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N,N-Diisopropyl-O-p-nitrophenyl-P-methylphosphonoamidite: Novel Difunctional P^{III} Reagent in Oligonucleoside Methylphosphonate Synthesis Containing 4-Nitrophenoxy Group

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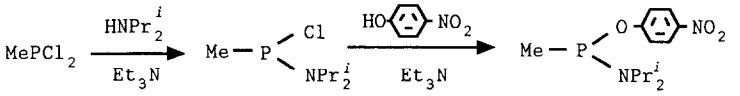
Abstract: First example of the P^{III} coupling reagent containing 4-nitrophenoxy group is described. Its usefulness is demonstrated by synthesis of dinucleoside methylphosphonate and methylthiophosphonate.

The biological importance of oligonucleoside methylphosphonates is well established[1]. In order to widen the scope of reactions leading to this class of compounds we became interested in phosphitylating reagents containing the p-nitrophenoxy group.

The p-nitrophenoxy group attached to the tetracoordinate phosphorus center exhibits a high propensity to act as a leaving group in nucleophilic displacement reactions. This ability has been widely used in phosphorus chemistry and biochemistry.[2] In contrast potentialities connected with a use of the p-nitrophenoxy group in phosphitylation procedures have not been previously disclosed.

A very efficient synthesis of 3',5'-dithymidyl methylphosphonate and its sulfur analogue is reported herein as an illustration of a novel internucleoside coupling procedure based on tricoordinate phosphorus reagents containing the p-nitrophenoxy group. Therefore we used the N,N-Diisopropyl-O-p-nitrophenyl-P-methylphosphonoamidate **1** which is a stable crystalline compound and is readily available by the following one-flask procedure:

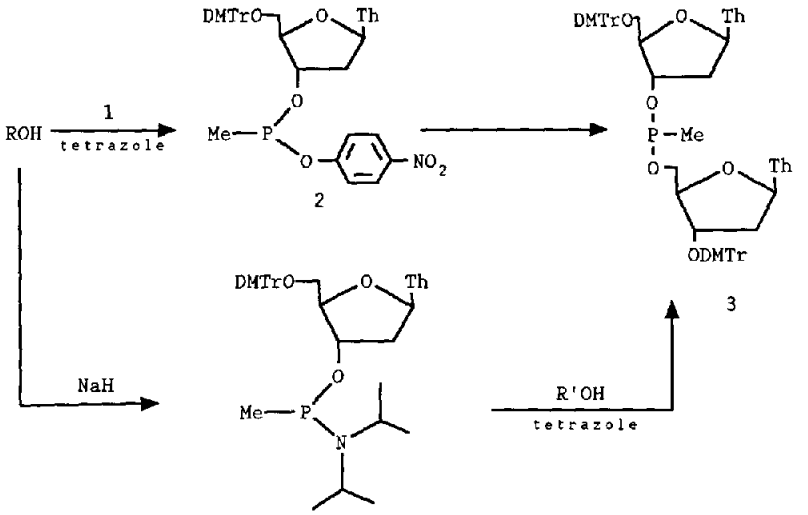


1

The total yield of the crystalline pure **1** is over 90%. This difuncionnal reagent is stable at ambient temperature and can be stored without any sign of decomposition due to deoxygenation of the nitro group by the tricoordinate phosphorus.[3]

Two pathways of employing **1** as coupling reagent are herein demonstrated. The first one is based on an activation of the diisopropylamino

group by the tetrazole[4] and subsequent condensation with the 5'-protected thymidine, leading to the compound 2. The second condensation of 2 with the 3'-protected thymidine in the presence of sodium hydride[5] leads to the methylphosphinate 3. Both reactions are very efficient and total yield is almost quantitative.

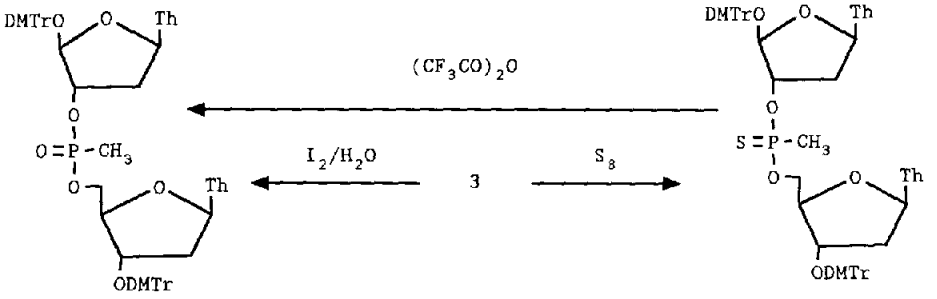


ROH: 5'-O-dimethoxytritylthymidine

R'OH: 3'-O-dimethoxytritylthymidine

Scheme 1

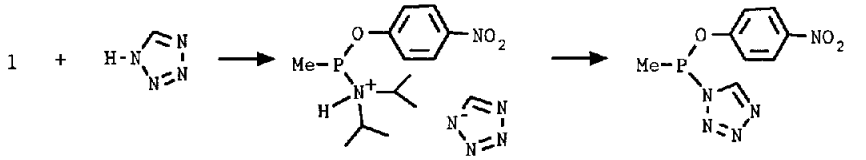
The methylphosphinite 3 can be efficiently oxidized by iodine-water system.[4] Fast addition of elemental sulfur leads to the methyltiophosphonate 6 in quantitative yield.



