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Use of Ammonium Aryl *H* - Phosphonates in the Preparation of Nucleoside *H* - Phosphonate Building Blocks

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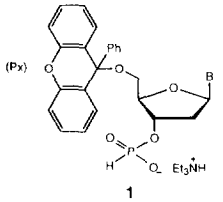
Abstract: Ammonium 4-methylphenyl *H*-phosphonate **4c** was used in the conversion of 5'-*O*-(dimethoxytrityl)-2'-deoxynucleoside derivatives **6** into the corresponding 3'-*H*-phosphonates **2**, which were isolated in a high state of purity and in high yield. Copyright © 1996 Elsevier Science Ltd

Three principal methods, namely the phosphotriester[1], phosphoramidite[2] and *H*-phosphonate[3] approaches, have been found to be effective for the chemical synthesis of oligonucleotides. While the phosphotriester approach has been used mainly for synthesis in solution, the phosphoramidite and *H*-phosphonate approaches have been widely used in solid phase synthesis. However, we believe that *H*-phosphonate building blocks (e.g. **1** and **2**) and possibly the *H*-phosphonate approach itself may well prove to



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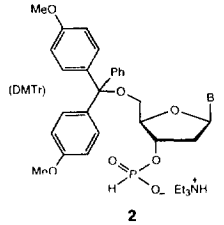

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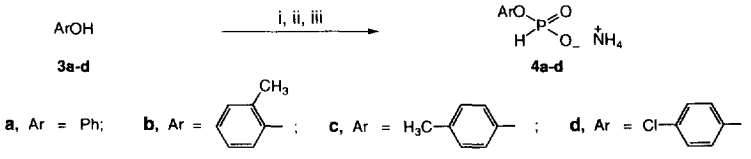
be most useful in the synthesis of oligonucleotides in solution. Indeed, we have recently developed[4] a solution synthesis of phosphorothioate analogues of oligonucleotides, based on *H*-phosphonate building blocks **1**. In the course of the latter study, we found that the published procedures[5] for the preparation of the required building blocks **1** and **2** were not entirely suitable for our purposes. We now report what we believe to be a particularly convenient and effective procedure for the conversion of alcohols into the corresponding *H*-phosphonate monoesters.

As indicated in Scheme 1, the phosphorus-containing reagents used in the present work, namely ammonium aryl *H*-phosphonates **4a** - **d**, were readily prepared by a modification of Hammond's procedure[6].



Scheme 1 *Reagents and conditions*: i, PCl_3, 160°C, 3h; ii, Me_3C•OH, 100°C, 30min; iii, conc. aq. NH_3 (*d* 0.88), 0°C, 1h

The appropriate phenol was heated with phosphorus trichloride, first under gentle reflux and then at 160°C. After treatment with *tert*-butanol (to remove hydrogen chloride), the products were allowed to react with concentrated aqueous ammonia at 0°C. The required ammonium salts **4a** - **d** were obtained and isolated as stable crystalline solids in 84, 86, 87 and 83% yields, respectively[7].

The procedure used for converting 5'-*O*-(dimethoxytrityl)-2'-deoxynucleosides **6** into the triethylammonium salts of the corresponding 3'-*H*-phosphonates **2** is indicated in outline in Scheme 2. The ammonium salts **4** were first solubilised (presumably as the corresponding triethylammonium salts **5**) by evaporation in the presence of triethylamine from pyridine solution (Scheme 2a). The putative triethylammonium salts **5** were then allowed to react (Scheme 2b) with appropriate nucleoside derivatives **6** in the presence of pivaloyl chloride[8] in pyridine solution at -35°C to give what were assumed to be intermediate *H*-phosphonate diesters **7**. As dialkyl *H*-phosphonates are very susceptible to base-catalysed hydrolysis[9], aryl alkyl *H*-phosphonates would be expected to undergo hydrolysis under exceptionally mild basic conditions. Indeed, when the intermediate diesters were allowed to stand in aqueous pyridine solution at room temperature for 1-2 h, the desired *H*-phosphonate monoesters **2** were obtained as virtually the sole nucleotide or nucleoside products. All four of the above ammonium salts **4a** - **d** were used in the conversion of 5'-*O*-(dimethoxytrityl)thymidine **6**; B = **8** into its 3'-*H*-phosphate **2**; B = **8**, and it was found that the purest product was obtained when ammonium 4-methylphenyl *H*-phosphonate **4c** was used. In this way, triethylammonium thymidine 3'-*H*-phosphonate **2**; B = **8** was prepared (Table 1, entry no. 1) and isolated as

