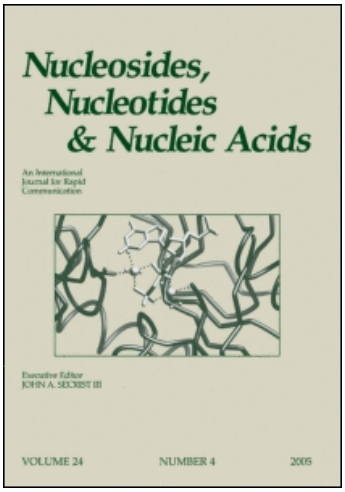

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Study on Reactivity and Protection of the α-Hydroxyphosphonate Moiety in 5'-Nucleotide Analogues: Formation of the 3'-O-P-C(OH)-C4' Internucleotide Linkage

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Study on Reactivity and Protection of the α-Hydroxyphosphonate Moiety in 5′-Nucleotide Analogues: Formation of the 3′-O-P-C(OH)-C4′ Internucleotide Linkage

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ABSTRACT

The recently described epimeric nucleosidyl-5′-*C*-phosphonates (α-hydroxyphosphonates) represent novel nucleotide analogues that can be incorporated into chimeric oligonucleotides by the phosphotriester condensation method. In order to prepare suitable protected monomer(s) we have studied condensation reaction between protected 2′-deoxythymidine and 2′-deoxythymidinyl-5′-*C*-phosphonate, both as model compounds, in dependence on the nature of the 5′-hydroxyl protecting group. We have found that the *O*-acetyl group is unstable in the presence of TPSCl or MSNT used as condensing agents for activation of the phosphorus moiety. This instability negatively influences the scope of the condensation process. On the other hand, introduction of the *O*-methoxycarbonyl group gave excellent results. The *O*-methoxycarbonyl group does not participate in the

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condensation process, and its quantitative introduction into the nucleotide analogues is accomplished using a novel acylating agent, methoxycarbonyl tetrazole.

Key Words: Nucleotides; Oligonucleotides; α-hydroxyphosphonates; Acylation; Protecting groups.

INTRODUCTION

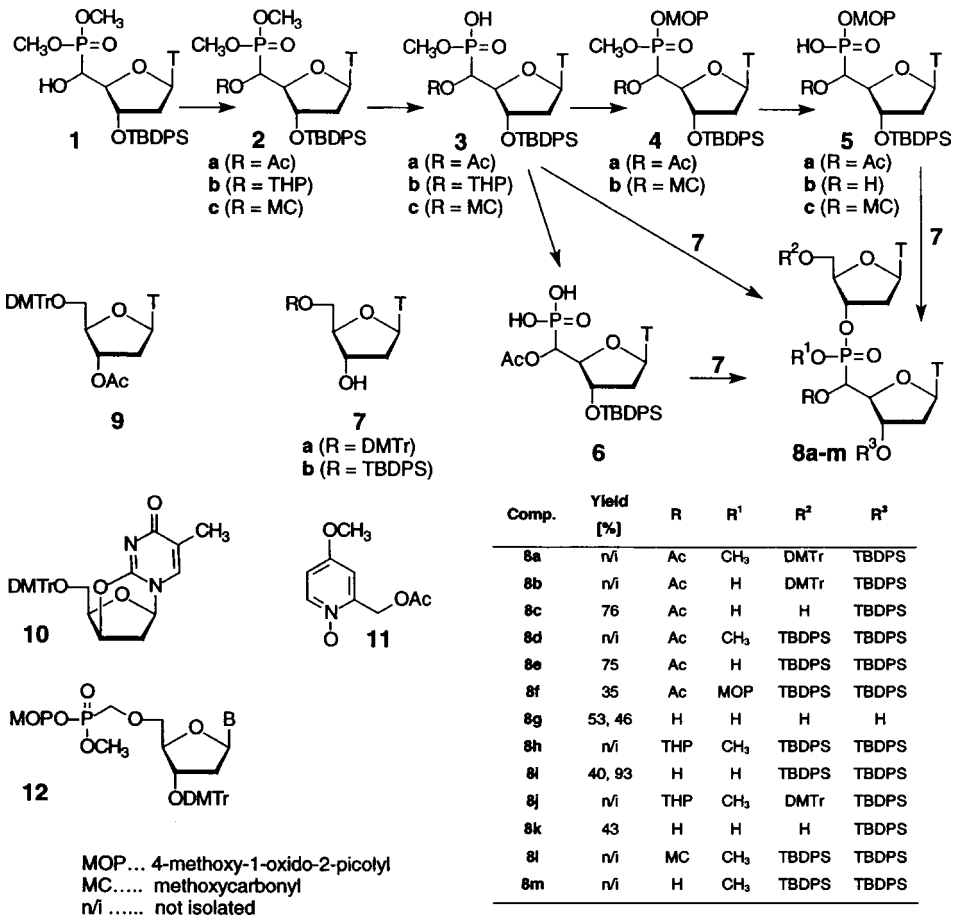
Isopolar phosphonate-based nucleotide analogues containing a bridging *P-C* bond instead on the ester *P-O* linkage have attracted attention for many years.[1–10] Recently we reported the synthesis of a novel type of isopolar 5′-nucleotide analogues[9] related to nucleoside 5′-monophosphates. These compounds with an α-hydroxyphosphonate moiety located on the 5′-carbon of the 2′-deoxyribo-nucleoside and ribonucleoside ring exist as epimeric pairs. Their simple, high-yield synthesis via nucleophilic addition of various phosphorous acid esters to nucleoside 5′-aldehydes provides a convenient route to the use of these compounds, after suitable protection, as monomers for the oligonucleotide construction.

This work describes the formation of dinucleoside phoshonates by triester-like condensation of the protected 2′-deoxythymidinyl-5′-*C*-phosphonate with nucleoside dependent on the nature of the *O*-protecting group of α-hydroxyphosphonate moiety. The configuration of the formed dimers containing a chiral 3′-*O*-*P*(*HO*)-5″ internucleotide linkage was assigned from NMR spectra. In addition, the chemical stability and enzymatic resistance of the modified internucleotide linkage is briefly discussed.

RESULTS AND DISCUSSION

The preparation of dinucleoside monophosphate analogues by reaction of the protected nucleoside 5′-phosphonate with nucleoside by the phosphotriester methodology must involve, as the first step, the protection of the hydroxyl group of the 5′-α-hydroxyphosphonate moiety (Sch. 1).

Thus, treatment of dimethyl ester[9] **1** with acetic anhydride in pyridine followed by hydrolysis of one methyl ester group of the diester **2a** in 60% aqueous pyridine resulted in quantitative yield of the 5′-*O*-acetyl derivative **3a**. The condensation of this compound with an excess of 5′-*O*-dimethoxytritylthymidine (**7a**) using mesitylenesulfonyl nitrotriazolide[11] (MSNT) in the presence of 4-methoxypyridine-1-oxide (MPNO) as a nucleophilic catalyst[12] afforded, after demethylation in aqueous pyridine and removal of DMTr group in 80% acetic acid, dimer **8c** as a mixture of epimers (9:91) in a 70% yield (related to 3a). In contrast to the literature data[12] the reaction proceeded extremely slowly and prolongation of the reaction time led only to detritylation and decomposition of the formed **8a**. Neither pre-activation of the P-component **3a** with MSNT nor the use of 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinance (CDOP)[13] instead of MSNT increased the yield. The use of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) as the condensing agent has led to a very complex mixture of products. A similar reaction of phosphonate **3a** with the

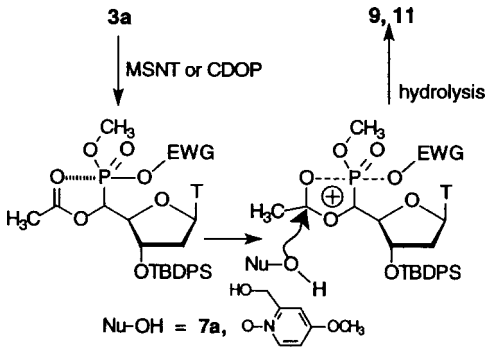


Comp.	Yield [%]	R	R^1	R^2	R^3
8a	n/i	Ac	CH_3	DMTr	TBDPS
8b	n/i	Ac	H	DMTr	TBDPS
8c	76	Ac	H	H	TBDPS
8d	n/i	Ac	CH_3	TBDPS	TBDPS
8e	75	Ac	H	TBDPS	TBDPS
8f	35	Ac	MOP	TBDPS	TBDPS
8g	53, 46	H	H	H	H
8h	n/i	THP	CH_3	TBDPS	TBDPS
8i	40, 93	H	H	TBDPS	TBDPS
8j	n/i	THP	CH_3	DMTr	TBDPS
8k	43	H	H	H	TBDPS
8l	n/i	MC	CH_3	TBDPS	TBDPS
8m	n/i	H	CH_3	TBDPS	TBDPS

Scheme 1.

