Aryl *H*-Phosphonates. 6. Synthetic Studies on the Preparation of Nucleoside *N*-Alkyl-*H*-phosphonamidates

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Various approaches to the synthesis of nucleoside *H*-phosphonamidates have been investigated. Direct couplings of nucleoside *H*-phosphonates with amines have been hampered by extensive reactions of the condensing agents with amines. Preactivation of nucleoside *H*-phosphonates with pivaloyl chloride or chlorophosphates, followed by the addition of amines, notably diminished these side reactions. The most efficient and versatile route to nucleoside *N*-alkyl *H*-phosphonates was found to be aminolysis of the *in situ*-produced aryl nucleoside *H*-phosphonates with appropriate amines.

Introduction of modifications at the phosphorus center of natural products may be valuable in elucidating of mechanisms of enzymatic reactions.[1] Such knowledge is of prime importance for the development of new enzyme inhibitors of potential medical value[2] or for the design of natural product analogues[3] that are resistant to enzymatic degradation while keeping some other biologically important properties intact.

With this in mind, we have embarked on investigations of *H*-phosphonate analogues having a nitrogen atom in the bridging position at the phosphorus center, *i.e.*, nucleoside *H*-phosphonamidates.[4] *N*-Alkyl-*H*-phosphonamidates can be prepared *via* controlled hydrolysis of the corresponding tervalent derivatives (usually *N*-alkylphosphordiamidites[5]), and this method has recently been applied to the synthesis of some nucleoside *H*-phosphonamidates.[6] However, its main disadvantage is that synthesis of each type of *H*-phosphonamidate derivative requires preparation of a suitable phosphitylating reagent. Moreover, due to some inherent problems in the preparation of phosphoramidites derived from primary amines,[7] the method is restricted to *N*,*N*-dialkyl derivatives. To the best of our knowledge, there is only one report in the literature on the synthesis and application of nucleoside *H*-phosphonamidates[6,8] (*N*,*N*-diisopropyl-*H*-phosphonamidate) as building blocks in oligonucleotide synthesis.

In order to investigate in detail chemical properties of nucleoside *H*-phosphonamidates,[4] easy access to this type of compound with diverse structural features was necessary. Using ^{31}P NMR spectroscopy as a tool, we tried to delineate the most important factors affecting formation of *H*-phosphonamidates from various *H*-phosphonate intermediates. This enabled us to develop a new, efficient method for the preparation of nucleoside *N*-alkyl-*H*-phosphonamidates starting from the easily accessible corresponding *H*-phosphonate monoesters.

Results and Discussion

Condensations Promoted by Pivaloyl Chloride. Searching for a simple and versatile method for the synthesis of nucleoside *N*-alkyl-*H*-phosphonamidates of type **3** (Scheme 1), we first investigated the direct coupling of a nucleoside *H*-phosphonate with an appropriate amine **2a**−**f** in the presence of pivaloyl chloride (Piv-Cl). Unfortunately, such condensations invariably resulted in mixtures of unreacted substrate **1** (∼20%), nucleoside bispivaloyl phosphite[9] **4** (∼70%), and the desired *H*-phosphonamidates **3a**−**f** as minor products (∼10%, Table 1). Since the ratio of **1** to the acyl phosphite **4** increased with the increasing concentration of an amine used, it seemed likely that the low yields of the desired products **3a**−**f** were due to extensive acylation of the amine occurring under the reaction conditions. Analogous reactions with diisopropylamine (**2g**) failed to produce detectable amounts of the desired product **3g**, and only the formation of bispivaloyl phosphite **4** was observed.

