12 H, $C_{5'}$, $C_{6'}$, C_6, C_7, C_{10}, C_{11}-CH_2), 2.45 (q, 2 H, $C_{2'}$-CH_2, J = 7.1 Hz), 2.67 (t, 2 H, $C_{1'}$-CH_2, J = 7.1 Hz), 3.01 (d, 1 H, $C_{3'}$-CH_2, J = 7.1 Hz), 5.08–5.2 (m, 5 H, vinyl H); MS, m/e (rel intensity) 412 (5.0), 373 (7.5), 344 (12.5), 333 (15), 303 (27.5), 275 (25), 259 (20), 207 (30), 183 (70), 165 (45), 135 (15), 109 (20), 81 (52.5), 69 (100). Anal. Calcd for $C_{28}H_{44}S$: C, 81.55; H, 10.67. Found: C, 81.57; H, 10.61.

Hexyl 5,9,13-Trimethyl-4(*E*),8(*E*),12-tetradecatrien-1-ynyl Sulfide (27b). The title compound was obtained in 96% yield: IR (film) 2927.6, 2856.4, 2361.3, 2184, 1669.4, 1452.1, 11378.2, 1284, 834.4 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3 H, J = 6.5 Hz), 1.22–1.45 (m, 6 H, C_2, C_3, C_4, C_5-CH_2), 1.54 (s, 6 H, vinyl methyl), 1.59 (s, 3 H, vinyl methyl), 1.68 (s, 3 H, vinyl methyl), 1.95–2.12 (m, 8 H, $C_{6'}$, $C_{7'}$, $C_{10'}$, $C_{11'}$-CH_2), 2.66 (t, 2 H, -SCH_2, J = 6.5 Hz), 3.0 (d, 2 H, $C_{3'}$-CH_2, J = 6.5 Hz), 5.04–5.21 (m, 2 H, vinyl H), 5.19 (t, 1 H, $C_{4'}$-CH, J = 6.5 Hz); MS, m/e (rel intensity) 346 (2.3), 331 (4.7), 261 (38), 159 (30.9), 125 ((28.5), 105 (4.5), 91 (38), 81 (35.7), 69 (100); HRMS m/e calcd 346.2694, found 346.2703.

4′,8′-Dimethyl-3′(*E*),7-nonadienyl 5,9,13-Trimethyl-1(*E*/*Z*),4(*E*),8(*E*),12-tetradecatraienyl Sulfides (28a,b). General Method. To a suspension of $LiAlH_4$ (0.08 g, 2 mmol) in 2 mL of dry THF was added **27a** (0.41 g, 1 mmol) in 3 mL of THF. The reaction was heated for 3 h at 40 °C to 60 °C and then cooled to 0 °C. Excess hydride was destroyed by careful addition of 1 N HCl. The aqueous solution was diluted with brine and extracted with ether (6 × 30 mL). The combined etheral solution was washed with brine (2 × 20 mL), dried ($MgSO_4$), and concentrated in vacuo to give a crude product. Flash column chromatography using n-hexane gave 0.23 g (56%) of **28a,b**: IR (film) 2965.4, 2918, 2854.6, 2358.6, 1666.3, 1605.7, 1440.4, 1376.4, 1278.7, 1107.7, 834.2 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.59 (s, 15 H, vinyl methyl), 1.67 (s, 6 H, vinyl methyl), 1.97–2.07 (m, 12 H, $C_{5'}$, $C_{6'}$, C_6, C_7, C_{10}, C_{11}-CH_2), 2.3–2.33 (m, 2 H, $C_{2'}$-CH_2), 2.61–2.67 (m, 2 H, $C_{1'}$-CH_2), 2.77 (t, 2 H_{trans}, C_3-CH_2, J = 6.8 Hz), 2.82 (t, 2 H_{cis}, C_3-CH_2, J = 6.8 Hz), 5.08–5.15 (m, 5 H, vinyl H), 5.48–5.54 (dt, 1 H_{cis}, C_2-vinyl H, J = 9.5, 7 Hz), 5.56–5.63 (dt, 1 H_{trans}, vinyl H, J = 15, 7 Hz), 5.92 (dt, 1 H_{trans}, C_1-vinyl H, J = 15, 1.5 Hz), 55.923 (dt, 1 H_{cis}, C_1-vinyl H, J = 9.5 1.5 Hz); MS, m/e (rel intensity) 414 (38.7), 345 (51.6), 277 (25.8), 263 (22.5), 205 (25.8), 135 (22.5), 109 (38.7), 81 (19.3), 69 (100). Anal. Calcd for $C_{28}H_{46}S$: C, 81.15; H, 11.11. Found: C, 81.17; H, 11.21.

Hexyl 5,9,13-Trimethyl-1(*E*/*Z*),4(*E*),8(*E*),12-tetradecatraienyl Sulfides (28c,d). The title compounds were obtained in 54% yield: IR (film) 2940, 2900, 2840, 1660, 1600, 1435, 1370, 1100, 935, 825 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3 H, CH_3, J = 7 Hz), 1.22–1.41 (m, C_2, C_3, C_4, C_5-CH_2), 1.6 (s, 9 H, vinyl methyl), 1.67 (m, 3 H, vinyl methyl), 1.95–2.15 (m, 8 H, $C_{6'}$, $C_{7'}$, $C_{10'}$, $C_{11'}$-CH_2), 2.61–2.66 (m, 2 H, -SCH_2), 2.76 (t, 2 H_{trans}, $C_{3'}$-CH_2, J = 7 Hz), 2.81 (t, 2 H_{cis}, $C_{3'}$-CH_2, J = 7 Hz), 5.05–5.25 (m, 3 H, vinyl H), 5.47–5.53 (dt, 1 H_{cis}, C_2-CH, J = 9.5, 7 Hz), 5.54–5.61 (dt, 1 H_{trans}, C_2-CH, J = 15, 7 Hz), 5.90 (d, 1 H, C_1-CH, J = 9.5 Hz), 9.1 (d, 1 H, C_1-CH, J = 15 Hz); MS, m/e (rel intensity) 348 (26.6), 279 (23.3), 263 (333.3), 191 (40), 157 (26.6), 135 (20), 105 (20), 93 (100), 69 (73.3). Anal. Calcd for $C_{23}H_{40}S$: C, 79.24; H, 11.56. Found: C, 79.24; H, 11.39.

4′,8′-Dimethyl-3′(*E*),7′-nonadienyl 5,9,13-Trimethyl-1(*Z*),4(*E*),8(*E*),12-tetradecatraienyl Sulfide (28b). To a suspension of LAH (0.069 g, 1.8 mmol) in 5 mL of THF was added MeOH (0.02 mL, 0.5 mmol) at room temperature. The flask was cooled to -60 °C and CuBr·Me_2S (0.309 g, 1.5 mmol) was added. After 15 min, **27a** (0.25 g, 0.6 mmol) was added followed by 2 mL of HMPA at -60 °C. The reaction mixture was warmed to room temperature over 2.5 h and quenched with aqueous NH_4Cl. The precipitate was filtered through Celite and the filtrate washed with aqueous NH_4Cl (20 mL) and brine (2 × 20 mL). After drying ($MgSO_4$) and removal solvent, the product was flash chromatographed, using hexanes/ethyl acetate (49/1), to provide **28b** in (0.2 g) 80% yield: IR (film 2965.4, 2921.6, 2854.6, 2361.2, 1696.5, 1666.1, 1605.2, 1447.2, 1376.6, 1107.6, 834.2 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.59–1.65 (3s, 15 H, vinyl methyl), 1.68 (s, 6 H, vinyl methyl), 1.95–2.12 (m, 12 H, CH_2), 2.31 (q, 2 H, $C_{2'}$-CH_2, J = 7 Hz), 2.65 (t, 2 H, -SCH_2, J = 7 Hz), 2.82 (t, 2 H, C_3-CH_2, J = 7 Hz), 5.05–5.25 (m, 5 H, vinyl H), 5.48–5.55 (dt, 1 H, C_2-CH, J = 9.5, 7 Hz), 5.91–5.94 (dt, C_1-CH, J = 9.5, 1.5 Hz); MS, m/e (rel intensity) 414 (6.6), 345 (60), 277 (13.3) 263 (20), 135 (13.3), 121 (33.3), 101 (33.3), 81 (20), 69 (100). Anal. Calcd for $C_{28}H_{46}S$: C, 81.09; H, 11.18. Found: C, 79.91; H, 10.69.

Synthesis of 16a,b. General Method. Silver perchlorate (0.062 g, 0.3 mmol) was added to a solution of a mixture of **28a,b** (0.12 g, 0.289 mmol) in CH_3CN (2 mL) followed by iodomethane (0.15 mL, 0.982 mmol) at 0 °C. The mixture was stirred for 3 h at 0 to 5 °C. Silver iodide was removed by filtration and the filtrate concentrated in vacuo. The paste was washed with pentane (8 × 30 mL) to remove unreacted **28a,b** and the crude product was purified by column chromatography on Celite with CH_2Cl_2 as eluant to give 90 mg (59%) of a mixture of **16a,b**: ^{1}H NMR ($CDCl_3$) δ 1.75–1.9 (s, 21 H, vinyl methyl), 3.1–2.45 (m, 12 H, methylene), 2.55 (q, 2 H, $C_{2'}$-CH_2, J = 6.5 Hz), 3.05 (s, 3 H, (*Z*)-*S*-methyl), 3.07 (s, 3 H, (*E*)-*S*-methyl), 3.19–3.61 (m, 4 H, -SCH_2, C_3-CH_2), 5.1–5.83 (m, 5 H, vinyl H), 6.38 (dt, 1 H, C_1-vinyl H, J = 9, 1 Hz), 6.58 (dt, 1 H, C_1-vinyl, H, J = 15, 1.5 Hz), 6.83–6.89 (m, 1 H, C_2-H), 7.0–7.07 (dt, 1 H, C_2-H, J = 15, 5.5 Hz); MS, FAB (xenon/thioglycerol) 429 (M^+, 100).

