223. Phosphoramidites of Chiral (R_P)- and (S_P)-Configurated d(T[*P*-^{18}O]-A): Synthesis, Configurational Assignment, and Use as Dimer Blocks in Oligonucleotide Synthesis

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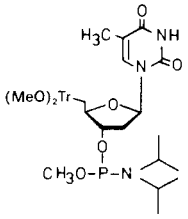
(16.VIII.85)

The *N*,*N*-diisopropylphosphoramidites **10a** and **10b** of appropriately protected chiral diastereoisomers of d(T[*P*-^{18}O]-A) (**8a** and **8b**, resp.), chiral by virtue of the isotope ^{18}O at the P-atom, have been synthesized. The ^{18}O-isotope was incorporated by oxidation of the phosphite triester **3** with $H_2[^{18}O]/I_2$. Separation of the diastereoisomers was accomplished by flash chromatography of the *O*-3′-deprotected phosphate triesters **5a**/**b**. The absolute configuration at the chiral P-atom was deduced from the methylation products of the fully deprotected diastereoisomers **8a** and **8b**. Phosphinylation of **5a** and **5b** yielded the configurationally pure phosphoramidites **10a** and **10b**, respectively, which were then employed in solid-phase synthesis to yield the self-complementary oligomers d(G-A-G-T-(R_p)-[P-^{18}O]-A-C-T-C) (**13**) and d(G-A-G-T-(S_P)-[*P*-^{18}O]-A-C-T-C) (**14**), respectively.

Introduction. – Nucleotides with chiral phosphate groups generated by O-labeling (='oxygen chiral phosphates') are useful probes for the elucidation of the stereochemical course of enzymatically catalyzed hydrolytic phosphodiester cleavage [1]. The first synthesis of a ^{18}O-labeled chiraly dinucleoside monophosphate has been reported by *Eckstein et al.* [2]. They were able to convert a diastereoisomerically pure phosphorthioate by S/O-exchange into a chiral ^{18}O-labeled phosphate. A direct approach for ^{18}O as well as ^{17}O-labeling of ribonucleoside and 2′-deoxyribonucleoside monophosphates generating chirality at the P-atom has been developed in our laboratory using phosphite intermediates and employing either $H_2[^{17}O]/I_2$ or $H_2[^{18}O]/I_2$ in the oxidation reaction [3]. Chromatographic separation of the diastereoisomeric triesters allowed the resolution of O-isotope-induced chirality.

In a previous publication we have reported on the synthesis and configurational assignment of d(C[*P*-^{18}O]-A) and d(T[*P*-^{18}O]-A) ((R_P)-configuration) [4][5]. In the course of this work, it became apparent that the separation of diastereoisomeric phosphate triesters was extremely difficult on the stage of fully protected dimers. However, detritylation at the 5′-position resulted in diastereoisomers which exhibited fairly different chromatographic mobilities and allowed preparative-scale separation employing flash chromatography. As a result of this, the ^{18}O-labeled chiral dinucleoside monophosphate **8a** (R_P) was isolated, and the configuration at the P-atom was assigned by combination of stereospecific enzymatic phosphodiester cleavage and ^{31}P-NMR spectroscopy on cyclo-dAMP methyl esters. Recently, this assignment has been confirmed by others which obtained the (R_P)- and (S_P)-diastereoisomers **8a** and **8b** from the corresponding chiral phosphorthioate by a nucleophilic displacement reaction [6]. However, this reaction is not entirely stereospecific. This difficulty does not arise in our method which leads to



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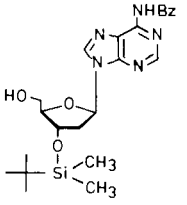

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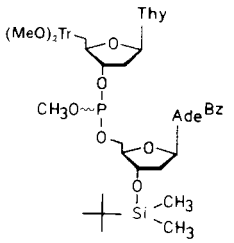

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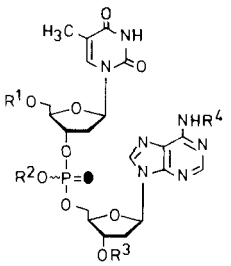
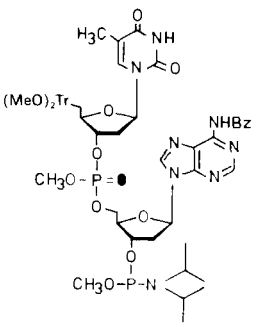
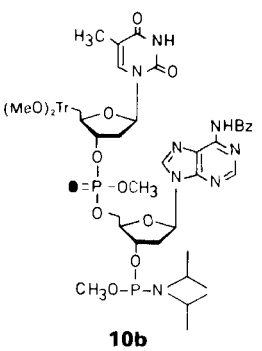


