Fluorination of Trimethylsilyl Phosphites and their Structural Analogues by Sulfuryl Chloride Fluoride: Simple Preparation of Phosphorofluoridates and Related Compounds, including Deoxynucleoside Phosphorofluoridates

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Trimethylsilyl esters of general formula $RR'POSiMe_3$ react in a quantitative and fully chemoselective way with sulfuryl chloride fluoride, $ClSO_2F$, under extremely mild conditions to give phosphorofluoridates $RO(R'O)P(O)F$ of high purity. This work provides easy access to all type of fluoridates from non-toxic starting materials. The synthetic usefulness of this method is illustrated by the synthesis of 3'- and 5'-mononucleoside phosphorofluoridates **10** and 3',5'-dinucleoside phosphorofluoridates **11** from the corresponding nucleoside trimethylsilyl phosphites.

Fluoro derivatives of phosphorus are of great importance in the chemistry and biochemistry of both elements. The incorporation of fluorine into biomolecules has frequently resulted in markedly changed biological properties. Simple phosphorofluoridates and their structural analogues are the classic inhibitors of serine proteases.[1] It can be reasoned that combination of nucleoside fragments with the phosphorofluoridate moiety could result in new properties with respect to selectivity of interaction with the active site of the serine hydroxy group. On the other hand, nucleotides containing a P–F linkage incorporated into oligonucleotides could be used in studies of biological functions of nucleic acids, including possibilities connected with the chirality of the phosphorofluoridate moiety.

Numerous pathways to phosphorofluoridates and their structural analogues have previously been described. In our opinion many of them are not suitable for application to sensitive molecules like nucleotides.

Recently, sulfuryl chloride fluoride, which is easy to prepare and handle, has been used as a fluorinating reagent in organophosphorus–fluorine chemistry.[2] The dialkyl phosphites and compounds of general formula $RR'P(O)H$ **1** react with sulfuryl chloride fluoride only in the presence of bases or as their alkali metal salts **2**.[2] Even under conditions where fluoro products are predominant, they are not readily separable from the corresponding chlorides [eqn. (1)].

$$\underset{\mathbf{2}}{RR'P{-}O^-\ M^+} + SO_2ClF \xrightarrow[-SO_2]{} \underset{\mathbf{3}}{RR'P(O)F} + RR'P(O)Cl \quad (1)$$

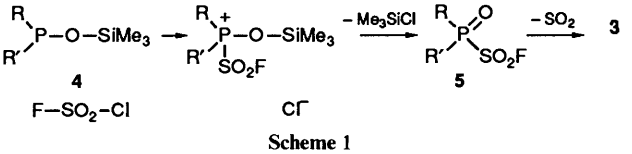
Compounds **1** can be readily transformed into their trimethylsilyl derivatives **4** by simple silylation procedures [eqn. (2)].[3] This reaction is based on the high affinity of the silicon centre towards oxygen and leads to highly reactive 'true' trico-ordinate phosphorus compounds.

$$\underset{\mathbf{1}}{RR'P(O)H} \xrightarrow{Me_3SiCl} \underset{\mathbf{4}}{RR'P{-}OSiMe_3} \quad (2)$$

We have found that trimethylsilyl esters **4** are reactive towards sulfuryl chloride fluoride in a fully chemoselective manner according to eqn. (3).[4] Phosphorofluoridates $RO(R'O)P(O)F$ of very high purity are formed in almost quantitative yield. The side-products are volatile and readily separable.

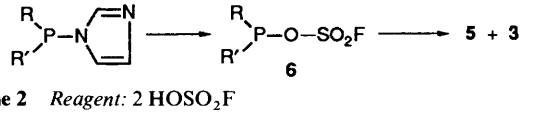
$$\underset{\mathbf{4}}{RR'POSiMe_3} + SO_2ClF \xrightarrow[CH_2Cl_2]{-50\ °C} \underset{\mathbf{3}}{RR'P(O)F} + Me_3SiCl + SO_2 \quad (3)$$

In our view, there are several contributing factors to this surprising chemoselectivity. The bond energies although higher for Si–F than for P–F, are not very different, and both heteroatoms are known to have a high affinity towards fluorine. It is more likely that in the sulfuryl chloride fluoride molecule the S–Cl bond should be broken more readily than the S–F bond. The mechanistic pathway proposed in our preliminary communication[4] and presented in Scheme 1 explains the 'high mobility' of the chlorine atom and 'low mobility' of the fluorine atom with respect to the silicon centre. However, our recent findings are not in favour of Scheme 1.



