Nucleoside H-Phosphonates. 17. Synthetic and ^{31}P NMR Studies on the Preparation of Dinucleoside H-Phosphonothioates

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Formation of H-phosphonothioate diesters *via* condensation of H-phosphonate monoesters with a hydroxylic component in the presence of various coupling agents and possible side reactions that may accompany this process were studied using ^{31}P NMR spectroscopy. Optimal reaction conditions, which eliminate or significantly suppress the side reactions, have been designed and assessed in syntheses of dinucleoside H-phosphonothioate diesters.

Introduction

The antisense and the antigene approaches to modulation of gene expression rely on formation of stable duplexes or triplexes between a target RNA or DNA sequence and a synthetic oligonucleotide. These powerful experimental techniques, which hold considerable medicinal promise for therapeutic intervention in many human genetic diseases,[1,2] require oligonucleotide analogues with good hybridization properties under physiological conditions (high affinity and high specificity) and with higher stability than their natural congener against degradation by cellular and serum nucleases. Among synthetic DNA and RNA, an important class of surrogates with modified internucleotidic linkages constitutes analogues in which one or two nonbridging oxygen atoms at the phosphorus center have been replaced by sulfur (oligonucleoside phosphorothioates[3] and oligonucleoside phosphorodithioates,[4] respectively). These oligonucleotide analogues are most conveniently accessible *via* sulfurization of the appropriate P(III) precursors,[5−11] however, approaches based on P(V) derivatives have also been developed.[12,13]

As a part of our research in H-phosphonate chemistry[14,15] directed toward development of new synthetic methods for biologically important phosphate esters and their analogues, we have recently begun synthetic studies on H-phosphonothioate esters.[9,16−18] To provide an easy access to this class of phosphonate analogues, we have developed two efficient methods for the preparation of nucleoside H-phosphonothioate monoesters[16,19] and showed that these compounds can be converted to the appropriate H-phosphonothioate diesters[9] *via* condensation with suitably protected nucleosides.

H-Phosphonothioate diesters are versatile synthetic intermediates, for example, they may serve as precursors to both phosphorothioate[9,20] and phosphorodithioate[9,20] diesters or to other analogues[21−23] that are rather difficult to obtain by other routes. Moreover, the possibility to control the stereochemistry at the phosphorus center during oxidation of H-phosphonothioate diesters with 3*H*-2,1-benzoxathiol-3-one 1-oxide[20] or with iodine[21] can provide a convenient entry to both diastereomers of P-chiral phosphate analogues, depending on the oxidation procedure chosen.

Substitution of one oxygen in H-phosphonate esters by sulfur has severe stereochemical and chemical consequences. At the monoester level,[16,19] it introduces chirality at the phosphorus and this may potentially be exploited in stereospecific synthesis of phosphorothioates and other chiral phosphate analogues. The presence of sulfur, however, makes two different nucleophilic centers (O and S) available for activation by a condensing agent. Lack of chemoselectivity in this process may cause a partial elimination of sulfur during the course of the reaction which can lead to formation of side products. At the diester level,[9,17,18] substitution of oxygen by sulfur at the phosphorus center causes the P−H bond in H-phosphonothioates to become noticeably more reactive than that in the oxygen congener. This, in principle, also is a potential source of side reactions.

Having in mind possible applications of H-phosphonothioates as precursor to various oligonucleotide analogues, we have undertaken systematic studies on this class of compounds. In this paper we address the

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problem of possible side reactions which may occur during preparation of H-phosphonothioate diesters *via* condensation of suitably protected nucleoside 3′-H-phosphonothioate monoesters with nucleosides. Various coupling agents and different reaction conditions for the condensation have been evaluated. These studies enabled us to develop an optimal synthetic protocol which eliminated or significantly suppressed side reactions during condensations to **3**. Dinucleoside H-phosphonothioate diesters **3a**−**d** containing different heterocyclic bases have been synthesised in 80−95% yields using the newly developed conditions.

Results and Discussion

Dinucleoside H-phosphonothioates **3** can be produced in a condensation reaction as in Scheme 1 using nucleoside H-phosphonothioates **1** as a nucleotidic material or, alternatively, by employing H-phosphonodithioate[8] monoesters for this purpose. Preliminary studies[18] showed, however, that possible advantages of using the latter derivatives are outnumbered by serious drawbacks, *e.g.*, lower reactivity of H-phosphonodithioates in condensations, formation of significant amounts of side products during coupling with nucleosides, and the limited number of coupling agents which may be used for their activation. For these reasons we decided to pursue our original approach,[9] which is based on H-phosphonothioates of type **1**, and to investigate it in more detail.