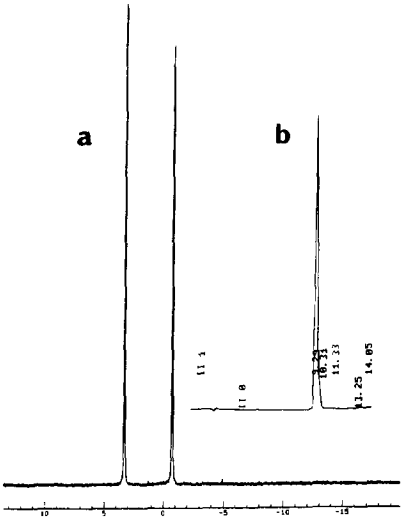
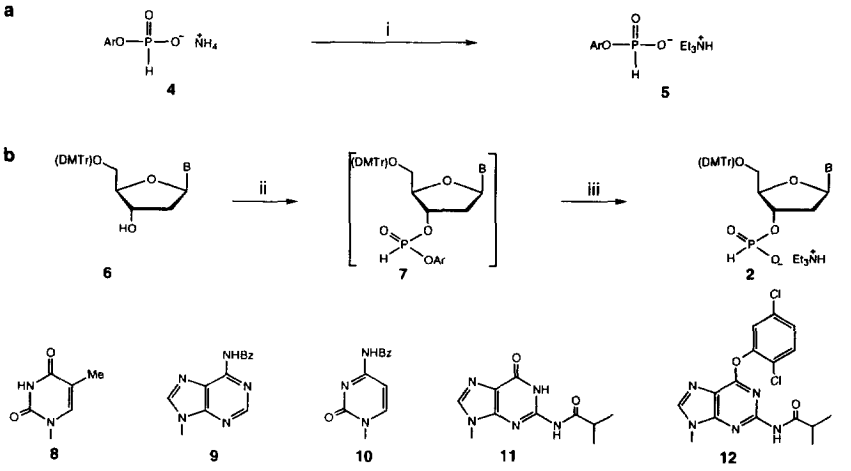


Figure 1. **a** ^{31}P NMR spectrum and **b** HPLC profile of 5'-*O*-(dimethoxytrityl)-4-*N*-benzoyl-2'-deoxycytidine 3'-*H*-phosphonate **2**; B = **10**. See also table 1 (entry no. 3).



Scheme 2 *Reagents and conditions*: i, Et_3N, C_5H_5N; ii, **5**, $Me_3C{\cdot}COCl$, C_5H_5N, - 35°C, 30 min; iii, a, H_2O, C_5H_5N, room temp., 1h, b, pH 7.0 phosphate buffer

a colourless glass in virtually quantitative yield. The triethylammonium 3'-*H*-phosphonates **2**; B = **9**, **2**; B = **10**, **2**; B = **11**, and **2**; B = **12** (entries nos. 2 - 5) were similarly prepared in a high state of purity by a general procedure[10] from 5'-*O*-dimethoxytrityl-6-*N*-benzoyl-2'-deoxyadenosine **6**; B = **9**, 5'-*O*-(dimethoxytrityl)-4-*N*-benzoyl-2'-deoxycytidine **6**; B = **10**, 5'-*O*-(dimethoxytrityl)-2-*N*-isobutyryl-2'-deoxyguanosine **6**; B = **11** and 6-*O*-(2,5-dichlorophenyl)-5'-*O*-(dimethoxytrityl)-2-*N*-isobutyryl-2'-deoxyguanosine[11] **6**; B = **12**, respectively. The ^{31}P NMR spectrum and HPLC profile of the *H*-phosphonate **2**; B = **10** derived from 2'-deoxycytidine is illustrated in Figure 1.

