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Synthesis of Some Pyridoxine and Pyridoxal Halophosphonates.

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A new series of pyridoxine and pyridoxal chloro and fluorophosphonates have been synthesized. Partially protected pyridoxine **1**, **2** or pyridoxal **4** reacted with methyl phosphonic dichloride in the presence of triethylamine at $-10\text{ }^{\circ}\text{C}$. Thus, the following chlorophosphonate derivatives were obtained: 3,4'-*O*-isopropylidene-pyridoxine-5'-*O*-methylchlorophosphonate (**5**), 4',5'-*O*-isobutylidene-pyridoxine-3-*O*-methylchlorophosphonate (**6**), and monoethylacetalpyridoxal-3-*O*-methylchlorophosphonate (**7**). Similarly, from adequate starting compounds **1**, **3** and unprotected pyridoxal (PL) in the reaction with methyl phosphonic difluoride and triethylamine, near $0\text{ }^{\circ}\text{C}$, the corresponding 3-*O* or 5'-*O*-methylfluorophosphonates were obtained; 3,4'-*O*-isopropylidene-pyridoxine-5'-*O*-methylfluorophosphonate (**8**), 4-methoxymethylpyridoxine-5'-*O*-methylfluorophosphonate (**9**) and pyridoxal-5'-*O*-methylfluorophosphonate (**10**).

INTRODUCTION

Pyridoxal-5'-phosphate (PLP) is a molecule which appears as a coenzyme in a group of enzymes such as aminotransferase,¹⁻³ glycogen phosphorylase,⁴ decarboxylase⁵ and many others. Extensive research has been carried out in order to clarify the catalytic activity of the enzymes, as well as to define

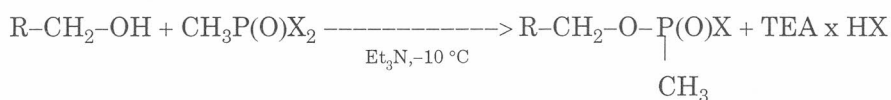
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the structural interrelationship and the significance of individual functional groups in the molecule, at position-4⁶⁻⁹ and position-5.¹⁰

In our recent work,^{11,12} a synthetic pathway for the fluorination of some pyridoxine derivatives (PN) or pyridoxal-5'-phosphate (PLP) is described.

A preliminary biological study¹¹ on the activity of the fluorinated coenzyme of aminotransferase, pyridoxal-5'-fluorophosphate (PL-FP), when reconstituted with an apoenzyme of aminotransferase, has proved that it functions as a coenzyme and is fully catalytically competent. This indicates that substitution of the hydroxyl group in the phosphate group for fluorine does not specifically impair the binding of the coenzyme to the protein. Furthermore, the fluorine presence in the phosphate moiety does not alter the coenzyme alignment in the protein cavity. It is believed that it has no deleterious effect on the coenzyme molecule's role in catalysis.

To investigate further the relationship of coenzyme structure in the phosphate moiety towards activity, we describe in this paper the syntheses of similar compounds to those of fluorophosphates^{11,12} with the P-F bond, the fluorophosphonates. These are compounds with an -O-P(O)XCH₃ group, where X- is a chloro or fluoro atom. The group is introduced at different positions of PN or PL molecules. The synthesis was carried out by the methods described by Tammelin *et al.*¹³⁻¹⁵ and Cheymol *et al.*¹⁶ where an aminoalcohol reacts with methyl phosphonic dichloride or methyl phosphonic difluoride, yielding the corresponding methylchloro or methylfluorophosphonates (Scheme 1).



X = Cl or F

Scheme 1.

