Studies on Reactions of Nucleoside H-Phosphonates with Bifunctional Reagents. Part 2. Stability of Nucleoside H-Phosphonate Diesters in the Presence of Amino Alcohols

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

H-Phosphonate diesters undergo transesterification with amino alcohols to afford as primary products the mixed and the symmetrical H-phosphonate esters. Alcohols react similarly but only in the presence of an external base or in a basic solvent. The rate and the course of transesterification strongly depend on the reaction conditions, the reactivity of the H-phosphonate diester used, and the nature of the amino alcohol.

Base-pairing properties of synthetic oligonucleotides are widely exploited in the detection of specific gene sequences, including those associated with human genetic disease, by a hybridisation technique.[1] Owing to some inherent problems connected with the handling of radioactive tracers, the molecular probes equipped with various labels (reporter groups) detectable by colorimetric, fluorescent, or enzymic methods are gaining interest as tools in molecular biology and medicine.[1,2] These applications, in turn, stimulate chemical studies directed towards efficient preparation of synthetic oligonucleotides with the ligands covalently bound to the heterocyclic bases or to the phosphate-sugar backbone.[3,4] Synthons carrying an anchor for a reporter group can be incorporated chemically[3,4] or enzymically[5,6] with the label being already on a nucleotide moiety[5] or to be attached post-synthetically[6] at the preselected position of an oligonucleotidic chain.

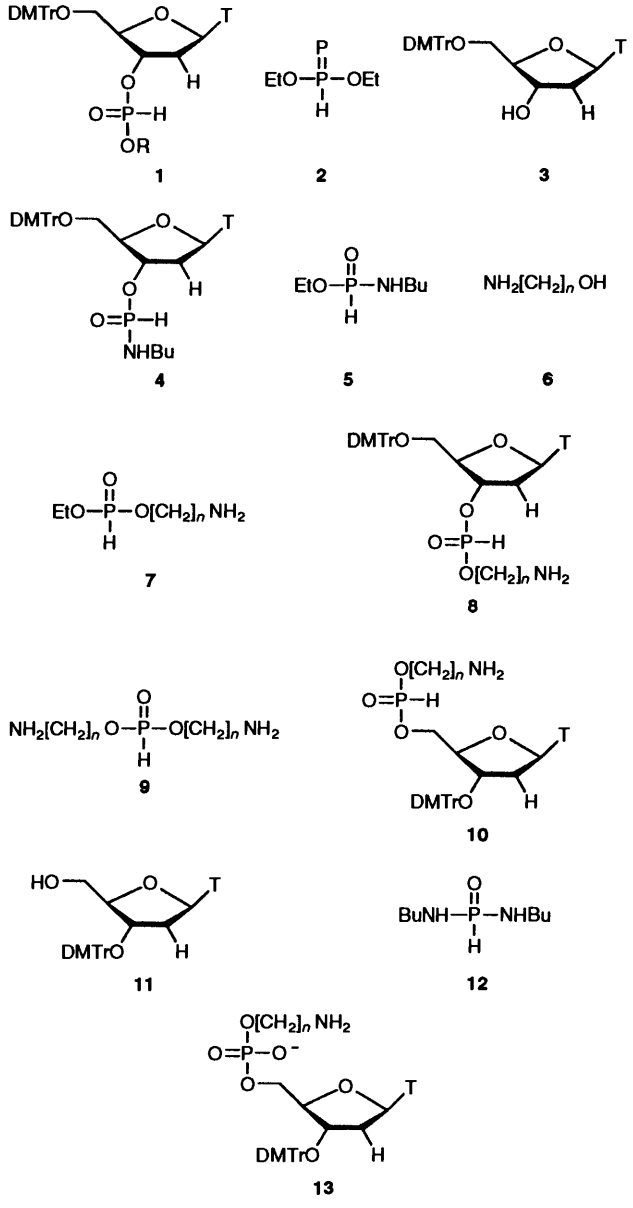
We have recently developed[7] a convenient method for functionalisation of nucleotides at the phosphorus centre *via* introduction of aminoalkyl moieties, amenable to the subsequent attachment of various reporter groups. The functionalisation was achieved by the reaction of nucleoside H-phosphonate monoesters with unprotected amino alcohols in the presence of 2-chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (NEP-Cl) as a condensing reagent. As an extension of these studies we have lately embarked on the oxidative esterification of nucleoside H-phosphonate diesters with various bifunctional reagents. Preliminary experiments with amino alcohols produced, however, variable results and strongy indicated that H-phosphonate diesters may react *per se* with these compounds (*e.g.*, to undergo transesterification) to afford, after oxidation, products different from those anticipated.

Only a few studies concerning transesterification of H-phosphonate diesters have been reported in the literature.[8–12] These involved thermal transesterification[8–11] (with or without a catalyst) of simple dialkyl H-phosphonates with higher alcohols (usually without solvent and with removal of a lower boiling alcohol by distillation) or the reactions catalysed by titanium tetraalkoxide[12] with the appropriate alcohol as solvent. Unfortunately, since the reaction conditions employed were very different from those commonly used in natural product synthesis, we found these studies to be of limited value for our purpose. Thus, to get some insight into a plausible route to the ligand-exchange process in phosphonic acid derivatives under various experimental conditions, especially those related to oligonucleotide synthesis on solid supports, we investigated in detail reactions of H-phosphonate diesters with alcohols, amines, amino alcohols, and mixtures of these reagents.