The high rates of condensations of H-phosphonothioate monoesters with nucleosides have the consequence that, similar to H-phosphonate diester syntheses, competing reactions of condensing agents with the hydroxylic component during the course of coupling can practically be neglected. The situation is, however, more complicated than in the synthesis of H-phosphonate diesters. Due to higher reactivity of H-phosphonothioate diesters, the condensation products are prone to subsequent reactions at the phosphorus center. In addition, the starting monoesters may react with a condensing agent in two different ways (O *vs* S activation) producing different products. For these reasons, the choice of a condensing agent in H-phosphonothioate diester synthesis is more critical than for H-phosphonates.

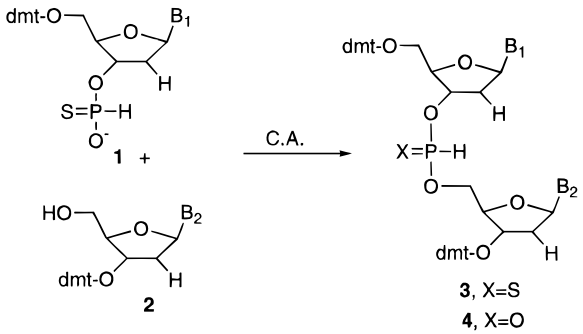
Different classes of condensing agents, *i.e.*, acyl chlorides, chlorophosphates, arylsulfonyl chlorides, and carbodiimides, have been evaluated from points of view of their (i) efficiency to promote formation of H-phosphonothioate diesters, (ii) chemoselectivity during the activation of H-phosphonothioate monoesters, and (iii) reactivity toward the P−H bond in H-phosphonothioate diesters.

To have uniform set of data, all condensations have been carried out in pyridine with variable amounts of a condensing agent, and progress of the reactions was monitored by ^{31}P NMR spectroscopy.

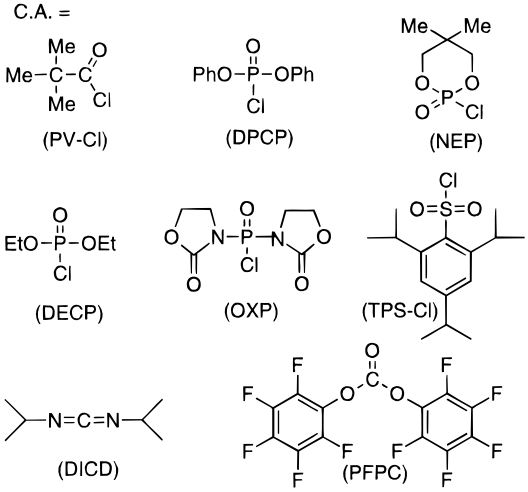
Pivaloyl Chloride as a Condensing Agent. Since pivaloyl chloride (PV-Cl) is commonly used as the coupling reagent in the automated synthesis of DNA and RNA fragments *via* the H-phosphonate approach,[15] it was our initial choice for the H-phosphonothioate diester formation (Scheme 1). Unfortunately, although the reagent promotes fast coupling, it may also react with the condensation product **3a** (δ_P ~71.3 and 72.7 ppm) leading to side products. These have been identified as the P-acylated dimer **5** (δ_P ~65.5 ppm, two singlets; isolation and ^{1}H NMR analysis) and the phosphite triester **6** (δ_P ~141.5 ppm; comparison with an authentic sample) (Scheme 2). The amounts of **5** and **6** varied, depending on the ratio of the reagents used. Condensation of equimolar amounts of H-phosphonothioate **1a** and the nucleosidic component **2**, in the presence of 3 equiv of PV-Cl, produced less than 2% of **6** and ca. 8% of the P-acylated product **5** (after 3 min). The signals related to **5** increased with time and amounted to ca. 25% of all nucleotidic material after 70 min. Under analogous conditions but with 2 equiv of nucleoside **2**, acylphosphonate **5** and the triester **6** were formed in ca. 5 and 8%, respectively (after 3 min). These amounts did not increase significantly upon standing, probably due to a complete consumption of PV-Cl in the acylation reaction of **2**. With the equimolar amounts of **1a** and **2** and 6-fold excess of PV-Cl, the H-phosphonothioate **3a** was completely P-acylated within 40 min, while the amount of phosphite **6** was ca. 5%.[24]

It is worth mentioning that in PV-Cl-promoted condensations to **3** we did not observe any formation of the H-phosphonate **4**. This indicates that activation of **1** with PV-Cl is chemoselective and occurs exclusively at the oxygen atom.

