A new method for the formation of the P–F bond

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Iodine-promoted desulfurization of phosphorothioate and phosphorodithioate diesters in the presence of triethylamine tris(hydrofluoride) (TAF) furnish a rapid and quantitative formation of the corresponding phosphorofluoridates and phosphorofluoridothioates; under the reaction conditions phosphoroselenoate and phosphoroselenothioate diesters undergo deselenization affording the phosphorofluoridate and phosphorofluoridothioate diesters, respectively.

The reaction of phosphorochloridate diesters with alkali metal fluorides in aprotic solvents is the method of choice for the preparation of simple dialkyl phosphorofluoridates.[1] However, in the synthesis of natural product derivatives containing the P–F bond, this approach is of limited value owing to the scant availability of the corresponding phosphorochloridates and the often detrimental reaction conditions.

Recently, we have reported on a new, simple and efficient method for the preparation of nucleoside phosphorofluoridate and nucleoside phosphorofluoridothioate diesters[2] and monoesters[3] *via* the iodine promoted oxidation of the corresponding H-phosphonates and H-phosphonothioates in the presence of triethylamine tris(hydrofluoride) (TAF). These studies have been directed towards the development of a synthetic procedure enabling the introduction of fluorine at a preselected phosphorus centre in an oligonucleotide, in order to investigate this kind of DNA surrogates as, *inter alia*, potential antisense/antigene therapeutics.

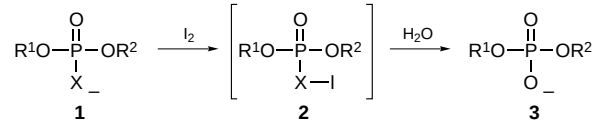
Although dinucleoside phosphorofluoridate and dinucleoside phosphorofluoridothioate diesters are stable solids, that can be purified by silica gel chromatography, the fluorine atom (particularly that in the phosphorofluoridate derivatives) may undergo nucleophilic substitution. This can happen, *e.g.* during the final deprotection of an oligonucleotide and thus would call for significant changes in a solid phase synthesis protocol (*e.g.* the use of more labile base protecting groups, a special procedure for the cleavage of a modified oligomer from the support). For these reasons we have been searching for a synthetic procedure allowing the introduction of fluorine into an unprotected oligonucleotide and in this way alleviating a potential problem of the degradation of a modified oligonucleotide (or the removal of fluorine) during the deprotection step or during its splitting from a support.

As a possible candidate for this purpose we considered phosphorothioate or phosphoroselenoate diesters bearing a sulfur or a selenium atom in the nonbridging position. Owing to the biochemical and medical importance of nucleoside phosphorothioates,[4] on the one hand, and the advances in the chemical synthesis of oligonucleotides, on the other, this class of compounds has recently emerged as most important oligonucleotide analogues.[5]

Since the reaction of iodine with sulfur is known to be a highly selective process[6–11] that can be carried out on unprotected oligonucleotides, we considered as a viable approach for the formation of the P–F bond an iodine-promoted desulfurization (or deselenization) of a suitable phosphorothioate (or phosphoroselenoate) diester in the presence of fluoride anions. The mechanism of this type of reactions (*e.g.* Scheme 1) is unknown but, most likely, it involves the initial formation of the corresponding oxaphosphoranesulfenyl (or oxaphosphoraneselenenyl) iodide (compounds of type **2**), which may undergo a subsequent nucleophilic substitution at the phosphorus centre.

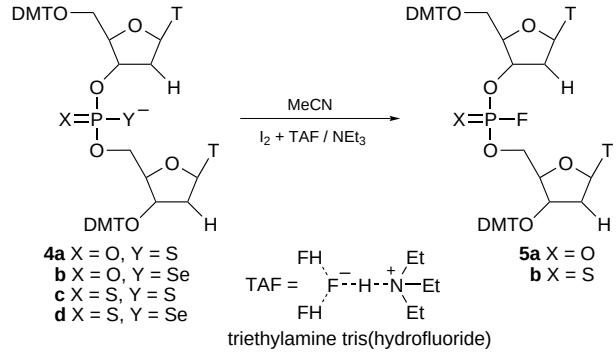
The efficacy of this approach to the formation of phosphorofluoridate diesters was evaluated by reacting the dinucleoside phosphorothioate **4a**[12] with iodine (2.5 equiv.) in acetonitrile in the presence of TAF (1 equiv.) and triethylamine (2 equiv.), Scheme 2. The reaction was fast and clean, as judged by ^{31}P NMR spectroscopy. Within 5 min the resonances due to the starting material **4a** (δ_P 57.5)† completely disappeared and only signals assigned to the expected product, the dinucleoside phosphorofluoridate **5a**[2] [δ_P −9.8, $^1J_{PF}$ = 978 (d), $^3J_{PH}$ = 7.3 Hz, (q) and δ_P −10.2, $^1J_{PF}$ = 985 (d), $^3J_{PH}$ = 7.3 Hz, (q); ratio *ca.* 1 : 1], were detected. No changes were observed in the reaction mixture when it was left to stand for 2–3 h. The phosphorofluoridate **5a** was also formed as the sole phosphorus-containing product (^{31}P NMR spectroscopy) when the dinucleoside phosphoroselenoate **4b**[7] (δ_P = 52.1[13]) was subjected to the reaction with iodine under analogous reaction conditions.

