

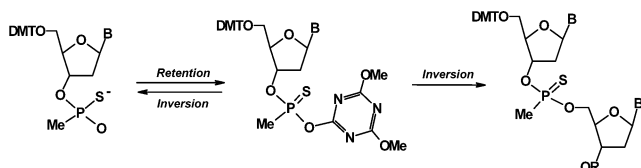
Chemoselective Activation of Nucleoside 3'-O-Methylphosphonothioates with 1,3,5-Triazinyl Morpholinium Salts

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Received July 9, 2007



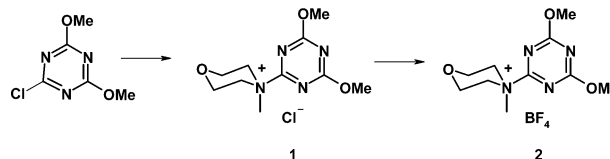
Chemoselective and stereospecific *O*-activation of 2'-deoxynucleoside 3'-*O*-methylphosphonothioates **5** with *N*-methyl-*N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)morpholinium salts results in formation with retention of configuration of 5'-*O*-DMT-2'-deoxynucleoside 3'-*O*-(4,6 dimethoxy-1,3,5-triazin-2-yl)methylphosphonothioates (**7**). Active esters **7** are convenient intermediates for hydrolytic interconversion of *R*_P-**5** into *S*_P-**5** and can be used as monomers for stereoselective synthesis of dinucleoside (3',5')-methyl phosphonothioates.

The concept of *active esters* and *superactive esters* as reactive intermediates in acylation reactions has been proven useful for the synthesis of peptides,¹ amides,² or α -lactams,³ and carboxylic acid esters⁴ as well as other compounds.⁵ Among many activators tested in these reactions, mostly derivatives of *N*-hydroxybenzotriazoles⁶ or azabenzotriazoles⁷ have been employed. Lately, very promising triazine-based coupling reagents (TBCRs) have been found to be more reactive than

other acylating reagents.⁸ Of special interest is application of chiral 1,3,5-triazinyl salts for asymmetric induction occurring during peptide synthesis.⁹

Some of these coupling reagents and their analogues have also been used previously in the phosphorylation of several alcohols and amines.¹⁰ Recently, we also demonstrated that both P^{III} and P^V compounds can be efficiently activated with 1,2,4 triazoles¹¹ or *N*-hydroxybenzotriazoles¹² in the esterification of methylphosphonates or phosphorothioates.

In our previous studies focused on large-scale synthesis of diastereomerically pure dinucleoside (3',5')-methylphosphonates (**3**) and methyl phosphonothioates (**4**), we established that the regioselective *S*-alkylations or *O*-acylations of diastereomerically pure nucleoside 3'-*O*-methyl phosphonothioates can provide monomers for this synthesis.¹³ Diastereomerically pure *S*-alkyl methylphosphonothioates react with nucleosides in the presence of DBU/LiCl in a stereospecific way to give with inversion of configuration diastereomerically pure **3**,¹⁴ whereas nucleoside 3'-*O*-methylphosphonothio-*O*-(2,4,6-trimethylphenyl)benzoates react with nucleosides stereoselectively with predominant inversion of configuration and afford the corresponding dinucleoside (3',5')-methylphosphonothioates **4**, usually with 75–80% diastereomeric purity.¹³



In such a context, we found it tempting to apply *N*-methyl-*N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)morpholinium chloride (**1**)¹⁵ or tetrafluoroborate **2**¹⁶ to an activation of nucleoside 3'-*O*-methylphosphonothioates (**5**).

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(15) Generous gifts of **1** and **2** from Prof. Z. Kamiński are greatly appreciated.

(16) Salts **2** are more stable and were reported (ref 8) as superior for peptide synthesis; however, in the activation of methylphosphonates they behave analogously to **1**.

