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A novel approach to the synthesis of lipophilic thymidinemonophosphoglucopyranosides as drug delivery systems [1]

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Abstract

The paper describes the synthesis of the hexadecyl phosphotriesters of thymidine 3′-(5′-deoxythymidin-5′-yl phosphate), thymidine 5′-(5′-deoxythymidin-5′-yl phosphate), thymidine 5′-(3′,5′-dideoxythymidin-5′-yl phosphate), thymidine 3′-[(methyl 6-deoxy-α-D-glucopyranosid-6-yl) phosphate], and thymidine 5′-[(methyl 6-deoxy-α-D-glucopyranosid-6-yl) phosphate]. The novel approach is based on the condensation of unprotected nucleosides or pyranosides with the lipophilic phosphodiesters 5′-*O*-dimethoxytritylthymidine 3′-(hexadecyl phosphate) and 3′-*O*-dimethoxytritylthymidine 5′-(hexadecyl phosphate) which were obtained in satisfactory yield from hexadecyl phosphorodichloridite and 5′-*O*-dimethoxytrityl- or 3′-dimethoxytrityl-thymidine. The latter was prepared in high yield from 5′-*O*-(4-nitrobenzoyl)thymidine, obtained from thymidine, 4-nitrobenzoic acid, and bis(2-oxooxazolidin-3-yl)phosphinic chloride, by a one-pot procedure. The introduction of the aliphatic chain in the early stage of the synthesis prevents the alkylation of the nucleobases and allows a regioselective phosphorylation of unprotected nucleosides and pyranosides. © 1996 Elsevier Science Ltd.

Keywords: Lipophilic phosphotriesters; Hexadecyl dinucleoside phosphates; Hexadecyl nucleoside glucopyranoside phosphates; Drug delivery systems

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[1] A preliminary account of part of this work was given in ref. [1].

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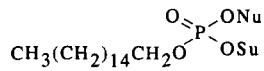
1. Introduction

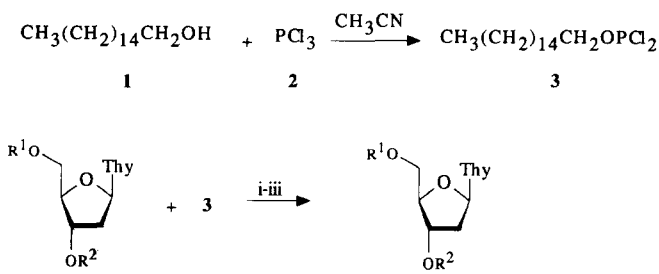
Modified nucleotide 5′-triphosphates can act as antiviral drugs and some of them are effective as inhibitors of HIV reverse transcriptase in AIDS therapy. A major drawback in the uptake of the active species is represented by its lack of penetration across cellular membranes, due to the presence of the highly charged triphosphate moiety. It has recently been proposed that a good delivery system for a nucleoside (Nu) across membranes could be represented by a phosphotriester molecule (**I**) bearing a carbohydrate group (Su), which gives the required hydrophilicity to the system and a hydrocarbon chain linked to the phosphate moiety which should ensure the adsorption and transport of the molecule [2].



I

A series of derivatives of **I** has been obtained by synthetic schemes whereby the aliphatic chain was introduced in the last step, by alkylation with 1-halohexadecane of the sodium salts of the appropriate phosphodiesters, prepared by direct phosphorylaton of protected pyranosides [2]. An alternative approach, based on the well-established phosphotriester method, is now proposed, aiming at the required phosphotriesters by condensation of phosphodiester synthons **6** and **7** (Scheme 1) with unprotected pyranosides [1].

The introduction of the hexadecyl group at the early stage of the synthesis has the advantage that alkylation of the pyrimidine nucleobase cannot take place, as was observed when the previous method was applied. Furthermore, the regioselectivity experienced in the functionalization of the primary hydroxyl group of nucleosides [3,4] and pyranosides [5] suggests that the secondary hydroxyl groups of the sugar units could



4 R^1= Dmt, R^2= H
5 R^1= H, R^2= Dmt
6 R^1= Dmt, R^2= $CH_3(CH_2)_{14}CH_2OPO_2^-$
7 R^1= $CH_3(CH_2)_{14}CH_2OPO_2^-$, R^2=Dmt

Scheme 1. i, Phosphitylation; ii, alkaline hydrolysis; iii, oxidation. Dmt = 4,4′-dimethoxytrityl.

