

New Approach to the Synthesis of Deoxyribonucleoside
Phosphoramidite Derivatives

Shoji HAMAMOTO and Hiroshi TAKAKU*

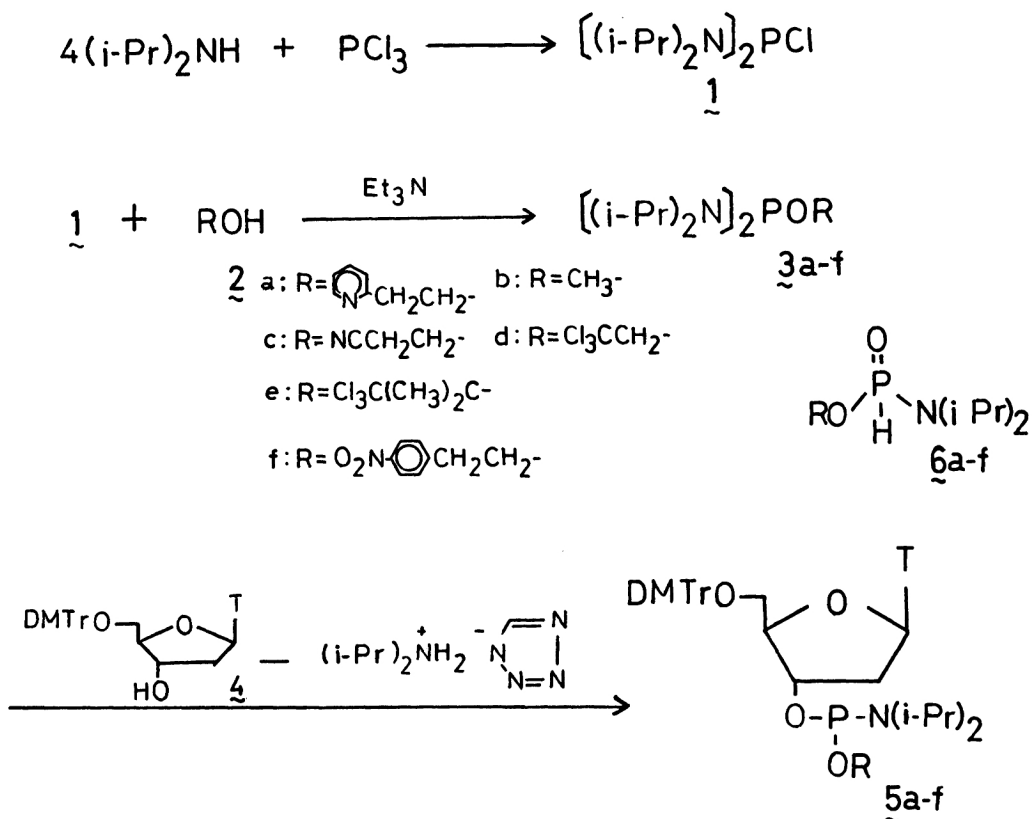
Laboratory of Bioorganic Chemistry, Chiba Institute of
Technology, Tsudanuma, Narashino, Chiba 275

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 Caruthers¹⁾ 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; ³¹P-NMR (CDCl₃, 85% H₃PO₄) 139.3 ppm. Found: C, 40.01; H, 7.82; N, 15.42; Cl, 19.50%. Calcd for C₆H₁₄N₂PCl: 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 mixture 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 $^{31}\text{P-NMR}^4$) was 97%) was treated with 5'-O-dimethoxytritylthymidine (4) (359 mg, 0.66 mmol) in the presence of diisopropylamine hydrotetrazolide^{2b)} (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 starting 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 appropriate 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-Pr) ₂ N] ₂ POR/DMTrT/Diisopropylamine hydrotetrazolide		Isolated yield %	³¹ P-NMR (ppm) (CDCl ₃)
5a	1.00 [ -CH ₂ CH ₂]	/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 ₂ CH ₂]	/1.00/1.00	80	148.5, 148.2
5d	1.50 [Cl ₃ CCH ₂]	/1.00/1.00	85	148.7, 148.3
5e	1.50 [Cl ₃ C(CH ₃) ₂ C]	/1.00/1.00	81	139.4, 139.1
5f	1.50 [(p)O ₂ NC ₆ H ₄ CH ₂ CH 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 ³¹P-NMR spectra of the above reaction mixture clearly demonstrated the selectivity 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 ³¹P-NMR spectra. No traces of 3',3'-dinucleoside phosphite triester was observed. A use of activator such as collidine hydrochloride⁶⁾ 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 products was checked by ³¹P-NMR (more than 95%). Furthermore, the ³¹P-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 retirement from Osaka University on March, 1986.

This research was supported by a Grant-in-Aid for Scientific Research from a Ministry of Education, Science and Culture.

References

- 1) a) S. L. Beaucage and M. H. Caruthers, *Tetrahedron Lett.*, 22, 1859 (1981); b) L. J. McBride and M. H. Caruthers, *ibid.*, 24, 245 (1983).
- 2) a) S. L. Beaucage, *Tetrahedron Lett.*, 25, 375 (1984); b) A. D. Barone, J.-Y. Tang, and M. H. Caruthers, *Nucleic Acids Res.*, 12, 4051 (1984).
- 3) J.-L. Fourrey and J. Varenne, *Tetrahedron Lett.*, 24, 1963 (1983), *ibid.*, 25, 4511 (1984); N. D. Sinha, J. Biernat, J. McManus, and H. Köster, *Nucleic Acids Res.*, 12, 4539 (1984); J. Nielson, J. E. Marugg, J. H. van Boom, J. Honnens, M. Taagaard, and O. Dahl, *J. Chem. Res. (S)*, 1986, 26.
- 4) ³¹P-NMR spectroscopy of 3a shows two peaks: a major peak at 129.3 ppm assigned to 3a and a minor peak at 140.0 ppm assigned to 1.
- 5) H. Takaku, T. Watanabe, and S. Hamamoto, submitted to *J. Org. Chem.*.
- 6) A, J, Jäger and J. Engels, *Tetrahedron Lett.*, 25, 1437 (1984).
- 7) Theoretically expected C, H, and N analyses were obtained for these compounds 5a-f. 2-(2-Pyridyl)ethyl (5a),⁴⁾ methyl (5b),^{a)} 2-cyanoethyl (5c),^{b)} 2,2,2-trichloroethyl (5d),^{c)} 2,2,2-trichloro,1,1-dimethylethyl (5e),^{d)} and 4-nitrophenylethyl (5f),^{e)} are used as the phosphate protecting groups for the oligodeoxyribonucleotide synthesis by the phosphite-triether method; a) E. E. van Tamelen and S. U. Daub, *J. Am. Chem. Soc.*, 99, 3526 (1977); M. D. Matteucci and M. H. Caruthers, *Tetrahedron Lett.*, 21, 719 (1980); b) N. D. Sinha, J. Biernat, and H. Köster, *ibid.*, 24, 5843 (1983); c) R. L. Letsinger, J. L. Finna, G. A. Heavner, and W. B. Lunsford, *J. Am. Chem. Soc.*, 97, 3278 (1975); K. K. Ogilvie and M. Nemer, *Can. J. Chem.*, 58, 1389 (1980); d) R. L. Letsinger, E. P. Groody, and T. Tanaka, *J. Am. Chem. Soc.*, 104, 6805 (1982); e) A. H. Beiter and W. Pfeleiderer, *Tetrahedron Lett.*, 25, 1307 (1984).

(Received June 17, 1986)