Base-Dependent Regioselective and P-Stereocontrolled Hydrolysis of Nucleoside 3′-*O*-(*O*-2,4,6-Trimethylbenzoyl Methanephosphonothioate)s

Lucyna A.Woźniak, Arkadiusz Chworoś, Jarosław Pyzowski, and Wojciech J. Stec*

Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Department of Bioorganic Chemistry, Sienkiewicza 112, 90-363 Łódź, Poland

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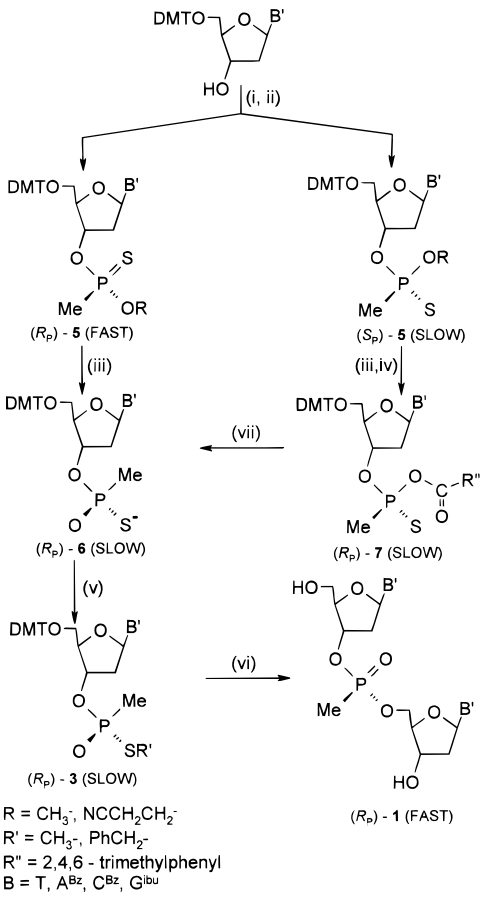
An early observation that oligonucleotides containing methanephosphonate linkages of (R_P) configuration at internucleotide positions possess higher affinity toward complementary DNA or RNA[1] facilitated studies on the synthesis of the chimeric oligonucleotide constructs with incorporated (R_P)-dinucleoside methanephosphonates (**1**).[2] Therefore, an approach to the stereoselective synthesis of **1**[3] appeared as the prerequisite for construction of the second generation of antisense molecules.[4] Recently, we have described a novel approach to the stereoselective synthesis of dinucleoside (3′,5′)-methanephosphonates (**1**) based upon the synthesis of the appropriately protected nucleoside 3′-*O*-methanephosphonoanilidothioates, their separation into diastereomeric species, and sequential stereospecific conversion of each single [(R_P) or (S_P)] diastereomeric methanephosphonoanilidothioate into (R_P)-5′-*O*-DMT-(N-protected)-nucleoside 3′-*O*-(*S*-alkyl methanephosphonothiolate) (**3**), followed by substitution of the thioalkyl group in (R_P)-**3** with the 5′-OH group of N-protected 3′-*O*-acetyl (or *tert*-butyldimethylsilyl) nucleoside (**2**).[5] To the best of our knowledge, this has been the first method allowing for the synthesis of one required diastereomer of dinucleoside (3′,5′)-methanephosphonates from either of the separated diastereomeric precursors.

In this paper we describe an alternative route leading exclusively to (R_P)-**3**. A corollary of this accomplishment is the simple synthesis of suitably protected nucleoside 3′-*O*-(*O*-alkyl methanephosphonothioate)s (**5**) (R = $-CH_3$, or preferably $-CH_2CH_2CN$). After their separation into diastereomers and subsequent O-dealkylation,[6] ammonium salts of diastereomerically pure (R_P)- and (S_P)-5′-*O*-DMT-nucleoside methanephosphonothioates (**6**) are obtained[7,8] (Scheme 1).

