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Stereospecific Coupling Reaction for Internucleotide Methyl Phosphonothioate Linkage

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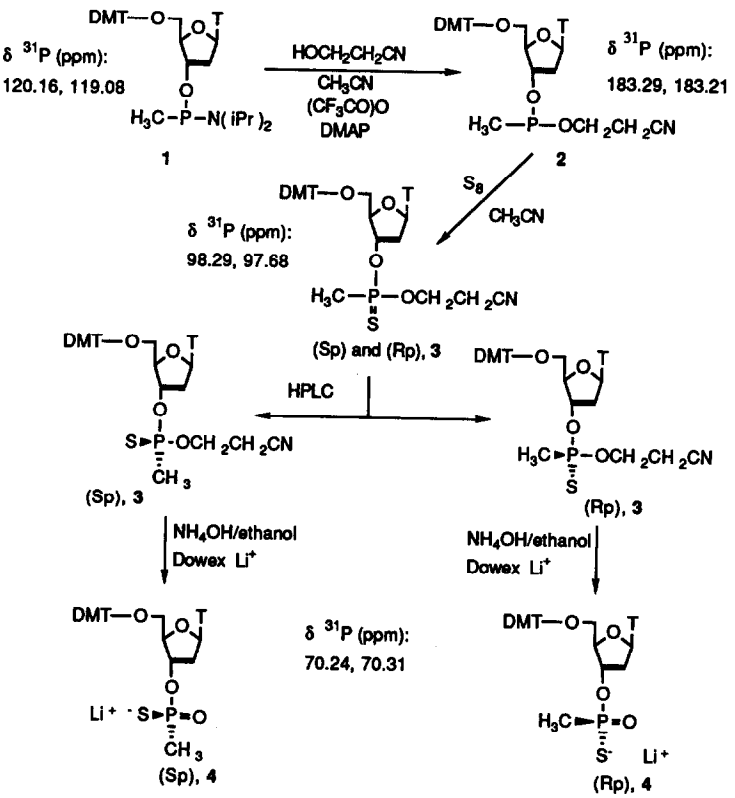
Abstract: The (Sp)- and (Rp)- diastereomers of 5'-dimethoxytritylthymidyl-(3'-5')-methylphosphono-5'-thio-3'-acetylthymidine were prepared by the stereospecific reaction of 5'-deoxy-5'-iodo-3'-acetylthymidine with the (Sp)- or (Rp)- diastereomers of 5'-dimethoxytritylthymidyl-3'-methylphosphonothioate (Li^+ salt) in DMF. At 50°C, the coupling reaction was complete within 6 hours.

Oligodeoxynucleotide derivatives with uncharged internucleotide groups, such as methylphosphonates[1] and alkyl phosphotriesters[2-4], are useful as probes of nucleic acid interactions with proteins and nucleic acids, as diagnostic probes, and as therapeutic agents[5-7]. These derivatives penetrate cell membranes easily and are highly resistant to nucleases[8-11]. However, this type of modification generates a chiral center at the phosphorus atoms, yielding nonequivalent diastereomeric oligodeoxynucleotide derivatives. Hybridization of the above oligodeoxynucleotide derivatives with complementary sequences of nucleic acids is highly dependent on the absolute configuration of the phosphorus centers[12-16]. Hence, stereoselective coupling reactions are being developed in several laboratories[17,18]. In this paper we report a stereospecific synthesis of P-S-C(5') modified dideoxynucleoside methylphosphonate.

First, both (Rp)-and (Sp)-isomers of the key intermediate, 5'-dimethoxytritylthymidyl-3'-methylphosphonothioate[19] (Li^+ salt) **4** were prepared according to scheme 1. 2-cyanoethylation of **1** was carried out in the presence of 4-(N,N-diethylamino)-pyridine (DMAP) and trifluoroacetic anhydride[20]. The reaction was followed with ^{31}P NMR spectroscopy; the chemical shifts of the reactants and products are given in scheme 1. The diastereomers of **3** were purified and separated by silica HPLC into two components designated **3-fast** and **3-slow**. The absolute configurations have not been determined. The cyanoethyl groups were removed with concentrated ammonium hydroxide: ethanol (1:2). The deprotected monomers were purified by silica HPLC. The ammonium cation was exchanged with Li^+ by using a Dowex 50W x 2 column, yielding the products **4-fast** and **4-slow**, whose structures were confirmed by ^1H NMR spectroscopy. 5'-deoxy-5'-iodo-3'-acetylthymidine (**5**) was prepared by reaction of 3'-acetylthymidine and methyltriphenoxyphosphonium iodide in DMF[21].

