syrup which crystallized on standing for several months at room temperature. This solid melted at 106°C. The solubility is of the order of 0.1 M in dioxane at room temperature, and this allows to remove coloured impurities originating from the phosphatase. Thus, a colourless compound was obtained with the expected composition, $C_7H_{14}O_6$. Both the sugar and its phosphate appeared homogeneous according to their ^{1}H-NMR spectra (Table 1). The configurations and conformations given in Scheme 1 are indicated by the following considerations: Formula **5** (Scheme 2) is a Fischer representation of the ketonic tautomer of this sugar, as expected from the known course of the aldolase reaction. There are two identical branches (*pro-R*) and (*pro-S*) on C-5. The correct name is 5-deoxy-5,5-di-*C*-hydroxymethyl-D-*threo*-pentulose, as there are only two permanent chiral centres. However, for the sake of clarity, we prefer to consider the cyclic tautomers as derivatives of 5-deoxyhexuloses (L-*xylo*, **6**) and (D-*arabino*, **7**). In these, the carbon atom of one of the branches, either the (*pro-R*) in **6**, or the (*pro-S*) in **7**, is considered as C-6 of a hexulose, the other hydroxymethyl group being a branch on C-5 of this hexulose.



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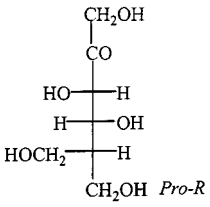
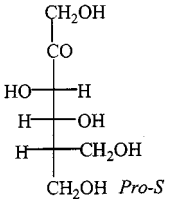


6 L-*xylo*



7 D-*arabino*

Scheme 2. Some drawings of the acyclic tautomer of hexulose **4**

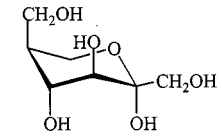
Four possible configurations can be drawn on paper for the pyranose hemiacetals derived from **5** (Scheme 3). Cyclization involving the (*pro-R*)-hydroxymethyl group would give the two anomeric L-*xylo*-hexopyranoses (α-L and β-L). Cyclization involving the (*pro-S*)-hydroxymethyl group would give the two D-*arabino* derivatives. These have been drawn in chair form, in the conformation presumed to be the most stable. Configurations at C-2 are imposed by two overwhelming factors: the anomeric effect and the enhanced conformational energy of a hydroxymethyl substituent when next to the ring oxygen atom in a pyranose.[7] The

Table 1. ^{1}H-NMR data for backbone and methyl protons of pyranoses and pyranosides[a]

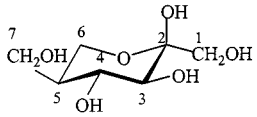
Compound Chemical shifts (δ)	**3**	**4**	**17**	**18**	**16**
1-H	3.44	3.44	4.07	3.62	3.60
1′-H	3.70	3.69	4.26	3.72	3.77
3-H		3.48	5.17	3.87	5.43[c]
4-H	3.62	3.68	5.35	3.93	5.43[c]
5-H[b]	1.84	1.86	2.33	2.16	2.22
6a-H	3.62	3.72	3.61	3.62	3.70
6e-H	3.95	3.77	3.85	3.86	3.94
7-H	3.52	3.59	4.1	3.53	3.57
7′-H			4.1	3.56	3.57
Me			3.27	3.29	3.16
Coupling constants (*J*, in Hz)					
$J_{1,1'}$	10	12	11.5	10	10
$J_{3,4}$		9	10	10	10
$J_{4,5}$	10	10	10	10	11
$J_{5,6a}$	9.5	10	12	10	11
$J_{5,6e}$	6	3.5	5	5	5
$J_{5,7}$	7				4
$J_{5,7'}$	5				
$J_{7,7'}$	11.5	12			

[a] Solvents: D_2O for **4**, D_2O with a drop of HNO_3 for **3**, $CDCl_3$ for the other compounds. – [b] Proton 5-H has 5 neighbours, which could lead in principle to a signal of 32 lines. – [c] $J/\Delta\nu > 2$.

