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Synthesis and Some Conformational Features of the 5'-Deoxy-5'-methylphosphonate Linked Dimer, 5'-Deoxy-5'-C-(phosphonomethyl)thymidin-3'-yl (Thymidin-5'-yl)methylphosphonate [p(CH_2)Tp(CH_2)T]

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Abstract: Efficient synthesis of the 5'-deoxy-5'-methylphosphonate linked thymidine dimer **11** [p(CH_2)Tp(CH_2)T] was developed *via* the 5'-deoxy-5'-C-(phosphonomethyl)-3'-silylated thymidine derivative **4** as a key intermediate. Conformational analysis of the sugar parts of the dimer showed that the deoxyribose residues exist in solution mainly in the S-type conformations but with a predominant contribution of antiperiplanar rotamers around the C4' - C5' bonds in both sugar units.

INTRODUCTION

The concept underlying the method of an artificial regulation of gene expression by the antisense approach is that a synthetic oligonucleotide upon binding to a complementary DNA or RNA sequence in cells may modulate biological functions of its target molecules and in this way is able to exert its therapeutic effect[1]. Feasibility of such an approach was for the first time demonstrated by Zamecnik and Stephenson[2], who showed that synthetic oligonucleotides complementary to some strategic regions of Rous sarcoma virus indeed inhibited replication of the viral DNA. The pharmacological value of oligonucleotides containing natural phosphodiester internucleotidic linkages is, however, seriously hampered due to their susceptibility towards cells and serum nucleases. To overcome this limitation a plethora of synthetic oligonucleotides having modified internucleotidic bonds has been developed[3]. These analogues may posses a phosphorus centre at which the non-bridging oxygen is replaced by, *e.g.*, a methyl group (methylphosphonates), one or two sulfurs (phosphorothio- or phosphorodithioates), *etc.*[3], or in which an entire sugar-phosphate backbone is substituted by a different group of atoms, *e.g.*, amide function to produce a peptide nucleic acid analogues[4].

Among chemically modified oligonucleotides, phosphorothioate and methylphosphonate derivatives have received most attention despite some serious problems connected with the presence of chiral phosphorus centers, which inevitably give rise to a large number of practically unresolvable diastereomers. The problem of chirality at the phosphorus centre in nucleoside C-phosphonate diesters may be circumvented by placing the P-C bond into the bridging position of the phosphonate group to produce achiral [(nucleosid-5' (or 3')-yl)methylphosphonates or 5'-(3'-)methylenephosphonates]. Although these kind of analogues have been

prepared for the first time some 25 year ago by Jones and Moffatt [5], they did not received much attention[6-8], probably due to some inconveniences in their preparation[9-11].

It is well established that some conformational features of nucleoside, nucleotides, and oligonucleotides[12], namely the distribution of rotamers around C4'-C5' bond (γ) are governed by the *gauche* effect of the C5'- O5' bond[13] and by involvement of the C5'-oxygen atom in hydrogen bonds with appropriate donor centers in the heterocylic bases[14]. One can thus anticipate that replacement of a polar P-O bond at the phosphorus centre by the P-C one in nucleotides may introduce substantial changes in electronic density at the phosphorus centre and these may be transmitted further to promote some conformational changes in the ribose residue. To study this problem, which may be essential for application of methylenephosphonates as potential therapeutics (*e.g.*, antisense analogues, enzyme inhibitors), we developed an efficient synthesis of the methylphosphonate linked dimer **11** and investigated some of the conformational features of its sugars moieties.

