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Synthesis of phosphorofluoridates and phosphorofluoridothioates *via* the phosphoramidite approach

Wojciech Dabkowski* and Izabela Tworowska†

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, 90-363, Łódź, Sienkiewicza 112, Poland. E-mail: wdabkow@bilbo.cbmm.lodz.pl; Fax: 0-48-426847126; Tel: 0-48-42-6843120

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We present a very efficient synthetic procedure leading to the phosphorofluoridates RO-P(O)(OH)F **1** or phosphorofluoridothioates RO-P(S)(OH)F **2**, which is based on the intermediary of fluorophosphoramidites (RO)P(F)N^{i}Pr$_2$ **5** [R = 9-(hydroxyethyloxymethyl)guaninyl), 3′azido-3′deoxythymidinyl, thymidinyl, anhydrothymidinyl, cholesteryl, N^6-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenosinyl]. The activation of the amino group was performed with trimethylchlorosilane (TMCS).

Introduction

Molecular biologists have become interested in compounds of types **1** and **2** (Fig. 1) as convenient surrogates for phosphate monoesters.[1]

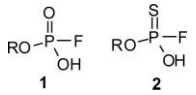

Fig. 1

Useful antiviral properties of this class of compounds have been disclosed.[2] The presence of a ^{19}F nucleus at the phosphorus center allows specific biological recognition by NMR spectroscopic techniques.[3] Anions derived from phosphorofluoridates RO-P(O)(OH)F **1**, in contrast to other phosphorohalidates, exhibit higher stability towards nucleophilic displacement of the halide group. This results from the strength of the P–F bond and the negative charge at the hydroxyl oxygen atom.[4] Wittmann was the first to prepare nucleoside phosphorofluoridate monoesters by reaction of nucleoside phosphate monoesters with 2,4-dinitrofluorobenzene.[1b] This method has been applied by other authors in nucleotide chemistry and sugar chemistry.[1c] Nucleoside 5′-*O*-phosphorofluoridates were obtained by coupling of the corresponding nucleoside with fluorophosphoric acid in the presence of *N*,*N*′-dicyclohexylocarbodiimide, 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) or mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole (MSNT).[1a,d] In the case of activation with TPSCl or MSNT, the reaction is likely to involve the intermediate formation of the phosphoro-sulfonic anhydride ArSO$_2$OP(O)(OH)F. Ribonucleotide analogues with a P–F linkage have been obtained by reaction of tetra-n-butylammonium fluoride (TBAF) with nucleoside-*O*-aryl-3-alkylthiophosphates.[1e] Most recently nucleoside phosphorofluoridate, phosphorofluoridothioate and phosphorofluoridodithioate monoesters were prepared *via* oxidation of the corresponding H-phosphonate and H-phosphonothioate monoesters with iodine in pyridine in the presence of trimethylchlorosilane, followed by reaction with triethylamine trishydrofluoride.[5] Phosphorofluoridate analogues of *myo*-inositol 1,4,5-tris(phosphate) were prepared by reaction of corresponding benzyl phosphates with 2-fluoro-1-methylpyridinium salt, the reaction proceeds with replacement of a benzyloxy group by a fluorine ligand.[6] 9-(Hydroxyethoxymethyl)guanine fluorophosphate was synthesized by the reaction of acyclovir with fluorophosphoric acid in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride.[1i] Stec *et al.* have found that the 5′-*O*- or 3′-*O*- protected thymidine 3′-*O*- or 5′-*O*-(2-thiono-1,3,2-oxathiaphospholanes) react with triethylammonium fluoride in the presence DBU leading to the appropriate phosphorofluoridothioates.[1h] All these methods have their advantages but are often inefficient or time-consuming and none of them has general application.

Results and discussion

We searched for a more general and efficient synthesis of phosphorofluoridates **1** and phosphorofluoridothioates **2** *via* the ″phosphoramidite approach″. The phosphoramidite approach is based on the relatively good stability of *N*,*N*-dialkylphosphoroamidites, which can be activated *in situ* by an appropriate activator, usually tetrazole. This paper is the continuation of our earlier studies on novel phosphitylating reagents containing *N*,*N*-dialkylamino and fluorine ligands. We have earlier reported a new approach to the synthesis of phosphorofluoridates **1** and phosphorofluoridothioates **2** that employs *O*-*tert*-butyl *N*,*N*-diisopropylfluorophosphoramidite [F-P(N^{i}Pr$_2$)O^{t}Bu] and *O*-(2-cyanoethyl) *N*,*N*-diisopropylfluorophosphoramidite [F-P(N^{i}Pr$_2$)OCH$_2$CH$_2$CN] reagents for phosphitylation of alcohols of biological interest.[7,8] In general, information about the chemistry and stereochemistry of organophosphorus esters containing the tricoordinate phosphorus atom attached directly to fluorine is scarce.[9] Known methods leading to this class of compounds require special techniques and are of limited value for application in bioorganic chemistry.[10] In this paper we describe an expedient synthetic method based on the displacement of aryloxy groups attached to the tricoordinate phosphorus center by the fluorine anion Fig. 2.

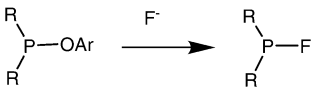

Fig. 2

The approach leading to compounds **1** and **2** involves phosphoramidite **3** as a key intermediate, which is readily available from commercial *N*,*N*-diisopropyldichlorophosphoramidite In the condensations described in Scheme 1 either trimethyl(4-nitro-phenoxy)silane or sodium 4-nitrophenolate can be employed. In both cases the phosphoramidite **3** is formed in over 90% yield.[11,12]

† Permanent address: Department of Biochemistry and Cell Biology, Rice University, MS-140, 6100 Main Street Houston, Texas 77251-1892, US.

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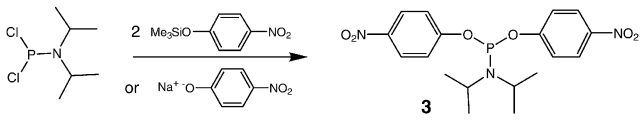
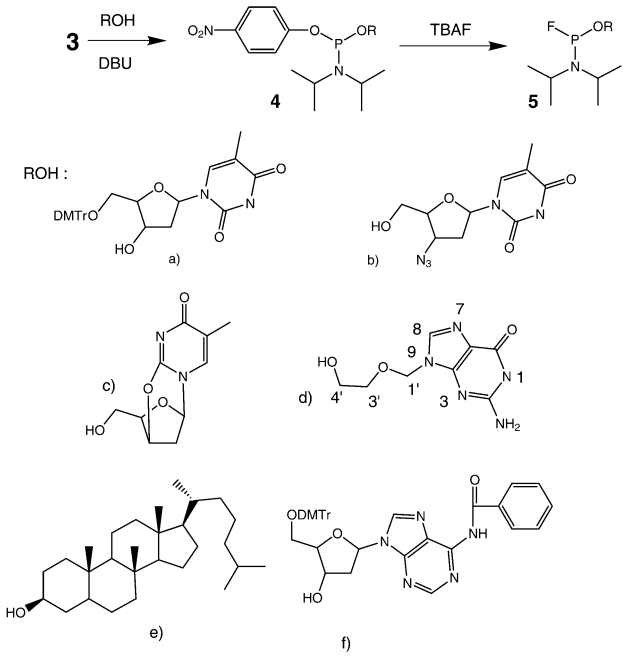


Scheme 1

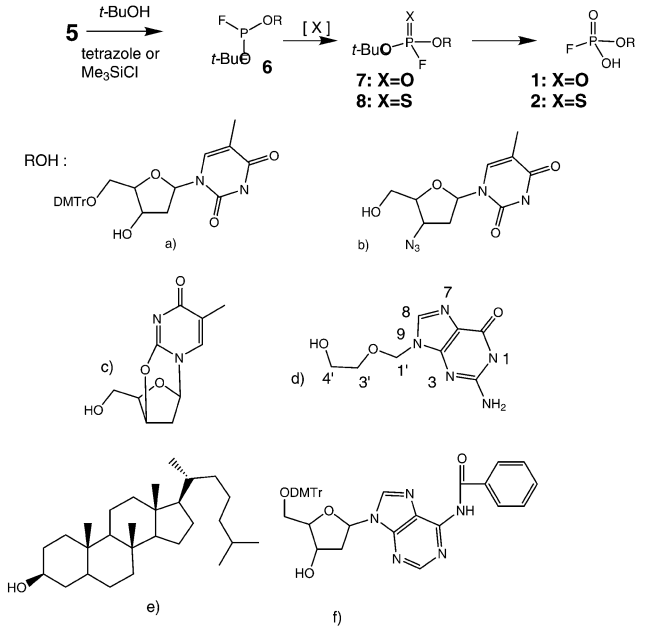
The phosphitylation of alcohols **a**, **b**, **c**, and **d** by the phosphoramidite **3** proceeds in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to the amidites **4** in excellent yield (Scheme 2).



Scheme 2

Treatment of aryl phosphoramidites **4** with a small excess of TBAF at room temperature afforded the phosphorofluoroamidites **5** (Scheme 2) in almost quantitative yield. The amidites **4** are formed as a (1 : 1) mixture of diastereomers, but their conversion into phosphorofluoroamidites **5** proceeds with some stereoselectivity. Their proportions remain unchanged for several days when the mixture is stored at room temperature. It is noteworthy that the observed stereochemical stability of fluorophosphoramidites **5** shows their low propensity to undergo nucleophilic fluorine–fluorine exchange. Another remarkable property of the amidites **4** and fluorophosphoramidites **5** is their stability. This desirable property allows them to be purified by chromatography on silica gel. The fluorophosphoramidites **5** can be stored unchanged at room temperature for several days. Their high stability in aqueous media is noticeably higher than that of other phosphorohalidites.[13] For example no visible changes are observed in aqueous acetonitrile (1 : 1 per volume) at room temperature within five hours. Coupling of amidites **5** with *tert*-butanol in the presence of tetrazole, or even better TMCS[14], leads to the phosphorofluoridites **6**. Subsequent oxidation by *tert*-butyl hydroperoxide or addition of elemental sulfur gives the corresponding phosphorofluoridates **7** or phosphorofluoridothioates **8** respectively (Scheme 3).

Removal of the protecting group at the phosphorus center takes place in the standard way. Thermal elimination of 2-methyl-1-propene from **7** or **8** gives the desired phosphorofluoridates **1** (X = O) or phosphorofluoridothioates **2** (X = S). All reactions described in Scheme 3 proceed fast at room temperature in solvents such as dichloromethane, acetonitrile or tetrahydrofuran. Yields of individual steps are very high. This synthesis can be performed as a one-flask procedure giving the final products as free acids or ammonium salts in over 90% yield.



Scheme 3

The phosphitylation described in Scheme 3, which is achieved by Me_3SiCl probably proceeds *via* formation of intermediate (RO)FPCl which reacts with alcohol R′OH to give ester (RO)(OR′)PF. This intermediate was clearly observed by ^{31}P NMR spectroscopy Fig. 3.

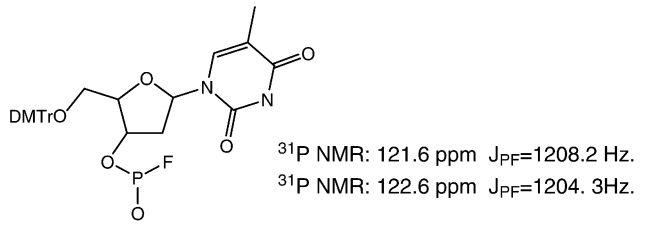
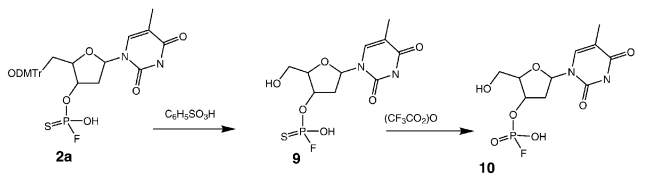


Fig. 3

The removal of the 4,4′-dimethoxytriphenylmethyl group (DMTr) by benzenesulfonic acid or other strong acids *e.g.* trichloroacetic acid proceeds without affecting the phosphoro–fluorine bond to give phosphorofluoridothioate **9** (Scheme 4). Compound **9** was converted by trifluoroacetyl anhydride into the corresponding oxo derivatives **10**[15,16] in almost quantitative yield (Scheme 4).