4–9a/b



10a



10b

● = ^{18}O

	R^1	R^2	R^3	R^4	
4a,b	$(MeO)_2Tr$	Me	(*t*-Bu)Me_2Si	Bz	**11** d(G[$(MeO)_2$Tr]-A-G-T-(R_P)-[*P*-^{18}O]-A-C-T-C)
5a,b	$(MeO)_2Tr$	Me	H	Bz	**12** d(G[$(MeO)_2$Tr]-A-G-T-(S_P)-[*P*-^{18}O]-A-C-T-C)
6a,b	H	Me	H	Bz	**13** d(G-A-G-T-(R_P)-[*P*-^{18}O]-A-C-T-C)
7a,b	H	H	H	Bz	**14** d(G-A-G-T-(S_P)-[*P*-^{18}O]-A-C-T-C)
8a,b	H	H	H	H	**4a–6a, 7b, 8b, 9a, 10a**: ($S_{P(V)}$)-diastereoisomer
9a,b[a])	H	Me	H	H	**4b–6b, 7a, 8a, 9b, 10b**: ($R_{P(V)}$)-diastereoisomer
					a,b: opposite configuration at P(V)

[a]) N^1-Methyladenine instead of adenine. In each **9a** and **9b**, the ^{18}O is located either on O = or MeO (4 species in all).

configurationally pure ^{18}O-labeled phosphate diastereoisomers. Dimers derived from **8** such as **10a** and **10b** are useful building blocks in oligonucleotide synthesis employing phosphoramidites either on solid support or in solution [7] [8].

According to the findings of *Cullis* [9], the oxidation of phosphite to phosphate triesters is a stereospecific process, and one could assume that chiral O-labeled phosphates can be prepared *via* this route. However, the condensation reaction between the phosphoramidite and the OH function of the next nucleoside is a nonstereospecific process leading to a mixture of diastereoisomers [10]. As a consequence, this route cannot be used to synthesize diastereoisomerically pure chiral O-labeled methyl phosphates. Therefore, the chiral O-labeled phosphate moiety had to be incorporated into dimer blocks such as **10a** or **10b** which then can be used for chain elongation.

Synthesis of the Phosphoramidites of (S_P)- and (R_P)- d(T[*P*-^{18}O]-A) (10a and 10b, resp.). – Our aim was to incorporate d(T-A) units, chiral at the P-atom, into oligonucleo-

tides. As a prerequisite, the synthesis of the phosphoramidites **10a** and **10b** had to be undertaken, hence the preparation of stereoisomerically pure and 3'-deprotected dimers such as **5a** and **5b**. In order to achieve this, the (*tert*-butyl)dimethylsilyl group was chosen for 3'-OH protection [11]. Condensation of the $(MeO)_2$Tr-thymidine phosphoramidite **1** with 3'-silylated N^6-benzoyl-2'-deoxyadenosine **2** in MeCN yielded the diastereoisomeric phosphit triesters **3**. These esters were not isolated but were oxidized with $H_2[^{18}O]$, I_2 to yield the diastereomeric phosphate triesters **4a/b**. All efforts to separate these fully protected diastereoisomers failed which was in accordance to earlier findings on compounds carrying a 3'-*O*-benzoyl group instead of a (*tert*-butyl)dimethylsilyl group [4][5]. The silyl residue was then selectively removed from **4a/b** by the action of Bu_4NF to yield compounds **5a/b**, which showed a fairly good separation on TLC. Preparative-scale separation of **5a/b** was accomplished on silica gel employing flash chromatography. The analytically pure diastereoisomers **5a** and **5b** were isolated in approximately 80% overall yield with a product ratio of *ca.* 1:1.

As TLC and ^{31}P-NMR spectra of **5a** and **5b** indicated (see *Table*), the phosphotriesters were stereoisomerically pure. Since $H_2[^{18}O]$ with an isotopic content of 90% ^{18}O, 7% ^{16}O, and 3% ^{17}O was used for oxidation, small downfield-shifted signals (4 Hz) were observed which belong to unlabeled material. The ^{17}O-labeled phosphotriesters were not detected due to the quadrupole moment of this isotope which broadens the P-signals [12][13]. From the peak areas of labeled and unlabeled material, a ^{18}O-content of 86% was calculated for **5a,b**.

Table. *Chemical Shifts in the ^{1}H-Decoupled ^{31}P-NMR Spectra of d(T[P-^{18}O]-A) Diastereoisomers and of the Phosphoramidites* **10a,b** *in $(D_6)Me_2SO$*[a])

Compound		P(III)		P(V) = O
4a[b])	(S_P)			0.23
4b[b])	(R_P)			0.03
5a	(S_P)			0.22
5b	(R_P)			0.04
10a	($S_{P(V)}$)	149.0	149.2	−0.53
10b	($R_{P(V)}$)	149.0	149.1	−0.87

[a]) δ's in ppm relative to H_3PO_4 as external standard.
[b]) Assignments are tentative because **4a/b** was not separated chromatographically.

In an earlier experiment, we had established the configuration at the P-atom of a 5'-deprotected d(T[*P*-^{18}O]-A) carrying a benzoyl protecting group at the 3'-position [4]. From this assignment it was shown that the diastereoisomer migrating faster on TLC possesses the (S_P)- and the slower migrating one the (R_P)-configuration. However, the chromatographic mobilities of **5a** and **5b** may be different with respect to the configuration at the P-atom.

