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Di-*tert*-butyl diethylphosphoramidite as the phosphitylating reagent in the preparation of 3-deoxy-3-*C*-methylene-D-*ribo*-hexose-6-phosphate and 3-deoxy-3-*C*-methylene-D-*erythro*-pentose-5-phosphate

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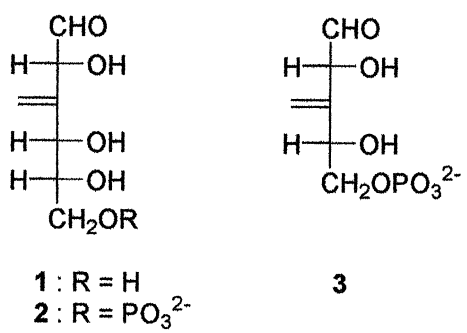
Abstract

3-Deoxy-3-*C*-methylene-D-*ribo*-hexose-6-phosphate and 3-deoxy-3-*C*-methylene-D-*erythro*-pentose-5-phosphate were prepared from a common intermediate 3-deoxy-3-*C*-methylene-1,2-*O*-isopropylidene-α-D-*ribo*-hexofuranose. The preparation of the phosphorylated unsaturated sugars employed di-*tert*-butyl diethylphosphoramidite as the phosphitylating reagent. The removal of all the protecting groups was done under acidic conditions in the ultimate step. The unsaturated sugar phosphates were competitive inhibitors but neither substrates nor inactivators of glucose-6-phosphate and ribose-5-phosphate isomerases.

Keywords: Carbohydrates; Enzyme inhibitors; Phosphoric acids and derivatives; Phosphorylation

1. Introduction

The glucose analogues, 3-deoxy-3-*C*-methylene-D-*ribo*-hexose (**1**) (Scheme 1) and 3-deoxy-3-*C*-fluoromethylene-D-*ribo*-hexose, are substrates of D-xylose isomerase (or D-glucose isomerase) (EC 5.3.1.5) isolated from *Streptomyces rubiginosus*.[1] The produced ketose derivatives inactivate the enzyme by an out/in mechanism and by a k_{cat} mechanism, respectively, with the remarkable partition ratio of 1.[1] The inactivation of the enzyme was due to the reaction with His-54. Based on the crystallographic structure of the native and the alkylated enzyme, a proton-transfer mechanism involving a histidine as the general base, was proposed (Scheme 2, **A**).[1] Later, other groups have proposed that the interconversion of aldose and ketose sugars follows rather through a hydride transfer mechanism (Scheme 2, **B**).[2] To get further information about the enzyme mechanism, we have extended our study to



Scheme 1. Structures of a k_{cat} inhibitor of D-xylose isomerase and of the putative k_{cat} inhibitors of phosphoglucose and phosphoribose isomerases.

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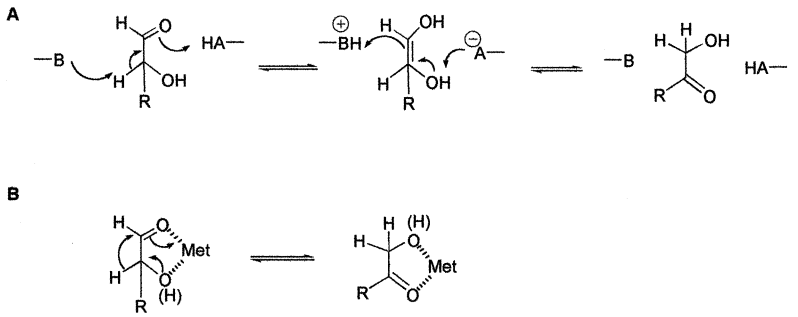


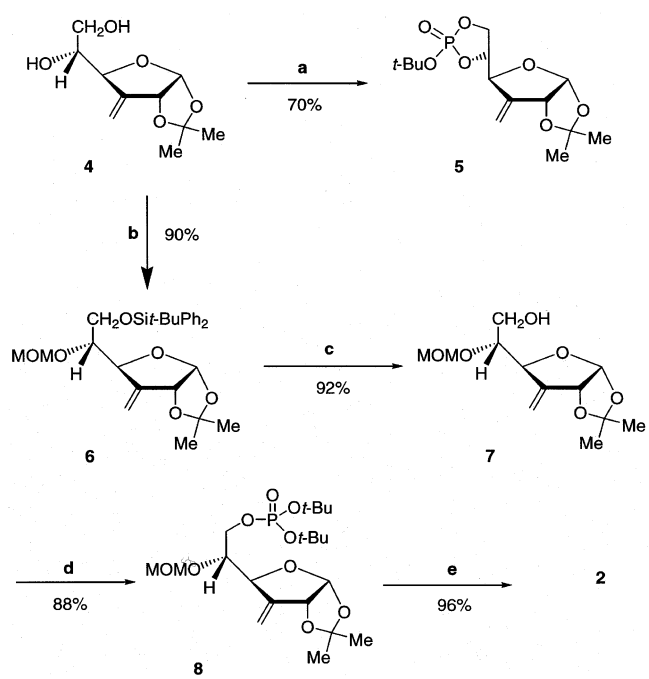
Scheme 2. Proposed enzymatic mechanisms for the isomerisation of an aldose to a ketose. The ene-diol and the hydride shift mechanisms are shown in **A** and **B**, respectively.

phosphoglucose isomerase (EC 5.3.1.9) and phosphoribose isomerase (EC 5.3.1.6). These enzymes perform the interconversion of glucose-6-phosphate and fructose-6-phosphate and of ribose-5-phosphate and ribulose-5-phosphate, respectively, via a proton-transfer mechanism.[3] For such a study, we required the phosphates of unsaturated carbohydrate derivatives **2** and **3**. We report here the synthesis of sodium salts of sugar phosphates **2** and **3** and the evaluation of their biological activity on phosphoglucose and phosphoribose isomerases.

