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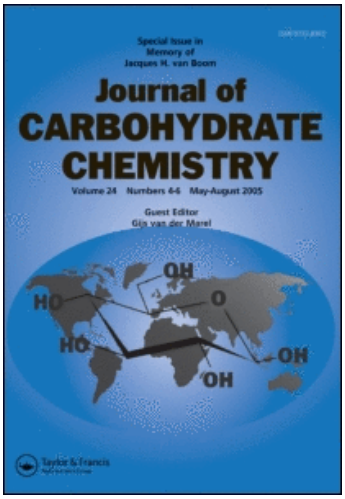

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SYNTHESIS AND BIOLOGICAL EVALUATION OF (2-HYDROXYETHYL) 2-DEOXY-α-D-*THREO*-PYRANOSIDE 3,4,2'-TRISPHOSPHATE, A MIMIC OF THE SECOND MESSENGER INOSITOL 1,4,5-TRISPHOSPHATE

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ABSTRACT

(2-Hydroxyethyl) 2-deoxy-α-D-*threo*-pentopyranoside 3,4,2'-trisphosphate (**3**) has been prepared starting from allyl-α-D-xylopyranoside. The suitably protected 2-deoxy intermediate obtained by judicious selective protection and deprotection has been phosphorylated using the phosphoramidite methodology. Final deprotection gave the expected analogue of *myo*-inositol 1,4,5-trisphosphate.

INTRODUCTION

Interest in *myo*-inositol phosphates is focused mainly on their intracellular activity as second messengers.[1] D-*myo*-inositol-1,4,5-trisphosphate [Ins(1,4,5)P_3] functions as an intracellular second messenger. The interaction with its receptor, IP_3-R, induces calcium mobilisation from intracellular stores. In order to find agonists and antagonists of IP_3-R,

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analogues of IP_3 have been synthesized.[2] We have recently introduced D-xylose-based analogue **1** of $InsP_3$ which is a full agonist of IP_3-R.[3] Other derivatives based on D-glucose and designed by analogy with the natural agonist adenophostin have been reported.[4] Sugar-based analogues are easier to prepare than inositol derivatives and are produced in enantiomerically pure form. They provide a platform for the exploration of structure-activity relationship. In connection with such a programme, we wanted to confirm the role of the hydroxyl group at C-2 of **1** which has been recognized as important in the $InsP_3$ series (6-OH). We report here the chemical synthesis of the 2-deoxy derivative **3**. The synthesis of 2-deoxy sugars is often based on glycal chemistry [5,6] or on the stepwise deoxygenation of a suitably protected derivative.[7] An elegant methodology based on anomeric radical migration has also been described.[8] The close reactivities of the equatorial alcohols of D-xylose preclude selective protection and also selective cyclic acetal formation. We took advantage of the formation of a six-membered bisacetal at positions 2,3 and 3,4 to reach compounds having the required free 2-OH group.

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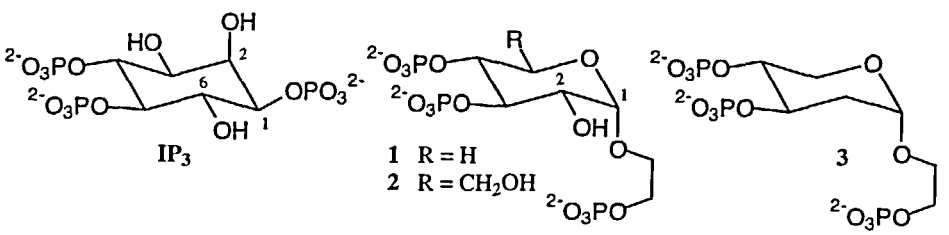
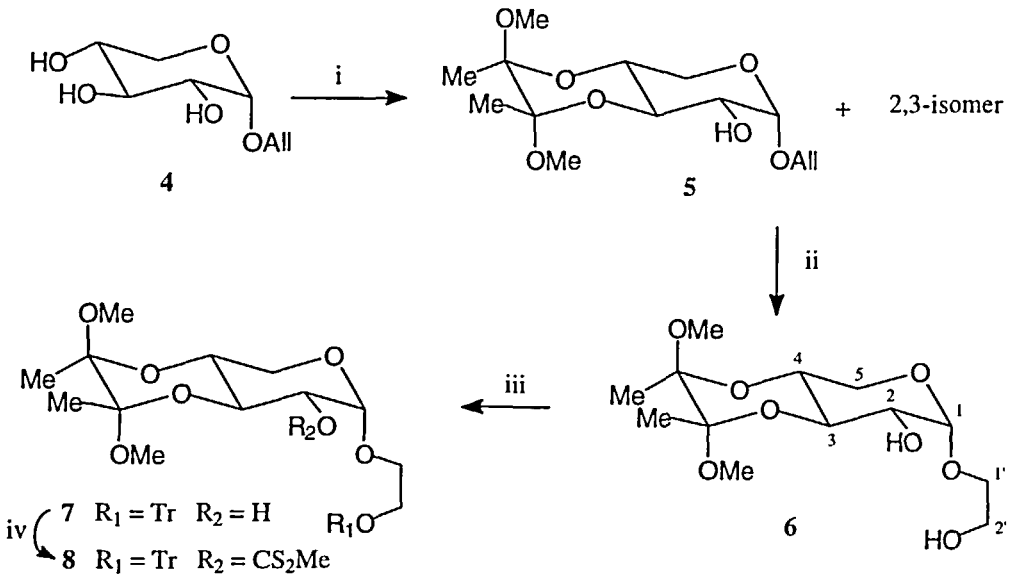
RESULTS AND DISCUSSION

