Novel phosphitylating reagents containing a phosphorus–fluorine bond and their application in efficient synthesis of phosphorofluoridates and phosphorofluoridothionates

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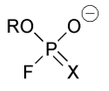
Novel phosphitylating reagents containing a P–F bond are obtained in excellent yield from commercial materials. They react with alcohols at 20 °C in neutral solvents in the presence of tetrazole, chlorotrimethylsilane or benzoyl chloride to give the intermediate phosphorofluoridites. Subsequent oxidation or sulfurization followed by the removal of protecting groups gives the phosphorofluoridates and thiophosphorofluoridates in over 90% total yield.

Letsinger and Lunsford revolutionized the chemical synthesis of oligonucleotides by introduction of the 'phosphite triester' methodology.[1] This approach became widely accepted when Beaucage and Caruthers developed phosphoroamidites as a new class of preferred phosphitylating intermediates.[2] Phosphorochloridites are so reactive that their isolation as stable enantiomers or diastereoisomers is very unlikely. In contrast, phosphorofluoridites should be chemically and stereochemically considerably more stable due to the strength of the phosphorus–fluorine bond.[3] This higher stability has been demonstrated by recent work from this laboratory.

Known routes to phosphorofluoridites derived from alcohols of biological interest are of limited value. Recently we discovered that aryloxy groups attached to a tricoordinate phosphorus center are readily replaced by fluoride anion (Fig. 1).[4,5]

Results and discussion

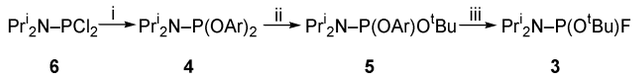
Best results were obtained using the 4-nitrophenyl group.[4] This is a surprisingly easy and efficient nucleophilic displacement reaction propelled by the high affinity of the fluoride anion for a phosphorus center. Under favorable structural circumstances this reaction can compete efficiently with hydrolysis. For example, in the case of *O*-nucleosidyl-*O*-(4-nitrophenyl) phosphoroamidites the replacement of the 4-nitrophenoxy group by fluoride proceeds in very high yield with the aid of commercial TBAF·3H_2O. It is most likely that hydrolysis is sterically inhibited. P^{III} compounds containing substituents with less steric hindrance require water-free fluorinating reagents if hydrolysis is to be avoided. Another important feature of our current studies is the demonstration of stereochemical stability of a number of phosphorofluoridite and other P^{III}–F derivatives.[6] As part of our search for convenient syntheses of P–F structures derived from alcohols of biological interest, we describe in this paper novel efficient phosphitylating reagents **3** [*O-tert*-butyl *N,N*-diisopropylfluorophosphoramidite F-P(N^iPr_2)O^{t}Bu] and **7** [*O*-(2-cyanoethyl) *N,N*-diisopropylfluorophosphoramidite F-P(N^iPr_2)OCH_2CH_2CN] in which the P^{III}–F bond is already present.



1a-e X = O
2a-e X = S

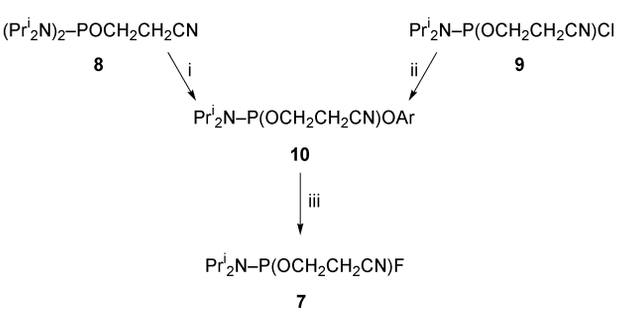
Fig. 1

The synthetic strategy for the preparation of *O-tert*-butyl *N,N*-diisopropylfluorophosphoramidite **3** is delineated in Scheme 1. Bis-*O,O*-(4-nitrophenyl) *N,N*-diisopropylphosphoramidite **4**[4] was allowed to react with one equivalent of *tert*-butyl alcohol in the presence of sodium hydride to give *O-tert*-butyl *O*-(4-nitrophenyl) *N,N*-diisopropylphosphoramidite **5**, which is finally transformed into the phosphitylating reagent *O-tert*-butyl *N,N*-diisopropylphosphorofluoramidite **3** by reaction with TBAF·3H_2O in 90% overall yield.



Scheme 1 *Reagents and conditions*: i, Me_3SiOAr, THF, 20 °C, 1 h; ii, ^{t}BuOH, NaH, THF, 20 °C, 5 h; iii, TBAF, THF, 20 °C, 10 min.

The synthesis of *O*-(2-cyanoethyl) *N,N*-diisopropylphosphorofluoramidite **7** starts from *O*-(2-cyanoethyl) *N,N,N′,N′*-tetraisopropylphosphorodiamidite **8** or *O*-(2-cyanoethyl) *N,N*-diisopropylchlorophosphoramidite **9** and proceeds in overall 95% yield (Scheme 2). Compounds **3** and **7** can be purified by distillation *in vacuo* or by silica gel chromatography. They also can be prepared in a one-flask procedure and used immediately. They are colorless liquids which can be stored at ambient temperature. Substrates **6**, **8** and **9** are commercially available. Phosphitylating reagents **3** and **7** react with alcohols



Scheme 2 *Reagents and conditions*: i, HOAr, THF, 20 °C, 3 h; ii, Me_3SiOAr, THF, 20 °C, 1 h; iii, TBAF, THF, 20 °C, 10 min.

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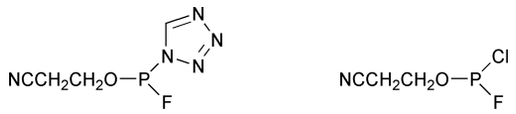


11 δ_P: 130.18, 115.27; $J_{P\text{-}F}$: 1208.65 Hz

12 δ_P: 128.18, 113.37; $J_{P\text{-}F}$: 1206.41 Hz

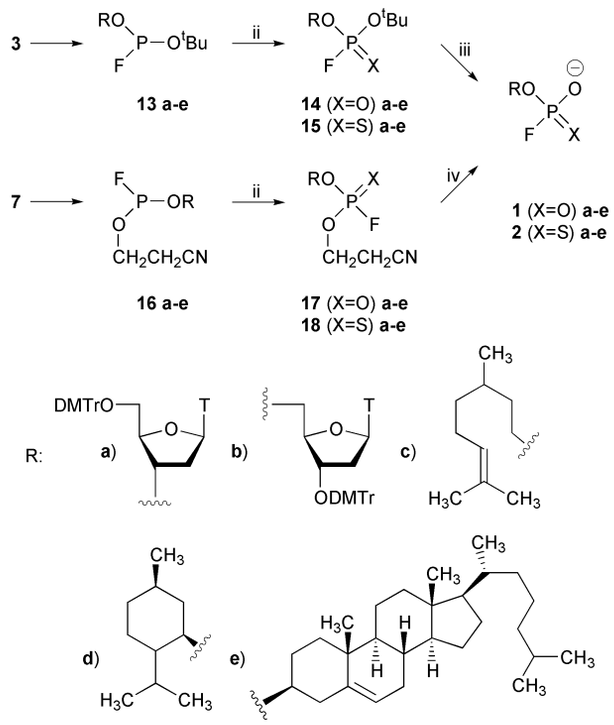
Fig. 2

in the presence of activators such as tetrazole, trimethylchlorosilane (TMSCl) or benzoyl chloride. The phosphorus–fluorine bond is not affected in this procedure. TMSCl proved to be a better activator than tetrazole, which must be used in large excess. The strongly electronegative fluorine does not affect the use of the activated amino ligand as a leaving group in phosphitylation procedures. This behavior differs from that of P^{III} amidites containing a CF_3 group attached to the phosphorus center.[7] It is noteworthy that examination of the phosphitylation reactions by ^{31}P NMR spectroscopy using fluorophosphoramidite **7** in the presence of tetrazole or TMSCl reveals the formation of intermediates **11** or **12** (Fig. 2) which is in agreement with the mechanistic scheme we proposed earlier.[8]

Nucleosidyl phosphorofluoridates **1** have been obtained by Wittmann[9] and used in biological studies. More recent approaches to the synthesis of this class of compounds come from several groups of workers.[10] The thioanalogues of **1** – phosphorofluoridothionates **2** – have been prepared for the first time in this laboratory.[11]

Our phosphorylation procedure leading to Wittmann's type of compounds is illustrated by the reactions of 5′-*O*-DMTr- and 3′-*O*-DMTr-protected thymidines with **3** and **7** followed by oxidation (sulfurization) of the intermediate phosphorofluoridites **13a**–**e** and **16a**–**e**. This leads to the corresponding phosphorofluoridates **14a**–**e**, **17a**–**e** and phosphorofluoridothionates **15a**–**e**, **18a**–**e** using *tert*-butyl hydroperoxide or by elemental sulfur in the presence of triethylamine. In the final step the *tert*-butyl group undergoes thermal elimination and the 2-cyanoethyl group is removed by β-elimination under standard conditions to give phosphorofluoridates **1a**–**e** or phosphorofluoridothionates **2a**–**e** as final compounds. This sequence of reactions is shown in Scheme 3. Conversion of **14** and **15** to **1** and **2** involve heating in acetonitrile solution under reflux, without base being present. The DMTr potective group at the 5′-*O* or 3′-*O* center is not removed under these conditions.

