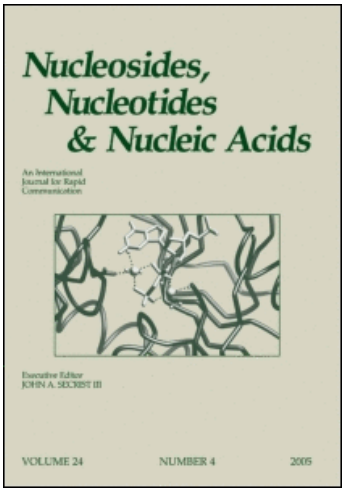

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New Efficient Synthesis of Thymidine Cyclic 3', 5'- Phosphorofluoridate and Its Sulfur Analogue via the Phosphoroamidite Route

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NEW EFFICIENT SYNTHESIS OF THYMIDINE CYCLIC 3', 5'-PHOSPHOROFLUORIDATE AND ITS SULFUR ANALOGUE VIA THE PHOSPHOROAMIDITE ROUTE‡

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Abstract: We describe the convenient synthesis of thymidine cyclic 3', 5'-phosphorofluoridate **6**, which is superior to that previously reported. Our procedure is based on a sequence of reactions utilizing **3** as the key substrate. Similar sequence of reaction leads to the sulfur analogues of **6** the thymidine cyclic 3', 5'-phosphorofluoridothioate **7**.

The presence of fluorine in biomolecules frequently changes their biological properties. This is equally true for compounds containing a phosphorus-fluorine bond. For example, Krayevsky and associates[1] have demonstrated that 5'-fluorophosphates inhibit the phosphorylation reaction catalyzed by adenylate kinases and effectively inhibit HIV reproduction in cell cultures, thus opening a new group of potential antiretroviral nucleoside derivatives. Cyclic nucleotides are of great importance for regulation of the activity of enzymes.[2]

In our extensive studies on modified nucleotides containing a P-F bond, we have described synthesis of the first thymidine cyclic phosphorofluoridate.[3] The efficiency of this synthesis, proceeding via the phosphorus-sulfonic anhydride, is low and requires tedious synthetic procedures. Here we report an efficient synthesis of thymidine cyclic 3',5'-phosphorofluoridate **6** and its sulfur analogue via the phosphoroamidite route. This synthesis is based on the new phosphitylating reagent **3** containing the P(III)-F group.

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‡ This paper is dedicated to the memory of Professor Alexander A. Krayevsky.

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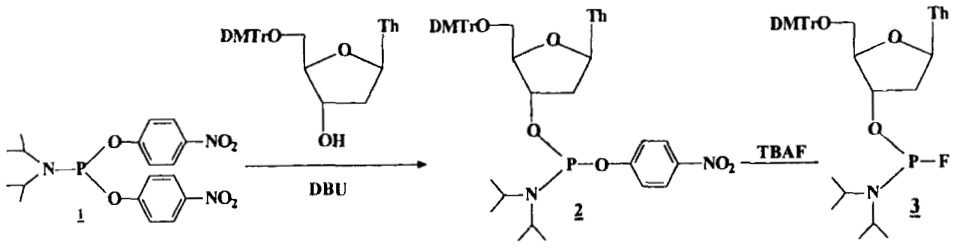
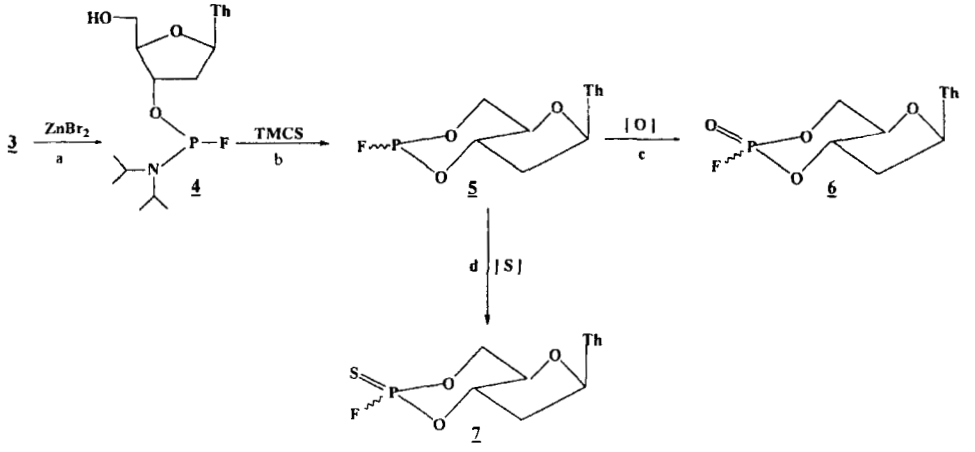
The key substrate **3** was prepared by the procedure described in Scheme 1.