Synthesis of 16c,d. These compounds were prepared in 90% yield: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 3 H, methyl, J = 7 Hz), 1.15–1.55 (m, 8 H, methylene), 1.85–1.95 (s, 12 H, vinyl methyl), 2.21–2.55 (m, 8 H, methylene), 3.05 (s, 3 H, (*Z*)-*S*-CH_3), 3.06 (s, 3 H, (*E*)-*S*-CH_3), 3.26–3.54 (m, 4 H, -SCH_2, C_3-CH_2), 5.45–5.7 (m, 2 H, vinyl H), 5.82 (t, 1 H, vinyl H, J = 6 Hz), 6.36 (d, 1 H, C_1-H, J = 9 Hz), 6.51 (d, 1 H, C_1-H, J = 16 Hz), 6.87–6.91 (m, 1 H, C_2-H), 7.03–7.09 (dt, 1 H, C_2-H, J = 16, 6.7 Hz); MS, FAB (xenon/thioglycerol) 363 (M^+, 100).

Synthesis of 16b. This was obtained in 93% yield: ^{1}H NMR ($CDCl_3$) δ 1.75–1.95 (s, 21 H, vinyl methyl), 2.11–2.48 (m, 12 H, methylene), 2.59 (q, 2 H, $C_{2'}$-CH_2, J = 6.5 Hz), 3.05 (s, 3 H, SCH_3), 3.25–3.51 (m, 4 H, SCH_2, C_3-CH_2), 5.38–5.60 (M, 4 H, vinyl H), 5.67–5.81 (m, 1 H, vinyl H), 6.36 (d, 1 H, C_1-H, J = 9.5 Hz), 6.83–6.89 (m, 1 H, C_2-H); MS FAB (xenon/thioglycerol) 429 (M^+, 100).

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Diastereomerically Pure R_P and S_P Dinucleoside H-Phosphonates: The Stereochemical Course of Their Conversion into *P*-Methylphosphonates, Phosphorothioates, and [^{18}O] Chiral Phosphates

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The dinucleoside H-phosphonates **7a,b**–**14a,b** were prepared by condensation of the phosphonates **1**–**4**[1] with the protected nucleosides **5** or **6** using pivaloyl chloride. The P-chiral dimers formed in about equal amounts were then separated. The fast-migrating dimers exhibited ^{31}P NMR chemical shifts located upfield compared to the slow

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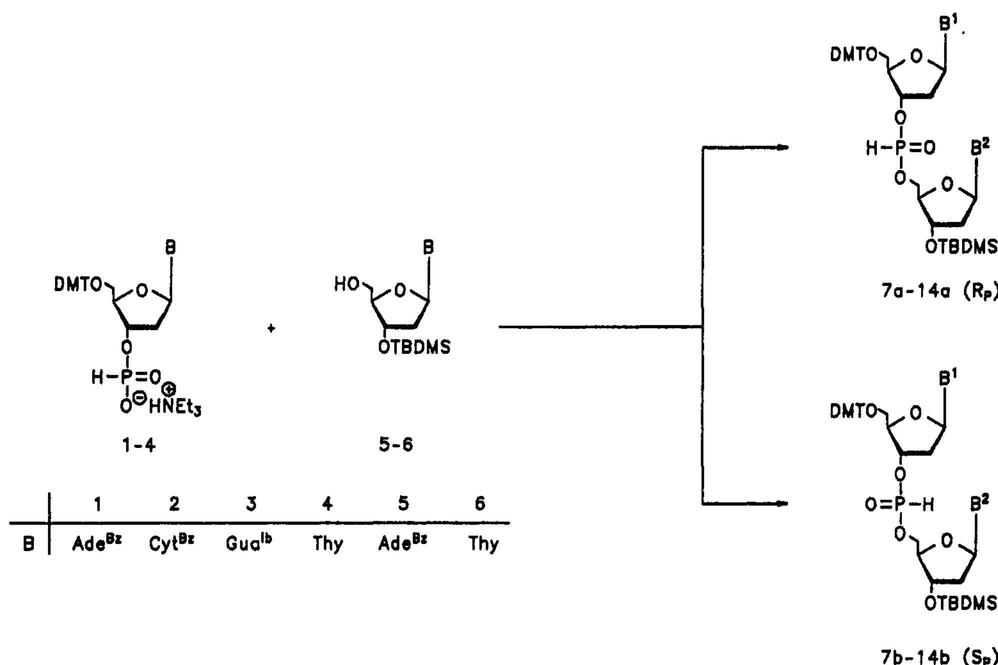
Scheme I



	7	8	9	10	11	12	13	14
B¹	Ade^Bz	Cyt^Bz	Gua^ib	Thy	Ade^Bz	Cyt^Bz	Gua^ib	Thy
B²	Ade^Bz	Ade^Bz	Ade^Bz	Ade^Bz	Thy	Thy	Thy	Thy

migrating compounds. Sulfurization of H-phosphonates **7a**, **10a**, **11a** and **7b**, **10b**, **11b** was stereospecific, yielding the optically pure phosphorothioates **27a–29a** and **27b–29b**, respectively. They were assigned by stereoselective hydrolysis with nuclease P1.[2] The stereospecific route found for sulfurization was also observed for the methylation. Optically pure *P*-methylphosphonates (**15a–20a** and **15b–20b**) were isolated and were assigned according to the recently published NOE data.[3] Configurational assignment of the H-phosphonates was made in analogy to the ^{31}P NMR chemical shifts of methylphosphonates. Methylation as well as sulfurization of dinucleoside H-phosphonates occurred under retention of configuration. Oxidation of the H-phosphonates **7a,b** or **10a,b** with I_2/[^{18}O]H_2O/pyridine, however, preferentially led to (S_P)-[^{18}O]d(ApA) (**30a**) or (S_P)-[^{18}O]d(TpA) (**31a**), regardless of the diastereomeric dinucleosides H-phosphonate diester used.

Studies on nonionic[4,5] or chirally labelled[2,6,7] DNA fragments containing modified internucleotidic linkage have made important contributions to the understanding of nucleic acid structure[8] and their interaction with nucleic acid binding enzymes[9,10] or antibiotics.[11] As deoxyribonucleoside methylphosphonates or phosphorothioates can reduce degradation of the internucleotide bond by cellular enzymes, these oligonucleotides represent a new therapeutic principle (antisense oligonucleotides).[12] In some cases only one of the diastereoisomers is resistant to enzymatic phosphodiester hydrolysis[2,13] (phosphorothioates) or binds specifically to its cognate strand[14,15] (methylphosphonates); ^{18}O chirally labeled phosphates[16–18] allow to follow the stereochemical course of phosphodiester hydrolysis without affecting the reaction rate by sterical interference. As a result the syntheses of oligonucleotides modified at phosphorous exhibiting a defined stereochemistry at the chiral center is required.

Recently, the synthesis of R_P and S_P ^{18}O chirally labeled d(TpA) diastereoisomers has been described using phosphoramidites as intermediates.[10,19] Dimeric phosphoramidites have been used to incorporate oxygen chiral phosphate into oligonucleotides.[20] Now, we present the syntheses, separation, and configurational assignment of a series of dinucleoside H-phosphonates.[21] Their conversion into *P*-methylphosphonates, phosphorothioates, and ^{18}O chirally labeled phosphates will be studied with respect to the stereochemistry.

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Table I. ^{31}P NMR Chemical Shifts[a] and Chromatographical Mobilities[b] of R_p and S_p Dinucleoside H-Phosphonates in $(CD_3)_2SO$

compd	chemical shift	S_P–R_P	R_f values
7a (R_P)	9.78		0.25
7b (S_P)	10.24	0.46	0.22
8a (R_P)	9.86		0.33
8b (S_P)	10.30	0.44	0.30
9a (R_P)	9.90		0.20
9b (S_P)	10.37	0.47	0.19
10a (R_P)	9.87		0.37
10b (S_P)	10.34	0.47	0.35
11a (R_P)	9.83		0.26
11b (S_P)	10.52	0.69	0.23
12a (R_P)	9.89		0.63
12b (S_P)	10.60	0.71	0.62
13a (R_P)	9.92		0.54
13b (S_P)	10.62	0.70	0.49
14a (R_P)	9.94		0.51
14b (S_P)	10.62	0.68	0.49

[a] Relative to 85% phosphoric acid as external standard. [b] TLC (silica gel; EtOAc).

Results and Discussion

Dinucleoside H-Phosphonates. The synthese of diastereomeric R_P and S_P configurated H-phosphonates **10a** and **10b** has been described recently.[21] We now report on the preparation and resolution of the 14 different diastereomeric dinucleoside H-phosphonates containing a 2′-deoxyadenosine or -thymidine moiety at the 3′-terminus and the four regular DNA constituents at the 5′-end. Condensation of compounds **1–4**[1] with 3′-silylated monomers **5** and **6**[22] in anhydrous pyridine using pivaloyl chloride as coupling reagent[23–25] yielded the diastereomeric dinucleoside H-phosphonates (**7a,b–9a,b** and **11a,b–14a,b**) in 80–85% yield. According to ^{31}P NMR spectra the ratio of diastereoisomers was 1:1. The diasteromeric mixtures were separated by column chromatography and the isomers isolated as amorphous foams.

The protected isomers with the larger R_f values (**7a–14a**) always exhibit ^{31}P NMR signals upfield from those of the slower migrating compounds (**7b–14b**). From Table I it is apparent that the change in chemical shift differences depends on the type of nucleoside located at the 3′-terminus. Values of around 0.4–0.5 ppm are found if the 3′-terminal nucleoside was dA and 0.6–0.7 in case of dT. This is in line with the expectation; the diastereotopic environment of the phosphorous toward the 3′-end of the dimer is much more important for the shielding (closer proximity) than the nucleoside residue of the 5′-side. It is consistent with the finding of almost identical shift differences within compounds **7–10** and **11–14**. They contain the four different nucleosides at the 5′-terminus. The faster migrating isomers (**7a–14a**) later assigned as R_P compounds and the slower migrating ones (**7b–14b**) (S_P; Table I) were then employed in the following reactions: (i) methylation, (ii) sulfurization, and (iii) oxidation.