Scheme 1



C.A. =

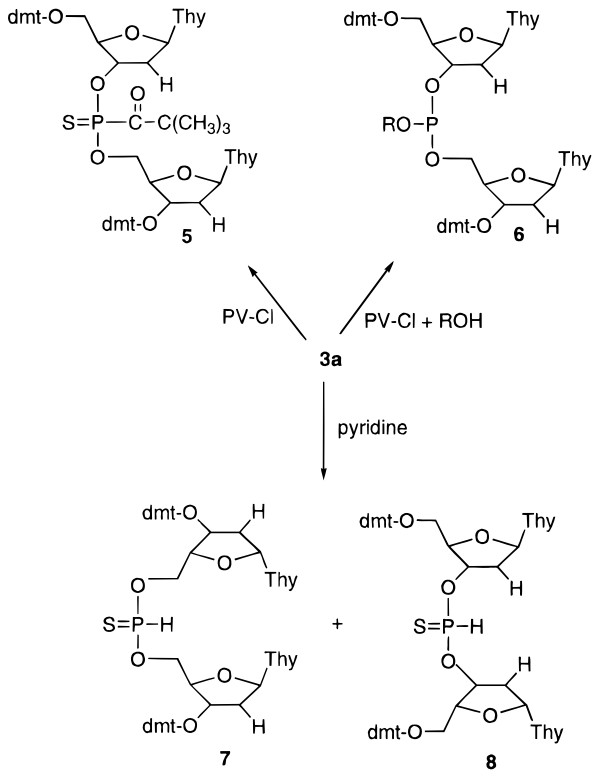


dmt - 4',4'-dimethoxytrityl
Thy - thymin-1-yl
^{pr}Cyd - N^4-propionylcytosin-1-yl
^{bu}Ade - N^6-butyryladenin-9-yl
^{phoac}Gua - N^2-phenoxyacetylguanin-9-yl

1a B_1 = Thy
1b, B_1 = ^{pr}Cyd
1c, B_1 = ^{bu}Ade
1d, B_1 = ^{phoac}Gua
2, B_2 = Thy

3a, B_2 = B_1 = Thy
3b, B_2 = Thy, B_1 = ^{pr}Cyd
3c, B_2 = Thy, B_1 = ^{bu}Ade
3d, B_2 = Thy, B_1 = ^{phoac}Gua
4a, B_2 = B_1 = Thy

Scheme 2[a]



[a] *R* = 5′-*O*-(dimethoxytrityl)thymidin-3′-yl. For other abbreviations, see Scheme 1.

Another interesting observation was that in all investigated reactions the ^{31}P NMR resonances due to **3a** were always accompanied by two very small singlets at higher and lower fields. These signals (δ_P ~70.3 and 73.6 ppm), whose intensities slowly increased with time, totally amounted to ca. 5% of the nucleotidic material after 1 h. Since they were present also in the reaction mixtures when other coupling agents were used, they could not be related to side products contained as an integral part a condensing agent moiety (see later in the text).

Chlorophosphates as Condensing Agents. Due to low reactivity of S-nucleophiles toward the phosphorus center,[25] we expected that the use of chlorophosphates to produce H-phosphonothioate diesters should alleviate problems connected with subsequent reactions of the condensation products **3** with a condensing agent. To this end we carried out condensations of H-phosphonothioate **1a** with a nucleosidic component (**2**) in the presence of diphenyl phosphorochloridate[26] (DPCP), 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane[27] (NEP), diethyl phosphorochloridate[27] (DECP), and bis(2-oxo-3-oxazolidinyl)phosphinic chloride[27,28] (OXP) (Scheme 1). All these reagents, which have been previously evaluated in the synthesis of H-phosphonate diesters, promoted clean formation of the H-phosphonothioates **3** when used in a 3 equiv excess over the starting material **1**. In the reactions when more than equimolar amounts of a hydroxylic component was used, some 5′-phosphorylation of **2** by DPCP was observed. Possible side products, which might have resulted from the subsequent reaction of **3a** with the condensing agents (phosphite triester **6** or hypophosphates[29]), were not observed. All the investigated chlorophosphates showed also a complete chemoselectivity in the activation process of **1**, as judged from the absence of H-phosphonate diesters of type **4** in the reaction mixtures.

The coupling reactions promoted by DPCP or DECP were complete in less than 5 min (time necessary to record the first ^{31}P NMR spectrum), while the two other chlorophosphates, NEP and OXP, required ~15 and ~40 min, respectively, to drive the reactions to completion. As we already observed in the PV-Cl-promoted condensation, the resonances due to the product **3a** were in all instances accompanied by two very small singlets at higher and lower fields. The intensities of these signals, compared to those of the diesters **3**, were stronger in the NEP- and OXP-promoted condensations than in the couplings where DPCP or DECP was used.

