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Synthesis of Methyl Esters of 3-Deoxy-D-*erythro*-2-hexulosonic acid (KDG) Analogs, Inducers of the Expression of Pectinase Genes in bacteria *Erwinia chrysanthemi*

Frederic Alessi, Alain Doutheau, Daniel Anker*

Laboratoire de Chimie Organique, Département de Biochimie, Institut National des Sciences Appliquées,
20 avenue A. Einstein, 69621 Villeurbanne, FRANCE.

Guy Condemine, Janine Robert-Baudouy

Laboratoire de Génétique Moléculaire des Microorganismes et des Interactions Cellulaires, URA-CNRS-1486,
Département de Biochimie, Institut National des Sciences Appliquées, 20 avenue A. Einstein, 69621 Villeurbanne, FRANCE.

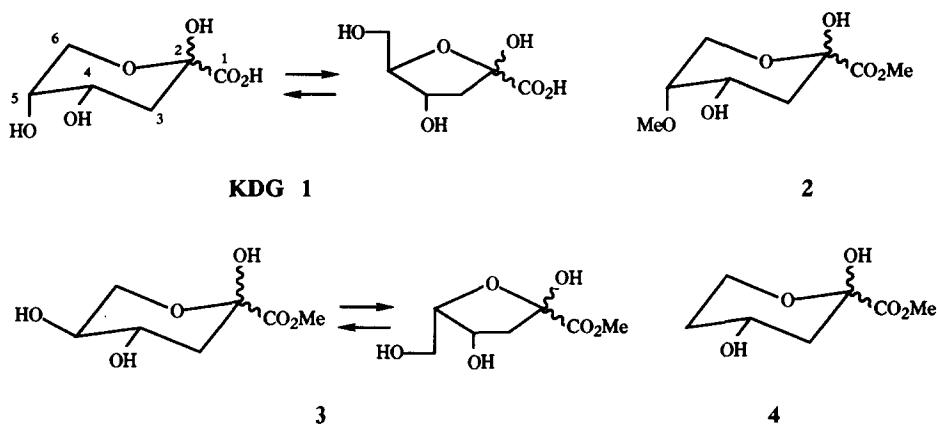
Abstract: Two title compounds, methyl 3-deoxy-L-*threo*-2-hexulosonate 3 and methyl 3,5-dideoxy-D-*glycero*-2-hexulosonate 4, were prepared from D-*glucono*-1,5-lactone and proved to be *gratuitous* inducers of the expression of pectinase genes in the phytopathogenic bacteria *Erwinia chrysanthemi*.

INTRODUCTION

Phytopathogenic bacteria of *Erwinia* type and, particularly, *E. chrysanthemi* have pectinolytic and cellulolytic properties which cause economically important damage in plants in the field or, after harvest, during storage. The degradation of pectins by the pectinases of this bacteria leads to the formation of 3-deoxy-D-*erythro*-2-hexulosonic acid or 2-keto-3-deoxy-D-gluconic acid (KDG) 1 which is then metabolized. This compound induces the expression of pectinase genes by binding to a specific site on the KdgR protein, a repressor of the expression of these genes. As part of a study of the functionalities which are necessary for inducers to recognize the repressor protein, we have previously shown¹ that the 5-*O*-methyl derivative 2 of KDG (scheme 1) was a *gratuitous* inducer i.e. it was recognized by the KdgR protein but not metabolized.

To complete this study concerning the importance of the C-5 hydroxyl function in the above recognition (is it useful as an acceptor for hydrogen bonding, can its stereochemistry be modified or is it useless ?) we have

now prepared two new analogs of KDG, methyl 3-deoxy-L-threo-2-hexulosonate **3** (the methyl ester of 5-*epi*-KDG) and methyl 3,5-dideoxy-D-glycero-2-hexulosonate **4** (the methyl ester of 5-deoxy-KDG).



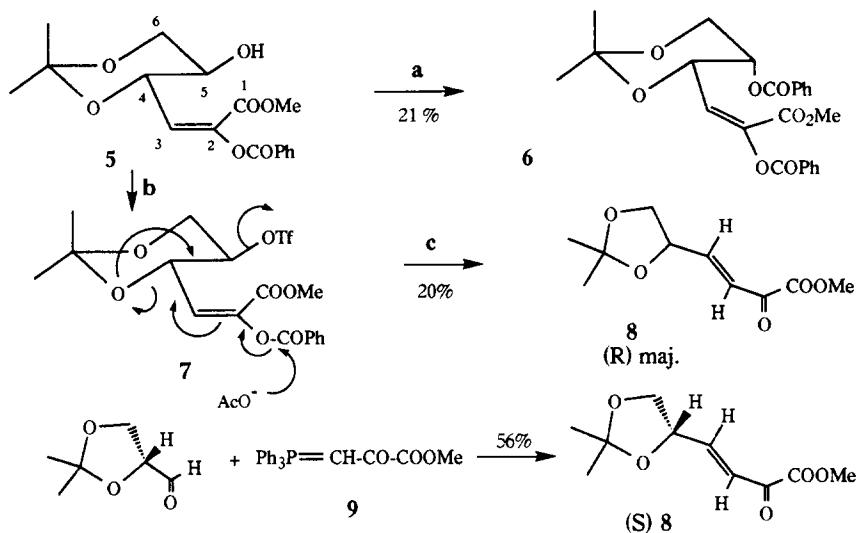
Scheme 1

RESULTS AND DISCUSSION

Since KDG exists as a mixture of four species (α and β pyranose and α and β furanose forms) in equilibrium it was obviously an inconvenient starting material for the preparation of C-5 analogs. We thus followed an approach similar to that used for the preparation of KDG or for its 5-*O*-methyl methyl ester **2**, starting from compound **5** obtained in four steps from D-glucono-1,5-lactone.²