Methylation of Dinucleoside H-Phosphonates. Methylation of the dinucleoside H-phosphonates **7a**, **10a**, **11a** and **7b**, **10b**, **11b** was studied. Butyllithium was used for the metalation of the H-phosphonate moiety followed by methylation with MeI at –15 °C in THF. TLC analyses showed that only one dinucleoside methylphosphonate was formed from a stereochemically pure H-phosphonate in both series. Upon purification the diastereoisomerically pure methylphosphonates **15a–17a** and **15b–17b** were isolated. According to the ^{1}H NMR spectra showing the NH signals for the lactam moiety of the dT residue and/or the amide function of benzoylated dA methylation at the heterocycles did not occur.

The diastereoisomers **15a–17a** and **15b–17b** were characterized by their NMR spectra. The loss of the ^1J coupling constant (720 Hz) in the ^{1}H NMR spectrum (coupled mode) indicates methylation at phosphorus. However, the chemical shifts of the protons are similar in both series of diastereoisomers. Significant changes are observed in the ^{31}P NMR spectra (Table II). In order to establish the absolute configuration of the dinucleoside methylphosphonates ^{1}H NOE difference spectroscopy was employed as described by Miller et al.[26] However, all efforts to observe NOEs of the environmental atoms of the methyl group failed.

During the preparation of this manuscript the 2D ROESY pulse technique was applied to determine the

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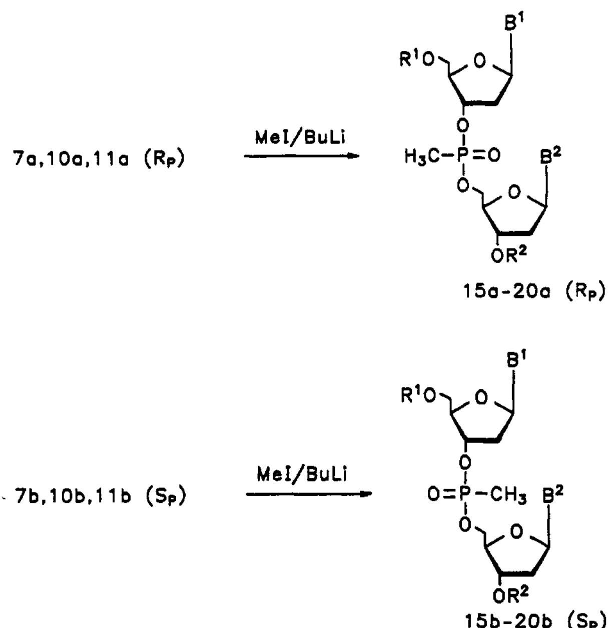
Scheme II



	B¹	B²	R¹	R²
15	Ade^{Bz}	Ade^{Bz}	DMT	TBDMS
16	Thy	Ade^{Bz}	DMT	TBDMS
17	Ade^{Bz}	Thy	DMT	TBDMS
18	Ade	Ade	H	H
19	Thy	Ade	H	H
20	Ade	Thy	H	H

Table II. ^{31}P NMR Shifts[a] and Chromatographical Mobilities[b] of R_P and S_P Methylphosphonates in $(CD_3)_2SO$

compd		chemical shift	S_P–R_P	R_f values
15a (R_P)[3]	fast	32.81		0.62[c]
15b (S_P)[3]	slow	33.11	0.30	0.60[c]
16a (R_P)[3]	fast	32.80		0.85[d]
16b (S_P)[3]	slow	33.11	0.31	0.83[d]
17a (R_P)[3]	fast	32.76		0.82[d]
17b (S_P)[3,30]	slow	33.11	0.35	0.78[d]

[a] Relative to 85% phosphoric acid as external standard. [b] TLC (silica gel, [c] CH_2Cl_2/MeOH, 95:5; [d] CH_2Cl_2/MeOH, 9:1).

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configuration of the diastereomeric dinucleoside methylphosphonates of ApT, TpT, ApA, and TpA.[3] For this purpose the ROEs from the P-CH_3 group of the nearest neighbours were measured: H-3′ of the S_P isomer and H-3′ as well as H-4′ of the R_P compound (5′-direction). According to the 2D ROESY data these assignments were conclusive in all cases.[3] However, the formerly proposed S_P configuration for ApA 1[26,27] was corrected into R_P.[3]

We have measured ^{31}P NMR spectra of the diastereomeric methylphosphonates **15a–17a** and **15b–17b** and have correlated the chemical shifts with their configuration and the chromatographic mobilities on TLC (Table II). All R_P-configurated dinucleoside methylphosphonates (**15a–17a**; fast migrating isomers on TLC) exhibit a ^{31}P NMR signal located upfield compared to the S_P compound (**15b–17b**). These assignments based on the ^{31}P NMR shifts are in agreement with those determined by the 2D ROESY technique. As there are other examples known from the literature[3,28] we regard this as a general phenomenon.

This finding may not only be valid for methylphosphonates but also for dinucleoside H-phosphonates. The ^{31}P NMR signals of the R_P isomers of compounds with identical chiral nucleoside ligands (H-phosphonates **7a**, **10a**, **11a** vs methylphosphonates **15a**, **16a**, **17a**; see Tables I and II) show upfield shielding ($(CD_3)_2SO$) compared to the other isomers. Therefore, we conclude that the H-phosphonates **7a**, **10a**, and **11a** with the highfield ^{31}P NMR signals have the R_P configuration the same as the unambiguously assigned methylphosphonates **15a–20a**.

In order to obtain the deprotected diastereoisomers **18a–20a** and **18b–20b** compounds **15a–17a** and **15b–17b** were (i) desilylated (1 M tetrabutylammonium fluoride in THF); (ii) detritylated (80% acetic acid), and (iii) debenzoylated (25% aqueous ammonia). The deprotected d-[Ap(Me)T] isomers (**20a** and **20b**) were compared regarding their HPLC retention times with HPLC data of Stec.[29] Our retention pattern was the same as reported earlier (R_P isomer, faster migrating); the assignment is in agreement with the single-crystal X-ray analysis of Saenger and co-workers.[30] Additional information about the stereochemistry of dinucleoside methylphosphonates can be drawn from the CD spectra.

According to the work of Miller diastereomeric dinucleoside methylphosphonates exhibit different CD spectra.[27] Similar results have been reported by Agarwal[31] and Stec.[32] Although the first configurational assignment for the methylphosphonates of CpG[31] were corrected by a later paper[33] and unambiguously assigned by Löschner and Engels,[3] it seems to us that this criterion is very useful if only small amounts of material is available. In the case of **18a–20a** (R_P) and **18b–20b** (S_P), only the R_P diastereoisomers show a pronounced CD spectrum whereas those of the S_P-configurated diastereoisomers are more flat.

Our synthetic route toward the synthesis of diastereomerically pure dinucleoside methylphosphonates differ from the method of Stec and co-workers.[32] We have carried out the resolution of diastereoisomers on the dimeric H-phosphonates whereas Stec made the resolution of 3′-*O*-[*O*-(4-nitrophenyl) methylphosphonates] before condensation. Due to the bulky nitrophenyl group the condensation reaction was stereospecific. This does not appear with methylphosphonoamidites[32,34] and dinucleoside H-phosphonates in the 2′-deoxy series. Recently, a diastereoselective synthesis of R_P-configurated dinucleoside methylphosphonates using methyldichlorophosphine as coupling reagent has been described.[35] The diastereomeric excess depends on the size of the nucleobases and the temperature. Only the R_P isomers are accessible by this route. The stereochemical induction at phosphorous is caused by the chiral centers of the sugar moiety.

Sulfurization of Dinucleoside H-Phosphonates. Sulfurization of **7a**, **10a**, **11a** or **7b**, **10b**, **11b** was carried out with octameric sulfur in pyridine–CS_2 (1:1).[36] The sulfurization of the silyl triesters **21a–23a** or **21b–23b** formed in situ was performed with sulfur in pyridine in the absence of CS_2. As indicated by TLC only one diastereomeric sulfurization product (R_P or S_P) was formed from an optically pure H-phosphonate. Two phosphorothioates appeared upon oxidation of the unresolved mixture of diastereoisomers. This confirms that the reaction is stereospecific. The results are in line with the sulfurization of other P-III compounds, e.g. dinucleoside phosphite triesters.[37]

Without isolation of intermediates, compounds **24a–26a** and **24b–26b** were deprotected separately by (i) desilylation (1 M Bu_4NF in THF), (ii) detritylation (80% acetic acid), and (iii) deacylation (25% aqueous ammonia). The deprotected phosphorothioates **27a–29a** and **27b–29b** were purified by ion-exchange chromatography (Sephadex A-25) and then applied to stereoselective phosphodiester hydrolysis. Enzymatic digestion with nuclease P1 was carried out on the two series of diastereoisomers. Only the phosphorothioates obtained upon sulfurization of the fast-migrating H-phosphonates (**7a**, **10a**, **11a**) were hydrolyzed by the enzyme. The other isomers (**7b**, **10b**, **11b**) were resistant. According to earlier observations only S_P-configurated phosphorothioates are substrates upon phosphorodiester hydrolysis.[2] Consequently, the configuration of compounds **27a–29a** was assigned as S_P; **27b–29b** are the R_P isomers. It has been shown already in case of other P-III compounds that sulfurization occurs with retention of configuration.[38,39] The same was found for dinucleoside H-phosphonates.