To establish the absolute configuration at the P-atom of compounds **5a** and **5b**, they were converted into the fully deprotected compounds **8a** and **8b**. First, the diastereoisomers **5a** and **5b** were separately treated with $ZnBr_2$ in nitromethane in order to remove the 5'-$(MeO)_2$Tr residue. The detritylation products **6a** and **6b** were not isolated but demethylated at the phosphate moiety with thiophenol/Et_3N. To remove S-containing impurities, the resulting diastereoisomers **7a** and **7b** were purified by silica-gel chromatography. Subsequent treatment with aq. ammonia removed the benzoyl group to give the chiral d(T[*P*-^{18}O]-A) diastereoisomers **8a** and **8b**, respectively. Ion-exchange chro-

matography on *DEAE-Sephadex* with Et_3NHCO_3 yielded the Et_3NH^+ salts of **8a** and **8b**. The configurational assignment of **8a** and **8b** was accomplished after methylation of the phosphate moiety. For this 1:1 mixtures of ^{18}O-labeled (**8a** or **8b**) and unlabeled material (d(T-A)) – both as Et_3NH^+ salts – were converted into the potassium salts. The 1:1

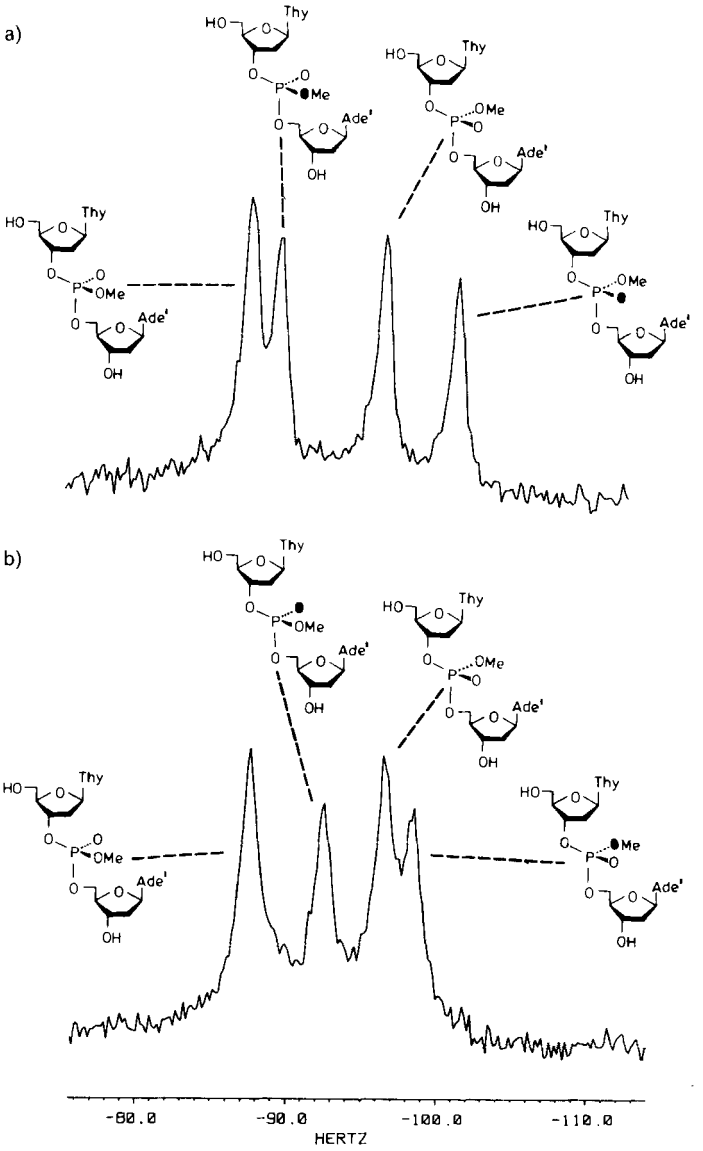


Fig. 1. *121-MHz-^{31}P-NMR spectra of 1:1 mixtures of the diastereoisomers of unlabeled d(T-A') methyl esters and d(T[P-^{18}O]-A') methyl esters* (each 5 mM) *in (D_6)Me_2SO containing 8-hydroxyquinoline* (10 mM). a) Methylation products of d(T-(S_P)-[*P*-^{18}O]-A) (**8b**)/d(T-A); b) methylation products of d(T-(R_P)-[*P*-^{18}O]-A) (**8a**)/d(T-A). Ade' = N^1-methyladenine, A' = N'-methyladenosin. Sweep width, 500 MHz; pulse width, 7 μs; aquisition time, 8 s; data collection in 16 K; number of scans: a) 250, b) 180.

