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ARYL H-PHOSPHONATES. PART IV. A NEW METHOD FOR INTERNUCLEOTIDE BOND FORMATION BASED ON TRANSESTERIFICATION OF ARYL NUCLEOSIDE H-PHOSPHONATE DIESTERS

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Abstract: Under mild reaction conditions nucleoside aryl H-phosphonate diesters undergo fast and efficient transesterification with suitably protected nucleosides, affording dinucleoside (3'-5') H-phosphonate diesters. Copyright © 1996 Elsevier Science Ltd

The synthetic approaches based on phosphoramidite[1,2] and H-phosphonate chemistry[3,4] presently almost entirely dominate the preparation of phosphorus-containing natural products and their analogues and, in particular, the field of oligonucleotide synthesis. Although these two methodologies are based on P(III) derivatives, there are fundamental differences[3] between them. These stem, in principle, from different co-ordination numbers of phosphorus in the starting materials and are also reflected in the distinctive ways in which formation of an internucleotidic bond is brought about. Nucleoside phosphoramidites are tricoordinated species and their transformation to the corresponding phosphite triesters relies on activation (usually *via* protonation) of a leaving group in the phosphoramidites, followed by nucleophilic attack on the phosphorus centre by a hydroxylic component[5,6]. In the H-phosphonate methodology, formation of internucleotidic bond was until now exclusively accomplished *via* a coupling agent promoted condensations of the corresponding H-phosphonate monoesters[7-10] with a nucleosidic component. The perennial problem of all synthetic procedures that involve condensing agents as activators is the possibility of occurrence of various side reactions. In the instance of the synthetic methods based on H-phosphonate chemistry, however, these undesired reactions pathways are heavily suppressed[11-13] due to favourable kinetics of the condensation step.

In the early stages of our studies in H-phosphonate chemistry[14,15] it was found that to secure an efficient formation of H-phosphonate diesters, the corresponding H-phosphonate monoester has to be activated by a condensing agent in the presence of a hydroxylic component. Since this requirement occasionally may impose limitations on this methodology, we have recently embarked on investigations directed towards development of synthetic methods that would not necessitate condensing agents to bring about formation of the H-phosphonate diester bond.

It became apparent during our studies on the transesterification of H-phosphonate diesters[16] that aryl nucleoside H-phosphonates are particularly prone to substitution with amino alcohols forming exclusively aminoalkyl nucleoside H-phosphonate diesters. These studies prompted us to consider aryl nucleoside H-phosphonates as possible substrates for internucleotide bond formation via transesterification reaction with a suitably protected nucleosidic component. An attractive feature of aryl nucleoside H-phosphonates as synthetic intermediates seemed to be the possibility of controlling their reactivity not only by the external factors (*e.g.*, solvent, nucleophilic catalysts) but also by changing the electron density at the phosphorus centre *via* introduction of suitable substituents on the aromatic rings.

To develop this into a synthetic method for internucleotidic bond formation, accessibility of the starting material, aryl nucleoside H-phosphonate diesters, was first investigated. To this end the nucleoside H-phosphonate monoester **1a**[17] was reacted in pyridine with phenol (2 equiv.) in the presence of pivaloyl chloride (PV-Cl, 3 equiv.) (standard reaction conditions for H-phosphonate diesters formation[18,19]). As judged from the ^{31}P NMR spectrum, the reaction produced the desired nucleoside phenyl H-phosphonate **2** [B_1= Thy, Ar = Ph, δ_P =3.41 and 3.50 ppm, $^1J_{HP}$ = 725.8 and 723.5 Hz (d), $^3J_{HP}$ = 8.2 Hz (d)] but simultaneously also the corresponding nucleoside bisacyl phosphite (*ca* 20%, δ_P ~123.3 ppm) was formed[20]. The presence of the latter intermediate (a doubly activated species[14]) indicated that under the reaction conditions phenol was apparently too weak a nucleophile to trap efficiently the primary reactive intermediate, the phosphono – acyl mixed anhydride[14]. To overcome this problem we carried out the coupling reactions in methylene dichloride – pyridine (9:1, v/v) mixture anticipating that this less basic reaction medium would slow down the double activation[14,21] of the H-phosphonate monoester **1a** and, at the same time, increase the nucleophilicity of phenol. Indeed, activation of the H-phosphonate **1a** with PV-Cl (1.2 equiv.) under these reaction conditions proceeded almost exclusively to give the corresponding phosphono-pivaloyl mixed anhydride [δ_P = 0.83 and 0.94 ppm, $^1J_{HP}$ = 745.2 and 746.2 Hz (d), $^3J_{HP}$ = 8.1 Hz (d)], and an analogous reaction in the presence of phenol (2 equiv.) produced the nucleoside phenyl H-phosphonate **2** (B_1= Thy, Ar = Ph) as the sole nucleotidic product (^{31}P NMR spectroscopy).