TPS-Cl-Promoted Condensations. When a mixture of **1a** and **2** (1.5 equiv) in pyridine was treated with incremental amounts of 2,4,6-triisopropylbenzenesulfonyl chloride[30] (TPS-Cl) (Scheme 1) and the ^{31}P NMR spectrum monitored, two singlets at ~55.5 ppm due to the starting material **1a** gradually disappeared. New absorptions for the product **3a**, the H-phosphonate **4a** (δ_P ~7.9 and 9.7 ppm), the desulfurized starting material (δ_P ~3.7 ppm), and for at least three additional compounds without P−H bonds (singlet at ~116.5 ppm, and two pair of singlets at ~68 and ~59 ppm) emerged simultaneously. The signals due to the product **3a** and the desulfurized starting material completely disappeared within 20 min while intensities of the other resonances increased. In an analogous reaction when 3 equiv of TPS-Cl was added in one portion, a similar pattern of ^{31}P NMR signals was observed and the desired product of the reaction, the H-phosphonothioate diester **3a**, completely disappeared within 15 min. A higher excess of the condensing agent (10 equiv) made the reaction proceed more rapidly (presence of **3a** could not be detected in the first ^{31}P NMR spectrum), and the final reaction mixture showed only the presence of the H-phosphonate **4a** and a compound resonating at ~69 ppm (two singlets).

In light of our previous studies on using TPS-Cl as a condensing agent in the synthesis of H-phosphonate diesters,[31] the above results can be interpreted as follows. TPS-Cl promoted rather fast formation of the H-phosphonothioate **3a**, but this compound, due to its higher susceptibility to oxidation[17,18] than that of the corresponding H-phosphonate diester, rapidly underwent subsequent reactions with the condensing agent producing compounds which lacked P−H bonds. No attempt was made to identify these products, but on the basis of the ^{31}P NMR data, it seems that they are analogous to those produced from H-phosphonate diesters and arylsulfonyl derivatives.[31,38] Since H-phosphonate diesters

(24) Extent of P-acylation and phosphite triester formation strongly depend on reaction conditions. For example, the PV-Cl-promoted condensation of **1a** + **2** was significantly cleaner in acetonitrile−2,6-lutidine (4:1, v/v) than in neat pyridine (P-acylation less than 5% and no phosphite triesters formation; ^{31}P NMR experiments). See also later in the text.

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4 were formed under these reaction conditions in significant amount (35−45%), we could conclude that the H-phosphonothioate monoesters **1** showed only a slight preference for the formation of O-activated species in the reactions with TPS-Cl.

Other Coupling Agents. Utility of carbodiimides as coupling agents for H-phosphonothioate monoesters was assessed in the diisopropylcarbodiimide (DICD) promoted condensation. To this end 3 equiv of DICD was added to a mixture of **1a** and **2** in pyridine containing 2 equiv of pyridinium hydrochloride as an activator (Scheme 1). This resulted in an immediate disappearance of **1a** (first ^{31}P NMR spectrum, ∼5 min) and formation of two nucleotidic compounds without sulfur at the phosphorus center, *i.e.*, **4** and the nucleoside H-phosphonate monoester (from desulfurization of **1a**) in ∼1:1 ratio. The amount of the H-phosphonothioate diester **3a** did not exceed 5% and remained unchanged during the course of the reaction (∼40 min). This indicated that the presence of the H-phosphonate **4** was not due to rapid formation of **3a** followed by its desulfurization but rather resulted, as expected,[32] from the predominant S-activation of the H-phosphonothioate **1a** by DICD. The nucleoside H-phosphonate monoester observed at the initial stages of the condensation was most likely formed by a partial hydrolysis of the S-activated species by spurious water, and it was gradually disappearing with time to form **4**.

We have also evaluated utility of bis(pentafluorophenyl) carbonate (PFPC), a coupling agent recently proposed for the synthesis of H-phosphonate diesters.[33] The formation of **3a** in a coupling reaction between **1a** and **2** promoted by this reagent (3 equiv) in pyridine (Scheme 1) was rather slow (85% completion after ∼40 min). Though the activation of **1** with this reagent was completely chemoselective (O-activation; no H-phosphonate derivatives present), nevertheless the reaction was accompanied by formation of unidentified side products, as judged from the presence of several small resonances at δ_P ∼50−60 ppm in the ^{31}P NMR spectrum.

Side Reactions Not Related to the Use of a Condensing Agent. The presence of two singlets (δ_P ∼70.3 and 73.6 ppm), placed almost symmetrically around the resonances due to **3a** in the ^{31}P NMR spectra of all investigated condensation reaction mixtures, was puzzling. Although low intensities of these signals (Figure 1a) prevented detailed NMR analysis, we nevertheless noticed that both of them possessed large $^1J_{PH}$ constants (∼670 Hz) indicative of a P−H bond. Since their chemical shifts were close to those of the diastereomers of **3a**, we assumed that atoms directly connected to the phosphorus centers should be the same in both instances. These eliminated, in principle, a vast number of possible structures, which might have resulted from reactions of **3** with condensing agents, and prompted us to consider a slow, spontaneous isomerization of the H-phosphonothioate diester **3a** as a source of this side product formation.

