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A New DMAP-Catalyzed Phosphonamidite Coupling Reaction for Synthesis of Oligonucleotide Methylphosphonate Derivatives

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Abstract: 4-N,N-dimethylaminopyridine (DMAP) catalyzed coupling of 5'-dimethoxy-tritylthymidyl-3'-methylphosphonoamidite and 5'-trifluoroacetyl-3'-acetylthymidine and subsequent oxidation produce dinucleoside methyl phosphonate in a new, less water sensitive coupling reaction.

Oligodeoxynucleoside methylphosphonates [1-4] are a new class of sequence specific inhibitors of gene expression potentially useful as antiviral or anticancer agents [5]. While pursuing synthetic work in this area we have developed a new nucleophile catalyzed phosphonoamidite coupling reaction indicated in Scheme 1. This scheme is based on the reaction of p-cresol trifluoroacetate with N,N-diethyl ethyl-phenylphosphinamide observed by Horner [6]. The activated nucleoside component for coupling, 5'-trifluoroacetyl-3'-acetylthymidine (**2**), was prepared by the reaction of thymidine 3'-acetate and trifluoroacetic anhydride in acetonitrile. The reaction is complete within five minutes, and is easily followed by ^{1}H NMR spectroscopy because of the 0.7 ppm downfield chemical shift for 5'- and 5"-protons due to 5'-esterification [7]. Compound 2 was purified by silica gel HPLC using toluene-acetonitrile (3:2) as eluent. Before the coupling reaction all components were dried by evaporation from anhydrous acetonitrile.

After 2.5 hrs of coupling, ^{31}P NMR indicated that the reaction mixture contained 11% starting monomer **1**, 73% dimer product **3**, 10% oxidized dimer **4**, and 6% of several byproducts, including hydrolyzed starting monomer. With prolonged reaction time the amount of by-products significantly increased. After oxidation with iodine, the diastereomers of dimer **4** were purified and isolated by silica gel HPLC. The structures were confirmed by NMR spectroscopy and by the comparison of HPLC retention time with those for authentic samples. N,N-diisopropyltrifluoroacetamide was not isolated but its presence in the reaction mixture was confirmed by ^{19}F NMR spectroscopy by comparing the ^{19}F chemical shift with that of an authentic sample.

The conditions for the reactions carried out are given in Table 1. It is clear that DMAP shows a dramatic accelerating effect on the reaction rate (5 days vs 3 hours). The advantage of this route is that trace amounts of water, sufficient to cause immediate total hydrolysis of starting phosphoroamidite **1**, via the typical tetrazole catalyzed route [3,4] lead to only small amounts of hydrolysis in the DMAP-catalyzed process. Tertiary amines also protect the starting phosphonoamidite **1** from hydrolysis but

Scheme 1

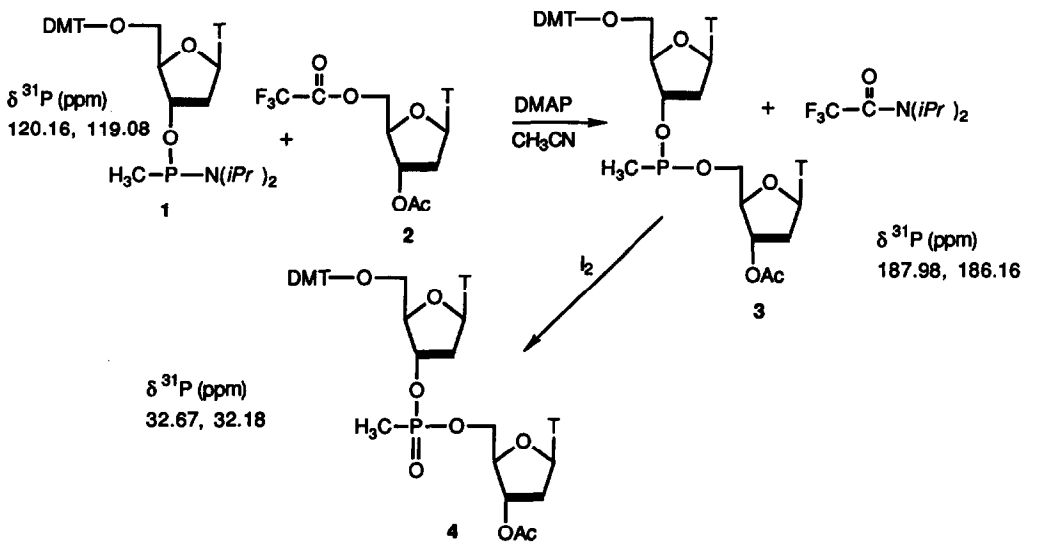


Table 1. Concentrations (M) and completion times for coupling reactions.