*Synthesis of 5-*epi*-KDG methyl ester (3)*

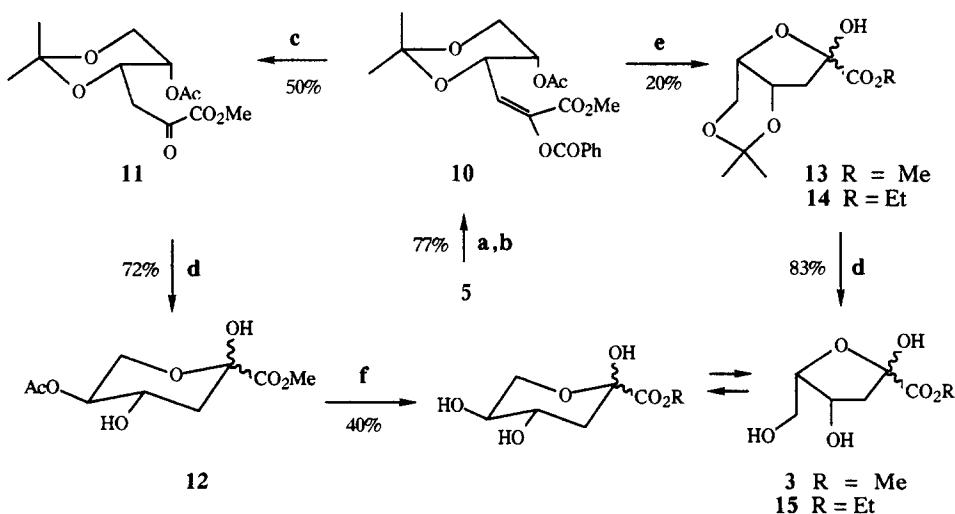
To obtain this compound the key step consisted in the epimerization of the C-5 in compound **5**. We first tried the Mitsunobu procedure³ using benzoic acid as a nucleophile (scheme 2). Under these conditions, the expected C-5 epimeric benzoate **6** was obtained but was contaminated with by-products from which it was hardly separated. Therefore we then turned to the S_N2 displacement of the triflate **7**. This compound was prepared from alcohol **5** using triflic anhydride and 2,6-di-*tert*-butylpyridine as a base.⁴ After disappearance of the starting material (t.l.c.) a solution of tetrabutylammonium acetate (2 eq) in acetone was added at room temperature. From the complex mixture thus obtained we were able to isolate the major compound **8** (20%). The formation of **8** from **7** can be explained by the mechanism depicted in scheme 2. The structure of **8** was established by comparison with an authentic sample prepared by a Wittig reaction between 2,3-*O*-isopropylidene-D-glyceraldehyde⁵ and phosphorane **9b** (scheme 2). The ¹H and ¹³C NMR spectra data of the compound thus obtained and those of **8** were identical. However if the optical rotation for the compound obtained from **7** (-3°) and for the compound prepared by the Wittig reaction ($+45^\circ$) were, as expected, of opposite sign, their absolute values differ notably. This result shows that a partial racemisation of (*R*) **8** occurred under the reaction conditions, the acetate ion acting as a base.



a) PPh_3 , DEAD, PhCOOH ; b) Tf_2O , 2,6-di-*tert*-butylpyridine, CH_2Cl_2 ; c) $\text{AcO}^- \text{Bu}_4\text{N}^+$, CH_3COCH_3

Scheme 2

We thought that the ring contraction leading to 8 was due to the high polarity of the solvent and the low nucleophilicity of the acetate ion. Therefore, in order to circumvent this undesired process and favour the $\text{S}_{\text{N}}2$ reaction, the crude triflate 7 was reacted with a large excess (10 eq) of tetrabutylammonium acetate in dichloromethane, a less polar solvent than acetone. As expected compound 10 was now formed in 77% yield (scheme 3).



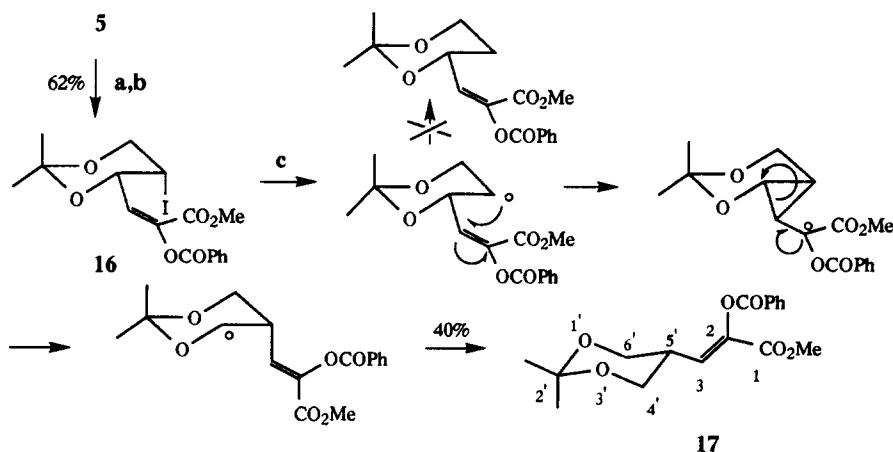
a) Tf_2O , 2,6-di-*tert*-butylpyridine, CH_2Cl_2 ; b) $\text{AcO}^- \text{Bu}_4\text{N}^+$, CH_2Cl_2 ; c) $\text{MeO}^- \text{Na}^+$, MeOH, 4 °C, 3 h; d) PPTS, MeOH; e) $\text{MeO}^- \text{Na}^+$, MeOH, 4 °C, 72 h; f) $\text{MeO}^- \text{Na}^+$, MeOH, 4 °C, 200 h.

Scheme 3

The methyl ester of 5-*epi*-KDG 3 was then obtained in two or three steps. A controlled (4 °C, 3 h) basic methanolysis of the enol benzoate led to a methanolic solution of the keto-ester 11⁷ whose acidification led to the simultaneous deprotection of the hydroxyl functions at C-4 and C-6 furnishing 12. Finally a Zemplen deprotection of the 5-acetoxy group led to 3 (scheme 3). This compound could be obtained by a slightly different sequence. Compound 10, when submitted to prolonged methanolysis (4 °C, 72 h), gave rise to 13 but in low yield (20%) probably because a β -elimination reaction took place. The acidic hydrolysis of 13 led to 3 which was more easily purified than the product obtained using the preceding sequence. We were not able however to obtain a satisfactory elemental analysis for compound 3. In as much as the preparation of 15, the ethyl ester analog of 3, had been recently reported⁸, we decided to prepare the former compound. The methyl ester 13 was first converted into the ethyl ester 14 (non isolated) whose hydroxyl functions were then deprotected. The spectral (¹H and ¹³C NMR) data and optical rotation of the compound thus obtained, in 80% yield for the two steps, were in perfect agreement with reported literature values.⁸ It is also of interest to note that the ¹³C NMR values of ester 3 are in good agreement (less than one ppm difference except for C-1) with the reported values for the enantiomeric acid obtained by enzymatic methods.⁹ The ¹H NMR values of these two compounds are also closely related.^{9,10} Finally, the chemical shifts and coupling constants of H-3e and H-3a in compound 3 are also very similar to those given for the same protons of the pyranose form of an analog : 3-deoxy-D-arabino-2-heptulosonic acid.¹¹

Synthesis of 5-deoxy-KDG methyl ester (4)

As for the synthesis of 5-*epi*-KDG, described above, compound 5 seemed to be an interesting precursor of the methyl ester of 5-deoxy-KDG 4 since only the C-5 hydroxyl was unprotected. Initially we attempted to carry out a radicalar deoxygenation.¹² However, when 1,1'-thiocarbonyldi-2,2'-pyridone was reacted with 5, we did not obtain the expected corresponding alkoxy(thiocarbonyl)-2-pyridone but only observed the 1,5 lactonisation of 5. Therefore we decided to reduce a halogenated compound. The 5-iodo derivative 16 was thus prepared using the triflate 7 as an intermediate. Unfortunately treatment of 16 with tributyltin hydride did not lead to the expected compound but to its achiral isomer 17 in 40% yield (scheme 4).