The known allyl α-D-xylopyranoside (**4**) was treated with 2,2,3,3-tetramethoxybutane[9] in methanol in the presence of a catalytic amount of (±)camphorsulfonic acid to give an equimolar mixture of the 2,3 and the 3,4 regioisomers. The desired crystalline compound **5** was easily obtained in 46% yield after column chromatography.[10] Ozonolysis of **5** followed by reductive work-up with sodium borohydride gave the expected 2-hydroxyethyl xyloside derivative **6** in 98% yield. The resulting primary hydroxyl group was selectively protected as a trityl ether to provide compound **7**. The C-2 hydroxyl group of **7** was then converted into its methyl xanthate **8**[11] which could be deoxygenated using the Barton-McCombie method[12] to afford compound **9** in 86% yield.



Scheme 1



Reagents: i) $MeC(OMe)_2C(OMe)_2Me$, CSA, MeOH, $(MeO)_3CH$, reflux, 90 min (46%); ii) O_3, CH_2Cl_2/MeOH, -70 °C, 90 min then $NaBH_4$ (98%); iii) TrCl, DMAP, pyridine, 80 °C, 30h, (71%); iv) NaH, CS_2, THF then MeI (90%).

Scheme 2

Having compound **9** in hand we were only two steps (acid hydrolysis and phosphorylation) away from our target trisphosphate compound **3**. Removal of the 3,4 bisacetal and the trityl groups by treatment of **9** with trifluoroacetic acid did not furnish the desired compound **15** but an inseparable mixture of two polar compounds. In order to establish the structure of these compounds, the mixture was acetylated and the corresponding acetates separated by preparative HPLC. The first eluting product was identified as the 1,3,4-tri-*O*-acetyl-2-deoxy-α-D-*threo*-pentopyranose (**10**),[8] the second product was its β isomer **11**. Removal of the protective groups also results in the cleavage of the glycosidic linkage to give back an α,β anomeric mixture of the 2-deoxy parent sugar. In order to overcome the acid lability of deoxy compound **9**, we carried out the deoxygenation step after acidic cleavage of the bisacetal and the trityl groups.

Hydrolysis of **8** with aqueous trifluoroacetic acid in dichloromethane gave compound **12** which was converted into its di-*O*-acetyl derivative **13** for purification and structure determination. Compound **13** was then reacted with tributyltin hydride and α,α azoisobutyronitrile (AIBN) in refluxing toluene to give the deoxygenated product **14** in

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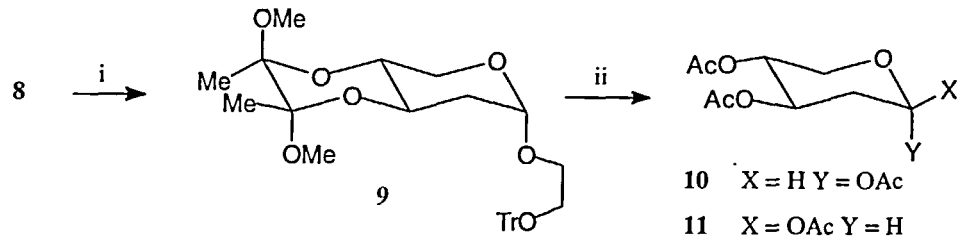

Reagents: i) Bu_3SnH, AIBN, toluene (86%); ii) 95% aqTFA-CH_2Cl_2 (1-1), room temp., 15 min then Ac_2O, pyridine.

