NUCLEOSIDE H-PHOSPHONATES. XXI. SYNTHETIC AND ^{31}P NMR STUDIES ON THE PREPARATION OF DINUCLEOSIDE H-PHOSPHONOSELENOATES

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Received March 1, 2006
Accepted April 14, 2006

Dedicated to Professor Antonín Holý on the occasion of his 70th birthday.

Using ^{31}P NMR spectroscopy as a tool, we investigated the condensation of H-phosphonoselenoate monoesters with a hydroxy component in the presence of various coupling agents. On this basis, synthetic protocols that eliminate or significantly suppress side reactions which might occur during the condensation in synthesis of H-phosphonoselenoate derivatives, have been developed. Absolute configuration of the produced dinucleoside H-phosphonoselenoates has been determined by stereochemical correlation analysis.

Keywords: H-Phosphonoselenoates; H-Phosphonates; Coupling agents; ^{31}P NMR spectroscopy; Absolute configuration assignment; Dinucleosides; Oligonucleotides.

As part of our research in H-phosphonate chemistry[1,2] directed toward development of new synthetic methods for biologically important phosphate esters and their analogues, we have recently embarked on synthetic studies on H-phosphonoselenoate esters. To provide an easy access to this new class of phosphonate analogues, we have developed two efficient methods for the preparation of nucleoside H-phosphonoselenoate monoesters[3,4] and showed that these compounds can be converted to the appropriate H-phosphonoselenoate diesters[5] by condensation with suitably protected nucleosides.

H-Phosphonoselenoates are a relatively unexplored class of phosphorus compounds[6–8]. Due to the presence of selenium bound to a phosphorus atom at +3 oxidation state, these compounds can be considered as novel, potentially useful synthetic intermediates for the preparation of new phosphate analogues or selenium-containing derivatives that might be rather difficult to obtain by classical procedures involving oxidation of P(III) com-

Collect. Czech. Chem. Commun. 2006, Vol. 71, No. 6, pp. 820–831

doi:10.1135/cccc20060820

pounds with selenium. Moreover, due to the possibility to control stereochemistry at the phosphorus centre during the oxidation step, P-chiral phosphate analogues with defined stereochemistry can be obtained.

Replacement of one oxygen in H-phosphonate esters by selenium has important stereochemical and chemical consequences. At the monoester level, similarly to H-phosphonothioate monoesters[9,10], it introduces chirality at the phosphorus and this may potentially be exploited in stereospecific synthesis of phosphoroselenoates and other chiral phosphate analogues. The presence of selenium, however, makes two different nucleophilic centers (O and Se) available for activation by a condensing agent. Lack of chemoselectivity in this process may cause a partial elimination of selenium in the course of the reaction, which can lead to formation of side products. At the diester level, substitution of oxygen by selenium at the phosphorus center causes the P–H bond in H-phosphonoselenoates to become noticeably more reactive than that in the oxygen congener. This, in principle, can also be a potential source of side reactions.

Having in mind possible applications of H-phosphonoselenoates as precursor to various oligonucleotide analogues, we have undertaken systematic studies on this class of compounds. In this paper we address the problem of possible side reactions which may occur in preparation of H-phosphonoselenoate diesters by condensation of suitably protected nucleoside 3′-H-phosphonoselenoate monoesters with nucleosides. Various coupling agents and reaction conditions for the condensation have been evaluated. These enabled us to develop optimal synthetic protocols which eliminated or significantly suppressed side reactions during synthesis of dinucleoside H-phosphonoselenoate derivatives. Absolute configuration of the produced H-phosphonoselenoate diesters has been determined by stereochemical correlation analysis.

