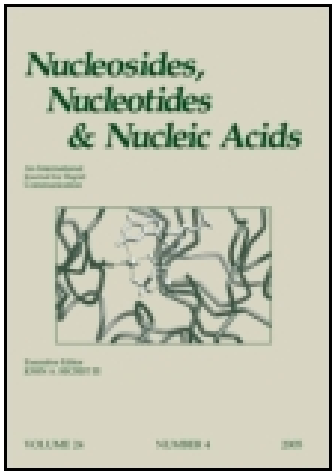

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Straightforward Synthesis of Lipophilic Thymidine Glucopyranosyl Monophosphates as Models for a Drug Delivery System Across Cellular Membranes

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STRAIGHTFORWARD SYNTHESIS OF LIPOPHILIC THYMIDINE GLUCOPYRANOSYL MONOPHOSPHATES AS MODELS FOR A DRUG DELIVERY SYSTEM ACROSS CELLULAR MEMBRANES

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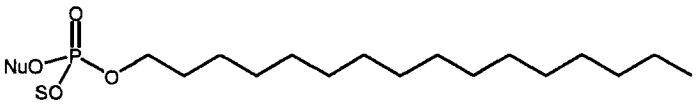
ABSTRACT: *O*-methyl (3'-6) and (5'-6) thymidinyl gluco- and mannopyranosides and (3'-6) thymidinyl glucofuranose can be synthesised in excellent yields by applying the phosphotriester method. The lipophilic phosphotriesters thus obtained are designed as carriers of nucleoside drugs across cellular membranes.

Introduction

2',3'-Dideoxynucleosides (such as ddT, ddC, ddA, ddG and ddI) and their modified analogues are very effective as antiviral drugs.[1,2] Some of them have officially been approved by FDA for clinical treatment of patients affected by AIDS and pathologies caused by other species of DNA and RNA viruses.[3-5] These nucleoside analogues can act as potent inhibitors of the viral replicative cycle after their anabolic activation into infected cells.[6] Therefore, penetration across cellular membranes is an extremely important step for the expression of the biological activity of the drug. The antiviral agent is then transformed by the intracellular encoded viral kinases into its 5-triphosphate form. In the case of HIV the latter derivative acts as selective inhibitor of the viral reverse transcriptase or as terminator during the viral DNA strand elongation.[7]

The lack of activity of the antiviral agent is related (i) to a successful transmembrane transport and (ii) to the efficiency of its enzyme-induced phosphorylation into infected cells. Highly polar and hydrophilic nucleotide analogues cannot penetrate through the cellular lipophilic bilayer, due to the presence of a charged monophosphate moiety.[8]



S = Carbohydrate; Nu = Nucleoside analogue

FIG. 1

Great effort has been paid to design lipophilic carriers suitable for the penetration of prodrugs into cells. Neutral lipophilic phosphotriester derivatives of nucleoside analogues have proved to be a very promising delivery system for the transmembrane transport either of antiviral or antibiotics agents.[9] Some of these models have been proposed for wide use in the clinical treatment by drug cocktails of retroviral infections.[10,11] Moreover, an extensive evaluation of membrane-permeable compounds has shown that hexadecyl glucopyranosyl phosphotriesters (Fig. 1) could represent a good delivery system for nucleoside analogues across cell walls.[12-14]

As reported by Huynh-Dinh's research group,[13] these systems provide some advantageous characteristics: (1) the carbohydrate could ensure the requested hydrosolubility into cellular milieu; (2) the hexadecyl chain is quite similar to the biological membrane constituents; its presence in the monophosphate moiety ensures high lipophilicity for the drug carrier; (3) the absence of charged functionality should allow an easy transport through the cellular barrier of the phosphotriester derivative, which in turn could undergo spontaneous or enzyme-assisted selective hydrolysis *in situ*, releasing the nucleoside analogue as active 5'-monophosphate.

Results and discussion

Our research group has recently indicated the phosphotriester chemistry as a valuable synthetic strategy for the preparation of the title compounds.[15] In a typical approach, the key thymidine long fatty chain phosphodiester synthons **1** and **2** (Fig. 2) can be obtained by a final oxidation step of the respective stable *H*-phosphonate intermediates. Nevertheless, tedious chromatographic procedures are necessary to separate in appreciable overall yields the desired compounds. We now present a more convenient, useful and rapid methodology completely based on the phosphotriester approach.[16]