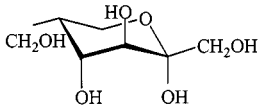
β-L-*xylo* configuration appears much favoured, while we may expect to observe the presence of small quantities of the β-D-*arabino* sugar.



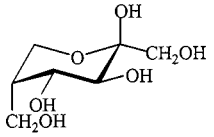
α-L-*xylo*



β-L-*xylo* (**4**)



α-D-*arabino*



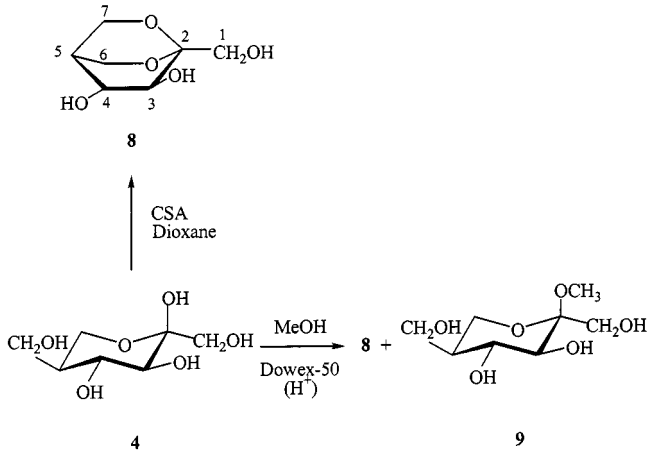
β-D-*arabino*

Scheme 3. Possible cyclic tautomers of hexulose **5**

In the ^{1}H-NMR spectrum of the pyranoses in Scheme 3 (see **4** in Scheme 3 for numbering), we expect the signal of 5-H to appear at high fields, well separated from the signals of the other skeleton protons. Then the β-D-*xylo* configuration should be easily recognized, as this is the only one in which this proton is axial and coupled to two axial protons. One further large coupling with one of the hydroxymethyl protons, 7-H or 7′-H, and two smaller couplings can be expected. In fact, the signal of 5-H in phosphate **3** (or the corresponding acid) was a broad multiplet at δ = 1.94, spanning 38 Hz. The signal of 5-H was very similar in the spectrum of the free sugar, identical in most respects with

that of **3**, in agreement with configuration **4**. The signal of 5-H, and the observation of coupling constants in the vicinity of 10 Hz between 3-H and 4-H, 4-H and 5-H, 5-H and 6a-H in the NMR spectrum of other derivatives described below, confirmed the permanence of this configuration and conformation in the transformation products (Table1). Furthermore the sharpness of the peaks of **3** and **4** indicated the homogeneity of the compound. Thus, of the four possible configurations for this product, only one was observed for conformational reasons. The cyclization reaction involves the (*pro-R*)-hydroxymethyl group.

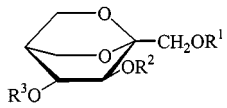
Sugar **4** was converted to an anhydro derivative **8** by treatment with acid in a non-hydroxylated solvent (Scheme 4). The reaction in a 0.1 M solution of camphorsulfonic acid in dioxane proceeded very slowly at room temperature after ca. 80% conversion. This reaction may be equilibrated. In the spectrum of the product (see Scheme 4 for the numbering) the signal of 5-H appears as an unresolved multiplet at $\delta = 2.10$, spanning only 12 Hz (Table 2). Only weak couplings were observed for protons 3-H, 4-H and 5-H. The same observations could be made in the spectra of derivatives **10** and **11** (Scheme 5) and confirm structure **8** for the *anhydro* sugar. Both prochiral branches of **4** are involved in this reaction. Observation of a model shows that this can be done in only one way and the given numbering for the carbon atoms of **8** has been chosen consistent with this origin: Thus, carbon atom 7 in **8** and carbon atom 7 in **4** both originate from the (*pro-S*)-carbon atom in **5**.