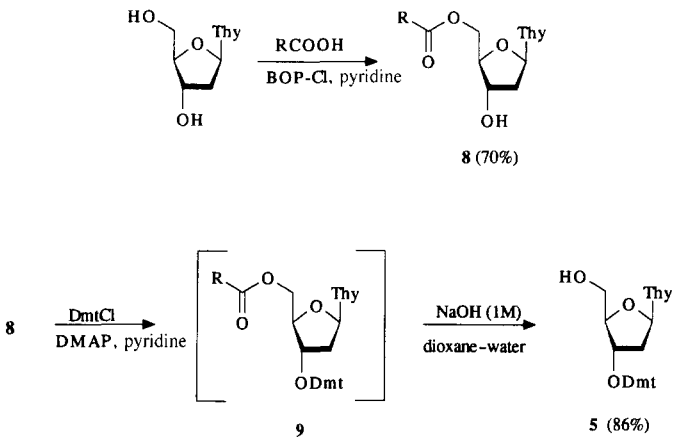
be left unprotected in the preparation of the target compounds **I** by means of **6** and **7** (Scheme 1). A further advantage of the proposed synthetic method could be the possibility of introducing a wide variety of monosaccharides for the evaluation of the role of the sugar moiety in cell surface recognition [6].

2. Results and discussion

The exploitation of the synthetic approach reported in Scheme 1 requires the formation of hexadecylphosphorodichloridite (**3**) from phosphorus trichloride (**2**) and 1-hexadecanol (**1**) by a literature method [7]. Crude **3** was obtained in bulk quantities and used without further purification in the next step. The formation of **6** and **7** requires an initial phosphitylation of thymidine to be performed selectively at positions 5′ or 3′ of the nucleoside which must be temporarily protected at the appropriate hydroxyl groups. 4,4′-Dimethoxytrityl (Dmt) was chosen as a protecting group either for the 3′- or the 5′-position of the nucleoside, to allow a mild deblocking of the precursors leading to the target molecules. The use of acid-labile groups should prevent the phosphotriester from partial degradation which might occur in the final deprotection if a base-labile derivative was employed for the 3′-position of the nucleoside.

3′-*O*-Monomethoxytrityl- [8] and 3′-*O*-dimethoxytrityl-thymidines [9] have previously been prepared from isobutyl thymidine 5′-carbonate or from the fully tritylated derivative, respectively. In the latter process, however, the pure 3′-derivative was obtained only after preparative TLC on silica gel. Regioselective protection of the primary hydroxyl group of nucleosides and glycosides can be achieved by means of bis(2-oxooxazolidin-3-yl)phosphinic chloride (BOP-Cl) [4,5]. This reaction can be carried out, in mild conditions, from commercially available carboxylic acids. A synthetic scheme was therefore evaluated, whereby the target 3′-*O*-dimethoxytritylthymidine (**5**) could be obtained by alkaline treatment of 5′-*O*-aroyl-3′-*O*-dimethoxytritylthymidine intermediates by a one-pot procedure. The evaluation of both reactivity and yields in the transformation of thymidine into **5**, as a function of the structure of the 5′-*O*-acyl group, showed that the 4-nitrobenzoyl group provides the best results (Scheme 2). The 5′-*O*-protected intermediate **8** was obtained, in satisfactory isolated yields, as a pure crystalline compound directly from the crude mixture. The crude, fully protected intermediate **9** was directly saponified in 7 min at ambient temperature, affording the target 3′-*O*-protected thymidine in good isolated yields. The formation of 3′-*O*-dimethoxytritylthymidine (**5**) by the procedure described above is a valuable alternative to the existing methods [8,9] since it can be obtained from commercially available compounds in three consecutive steps which do not require any chromatographic purification.