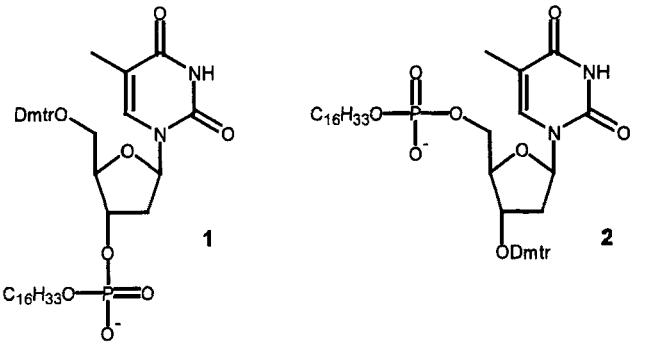


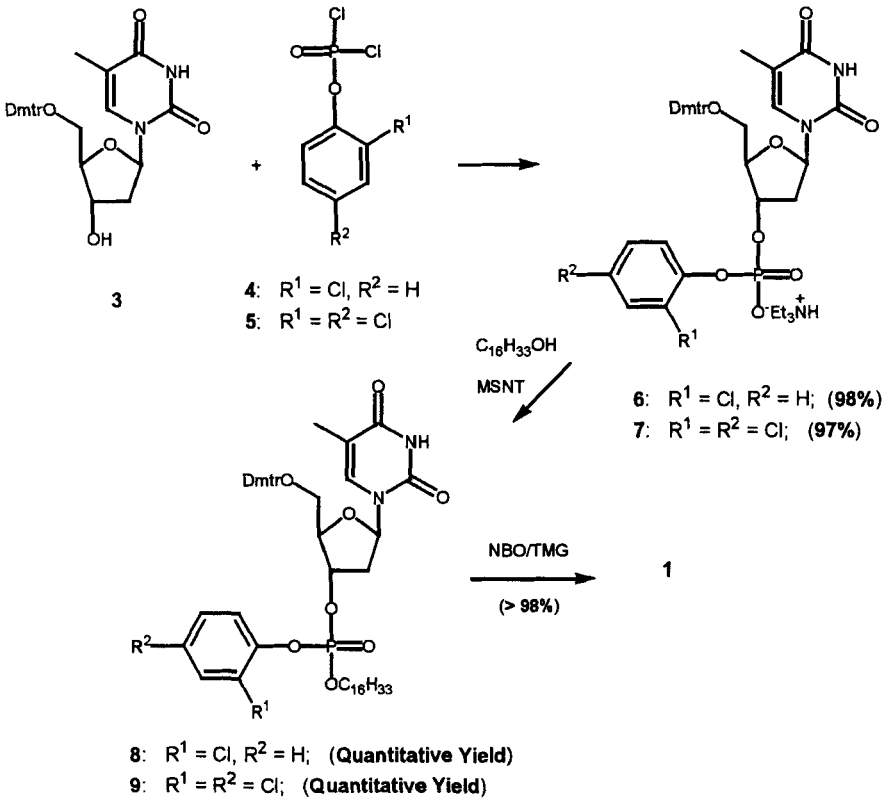
FIG. 2

In order to achieve the preparation of phosphodiesters **1** and **2**, 2-chlorophenyl- and 2,4-dichlorophenylphosphodichloridate (**4** and **5**, respectively; Scheme 1) were used as phosphorylating agents. Both bifunctional reagents have been proposed by Reese[16] and reported to be suitable systems for the formation of internucleotidic linkages.[17]

The effectiveness of this alternative synthetic strategy was checked by the preparation of the model compound **1**. 5'-*O*-(Dimethoxytrityl)thymidine[18] (**3**; Scheme 1), was treated with **4** to give the aryl monophosphate **6** in the form of the triethyl ammonium salt. The high purity of **6** was confirmed by reverse-phase high performance liquid chromatography.

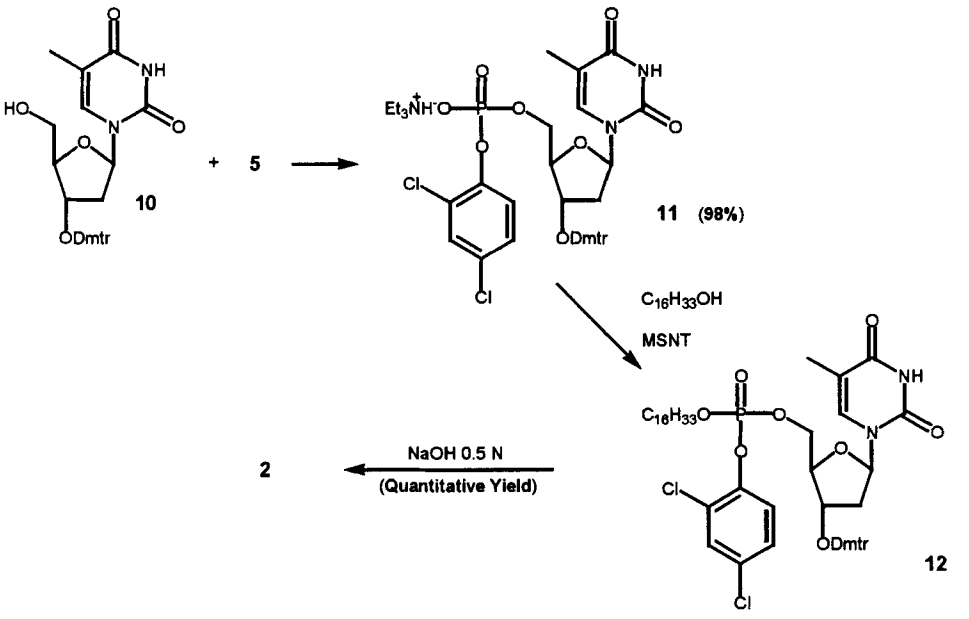
The phosphodiester **6** was then reacted at room temperature with hexadecanol, using 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT) as activating agent. The lipophilic aryl phosphotriester **8** was obtained in nearly quantitative yield and of high enough purity to be transformed into the hexadecyl monophosphate **1** by treatment with *syn*-2-nitrobenzaldoximate/1,1,3,3-tetramethylguanidinium (NBO/TMG), under the typical reaction conditions suggested by Reese and Zard for the deprotection of the phosphate moiety in short oligonucleotidic chains.[19,20] The use of this particular reagent resulted in a nearly quantitative and highly regioselective nucleophilic displacement of the aryl group from the monomer **8**. Undesirable cleavage of the other P-O ester bonds in **8** was limited to a small percentage (<0.2%), as evaluated by HPLC monitoring.

