New General Synthesis of Organophosphorus P–F Compounds *via* Reaction of Azolides of Phosphorus Acids with Acyl Fluorides: Novel Route to 2-Deoxynucleosidyl Phosphorofluoridates and Phosphorodifluoridates

Wojciech Dąbkowski,[a] Jan Michalski,*[,a] Jacek Wasiak[a] and Friedrich Cramer[b]
[a] *Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Sienkiewicza 112, 90-363 Łódź, Poland*
[b] *Max Planck Institut für Experimentelle Medizin, Herman-Rein Str. 3, D-3400 Göttingen, Germany*

Tetra- and tri-coordinate P–N-imidazole derivatives and their structural analogues react smoothly with acyl fluorides to give the corresponding P–F compounds in almost quantitative yield. This method has been successfully applied to produce 2-deoxynucleosidyl phosphorofluoridates and phosphorodifluoridates.

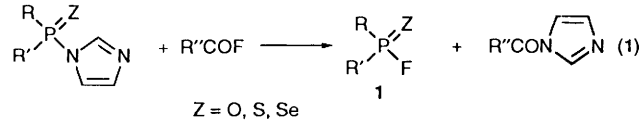
The importance of backbone-modified oligonucleotides in molecular biology makes such compounds targets of considerable interest.[1] Recently we have investigated synthetic routes leading to P–F modified nucleotides.[2] We have shown that trimethylsilyl phosphites derived from 2-deoxyribonucleosides react with sulfuryl chloride fluoride (SO_2ClF) to give nucleosidyl phosphorofluoridates.[2a] However, this highly efficient and selective method has some limitations. It is not suited for synthesis of systems containing two fluorine ligands at the phosphorus atom or for tricoordinate P–F derivatives.

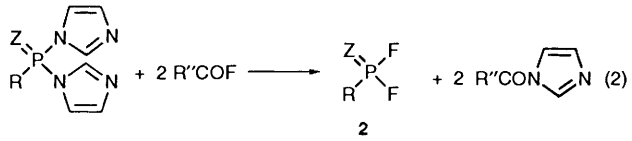
In this paper we report a full account of a novel approach towards the synthesis of phosphorus–fluorine systems based on the reaction of P–N-imidazole derivatives of tri- and tetracoordinate phosphorus acids with acyl fluorides.[3],† Applications of this methodology in nucleotide chemistry provides a unique access to a variety of P–F nucleotide derivatives.

Results and Discussion

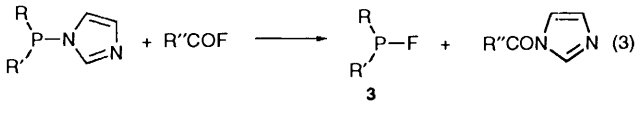
Model Studies.—The synthesis of the P–F compounds is illustrated by the following classes of reactions.

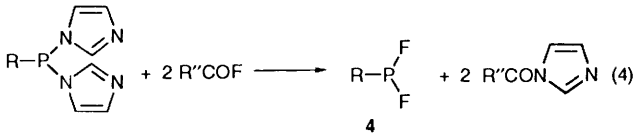
Preparation of tetracoordinate RR'P(Z)F and $RP(Z)F_2$ systems.





Preparation of tricoordinate RR'PF and RPF_2 systems.





In reactions (1)–(4) R''COF represents acyl fluoride. Benzoyl fluoride and oxalyl difluoride are the reagents of choice. The former is commercially available. The latter has the advantage of being transformed into poorly soluble oxalyl diimidazolide.

The reactions described by eqns. (1)–(4) are exothermic and proceed in neutral solvents at room temperature in almost quantitative yield. Triazolides and tetrazolides of phosphorus acids are also excellent substrates in the reaction with acyl fluorides. The yield and spectral data of compounds prepared in these model studies are given in Tables 1 and 2.

