Studies on Reactions of Nucleoside *H*-Phosphonates with Bifunctional Reagents. Part 1. Reaction with Amino Alcohols

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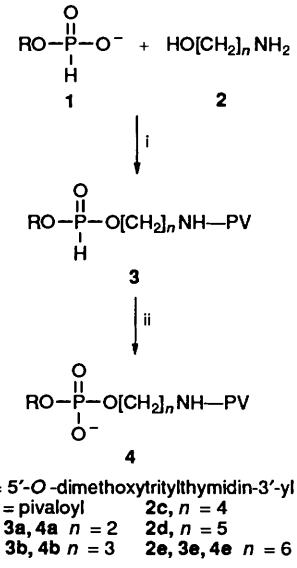
The reaction of nucleoside *H*-phosphonates with amino alcohols in the presence of condensing agents has been studied. Depending on the coupling procedure used and the length of the polymethylene bridge in the amino alcohols, different *H*-phosphonate diesters, cyclic phosphoramidite derivatives or phosphite triesters can be produced. Oxidation of these species with iodine under various experimental conditions has been studied and a general procedure for synthesis of nucleoside alkyl phosphodiesters has been developed.

Bifunctional reagents are commonly used as modifying agents for heterocyclic bases of nucleic acids in photochemical and structural studies of this class of compounds.[1] In the recent years modifications of heterocyclic bases[2a-e] with bifunctional reagents have been employed to produce anchors with active groups for the attachment of various types of reporter groups in molecular probes and primers. To a smaller extent, but for the same purposes, modifications of the sugar moiety of nucleosides has been exploited.[3] Another group of applications of bifunctional reagents involves modifications of a phosphate function in nucleotides[4a-e] or nucleotide analogues[4f-i] for the purpose of introduction of structural or electronic changes at preselected positions in molecular probes, primers and modified antisense DNA.

This paper presents our studies on the reaction of nucleoside *H*-phosphonate monoesters with unprotected or *in situ* protected amino alcohols in the presence of a condensing agent, and transformations occurring during oxidation of the produced *H*-phosphonate derivatives. These constituted the basis for the development of a synthetic method for the preparation of nucleotides carrying a reactive amino group suitable for further derivatization.



R = 5′-*O*-dimethoxytritylthymidin-3′-yl
PV = pivaloyl **2c**, *n* = 4
2a, **3a**, **4a** *n* = 2 **2d**, *n* = 5
2b, **3b**, **4b** *n* = 3 **2e**, **3e**, **4e** *n* = 6

Scheme 1 *Reagents and conditions:* i, PV-Cl; ii, oxidation.

Results and Discussion

Condensations Promoted by Pivaloyl Chloride.—Since amino alcohols bear two nucleophilic groups (amino and hydroxy functions) it was important to know about the degree of chemoselectivity during their condensation (O- *vs.* N-coupling) with nucleoside *H*-phosphonate monoesters in the presence of a condensing agent. The products of such a reaction, *H*-phosphonate or *H*-phosphonamidate diesters (or products of their oxidation, phosphodiesters or phosphoramidates) are usually easily distinguishable using ^{31}P NMR spectroscopy.

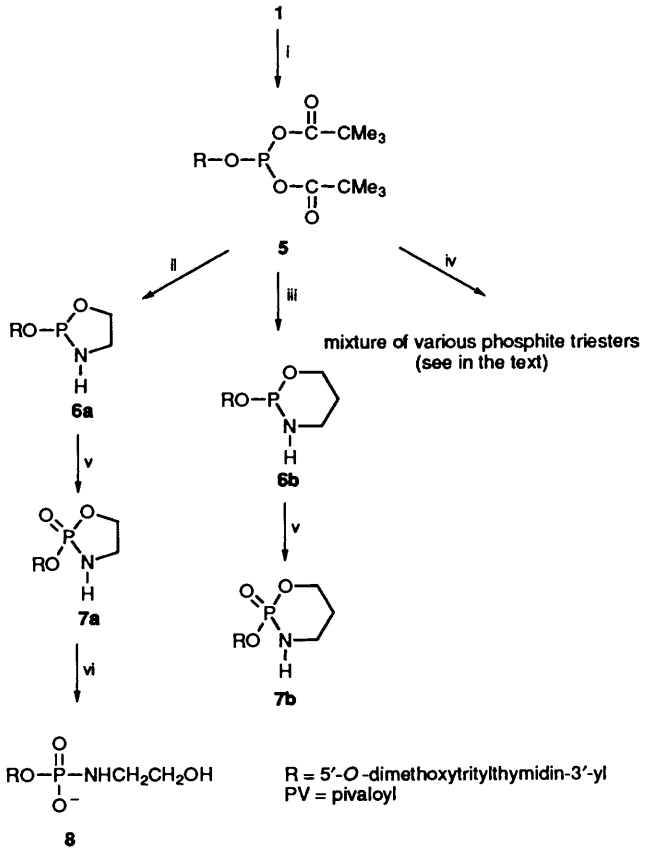
It was found that under standard condensation conditions (*i.e.*, activation of a nucleotide with a condensing agent in the presence of a hydroxylic component)[5] the nucleoside 3′-*H*-phosphonate **1** reacted with amino alcohols **2a**, **b**, **e** in the presence of pivaloyl chloride (PV-Cl) with complete chemoselectivity, affording, after oxidation[6] (iodine in aq. pyridine), the phosphodiesters of type **4** (Scheme 1) with an acylated amino function. No products with P–N bonds could be detected in the reaction mixtures (^{31}P NMR, TLC).

Most likely the coupling of amino alcohols with *H*-phosphonate monoesters and the acylation of the amino function of amino alcohol **2** with pivaloyl chloride are parallel reactions. The coupling is, however, probably slightly faster as judged from that occasionally detected in the reaction mixtures (^{31}P NMR) of small amounts of *H*-phosphonate diesters with free amino groups (compounds of type **10**).

