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Stereochemistry of the DBU/LiCl-Assisted Nucleophilic Substitution at Phosphorus in Nucleoside-3'-O-(*Se*-methyl Methanephosphonoselenolate)s[†]

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The crystal and molecular structure of diastereomerically pure *N*⁴-benzoyl-2'-deoxycytidine 3'-O-(*Se*-methyl methanephosphonoselenolate (FAST-eluted) (**4**, R = H, B = C^{Bz}) and 5'-O-pixylthymidine 3'-O-(*S*-methyl methanephosphonothiolate (FAST-eluted) (**5**) have been elucidated by X-ray crystallography. The absolute configuration at the phosphorus atom in both compounds is *S*_P. Each **FAST-4'** (R = DMT, B = C^{Bz}) and **FAST-5** (R = Px, B = Thy) in the process of DBU/LiCl-assisted condensation with 3'-O-acetyl-*N*⁴-benzoylcytidine and 3'-O-acetylthymidine gave after deprotection (*S*_P)-dicytidine-(3',5')-methanephosphonate and (*S*_P)-dithymidine-(3',5')-methanephosphonate, respectively. Unambiguous assignment of the absolute configuration at the phosphorus in **4** (R = H, B = C^{Bz}) and **5** (R = Px, B = Thy) allows for stereochemical correlation and the conclusion that DBU/LiCl-assisted nucleophilic substitution at phosphorus occurs with *net inversion of configuration*, in contrast to our earlier erroneous deduction.⁵ Moreover, the knowledge of the absolute configuration at the phosphorus atom in both **4** (R = H, B = C^{Bz}) and **5** (R = Px, B = Thy) allows for assignment of the absolute configuration at phosphorus in precursors of **4'** and **5**, such as 5'-O-DMT-nucleoside 3'-O-methanephosphonothioanilidates (**6**), methanephosphonoanilidates (**8**), and methanephosphonoselenoanilidates (**9**).

Introduction

(all-*R*_P)-Oligo(nucleoside methanephosphonate)s (**1**) and chimeric oligonucleotide constructs (**2**) containing incorporated (*R*_P)-dinucleoside-(3',5')-methanephosphonates (**3**) (Figure 1) constitute promising second-generation *antisense* mRNA molecules due to their resistance to nucleolytic degradation and their high affinity for complementary mRNA.¹

Stereocontrolled synthesis of **1** and **3** is still a matter of challenge in several research establishments, although the chimeric molecules **2** have become available from either chromatographic separation of dinucleoside-(3',5')-methanephosphonates (**3**), followed by incorporation of (*R*_P)-**3** into an oligonucleotide chain,² or the stereocontrolled synthesis of (*R*_P)-**3** from diastereomerically pure precursors such as 5'-O-DMT-nucleoside 3'-O-(4-nitrophenyl methanephosphonates),³ 5'-O-DMT-nucleoside-3'-O-(*O*-hexafluoroisopropyl methanephosphonates)⁴ or 5'-O-DMT-nucleoside-3'-O-(*Se*-methyl methanephosphono-

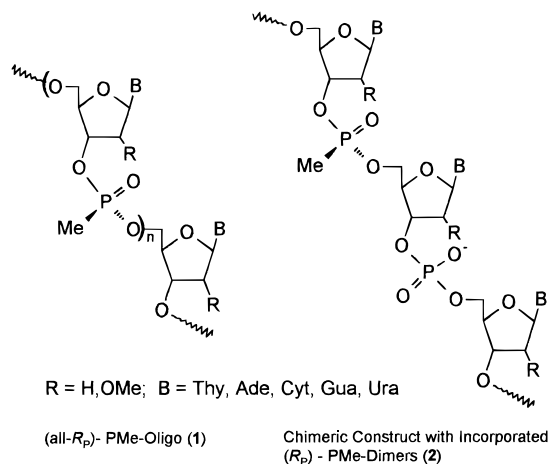


Figure 1. Schematic representations of (*R*_P)-dinucleoside (3',5')-methanephosphonates and *chimeric constructs*.

selenolates) (**4'**).⁵ Recently a novel, cost-effective stereospecific synthesis of (*R*_P)-**3** based upon the stereocontrolled synthesis of 5'-O-DMT-nucleoside-3'-O-(*S*-methyl methanephosphonothiolates) (**5**) has been reported from this laboratory.⁶

The synthesis of (*R*_P)-**3**, depicted in Scheme 1, relies upon the synthesis and separation into diastereomers of protected nucleoside 3'-O-methanephosphonothioanilidates (**6**).

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[†] This paper is dedicated to Professor Marian Mikolajczyk on the occasion of his 60th Birthday.

[‡] Technical University of Łódź.

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Scheme 1

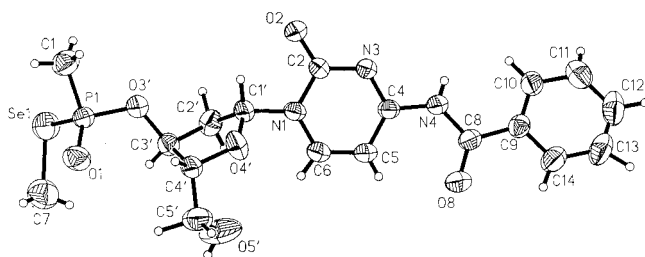
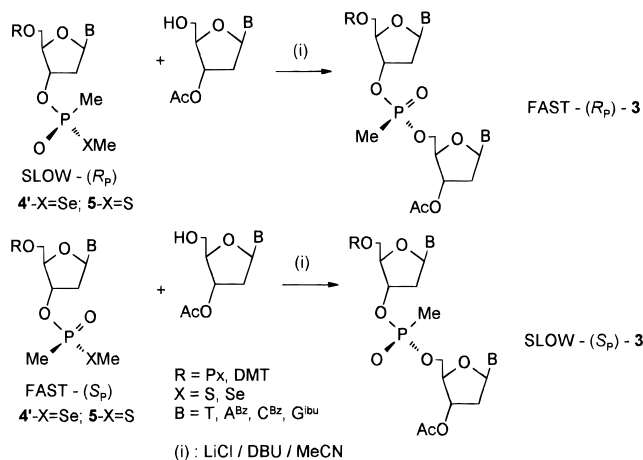


Figure 2. Molecular structure of **FAST-4** (R = H, B = C^{Bz}) showing 50% probability displacement ellipsoids.

The corollary of our current strategy is the use of both separated diastereomers of each precursor **6** for preparation of the desired (*R_p*)-diastereomer **5**.

Unfortunately, in our earlier work,⁵ the absolute configuration at the phosphorus in FAST- 5'-*O*-pixylthymidine 3'-*O*-(*Se*-methyl methanephosphonoselenolate) (**7**) was erroneously assigned, which led us to the incorrect conclusion that the process of DBU/LiCl-assisted condensation of 5'-*O*-pixylthymidine 3'-*O*-(*Se*-methyl methanephosphonoselenolate) **7** with 3'-*O*-acetylthymidine occurred with retention of configuration.