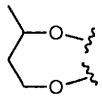
Applications in Nucleotide Chemistry.—Phosphoroimidazolidates **5** derived from 2-deoxynucleosides have recently become readily available.[16] The imidazolides react with benzoyl fluoride in dichloromethane solution at 20 °C to yield the phosphorofluoridates **6** in almost quantitative yield. P–F nucleotides prepared in this way are identical with those prepared by the reaction of dinucleosidyl trimethylsilyl phosphites with sulfuryl chloride fluoride.[17] The fluoridates **6** are formed without any stereoselectivity. In every case a characteristic doublet of doublets was observed in the ^{31}P NMR spectra and 1:1 proportions of the corresponding diastereoisomers was noted. The structure of compounds **6a** and **6b** was confirmed by their transformation under standard conditions into the deprotected dinucleosidyl phosphorofluoridates **7** and chemoselective hydrolysis in the presence of spleen phosphodiesterase and snake venom phosphodiesterase.[2c] The mononucleosidyl phosphorofluoridates **8**, **9** are identical with compounds prepared by the procedures of Wittman[18a] and Chattopadhyaya.[18b] All these chemical changes are shown in reaction (5).

Until now there has been no information available concerning nucleosidyl phosphorodifluoridates. It was reasoned that this type of compound can be prepared in the same manner as the monofluoro derivatives by transformation of the corresponding diimidazole derivative. To test this hypothesis, nucleosidyl phosphorodiimidazolidates were allowed to react with two mole equivalents of benzoyl fluoride. The main problem in this approach is the availability of the substrates.

The slow reaction of the 3'-*O*-dimethoxytritylthymidine **10** with phosphoryl **11a** (X = O) or thiophosphoryl triimidazolides **11b** (X = S) as described by Eckstein[19] and Stawiński[20] affords ~40–60% yields of the diimidazolides **12a**, **b** (X = O, S) contaminated with unchanged starting material [eqn. (6)]. To

† Ethyl(fluoro)phenylphosphine EtPhPF has been obtained by the reaction of (diethylamino)(ethyl)phenylphosphine with benzoyl fluoride (ref. 3*d*).

