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New Phosphitylating Reagent in the Nucleotide Chemistry Containing Two 4-Nitrophenoxy Leaving Groups. Remarkably Fast and Clean Phosphitylations Activated by DBU Leading to Thio- and Seleno-oligonucleotides

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Abstract: A new synthetic approach to modified oligonucleotides based on N,N'-diisopropyl-di-(4-nitrophenyl)phosphoroamidite 1 is described. The procedure involves displacement of either 4-nitrophenoxy or diisopropylamino ligands. The former proceeds very fast in the presence of DBU, the latter requires activation by tetrazole. This method allows rapid and almost quantitative construction of modified nucleotides derived from thio- and selenophosphorus acids.

Phosphitylating reagents in oligonucleotides synthesis have been widely used since the classic work of Letsinger.[1] Important improvements have been made by Caruthers[2] who introduced N,N-diisopropylamidites. In the Letsinger-Caruthers synthesis the amidite moiety acts as stabilizing factor and, when activated by tetrazole[2] or amine hydrochlorides,[3] as leaving group. Our recent experience shows that the desired stability of a phosphitylating reagent can be achieved by the presence of the 4-nitrophenoxy group which is remarkably readily displaced by nucleosides (alcohols) in the presence of a proper base. In our recent publications phosphitylating reagents containing one 4-nitrophenoxy group and the diisopropylamino group attached to the P^{III} center have been described.[4,5] These reagents has been used in oligonucleoside methylphosphonate synthesis[4] and in the synthesis of thio- and seleno-oligonucleotides.[5] Internucleoside linkages have been constructed either with the assistance of tetrazole activation or sodium hydride activation of the nucleoside substrate. 4-Nitrophenoxide activation of the intermediate P^{III} system oxidations and its ability to act as demethylating agent toward >P(X)OMe systems (X=O,S,Se) have been an integral parts of this preparative procedure.[4,5] Very high yields have been noted in single steps and in the one-flask multi-step synthesis leading to oligonucleotides. However, the strategy has some disadvantages. Activation by sodium hydride of the alcohol substrate in some solvents may lead to clotting, and individual synthetic steps are completed within several minutes. These properties can be considered somewhat unfavorable for applications in polymer support authomathized methodology.

In this paper we report two novel features of our strategy: a) application of the readily available and stable phosphitylating reagent 1 containing two 4-nitrophenoxy groups and b) conditions for extremely efficient activation of a nucleoside substrate in phosphitylation procedures by reagents containing 4-nitrophenoxy group. We have found that the 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) promotes displacement of the 4-nitrophenoxy group remarkably well, and that the 4-nitrophenol/DBU system shows very useful properties for addition of elemental sulfur and selenium to P^{III} compounds and ability to demethylate >P(X)OMe systems (X=O,S,Se). All these reactions are almost quantitative and are completed within seconds at room temperature in acetonitrile solution.

The protocols of our previous papers[4,5] can be greatly improved by activating with DBU rather than sodium hydride. Individual steps in which DBU is involved are so rapid in acetonitrile solution that the time necessary to obtain target compounds is limited by the performance of preparative manipulations. The overall yields are almost quantitative.

The compound 1 is readily available and shows high stability. The unique feature of