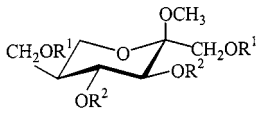
8 **4** **9**

Scheme 4. Reactions of hexulose **4** in acidic media

The rate of ring closure is higher than the rate of glycoside formation in methanol in the presence of an acidic resin at room temperature. Under some conditions (see Experimental Section) complete disappearance of **4** was observed after 2 h. The estimation of the composition of the medium at this stage from the relative intensities of the NMR signals of 5-H of **8** and **9** was misleading, as it greatly underrated the concentration of **9**. On the other hand, derivatization with *tert*-butylchlorodiphenylsilane[8] gave a mixture of three silyl ethers which were cleanly separated on a silica-gel column in the expected order of polarities. The first eluted product was the monoalcohol **12**, silylated on positions 1 and 4. This was indicated by the ^{1}H-NMR spectrum of the acetyl derivative **13**, on which the signal of the only displaced proton appears as a sharp peak. Compound **12** was isolated in 20% yield.



10 $R^1 = R^2 = R^3 = Ac$
11 $R^1 = R^2 = R^3 = Bn$
12 $R^1 = R^3 = SiPh_2tBu$, $R^2 = H$
13 $R^1 = R^3 = SiPh_2tBu$, $R^2 = Ac$
14 $R^1 = SiPh_2tBu$, $R^2 = R^3 = H$



15 $R^1 = SiPh_2tBu$, $R^2 = H$
16 $R^1 = SiPh_2tBu$, $R^2 = Ac$
17 $R^1 = R^2 = Ac$
18 $R^1 = R^2 = Bn$

Scheme 5. Derivatives of **8** and **9**

Table 2. ^{1}H-NMR data for backbone protons of anhydro derivatives[a]

Compound **2** Chemical shifts (δ)		**10**	**11**	**14**	**12**	**13**
1-H	3.50	3.86	3.38	3.67	3.64	3.46
1′-H	3.50	4.20	3.62	3.67	3.64	3.46
3-H	3.70	5.12	3.90	3.79	4.12	5.50
4-H	3.93	5.01	3.83	ca 4.1	3.92	3.92
5-H[b]	2.06	2.22	2.16	2.04	1.72	1.64
6a-H	4.07	4.06	3.93	3.91	3.79	3.87
6e-H	4.14	4.09	3.98		3.89	4.46
7-H	3.96	4.20	4.13	4.10	3.96	3.90
7′-H	3.96	4.25	4.27	4.22	4.39	3.62
Coupling constants (J, in Hz)						
$J_{1,1'}$	17	12.5	10.5	10.5	4	13
$J_{3,4}$	2.5	2.5				0
$J_{4,5}$	5				5	
$J_{5,6a}$					2.5	
$J_{5,6e}$					1	
$J_{6,6}$	9	7	9	9	10	9
$J_{7,7'}$		10	8.5	10	9	9

[a] Solvent: $CDCl_3$. Long-range couplings may be expected between 3-H and 5-H, 4-H and 6-H, 6′-H and 7′-H. Couplings of the order of 0.5−2 Hz are visible on the spectra, but have not been recorded here. Number 7 has been given to the only proton which cannot exhibit W-type long-range coupling. − [b] Broad signals: width at half heigth, 9−12 Hz.

The next eluted product was the methyl glycoside **15**, silylated on positions 1 and 7, obtained in 22% yield. This was characterized as the acetate **16**. In the ^{1}H-NMR spectrum of **16** the distance between the signals of 3-H and 4-H is smaller than their coupling and accordingly, a first-order analysis is not possible. The observed pattern corresponds to $\nu_3 - \nu_4 = 2$ Hz, $J_{3,4} = 10$ Hz, $J_{4,5} = 8$ Hz, that is, a

trans-diaxial disposition of 3-H, 4-H and 5-H. The most polar derivative was the *anhydro* sugar **14**, silylated at its primary position (19%). Thus, the total yield of derivatives of **8** is 39%, while the derivative of the methyl glycoside is obtained in 22% yield. Under the conditions of the derivatization (excess of reagent) we may assume that these figures truly reflect the proportions of **8** and **9** in the glycosidation medium after 2 h and that there is roughly twice as much *anhydro* sugar as methyl glycoside.

