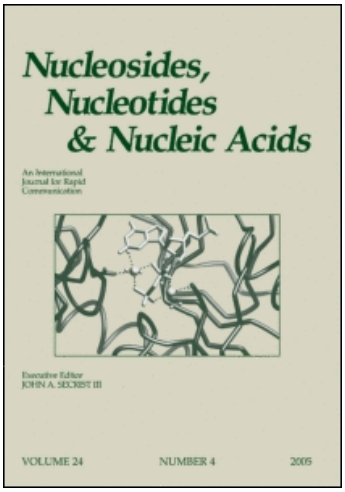

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Extension of the Applicability of δJ-Values for the Configurational Assignment of Diastereomeric Phosphate-Modified Dideoxynucleotides

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EXTENSION OF THE APPLICABILITY OF ΔJ-VALUES FOR THE CONFIGURATIONAL ASSIGNMENT OF DIASTEREOMERIC PHOSPHATE-MODIFIED DIDEOXYNUCLEOTIDES

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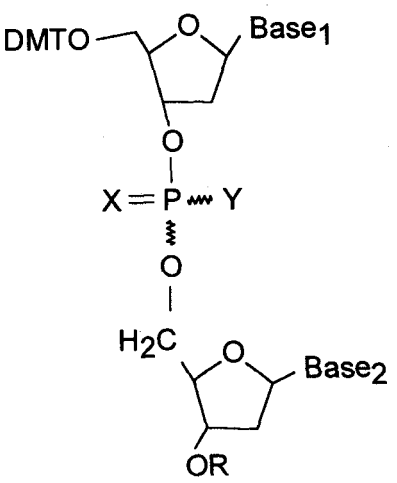
ABSTRACT

The stereochemical assignment of dinucleoside-S-p-nitrobenzyl-phosphorothioates by NMR spectroscopy is reported. It was found that the method based on the difference of the vicinal phosphorus-carbon coupling constants ($\Delta J = {}^3J_{C4',P} - {}^3J_{C2',P}$) can widely be applied for the determination of the configuration at the pohosphorus atom in phosphate-modified dideoxynucleotides.

INTRODUCTION

Phosphate-modified dideoxynucleotides are constituents of antisense oligo-deoxynucleotides that are potential therapeutic agents against viral deseases[1]. When synthesized, these compounds occur as mixtures of diastereomers due to the chiral phosphorus atom. Recently we have found that the configurational assignment of the two isomers can be readily deduced from the observed phosphorus-carbon coupling constants ($^3J_{C,P}$)[2,3]. Specifically, we have shown for some mono- and dinucleoside-phosphorothioates, mono- and dinucleoside-phosphoramidates and hydrogene-phosphonates[2,3] that the values of $\Delta J = {}^3J_{C4',P} - {}^3J_{C2',P}$ are larger in the isomers with R_P-configured phosphorus atom than those of the S_P diastereomers (see FIG. 1 for the corresponding dinucleoside phosphoramidates **1a,b** and phosphorothioates **2a,b**). We



1a, 1b X:O, Y:HN-Bu, $Base_1$, $Base_2$:Thymine. R:$(CH_3)_2Si\text{-}C(CH_3)_3$

2a, 2b X:S, Y:$OCH_2\text{-}CH_2\text{-}CN$, $Base_1$, $Base_2$:Thymine. R:$(CH_3)_2Si\text{-}C(CH_3)_3$

3a, 3b X:O, Y:$S\text{-}CH_2\text{-}C_6H_4\text{-}NO_2$(p), $Base_1$:Adenine, $Base_2$:Cytosine. R:H

4a, 4b X:O, Y:$S\text{-}CH_2\text{-}C_6H_4\text{-}NO_2$(p), $Base_1$:Cytosine, $Base_2$:Guanine. R:H

5a, 5b X:O, Y:$S\text{-}CH_2\text{-}C_6H_4\text{-}NO_2$(p), $Base_1$:Cytosine, $Base_2$:Guanine. R:$H\text{-}P(O)O^{(-)}{}^{(+)}NH(C_2H_5)_3$

6a, 6b X:O, Y:$S\text{-}CH_2\text{-}C_6H_4\text{-}NO_2$(p), $Base_1$:Adenine, $Base_2$:Cytosine, R:$H\text{-}P(O)O^{(-)}{}^{(+)}NH(C_2H_5)_3$

FIG. 1

interpreted this phenomenon as a result of the different preference of conformers in the diastereomers. However, the conformational properties are influenced, among other parameters, by the presence and nature of different substituents. Unfortunately, the meagre available $^{13}C\text{-}^{31}P$ data for diastereomeric modified nucleotide analogues have prevented generalization of the use of ΔJ values for configurational determination. Therefore we extended our studies on dinucleoside-S-p-nitrobenzyl-phosphorothioates (**3a-6b**, see FIG. 1). The side-chain and base-pairs differ in these compounds and also from those of the previously studied compounds. Therefore the new results when combined with the previous ones can afford expansive use of the ΔJ-method for the configurational assignment of diastereomeric phosphate-modified dideoxynucleotides.

RESULTS AND DISCUSSION

Enantiomerically pure dinucleoside-phosphorothioates were isolated by column chromatography. The assignment of the ^{1}H and ^{13}C spectra was achieved by decoupling, HETCOR and T-ROESY experiments. The spectral data are given in the 'Experimental'. In the case of the hydrogen-phosphonates (**5a-6b**) full assignment of the ^{1}H spectra was not possible due to the fast decomposition of these compounds in solution (^{13}C experiments, made from fresh solutions, were not suffering from signal overlapping).

