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Nucleosides, Nucleotides and Nucleic Acids

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Studies on Reactions of Nucleoside H-Phosphonates with Bifunctional Reagents. Part VI. Reaction with Diols

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STUDIES ON REACTIONS OF NUCLEOSIDE *H*-PHOSPHONATES WITH BIFUNCTIONAL REAGENTS. PART VI. REACTION WITH DIOLS^a

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ABSTRACT: Reactions of nucleoside *H*-phosphonates with various diols using different types of condensing agents have been studied. Depending on the coupling procedure and the length of a polymethylene chain of the diol, acyclic *H*-phosphonate diesters or cyclic phosphite triesters were formed. The course of oxidation with iodine to produce cyclic nucleoside alkyl phosphotriesters or hydroxyalkyl nucleoside phosphodiesters can be controlled by the amount of water present in the reaction medium.

INTRODUCTION

Functionalisation of oligonucleotides *via* attachment of various functionalities to different parts of these macromolecules, have received much attention during the past decade.¹ This approach enables linking oligonucleotides to other classes of biopolymers or to low molecular compounds (*e.g.* reporter groups, haptens) to produce a vast array of oligonucleotide conjugates for diverse therapeutic, diagnostic and research applications.²⁻⁵ By far the most common type of functionalisation consists of the introduction to the 5'- or 3'-end of an oligonucleotide of a suitable linker molecule bearing a terminal functional

^a This paper is dedicated to the late Professor Alexander Krayevsky

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group amenable for further attachment of a desired ligand.^{6,7} By varying the length of the linker one can control the proximity of a ligand vs an oligonucleotidic chain in order to minimise an interference of the ligand during the hybridization process.

As part of our interest in developing synthetic methodologies for P-modified oligonucleotides we have embarked on investigations of reactions of bifunctional reagents with nucleoside *H*-phosphonates, and have recently reported an efficient method for the introduction of an aminoalkyl moiety into oligonucleotides using unprotected amino alcohols.⁷⁻¹⁰ We have now extended these studies by including reactions of nucleoside *H*-phosphonates with unprotected diols as a viable route to hydroxyalkyl functionalised oligonucleotides. Although this type of nucleotide derivatives has not received much attention,¹⁰⁻¹² they represent a class of synthetic intermediates complementary to that of the corresponding aminoalkyl derivatives. In addition, hydroxyalkyl esters of *H*-phosphonates seem to be convenient model compounds for studying the cyclisation processes in phosphorus compounds as a function of (i) type of the phosphorus center involved, (ii) the length of a polymethylene spacer in the diol used and, in conjunction with our previous studies on nucleoside aminoalkyl *H*-phosphonates,^{8,9,13} (iii) can provide additional information on reactivity of O *vs* N nucleophiles in the ring closure reactions.

In this paper we present our studies on the reaction of nucleoside *H*-phosphonate monoesters with unprotected diols in the presence of various condensing agents and transformations occurring during oxidation of the *H*-phosphonate derivatives produced. These shed light on general reactivity of nucleoside hydroxyalkyl *H*-phosphonate diesters and constituted the basis for the development of new synthetic methods for the preparation of functionalised nucleotides bearing hydroxyalkyl moieties.

RESULTS AND DISCUSSION

While investigating the phosphonylation of a cis-diol system of 5'-O-protected ribonucleosides with *H*-phosphonate monoesters,¹⁴ we observed that *H*-phosphonate diesters bearing vicinal hydroxyl groups were significantly more susceptible to cyclisation than hydroxyalkyl phosphates diesters¹⁵ and instantaneously produced the corresponding cyclic *H*-phosphonates with the expulsion of the exocyclic hydroxylic component. These findings stimulated the recent interest in 2-hydroxyalkyl *H*-phosphonate diesters as

intermediates for dephosphonylation of nucleoside H-phosphonate monoesters¹⁶⁻¹⁸ and as a convenient model system for studying some mechanistic aspects of ribozymes catalysed reactions.¹⁹

To get a more detailed picture concerning the general reactivity of hydroxyalkyl *H*-phosphonate diesters and their oxidative transformations, the coupling reaction of 5'-O-dimethoxytritylthymidine 3'-*H*-phosphonate 1 with various diols **2a-d** was investigated first. Since the efficiency of formation of hydroxyalkyl *H*-phosphonate derivatives was strongly dependent on the reaction conditions, results will be discussed separately for each condensing agent used.