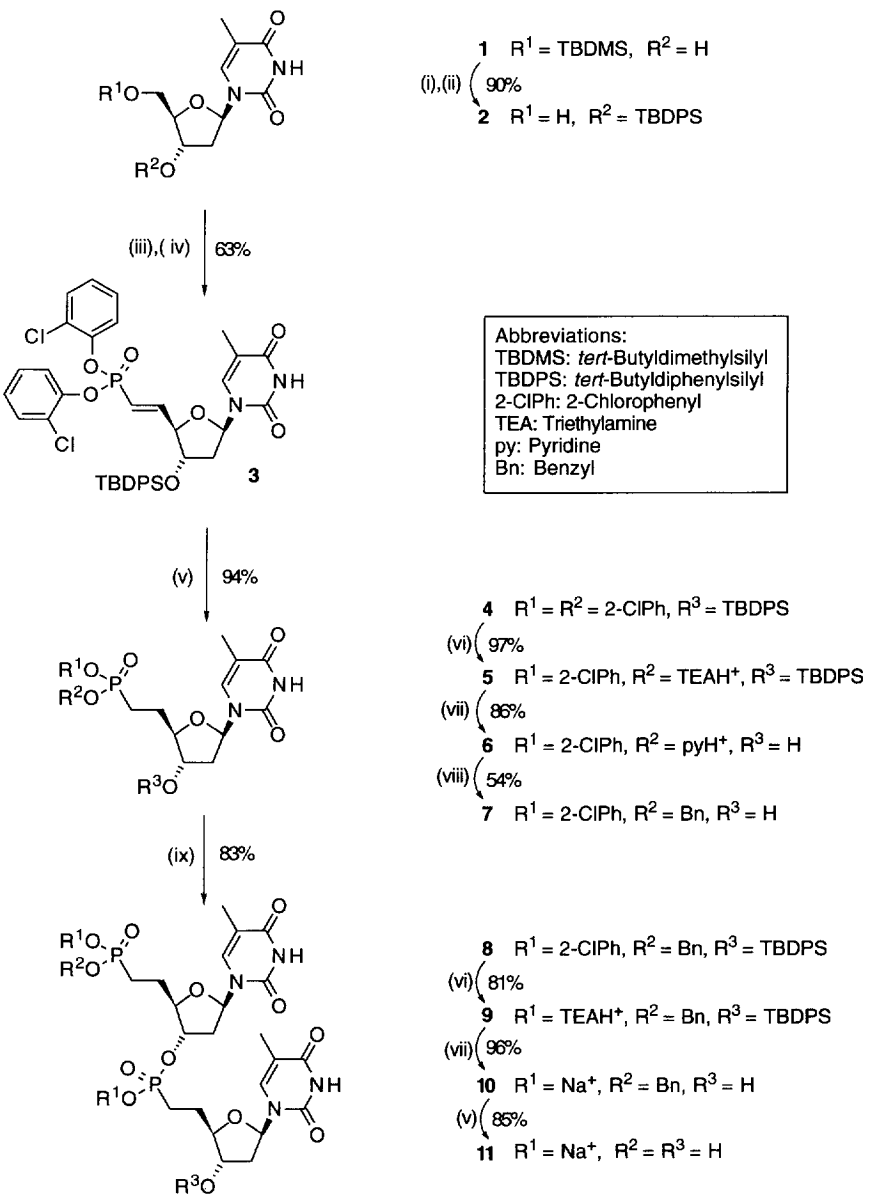
RESULTS AND DISCUSSION

Synthesis of the p(CH_2)Tp(CH_2)T dimer 11

For the purpose of conformational analysis we wanted to prepare a simplest model of 5'-deoxy-5'-methylphosphonate linked oligonucleotide, consisting of two repeating phosphonate units, namely the p(CH_2)Tp(CH_2)T dimer **11** (the abbreviation "p(CH_2)" stands for the =O_2(O)P-CH2- group). To minimize the number of synthetic steps we decided to make use of the phosphonate **4** as the key intermediate from which the nucleotidic (**5**) and the nucleosidic (**7**) components can be generated *via* a partial deprotection of the phosphonate and the 3'-hydroxyl functions, respectively. To secure the proper stability of the protecting groups throughout the synthesis, t-butyldiphenylsilyl group (TBDPS) for the hydroxyl functions and 2-chlorophenyl (2-ClPh) and benzyl (Bn) groups for the phosphonate functions, were chosen. The route to the precursor **4** commences (Scheme 1) with introduction to thymidine two silyl groups of different susceptibility to acids (TBDMS and TBDPS), removal of the more labile one (TBDMS) with acetic acid, followed by the one-pot oxidation of the 5'-hydroxyl group using DCC-DMSO[5] and the reaction of the produced aldehyde with the appropriate Wittig reagent to give the vinylphosphonate **3**. Catalytic hydrogenation of **3** smoothly afforded the key intermediate **4**, which upon treatment with 2-pyridinealdoximate[15] produced the nucleotidic component **5**. The nucleosidic component **7** was obtained from the partially deprotected phosphonate **5** by treatment with tetrabutylammonium fluoride (TBAF) to remove the 3'-silyl protecting group, followed by esterification of the phosphonate function with benzyl alcohol. The last operation was intended to introduce a suitable phosphonate protecting group which could be easily removed during the final deprotection *via* hydrogenation, to produce the 5'-terminal phosphonate group.

Formation of the fully protected phosphonate dimer **8** was accomplished *via* condensation of the nucleotidic (**5**) and the nucleosidic (**7**) components promoted by 2-chloro-2-oxo-5,5-dimethyl-1,3,2-dioxaphosphinane in the presence of a nucleophilic catalyst, 4-methoxypyridine-1-oxide. Total deprotection was carried out by a consecutive treatment of **8** with 2-pyridinealdoximate and TBAF, followed by the hydrogenolytic removal of the benzyl group, to produce the 5'-deoxy-5'-methylphosphonate linked dimer **11** having a phosphonate group in the 5'-position.

Scheme 1



(i) TBDPSCl, imidazole, DMF; (ii) AcOH/H_2O/THF; (iii) DCC, DMSO, pyridine, TFA; (iv) Di(2-chlorophenyl) (triphenylphosphoranylidene)methylphosphonate; (v) H_2, Pd/C; (vi) 2-Pyridinealdoxime, 1,1,3,3-tetramethylguanidine; (vii) $Bu_4NF \cdot 3\ H_2O$, THF; (viii) 2-Chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane, 4-methoxypyridine-1-oxide, BnOH; (ix) 2-Chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane, 4-methoxypyridine-1-oxide, **5**

Conformational analysis

^1H NMR Analysis. The dimer **11** was studied in D_2O at sample concentration of 20 mM at the temperatures 293, 323, and 353 K. The assignments of the non-exchangeable protons were done on the basis of DQF-COSY spectra. The 1D version of the homonuclear Hartman-Hahn (HOHAHA) experiment was used to obtain chemical shifts of the individual H2' and H2" protons. Detailed analysis of 1D ^1H NMR was done with a least-squares iterative spin-simulation program (geNMR ver. 3.5, IvorySoft 1992-1994). The chemical shifts values and the coupling constants derived from the simulated spectra are listed in Table 1. Since the stereospecific assignment of the H5' and H5" protons could not be done, we tentatively assigned the $J_{4'5'}$ and $J_{4'5''}$ as in Table 1 assuming that the Remin and Shugar's rule[16] applies. No attempt was made to assign the protons from both P-CH_2-CH_2- groups whose resonances were buried in a complicated multiplet at ~1.8 ppm.

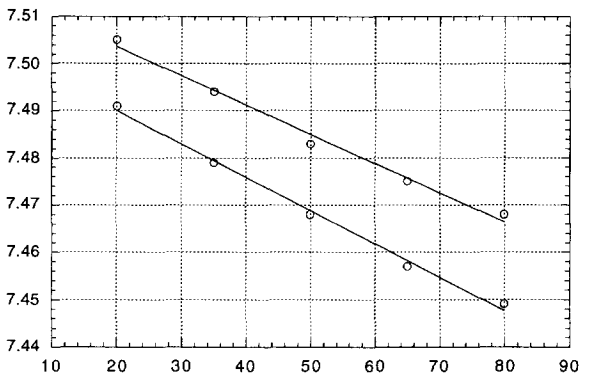
Table 1. ^1H NMR chemical shifts (in ppm) and vicinal coupling constants (in Hz) for the sugar ring protons of the dimer **11**

	p(CH$_2$)Tp(CH$_2$)T			p(CH$_2$)T***p(CH$_2$)T***		
	293 K	323 K	353 K	293 K	323 K	353 K
H1'	6.295	6.270	6.248	6.255	6.235	6.214
H2'	2.404	2.400	2.392	2.415	2.400	2.385
H2"	2.461	2.474	2.488	2.351	2.358	2.361
H3'	4.717	4.713	4.707	4.362	4.351	– [a]
H4'	4.112	4.106	4.100	3.962	3.963	3.962
$J_{1'2'}$	8.2	8.1	7.9	6.9	6.8	6.7
$J_{1'2''}$	6.1	6.1	6.0	6.8	6.8	6.7
$J_{2'2''}$	-14.0	-14.2	-14.2	-14.3	-14.2	-14.2
$J_{2'3'}$	5.8	6.1	6.1	7.5	7.2	7.3
$J_{2''3'}$	2.6	2.8	3.0	4.0	4.1	4.0
$J_{3'4'}$	2.8	2.9	3.2	4.2	4.2	4.0
$J_{4'5'}$	5.7	5.9	5.9	4.4	5.5	6.0
$J_{4'5''}$	8.0	7.6	7.7	8.1	7.6	7.1
$J_{3'P}$	8.3	8.1	7.4			

[a] Not determined due to overlapping with the residual HDO signal.