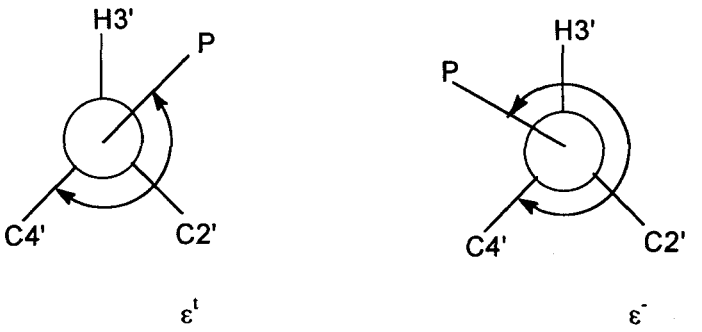
Preliminary information concerning the configuration of the phosphorus atom was obtained by comparing the chromatographical mobility of the corresponding diastereomeric 3''-levuline-protected phosphorothioates[7], the intermediates formed during the synthesis of the target compounds (see 'Experimental'). The final configurational assignment was achieved by T-ROESY experiments and by vicinal ^{13}C-^{31}P couplings. In the ROESY spectrum of compound **4b** the SCH_2 protons showed crosspeaks with $H_{3'}$ and $H_{4'}$ protons whereas in case of compound **4a** the SCH_2 gave crosspeaks with $H_{2'\alpha}$ and $H_{3'}$ protons. These results are in agreement with those of the diastereomeric methylphosphonates interpreted by Löschner et al[4] and allowed the determination of the absolute configuration of the phosphorus.

The spectral parameters which we found characteristic for the configuration of the phosphorus atom are listed in TABLE 1 together with the data of some of the previously studied dinucleoside-phosphorothioates and -phosphoramidates. It is important to note that according to the CIP rules, in the S-p-nitrobenzyl-phosphorothioates, compared to phosphorothioates and phosphoramidates, reversed configuration means similar sterical arrangement.

TABLE 1 clearly shows that the ΔJ value is diagnostic for the configuration of the phosphorus, i.e. $\Delta J_{SP} > \Delta J_{RP}$. This finding is readily explained by considering the different preference of conformers in the two diastereomers along the C3'-O3' bond. The two main rotamers are shown in FIG. 2. ^{3}J values in general monitor the corresponding torsion angles, according to the Karplus equation. As it was already discussed by Altona[5] for dinucleotides, while $^{3}J_{H3',P}$ is not expected to show much variation, $^{3}J_{C4',P}$ has large value in the ε^{t} (trans) conformer, and small value in the ε^{-} (gauche(-)) conformer, $^{3}J_{C2',P}$

TABLE 1.

Comp.	$^3J_{C4',P}$ [Hz]	$^3J_{C2',P}$ [Hz]	ΔJ [Hz]	$^4J_{H2b',P}$ [Hz]	$^3J_{H3',P}$ [Hz]	$^2J_{C3',P}$ [Hz]	δ(^{31}P) [ppm]	Config.	Solvent	Temp. [°C]
1a	5.1	4.5	0.6	1.5	7.0	4.7	9.69	S_P	$CDCl_3$	22
1b	7.3	2.7	4.6	<1	7.0	4.4	9.43	R_P	$CDCl_3$	22
2a	6.0	5.5	0.5	1.5	9.4	4.5	67.93	S_P	$CDCl_3$	22
	6.8	4.3	2.5	1.1					C_6D_6	50
2b	6.8	4.8	2.0	1.2	9.4	4.3	67.89	R_P	$CDCl_3$	22
	7.2	3.9	3.3	0.9					C_6D_6	50
3a	4.7	4.3	0.4	1.2	7.9	6.1	28.05	R_P	$CDCl_3$	22
	4.9	4.5	0.4	0.9	8.2	5.7			C_6D_6+DMSO-d_6	22
	4.9	4.9	0.0	0.9	8.4	6.2			C_6D_6+DMSO-d_6	50
3b	7.4	2.7	4.7	<0.8	8.0	5.3	28.37	S_P	$CDCl_3$	22
	7.3	n.d.	n.d.	<0.8	7.9	5.2			C_6D_6+DMSO-d_6	22
	7.3	2.9	4.4	<0.8	7.9	5.7			C_6D_6+DMSO-d_6	50
4a	7.0	3.0	4.0	0.9	7.7	6.9	27.03	R_P	$CDCl_3$	22
	5.7	4.4	1.3	1.2	8.2	6.2			C_6D_6+CD_3OD	22
	5.8	4.6	1.2	1.2	8.2	6.3			C_6D_6+CD_3OD	50
4b	8.6	3.0	5.6	<0.8	7.2	6.6	28.23	S_P	$CDCl_3$	22
	8.3	2.3	6.0	<0.8	7.7	6.1			C_6D_6+CD_3OD	22
	8.1	2.6	5.5	<0.8	7.7	6.1			C_6D_6+CD_3OD	50
5a	5.4	4.3	1.1	1.2	8.2	6.3		R_P	$CDCl_3$	22
	5.5	4.6	0.9	1.3	8.2	6.1	27.13		C_6D_6+CD_3OD	22
5b	7.0	n.d.	n.d.	<0.8	n.d.	6.5		S_P	$CDCl_3$	23
	6.2	3.7	2.5	0.8	7.9	6.4	27.54		C_6D_6+CD_3OD	22
	6.2	3.7	2.5	0.8	8.1	6.8			C_6D_6+CD_3OD	50
6a	5.0	5.4	-0.4	1.0	8.1	6.3	27.70	R_P	C_6D_6+DMSO-d_6	50
6b	7.1	n.d.	n.d.	<0.5	n.d.	5.2		S_P	$CDCl_3$	22
	7.0	2.3	4.7	<0.5	8.0	5.5	27.84		C_6D_6+DMSO-d_6	22
	7.1	2.6	4.5	<0.5	8.0	5.8			C_6D_6+DMSO-d_6	50