On the other hand, acetylation of the mixture obtained after several days of reaction at room temperature gave the tri-*O*-acetyl derivative **17** of the methyl glycoside in 66% yield from the crude sugar **4** (about 50% yield from DHAP). Configuration **17** is proved by ^{1}H-NMR spectroscopy. This result indicates that the methyl glycoside **9** is thermodynamically more stable that the anhydro sugar **8**. Zemplén deacetylation of **17** followed by benzylation in the usual way gave the tri-*O*-benzyl ether **18**, again with the same configuration.

Thus, the aldolase-mediated synthesis gave only one pyranose configuration, **4**. This configuration could be stabilized by conversion to the glycoside **9**. Under equilibrium conditions the *anhydro* derivative **8** was also obtained as a by-product. Compound **8** can be considered as a derivative of the same configuration as the free sugar, originating from the attack of the hemiacetal carbon atom by the branch on C-5. In fact, in the acidic medium of glycosidation, the acetal function in **8** might open in two ways, and lead, in principle, to an equilibrium mixture of the four possible methyl glycosides, but free enthalpy differences between these insure that **9** is the only one that can be isolated.

It is remarkable that both the starting materials are achiral and that aldolase is the only source of chirality. It should be noted that the discrimination between the prochiral branches of **5** is not a consequence of the use of an enzyme. It is well known, that the aldolase-mediated condensation of DHAP with racemic glyceraldehyde gives the D-*fructo* and L-*sorbo* configuration in roughly equal amounts. In our work, aldolase only insured an easy preparation of the branched-chain ketose, and its locking in the D series (the acyclic configuration being used to define the series, as it should).

The prevalence of a β-D-*xylo* configuration in an aldolase condensation has already been reported by Durrwachter et al.[9] Nevertheless, there is only a superficial similarity with the reaction reported in our paper. The substrate utilized by these authors is a mixture of enantiomers, (*R*,*S*)-2-(hydroxymethyl)-4-pentenal, added in excess. The product obtained under equilibrium conditions corresponds to the practically exclusive utilization of the (*S*) enantiomer. This is a consequence of the reversibility of the catalysis by aldolase, which regenerates DHAP from the less stable hexulose. Thus, in these experiments, the substrate is chiral, there is no de novo creation of a chiral centre at C-5, the presence of aldolase is necessary, and the maximum theoretical yield is 50% of the racemic substrate. On the other hand, in our experiment the substrate is achiral, *the enzyme does not participate* in the creation of chirality at C-5, the equilibrium conditions being achieved by the operation of the classical ring-chain tautomerism of sugars. The maximum theoretical yield is 100%. Furthermore, to reach the aldolase-mediated equilibrium in reasonable time, the above-mentioned authors have added much more enzyme, in fact 22 times as much as we have used per mol of final product.

From the preparative point of view, we foresee no bottleneck in the scaling up of this preparation. All the starting materials are easily available, and aldolase could be immobilized if necessary. Finally, we note that treatment of compound **15** or some analogue with $NaIO_4$ and $NaBH_4$ (the so-called Smith degradation of polysaccharides) would give a chiral tris(hydroxymethyl)methane. There is current interest in these compounds.[10]

Experimental Section

General: Melting points are uncorrected. − Optical rotations: JASCO DIP 370 polarimeter; 1-cm cells; all values measured at room temperature. − Thin-layer chromatography (TLC): Silica gel coated glass plates (Merck); detection by spraying with 5% H_2SO_4 in ethanol and charring. − Column chromatography: Open columns filled with 20 g of silical gel (particle size 60 μm) per g of product. − UV/Vis: Cary Varian 1E. − ^{1}H NMR: Bruker AC 250 (250 MHz); $CDCl_3$ as solvent, Me_4Si as internal standard; D_2O as solvent, $\delta_H = 4.80$. The chemical shifts of the protons bound to the carbon skeletons of the sugars are given in Table 1 and 2; the signals of substituents in derivatives, that is, acetyl, phenyl, benzyl, and *tert*-butyl are as predicted in locations and intensities and have not been reported. − Rabbit muscle aldolase, phosphatase, D-glycerophosphate dehydrase and ethanol dehydrase were Sigma products. DHAP was prepared by the method of Effenberger[6] and estimated with D-glycerophosphate dehydrase and NADH according to ref.[11] Ethanol in water was estimated by the reduction of NAD^+ in the presence of ethanol dehydrase, at pH = 10.0 in 0.1 M glycine buffer with the help of the equilibrium constant measured by Racker:[12] $k = [CH_3CHO][NADH]/[CH_3CH_2OH][NAD^+] = 0.115$.

