A New, Efficient Entry to Non-Lipophilic H-Phosphonate Monoesters – Preparation of Anti-HIV Nucleotide Analogues

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Abstract: Crystalline ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate was prepared by a new, simple method and it was used as the reagent of choice for the introduction of an H-phosphonate monoester functionality into non-lipophilic anti-HIV nucleoside analogues.

Keywords: H-phosphonates, phosphonylating agents, nucleoside analogues, nucleoside H-phosphonates, protecting groups, β-elimination.

INTRODUCTION

H-Phosphonate derivatives are very convenient and useful precursors in the synthesis of biologically active phosphates and their analogues, e.g. nucleotides [1], or phosphorylated sugar and carbohydrates [2]. Although there are several efficient protocols available for H-phosphonylation, they usually work well with lipophilic substrates. Typical example here is synthesis of 5'-*O*-tritylated nucleoside 3'-H-phosphonates, that can be performed using a variety of H-phosphonylating reagents [3-5]. However, problems are frequently encountered during synthesis of H-phosphonate monoesters derived from polar hydroxylic components, for example, anti-HIV active nucleoside analogues such as 2',3'-dideoxyadenosine (ddA) [6], 2',3'-dideoxyinosine (ddI) [7] or 2',3'-dideoxyuridine (ddU) [8]. In contrast to the tritylated nucleoside derivatives, aqueous work-up of the reaction mixtures after H-phosphonylation of dideoxynucleosides appeared to be troublesome, and the purification process requires a painstaking chromatography. To overcome these synthetic limitations we set out to develop a new H-phosphonylation protocol suitable for the introduction of an H-phosphonate monoester group into polar hydroxylic compounds.

RESULTS AND DISCUSSION

To this end, we searched for an H-phosphonylating reagent with a lipophilic handle, that would permit simple aqueous work-up of the reaction mixtures. As a viable candidate for this purpose we considered (9*H*-fluoren-9-yl)methyl H-phosphonic acid, synthesized by Z. W. Yang *et al.* [9] and used for the phosphonylation of 5'-*O*-tritylated nucleosides. Preliminary experiments showed that, indeed, the (9*H*-fluoren-9-yl)methyl group can provide the required lipophilicity during a multistep synthesis of H-phosphonate monoester derivatives. Unfortunately, the preparation of (9*H*-fluoren-9-yl)methyl H-phosphonic acid is far from simple and requires hazardous and commercially restricted reagents (e.g. phosphorus trichloride). To overcome these inconveniences we developed a new, efficient method for the synthesis of ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate, and used this reagent for the preparation of 5'-H-phosphonate monoesters derived from nucleoside analogues with established anti-HIV potency.

The synthesis of ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate was based on commercially available, non-toxic and cheap phosphonic acid (**1**), and (9*H*-fluoren-9-yl)methanol (**3**) (Scheme **1**). First, phosphonic acid (**1**) in pyridine was treated with pivaloyl chloride to produce quantitatively (<3 min, ^{31}P NMR) pyridinium H-pyrophosphonate (**2**) [10]. To this, (9*H*-fluoren-9-yl)methanol (**3**) was added and the desired (9*H*-fluoren-9-yl)methyl H-phosphonate (**4**) (pyridinium salt) was formed nearly quantitatively [the disappearance of (**3**), TLC analysis]. After simple work-up of the reaction mixture, ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate (**4**) was obtained as a crystalline compound in high yield (~ 90%).

In a typical experiment, a nucleoside analogue of type (**5**) was reacted with ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate (**4**) in CH_2Cl_2/pyridine 95:5 (v/v) [11] using PvCl [12, 13] as an activator. After ca 60 min the reaction was complete as indicated by ^{31}P NMR spectroscopy and TLC analysis. The excess of nucleosides (**5**) and polar remainings from the condensing agent were removed by simple extraction of the organic solution with water to afford crude H-phosphonate diesters (**6**), free from unreacted substrates (TLC, ^{31}P NMR; Table **1**).