We also checked the feasibility of using this approach for the synthesis of phosphorofluoridothioates. To this end, the dinucleoside phosphorodithioate **4c**[12] was treated with iodine (2.5 equiv.) in acetonitrile in the presence of TAF (1 equiv.) and triethylamine (2 equiv.). The reaction proceeded to completion within 5 min (^{31}P NMR) as was apparent from the disappearance of the starting material **4c** (δ_P 115.4) and a clean and quantitative formation of the phosphorofluoridothioate **5b**[2] [δ_P 62.0, $^1J_{PF}$ = 1076 (d), $^3J_{PH}$ = 8.6 Hz, (q) and δ_P 61.2, $^1J_{PF}$ = 1085 (d), $^3J_{PH}$ = 8.5 Hz, (q); ratio *ca.* 1 : 1]. We did not observe any desulfurization of **5b** under the reaction conditions. This was, in principle, expected in light of the known inertness of



X = S or Se
R^1 and R^2 = nucleoside moieties

Scheme 1



Scheme 2

phosphorothioate triesters (and probably other uncharged phosphorus compounds bearing the thiophosphoryl function) towards iodine.[6]

In dinucleoside phosphoroselenothioate diesters, there are two centres which may react with iodine, *i.e.* selenium and sulfur. Owing to differences in the standard reduction potentials of selenium and sulfur ($E°_{Se} = -0.924$ and $E°_{S} = -0.476$ V), it is the former which should undergo preferential oxidation with iodine. Indeed, the reaction of **4d**[7] (δ_P 104.2 and 103.7) with iodine in the presence of fluoride anion under the conditions as for **4c** afforded exclusively the phosphorofluoridothioate **5b**, as was apparent from the chemical shift value of the product and the absence of a characteristic $^1J_{PSe}$ coupling.

The above reactions were also run on a preparative scale. In a typical experiment, the dinucleoside phosphorothioate **4a** (triethylammonium salt, 0.05 mmol) was rendered anhydrous by repeated evaporation of the added acetonitrile and dissolution in the same solvent (3 ml) containing triethylamine (2 equiv.) and TAF (1 equiv.). A solution of iodine (2.5 equiv.) in acetonitrile (0.5 ml) was then added. After 10 min the reaction mixture was diluted with chloroform (10 ml) and extracted with aq. 10% $Na_2S_2O_3$ (1 × 10 ml). The organic phase was dried over $NaSO_4$, concentrated *in vacuo*, and the residue was chromatographed on a silica gel column using ethyl acetate–toluene (1 : 1, v/v) as eluent. Using this procedure the phosphorofluoridate **5a** was obtained in 70–85% yield‡ (starting from **4a** or **4b**) and the phosphorofluoridothioate **5b** in > 90% yield (starting from **4c** or **4d**). Compounds **5a** and **5b** prepared in this study were identical (TLC, ^{1}H and ^{31}P NMR and FABMS) with those synthesised *via* the iodine-promoted oxidation of the corresponding dinucleoside H-phosphonate and H-phosphonothioate in the presence of TAF.[2]

The relevance of the procedure to the preparation of oligonucleotide analogues was assessed by carrying out some preliminary ^{31}P NMR experiments on unprotected dinucleoside phosphorothioates. To this end, dithymidine phosphorothioate (obtained by the detritylation of **4a**, followed by trituration with diethyl ether) was subjected to the reaction with iodine (5 equiv.) in pyridine in the presence of fluoride anion (1 equiv. TAF). A clean formation of the corresponding dithymidine phosphorofluoridate [δ_P −9.4, $^1J_{PF}$ = 977 Hz (d) and δ_P −9.8 ppm, $^1J_{PF}$ = 985 Hz (d); ratio *ca.* 1 : 1] was observed (5 min, ^{31}P NMR). The detritylated dinucleoside phosphorodithioate derived from **4c** reacted similarly under the reaction conditions producing the corresponding phosphorofluoridothioate [δ_P 61.3, $^1J_{PF}$ = 1078 Hz (d) and δ_P 60.9, $^1J_{PF}$ = 1085 Hz (d); ratio *ca.* 1 : 1].

In conclusion, iodine promoted desulfurization or deselenization of the appropriate phosphorothioate and phosphoroselenoate diesters in the presence of fluoride anions offers a new means for the formation of the P–F bond under exceedingly mild reaction conditions and using easily accessible substrates. Since the method is based on a highly selective activation of sulfur or selenium centres by iodine, it is compatible with the presence of unprotected functional groups in a substrate molecule. This, and the possibility of replacing fluorine with other functionalities, makes the method of particular relevance to the synthesis of oligonucleotides and other analogues bearing labile modifications at the phosphorus centre.

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Footnotes

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† Signals for the two phosphorus diastereomers were not resolved.

‡ Lower yields were probably due to partial decomposition during silica gel chromatography.

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