Scheme 1



Chemoselective S-alkylation of (R_P)-**6** results in the formation of (R_P)-5′-*O*-DMT-(N-protected, except for thymine) nucleoside 3′-*O*-(*S*-alkyl methanephosphonothiolate)s (**3**) which, as depicted in Scheme 1, are used as the substrates for synthesis of the desired (R_P)-**1**. The opposite diastereomers, (S_P)-**6**, are treated with sterically hindered 2,4,6-trimethylbenzoyl chloride. The process of acylation is completely regioselective (exclusive *O*-benzoylation) and occurs with retention of configuration at the phosphorus atom, providing in high yield of (R_P)-5′-*O*-DMT-(N-substituted, except thymine) nucleoside 3′-*O*-(2,4,6-trimethylbenzoyl methanephosphonothioate)s (**7**). The hydrolysis of the mixed anhydrides (R_P)-**7** in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) occurs smoothly within 30−40 min giving (R_P)-methanephosphonothioates **6** in high (over 90%) preparative yields.[9] This process is fully stereospecific and *occurs with inversion of configuration at the phosphorus center*.[10] Therefore, the benzoylation of (S_P)-**6** followed by the DBU-assisted hydrolysis of the resulting (R_P)-**7** allows for the stereoconversion of (S_P)-**6** into (R_P)-**6** and the use of both separated diastereomers **6** for the stereoselective synthesis of slow-(R_P)-**3**[11] (Data are collected in Table 1).

* Author to whom correspondence should be addressed. Fax: (48-42) 681 97 44. E-mail: wjstec@bio.cbmm.lodz.pl.

(1) (a) Miller, P. S.; Annan, N. D.; McParland; K. B.; Pulford, S. M. *Biochemistry* **1982**, *21*, 2507−2512. (b) Leśnikowski, Z.; Jaworska, M.; Stec, W. J. *Nucleic Acids Res.* **1990**, *18*, 2109−2115.

(2) Reynolds, M. A.; Hogrefe, R. I.; Jaeger, J. A.; Schwartz, D. A.; Riley, T. A.; Marvin, W. B.; Daily, W. J.; Vaghefi, M. M.; Beck, T. A.;. Knowles, S. K.; Klem, R. E.; Arnold, L. J., Jr. *Nucleic Acids Res.* **1996**, *24*, 4584−4591.

(3) (a) Kaji, A.; Higuchi, H., Endo, T. *Biochemistry* **1990**, *29*, 8747−8753. (b) Loschner, T.; Engels, J. *Tetrahedron Lett.* **1989**, *30*, 5587−5590.

(4) (a) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544−584. (b) Englisch, U.; Gauss, D. H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 613−629.

(5) Stec, W. J.; Woźniak, L. A.; Pyzowski, J.; Niewiarowski, W. *Antisense Nucleic Acid Drug Dev.* **1997**, *7*, 383−397.

(6) Gray, M. D. M.; Smith, D. J. H. *Tetrahedron Lett.* **1980**, *21*, 859−862.

(7) (R_P)- and (S_P) assignments are in accordance with Cahn−Ingold−Prelog formalism,[10] but O-benzoylation changes the priorities of ligands surrounding phosphorus in compounds **6** and **7**.

(8) Cahn, R. S.; Ingold, C. K.; Prelog, V. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385−414.

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Table 1. Stereoconversion of Nucleoside 3′-*O*-Methanephosphonothioic Acids

		slow (R_P)-**7**			fast (S_P)-**7**		
B′	(S_P)-**6** ^{31}P NMR[a]	^{31}P NMR	^{1}H NMR[b]	(R_P)-**6** ^{31}P NMR	^{31}P NMR	^{1}H NMR[b]	FAB MS (calcd)
Thy	77.63	91.41	2.09 (15.9)	77.45	91.77	2.04 (15.9)	784.2 (784.2583)
C^{Bz}	75.77	91.50	2.08 (15.9)	75.66	91.57	2.05 (15.9)	873.2849 (867.3067)
A^{Bz}	77.46	91.06	2.14 (15.9)	77.27	91.49	2.07 (15.9)	896.9 (897.2961)
G^{iBu}	76.82	91.29	2.14 (15.9)	77.01	91.85	2.09 (15.9)	879.10 (879.3067)

[a] ^{31}P NMR in $CDCl_3$, as triethylammonium salt. [b] ^{1}H NMR in $CDCl_3$ ($^{2}J_{P-CH_3}$ in Hz).