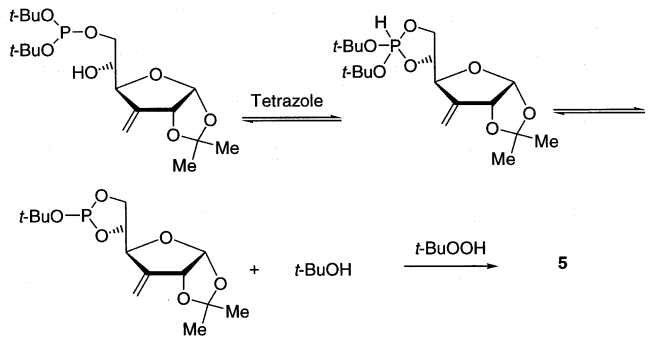
2. Results and discussion

Synthesis.—The synthesis of the two target compounds could be realised from the common starting material **4**.[4] Compound **4** was prepared by known procedures.[4,5] We first envisioned to prepare the 6-phosphate glucose derivative using the dibenzylphosphate strategy of Müller and Schmidt for the preparation of a sugar diphosphate nucleoside.[5] In their synthesis, the hydrogenolysis over palladium of a 1-dibenzylphosphate-3-*C*-methylene-hexopyranosyl intermediate afforded selectively the corresponding 1-phosphate-3-*C*-methylene-hexopyranosyl compound. We prepared the dibenzylphosphate derived from **4**. However, in our hands and under various conditions, reduction of the double bond occurred with hydrogenolysis of the benzyl protecting groups. To overcome this limitation, a new approach was elaborated and applied to **4** as shown in Scheme 3. We hoped that the acidic removal of the phosphate *tert*-butyl protecting groups could be compatible with the unsaturation[6] and the O-6-phosphate functionalities.[7]

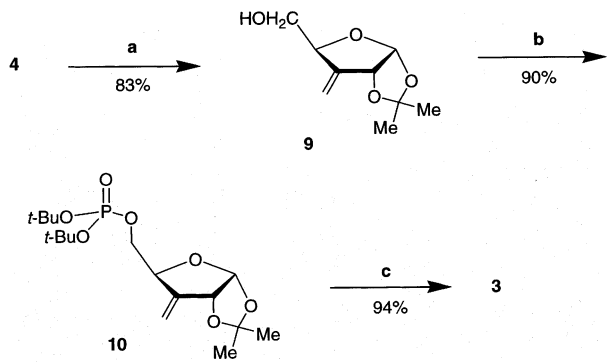
We initially expected to prepare selectively the di-*tert*-butyl phosphate at O-6 by treatment of diol **4** with di-*tert*-butyl diethylphosphoramidite[8] and 1*H*-tetrazole followed by oxidation with *tert*-butyl hydroperoxide. The unstable five membered cyclic phosphate **5**[9] was isolated instead of the desired compound. The proposed cyclic structure for **5** was supported by ^{1}H NMR and FABMS data. One plausible reason for the formation



Scheme 3. (a) (i) $(t\text{-BuO})_2Et_2NP$, 1*H*-tetrazole, THF, −25 °C to rt, 3 h; (ii) *t*-BuOOH, 4 equiv Et_3N. (b) (i) $t\text{-BuPh}_2SiCl$, $NEt(i\text{-Pr})_2$, DMF, 3 h; (ii) 4 equiv $ClCH_2OCH_3$, 5 equiv $NEt(i\text{-Pr})_2$, 4 °C to rt, 12 h. (c) Bu_4NF, THF, 1 h. (d) (i) $(t\text{-BuO})_2Et_2NP$, 1*H*-tetrazole, THF, −25 °C to rt, 3 h; (ii) 3 equiv H_2O_2, 9 equiv Et_3N. (e) (i) 0.3 equiv CF_3CO_2H, water, 7 days; (ii) 2 equiv NaOH, water.



Scheme 4. Proposed mechanism for the intramolecular transesterification.



Scheme 5. (a) (i) $NaIO_4$, 1:1 water–EtOH, 1 h; (ii) $NaBH_4$, 4 °C, 2 h. (b) (i) $(t\text{-BuO})_2Et_2NP$, 1*H*-tetrazole, THF, −25 °C to rt, 3 h; (ii) 3 equiv H_2O_2, 9 equiv Et_3N. (e) (i) 0.3 equiv CF_3CO_2H, water, 32 h; (ii) NaOH, water.

of by-product **5** is related to Watanabe's observation[10] that 1*H*-tetrazole catalysed transesterification of different trialkyl phosphite with an alcohol. However, decomposition of an alkyl di-*tert*-butyl phosphite to the corresponding alkyl *tert*-butyl hydrogenphosphonate was observed rather than transesterification. Nevertheless, the kinetically more favourable intramolecular reaction with the vicinal alcohol likely explains why transesterification occurred with the di-*tert*-butyl phosphite derived from **4** (Scheme 4). Acidic hydrolysis of the cyclophosphate triester gave a complex mixture of products that we were unable to separate. Thus, use of Perich and Johns phosphitylating reagent[8] required prior to protect the secondary vicinal alcohol. This was accomplished by the sequence of reactions depicted in Scheme 3.