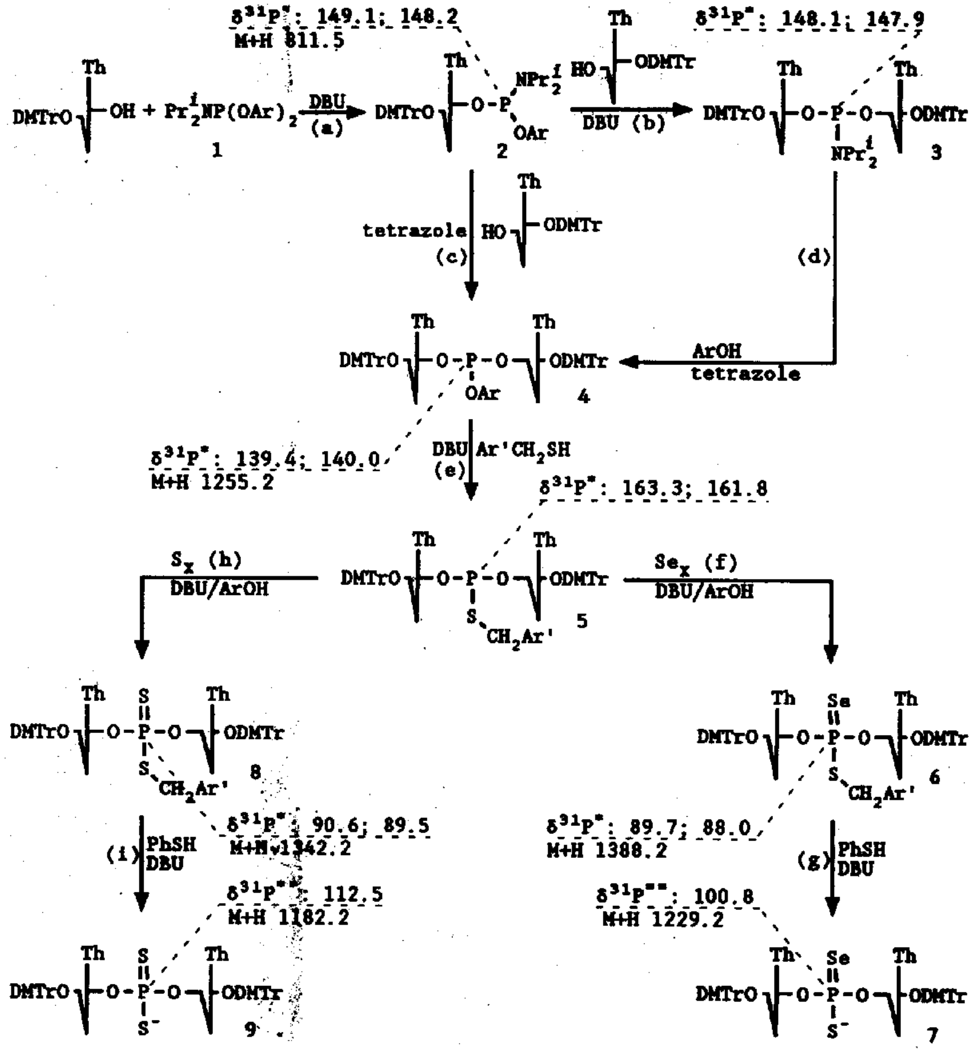
$$Pr^{i}_{2}N\text{-}PCl_{2} \xrightarrow{2\ ArONa} \underset{1}{Pr^{i}_{2}N\text{-}P(OAr)_{2}}$$

Ar –


1 is its ability to form internucleoside phosphite links by virtue of both 4-nitrophenoxy groups. Phosphitylation procedure by 1 in presence of DBU in acetonitrile solution proceeds stepwise in a fully selective manner. This desirable property allows highly effective coupling without tetrazole activation and leaves the diisopropylamino group available for further transformations. Application of the reagent 1 is illustrated in Scheme 1.

The activation by DBU is required in steps (a),(b),(e),(f),(g),(h) and (i). The phosphoroamidite 2 is a stable crystalline compound and can readily be obtained in bulk. The phosphoroamidite 3 prepared efficiently by our method is well known and can be used in a number of transformations.[6] Phosphitylations with an aid of diisopropylamino and 4-nitrophenoxy groups are complementary. The rates observed are distinctly in favour of the latter. The methodology described here can in our experience, be readily extended to formation of trimers and tetramers containing a variety of internucleoside linkages such as methylphosphonate, phosphate, thiophosphate, selenophosphate and thioselenophosphate. In spite of the preparative efficacy of the reactions, some details of the physical organic chemistry of this approach deserve better understanding. Typical reaction procedures are described below:

Preparation of N,N-Diisopropyl-di-O-4-nitrophenylphosphoamidite 1. The solution of N,N-diisopropyldichlorophosphoamidite (0.01 M) in dry THF (10 ml) was added dropwise at r.t. under a nitrogen atmosphere to the solution of sodium 4-nitrophenolate (0.025 M) in dry THF (50 ml) with stirring for 2 h. The sodium chloride was removed by filtration. The filtrate evaporated to dryness and the crude 1 purified by column chromatography [Kieselgel 60, Et_2O: n-pentane: triethylamine 50:30:5 v\v, Rf: 0.75] to give N,N-diisopropyl-di-O-4-nitrophenylphosphoamidite 1. [$\delta^{31}P$ ($CDCl_3$): 144.8 ppm; m.p. 120°-122°; pale yellow crystals, M^+: 407.2]. Yield of isolated 1: 95%.



Ar' = 2,4-dichlorophenyl
Ar = 4-nitrophenyl
Chemical shifts relative to external standard 85% H_3PO_4
* in $CDCl_3$
** in pyridine-d_5

Scheme 1

Synthesis of 5'-O-DMT-thymidine-3'-O-4-nitrophenyl-N,N-diisopropylphosphoamidite 2 (step a). The solution of 5'-O-dimethoxytritylthymidine (1.0 mM) and DBU (1.1 mM) in dry acetonitrile (10 ml) was added dropwise at r.t. under a nitrogen atmosphere to the solution of amidite 1 (1.1 mM) in dry acetonitrile (10 ml) with stirring for 10 min. The solution was evaporated to dryness and the crude 2 purified by column [Kieselgel 60, 1,2-dichloroethane: ethyl acetate 8:2 v/v Rf= 0.7] to give 5'-O-DMT-thymidine-3'-O-4-nitrophenyl-N,N-diisopropylphosphoamidite 2 [$\delta^{31}P$ $(CDCl_3)$: 149.1; 148.2; m.p. 105°-107°; pale yellow crystals. M+H (FAB MS) 812.5]. Yield of isolated 2: 95%.

One flask synthesis of dinucleosidylphosphoroselenothioate 7.

Step (a):	as described above.
Step (b):	the reaction of 2 with 3'-O-dimethoxytritylthymidine (1.1 mM) and DBU (1.1 mM) in acetonitrile (10 ml) gave 3 (1 min).
Step (c):	the reaction of 2 with 3'-O-dimethoxytritylthymidine (1.1 mM) in the presence of sublimed tetrazole (1.1 mM) in acetonitrile (10 ml) gave 4 (20 min.).
Step (d):	the reaction of 4-nitrophenol (1.1 mM) with 3 in the presence of sublimed tetrazole (2.2 mM) in acetonitrile (10 ml) gave 4 (15 min.).
Step (e):	the reaction of 2.4-dichlorbenzylmercaptan (1.1 mM) with 4 in the presence of DBu (2.2 mM) in acetonitrile (10 ml) gave 5 (30 sek).
Step (f):	addition of elemental selenium (1.1 mM) to 5 gave 6 (1 min.).
Step (g):	the reaction of 6 with thiophenol (1.1 mM) in the presence DBU (1.1 mM) gave 7 (10 h).

The reaction mixture was concentrated to dryness, dissolved in 1,2-dichloroethane, applied to a silica gel column, and fractionated using a gradient of methanol (0-20%) in 1,2-dichloroethane. The product 7 was isolated in 90% yield. By analogues protocol was synthesized dinucleosidylphosphorodithioate 9. All operations were performed at 20° in strictly dry acetonitrile under argon. Structures 6, 7, 8, 9 were confirmed by NMR spectroscopy and FAB mass spectroscopy.

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