Results and Discussion

Since amino alcohols have two different nucleophilic groups (–OH and –NH_2) we tried to evaluate first their relative reactivity toward the phosphorus centre in H-phosphonate diesters by carrying out reactions with compounds having only one of these functions. To this end 5′-*O*-(4,4′-dimethoxytrityl)-thymidin-3′-yl ethyl phosphonate **1a** was treated in pyridine with, separately, ethanol and butylamine. Ethanol (10 mol equiv.) [Scheme 1, reaction (a)] reacted in pyridine very slowly and afforded, after 96 h, only ~15% of diethyl phosphonate **2** (^{31}P NMR) and the equivalent amount of the dimethoxytritylated thymidine **3** (TLC). The reaction was very sluggish and after several weeks the starting material **1a** was still present in the reaction mixture. Butylamine (10 mol equiv.) reacted in pyridine [Scheme 1, reaction (b)] much faster with the H-phosphonate diester **1a**, and after 15 h (~67% completion) four new signals (three at δ_P ~ 13 and one at δ_P ~ 7.5) were observed in the ^{31}P NMR spectrum. The relative intensities of the signals and changes in their appearance during the course of the reaction indicated that the two resonances at δ_P 13.21 and 13.34 (~13%) might belong to a one-spin system of phosphorus diastereoisomers, and the singlet at δ_P 13.00 (~37%) to a one-spin system of phosphorus enantiomers (or to an achiral phosphorus centre). These considerations, together with the chemical-shift values and multiplicity of the signals in the {^{1}H}-coupled spectra, allowed us to assign these resonances to 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′yl *N*-butylphosphonamidate **4** and to ethyl *N*-butylphosphonamidate **5**, respectively. The fourth resonance (δ_P 7.51, 17%) was assigned to diethyl hydrogen phosphite **2** (comparison with the reference compound).

The H-phosphonamidates **4** and **5** were most likely produced as a result of substitution of the ethoxy or the nucleoside group, respectively, by butylamine in the H-phosphonate diester **1a**. The formation of diethyl phosphonate **2**, however, can be explained by assuming transesterification of compound **1a** with ethanol liberated during aminolysis of the starting material. Since the amount of diester **2** increased even when the starting material completely disappeared, this may suggest that compound **4** can be transformed into diester **2** *via* compound **1a** as an intermediate. The latter assumption was substantiated by findings that ethanol (10 mol equiv.) in the presence of butylamine (10 mol equiv.) reacted with nucleoside *N*-

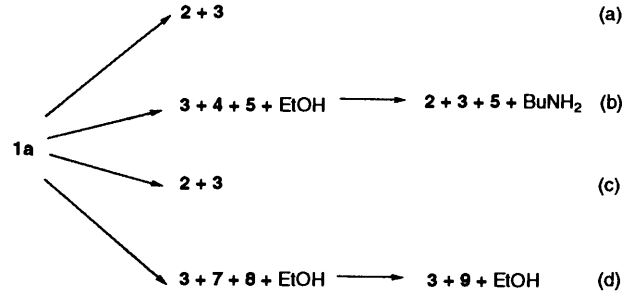


1a, R = Et
1b, R = 3′-*O*-(4,4′-dimethoxytrityl)thymidin-5′-yl
1c, R = *p*-chlorophenyl
6a–10a, **13a**, $n = 2$
6b–10b, **13b**, $n = 3$
6c–10c, **13c**, $n = 4$
6d–10d, **13d**, $n = 6$

Abbreviations:
DMTr -4,4′-dimethoxytrityl
T -thymin-1-yl

butylphosphonamidates of type **4** to afford ethyl *N*-butylphosphonamidate **5** and diethyl phosphonate **2** in the ratio 1 : 1 which remained unchanged over a period of several days.

The postulated transesterification occurring during the course of aminolysis of compound **1a** was substantially faster than the reaction **1a** + ethanol in pyridine. To clarify this point the mixture of ethanol (10 mol equiv.) and butylamine (10 mol equiv.) [Scheme 1, reaction (c)] was allowed to react with H-phosphonate **1a**. As expected, the same products were formed (compounds **2** and **3**) as with ethanol alone, but the reaction was much faster and went to completion within 10 h (^{31}P NMR, TLC). Absence of H-phosphonamidates in the reaction mixture and the observation that *N*,*O*-dialkyl H-phosphonamidates react with alcohols in pyridine rather slowly seem to indicate that the enhanced rate of transesterification of compound **1a** with ethanol in the presence of butylamine is due to base catalysis.



Scheme 1 Pathways for the reactions of the H-phosphonate diester **1a** with 10 mol equiv. of ethanol, butylamine, or various amino alcohols in pyridine. Reaction (a), EtOH; ~ 15% completion after 96 h. Reaction (b), $BuNH_2$; the starting material **1a** disappeared within 96 h. Reaction (c), $BuNH_2$, EtOH; over within 10 h. Reaction (d), $H_2N[CH_2]_nOH$; with **6a**, over within 2 h to give compounds **3**, EtOH and **9** exclusively. For substrates **6b**, **6c** and **6d** compound **1a** disappeared after 3, 8 and 8 h, respectively. After prolonged storage, the final phosphorus-containing product was the H-phosphonate **9**.