Scheme 1

Phosphoroamidite **1**, which is readily available from commercial reagents, reacts selectively with 5'*O*-DMTr thymidine in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) giving the desired fluoridoamidite **3** by nucleophilic replacement of the 4-nitroaryloxy group by the fluorine ligand with an aid of tetrabutylamonium fluoride (TBAF).[4,5] All these reactions proceed in THF or acetonitrile solution at ambient temperature and can be performed as a one-flask procedure. 5'-O-(4,4'-Dimethoxytrityl)thymidin-3'-yl-(N,N-diisopropylamino)-phosphorofluoridites **3**, purified by silica gel chromatography, is formed in 85% yield.

The fluorophosphoramidite **3** is readily converted into its 5'-hydroxy derivative **4** by removal of the DMTr group by $ZnBr_2$ without affecting the P(III)-F bond (Scheme 2, reaction a). The fluorophosphoramidite **4** undergoes cyclization in the presence of trimethylchlorosilane (TMCS) (reaction b). This catalyst proved to be superior to tetrazole and similar activators.[6] When a enriched mixture of diastereoisomers **4** (60:40) was used a (50:50) mixture of diastereoisomer **5** was formed. Lack of stereoselectivity by phosphoramidite in phosphitylations catalyzed by TMCS seems be a general phenomenon explicable by mechanistic features of its action.[6]



Scheme 2

The cyclic phosphorofluoridite **5** was oxidized by *tert*-butylhydroperoxide to give thymidine cyclic 3', 5'-phosphorofluoridate **6** as a (50:50) mixture of diastereoisomers (reaction c). Similarly the fluoridite **5** was converted into thymidine cyclic 3',5'-phosphorofluoridothionate **7** by the addition of elemental sulfur in the presence of diisopropylamine (reaction d). In this case to, a (50:50) mixture of diastereoisomers was formed. Compounds **5**, **6** and **7** are stable enough to be purified by silica gel column chromatography. So far we have not been able to separate them into single diastereisomers. Reactions described in Scheme 2 can be performed as one-flask procedure in over 90% yield.

The ^{31}P and ^{19}F NMR spectra of compounds **5**, **6** and **7** are shown in Table 1. The multiplicity of ^{31}P and ^{19}F signals indicate as formation of (1:1) diastereoizomeric mixtures and fully confirms their structure which is also supported by ^{13}C and spectra HRMS FAB.

Table 1

No	^{31}P NMR (ppm) ($CDCl_3$)	^{19}F NMR (ppm) ($CDCl_3$)	J_{P-F} (Hz.)
5	129.38, 113.99 128.24, 112.81	-50.95, -57.57 -51.44, -58.07	1246.8 1249.8
6	-10.91, -23.29	-73.69, -79.03	1004.26
7	60.19, 47.10 59.79, 46.76	-26.74, -32.35 -27.30, -32.93	1055.24 1059.61

This work opens new possibilities for synthesis of cyclic nucleotides. Efforts toward this goal are currently being made in our laboratory.