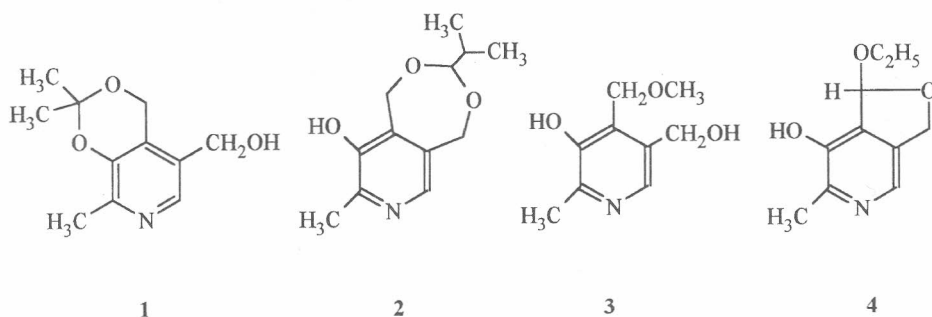
The main reason for the synthesis of chlorophosphonate derivatives was their capability to serve as starting products for the synthesis of fluorophosphonates, in the reaction with NaF. In our previous work,¹² in the reaction of dichlorophosphate and NaF in the presence of crown-ether, a number of difluorophosphates were obtained. Nevertheless, no similar method could be applied to the synthesis of fluorophosphonates due to the instability of starting chlorophosphonates 5-7.

Monard and Quinshon,¹⁷ Zeffert,¹⁸ and Benschop *et al.*¹⁹ have described another reaction procedure for the synthesis of methylfluorophosphonates, where, instead of methyl phosphonic difluoride, a mixture of both, methyl phosphonic dichloride and methyl phosphonic difluoride, in a molar ratio 1 : 1, was used. The reaction mechanism was proposed by Zeffert.¹⁸ During

the reaction, in its first step, a mixed reagent of an methylphosphonicfluorochloride ($\text{CH}_3\text{P}(\text{O})\text{ClF}$) is formed, which reacts further with the alcoholic group. The reaction proceeds in such a manner that, finally, a compound with a stronger P-F bond is formed.

RESULTS AND DISCUSSION

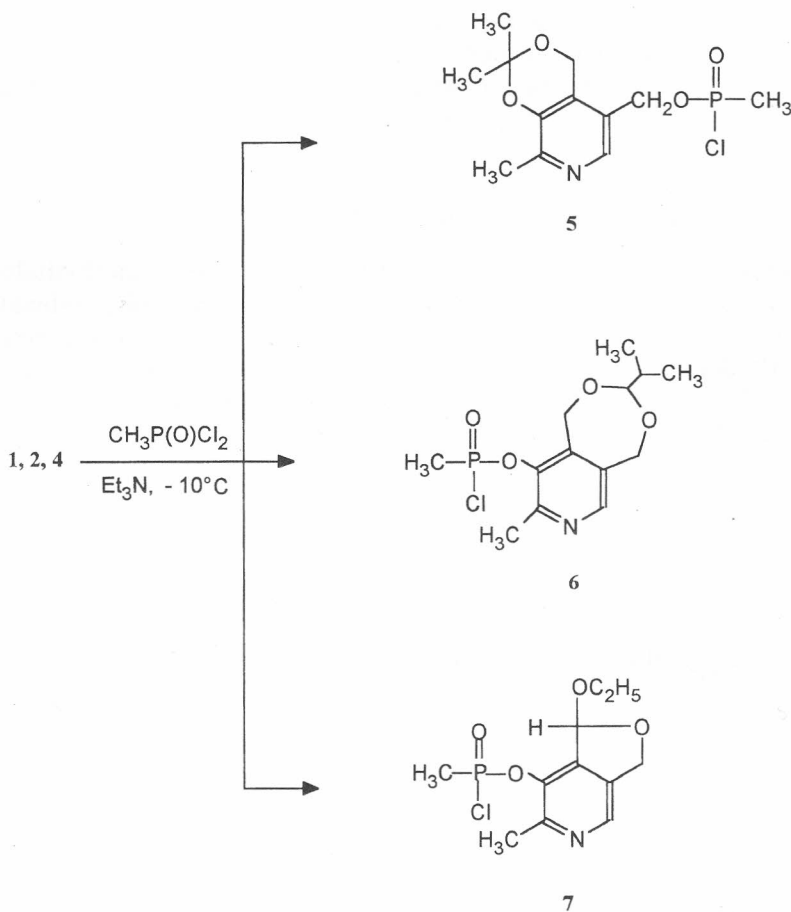
An experimental procedure for the synthesis of some methylhalophosphonates, starting with some protected pyridoxine (PN) and pyridoxal (PL) as well as with unprotected pyridoxal, is described. The following protected PN and PL derivatives were prepared as starting materials: 3,4'-O-isopropylidenepyridoxine (**1**),^{12,20} 4,5'-O-isobutylidenepyridoxine (**2**),^{21,22} 4'-O-methylpyridoxine (**3**),²³ and monoethylacetalpyridoxal (**4**).²⁴