5′-*O*-*tert*-butyldiphenylsilythymidine (**7b**) resulted in dimer **8d** that was converted to derivative **8e** (31 : 69 mixture of epimers) in a 75% yield. In the case of condensation of ester **3a** with nucleoside **7a**, we isolated considerable amounts of 3′-*O*-acetyl derivatice **9** and 2,3′-anhydrothymidine derivative **10.**

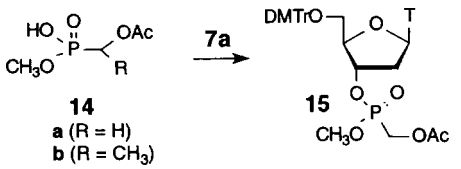
In order to increase the rate and yield of esterification of the phosphonate moiety with the nucleoside we prepared 4-methoxy-l-oxido-2-picolyl ester **5a** that should enhance the condensation rate via intramolecular catalysis.[13] Thus, monomethyl ester **3a** was esterified with 4-methoxy-l-oxido-2-pyridinemethanol (MOP) using CDOP as condensing agent and MPNO as catalyst.[12] Demethylation of the mixed diester **4a** in 60% aqueous pyridine afforded two epimeric pairs, determined by NMR as compound **5a** (41:59; 92%) and its deacetylated derivative **5b** (37:63; 8%) in overall 50% yield. Beside esters **5a**, **5b** we also isolated the 5′-*O*-acetylated phosphonic acid **6** and 4-methoxy-l-oxido-2-picolyl acetate (**11**). The condensation of the picolyl ester **5a** with **7b** in pyridine using TPSCl proceeded also very slowly to afford dimer **8f** in a low yield.



Scheme 2. Proposed mechanism of transacetylation during activation of phosphonate moiety.

The presence of compound **6** in the reaction mixture was a surprise because in aqueous pyridine we have never observed[14] simultaneous hydrolysis of both the methyl and picolyl ester groups of compound **12** lacking α-acetoxy moiety. Only the methyl group was always cleaved off with high selectivity and resulted monopicolyl ester was quite stable when heated in aqueous pyridine. Concerning these facts it is clear that the α-acetoxy group must play a crucial role in the hydrolysis of the picolyl ester group of diester **4a**. On the other hand, the unexpected formation of acetyl derivatives **9** and **11** could be explained by one of possible transacetylation mechanism as is proposed in Scheme 2. Possible formation of five-membered acetyloxonium intermediate would not be surprising because a similar intermediate is supposed to be formed at the activation of 1,2-di-*O*-acetyl derivatives of furanoses with Lewis acids during nucleosidation reaction.

To clarify more the participation of α-acetoxy group at the activation of the phosphorus moiety in compound **3a** or **5a** and transacetylation reaction, we prepared the simplest α-acetoxyphosphonates **14a** and **14b** differing in the nature of the acetoxy group (primary *vs.* secondary) (Sch. 3). Whereas the condensation of **14a** with **7a** using MSNT-MPNO[12] afforded epimeric nucleoside phosphonates **15** (50:50) in a high yield, a different result was obtained with the "secondary" acetoxy derivative **14b**. From the reaction mixture we isolated 3′-*O*-acetylated product **9** and its 3′-epimer, the 1-(3-*O*-acetyl-2-deoxy-5-*O*-dimethoxytrityl-β-D-*threo*-pentofuranosyl)thymine. Both these compounds were formed as the products of the transacetylation reaction (cf. Sch. 2) from the nucleoside **7a** and MSNT (the 3′-epimer *via* 2,2′-anhydro nucleoside). Although these findings confirmed our above-mentioned results, we still are not able to fully explain the nature of different



Scheme 3.

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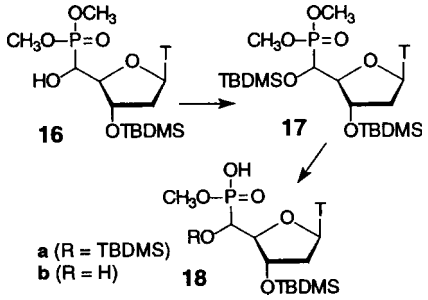
reactivities of primary (**14a**) and secondary (**3a**, **4a**, **5a**, **14b**) α-acetoxyphosphonates in the condensation reactions. We already observed differences in reactivity of other types of α-hydroxyphosphonate derivatives. Thus, α-tosyloxyphosphonates bearing a secondary tosyloxy group did not undergo nucleophilic substitution of tosyloxy group with alkoxide anion, whereas a tosyloxymethylphosphonate bearing primary tosyloxy group did.[15]

In the light of these findings we turned our attention towards other types of groups to protect the 5′-hydroxy group of diester **1** (Sch. 1). Thus, the 5′-*O*-tetrahydropyranyl derivative **3b** was prepared in 67% yield by treatment of **1** with dihydropyran and pyridinium 4-toluenesulfonate in dichloromethane followed by partial demethylation of formed **2b** in 60% aqueous pyridine. However, the use of the obtained 5′-*O*-tetrahydropyranyl derivative **3b** instead of the 5′-*O*-acetyl derivative **3a** in the condensation reaction with nucleoside **7b** under standard conditions[12] did not improve the yield of the dimer **8h**. After a partial deprotection of **8b** we afforded epimeric dimers **8i** (33:67) in a moderate yield of 52%. Similarly, the condensation of phosphonate **3b** with nucleoside **7a** afforded dimer **8j**. Its partial deprotection gave epimeric dimers **8k** (20:80; 43%). The tetrahydropyranyl group in dimer **8h** was removed by treatment with 0.02 M HCl in aqueous dioxane overnight, whereas the cleavage of the same group in dimer **8j** required treatment in 0.2 M HCl for several days.

As another type of group for protection of the 5′-hydroxyphosphonate moiety, we examined the *tert*-butyldimethylsilyl group. The reaction of dimethyl phosphonate **16** (Sch. 4) with an excess of *tert*-butylchlorodimethylsilane in the presence of imidazole in dimethylformamide proceeded very slowly. Neither heating at 40°C nor addition of DBU accelerated the silylation reaction. The expected 5′-*O*-*tert*-butyldimethylsilyl derivative **17** was obtained in overall 38% yield as an epimeric mixture (17:83) whose epimeric composition differed from that in the starting compound **16** (10:90). Recovery of the major epimer of **16** from the reaction mixture suggests that the minor epimer was silylated by the bulky *tert*-butyldimethylsilyl group faster than the major one. These findings are in a good agreement with the results obtained from conformational analysis of single epimers **16** performed in our laboratory earlier.[9] The silyl derivative **17** was subjected to partial demethylation in 60% aqueous pyridine to give the monoester **18a**. This demethylation, accompanied by complete and very fast hydrolysis of the 5′-*O*-*tert*-butyldimethylsilyl group, afforded



Scheme 4.

compound **18b** instead of silyl derivative **18a**. This unexpected hydrolysis of the silyl group seem to be accelerated by the presence of the phosphorus moiety; a migration of the silyl group from secondary 5′-hydroxyl to the hydroxyl of the phosphorus moiety and its fast hydrolysis as ester group is very likely the driving force of this hydrolytic process.