In summary, the procedures we describe are completely general and very simple. They make phosphorofluoridates **1a**–**e** and phosphorofluoridothionates **2a**–**e** readily available in excellent yield.



Scheme 3 *Reagents and conditions*: i, ROH, tetrazole, THF, 3 h or ROH, benzoyl chloride, THF, 24 h or ROH, Me_3SiCl, THF, 1 h; ii, *tert*-butyl hydroperoxide, THF, 1 h or sulfur in $(C_3H_7)_2NH$, THF, 2 h; iii, CH_3CN, reflux, 2 h; iv, Et_3N, THF, 6 h.

Experimental

The solvents were reagent grade and were distilled and dried by conventional methods before use. *N,N*-Diisopropylaminodichlorophosphine[12] **6**, *O*-2-cyanoethyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite[13] **8** and *O*-(2-cyanoethyl) *N,N*-diisopropylchlorophosphoramidite[14] **9** were synthesized according to published procedures. Tetrazole was purified by sublimation at 115 °C/0.2 mmHg. Chlorotrimethylsilane was purchased from Fluka. TBAF was purchased from Aldrich. Thin-layer chromatography (TLC) was performed on silica gel 60F-254 plates (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 ^{13}C NMR (50.288 and 75.47 MHz), relative to H_3PO_4 as external standard for ^{31}P NMR (80.96 and 121.49 MHz), and 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.

O-tert-Butyl *N,N*-diisopropylfluorophosphoramidite 3

To a solution of *O-tert*-butyl *O′*-(4-nitrophenyl) *N,N*-diisopropylphosphoramidite **5** (see below) (10 mmol) in dry THF (50 ml) was added a solution of TBAF (11 mmol) in THF (5 ml) at room temperature. After 10 min, tetrabutylammonium 4-nitrophenolate was removed by filtration. The filtrate was evaporated *in vacuo* and the residue was purified by column chromatography using CH_2Cl_2–$CH_3C(O)CH_3$ (10 : 3 v/v, R_f 0.87) as eluent to give pure *O-tert*-butyl *N,N*-diisopropylfluorophosphoramidite **3** in 95% yield, δ_P (80.96 MHz; $CDCl_3$) 148.85 (d, J_{PF} 1096.0); δ_F ($CDCl_3$) −69.0 (d, J_{PF} 1096.1); δ_H (200.13 MHz; $CDCl_3$), 1.17 {12 H, t, J 6.6, $N[CH(CH_3)_2]_2$}, 1.32 [9 H, s, $C(CH_3)_3$], 3.54–3.74 {2 H, m, $N[CH(CH_3)_2]_2$}.

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

(Route a). A solution of *N,N*-diisopropylaminodichlorophosphine **6** (10 mmol) in dry THF (10 ml) was added dropwise at room temperature under a nitrogen atmosphere to a stirred solution of sodium 4-nitrophenolate (25 mmol) in dry THF (50 ml) during 2 h. Once sodium chloride had been removed by filtration, the filtrate evaporated to dryness and the residue was purified by column chromatography (Et_2O–*n*-pentane–triethylamine 50 : 30 : 5 v/v, R_f 0.75) to give pure *O,O′*-bis-(4-nitrophenyl) *N,N*-diisopropylphosphoramidite **4** in 95% yield.

(Route b). A solution of trimethyl(*p*-nitrophenoxy)silane (20 mmol) in dry THF (20 ml) was added to a solution of *N,N*-diisopropylaminodichlorophosphine **6** (10 mmol) in dry THF (20 ml) at room temperature. The mixture was stirred for 1 h, then chlorotrimethylsilane and solvent were removed under reduced pressure to give pure phosphorodiamidite **4** in 97% yield, δ_P (80.96 MHz; $CDCl_3$) 144.8; δ_H (200.13 MHz; $CDCl_3$) 1.01 {12 H, d, J 6.8 $N[CH(CH_3)_2]_2$}, 3.46–3.65 {2 H, m, $N[CH(CH_3)_2]_2$}, 6.75 (4 H, d, J 9.13, Ph H_{ortho}), 7.86 (4 H, d, J 9.12, Ph H_{meta}); mp 120–122 °C; pale yellow crystals.

O-*tert*-Butyl *O*′-(4-nitrophenyl) *N,N*-diisopropylphosphoramidite 5

To a solution of *O,O*′-bis-(4-nitrophenyl) *N,N*-diisopropylphosphoramidite **4** (1 mmol) in dry THF (50 ml) were added *tert*-butyl alcohol (1 mmol) and NaH (1 mmol) at room temperature. After 5 h sodium 4-nitrophenolate was removed by filtration. The filtrate was evaporated *in vacuo* and the residue was purified by column chromatography using CH_2Cl_2–$CH_3C(O)CH_3$ (10 : 3 v/v) as eluent to give pure phosphoramidite **5** in 90% yield, δ_P (80.96 MHz; $CDCl_3$) 141.3; δ_H (300.13 MHz; $CDCl_3$) 1.03 {6 H, d, *J* 6.81, N[CH(CH_3)$_2$]$_2$}, 1.13 {6 H, d, *J* 6.79, N[CH(CH_3)$_2$]$_2$}, 1.24 [9H, s, C(CH_3)$_3$], 3.22–3.42 {2 H, m, N[C*H*(CH$_3$)$_2$]$_2$}, 6.82 (2 H, d, *J* 9.20, Ph H$_{ortho}$), 7.90 (2 H, d, *J* 9.13, Ph H$_{meta}$).

O-(2-Cyanoethyl) *N,N*-diisopropylfluorophosphoramidite 7

A solution of *O*-(2-cyanoethyl) *O*′-(4-nitrophenyl) *N,N*-diisopropylphosphoramidite **10** (see below) (10 mmol) in dry THF (50 ml) was treated with a solution of TBAF (11 mmol) in THF (5 ml) at room temperature. After 10 min, tetrabutylammonium 4-nitrophenolate was removed by filtration. The filtrate 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, R_f 0 : 9) as eluent to give pure *O*-(2-cyanoethyl) *N,N*-diisopropylfluorophosphoramidite **7** in 95% yield, δ_P (80.96 MHz; $CDCl_3$) 155.85 (d, J_{PF} 1118.1); δ_F ($CDCl_3$) −78.1 (d, J_{PF} 1118.4); δ_H (200.13 MHz; $CDCl_3$) 1.09 {12 H, d, *J* 6.82, N[CH(CH_3)$_2$]$_2$}, 1.56–1.74 (2 H, m, OCH$_2$CH_2CN), 3.22–3.42 {2 H, m, N[C*H*(CH$_3$)$_2$]$_2$}, 3.46–3.59 (2 H, m, OCH_2CH$_2$CN).

O-(2-Cyanoethyl) *O*′-(4-nitrophenyl) *N,N*-diisopropylphosphoramidite 10

(Route a). To a stirred mixture of *O*-(2-cyanoethyl) *N,N,N*′,*N*′-tetraisopropylphosphorodiamidite **8** (10 mmol) and tetrazole (11 mmol) in dry THF (50 ml) was added a solution of 4-nitrophenol (10 mmol) in dry THF (10 ml) at room temperature. After 3 h *N,N*-diisopropylammonium tetrazolide was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was purified by silica gel chromatography, eluting with CH_2Cl_2 (R_f 0.97). The fractions containing *O*-(2-cyanoethyl) *O*′-(4-nitrophenyl) *N,N*-diisopropylphosphoramidite **10** were collected and evaporated to give a yellow oil in 95% yield.

(Route b). A solution of trimethyl(*p*-nitrophenoxy)silane (10 mmol) in dry THF (10 ml) was added to a solution of *O*-(2-cyanoethyl) *N,N*-diisopropylchlorophosphoramidite **9** (10 mmol) in dry THF (50 ml) at room temperature. The mixture was stirred for 1 h, then chlorotrimethylsilane and solvent were removed under reduced pressure to give pure phosphoramidite **10** in 97% yield, δ_P (80.96 MHz; $CDCl_3$) 147.9; δ_H (300.13 MHz; $CDCl_3$) 1.15 {6 H, d, *J* 6.81, N[CH(CH_3)$_2$]$_2$}, 1.23 {6 H, d, *J* 6.80, N[CH(CH_3)$_2$]$_2$}, 2.70 (2 H, t, *J* 6.20, OCH$_2$CH_2CN), 3.59–3.83 {2 H, m, N[C*H*(CH$_3$)$_2$]$_2$}, 3.86–4.04 (2 H, m, OCH_2-CH$_2$CN), 7.12 (2 H, d, *J* 9.21, Ph H$_{ortho}$), 8.15 (2 H, d, *J* 6.16, Ph H$_{meta}$).

General procedure for the synthesis of *tert*-butyl phosphorofluoridites 13a–e

(Route a). To a mixture of *O*-*tert*-butyl *N,N*-diisopropylfluorophosphoramidite **3** (10 mmol) and tetrazole (11 mmol) in dry THF (50 ml) was added a solution of the required alcohol (10 mmol) in dry THF (20 ml). The reaction mixture was stirred for 3 h and monitored by TLC. *N,N*-Diisopropylammonium tetrazolide was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was chromatographed on silica gel, using a gradient of 0–10% $CH_3C(O)CH_3$ in CH_2Cl_2 as eluent, to give pure phosphorofluoridites **13a**–**e**.