Table 1 Yield and spectral data of compounds RR'P(X)F **1** and **3**

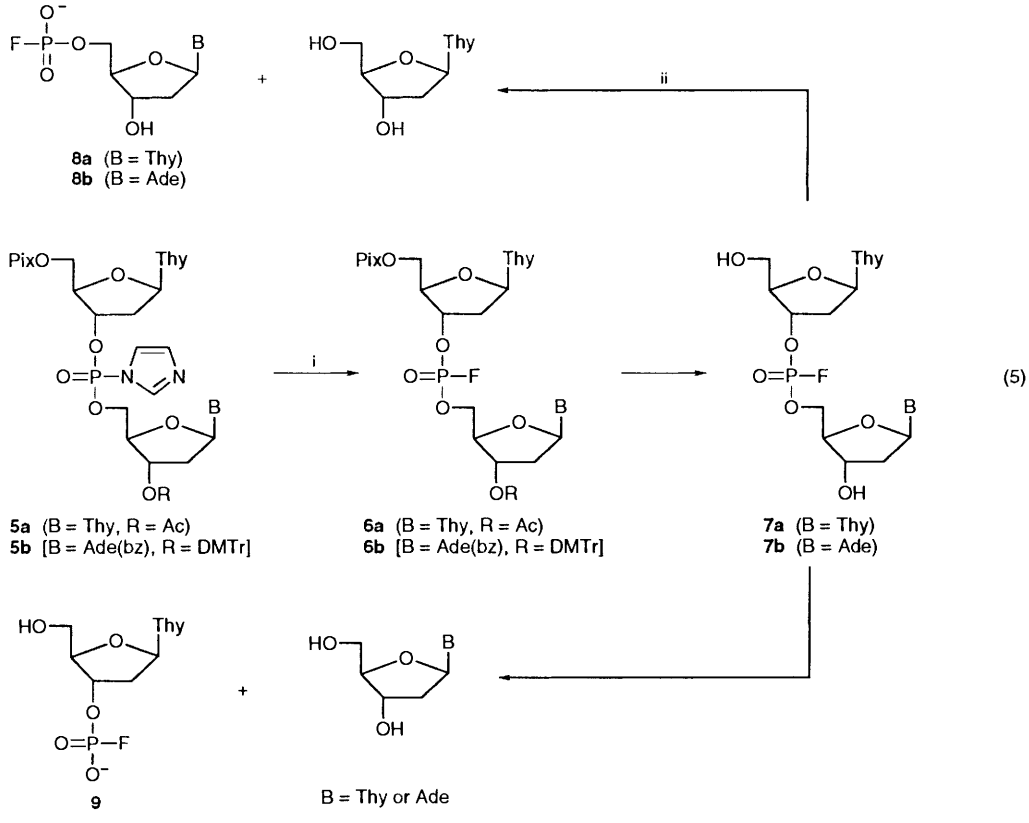
	R	R'	X	$\delta(^{31}P)$ NMR [a]	J_{P-F} (Hz)	Yield [b] (%)	Ref.	B.p. (°C)/ pressure (mmHg)
1a	EtO	EtO	O	−8.60	970.10	100*	4	58–60/12
1b	CF_3CH_2O	CF_3CH_2O	O	−10.22	995.10	99*	2*c*	70–72/0.1
1c	PhO	PhO	O	−19.73	995.99	100†	4*b*, 5	125–128/0.1
1d	Me	Me	O	66.3	990.0	100*	6	170–174
1e	Ph	Ph	O	39.6	1016	100†	7	140/3
1f	EtO	EtO	S	63.38	1072	95*	8	80–83/10
1g	Ph	Ph	S	102.0	1021	100†	9	166–170/1.5
1h	Bu^t	Ph	Se	139.0	1059.6	100†		Oil [c]
3a	Ph	Ph	*d*	169.1	1097	100*	10	128–130/0.01
3b			*d*	128.9, 105.0 (1:1)	1178, 1156	100*	11	40/25

[a] Relative to the external standard 85% H_3PO_4. Approximate ratios determined by ^{31}P NMR spectroscopy. [b] Product yield by ^{31}P NMR spectroscopy. * Method A. † Method B. [c] Product purified by silica gel chromatography using hexane as eluent. [d] Lone pair of electrons.

Table 2 Yield and spectral data of compounds $RP(X)F_2$ **2** and **4**

	R	X	$\delta(^{31}P)$ NMR [a]	J_{P-F} (Hz)	Yield [b] (%)	Ref.	B.p. (°C)/ pressure (mmHg)
2a	4-ClC_6H_4O	O	−15.4	956.2	95	12	80–82/10
2b	MeO	O	−15.2	957.2	100	13	60–63
2c	Me	O	26.4	1107.5	97	6	27–30/15
2d	Me	S	106.1	1145.8	100	14	60–63
4a	Ph	*c*	207.2	1166.2	100	15	30–31/15

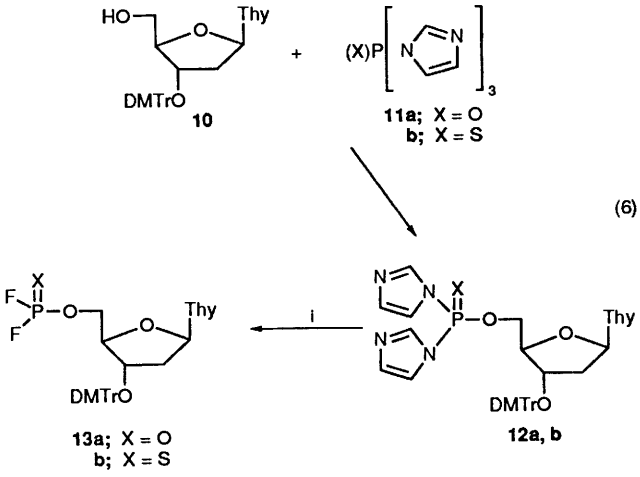
[a] Relative to the external standard 85% H_3PO_4. [b] Product yield by ^{31}P NMR spectroscopy. Lone pair of electrons.