Next we investigated the reaction of H-phosphonate diester **1a** with bifunctional reagents (amino alcohols) having two nucleophilic centres spaced by different numbers of methylene groups [Scheme 1, reaction (d)]. The reaction with 2-aminoethanol **6a** in pyridine was fast and went to completion in 2 h to afford bis-(2-aminoethyl)phosphonate **9a** (^{31}P NMR) and the nucleoside **3** (TLC). Experiments with a stepwise addition of 2-aminoethanol (1–10 mol equiv.) showed that, besides compound **9a**, the unsymmetrical H-phosphonate diester **7a** (δ_P ~ 8.4) was formed, which underwent further reaction to the symmetrical H-phosphonate **9a** (δ_P ~ 9.2) with the added amino alcohol **6a**. These results might suggest that the nucleoside moiety in compound **1a** is more susceptible to transesterification than is the ethoxy group. However, additional studies (see also the reaction compound **1b** with **6a** later in the text) showed that the absence of the H-phosphonate diester **8a** in the reaction mixture was probably due to its substantially faster conversion into the product **9a** (~ 15 times faster than that of diester **7a**), and not due to chemoselectivity in the transesterification. Both compounds, **7a** and **9a**, when kept in pyridine underwent further transformations (*e.g.*, into H-phosphonate monoesters).

3-Aminopropan-1-ol (**6b**, 10 mol equiv.) reacted similarly with the H-phosphonate **1a** but slightly slower, to afford after 3 h (^{31}P NMR) two phosphorus-containing products: the H-phosphonate diester **7b** (δ_P ~ 8.0) and the symmetrical H-phosphonate diester **9b** (δ_P ~ 8.4) (ratio 1 : 1). Monitoring of the progress of the reaction in time showed that, after 80 min, 73% of the substrate **1a** was converted into three products: **7b** (40%), **9b** (15%) and 3-aminopropyl 5′-*O*-(4,4′-dimethoxytrityl)-thymidin-3′-yl phosphonate diester **8b** (18%, two singlets at δ_P ~ 7.8). The presence of the latter species in the reaction mixture may indicate that the transesterification is not selective (the nucleoside *vs* the ethoxy group) in this instance as well.

For higher amino alcohols the rates of reactions with the H-phosphonate **1a** approached those of the equimolar mixtures of alcohols and amines. Thus, 4-aminobutan-1-ol **6c** or 6-aminohexan-1-ol **6d** reacted with compound **1a** with rates close to that of the ethanol and butylamine mixture (*vide supra*) to afford, after 5 h, three H-phosphonate diesters: **7c** (70%), **9c** (22%), **8c** (8%) and **7d** (51%), **9d** (35%), **8d** (14%), respectively. Upon prolonged storage of the reaction mixtures (several days), the symmetrical H-phosphonate diesters (compounds **9c** and **9d**, respectively) became the final products of the transesterification. For the H-phosphonate diesters bearing 4-aminobutyl groups (**7c**, **8c** and **9c**), similarly to the 2-aminoethyl derivatives **7a** and **9a** (*vide supra*), decomposition toward H-phosphonate monoesters was observed during this time. In all instances,

compound **3** was the only nucleosidic material detected in the reaction mixtures.

The decrease in reactivity of amino alcohols towards H-phosphonate diester **1a**, going from 2-aminoethanol to 6-aminohexan-1-ol, can be explained by an electron-withdrawing effect exerted by the protonated amino function ($-NH_3^+$). In 2-aminoethanol this effect is most pronounced owing to the close vicinity of the hydroxy and the amino groups and may result in generation, in the reaction mixture, of reactive zwitterionic species. This probably makes compound **6a** most reactive despite the lower basicity of its amino function ($pK_a \sim 9.51$[13]) compared with that the other amino alcohols ($pK_a \sim 9.96$[13] for **6b**, 10.35[13] for **6c** and 10.60[13] for **6d**). When the amino and the hydroxy groups in amino alcohols are separated by more than three methylene groups (*e.g.*, 4-aminobutan-1-ol **6c** and 6-aminohexan-1-ol **6d**), the inductive effect of the protonated amino function ceases and the reactivities of such bifunctional reagents approach those of equimolar mixtures and amines.

To test the correctness of this interpretation we investigated transesterification of the H-phosphonate **1a** with amino alcohols in the presence of triethylamine (TEA, $pK_a \sim 11.01$). Addition of an external strong amine should result in deprotonation of the $-NH_3^+$ function of the amino alcohol (a change from the electron-withdrawing to the electron-donating group) and also should increase ionisation of the hydroxy group. For 2-aminoethanol these two phenomena will have contradictory effects. Strong base may increase reactivity of compound **6a** owing to more efficient base catalysis, but this will be offset, or more likely exceeded, by the decrease in reactivity of the hydroxy group due to deprotonation of the vicinal $-NH_3^+$ group. The latter process should not affect, however, reactivity of the hydroxy group in compound **6d**. Thus, for 2-aminoethanol **6a** there should be a net decrease in the rate of transesterification while for 6-aminohexan-1-ol **6d** an enhanced reactivity toward compound **1a** is expected. Indeed, transesterification of the H-phosphonate **1a** in pyridine in the presence of TEA (10 mol equiv.) with 2-aminoethanol **6a** (10 mol equiv.) was found to be 1.5 times slower than the corresponding reaction in the absence of strong base, while a similar reaction for 6-aminohexan-1-ol **6d** (10 mol equiv.) in the presence of TEA was twice as fast.

Since the reactions of H-phosphonate diesters with amino alcohols can be relevant to modification of oligonucleotides on solid supports we decided to investigate also the susceptibility of a dinucleoside H-phosphonate diester (the simplest model of oligonucleoside H-phosphonates) to transesterification.