The intermediates, nucleoside *H*-phosphonates **3a**, **b**, **e**, were not isolated due to their partial instability during column chromatography (regular or silanized silica gel), but their presence in the reaction mixtures has been demonstrated by ^{31}P NMR spectroscopy (see Table 1). The potential synthetic utility of compounds **4a**, **b**, **e** seems to be rather limited because of the high stability of the amide bond that made it virtually impossible to convert them into synthetically useful compounds of type **12**.†

To suppress the acylation of the amino alcohol during condensation we attempted condensation with preactivation of the *H*-phosphonate monoester. To this end compound **1** was converted into the dipivaloyl phosphite **5**[5] (2.5 mol equiv. of PV-Cl in pyridine), which was then allowed to react with various amino alcohols **2** (Scheme 2).

† Attempted syntheses with acetic or trifluoroacetic anhydride as a condensing agent afforded complicated reaction mixtures (^{31}P NMR).



Scheme 2 *Reagents:* i, PV-Cl; ii, **2a**; iii, **2b**; iv, **2c**, **2d**, or **2e**; v, I_2; vi, water.

In principle, one can anticipate formation of several products (*e.g.*, symmetrical phosphite triesters, symmetrical phosphoramidites, cyclic phosphoramidites) depending on the chemoselectivity of the reaction (O *vs.* N attack of amino alcohols on the P^{III} centre in phosphite **5**) and a tendency to cyclization of some possible intermediates. Additional complications could arise if phosphite **5** itself would act as an acylating agent towards amino alcohols. It was found that the complexity of the reaction mixtures varied depending on the nature of the amino alcohol used. In the reaction of 2-aminoethanol **2a** with compound **5**, apparently one reaction pathway prevailed, as judged from formation of only one product (^{31}P NMR, two singlets at δ 138.07 and 138.02 ppm) irrespective of the excess of substrate **2a** used. On the basis of the chemical-shift value (see Table 1), multiplicity of signals in the $\{^1H\}$-coupled and $\{^1H\}$-decoupled spectra, and chemical reactivity, the product was tentatively identified as the cyclic phosphoramidite **6a** [2-(5′-*O*-dimethoxytritylthymidin-3′-yloxy)-1,3,2-oxazaphospholidine]. In accord with the assigned structure, compound **6a** was quantitatively converted with iodine in the presence of a limited amount of water into the cyclic phosphoramidate **7a** (^{31}P NMR, see Table 1), which underwent further selective hydrolysis to the final product, the acyclic phosphoramidate **8** (^1H and ^{31}P NMR). This is, to our knowledge, the first example of exclusive P–O bond cleavage under basic conditions in *N*-unsubstituted 1,3,2-oxazaphospholidin-2-one **7a**.*

Since regular coupling of amino alcohol **2a** with *H*-phosphonate **1** in the presence of pivaloyl chloride, followed by oxidation, affords exclusively the phosphodiester **4a**, the route *via* preacylation may be considered as a convenient entry to phosphoramidates of type **8** starting from the same reagents.

* Cyclic *N*-alkyl phosphoramidate triesters of type **7** hydrolyse with considerable P–N bond fission, while in *N*-phenyl derivatives fission of the endocyclic P–O bond occurs exclusively.[7]

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

Compound	Chemical shift[a] (δ/ppm)	$^1J_{PH}$ (Hz)	$^3J_{PH}$ (Hz)
3a	8.47, 8.36	706.8, 706.8	9.7 and 11.0[b]
3b	8.30, 8.26	706.3, 706.3	7.4 and 9.3[b]
3e	7.75, 7.67	701.9, 701.9	8.5 and 9.7[b]
4a	0.22		7.3 and 8.6[c]
4b	0.72		8.3 and 9.5[c]
4e	−0.31		6.1 and 7.3[c]
6a	138.07, 138.02		*d*
6b	134.04, 134.75		*d*
7a	26.20		*d*
7b	3.73, 3.70		*d*
7c	14.98, 14.87		*d*
8	8.18		*d*
10a	8.81, 8.47	738.9, 719.2	7.4 and 7.4[b]
10b	8.40, 8.20	711.8, 710.0	9.4 and 7.4[b]
10c	8.00, 7.88	706.3, 704.4	9.3 and 9.3[b]
10d	7.96, 7.82	704.4, 702.6	7.4 and 7.4[b]
10e	7.91, 7.77	704.4, 704.4	9.3 and 9.3[b]
11b	−13.10, −13.25 −13.45, −13.60		*d*
11c	−13.09, −13.25 −13.34, −13.45		*d*
11d	−13.07, −13.18 −13.31, −13.42		*d*
11e	−13.09, −13.25 −13.34, −13.45		*d*
12a	0.87		6.5[c]
12b	0.41		5.4[c]
12c	−0.50		7.4 and 7.4[c]
12d	−0.47		5.5 and 7.4[c]
12e	−0.46		7.4 and 7.4[c]