Interestingly, if the hydrolysis of mixed anhydrides (R_P)-**7** is performed in the presence of 4-(dimethylamino)-pyridine DMAP, (S_P)-nucleoside methanephosphonothioate **6** is recovered. The hydrolysis of the opposite diastereomer (S_P)-**7** in the presence of DMAP occurs with the same base-dependent stereochemistry, so this reaction proceeds with *retention of configuration* at the phosphorus atom.

The observation of variable stereochemistry of the base-promoted hydrolysis of mixed anhydrides **7** indicates that this reaction most probably proceeds via two different mechanisms and is dependent upon the nature of the catalyst. Strongly basic DBU ($pK_a = 11.6$)[12] promotes an attack of the hydroxyl oxygen at the phosphorus atom, while the weaker base, DMAP ($pK_a = 9.70$)[13] causes exclusive attack of water at the carbonyl function.[14] An influence of the base strength upon the site of attack of the water molecule on ambident electrophiles containing both carbonyl and methanephosphonothioyl centers (as in compounds **7**) has also been confirmed in the studies involving stereoselective incorporation of [^{18}O] into 5′-*O*-DMT-thymidine 3′-*O*-methanephosphonothioate **6**. In separate experiments, the hydrolysis of 5′-*O*-DMT-thymidine 3′-*O*-(2,4,6-trimethylbenzoyl methanephosphonothioate) (**7**) was performed in $H_2{}^{18}O$ (95% enriched), in the presence of DBU or DMAP. The reaction progress was monitored by TLC (5% MeOH in $CHCl_3$). After hydrolysis was complete (about 1 h), the reaction mixture was analyzed by both ^{31}P NMR and mass spectrometry. It was found that, in the DBU-assisted hydrolysis, oxygen [^{18}O] originating from $H_2{}^{18}O$ was almost exclusively incorporated into 5′-*O*-DMT-thymidine 3′-*O*-methanephosphonothioic acid **6**. The isotope chemical shift effect measured for **6** was $\Delta\delta = 3.7$ Hz. The product of hydrolysis was then S-alkylated and the value of the isotope effect was $\Delta\delta = 4.1$ Hz for ester **3**. Similar substitution-directing effects have also been observed for the DBU- or DMAP-assisted alcoholysis (details not given).

The effectiveness of our new stereoselective synthesis of (R_P)-nucleoside 3′-*O*-(*S*-methyl methanephosphonothiolate)s **3** is summarized in Table 2.

Analysis of the data included in Tables 1 and 2 indicates that the ^{31}P NMR chemical shift values are not always reliable parameters for distinguishing between the (R_P) and (S_P) diastereomers of **5**, **6**, and **3**. The apparent distinction between diastereomers is only possible via HPLC (or HP TLC) comparison of the given single diastereomer with the mixture of both diastereomers. The earlier assignments[17] of the absolute configuration at the phosphorus atom in both diastereomers of compound **6** (B = Thy) are, however, in agreement with our recent results.

Besides the practical application of the base-directed solvolysis of mixed anhydrides **7** for the cost-effective stereoselective synthesis of **1**, we wish to emphasize that the obtained results allow us to demonstrate a new Walden cycle in phosphorus chemistry[18] (Scheme 2).

