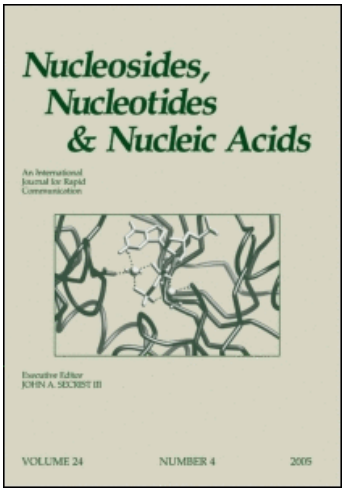

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The 1,1-Dianisyl-2,2,2-trichloroethyl Moiety as a New Protective Group for the Synthesis of Dinucleostde Trifluoromethylphosphonates

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THE 1,1-DIANISYL-2,2,2-TRICHLOROETHYL MOIETY AS A NEW PROTECTIVE GROUP FOR THE SYNTHESIS OF DINUCLEOSIDE TRIFLUOROMETHYLPHOSPHONATES

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Abstract: The new 1,1-Dianisyl-2,2,2-trichloroethyl moiety (DATE) is used as an acid and base stable protective group for nucleosides. 5′-*O*-DATE-thymidine and 3′-*O*-acetyl-thymidine are phosphorylated with $CF_3P(NR_2)_2$ to the corresponding thymidine trifluoromethylphosphonous amidites. These building blocks are coupled with appropriate protected thymidines to a dinucleotide trifluoromethylphosphonate.

For oligonucleotides to be effective as antisense agents, they need to penetrate cell membranes and to be resistant to degradation by nucleases. These requirements have stimulated efforts to prepare backbone-modified derivatives which might be capable of penetrating membranes more readily whilst retaining their resistance to degradation. One widely used approach to improve cell penetration involves removal of the negative charges to produce neutral backbones such as, for example, methyl phosphonates[1,2], phosphoramidates[2] or peptide nucleic acids (PNAs)[3].

Our approach to backbone modification is to replace the anionic phosphodiester groups with the neutral trifluoromethylphosphonates. These modified phosphonates should show similar steric, polar and electronic effects as a hydroxy group and additionally the lipophilicity should be enhanced.

The tetraalkyl trifluoromethylphosphorus diamides **1a/b** were synthesized by an improved procedure based on the work of Volbach[4]. Little is known about the stability of

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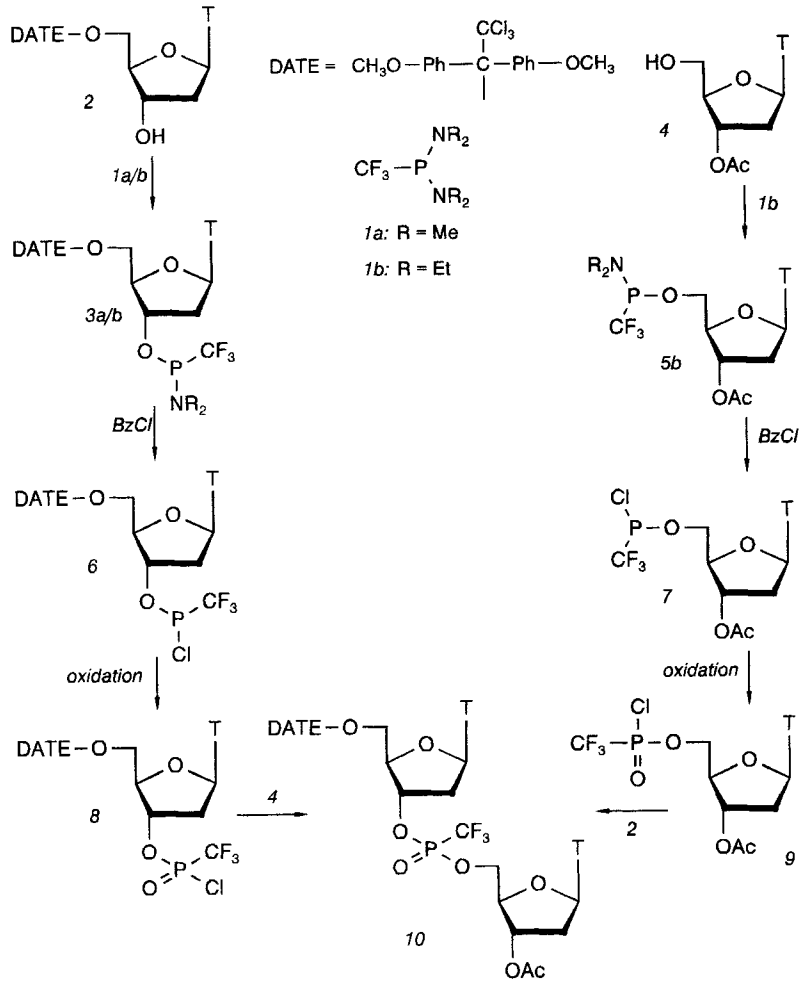
* *Dedicated to Dr. Yoshihisa Mizuno on the occasion of his 75th birthday.*