The synthesis of methylchlorophosphonates of PN and PL derivatives

Derivatives of pyridoxine **1**, **2** and pyridoxal **4** (1 mmole) react with a slight excess of methyl phosphonic dichloride (1,1 or up to 1.4 mmole) and Et_3N (4–10 mmole) at -10°C , yielding the corresponding methylchlorophosphonates, **5**, **6**, and **7**, in good yields (Scheme 2). This reaction shows that both the hydroxymethyl group at position 5' and the hydroxyl group at position 3 react with methyl phosphonic dichloride. This is why unprotected PN or PL, in the reaction with methyl phosphonic dichloride, gave a mixture of several components detected by TLC (solvent system a or b). No separation of these products could be achieved using the standard procedure for separation and purification.

The following methylchlorophosphonates were isolated: 3,4'-O-isopropylidenepyridoxine-5'-O-methylchlorophosphonate (**5**), 4',5'-O-isobutylidenepyridoxine-3-O-methylchlorophosphonate (**6**) and monoethylacetalpyridoxal-3-O-methylchlorophosphonate (**7**).



Scheme 2.

The reaction progress as well as the purity of the isolated methylchlorophosphonates **5**, **6**, and **7** were monitored by TLC. The isolated methylchlorophosphonates were difficult to purify. Therefore, analyses were carried out on freshly prepared samples (yellow oils) without purification. The reaction conditions and analytical data are listed in Table I.

The synthesis of methylfluorophosphonates of PN derivatives and PL

Similarly, in the reaction of **1**, **3**, or **PL**, where hydroxymethyl groups at position 5'- of the pyridine ring are available for the reaction with methyl phosphonic difluoride, 5'-O-methylfluorophosphonates **8**, **9** and **10** were obtained. (Scheme 3, Table II – method A).