Experimental

The solvents were reagent grade and were distilled and dried by conventional methods before use. NMR spectra were obtained on a Bruker AC 200 MHz spectrometers. δ-Values are reported in ppm relative to Me_4Si as standard for ^1H NMR (200.13 MHz) and ^{13}C NMR (50.33 MHz), relative to H_3PO_4 as external standard for ^{31}P NMR (81.014 MHz.), as relative to $CFCl_3$ as external standard for ^{19}F NMR (188.154 MHz). The singals are expressed as s (singlet), d (doublet), t (triplet) or m (multiplet). Coupling constants (*J*) are in Hz. The unprotected thymidine was purchased from Sigma. These compounds were reacted with 4,4'-dimethoxytriphenylmethyl chloride by standard procedures and 5'-*O*-DMT thymidine were isolated by column chromatography on silica gel. Trimethylchlorosilane was purchased from Fluka. Thin layer chromatography (TLC) was performed on plates silica gel 60F-254 (Merck).

The products were purified by flash chromatography on silica gel 60 (Merck; 0.063 mm, 230-400 mesh ASTM).

N,N-Diisopropyl-bis-O-4-nitrophenylphosphoamidite 1. A solution of *N,N*-diisopropyldichlorophosphoamidite (0.01 M) in dry THF (10 ml) was added dropwise at r.t. under a nitrogen atmosphere to a solution of sodium 4-nitrophenolate (0.025 M) in dry THF (50 ml) with stirring for 2 h. The sodium chloride was removed by filtration. The filtrate was evaporated to dryness and crude **1** purified by column chromatography (Et_2O: n-pentane: triethylamine 50:30:5 v/v, Rf: 0.75) to give *N,N*-diisopropyl-bis-*O*-4-nitrophenyl-phosphoamidite. Yield of isolated **1**: 95%. δ_P ($CDCl_3$) 144.8; δ_H ($CDCl_3$) 1.01 (12 H, d, *J* 6.8 $N[CH(C\underline{H}_3)_2]_2$), 3.46-3.65 (2 H, m, $N[C\underline{H}(CH_3)_2]_2$), 6.75 (4 H, d, *J* 9.13, Ph-H_{ortho}) 7.86 (4 H, d, *J* 9.12, Ph-H_{meta}), m.p. 120°C-122°C; pale yellow crystals; HRMS FAB [M-H] calcd. for $C_{18}H_{21}N_3O_6P$ 406.1168, found 406.1157

5'-O-(4,4'-Dimethoxytrityl)thymidin-3'-yl-O-4-nitrophenyl N,N-diisopropylphospho-amidite 2. The solution of 5'-*O*-(*4,4'*-dimethoxytrityl)thymidine (1.0 mmol) and DBU (1.1 mM) in dry acetonitrile (10 ml) was added dropwise at r.t. under a nitrogen atmosphere to the solution of amidite **1** (1.1 mmol) in dry acetonitrile (10 ml) with stirring for 10 min. The solution was evaporated to dryness and crude **2** purified by column chromatography (1,2-dichloroethane: ethyl acetate 8:2 v/v, Rf = 0.7) to give 5'-*O*-DMT-thymidin-3'-yl-*O*-4-nitrophenyl-*N,N*-diisopropylphosphoamidite **2** Yield 98 %; m.p. 105-107°; pale yellow crystals. δ_P ($CDCl_3$) 148.7, 147.8 (1:1); δ_H ($CDCl_3$) 1.18 1.21 (12 H, 2d, *J* 7.3, 6.9, CH_3 of isopropyl), 1.80 (3 H, s, 5-CH_3), 2.25 (1 H, m, H-2'), 2.75 (1 H, m, H-2''), 3.27-3.51 (4 H, m, 5', 5'' and CH of isopropyl), 3.67 (6 H, s, OCH_3 of DMTr), 4.24, 4.35 (1 H, m, H-4'), 5.55 (1 H, m, H-3'), 6.19, 6.43 (1 H, dd, *J* 8.1, 7.1 H-1'), 6.72 (4 H, 2d, *J* 8.6, 7.7 H-3, 3', 5, 5' of DMTr), 7.04-7.32 (11 H, ArH of DMTr except for H-3, 3', 5, 5' and 4-NO_2-Ph-H_{ortho}), 8.40 (2H, d, *J* 6.11 4-NO_2-Ph-H_{meta}); δ_C($CDCl_3$) 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-4 of 4-NO_2Ph), 128.11, 128.89, 130.32, 130.78, 131.66 (ArC of DMTr except for C-3, 3', 5, 5'), 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), 158.78 (C-4, 4' of DMTr), 162.31 (d, *J* 6.91, C-1 of 4-NO_2Ph); HRMS FAB [M-H] calcd. for $C_{43}H_{48}N_4O_{10}P$, 811.3108 found 811.3178.