This assumption was substantiated by the observation that intensities of the two singlets became comparable to those of the product **3a** when the reaction mixture, resulting from the coupling of equimolar amounts of **1a** and **2** in the presence of 3 equiv of PV-Cl in pyridine, was left standing overnight. In analogous condensations, when the nucleosidic component **2** was used in excess (2 equiv), the intensities of the singlets were ∼7:1, in favor of that resonating at the lower field (δ_P ∼73.6 ppm). Inasmuch as the reaction mixtures were heavily contaminated by a variety of other side products, we carried out similar experiments but with chlorophosphates as condensing agents to be able to extract more detailed ^{31}P NMR data. As anticipated, when the reaction mixtures from the DPCP-promoted condensations were left for 24 h, the whole nucleotidic material could be assigned only to these two singlets and the two resonances due to **3a** (Figure 1b). Since intensities of the two unassigned singlets varied, depending on the excess of the nucleosidic component used for the condensation, we assumed that they belonged to two compounds having two identical substituents at the phosphorus center rather than arising

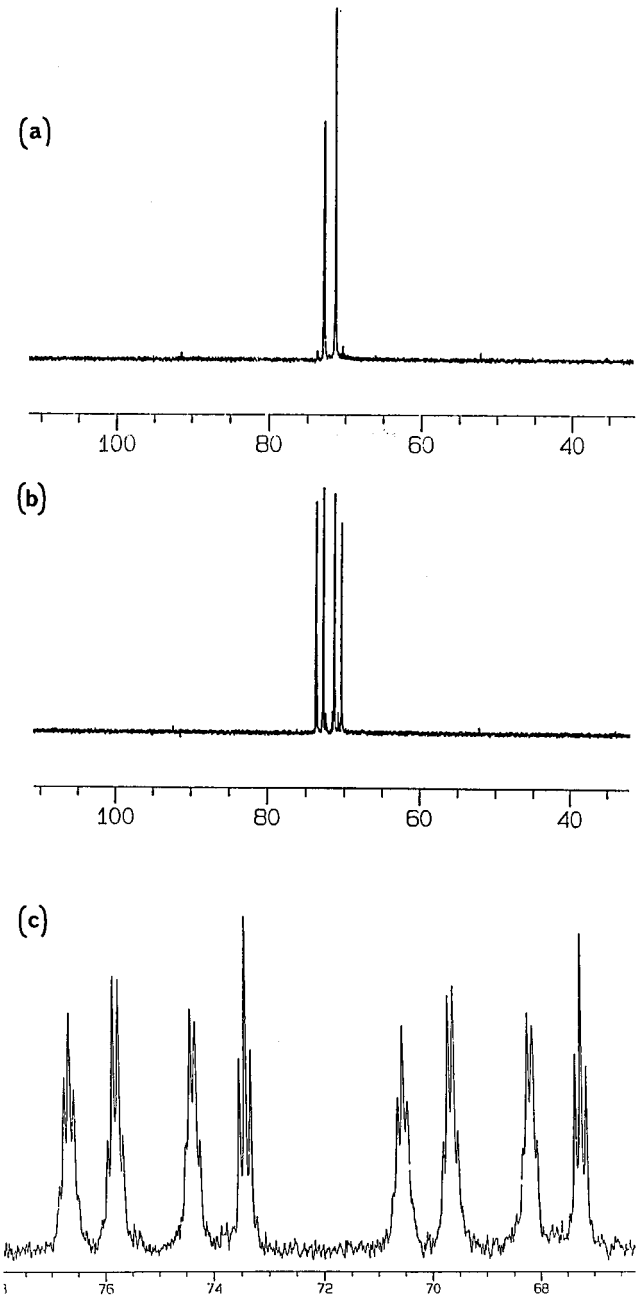

Figure 1. ^{31}P NMR spectra of the reaction between **1a** and **2** promoted by DPCP in pyridine: (a) {^{1}H}-decoupled spectrum after ∼5 min (the highest signals due to **3a**); (b) {^{1}H}-decoupled spectrum after 24 h; (c) ^{31}P−^{1}H coupled spectrum after 24 h.

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from a one-spin system of phosphorus diastereomers (*e.g.*, as in **3a**). The multiplicity of these signals in the $^{31}P-^{1}H$ coupled spectrum (Figure 1c) taken in conjunction with the chemical shift values [$\delta_P \sim 73.60$, $^1J_{PH} = 669.0$ Hz (d), $^3J_{PH} = 9.8$ Hz (quintet); $\delta_P \sim 70.33$, $^1J_{PH} = 676.3$ Hz (d), $^3J_{PH} = 11.6$ Hz (t)] indicated that these resonances can be assigned to two symmetrical dinucleoside H-phosphonothioate diesters **7** and **8**, respectively (Scheme 2).