Purity of the obtained (9*H*-fluoren-9-yl)methyl nucleoside H-phosphonate diesters (**6**) was in each case high and permited direct removal of the (9*H*-fluoren-9-yl)methyl group without recourse to chromatographic purification of intermediate (**6**). To this end, crude product (**6**) was treated with triethylamine in anhydrous acetonitrile [14] to furnish within 20 min at room temperature a clean formation of nu-

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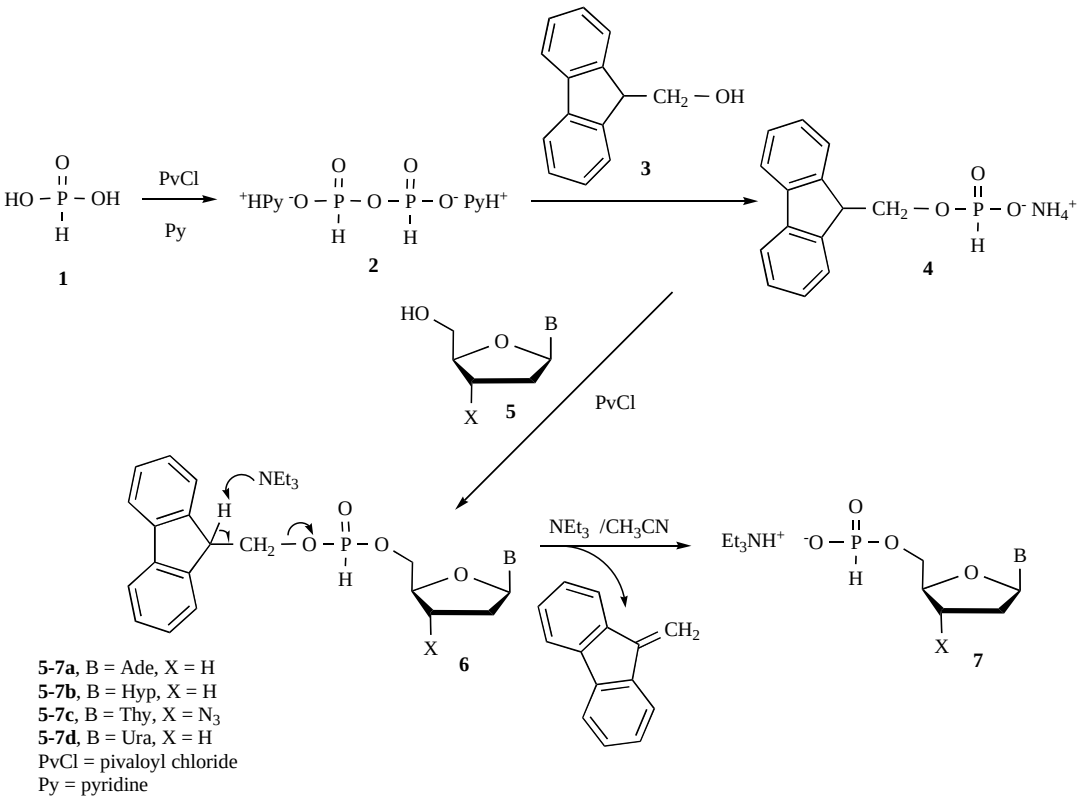

Scheme 1. Synthesis of ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate (**4**) and its use for H-phosphonylation of nucleosides (**5**).

Table 1. The ^{31}P NMR Data for Intermediates and the Final Products

Cpds	δ_P (ppm)	$J_{H\text{-}P}$ (Hz)	Cpds	δ_P (ppm)	$J_{H\text{-}P}$ (Hz)
4	3.85	$^1J_{H\text{-}P}$ 613.4; $^3J_{H\text{-}P}$ 7.3 (dt)	**7a**	2.40	$^1J_{H\text{-}P}$ 617.2; $^3J_{H\text{-}P}$ 6.4 (dt)
6a	8.65, 8.08[a]	$^1J_{H\text{-}P}$ 711.5; $^3J_{H\text{-}P}$ 8.3 (two dm)	**7b**	2.32	$^1J_{H\text{-}P}$ 601.6; $^3J_{H\text{-}P}$ 7.3 (dt)
6b	8.54, 8.09[a]	$^1J_{H\text{-}P}$ 710.5; $^3J_{H\text{-}P}$ 8.3 (two dm)	**7c**	2.83	$^1J_{H\text{-}P}$ 616.3; $^3J_{H\text{-}P}$ 6.4 (dt)
6c	8.62, 8.04[a]	$^1J_{H\text{-}P}$ 711.4; $^3J_{H\text{-}P}$ 8.3 (two dm)	**7d**	3.17	$^1J_{H\text{-}P}$ 615.4; $^3J_{H\text{-}P}$ 6.4 (dt)
6d	8.73, 8.14[a]	$^1J_{H\text{-}P}$ 711.4; $^3J_{H\text{-}P}$ 8.3 (two dm)			