1b + **6a–d** ⟶ **3** + **8** + **10** + **11** ⟶ **3** + **9a–d** + **11**

Scheme 2 Pathway for the reactions of the dinucleoside H-phosphonate diester **1b** with 10 mol equiv. of amino alcohols **6a–d** in pyridine compound **1b** reacted completely to give products **9a–d** within 15 min for **6a**, 2 h for **6b**, 3 h for **6c** and 5 h for **6d**

To this end the dinucleoside H-phosphonate diester **1b** [3′-*O*-(4,4′-dimethoxytrityl)thymidin-5′-yl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphonate] (Scheme 2) was allowed to react with 2-aminoethanol (10 mol equiv.) in pyridine and the progress of the reaction was followed by ^{31}P NMR spectroscopy. It was found that compound **1b** reacted faster than compound **1a** with 2-aminoethanol **6a** and after 15 min only one phosphorus-containing compound, the symmetrical H-phosphonate **9a** (^{31}P NMR) and two nucleosidic products, **3** and **11** (TLC analysis) were detected in the reaction mixture. The ^{31}P NMR spectra recorded at the early stages of the reaction (after *ca.* 5 min) revealed the presence of the starting material **1b** (40%), two nucleoside alkyl H-phosphonate diesters **8a** and **10a** (19 and 23%, respectively), and the symmetrical H-phosphonate **9a** (18%). Formation of compounds **8a** and **10a** in approximately equal amounts indicated essentially a lack of chemoselectivity in the transesterification of compound **1b**.

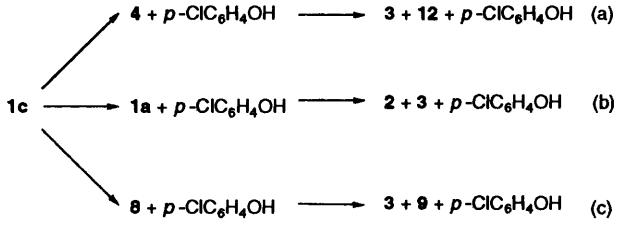
The reaction of compound **1b** with other amino alcohols **6b–d** proceeded similarly *via* the nucleoside alkyl H-phosphonate diesters **8b–d** and **10b–d** to the appropriate final products, the symmetrical H-phosphonate diesters **9b–d**. These reactions were also faster (2 h for **9b**, 3 h for **9c** and 5 h for **9d**) than those of the H-phosphonate **1a** with amino alcohols **6b–d**.

The substantial differences in rates of transesterification between compounds **1a**, **1b** and **2** seem to indicate changes in electrophilicity of the phosphorus centre in these compounds depending on the nature of the alkyl groups. For instance, 2-aminoethanol **6a** (10 mol equiv.) reacted in pyridine with the dinucleoside H-phosphonate **1b** and the nucleoside ethyl H-phosphonate diester **1a** within 15 and 120 min, respectively while the reaction with diethyl phosphonate **2** required a few weeks to go to completion. This can be the reason for a particular susceptibility of dinucleoside H-phosphonate diesters to transesterification with amino alcohols, although to explain this in detail further studies are needed.

The reaction of H-phosphonate diesters with primary amines or hydroxylic compounds can be of potential synthetic value for the preparation of H-phosphonamidates or asymmetrical H-phosphonate diesters, respectively. To achieve this, however, rather high chemoselectivity in the transesterification is required. For this purpose we investigated the reactions of the nucleoside aryl H-phosphonate diester [*p*-chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphonate] **1c** with alcohols and amines, assuming that the aryl group should undergo much faster replacement than the nucleoside moiety.

To this end the H-phosphonate diester **1c** was allowed to react with butylamine (5 mol equiv.) in pyridine [Scheme 3, reaction (a)]. The reaction was rapid (5 min) and afforded quantitatively the nucleoside H-phosphonamidate **4** (^{31}P NMR) as the sole product ($\delta_P \sim 13$). This compound with the added butylamine (10 mol equiv.) underwent slowly further aminolysis towards dibutylphosphonodiamidate **12** (70 h, ~ 50% completion).

Ethanol (2 mol equiv.) also reacted with compound **1c** [Scheme 3, reaction (b)] within several minutes to afford the



Scheme 3 Pathways for the reaction of the nucleoside *p*-chlorophenyl H-phosphonate **1c** with 5 mol equiv. of butylamine, 2 mol equiv. of ethanol and 1 mol equiv. of various amino alcohols in pyridine. Reaction (a), $BuNH_2$; over within 5 min. With an additional 10 mol equiv. of butylamine the half-life of aminolysis to H-phosphonodiamidate **12** was 76 h. Reaction (b), EtOH; over within 5 min. With 10 mol equiv. of ethanol the final phosphorus-containing product was the H-phosphonate diester **2**. Reaction (c), $H_2N[CH_2]_nOH$; over within 5 min. With 10 mol equiv. of **6a–d** the final phosphorus-containing product was the H-phosphonate diester **9**.

H-phosphonate diester **1a**. This compound, as described above, reacted further with an excess of ethanol (10 mol equiv.) to give diethyl phosphonate **2** and the nucleoside **3**.

