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Stereospecific conversion of H-phosphonates into phosphoramidates. The use of vicinal carbon-phosphorus couplings for configurational determination of phosphorus

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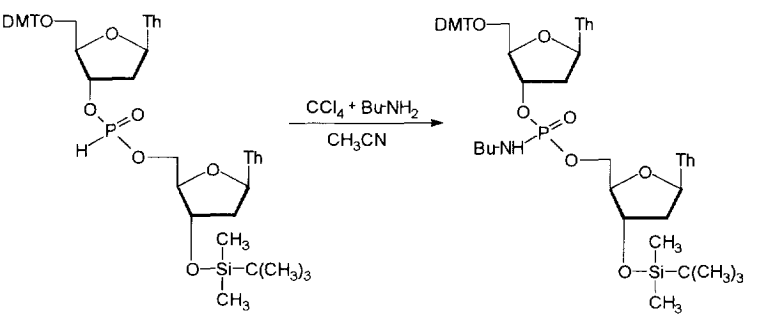
Abstract: Separated P-diastereomers of H-phosphonate diesters gave exclusively one isomer of amidates on treatment with CCl_4 + butylamine with inversion of configuration. NOE experiments and the trend observed in ^3J(C2-P) and ^3J(C4-P) couplings were applied for stereochemical assignment.

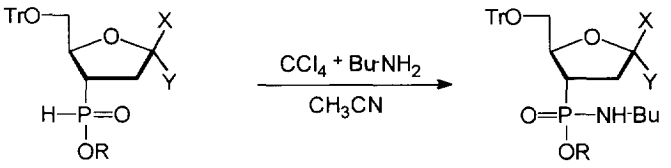
Increasing interest in phosphate-modified oligodeoxynucleotides (antisense oligodeoxynucleotides[1]) as potential new therapeutic agents[2] against viral diseases focused attention on the stereospecific synthesis of chiral phosphate derivatives[3] (phosphorothioates, methylphosphonates, phosphoramidates) which are considerably resistant to intracellular hydrolytic enzymes (DNases). Modification of charged phosphate diester moiety in the backbone of natural oligodeoxynucleotides to neutral phosphoramidates may as well facilitate their penetration through cellular membranes.

In the course of our investigations aimed at the synthesis of phosphoramidates adaptable to automated procedure on solid support we found that Todd's method[4] is not only exceptionally simple and effective for the conversion of H-phosphonate diesters into phosphoramidate diesters but it represents a highly stereospecific route as well. Treatment of the protected isomer (S_P or R_P) of dithymidinyl (3'→5') H-phosphonate **1** with carbon tetrachloride and n-butylamine in acetonitrile afforded exclusively one isomer of the corresponding amidate **2** (*uf* or *df*, resp) in high yield. (Symbols *uf* or *df* designate that isomer of a P-diastereomeric pair which gives a ^{31}P NMR signal *up*field or *down*field in $CDCl_3$ as compared with the other.)



1 (S_P or R_P) **2** (*uf* or *df*)

With the aim to elucidate the type of the stereospecificity observed (retention, inversion), we examined several simplified models exerting less entangled NMR spectra than **2**.