[1]	[2]	[DMAP]	[Tetrazole]	[DPEA][a]	Time (hrs)[b]
.061	.086	-	-	-	18
.061	.086	-	-	.35	>200
.061	.086	.18	-	-	3-4
.061	.086	-	.18	-	.1[d]
.42	.84[c]	.42	-	-	1

All reactions were run in acetonitrile at 24°C.

[a] diisopropylethylamine

[b] Approximate time for completion.

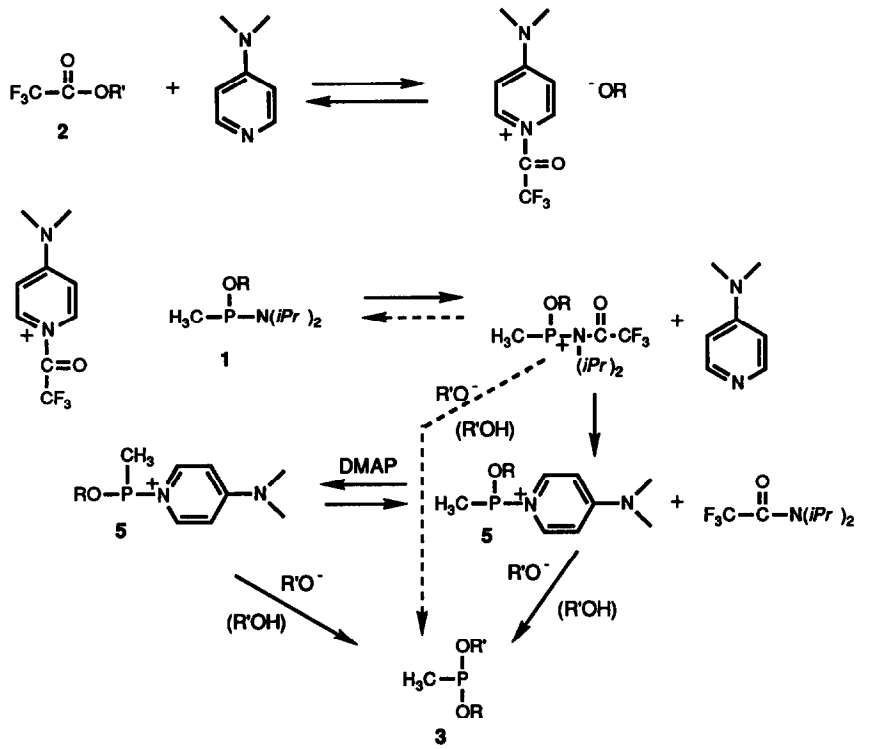
[c] 1-trifluoroacetyl-2-cyanoethanol, prepared in situ from 2-cyanoethanol (.84 M) and trifluoroacetic anhydride (.84 M) in CH_3CN.

[d] No coupling, but only hydrolysis observed.

dramatically reduced the rate of coupling. It is worthwhile to note that the rate of the coupling reaction of **1** with 3'-acetylthymidine is at least 100 times slower in the presence of DMAP than the same reaction with 5'-trifluoroacetyl-3'-acetylthymidine. This slow reaction probably represents a trace acid catalyzed route.

The stereospecificity of the coupling reaction (Scheme 1) was also of great interest due to the observed retention of configuration in the reaction of Horner [6]. However the reaction of chromatographically resolved diastereomers of compound **1** [8] in both catalyzed and uncatalyzed routes led to an equimolar mixture of diastereomers of dimer **3**. The same stereochemical result has been

Scheme 2



observed before for the typical tetrazole-mediated reaction [9-11], and is probably due to multiple displacement at phosphorus in intermediate **5** (Scheme 2). No racemization of the individual starting diastereomers was observed during the coupling reaction in the presence of DMAP (or tertiary amine) indicating that racemization occurred only after activation of phosphonoamidite 1. Scheme 2 indicates plausible pathways for DMAP-catalyzed reactions consistent with the observed lack of racemization of the phosphonamidite **1**. Further stereochemical studies will be pursued.

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7. ^{1}H NMR, ppm. dT(Ac) (4.6 mg in 330 µL CD_3CN: H6, 7.468; 5-CH_3, 1.900; H1', 6.101; H2',2", 2.25-2.43; H3', 5.175; H4', 4.035; H5',5", 3.724; $COCH_3$, 2.093. (CF_3)COdT(Ac) (4.6 mg in 330 µL CD_3CN plus 50 µL $(CF_3CO)_2O$): H6, 7.307; 5-CH_3, 1.925; H1', 6.101; H2',2", 2.37-2.59; H3', 5.231; H4', 4.299; H5',5", 4.591; $COCH_3$, 2.124.

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