a trifluoromethyl group attached to phosphorus that is why for the synthesis of a 3′-phosphorylated building block the 5′-hydroxy group is protected by means of the 1,1-dianisyl-2,2,2-trichloroethyl moiety (DATE)[5] as an extremely acid and base stable ß-haloalkyl ether. This ß-haloalkyl ethers are cleavable under neutral conditions by reductive fragmentation with the supernucleophile lithium cobalt(I)phthalocyanine or classically with the zinc method[5]. The reagents **1a/b** were used directly for the phosphorylation of the 5′-*O*-DATE- or 3′-*O*-acetyl protected nucleosides, respectively. Therefore, a conversion of **1a/b** to the corresponding chloro dialkyl trifluoromethyl-phosphorus monoamide [$CF_3P(Cl)NR_2$, R= Me, Et][6] is not necessary. But, however, an activation of **1a/b** is not possible with usually used salts of tetrazole. On the other hand a fourfold excess of 1*H*-tetrazole leads to a successful activation and reaction with the nucleosides **2** or **4** results in the formation of the nucleoside monoamidites **3 a/b** and **5 b**, respectively. The trifluoromethylphosphonous amidites **3 a/b** and **5 b** can be purified by flash chromatography and are stable for months at 0 °C. It is also possible to separate the diastereomers which are formed in a ratio 1:1.