3 **4**

	R	X	Y
a		H	OCH_3
b	$-CH_3$	OCH_3	H
c		OCH_3	H
d	$-CH_2Ph$	OCH_3	H
e		OCH_3	H

Unfortunately **3b** could not be separated even partially to diastereomers on silica gel probably due to the insufficient difference in the steric bulk of =O and -OCH_3 groups. On the contrary, clean separation of **3a**, **c**, **d**, **e** on silica gel column eluting with hexane-ethyl acetate mixture afforded pure diastereomers. It has been found (TLC, ^{31}P NMR) that *uf* isomer of H-phosphonates **3c**, **d**, **e** on treatment with 10 eq carbon tetrachloride and 5 eq n-butylamine in acetonitrile solution (ambient temperature, 5-10 min) afforded exclusively the *df* isomer of amidates **4c**, **d**, **e**, respectively. Under identical conditions the *df* isomer of H-phosphonates **3c**, **d**, **e** gave rise to the corresponding *uf* amidates **4c**, **d**, **e**. (Apparent deviation from this regularity in the case of **3a** → **4a** should be attributed to change in the configuration of the glycosidic OCH_3 group which on the α-side of the furanose ring may exert unpredictable influence upon the phosphorus resonance). Seela and Kretschmer[5] demonstrated that in a given solvent the conformity in the shielding of the ^{31}P NMR signals of constitutionally related H-phosphonate diastereomers arise from homochiral arrangement of the corresponding ligands at phosphorus, i. e., in the present case, *uf* isomers of **3c**, **d**, **e** all have the same S_P or R_P configuration, consequently the corresponding *df* isomers belong to the complementary R_P or S_P class. It is reasonable to assume that similar regularity holds true for amidates **4c**, **d**, **e**., however, the absolute configuration of these compounds cannot be anticipated.

Recently, the one- and two-dimensional NOE and ROESY methods[5-9] were succesfully applied to determine the configuration of the diastereomeric dinucleoside methylphosphonates, and the resulting stereochemical assignments were in agreement with X-ray and CD data available for some of the molecules[5,9].

Using the NOE difference method for both isomers of type **4d** distinguishable NOE enhancements were measured by irradiation of the NH resonance. This proton showed NOE correlation with H3 and H4 ring protons in *df*-**4d**, while irradiation of the NH multiplet in *uf*-**4d** gave, in addition to an effect on H3, a week NOE correlation with the H2 protons. Considering, that the small chemical shift difference between NH and H2 protons in $CDCl_3$ might result in false signals, the NOE experiment of *uf*-**4d** was repeated in deuterobenzene solution. The NOE effects were similar to those obtained in $CDCl_3$ solution (H3 : medium, H2 : week and no effect on the signal of H4). These observations, in agreement with those determined for the methylphosphonates, gave the R_P configuration for *df*-**4d** and S_P configuration for *uf*-**4d**. However, all efforts failed to observe discernible differences in NOE and ROE data for diastereomeric H-phosphonates *uf*-**3d** and *df*-**3d**.

By contrast, the ^3J(C2-P) and ^3J(C4-P) couplings provided information about the configuration. In all compounds studied here the ^3J(C4-P) couplings have always higher value than ^3J(C2-P) of the same molecule (Table 2.), revealing the preponderance of εgt conformation[10]. On the other hand, we have found that the difference of the above coupling values is characteristic for the stereochemistry (Table 3.). Thus for *df*-**4d** with R_P configuration the ΔJ between the ^3J(C4-P) and ^3J(C2-P) couplings was 4.5 Hz, while for *uf*-**4d** with S_P configuration the ΔJ = 3.1 Hz. These findings were conclusive in all phosphoramidates, i. e., in the *df*-**4** isomers the difference of the measured ^3J(C4-P) and ^3J(C2-P) couplings varied between 4.7 and 5.4 Hz, while for the *uf*-**4** isomers the ΔJ value was 3.0 - 3.7 Hz. Consequently, the configuration of all the *df*-**4** compounds was assigned as R_P, and the *uf*-**4** amidates are the S_P isomers. This observation is valid also for H-phosphonates. In the *uf* isomers of **3c**, **d**, **e** the ΔJ values are 2.6 -3.1 Hz, while in the *df* isomers this difference varies between 3.6 - 4.0 Hz, revealing the S_P configuration for the former and R_P configuration for the latter isomers (Table 3.). Since *df*-**4** and *uf*-**4** amidates are obtained from *uf*-**3** and *df*-**3** H-phosphonates, respectively, the trend observed in the coupling constant differences provides direct proof for the inversion of configuration in the amidate forming reaction.

Another spectral feature may help to identify the configuration of the *uf* and *df* isomers of compound **3a**, where ^{13}C NMR data were not measured. For **3c**, **d**, **e** we always observed higher ^1J(H-P) values for the S_P than for the R_P diastereomers (Table 3.). Therefore the S_P configuration is proposed for *df*-**3a** and R_P for the *uf*-**3a** isomer.

