

## Nucleoside 3'-methylphosphonofluoridates and Nucleoside 3'-methylfluorido-phosphonothioates. New Convenient intermediates in Large Scale Synthesis of Dinucleoside-(3',5')-methylphosphonates

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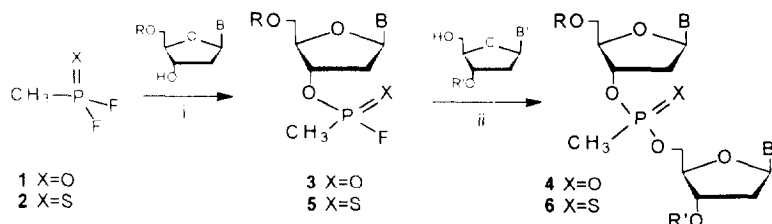
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**Abstract** Nucleoside 3'-methylphosphonofluoridates **3** and nucleoside 3'-methylfluoridophosphonothioates **5** for the first time are used directly in the synthesis of dinucleosidemethylphosphonates and methylphosphonothioates. The fluoridophosphonothioates **5** were separated into pure diastereomers.

Studies on oligonucleotide methylphosphonates originated with the pioneering work of Ts'o and Miller.<sup>1</sup> They became important antisense inhibitors of gene expression and met criteria to become useful as therapeutic agents.<sup>2</sup> Synthesis of oligonucleotides methylphosphonate via P(III) phosphorus intermediates has proved costly, inefficient and difficult to conduct for yields in excess of 2g.<sup>3</sup> In our search for a large scale synthesis of these compounds we concluded that a route via P(IV) coordinate phosphorus intermediates was feasible.

We noticed that alkylphosphonodifluoridates  $RP(O)F_2$  and their sulfur analogues  $RP(S)F_2$  react with alcohols in a chemoselective manner.<sup>4</sup> The rate of replacement of the first fluorine ligand is visibly faster than that of the second. We also anticipated that this selectivity would be even more pronounced in the reactions with 3'-OH nucleosides, due to the steric effect. Our experiments described in this communication showed these expectations to be correct.

Methylphosphonodifluoridate **1** or its sulfur analogue **2** have already been prepared by the reaction discovered earlier in this Laboratory from the commercially available dichlorides  $MeP(O)Cl_2$  or  $MeP(S)Cl_2$ .<sup>5</sup> Difluoridates **1** and **2** can be prepared in very high yield in one-flask procedures with the usual precautions taken in the synthesis of potentially toxic organophosphorus compounds.



Scheme 1. Reagents i)  $NEt_3$ ,  $CH_3CN$ , r.t., ii)  $NaH$  or  $DBU$ , THF, r.t.

When the 5'-O-protected nucleosides were allowed to react with difluoridates **1** or **2** in the presence of  $NEt_3$  in the proportion 1:1, the nucleoside 3'-methylphosphorofluoridates **3** or the nucleoside 3'-methylfluoridophosphonothioates **5** are formed respectively in over 95% yield (Table 1). Both fluoridates **3** and **5** are formed as 1:1 mixtures of diastereomers. Attempts to separate fluoridates **3** into pure diastereomers failed: we assumed that separation was prevented by their high reactivity that favour fast ligand-ligand

exchange. In contrast the thioanalogues **5** are stable and were separated into „slow” and „fast” diastereomers using silica-gel column chromatography.

Coupling of the fluoridate **3** or **5** in the presence of DBU or NaH with 3'-O-protected nucleosides led to the dinucleosidemethylphosphonates **4** or dinucleosidemethylphosphonothionates **6** respectively. condensation Yields of dinucleosidemethylphosphonates **4** and **6** were over 95%.

Table 1. Nucleotide derivatives 3,4,5 and 6 with corresponding  $^{31}\text{P}$ -NMR<sup>1</sup> and  $^{19}\text{F}$ -NMR<sup>2</sup> data.

Comp.	X	R	R <sup>1</sup>	B	B <sup>1</sup>	$^{31}\text{P}(\text{J}_{\text{P-F}})$ [ppm, Hz]	$^{19}\text{F}(\text{J}_{\text{F-P}})$ [ppm, Hz]
3a	O	DMTr	-	Th	-	35.17, 26.51 (1052.191) 34.50, 25.81 (1055.751)	
3b	O	DMTr	-	Ad <sup>(Bz)</sup>	-	37.68, 24.69 (1052.413) 37.00, 23.98 (1055.424)	
4a	O	DMTr	DMTr	Th	Th	32.22, 31.66	-
4b	O	DMTr	DMTr	Ad <sup>(Bz)</sup>	Th	32.95, 30.85	-
5a	S	DMTr	-	Ad <sup>(Bz)</sup>	-		
Fast						111.56, 98.12 (1089.31)	-37.92, -42.93 (1089.20)
Slow						111.45, 97.99 (1089.67)	-36.72, -43.01 (1089.93)
5b	S	DMTr	-	Th	-		
Fast						111.56, 98.12 (1089.2)	-37.11, -42.87 (1096.23)
Slow						111.32, 97.84 (1092.6)	-37.45, -43.26 (1093.78)
5c	S	TBDMS <sub>1</sub>	-	Th	-		
Fast						110.97, 97.48 (1093.1)	-43.09, -37.75 (1094.19)
Slow						111.28, 97.85 (1089.04)	-42.46, -36.67 (1089.89)
6a	S	TBDMS <sub>1</sub>	DMTr	Th	Th		
Fast						97.80	-
Slow						98.48	-
6b	S	DMTr	DMTr	Ad <sup>(Bz)</sup>	Th		
Fast						97.90	-
Slow						99.21	-

1.  $^{31}\text{P}$ -NMR (CDCl<sub>3</sub>, 81 014 Mhz. H<sub>3</sub>PO<sub>4</sub> external standard)

2.  $^{19}\text{F}$ -NMR (CDCl<sub>3</sub>, 188 154 Mhz. CFCl<sub>3</sub> external standard)

To summarize, we were successful in developing a simple procedure which is useful for large-scale preparation of dimers **4** and **6**. Work is in progress to use the pure diastereomers **5** in the stereoselective preparation of dimers **6** and other derivatives.

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## References and Notes

- On leave from the Kazan State University, Kazan, Russia.
- Miller, P.S., Yano, J., Carroll, C., Jayaraman, K., Ts'o, P.O.P. *Biochemistry* **1979**, 18, 5134.
- Miller, P. Antisense oligonucleoside methylphosphonates. In: Murray, J.A.H., Ed. *Antisense RNA and DNA*. New York: Wiley-Liss, 1992, p. 241-253.
- Hogrefe, R.I., Reynolds, H.A., Vaghefi, M.M., Young, K.M., Riley, T.A., Klem, R.E., Arnold Jr, L.J. An Improved Method for the Synthesis and Deprotection of Methylphosphonate Oligonucleotides. In *Methods in Molecular Biology*, vol. 20. *Protocols for Oligonucleotides and Analogs*, Ed. by S.Agrawal, 1993, Humana Press Inc., Totowa, NJ, p. 143.
- Horner, L., Gehring, R. *Phosphorus and Sulfur* **1982**, 12, 295.
- Dąbkowski, W., Michalski, J., Wasiak, J., Cramer, F. *J.Chem.Soc., Perkin Trans. 1*, **1994**, 817.

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