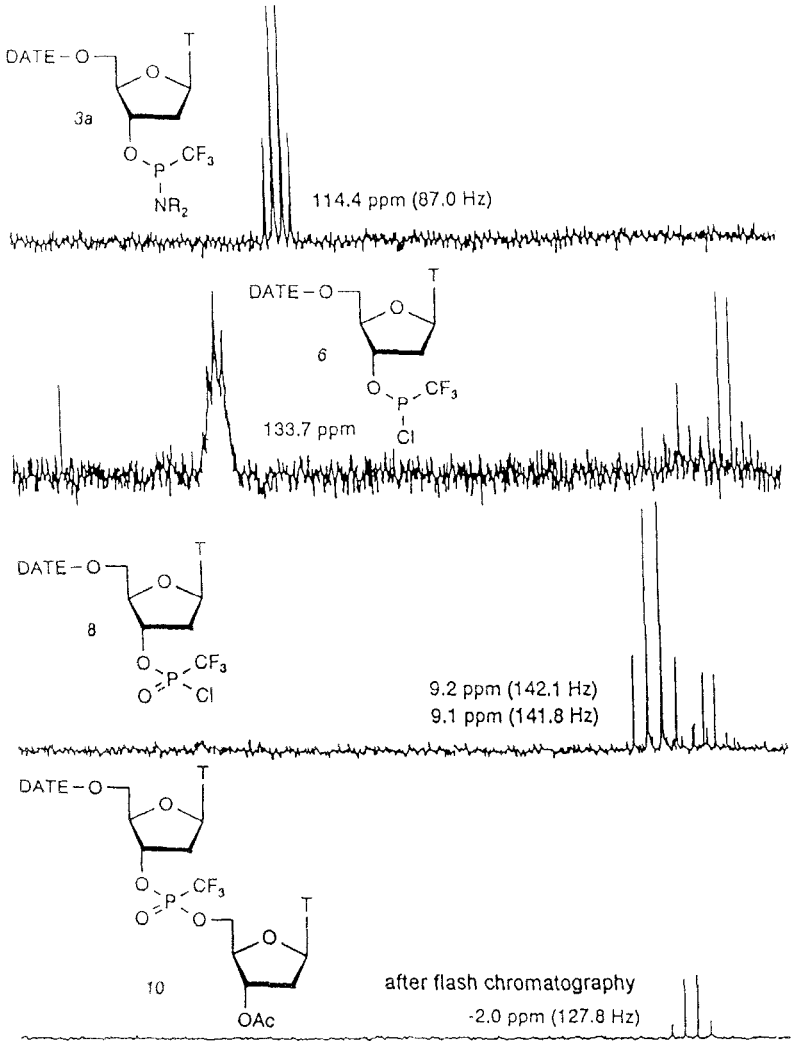
For the synthesis of the dinucleotide **10** starting from **3 a/b** or **5 b** several reagents for the activation were tested. With 5-(4′-nitrophenyl)-1*H*-tetrazole, 5-(2′,4′-dichlorophenyl)-1*H*-tetrazole, collidine hydrochloride and pyridine hydrochloride no reaction of the monoamidites **3 a/b** or **5 b** with the protected nucleosides **4** or **2** could be observed. Among the several tried acid chlorides (acetyl chloride, benzoyl chloride BzCl, tosyl chloride, 2,4,6-trimethylphenylsulfonyl chloride) only BzCl converts the amidites **3 a/b** or **5 b** to the corresponding chloridites **6** or **7** in a satisfactory manner. With an equimolar amount of BzCl the reaction time for complete conversion lies between 5 - 18 hours according to ^{31}P-NMR spectroscopy. The reaction proceeds faster with the dimethyl amidite **3 a** (5 h) in contrast to the diethyl amidite **3 b**. The reason for the low activity towards the common 1*H*-tetrazole activators is probably because of the strong electron withdrawing effect of the trifluoromethyl group which causes that the amidite nitrogen is not accessible to protonation.

The monochloridite building blocks **6** or **7** can be converted with another protected nucleoside to the corresponding P(III)-dinucleotides, but, however, these show ligand exchange by standing in solution for a while. A similar behavior was observed in the case of the corresponding methyl phosphonates[7]. Therefore, at first the chloridites **6** or **7** were



Scheme 1. Synthesis of dinucleotide **10** starting from 3′- or 5′-phosphorylated thymidine derivatives.

oxidized *in situ* with oxaziridines, which proved to be very mild oxidation reagents for nucleotides[8]. The final coupling step was performed in the presence of triethylamine or pyridine as acid scavangers to yield the fully protected trifluoromethylphosphonous dinucleotide **10**. The complete reaction sequence was monitored by ^{31}P-NMR spectroscopy (scheme 2).



Scheme 2. ^{31}P-NMR spectroscopic monitoring of the reaction of **3a** to **10.**

The chemical shifts but especially the coupling constants are the most characteristic features of the various intermediates and products. The low coupling yield is probably due to the extreme sensitivity of the chloridites **6** or **7** towards moisture. The diastereoisomers of **10** are not resolved in the ^{31}P-NMR spectra. But, however, in the ^{19}F-NMR spectra they appear clearly as a 1:1 mixture of diastereoisomers (see experimental section).

Further investigations towards the unusual activation behavior of the trifluoromethylphosphonous amidites and the exact cleavage conditions of the protecting groups are currently in work. Nevertheless, more research towards improved phosphorylating reagents probably on P(V)-base is necessary.