Mechanistic elucidation of the observed base-dependent regioselectivity of hydrolysis of mixed anhydrides **7** deserves further study, but the results presented here imply that DBU,[19] in contrast to DMAP,[20] exerts in the hydrolysis of **7** a function of the base strong enough to generate hydroxyl ions in aqueous solutions, which are then able to attack the phosphorus atom, leading to inversion of configuration at this center. DMAP, which is known to be less basic than DBU but much more nucleophilic, most probably attacks the carbonyl center with the formation of an adduct,[20] which is subsequently

(9) It has been known that mixed phosphorus/carboxylic anhydrides may exercise both acylating and phosphorylating properties, as demonstrated in the following: (a) Clark, V. M.; Hutchinson, D. V.; Kirby, A. J.; Warren, S. G. *Angew. Chem.* **1964**, *76*, 704−711; *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 678−685. (b) Lambie, A. J. *Tetrahedron Lett.* **1966**, *33*, 3709−3712. (c) Sokolova, N. I.; Nosova, V. V.; Velmoga, I. S.; Shabarova, Z. A. *Zh. Obshch. Khim.* **1975**, *45*, 1197. (d) Jackson, A. G.; Kenner, G. W.; Moore, G. A.; Ramage, R.; Thorpe, W. D. *Tetrahedron Lett.* **1976**, *40*, 3627−3630. (e) Drutsa, V. L.; Zarytova, Z. F.; Knorre, D. G.; Lebedev, A. V.; Sokolova, N. I.; Shabarova, Z. A. *Dokl. Akad. Nauk SSSR* **1977**, *223*, 595−597. (f) Jacobsen, E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1983**, *105*, 1619−1624.

(10) The assignment of the absolute configurations of compounds **6** by means of X-ray analysis of their derivatives **3** was independently supported by the stereoselective enzymatic digestion of fully deprotected **6** with Nuclease P1.[5]

(11) It has to be mentioned that other direct methods of synthesis of **3** do not allow for diastereoconversion of separated isomers, for example: Brill, W. K.-D.; Caruthers, M. H. *Tetrahedron Lett.* **1987**, *28*, 3205−3208. Brill, W. K.-D. *Tetrahedron Lett.* **1995**, *36*, 703−706.

(12) Nakatani, K.; Hashimoto, S. *Soc. Synth. Org. Chem.* **1975**, *33*, 925.

(13) Chakrabarty, M. R.; Handlosed, C. S.; Mosher, M. W. *J. Chem. Soc., Perkin Trans. 2* **1973**, 938−942.

(14) The regioselectivity of alcoholysis of mixed phosphilic/carboxylic anhydrides is still unpredictable and depends not only upon the kind of the base but also upon the nature of substituents at both phosphorus and the carbonyl center, as well as upon the solvolytic medium. For example, *O*,*O*-diethyl-*O*-trifluoroacetyl phosphate reacts with ethanol as an acetylating agent as promptly noticed by Wasiak et al.,[15] correcting our erroneous data.[16]

(15) Wasiak, J.; Heliński, J.; Dąbkowski, W.; Skrzypczyński, Z.; Michalski, J. *Pol. J. Chem.* **1995**, *69*, 1027−1032.

(16) Woźniak, L. A.; Pyzowski, J.; Wieczorek, M.; Stec, W. J. *J. Org. Chem.* **1994**, *59*, 5843−5846.

(17) (a) Niewiarowski, W.; Leśnikowski, Z.; Wilk, A.; Guga, P.; Okruszek, A.; Uznański, B.; Stec, W. J. *Acta Biochim. Pol.* **1987**, *34*, 217−231. (b) Lebedev, A. V.; Rife, P. J.; Seligsohn, H. W.; Wenzinger, G. R.; Wickstrom, E. *Tetrahedron Lett.* **1990**, *31*, 855−858. (c) Engels, J. W.; Loschner, T. *Nucleic Acid Res.* **1990**, *18*, 5083−5088.

(18) Mikołajczyk, M.; Michalski, J.; Omelańczuk, J. *Tetrahedron Lett.* **1965**, *23*, 1779−1784.

