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A Facile Access to Nucleoside Phosphorofluoridate, Nucleoside Phosphorofluoridothioate, and Nucleoside Phosphorofluoridodithioate Monoesters

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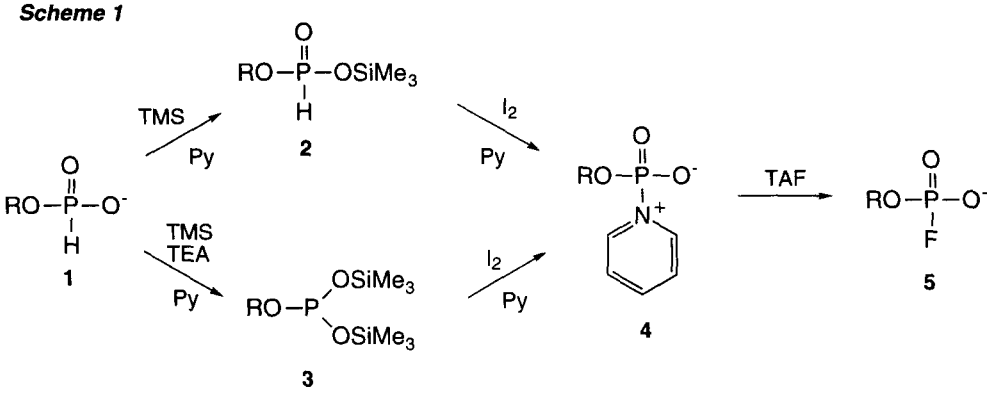
Abstract: Oxidation of H-phosphonate, H-phosphonothioate, or H-phosphonodithioate monoesters with iodine in pyridine in the presence of trimethylsilyl chloride, followed by addition of triethylamine trishydrofluoride (TAF) furnished rapid and quantitative formation of the corresponding phosphorofluoridate, phosphorofluoridothioate, or phosphorofluoridodithioate monoesters. Copyright © 1996 Elsevier Science Ltd

The extreme toxicity of some simple organic phosphorofluoridates[1] on the one hand, and particular chemical properties of the P-F bond[2,3] on the other, have stimulated extensive biological and chemical studies on fluorophosphate derivatives. However, natural product analogues carrying =P(O)-F or =P(S)-F functionality have received relatively little attention. Nucleoside phosphorofluoridates were prepared for the first time in the early sixties by Wittmann[4] and were used in several mechanistic studies of enzymatic reactions[5-7] as convenient surrogates for a phosphate monoester moiety. In recent years, with the advent of the antisense methodology for modulation of gene expression and intensive search for anti-HIV agents, the interest in nucleotide analogues containing fluorine bound phosphorus[8-15] is gaining a new momentum.

In contradistinction to phosphorofluoridate diesters[11-15], there are only a few synthetic methods available for the preparation of monoesters of fluorophosphoric acid. Some older procedures[16], which involve fission of polyphosphoric acid esters with liquid hydrogen fluoride, are not applicable for the preparation of phosphorofluoridate monoesters derived from natural products. Such compounds are usually accessible in the reaction of phosphate monoesters with 2,4-dinitrofluorobenzene[4] or *via* activation of the corresponding monoesters with trichloroacetonitrile in the presence of HF[17]. Lately, more efficient methods have been proposed for this purpose. They rely either on the condensation of fluorophosphoric acid with a hydroxylic component in the presence of a coupling agent[8,9] or on the selective removal of a t-butyl or 2-cyanoethyl group from the corresponding mixed phosphorofluoridate or phosphorofluoridothioate diesters[12].

Recently, we have reported[18] that iodine promoted oxidation of dinucleoside H-phosphonates or dinucleoside H-phosphonothioates in the presence of triethylamine trishydrofluoride (TAF) provides a new convenient entry to the corresponding phosphorofluoridate and phosphorofluoridothioate diesters. These studies encouraged us to investigate also the possibility of transforming nucleoside H-phosphonate or nucleoside H-phosphonothioate monoesters to the corresponding fluorophosphate or fluorothiophosphate monoesters by oxidation in the presence of fluoride anions. Since anionic H-phosphonate monoesters are known to be significantly more resistant to oxidation with iodine[19] than electrically neutral H-phosphonate diesters, we devised the synthetic protocol (see Scheme 1) that included a presilylation[19,20] of the starting material **1**.