The *tert*-butyldimethylsilyl group was reported as a selective protecting group of the primary alcohol of the 5,6-diol of a glycoside.[11] However, under basic conditions, migration of the silyl group to the vicinal secondary alcohol was observed.[11] For this reason we preferred the more bulky *tert*-butyldiphenylsilyl group because of its lower tendency to migrate.[12] Selective protections of the diol **4** were accomplished in one pot in 90% overall yield. Successive treatment with *tert*-butylchlorodiphenylsilane and Hünig's base,[13] and chloromethyl methylether[14] afforded the silyl ether at O-6 and the MOM acetal at O-5, respectively. Removal of the silyl moiety of ether **6** under standard conditions with the fluoride anion[12] furnished the alcohol **7**. Preparation of the 6-phosphotriester **8** was first tried using the original conditions.[8] Successive treatment of alcohol **7** with di-*tert*-butyl diethylphosphoramidite in the presence of 1*H*-tetrazole and *m*-chloroperoxybenzoic acid (mCPBA) afforded the desired compound along with a complex mixture of polar products as evidenced by TLC. De-*tert*-butylation of acid sensitive phosphate triesters was already observed. Surprisingly, it was mentioned for silica-gel chromatography but not for phosphite oxidation with mCPBA.[15] Decomposition of the phosphate triester **8** was avoided when triethylamine was added to neutralise 1*H*-tetrazole in excess and hydrogen peroxide was used instead of mCPBA. Under these conditions, the triester **8** was isolated in high yield. Removal of all the protecting groups of compound **8** was performed in the last step under acidic conditions with 0.3 equiv of trifluoroacetic acid in water. Deprotection of the phosphate triester and acetonide groups was faster than removal of the MOM group as evidenced by ^1H NMR. After completion, the volatiles were removed under vacuum to give the corresponding phosphate monoester that was pure enough to continue without chromatography. The disodium salt **2** was obtained in 96% yield after addition of two equivalents of sodium hydroxide.

The same methodology was used for the preparation of the second target compound **3** (Scheme 5).

The alcohol **9**[16] was obtained in two steps in 83% yield by oxidative cleavage of the diol **4** with sodium periodate and by reduction of the generated aldehyde with sodium borohydride.[17] Phosphorylation and removal of the protecting groups were realised as described

before. Phosphate **3** was isolated in high yield as the sodium salt.

Phosphate salts **2** and **3** were characterised by their spectroscopic and physical data. For instance in ^{31}P NMR, four resonances of phosphate **2** were detected on the proton-decoupled spectrum at 4.71 (50%), 4.67 (37%), 4.45 (2%) and 4.3 (11%) ppm. On the proton-coupled spectrum, only the three most abundant isomers were detected each as a triplet signal with a $^3J_{PH}$ coupling constant of 6.5, 5.6 and 6.9 Hz, respectively, in agreement with the $^3J_{PH}$ determined on other phosphoric mono-esters of primary alcohols.[18] In deuterated water, phosphate **2** exists likely as a mixture of both α and β pyranose and furanose anomers, the α and β pyranose anomers being probably the most abundant by analogy to glucose-6-phosphate.[19] On the other hand, two triplet signals were observed in the NMR spectrum for phosphate **3** at 1.32 (43%) and 1.18 (57%) ppm with $^3J_{PH}$ of 6.1 and 5.8 Hz, respectively. In deuterated water, phosphate **3** exits likely as a mixture of α and β furanose anomers as observed for ribose-5-phosphate.[20]

Enzymatic assays.—Phosphoglucose isomerase is assayed by a coupled enzymatic test with glucose-6-phosphate dehydrogenase.[21] So we have first studied the effect of 3-deoxy-3-*C*-methylene-D-*ribo*-hexose-6-phosphate (**2**) and 3-deoxy-3-*C*-methylene-*erythro*-pentose-5-phosphate (**3**) on the glucose-6-phosphate dehydrogenase activity. Both compounds **2** and **3** were neither substrate nor inactivator of this enzyme. On the other hand, they were reversible competitive inhibitors. Inhibition constants of 14 and 10 mM were found for compounds **2** and **3**, respectively. These inhibitions were too low to affect the activity of glucose-6-phosphate dehydrogenase during the determination of the activity of phosphoglucose isomerase in our experimental conditions.

The incubation of phosphoglucose isomerase in the presence of compounds **2** or **3** did not inactivate the enzyme. As mentioned above, the inactivation of xylose isomerase with 3-deoxy-3-*C*-methylene-*ribo*-hexose (**1**) and 3-deoxy-3-*C*-fluoromethylene-*ribo*-hexose requires the transformation of the compounds by the enzyme to the corresponding α,β-unsaturated ketoses. So the absence of inactivation of phosphoglucose isomerase could be due to the fact that both compounds **2** and **3** were not isomerised by the enzyme. Contrarily to xylose isomerase, phosphoglucose isomerase has a rather strict substrate specificity. The enzyme catalyses only the reversible isomerisation of glucose-6-phosphate and fructose-6-phosphate, but 3-deoxy-3-fluoro-D-glucose was reported to be transformed by the enzyme at a relatively slow rate.[22] The two analogues **2** and **3** inhibited reversibly phosphoglucose isomerase. Inhibition constants of 4–6 mM were found for both compounds.