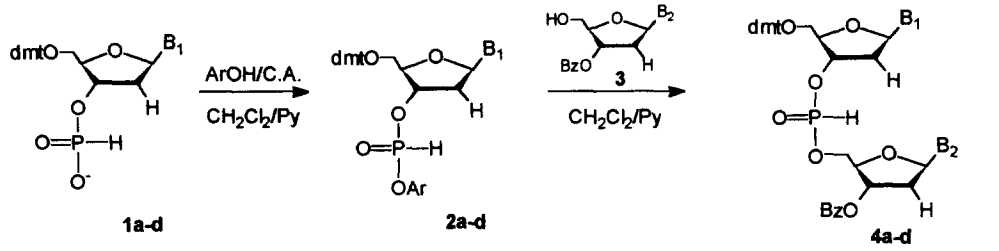
Reactivity of the *in situ* formed **2** (B_1= Thy, Ar = Ph) was probed in the reaction with suitably protected nucleoside **3** (1.2 equiv.). As judged from the ^{31}P NMR spectrum, the transesterification with the added **3** produced cleanly the expected dinucleoside H-phosphonate **4a** [δ_P =7.90 and 8.60 ppm, $^1J_{HP}$ = 757.8 and 756.7 Hz (d), $^3J_{HP}$ = 9.1 Hz (d)], but the reaction was rather slow (completion *ca* 3 h). Seeking a remedy for the low reactivity of phenyl nucleoside H-phosphonates we assessed the derivatives with substituted aryl rings as a starting material for the transesterification. Among various phenols investigated[22], the most promising seemed to be 2,4,6-trichlorophenol, and it was selected for the preparation of four aryl nucleoside H-phosphonates **2a–d** bearing various heterocyclic bases.

The aryl nucleoside H-phosphonates **2a–d**[23] were generated *in situ* as sole nucleotidic products from the corresponding nucleoside H-phosphonates **1a–d** and 2,4,6-trichlorophenol (2 equiv.) as described above for the phenyl derivative. The same reactions aided by 2-chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane[24,25] (NEP-Cl) as a coupling agent also led to the aryl H-phosphonates **2a–d**, but they were slower (required 20–60 min for the completion).

In a typical procedure for the formation of dinucleoside H-phosphonates **4**, a nucleoside H-phosphonate **1** (0.1 mmol/mL) in CH_2Cl_2-pyridine (9:1, v/v) was reacted with 2,4,6-trichlorophenol (2 equiv.) and PV-Cl (1.2 equiv.) for 5 min to produce the corresponding aryl nucleoside H-phosphonate **2** and to this, the nucleosidic

component **3** (1.5 equiv.) was added. The reactions were complete in less than 5 min (the first ^{31}P NMR spectrum recorded) and furnished clean formation of the dinucleoside H-phosphonates **4**[26].

Scheme 1.



