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NEW APPROACH TO THE SYNTHESIS OF DEOXYNUCLEOSIDE-PHOSPHORAMIDITE DERIVATIVES

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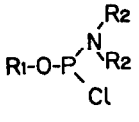
Summary
A new method for the synthesis of deoxyribonucleoside-phosphoramidite via an intermediate, 5'-O-dimethoxytritylthymidine-3'-O-diisopropylamino-phosphorochloridite, is reported. By using this method, several deoxyribonucleoside phosphoramidite derivatives with different protecting groups at phosphorus including P-S and P-N bond besides P-O bond were synthesized.

The phosphite-triester method for the oligodeoxynucleotide synthesis was introduced by Letsinger in 1975[1]. This technique was applied to the rapid synthesis of oligodeoxynucleotides[2] or mixed oligodeoxynucleotides[3] on polymer support. However the unstability of the activated intermediate (nucleoside phosphorochloridite derivative) to the moisture remained unsolved. The nucleoside-phosphoramidite derivatives were synthesized to overcome this problem[4]. Especially, diisopropylamino or morpholino-phosphoramidite derivatives are stable and even can be isolated by the silica gel column chromatography[5]. As to the protecting group, various phosphorus protecting groups[6] can be used though methyl group is generally used. Various phosphorodichloridite was prepared from phosphorus trichloride and corresponding alcohol, and converted to the phosphoramidite derivatives (I) by adding the corresponding amine. 3'-Hydroxy group of 5'-O-dimethoxytritylthymidine was then reacted with (I) to obtain 5'-O-dimethoxytritylthymidine-3'-phosphoramidite. The alkoxy group in (I) was then replaced.



(I)

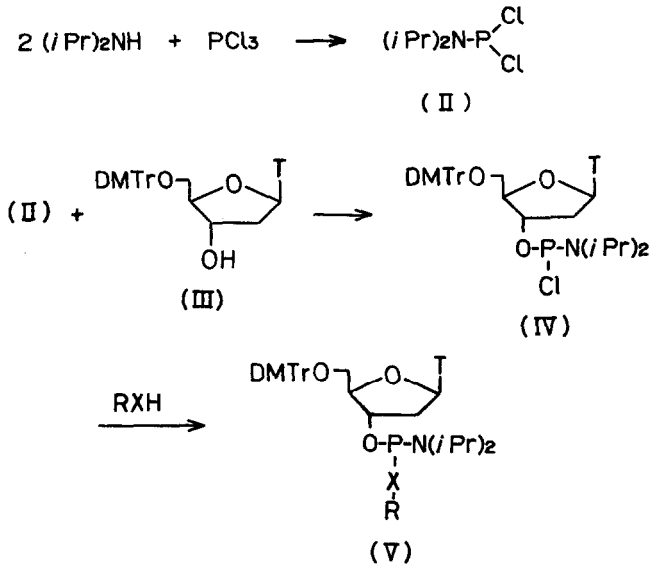


Figure.1. The scheme for the synthesis of compound (Ⅴ)

Table 1. The yields and analyses of compound (Ⅴ)

	X	R	Yield (%)	^{31}P-NMR
Ⅴ-a	O	-CH_3	71	146.5 , 145.9
b	O	-C_6H_4Cl(o)	81	145.0 , 144.6
c	O	-CH_2CH_2CN	57	148.5 , 148.2
d	O	-CH_2CCl_3	67	148.5 , 148.3
e	O	-$C(CH_3)_2CCl_3$	57	139.3 , 138.9
f	O	-$CH_2C_6H_5$	70	145.4 , 145.1
g	S	-C_6H_5	38	165.5 , 164.3
h	NH	-C_6H_5	64	114.8 , 114.5

Here, we will describe the versatile procedure for the synthesis of nucleoside phosphoramidites with various protecting group at phosphorus. Using this method, we demonstrate the synthesis of several nucleoside phosphoamidite derivatives. At first we prepared the intermediate phosphitilating reagent, diisopropylphosphordichloridite (II), as showed in Fig. 1. To a dry ether(70 ml) solution of phosphorus trichloride (21.8 ml, 0.25 mol) was added a dry ether (70 ml) solution of diisopropylamine (70.1 ml, 0.5 ml) with stirring at -10°C for 2.5 hr followed by additional 1 hr at r.t. After the precipitated salt was filtered off, the mixture was distilled at 72-73°C/7 mmHg to give compound (II) in 67 % yield. This compound shows as a single peak at 166.7 ppm on ^{31}P NMR spectroscopy[7]. We use this phosphitilating reagent (II) to synthesize 5'-O-dimethoxytrityl-thymidine-3'-O-o-chlorophenyl, diisopropylphosphoramidite (V b). To a CH_2Cl_2 (2 ml) solution of (II) (450 µl) containing isopropylethylamine (1 ml), a CH_2Cl_2 (3 ml) solution of dimethoxytritylthymidine (III) (0.5 mmol) was dropped slowly over a period of 10 min at -10°C under N_2. The mixture was stirred for additional 30 min., then o-chlorophenol (510 µl) in CH_2Cl_2 (3 ml) was added to the mixture. After 30 min stirring, the desired product (V b) was purified by silica gel column chromatography in 80% yield as white solid after the precipitation into n-hexane from its solution in CH_2Cl_2. This compound has more than 95 % purity judging from ^{31}P NMR spectra. IV b was further characterized by ^{1}H NMR and elemental analysis. The another dimethoxytritylthymidine phosphoramidite derivatives (V a-f) were also prepared with the same procedure as described in the above experiment. Methanol, 2,2,2-trichloroethanol, 2,2,2-trichloro,1,1-dimethylethanol, β-cyanoethanol and benzylalcohol were used instead of o-chlorophenol. The reaction was complete within 30 min except the bulky alcohol. In the case of 2,2,2-trichloro-1,1-dimethylethanol, the reaction was prolonged to 1 hr. After work-up and purification by silica gel column chromatography, compound (V a-f) were obtained as a white solid in yields of 57-81 %. The relatively low yield of compound (V) was due to the formation of dimer with 3'-3' linkage, which was separated by silica gel column chromatography, at the intermediate (IV) formation stage. To prevent the formation of dimer, it is essential that dimethoxytritylthymidine was added slowly to the reaction mixture. Another reason of the low yield of compounds (V) was their solubility in n-hexane even at low temperature. All these products were analyzed by ^{31}P NMR spectroscopy. Results are summarized in Table 1.

We investigated the synthesis of the analogues of nucleoside phosphoramidite derivatives which have P-S or P-N bond. After the intermediate compound (IV) was formed as shown in Fig. 1, thiophenol, or aniline diluted in CH_2Cl_2 was added to the reaction mixture. After 30 min

stirring and work-up, the mixture were separated by silica gel column chromatography to give compounds (V g,h) in yields of 38, and 64 % respectively. These compounds were characteized by ^{1}H and ^{31}P NMR.

In summary, using diisopropylaminophosphordichloridite (II), 5'-O-dimethoxytritylthymidine-3'-O-phosphoramidite derivatives which have various protecting groups at phosphorus could be synthesized in one-pot reaction. We suppose this procedure will make it easier to synthesize oligonucleotides with different type of protecting groups at phosphorus. With this technique, we will be able to make a modified oligonucleotides at phosphorus as well as common oligonucleotides.

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7) ^{31}P NMR spectra was measured in $CDCl_3$. Trimethylphosphate was used as the external standard.

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