ε^t ε^-

FIG. 2

displays opposite behaviour. Therefore, large ΔJ values correspond to the predominance of the ε^t conformer. Consequently, in the compounds of the present study for the S_P isomers the ratio of the ε^t conformer is dominant, while for the R_P isomers the ratio of the ε^- conformer increases.

Decomposition and signal-broadening in deuterochloroform solution in some cases prevented the exact determination of the relevant coupling constants (denoted with n.d. in Table I). The ^{13}C spectra run in deuterobenzene solutions and at elevated temperature afforded sharp doublets, thus the more accurate measurement of ^{13}C-^{31}P couplings. (One drop of CD_3OD or DMSO-d_6 was added to enhance the solubility.) Change of solvent and temperature both modify the conformational equilibrium about the C3'-O3' bond, consequently the vicinal couplings. It is to be noted, however, that even under altered conditions the isomers maintain their characteristic differences in the ΔJ values. These differences ($\Delta\Delta J$ values) were generally between 4-4.9 Hz. The possible explanation for the small ΔJ values in both diastereomers of thioates **2a** and **2b** is connected with the fact that in these molecules the phosphorus-sulfur bond has double bond character, while in all the other compounds of Table 1 the double bond is between the phosporus and oxygen atoms. The smaller ΔJ (and consequently the smaller $\Delta\Delta J$) values in **2a** and **2b** were interpreted by a substantial increase in the amount of the ε^- relative to the ε^t conformer[3].

For RNA model compounds a distinct base-sequence dependency of the magnitude of ε^t was observed, while for deoxynucleotides such effect was found only for the py-pu type (C-G) dinucleotide[5,10]. For the cases studied here this type of dependency is

expected to be influenced by the absolute configuration and solvent effect, since the extent of base overlap differs in the isomers. Aromatic solvent and the phenyl ring in the side chain both affect the base-stacking procedure, consequently the conformational equilibrium. It is worth mentioning that the only noteworthy difference upon changing the solvent was observed in the vicinal couplings of **4a** (also a py-pu type dimer).

The increased ratio of the ε^- conformer in the Rp isomers in comparison with the Sp isomers is also reflected in the coupling pattern of $H_{2'\beta}$. As it was demonstrated for oligonucleotides by Blommers et al[6] the four-bond coupling constant $^4J_{H2'\beta,P}$ is only resolved for the ε^- conformation where the four bonds involved are coplanar and in zig-zag (W) arrangement. In the compounds of the present study this coupling is not detectable or smaller for the S_P than for the R_P isomer.

The $^2J_{C3',P}$ coupling constants of the diastereomers show characteristic differences for compounds containing adenosine-cytosine base-pair (i.e. this coupling constant is larger in the R_P isomer than in the S_P). Moreover, in all compounds the ^{31}P chemical shift of the S_P isomer is larger than that of the R_P isomer. For the interpretation of these findings detailed conformational analysis is in progress to get insight into the presumable differences in the rotational behaviour of the diastereomers concerning the P-O3' bonds.

In summary, we have demonstrated that similarly to the ^{31}P chemical shift value, $^4J_{H2'\beta,P}$ value and the geminal phosphorus-carbon coupling constant $^2J_{C3',P}$, the difference of the vicinal phosphorus-carbon coupling constants ($\Delta J = {}^3J_{C4',P} - {}^3J_{C2',P}$) reflect characteristically the configuration of the phosphorus atom in dinucleoside-S-p-nitrobenzyl-phosphorothioates. Although, similarly to all the previously studied diastereomeric modified nucleotides[2,3] the vicinal ^{13}C-^{31}P coupling constants show variation with temperature and solvent, the ΔJ value remains diagnostic for the configuration of the phosphorus, i.e. $\Delta J_{SP} > \Delta J_{RP}$. Therefore we conclude that the determination of the ΔJ is a reliable and widely applicable method for the configurational assignment of diastereomeric phosphate-modified dideoxynucleotides.

EXPERIMENTAL

General. ^1H, ^{13}C and ^{31}P spectra were recorded in $CDCl_3$ and C_6D_6+DMSO-d_6 or C_6D_6+CD_3OD solutions at 295K and 323K using a Varian VXR 400 spectrometer.

Chemical shifts were referenced to internal TMS (^{1}H spectra), to external H_3PO_4 (^{31}P spectra), to DMSO (^{13}C spectra in C_6D_6+DMSO solution) or to CD_3OD (^{13}C spectra in C_6D_6+CD_3OD solution).

The T-ROESY experiments were run on a Varian Unity.plus (500MHz) spectrometer using 450 msec mixing time.

Materials.