(Route b). To a solution of **3** (10 mmol) and the required alcohol (10 mmol) in dry THF (30 ml) was added a solution of benzoyl chloride (1 eq.) in 10 ml of THF. After 24 h at room temperature the mixture was evaporated *in vacuo* and the residue was purified as above by column chromatography to obtain pure phosphorofluoridites **13a**–**e**.

(Route c). To a solution of **3** (10 mmol) in dry THF (20 ml) and an alcohol ROH (10 mmol) in dry THF (30 ml) was added a solution of chlorotrimethylsilane (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 eluent, to give pure phosphorofluoridites **13a**–**e**.

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] *O*-*tert*-butyl phosphorofluoridite 13a.** 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 [9 H, s, C(CH_3)$_3$], 1.41 (3 H, s, 5-CH_3), 2.40 (1 H, m, H-2′), 2.63 (1 H, m, H-2″), 3.45–3.59 (2 H, m, H-5′, -5″), 3.79 (6 H, s, OCH_3 of DMTr), 4.28–4.42 (1 H, m, H-4′), 5.24 (1 H, m, H-3′), 6.45 (1 H, dd, *J* 6.4, 6.4, H-1′), 6.84, 6.88 (4 H, 2d, *J* 8.6, 7.7, H-3, -3′, -5, -5′ of DMTr), 7.18–7.40 (9 H, ArH of DMTr); δ_C (75.47 MHz; $CDCl_3$) 11.58 (5-CH_3), 32.9 [C(*C*H$_3$)$_3$], 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).

***O*-[3′-*O*-(4,4′-Dimethoxytrityl)thymidin-5′-yl] *O*-*tert*-butyl phosphorofluoridite 13b.** Yield 92%, δ_P (121.49 MHz; $CDCl_3$) 131.8 (d, J_{PF} 1210.1), 133.3 (d, J_{PF} 1205.0); δ_F ($CDCl_3$) −51.4, (d, J_{PF} 1210.5), −52.0 (d, J_{PF} 1204.8); δ_H (300.13 MHz; $CDCl_3$) 1.33 [18 H, s, C(CH_3)$_3$], 1.90 (3 H, s, CH_3), 2.34 (1 H, m, H-2′), 2.51 (1 H, m, H-2″), 3.33 (1 H, d, H-5′), 3.67 (1 H, d, H-5′), 3.75 (6 H, s, 2 × OCH_3), 4.00 (1 H, s, H-4′), 4.30 (1 H, d, H-3′), 6.40 (1 H, m, H-1′), 6.79 (4 H, d, Ph), 7.20–7.60 (10 H, m, H-6 and Ph); δ_C (75.47 MHz, $CDCl_3$) (11.66), 11.70 (5-CH_3), 32.61 [C(*C*H$_3$)$_3$], 39.79 (C-2′), 55.29 (CH$_3$ of DMTr), 69.52 [*C*(CH$_3$)$_3$], 62.98, 63.41 (C-5′), 74.35, 75.01 (J_{POC} = 6.1 and 6.1, C-3′), 84.70 (C-1′), 85.14, 85.43 (J_{POCC} = 4.8 and 4.9 C-4′), 87.21, 87.30 (*tert*-C of DMTr), 111.43, 111.56 (C-5), 113 37 (C-3, -3′, -5, -5′ of DMTr), 128.04, 128.17, 130.15, 130.50, 131.54 (ArC of DMTr except for C-3, -3′, -5, -5′), 135.25 (C-6), 144.11 (C-1″ of DMTr), 149.35, 149.41 (C-2), 158.80 (C-4, -4′).

***O*-Citronellyl *O*-tert-butyl phosphorofluoridite 13c.** Yield 90%; δ_P (121.49 MHz; $CDCl_3$) 132.2 (d, J_{PF} 1211.0), 131.05 (d, J_{PF} 1211.5); δ_F ($CDCl_3$) −58.6 (d, J_{PF} 1211.1), −58.7 (d, J_{PF} 1211.9); δ_H (300.13 MHz; $CDCl_3$) 0.81 (3 H, d, *J* 6.01, CH_3CH), 1.09–1.42 (4 H, m), 1.29 [9 H, s, C(CH_3)$_3$], 1.53 (3 H, s, CH_3C=), 1.63 (3 H, s, CH_3C=), 1.82–2.29 (3 H, m, CH_3C*H*- and CH_2CH=C), 3.63 (2 H, m, CH_2O), 5.07 (1 H, t, *J* 6.01, CH=C); δ_C (75.47 MHz; $CDCl_3$) 17.67, 19.54, 25.41, 25.72, 29.11, 32.00, [C(*C*H$_3$)$_3$], 37.22, 39.77, 61.21, 69.77 [*C*(CH$_3$)$_3$] 124.71, 131.29.

***O*-(−)-Menthyl *O*-tert-butyl phosphorofluoridite 13d.** Yield 90%, δ_P (121.49 MHz; $CDCl_3$) 135.75 (d, J_{PF} 1207.0), 133.85 (d, J_{PF} 1201.9); δ_F ($CDCl_3$) −47.01 (d, J_{PF} 1207.2), −47.15 (d, J_{PF} 1201.5); δ_H (300.13 MHz; $CDCl_3$) 0.69 (3 H, d, *J* 6.93), 0.81 (3 H, d, *J* 6.52), 0.82 (3 H, d, *J* 7.03), 1.09 [9 H, s, C(CH_3)$_3$], 1.11–2.21 (9 H, m, H of cyclohexanol), 3.26 (1 H, t, *J* 10.44 OC*H*CH$_2$); δ_C (75.47 MHz, $CDCl_3$) 15.74, 20.86, 22.04, 22.85, 25.33, 31.50, 32.71 [C(*C*H$_3$)$_3$], 34.41, 44.83, 49.76, 70.94, 71.26 [*C*(CH$_3$)$_3$].

***O*-Cholesteryl *O*-*tert*-butyl phosphorofluoridite 13e.** Yield 95%, δ_P (121.49 MHz; $CDCl_3$) 130.9 (d, J_{PF} 1212.3), 131.1 (d, J_{PF} 1220.5); δ_F −55.95 (d, J_{PF} 1212.5), −55.90 (d, J_{PF} 1220.8); 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, br, 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).

General procedure for the synthesis of *O*-*tert*-butyl phosphorofluoridates 14a–e. A compound **13a**–**e** (10 mmol) was dissolved in dry THF (15 ml) and a solution of *tert*-butyl hydroperoxide (11 mmol) was added. The mixture was stirred 1 h at rt, concentrated *in vacuo*, and purified by column chromatography, using CH_2Cl_2–$CH_3C(O)CH_3$ (10 : 1 v/v) as eluent to give the corresponding *O*-*tert*-butyl phosphorofluoridates **14a**–**e**.

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] *O*-*tert*-butyl phosphorofluoridate 14a.** 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 [9 H, s, $C(CH_3)_3$], 1.91 (3 H, s, 5-CH_3), 2.14 (1 H, m, H-2′), 2.36 (1 H, m, H-2″), 3.35–3.69 (2 H, m, H-5′, -5″), 3.87 (6 H, s, OCH_3 of DMTr), 4.18–4.34 (1 H, m, H-4′), 5.22 (1 H, m, H-3′), 6.55 (1 H, dd, *J* 6.4, 6.4, H-1′), 6.88, 6.99 (4 H, 2d, *J* 8.6, 7.7, H-3, -3′, -5, -5′ of DMTr), 7.11–7.41 (9 H, 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).

***O*-[3′-*O*-(4,4′-Dimethoxytrityl)thymidin-5′-yl] *O*-*tert*-butyl phosphorofluoridate 14b.** Yield 98%, δ_P (121.49 MHz; $CDCl_3$) −9.85 (d, J_{PF} 985.2), −10.9 (d, J_{PF} 985.4); δ_F ($CDCl_3$) −77.9 (d, J_{PF} 985.0), −77.0 (d, J_{PF} 985.4); δ_H (300.13 MHz; $CDCl_3$) 1.50 [9 H, s, $C(CH_3)_3$], 1.81 (3 H, s, 5-CH_3), 2.00 (1 H, m, H-2′), 2.33 (1 H, m, H-2″), 3.30–3.71 (2 H, m, H-5′, -5″), 3.80 (6 H, s, OCH_3 of DMTr), 4.11–4.44 (1 H, m, H-4′), 5.17 (1 H, m, H-3′), 6.33 (1 H, dd, *J* 6.4, 6.4, H-1′), 6.66, 7.11 (4 H, 2d, *J* 8.6, 7.7, H-3, -3′, -5, -5′ of DMTr), 7.22–7.49 (9 H, ArH of DMTr); δ_C (75.47 MHz; $CDCl_3$) 10.99 (5-CH_3), 32.98 [$C(CH_3)_3$], 34.99 (C-2′), 53.49 (OCH_3 of DMTr), 64.00 (C-5′), 70.09 [$C(CH_3)_3$], 72.13, 72.45 (J_{POC} 6.1, 6.1, C-3′), 84.40 (C-1′), 85.23, 85.82 (J_{POCC} 4.8, 4.9, C-4′), 87.00, 87.23 (*tert*-C of DMTr), 111.44, 111.72 (C-5), 113.41 (C-3, -3′, -5, -5′ of DMTr), 128.10, 128.42, 130.01, 130.33, 132.00 (DMTr), 135.14, 135.62 (C-1, -1′ of DMTr), 135.88 (C-6), 144.44 (C-1″ of DMTr), 149.82, 150.11 (C-2), 158.27 (C-4, -4′ of DMTr).