Since both pivaloyl chloride and the reactive intermediate involved in the coupling reaction, the mixed anhydride **5**[9] (Scheme 1), may act as acylating agents, we attempted the synthesis of *H*-phosphonamidates **3** *via* generation of **5** in the absence of an amine. If N-acylation by Piv-Cl is more rapid than N-acylation by **5**, this approach should significantly increase yields of **3**. We could also gain some insight into the chemoselectivity of **5** in reactions with amines and other nucleophiles.[10]

This issue was addressed by activation of the nucleoside *H*-phosphonate **1** with Piv-Cl (1.5 molar equiv) in

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(1) Eckstain, F.; Gish, G. *TIBS* **1989**, 97−100.
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(3) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543−584.
(4) Sobkowska, A.; Sobkowski, M.; Stawiński, J.; Kraszewski, A. *Nucleosides Nucleotides* **1995**, *14*, 703−706.
(5) Zwierzak, A.; Koziara, A. *Tetrahedron* **1967**, *23*, 2243−2252.
(6) Wada, T.; Ishikawa, K.; Hata, T. *Tetrahedron* **1993**, *49*, 2043−2054.
(7) *Methoden der organischen Chemie (Houben-Weyl)*; Müller, E., Ed.; George Thieme Verlag: Stuttgart, 1964; Vol. XII/2, p 104.
(8) Formation of nucleoside *H*-phosphonamidates as side products during phosphitylation of nucleosides with various phosphoramidochloridites was also observed. See, e.g.: Uznanski, B.; Wilk, A.; Stec, W. J. *Tetrahedron Lett.* **1987**, *28*, 3401−3404. Marugg, J. E.; Burik, A.; Tromp, M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2271−2274.
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(10) These studies are in progress in our laboratories.

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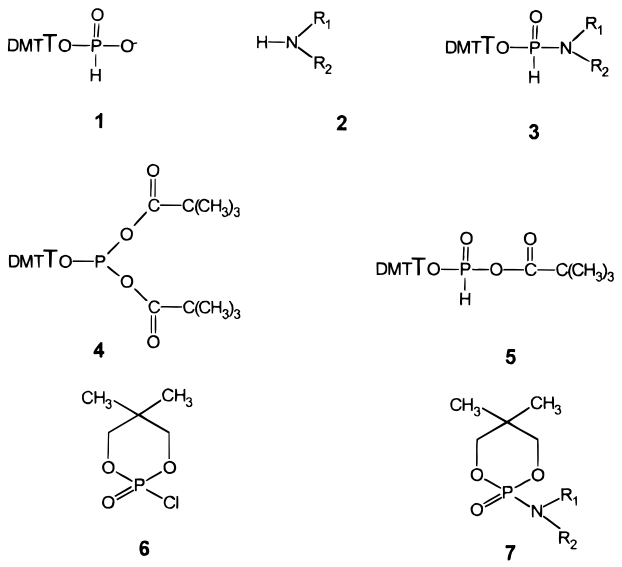
Scheme 1



DMTT = 5′-O-(4,4′-dimethoxytrityl)thymidin-3′-yl

2a, 3a, 7a, R_1 = H, R_2 = butyl
2b, 3b, 7b, R_1 = H, R_2 = *iso*-propyl
2c, 3c, 7c, R_1 = H, R_2 = *tert*-butyl
2d, 3d, 7d, R_1 = methyl, R_2 = butyl
2e, 3e, 7e, $R_1 = R_2$ = butyl
2f, 3f, 7f, $R_1,R_2 = (CH_2)_5$
2g, 3g, $R_1 = R_2$ = *iso*-propyl

Table 1. Amounts (%) of *H*-Phosphonamidates 3 Produced under Various Reaction Conditions As Estimated by ^{31}P NMR Spectroscopy

condensing agent or an intermediate	3a	3b	3c	3d	3e	3f	3g
Piv-Cl[a]	<10	<10	<10	<10	<10	<10	0
Piv-Cl[b]	73	84	85	78	83	74	0[f]
NEP[c]	4	42	81	10	68	3	>95
NEP[d]	62	67	96	55	92	51	>95
8[e]	>95	>95	>95	>95	>95	>95	0[f]