(19) The function of DBU as a "strong non-nucleophilic base" has been demonstrated. See: (a) Seebach, D.; Thaler, A.; Blaser, D.; Ko, S. Y. *Helv. Chim. Acta* **1991**, *74*, 1102−1118. (b) Muathen, H. A. *J. Org. Chem.* **1992,** *57*, 2740−2741. (c) Tawfik, D. S.; Eshhar, Z.; Bentolila, A.; Green, B. S. *Synthesis* **1993**, 968−972. (d) Bennua-Skalmowski, B.; Vorbruggen, H. *Tetrahedron Lett.* **1995**, *36*, 2611−2614. (e) Soloshonok, V. A.; Ono, T. *J. Org. Chem.* **1997**, *62*, 3030−3032.

(20) Hofle, G.; Steglich, W.; Vorbruggen, H. *Angew. Chem.* **1978**, *90*, 602−616. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 569−583.

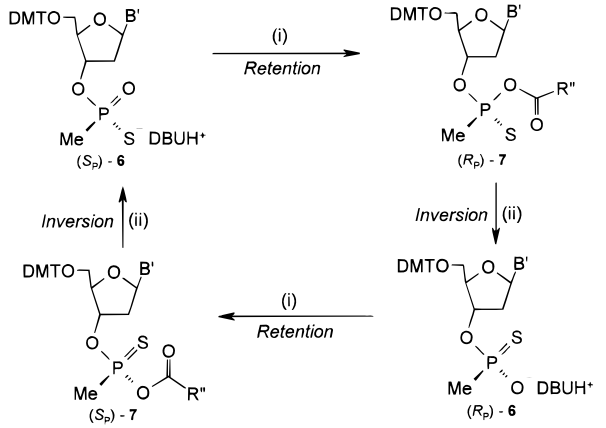
(21) *Oligonucleotide Synthesis. A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1984.

Table 2. Spectral Characteristics for 5′-*O*-DMT-nucleoside Methanephosphonothioates (*O*-alkyl 5), (*S*-alkyl 3), and 6

	5′-*O*-DMT-nucleoside 3′-*O*-(*O*-alkyl methanephosphonothioate) (R = CH_2CH_2CN) **5**			thioacid **6**		5′-*O*-DMT-nucleoside 3′-*O*-(*S*-alkyl methanephosphonothiolate) (R = CH_3) **3**			
nucleoside[a]	config[b]	^{31}P NMR (δ, ppm)	^{1}H NMR (δ, ppm; J_{PCH3} in Hz)	config (yield, %)[c]	^{31}P NMR (δ, ppm)[d]	config (yield, %)[e]	^{31}P NMR (δ, ppm)	HR FAB MS [M−H][f]	^{1}H NMR (δ, ppm; J_{P-H}, in Hz)[g]
thymidine (80)	(R_P)-**5** fast (0.58)	98.85	1.89; 15.54[h]	(R_P)-**6** (86)	72.67	(R_P)-**3** (95) slow	57.22	637.178	1.74; 15.78 (CH_3P, d) 2.19, 11.6 (CH_3S, d)
	(S_P)-**5** slow (0.48)	98.34	1.84; 15.58	(S_P)-**6** (87)	72.34	(S_P)-**3** (95) fast	57.21	(637.177)[i]	1.82; 15.67 (CH_3P, d) 2.18; 13.18 (CH_3S, d)
cytidine (67)	(R_P)-**5** fast (0.55)	98.90	1.88; 15.52	(R_P)-**6** (70)	72.97	(R_P)-**3** (90) slow	56.96	740.209	1.81; 15.63 (CH_3P, d) 2.21; 13.30 (CH_3S, d)
	(S_P)-**5** slow (0.43)	98.31	1.83; 15.60	(S_P)-**6** (65)	72.58	(S_P)-**3** (86) fast	57.01	(740.219)	1.83; 15.6 (CH_3P, d) 2.27; 13.3 (CH_3S, d)
adenosine (63)	(R_P)-**5** fast (0.58)	98.32	1.91; 15.57	(R_P)-**6** (85)	72.93	(R_P)-**3** (80) slow	57.32	764.4	1.85; 13.6 (CH_3P, d) 2.2; 12.08 (CH_3S, d)
	(S_P)-**5** slow (0.48)	98.47	1.87; 15.54	(S_P)-**6** (81)	72.70	(S_P)-**3** (75) fast	57.41	(764.261)	1.88; 15.07 (CH_3P, d) 2.1; 11.50 (CH_3S, d)
guanosine (51)	(R_P)-**5** fast (0.30)	98.47	1.85; 15.52	(R_P)-**6** (70)	72.06	(R_P)-**3** (85) slow	57.34	746.235	1.81; 20.38 (CH_3P, d) 2.10; 13.35 (CH_3S, d)
	(S_P)-**5** slow (0.20)	98.80	1.83; 15.61	(S_P)-**6** (75)	72.22	(S_P)-**3** (82) fast	57.47	(746.241)	1.82; 15.55 (CH_3P, d) 2.32; 13.19 (CH_3S, d)

