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STUDIES ON TRIFLUOROMETHYLPHOSPHONAMIDITE ANALOGUES AS BUILDING BLOCKS IN OLIGONUCLEOTIDE SYNTHESIS

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Abstract. *The phosphorylating reagents $CF_3P(NMe_2)Cl$ and $CF_3P(NEt_2)Cl$ are used to phosphorylate the 3'-hydroxyl moiety of several protected 2'-deoxynucleosides yielding the corresponding nucleoside trifluoromethyl phosphonamidites. Their scope and limitations towards amidite activation are investigated but, however, their behavior is completely different to commonly used nucleoside phosphorus amidites.*

Oligo(deoxy)ribonucleotides with one or more modified phosphorus centres have found growing interest as *antisense* probes[1]. The standard methods of nucleotide synthesis are applicable with more or less modifications to obtain phosphorothioates[2], -dithioates[3] and methylphosphonates[4] as the most common used analogues.

At the beginning of 1992 we started a program to investigate the reactivity of trifluoromethylphosphonous reagents. A trifluoromethyl group attached directly to phosphorus should show similiar steric, polar and electronegative effects as a hydroxyl moiety but has no negative charge. On the other side the lipophilicity should be much more enhanced as the methyl analogue being an advantage in respect of cell permeability.

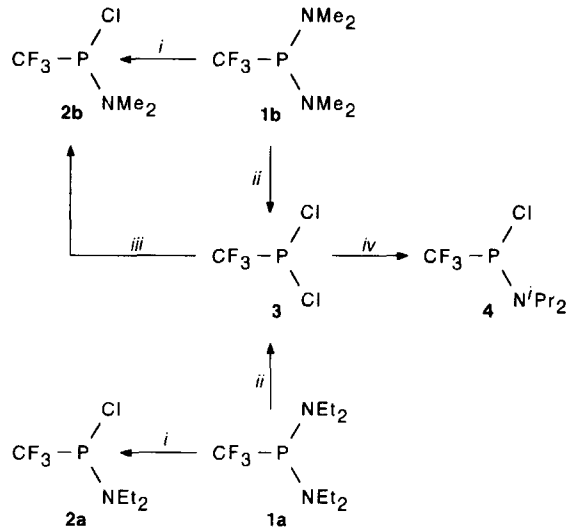
The first procedures to synthesize suitable phosphorylating reagents with a trifluoromethyl group at phosphorus have been extremely difficult and time consuming[5]. However, Volbach and Ruppert[6] have found an easy access to tetraethyl trifluoromethyl phosphorus diamide, $CF_3P(NEt_2)_2$ **1a.**

Casara *et al.*[7] treated trifluoromethylphosphonic acid[8] with protected nucleoside derivatives and obtained the corresponding 5'-O-trifluoromethylphosphonates. Some of these compounds exhibit a remarkable inhibitory activity against *avian myeloblastosis virus* (AMV) and recombinant HIV-1 reverse transcriptase[7].

Blackburn and Guo[9] recently described the synthesis of trifluoromethylphosphorus bistriazolides from CF_3PBr_2 and the coupling with 5'-O-dimethoxytritylthymidine and 2',3'-O-isopropylideneadenosine to the corresponding trifluoromethyl phosphonate.

Our investigations towards easy to handle nucleoside trifluoromethylphosphonamidites as building blocks in oligonucleotide synthesis led to the phosphorylating reagents **2**, which can prepared directly from the

bisamidites **1** with two equivalents of hydrogen chloride[10]. The tetramethyl trifluoromethylphosphorus-diamides **1** are synthesized in high yields in an improved procedure.



Reagents: *i*, 2 HCl/Et_2O (-78°C); *ii*, 4 HCl/Et_2O (-78°C); *iii*, $Me_2NCH_2NMe_2/Et_2O$ (0°C); *iv*, 2 $^iPr_2NH/Et_2O$ (0°C).