mixtures were treated with MeI to yield a mixture of the methyl esters **9a/b** and the corresponding unlabeled esters. These methylation products of d(T[*P*-^{18}O]-A) **8a**/d(T-A) and of d(T[*P*-^{18}O]-A) **8b**/d(T-A) show different ^{31}P-NMR-peak patterns (*Fig. 1b* and *1a*, resp.). In each, four signals are clearly resolved. Two appear from the unlabeled and two from the ^{18}O-labeled methyl esters. The ^{18}O-isotope is placed either in a bridging position or in a double bond. Since the doubly bonded ^{18}O-isotope generates a stronger upfield shift of the δ_P than the singly bonded [13], asymmetric patterns as shown in *Fig. 1a* and *1b* are observed. The methylation experiment was carried out previously with d(T-(R_P)-[*P*-^{18}O]-A) [4]. The ^{31}P-NMR pattern of the methylation products was essentially identical to that exhibited in *Fig. 1b*. Hence **8a** has (R_P)-configuration.

The sequence rules according to *Cahn, Ingold,* and *Prelog* (*CIP* rules) [14] give priority to atomic numbers over atomic mass numbers. Thus, applying the first criterion, the methylester **5a** has (S_P)- and **5b** has (R_P)-configuration at the P-atom. Unfortunately, it is not possible to define the absolute position of an O-isotope at a P-atom with the (*R*/*S*) nomenclature in any case; a change of the position of the ^{18}O-isotopes (*e.g.* in **5a,b**) is not considered by this nomenclature. In this case, the exact position of an ^{18}O-isotope can only be derived from the stereo formula or from an extended nomenclature. However, as one can see, the *CIP* rules define the absolute position of an ^{18}O-isotope at the P-center for the compounds **8a** and **8b**.

After the assignment of the absolute configuration, compounds **5a** and **5b** were phosphinylated at the 3'-position. Separate treatment with (*N*,*N*-diisopropylamino)methoxyphosphine according to *Kumar* and *Poonian* [15] generated the phosphoramidites **10a** and **10b**. Both compounds were obtained pure after flash chromatography on silica gel. The ^{31}P-NMR spectra of **10a** and **10b** (see *Table*) showed two well-resolved signals at about 149 ppm and another signal at *ca.* 0 ppm. The precursor molecules showed a signal at *ca.* 0 ppm. The phosphate groups of the (R_P)-configurated compounds resonate thereby at higher field than those with (S_P)-configuration. The splitting of the signals at 149 ppm is due to the diastereoisomeric phosphoramidite moiety [16]. As a result of the distance between the phosphoramidite and the phosphate group, the signal of the latter is not splitted in the ^{31}P-NMR spectrum.

Solid-phase Synthesis of the Oligomers 13 and 14. – To study the utility of the phosphoramidites **10a** and **10b**, the solid-phase synthesis of the octamers **13** and **14** was carried out. The synthesis was performed on a manual DNA synthesizer using commercially available *Fractosil-500*-bound *N*-benzoyl-5'-*O*-dimethoxytrityl-2'-deoxycytidine as polymeric support. The cycles for oligomerization followed a protocol developed by *Matteucci* and *Caruthers* [8] which was also used for the incorporation of modified bases into an oligonucleotide sequence [17][18]. In the third reaction cycle, the phosphoramidites **10a** and **10b** were employed as coupling blocks instead of the monomeric phosphoramidites. The coupling yield was determined by UV spectroscopy at 498 nm on the basis of the liberated dimethoxytrityl residue [19] and was higher than 95% in each coupling step. This demonstrated that the dimer blocks **10a** and **10b** are as applicable as monomeric phosphoramidites. After six cycles of oligomerization, the methyl groups of the phosphortriesters were split off by the action of thiophenol in dioxane/Et_3N. Treatment with aq. ammonia removed the protected oligomers from the solid support and resulted in base deprotection within 36 h. The 5'-$(MeO)_2$Tr-protected oligomers **11** and **12**

were purified on reverse-phase HPLC with Et_3NHOAc/MeCN. Detritylation was accomplished with 80% aq. AcOH. Purification on reverse-phase HPLC gave the oligomers **13** and **14** in approximately 25% yield based on the amount of polymer-bound *N*-benzoyl-5'-*O*-dimethoxytrityl-2'-deoxycytidine.

The structure of the oligomers was confirmed by enzymatic cleavage with snake-venom phosphodiesterase and subsequent dephosphorylation with alkaline phosphatase. The reaction mixture was analyzed on reverse-phase HPLC *(Fig. 2)* and confirmed the sequence of the oligomers on the amount of the incorporated nucleosides.

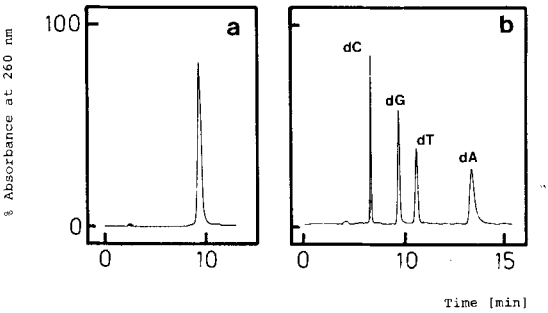


Fig. 2. *HPLC-elution profiles of a) the purified oligomer* **13** *(solvent system III) and b) the enzymatically hydrolyzed oligomer* **13** *(solvent system IV).* Digestion was performed with snake-venom phosphodiesterase followed by alkaline phosphatase (see also *Exper. Part*).