The phosphorylation or phosphitylation of nucleosides with polyfunctional agents suffers from the known drawbacks of the concomitant formation of polysubstituted derivatives [10]. The synthesis of the target compounds of structure type **I** requires, however, only the formation of a single phosphate bond. The control of the reaction conditions should therefore allow the use of the readily available reagent **3** in the formation of the synthons **6** and **7** (Scheme 1). Good yields of dinucleoside phosphates have, in fact, been reported with bifunctional phosphitylating [11,12] or phosphorylating

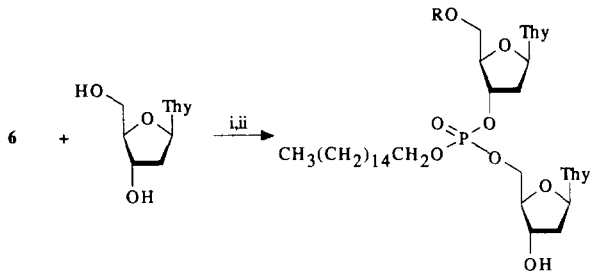


Scheme 2.

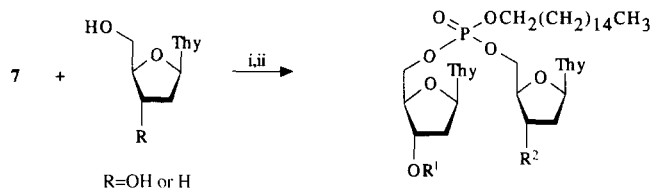
[13] agents and when (1,2,4-triazolyl)phosphine, a trifunctional species, was used in the stepwise synthesis of dinucleoside phosphorothioates [14].

5′-*O*-Dimethoxytritylthymidine (**4**) or 3′-*O*-dimethoxytritylthymidine (**5**) reacted with **3** in tetrahydrofuran at −78 °C in 15 min (Scheme 1). Treatment with aqueous 10% hydrogencarbonate followed by ethylethanoate extraction afforded the corresponding 3′- or 5′-phosphitylated species as stable H-phosphonate derivatives [15] which were used directly for the next step. The presence of the latter in the crude mixture was ascertained by fast atom bombardment mass spectrometry (FABMS) [16] through the appearance, in the negative (−) ionization mode, of the appropriate $[M - H]^-$ ions and of diagnostic fragments [17,18]. Conventional oxidation of the H-phosphonate intermediates with iodine afforded the sodium salts of the phosphodiesters **6** and **7**, in 65 and 61% isolated yields, based on **4** and **5**, respectively, after flash chromatographic purification of the crude reaction mixture. The presence of sodium as the counterion was ascertained by FABMS analysis in the positive (+) ionization mode. ^{31}P NMR spectra of **6** and **7** showed the presence of a single phosphodiester group at the expected chemical shift values (see Experimental section), whereas the FAB (−) spectra, taken in *m*-nitrobenzyl alcohol (NBA) matrix, provided the appropriate $[M - H]^-$ species at m/z 847. It seems worthwhile to mention that the presence of both the lipophilic chain attached at the phosphate moiety and the Dmt protecting group allows an easy purification of both phosphodiester salts by silica gel flash chromatography with trichloromethane–methanol (9:1, v/v) as eluent.

The synthesis of the target compounds of type **I** from **6** and **7** requires a condensation step to be performed according to the phosphotriester method widely exploited in oligonucleotide synthesis [19]. One of the objectives of the procedure presented here is the use of unprotected pyranosides, which might pose problems due to the availability on the substrates of many condensation sites represented by the primary and secondary hydroxyl groups. Therefore the establishment of appropriate reaction conditions for the formation of the phosphotriester linkage was first explored in the preparation of the dinucleoside phosphates **10b–12b** (Scheme 3). The latter, however, could have some



10a R = Dmt (84%)
10b R = H (95%)