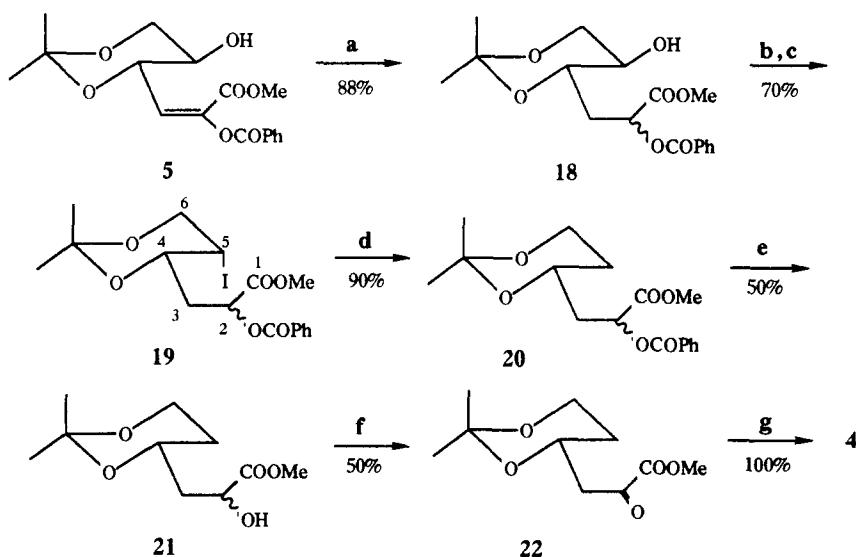


a) Tf₂O, 2,6-di-*tert*-butylpyridine, CH₂Cl₂; b) NaI, acetone; c) Bu₃SnH, AIBN cat., toluene, 75 °C.

Scheme 4

This compound resulted from a two-step rearrangement, the first one converting the initially formed radical into a more stabilized capto-dative one. The structure of 17 was ascertained from its ^1H NMR spectrum. Indeed we observed the eight signals of the AB part of a ABX system (δ 3.7 to 4.0 ppm, for 4H : 2 H-4' + 2 H-6'), a large multiplet ($\Sigma J = 35.2$ Hz) for the allylic H-5' at 2.9 ppm and a doublet ($^3J = 9.8$ Hz) for the vinylic proton H-3 at 6.62 ppm. Only the *Z* isomer was formed as indicated by the NMR shift of H-3 compared to those in related structures.^{13,14,15} The symmetry of 17 was confirmed by its lack of optical activity and by the presence of a single signal (at 62.7 ppm) for the two methylenic carbons C-4' and C-6' in the ^{13}C NMR spectrum.

Surprisingly, attempted hydrogenolysis of the carbon-iodine bond in compound 16 simultaneously with the hydrogenation of the carbon-carbon double bond to obtain 20 was unsuccessful leading only to degradation products. This compound 20 was then prepared, as a mixture of stereoisomers, by the sequence depicted in scheme 5. We first reduced the carbon-carbon double bond of 5 and then converted the resulting alcohols 18 into the corresponding triflates which were submitted to the action of sodium iodide. Finally the iodides 19 thus obtained were hydrogenolyzed in the presence of diisopropylethylamine¹⁶ to give 20. We checked that in the absence of hydrogen, compounds 19 did not undergo any elimination of the axial iodine. If such an elimination had occurred (involving axial proton at C-4) the further hydrogenation of the enol ether thus formed would have led to a partially, or totally, racemized 21. The methanolysis of the benzoate ester in diastereomers 20 followed by an oxydation of the resulting alcohols 21 using Czernecki *et al.* conditions¹⁷ led to the keto-ester 22 which was then protected to give the target compound 4.



a) H_2 , Pd/C; b) Tf_2O , 2,6-di-*tert*-butylpyridine, CH_2Cl_2 ; c) NaI, acetone; d) H_2 , Pd/C, N,N-diisopropylethylamine; e) $\text{MeO}^- \text{Na}^+$, MeOH; f) PDC, 3 Å molecular sieve, AcOH, CH_2Cl_2 ; g) PPTS, MeOH.

Scheme 5

CONCLUSION

Compounds **3** and **4** were obtained in four and five steps respectively from the intermediate alcohol **5**. They, as well as the acetylated derivative **12**, were tested and proved to be, as their free acids¹⁸, *gratuitous* inducers of pectinases in *Erwinia chrysanthemi*. These results provide evidence that the hydroxyl function in C-5 is not required for the recognition between inducers and the KdgR repressor protein. Therefore we can now envisage the preparation of affinity columns, by immobilizing inducers on supports using a spacer arm bound to this function. Such columns could then be used for the purification of the repressor protein.

EXPERIMENTAL

General methods. Solvents were distilled and dried before use. The reactions were monitored by t.l.c. on Silica Gel 60 F₂₅₄ (Merck) and detection was carried out by charring with a 5 % phosphomolybdic acid solution in ethanol containing 10 % H₂SO₄ and heating. Organic layers were dried on anhydrous MgSO₄. Flash column chromatographies were performed on silica gel Merck 60 H. The composition of eluents is expressed in parts by volumes. Melting points were determined with a Kofler hot-stage melting-point apparatus. Unless otherwise specified NMR spectra were recorded in CDCl₃ on a Bruker AM 200 (200 MHz for ¹H, 50.32 MHz for ¹³C) spectrometer. ¹H NMR (300 MHz) or ¹³C NMR (75.47 MHz) spectra were recorded on a Bruker AM 300. Chemical shifts are given in ppm downfield from internal Me₄Si. Coupling constants are expressed in Hertz and splitting pattern abbreviations are : s, singulet; d, doublet; t, triplet; m, multiplet; p, pseudo. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by "Service Central de Microanalyses du CNRS" (69390 Vernaison, France).