[a] Condensation of **1** in pyridine with the appropriate amine **2** (1.5 molar equiv) in the presence of Piv-Cl (3.0 molar equiv). [b] Preactivation of **1** with Piv-Cl (1.1 molar equiv) for 5 min in CH_2Cl_2−pyridine (9:1, v/v), followed by addition of the appropriate amine **2** (5.0 molar equiv). [c] Condensation of **1** with the appropriate amine **2** (5.0 molar equiv) in CH_2Cl_2−pyridine (9:1, v/v) in the presence of NEP (3.0 molar equiv). [d] Preactivation of **1** with NEP (3.0 molar equiv) for 10 min in CH_2Cl_2−pyridine (9:1, v/v) followed by addition of the appropriate amine **2** (5.0 molar equiv). [e] Produced in situ from **1** and 2,4,6-trichlorophenol in CH_2Cl_2−pyridine (9:1, v/v) in the presence of Piv-Cl (1.5 molar equiv) or NEP (3.0 molar equiv). [f] Products other than **3g** were formed (see the text also).

methylene chloride−pyridine (9:1, v/v) to produce **5** [^{31}P NMR, δ_P = 2.10 and 1.91 ppm, $^1J_{HP}$ = 749.0 Hz (d), $^3J_{HP}$ = 9.6 Hz (d); >95% yield], followed by the addition of an excess of amines **2a**−**f**. These reactions were rapid (<5 min) and afforded the corresponding nucleoside *N*-alkyl-*H*-phosphonamidates **3** in 73−85% yield (Table 1). We observed (^{31}P NMR), however, the appearance of resonances due to nucleoside *H*-phosphonate **1** (~20%) in the reaction mixtures, which may indicate that the attack of amines occurred predominantly, but not exclusively,[11] at the phosphorus center of **5**. This is worth stressing, since phosphoric−carboxylic anhydrides usually show opposite chemoselectivity and act exclusively as acylating agents.[12]

Diisopropylamine (**2g**) reacted with the mixed anhydride **5** differently than the other amines, affording as the only products the nucleoside bispivaloyl phosphite **4** and the nucleoside *H*-phosphonate **1** in the ratio ~1:1 (^{31}P NMR).

Condensations Promoted by 2-Chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (NEP). Since 2-chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (**6**) is known to be an efficient coupling agent for the formation of *H*-phosphonate diesters[13] and, due to steric hindrance, is rather unreactive as a phosphorylating agent, we assessed its potential utility in the synthesis of the *H*-phosphonamidates **3**.

In the reactions of nucleoside *H*-phosphonate **1** and amines **2a**−**f** with **6** instead of Piv-Cl as the condensing agent, the yields of the *H*-phosphonamidates **3** differed significantly depending on the amine used (Table 1). The highest yields were observed with moderately sterically hindered amines (*e.g.*, **2b**, **2c**, **2e**), while the lowest yields were observed with relatively nonhindered amines (*e.g.*, **2a**, **2d**, and **2f**). In all reaction mixtures, ^{31}P NMR spectroscopy revealed the presence of the cyclic phosphoroamidate **7** (Scheme 1), arising via reaction of the amines with **6** instead of **1**. This was confirmed in separate ^{31}P NMR experiments in which the reactivity of **2a**−**f** with **6** was found to be **2a** ≈ **2d** ≈ **2f** > **2b** ≈ **2c** ≈ **2e**. Diisopropylamine (**2g**) did not undergo detectable phosphorylation under these reaction conditions, and as a consequence, it produced the corresponding *H*-phosphonamidate **3g** with a yield >95%, although the reaction was rather slow.

The condensations involving preactivation of *H*-phosphonate **1** with condensing agent **6** (10 min), followed by the addition of amines **2a**−**f**, considerably improved the yields of *H*-phosphonamidates **3** (Table 1). This was particularly pronounced for the nonhindered amines **2a**, **2d**, and **2f** where yields of the corresponding *H*-phosphonamidates **3** increased 5- to 15-fold. In the instance of highly sterically hindered amine **2g**, both protocols (with and without preactivation) worked equally well, affording the nucleoside *H*-phosphonamidate **3g** in high yields.