TABLE I
Pyridoxine- and pyridoxal-*O*-methylchlorophosphonates 5-7

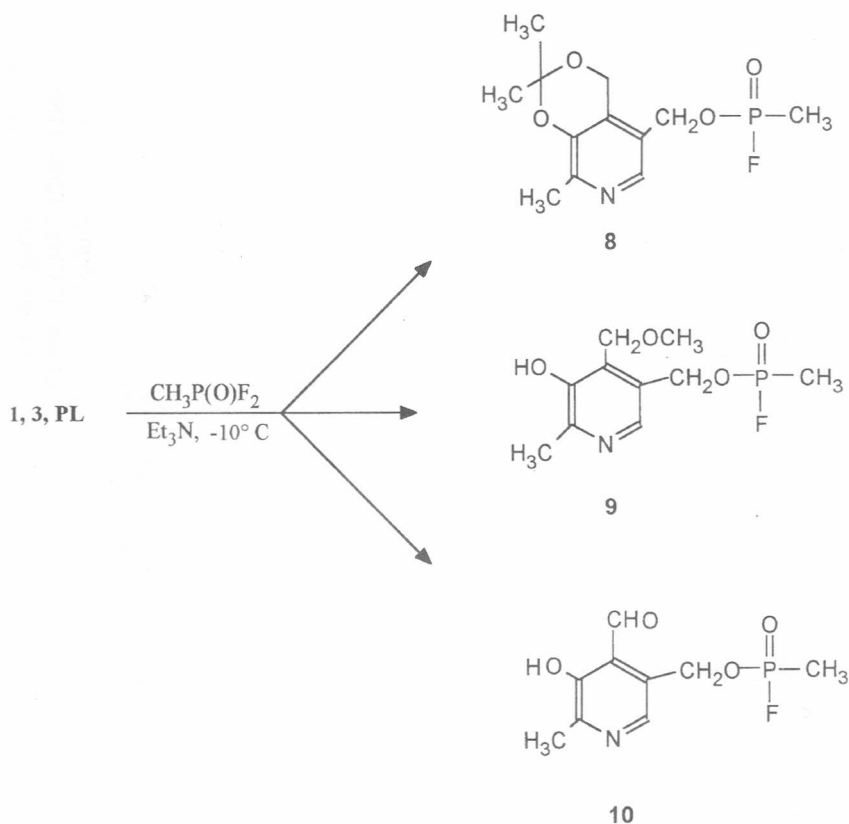
No	Ratio*	Prod.	Yield (%)	Molecular Formula	Analysis Calc./Found %C H	IR(Film) ν/cm^{-1}
1	1.1 4	5	85	C ₁₂ H ₁₇ NCIO ₄ P (305.699)	47.15 5.60 47.35 5.88	2960-2740 (-CH ₂ -CH ₃), 1610 (Py), 1320-1200 (P-CH ₃ , P=O), 1070 (C-O-C), 1030 (P-O)
2	1.1 6	6	80	C ₁₃ H ₁₉ NCIO ₄ P (319.726)	48.85 5.92 48.72 6.09	2980-2760(-CH ₂ -CH ₂ -CH ₃), 1600 (Py), 1310 (P-CH ₃), 1270-1220 (P=O), 1080(C-O-C), 1060 (P-O)
3	1.4 10	7	68	C ₁₁ H ₁₅ NCIO ₄ P (291.673)	45.30 5.18 44.92 5.58	2980, 2880 (-CH ₂ -CH ₂ -CH ₃), 1570 (Py), 1315-1200 (P-CH ₃ , P=O), 1060(C-O-C),1040 (P-O)

* Ratio of reagents /CH₃P(O)Cl₂ and TEA/ in mmole to one mmole of the starting compound.

TABLE II
Pyridoxine- and pyridoxal-*O*-methylfluorophosphonates 8-10

No	Ratio*	Meth. Prod.	Yield (%)	Molecular Formula	Analysis			IR(Film) ν/cm^{-1}
					Calc./ Found %C	H	N	
1	2.5 4	A	8	C ₁₂ H ₁₇ NFO ₄ P (289.247)	49.82	5.92	4.84	3000, 2930 (-CH ₂ -CH ₃), 1600 (Py),1315 (P-CH ₃), 1290-1220 (P=O),1080 (C-O-C), 1030 (P-O)
	1 4	B	8		49.69	6.01	4.57	
3	2 6	A	83	C ₁₀ H ₁₅ NFO ₄ P (263.210)	45.63	5.74	5.32	2940-2500 (-CH ₂ -CH ₃ ; / N ⁺ -H ⁺ ·O ⁻ / assoc.), 1600 (Py),1320(P-CH ₃),1280-1240 (P=O), 1070 (C-O-C), 1030 (P-O)
					45.38	5.92	5.09	
PL	2 6	A	10	C ₉ H ₁₁ NFO ₄ P (247.166)	43.73	4.48	5.66	2980 (-CH ₂ -CH ₃), 2740-2500(N ⁺ -H ⁺ ·O ⁻ , assoc.), 1570 (Py),1310 (P-CH ₃), 1260-1180 (P=O), 1080 (C-O-C),1060 (P-O)
					43.28	4.31	5.41	

* Ratio of reagents: CH₃P(O)F₂ and TEA (method A); CH₃P(O)F₂, CH₃P(O)Cl₂ and TEA (method B) in mmole to one mmole of the starting compound.