Similarly to model compounds, no difference was found between the ring-proton coupling constants of the diastereomeric dinucleosidyl phosphoramidates *uf*-**2** and *df*-**2**, (Table 1.). On the other hand we failed to find uniform relation between chromatographical mobilities and ^{31}P chemical shifts, moreover, direct correlation with literature spectral data for the precursor **1**[5] was hampered by the solvent dependence of the above parameters. However, since the phosphorus is identically bounded in **2** as in the model phosphoramidates, the ^3J(C2-P) and ^3J(C4-P) couplings can be compared in configurational terms. This comparison established the R_P configuration for *uf*-**2** and S_P configuration for *df*-**2** isomers (ΔJ = 4.6 and 0.6 Hz, respectively).

Table 1. ^{1}H chemical shifts (in $CDCl_3$, δ ppm from TMS) and coupling constants (Hz, in brackets)

	uf-**3d**	*df*-**3d**	*df*-**4d**	*uf*-**4d**	*uf*-**2**	*df*-**2**
H1	5.11(5.0 + 3.2)	5.08(4.8 + 3.5)	5.11(5.0 + 3.1)	5.14(5.0 + 3.1)	6.44 (9+5.5)	6.45(9+5.5)
H2	2.20(14.0+6.4+3.2) 2.25(14.0+5.1+5.0)	2.18 - 2.22	2.24(13.8+6.2+3.1) 2.30(13.8+5.0+5.0)	2.24(13.8+6.3+3.1) 2.31(13.8+5.0+5.0)	2.42 (14+9+1*+5.5) 2.71 (14+5.5+1.3)	2.42 (14+9+1.5*+5.5) 2.61 (14+5.5+1.3)
H3	5.00(6.4+5.1+3.5+8.5*)	4.99(6.2+5.5+3.5+8.5*)	4.89(6.2+5.0+3.2+6.8*)	4.93(overlapped)	5.14 (5.5+2.5+1.3+7.0*)	5.13 (5.5+2+1.3+7.0*)
H4	4.18(5.7+5.3+3.5)	4.18(5.5+5.5+3.5)	4.24(5.8+5.8+3.2)	4.25(5.6+5.6+3.2)	4.22 (2.8+2.5+2.5)	4.31 (2.5+2.5+2)
H5	3.18(10.0+5.3) 3.21(10.0+5.7)	3.21(5.5)	3.20(5.8)	3.17(9.9+5.6) 3.23(9.9+5.6)	3.34 (10.5+2.5) 3.57 (10.5+2.8)	3.41 (10.5+2.5) 3.51 (10.5+2.5)
H1'	-	-	-	-	6.19 (6.6 + 6.7)	6.18 (6.5+6.5)
H2'	-	-	-	-	2.19 (13+6.6+4) 2.27 (13+6.5+6.7)	2.09 (13+6.5+6.5) 2.25 (13+6.5+3.5)
H3'	-	-	-	-	4.45 (6.5+4+4)	4.36 (6.5+4+3.5)
H4'	-	-	-	-	3.99 (4+3.8+3.3+1.8*)	3.97 (4+4+3.8+1*)
H5'	-	-	-	-	4.13 (11.2+3.8+5.5*) 4.23 (11.2+3.3+6.0*)	4.05 - 4.41
-OCH_2Ph (or -OCH-)	4.98(11.8+9.7*) 5.05(11.8+9.7*)	5.3(11.9+9.8*) 5.08(11.9+9.8*)	4.98(7.8*)	4.90(11.8+8.1*) 4.95(11.8+8.1*)	- -	- -
-OCH_3	3.29	3.28	3.30	3.30	-	-
-PH	6.80 (707.3*)	6.83 (704.1*)	-	-	-	-
NH	-	-	2.42(7.0+7.0+10.6*)	2.46(7.0+7.0+10.6*)	overlapped	overlapped
N-$CH_2\alpha$	-	-	2.75	2.78	2.78	2.91
$CH_2\beta$+$CH_2\gamma$	-	-	1.15 - 1.35	1.20 - 1.40	1.20 - 1.45	1.25 - 1.50
CH_3	-	-	0.83	0.84	0.86	0.89