EXPERIMENTAL

The ^{1}H-NMR and ^{13}C-NMR spectra were obtained by a Bruker AM 360 in $CDCl_3$ with TMS as the internal standard with the ^{1}H-NMR at 360.13 MHz, and the ^{13}C-NMR at 90.556 MHz. The ^{19}F-NMR spectra were recorded by a Bruker AC 250 (235.34 MHz) or a Bruker AM 360 (338.86 MHz), ^{31}P-NMR spectra by the same instruments at 101.26 MHz and 145.79 MHz, respectively or by a Jeol JNM FX 90 (36.20 MHz). Mass spectra (FAB) were obtained on a Varian MAT CH-5 (70 eV) instrument in glycerol as matrix. Melting points are uncorrected and were determined with a Büchi SMP-20 apparatus. Elemental analyses were performed by the Microchemical laboratory of the Institute of Organic Chemistry, Technical University, Munich. Flash chromatography was done on a column of Silica gel 60, 15-40 μm (Merck). Thin layer chromatography was performed on Silica gel 60 F_{254} plates (Merck) and the compounds were detected by ultraviolet light. The solvents were purified and dried by the usual methods. Moisture and oxygen sensitive compounds are handled in flame-dried flasks under an atmosphere of dry nitrogen.

5′-*O*-(1,1-Dianisyl-2,2,2-trichloroethyl) thymidine (*2*): To a solution of 4.1 mmol (1.0 g) thymidine in 15 mL acetonitrile, 10 mL of pyridine, 7.5 mmol (2.84 g) DATE chloride[5] and 9.8 mmol (2.72 g) silver tosylate are added and stirred at ambient temperature for 20 h. The solvent and the excess of pyridine are evaporated. The residue is dissolved in $CHCl_3$ and washed with water. The aqueous phase is extracted with ether. The organic phases are collected and the solvent is evaporated. Traces of pyridine are coevaporated with toluene. The desired product is isolated by flash chromatography (CH_2Cl_2/MeOH 100:0 → 95:5, v/v). Yield: 1.9 g (78%). Mp.: 108 - 110 °C. R_f = 0.32 (CH_2Cl_2/MeOH 95:5, v/v). Anal. calcd. for $C_{26}H_{27}Cl_3N_2O_7$: C, 53.30; H, 4.64; N, 4.78. Found: C, 53.81; H, 4.97; N, 4.71. ^{1}H-NMR: δ = 1.49 (s, 3H, 5-CH_3); 2.30 (m, 1H, H-2′A); 2.44 (m, 1H, H-2′B); 3.70 (d, 1H, OH-3′, $J_{OH\text{-}3',\ H\text{-}3'}$ = 4.4 Hz, D_2O-exchange); 3.78 (s, 3H, OCH_3); 3.81 (s, 5H, OCH_3, H-5′); 4.10 (d, 1H, H-4′, J = 2.4 Hz); 4.76 (br s, 1H, H-3′); 6.47 (dd, 1H, H-1′, $J_{H\text{-}1',\ H\text{-}2'}$ = 8.2/5.7 Hz); 6.80 (d, 2H, m-H_{anisyl}, J = 9.0 Hz); 6.85 (d, 3H, m-H_{anisyl}, J = 8.9 Hz); 7.40 (s, 1H, H-6); 7.47 (d, 2H, o-H_{anisyl}, J = 8.9 Hz);

7.58 (d, 2H, o-H_{anisyl}, J = 8.9 Hz); 9.89 (br s, 1H, NH, D_2O-exchange). ^{13}C-NMR: δ = 11.9 (5-CH_3); 40.5 (C-2′); 55.3 (OCH_3); 66.5 (C-5′); 72.3 (C-3′); 84.5, 85.9 (C-1′, C-4′); 92.4 (DATE-C); 105.1 (CCl_3); 111.4 (C-5′); 112.5, 112.9 (m-C_{anisyl}); 129.5, 131.7 (ipso-C_{anisyl}); 131.8, 132.0 (o-C_{anisyl}); 135.6 (C-6); 159.5, 159.7 (p-C_{anisyl}); 150.8, 164.1 (C-2, C-4).

