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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*

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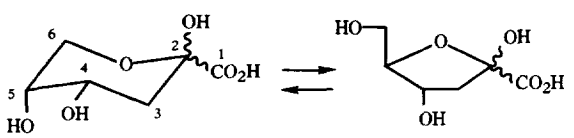
Abstract: Two title compounds, methyl 3-deoxy-L-*threo*-2-hexulosonate **3** and methyl 3,5-dideoxy-D-*glycero*-2-hexulosonate **4**, were prepared from D-*glucono*-1,5-lactone and proved to be *gratuitous* inducers of the expression of pectinase genes in the phytopathogenic bacteria *Erwinia chrysanthemi*.

INTRODUCTION

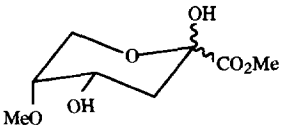
Phytopathogenic bacteria of *Erwinia* type and, particularly, *E. chrysanthemi* have pectinolytic and cellulolytic properties which cause economically important damage in plants in the field or, after harvest, during storage. The degradation of pectins by the pectinases of this bacteria leads to the 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 shown[1] 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).



KDG 1



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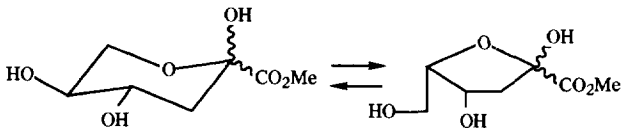

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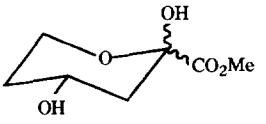


4

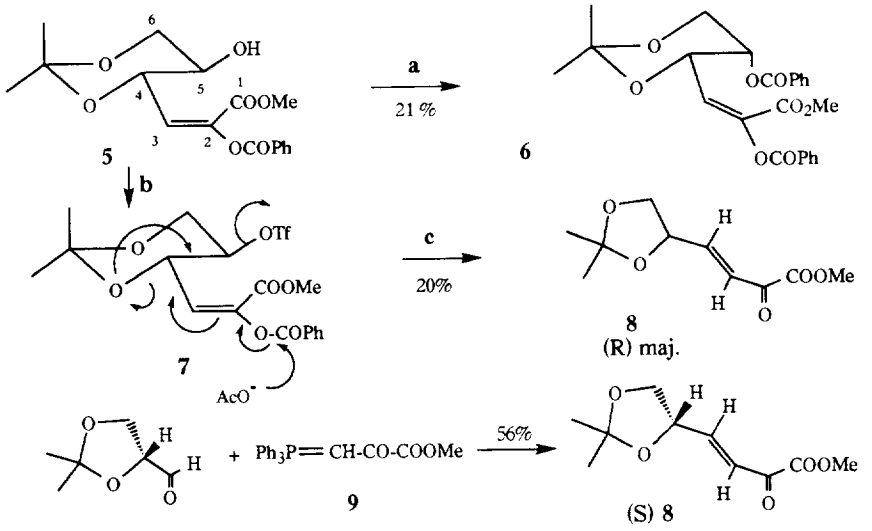
Scheme 1

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)

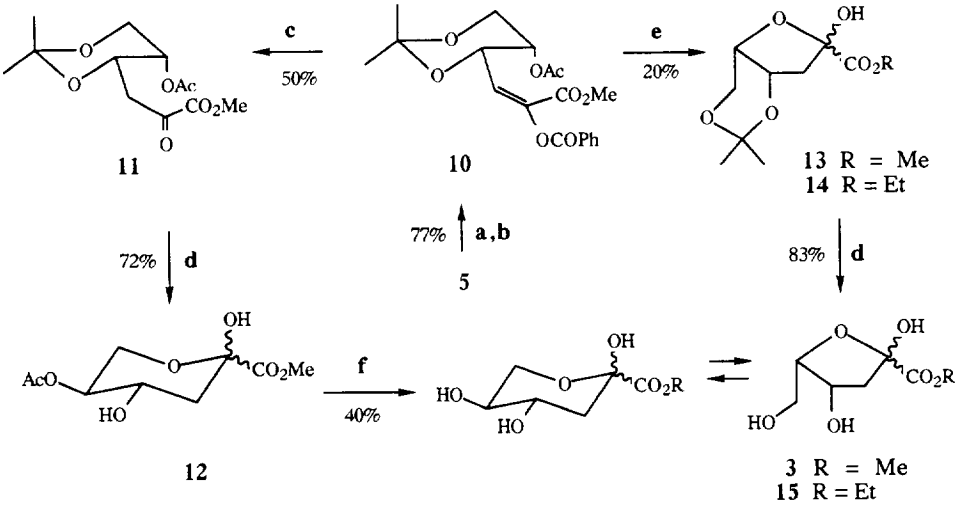
To obtain this compound the key step consisted in the epimerization of the C-5 in compound **5**. We first tried the Mitsunobu procedure[3] using benzoic acid as a nucleophile (scheme 2). Under these conditions, the expected C-5 epimeric benzoate **6** was obtained but was contaminated with by-products from which it was hardly separated. Therefore we then turned to the SN_2 displacement of the triflate **7**. This compound was prepared from alcool **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-glyceraldehyde[5] and phosphorane **9**[6] (scheme 2). The ^1H and ^{13}C 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°) were, as expected, of opposite sign, their absolute values differ notably. This result shows that a partial racemisation of (R) **8** occured 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) $AcO^-\ Bu_4N^+$, 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 SN_2 reaction, the crude triflate **7** was reacted with a large excess (10 eq) of tetrabutylammonium acetate in dichloromethane, a less polar solvant than acetone. As expected compound **10** was now formed in 77% yield (scheme 3).