Methyl 2,5-di-O-benzoyl-3-deoxy-4,6-O-isopropylidene-L-threo-E-2-hexenonate (6). A solution of diethyl azodicarboxylate (2.4 ml, 15 mmol) in anhydrous THF (8 ml) was added dropwise to a solution of **5**² (1.46 g, 4.34 mmol), triphenylphosphine (2.23 g, 5.7 mmol) and benzoic acid (1.4 g, 11.4 mmol) in anhydrous THF (35 ml). The mixture was heated at 50 °C for 50 h and then concentrated in vacuo. Three successive chromatographies of the crude product (acetone-petroleum ether: 6/1; then 10/1; then dichloromethane) afforded 400 mg (21 %) of **6** as a syrupy product not pure enough for analysis. $[\alpha]_{\text{D}}^{20} = +132^{\circ}$ (c = 1.9, CHCl₃). ¹H NMR : 8.14-8.00 (m, 4H, H ortho); 7.63-7.50 (m, 2H, H para); 7.50-7.40 (m, 4H, H meta); 6.16 (d, 1H, J_{3,4} = 7.5, H-3); 5.75 (dd, 1H, J_{4,3} = 7.5, J_{4,5} = 2.0, H-4); 5.17 (pq, 1H, J_{5,6e} = J_{5,6a} = J_{5,4} = 2.0, H-5); 4.30 (dd, 1H, J_{6e,6a} = 13.3, J_{6e,5} = 2.0, H-6e); 4.09 (dd, 1H, J_{6a,6e} = 13.3, J_{6a,5} = 2.0, H-6a); 3.78 (s, 3H, OMe) ; 1.59 (s, 3H, Me); 1.51 (s, 3H, Me). ¹³C NMR : 166.1, 164.8, 162.0 (3 COO); 138.5 (C-2); 133.8, 133.1, 130.8 (2C para, C-3); 130.2, 129.9, 128.5, 128.4 (4 C meta, 4C ortho); 130.1, 128.5 (2C ipso); 99.1 (CMe₂); 69.8, 67.9 (C-4, C-5); 62.6 (C-6); 52.5 (OMe); 29.2 (Me); 19.0 (Me).

Methyl 3,4-dideoxy-5,6-O-isopropylidene-D-glycero-E-3-eno-2-hexuloseonate (S-8). A solution of 2,3-O-isopropylidene-D-glyceraldehyde⁵ (690 mg, 5.3 mmol) and methoxyoxalyl methylenetriphenylphosphorane⁶ (2.2 g, 6.1 mmol) in toluene (130 ml) was left 24 h at room temperature. The solvent was evaporated to dryness, the residue was triturated with diethyl ether (30 ml) and the mixture cooled to

0 °C. The precipitated triphenylphosphine oxide was filtered off and washed with cold diethyl ether (2 x 10 ml). The combined filtrate and washing were concentrated to a yellow solid which was chromatographed (diethyl ether-petroleum ether: 1/2) giving the ketoester (**S**) **8** (530 mg, 56 %) as a pale yellow syrup. $[\alpha]_{\text{D}}^{20} = +45^{\circ}$ ($c = 1$, CHCl_3). $^1\text{H NMR}$: 7.13 (dd, 1H, $J_{4,3} = 15.8$, $J_{4,5} = 4.7$ H-4); 6.96 (dd, 1H, $J_{3,5} = 1.2$, $J_{3,4} = 15.8$, H-3); 4.77 (ptdd, 1H, $J_{5,6} = J_{5,6'} = 6.8$, $J_{5,4} = 4.7$, $J_{5,3} = 1.2$, H-5); 4.40 (dd, 1H, $J_{6,6'} = 8.3$, $J_{6,5} = 6.8$, H-6); 3.90 (s, 3H, OCH₃) 3.72 (dd, 1H, $J_{6',6} = 8.3$, $J_{6',5} = 6.8$, H-6'); 1.47 (s, 3H, CH₃); 1.43 (s, 3H, CH₃). $^{13}\text{C NMR}$: 182.9 (C-2); 162.5 (C-1); 150.0 (C-4); 125.0 (C-3); 110.9 (CMe₂); 75.6 (C-5); 69.0 (C-6); 53.4 (OMe); 26.8 (Me); 26.3 (Me).

Methyl 3,4-dideoxy-5,6-O-isopropylidene D and L glycerol-E-3-eno-2-hexulosonate (8). To a solution of alcohol **5** (270 mg, 0.8 mmol) and di-*tert*-butylpyridine (0.36 ml, 1.6 mmol) in dry CH_2Cl_2 (2.5 ml) kept at -15°C, was added trifluoromethanesulfonic anhydride (0.195 ml, 1.16 mmol). T.l.c (diethyl ether-petroleum ether, 1/1) showed the reaction to be complete after 25 min. A solution of tetra-*n*-butylammonium acetate (470 mg, 1.6 mmol) in acetone (5 ml) was then added and the reaction was monitored by t.l.c (diethyl ether-petroleum ether, 1/1). After 3.5 h the reaction mixture was diluted with diethyl ether (100 ml) washed with aqueous sodium hydrogenocarbonate (20 ml) and brine (20 ml). Aqueous layers were extracted with diethyl ether (2 x 20 ml). The combined organic layers were dried, filtered and evaporated to give a red-brown oil (1.15 g). The crude product was flash chromatographed (diethyl ether-petroleum ether: 1/2) to yield **8** as a colorless oil (140 mg, 46%). A second flash chromatography was required to obtain **8** pure. Its ^1H and ^{13}C NMR spectra were identical with those of (**S**) **8**. $[\alpha]_{\text{D}}^{20} = -3^{\circ}$ ($c = 1$, CHCl_3).

Methyl 5-O-acetyl-2-O-benzoyl-3-deoxy-4,6-O-isopropylidene-L-threo-hex-E-2-enonate (10). To a solution of triflate **7** prepared as described above from **3g** (9 mmol) of **5**, was added a solution of tetra-*n*-butylammonium acetate (32g, 108 mmol) in dichloromethane (16 ml). The reaction mixture was kept at 0 °C for 40 min, poured into diethyl ether (400 ml) and the solution was washed with saturated aqueous sodium hydrogenocarbonate (150 ml) and then brine (150 ml). The aqueous layer was extracted with diethyl ether (2 x 150 ml) and the combined organic layers were dried. Removal of the solvent under reduced pressure and flash chromatography of the residue (diethyl ether-petroleum ether: 1/4 and then 1/1) gave **10** (2.6 g, 77%) as a white solid. mp : 122 °C. $[\alpha]_{\text{D}}^{20} = +82^{\circ}$ ($c = 4$, CHCl_3). $^1\text{H NMR}$: 8.11-8.05 (m, 2H, H ortho); 7.70-7.56 (m, 1H, H para); 7.53-7.44 (m, 2H, H meta); 6.09 (d, 1H, $J_{3,4} = 7.2$, H-3); 5.62 (dd, 1H, $J_{4,3} = 7.2$, $J_{4,5} = 2.2$, H-4); 4.99 (pq, 1H, $J_{5,6a} = J_{5,6e} = 1.9$, $J_{5,4} = 2.2$, H-5); 4.21 (dd, 1H, $J_{6e,6a} = 13.3$, $J_{6e,5} = 1.9$, H-6e); 3.96 (dd, $J_{6a,6e} = 13.3$, $J_{6a,5} = 1.9$, 1H, H-6a); 3.77 (s, 3H, OMe); 2.14 (s, 3H, MeCO); 1.55 (s, 3H, Me); 1.47 (s, 3H, Me). $^{13}\text{C NMR}$: 170.8, 164.8, 161.9 (3 COO); 138.0 (C-2); 133.9, 131.4 (C para, C-3); 130.2, 128.6 (2 C meta, 2 C ortho); 128.5 (C ipso); 99.0 (CMe₂); 67.1, 66.8 (C-4, C-5); 62.6 (C-6); 52.5 (OMe); 29.1 (OCOMe); 21.0 (Me); 18.9 (Me). Anal. Calcd. for $\text{C}_{19}\text{H}_{22}\text{O}_8$: C, 60.32; H, 8.52. Found C, 60.18; H, 5.76.