Table 1. Preparation of triethylammonium 5'-*O*-(dimethoxytrityl)-2'-deoxyribonucleoside 3'-*H*-phosphonates **2**

Entry no.	Product **2** B =	Yield (%)	^{31}P NMR[a] ($J_{P,H}$)	t_R (min)[b]
1	**8**	99	1.3 (8.9 and 588.6)	8.84
2	**9**	98	1.2 (8.9 and 587.6)	9.65
3	**10**	98	1.3 (8.5 and 588.2)	10.31
4	**11**	97	1.1 (8.6 and 589.6)	8.4
5	**12**	99	1.3 (8.6 and 588.4)	12.09

[a]NMR spectra were measured in $(CD_3)_2SO$ solution at 145.8 MHz. [b]HPLC was carried out on a 25 cm x 4.6 mm Jones ODS 5μ column, which was eluted with 0.1 mol dm^{-1} triethylammonium acetate - acetonitrile mixtures (linear gradient 7 : 3 to 2 : 8 v/v over 10 min and then isocratic elution).

In our hands, the present approach to the preparation of *H*-phosphonate monoesters proved to be superior to other commonly used methods (including those involving the use of phosphorus trichloride / base / imidazole[3] or 1-*H*-1,2,4-triazole[14] and 2-chloro-4-*H*-1,3,2-benzodioxaphosphorin-4-one[15]) inasmuch as the results obtained were always reproducible, and very high yields of pure products were invariably obtained. Furthermore, ammonium 4-methylphenyl *H*-phosphonate **4c**, the required agent, is a readily-prepared, stable, crystalline solid.

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7. **Ammonium 4-methylphenyl *H*-phosphonate 4c** was prepared as follows:- 4-Methylphenol (43.26 g, 0.40 mol) and phosphorus trichloride (17.44 cm^3, 0.20 mol) were heated together, first under gentle reflux and then at 160°C for 3 h. The products were cooled to 100°C and *tert*-butanol (21.0 cm^3, 0.22 mol) was added with stirring. After 30 min, the products were evaporated under reduced pressure, cooled to 0°C and poured into a stirred solution of concentrated aqueous ammonia (*d* 0.88, 200 cm^3) in water (200 cm^3) at 0°C (ice-water bath). The reactants were allowed to warm up to room temperature. After 1 h, the products were evaporated under reduced pressure, redissolved in dry ethanol and the solution reevaporated. After this process had been repeated twice more, the residue was dissolved in ethanol (400 cm^3) and the resulting solution was filtered through a bed of celite (30 g). The residue was washed with ethanol (100 cm^3) and the combined filtrate and washings were concentrated (to less than half-volume) under reduced pressure until crystallisation occurred. Dry diethyl ether (400 cm^3) was then added dropwise over a period of *ca.* 1 h at room temperature. After a further period of *ca.* 2 h, colourless crystals of **ammonium 4-methylphenyl *H*-phosphonate 4c** (32.95 g, 87%) (Found: C, 44.25; H, 6.38; N, 7.39. $C_7H_{12}NO_3P$ requires: C, 44.45; H, 6.39; N, 7.40%), m.p. 160-161°C, were collected; δ_H [$(CD_3)_2SO$] 2.22 (3 H, s), 6.81 (1 H, d, *J* 603.4), 6.95 (2 H, d, *J* 8.4), 7.04 (2 H, d, *J* 8.3), 7.49 (4 H, br); δ_C [$(CD_3)_2SO$] 20.1, 120.2, (d, $J_{C,P}$ 4.8), 129.4, 131.1, 150.4 (d, $J_{C,P}$ 6.5); δ_P [$(CD_3)_2SO$] -1.0 (d, $J_{P,H}$ 603.4).
Ammonium phenyl *H*-phosphonate 4a was similary prepared in 84% isolated yield (Found C, 41.22; H, 5.71; N, 8.13. Calc. for $C_6H_{10}NO_3P$: C, 41.15; H, 5.76; N, 8.00%), m.p. 175-176°C (lit.[6] 172-173°C); δ_P [$(CD_3)_2SO$] -1.4 (d, $J_{P,H}$ 605.3).
Ammonium 2-methylphenyl *H*-phosphonate 4b was similarly prepared in 86% isolated yield (Found: C, 44.07; H, 6.32; N, 7.58. $C_7H_{12}NO_3P$ requires: C, 44.45; H, 6.39; N, 7.40%), m.p. 136-137°C; δ_P [$(CD_3)_2SO$] -1.6 (d, $J_{P,H}$ 603.6).
Ammonium 4-chlorophenyl *H*-phosphonate 4d was similarly prepared in 83% isolated yield (Found: C, 34.18; H, 4.37; N, 6.67. $C_6H_9ClNO_3P$ requires: C, 34.39; H, 4.33; N, 6.68%), m.p. 167-168°C; δ_P[$(CD_3)_2SO$] -1.4 (d, $J_{P,H}$ 607.7).