Since chemical shifts of H-6 proton of thymidine are known to be sensitive to stacking[17], we recorded ^{1}H NMR spectra of **11** at different temperatures. A seen from Fig. 1 the H-6 protons of both thymine residues experience an upfield shift with increasing temperature. The temperature dependence of δ_{H6} are smaller than those for TpT[18] but comparable to those of some modified dimers[18]. This may suggest that **11** adopts even at room temperature a conformation with a rather extended backbone.

Fig. 1. Plot of δ_{H6} of thymines *vs* temperature for the dimer **11**



*Determination of the deoxyribose conformations in **11**.* The geometry of the 5-membered ring is defined by the five endocyclic angles (Φ_0 - Φ_4) which according to the concept of pseudorotation[19,20] can be described as a cosine function of two parameters, the phase angle of pseudorotation (P) and the puckering amplitude (Φ_m) according to the equation:

$$\Phi_j = \Phi_m \cos(P + 4j\pi / 5), \quad j = 0 - 4 \qquad \text{(eq. 1)}$$

The proton-proton torsion angles (Φ_{HH}) are related to the above endocyclic angles[21] and can also be expressed as a function of P and Φ_m:

$$\Phi_{HH} = a + b\Phi_m \cos(P + phase) \qquad \text{(eq. 2)}$$

where a and b assume values of 0° and 1, respectively, in the trigonal symmetry approximation.

In contradistinction to cyclopentane, much of the conformational preferences of ribo- and deoxyribofuranosides originate from the presence of the ring oxygen which give rise to two conformational families, referred to as N (North) (C2'-*exo*, C3'-*endo*) and S (South) (C2'-*endo*, C3'-*exo*) conformations. Since the furanoside ring in nucleosides and their derivatives is assumed to be in a rapid two-state conformational equilibria involving these two conformations[22], the experimentally determined proton-proton coupling constants (J_{exp}) have to be considered as time averaged couplings, linearly related to the coupling constants of the individual conformers (J_S and J_N) and their molar fractions (X_S):

$$J_{exp} = x_S J_S + (1 - x_S) J_N \qquad \text{(eq. 3)}$$

Thus, for a complete conformational description of a furanoside ring one has to determine five independent parameters: the phase angle of pseudorotation and the puckering amplitude for the S (P_S, Φ_S,), for the N (P_N, Φ_N) conformations, and the molar fraction X_S. Recognizing that the coupling constants are related to the pseudorotational parameters[23] *via* equation 2 and the appropriately parametrized Karplus equation[24], the conformational analysis of furanosides can rely almost entirely on the proton-proton coupling constants deduced from NMR experiments[25]. Extraction of the pseudorotational parameters from the NMR data is greatly facilitated by using the program PSEUROT[21] which calculates the best fit of the parameters P_S, Φ_S, P_N, Φ_N, and X_S to the experimentally determined proton-proton coupling constants.

Since the number of conformational parameters is five, at least the same number of observables are needed for a furanoside ring. For each of the deoxyribofuranoside residue of **11**, five coupling constant, $J_{1'2'}$, $J_{1'2''}$, $J_{2'3'}$, $J_{2''3'}$ and $J_{3'4'}$ were obtained from the ^{1}H NMR spectra. To increase the accuracy, the spectra were recorded at three temperatures thus affording total fifteen observables for each deoxyribofuranoside moiety.

Inspection of the data in Table 1 shows that for both deoxyriboses the value of the sums of the appropriate coupling constants, $\Sigma 1' > 13$ Hz and $\Sigma 2' > \Sigma 2''$, are indicative[26] of the predominance of the S-type conformer. Since $\Sigma 2''$ is mainly influenced by the relative population of S and N conformers, one can calculate an approximate molar fraction for S (X_S) using the empirical formula, $X_S = (31.5\text{-}\Sigma 2'')/10.9$[26]. From Table 2 it is apparent that molar fractions X_S are practically invariant (variation less than 5%) over the temperature range studied, although they are different for both sugar residues. This also means that our system is not "overdetermined", and thus in practice[21], the number of observables used for the minimization by the program PSEUROT is equal to the number of parameters to be extracted.

Preliminary calculations showed that the pseudorotational parameters derived for the furanoside rings of **11** varied substantially depending on the starting conformation chosen. To minimize the number of possible solutions, one can try to make a system artificially "overdetermined" by constraining one or two parameters (usually those for the minor conformers or ones which can be guessed to assume certain values). For the dimer **11** there was no strongly biased conformational equilibria as judged from the approximate calculations using "the sum" rule[26] (see Table 2) and also no crystallographic data were available which could be used as aid for making constrainment. In this situation we decided to make no constrains and to treate the coupling constants determined at various temperatures as separate input data. Using a wide range of the starting conformations (P_N 0° ± 90°, Φ_N 25°-35°, P_S ± 180° ± 90°, Φ_S 25°-35°) and guided by the r.m.s.d. for the parameters derived, we obtained for the 3'-deoxyribofuranoside ring a dozen of conformations which clustered in a region of P 130° ± 10°, for major conformers, and at P -5° ± 15°, for minor ones. Then, when P and Φ_m of any of those conformations were used as the starting values for the minimization using data from the whole temperature interval, the program converged to conformations with a rather narrow range of pseudorotational parameters (see Table 2). The same procedure was used for the determination of pseudorotational parameters of the 5'-deoxyribose ring in **11**.

Data from Table 2 indicate that replacement of the oxygen in the C5' position by carbon in the dimer **11** does not have a significant influence on the deoxyriboses conformations, which are within the range find for unmodified nucleic acid constituents[12]. For both sugars, the S-type conformation prevails, however, it is more populated in the 5'-moiety of the dimer **11** than in the 3'-one. The later phenomenon is probably due to the presence of a bulky and polar substituent in the 3'-position of the upper unit of the dimer.