Scheme 3

91% yield. Phosphate units were introduced using the phosphoramidite method. After removal of the acetyl groups of **14**, the resulting trihydroxy product **15** was phosphitylated with bis(2-cyanoethyl) *N,N*-diisopropylphosphoramidite[13] in the presence of 1*H*-tetrazole in dichloromethane. Oxidation of the intermediate trisphosphite with t-butyl hydroperoxide gave the fully protected compound **16** in 66% overall yield which was cleanly deprotected by heating in a 0.5 M methanolic potassium hydroxide solution to yield the target trisphosphate **3**. Compound **3** was purified by ion exchange chromatography and isolated as its hexasodium salt.

The binding affinity of compound **3** for the IP_3 receptor of rat cerebellar membranes was evaluated *in vitro* with [^{3}H]IP_3 as a radioligand.[14] Compound **3** showed about 100 fold lower potency (IC_{50} = 24.5 μM) than compound **1** (IC_{50} = 220 nM) and 2000 fold lower potency than $InsP_3$ (IC_{50} = 13 nM) itself. Thus this study demonstrates the important role of the hydroxyl group at C-2 of **1** and contributes to define the minimal structural requirements for a good receptor affinity: the presence of the triad 4,5 *trans* bisphosphate[15] next to an hydroxyl group, this triad being flanked by a less important third phosphate.

CONCLUSION

We have described an expeditious synthesis of the trisphosphate **3** from D-xylose using a bisacetal protecting group which proved efficient to provide a free 2-OH derivative. It is worth observing that removal of this acetal without affecting the glycosidic bond is not possible on 2-deoxy sugars.

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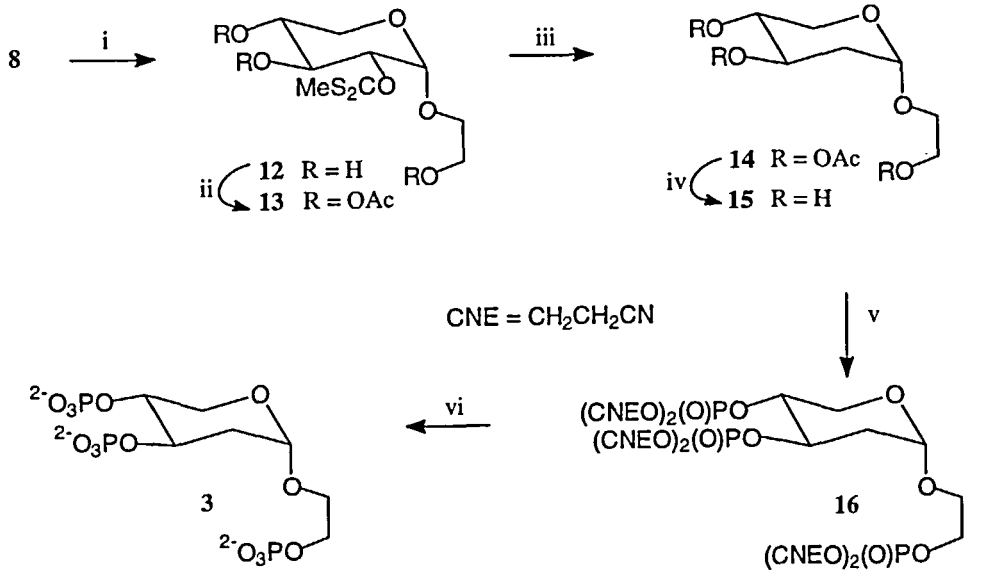

Reagents: i) 95% aq. TFA-CH_2Cl_2 (1-1), room temp. 10 min.; ii) Ac_2O, pyridine (93% from **8**); iii) Bu_3SnH, AIBN, toluene (90%); iv) MeONa, MeOH (98%); v) $(CNCH_2CH_2O)_2PNiPr_2$, 1*H*-tetrazole, CH_2Cl_2, room temp., 4h then *t*BuOOH, 0 °C (66%); vi) KOH-MeOH, 40 °C, 2.5 h (99%).