[a]Two diastereoisomers.

cleoside 5'-H-phosphonates (**7**) [β-elimination of the (9*H*-fluoren-9-yl)methyl group]. Products (**7**) were isolated by simple silica gel filtration using a stepwise gradient of methanol [0 – 30%] in methylene chloride containing Et_3N (3%). The yields of H-phosphonate monoesters (**7a-d**) were invariably high (72 - 90%) and purity of the isolated products was >98% (^{1}H, ^{13}C, and ^{31}P NMR spectroscopy, and HPLC analysis).

In conclusion, we have developed an efficient protocol for the introduction of an H-phosphonate monoester function into non-lipophilic nucleosides and this can be considered as a general method for phosphonylation of various types of polar hydroxylic compounds. It makes use of an H-phosphonylating reagent (**4**) with a lipophilic (9*H*-fluoren-9-yl)methyl handle that permits an aqueous work-up of the intermediate (9*H*-fluoren-9-yl)methyl H-phosphonate diesters (**6**). The efficacy of the developed method was demonstrated in the synthesis of various important anti-HIV active nucleotide analogues (**7a-d**), which were isolated in good yields. For the preparation of a phosphonylating reagent, ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate (**4**), a simple and efficient procedure was also developed.

EXPERIMENTAL

^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on 300 MHz or 400 MHz machines. The ^{31}P NMR experiments were carried out in 5 mm tubes using 0.1 M solutions of the phosphorus-containing compound. ^{31}P NMR chemical shifts are reported in ppm relative to 85% H_3PO_4 in water (external standard). Mass spectra were recorded with liquid secondary ion mass technique (LSIMS) using Cs^+ (12 keV) for ionisation. The amount of water in solvents was measured with Karl Fisher coulometric titration. Methylene dichloride was dried over P_2O_5, distilled, and kept over molecular sieves 4 Å until the amount of water was less than 10 ppm. Pyridine was stored over molecular sieves 4 Å until the amount of water was below 20 ppm. Triethylamine was distilled and stored over CaH_2. For column chromatography silica gel 60

(Merck) was used. For TLC analysis, the precoated plates (Merck silica gel 60 F_{254}) were used.

Synthesis of Ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate (4)

Phosphonic acid (4.1 g, 50 mmol) was rendered anhydrous by repeated evaporation of the added pyridine. The residue was dissolved in the same solvent (~ 150 mL) and treated with PvCl (3.5 mL, 0.55 molar equiv.) for 5 min. To the generated pyridinium H-pyrophosphonate, (9*H*-fluoren-9-yl)methanol (4.9 g, 25 mmol) was added, the reaction mixture was concentrated to ¼ of its initial volume, and was left at room temperature for 48 h (over weekend) or for 24 h at 40 °C (overnight). After cooling down the reaction flask on ice-bath, water (10 mL) was added, and upon standing for 10 min, the solvents were removed under reduced pressure. The viscous oil was dissolved in dichloromethane (150 mL), washed with brine (2 x 50 mL), and the organic layer was separated, dried over Na_2SO_4 anh., and evaporated. The (9*H*-fluoren-9-yl)methyl pyridinium H-phosphonate was converted into the ammonium salt by addition of propan-2-ol (50 mL) containing aqueous ammonia (5% v/v). Repeated evaporation of the added propan-2-ol caused spontaneous precipitation of (**4**) as a white solid. After filtration and drying, product (**4**) (ammonium salt, 6.2 g) was obtained as non-hygroscopic micro-crystals (m. p. 174-176 °C). ^{31}P NMR data are given in Table **1**.