Scheme 1

Imidazolides of trico-ordinate phosphorus acids are known to react with sulfonic acids to form anhydrides $RR'P{-}O{-}SO_2R''$, which isomerize into sulfonylphosphonates $RR'P(O){-}SO_2R''$.[5] The analogous approach was used in order to obtain the desired fluorosulfonylphosphonate **5** (Scheme 2).



Scheme 2 *Reagent:* $2\ HOSO_2F$

Intermediate formation of the anhydride **6**, which spontaneously isomerized into the fluorosulfonylphosphonate **5** contaminated with the fluoridate **3**, was confirmed by ^{31}P NMR spectroscopy as indicated in Table 1. The spectroscopic properties of the anhydride **6** and the fluorosulfonylphosphonate **5** are in good agreement with those observed for other com-

Table 1 ^{31}P NMR analysis of the reaction system presented in Scheme 2

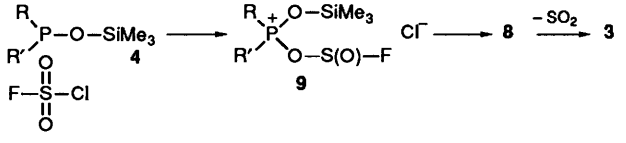
		6		5		3	
R	R′	δ	J_{P-F} (Hz)	δ	J_{P-F} (Hz)	δ	J_{P-F} (Hz)
EtO	EtO	119.8	5.4	24.7	14.3	−8.6	970.1
Bu^t	Ph	123.4	7.1	65.4	15.4	+58.6	1048.0

pounds of these classes.[5] The observed stability of compound **5**, and our failure to detect it when reaction (3) was monitored by ^{31}P NMR spectroscopy, excludes its involvement.

It is known that the reaction between the chloridate **7** and potassium fluorosulfinate, KSO_2F, proceeds with formation of the fluoridate **3**[6] [eqn. (4)].

$$\underset{\mathbf{7}}{RR'P(O)\text{–}Cl} + KSO_2F \xrightarrow{-KCl} \underset{\mathbf{8}}{RR'P(O)\text{–}O\text{–}SO\text{–}F} \xrightarrow{-SO_2} \mathbf{3} \quad (4)$$

It appears that the highly unstable anhydride **8** is formed and decomposes into the fluoridate **3** and sulfur dioxide. Another reaction in which the anhydride **8** is likely to be formed is that between dialkyl hydrogen phosphates and thionyl fluoride.[7] Taking into account these facts, we assume that this type of intermediate is likely to be formed in reaction (3). We postulate that the interaction between the phosphite **4** and sulfuryl chloride fluoride involves formation of the phosphorus–oxygen bond, with simultaneous rupture of the sulfur–chlorine bond, leading to the phosphonium salt **9**, which decomposes into the anhydride **8** and trimethylsilyl chloride. Finally, compound **8** decomposes into the fluoridate **3** and sulfur dioxide. The formation of the phosphonium salt **9** proceeds by oxidation of the phosphorus centre and reduction of the sulfur (Scheme 3). Concerted formation of a six-membered transition state for the reaction can also be considered. A radical pathway leading to the phosphonium salt **9** seems less likely, but a single-electron-transfer process can be envisaged.



Scheme 3

This new reaction provides easy access to all types of fluoridates **3** from non-toxic starting materials. The method can be used for the preparation of toxic fluoridates required in enzymatic studies, *e.g.* **3** (R = R′ = Pr^{i}O).

The procedure described in this paper leads to extremely pure fluoridates. Further purification by distillation is necessary only when the starting silyl ester is not of high purity. The structures and purity of the phosphorofluoridates RO(R′O)P(O)F were confirmed by ^{31}P NMR spectroscopy, mass spectroscopy and gas chromatography.

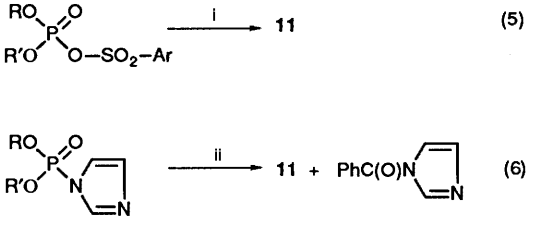
In 1963 Wittman prepared, for the first time, the nucleoside 5′-monophosphorofluorides by reaction of mononucleoside 5′-phosphates with 2,4-dinitrofluorobenzene.[8] This synthesis of nucleoside phosphorofluoridates **10** has also been employed by other authors.[9] Recently, mononucleoside phosphorofluoridates **10** have also been prepared as the dominant product from flouride ion fission of the phosphorus–sulfur bond.[10] The reported yields were usually low, and the method could not be extended to preparation of the dinucleoside phosphorofluoridates **11** (R,R′ = nucleosidyl).