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10. **Triethylammonium 5'-*O*-(dimethoxytrityl)-2'-deoxyribonucleoside 3'-*H*-phosphonates 2** were prepared as follows: Ammonium 4-methylphenyl *H*-phosphonate **4c** (2.84 g, 15.0 mmol), 5'-*O*-(dimethoxytrityl)-2'-deoxyribonucleoside derivative **6**; B = **8**, **9**, **10**, **11** or **12** (5.0 mmol) triethylamine (4.2 cm^3, 30 mmol) and dry pyridine (20 cm^3) were evaporated together under reduced pressure. The residue was coevaporated again with dry pyridine (20 cm^3). The residue was dissolved in dry pyridine (40 cm^3) and the solution was cooled to -35°C (industrial methylated spirits/dry ice bath). Pivaloyl chloride (1.85 cm^3, 15.0 mmol) was added dropwise to the stirred solution over a period of 1 min, and the reactants were maintained at -35°C. After 30 min, water (5 cm^3) was added, and the stirred mixture was allowed to warm up to room temperature. Potassium phosphate buffer (1.0 mol dm^{-3}, pH 7.0, 250 cm^3) was added to the products, and the resulting mixture was concentrated under reduced pressure until all of the pyridine had been removed. The residual mixture was partitioned between dichloromethane (250 cm^3) and water (200 cm^3). The organic layer was washed with triethylammonium phosphate buffer (0.5 mol dm^{-3}, pH 7.0, 3 x 50 cm^3), dried ($MgSO_4$) and then evaporated under reduced pressure. The residue was fractionated by short column chromatography on silica gel (25 g). Appropriate fractions, eluted with dichloromethane - methanol (95 : 5 to 90 : 10 v/v), were evaporated under reduced pressure to give the desired **triethylammonium 5'-*O*-(dimethoxytrityl)-2'-deoxyribonucleoside 3'-*H*-phosphonates 2**. The isolated yields obtained, and ^{31}P NMR and HPLC data are indicated in Table 1.

11. In the phosphotriester approach, we found[12,13] that it was advisable to protect guanine residues both with a 2-*N*-acyl and a 6-*O*-aryl group. As it is not yet clear whether such double protection is desirable in the *H*-phosphonate approach, we have prepared two 2'-deoxyguanosine *H*-phosphonate building blocks **2**; B = **11** and **2**; B = **12** with singly- and doubly-protected guanine residues, respectively.

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