Scheme 4

EXPERIMENTAL

General procedures. Optical rotations were determined at 20 °C with a Perkin Elmer model 141 automatic. NMR spectra were recorded at 25 °C with a Bruker AC250 spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal tetramethylsilane for ^1H NMR and indirectly to the central line of $CDCl_3$, δ 77.03 for ^{13}C NMR spectra; those in deuterium oxide are reported relative to external 2,3 dimethyl-2-silapentane-5 sulfate (DSS). Infrared spectra were recorded on a Perkin-Elmer model IRFT Spectrum 1000 spectrometer. All reactions were monitored by thin-layer chromatography on Kieselgel $60F_{254}$ (Merck) with detection by UV light and/or by charring with 15% sulfuric acid in ethanol. Elemental analyses and mass spectra were performed by the "Service Central d'Analyse du CNRS"at Vernaison (France).

(2-Hydroxyethyl) 3,4-*O*-[(2*S*,3*S*) (2,3-dimethoxybutane-2,3-diyl)]-α-D-xylopyranoside (6). A solution of compound **5**[10] (1.6 g, 5.25 mmol) in a dichloromethane-

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methanol mixture (65 mL, 1:1 v/v) was cooled to -70 °C and ozone was bubbled through the solution during 90 minutes. Sodium borohydride (0.79 g, 21 mmol) was added and the reaction mixture was allowed to warm up to room temperature. The solvent was evaporated and the residue was extracted with ethyl acetate. The organic layer was washed with hydrochloric acid (3N), water, dried over magnesium sulfate and concentrated to afford 1.6 g (98%) of compound **6** as a syrup. R_f 0.32 (CH_2Cl_2/acetone, 6:4); $[\alpha]_D$ +231 (*c* 0.6, $CHCl_3$); IR 3368 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.87 (d, 1H, J_{1-2} = 3.6 Hz, H-1); 3.48-3.97 (m, 9H, H-2, H-3, H-4, H-5ax, H-5eq, H-1', H-1", H-2', H-2"); 3.26; 3.33 (2s, 6H, 2×OCH_3); 2.03 (m, 2H, 2×OH); 1.30; 1.34 (2s, 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$) δ 99.0; 99.3; (2×CH_3COCH_3); 98.6 (C-1); 69.7 (C-3); 69.2 (C-2 or C-4); 68.9 (C-1'); 65.8 (C-2 or C-4); 60.6 (C-2'); 59.2 (C-5); 47.4 (2×OCH_3); 17.1; 17.3 (2×CH_3).

Anal. Calcd for $C_{13}H_{24}O_8$: C, 50.64; H, 7.85. Found: C, 50.76; H, 7.81.

(2-Trityloxyethyl) 3,4-*O*-[(2*S*,3*S*) (2,3-dimethoxybutane-2,3-diyl) -α-D-xylopyranoside (7). To a solution of compound **6** (1.1 g, 3.56 mmol) in pyridine (24 mL) was added trityl choride (1.89 g, 6.77 mmol) and DMAP (43 mg, 0.35 mmol). The stirred solution was heated at 80 °C during 30 h and the solvent was evaporated. The residue was extracted with dichloromethane. The extract was successively washed with hydrochloric acid (3N), water, dried over sodium sulfate and concentrated. The product was chromatographed on a column of silica gel with hexane-ethyl acetate (first 7:3 then 5:5 v/v) as eluent to give 1.4 g (71%) of compound **7** as a white foam. R_f 0.26 (Hex/AcOEt, 7:3); $[\alpha]_D$ +155 (*c* 1.04, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.38-7.53 (m, 6H, Ar); 7.17-7.36 (m, 9H, Ar); 4.91 (d, 1H, J_{1-2} = 3.6 Hz, H-1); 3.81-3.93 (m, 2H, H-3, H-1'); 3.59-3.80 (m, 4H, H-2, H-4, H-5', H-1"); 3.55 (dd, 1H, $J_{5-5'}$ = 9.7, J_{4-5} = 4.4 Hz, H-5); 3.18-3.43 (m, 8H, H-2', H-2", 2×OCH_3); 2.12 (d, 1H, $^3J_{H-OH}$ = 9.8 Hz, OH); 1.32; 1.38 (2s, 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$) δ 143.6 (C ipso); 128.6 (6C, Ar); 127.5 (6C, Ar); 126.7 (3C, Ar); 99.5; 99.2 (2C, 2×CH_3COCH_3); 98.8 (C-1); 86.3 (CPh_3); 70.7 (C-3); 69.6 (C-2 or C-4); 67.2 (C-1'); 65.9 (C-2 or C-4); 62.6 (C-2'); 59.7 (C-5); 48.0; 47.6 (2×OCH_3); 17.5; 17.3 (2×CH_3).