In this report, the results of the detailed X-ray studies of fast-eluted diastereomers of *N*⁴-benzoyl-2'-deoxycytidine-3'-*O*-(*Se*-methyl methanephosphonoselenolate) (**4**) (R = H, B = C^{Bz}) (Figure 2) and fast-eluted 5'-*O*-Px-thymidine 3'-*O*-(*S*-methyl methanephosphonothiolate) (**5**) (R = Px, B = Thy) (Figures 3 and 4) are presented. Both **FAST-5** (R = Px, B = Thy) and **FAST-4** (R = H, B = C^{Bz}) appeared to have the (*S_p*)-configuration. Because DBU/LiCl assisted condensation of FAST-(*S_p*)-**5** with 3'-acetylthymidine gives (*S_p*)-dithymidine-3',5'-methanephosphonate ((*S_p*)-**3**), this process occurs with inversion of configuration. Moreover, since each diastereomer **4'** and **5** is prepared in the Wadsworth–Emmons–Horner–type reaction^{7a} via stereoretentive conversion either from a single diastereomer of 5'-*O*-DMT-nucleoside 3'-*O*-methanephosphonothioanilide **6** or methanephosphonoanilide **8**, followed by alkylation, an unambiguous assignment of the absolute configuration at phosphorus in diastereomers **6** and **8** has become feasible.

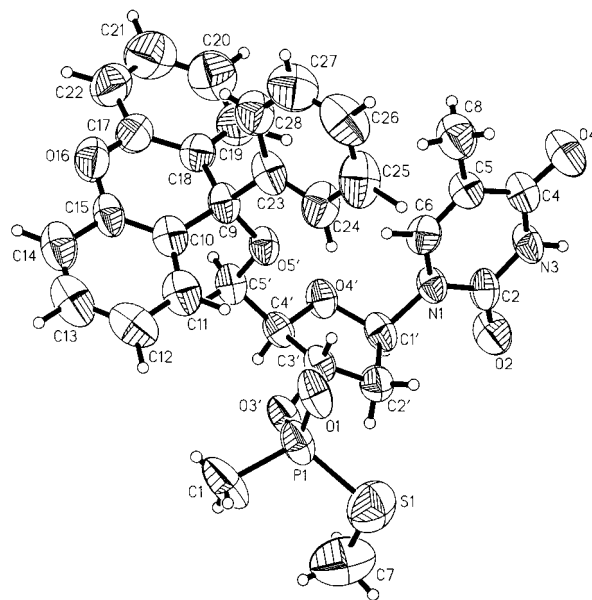


Figure 3. Molecular structure of **FAST-5** (R = Px, B = Thy) (molecule **a**) showing 50% probability displacement ellipsoids.

Results and Discussion

Synthesis of Selenoloester 4 (R = H, B = C^{Bz}) and Thiolester 5 (R = Px, B = Thy). The reaction of 5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine with MePCl₂ was performed in THF at -40 °C and was followed by addition of aniline and elemental selenium. After standard workup, **9** (R = DMT, B = C^{Bz}) was obtained in 71% yield as a mixture of two diastereomers (in ~1:1 ratio), which were separated into diastereomerically pure species by means of column chromatography (silica gel 60, 230–400 mesh) to give **FAST-9** (R = DMT, B = C^{Bz}) and **SLOW-9** (R = DMT, B = C^{Bz}). Each separated diastereomer **9** (R = DMT, B = C^{Bz}) in DMF or THF solution was activated with sodium hydride (1.3 molar equiv), and into the resulting slurry the stream of dry CO₂ was introduced. The obtained sodium 5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine 3'-*O*-methanephosphonoselenoate was, without isolation, alkylated with MeI (5 equiv) to give the corresponding 5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine 3'-*O*-(*Se*-methyl methanephosphonoselenolate) (**4'**); thus, from methanephosphonoselenoanilide **FAST-9** (R = DMT, B = C^{Bz}), slow-eluted ester **SLOW-4'** (R = DMT, B = C^{Bz}) was obtained, and methanephosphonoselenoanilide **SLOW-9** (R = DMT, B = C^{Bz}) was the substrate for preparation of fast-eluted *Se*-methyl ester **FAST-4'**. Ester **FAST-4'** (R = DMT, B = C^{Bz}) was deprotected by an acid treatment and purified by means of flash silica gel column chromatography, and pure **FAST 4** (R = H, B = C^{Bz}) was crystallized in darkness from EtOH–H₂O (95:5 v/v) solution.

Analogously, 5'-*O*-Px-thymidine gave the appropriate methanephosphonothioanilide (**6**) (R = Px, B = Thy) after treatment with MePCl₂ at -40 °C, followed by addition of aniline and elemental sulfur. After standard workup, **6** (R = Px, B = Thy) was obtained in 91% yield as a mixture of two diastereomers, which were separated into diastereomerically pure species by silica gel column chromatography to give **FAST-6** (R = Px, B = Thy) and **SLOW-6** (R = Px, B = Thy).

Each separated diastereomer **6** was converted (NaH/CO₂ treatment) into sodium 5'-*O*-Px-thymidine-3'-*O*-

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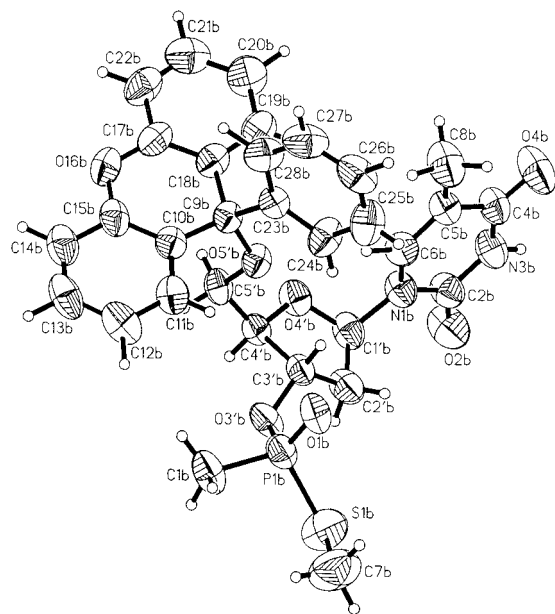


Figure 4. Molecular structure of **FAST-5** ($R = \text{Px}$, $B = \text{Thy}$) (molecule **b**) showing 50% probability displacement ellipsoids.

methanephosphonothioate and, without isolation, was alkylated with MeI (5 equiv) to give the corresponding 5'- O -Px-thymidine-3'- O -(*S*-methyl methanephosphonothiolate) (**5**) ($R = \text{Px}$, $B = \text{Thy}$). Compound **FAST-6** ($R = \text{Px}$, $B = \text{Thy}$) was converted into **SLOW-5** ($R = \text{Px}$, $B = \text{Thy}$), while **SLOW-6** ($R = \text{Px}$, $B = \text{Thy}$) was a substrate for preparation of **FAST-5** ($R = \text{Px}$, $B = \text{Thy}$). Fully protected **FAST-5** ($R = \text{Px}$, $B = \text{Thy}$) was crystallized from a CH_2Cl_2 -hexane mixture (slow evaporation of methylene chloride).