Methyl 5-O-acetyl-3-deoxy-4,6-O-isopropylidene-L-threo-2-hexulosonate (11). A solution of compound **10** (630 mg, 1.7 mmol) and sodium methoxide (10 mg, 0.2 mmol) in anhydrous methanol (60 ml) was kept at 0 °C for 3 h and then neutralized with Amberlite IR 120 (H⁺ form) resin. The reaction mixture was filtered and an aliquot was evaporated and flash chromatographed (diethyl ether-petroleum ether: 1/1) to give **11** contaminated by a small amount (about 10%) of **10**. $^1\text{H NMR}$: 4.67-4.62 (m, 2H, H-4, H-5); 4.15 (dd, $J_{6e,6a} =$

13.4, $J_{6e,5} = 2.2$, H-6e); 3.91-3.77 (m, 1H, H-6a); 3.87 (s, 3H, OCH₃); 3.13 (dd, 1H, $J_{3,3'} = 17.9$, $J_{3,4} = 7.3$, H-3); 2.95 (dd, 1H, $J_{3,3'} = 17.9$, $J_{3,4} = 5.3$, H-3'); 2.17 (s, 3H, MeCOO); 1.49 (s, 3H, Me); 1.40 (s, 3H, Me). ¹³C NMR : 190.8 (C-2); 171.0 (MeCOO); 160.9 (C-1); 99.2 (CMe₂); 66.8, 65.8 (C-4, C-5); 62.8 (C-6); 53.1 (OMe); 40.9 (C-3); 28.8 (Me); 21.2 (OCOMe); 18.9 (Me).

Methyl 5-O-acetyl-3-deoxy- α and β -L-threo-2-hexulopyranosonate (12). To the above remaining methanolic solution was added pyridinium *p*-toluenesulfonate (100 mg, 0.3 mmol) and the reaction mixture was heated at 55 °C for 8 h. The methanol was then evaporated and the residue was flash chromatographed (diethyl ether-petroleum ether: 3/2) to afford compound 12 (280 mg, 72%) as a syrupy product. $[\alpha]_D^{20} = +33^\circ$ (c = 1.7, CHCl₃). Anal. Calcd. for C₉H₁₄O₇ : C; 46.15; H; 5.98. Found C, 46.35; H, 5.91. 12 (β -anomer, 85%). ¹H NMR (D₂O) : 4.76 (m, 1H, H-5); 4.15 (ddd, 1H, $J_{4,3a} = 10.9$, $J_{4,5} = 9.0$, $J_{4,3e} = 5.0$, H-4); 3.97 (dd, 1H, $J_{6e,6a} = 11.4$, $J_{6e,5} = 5.3$, H-6e); 3.83 (s, 3H, OMe); 3.76 (dd, 1H, $J_{6a,6e} = 11.4$, $J_{6a,5} = 10.0$, H-6a); 2.32 (dd, 1H, $J_{3e,3a} = 13.4$, $J_{3e,4} = 5.0$, H-3e); 2.14 (s, 3H, COMe); 1.97 (dd, 1H, H-3a). ¹³C NMR (D₂O) : 174.0, 171.5 (2 COO); 95.6 (C-2), 72.8 (C-5); 66.0 (C-4); 60.6 (C-6); 54.0 (OMe); 39.0 (C-3); 20.8 (OCOMe). 12 (α -anomer, 15%). ¹³C NMR (D₂O) : 173.9, 171.5 (2 COO); 95.5 (C-2), 70.5 (C-5); 64.7 (C-4); 60.2 (C-6); 54.0 (OMe); 34.4 (C-3); 20.8 (OCOMe).

Methyl 3-deoxy-4,6-O-isopropylidene-L-threo-2-hexulofuranosonate (13). A solution of compound 10 (1g, 2.65 mmol) and sodium methoxide (60 mg, 1.1 mmol) in anhydrous methanol (70 ml) was kept at 0 °C for 3 days and then neutralized with Amberlite IR 120 (H⁺ form) resin. The resin was filtered off and the filtrate was concentrated to dryness and flash chromatographed (diethyl ether-petroleum ether: 1/1) to give 11 (55 mg, 8%) and 13 (110 mg, 21%) as colorless oils. 13 $[\alpha]_D^{20} = +11^\circ$ (c = 4.5, CHCl₃). 13 (major isomer, 75%). ¹H NMR : 4.57-4.50 (m, 1H, H-5); 4.47 (m, 1H, OH); 4.20-4.04 (m, 2H, H-4, H-6e); 4.02-3.95 (m, 1H, H-6a); 3.84 (s, 3H, OMe); 2.70 (d, 1H, $J_{3,3'} = 14.1$ Hz, H-3); 2.30 (d, 1H, $J_{3,3'} = 14.1$, H-3'); 1.47 (s, 3H, Me); 1.44 (s, 3H, Me). ¹³C NMR : 169.7 (C-1); 103.2 (C-2); 98.1 (CMe₂); 76.0, 70.7 (C-5, C-4); 60.8 (C-6); 53.1 (OMe); 43.3 (C-3); 28.9 (Me); 19.1 (Me). 13 (minor isomer, 25%). ¹H NMR : 4.57-4.50 (m, 1H, H-5); 4.47 (m, 1H, OH); 4.20-4.04 (m, 2H, H-4, H-6e); 4.02-3.95 (m, 1H, H-6a); 3.86 (s, 3H, OMe); 2.67 (d, 1H, $J_{3,3'} = 14.0$ Hz, H-3); 2.36 (dd, 1H, $J_{3,3'} = 14.0$, $J_{3,4} = 5.8$, H-3'); 1.44 (s, 3H, Me); 1.38 (s, 3H, Me). ¹³C NMR : 170.5 (C-1); 102.1 (C-2); 97.8 (CMe₂); 74.6, 70.4 (C-5, C-4); 57.9 (C-6); 53.3 (OMe); 43.5 (C-3); 28.2 (Me); 19.9 (Me). Anal. Calcd. for C₁₀H₁₆O₆ + 0.5 H₂O (hygroscopic): C; 49.79; H; 7.05. Found C, 50.04; H, 6.98.