***O*-Citronellyl *O*-*tert*-butyl phosphorofluoridate 14c.** Yield 98%, δ_P (121.49 MHz; $CDCl_3$) −8.00 (d, J_{PF} 979.01); δ_F ($CDCl_3$) −76.40 (d, J_{PF} 979.00), −76.95 (d, J_{PF} 973.5); δ_H (300.13 MHz; $CDCl_3$) 0.91 (3 H, d, *J* 6.01, CH_3CH), 1.07–1.44 (4 H, m), 1.38 [9 H, s, $C(CH_3)_3$], 1.57 (3 H, s, CH_3C=), 1.66 (3 H, s, CH_3C=), 1.85–2.29 (3 H, m, CH_3CH and CH_2CH=C), 3.65 (2 H, m, CH_2O), 5.27 (1 H, t, *J* 6.01, CH=C); δ_C (75.47 MHz; $CDCl_3$) 18.12, 19.68, 25.48, 25.77, 30.01, 32.56 [$C(CH_3)_3$], 37.45, 39.98, 61.67, 69.89 [$C(CH_3)_3$], 124.89, 131.45.

***O*-(−)-Menthyl *O*-*tert*-butyl phosphorofluoridate 14d.** Yield 95%, δ_P (121.49 MHz; $CDCl_3$) −10.25 (d, J_{PF} 982.91); δ_F ($CDCl_3$) −80.30 (d, J_{PF} 982.70), −79.90 (d, J_{PF} 985.90); δ_H (300.13 MHz; $CDCl_3$) 0.67 (3 H, d, *J* 6.90), 0.80 (3 H, d, *J* 6.49), 0.85 (3 H, d, *J* 7.14), 1.09 [9 H, s, $C(CH_3)_3$], 1.13–2.26 (9 H, m, H of cyclohexanol), 3.29 (1 H, t, *J* 10.39 $OCHCH_2$); δ_C (75.47 MHz, $CDCl_3$) 15.66, 21.11, 22.15, 22.99, 25.34, 31.48, 32.69 [$C(CH_3)_3$], 34.40, 44.91, 49.78, 71.00, 71.37 [$C(CH_3)_3$]

***O*-Cholesteryl *O*-*tert*-butyl phosphorofluoridate 14e.** 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, br, H-3), 5.28 (1 H, s, H-6); δ_C (75.47 MHz, $CDCl_3$) 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).

General procedure for the synthesis of *O*-*tert*-butyl thiophosphorofluoridates 15a–e

To a solution of phosphorofluoridate **13a**–**e** (10 mmol) in dry THF (15 ml) was added a saturated solution of sulfur in $(C_3H_7)_2NH$ (5 ml), with stirring, during 2 h at rt. The crude product *O*-*tert*-butyl thiophosphorofluoridate **15a**–**e** was chromatographed on silica gel, using a gradient of 0–10% $CH_3C(O)CH_3$ in CH_2Cl_2 as eluent.

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] *O*-*tert*-butyl thiophosphorofluoridate 15a.** 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 [9 H, s, $C(CH_3)_3$], 1.51 (3 H, s, 5-CH_3), 2.34 (1 H, m, H-2′), 2.63 (1 H, m, H-2″), 3.40–3.51 (2 H, m, H-5′, -5″), 3.71 (6 H, s, OCH_3 of DMTr), 4.22–4.44 (1 H, m, H-4′), 5.52 (1 H, m, H-3′), 6.14 (1 H, dd, *J* 6.4, 6.4, H-1′), 6.76, 6.98 (4 H, 2d, *J* 8.6, 7.7, H-3, -3′, -5, -5′ of DMTr), 7.13–7.39 (9 H, 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).

***O*-[3′-*O*-(4,4′-Dimethoxytrityl)thymidin-5′-yl] *O*-*tert*-butyl thiophosphorofluoridate 15b.** Yield 97%, δ_P (121.49 MHz; $CDCl_3$) 52.25, 51.08 (2d, J_{PF} 1082.2, J_{PF} 1081.9); δ_F ($CDCl_3$) −34.2, −35.0 (2d, J_{PF} 1082.7, J_{PF} 1085.9); δ_H (300.13 MHz; $CDCl_3$) 1.37 [9 H, s, $C(CH_3)_3$), 1.57 (3 H, s, 5-CH_3), 2.33 (1 H, m, H-2′), 2.69 (1 H, m, H-2″), 3.33–3.50 (2 H, m, H-5′, -5″), 3.69 (6 H, s, OCH_3 of DMTr), 4.00–4.59 (1 H, m, H-4′), 5.55 (1 H, m, H-3′), 6.11 (1 H, dd, *J* 6.4, 6.4, H-1′), 6.66, 7.00 (4 H, 2d, *J* 8.6, 7.7, H-3, -3′, -5, -5′ of DMTr), 7.10–7.41 (9 H, ArH of DMTr); δ_C (75.47 MHz; $CDCl_3$) 11.49 (5-CH_3), 33.04 [$C(CH_3)_3$], 35.60 (C-2′), 53.53 (OCH_3 of DMTr), 63.29 (C-5′), 69.76 [$C(CH_3)_3$], 71.42, 72.32 (J_{POC} 6.1, 6.1, C-3′), 84.78 (C-1′), 85.19, 85.43 (J_{POCC} 4.8, 4.9, C-4′), 87.00, 87.34 (*tert*-C of DMTr), 111.78, 111.99 (C-5), 113.57, (C-3, -3′, -5, -5′ of DMTr), 128.19, 128.30, 130.98, 131.09, 131.78 (DMTr), 135.28, 135.86 (C-1, -1′ of DMTr), 136.13 (C-6), 144.39 (C-1″ of DMTr), 149.71, 149.99 (C-2), 160.01 (C-4, -4′ of DMTr).

***O*-Citronellyl *O*-*tert*-butyl thiophosphorofluoridate 15c.** Yield 97%, δ_P (121.49 MHz; $CDCl_3$) 53.85 (d, J_{PF} 1047.19); δ_F −29.75 (d, J_{PF} 1047.20), −30.30 (d, J_{PF} 1050.90); δ_H (300.13 MHz; $CDCl_3$) 0.92 (3 H, d, *J* 6.01, CH_3CH), 1.05–1.47 (4 H, m), 1.41 [9 H, s, $C(CH_3)_3$], 1.50 (3 H, s, CH_3C=), 1.62 (3 H, s, CH_3C=), 1.83–2.31 (3 H, m, CH_3CH- and CH_2CH=C), 3.72 (2 H, CH_2O), 5.32 (1 H, t, *J* 6.01, CH=C); δ_C (75.47 MHz; $CDCl_3$) 18.09, 19.83, 25.54, 25.81, 30.43, 32.62 [$C(CH_3)_3$], 37.67, 40.04, 61.78, 71.00 [$C(CH_3)_3$], 124.94, 131.61.

***O*-(−)-Menthyl *O*-*tert*-butyl thiophosphorofluoridate 15d.** Yield 98%, δ_P (121.49 MHz; $CDCl_3$) 55.55 (d, J_{PF} 1045.19), 54.45 (d, J_{PF} 1045.70); δ_F −28.65 (d, J_{PF} 1045.20), −32.75 (d, J_{PF} 1045.70); δ_H (300.13 MHz; $CDCl_3$) 0.82 (3 H, d, *J* 6.90), 0.87 (3 H, d, *J* 6.55), 0.91 (3 H, d, *J* 7.13), 1.13–2.27 (9 H, m, H of cyclohexanol), 3.09 (1 H, t, *J* 11.11, OC*H*CH_2); δ_C (75.47 MHz; $CDCl_3$) 15.09, 21.11, 22.32, 23.01, 25.78, 31.52, 34.45, 44.95, 49.81, 70.96.

***O*-Cholesteryl *O*-*tert*-butyl thiophosphorofluoridate 15e.** 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, br, 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).

General procedure for the synthesis of *O*-(2-cyanoethyl) phosphorofluoridites 16a–e

(Route a). To a mixture of *O*-(2-cyanoethyl) *N,N*-diisopropylfluorophosphoramidite **7** (10 mmol) and tetrazole (11 mmol) in dry THF (50 ml) was added a solution of the required alcohol ROH (10 mmol) in dry THF (20 ml). The reaction mixture was stirred for 3 h and monitored 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 eluent to give the corresponding pure *O*-(2-cyanoethyl) phosphorofluoridite **16a**–**e**.

(Route b). To a solution of **7** (10 mmol) and an alcohol ROH (10 mmol) in dry THF (30 ml) was added a solution of benzoyl chloride (1 eq.) in 10 ml of THF. After 24 h at room temperature the mixture was evaporated *in vacuo* and the residue was purified by column chromatography (see route a) to obtain the pure *O*-(2-cyanoethyl) phosphorofluoridite **16a**–**e**.

(Route c). To a solution of **7** (10 mmol) in dry THF (20 ml) and an alcohol (10 mmol) in dry THF (30 ml) was added a solution of chlorotrimethylsilane (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 eluent, to give the corresponding pure *O*-(2-cyanoethyl) phosphorofluoridite **16a**–**e**.