As we have carried out sulfurization on the H-phosphonates **7**, **10**, and **11** but not on the other dimers **8**, **9**, and **12–14** one could argue that there is a change between the mobility of these compounds and their configuration at phosphorous. However, according to Table I a uniform relationship between the mobilities on TLC and the location of the ^{31}P NMR chemical shift were observed in all cases. As the sulfurization of the in situ formed silyl triesters **21a,b–23a,b** gave the same phosphorothioates as those of the corresponding H-phosphonates, a configurational change upon silylation is unlikely. The configurational change from R_P (H-

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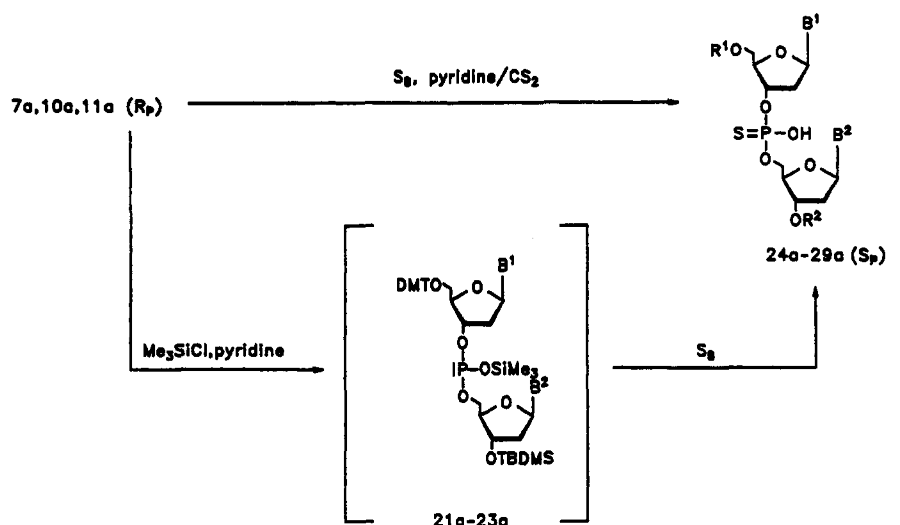
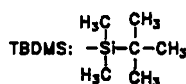
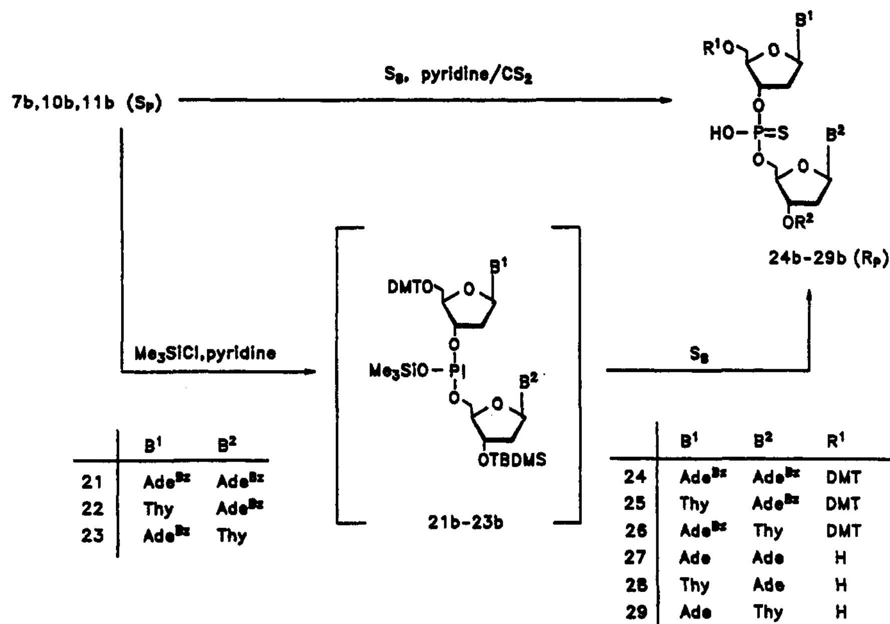
Scheme III







	B1	B2
21	Ade^Bz	Ade^Bz
22	Thy	Ade^Bz
23	Ade^Bz	Thy

	B1	B2	R1	R2
24	Ade^Bz	Ade^Bz	DMT	TBDMS
25	Thy	Ade^Bz	DMT	TBDMS
26	Ade^Bz	Thy	DMT	TBDMS
27	Ade	Ade	H	H
28	Thy	Ade	H	H
29	Ade	Thy	H	H

phosphonates) into S_P (phosphorothioates), and vice versa, is simply due to the CIP rules. As a consequence sulfurization as well as methylation of dinucleoside H-phosphonates occur under retention of configuration.

Oxidation of Dinucleoside H-Phosphonates. Oxidation of the dinucleoside H-phosphonates **10a** or **10b** with $I_2/[^{18}O]H_2O$ has been already described.[21] The same experiments have now been carried out with compounds **7a** and **7b**. The reaction was performed in pyridine with $I_2/[^{18}O]H_2O$ with and without silylation of the phosphoric ester moiety. Excess of I_2 was removed by aqueous Na_2SO_3 (1% solution). For configurational assignment the reaction mixtures were (i) desilylated (1 M Bu_4NF in THF), (ii) detritylated (80% acetic acid) followed by chromatographic purification (silica gel, solvent A), and (iii) debenzoylated (25% aqueous ammonia). Chromatographic purification on Sephadex A-25 (aqueous triethylammonium bicarbonate, 0–200 mM) afforded the R_P- and S_P-configurated diesters **32a/b** or **33a/b**.

Following the procedure of Lowe et al.,[6] the ^{18}O chirally labeled phosphorodiester of d(TpA) (**32a/b**) and of d(ApA) (**33a/b**) were methylated with MeI in $(CD_3)_2SO$ to give two diastereoisomers and two isotopomers (Figure 1). ^{31}P NMR spectra showed six signals which were assigned according to a similar pattern reported for TpA.[10,21] Integration showed that the S_P-configurated diester[39] was formed in preponderance compared to the R_P compound.[40]

The diastereomeric excess (de) of the S_P isomer was 55% in case of the oxidation of **7a** and 30% in case of **7b**. This was similar to that found for the dinucleoside H-phosphonates **10a** and **10b**.[21] It indicates that the stereochemical course of dinucleoside H-phosphonate oxidation with $I_2/[^{18}O]H_2O$/pyridine is not stereospecific. Earlier observations of Cullis[41] who used a 2:1 (S_P, R_P)

(40) S_P and R_P are referring to the position of $[^{18}O]$ and not to the methyl esters.

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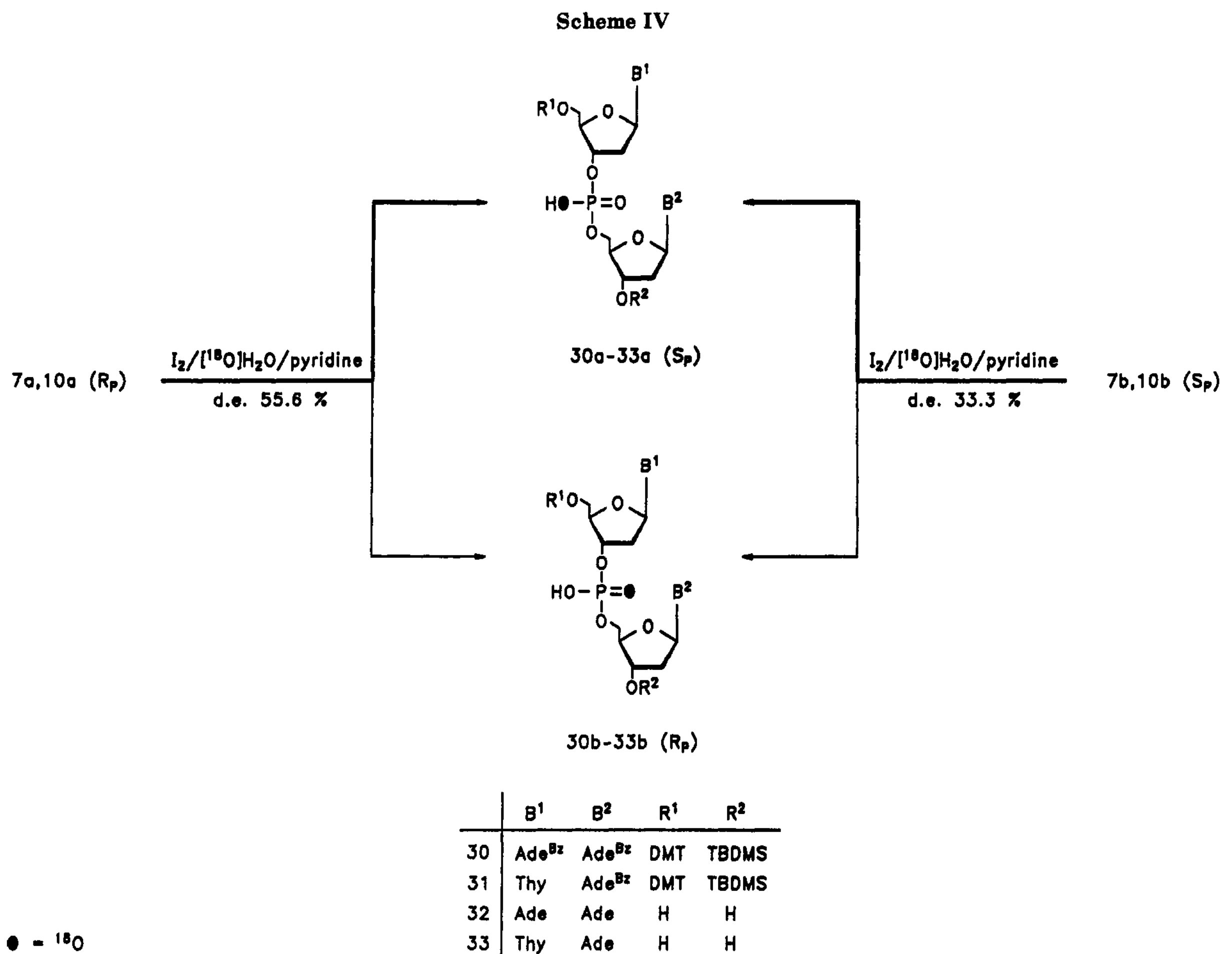
Scheme IV



	B¹	B²	R¹	R²
30	Ade^{Bz}	Ade^{Bz}	DMT	TBDMS
31	Thy	Ade^{Bz}	DMT	TBDMS
32	Ade	Ade	H	H
33	Thy	Ade	H	H

mixture of phosphite triesters of ApA upon oxidation with I_2–$H_2{}^{18}O$ are in agreement with our findings on the corresponding ApA phosphonate. However, the conclusion that the reaction proceeds with retention of configuration cannot be drawn from his experiments as according to our findings a similar result would have been found for the oxidation of an 1:2 (S_P, R_P) mixture. The S_P diastereoisomers are formed in preponderance regardless the configuration of the dinucleoside H-phosphonates which were employed in the reaction. According to that the oxidation process is not simple. We favor a reaction with an attack of I^+ on the tautomeric form of the H-phosphonate. This reaction may be stereospecific. Racemization may occur upon the hydrolysis of this intermediate by the action of water. As other chiral centers are present in the ligands the diastereotopic environment may control the stereochemical outcome of the hydrolysis products.