Recently, our attention has been focused on new methodology for the synthesis of non-ionic nucleoside phosphorofluoridates of the type **11**.

10 **11**

R, R′ = 3′- or 5′-nucleosidyl

Nucleoside 3′- and 5′-phosphorofluoridates **11** (R = 3′- or 5′-nucleosidyl, R′ = aryl) have been obtained by reaction of the nucleotide sulfonic anhydrides[11] with tetrabutylammonium fluoride (TBAF) [eqn. (5)][12] and from the nucleotide azolides by reaction with benzoyl flouride [eqn. (6)].[13]



Reagents: i, F^-; ii, PhCOF

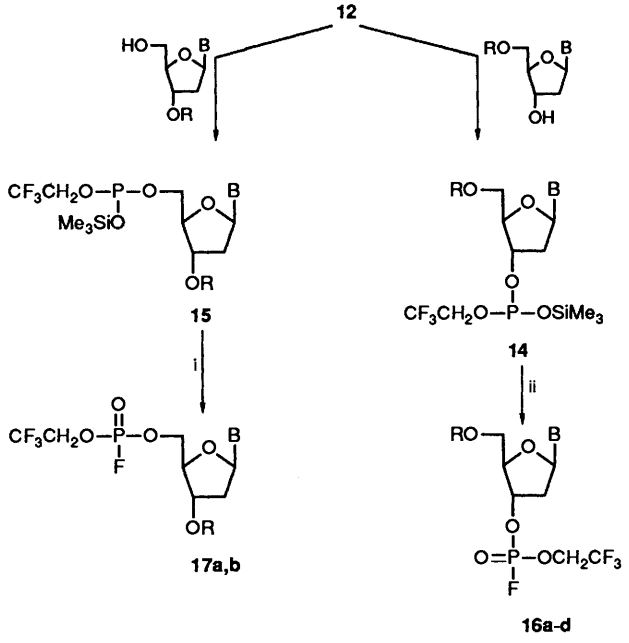
In this work we were able to demonstrate that the phosphorofluoridates **11** derived from nucleosides can be efficiently synthesized from the corresponding trimethylsilyl phosphites **12** or **13** and sulfuryl chloride fluoride. In this approach nucleotides derived from thymidine can be employed without protection of the nitrogen base. In other cases standard protection is sufficient. The starting mononucleoside trimethylsilyl phosphites **14**, **15**, **18** and **19** were synthesized by phosphitylating reagent containing a trimethylsiloxy group, *viz.* compounds **12** and **13**.[14,15]

$(CF_3CH_2O)_2P\text{–}OSiMe_3$ **12** $(Pr^i_2N)_2P\text{–}OSiMe_3$ **13**

Phosphitylation by the trimethylsilyl ester **12** proceeds without any activation (Scheme 4),[14] whereas reagent **13** requires activation by, *e.g.* tetrazole (Scheme 5).[15] The fact that phosphitylation by phosphorodiamidite **13** can be stopped at the stage of intermediate **18** or **19** can be explained by steric factors. Strictly anhydrous conditions and sublimed tetrazole are necessary to secure high yield.

Trimethylsilyl phosphites **14**, **15**, **18** and **19** prepared *in situ* were treated in dry pyridine solution with an excess of sulfuryl chloride fluoride at −30 °C, using a simple vacuum-line technique. The reaction can also be performed in other non-protic solvents in the presence of pyridine. The base is required to avoid deprotection of acid-sensitive groups.

The trimethylsilyl esters of the dinucleoside phosphites **22** were prepared with the aid of phosphitylating reagent **13** in two steps, without isolation of the intermediate compounds.[19] Both



R	BH
17a; 4,4′-dimethoxytrityl	N^6-benzoyladenine
b; Ac	thymine

R	BH
16a; $SiMe_2Bu^t$	N^6-benzoyladenine
b; Ac	thymine
c; 4,4′-dimethoxytrityl	N^6-benzoyladenine
d; 9-phenylxanthen-9-yl	thymine

Scheme 4 *Reagent:* i, SO_2ClF

steps were catalysed by tetrazole. Conversion into the dinucleoside phosphorofluoridates **23** was performed under conditions used for the preparation of mononucleoside phosphorofluoridates **16**, **17**, **20** and **21**.