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] *O*-(2-cyanoethyl) phosphorofluoridite 16a.** Yield 99%, δ_P (121.49 MHz; $CDCl_3$) 129.65 (d, J_{PF} 1218.5); δ_F ($CDCl_3$) −55.9 (d, J_{PF} 1218.3), −56.05 (d, J_{PF} 1220.3); δ_H (300.13 MHz; $CDCl_3$) 1.45 (3 H, s, 5-CH_3), 2.50 (1 H, m, H-2′), 2.61 (1 H, m, H-2″), 2.63 (2 H, t, *J* 6.7, OCH_2CH_2CN), 3.30–3.49 (2 H, m, H-5′, -5″), 3.75 (6 H, s, 2 × OCH_3 of DMTr), 4.12 (2 H, q, *J* 6.7, $POCH_2CH_2CN$), 4.26, 4.30 (1 H, m, H-4′), 5.14 (1 H, m, H-3′), 6.45, 6.48 (1 H, dd, *J* 8.5 and 7.1, H-1′), 6.85 (4 H, 2d, *J* 8.6 and 7.7, H-3, -3′, -5, -5′ of DMTr), 7.11–7.44 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (50.288 MHz; $CDCl_3$) 11.63, 11.72 (5-CH_3), 20.19 (OCH_2CH_2CN), 39.77 (C-2′), 55.30 (OCH_3 of DMTr), 60.11 (OCH_2CH_2CN), 62.99, 63.46 (C-5′), 74.37, 75.03 (J_{POC} 6.1 and 6.1, C-3′), 84.70 (C-1′), 85.17, 85.40 (J_{POC} 4.8 and 4.9, C-4′), 87.21, 87.30 (*tert*-C of DMTr), 111.50, 111.56 (C-5), 113.40, (C-3, -3′, -5, -5′ of DMTr), 127.50 (OCH_2CH_2CN), 128.00, 128.21, 130.11, 130.54, 131.50 (ArC of DMTr except for C-3, -3′, -5, -5′), 135.02, 135.10 (C-1, -1′ of DMTr), 135.35 (C-6), 144.10 (C-1″ of DMTr), 149.43, 149.49 (C-2), 158.80 (C-4, -4′ of DMTr).

***O*-[3′-*O*-(4,4′-Dimethoxytrityl]thymidin-5′-yl] *O*-(2-cyanoethyl) phosphorofluoridite 16b.** Yield 98%, δ_P (121.49 MHz; $CDCl_3$) 131.6 (d, J_{PF} 1219.1), 130.85 (d, J_{PF} 1220.0); δ_F ($CDCl_3$) −56.0 (d, J_{PF} 1218.3), −56.85 (d, J_{PF} 1220.3); δ_H (300.13 MHz; $CDCl_3$) 1.90 (3 H, s, 5-CH_3), 2.45 (1 H, ddd, *J* 13.8, 8.9 and 6.0, H-2″), 2.56 (1 H, m, H-2′), 2.66 (2 H, t, *J* 6.6, OCH_2CH_2CN), 3.25–3.34 (2 H, m, H-5′, -5″), 3.57 (6 H, s, 2 × OCH_3 of DMTr), 4.10 (2 H, q, *J* 6.5, $POCH_2CH_2CN$), 4.26, 4.31 (1 H, m, H-4′), 5.19 (1 H, m, H-3′), 6.45, 6.50 (1 H, dd, *J* 8.4 and 7.1, H-1′), 6.80 (4 H, 2d, *J* 8.8 and 7.1, H-3, -3′, -5, -5′ of DMTr), 7.21–7.49 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (50.288 MHz; $CDCl_3$) 11.60, 11.79 (5-CH_3), 20.21 (OCH_2CH_2CN), 39.69 (C-2′), 55.38 (OCH_3 of DMTr), 60.18 (OCH_2CH_2CN), 63.00, 63.49 (C-5′), 74.43, 75.13 (J_{POC} 6.7 and 6.9, C-3′), 84.74 (C-1′), 85.21, 85.44 (J_{POC} 4.9 and 5.0, C-4′), 87.28, 87.37 (*tert*-C of DMTr), 111.59, 111.65 (C-5), 113.41 (C-3, -3′, -5, -5′ of DMTr), 127.53 (OCH_2CH_2CN), 128.10, 128.22, 130.18, 130.55, 131.51 (ArC of DMTr except for C-3, -3′, -5, -5′), 135.08, 135.11 (C-1, -1′ of DMTr), 135.45 (C-6), 144.15 (C-1″ of DMTr), 149.46, 149.50 (C-2), 158.87 (C-4, -4′ of DMTr).

***O*-Citronellyl *O*-(2-cyanoethyl) phosphorofluoridite 16c.** Yield 95%, δ_P (121.49 MHz; $CDCl_3$) 131.05 (J_{PF} 1207.3), 132.25 (J_{PF} 1208.5); δ_F −56.35 (J_{PF} 1207.3), −56.75 (J_{PF} 1208.3); δ_H (300.13 MHz; $CDCl_3$) 0.80 (3 H, d, *J* 6.01, CH_3CH), 1.11–1.44 (4 H, m), 1.51 (3 H, s, CH_3C=), 1.60 (3 H, s, CH_3C=), 1.80–2.30 (3 H, m, CH_3CH and CH_2CH=C), 2.69 (2 H, 2t, *J* 6.0, OCH_2CH_2CN), 3.65 (2 H, m, CH_2O), 4.20–4.41 (2 H, m, OCH_2CH_2CN) 5.12 (1 H, t, *J* 6.01, CH=C); δ_C (75.47 MHz; $CDCl_3$) 17.62, 19.50, 19.99 (OCH_2CH_2CN), 25.41, 25.79, 29.83, 37.45, 39.64, 61.30, 63.24 (OCH_2CH_2CN), 115.48 (OCH_2CH_2CN), 124.69, 131.31.

***O*-(−)Menthyl *O*-(2-cyanoethyl) phosphorofluoridite 16d.** Yield 93%, δ_P (121.49 MHz; $CDCl_3$) 132.15 (J_{PF} 1206.3); δ_F −53.15 (J_{PF} 1206.9), −57.3 (J_{PF} 1209.2); δ_H (300.13 MHz; $CDCl_3$) 0.68 (3 H, d, *J* 6.91), 0.83 (3 H, d, *J* 6.50), 0.91 (3 H, d, *J* 7.03), 1.09–2.30 (9 H, m, of cyclohexanol), 2.32 (2 H, 2t, *J* 6.0, OCH_2CH_2CN), 3.38 (1 H, t, *J* 10.44, OC*H*CH_2), 4.20–4.47 (2 H, m, OCH_2CH_2CN); δ_C (75.47 MHz; $CDCl_3$) 15.09, 20.45 (OCH_2CH_2CN), 20.74, 22.33, 23.01, 25.53, 31.51, 34.52, 44.98, 49.82, 63.93 (OCH_2CH_2CN), 70.91, 115.71 (OCH_2CH_2CN).

***O*-Cholesteryl *O*-(2-cyanoethyl) phosphorofluoridite 16e.** Yield 96%, δ_P (121.49 MHz; $CDCl_3$) 131.1 (J_{PF} 1220.3), 131.4 (J_{PF} 1212.5); δ_F −55.9 (J_{PF} 1220.8), −56.0 (J_{PF} 1212.5); δ_H (300.13 MHz; $CDCl_3$) 0.63 (3 H, s, CH_3-18), 0.73 (3 H, d, *J* 6.4, CH_3-26), 0.78 (3 H, d, *J* 6.4, CH_3-27), 0.85 (3 H, d, *J* 6.4, CH_3-21), 0.95 (3 H, s, CH_3-19), 2.80 (2 H, 2t, *J* 6.0, OCH_2CH_2CN), 3.74 (1 H, d, *J* 8.0, H-7), 3.95 (1 H, br, H-3), 4.23–4.45 (2 H, m, OCH_2CH_2CN), 5.19 (1 H, s, H-6); δ_C (75.47 MHz; $CDCl_3$) 11.67 (C-18), 18.63 (C-19), 19.89 (OCH_2CH_2CN), 22.44 (C-26), 22.56 (C-27), 55.36 (C-14), 55.82 (C-17), 62.14, (OCH_2CH_2CN), 72.99 (C-7), 77.31 (C-3), 116.50 (OCH_2CH_2CN), 125.80 (C-6), 142.81 (C-5).

General procedure for the synthesis of *O*-(2-cyanoethyl) phosphorofluoridates 17a–e

To a solution of a compound **16a**–**e** (10 mmol) in dry THF (15 ml) was added *tert*-butyl hydroperoxide (11 mmol). The mixture was stirred 1 h at rt, and then was concentrated *in vacuo*. The residue was purified by column chromatography, using CH_2Cl_2–$CH_3C(O)CH_3$ (10 : 1 v/v) to give the corresponding *O*-(2-cyanoethyl) phosphorofluoridate **17a**–**e**.