Conclusion. Methylation of diastereomerically pure dinucleoside H-phosphonates is stereospecific and occurs under retention of configuration similar to the alkylation of menthyl phenylphosphinates.[42] Sulfurization follows the same route. Oxidation with I_2/[^{18}O]H_2O (pyridine), however, preferentially leads to the S_P-configurated isomer, regardless of which diastereoisomer of a dinucleoside phosphonate was used. Consequently, oxidation may serve as a tool to synthesize an almost S_P-configurated oxygen-labeled DNA fragment even by automated synthesis on solid support. It remains to be proven whether the stereochemical outcome is the same in the case of the oxidation of other dimeric dinucleoside H-phosphonates or oligomeric phosphonates and whether the stereochemical course is influenced by the solvent or the oxidation reagent.

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Experimental Section

General Methods. ^{31}P spectra were referenced to external 85% H_3PO_4. Thin-layer chromatography (TLC): silica gel SIL G-25 UV_{254} plates (Macherey-Nagel, FRG) and G 1440/LS 254 cellulose plates (Schleicher & Schüll, FRG). HPTLC: precoated HPTLC silica gel 60 F_{254} plates (Merck, FRG). Flash chromatography (FC) was performed at 0.8 bar with silica gel 60 H (Merck, FRG). Solvent systems: A, AcOEt/AcOH (998:2); B, CH_2Cl_2/MeOH (95:5); C, AcOEt/acetone (95:5); D, i-PrOH/NH_4OH (25%)/H_2O (3:1:1); E, CH_2Cl_2/MeOH/triethylamine (85:10:5); F, CH_2Cl_2/MeOH/triethylamine (85:14:1). Reverse-phase HPLC (LiChrosorb RP-18 column, Merck, FRG). The solvent system was 0.1 M Et_3NHOAc at pH 7.0 (A) and MeCN (B). Gradient I: 5–25% B for 20 min at a flow rate of 1 mL/min. ^{18}O-Enriched water (^{18}O, 90%; Ventron Ltd., Karlsruhe). Nuclease P1 (EC 3.1.30.1) from *Penicillium citrum* was obtained from Boehringer Mannheim (FRG). Organic layers were dried over Na_2SO_4.

General Procedure for Synthesis of Dinucleoside H-Phosphonates 7a,b–14a,b. The H-phosphonates 1–4[1] and the 3′-*O*-protected 2′-deoxyribonucleosides 5 or 6[22] (amounts see particular compound) were dried twice by coevaporation with anhydrous pyridine (20 mL) and dissolved in the same solvent (20 mL). Pivaloyl chloride (3-fold molar quantity) was added with stirring at room temperature, and the stirring was continued for 10 min (rt) under argon atmosphere. CH_2Cl_2 (50 mL) was added, and the solution was extracted with an aqueous $NaHCO_3$ solution (50 mL). The aqueous layer was extracted with CH_2Cl_2 (50 mL, twice). The organic layers were combined, dried, and evaporated. Colorless foam (1:1 mixture) which was separated by FC (solvent A, column 15 × 5 cm, if other column size used, see the particular compound).

(R_P)-N^6-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyadenoylyl-(3′→5′)-N^6-benzoyl-3′-*O*-[(1,1-dimethylethyl)di-

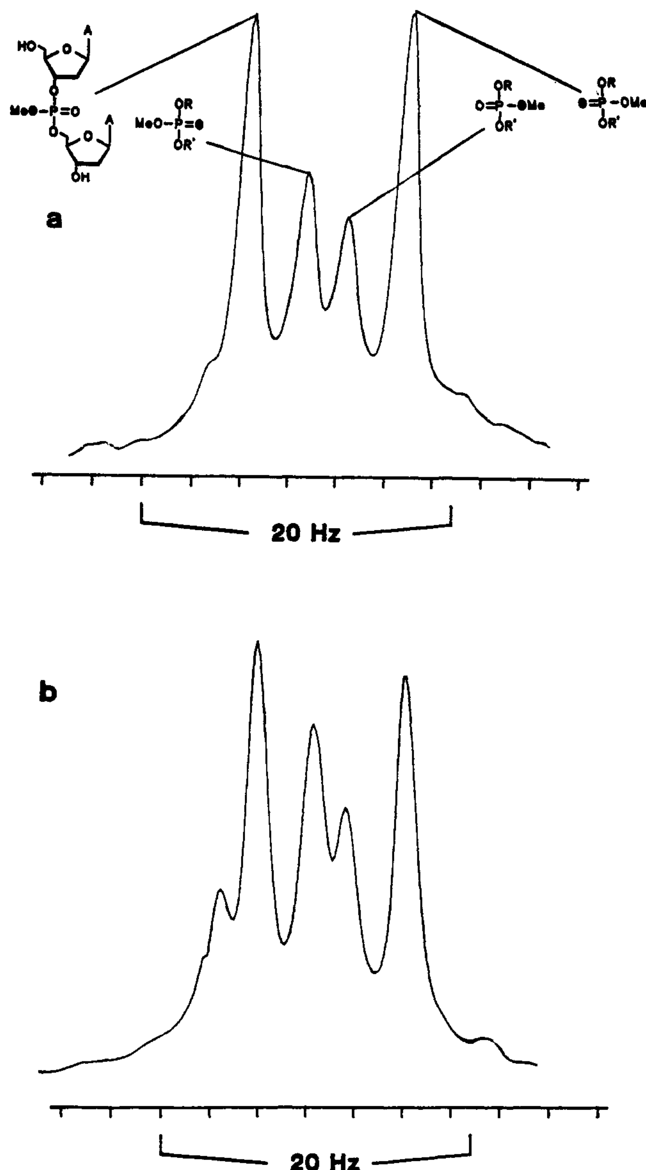

Figure 1. ^{31}P NMR spectra of diastereomeric [^{18}O]d(A'pA') methyl esters (15 mM) obtained from the H-phosphonates **7a** or **7b** by I_2/[^{18}O]H_2O oxidation followed by methylation. Solvent $(CD_3)_2SO$ containing 8-hydroxyquinoline. (a) oxidation of **7a**; (b) oxidation of **7b**.

methylsilyl]-2'-deoxyadenosine 3'-H-Phosphonate (7a). From **1** (823 mg, 1.0 mmol) and **5** (470 mg, 1.0 mmol) a **7a/b** mixture (905 mg, 77%) was obtained. Separation of 700 mg (0.6 mmol) gave **7a** (330 mg, 47%, fast migrating) as a colorless foam: HPTLC (silica gel, solvent A) R_f 0.25; UV (MeOH) λ_{max} 234, 275 nm; ^{1}H NMR ($(CD_3)_2SO$) δ 11.19 (2 H, m, NH), 8.70, 8.67, 8.58 (4 H, 3 s, 2-H and 8-H), 8.02–6.80 (arom H), 6.95 (1 H, d, J = 720 Hz, PH), 6.50 (2 H, m, 1'-H), 3.69 (6 H, s, OCH_3), 0.90 (9 H, s, $C(CH_3)_3$), 0.13 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 9.78. Anal. Calcd for $C_{61}H_{65}N_{10}O_{11}PSi$: C, 62.45; H, 5.58; N, 11.94. Found: C, 62.52; H, 5.75; N, 11.75.

(S_P)-N^6-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenoylyl-(3'→5')-N^6-benzoyl-3'-O-[(1,1-dimethylethyl)dimethylsilyl]-2'-deoxyadenosine 3'-H-Phosphonate (7b). The slow migrating zone afforded colorless amorphous **7b** (295 mg, 42%): HPTLC (silica gel, solvent A) R_f 0.22; UV (MeOH) λ_{max} 232, 278 nm; ^{1}H NMR ($(CD_3)_2SO$) δ 11.20 (2 H, s, NH), 8.74, 8.68, 8.58 (4 H, 3 s, 2-H and 8-H), 8.05–6.80 (arom H), 6.99 (1 H, d, J = 720 Hz, PH), 6.51 (2 H, m, 1'-H), 3.69 (6 H, s, OCH_3), 0.90 (9 H, s, $C(CH_3)_3$), 0.13 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 10.24.

