Diastereoisomerically Pure Dinucleosidylphosphorofluoridites and their Application in Stereospecific Synthesis of Dinucleosidylphosphorofluoridothionates

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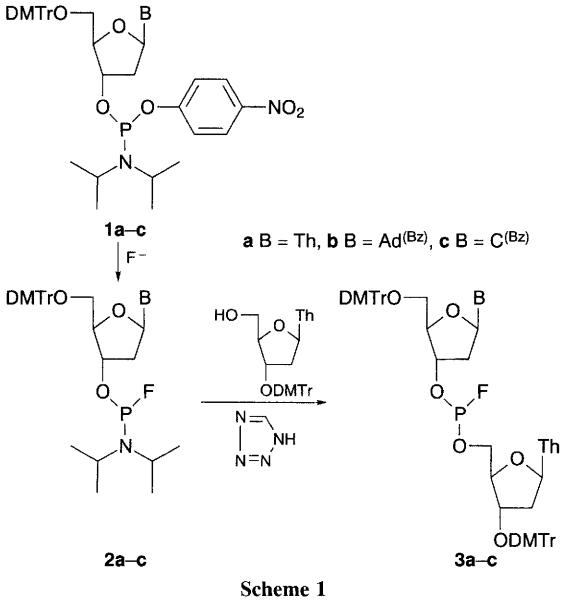
Dinucleosidylphosphorofluoridites with dA(Bz), dC(Bz) or dT at the 3′-end and dT at the 5′-end are synthesised, separated into their diastereoisomers and shown to be converted stereospecifically into the corresponding phosphorofluoridothionates high hydrolytic stability.

Since the classical work of Eckstein and others on chiral nucleosidylphosphorothioates the stereochemistry of backbone modified oligonucleotides became of considerable interest.[1] In the last few years four-coordinate P-chiral modified nucleotides have been employed in stereoselective synthesis of backbone modified oligonucleotides by Stec and Wilk[2a] and Leśnikowski.[2b] In contrast, stereoselective synthetic procedures based on synthons containing three-coordinate phosphorus centres have received considerably less attention.[2a,3] This situation can be ascribed to the chemical and stereochemical instability of many phosphorus(III) systems. The properties of three-coordinate phosphorus compounds can be varied by electronic and steric effects. A search for phosphorus(III) nucleotide systems combining stereochemical stability with the presence of a good leaving group would be of considerable interest. In an attempt to find suitable systems of this type we have examined nucleotides containing the 4-nitrophenoxy group attached to a P^{III} centre[4] and more recently to nucleotides accommodating fluoride at a three-coordinate phosphorus atom.[5] Here we report the synthesis of deoxynucleosidylphosphorofluoridates and their separation into pure diastereoisomers.

In general, little is known about the chemistry and stereochemistry of organophosphorus compounds containing a P^{III}–F functional centre. *trans*-2-Fluoro-4-methyl-1,3,2-dioxaphosphorinane has been prepared by Mikołajczyk *et al.*[6] while the first resolution of a free fluorophosphane MePhPF has been achieved only recently.[7] We have recently devised a strategy for the synthesis of nucleosidylphosphorofluoridites **2** based on the replacement of a 4-nitrophenoxy group attached to a P^{III} centre by fluoride.[5] In continuation of these studies we now report the first synthesis of dinucleosidylphosphorofluoridites **3a–c** which are surprisingly stable and can be readily separated into pure diastereoisomers. The synthetic pathway leading to the dinucleosidylphosphorofluoridites **3** is shown in Scheme 1.



Scheme 1

The nucleosidyl phosphoroamidofluoridites **2** are formed with some degree of stereoselectivity† and can be separated into pure diastereoisomers.[8] However, their coupling reaction in the presence of tetrazole under usual conditions affords diastereoisomers **3a–e** as 1 : 1 mixtures in almost quantitative yield. It is noteworthy that this coupling does not affect the phosphorus–fluorine bond. Chromatography of phosphorofluoridites **3a–c** on silica gel gives pure 'fast' isomers of high configurational stability.‡ Typical ^{31}P and ^{19}F NMR spectra of the 'fast' isomer of **3b** are shown in Fig. 1.

