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# BIS-(N,N-DIALKYLAMINO)-ALKOXYPHOSPHINES AS A NEW CLASS OF PHOSPHITE COUPLING AGENT FOR THE SYNTHESIS OF OLIGONUCLEOTIDES

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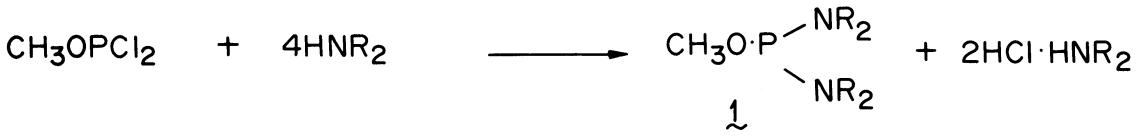
A new class of bifunctional phosphitylating agent which is stable but readily activatable for the efficient synthesis of oligonucleotides is described.

The phosphite triester method for the preparation of the internucleotide bonds is simple and fast.[1-4] The method involves the reaction of various mono- or bi-functional phosphite coupling agents[5-8] toward the 3' or 5' free hydroxyl group of suitably protected nucleosides, and thus a search of the effective coupling agents has been of the utmost importance in the synthesis of oligonucleotides. Although there have been reports of several classes of phosphite coupling agents,[7-10] there still remain some limitations in their practical applications. A serious problem in the use of these coupling agents is the inconvenient and costful preparations of individual coupling agents for each nucleoside applied.

In this communication, we report a new class of phosphorodiamidites, 1, bis-(N,N-dialkylamino)-methoxyphosphines, as a hitherto unrecognized class of simple, convenient and versatile coupling agent for the synthesis of oligonucleotides, especially in the solid phase synthetic system. In our approach, the highly reactive chlorines of methyl dichlorophosphite were replaced by dialkyl substituted secondary amines, and thus rendering the reagent less reactive in much the same way as for nucleoside phosphoramidites and tetrazolides.[6-9]

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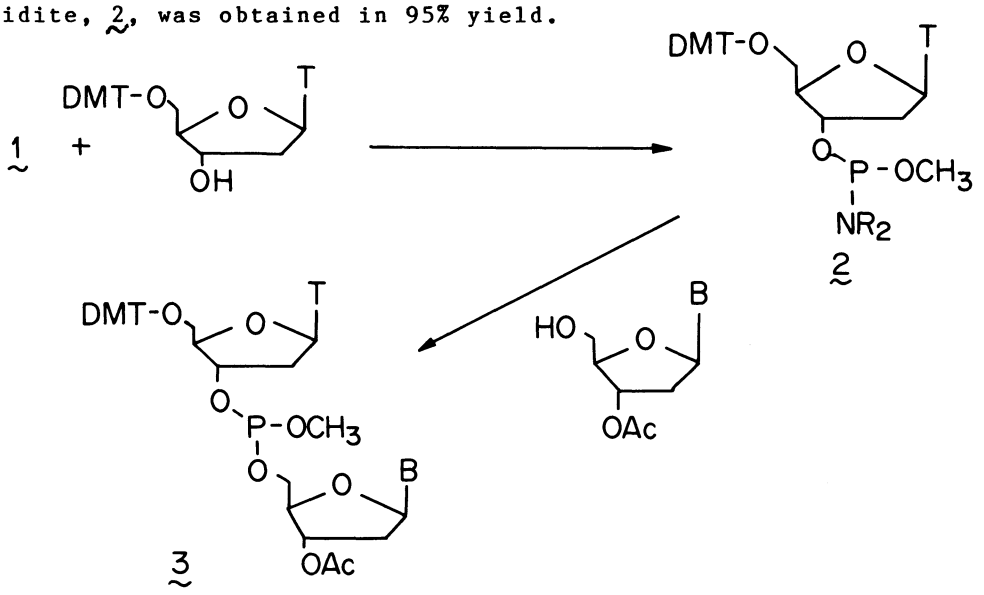
The major advantages of our new phosphitylating agent in the oligonucleotide synthesis are: 1) the new coupling agent is relatively stable under laboratory condition and is easy to prepare, 2) the synthetic system involving our new agent

requires only the readily available starting materials such as suitably protected nucleosides, 3) the coupling reaction is very fast and efficient in the presence of weakly acidic heterocyclic base such as 1H-tetrazole at room temperature.