X-ray studies. The molecular structures of methanephosphonoselenoester **FAST-4** ($R = \text{H}$, $B = \text{C}^{\text{Bz}}$) and methanephosphonothioester **FAST-5** ($R = \text{Px}$, $B = \text{Thy}$) were determined by a single-crystal X-ray analysis and are presented in Figures 2 [**FAST-4** ($R = \text{H}$, $B = \text{C}^{\text{Bz}}$)], 3, and 4 [**FAST-5** ($R = \text{Px}$, $B = \text{Thy}$)].

The compound **FAST-4** ($R = \text{H}$, $B = \text{C}^{\text{Bz}}$) consists of one molecule in the independent part of the elemental cell, while **FAST-5** ($R = \text{Px}$, $B = \text{Thy}$) consists of two formal **a** and **b** molecules in the independent part of the elemental cell, both possessing the absolute configuration at the chiral centers identical with the configuration of **FAST-4** ($R = \text{H}$, $B = \text{C}^{\text{Bz}}$), and determined as R_{C1} , S_{C3} , R_{C4} , and S_{P1} . Both the substitution of selenium for the sulfur atom, incorporation of the 5'-Px protecting group in **FAST-5**, as well as different crystallization conditions change significantly the structure of the elemental cell, thus creating enough space for four molecules of hexane to cocrystallize inside the elemental cell [structure **FAST-5** ($R = \text{Px}$, $B = \text{Thy}$)].

Crystal data and structure solution for **FAST-4** and **FAST-5** are collected in Table 1. Further detailed discussion of these results is presented in the Supporting Information.

Using the Cahn-Ingold-Prelog rules⁸ for the description of the absolute configuration at the phosphorus center, with the priority order $\text{MeSe}(\text{MeS}) > \text{nucleoside}$

3'- $O > O > \text{Me}$, the absolute configurations at phosphorus in compounds **FAST-4'** ($R = \text{DMT}$, $B = \text{C}^{\text{Bz}}$) and **FAST-5** ($R = \text{Px}$, $B = \text{Thy}$) were assigned as (S_{P}). These data were used as the foundation for reinvestigation of the stereochemical pathway of the substitution of the alkylseleno group in (*Se*-alkyl methanephosphonoselenolate)s and the *S*-alkyl group in (*S*-alkyl methanephosphonothiolate)s by alcohols, in particular by the 5'-OH group of nucleosides.

Condensation of FAST-4' ($R = \text{DMT}$, $B = \text{C}^{\text{Bz}}$) with 3'- O -acetyl-2'-deoxy-*N*⁴-benzoylcytidine. FAST-eluted 5'- O -DMT-*N*⁴-benzoyl-2'-deoxycytidine 3'- O -(*Se*-methyl methanephosphonoselenolate) ((S_{P})-**4'**) was condensed with 3'- O -acetyl-2'-deoxy-*N*⁴-benzoylcytidine. The reaction was performed in MeCN in the presence of DBU and LiCl (Scheme 1). The reaction progress was controlled by HP TLC. Upon completion (decease of (S_{P})-**4'**), the reaction mixture was diluted with chloroform, washed twice with 0.05 M citric acid, concentrated to dryness, and purified by silica gel column chromatography. The product of condensation, SLOW-*N*⁴-benzoyl-5'- O -DMT-2'-(deoxycytidylyl)-(3',5')-*N*⁴-benzoyl-3'- O -acetyl-2'-deoxycytidine-3'-methanephosphonate (**3**, $R = \text{DMT}$, $B = \text{C}^{\text{Bz}}$), was isolated, fully characterized (^{31}P NMR δ 33.00 ppm; ^1H NMR (CDCl_3) δ , 1.58 (d, 3H, $^2J_{\text{P-CH}_3}$ = 17.6, Hz, PCH_3); FAB-MS [$M - \text{H}$] 1067), and deprotected according to the standard procedures.⁵

After RP-HPLC purification, fully deprotected **3** ($R = \text{H}$, $B = \text{Cyt}$) was compared with the authentic sample of the diastereomeric mixture of d(C_{PMcC}) obtained on a solid support via *nonstereospecific* methanephosphonoamidite method and by co-injection on RP-HPLC it was found to be identical with the SLOW-eluted diastereomer, (S_{P})-**3**.^{9d}

In an analogous way, the reaction between FAST-(S_{P})-**5** and 3'- O -acetylthymidine was performed. The resulting fully protected dimer **SLOW-3** ($R = \text{DMT}$, $B = \text{Thy}$) was isolated, characterized (^{31}P NMR δ 34.14; ^1H NMR (CDCl_3) 1.57 (d, 3H, $^2J_{\text{P-CH}_3}$ = 17.6 Hz, PCH_3); FAB-MS [$M - \text{H}$] 887), and after deprotection compared with an authentic sample of MIX-**3** ($R = \text{H}$, $B = \text{Thy}$) prepared via the methanephosphonoamidite method. The product of condensation was identical, as proved by RP HPLC, with (S_{P})-**3**. The condensation reactions, under the same conditions, led to the opposite products, namely (R_{P})-**3**, when the opposite diastereomers **SLOW-4'** and **SLOW-5** were used as substrates.

The Stereochemistry of Condensation of Nucleoside 3'- O -(*Se*-Methyl methanephosphonoselenolate)s (4'**) and *S*-Methyl Methanephosphonothiolates (**5**) with 3'- O -Protected Nucleosides: A Correction.** As described above, **FAST-4'** and **FAST-5** were the precursors of **SLOW-3** while the condensation of **SLOW-4'** and **SLOW-5** with the appropriately protected nucleosides, under analogous conditions, gave **FAST-3** (Scheme 1).

Since all data coming from both X-ray and NMR studies confirmed the absolute configuration at phosphorus in **FAST-3** as (R_{P})-isomers and that of the **SLOW-3** as (S_{P})⁹ (Figure 1), the DBU/LiCl-assisted nucleophilic

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Table 1. Crystal Data and Structure Refinement for FAST-4 (R = H, B = C^{Bz}) and FAST-5 (R = Px, B = Thy)

	FAST-4	FAST-5
mol formula	C ₁₈ H ₂₂ N ₃ O ₆ PSe	C ₃₇ H ₄₅ N ₂ O ₇ PS
formula wt	486.32	692.78
cryst syst	monoclinic	monoclinic
space grp	<i>P</i> 2 ₁	<i>P</i> 2 ₁
<i>a</i> (Å)	9.419(2)	18.828(10)
<i>b</i> (Å)	7.911(2)	9.007(4)
<i>c</i> (Å)	13.668(3)	23.899(9)
β (deg)	94.69(3)	105.89(3)
<i>V</i> (Å ³)	1025.3(4)	3897.9(31)
<i>Z</i>	2	4
<i>D_c</i> (g/cm ³)	1.575	1.181
μ (cm ⁻¹)	35.81	15.06
cryst dimens (mm)	0.12 × 0.16 × 0.32	0.03 × 0.28 × 0.65
max 2 θ (deg)	150	146
radiatn, λ (Å)	Cu K α , 1.541 84	Cu K α , 1.541 78
scan mode	$\omega/2\theta$	$\omega/2\theta$
scan width (deg)	1.27 + 0.14 tan θ	1.25 + 0.14 tan θ
<i>hkl</i> ranges: (<i>h</i>)	−11 11	−23 23
(<i>k</i>)	−10 10	−11 0
(<i>l</i>)	−17 0	−29 29
EAC correction: (min)	0.8731	0.9026
max	1.0000	0.9994
avg	0.9328	0.9782
no. of reflns: (unique)	4217	8256
refine with <i>I</i> > 0 σ (<i>I</i>)	4038	6979
obsd with <i>I</i> > 2 σ (<i>I</i>)	3742	4267
no. of param refined	337	858
no. of restraints	0	18
largest diff. peak (e Å ⁻³)	0.423	0.430
largest diff. hole (e Å ⁻³)	−1.193	−0.252
<i>R</i> _{obs}	0.0576	0.0626
<i>wR</i> _{obs}	0.1434	0.1379
weighting coeff. ^a (<i>m</i>)	0.1156	0.0782
<i>S</i> _{obs}	1.088	1.144
shift/esd max	−0.001	−0.001
<i>R</i> _{int}	0.0530	0.0873
<i>T</i> _{meas.}	293(2)	293(2)
<i>F</i> 000	496	1472
abs structure	<i>R</i> _{CI'} , <i>S</i> _{C3'} , <i>R</i> _{C4'} , <i>S</i> _{P1}	<i>R</i> _{CI'} , <i>S</i> _{C3'} , <i>R</i> _{C4'} , <i>S</i> _{P1}
flack param χ	0.00(3)	0.01(3)