The pentose analogue **3** was also tested on phosphoribose isomerase. It was neither substrate nor inactivator. The enzyme was reversibly inhibited by compound **3**. The inhibition constant (about 27 mM) was much higher than the inhibition constants of known competitive inhibitors such as 4-phospho-D-erythronic acid ($K_i = 4.4$ μM) or 5-phospho-D-ribonic acid ($K_i = 119$ μM).[23]

In conclusion, the synthetic strategy presented here employed di-*tert*-butyl diethylphosphoramidite as the phosphitylating reagent for the preparation of unsaturated carbohydrate phosphates. It represents a valuable alternative to the dibenzylphosphate one, which failed in our hands. The phosphate and sugar protecting groups were removed at the last step under acidic conditions compatible with the unsaturation. The unsaturated sugar phosphates were competitive inhibitors but neither substrates nor inactivators of glucose-6-phosphate and ribose-5-phosphate isomerases.

3. Experimental

General methods.—Unless otherwise indicated, all reagents were obtained from commercial suppliers and were used without purification. All experiments sensitive to air and/or to moisture were carried out under an Ar atmosphere in an oven dried (120 °C) glassware assembled under a stream of Ar. Anhydrous solvents were freshly distilled before use: THF from sodium benzophenone ketyl radical, diisopropylethylamine from CaH_2 and DMF from P_2O_5. Analytical thin-

layer chromatography was performed on silica gel pre-coated TLC plates (E. Merck, 60, F_{254}). Products were isolated by flash chromatography on silica gel (E. Merck, 60, 230–400 mesh). Melting points were measured on a Reichert microscope and were uncorrected. $[\alpha]_D$ were measured on a Perkin–Elmer 241MC polarimeter. IR spectra were recorded on a Perkin–Elmer 881 or a Bruker FT IFS25. NMR spectra were recorded on a Brucker SY (200 or 300 MHz) apparatus. For ^{1}H NMR the residual proton signal of the deuterated solvent was used as an internal reference: for $CDCl_3$ (δ 7.26 ppm), C_6D_6 (δ 7.16), and D_2O (δ 4.90). For ^{13}C NMR, the ^{13}C central signal of the deuterated solvent was used as an internal reference; for $CDCl_3$ (δ 76.9 ppm), C_6D_6 (δ 128.0) and 2-methyl-2-propanol-d_{10} (δ 40.02). The chemical shifts are reported in ppm downfield from TMS. For ^{31}P NMR, H_3PO_4 (85%) was used as an external reference (δ 0 ppm). MS were measured on a LKB 9000S apparatus by electron impact (EI, 70 eV), on a Trio 2000 (FISONS, UK) apparatus by chemical ionisation (CI) or on a ZAB (FISONS, UK) apparatus by fast-atom bombardment (FAB). Microanalyses were performed by the Service de Microanalyses de Strasbourg.

*3-Deoxy-3-*C*-methylene-1,2-*O*-isopropylidene-α-*D*-ribo-hexofuranose* (**4**).—Diol **4** was obtained as described:[5] $[\alpha]_D^{21}$ +134° (*c* 4, $CHCl_3$); lit. $[\alpha]_D^{20}$ +119° (*c* 1, $CHCl_3$);[4] Anal. Calcd for $C_{10}H_{16}O_5$: C, 55.55; H, 7.46. Found: C, 55.50; H, 7.50.

*3-Deoxy-3-*C*-methylene-1,2-*O*-isopropylidene-α-*D*-ribo-hexofuranose-5,6-*tert*-butylphosphate* (**5**).—Compound **4** (486 mg, 2.25 mmol) (dried under high vacuum, $p = 100$ Pa, at 50 °C for 5 h) and anhyd 1*H*-tetrazole (dried by sublimation at $p = 100$ Pa in an oil bath at 85 °C. CAUTION, explosive, the oil bath temperature was kept below 100 °C[24]), were dissolved in dry THF (6 mL). The solution was cooled at −25 °C. A solution of di-*tert*-butyl diethylphosphoramidite[7] (586 mg, 2.35 mmol) in dry THF (2 mL) was added dropwise to the mixture during a 5 min period. After addition, the reaction temperature was allowed to raise slowly, in 3 h to rt. After 30 min, Et_3N (1.26 mL, 9 mmol) followed by a 70% solution of *t*-BuOOH in water (0.32 mL, 2.35 mL) were added to the cooled solution (−30 °C). The cooling bath was removed and stirring was continued until conversion was completed (TLC). The reaction was diluted with AcOEt (20 mL) and water. The phases were separated, the organic phase was washed with brine (10 mL), dried over Na_2SO_4, filtered and evaporated to dryness to give the crude extract of the unstable cyclophosphotriester **5** (720 mg) that decomposed quickly if subjected to SiO_2 chromatography. ^{1}H NMR analysis indicated essentially a mixture of two glucose derivatives where phosphate **5** was the major compound (70%). ^{1}H NMR (CD_2Cl_2): δ 5.80 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 5.49 (bs, 1 H, H-unsat), 5.41 (bs, 1 H, H-unsat), 4.91 (m, 1 H, H-2), 4.84 (m, 1 H, H-4), 4.60–4.15 (m, 3 H, H-5, H-6), 1.50 (s, 9 H, *t*-Bu), 1.47 (s, 3 H, Me), 1.35 (s, 3 H, Me); (FABMS, NBA): *m/z* 333 [M − 1], 277, 209, 153, 97.