5'-O-(4,4'-Dimethoxytrityl)thymidin-3'-yl-(N,N-diisopropylamino)phosphoro-fluoridite 3. To a solution of **2** (0.2 mmol)in 10 mL of dry THFwas added TBAF (0.25 mmol) at room temperature. After 10 min. tetrabutylammonium 4-nitrophenolate was removed by filtration. The filtrate was concentrated in vacuo and the residue was purified by column chromatography on silica gel, using CH_2Cl_2:CH_3COCH_3 (10:1 v/v) as an eluent to give pure fluoridite **3**. Yield 95%. δ_P ($CDCl_3$) 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 ($CDCl_3$) 1.20 1.21 (12 H, 2d, *J* 7.3, 6.9, CH_3 of isopropyl), 1.51 (3 H, s, 5-CH_3), 2.45 (1 H, m, H-2'), 2.68 (1 H, m, H-2"), 3.30-3.51 (4 H, m, 5', 5" and CH of isopropyl), 3.86 (6 H, s, OCH_3 of DMTr), 4.28, 4.32 (1 H, m, H-4'), 5.34 (1 H, m, H-3'), 6.40, 6.51 (1 H, dd, *J* 8.1, 7.1 H-1'), 6.82 (4 H, 2d, *J* 8.6, 7.7 H-3, 3', 5, 5' of DMTr), 7.10-7.45 (2 H, ArH of DMTr except for H-3, 3', 5, 5'); δ_C($CDCl_3$) 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); HRMS FAB [M-H] calcd. for $C_{37}H_{44}N_3O_7FP$ 692.2900, found 692.2912.

Thymidin-3'-yl-(N,N-diisopropylamino)phosphorofluoridite 4. To a saturated solution of $ZnBr_2$ (0.2 mmol) in 10 mL of CH_3NO_2 compound **3** was added (0.25 mmol) at room temperature, stirred for 4h and then concentrated *in vacuo*. Crude product was was purified by column chromatography on silica gel, using a gradient of 0-10% CH_3COCH_3 in CH_2Cl_2 to give fluoridite **4**. Yield 95%. δ_P ($CDCl_3$) 154.0 (d, J_{PF} 1115.8), 150.2 (d, J_{PF} 1115.0); δ_F ($CDCl_3$) -75.05 (d, J_{PF} 1116.9), -75.10 (d, J_{PF} 1114.6); δ_H ($CDCl_3$) 1.19 1.20 (12 H, 2d, *J* 7.2, 6.9, CH_3 of isopropyl), 1.50 (3 H, s, 5-CH_3), 2.40 (1 H, m, H-2'), 2.68 (1 H, m, H-2"), 3.32-3.50 (4 H, m, 5', 5" and CH of isopropyl), 3.86 (6 H, s, OCH_3 of DMTr), 4.28, 4.32 (1 H, m, H-4'), 5.34 (1 H, m, H-3'), 6.41, 6.55 (1 H, dd, *J* 8.1, 7.1 H-1'); δ_C(CDCl3) 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), 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'), 111.65, 111.76 (C-5), 135.45 (C-6), 149.30, 149.51 (C-2); HRMS FAB [M-H] calcd. for $C_{16}H_{26}N_3O_5FP$ 391.1672, found 391.1681.