In every P–F nucleotide **16**, **17**, **20**, **21** and **23**, a characteristic doublet of doublets was observed in the ^{31}P NMR spectrum, and 1:1 ratios of the corresponding diastereoisomers was noted. This means that there is no kinetic selection or thermodynamic preference favouring a particular diastereoisomer.

The dinucleoside phosphorofluoridates **23** undergo chemoselective hydrolysis in the presence of spleen phosphorodiesterase and snake venom phosphorodiesterase. Compound **23b** was readily deprotected under standard conditions [16] to give the corresponding phosphorofluoridate **23e** without affecting the phosphorus–fluorine bond. Enzymatic hydrolysis of compound **23e** provides additional proof of the structure of compound **23c** and shows the relatively high stability of the latter compound in aq. medium. The monofluoridates **24** and **25** (Scheme 6) are identical with compounds prepared recently by Chattopadhyaya.[10]

The mono- and di-nucleoside phosphorofluorides described in this paper can be considered as building units for oligonucleotides of biological interest. Our preliminary studies show that the phosphorus–fluorine bond is able to survive both deprotection and coupling procedures.

Experimental

Solvents and commercial reagents were purified by conventional methods before use. NMR spectra were measured on a Bruker HX-72 (36.4 Hz ^{31}P) spectrometer; positive chemical shifts are downfield from 85% H_3PO_4. *J*-Values are given in Hz. All reactions were performed under argon. Column chromatography was carried out on silica gel 60. Analytical TLC was performed on 60 F_{254} (Merck) plates developed in one of the following solvent systems: system A [CH_2Cl_2–MeOH (9:1)], system B [CH_2Cl_2–EtOAc–Et_3N (45:45:10)]. 3′- and 5′-*O*-Protected nucleosides were synthesized by known methods.[16] Imidazolides of trico-ordinate phosphorus acids were prepared following the methodology described by Bugerenko[3a] and Nesterov.[3b]

Reaction of Trimethylsilyl Ester **4** *with Sulfuryl Chloride Fluoride.*—In a typical experiment compound **4** (20 cm^3, 0.01 mol) in dry methylene dichloride (20 cm^3) was cooled to −50 °C and SO_2ClF (5% excess) added to the stirred and cooled mixture. The reaction mixture was kept for 1 h at −50 °C and then for an additional 1 h at 20 °C. The solvent, sulfur dioxide, and the excess of SO_2ClF were removed under reduced pressure. The structure and purity of phosphorofluoridates RO(R′O)P(O)F were confirmed by ^{31}P NMR spectroscopy and by comparison with authentic samples.

Diethyl phosphorofluoridate,[2b] b.p. 60 °C/12 mmHg (98%); $\delta_P(CDCl_3)$ −8.60 (J_{P-F} 964.1).

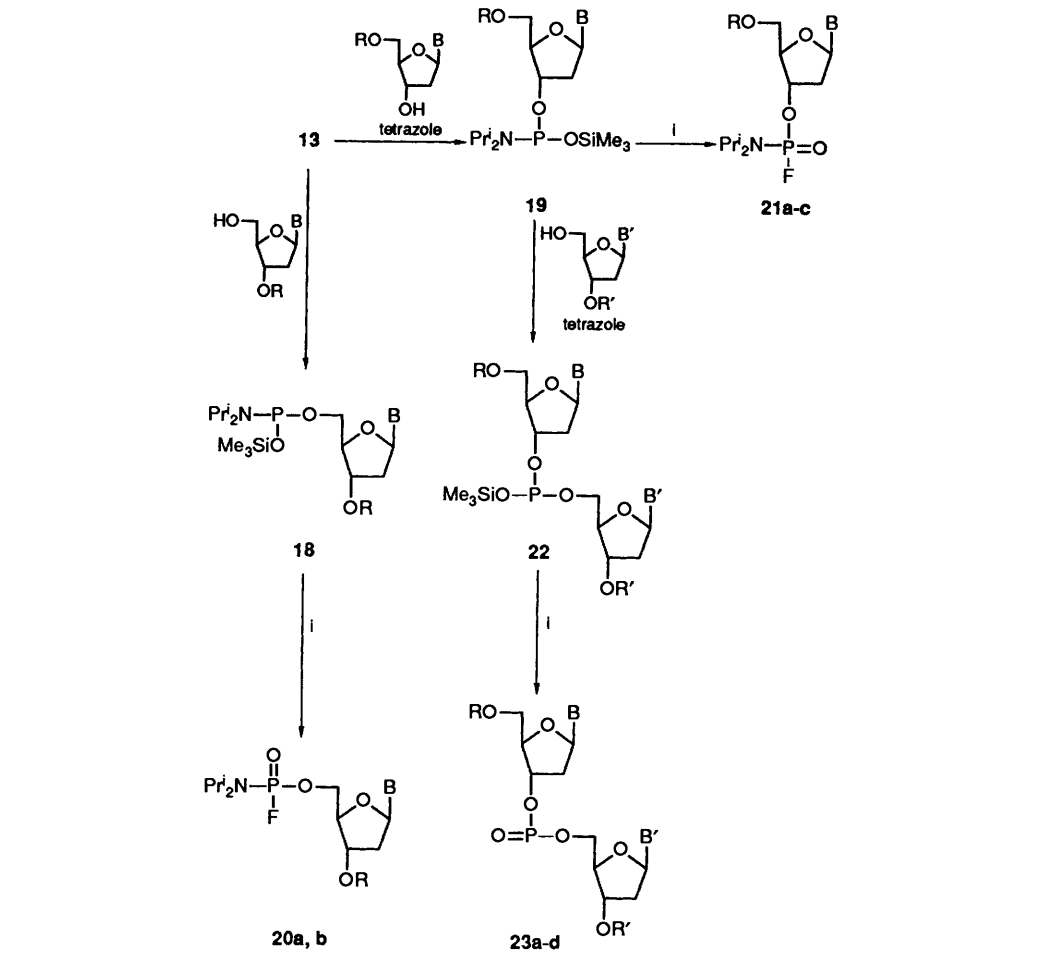
Bis(2,2,2-trifluoroethyl) phosphorofluoridate, b.p. 72 °C/0.1 mmHg (98%); $\delta_P(CDCl_3)$ −10.22 (J_{P-F} 995.1) (Found: C, 18.0; F, 50.3; P, 11.6. $C_4H_4F_7O_3P$ requires C, 18.20; F, 50.37; P, 11.73%).