^a Weighting scheme $w = [\sigma^2(F_o^2) + (mP)^2]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$.

substitution at phosphorus in methane phosphonothio- (seleno) derivatives with methylseleno or methylthio leaving groups occurs with *inversion of configuration*. These results indicate that the process of nucleophilic substitution at phosphorus in methanephosphonosele- nolates or methanephosphonothiolates occurs according to the *in-line mechanism*, where the transition state requires the location of the nucleophile attacking at phosphorus at the face opposite to the leaving group. Such a stereochemical result is not surprising, although it contrasts with some earlier results reported by several authors.¹⁰ Although in Inch's report the stereochemical diversity of S_N2(P) substitution is demonstrated as depending upon the mode of catalysis,¹¹ in this laboratory it has been demonstrated that the DBU-assisted 1,3,2-

oxathiaphospholane ring-opening condensation process¹² occurs according to the *adjacent type mechanism*¹³ and the cleavage of the P–S bond occurs with *retention of configuration*. Ab initio studies of this reaction profile suggest that the ring opening with retention of configuration is energetically favorable.¹⁴ In both discussed reactions the P–S bond is cleaved, and DBU is used as an activator, most probably being responsible for proton abstraction from the 5'-OH group of an attacking nucleoside. Interestingly, if in the reaction of **4'** or **5** with the 5'-OH of nucleoside DBU is used as a sole activator, condensation occurs at a much lower rate and is accompanied by partial loss of stereospecificity. Such observation may suggest that DBU, being usually considered as a strong nonnucleophilic base, without competitive nucleophile may also act as nucleophilic reagent.¹⁵ However, as demonstrated by Masamune,¹⁶ and

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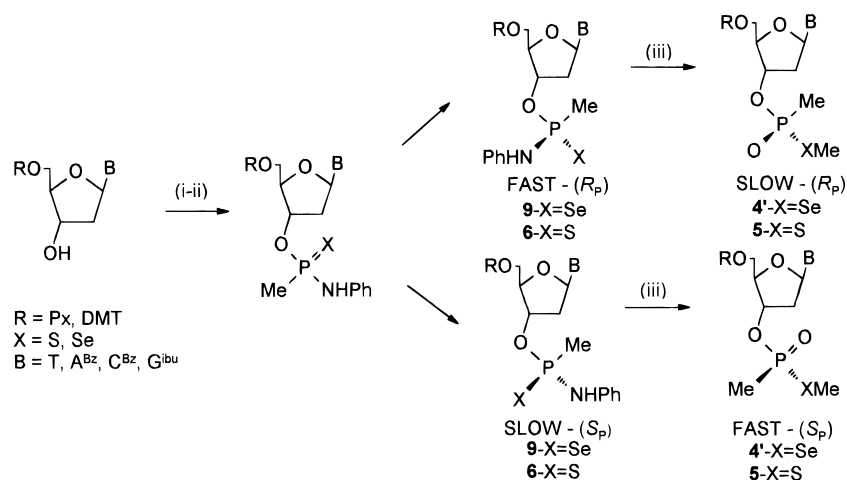
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Scheme 2^a

^a Key: (i) MePCl₂, Et₃N, -40 °C, THF, 1 h; (ii) aniline, elemental selenium or sulfur rt, 1–2 h; (iii) NaH/CO₂, DMF, followed by methyl iodide.

independently by Seebach,¹⁷ addition of lithium salts to DBU potentiates its basicity, so DBU/LiCl is a much more efficient catalyst of nucleophilic substitution at phosphorus. This explanation does not elucidate, however, the essential difference in stereochemistries of both reactions discussed here. The ring-opening condensations must occur with participation of the pentacoordinate intermediate due to its five-membered ring stabilization, and an attack of nucleophile at phosphorus occurs from the site opposite to the most electronegative ligand such as endocyclic oxygen. The requirement of an apical entry of nucleophile and an apical departure of the leaving group¹⁸ is violated here because pseudorotation is concerted with formation/cleavage of an equatorial bond in the TBP species. According to such a mechanistic proposal, nucleophilic substitution at phosphorus involved in the 1,3,2-oxathiaphospholane ring occurs with retention of configuration. In the process of nucleophilic substitution at phosphorus in **4'** and **5**, an attack of alkoxide must occur from the site opposite to the -SeMe or -SMe ligand via an S_N2(P)-type mechanism without involvement of the TBP intermediate. Besides enhancement of DBU basicity (pK_a 11.6 in water¹⁵ and 23.9 in MeCN¹⁹), the function of LiCl may be also considered in terms of coordination of lithium cation with methanephosphonate oxygen atom, additionally increasing the electrophilicity of phosphorus center. That assumption is lacking, however, any experimental evidence.

Correlational Analysis of the Absolute Configuration at Phosphorus in Methanephosphonothioanilidates (6**), Methanephosphonoanilidates (**8**), and Methanephosphonoselenoanilidates (**9**).** As described in our earlier paper,⁵ both diastereomeric 5'-O-DMT-thymidine 3'-O-(Se-methyl methanephosphonose-

lenolate)s (**4'**, R = DMT, B = Thy) were prepared via procedures analogous to those presented in Scheme 2. If 5'-O-DMT(or Px)-thymidine was used as the substrate for condensation with methyldichlorophosphine followed by substitution with aniline and elemental sulfur was used as an oxidant of transiently formed methanephosphonoanilidites, 5'-O-DMT (or Px)-thymidine 3'-O-methanephosphonothioanilidates **6** were obtained as major products. Chromatographic separation gave pure **FAST-6** and **SLOW-6** isomers.