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] *O*-(2-cyanoethyl) phosphorofluoridate 17a–e.** Yield 98%, δ_P (129.49 MHz; $CDCl_3$) −9.85 (d, J_{PF} 979.2); δ_F ($CDCl_3$) −78.7 (d, J_{PF} 979.6), −78.8 (d, J_{PF} 979.3); δ_H (300.13 MHz; $CDCl_3$) 1.91 (3 H, s, 5-CH_3), 2.58 (1 H, m, H-2′), 2.69 (1 H, m, H-2″), 2.71 (2 H, t,

J 6.7, OCH_2CH_2CN), 3.33–3.54 (2 H, m, H-5′, -5″), 3.77 (6 H, s, 2 × OCH_3 of DMTr), 4.11 (2 H, q, *J* 6.7, $POCH_2CH_2CN$), 4.256, 4.33 (1 H, m, H-4′), 5.14 (1 H, m, H-3′), 6.41, 6.49 (1 H, dd, *J* 8.5 and 7.1, H-1′), 6.88 (4 H, 2d, *J* 8.6 and 7.7, H-3, -3′, -5, -5′ of DMTr), 7.19–7.44 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (50.288 MHz; $CDCl_3$) 11.60, 11.78 (5-CH_3), 20.19 (OCH_2CH_2CN) 39.67 (C-2′), 55.43 (OCH_3 of DMTr), 60.91 (OCH_2CH_2CN), 62.88, 63.49 (C-5′), 74.32, 75.40 (J_{POC} 6.1 and 6.1, C-3′), 84.75 (C-1′), 85.27, 85.48 (J_{POC} 4.8 and 4.9, C-4′), 87.21, 87.33 (*tert*-C of DMTr), 111.45, 111.52 (C-5), 113.41 (C-3, -3′, -5, -5′ of DMTr), 127.45 (OCH_2CH_2CN), 128.09, 128.27, 130.17, 130.53, 131.57 (ArC of DMTr except for C-3, -3′, -5, -5′), 135.12, 135.18 (C-1, -1′ of DMTr), 135.31 (C-6), 144.17 (C-1″ of DMTr), 149.41, 149.54 (C-2), 158.78 (C-4, -4′ of DMTr).

***O*-[3′-*O*-(4,4′-Dimethoxytrityl)thymidin-5′-yl] *O*-(2-cyanoethyl) phosphorofluoridate 17b.** Yield 92%, δ_P (129.49 MHz; $CDCl_3$) −9.09 (d, J_{PF} 979.2); δ_F ($CDCl_3$) −77.7 (d, J_{PF} 979.6), −79.2 (d, J_{PF} 979.3); δ_H (300.13 MHz; $CDCl_3$) 1.88 (3 H, s, 5-CH_3), 2.47 (1 H, m, H-2′), 2.72 (1 H, m, H-2″), 2.86 (2 H, t, *J* 6.7, OCH_2CH_2CN), 3.52–3.71 (2 H, m, H-5′, -5″), 3.99 (6 H, s, 2 × OCH_3 of DMTr), 4.25 (2 H, q, *J* 6.7, $POCH_2CH_2CN$), 4.46, 4.54 (1 H, m, H-4′), 5.33 (1 H, m, H-3′), 6.43, 6.52 (1 H, dd, *J* 8.5 and 7.1, H-1′), 6.92 (4 H, 2d, *J* 8.6 and 7.7, H-3, -3′, -5, -5′ of DMTr), 7.15–7.50 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (50.288 MHz; $CDCl_3$) 11.66, 11.82 (5-CH_3), 20.21 (OCH_2CH_2CN), 39.69 (C-2′), 55.44 (OCH_3 of DMTr), 61.00 (OCH_2CH_2CN), 62.96, 63.57 (C-5′), 74.64, 75.84 (J_{POC} 6.1 and 6.1, C-3′), 84.81 (C-1′), 85.35, 85.56 (J_{POC} 4.8 and 4.9, C-4′), 87.09, 87.40 (*tert*-C of DMTr), 111.33, 111.74 (C-5), 113.56 (C-3, -3′, -5, -5′ of DMTr), 127.49 (OCH_2CH_2CN), 128.13, 128.27, 130.34, 130.87, 131.37 (ArC of DMTr except for C-3, -3′, -5, -5′), 135.20, 135.34 (C-1, -1′ of DMTr), 135.40 (C-6), 144.24 (C-1″ of DMTr), 149.89, 149.54 (C-2), 158.81 (C-4, -4′ of DMTr).

***O*-Citronellyl *O*-(2-cyanoethyl) phosphorofluoridate 17c.** Yield 94%, δ_P (121.49 MHz; $CDCl_3$) −9.45 (d, J_{PF} 978.01); δ_F ($CDCl_3$) −81.55 (d, J_{PF} 974.60), −81.60 (d, J_{PF} 978.60); δ_H (300.13 MHz; $CDCl_3$) 0.97 (3 H, d, *J* 6.01, CH_3CH), 1.18–1.46 (4 H, m), 1.54 (3 H, s, CH_3C=), 1.62 (3 H, s, CH_3C=), 1.82–2.34 (3 H, m, CH_3CH and CH_2CH=C), 2.71 (2 H, 2t, *J* 6.0, OCH_2CH_2CN), 3.71 (2 H, m, CH_2 O), 4.19–4.42 (2 H, m, OCH_2CH_2CN), 5.14 (1 H, t, *J* 6.01, CH=C); δ_C (75.47 MHz; $CDCl_3$) 16.54, 20.19 (OCH_2CH_2CN), 25.78, 26.01, 29.92, 37.76, 39.80, 61.54, 63.65 (OCH_2CH_2CN), 115.52 (OCH_2CH_2CN), 124.78, 131.50.

***O*-(−)-Menthyl *O*-(2-cyanoethyl) phosphorofluoridate 17d.** Yield 96%, δ_P (121.49 MHz; $CDCl_3$) −9.45 (d, J_{PF} 978.45), −9.40 (d, J_{PF} 974.60); δ_F ($CDCl_3$) −78.10 (d, J_{PF} 978.69), −80.05 (d, J_{PF} 974.80); δ_H (300.13 MHz; $CDCl_3$) 0.71 (3 H, d, *J* 6.94), 0.84 (3 H, d, *J* 6.52), 0.87 (3 H, d, *J* 7.03), 1.10 [9 H, s, $C(CH_3)_3$], 1.14–2.25 (9 H, m, H of cyclohexanol), 3.23 (1 H, t, *J* 10.44 $OCHCH_2$); δ_C (75.47 MHz, $CDCl_3$) 15.63, 20.92, 22.04, 22.91, 25.20, 31.41, 32.71 [$C(CH_3)_3$], 34.39, 44.80, 49.62, 70.81, 71.26 [$C(CH_3)_3$].

***O*-Cholesteryl *O*-(2-cyanoethyl) phosphorofluoridate 17e.** Yield 91%, δ_P (121.49 MHz; $CDCl_3$) −10.1 (d, J_{PF} 978.8); δ_F −79.3 (d, J_{PF} 979.4), −79.75 (d, J_{PF} 980.1); δ_H (300.13 MHz; $CDCl_3$) 0.60 (3 H, s, CH_3-18), 0.81 (3 H, d, *J* 6.4, CH_3-26), 0.80 (3 H, d, *J* 6.4, CH_3-27), 0.86 (3 H, d, *J* 6.4, CH_3-21), 1.00 (3 H, s, CH_3-19), 2.98 (2 H, t, *J* 6.2, OCH_2CH_2CN), 3.74 (1 H, d, *J* 8.0, H-7), 4.01 (1 H, br, H-3), 4.23–4.45 (2 H, m, OCH_2CH_2CN), 5.21 (1 H, s, H-6); δ_C (75.47 MHz, $CDCl_3$) 11.11 (C-18), 18.26 (C-19), 19.92 (OCH_2CH_2CN), 22.14 (C-26), 22.76 (C-27), 55.39 (C-14), 55.98 (C-17), 62.04 (OCH_2CH_2CN), 73.29 (C-7), 77.39 (C-3), 116.65 (OCH_2CH_2CN), 125.78 (C-6), 142.18 (C-5).

General procedure for the synthesis of *O*-(2-cyanoethyl) thiophosphorofluoridates 18a–e

A solution of a phosphorofluoridate **16a–e** in dry THF (10 ml) was treated a saturated solution of sulfur in $(C_3H_7)_2NH$ (5 ml) and the mixture was stirred for 2 h at rt. Crude product **18a–e** was chromatographed on silica gel, using a gradient of 0–10% $CH_3C(O)CH_3$ in CH_2Cl_2 as eluent.

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] *O*-(2-cyanoethyl) thiophosphorofluoridate 18a.** Yield 97%, δ_P (121.49 MHz; $CDCl_3$) 52.1 (d, J_{PF} 1089.0), 52.75 (d, J_{PF} 1081.0); δ_F ($CDCl_3$) −39.15 (d, J_{PF} 1089.0), −39.9 (d, J_{PF} 1080.2); δ_H (300.13 MHz; $CDCl_3$) 1.51 (3 H, s, CH_3-5, 2.45 (1 H, m, H-2′), 2.59 (1 H, m, H-2″), 2.61 (2 H, t, *J* 6.7, OCH_2CH_2CN), 3.29–3.51 (2 H, m, H-5′, -5″), 3.71 (6 H, s, 2 × OCH_3 of DMTr), 4.11 (2 H, q, *J* 6.7, $POCH_2CH_2CN$), 4.26, 4.33 (1 H, m, H-4′), 5.09 (1 H, m, H-3′), 6.44, 6.49 (1 H, dd, *J* 8.5 and 7.1, H-1′), 6.79 (4 H, 2d, *J* 8.6 and 7.7, H-3, -3′, -5, -5′ of DMTr), 7.09–7.38 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (50.288 MHz; $CDCl_3$) 11.53, 11.65 (5-CH_3), 21.21 (OCH_2CH_2CN), 39.69 (C-2′), 55.09 (OCH_3 of DMTr), 59.09 (OCH_2CH_2CN), 63.07, 63.46 (C-5′), 74.29, 75.21 (J_{POC} 6.1 and 6.1, C-3′), 84.68 (C-1′), 85.15, 85.75 (J_{POC} 4.8 and 4.9, C-4′), 87.21, 87.51 (*tert*-C of DMTr), 111.21, 111.43 (C-5), 113.09 (C-3, -3′, -5, -5′ of DMTr), 127.55 (OCH_2CH_2CN), 128.12, 128.34, 130.09, 130.76, 131.85 (ArC of DMTr except for C-3, -3′, -5, -5′), 135.78, 135.86 (C-1, -1′ of DMTr), 135.98 (C-6), 144.34 (C-1″ of DMTr), 149.43, 149.56 (C-2), 158.83 (C-4, -4′ of DMTr).