(R_P)-N^4-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidylyl-(3'→5')-N^6-benzoyl-3'-O-[(1,1-dimethylethyl)dimethylsilyl]-2'-deoxyadenosine 3'-H-Phosphonate (8a). From **2** (886 mg, 1.08 mmol) and **5** (470 mg, 1.0 mmol) a **8a/b** mixture (863 mg, 75%) was obtained as colorless foam. Separation gave colorless amorphous **8a** (406 mg; 47%, fast migrating): HPTLC (silica gel, solvent A) R_f 0.33; UV (MeOH) λ_{max} 234, 275 nm; ^{1}H NMR ($(CD_3)_2SO$) δ 11.25 (2 H, 2 s, NH), 8.73, 8.66 (2 H, 2 s, 2-H and 8-H), 8.16–6.86 (arom H), 6.87 (1 H, d, J = 720 Hz, PH), 6.49, 6.15 (2 H, 2 pt, 1'-H), 3.71 (6 H, s, OCH_3), 0.90 (9 H, s, $C(CH_3)_3$), 0.13 (6 H, s, $Si(CH_3)_2$). ^{31}P NMR ($(CD_3)_2SO$) δ 9.86. Anal. Calcd for $C_{60}H_{65}N_8O_{12}PSi$: C, 62.71; H, 5.70; N, 9.75. Found: C, 62.69; H, 5.96; N, 9.58.

(S_P)-N^4-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxycytidylyl-(3'→5')-N^6-benzoyl-3'-O-[(1,1-dimethylethyl)dimethylsilyl]-2'-deoxyadenosine 3'-H-Phosphonate (8b). The slow migrating zone afforded colorless amorphous **8b** (345 mg; 40%): HPTLC (silica gel, solvent A) R_f 0.30; UV (MeOH) λ_{max} 233, 272 nm; ^{1}H NMR ($(CD_3)_2SO$) δ 11.25 (2 H, 2 s, NH), 8.74, 8.66 (2 H, 2 s, 2-H and 8-H), 8.16–8.86 (arom H), 6.94 (1 H, d, J = 720 Hz, PH), 6.50, 6.14 (2 H, 2 pt, 1'-H), 3.71 (6 H, s, OCH_3), 0.90 (9 H, s, $C(CH_3)_3$), 0.13 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 10.30.

(R_P)-N^2-Isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosylyl-(3'→5')-N^6-benzoyl-3'-O-[(1,1-dimethylethyl)dimethylsilyl]-2'-deoxyadenosine 3'-H-Phosphonate (9a). From **3** (885 mg, 1.1 mmol) and **5** (470 mg, 1.0 mmol) a **9a/b** mixture (902 mg, 78%) was obtained. Separation of 500 mg (0.43 mmol) gave colorless amorphous **9a** (230 mg, 46%): HPTLC (silica gel, solvent A) R_f 0.20; UV (MeOH) λ_{max} 237, 275 nm; ^{1}H NMR ($(CD_3)_2SO$) δ 12.20, 11.65 (2 H, NH), 8.70, 8.65, 8.15 (3 H, 3 s, 2-H and 8-H), 8.11–6.80 (arom H), 6.92 (1 H, d, J = 720 Hz, PH), 6.49, 6.26 (2 H, 2 pt, 1'-H), 3.72 (6 H, 2 s, OCH_3), 1.1 (6 H, m, $C(CH_3)_2$), 0.89 (9 H, s, $C(CH_3)_3$), 0.12 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 9.90. Anal. Calcd for $C_{58}H_{68}N_{10}O_{12}PSi$: C, 60.25; H, 5.90; N, 12.10. Found: C, 60.41; H, 6.15; N, 11.75.

(S_P)-N^2-Isobutyryl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyguanosylyl-(3'→5')-N^6-benzoyl-3'-O-[(1,1-dimethylethyl)dimethylsilyl]-2'-deoxyadenosine 3'-H-Phosphonate (9b). Colorless amorphous **9b** (205 mg, 41%) was isolated from the slow migrating zone: HPTLC (silica gel, solvent A) R_f 0.19; UV (MeOH) λ_{max} 235, 252, 274 nm; ^{1}H NMR ($(CD_3)_2SO$) δ 8.73, 8.67, 8.11 (3 H, 3 s, 2-H and 8-H), 8.04–6.77 (arom H), 6.97 (1 H, d, J = 720 Hz, PH), 6.50, 6.23 (2 H, 2 pt, 1'-H), 3.73 (6 H, s, OCH_3), 1.1 (6 H, m, $C(CH_3)_2$), 0.90 (9 H, s, $C(CH_3)_3$), 0.12 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 10.37.

(R_P)-5'-O-(4,4'-Dimethoxytrityl)-2'-thymidylyl-(3'→5')-N^6-benzoyl-3'-O-[(1,1-dimethylethyl)dimethylsilyl]-2'-deoxyadenosine 3'-H-Phosphonate (10a). From **4** (781 mg, 1.1 mmol) and **5** (470 mg, 1.0 mmol) a **10a/b** mixture (900 mg, 85%) was isolated. Separation afforded colorless amorphous **10a** (414 mg, 46%): HPTLC (silica gel, solvent A) R_f 0.37; UV (MeOH) λ_{max} 233, 275 nm; ^{1}H NMR ($(CD_3)_2SO$) δ 11.39 (1 H, s, NH), 11.19 (1 H, s, NH), 8.72, 8.65 (2 H, 2 s, 2-H and 8-H), 8.03–6.66 (arom H), 6.88 (1 H, d, J = 720 Hz, PH), 6.48, 6.21 (2 H, 2 pt, 1'-H), 3.71 (6 H, s, OCH_3), 1.42 (3 H, s, CH_3), 0.90 (9 H, s, $C(CH_3)_3$), 0.13 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 9.87. Anal. Calcd for $C_{54}H_{62}N_7O_{12}PSi$: C, 61.18; H, 5.90; N, 9.24. Found: C, 61.23; H, 5.80; N, 9.11.

(S_P)-5'-O-(4,4'-Dimethoxytrityl)-2'-thymidylyl-(3'→5')-N^6-benzoyl-3'-O-[(1,1-dimethylethyl)dimethylsilyl]-2'-deoxyadenosine 3'-H-Phosphonate (10b). The slow migrating zone gave colorless amorphous **10b** (380 mg, 42%): HPTLC (silica gel, solvent A) R_f 0.35; UV (MeOH) λ_{max} 231, 276 nm; ^{1}H NMR ($(CD_3)_2SO$) δ 11.39 (1 H, s, NH), 11.20 (1 H, s, NH), 8.72, 8.65 (2 H, 2 s, 2-H and 8-H), 8.04–6.82 (arom H), 7.06 (1 H, d, J = 720 Hz, PH), 6.49, 6.21 (2 H, 2 pt, 1'-H), 3.71 (6 H, s, OCH_3), 1.43 (3 H, s, CH_3), 0.90 (9 H, s, $C(CH_3)_3$), 0.12 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 10.34.

(R_P)-N^6-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenoylyl-(3'→5')-3'-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3'-H-Phosphonate (11a). From **1** (1.3 g, 1.6 mmol) and **6** (570 mg, 1.6 mmol) a **11a/b** mixture (1.48 g; 88%) was isolated. From the fast migrating zone **11a** (720 mg, 49%) was obtained as colorless foam: HPTLC (silica gel, solvent A) R_f 0.59; UV (MeOH) λ_{max} 233, 275 nm; ^{1}H NMR ($(CD_3)_2SO$) δ 11.33 (1 H, s, NH), 11.20 (1 H, s, NH), 8.59 (2 H, s, 2-H and 8-H), 8.06–6.79 (arom H), 7.06 (1 H, d, J = 720 Hz, PH), 6.53, 6.17 (2 H, 2 pt, 1'-H), 3.71 (6 H, s, OCH_3), 1.76 (3 H, s, CH_3), 0.86 (9 H, s, $C(CH_3)_3$),

0.08 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 9.83. Anal. Calcd for $C_{54}H_{62}N_7O_{12}PSi$: C, 61.18; H, 5.90; N, 9.24. Found: C, 61.05; H, 6.09; N, 9.16.

(S_P)-N^6-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyadenoylyl-(3′→5′)-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3′-H-Phosphonate (11b). The slow migrating zone afforded **11b** (690 mg, 47%) as colorless foam: HPTLC (silica gel, solvent A) R_f 0.48; UV (MeOH) λ_{max} 234, 273 nm; ^1H NMR ($(CD_3)_2SO$) δ 11.34 (1 H, s, NH), 11.20 (1 H, s, NH), 8.59 (2 H, s, 2-H and 8-H), 8.06–6.78 (arom H), 7.07 (1 H, d, J = 720 Hz, PH), 6.53, 6.20 (2 H, 2 pt, 1′-H), 3.71 (6 H, s, OCH_3), 1.77 (3 H, s, CH_3), 0.86 (9 H, s, $C(CH_3)_3$), 0.08 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 10.52.

(R_P)-N^4-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxycytidylyl-(3′→5′)-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3′-H-Phosphonate (12a). **2** (1.0 g, 1.25 mmol) and **6** (455 mg, 1.28 mmol) gave a **12a/b** mixture (828 mg, 64%). Separation of 660 mg afforded **12a** (284 mg, 43%, fast migrating) as colorless foam: HPTLC (silica gel, solvent A) R_f 0.63; UV (MeOH) λ_{max} 236, 262, 306 nm; ^1H NMR ($(CD_3)_2SO$) δ 11.32 (1 H, s, NH), 11.28 (1 H, s, NH), 8.17–6.89 (arom H), 7.01 (1 H, d, J = 720 Hz, PH), 6.18 (2 H, m, 1′-H), 3.74 (6 H, s, OCH_3), 1.76 (3 H, s, CH_3), 0.87 (9 H, s, $C(CH_3)_3$), 0.08 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 9.89. Anal. Calcd for $C_{53}H_{62}N_5O_{13}PSi$: C, 61.44; H, 6.03; N, 6.76. Found: C, 61.39; H, 5.98; N, 6.69.