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TABLE 1. Stereospecific and Chemoselective Synthesis of Active Esters **7** (B = Thy)

reagents	solvent	time (h)	active ester 7	yield ^a (%)
substrate 5 , (mix <i>R_P/S_P</i> 2:1)	THF	12	<i>R_P/S_P</i> 1:2	95
substrate 5 (mix <i>R_P/S_P</i> 2:1)	MeCN	0.5	<i>R_P/S_P</i> 1:2	98
substrate 5 (SLOW <i>R_P</i>)	MeCN	0.5	SLOW <i>S_P</i>	98
substrate 5 (FAST <i>S_P</i>)	MeCN	0.5	FAST <i>R_P</i>	98

^a Calculated from the ³¹P NMR spectrum of the crude reaction mixture.

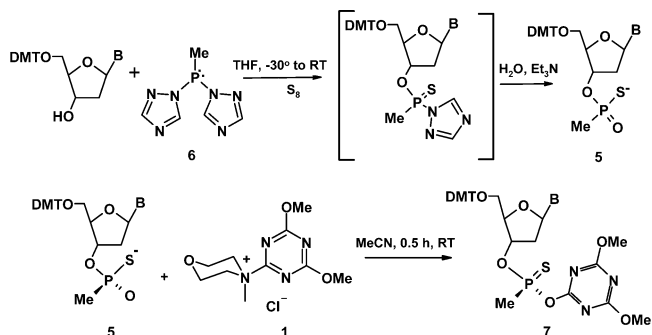
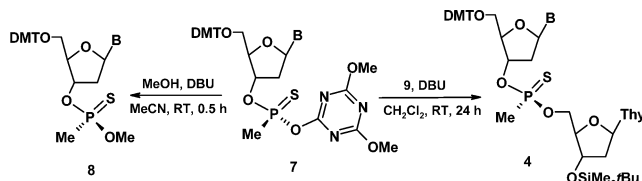
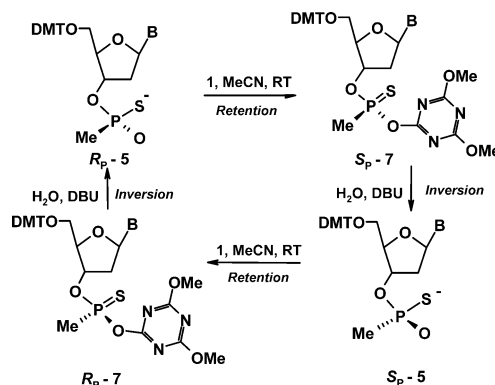
Diastereomerically pure 5'-O-DMT-2'-deoxynucleoside 3'-O-methylphosphonothioates (**5**) were synthesized in a one-pot synthesis, as described previously,¹¹ in the reaction of 5'-O-DMT-*N*-protected 2'-deoxynucleoside and bis(1,2,4-triazoyl) methylphosphonite (**6**) [³¹P NMR (MeCN-*d*₃) δ 73.47 ppm; ¹H NMR δ 2.25 (d, 3H, *J_{P-H}* = 15.6 Hz), 8.15, 9.05], followed by sulfurization of the intermediary nucleoside 3'-O-(1,2,4-triazoyl) methylphosphonite, hydrolysis of the corresponding protected nucleoside 3'-O-(1,2,4-triazoyl) methylphosphonothioate, and separation of the methylphosphonothioate **5** into diastereomers by silica gel column chromatography.

The activator **1** was generated in situ from equimolar amounts of 2-chloro-4,6-dimethoxy-1,3,5-triazine and *N*-methylmorpholine,¹⁷ since the crystalline form of **1** was unstable, even when stored at low temperatures.¹⁸ Freshly generated **1** reacted smoothly with diastereomerically pure **5** and resulted in almost quantitative formation of 5'-O-DMT-nucleoside 3'-O-(4,6-dimethoxy-1,3,5-triazin-2-yl) methylphosphonothioates (**7**) with retention of configuration at the phosphorus center.