***O*-[3′-*O*-(4,4′-Dimethoxytrityl)thymidin-5′-yl] *O*-(2-cyanoethyl) thiophosphorofluoridate 18b.** Yield 92%, δ_P (121.49 MHz; $CDCl_3$) 51.09 (d, J_{PF} 1089.0), 52.90 (d, J_{PF} 1081.0); δ_F ($CDCl_3$) −39.21 (d, J_{PF} 1089.0), −39.67 (d, J_{PF} 1080.2); δ_H (300.13 MHz; $CDCl_3$) 1.72 (3 H, s, CH_3-5), 2.38 (1 H, m, H-2′), 2.63 (1 H, m, H-2″), 2.81 (2 H, t, *J* 6.7, OCH_2CH_2CN), 3.20–3.47 (2 H, m, H-5′, -5″), 3.89 (6 H, s, 2 × OCH_3 of DMTr), 4.23 (2 H, q, *J* 6.7, $POCH_2CH_2CN$), 4.44, 4.56 (1 H, m, H-4′), 5.11 (1 H, m, H-3′), 6.14, 6.49 (1 H, dd, *J* 8.5 and 7.1, H-1′), 6.81 (4 H, 2d, *J* 8.6 and 7.7, H-3, -3′, -5, -5′ of DMTr), 7.00–7.28 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (50.288 MHz; $CDCl_3$) 11.19, 11.72 (5-CH_3), 21.21 (OCH_2CH_2CN), 39.79 (C-2′), 55.22 (OCH_3 of DMTr), 59.35 (OCH_2CH_2CN), 63.14, 63.87 (C-5′), 74.37, 75.89 (J_{POC} 6.1 and 6.1, C-3′), 84.22 (C-1′), 85.37, 85.67 (J_{POC} 4.8 and 4.9, C-4′), 87.09, 87.57 (*tert*-C of DMTr), 111.34, 111.59 (C-5), 113.24 (C-3, -3′, -5, -5′ of DMTr), 127.09 (OCH_2CH_2CN), 128.09, 128.19, 130.23, 130.82, 131.90 (ArC of DMTr except for C-3, -3′, -5, -5′), 135.00, 135.16 (C-1, -1′ of DMTr), 135.67 (C-6), 144.99 (C-1″ of DMTr), 149.53, 149.98 (C-2), 158.02 (C-4, -4′ of DMTr).

***O*-Citronellyl *O*-(2-cyanoethyl) thiophosphorofluoridate 18c.** Yield 92%; δ_P (129.49 MHz; $CDCl_3$) 53.05 (d, J_{PF} 1047.00); δ_F −28.42 (d, J_{PF} 1047.21), −29.60 (d, J_{PF} 1053.10); δ_H (300.13 MHz; $CDCl_3$) 0.92 (3 H, d, *J* 6.01, CH_3CH), 1.15–1.46 (4 H, m), 1.57 (3 H, s, CH_3C=), 1.65 (3 H, s, CH_3C=), 1.75–2.33 (3 H, m, CH_3CH and CH_2CH=C), 2.69 (2 H, 2t, *J* 6.0, OCH_2CH_2CN), 3.78 (2 H, m, CH_2O), 4.19–4.48 (2 H, m, OCH_2CH_2CN), 5.18 (1 H, t, *J* 6.01, CH=C); δ_C (75.47 MHz; $CDCl_3$) 17.09, 20.32 (OCH_2CH_2CN), 20.56, 25.91, 26.43, 29.99, 37.89, 39.91, 61.77, 63.78 (OCH_2CH_2CN), 115.61 (OCH_2CH_2CN), 124.99, 131.76.

***O*-(−)-Menthyl *O*-(2-cyanoethyl) thiophosphorofluoridate 18d.** Yield 95%; δ_P (129.49 MHz; $CDCl_3$), 53.60 (d, J_{PF} 1049.10), 54.90 (d, J_{PF} 1047.91); δ_F −26.75 (d, J_{PF} 1047.90), −26.90 (d, J_{PF} 1050.10); δ_H (300.13 MHz; $CDCl_3$) 0.77 (3 H, d, *J* 6.91), 0.82 (3 H, d, *J* 6.50), 0.89 (3 H, d, *J* 7.03), 1.11–2.23 (9 H, m, H of cyclohexanol), 2.71 (2 H, 2t, *J* 6.0, OCH_2CH_2CN), 3.33 (1 H, t, *J* 10.44, $OCHCH_2$), 4.21–4.45 (2 H, m, OCH_2CH_2CN); δ_C (75.47 MHz; $CDCl_3$) 15.33, 20.30 (OCH_2CH_2CN), 20.45,

22.15, 22.99, 25.47, 31.57, 34.48, 44.91, 49.78, 63.91 (OCH_2-CH_2CN), 70.89, 115.70 (OCH_2CH_2CN).

***O*-Cholesteryl *O*-(2-cyanoethyl) phosphorofluoridate 18e.** Yield 95%; δ_P (129.49 MHz; $CDCl_3$) 53.95 (d, J_{PF} 1050.0); δ_F −30.05 (d, J_{PF} 1050.6), −30.10 (d, J_{PF} 1051.1); δ_H (300.13 MHz; $CDCl_3$) 0.54 (3 H, s, CH_3-18), 0.75 (3 H, d, *J* 6.4, CH_3-26), 0.79 (3 H, d, *J* 6.4, CH_3-27), 0.89 (3 H, d, *J* 6.4, CH_3-21), 1.09 (3 H, s, CH_3-19), 2.82 (2 H, 2t, OCH_2CH_2CN), 3.79 (1 H, d, *J* 8.0, H-7), 3.95 (1 H, br, H-3), 4.35–4.50 (2 H, m, OCH_2CH_2CN), 5.31 (1 H, s, 6-H); δ_C (50.288 MHz; $CDCl_3$) 11.07 (C-18), 18.68 (C-19), 19.11 (OCH_2CH_2CN), 22.94 (C-26), 22.98 (C-27), 55.73 (C-14), 55.98 (C-17), 72.91 (C-7), 77.73 (C-3), 117.53 (OCH_2-CH_2CN), 125.75 (C-6), 143.01 (C-5).

General procedure for the synthesis of phosphoromonofluoridates 1a–e

(Route a). A solution of a compound **14a–e** in CH_3CN was refluxed for 2 h at 80 °C. Thermal elimination of the vinyl cyanide group gave the desired corresponding compound **1a–e**, which was purified by silica gel column chromatography [CH_2Cl_2–$CH_3C(O)CH_3$ (10 : 2 v/v)].

(Route b). A solution of a compound **17a–e** in dry THF (10 ml) was treated with Et_3N; after a further 6 h, the mixture was concentrated under reduced pressure and the residue was purified by silica gel chromatography [CH_2Cl_2–$CH_3C(O)CH_3$ (10 : 2 v/v)] to obtain the corresponding phosphoromonofluoridate **1a–e**.

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] hydrogen phosphorofluoridate 1a.** Yield 94%, δ_P (121.49 MHz; $CDCl_3$) −9.4 (d, J_{PF} 929); δ_F ($CDCl_3$) −80.05 (d, J_{PF} 929); δ_H (300.13 MHz; $CDCl_3$) 1.65 (3 H, s, CH_3-5), 2.55 (1 H, m, H-2′), 2.63 (1 H, m, H-2″), 3.33–3.54 (2 H, m, H-5′, -5″), 3.87 (6 H, s, 2 × OCH_3 of DMTr), 4.26, 4.33 (1 H, m, H-4′), 5.24 (1 H, m, H-3′), 6.14, 6.38 (1 H, dd, *J* 8.5 and 7.1, H-1′), 6.88 (4 H, 2d, *J* 8.6 and 7.7, H-3, -3′, -5, -5′ of DMTr), 7.13–7.54 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (75.47 MHz; $CDCl_3$) 11.16, 11.78 (5-CH_3), 38.88 (C-2′), 55.33 (OCH_3 of DMTr), 63.28, 63.57 (C-5′), 74.23, 75.10 (J_{POC} 6.1 and 6.1, C-3′), 84.73 (C-1′), 85.12, 85.34 (J_{POCC} 4.8 and 4.9, C-4′), 87.21, 87.30 (*tert*-C of DMTr), 111.05, 111.56 (C-5), 113.49 (C-3, -3′, -5, -5′ of DMTr), 128.11, 128.92, 130.61, 130.89, 131.85 (ArC of DMTr except for C-3, -3′, -5, -5′), 134.99, 135.19 (C-1, -1′ of DMTr), 135.82 (C-6), 144.87 (C-1″ of DMTr), 149.41, 150.49 (C-2), 158.83 (C-4, -4′ of DMTr).