The hexadecyl phosphodiester **1** can also be synthesised in an alternative way by sodium hydroxide hydrolysis of the phosphate protecting group. Various aqueous



Scheme 1

systems were checked to optimise the removal of the 2,4-dichloroaryl residue from the precursor **9** (Scheme 1) prepared by a synthetic tool similar to that previously indicated for **8**, using **5** as phosphorylating reagent. The best results were obtained by employing 0.5 N aqueous sodium hydroxide, in the presence of dioxane as co-solvent: the reaction was complete after 3 hours at room temperature and the resulting sodium salt of monophosphate **1** was obtained as a stable pale yellow glass in excellent yield without the need for chromatographic purification. More diluted aqueous NaOH (ranging from 0.01 to 0.2 N) proved to be less effective for this process, affording **1** in modest yield after reaction times more prolonged than in the case of the treatment performed with concentrated alkaline solutions. Moreover, very poor yields (58% max) of the phosphodiester **1** were obtained employing aqueous tertiary amines (such as

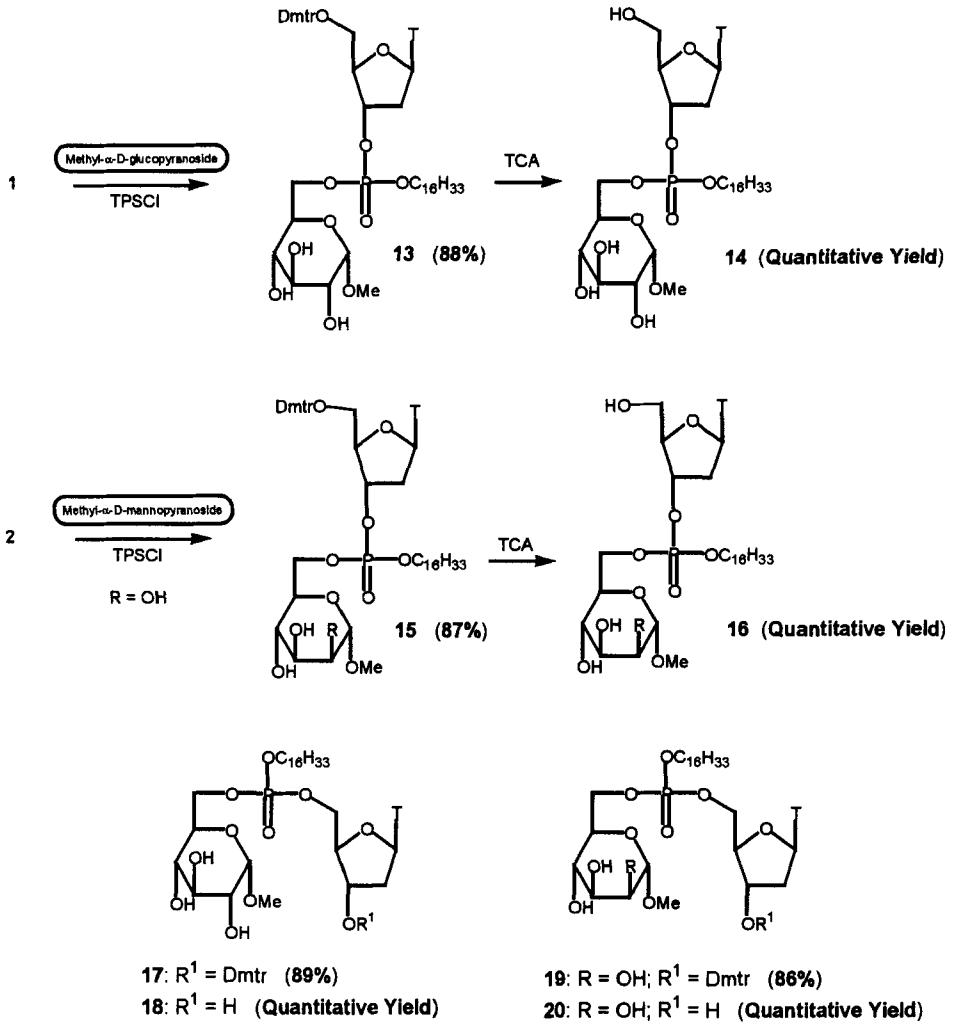


Scheme 2

triethylamine). Remarkably, the reaction conditions adopted in the alkaline hydrolysis of **8** do not involve any competitive cleavage of the other P-O linkages, thus affording a regiospecific displacement of the aryl masking group from the triphosphate **9**, as assessed by HPLC analysis of the crude mixture.

The proposed methodology was then exploited in the preparation of the hexadecyl thymidinyl 5'-phosphate **2** (Scheme 2). In particular, compound **2** can advantageously be obtained without chromatographic purification and on a large scale, starting from 3'-*O*-dimethoxytritylthymidine. The protected nucleoside **10**, synthesised according to a reported procedure,[15] was phosphorylated by means of the dichlorophenyl protected agent **5**. From the MSNT-activated condensation of the triethylammonium salt **11** with hexadecanol, the neutral phosphotriester **12** was recovered in quantitative yield and used in the next step without any further purification. Basic hydrolysis of **12** afforded the target phosphodiester **2** in excellent yield.