Phosphorylation of 5′-*O*-DATE-thymidine (*2*) or 3′-*O*-acetylthymidine (*4*) with tetraalkyl trifluoromethylphosphorus diamides *1a/b*: To 1 equiv. of the phosphorylating reagent **1a/b** in 5 mL of CH_2Cl_2, $CHCl_3$ or acetonitrile are added 4 equiv. of 1*H*-tetrazole and 1 equiv. of 5′-*O*-DATE-thymidine (**2**) or 3′-*O*-acetylthymidine (**4**) in portions. The reaction is monitored by TLC. Stirring is continued until no more nucleoside is consumed. The solvent is evaporated and the residue is treated with diethyl ether or benzene. After filtration of the dialkylammoniumtetrazolide and evaporation of the solvent the resulting foam is purified by flash chromatography (hexane/ethyl acetate/triethylamine 60:30:10 v/v/v). For the corresponding 5′-*O*-phosphorylated thymidine **5b** the chromatographic step is not necessary.

5′-*O*-(1,1-Dianisyl-2,2,2-trichloroethyl)-3′-*O*-(N,N-dimethylaminotrifluoromethyl-phosphine) thymidine (*3a*): Yield: 68%. R_f = 0.44, 0.51 (ethyl acetate/hexane/triethylamine 45:45:10, v/v/v; diastereomeric mixture 1:1). ^{1}H-NMR: δ = 1.89, 2.01 (s, 3H, Me-5); 2.25 (m, 1H, H-2′A); 2.52 (m, 1H, H-2′B); 2.71 (d, 6H, $^2J_{PH}$ = 8.6 Hz, NMe_2); 3.72 (s, 3H, OCH_3); 3.75 (m, 2H, H-5′); 3.78 (s, 3H, OCH_3); 4.00, 4.09 (m, 1H, H-4′); 4.84 (m, 1H, H-3′); 6.34 (m, 1H, H-1′); 6.72 (d, 2H, J = 8.4 Hz, m-H_{anisyl}); 6.74 (d, 2H, J = 8.4 Hz, m-H_{anisyl}); 7.19 (s, 1H, H-6); 7.39 (m, 2H, o-H_{anisyl}); 7.43 (d, 2H, J = 8.4 Hz, o-H_{anisyl}); 9.93 (s, br, 1H, NH). ^{19}F-NMR: δ = 9.0 ($^2J_{PF}$ = 84.8 Hz). ^{31}P-NMR: δ = 114.8 (q, $^2J_{PF}$ = 86.7 Hz); 114.9 (q, $^2J_{PF}$ = 86.7 Hz).

5′-*O*-(1,1-Dianisyl-2,2,2-trichloroethyl)-3′-*O*-(N,N-diethylaminotrifluoromethyl-phosphine) thymidine (*3b*): Yield: 75%. R_f = 0.42, 0.50 (ethyl acetate/hexane/triethylamine 45:45:10, v/v/v; diastereomeric mixture 1:1). ^{1}H-NMR: δ = 1.01 (tr, 6H, J = 7.1 Hz, NEt_2-CH_3); 1.41, 1.47 (s, 3H, Me-5); 2.27 (m, 1H, H-2′A); 2.43 (m, 1H, H-2′B); 3.08 (m, 4H, NEt_2-CH_2); 3.71 (s, 3H, OCH_3); 3.73 (m, 2H, H-5′); 3.77 (s, 3H, OCH_3); 4.05, 4.08 (m, 1H, H-4′); 4.82 (m, 1H, H-3′); 6.34 (m, 1H, H-1′); 6.73 (d, 2H, J =

8.4 Hz, m-H_{anisyl}); 6.78 (d, 2H, J = 8.4 Hz, m-H_{anisyl}); 7.20 (s, 1H, H-6); 7.38 (m, 2H, o-H_{anisyl}); 7.52 (d, 2H, J = 8.4 Hz, o-H_{anisyl}); 9.84 (s, br, 1H, NH). ^{19}F-NMR: δ = 8.66 (d, $^{2}J_{PF}$ = 88.6 Hz); 8.73 (d, $^{2}J_{PF}$ = 88.5 Hz). ^{31}P-NMR: δ = 113.0 (q, $^{2}J_{PF}$ = 88.6 Hz); 113.1 (q, $^{2}J_{PF}$ = 88.5 Hz). m/e (FAB): 778, 780, 782 ([M + ^{23}Na]$^{\oplus}$, 8 %); 343 ([DATE]$^{\oplus}$, 28 %).