Reagents: i, PhCOF; ii, snake venom phosphodiesterase; iii, spleen phosphodiesterase. DMTr = 4,4'-dimethoxytrityl. Pix = 9-phenylxanthen-9-yl.

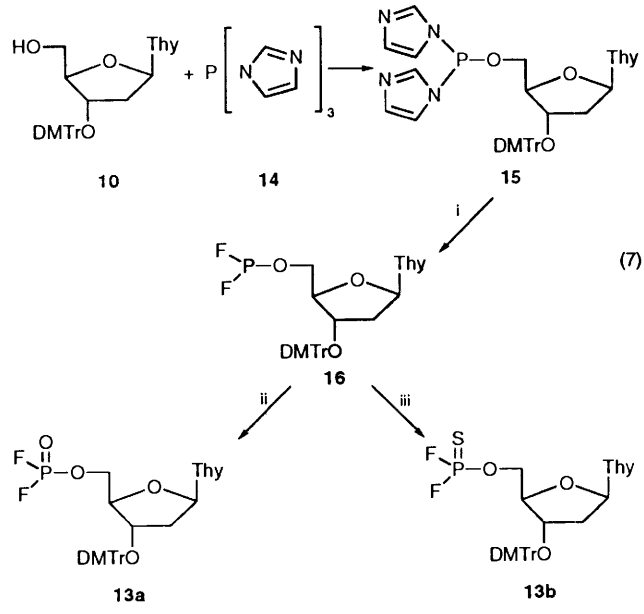
use these precursors in our fluorination procedure without prior purification it is necessary to neutralize the unchanged thymidine derivatives **10** by silylation. The amount of benzoyl fluoride necessary was calculated from ^{31}P NMR data taking into account quantities of both compounds **11** and **12** present in the reaction mixture. The volatile phosphoryl or thiophosphoryl trifluoride $P(X)F_3$ was removed by evaporation. The difluoridates **13** were separated by silica gel chromato-

graphy. Although the transformation of phosphorodiimidazolidates **12** into difluoridates **13** is almost quantitative, the total yield of pure product **13** is relatively low.



Reagent: i, PhCOF

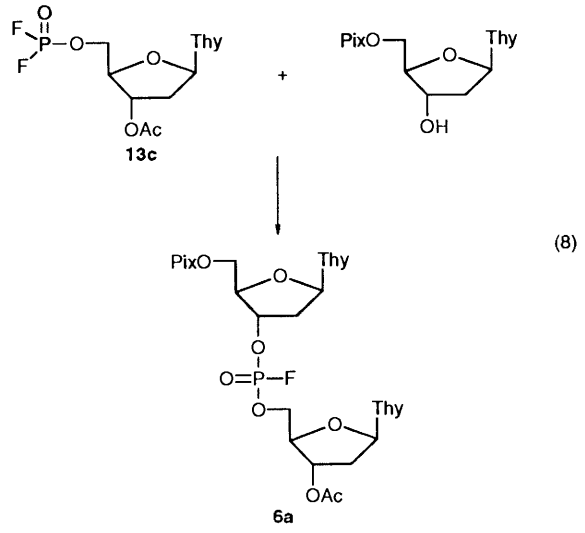
An alternative route to compounds **13** is based on tricoordinate phosphorus intermediates. Unstable triimidazolyl phosphine **14**,[3a,21] which must be prepared *in situ*, was shown by Stawiński to be an excellent reagent to prepare the diimidazolidite **15** in very high yield.[22] Compound **15** was allowed to react *in situ* with two mole equivalents of benzoyl fluoride to give highly pure difluoridite **16** in almost quantitative yield [reaction (7)].