5'-O-DMT-2'-deoxynucleoside 3'-O-(4,6 dimethoxy-1,3,5-triazin-2-yl) methylphosphonothioates were isolated and purified by silica gel column chromatography. Starting from the isomer *R_P*-**5** (SLOW eluting isomer, silica gel chromatography) the isomer SLOW-**7** was obtained, and the *S_P*-**5** was a precursor for FAST-**7**. These experiments established that the reaction was *O*-chemoselective and stereospecific, indicating an exclusive attack of the oxygen of an ambident methylphosphonothioate anion at the triazine ring.

The use of diastereomerically pure methylphosphonothioates **5** of known absolute configuration at the phosphorus permitted assignment of the absolute configuration of active esters **7** (Scheme 1, Table 1) and optimization of the esterification conditions.

The esters **7** are stable in the presence of methanol or water; however, in the presence of strong organic bases, e.g., *i*Pr₂NEt or, preferentially DBU, they react in stereospecific way, affording the corresponding 5'-O-DMT-2'-deoxynucleoside 3'-O-(*O*-methyl methylphosphonothioates) (**8**) and 5'-O-DMT 2'-deoxynucleoside 3'-O-methylphosphonothioates (**5**), respectively. Since the activation of *R_P*-methyl phosphonothioate **5** occurred exclusively at the phosphoryl oxygen atom (³¹P NMR evidence), the formation of *S_P*-*O*-methyl ester **8** confirmed the inversion of configuration of reaction of **7** with methanol in the presence of DBU.¹⁹ Analogously, starting from *S_P*-**7** ester, the corresponding *R_P*-**8** was formed (Scheme 2).

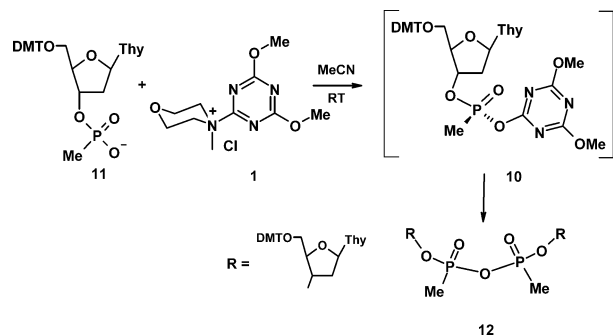
SCHEME 1. Synthesis of Active Esters **7****SCHEME 2.** DBU-Promoted Solvolysis of Active Esters **7****SCHEME 3.** Walden Cycle

The reaction between *R_P*-5'-O-DMT-thymidine 3'-O-(*O*-4,6-dimethoxy-1,3,5-triazin-2-yl) methylphosphonothioate (**7**, *R_P/S_P*-92:8) and 3'-O-*tert*-butyldimethylsilyl thymidine (**9**) activated with 2 equiv of DBU resulted in dithymidyl (3',5')-methylphosphonothioate (**4**, *R_P/S_P*-88:12) in 61% yield and high stereoselectivity (95%) after 24 h, whereas *A_{PMES}*-**4** was obtained in 52% yield under the same conditions.

Hydrolysis of the *R_P*-**7** in presence of DBU is also stereospecific and occurs with inversion of configuration (Scheme 3). This result was of particular interest since it allowed conversion of the diastereoisomer *S_P*-**5** quantitatively into its opposite diastereoisomer *R_P*-**5**.

Previously, we recognized the importance of such conversions (Walden closed stereochemical cycles) for a waste-free, stereoconvergent synthesis of dinucleoside (3',5')-methylphosphonates,²⁰ being used as the building blocks for the synthesis of chimeric oligonucleotides with predetermined sense of chirality at phosphorus of internucleotide methylphosphonate bonds.^{21,22} Reported here, easily prepared nucleoside 3'-O-(*O*-4,6-dimethoxy-1,3,5-triazin-2-yl) methylphosphonothioate(s) (**7**) constitute new

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SCHEME 4. Activation of Prochiral Thymidine Methylphosphonate (11)


of class of active esters that can be used for efficient synthesis of exclusively one diastereoisomer (either R_P or S_P) of methylphosphonothioates 5.