General Procedure for the Synthesis of Nucleoside H-phosphonate Monoesters of Type (7)

Nucleoside analogue of type (**5**) (1.05 mmol) and ammonium (9*H*-fluoren-9-yl)methyl -H-phosphonate (**4**) (1.0 mmol, 1 molar equiv.) were made anhydrous by repeated evaporation of the added pyridine. After this, the residue was dissolved in 10 mL of CH_2Cl_2/pyridine (95:5, v/v) and PvCl (1.5 molar excess) was added. H-Phosphonylation of nucleosides (**5**) was complete (^{31}P NMR, TLC) after ca 60 min. The reaction mixture was diluted with the same volume of methylene chloride, and the excess of nucleoside (**5**) and polar remainings from the condensing agent, were removed by extraction of the organic solution with water (one third of the total volume). The organic layer was separated, dried over Na_2SO_4 anh., and the volatiles were removed by evaporation. The remaining oily residue was dissolved in CH_3CN/Et_3N [2 : 1, v/v; 10 mL per 1 mmol of (**6**)] and kept 20 min at room temperature to effect a quantitative elimination of the (9*H*-fluoren-9-yl)methyl group. The reaction mixture was evaporated and products (**7**) were isolated by a silica gel filtration using a stepwise gradient of methanol (0 – 30% v/v) in methylene chloride containing Et_3N (3% v/v). Fractions containing pure product (**7**) were collected and evaporated, to furnish colourless, crispy foams. ^{31}P NMR data of products (**7**) are listed in Table **1**.

^{1}H, ^{13}C NMR and HRMS Data for Reagent (4) and Nucleoside H-phosphonate Monoesters (7a-d)

*Ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate (4)*

Yield 90%. ^{1}H NMR (DMSO-d_6) δ 7.86 (d, *J* = 7.4 Hz, 2H, ArH), 7.67 (d, *J* = 7.4 Hz, 2H, ArH), 7.42 (br s, 4H, exch. D_2O, NH_4^+), 7.39 (t, *J* = 7.4 Hz, 2H, ArH), 7.31 (t, *J* = 7.4 Hz, 2H, ArH), 6.59 (d, $J_{H\text{-}P}$ = 635.4 Hz, 1H, H-P), 4.15 (t, *J* = 7.2 Hz, 1H, 9-CH), 3.93 (dd, $^3J_{H\text{-}P}$ = 7.2 Hz, $J_{H\text{-}H}$ = 7.2 Hz, 2H, CH_2); ^{13}C NMR (D_2O) δ 143.75 (C-10 & C-13), 140.72 (C-11 & C-12), 127.6 (C-1 & C-8), 127.1 (C-4 & C-5), 124.95 (C-2 & C-7), 119.81 (C-3 & C-6), 65.52 (CH_2), 47.84 (C-9); HRMS [M $-NH_4^+$]$^-$ *m/z* 259,0502, calculated for $C_{14}H_{12}O_3P$ 259,0524.

2',3'-Dideoxyandenosin-5'-yl H-phosphonate Triethylammonium Salt (7a)

Yield 90%. ^{1}H NMR (D_2O) δ 8.28 (s, 1H, H-8), 8.05 (s, 1H, H-2), 6.61 (d, *J* = 637.8 Hz, H-P), 6.19 (dd, *J* = 6.8 Hz, *J* = 3.2 Hz, 1H, 1'-H), 4.46-4.39 (m, 1H, 4'-H), 4.11-4.06 & 3.96-3.90 (2m, 2H, 5',5"-H_2), 3.15 (q, *J* = 7.2 Hz, 6H, C$\underline{H}_2$CH$_3$), 2.65-2.55 & 2.47-2.41 (2m, 2H, 3',3"-H_2), 2.27-2.19 & 2.13-2.03 (2m, 2H, 2',2"-H_2), 1.24 (t, *J* = 7.2 Hz, 9H, CH$_2$C$\underline{H}_3$). ^{13}C NMR (D_2O) δ 154.50 (C-4), 151.55 (C-2), 147.84 (C-6), 139.65 (C-8), 118.22 (C-5), 84.78 (C-1'), 80.74 (C-5'), 64.44 (C-4'), 46.57 ($\underline{C}$H$_2$CH$_3$), 31.57 (C-2'), 25.65 (C-3'), 8.16 (CH$_2\underline{C}$H$_3$). HRMS [M – Et_3NH^+]$^-$ *m/z* 298.0719, calculated for $C_{10}H_{13}N_5O_4P$ 298.0705.