4 was coupled with either CH_3I or **5** according to scheme 2, similarly to the synthesis of 5'-thio

Scheme 1



Scheme 2

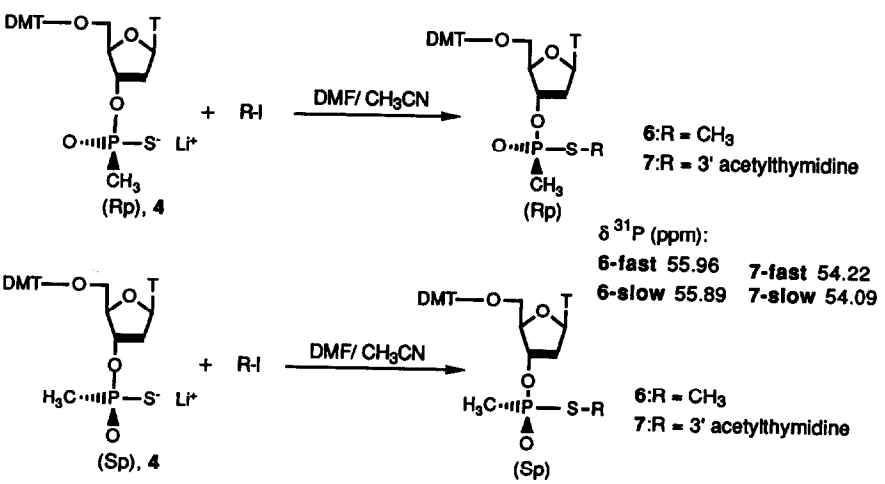


Table 1: Dependence of Coupling Rate on Concentration and Temperature.

[4]		[5]	[CH_3I]	Temp.(°C)	Completion Time
0.032	**fast**	0.033	-	24	>10 days
0.096	"	-	0.26	24	0.5 hrs
0.033	**slow**	0.033	-	24	>10 days
0.093	"	-	0.26	24	0.5 hrs
0.141	"	0.19	-	24	4 days
0.030	"	0.064	-	24	8 days
0.070	"	0.095	-	50	<6 hrs
0.035	"	0.076	-	50	<6 hrs
0.141	"	0.19	-	50	<6 hrs

For coupling with **5**, solvent was DMF; for coupling with CH_3I, solvent was acetonitrile, and **4-fast** and **4-slow** were ammonium salts.

oligodeoxynucleotides[22]. The coupling reaction with CH_3I was completed within 30 minutes . The products **6** were purified by silica HPLC with 2% EtOH in $CHCl_3$, yielding either **6-fast** (7 min.) or **6-slow** (10 min.) whose structures were confirmed by ^1H NMR spectroscopy.

The rate of coupling of **4** with **5** was shown to be greatly dependent on temperature and concentration (Table.1). At low concentrations ([**4**]= [**5**]= 0.03 M) and room temperature, the reactions required more than 10 days for completion. However, at room temperature and high concentrations ([**4**]= 0.14 M, [**5**] = 0.19 M) the reaction was complete in less than four days. In contrast, at 50°C, the reactions were complete within 6 hrs., at all concentrations tested. The products were purified by silica HPLC with 3% EtOH in $CHCl_3$ yielding either **7-fast** (8 min.) or **7-slow** (10 min.), whose structures were confirmed with ^1H NMR spectroscopy. Although 50-60% yields of **7** were obtained as dry powders after purification and precipitation, the ^{31}P NMR and silica HPLC data implied that the coupling reaction was nearly quantitative. Even with the very long reaction time there were no indications of by-products in ^{31}P NMR spectra.

In both coupling reactions, with either CH_3I or **5**, **3-fast** yielded only **6-fast** or **7-fast**, while **3-slow** yielded only **6-slow** or **7-slow**, without racemization. Similar stereospecific coupling was obtained for the reaction of 5'-dimethoxytrityl-thymidyl-3'-methylthiophosphonate (prepared from separated diastereomers of the S-aryl derivative[23]) and 5'-tosylthymidine in the presence of sodium iodide or tetrabutylammonium iodide as a catalyst[18], but the rates of the reactions were at least 10 times slower.

The next goals of our research are to develop a scheme for solid phase synthesis following the same reaction pathway and to develop an alternative scheme that yields a methylphosphonate P-O-C(5').

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