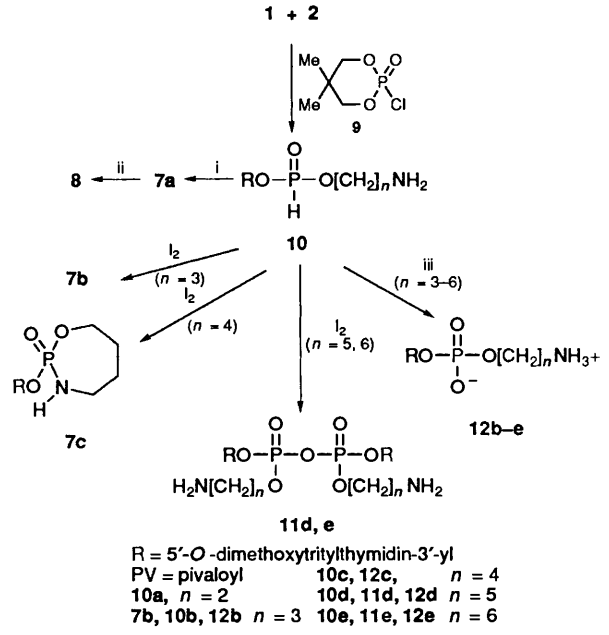
[a] Spectra in pyridine with heteronuclear decoupling (2% or 5% H_3PO_4 in D_2O as an external reference). [b] Two partially overlapping doublets of triplets. [c] Partially overlapping dt. [d] Unresolved symmetrical multiplet.

The reaction of 3-aminopropan-1-ol **2b** with the dipivaloyl phosphite **5** afforded also one product. The ^{31}P NMR spectrum of the reaction mixture showed two singlets (δ 134.04 and 134.75 ppm) characteristic for one spin system of trivalent phosphorus diastereoisomers, which we assigned to the cyclic phosphoramidite **6b** [2-(5′-*O*-dimethoxytritylthymidin-3′-yloxy)-1,3,2-oxazaphosphinane]. In agreement with the assigned six-membered ring structure of compound **6b**, addition of iodine in pyridine (with a limited amount of water) resulted in formation of the cyclic phosphoramidate **7b** (Scheme 2). This compound was stable during standard work-up and was isolated as a pure solid. Its structure was confirmed by ^1H and ^{31}P NMR, MS and TLC analyses.

The reactions of compound **5** in pyridine with amino alcohols having a longer polymethylene spacer (4-aminobutan-1-ol **2c**, 5-aminopentan-1-ol **2d** and 6-aminohexan-1-ol **2e**) gave exceedingly complicated reaction mixtures as judged from ^{31}P NMR spectra of their products. No attempt was made to identify the observed intermediates. However, from the absence of species containing P–N bonds (^{31}P NMR spectra after oxidation with iodine) one can infer that higher amino alcohols have no tendency to form cyclic phosphoramidites in the reaction with the dipivaloyl phosphite **5** nor to undergo intramolecular cyclization during oxidation to phosphoramidates (see also later in the text).

Condensation Promoted by 2-Chloro-5,5-dimethyl-2-oxo-1,3,-2-dioxaphosphinane.—Owing to difficulties in obtaining nucleo-

side phosphodiesters with free amino functions (compounds of type **12**) by using pivaloyl chloride as a condensing agent [regular coupling (Scheme 1) or coupling with preactivation (Scheme 2)] we decided to try 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane[8] **9** for this purpose (Scheme 3). Since



Scheme 3 *Reagents:* i, I_2; ii, water; iii, I_2, then water.

the cyclic chlorophosphate **9** is known to be a rather efficient activating agent for *H*-phosphonate monoesters[9] but is unreactive as a phosphorylating agent, its use should secure an efficient formation of *H*-phosphonate diesters with minimal phosphorylation of the amino group in the alkyl chain.

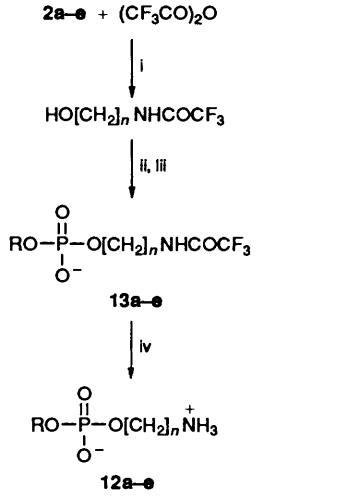
Indeed, the reaction of *H*-phosphonate **1** with 2-aminoethanol **2a** in the presence of the chlorophosphate **9** as coupling agent afforded the *H*-phosphonate diester **10a** with a free amino function in almost quantitative yield (^{31}P NMR). Unfortunately, oxidation of the *H*-phosphonate diester **10a** with aq. iodine resulted in the formation of the cyclic nucleoside phosphoramidate **7a**, which hydrolysed (as was discussed above) quantitatively with selective endocyclic P–O bond scission to the phosphoramidate **8**. The intramolecular cyclization during oxidation of compound **10a** apparently proceeds *via* the phosphoroiodate as intermediate and this course of the reaction is not affected by the amount of water present in the reaction mixture.

3-Aminopropan-1-ol **2c** and 4-aminobutan-1-ol **2d** reacted similarly with compound **1** to form the corresponding nucleoside *H*-phosphonate diesters **10b** and **10c** but, in contradistinction to **10a**, their oxidation afforded different products depending on the amount of water present. Under anhydrous conditions compounds **10b** and **10c** were oxidized to hydrolytically stable cyclic phosphoramidates **7b** and **7c*** (^{31}P NMR), while in the presence of a limited amount of water (~2.0 mol equiv.) two products were always formed (^{31}P NMR): cyclic phosphoramidates **7b** and **7c** (oxidative cyclization) and the symmetrical pyrophosphates **11b** and **11c**, respectively (Table 1). The ratio **7b(c)**:**11b(c)** varied and it decreased with an increasing amount of water present during oxidation. The pyrophosphates **11b** and **11c** are not very reactive species but when left in pyridine they generated slowly, most likely as a result of an intramolecular ring closure, equimolar amounts of cyclic phosphoramidates of type **7** and acyclic phosphodiesters (**12b** and **12c**). These reactions went to completion within 2 and 6 days, respectively.