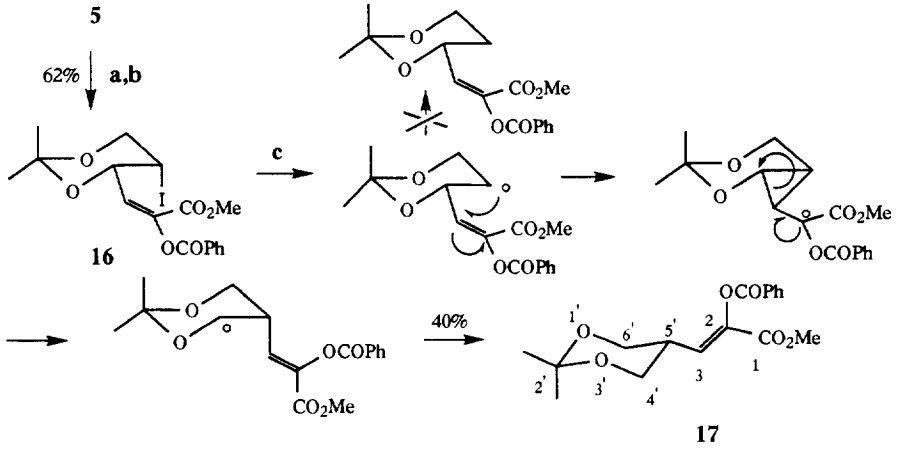
a) Tf_2O, 2,6-di-*tert*-butylpyridine, CH_2Cl_2; b) $AcO^-\ Bu_4N^+$, CH_2Cl_2; c) MeO^-Na^+, MeOH, 4 °C, 3 h; d) PPTS, MeOH; e) MeO^-Na^+, MeOH, 4 °C, 72 h; f) MeO^-Na^+, MeOH, 4 °C, 200 h.

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 **11**[7] 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 reported[8], 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 (^{1}H and ^{13}C 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 ^{13}C 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 ^{1}H 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. Initialy 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).

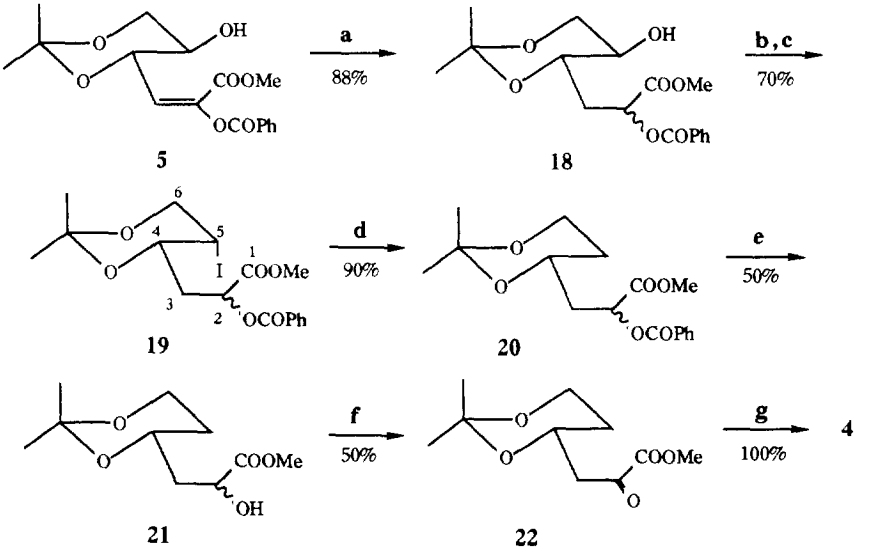


a) Tf_2O, 2,6-di-*tert*-butylpyridine, CH_2Cl_2; b) NaI, acetone; c) Bu_3SnH, AIBN cat., toluene, 75 °C.