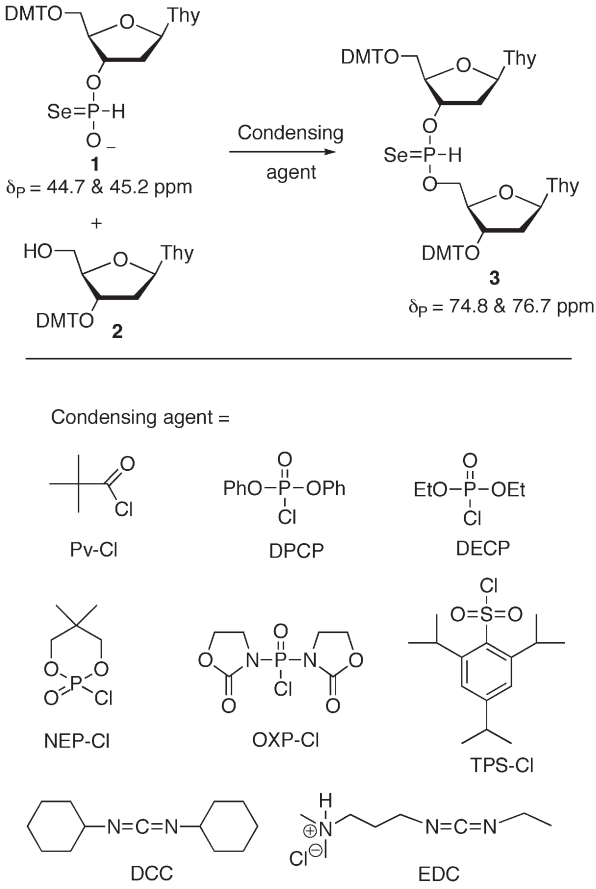
RESULTS AND DISCUSSION

Dinucleoside H-phosphonoselenoates **3** can be produced in a condensation reaction (Scheme 1) using nucleoside H-phosphonoselenoates **1** as a nucleotidic material. It was expected, however, that this rather straightforward reaction for H-phosphonates, might be synthetically more complex in the case of H-phosphonoselenoate derivatives. Similarly to the corresponding thio analogues, H-phosphonoselenoate diesters may be more prone to subsequent reactions during condensation, due to higher reactivity of the P–H bond. In addition, the starting monoesters may react with condensing agents in two different ways (O vs Se activation) producing different prod-

ucts. For these reasons, the choice of a condensing agent in H-phosphonoselenoate diester synthesis can be more critical than in the case of H-phosphonate or H-phosphonothioate derivatives.

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

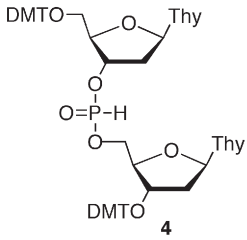
To have a uniform set of data, all condensations have been carried out in pyridine with variable amounts of a condensing agent, and progress of the reactions was monitored by ^{31}P NMR spectroscopy.



SCHEME 1

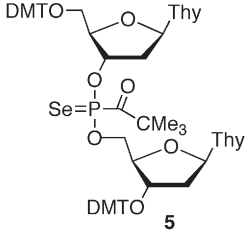
Pivaloyl Chloride as a Condensing Agent

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[11], it was our initial choice for the H-phosphonoselenoate diester formation (Scheme 1). Unfortunately, in the reaction of H-phosphonoselenoate **1** (1.1 equivalents) with nucleoside **2** in the presence of 3 equivalents of Pv-Cl in pyridine, the starting material **1** (δ_P 44.7 and 45.2 ppm) rapidly disappeared (<5 min) but there was no signals that could be assigned to the desired H-phosphonoselenoate diester **3** (two resonances expected at ca. δ_P 76 ppm with $^1J_{PH}$ ca. 650 Hz). Instead, the major products formed gave rise to a signal at δ_P 140.0 (ca. 30%) and two singlets at δ_P 72.6 and 72.3 (ca. 30%). The former signal was tentatively assigned to trinucleoside phosphite **7** (Chart 1), and the two latter signals, on the basis of their chemical shifts and the absence of a P–H bond, to diastereomeric P-acylated derivatives **5**. Among other side products observed in this coupling reaction