In order to prepare (3'-6) and (5'-6) lipophilic thymidinyl glucosyl phosphotriesters, we have exploited the condensation reactions of both charged phosphates **1** and **2** with



Scheme 3

different carbohydrates. All the esterifications were carried out using methyl α-D-glucopyranoside or methyl α-D-mannopyranoside, in the presence of triisopropyl sulfonyl chloride (TPSCl) as activating agent (Scheme 3). The two carbohydrate residues were selected to prepare differently designed lipophilic transmembrane carrier models, since the sugar moiety has proved to play an important role in the interaction with the specific pyranosyl or mannosyl receptors of target cells.[21,22]

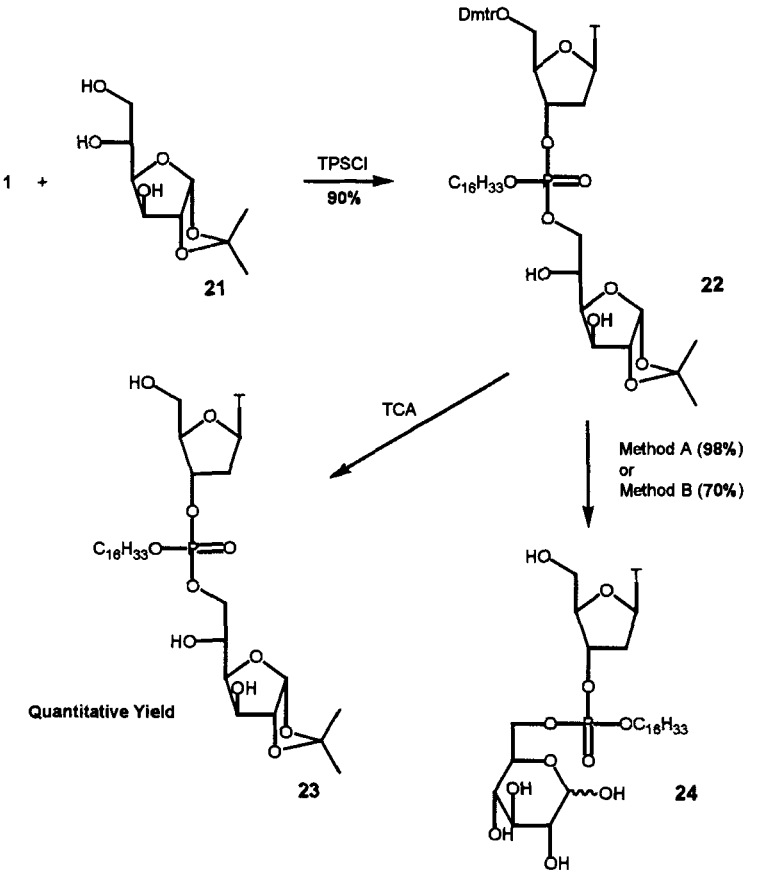
Compounds **13** and **15** were obtained in very high yields after column chromatography. All the investigated reactions went to completion after 3 hours at 40 °C in dry pyridine as solvent. These processes were also characterised by total regioselectivity: therefore, only the primary alcoholic function of both sugars was involved in the P-O bond formation, as demonstrated by ^{1}H and ^{31}P-NMR and by negative ion FAB mass spectrometric analysis.

Finally, the desired lipophilic models were obtained by reacting **13** and **15** with diluted trichloroacetic acid (TCA) in chloroform. The mild conditions adopted for the acidolysis allowed selective removal of the dimethoxytrityl protecting group, without altering the structure of the phosphotriester moiety. The (3'-6) phoshotriesters **14** and **16** were separated in nearly quantitative yields and good degree of purity. Analytical samples were obtained by precipitation of the respective crude from boiling chloroform/*n*-pentane solutions.

By analogy with the above reported procedure, the isomeric (5'-6) derivatives **17** and **19** were synthesised. In this case too, site-specificity of the phosphorylation of the glucoside moiety was observed, whereas the purification of both products was achieved by flash column chromatography. The target molecules **18** and **20** were obtained in nearly quantitative yields by applying the same methodology (Scheme 3).

Having exploited the excellent yields and the complete site-selectivity of the phosphorylation of the saccharide moiety, the method was extended to the synthesis of lipophilic carriers containing carbohydrate residues bearing the free semiketal anomeric function. These analogues belong to a new class of substrates that have shown high effectiveness *in vivo* against dangerous modifications of the genetic code.[23] Simultaneous presence of the lipophilic long chain could also provide these special tailored phosphotriesters to potential delivery system in a prooligonucleotide approach.[24-26] The latter compounds cannot be obtained from **14**, **16**, **18** and **20**, due to the stability of the methyl aglycone under acid conditions.