The mechanism of formation of **7** and **8** is unknown. We can only speculate that these compounds are formed in a pyridine-mediated ligand-exchanged process, which leads to scrambling of substituents at the phosphorus center of the H-phosphonothioate diester **3a**. Detailed studies of this phenomenon are subjects of separate investigations in this laboratory. However, in connection with the present synthetic studies we made a preliminary attempt to suppress this ligand exchange process. To this end we investigated stability of the H-phosphonothioate **3a** in acetonitrile containing various amounts of 2,6-lutidine. In neat acetonitrile no ligand exchange could be detected by ^{31}P NMR spectroscopy, and in the presence of 20% 2,6-lutidine, it did not exceed ~3% after 24 h.

Preparation of Dinucleoside H-Phosphonothioate Diesters 3a−d. On the basis of the above ^{31}P NMR studies we concluded that among the various condensing agents investigated only chlorophosphates secured clean formation of H-phosphonothioate diesters in a coupling reaction between nucleoside H-phosphonothioate monoesters **1** and the appropriate hydroxylic component **2** (Scheme 1). To minimize the ligand exchange process observed for H-phosphonothioate diesters **3** in pyridine, the condensation time should be as short as possible and neat pyridine should be replaced by a mixture of solvents containing only a limited amount of pyridine or other base. To fulfil these requirements we selected for further synthetic studies chlorophosphates that performed couplings most rapidly and cleanly (*i.e.*, DPCP and DECP) and as a solvent mixture, acetonitrile−pyridine (4:1, v/v). Under these reaction conditions, using either DPCP or DECP, the condensation of H-phosphonothioate monoesters **1** to form the corresponding diesters **3** could be effected within 5 min (^{31}P NMR) without the formation of detectable amounts of side products. Condensations to **3** in acetonitrile containing 10 equiv of 2,6-lutidine also proceeded rapidly with DPCP, but they were rather sluggish when DECP was used as a coupling agent (~30% completion after 15 min). No scrambling of ligands in **3** during the course of the reaction could be detected under these conditions (^{31}P NMR spectroscopy).

For a prospective solid phase synthesis of oligonucleoside H-phosphonothioates, the latter reaction conditions (DPCP or DECP in acetonitrile with variable amounts of 2,6-lutidine) may constitute a reasonable starting point to formulate a working synthetic protocol. However, for the preparation of H-phosphonothioate diesters using "solution chemistry", we found it more convenient to use a limited amount of pyridine as a base and as a cosolvent. These alleviated the problem of a nuisance interference from 2,6-lutidine during chromatography, while keeping the ligand exchange process in **3** below the detection level of ^{31}P NMR spectroscopy. All preparative syntheses of the dinucleoside H-phosphonothioate diesters **3a−d** were thus carried out in acetonitrile−pyridine (4:1, v/v) and afforded the desired products in 80−95% yields. Essentially, no differences in yields were observed when, instead of DECP, DPCP was used as a condensing agent. In separate experiments we also assessed the composition of the of crude reaction mixture resulting from the DPCP-promoted condensation of the H-phosphonothioate **1d** with **2** (the ^{31}P NMR spectra). No indication of phosphorylation of the heteroaromatic lactam system of guanine[31] was observed under the reaction conditions.

Experimental Section

Materials and Methods. Pyridine, 2,6-lutidine, acetonitrile, and triethylamine (TEA) were refluxed with CaH_2 and then distilled and stored over 4 Å molecular sieves or CaH_2 (TEA). Pivaloyl chloride, diethyl phosphorochloridate, diphenyl phosphorochloridate, 2,4,6-triisopropylbenzenesulfonyl chloride, bis(2-oxo-3-oxazolidinyl)phosphinic chloride, and diisopropylcarbodiimide were commercial grade (Aldrich).

The nucleoside H-phosphonothioate monoesters **1a−d** were prepared[19] via nonoxidative thiation of the corresponding H-phosphonates. Introduction of the dimethoxytrityl group was done by standard methods.[34] Protecting groups for the heterocyclic bases are the same as those proposed previously[35] for RNA synthesis *via* the H-phosphonate approach. 2-Chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane[36] (NEP) and bis(pentafluorophenyl) carbonate[33] (PFPC) were prepared according to published procedures. TLC analyses were carried out on Merck silica gel 60 F_{254} precoated plates using the following eluents: toluene−methanol (9:1 v/v; system A); chloroform−methanol (9:1 v/v; system B).

The ^{31}P NMR experiments concerning the formation of H-phosphonothioate diesters **3** from **1** and **2** were carried out in 10-mm tubes using 0.05 mmol of phosphorus-containing compounds (**1**) in 2 mL of a solvent. H_3PO_4 (2%) in D_2O was used as external standard (coaxial inner tube). Amounts of the hydroxylic component (**2**) and a coupling agent and the solvent composition are as indicated in the text. The values of the chemical shifts for the intermediates produced *in situ* in some experiments varied (±1 ppm) depending on the reaction conditions.