The high configurational stability of phosphorofluoridites **3** can be explained by the presence of the electronegative fluorine ligand and steric hindrance exerted by the nucleosidyl groups.[9]

To underline the desirable features of phosphorofluoridites **3**, their use in the stereospecific synthesis of dinucleosidylphosphorofluoridothionates **3** and their hydrolytic stability can be cited. This is illustrated by the reaction of the phosphorofluoridite **3a** with bisbenzoyl disulfide which leads to a single diastereoisomer **4a**§ (Scheme 2), which most likely proceeds with retention of configuration at the chiral phosphorus atom.[6]

As expected, compounds **4** containing a thiophosphoryl centre are distinctly more resistant towards hydrolysis and other nucleophilic displacements than their oxo analogues.[10] Hydrolytic susceptibility of phosphorofluoridates and phosphorofluoridothionates is strongly influenced by the presence of fluoride ions.[11] The same phenomenon was also observed in our

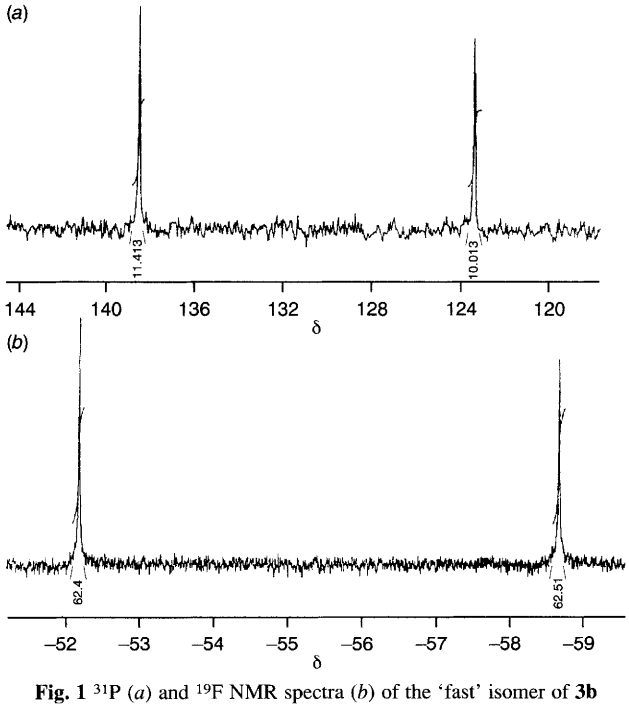
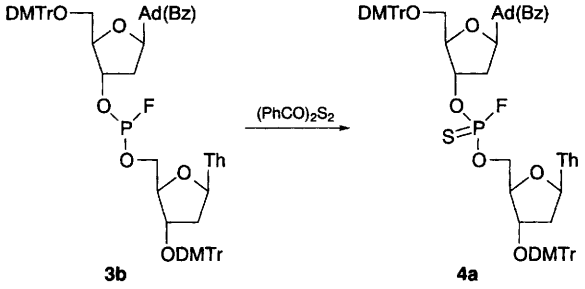


Fig. 1 ^{31}P (*a*) and ^{19}F NMR spectra (*b*) of the 'fast' isomer of **3b**



Scheme 2

studies.¶ The unexpected high stability of phosphorus(III) phosphorofluoridites **3** towards hydrolysis is somewhat surprising. The structures of **3a–c** and **4b** were confirmed using ^{31}P and ^{19}F NMR spectroscopy.‖ They are also in accord with FAB MS data.

In conclusion, we have reported a highly efficient synthesis of diastereoisomeric dinucleosidylphosphorofluoridites **3** and their use in stereospecific preparation of dinucleosidylphosphorofluoridothionates **4**. The latter type of compounds are potentially useful for a 'antisense' approach in constructing antiviral drugs.

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Footnotes

† Compounds **2a–c** were obtained from the corresponding deoxynucleosid-3′-yl-*O*-(4-nitrophenyl)-*N*,*N*-diisopropylphosphoramidites **1a–c** by treatment with tetrabutylammonium fluoride.