Stoichiometric amounts of amino alcohols **6a–d** and compound **1c** reacted in pyridine quantitatively (*ca.* 5 min) to the respective nucleoside H-phosphonate diesters **8a–d** [Scheme 3, reaction (c)]. With an excess of amino alcohols (10 mol equiv.) the reaction proceeded further to give the symmetrical

Table 1 ^{31}P NMR data of the intermediates and the final products

Compound	Chemical shift[a] (δ_P)	$^1J_{PH}$ (Hz)	$^3J_{PH}$ (Hz)
1a	7.44, 7.50	704.5	8.3[b]
1b	8.11, 9.47	715.5, 714.7	8.4[b]
1c	4.58	734.1	8.4[c]
4	13.21, 13.34	640.5, 638.6	11.2[d]
5	12.96	618.8	10.2[d]
7a	8.44	692.4	9.3[d]
7b	8.02	687.7	9.2[d]
7c	7.83	687.7	9.2[d]
7d	7.86	686.8	9.2[d]
9a	9.21	698.9	8.8[d]
9b	8.44	688.7	8.4[d]
9c	8.10	686.8	8.3[d]
9d	8.13	686.9	8.3[d]
10a	9.13, 10.04	707.1, 706.3	8.4[d]
10b	8.71, 9.64	688.7, 699.7	8.4[d]
10c	8.47, 9.43	699.8, 691.5	8.3[d]
10d	8.47, 9.44	699.2, 698.9	8.3[d]
12	11.01	556.15	[e]
13a	1.43		[e]
13b	1.25		[e]
13c	−0.57		[e]
13d	−0.40		[e]

[a] Spectra in pyridine with heteronuclear decoupling (2% H_3PO_4 in D_2O as external reference). [b] Doublet of quartets. [c] Doublet of doublets. [d] Doublet of quintets. [e] Multiplet. ^{31}P NMR data for nucleoside H-phosphonate diesters of type **8** have already been published.[7]

H-phosphonate diesters of type **9**, similarly as in the transesterification of compounds **1a** and **1b**. It is worth noticing that despite the high reactivity of compound **1c** toward amines, no formation of H-phosphonamidates was observed in these reactions. Lack of significant differences in the reactivity between 2-aminoethanol **6a** and higher homologues of amino alcohols (compounds **6b–d**) in the transesterification of compound **1c**, in contrast to the reactions with the analogues **1a** or **1b**, is probably due to the enhanced electrophilicity of the phosphorus centre in compound **1c** caused by the presence of an electron-withdrawing aryl group.

In conclusion, H-phosphonate diesters may react with amines, alcohols, and amino alcohols to afford H-phosphonamidates and symmetrical H-phosphonate diesters, respectively. The rate and course of aminolysis and transesterification depend on the reaction conditions, reactivity of the H-phosphonate diester and the nature of the amino alcohol. These one should bear in mind when designing chromatographic conditions for separation of H-phosphonate diesters or when oligonucleotides containing internucleosidic H-phosphonate bonds are subjected to various reactions on a solid support (*e.g.*, during modification of oligonucleotides at phosphorus centres). On the other hand, the transesterification of nucleoside alkyl H-phosphonates may be of some synthetic value as a means for removal of the phosphonyl group from nucleoside H-phosphonates (dephosphonoylation) under mild conditions. It seems that further exploration of nucleoside aryl H-phosphonate diesters may extend synthetic applications of H-phosphonates[14] in nucleotide chemistry, *e.g.*, by providing a convenient entry to nucleoside H-phosphonamidates or to internucleotidic bond formation. These studies are in progress in this laboratory.

Experimental

Materials and Methods.—^1H and ^{31}P NMR spectra were recorded on a Varian Unity BB VT spectrometer. *J*-Values are given in Hz. The ^{31}P NMR experiments were carried out in 5 mm tubes using 0.1 mmol of phosphorus-containing compounds in pyridine (0.7 cm^3). Mass spectra were recorded on a JEOL MS SX 102 spectrometer with *m*-nitrobenzyl alcohol as matrix. TLC analyses (Merck silica gel 60 F_{254} precoated plates) were carried out in saturated chambers using the following solvent systems: A, chloroform–methanol (9:1, v/v); B, chloroform–propan-2-ol (95:5, v/v); C, chloroform–methanol–TEA (80:15:5, v/v); D, propan-1-ol–water–25% aq. ammonia (85:10:5, v/v). The R_f-values reported are relative to 5′-*O*-(4,4′-dimethoxytrityl)thymidine (systems A and B), and relative to 5′-*O*-(4,4′-dimethoxytrityl)thymidine hydrogen 3′-phosphonate (systems C and D).

Pyridine (Lab Scan Ltd.) was stored over molecular sieves 4 Å until the amount of water was below 20 ppm. Ethanol (POCH) was dried by the standard procedure[15] and stored over molecular sieves 3 Å (water content below 10 ppm). The amount of water in solvents was measured with Karl Fischer coulometric titration using Metrohm 684 KF coulometer. Butylamine (Fluka) was dried with KOH, distilled and stored over molecular sieves 3 Å. 2-Aminoethanol **6a** (Fluka) and 3-aminopropan-1-ol **6b** (Aldrich) were commercial grades and were distilled before use. 4-Aminobutan-1-ol **6c** (Merck) and 6-aminohexan-1-ol **6d** (Aldrich), diethyl phosphonate **5** (Aldrich), diphenyl phosphonate (Aldrich) (all commercial grades) were used without additional purification. 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl ethyl phosphonate[16] **1a**, 3′-*O*-(4,4′-dimethoxytrityl)thymidin-5′-yl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphonate[17] **1b** and *p*-chlorophenyl 5′-*O*-(4,4′-dimethoxytrityl)thymidin-3′-yl phosphonate[18] **1c** were synthesized according to published procedures. The nucleoside aryl H-phosphonate diester **1c** was synthesized and used directly in transesterification experiments.

In most instances, the intermediates and the final products (**5**, **7–10**) of the investigated reactions were unstable compounds and it was difficult to isolate them in a pure state by column chromatography on a regular or silanised silica gel. Their chemical structures were inferred from ^{31}P NMR spectroscopy data (see Table 1) and from spectroscopic comparison with compounds **5**, **7**, **8** and **10** which were produced *in situ* by coupling of the appropriate H-phosphonate monoesters with butylamine or amino alcohols **6a–d** in the presence of NEP-Cl as described previously.[7] The aminoalkyl nucleoside H-phosphonate diesters of type **10** were oxidised and isolated as stable phosphodiesters **13a–d**. The reference compounds of type **9** and **12** were synthesized by transesterification[19] or total aminolysis of diphenyl hydrogen phosphite with amino alcohols **6a–d** or butylamine, respectively.