An analogous approach eventually leading to diastereomers of 5'-O-DMT-thymidine 3'-O-(4,6-dimethoxy-1,3,5-triazin-2-yl methylphosphonate) (**10**), prepared from the *P*-prochiral **11**, was appealing in the context of the expected diastereoselective activation and possible separation of formed diastereomers **10** (Scheme 4). We found, however, that instead of the expected ester **10** (^{31}P NMR δ 29.09, 29.12 ppm, diastereomeric ratio ca. 1:1, observed about 5%), the corresponding dithymidyl (3',3')-pyrophosphonate (**12**) [^{31}P NMR δ 24.85, 24.98, 25.1 ppm, 1:2:1 ratio, 85%] was observed as the foremost product. Pyrophosphonate **12** was, most probably, produced in reaction of the active ester **10** with methylphosphonic acid **11**. Different conditions tested for this reaction did not improve the yield of the required **10**, observed only transiently and at low concentrations during the course of the reaction.²³

In conclusion, we established that *N*-methyl-*N*-4,6-dimethoxy-1,3,5-triazinylmorpholinium salts **1** or **2** can be applied as activators of nucleoside methylphosphonates **11** and their thiocongeners **5**.²⁴ In the case of methylphosphonothioates **5**, the reaction is both chemoselective and stereospecific leading to stable active esters **7**, converted in base-catalyzed reactions with *O*-nucleophiles, including water and alcohols into the corresponding esters (or acids) with inversion of configuration. Therefore, esters **7** can constitute convenient tools in chirotechnology for exchange of the *P*-chirality in these methylphosphonothioates **5**, but at this stage, they are not competitive monomers for synthesis of modified oligonucleotides.

Experimental Section

Reactions were carried out under positive pressure of dry argon. Solvents and reagents were purified according to standard laboratory techniques and distilled directly into reaction vessels. Column chromatography and TLC analyses were performed on silica gel (240–400 mesh) and silica gel HP TLC precoated F₂₅₄ plates, respectively. NMR spectra were recorded at 500.13 MHz (^1H) and 202.46 MHz (^{31}P). Chemical shifts (δ) are reported relative to TMS (^1H) and 80% H_3PO_4 (^{31}P) as external standards.

Synthesis of *N*-Methyl-*N*-[4,6-dimethoxy-1,3,5-triazin-2-yl]-morpholinium chloride (1**).** 2-Chloro-4,6-dimethoxy-1,3,5-triazine (4.4 g, 25 mmol) and *N*-morpholine (2.75 mL, 25 mmol) were dried

separately as benzene solutions over activated molecular sieves 4 Å for 3 days. To a solution of triazine in benzene (15 mL), cooled in an water–ice bath, was added dropwise a solution of morpholine. The precipitated salt was washed with petroleum ether, dried, and used without further purification. Yield: 71%.

1: ^1H NMR (CDCl_3) δ 3.01 (s, 5H), 3.72 (t, 4H), 3.83 (t, 4H), 3.95 (s, 6H). Anal. ($\text{C}_{10}\text{H}_{17}\text{N}_4\text{O}_3\text{Cl}$): C, 43.15; H, 6.18; Cl, 6.34; N, 21.05. Calcd: C, 43.39; H, 6.19; Cl, 12.81; N, 20.24.

Synthesis of 5'-O-DMT-thymidine 3'-O-Methylphosphonothioate (5**).** Into a solution of 1,2,4-triazole (0.345 g, 5 mmol, 2.5 equiv) and triethylamine (0.85 mL, 6 mmol, 3 equiv) in dry THF (10 mL), cooled in ice bath, was added methyldichlorophosphine (2.2 mmol, 1.1 equiv) with vigorous stirring, and the reaction mixture was stirred for 20 min at this temperature. The immediate formation of white precipitate was observed. 5'-O-DMT-thymidine (1.09 g, 2 mmol) dissolved in THF (10 mL) was added to this mixture dropwise at 0 °C, and the ice bath was removed. Stirring was continued for 30 min. After this time, sulfur was added, and the reaction mixture was allowed to sit overnight. Triethylamine and water (1:4 v/v) were added to the reaction mixture until the solution became clear, and stirring was continued for an additional 0.5 h. After this time, the reaction mixture was diluted with NaHCO_3 (0.1 M) and extracted three times with chloroform. The combined organic fractions were additionally washed with NaHCO_3 and water, dried with MgSO_4 , and concentrated. The obtained product (as a foam) was dissolved in a small amount of CH_2Cl_2 and precipitated to hexane or petroleum ether. The product (mixture of diastereomers in ca. 1:1 ratio, 1.18 g) was separated by short path column chromatography on silica gel (40 g) using a mixture of chloroform and ethanol (19:1, v/v) containing 1% of triethylamine as an eluent. The R_P - and S_P -**5** were separated by the column chromatography on fine silica gel (50 g) using a mixture of chloroform and ethanol (39:1, v/v) containing up to 5% of Et_3N as eluent.²⁵