Methyl 3-deoxy-L-threo-2-hexulose (3). From 12 : a solution of compound 12 (70 mg, 0.31 mmol) and sodium methoxide (3 mg, 0.06 mmol) in anhydrous methanol (10 ml) was stirred at +4 °C for 8 days and then neutralized with Amberlite IR 120 (H⁺ form). The resin was filtered off and the filtrate was evaporated to dryness and flash chromatographed (methanol-dichloromethane: 1/15) to give 3 (22 mg, 38%) as a colorless syrup. From 13 : a solution of compound 13 (70 mg, 0.3 mmol) and pyridinium paratoluenesulfonate (12 mg, 0.04 mmol) in anhydrous methanol (2 ml) was heated at 50 °C for 2 h and the solvent was removed in vacuo. The residue was then flash chromatographed (methanol-dichloromethane: 1/15) to afford 44 mg (83 %) of a colorless syrup. The compound 3 thus obtained was purer (¹³C NMR) than this obtained from 12. $[\alpha]_D^{20} = +1.5^\circ$ (c = 2.2, CHCl₃, 2 h). NMR data of the predominant form in D₂O (α pyranose) : ¹H NMR : 3.97-3.44 (m, 4H, H-

4, H-5, H-6, H-6'); 3.82 (s, 3H, OCH₃); 2.26 (dd, 1H, J_{3e,3a} = 13.2, J_{3e,4} = 4.9, H-3e); 1.83 (dd, 1H, J_{3a,3e} = 13.2, J_{3a,4} = 11.3, H-3a). ¹³C NMR : 171.8 (C-1); 95.9 (C-2); 70.6, 68.7 (C-4, C-5); 63.6 (C-6); 54.0 (OMe); 38.8 (C-3).

Ethyl 3-deoxy-L-threo-2-hexuloseonate (15). Compound 13 (90 mg, 0.39 mmol) was dissolved in ethanol (2 ml) freshly distilled from magnesium ethoxide and a solution of 5 mg (0.05 mmol) of sodium ethoxide in ethanol (0.5 ml) was added. After standing at room temperature for 10 min the solution was neutralized by stirring with Dowex 50x8-200 (H⁺ form) resin (170 mg, 0.7 mmol). The resin was filtered off and to the solution of 14 thus obtained was added pyridinium *p*-toluenesulphonate (15 mg, 0.05 mmol) and the reaction mixture was heated at 55 °C for 2 h. Removal of the solvent in vacuo provided a clear oil which was flash chromatographed (methanol-dichloromethane: 1/15) to afford 68 mg (80%) of 15 as a colorless oil. ¹H (300 MHz) and ¹³C NMR (75.47 MHz) data are identical with those of literature⁷. [α]_D²⁵ = - 5°(c = 1, CHCl₃, 18h); [α]_D²⁵ = - 6.7°(c = 1, CHCl₃, 20 min); lit.⁸: [α]_D²⁵ = - 7°(c = 1, CHCl₃, 20 min¹⁹).

Methyl 2-O-benzoyl-3,5-dideoxy-5-iodo-4,6-O-isopropylidene-L-threo-hex-E-2-enonate (16). To a solution of 5 (500 mg, 1.5 mmol) and 2,6-di-*tert*-butylpyridine (0.65 ml, 3 mmol) in anhydrous dichloromethane (2 ml) at 0°C, was added trifluoromethanesulfonic anhydride (0.37 ml, 2.2 mmol). After 5 min a solution of sodium iodide (1.12 g, 7.5 mmol) in anhydrous acetone (5 ml) was added to the mixture. After 1 h the mixture was diluted with diethyl ether (200 ml), washed with saturated aqueous sodium hydrogenocarbonate (50 ml) and then with aqueous sodium thiosulfate (20 ml). The aqueous layers were extracted with diethyl ether (2 x 50 ml) and the combined organic layers were dried. Removal of the solvent under reduced pressure followed by flash chromatography (diethyl ether-petroleum ether: 1/2) of the residue provided 415 mg (62%) of a white solid. mp 112°C. [α]_D²⁰ = + 50°(c = 2.7, CHCl₃). ¹H NMR : 8.13-8.00 (m, 2H, H ortho); 7.67-7.55 (m, 1H, H para); 7.57-7.39 (m, 2H, H meta); 5.96 (d, 1H, J_{3,4} = 6.5, H-3); 4.82 (dd, 1H, J_{4,3} = 6.5, J_{4,5} = 2.0, H-4); 4.52 (m, 1H, H-5); 4.32 (dd, 1H, J_{6e,6a} = 13.3, J_{6e,5} = 2, H-6e); 4.02 (dd, 1H, J_{6a,6e} = 13.3, J_{6a,5} = 1.6, H-6a); 3.75 (s, 3H, OMe); 1.52 (s, 3H, Me); 1.51 (s, 3H, Me). ¹³C NMR : 165.3, 162.6 (2 COO); 138.0 (C ipso); 137.0, 134.4 (C-3, C para); 130.2, 128.9 (2C meta, 2C ortho); 128.8 (C-2); 100.4 (CMe₂); 68.4 (C-4); 67.9 (C-6); 53.0 (OMe); 31.3 (C-5); 30.0 (Me); 20.7 (Me). Anal. Calcd. for C₁₇H₁₉O₆I : C, 45.74; H, 4.26; I, 28.47. Found C, 45.77; H, 4.08; I, 28.33.