[a] In parentheses yields of compounds **5** are given. [b] Relative mobilities of diastereomers on HP TLC analytical plates (E. Merck). Developing system: $CHCl_3$/MeOH (96:4 v/v, twice). [c] Yields of conversion **5** → **6** (after purification). [d] Spectra registered in DMF/C_6D_6 (sodium salt, precipitated from petroleum ether). [e] In parenthesis, yields of conversions **6** → **3** are given. [f] HR MS analysis was performed for 1:1 mixture of diastereomers **3**. Calculated molecular weights are given in parentheses. [g] Data are given for P−CH_3 protons, in $CDCl_3$. [h] Data are given for P−CH_3 and P−SCH_3 protons, in $CDCl_3$, appropriately. [i] [M−CH_3] peak was observed.

Scheme 2



hydrolyzed, restoring parent **6** with unchanged configuration at phosphorus.

Experimental Section

General. NMR spectra were recorded at 300.13 (^{1}H) and 121.47 (^{31}P) MHz. Chemical shifts are reported (δ) relative to TMS (^{1}H) and 80% H_3PO_4 (^{31}P) as external standards. 2D ^{1}H-^{1}H NMR (NOESY) correlations were applied for identification of signals in NMR spectra. Positive chemical shift values were assigned for compounds resonating at lower fields than the 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 mass spectra were recorded for 1:1 mixtures of diastereomers. Column chromatography and HP TLC analyses were performed on silica gel (240−400 mesh) and precoated F_{254} silica gel plates, respectively. Solvents and reagents were purified according to the standard laboratory techniques and stored under argon. MeCN was distilled from CaH_2 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.

General Procedure for Preparation of 5-*O*-DMT-(N-Protected) Nucleoside 3′-*O*-(*O*-Alkyl Methanephosphonothioate)s (5) from MeP(S)Cl_2. To a solution of MeP(S)Cl_2 (2 mmol) in dry pyridine (15 mL), a solution of 5′-*O*-DMT-(N-protected, except thymine) nucleoside (1 mmol), synthesized according to the literature[21] in pyridine (5 mL), was added. The reaction mixture was stirred for 15 min at 55 °C and cooled to ambient temperature, followed by an addition of 4−6 mmol of alcohol. After 30 min, the reaction was completed, the reaction mixture was concentrated under reduced pressure to about 1/3 of the original volume, and the oily residue was dissolved in $CHCl_3$, (30 mL) and washed 3 times with citric acid (0.1 M, 15 mL). The organic layer was dried over anhydrous $MgSO_4$, concentrated, and purified by means of silica gel column chromatography [elution with chloroform containing petroleum ether (20−5%)]. Appropriate fractions of products fast-**5** (isomer faster migrating during a silica gel chromatography) and slow-**5** (isomer slower migrating during a silica gel chromatography)-(R = $NCCH_2CH_2$) were collected and concentrated to give pure diastereomers as colorless foams. Their characteristics are given in Table 2.