B = Thy: ^{31}P NMR (CDCl_3) δ (FAST- R_P -**5**) 77.18; (SLOW- S_P -**5**) 77.46 ppm.

FAST- R_P -5** (B = Thy)** (0.44 g, 30% yield): TLC R_f 0.50 (chloroform–ethanol (19:1, v/v) containing 5% of Et_3N); ^1H NMR (CDCl_3) δ 1.26 (t, J 7.3 Hz, 9H), 1.34 (d, J 1.2 Hz, 3H), 1.70 (d, J 14.3 Hz, 3H), 2.40 (m, 1H), 2.94 (m, 1H), 3.05 (q, J 7.3 Hz, 6H), 3.40 (dd, J 10.4, 2.3 Hz, 1H), 3.54 (dd, J 10.4, 2.3 Hz, 1H), 3.78 (s, 6H), 4.36 (m, 1H), 5.38 (m, 1H), 6.47 (dd, J 8.5, 5.7 Hz, 1H), 6.82 (m, 4H), 7.33 (m, 9H), 7.62 (d, J 1.2 Hz, 1H), 8.26 (br s, 1H), 12.44 (br s, 1H); MS-FAB ($\text{M} - \text{H}$)[−] 637.4 (calcd 637.603).

SLOW- S_P -5** (B = Thy)** (0.41 g, 28% yield): TLC R_f 0.42 (chloroform–ethanol (19:1, v/v) with 5% of Et_3N); ^1H NMR (CDCl_3) δ 1.24 (t, J 7.3 Hz, 9H), 1.39 (d, J 1.1 Hz, 3H), 1.60 (d, J 14.4 Hz, 3H), 2.38 (m, 1H), 2.71 (m, 1H), 2.95 (q, J 7.3 Hz, 6H), 3.43 (d, J 2.7 Hz, 2H), 3.78 (s, 6H), 4.23 (m, 1H), 5.35 (m, 1H), 6.42 (dd, J 8.2, 5.8 Hz, 1H), 6.82 (m, 4H), 7.32 (m, 9H), 7.62 (d, J 1.1 Hz, 1H); MS-FAB ($\text{M} - \text{H}$)[−] 637.4 (calcd 637.603).

B = Ade^{Bz}: ^{31}P NMR (CDCl_3) δ (FAST- R_P -**5**) 77.52; (SLOW- S_P -**5**) 77.59 ppm.

B = Cyt^{Bz}: ^{31}P NMR (CDCl_3) δ (FAST- R_P -**5**) 77.02; (SLOW- S_P -**5**) 77.24 ppm.

B = Gua^{ibu}: ^{31}P NMR (CDCl_3) δ (FAST- R_P -**5**) 76.98; (SLOW- S_P -**5**) 77.04 ppm.

Synthesis of 5'-O-DMT-thymidine 3'-O-(4,6-Dimethoxy-1,3,5-triazin-2-yl) Methylphosphonothioate (7**).** Substrate SLOW- R_P -**5** (B = Thy, ^{31}P NMR 77.27) and *N*-methyl-*N*-[4,6-dimethoxy-1,3,5-triazin-2-yl]morpholinium chloride [**1**] (2 equiv) were stirred at rt for 0.5 h in dry MeCN. After the reaction was complete (TLC control), the reaction mixture was concentrated and applied to a silica gel column. Product SLOW- S_P -**7** (^{31}P NMR 95.68 ppm) was eluted with 2% methanol in CHCl_3 in 88% yield. Analogously,

(23) The attempts to obtain the active ester **10** in a reaction of the stable ester **7** with tBuOOH or SeO_2 under mild anhydrous conditions have failed.