*Preparation of compounds **3a,3b,4a,4b**.* The corresponding 3''-levuline-protected phosphorothioates were prepared via dinucleoside H-phosphonates with sulfurization with elementar sulfur in pyridine-CS_2 (1:1)[7]. Diastereomerically pure R_P and S_P compounds formed in 1:1 ratio were separated by coloumn chromatography on silica gel eluting with [chloroform +triethylamine(1%) + methanol (0-10%)]. The separated isomers were deprotected with hydrazine hydrate-pyridine-acetic acid[8] to give the 3''-deprotected phosphorothioates after silica gel coloumn chromatography with chloroform +triethylamine(1%) + methanol (0-10%). To the dry dichloromethane (40ml) solution of the the 3''-deprotected phosphorothioates (1 mmol) pyridine (10 mmol) and 4-nitrobenzyl bromide (2 mmol) were added. The reaction mixture was stirred at room temperature overnight {TLC: [chloroform + triethylamine (1%)]:methanol=9:1} then cooled in the refrigerator. The solid was filtered and washed with dichloromethane (2x5 ml) and the mother liquor was evaporated to dryness. The residue was purified on silica gel coloumn using {[chloroform+triethylamine(1%)]:methanol=30:1} as eluting agent. Yields: **3a** (67%), **3b** (70%), **4a** (59%), **4b** (60%).

NMR data.

3a: ^{1}H NMR ($CDCl_3$, 65°C): δ 2.202 ppm (1H, J_{gem}= -14.0, $J_{2'',\beta,3''}$=6.7, $J_{1'',2''\beta}$=5.7 Hz; $H_{2''\beta}$) , 2.635 (1H, J_{gem}= -14.0, $J_{1'',2''\alpha}$=6.5, $J_{2''\alpha,3''}$=5.0 Hz; $H_{2''\alpha}$), 2.699 (1H, J_{gem}= -14.2, $J_{1',2'\alpha}$=5.8, $J_{2'\alpha,3'}$=2.1 Hz; $H_{2'\alpha}$), 3.108 (1H, J_{gem}= -14.2, $J_{1',2'\beta}$ =8.2, $J_{2'\beta,3'}$=5.6, $J_{2'\beta,P}$=1.5 Hz; $H_{2'\beta}$) , 3.405 (1H, J_{gem}=-10.5, $J_{4',5'A}$=4.5 Hz; $H_{5'A}$), 3.462 (1H, J_{gem}=-10.5, $J_{4',5'B}$=5.0 Hz; $H_{5'B}$), 4.149 (1H, $J_{3'',4''}$=4.6, $J_{4'',5''B}$=4.3, $J_{4'',5''A}$=3.5, $J_{4'',P}$=1.5 Hz; $H_{4''}$), 4.16 (2H, SCH_2), 4.264 (1H, J_{gem}=-11.4, $J_{5''A,P}$=7.4, $J_{4'',5''A}$=3.5 Hz; $H_{5''A}$), 4.365 (1H, J_{gem}=-11.4, $J_{5''A,P}$=8.5, $J_{4'',5''B}$=4.3 Hz; $H_{5''B}$), 4.385 (1H, $J_{2'',\beta,3''}$=6.7, $J_{2''\alpha,3''}$=5.0,

$J_{3'',4''}$=4.6 Hz; $H_{3''}$), 4.437 (1H, $J_{4',5'B}$=5.0, $J_{4',5'A}$=4.5, $J_{3',4'}$=2.0 Hz; $H_{4'}$), 5.333 (1H, $J_{3',P}$=8.4, $J_{2'\beta,3'}$=5.6, $J_{2'\alpha,3'}$=2.1, $J_{3',4'}$=2.0 Hz; $H_{3'}$), 6.187 (1H, $J_{1'',2''\alpha}$ =6.5, $J_{1'',2''\beta}$ =5.7 Hz; $H_{1''}$), 6.421 (1H, $J_{1',2'\beta}$ =8.2, $J_{1',2'\alpha}$=5.8 Hz; $H_{1'}$), 7.498 (1H, $J_{C5,C6}$=7.4 Hz; H_{C5}), 7.987 (1H, $J_{C5,C6}$=7.4 Hz; H_{C6}), 8.152 (1H, s; H_{A2}), 8.623 (1H, s; H_{A8}).
^{13} C NMR data ($CDCl_3$, 22°C): δ 34.43 ppm ($J_{C,P}$=4.0 Hz; SCH_2), 37.94 ($J_{C,P}$=4.3 Hz; $C_{2'}$), 79.46 ($J_{C,P}$=6.1 Hz; $C_{3'}$), 84.85 ($J_{C,P}$=7.5 Hz; $C_{4''}$), 85.03 ($J_{C,P}$=4.7 Hz; $C_{4'}$).