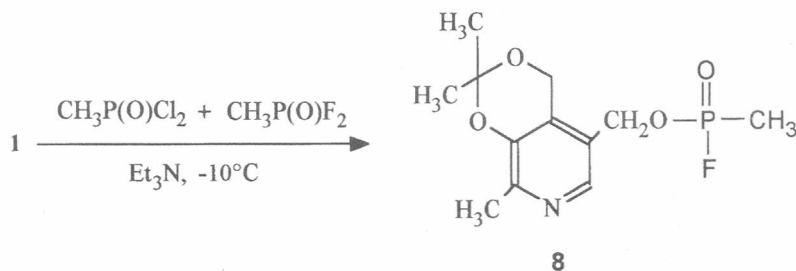
Scheme 3.

The following methylfluorophosphonates were prepared: 3,4'-O-isopropylidene-pyridoxine-5'-O-methylfluorophosphonate (**8**); 4'-O-methylpyridoxine-5'-O-methylfluorophosphonate (**9**) and pyridoxal-5'-O-methylfluorophosphonate (**10**).

In the reaction with a mixed reagent (methyl phosphonic dichloride and methyl phosphonic difluoride; method B) and compound **3** or **PL**, the corresponding **9** and **10** were obtained in similar yields (between 60 to 86 percent). Only compound **1** and mixed reagents gave 3,4'-O-isopropylidene-5'-O-methylfluorophosphonate (**8**) in a better yield (93%) than with methyl phosphonic difluoride alone (Scheme 4).

The reaction conditions and analytical data are listed in Table II.

An attempt to prepare 3-O-methylfluorophosphonates starting from **2**, **3** or **4** derivatives, with the free hydroxyl group at position 3, by using both single and mixed fluorinated agents, failed.



Scheme 4.

NMR SPECTRA

^{13}C NMR spectra were recorded in deuterated solvents (CDCl_3). Chemical shifts are reported as δ/ppm versus TMS as internal standard. The resonance of C-5' is in the range from 63 to 68 ppm, and the aromatic carbons from 98 to 151 ppm. The ^{13}C NMR chemical shifts for compounds **8**, **9** and **10** are listed in Table III.

TABLE III

^{13}C NMR chemical shifts of PN and PL derivatives **8–10** (CDCl_3/TMS)

Compound	Chemical shifts, δ/ppm
8	148.89 (C-6); 145.85 (C-3); 139.31 (C-2); 125.88 (C-4); 123.86 (C-5) $^3J_{\text{CP}} = 5.86$ Hz (d) 100.31(O-C-O); 63.00 (C-5'); $^2J_{\text{CP}} = 5.86$ Hz (d); 57.98 (C-4'); 17.81 (C-2'); 10.33 ($\text{CH}_3\text{-P}$); $^1J_{\text{PC}} = 146.53$ Hz (d) $^2J_{\text{CP}} = 26.37$ Hz (d)
9	151.16 (C-3); 148.83 (C-6); 138.26(C-2); 133.18 (C-5); $^3J_{\text{CP}} = 14.65$ Hz (d); 127.60 (C-4) $^4J_{\text{CP}} = 7.32$ Hz (d); 67.03 (C-4'); 63.65 (C-5') $^2J_{\text{CP}} = 5.86$ Hz (d); 45.78 (OCH_3); 18.98 (C-2'); 12.61 ($\text{CH}_3\text{-P}$) $^1J_{\text{CP}} = 145.30$ Hz (d) $^2J_{\text{CP}} = 36.57$ Hz (d)
10	174.22 (C-4'); 146.38 (C-6); 144.57 (C-3); 135.25 (C-5) $^3J_{\text{CP}} = 5.86$ Hz (d); 130.38 (C-2); 98.05 (C-4); 68.55 (C-5'); 18.05 (C-2'); 12.22 ($\text{CH}_3\text{-P}$) $^1J_{\text{PC}} = 143.60$ Hz (d) $^2J_{\text{CP}} = 38.09$ Hz (d)

Chemical shifts in the ^{19}F NMR of C-5'-O-substituted compounds in the range from (-)54 to (-)59 ppm and doublet splitting of signals, due to $I = 1/2$ of ^{31}P nucleus, indicate the P-F bond. Coupling constants are in the range of 940–1043.5 Hz. These values are in good agreement with the data published for some phosphofluorides.²⁶ The ^{19}F NMR data of compounds **8–10** are listed in Table IV.