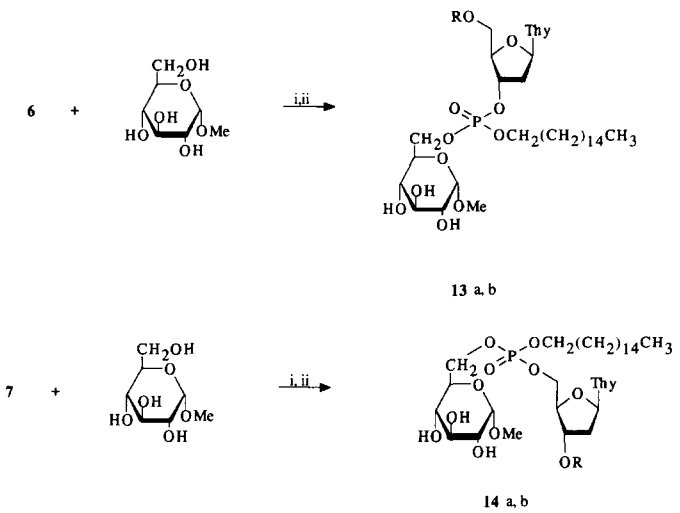
R=OH or H

11a R^1=Dmt, R^2=OH (82%)
11b R^1=H, R^2=OH (95%)
12a R^1=Dmt, R^2=H (84%)
12b R^1=R^2=H (95%)

Scheme 3. i, TPS-Cl, 1-methylimidazole, pyridine, 2 h, 45 °C; ii, 5% CCl_3CO_2H–CH_2Cl_2, 15 min.

synthetic interest either as building blocks to be incorporated into modified DNA strands (**10a**) or as a delivery system for the potential drug deoxythymidine as in **12b**. The best results in the activation of **6** and **7** and coupling with the appropriate nucleosides (Scheme 3) were obtained with the system 2,4,6-tri-isopropylbenzenesulfonyl chloride (TPS-Cl)–1-methylimidazole in anhydrous pyridine, recently applied in the formation of phosphorodithioate [20] and phosphorothioate [21] internucleotidic linkages.

Good isolated yields of the partially protected dinucleoside phosphates **10a–12a** were obtained after 2 h at room temperature and were converted into the unprotected isomers **10b–12b** by mild acid treatment. The structures of the latter were determined from their ^{31}P, ^1H NMR, and FAB (−) spectra. Proton NMR analysis proved to be very useful in the differentiation of the isomeric dinucleoside phosphates **10b** and **11b**. The latter showed a single doublet of doublets due to the thymidine H-1′ and a single resonance for the phosphorus atom at 6.20 and −0.98 ppm, respectively, as a consequence of the symmetry of the molecule. The 3′-5′-linked isomer **10b** displayed, as expected, overlapping signals for the same proton (6.12–6.20) and two resonances for the chiral phosphorous atom at −1.070 and −1.053 ppm, respectively. FABMS (−) was very helpful in the structure determination of **12b** since the spectrum of the stable ions showed, besides the formation of the appropriate $[M-H]^-$ species at m/z 753, the



Scheme 4. i, TPS-Cl, 1-methylimidazole, pyridine, 2 h, 45°C; ii, 5% CCl_3CO_2H–CH_2Cl_2, 15 min.

fragments at m/z 545 and 529 due to the competitive loss of deoxythymidine and thymidine neutral fragments, respectively.

The results obtained in the formation of the dinucleoside phosphates **10b** and **11b** prove that the phosphodiesters **6** and **7** can be used as synthons in the regioselective phosphorylation of thymidine at the primary hydroxyl function. The method was therefore applied to the preparation of **13b** and **14b** (Scheme 4) as models of a drug delivery system across cellular membranes. Methyl α-D-glucopyranoside was chosen as the carbohydrate unit because of the number of studies [2] reported for the biological activity of systems similar to **13b**. Good yields of thymidine 3′-[hexadecyl (methyl 6-deoxy-α-D-glucopyranosid-6-yl) phosphate] (**13b**) and thymidine 5′-[hexadecyl (methyl 6-deoxy-α-D-glucopyranosid-6-yl) phosphate] (**14b**) were obtained by applying the methodology discussed so far (Scheme 4).