Table 2. Pseudorotational parameters for the deoxyribose residues in the dimer **11**

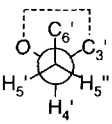
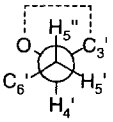
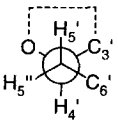
Pseudorotational parameters[a]	***p(CH$_2$)T***p(CH$_2$)T	p(CH$_2$)T***p(CH$_2$)T***	Molar fraction of the major conformer	***p(CH$_2$)T***p(CH$_2$)T	p(CH$_2$)T***p(CH$_2$)T***
P_S	150°	130° ± 3°			
Φ_S	29°	31° ± 1°	%X_S (293 K)	82 (80)[c]	60-65 (59)
			%X_S (323 K)	81 (77)	60-64 (59)
P_N	-7°	-10° ± 4°	%X_S (353 K)	78 (76)	60-64 (60)
Φ_N	52°	29° ± 2°			
r.m.s.d. [b]	0.08 Hz	0.18 Hz			

[a] Calculated using the program PSEUROT 5.4[27].
[b] Deviations between the five experimental coupling constants and their values calculated from the determined pseudorotational parameters (output from the PSEUROT 5.4).
[c] In parentheses, molar fractions calculated according to the formula: (31.5-Σ2")/10.9 [26].

Determination of rotamer population around the C4'-C5' bonds. The conformation around the C4'-C5' bond in terms of three rotamers population [gauche$^+$ (γ^+) or + synclinal (+sc), gauche$^-$ (γ^-) or -synclinal (-sc), and trans (γ^t) or antiperiplanar (ap), see Table 3] can be determined by analysis of the vicinal coupling constants $^3J_{4'5'}$ and $^3J_{4'5''}$. Assuming a rapid interconversion between these rotamers, the observed ^{1}H NMR coupling constants can be regarded as weighted time-averages for the three limiting rotamers and their populations (Table 3). From the assumed geometries, which have been derived from the statistical analysis of crystallographic data for furanosides[28], and using the appropriately parametrized Karplus equation[28], the limiting coupling constants for the individual rotamers can be calculated (Table 3). In the phosphonate dimer **11**, the atoms attached to the C5' are carbons, in contradistinction to dimers containing natural internucleotidic bond where oxygen are present in these positions. Since magnitudes of the vicinal coupling constants depend not only on the torsion angles but also upon electronegativity[24] of the substituents, an appropriate electronegativity factor $\Delta\chi = 0.4$ (according to the Huggin's scale[29]) was used for calculations of the limiting coupling constants.

Values for the $^3J_{4'5'}$ and $^3J_{4'5''}$ coupling constants in Table 1, as well as their differences, are significantly larger than those for the unmodified TpT dimer[18]. These may be due to changes in electronegativity of the substituents (carbon at the C5' in **11** *vs* oxygen in TpT) and/or due to different orientation of the -C-P- (-O-P- in TpT) fragments relative to the deoxyribose rings. Using the experimental coupling constants from Table 1 and the calculated coupling for the limiting conformers, population of the rotamers around the C4'-C5' bonds in the dimer **11** were calculated by solving the system of linear equations (Table 3).

Table 3. The rotamers distribution (in %)[a] around the $C_{4'}$ - $C_{5'}$ bonds (γ) at various temperatures in the p(CH_2)Tp(CH_2)T phosphonate dimer **11**

		γ^+ (+sc)	γ^t (ap)	γ^- (-sc)
The staggered rotamers around $C_{4'}$ - $C_{5'}$ bond				
Limiting J' and J'' coupling constants for the rotamers[b]		$J_{4',5'}$ = 3.3 Hz $J_{4',5''}$ = 2.5 Hz	$J_{4',5'}$ = 1.8 Hz $J_{4',5''}$ = 11.4 Hz	$J_{4',5'}$ = 11.6 Hz $J_{4',5''}$ = 3.0 Hz
$H_{4'}$ - $H_{5'}$ ($H_{5''}$) torsion angles Φ[c]		$\Phi_{H4',H5'}$ = -64° $\Phi_{H4',H5''}$ = 55°	$\Phi_{H4',H5'}$ = 64° $\Phi_{H4',H5''}$ = -178°	$\Phi_{H4',H5'}$ = 174° $\Phi_{H4',H5''}$ = -68°
p(CH₂)Tp(CH_2)T	293 K	1 (5)[d]	59 (32)	40 (63)
	323 K	4 (7)	55 (35)	41 (58)
	353 K	2 (6)	56 (35)	41 (59)
p(CH_2)T***p(CH₂)T***	293 K	14 (21)	62 (18)	24 (61)
	323 K	8 (12)	55 (31)	37 (57)
	353 K	9 (11)	49 (37)	42 (52)

[a] Calculated from the set of equations:

$$^3J_{4'5'} = x_{\gamma^+}J'_{\gamma^+} + x_{\gamma^-}J'_{\gamma^-} + x_{\gamma^t}J'_{\gamma^t}$$

$$^3J_{4'5''} = x_{\gamma^+}J''_{\gamma^+} + x_{\gamma^-}J''_{\gamma^-} + x_{\gamma^t}J''_{\gamma^t}$$

$$x_{\gamma^+} + x_{\gamma^-} + x_{\gamma^t} = 1$$

[b] Calculated from the Karplus equation parametrized by Haasnoot *et al.*[28]:

$$^3J_{HH} = 13.22\cos^2\Phi - 0.99\cos\Phi + \sum\{0.87 - 2.46\cos^2(\zeta_i\Phi + 19.9|\Delta\chi_i|)\}\Delta\chi_i$$

where ζ = +1 or -1 depending on the orientation of the substituent with respect to its geminal coupled proton; $\Delta\chi$ is the difference in electronegativity between the substituent and hydrogen in Huggins' electronegativity scale.

[c] The proton-proton torsion angles according to Haasnoot *et al.*[28] for the staggered conformation as deduced from the statistical analysis of crystallographic data of furanose systems.

[d] In parentheses the rotamers population if the assignment of H5' and H5" would be interchanged.