Methyl 2-benzoyloxy-3-(2',2'-dimethyl-1',3'-dioxan-5'-yl)-prop-Z-2-enoate (17). Compound 16 (200 mg, 0.44 mmol) was dissolved in toluene (2.2 ml). To the solution tributyltin hydride (0.29 ml, 1.05 mmol) and a catalytic amount of AIBN were added and the mixture was heated at 75 °C for 24 h. Removal of the solvent under reduced pressure followed by flash chromatography (diethyl ether-petroleum ether: 1/3) gave a syrupy product which crystallised by adding petroleum ether. Filtration and washing with petroleum ether provided 60 mg (42%) of 17 as a white solid. mp 119 °C. [α]_D²⁰ = 0. ¹H NMR : 8.15-8.05 (m, 2H, H ortho); 7.0-7.60 (m, 1H, H para); 7.59-7.48 (m, 2H, H meta); 6.62 (d, 1H, J_{3,5'} = 9.8, H-3); 3.93 (dd, 2H, J_{4'e,4'a} or J_{6'e,6'a} = 11.8, J_{4'e,5'} or J_{6'e,5'} = 4.7, H-4'e, H-6'e); 3.79 (dd, 2H, J_{4'a,4'e} or J_{6'a,6'e} = 11.8, J_{4'a,5'} or J_{6'a,5'} = 8.0, H-4'a, H-6'a); 3.78 (s, 3H, OMe); 2.88 (m, 1H, H-5'); 1.45 (s, 3H, Me); 1.39 (s, 3H, Me). ¹³C NMR (75.47 MHz): 164.5, 161.9 (2 COO); 139.9 (C-2); 134.0 (C para); 130.3, 128.6 (2C meta, 2C ortho); 128.2,

128.1 (C-3, C ipso); 97.9 (CMe₂); 62.7 (2C, C-4', C-6'); 52.5 (OMe); 33.2 (C-5'). 25.8 (Me); 21.7 (Me). Anal. Calcd. for C₁₇H₂₀O₆: C, 63.75; H, 6.25. Found C, 64.07; H, 6.20.

Methyl 2-O-benzoyl-3-deoxy-4,6-O-isopropylidene-D-(ribo and arabo)-hexonate (18). A solution of the alcohol **5** (4.4 g, 13.1 mmol) containing 0.2 ml (1.4 mmol) of triethylamine in methanol (30 ml) was hydrogenated over palladium on charcoal (10%, 600 mg) under a pressure of 2 atm at room temperature for 7 h. The catalyst was then filtered off and the filtrate was concentrated in vacuo to give a mixture of 2 diastereoisomers in a 6/4 ratio as a colorless oil. ¹H NMR : 8.15-8.00 (m, 2H, H ortho); 7.65-7.52 (m, 1H, H para); 7.51-7.43 (m, 2H, H meta); 5.52-5.43 (m, 1H, H-2); 3.97-3.49 (m, 4H, H-4, H-5, H-6, H-6'); 3.77 and 3.75 (2s, 3H, 2 x OMe); 2.71 (broad s, 1H, OH); 2.59-2.44 (m, 1H, H-3); 2.26-2.01 (m, 1H, H-3'); 1.44, 1.39, 1.32, 1.30 (4s, 6H, 4 x Me). ¹³C NMR : major isomer : 170.50, 165.9 (2 COO); 133.5 (C para). 129.9, 129.2 (2C meta, 2C ortho); 129.3 (C ipso); 98.9 (CMe₂); 69.8, 68.9, 63.7 (C-2, C-4, C-5); 64.9 (C-6); 52.4 (OMe); 34.1 (C-3); 28.4 (Me); 19.2 (Me). minor isomer: 171.2, 165.9 (2 COO); 133.4 (C para). 129.9, 129.2 (2C meta, 2C ortho); 129.4 (C ipso); 98.8 (CMe₂); 70.4, 69.6, 67.1 (C-2, C-4, C-5); 52.2 (OMe); 34.0 (C-3); 28.4 (Me); 19.0 (Me). Anal. Calcd. for C₁₇H₂₂O₇: C, 60.36; H, 6.51. Found C, 59.85; H, 6.88.

Methyl 2-O-benzoyl-3,5-dideoxy-5-iodo-4,6-O-isopropylidene-L-(lyxo and xylo) hexonate (19). In the same manner as described for the preparation of **16**, the mixture of diastereoisomers **19** was prepared from **18** (1.85 g, 5.5 mmol) to afford 1.71 g (70%) of a pale yellow syrup. The products were not stable enough for elemental analysis. ¹H NMR : 8.11-8.05 (m, 2H, H ortho); 7.65-7.52 (m, 1H, H para); 7.51-7.43 (m, 2H, H meta); 5.50-5.35 (m, 1H, H-2); 4.32-3.98 (m, 3H, H-4, H-6, H-6'); 3.78 and 3.77 (2s, 3H, 2 x OMe); 3.37-3.16 (m, 1H, H-5); 2.42-2.22 (m, 1H, H-3); 2.07-1.89 (m, 1H, H-3'); 1.46, 1.44, 1.41, 1.30 (4s, 6H, 4 Me). ¹³C NMR : major isomer : 170.4, 165.7 (2 x COO); 133.5 (C para); 129.8, 128.5 (2C meta, 2C ortho); 129.1 (C ipso); 100.0 (CMe₂); 68.9, 67.0 (2C, C-2, C-4); 67.9 (C-6); 52.5 (OMe); 40.6 (C-3); 34.1 (C-5); 29.2 (Me); 20.0 (Me). minor isomer: 169.8, 165.7 (2 x COO); 133.5 (C para); 129.8, 128.5 (2C meta, 2C ortho); 129.3 (C ipso); 99.8 (CMe₂); 69.0, 67.2 (2C, C-2; C-4); 67.9 (C-6); 52.2 (OMe), 40.6 (C-3); 34.1 (C-5); 29.2 (Me); 19.8 (Me).

Methyl 2-O-benzoyl-3,5-dideoxy-4,6-O-isopropylidene-D-(erythro and threo) hexonate (20). A solution of the iodide **19** (1.5 g, 3.4 mmol) containing 0.73 ml (4.2 mmol) of N,N diisopropylethylamine in methanol (35 ml) was hydrogenated over palladium on charcoal (10%, 350 mg) under a pressure of 2 atm at room temperature for 45 min. The catalyst was then filtered off and the filtrate concentrated to a syrup which was flash chromatographed (diethyl ether-petroleum ether: 1/1) to furnish the diastereoisomeric mixture **20** (1.0 g, 93%) as a colorless oil. ¹H NMR : 8.13-8.07 (m, 2H, H ortho); 7.64-7.56 (m, 1H, H para); 7.51-7.43 (m, 2H, H meta); 5.54-5.41 (m, 1H, H-2); 4.26-3.80 (m, 3H, H-4, H-6, H-6'); 3.76, 3.75 (2s, 3H, 2 x OMe); 2.32-1.98 (m, 2H, H-5e, H-3); 1.80-1.59 (m, 1H, H-5); 1.52-1.40 (m, 1H, H-3'); 1.44, 1.36, 1.32 (3s, 6H, 4 x Me). ¹³C NMR : major isomer : 171, 165.8 (2 x COO); 133.4 (C para); 129.8, 128.5 (2C meta, 2C ortho); 129.5 (C ipso); 98.6 (CMe₂); 69.1, 64.6 (C-2, C-4); 59.8 (C-6); 52.3 (OMe); 37.9 (C-3); 31.3 (C-5); 29.8 (Me); 19.0 (Me). minor isomer : 170.3, 165.8 (2 x COO), 133.4 (C para); 129.8, 128.5 (2C meta; 2C ortho); 129.8 (C ipso); 98.5 (CMe₂); 69.5, 65.1 (C-2, C-4); 59.7 (C-6); 52.3 (OMe); 37.8 (C-3); 31.1 (C-5); 29.7 (Me); 18.8 (Me). Anal. Calcd. for C₁₇H₂₂O₆: C, 63.35; H, 6.83. Found C, 63.32; H, 6.94.