(S_P)-N^4-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxycytidylyl-(3′→5′)-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3′-H-Phosphonate (12b). The slow migrating zone gave **12b** (245 mg, 37%) as colorless foam: HPTLC (silica gel, solvent A) R_f 0.62; UV (MeOH) λ_{max} 233, 262, 305 nm; ^1H NMR ($(CD_3)_2SO$) δ 11.33 (1 H, s, NH), 11.27 (1 H, s, NH), 8.17–6.67 (arom H), 7.02 (1 H, d, J = 720 Hz, PH), 6.17 (2 H, m, 1′-H), 3.73 (6 H, s, OCH_3), 1.77 (3 H, s, CH_3), 0.85 (9 H, s, $C(CH_3)_3$), 0.07 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 10.60.

(R_P)-N^4-Isobutyryl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyguanosylyl-(3′→5′)-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3′-H-Phosphonate (13a). From **3** (1.5 g, 1.86 mmol) and **6** (689 mg, 1.93 mmol) a **13a/b** mixture (1.69 g, 87%) was obtained. Separation afforded **13a** (760 mg, 45%, fast migrating) as colorless foam: HPTLC (silica gel, solvent A) R_f 0.54; UV (MeOH) λ_{max} 237, 262, 280 nm; ^1H NMR ($(CD_3)_2SO$) δ 12.07, 11.59, 11.35 (3 H, 3 s, NH), 8.12, 7.49 (2 H, 2 s, 8-H and 6-H), 7.33–6.77 (arom H), 7.04 (1 H, d, J = 720 Hz, PH), 6.27, 6.15 (2 H, 2 pt, 1′-H), 3.72 (6 H, s, OCH_3), 1.73 (3 H, s, CH_3), 1.13 (6 H, d, $C(CH_3)_2$), 0.85 (9 H, s, $C(CH_3)_3$), 0.07 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 9.92. Anal. Calcd for $C_{51}H_{64}N_7O_{13}PSi$: C, 58.78; H, 6.19; N, 9.41. Found: C, 58.33; H, 6.16; N, 8.96.

(S_P)-N^4-Isobutyryl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyguanosylyl-(3′→5′)-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3′-H-Phosphonate (13b). The slow migrating zone afforded **13b** (659 mg, 39%) as a colorless foam: TLC (silica gel, solvent A) R_f 0.49; UV (MeOH) λ_{max} 235, 260, 280 nm; ^1H NMR ($(CD_3)_2SO$) δ 12.07, 11.61, 11.36 (3 H, 3 s, NH), 8.14, 7.49 (2 H, 2 s, 8-H and 6-H), 8.33–6.76 (arom H), 7.05 (1 H, d, J = 720 Hz, PH), 6.29, 6.19 (2 H, pq, 1′-H), 3.73 (6 H, s, OCH_3), 1.76 (3 H, s, CH_3), 1.16 (6 H, d, $C(CH_3)_2$), 0.85 (9 H, s, $C(CH_3)_3$), 0.07 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 10.66.

(R_P)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-(3′→5′)-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3′-H-Phosphonate (14a). From **4** (1.15 g, 1.62 mmol) and **6** (570 mg, 1.6 mmol) a **14a/b** mixture (1.27 g; 84%) was obtained. Separation afforded **14a** (559 mg, 44%) as colorless foam: HPTLC (silica gel, solvent A) R_f 0.51; UV (MeOH) λ_{max} 236, 265 nm; ^1H NMR ($(CD_3)_2SO$) δ 11.38, 11.32 (2 H, 2 s, NH), 7.48–6.87 (arom H), 7.01 (1 H, d, J = 720 Hz, PH), 6.23, 6.14 (2 H, 2 pt, 1′-H), 3.72 (6 H, s, OCH_3), 1.75 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 0.86 (9 H, s, $C(CH_3)_3$), 0.07 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 9.94. Anal. Calcd for $C_{47}H_{59}N_4O_{13}PSi$: C, 59.61; H, 6.28; N, 5.92. Found: C, 59.67; H, 6.34; N, 5.85.

(S_P)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-(3′→5′)-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3′-H-Phosphonate (14b). The slow migrating zone gave **14b** (508 mg, 40%) as colorless foam: HPTLC (silica gel, solvent A) R_f 0.49; UV (MeOH) λ_{max} 234, 265 nm; ^1H NMR ($(CD_3)_2SO$) δ 11.40, 11.33 (2 H, 2 s, NH), 7.47–6.86 (arom H), 7.02 (1 H, d, J = 720 Hz, PH), 6.23, 6.14 (2 H, 2 pt, 1′-H), 3.73 (6 H, s, OCH_3), 1.76 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 0.85 (9 H, s, $C(CH_3)_3$), 0.07 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 10.62.

Methylation of the 3′-H-Phosphonates. To a stirred solution of the H-phosphonates **7a,b**, **10a,b**, **11a,b** (0.085 mmol, each) in anhydrous THF (10 mL) was added BuLi (0.2 mL, 1.6 M in hexane) at −15 °C (ice/salt bath). After 5 min MeI (0.1 mL, 0.4 mmol; in 10 mL of THF) was added dropwise. The mixture was stirred for 30 min at −15 °C, the bath was removed, and the reaction mixture was allowed to come to room temperature. Upon evaporation the residue was dissolved in EtOAc (35 mL) and extracted with water (twice). The organic layer was dried and filtered, and the solvent was removed and applied to FC (silica gel 60 H, 5 × 4 cm, solvent A), furnishing a colorless foam that contained the methylphosphonates (**15a–17a** and **15b–17b**).

(R_P)-N^6-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyadenoylyl-(3′→5′)-N^6-benzoyl-3′-O-[(1,1-dimethylethyl)dimethylsilyl]-2′-deoxyadenosine 3′-Methylphosphonate (15a). From **7a** (100 mg, 0.090 mmol) colorless amorphous **15a** (74 mg, 73%) was obtained: TLC (silica gel, solvent B) R_f 0.62; UV (MeOH) λ_{max} 235, 275 nm; ^1H NMR ($(CD_3)_2SO$) δ 11.19 (2 H, 2 s, NH), 8.71, 8.66, 8.59, 8.58 (4 H, 4 s, 2-H and 8-H), 8.03–6.81 (arom H), 6.49 (2 H, m, 1′-H), 3.70 (6 H, s, OCH_3), 1.46 (3 H, d, J = 17.3 Hz, PCH_3), 0.89 (9 H, s, $C(CH_3)_3$), 0.13 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 32.71 ($^2J(P,CH_3)$ = 17.1 Hz, $^3J(P,H)$ = 7.6 Hz). Anal. Calcd for $C_{62}H_{67}N_{10}O_{11}PSi$: C, 62.72; H, 5.69; N, 11.80. Found: C, 62.55; H, 5.72; N, 11.62.

(S_P)-N^6-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyadenoylyl-(3′→5′)-N^6-benzoyl-3′-O-[(1,1-dimethylethyl)dimethylsilyl]-2′-deoxyadenosine 3′-Methylphosphonate (15b). From **7b** (100 mg, 0.09 mmol) colorless amorphous **15b** (71 mg, 70%) was isolated: TLC (silica gel, solvent B) R_f 0.60; UV (MeOH) λ_{max} 234, 275 nm; ^1H NMR ($(CD_3)_2SO$) δ 11.16 (2 H, 2 s, NH), 8.71, 8.67, 8.59, 8.58 (4 H, 4 s, 2-H and 8-H), 8.05–6.77 (arom H), 6.49 (2 H, m, 1′-H), 3.70 (6 H, s, OCH_3), 1.32 (3 H, d, J = 17.4 Hz, PCH_3), 0.89 (9 H, s, $C(CH_3)_3$), 0.12 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 33.11 ($^2J(P,CH_3)$ = 17.4 Hz, $^3J(P,H)$ = 7.6 Hz).

(R_P)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-(3′→5′)-N^6-benzoyl-3′-O-[(1,1-dimethylethyl)dimethylsilyl]-2′-deoxyadenosine 3′-Methylphosphonate (16a). From **10a** (88 mg, 0.08 mmol) colorless amorphous **16a** (72 mg, 81%) was isolated: TLC (silica gel, solvent B) R_f 0.85; UV (MeOH) λ_{max} 234, 274 nm; ^1H NMR ($(CD_3)_2SO$) δ 11.37, 11.19 (2 H, 2 s, NH), 8.72, 8.62 (2 H, 2 s, 2-H and 8-H), 8.04–6.85 (arom H), 6.48, 6.20 (2 H, 2 pt, 1′-H), 3.71 (6 H, s, OCH_3), 1.47 (3 H, d, J = 17.0 Hz, PCH_3), 1.35 (3 H, s, CH_3), 0.89 (9 H, s, $C(CH_3)_3$), 0.11 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 32.80 ($^2J(P,CH_3)$ = 17.0 Hz, $^3J(P,H)$ = 7.6 Hz). Anal. Calcd for $C_{55}H_{64}N_7O_{12}PSi$: C, 61.50; H, 6.01; N, 9.13. Found: C, 61.69; H, 6.12; N, 8.92.

(S_P)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-(3′→5′)-N^6-benzoyl-3′-O-[(1,1-dimethylethyl)dimethylsilyl]-2′-deoxyadenosine 3′-Methylphosphonate (16b). From **10b** (88 mg, 0.08 mmol) colorless amorphous **16b** (75.5 mg, 85%) was isolated: TLC (silica gel, solvent B) R_f 0.83; UV (MeOH) λ_{max} 272, 232 nm. ^1H NMR ($(CD_3)_2SO$) δ 11.37, 11.19 (2 H, 2 s, NH), 8.71, 8.62 (2 H, 2 s, 2-H and 8-H), 8.05–6.64 (arom H), 6.47, 6.20 (2 H, 2 pt, 1′-H), 3.71 (6 H, s, OCH_3), 1.47 (3 H, d, J = 17 Hz, PCH_3), 1.42 (3 H, s, CH_3), 0.89 (9 H, s, $C(CH_3)_3$), 0.11 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) δ 33.11 ppm.