Condensations promoted by pivaloyl chloride.

Compared to amino alcohols⁸ that have two functional groups with different reactivity (N vs O chemoselectivity), diols with two equivalent hydroxyl groups might seem to be simpler reaction systems. However, attempts to produce nucleoside hydroxyalkyl *H*-phosphonates in pyridine using equimolar amounts of *H*-phosphonate 1 and diol 2 in the presence of Pv-Cl (3 equiv.; standard condensation conditions²⁰) afforded (< 3 min) complicated mixtures of products (³¹P NMR spectroscopy), irrespective of the diol used. Since chemical shifts of the compounds produced clustered between 2-10 ppm and showed the presence of a hydrogen atom directly bound to the phosphorus center, we assumed that these mixtures contained various amounts of the desired nucleoside hydroxyalkyl *H*-phosphonates 10 and their O-pivaloylated and O-phosphonylated derivatives.

With ethylene glycol 2a, an additional signal (ca 25%) at $\delta_P \sim 23$ ppm appeared probably indicating a spontaneous cyclisation¹⁴ of the initially formed 2-hydroxyethyl nucleoside *H*-phosphonate 10a to produce cyclic *H*-phosphonate 12 (*vide infra*). Using less pivaloyl chloride caused incomplete condensations and did not eliminate the formation of the corresponding O-pivaloylated and O-phosphonylated side products. To suppress Oacylation of the diols during condensation we attempted condensation with preactivation of *H*-phosphonate monoester 1 (Scheme 1). We anticipated that bispivaloyl phosphite 3 formed during the preactivation,²¹ should react with the added diol 2 to produce various proportions of cyclic *vs* acyclic phosphites,²² depending on an excess and the tendency of a diol to cyclisation.



SCHEME1. (i), 2a; (ii), 2b; (iii), 2c, 1 equiv.; (iv), 2d, 1 equiv.; (v), 2c or 2d, > 3 equiv.

Thus, *H*-phosphonate 1 was converted into dipivaloyl phosphite 3^{21} (2.5 equiv. of Pv-Cl in pyridine), which was then allowed to react with various diols 2 (Scheme 1). Although phosphites 4 and 5 formed in these reactions were too unstable to be isolated,²³ ³¹P NMR spectroscopy permitted their tentative identification. The assignment of ³¹P NMR resonances to cyclic 4 vs acyclic 5 phosphites was based on the well established correlation between chemical shifts of tervalent P(III) compounds and the O–P–O bond angles²⁴ which predicts, that phosphites containing phosphorus in five- and six-membered rings should resonate at ca 6-11 ppm to higher fields (*e.g.* $\delta_P \sim 131$ and 128 ppm,

respectively) than the acyclic congeners ($\delta_P \sim 138$ ppm). We found, that ethylene glycol 2a and 1,3-propanediol 2b, irrespective of the excess used, reacted with intermediate 3 exclusively producing (< 3 min) species resonating at 134.4 and 129.8 ppm, respectively. These resonances we tentatively assigned on the bases of their chemical shifts and the splitting pattern²⁵ (Table 1) to 1,3,2-dioxaphospholanes 4a and 1,3,2-dioxaphosphinane 4b,²⁶ respectively.

The exclusive formation of cyclic species in these reactions indicated a strong tendency to cyclisation of the initially formed hydroxyalkyl phosphites, and this trend was also observed when 1,4-butanediol and 1,5-pentanediol were used for the reaction with bispivaloyl phosphite 3. However, in these instances, the respective cyclic phosphites 4c ($\delta_P = 132.1$ ppm) and 4d ($\delta_P = 132.0$ ppm), were formed as major products (>95%) only when an equimolar amount of the diols was used for the reaction. With 3-fold (or more) excess of 2c and 2d, the intermolecular reactions prevailed, and acyclic phosphites 5c ($\delta_P = 138.9$ ppm) and 5d ($\delta_P = 139.0$ ppm), respectively, were produced almost exclusively.