Reexamination of the results of X-ray analysis, leading to the correction of our former erroneous assignment of the absolute configuration of 5'-O-pixylthymidine 3'-O-(Se-methyl methanephosphonoselenolate) (**7**)⁵ (**FAST**-eluted isomer has (S_p) configuration) and additional X-ray data of **4** (R = H, B = C^{Bz}) and **5** (R = Px, B = Thy) allow us to correlate the absolute configuration of compounds **4'** and **5** with the mobility of diastereomers in HPLC and with the sense of the chemical shift in ³¹P NMR. It is also possible to assign the absolute configurations at phosphorus of precursors of compounds **4'** and **5**, the corresponding methanephosphonoselenoanilidates **9**, and sulfur analogues **6** (Scheme 3).

The competitive route to thioesters **5** involves the utilization of methanephosphonoanilidates **8**, which can be obtained directly in the reaction of appropriately protected nucleosides with methanephosphonodichloride, followed by a substitution of chlorine by aniline in transiently formed methanephosphonochloridates.²⁰ Additionally, as demonstrated at Scheme 3, the stereoretentive oxidation of methanephosphonothioanilidates **6** and methanephosphonoselenoanilidates **9** to methanephosphonoanilidates **8** permits for the correlation of the absolute configuration of **8** with the data obtained from X-ray analysis of **4** (R = H, B = C^{Bz}) and **5** (R = Px, B = Thy). The oxidation of **FAST-6** or **FAST-9**, accomplished under very mild conditions [2-fold excess of 0.1 M OXONE (potassium peroxymonosulfate), buffered at pH 7 in THF–MeOH solution) within 15 min leads exclusively to the **FAST-8**, while **SLOW-6** and **SLOW-9** are oxidized to **SLOW-8**. This oxidation proceeds with full

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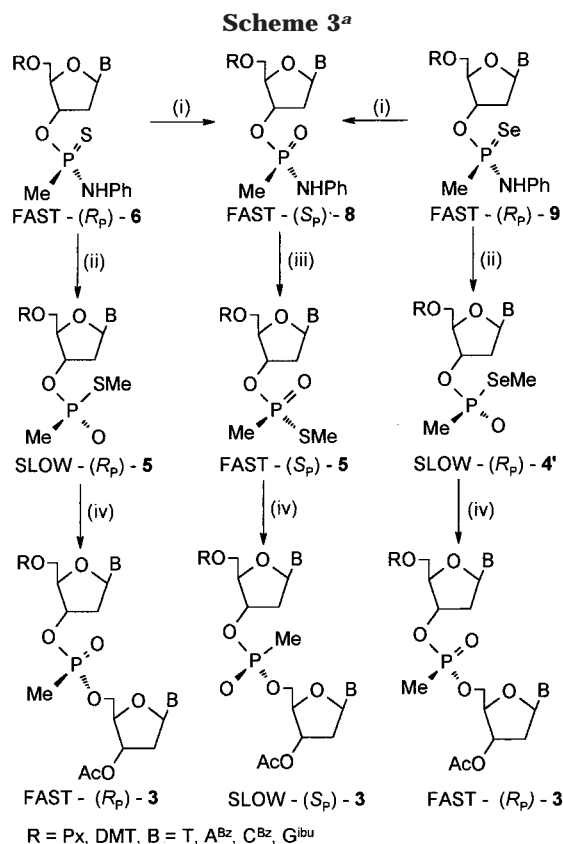
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^a Key: (i) Oxone; (ii) NaH/CO₂, DMF, followed by methyl iodide; (iii) NaH/CS₂, DMF, followed by methyl iodide; (iv) 5'-OH nucleoside, DBU/LiCl.

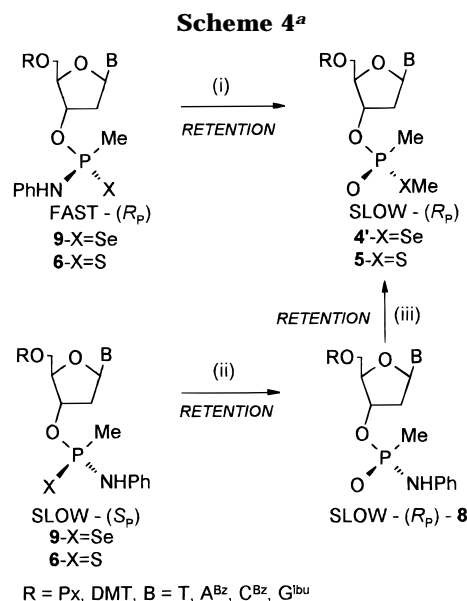
retention of configuration, as proven in model studies in which 1,3,2-dioxaphosphorinanes were used (data not shown).²¹

SLOW-eluted diastereomers **4'** and **5** both have the (*R_p*)-configuration. The reactions of activation of oxo-, thio-, and selenoanilidates by means of NaH and further reactions with electrophiles (CO₂, CS₂, and CSe₂)^{7b} (PN → PS, PN → PO, and PN → PSe conversions) leading to corresponding phosphylthio- and phosphylselenoates, respectively, are well documented and proceed with full retention of configuration.⁷ The *S*- and *Se*-alkylations take place beyond the stereogenic center so they cannot change the P-absolute configurations in thio- and selenoalkyl esters, as related to the precursors **6** and **9**.

In the conversion of methanephosphonoanilidates- (*R_p*)-**8** to *S*-alkyl esters **5** (Scheme 4), sulfur in the ester **5** originates from CS₂ introduced during the Wadsworth–Emmons–Horner type reaction.^{7a} Thus, the sequence of reactions presented in Scheme 4 correlating in one stereochemical cycle monomers **4'** or **5** with their precursors **6**, **8**, and **9**, respectively, creates an open, four-membered, diligostatic, antipodal stereochemical cycle with three retentions of configuration and one ligand metathesis involved.²²

Experimental Section

General Methods. NMR spectra were recorded either at 300.13 MHz (¹H) and 121.47 MHz (³¹P) or at 500.133 MHz (¹H) and 202.47 MHz (³¹P). Chemical shifts are reported (δ) relative



^a Key: (i) NaH/CO₂, DMF, followed by methyl iodide; (ii) Oxone; (iii) NaH/CS₂, DMF, followed by methyl iodide.

to TMS (¹H) and 80% H₃PO₄ (³¹P) as external standards. 2D ¹H–¹H NMR (NOESY) correlations were applied for identification of signals in ¹H NMR spectra. Unless otherwise specified, spectra were measured in CDCl₃ at 295 K. The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet. Positive chemical shift values were assigned for compounds resonating at lower fields than standards. Mass spectra were recorded on a mass spectrometer with a Cs⁺ gun operating at 13 keV using a 3-nitrobenzyl alcohol matrix. High-resolution MS spectra were recorded for 1:1 mixtures of diastereomers. High-pressure liquid chromatography was performed by the use of a reversed-phase column (C-18, 5 μm, 25 cm, 4.6 mm) and the following solvent system: 0.1 M triethylammonium bicarbonate (TEAB) at pH = 7.0 (A) and 40% MeCN in 0.1 M TEAB (B). Column chromatography and HP-TLC analyses were performed on silica gel (240–400 mesh) and precoated F₂₅₄ silica gel plates, respectively. Solvents and reagents were purified according to the standard laboratory techniques and stored under argon. MeCN was distilled from CaH₂ directly to the reaction vessels. LiCl was recrystallized from MeOH and dried under vacuum (150 °C/0.05 mmHg) for several days. Reactions involving air- or moisture-sensitive compounds were carried out in glassware that was dried either in an oven or under high vacuum and then placed under a positive pressure of argon.