General Procedure for Synthesis of Dinucleoside H-Phosphonothioate Diesters 3. A suitably protected nucleoside 3′-H-phosphonothioate (**1a-d**, triethylammonium salt, 0.5 mmol) and the nucleosidic component **2** (0.6 mmol) were rendered anhydrous by evaporation of added pyridine. The residue was dissolved in acetonitrile−pyridine (4:1 v/v, 15 mL) and treated with diethyl phosphorochloridate (DECP, 3 equiv) (for **1a−d**) or diphenyl phosphorochloridate (DPCP, 3 equiv) (for **1a** and **1d**) during ca. 5 min (TLC analysis).

The reaction was quenched with saturated sodium chloride (1 mL) and partitioned between toluene (2 × 50 mL) and brine (30 mL). The organic phase was evaporated, and the residue was purified on a silica gel column using ethyl acetate−toluene (1:1, v/v) containing 0.02% triethylamine. This chromatographic system[37] was also suitable for separation of the diastereomers of **3a−d**. Fractions containing the desired product were pooled, concentrated under reduced pressure, and dried overnight on a vacuum line.

5′-*O*-(Dimethoxytrityl)thymidin-3′-yl 3′-*O*-(Dimethoxytrityl)thymidin-5′-yl H-Phosphonothioate (3a).

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(37) One should exercise some caution during chromatography of the reaction mixtures resulting from the DECP-promoted condensations, since tetraethyl pyrophosphate has a chromatographic mobility similar to that of the dimers **3**. In cases where difficulties arise in getting rid of this contamination, a purification using reversed phase chromatography on the silanized silica gel is recommended.

(38) Singlet at 116.5 ppm is most likely due to the corresponding dinucleoside *S*-aryl phosphorodithioate, two singlet at ~69 ppm due to the dinucleoside phosphorothiochloridate, and two singlets at ~59 ppm, due to the dinucleoside phosphorothioate.

Table 1. ^{1}H NMR Chemical Shift Values (in ppm) of Some Diagnostic Signals of Dinucleoside H-Phosphonothioates 3[a]

	P−H ($^1J_{PH}$)	1′-H	3′-H	2-H	5-Me	6-H	8-H
3a (R_P)	7.64 (668.2 Hz), d	6.35	5.47		1.50, 1.85[c]	7.59	
3a (S_P)	7.69 (664.6 Hz), d	6.40	5.45		1.47, 1.88[c]	7.57	
3b (R_P)	7.62 (671.5 Hz), d	6.23	5.33		1.85	8.09	*b*
3b (S_P)	7.69 (662.7 Hz), d	6.30	5.34		1.88	8.06	*b*
3c (R_P)	7.65 (667.0 Hz), d	6.35	5.50	8.10	1.88		8.57
3c (S_P)	7.70 (667.1 Hz), d	6.38	5.50	8.13	1.90		8.62
3d (R_P)	7.71 (668.2 Hz), d	6.10	5.45		1.86		7.74
3d (S_P)	7.72 (664.6 Hz), d	6.15	5.45		1.89		7.83

[a] Signals from the methoxy grops of the dmt moieties appeared at ~3.7 ppm. [b] Signals 5-H buried in multiplets of the aromatic protons. [c] Protons in the thymidin-5′-yl unit of the dimer.

Yield: 95%. **3a** (R_P)[39] (faster moving isomer): R_f = 0.20 (system A); ^{31}P NMR (EtOAc, δ in ppm) 71.38 ($^1J_{PH}$ = 673.9 Hz, $^3J_{PH}$ = 9.8 Hz, dq). **3a** (S_P) (slower moving isomer): R_f = 0.18 (system A); ^{31}P NMR (EtOAc, δ in ppm) 72.32 ($^1J_{PH}$ = 670.8 Hz, $^3J_{PH}$ = 10.0 Hz, dq). For ^{1}H NMR data, see Table 1. HRMS(FAB) (mixture of diastereomers): calcd for $C_{62}H_{62}O_{14}N_4PS$ (M − H$^+$) 1149.3721, found (M − H$^+$) 1149.3748.

5′-*O*-(Dimethoxytrityl)-*N*^{4}-propionyldeoxycytidin-3′-yl 3′-*O*-(Dimethoxytrityl)thymidin-5′-yl H-Phosphonothioate (3b). Yield: 85%. **3b** (R_P) (faster moving isomer): R_f = 0.22 (system A); ^{31}P NMR (EtOAc, δ in ppm) 71.15 ($^1J_{PH}$ = 674.6 Hz, $^3J_{PH}$ = 9.9 Hz, dq) For the ^{1}H NMR data, see Table 1. **3b** (S_P) (slower moving isomer): R_f = 0.20 (system A); ^{31}P NMR (EtOAc, δ in ppm) 72.19 ($^1J_{PH}$ = 668.9 Hz, $^3J_{PH}$ = 9.8 Hz, dq). For ^{1}H NMR data, see Table 1. HRMS(FAB) (mixture of diastereomers): calcd for $C_{64}H_{65}O_{14}N_5PS$ (M − H$^+$) 1190.3987, found (M − H$^+$) 1190.4012.