The ease of formation of cyclic phosphites **4a-d** in the reaction of bispivaloyl phosphite **3** and the corresponding diols **2** prompted us to apply these findings to the development of a new entry to cyclic phosphate triesters from the corresponding *H*-phosphonate monoesters. To this end, the *in situ* produced cyclic phosphites **4a-d** were oxidised with iodine in 2% aqueous pyridine²⁷ and progress of the reaction was followed by ³¹P NMR spectroscopy. For phosphites **4b-d**, the integrity of ring systems was preserved and the corresponding phosphinane **6a** ($\delta_P = -8.4$ ppm), phosphepane **6c** ($\delta_P = 2.2$ ppm) and phosphocane **6d** ($\delta_P = 1.8$ ppm) were obtained in overall yields of 50-70% after column chromatography. Oxidation of five-membered phosphite **4a** resulted in the formation of an open-chain hydroxyalkyl phosphate **8a** ($\delta_P = 1.7$ ppm) exclusively. However, using a limited amount of water (1.5 equiv) for oxidation with iodine, intermediacy of the expected phospholane **6a** ($\delta_P = 16.8$ ppm) could be confirmed.

Condensation promoted by 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane (NEP-Cl).

Although pivaloyl chloride proved to be a convenient reagent for converting Hphosphonate monoester 1 into cyclic phosphites 4, it failed to produce hydroxyalkyl H- phosphonates in reasonable yields due to extensive occurrence of side reactions (vide supra). We therefore turned our attention to 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane 9 (NEP-Cl), a mild condensing agent, which can effect the formation of H-phosphonate diesters,²⁸ but which due to steric hindrance at the phosphorus center phosphorylates alcohols very slowly.²⁹



SCHEME 2

As expected, condensations of equimolar amounts of nucleoside *H*-phosphonate **1** and diols **2b–d** in pyridine in the presence of chlorophosphate **9** (2.5 equiv) furnished rapid (< 3 min) and clean formation (³¹P NMR spectroscopy) of the corresponding hydroxyalkyl nucleoside *H*-phosphonates **10b-d** (Scheme 2). With ethylene glycol **2a**, the desired hydroxyethyl *H*-phosphonate **10a** was formed under the reaction conditions as a minor product only (ca 10%, $\delta_P = 8.6$ ppm), while the main phosphorus-containing species was a compound resonating at $\delta_P = 23.1$ ppm. This, together with the TLC analysis which revealed the presence of 5'-O-4,4'-dimethoxytritylthymidine (ca 90%) suggested that the compound resonating at 23.1 ppm most likely was phospholane **12**,³⁰ formed *via* a spontaneous cyclisation of 2-hydroxyethyl *H*-phosphonate **10a**. The strong tendency of 2-hydroxyethyl *H*-phosphonate diesters to cyclisation with the expulsion of the exocyclic hydroxylic component (in this instance nucleoside **11**), prevents the synthesis of 2-hydroxyalkyl phosphonate diesters on this way. However, this type of derivatives are accessible from *H*-phosphonate monoesters *via* cyclic phosphite **4a** as an intermediate (*vide supra*).

In this context one should note that transforming alkyl (e.g. nucleoside) H-phosphonate monoesters into their 2-hydroxyethyl H-phosphonate diesters can be a

convenient method for removal of the *H*-phosphonate moiety from various *H*-phosphonate monoesters.¹⁶⁻¹⁸ Relevant to the use of cis-diols for this purpose is the observation that 2-hydroxyethyl *H*-phosphonate **10a** and the phospholane **12** are in equilibrium which can be shifted towards acyclic *H*-phosphonate **10a** by addition of nucleoside **11** to the reaction mixture (Scheme 2).