The FAB (−) spectra of both **13b** and **14b** were highly diagnostic, since the breakdown pattern of the $[M-H]^-$ species at m/z 847 shows the competitive release of the three units attached to the phosphotriester moiety (see Experimental section). Mass spectral data, however, cannot give definitive proof that the isolated compounds do not contain traces of isomers due to the phosphorylation of one of the secondary hydroxyl groups of the glucoside which might overlap with the main product of the reaction during the chromatographic purification. The ^{31}P NMR spectra of the 6-3′- and of the 6-5′-linked isomers **13b** and **14b** displayed two distinct resonances at −0.21 and −0.38 ppm and at −0.63 and −0.78 ppm, respectively. The ^1H NMR analysis gave additional evidence on the purity of the isolated compounds since, in the low-field portion of the spectra, a clean doublet of doublets due to the anomeric proton of the deoxyribose unit was present at 6.22 ppm for both compounds.

An indirect proof of the regioselectivity experienced in the phosphorylation of the pyranoside with the phosphodiesters **6** and **7** was obtained in the reaction of the same nucleotides with methyl 6-*O*-(2,6-dichlorobenzoyl)-α-D-glucopyranoside, prepared as previously described [5]. Under the same experimental conditions as reported above no reaction products were obtained after 24 h at room temperature. Steric hindrance, presumably, prevents the phosphorylation of the secondary hydroxyl groups of the substrate which was blocked at the *O*-6 position.

3. Experimental

General.—Bis(2-oxooxazolidin-3-yl)phosphinic chloride (BOP-Cl) was prepared as previously reported [22]. Precoated silica gel plates were used for TLC, and Kieselgel 60 H without gypsum was used for short column chromatography. NMR spectra were measured at 300 MHz (Me_4Si was used as internal standard for ^1H NMR). FAB mass spectra were obtained on a B–E type sector instrument, using a commercial gun operated with a neutral xenon beam of 8 keV.

Hexadecyl phosphorodichloridite (**3**) [7].—A warm solution of hexadecanol (**1**; 2.42 g, 0.01 mol) in dry MeCN (20 mL) was added dropwise during 5 min into a solution of PCl_3 (**2**; 6.12 mL, 0.07 mol) in dry MeCN (30 mL). The mixture was stirred at room temperature for 30 min. Evaporation of the solvent and of the excess of **2** afforded a quantitative yield of crude **3** which was used in the next step without further purification.

5′-O-(4-Nitrobenzoyl)thymidine (**8**).—BOP-Cl (3.41 g, 13.39 mmol) was added to a solution of 4-nitrobenzoic acid (1.46 g, 8.74 mmol) in 2:1 dry pyridine–CH_2Cl_2 (30 mL) and the mixture was stirred at room temperature for 15 min. A solution of thymidine (1.07 g, 4.42 mmol) in dry pyridine (10 mL) was then added and the resulting mixture was left under stirring at room temperature until a nearly complete conversion of the nucleoside was achieved (TLC, 9:1 $CHCl_3$–MeOH). Aq 10% $NaHCO_3$ (50 mL) was then added and the mixture was extracted with $CHCl_3$ (3 × 30 mL). The organic layers, evaporated to dryness (the pyridine present was removed by azeotropic distillation with toluene), afforded a residue which after crystallization from $CHCl_3$ afforded **8** (1.09 g, 68%): mp 180–181 °C; ^1H NMR ($CDCl_3$): δ 1.65 (s, 3 H, Me-5), 2.17 (ddd, 1 H, *J* 6.9, 3.8, and 13.6 Hz, H-2′a), 4.07 (ddd, 1 H, *J* 3.8, 5.9, and 4.0 Hz, H-4′), 4.41 (ddd, 1 H, *J* 4.5, 4.0, and 6.7 Hz, H-3′), 4.47 (dd, 1 H, *J* 4.5 Hz, HO-3′), 6.23 (dd, 1 H, *J* 6.7 and 6.9 Hz, H-1′), 7.45 (s, 1 H, H-6), 8.25 (d, 2 H, Ar), 8.29 (d, 2 H, Ar), 11.35 (s, 1 H, NH-3); FABMS [NBA (+)]: *m/z* 392 ($[M + H]^+$, 25.7%), 266 {$[(M - Thy) + H]^+$, 7.3%}, 127 ($[Thy + H]^+$, 100%). Anal. Calcd for $C_{17}H_{17}N_3O_8$: C, 52.13; H, 4.38; N, 10.74. Found: C, 52.19; H, 4.47; N, 10.83.