Anal. Calcd for $C_{32}H_{38}O_8$: C, 69.80; H, 6.90. Found: C, 69.60; H, 6.75 .

(2-Trityloxyethyl) 3,4-*O*-[(2*S*,3*S*) (2,3-dimethoxybutane-2,3-diyl)]-2-*O*-(methylxanthyl) -α-D-xylopyranoside (8). A mixture of **7** (0.6 g, 1.08 mmol) and sodium hydride (60% dispersion in mineral oil, 87 mg, 2.17 mmol) in dry tetrahydrofuran (17 mL) was stirred at room temperature during 0.5 h, at which time carbon disulfide (0.13

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mL, 2.17 mmol) was added dropwise. The reaction mixture was stirred for 1 h and an excess of methyl iodide (0.2 mL) was added. The resulting solution was kept with stirring for 0.5 h, diluted with diethyl ether (10 mL) and quenched by careful addition of ice. Diethyl ether and water were added and the organic layer was washed with water, dried over magnesium sulfate and concentrated. Purification by column chromatography using hexane-ethyl acetate (9:1 v/v) as eluent afforded 0.622 g (90%) of compound **8** as a white foam. R_f 0.26 (Hex/AcOEt, 9:1); $[\alpha]_D$ +165 (*c* 0.52, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.41-7.53 (m, 6H, Ar); 7.15-7.37 (m, 9H, Ar); 5.60 (dd, 1H, J_{2-3} = 10.2 Hz, H-2); 5.36 (d, 1H, J_{1-2} = 3.7 Hz, H-1); 4.42 (dd, 1H, J_{3-4} = 9.5 Hz, H-3); 3.83-3.97 (m, 2H, H-5, H-1'); 3.51-3.64 (m, 2H, H-5', H-1"); 3.22-3.38 (m, 7H, H-2', 2×OCH_3); 3.14 (ddd, 1H, H-2"); 2.47 (s, 3H, SCH_3); 1.33 (s, 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$) δ 215.4 (OC=SS); 143.9 (C ipso); 128.7 (6C, C Ar); 127.8(6C, C Ar); 126.9 (3C, C Ar); 100.0; 99.9 (2C, 2×CH_3COCH_3); 95.5 (C-1); 86.2 (CPh_3); 78.3 (C-2); 67.5 (2C, C-3, C-1'); 66.5 (C-4); 62.8 (C-2'); 59.5 (C-5); 48.0; 47.9 (2C, 2×OCH_3); 19.0 (SCH_3); 17.8; 17.6 (2C, 2×CH_3).

Anal. Calcd for $C_{34}H_{40}O_8S_2$: C, 63.73; H, 7.85; S, 10.01. Found: C, 63.82; H, 7.75; S, 9.94.

(2-Trityloxyethyl) 2-deoxy-3, 4-*O*-[(2*S*,3*S*) (2,3-dimethoxybutane-2,3-diyl)]-α-D-*threo*-pentopyranoside (9). A solution of freshly prepared[16] tributyltin hydride (0.42 mL, 1.52 mmol) in toluene (2 mL) was dropwise added to a solution of the thioester **8** (0.85 g, 1.32 mmol) and AIBN (0.2 g, 0.13 mmol) in refluxing toluene (11 mL). After completion of the reaction (TLC analysis) the solvent was evaporated. Purification by column chromatography using hexane-ethyl acetate (9:1 v/v) as eluent afforded 0.6 g (86%) of compound **9** as a white foam. R_f 0.5 (Hex/AcOEt, 85:15); $[\alpha]_D$ +149 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.40-7.54 (m, 6H, Ar); 7.16-7.37 (m, 9H, Ar); 4.97 (dd, 1H, J_{1-2ax} = 2.9 Hz, H-1); 4.13 (ddd, 1H, J_{3-4} = 8.8 Hz, H-3); 3.52-3.83 (m, 5H, H-4, H-5, H-5', H-1', H-1"); 3.12-3.37 (m, 8H, H-2', H-2", 2×OCH_3); 2.06 (ddd, 1H, J_{2eq-3} = 5.1, $J_{2eq-2ax}$ = 12.4 Hz, H-2eq) ; 1.78 (ddd, 1H, J_{2ax-3} = 11.7 Hz, H-2ax); 1.28 (s, 6H, 2×CH_3). ^{13}C NMR ($CDCl_3$) δ 143.8 (C ipso); 128.4 (6C, C Ar); 127.3 (6C, C Ar); 126.5 (3C, C Ar); 99.6; 99.5 (2C, 2×CH_3COCH_3); 97.7 (C-1); 86.2 (CPh_3); 68.2 (C-4); 66.1 (C-1'); 65.0 (C-3); 62.8 (C-2'); 60.2 (C-5); 47.6 (2C, 2×OCH_3); 34.4 (C-2) ; 17.7; 17.6 (2C, 2×CH_3).