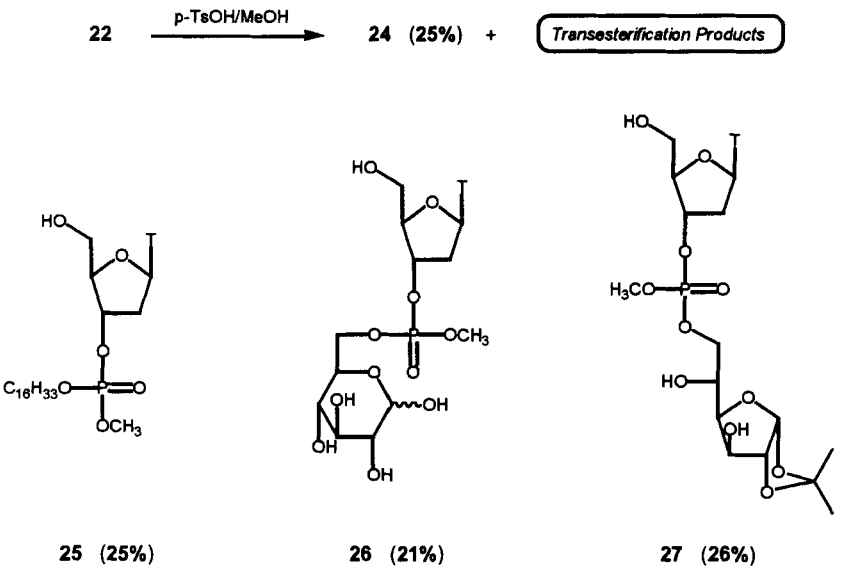
When the phosphodiester **1** was allowed to react with 1,2-*O*-isopropylidene-α-D-glucofuranoside (**21**; Scheme 4), the protected phosphotriester **22** was obtained in very good yield after column chromatography. Under mild acidic conditions (diluted TCA), deblocking of 5' hydroxyl function took place to give **23** without modifying the protected furanose structure. A stronger acid treatment was needed for a fully removal



Scheme 4

of the acid-labile groups from **22**, thus affording the thermodynamic product **24**. Excellent yields of the target phosphotriester **24** were obtained without chromatographic purification by treatment with 10% aqueous trifluoroacetic acid at 0 °C (method A); satisfactory yield of the same compound can be obtained using Amberlite® 120 IR (H^+ form) (method B). ^{1}H-NMR decoupling experiments and ^{31}P-NMR analysis confirmed the existence of the expanded pyranose ring in the desired phosphotriester structure.

An approximately equimolar yield of the phosphotriester **24** (25%) and of the transesterified analogues **25** (25%), **26** (21%) and **27** (27%) was obtained when the acid deblocking was carried out with catalytic amount of p-toluensulphonic acid in methanol (Scheme 5).



Scheme 5

Conclusions

The well established phosphotriester method was applied to the synthesis of (3'-6) and (5'-6) nucleotidyl-glucopyranosides bearing a fatty alcoholic chain at the phosphate linkage. The appropriate selection of the protected sugar unit allows the formation of (3'-6) thymidinyl glucopyranose. All the synthetic steps have provided good to excellent yields of the target compounds which might be useful as prodrugs, since they should provide the transport of a nucleoside unit across cellular membrane.

Experimental

All the reagents were purchased from Aldrich Chemical Co. and used without any further purification. All the solvents were dried using NaH or $LiAlH_4$ and distilled prior to use. Pyridine was twice redistilled from benzoyl chloride and CaH_2. All the reactions were carried out under inert atmosphere (dry N_2).

Mass spectra were obtained by a Vacuum Generators ZAB-2F instrument, using a gun operating with a neutral xenon beam of 8 KeV and at a total current of 10 μA. ^{31}P and ^{1}H NMR spectra were recorded at 300 MHz on a Bruker AC 300 spectrometer. For

proton analysis tetramethylsilane was used as internal standard; in ^{31}P analysis, δ values are referred to a 70% H_3PO_4 external standard.

Merck silica gel on aluminum precoated plates (60-F_{254}) were used for TLC analysis; the spots were also visualised with by charring after spraying with an ethanolic 20% sulphuric acid solution. Kieselgel 60 H without gypsum was used for short column chromatography. Analytical reverse-phase HPLC was performed on a Hewlett-Packard HP-1090 Series II instrument, equipped with an UV HP-1050 detector operating at 254 nm, and a Hewlett-Packard HP-ODS Hypersyl column (5 μm, 100x2.1 mm) with a 20-min gradient of 0-15% CH_3CN in 0.1 M triethylammonium hydrogen carbonate (pH = 7.5; flux rate: 0.1 ml/min).

Phosphodiester salts 6,7 and 11. The appropriate phosphorylating agent **4** or **5** (5 mmol) was added to a magnetically stirred solution of 1,2,4-triazole (11 mmol) and dry triethylamine (10 mmol) in freshly distilled THF (60 ml). After 15 min at room temperature, the protected nucleoside **3** or **10** (2 mmol) and 1-methylimidazole (8 mmol) were added. Complete conversion of the nucleoside was achieved after 10 min (TLC: $CHCl_3$/MeOH, 90:10). The resulting mixture was then quenched by adding distilled water (0.5 ml) and triethylamine (20 mmol) and the solvent was removed under reduced pressure conditions. The recovered crude yellow oil was partitioned between aqueous 10% $NaHCO_3$ and $CHCl_3$ (3x20 ml). The organic layers were washed twice with brine (20 ml), then separated and dried (Na_2SO_4). Evaporation to dryness of the solvent afforded the triethylammonium salt of monophosphates **6**, **7** and **11** with excellent purity.

Compound **6**: pale yellow glass solid (yield 98%). ^{31}P NMR ($CDCl_3$): δ -5.79 ppm; FAB MS (NBA, -): *m/z* 733 ([M-H]$^-$, 100%), 699 (3.1), 623 (2.5), 431 (49.4), 207 (81), 127 (37), 125 (95) and 97 (32.1). Anal. Calcd for $C_{43}H_{51}N_3O_{10}PCl$: C, 61.80; H, 6.11; N, 5.03; P, 3.71. Found: C, 61.82; H, 6.06; N, 4.99; P, 3.77.