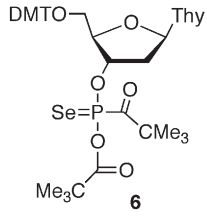
4

δ_P = 9.6 & 8.0 ppm



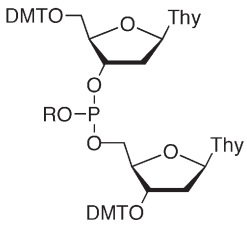
5

δ_P = 72.6 & 72.3 ppm



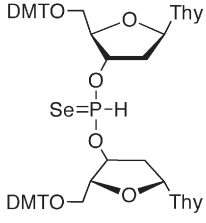
6

δ_P = 53.6 & 53.4 ppm



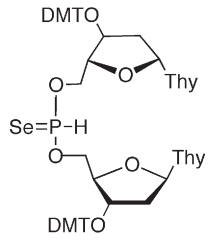
7, R = -T-DMT
δ_P = 140.0 ppm

8, R = Pivaloyl
δ_P = 133.8 & 132.9 ppm



9

δ_P = 70.3 ppm



10

δ_P = 73.8 ppm

Thy = thymin-1-yl

CHART 1

there were diastereomeric H-phosphonate diesters **4** (ca. 15%, δ_P 9.6 and 8.0 ppm, $^1J_{PH}$ = 714 and 7.17 Hz), dinucleoside acyl phosphite **8** (ca. 10%, δ_P 133.8 and 132.9 ppm) and probably P-acylated mixed anhydride **6** (ca. 10%, δ_P 53.6 and 53.4 ppm).

The observed product distribution in this reaction was consistent with a transient formation of H-phosphonoselenoate **3**, which in the presence of pivaloyl chloride underwent rapid P-acylation (to form **5**) or activation on the selenium centre to form ultimately phosphite derivatives **7** and **8**. Since H-phosphonoselenoate **1** was used in a slight excess, its unreacted part could account for the presence of P-acylated mixed anhydride **6**.

As to the formation of H-phosphonate diester **4**, two reaction pathways were possible: activation of the selenium centre in H-phosphonoselenoate monoester **1** by pivaloyl chloride and elimination of selenium upon coupling with a nucleoside, or conversion of pivaloyl phosphite **8** into H-phosphonate diester **4** by hydrolysis with adventitious water or via deacylation. Irrespective of the reaction pathways involved, it seemed that H-phosphonoselenoate diesters **3** were significantly more prone to side reactions than the corresponding H-phosphonothioates[12]. For the latter compounds, under analogous reaction conditions, the side reactions amounted to ca. 10% and the desired H-phosphonothioate diesters were formed as major products of the reaction. Also, in the case of H-phosphonothioate diesters, there were no H-phosphonate diesters formed, the presence of which might indicate incomplete chemoselectivity during activation.

A dramatic improvement was observed when the above coupling reaction was carried out in the presence of 1.5 equivalents of pivaloyl chloride. In this case the desired H-phosphonoselenoate **3** (δ_P 74.8 and 76.7 ppm, $^1J_{PH}$ = 648 and 644 Hz, $^1J_{PSe}$ = 877 and 879 Hz) was formed as a major product (ca. 80%) together with small amounts of phosphite triester **7** (<5%), H-phosphonate diester **4** (ca. 5%), and compound **6** (ca. 5%). Similarly to H-phosphonothioate diesters[12], the ^{31}P NMR resonances due to **3** were also accompanied by two very small signals at the higher and lower field. These signals (δ_P 70.3 and 73.8 ppm), which increased in the course of time and amounted to ca. 5% of the total nucleotidic material after 5 min, were assigned to the ligand exchange products, symmetrical dinucleoside H-phosphonoselenoates **9** and **10**.

The mechanism of the formation of **9** and **10** is not known. We can only speculate that these compounds are formed in a pyridine-mediated ligand-exchange process, which leads to scrambling of the substituents at the phosphorus centre of the H-phosphonoselenoate diesters **3** [13]. Consistent with this, the ligand exchange process was significantly suppressed upon

changing the reaction medium to acetonitrile–pyridine (4:1, v/v), and practically eliminated upon replacement of pyridine by 2,6-lutidine. The latter reaction was rapid and rather clean. Unfortunately, although H-phosphonates **4** were formed only in trace amounts (<2%), there was an increase in the P-acylation of H-phosphonoselenoate **3** (formation of acylphosphonate **5**, ca. 10%), most likely due to higher basicity of 2,6-lutidine than that of pyridine.