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 (ΣJ = 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 diisopropylethylamine[16] 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 occured (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 alcools **21** using Czernecki *et al.* conditions[17] led to the keto-ester **22** which was then deprotected 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) MeO^-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 acids[18], *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 F_{254} (Merck) and detection was carried out by charring with a 5 % phosphomolybdic acid solution in ethanol containing 10 % H_2SO_4 and heating. Organic layers were dried on anhydrous $MgSO_4$. 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 $CDCl_3$ on a Bruker AM 200 (200 MHz for ^1H, 50.32 MHz for ^{13}C) spectrometer. ^1H NMR (300 MHz) or ^{13}C NMR (75.47 MHz) spectra were recorded on a Bruker AM 300. Chemical shifts are given in ppm downfield from internal Me_4Si. 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. $[\alpha]_D^{20}$ = +132° (c = 1.9, $CHCl_3$). ^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). ^{13}C 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 ($\underline{C}Me_2$); 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-hexulosonate (S-8). A solution of 2,3-*O*-isopropylidene-D-glyceraldehyde[5] (690 mg, 5.3 mmol) and methoxyoxalyl methylenetriphenylphosphorane[6] (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. $[\alpha]_D^{20}$ = + 45° (c = 1, $CHCl_3$). ^1H 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, OCH_3) 3.72 (dd, 1H, $J_{6',6}$ = 8.3, $J_{6',5}$ = 6.8, H-6'); 1.47 (s, 3H, CH_3); 1.43 (s, 3H, CH_3). ^{13}C NMR : 182.9 (C-2); 162.5 (C-1); 150.0 (C-4); 125.0 (C-3); 110.9 ($\underline{C}Me_2$); 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 *glycero*-E-3-eno-2-hexulosonate (8). To a solution of alcool **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 yied **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**. $[\alpha]_D^{20}$ = - 3° (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. $[\alpha]_D^{20}$ = + 82° (c = 4, $CHCl_3$). ^1H 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}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 ($\underline{C}Me_2$); 67.1, 66.8 (C-4, C-5); 62.6 (C-6); 52.5 (OMe); 29.1 (OCO$\underline{Me}$); 21.0 (Me); 18.9 (Me). Anal. Calcd. for $C_{19}H_{22}O_8$: 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**. ^1H 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, OCH_3); 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). ^{13}C NMR : 190.8 (C-2); 171.0 (MeCOO); 160.9 (C-1); 99.2 (CMe_2); 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° (c = 1.7, $CHCl_3$). Anal. Calcd. for $C_9H_{14}O_7$: C; 46.15; H, 5.98. Found C, 46.35; H, 5.91. **12** (β-anomer, 85%). ^1H NMR (D_2O) : 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). ^{13}C NMR (D_2O) : 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%). ^{13}C NMR (D_2O) : 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°(c = 4.5, $CHCl_3$). **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). ^{13}C NMR : 169.7 (C-1); 103.2 (C-2); 98.1 (CMe_2); 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). ^{13}C NMR : 170.5 (C-1); 102.1 (C-2); 97.8 (CMe_2); 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 $C_{10}H_{16}O_6$+ 0.5 H_2O (hygroscopic): C; 49.79; H, 7.05. Found C, 50.04; H, 6.98.

Methyl 3-deoxy-L-*threo*-2-hexulosonate (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 (^{13}C NMR) than this obtained from **12**. $[\alpha]_D^{20}$ = +1.5° (c = 2.2, $CHCl_3$, 2 h). NMR data of the predominent form in D_2O (α pyranose) : ^1H NMR : 3.97-3.44 (m, 4H, H-

4, H-5, H-6, H-6'); 3.82 (s, 3H, OCH_3); 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). ^{13}C 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-hexulosonate (15). Compound **13** (90 mg, 0.39 mmol) was dissolved in ethanol (2 ml) freshly distillated 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 ^{13}C NMR (75.47 MHz) data are identical with those of literature[7]. $[\alpha]_D^{25}$ = - 5°(c = 1, $CHCl_3$, 18h); $[\alpha]_D^{25}$ = - 6.7°(c = 1, $CHCl_3$, 20 min); lit.[8] : $[\alpha]_D^{25}$ = - 7°(c = 1, $CHCl_3$, 20 min[19]).

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. $[\alpha]_D^{20}$ = + 50°(c = 2.7, $CHCl_3$). ^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). ^{13}C 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 ($\underline{C}Me_2$); 68.4 (C-4); 67.9 (C-6); 53.0 (OMe); 31.3 (C-5); 30.0 (Me); 20.7 (Me). Anal. Calcd. for $C_{17}H_{19}O_6I$: 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 roduct 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. $[\alpha]_D^{20}$ = 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). ^{13}C 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 $C_{17}H_{20}O_6$: 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 *arabino*)-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 diasteroisomers in a 6/4 ratio as a colorless oil. ^{1}H 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). ^{13}C 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 $C_{17}H_{22}O_7$: 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 diasteroisomers **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.^{1}H 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). ^{13}C 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. ^{1}H 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). ^{13}C 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 $C_{17}H_{22}O_6$: 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). ^{13}C 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 $C_{10}H_{18}O_5$: 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 CH_2Cl_2, 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° (c = 2.7, $CHCl_3$). ^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). ^{13}C 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 $C_{10}H_{16}O_5$: 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° (c = 2.4, $CHCl_3$, 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). ^{13}C 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, ΣJ = 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). ^{13}C 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 $C_7H_{12}O_5$: C, 47.73; H, 6.82. Found : C, 48.01; H, 6.89.

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