In our search for the protecting groups of the 5′-hydroxyl of the 5′-hydroxyphosphonate moiety we also examined the methoxycarbonyl group that could be easily attached to the hydroxyl and would not participate during activation of the phosphorus moiety. Thus, the treatment of phosphonate **1** with a large excess of MCC and DMAP in pyridine at 50°C for 6 d afforded the 5′-*O*-methoxycarbonyl derivative **3c** in a moderate yield (54%; 32% of **1** recovered). As a side reaction, we also observed partial demethylation. A comparison of the epimer ratio (determined by NMR spectroscopy) in the product **3c** (36:64), starting phosphonate **1** (22:78) and the recovered compound **1** (8:92) showed again the reaction preference for the minor isomer. A crucial improvement of the methoxycarbonylation was achieved using *N*-methoxycarbonyl tetrazole[16] (MCT), a novel acylating reagent prepared in analogy to the known *N*-benzoyl tetrazole.[17,18] Thus, the treatment of phosphonate **1** with MCT (2 equiv) in tetrahydrofuran containing DMAP led to complete consumption of the starting compound **1** within 5 min at rt. The workup and isolation afforded dimethyl phosphonate **2c** (27:73; 71%) and monomethyl ester **3c** (24%). Conversion of **2c** to **3c** in 60% aqueous pyridine afforded the monoester **3c** in overall 95% yield. The condensation of the 5′-*O*-methoxycarbonyl derivative **3c** with nucleoside **7b** under standard conditions[12] smoothly afforded the fully protected dimer **8l** that was not isolated but immediately subjected to the removal of the methyl ester group in aqueous pyridine as well as the methoxycarbonly group in a mixture methanol-Et_3N-water overnight. The partially deprotected dimer **8i** was obtained in excellent (93%) yield. Also the transformation of the 5′-*O*-methoxycarbonyl derivative **3c** into the picolyl ester **4b** according to the standard procedure[13] provided this compound in 96% yield.

Although both the acetyl and tetrahydropyranyl protecting groups of the α-hydroxyphosphonate moiety are acceptable for the synthesis of very short oligonucleotides in solution, they are not suitable for the synthesis of longer oligonucleotides on the solid phase where the interference of the protecting group must not affected the efficiency of coupling steps. We found that the methoxycarbonyl group is the group of choice because of its quantitative introduction and no participation during condensation step using triester method. Solid phase synthesis of longer oligonucleotides composed of nucleoside 5′-hydroxyphosphonates will be the subject of our further investigation.

Concerning the chemical stability of the 3′-*O*-*P*-(*HO*)-5′ internucleotide linkage in dimer **8g**, this compound is quite stable under standard deprotection conditions used in the solid phase synthesis of oligonucleotides (37% aqueous ammonia; 55°C; 16 h) and in 0.05 M aqueous NaOH for several days as determined by analytical reversed-phase HPLC. Complete resistance of the modified internucleotide linkage in both individual epimers **8g** against nucleases was observed in an L-1210 cell free extract under conditions leading to complete cleavage of the natural dimer TpT.

Determination of configuration at the C5′ carbon atom is closely connected with the conformation around the C4′-C5′ bond. Small vicinal couplings J(H4′,H5′) and

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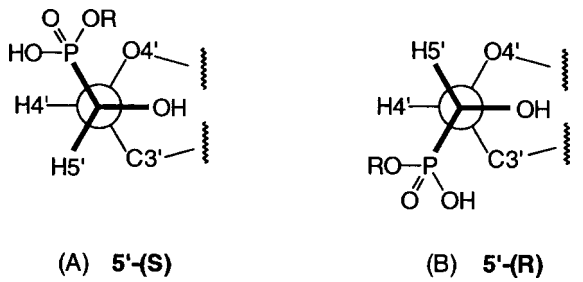

Figure 1. Preferred conformations of 5′-(*S*) and 5′-(*R*) isomers of compound **8g**.

J(P,H4′) observed in the 5′-diastereoisomers of compound **8g** (2.1–3.0 Hz, see Table 2) indicate that they adopt the preferred conformation (A) and (B) with a gauche orientation of atoms H4′/H5′ and P/H4′. Distinguishing between 5′-(R) and 5′-(*S*)-isomers is then possible on the basis of J(P,C3′) couplings. The large value J(P,C3′) = 11.7 Hz in the major isomer corresponds to the 5′-(*S*) configuration (conformer A) with *trans*-orientation P/C3′ while J(P,C3′)∼0 Hz in the minor isomer corresponds to the 5′-(*R*) configuratoin (conformer B) with *gauche*-P/C3′ orientation. We should also notice a large difference observed between geminal couplings J(P,C4′) in 5′-diastereoisomers (∼0 Hz in 5′ (*S*)-isomer in comparison with 10.2 Hz in 5′(*R*)-isomer) probably due to a different orientation of phosphorus and the oxygen atom O4′. Configuration at C5′ in compounds **8c**, **8e**, **8j** and **8k** follow from their chemical correlations with diastereoisomers of **8g**.

The observed NOE contacts of thymine proton H-6 with furanose ring protons H-1′,H-2′ and H-3′ indicate a preferred *anti*-orientation of both thymine rings in diastereoisomers (*S*)-**8g** and (*R*)-**8g**. Vicinal coupling constants together with a two-state model and generalized Karplus relation[19] have been used for estimation of conformation behaviour of furanose rings. It was found that the equilibrium between *C3′-endo* and *C2′-endo* forms in diasteroisomers (*S*)-**8g** and (*R*)-**8g** is shifted significantly towards the *C2′-endo* form for 5′-residues (*ca* 15:85 and 25:75 in (*S*)-**8g** and (*R*)-**8g**) but much less for 3′-residues (*ca* 45:55 and 35:65 in (*S*)-**8g** and (*R*)-**8g**). The only slightly different sets of vicinal coupling constants indicate a similar conformational behaviour for other epimers **8**.

EXPERIMENTAL

Unless stated otherwise, the solvents were evaporated at 40°C and 2 kPa and the products were dried over phosphorus pentoxide at 50–70°C and 13 Pa. The course of the reaction was checked by TLC on silica gel Silufol UV 254 foils (Kavalier Glassworks, Votice, Czech Republic) and the products were detected both by UV monitoring and by spraying with a 1% ethanolic solution of 4-(4-nitro-benzyl)pyridine [after short heating and exposing to ammonia vapours the product (dialkyl phosphonate) affords an intense blue spot]. Preparative column chromatography (PLC) was carried out on silica gel (40–60 μm; Fluka); the amount of adsorbent was 20–40 times the weight of the separated mixture, elution rate 40 mL/min. PLC and TLC were

carried out in the following solvent systems (v/v): chloroform–ethanol 9:1 (Cl); ethyl acetate-acetone-ethanol-water 4:1:1:1 (H1); 2-propanol–concentrated aqueous ammonia–water 7:1:2 (I, for charged compounds); TI – 50% EtOAc-toluene, T2 – 20% EtOAc-toluene. Preparative reversed-phase chromatography (PRPC) was carried out on a spherical octadecyl silica column (25 × 300 mm, 20–40 μm, IOCB Prague); compounds were eluted with a linear gradient of methanol in water at 10 mL/min. Chromatography on DEAE-Sephadex A-25 was performed with linear gradient of 0-0.2 M triethylammonium hydrogen carbonate (TEAB) in water. HPLC analysis was performed on a column of reverse phase (4.6 × 150 mm) Nucleosil 100-5 C18 (Macharey-Nagel), either isocratically at various concentrations of methanol in 0.1 M triethylammonium acetate (TEAA) or by gradient of methanol in the same buffer. The electrophoresis was made on Whatman No. 1 in 0.1 M TEAB (pH 7.5) at 20 V/cm. UV spectra were taken on PYE-Unicam SP 8000 spectrophotometer in water or in a methanol-water mixture (1:1, v/v) at pH 2, pH 7, and pH 12. Mass spectra (m/z) were recorded on ZAB-EQ (VG Analytical) instrument, using FAB (ionisation by Xe, accelerating voltage 8 kV) technique with glycerol and thioglycerol as matrices. NMR spectra were measured on a Varian UNITY-500 spectrometer (^{1}H at 500 MHz; ^{13}C at 125.7 MHz frequency) in d_6-DMSO and/or D_2O at 20°C. The signals were referenced either to solvent signal (converted to δ scale using relations δ_H(DMSO) = 2.50 and δ_C(DMSO) = 39.7 ppm) or to DSS (in D_2O). Proton 2D-COSY spectra were used for the structural assignment of coupled protons and 2D-ROESY spectra for detection of the NOE contacts. Carbon-13 chemical shifts and coupling constants J(C,P) were obtained from broadband proton-decoupled spectra using standard and attached proton test pulse sequences.