3b: ^{1}H NMR ($CDCl_3$, 65°C): δ 2.251 ppm (1H, J_{gem}= -14.4, $J_{2'',3''}$=6.7, $J_{1'',2''\beta}$ =5.5 Hz; $H_{2''\beta}$) , 2.644 (1H, J_{gem}= -14.4, $J_{1'',2''\alpha}$=6.6, $J_{2''\alpha,3''}$=5.3 Hz; $H_{2''\alpha}$), 2.808 (1H, J_{gem}= -14.3, $J_{1',2'\alpha}$=5.8, $J_{2'\alpha,3'}$=2.1 Hz; $H_{2'\alpha}$), 3.149 (1H, J_{gem}= -14.3, $J_{1',2'\beta}$=8.2, $J_{2'\beta,3'}$=5.7 Hz; $H_{2'\beta}$) , 3.366 (1H, J_{gem}=-10.4, $J_{4',5'A}$=4.6 Hz; $H_{5'A}$), 3.447 (1H, J_{gem}=-10.4, $J_{4',5'B}$=5.7 Hz; $H_{5'B}$), 4.143 (1H, $J_{3'',4''}$=5.1, $J_{4'',5''B}$=4.1, $J_{4'',5''A}$=3.3, $J_{4'',P}$=1.5 Hz; $H_{4''}$), 4.13 (2H, m, SCH_2), 4.306 (1H, J_{gem}=-11.4, $J_{5''A,P}$=8.3, $J_{4'',5''A}$=3.3 Hz; $H_{5''A}$), 4.337 (1H, $J_{4',5'B}$=5.7, $J_{4',5'A}$=4.6, $J_{3',4'}$=2.1 Hz; $H_{4'}$), 4.357 (1H, J_{gem}=-11.4, $J_{5''A,P}$=8.5, $J_{4'',5''B}$=4.1 Hz; $H_{5''B}$), 4.441 (1H, $J_{2''\beta,3''}$=6.7, $J_{2''\alpha,3''}$=5.3, $J_{3'',4''}$=5.1 Hz; $H_{3''}$), 5.374 (1H, $J_{3',P}$=7.9, $J_{2'\beta,3'}$=5.7, $J_{2'\alpha,3'}$=2.0, $J_{3',4'}$=2.1 Hz; $H_{3'}$), 6.201 (1H, $J_{1'',2''\alpha}$=6.6, $J_{1'',2''\beta}$=5.3 Hz; $H_{1''}$), 6.430 (1H, $J_{1',2'\beta}$=8.2, $J_{1',2'\alpha}$=5.8 Hz; $H_{1'}$), 7.45 (1H, $J_{C5,C6}$=7.4 Hz; H_{C5}), 8.040 (1H, $J_{C5,C6}$=7.4 Hz; H_{C6}), 8.152 (1H, s; H_{A2}), 8.592 (1H, s; H_{A8}).
^{13} C NMR data ($CDCl_3$, 22°C): δ 34.47 ppm ($J_{C,P}$=3.9 Hz; SCH_2), 37.94 ($J_{C,P}$=2.7 Hz; $C_{2'}$), 79.48 ($J_{C,P}$=5.3 Hz; $C_{3'}$), 84.76 ($J_{C,P}$=7.1 Hz; $C_{4''}$), 84.68 ($J_{C,P}$=7.4 Hz; $C_{4'}$).

4a: ^{1}H NMR ($CDCl_3$, 65°C): δ 2.256 ppm (1H, J_{gem}= -14.4, $J_{1',2'\beta}$ =7.9, $J_{2'\beta,3'}$=5.5, $J_{2'\beta,P}$=1.0 Hz; $H_{2'\beta}$), 2.436 (1H, J_{gem}= -13.7, $J_{1'',2''\alpha}$ =7.1, $J_{2''\alpha,3''}$=4.1 Hz; $H_{2''\alpha}$), 2.922 (1H, J_{gem}= -13.7, $J_{2''\beta,3''}$=6.4, $J_{1'',2''\beta}$ =6.4 Hz; $H_{2''\beta}$) , 2.984 (1H, J_{gem}= -14.4, $J_{1',2'\alpha}$ =5.5, $J_{2'\alpha,3'}$=2.4 Hz; $H_{2'\alpha}$), 3.350 (1H, J_{gem}=-10.8, $J_{4',5'A}$=3.6 Hz; $H_{5'A}$), 3.411 (1H, J_{gem}=-10.8, $J_{4',5'B}$=3.6 Hz; $H_{5'B}$), 4.039 (2H, $J_{CH2\text{-}P}$=15.2 Hz; SCH_2), 4.19-4.21 (2H, $H_{4''}$, $H_{5''A}$), 4.319 (1H, $J_{4',5'B}$=3.6, $J_{4',5'A}$=3.6, $J_{3',4'}$=2.2 Hz; $H_{4'}$), 4.69 (1H; $H_{5''B}$), 4.873 (1H, $J_{2''\beta,3''}$=6.4, $J_{2''\alpha,3''}$=4.1, $J_{3'',4''}$=3.2 Hz; $H_{3''}$), 5.105 (1H, $J_{3',P}$=7.7, $J_{2'\beta,3'}$=5.5, $J_{2'\alpha,3'}$=2.4, $J_{3',4'}$=2.2 Hz; $H_{3'}$), 6.209 (1H, $J_{1',2'\beta}$ =7.9, $J_{1',2'\alpha}$ =5.5 Hz; $H_{1'}$), 6.208 (1H, $J_{1'',2''\alpha}$ =7.1, $J_{1'',2''\beta}$ =6.4 Hz; $H_{1''}$), 7.495 (1H, $J_{C5,C6}$=7.5 Hz; H_{C5}), 7.744 (1H, s; H_{G8}), 8.034 (1H, $J_{C5,C6}$=7.5 Hz; H_{C6}).

^{13} C NMR data ($CDCl_3$, 22°C): δ 33.90 ppm ($J_{C,P}$=3.8 Hz; SCH_2), 40.05 ($J_{C,P}$=3.0 Hz; $C_{2'}$), 79.48 ($J_{C,P}$=6.9 Hz; $C_{3'}$), 84.84 ($J_{C,P}$=8.8 Hz; $C_{4''}$), 85.21 ($J_{C,P}$=7.0 Hz; $C_{4'}$).