Yields of pure isomers, obtained during purifications, are given in brackets.

General Procedure for Preparation of 5'-O-DMT-(*N*-protected) 2'-Deoxynucleoside 3'-O-Methanephosphonothioanilidates (6**) and Methanephosphonoselenoanilidates (**9**).** To a solution of methyldichlorophosphine (0.23 g, 2 mmol) in THF (10 mL) and triethylamine (0.40 g, 4 mmol) cooled to –40 °C by external cooling (dry ice–acetone) was added dropwise, under magnetic stirring, a solution of 5'-O-DMT (or Px) (*N*-protected, except thymidine) nucleoside (1 mmol) in THF (10 mL). After removal of external cooling, stirring was continued until the reaction mixture reached ambient temperature. Then aniline (0.28 g, 3 mmol) together with elemental selenium (or sulfur) (2.5 mmol) were added in one portion. Stirring was continued overnight. Excess selenium (or sulfur) was removed by filtration, and solvents were partially evaporated to reduce the volume of the reaction mixture to ca. 5 mL. The residue was diluted with chloroform, and the resulting solution was washed with saturated aqueous NaHCO₃. The organic fraction was dried over anhyd MgSO₄ and concentrated. Product **6** or **9** was isolated by silica gel chromatography (elution with a gradient of EtOH in CHCl₃).

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Appropriate fractions were combined and concentrated under reduced pressure.

(*R_p*,*S_p*)-5'-*O*-DMT-thymidine 3'-*O*-(methanephosphonothioanilide) (6) was obtained from 5'-*O*-DMT-thymidine (0.54 g, 1 mmol) as a colorless foam, total yield 93% (0.66 g). **FAST-(*R_p*)**: yield 45%; ³¹P NMR δ 79.43; ¹H NMR δ 2.04 (d, 15.3, 3H, PCH₃), 2.37 (m, 2H, H^{2'}, H^{2''}), 3.53 (m, 2H, H^{5'}, H^{5''}), 4.41 (d, 1.4, 1H, H^{4'}), 5.27 (d, 9.4, 1H), 6.36 (dd, 6.8, 6.8, 1H, H^{1'}); FAB-MS found [M - H] 712.3 (calcd 712.225). **SLOW-(*S_p*)**: yield 30%; ³¹P NMR δ 79.58; ¹H NMR δ 2.01 (d, 15.3, 3H, PCH₃), 2.42 (m, 4.2, 2.9, 1H, H^{2'}), 2.73 (m, 1H, H^{2''}), 3.29 (dd, 2.6, 10.6, 1H, H^{5'}), 3.17 (dd, 2.6, 10.7, 1H, H^{5''}), 4.17 (d, 1.9, 1H, H^{4'}), 6.48 (dd, 9.1, 9.0, 1H, H^{1'}); FAB-MS found [M - H] 712.3.

5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine 3'-*O*-methanephosphonothioanilide (6) was obtained from 5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine (0.633 g, 1 mmol). A mixture of diastereomers (about 1:1 ratio) was obtained as a colorless foam, total yield 70% (0.56 g). **FAST-(*R_p*)**: yield 28%; ³¹P NMR δ (CH₂Cl₂/C₆D₆) 79.33; ¹H NMR δ 2.05 (d, 15.3, 3H, PCH₃), 2.27 (dd, 6.5, 13.7, 1H, H^{2'}), 2.77 (dd, 5.1, 11.7, 1H, H^{2''}), 3.54 (dd, 2.54, 11.1, 1H, H^{5'}), 3.60 (dd, 3.2, 11.1, 1H, H^{5''}), 4.59 (m, 2.4, 1H), 6.36 (dd, 6.6, 6.2, 1H, H^{1'}); FAB-MS found [M - H] 801.3 (calcd 802.590). **SLOW-(*S_p*)**: yield 32%; ³¹P NMR δ (CH₂Cl₂/C₆D₆) 79.84; ¹H NMR δ 2.02 (d, 15.3, 3H, PCH₃); FAB-MS found [M - H] 801.3.

(*R_p*,*S_p*)-5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine 3'-*O*-methanephosphonoselenoanilide (9) was obtained from 5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine (0.633 g, 1 mmol). A mixture of diastereomers (1:1.2 ratio) was obtained as a colorless foam, total yield 70% (0.56 g). **FAST-(*R_p*)**: yield 30%; ³¹P NMR δ 76.65, ¹J_{P-Se} = 820 Hz; ¹H NMR δ 2.06 (d, 14.8, 3H, PCH₃), 2.3 (m, 1H, H^{2'}), 2.55 (m, 1H, H^{2''}), 3.41 (dd, 2.4, 10.7, 1H, H^{5'}), 3.32 (dd, 2.5, 10.6, 1H, H^{5''}), 4.15 (m, 1H), 5.5 (m, 1H), 6.45 (m, 1H, H^{1'}); FAB-MS found [M - H] 849.4 (⁸⁰Se) (calcd 849.5). **SLOW-(*S_p*)**: yield 25%; ³¹P NMR δ 76.29 (¹J_{P-Se} = 820 Hz); ¹H NMR δ 1.96 (d, 13.54, 3H, PCH₃), 2.37 (m, 1H, H^{2'}), 2.5 (m, 1H, H^{2''}), 2.94 (dd, 2.5, 10.5, 1H, H^{5'}), 3.1 (dd, 3.0, 10.5, 1H, H^{5''}), 4.35 (m, 1H), 5.25 (m, 1H), 6.38 (m, 1H, H^{1'}); FAB-MS found [M - H] 849.4 (⁸⁰Se).

General Procedure for Preparation of 5'-*O*-DMT-nucleoside 3'-*O*-Methanephosphonothioanilides (8) from Methanephosphonodichloridate. To a solution of MeP(O)-Cl₂ (2 mmol) in dry pyridine (15 mL) was added a solution of 5'-*O*-DMT-nucleoside (1 mmol) in pyridine (5 mL). The reaction mixture was stirred at room temperature for 15 min, and aniline (5 mmol, 0.465 g) was added. After the reaction was completed (30 min), all solvents and reagents were evaporated to ca. 1/3 volume.

The residue was dissolved with CHCl₃ (20 mL) and extracted with citric acid (0.05 M, 10 mL, three times). Combined organic layers were concentrated and purified by silica gel column chromatography [eluent: chloroform-EtOH (0-3%)]. Appropriate fractions of **FAST-8** and **SLOW-8** were collected and concentrated to give pure **8** as colorless foams.