Scheme 1



1a-5a, R = 5'-O-dimethoxytritylthymidin-3'-yl
1b-5b, R = 3'-O-dimethoxytritylthymidin-5'-yl

TMS - trimethylsilyl chloride
TEA - triethylamine
Py - pyridine
TAF - triethylamine trishydrofluoride

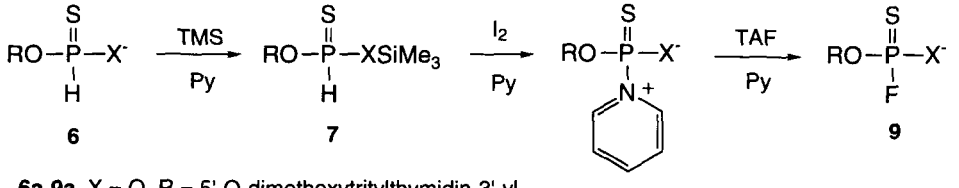
To check the efficacy of such an approach, the nucleoside H-phosphonate **1a** was treated in pyridine with TMS (3 equiv., 15 min) followed by the addition of iodine (1.5 equiv.) and triethylamine trishydrofluoride (TAF, 2 mole equiv). ^{31}P NMR spectroscopy revealed that in neat pyridine the monosilylated species **2a** (δ_P = -2.92 and -3.08 ppm, $^1J_{HP}$ = 703 and 702 Hz) was formed exclusively upon silylation of **1a** with TMS (see Scheme 1) but the subsequent addition of iodine and TAF resulted in complete regeneration of the starting H-phosphonate monoester **1a** (δ_P = 2.99 ppm, $^1J_{HP}$ = 613 Hz). This indicated that apparently the desilylation of **2a** (by fluoride) was faster than its oxidation with iodine. In the other reaction, when TEA was used during the presilylation step, the ^{31}P NMR spectrum showed the formation of the bis-silyl phosphite **3a**[19] (δ_P = 117.96 ppm, $^3J_{HP}$ = 8.6 Hz). This, upon addition of iodine and TAF within ca 5 min afforded exclusively the phosphorofluoridate **5a** (δ_P = -6.28 ppm, $^1J_{FP}$ = 930 Hz and $^3J_{HP}$ = 7.3 Hz).

In separate experiments we found that both silyl derivatives, **2a** and **3a**, when treated in pyridine with iodine (1,5 equiv.), produced rapidly (< 5 min) as the sole nucleotidic product the putative pyridine adduct of metaphosphate **4a**[19] (δ_P = -5.07 ppm, $^3J_{HP}$ = 7.3 Hz), which upon addition of TAF afforded quantitatively the phosphorofluoridate **5a**. These two reaction steps, oxidation and treatment with TAF, have to be carried out separately when the monosilylated species **2** is involved as an intermediate, or may be executed simultaneously,

if the transformation proceeds *via* the bis-silyl phosphite **3**. Using one of these procedures even the nucleoside 5'-phosphorofluoridate **5b** (δ_P = -5.40 ppm, $^1J_{FP}$ = 929 Hz) was produced quantitatively from the corresponding 5'-H-phosphonate **1b** (δ_P = 4.01 ppm, $^1J_{HP}$ = 609 Hz).

Analogous studies were also carried out on nucleoside H-phosphonothioates[21] **6**. From the ^{31}P NMR spectra it became apparent that these compounds, in contradistinction to H-phosphonates **1**, underwent in pyridine only transformation to the monosilylated species of type **7**, even in the presence of triethylamine and a large excess of TMS.