It is apparent from Table 3 that the preferred rotamers around both C4'-C5' bonds in the phosphonate dimer **11**, are those in which the phosphonate functions (-C-P-) are positioned away from the deoxyribose rings (γ^t and γ^- rotamers). These are the rotamers which are the least populated in the natural nucleic acid

constituents containing oxygen at the C5'. One should note that since the difference between the $^3J_{4'5'}$ and $^3J_{4'5''}$ for the γ^+ rotamer, in contradistinction to the γ^t and γ^- ones, is small, the population of the former one is rather insensitive to changes in the stereochemical assignment of the 5' and 5'' protons. If the assignment would be opposite to that as in Table 1, then again the most populated rotamers would be γ^t and γ^-, with the latter one being the predominant one (the values in parentheses in Table 3). Since both phosphonate functions (the 5'-terminal and the internucleoside ones) tend to adopt a similar preferred conformation around the C4'-C5' bonds it seems that the degree of esterification does not influence the rotamers population. The conformational purity around C4'-C5' bond is higher for the 3'-residue (which can be regarded being more rigid) but with increasing of temperature all rotamers are becoming more evenly populated although the clear preference for the γ^t and γ^- remains.

The marked preference in nucleoside and nucleotides for the γ^+ rotamer is most likely due to *gauche* effect[13] of two polar bonds, C5'-O5' and C4'-O4', and due to stabilization arising from intramolecular hydrogen bonds between the C5'-oxygen and heterocyclic bases[14,30]. In the dimer **11**, replacement of the C5'-oxygen by carbon eliminates both sources of possible stabilizations for the γ^+ rotamer and thus makes the γ^t and γ^- rotamers most populated. A similar trends in shifting a population of the rotamers toward γ^t and γ^- was also observed for some dinucleoside analogues (*e.g.*, sulfonate-, sulfonamide-, acetamide-linked nucleosides) having a less electronegative than oxygen atom at the C5'-position[31].

It is worth mentioning that conformational changes introduced to a furanoside ring by some substituents in the 3'-position (*e.g.*, azido, fluoro) may cause a significant alteration of the rotamers population around the C4'-C5' bond in favour of the γ^t conformer, even in those instances when the C5'-oxygen is present[32,33]. This became apparent from the solid state structures of some nucleoside analogues with an established antiviral potency (*e.g.*, AZT[30]) and it was rationalized in terms of transmission of the conformational changes exerted by the 3'-azido group on the deoxyribofuranoside ring, to the exocyclic hydroxymethyl function. Since in the phosphonate dimer **11** both furanoside rings have the expected S-type conformations but unusual rotamers distribution around the C4'-C5' bonds, it is apparent that the reversed transmission of the conformational changes does not operate. Van Roey *et al.*[34,35] hypothesized that an increased population of the γ^t rotamer may facilitate phosphorylation of the 5'-hydroxyl function and this may account for variations in antiviral activity among nucleoside analogues.

Conclusions

The 5'-deoxy-5-methylphosphonate linked dimer **11** with two phosphonate functions was synthesized *via* the methylphosphonate derivative **4** as a key intermediate. The conformational analysis of the sugar parts of the dimer revealed that replacement of the C5'-oxygen by carbon did not introduce significant conformational changes to the deoxyribose rings, which exist preferentially in the S-type conformations. The equilibrium constants for the N/S equilibria were found to be rather insensitive to temperature, within the range investigated. As judging from the temperature dependence of the H-6 chemical shifts, the dimer **11** adopts a rather extended conformation at room temperature. In contradistinction to natural nucleic acid components, the most populated rotamers around the C4'-C5' bonds in the phosphonate **11** are γ^t and γ^-. One may hypothesize, that these unusual rotamers distribution in nucleotide analogues containing P-C-C function should be preserved after phosphorylation of the phosphonate group (conversion to di- and triphosphate

analogues). In light of the observed correlation between biological activity and the rotamers distribution around the C4'-C5' bond in some nucleoside analogues[34,35], it is possible that the structural motif present in the 5'-methylenephosphonate analogues, namely the P-C-C function, can be of biological importance for modulation of the interactions with DNA synthesizing enzymes.

EXPERIMENTAL PART

Materials and Methods

Pyridine was dried by refluxing with CaH_2 overnight followed by distillation, redistillation from *p*-toluenesulfonyl chloride and stored over molecular sieves (4 Å). Dioxane was dried by distillation from $LiAlH_4$ and stored over Na-wire. Tetrahydrofuran was dried by distillation from $LiAlH_4$ directly before use. Chloroform was passed through basic Al_2O_3 prior to use. DMF and DMSO were made anhydrous by distillation from CaH_2 at reduced pressure (ca. 10 mm Hg) and stored over molecular sieves (4 Å).

5'-*O*-*tert*-Butyldimethylsilylthymidine[36] and 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane[37] were prepared by the published procedures. *tert*-Butyldiphenylsilyl chloride, imidazole, oxalic acid dihydrate, 2-pyridinealdoxime, and tetrabutylammonium fluoride trihydrate were commercial grades (Aldrich). 4-Methoxypyridine-1-oxide hydrate (Aldrich) was dried over P_2O_5 overnight at 70 °C at 0.2 mm Hg and 1,3-dicyclohexylcarbodiimide (Aldrich) and 1,1,3,3-tetramethylguanidine (Aldrich) were vacuum distilled. 1M Triethylammonium bicarbonate buffer (pH ~ 7) (TEAB) was prepared by passing carbon dioxide through an aqueous solution containing the appropriate amount of triethylamine. For column chromatography, silica gel (35-70 μm) from Amicon Europe was used, and the columns were run in the flash mode. All evaporations were carried out under reduced pressure using rotatory evaporator, unless stated otherwise. Yields reported refer to products obtained after drying at $p < 1$ mm Hg for at least 24 h.

NMR and MS analysis

^1H, ^{13}C, and ^{31}P NMR spectra were recorded on a Jeol GSX-270 FT spectrometer. ^1H NMR spectra of **11** were recorded on a Jeol α-400 FT spectrometer using 32K data points and the FIDs were zero filled to 64K before processing. Chemical shifts are given in ppm relative to tetramethylsilane (^1H, $CDCl_3$, 25 °C) or sodium 3-(trimethylsilyl)-*2,2,3,3-d*$_4$-propionate (^1H, D_2O, 50 °C) or $CDCl_3$ (δ = 77.17 ppm, ^{13}C, $CDCl_3$, 25 °C) or dioxane (δ = 67.40 ppm, ^{13}C, D_2O, 50 °C) or 2% H_3PO_4 in H_2O (^{31}P, $CDCl_3$, 25 °C or D_2O, 50 °C). High resolution FAB mass spectra were recorded on a Jeol SX-102 instrument.