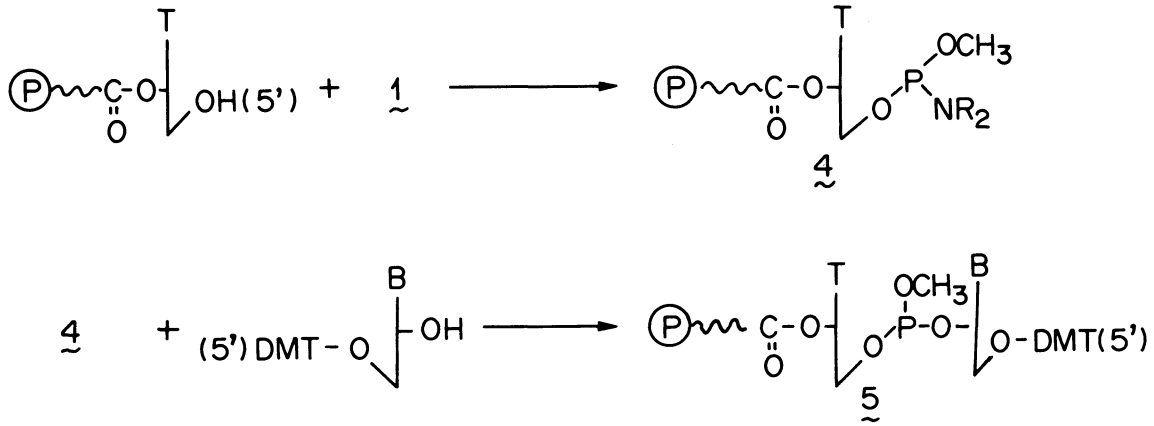
The new phosphitylating agent, 1, was prepared conveniently from the reaction of methyl dichlorophosphite with 4 molar equivalents of a series of dialkylamines ( R = methyl, ethyl or isopropyl) in anhydrous ether at room temperature. The resulting amine hydrochloride salt was removed by filtration, and the ethereal fraction was distilled under reduced pressure to give an oily product of 1. The yields and boiling points of the new coupling agents 1 were as follows: 1a, R = methyl, 80%, bp 60-62 °C (40 Torr); 1b, R = ethyl, 85%, bp 96-102 °C (50 Torr); 1c, R = isopropyl, 78%, bp 86-88 °C (30 Torr). For a valid comparison, 1a was also prepared from the reaction of tetramethyl phosphorodiamidous chloride with methanol in the presence of 2,4-lutidine.[11] The $^1$H-NMR of 1a showed a doublet at 3.8 ppm with J = 12 Hz and two singlets at 2.8 and 2.6 ppm. The purity and stability of 1 were demonstrated by phosphorous-NMR based on the δ value of -138 ppm ($CDCl_3$) with respect to the internal standard of 5% aqueous phosphoric acid (v/v). Our new agent showed a reasonable stability toward hydrolysis and air oxidation, and thus can be stored for several months under nitrogen atmosphere without any change.

The reaction of the agent 1a with the 3' or 5'-OH group of suitably protected deoxynucleosides yielded the corresponding nucleoside phosphoramidites essentially in quantitative yield in the presence of 1H-tetrazole. When an equimolar mixture of 1a and 5'-O-dimethoxytrityl thymidine (5'-ODMT-dT) is treated with 1.4 molar equivalents of 1H-tetrazole in acetonitrile at room temperature, the nucleoside phosphoramidite, 2, was obtained in 95% yield.



Further reaction of the intermediate, 2, with 3'-O-acetyl thymidine (3'-OAc-dT) in 1H-tetrazole/acetonitrile led to the formation of a 3',5'-dinucleoside phosphite triester, 3, which was identified as 3',5'-(TpT) by HPLC analysis using Lichrosorb Si-60 column after usual oxidation and deprotection procedures.[8)]

In addition, a trial experiment for the solid phase synthesis of dinucleotide was also performed on the derivatized silica gel.[12)] At the start, the 3'-OH group of 5'-ODMT-dT was attached covalently to the carboxy end of the derivatized silica gel (Vydak TP, 100mg) by the DCC coupling method, and the resulting gel was treated with 0.2M zinc bromide in nitromethane to remove the DMT blocking group from the 5' position of the gel bound nucleoside.




In the next step, the gel-bound nucleoside was suspended in anhydrous acetonitrile (5 mg gel/ml), and was treated with 5 μmol each of the agent 1a and 1H-tetrazole under nitrogen gas. The reaction mixture was shaken for 10 min, and then excess amounts of 1a and tetrazole were removed by filtration. After washing the gel twice with acetonitrile, the gel-bound nucleoside phosphoramidite, 4, was obtained in pure form. Further reaction of 4 with excess amounts of a series of suitably protected 5'-ODMT-deoxynucleosides in 1H-tetrazole/acetonitrile at room temperature resulted in the formation of the gel-bound 3',5'-dinucleoside phosphite ester, 5, which in turn, was converted easily to the corresponding internucleoside phosphate ester by mild iodine oxidation.[8)] Up to this stage, the yield of the gel-bound dinucleotide was about 95% based on the analysis of released dimethoxytrityl cations at 498 nm. By the treatment of the gel-bound dinucleotides with concentrated $NH_4OH$ at 50 °C for 12 h followed by treating with 80% acetic acid, the 3',5'-dinucleotides were obtained in 80-90% yields, and their purities were confirmed by reverse phase HPLC analysis and by computer simulated UV spectrum analysis.

In addition, the validity of our new coupling agent was also demonstrated by the preparation of a series of oligonucleotides; i.e., the yields of the solid phase synthesis for d(TpT), d(TpTpT), d(GpTpTpT), d(ApGpTpT), and d(ApApApTpTpT) were 85, 80, 82, 75, and 78 %, respectively.

In conclusion, our new phosphitylating agent displays its potential utility as a class of the most convenient and efficient bifunctional phosphitylating agent for the synthesis of oligonucleotides, especially in the solid phase synthetic system. Its preparation is very simple, and does not require any costful nucleosides. Furthermore, the new agent is found to be quite stable under normal laboratory conditions, but is activatable readily in the presence of heterocyclic bases such as 1H-tetrazole, and thus its reaction toward the suitably protected nucleosides led to the formation of the 3',5'-oligonucleotides in good yields.

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