Reactions of Aryl Nucleoside *H*-Phosphonate with Amines. To overcome the problems connected with the use of acyl chlorides or chlorophosphates as condensing agents, we attempted the synthesis of nucleoside *H*-phosphonamidates **3** *via* aryl nucleoside *H*-phosphonates **8** as intermediates. These compounds are known to be exceptionally prone to nucleophilic substitution at the phosphorus center[14] and can be conveniently prepared *in situ* by reaction of appropriate phenols with *H*-phosphonate monoesters (Scheme 2). Possible advantages of using aryl *H*-phosphonates as synthetic intermediates are that (i) there is only one electrophilic center in these compounds, which eliminates problems of chemoselectivity in reactions with nucleophiles, and (ii) if necessary, the electrophilicity of the phosphorus atom can be controlled by changing substituents on the aryl ring.

(11) At this stage of the investigations, no attempt was made to isolate the expected acylated amines from the reaction mixtures.

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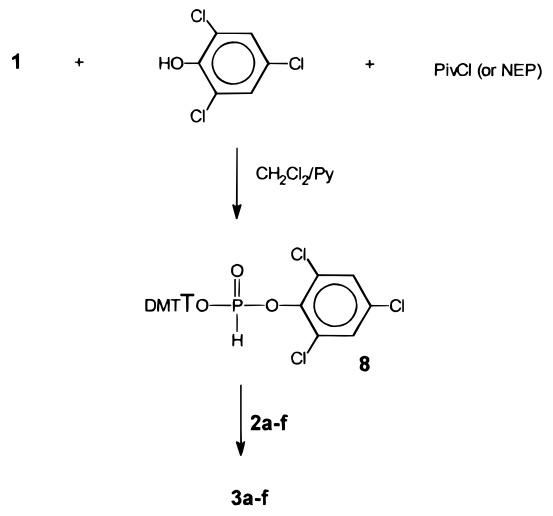
Scheme 2



DMTT = 5′-O-(4,4′-dimethoxytrityl)thymidin-3′-yl
Piv = pivaloyl
NEP = 2-chloro-5,5-dimethyl-2-oxo-2-λ^5-1,3,2-dioxaphosphinane

Table 2. ^{31}P NMR Data of Nucleoside *H*-Phosphonamidates of Type 3 and Other Products

compd	δ_P[a] (ppm)	$^1J_{HP}$ (Hz)	$^3J_{HP}$ (Hz)
3a	12.83	637.7	10.2[b]
	13.22	640.5	
3b	10.64	645.1	c
	11.14	641.4	
3c	8.59	635.9	*c*
	9.00	631.2	
3d	15.13	645.1	*c*
	15.42	643.3	
3e	14.86	642.3	9.3[d]
	15.30	640.5	9.3[d]
3f	12.56	647.0	8.3[d]
	12.89	644.2	8.3[d]
3g	12.98	641.4	c
	13.32	636.8	
8	1.60	758.2	9.3[e]
	1.10	759.1	9.3[e]

[a] Spectra recorded in CH_2Cl_2−pyridine (9:1, v/v) with heteronuclear decoupling (H_3PO_4 in D_2O as an external reference). [b] Doublets of quartets. [c] Doublets of poorly resolved multiplets. [d] Doublets of multiplets. [e] Two doublets.

The efficacy of this approach was assessed by forming the nucleoside 2,4,6-trichlorophenyl *H*-phosphonate diester **8** *in situ* (^{31}P NMR, Table 2) and allowing the latter to react with amines (**2a**−**f**). The reactions were fast (<3 min, ^{31}P NMR) and afforded the nucleoside *H*-phosphonamidates **3a**−**f** (Scheme 2) quantitatively. They were isolated in satisfactory yields, and their structures confirmed by ^{1}H and ^{31}P NMR (Table 2), MS spectroscopy, and TLC analysis.

Diisopropylamine (**2g**) did not yield the expected nucleoside *H*-phosphonamidate **3g** but, instead, gave other products depending the coupling agent used to generate the aryl nucleoside *H*-phosphonate **8**. This is the subject of separate investigations in these laboratories.

