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New Approach to the Synthesis of Deoxyribonucleoside Phosphoramidite Derivatives

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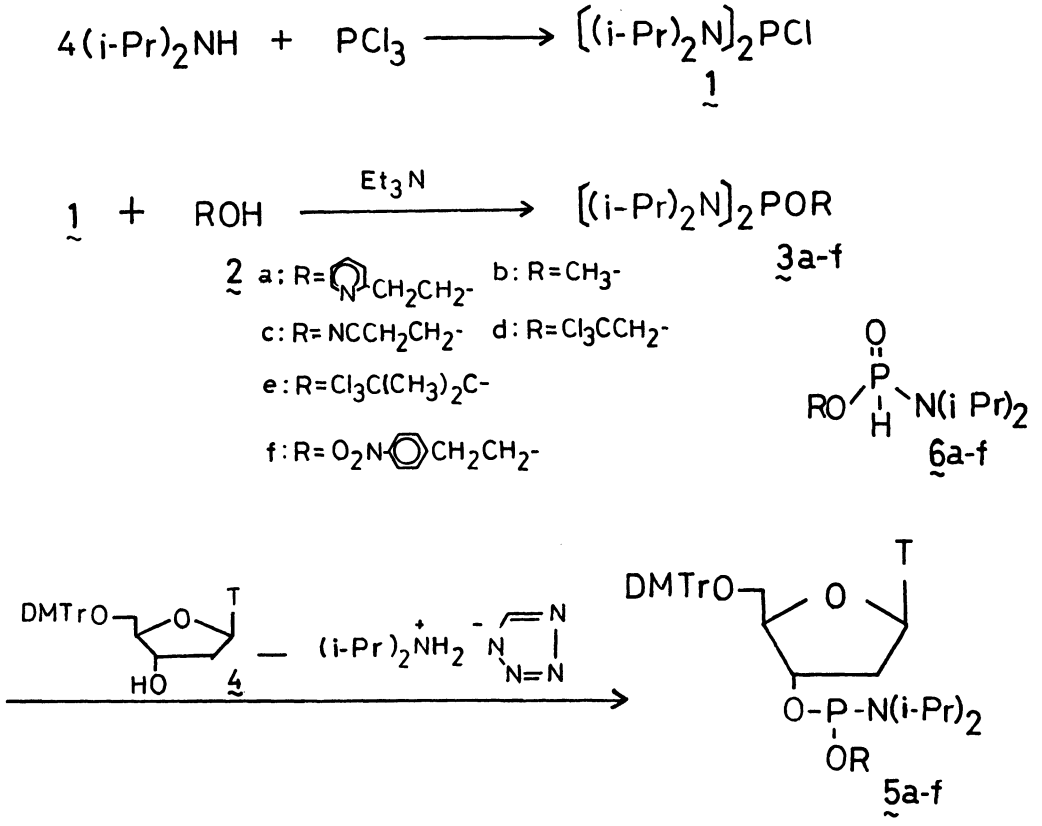
The deoxyribonucleoside phosphoramidites with various protecting groups at phosphorus have been prepared rapidly in good yields in one-pot reaction from bis(diisopropylamino)chlorophosphine as a new phosphitylating agent without isolation of bis(diisopropylamino)alkoxyphosphines.

The introduction of deoxyribonucleoside phosphoramidite derivatives by Caruthers[1] has lead to the most suitable synthetic method in polymer supported oligodeoxyribonucleotide synthesis. A new phosphitylating agent, bis(dialkylamino)alkoxyphosphines for synthesizing the deoxyribonucleoside phosphoramidites as reported by Beaucage[2a] and Caruthers[2b] made it more effective. Although, these phosphoramidite derivatives can be prepared by existing method from the appropriately protected deoxyribonucleoside and bis(dialkylamino)alkoxyphosphines, dichloroalkoxyphosphines used in forming bis(dialkylamino)alkoxyphosphines, are easily react with trace amounts of water and air.[1a] Moreover, bis(dialkylamino)alkoxyphosphines are not easy to prepare in view of their unstability during purification by distillation which requires very high vacuum.[3] We have recently solved this problem by developing a high efficient synthetic method and a new phosphitylating agent, bis(diisopropylamino)chlorophosphine (1) for the synthesis of deoxyribonucleoside phosphoramidites with various protecting groups at phosphorus. This phosphitylating agent 1 is more stable than dichloroalkoxyphosphines, and is stored for several months at -20 °C.

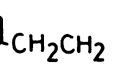
At first we prepared the phosphitylating agent, bis(diisopropylamino)chlorophosphine (1) as shown in Scheme 1. A solution of phosphorus trichloride (25.5 ml, 0.29 mol) in dry ether (50 ml) was added dropwise to a solution of diisopropylamine (162 ml, 1.15 mol) in dry ether (500 ml) with continuous stirring and cooling in an ice bath. After 30 min, the reaction mixture was gradually warmed to the room temperature and then boiled under reflux for 12 h. After cooling to the room temperature, the reaction mixture was filtered, and the precipitated salt was washed with ether. The filtrate and washings were combined and concentrated. The residue was fractionally distilled from cesium fluoride[1b] to give 92 g (60%) of 1: bp 129-133 °C/1 mmHg; ^{31}P-NMR ($CDCl_3$, 85% H_3PO_4) 139.3 ppm. Found: C, 40.01; H, 7.82; N, 15.42; Cl, 19.50%. Calcd for $C_6H_{14}N_2PCl$: C, 39.89; H, 7.81; N, 15.51; Cl, 19.63%.