The oligomers **13** and **14** are self-complementary and should form duplexes under appropriate salt conditions. This was proven by melting experiments measured by UV spectroscopy at 260 nm in H_2O containing 10 mM *Tris*-HCl (pH 7.9) and 6 mM $MgCl_2$ at an oligonucleotide concentration of 50 μM. Both oligomers exhibited sigmoidal melting profiles with T_m's of 31 °C. Further experiments which make use of the ^{18}O-labeled chiral phosphate moiety of **13** and **14** are under investigation.

We thank *D. Kaiser* for measurement of the ^{31}P-NMR spectra and Dr. *H. Rosemeyer* for critical reading of the manuscript. Financial support by the *Deutsche Forschungsgemeinschaft* and the *Stiftung Volkswagenwerk* is gratefully acknowledged.

Experimental Part

General. Pyridine and 2,6-dimethylpyridine were distilled from *p*-toluene sulfonyl chloride, redistilled from CaH_2, and stored over 4-Å molecular sieves. CH_2Cl_2 and MeCN were distilled from CaH_2 and stored over 3-Å molecular sieves. THF *(Goldmarke)* was purchased from *Sigma* (St. Louis, USA). DMF was dried over BaO and redistilled under reduced pressure. Tetrazole, 4-(dimethylamino)pyridine, and CCl_3COOH were sublimated under reduced pressure. The phosphoramidites were prepared from the 5'-tritylated and appropriately base-protected nucleosides $bzA_d[(MeO)_2Tr]$, $ibG_d[(MeO)_2Tr]$, $bzC_d[(MeO)_2Tr]$, and $T_d[(MeO)_2Tr]$ (*Biosyntech*, Hamburg, FRG) according to the procedure of *McBride* and *Caruthers* [16]. *Fractosil-500* polymeric support was purchased from *Biosyntech* (Hamburg, FRG). Chloro(diisopropylamino)methoxyphosphine was prepared as described [16] and stored at −18°. (*tert*-Butyl)dimethylsilyl chloride was purchased from *Petrarch Systems Inc.* (Lewittown, Pennsylvania, USA). Snake-venom phosphodiesterase (EC 3.1.16.1., *Crotallus durissus*) and alkaline phosphatase (EC 3.1.3.1., *E.coli*) were obtained from *Boehringer* (Mannheim, FRG). ^{18}O-Enriched H_2O (^{18}O:90%; ^{17}O:3%;

^{16}O:7%) was purchased from *Ventron Ltd.* (Karlsruhe, FRG). Oligonucleotide synthesis was carried out on a manual DNA synthesizer from *Labor Service* (Mannheim, FRG). TLC: HP-TLC plates silica gel *60 F_{254}* (*Merck*, FRG) for the anal. separation of **5a**/**b**. Column flash chromatography: 0.5–1.0 bar, silica gel *60-H* (*Merck*, FRG); solvent proportions in *v/v*. Anion-exchange: *DEAE-Sephadex A-25* (*Pharmacia*, Sweden); UV detector (254 nm). Reverse-phase HPLC was performed on prepacked columns (*Merck, Lichrosorb RP-18*, 4 × 250, 7 μm) using a *LKB* system with two pumps (model *2150*), a variable wavelength monitor (model *2152*), and a controller (model *2151*) connected with an integrator (*Hewlett Packard 3390 A*). The solvent systems for HPLC consisting of 0.1M Et_3NHOAc pH 7.0 (A) and MeCN (B) were used in the following order: solvent system I:9% B; II:25% B; III:15 min (5–20% B); IV:6% B. Flow rates for the systems I–III were 1 ml/min and 0.5 ml/min for the system IV. M.p.: *Linström* apparatus (*Wagner* and *Munz*, FRG). Melting curves were measured in *Teflon*-stoppered cuvettes with 2-mm-light-path length in a thermostatically controlled cell holder with a *Shimadzu-210-A* recording spectrophotometer connected with a *Kipp* and *Zonen BD 90* recorder. The increase of absorbance at 260 nm as a function of time was recorded while the temp. of the soln. was increased linearly with time at a rate of 20°/h using a *Lauda-PM-350* programmer and a *Lauda RCS 6* bath equipped with a *R-22* unit (*MWG Lauda*, Lauda-Königshofen, FRG). The actual temp. was measured in the reference cell with a Pt resistor. UV: *Uvikon 810* spectrophotometer (*Kontron*, Switzerland). NMR: *Bruker-WM-250* or -WM-300 spectrometer; δ's in ppm relative to 85% H_3PO_4 for the ^{31}P-nucleus, they are positive if downfield with respect to the standard; ^{31}P-NMR in $(D_6)Me_2SO$ containing 8-hydroxyquinoline (10mM). Elemental analyses were performed by *Mikroanalytisches Labor Beller* (Göttingen, FRG).