5′-*O*-(Dimethoxytrityl)-*N*^{6}-butyryldeoxyadenosin-3′-yl 3′-*O*-(Dimethoxytrityl)thymidin-5′-yl H-Phosphonothioate (3c). Yield: 81%. **3c** (R_P) (faster moving isomer): R_f = 0.22 (system A); ^{31}P NMR (EtOAc, δ in ppm) 71.13 ($^1J_{PH}$ = 672.6 Hz, $^3J_{PH}$ = 9.8 Hz, dq). For ^{1}H NMR data, see Table 1. **3c** (S_P) (slower moving isomer): R_f = 0.19 (system A); ^{31}P NMR (EtOAc, δ in ppm) 72.00 ($^1J_{PH}$ = 670.8 Hz, $^3J_{PH}$ = 10.4 Hz, dq). For ^{1}H NMR data, see Table 1. HRMS(FAB) (mixture of diastereomers): calcd for $C_{66}H_{67}O_{13}N_7PS$ (M − H$^+$) 1228.4255, found (M − H$^+$) 1228.4229.

5′-*O*-(Dimethoxytrityl)-*N*^{2}-(phenoxyacetyl)deoxyguanosin-3′-yl 3′-*O*-(Dimethoxytrityl)thymidin-5′-yl H-Phosphonothioate (3d). Yield: 82%. **3d** (R_P) (faster moving isomer): R_f = 0.24 (system A) ^{31}P NMR (EtOAc, δ in ppm) 71.58 ($^1J_{PH}$ = 674.63 Hz, $^3J_{PH}$ = 10.7 Hz, dq). For ^{1}H NMR data, see Table 1. **3d** (S_P) (slower moving isomer): R_f = 0.22 (system A) ^{31}P NMR: (EtOAc, δ in ppm) 72.11 ($^1J_{PH}$ = 675.1 Hz, $^3J_{PH}$ = 9.2 Hz, dq). For ^{1}H NMR data, see Table 1.

HRMS(FAB) (mixture of diastereomers): calcd for $C_{70}H_{67}O_{15}N_7PS$ (M − H$^+$) 1308.4154, found (M − H$^+$) 1308.4187.

Synthesis of 5′-*O*-(Dimethoxytrityl)thymidin-3′-yl 3′-*O*-(Dimethoxytrityl)thymidin-5′-yl 2,2,2-Trimethylacetylphosphonothioate (5). 5′-*O*-(Dimethoxytrityl)thymidine 3′-H-phosphonothioate **1a** (triethylammonium salt, 0.3 mmol) and 3′-*O*-(dimethoxytrityl)thymidine **2** (0.33 mmol) were rendered anhydrous by evaporation of added pyridine. The residue was dissolved in pyridine (2 mL) and treated with pivaloyl chloride (6 equiv) during 1 h. The reaction was quenched with brine, the mixture was extracted with methylene chloride, and the P-acylated product **5** was isolated by silica gel chromatography using ethyl acetate−toluene (1:1, v/v) containing 0.02% TEA as eluent. ^{31}P NMR parameters of **5** were found to be identical with those of the postulated side product observed by ^{31}P NMR spectroscopy during the coupling reaction of **1a** with **2** promoted by PV-Cl (see the text).

5: R_f 0.23 and 0.19 (system A) or 0.61 and 0.58 (system B); ^{31}P NMR (EtOAc, δ in ppm) 65.88 ($^3J_{PH}$ = 8.6 Hz, q), 65.56 ($^3J_{PH}$ = 7.4 Hz, q); ^{1}H NMR ($CDCl_3$, δ in ppm, mixture of diastereomers, selected signals) 7.05 (1H, 6-H), 6.32 (2H, m, 1′-H and 1′-H*), 5.46 (1H, m, 3′-H), 1.84 (3H, s, 5-Me*) 1.46 (3H, s, 5-Me), 1.27 and 1.22 (9H, 2s, Me_3C). HRMS(FAB) (mixture of diastereomers): calcd for $C_{65}H_{66}O_{13}N_4PS$ (M − H$^+$) 1173.4085, found (M − H$^+$) 1173.4028.

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(39) A tentative assignment of the configurations at the phosphorus center was done on the basis of the relative mobilities of the isomers on silica gel and the relative positions of their resonances in ^{31}P NMR spectra.[11,20,21]