1a-d **2a-d** **4a-d**

1a, B_1 = Thy
1b, B_1 = ^{bz}Ade
1c, B_1 = ^{bz}Cyt
1d, B_1 = ^{ibu}Gua

2a, B_1 = Thy, Ar = 2,4,6-trichlorophenyl
2b, B_1 = ^{bz}Ade, Ar = 2,4,6-trichlorophenyl
2c, B_1 = ^{bz}Cyt, Ar = 2,4,6-trichlorophenyl
2d, B_1 = ^{ibu}Gua, Ar = 2,4,6-trichlorophenyl

3. B_2 = ^{bz}Cyt

4a, B_1 = Thy, B_2 = ^{bz}Cyt
4b, B_1 = ^{bz}Ade, B_2 = ^{bz}Cyt
4c, B_1 = ^{bz}Cyt, B_2 = ^{bz}Cyt
4d, B_1 = ^{ibu}Gua, B_2 = ^{bz}Cyt

Abbreviations: dmt - 4,4'-dimethoxytrityl; Bz - benzoyl; Thy - thymin-1-yl; ^{bz}Ade - N^6-benzoyladenin-9-yl; ^{bz}Cyt - N^4-benzoylcytosin-1-yl; ^{ibu}Gua - N^2-isobutyrylguanin-9-yl; Py - pyridine; C.A. - condensing agent.

From the TLC experiments[27] it was found that all the condensations were actually complete in *ca* 3 min. When carried out on a preparative scale, the same reactions, followed by oxidation with iodine-water[28], afforded after purification (silica gel 60H, column chromatography) the corresponding dinucleoside monophosphates in yields > 90%. No 5' -O-pivaloylated product was found in the reaction mixture.

In conclusion, transesterification of aryl nucleoside 3'-H-phosphonates **2** with suitably protected nucleosides provides a new means for the formation of 3' – 5' H-phosphonate internucleotidic linkages. Some advantageous feature of the methods are: (i) the reaction is rapid and efficient, (ii) it can be carried out under mild reaction conditions, and (iii) the starting material, aryl nucleosides H-phosphonates **2**, can be conveniently generated *in situ* from easily accessible corresponding nucleoside H-phosphonate monoesters **1**. The method seems to be rather general one and thus applicable to the preparation of other phosphorus-containing natural products and their analogues. Since its underlying principle is different from that of coupling procedures based on condensing agents, the method should also expand the synthetic potential of H-phosphonate esters.

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23. **2a**, δ_P = 1.10 and 1.60 ppm, $^1J_{HP}$ = 758.2 and 759.1 Hz (d), $^3J_{HP}$ = 9.3 Hz (d); **2b**, δ_P = 0.98 and 1.56 ppm, $^1J_{HP}$ = 757.8 and 755.5 Hz (d), $^3J_{HP}$ = 9.1 Hz (d); **2c**, δ_P = 1.27 and 1.69 ppm, $^1J_{HP}$ = 759.0 Hz (d), $^3J_{HP}$ = 9.1 Hz (d); **2d**, δ_P = 1.38 and 2.10 ppm, $^1J_{HP}$ = 757.8 and 756.7 Hz (d), $^3J_{HP}$ = 9.1 Hz (d).
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26. **4a**, δ_P =7.90 and 8.60 ppm, $^1J_{HP}$ = 720.1 and 720.0 Hz (d), $^3J_{HP}$ = 8.0 Hz (q); **4b**, δ_P =7.59 and 8.21 ppm, $^1J_{HP}$ = 719.0 Hz (d), $^3J_{HP}$ = 6.9 Hz (q); **4c**, δ_P =7.59 and 8.29 ppm, $^1J_{HP}$ = 721.3 and 719.0 Hz (d), $^3J_{HP}$ = 8.0 Hz (q); **4d**, δ_P =8.05 and 8.65 ppm, $^1J_{HP}$ = 720.1 and 716.7 Hz (d), $^3J_{HP}$ = 8.0 Hz (q).
27. In a typical experiment, **3** (0.5 equiv.) was added to the *in situ* produced **2a**. Aliquots were taken after 1, 2, 3, and 4 min, oxidised with iodine-water, and analysed on silica gel plates using $CHCl_3$-MeOH (9:1, v/v) as an eluent.
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