Trifluoromethylphosphorus dichloride **3** is obtained under difficulties from the reaction of **1** with excess PCl_3, based on the work of Volbach[6]. Therefore it is preferable to use hydrogen chloride in ether for the exchange reactions and to utilize this solution for subsequent reactions.

Thus we have synthesized the mono chloridite **2b** from **1a** via the dichloridite **3**. The reaction of **3** with equimolar amounts of N,N,N',N'-tetramethyldiaminomethane[11] in ether at 0° C yields **2b** quantitatively. With two equivalents of diisopropylamine the N,N-diisopropylamino trifluoromethylphosphorus chloridite **4** was prepared.

The phosphonochloridites **2** are suitable in synthesizing the corresponding nucleoside trifluoromethyl-phosphonamidites **5**, which are obtained in yields up to 75% after flash chromatography. It is also possible to separate the diastereomers which are formed in a ratio 1:1.

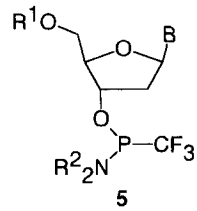
General procedure for the synthesis of protected 2'-O-deoxynucleoside-3'-O-dialkyl trifluoromethyl-phosphonamidites **5** (see Table 1):

To a solution of the protected 2'-deoxynucleoside in dichloromethane, chloroform or acetonitrile are added each 1.5 equivalents of triethylamine and phosphorylating reagent **2a** or **2b**. Stirring is continued for 12h at room temperature. The solvent is removed *in vacuo* and the residue is dissolved in diethyl ether. After filtration to remove the amine hydrochloride and evaporation of the ether the resulting foam is purified by flash chromatography.. Coevaporating of the obtained oils with dichloromethane in a high vacuum system yields the fully protected nucleoside trifluoromethylphosphonamidites **5**.

Table 1. Nucleotide derivatives **5** and **6** with corresponding ^{31}P-NMR and ^{19}F-NMR data

Comp.	R^1	R^2	B	$^{31}P(J_{PF})$ [ppm;Hz]	$^{19}F(J_{FP})$ [ppm;Hz]
5a[12]	DMTr	Me	T	114.3 (87.1); 114.4 (871.)	7.4 (86.9)
5b	Tr	Et	T	113.7 (86.5); 113.8 (86.9)	8.6 (89.0); 8.7 (88.6)
5c	Tr	Me	A^{Bz}	113.8 (86.3); 114.2 (87.0)	7.1 (87.0); 7.2 (86.3)
5d	Tr	Me	C^{Bz}	114.2 (87.1); 114.7 (87.5)	8.9 (86.9); 9.0 (87.5)
5e	TBDMS	Me	T	113.8 (86.7); 114.1 (87.1)	6.9 (86.0); 6.9 (86.8)
5f*	DATE	Me	T	114.4 (87.0)	
6a[13]	DATE		T	-2.0 (127.8)	5.5 (128.4); 6.2 (127.5)
6b	TBDMS		T	-2.7 (128.2); -2.9 (127.0)	

* DATE= 1,1-dianisyl-2,2,2-trichloroethyl-



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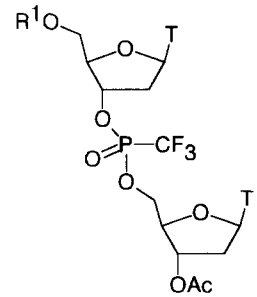

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Unfortunately, these building blocks **5** do not behave as expected by activation with common tetrazoles in presence of a second nucleoside. But reaction with equivalent amounts of benzoyl chloride and subsequent oxidation with 2,4-dichlorophenyl(N-tosyl)oxaziridine[14] leads to the corresponding phosphonochloridates according to ^{31}P-NMR data. Coupling with 3'-O-acetylthymidine and further purification by flash chromatography yields the dinucleoside trifluoromethylphosphonates **6**.