To get more detailed insight into this reaction, phospholane 12 was produced *in situ* as a single phosphorus-containing species by reacting equimolar amounts of diphenyl *H*-phosphonate and ethylene glycol 2a in pyridine. The product 12 was subjected to reactions with various hydroxylic compounds (Scheme 3). The addition of 1 equiv. of nucleoside 11 to such a reaction mixture resulted in the formation of hydroxyethyl *H*-phosphonate 10a (ca 10%), while ethanol (5 equiv.) completely converted (³¹P NMR spectroscopy) phospholane 12 into *H*-phosphonate diester 15 ($\delta_P = 8.1$ ppm). In agreement with the postulated equilibrium, the removal of ethanol from the reaction mixture by evaporation, restored the initial phospholane 12. Similarly, the addition of 2-hydroxyethyl *H*-phosphonate 14 ($\delta_P = 6.1$ ppm), which upon the removal of water and

,

additions of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl) afforded the initial phospholane 12.

Formation of nucleoside hydroxyalkyl phosphate from the corresponding *H*-phosphonate diesters.

Having at hand an efficient method for the generation of hydroxyalkyl *H*-phosphonate diesters, we investigated susceptibility to cyclisation of **10b-d** during oxidation with iodine under various experimental conditions (Scheme 4).



R = 5'-O-(4,4-dimethoxytrityl)thymidin-3'-yl

SCHEME 4

To this end, hydroxyalkyl *H*-phosphonates **10b-d** (produced *in situ* in pyridine using the reaction conditions as described above) were treated with iodine (1.1 equiv.) in anhydrous or aqueous pyridine. We found that oxidation with iodine under anhydrous conditions furnished rapid and clean formation of the corresponding cyclic phosphates **6b-d**, which were isolated by column chromatography in overall yields 50-70%.

The product distribution during oxidation of **10b-d** with iodine under aqueous conditions depended on the length of a polymethylene chain in the hydroxyalkyl derivatives and the amount of water in the reaction medium. Thus, 3-hydroxypropyl derivative **10b** exclusively produced the phosphinane **6b**, irrespective of the excess of water used,³¹ suggesting that intramolecular cyclisation of the produced iodophosphate intermediate²⁷ is strongly favoured over the intermolecular reaction with water. 4-Hydroxybutyl nucleoside *H*-phosphonate diester **10c**, when oxidised in the presence of 60 equiv. of water produced the expected 4-hydroxybutyl nucleoside phosphate **8c** and the

corresponding cyclic phosphate in the ratio 7 : 3. For 5-hydroxypentyl derivative 10d, an intermolecular hydrolysis (30 molar equiv. of water) of the intermediate iodophosphate was apparently the sole reaction (31 P NMR spectroscopy) as judged from the exclusive formation of the desired 5-hydroxypentyl nucleoside phosphate 8d under these reaction conditions. Both nucleoside hydroxyalkyl phosphates 8c and 8d were isolated by silica gel chromatography in satisfactory yields (>70%).

Tendency to cyclisation of hydroxyalkyl vs aminoalkyl phosphorus compounds

The findings from this work should be compared to those of oxidation of the corresponding aminoalkyl derivatives with iodine⁸ as they shed light on a tendency to cyclisation of phosphorus compounds containing hydroxyalkyl vs aminoalkyl moiety. At the level of H-phosphonate diesters, the 2-aminoethyl analogue of 10a in pyridine did not show any tendency to form 2-oxo-1,3,2-oxazaphospholidine⁸, while 2-hydroxyethyl Hphosphonate 10a under analogous conditions underwent almost complete cyclisation with the formation of phospholane 12. Also, 3-hydroxypropyl H-phosphonate 10b exclusively underwent intramolecular cyclisation during oxidation with iodine, irrespective of the amount of water used, while the 3-aminopropyl counterpart during oxidation under aqueous conditions exclusively produced the acyclic 3-aminopropyl phosphate derivative. ω -Aminoalkyl *H*-phosphonates with 4-5 methylene groups in the alkyl chain did not show any tendency to form cyclic phosphoramidates during oxidation with iodine under anhydrous conditions, and instead produced exclusively the corresponding symmetrical pyrophosphates⁸. In contradistinction to these, 4-hydroxybutyl- and 5-hydroxypentyl derivatives 10c and 10d underwent smooth cyclisation to the corresponding cyclic phosphates 6c and 6d, respectively, when oxidised with jodine in the absence of water.