*6-*O*-tert-Butyldiphenylsilyl-3-deoxy-5-*O*-methoxymethyl-3-*C*-methylene-1,2-*O*-isopropylidene-α-*D*-ribo-hexofuranose* (**6**).—To a stirred solution under Ar of compound **4** (0.649 g, 3 mmol) (dried by azeotropic distillation with 2 × 2 mL dry pyridine) in dry DMF (3 mL) were added *t*-$BuPh_2SiCl$ (0.831 mL, 3.15 mmol) and dry $NEt(i\text{-}Pr)_2$ (0.810 mL, 4.75 mmol). The reaction evolution was monitored by TLC with 7:3 hexane–ether as eluents. After 3 h, the reaction was cooled in an ice bath, $NEt(i\text{-}Pr)_2$ (2.76 mL, 15 mmol) was added followed by a dropwise addition of $ClCH_2OCH_3$ (0.975 mL, 13 mmol) (CAUTION, carcinogen and highly toxic[24]). Stirring was continued 1 h at 4 °C and overnight at rt. The reaction was diluted with water (2 mL) and Et_2O (30 mL), the phases were separated, the aqueous phase was extracted twice with Et_2O (2 × 15 mL). The combined organic phases were washed with brine (20 mL), dried over $MgSO_4$, filtered and evaporated to dryness to give an orange oil. Flash chromatography over SiO_2 with 17:5 then 4:1 hexane–ether as eluents furnished compound **6** as a colourless oil (1.28 g, 90%). $[\alpha]_D^{21}$ +61° (*c* 4.3, $CHCl_3$); IR (neat): *v* 3070 and 3049 (w), 1959,1895 and 1830 (w), 1589 (w), 1468 (m), 1427 (m), 1213 (m), 1019 (bs) cm^{-1}; ^{1}H NMR

($CDCl_3$): δ 7.69–7.63 (m, 4 H, Ph), 7.44–7.34 (m, 6 H, Ph), 5.77 (d, 1 H, $J_{1,2}$ 4.3 Hz, H-1), 5.40 (dd, 1 H, $J_1 = J_2$ 1.7 Hz, H-olef), 5.18 (dd, 1 H, $J_1 = J_2$ 1.7 Hz, H-olef), 5.02 (bs, 1 H, H-4), 4.85 (bdd, 1 H, H-2), 4.73 (d, 1 H, *J* 6.6 Hz, CH_2O), 4.69 (d, 1 H, CH_2O), 3.90–3.62 (m, 3 H, H-5, H-6), 3.3 (s, 3 H, OMe), 1.47 (s, 3 H, Me), 1.38 (s, 3 H, Me), 1.04 (s, 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$): δ 146.4 (q), 135.6 (t), 133.4 (q), 133.2 (q), 129.8 (t), 127.8 (t), 112.6 (q), 112.5 (s), 105.0 (t), 96.9 (s), 81.9 (t), 80.8 (t), 79.7 (t), 63.0 (s), 55.8 (p), 27.7 (p), 26.9 (p), 19.2 (q); MS (IE, 70 eV) 441 ($M^+ -$ 33, 8), 383 (4), 353 (8), 351 (4), 323 (8), 321 (7), 176; Anal. Calcd for $C_{28}H_{38}O_6Si$: C, 65.78; H, 8.07. Found: C, 65.92; H, 8.05.

*3-Deoxy-5-*O*-methoxymethyl-3-*C*-methylene-1,2-*O*-isopropylidene-α-*D*-ribo-hexofuranose* (**7**).—To a solution under Ar of compound **6** (1.19 g, 2.5 mmol) in dry THF (5 mL) was added $Bu_4NF{\cdot}3\ H_2O$ (0.867 mg, 2.75 mmol). After 1 h stirring, water (5 mL) was added and the reaction medium was extracted twice with AcOEt (2 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over $MgSO_4$, filtered and evaporated to dryness. Flash chromatography over SiO_2 with 1:1 hexane–ether and ether gave compound **7** (0.60 g, 92%) which crystallised upon standing in the refrigerator. Mp 47–48 °C; $[\alpha]_D^{21}$ + 170° (*c* 4.1, $CHCl_3$); IR (neat): *v* 3472 (bs),3086 (w), 1644 (w), 1457 (m), 1414 (m), 1377 (s), 1213 (s), 1019 (bs) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.82 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1), 5.48 (dd, 1 H, J_1 2, J_2 1.3 Hz, H-olef), 5.29 (dd, 1 H, $J_1 = J_2$ 2 Hz, H-olef), 4.93–4.83 (m, 2 H, H-2, H-4), 4.79 (d, 1 H, *J* 7 Hz, CH_2O), 4.75 (d, 1 H, CH_2O), 3.73–3.62 (m, 3 H, H-5, H-6), 3.45 (s, 3 H, OMe), 1.49 (s, 3 H, Me), 1.38 (s, 3 H, Me); ^{13}C NMR ($CDCl_3$): δ 146.6 (q), 112.8 (s), 104.6 (t), 97.3 (s), 82.9 (t), 81.8 (t), 80.0 (t), 62.1 (s), 55.9 (p), 27.5 (p), 27.4 (p); MS (IE, 70 eV) 245 ($M^+ - 15$, 9), 213 (8), 173 (11), 171 (37), 155 (55), 142 (59), 97 (100); Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.11; H, 7.93.

