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

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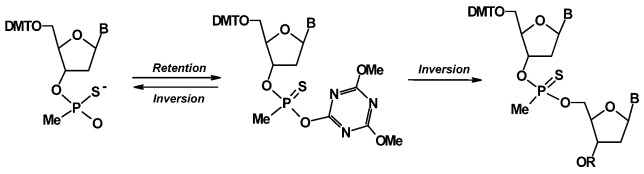
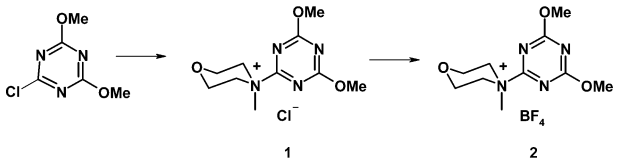


Chemoselective and stereospecific *O*-activation of 2′-deoxynucleoside 3′-*O*-methylphosphonothioates **5** with *N*-methyl-*N*-4,6-dimethoxy-1,3,5-triazin-2-yl morpholinium salts results in formation with retention of configuration of 5′-*O*-DMT-2′-deoxynucleoside 3′-*O*-(4,6 dimethoxy-1,3,5-triazin-2-yl 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 esters[4] as well as other compounds.[5] Among many activators tested in these reactions, mostly derivatives of *N*-hydroxybenzotriazoles[6] or azabenzotriazoles[7] 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 triazoles[11] or *N*-hydroxybenzotriazoles[12] 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 R_P/S_P 2:1)	THF	12	R_P/S_P 1:2	95
substrate **5** (mix R_P/S_P 2:1)	MeCN	0.5	R_P/S_P 1:2	98
substrate **5** (SLOW R_P)	MeCN	0.5	SLOW S_P	98
substrate **5** (FAST S_P)	MeCN	0.5	FAST R_P	98

[a] Calculated from the ^{31}P 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**) [^{31}P 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.

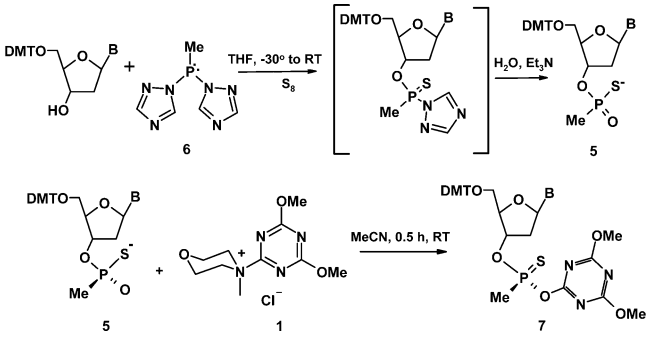
The activator **1** was generated in situ from equimolar amounts of 2-chloro-4,6-dimethoxy-1,3,5-triazine and *N*-methylmorpholine,[17] since the crystalline form of **1** was unstable, even when stored at low temperatures.[18] Freshly generated **1** reacted smoothly with diastereomerically pure **5** 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.

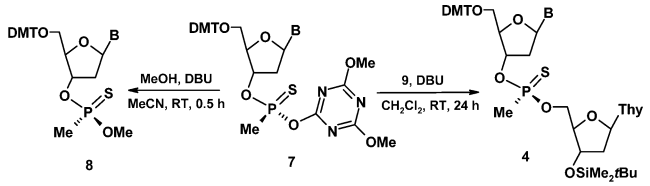
The use of diastereomerically pure methylphosphonothioates **5** of known absolute configuration at the phosphorus permitted assignment of the absolute configuration of active esters **7** (Scheme 1, Table 1) and optimization of the esterification conditions.

The esters **7** are stable in the presence of methanol or water; however, in the presence of strong organic bases, e.g., iPr_2NEt 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 (^{31}P 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).

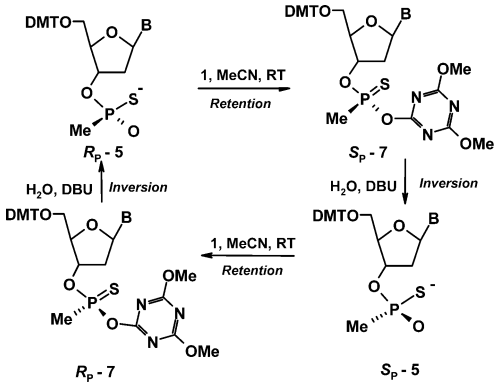
SCHEME 1. Synthesis of Active Esters 7



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 $A_{PMeS}T$-**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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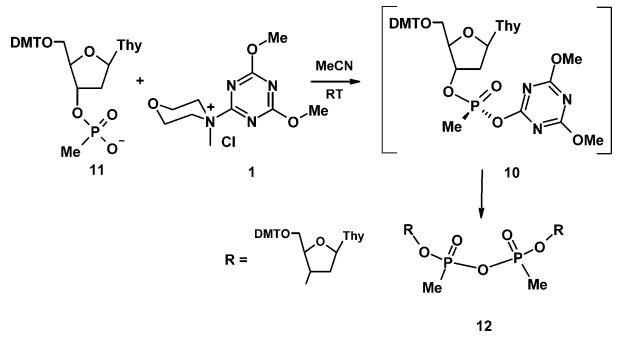
SCHEME 4. Activation of Prochiral Thymidine Methylphosphonate (11)