(24) Our observations suggest that methyl phosphonoselenoates are, in contrast, activated in a nonselective way, and both *Se*-esters and *O*-esters are formed.

(25) Misiura, K; Stec, W. J. *Synlett* **2004**, 2143–2146.

from FAST-**R_p**-5 (³¹P NMR: 77.46 ppm) was obtained pure FAST-**R_p**-7 (³¹P NMR 96.23 ppm) in 90% yield.

S_p-7 (B = Thy): ³¹P NMR (CDCl₃) δ 95.68 ppm; ¹H NMR (CDCl₃) δ 1.40 (s, 3H, CH₃-C5), 2.15 (d, ²J_{P-H} = 16 Hz, 3H, CH₃-P), 2.55 (m, 1H, H2'), 2.62 (dd, 1H, H2'), 3.36 (dd, 1H, H5'), 3.60 (dd, 1H, H5'), 3.78 (s, 6H, CH₃-O-Tr), 4.01 (s, 6H, CH₃-O-Ph), 4.27 (m, 1H, H4'), 6.13 (dd, 1H, H3'), 6.48 (dd, 1H, H1'), 6.82–7.43 (m, 13H, aromatic), 7.62 (d, 1H, H6), 8.09 (s, 1H, N-H); MS FAB [M + 1]⁺ = 778.0 (calcd 777.79).

R_p-7 (B = Thy): ³¹P NMR (CDCl₃) δ 96.23 ppm; ¹H NMR (CDCl₃) δ 1.41 (s, 3H, CH₃-C5), 2.17 (d, ²J_{P-H} = 16 Hz, 3H, CH₃-P), 2.57 (m, 1H, H2'), 2.62 (dd, 1H, H2'), 3.36 (dd, 1H, H5'), 3.60 (dd, 1H, H5'), 3.78 (s, 6H, CH₃-O-Tr), 4.01 (s, 6H, CH₃-O-Ph), 4.27 (m, 1H, H4'), 6.13 (dd, 1H, H3'), 6.42 (dd, 1H, H1'), 6.84–7.40 (m, 13H, aromatic), 7.62 (d, 1H, H6), 8.09 (s, 1H, N-H); MS FAB [M + 1]⁺ = 778.0 (calcd 777.79).

B = Ade^{Bz}: ³¹P NMR δ (FAST-**R_p**-7) 96.02; (SLOW-**S_p**-7) 95.89 ppm; yield 95%.

B = Cyt^{Bz}: ³¹P NMR δ (FAST-**R_p**-7) 95.79; (SLOW-**S_p**-7) 95.50 ppm; yield 97%.

B = Gua^{ibu}: ³¹P NMR δ (FAST-**R_p**-7) 96.15; (SLOW-**S_p**-7) 96.01 ppm; yield 95%.

Synthesis of 5'-O-DMT-nucleoside 3'-O-(O-Methyl) Methylphosphonothioate (8). To a solution of ester **7** (20 mg) dried overnight on high vacuum and dissolved in chloroform were added methanol (15 μL) and DBU (5 μL), and stirring was continued for 30 min. The reaction mixture was washed with 0.05% citric acid and water, and the organic layer was separated, dried with MgSO₄, and concentrated. The foamy residue was dissolved in CH₂Cl₂ and precipitated to hexane. It did not require further purification.