4b: ^{1}H NMR ($CDCl_3$, 65°C): δ 2.249 ppm (1H, J_{gem}= -14.3, $J_{1',2'\beta}$ =7.6, $J_{2'\beta,3'}$=5.7 Hz; $H_{2'\beta}$), 2.454 (1H, J_{gem}= -13.7, $J_{1'',2''\alpha}$ =6.7, $J_{2''\alpha,3''}$=4.6 Hz; $H_{2''\alpha}$), 2.775 (1H, J_{gem}= -13.7, $J_{2''\beta,3''}$=6.3, $J_{1'',2''\beta}$ =6.3 Hz; $H_{2''\beta}$), 2.942 (1H, J_{gem}= -14.3, $J_{1',2'\alpha}$ =5.6, $J_{2'\alpha,3'}$=2.4 Hz; $H_{2'\alpha}$), 3.325 (1H, J_{gem}=-10.7, $J_{4',5'A}$=3.6 Hz; $H_{5'A}$), 3.379 (1H, J_{gem}=-10.7, $J_{4',5'B}$=3.7 Hz; $H_{5'B}$), 3.979 (2H, $J_{CH2\text{-}P}$=15.4 Hz; SCH_2), 4.208 (1H, $J_{4',5'B}$=3.7, $J_{4',5'A}$=3.6, $J_{3',4'}$=2.4 Hz; $H_{4'}$), 4.208 (1H, $J_{4'',5''B}$=4.9, $J_{4'',5''A}$=4.1, $J_{3'',4''}$=3.9, $J_{4'',P}$=1.2 Hz; $H_{4''}$), 4.292 (1H, J_{gem}=-11.0, $J_{5''A,P}$=7.4, $J_{4'',5''A}$=4.1 Hz; $H_{5''A}$), 4.358 (1H, J_{gem}=-11.0, $J_{5''A,P}$=6.9, $J_{4'',5''B}$=4.9 Hz; $H_{5''B}$), 4.706 (1H, $J_{2''\beta,3''}$=6.3, $J_{2''\alpha,3''}$=4.6, $J_{3'',4''}$=3.9 Hz; $H_{3''}$), 5.125 (1H, $J_{3',P}$=7.2, $J_{2'\beta,3'}$=5.7, $J_{2'\alpha,3'}$=2.4, $J_{3',4'}$=2.4 Hz; $H_{3'}$), 6.172 (1H, $J_{1',2'\beta}$ =7.6, $J_{1',2'\alpha}$ =5.6 Hz; $H_{1'}$), 6.190 (1H, $J_{1'',2''\alpha}$ =6.7, $J_{1'',2''\beta}$ =6.3 Hz; $H_{1''}$), 7.30 (1H, $J_{C5,C6}$=7.5 Hz; H_{C5}), 7.821 (1H, s; H_{G8}), 8.011 (1H, $J_{C5,C6}$=7.5 Hz; H_{C6}).
^{13} C NMR data ($CDCl_3$, 22°C): δ 33.94 ppm ($J_{C,P}$=3.8 Hz; SCH_2), 40.51 ($J_{C,P}$=3.0 Hz; $C_{2'}$), 78.64 ($J_{C,P}$=6.7 Hz; $C_{3'}$), 84.75 ($J_{C,P}$=8.6 Hz; $C_{4''}$), 84.95 ($J_{C,P}$=8.6 Hz; $C_{4'}$).

Preparation of H-phosphonates. **5a**,**5b**,**6a** and **6b** were prepared by the method of Sakatsume[9]. Dinucleoside S-p-nitrobenzyl ethers **3a**,**3b**,**3c** and **3d** (0.25 mmol) were dissolved in dry dichloromethane (2.5 ml) then triethylamine (1.5 mmol) and tris-(1,1,1,3,3,3-hexafluoro-2-propyl)phosphite (0.5 mmol) were added. After 2 hours of stirring at room temperature 1 M triethylammonium bicarbonate (10 ml) was added to the reaction mixture and stirring was continued for additional 30 min. The reaction mixture was extracted with dichloromethane (2x3 ml), dried (Na_2SO_4) and evaporated. The residue was dissolved in dichloromethane (1 ml) and ether (15 ml) was added to precipitate the crude product. Crude products were purified on silica gel coloumn eluting with chloroform +triethylamine(1%) + methanol (0-10%). The appropriate fractions were washed with 1 M triethylammonium bicarbonate and dried (Na_2SO_4). **5a** (70%), **5b** (68%), **6a** (70%) and **6b** (79%) were obtained after evaporation of the solvent.

