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A New Entry to Nucleoside Phosphorofluoridate and Nucleoside Phosphorofluoridothioate Diesters

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Abstract: Oxidation of H-phosphonate or H-phosphonothioate diesters with iodine in the presence of triethylamine trishydrofluoride furnished a rapid and quantitative formation of the corresponding phosphorofluoridate or phosphorofluoridothioate diesters.

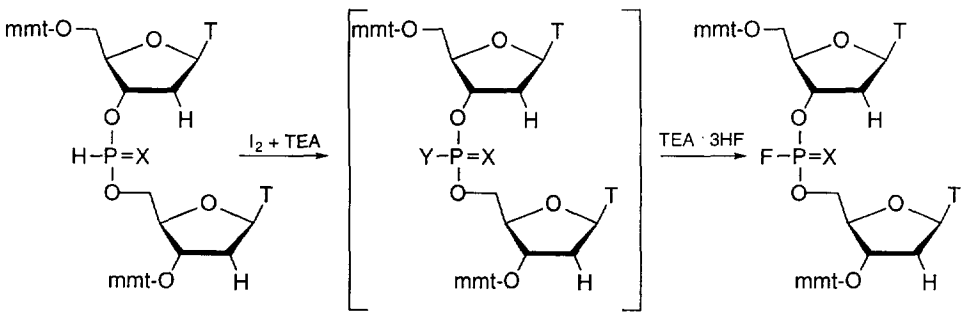
Recent years have witnessed a tremendous revival of fluoroorganic chemistry[1] not least due to finding of molecules with useful biological activity. This includes the chemistry of fluorophosphates. Nucleoside phosphorofluoridates have been synthesised for the first time by Wittmann[2] some thirty years ago, but it is only recently that the synthetic[3-16] and biological[17,18] potential of this class of compounds has begun to be explored. The attractive features of nucleoside phosphorofluoridate diesters, which bear a high resemblance to natural internucleotidic linkage, are that they may provide new means for a covalent attachment of molecular probes to proteins or that the fluoride may serve as a reporter group for probing conformational properties of nucleic acid fragments. In addition, these compounds themselves can be considered as potential therapeutics, *e.g.*, for the antisense/antigene modulation of gene expression.

Replacement of a phosphorus-bound chlorine by fluoride using variety of experimental procedures is by far the most common way for the preparation of fluorophosphoric acids or simple alkyl fluorophosphate diesters[19]. However, the reported methods suffer from several disadvantages when it comes to the preparation of natural products derivatives containing P-F bond. The major obstacles are poor availability of the corresponding phosphorochloridates and the elevated temperature usually required to effect the replacement. To overcome these limitations, new synthetic routes *via* P(V) derivatives (with, *e.g.*, azolides[3,9], thiomethyl[11,20], or selenomethyl[15] as a leaving group) and *via* tervalent P(III) compounds (*e.g.*, using dinucleoside silyl phosphites[4,5,7], dinucleoside phosphorofluoridites[9,10,12], or phosphitylating agents bearing fluorine[14]) have been pursued. Efficiency of these approaches has been demonstrated in the synthesis of ribo- and deoxyribonucleoside cyclic 3',5'-phosphorofluoridates[11,16], dinucleoside phosphorofluoridates[9,15] and phosphorofluoridothioates[12].

In the literature there are only a few procedures reported for the preparation of simple alkyl fluorophosphate diesters using H-phosphonate diesters as starting materials[21,22]. They are based on chlorination of the corresponding phosphonic acid diesters, followed by exchange of chlorine in the produced phosphorochloridate using potassium fluoride. The exchange process usually requires elevated temperature, prolonged reaction time, and due to heterogeneity of the reaction mixtures, also vigorous stirring[21,22]. These features make the procedures unsuitable for the preparation of nucleoside phosphorofluoridates both in solution and on solid support.

As a part of our research in H-phosphonate chemistry we have recently begun investigations directed towards development of synthetic methods that could afford, under mild, homogeneous reaction conditions, and with a minimum number of synthetic operations, nucleoside fluorophosphate and fluorothiophosphate derivatives. As a viable approach for this purpose we considered an iodine-promoted oxidation of H-phosphonate[23] and H-phosphonothioate diesters in the presence of fluoride anion. The rationale behind it was a known easy oxidation of nucleoside H-phosphonate or H-phosphonothioate[24] diesters that should secure fast generation of the corresponding iodophosphate or iodothiophosphate[25] intermediate, and these, having a good leaving group (iodide), should rapidly react further with fluoride anion affording the fluorophosphate or fluorothiophosphate derivatives (Scheme 1).

Scheme 1



1a, X = O
1b, X = S

2a, X = O, Y = I
2b, X = S, Y = I

3a, X = O
3b, X = S

Abbreviations: mmt - monomethoxytrityl; T - thymidin-1-yl; TEA - triethylamine

The choice of a source of nucleophilic fluoride is a perennial problem in substitution reactions at phosphorus (especially for those in organic solvents) and for the most part is a compromise between solubility, reactivity, homogeneity of the reaction mixtures, *etc.* Since the exchange of iodide by fluoride in iodophosphates or iodothiophosphates should be a facile process[26], we decided to use as a source of fluoride in our synthetic protocol very mild and soluble in organic solvents reagent, triethylamine trishydrofluoride[27,28] (TAF).