Scheme 4

Conclusion

In summary, the procedures we describe are completely general and simple. They make phosphorofluoridates and phosphorofluoridothioates derived from alcohols of biological interest readily available. This paper also illustrates the excellence of TMCS as an activator in phosphitylating procedures utilizing phosphoramidites.

Experimental

The solvents were reagent grade and were distilled and dried by conventional methods before use. *N*,*N*-Diisopropyldichlorophosphoramidite was synthesized according to procedure described previously.[17] Tetrazole was purified by sublimation at 115 °C/0.2 mmHg. Trimethylchlorosilane was purchased from Fluka. TBAF was purchased from Aldrich. Thin layer chromatography (TLC) was performed on silica gel plates 60F-254 (Merck). The products were purified by flash chromatography on silica gel 60 (Merck 0.063 mm, 230–400 mesh ASTM). NMR spectra were obtained on Bruker AC 200 and MSL 300 MHz spectrometers. δ-Values are reported in ppm relative to Me_4Si as standard for ^1H NMR (200.13 and 300.13 MHz) and relative to H_3PO_4 as external standard for ^{31}P NMR (80.96 and 121.49 MHz), as relative to $CFCl_3$ as external standard for ^{19}F NMR (188.15 MHz). The signals are expressed as s (singlet), d (doublet), t (triplet) or m (multiplet). Coupling constants (J) are in Hz.

Bis(*O*-4-nitrophenyl)-*N*,*N*-diisopropylphosphoramidite 3

(Route a) A solution of *N*,*N*-diisopropyldichlorophosphoramidite (10 mmol) in dry THF (10 ml) was added dropwise at room temperature under a nitrogen atmosphere to a solution of sodium 4-nitrophenolate (20 mmol) in dry THF (50 ml) with stirring for 2 h. The sodium chloride was removed by filtration. The filtrate was evaporated to dryness and **3** was purified by column chromatography (Et_2O–n-pentane–triethylamine 50 : 30 : 5 v \ v, R_f: 0.75) Yield 95%. (Route b) A solution of trimethyl(*p*-nitrophenoxy)silane (20 mmol) in dry THF (20 ml) was added to a solution of *N*,*N*-diisopropyldichlorophosphoramidite (10 mmol) in dry THF (20 ml) at room temperature. The mixture was stirred for 1 h, then trimethylchlorosilane and solvent were removed under reduced pressure to give pure phosphoramidite **3**. Yield 97%. δ_P (80.96 MHz, $CDCl_3$) 144.8; δ_H (200.13 MHz, $CDCl_3$) 1.01 (12H, d, J 6.8, $N[CH(CH_3)_2]_2$), 3.46–3.65 (2H, m, $N[CH(CH_3)_2]_2$), 6.75 (4H, d, J 9.13, Ph-H_{ortho}), 7.86 (4H, d, J 9.12, Ph-H_{meta}), mp 120–122 °C; pale yellow crystals, FAB(M + 1) calculated for $C_{18}H_{22}N_3O_6P$: 407.13, found: 408.50.

General procedure for the synthesis of compounds 4

A solution of the corresponding alcohol (1.0 mmol) and DBU (1.0 mmol) in dry acetonitrile (15 mL) was added dropwise at room temperature under a nitrogen atmosphere to a solution of bis(*O*-4-nitrophenyl)-*N*,*N*-diisopropylphosphoramidite **3** (1.1 mmol) in dry acetonitrile (15 mL) with stirring for 10 min. The mixture was evaporated to dryness and the resulting residue was purified by column chromatography using CH_2Cl_2 : $CH_3C(O)CH_3$ (10 : 3 v/v) as an eluent to give pure **4**.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl) *O*-(4-nitrophenyl)-*N*,*N*-diisopropylphosphoramidite 4a.** Yield 98%; δ_P (121.49 MHz, $CDCl_3$) 148.7, 147.8 (1 : 1); δ_H (300.13 MHz, $CDCl_3$) 1.18, 1.21 (12H, 2d, J 7.3, 6.9, CH_3 of isopropyl), 1.80 (3 H, s, 5-CH_3), 2.25 (1H, m, H-2′), 2.65 (1H, m, H-2″), 3.47 (4H, m, 5′, 5″ and CH of isopropyl), 3.67 (6H, s, OCH_3 of DMTr), 4.30 (1H, m, H-4′), 5.55 (1H, m, H-3′), 6.19, 6.43 (1H, dd, J 8.1, 7.1 H-1′), 6.72 (4H, 2d, J 8.6, 7.7 H-3, 3′, 5, 5′ of DMTr), 7.04–7.32 (11H, ArH of DMTr except for H-2, 2′, 6, 6′ and 4-NO_2-Ph-H_{ortho}), 7.58 (1H, s, H-6), 8.40 (2H, d, J 6.11 4-NO_2-Ph-H_{meta}); δ_C(57.47 MHz, $CDCl_3$) 11.00, 11.77 (5-CH_3), 22.22, 22.54 (J_{PNCC} 8.6, 6.1, CH_3 of isopropyl), 40.01 (C-2′), 45.11, 45.32 (J_{PNC} 7.0, 6.1, CH of isopropyl), 55.22 (OCH_3 of DMTr), 63.07, 63.39 (C-5′), 74.61, 75.34 (J_{POC} 6.1, 6.1 C-3′), 84.75 (C-1′), 85.45, 85.78 (J_{POCC} 4.8, 4.9, C-4′), 87.44, 87.59 (tert-C of DMTr), 111.76, 111.95 (C-5), 113.76, (C-3, 3′, 5, 5′ of DMTr), 118.02 (d, J 10.00, C-2 of 4-NO_2Ph), 120.98 (C-3 of 4-NO_2Ph), 128.11, 128.89, 130.32, 130.78, 131.66 (ArC of DMTr except for C-2, 2′, 6, 6′), 135.08, 135.34 (C-1, 1′ of DMTr), 135.45 (C-6), 139.01 (C-4 of 4-NO_2Ph), 144.89 (C-1″ of DMTr), 149.01, 149.89 (C-2), 150.32 (C-4), 158.78 (C-4, 4′ of DMTr), 162.31 (d, J 6.91, C-1 of 4-NO_2Ph), FAB(M + 1) calculated for $C_{43}H_{49}N_4O_{10}P$: 812.86, found: 813.00.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(4-nitrophenyl) *N*,*N*-diisopropylphosphoramidite 4b. Yield 92%; δ_P (121.49 MHz, $CDCl_3$) 147.3, 147.8 (1 : 1); δ_H (300.13 MHz, $CDCl_3$) 1.18, 1.21 (12H, 2d, J 7.3, 6.9, CH_3 of isopropyl), 1.92 (3H, d, J 1.1 5-CH_3), 2.25 (2H, m, H-2′, H-2″), 3.37–3.50 (4H, m, 5′, 5″ and CH of isopropyl), 4.14 (1H, m, H-4′), 4.45 (1H, m, H-3′), 6.19, 6.43 (1H, dd, J 8.1, 7.1 H-1′), 7.14 (2H, d, J 6.18, 4-NO_2-Ph-H_{ortho}), 7.90 (1H, s, H-6), 8.40 (2H, d, J 6.1, 4-NO_2-Ph-H_{meta}); δ_C (57.47 MHz, $CDCl_3$) 12.55, 12.77 (5-CH_3), 22.32, 22.54 (J_{PNCC} 8.6, 6.1, CH_3 of isopropyl), 37.41 (C-2′), 45.11, 46.38 (J_{PNC} 7.0, 6.1, CH of isopropyl), 63.07, 65.41 (C-5′), 76.50, 77.34 (J_{POC} 6.1, 6.1 C-3′), 84.75 (C-1′), 85.45, 85.78 (J_{POCC} 4.8, 4.9, C-4′), 111.76, 111.95 (C-5), 118.02 (d, J 10.00, C-2 of 4-NO_2Ph), 122.98 (C-3 of 4-NO_2Ph), 135.45 (C-6), 139.01 (C-4 of 4-NO_2Ph), 149.01, 149.89 (C-2), 152.13 (C-4), 162.31 (d, J 6.91, C-1 of 4-NO_2Ph), FAB(M + 1) calculated for $C_{22}H_{30}N_7O_7P$: 535.50, found: 536.21.

2,3′-Anhydrothymidin-5′-yl *O*-(4-nitrophenyl) *N*,*N*-diisopropylphosphoramidite 4c. Yield 98%; δ_P (121.49 MHz, $CDCl_3$) 150.3, 150.8 (1 : 1); δ_H (300.13 MHz, $CDCl_3$) 1.18, 1.21 (12H, 2d, J 7.3, 6.9, CH_3 of isopropyl), 1.80 (3H, s, 5-CH_3), 2.25 (1H, m, H-2′), 2.45 (1H, m, H-2″), 3.37–3.51 (4H, m, 5′, 5″ and CH of isopropyl), 4.49 (1H, m, H-4′), 5.55 (1H, m, H-3′), 6.19, 6.43 (1H, dd, J 8.1, 7.1, H-1′), 7.14 (2H, d, J 6.18, 4-NO_2-Ph-H_{ortho}), 7.58 (1H, s, H-6), 8.40 (2H, d, J 6.1,1 4-NO_2-Ph-H_{meta}); δ_C(57.47 MHz, $CDCl_3$) 12.76, 12.97 (5-CH_3), 20.42, 20.74 (J_{PNCC} 8.5, 5.9, CH_3 of isopropyl), 32.01 (C-2′), 42.00, 42.12 (J_{PNC} 7.0, 6.1, CH of isopropyl), 53.07, 53.39 (C-5′), 77.42, 77.84 (C-3′), 81.75 (C-4′), 85.91 (C-1′), 111.76, (C-5), 118.02 (d, J 10.00, C-2 of 4-NO_2Ph), 120.98 (C-3 of 4-NO_2Ph), 135.45 (C-6), 139.01 (C-4 of 4-NO_2Ph), 149.01, 149.89 (C-2), 162.31 (d, J 6.91, C-1 of 4-NO_2Ph), 171.09 (C-4) FAB(M + 1) calculated for $C_{22}H_{29}N_4O_7P$: 492.47, found: 493.55.

***O*-(9 [(2-Hydroxyethyloxy)methyl]guanin-4′-yl) *O*-(4-nitrophenyl) *N*,*N*-diisopropylphosphoramidite 4d.** Yield 85%; δ_P (121.49 MHz, $CDCl_3$) 145.0, 145.5 (1 : 1); δ_H (300.13 MHz, $CDCl_3$) 1.18, 1.21 (12 H, 2d, J 7.3, 6.9, CH_3 of isopropyl), 3.37–3.50 (2H, m, CH of isopropyl), 3.69 (2H, m, H-3′), 4.10 (2H, m, H-4′), 5.50 (2H, s, H-1′), 7.14 (2H, d, J 6.18, 4-NO_2-Ph-H_{ortho}), 7.90 (1H, s, H-8), 8.40 (2H, d, J 6.1,1 4-NO_2-Ph-H_{meta}); δ_C(57.47 MHz, $CDCl_3$) 24.87, 25.54 (J_{PNCC} 8.8, 6.9, CH_3 of isopropyl), 45.81, 46.08 (J_{PNC} 7.5, 6.1, CH of isopropyl), 65.1 (d, J_{COP} = 5.9 C-4′), 71.2 (d, J_{CCOP} = 7.4, C-3′). 73.5 (C-1′), 112.3 (C-5), 118.44 (d, J 10.00, C-2 of 4-NO_2Ph), 121.19 (C-3 of 4-NO_2Ph) 137.7 (C-8), 151.3 (C-4), 158.2 (C-2), 160.31 (d, J 6.91, C-1 of 4-NO_2Ph), 163.1 (C-6). FAB(M + 1) calculated for $C_{20}H_{28}N_7O_6P$: 493.46, found: 494.39.