By observing trends in product distribution upon varying the amount of pivaloyl chloride and reaction conditions, one can conclude that apparently the activation step of H-phosphonoselenoates **1** is not completely chemoselective (selenium activation might occurs to some extent) and that, due to rather high reactivity of the P–H bond, this coupling agent might have a limited application in H-phosphonoselenoate diesters synthesis.

Chlorophosphates as Condensing Agents

Due to low reactivity of S-nucleophiles towards the phosphorus centres[14], we expected that the use of chlorophosphates to produce H-phosphonoselenoate diesters should alleviate problems connected with subsequent reactions of **3** with condensing agents. To this end we carried out condensations of H-phosphonoselenoate **1** with a nucleosidic component **2** in the presence of diphenyl phosphorochloridate[15] (DPCP), diethyl phosphorochloridate[16] (DECP), 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane[16] (NEP-Cl), and bis(2-oxo-oxazolidin-3-yl)phosphinic chloride[16,17] (OXP-Cl) (Scheme 1).

Indeed, all these reagents, which have previously been evaluated in the synthesis of H-phosphonate[16] and H-phosphonothioate diesters[12], promoted also clean formation of the H-phosphonoselenoates **3** when used in a 3 equivalents excess over the starting material **1**. Possible side products, which might have resulted from the subsequent reaction of **3** with the condensing agents (formation of phosphite triester **7** or hypophosphates[18]), 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 all chlorophosphates investigated were very fast in pyridine (<5 min), but differed in kinetics when the reactions were carried out in the presence of limited amounts of pyridine.

DPCP and DECP as condensing agents gave indistinguishable results both in terms of kinetics and purity of the produced H-phosphonoselenoates **3**. Both of them promoted rapid (<5 min) condensations in neat pyridine and

in acetonitrile containing only 5 equivalents of the nucleophilic catalyst. In the reactions in pyridine, traces of the ligand exchange products (compounds **9** and **10**) could be observed after 5 min, but when the amount of pyridine was reduced to few equivalents, these side products could not be detected even after 30 min (^{31}P NMR spectroscopy). For the DECP-promoted condensations in neat pyridine, formation of unidentified side product (δ_P 101.7 ppm, ca. 5%) was usually observed.

A sterically hindered chlorophosphate, NEP-Cl, in neat pyridine promoted rapid (<5 min) and clean condensation, but in acetonitrile containing only 5 equivalents of pyridine, the condensation time increased to 60 min. For both reactions, the ligand exchange products **9** and **10** were observed (<5%), due to high concentration of pyridine or the extended reaction time (for the reactions with limited amounts of pyridine). Similarly to the reactions in neat pyridine in which DECP was used, also in this case, a by-product resonating at δ_P 100.7 ppm (ca. 2%), was formed.

The last chlorophosphate investigated, OXP-Cl showed like NEP-Cl, a sensitivity to the amount of pyridine present in the reaction mixture, but to smaller extent. For this condensing agent, the reaction time in acetonitrile in the presence of 5 equivalents of pyridine was ca. 30 min, and this apparently secured a clean coupling without formation of noticeable amounts of the ligand exchange products (^{31}P NMR spectroscopy).

TPS-Cl-Promoted Condensations

The reaction of H-phosphonoselenoate **1** (1.1 equivalents) with nucleoside **2** in pyridine in the presence of 3 equivalents of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl) produced after 5 min a complex mixture of products, none of them containing a P–H bond. In the light of our previous investigations of TPS-Cl as a condensing agent in the synthesis of H-phosphonate diesters[19], and the fact that arenesulfonyl derivatives did not show chemoselectivity in activation of the corresponding H-phosphonothioate derivatives[12], studies with this class of condensing agents were not pursued further.