Methyl 3,5-dideoxy-4,6-O-isopropylidene-D-(erythro and threo) hexonate (21). To a solution of compounds **20** (1.08 g, 3.35 mmol) in dry methanol (25 ml), sodium methoxide (40 mg, 0.74 mmol) was added. After standing at room temperature for 2h, the solution was neutralized by stirring with Amberlite IR 120 (H⁺ form) resin and filtered. The filtrate was concentrated *in vacuo* and the residue was flash chromatographed (diethyl ether-petroleum ether: 1/2) to afford 320 mg (50%) of the diastereoisomeric mixture **21** as a colorless oil. ¹H NMR : 4.46-3.80 (m, 4H, H-2,H-4, H-6e, H-6a); 3.78, 3.76 (2s, 3H, 2 x OMe); 3.44-3.35 (m, 1H, OH); 2.10-1.53 (m, 3H, H-3, H-5a, H-5e); 1.43 (m, 1H, H-3); 1.47, 1.42, 1.38, 1.32 (4s, 6H, 4 Me). ¹³C NMR : major isomer : 175.3 (C-1); 98.6 (CMe₂); 68.2, 66.2 (C-4, C-2); 59.8 (C-6); 52.3 (OMe); 40.3 (C-3); 31.3 (C-5); 29.9 (Me); 19.1 (Me). minor isomer : 175.2 (C-1); 98.3 (CMe₂); 67.6, 65.0 (C-4, C-2); 59.9 (C-6); 52.2 (OMe); 39.8 (C-3); 30.9 (C-5); 29.8 (Me); 18.9 (Me). Anal. Calcd. for C₁₀H₁₈O₅: C, 55.04; H, 8.26. Found C, 55.10; H, 8.17.

Methyl 3,5-dideoxy-4,6-O-isopropylidene-D-glycero-2-hexulosonate (22). To a solution of 180 mg (0.8 mmol) of alcohols **21** and 550 mg (1.5 mmol) of PDC in 4 ml of CH₂Cl₂, 1.2 g of freshly activated 3Å molecular sieve powder was added, followed by 80 ml of AcOH. The mixture was stirred magnetically at room temperature and the reaction was followed by t. l. c. (ethyl acetate-petroleum ether, 1/1). After 4 h the reaction mixture was stirred with celite (500 mg) for about 20 min, filtered on silica gel (ethyl acetate-petroleum ether: 1/3) and the filtrate was evaporated to give 90 mg (50%) of **22** as a clear oil. $[\alpha]_D^{25} = -11^\circ$ (c = 2.7, CHCl₃). ¹H NMR : 4.54-4.39 (m, 1H, H-4); 4.0 (ptd, 1H, J_{6a,6e} = J_{6a,5a} = 11.7, J_{6a,5e} = 3.7, H-6a); 3.87 (s, 3H, OMe); 3.86-3.73 (m, 1H, H-6e); 3.06 (dd, 1H, J_{3,3'} = 17.0, J_{3,4} = 7.4, H-3); 2.88 (dd, 1H, J_{3,3'} = 17.0, J_{3',4} = 5.2, H-3'); 1.76-1.35 (m, 2H, H-5a, H-5e); 1.45 (s, 3H, Me); 1.32 (s, 3H, Me). ¹³C NMR : 190.0 (C-2); 161.3 (C-1); 98.6 (CMe₂); 65.1 (C-4); 59.6 (C-6); 52.9 (OMe); 45.8 (C-3); 29.9 (C-5); 29.7 (Me); 19.0 (Me). Anal. Calcd. for C₁₀H₁₆O₅: C, 55.56; H, 7.41. Found C, 55.76; H, 7.55.

Methyl 3,5-dideoxy-D-glycero-2-hexulopyranosonate (4). A solution of compound **22** (90 mg, 0.42 mmol) and pyridinium paratoluenesulfonate (12 mg, 0.04 mmol) in anhydrous methanol (4 ml) was heated at 60 °C for 15 min and the solvent was then removed *in vacuo*. The residue was flash chromatographed (petroleum ether-acetone: 2/1) to afford 75 mg (100%) of a colorless syrup. $[\alpha]_D^{20} = -1.1^\circ$ (c = 2.4, CHCl₃, 18 h). β anomer. ¹H NMR (300 MHz): 4.16 (ptt, 1H, J_{4,5a} = J_{4,3a} = 11.1, J_{4,5e} = J_{4,3e} = 4.6, H-4); 4.01 (ddd, 1H, J_{6a,5a} = 12.6, J_{6a,6e} = 11.6, J_{6a,5e} = 2.3, H-6a); 3.88-3.73 (m, 1H, H-6e); 3.84 (s, 3H, OMe); 2.13 (ddd, 1H, J_{3e,3a} = 12.6, J_{3e,4} = 4.6, J_{3e,5e} = 1.8, H-3e); 1.94, (m, 1H, H-5e) ; 1.87 (dd, 1H, J_{3a,3e} = 12.6, J_{3a,4} = 11.1, H-3a); 1.65 (ptdd, 1H, J_{5a,5e} = J_{5a,6a} = 12.6, J_{5a,4} = 11.1, J_{5a,6e} = 5.1, H-5a). ¹³C NMR : 170.7 (C-1); 95.5 (C-2); 63.9 (C-4); 60.5 (C-6); 53.3 (OMe); 39.9, 34.5 (C-3, C-5). α anomer. ¹H NMR : 4.33 (ptd, 1H, J_{6a,5a} = J_{6a,6e} = 12.2, J_{6a,5e} = 2.6, H-6a) ; 4.27 (m, J = 12.5, H-4); 3.88-3.73 (m, 2H, H-6e); 3.83 (s, 3H, OMe); 2.19 (dd, 1H, J_{3a,3e} = 14.0, J_{3a,4} = 3.3, H-3a); 2.01 (m, 1H, H-3e); 2.0-1.90 (m, 1H, H-5a); 1.74 (m, 1H, H-5e). ¹³C NMR (75.47 MHz): 170.1 (C-1); 95.1 (C-2); 63.7 (C-4); 56.5 (C-6); 53.2 (OMe); 36.2, 31.5 (C-3, C-5). Anal calcd for C₇H₁₂O₅ : C, 47.73; H, 6.82. Found : C, 48.01; H, 6.89.

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