In conclusion, the most important factors affecting the synthesis of nucleoside *H*-phosphonamidates **3** *via* condensations mediated by a coupling agent are (i) reactivity of amines toward coupling reagents, (ii) the chemoselectivity of amines with the reactive species formed during the activation process, and (iii) the steric hindrance of the amines. These factors usually cause yields of *H*-phosphonamidate **3** to be higher when the condensation involves the preactivation of *H*-phosphonate monoesters. Using a preformed aryl nucleoside *H*-phosphonate diester such as **8** as a substrate alleviates most of the problems connected to the use of coupling agents. The ease of preparation of **8** and its high susceptibility to nucleophilic substitution at the phosphorus atom make aryl *H*-phosphonates valuable substrates in the synthesis of nucleoside *H*-phosphonamidates carrying primary and unhindered secondary amine moieties. For sterically hindered amines, however, use of chlorophosphate **6** to mediate coupling reactions of *H*-phosphonate monoesters and an amine is the method of choice.

Experimental Section

^{1}H and ^{31}P NMR spectra were recorded at 300 MHz on a Varian Unity BB VT spectrometer. The ^{31}P NMR experiments were carried out in 5 mm tubes using 0.1 mmol/0.7 mL of phosphorus-containing compound in methylene chloride containing 10% pyridine. TLC analyses (Merck silica gel 60 F_{254} precoated plates) were carried out in saturated chambers using following solvent systems: (A) CH_2Cl_2/MeOH, 9:1 (v/v); (B) ethyl acetate/toluene, 9:1 (v/v). The amount of water in the solvents was measured by Karl Fisher coulometric titration using a Metrohm 684 KF coulometer. Methylene chloride (POCH) was dried over P_2O_5, distilled, and kept over molecular sieves 4A until the amount of water was less than 10 ppm. Pyridine (Lab. Scan. Ltd.) was stored over molecular sieves 4A until the amount of water was below 20 ppm. *n*-Butylamine (Fluka) was dried over KOH, distilled, and stored over molecular sieves 3A. Isopropylamine (Aldrich), *tert*-butylamine (Aldrich), *N*-butyl-*N*-methylamine (Merck), piperidine (Aldrich), *N*,*N*-dibutylamine (Fluka), and *N*,*N*-diisopropylamine (Fluka) were distilled and stored over molecular sieves 4A. 2,4,6-Trichlorophenol (Aldrich, commercial grade) and 4,4′-dimethoxytrityl chloride (Fluka) were commercial grades. Pivaloyl chloride (Merck) was distilled before use. For column chromatography, silica gel 60 or silica gel silanized (Merck) was used. 5′-*O*-(4,4′-Dimethoxytrityl)thymidine 3′-*H*-phosphonate[15] and 2-chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane[16] (NEP) were prepared according to published procedures.

General Procedure for the Synthesis of Nucleoside 3′-*H*-Phosphonamidates of Type 3. 5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *H*-phosphonate (**1**) and 2,4,6-trichlorophenol (2.0 molar equiv) were dried by azeotropic distillation with pyridine, and the residue was dissolved in methylene chloride containing 10% pyridine (1 mmol/10 mL). To this was added pivaloyl chloride (1.5 molar equiv) to produce the aryl nucleoside *H*-phosphonate **8** (5 min), followed by addition of the appropriate amine **2** (5.0 molar equiv). After 5 min, the reaction mixture was diluted with methylene chloride (3-fold volume), triethylamine (5.0 molar equiv) was added, and the mixture was extracted with brine. The organic phase was separated and dried over anhydrous Na_2SO_4, and after evaporation the residue was chromatographed on a silica gel RP-2 column using a linear gradient of acetone (0−20% v/v) in CH_2Cl_2. Compounds **3** were obtained as white solids after freeze-drying from benzene. ^{31}P NMR data of the products are listed in Table 2.