*3'-O-(*tert*-Butyl)dimethylsilyl-N^6-benzoyl-2'-deoxyadenosine* (**2**). To a soln. of 5'-*O*-(4,4'-Dimethoxytrityl)-N^6-benzoyl-2'-deoxyadenosine (2.0 g, 3.04 mmol) in DMF (20 ml), imidazole (1.0 g, 14.6 mmol) and (*tert*-butyl)dimethylsilyl chloride (1.0 g, 6.63 mmol) were added. After stirring at r.t. for 5 h, the soln. was poured into 5% aq. $NaHCO_3$ soln. (150 ml) and the silylated nucleoside extracted with CH_2Cl_2 (3 × 50 ml). The soln. was dried over Na_2SO_4, and the solvent removed *in vacuo*. The oily residue, dissolved in CH_3NO_2/MeOH 95:5 20 ml, was treated with anh. $ZnBr_2$ (5.0 g, 22 mmol) and stirred at r.t. for 1 h. The red soln. was diluted with 5% aq. NH_4OAc (100 ml) and extracted with CH_2Cl_2 (3 × 50 ml). The org. layer was dried over Na_2SO_4, evaporated to a small volume, and applied to a silica-gel column (15 × 5 cm). Flash chromatography employing CH_2Cl_2/acetone 85:15 yielded **2**, which was isolated as a colorless foam (1.21 g, 80%). Recrystallization from Et_2O/hexane gave colorless crystals with m.p. 148° ([11]: 128–131°). UV: identical with published data [11].

*(R$_P$/S$_P$)-5'-O-(4,4'-Dimethoxytrityl)thymidylyl-[^{18}O](3'→5')-N^6-benzoyl-3'-O-(*tert*-butyl)dimethylsilyl-2'-deoxyadenosine Methyl Ester* (**4a**/**b**). The phosphoramidite **1** [16] (988 mg, 1.40 mmol) and **2** (496 mg, 1.00 mmol) were dried by evaporation with toluene (2 × 5 ml). Then, freshly sublimated tetrazole (350 mg, 5.00 mmol) was added. The reactants were dissolved by addition of MeCN (5 ml). The clear pale soln. was stirred at r.t. for 1 h. Oxidation of the resulting phosphite **3** was accomplished by the addition of I_2 (390 mg, 1.50 mmol) in MeCN/2,6-dimethylpyridine/$H_2[^{18}O]$ 3:2:1 *(v/v/v)*. The soln. was stirred for another 30 min and was then diluted with AcOEt (100 ml). The org. layer was separated and washed with 1% aq. $NaHSO_3$ soln. (20 ml), dried over Na_2SO_4, filtered, and evaporated to dryness. The residue in CH_2Cl_2 was applied to a silica-gel column (10 × 5 cm). Flash chromatography with CH_2Cl_2/acetone 7:3 yielded **4a**/**b** as colorless foam (902 mg, 84%). TLC (silica gel, CH_2Cl_2/acetone, 7:3): R_f 0.53. UV (CH_2Cl_2): 276 (26500). ^{31}P-NMR ($(D_6)Me_2SO$: 0.30 (*s*), 0.26 (*s*), 0.11 (*s*), 0.07 (*s*) (0.1:1.0:0.1:1). Anal. calc. for $C_{55}H_{64}N_7O_{13}PSi$: C 60.59, H 5.91, N 8.99; found: C 61.38, H 6.14, N 9.05.

Desilylation of **4a**/**b** *and Separation of the Diastereoisomers* **5a**/**b**. To a stirred soln. of **4a**/**b** (850 mg, 0.79 mmol) in THF (8 ml), a 1M soln. of Bu_4NF in THF (3 ml) was added. After stirring for 1 h, the mixture was evaporated to dryness *in vacuo* and partitioned between CH_2Cl_2 and H_2O. The org. layer was separated, dried over Na_2SO_4, evaporated to a small volume, and applied to a silica-gel column (40 × 3.5 cm). Flash chromatography with CH_2Cl_2/i-PrOH/EtOH 90:6:4 separated the products into two zones. From the fast migrating zone, *(R$_P$)-5'-O-(4,4'-dimethoxytrityl)thymidylyl-[^{18}O](3'→5')-N^6-benzoyl-2'-deoxyadenosine Methyl Ester* (**5b**; 288 mg, 38%) was obtained as colorless amorphous solid. TLC (silica gel, CH_2Cl_2/i-PrOH/EtOH 90:6:4): R_f 0.26. UV (CH_2Cl_2): 275 (26400). ^{31}P-NMR ($(D_6)Me_2SO$): 0.044, 0.004 (0.1:1.0).

The slowly migrating zone contained *(S$_P$)-5'-O-(4,4'-dimethoxytrityl)thymidylyl-[^{18}O](3'→5')-N^6-benzoyl-2'-deoxyadenosine Methyl Ester* (**5a**; 266 mg, 35%) which was obtained as a colorless amorphous solid. TLC (silica gel, CH_2Cl_2/i-PrOH/EtOH 90:6:4): R_f 0.23. UV (CH_2Cl_2): 275 (25700). ^{31}P-NMR ($(D_6)Me_2SO$): 0.26, 0.22 (1:10).