***O*-Cholesteryl *O*-(4-nitrophenyl) *N*,*N*-diisopropylphosphoramidite 4e.** Yield 95%; δ_P (121.49 MHz, $CDCl_3$) 140.2, 143.1 (1 : 1); δ_H (300.13 MHz, $CDCl_3$) 1.18, 1.20 (12 H, 2d, J 7.0, 6.9, CH_3 of isopropyl), 3.37–3.50 (2H, m, CH of isopropyl), 0.59 (3 H, s, CH_3-18), 0.70 (3 H, d, J 6.4, CH_3-26), 0.77 (3 H, d, J 6.4, CH_3-27), 0.88 (3 H, d, J 6.4, CH_3-21), 0.92 (3 H, s, CH_3-19), 3.77 (1 H, d, J 8.0, H-7), 3.99 (1 H, b, H-3), 5.27 (1 H, s, H-6), 7.10 (2H, d, J 6.18, 4-NO_2-Ph-H_{ortho}), 7.67 (1H, s, H-8), 8.46 (2H, d, J 6.1,1 4-NO_2-Ph-H_{meta}); δ_C (75.47 MHz, $CDCl_3$) 13.70 (C-18), 17.66 (C-19), 24.44 (C-26), 26.66 (C-27), 51.43 (C-14), 57.79 (C-17), 72.09 (C-7), 77.93 (C-3), 118.44 (d, J 10.00, C-2 of 4-NO_2Ph), 121.19 (C-3 of 4-NO_2Ph), 125.88 (C-6), 142.98 (C-5), FAB(M + 1) calculated for $C_{39}H_{63}N_2O_4P$: 654.92, found: 654.01.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)-N^6-benzoyl-2′-deoxyadenosin-3′-yl) *O*-(4-nitrophenyl) *N*,*N*-diisopropylphosphoramidite 4f.** Yield 97%; δ_P (121.49 MHz, $CDCl_3$) 148.7, 147.8; δ_H

(300.13 MHz, $CDCl_3$) 1.25, 1.26 (12 H, 2d, *J* 6.9, 6.3 CH_3 of isopropyl), 2.83 (1 H, m, H-2′), 3.09 (1 H, m, H-2″), 3.43 and 3.61 (4 H, m, H-5′, 5″ and CH of isopropyl), 3.77 (6 H, s, 2 × OCH_3 of DMTr), 4.19–4.31 (1 H, m, H-4′), 5.22–5.31 (1 H, m, H-3′), 6.45–6.47(1 H, dd, *J* 5.5, *J* 8.7, H-1′), 6.71, 6.82 (4 H, 2d, *J* 8.6, 8.3, H-3, 3′, 5, 5′ of DMTr), 7.09–7.36 (7H, m, H-2″, 3″, 4″, 5″ 6″ of DMTr and 4-NO_2Ph-H_{ortho}), 7.39, 7.41 (4 H, 2d, *J* 8.6, 8.3, H-2, 2′, 6, 6′ of DMTr), 7.49 (2 H, m, H-5, 6 of benzoyl), 7.65 (1 H, tt, *J* 7.3, 1.3 H-4 of benzoyl), 8.09, 8.17 (1 H, 2s, H-2), 8. 29 (2 H, d, *J* 6.00, 4-NO_2Ph-H_{meta}), 8.78, 8.81 (1 H, 2s, H-8), 9.76 (1 H, bs, NH-6); δ_C (75.47 MHz, $CDCl_3$) 22.91, 22.98 (J_{PNCC} 7.3, 4.9, CH_3 of isopropyl), 40.01, 40.23 (C-2′), 45.31, 45.43 (J_{PNC} 6.1, 4.9, C*H* of isopropyl), 55.28 (OCH_3 of DMTr), 63.13, 63.45 (C-5′), 74.59, 74.91 (C-3′), 84.60, 84.71 (C-1′), 85.40, 85.71 (C-4′), 86.70, 86.81 (tert-C of DMTr), 113.19 (C-3, 3′, 5, 5′ of DMTr), 119.20 (d, *J* 10.52, C-2 of 4-NO_2Ph), 123.41 (C-5), 125.27 (C-3 of 4-NO_2Ph), 127.00, 130.33 (C-2″, 3″, 4″, 5″, 6″ of DMTr), 127.18 (C-2,6 of benzoyl), 128.19 (C-2, 2′, 6, 6′ of DMTr), 128.79 (C-3, 5 of benzoyl), 132.70 (C-4 of benzoyl), 133.73 (C-1 of benzoyl), 135.44, 135.51 (C-1, 1′ of DMTr), 136.00 (C-4 of 4-NO_2Ph), 141.43, 141.48 (C-2), 144.31 (C-1″ of DMTr), 149.67 (C-6), 152.72 (C-8), 158.24 (C-4, 4′ of DMTr), 160.72 (d, *J* 6.85, C-1 of 4-NO_2Ph), 164.00 (C=O of benzoyl); FAB(M + 1) calculated for $C_{50}H_{52}N_7O_9S$: 925.99 found: 926.43.

Preparation of fluorophosphoramidite compounds 5

To a solution of aryl phosphoramidite **4** (1.0 mmol) in dry THF (10 mL) was added TBAF (1.2 mmol) at room temperature. After 10 min. tetra-n-butylammonium 4-nitrophenolate was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was purified by column chromatography using CH_2Cl_2 : CH_3COCH_3 as an eluent to give pure fluorophosphoramidite **5**.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl)-*N*,*N*-diisopropylfluorophosphoramidite 5a.** Yield 97%; δ_P (121.49 MHz, $CDCl_3$) 156.0 (d, J_{PF} 1116.8), 155.8 (d, J_{PF} 1115.6); δ_F ($CDCl_3$) −76.35 (d, J_{PF} 1116.9), −77.0 (d, J_{PF} 1115.6); δ_H (300.13 MHz, $CDCl_3$) 1.20, 1.21 (12H, 2d, *J* 7.3, 6.9, CH_3 of isopropyl), 1.51 (3H, s, 5-CH_3), 2.45 (1H, m, H-2′), 2.68 (1H, m, H-2″), 3.30–3.51 (4H, m, 5′, 5″ and CH of isopropyl), 3.86 (6H, s, OCH_3 of DMTr), 4.28, 4.32 (1H, m, H-4′), 5.34 (1H, m, H-3′), 6.40, 6.51 (1H, dd, *J* 8.1, 7.1 H-1′), 6.82, 6.84 (4H, 2d, *J* 8.6, 7.7 H-3, 3′, 5, 5′ of DMTr), 7.10–7.45 (2H, m ArH of DMTr except for H-3, 3′,5, 5′); δ_C(57.47 MHz, $CDCl_3$) 11.60, 11.77 (5-CH_3), 22.58, 22.98 (J_{PNCC} 8.6, 6.1, CH_3 of isopropyl), 39.78 (C-2′), 45.20, 45.28 (J_{PNC} 7.0, 6.1, CH of isopropyl), 55.31 (OCH_3 of DMTr), 62.71, 63.39 (C-5′), 74.42, 75.12 (J_{POC} 6.1, 6.1 C-3′), 84.69 (C-1′), 85.23, 85.50 (J_{POCC} 4.8, 4.9, C-4′), 87.29, 87.41 (tert-C of DMTr), 111.65, 111.76 (C-5), 113.32, (C-3, 3′, 5, 5′ of DMTr), 128.00, 128.21, 130.32, 130.54, 131.61 (ArC of DMTr except for 3, 3′, 5, 5′-C), 135.10, 135.19 (C-1, 1′ of DMTr), 135.45 (C-6), 144.12 (C-1″ of DMTr), 149.30, 149.51 (C-2), 158.91 (C-4, 4′ of DMTr); FAB(M + 1) calculated for $C_{37}H_{45}FN_3O_7P$: 693.76, found: 693.87.

3′-Azido-3′-deoxythymidin-5′-yl *N*,*N*-diisopropylfluorophosphoramidite 5b. Yield 90%; δ_P (121.49 MHz, $CDCl_3$) 150.0 (d, J_{PF} 1116.8), 153.2 (d, J_{PF} 1110.6); δ_F ($CDCl_3$) −75.28 (d, J_{PF} 1116.9), −76.09 (d, J_{PF} 1135.6); δ_H (300.13 MHz, $CDCl_3$) 1.18, 1.21 (12H, 2d, *J* 7.3, 6.9, CH_3 of isopropyl), 1.80 (3H, d, *J* 1.1, 5-CH_3), 2.05 (2 H, m, H-2′, H-2″), 3.37–3.50 (4H, m, 5′, 5″ and CH of isopropyl), 4.24 (1H, m, H-4′), 4.35 (1H, m, H-3′), 7.26, 7.30 (1H, dd, *J* 8.1, 7.1 H-1′); δ_C(75.47 MHz, $CDCl_3$) 14.11, 14.34 (5-CH_3), 19.60, 19.98 (J_{PNCC} 8.5, 5.9, CH_3 of isopropyl), 37.27 (C-2′), 42.42, 42.54 (J_{PNC} 7.0, 6.1, CH of isopropyl), 64.54 (C-3′), 66.78 (J_{POC} 6.1, C-5′), 82.08 (C-1′), 84.88 (C-4′), 118.57 (C-5), 135.37 (C-6), 146.03 (C-4) 149.51, 149.63 (C-2) FAB(M + 1) calculated for $C_{16}H_{26}FN_6O_4P$: 416.40, found: 416.49.

2,3′-Anhydrothymidin-5′-yl *N*,*N*-diisopropylfluorophosphoramidite 5c. Yield 90%; δ_P (121.49 MHz, $CDCl_3$) 157.0 (d, J_{PF} 1110.8), 155.3 (d, J_{PF} 1118.6); δ_F ($CDCl_3$) −76.85 (d, J_{PF} 1110.9), −77.9 (d, J_{PF} 1117.0); δ_H (300.13 MHz, $CDCl_3$) 1.18, 1.21 (12H, 2d, *J* 7.3, 6.9, CH_3 of isopropyl), 1.80 (3H, s, 5-CH_3), 2.05 (1H, m, H-2′), 2.25 (1H, m, H-2″), 3.37–3.51 (4H, m, 5′, 5″ and CH of isopropyl), 4.32 (1H, m, H-4′), 6.00 (1H, m, H-3′), 6.34, 6.55 (1H, dd, *J* 8.1, 7.1 H-1′), 7.60 (1H, s, H-6); δ_C (57.47 MHz, $CDCl_3$) 13.23 (5-CH_3), 20.42, 20.74 (J_{PNCC} 8.5, 5.9, CH_3 of isopropyl), 32.91 (C-2′), 42.10, 42.22 (J_{PNC} 7.0, 6.1, CH of isopropyl), 61.86 (C-5′), 77.42, (C-3′), 81.99 (C-4′), 87.91 (C-1′), 116.76, (C-5), 136.95 (C-6), 150.01 (C-2), 172.09 (C-4); FAB(M + 1) calculated for $C_{16}H_{25}FN_3O_4P$: 373.37, found: 374.37.

***O*-(9 [(2-Hydroxyethyloxy)methyl]guanin-4′-yl) *N*,*N*-diisopropylfluorophosphoramidite 5d.** Yield 85%; δ_P (121.49 MHz, $CDCl_3$) 155.0 (d, J_{PF} 1118.2), 157.9 (d, J_{PF} 1119.9); δ_F($CDCl_3$) −78.1 (d, J_{PF} 1118.6), −79.0 (d, J_{PF} 1120.3); δ_H (300.13 MHz, $CDCl_3$) 1.18, 1.21 (12H, 2d, *J* 7.3, 6.9, CH_3 of isopropyl), 3.37–3.50 (2H, m, CH of isopropyl), 3.90 (2H, s, H-3′), 4.10 (2H, m, H-4′), 5.50 (2H, s, H-1′), δ_C(57.47 MHz, $CDCl_3$) 22.22, 23.32 (J_{PNCC} 8.0, 6.2, CH_3 of isopropyl), 48.11, 49.92 (J_{PNC} 7.3, 5.8, CH of isopropyl), 61.4 (d, J_{COP} = 5.9 C-4′), 72.2 (d, J_{CCOP} = 7.4, C-3′). 73.3 (C-1′), 116.1 (C-5), 139.0 (C-8), 150.1 (C-4), 156.1 (C-2), 161.8 (C-6); FAB(M + 1) calculated for $C_{14}H_{24}FN_6O_3P$: 374.36, found: 375.60.