Diisopropyl phosphorofluoridate,[18] b.p. 60 °C/7 mmHg (97%); $\delta_P(CDCl_3)$ −10.26 (J_{P-F} 968.3).

Diphenyl phosphorofluoridate,[19] b.p. 120–125 °C/0.1 mmHg (90%); $\delta_P(CDCl_3)$ −19.73 (J_{P-F} 995.9).

tert-Butyl(phenyl)phosphinic fluore,[2b] b.p. 68–70 °C/0.2 mmHg (98%); $\delta_P(CDCl_3)$ +58.63 (J_{P-F} 1048.0).

General Procedure for the Synthesis of Nucleoside 2,2,2-*Trifluoroethyl Phosphorofluoridates* **10**, **17**.—To a solution of 5′- or 3′-*O*-protected deoxyribonucleoside (1.0 mmol), dried by repeated coevaporation with anhydrous pyridine, in dry pyridine (3 cm^3) was added bis(2,2,2-trifluoroethyl) trimethylsilyl phosphite **12**[14] (1.5 mmol) After 5 h, SO_2ClF (excess) was added to the stirred mixture at −50 °C. The stirred reaction mixture was gradually warmed to room temperature for an additional 1 h. The solvent and the sulfur dioxide were removed

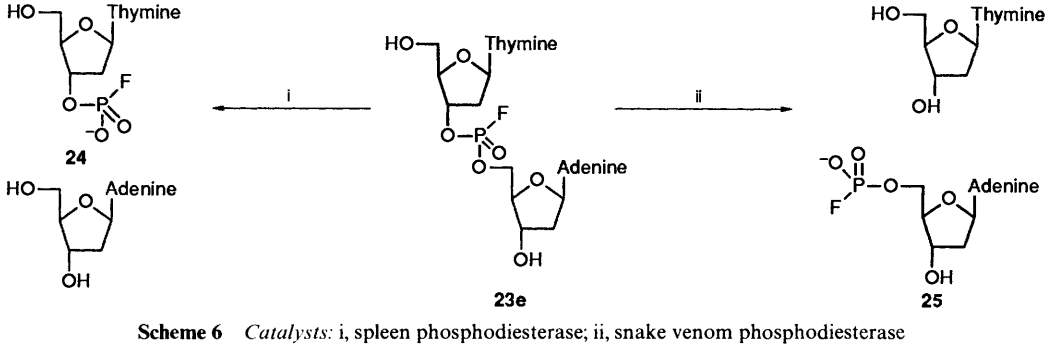


	R	BH
20a;	4,4′-dimethoxytrityl	N^6-benzoyladenine
b;	Ac	thymine

	R	BH
21a;	4,4′-dimethoxytrityl	N^6-benzoyladenine
b;	Ac	thymine
c;	9-phenylxanthen-9-yl	thymine