Spectral simulation

The ^1H chemical shifts and coupling constants in Table 1 were obtained from the iterative spin-spin simulation using a commercial package, geNMR ver. 3.5, IvorySoft 1992-1994. Approximate values for coupling constants and chemical shifts deduced from 1D ^1H NMR spectra were used as input data and the parameters were adjusted to achieve the best agreement between the experimental and the simulated spectrum.

Conformational analysis

The pseudorotational analysis of the deoxyribose moieties in the dimer **11** was performed using the program PSEUROT 5.4[27]. As input data the proton-proton coupling constants derived from 1D NMR spectra and refined by spin-spin simulation were used.

Di(2-chlorophenyl) (triphenylphosphoranylidene)methylphosphonate

Di(2-chlorophenyl) chloromethylphosphonate[38] (64.1 g, 182 mmol) and triphenyl phosphine (45.8 g, 175 mmol) were stirred at 160 °C for 16 h. Heating was discontinued and the reaction mixture solidified. The crude phosphonium salt was dissolved in dichloromethane (400 mL), transferred to a separatory funnel and treated with 2 M NaOH (200 mL). After separation of the layers, the organic phase was dried over Na_2SO_4 and evaporated to leave the ylide as a light yellow oil. The crude ylide was dissolved in ethyl acetate (500 mL) with heating and allowed to slowly cool down to room temperature. Crystals obtained were recrystallized from ethyl acetate (500 mL) to give the title compound as off-white crystals. Yield 53.3 g (52.7 %).

3'-*O*-*tert*-Butyldiphenylsilylthymidine (2)

5'-*O*-*tert*-Butyldimethylsilylthymidine (14.26 g, 40.0 mmol) and imidazole (5.99 g, 88.0 mmol) were dissolved in DMF (80 mL) and *tert*-butyldiphenylsilyl chloride (11.4 mL, 44.0 mmol) was added. The reaction mixture was stirred at room temperature for 3 h, poured into toluene (400 mL) and the DMF removed by extraction with H_2O (4 × 100 mL). The organic phase was dried over Na_2SO_4 and evaporated to give the crude disilylated nucleoside as a white foam. This was dissolved in THF (80 mL) and under stirring mixture of AcOH-H_2O (3:1, v/v, 320 mL) was added. The progress of desilylation was monitored by TLC. After 16 h the reaction mixture was evaporated and traces of acetic acid were removed by evaporation of added toluene (3 × 200 mL). Crude 3'-*O*-*tert*-butyldiphenylsilylthymidine was obtained as a white foam. Purification by silica gel column chromatography using a stepwise gradient of MeOH (0-5 %) in $CHCl_3$ as eluent afforded the title compound as a white foam. Yield 17.39 g (90.5 %). $\delta_H(CDCl_3)$ 1.09 (9 H, s), 1.85 (3 H, s), 2.1-2.3 (2 H, m), 3.24 (1 H, d, *J* 11.4), 3.63 (1 H, d, *J* 11.4), 3.98 (1 H, m), 4.45 (1 H, m), 6.23 (1 H, pt, *J* 7.0), 7.3-7.7 (11 H, m) and 8.39 (1 H, br s); $\delta_C(CDCl_3)$ 12.6, 19.1, 27.0, 40.4, 62.1, 73.1, 86.9, 87.8, 111.1, 128.0, 130.2, 130.3, 133. 2, 133.4, 135.8, 137.0, 150.5 and 163.9; HRMS Found: $(M-H)^-$, 479.2032. $C_{26}H_{32}N_2O_5Si$ requires *M*-H, 479.2002

Di(2-chlorophenyl) [1'-(2',5',6'-trideoxy-3'-*O*-*tert*-butyldiphenylsilyl-*β*-D-*ribo*-hex-5-enofuranosyl)-1-thymin]-6'-phosphonate (3)

2 (4.81 g, 10.0 mmol), 1,3-dicyclohexylcarbodiimide (6.19 g, 30.0 mmol) and pyridine (0.81 mL, 10 mmol) were dissolved in DMSO (40 mL) followed by addition of trifluoroacetic acid (0.38 mL, 5.0 mmol). After

stirring for 22 h at RT, di(2-chlorophenyl) (triphenylphosphoranylidene)methylphosphonate (8.66 g, 15.0 mmol) was added and stirring was continued for 6 h. Excess carbodiimide was hydrolysed by careful addition of a solution of oxalic acid dihydrate (2.52 g, 20.0 mmol) in MeOH (10 mL). The urea formed was removed by filtration and washed with toluene (150 mL). The combined filtrates were extracted with H_2O (4 × 100 mL), dried over Na_2SO_4, and evaporated. Silica gel column chromatography using toluene-ethyl acetate (2:1, v/v) as eluent afforded the title compound as a white foam. Yield 4.91 g (63.1 %). $\delta_H(CDCl_3)$ 1.09 (9 H, s), 1.73 (1 H, ddd, *J* -13.2, 8.3, 5.7), 1.86 (3 H, s), 2.28 (1 H, ddd, *J* -13.2, 5.7, 1.7), 4.20 (1 H, m), 4.50 (1 H, m), 6.04 (1 H, ddd, *J* -22.5, 17.1, 1.7), 6.49 (1 H, dd, 8.3, 5.7), 6.66 (1 H, ddd, *J* 24.4, 17.1, 4.1), 7.0-7.6 (19 H, m) and 8.17 (1 H, s); $\delta_C(CDCl_3)$ 12.7, 19.1, 27.0, 39.3, 76.0, 85.0, 85.9, 86.2, 111.9, 115.0, 117.8, 122.5, 122.6, 125.4, 125.7, 125.8, 125.9, 126.4, 128.1, 128.2, 128.2, 129.1, 130.5, 130.7, 130.8, 132.6, 134.7, 135.8, 146.1, 146.2, 150.5, 151.4, 151.5 and 163.6; $\delta_P(CDCl_3)$ 10.93; HRMS Found: $(M-H)^-$, 775.1530. $C_{39}H_{39}N_2O_7Cl_2PSi$ requires *M*-H, 775.1563

Di(2-chlorophenyl) (3'-*O*-*tert*-butyldiphenylsilylthymidin-5'-yl)methylphosphonate (4)