In conclusion, we found that hydroxyalkyl groups when attached to the activated P(III) center (e. g. pivaloyl phosphite) have a strong tendency to cyclisation and efficiently form five-, six-, seven- and eight-membered phosphorus-containing rings. These findings were exploited for the preparation of various cyclic nucleoside phosphotriesters **6a-d**. We also developed an efficient method for the formation of *H*-phosphonate diesters **10** containing hydroxyalkyl moiety and found that this type of compounds were stable under neutral and weakly basic conditions, except the 2-hydroxyethyl derivatives (**10a**) that

underwent spontaneous cyclisation with the expulsion of the exocyclic hydroxylic component. During oxidation with iodine, hydroxyalkyl *H*-phosphonates 10 underwent cyclisation to produce the corresponding phosphotriesters 6 or afforded ω -hydroxyalkyl phosphates 8, depending on the length of a polymethylene chain in the alkyl group and the reaction conditions used.

EXPERIMENTAL PART

Material and Methods

¹H and ³¹P spectra were recorded on a Varian Unity 300 BB VT spectrometer. The ³¹P NMR experiments were carried out at 25 °C in 5 mm tubes using 0.1 M solution of phosphorus-containing compounds in pyridine (0.6 mL) and the spectra were referenced to 2% H₃PO₄ in D₂O (external standard). Mass spectra were recorded on a JEOL MS SX 102 spectrometer with *m*-nitrobenzyl alcohol. TLC analyses were carried out on Merck silica gel 60 F₂₅₄ precoated plates using the following solvent systems: A – dichloromethane - methanol (9:1 v/v); B – dichloromethane - 2-propaneol (9:1, v/v); C – dichloromethane - methanol – triethylamine (85:10:5, v/v/v); D – *n*-propaneol – water – 25% aq. ammonia (85:10:5, v/v/v). TLC mobilities are reported relative to 5'-O-dimethoxytritylthymidine (*R*_T, systems A and B), and 5'-O-dimethoxytritylthymidine 3'-*H*-phosphonate (*R*_{TPH}, systems C and D).

Pyridine (LabScan Ltd.) was stored over molecular sieves 4A until the amount of water was below 20 ppm (Karl Fischer coulometric titration with Metrohm 684 KF coulometer). Pivaloyl chloride (Merck) and ethylene glycol (POCh, Poland) were of commercial grade and were distilled before use. 1,3-Propanediol, 1,4-butanediol, and 1,5-pentanediol (all from Fluka) were used without any additional purification. 5'-O-Dimethoxytritylthymidine 3'-H-phosphonate **1** was always dried by evaporation of added pyridine prior to reactions.

Identities of the isolated compounds were confirmed by ¹H, ³¹P NMR, and HRMS spectroscopy, and their purity was assessed by ¹H NMR spectroscopy. 5'-O-Dimethoxytritylthymidine 3'-H-phosphonate 1³² and 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane 9²⁹ were obtained according to published methods. The reference compounds were prepared as follows: 2-oxo-1,3,2-dioxaphospholane 12, by the reaction

of equimolar amounts of diphenyl *H*-phosphonate and ethylene glycol in pyridine; 2hydroxyethyl *H*-phosphonate 14, by reacting of equimolar amounts of ethyl *H*phosphonate and ethylene glycol in pyridine in the presence of NEP-Cl (2.5 equiv.), followed by the addition of water; ethyl 2-hydroxyethyl *H*-phosphonate 15, analogously to *H*-phosphonate 10a.

Synthesis of [5'-O-(4,4'-dimethoxytrityl)-thymidin-3'-yloxy]-1,3,2-dioxaphospholane