Compound **7**: pale yellow glass solid (yield 97%). ^{31}P NMR ($CDCl_3$): δ -5.94 ppm; FAB MS (NBA, -): *m/z* 767 ([M-H]$^-$, 100%), 733 (10.1), 623 (2.3), 465 (27.3), 321 (13), 241 (53), 161 (87), 125 (92) and 97 (85.3). Anal. Calcd for $C_{43}H_{50}N_3O_{10}PCl_2$: C, 59.37; H, 5.75; N, 4.83; P, 3.53. Found: C, 59.32; H, 5.78; N, 4.78; P, 3.51.

Compound **11**: pale yellow glass solid (yield 98%). ^{31}P NMR ($CDCl_3$): δ -5.85 ppm;

FAB MS (NBA, -): *m/z* 767 ([M-H]$^-$, 100%), 607 (3.5), 465 (48), 303 (59.2), 241 (27), 161 (63), 125 (31) and 97 (78.3). Anal. Calcd for $C_{43}H_{50}N_3O_{10}PCl_2$: C, 59.37; H, 5.75; N, 4.83; P, 3.57. Found: C, 59.41; H, 5.71; N, 4.89; P, 3.59.

Phosphotriesters 8, 9 and 12. To a solution of the appropriate nucleotide **6**, **7** or **11** (2 mmol) and hexadecanol (2.5 mmol) in dry pyridine (20 ml) was added 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole (MSNT; 5 mmol) and the resulting mixture was kept under magnetic stirring for 30 min at room temperature. After complete conversion of the starting nucleotide (TLC: $CHCl_3$/MeOH, 95:5), the solution was poured into aqueous 10% $NaHCO_3$ (15 ml) and brine (15 ml), then extracted with $CHCl_3$ (5x20 ml). The organic layers, evaporated to dryness under vacuum (pyridine traces were removed by azeotropic distillation with toluene) afforded aryl phosphotriesters **8**, **9** or **12**. All the products were obtained in nearly quantitative yield and were characterised by a very high purity grade.

Compound **8**: pale yellow oil. ^{31}P NMR ($CDCl_3$): δ -6.61 and –6.89 ppm; FAB MS (NBA, -): *m/z* 957 ([M-H]$^-$, 8%), 847 (3.2), 733 (3.2), 655 (13), 545 (5.3), 431 (100), 321 (17), 127 (52.1), 125 (29.4) and 97 (35.5). Anal. Calcd for $C_{53}H_{68}N_2O_{10}PCl$: C, 66.39; H, 7.10; N, 2.51; P, 3.24. Found: C, 66.27; H, 7.02; N, 2.40; P, 3.22.

Compound **9**: pale yellow oil. ^{31}P NMR ($CDCl_3$): δ -6.79 and –6.92 ppm; FAB MS (NBA, -): *m/z* 991 ([M-H]$^-$, 7.8%), 847 (2.9), 767 (6.1), 689 (5.3), 623 (2.0), 545 (68.7), 465 (100), 321 (52.3), 241 (10.5), 161 (61), 125 (33) and 97 (28.5). Anal. Calcd for $C_{53}H_{67}N_2O_{10}PCl_2$: C,64.11; H, 7.10; N, 2.51; P, 3.13. Found: C, 64.02; H, 7.02; N, 2.54; P, 3.09.

Compound **12**: pale yellow oil. ^{31}P NMR ($CDCl_3$): δ -6.88 and –7.03 ppm; FAB MS (NBA, -): *m/z* 991 ([M-H]$^-$, 7.2%), 957 (11), 847 (30), 767 (32), 733 (1.8), 689 (3.9), 545 (78.8), 465 (100), 431 (7.2), 321 (16.5). 241 (7.9), 161 (63), 125 (37.8) and 97 (15). Anal. Calcd for $C_{53}H_{67}N_2O_{10}PCl_2$: C,64.11; H, 7.10; N, 2.51; P, 3.13. Found: C, 64.12; H, 7.02; N, 2.48; P, 3.11.

Phosphodiester salt 1. To a magnetically stirred solution of the aryl phosphotriester **8** (2 mmol) in dioxane/water (60 ml; 1:2) were added *syn*-2-nitrobenzaldoxime (20 mmol) and 1,1,3,3-tetramethylguanidine (22 mmol). Complete conversion of **8** was detected after 3 h at room temperature (TLC: $CHCl_3$/MeOH, 80:20). The reaction

mixture was then concentrated under reduced pressure conditions. The resulting aqueous residue was diluted with distilled water (20 ml) and extracted with diethyl ether (5x20 ml). The ethereal layers were discarded and the mother liquor was further extracted with $CHCl_3$ (5x20 ml). The collected organic fractions were dried (Na_2SO_4) and evaporated to dryness. Pure monophosphate sodium salt **1** was recovered in nearly quantitative yield. Compound **1** was obtained too in an alternative manner as described below.

The aryl phosphotriester **9** (2 mmol), dissolved in dioxane (40 ml), was treated with aqueous 0.5 N NaOH (20 ml), under vigorous magnetic stirring at room temperature. After 3 h complete conversion of **9** was observed and the mixture extracted with $CHCl_3$ (3x20 ml). The collected organic layers were washed with brine (20 ml), dried (Na_2SO_4) then evaporated to dryness. Removal of the solvent afforded pure **1** in 92% total yield.
1: pale yellow glass solid. ^{31}P NMR ($CDCl_3$): δ -3.77 ppm; FAB MS (NBA, -): *m/z* 847 ([M-H]$^-$, 100%), 623 (3.1), 567 (6.7), 545 (2.3), 321 (18.2). 125 (28.8) and 97 (12.8). Anal. Calcd for $C_{47}H_{64}N_2O_{10}PNa$: C, 64.83; H, 7.36; N, 2.76; P, 3.56. Found: C, 64,77; H, 7.29; N, 2.69; P, 3.57.