Each transesterification experiment was carried out on a 0.1–0.3 mmole scale and progress of the reaction was monitored with ^{31}P NMR spectroscopy. In all reactions **1a–c** + **6a–d** the corresponding nucleosidic component(s) was (were) isolated after silica gel chromatography in over 90% yield.

5′-O-(4,4′-*Dimethoxytrityl*)*thymidin*-3′-*yl* 3′-N-*Butylphosphonamidate* **4**.—The nucleoside aryl H-phosphonate diester **1c** (0.5 mmol) was treated with butylamine (0.25 cm^3, 2.5 mmol) in pyridine (5 cm^3) within 5 min. The reaction mixture was diluted with methylene dichloride (10 volume excess) and washed with brine. The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated to give a viscous oil. The product **4** was separated from polar impurities by quick filtration through a silica gel column (product **4** was unstable during chromatography as indicated by its low yield after isolation) by using methylene dichloride containing methanol (10%) as a solvent system. The appropriate fractions were evaporated, and the residue was dissolved in methylene dichloride and precipitated into hexane. The precipitate was filtered off and, after being dried *in vacuo* over molecular sieves 4 Å, *compound*

4 was obtained as a powder (0.083 g, 25%) (Found: C, 63.2; H, 6.2; N, 6.3. $C_{35}H_{42}N_3O_8P$ requires C, 63.34; H, 6.38; N, 6.33%); R_f 1.16 (system A), 1.34 (system B); $\delta_H(CDCl_3)$ 0.88 and 0.92 (3 H, 2t, *J* 7.2 and 7.5, CH_2Me), 1.24–1.51 (4 H, m, $CH_2CH_2CH_2Me$), 1.39 and 1.41 (3 H, 2d, *J* 1.2 and 5, Me), 2.43 and 2.57 (2 H, m, 2'-H_2), 2.80 and 2.89 (2 H, m, $NHCH_2CH_2$), 3.38 and 3.52 (2 H, m, 5'-H_2), 3.79 (6 H, s, OMe), 4.24 (1 H, m, 4'-H), 5.16 (1 H, m, 3'-H), 5.83 and 7.99 (1 H, 2d, *J* 646.8 and 6.48.6, PH), 6.46 (1 H, m, 1'-H), 6.84 (4 H, d, *J* 9.0 and 3-, 3'-, 5-, and 5'-H of DMTr), 7.24–7.39 (9 H, m, ArH or DMTr except 3-, 3'-, 5- and 5'-H), 7.56 and 7.58 (1 H, 2d, *J* 1.2, 6-H) and 8.6 (1 H, br m, NH, exch. with D_2O); ^{31}P NMR data: Table 1.

General Procedure for Synthesis of Monohydrochlorides of Bis(aminoalkyl) H-Phosphonate Diesters of Type **9**.—Diphenyl phosphonate (1 mol equiv.), dissolved in acetonitrile (1 mmol cm^{-3}), was treated with an amino alcohol **6a–d** (2 mol equiv.) within 5 min. Gaseous, anhydrous hydrogen chloride was passed through the solution and a crude product of type **9** (monohydrochloride as judged from the amount of AgCl precipitated after treatment with aq. $AgNO_3$) was precipitated as a viscous oil. The solution was decanted and the remaining oil obtained after washing with acetonitrile was dissolved in a minimum volume of dimethyl sulfoxide and was reprecipitated with an excess of acetonitrile (10 volume excess) to afford pure products of type **9**, again as viscous oils. The solvent was decanted, the remaining oil was washed with acetonitrile, and the residual solvent was removed by evaporation under reduced pressure.

Bis(2-aminoethyl) phosphonate **9a** (*hydrochloride*). Yield 33% [Found: MH^+, 169. $C_4H_{14}ClN_2O_3P$ requires (M^+ – Cl), 169]; $\delta_H[(CD_3)_2SO]$ 3.11 (4 H, br m, $CH_2NH_3^+$), 4.31 (4 H, m, $POCH_2$) and 5.81 and 8.23 (1 H, d, *J* 720.6, HP). For ^{31}P NMR data, see Table 1.

Bis(3-aminopropyl) phosphonate **9b** (*hydrochloride*). Yield 43% [Found: MH^+, 197. $C_6H_{18}ClN_2O_3$ requires (M^+ – Cl), 197]; $\delta_H[(CD_3)_2SO]$ 1.96 (4 H, m, $CH_2CH_2CH_2$), 2.87 (4 H, t, *J* 7.2, $CH_2NH_3^+$), 4.12 (4 H, m, $POCH_2$) and 5.73 and 8.08 (1 H, d, *J* 705.3, HP). For ^{31}P NMR data, see Table 1.

Bis(4-aminobutyl) phosphonate **9c** (*hydrochloride*). Yield 45% [Found: MH^+, 225. $C_8H_{22}ClN_2O_3$ requires (M^+ – Cl), 225]; $\delta_H[(CD_3)_2SO]$ 1.45 (4 H, m, $CH_2CH_2NH_3^+$), 1.60 (4 H, m, $POCH_2CH_2$), 2.79 (4 H, m, $CH_2NH_3^+$), 4.02 (4 H, m, $POCH_2$) and 5.71 and 8.04 (1 H, d, *J* 697.2, HP). For ^{31}P NMR data, see Table 1.

