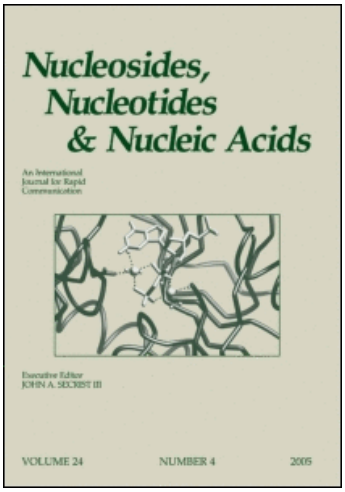

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Methylphosphonamidites: Preparation and Application in Oligodeoxynucleoside Methyl-Phosphonate Synthesis

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METHYLPHOSPHONAMIDITES: PREPARATION AND APPLICATION IN OLIGODEOXYNUCLEOSIDE METHYLPHOSPHONATE SYNTHESIS

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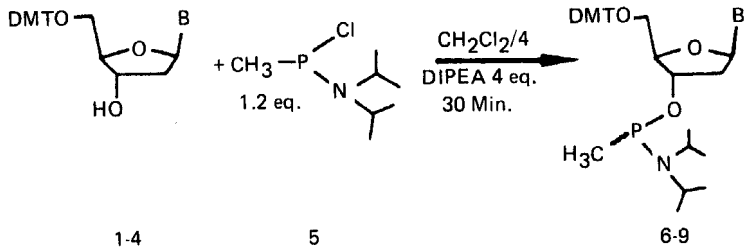
Abstract: The synthesis of methylphosphonamidites (Fig. 1) is described for all four deoxynucleosides. The T-amidte **6** was used on an automated DNA-synthesizer to show the usefulness in the solid phase synthesis of oligodeoxynucleoside methylphosphonates.

Anti-sense oligodeoxynuleotides bearing a methyl group at the phosphorous backbone are capable of inhibiting mRNA translation in mammalian cells. The inhibition was shown for vesicular stomatitis virus[1] and herpes simplex[2] protein synthesis in infected cells.

To synthesize oligomers of any given sequence and length in short time and high yield the solid phase approach is necessary. Recently these problems were partially solved by Miller with methylphosphon-imidazolides as synthetic intermediates[3]. Novel investigations showed the applicability of suitable protected 3'-O-(2'-deoxynucleoside) N,N-diisopropylamino methylphosphonamidites[4] (Fig. 1, **6-9**) to introduce methylphosphonate linkages into a oligodeoxynucleotide by solid phase synthesis[5,6].

The amidites were prepared closely related to the methods used for the synthesis of the corresponding alkoxy phosphoramidites[7,8]. The protected nucleosides (1 mmol) were diluted with stirring in dry CH_2Cl_2 containing 4 eq. diisopropylamin. A slight excess of chloro N,N-diisopropyl methylphosphine **5**[8] was added with a syringe at 4° according to Fig. 1. After 30 min. thin layer chromatography (aceton/hexane/triethylamine 50:50:1) revealed complete



1-4 5 6-9

B	T	A^bz	C^bz	G^Ibu
Nr.	1	2	3	4
	6	7	8	9

FIG. 1

T 6	A 7	C 8	G 9
120,6/121,1	120,2/122,0	121,2/122,8	119,7/1211

TAB.1: ^{31}P shifts (ppm) in CH_2Cl_2 versus 85% H_3PO_4

reaction. The solution was transferred with 100 ml CH_2Cl_2 into a separation funnel and extracted twice with 20 ml 5% aqueous $NaHCO_3$ and dried over Na_2SO_4. After concentration to a white foam the residue was dissolved in ethylacetate and precipitated into cold pentane (-60°), collected by filtration and dried in vacuo. The yield was between 50 and 85%. The amidites were satisfactory pure (>95%) as shown by ^{31}P-NMR (Tab. 1) and comparison with commercially available material. Impurities resulting from hydrolysis and oxidation were in the range of 1-3% per each.