Phosphodiester salt 2. Basic hydrolysis of the aryl phosphotriester **12**, performed as previously reported for **1**, afforded pure **2** in form of sodium salt as a pale yellow glass solid (yield 95%). ^{31}P NMR ($CDCl_3$): δ -3.75 ppm; FAB MS (NBA, -): *m/z* 847 ([M-H]$^-$, 100%), 623 (3.8), 567 (12.8), 545 (4.8), 321 (18.7), 125 (34.4) and 97 (22.2). Anal. Calcd for $C_{47}H_{64}N_2O_{10}PNa$: C, 64.83; H, 7.36; N, 2.76; P, 3.56. Found: C, 64,79; H, 7.31; N, 2.71; P, 3.54.

Phosphotriesters 13, 15, 17, 19 and 22. Nucleotide **1** or **2** (1 mmol) was allowed to react with the appropriate carbohydrate (1.12 mmol) in dry pyridine (15 ml), in the presence of TPSCl (10 mmol) and 1-methylimidazole (10 mmol). Complete conversion of the nucleotide was achieved after 2-4 h at 40 °C (TLC: CH_2Cl_2/n-PrOH, 90:10). Aqueous 10% $NaHCO_3$ was then added and the mixture was extracted with diethyl ether (3x20 ml). The organic phase was washed once with brine (15 ml), dried (Na_2SO_4) and evaporated to dryness. After removal of pyridine residues by azeotropic distillation with toluene, the crude material was purified by short column chromatography to give the title phosphotriesters.
Compound **13**: pale yellow glass solid (yield 88%). ^{31}P NMR ($CDCl_3$): δ -1.23 and -1.44 ppm; FAB MS (NBA, -): *m/z* 1023 ([M-H]$^-$, 12.1%), 847 (35), 799 (2.4), 721 (2.2),

545 (25.1), 497 (100), 321 (26), 125 (57) and 97 (13.8). Anal. Calcd for $C_{54}H_{77}N_2O_{15}P$: C, 64.83; H, 7.36; N, 2.76; P, 3.03. Found: C, 64.79; H, 7.26; N, 2.54; P, 3.00.

Compound **15**: pale yellow glass solid (yield 87%). ^{31}P NMR ($CDCl_3$): δ -1.41 and -1.77 ppm; FAB MS (NBA, -): *m/z* 1023 ([M-H]$^-$, 12.8%), 847 (37.2), 799 (3.8), 721 (5.4), 545 (22.1), 497 (100), 321 (26.4), 125 (52.9) and 97 (15.8). Anal. Calcd for $C_{54}H_{77}N_2O_{15}P$: C, 64.83; H, 7.36; N, 2.76; P, 3.03. Found: C, 64.59; H, 7.29; N, 2.64; P, 3.04.

Compound **17**: pale yellow glass solid (yield 89%). ^{31}P NMR ($CDCl_3$): δ -1.37 and -1.56 ppm; FAB MS (NBA, -): *m/z* 1023 ([M-H]$^-$, 11.7%), 889 (15), 847 (100), 799 (9.8), 769 (15.3), 652 (13.3), 623 (8.5), 545 (19.1), 497 (73.5), 363 (18), 321 (18.8), 125 (32.5) and 97 (22.1). Anal. Calcd for $C_{54}H_{77}N_2O_{15}P$: C, 64.83; H, 7.36; N, 2.76; P, 3.03. Found: C, 64.72; H, 7.30; N, 2.66; P, 3.01.

Compound **19**: pale yellow glass solid (yield 86%). ^{31}P NMR ($CDCl_3$): δ -1.52 and -1.87 ppm; FAB MS (NBA, -): *m/z* 1023 ([M-H]$^-$, 9.3%), 889 (21), 847 (100), 799 (10.2), 769 (14.3), 652 (12.5), 623 (8.8), 545 (21.1), 497 (65.5), 363 (17.8), 321 (20), 125 (31.9) and 97 (22.9). Anal. Calcd for $C_{54}H_{77}N_2O_{15}P$: C, 64.83; H, 7.36; N, 2.76; P, 3.03. Found: C, 64.77; H, 7.32; N, 2.68; P, 3.03.

Compound **22**: pale yellow glass solid (yield 90%). ^{31}P NMR ($CDCl_3$): δ -1.33 and -1.59 ppm; FAB MS (NBA, -): *m/z* 1051 ([M-H]$^-$, 4.8%), 847 (31.3), 825 (7.8), 623 (5.4), 545 (12.7), 523 (100), 321 (65), 125 (87) and 97 (73.4). Anal. Calcd for $C_{56}H_{79}N_2O_{15}P$: C, 63.94; H, 7.52; N. 2.27; P, 2.95. Found: C, 63.87; H, 7.44; N. 2.20; P, 2.97.