Method A: Condensation of the P- and OH-components by MSNT in the Presence of MPNO. A mixture of triethylammonium salt of 5′-*O*-protected phosphonate monoester (1 mmol), 5′-*O*-protected thymidine (2 mmol) and MPNO (5 mmol), pre-dried by co-evaporation with toluene and dichloromethane (2 × 20 mL), was treated in dichloromethane (12 mL) with MSNT (6 mmol) at rt under exclusion of moisture. After 40 min (TLC in Cl and H1) the reaction mixture was quenched with 2M-TEAB (10 mL), stirred for 15 min, then diluted with chloroform and the organic layer was washed with 1M-TEAB (3 × 200 mL) and dried over anhydrous Na_2SO_4. The product was purified on silica gel by elution with gradient of 1–10% of ethanol in chloroform; triethylamine (5 mL per 100 g of silica gel) was added to the suspension of silica gel when dimethoxytrityl derivatives were isolated.

Dimethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-methoxycarbonylthymidinyl-5′-*C*-phosphonate (2c). To a stirred solution of sublimed tetrazole (700 mg, 10 mmol) and Et_3N (1.39 mL, 10 mmol) in THF (30 mL) was added dropwise, under an argon atmosphere, methoxycarbonyl chloride (0.77 mL, 10 mL). After 90 min the suspension was filtered under argon and a part of the methoxycarbonyl tetrazole solution (8 mL, 2.65 mmol) was added under stirring to a mixture of α-hydroxy-phosphonate (**1**) (*R*/*S* 34/66) (390 mg, 0.66 mmol) and DMAP (324 mg, 2.65 mmol) dissolved in THF (2 mL). After 15 min (TLC in Cl and H1) the reaction mixture was quenched by addition of methanol (0.2 mol) and the solvent evaporated *in vacuo*. The product was purified on silica gel by elution with linear gradient of ethanol in

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chloroform. Yield 303 mg (71%, white foam) of **2c** (*R*/*S* 27/73). HR FAB calcd for $C_{30}H_{40}N_2O_{10}Psi$ 647.2190 $(M+H)^+$, found 647.2197. ^1H NMR (DMSO, 500 MHz) **2c**-(***R***): δ 11.37 (1H, bs, NH), 7.58 (1H, q, J(6,CH_3) = 1.2 Hz, H-6), 7.60 (4H, m, *o*-Ar-H), 7.50 (2H, m, *p*-Ar-H), 7.46 (4H, m, *m*-Ar-H), 6.30 (1H, dd, J(1′,2′) = 9.1, J(1′,2″) = 5.5 Hz, H-1′), 5.16 (1H, dd, J(5′,4′) = 6.0, J(5′,P) = 10.0 Hz, H-5′), 4.49 (1H, dt, J(3′,2″) = J(3′,4′) = 2.1, J(3′,2′) = 5.6 Hz, H-3′), 4.23 (1H, m, H-4′), 4.10 (3H, s, $COOCH_3$), 3.58 and 3.55 (2 × 3H, 2 × d, J(OCH_3,P) = 10.8 Hz, $P(OCH_3)_2$), 2.08 (1H, ddd, J(2′,1′) = 9.1, J(2′,2″) = 13.5, J(2′,3′) = 5.6 Hz, H-2′), 1.98 (1H, ddd, J(2″,1′) = 5.5 J(2″,2′) = 13.5, J(2″,3′) = 2.1 Hz, H-2″), 1.73 (3H, d, J(CH_3,6) = 1.2 Hz, CH_3), 1.03 (9H, s, t-But). **2c**-(***S***): δ 11.35 (1H, bs, NH), 7.35 (1H, q, J(6,CH_3) = 1.2 Hz, H-6)), 7.60 (4H, m, *o*-Ar-H), 7.50 (2H, m, *p*-Ar-H), 7.46 (4H, m, *m*-Ar-H), 6.34 (1H, dd, J(1′,2′) = 8.3, J(1′,2″) = 5.8 Hz, H-1′), 4.85 (1H, dd, J(5′,4′) = 4.3, J(5′,P) = 11.5 Hz, H-5′), 4.46 (1H, ddd, J(3′,2″) = 2.7, J(3′,2′) = 5.5, J(3′,4′) = 2.3 Hz, H-3′), 4.24 (1H, m, H-4′), 3.71 (3H, s, $COOCH_3$), 3.59 and 3.57 (2 × 3H, 2 × d, J(OCH_3,P) = 10.8 Hz, $P(OCH_3)_2$), 2.17 (1H, ddd, J(2″,1′) = 5.8, J(2″,2′) = 13.6, J(2″,3′) = 2.7 Hz, H-2″), 2.01 (1H, ddd, J(2′,1′) = 8.3, J(2″,2′) = 13.6, J(2′,3′) = 5.5 Hz, H-2′), 1.71 (3H, d, J(CH_3,6) = 1.2 Hz, CH_3), 1.04 (9H, s, t-But).

Methyl-5′-*O*-acetyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidinyl-5′-*C*-phosphonate (3a). α-Hydroxyphosphonate **1** (*R*/*S* 20/80)(1.353 g, 2.30 mmol) was treated with acetic anhydride (1.08 mL, 11.50 mmol) in pyridine (20 mL) in the presence of DMAP (50 mg, 0.41 mmol) at rt under exclusion of moisture until the starting compound disappeared (18 h; TLC in Cl and H1). Water (13.3 mL) was added to obtain a final concentration of pyridine equal 60%, and the mixture was heated at 50°C overnight. The solution was concentrated *in vacuo*, the residue co-distilled with ethanol (4 × 20 mL) and treated with Dowex 50×2 (Et_3NH^+ form) in 75% aqueous ethanol to remove *N*-methylpyridinium cations. The crude product was then purified by PRPC. Yield 1.535 g (93%, white foam) of triethylammonium salt of **3a** (*R*/*S* 20/80). HR FAB calcd for $C_{29}H_{37}N_2NaO_9Psi$ 639.1904 $(M+Na)^+$, found 639.1846. ^1H NMR (DMSO, 500 MHz) **3a**-(***R***): δ 11.27 (1H, bs, NH), 10.07 (1H, bs, P-OH), 7.80 (1H, bs, H-6), 7.65-7.59 (4H, m, Ar-H), 7.50-7.41 (6H, m, Ar-H), 6.21 (1H, dd, J(1′,2′) = 8.7, J(1′,2″) = 5.4 Hz, H-1′), 4.99 (1H, dd, J(5′,4′) = 4.2, J(5′,P) = 11.7 Hz, H-5′), 4.71 (1H, m, H-3′), 4.31 (1H, ddd, J(4′,3′) = 1.2, J(4′,5′) = 4.2, J(4′,P) = 13.4 Hz, H-4′), 3.30 (3H, d, J(P,OCH) = 10.0 Hz, P-OCH_3), 2.00 (1H, m, H-2″), 1.95 (1H, m, H-2′), 1.90 (3H, s, OAc), 1.71 (3H, d, J(CH_3, H-6) = 1.0 Hz, CH_3), 1.03 (9H, s, t-But). **3a**-(***S***): δ 11.27 (1H, bs, NH), 10.07 (1H, bs, P-OH), 7.92 (1H, q, J(6,CH_3) = 1.0 Hz, H-6), 7.65-7.59 (4H, m, Ar-H), 7.50-7.41 (6H, m, Ar-H), 6.25 (1H, dd, J(1′,2′) = 8.7,J(1′,2″) = 5.4 Hz, H-1′), 4.85 (1H, dd, J(5′,4′) = 4.2, J(5′,P) = 11.5 Hz, H-5′), 4.67 (1H, m, H-3′), 4.27 (1H, ddd, J(4′,3′) = 1.5, J(4′,5′) = 4.2, J(4′,P) = 8.1 Hz, H-4′), 3.33 (3H, d, J(P,OCH) = 10.2 Hz, Hz, P-OCH_3), 2.02 (1H, ddd, J(2″,1′) = 5.4, J(2″,2′) = 13.2, J(2″,3′) = 5.0 Hz, H-2″), 1.93 (1H, ddd, J(2′,1′) = 8.7, J(2′,2′) = 13.2, J(2′,3′) = 5.0 Hz, H-2′), 1.92 (3H, s, OAc), 1.72 (3H, d, J(CH_3,6) = 1.0 Hz, CH_3), 1.03 (9H, s, t-But).

Methyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-tetrahydropyranylthymidinyl-5′-*C*-phosphonate (3b). α-Hydroxyphosphonate **1** (*R*/*S* 25/75) (708 mg, 1.20 mmol)

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and dihydropyran (0.38 mL, 4.20 mmol) were treated in dichloromethane (12 mL) with pyridinium 4-toluensulfonate (301 mg, 1.20 mmol) at rt under stirring and exclusion of moisture. Further portions of dihydropyran (4 × 0.2 mL) and 0.5 M solution of pyridinium 4-toluensulfonate in dichloromethane (2 × 2.4 mL) were added within next 4 d (TLC in Cl). The reaction mixture was diluted by aqueous 2M-TEAB (2 mL), stirred for 10 min then diluted with chloroform (100 mL) and extracted with 1M-TEAB (3 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by silica gel chromatography using a linear gradient of ethanol in chloroform and the resulting protected dimethyl phosphonate was treated with 60% aqueous pyridine (20 mL) at 50°C for 48 h (TLC in C1 and H1). The solution was concentrated, the residue co-distilled with ethanol (4 × 20 mL), and treated with Dowex 50 × 2 (Et_3NH^+ form) in 75% aqueous ethanol to remove *N*-methylpyridinium cations. The product was finally purified by PRPC. Yield 606 mg (67%, white foam) of triethylammonium salt of **3b** (ratio of epimers was not determined). HR FAB calcd for $C_{38}H_{59}N_3O_9PSi$ 760.3758 $(M + Et_3N + H)^+$, found 760.3876.

Methyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-methoxycarbonylthymidinyl-5′-*C*-phosphonate (3c). α-Hydroxyphosphonate **1** (*R*/*S* 22/78) (441 mg, 0.75 mmol) was treated with methoxycarbonyl chloride (0.40 mL, 5.25 mmol) in pyridine (7 mL) in the presence of DMAP (183 mg, 1.50 mmol) at rt under stirring and exclusion of moisture for 3 d (TLC in Cl and H1). Then, further portions of methoxycarbonyl chloride (0.40 mL, 5.25 mmol) and DMAP (92 mg, 0.75 mmol) were added and the reaction mixture was heated at 50 °C for the next 2 d. The reaction mixture was quenched by addition of water (1 mL), and the product was partioned between 1 M TEAB (20 mL) and chloroform (100 mL). The organic layer was washed with 1M TEAB (3 × 20 mL) and dried over anhydrous Na_2SO_4. The product was purified on silica gel by elution with linear gradient of H3 in ethyl acetate followed by H1 in H3). Yield 289 mg (54%, white foam) of pyridinium salt of **3c** (*R*/*S* 36/64). HR FAB calcd for $C_{29}H_{38}N_2O_{10}PSi$ 633.2033 $(M + H)^+$, found 633.1987. ^1H NMR (DMSO, 500 MHz) **3c**-(***R***): δ 11.25 (1H, bs, NH), 7.96 (1H, bs, H-6), 7.60 (4H, m, Ar-H), 7.40 (6H, m, Ar-H), 6.21 (1H, dd, J(1′,2′) = 8.5, J(1′,2″) = 5.5 Hz, H-1′), 4.85 (1H, dd, J(5′,4′) = 4.0, J(5′,P) = 11.0 Hz, H-5′), 4.70 (1H, m, H-3′), 4.35 (1H, m, H-4′), 3.66 (3H, s, OCH_3), 3.32 (3H, d, J(OCH,P) = 10.0 Hz, P-OCH_3), 2.00 (1H, m, H-2″), 1.85 (1H, m, H-2′), 1.71 (3H, bs, J(CH_3,6) = 1.0 Hz, CH_3), 1.02 (9H, s, t-But). **3c**-(***S***): δ 11.25 (1H, bs, NH), 7.92 (1H, bs, H-6), 7.60 (4H, m, Ar-H), 7.40 (6H, m, Ar-H), 6.24 (1H, dd, J(1′,2′) = 8.2, J(1′,2″) = 5.6 Hz, H-1′), 4.73 (1H, dd, J(5′,4′) = 4.5, J(5′,P) = 11.8 Hz, H-5′), 4.62 (1H, m, H-3′), 4.37 (1H, ddd, J(4′,3′) = 2.1, J(4′,5′) = 4.5, J(4′,P) = 6.0 Hz, H-4′), 3.64 (3H, s, OCH_3), 3.37 (3H, d, J(OCH,P) = 10.3 Hz, P-OCH_3), 2.00 (1H, ddd, J(2″,1′) = 5.6, J(2″,2′) = 13.2, J(2″,3′) = 2.6 Hz, H-2″), 1.82 (1H, ddd, J(2′,1′) = 8.2, J(2″,2′) = 13.2, J(2′,3′) = 5.2 Hz, Hz, H-2′), 1.70 (3H, d, J(CH_3,6) = 1.0 Hz, CH_3), 1.02 (9H, s, t-But).

(4-Methoxy-1-oxido-2-picolyl)-5′-*O*-acetyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidinyl-5′-*C*-phosphonate (5a). To a solution of triethylammonium salt of monoester **3a** (*R*/*S* 25/75) (310 mg, 0.43 mmol), 4-methoxy-1-oxido-2-pyridinemethanol (74 mg, 0.48 mmol) and MPNO (162 mg, 1.29 mmol) (co-evaporated several times

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with pyridine) in pyridine (4.5 mL) was added at rt and under exclusion of moisture CDOP (239 mg, 1.29 mmol). After 2 h (TLC in C1 and H1) the reaction mixture was diluted with saturated aqueous solution of $NaHCO_3$ (10 mL) and chloroform (50 mL), the layers were separated, after shaking, by centrifugation and the organic layer was washed with solution of $NaHCO_3$ (4×10 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was treated with 60% aqueous pyridine (15 mL) at 50°C for 24 h. The solution was concentrated *in vacuo*, the residue co-distilled with ethanol (4×20 mL) and treated with Dowex 50×2 (Et_3NH^+ form) in 75% aqueous ethanol to remove *N*-methylpyridinium cations. The product was purified by PRPC. Yield 210 mg (58%, white foam) of triethylammonium salt of **5a** (*R*/*S* 41/59). HR FAB calcd for $C_{35}H_{43}N_3O_{11}PSi$ 740.2405 $(M+H)^+$, found 740.2476. ^{1}H NMR (DMSO, 500 MHz) **5a**-(***R***): δ 11.20 (1H, bs, NH), 10.05 (1H, bs, P-OH), 7.73 (1H, bs, H-6), 8.16 (1H, d, J = 7.3 Hz, Ar-H-pic), 7.05 (1H, d, J = 3.5 Hz, Ar-H-pic), 6.97 (1H, dd, J = 7.3 and 3.5 Hz, Ar-h-pic), 7.61 (4H, m Ar-H), 7.42 (6H, m, Ar-H), 6.20 (1H, dd, J(1′,2′) = 8.5, J(1′,2″) = 5.5 Hz, H-1′), 4.98 (1H, dd, J(5′,4′) = 4.5, J(5′,P) = 12.4 Hz, H-5′), 4.77 (1H, m, H-3′), 4.49 (1H, m, H-4′), 3.65 (3H, s, OCH_3), 1.95 (1H, m, H-2″), 1.90 (3H, s, OAc), 1.85 (1H, m, H-2′), 1.69 (3H, d, J(CH_3,6) = 1.2 Hz, CH_3), 1.02 (9H, s, t-But). **5a**-(***S***): δ 11.20 (1H, bs, NH), 10.05 (1H, bs, P-OH), 7.82 (1H, bs, H-6), 8.14 (1H, d, J = 7.1 Hz, Ar-H-pic), 7.02 (1H, d, J = 3.5 Hz, Ar-H-pic), 6.93 (1H, dd, J = 7.1 and 3.5 Hz, Ar-H-pic), 7.61 (4H, m, Ar-H), 7.42(6H, m, Ar-H), 6.23 (1H, dd, J(1′,2′) = 8.4, J(1′,2″) = 5.6 Hz, H-1′), 4.99 (1H, dd, J(5′,4′) = 4.4, J(5′,P) = 12.0 Hz, H-5′), 4.92 (2H, d, J(OCH,P) = 8.2 Hz, P-OCH_2-pic), 4.63 (1H, m, H-3′), 4.41 (1H, ddd, J(4′,3′) = 1.6, J(4′,5′) = 4.4, J(4′,P) = 6.2 Hz, H-4′), 3.67 (3H, s, OCH_3), 1.98 (1H, ddd, J(2″,1′) = 5.6, J(2″,2′) = 13.2, J(2″,3′) = 2.0 Hz, H-2″), 1.89 (3H, s, OAc), 1.86 (1H, m, H-2′), 1.72 (3H, d, J(CH_3,6) = 1.0 Hz, CH_3), 1.02 (9H, s, t-But).