***O*-Cholesteryl *N*,*N*-diisopropylfluorophosphoramidite 5e.** Yield 95%, δ_P (121.49 MHz, $CDCl_3$) 150.0 (d, J_{PF} 1110.2) 156.9 (d, J_{PF} 1115.9); δ_F($CDCl_3$) −77.0 (d, J_{PF} 1110.6) −79.8 (d, J_{PF} 1116.3); δ_H (300.13 MHz, $CDCl_3$) 1.20, 1.22 (12 H, 2d, *J* 7.0, 6.9, CH_3 of isopropyl), 3.37–3.50 (2H, m, CH of isopropyl), 0.60 (3 H, s, CH_3-18), 0.73 (3 H, d, *J* 6.4, CH_3-26), 0.75 (3 H, d, *J* 6.4, CH_3-27), 0.88 (3 H, d, *J* 6.4, CH_3-21), 0.90 (3 H, s, CH_3-19), 3.89 (1 H, d, *J* 8.0, H-7), 4.23 (1 H, b, H-3), 5.34 (1 H, s, H-6); δ_C (75.47 MHz, $CDCl_3$) 13.70 (C-18), 17.66 (C-19), 20.02, 21.31 (J_{PNCC} 8.0, 6.2, CH_3 of isopropyl), 24.44 (C-26), 26.66 (C-27), 49.91, 50.99 (J_{PNC} 7.3, 5.8, CH of isopropyl), 51.43 (C-14), 57.79 (C-17), 72.09 (C-7), 77.93 (C-3), 125.88 (C-6), 142.98 (C-5); FAB(M + 1) calculated for $C_{33}H_{59}FNOP$: 535.82, found: 536.01.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)-N^6-benzoyl-2′-deoxyadenosin-3′-yl) *N*,*N*-diisopropylfluorophosphoramidite 5f.** Yield 95%; δ_P (121.49 MHz, $CDCl_3$) 156.3 (d, J_{PF} 1114.7), 155.6 (d, J_{PF} 1118.6); δ_F ($CDCl_3$) −76.25 (d, J_{PF} 1114.5), −77.35 (d, J_{PF} 1118.9); δ_H (300.13 MHz, $CDCl_3$), 1.22, 1.25 (12 H, 2d, *J* 7.04, 7.33, CH_3 of isopropyl), 2.80 (1 H, m, H-2′), 3.17 (1 H, m, H-2″), 3.31 and 3.56 (4 H, m, H-5′ 5″ and CH of isopropyl), 3.80 (6 H, s, 2 × OCH_3 of DMTr), 4.20–4.39 (1 H, m, H-4′), 5.19–5.30 (1 H, m, H-3′), 6.40–6.45(1 H, dd, *J* 5.5, 8.7, H-1′), 6.68, 6.77 (4 H, 2d, *J* 8.6, 8.3, H-3, 3′, 5, 5′ of DMTr), 7.06–7.31 (5 H, m, H-2″, 3″, 4″, 5″, 6″ of DMTr), 7.31, 7.39 (4 H, 2d, *J* 8.6, 8.3, H-2, 2′, 6, 6′ of DMTr), 7.67 (2 H, m, H-5, 6 of benzoyl), 7.61 (1 H, tt, *J* 7.3 1.3, H-4 of benzoyl), 8.11, 8.17 (1 H, 2s, H-2), 8.72, 8.76 (1 H, 2s, H-8), 9.99 (1 H, bs, NH-6); δ_C(75.47 MHz, $CDCl_3$) 22.22, 22.47 (CH_3 of isopropyl), 38.29, 39.53 (C-2′), 44.91, 46.01 (CH of isopropyl), 55.19 (OCH_3 of DMTr), 63.22, 63.45 (C-5′), 74.82, 74.91 (C-3′), 84.43, 84.74 (C-1′), 85.89, 85.99 (C-4′), 113.34 (C-3, 3′, 5, 5′ of DMTr), 123.51 (C-5), 127.34, 130.69 (C-2″, 3″, 4″, 5″, 6″ of DMTr), 127.65 (C-2,6 of benzoyl), 128.10 (C-2, 2′, 6, 6′ of DMTr), 128.90 (C-3, 5 of benzoyl), 132.43 (C-4 of benzoyl), 133.71 (C-1 of benzoyl), 135.40, 135.49 (C-1, 1′ of DMTr), 141.33, 141.56 (C-2), 144.63 (C-1″ of DMTr), 149.72 (C-6), 152.90 (C-8), 158.36 (C-4, 4′ of DMTr), 164.87 (C=O of benzoyl); FAB(M + 1) calculated for $C_{44}H_{48}FN_6O_6P$: 806.88 found: 807.21.

General procedure for the synthesis of *tert*-butylphosphorofluoridites 6 (Route a)

To a mixture of *N*,*N*-diisopropylfluorophosphoramidite **5** (1.0 mmol) and tetrazole (1.1 mmol) in dry THF (30 ml) was added a solution of *tert*-butanol (1.0 mmol) in dry THF (20 ml). The reaction mixture was stirred for 3 h and controlled by TLC. *N*,*N*-Diisopropylammonium tetrazolide was removed by filtration. The filtrate was concentrated *in vacuo* and was chromatographed on silica gel, using a gradient of 0–10% $CH_3C(O)CH_3$ in CH_2Cl_2 as an eluent to give pure 6. (Route b): To a solution of **5** (1.0 mmol) in dry THF (20 mL) and *tert*-butanol (1.0 mmol) in dry THF (20 ml) was added a solution of trimethylchlorosilane (3 mmol) in THF (10 ml). After 1 h the mixture was evaporated *in vacuo* and the residue was purified by column chromatography, using CH_2Cl_2 : $CH_3C(O)CH_3$ (10 : 1 v/v) as an eluent, to give pure **6**.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl) *O*-(*tert*-butyl) phosphorofluoridite 6a.** Yield 90%, δ_P (121.49 MHz, $CDCl_3$) 131.75 (d, J_{PF} 1210.5), 133.25 (d, J_{PF} 1204.0); δ_F −51.35 (d, J_{PF} 1210.5), −52.00 (d, J_{PF} 1204.8); δ_H (300.13 MHz, $CDCl_3$) 1.33 (9H, s, $C(CH_3)_3$), 1.41 (3H, s, 5-CH_3), 2.40 (1H, m, H-2′), 2.63 (1H, m, H-2″), 3.45–3.59 (2H, m, H-5′, 5″), 3.79 (6H, s, OCH_3 of DMTr), 4.28–4.42 (2H, m, H-4′), 5.24 (1H, m, H-3′), 6.45, (1H, dd, *J* 6.4, 6.4, H-1′), 6.84, 6.88 (4H, 2d, *J* 8.6, 7.7, H-3, 3′, 5, 5′ of DMTr), 7.18–7.40 (9H, ArH of DMTr); δ_C (75.47 MHz, $CDCl_3$) 11.58 (5-CH_3), 32.9 (C(CH_3)$_3$), 35.88 (C-2′), 53.39 (OCH_3 of DMTr), 63.26 (C-5′), 69.9 ($C(CH_3)_3$), 71.37, 72.03 (J_{POC} 6.1, 6.1, C-3′), 84.70 (C-1′), 85.14, 85.43 (J_{POCC} 4.8, 4.9, C-4′), 87.30, 87.36 (tert-C of DMTr), 111.57, 111.66 (C-5), 113.39, (C-3, 3′, 5, 5′ of DMTr), 128.06, 128.17, 130.19, 130.50, 131.42 (DMTr), 135.10, 135.19 (C-1,1′ of DMTr), 135.27 (C-6) 144.04 (C-1″ of DMTr), 149.35, 149.45 (C-2), 158.87 (C-4, 4′ of DMTr); FAB(M + 1) calculated for $C_{35}H_{40}FN_2O_8P$: 666.69, found: 666.70.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(tert-butyl) phosphorofluoridite 6b. Yield 89%; δ_P (121.49 MHz, $CDCl_3$) 136.0 (d, J_{PF} 1210.8), 138.8 (d, J_{PF} 1215.6); δ_F ($CDCl_3$) −56.35 (d, J_{PF} 1210.9), −57.70 (d, J_{PF} 1220.6); δ_H (300.13 MHz, $CDCl_3$), 1.92 (3H, d, *J* 1.1 5-CH_3), 2.25 (2H, m, H-2′, H-2″), 3.37–3.50 (2H, m, 5′, 5″), 4.14 (1H, m, H-4′), 4.45 (1H, m, H-3′), 7.26, 7.29 (1H, dd, *J* 8.1, 7.1 H-1′); δ_C(75.47 MHz, $CDCl_3$) 12.37, 12.89 (5-CH_3), 30.19 (C(CH_3)$_3$), .37.27 (C-2′), 64.00 (C-3′), 66.64 (J_{POC} 6.1, C-5′), 71.23 ($C(CH_3)_3$), 82.08 (C-1′), 84.88 (C-4′), 111.43 (C-5), 135.37 (C-6), 151.83 (C-4) 152.91, 153.33 (C-2); FAB(M + 1) calculated for $C_{14}H_{21}FN_5O_5P$: 389.33, found: 384.72.

2,3′-Anhydrothymidin-5′-yl *O*-(*tert*-butyl) phosphorofluoridite 6c. Yield 98%; δ_P (121.49 MHz, $CDCl_3$) 130.55 (d, J_{PF} 1209.5), 130.95 (d, J_{PF} 1214.0); δ_F −50.25 (d, J_{PF} 1210.9), −51.11 (d, J_{PF} 1214.8); δ_H (300.13 MHz, $CDCl_3$) 1.53 (9H, s, $C(CH_3)_3$) 2.00 (3 H, s, 5-CH_3), 2.34 (1H, m, H-2′), 2.97 (1H, m, H-2″), 3.33–3.53 (2H, m, 5′, 5″), 4.74 (1H, m, H-4′), 5.61 (1H, m, H-3′), 6.27, 6.42 (1H, dd, *J* 8.1, 7.1 H-1′), 7.60 (1H, s, H-6); δ_C(57.47 MHz, $CDCl_3$) 11.23 (5-CH_3), 30.19 (C(CH_3)$_3$), 33.91 (C-2′), 60.86 (C-5′), 65.39 ($C(CH_3)_3$), 77.42, (C-3′), 82.19 (C-4′), 86.61 (C-1′), 116.06, (C-5), 134.55 (C-6), 153.61 (C-2), 170.79 (C-4); FAB(M + 1) calculated for $C_{14}H_{20}FN_2O_5P$: 346.30, found: 347.32.

***O*-(9-[(2-Hydroxyethyloxy)methyl]guanin-4′-yl) *O*-(*tert*-butyl) phosphorofluoridite 6d.** Yield 85%; δ_P (121.49 MHz, $CDCl_3$) 131.0 (d, J_{PF} 1218.8), 137.3 (d, J_{PF} 1220.3); δ_F($CDCl_3$) −48.9 (d, J_{PF} 1219.6), −50.0 (d, J_{PF} 1222.3); δ_H (300.13 MHz, $CDCl_3$) 1.43 (9 H, s, $C(CH_3)_3$) 3.50 (2H, m, H-3′), 4.50 (2H, m, H-4′), 5.50 (2H, s, H-1′), 8.20 (1H, s, H-8), δ_C (57.47 MHz, $CDCl_3$) 30.12 (C(CH_3)$_3$) 57.4 (d, J_{COP} = 5.9 C-4′), 65.22 ($C(CH_3)_3$), 72.1 (d, J_{CCOP} = 7.4, C-3′), 77.3 (C-1′), 116.3 (C-5), 134.2 (C-8), 151.3 (C-4), 157.3 (C-2), 159.1 (C-6); FAB(M + 1) calculated for $C_{12}H_{19}FN_5O_4P$: 347.29, found: 348.31.