5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *N*-butyl-*H*-phosphonamidate (3a): yield 52%; R_f = 0.7 (A), 0.19 (B); ^{1}H NMR δ_H ($CDCl_3$) 0.88, 0.92 (3H, 2t, J = 7.2 Hz, 7.5 Hz, CH_2CH_3), 1.24−1.51 (4H, m, $CH_2CH_2CH_2CH_3$), 1.39, 1.41 (3H, 2d, J = 1.2 Hz, 5-CH_3), 2.43, 2.57 (2H, m, 2′,2″-H_2), 2.8, 2.89 (2H, m, $NHCH_2CH_2$), 3.38, 3.52 (2H, m, 5′,5″- H_2), 3.79 (6H, s, OCH_3), 4.24 (1 H, m, 4′-H), 5.16 (1H, m, 3′-H), 6.46 (1H, m, 1′-H), 6.91 and 6.94 (1H, 2d, $^1J_{HP}$ = 645.5, 647.9 Hz, PH), 6.84

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(4H, d, J = 9.0 Hz, 3,3′,5- and 5′-H of DMT), 7.24 −7.39 (9H, m, ArH of DMT except of 3,3′,5- and 5′-H), 7.57 (1H, m, 6-H), 8.6 (1 H, br m, NH, exch. D_2O) (multiplicity of some signals due to the presence of the P-diastereomers); HRMS m/z 686.2606, calcd for $C_{35}H_{42}N_3O_8PNa$ 686.2607. Anal. Calcd for $C_{35}H_{42}N_3O_8P$: C, 63.32; H, 6.38; N, 6.33. Found: C, 63.29; H, 6.38; N, 6.16.

5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *N*-isopropyl-*H*-phosphonamidate (3b): yield 51%; R_f = 0.68 (A), 0.15 (B); ^{1}H NMR δ_H ($CDCl_3$) 1.07, 1.14, 1.19 (6H, 3d, J = 6.3 Hz, $CHCH_3$), 1.38, 1.41 (3H, 2s, 5-CH_3), 2.43, 2.59 (2H, m 2′,2″-H_2), 3.39 (1H, m, $CHCH_3$), 3.37−3.53 (3H, m, 5′,5″-H_2 and CH of isopropyl), 3.79 (6H, s, OCH_3), 4.26 (1H, m, 4′-H), 5.16 (1H, m, 3′-H), 6.46 (1H, m, 1′-H), 6.94 and 6.98 (1H, 2d, $^1J_{HP}$ = 641.6, 644.0 Hz, PH), 6.83 (4H, d, J = 8.7 Hz, 3,3′,5- and 5′-H of DMT), 7.24−7.39 (9H, m, ArH of DMT except of 3,3′,5- and 5′-H), 7.58 (1H, m, 6-H), 8.47 (1H, br m, NH, exch. D_2O) (multiplicity of some signals due to the presence of the P-diastereomers); HRMS m/z 672.2449, calcd for $C_{34}H_{40}N_3O_8PNa$ 672.2451. Anal. Calcd for $C_{34}H_{40}N_3O_8P$: C, 62.84; H, 6.21; N, 6.47. Found: C, 62.76; H, 6.19; N, 6.29.

5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *N*-*tert*-butyl-*H*-phosphonamidate (3c): yield 60%; R_f = 0.70 (A), 0.17 (B); H NMR δ_H ($CDCl_3$) 1.25, 1.29 [9H, 2s, $C(CH_3)_3$], 1.37, 1.41 (3H, 2s, 5-CH_3), 2.44, 2.61 (2H, 2m, 2″,2″-H_2), 3.40, 3.52 (2H, 2m, 5′,5″-H_2), 3.79 (6H, s, OCH_3), 4.26, 4.29 (1H, 2m, 4′-H), 5.16 (1H, m, 3′-H), 7.02, 7.08 (1H, 2d, $^1J_{HP}$ = 610.7, 642.5 Hz, PH), 6.48 (4H, d, J = 8.7 Hz, 3,3′,5- and 5′-H of DMT), 7.20−7.36 (9H, m, ArH of DMT except of 3,3′,5- and 5′-H), 7.58 (1H, m, 6-H) and 8.52 (1H, br, NH, exch. D_2O) (multiplicity of some signals due to the presence of the P-diastereomers); HRMS m/z 686.2599, calcd for $C_{35}H_{42}N_3O_8PNa$ 686.2607. Anal. Calcd for $C_{35}H_{42}N_3O_8P$: C, 63.32; H, 6.38; N, 6.33. Found: C, 63.27; H, 6.47; N, 6.21.