4a. To *H*-phosphonate 1 [triethylammonium (TEAH⁺) salt, 1 mmol] dissolved in pyridine (5 mL), pivaloyl chloride (2.5 equiv.) was added, followed by (after 5 min) ethylene glycol **2a** (1.1 equiv.). After another 5 min the solvent was evaporated and the residue was dissolved in dichloromethane and washed briefly with saturated aqueous NaHCO₃. The organic layer was separated, dried with anhydrous Na₂SO₄ and evaporated to dryness. Compound **4a** was purified by short-column chromatography on silica gel with 0.5% methanol in dichloromethane as eluent, precipitated from hexane – diethyl ether 1:1 and dried *in vacuo*. White powder (purity >95%, ¹H NMR). Yield: 43%. *R*_T 1.45 (system B), δ_{H} (CDCl₃; Me₄Si) 1.71 (3H, s, 5-CH₃), 2.47 and 2.66 (2H, 2 m, 2'- & 2"-H), 3.40 and 3.47 (2H, 2m, 5'- & 5"-H), 3.81 (6H, s, OCH₃), 4.22 (4H, m, POCH₂), 4.31 (1H, m, 4'-H), 5.08 (1H, m, 3'-H), 6.50 (1H, m, 1'-H), 6.82 (4H, d, *J* 8.7, 3, 3', 5, 5'-H of DMTr), 7.20–7.37 (9H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.58 (1H, br s, 6-H) and 8.70 (1H, br s, N³H, exch. with D₂O). FAB MS [MH]⁺ found 635. C₃₃H₃₆N₂O₉P requires 635.6. For ³¹P NMR data, see Table 1.

General procedure for the synthesis of cyclic phosphate triesters 6b-d.

(A) Approach with preactivation of H-phosphonate 1 with pivaloyl chloride: The syntheses of phosphite triesters 4b - d were carried out essentially as described above for the preparation of phospholane 4a. The produced cyclic phosphites were subjected to oxidation with iodine (1.05 equiv.) in the presence of water (0.5% v/v) and after 15 min, excess of iodine was decomposed with ethanethiol. The mixtures were evaporated to dryness, dissolved in methylene chloride (15 mL), extracted with sat. NaHCO₃ (2 x 10 mL) and purified by a short-column silica gel chromatography using a linear gradient of

Compound	Chemical shift ^a (in ppm)	¹ J _{PH} (Hz) ^b	³ Ј _{РН} (Нz) ^b
1	2.89	628.5 (d)	9.3 (d)
3	123.3	_	9.2 (d)
4 a	134.4	-	8.7 (p. q)
4b	129.5	-	9.2 (p. q)
4 c	132.1	-	9.3 (p. sex)
4d	131.9	-	(m)
5c	138.9	-	(m)
5d	139.0	-	(m)
6a	16.8	-	8.6 (p. sex)
6 b	-8.4	-	(m)
6с	2.2	-	4.7; 7.6 (dqui)
6d	1.8	-	(m)
7c	1.4	-	(m)
7d	1.5	-	(m)
8a	1.7	-	(m)
8c	0.4	-	7.4 (p. q)
8d	0.4	-	5.6 (p. q)
10a	8.6	714.7 (d)	7.6 (p. q)
10b	7.9; 8.0	703.5 (d)	8.4 (2 p. q)
10c	7.7	701.7 (d)	5.5; 7.4 (dt)
10d	7.7; 7.8	701.1 (d)	6.0; 7.7 (2q)
12	23.1	715.6 (d)	10.4 (qui)
14	6.1	628.4 (d)	9.7 (t)
15	8.8	694.3 (d)	9.2 (p. qui)

TABLE 1. The ³¹P NMR data of some intermediates and the final products

^a Spectra in pyridine (2% H_3PO_4 in D_2O as external reference).

^b Abbreviations: d, doublet; dt, doublet of triplets; dqui, doublet of quintets; m, multiplet; p. q, pseudo quartet; p. qui, pseudo quintet; p. sex, pseudo sextet; qui, quintet.

methanol in methylene chloride. Precipitation from hexane – diethyl ether (1:1, v/v) furnished white powders (purity > 97%, ¹H NMR).

[5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yloxyJ-2-oxo-1,3,2-dioxaphosphinane 6b. Yield 63%. R_T : 1.33 (system A), 1.38 (system B). δ_H (CDCl₃; Me₄Si) 1.46 (3H, s, 5-CH₃), 1.70 and 2.20 (2H, 2m, CH₂CH₂CH₂), 2.50 and 2.66 (2H, 2 m, 2'- & 2"-H), 3.49 (2H, m, 5'- & 5"-H), 3.75 (6H, s, OCH₃), 4.29 (4H, m, POCH₂), 4.33 (1H, m, 4'-H), 5.26 (1H, m, 3'-H), 6.56 (1H, m, 1'-H), 6.85 (4H, d, *J* 8.7, 3, 3', 5, 5'-H of DMTr), 7.23–7.44 (9H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.57 (1H, br s, 6-H) and 8.60 (1H, br s, N³H, exch. with D₂O). HRMS [MH]⁺ found 665.2296. C₃₄H₃₈N₂O₁₀P requires 665.2264. For ³¹P NMR data, see Table 1.