***O*-Cholesteryl *O*-(*tert*-butyl) phosphorofluoridite 6e.** Yield 95%, δ_P (121.49 MHz, $CDCl_3$) 130.9 (d, J_{PF} 1212.3 Hz), 131.1 (d, J_{PF} 1220.5 Hz); δ_F −55.95 (d, J_{PF} 1212.5 Hz), −55.90 (d, J_{PF} 1220.8 Hz); δ_H (300.13 MHz, $CDCl_3$) 0.61 (3 H, s, CH_3-18), 0.69 (3 H, d, *J* 6.4, CH_3-26), 0.77, (3 H, d, *J* 6.4, CH_3-27), 0.85 (3 H, d, *J* 6.4, CH_3-21), 0.92 (3 H, s, CH_3-19), 1.30 [9 H, s, $C(CH_3)_3$], 3.77 (1 H, d, *J* 8.0, H-7), 3.91 (1 H, b, H-3), 5.20 (1 H, s, H-6); δ_C (75.47 MHz, $CDCl_3$) 11.72 (C-18), 18.56 (C-19), 22.44 (C-26), 22.66 (C-27), 32.99 [C(CH_3)$_3$], 55.43 (C-14), 55.78 (C-17), 69.71 [$C(CH_3)_3$], 72.99 (C-7), 77.43 (C-3), 125.88 (C-6), 142.98 (C-5); FAB(M + 1) calculated for $C_{31}H_{54}FO_2P$: 508.75, found: 507.92.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl) *O*-(*tert*-butyl) phosphorofluoridite 6f.** Yield 90%, δ_P (121.49 MHz, $CDCl_3$) 132.2 (d, J_{PF} 1210.6 Hz), 131.1 (J_{PF} 1216.0 Hz); δ_F −50.55 (d, J_{PF} 1210.9 Hz), −51.10 (d, J_{PF} 1204.7); δ_H (300.13 MHz, $CDCl_3$) 1.30 (9 H, s, $C(CH_3)_3$), 2.55 (1 H, m, H-2′), 3.07 (1 H, m, H-2″) 3.44 and 3.54 (2 H, m, H-5′5″), 3.77 (6 H, s, 2 × OCH_3 of DMTr), 4.20–4.31 (1 H, m, H-4′), 5.25–5.36 (1 H, m, H-3′), 6.44 (1 H, dd, *J* 7.1 Hz, H-1′), 6.77, 6.80 (4 H, 2d *J* 8.6, *J* 8.3 Hz, H-3, 3′, 5, 5′ of DMTr), 7.11–7.30 (5 H, m, H-2″, 3″, 4″, 5″, 6″ of DMTr), 7.30, 7.40 (4 H, 2d, *J* 8.6 and 8.3 Hz, H-2, 2′, 6, 6′ of DMTr), 7.55 (2 H, m, H-5, 6 of benzoyl), 7.60 (1 H, tt, *J* 7.3 and 1.3 Hz, H-4 of benzoyl), 8.15, 8.18 (1 H, 2s, H-2), 8.71, 8.74 (1 H, 2s, H-8), 9.11 (1 H, bs, NH-6); δ_C(75.47 MHz, $CDCl_3$) 32.5 (C(CH_3)$_3$) 39.20, 39.40 (C-2′), 55.22 (OCH_3 of DMTr), 63.09, 63.30 (C-5′), 65.29 ($C(CH_3)_3$), 74.60, 74.81 (C-3′), 84.60, 84.65 (C-1′), 85.50, 85.66 (C-4′), 86.75, 86.79 (tert-C of DMTr), 113.27 (C-3, 3′, 5, 5′ of DMTr), 123.38 (C-5), 127.11, 130.03 (C-2,″ 3,″ 4,″ 5,″ 6″ of DMTr), 127.67 (C-2,6 of benzoyl), 128.17 (C-2, 2′, 6, 6′ of DMTr), 128.86 (C-3, 5 of benzoyl), 132.76 (C-4 of benzoyl), 133.70 (C-1 of benzoyl) 135.39, 135.45 (C-1, 1′ of DMTr), 141.31, 141.47 (C-2), 144.30 (C-1″ of DMTr), 149.60 (C-6), 153.11 (C-8), 158.55 (C-4, 4′ of DMTr), 164.47 (C=O of benzoyl); FAB(M + 1) calculated for $C_{42}H_{43}FN_5O_7P$: 779.81 found: 779.11.

General procedure for the synthesis of compounds 7

Compound **6** (1.0 mmol) was dissolved in dry THF (25 ml) and a solution of *tert*-butyl hydroperoxide (1.1 mmol) was added. The mixture was stirred for 1 h at rt, then was concentrated *in vacuo* and purified by column chromatography, using CH_2Cl_2 : $CH_3C(O)CH_3$ (10 : 1 v/v) as an eluent to give **7**.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl) *O*-(*tert*-butyl) phosphorofluoridate 7a.** Yield 98%, δ_P (121.49 MHz, $CDCl_3$) −10.95 (d, J_{PF} 985.2), −11.3 (d, J_{PF} 985.4); δ_F ($CDCl_3$) −77.2 (d, J_{PF} 985.0), −77,3 (d, J_{PF} 985.4); δ_H (300.13 MHz, $CDCl_3$) 1.53 (9H, s, $C(CH_3)_3$), 1.91 (3H, s, 5-CH_3), 2.14 (1H, m, H-2′), 2.36 (1 H, m, H-2″), 3.35–3.69 (2H, m, H-5′, 5″), 3.87 (6H, s, OCH_3 of DMTr), 4.18–4.34 (2H, m, H-4′), 5.22 (1H, m, H-3′), 6.55, (1H, dd, *J* 6.4, 6.4, H-1′), 6.88, 6.99 (4H, 2d, *J* 8.6, 7.7, H-3, 3′, 5, 5′ of DMTr), 7.11–7.41 (9H, ArH of DMTr); δ_C (75.47 MHz, $CDCl_3$) 11.78 (5-CH_3), 32.89 (C(CH_3)$_3$), 35.81 (C-2′), 53.43 (OCH_3 of DMTr), 63.66 (C-5′), 69.19 ($C(CH_3)_3$), 71.73, 72.03 (J_{POC} 6.1, 6.1, C-3′), 84.37 (C-1′), 85.18, 85.43 (J_{POCC} 4.8, 4.9, C-4′), 87.13, 87.63 (tert-C of DMTr), 111.51, 111.69 (C-5), 113.39, (C-3, 3′, 5, 5′ of DMTr), 128.16, 128.23, 130.21, 130.52, 131.24 (DMTr), 135.14, 135.26 (C-1,1′ of DMTr), 135.71 (C-6), 144.24 (C-1″ of DMTr), 149.22, 149.56 (C-2), 158.38 (C-4, 4′ of DMTr), FAB(M + 1) calculated for $C_{35}H_{40}FN_2O_9P$: 682.69, found: 681.87.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(*tert*-butyl) phosphorofluoridate 7b. Yield 92%; δ_P (121.49 MHz, $CDCl_3$) −9.78 (d, J_{PF} 999.5), −10.99 (d, J_{PF} 1000.0); δ_F −50.55 (d, J_{PF} 1002.5), −51.00 (d, J_{PF} 1002.0); δ_H (300.13 MHz, $CDCl_3$) 1.45 (9H, s, $C(CH_3)_3$), 1.92 (3H, d, *J* 1.1 5-CH_3), 2.25 (2H, m, H-2′, H-2″), 3.37–3.50 (2H, m, 5′, 5″), 4.14 (1H, m, H-4′), 4.45 (1H, m, H-3′), 7.26,7.29 (1H, dd, *J* 8.1, 7.1 H-1′); δ_C(75.47 MHz, $CDCl_3$) 15.42, 15.97 (5-CH_3), 30.19 (C(CH_3)$_3$), 32.56 (C-2′), 64.22 (C-3′), 69.22 (J_{POC} 6.9,

C-5′), 71.63 ($C(CH_3)_3$) 81.78 (C-1′), 84.88 (C-4′), 101.43 (C-5), 139.22 (C-6), 150.03 (C-4) 152.91 (C-2); FAB(M + 1) calculated for $C_{14}H_{21}FN_5O_6P$: 405.33, found: 405.89.

2,3′-Anhydrothymidin-5′-yl *O*-(*tert*-butyl) phosphorofluoridate 7c. Yield 82%; δ_P (121.49 MHz, $CDCl_3$) −11.00 (d, J_{PF} 992.5), −11.99 (d, J_{PF} 1001.0); δ_F −50.15 (d, J_{PF} 1000.1), −51.21 (d, J_{PF} 1002.0); δ_H (300.13 MHz, $CDCl_3$) 1.50 (9H, s, $C(CH_3)_3$), 1.89 (3H, s, 5-CH_3), 2.25 (1H, m, H-2′), 2.45 (1H, m, H-2″), 3.37–3.51 (2H, m, 5′, 5″), 4.49 (1H, m, H-4′), 5.55 (1H, m, H-3′), 6.49, 6.53 (1H, dd, *J* 8.1, 7.1 H-1′), 7.65 (1H, s, H-6); δ_C (57.47 MHz, $CDCl_3$) 16.23 (5-CH_3), 32.67 ($C(CH_3)_3$) 39.93 (C-2′), 64.16 (C-5′), 65.39 ($C(CH_3)_3$), 74.02, (C-3′), 81.19 (C-4′), 88.61 (C-1′), 120.06 (C-5), 139.55 (C-6), 161.61 (C-2), 169.99 (C-4); FAB(M + 1) calculated for $C_{14}H_{20}FN_2O_6P$: 362.30, found: 363.39.

***O*-(9 [(2-Hydroxyethyloxy)methyl]guanin-4′-yl) *O*-(*tert*-butyl) phosphorofluoridate 7d.** Yield 85%; δ_P (121.49 MHz, $CDCl_3$) −8.43 (d, J_{PF} 985.2), −10.5 (d, J_{PF} 991.4); δ_F ($CDCl_3$) −77.55 (d, J_{PF} 983.8), −78.96 (d, J_{PF} 998.2); δ_H (300.13 MHz, $CDCl_3$) 1.41 (9 H, s, $C(CH_3)_3$), 3.51 (2H, m, H-3′), 4.61 (2H, m, H-4′), 5.50 (2H, s, H-1′), 8.25 (1H, s, H-8); δ_C(57.47 MHz, $CDCl_3$) 32.9 ($C(CH_3)_3$), 59.2 (d, J_{COP} = 5.9 C-4′), 69.27 ($C(CH_3)_3$), 71.6 (d, J_{CCOP} 7.4, C-3′), 74.5 (C-1′), 111.3 (C-5), 139.7 (C-8), 151.3 (C-4), 153.9 (C-2), 163.1 (C-6); FAB(M + 1) calculated for $C_{12}H_{19}FN_5O_5P$: 363.29, found: 364.40.