Carbodiimides as Coupling Agents

Two carbodiimides, namely dicyclohexyl (DCC) and 1-[3-(dimethylamino)-propyl]-3-ethyl (EDC) derivatives (Scheme 1), were evaluated in reactions of H-phosphonoselenoate monoesters **1** (1.1 equivalents) with nucleoside **2** promoted by condensing agents. The reactions were carried out in pyridine

or in acetonitrile–pyridine (4:1, v/v) using pyridine hydrochloride (3 equivalents) as acid catalyst. With 3 equivalents of the condensing agents, the reactions were complete in less than 5 min affording as major product (98%) dinucleoside H-phosphonate **4**, irrespective of the kind of the carbodiimide or the reaction conditions used. Always, a small amount of H-phosphonoselenoate **3** (1–2%) was present in the reaction mixtures, indicating an incomplete chemoselectivity during the activation step. Occasionally, also variable amounts (0–1%) of the corresponding nucleoside H-phosphonate monoester were formed during the coupling reactions, most likely due to the presence of adventitious water.

Since EDC is commercially available in the form of its hydrochloride, we evaluated this condensing agent without external acid catalyst added. The coupling reaction of H-phosphonoselenoate **1** and nucleoside **2** in acetonitrile–pyridine (4:1, v/v) under such conditions was significantly slower (30 min for the disappearance of **1**) but showed improved chemoselectivity. Unfortunately, although the reaction produced H-phosphonate **4** (ca. 70%) without detectable amounts of H-phosphonoselenoate diester **3**, a significant portion of **1** (ca. 30%) was usually converted to the corresponding nucleoside H-phosphonate monoester.

To conclude this part, on the basis of the above ^{31}P NMR studies we found that among the various condensing agents investigated only chlorophosphates secured clean formation of H-phosphonoselenoate diesters **3** in the coupling reaction between nucleoside H-phosphonothioate monoesters **1** and the appropriate hydroxy component **2** (Scheme 1), with complete chemoselectivity in the activation step. To minimize the ligand exchange process, the condensation time should be as short as possible and the reactions should be preferably carried out in neutral solvents containing limited amounts of pyridine.

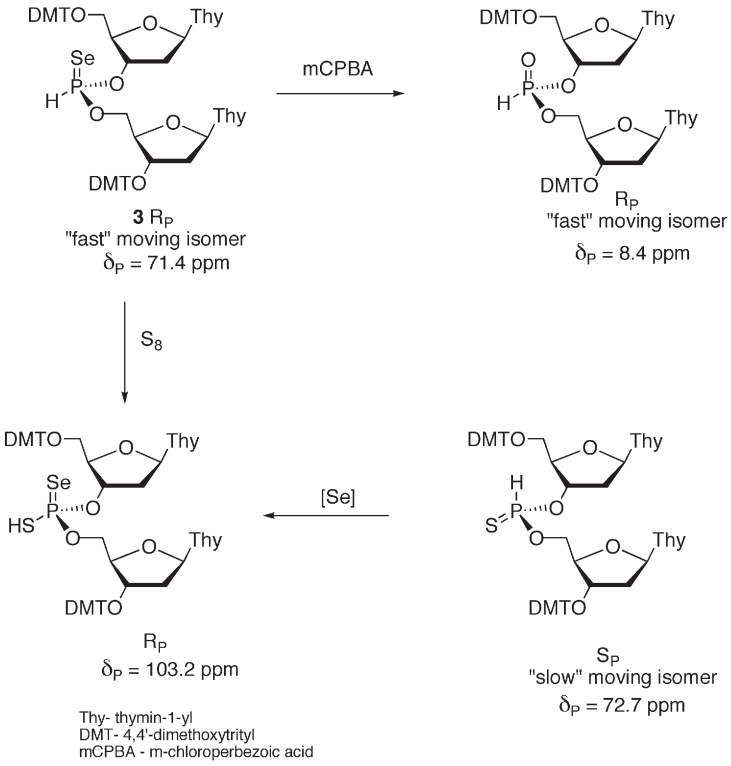
Taking this into consideration, dinucleoside H-phosphonoselenoate **3** was synthesised on a preparative scale in acetonitrile containing pyridine (5 equivalents) using DPCP or DECP as condensing agents. After work-up and chromatography on a silica gel column, compound **3** (1:1 mixture of the diastereomers) was obtained in a yield higher than 80% yield. The P-diastereomers of **3** showed sufficiently large differences in mobility on silica gel to separate them by the chromatography.

