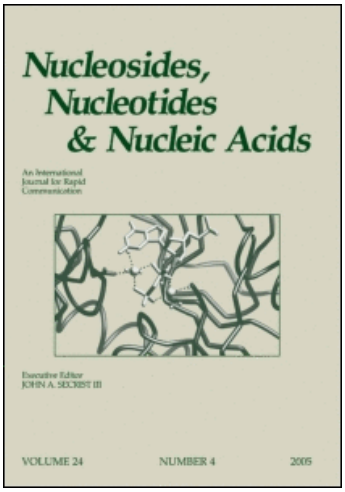

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Michal Sobkowski[a]; Malgorzata Wenska[a]; Adam Kraszewski[a]; Jacek Stawiński[ab]
[a] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland [b] Department of Organic Chemistry, Stockholm University, Arrhenius Laboratory, Stockholm, Sweden

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

Michał Sobkowski[b], Małgorzata Wenska[b], Adam Kraszewski[b,c] * and Jacek Stawiński[b,d]
[b]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland
[d]Stockholm University, Arrhenius Laboratory, Department of Organic Chemistry, S-10691, Stockholm, Sweden

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

[c] e-mail: akad@ibch.poznan.pl

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 diesters[15] 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

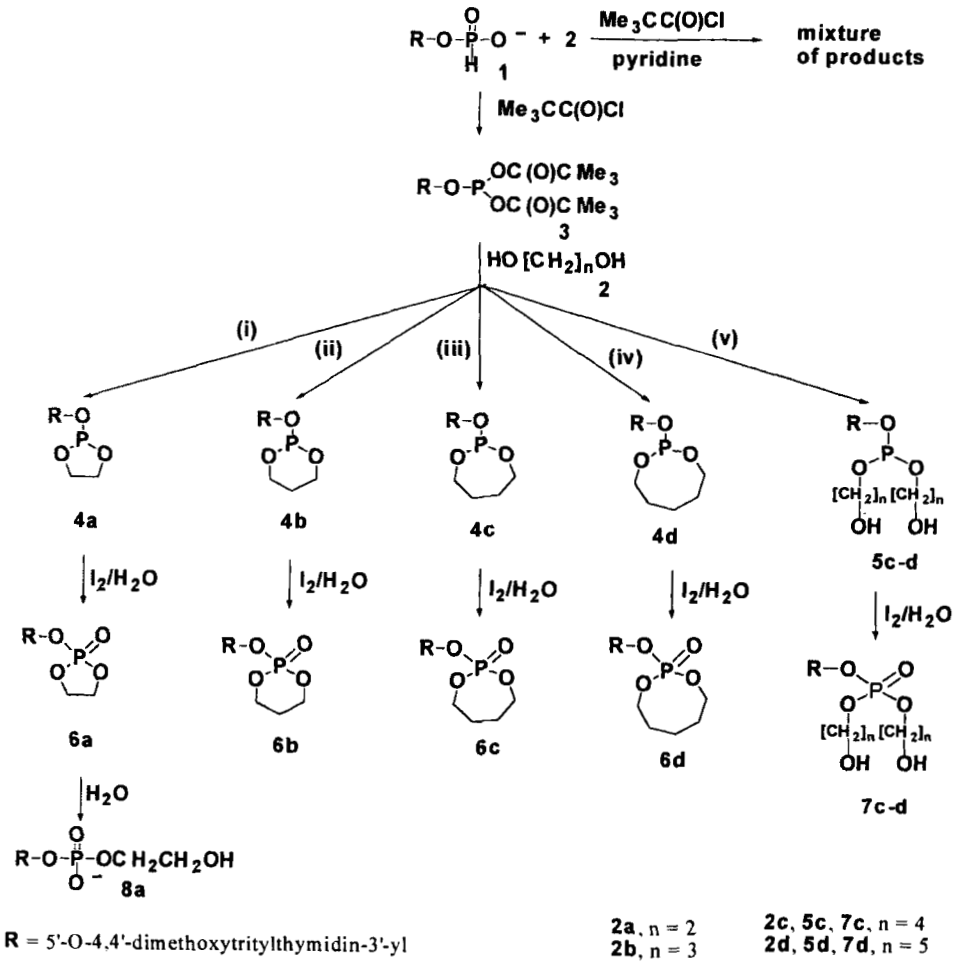
intermediates for dephosphonylation of nucleoside *H*-phosphonate monoesters[16-18] and as a convenient model system for studying some mechanistic aspects of ribozymes catalysed reactions.[19]

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 alcohols[8] 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 conditions[20]) afforded (< 3 min) complicated mixtures of products (^{31}P 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 δ_P ~23 ppm appeared probably indicating a spontaneous cyclisation[14] 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 O-acylation 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] ^{31}P NMR spectroscopy permitted their tentative identification. The assignment of ^{31}P 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 angles[24] 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 (δ_P ~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 pattern[25] (Table 1) to 1,3,2-dioxaphospholanes **4a** and 1,3,2-dioxaphosphinane **4b**,[26] respectively.