* couplings with phosphorus

Table 2. ^{13}C NMR data of relevant carbons (in $CDCl_3$, chemical shifts in δ ppm with TMS as internal standard, coupling constants in Hz)

	uf-**3d**	*df*-**3d**	*df*-**4d**	*uf*-**4d**	*uf*-**2**	*df*-**2**
C1	86.80	86.78	86.76	86.74	84.33	84.42
C2	40.20 ($^3J_{C2,P}$ = 3.6)	40.12 ($^3J_{C2,P}$ = 3.0)	40.10 ($^3J_{C2,P}$ = 3.0)	40.00 ($^3J_{C2,P}$ = 3.6)	39.17 ($^3J_{C2,P}$ = 2.7)	39.14 ($^3J_{C2,P}$ = 4.5)
C3	77.01 ($^2J_{C3,P}$ = 5.6)	76.99 ($^2J_{C3,P}$ = 5.4)	77.25 ($^2J_{C3,P}$ = 5.1)	77.28 ($^2J_{C3,P}$ = 5.4)	77.62 ($^2J_{C3,P}$ = 4.4)	77.73 ($^2J_{C3,P}$ = 4.7)
C4	83.56 ($^3J_{C4,P}$ = 6.2)	83.46 ($^3J_{C4,P}$ = 6.9)	83.77 ($^3J_{C4,P}$ = 7.5)	83.91 ($^3J_{C4,P}$ = 6.7)	84.61 ($^3J_{C4,P}$ = 7.3)	85.02 ($^3J_{C4,P}$ = 5.1)
C5	63.82	63.97	64.58	64.39	63.42	63.49
C1'	-	-	-	-	85.76	85.66
C2'	-	-	-	-	41.03	41.22
C3'	-	-	-	-	71.31	71.80
C4'	-	-	-	-	85.09 ($^3J_{C4',P}$ = 7.8)	85.27 ($^3J_{C4',P}$ = 7.4)
C5'	-	-	-	-	65.12 ($^2J_{C5',P}$ = 4.9)	65.71 ($^2J_{C5',P}$ = 5.5)
OCH_2PH or OCH	67.48 ($^2J_{C,P}$ = 5.6)	67.43 ($^2J_{C,P}$ = 5.5)	67.87 ($^2J_{C,P}$ = 5.1)	67.91 ($^2J_{C,P}$ = 5.1)	-	-
OCH_3	55.42	55.39	55.44	55.44	-	-
N-$CH_2\alpha$	-	-	41.14	41.13	40.40	40.48
N-$CH_2\beta$	-	-	33.69 ($^3J_{C,P}$ = 5.9)	33.66 ($^3J_{C,P}$ = 6.3)	33.65 ($^3J_{C,P}$ = 6.1)	33.76 ($^3J_{C,P}$ = 6.3)
N-$CH_2\gamma$	-	-	19.69	19.70	19.65	19.71
CH_3	.	.	13.72	13.72	13.69	13.62

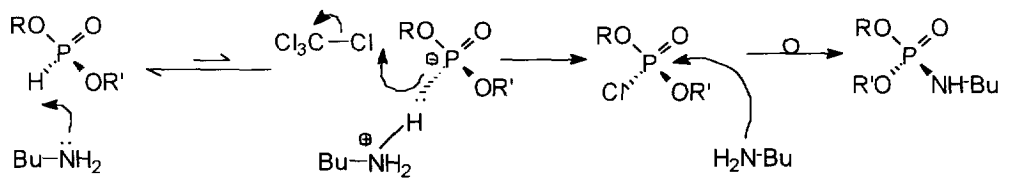
We can observe a similar trend in the pertinent couplings of some diastereomeric phosphate methyl esters[11], where the ΔJ value was 4.3 Hz for the R_P isomers and 1.8 - 2.4 Hz for the S_P isomers.