	R	R′	BH	B′H
23a;	4,4′-dimethoxytrityl	4,4′-dimethoxytrityl	N^6-benzoyladenine	N^6-benzoyladenine
b;	4,4′-dimethoxytrityl	Ac	N^6-benzoyladenine	thymine
c;	9-phenylxanthen-9-yl	4,4′-dimethoxytrityl	thymine	N^6-benzoyladenine
d;	9-phenylxanthen-9-yl	Ac	thymine	thymine

Scheme 5 *Reagent:* i, SO_2ClF



Scheme 6 *Catalysts:* i, spleen phosphodiesterase; ii, snake venom phosphodiesterase

by evaporation under reduced pressure. The products were purified by chromatography on silica gel.

N^6-Benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosin-3′-yl 2,2,2-trifluoroethyl phosphorofluoridate **16a**, 92%; R_f 0.7 (system A); $\delta_P(CDCl_3)$ −9.90 and −9.97 (J_{P-F} 980.4, 970.5).

5′-*O*-Acetylthymidin-3′-yl 2,2,2-trifluoroethyl phosphorofluoridate **16b**, 95%; R_f 0.6 (system A); $\delta_P(CDCl_3)$ −9.71 and −9.64 (J_{P-F} 980.1, 975.4).

N^6-Benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)adenosin-3′-yl 2,2,2-trifluroethyl phosphorofluoridate **16c**, 91%, R_f 0.6 (system B); $\delta_P(CDCl_3)$ −9.60 and −9.40 (J_{P-F} 987.4, 980.5).

5′-*O*-(9-phenylxanthen-9-yl)thymidin-3′-yl 2,2,2-trifluoroethyl phosphorofluoridate **16d**, 90%; R_f 0.7 (system B); δ_P −9.60 and −9.58 (J_{P-F} 970.5, 971.4).

N^6-Benzoyl-2′-deoxy-3′-*O*-(4,4′-dimethoxytrityl)adenosin-5′-yl 2,2,2-trifluoroethyl phosphorofluoridate **17a**, 90%; R_f

0.5 (system B); $\delta_P(CDCl_3)$ −9.71 and −9.73 (J_{P-F} 981.4, 980.1).

3′-*O*-Acetylthymidin-5-yl 2,2,2-trifluoroethyl phosphorofluoridate **17b**, 92%; R_f 0.5 (system A); $\delta_P(CDCl_3)$ −9.64 and −9.68 (J_{P-F} 970.7, 970.0).

Synthesis of Trimethylsilyl N,N,N′,N′-*Tetraisopropylphosphorodiamidite* **13**.—To a solution of chlorobis(diisopropylamino)phosphine[20] (0.1 mol) and pyridine (0.1 mol in tetrahydrofuran (THF) (50 cm^3) was added at 5 °C a mixture of water (0.1 mol) in THF (10 cm^3). The reaction mixture was gradually warmed to room temperature and was stirred for an additional 2 h, after which the precipitated salt was filtered off. The filtrate was evaporated to dryness to give bis-(*N*,*N*-diisopropylamino) hydrogen phosphite [$\delta_P(CDCl_3)$ 7.11, J_{P-H} 548.9].

Subsequently this intermediate (0.1 mol), which did not need to be purified, was dried by coevaporation with anhydrous pyridine and was then dissolved under argon in dry THF (50 cm^3) containing triethylamine (0.11 mol). Freshly distilled trimethylsilyl chloride (0.11 mol) in dry THF (20 cm^3) was added at room temperature. After 2 h triethylammonium chloride was removed by filtration. The filtrate was evaporated to dryness, and the crude product was purified by distillation to give **13** (95%), b.p. 100–102 °C/0.01 mmHg; $\delta_H(CDCl_3)$ 0.3 (9 H, s), 0.9 (2 H, d) and 3.0–3.7 (4 H, m); $\delta_P(CDCl_3)$ +108.23.

General Procedure for the Preparation of Nucleoside N,N-*Diisopropylphosphoramidofluoridates* **20**, **21**.—The protected deoxyribonucleoside (1.0 mmol) was dried by coevaporation with dry pyridine (3 cm^3) and was then dissolved under nitrogen in dry CH_2Cl_2 (2.5 cm^3). A solution of tetrazole (1.0 mmol) in dry CH_2Cl_2 (2.5 cm^3) was added to the stirred mixture at 20 °C, followed by trimethylsilyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (1.0 mmol). After the mixture had been stirred for 30 min the precipitate of diisopropylammonium tetrazolide was removed by filtration, and the solvent was removed under reduced pressure. The filtrate was evaporated to dryness, and the crude product **18** or **19** was dissolved in dry pyridine. Excess of SO_2ClF was added at −50 °C. The resulting solution was gradually warmed to room temperature and stirred for 1 h. The solvent, sulfur dioxide, and excess of SO_2ClF were removed under reduced pressure. The products were purified by chromatography on a silica gel column.