***O*-Cholesteryl *O*-(*tert*-butyl) phosphorofluoridate 7e.** Yield 96%, δ_P (121.49 MHz, $CDCl_3$) −8.65 (d, J_{PF} 971.8); δ_F −72.30 (d, J_{PF} 971.2), −72.35 (d, J_{PF} 971.0); δ_H (300.13 MHz, $CDCl_3$) 0.56 (3 H, s, CH_3-18), 0.66 (3 H, d, *J* 6.4, CH_3-26), 0.75 (3 H, d, *J* 6.4, CH_3-27), 0.81 (3 H, d, *J* 6.4, CH_3-21), 0.97 (3 H, s, CH_3-19), 1.34 [9 H, s, $C(CH_3)_3$], 3.57 (1 H, d, *J* 8.0, H-7), 3.89 (1 H, b, H-3), 5.28 (1 H, s, H-6); δ_C ($CDCl_3$) 11.67 (C-18), 18.46 (C-19), 22.94 (C-26), 23.26 (C-27), 33.09 [$C(CH_3)_3$] 55.43 (C-14), 55.38 (C-17), 69.91 [$C(CH_3)_3$], 73.01 (C-7), 77.48 (C-3), 125.58 (C-6), 143.18 (C-5); FAB(M + 1) calculated for $C_{31}H_{54}FO_3P$: 524.75, found: 525.72.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)-N^6-benzoyl-2′-deoxyadenosin-3′-yl) *O*-(*tert*-butyl) phosphorofluoridate 7f.** Yield 91%, δ_P (121.49 MHz, $CDCl_3$) −10.50 (d, J_{PF} 985.6), −11.00 (d, J_{PF} 989.9); δ_F −76.9 (d, J_{PF} 987.9), −77.8 (d, J_{PF} 990.1); δ_H (300.13 MHz, $CDCl_3$) 1.57 (9 H, s, $C(CH_3)_3$), 2.35 (1 H, m, H-2′), 3.17 (1 H, m, H-2″), 3.24 and 3.50 (2 H, m, H-5′5″), 3.81 (6 H, s, 2 × OCH_3 of DMTr), 4.29–4.33 (1 H, m, H-4′), 5.12–5.60 (1 H, m, H-3′), 6.24 (1 H, dd, *J* 7.1 Hz, H-1′), 6.65, 6.79 (4 H, 2d, *J* 8.6, *J* 8.3 Hz, H-3, 3′, 5, 5′ of DMTr), 7.11–7.32 (5 H, m, H-2″, 3″, 4″, 5″, 6″ of DMTr), 7.36, 7.44 (4 H, 2d, *J* 8.6, and 8.3 Hz, H-2, 2′, 6, 6′ of DMTr), 7.57 (2 H, m, H-5, 6 of benzoyl), 7.62 (1 H, tt, *J* 7.3 and 1.3 Hz, H-4 of benzoyl), 8.11, 8.20 (1 H, 2s, H-2), 8.77, 8.79 (1 H, 2s, H-8), 9.34 (1 H, bs, NH-6); δ_C($CDCl_3$), 33.5 ($C(CH_3)_3$) 39.42, 39.49 (C-2′), 55.62 (OCH_3 of DMTr), 63.29, 63.43 (C-5′), 65.59 ($C(CH_3)_3$), 74.67, 74.89 (C-3′), 84.76, 84.86 (C-1′), 85.55, 85.96 (C-4′), 86.87, 86.97 (tert-C of DMTr), 113.32 (C-3, 3′, 5, 5′ of DMTr), 123.43 (C-5), 127.31, 130.33 (C-2″, 3″, 4″, 5″, 6″ of DMTr), 127.87 (C-2,6 of benzoyl), 128.31 (C-2, 2′, 6, 6′ of DMTr), 128.86 (C-3, 5 of benzoyl), 132.97 (C-4 of benzoyl), 133.77 (C-1 of benzoyl) 135.53, 135.85 (C-1, 1′ of DMTr), 141.43, 141.64 (C-2), 144.37 (C-1″ of DMTr), 149.76 (C-6), 153.67 (C-8), 158.07 (C-4, 4′ of DMTr), 160.92 (C=O of benzoyl); FAB(M + 1) calculated for $C_{42}H_{43}FN_5O_8P$: 795.81 found: 795.68.

General procedure for the synthesis of compounds 8

To a solution of **6** (1.0 mmol) in dry THF (15 ml) was added the saturated solution of sulfur in $(C_3H_7)_2NH$ (5 ml) and stirred for 2 h at rt. Crude product **8** was chromatographed on silica gel, using a gradient of 0–10% $CH_3C(O)CH_3$ in CH_2Cl_2 as an eluent.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl) *O*-(*tert*-butyl) thiophosphorofluoridate 8a.** Yield 97%, δ_P (121.49 MHz, $CDCl_3$) 53.45, 53.10 (2d, J_{PF} 1082.2, J_{PF} 1081.9); δ_F ($CDCl_3$) −34.6, −35.2 (2d, J_{PF} 1082.7, J_{PF} 1085.9); δ_H (300.13 MHz, $CDCl_3$) 1.40 (9H, s, $C(CH_3)_3$), 1.51 (3H, s, 5-CH_3), 2.34 (1H, m, H-2′), 2.63 (1H, m, H-2″), 3.40–3.51 (2H, m, H-5′, 5″), 3.71 (6H, s, OCH_3 of DMTr), 4.22–4.44 (2H, m, H-4′), 5.52 (1H, m, H-3′), 6.14 (1 H, dd, *J* 6.4, 6.4, H-1′), 6.76, 6.98 (4H, 2d, *J* 8.6, 7.7, H-3, 3′, 5, 5′ of DMTr), 7.13–7.39 (9H, ArH of DMTr); δ_C (75.47 MHz, $CDCl_3$) 11.51 (5-CH_3), 32.99 ($C(CH_3)_3$), 35.58 (C-2′), 53.41 (OCH_3 of DMTr), 63.31 (C-5′), 69.18 ($C(CH_3)_3$), 71.31, 72.23 (J_{POC} 6.1, 6.1, C-3′), 84.67 (C-1′), 85.21, 85.43 (J_{POCC} 4.8, 4.9, C-4′), 87.13, 87.26 (tert-C of DMTr), 111.60, 111.76 (C-5), 113.41 (C-3, 3′, 5, 5′ of DMTr), 128.11, 128.23, 130.20, 130.49, 131.42 (DMTr), 135.09, 135.12 (C-1, 1′ of DMTr), 135.32 (C-6), 144.12 (C-1″ of DMTr), 149.45, 149.55 (C-2), 159.87 (C-4, 4′ of DMTr); FAB(M + 1) calculated for $C_{35}H_{40}FN_2O_8PS$: 698.75, found: 698.00.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(*tert*-butyl) thiophosphorofluoridate 8b. Yield 79%; δ_P (121.49 MHz, $CDCl_3$) −59.34 (d, J_{PF} 999.5), −53.99 (d, J_{PF} 1000.0); δ_F −50.55 (d, J_{PF} 1212.5), −51.00 (d, J_{PF} 1202.0); δ_H (300.13 MHz, $CDCl_3$) 1.45 (9H, s, $C(CH_3)_3$), 1.92 (3H, d, *J* 1.1 5-CH_3), 2.25 (2H, m, H-2′, H-2″), 3.37–3.50 (2H, m, 5′, 5″), 4.14 (1H, m, H-4′), 4.45 (1H, m, H-3′), 7.26, 7.29 (1 H, dd, *J* 8.1, 7.1 H-1′); δ_C(75.47 MHz, $CDCl_3$) 19.43, 20.11 (5-CH_3), 31.99 ($C(CH_3)_3$), 33.14 (C-2′), 64.97 (C-3′), 66.91 (J_{POC} 7.9, C-5′), 77.77 ($C(CH_3)_3$), 89.22 (C-1′), 95.97 (C-4′), 111.93 (C-5), 139.66 (C-6), 151.83 (C-4) 154.51, 154.87 (C-2); FAB(M + 1) calculated for $C_{14}H_{21}FN_5O_5PS$: 421.39, found: 422.00.

2,3′-Anhydrothymidin-5′-yl *O*-(*tert*-butyl) thiophosphorofluoridate 8c. Yield 86%; δ_P (129.49 MHz, $CDCl_3$) 60,32 (d, J_{PF} 999.0), 61.99 (d, J_{PF} 1054.1); δ_F ($CDCl_3$) −51.2 (d, J_{PF} 1054.1), −50.4 (d, J_{PF} 1054.2); δ_H (300.13 MHz, $CDCl_3$) 1.45 (9H, s, $C(CH_3)_3$), 1.78 (3H, s, 5-CH_3), 2.25 (1H, m, H-2′), 2.45 (1H, m, H-2″), 3.47 (2H, m, 5′, 5″), 4.49 (1H, m, H-4′), 5.05 (1H, m, H-3′), 6.19, 6.43 (1H, dd, *J* 8.1, 7.1 H-1′), 7.65 (1H, s, H-6); δ_C(57.47 MHz, $CDCl_3$) 16.99 (5-CH_3), 41.93 (C-2′), 60.16 (C-5′), 65.99 ($C(CH_3)_3$), 74.92 (C-3′), 81.99 (C-4′), 86.60 (C-1′), 122.06 (C-5), 141.55 (C-6), 160.65 (C-2), 172.09 (C-4); FAB(M + 1) calculated for $C_{14}H_{20}FN_2O_5PS$: 378.36, found: 379.39.

***O*-(9-[(2-Hydroxyethyloxy)methyl]guanin-4′-yl) *O*-(*tert*-butyl) thiophosphorofluoridate 8d.** Yield 85%; δ_P (121.49 MHz, $CDCl_3$) 51.19 (d, J_{PF} 1090.1), 52.6 (d, J_{PF} 1090.3); δ_F ($CDCl_3$) −40.11 (d, J_{PF} 1090.1), −40.34 (d, J_{PF} 1090.5); δ_H (300.13 MHz, $CDCl_3$) 1.66 (9H, s, $C(CH_3)_3$), 3.52 (2H, m, H-3′), 4.55 (2H, m, H-4′), 5.55 (2H, s, H-1′), δ_C(57.47 MHz, $CDCl_3$) 33.33 ($C(CH_3)_3$), 60.4 (d, J_{COP} 5.9, C-4′), 67.11 ($C(CH_3)_3$), 70.6 (d, J_{CCOP} 7.4, C-3′), 72.3 (C-1′), 116.3 (C-5), 137.7 (C-8), 151.3 (C-4), 156.9 (C-2), 161.8 (C-6); FAB(M + 1) calculated for $C_{12}H_{19}FN_5O_4PS$: 379.35, found: 380.43.

***O*-Cholesteryl *O*-(*tert*-butyl) thiophosphorofluoridate 8e.** Yield 98%, δ_P (121.49 MHz, $CDCl_3$) 55.0 (d, J_{PF} 1047.9), 53,25 (d, J_{PF} 1052.8); δ_F −29.75 (d, J_{PF} 1052.2), −30.3 (d, J_{PF} 1050.80); δ_H (300.13 MHz, $CDCl_3$) 0.51 (3 H, s, CH_3-18), 0.66 (3 H, d, *J* 6.4, CH_3-26), 0.70 (3 H, d, *J* 6.4, CH_3-27), 0.81, (3 H, d, *J* 6.4, CH_3-21), 0.89 (3 H, s, CH_3-19), 1.31 [9 H, s, $C(CH_3)_3$], 3.76 (1 H, d, *J* 8.0, H-7), 4.01 (1 H, b, H-3), 5.21 (1 H, s, H-6); δ_C (75.47 MHz, $CDCl_3$) 11.68 (C-18), 18.60 (C-19), 22.32 (C-26), 22.51 (C-27), 33.01 [$C(CH_3)_3$], 55.21 (C-14), 55.81 (C-17), 70.01 [$C(CH_3)_3$], 73.12 (C-7), 77.39 (C-3), 126.01 (C-6), 142.98 (C-5); FAB(M + 1) calculated for $C_{31}H_{54}FO_2PS$: 540.81, found: 540.84.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)-N^6-benzoyl-2′-deoxyadenosin-3′-yl) *O*-(*tert*-butyl) thiophosphorofluoridate 8f.** Yield 98%, δ_P (121.49 MHz, $CDCl_3$) 52.55 (d, J_{PF} 1081.6), 52.05 (d, J_{PF}

1084.0); δ_F −34.0 (d, J_{PF} 1081.9), −34,25 (d, J_{PF} 1084.0); δ_H (300.13 MHz, $CDCl_3$) 1.53 [9 H, s, $C(CH_3)_3$], 2.15 (1 H, m, H-2′), 3.17 (1 H, m, H-2″), 3.54 and 3.64 (2 H, m, H-5′ 5″), 3.79 (6 H, s, 2 × OCH_3 of DMTr), 4.19–4.30 (1 H, m, H-4′), 5.21–5.41 (1 H, m, H-3′), 6.39 (1 H, dd, *J* 7.1 Hz, H-1′), 6.73, 6.81 (4 H, 2d *J* 8.6, *J* 8.3 Hz, H-3, 3′, 5, 5′ of DMTr), 7.10–7.34 (5 H, m, H-2″, 3″, 4″, 5″, 6″ of DMTr), 7.29, 7.41 (4 H, 2d, *J* 8.6, and 8.3 Hz, H-2, 2′, 6, 6′ of DMTr), 7.49 (2 H, m, H-5, 6 of benzoyl), 7.61 (1 H, tt, *J* 7.3 and 1.3 Hz, H-4 of benzoyl), 8.10, 8.21 (1 H, 2s, H-2), 8.68, 8.71 (1 H, 2s, H-8), 9.31 (1 H, bs, NH-6); δ_C(75.47 MHz, $CDCl_3$), 33.2 ($C(CH_3)_3$), 39.22, 39.41 (C-2′), 55.12 (OCH_3 of DMTr), 63.11, 63.45 (C-5′), 66.01 ($C(CH_3)_3$), 74.58, 74.91 (C-3′), 84.57, 84.35 (C-1′), 85.47, 85.69 (C-4′), 86.64, 86.83 (tert-C of DMTr), 113.52 (C-3, 3′, 5, 5′ of DMTr), 123.41 (C-5), 127.09, 130.12 (C-2″, 3″, 4″, 5″, 6″ of DMTr), 127.52 (C-2,6 of benzoyl), 128.27 (C-2, 2′, 6, 6′ of DMTr), 128.91 (C-3, 5 of benzoyl), 132.23 (C-4 of benzoyl), 133.51 (C-1 of benzoyl), 135.79, 136.11 (C-1, 1′ of DMTr), 142.31, 142.47 (C-2), 145.30 (C-1″ of DMTr), 150.01 (C-6), 153.32 (C-8), 158.47 (C-4, 4′ of DMTr), 164.12 (C=O of benzoyl); FAB(M + 1) calculated for $C_{42}H_{43}FN_5O_7PS$: 811.88 found: 811.89.