Scheme 2



6 **7** **8** **9**

6a-9a, X = O, R = 5'-O-dimethoxytritylthymidin-3'-yl
6b-9b, X = O, R = 3'-O-dimethoxytritylthymidin-5'-yl
6c-9c, X = S, R = 5'-O-dimethoxytritylthymidin-3'-yl

To check a feasibility of the oxidative transformation of **6** to the phosphorofluoridothioate monoesters **9**, the nucleoside H-phosphonothioate **6a** (δ_P = 55.07 and 54.48 ppm, $^1J_{HP}$ = 570 and 572 Hz) was silylated with TMS (3 equiv.) in pyridine to produce the silyl derivative **7a** (δ_P = 57.03 and 56.42 ppm, $^1J_{HP}$ = 658 and 660 Hz), and this was treated with iodine (1.5 equiv) under anhydrous conditions (Scheme 2). The ^{31}P NMR spectra showed a clean conversion of **7b** to the putative pyridine adduct of metathiophosphate **8a** (δ_P = 54.88 ppm, $^3J_{HP}$ = 9.7 Hz), which upon addition of TAF (1.5 mole equiv.) produced the phosphorofluoridothioate **9a** (δ_P = 55.12 ppm, $^1J_{FP}$ = 1049. Hz, $^3J_{HP}$ = 9.7 Hz)[22] as the sole nucleotidic product. Similarly, the 5'-phosphorofluoridothioate **9b** (δ_P = 55.82. ppm, $^1J_{FP}$ = 1049 Hz)[22] was formed quantitatively from the corresponding nucleoside 5'-H-phosphonothioate **6b** (δ_P = 55.92 ppm, $^1J_{HP}$ = 571 Hz) using the same reactions sequence[23].

We assessed the method also in the synthesis of a new nucleotide analogue, the nucleoside phosphorofluoridodithioate **9c**. To this end, the H-phosphonodithioate[24] **6c** (δ_P = 85.38 ppm, $^1J_{HP}$ = 532 Hz) was treated in pyridine with iodine (1.5 equiv.) in the presence of TAF (1 mole equiv.). The reaction was fast and afforded the desired phosphorofluoridodithioate **9c** (δ_P = 119.97 ppm, $^1J_{FP}$ = 1101 Hz and $^3J_{HP}$ = 12.2 Hz) as a major product (ca 85%). The stepwise addition of iodine and TAF to **6a** produced the putative dithiometaphosphate derivative **8c** (δ_P = 117.87 ppm, $^3J_{HP}$ = 12.2 Hz) as an intermediate (^{31}P NMR) and this reacted further with the added fluoride to form the product **9c**.

In conclusion, the iodine promoted oxidation of H-phosphonate, H-phosphonothioate, and H-phosphonodithioate monoesters in the presence of TMS and triethylamine trishydrofluoride (TAF) provide a new entry to the corresponding phosphorofluoridate, phosphorofluoridothioate, and phosphorofluoridodithioate

monoesters, respectively. The transformations seem to be rather general ones and thus applicable to the preparation of other phosphorofluoridate and their analogues of natural products.

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22. After purification the diastereomers visible in the ^{31}P NMR spectrum in chloroform.
23. *Typical procedure:* The nucleoside H-phosphonothioate **6a** (triethylammonium salt, 0.1 mM) was treated in pyridine (4 mL) with trimethylsilyl chloride (0.3 mM) and iodine (0.15 mM) for 10 min, and to this, triethylamine trishydrofluoride (TAF) was added (0.3 mM). After 15 min the reaction mixture was diluted with chloroform (15 mL) and extracted with aq. 10% $Na_2S_2O_3$ (1 x 15 mL) and then with brine (2 x 15 mL). The organic phase was dried (Na_2SO_4) and concentrated in *vacuo*. The resulting oily residue was chromatographed on a silica gel column using a stepwise gradient of methanol in chloroform (0-10%) containing 0.5% of triethylamine. Fractions containing the desired product were concentrated and dried overnight under vacuum line to afford triethylammonium salt of **9a** as a white foam. Yield, 67 mg (90%). Analogously, the phosphorofluoridates **5a**, **5b**, the phosphorofluoridothioate **9b** (yields > 90%) and the phosphorofluoridodithioate **9c** (yield (88%), were prepared. They were characterized by TLC, ^1H NMR, ^{31}P NMR, and FAB MS.
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