NMR data

5a: ^{1}H NMR (C_6D_6+CD_3OD, 22°C): δ 2.256 ppm (1H, J_{gem}= -14.5, $J_{1',2'\beta}$ =6.8,

$J_{2',\beta,3'}$=5.6, $J_{2',\beta,P}$=1.3 Hz; $H_{2',\beta}$), 2.59 (1H, J_{gem}= -13.8, $J_{1'',2''\alpha}$ =7.4, $J_{2''\alpha,3''}$=5.6 Hz; $H_{2''\alpha}$), 2.838 (1H, J_{gem}= -14.4, $J_{1',2'\alpha}$ =6.0, $J_{2'\alpha,3'}$=3.1 Hz; $H_{2'\alpha}$), 2.923 (1H, J_{gem}= -13.8, $J_{1'',2''\beta}$ =5.3, $J_{2''\beta,3''}$=7.2 Hz; $H_{2''\beta}$), 3.4 (in $CDCl_3$, 22°C) (2H, m; $H_{5'A}$, $H_{5'B}$), 3.88 (2H, m; SCH_2), 4.372 (in $CDCl_3$, 22°C) (1H, $J_{4'',5''B}$=2.8, $J_{4'',5''A}$=2.8, $J_{3'',4''}$=2.8 Hz; $H_{4''}$), 4.47 (1H, m; $H_{4'}$), 4.66 (2H, m; $H_{5''}$), 5.229 (1H, $J_{3',P}$=8.2, $J_{2',\beta,3'}$=5.6, $J_{2',\alpha,3'}$=3.1, $J_{3',4'}$=2.9 Hz; $H_{3'}$), 5.372 (1H, m; $H_{3''}$), 6.112 (1H, $J_{1'',2''\alpha}$ =7.4, $J_{1'',2''\beta}$ =5.3 Hz; $H_{1''}$), 6.219 (1H, $J_{1',2'\beta}$ =6.8, $J_{1',2'\alpha}$ =6.0 Hz; $H_{1'}$), 7.167 (1H, $J_{H,P}$=625.2; H(P)), 7.532 (1H, $J_{C5,C6}$=7.5 Hz; H_{C5}), 7.809 (1H, s; H_{G8}), 8.034 (1H, $J_{C5,C6}$=7.5 Hz; H_{C6}).

^{13}C NMR data (C_6D_6+DMSO-d_6, 50°C): δ 33.55 ppm ($J_{C,P}$=3.7 Hz; SCH_2), 38.33 ($J_{C,P}$=3.8 Hz; $C_{3''}$), 39.21 ($J_{C,P}$=4.1 Hz; $C_{2'}$), 67.11 ($J_{C,P}$=6.2 Hz; $C_{5''}$), 72.86 ($J_{C,P}$=4.2 Hz; $C_{3''}$), 77.92 ($J_{C,P}$=6.1 Hz; $C_{3'}$), 84.00 (dd; $C_{4''}$), 84.67 ($J_{C,P}$=5.6 Hz; $C_{4'}$).

5b: ^{1}H NMR (C_6D_6+DMSO-d_6, 50°C): δ 2.313 ppm (in C_6D_6+CD_3OD, 22°C) (1H, m, $H_{2',\beta}$), 2.617 (in C_6D_6+CD_3OD, 22°C) (1H, J_{gem}= -14.0, $J_{1'',2''\alpha}$ =7.2, $J_{2''\alpha,3''}$=5.3 Hz; $H_{2''\alpha}$), 2.826 (1H, J_{gem}= -14.5, $J_{1',2'\alpha}$ =6.0, $J_{2'\alpha,3'}$=2.3 Hz; $H_{2'\alpha}$), 3.546 (2H, $J_{4',5'}$=4.2 Hz; $H_{5'}$), 4.059 (2H, J_{CH2-P}=14.8 Hz; SCH_2), 4.476 (1H, $J_{4'',5''B}$=4.2, $J_{4'',5''A}$=4.2, $J_{3'',4''}$=3.3 Hz; $H_{4''}$), 4.50-4.60 (3H, $H_{4'}$, $H_{5''}$), 5.29 (1H, m; $H_{3''}$), 5.322 (1H, $J_{3',P}$=7.9, $J_{2',\beta,3'}$=5.7, $J_{2',\alpha,3'}$=2.4, $J_{3',4'}$=2.0 Hz; $H_{3'}$), 6.420 (1H, $J_{1'',2''\alpha}$ =6.5, $J_{1'',2''\beta}$ =6.5 Hz; $H_{1''}$), 6.480 (1H, $J_{1',2'\beta}$ =7.7, $J_{1',2'\alpha}$ =6.0 Hz; $H_{1'}$), 7.215 (1H, $J_{H,P}$=608.6; H(P)), 7.685 (in C_6D_6+CD_3OD, 22°C) (1H, $J_{C5,C6}$=7.6 Hz; H_{C5}), 8.110 (1H, $J_{C5,C6}$=7.5 Hz; H_{C6}), 8.321 (1H, s; H_{G8}).

^{13}C NMR data (C_6D_6+CD_3OD, 50°C): δ 34.20 ppm ($J_{C,P}$=3.9 Hz; SCH_2), 38.66 ($J_{C,P}$=3.7 Hz; $C_{2''}$), 40.44 ($J_{C,P}$=3.7 Hz; $C_{2'}$), 67.55 ($J_{C,P}$=6.6 Hz; $C_{5''}$), 73.05 ($J_{C,P}$=4.6 Hz; $C_{3''}$), 79.73 ($J_{C,P}$=6.8 Hz; $C_{3'}$), 84.63 ($J_{C,P}$=8.6 and 5.1 Hz; $C_{4''}$), 87.25 ($J_{C,P}$=6.2 Hz; $C_{4'}$).