TABLE IV
 ^{19}F NMR of methylfluorophosphonates **8–10**
 ($\text{CDCl}_3/\text{CFCl}_3$)

Compound	Chemical Shifts, δ/ppm	$J(\text{P-F})/\text{Hz}$
8	-54.7 (d)	960
	-58.0 (d)	1040.5
	-59.3 (d)	1043.5
9	-54.3 (d)	959
	-57.7 (d)	1043
	-59.0 (d)	1034
10	-54.5 (d)	940

EXPERIMENTAL

Reagents and materials: Pyridoxine hydrochloride was kindly donated by »Pli-va«, Pharmaceutical, Chemical, Food and Cosmetic Industry, Zagreb, Croatia. Pyridoxal hydrochloride, anhydrous, was purchased from »Sigma« Chemical Co. Thin layer chromatography (TLC) was performed on Macherey Nagel, Sil-NMR/UV 254 polygram plates, and Whatman, PE Sil G/UV polyester plates, Darmstadt, Germany.

General remarks; Melting points (m.p.), not corrected, were determined on a Totoli apparatus. TLC solvent systems were used, (a) dichloromethane-methanol (9 : 1) or (b) isopropanol-ammonia-water (7 : 1 : 2). Spots were detected with a UV lamp (254 and 366 nm) or in iodine vapour. IR spectra were recorded as films on a Perkin Elmer 457 grating spectrometer. ^{13}C NMR spectra were recorded on a JEOL FX-100 spectrometer in CDCl_3 and $(\text{CD}_3)_2\text{CO}$. ^{19}F NMR spectra were recorded on a Varian 80 A FT NMR spectrometer operating at 74.84 MHz. Spectra widths of 8000–2000 Hz and 16 K addresses were used, giving digital resolution of ± 0.02 ppm for chemical shifts and ± 1 Hz coupling constants. The samples were dissolved in $\text{DMSO}-d_6$ or CDCl_3 using deuterium lock. ^{19}F NMR chemical shifts, expressed in δ/ppm , are referenced to CFCl_3 as internal standard.

Abbreviations: r.t. room temperature; Et_3N , triethylamine; PL, pyridoxal; PN, pyridoxine; TLC, thin layer chromatography.

Methylchlorophosphonates of pyridoxine and pyridoxal derivatives.

General procedure. Chosen PN derivatives **1**, **2** and **4** (1 mmole), together with Et_3N (4–10 mmole) in 5 ml of chloroform, were slowly added under vigorous stirring to a cooled solution (-10°C) of methyl phosphonic dichloride (1.1–1.4 mmole), dissolved in chloroform (2–3 ml), for 30 minutes. Stirring was continued an additional 1–1.5 hrs at room temperature. The course of reaction was monitored by TLC (a or b), and the disappearance of the starting compound indicated the end point of the reaction.

The resulting solution was concentrated in vacuum; dry ether was added to the residue and the precipitated Et₃N hydrochloride was filtered off. The ether was flash evaporated, yielding a pale yellow oily residue. The raw products thus obtained were kept in tightly closed bottles and refrigerated. The purity of isolated compounds was determined by TLC, elemental analyses and IR (film) spectra. Table I.

*3-O,4'-O-isopropylidene*pyridoxine-5'-O-methylchlorophosphonate (**5**) was obtained starting from compound **1** (209 mg, 1 mmole) dissolved together with Et₃N (0.6 ml, 4 mmole) in 5 ml of chloroform in reaction with methyl phosphonic dichloride (145 mg, 1.1 mmole, in 2 ml of chloroform). Yield: 260 mg (85%).