General procedure for the synthesis of compounds 1 or 2

The solution of compound **7** (1.0 mmol) or **8** (1.0 mmol) in CH_3CN (30 ml) was refluxed for 2 h at 80 °C. Thermal elimination of a 2-methylpropene group give the corresponding compound **1** or **2** which was purified by column chromatography ($CH_3C(O)CH_3$–CH_2Cl_2 (10 : 2 v/v)).

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl) phosphoromonofluoridate 1a.** Yield 96%; δ_P (121.49 MHz, $CDCl_3$–CD_3OD 10 : 3) −9.3 (d, J_{PF} 930); δ_F ($CDCl_3$–CD_3OD 10 : 3) −80.01 (d, J_{PF} 929); δ_H (300.13 MHz, $CDCl_3$–CD_3OD 10 : 3) 1.71 (3 H, 5-CH_3), 2.51 (1H, m, H-2′), 2.67 (1H, m, H-2″), 3.30–3.51 (2 H, m, H-5′, 5″), 3.78 (6H, s, OCH_3 of DMTr), 4.22, 4.37 (1H, m, H-4′), 5.19 (1H, m, H-3′), 6.23, 6.50 (1H, 2d, *J* 8.1, 7.1 H-1′), 6.79 (4H, 2d, *J* 8.6, 7.7, H-3, 3′, 5, 5′ of DMTr), 7.21–7.39 (2H, ArH of DMTr except for H-3, 3′, 5, 5′); δ_C (50.288 MHz, $CDCl_3$–CD_3OD 10 : 3) 19.33, 20.17 (5-CH_3), 42.76 (C-2′), 55.05 (OCH_3 of DMTr), 60.98, 62.45 (C-5′), 72.33, 73.34 (J_{POC} 6.0 6.9 3′-C), 83.43 (C-1′), 87.19, 87.99 (J_{POCC} 4.9, 5.4, C-4′), 87.39, 87.97 (tert-C of DMTr), 110.06, 110.59 (C-5), 113.48 (3, 3′, 5, 5′ of DMTr), 128.09, 129.66, 130.54, 130.87, 131.68 (ArC of DMTr except for C-3, 3′, 5, 5′), 137.39, 137.99 (C-1, 1′-C of DMTr), 140.82 (6-C), 142.11 (C-1″ of DMTr), 150.12, 150.44 (C-2), 160.11 (C-4, 4′ of DMTr) FAB(M − 1) calculated for $C_{31}H_{32}FN_2O_9P$: 626.58, found: 625.01.

3′-Azido-3′-deoxythymidin-5′-yl phosphoromonofluoridate 1b[1a]. Yield 80%; δ_P (121.49 MHz, $CDCl_3$–CD_3OD 10 : 3) −9.34 (d, J_{PF} 1012.1); δ_F ($CDCl_3$–CD_3OD 10 : 3) −50.55 (d, J_{PF} 1012.5); δ_H (300.13 MHz, $CDCl_3$–CD_3OD 10 : 3) 1.87 (3H, d, *J* 1.1, 5-CH_3), 2.65 (2H, m, H-2′, H-2″), 3.37–3.78 (2H, m, 5′, 5″), 4.21 (1H, m, H-4′), 4.45 (1H, m, H-3′), 7.30, 7.32 (1H, dd, *J* 8.1, 7.1 H-1′), δ_C(75.47 MHz, $CDCl_3$–CD_3OD 10 : 3) 12.37, 12.89 (5-CH_3), 39.07 (C-2′), 64.32 (C-3′), 69.01 (J_{POC} 6.1, C-5′), 82.18 (C-1′), 85.28 (C-4′), 111.03 (C-5), 137.17 (C-6), 151.13 (C-4) 149.11, 149.63 (C-2); FAB(M − 1) calculated for $C_{10}H_{13}FN_5O_6P$: 349.22, found: 348.07.

2,3′-Anhydrothymidin-5′-yl phosphoromonofluoridate 1c. Yield 92%; δ_P (121.49 MHz, $CDCl_3$–CD_3OD 10 : 3) −8.78 (d, J_{PF} 1090.0); δ_F ($CDCl_3$–CD_3OD 10 : 3) −49.55 (d, J_{PF} 1092.5); δ_H (300.13 MHz, $CDCl_3$–CD_3OD 10 : 3)1.78 (3H, s, 5-CH_3), 2.35 (1H, m, H-2′), 2.45 (1H, m, H-2″), 3.61 (2H, m, 5′, 5″), 4.49 (1H, m, H-4′), 5.55 (1H, m, H-3′), 6.43 (1H, dd, *J* 8.1, 7.1 H-1′), 7.60 (1H, s, H-6); δ_C(57.47 MHz, $CDCl_3$–CD_3OD 10 : 3) 17.93 (5-CH_3), 32.93 (C-2′), 60.16 (C-5′), 75.92, (C-3′), 80.69 (C-4′), 81.71 (C-1′), 120.96, (C-5), 137.35 (C-6), 165.23 (C-2), 172.22 (C-4), FAB(M − 1) calculated for $C_{10}H_{12}FN_2O_6P$: 306.19, found: 305.99.

***O*-(9-[(2-Hydroxyethyloxy)methyl]guanin-4′-yl) phosphoromonofluoridate 1d[1i].** Yield 85%; δ_P (121.49 MHz, $CDCl_3$–CD_3OD 10 : 3) −6.09 (d, J_{PF} 933); δ_F ($CDCl_3$–CD_3OD 10 : 3) −79.06 (d, J_{PF} 932); δ_H (300.13 MHz, $CDCl_3$–CD_3OD 10 : 3) 3.71 (2H, m, H-3′), 4.10 (2H, m, H-4′), 5.50 (2H, s, H-1′), 7.90 (1H, s, H-8); δ_C(57.47 MHz, $CDCl_3$–CD_3OD 10 : 3) 67.1 (d, J_{COP} 5.2, C-4′), 71.0 (d, J_{CCOP} = 7.4, C-3′), 80.9 (C-1′), 119.9 (C-5), 140.3 (C-8), 151.6 (C-4), 154.0 (C-2), 157.2 (C-6); FAB(M − 1) calculated for $C_8H_{11}FN_5O_5P$: 307.18, found: 306.00.

***O*-Cholesteryl phosphoromonofluoridate 1e.** Yield 90%, δ_P (121.49 MHz, $CDCl_3$) −7.95 (d, J_{PF} 925.81); δ_F −75.45 (d, J_{PF} 925.80); δ_H (300.13 MHz, $CDCl_3$) 0.65 (3 H, s, CH_3-18), 0.76 (3 H, d, *J* 6.4, CH_3-26), 0.82 (3 H, d, *J* 6.4, CH_3-27), 0.89 (3 H, d, *J* 6.4, CH_3-21), 1.10 (3 H, s, CH_3-19), 3.81 (1 H, d, *J* 8.0, H-7), 3.99 (1 H, b, H-3), 5.20 (s, 1 H, H-6); δ_C (300.13 MHz, $CDCl_3$) 11.00 (C-18), 18.41 (C-19), 22.09 (C-26), 22.99 (C-27), 55.48 (C-14), 60.00 (C-17), 73.31 (C-7), 77.42 (C-3), 126.01 (C-6), 142.89 (C-5); FAB(M − 1) calculated for $C_{27}H_{46}FO_3P$: 468.64, found: 467.02.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl) phosphoromonofluoridate 1f.** Yield 92%, δ_P (121.49 MHz, $CDCl_3$) −8.1 (d, J_{PF} 931); δ_F −73.2 (d, J_{PF} 930); δ_H (300.13 MHz, $CDCl_3$) 2.66 (1 H, m, H-2′), 3.30 (1 H, m, H-2″), 3.21 and 3.45 (2 H, m, H-5′5″), 4.00 (6 H, s, 2 × OCH_3 of DMTr), 4.13–4.43 (1 H, m, H-4′), 5.20–5.59 (1 H, m, H-3′), 6.19 (1 H, dd, *J* 7.1 Hz, H-1′), 6.60, 6.71 (4 H, 2d *J* 8.6, *J* 8.3 Hz, H-3, 3′, 5, 5′ of DMTr), 7.09–7.39 (5 H, m, H-2″, 3″, 4″, 5″, 6″ of DMTr), 7.42, 7.49 (4 H, 2d, *J* 8.6 and 8.3 Hz, H-2, 2′, 6, 6′ of DMTr), 7.50 (2 H, m, H-5, 6 of benzoyl), 7.71 (1 H, tt, *J* 7.3 and 1.3 Hz, H-4 of benzoyl), 8.30, 8.59 (1 H, 2s, H-2), 8.81, 8.91 (1 H, 2s, H-8), 9.42 (1 H, bs, NH-6), δ_C(75.45 MHz, $CDCl_3$) 39.50, 39.56 (C-2′), 55.69 (OCH_3 of DMTr), 64.31, 64.51 (C-5′), 75.51, 75.91 (C-3′), 84.47, 84.99 (C-1′), 85.34, 86.11 (C-4′), 87.12, 87.00 (tert-C of DMTr), 115.39 (C-3, 3′, 5, 5′ of DMTr), 125.49 (C-5), 126.39, 131.42 (C-2″, 3″, 4″, 5″, 6″ of DMTr), 127.91 (C-2,6 of benzoyl), 129.29 (C-2, 2′, 6, 6′ of DMTr), 128.87 (C-3, 5 of benzoyl), 132.99 (C-4 of benzoyl), 133.71 (C-1 of benzoyl), 135.43, 135.89 (C-1, 1′ of DMTr), 141.49, 141.76 (C-2), 144.42 (C-1″ of DMTr), 149.75 (C-6), 153.43 (C-8), 158.79 (C-4, 4′ of DMTr), 165.11 (C=O of benzoyl); FAB(M − 1) calculated for $C_{38}H_{35}FN_5O_8P$: 739.70 found: 740.70.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl) thiophosphoromonofluoridate 2a.** Yield 98%, δ_P (129.49 MHz, $CDCl_3$–CD_3OD 10 : 3) 54.24 (d, J_{PF} 1054.0), 54.55 (d, J_{PF} 1054.1); δ_F ($CDCl_3$–CD_3OD 10 : 3) −30.2 (d, J_{PF} 1054.1), −30.4 (d, J_{PF} 1054.2); δ_H (300.13 MHz, $CDCl_3$–CD_3OD 10 : 3) 1.71 (3 H, 5-CH_3), 2.51 (1H, m, H-2′), 2.67 (1H, m, H-2″), 3.30–3.51 (2 H, m, H-5′, 5″), 3.78 (6H, s, OCH_3 of DMTr), 4.22, 4.37 (1H, m, H-4′), 5.19 (1H, m, H-3′), 6.23, 6.50 (1H, 2d, *J* 8.1, 7.1, H-1′), 6.79 (4H, 2d, *J* 8.6, 7.7, H-3, 3′, 5, 5′ of DMTr), 7.21–7.39 (2H, ArH of DMTr except for H-3, 3′, 5, 5′); δ_C (50.288 MHz, $CDCl_3$–CD_3OD 10 : 3) 10.45, 10.94 (5-CH_3), .40.03 (C-2′), 55.35 (OCH_3 of DMTr), 62.32, 63.03 (C-5′), 74.51, 75.29 (J_{POC} 6.1, 6.1, 3′-C), 84.71 (C-1′), 85.09, 85.75 (J_{POCC} 4.8, 4.9, C-4′), 87.33, 87.56 (tert-C of DMTr), 111.26, 111.71 (C-5), 113.48 (3, 3′, 5, 5′ of DMTr), 128.11, 128.36, 130.54, 130.87, 131.68 (ArC of DMTr except for C-3, 3′, 5, 5′), 135.32, 135.54 (C-1, 1′-C of DMTr), 135.75 (6-C), 144.89 (C-1″ of DMTr), 149.63, 149.97 (C-2), 158.91 (C-4, 4′ of DMTr); FAB(M − 1) calculated for $C_{31}H_{32}FN_2O_8PS$: 642.65, found: 641.87.