*3-Deoxy-5-*O*-methoxymethyl-3-*C*-methylene-1,2-*O*-isopropylidene-α-*D*-ribo-hexofuranose-6-di-*tert*-butylphosphate* (**8**).—A stirred solution under Ar of the alcohol **5** (0.6 g, 2.3 mmol) (dried under high vacuum, $p = 100$ Pa, at 50 °C for 3 h) and dry 1*H*-tetrazole (0.603 g, 8.6 mmol) in dry THF (20 mL) was cooled at −25 °C. To this solution was added dropwise di-*tert*-butyl diethylphosphoramidite (0.723 g, 2.9 mmol). After addition, the reaction temperature was allowed to rise slowly to rt. After 3 h stirring, the reaction was cooled in an ice bath and Et_3N (2.75 mL, 20 mmol) and 30% H_2O_2 in water (0.6 mL, 6 mmol) were added. Stirring was continued at rt until complete conversion (TLC). The reaction was diluted with water (5 mL) and AcOEt (20 mL), and the phases were separated. The aqueous phase was extracted with AcOEt (10 mL). The combined organic phases were washed with brine (30 mL), dried over Na_2SO_4, filtered and evaporated to dryness. Flash chromatography over SiO_2 with 1:4 hexane–ether and ether gave compound **8** (0.912 g, 88%), which crystallised upon standing in the refrigerator. Mp 48–49 °C; $[\alpha]_D^{23}$ + 100° (*c* 1.3, AcOEt); IR (KBr): *v* 3083 (w), 1638 (w), 1393 (m), 1268 (s), 1002 (bs) cm^{-1}; ^1H NMR (C_6D_6): δ 5.74 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1), 5.35 (m, 1 H, H-unsat), 5.32 (m, 1 H, H-unsat), 5.14 (m, 1 H, H-4), 4.68 (m, 1 H, H-2), 4.57 (d, 1 H, *J* 6.7 Hz, CH_2O), 4.54 (d, 1 H, CH_2O), 4.38–4.20 (m, 2 H, H-6), 3.98 (bdd, 1 H, J_1 5.6, J_2 3.6 Hz, H-5), 3.14 (s, 3 H, OMe), 1.46 (s, 3 H, Me), 1.41 (s, 9 H, *t*-Bu), 1.40 (s, 9 H, *t*-Bu), 1.31 (s, 3 H, Me); ^{13}C NMR (C_6D_6): δ 147.7 (q), 112.5 (s), 105.5 (t), 97.0 (s), 82.2 (t), 81.9 (q), 81.8 (q), 80.3 (t), 78.7 (d, $J_{C\text{-}5P}$ 8 Hz, t), 65.4 (d, $J_{C\text{-}6P}$ 5 Hz, s), 55.6 (p), 29.9 (p), 29.8 (p), 27.8 (p); (FABMS, NBA): 395 (M − 57, 100), 339 (12), 305 (11), 209 (50), 166 (53); Anal. Calcd for $C_{20}H_{37}O_9P$: C, 53.09; H, 8.24. Found: C, 53.12; H, 8.37.

*3-Deoxy-3-*C*-methylene-*D*-ribo-hexose-6-phosphate disodium salt* (**2**).—A stirred mixture of compound **8** (0.95 g, 2.1 mmol) in deionised water (5 mL) was degassed three times under the vacuum of a water pump and flushed under a stream of Ar. To the mixture was added CF_3COOH (0.05 mL, 0.65 mmol). After 12 h stirring, a solution was obtained. The reaction was monitored by ^1H NMR as followed. An aliquot (0.025 mL) of the reaction was taken and diluted with D_2O (0.45 mL) for NMR analysis. The water peak was suppressed by pre-saturation. Hydrolysis of

the protecting groups was detected by the disappearance of the signals corresponding to the methyls of acetonide, the *tert*-butyl ester and MOM group, respectively, at 1.34, 1.43, 1.49 and 3.4 ppm (reference spectrum for *t* 0 was measured in MeOH-d_4) and by the appearance of the new signals corresponding to the methyls of *tert*-BuOH, acetone and MeOH, respectively, at 1.2, 2.18 and 3.4 ppm. After 7 days, activated charcoal (100 mg) was added, the mixture was stirred for 1 h and filtered (Millex-GV® filter unit 22 μm). Freeze-drying gave a colourless foam which was dissolved in deionised water (1 mL). To the cooled degassed solution at 4 °C under Ar was added dropwise an aqueous solution of NaOH (0.1 N, 4 mL). After freeze-drying, the compound was dissolved in deionised water (1.5 mL) and abs EtOH (20 mL) was added, and the mixture was stored in the refrigerator. The supernatant was removed. Freeze-drying and vacuum drying ($p = 100$ Pa, P_2O_5) afforded the 6-phosphate glucose analogue **2** (575 mg, 96%). Mp (dec.) 112°C; $[\alpha]_D^{21}$ +43° (30 min), +41° (17 h) (*c* 4.2, water); IR (KBr): *v* 3396 (bs), 1657 (m), 1095 (s), 978 (s) cm^{-1};^{1}H NMR (D_2O): δ 6.14 (d, *J* 4 Hz, 1%), 5.86 (bs, 1%), 5.76 (bs, 1%), 5.65–5.35 (complex, 24%), 4.67 (d, *J* 8 Hz, 3%), 4.52–4.00 (complex, 51%), 3.94 and 3.88 (2 bs, 8%), 3.70–3.52 (complex, 11%) due to the water signal, information around 4.9 ppm was missed; ^{13}C NMR (water + 5% *tert*-BuOH-d_{10} + 2.5% 4 mM EDTA, Na_3) showed two isomers: δ 149.2 (q), 147.7 (q), 107.4 (s), 107.2 (s), 100.7 (t), 94.9 (t), 84.4 (s), 81.2 (d, $J_{C\text{-}5P}$ 7 Hz, t), 76.1 (d, $J_{C\text{-}5P}$ 8 Hz, t), 75.0 (t), 72.5 (t), 69.6 (t), 69.3 (t), 65.8 (d, $J_{C\text{-}6P}$ 5 Hz, t); ^{31}P NMR (D_2O + 3% 4 mM EDTA, Na^3) showed four isomers: δ 4.72 (t, $J_{H\text{-}6P}$ 6.5 Hz, 50%), 4.67 (t, $J_{H\text{-}6P}$ 6.5 Hz, 37%), 4.45 (2%), 4.30 (t, $J_{H\text{-}6P}$ 6.5 Hz, 11%); (FABMS, thioglycerol) 277 (M − 23, 100), 255 (58), 237 (52); Anal. Calcd for $C_7H_{11}Na_2O_8P$: C, 28.00; H, 3.96. Found: C, 27.90; H, 4.06.