(S$_P$)-5'-O-(4,4'-Dimethoxytrityl)thymidylyl-[^{18}O](3'→5')-N^6-benzoyl-3'-O-[(N,N-diisopropylamino)methoxyphosphino]-2'-deoxyadenosine Methyl Ester (**10a**). Under reduced pressure, **5a** (250 mg, 0.26 mmol) was dried by evaporation with toluene (5 ml). The residue was then dissolved in CH_2Cl_2 (3 ml), and (*N*,*N*-diisopropylamino)methoxyphosphine (80 mg, 0.40 mmol) was added under dry N_2. The mixture was stirred for 30 min, diluted

with CH_2Cl_2, and extracted with a 2% aq. $NaHCO_3$ soln. The org. phase was dried over Na_2SO_4, concentrated to a small volume, and applied to a silica-gel column (20 × 1.5 cm). Flash chromatography with CH_2Cl_2/EtOAc/Et_3N 45:45:10 yielded **10a** (219 mg, 75%) as a colorless amorphous solid. TLC (silica gel, CH_2Cl_2/EtOAc/Et_3N 45:45:10): R_f 0.45. UV (CH_2Cl_2): 275 (28900). ^{31}P-NMR (121.5 MHz, $(D_6)Me_2SO$): −0.53 (phosphate), 149.0, 149.2 (phosphite). Anal. calc. for $C_{56}H_{66}N_8O_{14}P_2$: C 59.15, H 5.85, N 9.85; found: C 59.34, H 6.04, N 9.82.

(R$_P$)-5′-O-(4,4′-Dimethoxytrityl)thymidylyl-[^{18}O](3′→5′)-N^6-benzoyl-3′-O-[(N,N-diisopropylamino)methoxyphosphino]-2′-deoxyadenosine Methyl Ester (**10b**). As described for **10a**, **5b** (250 mg, 0.26 mmol) was phosphinylated to give **10b** as a colorless amorphous solid (204 mg, 70%). TLC (silica gel, CH_2Cl_2/EtOAc/Et_3N 45:45:10): R_f 0.45. UV (CH_2Cl_2): 275 (28400). ^{31}P-NMR (121.5 MHz, $(D_6)Me_2SO$): −0.87 (phosphate), 149.0, 149.1 (phosphite). Anal. calc. for $C_{56}H_{66}N_8O_{14}P_2$: C 59.15, H 5.85, N 9.85; found: C 59.26, H 5.91, N 9.98.

(R$_P$)-Thymidylyl-[^{18}O](3′→5′)-2′-deoxyadenosine Triethylammonium Salt (**8a**·*Et$_3$N*). To **5a** (200 mg, 0.21 mmol), a sat. soln. of $ZnBr_2$ in nitromethane/MeOH 95:5 (3 ml) was added. Upon stirring for 45 min at r.t., the reaction was stopped by the addition of 5% aq. NH_4OAc (10 ml). The aq. layer was saturated with NaCl, and **6a** was extracted with CH_2Cl_2/EtOH 9:1 (5 × 50 ml). The org. layers were dried over Na_2SO_4 and evaporated. Without further purification, the residue was suspended in dioxane/Et_3N/thiophenol 2:1:1 (5 ml). The mixture was stirred for 5 h at r.t. Deprotection was monitored on TLC (silica gel, $CHCl_3$/MeOH 4:1). The mixture was concentrated to a small volume under reduced pressure and chromatographed on a silica-gel column (15 × 2 cm) with CH_2Cl_2 (elution of excess of thiophenol) and CH_2Cl_2/MeOH/Et_3N 7:2:1 (elution of **7a**): **7a** as colorless amorphous solid (119 mg, 0.156 mmol) in 76% yield as Et_3NH^+ salt. To achieve deblocking of the benzoyl group, the product was dissolved in 25% aq. NH_3 soln. (10 ml) and stored at r.t. for 24 h. The soln. was evaporated and the residue dissolved in H_2O (10 ml). The resultant was applied to a *Sephadex-A-25* column (15 × 2 cm) and eluted with a linear gradient of $Et_3NH^+HCO_3^-/H_2O$ (20–200 mM). The slowly migrating zone containing **8a** was evaporated. To remove an excess of $Et_3NH^+HCO_3^-$, the residue was coevaporated 5× with H_2O (50 ml). After lyophilization, **8a**·Et_3N was isolated as an amorphous colorless solid (80 mg, 0.122 mmol) in 60% yield. This material was pure (99%) according to reverse-phase HPLC (*RP 18* column, solvent system I, t_R 5.6 min) and identical with commercially available material.

(S$_P$)-Thymidylyl-[^{18}O](3′→5′)-2′-deoxyadenosine Triethylammonium Salt (**8b**·*Et$_3$N*). Compound **5b** (200 mg, 0.205 mmol) was converted into **8b** as described for **8a** affording **8b**·Et_3N as colorless solid in 60% yield.