Anal. Calcd for $C_{32}H_{38}O_7$: C, 71.88; H, 7.10. Found: C, 71.50; H, 7.06.

1,3,4-Tri-*O*-acetyl-2-deoxy-β-D-*threo*-pentopyranose (11). R_f 0.73 (Hex/AcOEt , 4:6); $[\alpha]_D$ -21 (*c* 0.62, $CHCl_3$); IR 1742 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 5.96 (dd, 1H, H-1); 4.98 (ddd, 1H, J_{3-4} = 5.8 Hz, H-3); 4.83 (ddd, 1H, J_{4-5ax} = J_{4-5eq} = 5.1 Hz, H-4); 4.18

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(dd, 1H, $J_{5eq\text{-}5ax}$ = 12.8 Hz, H-5eq); 3.62 (dd, 1H, H-5ax); 2.29 (ddd, 1H, $J_{2eq\text{-}2ax}$ = 14.6, $J_{1\text{-}2eq}$ = 3.6, $J_{2eq\text{-}3}$ = 4.4 Hz, H-2eq); 2.17; 2.11 and 2.07 (3s, 9H, 3×$COCH_3$); 1.94 (ddd, 1H, $J_{1\text{-}2ax}$ = 4.4, $J_{2ax\text{-}3}$ = 6.6 Hz, H-2ax). ^{13}C NMR ($CDCl_3$): 169.6; 169.5; 169.0 (3C, 3×C=O); 90.5 (C-1); 67.6 (C-4); 66.7 (C-3); 61.0 (C-5); 30.3 (C-2); 21.0; 20.8 (3C, 3×CH_3CO).

(2-Acetoxyethyl) 3,4-di-*O*-acetyl-2-methylxanthate-α-D-xylopyranoside (13). To a solution of compound **8** (2.3 g, 3.57 mmol) in dichloromethane (7 mL) was added trifluoroacetic acid in water (95% v/v, 7 mL). The solution was stirred at room temperature for 10 min then the solvent was evaporated. The residue was dissolved in ethyl acetate and neutralised with $NaHCO_3$. The solid was filtered off and the filtrate was concentrated. The residue was dissolved in ethyl acetate (150 mL) and washed with water. The organic layer was dried over sodium sulfate and concentrated. The crude pruduct was acetylated using pyridine (9 mL) and acetic anhydride (4.5 mL). After usual work up, purification by column chromatography using hexane-ethyl acetate (7:3 v/v) as eluent gave 1.36 g (93%) of **13** as a syrup. R_f 0.46 (Hex/AcOEt, 6:4); $[\alpha]_D$ +77 (*c* 3, $CHCl_3$); IR 1745 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.56-5.72 (m, 2H, H-2, H-3); 5.23 (d, 1H, $J_{1\text{-}2}$ = 2.9 Hz, H-1); 5.01 (ddd, 1H, H-4); 4.16-4.29 (m, 2H, H-2', H-2"); 3.76-3.92 (m, 2H, H-5, H-1'); 3.61-3.75 (m, 2H, H-5', H-1"); 2.52 (s, 3H, SCH_3); 2.05, 2.03, 2.01 (3s, 9H, 3×$COCH_3$); ^{13}C NMR ($CDCl_3$) δ 200.0 (C=S); 169.87 (C=O); 95.2 (C-1); 77.8 (C-2); 69.3 (C-3); 69.1 (C-4); 66.3 (C 1'); 63.2 (C-2'); 58.4 (C-5); 20.7, 20.6 (3C, 3×CH_3CO); 18.8 (SCH_3).

Anal. Calcd for $C_{15}H_{23}O_9S_2$: C, 43.79; H, 5.63; S, 15.58. Found: C, 43.87; H, 5.71; S, 15.45.