5'-*O*-DMT-thymidine 3'-*O*-(methanephosphonothioanilide) (8) was obtained from 5'-*O*-DMT-thymidine (0.54 g, 1 mmol) and purified and separated by column chromatography (CHCl₃-2% EtOH), total yield 91%. **FAST-(*R_p*)**: yield 45%; ³¹P NMR δ 30.52; ¹H NMR δ 1.66 (d, 16.9, 3H, PCH₃), 2.31 (dd, 5.7, 14.3, 1H, H^{2'}), 2.47 (dd, 6.5, 13.7, 1H, H^{2''}), 3.50 (m, 2.3, 2.4, 2H, H^{5'}, H^{5''}), 6.47 (dd, 8.9, 9.0, 1H, H^{1'}); HR FAB-MS found [M - H] 696.244 (calcd 696.247). **SLOW-(*S_p*)**: yield 45%; ³¹P NMR δ 30.12; ¹H NMR δ 1.70 (d, 16.9, 3H, PCH₃), 2.40 (m, 6.1, 14.6, 1H, H^{2'}), 2.76 (m, 5.4, 13.8, 1H, H^{2''}), 3.27 (dd, 10.7, 2.9, 1H, H^{5'}), 4.02 (dd, 10.6, 2.6, 1H, H^{5''}), 6.52 (dd, 8.9, 9.0, 1H, H^{1'}); HR FAB-MS found [M - H] 696.244 (calcd 696.247).

5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine 3'-*O*-methanephosphonothioanilide (8) was obtained from 5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine (0.63 g, 1 mmol) and purified and separated into diastereomers by single column chromatography (CHCl₃-3% EtOH), total yield 90%. **FAST-(*R_p*)**: yield 45%; ³¹P NMR δ 30.12; ¹H NMR δ 1.51 (d, 16.5, 3H, PCH₃), 2.13 (m, 1H, H^{2'}), 2.36 (1H, H^{2''}), 3.17 (m, 2H, H^{5'}, H^{5''}), 4.18

(m, 1H, H^{4'}), 4.95 (m, 1H, H^{3'}), 6.3 (t, 6.1, 12.8, 1H, H^{1'}); HR FAB-MS found [M - H] 785.270 (calcd 785.274). **SLOW-(*S_p*)**: yield 40%; ³¹P NMR δ 29.95; ¹H NMR δ 1.56 (d, 15.6, 3H, PCH₃), 2.28 (m, 1H, H^{2'}), 2.50 (m, 1H, H^{2''}), 3.31 (m, 2H, H^{5'}, H^{5''}), 4.15 (m, 1H, H^{4'}), 4.94 (m, 1H, H^{3'}), 6.15 (dd, 7.1, 13.7, 1H, H^{1'}); HR FAB-MS found [M - H] 785.270.

General Procedure for Preparation of Diastereomerically Pure (*R_p*)- and (*S_p*)-5'-*O*-DMT-(*N*-protected)-2'-deoxynucleoside 3'-*O*-(*S*-Methyl methanephosphonosenolates) (4) or *S*-Methyl Methanephosphonothiolates (5). To a stirred solution of corresponding **6** or **9** [1 mmol, dried twice by coevaporation with dry toluene (5 mL)] in DMF (10 mL) was added NaH (1.2 molar equiv of 50% suspension in mineral oil) in a few portions. Stirring was continued until evolution of hydrogen had ceased, and then a stream of gaseous CO₂ (dried over P₂O₅) was passed through the slurry for ca. 4 h. The resulting sodium salt of the corresponding nucleoside 3'-*O*-methanephosphonoselenoic(thioic) acid was treated with MeI (5 equiv), and the progress of alkylation was followed by TLC. Upon completion of the reaction, solvents and excess MeI were removed by evaporation under reduced pressure. The solid residue was dissolved in CHCl₃ (20 mL) and washed twice with saturated NaHCO₃. The combined organic layers were dried over anhydrous MgSO₄ and concentrated. Ester **4'** or **5** was purified and separated into (*R_p*)- and (*S_p*)-diastereomers by silica gel column chromatography (Kieselgel 60, 230-400 mesh, 3 g of **5** per 90 g of silica gel). The appropriate fractions, eluted with CHCl₃-EtOH (1-5% EtOH), were combined and evaporated under reduced pressure.

5'-*O*-Px-thymidine 3'-*O*-(*S*-Methyl methanephosphonothiolate) (5). **SLOW-(*R_p*)** was obtained from **FAST-6** (R = Px, B = Thy): yield 86%; ³¹P NMR δ 57.76; ¹H NMR δ 1.81 (d, 15.6, 3H, PCH₃), 2.29 (d, 13.2, 3H, SCH₃), 2.45 (m, 1H, H^{2'}), 2.57 (m, 1H, H^{2''}), 3.47 (m, 2H, H^{5'}, H^{5''}), 4.38 (d, 1.2, 1H, H^{4'}), 5.29 (m, 1H, H^{3'}), 6.46 (dd, 5.4, 8.9, 1H, H^{1'}); FAB-MS found [M - H] 606.1 (calcd). **FAST-(*S_p*)** was obtained from **SLOW-6** (R = Px, B = Thy): yield 80%; ³¹P NMR δ 58.61; ¹H NMR δ 1.82 (d, 15.7, 3H, PCH₃), 2.18 (d, 3H, SCH₃), 2.45 (m, 1H, H^{2'}), 2.62 (m, 1H, H^{2''}), 3.44 (m, 2H, H^{5'}, H^{5''}), 4.18 (d, 1.9, 1H, H^{4'}), 5.34 (m, 1H, H^{3'}), 6.47 (dd, 5.6, 8.7, 1H, H^{1'}); FAB-MS found [M - H] 606.1.

5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine 3'-*O*-(*S*-Methyl methanephosphonothiolate) (5, R = DMT, B = C^{Bz}). **SLOW-[*R_p*]** was obtained from **FAST-7** (R = DMT, B = C^{Bz}) (0.82 g, 1 mmol): yield 82% (0.67 g); ³¹P NMR δ 55.41; ¹H NMR δ 1.82 (d, 15.6, 3H, PCH₃), 2.21 (d, 13.2, 3H, SCH₃), 2.92 (m, 2H, H^{2'}, H^{2''}), 3.48 (m, 2H, H^{5'}, H^{5''}), 4.31 (d, 3.0, 1H, H^{4'}), 5.28 (m, 1H, H^{3'}), 6.39 (t, 12.8, 1H, H^{1'}); HR FAB-MS found [M - CH₃] 726.204 (calcd 726.203). **FAST-(*S_p*)** was obtained from **SLOW-7** (R = DMT, B = C^{Bz}) (0.82 g, 1 mmol): yield 80% (0.65 g); ³¹P NMR δ 55.41; HR FAB-MS found [M - CH₃] 726.204.

5'-*O*-DMT-thymidine 3'-*O*-(*S*-methyl methanephosphonoselenolate) (4', R = DMT, B = Thy) was obtained from **9** (R = DMT, B = Thy) (0.760 g, 1 mmol). Total yield of both isomers (*R_p*) and (*S_p*): 86%. **SLOW-(*R_p*)**: yield 40%; ³¹P NMR δ 49.76 (¹J_{P-Se} = 430 Hz); ¹H NMR δ 1.91 (d, 15.1, 3H, PCH₃), 2.16 (d, 11.5, 3H, SeCH₃); HR FAB-MS found [M - CH₃] 685.124 (calcd [M - CH₃] 685.140). **FAST-(*S_p*)**: yield 46%; ³¹P NMR δ 49.72 (¹J_{P-Se} = 428 Hz); ³¹P NMR δ 1.93 (d, 15.2, 3H, PCH₃), 2.18 (d, 11.6, 3H, PSeCH₃); HR FAB-MS found [M - CH₃] 685.124.