2',3'-Dideoxyinosin-5'-yl H-phosphonate Triethylammonium Salt (7b)

Yield 72%. ^{1}H NMR (D_2O) δ 8.34 (s, 1H, 8-H), 8.18 (s, 1H, 2-H), 6.63 (d, *J* = 637.8 Hz, 1H, H-P), 6.34 (dd, *J* = 6.6 Hz, *J* = 2.7 Hz, 1-H, 1'-H), 4.14-4.08 (m, 1H, 4'-H), 4.02-3.90 (m, 2H, 5',5"-H_2), 3.21 (q, *J* = 7.2 Hz, 6H, C$\underline{H}_2$CH$_3$), 2.68-2.62 & 2.60-2.54 (2m, 2H, 3',3"-H_2), 2.30-2.23 & 2.3-2.14 (2m, 2H, 2',2"-H_2), 1.29 (t, J = 7.2 Hz, 9H, CH$_2$C$\underline{H}_3$). ^{13}C NMR (D_2O) δ 158.10 (C-4), 147.96 (C-6), 145.78 (C-8), 139.36 (C-2), 123.42 (C-5), 85.16 (C-5'), 81.02 (C-1'), 64.16 (C-4'), 46.60 ($\underline{C}$H$_2$CH$_3$), 31.66 (C-2'), 23.71 (C-3'), 8.20 (CH$_2\underline{C}$H$_3$); HRMS [M – Et_3NH^+]$^-$ *m/z* 299.0517, calculated for $C_{10}H_{12}N_4O_5P$ 299.0545.

3'-Azido-3'-dideoxythymidin-5'-yl H-phosphonate Triethylammonium Salt (7c)

Yield 85%. ^{1}H NMR (D_2O) δ 7.71 (s, 1H, H-6), 6.80 (d, *J* = 638.2 Hz, 1H, H-P), 6.26 (t, 1H, *J* = 6.6 Hz, 1H, 1'-H), 4.53-4.49 (m, 1H, 3'-H), 4.21-4.18 (m, 1H, 4'-H), 4.14-4.09 (m, 2H, 5',5"-H_2), 3.21 (q, *J* = 7.2 Hz, 6H, C$\underline{H}_2$CH$_3$), 2.51 (t, *J* = 6.4 Hz, 2H, 2',2"-H_2), 1.92 (s, 3H, 5-CH$_3$), 1.30 (t, *J* = 7.2 Hz, 9H, CH$_2$C$\underline{H}_3$). ^{13}C NMR (D_2O) δ 166.39 (C-4), 151.54 (C-2), 137.19 (C-6), 111.57 (C-5), 84.92 (C-1'), 82.89 (C-4'), 64.31 (C-5'), 60.48 (C-3'), 46.65 ($\underline{C}$H$_2$CH$_3$), 36.27 (C-2'), 11.61 (5-CH$_3$), 8.41 (CH$_2\underline{C}$H$_3$); HRMS [M – Et_3NH^+]$^-$ *m/z* 330.0576, calculated for $C_{10}H_{13}N_5O_6P$ 330.0603.

2',3'-Dideoxyuridin-5'-yl H-phosphonate Triethylammonium Salt (7d)

Yield 88%. ^{1}H NMR (D_2O) δ 7.94 (d, *J* = 8.0 Hz, 1H, 6-H), 6.76 (d, *J* = 637.4 Hz, 1H, H-P), 6.10 (dd, 1H, *J* = 7.2 Hz, J = 3.2 Hz, 1H, 1'-H), 5.88 (d, *J* = 8.0 Hz, 1H, 5-H) 4.36-4.33 (m, 1H, 4'-H), 4.18-4.13 & 4.02-3.97 (2m, 2H, 5',5"-H_2), 3.20 (q, *J* = 7.2 Hz, 6H, C$\underline{H}_2$CH$_3$), 2.48-2.42 (m, 1H, 3'- or 3"-H), 2.20-2.09 (m, 2H, 2', 2"-H_2), 2.02-1.92 (m, 1H, 3'- or 3"-H), 1.30 (t, *J* = 7.2 Hz, 9H, CH$_2$C$\underline{H}_3$). ^{13}C NMR (D_2O) δ 166.26 (C-4), 151.55 (C-2), 142.10 (C-6), 101.69 (C-5), 86.39 (C-1'), 80.50 (C-5'), 64.31 (C-4'), 46.62 ($\underline{C}$H$_2$CH$_3$),

31.24 (C-2'), 26.50 (C-3'), 8.40 ($CH_2\underline{C}H_3$); HRMS [M – Et_3NH^+]$^-$ *m/z* 275.0408, calculated for $C_9H_{12}N_2O_6P$ 275.0433.

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