Further purification is possible with flash chromatography on silica gel under N_2 pressure using the thin layer solvent conditions above. The amidites were stable as a solid at -20° for at least five month without detectable decomposition.

Solid phase synthesis was performed with an Applied Biosystems (Abi) DNA synthesizer (Model 380 A). The amidites were dissolved in CH_3CN as 94 mM solutions. For G 20% CH_2Cl_2 in CH_3CN was used to achieve solubility. A 10 fold molar excess of amidites were used per coupling step. The coupling time was succesfully reduced during our experiments starting with 60 seconds within published

STEP	TIME (min)
Deprotect with 3% TCA/CH_2Cl_2	5x0,16
Wash with CH_3CN	3,0
Couple with 94mM amidite in CH_3CN (20% CH_2Cl_2 for G)	0,5
Wash with CH_3CN	1,5
Cap with Ac_2O,DMAP,THF,Lutidine	1,0
Oxidation with J_2,H_2O,Lutidine,THF	0,5
Wash with CH3CN	2,0
	9,3

TAB.2 1 umol rection cycle for oligonucleoside methylphosphonates (Abi DNA-synthesizer Model 380 A and LCAA/CPG with 50umol/gr)

coupling times (1 min[5] / 2 min[6]). The final coupling time of 30 sec. is the same as used for our β-cyanoethyl phosphoramidites. The coupling yields per step were 96-97% using the trityl essay and commercially available 500 Å LCAA/CPG material attached with the first nucleoside. The whole cycle is shown in Tab. 2.

The described oligomer below (Fig. 3) bears a phosphate group at the 5'-end between the first and second nucleoside. This in general increase solubility and allows either phosphorylation of the 5'-OH for sequencing or purification with ion exchange columns or PAA-gel electrophoresis[3].
Cleavage and deprotection from the support was done with 0,4 ml ethylendiamine (EDA)/ethanol at 55° for 50 minutes. After concentration to dryness several coevaporations with ethanol are necessary to remove all traces of EDA. Otherwise degradation occurs by dilution with water. Using Tr-ON chemistry the resulting oligomer was easily separated with RP-HPLC from chain scissions (Fig. 2). After deprotection with 80% AcOH (0,4 ml,20 min,20°C the final oligomer was obtained after RP-chromatography. The different mobilities of the TR-ON (ret. time 23,2 min) and the final TR-OFF (ret. time 16.5 min) oligomer are compared in Fig. 3).

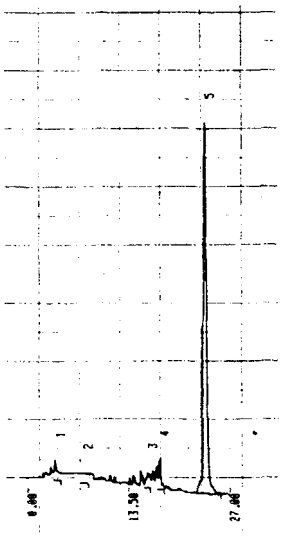


FIG.2 DMTO-ATpTpTpTpTpTpTpT-OH
p = Methylphosphonate

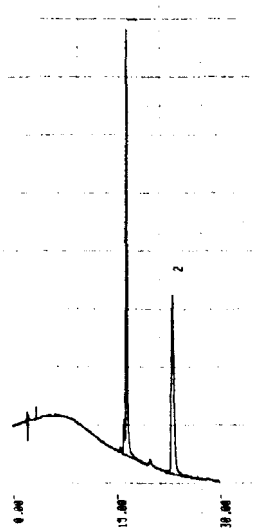


FIG.3 DMTO-ATpTpTpTpTpTpTpT-OH (2)
HO-ATpTpTpTpTpTpTpT-OH (1)

HPLC: Waters Delta Prep 3000, u-Bondapack C-18 3,9x250 mm,
Gradient 5-50% CH_3CN/TEAA 0,1m pH 7/ 30 min/ 1,2 ml/min

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