(R_P)-N^6-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyadenoylyl-(3′→5′)-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3′-Methylphosphonate (17a). Compound **11a** (50 mg, 0.05 mmol) was treated with BuLi (0.1 mL) and MeI (0.05 mL) as described to give colorless amorphous **17a** (42.5 mg, 84%): TLC (silica gel, solvent B) R_f 0.82; ^1H NMR ($(CD_3)_2SO$) δ 11.38 (1 H, s, NH), 11.19 (1 H, s, NH), 8.71, 8.62 (2 H, 2 s, 2-H and 8-H), 8.04–6.64 (arom H), 6.48, 6.20 (2 H, 2 pt, 1′-H), 3.70 (6 H, s, OCH_3), 1.39 (3 H, d, J = 17.4 Hz, PCH_3), 1.28 (3 H, s, CH_3), 0.89 (9 H, s, $C(CH_3)_3$), 0.11 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) 32.76.

(S_P)-N^6-Benzoyl-5′-O-(4,4′-dimethoxytrityl)-2′-deoxyadenoylyl-(3′→5′)-3′-O-[(1,1-dimethylethyl)dimethylsilyl]thymidine 3′-Methylphosphonate (17b). Compound **11b** (50 mg, 0.05 mmol) was treated with BuLi (0.1 mL) and MeI (0.05 mL) as described. Colorless amorphous **17b** (39.0 mg, 77%) was isolated: TLC (silica gel, solvent B) R_f 0.78; ^1H NMR ($(CD_3)_2SO$) 8.70, 8.64 (2 H, 2 s, 2-H and 8-H), 8.11–6.76 (arom H), 6.48, 6.23 (2 H, 2 pt, 1′-H), 3.70 (6 H, s, OCH_3), 1.45 (3 H, d, J = 17.5 Hz,

PCH_3), 1.12 (3 H, s, CH_3), 0.89 (9 H, s, $C(CH_3)_3$), 0.11 (6 H, s, $Si(CH_3)_2$); ^{31}P NMR ($(CD_3)_2SO$) 33.11.

Deprotection of the Methylphosphonates. Compounds **15a–17a** or **15b–17b** (50 mg) were dissolved in THF (5 mL). Bu_4NF (5 mL, 1 M in THF) was added, and the solution was stirred (rt, 30 min). Upon evaporation the residue was dissolved in CH_2Cl_2 (20 mL) and extracted with water. The organic layer was dried, the solvent was evaporated, and the residue was dissolved in 80% aqueous AcOH (rt, 10 min). The acid was removed under reduced pressure, and the residue was partitioned between water (25 mL) and CH_2Cl_2 (25 mL). The aqueous phase was evaporated; the residue was dissolved in 25% aqueous NH_3 (40 mL) and stored (3 h; 50 °C water bath). After evaporation compounds **18a–20a** and **18b–20b** were purified on TLC cellulose plates (solvent D). The main zones containing the methylphosphonates were pooled, dissolved in MeOH, and filtered. The filtrate was evaporated affording **18a–20a** and **18b–20b** as glassy solids (40 and 50% yield). Compounds **18a,b**, **19a,b**, and **20a,b** were identified by comparing their ^1H NMR spectra with those already published[3,26,27] and mobilities on HPLC (**20a,b**).[29]

(R_P)-Adenoylyl-(3'→5')-2'-deoxyadenosine Methylphosphonate (18a):[3,26,27] ^1H NMR (D_2O) δ 8.21, 8.01, 7.97, 7.81 (4 H, 4 s, 8-H and 2-H), 6.26, 6.07 (2 H, 2 pt, 1'-H), 1.67 (3 H, d, J = 17.6 Hz, PCH_3); ^{31}P NMR (D_2O) δ 36.49.

(S_P)-Adenoylyl-(3'→5')-2'-deoxyadenosine Methylphosphonate (18b):[3,26,27] ^1H NMR (D_2O) δ 8.20, 8.00, 8.95, 7.79 (4 H, 4 s, 8-H and 2-H), 6.25, 6.05 (2 H, 2 pt, 1'-H), 1.64 (3 H, d, J = 17.6 Hz, PCH_3); ^{31}P NMR (D_2O) δ 36.50.

(R_P)-Thymidylyl-(3'→5')-2'-deoxyadenosine Methylphosphonate (19a):[3,26] ^1H NMR (D_2O) δ 8.28, 8.08 (2 H, 2 s, 8-H and 2-H), 7.30 (1 H, s, 6-H), 6.36, 5.95 (2 H, 2 pt, 1'-H), 1.57 (3 H, d, J = 17.5 Hz, PCH_3).

(S_P)-Thymidylyl-(3'→5')-2'-deoxyadenosine Methylphosphonate (19b):[3,26] ^1H NMR (D_2O) δ 8.29, 8.08 (2 H, 2 s, 8-H and 2-H), 7.29 (1 H, s, 6-H), 6.35, 5.95 (2 H, 2 pt, 1'-H), 1.58 (2 H, d, J = 17.4 Hz, PCH_3).

General Procedure for Sulfurization of the H-Phosphonates and Deprotection. Method a. The H-phosphonates **7a,b**, **10a,b**, **11a,b** (0.1 mmol, each) were dissolved in dry pyridine (5 mL). *N,O*-Bis(trimethylsilyl)acetamide (1 mL, 5 mmol) and $(Et)_3N$ (0.5 mL) were added, and the solution was stirred at rt for 15 min. Sulfur (200 mg, 6.25 mmol) was added, and the mixture was stirred for another 4 h. Upon filtration the solution was evaporated, and the residue was dissolved in EtOAc and extracted with water. The organic layer was dried and evaporated. FC (silica gel 60 H, solvent E) afforded colorless amorphous **12a,b–14a,b**, which were deprotected as described for the methylphosphonates.

Method b. The H-phosphonates (0.1 mmol) were dissolved in pyridine/CS_2 (1:1, 5 mL). After addition of sulfur (50 mg, 1.5 mmol) the mixture was stirred at rt for 4 h. The solvent was evaporated; the residue was dissolved in EtOAc and extracted with water. The organic phase was dried and deprotected as described for the methylphosphonates. Compounds **27a–29a** and **27b–29b** were identified by their mobilities on HPLC (UV detection at 260 nm).[43] Retention times in solvent system I; t_R(**27b**) = 11.0 min; t_R(**27a**) = 11.7 min; t_R(**28b**) = 12.0 min; t_R(**28a**) = 12.5 min; t_R(**29b**) = 12.4 min; t_R(**29a**) = 13.0 min.

Nuclease P1 Hydrolyses on the Phosphorothioates 21a,-b–23a,b. The deprotected phosphorothioates (25 μmol each) were dissolved in a mixture of NH_4SO_4 (30 mmol, pH 5.3, 0.38 mL) and $ZnSO_4$ (10 mmol, 0.02 mL). Nuclease P1 (100 units) was added. This mixture was stored at 37 °C; samples were taken every 4 h and applied to reversed-phase HPLC (5–25% MeCN, 20 min, 260 nm). Compounds **21b–23b** were resistant to hydrolysis within 40 h. The hydrolyses of **21a–23a** gave two products. **21a:** t_R = 6.7 and 8.5 min. **22a:** t_R = 5.5 and 8.2 min. **23a:** t_R = 4.4 and 6.0 min.

General Procedure for the Oxidation of the H-Phosphonates 7a,b, 10a,b with I_2/[^{18}O]H_2O/Pyridine. The H-phosphonates **7a**, **7b**, **10a**, or **10b** (0.57 mmol) were dried by coevaporation with pyridine and dissolved in pyridine (10 mL). After addition of I_2/[^{18}O]H_2O (215 mg, 0.85 mmol; 1 mL) dissolved in pyridine (5 mL) the mixture was stirred for 15 min at rt. Excess of iodine was reduced by extraction with 1% aqueous Na_2SO_3 solution. The organic phase was extracted with triethylammonium bicarbonate (TBC) buffer (200 mM), washed with water, and dried. The residue was dissolved in CH_2Cl_2 and purified by FC (silica gel 60H, solvent F). ^{18}O Chirally labeled **30a/b** or **31a/b** were deprotected as described for the methylphosphonates. Chromatographical purification was carried out on Sephadex A-25 (aqueous triethylammonium bicarbonate; 0–200 mM) to give the triethylammonium salts of (R_P/S_P)-[^{18}O]-2'-deoxyadenoylyl(3'→5')-2'-deoxyadenosine (**32a/b**) or (R_P/S_P)-[^{18}O]-2'-deoxythymidylyl(3'→5')-2'-deoxyadenosine (**33a/b**). For configurational analysis the **32a/b** mixture (10 μmol) was methylated as described for UpA[7] to give the methyl esters as a solution in $(CD_3)_2SO$: ^{31}P NMR ($(CD_3)_2SO$) δ 0.22, 0.20, 0.18, 0.17 ppm. The same reaction was carried out on **33a/b** (10 μmol): ^{31}P NMR ($(CD_3)_2SO$) δ 0.22, 0.20, 0.19, 0.17 ppm.

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Building Blocks for Oligonucleotide Analogues with Dimethylene Sulfide, Sulfoxide, and Sulfone Groups Replacing Phosphodiester Linkages

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Two routes are presented for the synthesis of 3',5'-bishomodeoxyribonucleosides, building blocks needed to synthesize oligodeoxynucleotide analogues where the OPO_2O groups are replaced by CH_2SCH_2, CH_2SOCH_2, and $CH_2SO_2CH_2$ units. Two of these have been coupled to create an uncharged analogue of a dinucleotide. As isosteric, achiral, and nonionic analogues of natural oligonucleotides stable to both enzymatic and chemical hydrolysis, such molecules have potential application as probes in the laboratory, in studies of the role of individual genes in biological function, and as "antisense" oligonucleotide analogues for the treatment of diseases.

Oligonucleotides with a defined sequence can bind to complementary single-stranded oligonucleotides and disrupt their biological activity.[1] In recent years, it has become widely recognized that this specific interaction

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