Bis(6-aminohexyl) phosphonate **9d** (*hydrochloride*). Yield 32% [Found: MH^+, 281. $C_{10}H_{26}ClN_2O_3P$ requires (M^+ – Cl), 281]; $\delta_H[(CD_3)_2SO]$ 1.33 (8 H, m, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.59 (8 H, m, $CH_2CH_2CH_2CH_2CH_2CH_2$), 2.74 (4 H, t, *J* 7.5, $CH_2NH_3^+$), 4.00 (4 H, m, $POCH_2$) and 5.67 and 7.99 (1 H, d, *J* 694.5, HP). For ^{31}P NMR data, see Table 1.

2-Aminoethyl 3'-O-(4,4'-*Dimethoxytrityl*)*thymidin*-5'-*yl Hydrogen Phosphate* **13a**.—2-Aminoethanol (0.55 mmol) was treated with trifluoroacetic anhydride (0.55 mmol) in pyridine (2.5 cm^3) and to this was added a solution of 3'-*O*-(4,4'-dimethoxytrityl)thymidin-5'-yl hydrogen phosphonate (0.50 mmol) in a minimal amount of pyridine, followed by pivaloyl chloride (1.5 mmol). After 10 min, a mixture of iodine (0.55 mmol) in 20% aq. pyridine (2.5 cm^3) was added and oxidation was continued for 15 min. Excess of iodine was decomposed with ethanethiol, the solvent was evaporated off, and the remaining oil was dissolved in pyridine (2.5 cm^3). To this was added an equal volume of 25% aq. ammonia and the reaction mixture was left overnight at room temperature. After evaporation the product **13a** was isolated by silica gel column chromatography using a linear gradient (0–10%) of methanol in methylene dichloride containing triethylamine (5%). Pure *product* **13a** was precipitated from a minimum volume of methylene dichloride into hexane–diethyl ether (1:1, v/v). The precipitate was filtered off, and dried over molecular sieves 4 Å to constant weight (0.15 g, 45%) (Found: C, 57.2; H, 6.0; N, 6.1. $C_{33}H_{38}N_3O_{10}P \cdot 1.5\ H_2O$ requires C, 57.04; H, 5.95; N, 6.05%); R_f 0.23 (system C), 0.56 (system D); $\delta_H(CDCl_3)$ 1.71 (3 H, s, Me), 1.73 (2 H, m, 2'-H_2), 2.98 (2 H, m, $CH_2NH_3^+$), 3.53 (2 H, m, $POCH_2$), 3.70 (6 H, s, OMe), 3.83 (1 H, m, 4'-H), 4.31 (1 H, m, 3'-H), 6.18 (1 H, m, 1'-H), 6.79 (4 H, d, *J* 7.2, 3-, 3'-, 5- and 5'-H of DMTr), 7.18–7.42 (9 H, m, ArH of DMTr except 3-, 3'-, 5- and 5'-H), 7.44 (1 H, s, 6-H), 8.26 (1 H, br s, N^3H, exch. with D_2O). For ^{31}P NMR data, see Table 1.

General Procedure for Synthesis of Nucleoside Aminoalkyl Phosphodiesters **13b–d**.—A 3'-*O*-(4,4'-dimethoxytrityl)-thymidin-5'-yl phosphonate (1 mol equiv.) and the appropriate amino alcohol **6b–d** (1.1 mol equiv.) were treated in pyridine (5 cm^3) with NEC-Cl (2.5 mol equiv.) during 10 min. To this mixture was added water to a final concentration of 5% and the H-phosphonate diester of type **10** was oxidised with iodine (1.05 mol equiv.) during 15 min. Excess of iodine was decomposed with ethanethiol, solvent was evaporated off, and the remaining oil was dissolved in methylene dichloride and the solution was washed with 5% aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated. Purification was carried out as described above for compound **13a**.

3-Aminopropyl 3'-O-(4,4'-*dimethoxytrityl*)*thymidin*-5'-*yl hydrogen phosphate* **13b**. 78% (Found: C, 57.55; H, 6.0; N, 6.0. $C_{34}H_{40}N_3O_{10}P \cdot 1.5\ H_2O$ requires C, 57.61; H, 6.12; N, 5.93%); R_f 0.13 (solvent C), 0.34 (solvent D); $\delta_H(CDCl_3)$ 1.78 (3 H, s, Me), 1.88 (2 H, m, 2'-H_2), 1.98 (2 H, m, $POCH_2CH_2$), 2.98 (2 H, m, $CH_2NH_3^+$), 3.54 (2 H, m, $POCH_2$), 3.73 (6 H, s, OMe), 3.78 (2 H, m, 5'-H_2), 3.85 (1 H, m, 4'-H), 6.30 (1 H, m, 1'-H), 6.79 (4 H, d, *J* 8.2, 3-, 3'-, 5- and 5'-H of DMTr), 7.18–7.42 (9 H, m, ArH of DMTr except 3-, 3'-, 5- and 5'-H), 7.44 (1 H, s, 6-H) and 8.26 (1 H, br s, exch. with D_2O). For ^{31}P NMR data, see Table 1.