*Absolute Configuration of Diastereomeric H-Phosphonoselenoates **3***

As a final stage of these investigations, assignment of the absolute stereochemical configuration at the phosphorus atom in H-phosphonoselenoate

diesters **3** was undertaken. The diastereomeric H-phosphonate and the H-phosphonothioate diesters with known configuration were used as references[20–22]. The absolute configuration of H-phosphonate diesters was determined previously on the basis of sulfurization (proceeding with retention of configuration), followed by stereospecific degradation of the produced phosphorothioates with SVPDE and P1 nuclease[21,22]. By employing desulfurization with *m*-chloroperbenzoic acid (mCPBA), the configuration of H-phosphonothioates was correlated with that of the corresponding H-phosphonates[20].

For H-phosphonoselenoate diesters, we performed a similar stereochemical correlation analysis in order to determine the absolute configuration of the dinucleoside H-phosphonoselenoates **3**. Thus, treatment of the diastereomer of H-phosphonoselenoate **3** demonstrating higher mobility on silica gel ("fast" isomer) with mCPBA yielded the corresponding H-phosphonate diester **11** with R_P configuration[20–22] (Scheme 2). Since



SCHEME 2

deselenization with mCPBA most likely occurs with retention of configuration around the phosphorus atom, this defines R_P configuration for the "fast" isomer of **3**.

To substantiate this assignment, the "fast" H-phosphonoselenoate **3** was subsequently sulfurized using elemental sulfur. This afforded the corresponding (R_P)-phosphorothioselenoate diester **13** [20], which was identical to the product obtained via selenization of the (S_P)-H-phosphonothioate **12** (Scheme 2).

A similar correlation analysis performed on the "slow" isomer of **3** confirmed these assignments. Thus, we can conclude that the "fast" H-phosphonoselenoate **3** has the R_P configuration, while the "slow" isomer, S_P.

In conclusion, using ^{31}P NMR spectroscopy we investigated the condensation of nucleoside H-phosphonoselenoate monoester **1** with a nucleosidic component under various experimental conditions, and on this basis developed an efficient protocol for the preparation of dinucleoside H-phosphonoselenoate diesters **3**. Absolute configuration of these P-chiral compounds was determined by stereochemical correlation analysis.

EXPERIMENTAL

Materials and Methods

Pyridine, 2,6-lutidine, acetonitrile, and triethylamine (TEA) were refluxed with CaH_2, then distilled and stored over 4Å molecular sieves or CaH_2 (TEA). Pivaloyl chloride (Pv-Cl), diethyl phosphorochloridate (DECP), diphenyl phosphorochloridate (DPCP), 2,4,6-triisopropylbenzenesulfonyl chloride (TPS-Cl), bis(2-oxo-oxazolidin-3-yl)phosphinic chloride (OXP-Cl), dicyclohexylcarbodiimide (DCC), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC), were of commercial grade (Aldrich).

Nucleoside H-phosphonoselenoate monoester **1** was prepared by phosphinylation of 5′-*O*-(4,4′-dimethytrityl)thymidine, followed by selenization of the produced nucleoside phosphinate[5]. 2-Chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane (NEP-Cl) was obtained using the published procedure[23]. 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). ^1H, ^{13}C and ^{31}P NMR specra were measured in $CDCl_4$; chemical shifts (δ scale) are given in ppm, coupling constants (*J*) in Hz. The ^{31}P NMR experiments concerning the formation of H-phosphonoselenoate 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). The amounts of the hydroxy 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. A tentative assignment of structure of intermediate or by-products **4**–**10** observed during the coupling reaction was done on the basis of known or expected ^{31}P NMR chemical shifts, the observed splitting patterns or comparison with authentic samples.