(4-Methoxy-1-oxido-2-picolyl)-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-methoxycarbonylthymidinyl-5′-*C*-phosphonate (5c). Esterification of the monester **3c** (*R*/*S* 25/75) (280 mg of pyridinium salt, 0.39 mmol) by 4-methoxy-1-oxido-2-pyridinemethanol (67 mg, 0.43 mmol) in the presence of MPNO (148 mg, 1.18 mmol) and CDOP (218 mg, 1.18 mmol) as the condensing agent was performed in pyridine (4 mL) for 60 min according to the procedure described for compound **5a**. The crude product was purified on silica gel by elution with linear gradient of H3 in ethyl acetate followed by H1 in H3). Yield 324 mg (96%, white foam) of triethylammonium salt of **5c** (*R*/*S* 41/59). HR FAB calcd for $C_{35}H_{42}N_3NaO_{12}PSi$ 778.2173 $(M+Na)^+$, found 778.1893. ^{1}H NMR (DMSO, 500 MHz) **5c**-(***R***): δ 11.20 (1H, bs, NH, 8.11 (1H, d, J = 7.3Hz, Ar-H-pic), 8.00 (1H, bs, H-6), 7.60 (4H, M, Ar-H), 7.40 (6H, m, Ar-H), 6.99 (1H, d, J = 3.5 Hz, Ar-H-pic), 6.89 (1H, dd, J = 7.3 and 3.5 Hz, Ar-H-pic), 6.23 (1H, dd, J(1′,2′) = 8.5, J(1′,2″) = 6.0 Hz, H-1′), 4.95-4.68 (4H, m, H-3′, H-5′ and P-OCH_2-pic), 4.47 (1H, m, H-4′), 3.77 and 3.58 (2×3H, $2 \times$ s, $2 \times OCH_3$), 1.02 (9H, s, t-But). **5c**-(***S***): δ 11.20 (1H, bs, NH), 8.08 (1H, d, J = 7.1 Hz, Ar-H-pic), 7.90 (1H, bs, H-6), 7.60 (4H, m. Ar-H), 7.40 (6H, m, Ar-H), 6.98 (1H, d, J = 3.5 Hz, Ar-H-pic), 6.93 (1H, dd, J = 7.1 and 3.5 Hz, Ar-H-pic), 6.26 (1H, t, J(1′,2″) = J(1′,2″) = 7.0 Hz, H-1′), 4.95 - 4.68 (4H, m, H-3′, H-5′ and P-OCH_2-pic), 4.36

(1H, m, H-4′), 3.76 and 3.59 (2 × 3H, 2 × s, 2 × OCH_3), 1.90 and 1.64 (2 × 1H, 2 × m, H-2′ and H-2″), 1.65 (3H, bs, CH_3), 1.02 (9H, s, *t*-But).

(2′,3′-Dideoxythymidin-3′-yl)-5″-*O*-acetyl-3″-*O*-*tert*-butyldiphenylsilyl-2″-deoxythymidinyl-5″-*C*-phosphonate (8c). Triethylammonium salt of monoester **3a** (*R*/*S* 18/82) (275 mg, 0.38 mmol) and 5′-*O*-dimethoxytritylthymidine (**7a**) (416 mg, 0.76 mmol) were condensed according to **Method A**. After extraction, the solvent was evaporated and the residue treated with 60% aqueous pyridine (20 mL) at 50°C for 20 h. The solution was concentrated in vacuo, the residue co-distilled with ethanol (4 × 20 mL) and treated with Dowex 50 × 2 (pyridinium form) in 70% aqueous ethanol to remove *N*-methylpyridinium cations. Because double chromatography on silica gel (using linear gradient of H3 in ethyl acetate followed by H1 in H3) did not afford pure dimer **8b**, the crude product (461 mg) was detritylated in 80% acetic acid (100 mL) for 30 min. The acid was evaporated at low temperature, the residue co-distilled three times with water and the dimer **8c** purified on a silica gel in the solvent system mentioned above. Yield 240 mg (76%, white foam) of **8c** (*R*/*S* 9/91). HR FAB calcd for $C_{38}H_{47}N_4NaO_{13}PSi$ 849.2544 $(M + Na)^+$, found 849.2381. ^{1}H NMR data – see Table 1, 2.

(5′-*O*-*tert*-Butyldiphenylsilyl-2′,3′-dideoxythymidin-3′-yl)-5″-*O*-acetyl-3″-*O*-*tert*-butyldiphenylsilyl-2″-deoxythymidinyl-5″-*C*-phosphonate (8e). Triethylammonium salt of monoester **3a** (*R*/*S* 20/80) (448 mg, 0.62 mmol) and 5′-*O*-*tert*-butyldiphenylsilylthymidine (**7b**) (600 mg, 1.25 mmol) were condensed according to **Method A**. After extraction of the product and evaporation of the solvent, the residue was treated with 60% aqueous pyridine (20 mL) at 50°C for 17 h. The solution was concentrated *in vacuo*, the residue co-distilled with ethanol (4 × 20 mL) and treated with Dowex 50 × 2 (pyridinium form) in ethanol to remove *N*-methyl pyridinium cations. Chromatography on silica gel by elution with linear gradient of H3 in ethyl acetate followed by H1 in H3 afforded 542 mg (75%, white foam) of triethylammonium salt of **8e** (*R*/*S* 31/69). HR FAB calcd for $C_{54}H_{65}N_4NaO_{13}PSi_2$ 1087.3722 $(M + Na)^+$, found 1087.3633. ^{1}H NMR data – see Table 1, 2.

(5′-*O*-*tert*-Butyldiphenylsilyl-2′,3′-dideoxythymidin-3′-yl)-4-methoxyl-1-oxido-2-picolyl)-5″-*O*-acetyl-3″-*O*-*tert*-butyldiphenylsilyl-2″-deoxythymidinyl-5″*C*-phosphonate (8f). Triethylammonium salt of picolyl ester **5a** (*R*/*S* 41/59) (200 mg, 0.24 mmol) and 5′-*O*-*tert*-butyldiphenylsilylthymidine (7b) (180 mg, 0.36 mmol), pre-dried by co-evaporation with pyridine (2 × 15 mL), was treated with TPSCl (218 mg, 0.72 mmol) in pyridine (4 mL) under exclusion of moisture at rt. After 70 min (TLC in H1), the reaction mixture was cooled in an ice bath, quenched by addition of water (1 mL), stirred for 5 min and then partitioned between saturated solution of $NaHCO_3$ (20 mL) and chloroform (100 mL). The layers were separated by centrifugation; the organic part was washed several times with saturated solution of $NaHCO_3$, dried over anhydrous Na_2SO_4 and finally evaporated. The residue was purified by silica gel chromatography; elution with linear gradient of ethanol in chloroform. Yield 106 mg (35% white form) of **8f** (*R*/*S* 48/52). HR FAB calcd for $C_{61}H_{73}N_5O_{15}PSi_2$ 1202.4379 $(M + H)^+$, found 1202.4352.

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(2′,3′-Dideoxythymidin-3′-yl)-2″-deoxythymidinyl-5″-*C*-phosphonate (8g). Triethylammonium salt of **8e** (*R*/*S* 31/69) (500 mg, 0.43 mmol) was treated with a mixture of methanol-concentrated aqueous ammonia (2:3; 50 mL) at rt overnight (TLC in H1) and then, after evaporation of the solvents and co-distillation of the residue with ethanol (3 × 20 mL) and toluene (2 × 10 mL), with 0.5 M *tetra*-*n*-butylammonium fluoride in THF (5 mL, 2.5 mmol). The mixture was set aside for 20 h at rt under exclusion of moisture (TLC in H1 and I). After evaporation of the solvent and treatment with Dowex 50 × 2 (Et_3NH^+ form) in 50% aqueous ethanol to remove *tetra*-*n*-butylammonium cations, the deprotected product was purified on DEAE-Sephadex. Freeze-drying from water afforded 177 mg (85%, white foam) of epimer (***S***)-**8g** and 50 mg (72%, white foam) of epimer (***R***)-**8g**. HR FAB calcd for $C_{26}H_{43}N_5O_{12}P$ 648.2646 $(M + Et_3N + H)^+$, found 648.2682. ^1H and ^{13}C NMR data – see Table 1 and 2.