Higher amino alcohols (5-aminopentan-1-ol **2d**, 6-aminohexan-1-ol **2e**) also afforded the corresponding nucleoside *H*-phosphonates **10d** and **10e** upon condensation with compound **1** aided by the chlorophosphate **9** (^{31}P NMR). However, these *H*-phosphonate diesters, in contradistinction to **10a–c**, upon oxidation with iodine in pyridine afforded exclusively the corresponding symmetrical pyrophosphates **11d** and **11e**. These compounds, upon prolonged storage in pyridine, underwent very slow, nonspecific decomposition.

It is worth mentioning that all nucleoside *H*-phosphonate diesters **10b–e** (but not **10a**), when oxidized with iodine in the presence of an excess of water (5% aq. pyridine), afforded exclusively the corresponding nucleoside phosphodiesters **12b–e** with free amino function (total yield after purification 62–78% as calculated from initial amount of **1**).

Condensations with Preacylation of Amino Alcohols.—Owing to the involvement of the amino group of the 2-aminoethyl residue in intramolecular cyclization during oxidation of the *H*-phosphonate diester **10a** with iodine, and also owing to chemoselective P–O bond scission during hydrolysis of compound **7a** (Schemes 2 and 3), it was not possible to obtain, starting from unprotected aminoethanol **2a**, the phosphodiester **12a** with a free amino function. Since the *H*-phosphonate **3a** with N-pivaloylated amino function did not show any tendency to undergo intramolecular cyclization during oxidation with iodine, we assumed that transient protection of the amino group of 2-aminoethanol **2a** with a very base-labile group would solve the problem (Scheme 4).



Scheme 4 *Reagents and conditions:* i, preacylation; ii, **1** + PV-Cl; iii, I_2, water; iv, NH_4OH.

To this end 2-aminoethanol **2a** was treated with an equimolar amount of trifluoroacetic anhydride (TFAA) in pyridine, and to this mixture the nucleoside *H*-phosphonate **1**, followed by pivaloyl chloride, were added. After *ca.* 10 min the crude reaction mixture was treated with aq. iodine to afford the phosphodiester **13a**, which was the single phosphorus-contain-

* The cyclic phosphoramidate **7c** [2-(5′-*O*-dimethoxytritytlthymidin-3′-yloxy)-1,3,2-oxazaphosphepan-2-one] resonates in the ^{31}P NMR spectrum at a rather unusually low field (two singlets from two diastereoisomers at δ ~14 ppm). This may probably be due to a particular conformation adopted by the seven-membered ring in compound **7c**.

ing species found (^{31}P NMR). A similar sequence of the reactions lead also to phosphodiesters **13b–e** containing longer *N*-trifluoroacetylated aminoalkyl residues. The N-protective groups from **13a–e** were removed by treatment with ammonia and the final products, the nucleoside phosphodiesters **12a–e** with free aminoalkyl groups, were obtained in > 80% yield after short-column silica gel chromatography.[10] Since none of the intermediates has to be isolated during the course of synthesis, this approach with *in situ* trifluoroacetylation of the starting amino alcohols can be considered as a general method for the preparation of nucleoside aminoalkyl phosphodiesters.

In conclusion, although the amino function of amino alcohols does not interfere with the coupling to *H*-phosphonate diesters, it may participate in some inter- or intra-molecular reaction during the subsequent oxidation with iodine and this may lead to some synthetic complications. For this reason we consider the synthetic approach involving a preacylation of amino alcohols to be a method of choice for the preparation of nucleoside phosphodiesters bearing a free alkyl amino function. For special purposes, however, condensation of *H*-phosphonate monoesters with unprotected amino alcohols in the presence of the chlorophosphate **9** can be a viable alternative.

Experimental

Materials and Methods.—^{1}H and ^{31}P NMR spectra were recorded on JEOL GSX-270 FT and Varian Unity 300 BB VT spectrometers. *J* Values are given in Hz. The ^{31}P NMR experiments were carried out at 25 °C in 10 mm (JEOL) and in 5 mm (Varian) tubes using 0.1 mmol 1 cm^{-3} concentrations of phosphorus-containing compounds in pyridine (2 cm^{3}; JEOL or 0.7 cm^{3}; Varian). Mass spectra were recorded on a JEOL MS SX 102 spectrometer with *m*-nitrobenzyl alcohol as a matrix and with sodium acetate as a source of sodium ions. TLC was carried out on Merck silica gel 60 F_{254} precoated plates using the following solvent systems: A—chloroform–methanol (9:1 v/v); B—chloroform–propan-2-ol (9:1); C—chloroform–methanol–triethylamine (85:10:5); D—propan-1-ol–water–25% aq. ammonia (85:10:5). TLC analysis was carried out in a saturated chamber, and R_f values reported are relative to 5′-*O*-dimethoxytritylthymidine (systems A and B), and to 5′-*O*-dimethoxytritylthymidine 3′-*H*-phosphonate (systems C and D).

Pyridine (Lab Scan Ltd.) was stored over molecular sieves 4 A until the amount of water was below 20 ppm (Karl Fischer coulometric titration with Metrohm 684 KF coulometer). Pivaloyl chloride (Merck), 2-aminoethanol **2a** (Fluka) and 3-aminopropan-1-ol **2b** (Aldrich) were of commercial grade and were distilled before use. 4-Aminobutan-1-ol **2c** (Merck), 5-aminopentan-1-ol **2d** (Fluka) and 6-aminohexan-1-ol **2e** (Aldrich) (all of commercial grade) were used without any additional purification. 5′-*O*-Dimethoxytritylthymidine 3′-*H*-phosphonate **1**[11] and 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane **9**[8] were prepared according to published methods.