Reagents and conditions: i, PhCOF; ii, [O]; iii, [S]

This reaction must be performed in a moisture- and oxygen-free atmosphere. The difluoridate **16** undergoes easy aerial oxidation to form the difluoridate **13a** and adds elemental sulfur to give the difluoride **13b**.

It is known that compounds of the general formula -P(O)F_2 are more reactive than the monofluoro derivatives >P(O)F and one fluorine ligand can be exchanged selectively in reactions with nucleophilic species. This property is demonstrated in the coupling reaction between acetoxy difluoridate **13c** and 5′-*O*-(9-phenylxanthen-9-yl)thymidine activated by the presence of caesium fluoride [eqn. (8)].



Reagent: CsF

The dinucleosidyl phosphorofluoridate **6a**, formed in very good yield, is identical with the same compound prepared by other methods.

Final Remarks.—We have shown that tri- and tetra-coordinate P–N-imidazole compounds and analogous systems react very readily with acyl fluorides, providing easy access to a variety of P–F derivatives including 2-deoxyribonucleotides. This work illustrates once again the synthetic utility of the acyl imidazole derivatives first used by Staab and his colleagues.[3a]

Experimental

All experiments were carried out using rubber gloves under an effective hood.

Synthesis of Monofluorides **1** *and* **3**.—*Method A.* In a typical synthesis of monofluorides **1** and **3**, benzoyl fluoride (1.0 mmol) was added to a stirred solution of the corresponding azolide (1.0 mmol) in dry CH_2Cl_2 (20 cm^3) at 0 °C and the reaction mixture was kept for 1 h at room temp. The solvent was removed under reduced pressure and the residue was treated with a small amount of dry 1,4-dioxane. The precipitated benzoylazolide was filtered off. The residue was purified by distillation *in vacuo*.

Method B. The solution of azolide (1.0 mmol) in dry tetrahydrofuran (THF) or CH_2Cl_2 (20 cm^3) was frozen in liquid nitrogen. Oxalyl difluoride was introduced using vacuum-line techniques and the reaction mixture was allowed to warm up to room temperature during 30 min. The precipitated oxalyldiimidazole was filtered off. After evaporation of the solvent, the crude product was purified by distillation *in vacuo*.

Synthesis of Difluorides **2** *and* **4**.—Difluorides were prepared by procedures analogous to those used for the monofluorides (procedure A) using 2 equiv. of benzoyl fluoride.

General Procedure for the Preparation of Dinucleoside Phosphorofluoridates **6a**, **b**.—A solution of benzoyl fluoride (0.3 mmol) in dry CH_2Cl_2 (5 cm^3) was added at 0 °C to a solution of the phosphoroimidazolide (0.3 mmol) in dry CH_2Cl_2 (10 cm^3). The mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure and the residue was treated with a minimum amount of dry 1,4-dioxane. The precipitated benzoylimidazole was filtered off. The products were purified by chromatography on silica gel.

3'-O-*Acetylthymidin-5'-yl* 5'-O-(9-*phenylxanthen-9-yl*)*thymidin-3'-yl phosphorofluoridate* **6a**. 97%, R_f 0.5 [CH_2Cl_2–EtOAc–Et_3N (45:45:10)]; $\delta_P(C_5D_5N)$ −8.92 and −8.90, J_{P-F} 975.4 and 976.3; FAB$^+$ *m/z* 847.9 (M + H).

N^6-*Benzoyl*-3'-O-(4,4'-*dimethoxytrityl*)-2'-*deoxyadenosin*-5'-*yl* 5'-O-(9-*phenylxanthen*-9-*yl*)*thymidin*-3'-*yl phosphorofluoridate* **6b**. 98%, R_f 0.6 [CH_2Cl_2–EtOAc–Et_3N (40:40:10)]; $\delta_P(C_5D_5N)$ −8.71 and −8.90, J_{P-F} 976.1 and 981.2; FAB$^+$ *m/z* 1221.4 (M + H).