Deprotected phosphotriesters 14, 16, 18, 20 and 23. The dimethoxytrityl group was removed by treating the appropriate phosphotriester **13**, **15**, **17**, **19** or **22** (1 mmol) with 5% trichloroacetic acid in $CHCl_3$ (12 ml). The mixture was kept at room temperature for 10 min (TLC: $CHCl_3$/MeOH, 85:15). Aqueous 10% $NaHCO_3$ was then added and the mixture was extracted with $CHCl_3$ (3x10 ml). The combined organic layers were dried (Na_2SO_4) and evaporated to dryness to give a pale yellow solid which was washed several times with *n*-pentane. All the title compounds were obtained in quantitative yields as white solids in very high purity.

Compound **14**: ^{31}P NMR (DMSO-d_6): δ -0.76 and -0.87 ppm; FAB MS (NBA, -): *m/z* 721 ([M-H]$^-$, 8.5%), 545 (12.7), 497 (100), 321 (62), 241 (93), 125 (73) and 97 (35.8).

Anal. Calcd for $C_{33}H_{59}N_2O_{13}P$: C, 54.85; H, 8.17; N, 3.32; P, 4.29. Found: C, 54.65; H, 8.08; N, 3.12; P, 4.25.

Compound **16**: ^{31}P NMR (DMSO-d_6): δ -0.75 and -0.88 ppm; FAB MS (NBA, -): *m/z* 721 ([M-H]$^-$, 8.9%), 545 (13.5), 497 (100), 321 (61.1), 241 (91), 125 (75) and 97 (35.4). Anal. Calcd for $C_{33}H_{59}N_2O_{13}P$: C, 54.85; H, 8.17; N, 3.32; P, 4.29. Found: C, 54.67; H, 8.13; N, 3.29; P, 4.31.

Compound **18**: ^{31}P NMR (DMSO-d_6): δ -0.87 and -0.96 ppm; FAB MS (NBA, -): *m/z* 721 ([M-H]$^-$, 7.9%), 545 (14.7), 497 (100), 321 (65.9), 241 (90.4), 125 (78.2) and 97 (36). Anal. Calcd for $C_{33}H_{59}N_2O_{13}P$: C, 54.85; H, 8.17; N, 3.32; P, 4.29. Found: C, 54.73; H, 8.12; N, 3.16; P, 4.31.

Compound **20**: ^{31}P NMR (DMSO-d_6): δ -0.85 and -0.92 ppm; FAB MS (NBA, -): *m/z* 721 ([M-H]$^-$, 8.3%), 545 (13.1), 497 (100), 321 (58.8), 241 (91), 125 (71.2) and 97 (35.3). Anal. Calcd for $C_{33}H_{59}N_2O_{13}P$: C, 54.85; H, 8.17; N, 3.32; P, 4.29. Found: C, 54.65; H, 8.04; N, 3.13; P, 4.27.

Compound **23**: ^{31}P NMR (DMSO-d_6): δ -0.82 and -0.99 ppm; FAB MS (NBA, -): *m/z* 747 ([M-H]$^-$, 3.9%), 545 (10.5), 523 (100), 321 (34.5), 125 (63.9) and 97 (33.9). Anal. Calcd for $C_{35}H_{61}N_2O_{13}P$: C, 56.15; H, 8.16; N, 3.95; P, 4.15. Found: C, 56.07; H, 8.11; N, 3.89; P, 4.13.

Phosphotriester 24. *Method A.* Phosphotriester **22** (0.1 mmol) was allow to react with 10% trifluoroacetic acid in $CHCl_3$ (4.5 ml) and distilled water (0.5 ml). After 3 h at 5 °C, the mixture was heated at 45 °C until complete conversion of **22** (TLC: $CHCl_3$/MeOH, 70:30). The solvent was evaporated to dryness and the residue was dispersed in aqueous 10% $NaHCO_3$ (3 ml) and brine (1 ml). The resulting solution was then extracted with $CHCl_3$ (3x5 ml) and the collected organic layers were dried ($MgSO_4$). Removal of the solvent under vacuum afforded pure **24** as white solid (yield 98%).

Method B. To a suspension of **22** (0.1 mmol) in distilled water (5 ml) was added Amberlite® 120 IR (H+ form; 90 mg) and the mixture was allowed to react at room temperature for 30 min. The heterogeneous mixture was then heated at 45 °C until complete conversion of **22** was achieved. After cooling at room temperature, the resin was filtered by means of a short pad of Celite 545 (eluant: MeOH, 50 ml). The solvent was then evaporated to dryness and the recovered crude material was purified by short

column chromatography to give 24 (yield 70%). ^{31}P NMR (DMSO-d_6): δ -0.64, -0.70, -0.76 and -0.80 ppm; ^1H NMR (DMSO-d_6): diastereomeric mixture; glucose, δ 6.77 ppm [d, anomeric$_{\alpha(\beta)}$ OH; 1 H], 6.72 [d, anomeric$_{\beta(\alpha)}$ OH; 1 H], 4.75 (d, H_1, J = 8.7 Hz; 1 H) and 4.67 (d, H_1, J = 2.7 Hz; 1 H); FAB MS (NBA, -): *m/z* 707 ([M-H]$^-$, 5.5%), 545 (73.5), 483 (27.8), 465 (10.2), 419 (18), 321 (100), 259 (7.2), 125 (45.4) and 97 (43.8). Anal. Calcd for $C_{32}H_{57}N_2O_{13}P$: C, 54.24; H, 8.05, N, 29.38; P, 4.38. Found: C, 54.14; H, 8.12, N, 29.27; P, 4.36.

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