N^6-Benzoyl-2′-deoxy-3′-*O*-(4,4′-dimethoxytrityl)adenosin-5′-yl *N*,*N*-diisopropylphosphoramidofluoridate **20a**, 81%; R_f 0.4 (system B); $\delta_P(CDCl_3)$ +21.4 and +20.9 (J_{P-F} 1070.1, 1071.2).

3′-*O*-Acetylthymidin-5-yl *N*,*N*-diisopropylphosphoramidofluoridate **20b**, 92%; R_f 0.5 (system B); $\delta_P(C_5D_5N)$ +20.4 and +19.4 (J_{P-F} 1070.0, 1060.0).

N^6-Benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)adenosin-3′-yl *N*,*N*-diisopropylphosphoramidofluoridate **21a**, 90%; R_f 0.6 (system B); $\delta_P(CDCl_3)$ +21.7 and +21.5 (J_{P-F} 1075.4, 1070.3).

5′-*O*-Acetylthymidin-3′-yl *N*,*N*-diisopropylphosphoramidofluoridate **21b**, 95%; R_f 0.6 (system A); $\delta_P(C_5D_5N)$ +20.4 and +20.1 (J_{P-F} 1060.3, 1058.4).

5′-*O*-(9-Phenylxanthen-9-yl)thymidin-3′-yl *N*,*N*-diisopropylphosphoramidofluoridate **21c**, 94%; R_f 0.6 (system A); $\delta_P(CDCl_3)$ +20.7 and +20.4 (J_{P-F} 1070.4, 1065.1).

General Procedure for the Preparation of Dinucleoside Phosphorofluoridates **23**.—A solution of the phosphoramidite **19** (0.3 mmol) in dry acetonitrile (5 cm^3) was added to the 3′-*O*-protected nucleoside (0.1 mmol) and tetrazole (0.33 mmol), also in dry acetonitrile (3 cm^3). The mixture was stirred for 20 min. It was then cooled to −50 °C, SO_2ClF (excess) was added, and the resulting solution was gradually warmed to room temperature and was then stirred for 1 h. The solvent, sulfur dioxide, and excess of SO_2ClF were removed under reduced pressure. The products were purified by chromatography on silica gel.

N^6-Benzoyl-2′-deoxy-3′-*O*-(4,4′-dimethoxytrityl)adenosin-5′-yl N^6-benzoyl-2′-*O*-(4,4′-dimethoxytrityl)adenosin-3′-yl phosphorofluoridate **23a**, 98%; R_f 0.4 (system A); $\delta_P(C_5D_5N)$ −8.60 and −8.00 (J_{P-F} 977.1, 975.2).

3′-*O*-Acetylthymidin-5′-yl N^6-benzoyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)adenosin-3′-yl phosphorofluoridate **23b**, 95%; R_f 0.4 (system A); $\delta_P(C_5D_5N)$ +8.70 and −8.90 (J_{P-F} 975.1, 980.3).

N^6-Benzoyl-2′-deoxy-3′-*O*-(4,4′-dimethoxytrityl)adenosin-5′-yl 5′-*O*-(9-phenylxanthen-9-yl)thymidin-3′-yl phosphorofluoridate **23c**, 98%; R_f 0.6 (system A); $\delta_P(C_5D_5N)$ −8.71 and −8.90 (J_{P-F} 976.1, 981.2).

3′-*O*-Acetylthymidin-5′-yl 5′-*O*-(9-phenylxanthen-9-yl)thymidin-3′-yl phosphorofluoridate **23d**, 97%; R_f 0.5 (system B); $\delta_P(C_5D_5N)$ −8.92 and −8.90 (J_{P-F} 975.4, 976.3).

Enzymatic Digestion of Thymidin-3′-yl 2′-Deoxyadenosin-5′-yl Phosphorofluoridate **23e**.—Samples of the dinucleotide **23e** were incubated respectively with spleen phosphodiesterase (Boehringer) in buffer (0.1 mol dm^{-3} NH_4OAc, pH 5.7) and with snake venom phosphodiesterase (Boehringer) in buffer [0.1 mol dm^{-3} Tris-HCl (pH 9), 0.1 mol dm^{-3} NaCl, 15 mmol dm^{-3} $MgCl_2$] for 3 h at 37 °C. Hydrolytic degradation of compound **23e** by spleen phosphodiesterase led to the thymidine-3′-yl phosphorofluoridate **24** [$\delta_P(D_2O)$ −7.8, J_{P-F} 947] and adenosine: that by snake venom phosphodiesterase led to adenosin-5′-yl phosphorofluoridate **25** [$\delta_P(D_2O)$ −6.47, J_{P-F} 928] and thymidine.