Silica gel short-column chromatography (99:1 $CHCl_3$–MeOH) of the residue obtained after evaporation of the mother liquor afforded further **8** (80 mg, 4.6%).

3′-O-Dimethoxytrityl-5′-O-(4-nitrobenzoyl)thymidine (**9**).—4,4′-Dimethoxytrityl chloride (Dmt-Cl, 1.34 g, 3.9 mmol) and 4-dimethylaminopyridine (DMAP; 52 mg, 0.43 mmol) were added to a solution of **8** (1.04 g, 2.66 mmol) in dry pyridine (13 mL) and

the mixture was stirred at room temperature for 49 h until complete conversion of the starting material was achieved (TLC, 19:1 $CHCl_3$–MeOH). MeOH (1 mL) was added and the solution was stirred for an additional 15 min. The crude solution was then partitioned between aq 10% $NaHCO_3$ (50 mL) and diethyl ether (3 × 30 mL). The organic layer, evaporated to dryness, afforded quantitative yields of **9** as a yellow solid, which was used for the next step without further purification; FABMS [NBA (+)]: *m/z* 694 ($[M + H]^+$, 10.2%), 303 (Dmt^+, 100%).

3′-O-Dimethoxytritylthymidine (**5**) [8].—2M NaOH solution (5.0 mL) was added to a solution of **9** in dioxane (5.0 mL) and the mixture was left under stirring at room temperature until complete conversion of the starting product was achieved (TLC, 19:1 $CHCl_3$–MeOH). After 7 min, the solution was partitioned between ethyl ether (2 × 15 mL) and water (40 mL). The organic layers were discarded and the aqueous layers were extracted with $CHCl_3$ (3 × 10 mL). The $CHCl_3$ layers were dried and evaporated to dryness to afford pure **5** (86% based on **8**); mp 169–173 °C; ^1H NMR ($CDCl_3$): δ 1.35 (m, 1 H, H-2′a), 1.68 (m, 1 H, H-2′b), 1.66 (s, 3 H, Me-5), 3.17 (dd, 1 H, $J_{5'a,4'}$ 2.5, $J_{5'a,5'b}$ 11.7 Hz, H-5′a), 3.34 (dd, 1 H, $J_{5'b,4'}$ 3.0 Hz, H-5′b), 3.73 (s, 6 H, 2 × MeO), 3.75 (m, 1 H, H-4′), 4.21 (m, 1 H, H-3′), 6.22 (dd, 1 H, $J_{1',2'a}$ 5.34, $J_{1',2'b}$ 8.95 Hz, H-1′), 6.93 (d, 4 H, Ar), 7.35 (m, 9 H, Ar), 7.45 (s, 1 H, H-6), 8.32 (s, 1 H, NH); FABMS [NBA (−)]: *m/z* 543 ($[M - H]^-$, 64.1%), 125 (Thy^-, 100%). The analytical sample was obtained by crystallization from benzene. Anal. Calcd for $C_{31}H_{32}N_2O_7$: C, 68.37; H, 5.92; N, 5.14. Found: C, 68.44; H, 5.85; N, 5.28.

Synthesis of 5′-O-dimethoxytritylthymidine 3′-(hexadecyl phosphate) (**6**) *and 3′-O-dimethoxytritylthymidine 5′-(hexadecyl phosphate)* (**7**).—*5′-O*-Dimethoxytritylthymidine (**4**, 1.0 mmol), or *3′-O*-dimethoxytritylthymidine (**5**, 1.0 mmol), in dry tetrahydrofuran (THF, 5.0 mL) was added during 15 min to a solution of **3** (1.0 mmol) in dry THF (1.0 mL) containing *sym*-collidine (4.0 mL) at −78 °C. After 15 min, the solution was poured into aq 10% $NaHCO_3$ (30 mL) and extracted with EtOAc (3 × 20 mL). The organic layers, dried with Na_2SO_4 and evaporated to dryness, afforded a pale-yellow foam (containing the expected H-phosphonate derivatives, as confirmed by FABMS). The crude material thus obtained was treated with I_2 (4.0 mmol) in 1:1:1 THF–pyridine–water (15 mL). After 15 min, the mixture was diluted with EtOAc (20 mL) and washed with brine (40 mL) containing traces of $Na_2S_2O_5$. The aqueous layers were extracted with EtOAc (3 × 20 mL) and the combined organic layers, dried and evaporated to dryness, afforded a pale-yellow foam. The nucleotides **6** and **7** were obtained in 65 and 61% overall yield, based on **4** and **5**, respectively, after short-column chromatography with 9:1 $CHCl_3$–MeOH. It was assumed that Na^+ was the counterion of the salts **6** and **7** [FABMS (+) evidence]. For **6**: ^{31}P NMR (Me_2SO, Me_2SO-d_6 lock): δ −3.97; FABMS [NBA (−)]: *m/z* 847 (M^-, 19.6%). For **7**: ^{31}P NMR (Me_2SO, Me_2SO-d_6 lock): δ −3.58; FABMS [NBA (−)]: *m/z* 847 (M^-, 22%).