Scheme 2

The methylphosphonate 5 can also be obtained by the reaction of 6 with trifluoroacetic anhydride.[6] Structure assignment for compounds presented

in the Schemes 1 and 2 is straightforward. Compounds 3, 4, 5 and 6 are known and their properties correspond to those described by other authors.[7] The p-nitrophenylmethylphosphinate 2 was fully characterized by its ^{31}P n.m.r. spectrum. Advantage was taken of the fact that liberation of the p-nitrophenoxide ion can be followed by light absorption in the characteristic visible region. The reaction of the phosphonoamidite 1 with tetrazole proceeds via two discrete steps 7 and 8 which were clearly observed by ^{31}P N.m.r. spectroscopy.[8] The 8 acts as phosphitylating



7 8

δ ^{31}P n.m.r. 181.6 ppm δ ^{31}P n.m.r. 186.6 ppm

reagent and its signal ceases to be visible after addition of nucleoside. Both pathways A and B do not need isolation of the intermediate mononucleoside and can performer as one flask procedure. In conclusion we have described the first example of P^{III} coupling reagent containing p-nitrophenoxy leaving group. We are investigating similar reagents of potential applicability in nucleotide chemistry.
It seems that this method can be applied in the authomatic oligonucleotides synthesis.

N,N-Diisopropyl-O-p-nitrophenyl-P-methylphosphonoamidite 1: A solution of diisopropylamine (0.01 M) and triethylamine (0.01 M) in dry THF (10 ml) was added dropwise to a solution of dichloromethylphosphine (0.01 M) at -10°C with stirring which was continued 1h at r.t. A solution of 4-nitrophenol (0.01 M) and triethylamine (0.01 M) in dry THF (20 ml) was added at r.t. with vigorous stirring for 2h. The precipitated triethylamine hydrochloride was filtered off. The filtrate was evaporated to dryness and the crude 1 was purified by column chromatography [Kieselgel 60, Et_2O:n-pentane:NEt_3 50:30:5 v/v R_f: 0.4] to give N,N-diisopropyl-O-p-nitrophenyl-P-methylphosphonoamidite 1 [δ ^{31}P (C_5D_5N): 128.7 ppm, m.p. 60-62°C]. Yield of the isolated 1: 95%.

3'-[5'-O-4,4'-dimethoxytriphenylmethylthymidyl]-5'-(3'-O-dimethoxytriphenylmethyl)thymidine methyl phosphinate 3. Route A. The reagent 1 (1.1 mM) was treated with 5'-O-dimethoxytritylthymidine (1.0 mM) in presence of the tetrazole (1.0 mM) in dry THF (30 mL) at r.t. The reaction was monitored by ^{31}P n.m.r. spectroscopy and completed within 0.5 h. The precipitate of diisopropylamine tetrazolide was filtered off. The solution of 2 was added dropwise at r.t. for 0.5 h to the 3'-O-dimethoxytritylthymidine (1 mM) and NaH (1 mM) suspended in dry THF (30 mL). After 0.5h sodium 4-nitrophenolate was filtered off. The filtrate was evaporated to give 3 [δ ^{31}P (C_5D_5N): 184.0, 186.2 ppm 1:1]. Quantitative yield (^{31}P n.m.r.).

Route B. The 5'-O-dimethoxytritylthymidine (1 mM) and NaH (1 mM) were suspended and stirred in dry THF (30 mM). A solution of the **1** (1.1 mM) at r.t. was added dropwise. The reaction was completed within 0.5h. The sodium 4-nitrophenolate was filtered off. The ^{31}P n.m.r. [$\delta^{31}P$ (C_5D_5N): 119.2, 119.9 ppm, 1:1]. Quantitative yield of **4** (^{31}P n.m.r.). A solution of 3'-O-dimethoxytritylthymidine (1.0 mM) and tetrazole (1.0 mM) in dry THF (30 ml) was added at the r.t. to the solution of **4** with stirring. After 0.5h precipitate of diisopropylamine tetrazolide was filtered off and the filtrate evaporated to give **3** [$\delta^{31}P$ (C_5D_5N): 184.0, 186.2 ppm 1:1], 95% by ^{31}P n.m.r. spectroscopy.

3'[5'-O-4,4'-dimethoxytritylphenylmethyl)thymidyl]thymidine methylphosphonates **5**. Route A. A solution of **3** (1 mM) in dry THF (10 mL) was treated with 0.1 M of iodine in THF-pyridine-H_2O (4:3:3 v/v) for 15 min. The crude **5** was pure by TLC on silica gel in CH_2Cl_2:EtOAc:Et_3N (9:9:2) v/v) and characterized by ^{31}P n.m.r. [$\delta^{31}P$ (C_5D_5N): 32.5, 33.0 ppm, 1:1]. Quantitative yield (^{31}P n.m.r.).

Route B. P-methylphosphorothionate **6** was prepared by addition of equivalent amount of sulfur in dry THF solution [$\delta^{31}P$ (C_5D_5N): 97.4, 98.0 ppm, 1:1]. Quantitative yield (^{31}P n.m.r.). A solution of **6** (1.0 mM) in dry THF (10 mL) was allowed to react with $(CF_3CO)_2O$ (1.0 mM) at r.t. and kept overnight. The crude methylphosphonate **5** was purified by TLC on silica gel in CH_2Cl_2:EtOAc:Et_3N (9:9:2 v/v). The ^{31}P n.m.r. spectroscopy showed two signals corresponding [$\delta^{31}P$ (C_5D_5N): 34.4, 33.0 ppm, 1:1].Yield 95% (^{31}P n.m.r.).

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