*3-Deoxy-3-*C*-methylene-1,2-*O*-isopropylidene-α-*D*-erythro-pentofuranose* (**9**).—A solution of diol **4** (1.15 g, 5.3 mmol) in EtOH (10 mL) was added dropwise to a solution of $NaIO_4$ (1.3 g, 6.05 mmol) in water (10 mL) under vigorous stirring. After 1 h stirring, ethylene glycol (0.1 mL) was added to the mixture. The mixture was filtered and the solid was washed twice with EtOH (2 × 5 mL). The filtrate was used directly for reduction. It was cooled in an ice bath and $NaBH_4$ (0.2 g, 5.3 mmol) was added portionwise. After 2 h stirring, excess $NaBH_4$ was decomposed by slow addition of glacial acetic acid (0.914 mL, 16 mmol) to the cold reaction medium. The volume was reduced under vacuum to about 5 mL (pale orange). The compound was extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with aq $Na_2S_2O_4$ (10%, 5 mL). The thiosulfate solution was extracted with AcOEt (10 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, filtered and evaporated to dryness to give **9** as a pale yellow oil (0.814 g, 83%). Compound **9** was homogeneous by TLC (AcOEt as eluent) with spectroscopic data identical to the lit.[16] $[\alpha]_D^{21}$ +170° (*c* 4, $CHCl_3$); lit. $[\alpha]_D^{21}$ +151° (*c* 0.8, $CHCl_3$);[16] IR, ^{1}H NMR lit.;[16] ^{13}C NMR ($CDCl_3$): δ 145.6 (q), 112.6 (q), 112.2 (s), 104.4 (t), 82.1 (t), 80.0 (t), 63.5 (s), 27.4 (p), 27.1 (p); MS (CI, CH_4) 187 (M^+ + 1, 30), 170 (12), 155 (14), 129 (100), 111 (84).

*3-Deoxy-3-*C*-methylene-1,2-*O*-isopropylidene-α-*D*-erythro-pentofuranose-5-di-tert-butylphosphate* (**10**).—The triester **10** was prepared as described for **6**. Flash chromatography over SiO_2 (treated with 1% Et_3N) with 1:9 hexane–ether as eluents gave compound **10** (90%). Mp 61–62 °C; $[\alpha]_D^{21}$ +88° (*c* 4, AcOEt); IR (neat): *v* 2985(s), 2936 and 2876 (m), 1277 (s), 990 (bs) cm^{-1}; ^{1}H NMR (C_6D_6): δ 5.79 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1), 5.18 (dd, 1 H, J_1 2.4, J_2 1.2 Hz, H-unsat), 4.97 (dd, 1 H, *J* 1.3 Hz, H-unsat), 4.85 (m, 1 H, H-4), 4.55 (dd, 1 H, H-2), 4.20–3.94 (m, 2 H, H-5), 1.45 (s, 3 H, Me), 1.41 (s, 18 H, *t*-Bu), 1.25 (s, 3 H, Me); ^{13}C NMR (C_6D_6): δ 147.2 (q), 112.5 (q), 112.0 (s), 105.3 (t), 82.1 (t), 81.8 (q), 81.7 (q), 78.8 (d, $J_{C\text{-}4P}$ 9.7 Hz, t), 68.2 (d, $J_{C\text{-}5P}$ 5.6 Hz, s), 29.9 (p), 29.8 (p), 27.8 (p), 27.5 (p); (FABMS, thioglycerol) 321 (M − 57, 100), 265 (15), 232 (7), 209 (65), 166 (63); Anal. Calcd for $C_{17}H_{31}O_7P$: C, 53.96; H, 8.26. Found: C, 53.96; H, 8.28.