General Procedure for Synthesis of Nucleoside N-*Pivaloylaminoalkyl Phosphodiesters of Type* **4**.—5′-*O*-Dimethoxytritylthymidine 3′-*H*-phosphonate **1** [triethylammonium ($TEAH^+$) salt, 1 mol equiv.] was dried by evaporation of added pyridine and was then dissolved in the same solvent (1 mmol/5 cm^{3}). To this were added an appropriate amino alcohol (1 mol equiv.) followed by pivaloyl chloride (3 mol equiv.). After *ca.* 10 min (TLC, system A) water (up to ~ 5% concentration) and iodine (1.05 mol equiv.) were added and the oxidation was continued for 20 min. Excess of iodine was decomposed with ethanethiol and solvents were removed by evaporation under reduced pressure. The oily residue was dissolved in methylene dichloride, and washed with 5% aq. $NaHCO_3$, and the organic layer was dried over anhydrous Na_2SO_4, and finally evaporated to dryness. Products of type **4** were isolated by short-column chromatography using a linear gradient of methanol in chloroform containing 5% triethylamine. Fractions containing pure products were collected and evaporated. The obtained glass was dissolved in chloroform and precipitated into hexane–diethyl ether (1:1). The solid was filtered off and dried *in vacuo* over molecular sieves 4A.

5′-O-*Dimethoxytritylthymidin*-3′-*yl* 2-(*pivaloylamino*)*ethyl phosphate* **4a**·$TEAH^+$. Yield 87% (Found: C, 62.1; H, 7.1; N, 6.1; P. 3.6. $C_{44}H_{61}N_4O_{11}P$ requires C, 61.95; H, 7.20; N, 6.57; P, 3.63%); R_f 0.47 (system C), 0.59 (system D); $\delta_H(CDCl_3)$ 1.17 (9 H, s, CMe_3), 1.31 [9 H, t, *J* 7.33, $N(CH_2Me)_3$], 1.35 (3 H, s, 5-Me), 2.33 and 2.62 (2 H, 2 m, 2′-H_2), 3.03 [6 H, q, *J* 7.33, $N(CH_2Me)_3$], 3.38 (2 H, m, CH_2CH_2NH), 3.49 (2 H, m, 5′-H_2), 3.78 (6 H, s, OMe), 3.89 (2 H, m, $POCH_2CH_2$), 4.31 (1 H, m, 4′-H), 4.98 (1 H, m, 3′-H), 6.43 (1 H, m, 1′-H), 6.82 (4 H, d, *J* 8.9, 3, 3′, 5, 5′-H of DMTr), 7.24–7.37 (9 H, m, ArH of DMTr except 3, 3′, 5, 5′-H), 7.61 (1 H, s, 6-H) and 8.84 (1 H, br s, N^3H, exch. with D_2O). For ^{31}P NMR data, see Table 1.

5′-O-*Dimethoxytritylthymidin*-3′-*yl* 3-(*pivaloylamino*)*propyl phosphate* **4b**·$TEAH^+$. Yield 84% [Found: MH^+, 867; $(MH^+ - TEAH^+)Na^+$, 788; $(M - TEAH^+)2Na^+$, 810. $C_{45}H_{63}N_4O_{11}P$ requires MH^+, 867]; R_f 0.52 (system C), 0.59 (system D); $\delta_H(CDCl_3)$ 1.17 (9 H, s, CMe_3), 1.32 (3 H, s, 5-Me), 1.32 [9 H, t, *J* 7.33, $N(CH_2Me)_3$], 1.70 (2 H, dt, *J* 5.86, $CH_2CH_2CH_2$), 2.35 and 2.66 (2 H, 2 m, 2′-H_2) 3.05 [6 H, q, *J* 7.33, $N(CH_2Me)_3$], 3.37 (2 H, m, CH_2CH_2NH), 3.49 (2 H, m, 5′-H_2), 3.78 (6 H, s, OMe), 3.86 (2 H, m, $POCH_2$), 4.31 (1 H, m, 4′-H), 4.97 (1 H, m, 3′-H), 6.43 (1 H, m, 1′-H), 6.88 (4 H, d, *J* 8.7, 3, 3′, 5, 5′-H of DMTr), 7.25–7.39 (9 H, m, ArH of DMTr except 3, 3′, 5, 5′-H), 7.62 (1 H, s, 6-H) and 8.86 (1 H, br s, N^3H, exch. with D_2O). For ^{31}P NMR data, see Table 1.

5′-O-*Dimethoxytritylthymidin*-3′-*yl* 6-(*pivaloylamino*)*hexyl phosphate* **4e**·$TEAH^+$. Yield 72% (Found: C, 63.0; H, 7.5; N, 6.0; P, 3.4. $C_{48}H_{69}N_4O_{11}P$ requires C, 63.41; H, 7.65; N, 6.16; P. 3.41%); R_f 0.52 (system C), 0.59 (system D); $\delta_H(CDCl_3)$ 1.18 (9 H, s, CMe_3), 1.32 (3 H, s, 5-Me), 1.32 [9 H, t, *J* 7.20, $N(CH_2Me)_3$], 1.32 (4 H, m, $OCH_2CH_2CH_2CH_2CH_2CH_2NH$), 1.45 (2 H, dt, *J* 6.90, $CH_2CH_2CH_2NH$), 1.57 (2 H, dt, *J* 6.90, $POCH_2CH_2CH_2$), 2.35 and 2.66 (2 H, 2 m, 2′-H_2), 3.04 [6 H, q, *J* 7.20, $N(CH_2Me)_3$], 3.19 (2 H, dt, *J* 6.60 and 6.90, CH_2CH_2NH), 3.40 (2 H, m, 5′-H), 3.78 (6 H, s, OMe), 3.80 (2 H, m, $POCH_2CH_2$), 4.32 (1 H, m, 4′-H), 5.78 (1 H, m, 3′-H), 6.45 (1 H, m, 1′-H), 6.82 (4 H, d, *J* 8.9, 3, 3′, 5, 5′-H of DMTr), 7.24–7.40 (9 H, m, ArH of DMTr except 3, 3′, 5, 5′-H), 7.61 (1 H, s, 6-H) and 8.91 (1 H, br s, N^3H, exch. with D_2O). For ^{31}P NMR data, see Table 1.