*4'-O,5'-O-isobutylidene*pyridoxine-3-O-methylchlorophosphonate (**6**). Methyl phosphonic dichloride (145 mg, 1.1 mmole) in 2 ml of chloroform and PN derivative **2**, (223 mg, 1 mmole), together with Et₃N (0.9 ml, 6 mmole), in chloroform (5 ml), at -10 °C gave compound **6**. Yield: 255 mg (80%)

Monoethylacetal pyridoxal-3-O-methylchlorophosphonate (**7**) was obtained from PL derivative as hydrochloride **4**, (231 mg, 1 mmole), Et₃N (1.5 ml, 10 mmole) and methyl phosphonic dichloride (190 mg, 1.4 mmole) according to the general procedure. Yield: 199 mg (68%).

Methylfluorophosphonates of pyridoxine derivatives and pyridoxal

METHOD A

To the solution of methyl phosphonic difluoride (2–2.5 mmole, 0.14–0.17 ml) in dry chloroform, a mixture of protected PN **1** or **3** derivative or unprotected PL (1 mmole) and Et₃N (4–6 mmole), in chloroform, was added dropwise, at -10 °C, during 20–50 minutes. When addition was completed, the reaction was continued under stirring for another 30 min. at rt. The end point of the reaction was determined by TLC. The excess of solvent was evaporated and Et₃N hydrogen fluoride was precipitated with ether. The products were isolated from the reaction mixture in a similar manner as chloroderivatives. Thus, compounds **8**, **9** and **10** were isolated as purple oily products, which were more stable than chlorophosphonate derivatives. Elemental analysis and IR (film) data of TLC pure products are listed in Table II.

*3,4'-O-isopropylidene*pyridoxine-5'-O-methylfluorophosphonate (**8**) was synthesized in the reaction of methyl phosphonic difluoride (0.17 ml, 2.5 mmole) in chloroform (3–5 ml) and the mixture of compound **1** (209 mg, 1 mmole) and Et₃N (0.6 ml, 4 mmole) dissolved in 3 ml of chloroform, under stirring at -10 °C. Yield: 174 mg (60%).

*4'-O-methyl*pyridoxine-5'-O-methylfluorophosphonate (**9**). Into the solution of methyl phosphonic difluoride in chloroform (0.14 ml, 2 mmole, 3 ml), a mixture of compound **3**, as hydrochloride (220 mg, 1 mmole) and Et₃N (0.9 ml, 6 mmole) in 5 ml of chloroform, was added under vigorous stirring, at -10 °C, during 20 minutes. The purity of the dark oily product was determined by TLC (b). Yield: 220 mg (83.5%).

*Pyridoxal-5'-O-methyl*fluorophosphonate (**10**). The solution of pyridoxal hydrochloride (204 mg, 1 mmole) and Et₃N (0.9 ml, 6 mmole) in chloroform (6 ml) was added to methyl phosphonic difluoride (0.14 ml, 2 mmole) in chloroform under stirring at -10 °C, during 30 minutes. Evaporation of solvents gave an oily TLC(b) pure residue. Yield: 212 mg (86%).

METHOD B

3,4'-O-isopropilidenepyridoxine-5'-O-methylfluorophosphonate (8). A mixture of equal quantity of methyl phosphonic dichloride (128 mg, 1 mmole) and methyl phosphonic difluoride (100 mg, 0.072 ml, 1 mmole) was dissolved in 2 ml of dry chloroform and cooled to -10°C . Into the mixture, while stirring vigorously, a solution of **1** (1 mmole) and Et_3N (0.6 ml, 4 mmole) in dry chloroform (3 ml) was successively added, during 30 minutes. The stirring was continued for another 30 minutes at r.t. The solvent was evaporated and then 5 ml of dry ether was added. The precipitated Et_3N -hydrofluoride was filtered off and the ether was flash-evaporated. The purity of the oily residue was determined by TLC (b). Yield: 270 mg (93%).

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