*3-Deoxy-3-*C*-methylene-*D*-erythro-pentose-5-phosphate disodium salt* (**3**).—Acidic hydrol-

ysis of the phosphate triester **10** was realised and monitored as described for **6** (32 h reaction time). Compound **3** was isolated quantitatively as the sodium salt. Mp (dec.) 90 °C; $[\alpha]_D^{21}$ +96° (15 min), +93° (24 h) (*c* 2.7, water); IR (KBr): *v* 3432 (bs), 1626 (m), 1005 (m) cm^{-1}; ^1H NMR (D_2O + 5% 4 mM EDTA, Na_3): δ 5.65–5.46 (complex, 50%), 5.34 (d, *J* 2 Hz, 7%), 4.19–3.93 (complex, 43%) due to the water signal, information around 4.9 ppm was missed; ^{13}C NMR (D_2O + 2.5% 4 mM EDTA, Na_3) showed two isomers: δ 146.7 (q), 146.5 (q), 113.7 (s), 110.0 (s), 102.1 (t), 96.3 (t), 79.7 (d, $J_{C\text{-}4P}$ 7.9 Hz, t), 78.9 (d, $J_{C\text{-}4P}$ 7.9 Hz, t), 78.0 (t), 73.2 (t), 69.2 (d, $J_{C\text{-}5P}$ 5.1 Hz, s), 68.3 (d, $J_{C\text{-}5P}$ 5.1 Hz, s); ^{31}P NMR (D_2O + 2.5% 4 mM EDTA, Na_3) showed two isomers: δ 1.32 (t, $J_{H\text{-}6P}$ 6.1 Hz, 43%), 1.18 (t, $J_{H\text{-}6P}$ 5.8 Hz, 57%); (FABMS, thioglycerol) 247 (M − 23, 65), 225 (100), 197 (25), 165 (27); Anal. Calcd for $C_6H_{10}NaO_7P{\cdot}H_2O$: C, 27.08; H, 4.55. Found: C, 26.95; H, 4.35.

Determination of the activity of glucose-6-phosphate dehydrogenase.—Glucose-6-phosphate (1.2 mM), $NADP^+$ (4.3 mM) and glucose-6-phosphate dehydrogenase from bakers yeast (125 ng/mL) were incubated at 30 °C in a 0.1 M triethanolamine–HCl buffer pH 7.7 (total vol 1 mL). The activity was measured by following the formation of NADPH at 340 nm with an Uvicon 933 spectrophotometer (Kontron). 3-Deoxy-3-*C*-methylene-D-*ribo*-hexose-6-phosphate (**2**), 3-deoxy-3-*C*-methylene-D-*erythro*-pentose-5-phosphate (**3**) and glucose-6-phosphate dehydrogenase were incubated at a concentration of 4 mM with the enzyme (2.5 μg/mL) and $NADP^+$ (4.3 mM) to control if the two sugar phosphates were substrates of glucose-6-phosphate dehydrogenase. The formation of NADPH was followed at 340 nm. To test if they were able to inactivate glucose-6-phosphate dehydrogenase, compounds **2** and **3** (20 mM) were incubated with glucose-6-phosphate dehydrogenase (2.5 μg/mL) in the absence or in the presence of $NADP^+$ (4.3 mM) in a 0.1 M triethanolamine–HCl, 6.5 mM $MgCl_2$ buffer pH 7.8 at 30 °C. At given time aliquots (0.5 μL) were withdrawn to determine the residual enzymatic activity. Reversible inhibition tests were undertaken by determining the enzymatic rate with glucose-6-phosphate dehydrogenase (125 ng/mL), glucose-6-phosphate (0.12 mM) and $NADP^+$ (4.3 mM) in the presence of the analogues at different concentrations (10–40 mM). The influence of ribose-5-phosphate on the activity of the dehydrogenase was studied similarly.

Determination of the enzymatic activity of phosphoglucose isomerase.—The activity was measured using the coupled test with glucose-6-phosphate dehydrogenase.[21] The concentration of phosphoglucose isomerase from rabbit muscle (Sigma) was determined at 280 nm assuming an absorption coefficient $A^{1\%}$ 13.2 and a molecular weight of 132,000 Da for the dimer.[25] For 3-deoxy-3-*C*-methylene-D-*ribo*-hexose-6-phosphate (**2**), 3-deoxy-3-*C*-methylene-D-*erythro*-pentose-5-phosphate (**3**) and phosphoglucose isomerase, inactivation tests were performed by incubating the analogues at different concentrations (1–100 mM) with phosphoglucose isomerase in a 0.1 M triethanolamine–HCl buffer pH 7.7 at 30 °C and measuring the residual enzymatic activity in function of time. Reversible inhibition tests were undertaken with the analogues and ribose-5-phosphate (10–40 mM). The concentration of fructose-6-phosphate was 1.5 mM.

Enzymatic activity of phosphoribose isomerase.—The enzymatic activity was determined by following the absorbance increase at 282 nm due to the formation of ribulose-5-phosphate from ribose-5-phosphate (ε 58.6 $M^{-1}cm^{-1}$).[26] Ribose-5-phosphate (50 mM) was incubated with phosphoribose isomerase from spinach (Sigma) (1 μg/mL) at 30 °C in a triethanolamine–HCl buffer pH 7.7 (total volume 500 μl) for 3-deoxy-3-*C*-methylene-D-*erythro*-pentose-5-phosphate (**3**) and phosphoribose isomerase, the ribose-5-phosphate analogue was added in the medium to test if the enzyme is able to isomerise it in the ketose derivative. The reaction was followed by taking the absorption spectrum (240–320 nm) as a function of time. To test if compound **3** is an irreversible inhibitor of phosphoribose isomerase, the ribose-5-phosphate analogue (50 mM) was incubated with the enzyme (1 mg/mL) in a 0.1 M triethanolamine–HCl buffer pH 7.8 at 37 °C. At given time, aliquots (0.5

μL) were withdrawn to test the residual enzymatic activity. The isomerase rate was measured with phosphoribose isomerase (1 μg/mL) and ribose-5-phosphate (10 mM) in the presence of the ribose-5-phosphate analogue at different concentrations (10–40 mM) to study the reversible inhibition.

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