To a solution of **3** (4.67 g, 6.00 mmol) in ethanol (120 mL), Pd/C (10 %) (1.2 g) was added and the reaction mixture stirred under an atmosphere of hydrogen. After 24 h, when the calculated amount of hydrogen had been consumed, the catalyst was removed by filtration through Celite® and the solvent evaporated. The title compound was obtained as a white foam. Yield 4.39 g (93.8 %). $\delta_H(CDCl_3)$ 1.08 (9 H, s), 1.82 (3 H, s), 1.8-2.4 (6 H, m), 3.93 (1 H, m), 4.12 (1 H, m), 6.35 (1 H, pt, *J* 6.6), 7.0-7.7 (19 H, m) and 8.14 (1H, s); $\delta_C(CDCl_3)$ 12.6, 19.1, 22.2, 24.3, 26.6, 26.7, 27.0, 40.0, 75.7, 84.7, 86.0, 86.3, 111.6, 122.4, 125.7, 125.8, 126.3, 128.1, 129.9, 130.3, 130.4, 130.8, 133.0, 134.9, 135.8, 136.2, 146.1, 146.3, 150.4 and 163.8; $\delta_P(CDCl_3)$ 25.90; HRMS Found: $(M-H)^-$, 777.1712. $C_{39}H_{41}N_2O_7Cl_2PSi$ requires *M*-H, 777.1719

2-Chlorophenyl (3'-*O*-*tert*-butyldiphenylsilylthymidin-5'-yl)methylphosphonate, triethylammonium salt (5)

To a solution of **4** (3.90 g, 5.00 mmol) in dioxane-H_2O (3:1, v/v, 100 mL), 2-pyridinealdoxime (1.22 g, 10.0 mmol) and 1,1,3,3-tetramethylguanidine (1.25 mL, 10.0 mmol) were added, and the reaction mixture was stirred at room temperature for 3 h. The mixture was partitioned between 1 M TEAB (100 mL) and $CHCl_3$ (2 × 100 mL). The combined organic phases were dried over Na_2SO_4, evaporated and the residue was purified by silica gel chromatography using a stepwise gradient of MeOH (0-12 %) in $CHCl_3$-Et_3N (995:5, v/v). The title compound was obtained as a white foam. Yield 3.74 g (97.1 %). $\delta_H(CDCl_3)$ 1.06 (9 H, s), 1.25 (9 H, t, *J* 7.3), 1.4-1.9 (5 H, m), 1.83 (3 H, s), 2.21 (1 H, m), 2.98 (6 H, q, *J* 7.3), 3.98 (1 H, m), 4.09 (1 H, m), 6.35 (1 H, dd, *J* 7.9, 5.8), 6.9-7.6 (15 H, m) and 8.12 (1 H, br s); $\delta_C(CDCl_3)$ 8.5, 12.5, 19.1, 23.3, 25.4, 27.0, 28.6, 39.7, 45.3, 76.0, 85.1, 87.7, 88.0, 111.0, 122.2, 123.4, 125.3, 125.4, 127.5, 127.9, 129.9, 130.1, 133.1, 133.3,

135.3, 135.7, 149.2, 149.3, 150.2 and 163.9; $\delta_P(CDCl_3)$ 22.11; HRMS Found: M^-, 667.1809. $C_{33}H_{37}N_2O_7ClPSi$ requires *M*, 667.1796

2-Chlorophenyl (thymidin-5'-yl)methylphosphonate, pyridinium salt (6)

To a solution of **5** (2.70 g, 3.50 mmol) in a freshly distilled THF (28 mL), tetrabutylammonium fluoride trihydrate (4.42 g, 14.0 mmol) was added and the reaction mixture was stirred for 4 h. After addition of water (7 mL) and removal of the organic solvent, the residue was partitioned between diethyl ether (35 mL) and H_2O (35 mL). The aqueous phase was concentrated and passed through a Dowex® 50W (pyridinium form) column using water as an eluent. The appropriate fractions were pooled, diluted with water to 50 mL and repeatedly lyophilised to afford the title compound as a white solid. Yield 1.54 g (86.3 %). $\delta_H(D_2O)$ 1.3-2.0 (6 H, m), 1.75 (3 H, s), 3.89 (1 H, m), 4.24 (1 H, m), 6.14 (1 H, pt, *J* 6.8) and 7.0-8.7 (10 H, m); $\delta_C(D_2O)$ 12.3, 23.4, 25.4, 28.0, 28.1, 38.7, 74.1, 85.8, 87.3, 87.6, 112.4, 123.0, 125.8, 126.0, 126.1, 128.2, 128.9, 131.0, 137.8, 141.9, 148.0, 148.5, 148.6, 152.3, 167.0; $\delta_P(D_2O)$ 25.44; HRMS Found: M^-, 429.0631. $C_{17}H_{19}N_2O_7ClP$ requires *M*, 429.0618

Benzyl 2-chlorophenyl (thymidin-5'-yl)methylphosphonate (7)

6 (1.02 g, 2.00 mmol) and 4-methoxypyridine-1-oxide (751 mg, 6.00 mmol) were dried by evaporation of added pyridine (20 mL) and dissolved in pyridine/benzyl alcohol (1/1, 20 mL). To this 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane (1.11 g, 6.00 mmol) was added, the reaction mixture stirred for 5 min, poured into aqueous 0.5 M sodium bicarbonate (100 mL) and extracted with $CHCl_3$ (2 × 100 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated to an oily residue. Two consecutive purifications by silica gel column chromatography using a stepwise gradient of MeOH (0-6 %) in $CHCl_3$ afforded the title compound as a white foam. Yield 566 mg (54.3 %). $\delta_P(CDCl_3)$ 29.82; HRMS Found: $(M-H)^-$, 519.1088. $C_{24}H_{26}N_2O_7ClP$ requires *M*-H, 519.1088

(Benzyl 2-chlorophenyl 5'-deoxy-5'-*C*-phosphonomethyl)thymidin-3'-yl 2-chlorophenyl (3'-*O*-*tert*-butyldiphenylsilyl-thymidin-5'-yl)methylphosphonate (8)