Table 3. ^{13}C and ^{31}P spectral characteristics of diastereomers

		^{31}P	ΔJ ($J_{C4,P} - J_{C2,P}$) (Hz)	$^{1}J_{H\text{-}P}$ (Hz)	config.
4a	*df*	8.34	5.4	-	R_P
	uf	8.16	3.4	-	S_P
3a	*df*	6.51	*	702.0	S_P
	uf	6.46	*	699.5	R_P
4c	*df*	8.15	4.7	-	R_P
	uf	7.83	3.7	-	S_P
3c	*df*	6.45	4.0	693.6	R_P
	uf	5.98	3.0	698.0	S_P
4d	*df*	9.23	4.5	-	R_P
	uf	8.96	3.1	-	S_P
3d	*df*	7.66	3.9	704.1	R_P
	uf	7.30	2.6	707.3	S_P
4e	*df*	8.10	5.2	-	R_P
	uf	7.78	3.2	-	S_P
3e	*df*	6.03	3.6	693.9	R_P
	uf	5.57	3.1	698.2	S_P
2	*df*	9.69	0.6	-	S_P
	uf	9.43	4.6	-	R_P

* not measured

Probable mechanism of amidate forming reaction implies deprotonation of the H-phosphonate by amine base as the first step. This view is supported by the fact that the mixture of H-phosphonate diester **1** and carbon tetrachloride in acetonitrile remains unchanged up to the addition of butylamine which in turn causes immediate conversion to amidate **2**. Configurational stability of P-anions derived from H-phosphonate diesters has been documented by Seela and Kretschmer[5] who found that treatment of diastereomerically pure H-phosphonate diesters with butyllithium followed by the addition of methyl iodide gave only one isomer of methylphosphonate diesters with retention of configuration. Based on this fact it is reasonable to assume that the intermediary phosphoric diester chloridate is formed with retention of configuration and converted by butylamine to the corresponding amidate with inversion.



Experimental part

^1H, ^{13}C, and ^{31}P NMR spectra were recorded with a Varian VXR 400 spectrometer, with TMS as internal standard or with H_3PO_4 as external standard. ^{13}C-^1H correlation experiments were carried out to facilitate ^{13}C signal assignments. The componds (10 - 13 mg) were dissolved in 0.6 ml deuterochloroform. All the spectra were recorded at ambient temperature. The ^{13}C spectral width for compounds **3b, c, e** and **4a, b, c, e** was 125000 Hz and for compounds **3d, 4d, 2** was 5000 Hz (covered by 32 K data points). Zero-zero filling was applied for resolution enhancement giving digital resolutions 0.39 Hz and 0.16 Hz, respectively.

Protected dithymidinyl (3'→5') H-phosphonate (**1**) was prepared according to Seela and Kretschmer[5]. Carbon tetrachloride and butylamine were refluxed over calcium hydride, distilled and stored over 4A molecular sieve. Acetonitrile was twice distilled from P_2O_5, then from calcium hydride and stored on 4A molecular sieve.

General procedure for the preparation of H-phosphonate diesters

5-O-Trityl-2-deoxy-β-methylriboside (2 mmol) was dissolved in 20 ml CH_2Cl_2 containing 0.1 ml triethylamine. To this solution was added 0.63 ml (2.1 mmol) tris(1,1,1,3,3,3-hexafluoro-2-propyl) phosphite[12] with vigorous stirring. After 15 min 25 ml 1 M aqueous triethylammonium bicarbonate were poured into the rection mixture and stirring continued for 1 hour. Phases were separated, the aqueous part extracted with 2 x 10 ml CH_2Cl_2 and dried over $MgSO_4$. Evaporation of the solvent gave a syrup which was purified by chromatography on silica gel eluting with chloroform-methanol 10:1 containing 1% triethylamine. The solid foam which was obtained from appropriate fractions (0.94 - 0.96 g, 90 - 92%) was dissolved in 20 ml CH_2Cl_2, 3 mmol of the corresponding alcohol and 6 mmol N-methylimidazole were added followed by 3 mmol p-toluenesulfonyl chloride. The reaction was monitored by TLC (hexane-ethyl acetate) and was found to be complete in 5 to 10 min. The reaction mixture was washed with 2 x 10 ml H_2O, dried over $MgSO_4$ and subjected to chromatography on silica gel eluting with hexan-ethyl acetate 1:1. In the case of β-riboside the more polar isomers gave upfield ^{31}P signals (*uf*-isomers) while the less polar ones resonate downfield (*df*-isomers), however, this rule is violated in the case of α-riboside.