***O*-[3′-*O*-(4,4′-Dimethoxytrityl)thymidin-5′-yl] hydrogen phosphorofluoridate 1b.** Yield 92%, δ_P (121.49 MHz; $CDCl_3$) −6.4 (d, J_{PF} 929); δ_F ($CDCl_3$) −80.15 (d, J_{PF} 929); δ_H (300.13 MHz; $CDCl_3$) 1.75 (3 H, s, CH_3-5), 2.50 (1 H, m, H-2′), 2.60 (1 H, m, H-2″), 3.30–3.58 (2 H, m, H-5′, -5″), 3.91 (6 H, s, 2 × OCH_3 of DMTr), 4.36, 4.44 (1 H, m, H-4′), 5.77 (1 H, m, H-3′), 6.32, 6.47 (1 H, dd, *J* 8.5 and 7.1, H-1′), 6.90 (4 H, 2d, *J* 8.6 and 7.7, H-3, -3′, -5, -5′ of DMTr), 7.09–7.50 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (75.47 MHz; $CDCl_3$) 11.22, 11.98 (5-CH_3), 38.96 (C-2′), 55.41 (OCH_3 of DMTr), 63.31, 63.50 (C-5′), 74.34, 75.90 (J_{POC} 6.1 and 6.1, 3′-C), 84.79 (C-1′), 85.21, 85.47 (J_{POCC} 4.8 and 4.9, C-4′), 87.33, 87.42 (*tert*-C of DMTr), 111.15, 111.50 (C-5), 113.51 (C-3, -3′, -5, -5′ of DMTr), 128.32, 128.99, 130.73, 130.94, 131.92 (ArC of DMTr except for C-3, -3′, -5, -5′), 135.00, 135.32 (C-1, -1′ of DMTr), 135.90 (C-6), 144.93 (C-1″ of DMTr), 149.49, 150.57 (C-2), 158.90 (C-4, -4′ of DMTr).

***O*-Citronellyl hydrogen phosphorofluoridate 1c.** Yield 93%, δ_P (121.49 MHz; $CDCl_3$) −8.85 (d, J_{PF} 974.01); δ_F −81.40 (d, J_{PF} 978.40); δ_H (300.13 MHz; $CDCl_3$) 0.88 (3 H, d, *J* 6.01, CH_3CH), 1.09–1.42 (4 H, m), 1.51 (3 H, s, CH_3C=), 1.62 (3 H, s, CH_3C=), 1.79–2.33 (3 H, m, CH_3CH and CH_2CH=C), 3.65 (2 H, m, CH_2-O), 5.16 (1 H, t, *J* 6.01, CH=C); δ_C (75.47 MHz; $CDCl_3$) 17.56, 19.98, 24.98, 25.82, 30.24, 37.87, 39.71, 61.89, 124.91, 131.64.

***O*-(−)Menthyl hydrogen phosphorofluoridate 1d.** Yield 94%, δ_P (121.49 MHz; $CDCl_3$) −8.10 (d, J_{PF} 942.51); δ_F −76.80 (d, J_{PF} 942.90); δ_H (300.13 MHz; $CDCl_3$) 0.98 (3 H, d, *J* 6.94), 1.01 (3 H, d, *J* 6.50), 0.12 (3 H, d, *J* 7.03), 1.14–2.32 (9 H, m, H of cyclohexanol), 3.43 (1 H, t, *J* 10.44, $OCHCH_2$); δ_C (75.47 MHz; $CDCl_3$) 15.45, 21.45, 22.34, 22.91, 25.43, 31.78, 34.44, 44.91, 49.78, 70.95.

***O*-Cholesteryl hydrogen phosphorofluoridate 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, br, H-3), 5.20 (s, 1 H, H-6); δ_C (75.47 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).

General procedure for the synthesis of thiophosphorofluoridates 2a–e

(Route a). A solution of a compound **15a–e** in CH_3CN was refluxed 2 h at 80 °C. Thermal elimination of the vinyl cyanide group gave the corresponding compound **2a–e**, which was purified by column chromatography [$CH_3C(O)CH_3$–CH_2Cl_2 (10 : 2)].

(Route b). The β-elimination of the *O*-(2-cyanoethyl) group from a substrate **18a–e** gave the desired product thiophosphorofluoridate **2a–e**, which was purified by column chromatography, using the same eluent as for compounds **1a–e**.

***O*-[5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl] hydrogen thiophosphorofluoridate 2a.** Yield 96%; δ_P (129.49 MHz; $CDCl_3$) 54.35 (d, J_{PF} 1054.0), 54.45 (d, J_{PF} 1054.1); δ_F (200.13 MHz, $CDCl_3$) −30.2 (d, J_{PF} 1054.1), −30.4 (d, J_{PF} 1054.2); δ_H (200.13 MHz; $CDCl_3$) 1.71 (3 H, CH_3-5), 2.51 (1 H, m, H-2′), 2.67 (1 H, m, H-2″), 3.30–3.51 (2 H, m, H-5′, -5″), 3.78 (6 H, s, OCH_3 of DMTr), 4.22, 4.37 (1 H, m, H-4′), 5.19 (1 H, m, H-3′), 6.23, 6.50 (1 H, 2d, *J* 8.1, 7.1, H-1′), 6.79 (4 H, 2d, *J* 8.6, 7.7, H-3, -3′, -5, -5′ of DMTr), 7.21–7.39 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (50.288 MHz; $CDCl_3$) 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, C-3′), 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 (C-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′ of DMTr), 135.75 (C-6), 144.89 (C-1″ of DMTr), 149.63, 149.97 (C-2), 158.91 (C-4, -4′ of DMTr).

***O*-[3′-*O*-(4,4′-Dimethoxytrityl)thymidin-5′-yl] hydrogen thiophosphorofluoridate 2b.** Yield 94%; δ_P (129.49 MHz; $CDCl_3$) 54.00 (d, J_{PF} 1054.0), 54.49 (d, J_{PF} 1054.1); δ_F ($CDCl_3$) −30.82 (d, J_{PF} 1054.1), −30.94 (d, J_{PF} 1054.2); δ_H (200.13 MHz, $CDCl_3$) 1.77 (3 H, CH_3-5), 2.61 (1 H, m, H-2′), 2.73 (1 H, m, H-2″), 3.26–3.64 (2 H, m, H-5′, -5″), 3.82 (6 H, s, OCH_3 of DMTr), 4.34, 4.41 (1 H, m, H-4′), 5.22 (1 H, m, H-3′), 6.20, 6.52 (1 H, 2d, *J* 8.1, 7.1, H-1′), 6.81 (4 H, 2d, *J* 8.6 7.7, H-3, -3′, -5, -5′ of DMTr), 7.18–7.43 (2 H, ArH of DMTr except for H-3, -3′, -5, -5′); δ_C (50.288 MHz; $CDCl_3$) 10.11, 10.99 (5-CH_3), 40.22 (C-2′), 55.47 (OCH_3 of DMTr), 62.89, 63.56 (C-5′), 74.48, 75.00 (J_{POC} 6.1, 6.1, C-3′), 84.93 (C-1′), 85.35, 85.98 (J_{POCC} 4.8, 4.9, C-4′), 87.52, 87.88 (*tert*-C of DMTr), 111.33, 111.99 (C-5),

113.56 (C-3, -3′, -5, -5′ of DMTr), 128.34, 128.58, 130.63, 130.90, 131.54 (ArC of DMTr except for C-3, -3′, -5, -5′), 135.41, 135.67 (C-1, -1′ of DMTr), 135.84 (C-6), 144.83 (C-1″ of DMTr), 149.77, 150.00 (C-2), 159.04 (C-4, -4′ of DMTr).

***O*-Citronellyl hydrogen thiophosphorofluoridate 2c.** Yield 95%; δ_P (129.49 MHz; $CDCl_3$) 52.15 (d, J_{PF} 1051.60); δ_F ($CDCl_3$) −31.62 (d, J_{PF} 1051.60), −31.65 (d, J_{PF} 1050.30); δ_H (300.13 MHz; $CDCl_3$) 0.89 (3 H, d, *J* 6.01, C*H*$_3$CH), 1.11–1.35 (4 H, m), 1.49 (3 H, s, CH_3C=), 1.73 (3 H, s, CH_3C=), 1.88–2.39 (3 H, m, CH_3CH and CH_2CH=C), 3.77 (2 H, m, CH_2O), 5.89 (1 H, t, *J* 6.01, CH=C); δ_C (75.47 MHz; $CDCl_3$) 17.04, 21.01, 25.93, 26.22, 30.01, 37.76, 39.95, 61.54, 124.91, 131.76.

***O*-(−)-Menthyl hydrogen thiophosphorofluoridate 2d.** Yield 98%; δ_P (129.49 MHz; $CDCl_3$), 60.20 (d, J_{PF} 1049.11), 60.90 (d, J_{PF} 1053.65); δ_F ($CDCl_3$) −37.30 (d, J_{PF} 1055.80), −40.70 (d, J_{PF} 1049.80); δ_H (300.13 MHz; $CDCl_3$) 0.80 (3 H, d, *J* 6.95), 0.89 (3 H, d, *J* 6.50), 0.91 (3 H, d, *J* 7.13), 1.15–2.23 (9 H, m, H of cyclohexanol), 3.30 (1 H, t, *J* 10.00, OC*H*CH_2); δ_C (75.47 MHz; $CDCl_3$) 15.29, 20.65, 22.09, 22.30, 25.89, 31.37, 34.48, 44.54, 49.78, 70.80.

***O*-Cholesteryl hydrogen thiophosphorofluoridate 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, br, H-3), 5.09 (1 H, s, H-6); δ_C (75.47 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).

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