Synthesis of 5′-O-*Dimethoxytritylthymidin*-3′-*yl* N-(2-*Hydroxyethyl*) *Phosphoramidate* **8**·$TEAH^+$.—A. *Approach with the preactivation of compound* **1**. A solution of 5′-*O*-dimethoxytritylthymidine 3′-*H*-phosphonate **1** (triethylammonium salt, 1 mol equiv.) in pyridine (concentration 1 mmol/5 cm^{3}) was treated with pivaloyl chloride (3 mol equiv.). To this was added 2-aminoethanol **2a** (1.1 mol equiv.) and after 5 min the reaction mixture was treated with iodine (1.05 mol equiv.) in the presence of water (0.5% concentration). After 15 min, excess of iodine was decomposed with ethanethiol, the solvent was removed by evaporation, and the residue was dissolved in chloroform (50 cm^{3}/1 mmol of **1**). The organic phase was washed with 5% aq. $NaHCO_3$, dried over anhydrous Na_2SO_4, and finally evaporated to leave a solid glass. Purification of compound **8** was performed by short-column chromatography on silica gel 60 using a linear gradient of methanol in chloroform containing 5% of triethylamine. After evaporation of the appropriate fractions, the residue was dissolved in chloroform and precipitated into hexane–diethyl ether (1:1). The precipitate was

filtered off and, after drying *in vacuo* over molecular sieves 4A, compound **8** was obtained as a powder (57%). For analytical data, see below.

B. *Condensation with the aid of* 2-*chloro*-5,5-*dimethyl*-2-*oxo*-1,3,2-*dioxaphosphinane* **9**. The *H*-phosphonate **1** (1 mol equiv.) and 2-aminoethanol **2a** (1.05 mol equiv.) in pyridine (5 cm^3/1 mmol of **1**) were treated with the chlorophosphate **9** (2.5 mol equiv.) during 10 min. To this was added water to a final concentration of 5% and the reaction mixture was oxidized with iodine (1.05 mol equiv.) for 15 min. Further work-up was as above in the approach with preactivation (A) (yield 69%) [Found: MH^+, 769, $(MH^+ - TEAH^+)Na^+$, 690; $(M - TEAH^+)2Na^+$, 712. $C_{39}H_{53}O_{10}N_4P$ requires MH^+, 769]; R_f 0.22 (system C), 0.37 (system D); $\delta_H(CDCl_3)$ 1.30 [9 H, t, *J* 7.33, $N(CH_2Me)_3$], 1.34 (3 H, s, 5-Me), 2.35 and 2.66 (2 H, 2 m, 2'-H_2), 2.85 (2 H, m, $PNHCH_2$), 3.04 [6 H, q, *J* 7.33, $N(CH_2Me)_3$], 3.49 (2 H, m, 5'-H_2), 3.53 (2 H, m, CH_2OH), 3.77 (6 H, s, OMe), 4.24 (1 H, m, 4'-H), 4.91 (1 H, m, 3'-H), 6.36 (1 H, m, 1'-H) 6.82 (4 H, d, *J* 8.8, 3, 3', 5, 5'-H of DMTr), 7.25–7.41 (9 H, m, ArH of DMTr except 3, 3', 5, 5'-H) and 7.62 (1 H, s, 6-H). ^{31}P NMR data, see Table 1.

Synthesis of 2-(5'-O-*Dimethoxytritylthymidin*-3'-*yloxy*)-1,3,2-*oxazaphosphinan*-2-*one* **7b**.—A. *Approach with the preactivation of compound* **1**.—The reaction was carried out essentially as described above for the preparation of phosphoramidate **8** (approach A), with the exception that 3-aminopropan-1-ol **2b** was used instead of 2-aminoethanol **2a**. The product **7b** was purified by short-column chromatography on silica gel 60 with a linear gradient of methanol in chloroform (69%) yield). For analytical data, see below.

B. *Condensation with the aid of* 2-*chloro*-5,5-*dimethyl*-2-*oxo*-1,3,2-*dioxaphosphinane* **9**. The reaction was carried out essentially as described above for the preparation of compound **8** (approach B), with the exception that 3-aminopropan-1-ol **2b** was used instead of 2-aminoethanol **2a**, and oxidation with iodine was carried out under anhydrous conditions (purification as above for compound **7b**, approach A). Yield was 66% [Found: MH^+, 664; MNa^+, 686; $(M - H^+)2Na^+$, 708. $C_{34}H_{38}N_3O_9P$ requires MH^+, 664]; R_f (two diastereoisomers) 0.58 and 0.62 (system A), 0.58 and 0.66 (system B); $\delta_H(CDCl_3)$ 1.73 (3 H, s, 5-Me), 1.65 and 2.00 (2 H, 2 m, $CH_2CH_2CH_2$), 2.43 and 2.65 (2 H, 2 m, 2'-H_2), 3.18 and 3.25 (2 H, 2 m, $PNHCH_2$), 3.41 and 3.51 (2 H, 2 m, 5'-H_2), 3.79 (6 H, s, OMe), 4.18 and 4.29 (2 H, 2 m, $POCH_2$), 4.29 (1 H, m, 4'-H), 5.12 (1 H, m, 3'-H), 6.47 (1 H, m, 1'-H), 6.83 (4 H, d, *J* 8.7, 3, 3', 5, 5'-H of DMTr), 7.24–7.39 (9 H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.57 (1 H, br s, 6-H) and 8.81 (1 H, br s, N^3H, exch. with D_2O).