General procedure for the preparation of amidates

H-phosphonate diester (0.5 mmol) obtained in the above procedure was dissolved in 10 ml dry acetonitrile. To this solution were added 0.5 ml (5 mmol) carbon tetrachloride and 0.25 ml (2.5 mmol) n-butylamin succesively. The reaction was complete in 5 - 10 min as evidenced by TLC (EtOAc + 0.1% AcOH, in the case of **2**, hexane-ethyl acetate 1:1, in the cases of **4**). The solvent was removed by evaporation on reduced pressure and the residue subjected to column chromatography on silica gel eluting with EtOAc (in the case of **2**) or hexane-ethyl acetate 2:1 (in the cases of **4**). Amidates were obtained in 90 - 92 % yield.

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References

1. Uhlmann, E. and Peyman, A. *Chemical Reviews* **1990**, *90*, 543 - 584
2. Milligan, J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923 - 1937
3. Stec, W. J.; Wilk, A. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 709 -722
   Lesnikowski, Z. J. *Bioorganic Chemistry* **1993**, *21*, 127 - 155
   Suska, A.; Grajkowski, A.; Wilk, A.; Uznanski, B.; Blaszczyk, J.; Wierczorek, M.; Stec, W. J. *Pure & Appl. Chem.* **1993**, *65*, 707 -714
   Stec, W. J.; Grajkowski, A.; Koziolkiewivz, M.; Uznanski, B. *Nucleic Acids Res.* **1991**, 19, 5883 - 5888
   Wozniak, L. A.; Pyzowski, J.; Wieczorek, M.; Stec, W. J. *J. Org. Chem.* **1994**, *59*, 5843 -5846
   Patil, S. V.; Mane, R. B.; Salunkhe, M. M. *Bioorganic & Medicinal Chemistry Lett.* **1994**, *4*, 2663 - 2666
4. Atherton, F. R.; Opeshaw, H. J.; Todd, A R. *J. Chem. Soc.* **1945**, 660 - 663
   Atherton, F. R.; Todd, A. R. *J. Chem. Soc.* **1947**, 674 - 678
   Steinberg, G. M. *J. Org. Chem.* **1950**, *15*, 637 - 647
5. Seela, F. and Kretschmer, U. *J. Org. Chem.* **1991**, *56*, 3861 - 3869
6. Kan, L. S.; Cheng, D. M.; Miller, P. S.; Yano, J. and Ts'o, P. O. P. *Biochemistry* **1980**, *19*, 2122-2132
7. Wang, C.; Wang, L.; Yang, X.; Jiang, T. and Zhang, L. *Nucleic Acids Res.* **1993**, *21*, 3245-3248
8. Lebedev, A. V.; Frauendorf, A.; Vyazovkina, E. V. and Engels, J. W. *Tetrahedron* **1993**, *49*, 1043-1052
9 Löschner, T. and Engels, J. W. *Nucleic Acids Res.* **1990**, *18*, 5083 - 5088
10. Wijmenga, S. S.; Mooren M. M. W. and Hilbers, C. W. *NMR of Nucleic Acids; From Spectrum to Structure;* Roberts, G. C. K. Ed; Oxford University Press, 1993; pp. 217 - 288
11. Herdering, W. and Seela, F. *J. Org. Chem.* **1985**, *50*, 5314 - 5323
12. Hosaka, H.; Suzuki, Y.; Sato, H.; Gug-Kim, S.; Takaku, H. *Nucleic Acids Res.* **1991**, *19*, 2935 - 2940

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