Synthesis of **10b–14b**.—*General procedure.* Nucleotide **6** or **7** (1.0 mmol) was allowed to react at 45 °C with the appropriate nucleoside or with methyl α-D-glucopyranoside (1.2 mmol) in dry pyridine (15.0 mL) in presence of TPS-Cl (3.4 mmol) and 1-methylimidazole (7.0 mmol). The reaction was stopped after 2 h at room temperature. Usual workup afforded crude products which were purified by silica gel short-column chromatography. The dimethoxytrityl group was removed by conventional treatment

with 5% trichloroacetic acid in CH_2Cl_2 in 15 min and the deblocked products were purified by short-column chromatography.

Thymidine 3′-(5′-deoxythymidin-5′-yl hexadecyl phosphate) (**10b**, 80%); ^{31}P NMR (Me_2SO, Me_2SO-d_6 lock): δ −1.070, −1.05; FABMS [NBA (−)]: m/z 769 ([M − H]$^-$, 4.9%), 545 (68.5%), 321 (22%), 125 (100%).

Thymidine 5′-(5′-deoxythymidin-5′-yl hexadecyl phosphate) (**11b**, 79%); ^{31}P NMR (Me_2SO, Me_2SO-d_6 lock): δ −0.98; FABMS [NBA (−)]: m/z 769 ([M − H]$^-$, 6.0%), m/z 545 (71%), 321 (22.8%), 125 (100%).

Thymidine 5′-(3′,5′-dideoxythymidin-5′-yl hexadecyl phosphate) (**12b**, 78%); ^{31}P NMR (Me_2SO, Me_2SO-d_6 lock): δ 0.428, −0.424; FABMS [NBA (−)]: m/z 753 ([M − H]$^-$, 7.9%), 545 (68.5%), 529 (90%), 125 (100%).

Thymidine 3′-[hexadecyl (methyl 6-deoxy-α-D-glucopyranosid-6-yl) phosphate] (**13b**, 85%); ^{31}P NMR (Me_2SO, Me_2SO-d_6 lock): δ −0.21, −0.38; FABMS [NBA (−)]: m/z 721 ([M − H]$^-$, 5.9%), 545 (48.7%), 497 (89%), 125 (100%).

Thymidine 5′-[hexadecyl (methyl 6-deoxy-α-D-glucopyranosid-6-yl) phosphate] (**14b**, 83%); ^{31}P NMR (Me_2SO, MeSO-d_6 lock): δ −0.63, −0.78; FABMS [NBA (−)]: m/z 721 ([M − H]$^-$, 3.7%), 545 (57.4%), 497 (82%), 125 (100%)

4. Conclusions

The results presented above show that the formation of lipophilic phosphotriesters bearing nucleoside and carbohydrate groups does not require the extensive use of protecting groups on the interacting substrates. The key step in the above reported method is represented by the preparation of the phosphodiester synthons **6** and **7** by a straightforward procedure requiring a single purification step which poses no problems, due to the lipophilicity of the molecules. The regioselectivity observed in the formation of the triester bond could be extended to the introduction of unprotected nucleosides and pyranosides different from those here examined. Finally, the introduction of the hexadecyl group at an early stage of the synthesis prevents the alkylation of the nucleobase and facilitates all of the isolation procedures leading to the target compounds.

Acknowledgements

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