1,1-Diethoxy-3-hydroxy-2-(hydroxymethyl)propane: A solution of 50.4 g (0.192 mol) of diethyl diethoxymethylmalonate in 100 mL of ether was added dropwise in the course of 2 h to a solution of 11 g (0.29 mol) of $LiAlH_4$ in 200 mL of ether, and the mixture was refluxed for 16 h. Then 11 mL of water, 11 mL of 15% aqueous NaOH, and 11 mL of water were added in succession. The suspension was filtered, and the precipitate was extracted twice with boiling ether (4 h). Distillation of the combined ether solutions gave 28.3 g of the diethyl acetal (82%), liquid, b.p. 93°C (0.05 Torr). − ^{1}H NMR (D_2O): δ = 1.18 (t, 6 H, J = 7 Hz, 2 Me), 1.94 [dq 1 H, J = 6.5 and 7.5 Hz, $CH(CH_2OH)_2$], 3.55−3.62 (m, 8 H), 4.63 [d, 1 H, J = 6.5 Hz, $CH(OEt)_2$].

5-Deoxy-5-*C*-hydroxymethyl-β-L-*xylo*-hex-2-ulopyranose 1-(Barium Phosphate) (3): The above acetal (17.6 g; 96.8 mmol) was added to 251 mL of a 0.123 M solution of DHAP in water, and the pH was raised to 2.00 with aqueous KOH. After 2 h at room temperature, the pH was raised to 7.19 with 1 M KOH, 5 mg of NaN_3, 29 mg of tricyclohexylammonium phosphoglycollate and 1 mL (275 units) of aldolase suspension were added. After 64 h at room temperature, the amount of DHAP had dropped to 5.3 mmol. Then the pH was raised to 8.4 with 1 M KOH. Barium acetate (15.24 g; 0.6 mol) was added, the pH which had dropped to 7.0 was again adjusted to 8.4 with 1 M KOH. Barium phosphate was removed by filtration, and

800 mL of 95% ethanol was added to the filtered solution. The precipitate was collected, washed with 150 mL of ethanol/water (4:1), ethanol and ether, and air-dried to give 8.03 g of crude product, which, from its NMR spectrum still contained 9.4% of barium acetate ($\delta = 1.86$ in D_2O, $CH_3CO_2^-$, shifting to $\delta = 2.00$ by acidification with HNO_3). The corrected yield of **3** was 58% (70% accounting for recovered DHAP). This product was used as such for the next step.

5-Deoxy-5-*C*-hydroxymethyl-β-L-*xylo*-hex-2-ulopyranose (4): A solution of 7.6 g (18.6 mmol) of **3**, dissolved in a little water, was passed through a 90 meq column of Dowex-50 (H^+) ion-exchange resin. The effluent was concentrated to 40 mL at 45−50°C, the volume was adjusted to 100 mL, and the pH was brought to 4.25 with 1 M KOH. Wheat germ phosphatase (120 units) was added and the solution was kept for one week at room temperature under a layer of toluene. Then it was heated to 90°C for a few minutes, cooled and separated from the coagulated proteins by filtration, and deionized by successive passages through a 25 meq Dowex-50 (H^+) and a 30 meq Dowex-1 (AcO^-) ion-exchange resin column. Concentration gave 3.0 g (83.6%) of **4**, as a hygroscopic syrup slowly crystallizing in a few months at room temperature. Dissolution in dioxane at 80°C (10 mL per mmol), filtration and concentration removed some coloured impurities; m.p. 106°C. − TLC [2-propanol/water (3:1)]: $R_f = 0.71$; [methanol/dichloromethane (1:1)]: $R_f = 0.51$; [methanol/dichloromethane (1:4)]: $R_f = 0.11$.− $[\alpha]_D = -24.3$ ($c = 2.0$, H_2O). − $C_7H_{14}O_6$ (194): calcd. C 43.30, H 7.22, O 49.48; found C 43.11, H 7.25, O 49.22.