In this work we describe an easy access to trifluoromethylphosphonamidite building blocks of nucleosides, their unconventional activation and reaction after oxidation with another nucleoside to a fully protected dinucleoside trifluoromethylphosphonate, a new class of compounds.

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12. **5a:** ^{1}H-NMR ($CDCl_3$, 360 MHz): δ= 1.40 (s, 3 H, CH_3, Me-5); 2.28 (m, 1 H, H-2'.); 2.48 (m,1 H, H-2'); 2.52 (d, 6 H, $^{2}J_{PH}$= 8.6, 2 CH_3, NMe_2); 3.32 (dd 1 H, ^{2}J= 10.8, $^{3}J_{4'5'}$= 2.9, H-5'); 3.44 (dd 1 H, ^{2}J= 10.8, $^{3}J_{4'5'}$= 2.9, H-5'); 3.71 (s, 6 H, 2 CH_3, DMTr); 4.01 (m, 1 H, H-4'); 4.61 (m, 1 H, H-3'); 6.32 (dd, 1 H, $^{3}J_{1'2'}$= 7.2, 6.1, H-1'); 6.76 (d, 4 H, ^{3}J= 8.6, 4 CH, m-An, DMTr); 7.19- 7.36 (m, 9 H, 9 CH, m-An, Ph, DMTr), 7.48 (s, 1 H, H-6); 9.13 (s, br, 1 H, NH);.
    R_F: 0.50 (ethylacetate/hexane/triethylamine 45:45:10 v/v/v); faster diastereomer.
    ^{1}H-NMR ($CDCl_3$, 360 MHz): δ= 1.39 (s, 3 H, CH_3, Me-5); 2.28 (m, 1 H, H-2'.); 2.37 (m,1 H, H-2'); 2.70 (d, 6 H, $^{2}J_{PH}$= 8.6, 2 CH_3, NMe_2); 3.31 (dd 1 H, ^{2}J= 10.8, $^{3}J_{4'5'}$= 2.9, H-5'); 3.42 (dd 1 H, ^{2}J= 10.8, $^{3}J_{4'5'}$= 2.8, H-5'); 3.72 (s, 6 H, 2 CH_3, DMTr); 4.11 (m, 1 H, H-4'); 4.63 (m, 1 H, H-3'); 6.28 (dd, 1 H, $^{3}J_{1'2'}$= 7.2, 6.1, H-1'); 6.76 (d, 4 H, ^{3}J= 8.6, 4 CH, m-An, DMTr); 7.19- 7.38 (m, 9 H, 9 CH, m-An, Ph, DMTr), 7.57 (s, 1 H, H-6); 9.33 (s, br, 1 H, NH);
    R_F: 0.44 (ethylacetate/hexane/triethylamine 45:45:10 v/v/v); slower diastereomer.
13. **6a:** $C_{39}H_{41}Cl_3F_3N_4O_{14}P$= 984.11; ^{1}H-NMR ($CDCl_3$, 360 MHz): δ= 1.49, 1.51 (2 s, 3 H); 1.87, 1.89 (2 s, 3 H); 2.12, 2.14 (2 s, 3 H); 2.21- 2.60 (m, 4 H.); 3.70- 3.90 (m, 2 H); 3.78, 3.84 (2 s, 6 H); 4.17- 4.26 (m, 2 H); 4.62 (m, 1 H); 4.98 (m, 1 H); 5.19 (m, 1 H); 5.60 (m, 1 H); 6.37, 6.41 (2 m, 2 H); 6.70- 6.93 (m, 4 H); 7.18- 7.88 (m, 6 H); 8.93, 8.94, 9.12, 9.19 (4 s, br, 2 H);
    R_F: 0.41 (chloroform/ethanol/triethylamine 90:10:1 v/v/v); m/e (FAB): 1005 ($[M+ {}^{23}Na]^{+}$, 7 %); 343 ($[DATE]^{+}$, 44 %); Yield: 26%.
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