[5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yloxyJ-2-oxo-1,3,2-dioxaphosphepane 6c. Yield 61%. R_T : 1.34 (system A), 1.39 (system B). $\delta_H(300 \text{ MHz}; \text{CDCl}_3; \text{ Me}_4\text{Si})$ 1.41 (3H, s, 5-CH₃), 1.89 (4H, m, CH₂CH₂CH₂CH₂), 2.43 and 2.62 (2H, 2 m, 2'- & 2"-H), 3.47 (2H, m, 5'- & 5"-H), 3.76 (6H, s, OCH₃), 4.13 (4H, m, POCH₂), 4.30 (1H, m, 4'-H), 5.30 (1H, m, 3'-H), 6.54 (1H, m, 1'-H), 6.84 (4H, d, *J* 8.1, 3, 3', 5, 5'-H of DMTr), 7.27–7.42 (9H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.57 (1H, br s, 6-H) and 8.61 (1H, br s, N³H, exch. with D₂O). HRMS [MH]⁺ found 679.2448. C₃₅H₄₀N₂O₁₀P requires 679.2421. For ³¹P NMR data, see Table 1.

[5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yloxy]-2-oxo-1,3,2-dioxaphosphocane 6d. Yield 52%. R_T : 1.34 (system A), 1.38 (system B). δ_{ll} (CDCl₃; Me₄Si) 1.41 (3H, s, 5-CH₃), 1.73 (4H, m, POCH₂<u>CH₂</u>), 1.90 (2H, m, POCH₂CH₂<u>CH₂</u>), 2.40 and 2.65 (2H, 2 m, 2'- & 2"-H), 3.46 (2H, m, 5'- & 5"-H), 3.78 (6H, s, OCH₃), 4.06 (4H, m, POCH₂), 4.29 (1H, m, 4'-H), 5.22 (1H, m, 3'-H), 6.51 (1H, m, 1'-H), 6.84 (4H, d, J 9.0, 3, 3', 5, 5'-H of DMTr), 7.26–7.41 (9H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.56 (1H, br s, 6-H) and 8.61 (1H, br s, N³H, exch. with D₂O). HRMS [MH]⁺ found 693.2599. C₃₆H₄₂N₂O₁₀P requires 693.2577. For ³¹P NMR data, see Table 1. (B) Approach involving condensation with the aid of 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane 9. The *H*-phosphonate 1 (1 mmol) and diol 2 (1.05 equiv.) dissolved in pyridine (5 mL) were treated with NEP-Cl 9 (2.5 equiv.) during 10 min. To this was added water to a final concentration of 5% and the reaction mixture was oxidised with iodine (1.05 equiv.) for 15 min. Further work-up as in approach (A). White powders (purity > 97%; ¹H NMR).

[5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yloxy]-2-oxo-1,3,2-dioxaphosphinane 6b. Yield 58%. For analytical data, see approach (A).

[5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yloxy]-2-oxo-1,3,2-dioxaphosphepane 6c. Yield 68%. For analytical data, see approach (A).

[5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yloxy]-2-oxo-1,3,2-dioxaphosphocane 6d. Yield 45%. For analytical data, see approach (A).