Thymidine cyclic 3',5-phosphorofluoridite 5. To a solution of compound **4** (0.2 mmol) in 10 mL of dry THF was added TMCS (0.25 mmol) at room temperature. After 1h min the reaction mixture was concentrated in vacuo and the residue was purified by column chromatography on silica gel, using gradient of 0-10% CH_3COCH_3 in CH_2Cl_2 to give pure fluoridite **5** (δ_P see Table 1). Yield 97%. δ_C(CDCl3) 11.60, 11.77 (5-CH_3), 34.78 ($J_{PC2'}$ 8.1, C-2'), 67.71, ($J_{PC5'}$ 6.7, C-5'), 75.35 ($J_{PC4'}$ 4.3 C-4'), 75.55 ($J_{PC3'}$ 4.0, C-3'), 84.09 (C-1'), 115.65, (C-5), 135.95 (C-6), 150.13 (C-2) 163.20 (C-4); HRMS FAB [M-H] calcd. for $C_{10}H_{11}N_2O_5FP$ 289.0390, found 289.0398.

Thymidine cyclic 3',5'-phosphorofluoridate 6. To a solution of phosphorfluoridite **5** (1.0 mmol) in dry acetonitrile (10 ml) was added dropwise at r.t. under a nitrogen atmosphere a solution of *tert*-butylhydroperoxide (1.1 mmol) in CH_2Cl_2 (10 ml) with stirring for 10 min. After 3h the solution was evaporated to dryness and crude **6** purified by column chromatography (1,2-dichloroethane: ethyl acetate 8:2 v/v, Rf = 0.35) to give **6**.(δ_P see Table 1) Yield 95%.δ_C(CDCl3) 11.61, 11.75 (5-CH_3), 34.38 ($J_{PC2'}$ 8.1, C-2'), 68.79, ($J_{PC5'}$ 6.7, C-5'), 73.45 ($J_{PC4'}$ 4.3 C-4'), 74.75 ($J_{PC3'}$ 4.0, C-3'), 82.39 (C-1'), 117.25, (C-5), 145.25 (C-6), 151.13 (C-2) 162.21 (C-4); HRMS FAB [M-H] calcd. for $C_{10}H_{11}N_2O_6FP$ 305.0339, found 305.0378.

Thymidine cyclic 3',5'-phosphorofluoridothioate 7. To a solution of phosphoro-fluoridite **5** (1.0 mmol) in dry acetonitrile (10 ml) was added dropwise at r.t. under a nitrogen atmosphere a solution of sulphur in $(C_3H_7)_2NH$ (10 ml) with stirring for 4h. The mixture reaction was concentrated in vacuo and the residue was purified by column chromatography on silica gel, using gradient of 0-10% CH_3COCH_3 in CH_2Cl_2 to give pure phosphoro-fluoridothioate **7** (δ_P see Table 1). Yield 97%.δ_C(CDCl3) 10.61, 10.55 (5-CH_3), 35.98 ($J_{PC2'}$ 8.1, C-2'), 67.49, ($J_{PC5'}$ 6.7, C-5'), 70.45 ($J_{PC4'}$ 4.3 C-4'), 75.95 ($J_{PC3'}$ 4.0, C-3'), 81.49 (C-1'), 115.35, (C-5), 143.25 (C-6), 147.13 (C-2) 164.11 (C-4); HRMS FAB [M-H] calcd. for $C_{10}H_{11}N_2O_5FSP$ 321.0110, found 321.01121.

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