(5′-*O*-*tert*-Butyldiphenylsilyl-2′,3′-dideoxythymidin-3′-yl)-3″-*O*-*tert*-butyldiphenylsilyl-2″-deoxythymidinyl-5″-*C*-phosphonate (8i). Procedure 1: Triethylammonium salt of phosphonate monoester **3b** (ratio of epimers not determined) (300 mg, 0.39 mmol) and 5′-*O*-*tert*-butyldiphenylsilylthymidine (**7b**) (375 mg, 0.78 mmol) were condensed according to **Method A**. Yield 227 mg (52%) of **8h**. HR FAB calcd for $C_{58}H_{73}N_4NaO_{13}PSi_2$ 1143.4348 $(M + Na)^+$, found 1143.4457. This dimer **8h** was treated with 60% aqueous pyridine (20 mL) at 50°C overnight to remove the methyl ester protecting group (TLC in C1 and H1). The solution was concentrated in vacuo, the residue co-distilled with ethanol (4 × 20 mL) and treated with Dowex 50 × 2 (Et_3NH^+ form) in 70% aqueous ethanol to remove *N*-methylpyridinium cations. The residue was, after evaporation, treated with 0.02 M hydrochloric acid in 50% aqueous dioxane (50 mL) at rt for 1 d (TLC in H1). The reaction mixture was neutralized with Et_3N and the solvent was evaporated. The product was purified by PRPC. Yield 175 mg (40%, white foam) of triethylammonium salt of **8i** (*R*/*S* 33/67). HR FAB calcd for $C_{52}H_{63}N_4NaO_{12}PSi_2$ 1045.3616 $(M + Na)^+$, found 1045.3514. ^1H NMR data – see Table 1 and 2.

Procedure 2: Pyridinium salt of phosphonate monoester **3c** (*R*/*S* 36/64) (191 mg, 0.27 mmol) and 5′-*O*-*tert*-butyldiphenylsilylthymidine (**7b**) (260 mg, 0.54 mmol) were condensed according **Method A** for 20 min. Isolation on silica gel afforded both fully protected dimer **8a** and demethylated product **8b**. Both products were combined and treated with methanol-Et_3N-water mixture (2:5:5; 12 mL) overnight. Under these conditions, both methyl and methoxycarbonyl group were removed. The product was purified by PRPC. Yield 281 mg (93%, white foam) of triethylammonium salt of **8i** (*R*/*S* 38/62).

(2′,3′-Dideoxythymidin-3′-yl)-3″-*O*-*tert*-butyldiphenylsilyl-2″-deoxythymidinyl-5″-*C*-phosphonate (8k). Triethylammonium salt of phosphonate monoester **3b** (epimeric ratio not determined) (250 mg, 0.38 mmol) and 5′-*O*-dimethoxytritylthymidine (**7a**) (416 mg, 0.76 mmol) were condensed according to **Method A**. After extraction and evaporation, the residue was treated with 60% aqueous pyridine (20 mL) at 50°C overnight. The solution was concentrated in vacuo, the residue co-distilled with ethanol (4 × 20 mL) and treated with 0.2 M HCl in 50% aqueous dioxane (50 mL) at rt for 3 d (TLC in H1). The reaction mixture was neutralized with Et_3N and the

Table 1. Proton NMR parameters of epimers **8**.

		5′-residue									3′-residue							
		Chemical shifts																
Comp.	Solv.[a]	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	H-5″	H-6	CH_3	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	H-6	CH_3
(***S***)-**8c**[b]	**S**	6.08	2.06	(2H)	4.73	3.84	3.57	3.54	7.68	1.78	6.22	1.86	1.98	4.62	4.35	4.90	7.78	1.71
(***R***)-**8c**[b]	**S**	6.09	2.09	(2H)	4.76	3.80	[c]	[c]	7.70	1.71	6.18	1.90	2.02	4.70	[c]	5.02	7.84	1.69
(***S***)-**8e**[c,d]	**S**	6.16	2.18	2.10	4.91	4.01	3.87	(2H)	7.74	1.67	6.18	1.84	1.99	4.58	4.40	4.97	7.58	1.36
(***R***)-**8e**[c,d]	**S**	6.14	2.07	2.22	4.87	3.95	3.88	3.82	7.86	1.69	6.20	1.92	2.02	4.73	4.39	5.03	[c]	1.39
(***S***)-**8g**	**S**	6.10	2.30	(2H)	4.54	4.05	4.17	4.15	7.76	1.91	6.21	2.38	2.30	4.58	4.24	4.08	7.72	1.85
(***R***)-**8g**	**S**	6.15	[c]	[c]	4.35	4.15	4.24	4.21	7.58	1.92	6.23	[c]	[c]	4.38	4.25	4.09	7.75	1.91
(***S***)-**8g**	**W**	6.22	2.32	2.53	4.93	4.14	3.77	3.73	7.63	1.88	6.25	2.39	2.36	4.57	4.17	4.02	7.90	1.81
(***R***)-**8g**	**W**	6.33	2.42	2.55	4.94	4.21	3.86	3.81	7.70	1.90	6.30	~2.35	~2.35	4.77	4.25	4.06	7.82	1.91
(***S***)-**8i**[d]	**S**	6.14	2.06	2.18	4.90	4.02	3.85	(2H)	7.60	1.66	6.18	1.92	1.80	4.54	4.27	3.75	7.70	1.36
(***R***)-**8i**[d]	**S**	6.16	2.08	2.23	4.94	3.96	3.88	3.83	[c]	1.71	6.26	2.08	1.95	4.84	4.36	3.83	[c]	1.40
(***R***)-**8k**[d]	**S**	6.02	[c]	2.25	4.80	3.93	3.55	3.52	7.70	1.76	6.13	[c]	[c]	4.46	3.60	3.83	7.78	1.77

		Coupling constants																	
		1′,2′	1′,2″	2′,2″	2′,3′	2″,3′	3′,4′	4′,5′	4′,5″	5′,5″	H3′,P	1′,2′	1′,2″	2′,2″	2′,3′	2″,3′	3′,4′	4′,5′	H4′,P	H5′,P
(*S*)-**8c**	**S**	7.6	6.2	[c]	6.2	3.4	2.9	3.5	3.5	11.8	6.8	8.6	5.4	13.2	5.4	2.2	7.2	4.3	2.2	11.8
(*R*)-**8c**	**S**	6.8	6.8	[c]	∼7.0	−3.0	2.8	3.2	3.2	[c]	−7.0	8.8	5.6	13.2	5.0	2.0	[c]	3.5	[c]	11.8
(*S*)-**8e**	**S**	8.7	5.7	13.4	5.6	2.0	2.2	2.8	2.8	–	7.3	8.8	5.7	13.4	5.1	2.6	7.2	3.8	2.2	12.1
(*R*)-**8e**	**S**	9.3	5.1	13.6	5.8	2.0	2.4	2.4	4.0	11.5	7.0	8.5	5.7	[c]	5.0	2.0	[c]	4.4	[c]	11.5
(*S*)-**8g**	**S**	6.9	6.9	[c]	6.7	3.7	3.7	3.2	3.2	11.6	7.0	6.7	6.5	14.2	6.7	3.8	5.8	2.4	2.4	12.7
(*R*)-**8g**	**S**	6.6	6.6	[c]	6.8	2.8	2.8	4.9	3.3	11.6	6.8	6.6	6.6	[c]	6.6	4.1	4.1	2.1	3.0	12.8
(*S*)-**8g**	**W**	8.7	5.8	13.9	6.0	2.4	2.6	3.5	4.8	12.5	7.5	∼6.5	∼6.5	14.0	6.0	−5.8	∼5.8	2.45	−2.4	12.8
(*R*)-**8g**	**W**	7.8	6.2	14.3	6.5	3.2	3.2	3.4	4.7	12.6	7.5	∼6.9	−6.9	[c]	[c]	[c]	∼3.0	2.9	∼3.0	8.5
(*S*)-**8i**	**S**	8.5	5.6	13.6	5.4	2.4	2.4	2.7	2.7	–	7.3	9.0	5.6	13.7	5.4	3.0	6.0	4.0	2.5	11.2
(*R*)-**8i**	**S**	8.5	5.6	13.4	5.6	2.4	2.7	2.7	4.0	11.5	7.0	9.3	5.1	14.4	5.9	2.0	4.0	2.7	2.0	11.0
(*S*)-**8k**	**S**	6.9	5.9	13.4	6.9	3.2	2.5	3.5	3.5	–	7.1	7.1	6.5	13.6	6.9	6.6	6.3	3.8	3.8	12.0
(*R*)-**8k**	**S**	7.0	6.0	[c]	−7.0	−3.0	3.3	5.3	3.3	11.7	−7.0	7.0	7.0	[c]	6.8	6.8	6.0	[c]	3.0	11.7

[a]S = DMSO, W = D_2O.
[b]1.99 (3H, s, OAc).
[c]The value of parameter was not determined.
[d]7.61 (m, 4H, Ar-H), 7.45 (m, 6H, Ar-H), 1.02 (s, 9H, t-But).