(2-Acetoxyethyl) 3,4-di-*O*-acetyl-2-deoxy-α-D-*threo*-pentopyranoside (14). Compound **13** (0.82 g, 2 mmol) was converted into **14** using the procedure described for the preparation of **9**. Purification by column chromatography using hexane-ethyl acetate (7:3 v/v) as eluent afforded 0.55 g (90%) of compound **14** as a gum. R_f 0.43 (Hex/AcOEt, 6:4); $[\alpha]_D$ +69 (*c* 1.08, $CHCl_3$); IR 1740 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.25 (dd, 1H, $J_{3\text{-}4}$ = 8.4 Hz, H-3); 4.79-4.92 (m, 2H, H-1, H-4); 4.12-4.33 (m, 2H, H 2', H-2"); 3.72-3.90 (m, 2H, H-5, H-1'); 3.56-3.71 (m, 2H, H-5', H-1"); 2.20 (ddd, 1H, $J_{2eq\text{-}2ax}$ = 13.2, $J_{2eq\text{-}3}$ = 5.2 Hz, H-2eq); 2.07, 2.04, 2.02 (3s, 9H, 3×$COCH_3$) ; 1.77 (ddd, 1H, $J_{2ax\text{-}3}$ = 10.2 Hz, H-2ax); ^{13}C NMR ($CDCl_3$) δ 170.5, 169.7, 169.5 (3C, 3×C=O); 96.7 (C-1); 69.0 (C-4); 67.9 (C-3); 65.0 (C-1'); 62.8 (C-2'); 59.5 (C-5); 33.9 (C-2); 20.6, 20.4 (3C, 3×CH_3CO).

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Anal. Calcd for $C_{13}H_{20}O_8$: C, 51.31; H, 6.62. Found: C, 51.25; H, 6.79.

(2-Hydroxyethyl) 2-deoxy-α-D-*threo*-pentopyranoside (15). Compound **14** (0.53 g, 1.74 mmol) was deacetylated using sodium methoxide in methanol to furnish 0.3 g (98%) of compound **15** as a gum. R_f 0.14 (AcOEt /MeOH, 95:5); $[\alpha]_D$ +111 (*c* 0.56, MeOH); IR 3368 (OH) cm^{-1}; ^1H NMR (D_2O) δ 5.01 (dd, 1H, H-1); 3.91 (ddd, 1H, J_{3-4} = 8.3 Hz, H-3); 3.67-3.83 (m, 4H, H-5, H-1', H-2', H-2"); 3.47-3.65 (m, 3H, H-4, H-5', H-1"); 2.01 (ddd, 1H, $J_{2eq-2ax}$ = 13.1, J_{1-2eq} = 2.2, J_{2eq-3} = 4.7 Hz, H-2eq); 1.50 (ddd, 1H, J_{2ax-3} = 10.7, J_{1-2ax} = 3.0 Hz, H-2ax); ^{13}C NMR (D_2O) δ 100.4 (C-1); 73.1 (C-4); 71.3 (C-1'); 70.9 (C-3); 64.8 (C-5); 63.3 (C-2'); 39.2 (C-2).

Anal. Calcd for $C_7H_{14}O_5$: C, 47.19; H, 7.92. Found: C, 47.35; H, 7.84.