5 (1.02 g, 1.33 mmol), **7** (521 mg, 1.00 mmol), and 4-methoxypyridine-1-oxide (501 mg, 4.00 mmol) were dried by evaporation of added pyridine (20 mL) and dissolved in pyridine (20 mL). To this 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane (738 mg, 4.00 mmol) was added, the reaction mixture stirred for 2 h, partitioned between aqueous 0.5 M sodium bicarbonate (50 mL) and $CHCl_3$ (50 mL), and extracted with $CHCl_3$ (2 × 50 mL). The combined organic extracts were dried over Na_2SO_4, concentrated to an oil, and traces of pyridine were removed by evaporation of added toluene (2 × 50 mL). Silica gel column chromatography using a stepwise gradient of MeOH (0-4 %) in $CHCl_3$ afforded the title compound as a white

foam. Yield 972 mg (82.9 %). $\delta_P(CDCl_3)$ 28.70, 28.72, 28.96, 28.98, 29.80 and 29.82; HRMS Found: $(M-H)^-$, 1169.2913. $C_{57}H_{62}N_4O_{13}Cl_2P_2Si$ requires *M*-H, 1169.2857

(Benzyl 5'-deoxy-5'-*C*-phosphonomethyl)thymidin-3'-yl (3'-*O*-*tert*-butyldiphenylsilyl-thymidin-5'-yl)methylphosphonate, bis-triethylammonium salt (9)

To a solution of **8** (879 mg, 0.75 mmol) in dioxane-H_2O (3:1, 150 mL), 2-pyridinealdoxime (733 mg, 6.00 mmol) and 1,1,3,3-tetramethylguanidine (751 μL, 6.00 mmol) were added and the reaction mixture was stirred at RT. When the ^{31}P-NMR analysis shown a complete removal of the 2-chlorophenyl groups (ca 1 h) the mixture was partitioned between 1 M TEAB (25 mL) and $CHCl_3$ (2 × 25 mL). The combined organic phases were dried over Na_2SO_4 and evaporated. Purification by silica gel chromatography using a stepwise gradient of MeOH (0-30 %) in $CHCl_3$-Et_3N (995:5, v/v) afforded the title compound as a white foam. Yield 696 mg (80.5 %). $\delta_H(CDCl_3)$ 1.06 (9 H, s), 1.21 (18 H, br), 1.4-2.2 (11 H, m), 1.86 (1 H, s), 1.91 (1 H, s), 2.44 (1 H, m), 2.90 (12 H, br), 4.02 (2 H, m), 4.17 (1 H, m), 4.56 (1 H, m), 4.94 (2 H, d, *J* 7.3), 6.11 (1 H, pt, *J* 7.0), 6.24 (1 H, dd, *J* 8.1, 5.5) and 7.0-7.7 (17 H, m); $\delta_C(CDCl_3)$ 9.0, 12.6, 19.1, 22.8, 23.2, 24.8, 25.2, 27.0, 28.5, 39.0, 39.3, 45.3, 65.8, 65.9, 76.0, 76.1, 84.6, 86.0, 86.5, 86.7, 87.6, 87.9, 110.8, 111.1, 127.3, 127.4, 127.9, 128.2, 130.0, 133.2, 133.3, 135.3, 135.8, 136.2, 139.3, 139.4, 150.5, 150.6, 164.1 and 164.3; $\delta_P(CDCl_3)$ 23.35 and 24.31; HRMS Found: $(M+H)^-$, 949.3042. $C_{45}H_{54}N_4O_{13}P_2Si$ requires *M*+H, 949.3010

(Benzyl 5'-deoxy-5'-*C*-phosphonomethyl)thymidin-3'-yl (thymidin-5'-yl)methylphosphonate, disodium salt (10)

9 (577 mg, 0.50 mmol) was dissolved in freshly distilled THF (4 mL) was treated with tetrabutylammonium fluoride trihydrate (630 mg, 2.00 mmol) for 4 h. Water was added (1 mL), THF removed by evaporation, and the residue was partitioned between diethyl ether (5 mL) and H_2O (5 mL). The aqueous phase was concentrated and passed through a Dowex® 50W (Na^+) column using water as an eluent. The appropriate fractions were pooled, concentrated and applied on a column of Sephadex® G10 resin. Elution with H_2O followed by lyophilization of nucleotide-containing fractions afforded the title compound as a white solid. Yield 363 mg (96.0 %). $\delta_H(D_2O)$ 1.6-2.0 (8 H, m), 1.87 (6 H, s), 2.2-2.5 (4H, m), 3.93 (1 H, m), 4.02 (1 H, m), 4.31 (1 H, m), 4.61 (1 H, m), 4.90 (2 H, d, *J* 7.3), 6.19 (2 H, pt, *J* 6.8) and 7.3-7.5 (7 H, m); $\delta_C(D_2O)$ 12.3, 22.6, 23.3, 24.6, 25.3, 27.9, 27.9, 28.0, 28.1, 38.3, 38.8, 66.8, 66.9, 74.0, 76.6, 76.7, 85.7, 85.9, 86.7, 86.8, 87.0, 87.0, 87.1, 87.4, 112.3, 112.4, 128.3, 128.8, 129.4, 137.8, 138.0, 138.7, 152.3, 167.0 and 167.0; $\delta_P(D_2O)$ 26.48 and 26.92; HRMS Found: $(M+H)^-$, 711.1860. $C_{29}H_{36}N_4O_{13}P_2$ requires *M*+H, 711.1832

(5'-Deoxy-5'-*C*-phosphonomethyl)thymidin-3'-yl (thymidin-5'-yl)methylphosphonate, disodium salt (11)

To a solution of **10** (189 mg, 0.25 mmol) in EtOH-H_2O (1:1, v/v, 25 mL) Pd/C (10 %, 60 mg) was added, and the reaction mixture was stirred under an atmosphere of hydrogen until ^{31}P- NMR analysis indicated a complete debenzylation (ca 30 min). The catalyst was then removed by filtration through Celite® and the solvents evaporated. After a final desalting on a Sephadex® G10 column using water as an eluent, followed by lyophilization, the title compound was obtained as a white solid. Yield 142 mg (85.2 %). $\delta_H(D_2O)$ 1.6-2.0 (8 H, m) 1.90 (3 H, s), 1.92 (3H, s), 7.47 (1 H, s), 7.48 (1 H, s) (For the sugar rings protons, see Table 1); $\delta_C(D_2O)$ 12.3, 23.2, 24.2, 25.3, 26.2, 27.8, 27.8, 28.4, 28.4, 38.0, 38.7, 73.9, 76.6, 76.7, 85.7, 87.0, 87.1, 87.3, 87.4, 112.4, 112.6, 137.9, 138.1, 152.4, 167.1 and 167.1; $\delta_P(D_2O)$ 24.25 and 26.55; HRMS Found: $(M+H)^-$, 621.1379. $C_{22}H_{30}N_4O_{13}P_2$ requires *M*+H, 621.1363

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