4-Aminobutyl 3'-O-(4,4'-*dimethoxytrityl*)*thymidin*-5'-*yl hydrogen phosphate* **13c**. 85% (Found: C, 58.25; H, 6.2; N, 5.7. $C_{35}H_{42}N_3O_{10}P \cdot 1.5\ H_2O$ requires C, 58.15; H, 6.28; N, 5.82%); R_f 0.18 (solvent C), 0.30 (solvent D); $\delta_H(CDCl_3)$ 1.61 (2 H, m, $CH_2CH_2NH_3^+$), 1.75 (2 H, m, $POCH_2CH_2$), 1.80 (2 H, m, 2'-H_2), 1.81 (3 H, s, Me), 2.86 (2 H, m, $CH_2NH_3^+$), 3.51 (2 H, m, $POCH_2$), 3.74 (6 H, s, OMe), 3.78 (2 H, m, 5'-H_2), 3.93 (1 H, m, 4'-H), 4.32 (1 H, m, 3'-H), 6.30 (1 H, m, 1'-H), 6.80 (4 H, d, *J* 8.2, 3-, 3'-, 5- and 5'-H of DMTr), 7.17–7.46 (9 H, m, ArH of DMTr except 3-, 3'-, 5- and 5'-H), 7.48 (1 H, s, 6-H) and 8.21 (1 H, br s, N^3H, exch. with D_2O). For ^{31}P NMR data, see Table 1.

6-Aminohexyl 3'-O-(4,4'-*dimethoxytrityl*)*thymidin*-5'-*yl hydrogen phosphate* **13d**. 51% (Found: C, 58.9; H, 6.6; N, 5.7. $C_{37}H_{46}N_3O_{10}P \cdot 1.5\ H_2O$ requires C, 59.19; H, 6.58; N, 5.60%); R_f 0.17 (solvent C), 0.41 (solvent D); $\delta_H(CDCl_3)$ 1.36 (4 H, m, $POCH_2CH_2CH_2CH_2CH_2CH_2NH_3^+$), 1.48 (2 H, m, $CH_2CH_2NH_3^+$), 1.61 (2 H, m, $POCH_2CH_2$), 1.75 (2 H, m, 2'-H_2), 1.82 (3 H, s, Me), 2.81 (2 H, m, $CH_2NH_3^+$), 3.52 (2 H, m, $POCH_2$), 3.65 (2 H, m, 5'-H_2), 3.78 (6 H, s, OMe), 3.88 (1 H, m, 4'-H), 4.31 (1 H, m, 3'-H), 6.34 (1 H, m, 1'-H), 6.80 (4 H, d, *J* 8.1, 3-, 3'-, 5- and 5'-H of DMTr), 7.15–7.41 (9 H, m, ArH of DMTr except 3-, 3'-, 5- and 5'-H) and 7.53 (1 H, s, 6-H). For ^{31}P NMR data, see Table 1.

Acknowledgements

We are indebted to Prof. Per J. Garegg for his interest, and to the State Committee for Scientific Research, Republic of Poland and to the Swedish Natural Science Research Council for financial support.

References

1 J. A. Matthews and L. J. Kricka, *Anal. Biochem.*, 1988, **169**, 1.
2 R. Teoule, *Nucleosides, Nucleotides*, 1991, **10**, 129.
3 R. Kierzek and W. T. Markiewicz, *Nucleosides, Nucleotides*, 1987, **6**, 403; J. Tesler, K. A. Cruickshank and T. L. Netzel, *J. Am. Chem. Soc.*, 1989, **111**, 6966.
4 R. F. Atherton, H. T. Openshaw and A. R. Todd, *J. Chem. Soc.*, 1945, 660; O. S. Bashuk, V. F. Zarytova and A. S. Levina, *Bioorg. Khim.*, 1988, **14**, 606; S. Agrawal, *Tetrahedron Lett.*, 1989, **30**, 7025; U. Asseline, E. Bonfils, R. Kurfurst, M. Chasignol, V. Roig and N. Thuong, *Tetrahedron*, 1992, **48**, 1233.
5 I. C. Gillam and G. M. Tener, *Anal. Biochem.*, 1986, **157**, 199.
6 H. Eshaghpour, D. Soll and D. M. Crothers, *Nucleic Acids Res.*, 1979, **7**, 1485.
7 A. Kraszewski, M. Sobkowski and J. Stawiński, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1699.
8 E. Müller, *Methoden der Organischen Chemie* (*Houben-Weyl*), George Thieme Verlag, Stuttgard, 1964, vol. XII/2, p. 35.
9 K. Troev, E. Tashev and G. Borisov, *Phosphorus Sulfur*, 1982, **12**, 313.
10 K. Troev and G. Borisov, *Phosphorus Sulfur*, 1987, **29**, 129.
11 E. Tashev, S. Shenkov, K. Troev and G. Borissov, *Phosphorus Sulfur Silicon*, 1991, **56**, 225.
12 M. Froneman and T. A. Modro, *Synthesis*, 1991, 201.
13 D. D. Perrin, *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworth, London, 1965.
14 J. Stawiński, in *Handbook of Organophosphorus Chemistry*, ed. R. Engel, Marcel Dekker, New York, 1992, p. 377.
15 A. I. Vogel, *Practical Organic Chemistry*, Longmans and Green, London, 3rd edn., 1956.
16 P. J. Garegg, T. Regberg, J. Stawinski and R. Strömberg, *Nucleosides, Nucleotides*, 1987, **6**, 655.
17 F. Seela and U. Kretschmer, *J. Org. Chem.*, 1991, **56**, 3861.
18 P. J. Garegg, T. Regberg, J. Stawiński and R. Strömberg, *Chem. Scr.*, 1986, **26**, 59.
19 F. Himmelsbach and W. Pfleiderer, *Tetrahedron Lett.*, 1982, **23**, 4793.

Paper 4/00357H
Received 20*th January* 1994
Accepted 16*th March* 1994