Synthesis of 2-(5'-O-*Dimethoxytritylthymidin*-3'-*yloxy*)-1,3,2-*oxazaphosphepan*-2-*one* **7c**.—The cyclic phosphoramidate **7c** was prepared analogously to compound **7b** (approach B) with the exception that instead of 3-aminopropan-1-ol **2b**, 4-aminobutan-1-ol **2c** was used for the condensation with the *H*-phosphonate monoester **1**. Yield was 53% [Found: MH^+, 678; MNa^+, 700; $(M - H^+)2Na^+$, 722. $C_{35}H_{40}N_3O_9P$ requires MH^+, 678]; R_f (two diastereoisomers) 0.53 and 0.58 (system A), 0.58 and 0.66 (system B); $\delta_H(CDCl_3)$ 1.65 (3 H, s, 5-Me), 1.66 and 1.82 (4 H, 2 m, $CH_2CH_2CH_2CH_2$), 2.40 and 2.63 (2 H, m, 2'-H_2), 2.91 and 3.0 (2 H, 2 m, $PNHCH_2$), 3.40 and 3.51 (2 H, 2 m, 5'-H_2), 3.79 (6 H, s, OMe), 4.10 (1 H, m, 4'-H), 4.08 and 4.20 (2 H, 2 m, $POCH_2$), 5.17 (1 H, m, 3'-H), 6.45 (1 H, dt, *J* 5.62, 1'-H), 6.83 (4 H, d, *J* 8.7, 3, 3', 5, 5'-H of DMTr), 7.23–7.39 (9 H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.58 (1 H, s, 6-H) and 8.50 (1 H, br s, N^3H, exch. with D_2O).

General Procedure for the Synthesis of Nucleoside Aminoalkyl Phosphodiesters **12b–e** (*an Approach with Unprotected Amino Alcohols*).—The individual nucleoside *H*-phosphonate diesters **10b–e** were prepared analogously as described for compound **7b** (approach B) by condensation of substrate **1** with the appropriate amino alcohol in the presence of the cyclic chlorophosphate **9** as a condensing agent. Oxidation with iodine (1.05 mol equiv.) was carried out during 15 min after addition of water to ~5% concentration. Excess of iodine was decomposed with ethanethiol, and $NaHCO_3$ (5% aq. solution) was added to neutralize acidic components present in the mixtures. The reaction mixtures were concentrated to leave heavy oils by evaporation of added pyridine and the residues were dissolved in a minimal amount of chloroform containing 5% triethylamine. The undissolved material (inorganic salts) was filtered off and the filtrates were loaded onto a silica gel column equilibrated with the same solvent mixture. A gradient of methanol in chloroform (containing 5% triethylamine) was used as the solvent system. Fractions containing pure phosphodiesters **12b–e** were collected and evaporated, and the oily residues were precipitated from chloroform solutions into hexane–diethyl ether (1:1). The precipitates were collected by filtration and were dried *in vacuo* over molecular sieves 4A to constant weight.

3-*Aminopropyl* 5'-O-*dimethoxytritylthymidin*-3'-*yl phosphate* **12b**. Yield 73% [Found: MH^+, 682; MNa^+, 704; $(M - H^+)$-$2Na^+$, 726. $C_{34}H_{40}N_3O_{10}P$ requires MH^+, 682]; R_f 0.02 (system C), 0.18 (system D); $\delta_H(CDCl_3)$ 1.32 (3 H, s, 5-Me), 1.88 (2 H, m, $POCH_2CH_2CH_2$) 2.27 and 2.62 (2 H, 2 m, 2'-H_2), 3.02 (2 H, m, $CH_2NH_3^+$), 3.33 (2 H, m, 5'-H_2), 3.72 (6 H, s, OMe), 3.89 (2 H, m, $POCH_2$), 4.20 (1 H, m, 4'-H), 4.88 (1 H, m, 3'-H), 6.27 (1 H, m, 1'-H) 6.78 (4 H, d, *J* 9.0, 3, 3', 5, 5'-H of DMTr), 7.18–7.35 (9 H, m, ArH of DMTr except 3, 3', 5, 5'-H) and 7.53 (1 H, s, 6-H). For ^{31}P NMR data, see Table 1.

4-*Aminobutyl* 5'-O-*dimethoxytritylthymidin*-3'-*yl phosphate* **12c**. Yield 62% [Found: MH^+, 696; MNa^+, 718; $(M - H^+)$ $2Na^+$, 740. $C_{35}H_{42}N_3O_{10}P$ requires MH^+, 696]; R_f 0.03 (system C), 0.15 (system D); $\delta_H(CDCl_3)$ 1.35 (3 H, s, 5-Me), 1.62 (2 H, m, $CH_2CH_2NH_3^+$), 1.79 (2 H, m, $POCH_2CH_2$), 2.25 and 2.66 (2 H, 2 m, 2'-H_2), 2.9 (2 H, m, $CH_2NH_3^+$), 3.30 (2 H, m, 5'-H_2), 3.72 (6 H, s, OMe), 3.79 (2 H, m, $POCH_2$), 4.23 (1 H, m, 4'-H), 4.89 (1 H, m, 3'-H), 6.29 (1 H, m, 1'-H), 6.79, (4 H, d, *J* 8.7, 3, 3', 5, 5'-H of DMTr), 7.20–7.36 (9 H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.55 (1 H, s, 6-H) and 8.30 (1 H, br s, N^3H, exch. D_2O). For ^{31}P NMR data, see Table 1.