B = Thy-**8**: ³¹P NMR (CDCl₃) δ 99.66 ppm, ¹H NMR (CDCl₃) δ 1.43 (s, 3H, CH₃-C5), 1.79 (d, ²J_{P-H} = 15.5 Hz, 3H, P-CH₃), 2.42 (m, 1H, H2'), 2.53 (dd, 1H, H2'), 3.42 (dd, 1H, H5'), 3.49 (dd, 1H, H5'), 3.72 (d, ³J_{P-H} = 13.9 Hz, 3H, CH₃-O-P) 3.79 (s, 6H, CH₃-O-Ph), 4.19 (d, 1H, H4'), 5.48 (m, 1H, H3'), 6.43 (dd, 1H, H1'), 6.84–7.31 (m, 13H, aromatic), 7.60 (d, 1H, H6) 8.09 (d, 1H, N-H).

Dithymidine (3',5')-Methylphosphonothioate (4). Substrates **5** (B = Thy, 10 mg, 96.23 ppm, 95.68 ppm, diastereomeric ratio:

92:8) and **9** (12 mg, 2 equiv) were stirred with DBU (17 μL, 4 equiv) for 24 h in CH₂Cl₂ (0.5 mL) at rt. After overnight stirring, the solvent was reduced to one-third its volume, and the residue was dissolved with methylene chloride and washed twice with diluted citric acid (0.01 M) and water. The organic layer was dried with MgSO₄, concentrated to dryness, and coevaporated twice with toluene. The residue was dissolved in a small volume of dry THF and treated with Et₃N × 3HF/Et₃N (3:1 v/v). After deprotection was completed (3–4 h), the reaction mixture was diluted with chloroform, washed twice with NaHCO₃ (0.1 M), concentrated, and subjected to a silica gel column chromatography (gradient 0–4% EtOH in chloroform, with addition of 0.05% of Et₃N). The product **4** was obtained in 61% as a mixture of diastereomers (97.65 ppm, 98.27 ppm; diastereomeric ratio 88:12).

FAST-(**S_p**)-**4**: (*R_f* = 0.5, CHCl₃–EtOH, 9:1); ³¹P NMR (202.46 MHz, CDCl₃) δ 98.2; ¹H NMR (500 MHz, CDCl₃) δ 1.44 (s, 3H), 1.62 (d, ²J_{PH} = 17.6, 3H, P-CH₃), 2.51 (m, 2H), 2.68 (m, 1H), 3.10 (m, 1H), 3.8 (s, 6H, (Thy) CH₃O-DMT), 3.90 (m, 2H), 4.42 (m, 1H), 4.54 (m, 1H), 5.35 (m, 1H), 6.09 (m, 1H, H1'), 7.98 (1H, H6), 8.16 (1H, H6); FAB[–]MS [M – H][–] 962.6 (C₄₆H₅₅N₅O₁₄PS calcd 963.99); [α]_D = +50.7 (*c* = 0.1, CHCl₃).

SLOW-(**R_p**)-**4**: (*R_f* = 0.45, CHCl₃–EtOH, 9:1); ³¹P NMR (CDCl₃) δ 98.0; ¹H NMR (CDCl₃) δ 1.43 (s, 3H), 1.56 (d, ²J_{PH} = 17.6, 3H, P-CH₃), 2.29 (m, 1H), 2.40 (m, 1H), 2.67 (m, 1H), 2.75 (m, 1H), 3.36 (dd, 1H), 3.51 (dd, 1H), 3.81 (s, 6H, CH₃O-DMT), 4.21 (m, 2H), 4.37 (dd, 2H), 4.51 (m, 1H), 5.19 (m, 1H, H2'), 6.23 (m, 1H, H1'), 6.37 (m, ³J_{HH} = 3.43, 1H, H1'), 7.98 (d, ³J_{HH} = 7.7, 1H, H6), 8.16 (d, ³J_{HH} = 7.5, 1H, H6); FAB[–]MS [M – H][–] 962.4 (calcd 963.99); [α]_D = 81.9 (*c* = 0.13, CHCl₃).

Acknowledgment. This work was financially assisted by the State Committee for Scientific Research (KBN-Grant 4 TO9A 073 25 for L.A.W.)

Supporting Information Available: General synthetic procedures and NMR and MS spectra of compounds **6–11**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO7014906