General Procedure for the Preparation of Nucleoside Phosphorodifluoridate **13a** *and Phosphorothiodifluoridate* **13b**.—*Method A*. 3'-*O*-Dimethoxytritylthymidine (0.01 mmol) was dissolved in dry pyridine (5 cm^3) and compound **11a** or **11b** (0.012 mmol) was added at room temperature. After 2 h the reaction mixture was cooled to 10 °C and a solution of benzoyl fluoride (0.036 mmol) in dry CH_2Cl_2 (10 cm^3) was added. The mixture was stirred for 2 h at room temperature. The solvent, $P(O)F_3$, and excess of PhC(O)F were removed under reduced pressure. The product was purified by column chromatography.

Method B. A solution of 3'-dimethoxytritylthymidine (0.01 mmol) in anhydrous CH_2Cl_2 (20 cm^3) was added at 0 °C to a solution of triimidazolylphosphine (0.012 mmol) in dry CH_2Cl_2. The mixture was stirred for 10 min at room temp., cooled to 0 °C, and a solution of benzoyl fluoride (0.036 mmol) in CH_2Cl_2 (10 cm^3) was then added dropwise. The reaction mixture was kept at room temp. for 1 h. A solution of 3'-*O*-dimethoxytritylthymidine difluorophosphite **16** was treated with a solution of iodine in THF–pyridine–water (4:3:3 v/v) (0.1 mol dm^{-3}) for 15 min. The solvents and excess of PhC(O)F were removed under reduced pressure. The crude phosphorodifluoridate **13a** was purified by column chromatography.

The nucleoside phosphorothiodifluoridate **13b** was prepared by addition of an equivalent amount of sulfur to the solution of difluorophosphite **16** in pyridine.

3'-O-*Dimethoxytritylthymidine phosphorodifluoridate* **13a**. 95%, R_f 0.71 (EtOAc); $\delta_P(CD_3CN)$ −14.13 (t), J_{P-F} 956; FAB$^+$ *m/z* 628.3 (M + H).

3'-O-*Dimethoxytritylthymidine phosphorothiodifluoridate* **13b**. 95%, R_f 0.66 (EtOAc); $\delta_P(CD_3CN)$ +47.7 (t), J_{P-F} 1095.9; FAB$^+$ *m/z* 644.3 (M + H).

3'-O-*Acetylthymidin-5'-yl* 5'-O-(9-*Phenylxanthen-9-yl*)*thymidin-3'-yl Phosphorofluoridate* **6a**.—Equimolar amounts of 5'-*O*-(9-phenylxanthen-9-yl)thymidine (0.1 mmol) and CsF (0.1 mmol) were suspended in dry acetonitrile (5 cm^3) and a solution of 3'-*O*-acetylthymidine phosphorodifluoridate **13c** (0.1 mmol) and NEt_3 (0.1 mmol) in dry acetonitrile (5 cm^3) was added at room temperature. After 2 h, ^{31}P NMR spectroscopy showed complete conversion of phosphorodifluoridate **13c** into dinucleoside phosphorofluoridate **6a**. The reaction mixture was evaporated to dryness and the crude product was purified by column chromatography.

Enzymic Digestion of 2'-Deoxyadenosin-5'-yl Thymidin-3'-yl Phosphorofluoridate **7b**.—Samples of the dinucleotide **7b** were incubated respectively with spleen phosphodiesterase (Boehringer) in buffer (0.1 mol dm^{-3} aq. 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 **7b** by spleen phosphodiesterase led to thymidin-3'-yl phosphorofluoridate **9** [$\delta_P(D_2O)$ −7.8, J_{P-F} 947; FAB$^+$ *m/z* 322.3 (M + H)] and 2'-deoxyadenosine.