[(2-Dicyanoethoxyphosphoryl)ethyl] 2-deoxy-3,4-bis[(2-cyanoethoxy) phosphono]-α-D-*threo*-pentopyranoside (16). Compound **15** (0.142 g, 0.8 mmol) was dissolved in *N,N* dimethylformamide (1 mL) and diluted with dichloromethane (12 mL) then 1*H*-tetrazole (0.67 g, 9.56mmol) and bis(2-cyanoethyl) *N,N*-diisopropyl phosphor-amidite[13] (1.62 g, 5.97 mmol) were added to the solution. The reaction mixture was stirred under argon at room temperature during 4 h then cooled to 0 °C and *t*BuOOH (70% solution in water, 1 mL) was added. The solution was allowed to warm to room temperature and was then stirred for 15 min. The clear solution was diluted with dichloromethane (100 mL) and this solution was washed successively with water (10 mL), saturated aqueous $NaHCO_3$ solution (2 × 10 mL) and water (2 × 10 mL), dried over magnesium sulfate and concentrated. The residue was purified by column chromatography using ethyl acetate-methanol (from 96:4 to 88:12 v/v) as eluent to afford 0.390 g (66%) of compound **16** as a gum. R_f 0.2 (AcOEt /MeOH, 85:15); $[\alpha]_D$ +22 (*c* 1.68, $CHCl_3$); IR 2251 (CN); 1278 (P=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.98 (dd, 1H, J_{1-2eq} = 2.2 Hz, H-1); 4.83 (dddd, 1H, H-3); 4.26-4.50 (m, 15H, H-4, H-2', H-2", 6×CH_2CH_2CN); 4.03 (dd, 1H, $J_{5eq-5ax}$ = 11,2, J_{4-5eq} = 5.3 Hz, H-5eq); 3.89 (dddd, 1H, $J_{1'-1"}$ = 11.9, $J_{1'-2'} = J_{1'-2"}$ = 4.4, $^4J_{H-P}$ = 1.6 Hz, H-1'); 3.78 (dd, 1H, J_{4-5ax} = 10.7 Hz, H-5ax); 3.68 (dddd, 1H, $J_{1"-2'} = J_{1"-2"}$ = 4.4, $^4J_{H-P}$ = 1.3 Hz, H-1"); 2.75-2.89 (m, 12H, 6×CH_2CH_2CN); 2.50 (ddd, 1H, $J_{2eq-2ax}$ = 13.2, J_{2eq-3} = 4.7 Hz, H-2eq); 1.95 (ddd, 1H, J_{2ax-3} = 11.2, J_{1-2ax} = 3.4 Hz, H-2ax); ^{13}C NMR ($CDCl_3$) δ 117.2; 117.1; 117.0 (CN); 96.7 (C-1); 74.7 (dd, $^3J_{C-P}$ = 5.2, $^4J_{C-P}$ = 1.0 Hz, C-4); 74.4 (dd, $^3J_{C-P}$ = 5.2, $^4J_{C-P}$ = 1.0 Hz, C-3); 67.3 (d, $^2J_{C-P}$ = 5.7 Hz, C-2"); 66.1 (d, $^3J_{C-P}$ = 6.7 Hz, C-1'); 62.5-63.0 (6d, 6C, $^2J_{C-P}$ = 4.9 Hz, 6×CH_2CH_2CN); 60.1 (C-5); 35.9 (C-2); 19.5-19.7 (6d, 6C, 6×CH_2CH_2CN). ^{31}P NMR ($CDCl_3$) δ -1.30;-1.91;-1.95 (3s, 3P); Positive-ion FAB-MS: *m/z* 737.1 $(M+H)^+$

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Anal. Calcd for $C_{25}H_{35}N_6O_{14}P_3$: C, 40.77; H, 4.79; N, 11.41; P, 12.62. Found: C, 40.90; H, 4.85; N, 11.34; P, 12.51.

(2 Hydroxyethyl) 2-deoxy-α-D-*threo*-pentopyranoside 3,4,2' trisphosphate (3). Compound **16** (0.2 g, 0.27 mmol) was dissolved in a methanolic potassium hydroxide solution (0.5 M, 4.5 mL) and heated at 40 °C during 2.5 h. The reaction mixture was cooled to room temperature and neutralised to pH 7 with Dowex 50W (H^+) cation-exchange resin. The resin was filtered off and washed twice with methanol. The filtrate was concentrated to dryness. The residue was dissolved in water (3 mL) and applied to a column of Bio-Rad Chelex 100 resin (Na^+ form). The column was eluted with water, fraction containing compound **3** were combined and freeze dried to give 0.146 g (99%) of compound **3** as a white powder. $[\alpha]_D$ +20 (*c* 1.02, H_2O); IR (KBr) 1096 (P-O) cm^{-1}; ^1H NMR (D_2O; 400 MHz) δ 4.90 (dd, 1H, $J_{1\text{-}2eq}$ = 2.8, $J_{1\text{-}2ax}$ = 5.4 Hz, H-1); 4.32 (dddd, 1H, H-3); 3.77-4.00 (m, 5H, H-4, H-5, H-1', H-2', H-2"); 3.59-3.75 (m, 2H, H-5', H-1"); 2.08 (ddd, 1H, $J_{2eq\text{-}2ax}$ = 13.7, $J_{2eq\text{-}3}$ = 4.5 Hz, H-2eq); 1.91, (ddd, 1H, $J_{2ax\text{-}3}$ = 7.5 Hz, H-2ax).^{13}C NMR (D_2O) δ 100.6 (C-1); 73.4 (2C, C-3, C-4); 70.7 (d, $^3J_{C\text{-}P}$ = 7.3 Hz, C-1'); 66.2 (d, $^2J_{C\text{-}P}$ = 3.7 Hz, C-2'); 65.8 (C-5); 37.0 (C-2); ^{31}P NMR (D_2O) δ -6.54;-5.75;-5.18 (3s, 3P); Negative mode ESI-MS:*m/z* 417 $(M\text{-}H)^-$.

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