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 methylphosphonotioates **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 F_{254} plates, respectively. NMR spectra were recorded at 500.13 MHz (^{1}H) and 202.46 MHz (^{31}P). Chemical shifts (δ) are reported relative to TMS (^{1}H) 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: ^{1}H NMR ($CDCl_3$) δ 3.01 (s, 5H), 3.72 (t, 4H), 3.83 (t, 4H), 3.95 (s, 6H). Anal. ($C_{10}H_{17}N_4O_3Cl$): 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}PNMR ($CDCl_3$) 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); ^{1}H 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 (M − 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); ^{1}H 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 (M − 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** (^{31}P NMR: 77.46 ppm) was obtained pure FAST-R_P-**7** (^{31}P NMR 96.23 ppm) in 90% yield.

S_P-**7** (B = Thy): ^{31}P NMR ($CDCl_3$) δ 95.68 ppm; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3H, CH_3-C5), 2.15 (d, $^2J_{P-H}$ = 16 Hz, 3H, CH_3-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, CH_3-O-Tr), 4.01 (s, 6H, CH_3-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): ^{31}P NMR ($CDCl_3$) δ 96.23 ppm; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 3H, CH_3-C5), 2.17 (d, $^2J_{P-H}$ = 16 Hz, 3H, CH_3-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, CH_3-O-Tr), 4.01 (s, 6H, CH_3-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}: ^{31}P NMR δ (FAST-R_P-**7**) 96.02; (SLOW-S_P-**7**) 95.89 ppm; yield 95%.

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

B = Gua^{ibu}: ^{31}P 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 $MgSO_4$, and concentrated. The foamy residue was dissolved in CH_2Cl_2 and precipitated to hexane. It did not require further purification.

B = Thy-**8**: ^{31}P NMR ($CDCl_3$) δ 99.66 ppm, ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 3H, CH_3-C5), 1.79 (d, $^2J_{P-H}$ = 15.5 Hz, 3H, P-CH_3), 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, CH_3-O-P) 3,79 (s, 6H, CH_3-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 (17μL, 4 equiv) for 24 h in CH_2Cl_2 (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 $MgSO_4$, concentrated to dryness, and coevaporated twice with toluene. The residue was dissolved in a small volume of dry THF and treated with $Et_3N \times 3HF/Et_3N$ (3:1 v/v). After deprotection was completed (3−4 h), the reaction mixture was diluted with chloroform, washed twice with $NaHCO_3$ (0.1 M), concentrated, and subjected to a silica gel column chromatography (gradient 0−4% EtOH in chloroform, with addition of 0.05% of Et_3N). 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, $CHCl_3$−EtOH, 9:1); ^{31}P NMR (202.46 MHz, $CDCl_3$) δ 98.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 3H), 1.62 (d, $^2J_{PH}$ = 17.6, 3H, P-CH_3), 2.51 (m, 2H), 2.68 (m, 1H), 3.10 (m, 1H), 3.8 (s, 6H, (Thy) CH_3O-DMT), 3.90 (m, 2H), 4.42 (m, 1H), 4.54 (m, 1H), 5.35 (m, 1H), 6.09 (m,1H, *H*1′), 7.98 (1H, *H*6), 8.16 (1H, *H*6); FAB$^-$MS [M − H] 962.6 ($C_{46}H_{55}N_5O_{14}PS$ calcd 963.99); $[\alpha]_D$ = +50.7 (c = 0.1, $CHCl_3$).

SLOW-(R_p)-**4**: (R_f = 0.45, $CHCl_3$−EtOH, 9:1); ^{31}P NMR ($CDCl_3$) δ 98.0; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 3H), 1.56 (d, $^2J_{PH}$ = 17.6, 3H, P-CH_3), 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, CH_3O-DMT), 4.21 (m, 2H), 4.37 (dd, 2H), 4.51 (m, 1H), 5.19 (m, 1H, *H*2′), 6.23 (m, 1H, *H*1′), 6.37 (m, $^3J_{HH}$ = 3.43, 1H, *H*1′), 7.98 (d, $^3J_{HH}$ = 7.7, 1H, *H6*), 8.16 (d, $^3J_{HH}$ = 7.5, 1H, *H*6); FAB$^-$MS $[M - H]^-$ 962.4 (calcd 963.99); $[\alpha]_D$ = 81.9 (c = 0.13, $CHCl_3$).

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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.

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