Separation of esters **5** (R = OMe) was more difficult, so they were often collected as a mixture of diastereomers and further separated for analytical reasons. Alternatively, separation can be directly achieved for methanephosphonothioates **6** (silica gel column chromatography**,** silica gel 240−400 mesh, gradient 3−6% MeOH in $CHCl_3$, with 0.5% Et_3N).

Fast(R_p)-**5** (B = T, R = OMe): ^{31}P NMR δ 98.91 ($CDCl_3$); ^{1}H NMR ($CDCI_3$) δ 1.77 (9.68, d, 3H, PCH_3), 3.74 (8.22, d, 3H, $POCH_3$), HR FAB [M − H] 651.186 (calcd 651.193). Slow(S_p)-**5** (B = Thy, R = OMe): ^{31}P NMR δ 99,00 ($CDCI_3$); ^{1}H NMR ($CDC1_3$) δ 1.85 (10.63, 3H, PCH_3), 3.70 (7.35, d, 3H, $POCH_3$). Fast(R_p)-**5** (C = C^{Bz}, R = OMe): ^{31}P NMR δ 98.77 ($CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.76 (11.05, d, 3H, PCH_3), 3.72 (14.00 d, 3H, $POCH_3$); HR FAB [M − H] 740.215 (calcd 740.219). Slow(S_p)-**5** (B = C^{Bz}, R = OMe): ^{31}P NMR δ 99.37 ($CDCI_3$); ^{1}H NMR ($CDCI_3$) δ 1.84 (14.01, 3H, PCH_3), 3.70 (7.35, d, 3H, $POCH_3$). Fast(R_p)-**5** (B = A^{Bz}, R = OMe): ^{31}P NMR δ 98.91 ($CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.81 (15.49, d, 3H, PCH_3), 3.74 (14.07,d, 3H, $POCH_3$); HR FAB [M − H] 764.238 (calcd 764.261). Slow(S_p)-**5** (R = A^{Bz}, R = OMe): ^{31}P NMR δ 99.00 ($CDCI_3$); ^{1}H NMR ($CDCl_3$) δ 1.89 (15.53, 3H, PCH_3), 3.62 (13.99, d, 3H, $POCH_3$). Fast(R_P)-**5** (B = G^{ibu}, R = OMe): ^{31}P NMR δ 99.23 ($CDCI_3$); ^{1}H NMR ($CDCI_3$) δ 1.76 (15.49, d, 3H, PCH_3), 3.51 (14.01, d, 3H, $POCH_3$); HR FAB [M − H]: 746.238 (calcd 746.241).

General Procedure for Preparation of 5′-*O*-DMT-(N-Protected Except Thymine) Nucleoside 3′-*O*-(*O*-2,4,6-Trimethylbenzoyl Methanephosphonate)s (7). *N*-Methyl-*N*-*tert*-butylammonium salts of (S_P)-5′-*O*-DMT-nucleoside methanephosphonothioates (**6**) (0.5 mmol) obtained from **5** according to methodology described earlier,[8] were twice coevaporated with pyridine, and left overnight on high vacuum, and redissolved in dry pyridine (8 mL) with Et_3N (2 mmol), and to this solution 2,4,6-trimethylbenzoyl chloride (1−1.5 mmol) was added in one portion. After 30 min, the reaction mixture was concentrated to 1/4 of the original volume, diluted with $CHCl_3$ (15 mL), and washed with 0.05 M citric acid. The organic layer was dried over $MgSO_4$ and concentrated to dryness. ^{31}P NMR spectra confirmed complete conversion to **7**. Compounds **7** were purified by means of silica gel column chromatography. Data are collected in Table 1.