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-thymidin-3'-yl 2-hydroxyethyl phosphate TEAH⁺ 8a. To the reaction mixture containing phospholane 4a (see above) was added water (1.5 equiv.) and iodine (1.05 equiv.). After 15 min the amount of water was increased to 10% and the reaction mixture was worked-up as above. Pure phosphate 8a was isolated by short-column chromatography on silica gel using a linear gradient of methanol in dichloromethane containing 1% of triethylamine. The product was precipitated from hexane – diethyl ether 1:1 and dried *in vacuo*. Yield 41%. R_{TPH} : 0.31 (system C), 0.41 (system D). δ_H (CDCl₃; Me₄Si) 1.31 [9H, t, J 7.5, N(CH₂CH₃)₃] 1.35 (3H, s, 5-CH₃), 2.36 and 2.67 (2H, 2 m, 2'- & 2"-H), 3.06 [6H, q, J 7.2, N(CH₂CH₃)₃], 3.42 (2H, m, 5'- & 5"-H), 3.70 (2H, t, J 4.2 <u>CH₂OH</u>), 3.78 (6H, s, OCH₃), 3.94 (2H, m, POCH₂), 4.30 (1H, m, 4'-H), 5.04 (1H, m, 3'-H), 6.41 (1H, m, 1'-H), 6.83 (4H, d, J 9.0, 3, 3', 5, 5'-H of DMTr), 7.25–7.40 (9H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.60 (1H, br s, 6-H). HRMS [MH–TEA]⁺ found 699.2234. C₃₃H₃₈N₂O₁₁P requires 699.2213. For ³¹P NMR data, see Table 1.

General procedure for the synthesis of nucleoside hydroxyalkyl phosphates 8c-d.

The syntheses were carried out essentially as described above for the preparation of cyclic triesters of type 6 [approach (B)] with the exception that oxidation was carried out with iodine in 10% aqueous pyridine.

5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yl 4-hydroxybutyl phosphate TEAH⁺, 8c.

Yield: 71%. R_{TF} 0.33 (system C), 0.45 (system D). δ_{H} (300 MHz; CDCl₃; Me₄Si) 1.29 [9H, t, *J* 7.5, N(CH₂<u>CH₃</u>)₃] 1.34 (3H, s, 5-CH₃), 1.48 (2H, m, <u>CH₂</u>CH₂OH), 1.80 (2H, m, POCH₂<u>CH₂</u>), 2.39 and 2.49 (2H, 2 m, 2'- & 2"-H), 3.11 [6H, q, *J* 7.2, N(<u>CH₂</u>CH₃)₃], 3.34 and 3.45 (2H, m, 5'- & 5"-H), 3.57 (2H, t, *J* 4.1 <u>CH₂</u>OH), 3.82 (6H, s, OCH₃), 4.03 (2H, m, POCH₂), 4.20 (1H, m, 4'-H), 5.01 (1H, m, 3'-H), 6.52 (1H, m, 1'-H), 6.78 (4H, d, *J* 9.0, 3, 3', 5, 5'-H of DMTr), 7.22–7.41 (9H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.65 (1H, br s, 6-H). HRMS [MH–TEA]⁺ found 697.2575. C₃₅H₄₂N₂O₁₁P requires 697.2526. For ³¹P NMR data, see Table 1.

5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yl 5-hydroxypentyl phosphate TEAH⁺, 8d.

Yield: 75%. R_{TF} 0.38 (system C), 0.50 (system D). δ_H (CDCl₃; Me₄Si) 1.29 [9H, t, *J* 7.5, N(CH₂<u>CH</u>₃)₃], 1.39 (2H, m, CH₂CH₂CH₂CH₂CH₂), 1.44 (3H, s, 5-CH₃), 1.53 (2H, m, <u>CH</u>₂CH₂OH), 1.78 (2H, m, POCH₂<u>CH</u>₂), 2.38 and 2.46 (2H, 2 m, 2'- & 2"-H), 3.21 [6H, q, *J* 7.2, N(C<u>H</u>₂CH₃)₃], 3.33 and 3.48 (2H, m, 5'- & 5"-H), 3.61 (2H, t, *J* 4.1 <u>CH</u>₂OH), 3.78 (6H, s, OCH₃), 4.10 (2H, m, POCH₂), 4.22 (1H, m, 4'-H), 5.12 (1H, m, 3'-H), 6.49 (1H, m, 1'-H), 6.73 (4H, d, *J* 9.0, 3, 3', 5, 5'-H of DMTr), 7.20–7.39 (9H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.66 (1H, br s, 6-H). HRMS [MH–TEA]⁺ found 711.2701. C₃₆H₄₄N₂O₁₁P requires 711.2682. For ³¹P NMR data, see Table 1.

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