5′-*O*-(4,4′-Dimethoxytrityl)thymidin-3′-yl 3′-*O*-(4,4′-Dimethoxytrityl)thymidin-5′-yl H-Phosphonoselenoate (**3**)

5′-*O*-(4,4′-Dimethoxytrityl)thymidin 3′-H-phosphonoselenoate **1** (triethylammonium salt, 0.22 mmol) and 3′-*O*-(4,4′-dimethoxytrityl)thymidine **2** (0.2 mmol) were dried by repeated evaporation of the added pyridine. The oily residue was dissolved in acetonitrile (4 ml) containing pyridine (1 mmol), and then diphenyl chlorophosphate (0.5 mmol) was added. After 5 min, the reaction was quenched with saturated sodium chloride solution (1 ml) and the mixture was partitioned between toluene (2 × 50 ml) and brine (20 ml). The organic phase was dried, evaporated and the residue was purified on a silica gel column using ethyl acetate–toluene (1:1, v/v) containing 0.01% triethylamine as eluent. The product was isolated as a white solid in 80% yield (purity > 98%, ca. 1:1 mixture of diastereoisomers; ^{1}H NMR spectroscopy). FAB HRMS $[M]^+$, found: 1221.3145; $C_{62}H_{63}N_4NaO_{14}PSe$ requires 1221.3141.

Compound **3** could be separated into P-diastereomers. These are referred to as “faster” and “slower” moving diastereomer, according to their chromatographic mobilities on silica gel.

Faster moving diastereoisomer (R_P)-**3**, R_F 0.22 (system A): ^{1}H NMR: 9.61 (s, 1 H, NH); 9.51 (s, 1 H, NH); 8.18 (d, $^1J_{PH}$ = 649, 1 H); 7.36 (m, 20 H); 6.85 (m, 8 H); 6.38 (dd, ^3J = 8.3 and 6.0, 1 H); 6.29 (dd, ^3J = 8.2 and 6.1, 1 H); 5.53 (m, 1 H); 4.28 (m, 1 H); 4.17 (m, 1 H); 4.1 (m, 1 H); 3.79 (s, 6 H); 3.78 (s, 6 H); 3.62 (m, 2 H); 3.44 (m, 2 H); 2.36 (m, 2 H); 2.02 (m, 1 H); 1.88 (s, 3 H); 1.77 (m, 1 H); 1.48 (s, 3 H). ^{31}P NMR: 71.36 ($^1J_{PH}$ = 649, $^3J_{PH}$ = 9.95, $^1J_{PSe}$ = 878). ^{13}C NMR: 164.17, 164.16, 159.09, 159.05, 150.79, 150.65, 145.10, 144.39, 136.22, 136.19, 135.95 135.45, 135.28, 130.52, 130.45, 130.29, 128.57, 128.51, 128.35, 127.50, 113.74, 113.73, 113.65, 112.00, 111.57, 87.74, 87.54, 86.34, 84.94 (J = 6.44), 84.66, 84.22 (J = 7.73), 78.41, 74.32, 66.83, 66.77, 63.41, 55.52, 39.43, 39.12, 12.78, 12.04.

Slower moving diastereoisomer (S_P)-**3**, R_F 0.18 (system A): ^{1}H NMR: 9.57 (s, 1 H, NH); 9.37 (s, 1 H, NH); 8.25 (d, $^1J_{PH}$ = 645, 1 H); 7.38 (m, 20 H); 6.84 (m, 8 H); 6.38 (m, 1 H); 6.18 (dd, ^3J = 8.6 and 5.9, 1 H); 5.53 (m, 1 H); 4.37 (m, 1 H); 4.21 (m, 1 H); 4.09 (m, 1 H); 3.98 (m, 1 H); 3.78 (s, 12 H); 3.66 (m, 1 H); 3.45 (m, 1 H); 3.35 (m, 1 H); 2.53 (m, 1 H); 2.38 (m, 1 H); 1.89 (m, 1 H); 1.83 (s, 6 H); 1.70 (m, 1 H). ^{31}P NMR: 73.24 ($^1J_{PH}$ = 645, $^3J_{PH}$ = 10.6, $^1J_{PSe}$ = 879). ^{13}C NMR: 162.22, 159.06, 158.96, 158.94, 150.77, 145.37, 137.39, 136.57, 136.51, 136.17, 135.42, 130.51, 130.47, 130.30, 128.57, 128.53, 128.46, 128.37, 128.30, 128.23, 127.50, 127.33, 113.68, 113.58, 111.17, 87.46, 86.85, 85.17, 74.54, 62.76, 55.52, 55.49, 38.95, 12.67.

Financial support from the Swedish Research Council is gratefully acknowledged.

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