5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *N*-butyl-*N*-methyl-*H*-phosphonamidate (3d): yield 56%; R_f = 0.76 (A), 0.24 (B); ^{1}H NMR δ_H ($CDCl_3$) 0.89, 0.93 (3H, 2t, J = 7.2 Hz, CH_2CH_3), 1.27 (2H, m, CH_2CH_3), 1.38, 1.40 (3H, 2s, 5-Me), 1.48 (2H, m, $CH_2CH_2CH_3$), 2.44, 2.58 (2H, 2m, 2′,2″-H_2), 2.52, 2.62 (3H, 2d, J = 10.8 Hz, NCH_3), 2.93 (2H, m, NCH_2CH_2), 3.38, 3.52 (2H, 2m, 5′, 5″-H_2), 3.79 (6H, s, OCH_3), 4.21, 4.25 (1H, 2m, 4′-H), 5.08 (1H, m, 3′-H), 6.44 (1H, m, 1′-H), 6.74 and 6.78 (1H, 2d, $^1J_{HP}$ = 646.1, 647.3 Hz, PH), 6.84 (4H, d, J = 8.7 Hz, 3,3′,5- and 5′-H of DMT), 7.24−7.39 (9H, m, ArH of DMT except of 3,3′,5- and 5′-H), 7.57 (1H, m, 6-H) and 8.24 (1H, br s, NH, exch. D_2O) (multiplicity of some signals due to the presence of the P-diastereomers); HRMS m/z 700.2763, calcd for $C_{36}H_{44}N_3O_8PNa$ 700.2764. Anal. Calcd for $C_{36}H_{44}N_3O_8P$: C, 63.78; H, 6.55; N, 6.20. Found: C, 63.63; H, 6.84; N, 6.28.

5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *N*,*N*-dibutyl-*H*-phosphonamidate (3e): yield 60%; R_f = 0.79 (A), 0.39 (B); ^{1}H NMR δ_H ($CDCl_3$) 0.89, 0.92 (6H, 2t, J = 7.2 Hz, CH_2CH_3), 1.27 (4H, m, $CH_2CH_2CH_3$), 1.35, 1.39 (3H, 2s, 5-CH_3), 1.45 (4H, m, $NCH_2CH_2CH_2$), 2.44, 2.57 (2H, 2m, 2, 2″-H_2), 2.95 (4H, m, NCH_2CH_2), 3.37−3.51 (2H, m, 5′, 5″-H_2), 3.79 (6H, s, OCH_3), 4.24 (1H, m, 4′-H), 5.12 (1H, m, 3′-H), 6.46 (1H, m, 1′-H), 6.76, 6.82 (1H, 2d, $^1J_{HP}$ = 642.2, 630.8 Hz, P-H), 6.84 (4H, d, J = 8.7 Hz, 3,3′,5- and 5′-H of DMT), 7.21−7.39 (9H, m, ArH of DMT except of 3,3′,5- and 5′-H), 7.58 (1H, m, 6-H), 8.48 (1H, br s, NH, exch. D_2O) (multiplicity of some signals due to the presence of the P-diastereomers); HRMS m/z 742.3227, calcd for $C_{39}H_{50}N_3O_8PNa$ 742.3233. Anal. Calcd for $C_{39}H_{50}N_3O_8P$: C, 65.06; H, 7.01; N, 5.84. Found: C, 64.82; H, 7.21; N, 5.63.

5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *N*-piperidyl-*H*-phosphonamidate (3f): yield 64%; R_f = 0.74 (A), 0.18 (B); ^{1}H NMR δ_H ($CDCl_3$) 1.37, 1.41 (3H, 2s, 5-CH_3), 1.35−1.65 (6H, m, $CH_2CH_2CH_2CH_2CH_2$), 2.42, 2.57 (2H, 2m, 2′,2″-H_2), 3.00 (4H, m, NCH_2, NCH_2), 3.37, 3.52 (2H, m, 5′,5″-H_2), 3.79 (6H, s, OCH_3), 4.20, 4.25 (1H, 2m, 4′-H), 5.09 (1H, m, 3′-H), 6.47 (1H, m, 1′-H), 6.72 and 6.76 (1H, 2d, $^1J_{HP}$ = 638.3, 649.4 Hz, PH), 6.83 (4H, d, J = 8.7, 3,3′,5- and 5′-H of DMT), 7.24−7.39 (9H, m, ArH of DMT except of 3,3′,5- and 5′-H), 7.58 (1H, m, 6-H) and 8.42 (1H, br s, NH, exch. with D_2O) (multiplicity of some signals due to the presence of P-diastereomers); HRMS m/z 698.2608, calcd for $C_{36}H_{42}N_3O_8PNa$ 698.2607. Anal. Calcd for $C_{36}H_{42}N_3O_8P$: C, 63.97; H, 6.27; N, 6.22. Found: C, 63.62; H, 6,.23; N, 6.22.