Hydrolysis of compound **7b** by snake venom phosphodiesterase led to 2'-deoxyadenosin-5'-yl phosphorofluoridate **8b** [$\delta_P(D_2O)$ −6.47, J_{P-F} 928; FAB$^+$ *m/z* 331.2 (M + H)] and thymidine.

Acknowledgements

This work was partly supported by the State Committee for Scientific Research, Poland (Grant no. 2-2664-92-03).

References

1 E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90**, 563.
2 (*a*) W. Dabkowski, F. Cramer and J. Michalski, *Tetrahedron Lett.*, 1987, **28**, 3561; (*b*) M. Michalski, W. Dabkowski, A. Łopusiński and F. Cramer, *Nucleosides, Nucleotides*, 1991, 283; (*c*) W. Dabkowski, F. Cramer and J. Michalski, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1447.
3 (*a*) H. A. Staab, *Angew. Chem.*, 1962, **12**, 407; (*b*) W. Dabkowski, J. Michalski and Z. Skrzypczyński, *Phosphorus Sulfur*, 1986, **26**, 321; (*c*) Reactions of P^{III} amides with acyl chlorides leading to P^{III} chlorides have been described by R. F. Hudson and C. Brown, *Phosphorus*, 1973, **2**, 287; (*d*) L. Horner and M. Jordan, *Phosphorus Sulfur*, 1980, **8**, 235.
4 (*a*) A. Łopusiński and J. Michalski, *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 294; (*b*) W. Dabkowski and J. Michalski, *J. Chem. Soc., Chem. Commun.*, 1987, 755.
5 L. Horner, *Phosphorus Sulfur*, 1981, **11**, 155.
6 A. V. Fokin, *Izv. Akad. Nauk. SSSR, Ser. Khim.*, 1976, 2435.
7 E. V. Nikitin, *Zh. Obshch. Khim.*, 1982, **52**, 2721.
8 Zh. M. Ivanova, *Zh. Obshch. Khim.*, 1968, **38**, 551.
9 R. J. Schmutzler, *J. Inorg. Nucl. Chem.*, 1963, **25**, 335.
10 T. J. Hall and J. H. Hargis, *J. Org. Chem.*, 1986, **51**, 4185.
11 A. Okruszek and W. J. Stec, *Z. Naturforsch., Teil B*, 1976, **31**, 354.
12 F. Effenberger, *Synthesis*, 1981, 70.
13 G. S. Reddy, *Z. Naturforsch., Teil B*, 1970, **25**, 1199.
14 H. L. Boter and A. J. J. Ooms, *Recl. Trav. Chim. Pays-Bas*, 1966, **85**, 21.
15 R. J. Schmutzler, *Chem. Ber.*, 1965, **98**, 552.
16 W. Dabkowski, J. Michalski and Q. Wang, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 522.
17 W. Dabkowski, F. Cramer and J. Michalski, *Tetrahedron Lett.*, 1988, **29**, 3301; see also ref. 2*c*.
18 (*a*) R. Wittman, *Chem. Ber.*, 1963, **96**, 771; (*b*) Ch. Sund and J. Chattopadhyaya, *Tetrahedron*, 1989, **45**, 7523.
19 F. Eckstein, *J. Am. Chem. Soc.*, 1966, **88**, 4292; 1968, **90**, 5337.
20 A. Kraszewski and J. Stawiński, *Tetrahedron Lett.*, 1980, **21**, 2935.
21 T. Shimidzu, K. Yamana, A. Murakami and K. Nakamichi, *Nucleic Acids Res. Symp. Ser.*, 1980, **8**, 81.
22 P. J. Garegg, T. Regberg, J. Stawiński and R. Strömbert, *Chem. Scr.*, 1985, **25**, 280.

Paper 3/06181G
Received 15*th October* 1993
Accepted 17*th November* 1993