Next, the new phosphitylating agent 1 was examined for a new approach to the synthesis of 5'-O-dimethoxytritylthymidine-3'-O-alkyl-N,N-diisopropylamino phosphoramidites (5). To a dry ether solution of 1 (2.66 g, 1.0 mmol/10 ml), 2-pyridylethanol (1.12 ml, 1.0 mmol) and triethylamine (1.33 ml, 1.0 mmol) were added by using a microsyringe at 0 °C under a nitrogen atmosphere. The reaction mxiture was gradually warmed to the room temperature and stirred for 16 h. Petroleum ether was subsequently added and the precipitated salt was removed by filtration. All the volatile components of the filtrate were removed under high vacuum. The residue, bis(diisopropylamino)-2-pyridylethoxyphosphine (3a) (purity as estimated by ^{31}P-NMR[4] was 97%) was treated with 5'-O-dimethoxytritylthymidine (4) (359 mg, 0.66 mmol) in the presence of diisopropylamine hydrotetrazolide[2b] (113 mg, 0.66 mmol) in dry CH_2Cl_2 (3 ml) at room temperature. After 30 min, TLC analysis showed complete conversion of strating material 4 [Rf 0.25, methylene chloride-ethyl acetate-triethylamine (45:45:10, v/v)] into Rf 0.57 material. The solution was washed with aqueous saturated solution of NaCl (3 X 10 ml). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The residue was applied to a column of silica gel and eluted with a methylene chloride-ethyl acetate-triethylamine (45:45:10, v/v). The appropritate fractions of 5'-O-dimethoxytritylthymidine-3'-O-2-(2-pyridyl)ethyl-N,N-diisopropylamino phosphoramidite (5a)[5] was concentrated under a high vacuum and 5a was isolated as a white powder (522 mg, 89%) by precipitation from cold hexane (-78 °C).



Scheme 1.

Table 1. Synthesis of 5'-O-Dimethoxytritylthymidine-3'-O-Alkyl-N,N-Diisopropylamino Phosphoramidite Derivatives (5a-f)

	Molar ratio of $[(i\text{-}Pr)_2N]_2POR$/DMTrT/Diisopropylamine hydrotetrazolide		Isolated yield %	^{31}P-NMR (ppm) ($CDCl_3$)
5a	1.00 []	/1.00/0.05	50	147.4, 147.1
	1.50	/1.00/0.05	70	
	1.50	/1.00/0.50	72	
	1.50	/1.00/1.00	89	
	2.00	/1.00/2.00	83	
	1.50	/1.00/1.00 (collidine hydrochloride)	52	
5b	1.50 [CH_3]	/1.00/1.00	85	148.5, 148.1
5c	1.50 [$NCCH_2CH_2$]	/1.00/1.00	80	148.5, 148.2
5d	1.50 [Cl_3CCH_2]	/1.00/1.00	85	148.7, 148.3
5e	1.50 [$Cl_3C(CH_3)_2C$]	/1.00/1.00	81	139.4, 139.1
5f	1.50 [$(p)O_2NC_6H_4CH_2CH_2$]	/1.00/1.00	83	147.8, 147.3

The yields of 5 depended on a molar ratio of the phosphitylating agent 3, nucleoside 4, and diisopropylamino hydrotetrazolide as an activator. The best result was obtained when the reaction was carried out with a molar ratio of 1.5:1.0:1.0 (2/4/diisopropylamine hydrotetrazolide). The ^{31}P-NMR spectra of the above reaction mixture clearly demonstrated the selectively of the phosphitylation. In addition to the main peaks characteristic of 5a, there was a minor peak at 13.4 ppm assigned to phosphoamidous acid 6a resulting from hydrolysis of 3a in the ^{31}P-NMR spectra. No traces of 3',3'-dinucleoside phosphite triester was observed. A use of activator such as collidine hydrochloride[6] in place of diisopropylamine hydrotetrazolide gave poor yield of 5a (Table 1).

In a similar manner, other 5'-O-dimethoxytritylthymidine 3'-O-alkyl-N,N-diisopropylamino phosphoramidite derivatives (5b-f)[7] were obtained in good yields as shown in Table 1. It is found that this procedure is much more effective for the synthesis of 5 than the procedure reported by Beaucage[2a] and Caruthers.[2b] The purity of the procucts was checked by ^{31}P-NMR (more than 95%). Furthermore, the ^{31}P-NMR spectra of 5 (Table 1) showed two signals corresponding to a diastereomeric mixture of phosphoramidites.

In conclusion, we have established a new approach to the synthesis of 5'-O-dimethoxytrityldeoxyribonucleoside-3'-O-phosphoramidite derivatives with

different type of protecting groups at phosphorus in one-pot reaction from 1 without isolation of bis(diisopropylamino)alkoxyphosphines. Further, our new phosphitylating agent 1 is easy to prepare and is more stable under the normal laboratory conditions than other dichloroalkoxyphosphines.

This paper is dedicated to Professor Morio Ikehara for the occasion of his retirment from Osaka University on March, 1986.

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