‡ *Example of experimental procedure for the synthesis of phosphorofluoridite* **3b** d[DMTrA^{Bz} P$_F^{III}$TDMTr]. To a solution of 5′-*O*-DMTr-*N*^{6}-benzoyl-2′-deoxyadenosine-3′-yl-(*N*,*N*-diisopropylamino)phosphorofluoridite **2b** (0.1 mmol) in 10 ml of dry THF was added a solution of 3′-*O*-(4,4′-dimethoxytrityl)thymidine (0.1 mmol) and tetrazole (0.6 mmol) in 15 ml of dry THF. After 10 min, *N*,*N*-diisopropylammonium tetrazolide was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was dissolved in 3 ml of CH_2Cl_2. This solution was applied to a chromatography column (250–400 mesh, silica gel Merck 9385) and was eluted (1 ml min^{-1}) with CH_2Cl_2–Me_2CO (10:2 *v*/*v*). The fractions containing diastereoisomers of dinucleoside phosphorofluoridite were collected and evaporated. The diastereoisomeric purity of the 'fast' isomer was 100% according to ^{31}P and ^{19}F NMR spectroscopy (**3b** R_f = 0.30). The residue consisted of a mixture enriched in the 'slow' isomer. A similar procedure was used for the separation of 'fast' isomers of DMTrThP$_F^{III}$ThDMTr (**3a** R_f = 0.30), and DMTrC^{Bz}P$_F^{III}$ThDMTr **3c** (R_f = 0.32).

§ *Synthesis of* d[DMTrA^{Bz}P$_F$(S)TDMTr] **4b**. To the solution of **3b** (0.1 mmol) in dry THF (10 ml) was added the bisbenzoyl disulfide (0.1 mmol) in dry THF (10 ml) at room temperature. After 1 h the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (230–400 mesh, Merck 9385) using CH_2Cl_2–Me_2CO (10:2 *v*/*v*) as eluent to give the desired phosphorofluoridothionate. Yield 95%, R_f = 0.25.

¶ Phosphorofluoridite **3b** (1.8×10^{-5} mmol) was dissolved in 1.5 ml of MeCN–water (10:3 *v*/*v*); after 3 h ^{31}P NMR spectroscopy showed only the presence of **3b**. In a separate experiment, **3b** was dissolved in 1.5 ml of MeCN–water (10:3 *v*/*v*) and 0.1 ml of 1.1 mol dm^{-3} NBu_4F in THF was added. After 3 h, ^{31}P NMR spectroscopy showed only the presence of the dinucleosidyl *H*-phosphonate [δ_P ($CDCl_3$) 10.21, 9.42, J_{P-H} = 723 Hz]. An identical procedure was carried out with phosphorofluoridothionate **4b**. Again no reaction was observed in the absence of NBu_4F after 3 h, while in its presence the ^{31}P NMR spectrum taken after 3 h showed complete hydrolysis to the deoxydinucleosidyl phosphorothioate d[DMTrA^{Bz}-P(S)(O$^-$)TDMTr] [δ_P ($CDCl_3$) 55.95, 55.14].

‖ *Selected spectroscopic data* ^{31}P NMR ($CDCl_3$, 81.014 MHz, H_3PO_4 external standard), ^{19}F NMR ($CDCl_3$, 188.154 MHz, $CFCl_3$ external standard), J_{P-F}/Hz. **3a**: fast isomer; ^{31}P NMR, δ 138.42, 123.35; ^{19}F NMR, δ 53.91, −60.21, J_{P-F} = 1220.69 Hz; slow isomer; ^{31}P NMR, δ 139.72, 124.61; ^{19}F NMR, δ −53.61, −60.07, J_{P-F} = 1224.03. **3b**; fast isomer; ^{31}P NMR, δ 138.50, 123.47; ^{19}F NMR, δ −52.18, −58.66, J_{P-F} = 1221.24; slow isomer; ^{31}P NMR, δ 140.04, 124.90; ^{19}F NMR, δ −52.77, −59.29, J_{P-F} = 1226.99. **3c**: fast isomer; ^{31}P NMR, δ 138.9, 123.81; ^{19}F NMR, δ −55.34, −61.83, J_{P-F} = 1222.07; slow isomer; ^{31}P NMR, δ 139.19, 124.12; ^{19}F NMR, δ −53.08, −59.56, J_{P-F} = 1221.80. **4a**: ^{31}P NMR, δ 68.16, 54.84, ^{19}F NMR, δ −40.62, −46.35, J_{F-P} = 1086.02.

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