2,7-Anhydro-5-deoxy-5-*C*-hydroxymethyl-β-L-*xylo*-hex-2-ulopyranose (8): The addition of 194 mg of **4** to 11 mL of a 0.1 M camphorsulfonic acid solution in dioxane gave a clear solution which was kept for 2 d at room temperature. Then K_2CO_3 (415 mg; 3 mmol) was added and the suspension was stirred for 2 d at room temperature, then filtered, and dioxane was removed from the liquid phase by evaporation. TLC examination indicated that the residue was a mixture of **4** and **8** in a ratio of ca 3:1. Chromatography dichloromethane/methanol (6:1)] separated **8** (95 mg; 54%) m.p. 135−137°C. − TLC dichloromethane/methanol (4:1)]: $R_f = 0.33$. − $[\alpha]_D = -3.4$ ($c = 1$, methanol). − $C_7H_{12}O_5$ (176): calcd. C 47.72, H 6.82, O 45.45; found C 47.61, H 6.71, O 45.29.

1,3,4-Tri-*O*-acetyl-2,7-anhydro-5-deoxy-5-*C*-hydroxymethyl-β-L-*xylo*-hex-2-ulopyranose (10): Prepared from **8** in the usual way (pyridine/acetic anhydride) and purified by column chromatography [light petroleum ether/ethyl acetate (8:1)]. Syrup; TLC light petroleum ether/ethyl acetate (1:1)]: $R_f = 0.46$. − $[\alpha]_D = -9.2$ ($c = 0.98$, dichloromethane). − $C_{13}H_{18}O_8$ (302): calcd. C 51.68, H 6.12; found C 51.96, H 6.12.

2,7-Anhydro-1,3,4-tri-*O*-benzyl-5-deoxy-5-*C*-hydroxymethyl-β-L-*xylo*-hex-2-ulopyranose (11): Prepared from **8** in the usual way (NaH and benzyl bromide in DMF) and purified by column chromatography [light petroleum ether/ethyl acetate (4:1)]. Syrup; TLC [light petroleum ether/ethyl acetate (1:1)]: $R_f = 0.74$. − $[\alpha]_D = -0.6$ ($c = 1.8$, dichloromethane). − $C_{28}H_{30}O_5$ (446): calcd. 75.33, H 6.72, O 17.93; found C 75.19, H 7.01, O 17.8.

Study of the First Steps of the Glycosidation Reaction: Sugar **4** (304 mg; 1.57 mmol) was dissolved in a suspension of 1 mL of Dowex-50 (H^+) ion-exchange resin in 10 mL of methanol. The mixture was stirred at room temperature for 2 h, when TLC [methanol/dichloromethane (2:3)] indicated the disappearance of the starting material. The resin was filtered off and the methanol evaporated. The residue was dissolved in 1.5 mL of DMF and 0.81 mL of *tert*-butylchlorodiphenylsilane, and 0.4 g of imidazole were added.[8] After 6 h standing at room temperature, the mixture was partitioned between water and diethyl ether . The silyl ethers in the ethereal extract (1.118 g) were separated by chromatography. Elution with light petroleum ether/ethyl acetate (8:1) gave 204 mg of 2,7-*anhydro*-1,4-di-*O*-*tert*-butyldiphenylsilyl-5-deoxy-5-*C*-hydroxymethyl-β-L-*xylo*-hex-2-ulopyranose (**12**; 0.31 mmol; 20%). − TLC ixght petroleum ether/ethyl acetate (4:1)]: $R_f = 0.63$. − $[\alpha]_D = -5.2$ ($c = 0.89$, dichloromethane). − $C_{39}H_{48}O_5Si_2$ (652): calcd. C 71.78, H 7.36; found C 71.79, H 7.61. − Elution with light petroleum ether/ethyl acetate (4:1) gave 240 mg (0.35 mmol; 22%) of the bis(ether) **15** which was characterized as the diacetate (see below). Finally, elution with ethyl acetate gave 355 mg of a mixture which was further purified by chromatography [light petroleum ether/ethyl acetate (1:1)] to give 123 mg (0.30 mmol; 19%) of 2,7-*anhydro*-1-*O*-*tert*-butyldiphenylsilyl-5-deoxy-5-*C*-hydroxymethyl-β-L-*xylo*-hex-2-ulopyranose (**14**) as a syrup which crystallized on standing, m.p. 120−123°C. − TLC [light petroleum ether/ethyl acetate (1:1)]: $R_f = 0.23$. − $[\alpha]_D = -20.8$ ($c = 1.25$, dichloromethane). − $C_{23}H_{30}O_5Si$ (414): calcd. C 66.66, H 7.25; found C 66.66, H 7.39. − Acetylation of **12** (acetic anhydride/pyridine) gave the 3-*O*-acetyl derivative **13**. Acetylation of **15** gave methyl 3,4-di-*O*-acetyl-1-*O*-*tert*-butyldiphenylsilyl-5-*tert*-butyldiphenylsilyloxymethyl-5-deoxy-β-L-*xylo*-hex-2-ulopyranoside (**16**; 100%), which was crystallized from pentane, m.p. 90−98°C. − $[\alpha]_D = 24.8$ ($c = 1.7$, dichloromethane). − $C_{44}H_{56}O_8Si$ (768): calcd. C 68.75, H 7.29; found C 68.83, H 7.39.