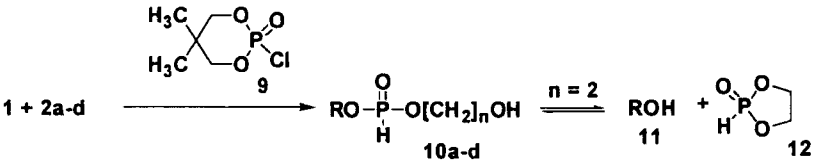
The exclusive formation of cyclic species in these reactions indicated a strong tendency to cyclisation of the initially formed hydroxyalkyl phosphites, and this trend was also observed when 1,4-butanediol and 1,5-pentanediol were used for the reaction with bispivaloyl phosphite **3**. However, in these instances, the respective cyclic phosphites **4c** (δ_P = 132.1 ppm) and **4d** (δ_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** (δ_P = 138.9 ppm) and **5d** (δ_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 pyridine[27] and progress of the reaction was followed by ^{31}P NMR spectroscopy. For phosphites **4b-d**, the integrity of ring systems was preserved and the corresponding phosphinane **6a** (δ_P = - 8.4 ppm), phosphepane **6c** (δ_P = 2.2 ppm) and phosphocane **6d** (δ_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** (δ_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** (δ_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 *H*-phosphonate 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]



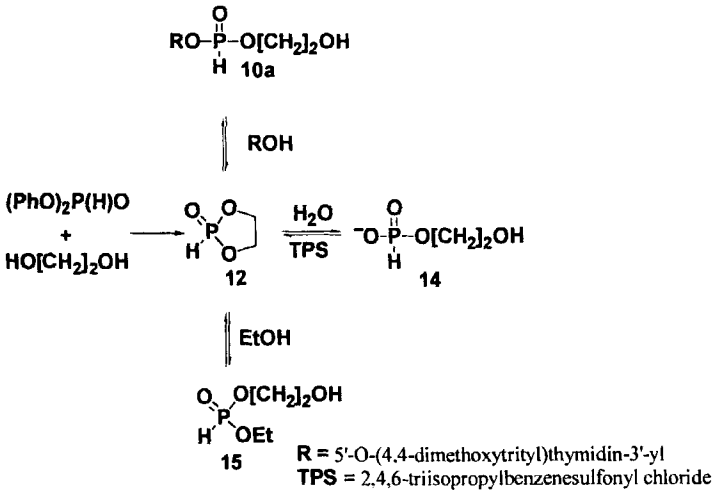
R = 5'-O-(4,4-dimethoxytrityl)thymidin-3'-yl
10a, n = 2; **10b**, n = 3; **10c**, n = 4; **10d**, n = 5

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 (^{31}P 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%, δ_P = 8.6 ppm), while the main phosphorus-containing species was a compound resonating at δ_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).



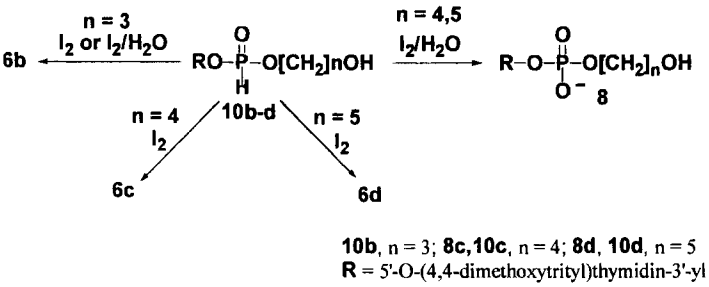
SCHEME 3

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 (^{31}P NMR spectroscopy) phospholane **12** into *H*-phosphonate diester **15** (δ_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 water (10 equiv) caused an immediate hydrolysis of **12** with the formation of 2-hydroxyethyl *H*-phosphonate **14** (δ_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).



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 intermediate[27] 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 iodine[8] 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-oxazaphospholidine[8], while 2-hydroxyethyl *H*-phosphonate **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 pyrophosphates[8]. 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 iodine 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

^{1}H and ^{31}P spectra were recorded on a Varian Unity 300 BB VT spectrometer. The ^{31}P 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% H_3PO_4 in D_2O (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 F_{254} 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 ^{1}H, ^{31}P NMR, and HRMS spectroscopy, and their purity was assessed by ^{1}H NMR spectroscopy. 5'-*O*-Dimethoxytritylthymidine 3'-*H*-phosphonate **1**[32] and 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane **9**[29] 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; 2-hydroxyethyl *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 $NaHCO_3$. The organic layer was separated, dried with anhydrous Na_2SO_4 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 ($CDCl_3$; Me_4Si) 1.71 (3H, s, 5-CH_3), 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, OCH_3), 4.22 (4H, m, $POCH_2$), 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, N^3H, exch. with D_2O). FAB MS $[MH]^+$ found 635. $C_{33}H_{36}N_2O_9P$ requires 635.6. For ^{31}P 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. $NaHCO_3$ (2 x 10 mL) and purified by a short-column silica gel chromatography using a linear gradient of

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

Compound	Chemical shift[a] (in ppm)	$^{1}J_{PH}$ (Hz)[b]	$^{3}J_{PH}$ (Hz)[b]
1	2.89	628.5 (d)	9.3 (d)
3	123.3	–	9.2 (d)
4a	134.4	–	8.7 (p. q)
4b	129.5	–	9.2 (p. q)
4c	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)
6b	-8.4	–	(m)
6c	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)

[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%, ^{1}H NMR).