Reaction of Imidazolides of Trico-ordinate Phosphorus Acids with $HOSO_2F$.—To a stirred solution of an imidazolide (1 mmol) in methylene dichloride (5 cm^3) at −30 °C was added dropwise a solution of $HOSO_2F$ (2 mmol) in methylene dichloride (5 cm^3). The reaction mixture was then stirred for 10 min at −30 °C, after which its ^{31}P NMR spectrum showed one major signal, corresponding presumably to compound **6**. The reaction mixture was then gradually warmed to room temperature, after which its ^{31}P NMR spectrum showed two signals, corresponding to products **5** and **3**.

References

1 L. A. Mounter, B. A. Shipley and M.-E. Mounter, *J. Biol. Chem.*, 1963, **238**, 1979; N. S. Sampson and P. A. Bartlett, *Biochemistry*, 1991, **30**, 2255.
2 A. Lopusiński and J. Michalski, (*a*) *J. Am. Chem. Soc.*, 1982, **104**, 290; (*b*) *Angew. Chem.*, 1982, **94**, 302.
3 (*a*) E. F. Bugerenko, E. A. Chernyshev and E. M. Popov, *Bull. Acad. Sci. USSR*, 1966, 1334; (*b*) L. V. Nesterov, N. E. Kvepysheva, R. A. Sabirova and G. N. Romanova, *J. Gen. Chem. USSR* (*Engl. Transl.*), 1971, **41**, 2449.
4 Preliminary communication, W. Dąbkowski and J. Michalski, *J. Chem. Soc., Chem. Commun.*, 1987, 755.
5 W. Dąbkowski, J. Michalski and Z. Skrzypczyński, *J. Chem. Soc., Chem. Commun.*, 1982, 1260.
6 F. Seel, K. Ballreich and R. Schmutzler, *Chem. Ber.*, 1962, **95**, 199.
7 W. T. Konieczko, A. Lopusiński and J. Michalski, *Phosphorus, Sulfur Silicon Relat. Elem.*, 1989, **42**, 103.
8 R. Wittman, *Chem. Ber.*, 1963, **96**, 771.
9 F. Eckstein, W. Bruns and A. Parmeggiani, *Biochemistry*, 1975, **14**, 5225; S. G. Withers and N. B. Madsen, *Biochem. Biophys. Res. Commun.*, 1980, **97**, 513; H. G. Vogel and W. A. Bridger, *Biochemistry*, 1982, **21**, 394.
10 Ch. Sund and J. Chattopadhyaya, *Tetrahedron*, 1989, **45**, 7523.
11 W. Dąbkowski, Z. Skrzypczyński, J. Michalski, N. Piel, L. W. McLaughlin and F. Cramer, *Nucleic Acids Res.*, 1984, **12**, 9123.
12 W. Dąbkowski and J. Michalski, unpublished results.
13 W. Dąbkowski, F. Cramer and J. Michalski, *Tetrahedron Lett.*, 1987, **28**, 3561.

14 K. Imai, T. Ito, S. Kondo and T. Takaku, *Nucleosides, Nucleotides*, 1985, **4**, 669.
15 W. Dąbkowski, F. Cramer and J. Michalski, *Tetrahedron Lett.*, 1988, **29**, 330; W. Dąbkowski, J. Michalski and Q. Wang, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 522.
16 A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 1953, 951; J. B. Chattopadhyaya and C. B. Reese, *J. Chem. Soc., Chem. Commun.*, 1978, 639; R. A. Jones in *Oligonucleotide Synthesis, A Practical Approach*, ed. M. J. Gait, IRL Press, Oxford, 1984, p. 23.
17 W. Dąbkowski, J. Michalski and Z. Skrzypczyński, *Phosphorus Sulfur Relat. Elem.*, 1986, **26**, 321.
18 A. H. Ford-Moore, L. J. Lermit and C. Stratford, *J. Chem. Soc.*, 1953, 1776.
19 A. Hood and W. Lange, *J. Am. Chem. Soc.*, 1950, **72**, 4956.
20 R. B. King and P. M. Sundaram, *J. Org. Chem.*, 1984, **49**, 1784.

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