6a: ^{1}H NMR (C_6D_6+DMSO-d_6, 50°C): δ 2.245 ppm (1H, J_{gem}= -13.7, $J_{1'',2''\beta}$ =6.7, $J_{2''\beta,3''}$=6.7 Hz; $H_{2''\beta}$), 3.233 (1H, J_{gem}= -14.2, $J_{1',2'\beta}$ =7.8, $J_{2',\beta,3'}$=5.9, $J_{2',\beta,P}$=1.3 Hz; $H_{2',\beta}$), 3.467 (1H, J_{gem}=-10.3, $J_{4',5'A}$=5.3 Hz; $H_{5'A}$), 3.523 (1H, J_{gem}=-10.3, $J_{4',5'B}$=5.4 Hz; $H_{5'B}$), 4.08 (2H, m; SCH_2), 4.38-4.55 (3H, m; $H_{4''}$, $H_{5''}$), 4.59 (1H, $J_{4',5'B}$=5.4,

$J_{4',5'A}$=5.3, $J_{3',4'}$=2.3 Hz; $H_{4'}$), 5.00 (1H, m; $H_{3''}$), 5.470 (1H, $J_{3',P}$=8.1, $J_{2'\beta,3'}$=5.9, $J_{2'\alpha,3'}$=2.3, $J_{3',4'}$=2.3 Hz; $H_{3'}$), 6.330 (1H, $J_{1'',2''\beta}$ =6.7, $J_{1'',2''\alpha}$ =6.0 Hz; $H_{1''}$), 6.496 (1H, $J_{1',2'\beta}$ =7.8, $J_{1',2'\alpha}$ =6.2 Hz; $H_{1'}$), 7.060 (1H, $J_{H,P}$=606.6; H(P)), 7.484 (1H, $J_{C5,C6}$=7.6 Hz; H_{C5}), 8.021 (1H, $J_{C5,C6}$=7.6 Hz; H_{C6}) 8.461 (1H, s; H_{A2}), 8.565 (1H, s; H_{A8}).

^{13}C NMR data (C_6D_6+DMSO-d_6, 50°C): δ 33.72 ppm ($J_{C,P}$=4 Hz; SCH_2), 36.44 ($J_{C,P}$=5.4 Hz; $C_{2'}$), 38.50 ($J_{C,P}$=3.8 Hz; $C_{2''}$), 66.87 ($J_{C,P}$=5.8 Hz; $C_{5''}$), 71.96 ($J_{C,P}$=4.2 Hz; $C_{3''}$), 78.88 ($J_{C,P}$=6.3 Hz; $C_{3'}$), 86.47 ($J_{C,P}$=5.0 Hz; $C_{4'}$).

6b: ^{1}H NMR (C_6D_6+DMSO-d_6, 50°C): δ 2.399 ppm (1H, J_{gem}= -13.9, $J_{1'',2''\beta}$ =6.6, $J_{2''\beta,3''}$=6.7 Hz; $H_{2'\beta}$), 2.778 (1H, J_{gem}= -13.9, $J_{1'',2''\alpha}$ =6.3, $J_{2''\alpha,3''}$=3.9 Hz; $H_{2''\alpha}$), 2.869 (1H, J_{gem}= -14.2, $J_{1',2'\alpha}$ =6.1, $J_{2'\alpha,3'}$=2.4 Hz; $H_{2'\alpha}$), 3.485 (1H, J_{gem}= -14.2, $J_{1',2'\beta}$ =8.1, $J_{2'\beta,3'}$=5.6; $H_{2'\beta}$), 3.582 (1H, J_{gem}=-10.2, $J_{4',5'A}$=5.3 Hz; $H_{5'A}$), 3.688 (1H, J_{gem}=-10.2, $J_{4',5'B}$=6.0 Hz; $H_{5'B}$), 4.20 (2H, m; SCH_2), 4.58 (1H, m; $H_{4''}$), 4.541 (1H, $J_{4',5'B}$=6.0, $J_{4',5'A}$=5.3, $J_{3',4'}$=2.3 Hz; $H_{4'}$), 5.153 ($J_{3'',P}$=9.1, $J_{2''\beta,3''}$=6.7, $J_{2'\alpha',3''}$=3.9, $J_{3'',4''}$=3.8 Hz; $H_{3''}$), 5.672 (1H, $J_{3',P}$=8.0, $J_{2'\beta,3'}$=5.6, $J_{2'\alpha,3'}$=2.4, $J_{3',4'}$=2.3 Hz; $H_{3'}$), 6.490 (1H, $J_{1'',2''\beta}$ =6.6, $J_{1'',2''\alpha}$ =6.3 Hz; $H_{1''}$), 6.736 (1H, $J_{1',2'\beta}$ =8.1, $J_{1',2'\alpha}$ =6.1 Hz; $H_{1'}$), 7.212 (1H, $J_{H,P}$=607.3; H(P)), 7.64 (1H; H_{C5}), 8.188 (1H, $J_{C5,C6}$=7.6 Hz; H_{C6}) 8.669 (1H, s; H_{A2}), 8.694 (1H, s; H_{A8}).

^{13}C NMR data (C_6D_6+DMSO-d_6, 50°C): δ 33.73 ppm ($J_{C,P}$=3.8 Hz; SCH_2), 36.39 ($J_{C,P}$=2.6 Hz; $C_{2'}$), 39.56 ($J_{C,P}$=4.6 Hz; $C_{2''}$), 66.94 ($J_{C,P}$=7.0 Hz; $C_{5''}$), 72.04 ($J_{C,P}$=4.3 Hz; $C_{3''}$), 79.01 ($J_{C,P}$=5.8 Hz; $C_{3'}$), 84.49 ($J_{C,P}$=7.0 and 4.7 Hz; $C_{4''}$), 84.19 ($J_{C,P}$=7.1 Hz; $C_{4'}$).

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