5-*Aminopentyl* 5'-O-*dimethoxytritylthymidin*-3'-*yl phosphate* **12d**. Yield 78% [Found: MH^+, 710; MNa^+, 732; $(M - H^+)$-$2Na^+$, 754. $C_{36}H_{44}N_3O_{10}P$ requires MH^+, 710]; R_f 0.02 (system C), 0.16 (system D); $\delta_H(CDCl_3)$ 1.40 (3 H, s, 5-Me), 1.51 (2 H, m, $CH_2CH_2CH_2CH_2CH_2$), 1.59 (2 H, m, CH_2-CH_2NH_3), 1.71 (2 H, m, OCH_2CH_2), 2.20 and 2.62 (2 H, 2 m, 2'-H_2), 2.98 (2 H, m, $CH_2NH_3^+$), 3.34 (2 H, m, 5'-H_2), 3.75 (6 H, s, OMe), 3.79 (2 H, m, $POCH_2$), 4.25 (1 H, m, 4'-H), 4.93 (1 H, m, 3'-H), 6.81 (4 H, d, *J* 8.8, 3, 3', 5, 5'-H of DMTr), 7.23–7.37 (9 H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.51 (1 H, s, 6-H) and 8.38 (1 H, br s, N^3H, exch. with D_2O). For ^{31}P NMR data, see Table 1.

6-*Aminohexyl* 5'-O-*dimethoxytritylthymidin*-3'-*yl phosphate* **12e**. Yield 69% [Found: MH^+, 724; MNa^+, 746; $(M - H^+)$-2Na, 768. $C_{37}H_{46}N_3O_{10}P$ requires MH^+, 724]; R_f 0.05 (system C), 0.19 (system D); $\delta_H(CDCl_3)$ 1.32 (4 H, m, $POCH_2$-$CH_2CH_2CH_2CH_2CH_2NH_3^+$), 1.33 (3 H, s, 5-Me), 1.54 (2 H, m, $CH_2CH_2NH_3^+$), 1.67 (2 H, m, $POCH_2CH_2$), 2.23 and 2.63 (2 H, 2 m, 2'-H_2), 2.86 (2 H, m, $CH_2NH_3^+$), 3.30 (2 H, m, 5'-H_2), 3.7 (2 H, m, $POCH_2$), 3.73 (6 H, s, OMe), 4.24 (1 H, m, 4'-H), 4.93 (1 H, m, 3'-H), 6.34 (1 H, m, 1'-H), 6.80 (4 H, d, *J* 8.7, 3, 3', 5, 5'-H of DMTr), 7.24–7.35 (9 H, m, ArH of DMTr except 3, 3', 5, 5'-H), 7.57 (1 H, s, 6-H) and 8.33 (1 H, br s, N^3H, exch. with D_2O). For ^{31}P NMR data, see Table 1.

General Procedure for the Synthesis of Nucleoside Aminoalkyl Phosphodiesters **12a–e** *(an Approach with Preacylation of Amino Alcohols).*—The appropriate amino alcohol **2a–e** (1.1 mol equiv.) was treated with TFAA (1.1 mol equiv.) in pyridine (5 cm^3/1 mmol of amino alcohol). The reaction mixture was treated with a solution of the nucleoside *H*-phosphonate **1** in a minimal amount of pyridine, and, after homogenization, pivaloyl chloride (3.0 mol equiv.) was added. After 10 min, iodine (1.05 equiv.) in 20% aq. pyridine (5 cm^3/1 mmol) was added and oxidation was continued for 15 min. Excess of iodine was decomposed with ethanethiol, and the reaction mixture was concentrated to an oil, which was dissolved in pyridine (5 cm^3/1 mmol of **1** used). To this was added an equal volume of 25% aq. ammonia and the reaction mixture was left overnight at room temperature. Isolation of the phosphodiesters **12a–e** was carried out as described above for **12b–e**.

2-*Aminoethyl* 5′-O-*dimethoxytritylthymidin*-3′-*yl phosphate* **12a**. Yield 88% [Found: MH^+, 668; MNa^+, 690; $(M - H^+)$-$2Na^+$, 712. $C_{33}H_{38}N_3O_{10}P$ requires MH^+, 668]; R_f 0.07 (system C), 0.29 (system D); $\delta_H(CDCl_3)$ 1.31 (3 H, s, 5-Me), 2.25 and 2.67 (2 H, 2 m, 2′-H_2), 3.11 (2 H, m, $CH_2NH_3^+$), 3.31 (2 H, m, 5′-H_2), 3.69 (6 H, s, OMe), 4.03 (2 H, m, $POCH_2$), 4.20 (1 H, m, 4′-H), 4.85 (1 H, m, 3′-H), 6.20 (1 H, m, 1′-H), 6.76 (4 H, d, *J* 8.1, 3, 3′, 5, 5′-H of DMTr), 7.20–7.33 (9 H, m, ArH of DMTr except 3, 3′, 5, 5′-H), 7.51 (1 H, s, 6-H) and 8.06 (1 H, br s, N^3H, exch. with D_2O). For ^{31}P NMR data, see Table 1.

3-*Aminopropyl* 5′-O-*dimethoxytritylthymidin*-3′-*yl phosphate* **12b**. Yield 82%. Spectral and analytical data as above.

4-*Aminobutyl* 5′-O-*dimethoxytritylthymidin*-3′-*yl phosphate* **12c**. Yield 73%. Spectral and analytical data as above.

5-*Aminopentyl* 5′-O-*dimethoxytritylthymidin*-3′-*yl phosphate* **12d**. Yield 81%. Spectral and analytical data as above.

6-*Aminohexyl* 5′-O-*dimethoxytritylthymidin*-3′-*yl phosphate* **12e**. Yield 80%. Spectral and analytical data as above.

Acknowledgements

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

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Paper 3/00128H
Received 8*th January* 1993
Accepted 2*nd April* 1993