To evaluate feasibility of such an approach, we carried out preliminary experiments on diethyl H-phosphonate (DEP) and diethyl H-phosphonothioate (DEPS). To this end DEP (0.1 mM) in acetonitrile (2 mL) containing triethylamine (TEA, 4 equiv.), TAF (1 mole equiv.), was reacted with iodine (1.5 equiv), and the ^{31}P

NMR spectrum monitored. The reaction was rapid and clean. In the first ^{31}P NMR spectrum recorded (after ca 5 min), only signals assigned to the expected diethyl phosphorofluoridate were present [δ_P = -8.39 ppm, $^1J_{PF}$ = 966.8 Hz (d), $^3J_{PH}$ = 8.6 Hz, (q)]. Under the same reaction conditions also the thiophosphonate analogue, DEPS (δ_P = 70.44 ppm), underwent in less than 5 min (the first ^{31}P NMR spectrum) a smooth and clean conversion to the corresponding diethyl phosphorofluoridothioate [δ_P = 62.95 ppm, $^1J_{PF}$ = 1068.1 Hz (d), $^3J_{PH}$ = 10.5 Hz, (q)]. In the analogous reactions in pyridine (without TEA), DEPS was converted to the corresponding fluorothiophosphate within 5 min, but DEP reacted significantly slower (~75% conversion to diethyl phosphorofluoridate after 20 min). In separate experiments we found that all reactions proceeded quantitatively also with 0.3 mole equiv. of TAF, indicating that all fluorine in the reagent was available for the exchange.

The efficacy of the replacement of iodide by fluoride, in the *in situ* generated phosphoroiodidates and phosphoroiodidothioates, was also evaluated for the generation of dinucleoside derivatives **3a** and **3b** (Scheme 1). When the dinucleoside H-phosphonate **1a**[25] (δ_P = 8.39 and 9.37 ppm, mixture of the diastereomers) was subjected to the oxidation with iodine (1.5 equiv) in acetonitrile in the presence of TEA (4 equiv) and TAF (0.5 mole equiv., 1.5 F^- equiv.), the first ^{31}P NMR spectrum recorded (after ca 5 min) indicated a complete conversion of the starting material to the mixture of fluorophosphate diastereomers **3a** [δ_P = - 9.84 ppm, $^1J_{PF}$ = 977.7 Hz (d), $^3J_{PH}$ = 7.3 Hz, (q) and δ_P = - 10.24 ppm, $^1J_{PF}$ = 985.1 Hz (d), $^3J_{PH}$ = 7.3 Hz, (q)]. The analogous reaction using the dinucleoside H-phosphonothioate **1b**[25] (δ_P = 70.10 and 71.40 ppm, mixture of the diastereomers) as a starting material, also afforded in less than 5 min exclusively the corresponding dinucleoside phosphorofluoridothioate **3b** [δ_P = 61.98 ppm, $^1J_{PF}$ = 1076.7 Hz (d), $^3J_{PH}$ = 8.6, (q) and δ_P = 61.16 ppm, $^1J_{PF}$ = 1085.2 Hz (d), $^3J_{PH}$ = 8.5 Hz, (q)]. No intermediate (*e.g.*, the iodophosphate **2a** or the iodothiophosphate **2b**) could be detected by ^{31}P NMR spectroscopy under the reaction conditions. Both reactions proceeded rapidly and cleanly in neat pyridine as well (completion in less than 5 min).

We also investigated the possibility to effect the transformations of H-phosphonate and H-phosphonothioate diesters to the corresponding fluoro- and fluorothiophosphates, respectively, by using the Atherton-Todd oxidation[29] in the presence of TAF. We found that the replacement of carbon tetrachloride[29] (6 equiv.) for iodine in the reaction of DEPS in acetonitrile (1 mole equiv. TAF and 4 equiv. TEA), furnished formation of the corresponding phosphorofluoridothioate in ca 5 min. The generation of diethyl phosphorofluoridate from DEP under analogous reaction conditions was slower and required ca 50 min for completion. Attempted oxidation of DEP with CCl_4 in the presence of TAF in neat pyridine afforded after 24 h a complicated mixture of products. In contradistinction to this, the thio derivative DEPS, produced under analogous reaction conditions smoothly diethyl phosphorofluoridothioate (completion within ca 10 min). The H-phosphonate **1a** and the H-phosphonothioate **1b** showed a similar to DEP and DEPS, respectively, reactivity pattern. In the reactions in acetonitrile (6 equiv. CCl_4, 4 equiv. TEA, 1 mole equiv. TAF), the formation of **3a** and **3b** was completed in ca 20 and 5 min, respectively. The H-phosphonothioate **1b** reacted also smoothly to the fluoro derivative **3b** (completion within ca 5 min) when acetonitrile and TEA were replaced by neat pyridine. The H-phosphonate **1a** afforded under such reaction conditions a complicated mixture of products.

In conclusion, oxidation of H-phosphonate or H-phosphonothioate diesters with iodine (or with CCl_4) in the presence of triethylamine trishydrofluoride (TAF) as a source of nucleophilic fluoride, provides a convenient entry to phosphorofluoridate and phosphorofluoridothioate diesters. The transformation is clean, fast, and can be carried out as an "one pot" operation under homogeneous reaction conditions.

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