3′-*O*-Acetyl-5′-*O*-(N,N-diethylaminotrifluoromethylphosphine) thymidine (*5b*): Yield: 77%. R_f = 0.43 (ethyl acetate/hexane/triethylamine 45:45:10, v/v/v; diastereomeric mixture 1:1). ^{1}H-NMR: δ = 1.12, 1.13 (2 tr, 6H, J = 7.1 Hz, NEt_2); 1.91 (s, 3H, Me-5); 2.11 (s, 3H, Ac); 2.11 (m, 1H, H-2′); 2.83 (m, 1H, H-2′); 3.14 (m, 4H, NEt_2); 4.01 (m, 2H, H-5′); 4.16 (m, 1H, H-4′); 5.25 (m, 1H, H-3′); 6.42 (m, 1H, H-1′); 7.36, 7.44 (2 s, 1H, H-6); 9.87 (s, br, 1H, NH). ^{19}F-NMR: δ = 7.30 (d, $^{2}J_{PF}$ = 84.6 Hz); 7.40 (d, $^{2}J_{PF}$ = 85.6 Hz). ^{31}P-NMR: δ = 114.0 (q, $^{2}J_{PF}$ = 85.8 Hz); 115.0 (q, $^{2}J_{PF}$ = 85.0 Hz).

Synthesis of the dinucleotide *10*: To a solution of 1 equivalent (0.5 to 1 mmol) of 3′- or 5′-nucleoside phosphonous amidites **3a/b** or **5b** in 2 mL of $CDCl_3$ is added 1 equivalent of freshly distilled benzoyl chloride (BzCl) at room temperature. The mixture is allowed to stand at ambient temperature until **3a/b** or **5b**, respectively, is consumed (5 - 18 h; controlled by ^{31}P-NMR). The solution is cooled to -78 °C and 1.5 equivalents of 3-(2,4-dichlorophenyl)-2-toluenesulfonyl oxaziridine[9] are added. The solution is allowed to come to room temperature and is mixed with 1 equivalent 3′- or 5′-protected nucleoside **4** or **2**, respectively, and 1 equivalent of base in $CDCl_3$. After evaporation of the solvent the residue is subjected to flash chromatography. Yield: 26%. R_f = 0.41 ($CHCl_3$ / EtOH / triethylamine 90:10:1, v/v/v; diastereomeric mixture 1:1). ^{1}H-NMR: δ = 1.49, 1.51 (2s, 3H); 1.87, 1.89 (2s, 3H); 2.12, 2.14 (2s, 3H); 2.21-2.60 (m, 4H); 3.70-3.90 (m, 2H); 3.78, 3.84 (2s, 6H); 4.17-4.26 (m, 2H); 4.62 (m, 1H); 4.98 (m, 1H); 5.19 (m, 1H); 5.60 (m, 1H); 6.37, 6.41 (2m, 2H); 6.70-6.93 (m, 4H); 7.18-7.88 (m, 6H); 8.93, 8.94, 9.12, 9.19 (4s, br, 2H). ^{19}F-NMR: δ = -5.46 (d, $^{2}J_{PF}$ = 128.4 Hz); -6.23 (d, $^{2}J_{PF}$ = 127.5 Hz). ^{31}P-NMR: δ = -2.0 (q, $^{2}J_{PF}$ = 127.8 Hz). m/e (FAB): 1005 ([M + ^{23}Na]$^{\oplus}$, 7 %); 343 ([DATE]$^{\oplus}$, 44 %).

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