Methyl 5-*C*-Acetoxymethyl-1,3,4-tetra-*O*-acetyl-5-deoxy-β-L-*xylo*-hex-2-ulopyranoside (17): Dowex-50 ion-exchange resin in the H^+ form was stirred for 2 d in anhydrous methanol, with frequent removal of the liquid. A sample of this resin (1.8 mL) was added to a solution of 842 mg (4.42 mmol) of **4** in dry methanol (20 mL), and the mixture was stirred for 3 d at room temperature. The resin was filtered off, 0.2 mL of pyridine was added to the solution which was concentrated to dryness. To the resin were added 3.4 mL of acetic anhydride and 4.3 mL of pyridine, and the mixture was kept for 2 d at room temperature, then concentrated to dryness to give a crude product (1.372 mg) which was purified by chromatography [methanol/dichloromethane (1:100)]. Elution first gave 376 mg of a fraction which was a 1.4:1 mixture of **17** and **10**, as estimated from the intensities of the signals of 3-H and 4-H in the NMR spectrum. Continued elution gave 848 mg (52%) of **17** as a syrup. − TLC [methanol/dichloromethane (2.5:100)]: $R_f = 0.37$. − $[\alpha]_D = -13.2$ ($c = 2.1$, dichloromethane). − $C_{16}H_{24}O_{10}$ (376): calcd. C 51.07, H 6.45, O 42.31; found C 51.25, H 6.56, O 42.63.

Methyl 1,3,4-Tri-*O*-benzyl-5-*C*-5-benzyloxymethyl-β-L-*xylo*-hex-2-ulopyranoside (18): To a solution of sodium methoxide, prepared from 56 mg of Na in 5.6 mL of methanol, was added 400 mg (1.06 mmol) of **17**. The solution was kept for 4 h at room temperature, refluxed for 90 min and concentrated to dryness. Benzylation in 11 mL of DMF, with 1.0 mL (8.5 mmol) of benzyl bromide and 250 mg (6.3 mmol) of 60% NaH suspension in oil, by heating at 90°C for 4 h, was incomplete. The product was recovered as described for **18** at the end of this section, dissolved in 12 mL of DMF, and heated for 6 h at 95°C in the presence of 300 mg of NaH suspension. Methanol (1 mL) was added, the volatiles were evaporated, and the residue was dissolved with ether which was washed with water to neutrality. Purification by chromatography [light petroleum ether/ethyl acetate (7:1)] separated **18** (428 mg; 71%). − $[\alpha]_D = -8.6$ ($c = 1.2$, dichloromethane). − $C_{36}H_{40}O_6$ (568): calcd. C 76.05, H 7.04, O 16.90; found C 75.97, H 7.11, O 17.08.

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