5'-*O*-DMT-*N*⁴-benzoyl-2'-deoxycytidine 3'-*O*-(*S*-Methyl methanephosphonoselenolate) (4', R = DMT, B = C^{Bz}). **SLOW-(*R_p*)** was obtained from **FAST-9** (R = DMT, B = C^{Bz}) (1 mmol): yield 70%; ³¹P NMR δ 49.76 (¹J_{P-Se} = 430 Hz); ¹H NMR δ 1.92 (d, 15.1, 3H, PCH₃), 2.15 (d, 11.6, 3H, SeCH₃), 2.40 (m, 1H, H^{2'}), 2.95 (n, 1H, H^{2''}), 3.50 (m, 2H, H^{5'}, H^{5''}), 4.32 (dd, 2.9, 1H, H^{3'}), 5.31 (m, 1H, H^{4'}), 6.41 (dd, 6.3, 6.4, 1H, H^{1'}); HR FAB-MS found [M - H] 788.159 (calcd 788.164). **FAST-(*S_p*)** was obtained from **SLOW-9** (R = DMT, B = C^{Bz}) (1 mmol): yield 72%; ³¹P NMR δ 49.72 (¹J_{P-Se} = 428 Hz); ¹H NMR δ 1.92 (d, 15.1, 3H, PCH₃), 2.19 (d, 11.6, 3H, SeCH₃), 2.38 (m, 1H, H^{2'}), 2.95 (m, 1H, H^{2''}), 3.44 (dd, 3.3, 11.3, 1H,

H5'), 3.49 (dd, 2.7, 10.7, 1H, H5''), 4.54 (dd, 2.2, 1H, H3'), 5.26 (m, 1H, H4'), 6.33 (dd, 5.9, 1H, H1'); FAB-MS found [M - H] 788.2 (⁸⁰Se).

Deprotection of 4' (R = DMT, B = C^{Bz}). FAST-4' (0.07 g) was dissolved in 3% trichloroacetic acid in CH₂Cl₂ (2 mL) and stirred at room temperature for 3 min. The reaction was quenched with NaHCO₃, and **4** (R = H, B = C^{Bz}) was extracted with CHCl₃, dried with MgSO₄, and concentrated under reduced pressure. Compound **FAST-4** (R = H, B = C^{Bz}) was purified by silica gel column chromatography and crystallized from EtOH/H₂O solution (95:5 v/v). Obtained crystals (mp >200 °C dec) were subjected to X-ray and spectrometric analyses: ³¹P NMR (DMSO-*d*₆) δ 50.70 (¹J_{P-Se} = 522.6 Hz); ¹H NMR δ 1.97 (d, 15.15, 3H, PCH₃), 2.19 (d, 11.4, 3H, SeCH₃), 2.33 (dd, 6.6, 14.1, 1H, H2'), 2.62 (dd, 6.0, 14.0, 1H, H2''), 3.67 (m, 2H, H5', H5''), 4.26 (dd, 2.2, 1H, H3'), 5.08 (m, 1H, H4'), 6.20 (dd, 7.3, 6.2, 1H, H1'); HR FAB-MS found [M - H] 486.033 (calcd 486.035).

General Procedure for Preparation of Dinucleoside (3',5')-Methanephosphonates (3). The corresponding diastereomerically pure **4'** or **5** (0.3 mmol), and 3'-*O*-acetyl (*N*-protected)-2'-deoxynucleoside (0.1 mmol) were coevaporated twice with dry pyridine (5 mL) and left overnight under high vacuum. LiCl (freshly dried at 150 °C/0.1 mmHg, 0.125 g, 3 mmol) was added, and the resulting mixture was dissolved in dry acetonitrile (5 mL). To this solution was added DBU (0.456 g, 3 mmol) in acetonitrile (1.5 mL) in one portion. After the reaction was completed (2 h; disappearance of **4'** or **5** was followed by HP-TLC), the solvent was evaporated, and the oily residue was added dropwise to cold hexane. The solid precipitate was collected by centrifugation and redissolved in CHCl₃ and the solution extracted twice with citric acid (0.05 M). The combined organic layers were dried over anhyd MgSO₄ and concentrated, and crude **3** was purified by column chromatography. The appropriate fractions, eluted with a gradient of CHCl₃-EtOH (3-10% EtOH), were collected, combined, and concentrated under reduced pressure.

(R_p)-5'-*O*-DMT-thymidylyl-(3',5')-3'-*O*-acetylthymidine 3'-methanephosphonate (3) was obtained from **(R_p)-5** (R = DMT, B = Thy) and 3'-*O*-acetylthymidine: yield 92%; ³¹P NMR δ 33.00; ¹H NMR δ 1.58 (d, 17.6, 3H, PCH₃); FAB-MS found [M - H] 887.3 (calcd 887.290).

(S_p)-5'-*O*-DMT-thymidylyl-(3',5')-3'-*O*-acetylthymidine 3'-methanephosphonate (3) was obtained from **(S_p)-5**

(R = DMT, B = Thy) and 3'-*O*-acetylthymidine: yield 86%; ³¹P NMR δ 34.14; ¹H NMR δ 1.57 (d, 17.6, 3H, PCH₃), 2.43 (m, 2H, H2', H2''), 2.56 (m, 2H, H22', H22''), 3.44 (m, 2H, H15', H15''), 4.01 (m, 2H, H25', H25''), 4.16 (m, 1H, H14'), 4.23 (m, 1H, H24'), 5.08 (m, 5.0, 1H, H13'), 5.22 (m, 1H, H23'), 6.34 (dd, 8.7, 3.2, 1H, H11'), 6.58 (dd, 8.5, 2.9, 1H, H21'); FAB-MS found [M - H] 887.3.

(R_p)-N⁴-Benzoyl-5'-*O*-DMT-2'-deoxycytidylyl-(3',5')-N⁴-benzoyl-3'-*O*-acetyl-2'-deoxycytidine 3'-methanephosphonate (3) was obtained from **(R_p)-4'** (R = DMT, B = C^{Bz}) and 3'-*O*-acetyl-N⁴-benzoyl-2'-deoxycytidine: yield 82%; ³¹P NMR δ 32.98; ¹H NMR δ 1.65 (d, 17.5, 3H, PCH₃); FAB-MS found [M - H] 1067 (calcd 1067.359).

(S_p)-N⁴-Benzoyl-5'-*O*-DMT-2'-deoxycytidylyl-(3',5')-N⁴-benzoyl-3'-*O*-acetyl-2'-deoxycytidine 3'-methanephosphonate (3) was obtained from **(S_p)-4'** (R = DMT, B = C^{Bz}) and 3'-*O*-acetyl-N⁴-benzoyl-2'-deoxycytidine: yield 67%; ³¹P NMR δ 33.06; ¹H NMR δ 1.66 (d, 17.5, 3H, PCH₃); FAB-MS found [M - H] 1067.

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Supporting Information Available: X-ray crystallographic data for compounds listed in Table 1, including tables of bond lengths, bond angles, atomic coordinates, temperature factors, bond distances, angles, intermolecular contacts, and hydrogen-bond parameters, five additional figures (stereoviews of accurate positions of molecules in the elemental cells and the Newmann projections), and discussion (31 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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