Hydrolysis of Mixed Anhydrides 7 with $H_2{}^{18}O$ and DBU. Mixed anhydride (R_P)-**7** (B = Thy, R′ = 2,4,6-trimethylphenyl) (0.05 mmol) was dissolved in MeCN (1 mL) with 0.05 mL of $H_2{}^{18}O$ (95% ie) and DBU (0.2 mmol). After 4 h, a ^{31}P NMR spectrum of the reaction mixture revealed the presence of a single product (R_P)-**6** [^{31}P NMR (MeCN-d_3) δ 74.6 ppm]. MS analysis of the mixture of unseparated products confirmed the presence of a species with a molecular ion [found FABMS [M − H] 639.3 (calcd 639.2)], indicating the incorporation of [^{18}O] into (R_P)-**6**. The molecular ion of 2,4,6-trimethylbenzoic acid [found FAB MS [M − 1] 163.2 (calcd 163.2)], which was not enriched in [^{18}O] isotope, was also detected. The reaction product was treated with MeI (0.1 mL), and the isolated (R_P)-**3** was identical to a genuine sample prepared independently.[5] The diastereomeric purity of slow-(R_P)-**3** was 95% [^{31}P NMR ($CDCl_3$) δ 58.6].

Hydrolysis of Mixed Anhydrides 7 with $H_2{}^{18}O$ and DMAP. Mixed anhydride (R_P)-**7** (B = Thy, R′ = 2,4,6-trimethylphenyl) (0.05 mmol) was dissolved in MeCN (1 mL) with 0.05 mL of $H_2{}^{18}O$ (95% ie) and DMAP (0.2 mmol). After 2 h, a ^{31}P NMR spectrum of the reaction mixture revealed the presence of a single product of hydrolysis, (S_P)-**6** [^{31}P NMR (MeCN-d_3) δ 74.3; yield +99%; found FAB MS [M − H] 637.3 (calcd 637.2)]. The incorporation of [^{18}O] into benzoic acid was confirmed by means of MS [found FAB MS [M − 1] 166.1 (calcd 166.2)]. The reaction product, (S_P)-**6**, was treated with MeI (0.1 mL), and the isolated (S_P)-**3** was identical to a genuine sample prepared independently.[5] The diastereomeric purity of slow (S_P)-**3** was 95% [^{31}P NMR ($CDCl_3$) δ 58.6].

Stereoconversion of the Nucleoside 3′-*O*-Methanephosphonothioate (S_P)-6 into (R_P)-6 via Mixed Anhydride (R_P)-7. The triethylammonium salt of (S_P)-nucleoside 3′-*O*-methanephosphonothioate (**6**) was coevaporated with pyridine and dried overnight on high vacuum. To a pyridine solution, 2,4,6-trimethylbenzoyl chloride (1.2 equiv) was added in one portion. The reaction progress was controlled by TLC (5% MeOH/$CHCl_3$). After the reaction was complete (about 30 min), the reaction mixture was concentrated and the crude viscous liquid was diluted with $CHCl_3$ and washed with 0.05 M citric acid. The combined organic layers were dried with $MgSO_4$ and concentrated. Product (R_P)-**7** was purified by silica gel column chromatography (chloroform as eluent). The pure mixed anhydride (R_P)-**7** was dissolved in MeCN, followed by addition of DBU (20 equiv) and water (100 equiv). After 2 h, the reaction mixture was concentrated, dissolved with chloroform, and washed with 0.05 M citric acid. The obtained (R_P)-**7** was purified by means of flash silica gel column chromatography (4% EtOH/$CHCl_3$) or used without purification for S-alkylation (data are collected in Table 1).

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Supporting Information Available: Copies of ^1H NMR spectra and high-resolution mass spectra (FAB) of two mixed anhydrides (10 pages). This material is contained in libraries on microfiche, immediately follows this article in microfilm version of the journal, and can be ordered from ACS; see any current masthead page for ordering information.

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