3′-Azido-3′-deoxythymidin-5′-yl thiophosphoromonofluoridate 2b. Yield 96%, δ_P (129.49 MHz, $CDCl_3$–CD_3OD 10 : 3) 54.35 (d, J_{PF} 1054.0), 54.45 (d, J_{PF} 1054.1); δ_F ($CDCl_3$–CD_3OD 10 : 3) −30.2 (d, J_{PF} 1054.1), −30.4 (d, J_{PF} 1054.2); δ_H (300.13 MHz, $CDCl_3$–CD_3OD 10 : 3), 1.87 (3H, d, *J* 1.1, 5-CH_3), 2.65 (2H, m,

H-2′, H-2″), 3.37–3.78 (2H, m, 5′, 5″), 4.21 (1H, m, H-4′), 4.45 (1H, m, H-3′), 7.30, 7.32 (1H, dd, *J* 8.1, 7.1 H-1′); δ_C(75.47 MHz, $CDCl_3$–CD_3OD 10 : 3) 12.37, 12.89 (5-CH_3), 37.27 (C-2′), 64.00 (C-3′), 66.64 (J_{POC} 6.1, C-5′), 82.08 (C-1′), 84.88 (C-4′), 111.43 (C-5), 135.37 (C-6), 150.03 (C-4), 149.51, 149.63 (C-2); FAB(M − 1) calculated for $C_{10}H_{13}FN_5O_5PS$: 365.28, found: 364.98.

2,3′-Anhydrothymidin-5′-yl thiophosphoromonofluoridate 2c. Yield 96%, δ_P (129.49 MHz, $CDCl_3$–CD_3OD 10 : 3) 50.62 (d, J_{PF} 1072.0), 50.95 (d, J_{PF} 1074.1); δ_F ($CDCl_3$–CD_3OD 10 : 3) −33.3 (d, J_{PF} 1074.1), −35.9 (d, J_{PF} 1084.2); δ_H (300.13 MHz, $CDCl_3$–CD_3OD 10 : 3),1.88 (3H, s, 5-CH_3), 2.33 (1H, m, H-2′), 2.63 (1H, m, H-2″), 3.82 (2H, m, 5′, 5″), 4.82 (1H, m, H-4′), 6.78 (1H, m, H-3′), 7.00 (1H, dd, *J* 8.1, 7.1 H-1′), 7.69 (1H, s, H-6) δ_C(57.47 MHz, $CDCl_3$–CD_3OD 10 : 3) 12.93 (5-CH_3), 41.93 (C-2′), 59.96 (C-5′), 75.72, (C-3′), 81.19 (C-4′), 80.05 (C-1′), 121.26, (C-5), 135.35 (C-6), 160.13 (C-2), 170.99 (C-4); FAB(M − 1) calculated for $C_{10}H_{12}FN_2O_5PS$: 322.25, found: 321.00.

***O*-(9-[2-Hydroxyethyloxymethyl]guanin-4′-yl) thiophosphoromonofluoridate 2d.** Yield 85%; δ_P (121.49 MHz, $CDCl_3$–CD_3OD 10 : 3) 50.12 (d, J_{PF} 1051.4), 51.92 (d, J_{PF} 1051.8); δ_F ($CDCl_3$–CD_3OD 10 : 3) −30.10 (d, J_{PF} 1051.66), −30.20 (d, J_{PF} 1051.67); δ_H (300.13 MHz, $CDCl_3$–CD_3OD 10 : 3) 3.51 (2H, m, H-3′), 4.61 (2H, m, H-4′), 5.50 (2H, s, H-1′), 8.25 (1H, s, H-8); δ_C(57.47 MHz, $CDCl_3$–CD_3OD 10 : 3) 67.4 (d, J_{COP} 5.2 C-4′), 71.3 (d, J_{CCOP} 7.4, C-3′), 71.9 (C-1′), 118.8 (C-5), 138 (C-8), 151.6 (C-4), 153.7 (C-2), 156 (C-6); FAB(M − 1) calculated for $C_8H_{11}FN_5O_4PS$: 323.24, found: 323.11.

***O*-Cholesteryl thiophosphoromonofluoridate 2e.** Yield 96%, δ_P (129.49 MHz, $CDCl_3$) 53.95 (d, J_{PF} 1050.10); δ_F ($CDCl_3$) −30.05 (d, J_{PF} 1050.60), −30.10 (d, J_{PF} 1051.10); δ_H (300.13 MHz, $CDCl_3$) 0.62 (3 H, s, CH_3-18), 0.79 (3 H, d, *J* 6.4, CH_3-26), 0.88 (3 H, d, *J* 6.4, CH_3-27), 0.94 (3 H, d, *J* 6.4, CH_3-21), 1.31 (3 H, s, CH_3-19), 3.34 (1 H, d, *J* 8.0, H-7), 4.07 (1 H, b, H-3), 5.09 (1 H, s, H-6); δ_C (300.13 MHz, $CDCl_3$) 10.94 (C-18), 18.00 (C-19), 22.84 (C-26), 22.99 (C-27), 55.39 (C-14), 56.11 (C-17), 73.39 (C-7), 77.55 (C-3), 125.91 (C-6), 142.33 (C-5); FAB(M − 1) calculated for $C_{27}H_{46}FO_2PS$: 484.70, found: 485.97.

***O*-(5′-*O*-(4,4′-Dimethoxytrityl)-N^6-benzoyl-2′-deoxyadenosin-3′-yl) thiophosphoromonofluoridite 2f.** Yield 90%, δ_P (129.49 MHz, $CDCl_3$) 54.22 (d, J_{PF} 1053.0), 53.18 (d, J_{PF} 1053.1); δ_F ($CDCl_3$) −30.33 (d, J_{PF} 1053.2), −30.49 (d, J_{PF} 1053.2); δ_H (50.288 MHz, $CDCl_3$) 2.57 (1 H, m, H-2′), 3.07 (1 H, m, H-2″), 3.39 and 3.63 (2 H, m, H-5′5″), 3.92 (6 H, s, 2 × OCH_3 of DMTr), 4.00–4.46 (1 H, m, H-4′), 5.25–5.41 (1 H, m, H-3′), 6.38 6.51 (1 H, dd, *J* 5.5, 8.7, H-1′), 6.71, 6.92 (4 H, 2d, *J* 8.6 8.3, H-3, 3′, 5, 5′ of DMTr), 7.10–7.44 (5 H, m, H-2″, 3″, 4″, 5″, 6″ of DMTr), 7.28, 7.41 (4 H, 2d, *J* 8.6, 8.3 H-2, 2′, 6, 6′ of DMTr), 7.77 (2 H, m, H-5, 6 of benzoyl), 7.88 (1 H, tt, *J* 7.3 1.3, H-4 of benzoyl), 8.30, 8.42 (1 H, 2s, H-2), 8.88, 8.92 (1 H, 2s, H-8), 10.11 (1 H, bs, NH-6); δ_C(50.288 MHz, $CDCl_3$) 40.22, 40.43 (C-2′), 55.66 (OCH_3 of DMTr), 63.24, 63.76 (C-5′), 74.91, 75.09 (C-3′), 84.54, 84.69 (C-1′), 85.50, 85.83 (C-4′), 86.89, 86.99 (tert-C of DMTr), 113.11 (C-3, 3′, 5, 5′ of DMTr), 123.00 (C-5), 127.23, 130.78 (C-2″, 3″, 4″, 5″, 6″ of DMTr), 128.00 (C-2,6 of benzoyl), 128.19 (C-2, 2′, 6, 6′ of DMTr), 128.99 (C-3, 5 of benzoyl), 132.93 (C-4 of benzoyl), 133.53 (C-1 of benzoyl), 135.88, 135.93 (C-1, 1′ of DMTr), 141.31, 141.58 (C-2), 144.40; FAB(M − 1) calculated for $C_{38}H_{35}FN_5O_7PS$: 755.77 found: 755.98.

Reaction of thiophosphorofluoridates 8a with benzenesulfonic acid and trifluoroacetic anhydride

To a solution of **2a** (1.0 mmol.) in THF (10 mL) was added a solution of benzenesulfonic acid (1.1 mmol) in THF (10 mL) at room temperature. After 1 h the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography using CH_2Cl_2–$CH_3C(O)CH_3$ (10 : 3 v/v) as an eluent to give **9**. The resulting **9** (10 mmol) was dissolved in THF (10 mL) and trifluoroacetic anhydride (15 mmol) was added at room temperature. After 4 h, the solution was treated with methanol (20 mL). The solution was concentrated under reduced pressure. The residue was purified by column chromatography using CH_2Cl_2–$CH_3C(O)CH_3$ (10 : 3 v/v) as an eluent to give **10**.

***O*-(Thymidin-3′-yl) thiophosphorofluoridate 9.** Yield 98%, δ_P (121.49 MHz, D_2O) 53.65 (d, J_{PF} 1050.0), 53.50 (d, J_{PF} 1049.9); δ_F (D_2O) −29.40 (d, J_{PF} 1050.1), −29.65 (d, J_{PF} 1049.4); δ_H (300.13 MHz, D_2O) 1.43 (3H, s, 5-CH_3), 2.49 (1H, m, H-2′), 2.63 (1H, m, H-2″), 3.30–3.49 (4H, m, 5′, 5″), 4.28, 4.32 (2H, m, H-4′), 5.14 (1H, m, H-3′), 6.45, 6.49 (1H, 2d, *J* 8.1, 7.1 H-1′); δ_C (75.47 MHz, D_2O) 11.63, 11.72 (5-CH_3), 39.74 (C-2′), 63.00, 63.46 (C-5′), 74.47, 75.23 (J_{POC} 6.1, 6.1, C-3′), 84.70, (C-1′), 85.14, 85.43 (J_{POC} 4.8 and 4.9, C-4′), 149.33, 149.44 (C-2).

***O*-(Thymidin-3′-yl) phosphorofluoridate 10.** Yield 97%, δ_P (121.49 MHz, D_2O) −9.3 (d, J_{PF} 927.7); δ_F (D_2O) −75.0 (d, J_{PF} 927.0); δ_H (300.13 MHz, D_2O) 1.61 (3H, s, 5-CH_3), 2.54 (1 H, m, H-2′), 2.63 (1H, m, H-2″), 3.35–3.49 (2H, m, H-5′, 5″), 4.28, 4.32 (2H, m, H-4′), 5.14 (1H, m, H-3′), 6.45, 6.48 (1H, 2d, *J* 8.1, 7.1, H-1′), δ_C(75.47 MHz, D_2O) 11.72, 11.89 (5-CH_3), 39.73 (C-2′), 63.00, 63.46 (C-5′), 74.37, 75.03 (J_{POC} 6.1 and 6.1, C-3′), 84.71 (C-1′), 85.14, 85.43 (J_{POCC} 4.8 and 4.9, C-4′), 149.51, 149.63 (C-2).

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