Methylation of d(T-(R$_P$)-[P-^{18}O]-A) (**8a**) *for Configurational Analysis.* A soln. of **8a**·Et_3N (10 mg, 15 μmol) and an equal amount of unlabeled d(T-A)·Et_3N in H_2O (8 ml) was stirred with the anion exchanger *Dowex XW* (2 ml, K^+ form) for 30 min. The soln. was filtered from the resin, and filtrate and washings were evaporated to a small volume. [18]Crown-6 was added (20 mg, 76 μmol), and the soln. was thoroughly evaporated *in vacuo*. The residue was dissolved in anh. DMF (5 ml) and coevaporated (3×) with DMF to remove traces of H_2O. The residue was then dissolved in $(D_6)Me_2SO$ (400 μl), and MeI (200 μl) was added[1]). After the mixture had been stirred for 12 h, the MeI was removed *in vacuo* to give **9a**/**b** and the corresponding unlabeled methyl esters. To the residue, 8-hydroxyquinoline (5 mg, 34 μmol) was added. After dilution with $(D_6)Me_2SO$ (2.5 ml), the soln. was filtered and the spectrum recorded. ^{31}P-NMR (121.5 MHz, $(D_6)Me_2SO$): −0.726 (CH_3O–P=O) and −0.767 (CH_3O–P=^{18}O) for the (*S*$_P$)-diastereoisomers; −0.801 (O=P–OCH_3) and −0.818 (O=P–$^{18}OCH_3$) for the (*R*$_P$)-diastereoisomers *(Fig. 1b)*.

Methylation of d(T-(S$_P$)-[P-^{18}O]-A) (**8b**). Compound **8b** (10 mg, 15 μmol) and an equal amount of unlabeled material was converted into the methyl esters **9a**/**b** and the corresponding unlabeled methyl esters as described above. ^{31}P-NMR (121.5 MHz, $(D_6)Me_2SO$): −0.726 (CH_3O–P=O) and −0.743 ($CH_3{}^{18}O$–P=O) for the (*S*$_P$)-diastereoisomers; −0.801 (O=P–OCH_3) and −0.842 (^{18}O=P–OCH_3) for the (*R*$_P$)-diastereoisomers *(Fig. 1a)*.

Solid-phase Synthesis of the Chiral [^{18}O]Oligomers **13** *and* **14**. *Fractosil-500*-linked N^4-benzoyl-5′-*O*-dimethoxytrityl-2′-deoxycytidine (50 mg; 32 μmol nucleoside per gram of support) were charged into the reactor of the manual DNA synthesizer. The reaction cycles for oligomerization in the order detritylation/coupling/capping/oxidation followed a protocol developed for the phosphit triester technique on solid support [8]. The phosphoramidites **10a** and **10b** were employed in the third reaction cycle: A soln. of 60 mg (52 μmol) of **10a** or **10b** in 400 μl of a 0.1M soln. of tetrazole in abs. MeCN was taken onto the solid-support for 30 min. The coupling yield determined by UV at 498 nm on the basis of the liberated $(MeO)_2Tr^+$ after treatment with 3% CCl_3COOH in abs. CH_2Cl_2 was higher than 95% in each coupling step. After six cycles of oligomerization, the polymer support was dried with Ar, and the reactor was filled with 3 ml of thiophenol/Et_3N/dioxane 1:1:2 for 30 min at r.t. After washing with MeOH (20 ml) and Et_2O (20 ml), the support was dried with Ar and suspended in 25% aq. NH_3 (5 ml) at 60° for 36 h.

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[1]) Excess of MeI causes also N(1) methylation of the adenine moiety. This does not obscure configurational analysis [6].

Evaporation and purification on reverse-phase HPLC (*RP-18* column, solvent system II, t_R 10.8 min) yielded the 5'-protected oligomers **11** and **12**, respectively. After evaporation, the oligomers were dissolved in 80% aq. AcOH (5 ml) at r.t. for 30 min. The acid was removed under reduced pressure. Extraction of the trityl residue with Et_2O/H_2O and subsequent purification on reverse-phase HPLC (*RP-18* column, solvent system III, t_R 9.5 min) gave 66 A_{260} units of the oligomer **13** and 70 A_{260} units of **14** which represented a yield of 24 and 25%, respectively. The compounds were lyophilized, dissolved in 1 ml, of H_2O, and stored frozen at −20°.

Enzymatic Hydrolysis of the Oligomers **13** *and* **14**. The oligomer (*ca.* 0.4 A_{260} units) was dissolved in 100 µl of 0.1M *Tris*-HCl buffer pH 8.5 and treated with snake-venom phosphodiesterase (5 µg) at 37° for 1 h. The mixture was analyzed on reverse-phase HPLC (*RP-18*, solvent system IV). Quantification of the material was made on the basis of the peak areas which were divided by the extinction coefficients of the containing nucleoside *(Fig. 2)*.

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