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Table 2. ^{13}C NMR chemical shifts and coupling constants J(P,C) of epimers **8g** in D_2O.

	Carbon-13 chemical shifts										Coupling constants			
	5′-residue/3′-residue										5′-residue/3′-residue			
Comp.	C-1′	C-2′	C-3′	C-4′	C-5′	C-2	C-4	C-5	C-6	CH_3	P,C2′	P,C3′	P,C4′	P,C5′
(***S***)-**8g**	84.78	37.25	74.87	85.84	60.89	151.23	165.98	111.24	138.03	11.13	−4.7	5.9	4.7	
	84.37	38.04	70.36	85.32	67.28	151.19	165.88	110.50	136.96	11.13		11.7	−0	160.2
(***R***)-**8g**	84.67	37.73	73.91	85.68	60.70	151.45	166.16	111.20	137.36	11.16	2.5	5.9	4.5	
	84.16	38.38	69.66	86.50	68.19	151.36	166.16	111.06	137.18	11.16		−0	10.2	156.2

product was purified by PRPC. Yield 145 mg (43%, white foam) of triethylammonium salt of **8k** (*R*/*S* 20/80). HR FAB calcd for $C_{36}H_{45}N_4NaO_{12}PSi$ 807.2439 $(M + Na)^+$, found 807.2438. ^1H NMR data – see Table 1, 2.

(2′-Deoxy-5′-*O*-dimethoxytrityl-3′-thymidinyl)-(methyl)-acetoxymethylphosphonate (15). Dimethyl hydroxymethylphosphonate (1.27 g, 9.20 mmol), pre-dried by co-distillation with pyridine (2 × 15 mL), was treated with acetic anhydride (1.72 mL, 18.20 mmol) in pyridine (20 mL) at rt under exclusion of moisture until the starting compound disappeared (1 d; TLC in H3). The reaction mixture was diluted with water (13.3 mL) to obtain final concentration of pyridine 60%, heated at 50°C for 11 h and then concentrated in vacuo. The residue was co-distilled with ethanol (4 × 20 mL) and treated with Dowex 50 × 2 (H^+ form) in water to remove N-methylpyridinium cations and traces of pyridine. The suspension was filtered, the filtrate evaporated and the residue co-distilled several times with water to remove the acetic acid and then with ethanol and toluene. A solution of the acetoxy derivative **14a** in dichloromethane (50 mL) and triethylamine (1.25 mL, 9 mmol) was evaporated and the residue dried in vacuo. Triethylammonium salt of phosphonate monoester **14a** (538 mg, 2 mmol) and 5′-*O*-dimethoxytritylthymidine (**7a**) (1.30 g, 2.4 mmol) were condensed for 3 h and worked up according to **Method A**. Yield 622 mg (45%, light yellow foam) of 15 (*R*/*S* 50/50). HR FAB calcd for $C_{35}H_{39}N_2NaO_{11}P$ 717.2189 $(M + Na)^+$, found 717.2233. **15**-(***R*,S**): ^1H NMR (DMSO, 500 MHz) δ 11.39 (1H, bs, NH), 7.50 (1H, bs, H-6), 7.40 - 7.20 (9H, m, Ar-H), 6.89 (4H, m, Ar-H), 6.22 and 6.21 (1H, bs, 2 × t, J(1′,2′) = J(1′,2″) = 6.9 Hz, H-1′), 5.14 (1H, m, H-3′), 4.47 and 4.44 (2H, 2 × d, J(CH,P) = 8.5 Hz, P-CH_2), 4.14 (1H, m, H-4′), 3.74 (3H, s, OCH_3), 3.69 and 3.62 (3H, 2 × d, J(OCH,P) = 11.0 Hz, P-OCH_3), 3.27 (2H, m, H-5′ and H-5″), 2.45 (2H, m, H-2′ amd H-2″), 2.06 and 2.00 (3H, 2 × s, OAc), 1.46 (3H, bs, CH_3).

Dimethyl-3′,5′di-*O*-*tert*-butylmethylsilyl-2′-deoxythymidinyl-5′-*C*-phosphonate (17). α-Hydroxyphosphonate (**16**) (*R*/*S* 10/90) (613 mg, 1.32 mmol) and imidazole (449 mg, 6.60 mmol), pre-dried by co-distillation with DMF (2 × 20 mL), were treated with *tert*-butylchlorodimethylsilane (239 mg, 1.58 mmol) in DMF (10 mL) at rt under exclusion of moisture. After 3 d, a further portion of *tert*-butylchlorodimethylsilane (478 mg, 3.16 mmol) was added and the reaction mixture was heated at 40°C for 2 d (TLC in C1). The reaction mixture was quenched with methanol (1 mL), evaporated and the residue partitioned between 1M-TEAB (20 mL) and chloroform (100 mL). The organic layer was washed with 1M-TEAB (3 × 20 mL) and dried over anhydrous $MgSO_4$. The product was purified on silica gel (elution with linear gradient of ethanol in chloroform). Yield 290 mg (38%,white foam) of **17** (*R*/*S* 17/83). HR FAB calcd for $C_{24}H_{48}N_2O_8PSi_2$ 579.2687 $(M + H)^+$, found 579.2613. ^1H NMR (DMSO, 500 MHz) **17**-(***R***): δ 11.38 (1H, s, NH), 7.66 (1H, q, J(6,CH_3) = 1.2 Hz, H-6), 6.19 (1H, dd, J(1′,2″) = 6.6,J(1′, 2′) = 9.5 Hz, H-1′), 4.56 (1H, dt, J(3′,2′) = J(3′,4) = 4.0 Hz, J(3′,2″) = 6.6 Hz, H-3′), 4.20 (1H, dd, J(5′,4′) = 4.6, J(5′,P) = 9.3 Hz, H-5′), 3.85 (1H, dt, J(4′3′) = J(4′,5′) = 4.3 Hz, J(4′,P) = 19.3 Hz, H-4′), 3.64 (3H, d, J(P,OCH) = 10.5 Hz, P-OCH_3), 3.62 (3H, d, J(P,OCH) = 10.5 Hz, P-OCH_3), 2.31 (1H, dt, J(2″,1′) = J(2″,3′) = 6.6 Hz, J(2″,2′) = 14.2 Hz, H-2″), 2.08 (1H, m, H-2′), 1.78 (3H, d, J(CH_3,H-6) = 1.2 Hz, CH_3), 0.88

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(9H, s, *t*-But), 0.86 (9H, s, *t*-But), 0.11 (3H, s, $SiCH_3$), 0.10 (3H, s, $SiCH_3$). **17**-(***S***): 11.38 (1H, s, NH), 7.76 (1H, q, $J(6,CH_3) = 1.2$ Hz, H-6), 6.17 (1H, dd, $J(1',2') = 9.3$, $J(1',2'') = 5.4$ Hz, H-1′), 4.43 (1H, dd, $J(5',4') = 3.2$, $J(5',P) = 10.7$ Hz, H-5′), 4.43 (1H, bd, $J(3',2'') = J(3',4') = 1.0$, $J(3',2') = 5.0$ Hz, H-3′), 4.10 (1H, bt, $J(4',5') = J(4',P) = 3.2$ Hz, H-4′), 3.68 (3H, d, $J(P,OCH) = 10.5$ Hz, P-OCH_3), 3.63 (3H, d, $J(P,OCH) = 10.5$ Hz, P-OCH_3), 2.11 (1H, bdd, $J(2'',1') = 5.4$, $J(2'',3') = 1.0$ Hz, $J(2'',2') = 13.2$ Hz, H-2″), 2.02 (1H, ddd, $J(2',3') = 4.9$, $J(2',1') = 9.3$, $J(2',2'') = 13.2$ Hz, H-2′), 1.77 (3H, d, $J(CH_3,H\text{-}6) = 1.2$ Hz, CH_3), 0.88 (9H, s, *t*-But), 0.87 (9H, s, *t*-But), 0.16 (3H, s, $SiCH_3$), 0.11 (3H, s, $SiCH_3$)

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