5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl *N*,*N*-diisopropyl-*H*-phosphonamidate (3g). 5′-*O*-(4,4′-Dimethoxytrityl)-thymidin-3′-yl *H*-phosphonate (**1**) was dried by evaporation of added pyridine, and after it was dissolved in methylene chloride containing 10% pyridine (1 mmol/10 mL), diisopropylamine (5.0 molar equiv) and 2-chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (**6**) (3.0 molar equiv) were added. After 1 h, the reaction mixture was diluted with methylene chloride (5-fold of the initial volume) and washed with saturated aqueous $NaHCO_3$. The organic layer was separated and dried over anhydrous Na_2SO_4, and solvents were removed by evaporation under reduced pressure. Product **3g** was purified by silica gel column chromatography using a linear gradient of acetone in methylene chloride (0−20% v/v). Fractions containing the desired product were collected and evaporated. The *H*-phosphonamidate **3g** was obtained as white solid after lyophilization from benzene: yield 69%; R_f = 0.74 (A), 0.29 (B); ^{1}H NMR ($CDCl_3$) (multiplicity of some signals are due to the presence of P-diastereoisomers) δ_H 1.22, 1.23 (12H, 2d, J = 6.6, 6.9 Hz, CH_3 of isopropyl) 1.35, 1.39 (3H, 2d, J = 1.2, 0.9 Hz, 5-CH_3), 2.46, 2.58 (2H, 2m, 2′,2″-H_2), 3.37−3.54 (4H, m, 5′,5″-H_2 and CH of isopropyl), 3.79 (6H, s, OCH_3), 4.27 (1H, m, 4′-H), 5.12 (1H, m, 3′-H), 6.46 (1H, m, 1′-H), 6.83 (4H, d, J = 8.9 Hz, 3,3′,5- and 5′-H of DMT), 6.85, 6.93 (1H, 2d, $^1J_{HP}$ = 636.8, 637.4 Hz, PH) 7.21−7.39 (9H, m, ArH of DMT except of 3,3′,5- and 5′-H), 7.6 (1H, m, 6-H), 8.3 (1H, br s, NH, exch. D_2O) (multiplicity of some signals due to the presence of P-diastereomers); ^{31}P NMR data, see Table 2; HRMS m/z 714.2922, calcd for $C_{37}H_{46}N_3O_8PNa$ 714.2920. Anal. Calcd for $C_{37}H_{46}N_3O_8P$: C, 64.23; H, 6.71; N 6.08. Found: C, 64.19; H, 6.58; N, 5.92.

Reaction of 2-Chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (6) with Amines 2a−f. 2-Chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (**6**) (1.0 molar equiv) in CH_2Cl_2−pyridine (9:1 v/v) (1.0 mmol/10 mL) was allowed to react with the appropriate amine **2** (5.0 molar equiv) to produce the corresponding phosphoramidates. In all instances, their ^{31}P NMR spectra were identical with those of side products of type **7** formed during the condensations of **1** with amines **2a**−**f** mediated by **6**; ^{31}P NMR **7a**, δ_P 5.70 ppm (br m); **7b**, δ_P 4.25 ppm (br m); **7c**, δ_P 3.50 (br m); **7d**, δ_P 6.81 ppm (br m); **7e**, δ_P 7.51 ppm (br m); **7f**, δ_P 5.12 ppm (br m).

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