[5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yloxy]-2-oxo-1,3,2-dioxaphosphinane **6b.** Yield 63%. R_T: 1.33 (system A), 1.38 (system B). δ_H ($CDCl_3$; Me_4Si) 1.46 (3H, s, 5-CH_3), 1.70 and 2.20 (2H, 2m, $CH_2\underline{CH_2}CH_2$), 2.50 and 2.66 (2H, 2 m, 2'- & 2"-H), 3.49 (2H, m, 5'- & 5"-H), 3.75 (6H, s, OCH_3), 4.29 (4H, m, $POCH_2$), 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, N^3H, exch. with D_2O). HRMS $[MH]^+$ found 665.2296. $C_{34}H_{38}N_2O_{10}P$ requires 665.2264. For ^{31}P NMR data, see Table 1.

[5'-O-(4,4'-Dimethoxytrityl)-thymidin-3'-yloxy]-2-oxo-1,3,2-dioxaphosphepane **6c.** Yield 61%. R_T: 1.34 (system A), 1.39 (system B). δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.41 (3H, s, 5-CH_3), 1.89 (4H, m, $CH_2\underline{CH_2}\underline{CH_2}CH_2$), 2.43 and 2.62 (2H, 2 m, 2'- & 2"-H), 3.47 (2H, m, 5'- & 5"-H), 3.76 (6H, s, OCH_3), 4.13 (4H, m, $POCH_2$), 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, N^3H, exch. with D_2O). HRMS $[MH]^+$ found 679.2448. $C_{35}H_{40}N_2O_{10}P$ requires 679.2421. For ^{31}P 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_H ($CDCl_3$; Me_4Si) 1.41 (3H, s, 5-CH_3), 1.73 (4H, m, $POCH_2\underline{CH_2}$), 1.90 (2H, m, $POCH_2CH_2\underline{CH_2}$), 2.40 and 2.65 (2H, 2 m, 2'- & 2"-H), 3.46 (2H, m, 5'- & 5"-H), 3.78 (6H, s, OCH_3), 4.06 (4H, m, $POCH_2$), 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, N^3H, exch. with D_2O). HRMS $[MH]^+$ found 693.2599. $C_{36}H_{42}N_2O_{10}P$ requires 693.2577. For ^{31}P 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%; ^{1}H 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 ($CDCl_3$; Me_4Si) 1.31 [9H, t, *J* 7.5, $N(CH_2\underline{CH_3})_3$] 1.35 (3H, s, 5-$CH_3$), 2.36 and 2.67 (2H, 2 m, 2'- & 2"-H), 3.06 [6H, q, *J* 7.2, $N(\underline{CH_2}CH_3)_3$], 3.42 (2H, m, 5'- & 5"-H), 3.70 (2H, t, *J* 4.2 $\underline{CH_2}OH$), 3.78 (6H, s, OCH_3), 3.94 (2H, m, $POCH_2$), 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. $C_{33}H_{38}N_2O_{11}P$ requires 699.2213. For ^{31}P 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; $CDCl_3$; Me_4Si) 1.29 [9H, t, *J* 7.5, $N(CH_2\underline{CH_3})_3$] 1.34 (3H, s, 5-$CH_3$), 1.48 (2H, m, $\underline{CH_2}CH_2OH$), 1.80 (2H, m, $POCH_2\underline{CH_2}$), 2.39 and 2.49 (2H, 2 m, 2'- & 2"-H), 3.11 [6H, q, *J* 7.2, $N(\underline{CH_2}CH_3)_3$], 3.34 and 3.45 (2H, m, 5'- & 5"-H), 3.57 (2H, t, *J* 4.1 $\underline{CH_2}OH$), 3.82 (6H, s, OCH_3), 4.03 (2H, m, $POCH_2$), 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. $C_{35}H_{42}N_2O_{11}P$ requires 697.2526. For ^{31}P 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 ($CDCl_3$; Me_4Si) 1.29 [9H, t, *J* 7.5, $N(CH_2\underline{CH_3})_3$], 1.39 (2H, m, $CH_2CH_2\underline{CH_2}CH_2CH_2$), 1.44 (3H, s, 5-$CH_3$), 1.53 (2H, m, $\underline{CH_2}CH_2OH$), 1.78 (2H, m, $POCH_2\underline{CH_2}$), 2.38 and 2.46 (2H, 2 m, 2'- & 2"-H), 3.21 [6H, q, *J* 7.2, $N(\underline{CH_2}CH_3)_3$], 3.33 and 3.48 (2H, m, 5'- & 5"-H), 3.61 (2H, t, *J* 4.1 $\underline{CH_2}OH$), 3.78 (6H, s, OCH_3), 4.10 (2H, m, $POCH_2$), 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. $C_{36}H_{44}N_2O_{11}P$ requires 711.2682. For ^{31}P NMR data, see Table 1.

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